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Rifamycin antibiotics—new compounds and synthetic methods. Part 3: Study of the reaction of 3-formylrifamycin SV with primary amines and ketones

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ABSTRACT

In the third stage of our study concerning the search for new antibacterial rifamycin antibiotics, the reactions of 3-formylrifamycin SV (**1**) with a range of primary alkylamines and ketones of general structure $R_1{-}CH_2{-}CO{-}R_2$ (R_1=H or alkyl and R_2=alkyl or aryl) has been investigated. A new synthetic method for the preparation of a new group of rifamycin derivatives with an α,β-unsaturated imine substituent at C-3 has been developed.

These compounds showed a tendency to reversibly isomerise in organic solvents and, in the presence of water, to rapidly hydrolyse. The structures of four isolated microcrystalline compounds **2**, **3**, **4**, **5** and a reaction's mechanism have been proposed on the basis of mass spectrometry results as well as (1D) and (2D) ^1H and ^{13}C NMR analysis. The new synthetic route reported herein is a promising pathway to new reactive rifamycins displaying broader capabilities than the plain 3-formylrifamycin SV.

1. Introduction

Rifamycins belong to the class of ansamycins,[1] playing an important role as antibiotics in therapeutics against tuberculosis, leprosy and various other mycobacterial infections.[2,3] The most efficient and well established rifamycins are rifampicin, rifabutin, rifapentine and rifaximin,[4] however the increasing microbial resistance found with these commercially available rifamycins calls for new therapeutic strategies and/or new rifamycin sorts.[5,6] In the first part of our work dealing with new antibacterial rifamycins, the products of the reaction of 3-formylrifamycin SV (**1**) with ammonia or primary alkylamines were investigated.[7,8] In the second part, we characterized the derivatives obtained from the reaction of rifamycin **1** or 3-formyl-25-*O*-desacetyl rifamycin SV with ammonia and acetone.[9,10]

The third part of this work concerns the study of the reaction of 3-formylrifamycin SV (**1**) with selected primary alkylamines and ketones of general structure $R_1{-}CH_2{-}CO{-}R_2$, where R_1=H or alkyl, R_2=alkyl, aryl. The synthesis of related α,β-unsaturated ketones via cross aldol synthesis (Claisen–Schmidt condensation) of one of the 3-alkenylrifamycin groups was the initial goal of these studies.[11]

Several groups of 3-alkenylrifamycin SV derivatives were produced in the 70's of the last century by R. Cricchio and co-workers.[12] In the first synthesis' step, 3-formylrifamycin SV (**1**) was, however, subjected, not to the aldol reaction but to the Wittig reaction with phosphorus ylides, e.g., acetyl–methylenetriphenyl phosphine, obtaining various 3-(3-oxo-alkylidenyl)–rifamycins SV. In a subsequent synthesis step these derivatives were condensed with appropriate hydroxylamine-, phenylhydrazine-, *N*-amino-N′-alkylpiperazine- or hydrazine derivatives, obtaining new compounds displaying an enlarged substituent at carbon C3 of the chromophore, with Schiff base, hydrazone or oxime characteristics. All these new groups of derivatives were tested in view of antibacterial and antiviral activity. Some substances, especially the oxime derivatives, showed good activity against gram-positive bacteria, comparable to that of already reported classes of rifamycins. The three most effective derivatives showed good inhibiting action towards several RDDP (RNA-dependent DNA polymerases) chosen for virus tests (inhibition level of 50–80% at concentrations 20 μg/ml).

In the published patent,[13] Cricchio claimed, besides the method of obtaining 3-(3-oxo-alkylidenyl)-rifamycins SV via the use of corresponding ylides, also the synthesis of these rifamycin SV derivatives via an aldol-like synthesis reacting **1** with ketones displaying a $R_1{-}CH_2{-}CO{-}R_2$ structure, where R_1=H or alkyl and R_2=H, alkyl, aryl. However this second method was only illustrated with one example, where **1** condensates with acetaldehyde, the reaction running in tetrahydrofurane in the presence of pyridine and acetic acid at ca. 5 °C. Cricchio mentioned various bases, including alkali metals hydroxides, alkoxides and amines as well as ammonium acetate or alkali metals acetates as potential catalysts for this kind of reactions.

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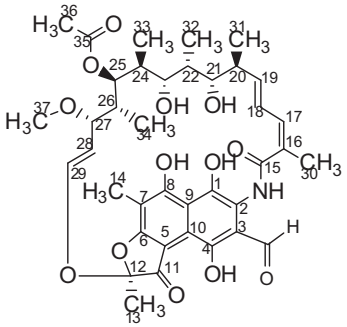
2. Results and discussion

2.1. Generalities on the reaction

In order to perform a screening of the cross aldol reaction with 3-formylrifamycin SV (**1**, Fig. 1), we selected aliphatic and aliphatic–aromatic ketones (Table 1) bearing in their structure at least one methylene or methyl group in α position of the carbonyl group. According to the literature, the presence of this methylene group is a prerequisite for a dehydration of the intermediately formed β-hydroxyketones to eventually yield the desired α,β-unsaturated ketone derivatives (vide infra, section 2.4).[14]



1 $C_{38}H_{47}NO_{13}$ M 726

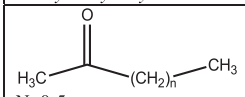
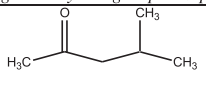
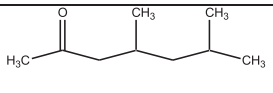
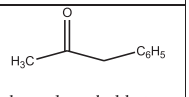
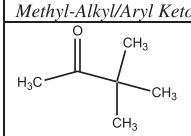
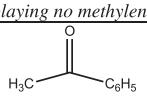
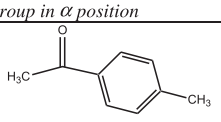
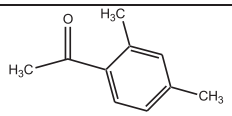
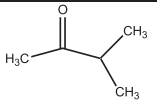
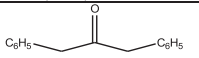
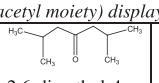
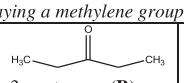
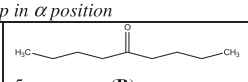
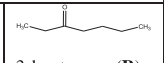
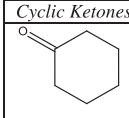
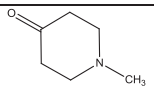
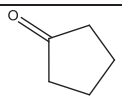
Fig. 1. Structure of 3-formylrifamycin SV.

The presence of 'acidic' protons in α position of the carbonyl group is paramount to observe a significant reaction of aldehyde **1** with primary amines and ketones, as confirmed by the experiments carried out. However, in a 'standard' cross aldol reaction, an amine usually plays the part of the catalyst: abstracting a proton from the methylene in C-α of the ketone to form a reactive carboanion.[9,15] It was found in our case that, amines, besides activating the acidic methylene group, are also directly engaged in the reaction, forming reactive α,β-unsaturated imines. Therefore, various primary aliphatic amines, with linear, branched and cyclic alkyl groups, were used to assess the influence of the amine's substituents on the reaction progress (vide infra, section 2.3).

A wide range of polar solvents were also tested, revealing that lower alcohols, and especially methanol, are the most suited solvents to carry out the synthesis. A rapid reaction together with a good selectivity and easy crystallization of the main product were also observed when using ethylene glycol as solvent, nevertheless the complete removal of this solvent during the drying step revealed to be quite tedious. In some cases it was also possible to run the reaction and crystallize the product in chloroform. In all cases, the reaction has to be performed in a narrow temperature window: at temperatures lower than 35 °C the reactions proceeded very slowly or did not proceed at all, whereas above 45 °C the hydrolysis of imines **E** to the corresponding α,β-unsaturated ketones **F** considerably increased.

The addition of an acid had a positive effect on the rate of the reaction, which is in agreement with the related literature.[16] Among the common carboxylic and sulfonic acids tested as catalysts (acetic acid, trichloroacetic acid, L-tartaric acid, citric acid, methanesulfonic acid, *p*-toluenesulfonic acid), the best results were obtained with

Table 1
List of the investigated ketones with the final product types (**E**, **B**, **F** as in Scheme 1 or complex mixture: **X**)

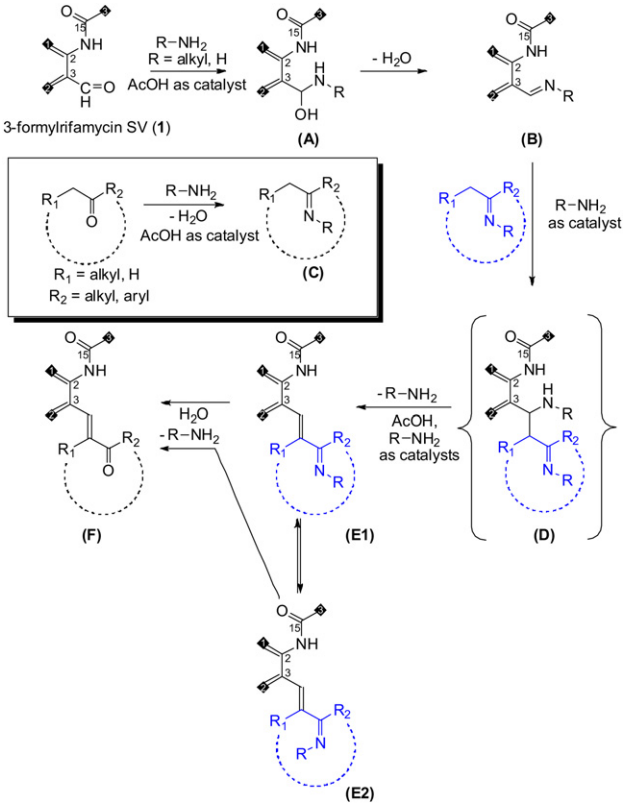
Methyl-Alkyl/Aryl Ketones displaying a methylene group in α position			
 N=0-5 acetone (**F**) ; 2-butanone (**E**→**F**) ; 2-pentanone (**E**→**F**) ; 2-hexanone (**E**→**F**) ; **2-heptanone** (**E2**, reaction with cyclohexylamine: compound **4**) ; **2-octanone** (**E2,** reaction with isopropylamine: compound **5**)	 **4-methyl-2-pentanone** (**E1;** reaction with isopropylamine: compound **2** ; reaction with propylamine: compound **3**)	 4,6-dimethyl-2-heptanone (**E1**)	 benzyl-methyl ketone (**E**→**F**)
Methyl-Alkyl/Aryl Ketones displaying no methylene group in α position			
 *tert*-butyl-methyl ketone (**B**)	 acetophenone (**X**)	 *p*-methylacetophenone (**X**)	 2,4-dimethyl-acetophenone (**E**)
Methyl-Alkyl Ketones displaying a methine group in α position			
 3-methyl-2-butanone (**E**→**F** slow)			
Dialkyl/Aryl Ketones (without acetyl moiety) displaying a methylene group in α position			
 1,3-diphenyl-2-propanone (**B**)	 2,6-dimethyl-4-heptanone (**B**)	 3-pentanone (**B**)	 5-nonanone (**B**)	 3-heptanone (**B**)
Cyclic Ketones			
 cyclohexanone (**X**)	 4-methylpiperidone (**F**)	 cyclopentanone (**X**)	

acetic acid. The action of the acid is discussed more in detail in the paragraph dealing with the reaction mechanism (section 4).

Reaction progress and purity of the isolated rifamycin derivatives were controlled by thin layer chromatography (TLC), an 'old-fashioned' analytic method, which works surprisingly well in the tracking and quantification of antibiotics.[17,18]

2.2. Evolution of the reaction and isolation of the main products

According to the standard procedure, the solvent (mainly methanol) was poured into the reaction flask, followed by the addition of acetic acid, amine, 3-formylrifamycin SV (**1**) and the respective ketones, the mixture being stirred around 40 °C for one to 4 h. In a typical reaction's course, the control chromatograms (TLC—see experimental part) of the reaction mixture showed over the usual reaction time a gradual decay of the initial red spot characteristic of the 3-formylrifamycin SV and the appearance of: (i) first a violet spot, probably related to an intermediate imine of structure **B**, (Scheme 1) and (ii) an intense blue spot of product **E1**, which slowly dominated in the system.



Scheme 1. Mechanism proposal for the reaction of 3-formylrifamycin SV (**1**) with primary amines and ketones.

This fraction was identified as a rifamycin SV derivative with an α,β-unsaturated imine substituent at C-3. Simultaneously, the formation of the second product, **E2**, affording a navy blue spot on the chromatogram (usually smaller than that of **E1**) was observed, **E2** being an isomer of **E1** (Scheme 1).

Substance **E2**—slightly soluble in methanol, started to crystallize, sometimes already in the reaction mixture. In a further reaction step imines **E1** and **E2** underwent a gradual hydrolysis to form the α,β-unsaturated ketone **F**, giving a burgundy-red spot on the chromatogram.

Fortunately the various substances spotted using the TLC method could be easily differentiated thanks to the broad range of retention coefficients, displaying usually the arrangement R_f**B**>R_f**E2**>R_f**E1**>R_f**1**>R_f**F**.

The reaction was regarded as completed when substrate **1** was consumed and the concentration of intermediate imine **B** was low. Afterwards the reaction mixture was cooled to 0–5 °C and usually left at this temperature for about 12 h. In many cases a 'navy-blue' main product was obtained identified as type **E2** imine (TLC), which was filtered off, washed with cold methanol, and then dried under vacuum at 40 °C. When 4-methyl-2-pentanone (isobutyl-methyl ketone) was used in the reaction, type **E2** imines did not crystallize from methanol, even at low temperature, and blue imines of **E1** type were the dominating products in the reaction mixture. The final reaction mixture was diluted with water and extracted with chloroform. A number of blue microcrystalline imines of the **E1** type were obtained from the chloroform concentrates. Unfortunately, in neither cases, **E1** or **E2**, was it possible to isolate single crystals good enough for a supporting X-ray diffraction structural determination.

On the whole, it was found that:

In solutions containing **E2** imines (solvent: MeOH, $CHCl_3$, THF and DMSO), an isomerisation of these compounds proceeds giving **E1**, as recorded via TLC where besides a dark blue spot of **E2**, a blue spot of **E1** increasingly appears. The rate of this isomerisation is related to the structure of imine **E2**, allowing in some cases to study comprehensively the structure also via time-consuming NMR-techniques (e.g., 2D correlation spectroscopies). The ^{1}H and ^{13}C NMR spectra in DMSO-d_6 of the slowly isomerising imine **4** of type **E2**, 3-(3′-isopropylimino) nonenylrifamycin SV, (Fig. 3) were recorded at ambient temperature at a time not exceeding 4 h, shows one type of imine in solution. Overnight the same sample clearly exhibits two sets of signals characteristic of a mixture of isomers. Conversely, in analogous solutions of imine **E1**, the **E2** isomer slowly appears.

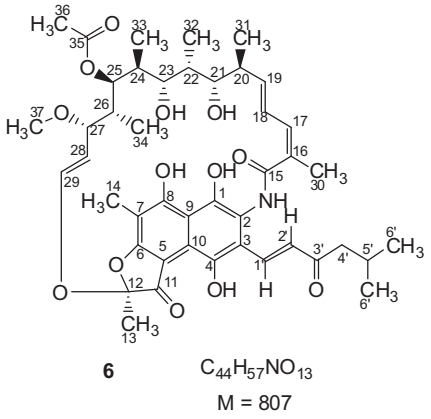


Fig. 2. Structure of the α,β-unsaturated ketone 6 of type F.

The presence of water traces in the solutions, besides the described **E1**↔**E2** isomerisation, leads to a slow hydrolysis of **E1**/**E2** to yield **F** (α,β-unsaturated ketone). Another product **B** (most likely also an imine) appears as a result of a gradual conversion of **E** in **B** (TLC).

Ketones of type **F** (e.g., compound **6**: 3-(5′-Methyl-3′-oxo)hexenylrifamycin SV; Fig. 2) do not react in the presence of primary amines to yield imines of type **E** again, indicating that the hydrolysis of **E** is not reversible.

2.3. Effect of amine concentration and structure

In the study at hand, primary alkylamines with linear chains (n-propylamine, n-butylamine and n-hexylamine), branched chains (isopropylamine and isobutylamine) and cyclic chain (cyclohexylamine) as well as hydroxylalkylamine—(ethanolamine) were used.

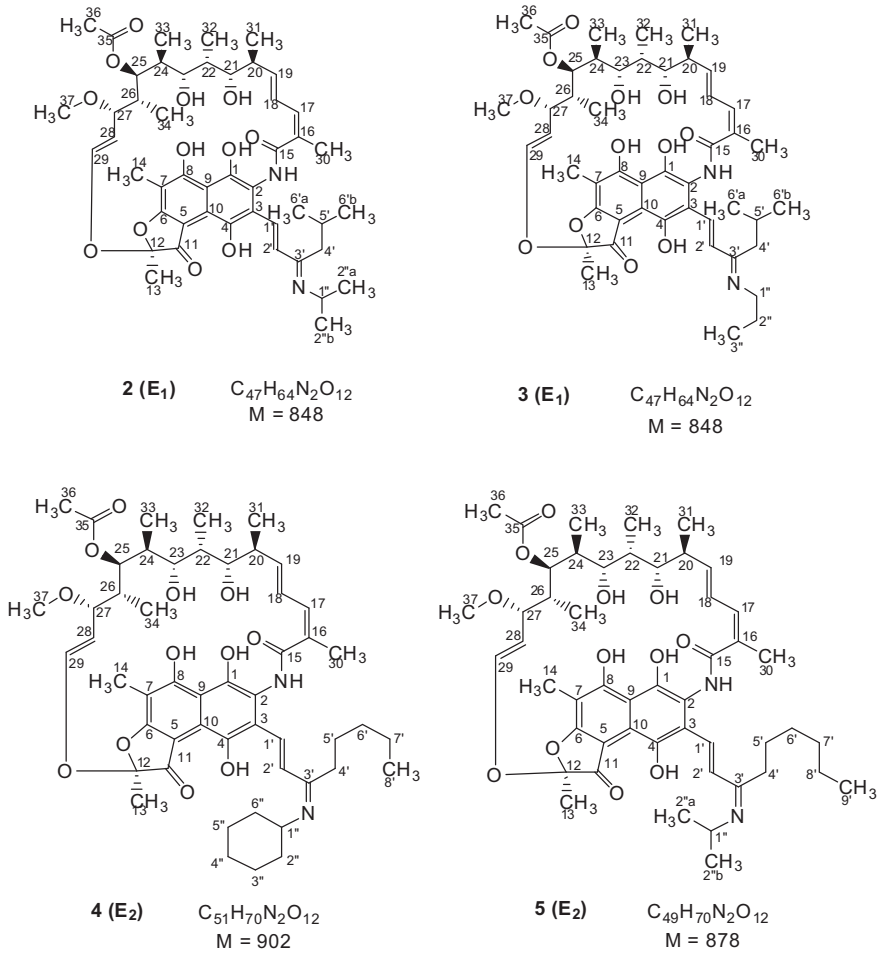


Fig. 3. Structures of four representative α,β-unsaturated imines of type E1 and E2.

All the amines studied formed products with a general iminic structure **E**, albeit the imimes formed using ethanolamine showed the greatest tendency towards fast hydrolysis. The best yield and selectivity towards imines of type **E** was achieved when using 5 mmol of amine per 1 mmol of **1**. An amine deficit caused the suppression of the **B** to **E** transformation (Scheme 1).

2.4. Effect of ketone concentration and structure

In a majority of cases, the optimal yields for the imines **E** were achieved when using about 2.5 mmol of ketones for 1 mmol of **1**. Although an increase of the ketone amount usually accelerates the formation of imines, this procedure was only used in cases where the synthesis proceeded too slowly. The reason for this is that a larger amount of unreacted ketone in the reaction mixture affected negatively the crystallization of imine **E2** from this mixture.

It was found that the synthesis' efficiency of α,β-unsaturated imines (**E1** or **E2**) was related to the nature of the ketone. Three main ways of reaction can be distinguished for the investigated system (Table 1):

1. The formation of imines **E1** or **E2** proceeded quickly, the products crystallizing rapidly from the reaction mixture in the case of **E2**, or, considering product **E1**, from the concentrated chloroform extract (see 2.2).
2. The reaction delivered no crystalline materials: neither product **E1** nor **E2** was obtained. This is most likely due to a partial hydrolysis to form α,β-unsaturated ketone **F** that hinders the crystallisation of **E2** or **E1**.
3. The reaction stopped at the step of imine B.

To find a relationship between structure of the ketones and reaction progress, comparative attempts of reacting **1** with n-butylamine and a broad range of selected ketones were performed (see Table 1 for overview).

Symmetric dialkyl ketones as well as cyclic ketones like cyclopentanone, cyclohexanone and 4-methylpiperidone were also tested in the presence of acetic acid in methanol. To extend this series two alkyl-aryl ketones, *p*-methylacetophenone and 2,4-dimethyl-acetophenone were also used in this synthesis. These studies were also supplemented in some cases by reacting **1** with definite ketones and other primary alkylamines.

On the basis of chromatographic monitoring (TLC) of the reaction and some NMR knowledge of the system (vide infra, section 3), the following observations were made:

The nature of the alkyl substituent in the methyl–alkyl ketones shows a considerable effect on the synthesis of rifamycins displaying an α,β-unsaturated imine structure **E** (Table 1 for an overview of the reaction's outcome):

1. When a methylene group is present in α position of the carbonyl carbon, the reaction proceeded very rapidly and the desired product—α,β-unsaturated imine **E** was formed. This compound underwent in some cases (with e.g., 2-butanone, 2-pentanone 2-hexanone or benzyl–methyl ketone) a rapid hydrolysis to α,β-unsaturated ketone **F** and the further isolation of the crystalline imines failed. However, some imines **E2** (based on 2-heptanone and 2-octanone) as well as of imines **E1**

(involving e.g., 4-methyl-2-pentanone), could be isolated. The C=C double bond in the new substituent at carbon C-3 of the isolated compounds was always preferentially formed involving the methyl group carbon of these ketones (NMR).
2. When a methine group is present at the α position, as e.g., in 3-methyl-2-butanone, the formation of imine **E** proceeds much slower than that in the above described system. This product undergoes relatively rapidly hydrolysis to yield **F**, and hence crystalline imines **E**, derivatives of such ketones, have not been isolated.
3. When a quaternary carbon atom is present in the alkyl group in α position as, e.g., in methyl-*tert*-butyl ketone, only the formation of imine **B** can be observed as well as the formation of the α,β-unsaturated ketone, **F**.

From the above observations it results that increasing the steric hindrance of the group bound to the ketone hinders the formation of the imine **E**, probably due to an enhanced affinity of the β-aminoimine **D** towards hydrolysis.

Interestingly only the alkyl-methyl ketones used in the study formed a product displaying an imine **E** structure, in other cases the synthesis seems to be stopped at stage **B**. Acetone as a particular case reacts rapidly to yield the related α,β-unsaturated ketone **F**.

From the different methyl-aryl ketones investigated only 2,4-dimethylacetophenone yield the desired α,β-unsaturated imine **E** in crystalline form; the others ketones delivering only complex mixtures after rapid reaction with **1** and n-butylamine.

Cyclopentanone and cyclohexanone yield, in the reaction of **1** with monoalkylamines, a mixture of compounds which could not be separated. Only 4-methylpiperidone reacted with **1** and butylamine to yield the corresponding α,β-unsaturated ketone **F**, which was isolated as microcrystalline solid.

On the whole, these studies resulted in the synthesis and isolation as microcrystalline materials of a number of α,β-unsaturated imines of structure **E** (*N*-substituted 3-(3′-imino)alkylidenylrifamycins SV, yields ranging from 20 to 70%). In many cases, the final products isolated from methanol displayed traces of isomers **E2**, besides the major product isomer **E1**.

In order to convert the isolated imines **E1** and **E2** into the related α,β-unsaturated ketones **F**, they were heated in slightly alkaline aqueous-methanol solutions (see experimental part) until the chromatographic monitoring of the mixture showed a conversion of **E** to **F**. Products of structure **F** were most often obtained in a crystalline form from chloroform solutions. This synthetic method, yielding ketones from imines of structure **E** is much easier than that described formerly in the literature.[20]

3. Analytical studies—determination of the structure of rifamycins 2, 3, 4, 5 and of the hydrolysis product 6

A comprehensive analytical study is somewhat hindered by the lability of some of the intermediates. The isomeric imines of type **E1** or **E2**, isolated from the reaction mixtures, generally have a microcrystalline structure and can contain traces of impurities, mainly the second isomer, or rarely, the hydrolysis products, the corresponding α,β-unsaturated ketones **F**. The general low solubility in organic solvents (possibly due to the formation of stable rifamycin-solvent adducts during the synthesis[19]), as well as the high reactivity of the C=N double bond prone to isomerisation and hydrolysis, might be an explanation for the evident difficulty to get single crystals suitable for X-ray diffraction studies.

3.1. NMR studies

Among several crystalline **E1** imines, the imine **2**, product of the reaction of **1** with isopropyl−methyl ketone and isopropylamine, and similarly imine **3**, product of the reaction of **1** with isopropyl−methyl ketone and propylamine, provided very clear NMR spectra due to a high purity and exceptional stability in DMSO-d_6. Together with mass spectrometry and IR studies, the proposed structure of this compound could be determined (Fig. 3).

On the other hand, NMR spectra in DMSO-d_6 of various type **E2** imines, despite a high purity (TLC) of the materials, were quite complex due to an overlapping of the signal of both isomers, the second isomer being rapidly formed in solution.

This confirmed the isomerisation of **E2** in **E1** observed by means of TLC. Working under argon and with 'bone-dry' deuteriated solvent, it was possible to gather enough exploitable NMR data of the type **E2** imines, **4** and **5**, to establish their structures. To supplement the analytical studies performed on type **E1** and **E2** imines, a similar NMR analysis was run on a α,β-unsaturated ketone of type **F**, **6**, which confirmed the proposed structure.

In general, the assignment of NMR signals in ^{1}H and ^{13}C spectra of rifamycins **2**, **3**, **4**, **5** and **6** (hydrolysis product of **2**) was made correlating the information of 1D and 2D-NMR spectra: ^{1}H,^{1}H COSY, ^{1}H,^{13}C HSQC and ^{1}H,^{13}C HMBC. In the case of **2**, DEPT spectra were additionally recorded together with ^{1}H spectra before and after addition of D_2O to identify the mobile protons of the naphthol chromophore. The recorded chemical shifts were compared with literature data concerning NMR studies of rifamycins[20–25] and our own NMR database of rifamycin derivatives.[7,8] To avoid needless redundancy, we will only describe in detail the structure characterization of one **E1** derivative (**2**) and of a related hydrolysis product, **6**. The complete characterization of derivatives **3**, **4** and **5** can be found in the Supplementary data together with the related NMR-correlation tables.

3.2. NMR study of rimamycin 2: properties of the substituent at carbon C-3 (Fig. 3, Table 2)

The signal at 117.1 ppm in the ^{13}C NMR spectrum was assigned to C-1′. In the ^{1}H NMR spectrum the signal of the 1′-H proton occurs as a doublet at 7.94 ppm. The signal at 148.7 ppm (overlapping with the C-1 signal) was assigned to C-2′. In the ^{1}H NMR spectrum, the signal of 2′-H at 7.81 ppm appears as a doublet. In the ^{1}H,^{1}H COSY spectrum a clear correlation signal 1′-H/2′-H $J_{1',2'}$=15.6 Hz was found. The high coupling constant indicates a *trans* geometry of protons 1′-H and 2′-H.[26a] On the basis of the correlations found in 2D-NMR spectra, the low-field signal at 177.9 ppm was assigned to the imine carbon atom C-3′, the signal being located in the region characteristic of the iminic C=N bond (~150−180 ppm).[26b] The methylene group C-4′ was associated to the signal at 40.7 ppm (phased-down in DEPT 135 spectrum). The magnetically non-equivalent methylene group 4′-H_α and 4′-H_β protons in the ^{1}H NMR spectrum afford multiplets at 2.68 and 2.24 ppm, respectively. In the ^{13}C NMR spectrum the signal at 28.9 ppm was assigned to C-5′, and related to the signal at 2.00 ppm (5′-H), overlapping the signals of 30-3H and 36-3H. The signals at 22.2 and 20.8 ppm in the ^{13}C NMR spectrum were assigned to the non-equivalent methylene group carbon atoms C-6′$_\alpha$ and C-6′$_\beta$, respectively, and the signals at 0.83 and 0.77 ppm in the ^{1}H NMR spectrum were assigned to the 6′$_\alpha$-3H and 6′$_\beta$-3H protons of these methylene groups. This non-equivalency of carbon C-6′$_\alpha$ and C-6′$_\beta$ atoms correlated by the proton data, indicates clearly an inhibition of the rotation around bond C-4′−C-5′, caused by the steric hindrance of the neighbouring isopropyl group at the imine nitrogen (Table 2).

The signal at 48.6 ppm was assigned to the ternary C-1″ carbon atom bound to the imine nitrogen atom of the substituent at C-3. The signals of methylene groups C-2″$_\alpha$ and C-2″$_\beta$ carbons overlap at 21.1 ppm, whereas the protons' signals of these groups, 2″$_\alpha$-3H i 2″$_\beta$-3H, are separated, appearing as doublets at 1.31 and 1.26 ppm. This suggests a constrained geometry around the N−C-1″ bond via the presence of a bulky alkyl fragment at C-3′.

Table 2
^{13}C and ^{1}H NMR data of **2** 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
		$N_{(amide)}$–H	9.07	s			C-1, C-2, C-15
C-1	148.7[a]						
		1-OH	15.75	s			C-1, C-2
C-2	116.6						
C-3	119.0						
C-4	151.7						
		4-OH	14.14	s			C-3, C-4
C-5	99.2						
C-6	171.6						
C-7	103.3						
C-8	183.7						
		8-OH	11.59	br		1″-H lr.	
C-9	117.3[a]						
C-10	117.3[a]						
C-11	185.5						
C-12	108.7						
C-13	21.9	3 13-H	1.63	s			C-11, C-12
C-14	7.3	3 14-H	1.90	s			C-6, C-8
C-15	168.1						
C-16	131.5						
C-17	131.9	17-H	6.34	d	(17,18)=11.2	18-H, 3 30-H lr.	
C-18	125.4	18-H	6.78	dd	(18,19)=16.0	17-H, 19-H	
C-19	139.5	19-H	6.04	dd	(19,20)=7.0	18-H	
C-20	37.8	20-H	2.24	m[a]		21-H, 3 31-H	
C-21	71.8	21-H	3.63	m		20-H, 21-OH	
		21-OH	5.08	d[a]	(21-OH,21)=3.6	21-H	C-21, C-22
C-22	38.1	22-H	1.21	m[a]		23-H, 3 32-H	
C-23	75.6	23-H	2.75	m[a]		22-H, 23-OH	C-24
		23-OH	3.88	d	(23-OH,23)=8.8	23-H	
C-24	32.4	24-H	1.54	m		3 33-H	
C-25	73.1	25-H	5.05	dd	(25,26)=10.8	26-H	C-23, C-24, C-26, C-34 C-35
C-26	40.3	26-H	0.96	m[a]		25-H, 3 34-H	
C-27	76.2	27-H	3.20	d	(27,28)=8.8?	28-H	
C-28	117.6	28-H	4.86	dd[a]	(28,29)=12.8	27-H, 29-H	
C-29	143.0	29-H	6.24	d		28-H	C-12, C-27, C-28
C-30	20.5	3 30-H	1.92	s[a]		17-H lr.	C-16
C-31	17.6	3 31-H	0.67	d	(31,20)=6.4	20-H	C-19, C-20, C-21
C-32	8.8	3 32-H	0.35	d	(32,22)=6.4	22-H	C-22, C-23, C-25
C-33	10.8	3 33-H	0.85	d[a]	(33,24)=6.8	24-H	C-21, C-23, C-24
C-34	8.9	3 34-H	−0.34	d	(34,26)=6.4	26-H	C-26, C-27
C-35	169.2						
C-36	20.0	3 36-H	1.92	s[a]			C-35
C-37	55.5	3 37-H	2.84	s			C-27
C-1′	117.1	1′-H	7.94	d	(1′,2′)=15.6	2′-H	C-2, C-3′
C-2′	148.7[a]	2′-H	7.81	d		1′-H	C-1′, C-3′
C-3′	177.9						
C-4′	40.7	4′-H_α	2.68	dd[a]		4′-H_β, 5′-H	
		4′-H_β	2.24	[a]		4′-H_α, 5′-H	
C-5′	28.9	5′-H	2.00	[a]		4′-H_α, 4′-H_β, 3 6′$_\alpha$-H, 3 6′$_\beta$-H	C-6′$_\beta$
C-6′$_\alpha$	22.2	3 6′$_\alpha$-H	0.83	d[a]	(6′$_\alpha$,5′)=6.4	5′-H	C-5′, C-6′$_\beta$
C-6′$_\beta$	20.8	3 6′$_\beta$-H	0.77	d	(6′$_\beta$,5′)=6.4	5′-H	C-5′, C-6′$_\alpha$
C-1″	48.6	1″-H	4.24	m	(1″,8-OH)≈3.0	8-OH lr., 3 2″$_\alpha$-H, 3 2″$_\beta$-H	
C-2″$_\alpha$	21.1[a]	3 2″$_\alpha$-H	1.31	d[a]	(2″$_\alpha$,1″)=6.4	1″-H, 3 2″$_\beta$-H	C-1″, C-2″$_\beta$
C-2″$_\beta$	21.1[a]	3 2″$_\beta$-H	1.26	d[a]	(2″$_\beta$,1″)=6.4	1″-H, 3 2″$_\alpha$-H	C-1″, C-2″$_\alpha$

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

3.3. NMR study of rimamycin 2: characterization of the ansa-backbone

In the low-field part of the ^{1}H NMR spectrum (16.0–8.0 ppm) the signals of four mobile protons (all disappear after H→D exchange) were found. The signal at 15.75 ppm (s) is assigned to the 1-OH proton. In the $^{1}H,^{13}C$ HMBC spectrum 1-OH/C-1, 1-OH/C-2 correlations are observed. The proton giving a signal at 14.14 ppm, showing a long range coupling with C-3 (119.0) and C-4 (151.7), belongs to the 4-OH hydroxyl group. The signal at 9.07 ppm in the ^{1}H NMR spectrum, showing long range coupling with the C-1 (148.7 ppm) and C2 signals (168.1) was assigned to the amide group proton $N_{(amide)}$–H. The remaining broad signal (11.59 ppm) was assigned to the 8-OH proton. The 8-OH/1′-H correlation observed in the $^{1}H,^{1}H$ COSY spectrum, the low-field position of 1′-H signal (4.24 ppm) and the small difference in chemical shifts of carbon C-8 (183.7 ppm) and C-11 (185.5 ppm) signals result probably from a tautomeric equilibrium, analogous to that described in details in our previous publication.[9]

Considering the whole NMR data set, none of the analytically studied compounds, either **E1**- or **E2**-type rifamycin, displayed an enamine structure. This is quite obvious studying HMBC and HMQC spectra of the compounds. On the one hand, no ^{1}H-signals characteristic of enamine protons –C*H*=C-3′–N*H*–R could be detected and, on the other hand, clear C/H correlations were observed indicating the presence of two protons at C-4′ carbon.

The substituents containing protons 1′-H and 2′-H, in both **2** and **3** (blue imines of type **E1**) as well as in **4** and **5** (dark blue imines of

type **E2**) have a *trans* arrangement related to the double bond C-1′=C-2′.

It seems reasonable to think that having two possible configurations at the imine bond C-3′=N (*syn* (Z) or *anti* (E)) explains the presence of the two isomeric forms **E1** and **E2**. According to Kalinowski,[26b] the NMR signals of the C=N bond found in isomeric imines display, for the *anti* configuration a low-field shift of the carbon in α-position of the imine carbon, i.e., C-4′ in **2**–**5**, relative to the signal recorded for the the *syn* (Z) arrangement.

Prolonging the recording time of the ^{13}C NMR spectrum of **5** (max. 30 h), enabled the **E2**→**E1** isomerisation to take place, giving a second set of signals. Especially a clear decrease in the **E2** signals assigned to C-4′ (27.0 ppm) and of C-6′ (29.0 ppm) and the appearance of two distinct new signals at 29.5 ppm and 32.9 ppm, assigned to the structure **E1** could be noticed. Interestingly in dry solutions, no hydrolysis from **E** to **F** occurred as confirmed by the lack of NMR signal characteristic of a ketone ca. 200 ppm. Hence, it seems logical that the configuration of the double bond in imines **5** and **4** as well as in other **E2** type imines is of the *anti* type and, conversely the double bond in type **E1** imines should display a *syn* configuration.

3.4. NMR study of rifamycin 6 (type F): properties of the substituent at the C-3 atom (Fig. 2, Table 3)

In the ^{13}C NMR spectrum, the signal at 130.0 was assigned to C-1′ whereas proton 1′-H was found at 7.34 ppm as a doublet. In the ^{1}H,^{13}C HMBC spectrum a 1′-H/C-2 and 1′-H/C-3′ correlations were found. In the ^{1}H,^{1}H COSY spectrum a clear 1′-H/2′-H, correlation signal was found, and the high value of coupling constant $J_{1',2'}$=16.0 Hz proves a *trans* configuration of protons 1′-H and 2′-H. The characteristic low-field signal at 200.0 ppm was attributed to the carbonyl carbon C-3′ as well as the methylene C-4′ to the signal at 49.8 ppm. In the ^{1}H NMR spectrum the magnetically non-equivalent methylene groups 4′-H$_\alpha$ and 4′-H$_\beta$ give multiplets at 2.44 and 2.55 ppm. The ^{13}C signal at 24.7 ppm was assigned to C-5′ while the related proton 5′-H could be found as a multiplet at 2.03 ppm, overlapping with signals of 30-H and 36-H in the 1H spectrum. The signals at 22.4 and 22.3 ppm were assigned to methylene groups C-6′$_\alpha$ and C-6′$_\beta$ in the ^{13}C NMR spectrum whilst the ^{1}H-signals of these groups overlap at 0.84 ppm (6′$_\alpha$-3H and 6′$_\beta$-3H as doublets). Contrary to the situation found in **2**, the rotation around the C-4′–C-5′ bond is only slightly inhibited by the change of the alkyl substituents around C4′–C5′, the steric hindrance being mainly limited to an interaction involving isopropyl fragment and ketone group.

3.5. Selected elements of NMR analysis of the rifamycin 6 ansa-frame

In the low-field part of the ^{1}H NMR spectrum a broad signal characteristic of a mobile proton was found at 13.04 ppm, probably 8-OH, whereas a singlet at 8.92 ppm, showing H/C long range coupling with C-1, C-2 and C-15 was assigned to the proton of the amide group present in the *ansa* chain. The broad bulge around 8.0 ppm noticed in the baseline of the ^{1}H NMR spectrum is most likely due to rapid exchange phenomena involving four mobile protons: 1-OH, 4-OH, 21-OH and 23-OH (the latter two signals and the baseline hump disappeared after deuterium exchange via D_2O addition).

4. Mechanism proposal for the reaction of 1 with primary amines and ketones

As summarized in Scheme 1, a chain of successive reactions is initiated by the condensation of 3-formylrifamycins SV (**1**) with an alkylamine catalyzed by acetic acid. The so-formed α-aminoalcohol **A** affords, after abstraction of a water molecule, the violet-blue imine **B**, observed chromatographically (TLC). The structure of similar imines was determined in earlier studies on the condensation of **1** and monoalkylamines.[7]

Simultaneously, the starting ketone present in the system forms an imine **C** via acid-catalyzed reaction with the starting alkylamine.[16,27] The reaction of imines **B** and **C** in the following stage leads to the formation of a β-amino-imine **D**. This transient specie quickly rearranges to eliminate an alkylamine molecule, thus forming the α,β-unsaturated imine **E**. This imine occurs in the reaction mixture in two isomeric forms, **E1** and **E2**, however, a reversible **E1**↔**E2** isomerisation proceeds here. In many cases, the **E2** isomer, weakly soluble in methanol, starts to crystallize already from the hot reaction mixture. The isolation of **E2** crystals during the reaction process displaces logically the **E1**↔**E2** transformation towards **E2** as well as the entire synthesis route from **1** to **E**.

The formation of the α,β-unsaturated ketone **F** can be tentatively explained by the gradual hydrolysis of **E1** and **E2** caused by the presence of water, generated in situ during the formation of **B** and **C** (Scheme 1). However, studying the literature, one might oppose that the synthesis to the α,β-unsaturated ketone **F** can proceed according to an other wide-spread mechanism: following the classic aldol reaction, a related β-hydroxyketone **G** would be the product of a reaction between ketone and starting aldehyde **1** (see Scheme 2 for overview).[28] Albeit this compound **G** was not directly observed in the reaction mixture, its formation cannot be completely ruled out. The α,β-unsaturated ketone **F** (**G**→**F** transformation, Scheme 2) would readily form via elimination of a water molecule. On the other hand, speaking against this aldol-like condensation is the fact that ketone **F** is formed relatively late in the reaction course and that an increase of its concentration is clearly accompanied (TLC) by a decrease of the concentration of imine **E**. This supports the **E** to **F** route.

Considering other mechanistical possibilities, another reaction can be also taken into account (Scheme 2): The β-aminoketone **H**, formed from imine **B** and the starting ketone, can react with the starting amine to yield β-aminoimine **D**. This compound consecutively abstracts an amine and a water molecule to produce compound **F** (**1**→**B**→**H**→**D**→**E**→**F** reaction route). However confronting this mechanism to the findings of the TLC analysis, it is quite obvious that the proposed intermediates **H** and **D** have to be observed as distinct spots on the chromatograms:

If the hypothetical compound **H** was formed, it should be most likely detectable, since a very rapid occurrence of the **H**→**D** process is doubtful (an analogous **1**→**B** reaction takes 1–4 h to be completed). In the reaction system at hand no spot is observed that could be assigned to **H**. Moreover, **H** would convert directly into the α,β-unsaturated ketone **F**, splitting off an amine molecule instead of forming **E** (**B**→**H**→**F** transformation). This again widely diverges from the TLC findings observed during the conversion of **B** to **F**.

Literature reports have been found describing pathways analogous to the main synthetic route proposed in Scheme 1. For instance the acid-catalysed, aldol type condensation of two imine molecules having protons in α-position of the imino group to form β-aminoimines, these compounds losing an amine to form the corresponding α,β-unsaturated imine.[29–31]

5. Conclusions

In the reaction of 3-formylrifamycin SV (**1**) with primary alkylamines and ketones displaying a R$_1$–CH$_2$–CO-R$_2$ structure, where R$_1$=H or alkyl and R$_2$=alkyl, aryl (Scheme 1), a new group of rifamycin derivatives, displaying α,β-unsaturated imines substituents (**E1** and **E2**) at carbon C-3 could be isolated and fully characterized via NMR and MS-methods. These compounds showed a tendency to reversibly isomerise in organic solvents and, in the presence of water, to rapidly hydrolyse. Only some derivatives presenting

Table 3
^{13}C and ^{1}H NMR data of **6** in (D_6)DMSO

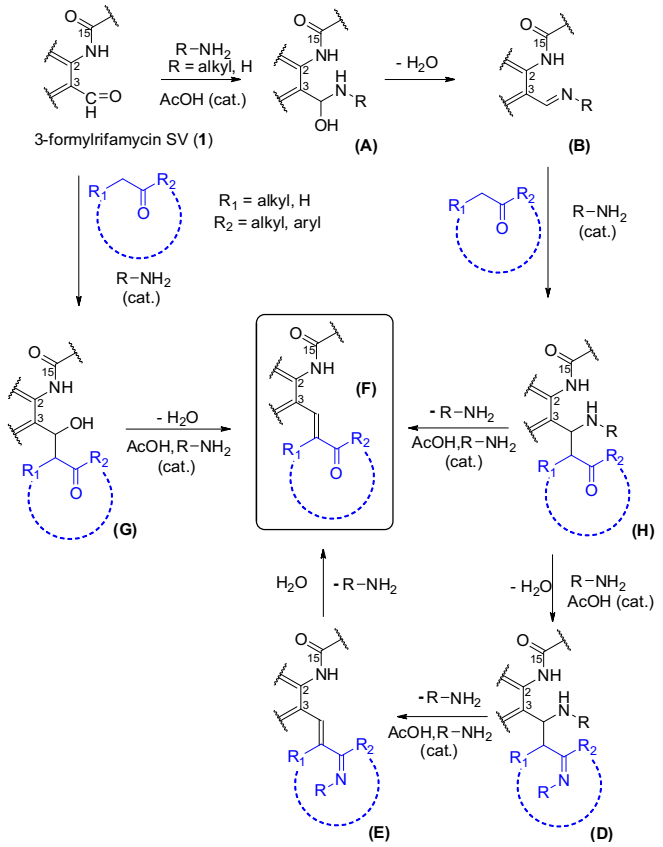
Atom C	δ	Atom H	δ	Multipl.	$J_{H,H}$	$^{1}H,^{1}H$ COSY correlations	$^{1}H,^{13}C$ HMBC correlations
		$N_{(amide)}$–H	8.92	s			C-1, C-2, C-15
C-1	147.5						
		1-OH	[b]				
C-2	118.4[a]						
C-3	118.5[a]						
C-4	148.3						
		4-OH	[b]				
C-5	99.8						
C-6	172.1						
C-7	102.1						
C-8	181.7						
		8-OH	13.04 br				
C-9	114.8						
C-10	118.5[a]						
C-11	186.4						
C-12	108.7						
C-13	22.0	3 13-H	1.67	s			C-11, C-12
C-14	7.5	3 14-H	1.94	s[a]			C-6, C-8
C-15	168.2						
C-16	131.7[a]						
C-17	131.8[a]	17-H	6.31	d	(17,18)=11.0	18-H, 3 30-H lr.	
C-18	126.4	18-H	6.83	dd	(18,19)=16.0	17-H, 19-H	C-17
C-19	138.8	19-H	6.03	dd	(19,20)=7.4	18-H, 20-H	
C-20	37.7	20-H	2.21	m[a]		19-H, 21-H, 3 31-H	
C-21	72.8	21-H	3.67	m		20-H	
		21-OH	[b]				
C-22	38.1	22-H	1.24	m		23-H, 3 32-H	
C-23	75.8	23-H	2.76	m		22-H, 24-H	
		23-OH	[b]				
C-24	32.3	24-H	1.57	m		23-H, 3 33-H	
C-25	73.2	25-H	5.07	dd	(25,26)=10.8	26-H	C-35
C-26	40.0[a]	26-H	1.03	m[a]		25-H, 3 34-H	
C-27	76.2	27-H	3.24	d	(27,28)=8.2	28-H, 29-H lr.	
C-28	117.7	28-H	4.89	dd	(28,29)=12.8	27-H, 29-H	
C-29	142.9	29-H	6.25	d		27-H lr., 28-H	C-12, C-27, C-28
C-30	20.3	3 30-H	1.96	s[a]		17-H lr.	C-15, C-16
C-31	17.3	3 31-H	0.77	d	(31,20)=6.8	20-H	C-19, C-20, C-21
C-32	9.1	3 32-H	0.42	d	(32,22)=6.8	22-H	C-22, C-23, C-25
C-33	10.7	3 33-H	0.87	d[a]	(33,24)=6.8	24-H	C-21, C-23, C-24
C-34	8.9	3 34-H	−0.40	d	(34,26)=6.8	26-H	C-26, C-27
C-35	169.3						
C-36	20.6	3 36-H	1.95	s[a]			C-35
C-37	55.6	3 37-H	2.88	s			C-27
C-1′	130.0	1′-H	7.34	d	(1′,2′)=16.0	2′-H	C-2, C-3′
C-2′	136.5	2′-H	7.58	d		1′-H	C-3, C-3′
C-3′	200.0						
C-4′	49.8	4′-H_α	2.44	dd[a]	($4'_\alpha 4'_\beta$)=12.8	4′-H_β, 5′-H	C-5′, C-$6'_\alpha$, C-$6'_\beta$
		4′-H_β	2.25	dd[a]	($4'_\alpha$,5′)=6.8	4′-H_α, 5′-H	C-5′, C-$6'_\alpha$, C-$6'_\beta$
C-5′	24.7	5′-H	2.03	m[a]	(5′$4'_\beta$)=6.8	4′-H_α, 4′-H_β, 3 $6'_\alpha$-H, 3 $6'_\beta$-H	C-$6'_\alpha$, C-$6'_\beta$
C-$6'_\alpha$	22.4[a]	3 $6'_\alpha$-H	0.84	d[a]		5′-H	C-5′, C-$6'_\beta$
C-$6'_\beta$	22.3[a]	3 $6'_\beta$-H	0.84	d[a]		5′-H	C-5′, C-$6'_\alpha$

Abbreviations: s: singlet, d: doublet, m: multiplet, br: broad, lr: long range correlation.
[a] This signal overlapped with another signal.
[b] Shared signal—broad bulge of the spectrum baseline with a maximum at ca. 8.0 ppm.

definite structural characteristics could be isolated in a crystalline, stable form: the compounds displaying substituents R_2=CH_2-A, with A=alkyl, R_2=aryl, and with R_3=H. Representative for this class of compounds, two type-**E1** and two type-**E2** rifamycins as well as a connected hydrolysis product, a α,β-unsaturated ketone, were reported in this contribution. The general synthetic method to obtain these *N*-substituted 3-(3′-imino)alkylidenylrifamycins SV is the subject of an ongoing patent application. Some of the new *N*-substituted 3-(3′-imino) alkylidenylrifamycins SV, among others compound **2**, were tested in vitro for tuberculostatic activity against different strains of *Mycobacterium* and compared to the main rifamycin antibiotics: rifampicin (RMP) and rifabutin (RBT). For the test standard strain of *Mycobacterium tuberculosis—M. tbc H$_{37}$Rv* and *M. tbc Bovis* were used, as well as different strains of MOTT (Mycobacteria Other Than *M. Tuberculosis*) sensitive or resistant to RMP and RBT. In spite of the already mentioned trend towards hydrolysis, all the tested compounds showed a marked anti-tuberculous activity and activity against MOTT, although not higher than the activity of the reference materials.[32]

Imines **E1** (generally—blue, crystallizing from chloroform) and **E2** (generally—dark blue, crystallizing from methanol) are most probably geometric isomers, differing in the configuration (*syn* and *anti)* of substituents R_2 and R at the imine bond (Scheme 1). The observed shifts in ^{13}C NMR spectra of the signals attributed to C(α)–C=N–, e.g., for **4** of type **E2** in $CDCl_3$ solution, correlate with the gradual isomerisation to form **E1** and suggests that these substituents have an *anti* arrangement in isomers **E1** and a *syn* one in isomer **E2**.

Along these lines, it could be noticed that structure **E1** generally shows higher stability in solution, probably due an energetically more favourable spatial arrangement of the substituents at the imine C=N bond.



Scheme 2. Alternative routes of reaction of **1** with primary amines and ketones.

Due to a general propensity to hydrolysis, the new rifamycin SV derivatives, exhibiting α,β-unsaturated iminic substituents at C-3, are not directly usable as potential medicines, but they are definitely valuable reactive derivatives for further rifamycin modifications.

In the reaction system studied here, imines **E** play a role as intermediates in the synthesis of rifamycins displaying α,β-unsaturated ketones at C-3 (compound **F**). The general mechanism of formation, proposed in Scheme 1, is different from the one described for classical aldol reactions, the key-step in our case being the formation of imino-derivatives, **B** and **C**.

The condensation of **B**- and **C**-type imines, formed in situ seems to be a promising, although hitherto rarely employed route to form α,β-unsaturated imines or, via further hydrolysis, to form the related α,β-unsaturated ketones.

6. Experimental part

6.1. General

Available commercial reactants were used without additional purification: 3-formylrifamycin SV (**1**) (provided by Polfa–Tarchomin), ketones, primary amines, carboxylic or sulfonic acids (all—synthesis grade purity: >98%) as well as solvents (all—99% min.). TLC chromatograms were developed on aluminium plates covered with silicagel 0.2 mm 60 F_{254}, using $CHCl_3$/MeOH (9:1 *v*/*v*) as the mobile phase.

The ^1H and ^{13}C NMR were recorded operating at 399.91 and 100.56 MHz, respectively, by means of a Varian Inova$_{Unity}$ 400 spectrometer equipped with an Oxford Magnet (9,4T). All samples were dissolved in ca. 0.5 ml of DMSO-d_6. Chemical shifts are reported in parts per million, and *J*-coupling constants are expressed in Hertz. The calibration was performed using the remaining signal of the non-deuteriated part of the solvent (2.50 ppm) and the C-D characteristic coupling pattern of the deuteriated solvent in ^{13}C (39.5 ppm), when necessary a supplementary calibration with TMS as internal standard has been done. The 2D, short- and long range correlated C,H- spectra (gHSQC and gHMBC, respectively) were recorded using standard pulse sequences with z-gradients, as provided by Varian with the VNMR 6.1C control and processing software. Mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded by employing a Mariner (PerSeptive Biosystems) mass scectrometer using the electro spray ionization (ESI) technique and are reported as *m/e* (relative intensity). Accurate masses are reported for the molecular ion (M+1) or a suitable fragment ion. For this analysis the samples were dissolved in CH_3OH. IR spectra were recorded on a Perkin–Elmer PE-577 instrument using KBr pellets. The new rifamycin derivatives have no definite melting points—they do not melt (up to 300 °C) and slowly decompose at temperatures over 160 °C.[9]

6.2. General synthetic procedure for α,β-unsaturated imines E

1.6 g of 3-formylrifamycin SV (2 mmol) was added to a solution containing: 15.0 ml of methanol, 0.6 g of acetic acid, 5–10 mmol of amine and 5–20 mmol of ketone. The system was stirred at 38–45 °C, chromatographically (TLC) monitoring the reaction progress. (The red spot of 3-formylrifamycin SV gradually disappears whereas two products spots appear and then enlarge—a blue one for type **E1** imine and a navy blue one for imine **E2**, with R_f **E1**<R_f **E2**).

After disappearance of 3-formylrifamycin SV or reaction inhibition via hydrolysis, only an increase in intensity of the red spot of ketone **F**—(hydrolysis of **E**; R_fF<R_fE1) was observed, the process was stopped by cooling down the system to room temperaure. Reaction time: 0.5–4 h.

A) When the α,β-unsaturated imines crystallized from methanol in the form of type **E2** isomer, e.g., **3** and **4**, the post-reaction mixture was left for 16 h at ~5 °C. The crystalline dark blue product was filtered off, washed with cold (~5 °C) methanol, and then with hexane followed by drying under vacuum.

B) When α,β-usaturated imines did not crystallize from methanol (this concerns derivatives of 4-methyl-2-pentanone (isobutylmethyl ketone) and 2,4-dimethyl-6-heptanone), 40 ml of chloroform and 60 ml of water were added to the post-reaction mixture. After mixing the reaction mixture at 40–45 °C, the chloroform phase was separated and washed with 120 ml of water. The organic phase was then concentrated to a volume of 10–20 ml, and left for 16 h at ~5 °C. The crystalline blue product—α,β-unsaturated imine as **E1** isomer—was filtered off, washed with cold (~5 °C) chloroform, then hexane and finally dried under vacuum.

6.3. Synthesis of the α,β-unsaturated imines 2, 3, 4, 5

Synthesis of 3-(3′-Isopropylimino-5′-methyl)hexenylrifamycin SV (**2**) (of type **E1**): 1.6 g of 3-formylrifamycin SV (2 mmol) was added to a solution containing 15.0 ml of methanol, 0.6 g of acetic acid, 0.6 g (~10 mmol) of isopropylamine and 2.0 g (~20 mmol) of 4-methyl-2-pentanone. The system was stirred at 40–45 °C for 4 h. The crystalline blue product was separated according to *6.2-B*. 1,2 g of **2** was obtained. Yield 70.6%. See Table 2 for ^{13}C and ^1H NMR spectroscopic data. MS (ESI): *m*/*z* (%): 849.5 (100.0) $[M+H]^+$, 871.4 (91.7) $[M+Na]^+$, 812.4 (20.8) $[M+Na-C_3H_7NH_2]^+$, 790.4 (7.1) $[M+H-C_3H_7NH_2]^+$. HRMS calcd for $[M+Na]^+$ $C_{47}H_{64}N_2O_{12}Na$

871.4351; found 871.4376 (error: 2.7 ppm). IR: 3400, 3240, 2960, 1700, 1600, 1535, 1470 cm^{-1}.

Synthesis of 3-(5′-Methyl-3′-propylimino) hexenylrifamycin SV (**3**) (of type **E1**): 1.6 g of 3-formylrifamycin SV (2 mmol) was added to a solution containing: 15.0 ml of methanol, 0.6 g of acetic acid, 0.6 g (~10 mmol) of propylamine and 2.0 g (~20 mmol) of 4-methyl-2-pentanone. The system was stirred at 40–45 °C for 4 h, The crystalline blue product was separated according to *5.2-B*. 1,05 g of 3 was obtained. Yield 61.8%. See Supplementary data for ^{13}C and ^{1}H NMR spectroscopic data. MS (ESI): *m/z* (%): 871.5 (100.0) $[M+Na]^+$, 812.4 (71.5) $[M+Na-C_3H_7NH_2]^+$, 849.5 (45.5) $[M+H]^+$, 790.4 (15.2) $[M+H-C_3H_7NH_2]^+$. HRMS calcd for $[M+Na]^+$ $C_{47}H_{64}N_2O_{12}Na$ 871.4351; found 871.4362 (error: 1.2 ppm). IR: 3409, 3246, 2968, 1704, 1596, 1472, 1247 cm^{-1}.

Synthesis of 3-(3′-Cyclohexylimino)octenylrifamycin SV (**4**) (of type **E2**): 1.6 g of 3-formylrifamycin SV (2 mmol) was added to a solution containing: 15.0 ml of methanol, 0.6 g of acetic acid, 1.0 g (~10 mmol) of cyclohexylamine and 2.3 g (~20 mmol) of 2-heptanone. The system was stirred at ~45 °C for 2/3 h. The crystalline dark blue product was separated according to *6.2-A*. 0.92 g of **4** was obtained. Yield 51.0%. See Supplementary data for ^{13}C and ^{1}H NMR spectroscopic data. MS (ESI): *m/z* (%): 925.5 (100.0) $[M+Na]^+$, 908.5 (38.1) $[M+H]^+$, 826.4 (32.7) $[M+Na-C_6H_{11}NH_2]^+$. HRMS calcd for $[M+Na]^+$ $C_{51}H_{70}N_2O_{12}Na$ 925.4821; found 925.4830 (error: 1.0 ppm). IR: 3458 (br), 2936, 2864, 1716, 1599, 1473, 1237 cm^{-1}.

Synthesis of 3-(3′-Isopropylimino)nonenylrifamycin SV (**5**) (of type **E2**): 1.6 g of 3-formylrifamycin SV (2 mmol) was added to a solution containing: 15.0 ml of methanol, 0.6 g of acetic acid, 0.6 g (~10 mmol) of isopropylamine and 2.5 g (~20 mmol) of 2-octanone. The system was stirred at ~40 °C for 3.0 h. The crystalline dark blue product was separated according to *5.2-A*. 1.16 g of **5** was obtained. Yield 66.3%. See Supplementary data for ^{13}C and ^{1}H NMR spectroscopic data. MS (ESI): *m/z* (%): 899.5 (100.0) $[M+Na]^+$, 840.4 (64.8) $[M+Na-C_3H_7NH_2]^+$, 877.5 (37.0) $[M+H]^+$ HRMS calcd for $[M+Na]^+$ $C_{49}H_{68}N_2O_{12}Na$ 899.4664; found 899.4638 (error: 2.95 ppm). IR: 3412, 3265, 2972, 2935, 1720, 1728, 1599, 1472, 1241 cm^{-1}.

Synthesis of 3-(5′-Methyl-3′-oxo)hexenylrifamycin SV (**6**) (of type **F**):

To 0.85 g (1 mmol) of **2** in 80 ml of methanol, 100 ml of an aqueous solution containing 5.0 g of $Na_2HPO_4{\times}12H_2O$ was slowly added (attention: a too rapid mixing of the solutions leads to a substantial foaming) and the homogeneous system obtained was stirred at 40–45 °C. The reaction progress was monitored chromatographically (TLC), observing the gradual disappearance of the initial imine (**2**) blue spot, and the increase of the product red spot Rf**6**<Rf**2**. After 4 h methanol was distilled off under vacuum (p=200 mmHg, temp=60 °C), and the remaining aqueous suspension was extracted twice with chloroform (50 ml+30 ml). The chloroform extract was washed twice with 200 ml of water, while adjusting pH of the aqueous phase during first washing with diluted H_2SO_4 to reach pH~2.0. The organic phase was dried with anhydrous sodium sulfate, and after filtering off the drying agent, concentrated to a volume of ~15 ml, and left for 16 h at ~0 °C. The fine crystalline red product was filtered off, washed with cold (~0 °C) chloroform and hexane and dried under vacuum. Yield 0.28 g (34.7%). See Table 3 for ^{13}C and ^{1}H NMR spectroscopic data. MS (ESI) (negative ionization): *m/z* (%): 806.4 (100.0) $[M-H]^-$. HRMS calcd for $[M-H]^-$ $C_{44}H_{56}NO_{13}$ 806.37462; found 806.37679 (error: 2.6935 ppm). IR: 3408 (br.), 2968, 1723, 1594, 1372, 1240 cm^{-1}.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2012.04.071. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Wrona, I. E.; Agouridas, V.; Panek, J. S. *C. R. Chim.* **2008**, *11*, 1483.
2. Lal, R.; Lal, S. *BioEssays* **1994**, *16*, 211.
3. Floss, H. G.; Yu, T. W. *Chem. Rev.* **2005**, *105*, 621.
4. Aristoff, P. A.; Garcia, G. A.; Kirchhoff, P. D.; Hollis Showalte, H. D. *Tuberculosis* **2010**, *90*, 94.
5. Mitchison, D. A.; Fourie, P. B. *Tuberculosis* **2010**, *90*, 177.
6. Cole, S. T. *Res. Microbiol.* **1996**, *147*, 48.
7. Bujnowski, K.; Synoradzki, L.; Dinjus, E.; Zevaco, T.; Augustynowicz-Kopeć, E.; Zwolska, Z. *Tetrahedron* **2003**, *59*, 1885.
8. Bujnowski K.; Synoradzki L.; Bojarska-Dahlig H.; Dinjus E.; Leitner W.; Gassner F., to *Warsaw University of Technology*, PL Pat. **2003**, *184*, 862.
9. Bujnowski, K.; Synoradzki, L.; Zevaco, T.; Dinjus, E.; Augustynowicz-Kopeć, E.; Zwolska, Z. *Eur. J. Org. Chem.* **2006**, 4155.
10. Bujnowski K.; Synoradzki L.; Bojarska-Dahling H.; Dinjus E.; Gassner F., to *Warsaw University of Technology*, PL Pat. **2003**, *185*, 662.
11. Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry Part B: Reactions and Synthesis*; Plenum: New York, NY and London, 1990, pp 58–60.
12. Cricchio, R.; Tamborini, G.; Bravo, P.; Gaudiano, G. *Il Farmaco—Ed. Sc.* **1974**, *29*, 358.
13. Cricchio R., to Gruppo Lepetit S.p.A., U.S. Patent 1,389,100, **1972**.
14. Carey, F. A. *Organic Chemistry*; The McGraw-Hill Companies: 1996, pp 741–747.
15. March, J. *Advanced Organic Chemistry*; John Wiley: 1992, pp 937–945.
16. Hammet, L. P. *Physical Organic Chemistry*; McGraw-Hill Book: New York, NY, 1940; p 333.
17. I.Choma, Antibiotics: TLC Analytics in Encyclopedia of Chromatography, 3rd ed., Ed. J.Cazes, vol. 1; pp 89–95.
18. Kanfer, I.; Skinner, M. F.; Walker, R. B. *J. Chromatogr., A* **1998**, *812*, 255.
19. Henwood, S. Q.; Liebenberg, W.; Tiedt, L. R.; Lotter, A. P.; De Villiers, M. M. *Drug Dev. Ind. Pharm.* **2001**, *27*, 1017.
20. Bujnowski K.; Synoradzki L.; Bojarska-Dahlig H.; Dinjus E., to *Warsaw University of Technology*, PL Pat. **2005**, *188*, 889.
21. Taguchi, M.; Yamana, Y.; Aikawa, N.; Trukamoto, G. *Chem. Pharm. Bull.* **1988**, *36*, 4157.
22. Taguchi, M.; Aikawa, N.; Tsukamoto, G. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2431.
23. Santos, L.; Fant, F.; Madeiros, M. A.; Borremans, F. A. M.; Costa, M. C.; Curto, M. J. M. *Magn. Reson. Chem.* **2000**, *38*, 937.
24. Santos, L.; Medeiros, M. A.; Santos, S.; Costa, M. C.; Tavares, R.; Curto, M. J. M. *J. Mol. Struct.* **2001**, *563–564*, 61.
25. Rubio, E.; Merino, I.; Garcia, A. B.; Cabal, M. P.; Ribas, C.; Bayod-Jasanada, M. *J. Mol. Struct.* **2005**, *43*, 269.
26. (a) Friebolin, H. In *Basic One- and Two-Dimensional NMR Spectroscopy*; VCH Verlagsgesellschaft mbH: Weinheim, 1993; pp 88–91; (b) Kalinowski, H. O.; Berger, S.; Braun, S. In *^{13}C NMR Spektroskopie*; Georg Thieme: Stuttgart, 1984; pp 220–227.
27. John McMurry in Organic Chemistry, 5th ed.; Brooks/Cole A Division of Thomson Learning: 2000, pp 770–774.
28. Carey, F. A.; Sundberg, R. J. In *Advanced Organic Chemistry: Part A: Structure and Reactivity Plenum: New York, NY and London, 1990; p 461.*
29. Kharash, M. S.; Richlin, J.; Mayo, F. R. *J. Am. Chem. Soc.* **1940**, *62*, 494.
30. Emerson, W. S.; Hess, S. M.; Uhle, F. C. *J. Am. Chem. Soc.* **1941**, *63*, 872.
31. Crampton, M. R.; Lord, S. D.; Millar, R. *J. Chem. Soc., Perkin Trans. 1* **1997**, *2*, 909.
32. Bujnowski K.; Synoradzki L.; Dinjus E.; Zevaco T., to *Warsaw University of Technology*, Process for the preparation of *N*-substituted 3-(3′-imino) alkylidenylrifamycin SV Pat. Appl. PL391473 (A1), 2010.