NEW OLEANDOMYCIN 9-OXIMES

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY

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A series of the novel oleandomycin 9-oximes has been prepared and characterized by spectroscopic data and X-ray analysis. The antibacterial *in vitro* activities of the oximes (**6**~**10**) were compared with that of oleandomycin (**1**). Among the novel derivatives the most active compound was 8(*R*)-methyloleandomycin-9-oxime (**9**) in contrast to its 8(*S*)-isomer (**10**) which possessed only low potency. Some preliminary pharmacokinetic data of **9** confirmed its activity. Compound **9** has been advanced to further biological study.

Introduction of the C-9 hydroxyimino group in the aglycone ring of erythromycin A[1] changed its biological and physico-chemical properties[2] and opened the possibilities for the preparation of the novel semisynthetic, highly promising macrolides, azithromycin[3~5] and roxithromycin.[6] Consequently, we were interested in examining the influence of the same functional group on the biological values of the erythromycin-related antibiotic, oleandomycin and some of its derivatives. Here we report our results related to the novel oleandomycin C-9 oximes.

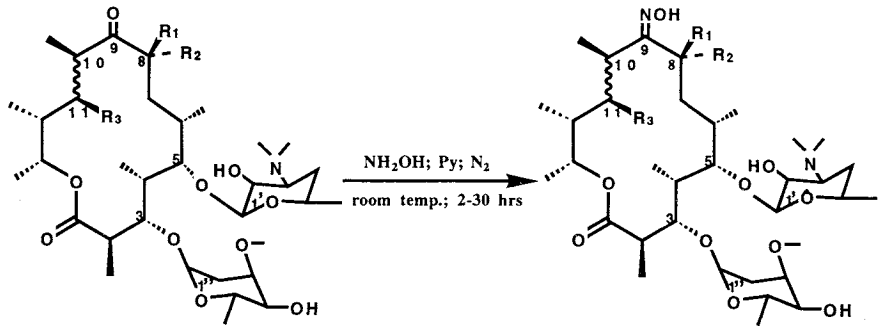
Chemistry

Oleandomycin (**1**) is a 14-membered macrolide antibiotic characterized with two carbohydrates, β-D-desosamine and α-L-oleandrose. The exocyclic epoxide at C-8 of **1** is a unique feature, which does not appear in any of the other known polyoxo macrolides. Previous studies[7,8] established the extreme sensitivity of **1** to both, acid and basic conditions. The acid treatment causes the opening of the C-8 epoxide, the cleavage of the C-3 sugar, α-L-oleandrose, as well as some remarkable macrolide ring contractions. The base treatment of **1** induces dehydration across C-10~C-11 bond to give anhydrooleandomycin (**2**).

Reductive deoxygenation of the C-8 epoxide group using chromium (II) chloride,[9] followed by catalytic hydrogenation of the resulting 8-methylene oleandomycin (**3**), gave a mixture of 8(*R*)- and 8(*S*)-methyl-isomers.[10] The mixture thus obtained we subjected to silica gel column chromatography ($CHCl_3$ - MeOH, 85 : 15) to yield homogenous 8(*R*)- (**4**) and 8(*S*)-methyl-isomers (**5**) with Rf values 0.63 and 0.67 on the TLC ($CHCl_3$ - MeOH - conc NH_4OH, 6 : 1 : 0.1), respectively.

Our initial attempts to prepare oximes (**6**)~(**10**) under conditions described for erythromycin A[1] were unsuccessful. The reaction was sluggish at room temperature, while prolonged treatment at elevated temperature resulted in the extensive decomposition of the starting C-9 ketones (**1**)~(**5**). The performance

Scheme 1. Synthesis of oleandomycin oximes.



1~5 6~10

	R_1	R_2	R_3	~~~
1; **6**	=O		OH	Single bond
2; **7**	=O		—	Double bond
3; **8**	$=CH_2$		OH	Single bond
4; **9**	CH_3	H	OH	Single bond
5; **10**	H	CH_3	OH	Single bond

Table 1. Some diagnostic ^{13}C NMR chemical shifts (δ)[a] for oleandomycin 9-oximes **6**, **7** and **8** in comparison with corresponding ketones **1**, **2** and **3**.

Carbon No.	δ					
	1[b]	**6**	**2**	**7**	**3**	**8**
C-7	42.1 t	43.0 t	42.0 t	43.6 t	42.1 t	43.6 t
C-8	63.1 s	56.1 s	61.5 s	56.3 s	149.7 s	141.4 s
C-8a	49.4 t	51.1 t	50.1 t	51.2 t	120.8 t	116.4 t
C-9	208.7 s	159.3 s	198.3 s	157.3 s	207.1 s	163.3 s
C-10	45.7 d	38.3 d	136.9 s	130.1 s	43.1 d	38.1 d
C-11	69.6 d	69.0 d	142.3 d	135.0 d	70.1 d	70.5 d

[a] Chemical shifts are in ppm downfield from TMS, measured in $CDCl_3$ at 75 MHz.
[b] Ref 8).

of the reaction in the desired position, leaving the rest of the molecule intact, was realized by treatment of compounds **1**~**5** with a 5 molar excess of hydroxylamine hydrochloride in pyridine as a solvent and as a base (Scheme 1).

The structure of the novel 9-oximino oleandomycins **6**~**10** has been well elucidated on the bases of their EI-MS, ^1H and ^{13}C NMR spectra. Increase of molecular ions for 15 in comparison with the parent ketones **1**~**5** was in agreement with the replacement of the C-9 keto group with a hydroxyimino one. The disappearance of the carbonyl absorption at about 290 nm in the UV spectra of **6**~**10** confirmed this modification. Further evidence was obtained from ^1H NMR spectra. In DMSO-d_6 compounds **6**~**10** showed N–OH proton absorption exchangeable with D_2O in 10.28~10.97 ppm region. Their ^{13}C NMR spectra (Table 1) also confirmed the presence of the corresponding C-9 oxime carbons. The other chemical shifts were consistent with the assigned structures. The ketoximes **6** and **7** appeared to be single isomers, which are most reasonably believed to have the C-9 oxime group *anti* to the C-8 quaternary carbon.[11]

Table 2. ^{1}H NMR chemical shifts (δ)[a] of **9** and **10** in comparison with ketones **4** and **5**.

Proton No.		4	9	5	10
2-H	dq	2.83	2.77	2.79	2.81
3-H	dd	3.69	3.71	3.56	3.83
4-H	ddq	1.62	1.32	1.68	1.71
5-H	dd	3.45	3.48	3.25	3.43
6-H	m	1.72	1.68	1.76	1.86
7a-H	dd	ND	1.44	1.76	1.27
7b-H	dd	ND	ND	1.67	0.92
8-H	ddq	2.61	3.73	2.90	2.66
9N-OH		—	10.61	—	10.40
10-H	dq	2.88	2.56	2.86	2.52
11-H	dd	3.57	3.17	3.82	3.80
12-H	ddq	1.66	1.55	1.45	1.64
13-H	dq	5.43	5.62	5.43	5.46
2-CH_3	d	1.21	1.17	0.99	1.15
4-CH_3	d	1.22	1.22	0.90	1.07
6-CH_3	d	1.17	1.11	1.13	1.15
8-CH_3	d	1.15	1.04	1.02	0.98
10-CH_3	d	1.02	1.02	1.19	1.04
12-CH_3	d	0.88	0.86	1.07	0.92
13-CH_3	d	1.29	1.24	1.31	1.27
1″-H	dd	4.98	5.00	4.97	4.98
2″a-H	ddd	2.37	2.35	2.32	2.35
2″b-H	ddd	1.55	1.51	1.49	1.50
3″-H	ddd	3.49	3.46	3.44	3.43
4″-H	dd	3.17	3.16	3.15	3.16
5″-H	dq	3.76	3.75	3.76	3.76
5″-CH_3	d	1.31	1.29	1.29	1.29
3″-OCH_3	s	3.41	3.41	3.41	3.42
1′-H	d	4.23	4.23	4.19	4.27
2′-H	dd	3.26	3.31	3.25	3.28
3′-H	ddd	2.57	2.65	2.59	2.72
4′a-H	ddd	1.78	1.72	1.72	1.75
4′b-H	ddd	1.30	1.32	1.24	1.27
5′-H	ddq	3.51	3.51	3.52	3.53
5′-CH_3	d	1.23	1.24	1.24	1.21
3′-$N(CH_3)_2$	s	2.34	2.37	2.34	2.40

[a] Values in ppm from TMS, measured in $CDCl_3$ at 300 MHz, as determined from ^{1}H-^{1}H 2D homonuclear shift correlated experiments.

Table 3. ^{13}C NMR chemical shifts (δ)[a] of **9** and **10** in comparison with ketones **4** and **5**.

Position	4	9	5	10
C-1	177.1	176.2	177.5	176.8
C-2	44.9	44.8	45.6	45.5
C-3	81.2	79.5	79.7	80.5
C-4	36.3	34.5	36.0	31.8
C-5	84.5	85.2	85.2	85.6
C-6	43.3	43.9	44.0	42.7
C-7	34.5	33.6	38.0	29.3
C-8	45.0	27.8	46.6	34.0
C-9	216.8	168.8	216.8	165.5
C-10	42.1	32.6	46.7	44.1
C-11	70.4	72.0	70.8	70.0
C-12	42.2	41.6	41.4	42.7
C-13	70.5	70.0	69.1	71.6
2-CH_3	14.6	14.3	14.2	14.2
4-CH_3	19.7	19.0	19.9	18.8
6-CH_3	9.4	9.8	10.8	15.7
8-CH_3	16.5	18.7	22.4	15.2
10-CH_3	7.7	12.3	6.6	8.2
12-CH_3	8.7	8.6	9.0	9.1
13-CH_3	18.3	17.9	17.4	10.6
C-1″	99.5	98.5	99.2	99.5
C-2″	34.0	33.9	34.0	34.0
C-3″	77.9	78.1	78.0	78.1
C-4″	76.0	75.8	76.1	76.0
C-5″	68.8	69.0	68.6	69.0
5″-CH_3	17.8	18.7	18.2	17.7
3″-OCH_3	56.4	56.4	56.5	56.4
C-1′	104.4	104.2	104.6	104.6
C-2′	70.5	70.7	70.6	70.5
C-3′	65.7	65.5	65.4	65.5
C-4′	28.9	29.3	28.9	29.0
C-5′	69.1	69.0	69.0	68.7
5′-CH_3	21.2	21.2	21.2	21.2
3′-$N(CH_3)_2$	40.3	40.3	40.3	40.3

[a] δ Values in ppm from TMS, measured in $CDCl_3$ at 75 MHz, as determined from ^{1}H-^{13}C 2D heteronuclear shift correlated experiments.

NOEs between N–OH and 10-H in **6** and **7** confirmed their spatial proximity. The resonance of both α-carbons in **8**~**10** shifted upfield on an oxime formation, with the effect for C-8 carbon being greater than for C-10. Such displacement suggested an *E*-isomer.[12] The structures of **9** and **10** were established as the C-8 enantiomers by means of the homonuclear ^{1}H-^{1}H and heteronuclear ^{1}H-^{13}C 2D NMR spectroscopy. Comparison of their ^{1}H chemical shifts showed differences similar to those for 8-*epi*-erythromycins.[13] Table 2 shows that the 8-H signal in **9** is 1.07 ppm downfield from that in **10**. In the ^{13}C NMR spectra (Table 3) C-8 resonated at 27.8 and 34.0 ppm, which was in accord with the predicted conformations 8(*R*)- for **9** and 8(*S*)- for **10** isomer, respectively.

The X-ray structure analysis was used to determine unambiguously the molecular configuration and conformation of **10** (Fig. 1). The conformation of 14-membered macrocycle is shown in polar diagram

Fig. 1. The ORTEP plot with the aglycon atom numbering of **10**.

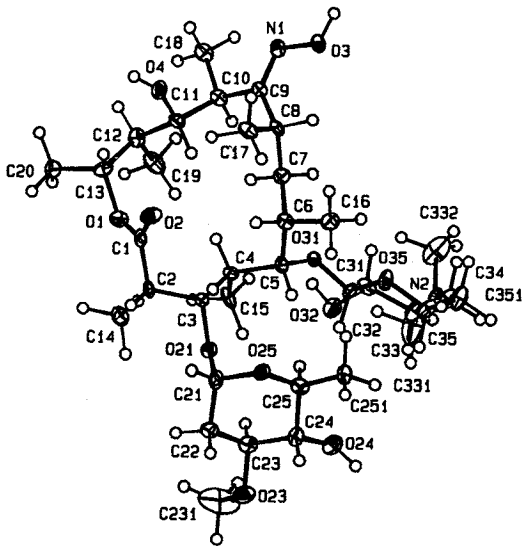


Fig. 2. Polar diagram illustrates the conformation of the macrocyclic ring of **10**; the values of torsion angles are plotted for the ring bond sequence starting with the C1-O1 bond (1).

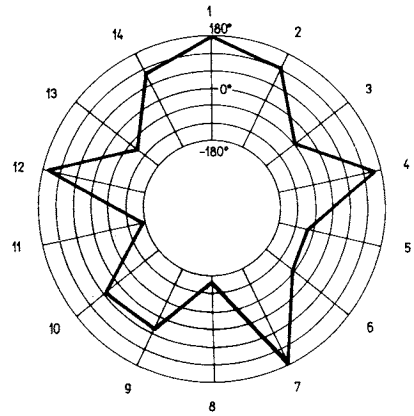


Table 4. Antibacterial *in vitro* activity of novel oximes **6**~**10** compared to oleandomycin (**1**).

Organisms (No. of strains)	% of strains inhibited	MIC (μg/ml)					
		1	**6**	**7**	**8**	**9**	**10**
Micrococcus luteus ATCC 9341		0.1	1.0	20.0	1.0	0.1	1.0
Staphylococcus aureus ATCC 6538 P		0.5	4.0	>20.0	8.0	0.5	8.0
Escherichia coli ATCC 10536		4.0	2.0	>20.0	20.0	2.0	8.0
Staphylococcus aureus (14)	50	1.0	4.0	20.0	8.0	0.5	8.0
S. saprophyticus (4)	50	1.0	4.0	>20.0	4.0	0.5	8.0
Streptococcus faecalis (9)	50	0.5	2.0	>20.0	4.0	0.25	16.0
S. pneumoniae (4)	90	0.5	0.25	8.0	0.25	0.25	2.0
Group B Streptococci (5)	90	2.0	2.0	16.0	4.0	0.25	4.0
Haemophilus influenzae (5)	90	0.5	NT	NT	NT	0.1	NT
Escherichia coli (4)	100	>20.0	>20.0	>20.0	>20.0	20.0	>20.0
Klebsiella pneumoniae (2)	100	20.0	>20.0	>20.0	>20.0	20.0	>20.0
Neisseria sicca (1)	100	16.0	8.0	>20.0	8.0	2.0	8.0

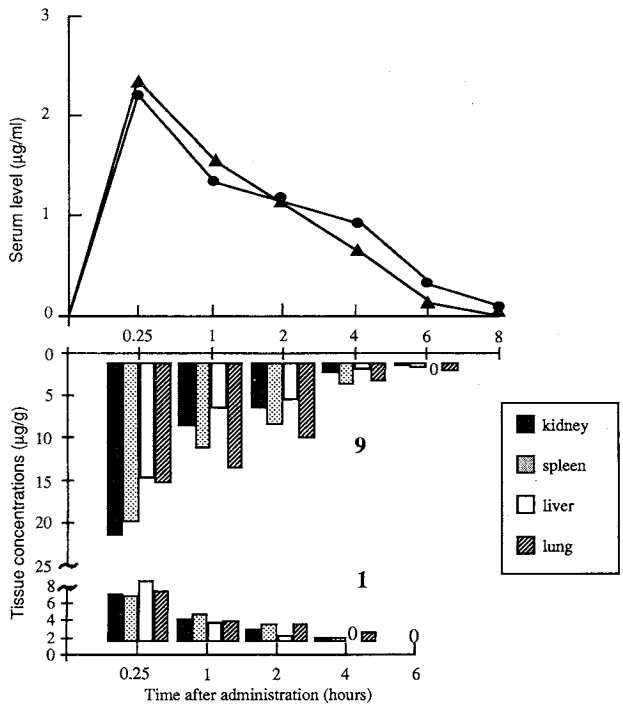
Method: Determined by tube dilution method using brain heart infusion medium.
Incubation: 24~48 hours, at 37°C.
Inoculum size: 10^{-5}~10^{-6} cfu/ml.
NT: Not tested.

of Fig. 2. The known absolute configuration of β-D-desosamine and α-L-oleandrose has been used as an internal standard to determine the absolute configuration of aglycon chiral centers including a novel chiral center at C-8. Thus, the absolute configuration is 2*R*, 3*S*, 4*R*, 5*S*, 6*S*, 8*S*, 10*R*, 11*S*, 12*S*, 13*R*. The absolute configuration of aglycon in **10** is in agreement with literature but in 11,4″-bis[*O*-(*p*-bromobenzoyl)]-oleandomycin C-12 was assigned to be *R*.[14] However, the *R* assignment was not correct due to the unproper priority at the chiral C-12 atom.

Biological Properties

Table 4 shows *in vitro* antibacterial activity of the novel 9-oximino oleandomycines **6**~**10** in comparison

Fig. 3. Comparison of serum and tissue concentrations between **1** and **9**.
(▲) **1**, (●) **9**.



with oleandomycin **1**. Of the five oximes, compound **9** is equal to or 2~8 fold more active than **1**. Group B Streptococci, *Heamophilus influenzae* were very susceptible to compound **9** which inhibited 90% of isolates at 0.25 and 0.1 μg/ml, respectively. Compounds **6** and **8** are equal to or 2~4 fold less active than **1**, while compounds **7** and **10** are much less active. Gram-negative bacteria were usually resistant to all compounds. It should be pointed out the expected superiority of the 8(*R*)-epimer **9** to 8(*S*)- **10**, since generally they show higher biological activities than 8(*S*) relatives.[15]

After having considered MIC-values, we selected the compound **9** for further biological studies. Pharmacokinetic properties of **9** and **1** were compared after single *i.v.* administration of 25 mg/kg/rats. Serum concentration of both compounds was approximately identical (Fig. 3). In contrast to the blood level results, compound **9** produced significantly higher and more prolonged tissue levels than did **1**. Fig. 3 indicates higher concentrations of **9** in lung, liver, kidney and spleen. Its superior *in vivo* pharmacokinetic profile relative to oleandomycin suggests considerable potential as an improved alternative to **1**.

Experimental

MPs were taken on a Fisher-Johns apparatus and are uncorrected. Electron impact mass spectra were measured on a Shimadzu GC MS-QP 1000 mass spectrometer at 20 eV and a source temperature of 250°C. UV spectra were recorded with a Shimadzu UV 240 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or in DMSO on JEOL FX-100 or GEM-300 spectrometers. TLC was performed on Merck Silica gel 60 plates using solvent systems A ($CHCl_3$-CH_3OH-conc NH_4OH, 6:1:0.1) and B (CH_2Cl_2-CH_3OH-conc NH_4OH, 90:9:1.5). Merck Silica gel 60 (70~230 mesh) was used for column chromatography.

Table 5. Crystal data and details of structure determination of **10**.

a) Crystal data		θ_{min}, θ_{max} (°) for cell det.	7~14
Formula	$C_{35}H_{64}N_2O_{11}\cdot 3H_2O$	No. of reflections for cell det.	25
MW	742.943	θ_{min}, θ_{max}	2~25
a (Å)	9.077(5)	$\omega/2\theta$ scan (°)	$\Delta\omega = 0.80 + 0.35 \tan\theta$
b (Å)	14.789(5)	$h\,k\,l$ limits	0→10, 0→17, 0→36
c (Å)	30.648(8)	Reflections measured	4104
V (Å^3)	4114(3)	Reflections observed with $I > 2\sigma(I)$	2224
D_{calc} (g/cm^3)	1.204	c) Refinement	
Z	4	No. of parameters	552
Crystal system	Orthorhombic	Quantity minimized, $\Sigma w\,\|\,\|F_o\|-\|F_c\|\,\|^2$	$w = k/(\sigma^2(F_o)+0.0006(F_o^2))$ $k = 1.5001$
Space group	$P2_12_12_1$	R, R_w	0.0529, 0.0487
Crystal size (mm)	0.56×0.2×0.1	Max. parameter shift, (Δ/σ)max	−0.612 (O43, water molecule)
Linear absorption coefficient (cm^{-1})	0.86	Residual electron density, $(\Delta\rho)_{max}$, $(\Delta\rho)_{min}$ (eA^{-3})	0.30, −0.29
F ($\phi\phi\phi$)	1624		
b) Data collection			
Diffractometer	Enraf-Nonius-CAD4		
Radiation	MoK_α (λ=0.7173 Å) graphite-monochromator		
Temperature (K)	133 (3)		

In Vitro and *In Vivo* Assay

MICs were determined on brain heart infusion agar by the method of ERRICSSON and SHERRIS.[16] Serum levels were determined in male Fisher rats (210~250 g), after a single iv dose of 25 mg/kg. Blood samples were obtained from the jugular vein. Determination of antibiotic levels was carried out by using a microbiological assay and *Micrococcus luteus* ATCC 9341 as the test strain. For determination of tissue levels small pieces of the organs were dried under vacuum for 2 days and then finely ground in a mortar. Dry powders were extracted three times with methanol. The extracts were combined and the organic solvent was evaporated. Residues were solubilized back in 0.1 M phosphate buffer, pH 8, and assayed for antibiotic contents.

X-Ray Analysis of **10**

The crystals were prepared by dissolving 10 mg of **10** in 0.5 ml of methanol and by adding about 30 drops of water. By slow evaporation of a solution over a few days at room temperature, the crystals suitable for X-ray analysis were obtained. During data collection at low temperature the crystal was frozen in an oil drop.

Crystallographic data and details of data collection and refinement are listed in Table 5. Data reduction was performed by the ENRAF-Nonius SDP/VAX package,[17] Lorentz and polarization effects were corrected. The structure was solved by direct methods using programme SHELX86.[18] The non-H atoms were refined anisotropically using SHELX77;[19] details of the refinement procedure are listed in Table 5c. The scattering factors were those included in the programme. Molecular geometry was calculated by the programme package EUCLID.[20] Drawing was prepared by ORTEP.[21]

Oxime Derivatives (**6**)~(**10**). General Method

Oleandomycin derivatives **1**~**5** (1 equiv) were dissolved in dry pyridine, $NH_2OH\cdot HCl$ (5 equiv) was added, and the reaction mixture was stirred under an inert atmosphere, at room temperature until TLC was indicated the completion of reaction (2~30 hours). The isolation of the obtained oximes **6**~**10** was performed by extraction with $CHCl_3$ within a pH-range of 7.0~8.5 and finally by evaporation of the organic solvents to dryness. The crude products were chromatographed on a silica gel column using solvent system A.

Oleandomycin-9-oxime (**6**)

Reaction time 2 hours; Yield 70%; MP 148~150°C; TLC, system A, Rf 0.51; EI-MS m/z 702 (M^+);

^{1}H NMR (90 MHz, DMSO-d_6) δ 2.23 (6H, s, 3'-N(CH_3)$_2$), 3.33 (3H, s, 3''-OCH_3), 10.82 (=NOH); ^{13}C NMR ($CDCl_3$): Table 1.

Anal Calcd for $C_{35}H_{62}N_2O_{12}$: C 59.81, H 8.89, N 3.99.
Found: C 59.49, H 9.12, N 3.75.

Anhydrooleandomycin-9-oxime (**7**)

Reaction time 18 hours; Yield 93%; TLC, system A, Rf 0.52; EI-MS *m/z* 684 (M^+); ^{1}H NMR (90 MHz, DMSO-d_6) δ 2.21 (6H, s, 3'-N(CH_3)$_2$), 3.34 (3H, s, 3''-OCH_3), 10.91 (=NOH); ^{13}C NMR ($CDCl_3$): Table 1.

Anal Calcd for $C_{35}H_{60}N_2O_{11}$: C 61.38, H 8.83, N 4.09.
Found: C 61.61, H 8.65, N 4.31.

8-Methylene-oleandomycin-9-oxime (**8**)

Reaction time 2 hours; Yield 73%; MP 126~128°C; TLC, system A, Rf 0.58; EI-MS *m/z* 686 (M^+); ^{1}H NMR (90 MHz, DMSO-d_6) δ 2.29 (6H, s, 3'-N(CH_3)$_2$), 3.34 (3H, s, 3''-OCH_3), 10.28 (=NOH); ^{13}C NMR ($CDCl_3$): Table 1.

Anal Calcd for $C_{35}H_{62}N_2O_{11}$: C 61.20, H 9.10, N 4.08.
Found: C 61.52, H 8.79, N 3.85.

8(*R*)-Methyloleandomycin-9-oxime (**9**)

Reaction time 30 hours; Yield 57%; MP 147~150°C; TLC, system A, Rf 0.57; EI-MS *m/z* 688 (M^+); ^{1}H NMR (90 MHz, DMSO-d_6): Table 2; ^{13}C NMR ($CDCl_3$): Table 3.

Anal Calcd for $C_{35}H_{64}N_2O_{11}$: C 61.02, H 9.37, N 4.07.
Found: C 61.42, H 9.69, N 3.80.

8(*S*)-Methyloleandomycin-9-oxime (**10**)

Reaction time 5 hours; Yield 30%; MP 134~135°C; TLC, system A, Rf 0.48; EI-MS *m/z* 688 (M^+); ^{1}H NMR (90 MHz, DMSO-d_6): Table 2; ^{13}C NMR ($CDCl_3$): Table 3.

Anal Calcd for $C_{35}H_{64}N_2O_{11}$: C 61.02, H 9.37, N 4.07.
Found: C 59.68, H 9.09, N 4.34.

Acknowledgments

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