Synthesis of 4″-Deoxy Motilides: Identification of a Potent and Orally Active Prokinetic Drug Candidate

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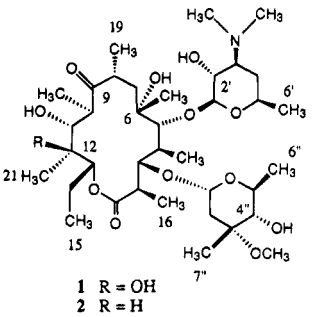
As an approach to discovering highly potent motilides with oral activity, novel 4″-deoxy derivatives of 8,9-anhydroerythromycin 6,9-hemiacetal were designed, synthesized, and evaluated for their gastrointestinal prokinetic activities. These compounds were orders of magnitude more potent than their 4″-hydroxy analogs in inducing smooth muscle contractions in an *in vitro* rabbit duodenal assay. Removal of the 12-hydroxy group, which was aimed at improving oral bioavailability, also afforded further potentiation in *in vitro* activity, leading to the identification of 8,9-anhydro-4″-deoxy-3′-*N*-desmethyl-3′-*N*-ethylerythromycin B 6,9-hemiacetal (ABT-229) as a potential prokinetic drug. ABT-229 was >300 000 times more potent than erythromycin *in vitro* and had 39% oral bioavailability in dog compared to its 4″,12-dihydroxy congener (EM-523), which was only 400 times more potent than erythromycin and had relatively low (1.4%) oral bioavailability.

Introduction

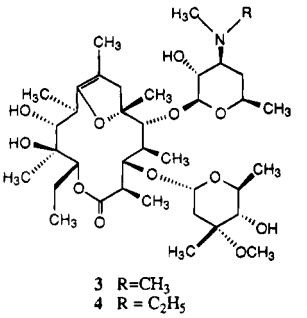
Omura *et al.*[1] have described the modification of erythromycin A **1** to produce a series of 8,9-anhydro 6,9-hemiacetals (enol ethers), represented by **3**,[2] which stimulate gastrointestinal (GI) motility in dog but, unlike **1**, lack antibacterial activity. Tsuzuki *et al.*[3] have proposed the name "motilides" for this class of erythromycin derivatives. Studies by Itoh *et al.*,[4] Depoortere *et al.*[5] and Satoh *et al.*[6] demonstrate that these compounds as well as their progenitor **1** bind to receptors of the peptide hormone motilin in the GI tract and mimic its physiological activity. Hence these compounds induce a pattern of antral and intestinal smooth muscle contractions that are characteristic of motilin-induced phase 3 activity of the migrating myoelectric complex. As this pharmacological action may be useful in the treatment of certain GI motility disorders, members of this class of compounds, e.g., EM-523[7] (**4**) and its 3′-*N*-isopropyl analog[8] EM-574, have been studied as potential prokinetic drugs.

Studies in our laboratories indicate that **4** has very low (1.4%) oral bioavailability in the dog. A plausible explanation is that enol ethers of erythromycin, such as **3** and **4**, undergo rapid reaction involving the 12-OH to produce 6,9:9,12-spiroacetals like **5** under acidic conditions as in the stomach.[9,10] Unlike **3** and **4**, spiroacetals such as **5** have weak prokinetic activities. For example, the pED_{50} of **5** in the *in vitro* assay (*vide infra*) was 5.78, compared to 8.40 for **3**. These factors may account for the lack of efficacy when **4** was administered orally to dogs.[11]

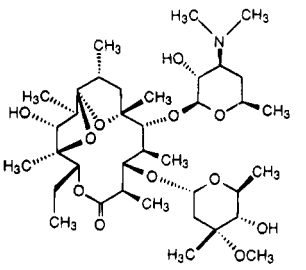
The objective of this study was to design and synthesize novel motilides with both more potent activity than **4** and oral efficacy in the dog. A compound meeting these criteria would merit further investigation as a potential prokinetic drug for the treatment of GI motility disorders including gastroesophageal reflux disease,



1 R = OH
2 R = H



3 R=CH_3
4 R = C_2H_5



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diabetic gastroparesis, nonulcerative dyspepsia, irritable bowel syndrome, and paralytic ileus.

Sunazuka *et al.*,[12] Omura *et al.*[1] and Tsuzuki *et al.*[3] have extensively studied the structure–activity relationships (SAR) of motilides and have reported that the enol ether moiety was important for high prokinetic potency and that modifications of the 3′-amino group further improved the smooth muscle stimulatory activity. On the other hand, acylation of the 4″-OH led to compounds with greatly diminished activity. In our quest to break new ground in the prokinetic area, we opted to extend the SAR by investigating a more drastic modification of the 4″-position, i.e., deoxygenation, to assess the importance of a free OH group at that position to biological activity.

As acid-catalyzed conversion to spiroacetals may contribute to low oral bioavailability in compounds such as **4**, we envisioned two approaches to preventing this reaction: removal of the 12-OH or reduction of the enol

† It is with deep regret that we announce the loss of our friend, colleague, and talented chemist, Dr. Leslie Alan Freiberg, who died on June 5, 1994.

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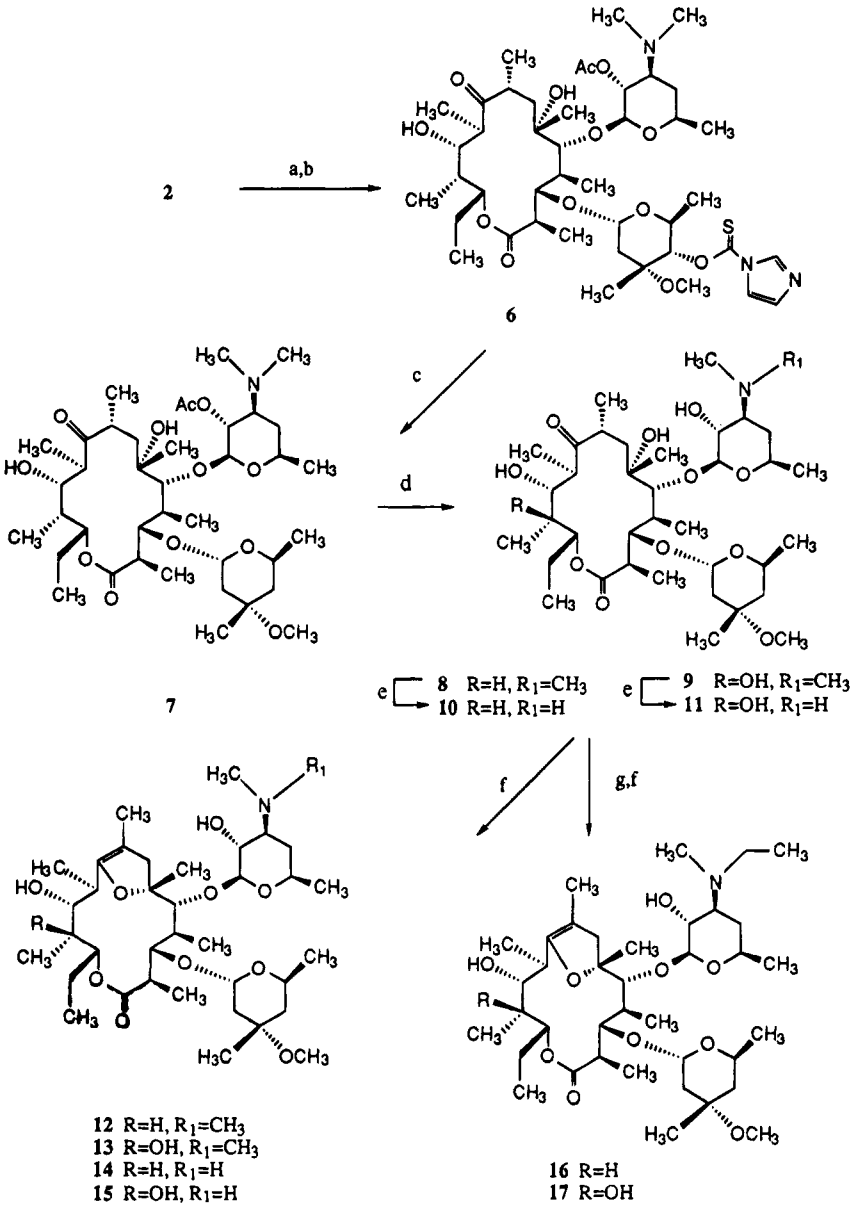
Scheme 1



[a] (a) Ac_2O/CH_2Cl_2; (b) TCDI/DMAP/CH_2Cl_2; (c) Bu_3SnH/AIBN/tol; (d) CH_3OH, reflux; (e) I_2/NaOAc/$CH_3OH/h\nu$; (f) $Cl_2CHCOOH/CH_3CN$; (g) $[(CH_3)_2CH]_2NC_2H_5/CH_3CN$.

ether moiety to a tetrahydrofuranyl system. We recently presented preliminary results from the latter approach,[13] but in this paper, we report the synthesis and *in vitro* evaluation of new 4″-deoxy and 4″,12-dideoxy congeners of **3**. Results of preliminary evaluations of oral bioavailability and efficacy of **16**, the most potent compound reported in this paper, are also discussed. Compound **16** has been code-named ABT-229.

Chemistry

Synthesis of the reference compounds **3, 4, 18,** and **19**,[3,14] as well as 4″-deoxyerythromycin A (**9**),[15] have been reported previously. The reported methodologies were followed with only slight modifications. Preparation of the novel 4″-deoxy analogs started from **1** or, in the case of the 4″,12-dideoxy congeners, from erythromycin B (**2**), the naturally occurring 12-deoxy analog of erythromycin A. Thus, after selective protection of the 2′-OH by acetylation under neutral conditions, treatment of the 2′-*O*-acetate of **2** with thiocarbonyldiimidazole resulted in thionoacylation of the 4″-OH group (Scheme 1) to give **6**. Treatment of **6** with tri-*n*-butyltin hydride provided 2′-*O*-acetyl-4″-deoxyerythromycin B (**7**), which was deprotected by treatment with CH_3OH to give 4″-deoxyerythromycin B (**8**).

The 4″-deoxy compounds **8** and **9** were converted to their respective enol ethers **12** and **13** by treatment with dichloroacetic acid.[14] Alternatively, these substrates were *N*-demethylated using conditions described by Freiberg[16] to provide substrates for further modifications of the 3′-amino group. This latter process afforded 4″-deoxy-3′-*N*-desmethylerythromycins B and A, **10** and **11,** respectively. Compounds **10** and **11** were converted to their corresponding enol ether derivatives **14** and **15** as described above.

To provide the 3′-*N*-desmethyl-*N*-ethyl congeners **16** and **17,** compounds **10** and **11** were *N*-alkylated using ethyl iodide in the presence of *N,N*-diisopropylethylamine. The resulting *N*-ethyl intermediates were not

purified further but converted into the desired products with dichloroacetic acid. The foregoing route provided compounds for studying the effects of 4″- and 12-deoxygenations, as well as a limited assessment of effects of 3′-amine modification on the activity of the 4″,12-dideoxy congener.

Biological Activity

Prokinetic activity was studied *in vitro* as smooth muscle contractility in isolated rabbit duodenum.[17] Longitudinal smooth muscle of the duodenum of male New Zealand white rabbits was bluntly separated from circular smooth muscle in a balanced electrolyte solution (pH 7.4). Isolated muscle (~30 mg) was mounted in a tissue bath and attached to force transducers for measurement of contractile activity. A dose-response curve was generated with the test compound and results expressed ($n = 2-3$) as fractional activity relative to the response observed in the presence of 10^{-6} M methacholine. From the dose-response profile, a pED_{50} (−log concentration yielding half-maximal contraction) was calculated as a comparative parameter for evaluating contraction induction potency. Results are also expressed as the differences between pED_{50}s for test compound versus erythromycin ($pED_{50} = 5.85$).

In vivo activity was assessed in fasted conscious male beagle dogs.[18] The animals were surgically prepared by applying strain gauge transducers to the serosal surfaces of the stomach antrum, duodenum, and jejunum. Smooth muscle motility responses were recorded and the results scored as the area under the force/time curve for 60 min following oral dosing. Dogs were dosed by gavage with an aqueous solution of the lactobionate salt of the test compound or a reference compound. Dose-response curves were generated by repeated dosing in the same animal on successive days with normalization to a dose common to all animals studied for purposes of interanimal comparisons.

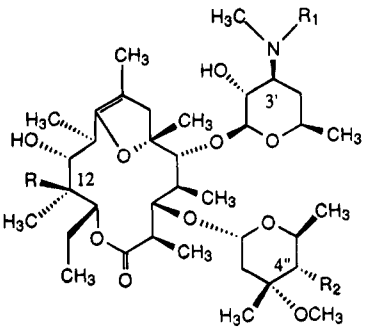
Pharmacokinetics

Selected test compounds, prepared as 2 mg/mL solutions of the lactobionate salt, were administered to groups of fasted beagle dogs (male/female) either at a 0.5 mg/kg iv or a 1 mg/kg po dose. Blood samples were obtained from each animal at selected times over the 12 h postdosing interval. The parent compounds were extracted from an alkalinized plasma aliquot using a mixture of EtOAc and C_6H_{14} (1:1). The desired compounds were separated from plasma contaminants on a 10 cm × 4.6 mm 3 μm Spherisorb ODS-AQ column (YMC inc.) using an CH_3CN:MeOH:buffer (0.01 M $(CH_3)_4NOH$ in 0.05 M KH_2PO_4 adjusted to pH 6.9) mobile phase at a flow rate of 1.0 mL/min. Analysis and quantitation was by electrochemical detection in the oxidative mode (DET1 = +0.40 V, DET2 = +0.85 V, GUARD = +0.90 V). The assay was linear (correlation coefficient >0.99) over the concentration range 0−500 ng/mL with a pooled standard deviation <7.5% from the analysis of quadruplicate standards at eight separate concentrations with an estimated limit of quantitation of ~7 ng/mL.

Results and Discussion

Removal of the 4″-OH did not lead to loss of activity but in fact led to an increase in *in vitro* potency. Even in the case of compounds still bearing a keto group at C-9, e.g., **8** and **9**, the pED_{50}s were 6.33 and 6.61, respectively, compared to 5.85 for **1**. As in the previously reported SARs,[1,3] conversion of **8** and **9** to their enol ether analogs **12** and **13** resulted in dramatic increases in potency. In the enol ether series (Table 1), there was a slight increase in potency when the 4″-OH of the *N,N*-dimethyl derivative **3** was removed to provide **13**. In the other cases, such as the 3′-*N*-desmethyl derivatives **18** and **15**, the 3′-*N*-desmethyl-*N*-ethyl analogs **4** and **17**, and the 12-deoxy-3′-*N,N*-dimethyl compounds **19** and **12**, 4″-deoxygenation led to as much as a thousand fold increase in potency. In a recent communication, Koga[19] *et al.* reported the effect of 4″-deoxygenation on a series of 11-deoxy-12-*O*-methyl-11-oxo-8,9-anhydroerythromycin A hemiacetals. This modification also resulted in increases in *in vitro* potency relative to the corresponding 4″-OH compounds; however, the improvements were rather modest (6−11-fold increase) compared to those observed in our series.

Table 1. *In Vitro* Activities of 4″-Hydroxy, 4″-Deoxy, and 4″,12-Dideoxy Analogs



				in vitro prokinetic activity	
compd	R	R_1	R_2	pED_{50}	activity relative to erythromycin A (log)
3	OH	CH_3	OH	8.40	2.55
4	OH	C_2H_5	OH	8.45	2.60
12	H	CH_3	H	11.26	5.41
13	OH	CH_3	H	8.41	2.56
14	H	H	H	>12.0	>6.15
15	OH	H	H	9.74	3.89
16	H	C_2H_5	H	>12.0	>6.15
17	OH	C_2H_5	H	11.15	5.30
18	OH	H	OH	7.1	1.30
19	H	CH_3	OH	8.68	2.83

The 12-deoxy congeners were also uniformly more potent than their 12-hydroxy counterparts. The increase in activity was modest when the 4″-position had a hydroxy group, e.g., **3** *vs* **19**, but large for the 4″,12-dideoxy congeners. This is illustrated by the differences in activity between the *N,N*-dimethyl **13** and **12**, the 3′-*N*-methyl **15** and **14** as well as the 3′-*N*-methyl-*N*-ethyl derivatives **17** and **16**. The increase in potency accorded by deoxygenation at both 4″ and 12 was more than additive and quite unexpected. This is clearly illustrated by comparisons between the pairs **3**:**12**, **4**:**16**, and **18**:**14**.

As in the case of the motilides reported by Sunazuka,[12] modification of the 3′-amine also influenced activity in the 4″,12-dideoxy series. In this case, however, the secondary amine **14** was as potent as the 3″-*N*-methyl-*N*-ethyl derivative **16** and more potent than the *N,N*-dimethyl homolog **12**. This SAR differed from that of the 4″-OH congeners, in which the second-

Table 2. Pharmacokinetics of Selected Compounds in Dog

compd	pharmacokinetics	
	$t_{1/2}$ (h)	bioavailability (%)
4	2.4	1.4
14	2.0	23.8
16	5.5	39.0
17	2.6	2.7

ary amine **18** was much weaker in activity compared to the corresponding tertiary amines **3** and **4**. It is difficult therefore to predict the effect of *N*-substituents in the 4″-deoxy series, based on the results of these studies.

Compounds **14** and **16** fulfilled our requirements for evaluation of bioavailability and *in vivo* activity in order to test the hypothesis behind these studies. The compounds were orders of magnitude more potent than EM-523 and lacked a 12-OH group; therefore, they were expected to exhibit better oral bioavailabilities than EM-523. Table 2 shows the pharmacokinetic data for **14** and **16** compared with the 12-OH congeners **4** and **17**, compound **17** being the most potent compound in this communication with a 12-OH. Of significance are the bioavailabilities of the compounds, which were poor for the 12-OH compounds **4** and **17** but much better for the 12-deoxy analogs **14** and **16**. On the basis of its potent *in vitro* activity, improved oral bioavailability and superior $t_{1/2}$, the *in vivo* activity of **16** was studied by measuring GI motility after oral administration in the dog. Compound **16** was very potent, with an oral ED_{50} of 0.50 μg/kg in conscious dogs.

Conclusion

Compound **16** represents a novel class of motilides with *in vitro* prokinetic activity that is approximately 300 000 times higher than **1**. The compound demonstrates good oral bioavailability and efficacy in dog. These properties qualify **16** for further evaluation as a potential drug for the treatment of GI motility disorders.

Experimental Section

NMR spectra were recorded on a GE QE 300 at 300 MHz for ^{1}H and at 75.48 MHz for ^{13}C with chemical shifts in ppm downfield from an internal TMS standard. D_2O was added to remove exchangeable protons from the ^{1}H-NMR spectra. Coupling constants are given in hertz. MS were recorded with a Finnigan SSQ 700 instrument. Melting points were determined on a Mel Temp II and are uncorrected. Optical rotations were measured at the sodium D line with a Perkin-Elmer 241 polarimeter at 25 °C. The progress of all reactions were monitored by TLC on E. Merck precoated silica gel (0.2 mm layer) plates containing a fluorescent indicator (Merck, 5539). Detection was first by UV (254 nm) and then by charring with a solution of ammonium molybdate tetrahydrate (12.5 g) and cerium sulfate tetrahydrate (5.0 g) in 10% aqueous sulfuric acid (500 mL). Flash chromatography was performed using silica gel (230–400 mesh, Merck). Reaction solvents were predistilled over appropriate drying agents just prior to use. Erythromycin A and B were available from the Chemical and Agricultural Products Division of Abbott Laboratories.

2′-*O*-Acetyl-4″-(imidazolylthiocarbonyl)erythromycin B (6). Acetic anhydride (0.4 mL, 4.3 mmol) was added to **2** (2.82 g, 3.9 mmol) in CH_2Cl_2 (100 mL) at 0 °C. After 15 min, the solution was allowed to warm to room temperature and stirred for 12 h. Saturated $NaHCO_3$ (100 mL) was added and the organic phase washed with H_2O (3 × 100 mL), dried ($MgSO_4$), and evaporated *in vacuo* to provide crude 2′-*O*-acetylerythromycin B. This intermediate was redissolved in CH_2Cl_2 (100 mL). 4-(Dimethylamino)pyridine (DMAP, 714 mg, 5.8 mmol) and 1,1′-thiocarbonyldiimidazole (TCDI, 1.04 g, 5.8 mmol) were subsequently added, and the mixture was stirred for 14 h. The mixture was diluted with 50 mL of CH_2Cl_2 and washed with aqueous $NaHCO_3$ (10%, 150 mL) and with H_2O (3 × 150 mL), then dried (Na_2SO_4), and concentrated *in vacuo*. The residue was recrystallized from CH_3CN to yield 2.60 g (77%) of solid **6**: mp 142 °C; $[\alpha]_D$ −101° (*c* 0.51, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86 (d, *J* = 7.8, 3H), 0.87 (t, *J* = 9.0, 3H), 0.99 (d, *J* = 6.0, 6H), 1.0 (d, *J* = 9.0, 3H), 1.15 (d, *J* = 5.7, 3H), 1.20 (s, 3H), 1.22 (d, 6H), 1.30 (m, 1H), 1.41 (s, 3H), 1.43–1.85 (m, 5H), 2.08 (s, 3H), 2.10 (m, 1H), 2.30 (s, 6H), 2.46 (bd, 1H), 2.68 (m, 2H), 2.75–3.05 (m, 2H), 3.4 (s, 3H), 3.5 (m, 2H), 3.73 (bd, 1H), 3.97 (bd, 1H),4.52 (m, 1H), 4.70 (d, *J* = 7.5, 1H), 4.75 (m, 1H), 5.02 (d, *J* = 4.5, 1H), 5.38 (m, 1H), 5.50 (d, *J* = 9.0, 1H), 7.05 (m, 1H), 7.60 (m, 1H), 8.30 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.04, 9.16, 9.40, 10.35, 18.08, 18.21, 21.06, 21.20, 21.38, 25.58, 27.14, 29.97, 35.40, 37.32, 38.42, 38.57, 39.88, 40.62, 44.47, 44.99, 49.37, 63.32, 63.65, 67.88, 69.35, 71.50, 73.15, 74.85, 75.04, 80.41 83.55, 86.80, 95.98 100.43, 117.71, 130.86, 136.68, 169.87, 175.55, 184.26, 220.27; MS *m/e* 870 (M^+). Anal. ($C_{43}H_{71}N_3O_{13}S$·0.25 H_2O) C, H, N, S.

2′-*O*-Acetyl-4″-deoxyerythromycin B (7). Azobisisobutyronitrile (AIBN, 72 mg, 4 mmol) was added to a solution of **6** (3.8 g, 4.36 mmol) in toluene (100 mL) and under N_2. *n*-Bu_3SnH (3.5 mL, 120 mmol) was added. The mixture was heated at 100 °C for 30 min and allowed to stir at room temperature for a further 15 min. Solvent was removed and the residue redissolved in CH_3CN (500 mL). The solution was washed with hexane (5 × 200 mL) and evaporated *in vacuo* to yield a residue which was recrystallized from CH_3CN to yield 2.53 g (78%) of **7**: mp 96 °C; $[\alpha]_D$ −57.5° (*c* 0.16, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85 (d, *J* = 7.5, 3H), 0.86 (t, *J* = 7.4, 7.4, 3H), 0.98 (d, *J* = 6.0, 3H), 1.0 (d, *J* = 6.0, 3H), 1.15 (s, 3H), 1.16 (d, *J* = 7.8, 3H), 1.18 (d, *J* = 6.3, 3H), 1.19 (d, *J* = 6.3, 3H), 1.20 (d, *J* = 7.5, 3H), 1.29 (d, *J* = 12.3, 1H), 1.30 (d,d, *J* = 11.7, 12.0, 2H), 1.42 (s, 3H), 1.48 (m, 1H), 1.5–1.9 (m, 6H), 2.04 (m, 1H), 2.05 (s, 3H), 2.20 (m, 1H), 2.25 (m, 1H), 2.26 (s, 6H), 2.6–2.75 (m, 2H), 2.88 (m, 1H), 3.0 (m, 1H), 3.3 (s, 3H), 3.5 (m, 1H), 3.57 (d, *J* = 9, 1H), 3.77 (m, 1H), 3.98 (dd, *J* = 3.0, 9.3, 1H), 4.25 (m, 1H), 4.66 (d, *J* = 6.3, 1H), 4.75 (dd, *J* = 6.3, 9.0, 1H), 5.02 (bd, *J* = 3.9, 1H), 5.36 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 9.09, 9.19, 9.40, 10.39, 15.59, 18.33, 21.15, 21.51, 21.87, 25.46, 25.58, 27.12, 30.60, 34.30, 37.39, 38.67, 38.86, 39.85, 40.65, 44.58, 45.04, 45.25, 49.16, 61.51, 63.21, 67.88, 69.40, 70.47, 71.75 , 74.78, 75.17, 79.33, 83.01, 96.90, 100.22, 170, 176.01, 220.15; MS *m/e* 744 (M + H^+). Anal. ($C_{37}H_{69}NO_{12}$) C, H, N.

4″-Deoxyerythromycin B (8). A solution of **7** (2.0 g, 2.7 mmol) in CH_3OH (50 mL) was stirred at room temperature for 12 h. Solvent was removed *in vacuo* to afford 1.85 g (98% yield) of solid **8**: mp 131 °C; $[\alpha]_D$ −70.5° (*c* 0.88, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85 (d, *J* = 7.5, 3H), 0.87 (t, *J* = 7.5, 3H), 1.0 (d, *J* = 6.0, 3H), 1.14 (s, 3H), 1.15 (d, *J* = 9.0, 3H), 1.16 (d, *J* = 7.5, 3H), 1.17 (d, *J* = 6.0, 3H), 1.19 (d, *J* = 4.5, 3H), 1.21 (d, *J* = 6.0, 3H), 1.25 (m, 2H), 1.45 (s, 3H), 1.46 (m, 1H), 1.65 (m, 4H), 2.02 (m, 1H), 2.10 (m, 1H), 2.21 (m, 1H), 2.30 (m, 1H), 2.32 (s, 6H), 2.55 (m, 1H), 2.62 (m, 1H), 2.89 (m, 1H), 3.0 (m, 1H), 3.25 (m, 1H), 3.27 (s, 3H), 3.54 (m, 1H), 3.62 (d, *J* = 6.0, 1H), 3.80 (m, 1H), 4.05 (dd, *J* = 3.0, 6.0, 1H), 4.30 (m, 1H), 4.54 (d, *J* = 6.0, 1H), 5.0 (d, *J* = 6.0, 1H), 5.35 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 9.13, 9.29, 9.34, 10.39, 15.58, 18.54, 21.35, 21.88, 25.49, 25.57, 27.83, 28.82, 34.41, 38.10, 39.02, 39.47, 39.28, 40.23, 44.94, 45.12, 45.18, 49.34, 61.54, 65.26, 68.56, 69.50, 70.47 70.95, 74.95, 75.35, 79.51, 83.32, 97.32, 102.69, 176.26, 219.57; MS *m/e* 702 (M + H^+). Anal. ($C_{37}H_{67}NO_{11}$) C, H, N.

4″-Deoxy-3′-*N*-desmethylerythromycin B (10). NaOAc·$3H_2O$ (2.02 g, 14.8 mmol) and I_2 (776 mg, 2.7 mmol) were added sequentially to a methanolic (20 mL) solution of **8** (1.9 g, 2.7 mmol). The mixture was exposed to a flood lamp (150 W) and stirred for 4 h. Saturated aqueous $Na_2S_2O_7$ (10 mL) was added and the mixture concentrated to half its volume *in vacuo*. CH_2Cl_2 (60 mL) was added and the mixture washed with saturated $NaHCO_3$ (50 mL) and H_2O (2 × 50 mL) The organic phase was dried (Na_2SO_4) and evaporated *in vacuo* and the residue chromatographed (1% NH_4OH/10% MeOH/$CHCl_3$) to yield 1.5 g (81%) of solid **10**: mp 134–136 °C; $[\alpha]_D$ −33° (*c* 1.1, $CHCl_3$);

^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 7.5, 3H), 0.91 (d, J = 6.0, 3H), 0.93 (d, J = 6.3, 3H), 1.0 (d, J = 6.3, 3H), 1.09 (d, J = 7.5, 3H), 1.18 (s, 3H), 1.19 (d, J = 6.0, 3H), 1.20 (d, J = 6.0, 3H), 1.22 (d, J = 6.0, 3H), 1.24 (d, J = 6.3, 3H), 1.30 (m, 2H), 1.40 (s, 3H), 1.50 (m, 2H), 1.70 (m, 3H), 2.0 (m, 1H), 2.10 (m, 1H), 2.2 (m, 1H), 2.44 (s, 3H), 2.45 (m, 1H), 2.60 (m, 1H), 2.80 (m, 2H), 3.0 (m, 1H), 3.25 (s, 3H), 3.28 (dd, J = 10.5, 1H), 3.52 (d, J = 4.5, 1H), 3.60 (m, 1H), 3.85 (m, 1H), 4.05 (dd, J = 4.5, 9.0, 1H), 4.30 (m, 1H), 4.50 (d, J = 9.0, 1H), 5.0 (d, J = 6.0, 1H), 5.9 (dd, J = 6.0, 7.5, 1H); ^{13}C NMR ($CDCl_3$) δ 8.92, 9.21, 10.11, 10.34, 16.00, 18.94, 21.11, 21.49, 25.26, 25.37, 28.33, 28.89, 33.22, 34.99, 37.27, 38.89, 39.34, 40.41, 44.47, 45.36, 49.32, 60.07, 62.01, 68.74, 69.77, 70.55, 74.57, 74.66, 75.06, 79.17, 85.97, 98.15, 103.18, 176.27, 221.43; MS m/e 688 (M + H$^+$) Anal. ($C_{36}H_{65}NO_{11}$·0.25 H_2O) C, H, N.

4''-Deoxy-3'-*N*-desmethylerythromycin A (11). Compound **11** was prepared in a manner similar to **10** starting from **9** (1.0 g, 1.4 mmol). The procedure yielded 0.82 g (84%) of **11**: mp 132–134 °C; [α]$_D$ −79.3° (c 1.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85 (t, J = 9, 3H), 1.03 (d, J = 9, 3H), 1.07 (d, J = 9, 3H), 1.12 (s, 3H), 1.15 (d, J = 9.3, 3H), 1.18 (s, 3H), 1.2–1.35 (m, 3H), 1.42 (s, 3H), 1.46 (m, 1H), 1.55 (m, 2H), 1.85–2.25 (m, 2H), 2.40 (m, 1H), 2.41 (s, 3H), 2.55 (m, 1H), 2.71 (m, 1H), 2.82 (m, 1H), 3.1 (m, 1H), 3.25 (s, 3H), 3.60 (m, 2H), 3.78 (m, 1H), 3.83 (bs, 1H), 3.98 (dd, J = 3, 10.5, 1H), 4.29 (m, 1H), 4.50 (d, J = 7.5, 1H), 4.98 (d, J = 4.5, 1H), 5.07 (dd, J = 3.0, 12, 1H); ^{13}C NMR ($CDCl_3$) δ 9.83, 10.77, 11.79, 16.02, 16.30, 18.43, 21.15, 21.36, 25.45, 26.53, 33.23, 34.66, 37.41, 38.71, 38.82, 39.66, 45.00, 45.17, 45.55, 49.35, 60.16, 61.71, 68.47, 69.07, 70.51, 74.73, 74.81, 74.89, 77.01, 79.14, 84.25, 97.46, 102.59, 175.78, 222.1; MS m/e 704 (M + H$^+$). Anal. ($C_{36}H_{65}NO_{12}$) C, H, N.

8,9-Anhydro-4''-deoxyerythromycin B 6,9-Hemiacetal (12). Compound **8** (1.0 g, 1.4 mmol) was dissolved in CH_3CN (20 mL). Dichloroacetic acid (0.17 mL, 2.06 mmol) was added and the mixture stirred for 7 h. Et_3N (10 mL) was added and the mixture evaporated *in vacuo*. The residue was redissolved in CH_2Cl_2 (80 mL) and washed with 8% aqueous $NaHCO_3$ (2 × 50 mL). The organic phase was dried (Na_2SO_4) and evaporated *in vacuo*. The residue was chromatographed (0.5% NH_4OH/5% MeOH in $CHCl_3$) to yield 0.87 g (89%) of **12**: mp 122–124 °C; [α]$_D$ −24.7° (c 0.34, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85 (d, J = 7.5, 3H), 0.89 (t, J = 7.5, 3H), 1.05 (d, J = 6.0, 3H), 1.09 (d, J = 9.0, 3H), 1.14 (s, 3H), 1.15 (d, J = 6.3, 3H), 1.17 (d, J = 6.0, 3H), 1.19 (d, J = 5.7, 3H), 1.19–1.30 (m, 2H), 1.33 (s, 3H), 1.54 (s, 3H), 1.60–1.70 (m, 4H), 1.95 (bd, J = 15, 1H), 2.05 (m, 1H), 2.25 (m, 1H), 2.26 (s, 6H), 2.35 (m, 1H), 2.50 (m, 1H), 2.62 (dd, J = 15, 6, 1H), 2.70 (m, 1H), 3.20 (dd, J = 9, 1H), 3.21 (m, 1H), 3.28 (s, 3H), 3.40 (m, 1H), 3.62 (m, 1H), 3.90 (d, J = 6.0, 1H), 4.05 (dd, J = 3.0, 6.0, 1H), 4.35 (m, 1H), 4.58 (d, J = 7.5, 1H), 5.10 (bd, J = 7.5, 1H), 5.15 (bd, J = 6.0, 1H); ^{13}C NMR ($CDCl_3$) δ 8.80, 10.34, 12.00, 13.07, 14.79, 21.22, 21.53, 24.95, 25.70, 26.33, 28.92, 33.46, 33.56, 40.35, 42.42, 43.03, 43.50, 44.49, 45.97, 49.35, 61.56, 65.62, 68.19, 70.60, 70.95, 71.07, 76.01, 77.18, 79.89, 85.67, 95.46, 101.43, 102.42, 151.57, 178.46; MS m/e 684 (M + H$^+$). Anal. ($C_{37}H_{65}NO_{10}$) C, H, N.

8,9-Anhydro-4''-deoxyerythromycin A 6,9-Hemiacetal (13). Compound **13** was prepared in the same manner as **12**, starting from **9** (0.5 g, 0.7 mmol) to yield 0.42 g (86%) of **13**: mp 123 °C; [α]$_D$ −40° (c 0.93, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.90 (t, J = 9.0, 3H), 1.03 (d, J = 6.0, 3H), 1.04 (d, J = 6.0, 3H), 1.10 (d, J = 6.0, 3H), 1.12 (s, 3H), 1.18 (s, 3H), 1.19 (d, J = 6.0, 3H), 1.20 (d, J = 6.0, 3H), 1.21–1.5 (m, 2H), 1.35 (s, 3H), 1.58 (s, 3H), 1.6–1.0 (m, 4H), 2.26 (m, 1H), 2.40 (s, 6H), 2.62 (m, 1H), 2.72 (m, 1H), 2.80 (m, 1H), 3.10 (m, 1H), 3.22 (m, 1H), 3.25 (s, 3H), 3.50 (dd, J = 6.0, 9.0, 1H), 3.65 (m, 1H), 3.90 (d, J = 6.0, 1H), 4.05 (m, 1H), 4.35 (m, 1H), 4.60 (d, J = 9.0, 1H), 4.85 (dd, J = 3.0, 9.0, 1H), 5.20 (d, J = 4.5, 1H); ^{13}C NMR ($CDCl_3$) δ 8.7, 10.8, 11.9, 13.3, 15.0, 16.1, 25.7, 26.4, 28.8, 30.5, 33.4, 40.3, 42.6, 43.3, 44.7, 45.9, 49.4, 61.7, 65.7, 68.3, 69.8, 70.5, 70.9, 75.4, 76.0, 78.2, 79.7, 85.5, 95.4, 101.5, 102.5, 151.8, 178.0; MS m/e 700 (M + H$^+$), Anal. ($C_{37}H_{65}NO_{11}$·H_2O) C, H, N.

8,9-Anhydro-4''-deoxy-3'-*N*-desmethylerythromycin B 6,9-Hemiacetal (14). Compound **14** was prepared following the procedure described for **13**, starting from **10** (0.13 g, 0.19 mmol). This yielded 0.12 g (94%) of **14**: mp 118 °C; [α]$_D$ −30° (c 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85 (d, J = 7.8, 3H), 0.89 (t, J = 8.4, 3H), 1.02 (d, J = 8.1, 3H), 1.07 (d, J = 8.1, 3H), 1.14 (s, 3H), 1.16 (d, J = 9.0, 3H), 1.17 (d, J = 9.0, 3H), 1.20 (d, J = 6.0, 3H), 1.25 (m, 2H), 1.35 (s, 3H), 1.45 (m, 2H), 1.50–1.72 (m, 4H), 1.55 (s, 3H), 1.95 (m, 2H), 2.25 (m, 1H), 2.35–2.75 (m, 4H), 2.41 (s, 3H), 3.15 (dd, J = 7.5, 1H), 3.28 (s, 3H), 3.29 (m, 1H), 3.41 (dd, J = 9.0, 1H), 3.7 (m), 3.91 (d, J = 6, 1H), 4.05 (m, 1H), 4.35 (m, 1H), 4.55 (d, J = 6, 1H), 5.10 (bd, J = 7.5, 1H), 5.15 (bd, J = 4.5, 1H); ^{13}C NMR ($CDCl_3$) δ 7.53, 9.68, 11.07, 12.66, 12.86, 20.03, 20.99, 21.63, 25.79, 26.60, 32.62, 33.10, 33.60, 35.64, 37.32, 41.66, 43.63, 45.17, 46.03, 49.24, 49.41, 54.39, 60.53, 61.27, 67.91, 70.69, 74.83, 76.08, 79.15, 81.93, 87.45, 95.61, 101.72, 115.97, 177.69; MS m/e 670 (M + H$^+$). Anal ($C_{36}H_{63}NO_{10}$·0.25H_2O) C, H, N.

8,9-Anhydro-4''-deoxy-3'-*N*-desmethylerythromycin A 6,9-Hemiacetal (15). Compound **15** was prepared in a manner similar to **12** starting from **11** (0.63 g, 0.9 mmol). The procedure yielded 0.57 g (92%) of **15**: mp 90 °C; [α]$_D$ −25.2° (c 1.0, $CHCl_3$) ^{1}H NMR ($CDCl_3$) δ 0.90 (t, J = 9.0, 3H), 1.02 (d, J = 6.6, 3H), 1.05 (s, 3H), 1.09 (d, J = 6, 3H), 1.15 (s, 3H), 1.16 (s, 3H), 1.18 (d, J = 4.5, 3H), 1.19 (d, J = 6, 3H), 1.20 (d, J = 7.5, 3H), 1.25–1.50 (m, 2H), 1.35 (s, 3H), 1.58 (s, 3H), 1.6–2.0 (m, 4H), 2.26 (m, 1H), 2.42 (s, 3H), 2.55 (m, 2H), 2.71 (m, 1H), 2.80 (m, 1H), 3.12 (dd, J = 6.0, 12, 1H), 3.30 (s, 3H), 3.50 (d, J = 9.0, 1H), 3.70 (m, 1H), 3.92 (d, J = 6.0, 1H), 4.08 (m, 1H), 4.38 (m, 1H), 4.52 (d, J = 6.0, 1H), 4.85 (dd, J = 3.0, 10.5, 1H), 5.20 (d, J = 4.5, 1H); ^{13}C NMR ($CDCl_3$) δ 8.61, 11.2, 11.83, 13.60, 14.80, 15.99, 21.1, 21.41, 21.55, 25.82, 26.31, 31.6, 32.80, 33.0, 43.1, 41.50, 45.2, 45.88, 49.63, 62.0, 67.53, 70.3, 70.1, 70.53, 71.0, 75.0, 76.2, 78.4, 79.60, 85.35, 95.85, 101.10, 102.80, 151.73, 178.88; MS m/e 686 (M + H$^+$). Anal. ($C_{36}H_{63}NO_{11}$·0.5H_2O) C, H, N.

8,9-Anhydro-4''-deoxy-3'-*N*-desmethyl-3'-*N*-ethylerythromycin B 6,9-Hemiacetal (16). Compound **10** (1.3 g, 1.9 mmol) was dissolved in CH_3CN (30 mL) and the solution warmed to 50 °C. *N,N*-diisopropylethylamine (1.6 mL, 6.5 mmol) was added followed by ethyl iodide (1.5 mL, 19 mmol). The mixture was stirred for 3 h and diluted with CH_2Cl_2 (60 mL). The solution was washed sequentially with Sorensen's buffer (40 mL), H_2O (40 mL) and brine (40 mL). The organic phase was dried (Na_2SO_4) and evaporated to yield 1.13 g of a residue. The residue was redissolved in CH_3CN (40 mL). Dichloroacetic acid (0.26 mL, 2.9 mmol) was added and the mixture stirred for 4 h. Et_3N (20 mL) was added and solvent removed *in vacuo*. The residue was redissolved in CH_2Cl_2 (80 mL) and the solution washed sequentially with 8% aqueous $NaHCO_3$ (50 mL) and H_2O (2 × 50 mL). The organic phase was dried (Na_2SO_4) and evaporated to yield a residue which was chromatographed (0.5% NH_4OH/5% MeOH in $CHCl_3$) to afford 1.01 g (77%) of solid **16**: mp 94 °C; [α]$_D$ −30.9° (c 0.87, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85 (d, J = 7.8, 3H), 0.90 (t, J = 6, 3H), 1.03 (d, J = 9, 3H), 1.04 (d, J = 3.5, 3H), 1.10 (t, J = 6.0, 3H), 1.14 (s, 3H), 1.15 (d, J = 6.0, 3H), 1.16 (d, J = 9.0, 3H), 1.20 (d, J = 5.7, 3H), 1.28 (m, 3H), 1.35 (s, 3H), 1.45 (m, 2H), 1.58 (s, 3H), 1.60–1.75 (m, 4H), 1.92–2.5 (m, 2H), 2.43 (s, 3H), 2.28 (m, 1H), 2.38–2.80 (m, 5H), 3.20 (m, 1H), 3.28 (s, 3H), 3.30 (m, 1H), 3.40 (m, 1H), 3.62 (m, 1H), 3.90 (d, J = 7.5, 1H), 4.06 (m, 1H), 4.38 (m, 1H), 4.58 (d, J = 9.0, 1H), 5.12 (bd, J = 10.5, 1H), 5.18 (d, J = 6.3, 1H); ^{13}C NMR ($CDCl_3$) δ 8.85, 10.36, 11.99, 13.17, 13.85, 14.63, 21.26, 21.63, 24.96, 25.74, 26.36, 29.67, 33.51, 36.24, 42.43, 43.00, 43.26, 44.50, 46.03, 47.61, 49.34, 61.42, 64.68, 68.28, 70.59, 70.68, 71.57, 77.0, 80.12, 85.70, 95.59, 101.36, 102.44, 151.54, 178.31; MS m/e 698 (M + H$^+$). Anal. ($C_{38}H_{67}NO_{10}$) C, H, N.

8,9-Anhydro-4''-deoxy-3'-*N*-desmethyl-3'-*N*-ethylerythromycin A 6,9-Hemiacetal (17). Compound **17** was prepared in the same manner as **16**, starting from **11** (0.7 g, 1.0 mmol). The procedure yielded 0.55 g (78%) of solid **17**: mp 162 °C; [α]$_D$ −20.9° (c 0.27, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.90 (t, J = 9.0, 3H), 1.05 (d, J = 7.5, 3H), 1.06 (s, 3H), 1.08 (d, J = 9.0, 3H), 1.09 (t, J = 6.0, 3H), 1.12 (s, 3H), 1.13 (d, J = 6.0, 3H), 1.14 (d, J = 6.0, 3H), 1.20 (d, J = 6.0, 3H), 1.25–1.55 (m, 2H), 1.35 (s, 3H), 1.58 (s, 3H), 1.62 (m, 2H), 2.0 (m, 3H), 2.25 (s, 3H), 2.30 (m, 1H), 2.40 (m, 2H), 2.60 (m, 1H), 2.70 (m, 1H),

2.80 (m, 1H), 3.20 (dd, $J = 6.0, 9.0$, 1H), 3.29 (s, 3H), 3.45 (m, 1H), 3.65 (m, 1H), 3.90 (d, $J = 6.0$, 1H), 4.09 (m, 1H), 4.40 (m, 1H), 4.52 (d, $J = 7.5$, 1H), 4.89 (dd, $J = 3.0, 10.5$, 1H), 5.20 (d, $J = 4.5$, 1H); ^{13}C NMR ($CDCl_3$) δ 8.71, 10.95, 11.94, 13.35, 13.90, 14.93, 16.08, 21.14, 21.25, 21.51, 26.43, 29.61, 30.47, 33.41, 42.58, 43.35, 44.71, 45.97, 47.62, 49.38, 61.19, 64.79, 68.38 , 69.87, 70.50, 70.57, 75.42, 76.16, 78.24, 79.89, 85.47, 95.40, 101.51, 102.63, 151.75, 178.52; MS m/e 714 ($M + H^+$). Anal. ($C_{38}H_{67}NO_{11}$) C, H, N.

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