compounds was determined in overnight-fasted, conscious Heidenhain pouch dogs. Pentagastrin (Pentavolon-Ayerst) was used to stimulate acid output by continuous infusion into a superficial leg vein at doses earlier determined to stimulate near maximal acid output from the gastric pouch. Gastric juice was collected at 30-min intervals following the start of a pentagastrin infusion and measured to the nearest 0.1 mL. Ten collections were taken for each dog during an experiment. Acid concentration was determined by titrating 1.0 mL of gastric juice to pH 7.4 with 0.1 N sodium hydroxide with an Autoburette and a glass electrode pH meter (Radiometer).

Drug or vehicle was given intravenously or orally 90 min following the start of the pentagastrin infusion at a dose of 2 mg/kg or less. Gastric acid antisecretory effects were calculated by comparing the lowest acid output after drug administration with the mean acid output immediately before drug.

Acknowledgment. The expert synthetic chemical assistance of C. J. Mularski, D. E. Muse, L. T. Wint, and M. P. Zawistoski is gratefully acknowledged. J. P. Hakkinen's important contribution to the generation of the biological data is gratefully acknowledged as is the expert biological technical assistance of H. R. Bolen, F. W. Bangerter, P. G. Cosgrove, D. Hessinger, and J. T. MacAndrew.

Registry No. 1, 115027-84-4; 1·$C_2H_4O_2$, 123311-07-9; **2**, 123309-49-9; **3**, 123309-50-2; **3**·HCl, 123310-52-1; **4**, 123309-51-3; **4**·$C_2H_4O_2$, 123310-53-2; **5**, 123309-52-4; **6**, 123309-53-5; **7**, 115027-85-5; **7**·$C_2H_4O_2$, 115027-86-6; **8**, 123309-54-6; **9**, 123309-55-7; **10**, 123309-56-8; **11**, 123309-57-9; **12**, 123309-58-0; **13**, 123309-59-1; **13**·2HCl, 123310-54-3; **14**, 123309-60-4; **14**·HCl, 123310-55-4; **15**, 123309-61-5; **15**·HCl, 123310-56-5; **16**, 115027-94-6; **16**·$C_2H_4O_2$, 115027-95-7; **17**, 115027-68-4; **17**·$C_2H_4O_2$, 123310-57-6; **18**, 123309-62-6; **18**·HCl, 115027-61-7; **19**, 115027-72-0; **19**·$C_2H_4O_2$, 115027-73-1; **20**, 115027-74-2; **20**·$C_2H_4O_2$, 115027-75-3; **21**, 115027-62-8; **21**·$C_2H_4O_2$, 115027-63-9; **22**, 115027-77-5; **22**·HCl, 115027-76-4; **23**, 123309-63-7; **23**·HBr, 115027-78-6; **24**, 123309-64-8; **25**, 123309-65-9; **25**·HCl, 115027-49-1; **26**, 123309-66-0; **26**·HCl, 115027-51-5; **27**, 123309-67-1; **27**·HCl, 115027-50-4; **28**, 123309-68-2; **28**·HCl, 115027-52-6; **29**, 123309-69-3; **29**·HCl, 115027-47-9; **30**, 115027-66-2; **30**·$C_2H_4O_2$, 115027-67-3; **31**, 115027-64-0; **31**·$^1/_2C_2H_4O_2$, 123310-58-7; **32**, 115027-91-3; **32**·$C_2H_4O_2$, 115027-92-4; **33**, 115027-93-5; **34**, 123309-70-6; **35**, 115027-59-3; **35**·$C_2H_4O_2$, 115027-60-6; **36**, 115027-70-8; **36**·$C_2H_4O_2$, 123330-27-8; **37**, 123309-71-7; **37**·$2C_2H_4O_2$, 123310-59-8; **38**, 123309-72-8; **38**·HCl, 115027-81-1; **39**, 123309-73-9; **39**·HCl, 115027-82-2; **40**, 115027-87-7; **40**·$C_2H_4O_2$, 115027-88-8; **41**, 115027-89-9; **41**·$2C_2H_4O_2$, 115027-90-2; **42**, 123309-74-0; **42**·HCl, 123330-28-9; **43**, 123309-75-1; **43**·HBr, 123310-60-1; **44**, 123309-76-2; **44**·$^1/_2$HCl, 123310-61-2; **45**, 123309-77-3; **45**·HCl, 123310-62-3; **46**, 123309-78-4; **46**·HBr, 123310-63-4; **47**, 123309-79-5; **48**, 2507-81-5; **49**, 123309-80-8; **49**·HBr, 123310-64-5; **50**, 123309-81-9; **50**·HBr, 123310-65-6; **51**, 123309-82-0; **51**·HBr, 123310-66-7; **52**, 7120-02-7; **52**·HBr, 123310-67-8; **53**, 2438-34-8; **53**·HCl, 123310-68-9; **54**, 123309-83-1; **54**·HBr, 123310-69-0; **55**, 123309-84-2; **55**·HBr, 123310-70-3; **56**, 123309-85-3; **57**, 123309-86-4; **57**·HCl, 123310-71-4; **58**, 123309-87-5; **58**·HBr, 96996-07-5; **59**, 123309-88-6; **60**, 123309-89-7; **60**·HBr, 123310-72-5; **61**, 123309-90-0; **61**·2HCl, 123310-73-6; **62**, 7120-04-9; **62**·HBr, 123310-74-7; **63**, 123309-91-1; **63**·HBr, 123310-75-8; **64**, 123309-92-2; **64**·HBr, 123310-76-9; **65**, 123309-93-3; **65**·HBr, 123310-77-0; **66**, 24644-41-5; **66**·HBr, 123310-78-1; **67**, 123309-94-4; **68**, 72801-60-6; **69**, 112598-38-6; **69**·HBr, 112598-39-7; **70**, 83831-31-6; **70**·2HCl, 107880-80-8; **71**, 123309-95-5; **71**·HBr, 123310-79-2; **72**, 123309-96-6; **72**·2HCl, 123310-80-5; **73**, 123309-97-7; **73**·HBr, 123310-81-6; **74**, 123309-98-8; **75**, 91089-11-1; **75**·HBr, 96996-05-3; **76**, 123309-99-9; **76**·HBr, 123310-82-7; **77**, 123310-00-9; **77**·HBr, 123310-83-8; **78**, 123310-01-0; **78**·HBr, 123310-84-9; **79**, 123310-02-1; **79**·HBr, 123310-85-0; **80**, 123310-03-2; **80**·HCl, 123310-86-1; **81**, 123310-04-3; **81**·HCl, 123310-87-2; **82**, 123310-05-4; **82**·HBr, 96996-13-3; **83**, 123310-06-5; **83**·HBr, 96996-11-1; **84**, 2507-84-8; **84**·HCl, 123310-88-3; **85**, 123310-07-6; **85**·HBr, 123310-89-4; **86**, 123310-08-7; **86**·HBr, 123310-90-7; **87**, 123310-09-8; **87**·HBr, 123310-91-8; **88**, 123310-10-1; **88**·HCl, 123310-92-9; **89**, 123310-11-2; **89**·HCl, 123310-93-0; **90**, 123310-12-3; **90**·HBr, 123310-94-1; **91**, 123310-13-4; **92**, 123310-14-5; **92**·HBr, 115027-42-4; **93**, 123310-15-6; **93**·HBr, 115027-43-5; **94**, 123310-16-7; **94**·HBr, 115027-44-6; **95**, 123310-17-8; **95**·HCl, 115027-46-8; **96**, 123310-18-9; **96**·HCl, 115027-45-7; **97**, 115027-97-9; **97**·HCl, 65040-42-8; **98**, 123310-95-2; **98**·HCl, 115027-96-8; **99**, 115027-55-9; **100**, 115027-56-0; **101**, 123310-19-0; **101**·HCl, 115027-99-1; **102**, 123310-20-3; **102**·HCl, 115027-98-0; **103**, 123310-21-4; **103**·HCl, 115028-02-9; **104**, 123310-22-5; **104**·HCl, 115028-03-0; **105**, 123310-23-6; **105**·2HCl, 115027-31-1; **106**, 123310-24-7; **106**·2HCl, 123310-96-3; **107**, 123310-25-8; **107**·2HCl, 123310-97-4; **108**, 123310-26-9; **108**·2HCl, 123310-98-5; **109**, 115028-04-1; **110**, 123310-27-0; **110**·HCl, 115028-05-2; **111**, 123310-28-1; **111**·HCl, 115028-06-3; **112**, 123310-29-2; **112**·HCl, 115028-07-4; **113**, 123310-30-5; **113**·HCl, 115028-08-5; **114**, 123310-31-6; **114**·HCl, 115028-09-6; **115**, 123310-32-7; **115**·HCl, 115027-30-0; **116**, 123310-33-8; **116**·HCl, 115028-00-7; **117**, 123310-34-9; **117**·HCl, 115028-01-8; **118**, 115027-54-8; **119**, 115027-10-6; **120**, 123310-35-0; **120**·HCl, 115027-33-3; **121**, 123310-36-1; **121**·HCl, 115027-34-4; **122**, 123310-37-2; **122**·HCl, 115027-35-5; **123**, 123310-38-3; **123**·HCl, 115027-36-6; **124**, 123310-39-4; **124**·HCl, 115027-37-7; **125**, 123310-40-7; **125**·HCl, 115027-38-8; **126**, 123330-26-7; **126**·HCl, 115027-39-9; **127**, 123310-41-8; **127**·HCl, 115027-40-2; **128**, 115027-41-3; **129**, 3858-85-3; **129**·$C_2H_4O_2$, 123310-99-6; **130**, 123310-42-9; **130**·$C_6H_6O_3S$, 123311-00-2; **131**, 123310-43-0; **132**, 123310-44-1; **132**·HCl, 123311-01-3; **133**, 123310-45-2; **133**·HCl, 123311-02-4; **134**, 123310-46-3; **134**·HCl, 123311-03-5; **135**, 123310-47-4; **135**·HCl, 123311-04-6; **136**, 123310-48-5; **136**·HCl, 123311-05-7; **137**, 123310-49-6; **137**·HBr, 123311-06-8; **139**, 115027-23-1; **141**, 115027-28-6; **142**, 115027-29-7; **143**, 115027-11-7; **144**, 115027-14-0; **145**, 115027-13-9; **146**, 115027-12-8; **147**, 115027-15-1; **149**, 30030-91-2; **150**, 399-52-0; **151**, 115027-06-0; **152**, 1477-50-5; **153**, 36709-95-2; **154**, 504-66-5; **155**, 101190-73-2; **156**, 101857-92-5; **158**, 88964-74-3; **159**, 556-61-6; **160**, 72751-64-5; **161**, 123310-51-0; $CH_3(CH_2)_5NHCH_3$, 35161-70-7; $ClCH_2COCl$, 79-04-9; $CH_3(CH_2)_5NH_2$, 111-26-2; H^+,K^+-ATPase, 9000-83-3; H_2NCN, 420-04-2; $CH_3(CH_2)_5NHC(SMe)$=NMe·HI, 93484-22-1; pyrrol-2-ylcarboxaldehyde, 1003-29-8; (3,4,5,6-tetrahydropyrimidin-1-yl)cyanamide, 36982-81-7; *N*-hexyl-*N'*-methylthiourea, 53393-06-9.

Hypolipidemic Activity of Rifamycin Derivatives

Peter Traxler,* Wilhelm Kump, Klaus Mueller, and Werner Tosch

Pharmaceuticals Research Laboratories, Ciba-Geigy, Ltd., CH-4002 Basle, Switzerland. *Received March 2, 1989*

Series of 3-piperidinyl- and 3-piperazinylrifamycins and to a certain extent 3-hydrazonorifamycins all bearing lipophilic side chains were found to exert potent hypolipidemic activity in lowering both serum cholesterol and LDL-cholesterol in rats. Starting from 3-[*N'*-(2,4,6-trimethylbenzyl)-*N*-piperazinyl]rifamycin SV (compound **25**), a series of derivatives were synthesized with the aim of dissociating the hypolipidemic from the antibacterial activity, leading to the 8-*O*,*N*-dipivaloyl derivative of **25** (compound **48**), which is devoid of any antibacterial activity but shows about 50–60% reduction of LDL-cholesterol and 20–30% reduction of serum cholesterol at a dose of 10 mg/kg. Compound **48** was selected for further pharmacological evaluation.

The rifamycins, which were first isolated toward the end of the 1950s from cultures of the microorganism *Nocardia mediterranei*, are antibiotics active in vitro against Gram-positive and Gram-negative bacteria.[1] They are

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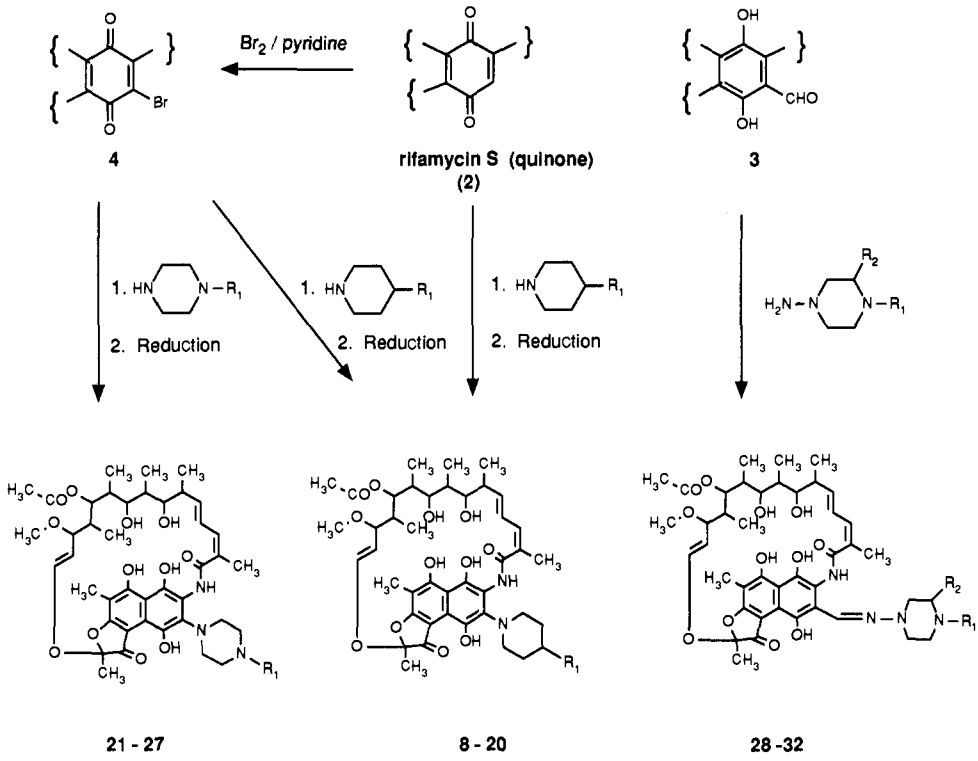
Scheme I

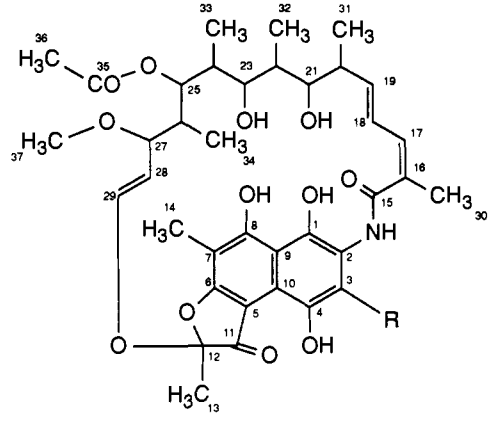


potent inhibitors of DNA-dependent RNA polymerase in bacteria.[2] Rifampin, or rifampicin (**1**) (Chart I), a semisynthetic derivative of rifamycin SV (**2**), was introduced on the market in 1967 under the trade name Rimactane. It is used as an orally active antituberculous agent, predominantly in countries of the Third World, in combined drug regimens given over a period of 6–9 months.

In the past, various effects of rifampicin (**1**) on lipid metabolism have been observed. Hypocholesterolemic activity in the monkey (*Macaca fascicularis*) was detected in the course of 3-month toxicity studies. Doses of 40 mg/kg lowered serum cholesterol by roughly 30%, and no significantly greater decrease was produced by doses of 80 or 120 mg/kg.[3] A slight hypocholesterolemic effect of 16% in cholesterol-fed rabbits has also been reported.[4] However, hypocholesterolemic activity of rifampicin in humans has not been observed so far. In a study in 19 healthy subjects who received 600 or 1200 mg of rifampicin daily for 14 days, no changes in plasma cholesterol or triglycerides were detected.[5] In mice receiving 40 mg/kg twice daily, a rifampicin-induced hyperlipidemia has been described.[6] In 20 patients with tuberculosis, treatment with 10 mg/kg rifampicin produced a time-dependent increase in serum cholesterol, reaching 12% after 1 week, whereas serum triglycerides and phospholipids remained unchanged.[6]

Unexpectedly, in the course of the biological characterization in our laboratories some antituberculous rifamycin derivatives, including especially those with *N*-alkylpiperazinyl substituents in position 3 of the chromophore, were found to exert a potent hypolipidemic action. We therefore made a systematic study of the structure–activity relationships with particular attention to the influence of various substituents in position 3 of the chromophore. In addition, we succeeded in separating the hypolipidemic from the antibacterial activity, leading to compound **48**, which is devoid of any antibacterial activity but shows good hypolipidemic properties.

Chart I



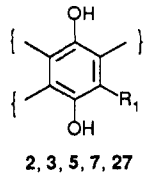
1 R = CH=N-N N–CH3
2 R = H
3 R = CHO
4 R = Br
5 R = NH_2
7 R = OH

Chemistry

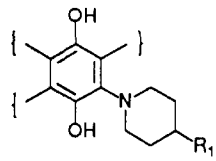
Series of 3-piperidinylrifamycins (**8–20**) and 3-piperazinylrifamycins (**21–27**) were synthesized from 3-

(1) Lettich, J.; Oppolzer, W.; Prelog. V. *Experientia* **1964**, *20*, 343.
(2) Wehrli, W.; Staehelin, M. *Antibiotics* Corcoran, J. W., Hahn, F. E., Eds.; Springer-Verlag: Berlin, Heidelberg, New York, 1974; Vol. III, p 252.
(3) Warner, S. D.; Stephenson, M. F. *Proc. Soc. Exp. Biol. Med.* **1971**, *137*, 194.
(4) Mathur, U.; Sharma, A. L.; Mishura, S. S.; Mathur, S. K. *J. Ind. Med. Assoc.* **1977**, *68*, 203.
(5) Ohnhaus, E. E.; Kirchhof, B.; Peheim, E. *Clin. Pharmacol. Ther.* **1979**, *25*, 591.
(6) Oberdisse, E.; Krause, I.; Goebel, D.; Radenbach, K. L. *Prax. Klin. Pneumol.* **1981**, *35*, 1059.

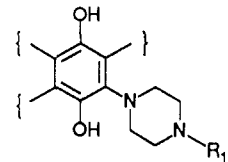
Table I. Hypolipidemic and Antibacterial Activities of C-3-Substituted Rifamycin Derivatives



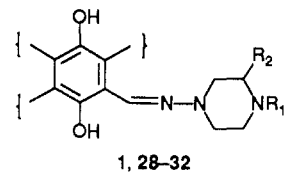
2, 3, 5, 7, 27



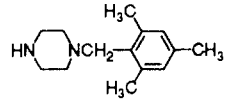
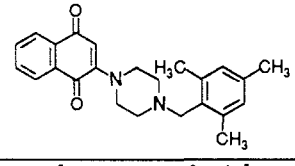
8–20



21–26



1, 28–32

compd	R_1	R_2	formula	mp, °C	dose,[a] mg/kg	% hypolipidemic activity vs control: serum chol	LDL-chol	HDL-chol	antibact. act.:[b] MIC, μg/mL
1	CH_3 (rimactane)	H	$C_{43}H_{58}N_4O_{12}$		100	−30 ± 4[f,g]	−31 ± 13	−27 ± 3[f]	0.003
2	H (rifamycin SV)		$C_{37}H_{47}NO_{12}$		50	+4 ± 2	+21 ± 9	+1 ± 5	0.001
3	CHO		$C_{38}H_{47}N_2O_{13}$		50	−5 ± 5	+19 ± 10	−14 ± 6	0.01
5	NH_2		$C_{37}H_{48}N_2O_{12}$		50	−2 ± 3	+19 ± 7	+6 ± 5	0.003
7	OH		$C_{37}H_{47}NO_{13}$		50	+4 ± 3	+41 ± 15	+15 ± 4	0.1
			3-Piperidinylrifamycins						
8	H		$C_{42}H_{56}N_2O_{12}$	201 dec	20	−32 ± 5[f]	−19 ± 19	−36 ± 2[f]	<0.1
9	OH		$C_{42}H_{56}N_2O_{13}$	199 dec	20	0 ± 4	+13 ± 17	−3 ± 3	<0.1
10	=O		$C_{42}H_{54}N_2O_{13}$	192 dec	20	+1 ± 6	+53 ± 16[f]	−9 ± 2	<0.1
11	CH_2OH		$C_{43}H_{58}N_2O_{13}$	208–209 dec	20	−14 ± 4[f]	−7 ± 7	−10 ± 5	<0.1
12	$COOC_2H_5$		$C_{45}H_{60}N_2O_{14}$	186–187	20	−16 ± 4[f]	+5 ± 7	−16 ± 5[f]	<0.1
13	$CONH_2$		$C_{43}H_{57}N_3O_{13}$	202 dec	20	−9 ± 5	+11 ± 5	−6 ± 7	<0.1
14	CH_3		$C_{43}H_{58}N_2O_{12}$	185–186 dec	20	−23 ± 3[f]	+23 ± 15	−30 ± 6[f]	<0.1
15	C_2H_5		$C_{44}H_{60}N_2O_{12}$	173 dec	20	−57 ± 7[f]	−51 ± 7[f]	−61 ± 4[f]	<0.1
16	$CH(CH_3)_2$		$C_{45}H_{62}N_2O_{12}$	175–177 dec	20	−61 ± 6[f]	−54 ± 5[f]	−61 ± 8[f]	<0.1
17	$C(CH_3)_3$		$C_{46}H_{64}N_2O_{12}$	185 dec	20	−51 ± 5[f]	−52 ± 6[f]	−48 ± 5[f]	<0.1
18	cyclopentyl		$C_{47}H_{64}N_2O_{12}$	188 dec	20	−42 ± 4[f]	−41 ± 13	−40 ± 4[f]	<0.1
19	CH_2-C_6H_5		$C_{49}H_{62}N_2O_{12}$	159–160 dec	20	−53 ± 5[f]	−66 ± 5[f]	−51 ± 3[f]	0.04
20	CH_2-cyclohexyl		$C_{49}H_{68}N_2O_{12}$	174 dec	20	−62 ± 3[f]	−31 ± 6[f]	−67 ± 1[f]	1
			3-Piperazinylrifamycins						
21	$CH_2CH(CH_3)_2$		$C_{45}H_{63}N_3O_{12}$	170 dec	50	−50 ± 5[f]	−64 ± 5[f]	−60 ± 2[f]	0.005
22	$(CH_2)_{11}CH_3$		$C_{53}H_{79}N_3O_{12}$	150 dec	20	−56 ± 4[f]	−68 ± 6[f]	−53 ± 3[f]	0.15
23	CH_2-C_6H_5		$C_{48}H_{61}N_3O_{12}$	171–172 dec	50	−29 ± 5[f]	−56 ± 3[f]	−36 ± 2[f]	0.004
24	CH_2-(4-Cl)C_6H_4		$C_{48}H_{60}N_3O_{12}Cl$	170–172 dec	50	−74 ± 2[f]	−85 ± 1[f]	−73 ± 3[f]	0.002
25	CH_2-(2,4,6-CH_3)C_6H_2[c]		$C_{51}H_{67}N_3O_{12}$	178–181 dec	20	−63 ± 4[f]	−79 ± 9[f]	−58 ± 5[f]	0.01
26	CH_2-(4-$C(CH_3)_3$)C_6H_4		$C_{52}H_{69}N_3O_2$	194 dec	20	−70 ± 3[f]	−70 ± 6[f]	−71 ± 1[f]	0.5
27			$C_{42}H_{55}N_3O_{14}$	>230 dec	50	−2 ± 5	+53 ± 7	+5 ± 4	0.5
			3-Hydrazonorifamycins						
28	cyclopentyl	H[d]	$C_{47}H_{64}N_4O_{12}$		50	−62 ± 4[f]	−60 ± 2[f]	−59 ± 9[f]	0.01
29	CH_2-(2,4,6(CH_3)$_3$)C_6H_2	H	$C_{52}H_{68}N_4O_{12}$	176–180	50	−81 ± 1[f]	−39 ± 4[f]	−79 ± 2[f]	0.05
30	CH_2-naphtyl	H	$C_{53}H_{64}N_4O_{12}$	196–200	50	−69 ± 1[f]	−35 ± 4[f]	−75 ± 1[f]	0.02
31	$-(CH_2)_4-$		$C_{46}H_{62}N_4O_{12}$	205–210 dec	50	−49 ± 3[f]	−27 ± 4[f]	−53 ± 1[f]	0.005
32	$-CH_2CH(C_2H_5)CH_2CH_2-$[e]		$C_{48}H_{66}N_4O_{12}$	173–180 dec	50	−62 ± 2[f]	−31 ± 5[f]	−69 ± 4[f]	0.005
33			$C_{14}H_{22}N_2$	122–125	50	−7 ± 5	0 ± 4	−5 ± 3	128
34			$C_{24}H_{26}N_2O_2$	138–140	50	−1 ± 2	+18 ± 6	+6 ± 2	>128

[a] Compounds were given for 4 days with two doses on the last day. [b] Against *S. aureus* 10B. [c] CGP 7040.[8-10] [d] Rifapentine (MDL 473).[11,12] [e] CGP 29 861.[10,27] [f] Statistically significant depression with $p < 0.05$ by Student's t test. [g] Values give mean ± SEM of four serum pools of pairs of rats.

bromorifamycin S (**4**) and the corresponding substituted piperidine or piperazine and 3-hydrazonorifamycins (**28–32**) from 3-formylrifamycin SV (**3**) and the corresponding hydrazine as outlined in Scheme I.

The naphthoquinone derivative (**34**) was obtained by reaction of 1,4-naphthoquinone with *N*-(2,4,6-trimethylbenzyl)piperazine (**33**) (Table I). 3,4-Imidazolylrifamycins (**35**, **36**) were obtained from 3-amino-4-iminorifamycin S (**6**) by reaction with *N*-benzyl-*N'*-formylpiperazine dimethyl acetal or 4-isobutylpiperidone (Scheme II). The 3,4-quinoxalinorifamycin (**37**) and the 3,4-benzoxazinorifamycin (**38**) were obtained by reaction of rifamycin S (**2**) with 3,4-diaminotoluene or 3-hydroxy-4-aminotoluene, respectively.

Starting from the quinone form of 3-[*N'*-(2,4,6-trimethylbenzyl)-*N*-piperazinyl]rifamycin SV (**25**), the following modifications were made (Scheme III): Hydrogenation of the two conjugated double bonds in the ansa ring with Pd/C gave a diastereomeric mixture of the tetrahydro derivative **39**; with PtO_2 as a catalyst, hexahydro derivative **40** was obtained (diastereomeric mixture). Reaction of **25** with diazomethane gave the 8-*O*-methyl

Table II. Hypolipidemic and Antibacterial Activities of C-3, C-4-Substituted Rifamycin Derivatives

compd	formula	mp, °C	dose,[a] mg/kg	% hypolipidemic activity vs control: serum chol	LDL-chol	HDL-chol	antibact act.:[b] MIC, μg/mL
35	$C_{49}H_{61}N_5O_{11}$	190 dec	50	−27 ± 5[d,e]	−23 ± 9[d]	−33 ± 4[d]	0.005
36[c]	$C_{48}H_{62}N_4O_{11}$		50	−7 ± 3	+14 ± 6	−21 ± 6	0.001
37	$C_{44}H_{51}N_3O_{11}$		50	−14 ± 4	−21 ± 7	not tested	<0.008
38	$C_{44}H_{60}N_2O_{12}$		50	−14 ± 3[d]	−21 ± 10	−21 ± 3[d]	0.001

[a] Compounds were given for 4 days with 2 doses on the last day. [b] Against *S. aureus* 10B. [c] Rifabutin (ansamycin).[13,14] [d] Statistically significant depression with $p < 0.05$ by the Student's t test. [e] Values given mean ± SEM of four serum pools of pairs of rats.

Table III. Hypolipidemic and Antibacterial Activities of Derivatives of 3-[(*N*-Trimethylbenzyl)piperazinyl]rifamycin SV **25**

compd	formula	mp, °C	dose,[a] mg/kg	% hypolipidemic activity vs control: serum chol	LDL-chol	HDL-chol	antibact. act.:[e] MIC, μg/mL	inhibn of RNA polymerase: IC_{50}, μg/mL
25	$C_{51}H_{67}N_3O_{12}$		10	−35 ± 7[c,d]	−43 ± 4[c]	−38 ± 6[c]	0.01	0.3
39	$C_{51}H_{71}N_3O_{12}$		10	−40 ± 3[c]	−59 ± 3[c]	−36 ± 4[c]	0.2	6.5
40	$C_{51}H_{73}N_3O_{12}$		10	−30 ± 4[c]	−32 ± 5[c]	−27 ± 4[c]	2	8.0
41	$C_{52}H_{69}N_3O_{12}$	157–162	10	−4 ± 4	+1 ± 5	−6 ± 5	1	nt[f]
42	$C_{51}H_{65}N_3O_{12}$	160–162	10	−11 ± 4	+1 ± 8	−15 ± 6	64	nt
43	$C_{51}H_{65}N_3O_{12}$	162–164 dec	10	−18 ± 6[c]	−1 ± 7	−23 ± 4[c]	2	nt
44	$C_{54}H_{71}N_3O_{12}$	>190 dec	10	−14 ± 4[c]	−6 ± 4	−27 ± 3[c]	8	nt
45	$C_{55}H_{71}N_3O_{14}$	165–169	10	−24 ± 3[c]	−19 ± 4	−24 ± 4[c]	4	nt
46	$C_{50}H_{63}N_3O_{11}$	>196 dec	10	0 ± 4	−9 ± 2	−4 ± 4	>128	>100
47	$C_{56}H_{73}N_3O_{13}$[b]	190–193 dec	20	−48 ± 4[c]	−32 ± 9[c]	−53 ± 5[c]	0.5	nt
48	$C_{61}H_{81}N_3O_{14}$[b]	157–163	10	−30 ± 4[c]	−55 ± 5[c]	−33 ± 1[c]	64	>100

[a] Compounds were given for 4 days with two doses on the last day. [b] Tested as quinone. [c] Statistically significant depression with $p < 0.05$ by Student's t test. [d] Values give mean ± SEM of four serum pools of pairs of rats. [e] Against *S. aureus* 10B. [f] Not tested.

ether (**41**). The two hydroxyl groups at C-21 and C-23 in the ansa chain were modified by three different methods: (i) Oxidation of **25** with pyridinium dichromate gave a mixture of the 21-keto and 23-keto derivatives **42** and **43** as main products, which were separated by silica gel chromatography. (ii) The 21,23-acetonide (**44**) was obtained from **25** with dimethoxypropane in acetone. (iii) Acetylation of **25** with acetic anhydride in pyridine gave a mixture of acetates with the 21,23-diacetyl compound (**45**) as the main product. Epoxide **46** with an additional ether ring within the ansa ring was formed when **25** was treated with formic acid. The pivaloyl groups were introduced into **25** with pivaloyl chloride in pyridine. In the first step, the 8-*O*-monopivaloyl compound **47** is formed. Further acylation under these conditions did not give the expected 8,21-dipivaloyl derivative as main product. To our surprise, the second pivaloyl group in compound **48** is attached to the amide nitrogen. Since the 8,21-dipivaloyl derivative was also isolated as a minor product, an extensive spectroscopic comparison (^{1}H NMR, ^{13}C NMR, ^{15}N NMR) of the two compounds was possible. The postulated structure of compound **48** is therefore well established. The structure determination of the 8-*O*,*N*-dipivaloyl derivative **48** and some of its rather unexpected rearrangement products by spectroscopic methods including X-ray analysis will be published elsewhere.[7]

Biological Results and Discussion

The compounds were tested for hypolipidemic activity in male rats by giving five or seven repetitive oral doses. Serum lipoproteins were separated by ultracentrifugation, and cholesterol was determined in the serum and in the low-density and the high-density lipoprotein (LDL and HDL) fractions. Serum triglycerides were routinely measured, but are shown only for selected compounds. Antibacterial activity in vitro was tested against *Staphylococcus aureus* S 10B. From selected compounds, the activity against DNA-dependant RNA polymerase from *Escherichia coli* was tested. The hypolipidemic and antibacterial activities are shown in Tables I–V.

Of all the rifamycin derivatives tested, the most potent compounds were found among the 3-piperidinylrifamycins (compounds **8**, **15–20**) and the 3-piperazinylrifamycins

(7) To be published elsewhere.

Table IV. Hypolipidemic Profile of Compound **48** and its Parent Compound (**25**)

compd	dose,[a] mg/kg	% serum lipids vs control: serum chol	LDL-chol	HDL-chol	serum triglycerides
		A. Normal Rat			
48	1	−5 ± 6[c]	−8 ± 20	0 ± 4	+14 ± 10
	3	−4 ± 3	−34 ± 10	−4 ± 5	+14 ± 10
	10	−19 ± 5[b]	−56 ± 5[b]	−21 ± 6	−17 ± 9
	30	−27 ± 4[b]	−51 ± 5[b]	−34 ± 4[b]	−22 ± 7[b]
25	1	0 ± 4	−11 ± 5	−8 ± 4	+11 ± 9
	3	−11 ± 4	−28 ± 10	−14 ± 3	−3 ± 9
	10	−42 ± 4[b]	−53 ± 5[b]	−46 ± 1[b]	−18 ± 12
	30	−72 ± 2[b]	−64 ± 5[b]	−69 ± 10[b]	−50 ± 7[b]
		B. Hypercholesterolemic RICO Rat			
48	3	−12 ± 3[b]	−35 ± 3[b]	−6 ± 7	−11 ± 8
	10	−29 ± 3[b]	−53 ± 7[b]	−24 ± 4[b]	−32 ± 3[b]
	30	−50 ± 2[b]	−69 ± 2[b]	−45 ± 3[b]	−47 ± 5[b]
25	3	−8 ± 3[b]	−29 ± 3[b]	−7 ± 3	+8 ± 10
	10	−30 ± 4[b]	−42 ± 5[b]	−30 ± 3[b]	−17 ± 7[b]
	30	−65 ± 4[b]	−68 ± 5[b]	−72 ± 5[b]	−55 ± 3[b]

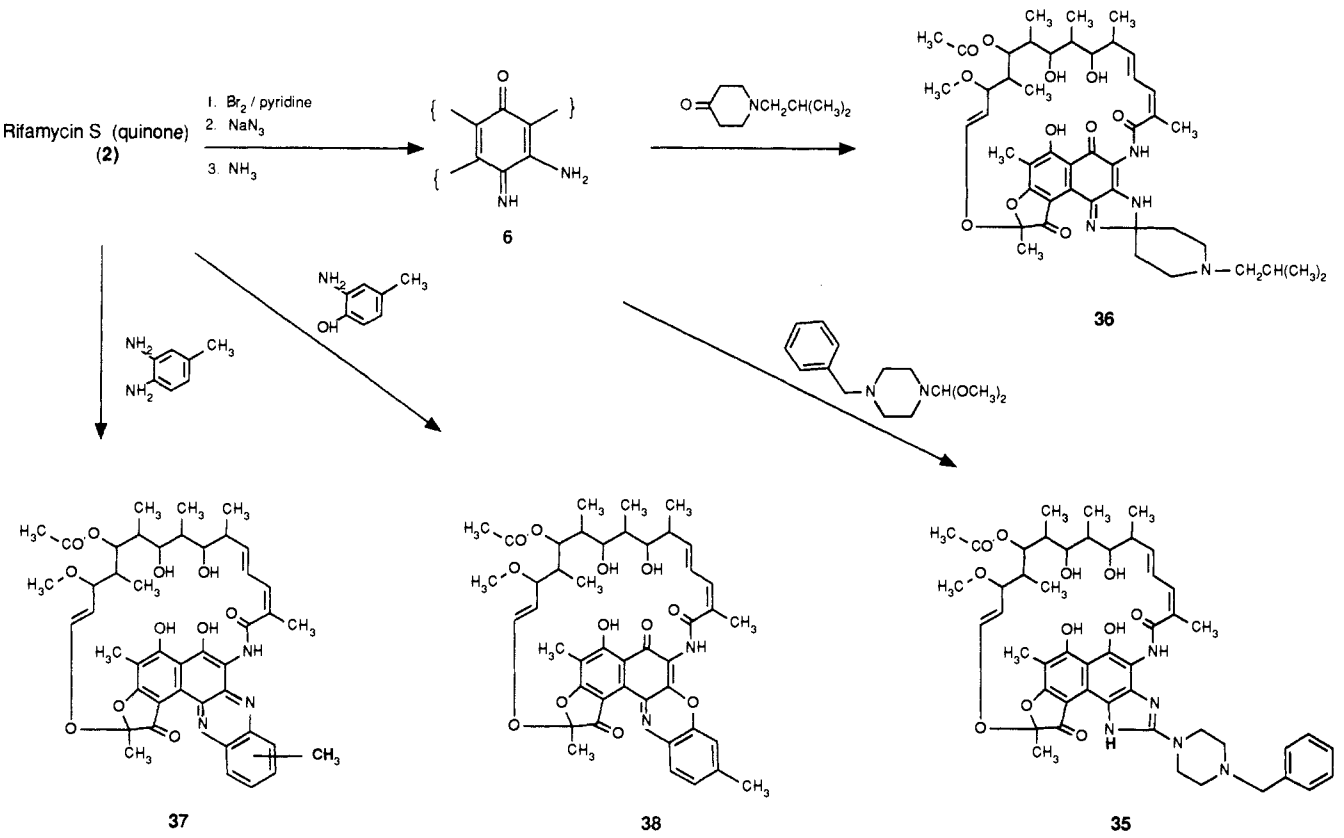
[a] Compounds were given for 7 days. [b] Statistically significant depression with $p < 0.05$ by Student's t test. [c] Values give mean ± SEM of four serum pools of pairs of rats.

Table V. Comparison of Compound **48** with Reference Compounds

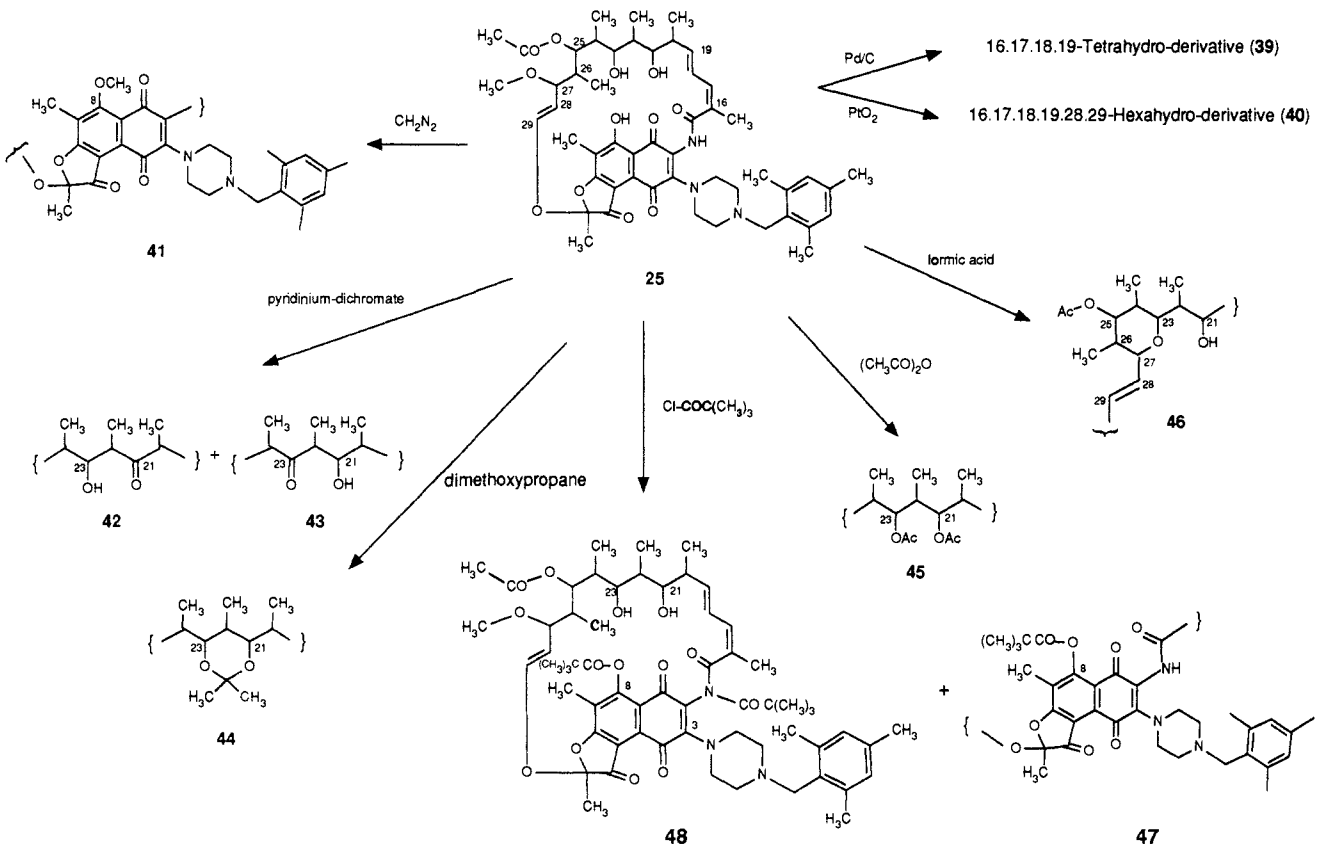
compd	dose,[a] mg/kg	% serum lipids vs control: serum chol	LDL-chol	HDL-chol
48	10	−16 ± 6[c,d]	−26 ± 6	−11 ± 4
	30	−33 ± 5[c]	−68 ± 6[c]	−27 ± 7[c]
clofibrate[b]	30	−15 ± 6	−8 ± 17	−13 ± 5
	100	−30 ± 4[c]	+14 ± 18	−34 ± 5[c]
nicotinic acid[b]	30	−6 ± 5	−32 ± 6[c]	−1 ± 1
	100	−10 ± 4[c]	−52 ± 5[c]	−13 ± 11

[a] Compounds were given for 7 days. [b] Reference compounds were commercially available. [c] Statistically significant depression with $p < 0.05$ by Student's t test. [d] Values give mean ± SEM of four serum pools of pairs of rats.

Scheme II



Scheme III



(compounds **21–26**) (Table I). In the 3-piperidinylrifamycin series, the most active compounds lowered serum cholesterol as well as LDL- and HDL-cholesterol concentrations by more than ~50% at doses of 5 × 50 mg/kg (compounds **15–17, 19, 20**). All these derivatives have a lipophilic alkyl substituent in position 4 of the piperidine moiety. On the other hand, derivatives with more polar substituents at the piperidine ring (e.g. hydroxy, hydroxymethyl, keto, ester, or amide (compounds **9–13**)) showed no hypolipidemic activity.

Among the 3-piperazinylrifamycins, again derivatives with lipophilic *N*-alkyl and especially *N*-benzyl substituents exerted the most pronounced effects on the lipid parameters (Table I). The most potent derivatives (compounds **21**, **22**, **24–26**) lowered serum cholesterol by 50–75% and LDL- and HDL-cholesterol by 53–85% at doses of 5 × 20 or 5 × 50 mg/kg. By contrast, compound **27**, without an *N*-alkylsubstituent but having an additional carboxyl function at the piperazine ring, showed no hypolipidemic effect, indicating that the lipophilic character of the piperazinyl side chain is important for lipid-lowering effects. By comparison, the minimum dose of rifampicin (**1**) needed to elicit a hypocholesterolemic effect was found to be 100 mg/kg (Table I), which is approximately 10 times the corresponding dose of the most potent derivatives. Neither *N*-(trimethylbenzyl)piperazine (**33**), the side chain of the potent hypolipidemic piperazinylrifamycin derivative **25**, which is a derivative presently under evaluation as a drug against opportunistic infections due to *Mycobacterium avium* complex (MAC) in AIDS patients,[8-10] nor 2-[*N*′-(2,4,6-trimethylbenzyl)-*N*-piperazinyl]-1,4-naphthoquinone (**34**) showed any lipid-lowering activity when tested alone, indicating that the ansa ring of the rifamycins is an important moiety for hypolipidemic activity.

Some 3-hydrazonorifamycins (compounds **28–32**), bearing lipophilic substituents at the piperazine ring, also showed hypolipidemic activity. This includes **28**, which is known as rifapentine (MDL-473, Merrel-Dow-Lepetit) and is an antituberculous drug at present in clinical phase II in various countries, including China.[11,12] These derivatives were, however, less potent than the piperidinyl- and piperazinylrifamycins particularly in lowering LDL-cholesterol: At doses of 5 × 50 mg/kg, serum cholesterol was lowered by 49–81% and LDL-cholesterol was lowered by 27–60%. It is noteworthy that compound **29** has the same lipophilic *N*-(trimethylbenzyl)piperazinyl side chain as that giving good hypolipidemic potency in the corresponding piperazinylrifamycin series (compound **25**).

Like the parent compound rifamycin SV (**2**), many other rifamycin derivatives, including 3-formylrifamycin SV (**3**), 3-aminorifamycin SV (**5**), 3-hydroxyrifamycin SV (**7**) (Chart I and Table I), and all the 3,4-substituted rifamycin derivatives (**35–38**) (Table II), showed no significant hypolipidemic activity. Although 3,4-imidazolorifamycin **35** and 3,4-imidazolinorifamycin **36** have an *N*-(alkylbenzyl)piperazinyl or *N*-alkylpiperidinyl side chain, respectively, they possess only marginal hypolipidemic activity. Compound **36** is known as rifabutin (ansamycin, LM 427, Farmitalia-Carlo Erba) and is at present in phase III trials for the treament of MAC infections in AIDS patients.[13-15]

Pharmacokinetic studies in animals have shown that some piperazinylrifamycins have much longer plasma half-lives than rifampicin. This is especially true of the (benzylpiperazinyl)rifamycins[8-10] and the lipophilic piperazinylhydrazones,[10] which display good hypolipidemic activity. Piperazinylrifamycin derivative **25** or hydrazonorifamycin **32** have plasma half-lives of more than 70 h in rats and 30 h in man, respectively.[8,10] The possibility that there may be a correlation between hypolipidemic potency and plasma half-life, however, remains to be investigated more thoroughly. Most rifamycin derivatives exhibiting a high degree of lipid-lowering activity were at the same time very potent antibacterials with MIC values <0.1 μg/mL, and their long-term use as hypolipidemic drugs would consequently lead to the development of resistance to rifampicin and disturbance of the gastrointestinal flora. To exclude these unwanted effects, the antibacterial and the hypolipidemic activity must therefore be dissociated. It is well-known that the antibacterial action of the rifamycins is due to inhibition of the bacterial RNA polymerase.[2] From structure–activity studies of the numerous derivatives, it is also known that the two hydroxyl groups at position C-21 and C-23 of the ansa chain together with the phenolic group at C-8 and the quinone/hydroquinone function at C-1 of the chromophore are essential for antibacterial activity. The three double bonds of the ansa chain are to a certain extent also important, because they fix the ansa ring into a conformation which is important for optimal interaction with the polymerase. Modification of these positions of the molecule was therefore the obvious approach to the problem of dissociating the hypolipidemic from the antibacterial activity. 3-[*N*′-(2,4,6-Trimethylbenzyl)-*N*-piperazinyl]rifamycin SV (**25**) was selected as a model compound for this investigation (Table III).

The tetrahydro and hexahydro derivatives (**39** and **40**) showed similar hypocholesterolemic potency but 100–200 times less antibacterial activity than the parent compound **25**. Their antibacterial activity was, however, still too marked for them to be of further interest. In the 8-*O*-methyl ether (**41**) as well as in the 21- and 23-keto derivatives (**42** and **43**), acetonide **44**, or cyclic ether derivative **46**, in which one or even more of the functional groups essential for antibacterial activity were blocked, the expected loss of antibacterial activity was observed. To our surprise, the hypolipidemic activity was also markedly decreased. These findings indicated that the hypolipidemic activity also has a high degree of structural specificity, comparable to that of the antibacterial activity. Although the metabolically unstable 21,23-diacetyl derivative **45** showed less hypolipidemic activity than the parent compound **25**, the reduction in antibacterial activity indicated that acylation with more bulky acyl groups might lead to useful compounds. In fact, the 8-*O*,*N*-dipivaloyl derivative (**48**) turned out to fulfill most of our requirements for dissociation of the antibacterial from the hypolipidemic activity (Tables III–V). With an MIC value of 64 μg/mL against various strains of *S. aureus* and an IC_{50} of >100 μg/mL for inhibition of DNA-dependent RNA polymerase of *E. coli*, it is devoid of any significant in vitro antibacterial activity. Both these values are more than 1000 times higher than the corresponding values of rifamycin derivatives with potent antibacterial activity (e.g. rifampicin (**1**) or compound **25**). In mice, infected with a strain of *S. aureus*, compound **48**, given in a single oral or

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subcutaneous dose of 200 mg/kg, displayed no antibacterial activity, whereas in the same experimental system, the parent compound **25**, into which compound **48** might be metabolized, showed ED_{50} values of 15 and 40 mg/kg for oral and subcutaneous administration, respectively. In addition, after a single or two, consecutive oral doses of 100 mg/kg of derivative **48** administered to rats, no antibacterial activity against *S. aureus* was found in the plasma. The limit of detection of compound **25** would have been 1.6 μg/mL. A direct comparison of the hypolipidemic activity of compound **48** and its parent compound **25** at doses between 1 and 30 mg/kg is shown in Table IV. In normal rats, the capacity to decrease LDL-cholesterol, the most important lipidic risk factor in coronary heart disease, was fully retained in **48**. The minimum effective dose of both compounds was around 3 mg/kg, and the effect reached a plateau of about 50–60% reduction at 10 mg/kg. The effect on serum and HDL-cholesterol was less marked, reaching a reduction of 34% at 30 mg/kg compared to the 69% reduction produced by compound **25**. Serum triglycerides were also decreased by both compounds at 10 and 30 mg/kg. The hypotriglyceridemic effect, however, was less pronounced than the hypocholesterolemic effect of both compounds. The two compounds **48** and **25** were also tested in a genetically hypercholesterolemic strain of rats (RICO).[16] The hypolipidemic effects in the RICO strain were similar to those in normolipidemic rats.

In comparison with known hypolipidemic drugs, compound **48** was 3 times more potent than clofibrate in lowering serum and HDL-cholesterol and 3 times more potent than nicotinic acid in lowering LDL-cholesterol.

On the basis of these interesting results, compound **48** was selected for further evaluation as a hypolipidemic drug.

Experimental Section

Biological Methods. Hypolipidemic Activity. Groups of eight male Wistar rats of 200–230-g body weight were treated orally with the drugs, dissolved in polyethylene glycol 400. Each experiment comprised one control group receiving the vehicle, one group receiving nicotinic acid at 50 mg/kg, and eight test groups. Two different schemes of treatment were used: either the drug was administered once daily at 8 a.m. on seven consecutive days and the animals were killed 24 h after the last administration or the animals received the drugs at 8 a.m. on four consecutive days and a second dose at 4 p.m. on the fourth day. In this case the animals were killed 16 h after the last dose. Food was withdrawn 16 h prior to death. The rats were killed by bleeding from the neck under anaesthesia with pentothal and serum was prepared. To isolate the lipoprotein fractions, the sera of pairs of rats were pooled, 0.05% EDTA and 0.01% thimerosal were added, and the lipoproteins were separated by ultracentrifugation using density limits of 1.006 and 1.040 g/cm^3 for VLDL and LDL, respectively.[17] The infranatant after removal of the LDL fraction was taken as the HDL fraction. Cholesterol and triglycerides were determined enzymatically with the reagent kits from Ames and Boehringer, respectively. The following concentrations of serum lipids were found in the control groups of 10 experiments (mean ± SEM): serum cholesterol 68.8 ± 2.7 mg/100 mL, LDL-cholesterol 8.2 ± 0.4 mg/100 mL, HDL-cholesterol 54.9 ± 2.2 mg/100 mL; and serum triglycerides 65.1 ± 2.0 mg/100 mL.

Selected compounds were tested in a genetically hypercholesterolemic strain of rats (RICO, Tif: RAI,f,SPF).[16] They showed the following concentration of serum lipids (mean ∓ SEM of 16 control animals): serum cholesterol 138.5 ∓ 3.5 mg/100 mL, LDL-cholesterol 24.4 ∓ 1.9 mg/100 mL, HDL-cholesterol 108.4 ∓ 3.1 mg/100 mL, and serum triglycerides 73.5 ∓ 3.5 mg/100 mL.

Antibacterial Activity in Vitro. The minimum inhibitory concentration (MIC) for *S. aureus* 10B (SA10B) in vitro was determined by the agar-dilution technique.[18] SA 10B was stored in the vapor phase of a liquid nitrogen vessel at −120 to −135 °C. It was thawed for 30 min before the beginning of the experiment and then used without subculturing. The surfaces of the plates of DST agar OXOID containing 2-fold dilutions of the drug were inoculated with 10^4 viable organisms, applied with the Steer's multiple-point replicator.[19] The plates were incubated overnight at 37 °C. The lowest drug concentration inhibiting bacterial growth by comparison with the control cultures was taken to be the MIC.

Antibacterial Activity in Vivo. Antibacterial Activity against *S. aureus* in Mice. Female NMRI mice (18–24 g) were infected by intraperitoneal injection with *S. aureus* 10B. The inoculum of the test organism was prepared from a standardized culture frozen in liquid nitrogen and diluted in BHI broth with or without 2% hog gastric mucin. The inoculum used corresponded to 3–30 times the LD_{50}. Groups of five mice at each dose level were treated once orally or subcutaneously with aqueous solutions of the test substance 30 min after infection. The ED_{50}, 50% protective dose value, expressed in mg/kg, was calculated by the probit method.

Metabolism in the Rat. Rats (Tif: RAI,f,SPF) had access to food and water ad libitum. Compound **48** (100 mg/kg) was orally administered by gavage as a suspension (1% in water, sodium bicarbonate, and ethanol). Blood was drawn by retroorbital puncture. In a first group of rats, blood was drawn 4, 8, and 24 h after administration of compound **48**. In a second group, a second dose of 100 mg/kg was given 24 h after the first dose and blood was drawn 4 and 8 h after the second dose. Blood was centrifuged in heparinized tubes and the antibacterial activity in the plasma was determined with the agar-diffusion method and *S. aureus* 10B as the test organism.

Inhibition of RNA Polymerase from *E. coli*. The inhibition of the DNA-dependent RNA polymerase from *E. coli* in vitro was assayed on ether-extracted cells of *E. coli* with increased permeability according to a method described in the literature.[2]

Chemistry. ^{1}H NMR spectra were recorded on a Varian XL-100-12, Varian HA-100-D, or Bruker Spectrospin HX-360 spectrometer with TMS as an internal standard. ^{13}C NMR spectra were recorded on a Varian XL-100-15 spectrometer. Fast atom bombardment mass spectra (FAB-MS) were recorded with a ZAB HF spectrometer (VG-Manchester). For column chromatography, silica gel Merck 60 (0.063 × 0.20 mm) was used. For TLC, silica gel plates F254 (Merck) were used. Since elemental analyses of rifamycins are usually unsatisfactory owing to inclusion of solvent in the crystals, the derivatives were characterized by their mass spectra and their typical ^{1}H NMR and ^{13}C NMR spectra. Purity of the derivatives was checked either by DC or HPLC analysis.

Rifampicin (**1**), rifamycin SV (**2**) and 3-formylrifamycin SV (**3**) were obtained by the production plant of Ciba-Geigy, Ltd. 3-Bromorifamycin S (**4**),[20] 3-aminorifamycin (**5**),[21] and 3-amino-4-deoxo-4-iminorifamycin S (**6**)[13] were prepared according to published procedures. 3-Hydroxyrifamycin SV (**7**) was isolated from a recombinant strain R-21 of *Nocardia mediterranei*.[22] The imidazolorifamycin (**35**)[23] and the imidazolinoderivative (**36**, rifabutin, ansamycin)[13] were prepared from **6** according to procedures described in the literature. Quinoxaline **37** and benzoxazine **38** were prepared from rifamycin S (**2**) and 3,4-diaminotoluene or 4-hydroxy-5-aminotoluene, respectively, as described in the literature.[24] Where not otherwise indicated, the physicochemical characterization of the rifamycin derivatives was done with the

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quinone form, whereas in the biological experiments the hydroquinone form was used. For oxidation of the hydroquinone to the quinone, an excess of MnO_2 in CH_2Cl_2 or a 10% aqueous solution of $K_3[Fe(CN)_6]$ in a 1:1 mixture of CH_3OH–10% $NaHCO_3$ was used. The quinones were reduced to their corresponding hydroquinones in CH_3OH with a 10% aqueous solution of sodium ascorbate. Both methods are described in the literature.[24]

General Method for the Preparation of 3-Piperidinyl- and 3-Piperazinylrifamycins. 3-Piperidinylrifamycins (**8–20**) and 3-piperazinylrifamycins (**21–27**) were prepared either from rifamycin S (**2**) or 3-bromorifamycin S (**4**), according to procedures described in the literature.[8,25,26]

3-[*N'*-(2,4,6-Trimethylbenzyl)-*N*-piperazinyl]rifamycin SV (25).[8] To a solution of 154 g (0.2 mmol) of 3-bromorifamycin S (**4**) in 850 mL of THF 52.5 g (0.23 mmol) were added *N*-(2,4,6-trimethylbenzyl)piperazine (**33**) and 29 mL (0.21 mmol) of triethylamine. The dark-blue suspension was stirred at room temperature for 3 h until no starting material was visible via TLC. CH_2Cl_2 (1700 mL) was added and the solution was washed with 1700 mL of 10% aqueous citric acid. The organic phase was washed with water, dried over Na_2SO_4 and evaporated. The dark, crude product was dissolved in 650 mL of warm ethanol, 360 mL of a 10% aqueous sodium ascorbate solution and 28 g of citric acid were added, and the mixture was stirred at room temperature for 5 min. After the addition of 300 mL of water, the product **25** started to crystallize. The crystals were filtered off, washed with cold ethanol/water 4:2, dried, and recrystallized from 95% aqueous ethanol. Compound **25** (135 g, 74% yield) of mp 178–181 °C was obtained in the hydroquinone form. MS (FAB) *m/z* 914 (M + H). Anal. ($C_{51}H_{67}N_3O_{12}$) C, H, N.

For spectroscopic characterization, the hydroquinone was oxidized to the quinone: MS (FAB) *m/z* 912 (M + H); ^{1}H NMR ($CDCl_3$) δ 0.20, 0.71, 0.87, 1.03 (4 × d, 12 H, CH_3-31,33,33,34), 1.74 (s, 3 H, CH_3-13), 2.08 (s, 6 H, CH_3-30 + CH_3-36), 2.26 (s, 6 H, CH_3-14 + 1 aromat-CH_3), 2.34 (s, 6 H, 2 aromat-CH_3), 2.45–2.73 (2 × m, 4 H, 2 piperazinyl-CH_2), 3.10 (s, 3 H, 37-OCH_3), 3.05–3.15 (m, 2 H, H-21 + H-23), 3.3 (m, 1 H, H-27), 3.4–3.9 (2 × m, 4 H, 2 piperazinyl-CH_2), 3.51 (d, 2 H, benzyl-CH_2), 5.10 (m, 2 H, H-25 + H-28), 6.06 (d, 1 H, H-29), 6.19 (m, 1 H, H-19), 6.32 (d, 1 H, H-17), 6.82 (s, 2 H, aromat H), 7.08 (m, 1 H, H-18), 7.44 (s,1 H, amide-NH), 13.39 (s, 1 H, OH-8); ^{13}C NMR ($CDCl_3$, selected data) δ 20.1 (aromat-CH_3) 20.9 (3 C, CH_3-30 + 2 aromat-CH_3), 51.2 (2 C) + 54.1 (2 C, 4 piperazinyl-C), 56.7 (benzyl-C), 57.5 (37-OCH_3), 73.4 (C-21), 74.4 (C-25), 77.3 (C-23), 78.3 (C-27), 117.2 (C-28), 125.8 (C-18), 130.0 (2 C) + 132.1 + 137.8 + 139.1 (2 C) (6 aromat C), 130.9 (C-16), 136.6 (C-17), 142.9 (C-19), 145.8 (C-29), 166.4 (C-8), 172.1 (C-15), 172.5 (C-6 + C-35), 179.5 (C-4), 185.2 (C-1), 193.1 (C-11). Anal. ($C_{51}H_{65}N_3O_{12}$) C, H, N.

Preparation of 3-Hydrazonorifamycins. The hydrazonorifamycins (**28–32**) were prepared from 3-formylrifamycin S (**3**) and the corresponding hydrazine according to methods described in the literature.[11,27]

Derivatives of 3-[*N'*-(2,4,6-Trimethylbenzyl)-*N*-piperazinyl]rifamycin SV (25). 16,17,18,19-Tetrahydro-3-[*N'*-(2,4,6-trimethylbenzyl)-*N*-piperazinyl]rifamycin SV (39). The hydroquinone of **25** (2.5 g, 2.75 mmol) in 200 mL of ethanol was hydrogenated over 200 g of Pd/C catalyst at room temperature for 4 h. The catalyst was filtered off and the filtrate was evaporated to dryness. A diastereometric mixture (2.5 g) of the tetrahydro derivative **39** was obtained. For physicochemical characterization, the hydroquinone was oxidized to the quinone: MS (FAB) *m/z* 916 (M + H), corresponding to $C_{51}H_{69}N_3O_{12}$; ^{1}H NMR ($CDCl_3$) δ 1.25 (d, 3 H, CH_3-30), 2.03 (s, 3 H, CH_3-36), 4.85 (d, 1 H, H-25), 5.18 (m, 1 H, H-28), 6.18 (d, 1 H, H-29), 7.46 (s, 1 H, amide-NH), 13.43 (s, 1 H, 8-OH), olefinic protons H-17,18,19 missing, all the other protons at chemical shifts similar to those in the parent compound **25**; ^{13}C NMR ($CDCl_3$, selected data) four additional signals of aliphatic carbons between δ 7 and 40, signals of olefinic protons C-16,17,18,19 missing, all the other C atoms at chemical shift similar to those in **25**.

16,17,18,19,28,29-Hexahydro-3-[*N'*-(2,4,6-trimethylbenzyl)-*N*-piperazinyl]rifamycin SV (40). The hydrodroquinone of **25** (3 g, 3.28 mmol) in 300 mL of ethanol was hydrogenated over 500 mg of PtO_2 catalyst at room temperature for 11 h. The catalyst was filtered off and the filtrate was evaporated. The residue was dissolved in CH_2Cl_2 and washed with 10% aqueous citric acid. The organic phase was dried and evaporated to a crude product. Chromatography on a silica gel column (ethyl acetate/cyclohexane) gave 1 g of the hexahydro product **40** as a diastereoisomeric mixture, which was oxidized to the quinone for physicochemical characterization: MS (FAB) *m/z* 918 (M + H), corresponding to $C_{51}H_{71}N_3O_{12}$; ^{1}H NMR ($CDCl_3$) δ 1.31 (d, 3 H, CH_3-30), 4.88 (d, 1 H, H-25), 7.51 (s, 1 H, amide-NH), 13.35 (s, 1 H, 8-OH), olefinic protons H-17,18,19,28,29 missing, all the other protons at chemical shifts similar to those in **25**; ^{13}C NMR ($CDCl_3$, selected data) six additional signals of aliphatic carbons between δ 7 and 40, signals of olefinic C-16,17,18,19,28,29 missing, all the other C atoms at chemical shifts similar to those in **25**.

8-*O*-Methyl-3-[*N'*-(2,4,6-trimethylbenzyl)-*N*-piperazinyl]rifamycin SV (41). Quinone **25** (2 g, 2.2 mmol) in 20 mL of ethyl ether was treated at 0 °C with 5 mL of a freshly prepared diazomethane solution in ether for 30 min. The solution was evaporated, redissolved in ethyl acetate, and washed with 10% aqueous citric acid. The organic phase was dried and evaporated, and the residue was chromatographed on silica gel with CH_2Cl_2/CH_3OH 99:1 as eluent to obtain 1 g of the *O*-methyl ether derivative **41** in the quinone form: MS (FAB) *m/z* 926 (M + H), corresponding to $C_{52}H_{67}N_3O_{12}$; ^{1}H NMR ($CDCl_3$) δ 2.09 (s, 3 H, CH_3-30), 3.07 (s, 3 H, 37-OCH_3), 3.92 (s, 3 H, additional 8-OCH_3), 5.1 (m, 2 H, H-25 + H-28), 6.06 (d, 1 H, H-29), 6.15 (m, 1 H, H-19), 6.32 (d, 1 H, H-17), 7.08 (m, 1 H, H-18), 7.55 (s, 1 H, amide-NH), 8-OH missing, all the other protons at chemical shifts similar to those in **25**; ^{13}C NMR ($CDCl_3$, selected data) δ 62.59 (8-OCH_3), 165.91 ± 166.08 (C-6 + C-8), all the other C-atoms at chemical shifts similar to those in **25**.

21-Keto-3-[*N'*-(2,4,6-trimethylbenzyl)-*N*-piperazinyl]rifamycin SV (42) and 23-Keto-3-[*N'*-(2,4,6-trimethylbenzyl)-*N*-piperazinyl]rifamycin SV (43). Quinone **25** (7 g, 8.42 mmol), dissolved in 140 mL of DMF, was oxidized with 15.9 g (42 mmol) of pyridinium dichromate at +5 °C for 7 h. The solution was concentrated in vacuo and ethyl acetate was added. The solution was washed several times with 10% aqueous citric acid and water, dried, and evaporated. Chromatography of the crude mixture of oxidizing products on a silica gel column with ethyl acetate/hexane 1:2 as eluent gave 950 mg of the 21-keto derivative **42** and 750 mg of the 23-keto derivative **43** in their quinone forms. 21-Keto derivative **42**: MS (FAB) *m/z* 910 (M + H), corresponding to $C_{51}H_{63}N_3O_{12}$; ^{1}H NMR ($CDCl_3$) δ 0.22, 0.75, 1.03, 1.25 (4 × d, 12 H, C-31,32,33,34), 5.07 (m, 2 H, H-25 + H-28), 5.40 (m, 1 H, H-19), 6.10 (d, 1 H, H-29), 6.19 (m, 1 H, H-19), 6.63 (m, 1 H, H-18), 7.34 (s, 1 H, amide-NH), 13.40 (s, 1 H, 8-OH), H-21 is missing, all the other protons at chemical shifts similar to those in **25**; ^{13}C NMR ($CDCl_3$, selected data) δ 73.87, 76.26, 77.00 (C-23,25,27), 211.13 (C-21), the signals of the other C-atoms at chemical shifts similar to those in **25**. 23-Keto derivative **43**: MS (FAB) *m/z* 910 (M + H); ^{1}H NMR ($CDCl_3$) δ 0.22, 1.06, 1.08, 1.25 (4 × d, 12 H, C-31,32,33,34), 4.93 (m, 1 H, H-28), 5.29 (d, 1 H, H-25), 5.61 (d, 1 H, H-29), 6.28 (d, 1 H, H-17), 6.46 (m, 1 H, H-18), 7.29 (s, 1 H, amide-NH), 13.28 (s, 1 H, 8-OH), H-23 is missing, all the other protons at chemical shifts similar to those in **25**; ^{13}C NMR ($CDCl_3$, selected data) δ 71.53, 76.26, 77.57 (C-21,25,27), 214.44 (C-23), the signals of the other C atoms at chemical shifts similar to those in **25**.

3-[*N'*-(2,4,6-Trimethylbenzyl)-*N*-piperazinyl]rifamycin SV 21,23-Acetonide (44). Acetonide **44** was prepared from 5 g (6.75 mmol) of quinone **25** with dimethoxypropane in acetone according to a procedure described for the preparation of rifamycin S 21,23-acetonide.[28] Chromatography of the crude product on silica gel gave 4 g of the desired acetonide **44** as the quinone: MS (FAB) *m/z* 952 (M + H), corresponding to $C_{54}H_{69}N_3O_{12}$); ^{1}H NMR ($CDCl_3$) two additional acetonide-CH_3 groups at δ 1.09, all the other protons at chemical shifts similar to those in **25**; ^{13}C NMR

(25) Bickel, H.; Kump, W. U.S. Patent 4.005.077, 1977.
(26) Kump, W. CH Patent 139674 (1973) and 10314 (1974).
(27) Traxler, P. CH Patent 22,1003/31 (1983) and U.S. Patent 4,681,938-A (1984).
(28) Kump, W.; Bickel, H. *Helv. Chim. Acta* **1973**, *56*(7), 2323.

($CDCl_3$) δ 25.2 + 25.5 (2 additional acetonide-CH_3), 99.4 (quat C of acetonide), 71.9 + 72.8 (C-21 + C-225), 77.4 (C-23), 79.4 (C-27), the signals of the other C-atoms at chemical shifts similar to those in **25**.

21,23-Diacetyl-3-[*N'*-(2,4,6-trimethylbenzyl)-*N*-piperazinyl]rifamycin SV (45). Quinone **25** (5 g, 5.47 mmol) in 50 mL of pyridine and 50 mL of acetic acid anhydride was left at room temperature for 3 h. The mixture was added to ice/water and extracted with CH_2Cl_2. The organic phase was dried and evaporated. For removal of the phenolic acetyl group, the crude product was dissolved in 50 mL of CH_3OH and 50 mL of a 10% aqueous Na_2CO_3 solution was added. CH_2Cl_2 was added after 15 min, the mixture was acidified with 10% aqueous citric acid, and the CH_2Cl_2 extract was dried and evaporated. From the crude mixture of various acetylation products, the 21,23-diacetyl derivative **45** (1.7 g) was obtained as quinone by chromatography on silica gel: MS (FAB) *m/z* 996 (M + H), corresponding to $C_{55}H_{69}N_3O_{14}$; ^1H NMR ($CDCl_3$) δ 0.15, 0.80, 0.88, 0.96 (4 × d, 12 H, CH_3-31,32,33,34), 4.52, 4.89 (2 × d, 2 H, H-21 + H-23), two additional acetyl-CH_3 at δ 2.00, the chemical shifts of the signals of the other protons are similar to those of compound **25**; ^{13}C NMR ($CDCl_3$, selected data) δ 35.54, 36.73, 39.41, 40.04 (C-20,22,24,26), 71.65, 76.20, 76.37, 78.82 (C-21,23,25,27), 170.18 (C-35), 170.35, 170.52 (2 additional acetyl-CH_3).

23-Dehydroxy-27-demethoxy-23,27-epoxy-3-[*N'*-(2,4,6-trimethylbenzyl)-*N*-piperazinyl]rifamycin SV (46). Epoxide **46** was prepared from 2.5 g (3.38 mmol) of quinone **25** in 40 mL of 99% formic acid according to the procedure described for the preparation of 23-dehydroxy-27-demethoxy-23,27-epoxyrifamycin S.[28] Chromatography of the crude product on silica gel gave 1.75 g of amorphous epoxide **46** as a quinone: MS (FAB) *m/z* 880 (M + H), corresponding to $C_{50}H_{61}N_3O_{11}$; ^1H NMR ($CDCl_3$, selected data) δ 5.0 (m, 1 H, H-28), 5.32 (m, 1 H, H-25), 5.71 (m, 1 H, H-29), 37-OCH_3 is missing.

8-*O*-Pivaloyl-3-[*N'*-(2,4,6-trimethylbenzyl)-*N*-piperazinyl]rifamycin S (47) and 8-*O*,*N*-Dipivaloyl-3-[*N'*-(2,4,6-trimethylbenzyl)-*N*-piperazinyl]rifamycin S (48). A solution of 25 g (27.5 mmol) of quinone **25** in 250 mL of pyridine and 35.5 mL of pivaloyl chloride was stirred at room temperature for 8 h until no starting material was detectable via TLC. To destroy excess pivaloyl chloride, 150 mL of methanol was then added and the solution was stirred for 1 h. The reaction mixture was evaporated in vacuo, the residue was dissolved in 300 mL of CH_2Cl_2 and filtered, and the filtrate was washed with 300 mL of water. The CH_2Cl_2 extract was dried and evaporated to dryness. The 8-*O*,*N*-dipivaloyl derivative **48** was obtained in the quinone form as wine-red crystals by crystallization from ether or ether/hexane: mp 157–163 °C; MS (FAB) *m/z* 1080 (M + H); ^1H NMR ($CDCl_3$) δ 0.71, 0.76, 0.85, 1.06 (4 × d, 12 H, CH_3-31,32,33,34), 1.42 + 1.46 (2 × s, 18 H, 6 pivaloyl-CH_3), 1.69 (s, 3 H, CH_3-13), 1.93 (s, 3 H, CH_3-36), 2.08 (s, 3 H, CH_3-30), 2.18 (s, 3 H, CH_3-14), 2.25 (s, 3 H, aromat-CH_3), 2.32 (s, 6 H, 2 aromat-CH_3), 2.38–2.67 (m, 4 H, 2 piperazinyl-CH_2), 2.97 (m, 1 H, H-23), 3.05 (m, 1 H, H-21), 3.15 (m, 1 H, H-27), 3.17 (s, 3 H, 37-OCH_3), 3.25–3.76 (m, 4 H, 2 piperazinyl-CH_2), 3.43 (d, 2 H, benzyl-CH_2), 5.02 (m, 1 H, H-28), 5.22 (d, 1 H, H-25), 5.42 (d, 1 H, H-29), 6.15 (m, 1 H, H-18), 6.17 (d, 1 H, H-17), 6.81 (s, 2 H, 2 aromat H), 8-OH and amide-NH are missing; ^{13}C NMR ($CDCl_3$, selected data) δ 19.7 (3 C, 3 aromat-CH_3), 20.7 (CH_3-30), 50.2 (2 C) + 52.5 (2 C) (4 piperazinyl-C), 54.9 (benzyl-C), 72.2 (C-21), 73.9 (C-25), 78.7 (C-23), 79.4 (C-27), 128.7 (2 C) + 131.3 + 135.7 + 137.5 (2 C) (6 aromat-C), 111.8 (C-28), 125.8 (C-16), 126.4 (C-18), 136.2 (C-17), 143.5 (C-29), 145.3 (C-19), 155.8 (C-8), 172.2 (C-15), 172.5 (C-35), 172.9 (C-6), 175.0 (C-1), 180.3 (C-4), 193.8 (C-11); signals of 8-*O*-pivaloyl group, 27.7 (3 C, $C(CH_3)_3$), 40.5 ($C(CH_3)_3$), 179.0 (piv-CO); signals of N-pivaloyl group, 29.4 (3 C, $C(CH_3)_3$), 45.3 ($C(CH_3)_3$), 190.4 (piv-CO). Anal. ($C_{61}H_{81}N_3O_{14}$) C, H, N.

The 8-*O*-monopivaloyl derivative **47** was obtained when the reaction was stopped after 2–4 h, or when less than an excess of pivaloyl chloride was used. Compound **47** was separated from the 8-*O*,*N*-dipivaloyl compound **48** by silica gel chromatography with ethyl acetate/hexane as eluent and crystallization from ether or ether/hexane: MS (FAB) *m/z* 996 (M + H); ^1H NMR ($CDCl_3$) δ 1.48 (s, 9 H, 3 piv-CH_3), 7.57 (s, 1 H, amide-NH), 8-OH is missing, all the other protons at chemical shifts similar to those in the parent compound **25**; ^{13}C NMR ($CDCl_3$, selected data) δ 22.36 (3C, $C(CH_3)_3$), 39.62 ($C(CH_3)_3$), 155.13 (C-8), 176.25 (8-piv-CO), 178.57 (C-4), 197.14 (C-1). Anal. ($C_{56}H_{73}N_3O_{13}$) C, H, N.

Acknowledgment. We thank Dr. H. Fuhrer and F. Raschdorf for spectral measurements; E. Lach, E. Seeber, R. Cortesi and R. Bachmann for technical assistance; and A. H. Kirkwood for reviewing the manuscript.

Registry No. 1, 13292-46-1; **2**, 6998-60-3; **3**, 13292-22-3; **4**, 57375-25-4; **5**, 59886-92-9; **6**, 62041-01-4; **7**, 38129-20-3; **8**, 17555-05-4; **9**, 51756-74-2; **10**, 123812-36-2; 11, 37839-09-1; **12**, 51756-73-1; **13**, 51756-72-0; **14**, 33019-90-8; **15**, 33024-21-4; **16**, 23589-18-6; **17**, 33997-52-3; **18**, 123812-37-3; **19**, 37839-20-6; **20**, 37839-21-7; **21**, 57184-22-2; **22**, 123812-38-4; **23**, 78813-61-3; **24**, 123812-39-5; **25** (hydroquinone derivative), 109006-01-1; **25** (quinone derivative), 122751-63-7; **26**, 123812-40-8; **27**, 123812-41-9; **28**, 61379-65-5; **29**, 120491-26-1; **30**, 123812-48-6; **31**, 100070-77-7; **32**, 100070-82-4; **33**, 41717-26-4; **34**, 123834-29-7; **35**, 82500-26-3; **36**, 72559-06-9; **37**, 123929-83-9; **38**, 7483-41-2; 16*S*-**39**, 123812-42-0; 16*R*-**39**, 123878-72-8; 16*S*-**40**, 123812-43-1; 16*R*-**40**, 123878-73-9; 41, 123812-44-2; **42**, 123834-30-0; **43**, 123834-31-1; 44, 123834-32-2; **45**, 123812-45-3; **46**, 123834-33-3; **47**, 123812-46-4; 48, 122751-61-5; 1-isobutylpiperazine, 5308-28-1; 1-dodecylpiperazine, 54722-40-6; 1-benzylpiperazine, 2759-28-6; piperazine-2-carboxylic acid, 2762-32-5; piperidine, 110-89-4; 4-piperidinol, 5382-16-1; 4-piperidinone, 41661-47-6; 4-hydroxymethylpiperidine, 6457-49-4; ethyl 4-piperidinecarboxylate, 1126-09-6; piperidine-4-carboxamide, 39546-32-2; 4-methylpiperidine, 626-58-4; 4-ethylpiperidine, 3230-23-7; 4-isopropylpiperidine, 19678-58-1; 4-*tert*-butylpiperidine, 1882-42-4; 4-cyclopentylpiperidine, 123812-47-5; 4-benzylpiperidine, 31252-42-3; 4-cyclohexymethylpiperidine, 78197-28-1; 4-cyclopentyl-1-piperazinamine, 61379-64-4; 4-[(2,4,6-trimethylphenyl)methyl]-1-piperazinamine, 55212-58-3; 4-(naphthylmethyl)-1-piperazinamine, 123812-49-7; 2-amino-octahydro-2*H*-pyrido[1,2-*a*]pyrazine, 67092-60-8; 2-amino-7-ethyloctahydro-2*H*-pyrido[1,2-*a*]pyrazine, 100070-67-5; pivaloyl chloride, 3282-30-2; 1,4-naphthalenedione, 130-15-4; 1-benzyl-4-(dimethoxymethyl)piperazine, 82500-25-2; 4-isobutylpiperidone, 72544-16-2; 3-hydroxy-4-aminotoluene, 95-84-1; 3,4-diaminotoluene, 496-72-0; 1-(4-chlorophenylmethyl)piperazine, 23145-88-2; 1-(4-*tert*-butylphenylmethyl)piperazine, 956-61-6.

Supplementary Material Available: An appendix listing analytical data for compounds **8–27**, **29–35**, and **39–48** (2 pages). Ordering information is given on any current masthead page.