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Chemical Modification of Reveromycin A and Its Biological Activities

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Abstract—Various derivatives of reveromycin A, a novel inhibitor of eukaryotic cell growth, were prepared and their inhibitory effects on both isoleucyl-tRNA synthetase activity and in vitro protein synthesis, and activities on the morphological reversion of *src*^{ts}-NRK cells were assayed. The C5 hydroxyl group and C24 carboxyl group are particularly important for these activities.

Reveromycins A–D (**1**–**4**, Fig. 1) are novel polyketide-type antibiotics isolated from the genus *Streptomyces* as inhibitors of mitogenic activity induced by the epidermal growth factor (EGF) in a mouse epidermal keratinocyte.[1] An inhibitor of mitogenic activity of EGF or transforming growth factor α (TGF- α), which shares the same receptor with EGF, may be the focal point for the development of a novel range of antitumor drugs. Therefore, reveromycins are possible new antitumor drugs with a novel mechanism of action. During the course of searching for the reveromycin A (1) target protein, we found several biological activities such as the morphological reversion of *src*^{ts}-NRK cells from spherical transformed cells to flat cells without any noticeable cytotoxicity (EC_{50} = 1.58 μg/mL), the antiproliferative activity against human tumor cell lines (IC_{50} = 1.3–2.0 μg/mL), antifungal activity (MIC = 2.0 μg/mL, pH 3), and in vitro protein synthesis of rabbit reticulocytes (IC_{50} = 40 nM).[2,3] Recently, the molecular target of **1** was identified as isoleucyl-tRNA synthetase (IleRS) using yeast genetics and biochemical studies.[4] The IC_{50} on this enzyme was 1.3 ng/mL, while other aminoacyl-tRNA synthetases were not inhibited.

The characteristic structural feature of **1** includes a 1,7-dioxaspiro[5.5]undecane moiety, that is, the 6,6-spiroacetal core bearing a hemisuccinate, two unsaturated carboxylic acid side chains and two alkyl groups.[5–7] Its strong biological activity as a potential drug and its synthetically challenging molecular architecture have attracted the attention of synthetic organic chemists, and the first asymmetric total synthesis of **1** was accomplished by our group.[8] However, the structure–activity relationships of **1** have not been systematically examined. We now report the synthesis of various derivatives of **1** and their biological activities.

Chemistry

The 6,6-spiroacetal core in **1** has two long alkenyl carboxylic acid side chains at C11 and C19 which might be in close proximity to each other (Fig. 2). Although the hemisuccinate of C18 *tert*-hydroxyl group and the C19

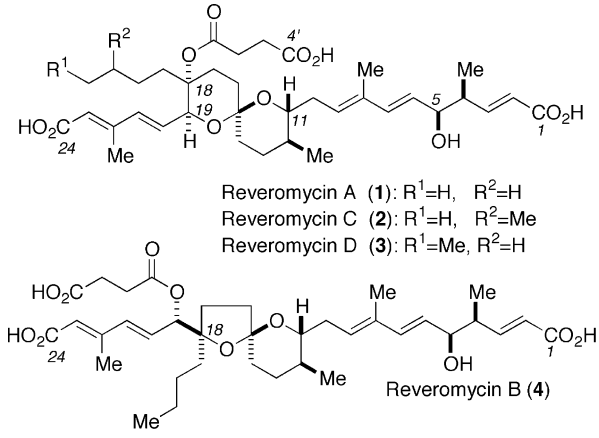

Figure 1. Structures of reveromycin A (**1**), C (**2**), D (**3**) and B (**4**).

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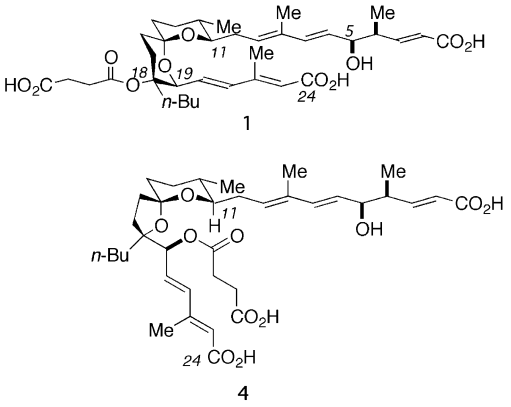


Figure 2. Possible conformations of reveromycin A (**1**) and B (**4**).

side chain are *trans*-diaxially oriented, we anticipated that the hydrogen bond between the C5 hydroxyl group and C24 carboxyl group might contribute to the stability of **1** in addition to the anomeric double stabilization. On the other hand, the antifungal activity of **1** (p*K*a 4.2) was markedly enhanced when the initial pH of the medium was decreased from 7.4 to 3.0.[2] However, the trimethyl ester derivative **8** (Scheme 1) was not active even at pH 3. These results suggested that the nondissociative form of the carboxylic acids is necessary for the antifungal activity. Based on these mentioned facts, the chemical modifications of the C5 hydroxyl group, C18 hemisuccinate and three carboxyl groups were designed to elucidate the structure–activity relationships of **1**.

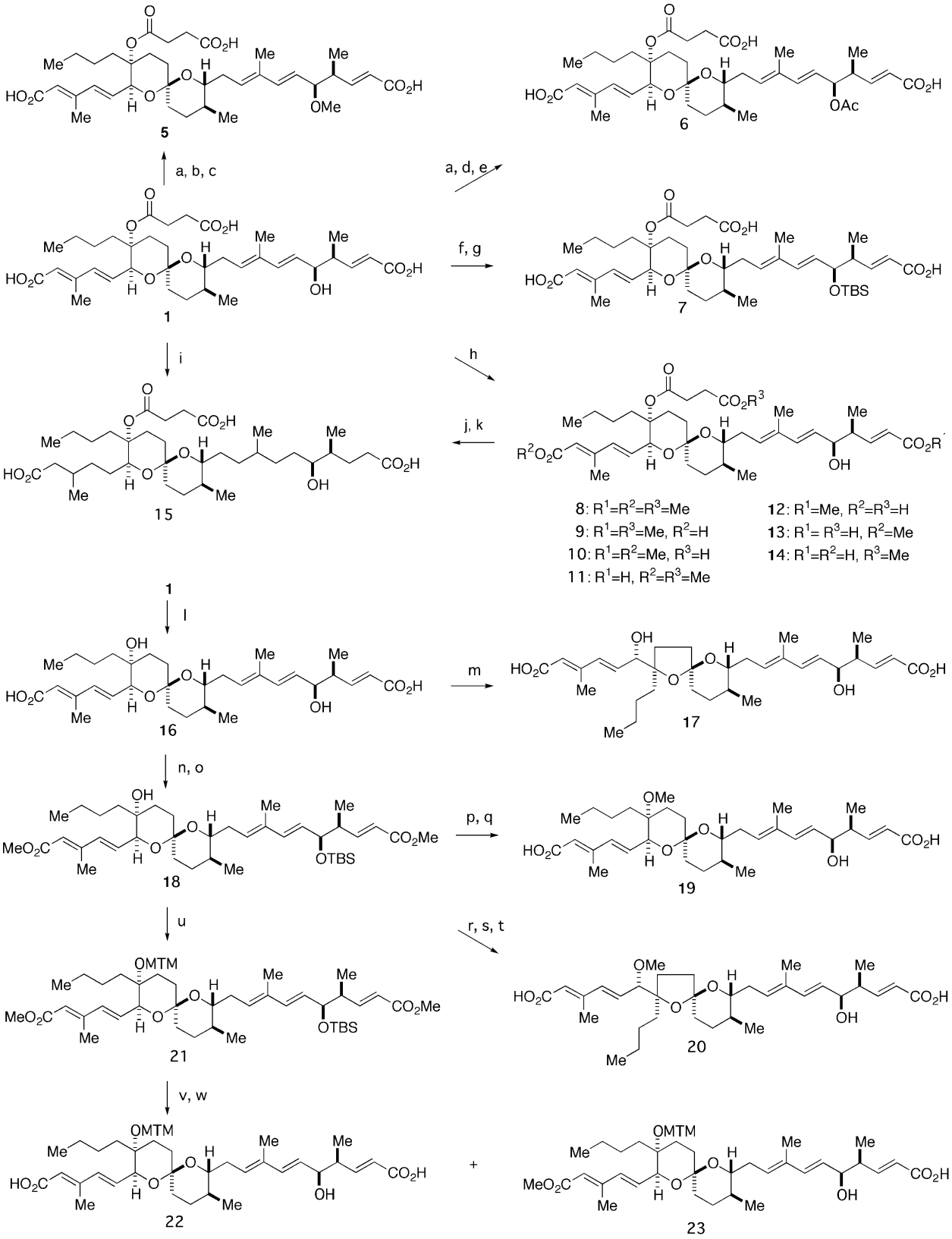
First, the C5 hydroxyl group in **1** was converted into the methoxy, acetoxy and silyloxy group to evaluate the correlation of the hydrogen bond to the C24 carboxyl group and the activity. Esterification of **1** with allyl alcohol in the presence of EDCI and DMAP at 1.5 Gpa[9] gave the triallyl ester in 36% yield. Methylation of the ester with MeOTf followed by the selective deprotection of the three allyl groups gave the C5 methoxy derivative **5** (81%, two steps) (Scheme 1). The C5 acetate **6** was obtained via the acetylation in a similar manner. The silylation of **1** with TBSCl followed by treatment with MeOH–THF afforded the C5 TBS ether **7** via hydrolysis of the silyl ester groups. The second task is preparation of the C24 ester derivatives. Although selective hydrolysis of the three methyl ester groups in **8** did not succeed, the esterification of 1 with 1 or 2 equivalents of $TMSCHN_2$[10] gave the C24 ester derivatives **8**, **10**, **11** and **13** and the C24 carboxylic acid derivatives **9**, **12** and **14**.[11] The catalytic hydrogenation of the ester **8** followed by deprotection of the methyl ester groups with LiSPr in HMPA[12] gave the saturated acids **15** in good yield, while the reduction of **1** afforded **15** in low yield. Next, the modification of the C18 hemisuccinyl group was carried out. The alkaline hydrolysis of **1** with LiOH gave the C18 hydroxyl derivative **16**[5] and then **16** was easily converted into the 5,6-spiroacetal derivative **17** using *p*-TsOH.[13] The esterification of **16** with $TMSCHN_2$ followed by silylation with TBSCl gave the C18 hydroxyl derivative **18**. The treatment of **18** with MeI-NaH[14] gave the C18 methoxy dicarboxylic acid accompanied with hydrolysis of the ester groups, and the acid was desilylated with TBAF to afford the C18 methoxy derivative **19**. The C18 hydroxyl derivative **18** having the 6,6-spiroacetal core was converted into the C19 methoxy derivative **20** having a 5,6-spiroacetal core in a similar manner. The C18 hydroxyl derivative **18** was also treated with DMSO-Ac_2O[15] to afford the C18 MTM ether derivative **21**. Then, **21** was partially hydrolyzed with LiOH and desilylated using TBAF to give the C18 MTM ether dicarboxylic acid **22** and monocarboxylic acid **23**.

Biological Activities and Discussion

We have already reported that reveromycin A (**1**) showed a strong inhibitory activity for the protein synthesis of the reticulocyte lysate system and morphological reversion activity on *src*^{ts}-NRK cells, while reveromycin B (**4**) showed little morphological reversion even at the concentration of 50 μg/mL.[2] It was also revealed that the molecular target of **1** was the isoleucyl-tRNA synthetase.[4] Therefore, we tested these biological activities of the designed compounds and the results are shown in Table 1.

Effects on both isoleucyl-tRNA synthetase activity and in vitro protein synthesis

Reveromycin A (**1**) strongly inhibited the IleRS activity and the IC_{50} on this enzyme was 1.3 ng/mL. The C5 methyl ether **5** having weak hydrogen bond weakly inhibited both the IleRS activity (IC_{50} = 165.0 ng/mL) and in vitro protein synthesis, while the C5 acetate **6** and the C5 TBS ether **7** did not inhibit both the activities. The triester **8** and the diesters **9**, **10** and **11** did not inhibit both the IleRS activity and in vitro protein synthesis. The C1 monoester **12** and C4′ monoester **14** having the C24 carboxyl group exhibited inhibitory effects on both the IleRS activity (IC_{50} = 92.8, 129.0 ng/mL, respectively) and in vitro protein synthesis. On the other hand, the C24 monoester **13** weakly inhibited the IleRS activity (IC_{50} = 490.8 ng/mL) and did not inhibit the in vitro protein synthesis. These results revealed that two of the three carboxyl groups in **1** are essential for the activities and the free hydroxyl group at C5 and the C24 carboxyl group are particularly important to show the strong activities. The saturated acid **15** did not show both the activities even though **15** contained all of the C5 hydroxyl group and three carboxyl groups. These results may be attributed to the free conformation of both substituents, in which formation of the hydrogen bond between the C5 hydroxyl group and C24 carboxyl group is hard. The C18 hydroxyl derivative **16** and the C18 methoxy derivative **19** strongly inhibited both the IleRS activity (IC_{50} = 41.7, 25.5 ng/mL, respectively) and in vitro protein synthesis. The C18 MTM ether derivative **22** also exhibited both the activities. However, the C24 ester derivative **23** of **22** did not show both the activities. It became clear that the C24 carboxyl group in **22** is essential and the C18 hemisuccinate group in 1 is important but not essential for these activities.



Scheme 1. (a) Allyl alcohol, EDCI, DMAP, CH_2Cl_2, 1.5 GPa, rt (36%); (b) MeOTf, 2,6-di-*t*-butyl-4-methylpyridine, CH_2Cl_2, 80 °C (75%); (c) $Pd(Ph_3P)_4$, Ph_3P, pyrrolidine, CH_2Cl_2, rt (93%); (d) Ac_2O, pyridine, DMAP, rt (76%); (e) $Pd(Ph_3P)_4$, Ph_3P, pyrrolidine, CH_2Cl_2, rt (85%); (f) TBSCl, imidazole, DMF, rt; (g) MeOH–THF, rt (71%, 2 steps); (h) $TMSCHN_2$, benzene–MeOH, rt; (i) H_2, Pd/C, MeOH, rt (47%); (j) H_2, Pd/C, EtOAc, rt; (k) LiSPr, HMPA, rt (73%, 2 steps); (l) 1N LiOH, rt (96%); (m) *p*-TsOH, MeOH, rt (96%); (n) $TMSCHN_2$, benzene–MeOH, rt; (o) TBSCl, imidazole, DMF, rt (92%, 2 steps); (p) NaH, MeI, THF, 0 °C rt; (q) TBAF, DMF, rt (36%, 2 steps); (r) *p*-TsOH, CH_2Cl_2, rt; (s) NaH, MeI, THF, 0 °C rt; (t) TBAF, DMF, rt (68%, 3 steps); (u) DMSO, Ac_2O, rt; (v) 1N LiOH, THF–MeOH, rt; (w) TBAF, DMF, rt (**22**: 50%; **23**: 16%, 3 steps).

Table 1. IC_{50} on IleRS activity, inhibition of in vitro protein synthesis, and morphological reversion of *src*^{ts}-NRK cells by reveromycin derivatives **1**, **4**–**17**, **19**, **20**, **22** and **23**

Compd	IC_{50} on IleRS activity (ng/mL)	Inhibition of in vitro protein synthesis (1 μg/mL)	Morphological reversion of *src*^{ts}-NRK cells concentration (μg/mL)							
			100	50	20	10	5	2	1	0.1
1	1.3	+++	+++[a]	+++	+++	++	+	−		
4	1000<	−	−							
5	165	++	−							
6	1000<	−	+++	+++	+++	++	−			
7	1000<	−	−							
8	1000<	−	−							
9	1000	−	+++	nt[b]	nt	+++	+++	+++	+++	+
10	1000<	−	+++	nt	nt	+++	+++	++	±	−
11	1000<	−	+++	+++	+++	+++	+	−		
12	92.8	+++	+++	+++	+++	+++	+	−		
13	490.8	−	+++	+++	++	±	−			
14	129	+++	+++	+++	+++	+++	+++	+++	±	
15	1000<	−	−							
16	41.7	+++	−							
17	1000<	−	−							
19	25.5	+++	+++	+++	++	++				
20	1000<	−	−							
22	219.2	+++	+++	++	±	−				
23	1000<	−	−							

[a]Rate of reversed cells was presented as follows: +++ ; > 80%, ++ ; 50–80%, + ; 20–50%, − ; < 20%.
[b]Not tested.

Morphological reversion of *src*^{ts}-NRK cells

Among all of the derivatives systematically prepared, the C1 monoester **12**, the C4′ monoester **14**, the C18 methoxy derivative **19** and the C18 MTM ether **22** having the C24 carboxyl group and the C5 hydroxyl group in common also exhibited morphological reversion activities on *src*^{ts}-NRK cells. However, the C18 hydroxyl derivative **16** did not show the morphological reversion. We assumed that **16** might be converted into the corresponding 5,6-spiroacetal derivative **17** in the medium or cells and lost its activity. In fact, the synthesized 5,6-spiroacetal **17** did not exhibit morphological reversion activity on *src*^{ts}-NRK cells and inhibitory effects on both the IleRS activity and in vitro protein synthesis. The 5,6-spiroacetal derivatives **4** and **20** also did not show any activities. It is important to note that both the unsaturated side chains in the 5,6-spiroacetal derivatives are separated and cannot form hydrogen bonds (Fig. 2). The IleRS inhibitory activity of the C5 methyl ether **5** are about 100-fold less than **1** and **5** did not show morphological reversion at the concentration of 100 μg/mL. The C5 acetate **6** and the methyl esters **9**, **10**, **11** and **13** induced strong morphological reversion of *src*^{ts}-NRK cells, although these esters scarcely inhibited the IleRS activity. It was suggested that these esters were hydrolyzed by cellular esterase to afford **1** after being taken into the cells and showed these activities.

Acknowledgements

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