## Notes

# CHEMICAL MODIFICATIONS OF THE ALIPHATIC BRIDGE OF ANSAMYCINS 2. SYNTHESIS AND ACTIVITY OF 23-*EPI*-25-DEACETYLRIFAMYCIN S

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Studies on structure-activity relationships of rifamycins and related antibiotics established that four hydroxyls (one of which, O(1), may be in the quinonic form) and mainly responsible for inhibiting binding of these antibiotics to the bacterial enzyme DNA-dependent RNA polymerase (DDRP)[1,2]. In active derivatives these four groups all point out on the same side of the molecule and display well-defined geometrical relationships to one another[3]. Two of the four hydroxyls, O(1)H and O(2)H, are phenolic, and the other two, O(9)H and O(10)H, are alcoholic. It was hypothesized that the two alcoholic hydroxyls bind the enzyme through the formation of hydrogen bonds. In support of this hypothesis the O(9), O(10) mono- and diacetyl derivatives of rifamycin SV, in which the two alcoholic hydroxyl groups are esterified, and the corresponding C(21) and C(23) keto derivatives, in which they are oxidized to ketones, were found to be microbiologically inactive[4,5]. However, the presence of an acetyl or keto group could affect by itself the ansa-conformation and, hence, the activity[3]. Therefore, in order to establish if and to what extent the hydroxyl on C(23) is essential for binding to the enzyme, we prepared 23-*epi*-25-deacetylrifamycin S. In that compound, in fact, only the configuration of C(23) is inverted with respect to the natural antibiotic, and the hydroxyl bound to it is no longer available for the inhibiting interaction.

The inversion of C(23) was carried out according to Scheme 1. The acetylation of rifamycin S (**1**) was already described in the work of KUMP and BICKEL[5], in which the main product of acetylation was 21,23-diacetylrifamycin S. 21-*O*-Acetylrifamycin S (**2**)[5] was obtained by us as the main derivative by shortening the reaction time to 3 days (yield 60%). Then **2** was transformed into 21 - *O* - acetyl - 23 - deoxy - 23 - oxorifamycin S (**3**) using pyridine dichlorochromate (PDC) in DMF (yield 50%). $NaBH_4$ reduction of **3** followed by $K_3[Fe(CN)_6]$ oxidation afforded a mixture of 21-*O*-acetylrifamycinols **4a** (yield 36%) and **4b** (yield 5%) epimeric at C(23), the most abundant isomer **4a** displaying the natural configuration (*R*). The oxidation of **4b** by $MnO_2$ for 4 days afforded 21-*O*-acetyl-23-*epi*-rifamycin S (**5**) (yield 70%). Hydrolysis of **5** in aqueous 2 N NaOH - dioxane, 1 : 1, at 0~5°C for 4 hours gave the 23-*epi*-25-deacetylrifamycin S (**6**) (yield 50%). The yield of this last reaction was rather low owing to the instability of the ansa bridge in alkaline media.

Compounds **3**, **4** were purified by column chromatography on silica gel 60 Merck (40~63 $\mu$m), and compounds **5**, **6** by preparative TLC on silica gel plates (Merck 60 $F_{254}$ 20×20×0.2 cm) eluting with mixtures of benzene - EtOAc.

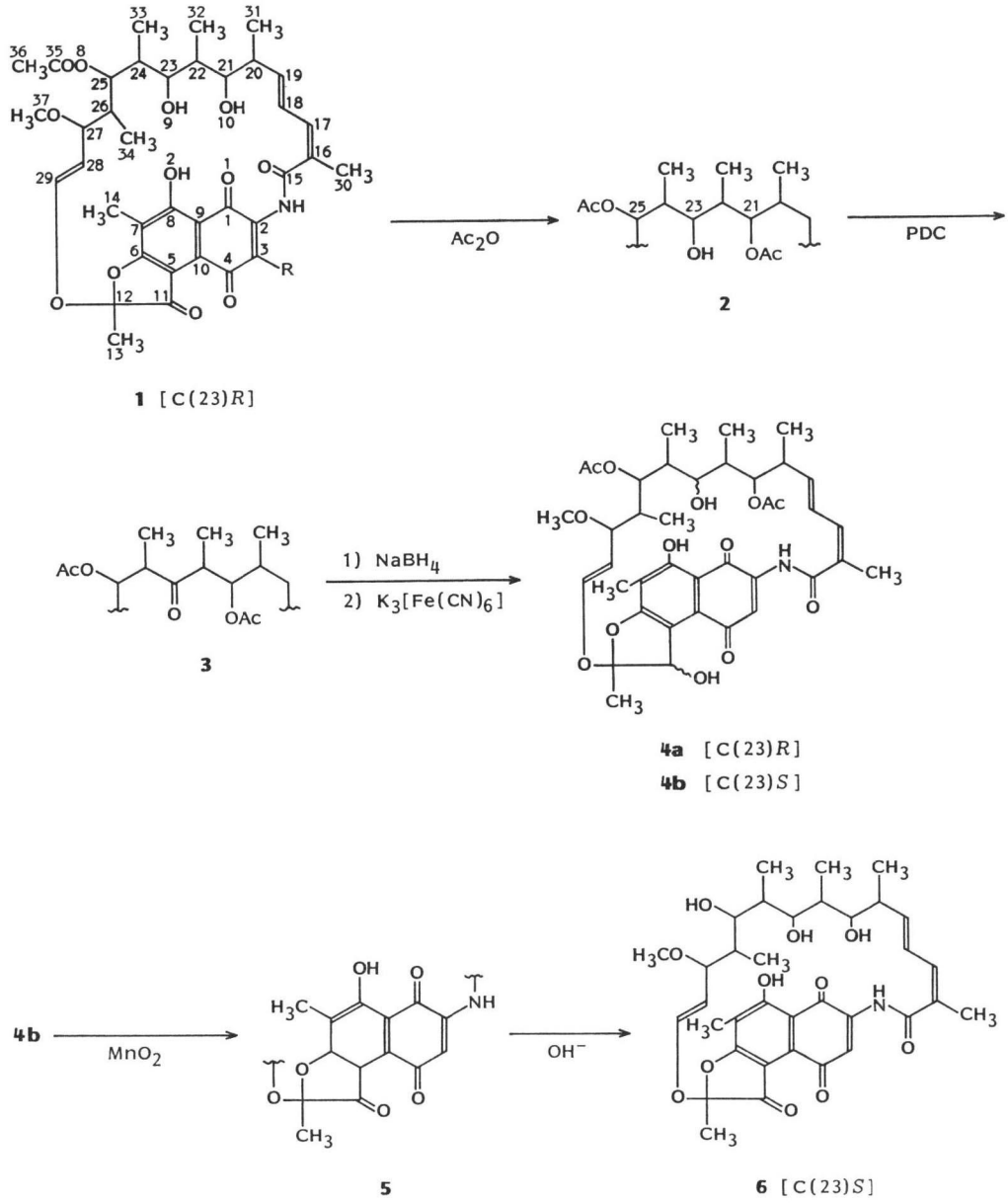
All compounds were crystallized from MeOH - $H_2O$ and gave indeterminate melting points indicating decomposition on heating. The elemental analyses were carried out for elements C, H, N and were in agreement with calculated values within 0.5% assuming that these compounds crystallize as monohydrate (Table 1).

A FAB-MS of **6** gave ($M^+ + 2$) 656.

The $^1H$ NMR spectra of **2**~**6** were registered at 200 MHz on a Brucker WP200 instrument.

All signals corresponding to the proposed formulas were found and assigned (Table 2). The corresponding vicinal coupling constants are rather similar in all compounds with the exception of those of H(22)-H(23) and H(23)-H(24), close to the center of inversion. The correlation of the vicinal coupling constants with an

Scheme 1.




**1** [C(23)*R*]

**2**

**3**

**4a** [C(23)*R*]

**4b** [C(23)*S*]

**5**

**6** [C(23)*S*]

Table 1. Elemental analyses for compounds **3**~**6** crystallized as monohydrate from MeOH - $H_2O$.

| Compound | Formula | MW | Calcd | | | Found | | |
|---|---|---|---|---|---|---|---|---|
| | | | C | H | N | C | H | N |
| **3** | $C_{39}H_{45}NO_{13}\cdot H_2O$ | 753.78 | 62.14 | 6.02 | 1.86 | 62.07 | 5.88 | 1.97 |
| **4a** | $C_{39}H_{49}NO_{13}\cdot H_2O$ | 757.83 | 61.81 | 6.52 | 1.85 | 61.45 | 6.08 | 1.97 |
| **4b** | $C_{39}H_{49}NO_{13}\cdot H_2O$ | 757.83 | 61.81 | 6.52 | 1.85 | 61.45 | 6.09 | 1.99 |
| **5** | $C_{39}H_{47}NO_{13}\cdot H_2O$ | 755.81 | 61.98 | 6.53 | 1.85 | 61.57 | 6.08 | 1.97 |
| **6** | $C_{35}H_{43}NO_{11}\cdot H_2O$ | 671.74 | 62.58 | 6.75 | 2.08 | 62.03 | 6.53 | 2.16 |

Table 2. $^1$H NMR data for **2**~**6** in $CDCl_3$ at 200 MHz, $\delta$ (ppm from $Me_4Si$), $J$ (Hz).

| 6 | 5 | 4b | 4a | 3 | 2 | | 2 | 3 | 4a | 4b | 5 | 6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8.41 | 8.04 | 8.24 | 8.30 | 8.16 | 8.05 | H-N$_{15}$ | | | | | | |
| | | | | | | C=O$_{16\ 30}$ | | | | | | |
| | | | | | | C-CH$_3$ (17) | 2.02 | | 2.01 | 1.93 | 2.04 | 2.02 |
| 6.25 | 6.05 | 6.04 | 6.08 | 6.13 | 6.06 | H-C$_{18}$ | | | | | | |
| (8.0) | (10.5) | (10.8) | (10.7) | (10.1) | (11.0) | | | | | | | |
| 6.10 | 6.25 | 6.18 | 6.23 | 6.09 | 6.23 | H-C$_{19}$ | | | | | | |
| (15.5) | (15.5) | (15.5) | (15.1) | (15.4) | (15.5) | | | | | | | |
| 5.74 | 5.84 | 5.86 | 5.85 | 5.78 | 5.85 | H-C$_{20\ 31}$ | | | | | | |
| (5.5) | (7.0) | (7.2) | (6.8) | (6.7) | (7.0) | | | | | | | |
| 2.25 | 2.55 | 2.56 | 2.50 | 2.23 | 2.46 | H-C-CH$_3$ (21) | 0.89 | 0.99 | 0.91 | 0.92 | 0.92 | 0.87 |
| (10.0) | (10.8) | (10.2) | (10.0) | (6.6) | (10.0) | | | | | | | |
| 3.17 | 4.92 | 4.86 | 5.08 | 5.21 | 5.06 | H-C-OH$_{22\ 32}$ | 1.80 | 1.89 | 1.92 | 2.00 | 1.83 | 4.32 |
| (3.0) | (2.0) | (2.0) | (2.0) | (5.6) | (2.5) | | | | | | | |
| 1.62 | 1.73 | 1.78 | 1.80 | 2.90 | 1.77 | H-C-CH$_3$ (23) | 1.02 | 0.92 | 1.04 | 0.96 | 0.99 | 0.93 |
| (6.5) | (9.5) | (8.1) | (2.4) | | (2.0) | | | | | | | |
| 3.33 | 3.28 | 3.24 | 2.87 | | 2.87 | H-C-OH$_{24\ 33}$ | 3.10 | | 3.31 | 2.12 | 2.47 | 4.08 |
| (1.0) | (2.0) | (1.0) | (10.3) | | (10.0) | | | | | | | |
| 1.29 | 1.96 | 2.05 | 2.05 | 2.53 | 1.98 | H-C-CH$_3$ (25) | 0.56 | 1.08 | 0.59 | 0.75 | 0.74 | 0.77 |
| (2.0) | (0) | (1.0) | (1) | (1.7) | (0.6) | | | | | | | |
| 2.94 | 4.64 | 4.73 | 5.01 | 5.09 | 4.97 | H-C-OAc$_{26\ 34}$ | 2.04 | 1.94 | 2.02 | 2.00 | 2.04 | |
| (10.0) | (10.8) | (10.6) | (10.3) | (10.4) | (10.2) | | | | | | | |
| 1.83 | 1.32 | 1.44 | 1.49 | 1.12 | 1.53 | H-C-CH$_3$ (27) | −0.11 | 0.04 | 0.03 | 0.02 | −0.12 | 0.31 |
| (4.0) | (2.5) | (2.0) | (3.0) | (1.9) | (2.9) | | | | | | | |
| 3.41 | 3.52 | 3.50 | 3.53 | 3.44 | 3.53 | H-C-OCH$_3$ (37) | 3.06 | 3.06 | 3.06 | 3.06 | 3.16 | 3.20 |
| (9.0) | (5.0) | (3.9) | (3.6) | (5.4) | (4.1) | | | | | | | |
| 5.14 | 5.09 | 5.03 | 5.02 | 5.10 | 5.06 | H-C$_{28}$ | | | | | | |
| (13.2) | (12.5) | (12.1) | (12.2) | (12.2) | (12.4) | | | | | | | |
| 6.32 | 6.12 | 5.96 | 5.94 | 6.03 | 6.05 | H-C$_{29}$ | | | | | | |
| | | | | | | O | | | | | | |
| | | | | | | $^{13}$CH$_3$ | 1.75 | 1.75 | 1.84 | 1.88 | 1.77 | 1.71 |
| | | | | | | $^{14}$CH$_3$ | 2.27 | 2.28 | 2.19 | 2.20 | 2.23 | 2.27 |
| | | | | | | $^{3}$CH | 7.70 | 7.84 | 7.80 | 7.82 | 7.88 | 7.80 |
| | | | | | | $^{2}$OH | 12.70 | | 12.66 | 12.67 | 12.73 | 12.50 |
| | | | | | | $^{11}$CH | | | 5.44 | 5.50 | | |
| | | | | | | $^{11}$COH | | | 5.41 | 5.42 | | |
| | | | | | | $J_{27,29}$ | 1.5 | 1.5 | 1.5 | 1.7 | 1.5 | |
| | | | | | | $J_{H-OH}$ (11 11) | | | 1.5 | 1 | | |
| | | | | | | $J_{H-(CH_3)}$ (30) | | 1.7 | 1.5 | 1.2 | | |
| | | | | | | $J_{H-OH}$ (23 23) | | | 3.8 | 4.0 | 4.0 | |

In parenthesis are indicated $J$ values.

optimized Karplus type equation[6] allowed us to derive the following dihedral angles: H(22)-H(23) ~50° to 60° and H(23)-H(24) ~−175° to 175° in **2**, **4a** and in 25-deacetylrifamycin S (**7**)[7]; H(22)-H(23) 140° to 150° and H(23)-H(24) ~80° in **4b**, **5** and **6**. The values of all other proton vicinal couplings (Table 2), show that the ansa chain has a preferential conformation similar to that found in other rifamycin derivatives[8].

Compound **6** was found to be inactive on isolated DDPR from *Escherichia coli* K12 (EC 2.7.7.6). Tests were performed according to

Table 3. Sensitivity of microorganisms ($10^4$ cells/ml) to **1**, **6** and **7**.

| Microorganism | MIC ($\mu$g/ml) | | |
|---|---|---|---|
| | **1** | **6** | **7** |
| *Escherichia coli* clinical isolate | >50 | >50 | >50 |
| *Staphylococcus aureus* FDA 209P | 0.005 | >50 | 0.05 |

standard procedures[9], using as enzyme concentration 10 nM, and antibiotic concentrations up to 100 nM. At the highest concentration **1** gave 90% inhibition, **7** gave about 50% inhibition, and **6** gave no inhibition.

A parallel test was also run on the activity of **6** *in vitro* in comparison with that of **1** and **7** (Table 3).

Since, the new semisynthetic rifamycin **6**, epimeric at C(23), proved to be inactive, and the NMR conformational analysis in solution did not indicate any other stereochemical variation in the ansa-chain that can explain the marked decrease of activity, it can be concluded that the availability of OH(23) is a requirement for efficient inactivating binding of the antibiotic to the bacterial enzyme.

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