21. Synthesis, Reactivity Studies, and X-Ray Crystal Structure of (11*R*)-25-*O*-Deacetyl-11-deoxo-11-hydroxy-21,23-*O*-isopropylidenerifamycin S

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The protected intermediate (11*R*)-25-*O*-deacetyl-11-deoxo-11-hydroxy-21,23-*O*-isopropylidenerifamycin S (**7**) has been synthesized starting from rifamycin S (**2**; *Scheme 2*), the former being a potential substrate for the preparation of new types of rifamicin-S derivatives modified at C(11) and/or C(25). The reactivity of **7** toward acylations has been studied under both base- and acid-catalyzed conditions. The compound either did not react or underwent unexpected reactions, and no acylation products could be isolated. The X-ray crystal structure of **7** reveals that both OH groups at C(11) and C(25) are hindered, and this is probably the reason, why other reactions take place faster than acylation.

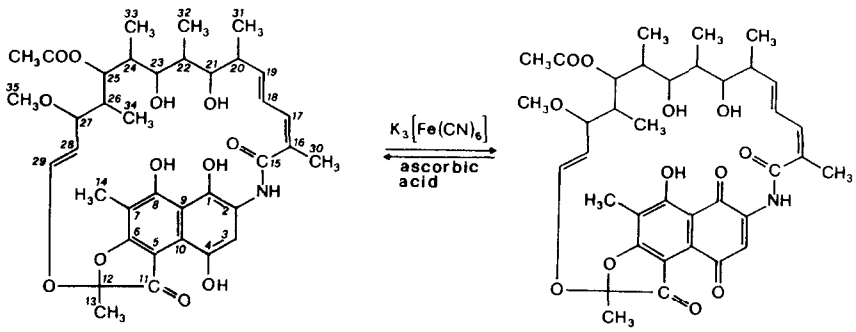
Introduction. – Rifamycins are antibiotics belonging to the group of naphthalenic ansamycins which show their activity by the specific inhibition of bacterial DNA-dependent RNA polymerase (DDRP) [1].

Rifamycin SV (= rifamycin; **1**; *Scheme 1*) a natural naphthohydroquinone, is very active against *Gram*-positive bacteria and mycobacteria and less active against *Gram*-negative bacteria. It shows some pharmacokinetic limitations (*i.e.* fast elimination *via* biliary route) [1]. Many derivatives have, therefore, been prepared starting from rifamycin S (= 1,4-dideoxy-1,4-dihydro-1,4-dioxorifamycin; **2**; *Scheme 1*) the naphthoquinone form of **1**, in the hope of extending the range of activity and of changing the pharmacokinetic behavior [1]. Modifications at C(3) and C(4) have been mostly exploited for the preparation of these derivatives. However, among all new products, only two, *i.e.* *Rifampicin* (**3**) [2] and recently *Rifaximin* (**4**) [3], have been introduced into therapy.

It has been shown that the oxygenated functions at C(1), C(8), C(21), and C(23) in **1** and its derivatives are directly involved in the interaction with the enzyme, and that the introduction of substituents at C(3), modulating the acidity of the OH–C(8)/OH–C(1) system, influences, with opposite effects, both the strength of the enzyme-drug binding [4] and the ability to penetrate the bacterial cell wall [5]. This may be one reason, why so many derivatives have shown no improvement with respect to rifamycin SV (**1**).

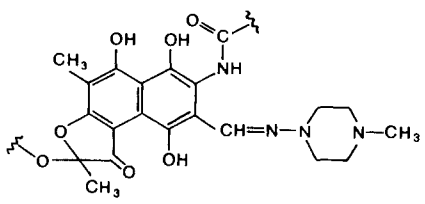
Established structure-activity relationships indicate other possible sites which can be used for the preparation of new derivatives. In particular, the hydrolysis of the AcO

Scheme 1

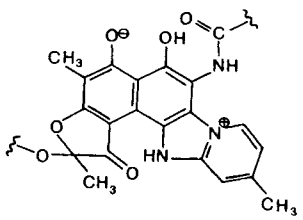


1 Rifamycin SV

2 Rifamycin S



3 Rifampicin



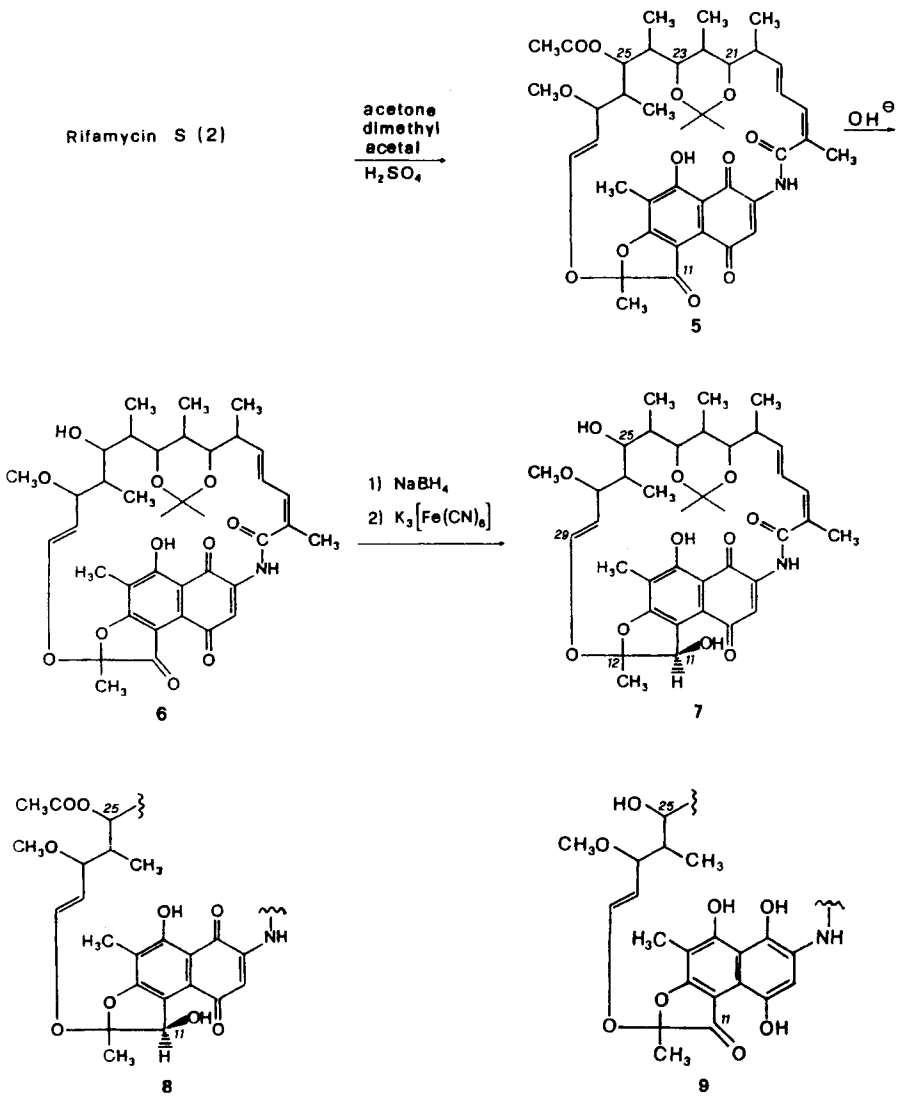
4 Rifaximin

group at C(25) and the reduction of the C(11)=O group result in the formation of secondary alcohols. These transformations by themselves have no significant influence on activity [1] [6], but the two new OH groups are potential substrates for the preparation of series of new derivatives such as esters, ethers, and urethanes.

The present paper reports on the synthesis, reactivity studies under acylating conditions, and X-ray crystal structure of (11*R*)-25-*O*-deacetyl-11-deoxo-11-hydroxy-21,23-*O*-isopropylidenerifamycin S (**7**), a derivative of rifamycin S (**2**) protected at C(21) and C(23), that can be functionalized at C(25) and/or at C(11).

Synthesis of (11*R*)-25-*O*-Deacetyl-11-deoxo-11-hydroxy-21,23-*O*-isopropylidenerifamycin S (7). – Since the OH groups on C(21) and C(23) are indispensable for the activity of rifamycins, it is necessary to protect them in order to prevent their participation during the formation of derivatives of rifamycin S (**2**). Hence, 21,23-*O*-isopropylidenerifamycin S, **5** [7] was prepared first *(Scheme 2)* and subsequently deacetylated to give 25-*O*-deacetyl-21,23-*O*-isopropylidenerifamycin S **6** [7]. Then, **6** was reduced with $NaBH_4$ to give the SV form of the 11-deoxo-11-hydroxyrifamycin derivative [7]. Since such hydroxyrifamycins are not stable in the SV form [7], this compound was not isolated, but directly oxidized with $K_3[Fe(CN)_6]$ [8] (see **1** → **2**) to give the desired (11*R*)-25-*O*-deacetyl-11-deoxo-11-hydroxy-21,23-*O*-isopropylidenerifamycin S **7** (^{1}H-NMR spectrum in *Fig. 1*). It has already been shown that reduction at C(11) is stereospecific giving only the (*R*)-epimer [6]. The configuration at C(11) in **7** is particularly important both in view of reactions with bifunctional reagents, aimed at the formation of C(11)–C(25)-bridged

Scheme 2



derivatives, and in consideration of the fact that the newly formed OH group in the epimeric (*S*)-configuration could interfere with the hypothesized π–π bonding interaction between the antibiotic chromophore rings and an aromatic amino acid on DDRP in the inhibition process.

The reaction sequence shown in *Scheme 2* proved to be the best one. In fact, deacetylation has to follow protection at C(21) and C(23) in order to prevent the formation of the 23,25-*O*-isopropylidene derivative [7]. Furthermore, the reduction should follow the deacetylation. In fact, when **5** was first reduced at C(11) to give (11*R*)-11-deoxo-11-hy-

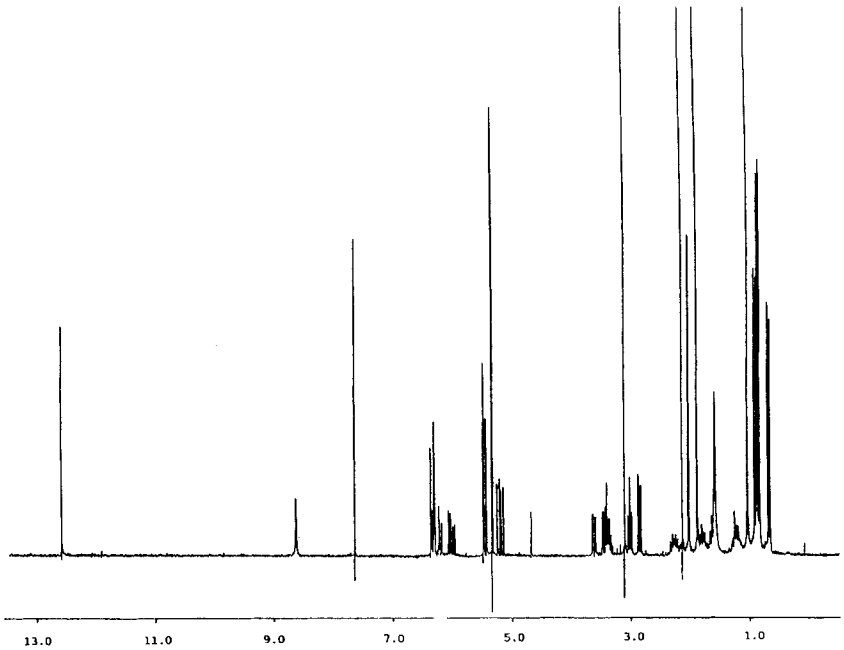


Fig. 1. *200-MHz ^{1}H-NMR spectrum of* **7**. 40 mM in CD_2Cl_2; δ in ppm from Me_4Si.

droxy-21,23-*O*-isopropylidenerifamycin S (**8**) and then deacetylated at C(25); 25-*O*-deacetyl-21,23-*O*-isopropylidenerifamycin SV (**9**) resulted instead of **7**, the basic conditions having caused a disproportion reaction between the C(11) and C(1)-to-C(4) moieties.

The ^{1}H-NMR data of compounds **5**–**9** are reported in *Table 1*.

Reactivity Studies. – *Base-Catalyzed Acylations.* Propionic and pivalic-acid esters of 25-*O*-deacetyl-21,23-*O*-isopropylidenerifamycin S (**6**) have already been prepared [7], while no reaction concerning the OH group at C(11) has been observed. Following the procedures described in [7], the synthesis of a pivaloyl derivative was tried starting from the 25-*O*-deacetyl-11-deoxo-11-hydroxy derivative **7** and using a large excess of pivaloyl chloride and anhydrous pyridine, the latter acting both as solvent and base reagent (room temperature for ten days). However, the medium was too basic, and the disproportion mentioned above (**8** → **9**) quickly took place yielding **9**.

We then sought for more controlled acylation conditions using 4-(dialkylamino)-pyridines as catalysts in the presence of Et_3N. In particular, we decided to try 4-(dimethylamino)pyridine and the more effective 4-(pyrrolidino)pyridine [8] and an acylating reagent such as pivalic or glutaric anhydride or chloride. However, reaction of **7** with pivalic anhydride in the presence of Et_3N and 4-(dimethylamino)pyridine, according to the conditions described in [8], led again to **9**.

Therefore, we decided to study first the acylation of **6** which is deacetylated but not reduced at C(11). The reaction of **6** with Et_3N, 4-(dimethylamino)pyridine, and pivalic anhydride gave no acylation products, but led to a compound, with 4-(dimethylamino)pyridine added to the rifamycin-S skeleton at C(3) giving rise to the SV form **10**

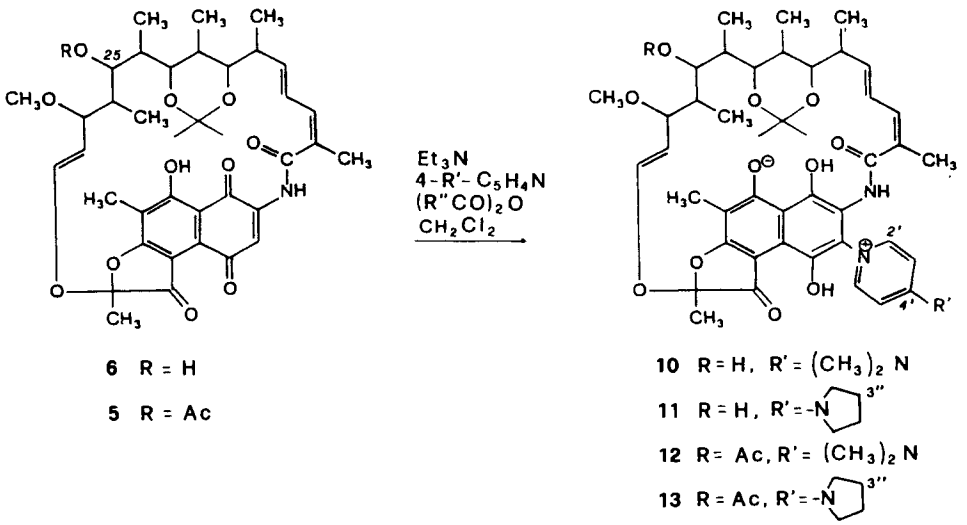
Table 1. *^{1}H-NMR Data* (in ppm rel. to Me_4Si) *in $CDCl_3$ for* **5**, **6**, *and* **8**, *in CD_2Cl_2 for* **7**, *and in D_2O for* **9**

	5		**6**		**7**		**8**		**9**	
NH–C(15)	8.158		8.253		8.632		8.405		7.450	
H–C(17)	6.170		6.214	(J = 11.3)	6.198	(J = 8.5)	6.159		*ca.* 6.2	
H–C(18)	6.170		6.203	(J = 15.5)	6.330	(J = 14.8)	6.109		*ca.* 6.2	
H–C(19)	6.170	(J = 6.0)	6.017	(J = 6.0)	6.007	(J = 6.3)	6.109		6.050	(J = 6.5)
H–C(20)	2.242	(J = 10.7)	2.056	(J = 10.3)	2.286	(J = 10.4)	2.311	(J = 11.0)	*ca.* 2.3	(J = 11.0)
H–C(21)	3.589	(J = 3.2)	3.485	(J = 3.4)	3.443	(J = 3.6)	3.578	(J = 3.3)	*ca.* 1.4	
H–C(22)	1.838	(J = 5.3)	1.599	(J = 1.8)	1.789	(J = 6.1)	1.612	(J = 8.8)	*ca.* 1.4	
H–C(23)	3.081	(J = 10.1)	3.110	(J = 8.0)	3.019	(J = 6.1)	3.064	(J = 5.2)	4.022	
H–C(24)	1.577	(J = 1.4)	1.640	(J = 5.2)	2.236	(J = 5.4)	2.043	(J = 2.0)	*ca.* 2	
H–C(25)	4.906	(J = 8.1)	3.436	(J = 8.8)	3.386	(J = 5.6)	5.039	(J = 6.1)	3.607	(J = 10.29)
H–C(26)	1.474	(J = 1.5)	1.405	(J = 3.0)	1.228	(J = 2.7)	1.451	(J = 2.0)	*ca.* 1.4	
H–C(27)	3.451	(J = 6.2)	3.654	(J = 8.8)	3.617	(J = 8.9)	3.289	(J = 7.4)	3.971	(J = 4.7)
H–C(28)	5.141	(J = 12.3)	5.237	(J = 12.1)	5.192	(J = 12.5)	5.095	(J = 12.5)	5.133	(J = 12.5)
H–C(29)	5.890		5.978		6.332		5.900		5.959	
CH_3(30)	2.055		2.107		2.037		2.041		2.060	
CH_3(31)	0.826		0.838		0.859		0.822		0.903	
CH_3(32)	0.846		0.911		0.681		0.814		1.005	
CH_3(33)	0.719		0.737		0.859		0.725		0.629	
RO–C(25)[a])	1.975		3.486		2.855		1.977			
CH_3(34)	0.416		0.737		0.912		0.646		–0.111	
CH_3(35)	2.954		3.125		3.111		2.932		3.150	
CH_3(13)	1.755		1.759		1.902		1.910		1.789	
CH_3(14)	2.309		2.242		2.140		2.214		1.921	
H–C(3)	7.860		7.790		7.650		7.746		7.446	
OH–C(8)	12.523		12.520		12.576		12.549			
H–C(11)					5.426		5.433			
OH–C(11)					5.474		5.433			
$(CH_3)_2C$	1.624		1.078		1.160		1.266		1.230	
	0.900		0.925		1.160		0.895		0.966	

[a]) R = Ac for **5** and **8**, R = H for **6**, 7, and **9**.

(Scheme 3), as usually observed for additions at C(3) [9] (^{1}H-NMR spectrum of **10** in *Fig. 2*). This reaction did not take place with **7**, since disproportion to the hydroquinone form **9** occurred rapidly, and the latter is not enough reactive for addition reactions at C(3) [9].

Scheme 3



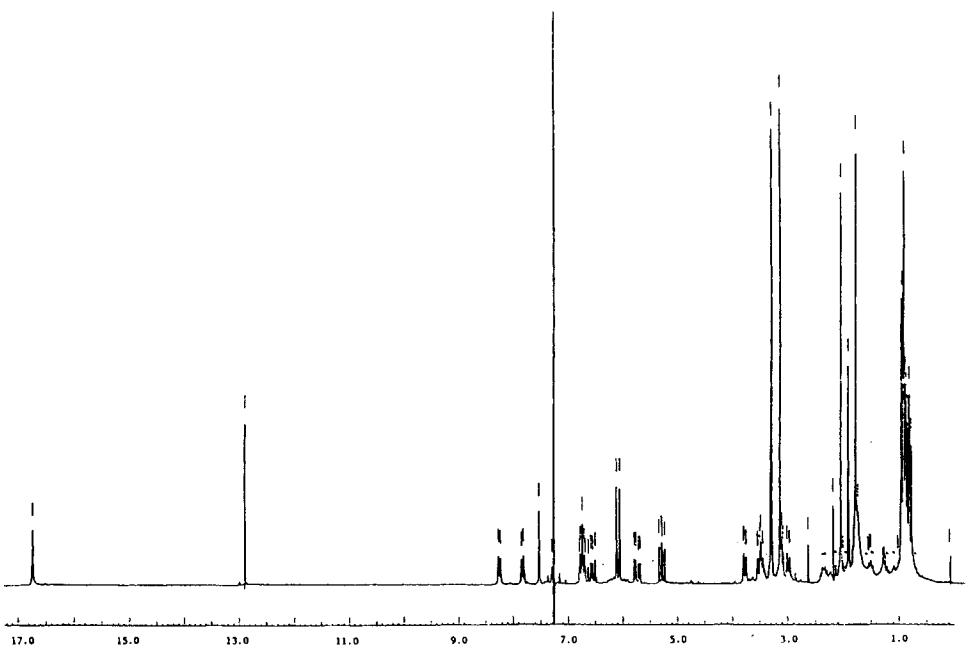


Fig. 2. *200-MHz ^{1}H-NMR spectrum of* **10**. 40 mM in CD_2Cl_2; δ in ppm from Me_4Si.

Table 2. *^{1}H-NMR Data* (in ppm. rel. to Me_4Si; $CDCl_3$) *of* **10–13**[a])

	10		**11**		**12**		**13**			**10**	**11**	**12**	**13**
NH–C(15)	7.525		7.532		7.467		7.688		CH_3(30)	1.896	1.903	1.955	1.966
H–C(17)	6.086	(J = 10.2)	6.098	(J = 10.1)	6.139		6.127	(J = 10.5)					
H–C(18)	6.565	(J = 15.8)	6.566	(J = 15.8)	6.588	(J = 10.9)	6.107	(J = 16.0)					
H–C(19)	5.738	(J = 6.0)	5.747	(J = 6.1)	5.940	(J = 5.1)	5.920	(J = 6.3)					
H–C(20)	n.a.	(J = 9.9)	n.a.		*ca.* 2.1	(J = 10.3)	2.287	(J = 10.2)	CH_3(31)	0.856	0.860	0.856	0.827
H–C(21)	3.514	(J = 3.2)	3.782		3.730	(J = 3.4)	3.741	(J = 3.5)					
H–C(22)	n.a.	(J = 4.3)	n.a.		*ca.* 1.6	(J = 5.4)	1.624	(J = 5.4)	CH_3(32)	0.895	0.926	0.836	0.861
H–C(23)	3.106	(J = 7.5)	*ca.* 3.1		3.146	(J = 10.4)	3.132	(J = 10.4)					
H–C(24)	n.a.		n.a.		*ca.* 2.2		2.393		CH_3(33)	0.808	0.823	0.733	0.731
H–C(25)	2.986	(J = 7.6)	3.133	(J = 7.3)	5.024	(J = 5.6)	5.028	(J = 5.5)	AcO–C(25)			1.997	1.955
H–C(26)	n.a.	(J = 2.8)	n.a.		*ca.* 1.6		1.530	(J = 1.0)	CH_3(34)	0.760	0.749	0.677	0.670
H–C(27)	3.780	(J = 8.3)	*ca.* 3.5	(J = 8.2)	3.308	(J = 8.1)	3.294	(J = 8.1)	CH_3(35)	3.131	3.130	2.864	2.854
H–C(28)	5.283	(J = 12.0)	5.270	(J = 12.2)	5.139	(J = 12.2)	5.138	(J = 12.1)					
H–C(29)	6.086		6.081		5.940		5.932						
$(CH_3)_2$N–C(4′)	3.288				3.270				CH_3(13)	1.764	1.767	1.763	1.763
H–C(2′)	8.250		8.193		8.026		7.982		CH_3(14)	2.035	2.025	2.134	2.133
H–C(6′)	7.827		7.861		7.853		7.818		OH–C(1)		12.878	12.986	12.878
H–C(3′), H–C(5′)	6.740		6.6		6.700		6.6		OH–C(4)		16.670	16.535	16.400
H–C(2″), H–C(5″)			3.5				3.5		$(CH_3)_2$C	0.391	0.905	0.856	0.860
H–C(3″), H–C(4″)			2.2				2.2			0.895	1.251	1.264	1.260

[a]) N.a. = not attributed.

The analogous reaction was tried using **6** and 4-(pyrrolidino)pyridine as catalyst, and in this case too, only the SV form **11** of the addition product was isolated *(Scheme 3)*.

For a better characterization of products **10** and **11**, the non-deacetylated protected rifamycin S **5** was submitted to the same reactions yielding the corresponding SV forms **12** and **13** *(Scheme 3)*, which are more stable than the deacetylated compounds **10** and **11**. It was shown that these additions take place even in absence of the anhydride, although the presence of the latter greatly accelerates the reaction rate.

The ^{1}H-NMR spectra of **10**–**13** *(Table 2)* show the characteristic signals of aminopyridines and the disappearance of the H–C(3) signal. The bulky substituent at C(3) of these rifamcin-SV derivatives, hindering the rotation around the amidic bond, leads to well-resolved spectra, even in $CDCl_3$, while broad peaks normally result under these conditions [10]. The appearance of just two peaks (phenolic OH groups) at lower field supports the hypothesis of a zwitterionic structure. The FAB-MS spectra of **10** and **11** are in agreement with the indicated structures and give molecular peaks at 857 ($M^+ - 2$) and 883 ($M^+ - 1$), respectively.

To avoid the undesired reaction at C(3), but using the same 4-(dialkylamino)pyridine catalysts, we decided to try a weaker base: on replacement of Et_3N by pyridine, neither **5** nor **6** underwent addition at C(3), proving that the presence of a base stronger than pyridine is necessary for the undesired addition. However, the addition of Ac_2O to the mixture of **6**, pyridine, and 4-(pyrrolidino)pyridine led to the formation of **5**, whereas the use of a bulkier reagent such as pivalic and glutaric anhydride did not give reaction products from **6** within 5 days.

To study also the reactivity of the OH group at C(11), the behavior of **7** in the presence of the reagents was studied by ^{1}H-NMR monitoring. Addition of the reagents to a solution of **7** in CD_2Cl_2 in the NMR tube (45 equiv. of pyridine, 0.1 equiv. of 4-(pyrrolidino)pyridine, and 6 equiv. of glutaryl chloride) resulted, within 6 h, neither in the above reported addition and disproportion nor in any decomposition.

The same conditions applied to the reaction of **7** with Ac_2O or cyclic anhydrides did not lead to any acylation products, and, after 48 h, eventually a mixture of decomposition products resulted.

Acid-Catalyzed Acylations. An alternative acylation mode using acid instead of base catalysis was investigated. The reaction of **7** in the presence of glutaric anhydride and $BF_3 \cdot Et_2O$ [11] yielded the three main products **14**–**16** after workup *(Scheme 4)*. ^{1}H- *(Table 3)* and ^{13}C-NMR spectra indicated that no acylation had occurred. Instead,

Scheme 4

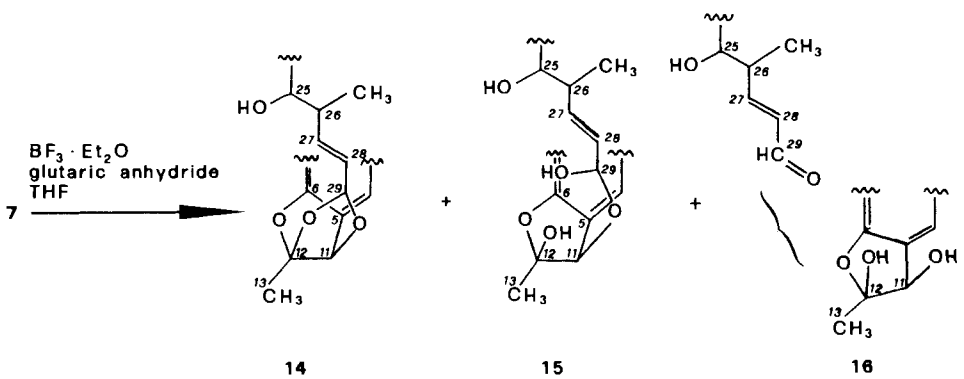


Table 3. *^{1}H-NMR Data* (in ppm rel. to Me_4Si; $CDCl_3$) *of* **14**–**16**[a])

	14		**15**		**16**			**14**	**15**	**16**
NH–C(15)	8.400		8.499		8.750		CH_3(30)	2.051	2.101	1.672
H–C(17)	6.236	(J = 10.2)	6.260		6.433	(J = 11.0)				
H–C(18)	6.084	(J = 15.6)	*ca.* 6.0		6.897	(J = 15.5)				
H–C(19)	6.287	(J = 6.9)	6.215		5.996	(J = 7.6)				
H–C(20)	2.273	(J = 7.3)	2.291		2.373	(J = 9.1)	CH_3(31)	0.894	1.001	1.023
H–C(21)	3.233		3.498		3.854	(J = 0.0)				
H–C(22)	1.427	(J = 10.5)	*ca.* 1.3	(J = 7.9)	1.748	(J = 2.6)	CH_3(32)	0.842	0.968	1.039
H–C(23)	3.277		3.179		3.376	(J = 7.8)				
H–C(24)	1.703	(J = 6.7)	*ca.* 1.6	(J = 5.1)	1.968	(J = 4.4)	CH_3(33)	0.808	0.582	1.010
H–C(25)	3.254	(J = 1.3)	3.811		3.560	(J = 10.3)	OH–C(25)	2.344	*ca.* 1.6	n.a.
H–C(26)	1.962		2.534	(J = 2.8)	2.429	(J = 7.4)	CH_3(34)	0.682	1.019	0.896
H–C(27)	5.268	(J = 16.3)	5.967	(J = 15.9)	7.056	(J = 15.7)				
H–C(28)	5.376		5.551	(J = 7.5)	6.129	(J = 7.8)				
H–C(29)	5.953		4.962							
H–C(11)	5.871		5.950		4.675		CH_3(13)	1.800	1.855	2.108
OH–C(11)					4.675		CH_3(14)	2.062	2.179	2.144
OH–C(12)	n.a.		2.796		5.156		H–C(3)	7.711	6.946	7.754
CH(29)=O					9.529		OH–C(8)	12.370	12.305	12.403
							$(CH_3)_2C$	1.187	1.220	1.306
								1.272	1.291	1.306

[a]) N.a. = not attributed.

reactions at the ether bond at C(27) under loss of the CH_3(35) group as well as at the enol ether bond C(29)–O(5)–C(12) had taken place.

Thus, the main product **14** results from the opening of the enol-ether bond C(29)–O–C(12) with formation of the corresponding vinyl alcohol, followed by its tautomerization to aldehyde (CH(29)=O), as described in the formation of rifarubins [7]. This opening of the ansa-bridge also results in the formation of an OH function at C(12). The aldehyde group CH(29)=O forms then an acetal with the two OH groups on C(11) and C(12). The opening of the ansa-bridge is accompanied by hydrolysis of CH_3(35)O–C(27) followed by elimination of H_2O and formation of the C(27)=C(28) bond (see ^{1}H,^{13}C heteronuclear correlation map in *Fig. 3*). The structure of compound **14** has been confirmed by X-ray analysis (our unpublished results).

The second product, **15**, is the hemiacetal form of **14**, in which C(29) is connected only to C(11). This has been confirmed by a NOESY experiment establishing a contact between H–C(29) and H–C(11), and no contact between H–C(29) and CH_3(13). Furthermore, **15** was not oxidized by MnO_2 [5], as it would be the case, had OH–C(11) been free.

The third product, **16**, is the ring-opened analog in which no acetal bond has been formed.

X-Ray Crystal Structure of 7. – In view of the unexpected difficulties to acylate the OH groups at C(25) and C(11) in **7**, and to establish the conformation of the molecule around these groups, the X-ray crystal structure was determined *(Fig. 4)*.

The structure shows that OH–C(25) is sterically hindered by the presence of CH_3O–C(27) and of CH_3(33) and CH_3(34). Thus, OH–C(25) is contained in a calyx,

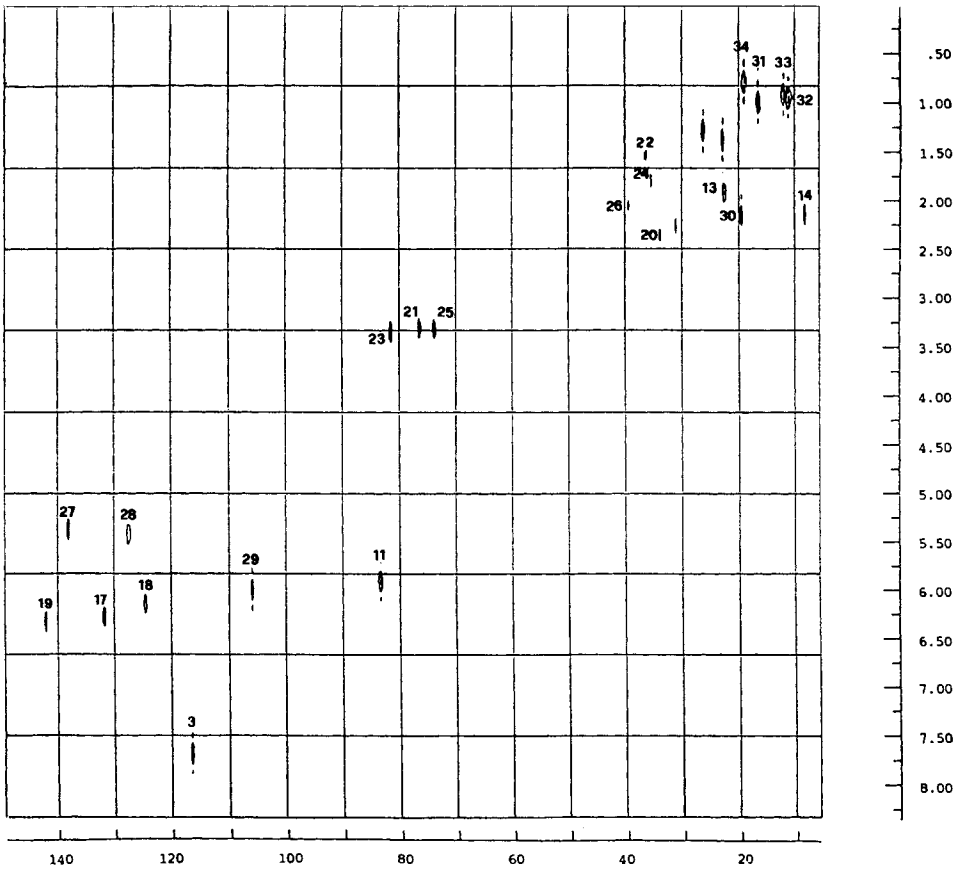


Fig. 3. *^1H,^{13}C Heteronuclear correlation map of* **14**. 50 mM in $CDCl_3$; δ in ppm from Me_4Si.

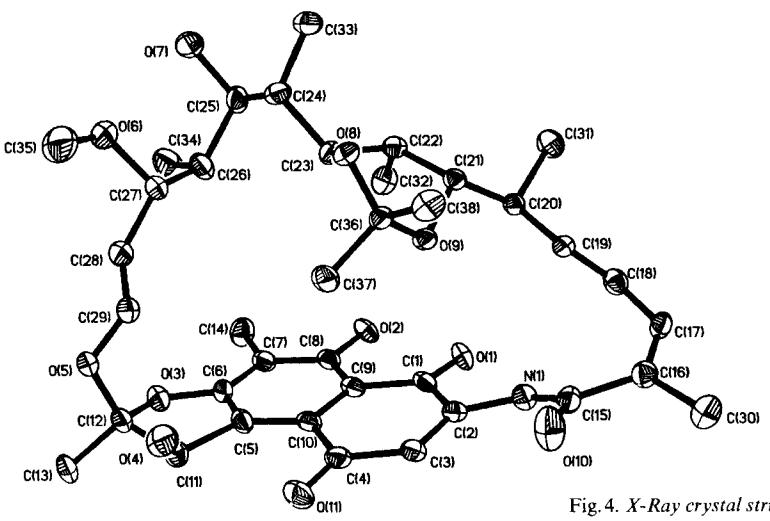
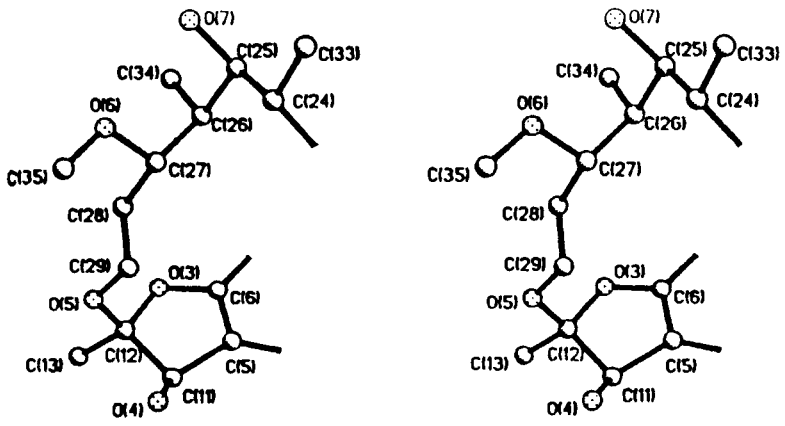


Fig. 4. *X-Ray crystal structure of* **7**



Distance [Å]	Distance [Å]
O(7)—C(33) 2.85	O(4)—C(29) 2.94
O(7)—C(34) 2.90	O(4)—O(5) 2.67
O(7)—O(6) 2.74	O(4)—O(7) 8.22
O(4)—C(13) 3.30	

Fig. 5. *Non-bonding interatomic distance* [Å] *around O(4) and O(7) as found in the crystal structure of* **7**

where O(6), C(33), and C(34) are at distances smaller than 3 Å from O(7) (see *Fig. 5*). Furthermore, the distance of O(4) from O(5) and C(29) are also lower than 3 Å, showing that OH–C(11) is hindered too *(Fig. 5)*.

The distance of 8.22 Å between O(4) and O(7) shows that bifunctional reagents such as glutaryl or adipoyl chlorides or the corresponding anhydrides would be of suitable length for the formation of a bridge connecting the OH groups at C(25) and C(11).

Conclusions. – The present study shows the low reactivity of the OH groups at C(25) and C(11) in the protected substrate (11*R*)-25-*O*-deacetyl-11-deoxo-11-hydroxy-21,23-*O*-isopropylidenerifamycin S (**7**) toward acylations. The formation of propionic and pivalic acid esters at C(25) from **6**, when the reaction was carried out in pyridine in the absence of a catalyst [7], indicates the ability of this solvent to favor a substrate conformation, in which at least OH–C(25) is sterically less hindered. However, the use of bulkier reagents such as anhydrides or chlorides containing 5–6 C-atoms did not lead to any acylation products. Furthermore, the use of pyridine in excess was incompatible with the stability of compounds reduced at C(11), even on contact for only a few h. The use of an (alkylamino)pyridine as catalyst brought no improvement, while the use of $BF_3 \cdot Et_2O$ led to decomposition of the substrate.

We also tried the esterification of **6** with malonic acid in the presence of DCCI under the conditions described for the preparation of a 25-*O* derivative of 3-morpholinorifamycin S [12], but no reaction occurred.

The study of the crystal structure of **7** confirmed that the difficulty to acylate the OH groups at C(25) and C(11) is mainly due to steric hindrance. This is most probably the

reason, why among hundreds of known rifamycin-S derivatives none contains any modification at C(11) other than that produced by carbonyl reduction. It can also be hypothesized that the removal of CH_3(35) should allow an easier access of the reagents, at least to OH–C(25).

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Experimental Part

1. *General.* TLC: plastic sheets, silica gel *60* F_{254} (0.2 mm) *Merck*. Column chromatography (CC): silica gel *60*, 0.063–0.200 mm *Merck*, or where specified, *Lichroprep RP18* 0.025–0.040 mm *Merck*. UV spectra: *Varian DMS90*; ^{1}H-NMR spectra: *Bruker AC200* spectrometer, 200.02 for ^{1}H and 50.33 MHz for ^{13}C; chemical shifts in ppm. rel. to Me_4Si (= 0 ppm). Elemental analysis for C, H, and N was in agreement with calculated values within ± 0.5%.

2. Preparation of *21,23-*O*-isopropylidenerifamycin S* (**5**) and the oxidation of the hydroquinone to the quinone forms were carried out as described in [7] [8].

3. *Deacetylation.* At 0°, 1 g of **5** was added to a 20% KOH soln. in abs. EtOH. The mixture was allowed to react at 0° for 3 h and then neutralized with an aq. citric-acid soln. After concentration of the volume, the product was extracted with $CHCl_3$, the combined extract dried (Na_2SO_4) and evaporated, and the residue submitted to CC ($CHCl_3$/AcOEt 1:1) yielding 60% of *25-*O*-deacetyl-21,23-*O*-isopropylidenerifamycin S* (**6**). TLC ($CHCl_3$/AcOEt 1:1): R_f 0.70. UV: 305 (4.02), 272 (4.18), 226 (4.30). ^{1}H-NMR: *Table 1.*

*25-*O*-Deacetyl-21,23-*O*-isopropylidenerifamycin SV* (**9**) was obtained from **8** (see below) under deacetylation conditions by disproportion. It was purified by CC on *RP18* with MeCN/H_2O 7:3. TLC (AcOEt/acetone 9:1): R_f 0.36. UV: 305 (4.02), 272 (4.18), 226 (4.30). ^{1}H-NMR: *Table 1.*

4. *Rifamycins Reduced at C(11).* To a soln. of 0.7 mmol of substrate in 50 ml of anh. EtOH, $NaBH_4$ (750 mg) was added gradually. After 1 h, MeOH (50 ml) was added and the mixture allowed to react for 30 min. After neutralization with an aq. citric-acid soln., the mixture was treated with a 33% aq. $K_3[Fe(CN)_6]$ soln. The product was extracted with $CHCl_3$ and the combined extract dried (Na_2SO_4) and evaporated.

*(11*R*)-11-Deoxo-11-hydroxy-21,23-*O*-isopropylidenerifamycin S* (**8**) was isolated by CC ($CHCl_3$/AcOEt 9:1). Yield 55%. TLC ($CHCl_3$/AcOEt 8:2): R_f 0.60. UV: 418 (3.53), 325 (3.94), 267 (4.08), 227 (4.45).

*(11*R*)-25-*O*-Deacetyl-11-deoxo-11-hydroxy-21,23-*O*-isopropylidenerifamycin S* (**7**) was isolated by CC (CH_2Cl_2/acetone 95:5). Yield 50%. TLC (CH_2Cl_2/acetone 95:5): R_f 0.60. UV: 417 (3.59), 325 (3.97), 228 (4.41). ^{1}H-NMR: *Table 1.* A COSYLR experiment was carried out for **7**.

5. *Reactions in the Presence of Et_3N and 4-(Dialkylamino)pyridines.* To 1.4 mmol of substrate in 30 ml of CH_2Cl_2, 2.8 mmol of Et_3N, 2.8 mmol of anhydride, and 4-(dialkylamino)pyridine were added. The mixture was allowed to react at r.t. under stirring for 48 h. For the acylation essays, 0.14 mmol of 4-(dialkylamino)pyridine were used. However, for a better characterization of the addition products, the same reactions were also carried out using 1 equiv. of catalyst. The mixture was then washed twice with a 5% aq. citric-acid soln., dried (Na_2SO_4), and evaporated.

*25-*O*-Deacetyl-3-[4-(dimethylamino)pyridinio]-21,23-*O*-isopropylidenerifamycin-8-*O*-ide SV* (**10**) was purified by CC (AcOEt/acetone 7:3). Yield 20%. TLC (AcOEt/acetone 7:3): R_f 0.43. UV: 450 (3.79), 306 (4.23), 215 (4.25).

*25-*O*-Deacetyl-3-[4-(pyrrolidin-1-yl)pyridinio]-21,23-*O*-isopropylidenerifamycin-8-*O*-ide SV* (**11**) was isolated by CC (AcOEt/acetone 8:2). Yield 20%. TLC (AcOEt/acetone 9:1): R_f 0.50. UV: 449 (3.72), 309 (4.21), 215 (4.21).

*3-[4-(Dimethylamino)pyridinio]-21,23-*O*-isopropylidenerifamycin-8-*O*-ide SV* (**12**) was isolated by CC (AcOEt/acetone 7:3). Yield 30%. TLC (AcOEt/acetone 7:3): R_f 0.60. UV: 448 (3.84), 307 (4.29), 218 (4.21).

*3-[4-(Pyrrolidin-1-yl)pyridinio]-21,23-*O*-isopropylidenerifamycin-8-*O*-ide SV* (**13**) was isolated by CC (AcOEt). Yield 30%. TLC ($CHCl_3$/AcOEt 6:4): R_f 0.40. UV: 445 (3.69), 309 (4.20), 216 (4.23).

^{1}H-NMR for **10**–**13**: *Table 2.* COSYLR experiments were carried out for **11** and **13**.

6. *Reactions in the Presence of BF_3.* To 300 mg of **7** in 5 ml of THF at 0° was added dropwise a soln. of 74 mg of anhydride and 0.030 ml of $BF_3 \cdot Et_2O$ in 4.8 ml of THF. After 1 h at 0°, the mixture was allowed to react at r.t. for further 3 h. After neutralization with 5% aq. $NaHCO_3$ soln. and extraction with $CHCl_3$, the combined org. phase was dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel with CH_2Cl_2/acetone 96:4. Three main products were isolated: *25-O-deacetyl-27,28-didehydro-27-demethoxy-11-deoxo-11,29-epoxy-28,29-dihydro-21,23-O-isopropylidenerifamycin S* (**14**; yield 60%), *25-O-deacetyl-27,28-didehydro-27-demethoxy-11-deoxo-12,29-deoxy-11,29-epoxy-28,29-dihydro-12,29-dihydroxy-21,23-O-isopropylidenerifamycin S* (**15**; 15%), and *25-O-deacetyl-27,28-didehydro-27-demethoxy-11-deoxo-12,29-deoxy-28,29-dihydro-11,12-dihydroxy-21,23-O-isopropylidene-29-oxorifamycin S* (**16**; 5%). TLC (CH_2Cl_2/acetone 9:1): R_f 0.84, 0.80, and 0.30, resp. UV (**14**): 324 (3.73), 265 (3.84), 225 (4.18). UV (**15**): 324 (3.81), 265 (4.08), 225 (4.34). UV (**16**): 324 (4.24), 274 (4.52), 222 (4.62). ^{1}H-NMR for **14–16**: *Table 3*. A magnitude-mode NOESY experiment was carried out for **15** (0.4 s mixing time). ^{13}C-NMR (**14**): 185.39 (C(1)); 139.63 (C(2)); 116.59 (C(3)); 182.14 (C(4)); 120.60 (C(5)); 165.10 (C(6)); 117.60 (C(7)); 165.56 (C(8)); 113.89 (C(9)); 126.22 (C(10)); 83.39 (C(11)); 108.24 (C(12)); 22.51 (C(13)); 8.19 (C(14)); 170.19 (C(15)); 132.71 (C(16)); 131.94 (C(17)); 124.66 (C(18)); 142.20 (C(19)); 33.73 (C(20)); 76.49 (C(21)); 35.22 (C(22)); 81.54 (C(23)); 36.24 (C(24)); 73.90 (C(25)); 39.24 (C(26)); 138.15 (C(27)); 127.43 (C(28)); 105.88 (C(29)); 19.46 (C(30)); 16.46 (C(31)); 11.20 (C(32)); 12.04 (C(33)); 19.06 (C(34)); 100.78, 26.26, 22.81 ($(CH_3)_2C$). ^{13}C-NMR (**15**): 183.88 (C(1)); 140.03 (C(2)); 114.34 (C(3)); 182.71 (C(4)); 118.02 (C(5)); 168.25 (C(6)); 116.97 (C(7)); 164.63 (C(8)); 112.33 (C(9)); 128.00 (C(10)); 82.40 (C(11)); 109.18 (C(12)); 22.12 (C(13)); 8.38 (C(14)); 169.71 (C(15)); 128.15 (C(16)); 134.30 (C(17)); 124.98 (C(18)); 143.25 (C(19)); 37.55 (C(20)); 73.54 (C(21)); 33.41 (C(22)); 71.98 (C(23)); 37.55 (C(24)); 81.39 (C(25)); 39.82 (C(26)); 143.94 (C(27)); 123.01 (C(28)); 104.25 (C(29)); 20.16 (C(30)); 19.02 (C(31)); 13.26 (C(32)); 13.70 (C(33)); 11.13 (C(34)); 101.02, 24.55, 23.58 ($(CH_3)_2C$).

COSYLR and ^{1}H,^{13}C heteronuclear correlation experiments were carried out for **14** and **15**. ^{1}H,^{13}C correlation (**14**, 50 mM in $CDCl_3$): ^1J(C,H) established for C(3), C(11), C(13), C(14), C(17), C(18), C(19), C(20), C(21), C(22), C(23), C(24), C(25), C(26), C(27), C(28), C(29), C(30), C(31), C(32), C(33), C(34).

7. *X-Ray Analysis of* **7**. Suitable crystals, hexagonal orange prisms, were grown from MeOH/H_2O at 4°. Crystal data: $C_{38}H_{49}NO_{11}$, M_r 695.8, space group tetragonal $P4_12_12$, $a = 12.221(2)$, $c = 49.55(1)$ Å, $V = 7404(2)$ Å^3, $F(000) = 2976$, $Z = 8$, $D_x = 1.249$ g·cm^{-3}, CuK_α, $\lambda = 1.54184$ Å, $\mu = 7.63$ cm^{-1}. A crystal of dimensions 0.4 × 0.3 × 0.1 mm was used for data collection and set on a *Nicolet R3m/V* diffractometer (graphite-monochromated CuK_α radiation). Intensity data were collected, the experimental conditions being: $(\sin\theta/\lambda)_{max} = 0.59$ Å^{-1}, ω-scan mode, scan range 1.2°, scan rate 1.0–14.65° min^{-1} (depending on reflection intensity), background count time half of the scan time. Accurate unit-cell parameters were determined by least-squares fit of the setting angles of 20 selected reflections with $60 \leqslant 2\theta \leqslant 64°$. There was no significant intensity variation for three standard reflections measured every hundred. Intensity data were corrected for average change in the intensity of reference reflections. *Lorentz* and polarization corrections were applied, but no absorption or extinction corrections were made. Of the 3714 unique reflections measured, 3147 with $I \geqslant 1.0\,\sigma(I)$ ($R_{int} = 5.43\%$) were considered as observed. The structure was solved by direct methods using the SHELXTL Plus [13] program. The SIR-CAOS program [14] was used for all further calculations. Difference *Fourier* syntheses, using only data with $\sin\theta/\lambda < 0.5$ Å^{-1}, computed at the end of the anisotropic least-squares refinement, showed all H-atoms in configurationally feasible positions. The final refinements were carried out by block-diagonal matrix with the H-atoms allowed to ride on the corresponding C-, N-, and O-atoms (452 parameters). The final R value was 0.066, $R_w = 0.096$ minimizing the function $w|\Delta F|^2$, with $w = (a + b|F_0| + c|F_0|^2)^{-1}$ ($a = 2(F_0)_{min} = 8.77724$, $b = 1.0$, $c = 2/F_o)_{max} = 0.00464$). At convergence, the maximum shift e.s.d. ratio was less than 0.59 and $S = 0.409$. Heights in final difference *Fourier* map $\rho_{max} = 0.20$, $\rho_{min} = -0.20$ e·Å^{-3}. Atomic scattering factors were taken from [15]. Final positional and equivalent isotropic thermal parameters U_{eq} have been deposited with the *Cambridge Crystallographic Data Centre*. A perspective view of the molecular structure of **7** was prepared using SHELXTL Plus [13] (see *Fig. 4*).

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