20, 129493-16-9; 21, 129493-17-0; I, 129520-72-5; Ia, 129520-71-4; II, 129493-20-5; IIa, 129493-19-2; III, 129520-73-6; IV, 129493-21-6; V, 129493-22-7; VI, 129493-23-8; VII, 129493-24-9; VIII, 129520-74-7; IX, 129520-75-8; X, 129493-25-0; XI, 129493-26-1; XII, 121199-55-1; XIII, 129493-27-2; XIV, 129520-76-9; XV, 129493-28-3; XVI, 129493-29-4; XVII, 129493-30-7; XVIII, 129493-31-8; XIX, 129493-32-9; XX, 129493-33-0; XXI, 129493-34-1; Arg(Tos)-NH_2·HCl, 129493-18-1; BOC-Arg(TOS), 13836-37-8; BOC-Pro, 15761-39-4; BOC-Orn(Tos), 18942-48-8; BOC-Asn-ONp, 4587-33-1; BOC-Val, 13734-41-3; BOC-Phe, 13734-34-4; BOC-D-Tyr(Et), 76757-92-1; Aaa-OH, 4942-47-6; BOC-D-Tyr(Me), 68856-96-2; Phaa-OH, 103-82-2; *t*-Baa-OH, 1070-83-3.

Synthesis and Structure–Activity Relationships of New 9-*N*-Alkyl Derivatives of 9(*S*)-Erythromycylamine[1]

Herbert A. Kirst,* Julie A. Wind, James P. Leeds, Kevin E. Willard, Manuel Debono, Rosanne Bonjouklian, James M. Greene, Kevin A. Sullivan, Jonathan W. Paschal, Jack B. Deeter, Noel D. Jones, John L. Ott, Anna M. Felty-Duckworth, and Fred T. Counter

Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285.
Received February 20, 1990

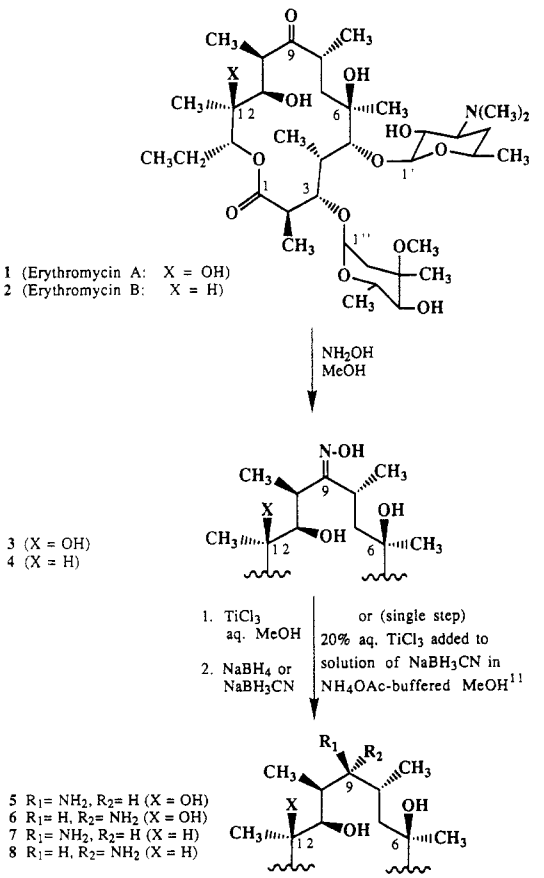
A series of new 9-*N*-alkyl derivatives of 9(*S*)-erythromycylamine has been synthesized by reductive alkylation of erythromycylamine with aliphatic aldehydes and sodium cyanoborohydride. Alternative syntheses employing hydrogenation methods have also been developed. These new 9-*N*-alkyl derivatives possess excellent antimicrobial activity in vitro and in vivo, especially when administered orally to treat experimental infections in mice. From structure–activity studies, 9-*N*-(1-propyl)erythromycylamine (LY281389) was selected as the most efficacious derivative. These methods have also been extended to the synthesis of some 9-*N*,*N*-dialkyl derivatives of erythromycylamine.

Introduction

9(*S*)-Erythromycylamine is a well-known semisynthetic derivative of erythromycin; it possesses excellent antimicrobial activity but is poorly absorbed after oral administration to humans.[2,3] Several approaches to improving the oral bioavailability of erythromycylamine have been investigated. Ketones and aromatic aldehydes have been condensed with erythromycylamine, yielding Schiff bases which can hydrolyze back to erythromycylamine;[3,4] however, these derivatives failed to increase serum concentrations of antibiotic after oral administration to humans despite good oral absorption in dogs.[3] In contrast to ketones and aromatic aldehydes, aliphatic aldehydes have been condensed with erythromycylamine to produce 9-*N*,11-*O*-oxazine derivatives.[3] Dirithromycin is a relatively new member of this oxazine class;[5] it produces high concentrations of antibiotic in tissues after oral administration and is currently being evaluated in clinical trials.[6,7]

We have recently reported that reductive amination of certain tylosin-related macrolides with dialkylamines produced a new series of antibiotics possessing an expanded spectrum of antimicrobial activity and good oral bioavailability.[8,9] In order to explore a similar approach

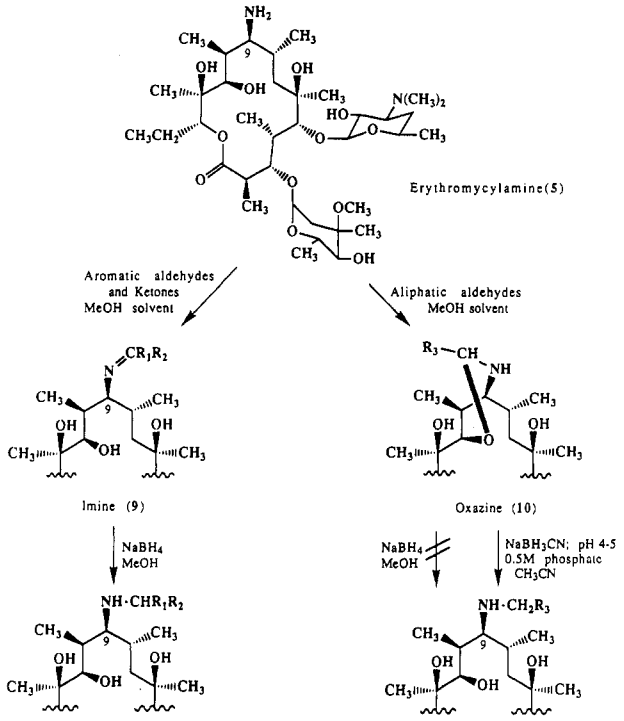
Scheme I. Synthesis of 9(*S*)-Erythromycylamine (5) and Related Compounds



(1) These results were initially presented at the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, New York, October 4–7, 1987; Session No. 45.
(2) Sakakibara, H.; Omura S. *Macrolide Antibiotics: Chemistry, Biology, and Practice*; Academic Press, Inc.: Orlando, FL 1984; pp 92–95.
(3) Massey, E. H.; Kitchell, B. S.; Martin, L. D.; Gerzon, K. *J. Med. Chem.* **1974**, *17*, 105.
(4) Cockerill, A. F.; Ellis, M. F.; Rackham, D. M.; Wildsmith, E. *J. Chem. Soc. Perkin Trans. 2* **1973**, 173.
(5) Luger, P.; Maier, R. *J. Cryst. Mol. Struct.* **1979**, *9*, 329.
(6) Bozler, G.; Heinzel, G.; Busch U. *Book of Abstracts* 8th International Symposium on Future Trends in Chemotherapy, Tirrenia (Pisa), Italy, March 28–30, 1988; 1988; p 30.
(7) Busch, U.; Lechner U. 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, California, October 23–26, 1988; Abstract No. 925.

0022-2623/90/1833-3086$02.50/0 © 1990 American Chemical Society

Scheme II. Synthesis of 9-*N*-Alkyl Derivatives of Erythromycylamine



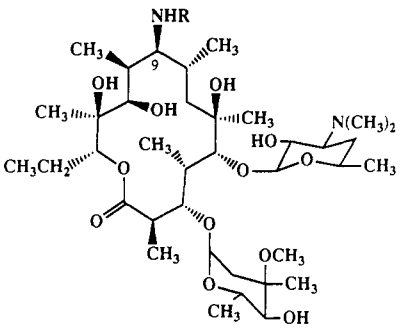
with erythromycin-related macrolides, the synthesis of some new 9-*N*-alkyl derivatives of erythromycylamine was undertaken.

Chemistry

Erythromycin does not react with primary amines (e.g., *n*-propylamine) and sodium cyanoborohydride under the standard conditions for direct reductive alkylation.[10] As a result, all synthetic routes to 9-amino derivatives of erythromycin have proceeded through the intermediacy of a C-9 oxime, hydrazone, or imine of erythromycin, whose reduction by sodium borohydride gives predominantly 9(*S*)-erythromycylamine (**5**, Scheme I).[2,11]

Prior to our work,[1,12] no 9-*N*,*N*-dialkyl derivatives and only certain selected 9-*N*-monoalkyl derivatives of erythromycylamine had been successfully prepared. Ketones and aromatic aldehydes were known to react with the 9-amino group of erythromycylamine, yielding Schiff bases (**9**, Scheme II) which were readily reduced by sodium borohydride to give the corresponding 9-*N*-alkyl or *N*-benzyl derivatives.[13] However, aliphatic aldehydes reacted with erythromycylamine in a divergent manner, giving 9-*N*,11-*O*-oxazine derivatives (**10**, Scheme II) instead of the anticipated Schiff bases;[3,14] these oxazine derivatives were not reduced by sodium borohydride.[3]

Scheme III. Structures of 9-*N*-Alkyl Derivatives of Erythromycylamine



Cpd. No.	9-N-Alkyl Substituent of Erythromycylamine	Cpd. No.	9-N-Alkyl Substituent of Erythromycylamine
11	Methyl	28	(Cyclohex-3-en-1-yl)methyl
12	Ethyl	29	2-(2,6,6-Trimethylcyclohex-1-enyl)ethyl
13	1-Propyl	30	(Cyclooctyl)methyl
14	1-Butyl	31	2-Methoxyethyl
15	1-Pentyl	32	3-Methoxy-1-propyl
16	3-Methyl-1-butyl	33	2-(2-Methoxyethoxy)ethyl
17	1-Hexyl	34	3-(Methylthio)-1-propyl
18	2-Ethyl-1-butyl	35	3-Cyano-1-propyl
19	1-Heptyl	36	3,7-Dimethyl-7-methoxy-1-octyl
20	1-Octyl	37	5-Hydroxy-1-pentyl
21	1-Decyl	38	3-Hydroxy-2,2-dimethyl-1-propyl
22	*cis*-4-Decen-1-yl	39	4-Amino-1-butyl
23	*trans*-4-Decen-1-yl	40	2-propyl
24	Undec-10-en-1-yl	41	Cyclopentyl
25	1-Dodecyl	42	Cyclohexyl
26	3-Phenylpropargyl	43	5-Diethylamino-2-pentyl
27	3-Phenyl-1-propyl	44	Benzyl

This limitation was partially circumvented by conjugate addition of the 9-amino group of erythromycylamine to various α,β-unsaturated ketones, esters, and nitriles, which provided a method for the synthesis of certain 9-*N*-alkyl derivatives bearing an additional functional group at C-3 of the 9-*N*-alkyl substituent.[13] This approach was later extended to include conjugate additions with nitroolefins or α-ketoaldehydes and then combined with subsequent modifications of the functional groups at the distant end of the 9-*N*-alkyl chain; by these means, additional compounds were prepared which possessed a functional group at the terminus of the 9-*N*-alkyl substituent of erythromycylamine.[15,16] However, a general solution to the direct synthesis of any 9-*N*-alkyl derivative of erythromycylamine from any simple or unsubstituted aliphatic aldehyde had not been previously reported.

We have now found that the 9-*N*,11-*O*-oxazine derivatives of erythromycylamine (**10**) are successfully converted into the corresponding 9-*N*-alkyl derivatives by reduction with sodium cyanoborohydride under mildly acidic conditions. These conditions were standardized with 0.5 M aqueous phosphate buffer to control the pH at 4–5 and acetonitrile as organic cosolvent. Under these conditions, a variety of oxazines were readily reduced to their respective 9-*N*-alkyl compounds. Subsequently, we found that these conditions could also be employed to convert erythromycylamine directly into its 9-*N*-alkyl derivatives, in a single step without the separate isolation of any intermediates.[12,17]

(8) Kirst, H. A.; Willard, K. E.; Debono, M.; Toth, J. E.; Truedell, B. A.; Leeds, J. P.; Ott, J. L.; Felty-Duckworth, A. M.; Counter, F. T.; Ose, E. E.; Crouse, G. D.; Tustin, J. M.; Omura, S. *J. Antibiotics* **1989**, *42*, 1673.
(9) Debono, M.; Willard, K. E.; Kirst, H. A.; Wind, J. A.; Crouse, G. D.; Tao, E. V.; Vicenzi, J. T.; Counter, F. T.; Ott, J. L.; Ose, E. E.; Omura, S. *J. Antibiotics* **1989**, *42*, 1253.
(10) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897.
(11) Leeds, J. P.; Kirst, H. A. *Synth. Commun.* **1988**, *18*, 777.
(12) Kirst, H. A.; Leeds, J. P.; Wind, J. A. EUCHEM Symposium on the Chemical Synthesis of Antibiotics, Aussois (Modane), France, May 2–6, 1988; Abstract No. 30.
(13) Ryden, R.; Timms, G. H.; Prime, D. M.; Wildsmith, E. *J. Med. Chem.* **1973**, *16*, 1059.
(14) Maier, R.; Woitun, E.; Wetzel, B.; Reuter, W.; Goeth, H.; Lechner, U. U. S. Patent 4,048,306, Sept. 13, 1977.
(15) Wetzel, B.; Woitun, E.; Maier, R.; Reuter, W.; Goeth, H.; Lechner, U. U. S. Patent 4,016,263, Apr. 5, 1977.
(16) Woitun, E.; Wetzel, B.; Maier, R.; Reuter, W.; Lechner, U.; Werner, R.; Goeth, H. U. S. Patent 4,256,736, Mar. 17, 1981.

Table I. Proton and ^{13}C NMR Assignments for Erythromycylamine and Its 9-*N*-Propyl Derivatives (**13** and **40**)

	erythromycylamine (**5**)		9-*N*-(1-Pr) deriv. (**13**)		9-*N*-(2-Pr) deriv. (**40**)	
position	proton[a]	^{13}C	proton[a]	^{13}C	proton[a]	^{13}C
1		176.93		177.38		177.24
2	2.79	44.35	2.85	44.72	2.77	44.90
3	4.12	78.28	4.16	79.33	4.12	79.38
4	1.90	42.47	1.94	40.77	1.91	40.82
5	3.72	83.39	3.61	83.96	3.53	84.80
6		74.10		73.91		73.90
7	1.71/1.34	37.79	1.50/1.35	37.20	1.54/1.35	37.06
8	2.00	33.76	2.19	31.82	2.15	32.33
9	2.72	63.44	2.19	71.14	2.27	67.56
10	1.90	30.98	2.08	29.99	2.01	29.84
11	3.80	70.18	3.84	70.27	3.79	70.46
12		75.48		75.87		75.80
13	4.85	77.38	4.71	77.94	4.72	78.03
14	1.86/1.44	21.88	1.89/1.45	21.62	1.90/1.47	21.77
15	0.90	11.18	0.88	11.12	0.87	11.20
2-Me	1.20	14.50	1.20	14.55	1.19	14.63
4-Me	1.13[b]	9.28	1.09	9.24	1.07	9.31
6-Me	1.24	24.55	1.27	25.82	1.26	25.23
8-Me	1.15[b]	20.21	1.04	21.23	1.01	22.42
10-Me	1.11[b]	16.13	1.15	16.34	1.10	16.35
12-Me	1.22	13.56	1.10	16.42	1.08	16.55
1′	4.60	102.51	4.49	103.01	4.46	103.16
2′	3.30	70.95	3.27	70.99	3.25	70.89
3′	2.44	65.29	2.43	65.40	2.44	65.34
4′	1.62/1.20	28.81	1.63/1.21	28.84	1.64/1.21	28.81
5′	3.59	69.21	3.51	69.01	3.50	69.06
6′	1.24	21.15	1.23	21.30	1.20	21.25
NMe_2	2.30	40.28	2.29	40.27	2.27	40.29
1″	5.05	95.47	4.99	96.01	4.95	96.11
2″	2.34/1.55	34.86	2.38/1.55	34.96	2.35/1.55	34.92
3″		72.82		72.71		72.71
4″	3.05	77.89	3.04	77.94	2.99	77.84
5″	4.01	65.83	4.04	65.63	4.03	65.64
6″	1.30	18.18	1.32	18.30	1.29	18.27
3″-Me	1.25	21.54	1.24	21.49	1.21	21.48
3″-OMe	3.35	49.23	3.33	49.33	3.30	49.36
propyl[c]			2.70/2.41	51.67	2.72	47.35
propyl[c]			1.45	22.79	1.00	21.86
propyl[c]			0.91	11.64	1.08	23.44

[a] Some assignments determined from $^1H/^{13}C$ correlation. [b] Assignments may be interchanged. [c] Assignments for appropriate 1-propyl and 2-propyl substituents.

The wide variety of 9-*N*-alkyl derivatives which have been synthesized is shown in Scheme III; 9-*N*-substituents include alkyl chains which are linear or branched, saturated or unsaturated, and which may be substituted with aromatic or nonaromatic rings or with functional groups such alkoxy, hydroxy, alkylthio, amino, alkylamino, cyano, or halo. Compounds were purified by chromatography as well as crystallization whenever possible; sample purity was ascertained by TLC analysis, elemental analysis, and proton NMR spectroscopy. Structures were assigned on the basis of their NMR and mass spectra; detailed assignments of the proton and ^{13}C NMR spectra for erythromycylamine and its 9-*N*-(1-propyl) (**13**) and 9-*N*-(2-propyl) (**40**) derivatives have been given in Table I. Further structural verification was obtained from a single-crystal X-ray diffraction study of the 9-*N*-(2-ethyl-1-butyl) derivative (**18**), whose structure is depicted in Figure 1.

The 9-*N*-alkyl compounds were converted into another set of oxazine derivatives by treatment with formaldehyde, thereby producing the corresponding 9-*N*-alkyl-9-*N*,11-*O*-methylene derivatives (Scheme IV). Although oxazines derived from formaldehyde were obtained in good yield,

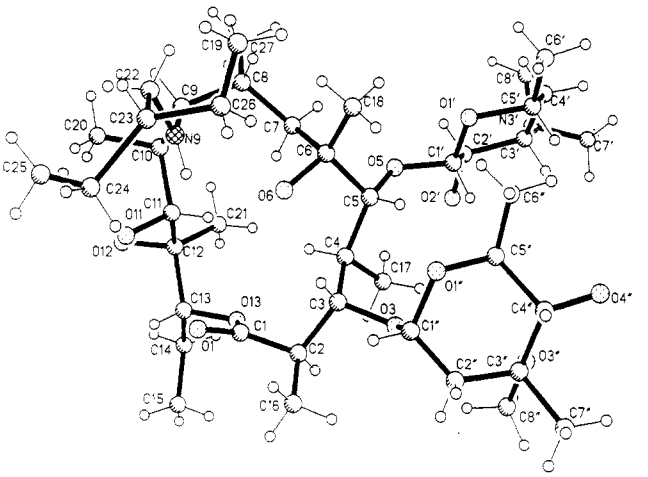
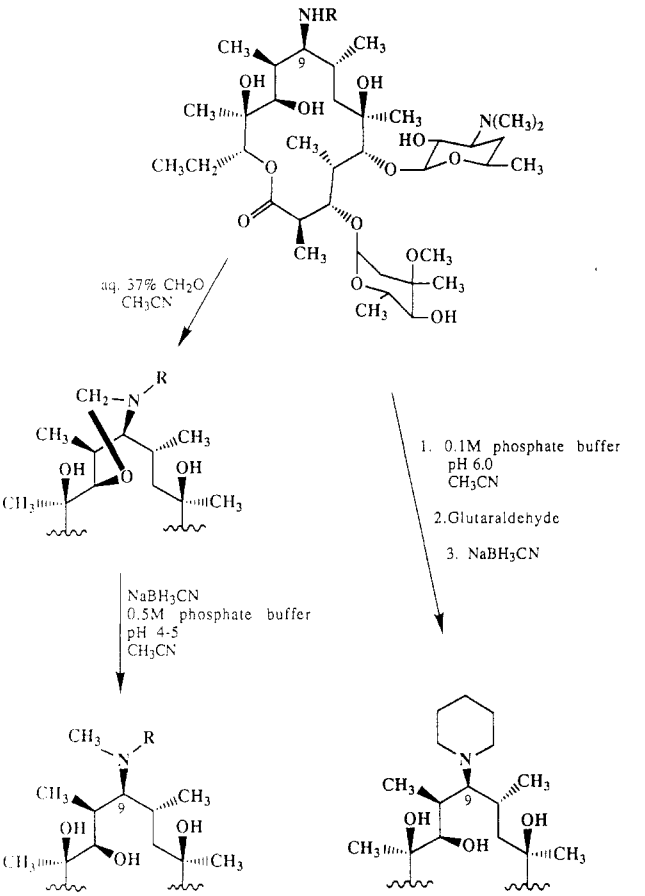


Figure 1. X-ray structure of 9-*N*-(2-ethyl-1-butyl)erythromycylamine (**18**).

Scheme IV. Synthesis of 9-*N*,*N*-Dialkyl Derivatives of Erythromycylamine

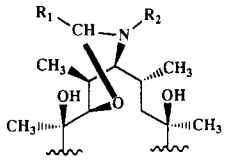


attempts to prepare analogous oxazines from 9-*N*-alkyl derivatives of erythromycylamine and other simple aldehydes (acetaldehyde, propionaldehyde) produced very little, if any, such adducts. The failure to form oxazines from aldehydes larger than formaldehyde is probably due to steric hindrance around the 9- and 11-positions of this highly substituted macrolide ring system.

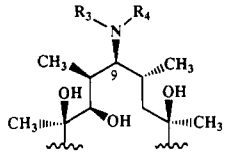
The 9-*N*-alkyl-9-*N*,11-*O*-methylene compounds were successfully reduced under the conditions described above to yield the respective 9-*N*-alkyl-9-*N*-methyl derivatives of erythromycylamine (Scheme IV). In addition to this set of tertiary amino derivatives, an azacyclic derivative was prepared by reductive amination of erythromycylamine with an α,ω-dialdehyde such as glutaraldehyde

(17) Kirst, H. A.; Wind, J. A.; Leeds, J. P.; Willard, K. E.; Debono, M.; Bonjouklian, R.; Greene, J. M.; Sullivan, K. A.; Ott, J. L.; Felty-Duckworth, A. M.; Counter F. T. 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, Texas, September 17–20, 1989; Abstract No. 1020.

Scheme V. Structures of 9-*N*,*N*-Dialkyl Derivatives of Erythromycylamine



Cpd.No.	R_1	R_2
10a	Ethyl	H
10b	$CH_3OCH_2CH_2OCH_2$	H
45	H	1-Propyl
46	H	2-Propyl
47	H	Cyclopentyl
48	H	5-NEt_2-2-Pentyl
49	H	Benzyl
50	Methyl	1-Propyl



Cpd.No.	R_3	R_4
51	Methyl	Methyl
52	Methyl	2-Propyl
53	Methyl	Cyclohexyl
54	NR_3R_4 = Piperidinyl	

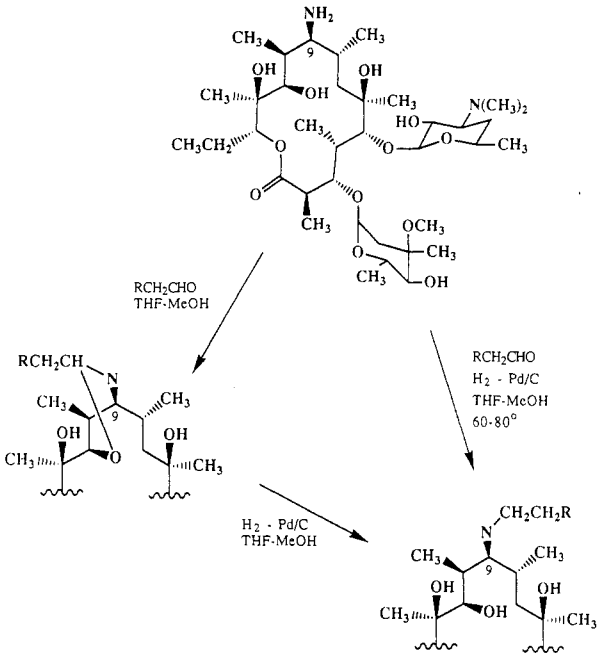
(Scheme IV). A similar series of derivatives from 9(*R*)-erythromycylamine has just recently been reported by another group.[18] Structures of the new 9-*N*,*N*-dialkyl derivatives of erythromycylamine are illustrated in Scheme V.

Although these procedures for reductive alkylation with sodium cyanoborohydride were very suitable for the synthesis of derivatives for antimicrobial evaluation and structure–activity studies, a more convenient method for larger scale synthesis was necessary. This was achieved by hydrogenation methods (Scheme VI). Reaction conditions for reductive alkylation of erythromycylamine with aldehydes ranged from 60 to 100 °C for 16–24 h at 50–100 psi hydrogen pressure under a catalyst load of 10–30% by weight of 5% Pd/C. More vigorous reaction conditions (higher temperature, pressure, and, particularly, catalyst load) accelerated the rate of reaction, but also led to more impurities. Best results were obtained with tetrahydrofuran-methanol mixtures as solvent, although ethyl acetate, dichloromethane, and acetonitrile have also been employed. Product was isolated by extractive procedures as described in the experimental section, which improved the purity of the product and eliminated the need for chromatography.

The course of the reaction was followed by HPLC, which showed almost instantaneous reaction between aldehyde and erythromycylamine to form oxazine **10**; this intermediate was then slowly reduced to the 9-*N*-alkyl derivative under the hydrogenation conditions. However, isolation of the oxazine is unnecessary, although it has been separately isolated and subsequently reduced under the same catalytic hydrogenation conditions to yield results identical with those from direct reductive alkylation of erythromycylamine with aldehydes. Incomplete reduction of the intermediate oxazine is a problem, because it is the major impurity (5–10%) at the end of the reaction. Inhibition of the catalyst by the basic substrate (erythromycylamine) is probably responsible for slowing or halting this catalytic process.

(18) Maring, C. J.; Klein, L. L.; Pariza, R. J.; Lartey, P. A.; Grampovnik, C. M.; Yeung, C. M.; Buytendorp, M.; Hardy, D. J. 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, Texas, September 17–20, 1989; Abstract No. 1023.

Scheme VI. Alternative Synthesis of 9-*N*-Alkyl Derivatives of Erythromycylamine



Antimicrobial Activity

In Vitro Evaluation. The new 9-*N*-substituted derivatives of erythromycylamine were initially evaluated for antimicrobial activity against a wide range of bacteria by standard agar-dilution methods. All of the compounds demonstrated in vitro activity; many of them exhibited excellent activity within the range of erythromycin and erythromycylamine (Table II). The spectrum of antimicrobial activity of these derivatives was very similar to that of erythromycin itself; good activity was observed against Gram-positive species but not against species of *Enterobacteriaceae* or *Pseudomonas*. Activity against Gram-negative pathogens of the respiratory tract, such as *Haemophilus influenzae*, was comparable to that of erythromycin and erythromycylamine.

In vitro activity of the 9-*N*,*N*-dialkyl derivatives which were prepared (compounds **51–54**) appeared to be weaker than that of the mono-*N*-alkyl derivatives; consequently, most of the effort was directed toward synthesis and evaluation of the latter group of compounds. The 9-*N*,11-*O*-methylene adducts of the 9-*N*-alkyl derivatives (compounds **45–50**) possessed in vitro activity essentially equivalent to that of their respective parent compounds (**13**, **40–44**); since these methylene adducts demonstrated no in vitro advantages and might liberate formaldehyde in vivo, further studies with this series did not appear warranted.

Among the mono-*N*-alkyl derivatives, in vitro activity began to diminish as the length of the alkyl chain exceeded 10 carbon atoms (compounds **24**, **25**). Among the various substituents on the alkyl chain which were investigated, only the polar, basic amino group appeared to lead to reduction of in vitro activity (compounds **39**, **43**, **48**). Otherwise, it was difficult to find significant differences with which to distinguish among the various derivatives.

In Vivo Evaluation. Since many of these new derivatives exhibited excellent antibiotic activity in vitro, it was necessary to employ in vivo evaluations in order to distinguish among members of the series. Because macrolide antibiotics are generally administered orally when used to treat many bacterial infections, initial in vivo evaluations were conducted by oral administration to mice which had

Table II. In Vitro Antibacterial[a] Activity of 9-*N*-Substituted Derivatives of Erythromycylamine

	MIC values,[b] μg/mL					
no.	S. aur X1	S. epi 222	S. pyog C203	S. pneum Park 1	S. faec X66	H. flu. CL
1	0.12	0.06	0.015	0.008	0.06	1.0
2	0.25	0.25	0.03	NT	0.06	4.0
3	0.25	0.06	0.008	0.008	0.06	0.5
4	0.5	0.25	0.015	0.008	1.0	1.0
5	0.25	0.25	0.06	0.015	0.25	1.0
6	2.0	1.0	0.5	0.25	NT	32.0
7	0.25	0.12	0.015	0.015	0.12	1.0
8	2.0	1.0	0.25	NT	1.0	16.0
10a	0.5	0.5	0.12	0.12	0.5	8.0
10b	0.5	0.5	0.12	0.06	0.25	2.0
11	1.0	0.25	0.06	NT	0.12	0.5
12	1.0	1.0	0.12	0.03	0.25	1.0
13	0.25	0.12	0.03	0.015	0.12	0.5
14	0.25	0.25	0.06	0.015	0.12	1.0
15	0.5	0.5	0.06	0.015	0.12	1.0
16	1.0	0.25	0.06	0.03	0.12	1.0
17	1.0	0.25	0.12	0.008	0.12	4.0
18	0.5	0.5	0.015	0.015	0.12	2.0
19	0.25	0.25	0.015	0.015	0.06	1.0
20	0.5	0.25	0.015	0.015	0.06	1.0
21	1.0	1.0	0.015	0.015	0.25	2.0
22	0.25	0.12	0.008	0.008	0.06	2.0
23	0.5	0.25	0.008	0.008	0.12	2.0
24	2.0	1.0	1.0	NT	0.12	2.0
25	4.0	4.0	0.5	0.06	1.0	8.0
26	1.0	0.5	0.008	0.008	0.25	4.0
27	1.0	0.5	0.015	0.015	0.06	2.0
28	1.0	0.25	0.06	0.008	0.25	4.0
29	1.0	1.0	0.5	NT	0.12	4.0
30	1.0	0.5	0.06	NT	0.06	NT
31	0.5	0.25	0.12	0.015	0.25	1.0
32	0.5	0.5	0.06	0.03	0.12	0.5
33	1.0	1.0	0.12	0.015	0.25	2.0
34	0.5	0.5	0.015	0.015	0.25	4.0
35	1.0	1.0	1.0	NT	0.12	2.0
36	0.5	0.25	0.015	NT	0.03	1.0
37	0.5	0.5	0.008	0.008	0.25	2.0
38	1.0	1.0	0.03	0.015	0.25	1.0
39	4.0	4.0	0.12	4.0	8.0	64
40	1.0	0.5	0.25	0.12	0.25	2.0
41	1.0	0.5	0.06	0.06	0.5	2.0
42	0.5	0.5	0.06	0.03	0.25	4.0
43	2.0	2.0	0.5	0.5	2.0	8.0
44	0.5	0.5	0.06	0.015	0.25	2.0
45	0.25	0.12	0.008	0.008	0.12	1.0
46	1.0	0.5	0.12	0.06	0.25	4.0
47	1.0	1.0	0.12	0.06	0.5	4.0
48	8.0	8.0	0.5	0.5	2.0	8.0
49	1.0	0.5	0.12	0.06	0.5	4.0
50	1.0	0.25	0.06	0.015	0.25	1.0
51	4.0	2.0	0.25	NT	0.5	2.0
52	8.0	8.0	0.5	0.5	2.0	32
53	16	8.0	1.0	0.12	8.0	32
54	16	8.0	1.0	1.0	4.0	2.0

[a] Abbreviations of organisms: *Staphylococcus aureus*, S. aur; *Staphylococcus epidermidis*, S. epi; *Streptococcus pyogenes*, S. pyog; *Streptococcus pneumoniae*, S. pneum; *Streptococcus faecalis*, S. faec; *Haemophilus influenzae*, H. flu. [b] NT means not tested due to lack of growth of organism on the day of testing.

been experimentally infected by one of three different pathogenic Gram-positive species (*Staphylococcus aureus*, *Streptococcus pyogenes*, or *Streptococcus pneumoniae*). Many members from the series of 9-*N*-alkyl derivatives demonstrated good efficacy against these experimental infections, treating the infections at concentrations comparable or somewhat superior to those used for erythromycin (Table III). The derivatives which appeared to be most consistently efficacious were those possessing short lipophilic alkyl chains of approximately two to six carbon atoms (compounds **12–18, 34, 40, 41, 44–46**). Results from

Table III. In Vivo Antibacterial Activity of 9-*N*-Substituted Derivatives of Erythromycylamine

	ED_{50} values, mg/kg × 2 po against bacterial infections in mice		
no.	*S. pyogenes*	*S. pneumoniae*	*S. aureus*
1	21	44	41
5	46	67	>100
10a	35	30	34
10b	43	32	>50
11	21	35	44
12	13	13	23
13	3	7	12
14	13	13	15
15	9	14	25
16	9	11	13
17	18	14	39
18	10	17	22
19	31	33	>50
20	23	46	>50
21	>50	>50	>50
25	>50	>50	>45
26	27	34	>50
27	14	17	37
28	15	9	20
29	>50	>50	>50
31	16	19	35
32	14	16	16
33	27	23	22
34	7	18	50
35	21	29	36
36	25	24	>46
37	43	>50	>50
38	20	46	>50
40	13	13	32
41	4	4	46
43	31	18	41
44	9	11	>50
45	5	5	7
46	7	6	9
47	10	9	>50
49	16	15	>50
51	22	44	>44
53	>50	>50	>50
54	24	21	>44

numerous in vivo studies suggested that the 9-*N*-(1-propyl) derivative (**13**) was consistently among the most efficacious compounds within the series.

In order to better evaluate these derivatives, several additional tests were conducted in which the most promising compounds were compared on a side-by-side basis for efficacy, by both oral and parenteral administration, against experimental bacterial infections (Table IV). Alkoxy-substituted alkyl groups are prominently featured within the structures of the new macrolide antibiotics roxithromycin and dirithromycin.[19] Consequently, the 1-propyl derivative (**13**) was compared with analogous derivatives containing the 2-methoxyethyl (**31**), 3-methoxy-1-propyl (**32**), and 2-(2-methoxyethoxy)ethyl (**33**) substituents. It should be noted that the latter is the product of reductive ring cleavage of dirithromycin. Although all of the compounds gave comparable results after subcutaneous administration, the 1-propyl derivative (LY281389, **13**) was consistently more effective than any of the alkoxy-substituted derivatives after oral dosing (Table IV).

9-*N*-(1-Propyl)erythromycylamine (**13**) was more orally effective than its 9-*N*,11-*O*-oxazine analogue (**9**), from which **13** could be prepared via reductive ring cleavage; dirithromycin, erythromycin, and erythromycylamine were also less active orally than was **13** in these experimental

(19) Kirst, H. A.; Sides, G. D. *Antimicrob. Agents Chemother.* **1989**, *33*, 1413.

Table IV. Comparative in Vivo Evaluation of 9-*N*-Substituted Derivatives of Erythromycylamine

expt. no.	no.	route of admin	ED_{50} values, mg/kg × 2, vs bacterial infections in mice: *S. pyogenes*	*S. pneumoniae*	*S. aureus*
1	**13**	po	9	14	19
	1	po	18	14	15
	31	po	16	25	44
	33	po	37	43	78
	13	sc	1.7	1.6	4.7
	1	sc	2.5	2.7	1.6
	31	sc	1.7	2.0	2.0
	33	sc	5.3	1.4	3.8
2	**13**	po	6	13	25
	31	po	16	19	35
	32	po	19	17	36
	13	sc	1.4	2.1	2.1
	31	sc	2.1	3.3	2.5
	32	sc	2.1	8.3	3.7
3	**13**	po	6	6	13
	1	po	12	>100	71
	5	po	27	28	50
	10a	po	19	17	20
	10b	po	34	23	27
	13	sc	1.7	1.8	6.1
	1	sc	2.8	3.5	4.1
	5	sc	1.1	<0.6	0.6
	10a	sc	<0.6	<0.6	0.8
	10b	sc	0.6	<0.6	1.0

Table V. Plasma Concentrations of Macrolides in Mice

compound	concentration,[b] μg/mL: 5 min[a]	20 min	40 min	1 h	2 h	4 h
Oral Administration, 20 mg/kg						
LY281389 (**13**)	0	0.01	0.036	0.08	0.07	0.06
erythromycin (**1**)	0.10	0.01	0.01	0	0	0
erythromycylamine (**5**)	0.05	0.02	0.01	0	0	0
Subcutaneous Administration, 20 mg/kg						
LY281389 (**13**)	1.58	0.75	0.47	0.39	0.11	0.06
erythromycin (**1**)	5.84	5.96	4.36	4.26	0.01	0
erythromycylamine (**5**)	8.48	10.54	4.02	2.26	1.32	0.28

[a] Time of sample after administration of compound. [b] Average of five mice.

infection models (Table IV). These results may be partially attributed to the greater stability of **13** to acidic conditions,[20] suggesting that it may provide good bioavailability even when administered to animals in solution, without enteric coatings or other protective measures commonly used with some macrolides. In contrast, erythromycylamine and its oxazine derivatives (**10a**, **10b**) were significantly more effective after parenteral administration, reflecting the excellent antibiotic activity but low oral bioavailability of these compounds in rodents.

A preliminary evaluation of plasma concentrations following administration to mice and rats indicated that LY281389 (**13**) yielded more prolonged concentrations of antibiotic after oral dosing than did either erythromycin or erythromycylamine (Tables V and VI). Such prolonged concentrations would be a desirable feature if it would permit lower and/or less frequent dosing with a new macrolide than is possible with erythromycin. In contrast to the relative order after oral dosing, erythromycin and erythromycylamine produced significantly higher serum concentrations than did **13** after subcutaneous dosing. These results correlate well with the results from the efficacy studies against experimental infections described above and suggest good bioavailability for LY281389 (**13**). The consistently high oral bioavailability of LY281389 (**13**) in animals has now been further confirmed.[20,21]

Table VI. Plasma Concentrations of Macrolides in Rats

compound	concentrations, μg/mL: 5 min[a]	20 min	40 min	1 h	2 h	4 h	8 h
Oral Administration, 20 mg/kg							
LY281389 (**13**)	0.08	0.04	0.01	0.01	0.28	0.08	0.06
erythromycin (**1**)	0.48	0.11	0.11	0.04	0.03	0.02	0.01
erythromycylamine (**5**)	0.13	0.08	0.01	0	0	0	0
Subcutaneous Administration, 20 mg/kg							
LY281389 (**13**)	0.04	0.07	0.14	0.14	0.18	0.23	0.24
erythromycin (**1**)	4.20	1.21	1.16	1.31	1.06	0.7	0
erythromycylamine (**5**)	0.45	0.6	0.71	0.91	1.21	0.69	0.31

[a] Time of sample after administration of compound.

Table VII. Tissue and Fluid Concentrations of Macrolides in Rats

tissue	concentrations, μg/mL or μg/gm: 0.5 h[a]	1 h	2 h	4 h	6 h
LY281389 (**13**)					
lung	0.31	2.25	4.90	5.46	5.08
liver	0.08	0.17	2.99	3.06	1.19
kidney	0.10	0.31	3.25	4.28	3.21
urine	3.92	3.78	4.80	18.63	11.3
serum	0.15	0.04	0.23	0.12	0.05
Erythromycin (**1**)					
lung	1.0	0.4	0.04	0.03	0.04
liver	0.15	0.03	0.04	0	0
kidney	0.17	0.15	0	0	0
urine	7.5	10.8	2.5	8.8	0.35
serum	0.01	0.4	0	0	0

[a] Time of sample after administration of compound by gavage at 20 mg/kg.

High and prolonged concentrations of macrolide antibiotics in various tissues and organs have recently been publicized, especially for the newer amino-macrolide antibiotics dirithromycin[6,7] and azithromycin.[22] In a preliminary experiment to examine tissue concentrations in rats, LY281389 produced substantially higher and more prolonged concentrations of antibiotic than did erythromycin in lung, liver, and kidney tissues as well as in urine (Table VII). Consequently, LY281389 demonstrated the necessary in vivo features for a new macrolide antibiotic which would be efficacious against bacterial infections upon oral administration of a significantly diminished amount or compound and/or frequency of dosage in comparison to other macrolides.

Experimental Section

Instrumentation. NMR spectra were obtained in $CDCl_3$ solution on a Bruker WH-360 or WM-270 or General Electric QE 300 NMR spectrometer; chemical shifts are given in ppm from internal TMS. Assignments were made using homonuclear decoupling, ^{13}C DEPT, and 2-D $^1H/^{13}C$ heteronuclear correlations (one bond and long range). Field desorption mass spectra were obtained on a Varian-MAT 731 spectrometer with carbon dendrite emitters. Melting points were taken on a Mel-temp apparatus and are uncorrected.

Materials. Erythromycin A and B were obtained from within Eli Lilly and Co. and were converted into compounds **3**–**10a** by

(20) Kirst, H. A.; Quay, J. F.; Johnston, S. R.; Buening, M. K.; Finch, L. S. 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, Texas, September 17–20, 1989; Abstract No. 1021.

(21) Quay, J. F.; Counter, F. T.; Kirst, H. A.; Lindstrom, T. D. 73rd Annual Meeting of the Federation of American Societies for Experimental Biology, New Orleans, Louisiana, March 19–23, 1989; Abstract No. 3829.

(22) Girard, A. E.; Girard, D.; English, A. R.; Gootz, T. D.; Cimochowski, C. R.; Faiella, J. A.; Haskell, S. L.; Retsema, J. A. *Antimicrob. Agents Chemother.* **1987**, *31*, 1948.

following published procedures.[3] Dirithromycin (**10b**) was provided by Karl Thomae GmbH.[5] Compounds **40–44** were prepared from erythromycylamine according to published methods.[13]

Chromatography. Thin-layer chromatography was performed using E. Merck silica gel plates with fluorescent indicator (F-254), developed with mixtures of dichloromethane–methanol–concentrated ammonium hydroxide (90:10:2 to 90:10:0.5); visualization was effected by iodine vapor or anisaldehyde reagents. Product purification was performed by preparative chromatography, using flash chromatography[23] (E. Merck grade 60 silica gel or Woelm basic alumina grade 3), silica gel plates in a Model 7924 Chromatotron (Harrison Research, Inc., Palo Alto, CA), or silica gel cartridges in a Waters Model 500 Prep LC system; product purity was ascertained by TLC, NMR, and elemental analysis.

X-ray Methods. 9-*N*-(2-Ethyl-1-butyl)erythromycylamine (**18**) crystallized in the orthorhombic space group $P22_12_1$ with a unit cell having dimensions of a = 10.832 (2) Å, b = 19.510 (4) Å, and c = 24.085 (3) Å and a calculated density of 1.069 g cm^{-3}. A total of 3916 reflections with 2θ less than 116.0° were measured on an automated four-circle diffractometer using monochromatic copper radiation. The structure was solved by using direct methods routine TREF of the SHELXTL program library and was refined by the least-squares method with anisotropic temperature factors for all atoms except hydrogen. All hydrogen atoms, except for the protons on the hydroxyl groups at O6, O11, O12, O2′, and O4′, were included at calculated positions. The final R factor was 0.12 for 2526 unique observed reflections.

In Vitro and In Vivo Evaluation Methods. Antibiotic susceptibility data in Table II was obtained by agar-dilution methods. Mouse-protection experiments in Tables III and IV were conducted by treating infected animals 1 and 5 h postinfection, either orally or subcutaneously, with 0.25 mL of a 10% aqueous ethanol solution of the antibiotic over a range of concentrations; tartaric acid was added if needed to help dissolve compounds. Peripheral plasma concentrations were determined by microbiological assay using *Micrococcus luteus* seeded in Difco Antibiotic Media 1. Zone sizes were measured with a Fisher zone reader, and antibiotic concentrations were calculated from the standard curve for the appropriate compound. Concentrations represent an average value from five mice per time period (Table V) or three rats per time period (Tables VI and VII).

Reductive Alkylation of Erythromycylamine and Product Characterization. Procedure 1: 9-*N*-(1-Ethyl)erythromycylamine (12). Erythromycylamine (5.0 g, 6.8 mmol) was dissolved in acetonitrile (20 mL, 4 mL/g) with warming as necessary. The resultant solution was removed from the heat and treated with acetaldehyde (0.45 g, 10.2 mmol, 1.5 equiv) and then 0.5 M sodium phosphate buffer solution (pH 4.5, 20 mL, 4 mL/g). After the resultant pH (ca. 7.5) had been lowered to 5.0 by careful addition of 6 N HCl, sodium cyanoborohydride (0.64 g, 10.2 mmol, 1.5 mol equiv) was added. The reaction was stirred at room temperature and monitored by TLC until it appeared to be completed. After 19 h, volatiles were evaporated under reduced pressure until an aqueous solution remained; after being made basic with either 1 N sodium hydroxide or saturation with sodium bicarbonate, the product was extracted into dichloromethane. The organic layer was separated, dried (sodium sulfate), filtered, and evaporated; the residue was purified by flash chromatography on silica gel, eluting with a linear gradient of 9:1 to 4:1 dichloromethane–methanol to yield 360 mg (7%) of **12** as a white, amorphous solid: FDMS m/e 762 (M$^+$). Anal. ($C_{39}H_{74}N_2O_{12}$) C, H, N.

9-*N*-(1-Propyl)erythromycylamine (13). Procedure 1 was followed with erythromycylamine (10.0 g) and propionaldehyde (1.2 g) for 3 h. The crude product was purified by preparative HPLC (Waters Prep 500), eluting with a linear gradient of dichloromethane to dichloromethane–methanol–concentrated ammonium hydroxide (94:5:1) to yield 5.9 g (56%) of **13**: NMR, Table I; FDMS m/e 776 (M$^+$). Anal. ($C_{40}H_{76}N_2O_{12}$) C, H, N.

9-*N*-(1-Butyl)erythromycylamine (14). Procedure 1 was followed with 7.0 g (9.5 mmol) of erythromycylamine and 1.0 g (14.3 mmol) of butyraldehyde; after 19 h, the reaction was not complete (TLC), so additional butyraldehyde (1.0 g, 14.3 mmol) was added. After another 5 h, the reaction was worked up and purified by HPLC as described for **13** to yield 1.3 g (17%) of 9-*N*-(1-butyl)erythromycylamine: FDMS m/e 790 (M$^+$). Anal. ($C_{41}H_{78}N_2O_{12}$) C, H, N.

9-*N*-(3-Phenyl-1-propyl)erythromycylamine (27). Procedure 1 was followed with erythromycylamine (10 g) and 3-phenylpropionaldehyde (2.7 g) for 10 min. The reaction mixture separated into two distinct phases, so the layers were separated; the organic layer was evaporated and the residue was dissolved in dichloromethane, while the aqueous layer was extracted as before. The crude product from the combined organic extracts was purified by preparative HPLC (Waters Prep 500), eluting with a linear gradient of dichloromethane to dichloromethane–methanol–concentrated ammonium hydroxide (87.5:7.5:5) to give 5.0 g (43%) of **27** as a solid white foam: ^{1}H NMR δ 7.18–7.24 (m, 5 H, phenyl); FDMS m/e 852 (M$^+$). Anal. ($C_{46}H_{80}N_2O_{12}$) C, H, N.

9-*N*-(Cyclohex-3-en-1-ylmethyl)erythromycylamine (28). Procedure 1 was followed with erythromycylamine (10 g) and 1,2,3,6-tetrahydrobenzaldehyde (2.2 g) for 1 h; product was purified by preparative HPLC (Waters Prep 500), eluting with a linear gradient of dichloromethane to dichloromethane–methanol (95:5) to yield 1.6 g (14%) of **28**: ^{1}H NMR δ 5.68 (m, 2 H, olefinic); FDMS m/e 829 (MH$^+$). Anal. ($C_{44}H_{80}N_2O_{12}$) C, H, N.

9-*N*-(2-Methoxyethyl)erythromycylamine (31). Procedure 1 was followed with erythromycylamine (3 g) and a solution which was prepared by stirring methoxyacetaldehyde dimethyl ketal (0.79 mL, 6.2 mmol) in 1 N HCl (6 mL) for 4 h. After mixing, the pH was adjusted to 5.0 (6 N HCl); after 10 min, sodium cyanoborohydride (390 mg) was added and the reaction was stirred for 1 h. Crude product (2.6 g) was separated by flash chromatography on silica gel, eluting with dichloromethane–methanol (24:1), to yield 1.1 g (34%) of **31**: ^{1}H NMR δ 3.28 (s, 3 H, OCH_3); FDMS m/e 793 (MH$^+$). Anal. ($C_{40}H_{76}N_2O_{13}$) C, H, N.

9-*N*-(3-Methoxy-1-propyl)erythromycylamine (32). Procedure 1 was followed with erythromycylamine (10 g) and 3-methoxypropionaldehyde (1.8 g) for 1 h. Product was purified by preparative HPLC (Waters Prep 500), eluting with a linear gradient of dichloromethane to dichloromethane–methanol–concentrated ammonium hydroxide (90.5:7.5:2) to give 2.4 g (22%) of **32**: ^{1}H NMR δ 3.34 (s, 3 H, OCH_3); FDMS m/e 806 (M$^+$). Anal. ($C_{41}H_{78}N_2O_{13}$) C, H, N.

9-*N*-[2-(2-Methoxyethoxy)ethyl]erythromycylamine (33). Dirithromycin (1.0 g, 1.2 mmol), prepared as previously described,[14] was dissolved in acetonitrile (5 mL) and 0.5 M, pH 4.5 potassium phosphate buffer solution (5 mL). The solution was adjusted to pH 5.0 with 6 N HCl and then treated with sodium cyanoborohydride (302 mg, 4.8 mmol). After stirring for 90 h, the crude product (1.5 g) was separated by flash chromatography on silica gel, eluting stepwise with dichloromethane and dichloromethane–methanol (24:1, then 23:2), to yield 450 mg (45%) of **33**: ^{1}H NMR δ 3.40 (s, 3 H, OCH_3); FDMS m/e 837 (MH$^+$). Anal. ($C_{42}H_{80}N_2O_{14}$) C, H, N.

9-*N*-[3-(Methylthio)-1-propyl]erythromycylamine (34). Procedure 1 was followed with erythromycylamine (10 g) and 3-(methylthio)propionaldehyde (2.1 g) for 5 h. The crude product was purified by preparative HPLC (Waters Prep 500), eluting with a linear gradient of dichloromethane to dichloromethane–methanol (9:1), to give 1.5 g (13%) of **34**: ^{1}H NMR δ 2.10 (s, 3 H, SCH_3); FDMS m/e 823 (MH$^+$). Anal. ($C_{41}H_{78}N_2O_{12}S$) C, H, N, S.

9-*N*-(3,7-Dimethyl-7-methoxy-1-octyl)erythromycylamine (36). Procedure 1 was followed with erythromycylamine (5 g) and 7-methoxy-3,7-dimethyloctanal (1.9 g) for 1 h. The product was purified by column chromatography on silica gel, eluting with a gradient of dichloromethane to dichloromethane–methanol (24:1), to yield 1.0 g (16%) of **36**: ^{1}H NMR δ 3.32 (s, 3 H, OCH_3); FDMS m/e 904 (M$^+$). Anal. ($C_{48}H_{92}N_2O_{13}$) C, H, N.

9-*N*-(3-Hydroxy-2,2-dimethyl-1-propyl)erythromycylamine (38). Procedure 1 was followed with erythromycylamine (5 g) and 3-hydroxy-2,2-dimethylpropionaldehyde (1.0 g, 10.2 mmol); after 5 h, the reaction was not complete, so additional sodium cyanoborohydride (10.2 mmol) was added and after another 1 h, additional aldehyde (10.2 mmol). After another 5 h, the product was isolated and crystallized from acetonitrile to yield 864 mg (16%) of **38**: ^{1}H NMR δ 0.84 and 0.92 (s, 3 H, each, 2,2-dimethyl);

(23) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

FDMS m/e 821 (MH^+). Anal. ($C_{42}H_{80}N_2O_{13}$) C, H, N.

Procedure 2: 9-*N*-(*cis*-4-Decen-1-yl)erythromycylamine (22). Procedure 2 followed procedure 1 except that after addition of sodium cyanoborohydride, the pH of the reaction mixture was readjusted from about 6 to 5.0 with 6 N HCl. Reductive alkylation of erythromycylamine (5 g) with *cis*-4-decenal was carried out for 2 h. The product was purified by flash chromatography on basic alumina grade 3, eluting stepwise with dichloromethane–chloroform (100:0, 1:1, 1:3, 0:100; 1 L each); crystallization from acetonitrile; and flash chromatography on silica gel, eluting with a linear gradient of chloroform to chloroform–methanol–concentrated ammonium hydroxide (89.5:10:0.5) followed by additional amounts of the latter solvent, to yield 1.4 g (23%) of **22**: ^1H NMR δ 5.38 (m, 2 H, olefinic); FDMS m/e 873 (MH^+). Anal. ($C_{47}H_{88}N_2O_{12}$) C, H, N.

9-*N*-(*trans*-4-Decen-1-yl)erythromycylamine (23). The procedure for **22** was followed using *trans*-4-decenal to yield 1.1 g (19%) of **23**; ^1H NMR δ 5.38 (m, 2 H, olefinic); FDMS m/e 873 (MH^+). Anal. ($C_{47}H_{88}N_2O_{12}$) C, H, N.

9-*N*-(3-Phenylpropargyl)erythromycylamine (26). The procedure for **22** was followed using 3-phenylpropargyl aldehyde to yield 0.8 g (14%) of **26**: ^1H NMR δ 3.54 and 3.77 (2 H, NCH_2); 7.3–7.4 (5 H, aryl); FDMS m/e 849 (MH^+). Anal. ($C_{46}H_{76}N_2O_{12}$) C, H, N.

9-*N*-(10-Undecen-1-yl)erythromycylamine (24). Procedure 2 was followed with erythromycylamine (5 g) and 10-undecenal for 1.5 h. The product was separated by flash chromatography on basic alumina, eluting with dichloromethane, a linear gradient of dichloromethane to chloroform, and additional chloroform; it was further purified by flash chromatography on silica gel, eluting with a linear gradient of chloroform to chloroform–methanol–concentrated ammonium hydroxide (89.5:10:0.5) followed by additional amounts of the latter solvent, to yield 1.6 g (27%) of **24**: ^1H NMR δ 4.96 and 5.82 (3 H, olefinic); FDMS m/e 886 (M^+). Anal. ($C_{48}H_{90}N_2O_{12}$) C, H, N.

9-*N*-(1-Pentyl)erythromycylamine (15). Procedure 2 was followed with erythromycylamine (5 g) and pentanal for 2 h. The product was purified by crystallization from acetonitrile to yield 1.14 g (21%) of **15**: mp 163 °C, FDMS m/e 804 (M^+). Anal. ($C_{42}H_{80}N_2O_{12}$) C, H, N.

9-*N*-(1-Heptyl)erythromycylamine (19). Procedure 2 was followed with erythromycylamine (5 g) and heptanal for 2 h. Product was purified by flash chromatography on basic alumina, eluting with dichloromethane, a linear gradient of dichloromethane to chloroform, and additional chloroform; it was then crystallized from acetonitrile to yield 0.78 g (14%) of **19**: mp 101 °C; FDMS m/e 833 (MH^+). Anal. ($C_{44}H_{84}N_2O_{12}$) C, H, N.

9-*N*-[2-(2,6,6-Trimethylcyclohex-1-enyl)ethyl]erythromycylamine (29). The procedure for **19** was followed with erythromycylamine (5 g) and 2-(2,6,6-trimethylcyclohex-1-enyl)-acetaldehyde for 2.5 h to yield 0.9 g (16%) of **29**: mp 172–175 °C; FDMS m/e 885 (MH^+). Anal. ($C_{48}H_{88}N_2O_{12}$) C, H, N.

9-*N*-(Cyclooctylmethyl)erythromycylamine (30). The procedure for **19** was followed using cyclooctane carboxaldehyde to yield 1.4 g (24%) of **30**: mp 200 °C; FDMS m/e 858 (M^+). Anal. ($C_{46}H_{86}N_2O_{12}$) C, H, N.

9-*N*-(3-Cyano-1-propyl)erythromycylamine (35). The procedure for **19** was followed using 3-cyanopropionaldehyde to yield 1.0 g (19%) of **35**: mp 135–140 °C; FDMS m/e 801 (MH^+). Anal. ($C_{41}H_{75}N_3O_{12}$) C, H, N.

9-*N*-(4-Amino-1-butyl)erythromycylamine (39). 9-*N*-(3-Cyano-1-propyl)erythromycylamine (500 mg, 0.6 mmol) was dissolved in methanol (50 mL) and hydrogenated at 60 psi over platinum oxide at 40 °C overnight. The catalyst was filtered and the filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography on basic alumina, eluting with a linear gradient from 10% to 20% dichloromethane–methanol, followed by a second flash chromatography on silica gel, eluting with a gradient of chloroform to chloroform–methanol–concentrated ammonium hydroxide (89.5:10:0.5) to yield 98 mg (20%) of **39**: FDMS m/e 806 (MH^+). Anal. ($C_{41}H_{79}N_3O_{12}$) C, H, N.

9-*N*-(5-Hydroxy-1-pentyl)erythromycylamine (37). Procedure 2 was followed with erythromycylamine (5 g) and 5-hydroxypentanal for 2 h. The product was separated by flash chromatography on basic alumina, eluting stepwise with dichloromethane, dichloromethane–chloroform (1:1, then 1:3), chloroform, and finally chloroform–methanol (99:1). The product was further purified by flash chromatography on silica gel, eluting with chloroform (250 mL) and then a linear gradient of chloroform to chloroform–methanol–concentrated ammonium hydroxide (89.5:10:0.5, 1.5 L) followed by 1 L of the latter solvent. Crystallization from acetonitrile yielded 0.80 g (14%) of **37**: mp 145 °C; FDMS m/e 821 (MH^+). Anal. ($C_{42}H_{80}N_2O_{13}$) C, H, N.

Procedure 3: 9-*N*-(3-Methyl-1-butyl)erythromycylamine (16). Procedure 3 followed procedure 2 except that two mol equiv of sodium cyanoborohydride were used rather than 1.5 mol equiv. Procedure 3 was followed with erythromycylamine (5 g) and 3-methylbutanal for 1.5 h. The product was separated by flash chromatography on silica gel, eluting first with chloroform and then a linear gradient of chloroform to chloroform–methanol–concentrated ammonium hydroxide (93.5:6:0.5) followed by additional amounts of the latter solvent. The product was further purified by flash chromatography on basic alumina, eluting with chloroform, to yield 2.1 g (38%) of **16**: FDMS m/e 805 (MH^+). Anal. ($C_{42}H_{80}N_2O_{12}$) C, H, N.

9-*N*-(1-Hexyl)erythromycylamine (17). Procedure 3 was followed with erythromycylamine (5.0 g) and hexanal for 45 min. The product was purified by preparative HPLC (Waters Prep 500), eluting with a linear gradient of hexane to ethyl acetate containing 1% triethylamine, followed by additional amounts of the latter solvent; it was then crystallized from chloroform–hexane to yield 1.33 g (24%) of **17**: mp 98 °C; FDMS m/e 819 (MH^+). Anal. ($C_{43}H_{82}N_2O_{12}$) C, H, N.

9-*N*-(2-Ethyl-1-butyl)erythromycylamine (18). Procedure 3 was followed with erythromycylamine (5 g) and 2-ethylbutanal for 1.25 h. The crude product was triturated with hexane and the finely divided powder was filtered. The precipitate was purified by flash chromatography on alumina, eluting with dichloromethane and then chloroform. The product was crystallized from chloroform–hexane to give 2.8 g (50%) of **18**: mp 190 °C; FDMS m/e 819 (MH^+). Anal. ($C_{43}H_{82}N_2O_{12}$) C, H, N.

9-*N*-(1-Decyl)erythromycylamine (21). Procedure 3 was followed with erythromycylamine (5 g) and decanal for 1 h. The product was separated by flash chromatography on silica gel, eluting first with chloroform and then a linear gradient of chloroform to chloroform–methanol–concentrated ammonium hydroxide (91.5:8:0.5) followed by additional amounts of the latter solvent to yield 1.3 g (22%) of **21**: FDMS m/e 875 (MH^+). Anal. ($C_{47}H_{90}N_2O_{12}$) C, H, N.

9-*N*-(1-Dodecyl)erythromycylamine (25). Procedure 3 was followed with erythromycylamine (5 g) and dodecanal for 1.5 h. The product was separated by flash chromatography on basic alumina, eluting with dichloromethane, a linear gradient of dichloromethane to chloroform, and additional chloroform. It was further purified by flash chromatography on silica gel, eluting first with chloroform and then a linear gradient of chloroform to chloroform–methanol–concentrated ammonium hydroxide (93.5:6:0.5) followed by additional amounts of the latter solvent, to yield 2.0 g (34%) of **25** as a white solid foam: FDMS m/e 903 (MH^+). Anal. ($C_{49}H_{94}N_2O_{12}$) C, H, N.

Procedure 4: 9-*N*-(1-Octyl)erythromycylamine (20). Procedure 3 was followed, with the exception that the pH was not readjusted after addition of sodium cyanoborohydride, using erythromycylamine (5 g) and octanal for 1.5 h. The product was purified by preparative HPLC (Waters Prep 500), eluting with a linear gradient of dichloromethane to dichloromethane–methanol–concentrated ammonium hydroxide (90.5:7.5:2). It was further purified by flash chromatography on basic alumina, eluting with chloroform to yield 0.63 g (11%) of **20** as a white amorphous solid: FDMS m/e 847 (MH^+). Anal. ($C_{45}H_{86}N_2O_{12}$) C, H, N.

9-*N*-Methylerythromycylamine (11) and 9-*N*,*N*-Dimethylerythromycylamine (51). Erythromycylamine (5.0 g, 6.8 mmol) was dissolved in acetonitrile (20 mL) and 0.5 M sodium phosphate buffer (pH 4.5, 20 mL) and then treated with 37% aqueous formaldehyde (6.8 mmol). The solution was adjusted to pH 5.0 with 1 N HCl and treated with sodium cyanoborohydride (640 mg, 10.2 mmol) for 30 min. After workup, the crude product was separated by flash chromatography on silica gel, eluting stepwise with dichloromethane and dichloromethane–methanol (24:1, then 23:2) to yield 1.97 g of material which contained the two products. The individual components were isolated by re-

versed-phase HPLC (Waters Prep 500), eluting with a linear gradient of aqueous triethylamine–phosphoric acid buffer (pH 3.0) to a 1:3 solution of acetonitrile–[aqueous triethylamine–phosphoric acid buffer (pH 3.0)], to yield 290 mg of 9-*N*-methylerythromycylamine (**11**) [^{1}H NMR δ 2.10 (s, 3 H, $NHCH_3$); FDMS *m/e* 749 (MH^+). Anal. ($C_{38}H_{72}N_2O_{12}$) C, H, N.] and 280 mg of 9-*N*,*N*-dimethylerythromycylamine (**51**) [^{1}H NMR δ 2.43 [s, 6 H, $N(CH_3)_2$]; FDMS *m/e* 763 (MH^+). Anal. ($C_{39}H_{74}N_2O_{12}$) C, H, N.].

9-*N*-(2-Propyl)-9-*N*,11-*O*-methyleneerythromycylamine (46). 9-*N*-(2-Propyl)erythromycylamine[13] (2.7 g, 3.5 mmol) was dissolved in acetonitrile (25 mL) with warming and then treated with 37% aqueous formaldehyde (1.8 mL). When conversion appeared to be complete after 2 h (TLC), solvent was evaporated under reduced pressure, and the residue was dissolved in ether; the solution was extracted with saturated sodium chloride solution, dried, filtered, and evaporated to yield 2.3 g (83%) of **46** as a white, solid foam: ^{1}H NMR δ 4.62 (2 H, NCH_2O); FDMS *m/e* 788 (M^+). Anal. ($C_{41}H_{76}N_2O_{12}$) C, H, N.

9-*N*-(1-Propyl)-9-*N*,11-*O*-methyleneerythromycylamine (45). 9-*N*-(1-Propyl)erythromycylamine (1.0 g, 1.3 mmol) was treated with formaldehyde (4 equiv) as described above; TLC analysis showed no change, but after workup of the reaction, it was determined that the starting material and product had coincidental R_f values. The product contained residual formaldehyde, so it was redissolved in dichloromethane, extracted with saturated sodium bicarbonate solution, dried, filtered, and evaporated, and this process was repeated again to yield 680 mg (67%) of **45**: FDMS *m/e* 788 (M^+). Anal. ($C_{41}H_{76}N_2O_{12}$) C, H, N.

9-*N*-Cyclopentyl-9-*N*,11-*O*-methyleneerythromycylamine (47). 9-*N*-Cyclopentylerythromycylamine[13] (1.3 g, 1.6 mmol) was dissolved in ethanol (10 mL) and treated with 37% aqueous formaldehyde (0.5 mL) at room temperature; additional formaldehyde was added after 24 and 48 h since the reaction was incomplete (TLC). Solvent was evaporated under reduced pressure and the residue was dissolved in ether, extracted with water, dried, filtered, and evaporated to yield 1.1 g (85%) of **47**: ^{1}H NMR δ 4.64 (2 H, NCH_2O); FDMS *m/e* 814 (M^+). Anal. ($C_{43}H_{78}N_2O_{12}$) C, H, N.

9-*N*-[5-(Dimethylamino)-2-pentyl]-9-*N*,11-*O*-methyleneerythromycylamine (48). 9-*N*-[5-(Dimethylamino)-2-pentyl]-erythromycylamine, prepared by following literature procedures[13] (1.0 g, 1.1 mmol), was dissolved in acetonitrile (5 mL) and treated with 37% aqueous formaldehyde (0.5 mL) for 3 days at room temperature. After evaporation of the solvent, the residue was dissolved in dichloromethane, and the solution was extracted with sodium bicarbonate solution, dried, filtered, and evaporated. The crude product was purified by chromatography (Chromatotron), loading in ethyl acetate solution and eluting stepwise with ethyl acetate–methanol mixtures (9:1, 83:17, and 4:1) to give 50 mg (5%) of **48** as a white, solid foam: ^{1}H NMR δ 4.59 (2 H, NCH_2O); FDMS *m/e* 887 (M^+). Anal. ($C_{47}H_{89}N_3O_{12}$) C, H, N.

9-*N*-Benzyl-9-*N*,11-*O*-methyleneerythromycylamine (49). 9-*N*-Benzylerythromycylamine[13] (1.0 g, 1.2 mmole) was dissolved in acetonitrile and treated with 37% aqueous formaldehyde at room temperature for 4.25 h. The white precipitate was collected, washed with acetonitrile, and dried in vacuo to give 220 mg (22%) of **49**: ^{1}H NMR δ 4.36 (2 H, NCH_2O); FDMS *m/e* 836 (M^+). Anal. ($C_{45}H_{76}N_2O_{12}$) C, H, N.

9-*N*-Propyl-9-*N*,11-*O*-ethylideneerythromycylamine (50). 9-*N*-Propylerythromycylamine (1.0 g, 1.3 mmol) was dissolved in acetonitrile and treated with acetaldehyde (0.15 mL, 2.6 mmol) at room temperature; after being stirred overnight, no evidence of reaction was observed, so additional acetaldehyde (0.15 mL) was added. After 2 h, a white precipitate formed, which was filtered to yield 140 mg (11%) of **50**: FDMS *m/e* 802 (M^+). Anal. ($C_{42}H_{78}N_2O_{12}$) H, N; C: calcd, 62.82; found, 61.46.

9-*N*-Methyl-9-*N*-(2-propyl)erythromycylamine (52). 9-*N*-(2-Propyl)-9-*N*,11-*O*-methyleneerythromycylamine (600 mg, 0.76 mmol) was dissolved in acetonitrile (4 mL) and 0.1 M, pH 5.5 potassium phosphate buffer solution (4 mL); the solution was adjusted to pH 6.0 with 1 N HCl and then treated with sodium cyanoborohydride (48 mg, 0.76 mmol) with stirring at room temperature. After 22 h, conversion was incomplete (TLC), so additional sodium cyanoborohydride (24 mg, 0.38 mmol) was added. After an additional 18 h, 1 N sodium hydroxide was added until precipitation was complete; solvent was evaporated, and the residue was partitioned between dichloromethane and saturated sodium bicarbonate. The organic layer was separated, dried, filtered, and evaporated and the residue (500 mg) was purified by flash chromatography on silica gel, eluting stepwise with dichloromethane and dichloromethane–methanol (9:1, then 4:1) to yield 100 mg (17%) of **52**: ^{1}H NMR δ 2.42 (s, 3 H, NCH_3); FDMS *m/e* 790 (M^+).

9-*N*-Methyl-9-*N*-cyclohexylerythromycylamine (53). 9-*N*-Cyclohexyl-9-*N*,11-*O*-methyleneerythromycylamine (2.3 g, 2.8 mmol) was dissolved in acetonitrile (12 mL) and 0.1 M, pH 5.5 potassium phosphate buffer solution (12 mL); the solution was adjusted to pH 6.0 with 1 N HCl and then treated with sodium cyanoborohydride (265 mg, 4.2 mmol) with stirring at room temperature for 1.5 h. After workup as for **52**, 2.1 g (90%) of **53** was obtained: FDMS *m/e* 830 (M^+). Anal. ($C_{44}H_{82}N_2O_{12}$) C, H, N.

9-Deoxo-9(*S*)-*N*-piperidinylerythromycin (54). Erythromycylamine (10.0 g, 13.6 mmol) was dissolved in acetonitrile (50 mL) and 0.1 M, pH 6.5 sodium phosphate buffer (50 mL) with warming. The solution was adjusted to pH 6.0 with 6 N HCl and then treated with glutaraldehyde (4.2 mL, 20.4 mmol). After stirring at room temperature for 3 h, sodium cyanoborohydride (1.3 g, 20.4 mmol) was added in four portions. After stirring for 1 h, solvent was evaporated under reduced pressure and the residue was partioned between ether and saturated sodium bicarbonate. The organic layer was separated, dried, filtered, and evaporated to give crude product (6.3 g); a portion (1.0 g) was purified by reversed-phase HPLC (Waters Prep 500), eluting with a linear gradient of 0.5% aqueous triethylamine to acetonitrile–0.5% aqueous triethylamine (3:7) to yield 300 mg of **54** as a white, solid foam: ^{1}H NMR δ 2.64 and 2.80 (m, 4 H, NCH_2); FDMS *m/e* 802 (M^+). Anal. ($C_{42}H_{78}N_2O_{12}$) C, H, N.

9-*N*-(1-Propyl)erythromycylamine (13) via Hydrogenation Methods. Pd/C (5%, 3.5 g) was added to a 1-L stainless steel hydrogenation vessel purged with nitrogen, and THF (35 mL) was added to wet the catalyst. Erythromycylamine (35.0 g, 47.6 mmol), propionaldehyde (5.2 mL, 72.1 mmol), methanol (220 mL), and THF (170 mL) were added, and the mixture was hydrogenated at 60 psi and 60 °C for 12–16 h. The reactor was then cooled, vented, and purged with nitrogen. The slurry was filtered over filter aid (Hyflo) and the catalyst was washed with THF (50 mL). The light green to colorless filtrate was evaporated under vacuum and the residue was dissolved in ethyl acetate (350 mL). The resultant solution was extracted twice with 0.5 M, pH 6.5 sodium phosphate buffer solution (350 mL). The combined aqueous extracts were adjusted to pH 7.0 with 5 N sodium hydroxide, and the product was extracted twice into dichloromethane (350 mL). The combined organic extracts were dried (sodium sulfate) and then filtered, and the filtrate was evaporated under vacuum. The residue was dissolved in acetone (210 mL) and the solution was warmed to 50 °C. Water (210 mL) was slowly added to the warm solution; after half of the water had been added, the solution was seeded. The slurry was cooled to 0–5 °C, filtered, and washed with acetone–water (1:1) to yield 9-*N*-(1-propyl)erythromycylamine (23.7 g, 64%). A second crystallization (20 g) from acetone–water (1:1) yielded 18.2 g (91%) of **13**, 96.7% pure by HPLC: mp 123–126 °C. Anal. ($C_{40}H_{76}N_2O_{12}$) C, H, N.

Acknowledgment. We thank J. L. Occolowitz and associates for mass spectra; T. K. Elzey for NMR assistance; L. L. Huckstep and R. J. Thomas for preparative HPLC separations; H. Michael, M. D. Newport, P. Lubbehusen, L. J. Thomas, P. A. Tarter, and M. E. Johnson for technical assistance in evaluation experiments; and Dr. G. M. Wild for a sample of erythromycin B.

Supplementary Material Available: Tables I–V listing atomic coordinates and equivalent isotropic displacement parameters, bond lengths, bond angles, anisotropic displacement parameters, and hydrogen atom coordinates and isotropic displacement parameters, respectively, and Figures 1 and 2 showing the X-ray structure of **18** and its stereoview (10 pages). Ordering information is given on any current masthead page.