selective recognition of (R)-PIA at the A_1AR. The N^6-substituted 2-chloroadenosines tend to be better stimulants of the adenylate cyclase of PC12 cell membranes than the corresponding deschloro adenosines.

Experimental Section

Melting points were measured on a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra of solutions of nucleosides in DMSO-d_6 obtained on a Varian EM 360L spectrograph were consistent with the assigned structures. M-H-W Laboratories, Tucson, AZ, performed the elemental analyses, which agreed to within ±0.4% of theoretical composition. Assays of purity by reverse-phase HPLC revealed that product accounted for >99% of the UV-absorbing material in samples submitted for assay.

2-Chloro-N^6-cyclopentyladenosine (2b). A mixture of 2,6-dichloro-9-(2,3,5-O-triacetyl-β-D-ribofuranosyl)purine (2.0 g, 4.5 mmol), cyclopentylamine (0.77 g, 9.0 mmol), N,N-diisopropylethylamine (1.6 mL, 9.2 mmol), and 70 mL of 100% ethanol was refluxed for 24 h. The resulting solution was cooled to 5–10 °C in an ice bath and saturated with dry ammonia. The solution was stirred at room temperature for 5 days. Evaporating the solvents in vacuo yielded a syrup, which was purified according to Table I.

Assays of Receptor Binding and Adenylate Cyclase. Inhibition of the binding of [^{3}H]-N^6-(1-phenyl-2(R)-propyl)adenosine ((R)-PIA) to the A_1AR in rat cerebral cortex membranes and of [^{3}H]-N-ethyladenosine-5'-uronamide (NECA) to rat striatal membranes were assayed as described.[11,14] Both assays employed binding in the presence of 5 mM theophylline to define unspecific binding, and in the assays of binding to the A_2AR, 50 nM CPA was present to block binding to the A_1AR. Calculations of K_i from measurements of IC_{50} employed the Cheng–Prusoff equation.[15] Previously described assays[16,17] measured A_2AR-mediated stimulation of the adenylate cyclase in membranes from PC12 rat pheochromocytoma cells.

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Registry No. **1b**, 41552-82-3; **1c**, 23589-16-4; **1d**, 38594-96-6; **1e**, 38594-97-7; **2a**, 146-77-0; **2b**, 37739-05-2; **2c**, 29204-70-4; **2d**, 23558-58-9; **2e**, 23559-45-7; 6-chloropurine riboside, 5399-87-1; 9-(2',3',5'-O-triacetyl-β-D-ribofuranosyl)purine, 3056-18-6; cyclopentylamine, 1003-03-8; (R)-1-phenyl-2-propylamine, 156-34-3; (S)-1-phenyl-2-propylamine, 51-64-9; adenylate cyclase, 9012-42-4.

(14) Jacobson, K. A.; Ukena, D.; Kirk, K. L.; Daly, J. W. [^{3}H]-Xanthine amine congener of 1,3-dipropyl-8-phenylxanthine: An antagonist radioligand for adenosine receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4089–4095.

(15) Cheng, Y. C.; Prusoff, W. H. Relationship between the inhibition constant (K_I) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

(16) Ukena, D.; Olsson, R. A.; Daly, J. W. Definition of subclasses of adenosine receptors associated with adenylate cyclase: interaction of adenosine analogs with inhibitory A_1 and stimulatory A_2 receptors. *Can. J. Physiol. Pharmacol.* **1987**, *65*, 365–376.

(17) Ukena, D., Daly, J. W.; Kirk, K. L.; Jacobson, K. A. Functionalized congeners of 1,3-dipropyl-8-phenylxanthine: potent antagonists for adenosine receptors that modulate membrane adenylate cyclase in pheochromocytoma cells, platelets and fat cells. *Life Sci.* **1985**, *38*, 797–807.

(18) Ukena, D.; Jacobson, K. A.; Padgett, W. L.; Ayala, C.; Shamim, M. T.; Kirk, K. L.; Olsson, R. A.; Daly, J. W. Species differences in structure–activity relationships of adenosine agonists and xanthine antagonists at brain A_1 adenosine receptors. *FEBS Lett.* **1986**, *209*, 122–128.

Synthesis and Antibacterial Activities of C-21 Functionalized Derivatives of (9R)-9-Amino-9-deoxoerythromycins A and B

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Selective protection of (9R)-9-amino-9-deoxoerythromycin A allowed for elimination of the 12-hydroxyl group to afford a versatile 12,21-olefin intermediate. Further modifications of the intermediate led to the syntheses of (9R)-9-deoxo-9-(N,N-dimethylamino)-12,21-epoxyerythromycin B, (9R)-9-deoxo-9-(N,N-dimethylamino)-21-hydroxyerythromycin A, and (9R)-9-deoxo-9-(N,N-dimethylamino)-21-hydroxyerythromycin B. All three compounds retained antibacterial activity against several organisms normally susceptible to (9R)-9-deoxo-9-(N,N-dimethylamino)erythromycin A. However, the 21-hydroxylated erythromycin A analogue was weaker in potency than the corresponding erythromycin B congener and much weaker than the epoxy derivative. This suggests that while substitution of a polar functionality at C-21 does not abolish antibacterial activity, introduction of vicinal polar groups at both C-12 and C-21 may lead to reduction in potency. Nevertheless, these 21-functionalized derivatives of (9R)-erythromycylamine provide an entry into novel analogues of the important macrolide antibiotic erythromycin.

Introduction

The macrolide antibiotic erythromycin A (1) has enjoyed successful clinical use for over 35 years. This longevity is due to its proven efficacy in Gram-positive infections and infections caused by organisms of emerging importance, such as *Legionella* and *Chlamydia*,[1] while showing a relative lack of toxicity. The success of 1 has led to several synthetic modifications aimed at improving its activity, antibacterial spectrum, and pharmacokinetics or at exploring its structure–activity relationships.[2] One such

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(1) Malmborg, A. S. The Renaissance of Erythromycin. *J. Antimicrob. Chemother.* **1986**, *18*, 293–299.

(2) Sakakibara, H.; Omura, S. Chemical Modification and Structure Activity Relationship of Macrolides. In *Macrolide Antibiotics Chemistry, Biology, and Practice*; Omura, S., Ed.; Academic Press: Orlando, FL, 1984; p 85.

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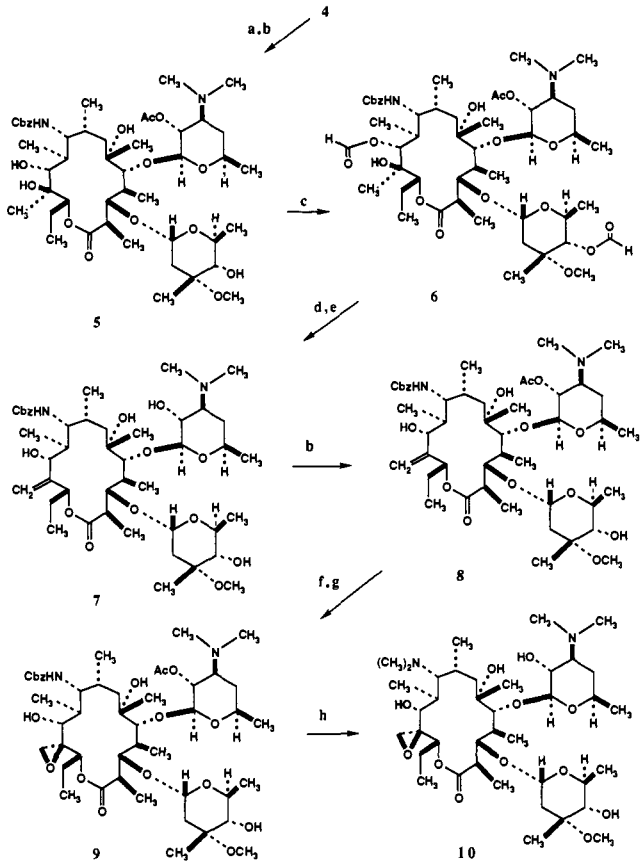
Scheme I

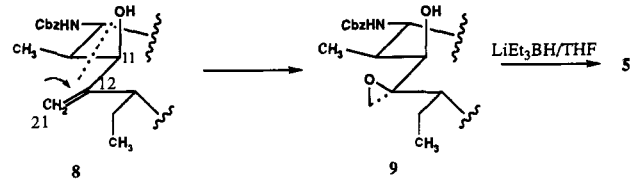


[a] (a) Cbz-NOS/CH_2Cl_2; (b) Ac_2O/CH_2Cl_2; (c) formic–acetic anhydride/DMAP/CH_2Cl_2; (d) $Et_3N/SOCl_2$/EtOAc; (e) MeOH/Et_3N; (f) MCPBA/CH_2Cl_2; (g) TPP/CH_2Cl_2; (h) H_2/Pd–C/HCHO/MeOH.

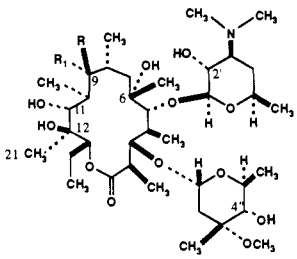
effort has been reported by Hauske,[3] in which the preparation of a 9,11-thionocarbonate derivative[4] of 9-dihydroerythromycin A (2) allowed for regioselective dehydration at C-12 and hence synthetic access to the relatively hindered and hitherto unexplored C-12,21 positions of 2.

Recent work reported from our laboratory[5,6] shows that replacement of the C-9 ketone of 1 with a (9*R*)-dimethylamino group (3) or a cyclic amino group in the *R* configuration leads to compounds which, unlike 2 or the analogous 9*S* congeners,[7-9] possess antibacterial activities

Scheme II



comparable to 1. The purpose of the present work was to explore the effects of C-12,21 modifications on the antibacterial activities of 3 and other (9*R*)-alkylamino derivatives. Hence, the objective was to synthesize a 12,21-didehydro intermediate, convert it to C-21 functionalized derivatives, and determine their antibacterial activities. In this paper, we report the synthesis and antibacterial activities of the 12,21-epoxide 10 and the C-21 hydroxyl derivatives 12 and 13.



1 R = R_1 = O

2 R = OH R_1 = H

3 R = H R_1 = $N(CH_3)_2$

4 R = H R_1 = NH_2

Results and Discussion

Chemistry. The synthetic targets were selected to allow for flexibility in substitutions on the 9-amino group (although in this paper only compounds with dimethyl substituents are reported) and ease of protecting and deprotecting the 4″- and 11-hydroxyl groups. Thus, as shown in Scheme I, the amino group of (9*R*)-erythromycylamine (4) was protected with a carbobenzyloxy group by treatment with *N*-[(benzyloxycarbonyl)oxy]succinimide. Subsequent selective acylation[10] of the 2′-hydroxyl group, using acetic anhydride, afforded 5. Treatment of 5 with formic–acetic anhydride resulted in selective formylation[11] of the 4″- and 11-hydroxyl groups. It was necessary to treat the crude reaction mixture with pH 5 buffer prior to attempted chromatography with the solvent system de-

(3) Hauske, J. R.; Guadliana, M.; Kostek, G.; Schulte, G. Aglycon Modifications of Erythromycin A: Regiospecific and Stereospecific Elaboration of the C-12 Position. *J. Org. Chem.* **1987**, *52*, 4622–4625.

(4) Hauske, J. R.; Guadliana, M.; Kostek, G. Aglycon Modifications of Erythromycin A and Erythromycin B: Regiospecific Nucleophilic Ring Opening of Cyclic Thionocarbonates. *J. Org. Chem.* **1983**, *48*, 5138–5140.

(5) Maring, C. J.; Klein, L. L.; Pariza, R. J.; Lartey, P. A.; Grampovnik, D. J.; Yeung, C. M.; Buytendorp, M.; Hardy, D. J. Synthesis and SAR of Derivatives of 9(R)-Erythromycylamine. *Program and Abstracts of the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Houston, TX, 1989; American Society for Microbiology: Washington, DC, 1989; p 1023.

(6) Hardy, D. J.; Vojtko, C.; Beyer, J. M.; Hensey, D. M.; Maring, C. J.; Klein, L. L.; Pariza, R.; Lartey, P. A.; Clement, J. J. In Vitro Evaluation of a New Series of (9R)-Amino-9-Deoxoerythromycin Analogs. *Program and Abstracts of the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy*; Houston, TX, 1989; American Society for Microbiology: Washington, DC, 1989; p 1024.

(7) Wilhelm, J. M.; Oleinick, N. L.; Corcoran, J. W. Interaction of Antibiotics with Ribosomes: Structure–Function Relationships and Possible Common Mechanism for the Antibacterial Action of the Macrolides and Lincomycin. In *Antimicrobial Agents and Chemotherapy—1967*; Hobby, G. L., Ed.; American Society for Microbiology: Ann Arbor, MI, 1968; p 236.

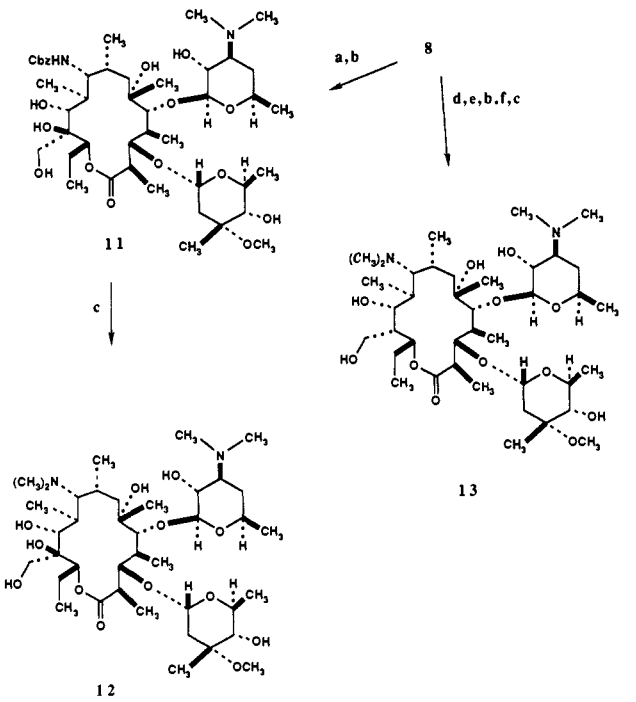
(8) Wiley, P. F.; Gerzon, K.; Flynn, E. H.; Sigal, M. V. Jr.; Weaver, O.; Quarck, U. C.; Chauvette, R. R.; Monahan, R. Erythromycin. X. Structure of Erythromycin. *J. Am. Chem. Soc.* **1957**, *79*, 6062–6070.

(9) Wetzel, L. O.; Woitun, E.; Maier, R.; Reuter, W.; Goeth, H.; Lechner, U. U.S. Patent 4 016 263, 1977.

(10) Stephens, V. C. Esters of Erythromycin II. Derivatives of Cyclic Anhydrides. In *Antiobiotics Annual 1953–1954*; Welch, H., Marti-Ibanex, F., Eds.; Medical Encyclopedia: New York, 1953; p 514.

(11) Jones, P. H.; Perun, T. J.; Rowley, E. K.; Baker, E. J. Chemical Modifications of Erythromycin Antibiotics. 3. Synthesis of 4″ and 11 Esters of Erythromycin A and B. *J. Med. Chem.* **1972**, *15*, 631–634.

Scheme III



[a] (a) OsO_4/THF; (b) MeOH reflux; (c) H_2/Pd–C/HCHO/MeOH; (d) BH_3–THF/THF; (e) H_2O_2/NaOH/THF/H_2O; (f) H_2/Pd–C/MeOH.

scribed below, since failure to do so resulted in the isolation of copious amounts of the cyclic 4″-formyl-11,12-methyl-orthoformate derivative[12] of **5**, presumably arising from silica gel catalyzed esterification of an intermediate cyclic orthoformate.

Unlike the 9,11-thionocarbonate[3] of **2**, treatment of **6** with thionyl chloride resulted in elimination at both C-12 and C-6, presumably because the stereochemical orientation of the (9*R*)-(carbobenzyloxy)amino group did not offer as much steric encumbrance to the C-6 position as a (9*S*)-9,11-thionocarbonate.[4] However, the elimination was still selective for the C-12 position, and the products could be conveniently separated, after removal of the ester protecting groups, to provide a good yield of the 12,21-didehydro derivative **7**. For purposes of further characterization and utility, the 2′-OH of compound **7** was selectively reacetylated to afford **8**.

Epoxidation of **8** with *m*-chloroperoxybenzoic acid (MCPBA) and subsequent reduction of the resulting 3′-*N*-oxide with triphenylphosphine proceeded smoothly to afford the 12,21-epoxide **9**. The MCPBA epoxidation was directed as expected,[13] both in terms of reactivity and stereoselectivity, by the allylic 11-hydroxyl group, as a similar reaction with the 11,4″-di-*O*-formyl derivative of **8** resulted only in oxidation of the 3′-dimethylamino group but failed to yield 12,21-epoxidation products. Further, only one diastereomeric epoxide could be isolated, which when treated with lithium triethylborohydride led to epoxide opening to afford **5**. These results are consistent with directed epoxidation (Scheme II) to retain the natural stereochemistry of erythromycin A at C-12. Hydrogenolysis of **9** in the presence of formalin and in methanol led to simultaneous removal of the carbobenzyloxy group, dimethylation of the 9-amine, and removal of the 2′-*O*-acetate to provide **10**.

Similarly, osmylation of **8** (Scheme III) proceeded smoothly to give a single 12,21-diol, which was not fully characterized at this stage but was treated with refluxing methanol to remove the 2′-*O*-acetate. The resulting mixture was more amenable to purification and provided the desired intermediate **11**. Subsequent hydrogenolysis with concomitant N-dimethylation, as described for **10**, led to **12**.

On the other hand, hydroboration of **8** using borane–THF[14] was sluggish. A large excess (10-fold) of reagent had to be used to drive the reaction to completion. This in turn led to a mixture of products which were difficult to purify to homogeneity, although the major component (characterized by MS and ^{1}H NMR) was the desired product. Other methods were not explored at this stage; instead, the partially purified intermediate was carried through subsequent synthetic steps to provide the desired 21-hydroxyerythromycin B derivative **13**, which could be purified to homogeneity at the end of the synthetic sequence (Scheme III). A major side reaction of the hydroboration step was the reduction of the lactone of the macrolide ring system, leading to the isolation[15] of the

Table I. Antibacterial Activities of C-21 Functionalized Derivatives of (9*R*)-9-Amino-9-deoxoerythromycins A and B

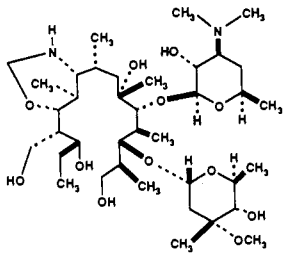
organism	strain	minimum inhibitory concentration (μg/mL) 3	10	12	13
Streptococcus pyogenes	EES61	0.05	0.05	1.56	0.39
Streptococcus bovis	A5169	0.1	0.02	3.1	0.78
Streptococcus agalactiae	CMX 508	0.1	0.05	3.1	0.78
Streptococcus pyogenes	930 CONST	>100	>100	>100	>100
Streptococcus pyogenes	2548 INDUC	100	6.2	100	50
Staphylococcus aureus	ATCC 6538P	0.39	0.78	25	6.2
Staphylococcus aureus	45	0.78	0.78	25	6.2
Staphylococcus aureus	CMX 553	0.78	0.78	25	6.2
Staphylococcus epidermidis	3519	0.39	0.39	25	6.2
Micrococcus luteus	ATCC 9341	0.1	0.1	6.2	0.78
Micrococcus luteus	ATCC 4698	0.1	0.1	3.1	0.78
Enterococcus faecium	ATCC 8043	0.2	0.02	12.5	1.56
Escherichia coli	JUHL	50	100	>100	>100
Escherichia coli	SS	0.1	0.39	1.56	0.39
Escherichia coli	DC-2	25	50	50	50
Escherichia coli	H560	12.5	25	25	25
Escherichia coli	KNK 437	50	100	>100	100
Enterobacter aerogenes	ATCC 13048	50	>100	>100	100
Klebsiella pneumoniae	ATCC 8045	25	100	>100	50
Pseudomonas aeruginosa	K799/WT	>100	>100	>100	50
Pseudomonas aeruginosa	K799/61	12.5	12.5	50	1.56
Pseudomonas cepacia	296I	>100	>100	>100	100
Acinetobacter calcoaceticus	CMX 669	12.5	12.5	>100	3.1

(12) ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3 H), 0.96 (d, 6 H), 1.08 (d, 3 H), 1.11 (m, 1 H), 1.16 (s, 3 H), 1.19 (d, 3 H), 1.21 (d, 3 H), 1.25 (s, 3 H), 1.30 (m, 1 H), 1.32 (m, 1 H), 1.48 (m, 1 H), 1.55 (m, 1 H), 1.65 (m, 2 H), 1.71 (m, 1 H), 1.93 (m, 1 H), 2.04 (s, 3 H), 2.26 (s, 6 H), 2.42 (d, 1 H), 2.75 (m, 2 H), 3.33 (s, 3 H), 3.34 (s, 3 H), 3.52 (m, 2 H), 3.72 (t, 1 H), 4.18 (d, 1 H), 4.33 (m, 2 H), 4.52 (d, 1 H), 4.75 (m, 3 H), 4.82 (d, 1 H), 5.12 (m, 4 H), 5.67 (s, 1 H), 7.28–7.39 (m, 5 H), 8.22 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 9.49, 10.49, 12.40, 13.32, 15.96, 18.46, 20.03, 21.33, 21.54, 22.13, 28.42, 28.50, 35.12, 36.72, 40.29, 40.37, 45.55, 49.38, 53.21, 60.84, 65.58, 65.67, 66.56, 69.09, 70.80, 72.58, 74.62, 77.93, 78.77, 80.10, 81.94, 83.49, 84.57, 84.62, 96.77, 103.56, 117.48, 127.89, 127.93, 128.46, 136.89, 156.93, 175.91; MS *m/e* 891 (M^+).

(13) Henbest, H. B.; Wilson, R. A. L. Aspects of Stereochemistry. Part I. Stereospecificity in Formation of Epoxides from Cyclic Allylic Alcohols. *J. Chem. Soc.* **1957**, 1958–1965.

(14) Knights, E. F.; Brown, H. C. Cyclic Hydroboration of 1,5-Cyclooctadiene. A Simple Synthesis of 9-Borabicyclo[3.3.1]-nonane, an Unusually Stable Dialkylborane. *J. Am. Chem. Soc.* **1968**, *90*, 5280–5281.

seco-1,13-diol **14** as the major byproduct in this synthetic route. Exploration of other methods for the synthesis of **13** may be warranted, in view of its interesting antibacterial activity, as discussed below.



14

Antibacterial Activity. The minimum inhibitory concentration (MIC) of each compound was determined (Table I) against a number of bacterial strains by standard agar dilution methods.[16] Modification of the C-21 position of **3** did not lead to abolition of antibacterial activity. The epoxide **10** had MICs and an antibacterial spectrum similar to those of **3**. However, introduction of a hydroxyl group at C-21, as in **12**, led to significant increase in MICs against all organisms tested. The loss of activity may suggest that hydrophilic groups cannot be tolerated at both C-12 and C-21 and that some lipophilic character is required at C-12 when C-21 is substituted with a hydroxyl group, since **13**, which has a C-21 hydroxyl group but lacks a C-12 hydroxyl, was more potent than **12**.

Conclusion

C-21 modified analogues of (9R)-erythromycylamine can be prepared by judicious selection of protecting groups to allow for elimination at C-12. The double bond can be epoxidized, osmylated, and hydroborated, thereby providing flexible synthetic access to the C-12,21 positions. Introduction of a polar functionality such as a hydroxyl group at C-21, while resulting in some loss of antibacterial potency, does not lead to a total loss of antibacterial activity or significant changes in the antibacterial spectrum. Hence, the C-21 position may provide another synthetic handle for modulating other parameters pertinent to efficacy, such as physicochemical properties or pharmacokinetics. The intermediates described in this paper, such as the protected C-12,21-epoxide and 21-hydroxyl derivatives, will be useful in further synthetic modifications to explore the structure–activity relationship of the macrolide class of antibacterial agents.

Experimental Section

NMR spectra were recorded at 300 MHz for ^1H and at 75.48 MHz for ^{13}C on a GE-Nicolet QE-300 spectrometer. Chemical shifts were measured as ppm from TMS as internal standard, and coupling constants were measured in hertz. Mass spectra were recorded on a Kratos MS 50 spectrometer. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. All analytical TLC and column chromatography were performed on E. Merck silica gel plates and silica gel 60 (230–400 mesh), respectively, from EM Science. Compound **4** was prepared as previously described.[5,9]

(9R)-2′-O-Acetyl-9-[N-(carbobenzyloxy)amino]-9-deoxoerythromycin A (5). *N*-[(Benzyloxycarbonyl)oxy]succinimide (7.1 g, 28.61 mmol) was added to (9*R*)-erythromycylamine (20.0 g, 27.25 mmol) in 200 mL of CH_2Cl_2 at room temperature. After 1.5 h, the solution congealed into a white mass. The solution was diluted with 200 mL of CH_2Cl_2, and after 4 h, TLC showed the reaction to be complete. Saturated sodium bicarbonate solution (200 mL), CH_2Cl_2 (200 mL), and methanol (100 mL) were added, the mixture was stirred, and insoluble material was filtered off. The filtrate was concentrated, and the remaining aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The extract was washed with brine (100 mL) and dried over $MgSO_4$ and filtered. The filtrate was concentrated, and the residue was chromatographed over silica gel (5% MeOH/0.5% NH_4OH in CH_2Cl_2 followed by 10% MeOH/1% NH_4OH in CH_2Cl_2) to yield 22.4 g (94%) of the desired 9-(carbobenzyloxy)amino intermediate. Acetic anhydride (2.5 mL, 0.027 mol) was added to a solution of a portion of the intermediate (21 g, 0.024 mol) in 400 mL of dry CH_2Cl_2 at room temperature. The reaction was stirred for 4 h, and the CH_2Cl_2 was removed in vacuo. The excess acetic anhydride was removed on the high-vacuum pump to give 19.0 g (87%) of **5**: mp 149–151 °C; $[\alpha]_D$ −49.6° (c 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.78 (t, *J* = 7.5, 3 H), 0.9–1.05 (m, 9 H), 1.11 (bs, 3 H), 1.19 (d, *J* = 7.5, 3 H), 1.22 (d, *J* = 6, 3 H), 1.26–1.35 (m, 10 H), 1.40–1.51 (m, 3 H), 1.62 (dd, *J* = 15, *J* = 5, 1 H), 1.73 (m, 1 H), 1.88 (bs, 1 H), 1.95 (m, 1 H), 2.03 (s, 3 H), 2.10–2.22 (m, 2 H), 2.26 (s, 6 H), 2.38 (d, *J* = 15, 1 H), 2.60–2.81 (m, 3 H), 3.06 (t, *J* = 9.5, 1 H), 3.33 (m, 1 H), 3.36 (s, 3 H), 3.50–3.60 (m, 2 H), 3.80 (m, 1 H), 3.97 (dd, *J* = 10.5, *J* = 6, 1 H), 4.08 (m, 1 H), 4.60 (bs, 1 H), 4.75–4.92 (m, 2 H), 4.97 (bs, 1 H), 5.10 (AB, *J* = 12, 2 H), 7.30–7.39 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 8.80, 10.63, 15.72, 18.03, 20.75, 21.14, 21.17, 21.46, 30.34, 34.80, 40.09, 44.78, 49.05, 62.40, 65.76, 66.36, 68.30, 71.71, 72.55, 74.41, 74.80, 77.68, 127.59, 127.66, 128.09, 128.17, 136.64, 157.60, 170.55, 176.27; IR (KBr) 3960–3440, 2965–2790, 1725, 1510, 1450, 1368, 1240, 1165, 1050, 1000 cm^{-1}; MS *m/e* 911 (M^+ + 1). Anal. ($C_{47}H_{78}N_2O_{15}\cdot 2H_2O$) C, H, N.

(9R)-2′-O-Acetyl-9-[N-(carbobenzyloxy)amino]-9-deoxo-4″,11-O-diformylerythromycin A (6). 4-(Dimethylamino)-pyridine (4.3 g, 35.4 mmol) was added to a stirring solution of **5** (16.6 g, 17.7 mmol) in 100 mL of CH_2Cl_2 at 0 °C. Formic–acetic anhydride (2.8 mL, 35.4 mmol) was added dropwise. After 15 min, the solution was warmed to room temperature and allowed to stir for 48 h. The reaction was quenched with saturated sodium bicarbonate solution (100 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The extract was washed with brine (100 mL) and dried ($MgSO_4$), and the solution was concentrated. The residue was redissolved in CH_2Cl_2 and washed with 10% aqueous HCl solution (50 mL). The aqueous layer was adjusted to pH 9 with NH_4OH and extracted with CH_2Cl_2 (3 × 50 mL). The combined CH_2Cl_2 extracts were dried ($MgSO_4$) and concentrated. The residue was redissolved in a minimum amount of THF and diluted with 5% NaH_2PO_4 solution. This homogeneous solution (pH = 5) was stirred for 20 min at room temperature and extracted with CH_2Cl_2 (3 × 50 mL). The organic extract was washed with brine (50 mL), dried ($MgSO_4$), and concentrated to give 15.0 g (88%) of solid **6**: $[\alpha]_D$ −59.0° (c 3.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, *J* = 7.5, 3 H), 0.95 (d, *J* = 7.5, 3 H), 1.06–1.27 (m, 15 H), 1.28–1.54 (m, 4 H), 1.68–1.76 (m, 3 H), 1.92 (m, 1 H), 2.03 (s, 3 H), 2.22 (m, 1 H), 2.29 (s, 6 H), 2.40–2.52 (m, 3 H), 2.67–2.88 (m, 2 H), 3.38 (s, 3 H), 3.52 (m, 1 H), 3.80 (m, 2 H), 4.20–4.30 (m, 2 H), 4.44 (dd, *J* = 9, *J* = 4, 1 H), 4.67 (d, *J* = 10, 1 H), 4.80–4.93 (m, 3 H), 5.00–5.12 (AB, *J* = 12, 2 H), 7.30–7.38 (m, 5 H), 8.18 (s, 1 H), 8.26 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 8.59, 11.13, 11.36, 17.55, 17.74, 21.04, 21.41, 21.59, 22.24, 31.29, 35.04, 40.72, 43.84, 48.96, 62.56, 63.35, 66.73, 68.20, 72.02, 72.78, 74.62, 75.29, 76.27, 78.20, 95.29, 127.98, 128.42, 136.58, 156.66, 160.30, 169.68, 175.04; IR (KBr) 3600–3440, 2980, 2940, 1725, 1510, 1450, 1370, 1240, 1220, 1180, 1175, 1050, 1005 cm^{-1}; MS *m/e* 967 (M^+ + 1).

(9R)-9-[N-(Carbobenzyloxy)amino]-12,21-didehydro-9-deoxoerythromycin B (7). Triethylamine (1.15 mL, 8.28 mmol) was added to a solution of **6** (2.0 g, 2.07 mmol) in 20 mL of dry

(15) ^1H NMR ($CDCl_3$) δ 0.83 (d, 3 H), 0.87 (d, 3 H), 0.98 (m, 6 H), 1.09 (d, 3 H), 1.20 (s, 3 H), 1.24 (d, 3 H), 1.26 (d, 3 H), 1.29 (s, 3 H), 1.41 (dd, 1 H), 1.52 (m, 3 H), 1.68 (m, 3 H), 1.90 (m, 2 H), 1.97 (m, 1 H), 2.05 (m, 1 H), 2.29 (s, 6 H), 2.32 (d, 1 H), 2.45 (d, 1 H), 2.50 (m, 1 H), 3.00 (d, 1 H), 3.28 (s, 3 H), 3.31 (m, 1 H), 3.37 (t, 1 H), 3.55 (m, 2 H), 3.63 (dd, 1 H), 3.90 (d, 1 H), 3.98 (d, 1 H), 4.03 (m, 3 H), 4.22 (d, 1 H), 4.41 (d, 1 H), 4.65 (d, 1 H), 4.83 (d, 1 H); ^{13}C NMR ($CDCl_3$) δ 5.16, 10.67, 10.84, 11.58, 17.87, 19.01, 21.15, 21.63, 25.42, 28.04, 28.61, 30.47, 30.80, 35.44, 37.39, 38.58, 40.26, 42.42, 44.64, 49.41, 59.14, 64.83, 65.25, 65.28, 66.31, 69.42, 70.74, 72.79, 73.19, 74.70, 77.93, 78.28, 79.88, 80.11, 88.20, 97.19, 104.88; MS *m/e* 751 (M^+ + 1).

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ethyl acetate at 0 °C. Thionyl chloride (0.17 mL, 2.28 mmol) was added rapidly via syringe. After 30 min at 0 °C, the reaction was quenched with saturated sodium bicarbonate solution and extracted sequentially with CH_2Cl_2 (3 × 20 mL) and ethyl acetate (30 mL). The combined extracts were washed with brine (50 mL), dried ($MgSO_4$), filtered, and concentrated. The crude residue was chromatographed over silica gel (1:1 hexanes/acetone) to yield 1.29 g (55%) of intermediate 2'-*O*-acetate-protected product as a white foam. Triethylamine (0.441 mL, 3.17 mmol) was added to a solution of a portion of the intermediate (600 mg, 0.633 mmol) in 10 mL MeOH. The solution was heated to reflux, allowed to stir for 18 h, cooled to room temperature, and concentrated in vacuo. The crude residue was chromatographed over silica gel (3% MeOH/0.5% NH_4OH in CH_2Cl_2 followed by 6% MeOH/0.5% NH_4OH in CH_2Cl_2) to yield 407 mg (76%) of solid **7**: $[\alpha]_D$ −39.6° (c 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.78 (d, *J* = 7.5, 3 H), 0.92 (t, *J* = 7.5, 3 H), 1.02 (d, *J* = 7, 3 H), 1.04 (d, *J* = 7, 3 H), 1.11 (d, *J* = 8, 3 H), 1.15 (m, 6 H), 1.16 (s, 3 H), 1.20 (m, 1 H), 1.21 (d, *J* = 8, 3 H), 1.50–1.82 (m, 7 H), 2.19 (d, *J* = 10, 1 H), 2.25 (m, 1 H), 2.30 (s, 6 H), 2.31 (m, 1 H), 2.59 (m, 2 H), 3.03 (m, 2 H), 3.32 (s, 3 H), 3.50–3.65 (m, 3 H), 3.86 (m, 2 H), 4.00 (dd, *J* = 12, *J* = 6, 1 H), 4.39 (bs, 1 H), 4.44 (bs, 1 H), 4.52 (d, *J* = 7.5, 1 H), 4.58 (d, *J* = 4.5, 1 H), 4.86 (d, *J* = 9, 1 H), 5.12 (AB, *J* = 12, 2 H), 5.19 (bs, 1 H), 5.43 (t, *J* = 7, 1 H), 5.49 (bs, 1 H), 7.30–7.39 (m, 5 H); ^{13}C NMR ($CDCl_3/CD_3OD$) δ 9.61, 9.69, 10.29, 12.65, 17.70, 17.86, 20.77, 21.23, 24.41, 27.67, 29.43, 29.46, 31.73, 34.65, 37.90, 40.07, 42.51, 44.10, 48.30, 48.60, 48.87, 49.10, 49.16, 49.45, 59.35, 64.67, 65.81, 66.73, 69.04, 70.51, 71.58, 72.63, 74.64, 77.58, 77.69, 79.02, 83.21, 95.78, 102.52, 114.51, 127.84, 127.90, 128.29, 147.36, 157.36, 175.75; IR (KBr) 2980, 2940, 1720, 1510, 1450, 1370, 1240, 1220, 1180, 1000 cm^{-1}; MS *m/e* 851 (M^+ + 1). Anal. ($C_{45}H_{74}N_2O_{13}\cdot H_2O$) C, H, N.

(9*R*)-2'-*O*-Acetyl-9-[*N*-(carbobenzyloxy)amino]-12,21-didehydro-9-deoxoerythromycin B (8). Acetic anhydride (35 μL, 0.373 mmol) was added to a solution of **7** (317 mg, 0.373 mmol) in 8 mL of dry CH_2Cl_2. The reaction was stirred for 20 h and concentrated. The residue was chromatographed over silica gel (3% MeOH/0.5% NH_4OH in CH_2Cl_2) to yield 365 mg (89%) of solid **8**: $[\alpha]_D$ −38.53° (c 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.77 (d, *J* = 7.5, 3 H), 0.92 (t, *J* = 7.5, 3 H), 0.96 (d, *J* = 7.5, 3 H), 1.08 (d, *J* = 7.5, 3 H), 1.16 (bs, 3 H), 1.18 (d, *J* = 7.5, 3 H), 1.24 (d, *J* = 6, 3 H), 1.25 (bs, 3 H), 1.32 (d, *J* = 6, 3 H), 1.37 (m, 1 H), 1.63–1.85 (m, 3 H), 2.05 (s, 3 H), 2.27 (s, 6 H), 2.30–2.40 (m, 2 H), 2.58–2.74 (m, 2 H), 3.04 (t, *J* = 9, 3 H), 3.37 (s, 3 H), 3.50–4.11 (m, 3 H), 3.75–3.82 (m, 2 H), 3.95 (dd, *J* = 9, *J* = 6, 1 H), 4.35 (bs, 1 H), 4.41 (bs, 1 H), 4.82–4.90 (m, 2 H), 5.12 (AB, *J* = 12, 2 H), 5.19 (bs, 1 H), 5.45 (t, *J* = 7.5, 1 H), 5.49 (bs, 1 H), 7.30–7.39 (m, 5 H); ^{13}C NMR (DMSO-d_6) δ 9.35, 10.32, 10.90, 12.73, 18.22, 19.37, 20.81, 21.07, 21.12, 24.58, 27.52, 30.54, 34.55, 37.22, 40.21, 42.91, 48.60, 62.07, 65.03, 65.16, 67.83, 71.19, 72.77, 74.02, 76.99, 77.41, 78.06, 79.16, 95.23, 99.27, 112.94, 127.65, 128.30, 137.46, 156.75, 169.07, 175.44; IR (KBr) 3680–3440, 2960, 2920, 1720, 1600, 1510, 1450, 1375, 1240, 1185, 1160, 1110, 1080, 1060, 1000 cm^{-1}; MS *m/e* 895 (M^+ + 1). Anal. ($C_{47}H_{76}N_2O_{14}\cdot H_2O$) C, H, N.

(9*R*)-2'-*O*-Acetyl-9-[*N*-(carbobenzyloxy)amino]-9-deoxo-12,21-epoxyerythromycin B (9). *m*-Chloroperoxybenzoic acid (73%) (267 mg, 1.13 mmol) was added to a solution of **8** (336 mg, 0.377 mmol) in 8 mL CH_2Cl_2 at room temperature. The solution was stirred for 4 h, and the excess oxidant was quenched with cyclohexene (76 μL, 0.754 mmol). The mixture was stirred for 15 min, excess saturated sodium bicarbonate solution was added, and the mixture was extracted with CH_2Cl_2 (3 × 25 mL). The organic phase was washed with brine, dried ($MgSO_4$), and concentrated. The residue was chromatographed over silica gel (5% MeOH/0.5% NH_4OH in CH_2Cl_2 followed by 10% MeOH/1% NH_4OH in CH_2Cl_2) to yield 271 mg (78%) of a white foam. Triphenylphosphine (581 mg, 2.22 mmol) was added to a stirring solution of the white foam (293 mg, 0.317 mmol) in 10 mL CH_2Cl_2 at room temperature. The reaction was stirred for 24 h and quenched with saturated sodium bicarbonate solution. The mixture was extracted with CH_2Cl_2 (3 × 25 mL) and ethyl acetate (30 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated. The crude residue was chromatographed over silica gel (3% MeOH/0.5% NH_4OH in CH_2Cl_2) to yield 231 mg (80%) of solid **9**: $[\alpha]_D$ −43.3° (c 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (d, *J* = 7.5, 3 H), 0.92 (t, *J* = 7.5, 3 H), 0.97 (d, *J* = 7.5, 3 H), 1.14 (s, 3 H), 1.18–1.44 (m, 18 H), 1.52–1.80 (m, 5 H), 1.86 (m, 1 H), 2.03 (s, 3 H), 2.14 (d, *J* = 10, 1 H), 2.27 (s, 6 H), 2.38 (d, *J* = 15, 1 H), 2.56–2.72 (m, 3 H), 3.05 (t, *J* = 9, 1 H), 3.17 (d, *J* = 4.5, 1 H), 3.38 (s, 3 H), 3.57 (m, 1 H), 3.63 (bs, 1 H), 3.71 (d, *J* = 6, 1 H), 3.88–3.95 (m, 2 H), 4.34 (bs, 1 H), 4.53 (d, *J* = 4.5, 1 H), 4.70 (m, 2 H), 4.79 (t, *J* = 7.5, 1 H), 4.88 (dd, *J* = 16, *J* = 7.5, 1 H), 5.04–5.14 (AB, *J* = 12, 2 H), 7.30–7.40 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 9.68, 11.09, 11.30, 11.79, 18.09, 20.91, 21.39, 21.71, 24.27, 26.22, 30.48, 30.78, 34.70, 35.72, 40.71, 43.17, 43.56, 46.82, 49.18, 59.82, 61.23, 63.28, 66.08, 66.87, 67.29, 69.22, 71.43, 72.90, 74.44, 77.85, 80.13, 95.25, 100.18, 128.08, 128.20, 128.51, 136.51, 169.75, 175.02; IR (KBr) 3540, 3440, 2980, 2940, 1740, 1510, 1460, 1380, 1240, 1220, 1180, 1160, 1120, 1080, 1060, 1000 cm^{-1}; MS *m/e* (M^+). Anal. ($C_{47}H_{76}N_2O_{15}\cdot H_2O$) C, H, N.

(9*R*)-9-Deoxo-9-(*N*,*N*-dimethylamino)-12,21-epoxyerythromycin B (10). Formalin (37%) (45 μL, 1.6 mmol) was added to a solution of **9** (139 mg, 0.161 mmol) in 5 mL of MeOH. Palladium on carbon catalyst (10%) (50 mg) was added, and the mixture was hydrogenated at 1 atm for 5 h. The reaction mixture was flushed with nitrogen and filtered through a plug of Celite, and the catalyst was washed repeatedly with CH_2Cl_2 and MeOH. The combined filtrate and washings were concentrated, and the residue was chromatographed over silica gel (10% MeOH/1% NH_4OH in CH_2Cl_2) to yield 64 mg (53%) of solid **10**: $[\alpha]_D$ −51.85° (c 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.95 (t, *J* = 7, 3 H), 1.12 (d, *J* = 7, 3 H), 1.20–1.35 (m, 18 H), 1.30 (d, *J* = 7, 3 H), 1.73 (t, 1 H), 2.18 (d, *J* = 10, 1 H), 2.36 (d, *J* = 15, 1 H), 2.49 (bs, 6 H), 2.62 (dd, *J* = 7, *J* = 3, 1 H), 2.77 (d, *J* = 4.5, 1 H), 2.91 (bs, 6 H), 3.04 (t, *J* = 9, 1 H), 3.16 (d, *J* = 4.5, 1 H), 3.21 (m, 1 H), 3.31 (s, 3 H), 3.41 (m, 1 H), 3.50–3.66 (m, 2 H), 3.96–4.05 (m, 2 H), 4.23 (d, *J* = 3, 1 H), 4.48 (m, 3 H), 4.77 (dd, *J* = 9, *J* = 4.5, 1 H); ^{13}C NMR ($CDCl_3$) δ 10.08, 11.37, 11.53, 12.17, 17.79, 21.15, 21.58, 23.48, 26.31, 28.61, 28.96, 33.91, 34.80, 40.35, 42.59, 43.34, 43.50, 44.65, 46.98, 49.34, 61.19, 65.47, 65.99, 69.61, 70.55, 72.80, 74.91, 77.15, 79.94, 95.63, 102.80, 175.13; IR (KBr) 3730, 3540–3460, 2965, 2945, 2860, 2790, 1735, 1600, 1450, 1380, 1180, 1160, 1110, 1090, 1050, 1000 cm^{-1}; MS *m/e* 761 (M^+ + 1). Anal. ($C_{39}H_{72}N_2O_{12}\cdot H_2O$) C, H, N.

(9*R*)-9-[*N*-(Carbobenzyloxy)amino]-9-deoxo-21-hydroxyerythromycin A (11). Osmium tetraoxide solution (2.5% in *t*-BuOH) (1.78 mL, 0.142 mmol) was added to a solution of **7** (115 mg, 0.29 mmol) in THF at room temperature. The solution was stirred for 24 h and diluted with water. Florisil (0.2 gm) and excess solid sodium dithionite were added, and the mixture was stirred for 2 h. The solution was diluted further with 30 mL of water and adjusted to pH = 9 with NH_4OH. The mixture was extracted with EtOAc (3 × 30 mL), washed with brine (50 mL), dried ($MgSO_4$), and concentrated. The residue was chromatographed over silica gel (3% MeOH/0.05% NH_4OH in CH_2Cl_2) to yield 50 mg (42%) of the 2'-*O*-acetyl intermediate as a white solid. A 120-mg portion (0.129 mmol) of the intermediate was heated to reflux in 5 mL of MeOH and allowed to stir for 24 h. The solution was concentrated, and the crude product was chromatographed over silica gel (5% MeOH/0.5% NH_4OH in CH_2Cl_2 followed by 10% MeOH/1% NH_4OH in CH_2Cl_2) to yield 74 mg (65%) of solid **11**: $[\alpha]_D$ −39.0° (c 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.89 (t, *J* = 7.5, 3 H), 0.91 (d, *J* = 7.5, 3 H), 1.01 (d, *J* = 7.5, 3 H), 1.09 (d, *J* = 7.5, 3 H), 1.18–1.31 (m, 15 H), 1.81–2.01 (m, 2 H), 2.10–2.25 (m, 2 H), 2.28 (s, 6 H), 2.30–2.51 (m, 2 H), 2.76 (t, *J* = 6, 1 H), 2.96 (m, 1 H), 3.05 (t, *J* = 9, 1 H), 3.26 (dd, *J* = 7.5, *J* = 3, 1 H), 3.33 (s, 3 H), 3.50–3.88 (m, 5 H), 4.02 (dd, *J* = 9, *J* = 6, 1 H), 4.17 (d, *J* = 6, 1 H), 4.49 (d, *J* = 7.5, 1 H), 4.87 (d, *J* = 9, 1 H), 4.93 (d, *J* = 4.5, 1 H), 5.10 (AB, *J* = 12, 2 H), 7.30–7.40 (m, 5 H); ^{13}C NMR ($CDCl_3/CD_3OD$) δ 9.35, 11.09, 11.48, 18.24, 21.25, 21.55, 22.30, 29.31, 34.92, 40.36, 49.22, 62.28, 64.87, 66.13, 66.76, 69.15, 71.04, 72.66, 74.94, 75.05, 75.49, 77.75, 78.55, 96.36, 127.99, 128.04, 136.72, 157.59, 176.56; IR (KBr) 3440, 2960, 2940, 1725, 1700, 1510, 1450, 1375, 1210, 1165, 1050, 1000 cm^{-1}; MS *m/e* 885 (M^+). Anal. ($C_{45}H_{76}N_2O_{15}\cdot H_2O$) C, H, N.

(9*R*)-9-Deoxo-9-(*N*,*N*-dimethylamino)-21-hydroxyerythromycin A (12). Formalin (37%) (15 μL, 0.532 mmol) was added to a solution of **11** (47 mg, 0.053 mmol) in 2 mL of MeOH at room temperature. Palladium on carbon (10%) (50 mg) catalyst was added, and the mixture was hydrogenated for 5 h at 1 atm. The mixture was filtered through Celite and was washed well with

CH_2Cl_2 and MeOH. The combined filtrate and washings were concentrated, and the residue was dissolved in 5% Na_2HPO_3 buffer (pH = 5). After 5 min, the aqueous solution was adjusted to pH = 9 with NH_4OH and extracted with ethyl acetate (3 × 50 mL). The extract was washed with brine (50 mL), dried ($MgSO_4$), and concentrated. The residue was chromatographed over silica gel (5% MeOH/0.5% NH_4OH in CH_2Cl_2 followed by 10% MeOH/1% NH_4OH in CH_2Cl_2) to yield 25 mg (61%) of solid **12**: $[\alpha]_D$ −44.90° (c 1.6, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.92 (t, J = 7, 3 H), 1.12 (t, J = 7, 6 H), 1.20–1.26 (m, 6 H), 1.33 (d, J = 7, 3 H), 1.35 (s, 3 H), 1.39 (m, 1 H), 1.54–1.73 (m, 3 H), 1.93 (m, 2 H), 2.25 (d, J = 12, 1 H), 2.28 (s, 3 H), 2.38 (d, J = 15, 1 H), 2.44 (bs, 3 H), 2.72 (m, 1 H), 3.03 (t, J = 9, 1 H), 3.25 (dd, J = 9, J = 7, 1 H), 3.31 (s, 3 H), 3.48–3.56 (m, 2 H), 3.72–3.84 (AB, J = 12, 2 H), 3.82 (s, 1 H), 4.05 (dd, J = 9, J = 6, 1 H), 4.35 (m, 1 H), 4.44 (d, J = 7, 1 H), 4.88 (d, J = 4.5, 1 H), 4.97 (dd, J = 10, J = 3, 1 H); ^{13}C NMR ($CDCl_3/CD_3OD$) δ 9.43, 11.00, 14.48, 17.82, 20.98, 21.15, 22.09, 29.43, 33.10, 34.72, 40.10, 43.74, 43.79, 44.79, 49.43, 61.83, 64.45, 65.60, 68.85, 70.90, 72.49, 73.94, 75.43, 76.24, 77.21, 77.50, 77.63, 78.44, 79.39, 96.21, 102.93, 176.89; IR (KBr) 3600–3460, 2965, 2935, 1725, 1600, 1450, 1380, 1160, 1105, 1050, 1005 cm^{-1}; HRMS calcd for $C_{39}H_{75}N_2O_{13}$ (MH^+) 779.5269, found 779.5252.

(9*R*)-9-Deoxo-9-(*N*,*N*-dimethylamino)-21-hydroxyerythromycin B (13). Borane–THF (1 M BH_3 solution in THF) (2.9 mL, 2.86 mmol) was added to a stirring solution of **8** (255 mg, 0.286 mmol) in 6 mL THF at 0 °C. The solution was warmed to room temperature and allowed to stir for 18 h. The reaction was quenched by portionwise addition of 15 mL of water, and 10% KOH solution was added dropwise until the pH was 8. Hydrogen peroxide (30% w/v) (0.29 mL, 2.86 mmol) was added via syringe, and the solution was stirred for 2 h. The solution was further diluted with 20 mL of water and adjusted to pH = 9.5 by addition of NH_4OH. The solution was extracted with EtOAc (3 × 20 mL), and the EtOAc extract was washed with brine (50 mL), dried ($MgSO_4$), and concentrated. The residue was redissolved in MeOH and heated to reflux for 20 h, and methanol was removed in vacuo. The residue was adhered onto silica gel and chromatographed over silica gel (5% MeOH/0.5% NH_4OH in CH_2Cl_2) to yield 160 mg of a mixture of two products, which was carried further. Palladium on carbon catalyst (10%) (50 mg) was added to a stirring solution of the mixture of products from above (160 mg) in MeOH (5 mL) under nitrogen. The flask was evacuated, and the mixture was hydrogenated at 1 atm for 30 min, filtered, and the residue was washed well with CH_2Cl_2 and MeOH. The combined filtrate and washings were concentrated, and the residue was chromatographed over silica gel (10% MeOH/1% NH_4OH in CH_2Cl_2 followed by 20% MeOH/2% NH_4OH in CH_2Cl_2) to give 80 mg of a mixture containing the desired intermediate. Formalin (37%) (162 μL, 2.17 mmol) was added to a stirring solution of the intermediate (80 mg, 0.108 mmol) in MeOH (5 mL) at room temperature. Palladium on carbon catalyst (10%) (50 mg) was added under nitrogen, and the flask was evacuated. The solution was hydrogenated at 1 atm for 5 h. The catalyst was filtered off and washed with CH_2Cl_2 and MeOH. The combined filtrate and washings were concentrated, and the residue was chromatographed over silica gel (7% MeOH/0.5% NH_4OH in CH_2Cl_2) to yield 55 mg (25% overall for three steps) of solid **13**: $[\alpha]_D$ −41.7° (c 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.94 (t, J = 7, 6 H), 1.13 (d, J = 7, 6 H), 1.23 (m, 9 H), 1.33 (d, J = 7, 3 H), 1.36 (s, 3 H), 1.50–1.72 (m, 9 H), 1.83 (m, 1 H), 1.90–2.00 (m, 2 H), 2.29 (s, 6 H), 2.32–2.50 (m, 6 H), 3.02 (t, J = 9, 1 H), 3.24 (dd, J = 12, J = 6, 1 H), 3.33 (s, 3 H), 3.42–3.55 (m, 2 H), 3.73 (dd, J = 12, J = 3, 1 H), 3.96 (dd, J = 14, J = 3, 1 H), 4.03 (dd, J = 10, J = 6, 1 H), 4.40 (d, J = 7, 1 H), 4.80 (d, J = 5, 1 H), 5.25 (m, 1 H); ^{13}C NMR ($CDCl_3/CD_3OD$) δ 10.00, 10.28, 14.00, 21.12, 21.40, 25.26, 29.16, 33.00, 34.98, 40.28, 44.07, 58.70, 64.98, 65.99, 69.16, 70.83, 70.57, 96.83, 103.93, 177.90; IR (KBr) 3600–3440, 2960, 2930, 2780, 1720, 1600, 1450, 1370, 1160, 1100, 1045, 1005 cm^{-1}; HRMS calcd for $C_{39}H_{74}N_2O_{12}$ (MH^+) 763.5320, found 763.5321.

Communications to the Editor

Examination of HIV-1 Protease Secondary Structure Specificity Using Conformationally Constrained Inhibitors

A key step in the maturation of retroviruses, including the human immunodeficiency virus (HIV), is the post-translational cleavage of the *gag* and *pol* gene product polyprotein fusions into their constituent functional proteins, including the HIV protease (HIV-PR).[1-3] A protease-deficient mutant HIV-1 strain has been reported to produce noninfectious virions of immature morphology.[4,5] Thus, the HIV-1 protease is essential to viral replication[6-9] and represents an ideal target for antiviral therapy. The HIV-1 protease has been classified as an aspartic protease that functions as a homodimer based on its crystal structure,[10-12] its inhibition by pepstatin,[13] and the conservation of the characteristic Asp-Thr-Gly active-site sequence.[14,15]

The conformation of the polyprotein has been shown to be important in HIV-1 protease processing, since denaturation renders the substrate resistant to proteolytic

(1) Ratner, L.; Haseltine, W.; Patarca, R.; Livak, K. J.; Starcich, B.; Josephs, S. F.; Doran, E. R.; Rafalski, J. A.; Whitehorn, E. A.; Baumeister, K.; Ivanoff, L.; Petteway, S. R.; Pearson, M. L.; Lautenberger, J. A.; Papas, T. S.; Ghrayeb, J.; Chang, N. T.; Gallo, R.; Wong-Staal, F. *Nature* 1985, *313*, 277–284.
(2) Yoshinaka, Y.; Katoh, I.; Copeland, T. D.; Smythers, G. W.; Oroszlan, S. *J. Virol.* 1986, *57*, 826–832.
(3) Yoshinaka, Y.; Katoh, I.; Copeland, T. D.; Oroszlan, S. *Proc. Natl. Acad. Sci. U.S.A.* 1985, *82*, 1618–1622.
(4) Katoh, I.; Yoshinaka, Y.; Rein, A.; Shibuya, M.; Odaka, T.; Oroszlan, S. *Virology* 1985, *145*, 280–292.
(5) Benveniste, R. E.; Eron, L. J.; Nagashima, K.; Gonda, M. A. *III International Conference on AIDS* 1987, Abstract MP13, p 12.
(6) Kohl, N. E.; Emani, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A. F.; Scolnick, E. M.; Sigal, I. S. *Proc. Natl. Acad. Sci. U.S.A.* 1988, *85*, 4686–4690.
(7) Krausslich, H. G.; Wimmer, E. *Annu. Rev. Biochem.* 1988, *57*, 701–754.
(8) Johnston, M. I.; Allaudeen, H. S.; Sarver, N. *Trends in Pharmacol. Sci.* 1989, *10*, 305–307.
(9) LeGrice, S. F. J.; Mills, J.; Mous, J. *EMBO J.* 1988, *7*, 2547–2553.
(10) Navia, M. A.; Fitzgerald, P. M. D.; McKeever, B. M.; Leu, C.-T.; Heimbach, J. C.; Herber, W. K.; Sigal, I. S.; Darke, P. L.; Springer, J. P. *Nature* 1989, *337*, 615–620.
(11) Lapatto, R.; Blundell, T.; Hemmings, A.; Overington, J.; Wilderspin, A.; Wood, S.; Merson, J. R.; Whittle, P. J.; Danley, D. E.; Geoghegan, K. F.; Hawrylik, S. J.; Lee, S. E.; Scheid, K. G.; Hobart, P. M. *Nature (London)* 1989, *342*, 299–302.
(12) Wlodawer, A.; Miller, M.; Jaskolski, M.; Salthyanarayana, B. K.; Baldwin, E.; Weber, I. T.; Selk, L. M.; Clawson, L.; Schneider, J.; Kent, S. B. H. *Science* 1989, *245*, 616–621.
(13) Seelmeier, S.; Schmidt, H.; Turk, V.; von der Helm, K. *Proc. Natl. Acad. Sci. U.S.A.* 1988, *85*, 6612–6616.
(14) Toh, H.; Ono, M.; Saigo, K.; Miyata, T. *Nature* 1985, *315*, 691.
(15) Pearl, L.; Taylor, W. *Nature* 1987, *329*, 351–354.

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