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Efficient Synthesis of MUC4 Sialylglycopeptide through the New Sialylation Using 5-Acetamido-Neuraminamide Donors

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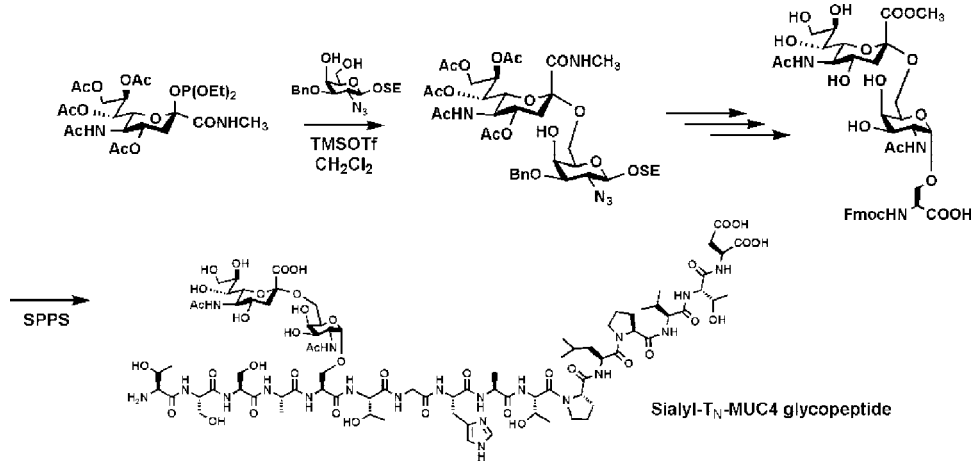

Sialylation reactions using a new sialyl donor, diethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-*O*-β-D-*glycero*-D-*galacto*-2-nonulopyranosylonamide phosphite (Neu5Ac-1-amide-2-phosphite) derivatives, and the synthesis of the sialyl-T_N-MUC4 glycopeptide are described. The sialylation was performed in CH_2Cl_2 solvent toward the 6-hydroxyl group of several monosugar acceptors and generated α-sialoside in good yield under low temperature and TMSOTf activation system. Amide derivatives of sialoside were easily converted into naturally occurring sialoside after hydrolysis of the amide group. Sialyl-α(2,6)-GalN_3 was also prepared by this new sialylation protocol, and then this sialoside was further converted into a Fmoc-protected sialyl-T_N serine derivative for solid-phase glycopeptides synthesis. The solid-phase glycopeptide synthesis using this sialyl-T_N serine derivative in which the sugar hydroxyl group was free afforded the target sialyl-T_N-MUC4 glycopeptide.

Introduction

Glycoprotein and glycopeptide play important roles in a large number of biological events, and these glycoconjugates frequently have sialic acid (Neu5Ac) at the terminal of those oligosaccharides.[1] It is known that sialyloligosaccharides exhibit several glycoforms, such as sialyl-T_N, α(2,3)-sialyl-T, α(2,6) sialyl-T, sialyl Lewis x, sialyl Lewis y, and sialyl Lewis a, and these have also been found in *O*-linked glycopeptides as tumor-associated glycopeptides.[2] These glycoconjugates have been of interest because of their potential as a cancer vaccine.[2] Synthesis of these *O*-linked glycopeptides has been carried out by chemical and chemoenzymatic methods.[3] The critical points in these synthetic methods are how to prepare an appropriate amount of sialyloligosaccharylamino acid and how to cleave the acid-labile sialylglycopeptide from its solid support. Hence, an annoying problem is the stereoselective construction of the

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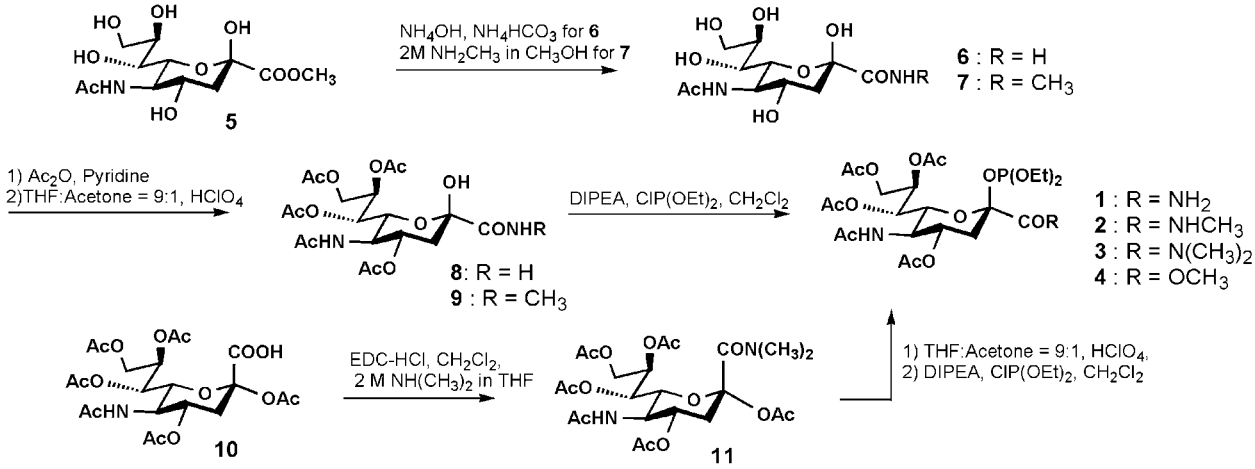
SCHEME 1. Synthesis of Amide Donors 1−3



naturally occurring α-sialyl linkage that is a thermodynamically unstable configuration compared with the unnatural β-sialyl linkage, because of the endoanomeric effect.

Beyond such inherent difficulties, sialylation reactions using a variety of sialyl donors have been reported.[4] These methods can sort out two phenomena, the solvent effect[5] and neighboring group effect.[6] The solvent effect is easily obtained in acetonitrile solvent. This method was reported by Kiso and Hasegawa[5a] and has been known as an easy way to obtain α-selectivity. Recently, modification of the acetamide group at the 5 position (Ac_2,[7a] TFA,[7b–d] N_3,[7e,f] Troc,[7g–i] Phth[7j] group) was found to elevate α-selectivity. On the other hand, the neighboring group effect type has also been used to achieve α-selectivity, traditionally introducing the auxiliary group at the 3-position[6a–e] or the 1-position.[6f–h] In addition, an oxazoline derivative at the 4 and 5 positions of Neu5Ac was found to be an excellent sialyl donor which does not require the solvent effect.[8]

So far, all of the sialyl donors reported have an ester group at the 1-position.[9]Therefore, we developed an interest in the ability of the amide group at the 1-position instead of an ester group (Scheme 1, **1**−**3**). It is known that the amide function reduces the electron-withdrawing effect comparing to that of the ester function. During our extensive investigation using 1-amide derivatives of Neu5Ac-donors, we found an interesting donor, 1-amide derivatives of the Neu5Ac-donor derivative, which can afford α-selectivity in CH_2Cl_2 solvent. Using this new sialylation method, we have studied the synthesis of sialyl-T_N and sialyl-T_N glycopeptide derivative. Herein, we report interesting new sialyl donors which can be prepared through a concise synthetic route with application for the synthesis of *O*-linked sialylglycopeptides.

Results and Discussion

Preparation of Sialyl Donor. In order to study the capacity of the amide group for the sialylation reaction, we chose three different amide functional groups, namely the amide ($CONH_2$), monomethylamide ($CONHCH_3$), and dimethylamide ($CON(CH_3)_2$) groups **1**-**3** shown in Scheme 1.

Each of the new sialyl donors having one of these amide groups was synthesized from the known compound, methyl 5-acetamido-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate (Neu5Ac-1-methyl ester, **5**).[10] For the synthesis of amide derivative **1**, Neu5Ac-1-methyl ester **5** was treated with NH_4OH solution containing NH_4HCO_3 to convert into Neu5Ac-1-$CONH_2$ **6** in 91% yield. Acetylation of compound **6** by pyridine/Ac_2O and subsequent selective de-*O*-acetylation at the 2 position by $HClO_4$ in THF/acetone solvent gave pentaacetyl-Neu5Ac-1-$CONH_2$ **8** in 80% overall yield. Compound **8** was then equipped with a diethyl phosphite group at the 2 position to be converted into 1-amide donor **1** (77% yield).

Synthesis of monomethyl donor **2** also started from compound **5**. Treatment with 2 M CH_3NH_2/CH_3OH solution gave Neu5Ac-1-$CONHCH_3$ **7** in 90% yield. Using the same manner of synthesis as in 1-amide donor **1**, pentaacetyl-Neu5Ac-1-$CONHCH_3$ **9** was prepared in 77% yield. Monomethyl amide **9** was then introduced as a diethyl phosphite group at the 2 position to be converted into monomethyl amide donor **2** (85% yield).

On the other hand, the dimethyl amide group was introduced by condensation reaction. 5-Acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonu-

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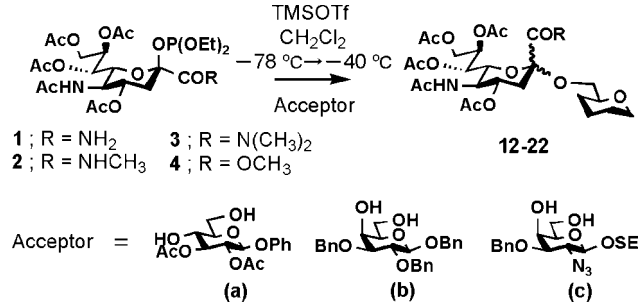
TABLE 1. Sialylation Using Amide Donors 1−3 and Conventional Methyl Ester Donor 4



entry	donor (R)	acceptor	product: yield (%) (α:β)[a]
1	**1** (NH_2)	**a**	**12**: 84 (7:1)
2	**1** (NH_2)	**b**	**13**: 79 (4.5:1)
3	**1** (NH_2)	**c**	**14**: 88 (4:1)
4	**2** ($NHCH_3$)	**a**	**15**: 90 (10:1)
5	**2** ($NHCH_3$)	**b**	**16**: 91 (5:1)
6	**2** ($NHCH_3$)	**c**	**17**: 92 (5.4:1)
7	**3** ($N(CH_3)_2$)	**b**	**18**: 43 (1:6)
8	**3** ($N(CH_3)_2$)	**c**	**19**: 46 (1:9)
9	**4** (OCH_3)	**a**	**20**: 75 (4:1)
10	**4** (OCH_3)	**b**	**21**: 80 (1:1)
11	**4** (OCH_3)	**c**	**22**: 80 (1:1)

[a] The ratios were determined by ^{1}H NMR.

losonic acid (hexaacetyl-Neu5Ac, **10**)[11] was treated with *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) and 2 M $NH(CH_3)_2$/THF solution to be converted to compound **11**. Selective de-*O*-acetylation at the 2 position of **11** and subsequent phosphitation afforded 1-dimethylamide donor **3** in 33% overall yield.

Sialylation Reaction. The new sialyl donors **1**−**3** were examined for the sialylation reaction with three acceptors (**a**−**c**) having a free hydroxyl group at the 6 position, and the results are summarized in Table 1.

Sialylation of glucoside acceptor **a** with amide donor **1** afforded sialoside **12** in 84% yield (α:β = 7:1, entry 1). In the case of a monomethyl amide donor **2** with acceptor **a**, it afforded sialoside **15** in 90% yield (α:β = 10:1, entry 4). Using acceptors **b** and **c**, naturally occurring Neu5Ac-α(2,6)-Gal and Neu5Ac-α(2,6)-GalNAc linkages were also obtained in good yield, respectively. Coupling of galactoside acceptor **b** with amide donor **1** provided sialoside **13** in 79% yield (α:β = 4.5:1, entry 2), and coupling of 2-N_3-galactoside acceptor **c**, which is a precursor of galactosamine, with amide donor **1**, provided sialoside **14** in 88% yield (α:β = 4:1, entry 3). Furthermore, the monomethyl amide donor **2** exhibited better α-selectivity and higher yield (y = 91%, α:β = 5:1, entry 5, and y = 92%, α:β = 5.4:1, entry 6). We also examined the same reaction using dimethylamide donor **3** toward acceptors **b** and **c**. Unexpectedly, these reactions showed β-selectivity rather than α-selectivity (α:β = 1:6, entry 7, and α:β = 1:9, entry 8). In order to evaluate the capacity of the amide group at the C(1) position, we also examined the sialylation reaction using a conventional methyl ester donor **4** in CH_2Cl_2.[5b] Of those results, even the best α:β ratio was 4:1, as shown in entry 9, which was a coupling reaction with acceptor **a**, and other reactions afforded a 1:1 ratio (entries 10 and 11). As a result, we concluded that the monomethyl amide derivative was shown to be optimum among sialyl donors investigated in this study.

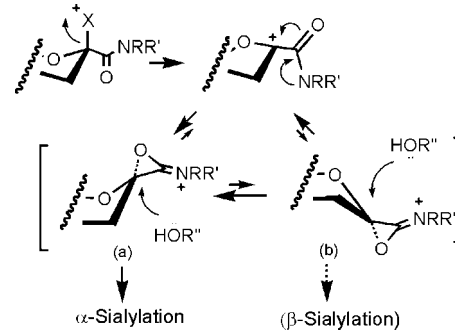
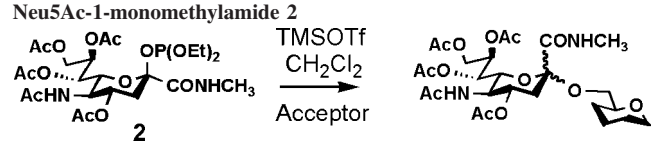
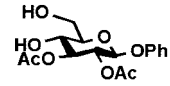
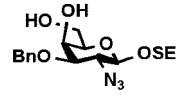
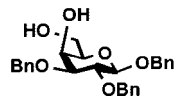


FIGURE 1. Presumable reaction mechanism of the new sialylation.

TABLE 2. Temperature Dependence of Sialylation Reaction Using Neu5Ac-1-monomethylamide 2



Entry	Acceptor	Temprature and α : β *		
		−50 °C	−78 °C	−87 °C
1		4.9 : 1	8.9 : 1	9 : 1
2		3 : 1	5.7 : 1	6.1 : 1
3		3.5 : 1	4.9 : 1	4.9 : 1

* The ratios were determined by ^{1}H NMR.

To gain insight into the origin of the stereoselectivity, we hypothesized the intermediacy of the three-membered cyclic intermediate generated by the interaction of the amide group with the C(2) position to stabilize the oxocarbenium ion intermediate (Figure 1).

This interaction may afford two possible intermediates, (a) or (b), on the α-side or β-side. According to the aforementioned result of the sialylation reaction, intermediate (a), which can generate an α-sialyl linkage, may be dominant. In the case of a dimethyl amide donor, which showed β-selectivity, approach of the acceptor from α-side might be disturbed by the steric hindrance of dimethyl group. For formation of this intermediate, oxygen of the amide group may interact with the cation at the 2 position instead of nitrogen, as shown in Figure 1, but we could not determine which atom exhibited the specific interaction. We also examined the sialylation reaction dependent on temperature using amide Neu5Ac-1-monomethylamide donor **2** (Table 2).

At −50 °C, the sialylation reaction afforded moderate α-selectivity (α:β = 3:1 ∼ 4.9:1). On the other hand, the sialylation at lower temperature such as −78 or −87 °C exhibited better α-selectivity (α:β = 4.9:1−9:1). In terms of these results, we hypothesize that the formation of intramolecular cyclization having a β-configuration, as shown in Figure 1a, may be faster than that of glycosylation. The lower temperature conditions decrease the accessibility of acceptor to the sialyl

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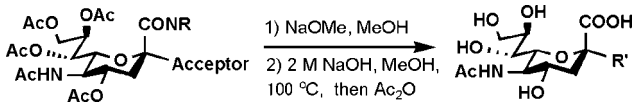
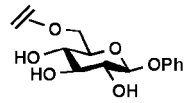
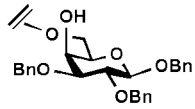
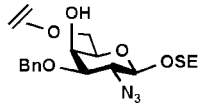
TABLE 3. Deamidation of 1-Amide Sialosides



Entry	R (compound)	R'	Product : Yield%
1	H (**12**α)		**23** : 80%
2	CH_3 (**15**α)		**23** : 85%
3	H (**13**α)		**24** : 84%
4	CH_3 (**16**α)		**24** : 75%
5	H (**14**α)		**25** : 89%
6	CH_3 (**17**α)		**25** : 92%

donor through solvent due to intermolecular reaction. Therefore, alcohol might attack the C(2) carbon from the α-side preferably after the formation of the thermodynamically stable intermediate (a) as shown in Figure 1.

We have also examined the sialylation reaction using Neu5Ac-1-amide donors having thiophenyl glycoside (SPh) at the 2 position. Although these donors also gave slight α-selectivity in CH_2Cl_2 solvent ($\alpha:\beta = 2:1-3:1$) under general activation conditions (NIS, TfOH), the reproducibility for α-selectivity was found to be poor. We speculated that the SPh group was not activated efficiently because TfOH might be trapped by the amide group. On the basis of the obtained data, a phosphite group was considered suitable for the amide donors. In particular, amide and monomethylamide donors afford tiny amounts of Neu5Ac-2-OH- and 2,3-en-Neu5Ac-type byproducts. Therefore, purification of sialoside was very easy on a silica gel column. In terms of sialylation toward secondary alcohol, Neu5Ac-1-monomethylamide **2** has not afforded sialyloligosaccharides in moderate yield. Therefore, we hope to optimize this sialylation toward sugar secondary alcohols. We also examined this sialylation in acetonitrile, but it afforded sialic acid derivative incorporated acetonitrile covalently to the 2 position. Therefore, we concluded that Neu5Ac-1-monomethylamide **2** cannot be used in acetonitrile solvent under the current conditions.

Since we obtained sufficient α-selectivity for the sugar primary alcohol, we examined the deamidation reaction at the 1 position by 2 M NaOH solution at 100 °C[12] followed by acetamidation with Ac_2O at the 5 position. This condition afforded several sialosides in good yield (two steps with an overall yield of ca. 80%, Table 3, and Supporting Information Figure S-1). As a result, we could obtain naturally occurring sialoside derivatives by use of amide derivatives of the Neu5Ac donor.

Synthesis of Sialyl-T_N Serine Derivative and Sialylglycopeptide. As we succeeded in finding an interesting new sialylation, we applied this sialylation reaction to the synthesis of naturally occurring sialylglycopeptide. In terms of the synthetic target, we selected MUC4 glycopeptide having the sialyl-T_N epitope (sialyl-T_N-MUC4 peptide).[13] MUC4 is one of the tumor-associated glycoproteins and has a variable number of tandem repeats of 16 amino acids with aberrant glycoforms. Like the MUC1 peptide, this tandem repeat glycopeptide is potentially an immune response target.[2,14]

In order to synthesize this target, we also prepared Fmoc-Ser(sialyl-T_N)-OH conjugate by use of this sialylation reaction (Scheme 2).[15]

Sialoside **25** was esterified at the 1 position by Dowex 50W-X8 in MeOH followed by acetylation with pyridine/Ac_2O to give compound **26** (75% yield). Conversion of the trimethylsilylethyl (SE) group at the 1-position of Gal-2-N_3 to a trichroloacetimidate group gave donor **28** (92% yield for affording **27**, 94% yield for affording **28**). Then, glycosylation of the benzyl-esterified serine derivative by donor **28** in the presence of TMSOTf gave sialyl-T_N derivative **29** in 69% yield.[16] Reduction of azido, benzyl ether, and benzyl ester of compound **29** by catalytic hydrogenation over Pd followed by acetoamidation with Ac_2O afforded compound **30** (93% overall yield). Then, the Boc-protected compound **30** was converted into Fmoc derivative **31** through deacetylation, deprotection of the Boc group, and introduction of the Fmoc group. As a result, we obtained Fmoc-Ser(sialyl-T_N-methyl ester)-OH **31** in 84% overall yield, which was ready to use in solid-phase glycopeptide synthesis.

In order to synthesize the sialyl-T_N-MUC4 glycopeptide by solid-phase peptide synthesis, we employed **31**, which has a free hydroxyl group. We did this because we have already demonstrated synthesis of N-linked glycopeptides by the use of an Fmoc-Asn-complex type oligosaccharide having a free sugar hydroxyl group.[17] Using this oligosaccharylamino acid with the free hydroxyl group has the major advantage of avoiding undesired side reactions, such as β-elimination of amino acids during base-deprotection steps for de-*O*-acetylation. However, deprotection for the ester group of *O*-linked sialyloligosaccharide requires more careful treatment under basic conditions. As far as we examined, the ester group of sialoside could be removed after 15 min, and this condition was expected to afford current synthetic target **32** in good yield. In addition, we also have already demonstrated the resistance of esterified sialyl-linkage to the 95% TFA condition, which is the general condition for the cleavage from the resin (Scheme 3).[17] Considering these, using the oligosaccharylamino acid with the free hydroxyl group has the advantage over using a protecting group because we do not need multiple deprotection steps for several functional groups on oligosaccharide.[17]

The MUC4-glycopeptide synthesis was performed on poly(ethyleneglycol)-poly(dimethylacrylamide) copolymer (PEGA) resin, which contains the acid-labile linker 4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid (HMPB).[17b] The first residue, aspartic acid, was introduced as Fmoc-Asp(tBu)-OH by activation with 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT) and *N*-methylinidazole. The second to eleventh residues were

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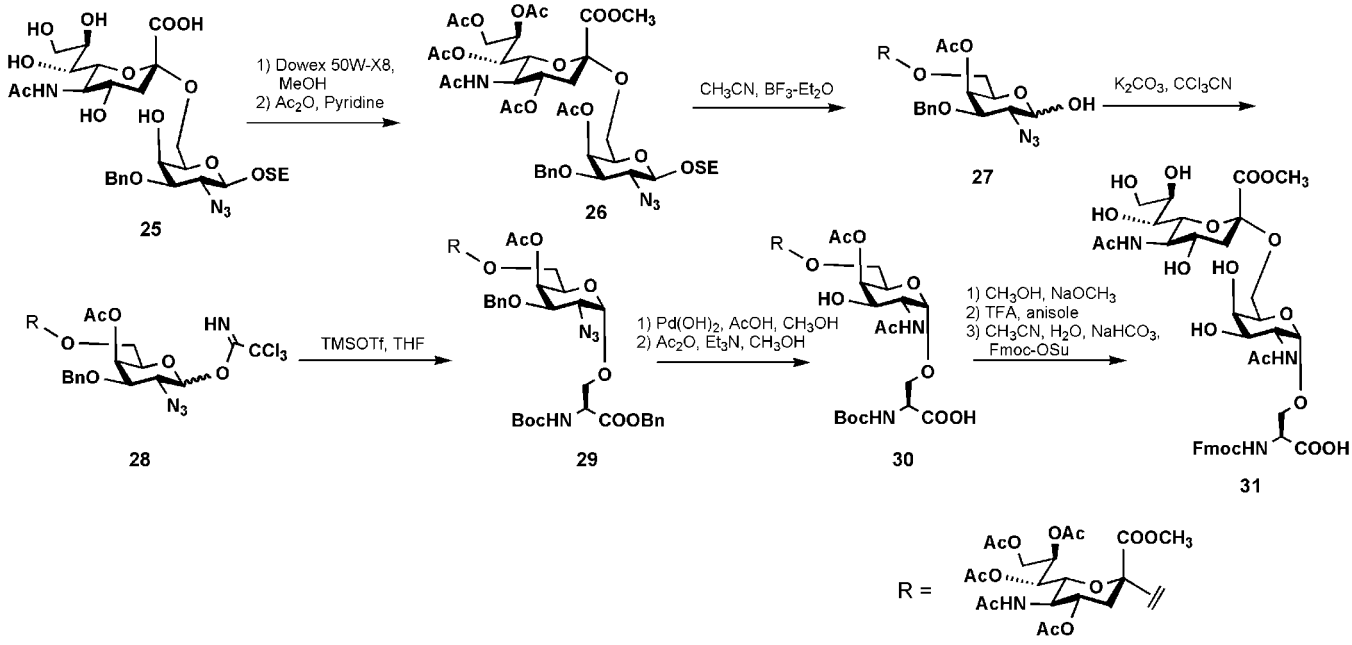
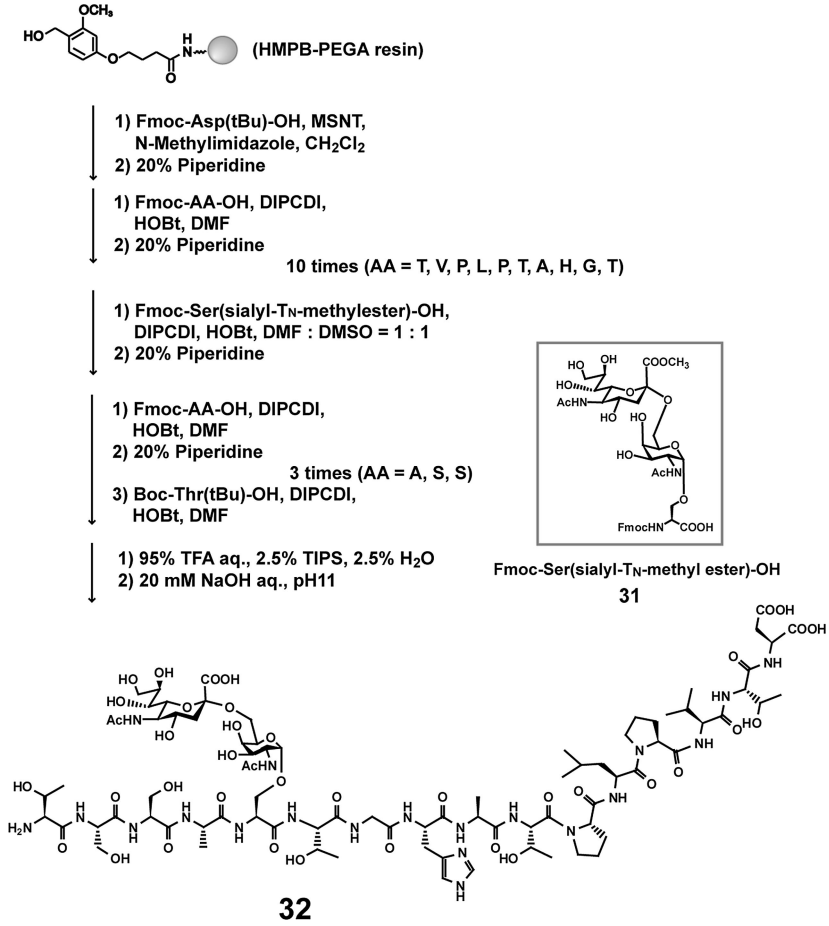
SCHEME 2. Synthesis of Sialyl-T_N Derivative 31



SCHEME 3. Solid-Phase Synthesis of Sialyl-T_N-MUC4 Glycopeptide 32



coupled by diisopropylcarbodiimide (DIPCDI) and hydroxybenzotriazole (HOBt) in DMF. At this point, **31** was introduced onto the resin by DIPCDI and HOBt. The following amino acid residues were incorporated by means of these same reagents, but 40 mM concentration of amino acids was essential to avoid esterfication of the sugar-hydroxyl group on sialyl-T_N.[17c] After construction of the glycopeptide, the glycopeptide was released from the resin by treatment with TFA/TIPS/H_2O (95:2.5:2.5) for 2 h. As expected, the sialyl linkage was stable throughout this treatment.

Brief saponification of the methyl ester was carried out by 200 mM NaOH solution for 10 min at 0 °C or 20 mM NaOH solution

for 1.5 h. As far as we analyzed by extensive HPLC analysis, ca. 5–7% of eliminated product was observed. Purification of the crude material by a reversed-phase HPLC column afforded sialyl-T_N-MUC4 peptide **32** (50% or 61% isolated yield).

Conclusion

We found a concise sialylation reaction using Neu5Ac-1-amide derivatives, and these donors exhibited good α-selectivity and yield. Simple modification by an amide group at the C(1) position of sialic acid instead of the ester group might afford an interesting intermediate to afford α-selectivity, and this reaction did not require acetonitrile solvent. Therefore, this finding enabled examination of sialylation in several different solvents and temperatures. This method was also developed for the synthesis of glycopeptides having a sialyl-T_N epitope. In order to obtain glycopeptides for measurement of the NMR spectrum and for bioassay, an adequate amount of Fmoc-Ser-O-oligosaccharide is essential. Our sialylation method satisfied this demand and afforded an appropriate amount of the MUC4 fragment having sialyl-T_N. Research is in progress to synthesize larger repetitive *O*-linked glycopeptides having sialyl-T_N.

Experimental Section

Diethyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosylonamide Phosphite (1) and Diethyl (Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-*O*-β-D-glycero-D-galacto-2-nonulopyranosylonamide) Phosphite (2). Compound **8** (110 mg, 0.240 mmol) was dissolved in CH_2Cl_2 (4.0 mL) at room temperature, and this solution was allowed to cool to 0 °C. To this solution were added diisopropylethylamine (245 μL) and diethyl chlorophosphite (104 μL), and then the mixture was stirred for 20 min at 0 °C. To this mixture was added MeOH (100 μL) for quenching of diethyl chlorophosphite at 0 °C. The mixture was diluted with $CHCl_3$ and washed with saturated $NaHCO_3$ solution and brine. The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by silica gel column chromatography (5% Et_3N solution of EtOAc/hexane = 3:1 → 5% Et_3N solution of EtOAc) afforded compound **1** (107 mg, 77%). Preparation of compound **2** from compound **9** (150 mg) was also performed by the same procedure described above (157 mg, 85%). Compound **1**: $[\alpha]^{24}_D = -22.7$ (c = 0.6, $CHCl_3$); ^{1}H NMR (400 MHz,$CDCl_3$) δ 6.83, (s, 1H), 5.37 (dd, 1H, J = 2.41 Hz, J = 7.23 Hz), 5.36 (s, 1H), 5.33–5.23 (m, 3H), 4.34 (dd, 1H, J = 2.68 Hz, J = 12.6 Hz), 4.22 (dd, 1H, J = 2.41 Hz, J = 11.0 Hz), 4.20–4.08 (m, 3H), 4.05 (dd, 1H, J = 6.69 Hz, J = 12.6 Hz), 4.02–3.85 (m, 4H), 2.70 (dd, 1H, J = 13.7 Hz, J = 4.82 Hz), 2.00 (1H), 2.15, 2.14, 2.05, 2.03. 1.90 (5 s, 15H), 1.28 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ 171.1, 170.8, 170.7, 170.4, 170.1, 169.5, 97.7, 71.7, 69.4, 68.8, 67.4, 62.6, 59.0, 58.3, 49.3, 37.3, 23.1, 21.1, 21.0, 20.9, 20.8, 16.7, 16.7; ^{31}P NMR ($CDCl_3$) δ 138.2; MALDI-HRMS calcd for $C_{23}H_{37}N_2NaO_{14}P$ [M + Na]$^+$ 619.1880, found 619.1870. (Compound **2**) $[\alpha]^{25}_D = -24.7$ (c = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz,$CDCl_3$) δ 6.89 (brd, 1H, J = 4.87 Hz), 5.37 (dd, 1H, J = 2.4 Hz, J = 6.84 Hz), 5.33–5.24 (m, 3H), 4.37 (dd, 1H, J = 2.62 Hz, J = 12.4 Hz), 4.22 (dd, 1H, J = 2.4 Hz, J = 10.7 Hz), 4.20–4.07 (m, 3H), 4.07 (dd, 1H, J = 6.77 Hz, J = 12.4 Hz), 4.00–3.80 (m, 4H), 2.87 (d, 3H, J = 4.87 Hz), 2.73 (dd, 1H, J = 13.6 Hz, J = 4.87 Hz), 1.92 (1H), 2.15, 2.13, 2.06, 2.03. 1.90 (5s, 15H), 1.27 (m, 6H); ^{13}C NMR (100 MHz,$CDCl_3$) δ 171.0, 170.8, 170.7, 170.3, 170.1, 167.7, 97.7, 71.8, 69.7, 68.8, 67.6, 62.6, 58.9, 58.1, 49.9, 37.5, 26.0, 23.1, 21.0, 20.8, 20.7, 20.7, 16.7, 16.7; ^{31}P NMR ($CDCl_3$) δ 138.1; MALDI-HRMS calcd for $C_{24}H_{39}N_2NaO_{14}P$ [M + Na]$^+$ 633.2037, found 633.2017.

Diethyl (dimethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-*O*-β-D-glycero-D-galacto-2-nonulopyranosylonamide) phosphite (3). Compound **11** (160 mg, 0.293 mmol) was dissolved in a solution of acetone and THF (1:9, 5.8 mL) containing 5% $HClO_4$, and this solution was stirred for 2 h at room temperature. The mixture was allowed to cool to 0 °C. This mixture was diluted with EtOAc, and the organic phase was washed with saturated $NaHCO_3$ solution and brine. The organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (4.9 mL, 60 mM) and the mixture was allowed to cool to 0 °C. To this solution was added diisopropylethylamine (300 μL, 1.76 mmol) and diethyl chlorophosphite (126 μL, 0.879 mmol) and this mixture was stirred for 15 min at 0 °C. To quench the reaction, MeOH was added to this mixture. The mixture was diluted with $CHCl_3$ and washed with saturated $NaHCO_3$ solution and brine. The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by silica gel column chromatography (5% Et_3N solution of EtOAc/hexane = 2:1) afforded compound **3** (60 mg, y = 33%): $[\alpha]^{25}_D = +7.50$ (c = 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.42 (d, 1H, J = 9.47 Hz), 5.38–5.30 (m, 2H), 5.17 (ddd, 1H, J = 2.74 Hz, J = 6.31 Hz, J = 6.73 Hz), 4.35 (dd, 1H, J = 2.74 Hz, J = 12.5 Hz), 4.04–3.82 (m, 4H), 4.30 (dd, 1H, J = 1.83 Hz, J = 10.6 Hz), 4.10 (dd, 1H, J = 6.32 Hz, J = 12.5 Hz), 4.08 (ddd, 1H, J = 10.3 Hz, J = 10.3 Hz, J = 10.3 Hz), 3.19 (s, 3H), 2.97 (s, 3H), 2.53 (dd, 1H, J = 14.0 Hz, J = 4.98 Hz), 2.29 (ddd, 1H, J = 14.0 Hz, J = 14.5 Hz, J = 0.85 Hz), 2.13, 2.09, 2.03, 2.03, 1.92 (5s, 15H), 1.27 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 170.7, 170.6, 170.5, 170.1, 166.10, 100.1, 72.2, 70.6, 69.6, 68.1, 62.7, 59.5, 58.3, 50.0, 38.3, 38.0, 37.2, 23.6, 21.3, 21.3, 21.1, 21.1, 17.3, 17.3; ^{31}P NMR ($CDCl_3$) δ 137.3; MALDI-HRMS calcd for $C_{25}H_{41}N_2NaO_{14}P$ [M + Na]$^+$ 647.2193, found 647.2215.

(Typical procedure for sialylation using amide-type phosphite donor 1–3). Each of donor (0.0503 mmol) and acceptor (0.100 mmol) was individually coevaporated with dry benzene twice and then dried up using vacuum pump. Both substrates were dissolved in CH_2Cl_2 (628 μL) containing activated 4Å molecular sieves (160 mg/1 mL) and this mixture was stirred for 2 h at room temperature. Then this mixture was allowed to cool to −78 °C. To this mixture was added TMSOTf (10% solution in CH_2Cl_2, 15 μL) and this mixture was stirred for 1 h. The mixture was allowed to warm to −40 °C and the mixture was stirred for additional 1 h. To this mixture was added Et_3N (15 μL) and then the mixture was diluted with $CHCl_3$ and then allowed to warm up to room temperature. The mixture was filtered and the filtrate was washed with saturated $NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue by gel permeation column chromatography followed by silica gel column chromatography (EtOAc → EtOAc/MeOH = 15:1) afforded desired sialoside.

(Typical deamidation reaction). To a solution of MeOH (450 μL) containing amide derivative of α-sialoside (0.0451 mmol) was added NaOMe (0.0451 mmol). This mixture was stirred at room temperature for 1 h and then the mixture was added ion-exchange resin IR-120. The mixture was filtered and the filtrate was concentrated in vacuo. Subsequently, the residue was dissolved in a solution of 2 M NaOH solution (450 μL) and MeOH (100 μL) and then this mixture was stirred for 2–8 h (depended on the substrate) at 100 °C. Then the mixture was allowed to cool to 0 °C and acetic anhydride was added to this mixture for acetamidation. After finish of acetamidation, the mixture was neutralized by AcOH and then directly concentrated in vacuo. Purification of the residue by C-18 reverse phase column chromatography (residue was subjected by H_2O and CH_3CN/H_2O = 50:50 was used as eluent) to obtain α-sialoside. The yields were shown in Table 3.

(Compound data of sialyl-T_N derivative 31). $[\alpha]^{21}_D = +49.9$ (c = 0.95, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 7.77 (d, 2H, J = 7.50 Hz), 7.65 (br, 2H), 7.39–7.28 (m, 4H), 4.79 (br, 1H), 4.43–4.39 (m, 3H), 4.23 (brt, 1H), 4.22 (dd, 1H, J = 3.65 Hz, J = 10.83 Hz), 3.91–3.83 (m, 6H), 3.83 (dd, 1H, J = 2.18 Hz, J = 11.69 Hz), 3.81–3.76 (m, 4H), 3.72 (dd, 1H, J = 10.83 Hz, J =

2.81 Hz), 3.67 (dd, 1H, $J = 4.48$ Hz, $J = 10.12$ Hz), 3.66 (m, 1H), 3.65 (dd, 1H, $J = 5.71$ Hz, $J = 11.48$ Hz), 3.61 (dd, 1H, $J = 10.46$ Hz, $J = 1.31$ Hz), 3.50 (dd, 1H, $J = 1.31$ Hz, $J = 9.04$ Hz), 2.67 (dd, 1H, $J = 12.87$ Hz, $J = 4.66$ Hz), 1.99, 1.95 (2s, 6H), 1.78 (dd, 1H, $J = 12.87$ Hz, $J = 12.24$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.1, 173.9, 173.6, 145.3, 142.6, 128.8, 128.2, 126.1, 121.0, 100.4, 99.9, 75.0, 72.4, 71.0, 70.3, 69.7, 69.6, 69.2, 68.6, 68.0, 64.9, 64.1, 55.9, 53.8, 53.4, 51.4, 48.5, 41.2 22.9, 22.7; MALDI-HRMS calcd for $C_{38}H_{49}N_3NaO_{18}$ $[M + Na]^+$ 858.2909, found 858.2907.

Solid-Phase Peptide Synthesis. The synthesis of MUC4 was performed on HMPB-PEGA resin (0.01 mol scale). The first amino acid, Fmoc-Asp(tBu)-OH (21 mg, 0.05 mmol), was attached quantitatively to the resin using MSNT (15 mg, 0.05 mol) and *N*-methylimidazole (3 μl, 0.0375 mol) in CH_2Cl_2 (500 μL). Coupling of amino acid from second to eleventh (T, V, P, L, P, T, A, H, G, T) was performed by use of Fmoc-amino acid (0.05 mmol), DIPCDI (0.05 mmol), and HOBt (0.05 mmol) in DMF (310 μL), and 20% piperidine/DMF was used for Fmoc deprotection. Then, coupling of compound **31** (17 mg, 0.02 mmol) was performed using DIPCDI (4.6 μL, 0.03 mmol) and HOBt (4 mg, 0.03 mmol) in DMF/DMSO = 1:1 solvent (1 mL) for 15 h followed by washing by DMF and deprotection of Fmoc by 20% piperidine/DMF for 10 min. The remaining amino acid was elongated manually by Fmoc-amino acid (A, S, S) or Boc-amino acid (T) (0.05 mmol), DIPCDI (0.05 mmol), and HOBt (0.05 mmol) in DMF (1.25 mL). After completion of chain assembly, the resin was treated with solution containing 95% TFA, 2.5% TIPS and 2.5% H_2O for 2 h at room temperature followed by filtering, and the filtrate was evaporated in vacuo. Purification of the residue by RP-HPLC with Vydac column C-18 (5 μm, 250 × 4.5 mm linear gradient of 9 → 49.5% containing 0.09% TFA in 0.1% TFA aqueous over 30 min) at a flow rate of 1.0 mL/min afforded the desired sialylglycopeptide (7.4 mg, 38% yield based on the first amino acid attached).

Saponification of Methyl Ester Sialylt$_N$-MUC4 Peptide: Condition A. Sialylglycopeptide (5 mg, 2.4 μmol) was dissolved in cooled 200 mM NaOH solution (400 μL) at 0 °C, and this mixture was stirred for 10 min. After neutralization by 200 mM HCl solution (400 μL), purification of the residue by RP-HPLC with Vydac column C-18 (5 μm, 250 × 4.5 mm linear gradient of 13.5 → 31.5% containing 0.09% TFA in 0.1% TFA solution over 60 min at a flow rate of 1.2 mL/min) afforded the desired sialylglycopeptide (2.5 mg, 50% yield). **Condition B.** Sialylglycopeptide (2.3 mg, 1.1 μmol) was dissolved in cooled 20 mM NaOH solution (225 μL) at room temperature, and this mixture was stirred for 1.5 h. After neutralization by 200 mM HCl solution (200 μL), purification of the residue by RP-HPLC with a Vydac column C-18 (5 μm, 250 × 4.5 mm linear gradient of 13.5 → 31.5% containing 0.09% TFA in 0.1% TFA aqueous over 60 min at a flow rate of 1.2 mL/min) afforded the desired sialylglycopeptide (1.4 mg, 61%): MALDI-HRMS calcd for $C_{83}H_{134}N_{20}O_{39}$ $[M + H]^+$ 2035.9195, found 2035.9210.

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Supporting Information Available: Full experimental details for the preparation of **1**−**3** and **31** and NMR spectra of all new compounds and coupling compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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