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α-Selectivity and Glycal Formation are Temperature Dependent in Glycosylation with Sialic Acid. Synthesis of a Neu5Acα(2-6)Gal Thioglycoside Building Block

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Summary: The glycosylation with the N-acetylneuraminic acid (Neu5Ac) donor **1** of the 6-OH position of D-galactose in various nitriles promoted by methylsulfenyl triflate at different temperatures is reported. The proportion of α-sialoside increased and glycal **5** decreased at lower temperatures. A short route to a Neu5Acα(2-6)Gal thioglycoside building block is also described.

Sialic acid residues are found at non-reducing ends of glycoproteins and glycolipids, known to be involved in several biological phenomena. For further evaluation of the biological role and development of pharmacological relevant compounds, the development of efficient preparative methods for sialosides is motivated. Recently chemical and enzymatic synthesis of sialyloligosaccharides has been reviewed[2]. Regarding chemical synthesis, two main problems were identified, the control of stereoselectivity to α-glycoside and the elimination reaction which gives the glycal derivative instead of glycosides. To solve these problems several new methods[2] have been suggested employing thioglycosides as glycosyl donors or using a 3-substituent such as OH-, Br-, PhS-, and PhSe in sialosyl halide derivatives. The use of a 3-substituent gives highest α-selectivity of glycosides and prevents glycal formation, but lowers the total yield due to the extra steps required for introduction and removal operations of the 3-substituent.

From a preparative point of view it would be preferable to use a non-transformed Neu5Ac derivative and control the α-selectivity and the glycal formation with external factors such as solvents, co-solvents, the temperature and different promoters. These factors have so far not been fully investigated.

In the present study we report the effect of temperature on α-selectivity and on glycal formation, in glycosylation with a Neu5Ac glycosyl donor and a galactose 6-OH acceptor . Glycosyl donor **1**[3] was designed as an S-glycoside due to the arsenal of activators available[4] and specifically an S-glycosyl xanthate due to the promising results previously reported[5] with such glycosyl derivatives. Furthermore **1** is an O-benzoylated derivative which enhances the chromatographic properties of the donor and of the products. The glycosyl acceptor **2**[6] was chosen as the 6-OH galactose derivative. Nitriles were chosen as solvent due to the possibility of forming glycosyl nitrilium ions as intermediates[7], thereby influencing the proportions of the products formed. A mixture of glycosyl donor **1** (29 μmol), glycosyl acceptor **2** (35 μmol), powdered

molecular sieves (3 Å, 100 mg) and solvent (3 mL) was stirred at room temperature for 1 h, then silver triflate (41 μmol) was added, and the mixture was flushed with dry nitrogen, sealed and cooled. The reaction was carried out at four different temperatures (see Table). Methylsulfenyl bromide (MSB)[8] (35 μmol, 4.7 M) in 1,2-dichloroethane was then injected in two equal portions at an interval of 30 min, after 4 h triethylamine (0.1 mL) was injected. Stirring was continued for 30 min and the temperature was allowed to attain 0 - 20 °C. The reaction mixture was filtered through a layer of Celite, washed with aqueous 1M H_2SO_4, aqueous $NaHCO_3$ and water, dried, and concentrated. The relative proportions of compounds **3**[9], **4**[9] and **5**[9] were measured from the areas of selected peaks in a ^{13}C NMR spectra: ∂ 93.0 (C-1 of **3**), 93.5 (C-1 of **4**) and 108.3 (C-3 of **5**). The results are shown in the Table.

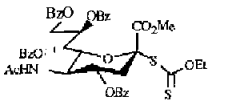
Table

		Relative proportions	
T (°C)	Solvent[a]	**3:4**	**(3+4) : 5**
20	A	0.6	0.2
0	A	1.0	0.7
-40	A	1.2	2.0
-70	B	8.7	1.0
-70	C	7.2	2.7
-70	D	5.7	2.0

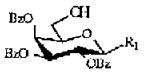
[a] A acetonitrile, B acetonitrile/dichloromethane 3:2, C propionitrile, D butyronitrile

As seen in the Table the α/β-ratio increases, and the ratio of sialylglycosides in relation to glycal formed is increased at lower temperatures. Thus both α–selectivity and glycal formation are dependent on the temperature under the conditions investigated here.

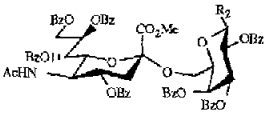
In a preparative experiment, where the glycosyl donor **1** was in excess, **1** (0.12 mmol), and acceptor **2** (0.1 mmol) were used and reacted in solvent B as above (except that MSB was injected in one portion) at -70 °C for 1 h (tlc showed that **1** was consumed). Diisopropylamine[10] was added, stirring was continued for 1 h, the temperature was allowed to attain -20 °C, and the products were isolated after filtration, concentration and silica gel chromatography. The yields were 47 % and 12 % of α-sialoside **3** and β-sialoside **4** respectively.



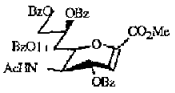
1



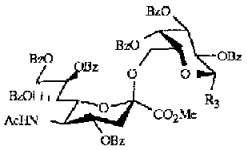
2 R_1= OBz **6** R_1= SEt



3 R_2= OBz **7** R_2= SEt



5



4 R_3= OBz **8** R_3= SEt

Furthermore a Neu5Acα(2-6)Gal building block would be useful for the synthesis of the corresponding sialyloligosaccharides. The use of thioglycosides as building blocks in oligosaccharide synthesis[4] is well established. Therefore the disaccharide **7** was chosen as a target. There are indications in the literature[5] that S-glycosyl xanthates are more reactive than thioethyl glycosides, and in addition the O-benzoylation of the thioethyl glycoside should also deactivate the thioethyl group for thiophilic attack of methylsulfenyl triflate. Therefore a direct condensation of S-glycosyl xanthate **1** with thioethyl glycoside acceptor **6**[11] was performed under the same conditions as for the preparative experiment of glycoside **3** described above. Indeed, the thioglycoside building block **7**[12] was obtained in 41 % yield. The β-glycoside **8**[12], glycal **5** were obtained in 18 % and 10 % yield respectively.

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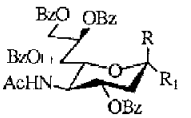
References and Notes

1. Present address HL: Symbicom AB, IDEON, S-223 70 Lund, Sweden.
2. K. Okamato, and T. Goto, *Tetrahedron*, **1990**,*46*, 5835-5857.
3. Preparation of **1**:



1 R = CO_2Me, R_1 = SC(=S)OEt **9** R = OBz, R_1 = CO_2Me **10** R = CO_2Me, R_1 = OBz

Methyl(5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranose (R. Kuhn, P. Lutz, and D. L. MacDonald, *Chem. Ber.*, **1966**, *99*, 611-617) was treated with benzoylchloride (6 equiv.)in anhydrous pyridine at -10 °C for24 h. Usual work up, and silica chromatography gave the corresponding β-benzoate **9**, in 71% yield,$[\alpha]_D$ +35° (c 0.5, chloroform); ^{13}C NMR ($CDCl_3$, 125 MHz) ∂ 23.2 ($NHCOCH_3$),36.6 (C-3), 50.6 (C 5), 53.1 (OCH_3), 62.6, 68.5, 68.5, 70.7, 72.0 (C-4,6-9), 97.8 (C-2), and 166.5 (C-1,$J_{C-1,H-3ax}$ 0.8 Hz, determined as described by: H. Hori, T. Nakajima, Y. Nishida, H. Ohrui, and H. Meguro, *Tetrahedron Lett.*, **1988**, *29(48)*, 6317-6320; M. F. Czarniecki, and E. R. Thornton, *J. Amer. Chem. Soc.*, **1977**, *99*, 8273-8279.). The α-benzoate **10** was also isolated in 11% yield.**9** was treated with $HBr_{(g)}$ in anhydrous dichloromethane at room temperature for 1h, concentration gave the corresponding β-bromide [^{13}C NMR ($CDCl_3$, 125 MHz) ∂ 42.0 (C-3), 92.0 (C-2), 166.0(C-1, $J_{C-1,H-3ax}$ 1.2 Hz)], which was directly treated with potassium ethylxanthogenate (1.2 equiv.) in anhydrous acetone for 3h at room temperature, followed by work up and silica chromatography to give **1** in 72 % yield, m.p. 201 °C (from dichloromethane/cyclohexane), $[a]_D$ +131° (c 0.5, chloroform); ^{13}C NMR ($CDCl_3$, 125 MHz) ∂ 13.3(OCH_2CH_3), 23.2 ($NHCOCH_3$), 37.4 (C-3), 49.9 (C-5), 53.3 (OCH_3), 62.9, 69.2, 69.3, 70.6, 71.5, 75.6 (C-4,6-9), 87.0 (C-2), 168.9 (C-1, $J_{C-1,H-3ax}$ 7.6 Hz), 207.4 (S-C=S)
4. P. Fügedi, H. Lönn, P. J. Garegg, and T. Norberg, *Glycoconjugate J.*, **1987**, *4*, 97-108.
5. A. Marra, and P. Sinaÿ, *Carbohydr. Res.*, **1990**, *195*, 303-308.
6. P. Kovac, C. P. J Glaudemans, W. Guo, and T. C. Wong, *Carbohydr. Res.*, **1985**, *140*, 299-311.
7. A. J. Ratcliffe, and B. Fraser-Reid, *J. Chem. Soc. Perkin Trans. 1*, **1990**, 747-750, and references therein; R. R. Schmidt, M. Behrendt, and A Toepfer, *Synlett*, **1990**, 694-696.
8. F. Dasgupta, and P. J. Garegg, *Carbohydr. Res.*, **1988**, *177*, C13-C17. Reaction of methylsulfenyl bromide with silver triflate gives methylsulfenyl triflate. This promoter was chosen due to its high reactivity, which is needed at low temperatures(-70 °C).

9. Analytical data for **3**: $[\alpha]_D$ +51° (c 0.5, chloroform); ^{13}C NMR ($CDCl_3$, 125 MHz) ∂ 23.2 ($NHCOCH_3$), 38.4 (C-3'), 50.4 (C-5') 52.5 (OCH_3), 62.8, 63.2(C-6,9'), 67.8, 68.4, 69.0, 69.0, 69.6, 71.7, 72.7, 72.8 (C-2-5,4',6'-8'), 93.0 (C-1), 99.9 (C-2'), 167.5 (C-1', $J_{C\text{-}1',H\text{-}3'ax}$ 5.9 Hz).
Analytical data for **4**: $[\alpha]_D$ +111° (c 0.5, chloroform); ^{13}C NMR ($CDCl_3$, 125 MHz) ∂ 23.1 ($NHCOCH_3$), 37.3
(C-3'), 48.5 (C-5') 52.9 (OCH_3), 62.9, 63.7 (C-6,9'), 68.4, 69.0, 69.8, 70.5, 71.3, 72.4, 73.0, 73.0 (C-2-5,4',6'-8') 93.5 (C-1) 99.9 (C-1'), 167.3 (C-1', $J_{C\text{-}1',H\text{-}3'ax}$ <1 Hz).
Analytical data for **5**: $[\alpha]_D$ +148° (c 0.5, chloroform); ^{13}C NMR ($CDCl_3$, 125 MHz) ∂ 23.2 ($NHCOCH_3$), 48.3 (C-5), 52.5 (OCH_3), 62.6 (C-9), 68.5, 68.9, 71.3, 76.4 (C-4,6-8) 108.3 (C-3), 145.5 (C-2), 161.7 (C-1).

10. The use of diisopropylamine instead of triethylamine suppress the formation of MeS-NAc derivatives originated from **3**, **4** and **5**. The MeS-NAc derivatives were tentatively assigned from their FAB-MS and ^{13}C NMR of isolated fractions from silica gel chromatography. S-Methylated acetamido groups have been observed before in hexopyranosides in glycosylation reactions, using DMTST [dimethyl(methylthio) sulfonium trifluoromethanesulfonate] as promoter (A. K. Tidén, *Chem. Commun. Stockholm. Univ.*, **1991**, *3*.) and thioglycosides as glycosyl donors.

11. **6** was prepared from the known ethyl 1-thio-3-D-galactoside (J. Fried, and D.E. Walz, *J. Am. Chem. Soc.*, **1949**, *71*, 140-143; R.J. Ferrier, and R.H. Furneaux, *Carbohydr. Res.*, **1976**, *52*, 63-68) analogously to **2**, see reference 6. Analytical data for **6**: $[\alpha]_D$ +194° (c 0.5, chloroform); ^{13}C NMR ($CDCl_3$, 125 MHz) ∂ 14.9 (SCH_2CH_3), 24.2(SCH_2CH_3), 60.6 (C-6), 68.3, 69.2, 72.8 (C-2-4), 77.9 (C-5), 84.1 (C-1).

12. Analytical data for **7**: $[\alpha]_D$ +65° (c 0.5, chloroform); ^{13}C NMR ($CDCl_3$, 125 MHz) ∂ 14.9 (SCH_2CH_3), 23.2 ($NHCOCH_3$), 24.4 (SCH_2CH_3), 38.4 (C-3'), 50.2 (C-5') 52.5 (OCH_3), 63.4, 63.7 (C 6,9'), 68.2, 68.4, 68.6, 69.0, 69.5, 72.8, 73.0, 75.3 (C-2-5,4',6'-8'), 84.0 (C-1), 100.2 (C-2'), 167.7 (C-1', $J_{C\text{-}1',H\text{-}3'ax}$ 7.6 Hz).
Analytical data for **8**: $[\alpha]_D$ +131° (c 0.2, chloroform); ^{13}C NMR ($CDCl_3$, 125 MHz) ∂ 14.7 (SCH_2CH_3), 23.0 ($NHCOCH_3$), 23.8 (SCH_2CH_3) 36.7 (C-3'), 48.5 (C-5') 52.8 (OCH_3), 61.6, 63.5 (C-6,9'), 68.2, 69.0, 69.7, 69.7, 71.9, 72.4, 72.5, 75.3 (C-2-5,4',6'-8'), 83.8 (C-1), 98.8 (C-2'), 167.2 (C-1', $J_{C\text{-}1',H\text{-}3'ax}$ 1.5 Hz).

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