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The first synthesis of glycan parts of lactoganglio- and neolactoganglio-series gangliosides[1]

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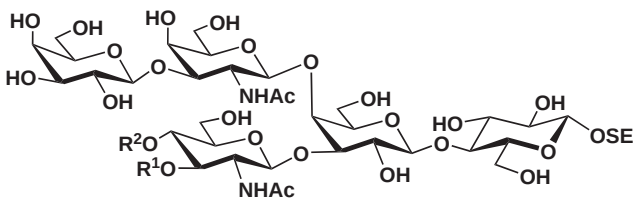
Abstract—The glycan parts of lactoganglio- and neolactoganglio-series of gangliosides were synthesized for the first time based on a well-designed synthetic strategy.

Glycosphingolipids are characteristic membrane components of eukariotic cells where they are found in the carbohydrate-rich glycocalix. Gangliosides are distinguished from other glycosphingolipids by containing one or more sialic acid residues, and have been recognized to play important roles in many biological processes.[2–5] We have succeeded[6a,b] in the total syntheses of a variety of gangliosides, e.g. ganglio-, lacto-, neolacto-, globo- and isoglobo-series gangliosides including their analogs and derivatives, and have contributed to the elucidation of their biological functions at the molecular level.

Recently, a novel series of gangliosides which contain the structural features of both lacto- and ganglio-series or neolacto- and ganglio-series, were isolated and termed as lactoganglio- (**1**)[7] and neolactoganglio-series (**2**)[8] gangliosides, respectively (Fig. 1). However, the biological functions of these molecules have not been elucidated because of limited insufficient availability of materials. In this paper, as a part of our studies to establish procedures to synthesize gangliosides, the successful construction of the glycan parts of lactoganglio- and neolactoganglio-series gangliosides is described.

The most important point in the synthesis of the title compounds is the construction of the core tetrasaccharide, GlcNAcβ1→3(GalNAcβ1→4)Galβ1→4Glc. Because the procedures employed for the synthesis of this tetrasaccharide by Ito et al.[9] are not applicable to further elongation of the sugar chain, which leads to the desired octasaccharides, we developed a novel strategy to construct a tetrasaccharide intermediate suitable for such elongation. Here, the synthesis of the neolactoganglio-series ganglioside (**2**) is described in detail, and the other target, lactoganglio-series ganglioside (**1**), was synthesized using the same procedure.

The NIS–TfOH promoted glycosylation[10] of **3**, which was prepared from 2-(trimethylsilyl)ethyl (2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[11] via its 3′,4′-orthoester[12] with the suitably protected glucosamine donor **4**[13] in dichloromethane at –20°C, gave the desired trisaccharide **5** in



1 R^1 = GM2 epitope, R^2 = H (lactoganglio)
2 R^1 = H, R^2 = GM2 epitope (neolactoganglio)

GM2 epitope:

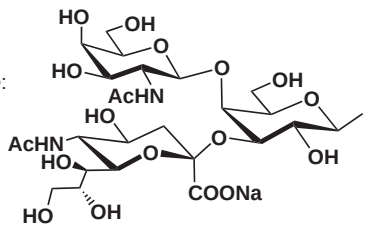


Figure 1. Structures of target compounds.

Keywords: lactoganglio-series gangliosides; neolactoganglio-series gangliosides.

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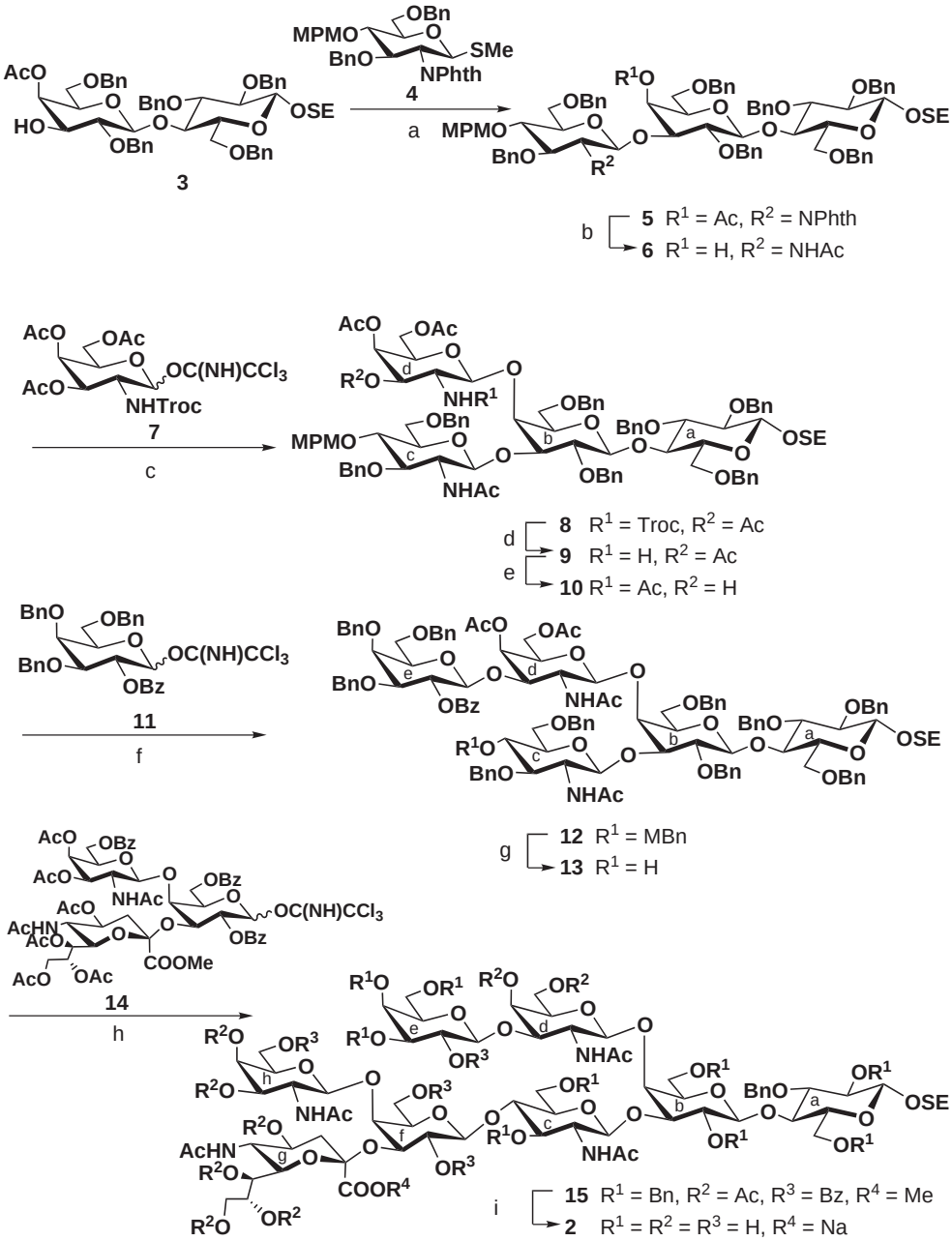

Scheme 1. *Reagents and conditions*: (a) NIS, TfOH, CH_2Cl_2, –20°C; (b) (i) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, (ii) Ac_2O, CH_2Cl_2; (c) TMSOTf, CH_2Cl_2, –10°C; (d) Zn, AcOH, 60°C; (e) Et_3N, 60°C; (f) TMSOTf, CH_2Cl_2, 0°C; (g) CAN, CH_3CN; (h) TMSOTf, CH_2Cl_2, 10°C; (i) (i) H_2, $Pd(OH)_2$, EtOH, (ii) NaOMe, MeOH, then 0.2 M KOH.

Table 1.

Target compounds	Reaction yield (%)							
	a	b	c	d+e	f	g	h	i
1	78	93	81	58	54	82	43	98
2	76	84	83	65	52	89	86	76

76% yield. Conversion of the phthalimido group to an acetamido group and the simultaneous deacetylation at O-4′ afforded **6**, which was successfully coupled with **7**[14] promoted by TMSOTf to give the core tetrasaccharide **8** (83%). In contrast to the 2,2,2-trichloroethoxycarbonyl (N-Troc) derivative of GalN (**7**), the *N*-phthaloyl derivative gave a poor yield (up to 20%) on coupling with the same glycosyl acceptor. In addition, the glycosylation of the GalN(1→4′)Lac acceptor with the GlcN donor, an alternative strategy, gave no product.

Treatment of **8** with zinc powder in acetic acid cleaved the Troc to give the free amine **9**, which then underwent acetyl migration from O-3 to N-2 of GalN on treatment with triethylamine (large excess) at 60°C, to afford the tetrasaccharide acceptor **10** (65% from **8**). In the ^{1}H NMR spectrum of **10**, H-3 of GalNAc appeared at δ 3.68, indicating the C-3 of the GalNAc was a free hydroxyl. The resulting hydroxyl of **10** was then galactosylated with **11**[15] by using the TMSOTf-promoted glycosylation procedure at 0°C to give the desired pentasaccharide **12** (52%). This pentasaccharide was converted, by removal of the *p*-methoxybenzyl (MPM) group with ceric ammonium nitrate (CAN) in acetonitrile, into the key glycosyl acceptor **13** (89%), which was then glycosylated with the GM2 epitope donor **14**. The donor **14** was obtained from 2-(trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,6-di-*O*-benzyl-β-D-galactopyranoside and methyl 6-*O*-benzyl-2-deoxy-3,4-*O*-isopropylidene-2-phthalimido-1-thio-β-D-galactopyranoside by employing the procedure reported for the synthesis of ganglioside GM1.[16] The TMSOTf-promoted glycosylation of the pentasaccharide acceptor **13** with GM2 epitope trisaccharide donor **14** in dichloromethane at 0°C gave the desired octasaccharide **15** (86%). Hydrogenolytic removal of the benzyl group in **15** over $Pd(OH)_2$ in ethanol, followed by removal of all the protecting groups under the basic condition and column chromatography on Sephadex LH-20, furnished the glycan part of neolactoganglio-series gangliosides (**2**) in high yield (Scheme 1).[17]

The other target, the glycan part of lactoganglio-series ganglioside (**1**), was also successfully synthesized in similar yields to those of **2**, except for the coupling of the GM2 epitope donor and the pentasaccharide acceptor (see Table 1), by employing the 3-*O*-*p*-methoxybenzylated GalN donor[18] in place of **4**.

In conclusion, a highly efficient synthesis of the glycan parts of novel, minor gangliosides, neolactoganglio- and lactoganglio-series has been achieved for the first time.

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17. Selected physical data: Compound **8**: $[\alpha]_D$ −14.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): 7.51–6.83 (m, 39H, Ph), 6.96 (d, 1H, $J_{NH,2}=10.07$, AcN*H*-2d), 5.40 (d, 1H, $J_{3,4}=3.0$ Hz, H-4d), 5.20 (d, 1H, $J_{1,2}=8.7$ Hz, H-1d), 5.06 (dd, 1H, $J_{2,3}=10.2$ Hz, $J_{3,4}=3.0$ Hz, H-3d), 3.96 (m, 1H, Me_3SiCH_2CHHO), 3.81 (s, 3H, *Me*OPH), 3.19 (m, 1H, H-5c), 2.23–1.59 (4s, 12H, AcN, 3AcO), 1.04 (2m, 2H, $Me_3SiCH_2CH_2O$). Compound **12**: $[\alpha]_D$ +18.5 (*c* 2.6, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): 8.02–6.85 (m, 59H, Ph), 6.08 (d, 1H, $J_{NH,2}=11.0$ Hz, AcN*H*-2d), 5.55 (t, 1H, $J_{1,2}=J_{2,3}=9.8$ Hz, H-2e), 5.34 (d, 1H, $J_{3,4}=3.00$ Hz, H-4d), 3.82 (s, 3H, *Me*OPh), 1.46–2.17 (4s, 12H, 2AcN, 2AcO), 1.03 (2m, 2H, $Me_3SiCH_2CH_2O$). Compound **15**: $[\alpha]_D$ +5.14 (*c* 1.4, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): 8.07–7.93 (m, 70H, Ph), 6.38–6.14 (2d, 2H, AcN*H*), 5.29 (m, 1H, H-8g), 5.25 (dd, 1H, $J_{6,7}=1.6$, $J_{7,8}=9.8$ Hz, H-7g), 5.06 (m, 1H, H-4h), 5.04 (d, 1H, $J_{1,2}=8.7$ Hz, H-1h), 3.84 (s, 3H, COOMe), 2.30–1.20 (12s, 36H, 4AcN, 8AcO), 1.01 (m, 2H, $Me_3SiCH_2CH_2$), Compound **2**: $[\alpha]_D$ +18.9 (*c* 0.38, CH_3OH); ^{1}H NMR (500 MHz; CD_3OD): 4.94 (d, 1H, $J_{1,2}=8.7$ Hz, H-1d), 4.81 (d, 1H, $J_{1,2}=8.7$ Hz, H-1h), 4.57 (d, 1H, $J_{1,2}=8.5$ Hz, H-1c), 4.47 (2d, 2H, H-1e, H-1f), 4.37 (d, 1H, $J_{1,2}=7.8$ Hz, H-1b), 4.29 (d, 1H, $J_{1,2}=8.0$ Hz, H-1a), 2.75 (dd, 1H, $J_{gem}=12.4$ Hz, $J_{3eq,4}=4.8$ Hz, H-3geq), 2.10–1.90 (4s, 12H, 4AcN), 1.88 (dd, 1H, $J_{gem}=12.4$ Hz, $J_{3ax,4}=11.5$ Hz, H-3gax), 1.01 (m, 2H, $Me_3SiCH_2CH_2$). FABMS $(M-Na)^-$ 1665.6 ($C_{64}H_{109}N_4NaO_{44}Si$).
18. Methyl 4,6-di-*O*-benzyl-2-deoxy-3-*O*-(4-methoxybenzyl)-2-phthalimido-1-thio-β-D-glucopyranoside.