Cladinose Analogues of Sixteen-membered Macrolide Antibiotics

V. Preparation of Unsubstituted L-Cladinose Analogues: Effect of Methylation of a 3″-Hydroxyl Group on the Bioactivity

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Sixteen-membered macrolide antibiotics[1] have been used in clinic because of their efficacy and safety. The design and synthesis of 16-membered macrolide derivatives considering deacylation[2] at a neutral sugar moiety *in vivo* would be an important approach to generate conceptually effective analogues in clinical use. As part of our program in this area we have recently designed and synthesized 4-*O*-alkyl-L-cladinose analogues of leucomycins[3~5], and their efficacy was demonstrated both *in vitro* and *in vivo*[6]. In this communication we wish to report the preparation and potency of unsubstituted L-cladinose analogues (**4**~**6**) of sixteen-membered macrolides, which were designed based on a structure of a neutral sugar in erythromycin. The reported compounds (**4**~**6**) exhibited dramatically enhanced activity *in vitro* in comparison with mycarose-type counterparts (Fig. 1). The greatly improved protecting effects *in vivo*[7] of **1** and its 9-*O*-acetyl derivative **8** (Fig. 2) could be explained with the unexpectedly improved *in vitro* activity of **4** determined as their major metabolite.

There are structural differences in neutral sugar moieties between sixteen- and fourteen-membered macrolide antibiotics. Although 14-membered macrolides possess mainly either cladinose or mycarose at the C-3 position, only mycarose is attached to the C-4′ position in 16-membered macrolides. Since a sixteen-membered macrolide having an unsubstituted α-L-cladinosyl residue has not been reported, studies of the compounds **4**~**6** would be challenging.

Effective transformations of **1** to the desired L-cladinose analogues (**4**~**6**) were done by appropriate microbial transformations[8] (Scheme 1). Oxidation of an allylic alcohol of **1**[7] afforded a dienone (**2**[9], 80% yield), MP 108°C, $[\alpha]_D^{17}$ −30° (*c* 0.6, MeOH), FD-MS *m/z* 826 $(M+H)^+$. A carbomycin B-type analogue of **2** (3″-*O*-methylcarbomycin B) has been synthesized by TATSUTA *et al.*[10] *via* glycosylation as a leading research. On the other hand, regioselective removal of a 3-*O*-propionyl group of **1** was achieved with an efficient biotransformation[11] using *Phialophora* sp. PF1083[12], furnishing a 3-OH derivative (**3**, 28% yield), MP 111~113°C, $[\alpha]_D^{17}$ −79° (*c* 1.0, MeOH), EI-MS *m/z* 771 $(M)^+$, ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.79 (1H, br d, J=11.0 Hz, 3-H) accompanied with recovered **1** (16%). Useful cleavage of the 3-*O*-acyl group in leucomycin family by synthetic chemistry has not been reported.

Next, these 4-*O*-acyl-L-cladinose derivatives (**1**~**3**) were converted to the corresponding unsubstituted L-cladinose analogues (**4**~**6**) respectively, using *Mucor spinescens* IAM 6071[13] or *Paecilomyces* sp. PF1108[9]. All new compounds provided satisfactory spectroscopic data (Table 1). One explanation concerning these incomplete conversion yields might involve steric hindrance of a 3″-*O*-methyl group of **1**~**3**. Bioconversion of a 4-*O*-acyl-L-mycarose derivative to the unsubstituted-L-mycarose analogue was proceeded more efficiently[13]. This difficulty about 4″-de-*O*-propionylation by biological medium of **1**~**3** might suggest that a 4″-*O*-acyl group of these cladionose analogues would be hardly cleaved by metabolism *in vivo* in comparison with that of the L-mycarose counterparts.

Antibacterial activities *in vitro* of the novel unsubstituted α-L-cladinosyl derivatives (**4**~**6**), compared with those of sixteen-membered macrolide antibiotics possessing L-mycarose, 4″-de-*O*-propionylmidecamycin A_1[13] (**7**), DOP[14] and leucomycin V[15] (LM-V) (Fig. 1), are shown in Table 2. As judged from MIC values, these cladinose analogues exhibited about four to sixteen times

Fig. 1. Sixteen-membered macrolide antibiotics possessing unsubstituted L-mycarose as a neutral sugar.

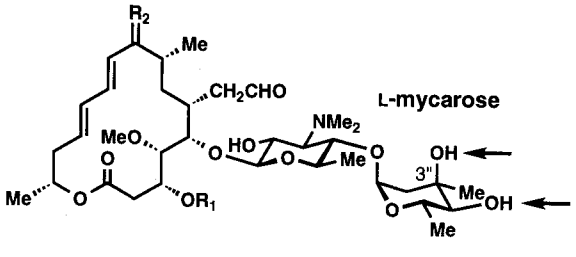
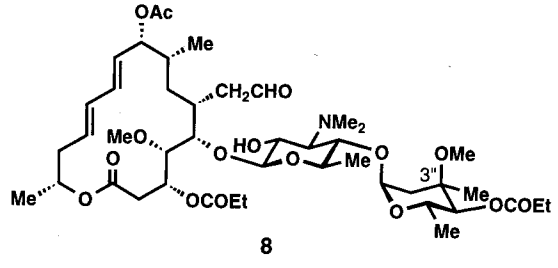
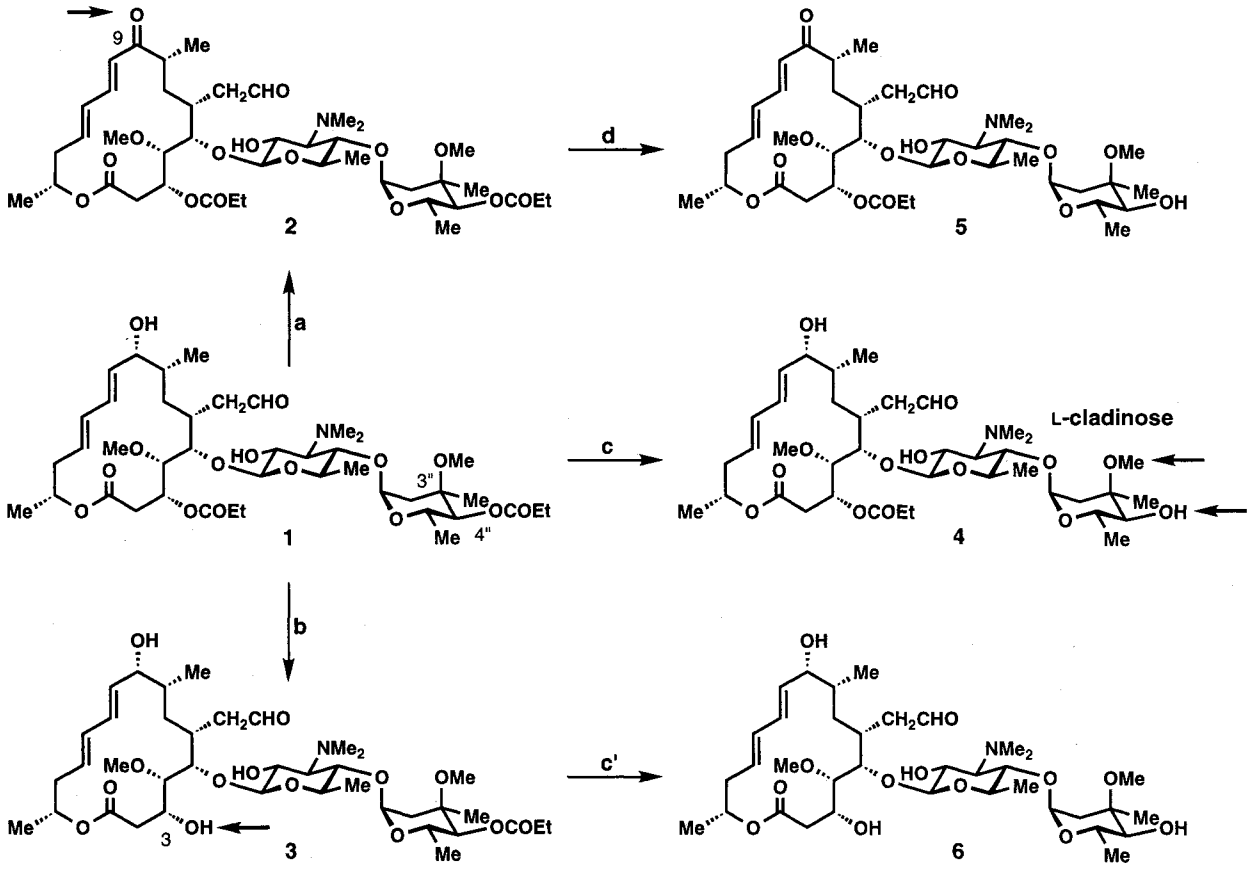


Fig. 2. Compound **8**: One of metabolically-programmed semisynthetic sixteen-membered macrolide antibiotics.



Scheme 1. Transformations of compound **1** to unsubstituted L-cladinose analogues (**4**~**6**)[a].



[a]**Methods and conditions: (a) CrO_3, aq pyr, 25°C, 2 h, 80%; (b) *Phialophora* sp. PF1083, 26°C, 10 days, 28% of 3 plus 16% recovered 1 ; (c) *Mucor spinescens* IAM 6071, 26°C, 8 days, 39% of 4 plus 27% recovered 1; (d) *Paecilomyces* sp. PF1108, 26°C, 9 days, 43%; (c') *Mucor spinescens* IAM 6071, 26°C, 9 days, 22% of 6 plus 17% recovered 3.**

Table 1. Physico-chemical properties of **4**~**6**.

Compound (**4**): MP 120~122°C; EI-MS *m/z* 771 $(M)^+$; $[\alpha]_D^{17}$ −56° (*c* 1.0, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (1H, br ddd, 7-H), 0.98 (3H, d, 19-H), 1.16 (3H, d, 6′-H), 1.22 (3H, t, 3-$OCOCH_2CH_3$), 1.22 (3H, s, 3″-CH_3), 1.23 (3H, d, 6″-H), 1.26 (3H, d, 16-H), 1.54 (1H, br dt, 7-H), 1.57 (1H, dd, 2″-H_{ax}), 1.89 (1H, m, 8-H), 2.15 (1H, dt, 14-H), 2.23 (1H, d, 2″-H_{eq}), 2.24 (1H, br d, 2-H), 2.32 (1H, br dd, 17-H), 2.39 (1H, t, 3′-H), 2.46 (1H, br dt, 14-H), 2.51 and 2.65 (each 1H, 2×dq, 3-$OCOCH_2CH_3$), 2.55 (6H, s, 3′-$N(CH_3)_2$), 2.76 (1H, dd, 2-H), 2.86 (1H, br dd, 17-H), 3.01 (1H, br t, 4″-H), 3.21 (1H, dd, 2′-H), 3.22 (3H, s, 3″-OCH_3), 3.26 (1H, br d, 4-H), 3.27 (1H, dq, 5′-H), 3.44 (1H, t, 4′-H), 3.58 (3H, s, 4-OCH_3), 3.88 (1H, br d, 5-H), 4.07 (1H, dd, 9-H), 4.18 (1H, dq, 5″-H), 4.51 (1H, d, 1′-H), 4.88 (1H, d, 1″-H), 5.03 (1H, ddq, 15-H), 5.14 (1H, br d, 3-H), 5.61 (1H, dd, 10-H), 5.79 (1H, ddd, 13-H), 6.08 (1H, br dd, 12-H), 6.67 (1H, dd, 11-H), 9.63 (1H, br s, 18-H).

Compound (**5**): MP 121~124°C; EI-MS *m/z* 769 $(M)^+$; $[\alpha]_D^{20}$ −27° (*c* 1.0, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ1.14 (3H, t, 3-$OCOCH_2CH_3$), 1.16 (3H, d, 6′-H), 1.20 (3H, d, 19-H), 1.22 (3H, s, 3″-CH_3), 1.23 (3H, d, 6″-H), 1.29 (3H, d, 16-H), 1.49 (1H, br t, 7-H), 1.57 (1H, dd, 2″-H_{ax}), 1.64 (1H, dt, 7-H), 1.78 (1H, br t, 6-H), 2.24 (1H, d, 2″-H_{eq}), 2.26 (1H, br d, 2-H), 2.58 (6H, br s, 3′-$N(CH_3)_2$), 2.75 (1H, br dd, 17-H), 2.78 (1H, dd, 2-H), 3.01 (1H, br d, 4″-H), 3.21 (1H, dd, 2′-H), 3.22 (3H, s, 3″-OCH_3), 3.27 (1H, dq, 5′-H), 3.30 (1H, br d, 4-H), 3.45 (1H, t, 4′-H), 3.60 (3H, s, 4-OCH_3), 3.89 (1H, br d, 5-H), 4.15 (1H, dq, 5″-H), 4.50 (1H, d, 1′-H), 4.85 (1H, ddq, 15-H), 4.89 (1H, d, 1″-H), 5.09 (1H, br d, 3-H), 6.22 (2H, m, 12-H, 13-H), 6.34 (1H, d, 10-H), 7.38 (1H, dd, 11-H), 9.54 (1H, s, 18-H).

Compound (**6**): MP 117~122°C; EI-MS *m/z* 715 $(M)^+$; $[\alpha]_D^{17}$ −74° (*c* 1.0, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 0.95 (1H, br ddd, 7-H), 0.99 (3H, d, 19-H), 1.19 (3H, d, 6′-H), 1.22 (3H, s, 3″-CH_3), 1.23 (3H, d, 6″-H), 1.31 (3H, d, 16-H), 1.57 (1H, dd, 2″-H_{ax}), 1.60 (1H, br dt, 7-H), 1.91 (1H, m, 8-H), 2.12 (1H, dt, 14-H), 2.23 (1H, d, 2-H), 2.24 (1H, d, 2″-H_{eq}), 2.34 (1H, br dd, 17-H), 2.40 (1H, t, 3′-H), 2.51 (1H, br dt, 14-H), 2.56 (6H, s, 3′-$N(CH_3)_2$), 2.70 (1H, dd, 2-H), 2.88 (1H, br dd, 17-H), 3.01 (1H, br t, 4″-H), 3.10 (1H, br d, 4-H), 3.22 (3H, s, 3″-OCH_3), 3.23 (1H, dd, 2′-H), 3.26 (1H, dq, 5′-H), 3.44 (1H, t, 4′-H), 3.55 (3H, s, 4-OCH_3), 3.80 (1H, br d, 3-H), 4.11 (1H, dd, 9-H), 4.11 (1H, br d, 5-H), 4.18 (1H, dq, 5″-H), 4.58 (1H, d, 1′-H), 4.89 (1H, d, 1″-H), 5.29 (1H, ddq, 15-H), 5.61 (1H, ddd, 13-H), 5.69 (1H, dd, 10-H), 6.04 (1H, br dd, 12-H), 6.27 (1H, dd, 11-H), 9.80 (1H, br s, 18-H).

Table 2. Antibacterial activities of L-cladinose analogues (**4**~**6**) and corresponding L-mycarose analogues (MIC, μg/ml).

Test organisms	**4**	**5**	**6**	**7**	DOP	LM-V
Staphylococcus aureus 209P JC-1	0.39	0.20	0.39	6.25	1.56	1.56
S. aureus M133	1.56	0.78	0.78	12.5	3.13	6.25
S. aureus M126	>100	>100	>100	>100	>100	>100
S. aureus MS15026	>100	>100	>100	>100	>100	>100
S. aureus MS15027	0.78	0.78	0.78	6.25	3.13	3.13
S. epidermidis ATCC14990	1.56	0.78	0.78	12.5	3.13	3.13
Micrococcus luteus ATCC9341	0.10	<0.025	0.05	0.39	0.20	0.20
Enterococcus faecalis W-73	0.78	0.39	0.78	12.5	3.13	6.25
Streptococcus pneumoniae IP692	0.10	0.05	0.10	0.78	0.39	0.39
S. pneumoniae Type I	0.10	<0.025	0.05	0.78	0.20	0.39
S. pyogenes Cook	0.20	0.10	0.10	1.56	0.39	0.39
Escherichia coli NIHJ JC-2	>100	>100	>100	>100	>100	>100
Klebsiella pneumoniae PCI602	>100	>100	>100	>100	>100	>100
Moraxella catarrhalis W-0500	3.13	1.56	1.56	25	12.5	12.5
M. catarrhalis W-0506	3.13	0.78	1.56	25	12.5	12.5
Haemophilus influenzae 9334	12.5	1.56	1.56	100	50	12.5
H. influenzae Type b	50	12.5	12.5	>100	100	50

4 *vs.* **7**: 3-*O*-acyl derivatives; **5** *vs.* DOP: 3-*O*-acyl-9-dehydro derivatives; **6** *vs.* LM-V: 3-OH derivatives.

higher activity than their counterparts respectively (for example **4** *vs.* **7**). This is the first report about 16-membered macrolides having unsubstituted cladinose at the C-4′ position, since it has been difficult to predict enhancement of *in vitro* activity by introducing a methyl group into the 3″-OH.

A neutral sugar moiety of natural sixteen-membered macrolide antibiotics and their acylated derivatives is converted to unsubstituted mycarose by metabolism in general. As reported partly in our previous paper[7], however, compound **1** and its 9-*O*-acetyl derivative (**8**) were mainly metabolized to an active metabolite **4** in mice. Actually, compound **4** was detected as a main metabolite in urine after oral administration of **8** in mice. Moreover, incubation of **8** with human liver S9 fraction gave **4** as one of major metabolites. These observations explained that the 4-*O*-acyl-L-cladinose analogues, compound **1**, **3** and their derivatives, for example **8**, could exhibit dramatically improved efficacy *in vivo* (In leucomycin family, compounds having an sp^3 carbon at the C-9 position exhibited superior efficacy *in vivo*[4,16] than those having an sp^2 carbon like compound **2**).

In conclusion, a series of sixteen-membered macrolides possessing an unsubstituted α-L-cladinose moiety were prepared *via* appropriate biotransformations. They showed antibacterial activity four to sixteen-fold more potent *in vitro* than the counterparts with the α-L-mycarose moiety. The clarified preliminary metabolic pathway of **1** and **3** and the described potency of their metabolites (**4** and **6**) open the way for eventually new metabolically-programmed sixteen-membered macrolide antibiotics.

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