SYNTHESES OF NEW IMMUNOSUPPRESSIVE MYRIOCIN ANALOGS, 2-*EPI*-MYRIOCIN, 14-DEOXOMYRIOCIN, *Z*-14-DEOXOMYRIOCIN, AND NOR-DEOXOMYRIOCINS : THEIR STRUCTURE-ACTIVITY RELATIONSHIPS

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Eight new myriocin analogs, 2-*epi*-myriocin, *Z*-14-deoxomyriocin, and nor-deoxomyriocins, and a known myriocin derivative, 14-deoxomyriocin, were synthesized from 2-deoxy-D-glucose *via* common intermediates in previous myriocin and *Z*-myriocin syntheses. The immunosuppressive activities of new myriocin analogs and *Z*-myriocin on mouse allogeneic mixed lymphocyte reaction were examined, and, by comparing with those of myriocin and 14-deoxomyriocin, some structure-activity relationships have been found.

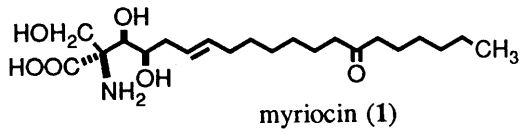
KEYWORDS myriocin; 2-*epi*-myriocin; *Z*-14-deoxomyriocin; nor-deoxomyriocin; immunosuppressive activity; structure-activity relationship

In the course of our studies on the effective utilization of natural carbohydrates as optically pure starting materials,[1] we have developed a versatile method for synthesizing a novel immunosuppressant, myriocin(**1**) together with its new analog, *Z*-myriocin(**2**).[2] This synthetic pathway comprised a stereoselective formation of the chiral α, α–disubstituted amino acid structure in myriocin(**1**) and *Z*-myriocin(**2**) from the isopropylidene ketone, which was prepared from 2-deoxy-D-glucose(**3**), using a modified Darzen reaction as a key step. Recently, immunosuppressive activity of myriocin(**1**) was reported to be almost two orders of magnitude more effective than cyclosporin A.[3] Furthermore, by comparison of the immunosuppressive activities for myriocin derivatives with that for myriocin(**1**), some relationships between their structures and activity have been reported, and 14-deoxomyriocin(**17**) have been found to show 5 to 10-fold more potent immunosuppressive activity than **1**.[4]

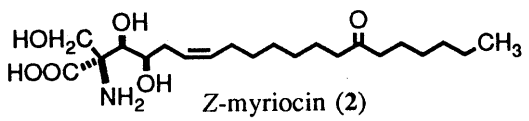
As our continuing synthetic studies aimed at the development of a new immunosuppressant, we have synthesized new myriocin analogs from carbohydrates.[5] In this paper, we describe the syntheses of eight new myriocin analogs such as 2-*epi*-myriocin(**11**), *Z*-14-deoxomyriocin(**21**), and nor-deoxomyriocins(**18~20**, **22~24**), and the known myriocin derivative 14-deoxomyriocin(**17**). In addition, the immunosuppressive activity of those myriocin analogs and *Z*-myriocin(**2**) was examined in comparison with those of myriocin(**1**) and 14-deoxomyriocin(**17**) in order to investigate the structure-activity relationships.

The synthesis of 2-*epi*-myriocin(**11**) started with the azido-aldehyde(**4**), which was the common synthetic intermediate in previous myriocin(**1**) and *Z*-myriocin(**2**) syntheses from 2-deoxy-D-glucose(**3**).[1] By oxidation of an aldehyde group in **4** with $NaClO_2$ in the presence of NH_2SO_3H in aq. dioxane and subsequent selective removal of the 1,3-*p*-methoxybenzylidene group with *p*-TsOH•H_2O, the 7, 3-lactone(**5**)[6] was obtained. Reduction of an azide group in **5** followed by benzoylation yielded the dibenzoate(**6**), which was converted to the 1-aldehyde(**7**) by successive de-*O*-benzoylation with 1% NaOMe-MeOH and PCC oxidation in CH_2Cl_2. Wittig reaction of **7** with the phosphonium salt(**8**)[2] in the presence of n-BuLi in t-BuOH-THF afforded the geometric mixture(**9**) (*E* : *Z* = 1 : 6). The photochemical geometrical isomerization reaction of **9** in the presence of diphenyldisulfide proceeded along deketalization at 14-position to provide a geometric mixture(**10**) (*E* : *Z* = 4 : 1). By the HPLC separation of **10** followed by deprotection and hydrolysis of the lactone ring, 2-*epi*-myriocin(**11**)[7] was obtained in 11.0% overall yield from 2-deoxy-D-glucose(**3**).

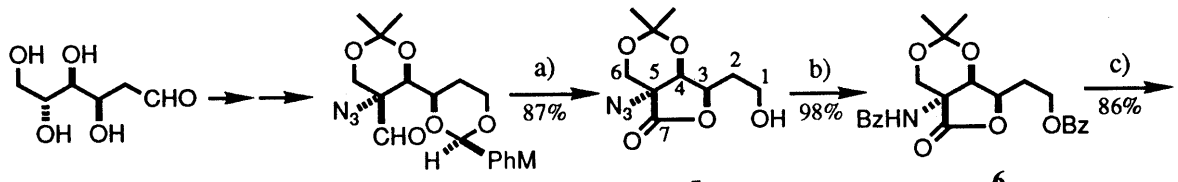
Furthermore, 14-deoxomyriocin(**17**), *Z*-14-deoxomyriocin(**21**), and nor-deoxomyriocins(**18~20**, **22~24**) were synthesized from the α, α–disubstituted amino acid derivative(**12**) which was also the synthetic intermediate of myriocin(**1**) and *Z*-myriocin(**2**).[2] Namely, Wittig reaction of **12** with the phosphonium salt(**13** : n=12) gave the geometric mixture(**14** : n=12) (*E* : *Z* = 1 : 7) which was subjected to HPLC separation to yield 6*Z*-form product(**14** : n=12, 74.9%) and 6*E*-form product(**14** : n=12, 10.7%). Treatment of the 6*Z*-form(**14** : n=12) with *p*-TsOH•H_2O



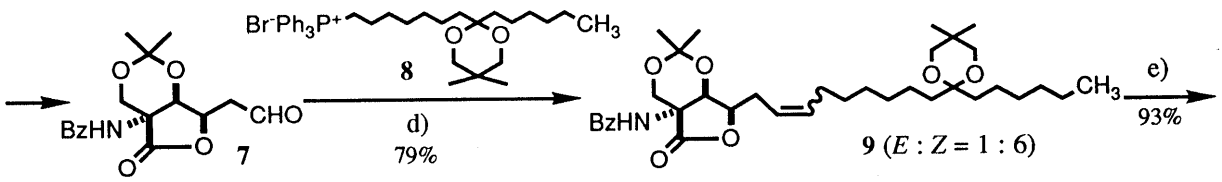
myriocin (1)



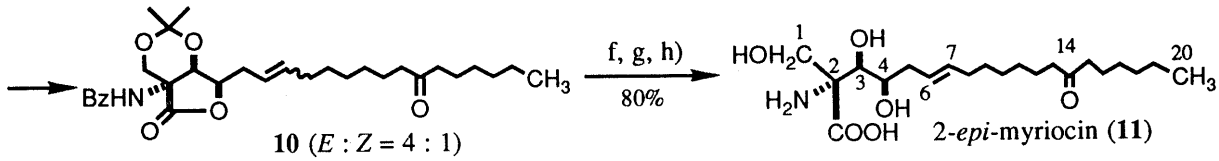
Z-myriocin (2)



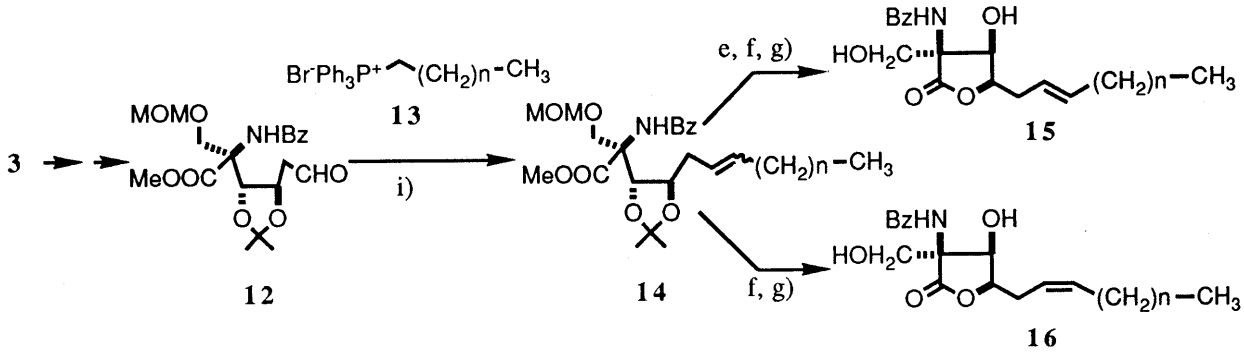
2-deoxy-D-glucose (3) 4 5 6



7 9 (E : Z = 1 : 6)



10 (E : Z = 4 : 1) 2-epi-myriocin (11)



12 14 15 16

17 : n=12 (14-deoxomyriocin)
18 : n=9 19 : n=6 20 : n=3

21 : n=12 (Z-14-deoxomyriocin)
22 : n=9 23 : n=6 24 : n=3

a) 1)$NaClO_2$/ NH_2SO_3H/ dioxane-H_2O, 2)*p*-TsOH•H_2O/ MeOH ; b) 1)H_2/ 10%Pd-C/ EtOH, 2)BzCl/ Py. ; c) 1)1% NaOMe/ MeOH, 2)PCC/ CH_2Cl_2 ; d) n-BuLi/ t-BuOH-THF ; e) *hv*/ PhSSPh/ cyclohexane ; f) HPLC separation ; g) *p*-TsOH•H_2O/ 70% aq. EtOH ; h)1N NaOH i) n-BuLi/ THF

Table I. Suppressive Effects of Myriocin(**1**) and Its Analogs(**2**, **11**, **17~24**) on Mouse Allogeneic Mixed Lymphocyte Reaction

Compounds	IC_{50} (μM)
myriocin (**1**)	0.00929
Z-myriocin (**2**)	0.00323
2-*epi*-myriocin (**11**)	0.00939
14-deoxomyriocin (**17**)	0.000662
Z-14-deoxomyriocin (**21**)	0.000457
E-trinor-deoxomyriocin (**18**)	0.00592
E-hexanor-deoxomyriocin (**19**)	4.38
E-nonanor-deoxomyriocin (**20**)	57.9
Z-trinor-deoxomyriocin (**22**)	0.00907
Z-hexanor-deoxomyriocin (**23**)	10.2
Z-nonanor-deoxomyriocin (**24**)	236

afforded the lactone(**16** : n=12, 77.4%), which was subjected to alkaline treatment to yield *Z*-14-deoxomyriocin(**21**)[8] in 60.1% yield. Similarly, 14-deoxomyriocin(**17**)[4] was efficiently prepared from **15**, obtained by successive photochemical geometrical isomerization(*E* : *Z* = 4 : 1) of **14**(n=12, *E* : *Z* = 1 : 7), HPLC separation, and deprotection steps. According to a similar procedure from **12** to **17** and **21**, various nor-deoxomyriocins(6*E*-form : **18**, **19**, **20** ; 6*Z*-form : **22**, **23**, **24**) were synthesized from **12** and the phosphonium salts(**13** : n=9, 6, 3) *via* the lactones(**15** or **16** ; n=9, 6, 3).

The effects of those synthesized myriocin analogs(**11**, **17~24**) and *Z*-myriocin(**2**) on mouse allogeneic mixed lymphocyte reaction were examined in comparison with those of myriocin(**1**) and 14-deoxomyriocin(**17**). As shown in Table I, *Z*-14-deoxomyriocin(**21**) showed the most potent suppressive activity on mouse allogeneic mixed lymphocyte reaction, and the following structure-activity relationships were found. 1) Since 2-*epi*-myriocin(**11**) shows almost as potent immunosuppressive activity as myriocin(**1**), the stereostructure at 2-positon of the amino acid moiety in **1** does not affect the activity. 2) Trinor-deoxomyriocins(**18**, **22**) exhibit potent activity similar to myriocin(**1**) and *Z*-myriocin(**2**), but their activities were less than those of 14-deoxomyriocin(**17**) and *E*-14-deoxomyriocin(**21**), respectively, while hexanor- and nonanor-deoxomyriocins(**19**, **20**, **23**, **24**) show a little activity. Based on this evidence, long carbon chain ($n \geqq 9$) bonded to the amino acid moiety is essential to the activity. 3) When the activities for myriocin analogs with twenty-membered carbon chains were compared, 6*Z*-isomers have more potent activity than 6*E*-ones. 4) Comparison of the activities for 6*E*-nor-deoxomyriocins(**18~20**) with those for 6*Z*-nor-deoxomyriocins(**22~24**) shows that, in the case of the nor-deoxomyriocins, each 6*Z*-isomer has less activity than 6*E*-isomer. We are currently working on the further characterization of structure-activity relationships and detailed investigation of immunosuppressive activity for synthesized myriocin analogs.

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6) All new compounds[**5~7**, **11**, **15**(n=12, 9, 6, 3), **16**(n=12, 9, 6, 3), **18~24**] were characterized by physicochemical properties, and full characteristics will be presented in our full paper. The molecular composition of the compounds given the chemical formula was determined by high-resolution FAB-MS measurement.

7) 2-*epi*-Myriocin(**11**), colorless fine crystals, mp 182-184°C, $[\alpha]_D$ +107°(MeOH), $C_{21}H_{39}NO_6$, IR(KBr) : 3197, 1711, 1671, 1032, 972 cm^{-1}, ^1H NMR(CD_3OD, δ) : 0.90(t, J=6.6, 20-H_3), 2.29(dd, J=6.9, 6.9, 5-H_2), 2.44(t-like, 13, 15-H_2), 3.78, 3.91(ABq, J=11.2, 2-CH_2OH), 3.80(t, J=6.9, 4-H), 4.00(s, 3-H), 5.40(dt, J=6.9, 16.1, 6-H), 5.54(dt, J=6.9, 16.1, 7-H), positive FAB-MS(m/z) : 402$(M+H)^+$.

8) *Z*-14-Deoxomyriocin(**21**), colorless fine crystals, mp 163-165°C, $[\alpha]_D$ -14.9°(MeOH), $C_{21}H_{41}NO_5$, IR(KBr) : 3431, 1632, 1458, 1387, 1262 cm^{-1}, ^1H NMR(DMSO-d_6, δ) : 0.85(t, J=5.9, 20-H_3), 1.24(22H, br s), 1.99(m, 8-H_2), 2.19(m, 5-H_2), 3.55(t-like, 4-H), 3.56, 3.68(ABq, J=10.8, CH_2OH), 3.60(br s, 3-H), 5.36(2H, m, 6, 7-H), negative FAB-MS(m/z) : 386$(M-H)^-$.

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