Preparation of 4-Pentenoic Acid Ester of Neu5Ac and 4-Pentenyl Glycoside of Neu5Ac and Their Application to Glycosylation

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Novel sialosyl donors, 4-pentenoic acid ester of *N*-acetylneuraminic acids (Neu5Ac) (1a) and 4-pentenyl glycoside of Neu5Ac (1b) were successfully prepared from the corresponding per-*O*-acetylated 2-hydroxy and 2-chloro derivatives of Neu5Ac, respectively and applied to the synthesis of *O*-sialosides.

Key words 4-pentenoic acid ester; 4-pentenyl glycoside; *N*-acetylneuraminic acids; *O*-sialylation; armed-disarmed methodology

N-Acetylneuraminic acids (Neu5Ac), sialic acids are most frequently found as α-glycosidically linked terminal residues of glycoproteins and glycolipids. There are a wide range of biological properties endowed by sialic acids on natural glycoconjugate structure and function, often involved in important cell surface communications and infection processes.[1—3] The development of the efficient method of *O*-sialylation has been a challenging task in the field of sialic acid chemistry.[4,5] Fraser-Reid and his co-workers introduced a 4-pentenyl group as a new and effective leaving group at the anomeric center of the glycosyl donor.[6] The attractiveness of 4-pentenyl glycosides is enhanced by the fact that they can be applied to the armed/disarmed methodology of oligosaccharide synthesis.[7] 4-Pentenyl glycosides are of interest for studying various biological and physiological phenomena of carbohydrates.[8] To the best of our knowledge, there has been no report on the synthesis of 4-pentenoic acid ester of Neu5Ac (**1a**). A few reports[9,10] on the synthesis of 4-pentenyl glycoside of Neu5Ac (**1b**) have been reported, however application of **1b** to *O*-sialylation has not been studied. As a part of our program aimed at the development of new *O*-sialylation,[11,12] we are interested in finding out whether **1a**, **b** would be suitable for stereoselective *O*-sialylation. This paper describes the synthesis of **1a**, **b** and their application to *O*-sialylation.

Results and Discussion

We first focused on an efficient synthesis of **1a** having 4-pentenoyloxy group at the 2-position of Neu5Ac as a leaving group. Treatment of per-*O*-acetylated Neu5Ac **2** with hydrogen chloride in AcCl–AcOH (1 : 1) gave 2-chloro derivative **3** in a quantitative yield. Compound **3** was treated with silver carbonate in acetone–H_2O (9 : 1) to afford 2-hydroxy derivative **4**[13] in 88% yield. Acylation of **4** was achieved by 4-pentenoic anhydride and pyridine and *N*,*N*-dimethylaminopyridine (DMAP) in CH_2Cl_2 to give **1a** in a quantitative yield as an anomeric mixture with β-anomer as the major product (Chart 1). Next, we focused on a search for an efficient method of synthesizing **1b**. When silver salicylate was used as a promoter in the presence of 4-penten-1-ol in CH_2Cl_2, **1b** was obtained in 80% yield.[9] The best result was obtained when the reaction was performed by Koenigs–Knorr condi-

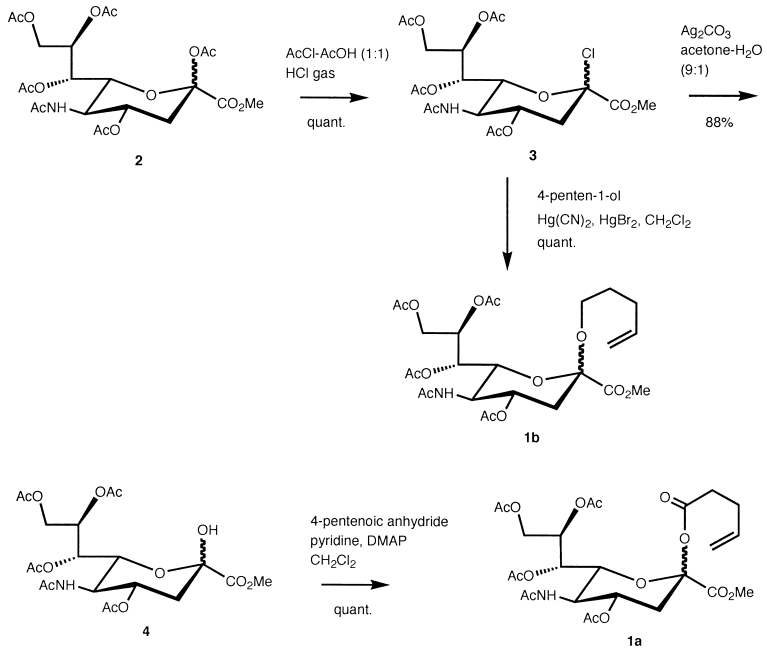

Chart 1. Preparation of 4-Pentenoic Acid Ester of Neu5Ac and 4-Pentenyl Glycoside of Neu5Ac

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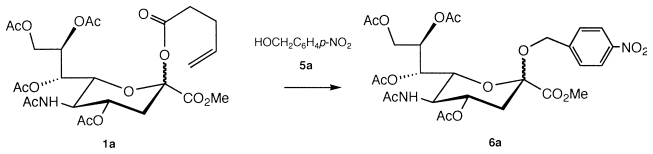
tion. Thus, the reaction of **3** with 4-penten-1-ol by using $Hg(CN)_2$ and $HgBr_2$ in CH_2Cl_2 afforded **1b** in a quantitative yield as an anomeric mixture ($\alpha/\beta=1:1$).

We studied the glycosylation of **1a** with *p*-nitrobenzyl alcohol **5a**, which is often used in biochemical studies as a hydrogen-sensitive linker.[14] The different reaction conditions including temperatures, promoters, and solvents were tested. The results are summarized in Table 1.

The reaction of **5a** with 1.5 molar equivalent of **1a** as a sialosyl donor using 3.0 molar equivalent of *N*-iodosuccinimide (NIS) and 0.5 eq of triethylsilyl triflate (TESOTf)[15] in CH_3CN at room temperature gave the expected glycosides **6a** in 68% yield with α-anomer as the major product ($\alpha/\beta=2:1$) (entry 1, Table 1). The α/β ratio was determined by integration of the H-3eq ^{1}H-NMR signal. Use of dimethyl(methylthio)sulfonium triflate (DMTST)[16] as promoters afforded **6a** in 48% yield ($\alpha/\beta=1:4$), while use of iodonium dicollidine perchlorate (IDCP)[17] gave a trace amount of **6a** (entries 2, 3, Table 1).

Table 1. Glycosylation Reactions Using 4-Pentenoic Acid Ester of Neu5Ac (**1a**)



Entry	Condition	Yield (%) ($\alpha:\beta$)[c]
1	NIS-TESOTf, CH_3CN, r.t.[a]	68 (2 : 1)
2	DMTST, CH_2Cl_2, r.t.[b]	48 (1 : 4)
3	IDCP, CH_3CN, r.t.[b]	Trace

a) With respect to **5a**, 1.5 eq of **1a** and 3.0 eq of NIS and 0.5 eq of TESOTf were used. *b*) With respect to **5a**, 1.5 eq of **1a** and 3.0 eq of the activator were used. *c*) Isolated yields. The anomeric ratios were determined on the basis of the integration ratios of the H-3*eq* signals of the glycosides in ^{1}H-NMR analysis.

Next, we studied the glycosylation of **1b** with *p*-nitrobenzyl alcohol **5a**. As summarized in Table 2, the reaction of **1b** with 1.5 molar equivalent of alcohol **5a** in the presence of 3.0 molar equivalent of NIS and 3.0 molar equivalent of TESOTf as a promoter in CH_3CN at $-25\,^\circ C$ gave **6a** in 83% yield, with an α/β ratio of 1 : 1 (entry 1). Further investigations using more biologically relevant acceptors, galactose diacetonide **5b** and benzyl 2,6-di-*O*-benzyl-β-D-galactopyranoside **5c** were performed with **1b**. Thus, the reaction of **1b** with **5b** promoted by 3.0 molar equivalent of NIS and 3.0 molar equivalent of trifluoromethanesulfonic acid (TfOH)[18] in CH_3CN at $-40\,^\circ C$ gave the corresponding $\alpha(2\rightarrow6)$-linked glycoside **6b** in 60% yield stereoselectively ($\alpha/\beta=11:1$, entry 2). The reaction of **1b** with **5b** did not proceed when the promoter was changed to AgOTf.[19] When ether was used as the solvent, decrement of both α-selectivity and yield was observed (entry 3). Finally, the glycosylation of **1b** with **5c** promoted by NIS-TfOH in CH_3CN at $-40\,^\circ C$ afforded a 4 : 1 mixture of the known α and β Neu5Ac-(2→3)-galactoside **6c**[20] in 37% (entry 4).

In conclusion, this study demonstrated the first example of utilization of **1a**, **b** in *O*-sialylation. Compound **1b** proved to be more efficient than **1a** as a sialyl donor. Further studies of

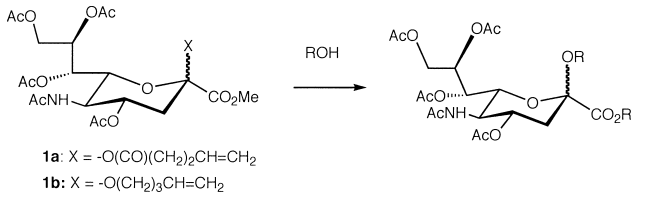
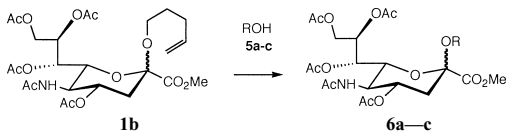
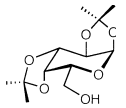
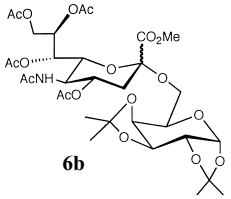
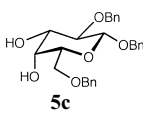
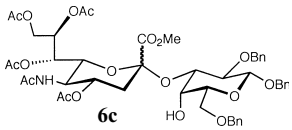


Fig. 1. 4-Pentenoic Acid Ester of Neu5Ac and 4-Pentenyl Glycoside of Neu5Ac

Table 2. Glycosylation Reactions Using 4-Pentenyl Glycoside of Neu5Ac (**1b**)



Entry	Alcohol	Condition[a]	Product	Yield (%) ($\alpha:\beta$)
1	$HOCH_2C_6H_4p$-NO_2 **5a**	NIS-TESOTf, CH_3CN, $-25\,^\circ C$	**6a**	83 (1 : 1)[b]
2	 **5b**	NIS-TfOH, CH_3CN, $-40\,^\circ C$	 **6b**	60 (11 : 1)[b]
3	**5b**	NIS-TfOH, ether, $-40\,^\circ C$	**6b**	33 (1 : 1)[b]
4	 **5c**	NIS-TfOH, CH_3CN, $-40\,^\circ C$	 **6c**	37 (4 : 1)[c]

a) With respect to **5**, 1.5 eq of **1b** and 3.0 eq of NIS and 3.0 eq of TESOTf or TfOH were used. *b*) Isolated yields. The anomeric ratios were determined on the basis of the integration ratios of the H-3*eq* signals of the glycosides in ^{1}H-NMR analysis. *c*) The anomeric ratio were determined by ^{1}H-NMR analysis.[20]

the approach to armed-disarmed methodology are now in progress.

Experimental

All melting points are uncorrected. ^{1}H-NMR spectra were recorded with a JEOL ECA-500 (500 MHz) spectrometer. ^{1}H chemical shifts are given in ppm relative to Me_4Si (δ=0) in $CDCl_3$ or CD_3OD as internal standards at ambient temperature. The abbreviations of signal patterns are as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Fast atom bombardment (FAB) mass spectra were obtained with a JEOL Mstation-700 mass spectrometer in the positive ion mode using NBA matrix. Column chromatography was performed on Silica Gel 60 (70—230 mesh, Merck). Thin-layer chromatography (TLC) was performed on aluminum sheets coated with Silica Gel 60F_{254} (Merck). The spots were visualized by spraying the plates with 5% aqueous sulfuric acid in MeOH and then heating.

Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2-(4-pentenoyl)-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosonate (1a) To a solution of compound **4**[13] (0.11 g, 0.22 mmol), pyridine (0.052 g, 0.66 mmol) and DMAP (0.027 g, 0.22 mmol) in dry CH_2Cl_2 (2 ml) was added 4-pentenoic anhydride (0.080 g, 0.44 mmol) with stirring at 0 °C under argon. After 1 h at the same temperature, the mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated to dryness, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH 10 : 1) to give **1a** (0.143 g, quant.) as amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.89 (3H, s, NHAc), 2.03, 2.06, 2.13, 2.14 (each 3H, s, OAc), 2.39–2.58 (6H, m, H-3*eq*, CH_2=$CHCH_2CH_2$–), 3.79 (3H, s, OCH_3), 4.12—4.16 (3H, m, H-5, H-6, H-9a), 4.51 (1H, dd, *J*=2.5, 12.5 Hz, H-9b), 5.02—5.38 (6H, m, H-4, H-7, H-8, NH, –$CHCH_2$), 5.87 (1H, m, –$CHCH_2$). Positive FAB-MS *m/z*: 574 $(M+H)^+$, 596 $(M+Na)^+$. HR-FAB-MS Calcd for $C_{25}H_{36}O_{14}N$ $(M+H)^+$: 574.2136. Found: 574.2148.

Methyl (4-Pentenyl 5-Acetoamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosid)onate (1b) A suspension of compound **3** (0.688 g, 1.35 mmol), 4-penten-1-ol (0.097 g, 1.13 mmol), 4 Å molecular sieves (1.0 g), and dry CH_2Cl_2 (5 ml) was stirred for 1 h at room temperature under Ar. The mixture was cooled to 0 °C, and $Hg(CN)_2$ (0.856 g, 3.39 mmol) and $HgBr_2$ (0.407 g, 1.13 mmol) were added to the stirring mixture. The solution was stirred for 20 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 and filtered through a Celite pad. The filtrate was then washed with 10% aqueous KI and then with saturated aqueous NaCl, and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo*, and the residue was purified by column chromatography (AcOEt) to afford **1b** (0.680 g, quant.) as amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.64 (4H, m, –CH_2CH_2CH=CH_2), 1.89 (3H, s, NHAc), 2.03, 2.06, 2.13, 2.14 (each 3H, s, OAc), 2.46 (1H, dd, *J*=4.4, 13 Hz, H-3*eq* of β-anomer), 2.59 (1H, dd, *J*=4.6, 12.9 Hz, H-3*eq* of α-anomer), 3.22 (2H, dd, OCH_2), 3.78 (3H, s, OCH_3), 4.01—4.12 (3H, m, H-5, CH=CH_2), 4.87—5.38 (7H, m, H-4, H-7, H-8, H-9b, NH, CH=CH_2), 5.80 (1H, m, CH=CH_2). Positive FAB-MS *m/z*: 560 $(M+H)^+$, 582 $(M+Na)^+$. HR-FAB-MS Calcd for $C_{25}H_{38}O_{13}N$ $(M+H)^+$: 560.2343. Found: 560.2357.

Methyl (4-Nitrobenzyl 5-Acetoamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosid)onate (6a) A suspension of **1a** (0.0513 g, 0.089 mmol), *p*-nitrobenzyl alcohol **5a** (0.0136 g, 0.089 mmol), 3 Å molecular sieves (0.25 g), and anhydrous CH_3CN (2 ml) was stirred for 1 h at room temperature under Ar. The mixture was cooled at −40 °C, and NIS (0.060 g, 0.267 mmol) and TESOTf (0.012 g, 0.267 mmol) were added to the stirring mixture. After being stirred overnight at the same temperature, the temperature was elevated to the room temperature followed by stirring for 1 h. After completion of the reaction, this reaction mixture was diluted with CH_2Cl_2 and filtrated through a Celite pad. The filtrate was washed with saturated aqueous $NaHCO_3$ and then with saturated aqueous NaCl, and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo*, and the residue was purified by column chromatography (AcOEt) to give glycoside **6a** (0.038 mg, 68%), which was identical with the authentic compound.[11]

With DMTST as a Promoter A suspension of **1a** (0.032 g, 0.056 mmol), *p*-nitrobenzyl alcohol **5a** (0.009 g, 0.056 mmol), 4 Å molecular sieves (0.20 g), and dry CH_2Cl_2 (2 ml) was stirred for 1 h at room temperature under Ar. To this mixture DMTST (0.014 g, 0.056 mmol) was added and the whole was stirred for 15 at room temperature. After all of the glycosyl donor was consumed, and the reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography (AcOEt) to give **6a** (0.017 g, 48%).

With 1b as a Glycosyl Donor A suspension of **1b** (0.030 g, 0.054 mmol), *p*-nitrobenzyl alcohol **5a** (0.012 g, 0.081 mmol), 4 Å molecular sieves (0.20 g), and dry CH_2Cl_2 (2 ml) was stirred for 1 h at room temperature under Ar. The mixture was cooled at −25 °C, and NIS (0.037 g, 0.16 mmol) and TESOTf (0.0428 g, 0.16 mmol) were added to the mixture. After being stirred overnight at the same temperature, the mixture was elevated to the room temperature followed by stirring for 1 h. The reaction mixture was diluted with CH_2Cl_2 and filtrated through a Celite pad. The filtrate was washed with saturated aqueous $NaHCO_3$ and then with saturated aqueous NaCl, and dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give a crude product, which was purified by column chromatography. Elution with EtOAc gave 0.028 g (83%) of **6a**.

1,2;3,4-Di-*O*-isopropylidene-6-*O*-[methyl(5-acetoamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosyl)onate]-α-D-galactopyranoside (6b) A suspension of **1b** (0.213 g, 0.38 mmol), **5b** (0.066 g, 0.25 mmol), 3 Å molecular sieves (0.25 g), and anhydrous CH_3CN (3 ml) was stirred for 1 h at ambient temperature under Ar. The mixture was cooled at −40 °C, and NIS (0.169 g, 0.75 mmol) and TfOH (0.113 g, 0.75 mmol) were added to the mixture. After being stirred for 15 h at the same temperature, the temperature was elevated to the room temperature followed by stirring for 1 h. The reaction mixture was diluted with CH_2Cl_2 and filtrated through a Celite pad. The filtrate was washed with 10% aqueous $Na_2S_2O_3$ and then with saturated aqueous NaCl, and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo*, and the residue was purified by column chromatography (AcOEt) to give **6b** (0.111 g, 60%) as amorphous powder. ^{1}H-NMR ($CDCl_3$) δ: 1.30, 1.31 (6H, s, isopropylidene), 1.40, 1.45 (3H, s, isopropylidene), 1.52, 1.54 (3H, s, isopropylidene), 1.86, 2.01, 2.02, 2.10, 2.11 (15H, s, OAc), 2.47 (1H, dd, *J*=5.2, 13.2 Hz, H-3*eq* of β-anomer), 2.59 (1H, dd, *J*=4.6 Hz, H-3*eq* of α-anomer), 3.76 (3H, s, OCH_3), 4.03—4.16 (2H, m, H-6b, H-9b), 4.21—4.28 (3H, m, H-6a, H-9b′), 4.57 (1H, dd, *J*=2.3, 8.0 Hz, H-2a), 4.82—4.87 (1H, m, H-8b), 5.24 (1H, t, *J*=8.6 Hz, H-5b), 5.30 (1H, dd, *J*=8.0, 1.7 Hz, H-7b), 5.36—5.39 (1H, m, H-4b), 5.48 (1H, d, *J*=5.2 Hz, H-1a). Positive FAB-MS *m/z*: 734 $(M+H)^+$. HR-FAB-MS Calcd for $C_{32}H_{48}O_{18}N$ $(M+H)^+$: 734.2871. Found: 734.2874.

With Ether as Solvent A suspension of **1b** (0.140 g, 0.25 mmol), **5b** (0.043 g, 0.17 mmol), 3 Å molecular sieves (0.22 g), and anhydrous ether (3 ml) was stirred for 1 h at ambient temperature under Ar. The mixture was cooled at −40 °C, and NIS (0.113 g, 0.50 mmol) and TfOH (0.075 g, 0.50 mmol) were added to the mixture. After being stirred for 15 h at the same temperature, the temperature was elevated to the room temperature followed by stirring for 1 h. The reaction mixture was diluted with CH_2Cl_2 and filtrated through a Celite pad. The filtrate was washed with 10% aqueous $Na_2S_2O_3$ and then with saturated aqueous NaCl, and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo*, and the residue was purified by column chromatography (AcOEt) to give **6b** (0.040 g, 33%).

Benzyl 2,6-Di-*O*-benzyl-3-*O*-[methyl(5-acetoamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranosyl)onate]-β-D-galactopyranoside (6c) A suspension of **1b** (0.121 g, 0.22 mmol), **5c** (0.065 g, 0.14 mmol), 3 Å molecular sieves (0.20 g), and anhydrous CH_3CN (2 ml) was stirred for 1 h at ambient temperature under Ar. The mixture was cooled at −40 °C, and NIS (0.097 g, 0.43 mmol) and TfOH (0.065 g, 0.43 mmol) were added to the mixture. After being stirred for 15 h at the same temperature, the temperature was elevated to the room temperature followed by stirring for 1 h. The reaction mixture was diluted with CH_2Cl_2 and filtrated through a Celite pad. The filtrate was washed with 10% aqueous $Na_2S_2O_3$ and then with saturated aqueous NaCl, and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo*, and the residue was purified by column chromatography (AcOEt) to give **6c** (0.046 g, 37%) as amorphous powder, which was identical with the authentic compound.[20] ^{1}H-NMR ($CDCl_3$) δ: 1.86, 1.94, 1.98, 1.99, 2.08 (each 3H, s, OAc), 2.51 (1H, dd, $J_{8,9b}$=4.6, $J_{9a,9b}$=13.2 Hz, H-3*eq*), 4.54 (1H, d, *J*=7.5 Hz, H-1a), 4.59 (2H, s, CH_2Ph), 4.66 (1H, d, *J*=12.5 Hz, CH_2Ph), 4.71 (1H, d, *J*=11.5 Hz, CH_2Ph), 4.83—4.87 (2H, m, H-4b, CH_2Ph), 4.95 (1H, d, *J*=12.5 Hz, CH_2Ph), 5.20—5.21 (1H, m, H-7b), 5.29 (1H, d, *J*=8.0 Hz, NH), 5.36—5.38 (1H, m, H-8b), 7.22—7.39 (15H, m, aromatic). Positive FAB-MS *m/z*: 924 $(M+H)^+$, 946 $(M+Na)^+$.

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