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SYNTHESES OF α -, β -MONOGLYCOSYLCERAMIDES AND FOUR DIASTEREOMERS OF AN α -GALACTOSYLCERAMIDE

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Abstract: To examine antitumor activities of monoglycosylceramide, we synthesized α -, β -galactosylceramides and α -, β -glucosylceramides which have the same ceramide portion, and four kinds of diastereomers of the ceramide portion in an α -galactosylceramide.

Agelasphines (AGLs) have been isolated from an extract of the marine sponge, *Agelas mauritianus*, as active substances in the course of screening of antitumor agents.^{1,2} Since sufficient amounts of the four kinds of AGLs (AGL-7a, AGL-9b, AGL-11, and AGL-13, Fig. 1) having α -galactosylceramide (α -GalCer) structures and AGL-10 which has a β -glucosylceramide (β -GluCer) structure were obtained, we examined the biological activities of five compounds using several assay systems, and found that α -GalCers showed stronger antitumor activities against B16-bearing mice and stimulatory effects of lymphocyte proliferation on allogeneic mixed lymphocyte reaction (MLR) than β -GluCer.

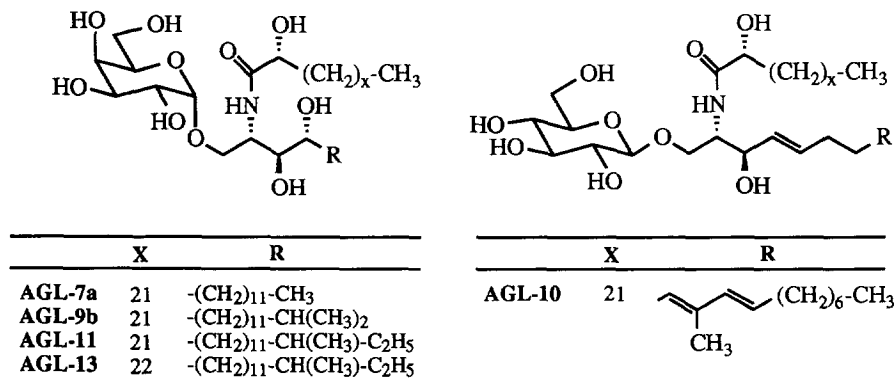


Fig. 1. Structures of AGL-7a, AGL-9b, AGL-11, AGL-13, and AGL-10.

These findings suggested that not only the manner of combination between sugar and ceramide but also the type of sugar combining to ceramide greatly affects the biological activities of monoglycosylated ceramides (MonoCers). To confirm this possibility, we synthesized four kinds of MonoCers (AGL-517,³ AGL-562,⁴ AGL-563,⁵ and AGL-564,⁶) which have the same ceramide and their structures are shown in Fig. 2.

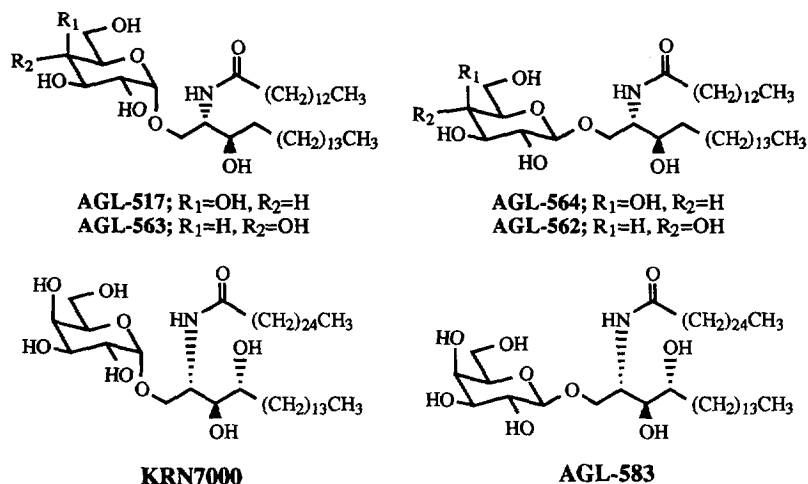
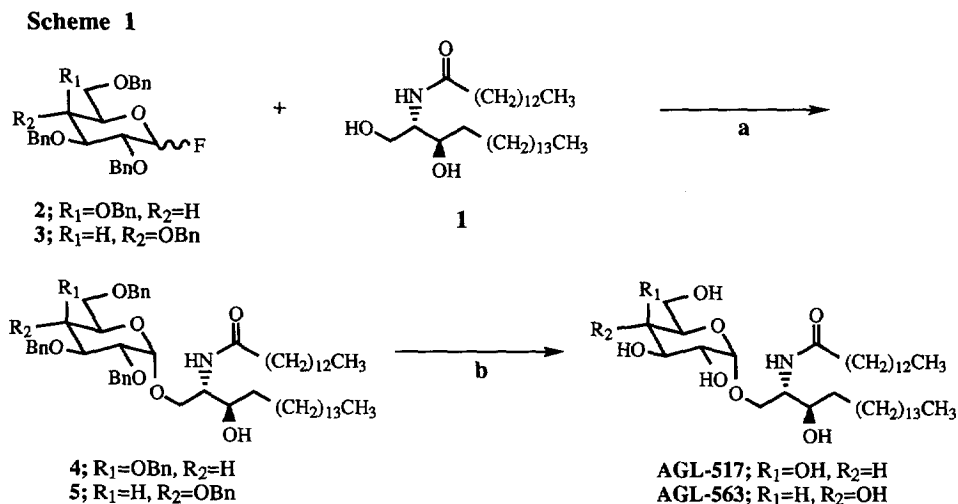


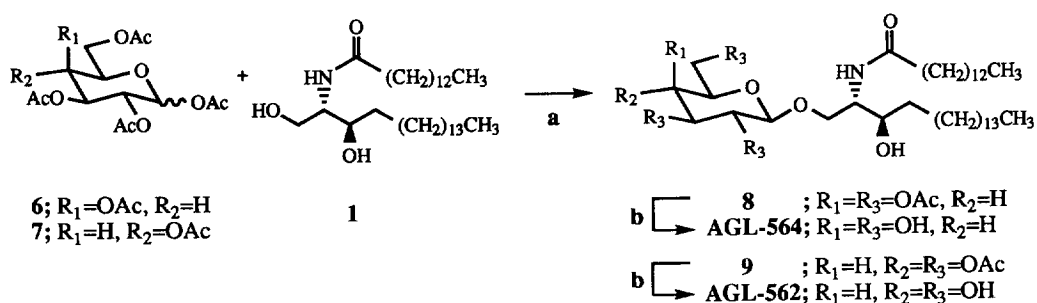
Fig. 2 Structures of AGL-517, AGL-564, AGL-563, AGL-562, KRN7000, and AGL-583.

As shown in Scheme 1, AGL-517 (α -GalCer) and AGL-563 (α -GluCer) were synthesized from a ceramide (1)⁷ under Mukaiyama's α -glycosidation condition⁸ and following hydrogenolysis.



(a) $SnCl_2$, $AgClO_4$, MS4A / THF; (b) H_2 , Pd-BaSO₄ / THF

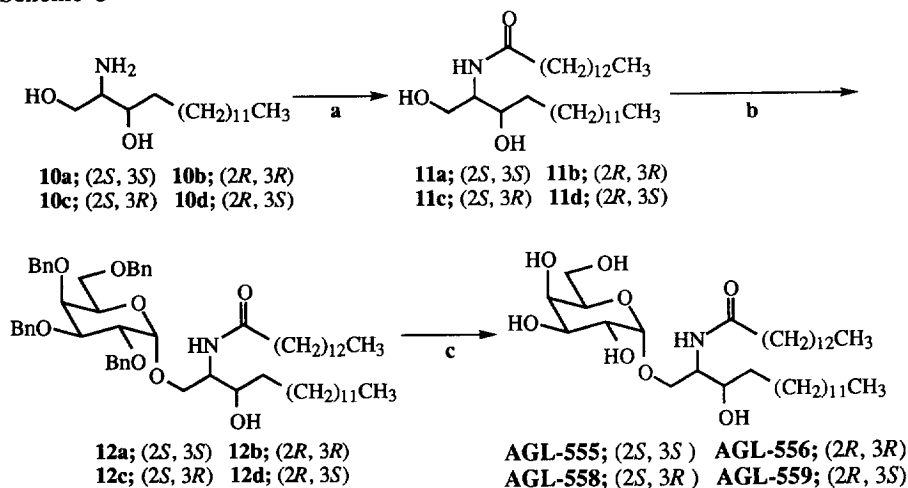
Scheme 2

(a) TMSOTf, MS4A / CH_2Cl_2 ; (b) NaOMe / MeOH-THF

AGL-564 (β -GalCer) and AGL-562 (β -GluCer) were synthesized as follows: the same ceramide (1) was treated with penta-*O*-acetyl sugars (6, 7) in the presence of trimethylsilyltriflate (TMSOTf) and molecular sieves 4A⁹ to afford the desired β -glycosides (8, 9). Deacetylation of 8 and 9 by treatment with sodium methoxide to give AGL-564 and AGL-562 (Scheme 2).

Furthermore we synthesized KRN7000¹⁰ (α -GalCer) and AGL-583¹¹ (β -GalCer) which have the different ceramide portion⁷ from AGL-517 using the same glycosidation methods as described for AGL-517 and AGL-564, and their structures are shown in Fig. 2.

Scheme 3



(a) *p*-nitrophenyl tetradecanoate, DMAP / THF; (b) 2, SnCl_2 , AgClO_4 , MS4A / THF;
 (c) H_2 , Pd-BaSO₄ / THF

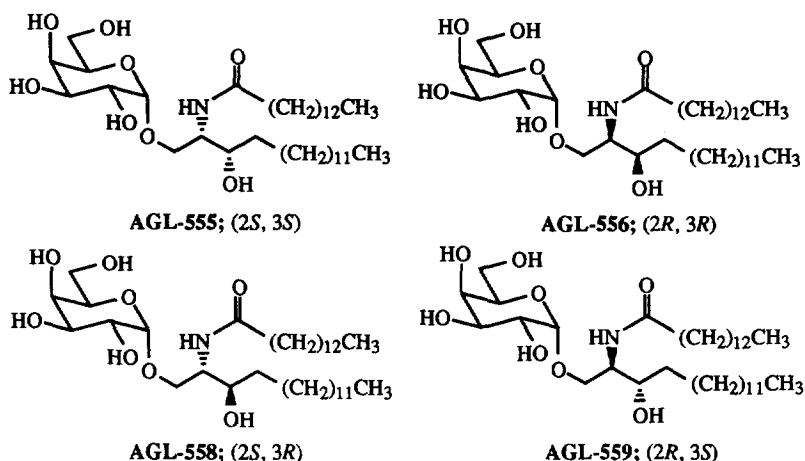


Fig. 3. Structures of AGL-555, AGL-556, AGL-558, and AGL-559.

In addition, we previously reported⁷ that, the structure of the ceramide portion in α -GalCer greatly affects their biological activities, i.e., (1) the longer fatty acid in ceramide moiety shows more marked antitumor activity in the range of C₁₄ - C₂₆, (2) the optimal length of long chain base in ceramide is C₁₈ in the range of C₁₁ - C₂₀, and (3) 3-hydroxyl group plays an important role in biological activities. However, the biological activities of the diastereomers of the ceramide portion have not been reported yet. To examine their biological activities, we synthesized four kinds of diastereomers having α -GalCer structures starting from dihydrosphingosines (**10a-d**)¹² by previously reported method^{2, 7} (Scheme 3). Fig. 3 shows the structures of four diastereomers which are AGL-555 (2*S*, 3*S*)¹³, AGL-556 (2*R*, 3*R*)¹⁴, AGL-558 (2*S*, 3*R*)¹⁵, and AGL-559 (2*R*, 3*S*)¹⁶.

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References and Notes

1. Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591-5592.
2. Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron*, **1994**, *50*, 2771-2784.
3. **AGL-517** physical data: $[\alpha]_{\text{D}}^{24} = +57.8^\circ$ (*c* 1.0, pyridine); m.p. 159.0-161.0 °C; FDMS *m/z* 674 M^+ ; ^1H NMR (500 MHz, $\text{C}_5\text{D}_5\text{N}$) δ (ppm) 8.52 (1H, d, *J* = 8.6 Hz), 5.45 (1H, d, *J* = 3.7 Hz), 4.69-4.76 (1H, m), 4.63-4.68 (1H, m), 4.40-4.58 (6H, m), 4.36 (1H, dd, *J* = 5.5, 10.0 Hz), 4.25-4.31 (1H, m), 2.48 (2H, t, *J* = 7.0 Hz), 1.80-1.95 (4H, m), 1.52-1.62 (1H, m), 1.18-1.43 (45H, m), 0.88 (6H, t, *J* = 6.7 Hz); Anal. $\text{C}_{38}\text{H}_{75}\text{NO}_8$ (C, H, N). calcd. 67.72, 11.22, 2.08, found. 67.41, 11.28, 2.03.
4. **AGL-562** physical data: $[\alpha]_{\text{D}}^{23} = -3.1^\circ$ (*c* 0.85, pyridine); m.p. 159.0-162.5 °C; FDMS *m/z* 675 ($\text{M}+1$) $^+$; ^1H NMR (500 MHz, $\text{C}_5\text{D}_5\text{N}$) δ (ppm) 8.40 (1H, d, *J* = 8.5 Hz), 4.97 (1H, d, *J* = 7.9 Hz), 4.78 (1H, dd, *J* = 4.9, 10.4 Hz), 4.68-4.75 (1H, m), 4.55 (1H, d, *J* = 11.6 Hz), 4.36 (1H, dd, *J* = 5.5, 11.6 Hz), 4.18-4.30 (4H, m), 4.06 (1H, t, *J* = 7.9 Hz), 3.94-3.98 (1H, m), 2.47 (2H, t, *J* = 7.3 Hz), 1.78-1.99 (4H, m), 1.52-1.61 (1H, m), 1.06-1.44 (45H, m), 0.88 (6H, t, *J* = 7.0 Hz); Anal. $\text{C}_{38}\text{H}_{75}\text{NO}_8$ (C, H, N). calcd. 67.72, 11.22, 2.08, found. 67.88, 11.39, 1.92.
5. **AGL-563** physical data: $[\alpha]_{\text{D}}^{23} = +57.4^\circ$ (*c* 0.46, pyridine); m.p. 162.0-163.0 °C; FDMS *m/z* 675 ($\text{M}+1$) $^+$; ^1H NMR (500 MHz, $\text{C}_5\text{D}_5\text{N}$) δ (ppm) 8.43 (1H, d, *J* = 8.6 Hz), 5.40 (1H, d, *J* = 3.1 Hz), 4.65-4.72 (1H, m), 4.22-4.60 (7H, m), 4.10-4.21 (2H, m), 2.43 (2H, t, *J* = 7.3 Hz), 1.76-1.93 (4H, m), 1.48-1.56 (1H, m), 1.10-1.42 (45H, m), 0.88 (6H, t, *J* = 6.7 Hz); Anal. $\text{C}_{38}\text{H}_{75}\text{NO}_8$ (C, H, N). calcd. 67.72, 11.22, 2.08, found. 67.84, 11.27, 1.96.
6. **AGL-564** physical data: $[\alpha]_{\text{D}}^{24} = +7.3^\circ$ (*c* 0.93, pyridine); m.p. 175.0-177.0 °C; FDMS *m/z* 675 ($\text{M}+1$) $^+$; ^1H NMR (500 MHz, $\text{C}_5\text{D}_5\text{N}$) δ (ppm) 8.31 (1H, d, *J* = 9.2 Hz), 4.88 (1H, d, *J* = 7.3 Hz), 4.80 (1H, dd, *J* = 4.9, 10.4 Hz), 4.64-4.70 (1H, m), 4.55 (1H, d, *J* = 3.1 Hz), 4.49 (1H, dd, *J* = 7.9, 9.2 Hz), 4.40-4.45 (2H, m), 4.10-4.25 (3H, m), 4.06 (1H, t, *J* = 5.8 Hz), 2.44 (2H, t, *J* = 7.3 Hz), 1.76-1.92 (4H, m), 1.48-1.57 (1H, m), 1.10-1.42 (45H, m), 0.88 (6H, t, *J* = 6.7 Hz); Anal. $\text{C}_{38}\text{H}_{75}\text{NO}_8$ (C, H, N). calcd. 67.72, 11.22, 2.08, found. 67.94, 11.37, 1.90.
7. Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Kobayashi, E.; Fukushima, H.; Koezuka, Y. Manuscript submitted for publication.
8. Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.*, **1981**, 431-432.
9. Ogawa, T.; Beppu, K.; Nakabayashi, S. *Carbohydr. Res.* **1981**, *93*, C6-C9.
10. **KRN7000** physical data: $[\alpha]_{\text{D}}^{23} = +43.6^\circ$ (*c* 1.0, pyridine); m.p. 189.5-190.5 °C; negative FABMS *m/z* 857 ($\text{M}-\text{H}$) $^-$; ^1H NMR (500 MHz, $\text{C}_5\text{D}_5\text{N}$) δ (ppm) 8.47 (1H, d, *J* = 8.5 Hz), 5.58 (1H, d, *J* = 3.7 Hz), 5.27 (1H, m), 4.63-4.70 (2H, m), 4.56 (1H, m), 4.52 (1H, t, *J* = 6.1 Hz), 4.37-4.47 (4H, m), 4.33 (2H, m), 2.45 (2H, t, *J* = 7.3 Hz), 2.25-2.34 (1H, m), 1.87-1.97 (2H, m), 1.78-1.85 (2H, m), 1.62-1.72 (1H, m), 1.26-1.45 (66H, m), 0.88 (6H, t, *J* = 6.7 Hz); Anal. $\text{C}_{50}\text{H}_{99}\text{NO}_9$ (C, H, N). calcd. 69.97, 11.63, 1.63, found. 69.77, 11.65, 1.53.
11. **AGL-583** physical data: $[\alpha]_{\text{D}}^{24} = -3.5^\circ$ (*c* 0.81, pyridine); m.p. 152.0-154.0 °C; negative FABMS *m/z* 857 ($\text{M}-\text{H}$) $^-$; ^1H NMR (500 MHz, $\text{C}_5\text{D}_5\text{N}$) δ (ppm) 8.44 (1H, d, *J* = 8.5 Hz), 5.11-5.17 (1H, m), 4.91 (1H, d, *J* = 7.3 Hz), 4.81 (1H, dd, *J* = 5.5, 10.4 Hz), 4.54 (1H, d, *J* = 3.1 Hz), 4.35-4.50 (6H, m), 4.17-4.24 (1H, m), 4.14 (1H, dd, *J* = 3.7, 9.8 Hz), 4.04 (1H, t, *J* = 6.1 Hz), 2.42 (2H, t, *J* = 7.3 Hz), 2.17-2.21 (1H, m), 1.63-1.92 (6H, m), 1.05-1.50 (64H, m) 0.86-0.89 [6H, (0.881, t, *J* = 6.7 Hz), (0.876, t, *J* = 6.7 Hz)]; Anal. $\text{C}_{50}\text{H}_{99}\text{NO}_9$ (C, H, N). calcd. 69.97, 11.63, 1.63, found. 70.12, 11.70, 1.51.
12. Matsuda, H.; Yamamoto, T.; Sato, T. Japanese Patent Laid-Open Publication No. 80617/1994.

13. **AGL-555** physical data: $[\alpha]_{\text{D}}^{23} +52.5^\circ$ (*c* 0.75, pyridine); m.p. 148.5-152.5 °C; FDMS *m/z* 646 M⁺; ¹H NMR (500MHz, C₅D₅N) δ (ppm) 8.10 (1H, d, *J* = 8.5 Hz), 5.46 (1H, d, *J* = 3.7 Hz), 4.75-4.82 (1H, m), 4.66 (1H, dd, *J* = 3.7, 9.8 Hz), 4.34-4.56 (7H, m), 4.12 (1H, t, *J* = 6.1 Hz), 4.07 (1H, dd, *J* = 6.1, 9.8 Hz), 2.49 (2H, t, *J* = 6.5 Hz), 1.63-1.92 (4H, m), 1.50-1.60 (1H, m), 1.20-1.42 (41H, m), 0.88 (6H, t, *J* = 6.7 Hz): Anal. C₃₆H₇₁NO₈ (C, H, N). calcd. 66.94, 11.08, 2.17, found. 67.01, 11.28, 1.93.
14. **AGL-556** physical data: $[\alpha]_{\text{D}}^{24} +80.7^\circ$ (*c* 0.27, pyridine); m.p. 149.0-150.5 °C; FDMS *m/z* 646 M⁺; ¹H NMR (500MHz, C₅D₅N) δ (ppm) 8.04 (1H, d, *J* = 8.6 Hz), 5.49 (1H, d, *J* = 3.7 Hz), 4.77-4.82 (1H, m), 4.68 (1H, dd, *J* = 3.7, 9.8 Hz), 4.65 (1H, bd, *J* = 2.4 Hz), 4.36-4.58 (6H, m), 4.16 (1H, dd, *J* = 6.7, 10.4 Hz), 2.50 (2H, t, *J* = 7.3 Hz), 1.64-1.92 (4H, m), 1.47-1.57 (1H, m), 1.20-1.42 (41H, m), 0.88 (6H, t, *J* = 7.0 Hz): Anal. C₃₆H₇₁NO₈ (C, H, N). calcd. 66.94, 11.08, 2.17, found. 67.12, 11.34, 2.09.
15. **AGL-558** physical data: $[\alpha]_{\text{D}}^{24} +74.3^\circ$ (*c* 0.35, pyridine); m.p. 153.5-156.0 °C; FDMS *m/z* 646 M⁺; ¹H NMR (500MHz, C₅D₅N) δ (ppm) 8.52 (1H, d, *J* = 8.6 Hz), 5.47 (1H, d, *J* = 3.7 Hz), 4.71-4.78 (1H, m), 4.67 (1H, dd, *J* = 3.7, 9.8 Hz), 4.34-4.60 (7H, m), 4.26-4.32 (1H, m), 2.48 (2H, dt, *J* = 1.2, 7.3 Hz), 1.80-1.95 (4H, m), 1.52-1.62 (1H, m), 1.20-1.42 (41H, m), 0.87 (6H, t, *J* = 6.8 Hz): Anal. C₃₆H₇₁NO₈ (C, H, N). calcd. 66.94, 11.08, 2.17, found. 66.99, 11.24, 2.01.
16. **AGL-559** physical data: $[\alpha]_{\text{D}}^{24} +62.0^\circ$ (*c* 0.50, pyridine); m.p. 145.0-147.0 °C; FDMS *m/z* 646 M⁺; ¹H NMR (500MHz, C₅D₅N) δ (ppm) 8.40 (1H, d, *J* = 8.5 Hz), 5.47 (1H, d, *J* = 3.7 Hz), 4.66-4.76 (3H, m), 4.10-4.62 (7H, m), 2.48 (2H, dt, *J* = 1.8, 7.3 Hz), 1.73-2.00 (4H, m), 1.52-1.62 (1H, m), 1.20-1.42 (41H, m), 0.88 (6H, t, *J* = 6.7 Hz): Anal. C₃₆H₇₁NO₈ (C, H, N). calcd. 66.94, 11.08, 2.17, found. 67.01, 11.30, 2.13.

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