SYNTHESIS, *IN VITRO* AND *IN VIVO* ACTIVITY OF NOVEL 9-DEOXO-9a-AZA-9a-HOMOERYTHROMYCIN A DERIVATIVES; A NEW CLASS OF MACROLIDE ANTIBIOTICS, THE AZALIDES

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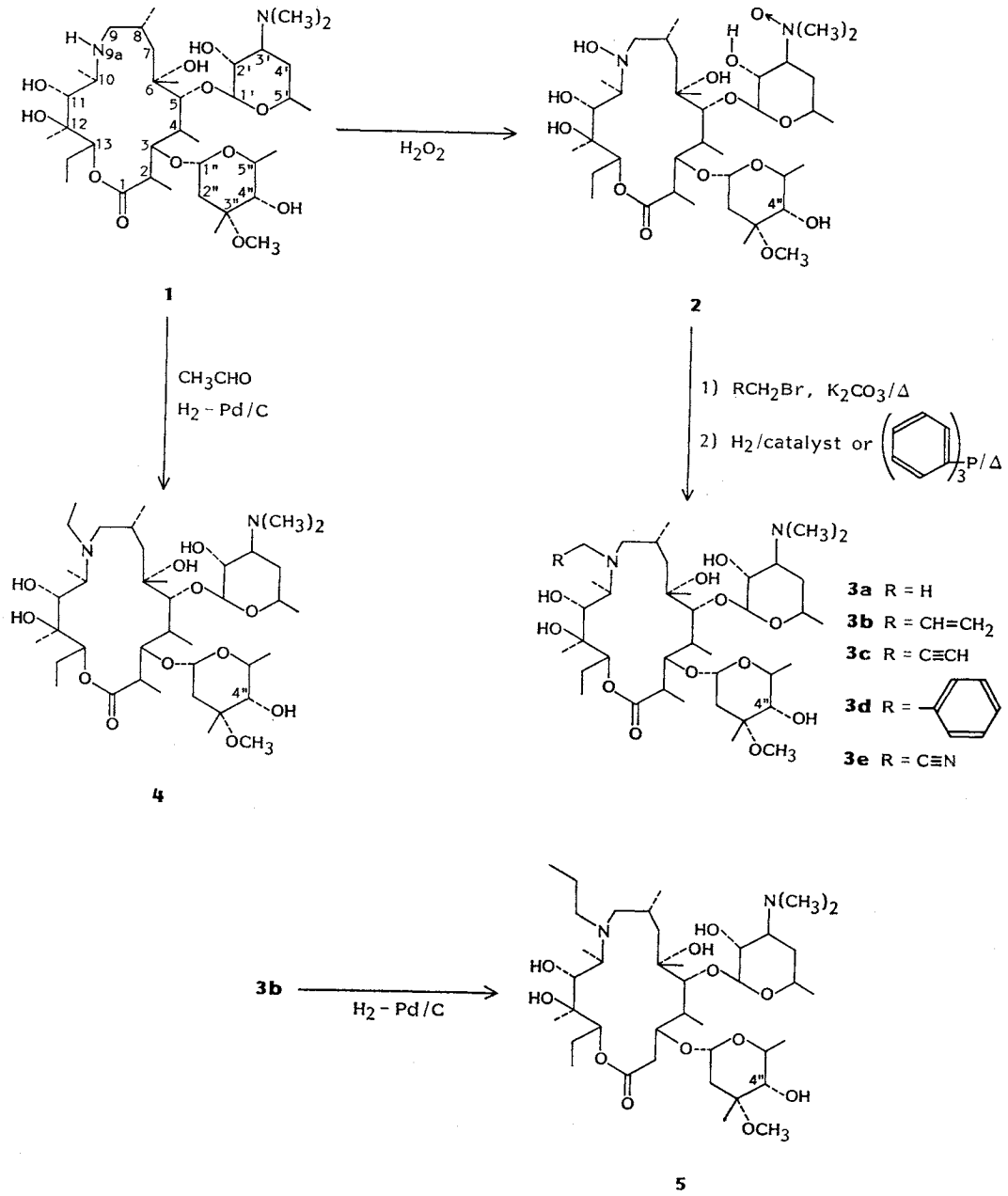
A series of erythromycin A-derived semisynthetic antibiotics, featuring incorporation of a basic nitrogen atom into a ring expanded (15-membered) macrocyclic lactone, have been prepared and biologically evaluated. Semisynthetic modifications focused upon (1) varied substitution at the macrocyclic ring nitrogen and (2) epimerization or amine substitution at the C-4″ hydroxyl site within the cladinose sugar. In general, the new azalides exhibit improved Gram-negative potency, expanding the spectrum of erythromycin A to fully include *Haemophilus influenzae* and *Neisseria gonorrhoeae*. When compared to erythromycin A, the azalides exhibit substantially increased half-life and area-under-the-curve values in all species studied. The overall *in vitro*/*in vivo* performance of *N*-methyl, C-4″ epimers **3a** and **9**; and C-4″ amine **11** identify these compounds as the most interesting erythromycin A-superior agents. Compound **3a** has been advanced to clinical study.

Erythromycin A is a widely used antibiotic in oral outpatient therapy, including pediatrics. It is frequently the agent of choice for treatment of respiratory, cutaneous, *Chlamydia*, and *Campylobacter* infections. However, erythromycin A is not indicated for the treatment of *Haemophilus influenzae* except with co-administration of sulfonamides. Erythromycin A is also unstable at gastric pH, and is poorly absorbed with oral dosing.

In our effort to expand the antimicrobial spectrum and to improve upon the pharmacokinetic properties of erythromycin A, the syntheses of erythromycin A-derived 15-membered aza-macrolides depicted in Schemes 1 and 2 were undertaken. Herein are presented the antibacterial profiles of the series, which features varied alkyl substitution at the 9a-aza site within the macrocyclic ring, and modifications at the C-4″ site within the cladinose sugar. Additionally, for selected compounds, anti-infective activity against *Staphylococcus aureus* in mice, and pharmacokinetic profiles in several species are presented.

Scheme 1.

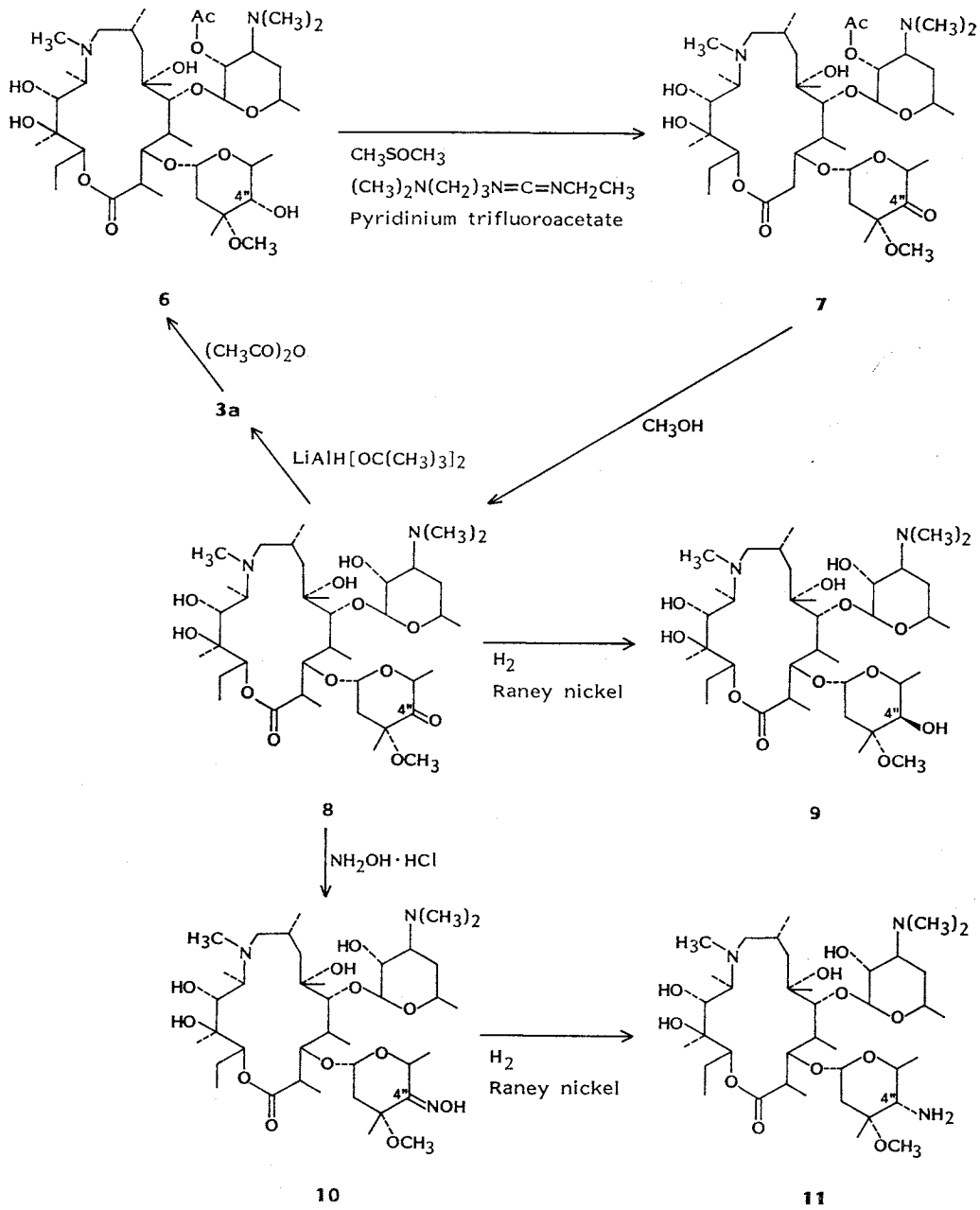


Chemistry

The syntheses of the novel 15-membered macrolides described in this paper, which are[1],† 9-deoxo-9a-aza-9a-homoerythromycin A derivatives, are depicted in Schemes 1 and 2. We refer to this novel class of 15-membered aza-macrolides as the azalides. In all cases, parent macrolide[2], 9-deoxo-9a-aza-9a-homoerythromycin A (compound **1**) and its C-4″ epimer **12**[3] served as starting materials. While the simple *N*-ethyl derivative **4** was prepared by a straightforward reductive amination of **1**

† Specifically, this nomenclature follows the instructive examples of p. 501 and p. 506 in ref 1.

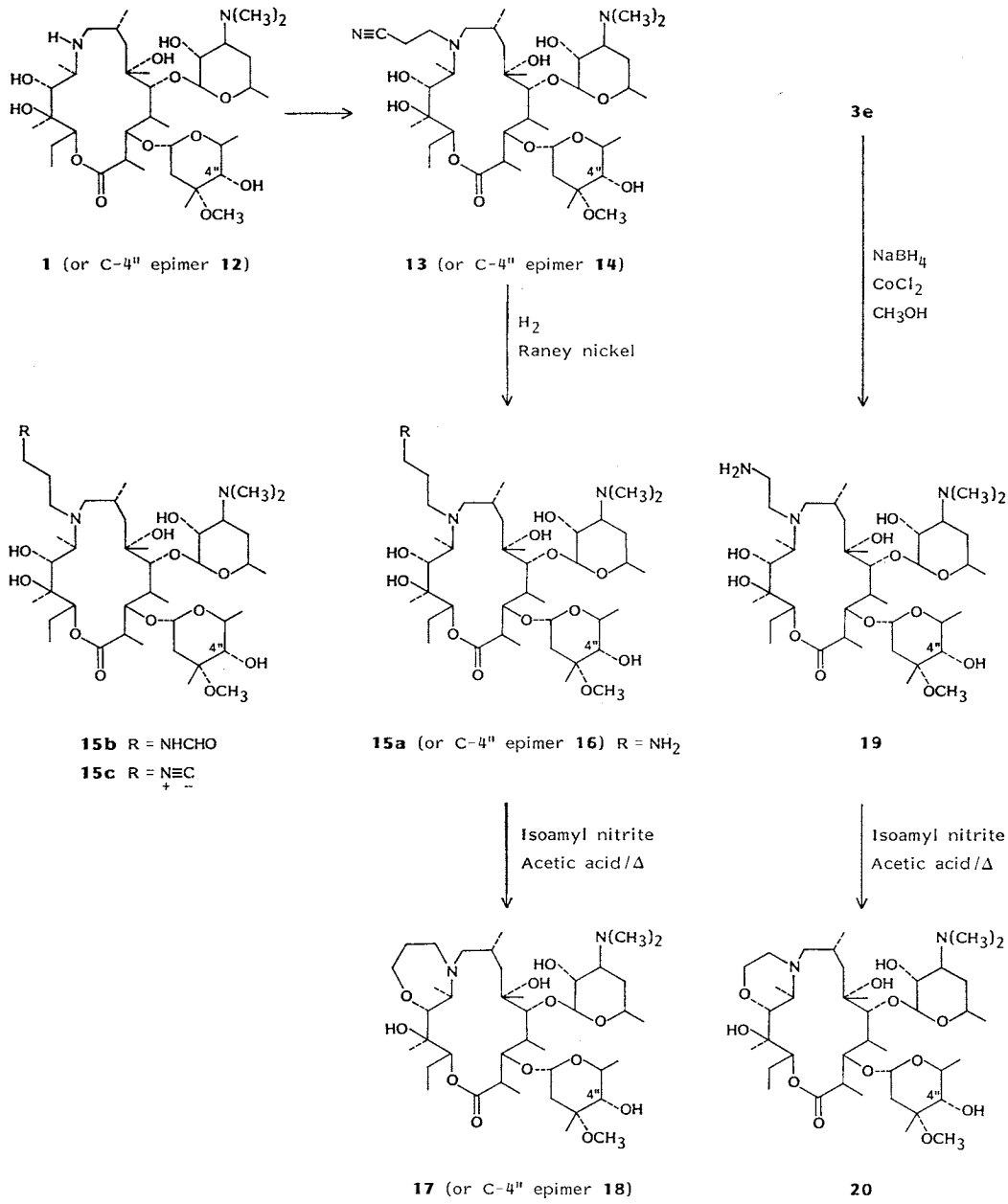
Scheme 1. (Continued)



with acetaldehyde, other *N*-alkyl analogs were prepared by alkyl halide reaction with *N*-oxide **2**, followed by catalytic hydrogenolysis or triphenylphosphine-induced deoxygenation (**2** to **3a**~**3e**)†. In the latter approach, oxygen served as a blocking group to prevent quaternization at the 3′-nitrogen

† Unbeknownst to us at the time of our **1**→**3a** synthesis, compound **3a** had been synthesized by alternative methods in yet unpublished work: S. DJOKIC and G. KOBREHEL (Pliva Pharmaceuticals, Zagreb, Yugoslavia): Novel derivatives of erythromycin A, procedures for their preparation, and their utilization as antibacterials. Belgium Patent 892,357, July 1, 1982.

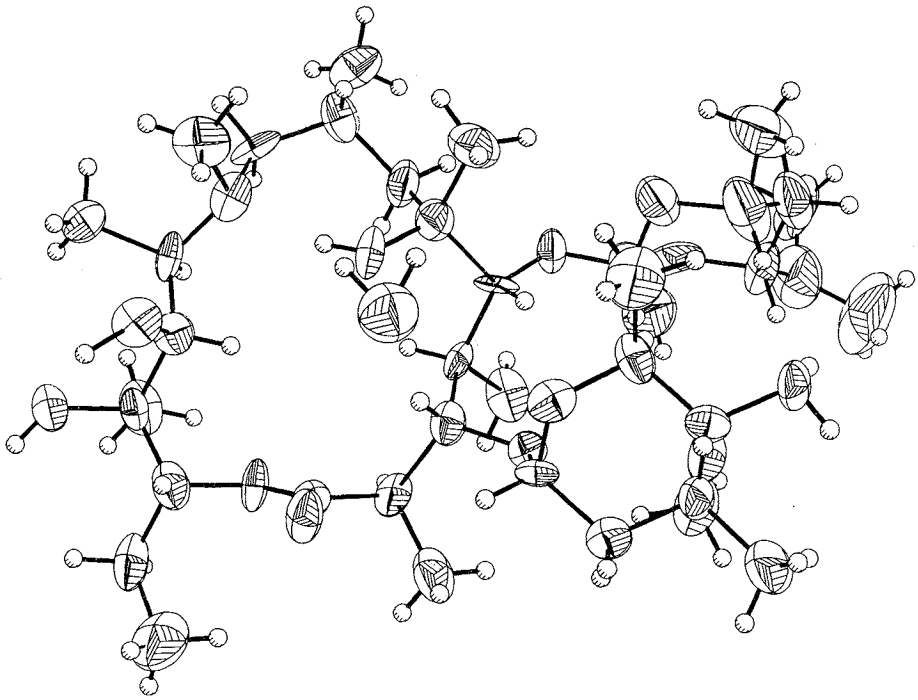
Scheme 2.



of the basic (desosamine) sugar.

Triphenylphosphine was utilized for deoxygenation in the **2** to **3** conversions where the desired products (**3b** and **3c**) are vulnerable to over-reduction by catalytic hydrogenation. *N*-Propyl derivative **5** was realized by catalytic reduction of the corresponding *N*-allyl compound **3b**; and also by a Barton-type deamination[4,5] of **15a**. In the latter approach to **5**, amine **15a** was first *N*-formylated with acetic-formic anhydride[6] to afford **15b** (Scheme 2). Dehydration of **15b** with *p*-toluenesulfonyl chloride in pyridine afforded the corresponding isonitrile **15c**, which was then deaminated to **5** by

Fig. 1. Molecular structure of compound **11**.



treatment with tri-*n*-butyltin hydride and azobisisobutylnitrile in refluxing xylene.

After protection of the C-2′ hydroxyl group by selective acetylation, *N*-methyl analog **3a** was efficiently oxidized (modified Moffat-Pfitzner conditions[7]) to the corresponding C-4″ ketone **7**. Deacetylation of **7** with methanol afforded ketone **8** in high yield; and catalytic hydrogenation of **8** (Raney nickel catalyst) afforded **9**, the C-4″ epimer of **3a** (overall yield from **3a**: 39%).

The stereochemical configuration at C-4″ in compound **9** is evident from NMR comparisons with the erythromycin A-derived epimer **3a**. Moreover, compound **9** is obtainable by an alternative synthesis[3] utilizing 4″-*epi*-erythromycin A[8] as its precursor. Acid methanolysis (HCl - MeOH) of the latter compound, as expected, affords the known[9,10] C-4″ axial hydroxyl-bearing α-methyl arcanoside sugar (F. C. SCIAVOLINO and M. A. GUADLIANA; privately communicated results, to be published). Treatment of ketone **8** with hydroxylamine afforded the corresponding C-4″ oxime **10**, which was converted to C-4″ equatorial amine **11** by catalytic hydrogenation (Raney nickel catalyst; yield from **3a**: 18%)[11]. The relative configuration of the 4″-amine in compound **11** was established *via* X-ray analysis (Fig. 1).

As shown in Scheme 2, parent compound **1** and its C-4″ epimer **12** served as starting materials for the synthesis of bicyclic macrolides **17** and **18** (the C-4″ epimer of **17**). Thus Michael condensation of **1** and **12** with acrylonitrile and subsequent reduction of the resulting cyanoethyl adducts (**13** and **14**), afforded the precursors to **17** and **18**, amines **15a** and **16**. Diazotization of **15a** or **16** with isoamyl nitrite in glacial acetic acid produced the corresponding 7-membered heterocyclic derivatives **17** and **18**, respectively. Similarly, treatment of amine **19** (prepared by sodium borohydride - cobaltous chloride[12] reduction of the *N*-cyanomethyl compound **3e**† with isoamyl nitrite - acetic acid afforded

† Attempts to reduce **3e** by catalytic hydrogenation produced exclusively hydrogenolysis product **1**.

Table 1. ^{13}C Chemical shift assignments of representative azalides[a].

Carbon[b]	3a	9	11	3c	15a	18
C-1	179.00 s	178.81 s	178.71 s	178.20	177.27 s	176.61 s
C-1′	102.84 d	102.59 d	102.96 d	102.83 d	102.97 d	102.62 d
C-1″	94.41 d	95.16 d	95.00 d	94.77 d	95.62 d	96.37 d
C-5	83.14 d	83.14 d	83.63 d	83.56 d	84.13 d	83.91 d
C-3	78.07 d	77.66 d	77.85 d	77.97 d	79.13 d	81.03 d
C-4″	77.64 d	77.52 d	62.44 d	77.63 d	78.11 d	79.60 d
C-13	77.44 d	74.47 d	77.46 d	77.52 d	77.73 d	77.79 d
C-12	74.08 s	74.32 s	72.81 s	74.88 s	74.75 s	74.30 d
C-6	73.67 s	74.16 s	73.72 s	73.86 s	74.54 s	74.12 s
C-11	73.35 d	73.56 d	73.99 d	74.02 d	74.42 d	74.25 d
C-3″	72.92 s	73.77 s	74.39 s	72.84 s	72.93 s	73.62 s
C-2′	70.80 d	71.07 d	71.08 d	70.77 d	71.08 d	71.19 d
C-9	70.00 t	70.20 t	70.21 t	63.31 t	64.56 t	68.73 t
C-5′	68.87 d	68.27 d	68.52 d	68.82 d	68.87 d	68.31 d
C-5″	65.79 d	65.60 d	66.63 d	65.60 d	65.66 d	65.46 d
C-3′	65.59 d	63.52 d	65.83 d	65.52 d	65.53 d	63.34 d
C-10	62.55 d	62.72 d	62.19 d	61.08 d	58.98 d	63.08 d
3″-OCH_3	49.44 q	49.20 q	49.42 q	49.36 q	49.43 q	49.21 q
C-2	45.43 d	45.49 d	45.28 d	44.75 d	45.10 d	45.07 d
C-4	42.56 d	42.76 d	42.08 d	43.05 d	41.08 d	39.93 d
C-7	42.14 t	42.25 t	42.53 t	42.02 t	40.76 t	42.34 t
$N(CH_3)_2$	40.32(2) q	40.36(2) q	40.39(2) q	40.29(2) q	40.40(2) q	40.35(2) q
NCH_3	36.00 q	36.10 q	36.35 q	—	—	—
C-2″	34.60 t	29.10 t	35.33 t	34.70 t	29.26 t	29.15 t
C-4′	28.79 t	29.10 t	29.00 t	28.65 t	29.15 t	29.08 t
6-CH_3	27.57 q	27.62 q	27.50 q	26.70 q	26.14 q	26.77 q
C-8	26.69 d	26.81 d	26.84 d	26.36 d	29.15 d	26.65 d
5′-CH_3	21.95 q	22.01 q	22.31 q	21.89 q	23.28 q	21.92 q
3″-CH_3	21.53 q	21.53 q	21.98 q	21.60 q	21.39 q	21.51 q
13-CH_2	21.30 t	21.47 t	21.35 t	21.54 t	21.08 t	20.96 t
2-CH_3	21.34 q	21.40 q	21.55 q	21.27 q	21.22 q	21.44 q
5″-CH_3	18.11 q	18.28 q	18.97 q	18.09 q	18.32 q	17.38 q
8-CH_3	16.30 q	16.40 q	16.31 q	16.58 q	16.56 q	16.98 q
12-CH_3	14.46 q	14.41 q	14.75 q	14.40 q	15.30 q	16.00 q
10-CH_3	11.17 q	11.27 q	11.25 q	11.23 q	10.99 q	10.75 q
13-CH_3	8.84 q	8.95 q	9.19 q	10.80 q	9.64 q	10.09 q
4-CH_3	6.98 q	7.07 q	7.43 q	9.46 q	7.00 q	6.75 q
$HC\equiv\underline{C}CH_2$	—	—	—	80.01 s	—	—
$HC\equiv C\underline{C}H_2$	—	—	—	74.03 d	—	—
$HC\equiv CCH_2$	—	—	—	37.33 t	—	—
$NH_2CH_2CH_2\underline{C}H_2$	—	—	—	—	48.54 t	—
$NH_2CH_2CH_2\underline{C}H_2$	—	—	—	—	39.80 t	—
$NH_2CH_2\underline{C}H_2CH_2$	—	—	—	—	35.14 t	—
$O\underline{C}H_2CH_2CH_2N$	—	—	—	—	—	67.56 t
$OCH_2\underline{C}H_2CH_2N$	—	—	—	—	—	47.18 t
$OCH_2CH_2\underline{C}H_2N$	—	—	—	—	—	30.00 t

[a] Chemical shifts are in ppm downfield of TMS. ^{13}C NMR spectra were taken in $CDCl_3$ solvent on a Bruker WM250 instrument, with multiplicities determined by distortionless enhancement by polarization transfer.

[b] For carbon numbering, see compound **1**, Scheme 1.

in analogous fashion, the six-membered heterocycle **20**. ^{13}C NMR assignments for six representative azalides are presented in Table 1.

Results and Discussion

In Vitro Studies

The azalides all have less potency than erythromycin A vs. Gram-positive isolates (Table 2). They also show cross resistance to erythromycin A-resistant *S. aureus* (Table 2) and *Streptococcus pyogenes* isolates (MIC >50 μg/ml for all macrolides). Better Gram-negative activity is observed with all the azalides, except **10**, **13**, **15a** and **16** (Table 2). The degree of Gram-negative activity appears to generally correlate with the increased hydrophilic nature of the compound. Compound **11**, the most basic of the experimental macrolides, demonstrates the greatest increase in Gram-negative potency; it is 30~60 times more potent than erythromycin A (Table 2). Comparison of compound **11** with erythromycin A against recent clinical isolates proves it to have broad spectrum activity (Table 3). Its MIC_{90} vs. *Escherichia coli* is 0.5 compared with >64 μg/ml for erythromycin A. It inhibits 90% of the *Enterobacter*, *Klebsiella* and *Citrobacter* species at 2 μg/ml compared with >64 for erythromycin A. It is not active against *Proteus* species. It is several times more potent than erythromycin A against *Neisseria gonorrhoeae* and *H. influenzae* (Tables 2 and 3), but four times less potent against Gram-positive clinical isolates (Table 3). A change in the stereochemical configuration of

Table 2. *In vitro* activity of aza-macrolides.

Compound	MIC (μg/ml)						
	Streptococcus pyogenes	*Staphylococcus aureus*			*Escherichia coli*		*Haemophilus influenzae*[b]
		ES	IER	CER	ES	ER	
Erythromycin A	≤0.025	0.10	6.25	>50	1.56	100	3.12
1	≤0.025	0.78	50	>50	0.78	12.5	1.56~0.78
3a[a]	≤0.025	0.39	25	>50	0.78	6.25	0.78
3b	≤0.025	0.20	3.12	>50	0.39	6.25	0.78~0.39
3c	≤0.025	0.20	12.5	>50	0.78	25	1.56
3d	≤0.025	0.39	12.5	>50	0.78	6.25	1.56
3e	≤0.025	0.78	50	>50	0.78	25	1.56
4	≤0.025	0.20	6.25	>50	0.39	6.25	0.78
5	≤0.025	0.39	12.5	>50	0.78	12.5	1.56
8	≤0.025	0.39	25	>50	0.20	3.12	0.78~1.56
9[a]	≤0.025	0.39	3.12	>50	0.78	3.12	0.39
10	0.10	1.56	>50	>50	0.39	50	3.12~6.25
11	≤0.025	0.78	12.5	>50	0.05	1.56	0.20
12	≤0.025	0.78	25	>50	0.78	12.5	1.56
13	0.05	0.39	25	>50	3.12	25	3.12
15a	0.78	1.56	>50	>50	1.56	50	25
16	0.20	3.12	>50	>50	3.12	50	25
17	≤0.025	0.20	25	>50	0.78	6.25	0.78
18	≤0.025	0.39	25	>50	0.39	3.12	0.78
20	0.05	0.39	25	>50	1.56	25	1.56

[a] MIC_{90} against *Neisseria gonorrhoeae*; 0.125 μg/ml.

[b] Average MIC from eight strains.

ES: Erythromycin A-susceptible, ER: erythromycin A-resistant, IER: inducible erythromycin A-resistant, CER: constitutive erythromycin A-resistant.

Table 3. *In vitro* potency of **11** compared to erythromycin A.

Organisms (No. of strains)	% of strains inhibited	MIC (μg/ml)	
		11	Erythromycin A
Staphylococcus aureus (10)	50	1	0.25
	90	2	0.5
S. epidermidis (14)	50	0.5	0.25
	90	1	0.25
Staphylococcus species erythromycin-resistant (12)	50	>64	>64
Streptococcus faecalis (15)	50	4	1
	90	>64	>64
Escherichia coli (22)	50	0.25	32
	90	0.5	>64
Enterobacter aerogenes (23)	50	1	>64
	90	2	>64
E. cloacae (31)	50	1	>64
	90	2	>64
Klebsiella pneumoniae (16)	50	1	>64
	90	2	>64
K. oxytoca (11)	50	1	>64
	90	2	>64
Serratia marcescens (18)	50	8	>64
	90	16	>64
Citrobacter freundii (19)	50	1	>64
	90	2	>64
Proteus mirabilis (14)	50	>64	>64
	90	>64	>64
P. vulgaris (12)	50	64	>64
	90	>64	>64
Neisseria gonorrhoeae PPNG (13)[a]	50	≤0.031	0.125
	90	0.062	0.25
Acinetobacter calcoaceticus (13)	50	0.125	2
	90	1	8
Pseudomonas aeruginosa (2)	100	>64	>64

[a] MIC_{50} for benzylpenicillin is >16 μg/ml.

the hydroxyl group at C-4″ does not lead to significant changes in *in vitro* activity (epimers **1**~**12**, **3a**~**9**, **15a**~**16**, **17** and **18** in Table 2).

Pharmacokinetics

Key oral pharmacokinetic properties of eight representative azalides compared with erythromycin A are presented in Table 4. Pharmacokinetics were determined in rats, mice, beagles, and monkeys to obtain as broad a profile as possible.

The oral pharmacokinetic properties of each azalide are generally superior to erythromycin A in the animal species studied. Most impressive data were obtained from beagles and rats. Outstanding pharmacokinetic features found in all animals are the long half-life and the large area-under-the-curve. In addition, the C_{max} values in rat studies with 50 mg/kg oral administration for all compounds are several-fold higher or equal to the MIC for key organisms (*S. aureus*, *S. pyogenes*, *H. influenzae*) included in the spectrum of an oral community use antibiotic. In contrast to the aza-macrolides, the C_{max} of erythromycin A in the rat (0.6 μg/ml) is considerably less than its average MIC against *H.*

Table 4. Oral pharmacokinetics of azalides in laboratory animals.

Azalide	Animal	Oral dose (mg/kg)	C_{max}[a] (μg/ml)	AUC (μg/ml·hour)	Half-life (hours)
3a	Rat	50	1.3	9.5	7.7
	Mouse	50	1.6	10.0	6.4
	Beagle	10	1.4	23.4	21.0
	Monkey	10	0.43	2.9	8.7
3b	Beagle	10	0.5	14.5	26.7
4	Rat	50	1.9	8.7	3.1
5	Beagle	10	0.9	17.9	23.3
9	Rat	50	2.6	9.2	5.6
	Mouse	50	3.1	9.0	2.0
	Beagle	10	1.5	19.0	18.9
	Monkey	10	0.5	2.2	9.5
11	Beagle	10	1.8	14.6	18.0
17	Rat	50	0.7	5.5	7.2
	Beagle	10	0.9	25.5	24.3
	Monkey	10	0.3	2.4	10.9
18	Rat	50	0.5	5.3	13.2
Erythromycin A	Rat	50	0.6	1.54	2.1
	Mouse	50	0.9	1.5	1.2
	Beagle	10	2.5	5.2	1.5
	Monkey	10	0.2	0.2	0.6

[a] C_{max}: Peak concentration of the antibiotic in serum/plasma.

influenzae (3.12 μg/ml). Extended studies with compound **3a** show tissue distributions superior to those of erythromycin A[13]. Additionally, in animal model infection studies — *e.g.*, localized thigh infection, anaerobic liver abscesses, *etc.* — the 9a-methyl derivative **3a** consistently and significantly outperforms erythromycin A[13].

In summary, the three best compounds overall are **3a**, **9**, and **11**. They possess an expanded and useful antimicrobial spectrum vs. *H. influenzae* and *N. gonorrhoeae*. Their superior oral *in vivo* pharmacokinetic profile relative to erythromycin A suggests considerable potential as an improved alternatives to that agent. Compound **3a** has been advanced to clinical study[13,14].

In Vivo Studies

Representative experimental macrolides having interesting *in vitro* potency against *S. aureus* ATCC 21351 were advanced to *in vivo* trial against an acutely-fatal infection produced by that organism in mice. These data, compared with erythromycin A, are presented in Table 5. *In vivo* activity after oral or parenteral administration was demonstrated with all of the compounds tested. Oral activity matches or exceeds that of erythromycin A in five examples (compounds **3a**, **8**, **9**, **11**, and **17**).

Only the parenteral activity of compound **12** exceeds or equals that of erythromycin A. In all cases, parenteral protection is greater than oral protection. Inspection of the Table 5 data clearly indicates that *in vitro* potency is not a paramount factor in eliciting *in vivo* potency. Compound **15a** has the highest MIC value (1.56 μg/ml), but its subcutaneous PD_{50} is lower than that of compounds having the lowest MIC value (0.2 μg/ml; compounds **4** and **17**).

One of the most striking structure-activity relationships involves comparison of the *in vivo* profiles of **1** and **12** with their respective 9a-alkylated counterparts **3a** and **9**. While **1** and **3a** exhibit equivalent

Table 5. Activity of azalides against susceptible *Staphylococcus aureus*[a] ATCC 21351 experimental infection in mice.

Azalide	PD_{50} (mg/kg dose)		po/sc
	po	sc	
1	>200	9.0	>22
3a	71	8.5	8
4	149.0	15.6	~10
8	100.0	43.0	2
9	69	22.4	3
11	86.0	10.3	8
12	>200	~2.4	>83
13	>200	18.0	11
15a	>200	10	20
17	100.0	30.0	3
Erythromycin A control	100.0	3.12	32

[a] MIC values (erythromycin A-susceptible strain) are presented in Table 1.

parenteral potency, only the 9a-methylated derivative **3a** is orally active (PD_{50}'s of >200 vs. 71 mg/kg, respectively). Similarly, parenterally potent compound **12** is devoid of oral activity; whereas its parenterally less active 9a-methylated counterpart (**9**) shows good (PD_{50} 69 mg/kg) oral activity. Also, the 9a-ethyl derivative **4** is moderately active orally, in contrast to its parent compound **1**. Thus, simple 9a-alkyl substitution appears to be a prerequisite for eliciting oral efficacy.

Experimental

IR spectra were recorded on a Perkin-Elmer 237B spectrophotometer. ^1H NMR spectra were recorded at 60 MHz on a Varian EM360 and at 250 MHz on a Bruker WM250 instrument. ^{13}C NMR at 100, 250, and 300 MHz were recorded respectively on Varian XL-100, Bruker WM250, and Varian XL-300 spectrometers. Low resolution mass spectra were recorded with a Finnigan 4510 GC instrument, while high resolution mass measurements were obtained with an AEI MS-30 spectrometer. All diffraction data was collected on a Nicolet R3m/μ diffractometer.

TLC measurements utilized Silica gel 60 F_{254} (0.25 mm thickness) plates (E. Merck, Darmstadt) developed with an atomized spray solution of vanillin (1 g) in EtOH - H_3PO_4 (100 ml of each), with intense post-spray heating of the plate.

In Vitro Studies, Serum/Plasma Level Determinations, and Acute Systemic Infection Studies

MICs were determined on brain heart infusion agar (Scott Laboratory Inc., Fiskeville, Rhode Island) as the basal medium by the method of ERICSSON and SHERRIS[15] using the multiple inoculator described by STEERS *et al.*[16]. The BHI was enriched and incubation conditions maintained as described previously for the growth of *H. influenzae* and *N. gonorrhoeae*[17]. For clinical isolates, MIC values were determined by the microtiter broth dilution technique as described previously[18].

In the serum/plasma level determinations, antibiotics were administered at either 10 or 50 mg/kg as an oral gavage in water - carboxymethyl cellulose - Tween 80. Plasma samples were obtained from the orbital sinus of CD rats and CD-1 mice using heparinized hematocrit tubes. Blood samples from beagle dogs were obtained from the jugular vein and from the femoral vein of cynomolgus monkeys. Sera were obtained by centrifugation of the samples at refrigerator temperatures. The number of animals used were dogs 10, rats 20, mice 20 and monkeys 4. Plasma/serum samples were then assayed using conventional agar diffusion methods using *Micrococcus luteus* ATCC 9341 as the bioassay organism.

The acute systemic infections were produced in mice by intraperitoneal inoculation of from one to ten 100% lethal doses of bacterial cultures suspended in 5% hog gastric mucin. Mice were treated

orally or subcutaneously, commencing 0.5 hour after challenge with subsequent treatments at 4 and 24 hours. The dosage range consisted of four different antibiotic concentrations in a 2-fold dilution series administered to 10 mice per dosage level. Percent survival was recorded after a 4-day observation period. After several experiments were completed, survival data were averaged and a 50%-protective dose expressed in mg/kg was calculated by the method of BATSON[19].

9-Deoxo-9a-aza-9a-hydroxy-9a-homoerythromycin A 3′-*N*-Oxide (**2**)

To a solution of **1** (10.0 g, 13.6 mmol) in 40 ml of MeOH, a total of 50 ml of 30% aqueous hydrogen peroxide (0.58 mol) was added dropwise while stirring over a 10-minute period. After stirring overnight at ambient temperature, the reaction mixture was poured onto a stirred slurry of ice (200 g), EtOAc (200 ml) and H_2O (100 ml). Excess hydrogen peroxide was quenched by cautious dropwise addition of saturated aqueous sodium sulfite until a negative starch-iodine test was indicated. The layers were separated, and the aqueous layer was extracted twice with 200 ml portions of EtOAc. The three organic extracts were combined, dried (anhydrous Na_2SO_4), and concentrated *in vacuo* to afford **2** as a colorless amorphous solid (8.6 g, 82% yield).

TLC Rf 0.20 (CH_2Cl_2 - MeOH - conc NH_4OH, 6:1:0.1); ^1H NMR (60 MHz, $CDCl_3$) δ 3.21 (6H, s, $(CH_3)_2NO$), 3.39 (3H, s, 3″-OCH_3); MS *m/z* 576.3654 (M−$C_8H_{16}O_4N$, $C_{29}H_{54}O_{10}N$), 418.2744 (M−$C_{16}H_{30}O_7N$, $C_{21}H_{40}O_7N$).

9-Deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (**3a**) (Method A)

To a well-stirred mixture of 4.83 g (6.3 mmol) of **2** in 100 ml of CH_2Cl_2 and 69.7 g (0.5 mol) of suspended anhydrous K_2CO_3, 15.7 ml (35.8 g, 0.25 mol) of iodomethane was added dropwise (under nitrogen) over several minutes. The mixture was then stirred under nitrogen at ambient temperature for 3.5 hours. The inorganic solids were removed by filtration. The filter cake was washed with CH_2Cl_2 (250 ml). The cake wash solution and filtrate were combined, and then stirred with H_2O (300 ml) while the pH was adjusted to 11 (6 N NaOH). The organic phase was separated, washed with an equal volume of H_2O, dried (anhydrous Na_2SO_4), and concentrated *in vacuo* to afford a colorless foam (4.36 g). The entire sample in absolute EtOH (150 ml) was hydrogenated (3.5 kg/cm² pressure, 8.0 g of 10% palladium-on-carbon catalyst; ambient temperature) for 1.25 hours. The catalyst was filtered, and the solvent was removed *in vacuo*, affording a colorless foam (4.3 g). The crude product was dissolved in CH_2Cl_2 (100 ml), and then stirred with H_2O (100 ml) while the pH was adjusted to 8.8 (6 N NaOH). The organic and aqueous layers were separated, and the aqueous layer was then extracted twice with 50 ml portions of CH_2Cl_2. The three organic extracts were combined, dried (anhydrous Na_2SO_4), and concentrated *in vacuo* to a colorless foam (3.0 g). The entire sample was taken up in EtOH, and H_2O was added until the solution became slightly turbid. Upon standing overnight, 1.6 g (34% yield) of **3a** crystallized from solution.

MP 136°C; ^1H NMR (250 MHz, $CDCl_3$) δ 3.29 (3H, s, 3″-OCH_3), 2.25 (3H, s, 9a-CH_3), 2.23 (6H, s, 3′-$N(CH_3)_2$); ^{13}C NMR Table 1; MS *m/z* 749.4 (M, $C_{38}H_{72}O_{12}N_2$), 590.4 (M−$C_8H_{16}O_2N$), 573.4 (M−$C_8H_{17}O_3N$), 432.3 (M−$C_{16}H_{30}O_5N$), 416.3 (M−$C_{16}H_{30}O_6N$), 158.0 (M−$C_{30}H_{56}O_{10}N$).

9-Deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (**3a**) (Method B)

A mixture of 200 mg (0.27 mmol) of ketone **8** and 76 mg (0.30 mol) of lithium tri-*tert*-butoxyaluminum hydride in 30 ml of anhydrous THF was stirred at ambient temperature for 50 minutes. The reaction was diluted with 30 ml of EtOAc - H_2O (1:1), and then concentrated *in vacuo* to a volume of 5 ml. After the addition of 30 ml of H_2O, the pH was adjusted to 6.0 (1 N HCl). The aqueous layer was separated, and the pH was adjusted to 9.5 (1 N NaOH). The basic solution was twice extracted with 30 ml portions of EtOAc. The combined organic extracts were dried (Na_2SO_4), and concentrated *in vacuo* to afford 120 mg (59% yield) of **3a** as a colorless foam, identical in all respects to the **3a** sample prepared by Method A.

9-Deoxo-9a-aza-9a-allyl-9a-homoerythromycin A (**3b**)

To a well-stirred mixture of 4.0 g (5.2 mmol) of **2** in 50 ml of $CHCl_3$ and 28 g (0.20 mol) of suspended anhydrous K_2CO_3, 25 g (17.9 ml, 0.21 mol) of allyl bromide in 10 ml of $CHCl_3$ was added dropwise over 5 minutes. Ambient temperature stirring was continued for 18 hours. Chromato-

graphy (200 g silica gel, 230~400 mesh, elution with $CHCl_3$ - 2-propanol - conc NH_4OH (8:2:0.1) afforded 0.72 g (colorless foam) of alkylated substrate, sufficiently pure for the next (deoxygenation) step. The entire sample was combined with 0.93 g (2.6 mmol) of triphenylphosphine in 21 ml of THF, and the resulting mixture was refluxed for 4 hours. Solvent removal *in vacuo* afforded an oily residue which was dissolved in 150 ml of EtOAc. An equal volume of H_2O was added and the pH was adjusted to 4.5 (6 N HCl). The separated aqueous phase was extracted with several 150 ml portions of EtOAc. Finally, the aqueous phase was combined with an equal volume of EtOAc, and the pH was elevated to 10.0 (10% K_2CO_3). The phases were separated, and the aqueous was extracted twice with 100 ml of EtOAc. The combined final (**3**) EtOAc extracts were dried (Na_2SO_4), and concentrated *in vacuo* to an amber foam (0.55 g). Flash chromatography (50 g silica gel, 230~400 mesh, elution with $CHCl_3$ - 2-propanol - conc NH_4OH, 15:1:0.1) afforded 408 mg (10% yield) of **3b** as a colorless amorphous solid.

TLC Rf 0.36 (CH_2Cl_2 - MeOH - conc NH_4OH, 9:1:0.1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 136.3 and 117.1 (olefinic carbons), 103.0, 95.2, 83.9, 78.5, 78.0, 77.9, 77.7, 74.8, 74.2, 72.8, 70.9, 68.8, 65.6, 64.3, 64.2, 61.2, 53.6, 49.4, 45.0, 41.9, 41.2, 40.3 (2), 35.0, 29.0, 27.8, 26.8, 22.0, 21.6, 21.5, 21.3, 18.3, 16.5, 15.0, 11.3, 9.7, 9.6; MS *m/z* 774.9 (M, $C_{40}H_{74}O_{12}N_2$), 616.4 (M$-C_8H_{16}O_2N$), 599.4 (M$-C_8H_{17}O_3N$), 458.2 (M$-C_{16}H_{30}O_5N$), 442 (M$-C_{16}H_{30}O_6N$), 157.9 (M$-C_{32}H_{58}O_{10}N$).

9-Deoxo-9a-aza-9a-propargyl-9a-homoerythromycin A (**3c**)

To a well-stirred mixture of 10.0 g (13.0 mmol) of **2** in 75 ml of $CHCl_3$ and 72 g (0.52 mol) suspended anhydrous K_2CO_3, 62 g (0.52 mol) of propargyl bromide was added dropwise over 15 minutes. Ambient temperature stirring was continued for 18 hours. The reaction mixture was filtered and concentrated *in vacuo* to a foam (8.5 g). The entire sample was dissolved in 75 ml of anhydrous THF. Triphenylphosphine (10.5 g, 0.04 mol) was added, and the mixture refluxed for 2 hours. Solvent was removed *in vacuo*, and the crude product was dissolved in 100 ml of EtOAc, which was then layered with an equal volume of H_2O. The pH was adjusted to 4.0 (6 N HCl). The separated aqueous layer was then stirred with 100 ml of fresh EtOAc while the pH was adjusted to 10.0 (6 N NaOH). Concentration *in vacuo* of the organic layer afforded 7.3 g of semi-purified product. Chromatography of the entire sample on silica gel (430 g, 230~400 mesh, elution with $CHCl_3$ - MeOH - conc NH_4OH, 15:1:0.1) afforded 1.67 g (17%) of purified product as a colorless amorphous solid.

TLC Rf 0.39 (CH_2Cl_2 - MeOH - conc NH_4OH, 9:1:0.1); ^{13}C NMR Table 1; MS *m/z* 772.9 (M, $C_{40}H_{72}O_{12}N_2$), 614.4 (M$-C_8H_{16}O_2N$), 597.4 (M$-C_8H_{17}O_3N$), 456.2 (M$-C_{16}H_{30}O_5N$), 440.3 (M$-C_{16}H_{30}O_6N$), 158.0 (M$-C_{32}H_{56}O_{10}N$).

9-Deoxo-9a-aza-9a-benzyl-9a-homoerythromycin A (**3d**)

A reaction mixture consisting of 2.00 g (2.6 mmol) of **2**, 18 g (0.105 mol) benzyl bromide, and 14 g (0.105 mol) of suspended anhydrous K_2CO_3 in 20 ml of $CHCl_3$, was stirred under nitrogen at ambient temperature for 18 hours. The mixture was then filtered, combined with 100 ml portions of $CHCl_3$ and H_2O, and the pH adjusted to 3.0 (6 N HCl). The separated organic layer was extracted with three 50-ml portions of dilute HCl (pH 3). The combined aqueous extracts were layered with 100 ml of $CHCl_3$ and the pH adjusted to 10 (10% K_2CO_3). The organic layer was washed with brine, dried (Na_2SO_4), and concentrated *in vacuo* to a foam (0.96 g). The entire sample was dissolved in 30 ml of EtOH and hydrogenated (0.53 kg/cm^2 pressure, 0.5 g of 5% palladium-on-carbon (50% water-wet by weight)) for 2 hours. Catalyst filtration and solvent removal afforded 0.66 g of crude product. Chromatography (30 g silica gel, 70~230 mesh, elution with $CHCl_3$ - 2-propanol - conc NH_4OH, 9:1:0.01) afforded 116 mg (5% yield) of **3d** as a colorless foam.

TLC Rf 0.33 (CH_2Cl_2 - MeOH - conc NH_4OH, 9:1:0.1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 139.7, 129.4 (2), 127.9 (2) and 126.7 (aromatic carbons), 103.6, 96.1, 85.6, 78.9, 78.0, 77.8, 75.4, 75.0, 74.8, 72.7, 70.8, 69.0, 65.9, 65.1, 58.7, 58.1, 49.4, 45.7, 41.6, 41.2, 40.4 (2), 35.0, 29.4, 29.2, 25.6, 22.0, 21.8, 21.5, 21.4, 21.2, 18.0, 16.7, 15.2, 11.4, 10.1, 8.4; MS *m/z* 825 (M, $C_{44}H_{76}O_{12}N_2$), 733.8 (M$-C_7H_7$), 666.6 (M$-C_8H_{16}O_2N$), 508.3 (M$-C_{16}H_{31}O_5N$), 158.0 (M$-C_{36}H_{60}O_{10}N$).

9-Deoxo-9a-aza-9a-cyanomethyl-9a-homoerythromycin A (**3e**)

A mixture consisting of **2** (0.75 g, 0.98 mmol) in 23 ml of $CHCl_3$, 5.4 g (39 mmol) of suspended

anhydrous K_2CO_3, and 4.64 g (39 mmol) of bromoacetonitrile was stirred for 18 hours at ambient temperature. The reaction was filtered, and the filtrate was concentrated *in vacuo* to a colorless oil. Trituration with Et_2O (350 ml) afforded a light yellow granular solid, isolated by filtration (0.48 g). The entire sample was dissolved in 3 ml of anhydrous THF. Triphenylphosphine (0.59 g, 2.3 mmol) was added, and the resulting solution was refluxed for 2 hours. The reaction was filtered and concentrated to an oil, which was dissolved in 40 ml of EtOAc. An equal volume of H_2O was added and the pH of the well-stirred mixture was adjusted to 2.0 (6 N HCl). The aqueous extract was separated, layered with 40 ml of fresh EtOAc, and the pH was adjusted to 9.0 (1 N NaOH). The organic phase was separated, dried (Na_2SO_4), and concentrated *in vacuo* to an amorphous solid (0.38 g). Chromatography of the entire sample (16 g silica gel, 32~63 mesh, elution with CH_2Cl_2 - MeOH - conc NH_4OH, 15 : 1 : 0.04) afforded 102 mg (13.5% yield) of **3e** as a colorless amorphous solid.

TLC Rf 0.61 ($CHCl_3$ - MeOH - conc NH_4OH, 6 : 1 : 0.1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 116.8 (C≡N), 102.8, 95.2, 83.7, 78.1, 77.9, 77.7, 77.2, 75.0, 74.4, 74.1, 72.8, 70.8, 68.8, 65.7, 65.5, 61.5, 49.3, 44.9, 42.5, 41.7, 40.3 (2), 37.2, 34.8, 28.8, 26.2, 26.0, 21.5, 21.4, 21.3, 21.1, 18.3, 16.5, 14.8, 11.2, 10.0, 9.5; MS *m/z* 773.5 (M, $C_{39}H_{71}O_{12}N_3$), 615.4160 (M−$C_8H_{14}O_3$), 615.3775 (M−$C_8H_{16}O_2N$), 159 (M−$C_{31}H_{56}O_9N_3$), 158 (M−$C_{31}H_{55}O_{10}N_2$).

9-Deoxo-9a-aza-9a-ethyl-9a-homoerythromycin A (**4**)

A solution of **1** (2.0 g, 2.72 mmol) and acetaldehyde (1.5 ml, 27 mmol) in 20 ml of EtOH and 2.3 ml of H_2O was hydrogenated (3.5 kg/cm² pressure, 2.0 g of 5% palladium-on-carbon catalyst (50% water-wet by weight)) for 18 hours. The catalyst was filtered, and the filtrate was concentrated *in vacuo* to a colorless foam. Chromatography on silica gel (30 g, 32~63 mesh, elution with CH_2Cl_2 - MeOH - conc NH_4OH, 10 : 1 : 0.04) afforded 1.00 g (48% yield) of **4** as a colorless amorphous solid.

TLC Rf 0.30 (CH_2Cl_2 - MeOH - conc NH_4OH, 6 : 1 : 0.1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.3, 103.0, 95.3, 83.8, 78.6, 78.1, 77.7, 75.0, 74.9, 74.1, 74.0, 72.9, 71.0, 68.7, 65.6, 63.9, 61.3, 49.4, 44.9, 43.4, 42.1, 41.1, 40.4 (2), 35.0, 29.0, 28.4, 27.1, 22.4, 21.6, 21.3 (2), 18.2, 16.7, 15.0, 12.3, 11.3, 9.6 (2); MS *m/z* 762.8 (M, $C_{39}H_{74}O_{12}N_2$), 604 (M−$C_8H_{16}O_2N$), 446.3140 (M−$C_{16}H_{30}O_5N$, $C_{23}H_{44}O_7N$), 430.3162 (M−$C_{16}H_{30}O_6N$, $C_{23}H_{44}O_6N$), 158 (M−$C_{31}H_{58}O_{10}N$).

9-Deoxo-9a-aza-9a-(*n*-propyl)-9a-homoerythromycin A (**5**) (Method A)

To a well-stirred solution of 5.0 g (6.24 mmol) of **15c** and 91 g (0.31 mol) of tri-*n*-butyltin hydride in 50 ml of xylenes (boiling range 139~141°C) heated to 125°C, azobisisobutylnitrile (5.12 g, 31.2 mmol) suspended in 50 ml of xylene was added dropwise over a period of 1 hour. On completion of the addition, the reaction mixture was maintained at 125°C for 45 minutes. EtOAc (75 ml) and H_2O (75 ml) were added, and the pH of the aqueous phase was adjusted to 4.5 (6 N HCl). After stirring for 20 minutes the phases were separated, and the organic phase was stirred for 20 minutes with 50 ml of fresh H_2O at pH 4.5. The two aqueous extracts were combined and washed with EtOAc (2× 30 ml). The aqueous layer was separated, combined with 50 ml of fresh EtOAc, and the pH was adjusted to 10 (10% K_2CO_3). The organic phase was separated and washed first with water, then with brine, and dried (anhydrous K_2CO_3). Solvent removal *in vacuo* afforded 3.9 g of amber foam. Chromatography of 3.2 g of the crude product (285 g of 230~400 mesh silica gel; eluting initially with 1 liter of $CHCl_3$ - MeOH - conc NH_4OH, 96 : 3.2 : 0.3 and then with $CHCl_3$ - MeOH - conc NH_4OH, 92 : 7.2 : 0.72) afforded 391 mg (10% yield) of **5** as a colorless foam.

TLC Rf 0.30 (CH_2Cl_2 - MeOH - conc NH_4OH, 9 : 1 : 0.1); ^{13}C NMR (300 MHz, $CDCl_3$) δ 177.9, 103.1, 95.3, 83.8, 78.7, 78.1, 77.8, 77.5, 75.0, 74.1, 74.0, 72.8, 70.9, 68.8, 65.6, 64.7, 61.3, 52.3, 49.4, 44.8, 41.9, 41.0, 40.4 (2), 35.0, 28.9, 28.5, 27.1, 22.4, 21.6, 21.5, 21.4, 20.3, 18.3, 16.5, 15.0, 12.1, 11.3, 9.6, 9.5; MS *m/z* 776.4 (M, $C_{40}H_{76}O_{12}N_2$), 618.4 (M−$C_8H_{16}O_2N$), 460.3 (M−$C_{16}H_{30}O_5N$), 158.1 (M−$C_{32}H_{60}O_{10}N$).

9-Deoxo-9a-aza-9a-(*n*-propyl)-9a-homoerythromycin A (**5**) (Method B)

A solution of 0.19 g (0.25 mmol) of **3b** in 5 ml of absolute EtOH was hydrogenated (3.5 kg/cm² pressure; 10% palladium-on-carbon catalyst) for 18 hours. The catalyst was filtered, and the filter cake was washed with 10 ml of EtOH. The cake wash solution and filtrate were combined, and then evaporated to a white solid. Chromatography of the entire sample (8 g silica gel, 32~63 mesh, eluting

initially with CH_2Cl_2 - MeOH - conc NH_4OH, 97:3:0.04) and then with CH_2Cl_2 - MeOH - conc NH_4OH, 95:5:0.4) afforded 37 mg (19% yield) of **5** which was identical in all respects to the sample of **5** prepared by Method A.

2'-Acetyl-9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (**6**)

To a solution of 6.4 g (8.6 mmol) of **3a** in 50 ml of EtOAc was added 1.32 g (13 mmol) of acetic anhydride. The mixture was stirred at ambient temperature for 18 hours. The reaction was diluted with 50 ml of H_2O, and then stirred for an additional 30 minutes. The pH of the aqueous layer was then adjusted to 2.5 (1 N HCl), and the organic and aqueous layers were separated. The pH of the aqueous solution was adjusted to 9.5 (1 N NaOH) and extracted with EtOAc. The pH 9.5 EtOAc extracts were combined, dried (anhydrous Na_2SO_4), and evaporated to yield 6.0 g (89% yield) of acetate **6** as a white amorphous foam. This material was used in all further reactions where required. Crystallization (Et_2O) afforded acetate **6** as a white solid.

MP 164~165°C; ^1H NMR (250 MHz, $CDCl_3$) δ 3.46 (3H, s, 3''-OCH_3), 2.30 (3H, s, 9a-CH_3), 2.25 (6H, s, 3'-$N(CH_3)_2$), 2.06 (3H, s, 2'-$COCH_3$); ^{13}C NMR (250 MHz, $CDCl_3$) δ 178.7, 169.9, 100.7, 94.7, 83.2, 78.2, 77.8, 77.6, 74.3, 74.0, 73.7, 73.1, 71.9, 70.2, 68.3, 65.7, 63.8, 62.4, 49.4, 45.2, 42.1, 41.9, 40.8, 36.3, 34.8, 30.6, 27.5, 26.7, 22.0, 21.7, 21.5, 21.3, 21.2, 18.3, 16.2, 14.8, 11.3, 8.9, 7.4; MS *m/z* 791 (M, $C_{40}H_{74}O_{13}N_2$), 615 (M$-C_8H_{15}O_4$), 590 (M$-C_{10}H_{18}O_2N$), 415 (M$-C_{18}H_{33}O_6N$), 200 (M$-C_{30}H_{56}O_{10}N$), 159 (M$-C_{32}H_{59}O_9N_2$), 127 (M$-C_{33}H_{62}O_{10}N)_2$.

2'-Acetyl-4''-dehydro-4''-oxo-9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (**7**)

A mixture of 7.5 g (9.5 mmol) of acetate **6**, 6.7 ml (95 mmol) of dimethyl sulfoxide, and 5.5 g (28 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide were combined at ambient temperature in 75 ml of CH_2Cl_2 under a nitrogen atmosphere. To this solution was added portionwise 5.5 g (28 mmol) of pyridinium trifluoroacetate over a 5-minute period. The reaction mixture was stirred at ambient temperature for 2 hours. To this solution was added an equal volume of H_2O, and the aqueous layer was extracted with EtOAc sequentially at pH 4.0, 6.5, and 9.5. The pH 9.5 EtOAc extracts were combined, dried (anhydrous Na_2SO_4), and evaporated to yield 6.2 g (82% yield) of ketone **7** as a white amorphous solid.

TLC Rf 0.6 (EtOAc - acetone - conc NH_4OH, 10:1:0.1); ^1H NMR (250 MHz, $CDCl_3$) δ 3.30 (3H, s, 3''-OCH_3), 2.24 (9H, s, 9a-CH_3, 3'-$N(CH_3)_2$), 2.00 (3H, s, 2'-$COCH_3$); ^{13}C NMR (250 MHz, $CDCl_3$) δ 211.1 (C-4'', C=O), 179.9, 169.5, 100.5, 95.6, 83.0, 78.1, 77.6, 74.5, 74.1, 73.5, 72.0, 71.5 (2), 70.0, 68.5, 63.1, 62.1, 51.1, 44.5, 41.7, 40.5 (2), 40.1, 37.2, 36.4, 30.5, 27.0, 26.5, 21.7, 21.4, 21.1, 21.0, 20.7, 16.3, 16.2, 15.0, 11.1, 8.9, 7.3; MS *m/z* 789.7 (M+1, $C_{40}H_{73}O_{13}N_2$), 673.5 (M$-C_6H_{11}O_2$), 588.4 (M$-C_{10}H_{18}O_2N$), 200.1 (M$-C_{30}H_{56}O_{10}N$), 157.1 (M$-C_{32}H_{59}O_9N_2$).

4''-Dehydro-4''-oxo-9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (**8**)

A solution of 0.93 g (1.2 mmol) of ketone **7** in 50 ml of MeOH was stirred at ambient temperature for 20 hours. Evaporation of the solvent yielded 0.82 g (94% yield) of ketone **8** as a white crystalline solid.

MP 133~134°C; TLC Rf 0.52 ($CHCl_3$ - MeOH - conc NH_4OH, 5:1:0.2); ^1H NMR (250 MHz, $CDCl_3$) δ 3.33 (3H, s, 3''-OCH_3), 2.38 (3H, s, 9a-CH_3), 2.29 (6H, s, 3'-$N(CH_3)_2$); ^{13}C NMR (250 MHz, $CDCl_3$) δ 208.1 (C-4'', C=O), 178.4, 103.7, 96.2, 84.5, 78.5, 77.8, 77.2, 74.9, 74.4, 73.6, 72.3, 71.0, 70.3, 69.3, 65.4, 62.2, 51.3, 44.6, 42.2, 40.4 (2), 40.1, 36.8, 36.6, 29.1, 27.0, 26.6, 21.8, 21.4, 21.0 (2), 16.3, 16.2, 15.3, 15.2, 11.2, 9.2, 7.5; MS *m/z* 746.4 (M, $C_{38}H_{70}O_{12}N_2$), 588.4 (M$-C_8H_{16}O_2N$), 573.4 (M$-C_8H_{15}O_4$), 158.1 (M$-C_{30}H_{56}O_{10}N$).

4''-*epi*-9-Deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (**9**)

A solution of 0.30 g (0.38 mmol) of ketone **8** in 10 ml of EtOH was hydrogenated (3.5 kg/cm^2 pressure, 50 mg of Raney nickel catalyst) for 18 hours. An additional 50 mg of Raney nickel was added to the mixture, and hydrogenation was continued for an additional 18 hours. The reaction mixture was filtered, and the filtrate evaporated. The residue was dissolved in 20 ml of EtOAc, and then stirred with 20 ml of H_2O while the pH was adjusted to 2.5 (2 N HCl). The aqueous layers was separated and then extracted with EtOAc at pH 4, 6.5, and 9.5 (pH adjusted with 2 N NaOH). The

pH 9.5 EtOAc extracts were combined, dried (anhydrous Na_2SO_4), and evaporated to yield 0.17 g (57% yield) of the C-4″ *epi*-alcohol **9** as a white amorphous solid.

TLC Rf 0.61 ($CHCl_3$ - acetone - conc NH_4OH, 6:6:0.4); ^1H NMR (250 MHz, $CDCl_3$) δ 3.32 (3H, s, 3″-OCH_3), 2.31 (3H, s, 9a-CH_3), 2.29 (6H, s, 3′-$N(CH_3)_2$); ^{13}C NMR Table 1; MS *m/z* 748.7 (M, $C_{38}H_{72}O_{12}N_2$), 590.5 (M−$C_8H_{16}O_2N$), 573.5 (M−$C_8H_{17}O_3N$), 432.4 (M−$C_{16}H_{30}O_5N$), 416.4 (M−$C_{16}H_{30}O_6N$), 158 (M−$C_{30}H_{56}O_{10}N$).

4″-Deoxo-4″-oximino-9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (**10**)

A mixture of 0.30 g (0.4 mmol) of ketone **8** dissolved in 15 ml of MeOH, and 0.14 g (0.20 mmol) of hydroxylamine hydrochloride was stirred at ambient temperature for 72 hours. The solvent was removed *in vacuo*, and the residue was dissolved in 30 ml of EtOAc - H_2O (1:1) mixture. The pH of the aqueous phase was adjusted to 9.5 (1 N NaOH). After stirring well, the EtOAc layer was separated from the aqueous layer, dried (anhydrous Na_2SO_4), and evaporated to yield 0.25 g (81% yield) of oxime **10** as a colorless amorphous solid.

TLC Rf 0.70 (acetone - $CHCl_3$ - conc NH_4OH, 8:4:0.2); ^1H NMR (250 MHz, $CDCl_3$) δ 3.20 (3H, s, 3″-OCH_3), 2.31 (3H, s, 9a-CH_3), 2.24 (6H, s, 3′-$N(CH_3)_2$); ^{13}C NMR (250 MHz, $CDCl_3$) δ 176.6, 157.8 (C-4″), 101.9, 94.7, 84.7, 82.2, 77.3, 75.2, 74.6, 74.2, 73.4, 71.2, 70.7, 68.6, 66.2, 65.9, 62.5, 50.1, 44.2, 42.0, 40.4 (2), 39.8, 37.6, 36.2, 29.7, 28.2, 26.6, 26.5, 21.5, 21.4, 21.1, 16.8, 16.3, 16.0, 11.0, 9.1, 7.2; MS *m/z* 761.7 (M, $C_{38}H_{71}O_{12}N_3$), 603.5 (M−$C_8H_{16}O_2N$), 573.5 (M−$C_8H_{14}O_4N$), 413.4 (M−$C_{16}H_{32}O_6N_2$), 158 (M−$C_{30}H_{57}O_9N_2$).

4″-Deoxo-4″-α-amino-9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A (**11**)

A solution of 0.8 g (1.05 mmol) of oxime **10** in 20 ml of EtOH was hydrogenated (3.5 kg/cm^2 pressure; 1.0 g of Raney nickel catalyst) at ambient temperature for 18 hours. Chromatography on silica gel using $CHCl_3$ - acetone (1:1) as eluant afforded 0.25 g (32% yield) of amine **11** as a colorless amorphous solid.

TLC Rf 0.33 ($CHCl_3$ - acetone - conc NH_4OH, 3:9:0.4); ^1H NMR (250 MHz, $CDCl_3$) δ 3.38 (3H, s, 3″-OCH_3), 2.31 (3H, s, 9a-CH_3), 2.28 (6H, s, 3′-$N(CH_3)_2$); ^{13}C NMR Table 1; MS *m/z* 748 (M, $C_{38}H_{73}O_{11}N_3$), 589 (M−$C_8H_{16}O_2N$), 432.3 (M−$C_{16}H_{31}O_4N_2$), 158 (M−$C_{30}H_{57}O_9N_2$).

9-Deoxo-9a-aza-9a-(β-cyanoethyl)-9a-homoerythromycin A (**13**)

A solution of **1** (1.0 g, 1.36 mmol) in 10.0 ml of acrylonitrile was refluxed for 6 hours and then stirred overnight at ambient temperature. The mixture was concentrated *in vacuo* to a tan foam. Chromatography (40 g silica gel, 70~230 mesh, elution with CH_2Cl_2 - MeOH - conc NH_4OH, 10:1:0.01) afforded 605 mg (56% yield) of **13** as a colorless foam.

TLC Rf 0.57 (CH_2Cl_2 - MeOH - conc NH_4OH, 6:1:0.01); ^{13}C NMR (300 MHz, $CDCl_3$) δ 177.6, 118.9 (C≡N), 103.0, 95.9, 84.4, 78.0, 77.8, 77.1, 75.8, 75.1, 74.7, 74.4, 72.7, 70.8, 68.8, 65.7, 65.3, 60.2, 49.3, 47.6, 45.1, 40.7, 40.6, 40.3 (2), 35.0, 29.1, 28.9, 26.2, 22.1, 21.5, 21.4, 21.3, 18.3, 17.3, 16.6, 15.4, 11.2, 9.7, 8.9; MS *m/z* 789.4 (M+1, $C_{40}H_{73}O_{12}N_3$), 629.7 (M−$C_8H_{16}O_2N$), 471.4 (M−$C_{16}H_{30}O_5N$), 455.4 (M−$C_{16}H_{30}O_6N$), 158.2 (M−$C_{32}H_{57}O_{10}N_2$).

4″-*epi*-9-Deoxo-9a-aza-9a-(β-cyanoethyl)-9a-homoerythromycin A (**14**)

A solution of **12** (11.6 g, 15.8 mmol) in 100 ml of acrylonitrile was refluxed for 19 hours, and then concentrated *in vacuo* to afford **14** (12.8 g, 98% yield) as an ivory foam. TLC inspection (CH_2Cl_2 - MeOH - conc NH_4OH, 6:1:0.1) showed a single (less polar, Rf 0.51) product.

^{13}C NMR (300 MHz, $CDCl_3$) δ 177.8, 119.0, 102.6, 96.1, 84.1, 78.5, 77.1, 76.7, 74.7, 74.4, 73.9, 73.8, 70.9, 68.4, 64.8, 64.6, 63.5, 60.8, 49.1, 47.0, 45.3, 41.4, 41.1, 40.2 (2), 29.4, 29.2, 28.4, 26.1, 22.2, 21.5, 21.3, 21.2, 17.2, 16.8, 16.6, 15.0, 11.1, 9.5, 8.8; MS *m/z* 788.3 (M^+, $C_{40}H_{73}O_{12}N_3$), 629.6 (M−$C_8H_{16}O_2N$), 471.3 (M−$C_{16}H_{30}O_5N$), 455.4 (M−$C_{16}H_{30}O_6N$), 158.1 (M−$C_{32}H_{57}O_{10}N_2$).

9-Deoxo-9a-aza-9a-(γ-aminopropyl)-9a-homoerythromycin A (**15a**)

A solution of 47 g (59.6 mmol) of **13** in 520 ml of EtOH was hydrogenated (3.5 kg/cm^2 pressure) using 47 g of Raney nickel catalyst (50% water-wet by weight) for 3 hours. The mixture was then charged with 25 g of fresh catalyst, and hydrogenation (3.5 kg/cm^2 pressure) was continued for an

additional 1.5 hours. Catalyst filtration and solvent removal *in vacuo* afforded a colorless foam. The crude product in 600 ml of EtOAc was stirred with 800 ml of H_2O while the pH was adjusted to 9.5 (6 N NaOH). The separated organic phase was dried (Na_2SO_4) and concentrated to a foam. Chromatography (800 g silica gel, 70~230 mesh, elution with $CHCl_3$ - MeOH - conc NH_4OH, 6:1:0.05) afforded 14.7 g (31% yield) of **15a**. Crystallization of a 1.1 g sample from Et_2O gave 545 mg of colorless crystals.

MP 180~183°C; TLC Rf 0.15 ($CHCl_3$ - MeOH - conc NH_4OH, 6:1:0.05); ^{13}C NMR Table 1; MS *m/z* 792.0 (M, $C_{40}H_{77}O_{12}N_3$), 633.6 (M−$C_8H_{16}O_2N$), 475.3 (M−$C_{16}H_{30}O_5N$), 157.9 (M−$C_{32}H_{61}O_{10}N_2$).

9-Deoxo-9a-(γ-formamidopropyl)-9a-aza-9a-homoerythromycin A (**15b**)

To a stirred solution of 3.0 g (3.8 mmol) of **15a** in 25 ml of CH_2Cl_2 cooled to 5°C, 370 mg (4.2 mmol) of acetic-formic anhydride in 5 ml of CH_2Cl_2 was added dropwise over 5 minutes. The reaction was then stirred at ambient temperature for 1 hour. After extraction with an equal volume of 10% aqueous K_2CO_3, the organic phase was separated, washed with brine, dried (Na_2SO_4) and concentrated *in vacuo* to afford **15b** (3.1 g, 100% yield) as a colorless foam.

^1H NMR (60 MHz, $CDCl_3$) δ 8.15 (1H, br s, *H*CONH), 6.76 (1H, br m, HCON*H*), 3.28 (3H, s, 3″-OCH_3), 2.25 (9H, two overlapping singlets, 9a-CH_3 and 3′-N(CH_3)$_2$); MS *m/z* 819.5 (M, $C_{41}H_{77}O_{13}N_3$), 645.5 (M−$C_8H_{16}O_3N$), 503.4 (M−$C_{16}H_{30}O_5N$), 487.2 (M−$C_{16}H_{30}O_6N$), 158.1 (M−$C_{33}H_{61}O_{11}N_2$).

9-Deoxo-9a-(γ-isonitrilopropyl)-9a-aza-9a-homoerythromycin A (**15c**)

To a stirred solution of 4.6 g (5.6 mmol) of **15b** in 30 ml of pyridine cooled to 5°C, a solution of 2.7 g (14 mmol) of *p*-toluenesulfonyl chloride in 10 ml of pyridine was added dorpwise over 10 minutes. The reaction was stirred for 1 hour at ambient temperature and then concentrated to dryness *in vacuo*. The residue was dissolved in 150 ml of CH_2Cl_2. An equal volume of H_2O was added, and the pH was adjusted to 10 (10% K_2CO_3). The organic phase was separated, washed with H_2O (2×100 ml) and brine (100 ml), dried (anhydrous K_2CO_3), and concentrated *in vacuo* to afford **15c** (5.0 g, 90% yield) as an amber foam.

IR ν_{max} (CCl_4) cm^{-1} 2140 (N≡C), 1725 (s, C=O); MS *m/z* 802.0 (M, $C_{41}H_{75}O_{12}N_3$), 643.5 (M−$C_8H_{16}O_2N$), 485.3 (M−$C_{16}H_{30}O_5N$), 158.0 (M−$C_{33}H_{59}O_{10}N_2$).

4″-*epi*-9-Deoxo-9a-aza-9a-(γ-aminopropyl)-9a-homoerythromycin A (**16**)

A solution of 12.8 g (16.2 mmol) of **14** in 250 ml of EtOH was combined with 12.8 g of Raney nickel catalyst (50% water-wet by weight) and hydrogenated (3.5 kg/cm^2 pressure) for 19 hours. The crude product obtained after catalyst filtration and solvent removal *in vacuo* was dissolved in 100 ml of CH_2Cl_2. After extraction with an equal volume of saturated $NaHCO_3$, the separated organic phase was dried (Na_2SO_4), and concentrated *in vacuo* to an ivory foam (10.5 g). Crystallization from warm Et_2O yielded 4.0 g (31% yield) of colorless crystals.

MP 135°C; TLC Rf 0.13 ($CHCl_3$ - MeOH - conc NH_4OH, 6:1:0.1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 102.2, 95.8, 83.4, 78.4, 77.4, 77.1, 75.8, 74.6, 74.1, 74.0, 71.0, 68.4, 65.7, 65.2, 63.8, 63.2, 58.6, 49.1, 48.3, 45.0, 41.2, 40.7, 40.3 (2), 40.0, 29.5, 29.0 (2), 26.0, 23.5, 21.6, 21.4, 21.2, 17.4, 16.5, 15.2, 11.0, 9.6, 6.9; MS *m/z* 792.1 (M, $C_{40}H_{77}O_{12}N_3$), 633.6 (M−$C_8H_{16}O_2N$), 475.3 (M−$C_{16}H_{30}O_5N$), 158.0 (M−$C_{32}H_{61}O_{10}N_2$).

9,11-Deoxo-9a-aza-[11-β,9a-(epoxypropano)]-9a-homoerythromycin A (**17**)

To a solution of **15a** (6.24 g, 7.90 mmol) in 128 ml of $CHCl_3$, 1.01 g (1.16 ml, 8.63 mmol) of isoamyl nitrite and 0.92 ml (0.97 g, 16.2 mmol) of glacial acetic acid were added, and the mixture was vigorously refluxed for 1 hour. The mixture was stirred with 150 ml of H_2O, and the pH was adjusted to 8.0 (saturated $NaHCO_3$). The separated organic phase was washed with an equal volume of H_2O, dried (Na_2SO_4), and concentrated *in vacuo* to a yellow foam. Formamide-treated silica gel was prepared by thoroughly mixing 360 ml of formamide, 1.8 liters of acetone, and 900 g of silica gel (230~400 mesh) and then removing solvent *in vacuo* on a rotary evaporator until a free-flowing powder was obtained. Chromatography of the crude product (5.8 g) on 900 g of formamide-impregnated silica

gel — eluting first with (2 liters) $CHCl_3$ - hexane (7 : 3), and then with $CHCl_3$ - hexane (8 : 3) — afforded **17** (0.79 g, 13% yield) as a colorless amorphous solid.

TLC Rf 0.36 (CH_2Cl_2 - MeOH - conc NH_4OH, 9 : 1 : 0.1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.1, 103.0, 96.1, 84.3, 80.42, 80.21, 78.0, 77.7, 74.1, 73.5, 72.7, 71.0, 70.9, 68.5, 67.2, 65.6, 65.4, 62.8, 49.3, 47.2, 44.9, 42.3, 40.3 (2), 39.8, 35.3, 29.0, 26.9 (2), 21.8, 21.5, 21.4, 21.2, 20.8, 18.5, 17.0, 16.2, 10.7, 9.9, 6.6; MS *m/z* 775 (M, $C_{40}H_{74}O_{12}N_2$), 617 (M−$C_8H_{16}O_2N$), 458.3045 (M−$C_{16}H_{30}O_5N$, $C_{24}H_{44}O_7N$), 442.3134 (M−$C_{16}H_{30}O_6N$, $C_{24}H_{44}O_6N$), 158 (M−$C_{32}H_{58}O_{10}N$).

4″-*epi*-9,11-Deoxo-9a-aza-[11-β,9a-(epoxypropano)]-9a-homoerythromycin A (**18**)

To a solution of **16** (3.37 g, 4.3 mmol) in 20 ml of $CHCl_3$, 0.66 ml (4.9 mmol) of isoamyl nitrite and 0.488 ml (8.52 mmol) of glacial AcOH were added, and the mixture was refluxed 1 hour. The reaction mixture was shaken with 50 ml of 10% aqueous K_2CO_3. The separated organic phase was then washed with brine, dried (Na_2SO_4), and concentrated *in vacuo* to a colorless foam. Formamide-treated silica gel was prepared by mixing well 120 ml of formamide, 600 ml of acetone, and 300 g silica gel (230~400 mesh), and then removing solvent *in vacuo* on a rotary evaporator until a free-flowing solid was obtained. The crude product was chromatographed on 300 g of formamide-impregnated silica gel, eluting with $CHCl_3$ - hexane (98 : 2). Thus **18** (344 mg, 10% yield) was isolated as a colorless amorphous solid. Crystallization of 196 mg from acetone - H_2O yielded 96 mg of colorless crystals.

MP 139~141°C; TLC Rf 0.34 (CH_2Cl_2 - MeOH - conc NH_4OH, 9 : 1 : 0.1); ^{13}C NMR Table 1; MS *m/z* 774.9 (M, $C_{40}H_{74}O_{12}N_2$), 616.4 (M−$C_8H_{16}O_2N$), 458.3142 (M−$C_{16}H_{30}O_5N$, $C_{24}H_{44}O_7N$), 442.3157 (M−$C_{16}H_{30}O_6N$, $C_{24}H_{44}O_6N$), 157.9 (M−$C_{32}H_{58}O_{10}N$).

9-Deoxo-9a-(β-aminoethyl)-9a-aza-9a-homoerythromycin A (**19**)

Sodium borohydride (1.17 g, 31 mmol) was added to a mixture of nitrile **3e** (2.4 g, 3.1 mmol), 72 ml of MeOH and anhydrous cobaltous chloride (0.79 g, 6.1 mmol) at room temperature. An exothermic reaction occurred, with considerable foaming. The mixture was stirred at room temperature for 2 hours, and then concentrated *in vacuo* to a black oily residue. The residue was taken up in a CH_2Cl_2 - H_2O (1 : 1) mixture (50 ml), and the mixture was stirred for 10 minutes after adjusting the pH to 2.5 (1 N HCl). The aqueous phase was separated, and then stirred with 25 ml of CH_2Cl_2 while the pH was adjusted to 9.5 (1 N NaOH). The organic phase was then separated, an equal volume of H_2O added, and the pH adjusted to 2.0 (1 N HCl). Again, the aqueous phase was separated and then stirred with 25 ml of fresh CH_2Cl_2 while the pH was raised to 9.5 (1 N NaOH). The organic phase was separated, dried (anhydrous Na_2SO_4), and concentrated *in vacuo* to a colorless foam (1.15 g). Chromatography of 1.05 g of the crude product (30 g silica gel, 70~230 mesh, $CHCl_3$ - MeOH - conc NH_4OH, 6 : 1 : 0.1) yielded 75 mg (3% yield) of **19** as a colorless amorphous solid.

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 102.9, 95.2, 84.0, 78.6, 78.0, 77.5, 77.3, 74.3, 74.0, 73.4, 72.8, 70.9, 68.9, 68.7, 65.5, 62.1, 53.3, 49.4, 45.1, 41.7, 41.6, 41.3, 40.3 (2), 35.0, 29.1, 28.9, 26.7, 22.6, 21.6, 21.3, 21.1, 18.4, 16.2, 15.2, 11.0, 9.7, 7.8; MS *m/z* 778.6 (M+1, $C_{39}H_{76}O_{12}N_3$), 619.4 (M−$C_8H_{16}O_2N$), 158.0 (M−$C_{31}H_{59}O_{10}N_2$).

9,11-Deoxo-9a-aza-[11-β,9a-(epoxyethano)]-9a-homoerythromycin A (**20**)

A mixture consisting of amine **19** (0.38 g, 0.49 mmol) in 4 ml of $CHCl_3$, isoamyl nitrite (0.072 ml, 0.54 mmol) and glacial AcOH (0.056 ml, 0.98 mmol) was refluxed for 1 hour. The addition of isoamyl nitrite and glacial AcOH (same amount of each just described) was repeated, and the mixture was refluxed for 2 hours. A mixture of $CHCl_3$ (10 ml) and saturated aqueous $NaHCO_3$ solution (15 ml) was added to the reaction, and the pH of the mixture was adjusted to 9.5 (1 N NaOH). The organic phase was separated, dried (anhydrous Na_2SO_4), and concentrated *in vacuo* to afford 0.37 g of a tan foam. Formamide-treated silica gel was prepared by adding 120 ml of formamide to a well-stirred 300 g silica gel (230~240 mesh) - 600 ml acetone slurry, and then removing solvent *in vacuo* on a rotary evaporator until a free-flowing powder was obtained. Chromatography of the crude product on 55 g of formamide-treated silica gel, and water washing followed by Na_2SO_4 drying of product-containing fractions (to remove formamide) afforded 26.5 mg (about 1% yield) of **20** as a white amor-

phous solid.

^{13}C NMR (250 MHz, $CDCl_3$) δ 177.7, 103.4, 96.0, 84.4, 79.5, 78.1, 78.0, 74.9, 74.3, 72.9, 71.0 (2), 68.9, 65.8, 65.6, 65.5, 63.1, 60.7, 50.0, 49.4, 45.1, 41.1, 40.6, 40.4 (2), 35.2, 29.7, 29.3, 26.6, 22.6, 21.6, 21.3, 20.9, 18.3, 16.4, 15.5, 11.2, 9.7, 8.5; MS *m/z* 602 ($M-C_8H_{17}O_2N$), 444.2942 ($M-C_{16}H_{30}O_5N$, $C_{23}H_{42}O_7N$), 428.3005 ($M-C_{16}H_{30}O_6N$, $C_{23}H_{42}O_6N$), 158 ($M-C_{31}H_{56}O_{10}N$).

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