C-2 Epimerisation in an Erythromycin Derivative. Preparation and NMR Spectroscopic Studies on (2*S*)-(*E*)-9-Deoxo-9-methoxyiminoerythromycin A

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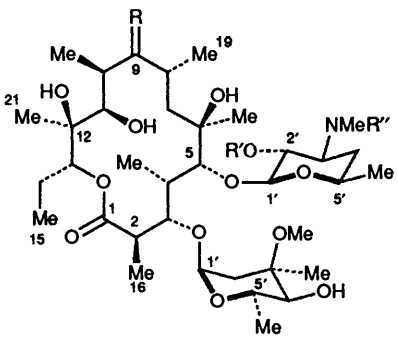
(2*S*)-(*E*)-9-Deoxo-9-methoxyiminoerythromycin A **9** has been prepared and characterised by NMR spectroscopy, including NOE experiments. In solution, **9** appears to adopt only the C-3 to C-5 'folded out' conformation. This is in contrast to (*E*)-9-deoxo-9-methoxyiminoerythromycin A **8**, itself, which exists in solution as a mixture of 'folded out' (major) and 'folded in' (minor) conformations in fast exchange. Epimerisation experiments suggest that, for the derivatives studied here, the 2-*epi* (2*S*) configuration is thermodynamically less stable than the natural (2*R*) configurations. The relative stabilities of the various configurations and conformations suggested by these experiments are supported by molecular mechanics calculations.

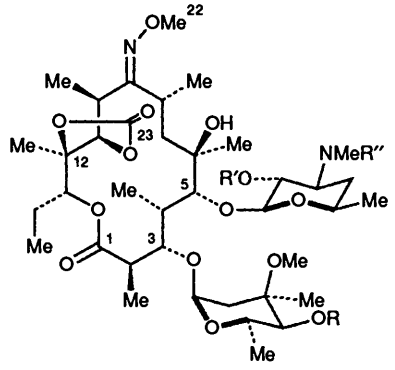
Valuable insights into the chemical, stereochemical and conformational behaviour of large-ring alicyclic compounds have been provided by work on modification of the lactone rings of the macrolide antibiotics. In this respect, one of the most studied of the natural macrolides is erythromycin A **1**,† and there have been numerous reports on chemical manipulation of the erythronolide functional groups.[1] Studies on modifying the stereochemistry of the aglycone, however, appear to be relatively rare. In the erythromycin B series,‡ 8-*epi*-erythromycin B[2] and 10-*epi*-erythromycin B[3] have been prepared, but these seem to be the only instances of stereochemical modification within the erythromycin aglycone.

In this paper, we describe the epimerisation of position 2 in a derivative of erythromycin A. These results may have particular interest in relation to recent reports on the C-2 alkylation and conformational analysis of 14-membered lactones.[4]



1 R = O; R′ = H; R″ = Me
2 R = NOMe; R′ = R″ = CO_2CH_2Ph

Our observation of C-2 epimerisation came about entirely by chance. We were interested[5] in preparing 9-deoxo-9-methoxyiminoerythromycin A 11,12-carbonate **5**, and one of our routes to this compound involved treatment of the methoxyimino derivative **2** with 1,1′-carbonyldiimidazole. Thus, compound **2** was treated with sodium hydride and carbonyldiimidazole in tetrahydrofuran (THF), and when all of **2** was consumed the mixture was treated with benzyl alcohol. This gave three products, two of which were readily identified as the cyclic-carbonate derivatives **3** (54%) and **4** (20%). The third product (16%) was isomeric with **3**, as shown by elemental analysis and mass spectrometry, and its ^{13}C NMR spectrum was very similar to that of **3**. To facilitate more detailed NMR studies, the unknown compound was deprotected by hydrogenation and reductively *N*-methylated.[6] Compound **3** was similarly converted into **5**.



3 R = R′ = R″ = CO_2CH_2Ph
4 R = H; R′ = R″ = CO_2CH_2Ph
5 R = R′ = H; R″ = Me

The ^1H and ^{13}C chemical shifts for **5** and its isomer were fully assigned, using the methods previously employed for derivatives of erythromycin.[7] The shifts for the C-8 and C-10 methine groups showed that both compounds had the *E* stereochemistry for the methoxyimino group.§[8] 2D ^1H and ^{13}C COLOC experiments[9] (correlation by long-range coupling, tuned for 2–4 bond interactions) suggested that the unknown isomer had the same carbon–oxygen aglycone framework as **5**; in particular, the presence of a correlation between the carbonate carbonyl and 11-H, and the absence of a correlation between

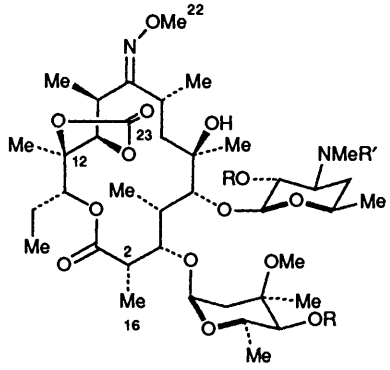
† The erythomycin structure consists of a 14-membered lactone aglycone, erythronolide, which carries an amino sugar, D-desosamine, and a neutral sugar, L-cladinose. Unprimed numbers are used for positions in the aglycone, primed numbers are used in the amino sugar, and double primed numbers are used in the neutral sugar.

‡ Erythromycin B differs from erythromycin A **1** in having no hydroxy group at position 12.

§ In addition to the δ_H differences noted in ref. 8, our experience with oximino ether derivatives of erythromycin has shown that *E* isomers give δ_C at 26–28 ppm for C-8 and δ_C at 33–36 ppm for C-10; *Z* isomers give δ_C at 31–34 ppm for C-8 and C-10.

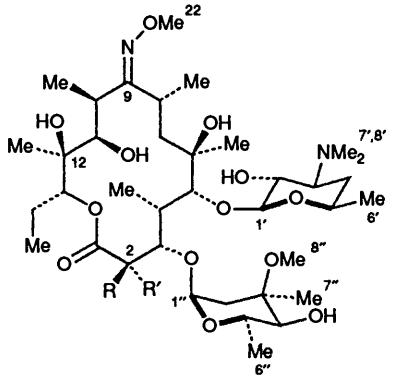
C-1 and 11-H, allowed us to discard the possibility of a ring-contracted aglycone.*

Comparison of the NMR spectra for **5** and its isomer revealed that the main differences occurred in the ^{13}C chemical shifts for C-1, C-2, C-4, C-5 and C-1″, and in the ^{1}H vicinal coupling constants for the 2-H to 5-H region. These observations, together with a consideration of the chemistry involved, suggested that the unknown isomer had structure **6**, in which the chirality has been inverted at C-2. The protected form of **6** therefore has structure **7**. This is the first time that C-2 epimerisation has been observed in a derivative of erythromycin.



6 R = H; R′ = Me
7 R = R′ = CO_2CH_2Ph

To confirm that the NMR effects observed for **6** were not caused by the carbonate group, the carbonate was removed by mild base hydrolysis to give (2*S*)-erythromycin methoxime **9**, and the NMR spectra for this compound were compared with those for erythromycin methoxime **8**. The NMR assignments for **5**, **6**, **8** and **9** are given in Tables 1–3. As can be seen, stereochemical inversion at C-2 gives rise to some expected changes in the chemical shifts and coupling constants for the C-1 to C-3 region, and some perhaps less expected changes for the C-3 to C-5 region. To try and rationalise the latter, we looked at the steric effects resulting from C-2 epimerisation. Thus, a study of molecular models showed that inverting the chirality at C-2 results in an easing of the steric congestion between the cladinose moiety and the C-2 methyl, and an increase in the steric congestion at the C-4 methyl (measured as changes in energy barriers to methyl group rotation [7b]). Small conformational shifts, driven by the changed steric environment, could therefore account for the observed NMR spectral changes in the C-3 to C-5 region.



8 R = Me; R′ = H
9 R = H; R′ = Me

* This type of compound might have been formed from **2** by a translactonisation reaction involving the 11-hydroxy group (ref. 10).

Table 1. ^{13}C NMR chemical shifts (δ_C in ppm) for compounds **5**, **6**, **8** and **9** in $CDCl_3$–Me_4Si

Carbon	5[a]	6[a]	8	9
1	175.7	172.6	175.4	172.5
2	45.1	42.6	44.7	42.6
3	79.3	79.2	79.9	77.4
4	40.4	37.4	39.2	36.5
5	83.7	86.3	83.2	83.6
6	75.6	74.9	75.4	74.3
7	38.4	39.6	37.9	37.6
8	26.2	26.8	26.4	26.2
9	166.5	165.1	171.6	171.5
10	33.3	33.7	32.9	32.8
11	83.5	84.1	70.6	70.4
12	85.1	85.9	74.3	75.2
13	76.0	77.7	77.0	77.7
14	22.3	23.4	21.2	21.3
15	10.4	10.9	10.7	10.9
16	15.7	16.6	16.1	11.9
17	9.2	11.0	9.2	11.0
18	26.7	26.5	27.0	27.3
19	19.2	20.6	18.3	18.8
20	15.3	14.1	14.5	14.4
21	13.4	14.6	16.3	16.4
22	61.7	61.7	61.8	61.7
23	154.3	153.6	—	—
1′	103.4	104.9	103.0	102.1
2′	71.0	70.7	71.0	71.0
3′	65.8	65.5	65.6	65.6
4′	29.2	29.2	28.8	28.9
5′	69.0	69.6	68.9	68.6
6′	21.3	21.2	21.4	21.4
7′,8′	40.4	40.4	40.3	40.3
1″	96.4	94.4	96.3	92.0
2″	35.1	35.4	35.1	35.3
3″	72.8	72.9	72.8	73.1
4″	78.1	78.0	78.1	78.1
5″	65.8	65.8	65.6	65.5
6″	18.5	18.0	18.6	18.1
7″	21.6	21.8	21.5	21.7
8″	49.5	49.4	49.5	49.4

[a] Spectrum run at 320 K.

Further evidence for the (2*S*) configuration in **9** was obtained from NOE experiments.[11] Thus, irradiation of 1″-H resulted in a very strong NOE on 2-H, but no effect on 16-H (Fig. 1). This is precisely the opposite of what is generally observed for erythromycin and its derivatives,[7b–d] and points very strongly to stereochemical inversion at C-2. Other NOE results (Table 4), from irradiation of 2-H, 3-H and 16-H, reinforced this conclusion.

NOE experiments also gave some indication of the probable conformation of compound **9** in solution. Irradiation of 11-H gave a strong NOE on 4-H (Fig. 1), indicative of the C-3 to C-5 'folded out' conformation [7c,d] but no NOE [11]3, suggesting that C-3 to C-5 'folded in' conformation is not significantly populated.† Other results (Table 4), involving irradiation of 3-H, 5-H and 8-H were also in accord with these conclusions.

† 'Folded out' conformations are characterised by having the C-3 to C-5 region positioned, relative to the C-9 to C-11 region, such that the attached sugars extend outwards, away from the centre of the aglycone; the X-ray structure of erythromycin A (ref. 12) provides an example of a 'folded out' conformation. 'Folded in' conformations are characterised by having the C-3 to C-5 region and the attached sugars folded inward towards C-11; the X-ray structure of (*E*)-11-*O*-(2-dimethylaminoethoxy)-methyl-9-deoxo-9-methoxyiminoerythromycin A [ref. 7(*d*)] provides an example of a 'folded in' conformation. Experimentally, 'folded out' conformations give rise to a strong NOE [11]4 and no (or very weak) NOE [11]3; for 'folded in' conformations the converse applies.

Table 2. ^{1}H NMR chemical shifts (δ_H in ppm) for compounds **5**, **6**, **8** and **9** in $CDCl_3$–Me_4Si

Proton	5[a]	6[a]	8	9
2	2.81	2.89	2.91	3.11
3	4.09	4.00	4.04	4.14
4	1.89	2.15	2.00	2.24
5	3.53	3.41	3.59	3.49
7	1.60, 1.50	1.60, 1.50	1.62, 1.58	1.60, 1.50
8	3.73	3.59	3.67	3.65
10	2.61	2.76	2.66	2.68
11	4.79	4.99	3.68	3.82
13	4.98	4.91	5.11	5.20
14	1.85, 1.55	1.85, 1.61	1.92, 1.50	1.95, 1.52
15	0.88	0.99	0.84	0.87
16	1.18	1.26	1.19	1.25
17	1.08	1.11	1.12	1.16
18	1.45	1.35	1.47	1.42
19	1.02	1.13	1.03	1.02
20	1.23	1.26	1.18	1.17
21	1.45	1.48	1.10	1.15
22	3.83	3.80	3.82	3.82
1′	4.40	4.34	4.44	4.51
2′	3.22	3.29	3.25	3.22
3′	2.49	2.53	2.52	2.51
4′	1.69, 1.23	1.70, 1.26	1.68, 1.25	1.70, 1.21
5′	3.49	3.59	3.51	3.53
6′	1.22	1.24	1.23	1.21
7′,8′	2.31	2.32	2.35	2.32
1″	4.86	4.86	4.92	4.80
2″	2.33, 1.57	2.19, 1.59	2.36, 1.40	2.25, 1.61
4″	3.05	3.00	3.03	3.06
5″	4.00	3.99	4.01	4.04
6″	1.28	1.24	1.30	1.26
7″	1.23	1.22	1.25	1.25
8″	3.29	3.28	3.32	3.36

[a] Spectrum run at 320 K.

Table 3. Vicinal coupling constants ($^3J_{HH}$/Hz) for the lactone ring protons in compounds **5**, **6**, **8** and **9**

^3J	5	6	8	9
$^3J_{2,3}$	8.5	2.5	9.5	5.3
$^3J_{3,4}$	1.4	7.2	1.6	2.0
$^3J_{4,5}$	7.7	3.3	7.5	4.2
$^3J_{7a,8}$	10.3	*a*	10.8	*ca.* 7
$^3J_{7e,8}$	3.5	*a*	2.5	*ca.* 2
$^3J_{10,11}$	0.7	4.0	1.2	<1.0

[a] Signals partially obscured.

It is interesting to compare these results with those for erythromycin methoxime **8**: this compound, like erythromycin **1**, appears to exist in solution in both 'folded out' (major) and 'folded in' (minor) conformations, which are in fast exchange. Some NOE results for **8** and **9** are given in Table 4.

Some further experiments were carried out to investigate the nature of the epimerisation reaction. Thus, the methoxyimino derivative **2** was treated with sodium imidazolide in THF to give two components, which were separated by chromatography. The more polar of these was the 2′,3′-cyclic carbamate derivative **10**, resulting from base-mediated reaction of the benzyloxycarbonyl groups. The other component, which was chromatographically identical to **2**, was deprotected and *N*-methylated, and the resulting methoxime was examined by ^{13}C NMR and HPLC. This showed that this product consisted of erythromycin methoxime **8** (*ca.* 85%) and its (2*S*) diastereoisomer

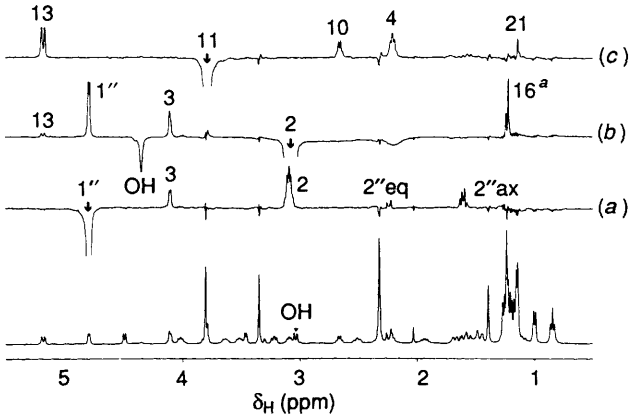


Fig. 1. Three 400 MHz ^{1}H NOE spectra for **9**, in $CDCl_3$, obtained by irradiation of (*a*) 1″-H, (*b*) 2-H and (*c*) 11-H. [a] NOE [2]16 plus INDOR [2]16.

Table 4. NOE results for compounds **8** and **9**

Proton irradiated	Observed NOE[a] 8	9
2-H	4-H (m), 16-H (m), 17-H (1)	3-H (m), 13-H (s), 16-H (m), 1″-H (1)
3-H	2-H (s), 4-H (m), 5-H (1), 1″-H(1)	2-H (m), 4-H (m), 5-H (1), 1″-H (m)
5-H	3-H (m), 18-H (m), 1′-H (1)	3-H (m), 18-H (m), 1′-H (m), 5″-H (m)
8-H[b]	3-H (s), 4-H (m), 7a-H (s), 7e-H (s),	7e-H (s), 18-H (s), 19-(H (m)
11-H[b]	10-H (m), 13-H (1), 18-H (m), 19-H (m), 21-H (m)	4-H (m), 10-H (m), 13-H (m), 21-H (s)
16-H	2-H (1), 3-H (s), 1″-H (1)	2-H (m), 1″-H (s)
1″-H	3-H (1), 16-H (s), 2″a-H (s), 2″e-H (m)	2-H (1), 3-H (m), 2″a-H (s), 2″e-H (m)

[a] s = small (<1%), m = medium (1–5%) and l = large (>5%) effect. [b] For compound **8**, absorptions for 8-H and 11-H are coincident.

9 (*ca.* 15%). Treatment of the (2*S*)-11,12-carbonate derivative **7** with sodium imidazolide also resulted in C-2 epimerisation. Again, it appeared that the natural (2*R*) configuration was favoured, with the ratio of isolated products **3**:**7** being *ca.* 4:1. In contrast, treatment of erythromycin methoxime **8** with sodium imidazolide for 24 h did not result in epimerisation.

The results so far suggest that for these derivatives the natural (*R*) configuration at C-2 is thermodynamically favoured over the (2*S*) configuration. Furthermore, for the (2*R*) compounds (*e.g.* **1** and **8**), the 'folded out' and 'folded in' conformations are close enough in energy for both of them to be detected in solution. For the (2*S*) compound **9**, however, the energy difference between the conformations is such that only the 'folded out' form is observed. These conclusions were supported by molecular mechanics calculations.[13] For convenience, these calculations were performed on the various forms of erythromycin A; the 'folded out' conformation of (2*S*)-erythromycin A was modelled[14] from the X-ray crystallographic structure of erythromycin,[12] and the 'folded in' conformations of erythromycin A and (2*S*)-erythromycin A were modelled from the X-ray structure of (*E*)-11-*O*-(2-dimethylaminoethoxy)methyl-9-deoxo-9-methoxyiminoerythromycin A.[7d] The results are given in Table 5. For erythromycin, the 'folded out' conformation is more stable than the 'folded in' conformation by *ca.* 0.5 kcal

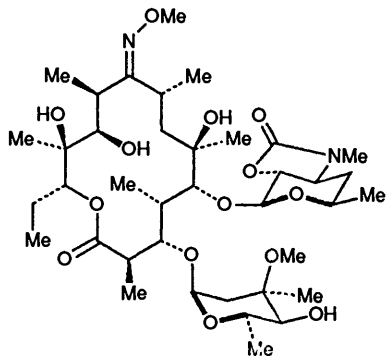


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Table 5. Molecular mechanics calculations for erythromycin A and (2*S*)-erythromycin A. Steric energies (kcal mol^{-1}) are relative to erythromycin A 'folded out' conformation

Compound (conformation)	Relative steric energy
Erythromycin A (folded out)	0.0
Erythromycin A (folded in)	0.49
(2*S*)-Erythromycin A (folded out)	0.96
(2*S*)-Erythromycin A (folded in)	3.43

mol^{-1},* whereas for (2*S*)-erythromycin the conformations differ by *ca.* 2.5 kcal mol^{-1}. For the 'folded out' conformations, erythromycin is more stable than its (2*S*) isomer by *ca.* 1 kcal mol^{-1}. Stereoscopic drawings of (2*S*)-erythromycin in the 'folded out' and 'folded in' conformations are shown in Figs. 2 and 3, respectively.

Finally, antibacterial testing *in vitro* showed that (2*S*)-erythromycin methoxime **9** was *ca.* half as active as erythromycin methoxime **8**. Similarly, in the 11,12-cyclic carbonate series, the (2*S*) compound **6** was two- to four-fold less active than the natural derivatives **5**.[15]

Experimental

M.p.s were determined using a Kofler hot-stage apparatus. IR spectra and specific rotations were recorded for solutions in chloroform. ^1H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra were recorded at 100 MHz on a Bruker AM 400 NMR spectrometer for solutions in $CDCl_3$ with $SiMe_4$ as an internal standard. MS (EI) 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) MS were recorded on the same instrument operating at 6 kV accelerating voltage with Xe atoms as the collision beam accelerated to 8 kV, and using 3-nitrobenzyl alcohol and sodium acetate as the matrix. Solutions were dried using sodium sulphate and solvents were removed by evaporation under reduced pressure using a rotary evaporator with bath temperature < 30 °C. Merck silica gel 60 was used for TLC and for column chromatography. HPLC was conducted 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 (0.067 mol dm^{-3}; pH 7) at a rate of 2 cm^3 min^{-1} and using a UV detector at 215 nm.

All new compounds gave ^{13}C NMR spectra which were in accord with the proposed structure.

Reaction of (E)-2′-O,3′-N-*Bis*(*benzyloxycarbonyl*)-N-*demethyl-9-deoxo-9-methoxyiminoerythromycin A* **2** *with* 1,1′-*Carbonyldiimidazole and Sodium Hydride.*—Compound **2** (1.0 g) in dry THF (20 cm^3) was treated with sodium hydride (50% dispersion in oil; 100 mg) and the mixture was stirred for 5 min. 1,1′-Carbonyldiimidazole (600 mg) was added and the mixture was stirred at 60 °C (bath temperature) for 30 min. The mixture was cooled to room temperature, treated with benzyl alcohol (1 cm^3) and stirred for 30 min. The mixture was diluted with ethyl acetate (100 cm^3) and was washed with water (50 cm^3), dil. HCl (50 cm^3) and water (50 cm^3). The solution was dried, the solvent was removed, and the residue was chromatographed using ethyl acetate–hexane to give, in order of elution, (*E*)-2′-*O*,3′-*N*,4″-*O*-tris(benzyloxycarbonyl)-11-*O*,12-*O*-carbonyl-*N*-demethyl-9-deoxo-9-methoxyiminoerythromycin A (**3**) (680 mg), its (2*S*) diastereoisomer **7** (190 mg) and (*E*)-2′-*O*,3′-*N*-bis(benzyloxycarbonyl)-11-*O*,12-*O*-carbonyl-*N*-demethyl-9-deoxo-9-methoxyiminoerythromycin A **4** (200 mg).

Compound **3** was obtained as colourless prisms from dichloromethane–hexane, m.p. 118–119 °C; $[\alpha]_D^{20}$ −44.3° (*c* 1.0 in $CHCl_3$); ν_{max}/cm^{-1} 3500, 1795, 1735 and 1690; FAB–MS *m/z* 1199 (MNa^+) (Found: C, 63.05; H, 7.2; N, 2.45. $C_{68}H_{84}N_2O_{20}$ requires C, 63.25; H, 7.2; N, 2.4%).

Compound **7** was obtained as colourless crystals from dichloromethane–hexane, m.p. 116–117 °C; $[\alpha]_D^{20}$ −51.6° (*c* 1.0 in $CHCl_3$); ν_{max}/cm^{-1} 3500, 1795, 1730 and 1690; FAB–MS *m/z* 1199 (MNa^+) (Found: C, 63.0; H, 7.15; N, 2.45. $C_{68}H_{84}N_2O_{20}$ requires C, 63.25; H, 7.2; N, 2.4%).

Compound **4** was obtained as a white foam, $[\alpha]_D^{20}$ −45.7° (*c* 1.0 in $CHCl_3$); ν_{max}/cm^{-1} 3550, 1790, 1735 and 1690; FAB–MS *m/z* 1065 (MNa^+) (Found: C, 62.35; H, 7.65; N, 2.65. $C_{54}H_{78}N_2O_{18}$ requires C, 62.15; H, 7.55; N, 2.7%).

(E)-11-O,12-O-*Carbonyl-9-deoxo-9-methoxyiminoerythromycin A* **5**.—Compound **3** (430 mg) in ethanol (25 cm^3)–acetate buffer (0.73 mol dm^{-3}; pH 4.8; 2 cm^3) was shaken with 10% palladium–carbon (120 mg) under hydrogen (1 atm) for 30 min. 37% aq. formaldehyde (2 cm^3) was added and the hydrogenation was continued for 1 h. The catalyst was removed by filtration and was washed with ethanol and water. The ethanol was evaporated under reduced pressure and the resulting solution was diluted with water and basified (pH 11) by adding solid potassium carbonate. The mixture was extracted with ethyl acetate (2 × 50 cm^3) and the extract was washed with water (50 cm^3) and dried. The solvent was removed to give the carbonate **5** as a white solid (260 mg, 90%). Crystallisation from dichloromethane–hexane gave colourless prisms, m.p. 229–231 °C (lit.,[5] 230–231 °C).

(2S)-(E)-11-O,12-O-*Carbonyl-9-deoxo-9-methoxyiminoerythromycin A* **6**.—Using the process for the preparation of compound **5**, compound **7** (180 mg) was converted into the carbonate **6**, which was obtained as a white solid (110 mg, 91%); $[\alpha]_D^{20}$ −38.5° (*c* 1.0 in $CHCl_3$); ν_{max}/cm^{-1} 3500, 3400, 1795 and 1720; *m/z* 788 (M^+) (Found: C, 59.65; H, 8.9; N, 3.6%; M^+, 788.4665. $C_{39}H_{68}N_2O_{14}$ requires C, 59.35; H, 8.7; N, 3.55%; M, 788.4671).

(2S)-(E)-9-*Deoxo-9-methoxyiminoerythromycin A* **9**.—Compound **6** (50 mg) in THF (4 cm^3) was treated with 0.1 mol dm^{-3} sodium hydroxide (1 cm^3) and the solution was kept for 41 h. The mixture was diluted with ethyl acetate (50 cm^3) and the solution was washed with water (2 × 20 cm^3) and dried. The solvent was removed and the resulting residue was chromatographed using 1:9:90 35% aq. ammonia–methanol–dichloro-

* 1 cal = 4.184 J.

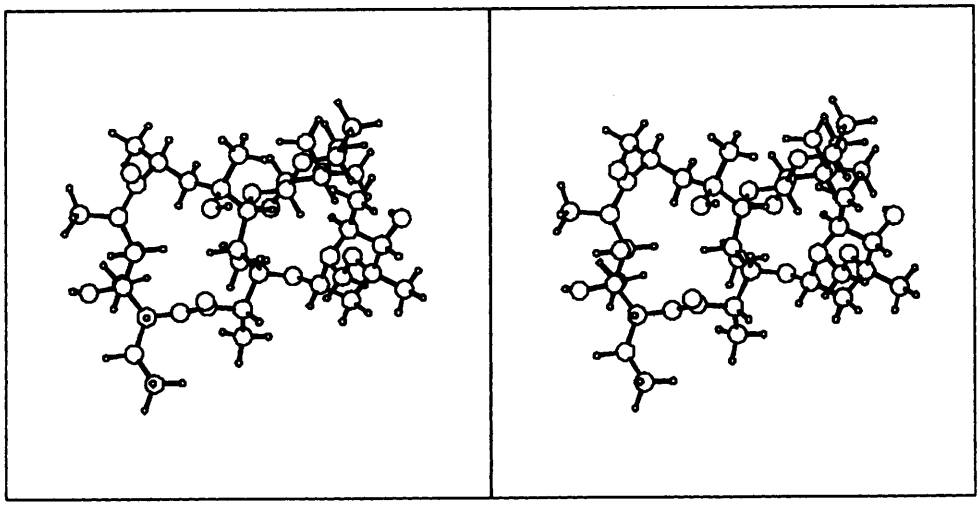

Fig. 2. Stereoscopic drawing of (2*S*)-erythromycin A modelled in the C-3 to C-5 'folded out' conformation.

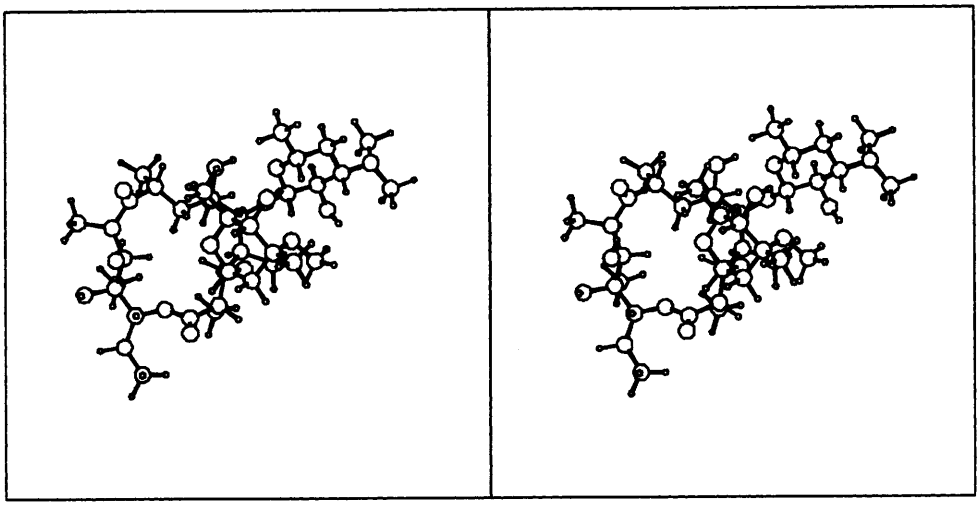

Fig. 3. Stereoscopic drawing of (2*S*)-erythromycin A modelled in the C-3 to C-5 'folded in' conformation.

methane to give the methoxyimino derivative **9** as a white solid (35 mg); $[\alpha]_D^{22}$ −54.9° (*c* 1.0 in $CHCl_3$); ν_{max}/cm^{-1} 3500, 3400 and 1720; *m/z* 762 (M^+) (Found: M^+, 762.4874. $C_{38}H_{70}N_2O_{13}$ requires M, 762.4878).

Reaction of (E)-2′-O,3′-N-*Bis(benzyloxycarbonyl)*-N-*demethyl*-9-*deoxo*-9-*methoxyiminoerythromycin A* **2** *with Sodium Imidazolide.*—Compound **2** (1.0 g) and imidazole (80 mg) were dissolved in dry THF (8 cm^3) and the solution was treated with sodium hydride (50% dispersion in oil; 60 mg). The mixture was stirred for 24 h, and was then diluted with ethyl acetate (100 cm^3) and washed with water (50 cm^3), dil. HCl (50 cm^3) and water. The solution was dried, the solvent was removed, and the residue was chromatographed using ethyl acetate–hexane to give compound **2** plus its (2*S*) diastereoisomer as a white solid (580 mg) and (*E*)-2′-O,3′-*N*-carbonyl-*N*-demethyl-9-deoxo-9-methoxyiminoerythromycin A **10** as colourless crystals (290 mg), m.p. 150–152 °C (ethyl acetate); $[\alpha]_D^{22}$ −77.0° (*c* 1.0 in $CHCl_3$); ν_{max}/cm^{-1} 3500, 3350, 1745 and 1720; δ_C 175.3 (C-1), 171.6 (C-9), 160.0 (C=O), 99.1 (C-1′) and 96.8 ppm (C-1″); *m/z* 774 (M^+); FAB-MS *m/z* 797 (MNa^+) (Found: C, 59.25; H, 8.7; N, 3.6%; M^+, 774.4530. $C_{38}H_{66}N_2O_{14}$ requires C, 58.9; H, 8.7; N, 3.6%; M, 774.4514).

The mixture of **2** and (2*S*)-**2** (450 mg) was deprotected and *N*-methylated (using the process described for the preparation of **5**) to give a white solid (320 mg). ^{13}C NMR and HPLC showed that this consisted of compound **8** (85%) and compound **9** (15%).

Reaction of (2S)-(E)-2′-O,3′-N,4″-O-*Tris(benzyloxycarbonyl)*-11-O,12-O-*carbonyl*-N-*demethyl*-9-*deoxo*-9-*methoxyiminoerythromycin A* **7** *with Sodium Imidazolide.*—Imidazole (200 mg) in dry THF (5 cm^3) was treated with sodium hydride (50% dispersion in oil; 100 mg) and the mixture was stirred under argon for 30 min. An aliquot of this solution (0.1 cm^3) was added to a solution of compound **7** (35 mg) in dry THF (2 cm^3). After 18 h, the solution was diluted with ethyl acetate (50 cm^3) and washed with water (20 cm^3), dil. HCl (20 cm^3) and water (20 cm^3). The solution was dried, the solvent was removed, and the residue was chromatographed using ethyl acetate–hexane to give compound **3** (15 mg) and compound **7** (4 mg), both as white solids.

NMR Spectroscopy.—All ^1H and ^{13}C NMR experiments were conducted using standard pulse sequences on a Bruker AM400 NMR spectrometer fitted with a 5 mm $^1H/^{13}C$ dual

probe. A sample concentration of 70–80 mg cm^{-3} in $CDCl_3$ was used. Experiments with compounds **5** and **6** were conducted at a slightly elevated temperature (320 K) in order to improve spectral resolution.

The 2D ^1H, ^{13}C COLOC experiment[9] was conducted using the standard Bruker program. Free induction decays were acquired (160 scans; 4 dummy scans) over 18.5 kHz, into a 8 K data block for 256 incremental values of the evolution time. The polarisation delay (D2) was 0.064 s, and the refocussing delay (D3) was 0.032 s. The data were zero-filled to an 8 K by 1 K data matrix prior to double Fourier transform.

The ^1H NOE data were acquired using a modification of the method of Hall and Sanders.[16] Sub-saturation power levels were used in order to enhance irradiation selectivity.

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