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In pursuit of an anticancer vaccine: a monomolecular construct containing multiple carbohydrate antigens†

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Abstract

The synthesis of a new type of anti-cancer vaccine candidate is presented. This compound contains the TF, Le^y, and Tn tumor antigens clustered in a monomolecular array. In addition to being a realistic mimic of 'micro-heterogeneous' mucins, this class of vaccine may trigger a multifaceted immune response convergent on a particular cancer type. © 2000 Elsevier Science Ltd. All rights reserved.

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The glyco-architecture of a cell surface can be highly complex. Mucin glycoproteins, for example, display clustered glycosylation motifs that are micro-heterogeneous.[1] These arise from non-identical glycosylation patterns on the side chains of sequentially arranged serine and threonine residues. While the deconvolution of the intricacies of mucins and related structures continues to be a focal point of glycobiology, certain carbohydrate antigens have been identified as markers for the onset of cancer. Some of these constructs have become targets for the development of anticancer vaccine therapies. We have advanced a set of vaccines that consist of a glycopeptide segment containing a carbohydrate antigen displayed in polyvalent form.[2] Such strategies rely on a single type of carbohydrate antigen to serve as the immunological homing device. Herein we report the synthesis of **1**, *which we view as the prototype of a new class of vaccines, containing multiple carbohydrate antigens clustered in a monomolecular array.*

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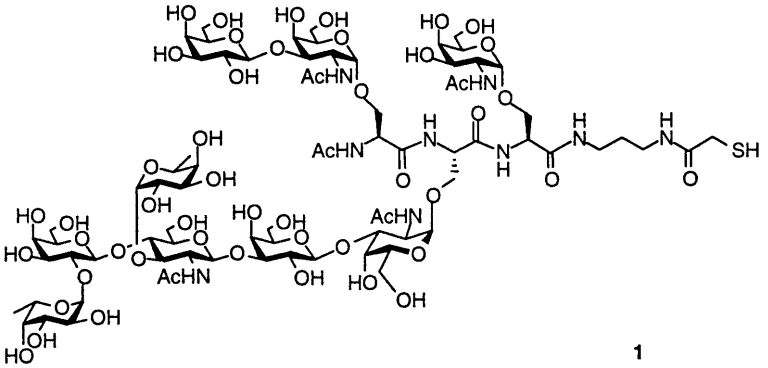

Compound **1** contains the Thompson–Friedenreich (TF) disaccharide, the Lewis^y (Le^y) determinant in hexasaccharide form, and the Tn monosaccharide tumor antigen. We have prepared glycoamino acids that contain these antigens by our recently introduced cassette methodology (**2**–**4**, Fig. 1).[3,4] Furthermore, vaccines based on clustered triads of **2**–**4**, e.g. (Le^y–Ser)$_3$, are being advanced through preclinical and clinical evaluation.[2] Presumably, not all tumor cells express the same carbohydrate antigens at all phases of cellular development. Since TF, Le^y, and Tn, have each been found on colon and prostate cancer, we were intrigued by the possibility of generating a single vaccine containing each of these tumor antigens. To test the feasibility of this principle, we chose **1** as our target. This molecule contains the clustered mucin motif linked via a diamine spacer to a terminal sulfydryl unit in order to allow attachment to a suitable carrier molecule.

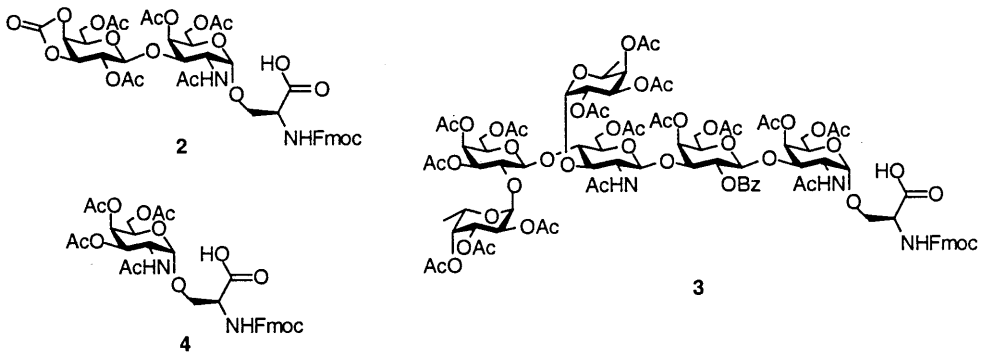

Figure 1. TF (**2**), Le^y (**3**), and Tn (**4**) glycoamino acid building blocks

Following our earlier protocols, a diamine spacer was incorporated early.[3,4] The synthesis of **1** commenced with the conversion of **5**[3] to **6** (Fig. 2). IIDQ activation of **3** in the presence of an excess of **5** gave the corresponding dipeptide. Subsequent removal of the Fmoc protecting group with morpholine provided the desired amine (**6**) in 72% overall yield.[5] Further coupling of **6** with **2**, again mediated by IIDQ, gave the fully protected tripeptide in 92% yield. Since removal of Fmoc with morpholine is incompatible with cyclic carbonate protection, we applied Joullie conditions to effect cleavage of the Fmoc group.[6] The resultant amine was further converted to the corresponding acetamide **7** in 90% yield (two steps).

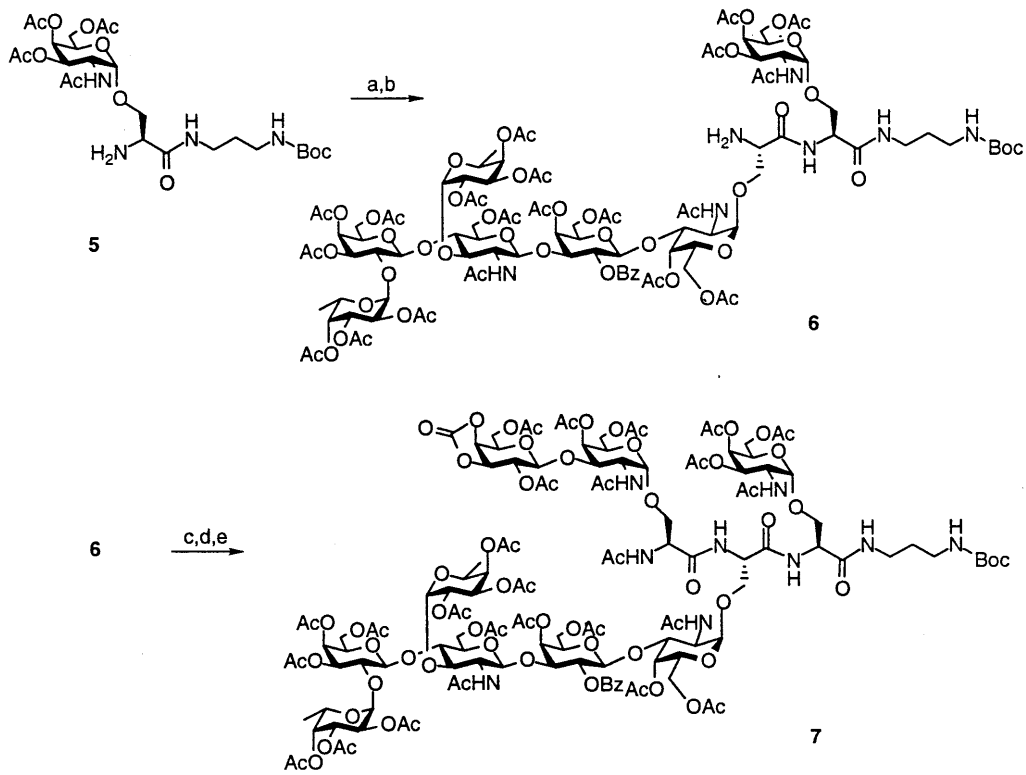

Figure 2. Synthesis of the protected peptide. Conditions: (a) **3**, IIDQ, CH_2Cl_2, rt. (b) morpholine, DMF, rt (72%, two steps). (c) **2**, IIDQ, CH_2Cl_2, rt (92%). (d) KF, 18-crown-6, DMF, rt. (e) Ac_2O, pyr, rt (90%, two steps)

In general, the routes to our most complex targets are designed to allow a single deprotection maneuver to give the desired final target. While **7** contains acetate, benzoate, and cyclic carbonate protection, we opted to forgo consolidation of the protecting group arrangement in hopes of simplifying the final sequence. Accordingly, the sulfhydryl linker was fashioned by removing the *t*-Boc followed by exposure of the crude product to the pentafluorophenyl ester of *S*-acetomercaptoacetate (pfp–O–SAMA) (**7**→**8**). With **8** in hand it remained only to remove the

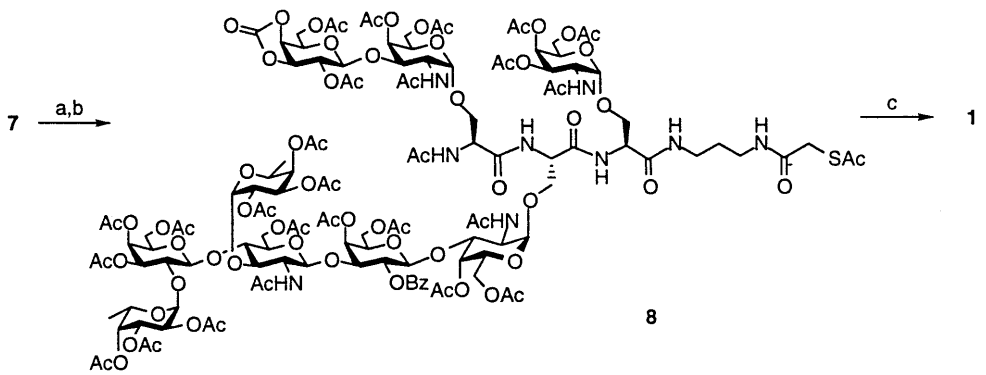

Figure 3. Installation of the sulfhydryl linker and global deprotection. Conditions: (a) TFA, CH_2Cl_2, rt. (b) pfp–O–SAMA, pyr, rt (69%, two steps). (c) CH_3OH, NH_2NH_2, rt (71%)

full complement of protecting groups. There is convincing evidence that the removal of acetates with methanolic hydrazine affords methyl acetate as the by-product.[7] With this in mind, we wondered if a cyclic carbonate might be cleaved under these conditions. We had noted previously that, while most standard deprotection conditions failed to remove the benzoate function from several related Le^y constructs, a mixture of hydrazine and methanol smoothly disengaged this otherwise recalcitrant protecting group.[4] In the event, treatment of **8** with 3:1 ratio of methanol:hydrazine afforded the globally deprotected 'pro-vaccine' candidate, **1** ($m/2z$ calcd for $Na_2C_{78}H_{131}N_9O_{51}S$: 1043.8; found: 1043.7), in 71% yield (Fig. 3).

There is mounting evidence, from our own early clinical trials and others, suggesting that vaccine strategies can mobilize the human immune system against specific tumor-associated carbohydrate antigens displayed on the surfaces of cancer cells. Compound **1** presages a new class of totally synthetic vaccine candidates. In addition to being a realistic mimic of 'micro-heterogeneous' mucins, such a construct has the potential to stimulate an immune response against each antigenic carbohydrate component. This in turn may trigger a multifaceted convergence on a particular cancer type. Preclinical evaluations of vaccines derived from **1** and related constructs are currently underway in anticipation of projected clinical trials.

Acknowledgements

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