136. Synthesis of Glycosylrifamycins, a New Type of Semisynthetic Rifamycins

by **Cecilia Bartolucci, Luciano Cellai*, Concetta Martuccio, Andrea Rossi,** and **Anna Laura Segre**

Istituto di Strutturistica Chimica 'G. Giacomello', CNR, CP 10, I–00016 Monterotondo Stazione, Roma

and **Simona Rizea Savu** and **Luigi Silvestro**

IBMP, Institut for Biomedical and Pharmaceutical Research, Schleifweg 3, D–90562 Heroldsberg

(25.IV.96)

Glycosylrifamycins, a new type of semisynthetic rifamycin derivatives, can be easily obtained by reaction of 3-(2-aminoethylthio)rifamycin SV (**2**) with a glycosyl compound carrying a coupling group, such as isothiocyanate or carboxy. We prepared *O*-acetylated and free glucopyranosyl and arabinopyranosyl derivatives of rifamycin S and SV (see **3**–**10**). Additionally, derivatives with D-saccharo-1,4-lactone and with shikimic acid were obtained (see **11**–**15**). Glycosylrifamycins show an interesting inhibitory power on *Gram*-positive bacteria (*Table*).

Introduction. – The appearence of new infectious diseases such as AIDS and the arise of drug resistance in previously sensitive microorganisms, as it is occurring with tuberculosis, prompted the search for new chemotherapeutics and antibiotics.

The rifamycins [1] belong to a family of antibiotics highly active on *Gram*-positive bacteria and on mycobacteria. The lead rifamycin, rifamycin SV (= rifamycin; *Scheme 1*), was discovered in the late 50's. Since then, hundreds of derivatives have been prepared with the aim of extending the spectrum of antibacterial action and of regulating absorption and pharmacokinetic *in vivo*. The most important antibacterial derivatives are rifampicin [2], useful in the systemic therapy of tuberculosis, leprosis, gonorrhea, and meningitis, rifamycin SV itself [3], useful for topical applications, rifabutin [4], currently of particular interest against opportunistic mycobacterial infections in AIDS, and rifaximin [5] used as gastrointestinal disinfectant and in zootechnic.

Among rifamycin-like natural products, there are some carrying additional OH groups which should make them more hydrophilic. *E.g.*, halomycins [6] carry at C(4) a hydroxylated pyrrolidine ring, and in some cases, also an OH group at C(20). Tolypomycins [7] are substituted at C(4) by a glycose moiety, named tolyposamine. Both types of compounds display interesting antibacterial properties. Nonetheless, there is no evidence that semisynthetic polyhydroxylated or glycosyl-substituted rifamycins have ever been considered for a developmental study.

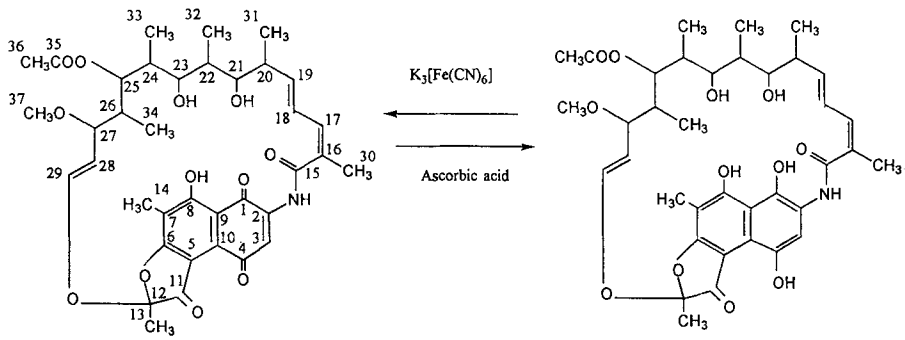
The presence of additional OH groups in a rifamycin derivative should make it much more hydrophilic and could, *e.g.*, address the pharmacokinetic route towards the renal and urinary compartment. Furthermore, the presence of a polyhydroxylated moiety or of a glycose moiety should influence the mechanism of transport into the bacterial cell.

Therefore, we undertook the synthesis of a few glycosylrifamycins and determined their activity on some bacterial strains, as a preliminary characterization of these new rifamycins.

Chemistry. – On the basis of the known structure-activity relationship (SAR) in rifamycins [1] and of the well established chemistry of their chromophore rings [8], it was decided to introduce a glycosyl group at C(3) of rifamycin S (= 1,4-dideoxy-1,4-dihydro-1,4-dioxorifamycin; **1**).

We first tried a direct reaction of rifamycin S (**1**; *Scheme 1*) with a D-glucosamine derivative. D-Glucosamine itself would reduce rifamycin S (naphthoquinone form) to rifamycin SV (naphthohydroquinone form) with a subsequent deactivation of C(3) towards a nucleophilic addition [8]. We, therefore, synthesized the 1,3,4,6-tetra-*O*-acetyl-*β*-D-glucosamine hydrochloride [9]. This compound, protected at the anomeric C-atom, is also soluble in organic aprotic solvents which are generally used in the semisynthesis of rifamycins.

Scheme 1

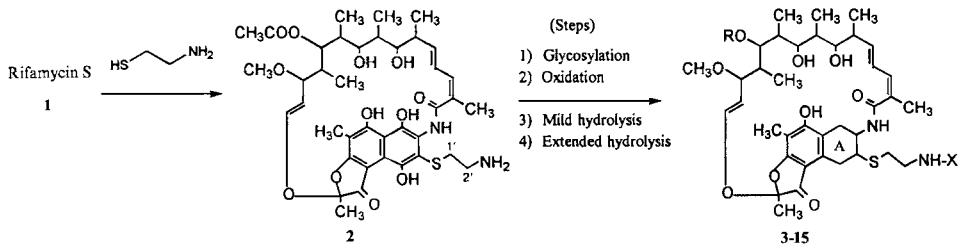


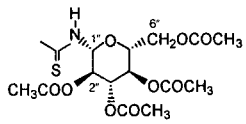
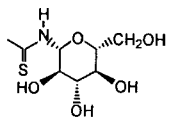
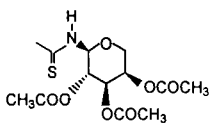
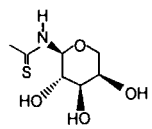
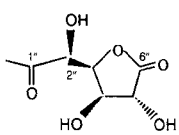
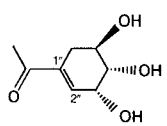
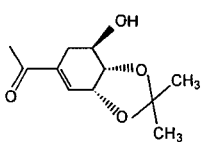
Rifamycin S (**1**) Rifamycin SV

Unfortunately, no reaction between rifamycin S (**1**) and 1,3,4,6-tetra-*O*-acetyl-*β*-D-glucosamine hydrochloride occurred in THF/Et_3N 9:1 in the presence of 2 equiv. of $(i\text{-}Pr)_2EtN$. This is probably due to steric hindrance which hampers the reaction between the amino group of the sugar and C(3) of the chromophore rings. Therefore, we adopted a new strategy, *i.e.*, the introduction of a spacer between the chromophore rings and the glycosyl group. Accordingly, we synthesized 3-(2-aminoethylthio)rifamycin SV (**2**) [10] and allowed it to react with 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl isothiocyanate, a commercially available substrate (*Scheme 2*). The obtained 3-{2-[*N*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)thioureido]ethylthio}rifamycin SV (**3**), in which the glycosyl moiety is attached to the rifamycin through a (thioureido)ethylthio spacer (*Scheme 2*), was oxidized to the S form **4** which was then deacetylated under mild conditions (20 mM KOH in MeOH) to yield compound **5** in which all OH groups of the glycopyranosyl moiety are free. Applying more drastic conditions (20% KOH in EtOH), we obtained the corresponding compound **6** which was also deacetylated at C(25) [11].

We tried the same reactions using another commercially available glycoside, the 2,3,4-tri-*O*-acetyl-α-D-arabinopyranosyl isothiocyanate, and succeeded in isolating 3-{2-[*N*-(2,3,4-tri-*O*-acetyl-α-D-arabinopyranosyl)thioureido]ethylthio}rifamycin SV (**7**), its S form **8**, the partially deacetylated **9**, and the completely deacetylated **10**, the analogs of compounds **3**–**6**.

Scheme 2



Product	Steps	A ring form[a])	R	X
3	1	SV	CH_3CO	
4	1, 2	S	CH_3CO	
5	1, 2, 3	S	CH_3CO	
6	1, 2, 4	S	H	
7	1	SV	CH_3CO	
8	1, 2	S	CH_3CO	
9	1, 2, 3	S	CH_3CO	
10	1, 2, 4	S	H	
11	1	SV	CH_3CO	
12	1	SV	CH_3CO	
13	1, 2	S	CH_3CO	
14	1	SV	CH_3CO	
15	1, 2	S	CH_3CO	

[a]) See *Scheme 1*.

Furthermore, **2** was reacted with yet another available compound, D-saccharo-1,4-lactone (= D-glucaro-1,4-lactone) monohydrate. After activation of the free carboxy group by DCC, the reaction with **2** yielded 3-[2-(D-saccharo-1,4-lacton-6-oylamino)-ethylthio]rifamycin SV (**11**). Finally, **2** was reacted with shikimic acid, under analogous conditions, to give 3-{2-{[(3*R*,4*S*,5*R*)-3,4,5-trihydroxycyclohex-1-en-1-ylcarbonyl]-amino}ethylthio}rifamycin SV (**12**), which was then oxidized to the S form **13**. Analogously, **2** was reacted with the 3,4-*O*-isopropylidene derivative of shikimic acid [12] to give rifamycin SV derivative **14** and, after oxidation, its S form **15**. In compounds **11–15**, the thioureido group of the derivatives **3–10** is replaced by an amide group.

Activity Measurements. – The *in vitro* activity of compounds **3–15** was tested on *Gram*-positive bacteria such as *Bacillus cereus* and *Micrococcus luteus*, and on *Gram*-negative bacteria such as *Escherichia coli*. As reference, the activity of *rifampicin* and penicillin G were assayed, too. The new compounds showed no activity on *Gram*-negative bacteria but an interesting activity on *Gram*-positive bacteria, in some cases comparable to that of *rifampicin* (see *Table*).

Table. MIC *Values*[a]) *for* **3–15** *on Examples of* Gram-*Positive* (*B. cereus* and *M. luteus*) *and* Gram-*Negative* (*E. coli*) *Bacteria*

	Bacillus cereus[b])	*Micrococcus luteus*[b])	*Escherichia coli*[b])
3	5–10	1–5	> 80
4	5–10	1–5	> 80
5	10–20	5–10	> 80
6	80	20–40	> 80
7	40–80	5–10	> 80
8	10–20	1–5	> 80
9	40–80	5–10	> 80
10	80	20–40	> 80
11	20–40	20–40	> 80
12	> 80	40–80	> 80
13	20–40	40–80	> 80
14	40–80	10–20	> 80
15	> 80	> 80	> 80
Rifampicin	1–5	1–5	20
Penicillin G	> 80	5–10	> 80

[a]) Minimal concentration [μg/ml] which inhibits the growth of microorganism in the culture broth (BHI) inoculated and incubated at 30° for 24 h.

[b]) Microorganism: *Bacillus cereus* NCIMB 50014, *Micrococcus luteus* ATCC 9341, *Escherichia coli* NCIMB 30034.

Conclusions. – Rifamycins are known to undergo a rigid internal conformational control, independently of the state of oxidation of the chromophore rings and of the nature of the substituents introduced at C(3) and/or C(4) [13]. Therefore, it can be excluded that the glycosyl substituents exert any effect on the conformation of the rifamycin molecule. It can also be excluded that the antibacterial activity of the above described derivatives can be influenced by the conformation of the glycosyl moiety itself.

The analysis of the data reported in the *Table* suggests then that the antibacterial activity of the present derivatives depends only on the number of free OH groups present

in each compound. The larger the number of free OH groups, the higher its polarity, the lower its antibacterial activity.

This is in agreement with known SAR [1] and indicates that the penetration of the bacterial cell wall by the glycosylrifamycins occurs by passive diffusion. No apparent active transport mechanism is exploited by these compounds so as to extend their spectrum of action to *Gram*-negative bacteria. According to literature, this has been achieved so far only in one case; with compound CGP 4832, obtained by esterification of 25-*O*-deacetyl-25-*O*-(2-carboxyacetyl)-3-morpholinorifamycin S with 3-(hydroxymethyl)-1-methylpiperidine [14].

Even though the number of compounds as well as of tested microorganisms is limited, it seems justified to say that glycosylrifamycins are a new type of interesting rifamycin S derivatives with a potential as antibacterial drugs against *Gram*-positive bacteria [15].

Furthermore, the synthesis of this new class of rifamycins points out a new strategy for the preparation of derivatives with potentially peculiar pharmacokinetic characteristics.

The authors wish to thank the Servizio NMR of the *CNR*, Research Area of Rome.

Experimental Part

General. TLC: glass plates, silica gel *60 F254* (0.25 mm) *Merck*. Column chromatography (CC): silica gel *60* (0.063–0.200 mm) *Merck*. UV Spectra: *Varian DMS90*; in H_2O unless specified. NMR Spectra: *Bruker-AMX* spectrometer, 600.13 MHz for ^1H and 150.9 MHz for ^{13}C; chemical shifts in ppm rel. to Me_4Si (= 0 ppm). All rifamycin derivatives show the characteristic spectral pattern and peak multiplicity previously described for the ansa-chain protons [13], without any remarkable variation in coupling constants. ^{13}C-NMR Spectra were obtained by the projections of 2D maps of reverse-detected $^1H,^{13}C$ heteronuclear correlated spectra [16]. The side-chain resonances were assigned through 2D spectra, mostly DQ-filtered COSY, but also by reverse-detected heteronuclear $^{13}C,^1H$ COSY. This was necessary particularly for the assignment of the CH_2CH_2 spacer; these resonances appear broad, probably due to slow motions near the magnetically anisotropic chromophore rings [17]. ESI-MS: *Perkin-Elmer SCIEX API III*; electrospray ionization (ESI) with ion-spray source, either as positive or as negative ions. FAB-MS: *VG 70-250 S*, in glycerol-thioglycerol matrix.

1. *3-(2-Aminoethylthio)rifamycin SV* (**2**) was prepared as described in [8]. ESI-MS: 772 ([M^-]).

2. *3-{2-[N-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)thioureido]ethylthio}rifamycin SV* (**3**). A soln. of **2** (1.0 g, 1.3 mmol) and 4-(dimethylamino)pyridine (20 mg) in anh. THF (20 ml) was added to 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (1.0 g, 2.6 mmol) in anh. THF (4 ml). The mixture was allowed to react at r.t. under stirring. The reaction was complete after 24 h. The solvent was evaporated and the residue submitted to CC ($CHCl_3$/MeOH 8:2). Yield 93%. UV/VIS ($c = 3 \cdot 10^{-5}$ M): 445 (4.02), 317 (4.25), 273 (4.43). ^1H-NMR (D_2O): 7.29 (*dd*, J = 16.2, 10.8, H–C(18)); 6.53 (*d*, J = 10.8, H–C(17)); 6.42 (*d*, J = 13.2, H–C(29)); 6.31 (*dd*, J = 16.2, 7.2, H–C(19)); 5.46 (br., H–C(1″)); 5.42 (*m*, H–C(3″)); 5.1 (*dd*, J = 13.2, 8.4, *ca.* 3 H, H–C(28), superimposed by *d* (J = 11.0, H–C(25)) and by *m* (H–C(4″))); 5.06 (*m*, H–C(2″)); 4.35 (*dd*, J = −12.6, 3.6, H_a–C(6″)); 4.16 (*dd*, J = −12.6, < 3, H_b–C(6″)); 4.07 (*m*, H–C(5″)); 4.04 (br., H_a–C(1′)); 3.9 (*d*, J = 9.6, H–C(21)); 3.63 (br., H_b–C(1′)); 3.60 (br., H_a–C(2′)); 3.48 (*d*, J = 8.4, H–C(27)); 3.23 (*s*, Me(37)); 3.14 (*d*, J = 10.3, H–C(23)); 3.08 (br., H_b–C(2′)); 2.48 (*m*, H–C(20)); 2.26 (*s*, Me(14)); 2.23–2.04 (6*s*, *ca.* 18 H, Me(30), Me(36), 4 Ac); 1.91 (*m*, H–C(22)); 1.46 (*m*, H–C(24)); 1.29 (*m*, H–C(26)); 1.03 (*d*, J = 7.0, Me(32)); 1.00 (*d*, J = 7.0, Me(31)); 0.84 (*d*, J = 7.0, Me(33)); −0.22 (*d*, J = 7.0, Me(34)). ESI-MS: 1162 (33, M^+), 1130 (100, [M − MeOH]$^+$).

3. *3-{2-[N-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)thioureido]ethylthio}rifamycin S* (**4**). A soln. of **3** (0.5 g) in AcOEt was treated with 33% aq. $K_3[Fe(CN)_6]$ soln. The org. phase was dried (anh. Na_2SO) and evaporated. The residue was purified by CC ($CHCl_3$/MeOH 9:1). Yield 90%. UV/VIS (H_2O/MeOH 9:1; $c = 5 \cdot 10^{-5}$M): 425 (3.58), 316 (4.08), 243 (4.30). ^1H-NMR ($CDCl_3$): 12.646 (*s*, OH–C(8)); 8.447 (*s*, NH–C(15)); 7.861 (*s*, NH–C(2′)); 6.814 (*d*, J = 8.6, NH–C(1″)); 6.612 (*dd*, J = 15.7, 10.5, H–C(18)); 6.411 (*d*, J = 10.5,

H–C(17)); 6.120 (*dd*, J = 15.7, 7.5, H–C(19)); 6.015 (*d*, J = 12.3, H–C(29)); 5.747 (br., H–C(1″)); 5.346 (*dd*, J = 9.5, 9.5, H–C(3″)); 5.079 (*dd*, J = 9.5, 9.8, H–C(4″)); 5.044 (*dd*, J = 12.3, 5.4, H–C(28)); 4.993 (*dd*, J = 9.8, 9.5, H–C(2″)); 4.976 (*dd*, J = 9.5, 0.9, H–C(25)); 4.302 (*dd*, J = −12.5, 4.4, H_a–C(6″)); 4.17 (br., H_a–C(2′)); 4.120 (*dd*, J = −12.5, 2.2, H_b–C(6″)); 3.930 (*d*, J = 4.2, OH–C(23)); 3.896 (*ddd*, J = 9.8, 4.4, 2.2, H–C(5″)); 3.710 (*dd*, J = 7.2, 0.9, H–C(21)); 3.688 (*s*, OH–C(21)); 3.62 (br., H_b–C(2′)); 3.55 (br., H_a–C(1′)); 3.465 (*dd*, J = 5.4, 3.1, H–C(27)); 3.24 (br., H_b–C(1′)); 3.100 (*s*, Me(37)); 3.040 (*ddd*, J = 10.1, 4.2, 2.0, H–C(23)); 2.333 (*m*, H–C(20)); 2.228 (*s*, Me(14)); 2.111 (*s*, Me(30)); 2.068–2.007 (5*s*, *ca.* 15 H, Me(36), 4 Ac); 1.811 (*m*, H–C(22)); 1.76 (*s*, Me(13)); 1.683 (*m*, H–C(26)); 1.69 (*m*, H–C(24)); 1.007 (*d*, J = 7.0, Me(32)); 0.881 (*d*, J = 7.0, Me(31)); 0.702 (*d*, J = 7.0, Me(33)); 0.211 (*d*, J = 7.0, Me(34)). ^{13}C-NMR ($CDCl_3$, ^{1}H-het. corr.): 143.9 (C(19)); 141.8 (C(29)); 137.2 (C(17)); 124.9 (C(18)); 116.7 (C(28)); 82.5 (C(1″)); 78.3 (C(27)); 77.3 (C(23)); 73.7 (C(25)); 73.7 (C(21)); 73.0 (C(3″)); 73.0 (C(5″)); 70.8 (C(2″)); 68.3 (C(4″)); 61.8 (C(6″)); 57.5 (C(37)); 44.2 (C(2′)); 38.8 (C(26)); 38.2 (C(20)); 37.2 (C(24)); 34.6 (C(1′)); 33.2 (C(22)); 21 (C(13), C(30), C(36), 4 *Me*COO); 17.5 (C(32)); 11.3 (C(31)); 10.4 (C(34)); 9.4 (C(33)); 7.8 (C(14)). ESI-MS: 1160 (6, M^+), 1128 (28, $[M-MeOH]^+$), 753 (18), 407 (100).

4. *3-{2-[N-(β-D-Glucopyranosyl)thioureido]ethylthio}rifamycin S* (**5**). A 0°, **4** (0.4 g, 0.35 mmol) was added to 20 mM KOH in MeOH. The mixture was allowed to react at 0° for 45 min, and then neutralized with 10% aq. citric acid soln. After evaporation, the residue was extracted in $CHCl_3$ and washed with cold brine. The org. phase was dried (Na_2SO_4) and evaporated and the residue submitted to CC ($CHCl_3$/MeOH 85:15→80:20). Yield 58%. UV/VIS ($c = 4 \cdot 10^{-5}$ M): 5.23 (3.54), 450 (3.60), 371 (3.58), 325 (4.42), 245 (4.53). ^{1}H-NMR (D_2O): 6.74 (br., H–C(18)); 6.57 (*d*, J = 10.9, H–C(17)); 6.30 (br., H–C(19)); 6.25 (*d*, J = 12.5, H–C(29)); 5.22 (*d*, J = 10.5, H–C(25)); 5.16 (*dd*, J = 12.5, 6.3, H–C(28)); 3.97–3.94 (*m*, *ca.* 3 H, H–C(21), H_a–C(2′), H–C(4″)); 3.88–3.84 (*m*, *ca.* 3 H, 2 H–C(6″), H_b–C(2′)); 3.69 (*m*, H–C(1″)); 3.59 (*m*, *ca.* 2 H, H–C(2″), superimposed at 3.57 (*m*, H–C(3″))); 3.48 (*m*, *ca.* 2 H, H–C(27), superimposed at 3.47 (*m*, H–C(5″))); 3.27–3.25 (br. *m*, 2 H–C(1′)); 3.24 (*d*, J = 10.6, H–C(23)); 3.13 (*s*, Me(37)); 2.50 (br. *m*, H–C(20)); 2.13 (*s*, Me(36), Me(14)); 2.07 (*s*, Me(30)); 1.98 (*m*, H–C(22)); 1.80 (*s*, Me(13)); 1.74 (br. *m*, H–C(24)); 1.60 (br., H–C(26)); 1.03 (*d*, J = 7.0, Me(32)); 0.99 (*d*, J = 7.0, Me(31)); 0.81 (*d*, J = 7.0, Me(33)); 0.24 (*d*, J = 7.0, Me(34)). ^{13}C-NMR (D_2O; ^{1}H-het. corr.): 143.7 (C(19), C(29)); 136.62 (C(17)); 126.52 (C(18)); 118.30 (C(28)); 78.38 (C(1″)); 78.24 (C(2″)); 77.82 (C(23)); 77.57 (C(4″)); 77.40 (C(3″)); 74.73 (C(25)); 73.18 (C(5″)); 70.36 (C(27)); 61.78 (C(6″), superimposed by C(21)); 57.42 (C(37)); 45.61 (C(2′)); 39.87 (C(26)); 38.58 (C(24)); 38.32 (C(20)); 33.51 (C(22)); 32.52 (C(1′)); 21.97 (C(13)); 21.16 (C(36)); 20.61 (C(30)); 18.74 (C(31)); 10.86 (C(32)); 10.17 (C(33)); 9.92 (C(34)); 8.87 (C(14)). ESI-MS: 990 (100, $[M-2]^+$), 395 (30).

5. *25-O-Deacetyl-3-{2-[N-(β-glucopyranosyl)thioureido]ethylthio}rifamycin S* (**6**). To **4** (0.5 g, 0.43 mmol) in abs. EtOH (15 ml) at 0°, 20% KOH in abs. EtOH (50 ml) was added. The mixture was allowed to react at 0° for 45 min and then worked up as described for **5** (CC ($CHCl_3$/MeOH 8:2)). Yield 37%. UV/VIS ($c = 4 \cdot 10^{-5}$ M): 534 (3.56), 380 (3.55), 330 (4.40), 250 (4.60). ^{1}H-NMR (D_2O): 6.591 (*dd*, J = 16.0, 10.8, H–C(18)); 6.443 (*d*, J = 10.8, H–C(17)); 6.288 (*dd*, J = 16.0, 7.2, H–C(19)); 6.190 (*d*, J = 12.3, H–C(29)); 5.149 (*dd*, J = 5.7, 12.3, H–C(28)); 4.060 (*d*, J = 5.7, H–C(27)); 3.963 (*m*, *ca.* 2 H, H–C(21), H_a–C(2′)); 3.93 (*dd*, J = −12.5, 2, H_a–C(6″)); 3.842 (*d*, J = 12.0, H–C(25)); 3.782 (*dd*, J = −12.5, 3.5, *ca.* 2 H, H_b–C(6″), superimposed by *m* (H_b–C(2′)); 3.598 (*dd*, J = 10.0, 10.0, *ca.* 2 H, H–C(4″), superimposed by *d* (J = 11.0, H–C(23)); 3.51–3.46 (*m*, *ca.* 4 H, H–C(1″), H–C(2″), H–C(3″), H–C(5″)); 3.25 (*m*, *ca.* 5 H, 2 H–C(1′), superimposed by *s* (Me(37))); 2.478 (*m*, H–C(20)); 2.080 (*s*, Me(30)); 2.032 (*s*, Me(14)); 2.002 (*m*, H–C(22)); 1.830 (*s*, Me(13)); 1.626 (*dd*, J = 10.5, 7.0, H–C(24)); 1.319 (br., H–C(26)); 1.051 (*d*, J = 7.0, Me(32)); 0.988 (*d*, J = 7.0, Me(31)); 0.694 (*d*, J = 7.0, Me(33)); 0.105 (br., Me(34)). ^{13}C-NMR (D_2O, ^{1}H-het. corr.): 142.7 (C(19)); 141.9 (C(29)); 136.0 (C(17)); 126.3 (C(18)); 119.0 (C(28)); 78.4 (C(27)); 78.0 (C(1″)); 77.2 (C(23)); 75.5 (C(21)); 72.8 (C(5″)); 70.9 (C(25)); 68.5 (C(2″), C(4″)); 60.4 (C(6″)); 57.5 (C(37)); 45.6 (C(2′)); 41.4 (C(26)); 38.5 (C(24)); 37.9 (C(20)); 33.4 (C(22)); 32.7 (C(1′)); 21.59 (C(13)); 21.52 (C(30)); 18.5 (C(31)); 11.6 (C(32)); 9.4 (C(34)); 9.3 (C(33)); 8.56 (C(14)). ESI-MS: 950 (33, M_r^+), 918 (100, $[M-MeOH]^+$), 670 (9).

6. *3-{2-[N-(2,3,4-Tri-O-acetyl-α-D-arabinopyranosyl)thioureido]ethylthio}rifamycin SV* (**7**). To **2** (1.0 g, 1.3 mmol) in anh. THF (20 ml), 2,3,4-tri-*O*-acetyl-α-D-arabinopyranosyl isothiocanate (1.0 g, 3.1 mmol) in anh. THF (2 ml) and 4-(dimethylamino)pyridine (30 mg) were added. The mixture was allowed to react at r.t. under stirring for 72 h. The solvent was evaporated and the residue submitted to CC ($CHCl_3$/MeOH 8:2). Yield 48%. UV/VIS ($c = 5 \cdot 10^{-5}$ M): 450 (3.97), 318 (4.08). ^{1}H-NMR (CDCl): 12.50 (*s*, OH–C(8)); 8.02 (*s*, NH–C(15)); 6.60 (*dd*, J = 11.3, 15, H–C(18)); 6.50 (*d*, J = 11.1, H–C(17)); 6.19 (*d*, J = 12.6, H–C(29)); 6.07 (*dd*, J = 5.0, 15.2, H–C(19)); 5.70 (br., H–C(1″)); 5.34 (br. *m*, H–C(3″)); 5.16–5.13 (*m*, H–C(28), H–C(4″)); 4.98 (*dd*, J = 8.7, H–C(2″)); 4.91 (*d*, J = 10.6, H–C(25)); 3.98 (*dd*, J = −13.0, < 2, H_b–C(5″)); 3.86 (*d*, J = −13.2, H_a–C(5″)); 3.77

(*m*, 2 H–C(1′)); 3.64 (*dd*, *J* = 6.8, H–C(27)); 3.58 (br., H–C(23)); 3.54 (*d*, *J* = 6.4, H–C(21)); 3.06 (*s*, Me(37)); 2.73 (*m*, H_a–C(2′)); 2.44 (br., H_b–C(2′)); 2.33 (br. *m*, H–C(20)); 2.25 (*s*, Me(14)); 2.16–2.02 (5*s*, Me(30), Me(36), 3 Ac); 1.82 (*s*, Me(13)); 1.70–1.54 (br., *ca.* 3 H, H–C(22), H–C(24), H–C(26)); 1.04 (*d*, *J* = 7.1, Me(32)); 0.91 (*d*, *J* = 7, Me(31)); 0.72 (*d*, *J* = 6.8, Me(33)); –0.29 (*d*, *J* = 6.9, Me(34)). ESI-MS: 1088 ([*M* – 2]$^-$).

7. *3-{2-[*N*-(2,3,4,Tri-*O*-acetyl-α-*D*-arabinopyranosyl)thioureido]ethylthio}rifamycin S* (**8**). A soln. of **7** (0.5 g) in AcOEt was treated with 33% aq. $K_3[Fe(CN)_6]$ soln. The org. phase was dried (Na_2SO_4) and evaporated and the residue purified by CC ($CHCl_3$/MeOH 9:1). Yield 90%. UV/VIS (*c* = 5·10^{-5} M): 443 (3.74), 318 (4.08). ^{1}H-NMR ($CDCl_3$): 12.663 (*s*, OH–C(8)); 8.394 (*s*, NH–C(15)); 6.556 (*dd*, *J* = 10.3, 15.3, H–C(18)); 6.339 (*d*, *J* = 10.6, H–C(17)); 6.035 (br., H–C(19)); 6.015 (br., H–C(29)); 5.826 (*d*, *J* = 9.5, NH–C(1″)); 5.30 (br., H–C(1″)); 5.184 (*dd*, *J* = 9.5, H–C(3″)); 5.113–5.091 (*m*, *ca.* 2 H, H–C(28), H–C(4″)); 5.017 (*dd*, *J* = 9.3, 9.5, H–C(2″)); 4.963 (*d*, *J* = 9.2, H–C(25)); 3.93 (*dd*, *J* = –13.0, 1.9, H–C(5″)); 3.75 (*d*, *J* = –13.0, H_a–C(5″)); 3.64–3.60 (*m*, *ca.* 2 H, H_b–C(2′), H–C(21)); 3.50 (*m*, H_a–C(2′)); 3.38 (*dd*, *J* = 4.6, H–C(27)); 3.15 (br. *m*, 2 H–C(1′)); 3.10 (*s*, Me(37)); 3.05 (br., H–C(23)); 2.28 (*s*, Me(14)); 2.25 (br., H–C(20)); 2.15 (*s*, Me(30)); 2.07–2.02 (4*s*, Me(36), 3 Ac); 1.81 (br., H–C(22)); 1.79 (*s*, Me(13)); 1.76–1.73 (br., *ca.* 2 H, H–C(24), H–C(26)); 0.99 (*d*, *J* = 7, Me(32)); 0.83 (*d*, *J* = 6.9, Me(31)); 0.69 (*d*, *J* = 6.8, Me(33)); 0.28 (*d*, *J* = 7, Me(34)). ^{13}C-NMR ($CDCl_3$; ^{1}H-het.corr.): 143.2 (C(29)); 141.9 (C(19)); 136.4 (C(17)); 125.3 (C(18)); 116.7 (C(28)); 80.9 (C(2″)); 78.4 (C(27)); 77.9 (C(23)); 74.0 (C(21)); 73.7 (C(25)); 71.2 (C(4″)); 69.8 (C(1″)); 68.4 (C(3″)); 65.4 (C(5″)); 57.3 (C(37)); 41.0 (C(2′)); 39.0 (C(26)); 38.3 (C(20)); 37.0 (C(24)); 36.1 (C(1′)); 32.8 (C(22)); 21.3 (C(13)); 20.8 (C(30), C(36), 3 *Me*COO); 17.6 (C(31)); 11.2 (C(32)); 10.7 (C(34)); 9.7 (C(33)); 7.8 (C(14)). ESI-MS: 1186 (100, [*M* –2]$^-$), 590 (31).

8. *3-{2-[*N*-(α-*D*-Arabinopyranosyl)thioureido]ethylthio}rifamycin S* (**9**). At r.t., 20 mM KOH in MeOH (30 ml) was added to **7** (0.4 g, 0.37 mmol). The mixture was allowed to react for 3 h. After neutralization with citric acid, the product was extracted with AcOEt. The org. phase was treated with 33% aq. $K_3[Fe(CN)_6]$ soln. and then dried (Na_2SO_4) and evaporated. The solid residue was submitted to CC ($CHCl_3$/MeOH 8:2). Yield 30%. UV/VIS (*c* = 5·10^{-5} M): 440 (3.45), 368 (3.58), 320 (4.04). ^{1}H-NMR (D_2O/(D_6)DMSO 9:1): 6.51 (br., H–C(18)); 6.32 (*d*, *J* = 11.3, H–C(17)); 6.06 (br., *ca.* 2 H, H–C(19), superimposed at 6.02 (*d*, *J* = 12.8, H–C(29))); 4.99 (*d*, *J* = 10.4, H–C(25)); 4.94 (*dd*, *J* = 6.3, 12.8, H–C(28)); 3.98 (*m*, H–C(4″)); 3.79–3.65 (br., *ca.* 4 H, H–C(2″) or H–C(3″) and 2 H–C(2′), superimposed at 3.66 (*d*, *J* = 13.1, H–C(21))); 3.56–3.51 (br. *m*, *ca.* 4 H, H–C(2″) or H–C(3″), H–C(1′), 2 H–C(5″)); 3.46 (*d*, *J* = 6.0, *ca.* 2 H, H–C(27), superimposed at 3.42 (br. *m*, H_b–C(1′))); 3.08 (br., H–C(1″)); 3.00 (*d*, *J* = 10.7, H–C(23)); 2.89 (*s*, Me(37)); 2.25 (br., H–C(20)); 2.10 (2*s*, Me(30), Me(36)); 1.56 (*s*, Me(13)); 1.32 (br., H–C(22)); 1.14 (br., H–C(26)); 0.99 (br., H–C(24)); 0.79 (*d*, *J* = 6.6, Me(32)); 0.76 (*d*, *J* = 6.4, Me(31)); 0.58 (*d*, *J* = 7.2, Me(33)); 0.15 (br., Me(34)). ESI-MS: 960 (100, [*M* – 2]$^-$), 772 (69).

9. *3-{2-[*N*-(α-*D*-Arabinopyranosyl)thioureido]ethylthio}-25-O-deacetylrifamycin S* (**10**). At r.t., 20 mM KOH in MeOH (30 ml) was added to **7** (0.4 g, 0.37 mmol). The mixture was allowed to react under stirring for 24 h and then worked up as described for **9**. Yield 30%. UV/VIS (*c* = 5·10^{-5} M): 445 (3.55), 373 (3.50), 323 (4.17). ^{1}H-NMR (D_2O): 6.65 (br., H–C(18)); 6.47 (*d*, *J* = 11.1, H–C(17)); 6.21 (br., H–C(19)); 6.21 (*d*, *J* = 12.6, H–C(29)); 5.11 (*dd*, *J* = 6.1, 12.5, H–C(28)); 3.97 (br., *ca.* 2 H, H–C(2″), superimposed at 3.95 (br., H–C(3″))); 3.88 (*d*, *J* = 9.2, *ca.* 2 H, H–C(21), superimposed at 3.86 (br., H_a–C(2′))); 3.82 (*d*, *J* = 12.6, H–C(25)); 3.78 (*d*, *J* = 10.0, H–C(27)); 3.73–3.63 (br., *ca.* 4 H, H_b–C(2′), H–C(4″), 2 H–C(5″)); 3.43 (*d*, *J* = 10.3, H–C(23)); 3.28 (br., H_a–C(1′)); 3.24 (br., *ca.* 5 H, H_b–C(1′), superimposed at 3.22 (br., H–C(1″)) and 3.19 (*s*, Me(37))); 2.40 (br., H–C(20)); 2.06 (*s*, Me(14)); 2.01 (*s*, Me(30)); 1.94 (br. *m*, H–C(22)); 1.74 (*s*, Me(13)); 1.54 (br., H–C(26)); 1.26 (br., H–C(24)); 0.98 (*d*, *J* = 6.8, Me(32)); 0.92 (*d*, *J* = 6.5, Me(31)); 0.64 (*d*, *J* = 6.8, Me(33)); 0.09 (br. *s*, Me(34)). ^{13}C-NMR (D_2O, ^{1}H-het. corr.): 142.5 (C(19)); 142.3 (C(29)); 126.2 (C(18)); 119.1 (C(28)); 78.6 (C(2″)); 77.9 (C(23)); 75.7 (C(21)); 74.0 (C(4″)); 71.0 (C(27)); 70.4 (C(3″)); 69.6 (C(1″)); 68.3 (C(25), C(5″)); 57.6 (C(7)); 45.9 (C(2′)); 41.5 (C(24)); 38.6 (C(26)); 38.0 (C(20)); 33.6 (C(22)); 32.8 (C(1′)); 21.9 (C(13)); 20.6 (C(30)); 18.6 (C(31)); 10.8 (C(32)); 9.8 (C(34)); 9.6 (C(33)); 8.7 (C(14)). ESI-MS: 920 (*M*$^-$).

10. *3-[2-(*D*-Saccharo-1,4-lacton-6-oylamino)ethylthio]rifamycin SV* (**11**). A soln. of DCC (2 g, 10 mmol) in anh. THF (20 ml) was added at 0°, under stirring, to D-glucaro-1,4-lactone monohydrate (1 g, 5.2 mmol) in anh. THF (30 ml). A soln. of **2** (1.1 g, 1.3 mmol) and 4-(dimethylamino)pyridine (30 mg) in anh. THF (20 ml) was added after 30 min. After 12 h, the mixture was filtered and the solvent evaporated. The solid residue was dissolved in AcOEt, the soln. washed with cold brine, dried (Na_2SO_4), and evaporated, and the residue purified by CC ($CHCl_3$/MeOH 8:2). Yield 49%. UV/VIS (*c* = 5·10^{-5} M): 450 (3.58), 320 (3.95). ^{1}H-NMR (D_2O): 7.36 (br., H–C(18)); 6.61 (*d*, *J* = 10.6, H–C(17)); 6.44 (*d*, *J* = 13.0, H–C(29)); 6.34 (*dd*, *J* = 10.9, 16.7, H–C(19)); 5.21 (*dd*, *J* = 12.5, 8.0, H–C(28)); 5.08 (*d*, *J* = 10.9, H–C(25)); 4.19 (br. *d*, H–C(2″)); 4.03 (*d*, *ca.* 2 H, H–C(5″), superim-

posed by *m* (H–C(3″))); 3.91 (*m*, H–C(4″)); 3.84 (*d*, J = 10.8, H–C(21)); 3.54 (*d*, J = 9.2, H–C(27)); 3.5 (very br., H_b–C(1′)); 3.36 (very br., H_a–C(1′)); 3.17 (br., H_a–C(2′)); 3.1 (br., *ca.* 2 H, H_b–C(2′), superimposed at 3.15 (*d*, J = 11.0, H–C(23))); 3.09 (*s*, Me(37)); 2.40 (*m*, H–C(20)); 2.17 (*s*, Me(14)); 2.13 (*s*, Me(30)); 2.10 (*s*, Me(36)); 2.02 (br., H–C(22)); 1.86 (*s*, Me(13)); 1.77 (*m*, H–C(24)); 1.37 (*m*, H–C(26)); 1.05 (*d*, J = 6.0, Me(31)); 1.03 (*d*, J = 7.2, Me(32)); 0.79 (*d*, J = 6.0, Me(33)); −0.20 (*d*, J = 6.0, Me(34)). ^{13}C-NMR (D_2O, ^{1}H-het. corr.): 137.0 (C(29)); 136.7 (C(17)); 136.3 (C(19)); 119.7 (C(18)); 78.7 (C(27)); 77.6 (C(23)); 75.4 (C(21)); 74.4 (C(4″)); 73.8 (C(2″)); 72.0 (C(3″), C(5″)); 57.5 (C(37)); 40.2 (C(1′)); 32.7 (C(24)); 32.5 (C(20)); 27.5 (C(2′)); 26.4 (C(26)); 26.0 (C(22)); 22.1 (C(36)); 19.6 (C(31)); 22.4 (C(13)); 15.0 (C(30)); 11.8 (C(32)); 10.6 (C(33)); 10.4 (C(34)); 8.6 (C(14)). ESI-MS: 963 (19, $[M-2]^+$), 947 (41, $[M-H_2O]^+$), 915 (100, $[M-H_2O-MeOH]^+$), 855 (17).

11. *3-{2-{[(3R,4S,5R)-3,4,5-Trihydroxycyclohex-1-en-1-ylcarbonyl]amino}ethylthio}rifamycin SV* (**12**). A soln. of shikimic acid (1 g, 5.7 mmol) and DCC (1.2 g, 5.7 mmol) in anh. THF (20 ml) at 0° was left under stirring for 45 min. Then **2** (4.4 g, 5.7 mmol) and 4-(dimethylamino)pyridine (70 mg) were added. After 16 h, the mixture was filtered and evaporated. The residue was treated with H_2O (20 ml), which was then washed with $CHCl_3$. The aq. phase was acidified with citric acid and extracted with $CHCl_3$. This latter org. phase was dried (Na_2SO_4) and evaporated to give pure **12**. Yield 74%. UV/VIS ($c = 5\cdot10^{-5}$ M): 455 (3.94), 322 (4.39), 275 (4.63). ^{1}H-NMR (D_2O): 7.281 (*dd*, J = 16.0, 11.0, H–C(18)); 6.592 (*d*, J = 11.0, H–C(17)); 6.397 (*d*, J = 8.6, H–C(29)); 6.331 (*dd*, J = 16.0, 7.7, H–C(19)); 6.068 (*m*, H–C(2″)); 5.124 (*dd*, J = 12.1, 8.6, H–C(28)); 4.995 (*d*, J = 10.9, H–C(25)); 4.020 (*m*, H–C(3″)); 3.947 (*m*, H_a–C(1′)); 3.876 (*m*, 1 H_b–C(1′)); 3.760 (*d*, J = 10.6, H–C(21)); 3.679 (*m*, H–C(5″)); 3.496 (*d*, J = 8.8, H–C(27)); 3.281 (*m*, H_a–C(2′)); 3.121 (*dd*, J = 10.6, 1.9, H–C(23)); 3.023 (*s*, Me(37)); 2.981 (*dd*, J = 9.2, 4.4, H–C(4″)); 2.751 (*m*, H_b–C(2′)); 2.604 (*dd*, J = −17.35, 5.5, H_a–C(6″)); 2.452 (*m*, H–C(20)); 2.106 (*s*, Me(30)); 2.086 (*s*, Me(14)); 2.046 (*s*, Me(36)); 1.801 (*s*, Me(13)); 1.532 (*m*, H_b–C(6″)); 1.288 (*m*, H–C(24)); 1.185 (*m*, H–C(26)); 1.000 (*d*, J = 6.9, Me(31)); 0.968 (*d*, J = 7.1, Me(32)); 0.765 (*d*, J = 6.8, Me(33)); −0.319 (*d*, J = 6.9, Me(34)). FAB-MS: 930 (4, $[M+1]^+$), 898 (6, $[M-MeOH]^+$), 490 (6).

12. *3-{2-{[(3R,4S,5R)-3,4,5-Trihydroxycyclohex-1-en-1-ylcarbonyl]amino}ethylthio}rifamycin S* (**13**). A soln. of **12** (0.5 g) in AcOEt was treated with 33% aq. $K_3[Fe(CN)_6]$ soln. The org. phase was dried (Na_2SO_4) and evaporated and the residue purified by CC ($CHCl_3$/MeOH 9:1). Yield 80%. UV/VIS ($c = 5\cdot10^{-5}$ M): 451 (3.49), 375 (3.45), 323 (4.19). ^{1}H-NMR ($CDCl_3$): 12.644 (*s*, OH–C(8)); 8.593 (*s*, NH–C(15)); 6.602 (br. H–C(2″)); 6.575 (*dd*, J = 15.1, 11.3, H–C(18)); 6.343 (*d*, J = 11.3, H–C(17)); 6.024 (*dd*, J = 15.1, 7.6, H–C(19)); 5.985 (*d*, J = 12.1, H–C(29)); 5.05–5.02 (*m*, *ca.* 2 H, H–C(28), H–C(25)); 4.401 (*m*, H–C(3″)); 3.99–3.95 (*m*, *ca.* 2 H, H–C(5″), H–C(21)); 3.678 (*d*, J = 9.8, *ca.* 2 H, H_a–C(2′), 1 OH); 3.597 (*dd*, J = 9.1, 4.2, H–C(4″)); 3.49–3.47 (*m*, *ca.* 2 H, H_a–C(1′), H_b–C(2′)); 3.429 (*m*, H–C(27)); 3.109 (*s*, Me(37)); 3.080 (*m*, *ca.* 2 H, H_b–C(1′), H–C(23)); 2.897 (*dd*, J = −17.3, 4.9, H_a–C(6″)); 2.269 (*s*, *ca.* 4 H, Me(36), H_b–C(6″)); 2.255 (*m*, H–C(20)); 2.074 (*s*, Me(30)); 1.78–1.70 (br. *m*, *ca.* 6 H, H–C(22), Me(13), H–C(26), H–C(24)); 0.981 (*d*, J = 6.9, Me(32)); 0.834 (*d*, J = 6.8, Me(31)); 0.703 (*d*, J = 6.7, Me(33)); 0.274 (*d*, J = 6.7, Me(34)). ^{13}C-NMR (CDCl, ^{1}H-het. corr.): 143.8 (C(29)); 141.3 (C(19)); 137.9 (C(17)); 131.3 (C(18), C(2″)); 103.8 (C(28)); 79.4 (C(23)); 75.8 (C(25)); 73.1 (C(5″)); 72.9 (C(4″)); 72.4 (C(27)); 67.3 (C(21)); 66.4 (C(3″)); 57.5 (C(37)); 41.8 (C(26)); 39.2 (C(2′)); 37.2 (C(24)); 36.7 (C(20)); 35.4 (C(1′)); 34.8 (C(22)); 32.6 (C(6″)); 22.2 (C(13)); 21.3 (C(36)); 21.0 (C(30)); 17.9 (C(31)); 17.1 (C(32)); 10.2 (C(33)); 10.0 (C(34)); 7.9 (C(14)). FAB-MS: 928 (8, $[M+1]^+$), 897 (24, $[M+1-MeO]^+$), 504 (44).

13. *3-{2-{[(3R,4S,5R)-5-Hydroxy-3,4-(isopropylidenedioxy)cyclohex-1-en-1-ylcarbonyl]amino}ethylthio}-rifamycin SV* (**14**). To a soln. of 5-hydroxy-3,4-(isopropylidenedioxy)cyclohex-1-ene-1-carboxylic acid (0.22 g, 1 mmol) in anh. THF (10 ml) at 0°, DCC (0.22 g, 1 mmol) was added. After 45 min, **2** (0.77 g, 1 mmol) and 4-(dimethylamino)pyridine (13 mg) were added. After 18 h at r.t., the mixture was filtered, the filtrate evaporated, and the residue redissolved in $CHCl_3$. The soln. was washed with 20% aq. citric acid soln. and then with brine, dried (Na_2SO_4), and evaporated, and the residue purified by CC ($CHCl_3$/MeOH 8:2). Yield 70%. UV/VIS ($c = 5\cdot10^{-5}$ M): 454 (4.06), 321 (4.31). ^{1}H-NMR (D_2O): 7.297 (*dd*, J = 15.9, 10.7, H–C(18)); 6.597 (*d*, J = 10.7, H–C(17)); 6.395 (*d*, J = 12.8, H–C(29)); 6.339 (*dd*, J = 16.1, 7.8, H–C(19)); 6.196 (*m*, H–C(2″)); 5.124 (*dd*, J = 12.8, 8.7, H–C(28)); 4.995 (*d*, J = 10.9, H–C(25)); 4.499 (*m*, H–C(3″)); 4.010 (*m*, H_a–C(1′)); 3.939 (*m*, H_b–C(1′)); 3.760 (*d*, J = 10.2, H–C(21)); 3.620 (*m*, H–C(4″)); 3.497 (*m*, H–C(27)); 3.354 (*m*, H–C(5″)); 3.271 (*m*, H_a–C(2′)); 3.123 (*dd*, J = 10.4, 2.0, H–C(23)); 3.022 (*s*, Me(37)); 2.738 (*m*, H_b–C(2′)); 2.464 (*m*, H–C(20)); 2.330 (*dd*, J = −16.95, 4.8, H_a–C(6″)); 2.084 (*s*, Me(14)); 2.032 (*s*, Me(36)); 1.961 (*m*, H–C(22)); 1.785 (*s*, Me(13)); 1.518 (*m*, H_b–C(6″)); 1.392 (*s*, Me_2C); 1.288 (*m*, H–C(24)); 1.187 (*m*, H–C(26)); 1.004 (*d*, J = 6.9, Me(31)); 0.970 (*d*, J = 7.1, Me(32)); 0.772 (*d*, J = 6.9, Me(33)); −0.132 (*d*, J = 6.9, Me(34)). FAB-MS: 970 (17, $[M+1]^+$), 938 (45, $[M+1-MeOH]^+$), 880 (24), 487 (58).

14. *3-{2-{[(3R,4S,5R)-5-Hydroxy-3,4-(isopropylidenedioxy)cyclohex-1-en-1-ylcarbonyl]amino}ethylthio}-rifamycin S* (**15**). A soln. of **14** (0.5 g) in AcOEt was treated with 33% aq. $K_3[Fe(CN)_6]$ soln. The org. phase was dried (Na_2SO_4) and evaporated and the residue purified by CC ($CHCl_3$/MeOH 8:2). Yield 70%. UV/VIS ($c = 5 \cdot 10^{-5}$ M): 462 (3.63), 376 (3.67), 324 (4.35). ^{1}H-NMR ($CDCl_3$): 12.637 (*s*, OH–C(8)); 8.458 (*s*, NH–C(15)); 6.662–6.618 (*m*, *ca.* 2 H, H–C(18), H–C(2″)); 6.362 (*d*, $J = 9.3$, H–C(17)); 6.075 (*dd*, $J = 15.7$, 7.5, H–C(19)); 5.998 (*d*, $J = 12.3$, H–C(29)); 5.039 (*dd*, $J = 12.3$, 5.3, H–C(28)); 5.005 (*d*, $J = 9.4$, H–C(25)); 4.732 (*m*, H–C(3″)); 4.078 (*dd*, $J = 8.9$, 4.6, H–C(4″)); 3.964 (*d*, $J = 4.1$, H–C(21)); 3.812 (*m*, H–C(5″)); 3.73–3.71 (*m*, *ca.* 2 H, H_a–C(1′), H_a–C(2′)); 3.518 (*m*, H_b–C(2′)); 3.462 (*m*, H–C(27)); 3.127 (*m*, H_b–C(1′)); 3.100 (*s*, Me(37)); 3.40 (*m*, H–C(23)); 2.822 (*dd*, $J = -17.0$, 4.5, H_a–C(6″)); 2.317 (*m*, H_b–C(6″)); 2.302 (*m*, H–C(20)); 2.276 (*s*, Me(14)); 2.073 (*s*, Me(30)); 2.067 (*s*, Me(36)); 1.75–1.68 (*m*, *ca.* 6 H, Me(13), H–C(22), H–C(24), H–C(26)); 1.444, 1.398 (2*s*, Me_2C); 1.001 (*d*, $J = 7.0$, Me(32)); 0.843 (*d*, $J = 6.9$, Me(31)); 0.696 (*d*, $J = 6.8$, Me(33)); 0.216 (*d*, $J = 7.0$, Me(34)). ^{13}C-NMR ($CDCl_3$, ^{1}H-het. corr.): 141.5 (C(29)); 141.0 (C(19)); 136.8 (C(17)); 128.6 (C(18), C(2″)); 116.4 (C(28)); 78.1 (C(27), C(4″)); 77.8 (C(23)); 73.8 (C(25)); 73.5 (C(5″)); 72.3 (C(3″)); 69.2 (C(21)); 57.7 (C(37)); 40.0 (C(2′)); 38.8 (C(26)); 38.5 (C(20)); 37.2 (C(24)); 34.7 (C(1′)); 32.9 (C(22)); 30.0 (C(6″)); 28.4, 25.9 (Me_2C); 21.4 (C(13)); 21.0 (C(36)); 21.0 (C(30)); 17.8 (C(31)); 11.3 (C(32)); 10.4 (C(34)); 9.8 (C(33)); 7.8 (C(14)). FAB-MS: 969 (18, $[M + 2]^+$), 938 (34, $[M + 2 - MeO]^+$), 487 (60).

15. *Activity Measurements.* The samples were dissolved in brain-heart-infusion (BHI) medium at a concentration 5 times higher than the highest concentration to be tested and then diluted with the same medium for the lower concentrations. The activity tests were run by diffusion in agar, incubating at 30° for 24 h and evaluating the growth of the microorganisms. Results: *Table.*

REFERENCES

[1] M. Brufani, 'The Ansamycins', in 'Topics in Antibiotic Chemistry', Ed. P.G. Sammes, Ellis Horwood Ltd., Chichester, 1977, Vol. 1, pp. 91–212; G.C. Lancini, W. Zanichelli, 'Structure-Activity Relationships in Rifamycins', in 'Structure-Activity Relationships among the Semisynthetic Antibiotics', Ed. D. Perlman, Academic Press, New York, 1977, pp. 531–596.

[2] N. Maggi, C.R. Pasqualucci, R. Ballotta, P. Sensi, *Chemotherapy* **1966,** *11,* 285.

[3] J. Leitich, V. Prelog, P. Sensi, *Experientia* **1967,** *23,* 505.

[4] E. Marchi, G. Mascellani, L. Montecchi, A.P. Venturini, M. Brufani, L. Cellai, *J. Med. Chem.* **1985,** *28,* 960.

[5] A. Sanfilippo, C. Della Bruna, L. Marsili, E. Morvillo, C.R. Pasqualucci, G. Schioppacassi, D. Ungheri, *J. Antibiot.* **1980,** *33,* 1193.

[6] A.K. Ganguly, Y.T. Liu, O.Z. Sarre, S. Szmulewicz, *J. Antibiot.* **1977,** *30,* 625.

[7] T. Kishi, M. Asai, M. Muroi, S. Arada, E. Mizuta, S. Terao, T. Miki, K. Mizuno, *Tetrahedron Lett.* **1969,** *2,* 91.

[8] W. Kump, H. Bickel, *Helv. Chim. Acta* **1973,** *56,* 2348.

[9] R.D. Guthrie, S.C. Smith, *Biochem. Prep.* **1971,** *13,* 1.

[10] N. Maggi, R. Pallanza, *Il Farmaco* **1967,** *12,* 307.

[11] W. Kump, H. Bickel, *Helv. Chim. Acta* **1973,** *56,* 2323.

[12] H.O.L. Fisher, G. Dangschat, *Helv. Chim. Acta* **1935,** *18,* 1206.

[13] L. Cellai, S. Cerrini, A.L. Segre, M. Brufani, W. Fedeli, A. Vaciago, *J. Org. Chem.* **1982,** *47,* 2652.

[14] W. Wehrli, W. Zimmermann, W. Kump, W. Tosch, W. Visher, O. Zak, *J. Antibiot.* **1987,** *40,* 1733.

[15] L. Cellai, Ital. Pat. 1995, application No. RM95 A000149.

[16] A. Bax, R.H. Griffey, B.L. Hawkins, *J. Magn. Reson.* **1983,** *55,* 301.

[17] C.W. Haigh, R.B. Mallion, *Progr. NMR Spectrosc.* **1980,** *13,* 303.