C-Glycosides

Synthesis of a C-Glycoside Analogue of sTn: An HIV- and Tumor-Associated Antigen**

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N-Acetylneuraminic acid occupies the nonreducing end of many naturally occurring glycoconjugates on the cell surface, including glycoproteins and glycolipids. The strategic location of glycoconjugates on the cell surface and the enormous structural information that they carry account for their major role in biological recognition in the control of both normal and pathological processes.[1] While the participation of sialic acid in these biological events has been established unequivocally in a large number of studies,[1] the linkage of sialic acid to glycoconjugates is one of the most labile glycosidic linkages, which renders these compounds less amenable to biological and biochemical studies.[2] Terminal sialic acid residues are easily cleaved in vitro under very mild acidic conditions and in vivo by neuraminidases.[3] The biological importance of sialic acid coupled with its lability towards hydrolysis prompted us to redesign the molecular architecture of glycoconjugates to afford a non-hydrolyzable glycosidic linkage to sialic acid. Such a stable linkage should contribute to our understanding of biological recognition and serve to enhance or suppress biological events at the molecular level. The replacement of the interglycosidic oxygen atom with a hydroxymethylene or methylene group affords a new class of hydrolytically stable C-glycoside analogues of glycoconjugates.[4] A method for the preparation of C-glycosides of ulosonic acids using SmI_2 was pioneered in our laboratory.[5]

The sialyl-Tn epitope [α-D-Neu5Ac-(2→6)α-D-GalNAc-(1→*O*)-Ser/Thr] (sTn, **1a**, Figure 1) is found on the HIV envelope glycoprotein gp120[6] and in tumor-associated antigens present in the glycoproteins on the surface of cancer cells, including those associated with carcinomas of the breast, prostate, pancreas, colon, ovary, lung, and stomach.[7] Synthetic vaccines based on the sTn epitope (O-glycoside) have been recently prepared[8] and shown to stimulate an immune response.[9]

Kishi and co-workers have demonstrated that C-glycosides and O-glycosides have similar conformational characteristics both in the free and bound states.[10] There are, nevertheless, differences in the population distribution of conformers adopted by C-glycosides and their natural counterparts.[11] However, the small energy differences among the various conformers allow them to interchange without any major energy conflicts, thus rendering the C-glycosides ideally suited as O-glycoside isosters for therapeutic evaluation. Our rationale for undertaking the synthesis of the C-glycoside analogue of sTn (**1b**, Figure 1) was to develop and test a new vaccine candidate against the elusive HIV or to prepare a better candicate for a cancer vaccine with a longer half-life in vivo.[9b]

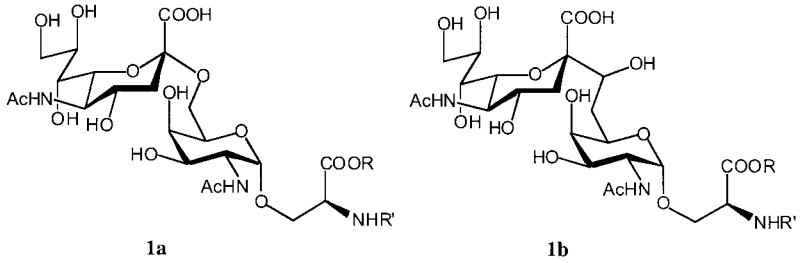

Figure 1. Tumor antigen sTn O-glycoside **1a** and C-glycoside analogue **1b**.

Fully protected sTn C-glycoside analogue **2** was prepared by C-glycosylation of the neuraminic acid sulfone donor **13** with an aldehyde acceptor **12** (Scheme 1). The donor **13** was prepared from neuraminic acid in four steps as previously reported.[12] The critical intermediate, aldehyde acceptor **12**, was prepared in 14 steps. Starting from the commercially available diisopropylidene galactose derivative **3**, the corresponding 6-iodo derivative **3a** was prepared and treated with KCN. The modest yield (30%) of this reaction might be due to unfavorable electronic and steric effects arising from the ring oxygen atom and the axially oriented oxygen at C-4, respectively. Reduction of the 6-cyano derivative **3b** with DIBAL-H afforded aldehyde **4** in moderate overall yield.[13] Quantitative reduction of aldehyde **4** with $NaBH_4$ in MeOH afforded the corresponding alcohol **5**. The acetal protecting groups were removed by treatment with Amberlite IR-120 (H^+) resin in water at 80 °C for 3 h to provide the 6-deoxy-D-galacto-heptopyranose **6** in quantitative yield. The one-carbon-extended galactal **7** was obtained in good yield from **6** by a one-pot, three-step procedure consisting of: a) peracetylation with acetic anhydride and catalytic HBr/HOAc; b) conversion of the anomeric acetate to the corresponding bromide with excess HBr/HOAc; and c) reductive elimination of the 1-bromo and 2-acetoxy groups with Zn/Cu.[14] Azidonitration[15] of **7** with excess ceric ammonium nitrate (CAN) and sodium azide in dry acetonitrile afforded primarily the 2-azido-1-nitrate addition product, which has the desired *galacto* configuration as unambiguously confirmed by ^1H NMR spectroscopy. Indicative are a large

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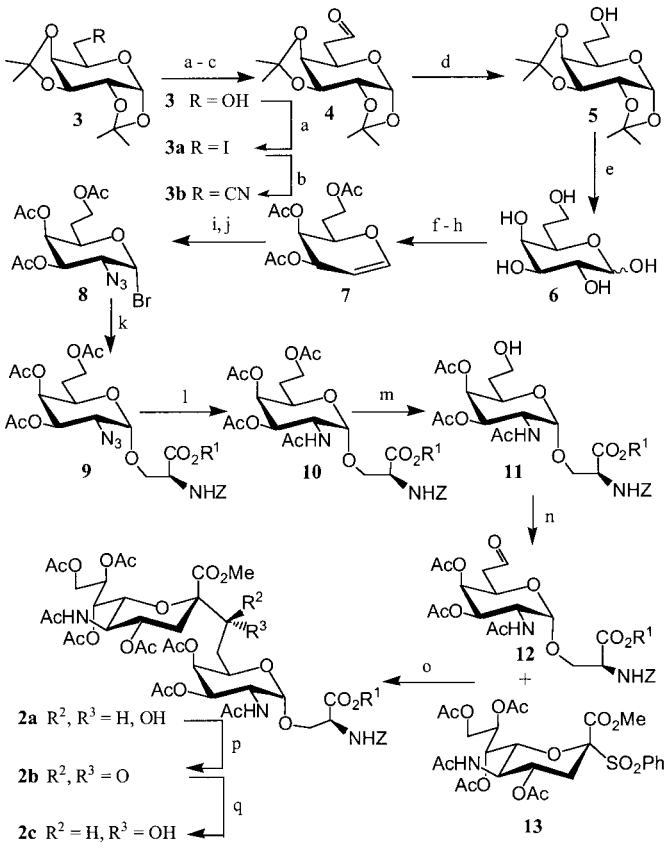

Scheme 1. Synthesis of sTn C-glycoside derivatives. a) Ph_3P, I_2, Im; b) KCN; c) DIBAL-H; d) $NaBH_4$; e) H^+ resin; f) Ac_2O, HBr/HOAc; g) excess HBr/HOAc; Zn/Cu; i) CAN, NaN_3; j) LiBr; k) Z-Ser-OBn, $AgClO_4$, 4Å molecular sieves; l) AcSH/Py; m) *R. toruloides*, sodium phosphate/sodium citrate buffer, pH 5; n) DMSO, ClCOCOCl, TEA; o) SmI_2; p) DMSO/Ac_2O; q) $Zn(BH_4)_2$. R^1 = Bn. Im = imidazole, DIBAL-H = diisobutylaluminunm hydride, Py = pyridine, TEA = triethylamine, DMSO = dimethyl sulfoxide.

coupling constant between the two vicinal protons H-2 and H-3 ($J_{2,3}$ = 11 Hz) and the chemical shift of H-2 (δ = 3.97 ppm). Treatment of the crude product with LiBr[15] in dry acetonitrile in the dark afforded **8**. Glycosylation of **8** with the N^{α}-benzyloxycarbonyl-protected OBn ester of L-serine, prepared as previously reported,[16] in the presence of silver perchlorate afforded the glycopeptide **9**. Separation of glycopeptide **9** from unreacted starting material, Z-Ser-OBn, was cumbersome since both compounds migrated with similar R_f values on silica. However, conversion of **9** to **10** by treatment with thioacetic acid/pyridine[17] resulted in a large change in the glycopeptide polarity and permitted removal of the unreacted amino acid acceptor from the desired glycopeptide product **10**.

Our efforts next focused on the selective deprotection of the C-7 primary acetyl group in **10**. Since chemical techniques are not sufficiently selective, we envisaged a selective enzymatic deacetylation using an esterase. The esterase from *Rhodosporidium toruloides* has been reported to selectively deprotect primary acetates in the presence of secondary acetates.[18] Treatment of **10** with this esterase at pH 5 in a sodium phosphate/sodium citrate buffer afforded **11** in quantitative yield. The site of the enzymatic deacetylation was unequivocally established as C-7 based on the upfield shift of H-7a and H-7b from δ = 4.19–4.06 in **10** to 3.62–3.74 ppm in **11** in the ^{1}H NMR spectra. Swern oxidation[19] of **11** afforded aldehyde acceptor **12**. Glycosylation of the neuraminic acid sulfone donor **13** with aldehyde acceptor **12** in the presence of freshly prepared SmI_2[20] afforded the fully protected sTn α-C-glycoside **2a**.

The ^{1}H NMR spectrum of **2a** indicated an *R*/*S* mixture (1:1) at the newly formed stereogenic bridge carbon C-7. Attempts were first made to remove the hydroxyl group at C-7 by standard reductive radical deoxygenation protocols. Compound **2a** was treated with $(imidazole)_2CS$ or $ClCS(PhF_5)$ then reduced with Ph_3SnH and azobisisobutyronitrile (AIBN). These reactions failed to yield the expected deoxygenated product probably due to unfavorable stereoelectronic factors. The C-7 hydroxyl group in **2a** was next converted to a triflate and subjected to nucleophilic reduction with hydride, but this resulted in decomposition of the triflate, presumably due to the unwanted rearrangement, followed by elimination—the result of the electron-rich functional groups close to the reactive center. Finally, chemoselective resolution to compound **2a** was undertaken by oxidation with DMSO/Ac_2O to keto-bridged compound **2b**, followed by stereoselective reduction with $Zn(BH_4)_2$ to regenerate the bridge hydroxyl function, gave **2c** in >90% *de* (Scheme 1). The configuration at the bridge carbon was established by ROESY. Chemical exchange of the proton of the hydroxyl group of the bridge carbon and the oxygen of the C-1 carboxyl group of Neu5Ac resulted in strong NOEs for H-3′e/H-7, H-7/H-6(proR), OH-7/H-6(proS), and H-6(proR)/H-5, consistent with the *R* configuration at C-7 in **2c** (Figure 2).

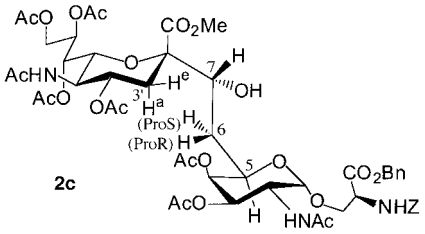

Figure 2. Stereochemical assignment of sTn *C*-glycoside **2c** at the bridge carbon C-7.

In summary, the synthesis of the C-glycoside analogue **2c** was achieved in 1 enzymatic and 17 chemical steps starting from diisopropylidene galactopyranose and sialic acid. Additional studies are underway aimed at conjugating the sTn C-glycoside hapten to KLH carrier protein to obtain a vaccine for biological evaluation.

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