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Chemical Synthesis of Normal and Transformed PSA Glycopeptides

Vadim Y. Dudkin,[†] Justin S. Miller,[†] and Samuel J. Danishefsky*[,†,‡]

Laboratory for Bioorganic Chemistry, The Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021, and Department of Chemistry, Columbia University, Havemeyer Hall, New York, New York 10027

Received August 18, 2003; E-mail: s-danishefsky@ski.mskcc.org

Localized cancer of the prostate gland can often be arrested, whereas progression to the metastatic state dramatically decreases quality of life and survival rates. The feasibility of early diagnosis of prostate tumors was enhanced with the identification of prostate specific antigen (PSA) as a cancer screening marker.[1−3] PSA, which is a glycoprotein secreted by the prostatic epithelium, manifests high tissue specificity.[4] It consists of 237 amino acid residues and possesses a single *N*-glycosylation site that typically carries an *N*-acetyllactosamine type glycan.[4] Despite the microheterogeneity of normal PSA, its carbohydrates appear to be of the dibranched type (e.g., **1**, Figure 1).[4,5] By contrast, glycans isolated from LnCaP prostatic cancer cells include tri- and even tetrabranched structures (e.g., **2** and **3**, Figure 1).[6] Since the distinctions between normal and "transformed" PSA are limited to glycan composition, they are invisible to current assays which employ antibodies that recognize the glycoprotein's conserved polypeptide domain.[7] Unfortunately, even state-of-the-art diagnostic methods based on PSA levels may fail to distinguish between pre-metastatic prostate cancer and benign prostatic hyperplasia.[8,9] Clinical measurements of PSA levels do not necessarily identify isoforms specific to malignant tissue.[10] This issue is often resolved through invasive biopsy procedures.

We envisioned that differentiated antibodies, sensitive to particular PSA glycoforms, could well form the basis of a new and potentially highly efficient diagnostic strategy to monitor not only the levels of PSA but also the likely aggressiveness of the disease. Furthermore, sensitive screening might enable the pinpointing of malignant transformations at an early stage of the disease, when serum PSA levels are particularly uninformative.

For such antibodies to be elicited, a source of defined and homogeneous PSA fragments bearing *N*-glycans with various degrees of branching is crucial. Challenging as it surely would be, it seemed to us that chemical synthesis might provide the best and most versatile solution to this need. To deal with the complexity of the targets, we hoped to chart new strategies for oligosaccharide assembly, stressing utmost convergency and stereochemical control. We report herein the first chemical synthesis of multibranched *N*-acetyllactosamine-type glycans and their incorporation into PSA glycopeptide fragments **1**−**3**.

In this introductory study, we selected the most common of the multibranched, *N*-acetyllactosamine-type PSA glycans as our targets.[4] Also, we chose not to prepare sialylated forms of the glycans, since these add significantly to the heterogeneiety of serum PSA.[11] Indeed, in the setting of diagnostic assays, samples are first subjected to sialidase digestion.

Earlier, we had found, in simple models, that a sequence consisting of Kochetkov amination[12] of an oligosaccharide bearing a free reducing end, followed by Lansbury aspartylation[13] and thence by native chemical ligation[14,15] (NCL), provides a way of building complex *N*-linked polypeptides.[16,17] As will be shown, these protocols served us well in a highly complex setting.

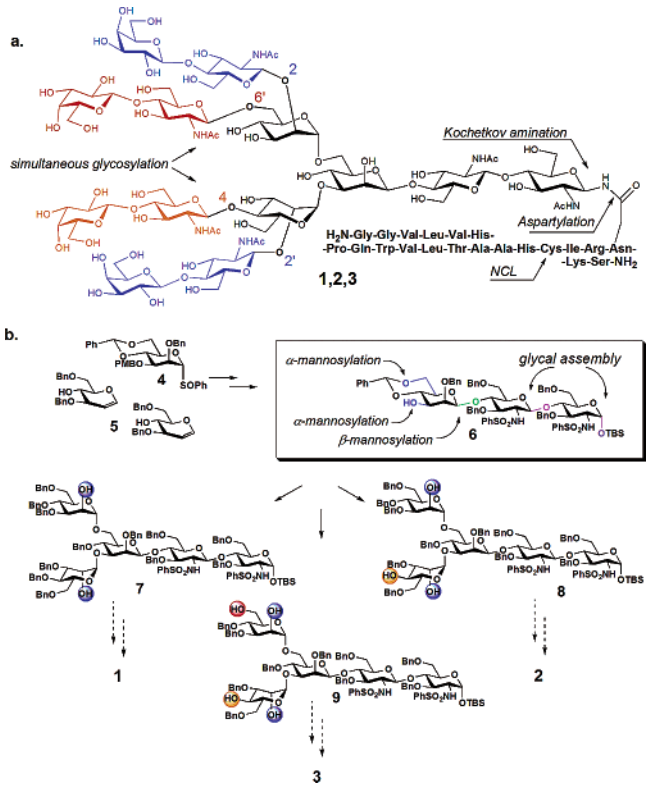

Figure 1. Structures of PSA$^{27-47}$ glycopeptides **1**−**3** (a) and their retrosynthetic analyses (b). **1**, "normal" dibranched PSA fragment with *N*-acetyllactosamines at 2,2′ shown in blue; **2**, tribranched at 2,4,2′ positions (additional orange branch); **3**, tetrabranched at 2,4,2′,6′ (additional orange and red branches).

The nexus of the problem, of course, centered around the carbohydrate domains featuring interwoven high mannose and lactosamine blocks. To solve the transformed PSA glycan construction problem, it would be necessary to go well beyond the preparation of symmetrically dibranched glycans (projecting from the 2 and 2′ positions of wing mannoses of the pentasaccacharide core system). While tribranched glycans isolated from natural sources have been used in glycopeptide preparation,[18] symmetrical dibranched structures represented the limit of previous chemical syntheses.[19−23] We set for ourselves the goal of creating a much more encompassing strategy which would pave the way for reaching larger, more branched and less symmetric constructs from a common intermediate (cf. **6**) with high stereoselection and maximum convergency.[24]

Our approach relied on a proposal that introduction of several *N*-acetyllactosamines can be accomplished in a single glycosylation

[†] The Sloan-Kettering Institute for Cancer Research.
[‡] Columbia University.

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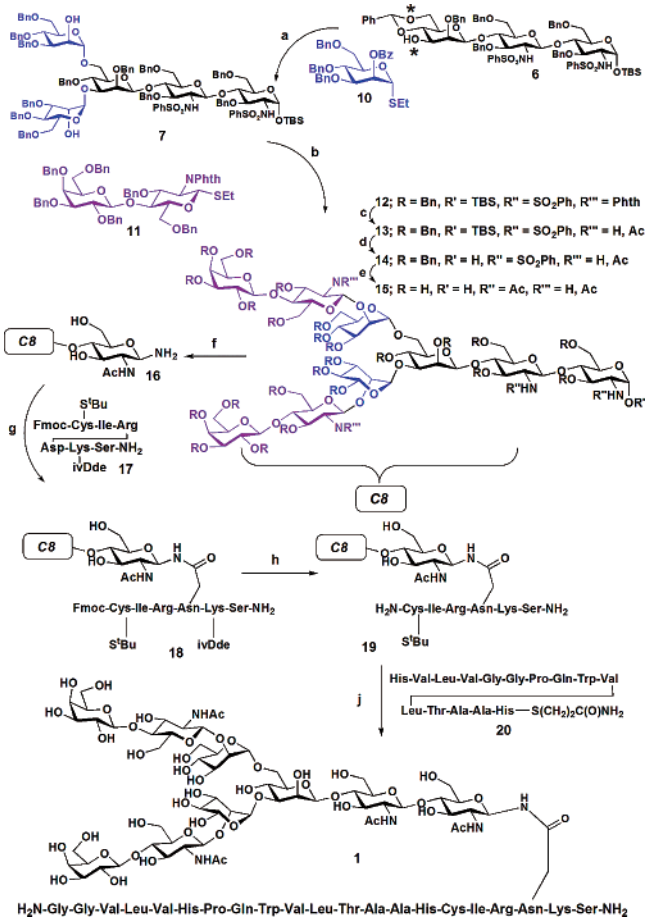
Scheme 1. Synthesis of Normal PSA Glycopeptide **1**[a]

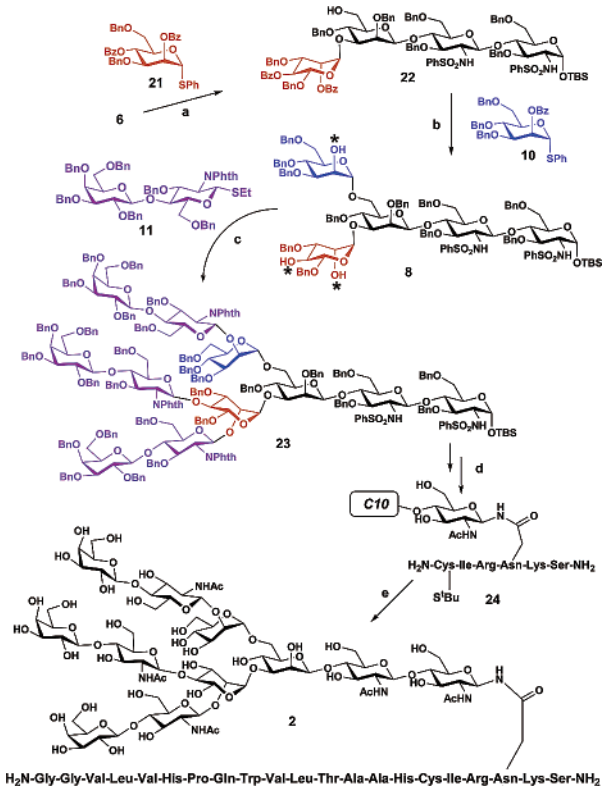


[a] Reagents and conditions: (a) i. BH_3·THF, Bu_2BOTf, THF, 72%; ii. **10**, $(BrC_6H_4)_3NSbCl_6$, MeCN, 74%; iii. NaOMe·MeOH, 89%; (b) MeOTf, DTBP, CH_2Cl_2, 60%; (c) i. ethylenediamine, *n*-BuOH·toluene, 90 °C, ii. Ac_2O/py, iii. NaOMe/MeOH, 72%; (d) TBAF/AcOH, THF, 76%; (e) i. Na/NH_3, −78 °C, ii. Ac_2O, iii. NaOMe/MeOH, 65%; (f) NH_4HCO_3/H_2O; (g) **17**, HATU, Hünig's base, DMSO, 61% from **15**; (h) $(NH_2)_2$, piperidine, DMF, 62%; (j) **20**, MES-Na, pH = 7.4, 17%.

event. This transformation has been demonstrated in a similar setting in simpler models.[25,26] The PSA glycan synthesis problem could then be translated into that of producing pentasaccharides **7**, **8**, and **9** with differentiated "free OH" acceptor sites. This retrosynthesis took us back to trisaccharide **6** as a common intermediate.[27] This key building block contains virtual (see benzylidene acetal) and identified acceptor loci.[28] By α-mannosylation with suitably differentiated α-mannosyl donors, permuted core pentassacharides **7**, **8**, and **9** quickly became accessible. The central intermediate trisaccharide **6** is smoothly assembled by a combination of glycal assembly in the context of sulfonamidoglycosylation and sulfonamidohydroxylation[29] (see **4** → AB rings of **6**) and Crich's β-mannosylation chemistry[30,31] (see **5** → ring C of **6**).[27] Building blocks **4** and **5** are prepared from D-glucal and mannose, respectively.

The validity of the concept was first field-tested in the context of a synthesis of the nontransformed-type glycan **1** (Scheme 1). Thus, trisaccharide **6** was prepared following the logic described above.[27] Reductive cleavage of the benzylidene acetal generated a diol that coupled at two points (see asterisks in **6**) with monoester-containing α-mannosyl donor **10** to assemble a pentasaccharide containing two esters. Cleavage of the two benzoates led to bis acceptor **7**. Indeed, two-fold glycosylation using donor **11** proceeded smoothly to establish the protected core system (**12**) corresponding

Scheme 2. Synthesis of Tribranched Transformed PSA Glycopeptide **2**[a]



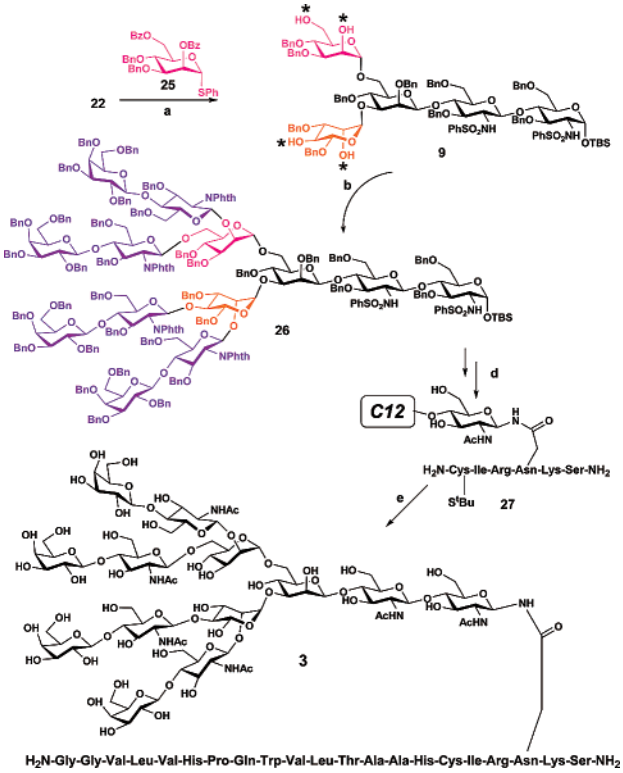
[a] Reagents and conditions.: (a) i. **21**, $(BrC_6H_4)_3NSbCl_6$, MeCN, 71%; ii. BH_3·THF, Bu_2BOTf, THF, 85%; (b) i. **10**, $(BrC_6H_4)_3NSbCl_6$, MeCN, ii. NaOMe/MeOH, 72%; (c) **11**, $(BrC_6H_4)_3NSbCl_6$, MeCN, 41%; (d) see Scheme 1, (c−h), 11% for 10 steps; (e) **20**, MES-Na, pH = 7.4, 38%

to **1**. The two phthalimides were then converted into acetamides, the anomeric hydroxyl group was liberated by desilylation, and the product was subjected to global deprotection (sodium in liquid ammonia). Here, we exploited our remarkable finding that the integrity of the reducing end hemiacetal is maintainable during global Birch debenzylation.[32] Amine-specific diacetylation afforded free glycan **15** as a mixture of anomers.[33] Free β-glycosylamine **16** was obtained from nonasaccharide **15** by a Kochetkov amination protocol. Coupling with excess hexapeptide **17** gave glycoconjugate **18**. The Fmoc and ivDde protecting groups in **18** were shed, and the resulting amine was subjected to NCL with pentadecapeptide thioester **20**. This sequence afforded the fully characterized normal PSA(27−47) glycopeptide fragment, presented as homogeneous nonasaccharide uneicosapeptide **1**.

Having tested our strategy in the control synthesis of **1**, we sought to apply these notions to the syntheses of **2** and **3**, as described in Schemes 2 and 3. The key point to be appreciated is that simple permutations in the processing and advancement of key trisaccharide **6**, and selection of the resident protection patterns in the α-mannosylation donors used in ring extension reactions of the strategic trisaccharide, build high diversity and high complexity at a stage where the systems are still of relatively modest size.

We first turned to the nonsymmetrically branched PSA glycan **2**. Here we extended, sequentially, the hydroxyls at C3 and C6 of the ring C system. Thus, we first accomplished α-mannosylation at C3,[34] using donor **21** bearing two ester linkages, leading to 4,6-benzylidene-protected tetrasaccharide. Controlled reductive cleavage of the benzylidene acetal[35] exposes the C6 hydroxyl of the C ring in **22**, which was α-mannosylated with the previously employed monoester α-mannosyl donor **10**. At this stage, the three esters were

Scheme 3. Synthesis of Tetrabranched Transformed PSA Glycopeptide **3**[a]



[a] Reagents and conditions: (a) i. **25**, $(BrC_6H_4)_3NSbCl_6$, MeCN, 74%, ii. NaOMe/MeOH, 92%; (b) **11**, $(BrC_6H_4)_3NSbCl_6$, MeCN, 19%; (d) see Scheme 1, (c−h), 32%; (e) **20**, MES-Na, pH = 7.4, 65%.

easily cleaved, thereby exposing trivalent acceptor system **8**. Three-fold β-lactosylation was accomplished using β-lactosamine donor **11** with stereodirecting phthalimide groups at C2′. Indeed, three such donors were incorporated, leading to the protected core system (**23**) corresponding to **2**. The steps for progressing from **23** to **2** were much as those worked out in advancing from **12** to **1** (vide supra).

Thus encouraged, we now undertook the challenge of reaching the highly branched system **3**. Toward this end, we revisited tetrasaccharide **22**. Reductive cleavage of the benzylidene acetal, as before, was now followed by mannosylation with **25**, bearing esters at C2′ and C6′. This reaction provided the required pentasaccharide, containing four acceptor sites momentarily masked as benzoate esters. The key hydroxy centers were smoothly unveiled (see **9**). At this stage, four-fold glycosylation was accomplished with lactosamine donor **11**, but this time in more modest yield. The tridecasaccharide core system (cf. **26**) was obtained, but this time in 19% yield.

Fortunately, the sequence from protected oligosaccharide to deprotected Kotchetkov amination product worked well, as did the introduction of **17** via aspartylation and deprotection (cf. **27**). Upon NCL with **19**, the tridecassacharide−uneicosapeptide glycoconjugate **3** was delivered in homogeneous form.

In summary, a universal strategy for the preparation of complex *N*-linked glycopeptides from a common precursor has been developed. This new methodology has proven its mettle in the preparation of normal and transformed PSA fragments. This particular project has now moved on from a purely chemical focus to the immunological realm. The next step involves production of selective antibodies through animal immunization; the results will be reported in due course.

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Supporting Information Available: Experimental procedures and compound characterization data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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