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LETTERS

A Novel and Versatile Glycosyl Donor for the Preparation of Glycosides of *N*-Acetylneuraminic Acid

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Abstract: An *N*-diacetyl derivative of neuraminic acid exhibits excellent glycosyl donor properties and is a very useful substrate for the preparation of glycosides of *N*-acetylneuraminic acid. The donor will be a key component in the preparation of a heptasaccharide derived from group B type III *Streptococcus*.

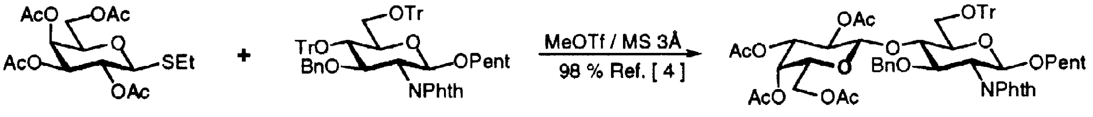
Group B type III *Streptococcus* is a major cause of bacterial meningitis in new-born infants.[1] It has been proposed[2,3] that pregnant mothers deficient in antibodies specific for the native, type III group B *Streptococcus* polysaccharide should be vaccinated with a polysaccharide vaccine. As part of a programme to develop an ELISA assay to detect antibody levels in pregnant women, we reported a high convergent synthesis of hexasaccharide **I** (Figure 1) which is derived from the capsular polysaccharide of group B type III *Streptococcus*.[4] However, the minimum structural element that can induce the production of protective antibodies needs an additional *N*-acetyl neuraminic unit (*e.g.* compound **II**, Figure 1).

β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-β-D-GlcNAc*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*
4
↑
1
(R→3)-β-D-Gal*p*

(**I**) R = H
(**II**) R = α-Neu5Ac(2→

Figure 1

An important step in the preparation of the hexasaccharide **I** was the coupling of thioglycoside **1** with di-*O*-trityl pentenylglycosyl acceptor **2** (pent = pentenyl) in the presence of methyl triflate (MeOTf) to give disaccharide **3**.[4] This glycosylation proceeded with very high regio- and stereoselectivity and it was demonstrated that the secondary trityl ether enhances the nucleophilicity of the C-4 alcohol (Scheme 1).



1 **2** **3**

Scheme 1

A similar glycosylation between the neuraminyl containing thioglycoside **7** and **2** to give trisaccharide **8** was planned as a key step in the synthesis of compound **II** (Scheme 2). The glycosyl donor **7** was conveniently prepared by an approach described by Hasegawa *et al.*[5] which entails a regio- and stereoselective coupling of **4**[6] with **5** to give disaccharide **6** which was converted into glycosyl donor **7** by a three step procedure.[7] However, MeOTf mediated glycosylation of **2** with **7** did not give the requisite trisaccharide **8** but FAB MS (m/z 1570 [M+Na$^+$] and NMR data (3.57, s, 3H, N*Me*) showed unambiguously that during the course of the glycosylation the *N*-acetyl group of the neuraminyl unit had been methylated to give compound **9**. Indeed, treatment of methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-non-2-ulo-pyranosid)onate

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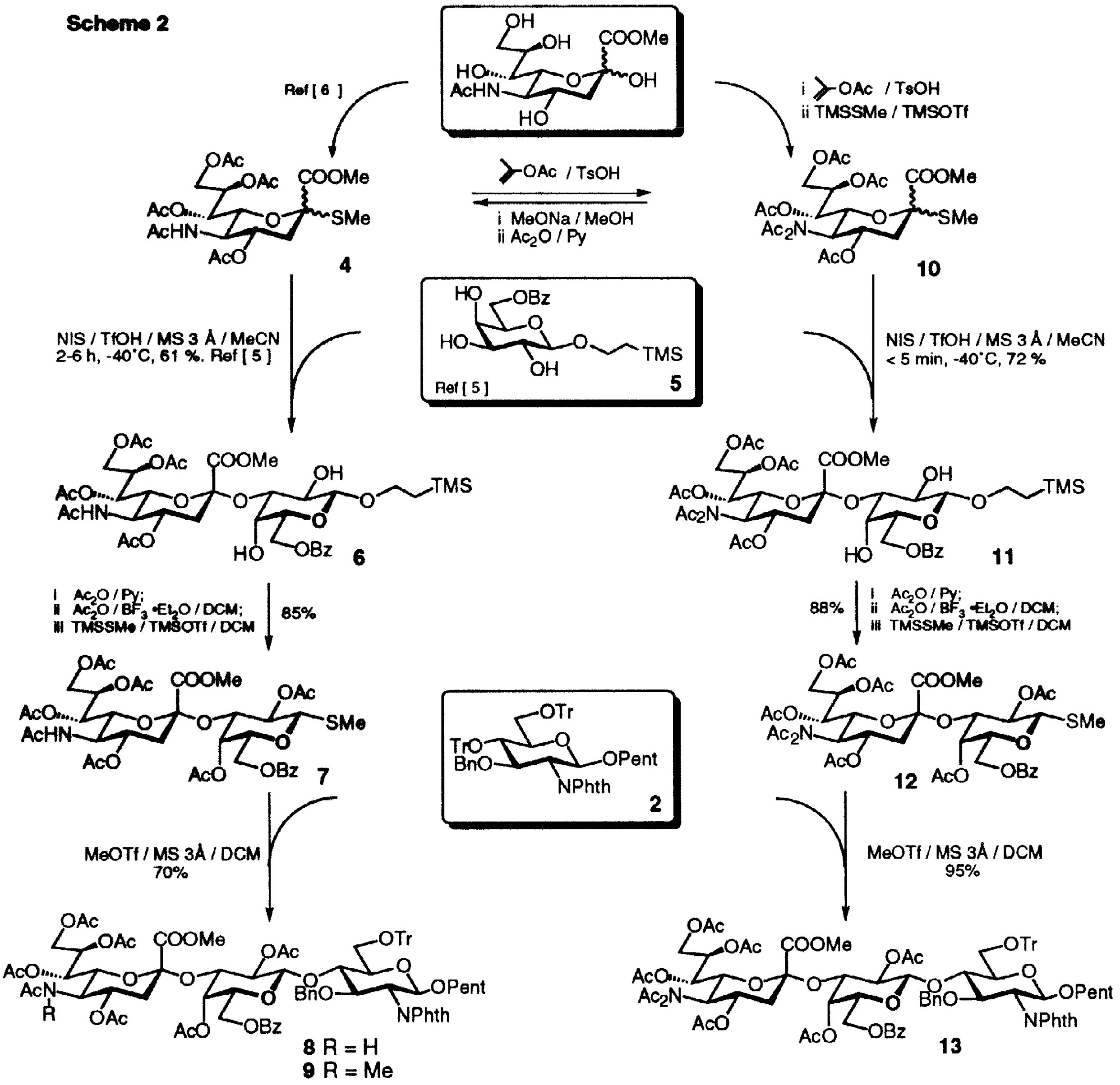
Scheme 2



(**4**) with MeOTf in acetonitrile at -40°C for 10 minutes gave the analogues *N*-methylated derivative in a quantitative yield. These reactions indicate that the acetamido moiety of **7** is of considerable nucleophilicity and reacts faster with MeOTf than its anomeric thiomethyl moiety.

During the investigations to address the above mentioned problems, we found that 5-*N*-diacetyl neuraminyl derivative **10** (Schemes 2 and 3) is significantly more reactive and gives higher yields in glycosylations than the corresponding mono-*N*-acetylated derivative **4**. Furthermore, the nucleophilicity of the *N*-diacetyl moiety is significantly reduced and is not methylated on treatment with methyl triflate and therefore **10** is an ideal substrate for the preparation of the heptasaccharide **II** and other sialyl containing oligosaccharides.

N-Diacetyl derivatives of carbohydrates have been described and for example Schmidt and co-workers reported that methylthio *N,N*-diacetyl glucosamine and galactosamine derivatives act as efficient glycosyl donors in DMTST promoted glycosylations.[8]

Several methods were attempted to prepare the *N*-diacetyl neuraminic acid **10**. As can be seen from Table 1, treatment of Neu5Ac with acetyl chloride and Hünigs base in acetonitrile gave almost no product formation. This result was rather surprising since these conditions gave excellent yields for the preparation of *N,N*-diacetyl

Table 1. *N*-Acetylation of Mono-*N*-acetyl Derivative **4** to Give Di-*N*-acetate **10**

Entry	Reagents	Temperature	Time	Yield
1	AcCl / i-Pr_2NEt / MeCN	RT	24 h	< 5%
2	Ac_2O / AcONa	140°C	20 h	55%
3	Ac_2O	140°C	20 h	70%
4	$CH_3(CH_2{=})COAc$ / TsOH	94°C	4 h	80%
5	$CH_3(CH_2{=})COAc$ / TsOH	65°C	16 h	99%

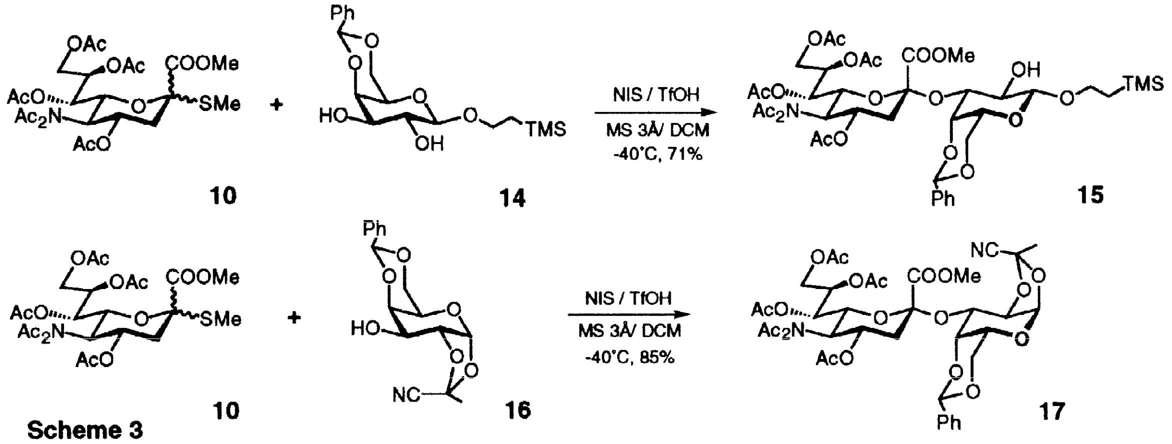
glucosamine and galactosamine derivatives. Acceptable yields were obtained by employing acetic anhydride as the acetylating reagent at evaluated temperatures (entries 2 and 3). However, a quantitative yield of **10** was obtained when mono-*N*-acetyl derivative **4** was treated with *iso*-propenylacetate in the presence of a catalytic amount of *p*-toluenesulphonic acid (TsOH) at 65°C (entry 5).[9] Compound **10** can be obtained directly from Neu5Ac by the *N*-acetylation followed by the reaction with TMSSMe in the presence of a catalytic amount of TMSOTf. Furthermore, transformation of the *N*-diacetyl moieties of into a mono-*N*-acetylated derivative with concominant *O*-deacetylation could easily be achieved by treatment with methanolic sodium methoxide (e.g. transformation **10** to **4**, Scheme 2).

Having successfully prepared **10**, attention was turned to its use as a glycosyl donor. Surprisingly, the trivial additional *N*-acetyl group dramatically improved the glycosyl donor properties of **10**. For example, coupling of **10** (1.7 eq.) with galactosyl acceptor **5** in the presence of NIS/TfOH (3.4 eq / 0.4 eq) in acetonitrile at -40°C gave, after a reaction time of < 5 minutes, the required disaccharide **11** in a yield of 65% together with the corresponding 2,4-lactone (34%). Thus, the total yield of dimeric product was almost quantitative. On the other hand, as described by Hasegawa *et al.*,[6] coupling of the mono-*N*-acetylated glycosyl donor **4** with acceptor **5** required 2 - 6 hours and yielded 61% of disaccharide **6**.

The formation of the undesirable lactone could easily be avoided by reducing the excess of donor and promoter and for example the employment of only 1.1 equivalents of **10** in the presence of NIS/TfOH (2eq. / 0.2 eq.) gave **11** in a 72 % yield and no lactone formation was observed.

O-Acetylation of **11** with acetic acid anhydride in pyridine followed by acetolysis of the anomeric TMSEt group with acetic anhydride in the presence of BF_3 etherate and finally conversion of the resulting anomeric acetate into methylthio glycoside by treatment with TMSSMe in the presence of a catalytic amount of TMSOTf gave **12**. Coupling of the glycosyl donor **12** with the ditritylated acceptor **2** in the presence of MeOTf yielded the trisaccharide **13** with absolute regio- and stereoselectivity in an excellent yield of 95%.[10,11] As expected no *N*-methylation of the diacetamido moiety had occurred.

N-Diacetyl derivative **10** proved also an efficient glycosyl donor for the glycosylation of other galactosyl acceptors (Scheme 3). As expected, coupling of **10** with benzylidene protected galactoside **14** under standard



Scheme 3

conditions gave the disaccharide **15** in an excellent yield of 71% as one anomer. In this case, no lactone formation was observed. Interestingly, coupling of the **10** with the 1,2-cyanoethylidene acceptor **16** also proceeded smoothly and **17** was isolated in a yield of 85% as a mixture of anomers. It is envisaged that disaccharide **17** would be a very useful derivative for the synthesis of **II** since it can immediately used as a donor for the glycosylation of **2**.

In conclusion, the glycosyl donor properties of the often employed thioglycoside of Neu5Ac[12] can dramatically be improved by conversion of its acetamido group into an *N*-diacetyl moiety. The latter transformation can quantitatively be performed by treatment of Neu5Ac with *i*-propenylacetate in the presence of a catalytic amount of TsOH. Removal of one of the *N*-acetyl groups and concomitant *O*-deacetylation can be accomplished by treatment with NaOMe in methanol. Preliminary results have shown that **10** is also an excellent glycosyl donor for the preparation of Neu5Ac(2-8)Neu5Ac and Neu5Ac(2-9)Neu5Ac fragments.

Acknowledgement
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9. General procedure for the preparation of NAc_2 derivatives of Neu5Ac: A mono-*N*-acetylated derivative (1 mmol) was dissolved in *i*-propenylacetate (5 ml) and TsOH monohydrate (0.05 mmol) was added. The reaction mixture was stirred at 65°C for 16 hours, then neutralised with Et_3N and evaporated to dryness. The crude product was purified by silica gel column chromatography.
10. Selected analytical data for trisaccharide **13**: *^{1}H NMR ($CDCl_3$, 500MHz):* 5.59 (m, 1H, =CH-), 5.54 (ddd, 1H, $J_{4'',5''}$ 10.5, H-4”), 5.42 (ddd, 1H, $J_{8'',9''a}$ 2.5, $J_{8'',9''b}$ 5, H-8”), 5,14 (dd, $J_{7'',8''}$ 9, H-7”), 5,13 (dd, 1H, H-4’), 5.08 (d, 1H, $J_{1,2}$ 8.5, H-1), 4.92 (dd, 1H, $J_{2',3'}$ 9.5, H-2’), 4.87 (d, 1H, $J_{1',2'}$ 7, H-1’), 4.73 (m, 2H, CH_2=), 4.59 (dd, 1H, $J_{6'',7''}$ 2, H-6”), 4.45 (dd, 1H, $J_{3',4'}$ 3, H-3’), 4.34 (dd, 1H, $J_{3,4}$ 8.5, H-3), 4.29 (dd, 1H, $J_{5'',6''}$ 10.5, H-5”), 4.22 (dd, 1H, $J_{9''a,9''b}$ 12.5, H-9”a), 4.20 (dd, 1H, $J_{2,3}$ 10.5, H-2), 4.15 (dd, 1H, $J_{4,5}$ 8.5, H-4), 4.14 (m, 2H, H-6’a, 6’b), 3.97 (dd, 1H, H-9”b), 3.77 (s, 3H, $COOCH_3$), 3.60 (m, 1H, $J_{5,6a}$ 2, $J_{5,6b}$ 6, H-5), 3.57 (dd, 1H, $J_{6a,6b}$ 10, H-6a), 3.22 (dd, 1H, H-6b), 2.60 (dd, 1H, $J_{3''e,3''a}$ 12.5, $J_{3''e,4''}$ 5, H-3”e), 2.35, 2.30 {2s, 6H, $N(COCH_3)_2$}, 1.61 (dd, 1H, $J_{3''a,4''}$ 12, H-3”a).
    ^{13}C NMR (CDCl3, 500MHz): 114.64 (CH_2=), 99.28 (C-1’), 98.08 (C-1), 97.26 (C-2”), 77.70 (C-3), 77.51 (C-4), 74.31 (C-5), 74.14 ($\underline{C}H_2Ph$), 71.83 (C-3’), 71.43 (C-2’), 70.36 (C-5’), 69.75 (C-6”), 68.45 (OCH_2), 67.70 (C-4’),* 67.64 (C-8”),* 67.01 (C-4”),$^{\neq}$ 66.89 (C-7”),$^{\neq}$ 62.88 (C-6), 61.95 (C-9”), 61.76 (C-6’), 56.20 (C-5”), 56.04 (C-2), 52.97 ($COO\underline{C}H_3$), 38.25 (C-3”), 28.03, 26.64 {$N(CO\underline{C}H_3)_2$}.
    MS (FAB): Found 1597.554644. Calc. 1597.557781. $C_{84}H_{90}N_2O_{28}Na$. *M.p.* 130-132°C (diethyl ether)
11. All new compounds gave statisfactory ^{1}H-NMR and high resolution Fab MS date.
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