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Immunostimulatory Activities of Monoglycosylated α-D-Pyranosylceramides

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Abstract—We compared the immunostimulatory effects of chemically synthesized α-galactosylceramides (α-GalCers), α-glucosylceramides (α-GluCers), 6″- monoglycosylated α-GalCer and 6″- or 4″-monoglycosylated α-GluCer and made the following observations: (1) the length of the fatty acid side chain in the ceramide portions greatly affects the immunostimulatory effects of α-GalCers and α-GluCers; (2) the configuration of the 4″-hydroxyl group of the inner pyranose moiety plays an important role in the immunostimulatory effects of monoglycosylated α-D-pyranosylceramides; (3) the free 4″-hydroxyl group of the inner pyranose of monoglycosylated α-D-pyranosylceramides plays a more important role in their immunostimulatory effects than the free 6″-hydroxyl group. © 1997 Elsevier Science Ltd.

Introduction

Since we showed that KRN7000, an α-D-galactopyranosylceramide (α-GalCer), has strong anti-tumor and anti-metastatic activities in mice inoculated with murine melanoma B16 cells via the enhancement of the antigen-presenting function of dendritic cells,[1,2] we have been interested in investigating the mechanism of this unique immunostimulatory effect. Recently, several kinds of mono- or diglycosylated α-GalCers were isolated from marine organisms,[3–5] and Costantino et al.[6] reported that the free 2″-hydroxyl group plays an essential role in the immunostimulatory effects of these α-GalCer derivatives. In addition, we reported that the 3″-hydroxyl group plays a more important role in the manifestation of the immunostimulatory effects of monoglycosylated α-GalCers than the 2″-hydroxyl group.[7] Although there are several findings concerning the roles of 2″- and 3″-hydroxyl groups in the immunostimulatory effects of monoglycosylated α-GalCers, there are no reports about the roles of other hydroxyl groups of the inner pyranose moieties of α-D-pyranosylceramides in their immunostimulatory activities.

We have previously compared the immunostimulatory effects of α-D-pyranosylceramides, including α-GalCer, α-D-glucopyranosylceramide (α-GluCer) and 6″-deoxy α-GalCer, which have the same ceramide portion, and found that the configuration of 4″-hydroxyl group of the pyranose moiety greatly influences the immunostimulatory effects, while the 6″-hydroxyl group does not.[8] In order to determine whether the configuration of 4″-hydroxyl group plays an important role in the activities of monoglycosylated α-D-pyranosylceramides we decided to compare the immunostimulatory effects of 6″-monoglycosylated α-GalCer and 6″-monoglycosylated α-GluCer. We therefore synthesized 6″-glycosylated α-GalCer, 6″-glycosylated α-GluCer and 4″-glycosylated α-GluCer to compare the roles of the free 4″- and 6″-hydroxyl groups in the immunostimulatory activities of monoglycosylated α-D-pyranosylceramides.

In this paper, we describe the syntheses of an α-GluCer and the monoglycosylated α-D-pyranosylceramides described above, which have common ceramide moieties and similar immunostimulatory effects. We will also discuss the roles of hydroxyl groups in 4″- and 6″-positions in the immunostimulatory activities of monoglycosylated α-D-pyranosylceramides.

Chemistry

Syntheses of α-GluCer and monoglycosylated α-D-pyranosylceramides

AGL-585 (α-GluCer) (Fig. 1), which has the same ceramide moiety as that of **KRN7000**, was synthesized by a coupling reaction of the suitable protected ceramide and glucosyl fluoride under Mukaiyama's α-

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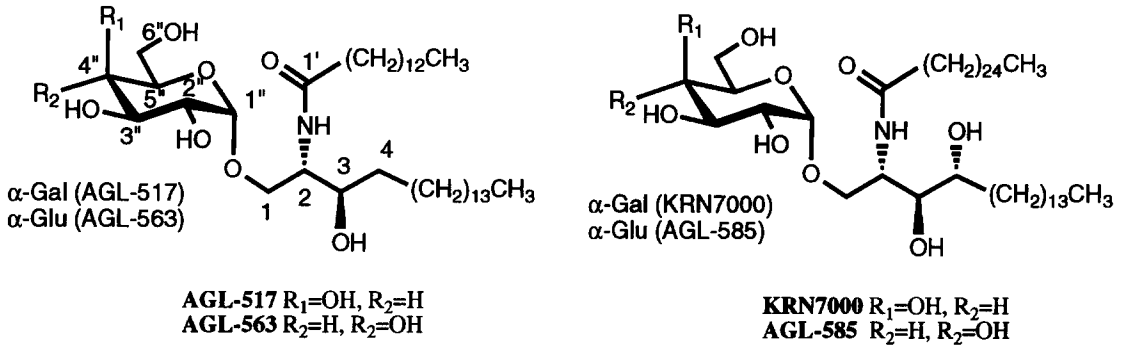
Key words: α-galactosylceramide, α-gluctosylceramide, monoglycosylated α-galactosylceramide, monoglycosylated α-glucosylceramide, immunostimulatory activity.



AGL-517 R_1=OH, R_2=H
AGL-563 R_2=H, R_2=OH

KRN7000 R_1=OH, R_2=H
AGL-585 R_2=H, R_2=OH

Figure 1. Structures of **AGL-517**, **AGL-563**, **KRN7000** and **AGL-585**.

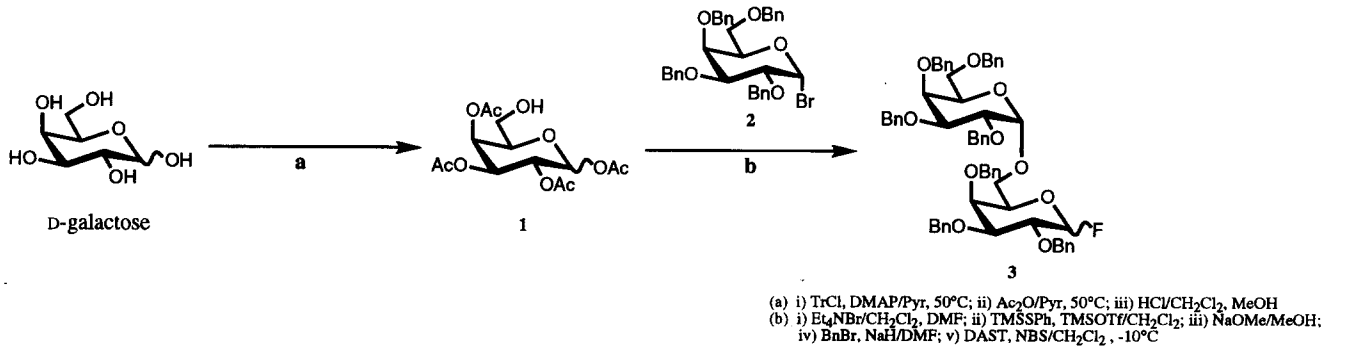
glycosidation condition[9] followed by hydrogenolysis, as we have previously reported.[10,11]

The disaccharide moiety of **AGL-586**, with a Galα1→6Gal structure, was synthesized as shown in Scheme 1. Briefly, a galactose acceptor (**1**) was derived from raw sugar through three steps: tritylation, acetylation, and selective deprotection of the primary hydroxyl group. This acceptor was glycosylated with a suitable protected D-galactose (**2**), with conversion of the anomeric acetoxy group to the thiophenyl group, followed by deacetylation, benzylation and fluorination with DAST (diethylaminosulfur trifluoride-triethylamine complex) to give digalactosyl fluoride (**3**). Scheme 2 shows the synthesis of three kinds of monoglycosylated α-D-pyranosylceramides. Briefly, **3** and diglycosyl fluorides, prepared from melibiose and maltose (**4** and **5**), were coupled with a ceramide (**6**), and the resulting α-glycosides were deprotected by hydrogenolysis to give the expected monoglycosylated α-D-pyranosylceramides; **AGL-586**, **AGL-584** and **AGL-588**.

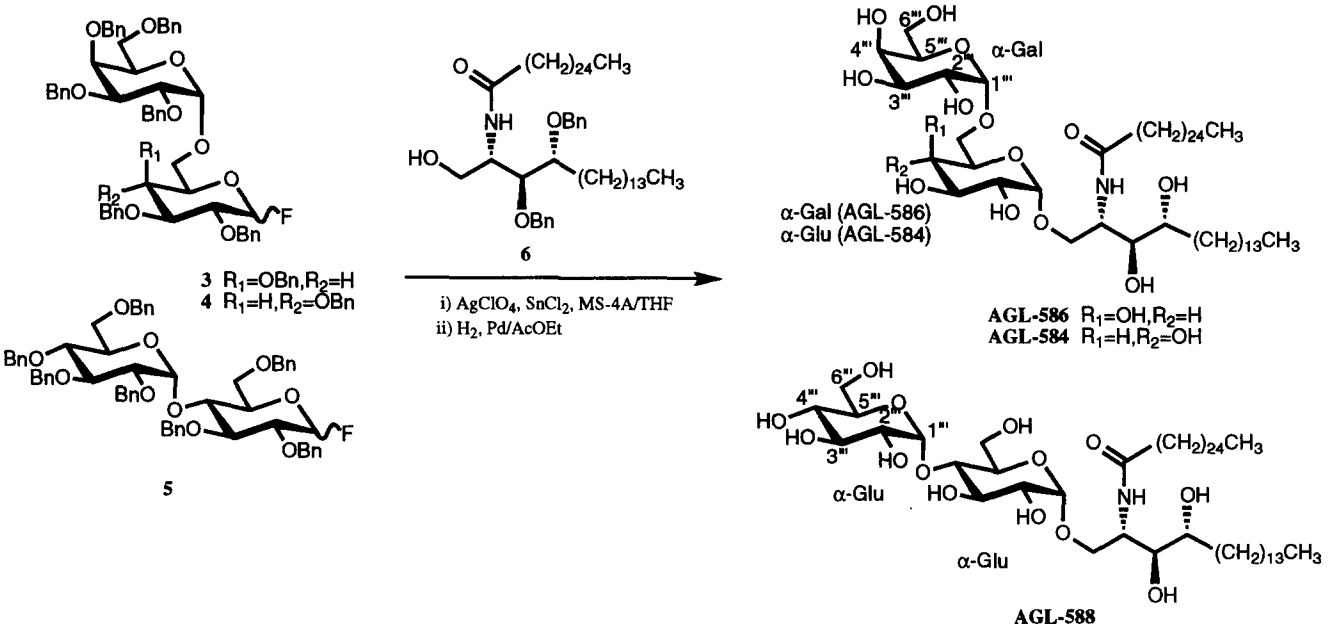
Results and Discussion

We compared the immunostimulatory effects of **AGL-517** (α-GalCer) and **AGL-563** (α-GluCer), which have the same ceramide portions (Fig. 1), on the proliferation of spleen cells. As shown in Table 1, **AGL-517** significantly stimulated spleen cell proliferation in a dose-dependent manner at concentrations 1–100 ng/mL, while **AGL-563** only showed significant stimulatory activity at a concentration of 100 ng/mL. To confirm this relationship between α-GalCers and α-GluCers, we also evaluated immunostimulatory effects of **KRN7000** (α-GalCer) and **AGL-585** (α-GluCer), which have the same ceramide portions, on the proliferation of spleen cells. As Table 1 shows, **KRN7000** markedly stimulated the proliferation in a dose-dependent manner and with significant stimulation at a concentration of 0.1 ng/mL. The stimulatory effect of **AGL-585** was weaker than that of **KRN7000**, but **AGL-585** also significantly stimulated spleen cell proliferation at a concentration of 0.1 ng/mL. These results strongly suggest that the ceramide moiety plays an important role in the immunostimulatory activities of α-GalCers and α-GluCers, and that the longer fatty acid side chains are responsible for the stronger immunostimulatory activities of α-D-pyranosylceramides. These results also demonstrated that the configuration of the 4″-hydroxyl group plays an important role in the immunostimulatory effects of α-D-pyranosylceramides.

We also reported that the 6″-hydroxyl group of α-GalCers does not have any effect.[8] In order to determine whether or not the configuration of the 4″-hydroxyl group in monoglycosylated α-D-MonoCers plays an important role, we synthesized 6″-glycosylated α-GalCer (**AGL-586**) and 6″-glycosylated α-GluCer (**AGL-584**), which have the same ceramide moiety as



Scheme 1. Synthetic procedure for digalactosyl fluoride.



Scheme 2. Synthetic procedure for **AGL-586**, **AGL-584** and **AGL-588**.

KRN7000, and examined their effects on the proliferation of spleen cells. **AGL-586** and **AGL-584** stimulated the proliferation of spleen cells, in a dose-dependent manner, at 0.1–100 ng/mL. The stimulatory effect of **AGL-586** was stronger than that of **AGL-584**, this parallels the relationship between **KRN7000** and **AGL-585** (Table 1). This result demonstrated that the configuration of the 4″-position of the inner pyranose moiety of these α-D-pyranosylceramide derivatives greatly affects their immunostimulatory potencies.

In addition, we synthesized 4″-monoglycosylated α-GluCer (**AGL-588**), which has the same ceramide moiety as the above four compounds, in order to compare the roles of the 4″- and 6″-hydroxyl groups in the immunostimulatory effects of monoglycosylated α-D-pyranosylceramides. As Table 1 shows, **AGL-588** stimulated the proliferation of spleen cells at concentrations 0.1–100 ng/mL, but the potency of **AGL-588** was weaker than that of **AGL-584**. This result strongly suggests that the free 4″-hydroxyl group plays a more significant role than the free 6″-hydroxyl group.

We also examined effects of all seven compounds on the allogeneic mixed leukocyte reaction (MLR). As Table 2 shows, their MLR response stimulatory activities paralleled their effects on the proliferation of spleen cells.

As mentioned above, we found that the inner pyranose moiety greatly affects the immunostimulatory potencies of monoglycosylated α-D-pyranosylceramides. It is well known that many kinds of monoglycosylated β-D-pyranosylceramides (lactosylceramide, etc.) occur naturally, but there is no report that these monoglycosylated β-D-pyranosylceramides have marked immunostimulatory effects. Taking these findings, and our previous reports[8] that α-GalCers have stronger immunostimula-

Table 1. Effects of α-galactosylceramide derivatives and α-glucosylceramide derivatives on the proliferation of spleen cells

	^{3}H-TdR incorporation (cpm, mean ± S.D.)			
Sample	**0.1**	**1**	**10**	**100 (ng/mL)**
Vehicle	2841 ± 285	3186 ± 423	2851 ± 290	2884 ± 238
AGL-517	2789 ± 210	4256 ± 477[a]	10541 ± 2089[b]	16295 ± 1215[c]
AGL-563	2803 ± 325	2618 ± 370	3193 ± 651	4111 ± 318[b]
KRN7000	9368 ± 1307[b]	15850 ± 644[c]	22733 ± 2862[c]	24535 ± 2331[c]
AGL-585	4276 ± 626[a]	11381 ± 1510[c]	14634 ± 956[c]	17731 ± 2076[c]
AGL-586	13974 ± 891[c]	18925 ± 1014[c]	23579 ± 1533[c]	25995 ± 529[c]
AGL-584	8254 ± 839[c]	15171 ± 2532[b]	16974 ± 976[c]	18348 ± 2744[c]
AGL-588	4007 ± 276[b]	8615 ± 775[c]	12848 ± 1299[c]	15101 ± 744[c]

Spleen cells (2.5×10^5 cells/100 μL/well) from BALB/c mice were plated in 96-well plate in triplicate and cultured with 0.1, 1, 10, 100 ng/mL of samples at 37 °C in 95 air, 5% CO_2 for two days, and then ^{3}H-TdR were pulsed for additional 8 h. ^{3}H-TdR uptake into cells was counted using a liquid scintillation counter. Statistical analysis was performed by two-sided unpaired Student's *t*-test. (a) $p < 0.05$; (b) $p < 0.01$; (c) < 0.001 (compared with vehicle-treated group).

Table 2. Effects of α-galactosylceramide derivatives and α-glucosylceramide derivatives on allogeneic MLR

Sample	^{3}H-TdR incorporation (cpm, mean ± S.D.)			
	0.1	1	10	100 (ng/mL)
Vehicle	5229 ± 699	5279 ± 607	5337 ± 710	4786 ± 543
AGL-517	5687 ± 565	5989 ± 232	11062 ± 1598[b]	13907 ± 955[c]
AGL-563	5349 ± 481	5395 ± 219	5576 ± 734	7398 ± 537[b]
KRN7000	10323 ± 2005[a]	14699 ± 1243[c]	16350 ± 715[c]	16169 ± 272[c]
AGL-585	8379 ± 1663[a]	12811 ± 912[c]	13613 ± 1155[c]	14669 ± 1024[c]
AGL-586	12739 ± 327[c]	13040 ± 547[c]	16489 ± 1317[c]	16571 ± 248[c]
AGL-584	9849 ± 778[b]	12706 ± 1681[b]	13815 ± 959[c]	14956 ± 179[c]
AGL-588	7831 ± 1349[a]	11939 ± 543[c]	13361 ± 1103[c]	14473 ± 1408[c]

Same volume (1.25×10^5 cells/50 μL/well) of responder cells (spleen cells from BALB/c mice) and stimulator cells (MMC-treated spleen cells from C57BL/6 mice) were plated in 96-well plate in triplicate. Other procedures were performed according to the methods described in Table 1. Statistical analysis was performed by two-sided unpaired Student's *t*-test. (a) $p < 0.05$; (b) $p < 0.01$; (c) $p < 0.001$ (compared with vehicle-treated group).

tory effects than β-GalCers, we conclude that the combination of inner sugar and ceramide also plays an important role in the manifestation of the immunostimulatory activities of monoglycosylated D-pyranosylceramides. To test this theory, it is necessary to compare the immunostimulatory effects of monoglycosylated α- and β-D-pyranosylceramides, which have the common ceramide structure.

This study suggests that: (1) the lengths of the fatty acid side chain in the ceramide portion greatly influences the immunostimulatory effects of α-D-pyranosylceramides; (2) the configuration of the 4″-hydroxyl group of the inner pyranose moiety plays an important role in the immunostimulatory effects of monoglycosylated α-D-pyranosylceramides; (3) the free 4″-hydroxyl group of the inner pyranose portion of monoglycosylated α-D-pyranosylceramides plays a more important role in their immunostimulatory effects than the free 6″-hydroxyl group.

Experimental

The physical properties of compounds synthesized in this study are shown below.

AGL-585. $[\alpha]^{23}_D$ + 43.9° (*c* 0.81; pyridine); mp 152.0–155.0 °C; negative HR FABMS *m/z* 856.7204 [(M-H)- calcd. for $C_{50}H_{98}NO_9$ 856.7242]; IR (KBr, cm^{-1}) 3380, 2920, 2850, 1632, 1552, 1468, 1130 and 1076; ^{1}H NMR (500 MHz, C_5D_5N) δ (ppm) 8.34 (1H, d, *J* = 8.5 Hz, NH), 7.31 (1H, bs), 7.10 (1H, bs), 7.04 (1H, bs), 6.47 (1H, d, *J* = 5.5 Hz), 6.32 (1H, m), 6.04 (1H, bs), 5.57 (1H, d, *J* = 3.7 Hz, H1″), 5.21–5.28 (1H, m, H2), 4.66 (1H, dd, *J* = 5.5, 11.0 Hz, H3), 4.54 (1H, bt, *J* = 9.2 Hz), 4.26–4.45 (6H, m), 4.08–4.22 (2H, m), 2.40 (2H, t, *J* = 7.3 Hz), 2.22–2.30 (1H, m), 1.77–1.94 (4H, m), 1.59–1.68 (1H, m), 1.24–1.47 (66H, m), 0.88 (6H, t, *J* =6.7 Hz, terminal methyl); ^{13}C NMR(125 MHz, C_5D_5N) δ (ppm) 173.2 (s, C1′), 101.0 (d), 76.7 (d), 75.4 (d), 74.6 (d), 73.5 (d), 72.3 (d), 71.9 (d), 68.1 (t), 62.7 (t), 51.3 (d), 36.8 (t), 34.4 (t), 32.1 (t), 30.4 (t), 30.1 (t), 30.05 (t), 30.02 (t), 29.93 (t), 29.88 (t), 29.82 (t), 29.76 (t), 26.5 (t), 26.4 (t), 23.0 (t), 14.3 (q, terminal methyl).

AGL-584. $[\alpha]^{23}_D$ + 82.7° (*c* 0.03; pyridine); mp 133.0–136.5 °C; negative HR FABMS *m/z* 1018.7833 [(M-H)- calcd. for $C_{56}H_{108}NO_{14}$ 1018.7776]; IR (KBr, cm^{-1}) 3400, 2950, 2870, 1645, 1535, 1475 and 1080; ^{1}H NMR (500 MHz, C_5D_5N + 1% D_2O, 24 °C) δ (ppm) 8.39 (1H, d, *J* = 8.6 Hz, NH), 5.39 (2H, m, H1″, H1), 5.11 (1H, m, H2), 4.62 (1H, dd, *J* = 5.3, 10.4 Hz, H3), 4.57 (1H, dd, *J* =3.7, 9.3Hz, H2), 4.53 (1H, t, *J* = 6.0 Hz, H1a), 4.48 (2H, m, H4, H3), 4.45 (1H, dd, *J* = 3.1, 6.7 Hz), 4.37–4.44 (3H, m), 4.32 (2H, m), 4.23 (2H, m, H1b, H4), 4.18 (1H, d, *J* = 9.0 Hz, H6a), 4.02 (2H, m, H6b, H2″), 2.35 (2H, m), 2.15 (1H, m), 1.65–1.86 (4H, m), 1.56 (1H, m), 1.02–1.38 (66H, m), 0.79 (6H, t, *J* = 6.7 Hz, terminal methyl); ^{13}C NMR (125 MHz, C_5D_5N + 1% D_2O, 24 °C) δ (ppm) 173.6 (s, C1′), 100.7 (d), 100.6 (d), 76.4 (d), 75.5 (d), 73.4 (d), 72.6 (d), 72.4 (d), 72.4 (d), 72.0 (d), 71.7 (d), 71.1 (d), 70.8 (d), 68.0 (t), 67.5 (t), 62.7 (t), 51.5 (d), 36.8 (t), 34.3 (t), 32.2 (t), 30.4 (t), 30.2 (t), 30.1 (t), 30.1 (t), 29.9 (t), 29.8 (t), 29.8 (t), 29.6 (t), 26.5 (t), 26.4 (t), 23.0 (t), 14.3 (q), terminal methyl).

AGL-586. $[\alpha]^{23}_D$ + 75.0° (*c* 1.07; pyridine); mp 127.0–131.0 °C; negative HR FABMS *m/z* 1018.7858 [(M-H)- calcd. for $C_{56}H_{108}NO_{14}$ 1018.7776]; IR (KBr, cm^{-1}) 3400, 2950, 2870, 1645, 1535, 1475 and 1080; ^{1}H NMR (500 MHz, C_5D_5N + 1% D_2O, 24 °C) δ (ppm) 8.52 (1H, d, *J* = 8.6 Hz, NH), 5.48 (2H, m, H1″,H1), 5.19 (1H, m, H2), 4.69 (1H, dd, *J* = 4.8, 10.3 Hz), 4.66 (2H, m), 4.54–4.63 (4H, m), 4.46–4.55 (2H, m), 4.36–4.46 (3H,m), 4.22–4.36 (4H, m), 2.46 (2H, m), 2.24 (1H, m), 1.90 (2H, m), 1.81 (2H, m), 1.67 (1H, m), 1.12–1.45 (66H, m), 0.86 (6H, t, *J* = 6.7 Hz, terminal methyl); ^{13}C NMR (125 MHz, C_5D_5N + 1% D_2O, 24 °C) δ (ppm) 173.7 (s, C1′), 101.0 (d), 100.9 (d), 76.3 (d), 72.7 (d), 72.6 (d), 71.7 (d), 71.4 (d), 71.0 (d), 70.7 (d), 70.7 (d), 70.5 (d), 70.3 (d), 68.1 (t), 67.8 (t), 62.5 (t), 51.6 (d), 36.9 (t), 34.3 (t), 32.2 (t), 30.5 (t), 30.2 (t), 30.1 (t), 30.1 (t), 30.0 (t), 29.9 (t), 29.8 (t), 29.6 (t), 26.5 (t), 26.5 (t), 23.0 (t), 14.3 (q, terminal methyl).

AGL-588. $[\alpha]^{23}_D$ + 60.1° (*c* 0.60; pyridine); mp 145.0–148.5 °C; negative HR FABMS *m/z* 1018.7753 [(M-H)- calcd. for $C_{56}H_{108}NO_{14}$ 1018.7776]; IR (KBr, cm^{-1}) 3400, 2950, 2870, 1645, 1535, 1475 and 1080; ^1H NMR (500 MHz, C_5D_5N + 1% D_2O, 23 °C) δ (ppm) 8.49 (1H, d, *J* = 8.6 Hz, NH), 5.85 (1H, d, *J* = 3.1 Hz), 5.48 (1H, d, *J* = 3.7 Hz), 5.20 (1H, m, H2), 4.44–4.65 (5H, m), 4.40 (1H, m), 4.22–4.36 (6H, m), 4.08–4.22 (3H, m), 4.04 (1H, dd, *J* = 3.1, 9.8 Hz), 2.41 (2H, t, *J* = 7.3 Hz), 2.25 (1H, m), 1.70–1.95 (4H, m), 1.64 (1H, m), 1.05–1.48 (66H, m), 0.86 (6H, t, *J* = 6.1 Hz, terminal methyl); ^{13}C NMR (125 MHz, C_5D_5N + 1% D_2O, 24 °C) δ (ppm) 173.3 (s, C1′), 103.3 (d), 100.7 (d), 81.7 (d), 76.6 (d), 75.5 (d), 75.3 (d), 74.8 (d), 74.6 (d), 73.0 (d), 72.9 (d), 72.4 (d), 71.9 (d), 68.3 (t), 62.7 (t), 61.7 (t), 51.2 (d), 36.8 (t), 34.4 (t), 32.2 (t), 30.5 (t), 30.2 (t), 30.1 (t), 30.1 (t), 30.0 (t), 29.9 (t), 29.9 (t), 29.8 (t), 29.7 (t), 26.5 (t), 26.4 (t), 23.0 (t), 14.4 (q, terminal methyl).

Biological method

Animals. Female C57BL/6 or BALB/c mice, 5–10 weeks old, purchased from Nippon SLC Co., Ltd were used in this study. Mice were maintained under our standard laboratory conditions.

Preparation of spleen cells. Mice were sacrificed, and the spleens were resected. The spleens were dissociated in 10% fetal calf serum (FCS, Gibco) RPMI 1640 (Gibco) medium, and RBC were lysed with Tris–NH_4Cl. The cells were washed three times using phosphate buffer saline (Nissui Pharmaceutical Co., Ltd), and viable cells were counted and resuspended in 10% FCS RPMI 1640 medium.

Spleen cell-proliferation assay. Spleen cells (2.5×10^5 cells/100 μL/well) suspended in 10% FCS RPMI 1640 medium and 0.1, 1, 10 and 100 ng/mL of samples (10 μL/well) were plated in 96-well round-bottomed plates (Nunclon) and were cultured at 37 °C in 95% air, 5% CO_2. Two days later, 0.5 μCi/well of tritium-thymidine (^3H-TdR, Du Pont/ NEN Research Products) was added to each well and the plate was incubated for additional 8 h. ^3H-TdR uptake into cells was measured using a liquid scintillation counter.

Allogeneic MRL assay. Spleen cells obtained from BALB/c mouse were used as responder cells, and MMC (50 μg/mL, 30 min.) treated spleen cells from C57BL/6 mice were used as stimulator cells in the allogeneic MLR assay. Equal volumes (1.25×10^5 cells/50 μL/mL) of responder cells and stimulator cells suspended in 10% FCS RPMI1640 medium, with the above samples, were plated in 96-well plates which were cultured at 37 °C in 95% air, 5% CO_2. Two days later, 0.5 μCi/well of ^3H-TdR was added into each well and the plate was incubated for additional 8 h. ^3H-TdR uptake into cells was measured using a liquid scintillation counter.

References

1. Kobayashi, E.; Motoki, K.; Uchida, T.; Fukushima, H.; Koezuka, Y. *Oncol. Res.* **1996**, *7*, 529.

2. Yamaguchi, Y.; Motoki, K.; Ueno, H.; Maeda, K.; Kobayashi, E.; Inoue, H.; Fukushima, H.; Koezuka, Y. *Oncol. Res.* **1996**, *8*, 399.

3. Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O. *Liebigs Ann. Chem.* **1995**, 1477.

4. Costantino, V.; Fattorusso, E.; Mangoni, A. *Liebigs Ann. Chem.* **1995**, 2133.

5. Cafieri, F.; Fattorusso, E.; Mahajnah, Y.; Mangoni, A. *Liebigs Ann. Chem.* **1994**, 1187.

6. Costantino, V.; Fattorusso, E.; Mangoni, A.; Rosa, M. D.; Ianaro, A.; Maffia, P. *Tetrahedron* **1996**, *52*, 1573–1578.

7. Uchimura, A.; Shimizu, T.; Nakajima, M.; Ueno, H.; Motoki, K.; Fukushima, H.; Natori, T.; Koezuka, Y. *Bioorg Med. Chem.* **1997**, *5*, 1447.

8. Motoki, K.; Morita, M.; Kobayashi, E.; Uchida, T.; Akimoto, K.; Fukushima, H.; Koezuka, Y. *Biol. Pharm. Bull.* **1995**, *18*, 1487.

9. Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.*, **1981**, 431.

10. Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sawa, T.; Yamaji, K.; Kobayashi, E.; Fukushima, H.; Koezuka, Y. *J. Med. Chem.* **1995**, *38*, 2176.

11. Morita, M.; Natori,T.; Akimoto, K.; Osawa, T.; Fukushima, H.; Koezuka, Y. *Bioorg Med. Chem. Lett.* **1995**, *5*, 699.

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