Synthetic Mucin Fragments: 2-(p-Trifluoroacetamidophenyl)ethyl 2-Acetamido-6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-3-O-(β-D-galactopyranosyl)-α-D-galactopyranoside and 2-(p-Trifluoroacetamidophenyl)ethyl 2-Acetamido-6-O-[2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-2-deoxy-3-O-(β-D-galactopyranosyl)-α-D-galactopyranoside

PER J GAREGG¹, MARTIN HARALDSSON¹, HANS LöNN² and THOMAS NORBERG²

¹Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden
²Organic Synthesis Department, BioCarb AB, S-223 70 Lund, Sweden

Received March 10, 1987.

Key words: oligosaccharide, synthesis, mucin

The title compounds were synthesized from methyl 2-azido-3-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-2-deoxy-1-thio-β-D-galactopyranoside, which was β-glycosylated in the 6-position with glucosamine or lactosamine derivatives.

The tri- and tetrasaccharide thioglycosides obtained were then converted to the 2-(p-trifluoroacetamidophenyl)ethyl α-glycosides and deprotected.

In glycoproteins such as the mucins, the majority of the oligosaccharide chains are O-glycosidically linked through a 2-acetamido-2-deoxy-galactosyl residue to serine or threonine in the peptide backbone. The structures of the carbohydrate moieties of various glycoproteins have been well documented [1, 2]. In mucins the O-linked oligosaccharides are derived by galactose, N-acetylgalactosamine, N-acetylneuraminic acid or larger oligosaccharide substitution of a basic β-D-Galp-(1-3)-α-D-GalNAc-p unit. This gives a series of structures, a number of these having the core elements depicted in Fig. 1.

In a research programme aimed at developing monoclonal antibodies which recognize mucin structures, representative synthetic oligosaccharides were needed. We now report syntheses of the trisaccharide 5 and the tetrasaccharide 10 in a form suitable for
attachment to proteins to form artificial antigens. The 2-(p-trifluoroacetamidophenyl)-ethyl linking arm is analogous to the 2-(p-nitrophenyl)ethyl group previously used by Schuerch et al. [3]. The synthesis, by a different route to ours, of the benzyl α-glycoside analogue of 5 has previously been reported [4]. In the present work, the strategy outlined [5-7] for block synthesis using thioglycoside intermediates was followed. Starting from the disaccharide thioglycoside 1, tri- (2) and tetrasaccharide (6) thioglycosides were assembled. These were then converted into glycosides of 2-(p-trifluoroacetamidophenyl)ethanol and deprotected.

**Results and Discussion**

Treatment of methyl 2-azido-3-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-4,6-O-benzylidene-2-deoxy-1-thio-β-D-galactopyranoside [8] with aqueous acetic acid gave the diol 1 in 91% yield. This compound was treated with 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyranosyl)-2,1-d-2-oxazoline [9] and anhydrous p-toluenesulfonic acid at 75°C to give the trisaccharide 2 in 70% yield. That glycosylation had occurred at the 6-position of the GalN₃ residue was indicated by the 13C-NMR spectrum of 2. Only two methylene signals (for Gal-6 and GlcNAc-6) were found below 65 p.p.m., i.e. GalN₃-6 had been shifted downfield from its position in 1, indicative [10] of alkyl or glycosyl substitution.

The trisaccharide 2 was treated with bromine in dichloromethane to give the corresponding bromide, which was directly used in a halide ion-promoted glycosidation of 2-(p-trifluoroacetamidophenyl)ethanol to give 3 in 82% yield. Treatment with hydrogen sulfide in pyridine/triethylamine converted the azido group of 3 to an amino group, and subsequent acetylation with acetic anhydride gave compound 4 (80% yield),