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GLYCOSIDATION OF SIALIC ACID

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CONTENTS

Abstract—Sialic acid is located at the non-reducing ends of carbohydrate chains of glycoproteins and glycolipids and plays an important role in biological processes. The synthesis of sialylated oligosaccharides has been encouraged in order to clarify their vital functions. In the synthesis of sialylated oligosaccharides, the most difficult problem remaining is the stereoselective glycosidation of sialic acid by the creation of the α-glycosidic linkage. This Report discusses enzymic and synthetic approaches to the glycosidation of sialic acid by new methodologies.

1. INTRODUCTION

Sialic acids are *N*-acyl derivatives of neuraminic acid, 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid such as *N*-acetylneuraminic acid (Neu5Ac, Fig. 1). Sialic acid residues are located at the non-reducing ends of glycoproteins and glycolipids, and play an important role in biological phenomena,[1,2] including the transport of ions, amino acids, and viruses through membranes.[3] Sialic acid is strongly and preferentially complexed with the calcium ion at pH 7 in a ratio of 1 : 1.[4] Gangliosides are animal glycerosphingolipids which contain sialic acid. They are recognized as binding sites for enzymes, hormones, toxins, lectins, bacteria and viruses.[5–7] They are principally located in the outer cell-surface of plasma membranes. In these situations, sialic acid serves as a carbohydrate with a central function and it is mainly responsible for the negative charge of cell surfaces. After Tsuji *et al.*[8] reported that the ganglioside, GQ_{1b} (Fig. 2), in a few nanomolar concentration, showed remarkable enhancement of cell growth and neurite outgrowth in neuroblastoma cell lines, then sialylglycoconjugates became important synthetic targets. Stereoselective

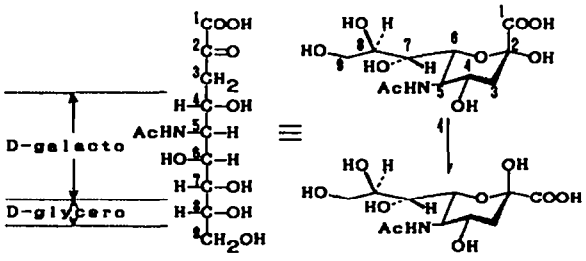


Fig. 1. Conformation of *N*-acetylneuraminic acid.

synthesis of Neu5Ac glycosides is of outstanding interest in order to provide tools for the study of biological functions. For these objectives, not only the naturally occurring α-glycosides but also the unnatural β-glycosides are targets.

The synthesis of Neu5Ac glycosides is challenging because the carboxyl group is attached to the anomeric position. This reduces its reactivity in glycosidation. Furthermore because it is a 3-deoxy sugar the formation of the 2,3-dehydro derivative may be observed when the anomeric hydroxyl group is activated. Many attempts have been made to overcome these difficulties because Neu5Ac is available from various sources.[9-12] This Report describes new methodologies for enzymic and synthetic approaches to glycosides of sialic acid.

2. ENZYMIC GLYCOSIDATION

The sialic acid, Neu5Ac, is synthesized *in vivo* from *N*-acetylmannosamine and pyruvic acid and this process is catalyzed by *N*-acylneuraminate pyruvate lyase (EC 4.1.3.3). CMP-sialic acid synthetase (EC 2.7.7.43) converts Neu5Ac to the activated form, CMP-Neu5Ac, which is incor-

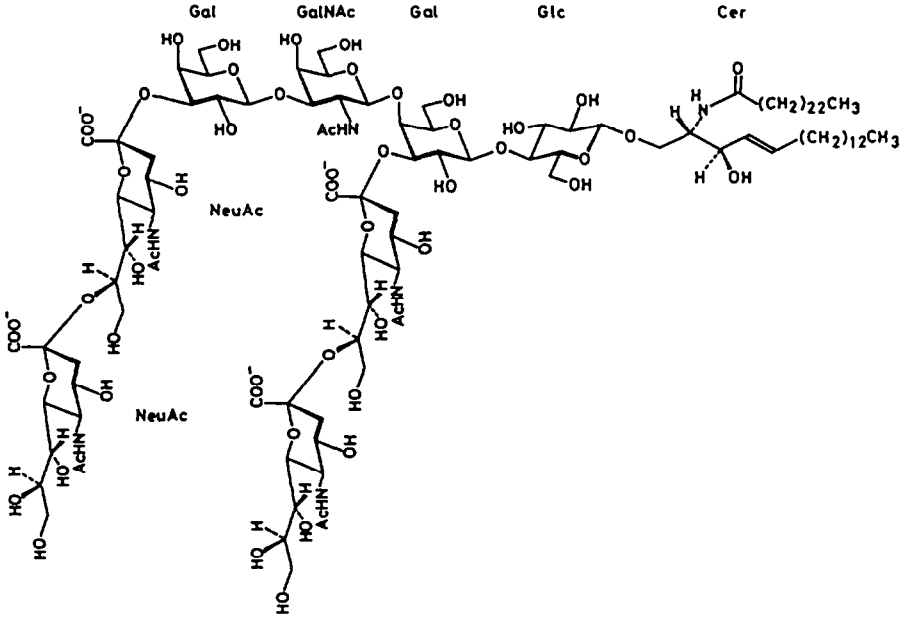
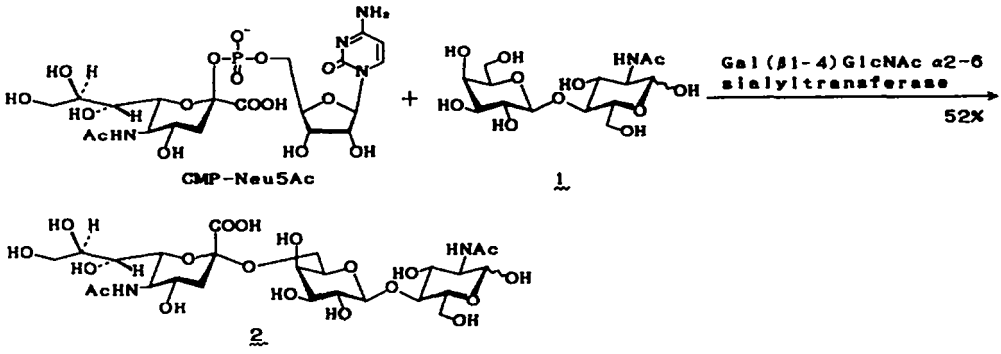


Fig. 2. Structure of ganglioside GQ_{1b}.

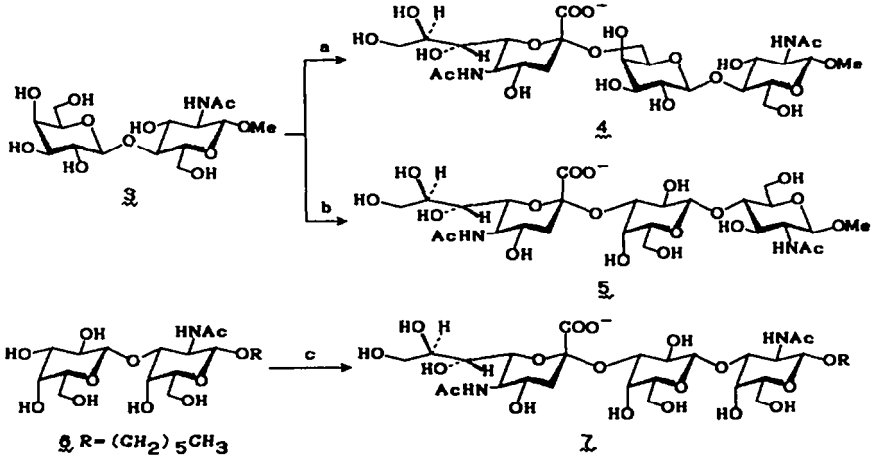


Scheme 1.

porated into the non-reducing ends of glycolipids and glycoproteins with the aid of sialyltransferases (EC 2.4.99.1).

Recently enzymic synthesis has been widely adopted as one of the useful routes to natural products with restriction of substrate. This technique is applicable to the glycosidation of sialic acids with α-glycosidic linkages. *N*-Acylneuraminate pyruvate lyase has the tolerance to catalyze the formation of various kinds of sialic acids having variants at C-5 not only from *N*-acetylmannosamine but also from many hexopyranoses.[13-16] CMP-sialic acid synthetase is also able to activate modified sialic acids at 5- and 9-positions (Fig. 1) (5-glycoloylamino, 5-hydroxy, 9-*O*-acetyl, 9-amino, 9-fluoro, etc.) to yield the corresponding CMP-sialic acids.[17-20] On the other hand sialyltransferases have a strict substrate specificity because they recognize the second sugar unit and the glycosidic linkage.

Thiem *et al.*[21] synthesized (52%) Neu5Ac(α2-6)Gal(β1-4)GlcNAc (**2**) in a 0.1 mmol scale from LacNAc (**1**) and CMP-Neu5Ac by the use of immobilized β-Gal α2-6 sialyltransferase (Scheme 1). Sabesan *et al.* have synthesized ten types of sialyloligosaccharides using three kinds of sialyltransferases by a skillful combination of chemical and enzymic methods (Scheme 2).[22] The di-



(a) Gal (β1-4) GlcNAc α2-6 sialyltransferase, CMP-Neu5Ac
(b) Gal (β1-3(4)) GlcNAc α2-3 sialyltransferase, CMP-Neu5Ac
(c) Gal (β1-3(4)) GlcNAc α2-3 sialyltransferase, CMP-Neu5Ac

Scheme 2.

sialylated tetrasaccharide was synthesized by double sialylation using different sialyltransferases.[23] CMP-Neu5Acs modified at the 9-position of Neu5Ac (Fig. 1) by for examples 9-NH_2 or 9-FITC can be transferred to the corresponding glycoconjugate by sialyltransferase.[19,20] Enzymic glycosidation does not require protecting groups and the immobilized enzyme can be used repeatedly. However, the disadvantage of the enzymic approach for glycosidation is that the individual enzyme has to be prepared and this can present difficulties. Furthermore this technique is not applicable for the synthesis of β-glycosides which are required for the investigation of the structure–function relations.

3. CHEMICAL GLYCOSIDATION

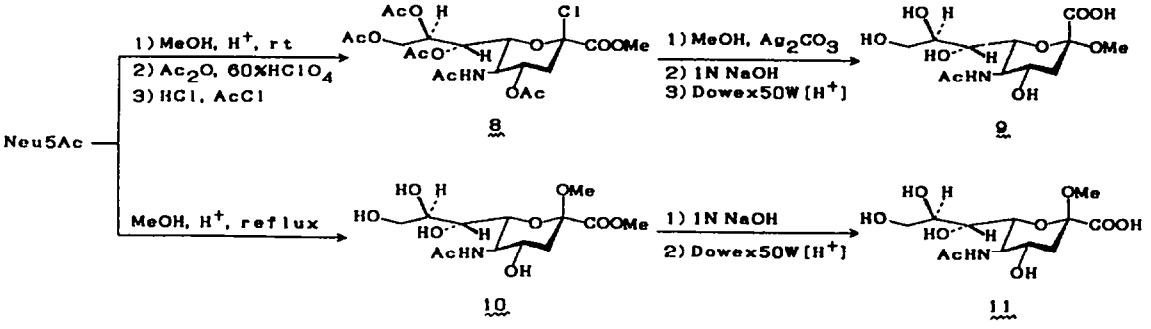
Organic synthesis can yield a reasonable amount of oligosaccharides of high purity.

3.1. *O-Glycosides from 2-deoxy-2β-halo-Neu5Ac derivatives*

Many groups have investigated the glycosidation of Neu5Ac with varying degrees of success. The first attempt was made in 1965 by Meindl *et al.*[24] who used 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-β-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid as the donor and succeeded in the synthesis (30–50%) of various glycosides using Koenigs–Knorr reaction conditions. The free acid can create problems so the corresponding methyl ester **8**, first prepared by Kuhn *et al.*,[29] has been widely used in glycosidation. The methyl α-glycoside **9** was obtained by treating **8** with silver carbonate in methanol: the methyl β-glycoside **11** was prepared by refluxing a methanol solution of Neu5Ac with acid (Scheme 3). This method is useful for the α-glycosidation of Neu5Ac with high reactive alcohols, phenols, and sugar alcohols (Table 1). Unfortunately in the case of less reactive acceptors such as secondary or hindered hydroxyl groups of sugars, the main product comes to be the 2,3-dehydro-Neu5Ac derivative **14** formed by intramolecular elimination. In order to raise the glycosidation yield and increase the α-selectivity a new promoter has been developed (Entry 16, 23). In addition the acceptor has been activated (Entry 67), the protecting groups of Neu5Ac were changed (Entry 68, 69), and minimization of the number of protecting groups in acceptor was examined in order to reduce steric bulk (Entry 89).

Since Neu5Ac is a 3-deoxy sugar it is difficult to avoid competitive elimination. Neighboring-group participation is not expected when **12** is used as a donor. As the anomeric carbon of Neu5Ac is quaternary, glycoside formation is inhibited. However, by using this method gangliosides GM_4[71] and GM_3[73,75] have been synthesized by Ogawa's group.

Among many glycosides obtained by this method, phenyl, indolyl and umbelliferyl glycosides (Entry 28–38) are artificial substrates which produce a dye or a fluorescence after sialidase treatment.



Scheme 3.

Table 1. Glycosidation of the acetyl protected donors 12 with R-OH

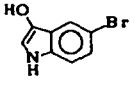
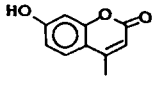
Entry	Donor X	Donor Y	Acceptor (R-OH)	Promoter	Glycosides(13) α(%)	Glycosides(13) β(%)	Ref
1	Cl	H	CH_3OH	Ag_2CO_3	40		24
2	Cl	H	$CH_3(CH_2)_4OH$	Ag_2CO_3	42		24
3	Cl	H	$CH_3(CH_2)_5OH$	Ag_2CO_3	47		24
4	Cl	H	$CH_3(CH_2)_9OH$	Ag_2CO_3	29		24
5	Cl	H	$AcOCH_2(CH_2)_2OH$	Ag_2CO_3	30		24
6	Cl	H	$AcOCH_2(CH_2)_4OH$	Ag_2CO_3	45		24
7	Cl	H	cyclohexanol	Ag_2CO_3	50		24
8	Cl	H	cyclohexylmethanol	Ag_2CO_3	30		24
9	Cl	H	m-Chlorobenzyl alcohol	Ag_2CO_3	48		24
10	Cl	H	m-bromobenzyl alcohol	Ag_2CO_3	47		24
11	Cl	H	m-iodobenzyl alcohol	Ag_2CO_3	30		24
12	Cl	H	p-methoxybenzyl alcohol	Ag_2CO_3	45		24
13	Cl	H	n-BuO_2CCH_2OH	Ag_2CO_3	31		25
14	Cl	H	MeO_2CCH_2OH	Ag_2CO_3		7[a]	25
15	Cl	H	$BnOCH_2CH_2OH$	Ag_2CO_3	24		26
16	Cl	H	CH_3OH	Ag polymaleate	63		27
17	Cl	H	$CH_2=CHCH_2OH$	Ag polymaleate	57		27
18	Cl	H	benzyl alcohol	Ag polymaleate	64		27
19	Cl	H	Z-$HNCH_2CH_2OH$	Ag polymaleate	58[a]		27
20	Cl	H	Z-$HNCH_2CH_2OH$	Ag polymaleate	14[a]		28
21	Cl	Me	CH_3OH	Ag_2CO_3	40-50[b]		29
22	Cl	Me	CH_3OH	Ag_2CO_3	30[b]		30
23	Cl	Me	CH_3OH	Ag salicylate	89		31
24	Cl	Me	CH_3CH_2OH	Ag salicylate	88		31
25	Cl	Me	$(CH_3)_2CHOH$	Ag salicylate	84		31
26	Cl	Me	$(CH_3)_3CCH_2OH$	Ag salicylate	67		31
27	Cl	Me	$CH_2=CHCH_2OH$	Ag salicylate	94		32
28	Cl	H	phenol	Ag_2CO_3	18	6	33
29	Cl	Me	p-nitrophenol	Ag_2CO_3	51		34
30	Cl	Me	p-nitrophenol[c]	----	57		35
31	Cl	Me	m-methoxyphenol	Ag_2CO_3	17	?	36
32	Cl	Me	2-hydroxypyridine[d]	----	19	5	28
33	Cl	Me	 c	----	16		37
34	Cl	Me		$CdCO_3$	61		38
35	Cl	Me		$CdCO_3$	0		39
36	Cl	Me	e	----	63		39
37	Cl	Me	c	Ag_2CO_3	50		40
38	Cl	Me	c	----	68		41

Table 1. (continued)

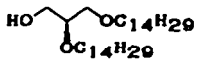
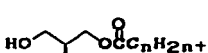
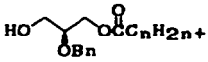
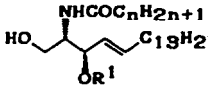
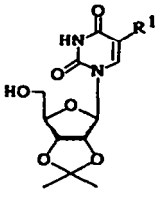
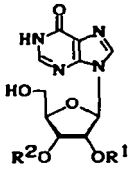
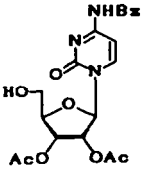
Entry	Donor X	Donor Y	Acceptor (R-OH)		Promoter	Glycosides(13) α(%)	Glycosides(13) β(%)	Ref
39	Cl	Me			$Hg(CN)_2$, $HgBr_2$	37	24	42
40	OAc	Me			TMSOTf	?	?	43
41	Cl	Me		n=7	$Hg(CN)_2$, $HgBr_2$	16	27	44
42	Cl	Me		n=11	$Hg(CN)_2$, $HgBr_2$	11	12	44
43	Cl	Me		n=15	$Hg(CN)_2$, $HgBr_2$	27	32	44
44	Cl	Me		n=15	$Hg(CN)_2$, $HgBr_2$	25	21	44
45	Cl	Me		R^1=Ac, n=17	AgOTf	27	30	45
46	Cl	Me		R^1=Bz, n=17	AgOTf	26	31	45
47	Cl	Me		R^1=Bz, n=23	AgOTf	21	25	45
48	Cl	Me	cholesterol		AgOTf	33	37	46,47
49	Cl	Me	cholesterol		AgOTf	30	30	48
50	Cl	Me		R^1=H	Ag_2CO_3	30	0	49,50
51	Cl	Me		R^1=H	$Hg(CN)_2$, $HgBr_2$	40	10	49,50
52	Cl	Me		R^1=F	$Hg(CN)_2$, $HgBr_2$	11	12	49,50
53	Cl	Me		R^1,R^2=isopropylidene	Ag_2CO_3	?	?	49
54	Cl	Me		R^1,R^2=Ac	AgOTf	9	22	51
55	Cl	Me			AgOTf	8	14	51

Table 1. (continued)

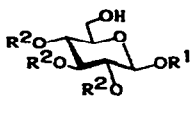
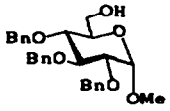
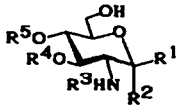
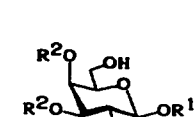
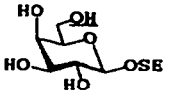
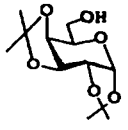
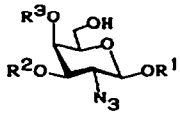
Entry	Donor X	Donor Y	Acceptor (R-OH)			Promoter	Glycosides(13) α(%)	Glycosides(13) β(%)	Ref
				R^1	R^2				
56	Cl	Me		Ac	Ac	Ag_2CO_3	17[a]		52
57	Cl	Me		Me	Bn	AgOTf	10	40	53
									
58	Cl	Me				AgOTf, Ag_2CO_3	0	22	54
59	Cl	Me				AgOTf	26	35	47
									
				R^1	R^2	R^3 R^4	R^5		
60	Cl	Me		H,OAc		Ac Ac	Ac	Ag_2CO_3	16[a]		52
61	Cl	Me		H	Bn	Ac $-Si(Pr)_2O(Pr)_2Si-$		AgOTf	12[f]	46[f]	54
62	Cl	Bn		Bn	H	$-COCH_2CHC_{11}H_{23}$ $OCOC_{13}H_{27}$	H	$Hg(CN)_2$- $HgBr_2$	23 (4)[g]	11 (0)	55
				R^1	R^2				
63	Cl	Me		Ac	Ac	Ag_2CO_3	18[a]		52
64	Cl	Me		Bn	Bn	Ag salicylate	65	3	56
65	Cl	Me		Bn	Bn	$Hg(CN)_2$, $HgBr_2$	36	48	57
66	Cl	Me		MBn	Bn	Ag salicylate	43		58
67	Cl	Me				$(n\text{-}Bu)_4N^+Br^-$	36[h]	23[h]	59
68	Cl	allyl				Ag_2CO_3	40	7	60,61
69	F	allyl				$BF_3 \cdot Et_2$	7	37	60,61
				R^1	R^2	R^3			
70	Cl	Me		Bn	Bn	Bn $Hg(CN)_2$,$HgBr_2$	42	36	62
71	Cl	Me		allyl	H	H AgOTf	41	8	63

Table 1. (continued)

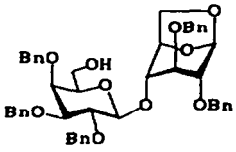
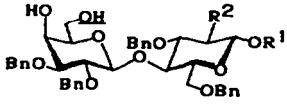
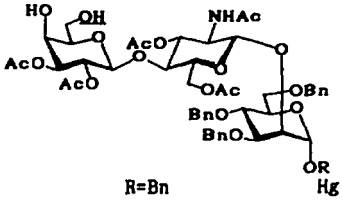
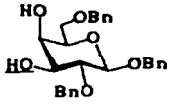
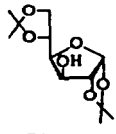
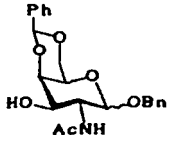
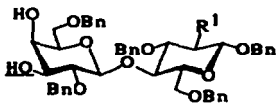
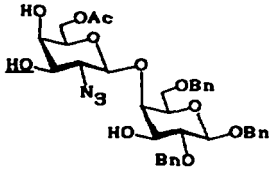
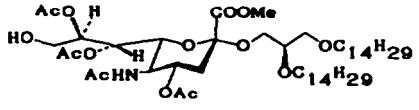
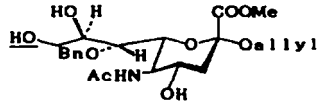
Entry	Donor X	Donor Y	Acceptor (R-OH)	Promoter	Glycosides(13) α(%)	Glycosides(13) β(%)	Ref
72	Cl	Me		$Hg(CN)_2$, $HgBr_2$	21	8	64
							
			R^1 R^2				
73	Cl	Me	Bn N_3	$Hg(CN)_2$, $HgBr_2$	22	23	57,65
74	Cl	Me	Ac NPhth	$Hg(CN)_2$, $HgBr_2$	25	27	66,67
							
75	Cl	Me	R=Bn	$Hg(CN)_2$, $HgBr_2$	34	30	68
76	Cl	Me	R=allyl	$Hg(CN)_2$, $HgBr_2$	48	33	69
77	Cl	Me		$Hg(CN)_2$, $HgBr_2$	6	6	70
78	Cl	Me		$Hg(CN)_2$, $HgBr_2$	12	19	71
79	Cl	Me		$AgClO_4$, Ag_2CO_3	10	8	72
80	Cl	Me		Ag_2CO_3	11[a]		52
81	Cl	Me		Ag_2CO_3	8		52

Table 1. (continued)

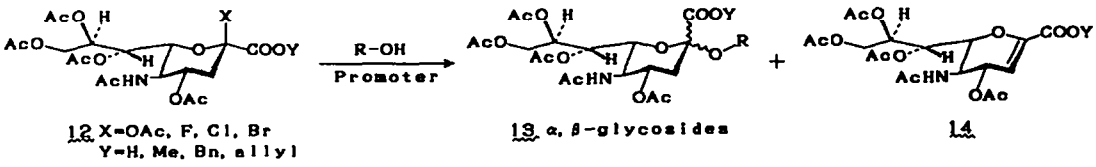
Entry	Donor X	Donor Y	Acceptor (R-OH)	Promoter	Glycosides(13) α(%)	Glycosides(13) β(%)	Ref
							
82	Cl	Me	R^1=OBn	$Hg(CN)_2$, $HgBr_2$	6	12	70,73
83	Br	Me	R^1=OBn	$AgClO_4$, Ag_2CO_3	16	19	74
84	Cl	Me	R^1=OBn	$Hg(CN)_2$, $HgBr_2$	7[h]	18[h]	75
85	Br	Me	R^1=N_3	$AgClO_4$, Ag_2CO_3	21	22	76
86	Br	Me	R^1=N_3	$AgClO_4$, Ag_2CO_3	?	?	77
87	Br	Me		$AgClO_4$, Ag_2CO_3	0	10	72
88	Cl	Me		$Hg(CN)_2$, $HgBr_2$	17	9	42
89	Cl	Me		$Hg(CN)_2$, $HgBr_2$	55	28	78

[a]Yield after complete deprotection. [b]Yield after deacetylation. [c]Sodium salt was used. [d]Silver salt was used. [e]Tetrabutylammonium salt was used. [f]Yield after desilylation followed by acetylation. [g]Hydroxyl groups of the donor were protected by chloroacetyl groups. [h]The acceptor was activated with dibutyltin oxide and yield was based on the acceptor. [i]The acetyl protected Neu5Gc methyl ester was used as a donor.

Glycosides of dialkyl glycerol, ceramide, and cholesterol (Entry 39, 40, 45–49) show strong neurite outgrowth activities in very low concentrations comparable with that of gangliosides.[43,79–82] The lyso-type glyceryl glycosides (Entry 41–44) have inhibitory activity towards phospholipases.[44] The nucleoside derivatives (Entry 50–55) influence the metastasis of lung cancer by inhibition of sialyltransferase.[83,84]

3.2. *Glycosidation using 3-substituted Neu5Ac derivatives*

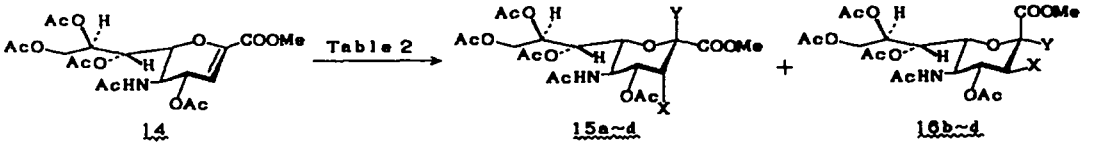
To increase the efficiency of glycosidation and influence α-selectivity the introduction of substituents into the 3-position of Neu5Ac have been examined to prevent the elimination reaction. This pioneer work was carried out by Okamoto *et al.*,[46,47,85–90] who used **14** and checked the reactivity of its 2,3-double bond. This gave new and useful glycosyl donors.



Scheme 4.

The compound **14** was easily prepared in high yield by treatment of peracetyl-Neu5Ac methyl ester with trimethylsilyl triflate (TMSOTf)[91-93] or by direct treatment of Neu5Ac methyl ester with acetic anhydride containing a catalytic amount of sulfuric acid.[94,95] The 2,3-double bond of **14** has high reactivity: chemical treatment easily gave adducts (Scheme 5, Table 2).[87] Bromination of **14** gave the 2β,3α-dibromide **15a** (Entry 1). The dibromide **15a** is a useful glycosyl donor. The 3-axial position is blocked by the bromo group and this prevents the elimination reaction (see below). Treatment of **14** with *N*-bromosuccinimide (NBS) gave two bromohydrins, the *trans*-diaxial adduct **15b** and the *trans*-diequatorial adduct **16b**. These were separable by a column chromatography and their stereochemistry was determined by $J_{3,4}$ values (3.7 and 11.0 Hz) in their ^{1}H-NMR spectra. In this bromohydrination, the product ratio was influenced by reaction temperature (Entry 2–4): low temperature gave the diequatorial adduct **16b** predominantly and the thermodynamically more stable adduct **15b** was the main product at higher temperatures. This phenomenon was reproducible in the iodohydrination (Entry 5, 6). Though haloglycosidation is a useful technique,[96] only the bromomethoxylation of **14** was successful.[97]

Compound **14** resisted direct epoxidation, so the *trans*-diaxial bromohydrin **15b** was converted to the epoxide **17** by treatment with base (Scheme 6). This means that the conformation $^2C_5$ is more

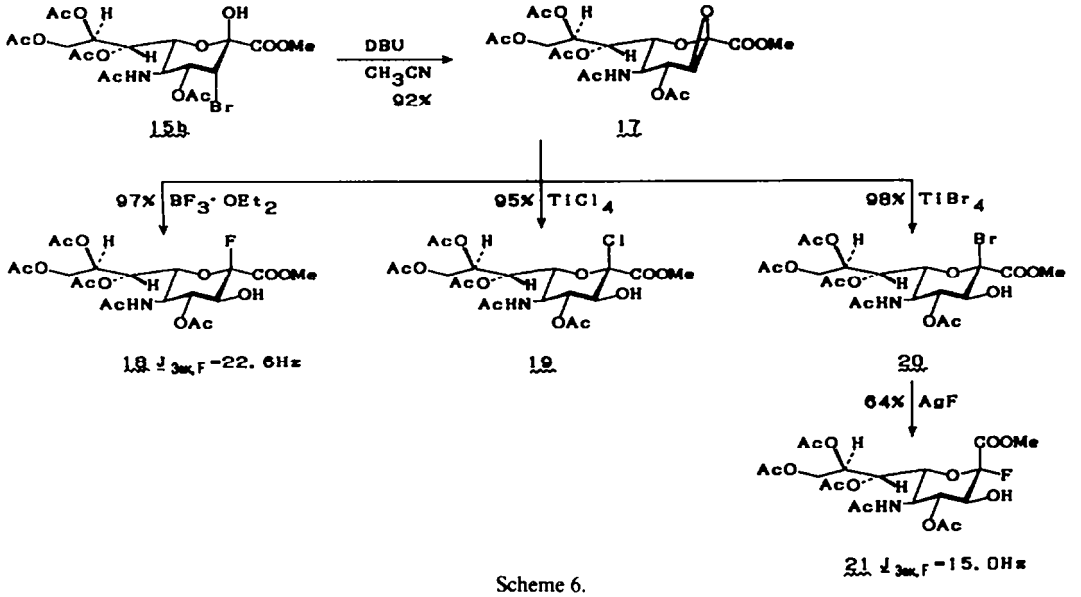


Scheme 5.

Table 2. Addition reaction of 14

Entry	Reaction System	Reaction Temp/°C	Reaction Time/h	X	Y	Product(yield/%[a]) 15	16
1	Br_2, CH_2Cl_2	0	0.2	Br	Br	15a(93)	
2	NBS, MeCN-H_2O	20	6.0	Br	OH	15b(39)	16b(59)
3	NBS, DMSO-H_2O	-20	0.5	Br	OH	15b(8)	16b(84)
4	NBS, MeCN-H_2O	80	0.2	Br	OH	15b(73)	16b(23)
5	NIS, MeCN-H_2O	20	8.0	I	OH	15c(56)	16c(38)
6	NIS, MeCN-H_2O	60	0.5	I	OH	15c(72)	16c(24)
7	NBS, MeOH	20	1.0	Br	OMe	15d(37)	16d(55)

[a]Isolated yield.

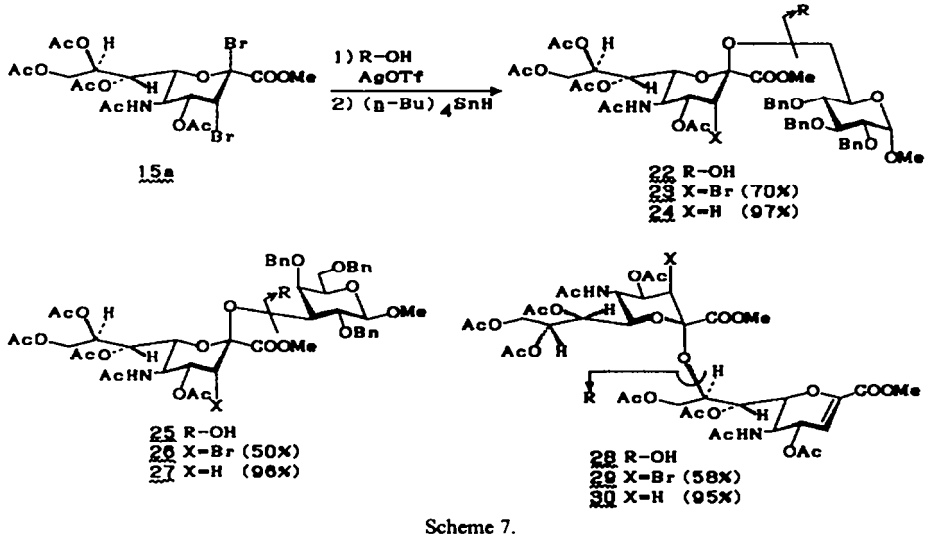


Scheme 6.

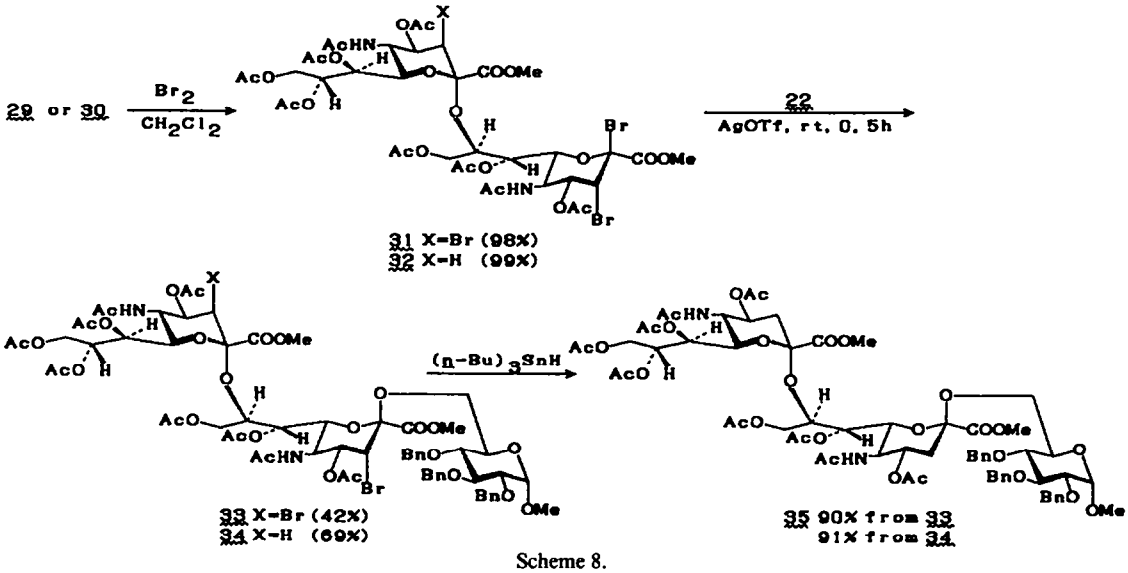
stable. The epoxide **17** was also subjected to the glycosidation reaction as a donor and gave the *cis*-halohydrins **18**, **19** and **20** in quantitative yields. For the determination of the anomeric configuration, the bromohydrin **20** was treated with silver fluoride and this gave the fluorohydrin **21** ($J_{3ax,F}$ = 15.0 Hz). This agreed with a reported value,[98] which was different from that reported for the β-isomer **18** (22.6 Hz).

In this way functionalization of the 2,3-double bond of **14** gave five new glycosyl donors, **15a**, **17**, **18**, **19** and **20**, and their glycosidation ability was examined.

Glycosidation of the 2β,3α-dibromide **15a** with the properly protected glucoside **22**, galactoside **25** and Neu5Ac derivative **28** in the presence of silver triflate (AgOTf) gave only the corresponding β-glycosides, **23**, **26** and **29**, due to steric protection of the α-face by the bromo-substituent (Scheme 7).[46,47] The 3α-bromo group of the glycosides obtained was easily reduced with tributylstannane to



Scheme 7.



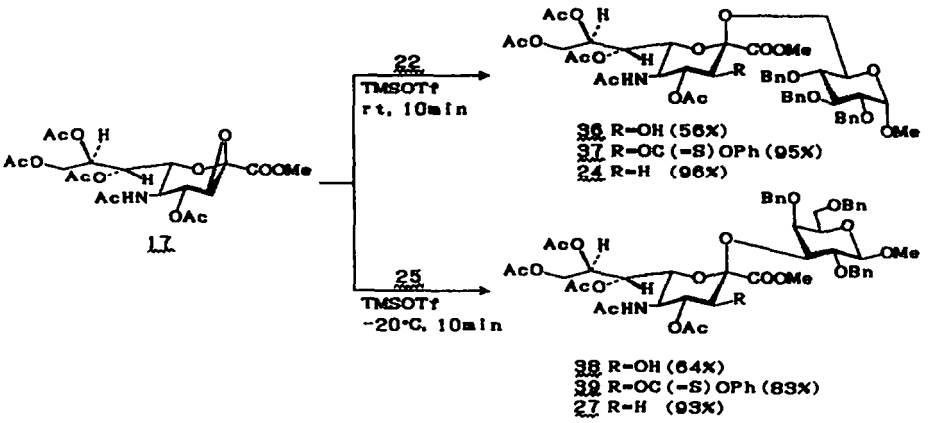
Scheme 8.

yield the corresponding glycosides, **24**, **27** and **30** in high yields. This is the simplest way for the preparation of β-glycosides stereospecifically. This is the first example of the synthesis of Neu5Ac(2–8)Neu5Ac having a β-linkage. This procedure will be applied to the stereospecific synthesis of unnatural sialylglycoconjugates which are required for structure–function studies. This method provides new tactics for the elongation of the sugar chain by repetition of the same operation (Scheme 8). The dineuraminyl saccharides, **29** and **30**, have a 2,3-double bond in the second Neu5Ac unit so this can be quantitatively converted to the corresponding tribromide **31** and dibromide **32** by treatment with bromine. These were used as glycosyl donors in the second glycosidation with the glucoside **22** giving the trisaccharides, **33** and **34**, the bromo groups of which were removed by reduction with tributylstannane giving the Neu5Ac(β2-8)Neu5Ac(β1-6)Glc derivative **35**.

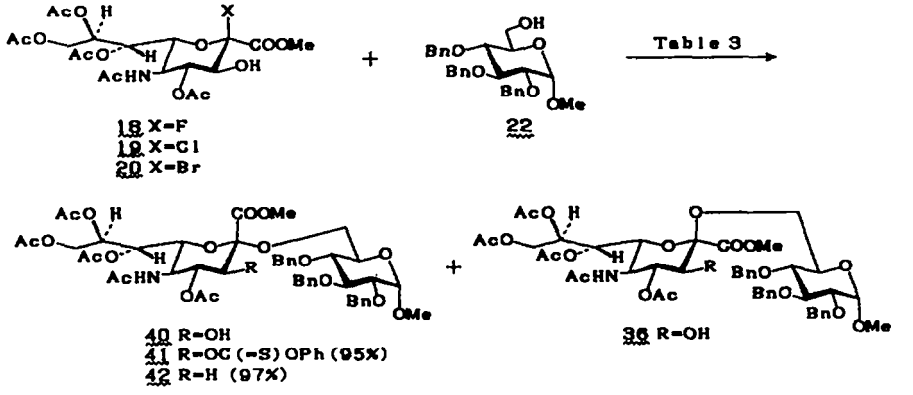
If the glycosidation reaction is of the S_N2 type then the epoxide **17** would be expected to yield α-glycosides. Treatment of **17** with methanol in the presence of acidic catalysts such as proton acid, Lewis acid, or [H^+] resin gave only the α-glycoside in nearly quantitative yield. On the other hand, when **17** was treated with the less reactive acceptors, **22** and **25**, in the presence of either antimony pentacholoride or TMSOTf then only the β-glycosides were obtained in moderate yields (Scheme 9).[88] To confirm the anomeric configurations, the hydroxy-glycosides, **36** and **38**, were thiocarbonylated and reduced with tributylstannane (the Robins' method[99]) giving the protected Neu5Ac(β2-6)Glc and Neu5Ac(β2-3)Gal derivatives, **24** and **27**, which were identical with the authentic samples obtained from the dibromide **15a**.

As shown in Scheme 10 and Table 3, the fluorohydrin **18** was recovered intact under glycosidation conditions in which the 2β-fluoro-Neu5Ac derivative **12** was glycosidated with the partly protected galactopyranose (Table 1, Entry 69). Glycosidation of the chlorohydrin **19** with two molar equivalents of **22** by the use of AgOTf gave a mixture of α- and β-glycosides. The chlorohydrin **19** was more reactive than the fluorohydrin **18** but glycosidation with the secondary alcohols of sugars did not proceed.[86,89]

The bromohydrin **20** was an efficient glycosyl donor. Even with equimolar amounts of the acceptor, the glycosidation yield did not decrease severely (Entry 6): α-selectivity increased when condensation was carried out at lower temperatures (Entry 7). This result demonstrates that kinetic



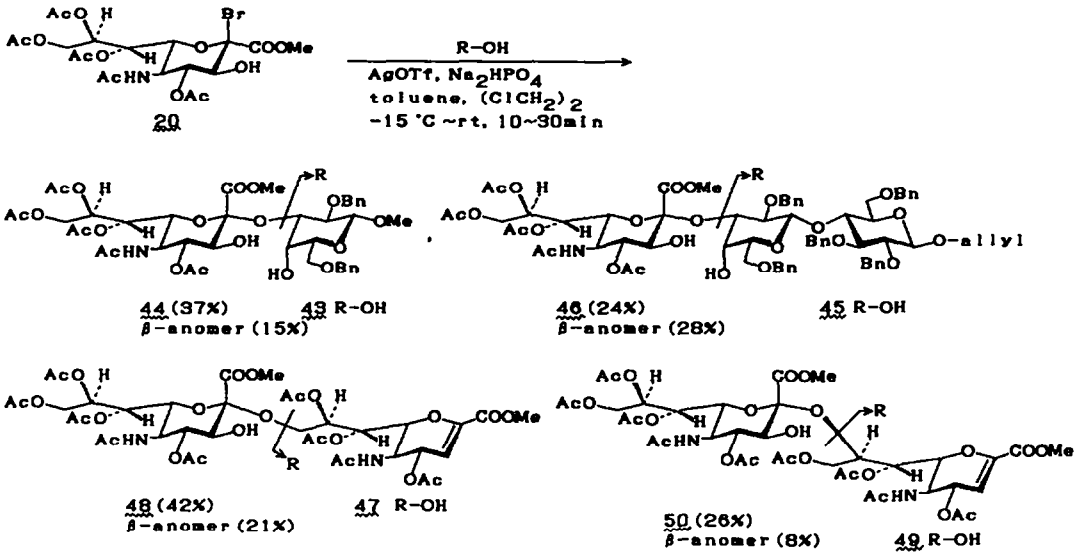
Scheme 9.



Scheme 10.

Table 3. Glycosylation of 22 with 18, 19, or 20

Entry	Donor	Acceptor (mol.equiv)	Promoter	Solvent	Reaction Temp	Reaction Time	Glycosides(%) 40(α)	Glycosides(%) 36(β)
1	18	2.0	$BF_3 \cdot OEt_2$	$(ClCH_2)_2$	rt	2.0h	no glycosides	
2	18	2.0	AgOTf	benzene	rt	1.0h	no glycosides	
3	19	2.0	AgOTf	benzene	rt	0.5h	33	25
4	20	2.0	AgOTf	benzene	rt	10min	38	50
5	20	2.0	$Hg(CN)_2, HgBr_2$	$(ClCH_2)_2$	rt	2.5d	6	32
6	20	1.0	AgOTf	benzene	rt	10min	28	53
7	20	1.0	AgOTf	toluene	-10°C	25min	64	15

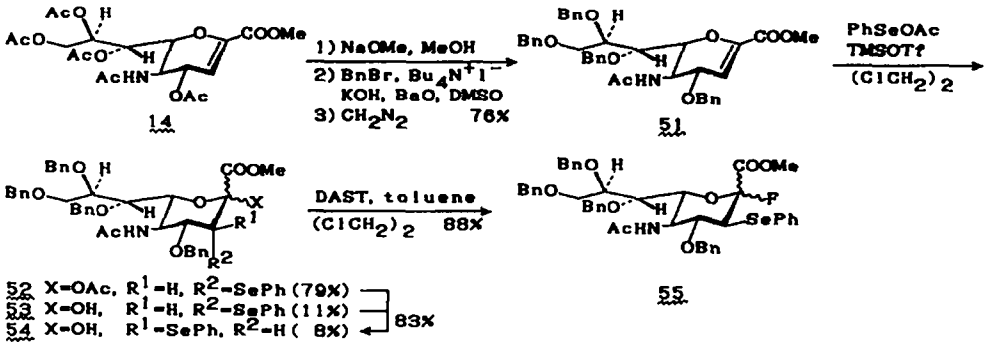


Scheme 11.

control is required to yield α-glycosides preferentially. The use of large excesses of the acceptor in glycosidation of the 2-halo-Neu5Ac derivative **12** makes this condensation very efficient. By this method glycosidation of **20** with various acceptors containing Gal, Lac and Neu5Ac was successful (Scheme 11).[85,86,89,90] Glycosidation with the 3,4-unprotected galactoside **43** in the presence of AgOTf gave the α-glycoside **44** (37%) together with the β-anomer (15%). When the 2,4,6-tri-*O*-benzylated β-galactoside was used, no glycoside was formed due to the steric bulk of the 4-*O*-benzyl group.[86,89] The lactoside **45** was glycosylated with **20** giving a mixture of α- and β-anomers because the reaction was carried out in toluene-1,2-dichloroethane (1 : 1) at higher temperatures due to the low solubility of **45**.

Dineuraminyl saccharide having the α2-9 linkage mode, which was found in the sugar moiety of the meningococcal serogroup C,[100,101] was synthesized with good stereoselectivity by glycosidation of the bromohydrin **20** with the acceptor **47** prepared from the Neu5Ac2en methyl ester in two steps.[85,90] The main objective in the synthesis of sialylglycoconjugates is how to make the α2-8 bond between two sialic acids. This linkage was synthesized for the first time by employing the above glycosidation method. Thus, **20** was treated with the 8-*O*-free Neu5Ac methyl ester **49** to give the α-anomer **50** (26%) and the corresponding β-anomer (8%). The hydroxyl groups of the dineuraminyl saccharides were removed by the Robins' reduction. This methodology established new tactics for the α-glycosidation of Neu5Ac instead of methods using the classical donor **12**.

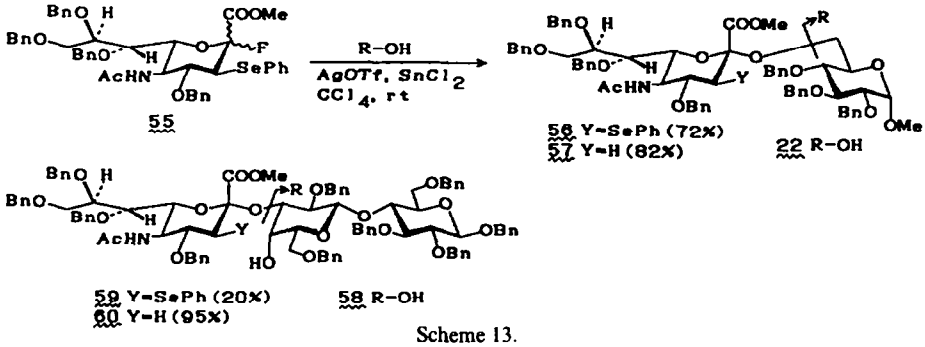
In order to raise α-stereoselectivity Ogawa's group developed an approach using the phenylselenenyl (PhSe)[102] and the phenylsulfenyl (PhS)[103] groups instead of the hydroxyl group as an auxiliary in the 3β-position of Neu5Ac. For introduction of the PhSe group the acetyl group of **14** was converted into the benzyl group (76%) by deacetylation, benzylation, followed by esterification (Scheme 12). The benzyl protected 2,3-dehydro derivative **51** was treated with PhSeOAc in the presence of TMSOTf giving a mixture of the acetoxy-selenide **52** and the hydroxy-selenides, **53** and **54**, which were converted by deacetylation and epimerization with sodium methoxide to the equilibrium mixtures of **53** and **54** in the ratio of 34 : 66. By repeating this epimerization of **53** the 3β-selenenyl compound **54** was obtained (83%). The DAST treatment of **54** gave the fluoride **55** in an anomeric mixture ($\alpha : \beta \geq 20 : 1$).[102]



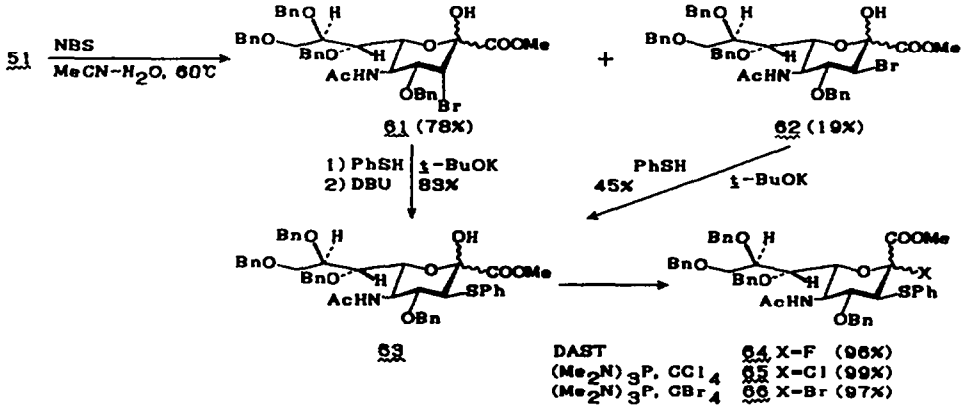
Scheme 12.

In the glycosidation of **55** the use of a combination of AgOTf and $SnCl_2$ as a promoter in carbon tetrachloride gave the best yield and α-stereoselectivity. When treated with the glucoside **22** only the α-glycoside **56** was obtained (72%). The side-product was the 2,3-dehydro compound **51** (Scheme 13). In the case of the lactoside **58** the desired α-glycoside **59** was obtained (20%). The regio-isomer, that is, 4′-*O*-glycoside was also formed (5%). The phenylselenenyl groups of the glycosides obtained could be directly removed by reduction with tributylstannane.

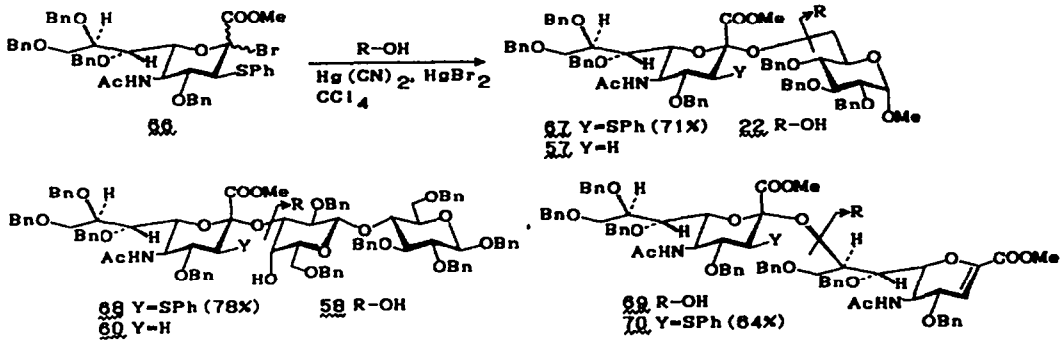
The PhS group was expected to provide more efficient neighboring-group assistance (Scheme 14). The 2,3-dehydro Neu5Ac methyl ester **51** was treated in a similar manner as **14** giving the



Scheme 13.



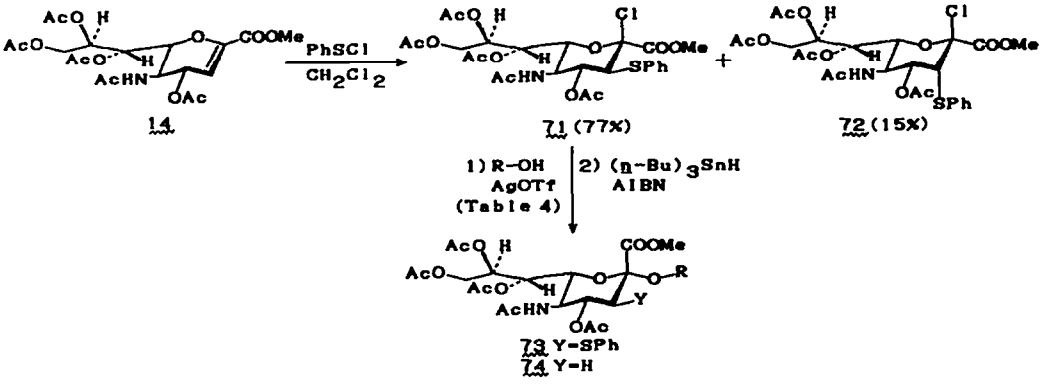
Scheme 14.



Scheme 15.

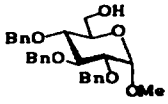
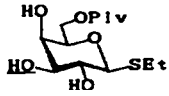
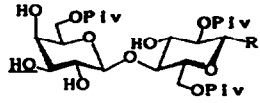
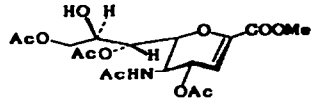
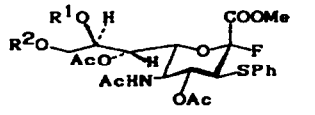
two bromohydrins, **61** and **62**. The 3-axial bromo-adduct **61** after phenylthiolation followed by epimerization with DBU yielded the 3β-PhS derivative **63**. The 3-equatorial bromo adduct **62** was directly converted to **63** by treatment with PhSH and potassium *t*-butoxide. Halogenation of **63** with either DAST, $(Me_2N)_3P\text{-}CCl_4$, or $(Me_2N)_3P\text{-}CBr_4$ gave the fluoro, chloro and bromo derivatives, **64**, **65**, and **66**, respectively.[103] Among these halohydrins, the most promising donor was the bromohydrin **66**. It is noteworthy that dramatic improvement of yield was observed in the glycosidation, especially, with the secondary alcohols, **58** and **69** (Scheme 15). The PhS group was also reduced with tributylstannane.[103] Recently by using this glycosidation method and the elongation technique of the sugar chain employed in the synthesis of the trisaccharide **35**, ganglioside GD_3 and GM_{1b} were synthesized in a stereoselective manner.[104,105] In conclusion, the 3β-PhS-Neu5Ac derivative among many donors so far examined gives the best glycosidation yield and α-stereoselectivity. Unfortunately it still leaves a problem that the preparation of the donor **66** requires multiple reaction steps.

Attempt to solve this problem was examined by Kondo *et al.* who used a direct introduction method of the 3β-PhS group by the addition of phenylsulfenyl chloride to the 2,3-double bond of Neu5Ac (Scheme 16).[106] The adducts were separated giving the major 3β- and the minor 3α-PhS derivatives, **71** and **72**. The former **71** was glycosidated with various acceptors in the presence of AgOTf to give the α-glycoside **73** in good yield (Table 4).[107] The 3β-PhS group of **73** was easily removed by reduction with tributylstannane affording the glycoside **74**. They also succeeded in the direct second glycosidation of the fluoro-dineuraminyl saccharide obtained in Entry 7 without the



Scheme 16.

Table 4. Glycosidation of 71 with R-OH[107]

Entry	Acceptor (R-OH)		Solvent[a]	Yield of 73 (%)
1			A	70
2			A	42
3		R=OCH_2CH_2TMS	A	70
4		R=SEt	A	68
5			B	50
6		R^1=Ac, R^2=H	A	45
7		R^1=H, R^2=Ac	C	42

[a]Solvents employed are: A, acetonitrile-toluene; B, 1,2-dichloroethane-toluene; C, dichloromethane-toluene.

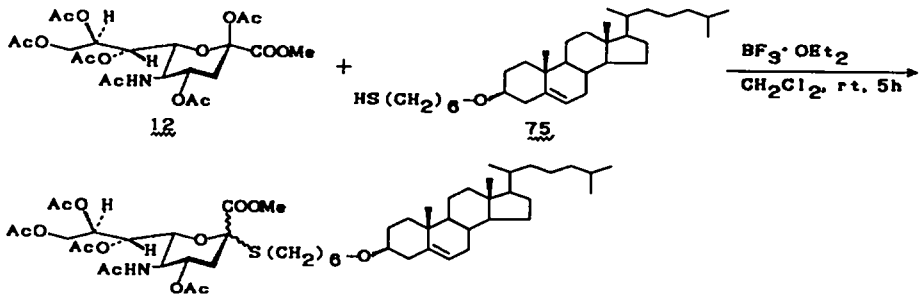
necessity of functionalization of the second Neu5Ac unit.[108] In this method the problem remaining is to raise the glycosidation yield for the synthesis of the dineuraminyl saccharide having α2-8 linkage. However, taking into account the studies mentioned above this problem may be solved if the corresponding 2-bromo donor can be synthesized.

3.3. S-*Glycosides and their conversion to* O-*glycosides*

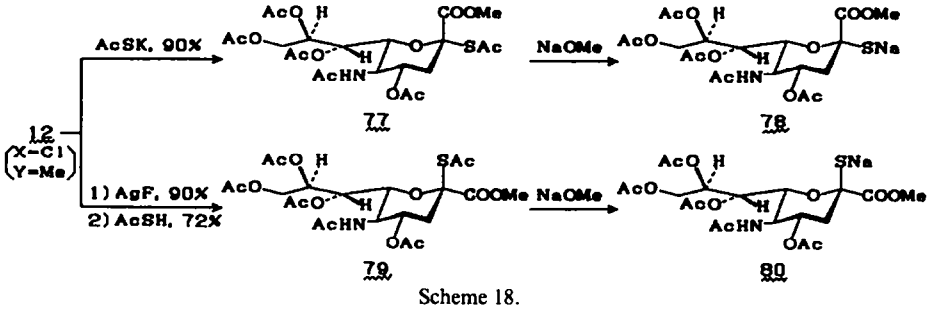
The replacement of an oxygen atom by a sulfur atom is often adopted to modify biological function. For example, 9- and 6-thio-sialic acids have been synthesized and the 2-azido derivative of the latter is a strong sialidase inhibitor.[109,110] An interesting aspect of 2-thiosialic acid is that epimerization at the anomeric position is not observed.

The first synthesis of thioglycosides was reported by Pivalova *et al.* but assignment of configuration at the anomeric positions of the product was doubtful.[111] In 1980 the Merck group synthesized the thioglycoside **76** for targeting studies (Scheme 17).[112] Thioglycosidation of the peracetyl Neu5Ac methyl ester **12** with **75** in the presence of $BF_3 \cdot OEt_2$ gave the β-glycoside **76** (21%) in preference to the α-glycoside.

For stereoselective thioglycosidation of Neu5Ac Hasegawa *et al.* developed a new method which uses the sodium salts of 2α- and 2β-thio-Neu5Acs, **78** and **80** (Scheme 18).[113,116] Thus, treatment

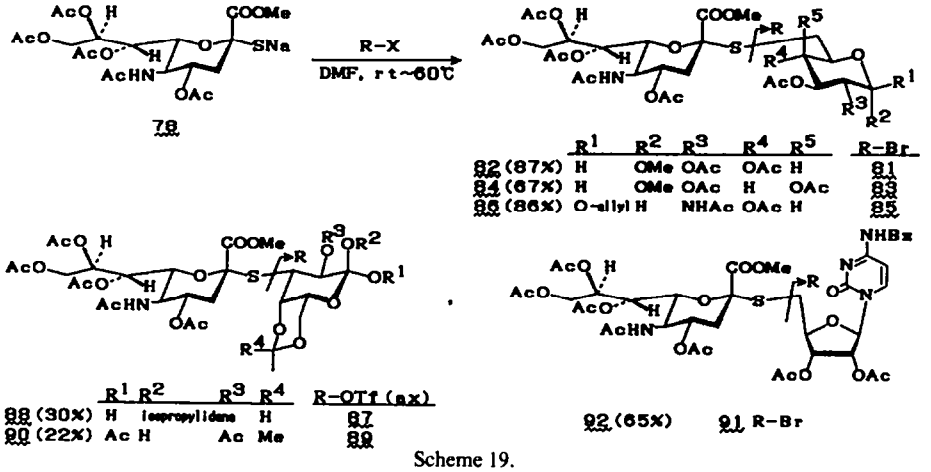


Scheme 17.



Scheme 18.

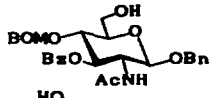
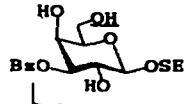
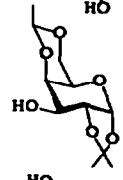
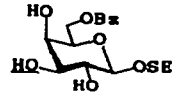
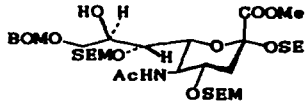
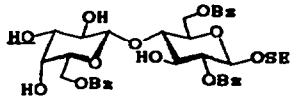
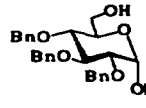
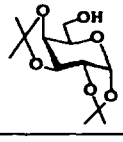
of **12** (X = Cl, Y = Me) with potassium thioacetate gave the 2α-thioacetate **77**. On the other hand, when **12** was treated with silver fluoride this yielded the α-fluoride which was subjected to thioacetoxylation with thioacetic acid and $BF_3 \cdot OEt_2$ giving the 2β-thioacetate **79**. When the thioacetates, **77** and **79**, were treated with equimolar amounts of sodium methoxide at −40°C this yielded the corresponding sodium salts, **78** and **80**, respectively. Alkyl 2α-thioglycosides were synthesized by condensing **78** with hexyl, dodecyl and octadecyl bromides.[113] In the same way, disaccharides were synthesized using 6-bromo-hexopyranoses, **81**, **83**, and **85**,[114] 3-*O*-trifluoromethanesulfonyl(Tf)gulopyranoses, **87** and **89**,[115] and 5′-bromocytidine derivative **91**[116] (Scheme 19). The β-thio-glycosides are also obtainable from the β-SNa **80** in the same manner. Using this method a photo probe, 4-azido-2-nitrophenyl α-thioglycoside was also synthesized.[117]



Scheme 19.

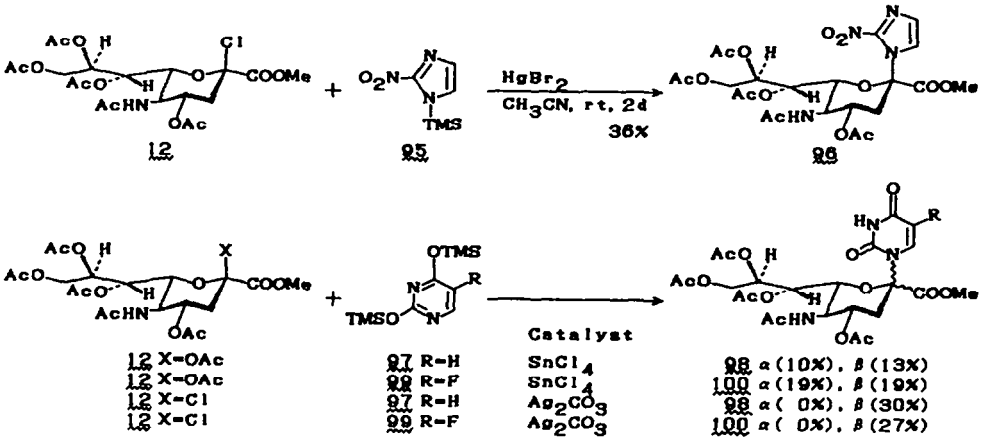
As a new *O*-glycosidation method, the thioglycosides were converted to *O*-glycosides[118–121] using mainly dimethyl(methylthio)sulfonium triflate (DMTST) by the method originally developed by Pavenscroft *et al.*[122,123] This method used the methyl and phenyl α-thioglycosides **93** obtained by methylation of **78** and by thiophenylation to the chloride **12** (X = Cl, Y = Me). As shown in Table 5, many *O*-glycosides **94** were synthesized by this method (Scheme 20). The ratio of α- and β-anomers in this reaction was influenced by the solvent: dichloromethane gave the β-anomer whereas acetonitrile gave the α-anomer predominantly. By reducing the number of protecting groups glycosidation yield and stereoselectivity were increased (Entry 7, 9, 11). Other promoters such as PhSeOTf and PhHgOTf were less effective. Ganglioside GM_3 have been synthesized by application of DMTST method.[124]

Table 5. Conversion of S-glycoside 93 into O-glycoside 94

Entry	Donor Y	Acceptor (R-OH)	Promoter	Glycosides(94) Yield(%)	α:β	Ref
1	Me	CH_3OH	DMTST[a]	quant	5:2	119
2	Me	$CH_3(CH_2)_3OH$	DMTST	quant	2:5	119
3	Me	$CH_3(CH_2)_7OH$	DMTST	90	1:1	119
4	Me	$CH_3(CH_2)_{15}OH$	DMTST	83	2:5	119
5	Me	cyclohexanol	DMTST	95	2:5	119
6	Me		DMTST	61	3:7	119
7	Me		DMTST	68[b]	1:0	120
8	Me		DMTST	50	6:5	119
9	Me		DMTST	43[b]	1:0	120
10	Me		DMTST	5	1:0	119
11	Me		DMTST	47[b]	1:0	120
12	Me		PhSeOTf	63	16:84	121
13	Me		PhSeOTf	70	41:59	121
14	Ph		PhHgOTf	24	5:1	118

[a]Dimethyl(methylthio)sulfonium triflate. [b]Yield based on the acceptor.

Scheme 20.



Scheme 21.

3.4. N-*Glycosides*

There are only two reports concerning *N*-glycosides of Neu5Ac (nucleoside type). Sakaguti *et al.* synthesized the 2-nitroimidazole nucleoside **96** as part of the study of radiosensitizer agents.[125] The modified Lowy–Davoll method was employed for the glycosidation of **12**. The imidazole **95** gave the only β-glycoside **96** (36%) (Scheme 21). Glycosidation of the peracetyl Neu5Ac methyl ester **12** with TMS protected uracils, **97** and **99**, in the presence of tin(IV) chloride afforded mixtures of the α- and β-nucleosides, **98** and **100**, respectively.[126] On the other hand when the chloride **12** was treated with **97** and **99** using the Ag_2CO_3 catalyst only the β-glycosides **98** and **100** were produced.

4. TOTAL SYNTHESIS

The total synthesis of Neu5Ac glycosides, which involves the construction of Neu5Ac moiety, is also an attractive target. By the use of an intramolecular oxymercuration–demercuration reaction ethyl β-glycoside[127] and sialyl disaccharides[128] have been synthesized. The stereoselective synthesis of sialyl conjugates has been reported by Danishefsky *et al.*[129] by a method which involves a hetero Diels–Alder reaction of the furyl diene and 2-(phenylseleno)propionaldehyde. These methods are not practical because multiple steps are required.

5. DETERMINATION OF THE ANOMERIC CONFIGURATION

In the glycosidation study of Neu5Ac, one of the difficult problems is how to determine the anomeric configuration. Several methods to solve this problem have been reported. Enzymic (sialidase)[29,30] and chemical[25,29,50,126] hydrolysis, CD[64,130–132] and ^{1}H-NMR[36,57,60,133] studies have led to the elucidation of the stereochemistry of the anomeric position. The difference in the hydrolysis

rates of α- and β-glycosidic bonds with sialidase or a dilute acid is applicable only to the non-protected glycosides. The CD method is not applicable to glycosides which have chromophores such as acyl groups. The NMR method is a rapid, simple, and nondestructive method and has been widely used. However, the usual methods ($J_{1,2}$ and $J_{C\text{-}1,H\text{-}1}$) to determine the anomeric configuration of aldopyranoses cannot be applied to Neu5Ac because the anomeric position bears no hydrogen atom. Therefore empirical rules for the acetyl protected Neu5Ac glycosides have been used: (1) α-H-3 eq > β-H-3 eq,[133] (2) α-H-4 < β-H-4,[31,57] (3) $\alpha\text{-}J_{7,8} > \beta\text{-}J_{7,8}$,[46,47,85,86,88-90] (4) α-|H-9′-H-9| < β-|H-9′-H-9|.[46,47,85,86,88-90] The rules 1 and 2 cannot be used for the special aglycones such as uracils. The rules 3 and 4 are applicable to the glycosides even if they are substituted at the 3-position of Neu5Ac.

Very recently the ^{13}C-NMR method has been developed as a general technique.[134] This is based on the Karplus relationship of $J_{C\text{-}1,H\text{-}3ax}$ of Neu5Ac. In the gated proton-decoupled or selective proton decoupled ^{13}C-NMR spectra, the α-anomer gives a doublet C-1 signal whereas the β-anomer gave a singlet. This method cannot be used for the C-3 axially substituted glycosides, but it is useful that a low frequency (100 MHz for ^{1}H) is sufficient for the measurement.

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