

Table 1. Hydrogenolysis conditions of benzyl and benzyldiene groups.

Entry	Substrate	Experimental conditions ^a	Product	Yield %
a	4	HCOONH ₄ - HCOOH EtOH, 4 hours	12	40 (35% starting material)
b	4	H ₂ -760 mmHg EtOH, 1 day	13	85
c	4	H ₂ -760 mmHg AcONa MeOH, 1 week	12	82
d	4	H ₂ -760 mmHg AcOH - AcONa EtOH - H ₂ O, 2 weeks	14	69
e	4	H ₂ -1550 mmHg AcOH - AcONa EtOH - H ₂ O, overnight	15	55 (20% starting material)
f	5	H ₂ -760 mmHg AcOH - AcONa EtOH - H ₂ O, 2 weeks	16	68
g	6	H ₂ -760 mmHg AcOH - AcONa EtOH - H ₂ O, 2 weeks	17	55
h	7	H ₂ -760 mmHg AcOH - AcONa EtOH - H ₂ O, 2 weeks	18	75
i	8	H ₂ -760 mmHg AcOH - AcONa EtOH - H ₂ O, 2 weeks	19	60
j	9	H ₂ -760 mmHg AcOH - AcONa EtOH - H ₂ O, 2 weeks	20	65

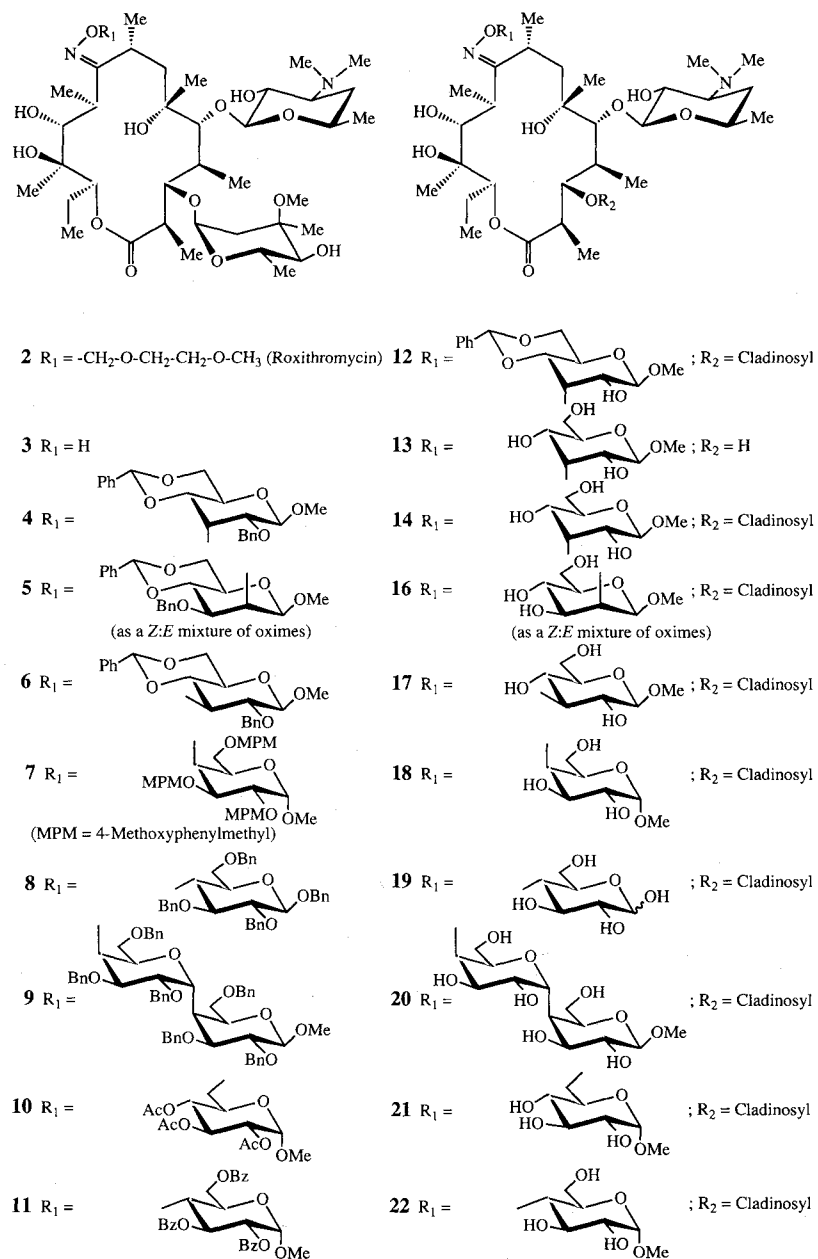
^a All reactions were carried out in the presence of a catalytic amount of Pd/C 10% w/w.

conditions¹²⁾. Treatment of compound **4** with ammonium formate and formic acid in the presence of palladium on charcoal 10% w/w (Pd/C 10% w/w) in refluxed MeOH afforded compound **12** in 40% yield together with a substantial amount (35%) of starting material and some degradation products (Table 1, entry a). Obviously, the benzyldiene acetal group was stable under these conditions. Thus, we turned to classical hydrogenolysis. Reaction of compound **4** under atmospheric pressure of hydrogen in the presence of a catalytic amount of Pd/C 10% w/w in EtOH allowed the desired cleavage of both the benzyl and benzyldiene groups. Unfortunately, the reaction was accompanied by the loss of the cladinoso moiety and compound **13** was obtained as a sole product in excellent yield (Table 1, entry b). This unexpected loss of cladinoso probably resulted from the acidification of the reaction mixture. To prevent its formation, the reaction was run in the presence of sodium acetate (Table 1, entry c). However, these conditions afforded the above mentioned compound **12** in rather improved yield. Slightly lowering the pH of the reaction medium was thought to diminish the stability of the benzyldiene acetal ring. Thus, deprotection was attempted in an acetic acid/sodium acetate buffer in a mixture of MeOH - H₂O according to the literature¹⁴⁾ (Table 1, entry d). Thus the fully deprotected compound **14** was isolated in 69% yield together with some degradations products. In fact, the reaction was slow and required two weeks to come to completion. An assay was run in a Parr apparatus under 1550 mmHg pressure of hydrogen to accelerate the

deprotection and, consequently, to reduce the decomposition which might be caused by prolonged stay of the substrates in the reaction vessel (Table 1, entry e). Disappointingly, the oximic linkage appeared to be unstable under these conditions. Starting compound **4** and erythromyclamine¹⁵⁾ **15** were the sole observed products in 20% and 55% yield, respectively, after 12 hours of reaction. The facile cleavage of the N-O bond was surprising if we refer to the usually applied conditions to prepare compound **15** from **3**¹⁵⁾. Previous conditions (*i.e.* entry d) were actually selected and applied for the deprotection of compounds **5**~**9**. Compounds **16**~**20** were obtained in 68, 55, 75, 60 and 65% yield, respectively. Finally, rapid sodium methanolate treatment of intermediates **10** and **11** afforded compounds **21** and **22** in 72 and 65% yield while minimizing lactone ring opening.

In Vitro Antibacterial Activity

The *in vitro* antibacterial activities, determined following standard broth microdilution techniques, are shown in Table 2. Compounds **13**, **19** and **20**, by far the most hydrophilic derivatives of the family, are essentially inactive. This absence of activity may be caused by cell membrane impermeability. The other compounds exhibit a more differentiated activity. On the one hand, they are only slightly active against Staphylococques, Enterococques and *Streptococcus agalactiae* compared to erythromycin A. On the other hand, their *in vitro* performance is more pronounced against strains (*e.g.* *Streptococcus pneumo-*



niae, *Moraxella catarrhalis* and *Haemophilus influenzae*) clinically treated with macrolides. Strong differences can however be noticed: compounds **17**, **18**, **21** and **22** are moderately active whereas compounds **14** and **16** are as or more active against these strains than erythromycin A (**1**). Surprisingly, the observed activity does not simply derive from the creation of an hydrophilic region but depends on more subtle structural changes such as the anomeric configuration of the newly introduced carbohydrate, the binding site on this carbohydrate and even on the nature (*i.e.* axial or equatorial) of this bond. For example, compound **21** is about two to four fold more active than compound **22** against the selected strains. These two compounds only differ from the carbon

of the glucose moiety at which the oxime is bond (at the C-6 position for compound **22** and at the C-4 position for compound **22**). The difference of activity of compounds **14** and **17** or **18** and **22** is even more striking and is only due to the orientation of the oximic linkage (either axial for compounds **14** and **18** or equatorial for compounds **17** and **22**).

Experimental

General

Meltings points were determined with a Reichert-Jung apparatus and are not corrected. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. Thin layer chromatography (TLC) was performed using

Table 2. *In vitro* activity of *O*-glycosyloxime derivatives^a.

<i>O</i> -Glycosyloxime derivative	13	14	16	17	18	19	20	21	22
Strains	Mueller Hinton broth + 10% lysed horse blood								
<i>Staphylococcus aureus</i> ATCC 29213	u	4	8	32	32	u	64	16	8
<i>S. aureus</i> 28	u	2	4	16	16	u	>64	8	4
<i>S. aureus</i> A8	u	8	8	32	32	u	>32	16	8
<i>S. epidermis</i> A10	u	2	4	16	16	u	32	8	4
<i>S. warneri</i> LG19	u	2	2	16	32	u	32	8	4
<i>Enterococcus faecalis</i> ATCC 29212	u	2	2	16	u	u	32	32	8
<i>E. faecalis</i> 995	u	4	4	64	u	u	u	64	16
<i>E. faecalis</i> 1072	u	8	8	64	u	u	64	16	8
<i>E. faecalis</i> 932	u	4	4	32	u	u	64	32	4
<i>Streptococcus agalactiae</i> 725	u	1	1	8	4	32	16	4	1
<i>S. agalactiae</i> 5942	u	1	2	8	8	32	16	4	1
<i>S. agalactiae</i> 280	u	1	1	8	8	32	32	4	2
<i>S. agalactiae</i> 288	u	0.5	1	4	4	32	32	4	0.5
<i>Streptococcus pneumoniae</i> Peni R	8	0.12	0.12	2	0.5	32	16	8	1
<i>S. pneumoniae</i> A14	32	0.5	0.5	4	2	32	8	nt	nt
<i>Moraxella catarrhalis</i> A04	u	4	4	32	32	64	32	8	4
	Mueller Hinton broth + Fildes extract								
<i>Haemophilus influenzae</i> A22	u	0.5	1	2	16	nt	nt	2	1

^a *In vitro* activity is the ratio of the geometric means of erythromycin A/geometric mean of the *O*-glycosyloxime derivatives' MIC of selected sensitive microorganisms. u=unactive; nt=not tested.

E. Merck plates of silica gel 60 with fluorescent indicator. Visualization was effected by spraying plates with 5% H₂SO₄ in ethanol followed by heating at 120~140°C. Column chromatography was made on the same support. THF was distilled over sodium/benzophenone, DMF, DMSO and pyridine were distilled over CaH₂. CH₂Cl₂ was distilled over P₂O₅ and MeOH over magnesium. Nuclear magnetic resonance (¹H and ¹³C NMR) spectra were recorded on Bruker WP 200, Bruker WP 300 and Bruker WP 300 spectrometers. Chemical shifts are recorded in ppm (δ) relative to tetramethylsilane as internal standard. FAB-MS spectra were obtained on a SM-80 spectrometer. High resolution mass spectra (HRMS) were run on a VG-ZAB-SEQ spectrometer by the Service Central d'Analyse, Vernaison. Elemental analyses were performed by the microanalytical laboratory at the ICSN, Gif sur Yvette.

General Procedure for the Hydrogenolysis of Protecting Groups (Compounds 4, 5, 6, 7, 8 and 9)

Substrate (1 equiv) was dissolved in EtOH-H₂O (15:1.75 ml/mmol of substrate) containing acetic acid (74 μl/mmol of substrate) and sodium acetate trihydrate (41.4 mg/mmol of substrate). 10% Pd/C w/w (150 mg/mmol of substrate) was then added. The reaction was run under atmospheric pressure of hydrogen at room temperature. After completion of the reaction, the catalyst was filtered off and washed with MeOH. The filtrate was concentrated under reduced pressure and the residue purified by flash chromatography on silica gel.

(*E*)-9-{*O*-[Methyl 4,6-*O*-(phenylmethylene)-β-D-allopyranosid-3-yl]oxime} of Erythromycin A (12)

To a solution of compound 4 (200 mg, 0.18 mmol) in MeOH (4 ml) were added sodium acetate (100 mg, 0.71 mmol) and Pd/C 10% w/w (60 mg). The mixture was stirred vigorously at room temperature under atmospheric pressure of hydrogen. After completion of the reaction, the catalyst was filtered and washed with MeOH. The filtrate was evaporated under reduced pressure and compound 12 (150 mg, 82%) was obtained after flash chromatography on silica gel (CH₂Cl₂-MeOH-NH₄OH, 10:1:0.05) followed by crystallization from Et₂O-heptane: R_f 0.32 (CH₂Cl₂-MeOH-NH₄OH, 10:1:0.05); mp 148~151°C; [α]_D²⁰ -41 (*c* 1, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 4.41 (1H, d, *J*=7.2 Hz, 1'-H), 4.54 (1H, d, *J*=8 Hz, 1'''-H), 5.04 (1H, *J*=10.9 Hz, 13-H), 5.50 (1H, s, H benzylidene acetal) and 7.28~7.48 (5H, m, Ph); ¹³C NMR (CDCl₃, 75 MHz): δ 14.5 (10-CH₃), 18.9 (8-CH₃), 27.0 (C-8), 33.2 (C-10), 57.5 (1'''-OCH₃), 64.6 (C-5'''), 69.4 (C-6'''), 70.7 (C-2'''), 77.9 (C-4'''), 80.7 (C-3'''), 101.7 (C benzylidene acetal), 103.3 (C-1'''), 126.3, 128.2, 129.1 and 137.6 (5 aromatic C) and 169.9 (C-9); FAB-MS (positive): *m/z* 1035 (M+Na)⁺ and 1013 (M+H)⁺.

FAB-HR-MS Calcd for C₅₁H₈₅N₂O₁₈, (M+H)⁺:

1013.5797.

Found:

1013.5818.

(*E*)-9-[*O*-(Methyl β-D-allopyranosid-3-yl)oxime] of 3-*O*-Decladinosylerythromycin A (13)

A mixture of compound 4 (200 mg, 0.18 mmol) and Pd/C 10% w/w (27 mg) in EtOH (10 ml) was stirred at

room temperature under atmospheric pressure of hydrogen. After completion of the reaction, the catalyst was filtered and washed with MeOH. The filtrate was concentrated under reduced pressure. Compound **13** (114 mg, 80%) was obtained after flash chromatography on silica gel (CH₂Cl₂ - MeOH - NH₄OH, 5 : 1 : 0.05) followed by crystallization from CHCl₃ - heptane: Rf 0.13 (CH₂Cl₂ - MeOH - NH₄OH, 5 : 1 : 0.05); mp 152 ~ 154°C; $[\alpha]_D^{20}$ -33 (*c* 1, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 1.00 (3H, d, *J* = 7 Hz, 8-CH₃), 2.00 (1H, broad dq, *J* = 4.6 and 7.6 Hz, 4-H), 2.21 (6H, s, N(CH₃)₂), 2.40 ~ 2.64 (3H, m, 2-H, 10-H and 3'-H), 3.17 (1H, dd, *J* = 7.2 and 10 Hz, 2'-H), 3.41 (1H, broad, s, 3-H), 3.48 (3H, s, 1''-OCH₃), 3.68 ~ 3.73 (3H, m, 8-H, 5'-H and 4''-H), 3.82 (1H, dd, *J* = 2.9 and 12 Hz, 6''-Heq), 4.41 (1H, d, *J* = 7.2 Hz, 1'-H), 4.56 (1H, d, *J* = 7.6 Hz, 1''-H), 4.77 (1H, t, *J* = 2 Hz, 3''-H) and 5.08 (1H, dd, *J* = 2.3 and 11 Hz, 11-H); ¹³C NMR (CDCl₃, 250 MHz): δ 14.9 (10-CH₃), 18.5 (8-CH₃), 25.6 (C-8), 33.7 (C-10), 57.1 (1''-OCH₃), 62.7 (C-6''), 68.4 (C-4''), 70.0 (C-2''), 75.3 (C-5''), 78.7 (C-3), 82.3 (C-3''), 106.0 (C-1'') and 171.4 (C-9); FAB-MS (positive): *m/z* 789 (M + Na)⁺ and 767 (M + H)⁺.

Anal FAB-HR-MS Calcd for C₃₆H₆₇N₂O₁₅, (M + H)⁺:
767.4541.

Found:
767.4545.

(E)-[O-(Methyl β-D-allopyranosid-3-yl)oxime] of Erythromycin A (14)

Deprotection of substrate **4** (200 mg, 0.18 mmol) was carried out following the general procedure to afford compound **14** (114 mg, 69%) after flash chromatography on silica gel (CH₂Cl₂ - MeOH - NH₄OH, 5 : 1 : 0.05) followed by crystallization from a mixture of Et₂O - heptane: Rf 0.14 (CH₂Cl₂ - MeOH - NH₄OH, 10 : 1 : 0.05); mp 153 ~ 155°C; $[\alpha]_D^{20}$ -73 (*c* 1, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 1.00 (3H, d, *J* = 7 Hz, 8-CH₃), 2.68 (1H, broad q, *J* = 6.9 Hz, 10-H), 3.00 (1H, d, *J* = 9.1 Hz, 4''-H), 3.22 (1H, dd, *J* = 7 and 10.4 Hz, 2'-H), 3.27 (3H, s, 3''-OCH₃), 3.51 (3H, s, 3''-OCH₃), 3.59 (1H, dd, *J* = 2.7 and 7.6 Hz, 2'''-H), 3.90 (1H, dd, *J* = 3.1 and 11.9 Hz, 6'''-Heq), 3.99 (1H, d, *J* = 9.4 Hz, 3-H), 4.40 (1H, d, *J* = 7 Hz, 1'-H), 4.58 (1H, d, *J* = 7.6 Hz, 1'''-H), 4.76 (1H, broad d, *J* = 2.7 Hz, 3'''-H), 4.89 (1H, d, *J* = 4.2 Hz, 1''-H) and 5.06 (1H, broad d, *J* = 10.9 Hz, 13-H); ¹³C NMR (CDCl₃, 62.5 MHz): δ 14.7 (10-CH₃), 19.0 (8-CH₃), 26.7 (C-8), 33.4 (C-10), 57.2 (1'''-OCH₃), 62.7 (C-6'''), 68.1 (C-4'''), 70.1 (C-2'''), 75.4 (C-5'''), 82.1 (C-3'''), 102.4 (C-1''') and 171.5 (C-9); FAB-MS (positive): *m/z* 947 (M + Na)⁺, 925 (M + H)⁺ and 767 [M + H - (Cladinose - H)]⁺.

FAB-HR-MS Calcd for C₄₄H₈₁N₂O₁₈, (M + H)⁺:
925.5484.

Found:
925.5526.

9-[O-(Methyl β-D-mannopyranosid-2-yl)oxime] of Erythromycin A (16)

Deprotection of compound **5** (200 mg, 0.18 mmol) was carried out following the general procedure to furnish an isomeric mixture (*E* and *Z*) of oximes **16** (114 mg, 68%) after flash chromatography on silica gel (CH₂Cl₂ - MeOH - NH₄OH, 10 : 1 : 0.05) as a foam: Rf 0.12 (CH₂Cl₂ - MeOH - NH₄OH, 10 : 1 : 0.05); ¹H NMR (CDCl₃, 300 MHz), (*E* isomer): δ 1.00 (3H, d, *J* = 7 Hz, 8-CH₃), 2.66 (1H, broad q, *J* = 7.1 Hz, 10-H), 3.00 (1H, dd, *J* = 8.9 and 9 Hz, 4''-OH), 3.22 (1H, dd, *J* = 7.1 and 10.3 Hz, 2'-H), 3.29 (3H, 3''-OCH₃), 3.32 (1H, broad dd, *J* = 4.8 and 9.5 Hz, 5'''-H), 3.51 (3H, s, 1'''-OCH₃), 3.67 (1H, broad s, 11-H), 3.69 (1H, dd, *J* = 2.9 and 9.5 Hz, 3'''-H), 3.79 (1H, t, *J* = 9.5 Hz, 4'''-H), 3.85 (1H, dd, *J* = 4.8 and 11.5 Hz, 6'''-Hax), 3.92 (1H, broad d, *J* = 11.5 Hz, 6'''-Heq), 3.96 (1H, broad d, *J* = 9.7 Hz, 3-H), 4.03 (1H, dq, *J* = 6.5 and 9.5 Hz, 5''-H), 4.36 (1H, d, *J* = 7.1 Hz, 1'-H), 4.48 (1H, broad s, 1'''-H), 4.52 (1H, broad s, *J* = 2.9 Hz, 2'''-H), 4.87 (1H, d, *J* = 4.2 Hz, 1''-H) and 5.04 (1H, broad d, *J* = 10.9 Hz, 13-H); ¹³C NMR (CDCl₃, 62.5 MHz), (*E* isomer): δ 14.8 (10-CH₃), 19.2 (8-CH₃), 26.4 (C-8), 33.0 (C-10), 57.1 (1'''-OCH₃), 62.7 (C-6'''), 68.8 (C-4'''), 74.7 (C-3'''), 76.4 (C-5'''), 80.7 (C-2'''), 101.3 (C-1''') and 170.2 (C-9); FAB-MS (positive): *m/z* 947 (M + Na)⁺ and 925 (M + H)⁺.

FAB-HR-MS Calcd for C₄₄H₈₁N₂O₁₈, (M + H)⁺:
925.5484.

Found:
925.5541.

(E)-[O-(Methyl β-D-glucopyranosid-3-yl)oxime] of Erythromycin A (17)

Deprotection of substrate **6** (140 mg, 0.13 mmol) was carried out following the general procedure to furnish compound **17** (64 mg, 55%) after flash chromatography on silica gel (CH₂Cl₂ - MeOH - NH₄OH, 10 : 1 : 0.05) as a foam: Rf 0.13 (CH₂Cl₂ - MeOH - NH₄OH, 10 : 1 : 0.05); $[\alpha]_D^{20}$ -73 (*c* 1, MeOH); ¹H NMR (CDCl₃, 300 MHz): δ 1.00 (3H, d, *J* = 7 Hz, 8-CH₃), 2.41 (1H, ddd, *J* = 2.4, 10.2 and 12.2 Hz, 3'-H), 2.58 (1H, broad q, *J* = 6.9 Hz, 10-H), 2.97 (1H, d, *J* = 9 Hz, 4''-H), 3.18 (1H, dd, *J* = 7.1 and 10.2 Hz, 2'-H), 3.24 (3H, s, 3''-OCH₃), 3.31 (1H, dd, *J* = 6 and 9.6 Hz, 5'''-H), 3.42 (1H, dd, *J* = 7.6 and 9.6 Hz, 2'''-H), 3.50 (3H, s, 1'''-OCH₃), 3.69 (1H, s, 11-H), 3.71 (1H, t, *J* = 9.6 Hz, 4'''-H), 3.88 (2H, d, *J* = 6 Hz, 6'''-H), 3.93 (1H, broad d, *J* = 9.5 Hz, 3-H), 4.00 (1H, dd, *J* = 6.2 and 9.7 Hz, 5''-H), 4.07 (1H, t, *J* = 9.6 Hz, 3'''-H), 4.25 (1H, d, *J* = 7.6 Hz, 1'''-H), 4.34 (1H, d, *J* = 7.1 Hz, 1'-H), 4.88 (1H, d, *J* = 4.2 Hz, 1''-H) and 5.04 (1H, dd, *J* = 2.3 and 10.5 Hz, 13-H); ¹³C NMR (CDCl₃, 75 MHz): δ 14.7 (10-CH₃), 19.0 (8-CH₃), 26.5 (C-8), 32.5 (C-10), 57.3 (1'''-OCH₃), 62.0 (C-6'''), 69.2 (C-4'''), 71.2 (C-2'''), 75.8 (C-5'''), 86.9 (C-3'''), 104.1 (C-1''') and 173.2 (C-9); FAB-MS (positive): *m/z* 947 (M + Na)⁺ and 925 (M + H)⁺.

FAB-HR-MS Calcd for $C_{44}H_{81}N_2O_{18}$, $(M+H)^+$:

925.5484.

Found:

925.5519.

(E)-9-[O-(Methyl α -D-galactopyranosid-4-yl)oxime] of Erythromycin A (18)

Compound **7** (350 mg, 0.27 mmol) was deprotected following the general procedure to furnish compound **18** (188 mg, 75%) after flash chromatography on silica gel (CH_2Cl_2 -MeOH- NH_4OH , 7:1:0.05) and crystallization from $CHCl_3$ -heptane as a white solid: Rf 0.20 (CH_2Cl_2 -MeOH- NH_4OH , 7:1:0.05); mp 152~154°C; $[\alpha]_D^{20}$ -18 (*c* 1, $CHCl_3$); 1H NMR ($CDCl_3$, 300 MHz): δ 1.00 (3H, d, $J=7$ Hz, 8- CH_3), 2.66 (1H, dq, $J=1.4$ and 6.9 Hz, 10-H), 3.18 (1H, dd, $J=7.2$ and 10.3 Hz, 2'-H), 3.28 (3H, s, 3''-OCH₃), 3.38 (3H, s, 1'''-OCH₃), 3.47 (1H, d, $J=7.8$ Hz, 5-H), 3.80 (1H, dd, $J=2.7$ Hz, and 9.8 Hz, 2'''-H), 3.87 (1H, dd, $J=3$ and 9.8 Hz, 3'''-H), 4.08 (1H, broad d, $J=9.5$ Hz, 3-H), 4.40 (1H, d, $J=7.2$ Hz, 1'-H), 4.62 (1H, broad d, $J=3$ Hz, 4'''-H), 4.87 (1H, d, $J=2.7$ Hz, 1'''-H), 4.95 (1H, d, $J=5$ Hz, 1''-H) and 5.03 (1H, dd, $J=2.4$ and 10.8 Hz, 13-H); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 14.5 (10- CH_3), 19.2 (8- CH_3), 26.5 (C-8), 32.8 (C-10), 55.6 (1'''-OCH₃), 60.9 (C-6'''), 70.5 (C-2''', C-3''' and C-5'''), 79.7 (C-4'''), 99.8 (C-1''') and 171.2 (C-9); FAB-MS (positive): m/z 947 $(M+Na)^+$ and 925 $(M+H)^+$.

FAB-HR-MS Calcd for $C_{44}H_{81}N_2O_{18}$, $(M+H)^+$:

925.5484.

Found:

925.5475.

(E)-9-[O-(D-Glucos-4-yl)oxime] of Erythromycin A (19)

Deprotection of substrate **8** (435 mg, 0.34 mg) was carried out following the general procedure to furnish compound **19** (187 mg, 60%), mainly as an anomeric mixture of the pyranosidic forms, after flash chromatography on silica gel (CH_2Cl_2 -MeOH- NH_4OH , 5:1:0.05) as a foam: Rf 0.22 (CH_2Cl_2 -MeOH- NH_4OH , 5:1:0.05); 1H NMR ($CDCl_3$, 300 MHz): δ 4.41 (1H, d, $J=7$ Hz, 1'-H), 4.51 (1H, t, $J=9.3$ Hz, 4'''-H), 4.63 (1H, d, $J=7$ Hz, 1'''-H β), 4.98 (1H, d, $J=5$ Hz, 1''-H), 5.06 (1H, broad d, $J=11$ Hz, 13-H) and 5.32 (1H, d, $J=3$ Hz, 1'''-H α); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 14.9 (10- CH_3), 18.7 (8- CH_3), 26.3 (C-8), 33.8 (C-10), 61.9 (C-6''' α and C-6''' β), 70.5 (C-5''' α), 71.9 (C-3''' α), 72.6 (C-2''' α), 74.4 (C-5''' β), 75.1 (C-2''' β), 75.5 (C-3''' β), 81.2 (C-4''' α), 81.3 (C-4''' β), 92.4 (C-1''' α), 98.7 (C-1''' β), 170.5 (C-9 α) and 170.9 (C-9 β); FAB-MS (positive): m/z 933 $(M+Na)^+$, 911 $(M+H)^+$ and 753 $[M+H-(Cladinose-H)]^+$.

FAB-HR-MS Calcd for $C_{43}H_{79}N_2O_{18}$, $(M+H)^+$:

911.5328.

Found:

911.5328.

(E)-9-{O-[(1-O-Methyl β -D-glucopyranosid-4-yl)- α -D-galactopyranos-4-yl]oxime} of Erythromycin A (20)

Deprotection of substrate **9** (400 mg, 0.2 mmol) was carried out following the general procedure to furnish compound **20** (174 mg, 65%) after flash chromatography on silica gel (CH_2Cl_2 -MeOH- NH_4OH , 5:1:0.05) and crystallization from Me_2CO as a white solid: Rf 0.15 (CH_2Cl_2 -MeOH- NH_4OH , 5:1:0.05); mp 195~199°C; $[\alpha]_D^{20}$ -8 (*c* 1, MeOH); 1H NMR ($DMSO-d_6$, 300 MHz): δ 1.00 (3H, d, $J=7$ Hz, 8- CH_3), 2.78 (1H, broad d, $J=6.9$ Hz, 10-H), 3.03 (1H, t, $J=8.5$ Hz, 4''-H), 3.33 (3H, s, 3''-OCH₃), 3.51 (3H, s, 1''''-OCH₃), 4.09 (1H, dq, $J=6.2$ and 9.4 Hz, 5''-H), 4.21 (1H, d, $J=7.7$ Hz, 1'-H), 4.59 (1H, d, $J=6.8$ Hz, 1''''-H), 4.72 (1H, t, $J=8.1$ Hz, 6''-Heq), 4.88 (2H, broad s, 1''-H and 2''-OH), 5.30 (1H, broad d, $J=10.7$ Hz, 13-H), 5.40 (1H, d, $J=5.2$ Hz, 2'-OH) and 5.49 (1H, d, $J=7.4$ Hz, 1'''-H); ^{13}C NMR ($DMSO-d_6$, 75 MHz): δ 13.4 (10- CH_3), 18.8 (8- CH_3), 25.8 (C-8), 30.8 (C-10), 55.8 (1''''-OCH₃), 59.9 (C-6'''), 60.3 (C-6'''), 69.1 (C-2'''), 69.4 (C-5'''), 71.6 (C-3'''), 73.9 (C-2'''), 75.1 (C-5'''), 76.0 (C-4'''), 77.1 (C-3'''), 83.4 (C-4'''), 100.5 (C-1'''), 101.1 (C-1''') and 168.4 (C-9); FAB-MS (positive): m/z 1087 $(M+H)^+$.

FAB-HR-MS Calcd for $C_{50}H_{91}N_2O_{23}$, $(M+H)^+$:

1087.6012.

Found:

1087.6041.

(E)-9-[O-(Methyl α -D-glucopyranosid-6-yl)oxime] of Erythromycin A (21)

To a stirred solution of compound **10** (178 mg, 0.17 mmol) in MeOH (1 ml) was added dropwise sodium methanolate (28 mg, 0.51 mmol) in MeOH (2.54 ml). The mixture was stirred for 10 minutes at room temperature and then, quenched with IRN 77 (H^+) ion exchange resin. The resin was filtered and washed with MeOH. The filtrate was concentrated under reduced pressure and the residue purified by flash chromatography on silica gel (CH_2Cl_2 -MeOH- NH_4OH , 10:1:0.05) to afford compound **21** (113 mg, 72%) as a foam: Rf 0.2 (CH_2Cl_2 -MeOH- NH_4OH , 10:1:0.05); $[\alpha]_D^{20}$ -29 (*c* 0.46, $CHCl_3$); 1H NMR ($CDCl_3$, 300 MHz): δ 1.02 (3H, d, $J=7$ Hz, 8- CH_3), 2.64 (1H, broad q, $J=6.7$ Hz, 10-H), 2.96 (1H, dd, $J=7.1$ and 10 Hz, 2-H), 3.03 (1H, dd, $J=1.2$ and 8.9 Hz, 4''-H), 3.26 (1H, dd, $J=7.3$ and 10.2 Hz, 2'-H), 3.32 (3H, s, 3''-OCH₃), 3.46 (3H, s, 1'''-OCH₃), 3.59 (1H, d, $J=7.8$ Hz, 5-H), 3.70 (1H, broad s, 11-H), 3.78~3.86 (1H, m, 8-H), 4.06 (1H, dq, $J=6.2$ and 9.7 Hz, 5''-H), 4.14 (1H, dd, $J=1.4$ and 10.3 Hz, 3-H), 4.34 (1H, broad d, $J=13.4$ Hz, 6'''-Hax), 4.40 (1H, d, $J=7.3$ Hz, 1'-H), 4.44 (1H, d, $J=13.4$ Hz, 6'''-Heq), 4.82 (1H, d, $J=1.8$ Hz, 1'''-H), 4.88 (1H, d, $J=4.6$ Hz, 1''-H) and 5.16 (1H, dd, $J=2$ and 9.5 Hz, 13-H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.9 (10- CH_3), 18.7 (8- CH_3), 26.8 (C-8), 34.0 (C-10), 55.4 (1'''-OCH₃), 71.1 and 71.2 (C-4''' and C-5'''), 72.4 (C-2'''), 72.8 (C-6'''), 75.4 (C-3'''), 100.7 (C-1''') and 171.6 (C-9); FAB-MS (positive): m/z 947 $(M+Na)^+$, 925 $(M+H)^+$ and 767 $[M+H-(Cladi-$

nose-H)]⁺.

FAB-HR-MS Calcd for C₄₄H₈₁N₂O₁₈, (M+H)⁺:

925.5484.

Found:

925.5477.

(E)-9-[O-(Methyl α-D-glucopyranosid-4-yl)oxime] of Erythromycin A (22)

To a stirred solution of compound **11** (335 mg, 0.27 mmol) in MeOH (1.5 ml) was added dropwise sodium methanolate (44 mg, 0.81 mmol) in MeOH (4 ml). The mixture was stirred for 15 minutes at room temperature and then, quenched with IRN 77 (H⁺) ion exchange resin. The resin was filtered and washed with MeOH. The filtrate was concentrated under reduced pressure and the residue purified by flash chromatography on silica gel (CH₂Cl₂-MeOH-NH₄OH, 10:1:0.05) to furnish compound **11** (162 mg, 65%) as a foam: Rf 0.16 (CH₂Cl₂-MeOH-NH₄OH, 10:1:0.05); [α]_D²⁰ -31 (c 0.83, CHCl₃); ¹H NMR (CDCl₃, 250 MHz): δ 0.98 (3H, d, J=7 Hz, 8-CH₃), 2.66 (1H, broad q, J=6.9 Hz, 10-H), 2.81 (1H, dd, J=7.5 and 10.2 Hz, 2-H), 2.92 (1H, d, J=9.7 Hz, 4''-H), 3.18 (1H, dd, J=7.5 and 10.2 Hz, 2'-H), 3.20 (3H, s, 3'''-OCH₃), 3.30 (3H, s, 1'''-OCH₃), 3.64 (1H, dd, J=4 and 10.9 Hz, 6'''-Hax), 4.34 (1H, d, J=7.5 Hz, 1'-H), 4.73 (1H, d, J=3.2 Hz, 1'''-H), 4.85 (1H, d, J=4.5 Hz, 1''-H) and 5.03 (1H, broad s, J=10.6 Hz, 13-H); ¹³C NMR (CDCl₃, 50 MHz): δ 14.7 (10-CH₃), 18.9 (8-CH₃), 26.6 (C-8), 33.1 (C-10), 55.4 (1'''-OCH₃), 62.1 (C-6'''), 70.6 (C-5'''), 72.4 and 72.6 (C-2''' and C-3'''), 80.5 (C-4'''), 99.7 (C-1''') and 170.9 (C-9); FAB-MS (positive): m/z 947 (M+Na)⁺ and 767 [M+H-(Cladinose-H)]⁺.

FAB-HR-MS Calcd for C₄₄H₈₁N₂O₁₈, (M+H)⁺:

925.5484.

Found:

925.5496.

In Vitro Antibacterial Activity

The *in vitro* activity in the tables is the ratio of the geometric mean of erythromycin A/geometric mean of the O-glycosyloximes derivatives MICs of 15 clinically isolated strains and 2 ATCC strains as reference. MIC were read after incubation at 37°C for 24 hours and measured by standard broth dilution methods. Concentration in the range 32~0.06 mg/liter were tested. Mueller Hinton broth supplemented with 10% lysed horse blood was used for all strains with the following exception: *Heamophilus* were grown in Mueller Hinton broth supplemented with Fildes extract.

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References

- 1) WASHINGTON, J. A., II & W. R. WILSON: Erythromycin A: A microbial and clinical perspective after 30 years of clinical use (first of two parts). *Mayo Clin. Proc.* 60: 189~203, 1985
- 2) WASHINGTON, J. A., II & W. R. WILSON: Erythromycin A: A microbial and clinical perspective after 30 years of clinical use (second of two parts). *Mayo Clin. Proc.* 60: 271~278, 1985
- 3) LARTEY, P. A. & R. FAGHIIH: Recent progress in the chemical modification of erythromycin. *In* Recent progress in the chemical synthesis of antibiotics and related microbial products. *Ed.*, G. LUKACS, Vol. 2, pp. 121~140, Springer-Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong, Barcelona, Budapest, 1993
- 4) DANAN, G.; V. DESCATOIRE & D. PESSAYRE: Self induction by erythromycin and its own transformation into metabolite forming an inactive complex with reduced cytochrome P450. *J. Pharmacol. Exp. Ther.* 218: 509~514, 1981
- 5) BABANY, G.; D. LARREY & D. PESSAYRE: Macrolide antibiotics as inducers and inhibitors of cytochrome P450 in experimental animals and man. *In* Progress in drug metabolism. *Ed.*, G. G. GIBSON, pp. 61~98, Taylor and Francis, London, 1988
- 6) SAKAKIBARA, H. & S. ŌMURA: Chapter 3. Chemical modification and structure-activity relationship of macrolides. *In* Macrolide antibiotics. Chemistry, biology and practice. *Ed.*, S. ŌMURA, pp. 85~125, Academic Press, New-York, 1984
- 7) DELAFORGE, M.; P. LADAM, G. BOUILLÉ, J. GHARBI-BENAROUS, M. JAOUEN & J. P. GIRAULT: pH effects on the N-demethylation and formation of the cytochrome P450 iron II nitrosoalkane complex for erythromycin derivatives. *Chem.-Biol. Interactions* 85: 215~227, 1992
- 8) DELAFORGE, M.; M. JAOUEN & D. MANSUY: Dual effects of macrolide antibiotics on rat liver cytochrome P-450. Induction and formation of metabolite-complexes: A structure-activity relationship. *Biochem. Pharmacol.* 32: 2309~2318, 1983
- 9) GHARBI-BENAROUS, J.; P. LADAM, M. DELAFORGE & J. P. GIRAULT: Conformational analysis of major metabolites of macrolide antibiotics roxithromycin and erythromycin A with different biological properties by NMR spectroscopy and molecular dynamics. *J. Chem. Soc., Perkin Trans. 2:* 2303~2315, 1993
- 10) GASC, J. C.; S. GOUIN D'AMBRIÈRES, A. LUTZ & J. F. CHANTOT: New ether oxime derivatives of erythromycin A. A structure-activity relationship study. *J. Antibiotics* 44: 313~330, 1991
- 11) GRANDJEAN, C. & G. LUKACS: S_N2 displacement of carbohydrate triflates by 9-oximes of erythromycin A and of a tylosin derivative. *J. Carbohydr. Chem.:* in the press.
- 12) WATANABE, Y.; M. KASHIMURA, T. ASAKA, T. ADACHI & S. MORIMOTO: Chemical modifications of erythromycin. X. Removal of benzyloxycarbonyl and 2-chlorobenzyl groups of erythromycin derivatives by use of catalytic transfer hydrogenation. *Heterocycles* 36: 761~768, 1993
- 13) WATANABE, Y.; S. MORIMOTO, T. ADACHI, M. KASHIMURA & T. ASAKA: Chemical modifications of erythromycin.

- XI. Selective methylation at the C-6 hydroxyl group of erythromycin A oxime derivatives and preparation of clarithromycin. *J. Antibiotics* 46: 647~660, 1993
- 14) NAGATE, T.; Y. WATANABE & S. ŌMURA: Chemical modification of erythromycins. II. Synthesis and anti-bacterial activity of *O*-alkyl derivatives of erythromycin A. *J. Antibiotics* 43: 286~294, 1990
- 15) MASSEY, E. H.; B. KITCHELL, L. D. MARTIN, K. GERZON & H. W. MURPHY: Erythromycylamine. *Tetrahedron Lett.* 2: 157~160, 1970