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Azalides: synthesis and antibacterial activity of novel 9a-*N*-(*N'*-substituted carbamoyl and thiocarbamoyl) derivatives of 9-deoxo-9a-aza-9a-homoerythromycin A

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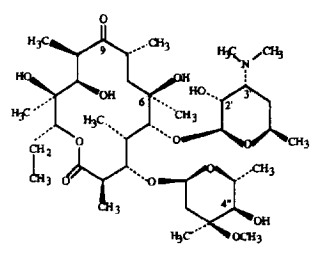
Summary — A series of 9a-*N*-(*N'*-substituted carbamoyl and thiocarbamoyl) derivatives of 9-deoxo-9a-aza-9a-homoerythromycin A was synthesized and structurally characterized by spectroscopic methods and X-ray crystallographic analysis. The new compounds were evaluated *in vitro* against a panel of Gram-positive and Gram-negative bacteria and their antibacterial activities compared with those of the parent 9a-amine **2** and azithromycin **3**. The results indicate that this type of structural modification offers no benefit over **2** and that the potency of the majority of these compounds *in vitro* decreased in comparison with **3**. Among the tested azalides, the *N'*-isopropylcarbamoyl derivative **4** and the *N'*-benzylthiocarbamoyl derivative **12** showed the most potent antibacterial *in vitro* activity.

azalide / 9-deoxo-9a-aza-9a-homoerythromycin A derivative / X-ray structure analysis / antibacterial activity

Introduction

Macrolide antibiotics are widely used antimicrobial agents in both clinical and veterinary medicine. A resurgence of interest in the discovery and development of new macrolides revealed a group of important semisynthetic antibiotics known as azalides [1–5]. The term azalide was originally introduced to denote a group of ring-expanded derivatives of erythromycin A **1** containing an additional basic nitrogen at the 9a-position of the 15-membered macrocyclic framework. As described in our previous papers, a stereospecific Beckmann rearrangement of 9(*E*)-erythromycin A oxime [1], followed by the reduction and reductive *N*-methylation of 9-deoxo-9a-aza-9a-homoerythromycin A **2** under Eschweiler–Clarke conditions provided azithromycin **3** [3, 4], the prototype azalide antibiotic with improved potency against Gram-negative pathogens and better pharmacokinetics than **1** [6–10]. Subsequent to the discovery of **3**, several new members of the family of 14-, 15- and 17-membered azalides were synthesized and screened in order to define the antibiotic potential of this class of compounds [11–15]. However, these attempts have not yet produced satisfactory results because of the poor antibacterial activities of the compounds obtained. Among the novel 15-membered azalides, only *O*-methyl derivatives of **3**, particularly 11-*O*-methylazithromycin [11] and the recently synthesized 8a-methyl positional isomer of **3** [13], showed *in vitro* potency comparable to that of **3**. As part of our screening program in the azalide chemistry we have recently disclosed the preparation of a number of 9a,11-cyclic carbamates of **2** [16]. The present paper deals with the synthesis, structure elucidation and antibacterial *in vitro* evaluation of a series of new 9a-*N*-(*N'*-substituted carbamoyl and thiocarbamoyl) derivatives of **2**.



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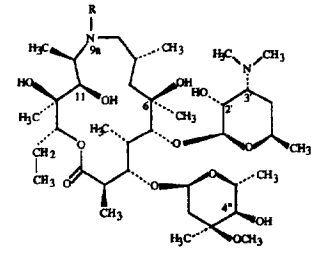

2 R = H; **3** R = CH_3

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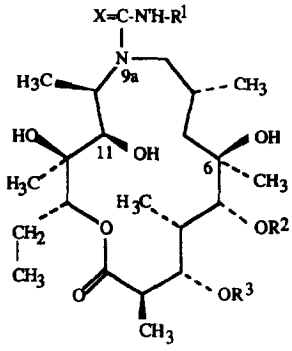

	X	R^1	R^2	R^3
4	=O	$CH(CH_3)_2$	D-desosamine	L-cladinose
6	=O	$CH(CH_3)_2$	H	H
7	=O	4-methyl-5-oxazolyl	D-desosamine	L-cladinose
8	=O	2-furyl	D-desosamine	L-cladinose
9	=O	4-pyridyl	D-desosamine	L-cladinose
10	=O	phenyl	D-desosamine	L-cladinose
11	=O	benzyl	D-desosamine	L-cladinose
12	=S	benzyl	D-desosamine	L-cladinose
13	=O	1-naphtyl	D-desosamine	Lcladinose

Fig 1. Structures of 9-deoxo-9a-*N*-(*N'*-substituted carbamoyl and thiocarbamoyl)-9a-aza-9a-homoerythromycins A **4–13.**

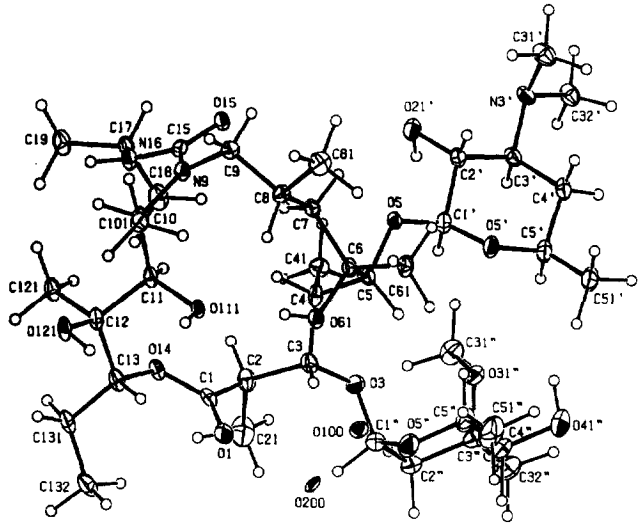

Fig 2. Molecular structure (Ortep plot) of **4** with atom numbering.

Chemistry

The addition of primary and secondary amines to isocyanates or isothiocyanates is an excellent method for the preparation of substituted ureas and thioureas, respectively [17]. When **2** was subjected to isopropylisocyanate in toluene, the reaction proceeded smoothly even at room temperature to form a less polar (by TLC, system A, see *Experimental protocols*) compound **4** in 86.2% yield (fig 1). The new bands at 1625 and 1515 cm^{-1} in the IR spectrum and an additional singlet at δ_C 158.2 in the ^{13}C-NMR spectrum of **4** indicated the presence of a carbamoyl group. The complex and overlapped ^1H- and ^{13}C-NMR spectra were elucidated by their ^1H-^1H and ^1H-^{13}C COSY experiments (table I). Although direct evidence for the $9_{ax,eq}$-H/9-C and 10-H/10-C cross peaks, based on 2D heteronuclear analysis, could not be established, the 2D NOESY analysis revealed clear NOEs N'H/9_{eq}-H and N'H/8-H. Further, the experimental $^3J_{2,3}$ value of 6.9 Hz, the observed NOE 11-H/4-H, and finally the missing NOE 11-H/3-H suggested that the solution-state conformation of **4** is predominantly a C-3 to C-5 'folded out' type [18]. To get more information and obtain less complex spectra we decided to synthesize the aglycone derivative **6** starting from 9-deoxo-9a-aza-9a-homoerythronolide A **5** [1]. The two overlapping methine protons at δ_H 4.12 in the ^1H NMR of **6** were attributed to 10-H and 5-H by the connectivities 10-H/10-Me and 5-H/4-H observed in its ^1H-^1H NMR COSY experiment. Additionally, the NOE contacts 9_{eq}-H/10-Me and 5-H/6-Me were detected. However, similarly to **4**, we did not establish the crosspeaks $9_{ax,eq}$-H/C-9 and 10-H/C-10, presumably due to a certain degree of shielding by the 9a-attached carbamoyl function.

The addition of 4-methyl-5-oxazolyl, 2-furyl, 4-pyridyl and phenyl groups to **2** was successfully performed by Curtius rearrangement of the corresponding acyl azides at elevated temperature, followed by the reaction of the *in situ* obtained isocyanates with the 9a-amino group of **2**. The spectroscopic data of the appropriate *N'*-arylcarbamoyl analogs **7–10** were in agreement with the assigned structures. Under similar reaction conditions as for **4**, compounds **11–13** were obtained. The structural assignment of *N'*-benzylcarbamoyl and *N'*-benzylthiocarbamoyl derivatives **11** and **12** is mainly based on their ^{13}C-NMR data, in particular on carbon singlets at δ_C 158.7 and δ_C 183.1, which were attributed to the 9a-NCO and 9a-NCS groups, respectively.

X-ray structure analysis of compound 4

The X-ray structure analysis was used to unambiguously determine the molecular structure, confor-

Table I. ^{1}H- and ^{13}C-NMR data for 9-deoxo-9a-*N*-(*N'*-isopropylcarbamoyl)-9a-aza-9a-homoerythromycin A **4** and its aglycone **6** in comparison with 9a-amines **2** and **5** (δ in ppm from TMS).

	^{1}H NMR				*^{13}C NMR*			
Position	**2**	**4**	**5**	**6**	**2**	**4**	**5**	**6**
	$CDCl_3$	*$CDCl_3$*	*Py-d_5*	*Py-d_5*	*$CDCl_3$*	*$CDCl_3$*	*Py-d_5*	*Py-d_5*
1	-	-	-	-	178.5	175.5	177.1	176.5
2	2.80	2.67	3.04	3.03	45.3	45.5	44.9	45.2
3	4.35	4.02	4.24	4.21	78.1	78.8	79.9	80.0
4	1.96	1.89	2.80	2.40	42.1	40.4	35.8	37.7
5	3.67	3.50	4.06	4.12	83.4	87.9	84.8	84.8
6	-	-	-	-	73.7	73.4	74.3	75.7
$7_{eq, ax}$	1.75, 1.38	1.62, 1.13	1.86, ND	2.21, 1.62	42.2	ND	40.9	40.1
8	1.74	2.29	1.90	2.63	29.9	27.4	30.0	29.7
$9_{eq, ax}$	3.04, 1.83	3.43, 2.52	3.02, 1.76	3.58, 3.02	57.3	ND	57.2	ND
9a-N*CO*	-	-	-	-	-	158.2	-	158.9
9a-NCO*N'H*	-	4.41	-	5.68	-	-	-	-
N'-*CH*	-	3.91	-	4.19	-	42.2	-	43.5
N'-CH*Me2*	-	1.14	-	1.19, 1.14	-	22.9	-	23.9
10	2.58	ND	2.80	4.12	56.7	ND	59.1	ND
11	3.46	3.76	4.13	4.17	73.2	77.1	75.5	76.9
12	-	-	-	-	73.8	74.1	73.9	76.3
13	4.73	5.00	5.52	5.79	77.2	74.4	77.8	78.0
$14_{eq, ax}$	1.91, 1.49	1.93, 1.47	2.21, 1.68	2.22, 1.73	21.1	21.8	22.1	23.2
15	0.90	0.91	0.85	0.95	11.2	10.8	11.4	11.8
16	1.21	1.24	1.55	1.51	15.0	14.8	16.5	16.3
17	1.06	1.06	1.39	1.47	9.4	9.5	8.5	8.3
18	1.30	1.29	1.54	1.70	27.4	22.7	27.0	27.2
19	0.94	1.04	0.92	1.08	21.9	20.5	21.7	20.5
20	1.15	1.31	1.51	1.62	14.9	12.2	14.3	13.9
21	1.08	1.15	1.42	1.63	16.2	16.9	17.6	19.1
1'	4.43	4.47	-	-	103.1	103.8	-	-
2'	3.22	3.35	-	-	70.9	70.3	-	-
3'	2.44	2.59	-	-	65.7	64.1	-	-
3'-N*Me2*	2.29	2.32	-	-	40.3	39.9	-	-
$4'_{eq, ax}$	1.66, 1.23	1.70, 1.28	-	-	28.7	29.1	-	-
5'	3.51	3.61	-	-	68.8	68.6	-	-
5'-Me	1.23	1.22	-	-	21.3	20.6	-	-
1"	5.04	4.85	-	-	94.9	96.0	-	-
$2"_{eq, ax}$	2.35, 1.58	2.33, 1.57	-	-	34.8	34.6	-	-
3"	-	-	-	-	72.9	72.3	-	-
3"-Me	1.25	1.23	-	-	21.6	20.9	-	-
3"-O*Me*	3.34	3.28	-	-	49.4	48.8	-	-
4"	3.04	3.04	-	-	77.9	77.0	-	-
5"	4.08	4.07	-	-	65.3	65.6	-	-
5"-Me	1.33	1.28	-	-	18.3	17.4	-	-

mation and absolute configuration of **4**. The molecular structure of **4** is shown in the Ortep [19] plot (fig 2) drawn with the thermal ellipsoids at the 30% probability level. The final atomic coordinates and equivalent isotropic thermal parameters of the non-hydrogen atoms are listed in table II. The bond lengths and bond angles are in agreement with the values observed for the analogous compounds. The conformation of the 15-membered macrocycle, described in the terms of the torsion angle values, is presented in the polar diagram (fig 3). Both sugar residues are in the chair conformation: α-L-cladinose in $^{1}C_4$ and β-D-desos-

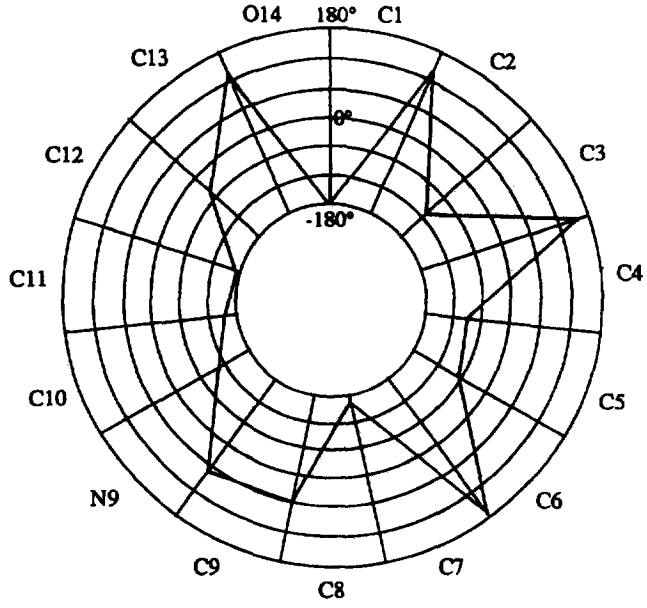

Fig 3. Polar diagram of the torsion angles values illustrates the conformation of the macrocyclic ring of **4**.

amine in $^4C_1$. The known absolute configurations of β-D-desosamine and α-L-cladinose have been used as the internal standards to determine the absolute configurations of the aglycone chiral centers. The absolute configurations are as follows: C2*R*, C3*S*, C4*S*, C5*R*, C6*R*, C8*R*, C10*R*, C11*R*, C12*S* and C13*R*.

Biological results

The preliminary antibacterial screening of the novel 9a-*N*-(*N'*-substituted carbamoyl) derivatives of **2** was performed by a standard dilution assay for the determination of minimum inhibitory concentrations (MICs) in accordance with NCCLS [20]. The MICs against a panel of Gram-positive and Gram-negative bacteria were determined in comparison with the starting 9a-amine **2** and azithromycin **3** and are presented in table III. Among the novel azalides, the *N'*-isopropylcarbamoyl derivative **4** and the *N'*-benzyl-thiocarbamoyl derivative **12** were the most active compounds but they exhibited 2–8 times lower potency in comparison with **2**. However, the antimicrobial *in vitro* activity of all the tested compounds strongly decreased in comparison with **3**. The data summarized in table III suggest that further research into *N'*-aryl side chain replacement analogs would not be warranted.

Experimental protocols

Chemistry

Melting points were determined with a Fisher-Johns apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer 257 G spectrophotometer. Electron impact mass spectra were obtained on a Shimadzu GCMS-QP 1000 spectrometer at temperature of 250°C. ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian-Gemini 300 instrument and chemical shifts are reported in δ values (ppm) relative to internal standard TMS for solvents $CDCl_3$, DMSO-d_6 and pyridine-d_5. TLC was performed on E Merck plates (Darmstadt, Germany) of silica gel 60 using solvent system A (EtOAc/*n*-hexane/Et_2NH, 100:100:20), or system B ($CHCl_3$/MeOH/conc NH_4OH, 6:1:0.1). Spots were visualized by spraying with 5% H_2SO_4/EtOH solution followed by heating at 110°C. Silica gel column chromatography was performed with silica gel 60 (70-230 mesh, E Merck, Darmstadt, Germany).

9-Deoxo-9a-N-(N'-isopropylcarbamoyl)-9a-aza-9a-homo-erythromycin A **4**

To a solution of **2** (7.27 g, 0.010 mol) in toluene (40 ml), 0.94 g (0.011 mol) of isopropylisocyanate was added and the reaction mixture was stirred at 25–30°C for 1 h. After removal of the solvent under reduced pressure, the residue was dissolved in $CHCl_3$ and precipitated with addition of Me_2CO to yield 7.0 g (86.2%) of a crude product **4**. Recrystallization from MeOH/water gave colorless crystals of **4** as a dihydrate; mp: 135–144°C. TLC, system A R_f 0.35, system B R_f 0.55. IR (KBr) cm^{-1} 1730, 1625, 1515, 1455, 1380, 1270, 1165, 1050, 950. ^{1}H NMR and ^{13}C NMR ($CDCl_3$): table I. Anal $C_{41}H_{77}N_3O_{13}\cdot 2H_2O$ (C, H, N).

9-Deoxo-9a-N-(N'-isopropylcarbamoyl)-9a-aza-9a-homo-erythronolide A **6**

To a solution of 9-deoxo-9a-aza-9a-homoerythronolide A **5** (4.2 g, 0.010 mol) in toluene (20 ml), 0.85 g (0.010 mol) of isopropylisocyanate was added and the reaction mixture was stirred for 30 min at 30°C. The solution was evaporated to dryness to give 4.57 g of crude **6**. Column chromatography of the residue on silica gel with $CHCl_3$/MeOH, 7:3 as the mobile phase, afforded 3.9 g (77.2%) of TLC-pure amorphous compound **6**; mp: 97–99°C. IR (KBr) cm^{-1} 1710, 1610, 1520, 1460, 1380, 1265, 1180, 1085, 960. ^{1}H NMR and ^{13}C NMR ($CDCl_3$): table I.

9-Deoxo-9a-N-[N'-(4-methyl-5-oxazolyl)carbamoyl]-9a-aza-9a-homoerythomycin A **7**

A mixture of **2** (4.8 g, 0.065 mol) and 1.0 g (0.066 mol) of 4-methyl-5-oxazolyl carboxylic acid azide in 30 ml dry toluene was heated for 15 min at reflux temperature and evaporated under reduced pressure. The residue was suspended in Me_2CO (20 ml), the suspension was stirred at room temperature for 30 min and the crystalline solid was filtered off to give 5.4 g (93.3%) of a crude compound **7**. Recrystallization of the residue from hot Me_2CO, the TLC pure **7** was obtained; mp: 181–183°C. TLC, system A R_f 0.14, system B R_f 0.49. IR (KBr) cm^{-1} 1730, 1680, 1655, 1490, 1460, 1380, 1170, 1050, 755, 660. ^{1}H NMR (pyridine-d_5, 50°C): 5.71 (1H, 13-H), 5.15 (1H, 1"-H), 4.94 (1H, 1'-H), 4.65 (1H, 10-H), 4.53 (1H, 5"-H), 4.30 (1H, 11-H), 4.07 (1H, 5-H), 3.96 (1H, 9_{eq}-H), 3.78 (1H, 9_{ax}-H), 3.50 (1H, 2'-H), 3.44 (3H, 3"-OCH_3), 3.21 (1H, 4"-H), 2.62 (1H, 3'-H), 2.40 (1H, 2"$_{eq}$-H), 2.35 (1H, 7_{eq}-H), 2.34 (1H,

Table II. Final atomic coordinates and equivalent isotropic thermal parameters of the nonhydrogen atoms for **4**.

Atom	*x*	*y*	*z*	$U_{eq}(Å^2)$	*Atom*	*x*	*y*	*z*	$U_{eq}(Å^2)$
O1	0.3754(4)	0.28880 *	0.2377(3)	0.0383(10)	C5"	0.7199(7)	0.4264(6)	0.0277(6)	0.0545(12)
O3	0.4883(4)	0.4991(4)	0.0920(3)	0.0368(10)	C6	0.7176(5)	0.4787(5)	0.3322(4)	0.0260(11)
O5	0.7193(4)	0.6322(4)	0.2719(3)	0.0253(9)	C7	0.6962(5)	0.5308(5)	0.4192(4)	0.0271(11)
O5'	0.8588(4)	0.6345(4)	0.1718(3)	0.0361(10)	C8	0.7216(5)	0.4772(5)	0.5054(4)	0.0259(11)
O5"	0.6138(6)	0.3789(4)	0.0486(4)	0.0613(11)	C9	0.6517(5)	0.5220(5)	0.5774(4)	0.0259(11)
O14	0.2611(3)	0.3799(4)	0.3186(3)	0.0284(9)	C10	0.4497(6)	0.4338(5)	0.5715(4)	0.0278(11)
O15	0.5183(3)	0.6735(3)	0.5120(3)	0.0261(9)	C11	0.3968(5)	0.4020(5)	0.4790(4)	0.0252(11)
O21'	0.7062(4)	0.8248(4)	0.2771(3)	0.0392(10)	C12	0.2767(5)	0.3449(5)	0.4747(4)	0.0303(11)
O31"	0.6236(4)	0.6018(4)	-0.0353(3)	0.0405(10)	C13	0.2485(5)	0.3032(5)	0.3815(5)	0.0299(11)
O41"	0.8103(5)	0.5059(5)	-0.0915(4)	0.0670(12)	C15	0.4640(5)	0.6043(5)	0.5393(4)	0.0238(11)
O61	0.6598(4)	0.3890(4)	0.3261(3)	0.0277(9)	C17	0.2712(5)	0.6864(5)	0.5031(5)	0.0311(11)
O111	0.4851(3)	0.3531(3)	0.4338(3)	0.0246(9)	C18	0.2536(6)	0.6744(6)	0.4035(5)	0.0420(12)
O121	0.2838(4)	0.2668(4)	0.5355(3)	0.0339(10)	C19	0.1494(6)	0.6894(6)	0.5425(5)	0.0409(11)
N3'	0.8704(5)	0.9344(4)	0.1928(3)	0.0311(10)	C21	0.2498(7)	0.4374(8)	0.1117(5)	0.0633(12)
N9	0.5204(5)	0.5211(4)	0.5632(3)	0.0256(10)	C31'	0.9776(6)	0.9390(6)	0.2564(5)	0.0436(12)
N16	0.3422(5)	0.6071(4)	0.5429(4)	0.0295(10)	C31"	0.5384(7)	0.6764(6)	-0.0411(5)	0.0496(12)
C1	0.3300(5)	0.36297 *	0.2520(4)	0.0300(11)	C32'	0.8850(6)	0.9980(6)	0.1198(5)	0.0368(12)
C1'	0.7577(6)	0.6818(5)	0.2022(4)	0.0304(11)	C32"	0.5742(9)	0.5520(8)	-0.1862(5)	0.0719(12)
C1"	0.5022(7)	0.4264(6)	0.0323(5)	0.0488(12)	C41	0.4750(6)	0.6427(5)	0.2143(4)	0.0302(11)
C2	0.3372(6)	0.4504(6)	0.1950(4)	0.0343(11)	C51'	1.0030(8)	0.6257(6)	0.0686(6)	0.0656(12)
C2'	0.7972(5)	0.7783(5)	0.2381(4)	0.0283(11)	C51"	0.8234(8)	0.3596(7)	0.0467(7)	0.0755(12)
C2"	0.4832(8)	0.4683(7)	-0.0621(5)	0.0573(12)	C61	0.8516(5)	0.4593(5)	0.3228(4)	0.0315(11)
C3	0.4728(6)	0.4680(5)	0.1798(4)	0.0293(11)	C81	0.8545(6)	0.4764(7)	0.5429(5)	0.0437(11)
C3'	0.8398(6)	0.8373(5)	0.1631(4)	0.0295(11)	C101	0.5265(6)	0.3574(5)	0.6227(4)	0.0314(11)
C3"	0.5932(7)	0.5212(6)	-0.0892(5)	0.0498(12)	C121	0.1744(5)	0.4078(5)	0.5015(5)	0.0337(11)
C4	0.5257(5)	0.5454(5)	0.2432(4)	0.0269(11)	C131	0.1222(5)	0.2618(5)	0.3630(5)	0.0354(11)
C4'	0.9389(6)	0.7829(6)	0.1218(5)	0.0410(12)	C132	0.1030(6)	0.2093(6)	0.2759(5)	0.0464(12)
C4"	0.7042(8)	0.4587(7)	-0.0700(5)	0.0581(12)	O100 #	0.7053(6)	0.2369(5)	0.2181(4)	0.0284(11)
C5	0.6664(5)	0.5414(5)	0.2544(4)	0.0244(11)	O200 #	0.7260(6)	0.0485(5)	0.2911(4)	0.0270(11)
C5'	0.8999(7)	0.6828(6)	0.0966(5)	0.0450(12)					

$U_{eq} = (1/3)\,\Sigma_i \Sigma_j U_{ij}\, a_i^*\, a_j^*\, a_i \cdot a_j$

*The origin was fixed by *y* coordinates of O1 anc C1. # Water molecules.

8-H), 2.15 [6H, 3'-N(CH_3)$_2$], 2.01 (3H, 6-CH_3), 1.97 (1H, 7_{ax}-H), 1.68 (3H, 10-CH_3), 1.51 (1H, $2"_{ax}$-H), 1.30 (3H, 3"-CH_3), 1.29 (3H, 12-CH_3), 1.09 (3H, 8-CH_3), 9.09 (1H, 9a-NCO*N'H*), 8.02 and 2.33 (4-methyl-5-oxazolyl protons). ^{13}C NMR (pyridine-d_5, 50°C) δ 177.2 (C-1), 157.2 (9a-N*CO*), 104.2 (C-1'), 96.9 (C-1"), 86.6 (C-5), 78.1 (C-4"), 77.1 (C-12), 76.8 (C-11), 75.8 (C-6), 74.0 (C-3"), 72.4 (C-2'), 66.6 (C-5"), 66.3 (C-3'), 50.1 (3"-OCH_3), 41.0 [3'-N(CH_3)$_2$], 36.2 (C-2"), 31.0 (6-CH_3), 29.1 (C-8), 21.1 (3"-CH_3), 19.6 (5"-CH_3), 14.4 (10-CH_3), 147.9, 142.2, 128.2 and 12.2 (4-methyl-5-oxazolyl carbons). EI-MS *m/z* 858 (M^+).

9-Deoxo-9a-N-[N'-(2-furyl)carbamoyl]-9a-aza-9a-homoerythromycin A **8**

By the method as described for **7**, the reaction of **2** (2.18 g, 0.003 mol) and 0.5 g (0.0036 mol) of 2-furylcarboxylic acid azide in toluene gave 2.1 g of a crude yellow product. Column chromatography of the residue on silica gel with $CHCl_3$/MeOH, 7:3 afforded 1.7 g (77.0%) of **8** as a colorless amorphous solid; mp: 155–159°C. TLC, system A R_f 0.26, system B R_f 0.57. IR ($CHCl_3$) cm^{-1} 1730, 1655, 1520, 1460, 1380, 1270, 1165, 1050, 1000, 955, 900, 830, 730. ^{1}H NMR (DMSO-d_6) δ 5.04 (1H, 13-H), 4.86 (1H, 11-H), 4.77 (1H, 1"-H), 4.47 (1H, 1'-H), 4.27 (1H, 10-H), 4.03 (1H, 5"-H), 3.47 (IH, 9_{eq}-H) 13.37 (1H, 5-H), 3.35 (3H, 3"-OCH_3), 3.25 (1H, 9_{ax}-H), 3.10 (1H, 2'-H), 2.91 (1H, 4"-H), 2.60 (1H, 3'-H), 2.50 (6H, 3'-N(CH_3)$_2$), 2.25 (1H, $2"_{eq}$-H), 2.07 (1H, 8-H), 1.41 (1H, 7_{eq}-H), 1.68 (3H, 10-CH_3), 1.51 (1H, $2"_{ax}$-H), 1.48 (3H, 5"-CH_3), 1.30 (3H, 6-CH_3), 1.20 (1H, 7_{ax}-H), 1.13 (3H, 3"-CH_3), 1.10 (3H, 12-CH_3), 0.91 (3H, 8-CH_3), 8.51 (9a-NCO*N'H*), 7.27, 6.35 and 6.03 (2-furyl protons). ^{13}C NMR (DMSO-d_6) δ 175.5 (C-1), 155.4 (9a-N*CO*), 101.9 (C-1'), 95.3 (C-1"), 84.4 (C-5), 77.5 (C-4"), 76.2 (C-13), 75.1 (C-12), 74.8 (C-11), 73.3 (C-6), 72.6 (C-3"), 70.7 (C-2'), 65.1 (C-5"), 64.5 (C-3'), 48.8 (3"-OCH_3), 40.3 (3'-N(CH_3)$_2$), 36.3 (C-7), 35.0 (C-2"), 27.7 (C-8), 27.6 (6-CH_3), 21.8 (C-14), 19.7 (8-CH_3), 18.3 (5"-CH_3), 13.2 (10-CH_3), 147.7, 136.5, 118.9 and 98.0 (2-furyl carbons). EI-MS *m/z* 843 (M^+).

Table III. MIC (μg/ml) values of 9-deoxo-9a-*N*-(*N'*-substituted carbamoyl and thiocarbamoyl)-9a-aza-9a-homoerythromycins A in comparison with 9a-amine **2** and azithromycin **3**.

Microorganism	*2*	*3*	*4*	*7*	*9*	*10*	*11*	*12*
Staphylococcus epidermidis ATCC 12228	3.12	0.78	6.25	6.25	25	3.12	6.25	6.25
Staphylococcus aureus ATCC 6538P	3.12	1.56	1.56	12.5	12.5	6.25	3.12	3.12
Micrococcus flavus ATCC 10240	1.56	–	3.12	12.5	12.5	6.25	3.12	1.56
Streptococcus faecalis ATCC 8043	3.12	0.78	3.12	6.25	6.25	3.12	3.12	1.56
Bacillus subtilis NCTC 8236	12.5	0.19	1.56	12.5	25	6.25	3.12	1.56
B pumilus NCTC 8241	12.5	1.56	6.25	12.5	12.5	6.25	3.12	1.56
B cereus ATCC 11778	3.12	0.78	6.25	12.5	12.5	12.5	6.25	6.25
Pseudomonas aeruginosa NCTC 10490	25	12.5	25	100	50	50	50	50
E coli ATCC 10536	3.12	1.56	12.5	25	12.5	12.5	25	12.5
Salmonella panama 6117	3.12	6.25	6.25	50	25	25	> 100	> 100
BHS-A *Streptococcus pyogenes* J-21	3.12	1.56	–	6.25	12.5	3.12	–	–
BHS-B *Streptococcus agalactiae* J-22	1.56	–	–	1.56	12.5	1.56	–	–

9-Deoxo-9a-N-[N'-(4-pyridyl)carbamoyl]-9a-aza-9a-homoerythromycin A **9**

By the method as described for **7**, the reaction of **2** (2.18 g, 0.003 mol) and 0.53 g (0.0036 mol) of isonicotinic acid azide in 15 ml toluene yielded 2.26 g of oily residue which was crystallized from MeOH/water to give 1.9 g (74.8%) of TLC-pure white crystals of **9**; mp: 149–153°C. TLC, system A R_f 0.08, system B R_f 0.44. IR ($CHCl_3$) cm^{-1} 1730, 1650, 1590, 1510, 1460, 1380, 1330, 1280, 1165, 1050, 1000, 955, 900, 830, 730. ^1H NMR (DMSO-d_6): 5.16 (1H, 13-H), 4.89 (1H, 1"-H), 4.52 (1H, 1'-H), 4.39 (1H, 10-H), 4.19 (1H, 5"-H), 3.68 (1H, 11-H), 3.53 (1H, 5-H), 3.51 (1H, 9_{eq}-H), 3.28 (1H, 9_{ax}-H), 3.05 (1H, 2'-H), 3.33 (3H, 3"-OCH_3), 3.00 (1H, 4"-H), 2.77 (1H, 2-H), 2.28 (1H, 8-H), 2.51 (1H, 3'-H), 1.88 (1H, 4-H), 2.37 (1H, $2"_{eq}$-H), 1.61 (1H, 7_{eq}-H), 2.34 (6H, 3'-N(CH_3)$_2$), 1.40 (3H, 6-CH_3), 1.23 (1H, 7_{ax}-H), 1.36 (3H, 10-CH_3), 1.63 (1H, $2"_{ax}$-H), 1.26 (3H, 5"-CH_3), 1.25 (3H, 3"-CH_3), 1.25 (3H, 12-CH_3), 1.04 (3H, 8-CH_3), 8.66 (9a-NCON'*H*), 8.25 and 7.35 (4-pyridyl protons). ^{13}C NMR (DMSO-d_6) δ 176.1 (C-1), 155.5 (9a-N*C*O), 102.2 (C-1'), 95.5 (C-1"), 84.3 (C-5), 77.6 (C-4"), 76.2 (C-13), 75.4 (C-12), 73.9 (C-6), 73.9 (C-11), 70.9 (C-2'), 65.0 (C-5"), 64.6 (C-3'), 48.9 (3"-OCH_3), 40.4 (3'-N(CH_3)$_2$), 39.0 (C-7), 34.9(C-2"), 27.9 (6-CH_3), 27.8 (C-8), 21.0 (5'-CH_3), 20.2 (8-CH_3), 18.5 (12-CH_3), 14.4 (10-CH_3), 149.8, 148.0, 113.9 (4-pyridyl carbons). EI-MS *m/z* 854 (M^+).

9-Deoxo-9a-N-(N'-phenylcarbamoyl)-9a-aza-9a-homoerythromycin A **10**

By the method as described for **7**, the reaction of **2** (2.0 g, 0.0027 mol) and 0.5 g (0.0034 mol) of benzoic acid azide in toluene gave 2.43 g of oily residue which was purified by silica-gel column chromatography with CH_2Cl_2/CH_3OH, 85:15 as the mobile phase to yield 1.4 g (61.4%) of **10** as an amorphous solid; mp: 126–130°C. TLC, system A R_f 0.34, system B R_f 0.63. IR (KBr) cm^{-1} 1730, 1645, 1600, 1539, 1510, 1455, 1380, 1315, 1240, 1165, 1045, 950, 895, 755, 690. ^1H NMR (DMSO-d_6) δ 5.05 (1H, 13-H), 4.79 (1H, 1"-H), 4.46 (1H, 1'-H), 4.27 (1H, 10-H), 4.05 (1H, 5"-H), 3.58 (1H, 11-H), 3.46 (1H, 5-H), 3.28 (1H, 9_{eq}-H), 3.16 (1H, 9_{ax}-H), 3.09 (1H, 2'-H), 3.23 (3H, 3"-OCH_3), 2.91 (1H, 4"-H), 2.16 (1H, 8-H) 2.61 (1H, 3'-H), 2.27 (1H, $2"_{eq}$-H), 1.58 (1H, 7_{eq}-H), 2.34 (6H, 3'-N(CH_3)$_2$), 1.30 (3H, 6-CH_3), 1.15 (1H, 7_{ax}-H), 1.40 (1H, 14_{ax}-H), 1.25 (3H, 10-CH_3), 1.53 (1H, $2"_{ax}$-H), 1.25 (3H, 5"-CH_3), 1.15 (3H, 3"-CH_3), 1.11 (3H, 12-CH_3), 0.94 (3H, 8-CH_3), 8.12 (9a-NCON'*H*), 7.35, 7.23 and 6.89 (Ph). ^{13}C NMR (DMSO-d_6) δ 175.6 (C-1), 156.1 (9a-N*C*O), 102.0 (C-1'), 95.4 (C-1"), 84.4 (C-5), 77.4 (C-4"), 76.2 (C-13), 75.1 (C-12), 73.7 (C-6), 72.7 (C-3"), 70.6 (C-2'), 65.0 (C-3'), 64.6 (C-5"), 48.9 (3"-OCH_3), 40.1 (3'-N(CH_3)$_2$), 35.0 (C-2"), 27.3 (C-8), 27.0 (6-CH_3), 20.0 (8-CH_3), 18.3 (5"-CH_3), 14.0 (10-CH_3), 149.8, 148.0 and 113.9 (Ph). EI-MS *m/z* 854 (M^+).

9-Deoxo-9a-N-(N'-benzylcarbamoyl)-9a-aza-9a-homoerythromycin A **11**

By the method as described for **4**, the reaction of **2** (7.27 g, 0.010 mol) and 1.33 g (0.010 mol) of benzylisocyanate in toluene (15 ml) gave 8.4 g of a crude product which on silica-gel column chromatography using solvent system $CHCl_3$/MeOH, 7:3 yielded 6.5 g of **11** as an amorphous solid (75.6%); mp: 142–144°C. TLC, system A R_f 0.35, system B R_f 0.62. IR (KBr) cm^{-1} 1730, 1630, 1525, 1410, 1380, 1270, 1165, 1045, 950, 895, 755, 700. ^1H NMR (DMSO-d_6) δ 5.02 (1H, 13-H), 4.79 (1H, 1"-H), 4.46 (1H, 1'-H), 4.05 (1H, 5"-H), 4.08 (1H, 10-H), 3.46 (1H, 5-H), 3.48 (1H, 9_{eq}-H), 3.09 (1H, 2'-H), 3.22 (3H, 3"-OCH_3), 2.91 (1H, 4"-H), 2.16 (1H, 8-H), 2.60 (1H, 3'-H), 2.27 (1H, $21"_{eq}$-H), 2.51 (1H, 9_{ax}-H), 2.34 (6H, 3'-N(CH_3)$_2$), 1.58 (1H, 7_{eq}-H), 1.52 (1H, $2"_{ax}$-H), 1.30 (3H, 6-CH_3), 1.25 (3H, 10-CH_3), 1.15 (1H, 7_{ax}-H), 1.15 (3H, 5"-CH_3), 1.15 (3H, 12-CH_3), 1.11 (3H, 3"-CH_3), 0.95 (3H, 8-CH_3), 7.29 (N'H-CH_2-*Ph*), 6.57 (*N'H*), 4.33 and 4.16 (N'H-*C*H_2). ^{13}C NMR (DMSO-d_6) δ 175.7 (C-1), 158.7 (9a-N*C*O), 102.2 (C-1'), 95.4 (C-1"), 84.5 (C-5), 77.5 (C-4"), 76.4 (C-13), 75.1 (C-12), 73.9 (C-6), 72.0 (C-3"), 70.2 (C-2'), 64.6 (C-3'), 65.1 (C-5"), 49.0 (3"-OCH_3), 40.2 (3'-N(CH_3)$_2$), 35.0 (C-2"), 28.0 (C-8), 24.4 (6-CH_3), 21.5 (3"-CH_3), 20.2 (8-CH_3), 14.0 (10-CH_3), 141.3, 128.1, 127.0 and 126.3 (Ph), 44.2 (N'H-*C*H_2). EI-MS *m/z* 854 (M^+).

Table IV. Crystal data and summary of experimental details for **4**.

Crystal data	
Molecular formula	$C_{41}H_{77}N_3O_{13}\cdot 2H_2O$
M_r	856.10
Crystal size (mm)	0.36 x 0.28 x 0.14
a (Å)	11.166(3)
b (Å)	14.193(3)
c (Å)	15.262(6)
β (°)	95.65(3)
V (Å^3)	2407(1)
D_{calc} (gcm^{-3})	1.18
Z	2
Crystal system	Monoclinic
Space group	$P2_1$
μ (cm^{-1})	7.0
F_{000} (electrons)	936
Data collection	
Diffractometer	Enraf-Nonius CAD-4
Radiation	Cu*K*α
T (K)	100(3)
No of reflections used for cell parameters and θ range (°)	18, 22–42
θ range for intensity measurement	2.59–74.33
hkl range	−13, 0; −17, 0; −19, 19
Scan	ω/2Θ
Δω	1.14 + 0.37tanΘ
No of measured reflections	6121
No of symmetric independent reflections	3883, I > 2σ(*I*)
Refinement	
No of variables	656
Quantity minimized	$\Sigma w (\|F_o - F_c\|)^2$; $w = 1$
R, *S*	0.061, 1.38
Final < shift/error>	≤ 0.05
Residual electron density $(\Delta\rho)_{max}$, $(\Delta\rho)_{min}$ (eÅ^{-3})	0.64, −0.28

9-Deoxo-9a-N-(N'-benzylthiocarbamoyl)-9a-aza-9a-homoerythromycin A ***12***

By the procedure as described for **4**, the reaction of 7.27 g (0.010 mol) of **2** and 1.50 g (0.010 mol) of benzylisothiocyanate in toluene (10 ml) gave 8.6 g of a crude product which on silica-gel column chromatography using solvent system $CHCl_3$/MeOH, 7:3 yielded 7.2 g (82.1%) of amorphous compound **12**; mp 119–122°C. TLC, system A R_f 0.37, system B R_f 0.68. IR (KBr) cm^{-1} 1730, 1630, 1525, 1410, 1380, 1270, 1165, 1045, 950, 895, 755, 700. ^{1}H NMR (DMSO-d_6) δ 5.04 (1H, 13-H), 4.80 (1H, 1"-H), 4.40 (1H, 1'-H), 4.06 (1H, 5"-H), 4.26 (1H, 10-H), 3.44 (1H, 5-H), 3.10 (1H, 2'-H), 3.23 (3H, 3"-OCH_3), 2.92 (1H, 4"-H), 2.13 (1H, 8-H), 2.56 (1H, 3'-H), 2.27 (1H, 2"$_{eq}$-H), 2.29 (6H, 3'-N(CH_3)$_2$), 1.38 (1H, 7$_{eq}$-H), 1.52 (1H, 2"$_{ax}$-H), 1.28 (3H, 6-CH_3), 1.24 (3H, 10-CH_3), 1.11 (1H, 7$_{ax}$-H), 1.19 (3H, 5"-CH_3), 1.06 (3H, 12-CH_3), 1.14 (3H, 3"-CH_3), 0.89 (3H, 8-CH_3), 7.29, 7.20 (N'H-CH_3-*Ph*), 6.57 (*N'H*), 4.90 and 4.70 (N'H-*CH*$_2$). ^{13}C NMR (DMSO-d_6) δ 175.7 (C-1), 183.1 (9a-N*CS*, 102.3 (C-1'), 95.2 (C-1"), 83.5 (C-5), 77.6 (C-4"), 76.3 (C-13), 75.1 (C-12), 73.8 (C-6), 72.0 (C-3"), 70.7 (C-2'), 64.6 (C-3'), 65.0 (C-5"), 49.0 (3"-OCH_3), 40.2 (3'-N(CH_3)$_2$), 34.9 (C-2"), 28.4 (6-CH_3), 21.2 (3"-CH_3), 20.1 (8-CH_3), 16.1 (10-CH_3), 140.1, 128.0, 127.5 and 126.5 (Ph), 49.3 (N'H-*CH*$_2$). EI-MS *m/z* 854 (M+).

9-Deoxo-9a-N-[N'-(1-naphthylcarbamoyl]-9a-aza-9a-homoerythromycin A ***13***

By the method as described for **4**, the reaction of 7.27 g (0.010 mol) of **2** and 1.7 g (0.010 mol) of l-naphthylisocyanate in toluene (40 ml) gave 9.0 g of an oily residue which on silica-gel column chromatography with $CHCl_3$/MeOH/conc NH_4OH, 6:1:0.1, yielded 7.8 g (86.6%) of **13**; mp: 134–137°C. TLC, system A R_f 0.33, system B R_f 0.65. IR ($CHCl_3$) cm^{-1} 1740, 1635, 1530, 1500, 1455, 1380, 1340, 1265, 1160, 1050, 1010, 960, 890, 795, 775, 735, 700. ^{1}H NMR ($CDCl_3$): δ 4.99 (1H, 13-H), 4.84 (1H, 1"-H), 4.41 (1H, 1'-H), 4.09 (1H, 3-H), 4.06 (1H, 5"-H), 3.58 (1H, 11-H), 3.56 (1H, 5-H), 3.89 (1H, 9$_{eq}$-H), 3.43 (1H, 5'-H), 3.32 (1H, 2'-H), 3.28 (3H, 3"-OCH_3), 2.95 (1H, 4"-H), 2.74 (1H, 9$_{ax}$-H), 2.71 (1H, 2-H), 2.56 (1H, 3'-H), 2.54 (1H, 8-H), 2.34 (1H, 2"$_{eq}$-H), 2.31 (6H, 3'-N(CH_3)$_2$), 1.99 (lH, 4-H), 1.95 (1H, 14$_{eq}$-H), 1.79 (1H, 7$_{eq}$-H), 1.64 (1H, 4'$_{eq}$-H) 1.54 (1H, 2"$_{ax}$-H), 1.53 (1H, 14$_{ax}$-H), 1.47 (3H, 10-CH_3), 1.32 (3H, 6-CH_3), 1.26 (3H, 2-CH_3), 1.22 (1H, 4'$_{ax}$-H), 1.21 (3H, 3"-CH_3), 1.20 (3H, 12-CH_3), 1.19 (3H, 5"-CH_3) 1.13 (3H, 8-CH_3), 1.09 (3H, 4-CH_3), 1.00 (3H, 5'-CH_3), 0.93 (3H, 15-CH_3), 8.12 (9a-NCO*N'H*), 7.92, 7.80, 7.63, 7.47 and 7.44 (1-naphthyl protons). ^{13}C NMR ($CDCl_3$) δ 176.0 (C-1), 157.7 (9a-N*CO*), 104.4 (C-1'), 96.7 (C-1"), 88.1 (C-5), 79.3 (C-3), 77.8 (C-13), 77.1 (C-4"), 74.7 (C-11), 74.4 (C-12), 74.1 (C-6), 72.3 (C-3"), 70.3 (C-2'), 68.8 (C-5'), 64.3 (C-3'), 65.6 (C-5"), 48.9 (3"-OCH_3), 46.2 (C-2), 40.0 (3'-N(CH_3)$_2$), 40.6 (C-4), 34.7 (C-2"), 29.0 (C-4'), 27.4 (C-8 and 6-CH_3), 21.6 (C-14), 21.0 (5'-CH_3 and 3"-CH_3), 20.6 (8-CH_3), 17.5 (5"-CH_3), 17.1 (12-CH_3), 15.2 (2-CH_3), 12.9 (10-CH_3), 10.9 (C-15), 9.9 (4-CH_3), 133.9, 133.8, 128.8.0, 127.9, 125.5, 125.4, 125.2, 124.4 and 120.8 (1-naphthyl carbons).

X-ray analysis of ***4***

The crystals suitable for X-ray analysis were grown from the MeOH/water solution by slow evaporation at room temperature over 3–4 weeks. Crystallographic data and details of data collection at liquid nitrogen temperature and refinement are listed in table IV. The data were corrected for Lorentz and polarization effects using the Enraf-Nonius SDP/VAX [21] package. The structure was solved by direct methods using the ShelX76 [22] program and refined using the ShelX76 [23] system of programs. Atomic scattering factors were those included in ShelX76 [23]. In the data set the *y* coordinates of O1 and C1 were used to fix the origin in the space group $P2_1$. The hydrogen atoms of the methyl groups (with the exception of those of C41, C18 and C32') and H2, H4, H5, H8, H1', H3', H1", H21" and H22" were calculated on stereochemical grounds and refined riding on their respective C atoms with an overall temperature factor. Other hydrogen atoms were located from the successive difference Fourier syntheses. The hydrogen atoms of the water molecules were not visible in a difference Fourier synthesis. The O-H and N-H distances were normal-

ized to the values of 0.983 and 1.009 Å, respectively, obtained from neutron diffraction [24]. The molecular geometry was calculated by the program package Euclid [25]. Calculations were performed on the Micro-Vax II and Silicon Graphics workstation Iris-4D25G computers of the X-ray laboratory, Ruđer Bosković Institute (Zagreb, Croatia).

Microbiology

MICs were determined in Mueller-Hinton broth (Difco-Laboratories, Detroit, MI) by a microtiter broth dilution procedure described by the National Committee for Clinical Laboratory Standards [20]. Panels were inoculated with each test organism to yield a final inoculum of 5×10^5 cfu/ml and were incubated aerobically at 37°C for 18 h. The MIC in the liquid medium was defined at the lowest concentration of antibacterial agent that inhibited development of visible growth in the microdilution wells. Organisms for quality control were obtained from the American Type Culture Collection, Rockville, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922. All strains were identified by standard procedures and maintained frozen at –70°C. Organisms for quality control were included in each assay.

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