Syntheses of α-Linked Derivatives of *N*-Acetyl Glucosamine and Gal-β(1-3)GalNAc (T Antigen) directly with the Natural *N*-Acetyl Protecting Group

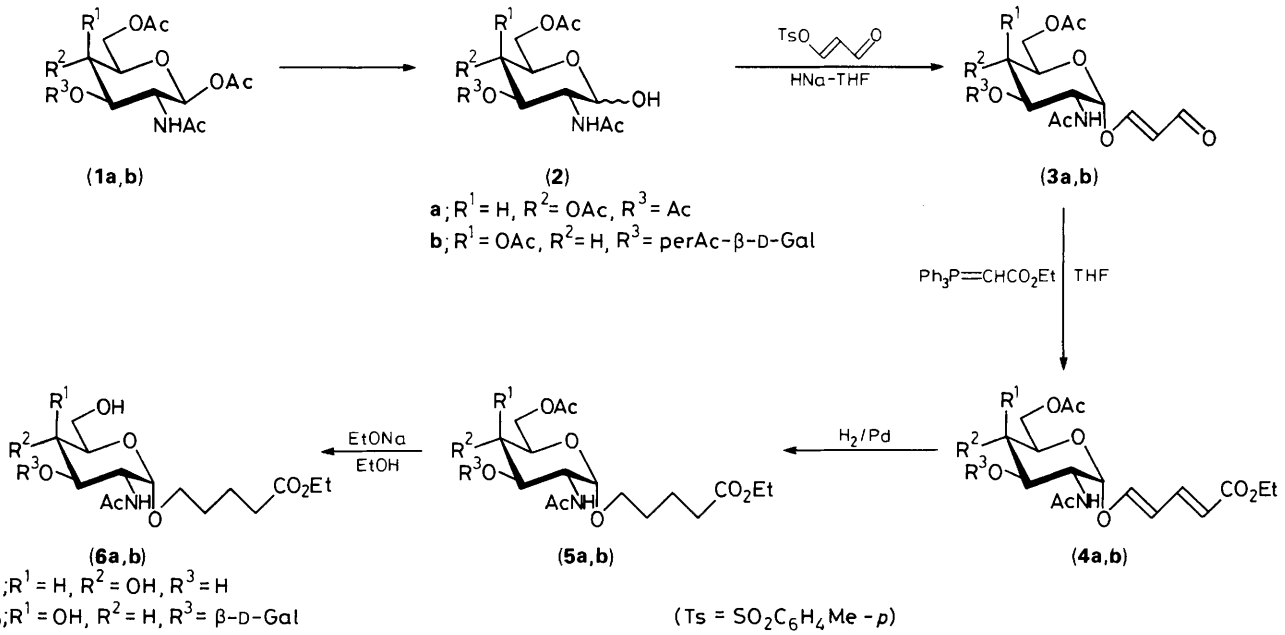
André Lubineau,* Hugues Bienaymé, and Joëlle Le Gallic

Laboratoire de Chimie Organique Multifonctionnelle (URA CNRS 0462), Université Paris-sud, 91405 Orsay Cedex, France

α-Linked spacer arms, suitable for coupling on a protein carrier, have been introduced directly on GlcNAc and on Gal-β(1-3)GalNAc in a 1,2-*cis* fashion through a new procedure involving a completely stereoselective Michael addition of the anomeric sodium alcoholate onto β-tosyloxy acrolein prepared *in situ* from the sodium salt of malonaldehyde and tosyl chloride.

In *N*-glycoproteins, the anchoring point of the carbohydrate chain on the protein backbone is the disaccharide Gal-β-(1-3)GalNac (the T antigen), which is covalently linked to serine or threonine through an α-galactosaminyl linkage. The finding that this structure occurs in tumour-associated antigen has caused a resurgence of interest in its investigation.[1] Synthetic antigens related to this disaccharide require conjugation with a biologically appropriate carrier through a spacer arm α-linked to the terminal *N*-acetyl galactosamine unit, for immunological application. However, the direct introduction of a linker on 2-acetamido sugars in a 1,2-*cis* fashion was said up to now to be impossible, and for instance, α-linked galactosamines are presently synthesized through azidochlorination of galactal followed by glycosidation which bypass the presence of the 2-acetamido participating group of *N*-acetyl galactosamine.[2,3] We describe in this communication a new methodology which allows such synthesis and which could possibly be used with oligosaccharides obtained from natural sources, which already possess the *N*-acetyl group, which cannot be removed for further elaboration.

Starting from 1,3,4,6-tetra-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranose (**1a**),[4] 1-*O*-deacetylation by hydrazine acetate[5] in *N*,*N*-dimethylformamide (DMF) (2 h, 20 °C, 60%) or, better, by transesterification using a lipase[6] from *Aspergillus niger* (AP6 from Amano, AcOEt–Pr^{i}OH–H_2O, 65 : 20 : 1, 93%) gave (**2a**) as a mixture of anomers. Michael addition of the sodium salt derived from (**2a**) [HNa, tetrahydrofuran (THF)] onto β-tosyloxy acrolein[7] (2 equiv.) prepared *in situ* from the sodium salt of malonaldehyde and tosyl chloride in THF, afforded the pure α-anomer (**3a**)† in 83% yield with complete stereoselectivity: no trace of the β-anomer was detected in the reaction conducted at room temp. for 1 h in the presence of a small amount of 18-crown-6 (1.0 mol %). The anomeric configuration was ascertained using ^{1}H NMR spectroscopy where H-1 exhibits a typical small coupling constant (3.5 Hz). We must emphasize at this stage the exceptional behaviour of 2-acetamido sugars in this type of reaction, as we have shown[8] that the sodium salt derived from 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose gave a mixture of α- and β-anomers (in a 2 : 1 ratio). Anomeric alcoholates have already been used for glycoside synthesis in reactions with triflate or trichloroacetonitrile to give, in the latter case, trichloroacetimidates which are precursors for 1,2-*trans*-glycoside synthesis in the case of the participating 2-*O*-acetyl group.[9] However, the method has not received any application in the case of sugars having a 2-acetamido protecting group because the corresponding trichloroacetimidate led to nonreactive intermediates such as oxazolines during activation



Scheme 1

under the usual acid catalysis.[10] Then, the three following reactions were conducted as usual. Wittig alkenation of the unsaturated aldehyde (**3a**) with ethoxycarbonyl methylenephosphorane (1.4 equiv.) in THF afforded the diene (**4a**)† in 86% yield as a single *E,E*-isomer. Catalytic reduction (H_2/Pd, AcOEt) gave (**5a**)† in a quantitative yield. Finally, Zemplen deacetylation (EtONa/EtOH) afforded (**6a**)† in 92% yield. The sugar was then linked to a protein carrier (BSA) *via* the acyl azide method[11] to give an artifical antigen bearing twenty one glucosamine units per mole of protein [as determined by the modified Morgan–Elson reaction[12] following acid hydrolysis of the synthetic glycoprotein in hydrochloric acid (4 M) for 4 h at 100 °C].

Armed with these positive results, the derivatization of the disaccharide Gal-β(1-3)GalNAc was next explored. Hydrazinolysis of (**1b**)[13] (α/β, 1 : 1) in the same conditions as for (**1a**) led to (**2b**) in 74% yield as a mixture of anomers. Compound (**2b**), which was not further characterized, was used directly for the Michael addition of its sodium salt (NaH in THF) onto the β-tosyloxy acrolein (1.5 equiv.) in the presence of a small amount of 18-crown-6 (1.0 mol %), to give (**3b**)† in 75% yield. Once again, only the α-anomer was obtained in the reaction. Wittig alkenation gave (**4b**) in 81% yield as the pure *E,E*-diastereoisomer. Finally, catalytic reduction (H_2/Pd, AcOEt, 100%) followed by the complete deprotection through the Zemplen procedure, afforded in quantitative yield the free disaccharide (**6b**)† having a linking arm with the desired α-configuration. Further extensions of this stereoselective Michael addition are currently under investigations.

We thank the C.N.R.S. and the University of Paris-Sud for financial support.

Received, 28th July 1989; Com. 9/03204E

† Satisfactory spectroscopic and analytical data were obtained for all new compounds. Physical and selected ^{1}H NMR data are given below. ^{1}H NMR spectra were recorded at 250 MHz in $CDCl_3$ [CD_3OD for (**6a**) and D_2O for (**6b**)] and were referenced with tetramethylsilane. (**3a**), m.p. 133 °C (AcOEt–hexane); $[\alpha]_D$ +177° (*c* 0.88, CH_2Cl_2) δ_H 5.46 (d, 1H, *J* 3.5 Hz, H-1), 5.92 (dd, 1H, *J* 8, 13 Hz, CH=C*H*CHO), 7.36 (d, 1H, *J* 13 Hz, C*H*=CHCHO), 9.44 (d, 1H, *J* 8 Hz, C*H*O). (**4a**), m.p. 157 °C (AcOEt–hexane); $[\alpha]_D$ +179° (*c* 0.96, CH_2Cl_2); δ_H 5.29 (d, 1H, *J* 3.5 Hz, H-1), 5.82 (d, 1H, *J* 15 Hz, CH=C*H*CO_2Et), 6.01 (t, 1H, *J* 12 Hz, OCH=C*H*), 6.81 (d, 1H, *J* 12 Hz, OC*H*=CH), 7.21 (dd, 1H, *J* 12, 15 Hz, C*H*=CHCO_2Et). (**5a**), m.p. 100 °C (AcOEt–Et_2O–hexane); $[\alpha]_D$ +87° (*c* 1, CH_2Cl_2); δ_H 4.83 (d, 1H, *J* 3.5 Hz, H-1). (**6a**) lyophilized white powder, $[\alpha]_D$ +118° (*c* 0.7, H_2O); δ_H 1.24 (t, 3H, *J* 7 Hz, OCH_2CH_3), 1.99 (s, 1H, $NHCOCH_3$), 2.35 (t, 2H, *J* 7 Hz, CH_2CO_2Et), 4.12 (q, 2H, *J* 7 Hz, OCH_2Me), 4.77 (d, 1H, *J* 3.5 Hz, H-1); (**3b**), colourless syrup, $[\alpha]_D$ +114° (*c* 1, CH_2Cl_2); δ_H 4.73 (d, 1H, *J* 8 Hz, H-1′). 5.63 (d, 1H, *J* 3.5 Hz, H-1), 5.85 (dd, 1H, *J* 8, 12 Hz, CH=C*H*CHO), 6.20 (d, 1H, *J* 8 Hz, NH), 7.40 (d, 1H, *J* 12 Hz, OC*H*=CHCHO), 9.44 (d, 1H, *J* 8 Hz, C*H*O). (**4b**), colourless syrup, δ_H 4.65 (d, 1H, *J* 8 Hz, H-1′), 5.40 (d, 1H, *J* 3.5 Hz, H-1), 5.77 (d, 1H, *J* 15 Hz, CH=C*H*CO_2Et), 5.93 (t, 1H, *J* 12 Hz, OCH=C*H*), 6.82 (d, 1H, *J* 12 Hz, OC*H*=CH), 7.20 (dd, 1H, *J* 12, 15 Hz, C*H*=CHCO_2Et). (**5b**), colourless syrup, $[\alpha]_D$ +53° (*c* 1, CH_2Cl_2); δ_H 4.65 (d, 1H, *J* 8 Hz, H-1′), 4.85 (d, 1H, *J* 3.5 Hz, H-1). (**6b**), m.p. 188 °C (EtOH–AcOEt); $[\alpha]_D$ +110° (*c*, 1, D_2O), δ_H 1.26 (t, 3H, *J* 7 Hz, OCH_2CH_3), 2.04 (s, 3H, $NHCOCH_3$), 2.43 (t, 2H, *J* 7 Hz, CH_2CO_2Et), 4.17 (q, 2H, *J* 7 Hz, CO_2CH_2Me), 4.48 (d, 1H, *J* 7.5 Hz, H-1′), 4.89 (d, 1H, *J* 3.5 Hz, H-1).

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