Probing Cell Surface "Glyco-Architecture" through Total Synthesis. Immunological Consequences of a Human Blood Group Determinant in a Clustered Mucin-like Context

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Blood group antigens are not confined to erythrocytes, also serving as terminal carbohydrate moieties on glycoproteins and glycolipids in many epithelial cells and their secretions.[1] Protein-bound blood group determinants are often encountered in a mucin-like context, *O*-linked via an *N*-acetylgalactosamine residue to hydroxyl groups of clustered serine or threonine residues.[2] Remarkably, altered expressions of certain blood-group antigens on tumor cells can serve as markers in a variety of carcinomas.[3] One such example is the enhanced presentation of the Lewis^y (Le^y) histo-blood determinant [Fucα1−2Galβ1−4(Fucα1−3)-GlcNAc] in mucin or glycolipid form on many human tumor cells including those found in colon, lung, breast, and ovarian cancers.[4] The isolation of homogeneous, structurally defined mucin segments, containing such clustered blood group determinants, from natural sources, is immensely complicated by microheterogeneity, compounding the difficulties associated with achieving proteolysis of glycoproteins at fixed points. The availability of realistic and homogeneous structurally defined mucin fragments would be of considerable advantage in facilitating biological and structural studies. The complexity of the many issues to be overcome in pursuit of a fully synthetic homogeneous blood group determinant in a clustered setting presented a clear challenge to the science of chemical synthesis. Herein we report a solution to the problem in the context of a total synthesis of an Le^y-containing glycopeptide in mucin form and immunological profiles of the fully synthetic construct.

Our designed Le^y-mucin mimic (see **1**, Figure 1) incorporates the following features: (i) display of the full Le^y tetrasaccharide, (ii) an intervening carbohydrate spacer group so that the structure and immunological integrity of the determinants are not altered by direct contact with the protein-like domain, and (iii) a carboxy terminus, culminating in the previously described immunostimulating Pam$_3$Cys moiety,[5,6] thereby circumventing the need for low-yielding conjugation to a carrier protein such as KLH. We directed particularly close attention to a blocking-group strategy that allowed for global deprotection of our sensitive glycopeptide constructs under mild conditions. Given the range of protecting groups necessary to support the synthesis, this requirement proved to be a major element of the undertaking.

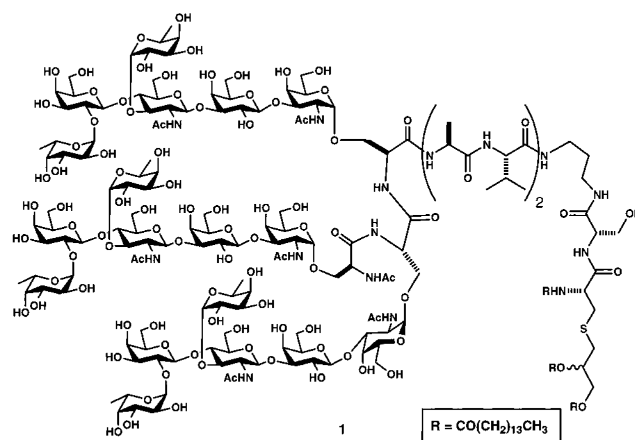

Figure 1.

The synthetic plan drew from previously described advances in the assembly of oligosaccharides.[7] Our recently developed "cassette" method[8,9] for solving the stereochemical problems associated with constructing α-serine (threonine) *O*-linked oligosaccharides was critical. This format utilizes an *N*-acetylgalactosamine synthon stereospecifically α-*O*-linked to a serine (or threonine) residue with an identified acceptor site. The acceptor cassette is joined to a target saccharide equipped with a donor function at its reducing end. Thus, the need for direct coupling of a serine side chain hydroxyl group to a complex saccharide donor is bypassed. The cassette method, unlike the classical approach, is highly convergent and stereoselective throughout the construction. The synthesis of **1** represents the most striking example of the method, in that design challenges included the coupling of large peracylated donors of low reactivity with acceptors also exhibiting only moderate reactivity profiles. Superimposed upon this challenge was the reduced stability of fucosylated oliogsaccharides α-*O*-linked to protected amino acids.

Le^y glycal[7,10] was prepared as shown (Scheme 1), and converted to the thioethyl donor **2** using previously described methodology.[11] At this stage, reaction of donor **2** with cassette acceptor **4**[7a] under NIS/TfOH conditions[12] afforded the coupled product bearing the

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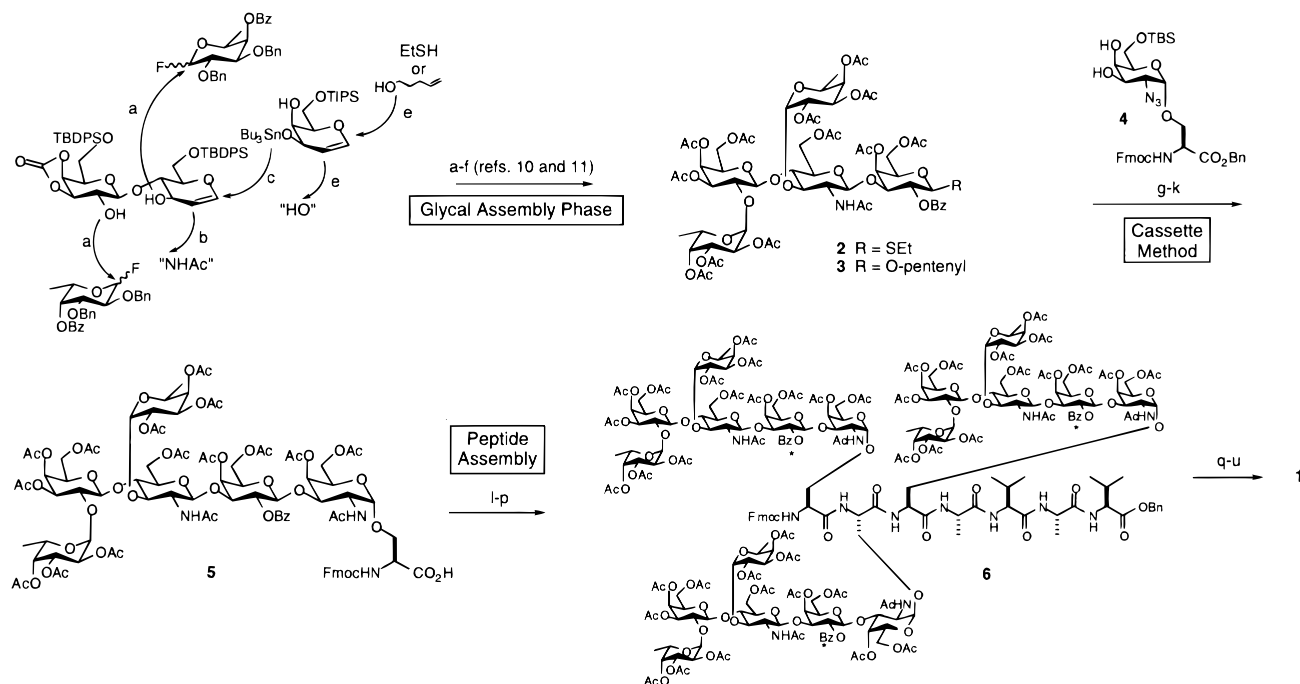
Scheme 1[a]



[a] (a−d) ref 10; (e) DMDO; EtSH, TFA (48%) or DMDO, pentenyl-OH, $ZnCl_2$ (94−96%); (f) BzCl, TEA, DMAP (87−93%); (g) NIS, TFA, **4** (R = SEt, 82%; R = pentenyl, 69%); (h) TBAF, AcOH; (i) Ac_2O, pyridine (96%, 2 steps); (j) AcSH, pyridine (77%); (k) H_2, Pd−C (quantitative); (l) HOAt, HAtU, collidine, H_2N-Ala-Val-Ala-Val-OBn (85%); (m) morpholine (quantitative); (n) HOAt, HAtU, collidine, **5** (77%); (o) morpholine (95%); (p) HOAt, HAtU, collidine, **5** (71%); (q) morpholine; (r) Ac_2O, TEA, DMAP (91%, 2 steps); (s) H_2, Pd−C, MeOH/H_2O; (t) hydrazine hydrate, MeOH (70%, 2 steps); (u) HOAt, HAtU, *i*Pr_2EtN, Pam_3Cys (**7**) (32%).

required serine α-*O*-linked to the complex carbohydrate domain. Alternatively, the Fraser-Reid-type pentenyl donor[13] **3**, formed by epoxide opening with pentenyl alcohol/$ZnCl_2$, functioned as an effective donor under the same coupling conditions.[12b] This latter method gains from improved efficiency in preparation of the donor from the glycal epoxide. Functional group management, as shown, led to acid **5**. Mucin construction required peptide couplings of highly complex glycosylamino acids. In the event, HOAt/HAtU methodology[14] allowed for efficient assembly of the linear heptapeptide mucin model precursor **6**. Following removal of the Fmoc-protecting group, the free amine was capped by acetylation. Hydrogenolytic cleavage of the benzyl ester exposed the fully protected C-terminal carboxyl. In the culminating global deprotection step, treatment with hydrazine hydrate in methanol smoothly cleaved the acetate and benzoate esters to afford the fully deprotected glycopeptide. The use of hydrazine was crucial, since the benzoate-protecting groups on the three galactose spacers (see asterisks in structure **6**) had resisted typical deprotection conditions. Finally, the lipid amine **7** was coupled to the acid terminus of the heptapeptide under the conditions shown, to afford the synthetic antigenic construct **1** ((M + 2 Na^+)/2e Calcd: 2404.6; Found: 2404.6).

The reactivities of Le^y-containing lipoglycopeptide **1**, as well as a Le^y-ceramide control compound,[15] with anti-Le^y antibody 3S193[16] were determined by ELISA assay. This antibody had been elicited against tumor cells that display the cell surface mucin motif. The α-*O*-linked hexasaccharide showed reactivity comparable to that of the Le^y-ceramide control. In addition, when mice were vaccinated with **1** formulated as an emulsion in Intralipid (Clintec Nutrition Co.), *with no additional adjuvant*, there resulted a strong IgM immune response, generating antibodies that strongly bind to natural mucin-related Le^y and Le^y-ceramide. *These biological results demonstrate that a realistic immunogenic mimic of a tumor-associated cell surface-displayed* Le^y *mucin has been achieved by total synthesis.*

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Supporting Information Available: Spectral characterizations of **1** as well as synthetic intermediates, ELISA determination of reactivities of **1** and Le^y-ceramide to anti-Le^y antibody 3S193, and ELISA determination of reactivities of antibodies generated by mouse immunizations with **1** against Le^y-mucin and Le^y-ceramide (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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