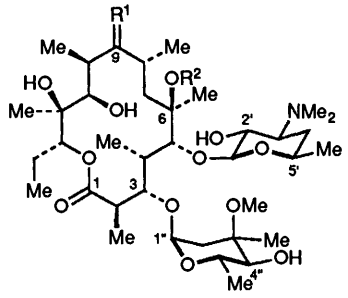
The Chemistry of Erythromycin. Reactions of Erythromycin A Imine and its 6-Methyl Ether with Aldehydes and Hydrazines

J. Sydney Davies, Eric Hunt,* and Iskander I. Zomaya
Beecham Pharmaceuticals, Research Division, Brockham Park, Betchworth, Surrey RH3 7AJ

Erythromycin imine (**3**) and its 6-methyl ether (**6**) are multifunctional *N*-unsubstituted imines, which, in contrast to most unsubstituted imines, are readily isolable and relatively stable towards hydrolysis. With aldehydes in ethanol, the imines react quite differently: the imine (**3**) reacts with aliphatic and aromatic aldehydes to give predominantly the 9,11-cyclic imines (**9**), whereas the ether (**6**) reacts with aliphatic aldehydes to give *N*-(1-ethoxyalkyl)imines (**13**) and with benzaldehyde to give a 9,12-epoxy Schiff's base derivative (**12**). The imines also differ in their reactivities towards hydrazine derivatives: the imine (**3**) readily reacts with monosubstituted hydrazines to form erythromycin hydrazone derivatives, whereas the ether (**6**), in common with erythromycin (**1**), is unreactive towards these reagents. A rationale for the different modes of reaction of compounds (**3**) and (**6**) is discussed.

Erythromycin A (**1**)† is one of the most important members of the macrolide group of antibiotics. It is also, in terms of its chemistry, one of the most studied of the natural macrolides,[1]



	R^1	R^2
(1)	O	H
(2)	NOH	H
(3)	NH	H
(4)	O	Me
(5)	NOH	Me
(6)	NH	Me

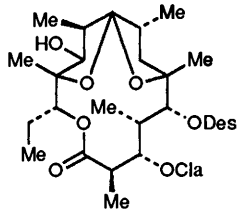
and in this respect reactions at the C-9 carbonyl group and subsequent modifications at this position have received considerable attention. Thus, the ketone (**1**) can be reduced using sodium borohydride[2] and the resulting 9-hydroxy group has been used in further chemical manipulations.[3–5] The C-9 carbonyl group also forms the normal carbonyl derivatives with hydrazine[2] and hydroxylamine,[6,8] but is inert towards semicarbazide and phenylhydrazine[2,7] and does not react with carbon nucleophiles.[3] Of the C-9 carbonyl derivatives the oxime (**2**) is probably the most useful. It has been used, for example, in the Beckmann rearrangement to give a series of ring-expanded 15-membered macrolides[9] and it can be reduced by the action of titanium(III) chloride to the imine (**3**).[7] This imine (**3**) is a crystalline, readily isolable compound and can itself be reduced using sodium borohydride[7] or sodium cyanoborohydride[10] to give the (9*S*)-amine (erythromycylamine A), which has also been used to make further derivatives.[11,12] Despite the fact that the imine (**3**) has been known for some time, its reduction to erythromycylamine is the only aspect of its chemistry which has been described. Our interest in this compound (**3**) was aroused by the fact that, in comparison with other unsubstituted imines, it is relatively stable towards hydrolysis, and thus offers a rare opportunity to study the chemistry of the unsubstituted imine group in a multifunctional compound. In this paper we describe some reactions of the 9-imino compound (**3**) and its 6-methyl ether derivative (**6**) with aldehydes and hydrazines.

Table 1. Half lives ($t_{0.5}$) for hydrolysis of imines (**3**) and (**6**) in aqueous methanol at 23 °C.

Compound	pH	$t_{0.5}$/h	Product
(**3**)	1.5	1.25	(**7**)
(**3**)	7	1.5	(**1**)
(**6**)	1.4	5.2	(**8**)
(**6**)	7	18.5	(**4**)
(**6**)	12.4	3.3	(**4**)

Erythromycin imine (**3**) was synthesised from the oxime (**2**) by the method of Timms and Wildsmith,[7] and the corresponding 6-methyl ether (**6**) was similarly prepared from the known[13] oxime (**5**). Both imines were readily isolated as stable, crystalline compounds, but were found to be susceptible to hydrolysis in aqueous solution. Thus, in aqueous methanol at pH 7, both imines were hydrolysed to their parent ketones, the hydrolysis of compound (**3**) being much more rapid than that of compound (**6**) (Table 1). The imine (**3**) was also hydrolysed quite rapidly in acid. In this case, the product was anhydroerythromycin A (**7**), which is also the product obtained from treating erythromycin (**1**) with dilute mineral acid.[14] Rather surprisingly, in the imine (**6**) the 9-imino group appeared to resist hydrolysis under acidic conditions. Although the imine (**6**) was hydrolysed at pH 1.4 (Table 1), the major product was not derived by hydrolysis of the 9-imino function but by hydrolytic cleavage of the neutral sugar to give the de-cladinosed 9-imine (**8**). Similar hydrolytic

† The erythromycin A structure consists of a 14-membered lactone aglycone, termed erythronolide A, substituted by a neutral sugar, L-cladinose, and an amino sugar, D-desosamine. Unprimed numbers are used for positions in the aglycone, primed numbers in the amino sugar, and double primed numbers in the neutral sugar. We use the abbreviations Cla for α-L-cladinosyl and Des for β-D-desosaminyl in structural formulae.



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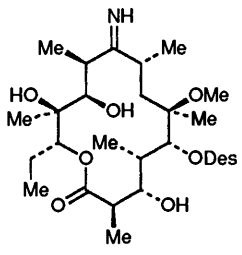
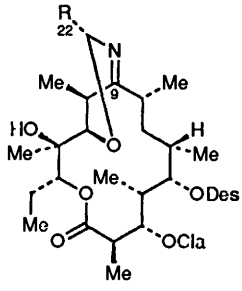


(8)

Cla = α-L-cladinosyl
Des = β-D-desosaminyl

cleavage of the neutral sugar has been reported for the ketone (**4**).[15]

The ^{13}C NMR spectrum of the imine (**3**) in $CDCl_3$ was quite complex and suggested that in this solvent the compound existed as a mixture of the 9-imino species (**3**) and at least two 9-amino ether tautomers [*e.g.* species (**14**) and (**15**)]. Thus, resonances which were attributed to C-9 were observed at δ 193.6 (9-imino structure) and at 98.8 and 95.3 (9-amino ether structures). Also, in addition to a cluster of signals at δ 75.8–74.8 owing to C-6 and C-12 carbons bearing hydroxy groups, two signals at δ 82.9 and 82.7 were assigned to C-6/C-12 carbons bearing ether oxygens. Groups of signals elsewhere in the spectrum, for example those owing to C-1 and C-3″, also pointed to the presence of three major species. In contrast to the above, the ^{13}C NMR spectrum of compound (**6**) showed only those signals expected for the 9-imino structure (**6**).



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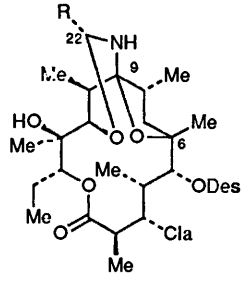


(10)

R
a; H
b; Me
c; Ph
d; *p*-MeC_6H_4
e; *p*-ClC_6H_4
f; *p*-$NO_2C_6H_4$
g; *p*-$MeOC_6H_4$

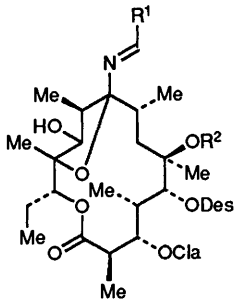
* The ^{13}C NMR spectrum of erythromycin (**1**) also shows signals for tautomeric species (hemiacetals) in addition to those for the ketone (**1**).[16] In this case, however, the signals owing to the hemiacetal structures are very much less pronounced. The spectrum of the ketone (**4**), on the other hand, shows no evidence for the presence of tautomeric forms.

† In compounds (**10**) the stereochemistry at C-9 is not known for certain, although in each case only one isomer appears to be present. From molecular modelling, the (9*S*) configuration would be suggested as being most likely.

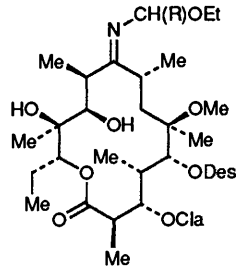
‡ In compounds (**9b–g**) and (**10b–g**) the stereochemistry at C-22 is not known for certain, although in each case only one isomer appears to be present. By analogy with (9*S*)-9-*N*,11-*O*-[2-(2-methoxyethoxy)ethylidene]erythromycylamine A, which has been examined by X-ray crystallography,[17] the (22*R*) configuration would be suggested.

§ In compounds (**11**) and (**12**) the stereochemistry at C-9 is unknown, although in each case only one isomer is produced.

Turning to the chemistry of the imines (**3**) and (**6**), we first investigated their reactions with aldehydes. Thus, erythromycin imine (**3**) reacted with aqueous formaldehyde in ethanol to give the 9,11-cyclic imine (**9a**) in 79% yield. The ^{13}C NMR spectrum of this product showed that in $CDCl_3$, compound (**9a**) is in tautomeric equilibrium with the 6,9-epoxy-9,11-cyclic amino ether (**10a**),† with the ratio of tautomers being *ca.* 1:1. Other aldehydes reacted analogously to give the 9,11-cyclic derivatives (**9b–g**) and (**10b–g**).‡ With benzaldehyde and 4-methylbenzaldehyde, minor products were also isolated from these reactions and were identified as the 9,12-epoxy Schiff's base derivatives (**11**).§ These reactions of the imine (**3**) have some parallels with the reactions of erythromycylamine A with aldehydes:[12] with aliphatic aldehydes erythromycylamine A gives 9,11-cyclic amino ethers, and with aromatic aldehydes it gives Schiff–base derivatives.



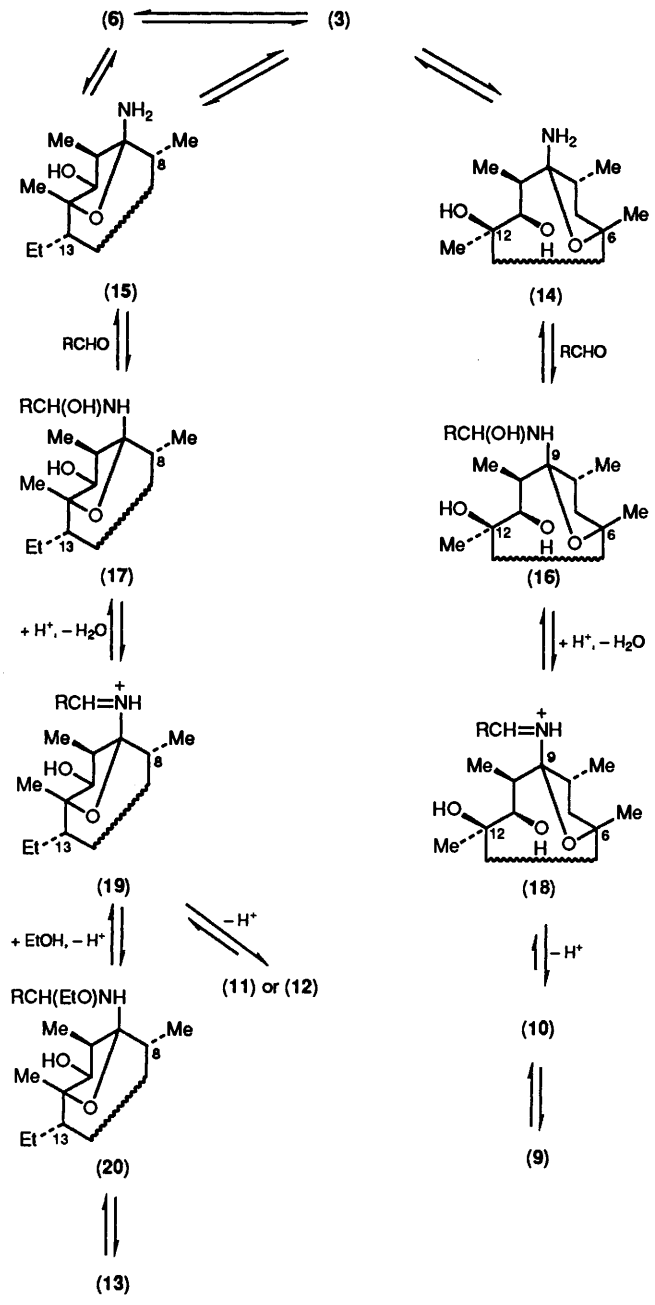
	R^1	R^2
(**11a**)	Ph	H
(**11b**)	*p*-MeC_6H_4	H
(**12**)	Ph	Me



(**13a**) R = H
(**13b**) R = Me

The reactions of the 6-methyl ether 9-imine (**6**) with aldehydes appeared to take a different course to those of compound (**3**). Thus, with aqueous formaldehyde in ethanol, compound (**6**) gave the *N*-ethoxymethylimine (**13a**), and with acetaldehyde in ethanol it gave the *N*-(1-ethoxyethyl) imine (**13b**). With methanol as solvent the analogous *N*-methoxymethyl and *N*-(1-methoxyethyl) imines were produced, whereas in a non-alcoholic solvent (*e.g.* tetrahydrofuran), imine (**6**) did not react with either aldehyde. With benzaldehyde in ethanol, the imine (**6**) gave the 9,12-epoxy Schiff's–base (**12**) as the only product (50%).§ This is analogous to the formation of compound (**11a**), as a minor product, in the reaction of the imine (**3**) with benzaldehyde. In none of the reactions of the imine (**6**) with aldehydes were any 9,11-cyclic derivatives detected.

The different reactions of the imines (**3**) and (**6**) with aldehydes can be rationalised if it is assumed that the imines react by way of their α-amino ether tautomers, (**14**) and (**15**). Obviously, for the imine (**3**) both of these tautomeric forms are available, whereas for the imine (**6**) only the tautomer (**15**) is possible. The proposed reactions are outlined in the Scheme. In the case of the tautomer (**14**), reaction of the 9-amino group with an aldehydic carbonyl would be expected[18] to give the α-hydroxy amine (**16**), which on acid catalysed dehydration would give the iminium intermediate (**18**). Intramolecular addition of the 11-hydroxy to the iminium group, followed by loss of a proton, would then give the 9,11-cyclic amino ether (**10**) in tautomeric equilibrium with the cyclic imine (**9**). Compounds (**9**) and (**10**) are the major products derived from the treatment of the imine (**3**) with aldehydes. In the case of the tautomer (**15**), treatment with an aldehyde would give the α-hydroxy amine (**17**) and hence the iminium species (**19**). In this case, however, it appears that the constraints imposed by the 9,12-oxygen bridge mean that the 11-hydroxy group is no longer positioned so that it can react with the 9-iminium



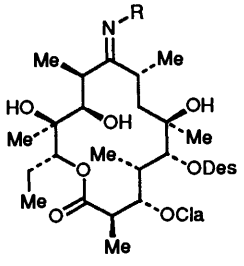
Scheme.

group.* For the reactions involving aliphatic aldehydes, therefore, the iminium group in species (**19**) reacts intermolecularly with the hydroxy group of the solvent (ethanol) to give the *N*-(1-ethoxyalkyl)amino ether (**20**), which tautomerises to the imine (**13**). This is the product from the treatment of the imine (**6**) with an aliphatic aldehyde and ethanol. In the case of aromatic aldehydes, the species (**19**) can simply lose a proton to give a conjugated Schiff-base, compounds (**11**) or (**12**). Presumably, the extra stability afforded by conjugation with the aromatic ring means that imines of this type are favoured over those corresponding to the imine (**13**). Compounds (**11**) and (**12**) are obtained from the reactions of aromatic aldehydes with the imines (**3**) and (**6**), respectively.

The derivatives (**9**)–(**13**), like the parent imines, were quite susceptible to hydrolysis in aqueous solution at pH 7, the final products of these hydrolyses being the ketones (**1**) [from compounds (**9**)–(**11**)] and (**4**) [from compounds (**12**) and (**13**)]. In the case of the 6-*O*-methyl compounds (**12**) and (**13**), the imine (**6**) was detected as an intermediate in the hydrolysis and, although it was not detected, it seems likely that the imine (**3**) is also an intermediate in the hydrolysis of compounds (**9**)–(**11**). We propose, therefore, that each of the derivatives (**9**)–(**13**) is initially hydrolysed to the parent imine, (**3**) or (**6**), by a reversal of the mechanism outlined in the Scheme,[20] and that this is then hydrolysed further to the corresponding ketone. The fact that the imine (**6**) was detected as an intermediate in some of these reactions is undoubtedly a result of its relatively slow hydrolysis to the ketone (**4**) (Table 1) compared with the initial hydrolyses of compounds (**12**) and (**13**) ($t_{0.5} < 1$ h). On the other hand, the imine (**3**) was not detected as an intermediate in the hydrolyses of compounds (**9**)–(**11**), presumably because its conversion into compound (**1**) occurs at a rate comparable to that of its formation (Tables 1 and 2). For the aromatic derivatives (**9c–g**) and (**10c–g**) the hydrolysis rates (first-order rate constants for disappearance of starting material) were measured at 37 °C (Table 2), and these results yielded a linear Hammett plot[21] with $\rho = -1.13$.

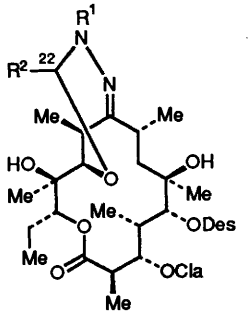
The *in vitro* antibacterial activities (testing conditions: pH 7.3; 37 °C; 18 h) of the imine derivatives reflected their relative ease of hydrolysis. For example, the antibacterial activities of the 9,11-cyclic imines (**9c–g**) and (**10c–g**) followed the order: (**9g**)/(**10g**) > (**9c**)/(**10c**) ≈ (**9d**)/(**10d**) > (**9e**)/(**10e**) ≫ (**9f**)/(**10f**).[22] In general, the less readily hydrolysed compounds showed the poorest antibacterial activity, implying that the imine derivatives have less intrinsic activity than either erythromycin (**1**) or its 6-methyl ether (**4**).

We then looked at the reactions of the imines (**3**) and (**6**) with nucleophiles in the form of various hydrazine derivatives. As stated earlier, erythromycin (**1**) reacts with hydrazine to form a 9-hydrazone but does not react with semicarbazide or phenylhydrazine. Erythromycin imine (**3**), on the other hand, did react with semicarbazide hydrochloride in aqueous 1,2-dimethoxyethane to give the semicarbazone (**21**) (37%). Phenylhydrazine hydrochloride reacted similarly to give the phenylhydrazone (**22**) (31%). The *E*-configuration was assigned to these products because of the known preference for this configuration in 9-imino derivatives of erythromycin.[23] The formation of phenylhydrazones from imines has been seen



R

(**21**) $NHCONH_2$
(**22**) NHPh
(**23**) NHMe



(**24**) $R^1 = Me$, $R^2 = H$
(**25**) $R^1 = R^2 = Me$

* We assume that in species (**19**) the configuration at C-9 is (*S*), which means that the 11-hydroxy and 9-iminium groups are disposed *trans* in a five-membered ring. This assumption is based on comparison with the 9,12-hemiacetal tautomer of erythromycin A 4″,11-diacetate (9-deoxo-12-deoxy-9-hydroxy-9,12-epoxyerythromycin A 4″,11-diacetate), which has been shown by NMR studies to have the (9*S*) configuration.[19]

Table 2. Half lives ($t_{0.5}$) and first order rate constants (k_1) for hydrolysis of imines (**9c–g**) in 1:1 methanol–phosphate buffer (0.067M; pH 7) at 37 °C.

	(9c)	(9d)	(9e)	(9f)	(9g)
$t_{0.5}$/h	6.45	4.7	12.1	53.0	3.3
10^{-5} k_1/s^{-1}	2.97	4.08	1.58	0.36	5.77

before,[24] and it has been noted[25] that imine derivatives of hindered ketones are often more reactive than their parents towards nitrogen nucleophiles. In contrast to the imine (**3**), the 6-methyl ether (**6**) did not react with either semicarbazide or phenylhydrazine under the above conditions. Under basic conditions (*ca.* pH 10), neither compound (**3**) nor compound (**6**) reacted with semicarbazide to give a semicarbazone derivative.

Erythromycin imine (**3**) also reacted with other mono-substituted hydrazines to give the expected hydrazone derivatives;[26] *e.g.* methylhydrazine gave the hydrazone (**23**) (75%). In common with erythromycin imine (**3**) and erythromycylamine A,[12] this methylhydrazone (**23**) was found to condense with simple aliphatic aldehydes to give 9,11-cyclic derivatives. Thus, formaldehyde reacted with (**23**) in the presence of acid to give the cyclic hydrazone (**24**) (68%), and acetaldehyde reacted similarly to give the hydrazone (**25**)* (48%). Clearly, during the formation of compounds (**24**) and (**25**) a change in configuration has occurred at the C=N bond; this could easily take place in the presence of mild acid, and might also involve intramolecular participation of the 6-hydroxy group [*cf.* compound (**14**)].[27] The hydrazones (**21**)–(**25**) were all quite active as antibacterials. However, as with other C-9 carbonyl derivatives of erythromycin, even in the best compounds this activity was less than that shown by compound (**1**) itself.[22]

In conclusion, we have seen that the differences between the reactions of erythromycin imine (**3**) and those of its 6-methyl ether (**6**) towards aldehydes can be explained by assuming that the imines react by way of their amino ether tautomers (**14**) and/or (**15**); the determining factor is that in imine (**3**) there is tautomerism involving the 9-imino group and the 6-hydroxy group, which is not possible in ether (**6**). The difference in the reactivities of compounds (**3**) and (**6**) towards acid hydrolysis and in the acid-catalysed reactions with hydrazine derivatives is more difficult to explain. Since there is no evidence to support the notion that the lactone ring conformations are different in compounds (**3**) and (**6**), it is tempting to speculate that the different behaviours of compounds (**3**) and (**6**) in these reactions is again a consequence of the presence in imine (**3**) of tautomerism involving the 6-hydroxy group. In other words, we would suggest that, as in its reactions with aldehydes, imine (**3**) reacts with dilute mineral acid and hydrazine derivatives by way of its amino ether tautomer (**14**).

Experimental

M.p.s were determined using a Kofler hot-stage apparatus. IR spectra and specific rotations were recorded for solutions in chloroform, and ^{13}C NMR spectra were recorded at 100 MHz for solutions in $CDCl_3$ with $SiMe_4$ as internal standard. Electron impact mass spectra were determined using a VG ZAB 1F mass spectrometer operating at 8 kV with 70 eV electrons and a source temperature of 200 °C. Fast atom bombardment (FAB) mass spectra were recorded on the same instrument operating at 6 kV accelerating voltage with xenon atoms as the collision beam accelerated to 8 kV, and using 3-nitrobenzyl alcohol and sodium acetate as the matrix. Except where stated otherwise, Merck silica gel 60 was used for TLC and for column chromatography with 1:9:90 35% aq. NH_3–MeOH–CH_2Cl_2 as eluant. Solutions were dried using sodium sulphate and solvents were removed by evaporation under reduced pressure using a rotary evaporator with bath temperature <30 °C. All new compounds gave ^{13}C NMR spectra which were in accord with the proposed structure.

9-Deoxo-9-iminoerythromycin A (**3**).—(9*E*)-Erythromycin A oxime (**2**) (2.0 g) was converted into the imine (**3**) using the method of Timms and Wildsmith.[7] The imine (**3**) was obtained as colourless crystals (1.6 g), m.p. (dichloromethane–hexane) 115–116 °C; $[\alpha]_D^{21}$ −55.0° (*c* 1.3); *m/z* (FAB) 755 ([*M* + Na]$^+$) (Found: C, 60.7; H, 9.5; N, 3.5. $C_{37}H_{68}N_2O_{12}$ requires C, 60.65; H, 9.35; N, 3.8%).

9-Deoxo-9-imino-6-O-methylerythromycin A (**6**).—(9*E*)-6-*O*-Methylerythromycin A oxime[13] (**5**) (1.0 g) in methanol (14 ml) was treated with ammonium acetate (2.0 g) and the mixture was stirred under nitrogen while 15% aqueous titanium(III) chloride (2.8 ml) was added dropwise during 10 min. The mixture was stirred under nitrogen for a further 6 h, and then poured into chloroform (100 ml). The mixture was stirred while 10% aqueous potassium carbonate (50 ml) was added, and the resulting mixture was filtered. The solid was washed with chloroform (2 × 30 ml). The layers were separated and the organic layer was washed with water (50 ml). The solution was dried and the solvent removed to give a white solid (0.91 g). Crystallisation from ethanol gave the *imine* (**6**) as colourless prisms (0.78 g), m.p. 137–138.5 °C; $[\alpha]_D^{20}$ −109.2° (*c* 1.0); ν_{max} 3 500, 1 720, and 1 625 cm^{-1}; δ_C 193.9 (C-9), 176.2 (C-1), 102.8 (C-1′), 96.3 (C-1″), 80.6 (C-5), 78.9 (C-6), 78.6 (C-3), 77.9 (C-4″), 77.2 (C-13), 74.5 (C-12), 72.8 (C-3″), 71.3 (C-2′), 69.7 (C-11), 68.6 (C-5′), 65.7 (C-5″), 65.1 (C-3′), 50.5 (6-OMe), 49.5 (3″-OMe), 45.2 (C-2), 40.3 (NMe_2), 39.0 (C-4), 38.1 (C-7), 35.1 (C-2″), 34.0 (C-8), 29.2 (C-4′), 25.4 (C-10), 21.5 (Me), 21.4 (Me), 21.1 (C-14), 20.0 (Me), 19.9 (Me), 18.7 (Me), 16.1 (Me), 16.0 (Me), 14.3 (Me), 10.7 (Me), and 9.3 (Me); *m/z* (FAB) 769 ([*M* + Na]$^+$) and 747 ([*M* + H]$^+$) (Found: C, 60.95; H, 9.85; N, 3.6. $C_{38}H_{70}N_2O_{12}$ requires C, 61.1; H, 9.45; N, 3.75%).

Hydrolysis Studies.—For studies at pH 7 the compound was dissolved in 1:1 methanol–phosphate buffer (0.067M; pH 7) to give a concentration of 1–5 mg ml^{-1}. For studies at pH other than 7, the compound was dissolved in 1:1 methanol–water (5 mg ml^{-1}) and the pH was adjusted by adding either 0.5M HCl or 0.1M sodium hydroxide. The hydrolysis reaction was followed using a Waters Associates HPLC apparatus equipped with a Waters 30 cm × 3.9 mm i.d. μBONDAPAK C18 column eluting with 9:5:4:2 acetonitrile–methanol–water–phosphate buffer (0.067M; pH 7) at a rate of 2 ml min^{-1} and using a UV detector at 215 nm. Half-lives ($t_{0.5}$) and first-order rate constants (k_1) were measured by following the reduction in concentration of starting material until at least half of the starting material had disappeared. Where appropriate, the identities of hydrolysis products were established by comparison with authentic samples using HPLC.

In the case of the 6-*O*-methyl 9-imine (**6**), the hydrolysis of the imine (10 mg) at pH 1.4 was conducted as above until all of the imine had reacted. The reaction mixture was diluted with ethyl acetate and the solution was washed with 10% aqueous potassium carbonate. The solution was dried and the solvent removed to yield the imine (**8**) as a colourless gum (7 mg), *m/z* (FAB) 611 (55%, [*M* + Na]$^+$), 589 (20%, [*M* + H]$^+$), 158 (100), and 116 (30).

* Compound (**25**) was obtained as a single isomer with the stereochemistry at C-22 unknown.

9-*Deoxo*-9-*imino*-9-N,11-O-*methylene-erythromycin A* (**9a**).—The imine (**3**) (1 g) in ethanol (10 ml) was treated with 38% aqueous formaldehyde (2 ml) and the solution was kept for 18 h. The ethanol was removed and the resulting residue dissolved in ethyl acetate and washed with water. The solution was dried, solvent was removed, and the residue was chromatographed to give the *imine* (**9a**) as an amorphous, white solid (0.8 g), ν_{max} 3 550, 1 720, and 1 650 cm^{-1}; the NMR spectrum showed that this compound existed as a 1:1 mixture of imine tautomer (**9a**) and amino ether tautomer* (**10a**); δ_C 180.6, 178.6, and 175.2 [C-1 and C-9 in (**9a**); C-1 in (**10a**)], 104.6 and 103.2 (C-1′), 97.2 [C-9 in (**10a**)], 96.7 and 96.6 (C-1″), 85.4 [C-6 in (**10a**)], 81.2 and 76.2 (C-22), 44.9 [C-7 in (**10a**)], 37.3 [C-7 in (**9a**)], and 21.1 and 20.5 (C-14); *m/z* 744 (M^+) (Found: M^+, 744.477. $C_{38}H_{68}N_2O_{12}$ requires *M*, 744.477).

9-*Deoxo*-9-N,11-O-*ethylidene*-9-*iminoerythromycin A* (**9b**).—The imine (**3**) (400 mg) in ethanol (10 ml) was treated with acetaldehyde (1.5 ml) and the solution was kept for 18 h. Work-up and chromatography as in the preparation of (**9a**) gave the *imine* (**9b**) as a white foam (200 mg); ν_{max} 3 550, 1 720, and 1 640 cm^{-1}; the NMR spectrum showed that this compound existed as a 1:1 mixture of imine tautomer (**9b**) and amino ether tautomer* (**10b**); *m/z* 758 (M^+) (Found: M^+, 758.493. $C_{39}H_{70}N_2O_{12}$ requires *M*, 758.493).

9-N,11-O-*Benzylidene*-9-*deoxo*-9-*iminoerythromycin A* (**9c**) *and* 9-(*Benzylideneamino*)-9-*deoxo*-12-*deoxy*-9,12-*epoxy-erythromycin A* (**11a**).—The imine (**3**) (1 g) in ethanol (7 ml) was treated with benzaldehyde (2 ml) and the solution was kept for 48 h. The solution was diluted with ethyl acetate and washed with water. The solution was dried, solvent removed, and the residue was chromatographed to give, in order of elution, the cyclic imine (**9c**) and the Schiff's–base (**11a**). The *cyclic imine* [**9c**) was obtained as a white foam (0.4 g); the NMR spectrum showed that this compound existed as a 1:1 mixture of imine tautomer (**9c**) and amino ether tautomer* (**10c**); *m/z* 820 (M^+) (Found: C, 64.5; H, 8.8; N, 3.3%; M^+, 820.512. $C_{44}H_{72}N_2O_{12}$ requires C, 64.35; H, 8.85; N, 3.4%; *M*, 820.508). The *Schiff's–base* (**11a**)† was obtained as a white foam (0.2 g), $[\alpha]_D^{20}$ −42.3° (*c* 1.0); ν_{max} 1 720 and 1 640 cm^{-1}; δ_C 176.2 (C-1), 157.4 (CH=N), 105.2 (C-1′), 100.4 (C-9), 97.4 (C-1″), 84.3 (C-12), 39.8 (C-7), and 24.9 (C-14); *m/z* 820 (M^+) (Found: M^+, 820.511. $C_{44}H_{72}N_2O_{12}$ requires *M*, 820.508).

Compounds (**9d**)–(**9g**) and (**11b**) were prepared similarly.[28]

9-(*Benzylideneamino*)-9-*deoxo*-12-*deoxy*-9,12-*epoxy*-6-O-*methylerythromycin A* (**12**).—The imine (**6**) (300 mg) in tetrahydrofuran (THF) (5 ml) was treated with ethanol (5 ml) and benzaldehyde (2 ml). The mixture was kept for 3 days, the solvent was removed and the oily residue was chromatographed on silanised silica gel using 1:1 and then 4:1 methanol–phosphate buffer (0.067M; pH 7). The Schiff's–base (**12**) was obtained as a white foam (150 mg), $[\alpha]_D^{20}$ −53.6° (*c* 1.0); ν_{max} 1 720 and 1 640 cm^{-1}; δ_C 176.1 (C-1), 156.3 (CH=N), 103.5 (C-1′), 100.8 (C-9), 97.7 (C-1″), 84.2 (C-12), 80.0 (C-6), 37.1 (C-7), and 24.2 (C-14); *m/z* (FAB) 857 ($[M + Na]^+$).

(9E)-9-*Deoxo*-9-(N-*ethoxymethyl*)*imino*-6-O-*methylerythromycin A* (**13a**).—The imine (**6**) (700 mg) in THF (5 ml) was treated with ethanol (5 ml) and 38% aqueous formaldehyde (1 ml). The mixture was kept for 18 h, evaporated to dryness and the residue was chromatographed to give the *imine* (**13a**) as a white, amorphous solid (300 mg), $[\alpha]_D^{20}$ −90.0° (*c* 1.0); ν_{max} 1 725 and 1 650 cm^{-1}; δ_C 183.1 (C-9), 174.9 (C-1), 102.4 (C-1′), 96.2 (C-1″), 81.4 (NCH_2O), 78.9 (C-6), 63.8 ($MeCH_2O$), 38.1 (C-7), and 21.1 (C-14); *m/z* (FAB) 827 ($[M + Na]^+$) (Found: C, 61.15; H, 9.5; N, 3.15. $C_{41}H_{76}N_2O_{13}$ requires C, 61.15; H, 9.5; N, 3.5%).

(9E)-9-*Deoxo*-9-[N-(1-*ethoxyethyl*)*imino*-6-O-*methylerythromycin A* (**13b**).—The imine (**6**) (250 mg) in THF (5 ml) was treated with ethanol (5 ml) and acetaldehyde (2 ml), and the mixture was kept for 3 days. The solvent was removed and the residue was chromatographed to give the imine (**13b**)‡ as a white foam (150 mg); *m/z* (FAB) 841 ($[M + Na]^+$).

(9E)-*Erythromycin A Semicarbazone* (**21**).—The imine (**3**) (200 mg) in 1,2-dimethoxyethane (2 ml) was treated with water (5 drops) and semicarbazide hydrochloride (33 mg). The mixture was stirred for 24 h, diluted with chloroform (50 ml), and washed with 10% aqueous potassium carbonate (20 ml) and water (2 × 20 ml). The solution was dried, the solvent was removed, and the residue was chromatographed to give a white solid (80 mg). Crystallisation of this from dichloromethane–hexane gave the *semicarbazone* (**21**) as colourless crystals, m.p. 185–186 °C; $[\alpha]_D^{20}$ −22.8° (*c* 1.0); ν_{max} 3 470, 3 370, 1 715, 1 675, 1 575, and 1 560sh cm^{-1}; δ_C 176.0 (C-1), 167.3 (C-9), 158.5 ($CONH_2$), 103.4 (C-1′), and 96.6 (C-1″); *m/z* (FAB) 813 ($[M + Na]^+$) (Found: C, 57.2; H, 8.8; N, 6.7. $C_{38}H_{70}N_4O_{13}$ requires C, 57.7; H, 8.9; N, 7.1%).

(9E)-*Erythromycin A Phenylhydrazone* (**22**).—The imine (**3**) (200 mg) in 1,2-dimethoxyethane (1 ml)–methanol (1 ml) was treated with phenylhydrazine hydrochloride (45 mg) and the mixture was stirred for 24 h. The mixture was diluted with ethyl acetate (50 ml) and the solution was washed with 10% aqueous potassium carbonate (20 ml) and water (2 × 30 ml). The solution was dried, the solvent removed, and the residue was chromatographed to give a pale yellow solid (70 mg). Crystallisation from ethyl acetate gave the *phenylhydrazone* (**22**) as colourless crystals, m.p. 150–152 °C; ν_{max} 3 400, 1 720, 1 600, and 1 490 cm^{-1}; δ_C 175.9 (C-1), 163.7 (C-9), 146.9 (Ph), 129.0 (Ph), 119.7 (Ph), 113.2 (Ph), 103.3 (C-1′), and 96.7 (C-1″); *m/z* 823 (M^+) (Found: C, 61.3; H, 8.85; N, 4.95%; M^+ 823.520. $C_{43}H_{73}N_3O_{12}\cdot H_2O$ requires C, 61.35; H, 9.0; N, 5.0%. $C_{43}H_{73}N_3O_{12}$ requires *M*, 823.519).

(9E)-*Erythromycin A Methylhydrazone* (**23**).—The imine (**3**) (280 mg) in methanol (3 ml) was treated with methylhydrazinium sulphate (65 mg) and triethylamine (30 mg). The solution was kept for 5 h, diluted with ethyl acetate (30 ml) and washed with 10% aqueous potassium carbonate (20 ml) and water (2 × 20 ml). The solution was dried and the solvent removed to yield a white solid. Crystallisation from acetone–water gave the *methylhydrazone* (**23**) as colourless crystals (220 mg), m.p. 128–129 °C; $[\alpha]_D^{20}$ −50.1° (*c* 1.0); ν_{max} 3 400, 1 720, and 1 600 cm^{-1}; δ_C 175.6 (C-1), 167.2 (C-9), 103.1 (C-1′), 97.1 (C-1″), 49.7 (OMe), 40.3 (NMe_2), and 39.2 (NHMe); *m/z* 761 (M^+) (Found: C, 59.75; H, 9.45; N, 5.25%; M^+, 761.504. $C_{38}H_{71}N_3O_{12}$ requires C, 59.9; H, 9.4; N, 5.5%; *M*, 761.504).

(9Z)-9-N′,11-O-*Methylene-erythromycin A Methylhydrazone* (**24**).—The methylhydrazone (**23**) (100 mg) in ethanol (4 ml) was treated with 37% aqueous formaldehyde (0.5 ml), acetic acid (0.5 ml), and 0.5M HCl (3 drops). The solution was kept for 2 h, diluted with ethyl acetate (50 ml), and washed with 10% aqueous potassium carbonate (30 ml) and water (2 × 30 ml). The solution was dried, the solvent was removed, and the residue was chromatographed to give the *hydrazone* (**24**) as a white foam (70 mg, 68%), m.p. 130–133 °C; $[\alpha]_D^{22}$ −150.0° (*c*

* A single isomer; the configuration is probably (9*S*).

† A single isomer, the stereochemistry at C-9 is unknown.

‡ A single isomer; the configuration of the 1-ethoxyethyl group is unknown.

1.0); ν_{max} 1 720 cm^{-1}; δ_C 175.4 (C-1), 171.0 (C-9), 103.0 (C-1′), 96.5 (C-1″), 87.0 (NCH_2O), 76.8 (C-12), 74.4 (C-11), 49.5 (OMe), 44.6 and 44.4 (C-2 and NMe), and 40.2 (NMe_2); *m/z* 773 (M^+, 12%), 615 (5), 174 (10), 158 (100), 116 (45), and 98 (45) (Found: C, 60.35; H, 9.45; N, 5.25%; M^+, 773.502. $C_{39}H_{71}N_3O_{12}$ requires C, 60.5; H, 9.25; N, 5.45%; *M*, 773.504).

(9Z)-9-N′,11-O-*Ethylidene-erythromycin A Methylhydrazone* (**25**).—Using the process described for the preparation of (**24**), but with acetaldehyde (0.3 ml) in place of 37% aqueous formaldehyde, the methylhydrazone (**23**) (100 mg) was converted into the *hydrazone* (**25**),* which was obtained as colourless crystals (50 mg), m.p. 129–132 °C (dichloromethane–hexane); $[\alpha]_D^{21}$ −178.4° (*c* 1.0); ν_{max} 1 720 cm^{-1}; δ_C 175.3 (C-1), 171.6 (C-9), 103.0 (C-1′), 96.5 (C-1″), 91.1 (NCHO), 76.9 (C-12), 74.2 (C-11), 49.5 (OMe), 42.7 (NMe), and 40.3 (NMe_2); *m/z* 787 (M^+, 25%), 629 (10), 174 (5), 158 (100), 116 (50), and 98 (40) (Found: C, 60.95; H, 9.4; N, 5.0%; M^+, 787.518. $C_{40}H_{73}N_3O_{12}$ requires C, 60.95; H, 9.35; N, 5.35%; *M*, 787.519).

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* A single isomer; the configuration at C-22 is unknown.

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