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Stereoselectivity Control in Anomeric *O*-alkylation. Application to the Synthesis of C_2 Symmetric Glycoconjugates.

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Abstract : Tetrabutylammonium salts strongly influence the stereoselectivity of *O*-anomeric alkylation and allows to shift from β to α sclectivity. Allyl glucosaminide **7** prepared in this way, was used to synthesize the new type of C_2 symmetric neoglycoconjugates **1a-c**. © 1997 Elsevier Science Ltd.

The glycosaminoglycans (GAGs), linear sulfated polymers of hexuronic acids and 2-amino sugars, are essential components of connective tissues and are also present at the cell surface. Their ability to bind and regulate the activity of various proteins, has been established.[1-5] Fragments of heparan sulfate/heparin (HS/HP), able to stabilize and enhance the activity of interferon γ (IFNγ), have been isolated. They are composed of two IFNγ high affinity binding sites (domain A and C, Fig. 1a), linked together by a larger domain (domain B, Fig. 1a) and thought to act by promoting the homodimerization of IFN γ.[5] GAGs neoconjugates, containing two HS/HP di- to octa-saccharides α linked to a polyethyleneglycol (PEG) spacer (Fig. 1b,c) would be useful tools to study GAGs/IFN γ interactions. We first focused on the preparation of α allyl glucosaminide **5a** α. Fischer glycosylation giving low yields,[6] we turned to the anomeric *O*-alkylation method developed by Schmidt and coworkers[7] and found that the stereochemistry of the anomeric *O*-alkylation may be controlled by the presence or absence of tetrabutylammonium salts (Scheme 1). The α allyl glucosaminide **7**, prepared in this way, was then used to synthesize compounds **1a-c**, designed to mimic HS/HP fragments as candidates for complexation of the C_2 symmetric IFNγ active dimer.[8] A similar GAGs neoconjugate approach has been recently used by van Boeckel *et al* to prepare selective factor Xa or thrombin inhibitors.[4]

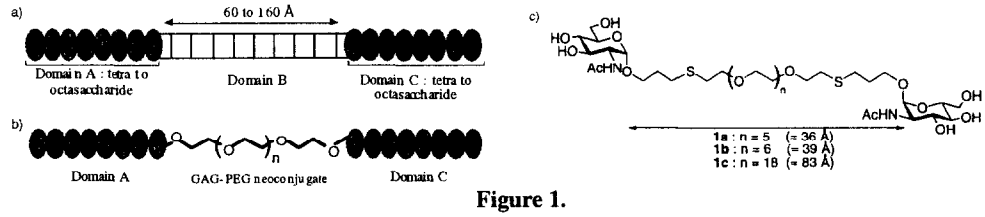

Figure 1.

Anomeric *O*-alkylation involves the generation of an anomeric alkoxide and its subsequent reaction with an electrophile (Scheme 1). This reaction generally gives high yields of glycosides, but the control of its stereochemical outcome remains problematic.[7] We have previously shown that the addition of 3,4,6-tri-*O*-

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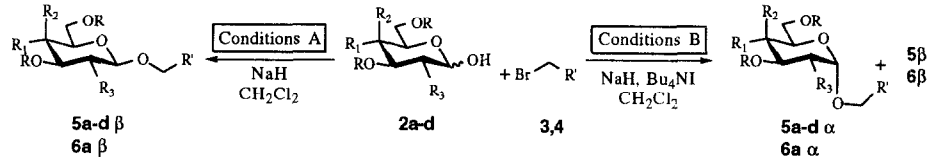
acetyl-*N*-acetyl-glucosamine **2a** as its sodium alkoxide onto tosylacrolein led, in THF, to the pure α glycoside.[9] In the same conditions, the reaction of **2a** alkoxide with allyl bromide **3** gave a mixture of α and β glycosides. We turned to methylene chloride as solvent and obtained the pure β glycoside in good isolated yields (table 1). In order to increase the alkoxide reactivity, we added tetrabutylammonium iodide to the reaction mixture, and obtained the α glycoside as the major product with an α/β ratio up to 98:2 (Scheme 1).

Scheme 1 :



5a-d β **6a β** **2a-d** **3,4** **5a-d α** **6a α**

3,5 : R' = $CH=CH_2$ **4,6** : R' = Ph

2a, 5a, 6a : R_1 = OAc, R_2 = H, R_3 = NHAc, R = Ac
2b, 5b : R_1 = OAc, R_2 = H, R_3 =OAc, R = Ac

2c, 5c : R_1 = H, R_2 =OAc, R_3 = OAc, R = Ac
2d, 5d : R_1 = OBn, R_2 = H, R_3 =OBn, R = Bn

The reaction conditions[10] were optimized for the acetylated glucosamine derivative **2a** and then applied to the other sugars **2b-d** and another electrophile, benzyl bromide **4**. When the reactions were carried out in methylene chloride with NaH as a base, the β glycosides **5a-c** β were obtained as the sole products in good isolated yields (conditions A, Table 1 and 2), excepted for the benzylated derivative **2d** which gave an α/β ratio of 7/93. When 2 equivalents tetrabutyl ammonium salts were added (conditions B), the α glycosides **5a-d** α were obtained as the major products in high yields, excepted for **2d** for which the α ratio was only raised from 7 to 38% (Table 1 and 2).

Table 1:

Reaction of sugars **2a-d** with allyl bromide **3**.

Sugar	Conditions	Eq. NaH	Time (h)	Isolated yield (%)	α:β[a]
2a	A	1.5	16	65-70	0:100
2a	B	1.5	16	87-90	98:2
2b	A	1.5	36	42-50[b]	0:100
2b	B	1.5	16	84-87	60:40
2c	A	1.5	36	86-84	0:100
2c	B	1.5	16	80-95	67:33
2d	A	4.5	36	84-86	7:93
2d	B	4.5	36	95-100	38:62

a) α:β ratio are based on isolated product for **5a** and determined by ^1H and ^{13}C for **5b-d**. b) This lower yield is du to acetyl migration.

Table 2 : Reaction of glucosaminyl derivative **2a** with various additives, electrophiles and solvents.

Electrophile	Conditions [a]	Isolated yield (%)	α:β[a]
3	A	65-70	0:100
3	B	87-90	98:2
4	A	84-88	0:100
4	B	88-90	86:14
3	B (THF)	81	55:45
3	B ($PhCH_3/CH_2Cl_2$)	79	78:22
4	B ($PhCH_3/CH_2Cl_2$)	86	86:14
3	B (10 mol% 18C6)	81	82:18

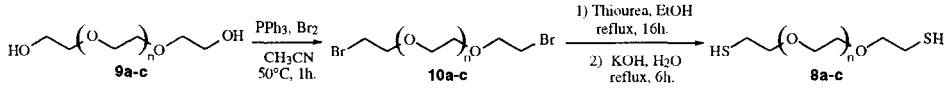
a)Solvent or additive specified when different from note 10. b) α:β ratio are based on isolated product for all compounds.

The origin of this effect does not take place in a bromine/iodine exchange in the electrophile since the use of allyl iodide, using condition A, lead to the β glucoside **5a** β, while tetrabutylammonium bromide gave similar results as iodide when using conditions B (data not shown). As already outlined by Schmidt *et al*,[11] a more polar solvent and lower reaction temperature enhance the α selectivity, but when we used conditions B with THF as solvent the α:β ratio dropped to 55:45. On the other hand, running the reaction for **2a** in a 1/1 CH_2Cl_2/toluene mixture did not improve the diastereoisomeric ratio, nor the use of crown ether to complex sodium ions (Table 2). We noticed that the reaction of **2d** needed three times more NaH than the other sugars

to go to completion in 36 hours (Table 1), suggesting that the limiting step in these reaction conditions must be the deprotonation of the anomeric hydroxyles by NaH. According to the mechanism of *O*-anomeric alkylation detailed by Schmidt, the β selectivity observed in CH_2Cl_2 may be explained by a rapid equilibrium between the α and β alkoxides and a higher reactivity of the β anion.[7a,b,11] The addition of Bu_4NI promotes the formation of tetrabutylammonium alkoxides which are more reactive than their sodium counterparts. The alkylation reaction may thus becomes more rapid than the alkoxides anomerization. The product ratio should then reflect the deprotonation kinetics and/or the sugar α/β equilibrium ratio.

Having in hand a method to prepare α allyl glucosaminide **5a** α, the glycoconjugates **1a-c** were synthesized using a radical coupling[7,12] between the free allyl glycoside **7**, prepared quantitatively from **5a** α by Zemplen deacetylation, and the various α,ω-bis-thio-PEGs **8a-c**. These functionnalized PEGs were prepared from commercials PEG 300 and PEG 900 polydisperses mixtures by a glycol --> dibromide --> dithiol sequence (Scheme 3) described for shorter PEGs (n<5).[13] At the dibromo-PEG **10a-c** stage, we were pleased to find that the polydisperses mixtures, obtained in 63 % to quantitative yields, could be resolved by flash chromatography to homogeneity for the PEG 300 products and enriched in fraction n=18 for PEG 900.[14] For example, homogeneous dibromo-PEG **10a** (n=5) and **10b** (n=6) were isolated in respective 22 and 13% yields. The dibromo compounds **10a-c** were then treated with thiourea to give the corresponding thiouronium salts, which were hydrolyzed by aqueous potassium hydroxide.[13c] After extractive workup and silica gel filtration, the α,ω-dithio-PEGs **8a-c** were isolated in 74-79% yields.

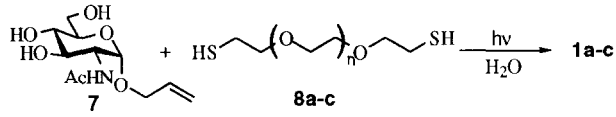
Scheme 2 :



8a, 9a, 10a : n = 5 ; **8b, 9b, 10b** : n = 6 ; **8c, 9c, 10c** : n = 18

Radical couplings between the allyl glucosaminide **7** and the α,ω-dithio-PEG **8a-c**, were carried in a quartz cuve under high pressure mercury lamp irradiation. We used 0.2 M solutions of α,ω-dithiol in water and 1.5 equivalent of allyl glycoside per thiol group. After C18 reverse phase flash chromatography the glycoconjugates **1a-c** were isolated in 74 to 85% yields.[15]

Scheme 3 :



We have thus developed a methodology, by preparing the new neoglycoconjugates **1a-c,** that will be further applied to larger oligosaccharides. The GAGs neoconjugates prepared in this way will be used as tools in GAG/cytokine interaction studies and are good candidates as cytokine activity modulators. In the course of this study, we have found that the stereochemistry of anomeric *O*-alkylation could be tuned from β to α selectivity by adding tetrabutylammonium salts. This finding extends the usefulness of anomeric *O*-alkylation as a method of glycosylation.

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10. **Conditions A.** A solution of 347 mg **2a** (1 mmol) in 1 mL CH_2Cl_2 was added to 60 mg NaH (1.5 mmol) in 860 µL allyl bromide **3** (10 mmol), or 1.2 mL benzyl bromide **4** (10 mmol) at -20°C. It should be noted that benzyl bromide is frozen at this temperature but redissolved after the addition of the CH_2Cl_2 solution. The temperature was raised to 20°C over 4 hours, and the mixture was kept at this temperature for additional 12 hours. The reaction was quenched by 30 µL of acetic acid, and directly purified by flash chromatography (tol./acet. 90/10-75/25 gradient). **Conditions B.** A solution of 2.50 g **2a** (7.2 mmol) in 14 mL CH_2Cl_2 was added dropwise to 430 mg NaH (10.8 mmol) and 5.32 g Bu_4NI (14.4 mmol) in 12 mL allyl bromide **3** (144 mmol) at -20°C. The reaction was continued as described in condition A. Other glycosides were obtained using the same procedure. For **2d**, which is only slightly soluble in CH_2Cl_2, NaH was added to a suspension of **2d** in CH_2Cl_2.
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14. Flash chromatography were performed on 16-40µ silica gel, using a 8/1/1 to 0/9/1 hexane/AcOEt/CH_2Cl_2 gradient for reaction with PEG 300 and 10/0 to 8/2 CH_2Cl_2/MeOH gradient for reactions with PEG 900.
15. When recovered **7** is also taken in account, yields based on coupled and recovered **7** were almost quantitative (86-93%). This later point is important since this methodology will be used to prepare neoglycoconjugates with more elaborated oligosaccharides. Selected data for : **1a.** ^{1}H NMR (D_2O) δ: 4.87 (d, 2H, *J* = 3.8 Hz), 3.90 (dd, 2H, *J* = 10.5, 3.8 Hz), 3.87 (dd, 2H, *J* = 12.0, 2.0), 3.85-3.68 (m, 32H), 3.55 (dt, 2H, *J* = 10.0, 6.0 Hz), 3.48 (dd, 2H, *J* = 10.0, 9.0 Hz), 2.79 (t, 4H, *J* = 6.5 Hz), 2.71 (t, 2H, *J* = 7.0 Hz), 2.05 (s, 6H), 1.90 (quintuplet, 4H, *J* = 6.5 Hz). From selective 1D-TOCSY:[16] δ: 3.81 (dd, *J* = 10.0, 6.0 Hz), 3.79 (dd, *J* = 12.0, 4.5 Hz), 3.73 (t, *J* = 6.5 Hz) 3.75 (dd, *J* = 10.5, 9.0 Hz), 3.71-3.68 (m). ^{13}C NMR (D_2O) δ: 174.5, 97.1, 72.1, 71.3, 70.2-69.6, 66.6, 60.8, 54.0, 30.8, 28.8, 28.5, 22.1. $[\alpha]_D^{30}$ = +97 (*c* 2.03, H_2O). Anal. Calcd. for $C_{36}H_{68}O_{18}N_2S_2$: C, 49.07; H, 7.78; N, 3.18; O, 32.70. Found: C, 48.85; H, 7.78; N, 2.99; O, 32.99. **1b.** ^{1}H NMR (D_2O) δ: 4.87 (d, 2H, *J* = 3.5 Hz), 3.90 (dd, 2H, *J* = 10.5, 3.5 Hz), 3.86-3.64 (m, 38H), 3.55 (dt, 2H, *J* = 10.0, 6.0 Hz), 3.48 (t, 2H, *J* = 9.0 Hz), 2.79 (t, 4H, *J* = 6.5 Hz), 2.71 (t, 2H, *J* = 7.0 Hz), 2.05 (s, 6H), 1.90 (quintuplet, 4H, *J* = 6.5 Hz). ^{13}C NMR (D_2O) δ: 174.5, 97.1, 72.1, 71.2, 70.2-69.6, 66.5, 60.7, 54.0, 30.8, 28.7, 28.4, 22.1. $[\alpha]_D^{30}$ = +91 (*c* 1.63, H_2O). Anal. Calcd. for $C_{38}H_{72}O_{19}N_2S_2$: C, 49.34; H, 7.84; N, 3.03. Found: C, 48.91; H, 7.67; N, 2.81. **1c.** ^{1}H NMR (D_2O) δ: 4.87 (d, 2H, *J* = 3.5 Hz), 3.90 (dd, 2H, *J* = 10.0, 3.5 Hz), 3.86-3.64 (m, 86H), 3.55 (dt, 2H, *J* = 10.0, 6.0 Hz), 3.48 (dd, 2H, *J* = 8.8, 9.8 Hz), 2.79 (t, 4H, *J* = 6.5 Hz), 2.71 (t, 2H, *J* = 7.0 Hz), 2.05 (s, 6H), 1.91 (quintuplet, 4H, *J* = 6.5 Hz).^{13}C NMR (D_2O) δ: 174.6, 97.2, 72.1, 71.2, 70.2-69.7, 66.6, 60.8, 54.0, 30.8, 28.8, 28.4, 22.2. $[\alpha]_D^{30}$ = +57 (*c* 2.13, H_2O). Anal. Calcd. for $C_{62}H_{120}O_{31}N_2S_2$ H_2O: C, 50.60; H, 8.36; N, 1.90. Found: C, 50.49; H, 8.23; N, 1.98.
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