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Rifamycin antibiotics—new compounds and synthetic methods. Part 1: Study of the reaction of 3-formylrifamycin SV with primary alkylamines or ammonia

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Abstract—Within the study covering the search for new methods of synthesis of rifamycin antibiotics, the reactions of 3-formylrifamycin SV (**2**) with primary amines or ammonia were studied. In the reaction of **2** with methylamine, unstable 3-methyliminomethylrifamycin SV (**8**) was formed, which was further oxidised to stable 3-methyliminomethylrifamycin S (**9**). In the reaction of **2** with ammonia, *N*,15-didehydro-15-deoxo-pyrimido-(4,5-*b*)rifamycin SV (**10**), a new compound with a chromophore system enlarged by a pyrimidine ring, was obtained. The product of its reduction with sodium borohydride—(**11**)—was also isolated. The structures of the compounds and an explanation of the synthesis mechanism have been proposed on the basis of mass spectrometry results as well as (1D) and (2D) ^{1}H- and ^{13}C NMR analysis. In vitro antituberculous activity of the new compounds have been investigated.

1. Introduction

The discovery of rifamycin antibiotics in the late 1950s[1] resulted, in 1965, in the synthesis of rifampicin (**3**),[2] introduced into medical care in 1968, which is still the most important agent in the treatment of tuberculosis, leprosy and diseases caused by the nontuberculous mycobacteria, e.g. mycobacteriosis (Scheme 1). Rifamycins are distinguished by a unique chemical structure, in particular, an aromatic chromophore with an *ansa* chain[3] and by a specific antibacterial action, which consists in selective blocking of the β-subunit of the DDRP enzyme (DNA-dependent RNA polymerase) responsible for controlling the rebuilding process of bacterial peptides.[4–10] **3** shows good in vitro and in vivo activities against *Mycobacterium tuberculosis*, *Mycobacterium kansasii* and *Mycobacterium marinum*. It has moderate activity against *Mycobacterium avium–intracellulare* complex and poor activity against rapid growers *Mycobacterium fortuitum* and *Mycobacterium chelonae*.[11] **3** is active against both intracellular and extracellular *M. tuberculosis*. Its use in combination with isoniazid in treatment regimes against tuberculosis allowed the shortening of therapy duration from 18–24 months to 6–9 months. The mutation in the rpoB gene, which is responsible for the coding in the β-subunit of the RNA polymerase, is the genetic basis for the occurrence of resistance phenomena to **3**.[12] In addition to their high activity towards a broad spectrum of Gram-positive bacteria and mycobacteria, rifamycins have also demonstrated the capability to subdue certain types of viruses.[13–15] It also has been determined that rifamycins exhibit a specific hypolipidemic action by decreasing lipid levels, especially cholesterol in blood plasma and lipoproteins.[16,17]

As a result of these unusual properties, the rifamycin derivatives are of great pharmaceutical interest. Therefore, new biologically active semisynthetic rifamycins are the targets of many studies. Clinical testing and studies of structure–biological activity relations have shown that the best activities are achieved if structural modifications are made to the rifamycin S (**5**) or to its hydroquinonic analogue, rifamycin SV (**1**), at the positions C-3 and C-4 of the aromatic chromophore. Another related compound, 3-formylrifamycin SV (**2**),[18,19] is of great importance as a key intermediate in the syntheses of semi-synthetic rifamycin derivatives, including **3**.

The purpose of the research carried out by our group is to search for new C-3-substituted derivatives of **2** exhibiting a

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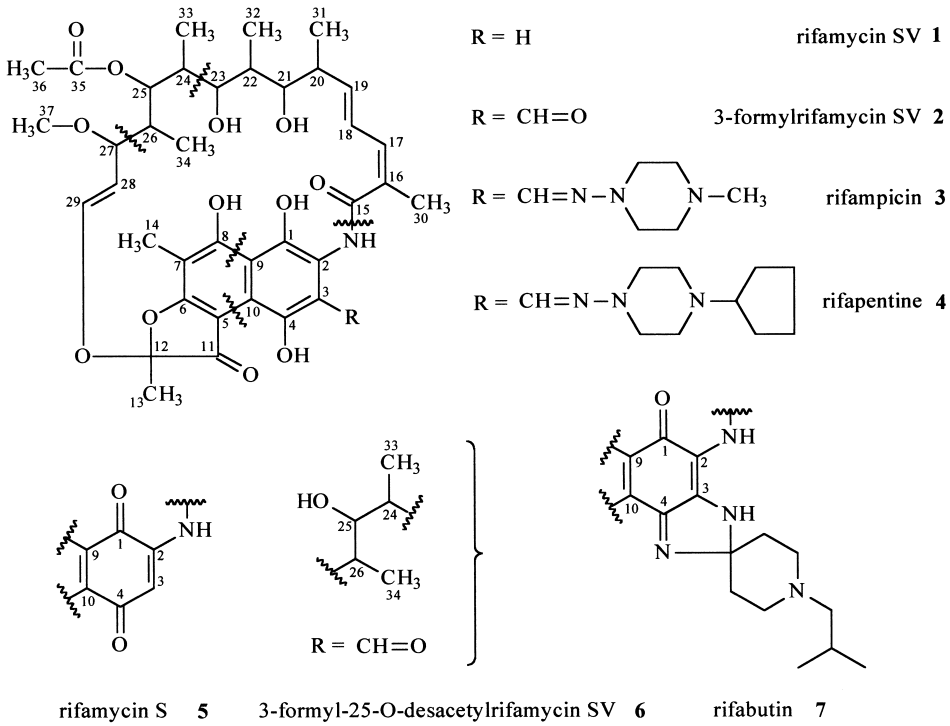


Scheme 1. Structures of some common rifamycin derivatives.

high antibacterial activity, especially towards *M. tuberculosis*. Three groups of reactions were studied in which **2** was the starting material:

1. reactions with primary alkylamines or ammonia,[20]
2. with gaseous ammonia and acetone,[21,22]
3. with ammonia or amines and ketones or aldehydes.[23]

The scope of this work covers the isolation and spectroscopic characterisation of the products formed, the study of the mechanisms involved in these new reactions as well as the evaluation of their activity through antibacterial activity tests. The results of the first part of the above mentioned studies are presented in this paper.

2. Results and discussion

2.1. Synthesis and purification

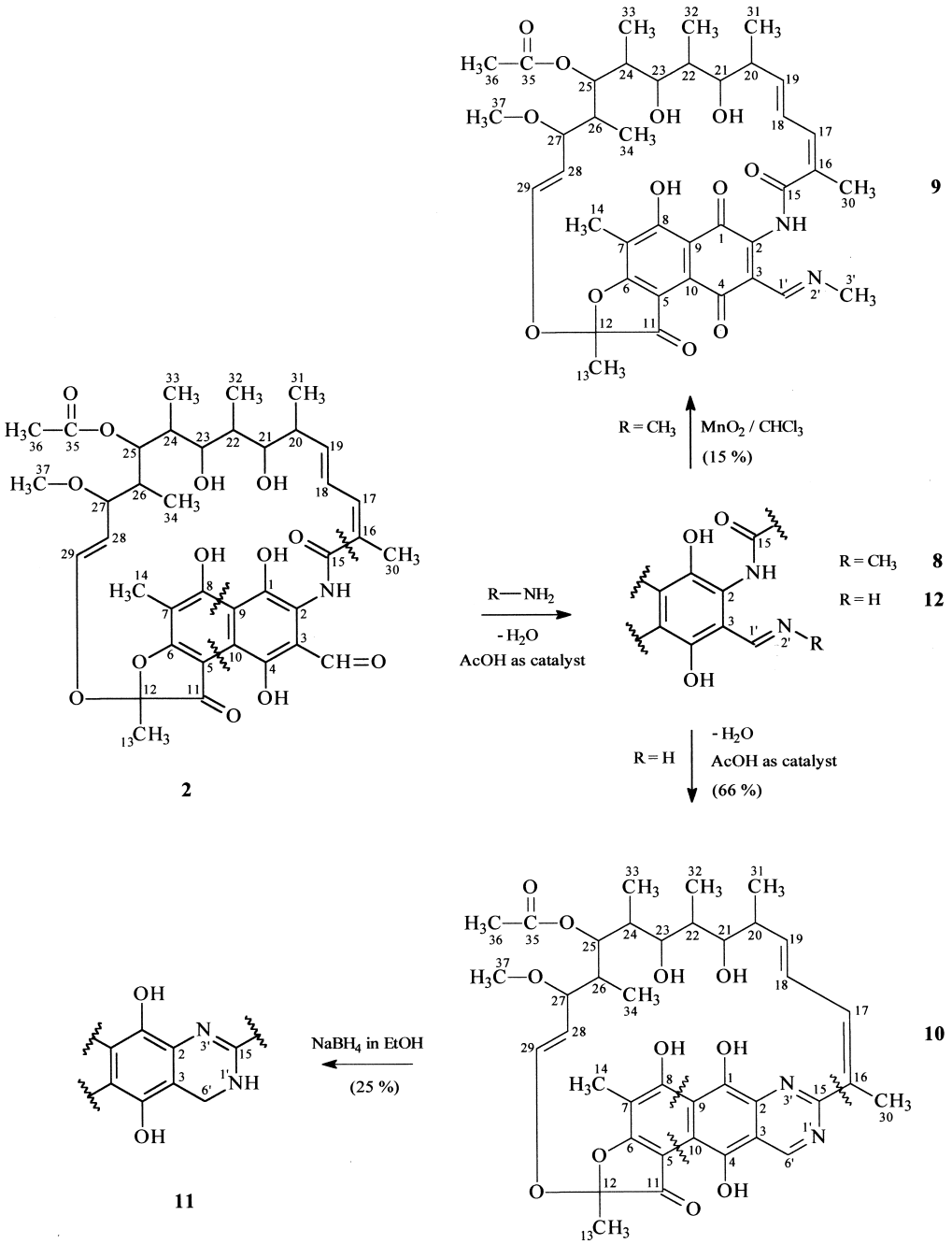
After the reaction of **2** with methylamine in chloroform in the presence of acetic acid as a catalyst, the formation of a product of presumed structure **8** was observed (TLC, intense blue spot). (A similar course was found if the process was carried out in lower aliphatic alcohol or tetrahydrofuran.) This unstable substance gradually decomposes during isolation from the reaction system, the hydrolysis to the starting aldehyde (**2**) being the main decomposition pathway observed (TLC). Compound (**8**) was isolated in the form of a crude precipitate, followed by oxidation with manganese dioxide in chloroform to obtain, after purification, an amorphous, olive coloured, substance (**9**) (quinonic analogue of **8**) (Scheme 2). Other monoalkylamines such as isopropylamine or *tert*-butylamine reacted analogously.

In the first step of the reaction of **2** with gaseous ammonia in chloroform in the presence of acetic acid, the formation of an intermediate was observed (probably of structure **12**) (TLC, intense blue spot), which quickly further reacted to a new substance (**10**) (TLC, violet-brown spot, R_f **10**$<R_f$ **12** (Scheme 2). Compound (**10**) was isolated from chloroform in the form of violet needles. (The formation of **10** was also observed when the reaction was carried out in lower aliphatic alcohols or tetrahydrofuran.) **10** was reduced with sodium borohydride in anhydrous ethanol and the orange coloured product (**11**) (TLC, orange spot, R_f **11**$>R_f$ **10**) was isolated in an amorphous form and purified (Scheme 2).

The structures of the new rifamycin compounds (**9**), (**10**), and of the already reported **11**[24,25] have been proposed on the basis of the mass spectrometry and ^{1}H, ^{13}C NMR-analysis in (D_6)DMSO solutions (Scheme 2, Tables 1–3). **8** and **9** can be included into the known group of 3-iminomethylrifamycin SV[2] and S derivatives. Criccho et al. obtained a broad range of substances of type **8** as hydrazone derivatives by the condensation of **2** with hydrazine substrates having a primary amine group.[26] Rifamycin S (**5**) was the starting material in another method of synthesis of rifamycin SV (**1**) 3-iminomethyl derivatives.[27] **11** was first synthesised by McCarthy et al.[24,25] in a reductive amination of **2** with ammonia in methanol in the presence of sodium cyanoborohydride and hydrogen chloride.

2.2. NMR studies

Generally, the assignments of proton and carbon signals of **9**–**11** were based on (1D) ^{1}H NMR and ^{13}C NMR spectra, on (2D) NMR ^{1}H,^{1}H COSY and ^{1}H,^{13}C COSY experiments (the latter were optimised for a single bond coupling



Scheme 2. Reaction of **2** with primary alkylamines and ammonia.

constant) and, in the case of **10** and **11**, $^{1}H,^{13}C$ HMBC correlation spectra (optimised for more than a single C–H bond interaction) (Tables 1–3). In addition, DEPT 135 spectra as well as ^{1}H NMR (H⟶D exchange) experiments recorded for all the samples were used. The rest of signal assignments were based on the literature[28–33] and our own spectroscopic database.

Compound **9**. In the DEPT 135 spectrum within the 100–160 ppm range, the five signals of all the carbon atoms of CH═ groups of the *ansa* chain were found. The sixth signal (159.5 ppm) was assigned to the C-1′ atom of the substituent at C-3. In comparison, the signal of the analogous carbon atom C-3–*C*H═N^{+} in the rifamycin derivative identified by Taguchi et al.[31] (reaction product of rifamycin S (**5**) with 1,3,5-tri-*t*-butylhexahydro-1,3,5-triazine) occurs at 154.3 ppm. After expansion of the described fragment of the DEPT 135 spectrum, separation of all the signals into two distinct peaks was observed, e.g. the signal of C-28 appears at 121.35 and 121.14 ppm, C-18 at 125.48 and 125.26 ppm. In the $^{1}H,^{13}C$, COSY spectrum a correlation signal H-1′/C-1′ was identified. In the ^{1}H NMR spectrum the signal corresponding to the H-1′ proton occurs in the form of two partially overlapping doublets at ca. 8.54 ppm. Two singlets are observed in this field after H⟶D exchange: a larger one (8.59 ppm) and a smaller one (8.49 ppm). The signal of the analogous C-3–CH═N^{+} proton in Tagushi's rifamycin derivative ($CDCl_3$) appears at 8.98 ppm,[31] and that of the C-3–CH═N proton in rifampicin ($CDCl_3$) at 8.25 ppm.[32]

Table 1. ^{13}C and ^{1}H NMR data of **9** in (D_6)DMSO

Atom C	δ	Atom H	δ	Multipl.	$J_{H,H}$	$^{1}H,^{1}H$ COSY correlations
		H–$N_{(amide)}$	10.71	br		3H-3′ lr
C-1	180.6					
C-2	111.0					
C-3	112.4					
C-4	179.9					
C-5	101.2					
C-6	167.3					
C-7	101.8					
C-8	177.3					
		HO-8	11.25	br		H-1′
C-9	112.4					
C-10	136.4					
C-11	190.8					
C-12	107.4					
C-13	21.8	3H-13	1.63	s		
C-14	7.3	3H-14	2.17	s		
C-15	169.5					
C-16	128.9					
C-17	136.9	H-17	6.52	a)		H-18
C-18	125.2	H-18	6.50	a)		H-17, H-19
C-19	142.2	H-19	6.24	a)		H-20
C-20	36.7	H-20	2.30	a)		H-19, H-21, H-31
C-21	77.7	H-21	3.08	dd		H-20, HO-21
		HO-21	4.47	s		H-21
C-22	37.6	H-22	1.37	a)		H-23, 3H-32
C-23	75.5	H-23	3.14	a)		H-22, H-24, HO-23
		HO-23	2.92	m		H-23
C-24	32.9	H-24	1.60	a)		H-23, 3H-33
C-25	72.5	H-25	5.08	dd	(25,24)<2.0	H-26
C-26	40.4	H-26	1.24	m	(25,26)=10.1	H-25, H-27, 3H-34
C-27	75.7	H-27	3.43	m	(27,28)=7.2	H-26, H-28
C-28	121.1	H-28	5.25	dd	(28,29)=12.6	H-27, H-29
C-29	145.5	H-29	6.30	a)		H-28
C-30	21.1	3H-30	2.09	s		
C-31	14.4	3H-31	0.85	a)		H-20
C-32	9.5	3H-32	0.53	d	(32,22)=6.0	
C-33	10.4	3H-33	0.88	a)	(33,24)=6.6	H-24
C-34	9.3	3H-34	−0.03	d	(34,26)=6.0	H-26
C-35	172.6					
C-36	20.6	3H-36	2.00	s		
C-37	56.0	3H-37	2.97	s		
C-1′	159.5	H-1′	8.56	m		HO-8, 3H-3′ lr
C-3′	37.5	3H-3′	3.28	m		H–$N_{(amide)}$ lr, H-1′ lr

Abbreviations: s: singlet, d: doublet, m: multiplet, br: broad, lr: long range correlation, a) this signal overlapped with another signal.

In the ^{13}C NMR spectrum the signal of the C-3′ carbon occurs at 37.5 ppm. In the $^{1}H,^{13}C$, COSY spectrum the correlation signal 3H-3′/C-3′ was identified. In the ^{1}H NMR spectrum the signal of the 3H-3′ protons occurs as a doublet at 3.41 ppm.

Two different size signals of proton H-1′ in the ^{1}H NMR spectrum after H⟶D exchange, as well as the splitting of signals in the carbon spectrum indicate that **9** occurs in the form of a mixture of two isomers, probably differing in the (*E*)- and (*Z*)-configuration of the C=N bond of the imino group. It appears that one of the two isomers quantitatively prevails.

In a (D_6)DMSO solution of **9** a polar tautomeric form (B) in which a proton is present at the nitrogen atom N-2′ probably dominates (Scheme 3). The correlation signals HO-8/H-1′, HO-8/3H-3′ and H-1′/3H-3′ in the $^{1}H,^{1}H$ COSY spectrum and the splitting of signals of H-1′, 3H-3′ and HO-8 protons in the ^{1}HNMR spectrum (Table 1) are a result of this tautomeric equilibrium. Thus, the chemical shift of the signal at 177.3 ppm in the ^{13}C NMR spectrum of **9**, tentatively assigned to C-8, is ca. 11 ppm low-field shifted with respect to the one found at 166.7 ppm in the spectrum of rifamycin S ($CDCl_3$).[29] A similar difference, of ca. 13 ppm, occurs for the chemical shifts of the C-1 signal of the sodium phenolate at 168.1 ppm and that of phenol alone at 155.4 ppm ($CDCl_3$).[33]

Compound **10**. On the basis of the correlation signals recorded in $^{1}H,^{13}C$ COSY and $^{1}H,^{13}C$ HMBC spectra, the signal (155.3 ppm) in the ^{13}C NMR spectrum was attributed to the C-6′ (sp_2) carbon atom. The chemical shift of this signal is analogous to the one found for the C-3–*C*H=N^+ carbon atom at 154.3 ppm in the substance produced by Taguchi et al.[31] and the chemical shift of the C-10–*C*-4=N-3 carbon signal at 155.9 ppm in the quinazoline ($CDCl_3$).[34] In the ^{1}H NMR spectrum of **10** the signal of the H-6′ proton occurs as a singlet (9.57 ppm), which does not disappear after H⟶D exchange. In the $^{1}H,^{13}C$ HMBC spectrum correlation signals are observed originating from long range couplings of the H-6′ protons with carbon atoms: C-1, C-2, C-3, C-4 and C-15 (Table 2).

Assignment 15 signals of quaternary carbon atoms in the

Table 2. ^{13}C and ^{1}H NMR data of **10** in (D_6)DMSO

Atom C	δ	Atom H	δ	Multipl.	$J_{H,H}$	$^{1}H,^{1}H$ COSY correlations	$^{1}H,^{13}C$ HMBC correlations
C-1	150.4						
		HO-1	16.38	s			
C-2	134.8						
C-3	113.1						
C-4	141.1						
		HO-4	13.16	s			C-2, 3, 4
C-5	98.1						
C-6	173.0						
C-7	100.1						
C-8	185.5						
		HO-8	7.09	br			
C-9	115.2						
C-10	113.6						
C-11	183.4						
C-12	108.8						
C-13	22.2	3H-13	1.67	s			C-11, 12
C-14	7.4	3H-14	1.95	s			C-6, 7, 8
C-15	160.2						
C-16	135.5						
C-17	130.0	H-17	6.28	d	(17,18)=10.9	H-18	C-16, 19
C-18	126.4	H-18	6.35	dd	(18,19)=15.7	H-17, H-19	C-16, 20
C-19	137.1	H-19	5.83	dd	(19,20)=6.8	H-18	
C-20	38.1	H-20	2.12	m	(20,21)=8.0	3H-31	
C-21	72.2	H-21	3.50	ddd	(21,22)=2.7		
		HO-21	4.47	d	(HO-21,21)=3.5	HO-23 lr	
C-22	37.4	H-22	1.13	a)	(22,23)=2.4	H-23, 3H-32	
C-23	75.8	H-23	2.79	ddd	(23,24)=10.0	H-22, HO-23	
		HO-23	4.32	d	(HO-23,23)=7.4	HO-21 lr, H-23	
C-24	32.9	H-24	1.51	m		3H-33	
C-25	73.0	H-25	5.04	dd	(25,24)<1.0	H-26	C-35
C-26	40.2	H-26	1.13	a)	(25,26)=11.0	H-25, 3H-34	
C-27	76.4	H-27	3.23	dd	(27,26)<1.0	H-28	C-28
C-28	116.8	H-28	4.92	dd	(27,28)=7.8	H-27, H-29	
C-29	142.4	H-29	6.20	d	(28,29)=12.7	H-28	C-12, 27, 28
C-30	20.6	3H-30	2.28	s			C-15, 16, 17
C-31	18.2	3H-31	0.85	d	(31,20)=7.0	H-20	C-19, 20, 21
C-32	7.7	3H-32	0.30	d	(32,22)=6.9	H-22	C-22
C-33	11.6	3H-33	0.81	d	(33,24)=7.0	H-24	C-24
C-34	8.4	3H-34	-0.37	d	(34,26)=7.0		C-25, 26, 27
C-35	169.7						
C-36	22.1	3H-36	1.98	s			C-35
C-37	55.8	3H-37	2.91	s			C-27
C-6′	155.3	H-6′	9.57	s			C-1, 2, 3, 4, 15

^{13}C NMR spectrum was made on the basis of the correlation signals found in the $^{1}H,^{13}C$ HMBC spectrum, utilising the literature[28–32] and our own spectroscopic data (Table 2). The C-15 signal (160.2 ppm) is high-field shifted by ca. 8–10 ppm with respect to the C-15 signal in various rifamycin SV derivatives, in which this carbon atom is a part of a tertiary or secondary amide group.[31,32] This has been ascribed to the formation of a new aromatic pyrimidine ring as a part of a substituted quinazoline system in which the C-15 carbon atom is bonded to two nitrogen atoms (Scheme 2). A similar chemical shift (160.7 ppm) was assigned for the signal of the N-1–C-2=N-3 carbon atom of quinazoline ($CDCl_3$).[34]

Despite careful drying of the sample under vacuum, supplementary signals were observed at 79.13 ppm in the ^{13}C NMR spectrum and at 8.31 ppm in the ^{1}H NMR spectrum, these signals can be attributed to the presence of chloroform in the crystals. This latter signal was absent when $CDCl_3$ instead of $CHCl_3$ was used for the synthesis and crystallisation of **10**. This proves the formation of a very stable adduct of **10** with chloroform.

An unusual process has been noticed in the aromatic chromophore of **10** in (D_6)DMSO solutions. The molecular, and thus magnetic, environment of C-8 and C-11 becomes similar (a small difference in the chemical shifts of atoms C-8 and C-11, 185.5 and 183.4 ppm, respectively, can still be detected in the ^{13}C NMR spectrum) (Table 2). This can be correlated to a quick migration of the HO-8 proton and the coexistence in solution of equal quantities of isomeric forms (C) and (D) (Scheme 3). Taguchi et al. drew attention to a similar phenomenon when interpreting the results of NMR studies of a rifamycin derivative obtained from the reaction of rifamycin S with 1,3,5-tri-*t*-butyl hexahydro-1,3,5-triazine ($CDCl_3$).[31] This process might result from the electron withdrawing influence of the newly formed pyrimidine ring, condensed at C-2 and C-3 of the chromophore, which consequently increases the acidity of the phenol hydroxyl group at C-8. In the ^{13}C NMR spectrum of rifampicin (**3**) ($CDCl_3$) the C-8 and C-11 signals occur at 169.2 and 195.4 ppm, respectively,[32] which indicates the absence of such a tautomeric equilibrium.

Compound **11**. The negative-phased signal of the C-6′ atom

Table 3. ^{13}C and ^{1}H NMR data of **11** in (D_6)DMSO

Atom C	δ	Atom H	δ	Multipl.	$J_{H,H}$	$^{1}H,^{1}H$ COSY correlations	$^{1}H,^{13}C$ HMBC correlations
C-1	142.6						
		HO-1	16.50	s			
C-2	117.9						
C-3	104.8						
C-4	141.5	HO-4	12.40	s			C-2,3,4,10
C-5	98.4						
C-6	171.7						
C-7	100.8						
C-8	183.8						
		HO-8	11.62	s			
C-9	113.3						
C-10	112.4						
C-11	186.0						
C-12	108.2						
C-13	21.7	3H-13	1.75	s a)			C-11,12
C-14	7.1	3H-14	2.03	s			C-6,8
C-15	157.3						
C-16	124.4						
C-17	123.1	H-17	6.17	a)		H-18	
C-18	134.3	H-18	6.53	dd		H-17, H-19	
C-19	141.6	H-19	6.08	a)		H-18	
C-20	39.5	H-20	2.28	m		H-21, 3H-31	
C-21	71.0	H-21	3.90	m		H-20	
		HO-21	4.83	d		HO-23 lr	
C-22	37.4	H-22	1.61	m		H-23, 3H-32	
C-23	75.8	H-23	3.14	a)		H-22, HO-23, H-24	
		HO-23	4.66	a)		HO-21 lr, H-23	C-21
C-24	33.7	H-24	1.75	a)		H-23, 3H-33	
C-25	73.0	H-25	5.30	dd	(25,24)<2.0	H-26	C-35
C-26	40.0	H-26	1.32	m	(25,26)=10.8	H-25, 3H-34	
C-27	76.4	H-27	3.44	d	(27,28)=7.0	H-28	C-28
C-28	116.3	H-28	5.06	dd	(28,29)=12.7	H-27, H-29	
C-29	142.2	H-29	6.28	d		H-28	C-12,28
C-30	20.2	3H-30	2.19	s			C-15,16
C-31	18.1	3H-31	0.99	d a)		H-20	C-20,21
C-32	8.2	3H-32	0.73	d	(32,22)=6.8	H-22	C-22
C-33	12.8	3H-33	1.02	d a)		H-24	C-24
C-34	8.2	3H-34	−0.02	d	(34,26)=6.7		C-26,27
C-35	170.0						
C-36	20.7	3H-36	2.14	s			C-35
C-37	56.0	3H-37	3.08	s			C-27
		H-1′	10.15	s			
C-6′	38.7	2H-6′	4.66	a)			C-4,10,15

in the newly formed dihydropyrimidine ring was found in the DEPT 135 spectrum of this substance at 38.7 ppm. In the ^{1}H NMR spectrum the signal of two protons bonded to C-6′ occurs as a doublet at 4.66 ppm—as a result of the coupling with H-1′ (Table 3).

Assignment of the quaternary carbon atoms present in **11** was made on the basis of the correlation signals found in the $^{1}H,^{13}C$ HMBC spectrum, by comparison with the spectrum of **10** and the literature data.[28–32] Thus, the signal at 157.3 ppm was assigned to the C-15 carbon atom in **11** (Table 3).

In the ^{1}H NMR spectrum of **11** four signals are present at low field assigned to mobile protons (these signals disappear after H→D exchange). Three signals were assigned to the three hydroxyl protons of the chromophore and one, at 10.15 ppm, to the H-1′ in the newly formed dihydropyrimidine ring (Table 3).

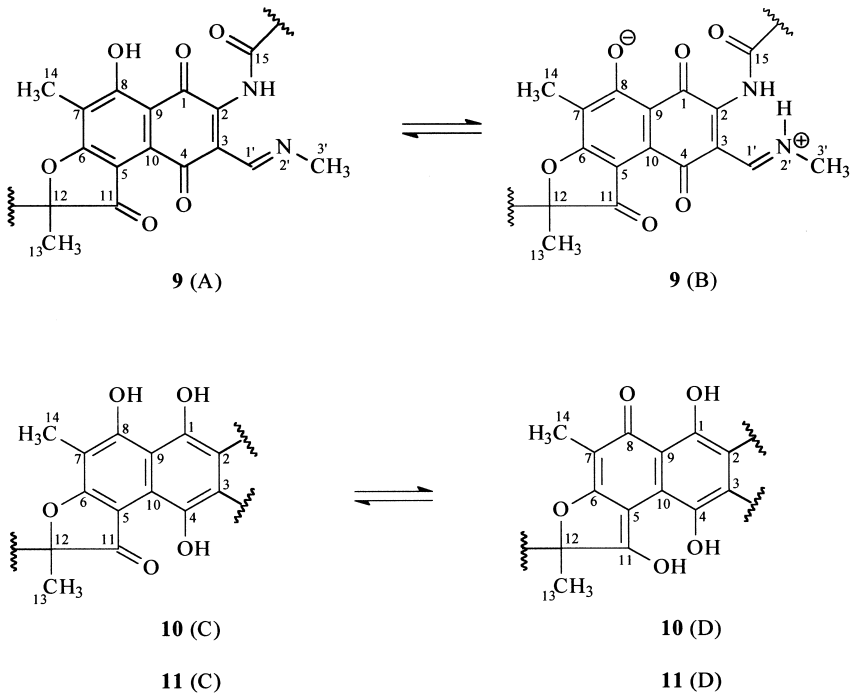
A tautomeric exchange probably occurs in a (D_6)DMSO solution of **11**, as found in the case of **10** (Scheme 3), leading to a similar chemical environment for carbons C-8 and C-11 which can again be seen in the ^{13}C NMR spectrum (183.8 and 186.0 ppm, respectively).

11, contrary to **10**, does not show a tendency to form an adduct with chloroform.

2.3. Synthesis of 10, mechanistic proposal

In the first stage of the reaction of **2** with ammonia an intermediate is formed, most probably an iminomethylrifamycin SV (**12**), which then undergoes an intramolecular cyclisation to form the stable new derivative (**10**), with the rifamycin chromophore enlarged by the newly formed pyrimidine ring (Scheme 2).

This intramolecular cyclisation does not occur when another substituent instead of a proton (e.g. an alkyl group) is present in the imino group of the intermediate (Scheme 2). The possibility for the imino group of the intermediate's carbocation to eliminate a proton is most probably required for the formation of a pyrimidine system in **10**.



9 (A) **9** (B)

10 (C) **10** (D)

11 (C) **11** (D)

Scheme 3. Tautomeric equilibria in (D_6)DMSO solution of **9**–**11**.

2.4. Initial studies on the in vitro antibacterial properties of 10 and 11

The obtained novel compounds were tested for antimicrobial and tuberculostatic activity in vitro against different strains of *M. tuberculosis*. The following strains have been used: (i) standard strain—*M. tbc* $H_{37}Rv$; (ii) some wild strains isolated from patients and sensitive to rifampicin (**3**)—*M. tbc* 234, *M. tbc* 243; (iii) some wild strains isolated from patients and resistant to rifampicin (**3**)—*M. tbc* 125, *M. tbc* 220, *M. tbc bovis*. They were also tested against different types of MOTT (Mycobacteria other than *M. tuberculosis*) sensitive or resistant to rifampicin (**3**): *M. kansasii*, *M. scrofulaceum*, *M. avium intracellulare*, *M. fortuitum*. **10** shows a marked antituberculous activity (MIC 0.5–1.0 μg/ml) against strains sensitive t o rifampicin although lower than **3** itself (Table 4). **11** proved to be much less active than **10**. All strains of *M. tuberculosis* resistant to **3** and all strains of MOTT tested were resistant to **3**, **10** and **11** at concentrations below 1.0 μg/ml.

3. Conclusion

Unstable 3-alkyliminomethyl derivatives of rifamycin SV, of type **8**, which are formed in the reaction of **2** with monoalkylamines, give stable quinonic 3-alkyliminomethyl compounds of type **9** after oxidation. Compound (**9**) probably occurs in the form of a mixture of two isomers, differing in the (*E*)-and (*Z*)-configuration of the substituents at the C-1′═N-2′ bond.

N,15-Didehydro-15-deoxo-pyrimido-(4,5-*b*)rifamycin SV (**10**)—a new derivative with an enlarged chromophore system is the reaction product of **2** with ammonia, in which the C-3 and C-15 carbon atoms of the backbone are linked by a newly formed –CH═N– group, building a pyrimidine ring. This results in a shortening of the *ansa* chain and leads to a molecular structure which differs from the structure of natural rifamycins.

The presented synthesis of **10** is also an interesting variant

Table 4. Antimycobacterial activity (MIC in μg/ml) of compounds **10** and **11** with reference to rifampicin (**3**). Protocol of the in vitro experiments

Strain	Compound	Concentration of compound (μg/ml)							
		0	0.03	0.06	0.12	0.25	0.5	1.0	3.1
M.tbc $H_{37}Rv$	**3**	+++	nt	nt	nt	nt	nt	nt	−
	10	+++	nt	nt	nt	nt	nt	nt	−
	11	nt	nt	nt	nt	nt	nt	nt	nt
M. tbc 234	**3**	+++	+++	++	+	−	−	−	nt
	10	+++	+++	+++	+++	++	+	−	nt
	11	+++	+++	+++	+++	+++	+++	+++	nt
M. tbc 234	**3**	+++	+++	+++	++	+	−	−	nt
	10	+++	+++	+++	++	+	−	−	nt
	11	+++	+++	+++	+++	+++	+++	+++	nt

(+)—growth, (−)—no growth, nt—not tested.

of the synthesis of substituted pyrimidine rings as parts of quinazoline systems. **10** is easily reduced with sodium borohydride in ethanol to yield **11**—a compound with a dihydropyrimidine ring as the main product (Scheme 2).

In a (D_6)DMSO solution of **9**–**11** specific tautomeric phenomena were observed (Scheme 3). **9**, as a result of the migration of the HO-8 proton to the N-2′ nitrogen atom, occurs mainly in the dipolar form (B). However, the migration of the HO-8 proton to the O=C-11 carbonyl group in **10** or **11** results in an equilibrium of the isomeric forms of (C) and (D). In introductory studies it was found that **10** shows clear action against *M. tuberculosis*.

4. Experimental

4.1. General

The reagents used: 3-formylrifamycin SV (**2**) (Polfa—Tarchomin) and methylamine, isopropylamine, *t*-butylamine, ammonia, acetic acid (all-synthesis grade purity). TLC: Merck Al-precoated silica gel 60 F_{254} (0.2 mm) plates, a $CHCl_3$/MeOH mixture (9:1) was used as the eluent. CC: glass columns, Merck silica gel 60 (0.040–0.063 mm), $CHCl_3$/MeOH (9:1) as the mobile phase. NMR: the ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 200 (^1H: 200.13 MHz, ^{13}C: 50.32 MHz) and Varian $Inova_{Unity}$ 400 spectrometer (^1H: 400.20 MHz, ^{13}C: 100.64 MHz) in (D_6)DMSO; chemical shift values δ in ppm relative to $SiMe_4$ as internal reference; J in Hz. In order to unequivocally attribute some coupling constants and chemical shifts (1D) ^1H NMR spectra were recorded on a Bruker AM 500 instrument (^1H: 500. 13 MHz). MS: mass spectra by the ESI technique, containing high resolution ESI MS, were recorded by employing a Mariner (PerSeptive Biosystems) mass spectrometer. For analysis, samples were dissolved in CH_3OH. IR: IR spectra (KBr pellets) were recorded on a Perkin–Elmer PE-577 (**9**) and Perkin–Elmer 16 PC (**10**, **11**) spectrometers. Mp determination: the new rifamycin derivatives have no definite melting points—they do not melt up to 300°C, at ca. 160°C decomposition starts. Biological tests: bacteriostatic properties of the compounds were studied on agar Petri plates, Youmans liquid medium containing 10% bovine serum. Rifampicin (**3**) (Ciba-Geigy) has been used as a reference drug.

4.1.1. 3-[(Methylimino)methyl]rifamycin S (9). 5.0 g (ca. 7 mmol) of 3-formylrifamycin SV (**2**) was dissolved in a solution of 40 ml chloroform and 1.6 g (ca. 27 mmol) acetic acid. The reaction mixture was stirred at 30–35°C and saturated with methylamine. After 2.5 h the starting aldehyde **2** reacted (TLC, $CHCl_3$/MeOH, 9:1) to yield the blue-violet substance (**8**) as the main product. The reaction mixture was washed twice with 150 ml of water. The remaining organic phase was dried with anhydrous sodium sulphate, and after separating the drying agent, evaporated to dryness, to obtain ca. 5.0 g of the crude product containing **8** as the main component. 3-Formylrifamycin SV (**2**) appeared among the precipitate components as a result of the partial hydrolysis of **8**. 1.0 g of the compound (**8**)/the crude reaction mixture was dissolved in 25.0 ml of chloroform and stirred with 0.6 g of manganese dioxide for 0.5 h. After this time an orange coloured oxidation product (**9**) was observed (TLC, $CHCl_3$/MeOH 9:1). Manganese dioxide was then filtered off from the reaction mixture. The chloroform filtrate was dried with anhydrous sodium sulphate and concentrated to ca. 4 ml. This concentrate was purified by CC ($CHCl_3$/MeOH 9:1 mixture as the eluent). The selected fractions containing **9** were washed with water, concentrated under vacuum to ca. 5 ml and diluted with *n*-hexane, to give **9** as an amorphous precipitate. Yield: 0.15 g of **9** (14.6%). ^{13}C and ^1H NMR data: Table 1. MS (ESI) *m/z*: 737.3 (100.0, $(M+H)^+$), 759.3 (66.7, $(M+Na)^+$). HRMS calcd for $(M+H)^+$ $C_{39}H_{48}N_2O_{12}$ 737.3280, found 737.3310, error 4.1 ppm. IR: 3460 (br.), 2970, 1730, 1650, 1590, 1405 cm^{-1}.

4.1.2. *N*,15-Didehydro-15-deoxo-pyrimido-(4,5-*b*)rifamycin SV (10). A solution of 5.8 g (ca. 8 mmol) of **2** in a mixture of 50 ml of chloroform and 2.0 ml (ca. 40 mmol) of acetic acid was saturated under stirring for 2 min with gaseous ammonia at 40–45°C. After 1 h of reaction at this temperature, the initial aldehyde (**2**) underwent complete conversion (TLC). The reaction mixture was first washed with 150 ml of water; the pH of the aqueous phase was adjusted during washing with 5% H_2SO_4 to ca. 6.0–7.0. The chloroform solution was then washed with 150 ml of aqueous Na_2HPO_4 (5%), and next dried with anhydrous sodium sulphate. The solution was evaporated to ca. 30 ml and left for 16 h at ca. 5°C. The crystalline violet product was filtered off, washed with cold (10°C) chloroform and dried under vacuum. Yield: 3.7 g of **10** (65.5%). ^{13}C and ^1H NMR data: Table 2, MS: (ESI) *m/z*: 727.3 (100.0, $(M_{oxidised}+Na)^+)^*$, 729.3 (65.5, $(M+Na)^+$), 751.3 (21.4, $(M+Na+MeOH)^+)^{**}$. HRMS calcd for $(M+Na)^+$ $C_{38}H_{46}N_2O_{11}Na$ 729.2994, found 729.3011, error 2.4 ppm). (*)—During MS analysis oxidation of **10** (hydroquinone) to the quinone form takes place. (**)—Ion of the adduct with methanol. IR: 3432 (sharp), 2968, 1712 (sharp), 1648, 1622 cm^{-1}.

4.1.3. *N*,15-Didehydro-15-deoxo-1′,6′-dihydropyrimido-(4,5-*b*)rifamycin SV (11). 1.0 g (ca. 1.4 mmol) of **10** in 25 ml of dry ethanol was stirred at room temperature with 0.1 g (ca. 2.6 mmol) of $NaBH_4$. After 2 h, the conversion of **10** was completed and the formation of a mixture of products with a dominance of **11** (TLC, $CHCl_3$/MeOH 9:1, orange spot, R_f **11**>R_f **10**) could be noticed. The reaction mixture was diluted with 30 ml of chloroform, followed by cautious addition of 50 ml aqueous Na_2HPO_4 (5%). The organic phase was then once again washed with 30 ml of Na_2HPO_4 (5%), dried with anhydrous sodium sulphate and evaporated to dryness, obtaining 0.8 g of a crude precipitate of **11**. It was then purified by CC ($CHCl_3$/MeOH 88:12). **11** was obtained as an amorphous yellowish powder after precipitation with *n*-hexane from concentrated selected fractions of the eluate. Yield: 0.25 g of **11** (24.9%). ^{13}C and ^1H NMR data Table 3. MS: (ESI) *m/z*: 709.3 (100.0, $(M+H)^+$). HRMS calcd for $(M+H)^+$ $C_{38}H_{49}N_2O_{11}$ 709.3331, found 709.3365, error 4.8 ppm. IR: 3416 (br.), 2974, 1714, 1646, 1602 cm^{-1}.

4.2. Biological tests

See Table 4.

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