X-RAY CRYSTAL STRUCTURE OF 4-DEOXY-3′-BROMOPYRIDO[1′,2′-1,2]IMIDAZO[5,4-c]RIFAMYCIN S

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This paper reports the determination of the X-ray molecular structure of 4-deoxy-3′-bromopyrido[1′,2′-1,2]imidazo[5,4-c]rifamycin S, carried out in order to unequivocally define the general structure of a new series of rifamycin SV derivatives, which are potent antibacterial agents, and are not absorbed at the gastroenteric level.

They have been prepared by Alfa Farmaceutici, Bologna, by condensing 2-aminopyridine derivatives to 3-bromorifamycin S. The solid state X-ray study has confirmed the structure proposed on the basis of ^{1}H NMR studies in solution. It has also shown that the newly formed pyridoimidazo system is in a mesomeric betaine form, the pyrido nitrogen being positively charged and the imidazo nitrogen being negatively charged.

This feature is believed responsible for the pharmacokinetic behavior of these new drugs, one of which, denoted either as rifamycin L 105 or rifaximin, is actually under clinical trial as a topical intestinal disinfectant.

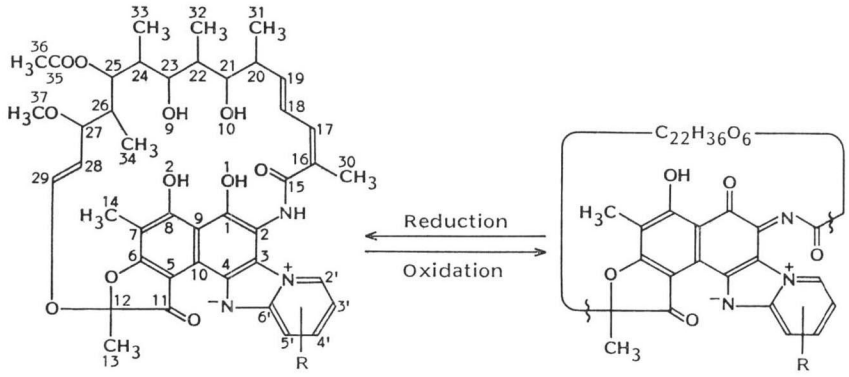
Rifamycin L 105 (Fig. 1, **1**) is a new antibacterial antibiotic, not absorbed at the gastroenteric level, currently under clinical evaluation as a topical intestinal disinfectant[1]. This new drug has been prepared, as one of a series of new rifamycin SV derivatives in the Research Laboratories of Alfa Farmaceutici, Bologna, by reacting 3-bromorifamycin S with 2-amino-4-picoline, and subsequently reducing with ascorbic acid. Previous chemical studies[2] on related compounds, and the ^{1}H NMR study of **1** and of the other similar derivatives[3] indicate that such a reaction leads to the formation of a pyridoimidazo system condensed at C_3-C_4 of the chromophore rings. The most probable general structure of the new derivatives appeared to be the one shown in Fig. 1. In order to verify this hypothesis, and to discover structural details which could help in understanding the peculiar pharmacokinetic properties of **1** an X-ray molecular structure of a related derivative, the 4-deoxy-3′-bromopyrido[1′,2′-1,2]imidazo[5,4-c]-rifamycin S (Fig. 1, **2**) was carried out.

The results of this structural study are reported in this paper.

^{1}H NMR Analysis

The spectrum of **2** in $CDCl_3$ has been assigned as shown in Table 1, by extensive spin decoupling, and resulted almost identical to that of **1** in the S form[3], therefore the two structures in solution must be also identical.

Fig. 1. General formulae of 4-deoxypyrido[1′,2′-1,2]imidazo[5,4-c]rifamycin S and SV derivatives.



(SV form)
Rifamycin L 105 (**1**) R = 4′-CH_3

(S form)
2 R = 3′-Br

Table 1. Assignment of the ^1H NMR spectrum of **2** in $CDCl_3$.

		N			
		C=O			
		C—CH_3 (16, 30)	2.27		
		HC (17)			
6.6		HC (18)			
		HC (19)			
	4.0	HC—CH_3 (20, 31)	0.58		
2.35					
	10.0	HC—OH (21)	3.46		
2.90					
	<1	HC—CH_3 (22, 32)	0.92		
1.40					
	2.0	HC—OH (23)	4.00	$^{13}CH_3$	1.75
2.85					
	10.0	HC—CH_3 (24, 33)	−0.03	$^{14}CH_3$	2.23
1.35					
	~1	HC—$OCOCH_3$ (25, 36)	2.03	^{1}OH	13.20
4.80					
	10.5	HC—CH_3 (26, 34)	−0.03	$^{2'}CH$	9.59
1.25					
	2.0	HC—OCH_3 (27, 37)	3.07	$^{4'}CH$	7.62
3.55					
	6.2	HC (28)		$^{5'}CH$	7.88
5.34					
	12.5	HC (29)		$J_{27,29}$	1.5
6.29				$J_{23,OH}$	5.0

Chemical shifts in ppm from TMS as 0, coupling constants in Hz. The spectra were run on the 200 MHz instrument of the CNR-Research Area of Rome.

X-Ray Crystal Structure

The molecular structure* of **2** is shown in Fig. 2. The atomic coordinates, bond distances and

* The observed amplitudes, calculated structure factors, and atomic thermal parameters are available on request from: The Director, Istituto di Strutturistica Chimica "G. Giacomello", C.N.R. C.P. 10-00016 Monterotondo Stazione, Roma, Italy.

Fig. 2. Molecular structure of 4-deoxy-3′-bromopyrido[1′,2′-1,2]imidazo[5,4-c]rifamycin S.

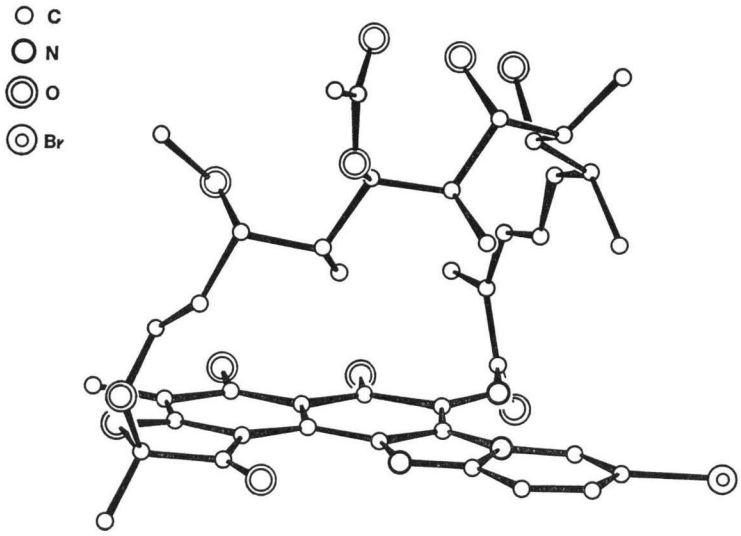


Table 2. Torsion angles (°) along the skeleton of the ansa-chain, calculated according to the convention of KLYNE and PRELOG[12].

	2	Rifamycin B[4]	Rifampicin[5]	3-Methoxycarbony-rifamycin S[6]	Rifamycin SV[7]
C(1)–C(2)–N–C(15)	−2	−32	−55	−141	167
C(2)–N–C(15)–C(16)	82	180	179	177	−171
N–C(15)–C(16)–C(17)	27	−43	−31	63	119
C(15)–C(16)–C(17)–C(18)	3	5	4	2	−3
C(16)–C(17)–C(18)–C(19)	179	168	155	169	−173
C(17)–C(18)–C(19)–C(20)	180	−175	−165	−179	−179
C(18)–C(19)–C(20)–C(21)	−116	−11	−19	−30	−52
C(19)–C(20)–C(21)–C(22)	180	170	169	180	176
C(20)–C(21)–C(22)–C(23)	−168	−179	−176	−178	−175
C(21)–C(22)–C(23)–C(24)	73	53	62	57	62
C(22)–C(23)–C(24)–C(25)	−148	174	165	−174	180
C(23)–C(24)–C(25)–C(26)	160	155	159	169	176
C(24)–C(25)–C(26)–C(27)	−178	174	153	180	174
C(25)–C(26)–C(27)–C(28)	−166	−170	−171	180	−179
C(26)–C(27)–C(28)–C(29)	−109	117	118	−110	−101
C(27)–C(28)–C(29)–O(5)	180	−168	−175	−176	−179
C(28)–C(29)–O(5)–C(12)	−115	49	65	−127	−118
C(29)–O(5)–C(12)–O(3)	−65	−79	−78	−52	−58

valence angles of the non-hydrogen atoms are deposited with the Crystallographic Data Centre, Cambridge, England.

The mean distances involving carbon atoms are 1.53 (3), 1.33 (2), 1.37 (4) Å for bonds $C(sp^3)$-$C(sp^3$ or $sp^2)$, $C(sp^2)$-$C(sp^2)$, and $C(sp^2)$-$C(sp^2)$ (aromatic), respectively; those involving hetero-atoms are 1.37 (4), 1.25 (1), 1.41 (5), 1.21 (2), and 1.88 (1) Å for bonds C–N, C=N, C–O, C=O, and C–Br, respectively. The mean values of valence angles are 110 (3), 120 (6), 118 (12), and 115 (6)° around atoms $C(sp^3)$, $C(sp^2)$, N, and O, respectively.

The conformation of the *ansa*-chain is described by the torsion angles reported in Table 2, together with those of other active rifamycins, such as rifamycin B[4], rifampicin[5], 3-methoxycarbonylrifamycin S[6], and rifamycin SV[7].

As found in the solid state, the conformation of the *ansa*-chain from C_{12} to C_{17} in **2** is comparable with those mentioned above. It is also similar to those found in solution for many other active ansamycins[8], particularly the three-dimensional arrangement of the four oxygen atoms O_1, O_2, O_9, and O_{10}. This arrangement is the feature assumed to be mainly responsible for the compounds' activity[9]. A different conformation is observed instead along C_2–N_1–C_{15}–C_{16}. Indeed the lack of the hydrogen atom on N_1 and the formation of the double bond between C_2 and N_1, as found in **2**, impose different conformational constraints on this junction of the *ansa*-chain.

The mean plane of the *ansa*-chain makes an angle of 123° with that of the chromophore ring. This distances between O_1, O_2, O_9, O_{10} are $O_1 \ldots O_2$=2.54, $O_1 \ldots O_9$=8.94, $O_1 \ldots O_{10}$=7.47, $O_2 \ldots O_9$=9.58, $O_2 \ldots O_{10}$=8.58, $O_9 \ldots O_{10}$=2.63 Å. The C_{23}-O_9 and C_{21}-O_{10} bonds are nearly parallel to the plane of the chromophore nucleus.

The crystal structure shows that the naphthoquinone nucleus and the newly formed pyrido-imidazolic ring system are coplanar. The root mean square distance of the seventeen constituent atoms from the plane through the entire chromophore system is 0.04 Å, with the maximum deviations occurring at C_3 (−0.04 Å) and at $C_{2'}$ (0.06 Å). In particular the geometry at $N_{1'}$ is quite planar, with $N_{1'}$ lying only 0.03 (1) Å outside the plane of its substituents.

The coplanarity of the atoms and the bond lengths clearly indicate that there is a large contribution by a mesomeric betaine structure, which suggests that a formal positive charge can be assigned to $N_{1'}$ and a negative charge to $N_{7'}$.

As a whole the molecular structure observed in the solid state* is stabilized by three intramolecular hydrogen bonds between $O_1 \ldots O_2$, (2.54 Å), $O_8 \ldots O_9$ (2.69 Å), $O_9 \ldots O_{10}$ (2.62 Å), in which the donors are O_2, O_9 and O_{10}, respectively. This hydrogen-bonding scheme has already been found both in the solid state[6] and in organic solutions for many rifamycin S and SV derivatives[9].

Experimental

Crude **2** was purified on a Silica Gel 60 column (40~63 μm) eluting with a mixture of $CHCl_3$ - MeOH, 95: 5. Well shaped red crystals of **2** were then obtained from a H_2O - MeOH solution. A crystal of dimensions 0.15×0.4×0.4 mm was used for the measurement of the cell constants and data collection. Crystal data: $C_{42}H_{46}N_3O_{11}Br \cdot 2H_2O$, monoclinic $P2_1$, a=9.581 (8), b=14.508 (8), c=16.752 (9) Å, β=97.31 (9)°, U=2310 Å^3, Z=2, D_c=1.27 gcm^{-3}, μ=10.1 cm^{-1}, MoKα radiation.

The intensities of 5281 reflections with $2\theta \leq 54°$ were measured using monochromatic MoKα radiation (λ=0.71069 Å) on a Syntex $P2_1$ diffractometer, in the ω-scan technique, with a variable scan rate between 0.5 and 29.3° minute^{-1}, (depending on the intensity), a scan range of 0.9°, and a background to peak ratio of 0.5. The intensities were corrected for Lorentz and polarization effects, but no correction was made for absorption. During the data collection four standard reflections were monitored every 100 measurements in order to check crystal alignment and stability. Of the 5281 independent reflections collected, 3163 had intensity $\geq 2\sigma(I)$ and were used in the structure determination. The crystal structure was solved by conventional heavy-atom methods. The atomic coordinates and anisotropic thermal parameters were refined by least-squares calculations in block-diagonal (9×9) approximation, to an R

* Two water molecules of crystallization, O_{12} and O_{13}, are linked by a hydrogen bond of 2.74 Å. In addition O_{12} acts as donor to O_{10} (2.87 Å) in the same asymmetric unit and to O_{11} (2.82 Å) of a screw-axis related molecule; O_{13} is donor to O_{12} and to O_2 (2.91 Å) of a third screw-axis related molecule.

value of 0.13.

At this stage the oxygen atoms of the water molecules of crystallization and the hydrogen atoms of **2** were found from difference Fourier syntheses. Refinement was brought to completion keeping the positions of the hydrogen atoms fixed with B values equal to that of the carrier atoms. The final reliability indices are R=0.089 and Rw=0.119. During refinement the quantity minimized was $\sum w|Fo-Fc|^2$, and the weight assigned to each reflection was $w=(a+|Fo|+b|Fo|^2)^{-1}$ with a=10.1 and b=0.007. The atomic scattering factors were taken from the International Tables for X-ray Crystallography[10]. All the calculations were carried out on the HP-1000 computer of the CNR Research Area of Rome, using a set of programs developed in this Institute[11].

Conclusions

The similarity in chemical composition and structure of the pyridoimidazo rifamycin S and SV derivatives[1], and the similarity of their ^{1}H NMR spectra[3], support the assumption that the main features of the molecular structure determined in the crystal on **2** are representative also of those of **1** and of the other related derivatives; that is, all must display analogous betaine structures.

This particular feature is believed to be the main factor determining the pharmacokinetic behavior of these drugs. In fact, the presence of the phenolic hydroxyls on C_1 and C_8, and the presence of the opposite charges on N_2 and N_3 make these molecules ionized at all pH values of the gastroenteric tract, and thus prevent their absorption, which is supposed to take place by a passive mechanism.

Acknowledgment

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SYNTHESES OF 23-DEOXY-23-*N*-ETHYL-23-(2-FLUORO-, 2,2-DIFLUORO-, AND 2,2,2-TRIFLUOROETHYL)AMINO DERIVATIVES OF MYCAMINOSYL TYLONOLIDE AND 4′-DEOXYMYCAMINOSYL TYLONOLIDE

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Derivatives of mycaminosyl tylonolide (**1**) and 4′-deoxymycaminosyl tylonolide (**2**) containing *N*-ethyl-2-fluoro-, 2,2-difluoro- and 2,2,2-trifluoroethylamino groups at their C-23 have been prepared by treating 23-deoxy-23-ethylaminomycaminosyl tylonolide diethyl acetal (**14**) and its 4′-deoxy analog **15** with 2-fluoro-, 2,2-difluoro- and 2,2,2-trifluoroethyl trifluoromethanesulfonates. The relationship between the antibacterial activity and the numbers of the fluorine atoms introduced in the final products is discussed.

23-Dialkylamino-23-deoxymycaminosyl tylonolides and their 4′-deoxy analogs[1] were found to show strong antibacterial activities against Gram-negative as well as Gram-positive bacteria. This remarkable extension of antibacterial spectrum may be due to the presence of a new basic nitrogen at C-23. This paper describes the effect on the antibacterial activity by conversion of the 23-diethylamino group of 23-deoxy-23-diethylaminomycaminosyl tylonolide (**3**) and its 4′-deoxy analog **4** into fluorine-containing diethylamino groups. Since fluorine is the most strongly electron-withdrawing atom with only slight increase in atomic volume in comparison to that of hydrogen, the replacement of one of the ethyl groups of the 23-diethylamino group of the compounds **3** and **4** with a fluorine-containing ethyl group will decrease the basicity of the nitrogen atoms at C-23 in comparison to that of **3** and **4** without significant change in the group volume at C-23. Thus, such fluorine-containing compounds are of interest to the study of the basicity (at C-23)-activity relationship.

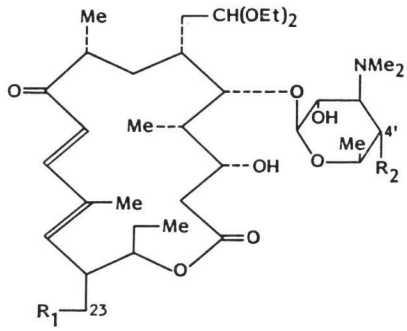
We commenced the experiment to prepare 23-deoxy-23-*N*-ethyl-23-(2,2,2-trifluoroethylamino)-mycaminosyl tylonolide diethyl acetal (**22**) by treating 23-deoxy-23-iodomycaminosyl tylonolide diethyl acetal[2] (**12**) with *N*-ethyl-2,2,2-trifluoroethylamine (**8**), a procedure being routinely used[1] in the syntheses of 23-dialkylamino-23-deoxy derivatives of mycaminosyl tylonolide (**1**) and its 4′-deoxy analog **2**. The reagent **8** was prepared by reduction of *N*-ethyltrifluoroacetamide (**7**) according to KANO *et al.*[3]

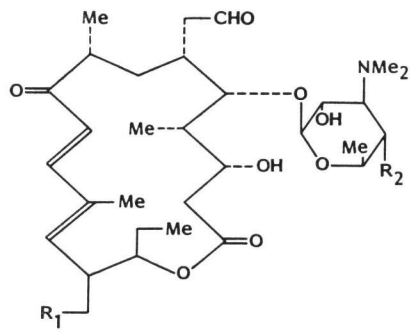
$$EtNH_2 \rightarrow Et(CRF_2CO)NH \rightarrow Et(CRF_2CH_2)NH$$

R=H **5** R=H **6**
R=F **7** R=F **8**

(**8** could also be prepared from **7** by the action of lithium aluminium hydride in ether in a similar yield). However, the reaction of **8** with **12** did not proceed at all only recovering the starting material **12**. Reaction of **12** with *N*-ethyl-2,2-difluoroethylamine (**6**) was next tried. The reagent **6** was prepared[3] likewise from *N*-ethyldifluoroacetamide (**5**), which in turn, was prepared by coupling ethylamine and

Chart 1.





	R_1	R_2
12	I	OH
13	I	H
14	NHEt	OH
15	NHEt	H
16	$NEt(CH_2CH_2F)$	H
18	$NEt(CH_2CHF_2)$	OH
20	$NEt(CH_2CHF_2)$	H
22	$NEt(CH_2CF_3)$	OH
24	$NEt(CH_2CF_3)$	H

	R_1	R_2
1	OH	OH
2	OH	H
3	NEt_2	OH
4	NEt_2	H
17	$NEt(CH_2CH_2F)$	H
19	$NEt(CH_2CHF_2)$	OH
21	$NEt(CH_2CHF_2)$	H
23	$NEt(CH_2CF_3)$	OH
25	$NEt(CH_2CF_3)$	H

$CH_2FCH_2OH \rightarrow CH_2FCH_2OSO_2CF_3$ **9**
$CHF_2CH_2OH \rightarrow CHF_2CH_2OSO_2CF_3$ **10**
$CF_3CH_2OH \rightarrow CF_3CH_2OSO_2CF_3$ **11**

difluoroacetic acid by use[4] of 2,2′-dibenzothiazolyl disulfide and triphenylphosphine (see Experimental). However, the reagent again did not react with **12**. The lack of reactivity is attributed to the remarkably decreased basicity of the reagents **6** and **8** caused by the fluorine atoms in comparison to diethylamine.

We, therefore, changed the synthetic pathway to attach the fluorine-containing ethyl groups to the nitrogens at C-23 of 23-deoxy-23-ethylaminomycaminosyl tylonolide diethyl acetal (**14**) and its 4′-deoxy analog **15**, both of the acetals being readily prepared from **12** and its 4′-deoxy analog **13** and ethylamine. The *N*-alkylations were carried out by use of 2,2,2-trifluoroethyl trifluoromethanesulfonate[5] (**11**), 2,2-difluoroethyl trifluoromethanesulfonate (**10**), and 2-fluoroethyl trifluoromethanesulfonate (**9**), all of them being prepared from the corresponding fluorine-containing alcohols and trifluoromethanesulfonyl chloride. *N*-(2-Fluoroethyl)-derivatization of **15** with **9** was carried out in the presence of triethylamine in benzene at room temperature to give the 23-*N*-(2-fluoroethyl) derivative (**16**). The *N*-(2,2-difluoroethyl)-derivatizations of **14** and **15** also readily proceeded to give **18** and **20**. However, *N*-(2,2,2-trifluoroethyl)-derivatization of **14** and **15** required a high temperature to give **22** and **24** (120°C for 1 ~ 2 hours in a pressure bottle; an improved procedure was also shown; see Experimental). Acidic hydrolysis of the resulting acetals (**16**, **18**, **20**, **22** and **24**) gave the final products (**17**, **19**, **21**, **23** and **25**).

The antibacterial spectra of the above compounds are shown in Table 1 with those of **3** and **4**. The results show that, against Gram-positive bacteria, there is not much difference in activity among the compounds including **3** and **4**, however, against Gram-negative bacteria and *Pseudomonas*, a significant gradual decrease in activity is observed with increase of the number of fluorine atoms. Thus, it is revealed that, for the latter group of bacteria, fluorination of the β-position of one of the

Table 1. Antibacterial spectra of the products synthesized, **3** and **4** (μg/ml).

Test organisms*	Compound Substituent at C-23	**3** NEt_2	**19** $NEt(CH_2CHF_2)$	**23** $NEt(CH_2CF_3)$	**4** NEt_2	**17** $NEt(CH_2CH_2F)$	**21** $NEt(CH_2CHF_2)$	**25** $NEt(CH_2CF_3)$
Staphylococcus aureus 193		0.78	<0.2	0.39	<0.2	0.39	<0.2	<0.2
〃	EMf**	>100	>100	100	12.5	100	100	100
〃	209P	0.78	0.39	0.39	<0.2	<0.2	<0.2	<0.2
〃	MS 9610	>100	>100	>100	>100	>100	>100	>100
Micrococcus luteus PCI 1001		<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Escherichia coli NIHJ		1.56	12.5	50	1.56	6.25	25	12.5
〃 K-12		3.12	25	50	3.12	6.25	12.5	12.5
〃 〃	ML 1629	6.25	>100	>100	3.12	12.5	50	100
〃 〃	ML 1410 R81	12.5	>100	>100	6.25	25	100	100
〃 〃	LA 290 R55	3.12	3.12	6.25	0.78	1.56	3.12	6.25
Klebsiella pneumoniae PCI 602		1.56	6.25	6.25	1.56	1.56	3.12	3.12
Salmonella enteritidis 1891		3.12	3.12	6.25	0.78	1.56	3.12	3.12
S. typhi T-63		3.12	50	>100	3.12	12.5	50	100
Proteus vulgaris OX-19		1.56	50	50	1.56	6.25	25	25
P. aeruginosa A3		12.5	25	50	12.5	50	50	50

* Agar dilution streak method (nutrient agar 37°C, 17 hours).

** Erythromycin-resistant strain.

diethylamino groups of **3** and **4** causes decrease in the antibacterial activity with decrease in basicity of the nitrogen at C-23.

Experimental

General

^{1}H NMR spectra were recorded at 90 MHz with a Varian EM-390 spectrometer, or at 250 MHz in the FT mode with a Bruker WM 250 spectrometer. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. UV spectrum was recorded with a Hitachi 200-10 spectrometer. Melting points were determined with a Kofler block and are uncorrected. Mass spectra were recorded with a Hitachi M-80 spectrometer. Thin-layer chromatography (TLC) were performed on Kieselgel 60 F-254 silica gel with detection by spraying with sulfuric acid, followed by slight heating. Column chromatography was performed on Kieselgel 60, 230~400 mesh (E. Merck).

N-Ethyldifluoroacetamide (**5**)

To a mixture of difluoroacetic acid (7 g), 2,2'-dibenzothiazolyl disulfide (25.4 g), and triphenylphosphine (20 g) in dichloromethane-*N*,*N*-dimethylformamide (1:1, 700 ml) were added 0.95 M ethylamine in acetonitrile (90 ml) and triethylamine (7.7 ml) and the solution was kept at room temp for 5 hours. The solution was washed with aq 2% hydrochloric acid, aq 2% sodium hydroxide, water, dried over sodium sulfate, and concentrated. The residue was distilled (4 Torr., bath temp 120°C) to give a syrup of **5**, 2.72 g (30%) (Ref[6] bp_3 60°C); ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, CH_3CH_2), 3.42 (quintet, 2H, CH_3CH_2NH), 5.91 (t, 1H, $^2J_{H,F}$=55 Hz, CF_2HCO).

N-Ethyl-2,2-difluoroethylamine (**6**)

Compound **5** (2.72 g) was treated with sodium borohydride (2.5 g) and titanium (IV) chloride (6.3 g) similarly as described for **8** to give a liquid of **6**, 0.5 g (21%), bp 40°C; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, CH_3CH_2), 2.73 (q, 2H, CH_3CH_2), 2.98 (dt, 2H, CHF_2CH_2), 5.86 (tt, 1H, CHF_2CH_2); $^3J_{H-1,H-2}$ =4 Hz, $^3J_{H-1,F}$=15 Hz, $^2J_{H-2,F}$=57 Hz.

N-Ethyltrifluoroacetamide (**7**)

To an ice-cold solution of trifluoroacetic anhydride (15 g) in dry ether (75 ml) was gently introduced ethylamine gas for 10 minutes. The weakly basic solution was concentrated. The residual syrup was distilled (bp_{15}, bath temp 60°C) to give a syrup of **7**, 8.0 g (80%); ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, CH_3CH_2NH), 3.43 (quintet, 2H, J=7 Hz, CH_3CH_2NH), 6~7 (br, 1H, NH).

N-Ethyl-2,2,2-trifluoroethylamine (**8**)

To a suspension of sodium borohydride (12 g) in dry 1,2-dimethoxyethane (330 ml) was gradually added titanium (IV) chloride (29 g). To the resulting blue solution, a solution of **7** (14.4 g) in 1,2-dimethoxyethane (100 ml) was added, and the mixture was stirred at room temp for 12 hours. Addition of water (1 liter) was followed by aq 28% ammonia (50 ml) and the reaction mixture was extracted with benzene. The extracts combined were treated with 0.5 M hydrochloric acid and the aq acidic layer separated was concentrated. To the concentrate (~15 ml), potassium hydroxide was carefully added and the mixture was distilled under atmospheric pressure to give a liquid of **8**, 3.9 g (30%), bp 66~68°C; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, J=7 Hz, CH_3CH_2), 2.73 (q, 2H, J=7 Hz, CH_3CH_2), 3.13 (q, 2H, $^3J_{H,F}$=9 Hz, CF_3CH_2).

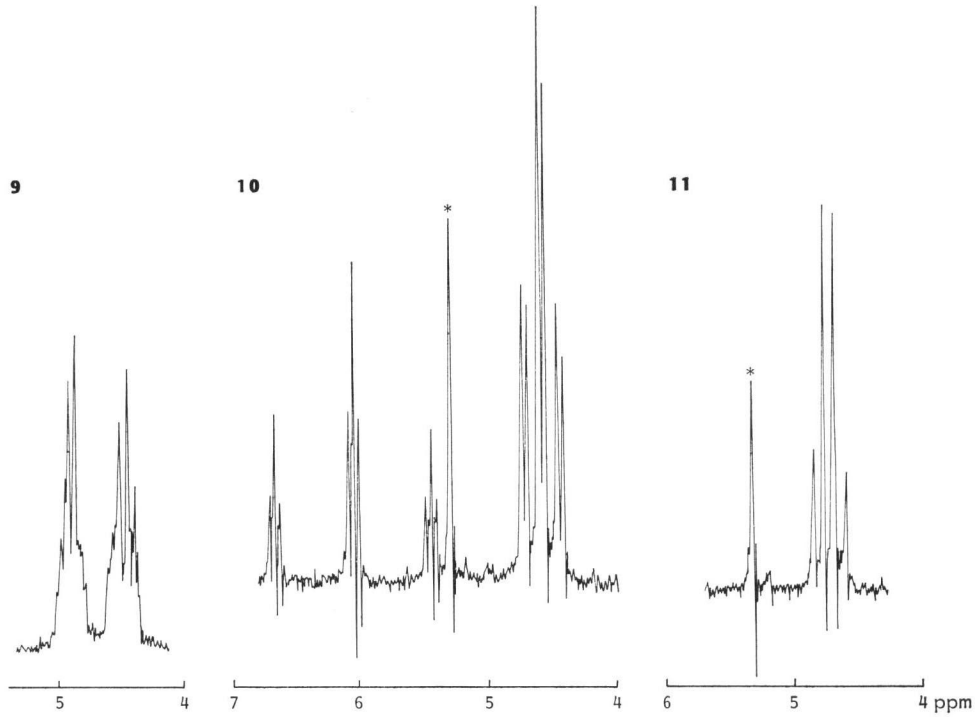
2,2-Difluoroethanol

Prepared from difluoroacetic acid according to HENNE and PELLEY[7], bp 95.5~96°C.

2-Fluoroethyl Trifluoromethanesulfonate (**9**)

To a cold solution (−30°C) of trifluoromethanesulfonyl chloride (15 g) in dichloromethane (15 ml) were added 2-fluoroethanol (2.9 g) and triethylamine (4.6 g), dropwise, in this order, and the mixture was stirred at 0°C for 30 minutes. Cold aq 5% hydrochloric acid was added untill strongly acidic, and the organic layer separated was dried over sodium sulfate. Evaporation followed by distillation (~12 Torr., bath temp 70°C) gave a liquid of **9**, 0.5 g.

Fig. 1. ^{1}H NMR spectra of **9**, **10** and **11** in $CDCl_3$ (* indicates the signals of CH_2Cl_2 contaminated).



2,2-Difluoroethyl Trifluoromethanesulfonate (**10**)

This was prepared from 2,2-difluoroethanol (2 g of the 66% solution in ether), trifluoromethanesulfonyl chloride (5 g) in dichloromethane (5 ml), and triethylamine (3.3 g) in a manner as described for **9**, 1.66 g (42%), bp 104~106°C.

2,2,2-Trifluoroethyl Trifluoromethanesulfonate (**11**)

2,2,2-Trifluoroethanol (3.3 g) was treated trifluoromethanesulfonyl chloride (5 g) and triethylamine (3.3 g) in a manner as described for **9**, and the fraction boiling at 94°C was isolated (3.58 g). The liquid was proved to be a ~85% solution of **11** containing dichloromethane (checked by the ^{1}H NMR spectrum), Ref[5] bp_{740} 89~91°C (prepared from CF_3SO_2F and CF_3CH_2OH).

23-Deoxy-23-ethylaminomycaminosyl Tylonolide Diethyl Acetal (**14**)

To a solution of **12**[2] (110 mg) in dry acetonitrile (2.2 ml) was added 1.55 M ethylamine in acetonitrile (1.1 ml) and the solution was heated at 80°C for 5 hours. Concentration gave a residue, that was dissolved in chloroform, and the solution was washed with satd aq sodium hydrogencarbonate and satd aq sodium sulfate, dried over sodium sulfate and concentrated. Column chromatography of the concentrate with chloroform - methanol - 28% aq ammonia (15: 1: 0.1) gave a solid of **14**, 71.4 mg (73%); $[\alpha]_D^{23}$ +53° (*c* 1, $CHCl_3$); TLC Rf 0.35 (*cf.* **12**, 0.57, with the above solvent system); UV λ_{max}^{MeOH} nm (ε) 281 (22,000); ^{1}H NMR ($CDCl_3$) δ 4.33 (d, 1H, H-1′), 5.70 (d, 1H, H-13), 6.35 (d, 1H, H-10), 7.33 (d, 1H, H-11).

Anal Calcd for $C_{37}H_{66}N_2O_{10}$: C 63.58, H 9.52, N 4.01.
Found: C 63.33, H 9.41, N 3.98.

4′,23-Dideoxy-23-ethylaminomycaminosyl Tylonolide Diethyl Acetal (**15**)

4′,23-Dideoxy-23-iodomycaminosyl tylonolide diethyl acetal[2] (**13**, 4.4 g), was treated in a manner as described for **14** to give a solid of **15**, 2.55 g (66%), $[\alpha]_D^{23}$ +39° (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$) δ 4.30 (d, 1H, H-1′), 5.70 (d, 1H, H-13), 6.35 (d, 1H, H-10), 7.32 (d, 1H, H-11).

Anal Calcd for $C_{37}H_{66}N_2O_9 \cdot \frac{1}{2}H_2O$: C 64.25, H 9.70, N 4.05.
Found: C 64.33, H 9.44, N 3.79.

4′,23-Dideoxy-23-(*N*-ethyl-2-fluoroethylamino)mycaminosyl Tylonolide Diethyl Acetal (**16**)

To an ice-cold solution of **15** (78 mg) and triethylamine (28 mg) in dry benzene (0.8 ml) was added 2-fluoroethyl trifluoromethanesulfonate (**9**, 50 mg) and the solution was kept at room temp for 1 hour. Additional triethylamine (56 mg×3) and **9** (100 mg×3) were added in every 1 hour. TLC ($CHCl_3$ - MeOH - 28% aq NH_3, 15: 1: 0.1) of the solution showed two spots at Rf 0.3 (**16**) and 0 (major, 3′-*N*-(2-fluoroethyl)-derivated product?, *cf.* **15**, 0.2). Concentration gave a residue, that was dissolved in chloroform and the solution was washed with satd aq sodium hydrogencarbonate and satd aq sodium sulfate, dried over sodium sulfate, and concentrated. Column chromatography of the residue with $CHCl_3$ - MeOH - 28% aq NH_3, 30: 1: 0.1 gave a solid of **16**, 18 mg (22%).

4′,23-Dideoxy-23-(*N*-ethyl-2-fluoroethylamino)mycaminosyl Tylonolide (**17**)

To a solution of **16** (22.4 mg) in acetonitrile (0.5 ml) was added 0.1 M aq hydrochloric acid (0.85 ml), and the solution was kept at room temp for 1 hour. Neutralization with sodium hydrogencarbonate was followed by extraction with chloroform (0.5 ml×3). The organic solution combined was washed with satd aq sodium sulfate, dried over sodium sulfate, and concentrated. Column chromatography of the residue with $CHCl_3$ - MeOH - 28% aq NH_3, 30: 1: 0.1 gave a solid, that was recrystallized from acetone - hexane to give prisms of **17**, 15 mg (75%), mp 151.5~152°C, $[\alpha]_D^{22}$ +22° (*c* 0.5, $CHCl_3$); MS *m/z* 654 (M, $C_{35}H_{59}FN_2O_8$), 550 [M−104 (=$CH_2NEt(CH_2CH_2F)$], 104; ^1H NMR ($CDCl_3$) δ 4.20 and 4.73 (each t, 2H in total, $^3J_{H,H}$=6 Hz, $^2J_{H,F}$=48 Hz, CH_2FCH_2), 5.80 (d, 1H, H-13), 6.32 (d, 1H, H-10), 7.43 (d, 1H, H-11), 9.85 (s, 1H, CHO).

Anal Calcd for $C_{35}H_{59}FN_2O_8$: C 64.19, H 9.08, N 4.28.
Found: C 64.04, H 9.11, N 4.29.

23-Deoxy-23-(*N*-ethyl-2,2-difluoroethylamino)mycaminosyl Tylonolide (**19**)

A mixture of **14** (63 mg), triethylamine (52 mg), and 2,2-difluoroethyl trifluoromethanesulfonate (**10**, 117 mg of 87.5% solution in dichloromethane) in dry benzene (0.63 ml) was kept at room temp for 4 hours. After addition of chloroform (6 ml), the solution was treated as described for **16**. Column chromatography of the crude product as described for **16** gave a solid of **18**, 59 mg (86%). To the solid in acetonitrile (1.2 ml) was added 0.1 M aq hydrochloric acid (2.3 ml) and the solution was treated as described for **17**. Crude product obtained was recrystallized from acetone - hexane to give **19** as prisms, 49 mg (92%); mp 187~190°C; $[\alpha]_D^{22}$ +16° (*c* 1, $CHCl_3$); MS *m/z* 688 (M, $C_{35}H_{58}F_2N_2O_9$), 565, 174; ^1H NMR ($CDCl_3$) δ 4.75 (dt, 1H, H-15), 5.69 (tt, 1H, $^2J_{H,F}$=57 Hz, $^3J_{H,H}$ =4.5 Hz, CHF_2CH_2), 5.73 (d, 1H, H-13), 6.26 (d, 1H, H-10), 7.32 (d, 1H, H-11), 9.70 (s, 1H, CHO).

Anal Calcd for $C_{35}H_{58}F_2N_2O_9 \cdot \frac{1}{2}H_2O$: C 60.24, H 8.52, N 4.01.
Found: C 60.26, H 8.56, N 4.01.

4′,23-Dideoxy-23-(*N*-ethyl-2,2-difluoroethylamino)mycaminosyl Tylonolide (**21**)

Compound **15** (83 mg) was treated as described for **19** to give **20** (46 mg, 51%). Hydrolysis of **20** as usual gave an amorphous solid of **21**, 38 mg (92%); $[\alpha]_D^{20}$+16° (*c* 1, $CHCl_3$); MS *m/z* 672 (M, $C_{35}H_{58}F_2N_2O_8$), 549, 122; ^1H NMR ($CDCl_3$) δ 4.74 (dt, 1H, H-15), 5.68 (tt, 1H, $^2J_{H,F}$=57.5 Hz, $^3J_{H,H}$ =3.7 Hz, CHF_2CH_2), 5.74 (d, 1H, H-13), 6.30 (d, 1H, H-10), 7.35 (d, 1H, H-11), 9.72 (s, 1H, CHO).

Anal Calcd for $C_{35}H_{58}F_2N_2O_8$: C 62.48, H 8.69, N 4.16.
Found: C 62.23, H 8.56, N 4.15.

23-Deoxy-23-(*N*-ethyl-2,2,2-trifluoroethylamino)mycaminosyl Tylonolide Diethyl Acetal (**22**)

A mixture of **14** (82 mg), triethylamine (0.13 ml), and 2,2,2-trifluoroethyl trifluoromethanesulfonate (**11**, 360 mg of 76% solution in dichloromethane) in dry benzene (0.82 ml) was heated, in a pressure bottle, at 120°C for 2 hours. Concentration gave a residue, that was treated as described for **16** to give, after column chromatography, a solid of **22**. Recrystallization from acetone - cyclohexane gave prisms, 64 mg (70%); mp 161~162°C; $[\alpha]_D^{23}$ +30° (*c* 1, $CHCl_3$).

Anal Calcd for $C_{39}H_{67}F_3N_2O_{10}$: C 59.98, H 8.65, N 3.59.
Found: C 59.74, H 8.65, N 3.38.

23-Deoxy-23-(*N*-ethyl-2,2,2-trifluoroethylamino)mycaminosyl Tylonolide (**23**)

Compound **22** (22 mg) was hydrolyzed as usual to give a solid of **23**, 19 mg (97%). Recrystalliza-

tion gave plates; mp 187~188°C; $[\alpha]_D^{20}$ +13° (*c* 1, $CHCl_3$); MS *m/z* 706 (M, $C_{35}H_{57}F_3N_2O_9$), 565, 516, 140 [$CH_2NEt(CH_2CF_3)$]; ^1H NMR ($CDCl_3$) δ 3.00 (q, 2H, *J*=9 Hz, CF_3CH_2), 4.75 (dt, 1H, H-15), 6.27 (d, 1H, H-10), 7.32 (d, 1H, H-11), 9.71 (s, 1H, H-20).

Anal Calcd for $C_{35}H_{57}F_3N_2O_9$: C 59.47, H 8.13, N 3.96.
Found: C 59.29, H 8.23, N 3.92.

4′,23-Dideoxy-23-(*N*-ethyl-2,2,2-trifluoroethylamino)mycaminosyl Tylonolide Diethyl Acetal (**24**)

Compound **15** (78 mg) was treated as described for **22** (reaction period, 1 hour) to give, after column chromatography, a solid of **24**, 37 mg (42%), and a by-product (24 mg).

Anal Calcd for $C_{39}H_{67}F_3N_2O_9 \cdot \frac{1}{2}H_2O$: C 60.54, H 8.80, N 3.62.
Found: C 60.24, H 8.99, N 3.50.

4′,23-Dideoxy-23-(*N*-ethyl-2,2,2-trifluoroethylamino)mycaminosyl Tylonolide (**25**)

Procedure A: Compound **24** (19 mg) was treated as usual to give a solid of **25**, 13 mg (72%); $[\alpha]_D^{25}$ +15° (*c* 1, $CHCl_3$); MS *m/z* 690 (M, $C_{35}H_{57}F_3N_2O_8$), 549, 140 [$CH_2NEt(CH_2CF_3)$]; ^1H NMR ($CDCl_3$) δ 4.74 (dt, 1H, H-15), 5.72 (d, 1H, H-13), 6.30 (d, 1H, H-10), 7.34 (d, 1H, H-11), 9.72 (s, 1H, H-20).

Anal Calcd for $C_{35}H_{57}F_3N_2O_8$: C 60.85, H 8.32, N 4.05.
Found: C 60.87, H 8.45, N 4.05.

Procedure B: A mixture of **15** (3.73 g), triethylamine (3.6 g), and **11** (11.4 g of 67% solution in dichloromethane) in dry benzene - *N*,*N*-dimethylformamide (1: 1, 19 ml) was heated at 50°C for 3 hours and then worked up as described for **24** and **25** (Procedure A) to give a solid of **25**, 1.67 g (44% based on **15**).

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