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Preliminary Communication

Synthesis of neoglycolipids containing a mucin-type core unit

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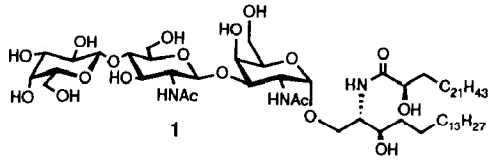
The cores of mucin-type carbohydrate chains have unique structures which have not been found in glycolipids. Typical structures of this type are T, Tn, and sialyl Tn antigens, which are considered to be tumor-associated antigens[1–4]. In contrast with the generation of monoclonal antibodies to glycolipids, it is difficult to produce specific antibodies against the carbohydrate portions of glycoproteins. To obtain monoclonal antibodies against mucin-type carbohydrate chains we established a synthetic route to neoglycolipid[5] antigens, such as **1** and **2**, which were designed to carry a mucin-type core unit on a ceramide foundation.

Boron trifluoride etherate-promoted glycosylation[6] of the 2-azido-2-deoxy-D-galactose derivative[7] **4** with lactosamine donor **3** in 1,2-dichloroethane was performed (Scheme 1) in the presence of activated, powdered molecular sieves



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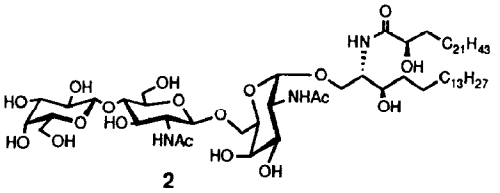

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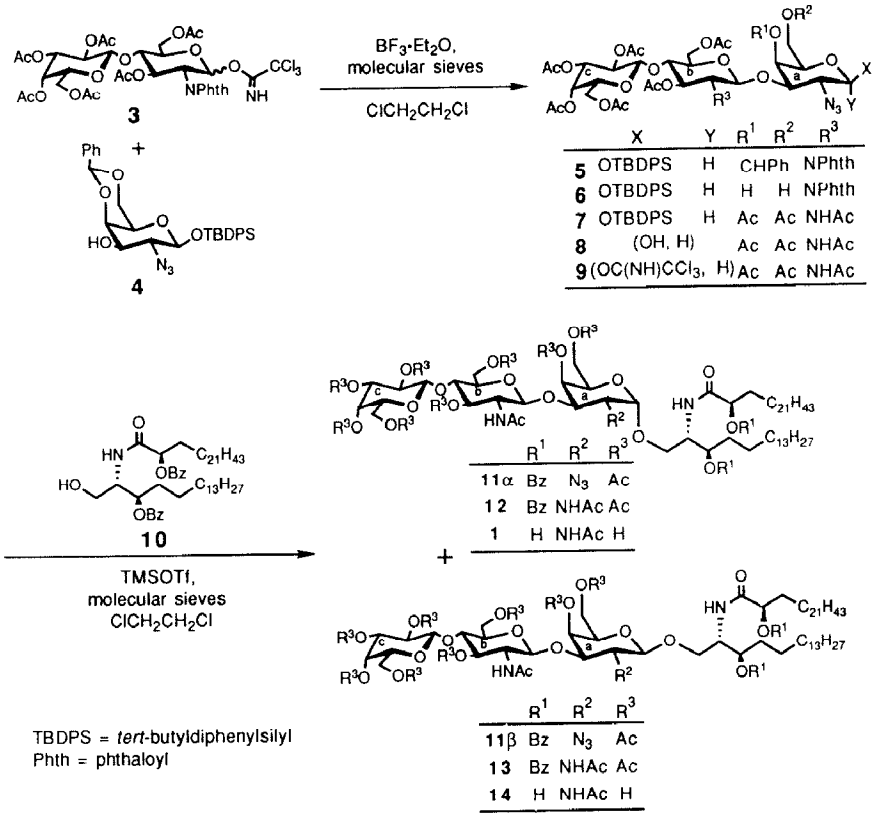


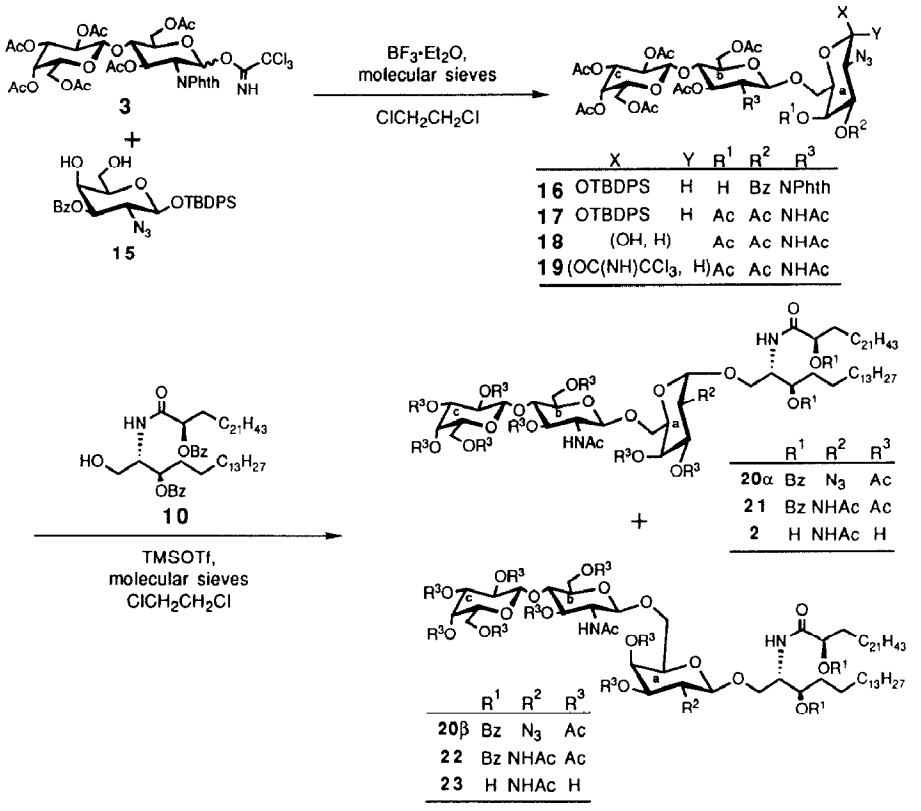
Scheme 1.

AW-300 at −20° to give an 84% yield of **5**, δ_H* 5.51 (d, 1 H, *J* 8.3 Hz, H-1b), 5.46 (s, 1 H, PhC*H*), 4.59 (d, 1 H, *J* 7.8 Hz, H-1c), and 4.30 (d, 1 H, *J* 7.8 Hz, H-1a); *m/z* ** 1236.8 (M^+). Removal of the benzylidene group[8] of **5** with CF_3CO_2H–CH_2Cl_2 gave an 80% yield of **6**, δ_H 5.49 (d, 1 H, *J* 8.8 Hz, H-1b), 4.56 (d, 1 H, *J* 7.8 Hz, H-1c), and 4.32 (d, 1 H, *J* 7.3 Hz, H-1a); *m/z* 1148.8 (M^+), which was converted into **7** in two steps (*i*, hydrazine hydrate–EtOH[9]; *ii*, Ac_2O–pyridine, 95% overall), δ_H 5.45 (d, 1 H, *J* 9.5 Hz, N*H*), 4.55 (d, 1 H, *J* 7.9 Hz, H-1b), 4.54 (d, 1 H, *J* 7.9 Hz, H-1c), and 4.31 (d, 1 H, *J* 7.7 Hz, H-1a); *m/z* 1167 $(M + Na)^+$. The TBDPS protective group was removed with Bu_4NF in the presence of acetic acid[10] to give an anomeric mixture **8**, δ_H 4.65 (d, 1 H, *J* 6.8 Hz, H-1b), 4.07 (d, 1 H, *J* 8.6 Hz, H-1aβ), and 4.54 (d, 1 H, *J* 8.3 Hz, H-1c); *m/z* 906.9 (M^+), which on treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene and CCl_3CN afforded a trisaccharide imidate **9** (54% overall yield from **7**), δ_H 8.76 (s, 1 H, C=N*H*), 6.44 (d, 1 H, *J* 3.4 Hz, H-1aα), 4.67 (d, 1 H, *J* 8.3 Hz, H-1b), and 4.52 (d, 1 H, *J* 7.8 Hz, H-1c). The glycosylation of protected ceramide **10** with **9** was promoted with

* Values of δ_H were measured for solutions in $CDCl_3$ unless noted otherwise.
** Values of *m/z* measured by FD-MS.

trimethylsilyl triflate in 1,2-dichloroethane at room temperature to give a mixture of **11**α and **11**β. The ceramide **10** was obtained in 4 steps from natural ceramide (type IV, purchased from Sigma Chemical Co., St. Louis, MO): *i*, tritylation; *ii*, benzoylation; *iii*, removal of the trityl group; and *iv*, reduction of the double bound by hydrogenation. As judged from the intensities of the NMR signals of H-1a (**11**α: δ_H 4.79, *J* 3.4 Hz; **11**β: 4.02, 7.8 Hz), the ratio of the α and β glycosides was 2:3. The anomers were not separated in this step. The azido groups of compounds **11**α and **11**β were transformed into acetamido groups in two steps (*i*, $NaBH_4$–$NiCl_2$ in ethanol[11]; *ii*, Ac_2O–pyridine) to give compounds **12** and **13**. These were separated by silica gel-column chromatography to give 17% of **12** and 24% of **13** from **10**. Compound **12** showed δ_H 7.32 (d, 1 H, CerN*H*), 4.70 (d, 1 H, *J* 7.8 Hz, H-1b), 4.63 (d, 1 H, *J* 3.9 Hz, H-1a), and 4.54 (d, 1 H, *J* 7.8 Hz, H-1c); **13** gave δ_H 6.88 (d, 1 H, *J* 8.1 Hz, CerN*H*), 4.82 (d, 1 H, *J* 8.3 Hz, H-1a), 4.51 (d, 1 H, *J* 7.8 Hz, H-1b), and 4.51 (d, 1 H, *J* 7.8 Hz, H-1c). Deprotection of both compounds **12** and **13** was carried out by treatment with NaOMe, to give compound **1** and the β isomer **14**. The structures of **1** and **14** were assigned from the reaction sequence, and established by their NMR data (pyridine-d_6–D_2O, 50°): **1**, δ_H 5.47 (d, 1 H, *J* 8.3 Hz, H-1b), 5.36 (d, 1 H, *J* 3.9 Hz, H-1a), and 4.97 (d, 1 H, *J* 7.8 Hz, H-1c); **14**, δ_H 5.52 (d, 1 H, *J* 8.3 Hz, H-1b), 5.16 (d, 1 H, *J* 8.3 Hz, H-1a), and 4.98 (d, 1 H, *J* 7.8 Hz, H-1c).

For the synthesis of the trisaccharide glycolipid **2** (Scheme 2), the 4,6-unprotected 2-azido-2-deoxygalactose derivative **15**, obtained from **4** in two steps (*i*, benzoyl chloride–pyridine; *ii*, CF_3CO_2H–CH_2Cl_2), was used as the acceptor. This glycosylation was promoted with boron trifluoride etherate in the presence of activated, powdered molecular sieves AW-300 in 1,2-dichloroethane at room temperature to give a 29% yield of **16**, δ_H 5.33 (d, 1 H, *J* 8.8 Hz, H-1b), 4.70 (dd, 1 H, $J_{3,4}$ 2.9 Hz, H-3a), 4.54 (d, 1 H, *J* 7.8 Hz, H-1c), and 4.40 (d, 1 H, *J* 7.8 Hz, H-1a). The 4-*O*-glycosylated regioisomer was not found in this reaction mixture. The conversion of **16** into glycotriosyl donor **19** was carried out (56% overall yield) as described for the preparation of **9** from **6**, via **17**, δ_H 5.22 (dd, 1 H, $J_{4,5}$ 0.5 Hz, H-4a), 5.13 (d, 1 H, *J* 10.3 Hz, N*H*), 4.66 (dd, 1 H, $J_{3,4}$ 2.9 Hz, H-3a), 4.48 (d, 1 H, *J* 7.8 Hz, H-1a), 4.45 (d, 1 H, *J* 7.8 Hz, H-1c), and 4.27 (d, 1 H, *J* 7.8 Hz, H-1b); *m/z* 1144.7 (M^+); and **18**, δ_H 4.85 (d, 1 H, *J* 6.8 Hz, H-1b), 4.72 (d, 1 H, *J* 8.6 Hz, H-1a), and 4.48 (d, 1 H, *J* 8.3 Hz, H-1c); *m/z* 906.9 (M^+). Compound **19** showed δ_H 9.30 (s, 0.5 H, C=N*H*), 8.78 (s, 0.5 H, C=N*H*), 6.40 (d, 0.5 H, *J* 3.4 Hz, H-1aα), and 5.65 (d, 0.5 H, *J* 7.8 Hz, H-1aβ). The coupling of **19** and protected ceramide **10** was promoted with trimethylsilyl triflate in 1,2-dichloroethane at room temperature to give a mixture of **20**α and **20**β, which were not separated in this step. As judged from the intensities of the NMR signals of H-1a (**20**α: δ_H 4.93, *J* 3.9 Hz; **20**β: 4.17, 8.3 Hz), the ratio of the α and β glycosides was 4:3. The azido groups of compounds **20**α and **20**β were converted into acetamido groups, and the products **21** and **22** were separated by silica gel-column chromatography to give 11% of **21** and 9% of **22** from **10**. The NMR data were: **21**, δ_H 6.80 (d, 1 H, *J* 9.8



	X	Y	R^1	R^2	R^3
16	OTBDPS	H	H	Bz	NPhth
17	OTBDPS	H	Ac	Ac	NHAc
18	(OH, H)		Ac	Ac	NHAc
19	$(OC(NH)CCl_3$, H)		Ac	Ac	NHAc

	R^1	R^2	R^3
20α	Bz	N_3	Ac
21	Bz	NHAc	Ac
2	H	NHAc	H

	R^1	R^2	R^3
20β	Bz	N_3	Ac
22	Bz	NHAc	Ac
23	H	NHAc	H

Scheme 2.

Hz, CerN*H*), 4.55 (d, 1 H, *J* 3.4 Hz, H-1a), 4.48 (d, 1 H, *J* 6.8 Hz, H-1b), and 4.47 (d, 1 H, *J* 7.3 Hz, H-1c); **22**, δ_H 7.10 (d, 1 H, *J* 8.1 Hz, CerN*H*), 4.71 (d, 1 H, *J* 8.3 Hz, H-1a), 4.57 (d, 1 H, *J* 7.3 Hz, H-1b), and 4.49 (d, 1 H, *J* 7.8 Hz, H-1c). Deprotection of both **21** and **22** was achieved by treatment with NaOMe, to give compound **2** and the β isomer **23**. The structures of **2** and **23** were supported by their NMR data (2:1 $CDCl_3$–CD_3OD): **2**, δ_H 4.52 (d, 1 H, *J* 3.4 Hz, H-1a), 4.19 (d, 1 H, *J* 7.8 Hz, H-1b), and 4.11 (d, 1 H, *J* 7.8 Hz, H-1c); **23**, δ_H 4.20 (d, 1 H, *J* 7.3 Hz, H-1b), 4.09 (d, 1 H, *J* 7.3 Hz, H-1c), and 4.07 (d, 1 H, *J* 7.3 Hz, H-1a).

In conclusion, we have established a facile route to the synthesis of mucin-type neoglycolipids **1** and **2** by using the imidates **9** and **19** as key intermediates.

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