JOURNAL OF combinatorial CHEMISTRY

Subscriber access provided by American Chemical Society

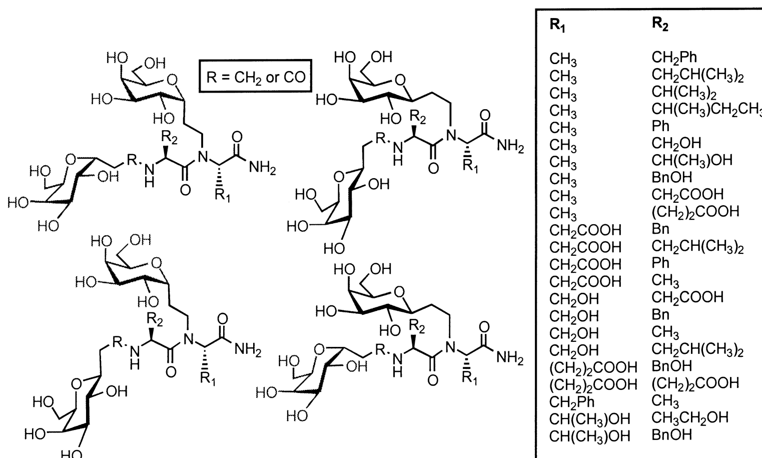
Article

Automated High-Throughput Synthesis of Artificial Glycopeptides. Small-Molecule Probes for Chemical Glycobiology

Prabhat Arya, Angela Barkley, and Karla D. Randell

J. Comb. Chem., **2002**, 4 (3), 193-198• DOI: 10.1021/cc020001i • Publication Date (Web): 08 March 2002

Downloaded from http://pubs.acs.org on March 20, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 6 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications High quality. High impact.

Journal of Combinatorial Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Articles

Automated High-Throughput Synthesis of Artificial Glycopeptides. Small-Molecule Probes for Chemical Glycobiology

Prabhat Arya,* Angela Barkley, and Karla D. Randell

Chemical Biology Program, Steacie Institute for Molecular Sciences, National Research Council of Canada, 100 Sussex Drive, Ottawa, Ontario, K1A 0R6, Canada

Received January 4, 2002

A fully automated method for the synthesis of artificial glycopeptides having two (similar or different) carbon-linked glycosyl moieties on a dipeptide scaffold has been developed. By use of this approach that combines the diversity of peptide/pseudopeptide and glycosides, different glycoside moieties can be incorporated onto the peptide/pseudopeptide backbone in a highly controlled manner. The approach utilizes a stepwise reductive amination with glycoside aldehyde derivatives (model **1**) or (ii) glycoside reductive amination followed by glycoside amide bond formation (model **2**). Further, an automated method has been utilized in the high-throughput library synthesis of 4 × 96 artificial glycopeptides. These libraries were tested as chemical probes/inhibitors of enzyme systems that convert a glucose moiety into rhamnose prior to incorporation of the rhamnose unit and the conversion of UDP-galactopyranose to UDP-galactofuranose via UDP-galactopyranose mutase enzyme during the biosynthesis of the mycobacterium cell wall.

Over the past few years, there has been a rise in interest in understanding the roles and functions of carbohydrates and carbohydrate conjugates at the chemical level. This has commonly become known as chemical glycobiology.[1] It is now well accepted that these derivatives play vital roles in a vast number of biological recognition events that range from cell−cell communication, fertilization, and cell growth and differentiation to pathological processes including cancer metastasis, inflammation, and microbial infections.[2] There are an immense number of glycoconjugate derivatives, and these are known to vary depending on stages of cell development, cell differentiation, and course of disease. It is becoming clear that the carbohydrate moieties of these glycoconjugates impact the architecture and function of underlying biomolecules and, as such, are also involved in important biological processes. Many examples have demonstrated that complex carbohydrates and their conjugates present on tumor cells possess unique structural features. Identification of these derivatives, followed by the development of chemically well-defined synthetic cancer vaccines is one such area of active research.[3]

A major obstacle in the field of *chemical glycobiology* is our inability to access rapidly chemically well-defined complex carbohydrates, carbohydrate conjugates, carbohydrate mimics, as well as carbohydrate-based small-molecule chemical probes.[4] For example, synthetic derivatives of cell-surface glycoconjugates serve as attractive tools in understanding cell-surface-based recognition events and provide a good starting point in developing a new class of therapeutic agents. Small-molecule-derived inhibitors of carbohydrate-processing enzymes (CPE) provide a means of controlling their biosynthetic pathways and offer new opportunities in carbohydrate-based therapeutic research.

Traditional synthesis of carbohydrates in any form is still a very challenging task, since there is no general method to produce complex carbohydrates as there is for proteins and nucleic acids. A typical synthesis consists of many protection and deprotection steps of multiple-hydroxyl groups, in addition to the fact that oligosaccharides are often branched rather than linear.[5] The linkages between monosaccharides can be either α or β, and the formation of these linkages requires different conditions for different sugar building blocks. Solid-phase synthesis and combinatorial synthesis are emerging as excellent tools for producing large numbers of diverse or focused compounds.[6] The use of glycomimetics has also been developed as an alternative to carbohydrate synthesis.[7] It has been shown that the terminal sugars (two to four residues) and their conformation are critical for biological activities. It is with this in mind that we chose to develop an automated, combinatorial approach[8] to obtain artificial glycopeptides as small-molecule probes or mimics containing a dipeptide backbone with two sugar moieties attached.

Our quest has been to develop methodologies for generating novel carbohydrate-based small molecules for use as chemical probes for the study of carbohydrate-processing

* To whom correspondence should be addressed. Phone: (613) 993 7014. Fax: (613) 952 0068. E-mail: Prabhat.Arya@nrc.ca.

10.1021/cc020001i CCC: $22.00 © 2002 American Chemical Society
Published on Web 03/08/2002

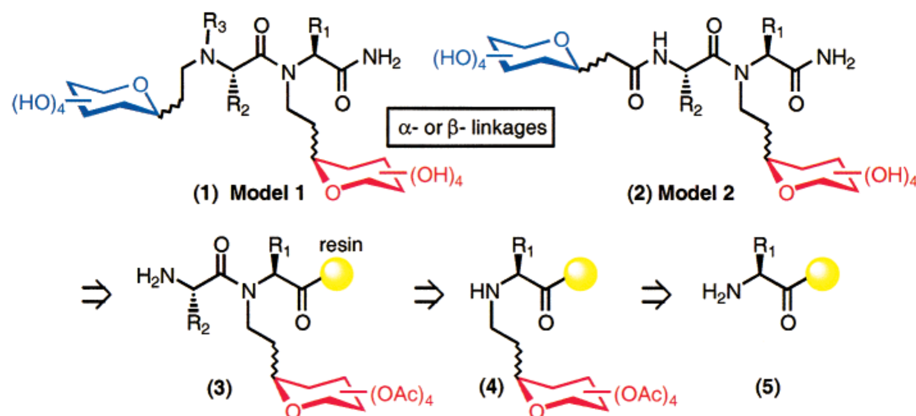


Figure 1. Retrosynthetic analysis: artificial glycopeptide models **1** and **2** with diversity of the peptide scaffold as well as glycosides moieties.

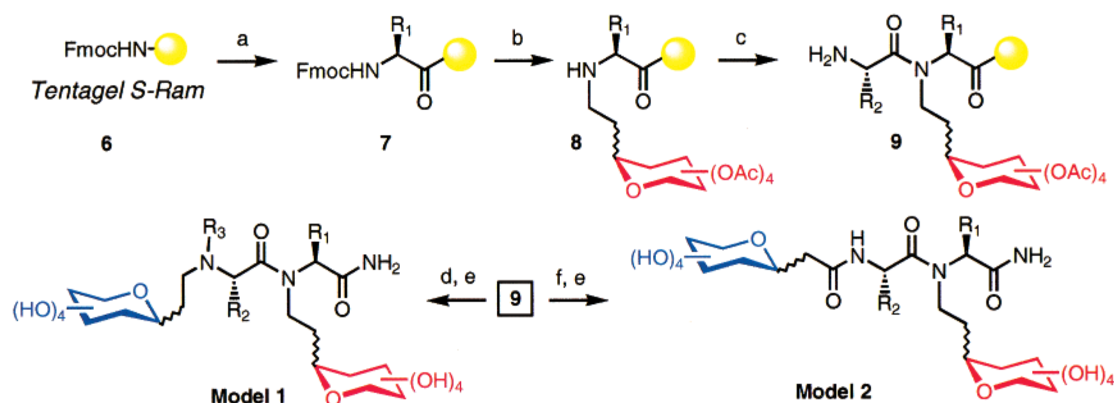
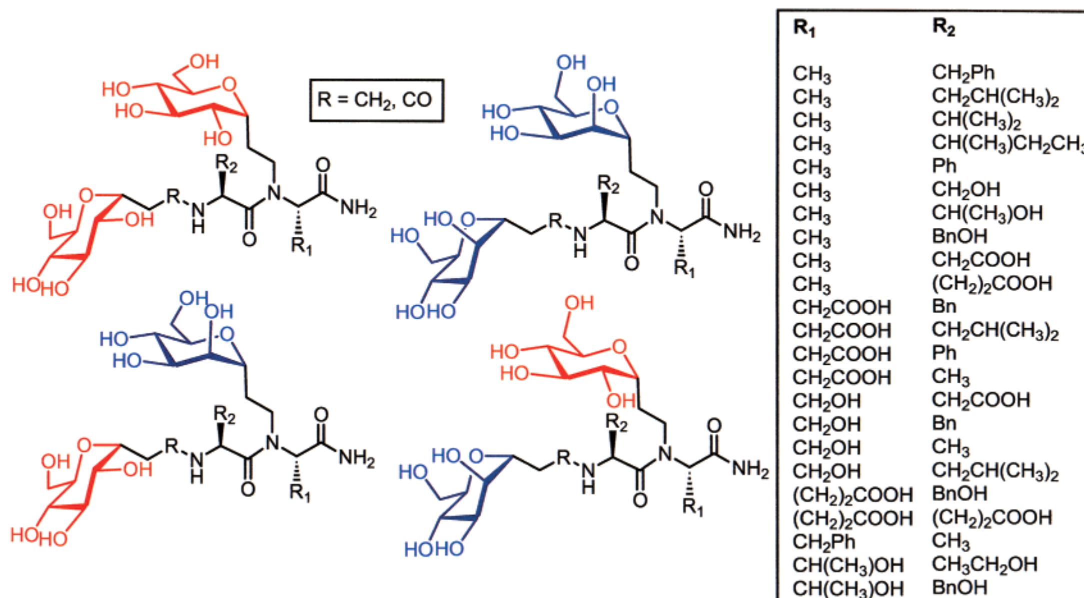


Figure 2. (a) (i) 20% piperidine, 0.5 h, repeat; (ii) 4.0 equiv of amino acid, 4.0 equiv of HBTU, 8.0 equiv of DIPEA, couple 3 × 25 h. (b) (i) 20% piperidine, 0.5 h, repeat; (ii) 1.5−2.5 equiv of α- or β-Gly-CHO, 2.5 equiv of $NaCNBH_3$, 0.5% AcOH, 5 h. (c) (i) 4.0 equiv of amino acid, 4.0 equiv of HATU, 8.0 equiv of DIPEA, couple 2 × 40 h; (ii) 2.0 equiv of amino acid, 2 equiv of HATU, 4.0 equiv of DIPEA, couple 4 × 40 h; (iii) 20% piperidine, 0.5 h, repeat. (d) (i) 1.5−2.5 equiv of α- or β-Gly-CHO, 2.5 equiv of $NaCNBH_3$, 0.5% AcOH, 5 h. (e) (i) hydrazine hydrate (1:11 in DMF), 4.5 h; (ii) 30% AcOH wash; (iii) 95% TFA:2.5% H_2O:2.5% TIS. (f) 1.5 equiv of α- or β-Gal-COOH, 1.5 equiv of HATU, 3.0 equiv of DIPEA, couple 2 × 40 h.

enzymes. We have developed a fully automated, combinatorial approach for rapidly accessing artificial glycopeptides as chemical probes.[9] It has been our hope that these high-throughput synthetic methods, which lead to artificial glycopeptides, will facilitate identification of useful chemical probes for chemical glycobiology and ultimately will provide inhibitors of carbohydrate-processing enzymes. In our design strategy, we have been interested in artificial glycopeptide derivatives that possess pertinent features of the underlying protein as well as the carbohydrate moiety, or glycoform of the glycoconjugates. This idea led to the development of carbohydrate diversity on a dipeptide/pseudo-peptide scaffold (Figure 1). Models **1** and **2** demonstrate the flexibility of this approach. The carbohydrates were incorporated as α- and β-linked *C*-glycosides, which are stable isosteres of native terminal sugars.[10] The versatility of this approach is reflected in the fact that a variety of sugars can be independently incorporated as peracetylated ethanal derivatives or ethanoic acid derivatives. Furthermore, these carbohydrate moieties may be present in the pyranose or furanose form. Figure 1 shows the four and five points of diversity that are utilized in this procedure. The building blocks (as carbon-linked sugar aldehydes and carbon-linked sugar acids) can be incorporated either at the N-terminal moiety or at the internal amide nitrogen of a short peptide/pseudo-peptide scaffold. This can be done in a highly flexible and controlled manner. By use of this approach, libraries of artificial glycopeptides are readily synthesized for probing carbohydrate−protein interactions. The libraries display two, i.e., homogeneous and heterogeneous, copies of carbohydrates, while the dipeptide scaffold may contribute to secondary interactions with the biological target.

A retrosynthetic analysis reveals that the two libraries, models **1** and **2**, can be obtained from a common precursor **3**. Both libraries contain a reductively aminated sugar at the internal nitrogen **4**, which is synthesized by coupling a sugar aldehyde to the free amine to form glycosylated amino acids. The next step is the coupling of a second amino acid to the secondary amine **3**. The amine is then diversified by either reductive amination to give libraries of type **1**, or sugar acid coupling leads to libraries of type **2**. Each of the steps of this synthesis has been optimized, including the reductive amination step being optimized for each of the monosaccharides used.

Artificial glycopeptide libraries of models **1** and **2** have been successfully synthesized (Figure 2) on TentaGel S RAM resin on a Multiple Organic Synthesizer (MOS). The success of the methodology was dependent on the optimization of the reductive amination reaction of the acetylated *C*-glycoside ethanal derivatives with the amino group of the anchored



R_1	R_2
CH_3	CH_2Ph
CH_3	$CH_2CH(CH_3)_2$
CH_3	$CH(CH_3)_2$
CH_3	$CH(CH_3)CH_2CH_3$
CH_3	Ph
CH_3	CH_2OH
CH_3	$CH(CH_3)OH$
CH_3	BnOH
CH_3	CH_2COOH
CH_3	$(CH_2)_2COOH$
CH_2COOH	Bn
CH_2COOH	$CH_2CH(CH_3)_2$
CH_2COOH	Ph
CH_2COOH	CH_3
CH_2OH	CH_2COOH
CH_2OH	Bn
CH_2OH	CH_3
CH_2OH	$CH_2CH(CH_3)_2$
$(CH_2)_2COOH$	BnOH
$(CH_2)_2COOH$	$(CH_2)_2COOH$
CH_2Ph	CH_3
$CH(CH_3)OH$	CH_3CH_2OH
$CH(CH_3)OH$	BnOH

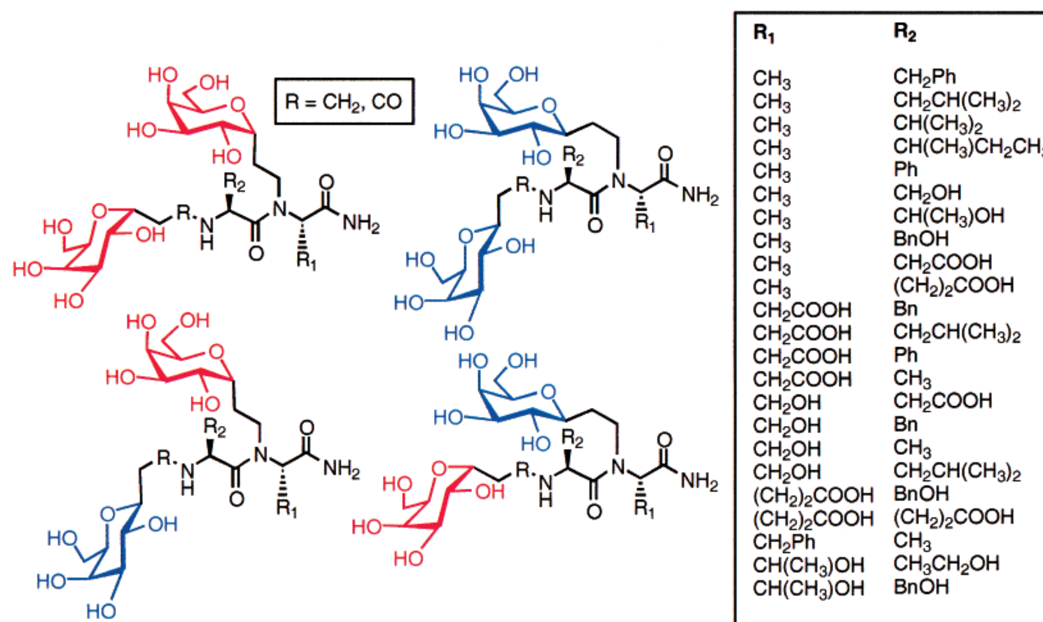
Figure 3. Artificial glycopeptide libraries (2 × 96 compounds; R = CH_2, model **1**; R = CO, model **2**) with α-glucoside and α-mannoside derivatives.

amino acid. After several attempts toward the synthesis of glycosyl amino derivative (see **3**, Figure 1) on solid phase, the reductive amination product could be obtained in high yields (70−95%) by using only fewer equivalents of the glycoside derivatives. Now, in our hand, this reaction requires only 1.5 equiv (α-mannoside derivative), 2.0 equiv (α-glucoside derivatives, *β*-galactoside derivatives) or 2.5 equiv (*β*-galactoside derivative) of the respective sugar derivatives, and the corresponding equivalents of sodium cyanoborohydride together with 0.5% glacial acetic acid. All the reagents were added in succession using trimethylorthoformate as the solvent and mixed for 4.5 h. This consistently gave high yields of the reductively aminated product without using the high concentrations of aldehyde (10 equiv) that is typically reported in the literature. The next challenging task was to optimize the coupling of the secondary amine having a glycoside derivative, with different amino acids. This coupling reaction turned out to be problematic for quite some time, and the products were formed in low yields. A significant amount of time has been spent to optimize this reaction in an automated synthesis method and to obtain the product with high yields (80−95%). The coupling of the resulting secondary amine to the next amino acid was accomplished with HATU reagent. The coupling of the acetylated ethanoic acid derivative of the respective sugar residue was also achieved with HATU reagent. Deacetylation of the sugar derivatives was achieved with a hydrazine hydrate/dimethylformamide mixture of pH 9−10 for 4.5 h. The time period as well as the pH was critical for on-bead deacetylation of the artificial glycopeptides. Cleavage of fully deprotected artifical glycopeptide from the resin was accomplished by mixing with trifluoroacetic acid for 2 h.

By use of the method discussed above, four 96-compound artificial glycopeptide libraries (Figures 3 and 4) have been synthesized in a fully automated manner. The artificial glycopetide library shown in Figure 3 contains two glycoside moieties (e.g., α-glucoside and α-mannoside). The synthesis of the first library (96 compounds) by automated solid-phase synthesis was obtained using model **1** as shown in Figure 1. Two glycoside derivatives were incorporated onto the peptide template by a stepwise reductive amination reaction. In the second library synthesis (see Figure 3), glycoside moieties were incorporated by the reductive amination reaction and the glycoside carboxyl acid coupling.

The compounds in artificial glycopeptide libraries were used as chemical probes in studying protein folding and trafficking, primarily of N-linked glycoproteins.[11] Work is in progress to test the ability of these derivatives to inhibit the reglucosylation of N-linked glycoprotein by a glucose-derived carbohydrate-processing enzyme.[12] Reglucosylation of N-linked glycoprotein appears to be a critical step in N-glycoprotein biosynthesis and protein folding and trafficking pathways.

In a second study, the artificial glycopeptide libraries were tested in enzyme systems that convert a glucose moiety into rhamnose prior to incorporation of the rhamnose unit during the biosynthesis of the mycobacterium cell wall.[13] The inhibition of this step may play an important role in the development of novel, carbohydrate-derived therapies to combat mycobacterium tuberculosis cell-wall biosynthesis. Further, inhibition of this biosynthetic pathway may lead to the development of compounds with a specific action because this particular biotransformation does not occur in mammalian systems. To date, few artificial glycopeptide derivatives as potential glycoside-based inhibitors containing at least one negatively charged amino acid residue have been identified. Detailed biological studies are in progress.[14] Furthermore, the artificial glycopeptide derivatives from the libraries shown in Figures 3 and 4 (models **1** and **2**) are being used as chemical probes or inhibitors for a study of the conversion of UDP-galactopyranose to UDP-galactofuranose via UDP-galactopyranose mutase enzyme.[14] The design and synthesis of inhibitors of this biosynthetic pathway is another interesting approach in the development of small-molecule-



R_1	R_2
CH_3	CH_2Ph
CH_3	$CH_2CH(CH_3)_2$
CH_3	$CH(CH_3)_2$
CH_3	$CH(CH_3)CH_2CH_3$
CH_3	Ph
CH_3	CH_2OH
CH_3	$CH(CH_3)OH$
CH_3	BnOH
CH_3	CH_2COOH
CH_3	$(CH_2)_2COOH$
CH_2COOH	Bn
CH_2COOH	$CH_2CH(CH_3)_2$
CH_2COOH	Ph
CH_2COOH	CH_3
CH_2OH	CH_2COOH
CH_2OH	Bn
CH_2OH	CH_3
CH_2OH	$CH_2CH(CH_3)_2$
$(CH_2)_2COOH$	BnOH
$(CH_2)_2COOH$	$(CH_2)_2COOH$
CH_2Ph	CH_3
$CH(CH_3)OH$	CH_3CH_2OH
$CH(CH_3)OH$	BnOH

Figure 4. Artificial glycopeptide libraries (2 × 96 compounds; R = CH_2, model **1**; R = CO, model **2**) with α-galactoside and β-galactoside derivatives.

based agents that may have the potential of blocking the growth of the mycobacterium tuberculosis cell-wall biosynthesis.

In summary, a fully automated method for the synthesis of artificial glycopeptides having two (similar or different) carbon-linked glycosyl moieties on a dipeptide scaffold has been achieved successfully. An automated method has been further utilized in the high-throughput library synthesis of 384 artificial glycopeptides, synthesized. It is hoped that further development of automated synthesis methods in the area of complex carbohydrates, glycoconjugates, and small-molecule carbohydrate mimics will provide rapid access to derivatives and that they may serve as useful tools in understanding glycobiology at the chemical level.

Experimental Procedure

1. Automated Synthesis of Artificial Glycopeptide Library by Multiple Organic Synthesizer (Figure 4). 1.a. Stepwise Double Reductive Amination Method. α- and β-Galactoside-Derived Artificial Glycopeptide Library (Model 1, R = CH_2, Figure 4). TentaGel S Ram (200 mg/well, 0.25 mmol/g, 0.05 mmol/well) was swelled in DMF (2 mL) for 30 min, mixing at 500 rpm. The wells were emptied, and the resin was washed with additional DMF (2 mL). Removal of the Fmoc group was effected using 20% piperidine in DMF (2 mL) for 30 min (repeat cycle). The resin was washed with DMF, MeOH (repeat two times), and then DMF. The first amino acid (4 equiv in 700 μL of DMF) was coupled using HBTU (4 equiv in 700 μL of DMF) and DIPEA (8 equiv in 600 μL of DMF), mixed for 25 h, emptied, and washed with DMF, and the cycle was repeated two times. The resin was washed with DMF, MeOH (repeat two times), and then DMF. Removal of the Fmoc group was effected using 20% piperidine in DMF (2 mL) for 30 min (repeat cycle). The resin was washed with DMF, MeOH (repeat two times), and then TMOF. The primary nitrogen on the amino acid was reductively aminated using sugar aldehyde (α-Gal 2.5 equiv or β-Gal 2.0 equiv in 800 μL of TMOF), $NaCNBH_3$ (α-Gal 2.5 equiv or β-Gal 2.0 equiv in 600 μL of MeOH), and AcOH (0.5% per volume in 600 μL of TMOF), mixed for 5 h, emptied, and washed with MeOH and DCM (repeat washings) and then with MeOH and DMF. The second amino acid (4.0 equiv in 700 μL of DMF) was coupled using HATU (4.0 equiv in 700 μL of DMF) and DIPEA (8.0 equiv in 600 μL of DMF), mixed for 40 h, emptied, and washed with DMF, DCM, and DMF, and the cycle was repeated. An additional set of couplings was carried out. The second amino acid (2.0 equiv in 700 μL of DMF) was coupled using HATU (2.0 equiv in 600 μL of DMF), HOAt (2.0 equiv in 200 μL of DMF), and DIPEA (4.0 equiv in 500 μL of DMF), mixed for 40 h, emptied, and washed with DMF, DCM, and DMF, and the cycle was repeated four times. The resin was washed with DMF, MeOH (repeat two times), and then DMF. Removal of the Fmoc group was effected using 20% piperidine in DMF (2 mL) for 30 min (repeat cycle). The resin was washed with DMF, MeOH (repeat two times), and then TMOF. The second amino acid was reductively aminated using sugar aldehyde (α-Gal 2.5 equiv, β-Gal 2.0 equiv in 800 μL of TMOF), $NaCNBH_3$ (α-Gal 2.5 equiv, β-Gal 2.0 equiv in 600 μL of MeOH), and AcOH (0.5 % per volume in 600 μL of TMOF), mixed for 5 h, emptied, and washed with MeOH, DCM (repeat washings). Deprotection of the acetyl groups was carried out using hydrazine hydrate (2 mL 1:11 in DMF), mixed for 4.5 h, emptied, and washed with DMF, MeOH, 30% AcOH, MeOH, 30% AcOH, and finally MeOH. The resin was dried thoroughly by emptying the rack 30 min × 8 times. The compounds were cleaved using 2 mL of 95% TFA (2.5% H_2O, 2.5% TIS), mixed for 2 h, emptied, and washed with TFA and then MeOH. Wash cycles consisted of the addition of solvent (2 mL) to each well, the sample was mixed for 3 min at 500 rpm, the wells were emptied, and the cycle was repeated.

1.b. Reductive Amination/Sugar Acid Coupling Method. α- and β-Galactoside-Derived Artificial Glycopeptide Library (Model 2, R = CO, Figure 4). TentaGel S Ram (200 mg/well, 0.25 mmol/g, 0.05 mmol/well) was swelled in DMF (2 mL) for 30 min, mixing at 500 rpm. The wells were emptied, and the resin was washed with additional DMF (2 mL). Removal of the Fmoc group was effected using 20% piperidine in DMF (2 mL) for 30 min (repeat cycle). The resin was washed with DMF, MeOH (repeat two times), and then DMF. The first amino acid (4.0 equiv in 700 μL of DMF) was coupled using HBTU (4.0 equiv in 700 μL of DMF) and DIPEA (8.0 equiv in 600 μL of DMF), mixed for 25 h, emptied, and washed with DMF, and the cycle was repeated two times. The resin was washed with DMF, MeOH (repeat 2 times), and then DMF. Removal of the Fmoc group was effected using 20% piperidine in DMF (2 mL) for 30 min (repeat cycle). The resin was washed with DMF, MeOH (repeat two times), and then TMOF. The primary nitrogen on the amino acid was reductively aminated using sugar aldehyde (α-Gal 2.5 equiv or β-Gal 2.0 equiv in 800 μL of TMOF), $NaCNBH_3$ (α-Gal 2.5 equiv or β-Gal 2.0 equiv in 600 μL of MeOH), and AcOH (0.5 % per volume in 600 μL of TMOF), mixed for 5 h, emptied, and washed with MeOH and DCM (repeat washings) and then with MeOH and DMF. The second amino acid (4.0 equiv in 700 μL of DMF) was coupled using HATU (4.0 equiv in 600 μL of DMF), HOAt (2.0 equiv in 200 μL of DMF), and DIPEA (8.0 equiv in 500 μL of DMF), mixed for 40 h, emptied, and washed with DMF, DCM, and DMF, and the cycle was repeated. An additional set of couplings was carried out. The second amino acid (2.0 equiv in 700 μL of DMF) was coupled using HATU (2.0 equiv in 600 μL of DMF), HOAt (2.0 equiv in 200 μL of DMF), and DIPEA (4.0 equiv in 500 μL of DMF), mixed for 40 h, emptied, and washed with DMF, DCM, and DMF, and the cycle was repeated four times. The resin was washed with DMF, MeOH (repeat wash), and then DMF. Removal of the Fmoc group was effected using 20% piperidine in DMF (2 mL) for 30 min (repeat cycle). The resin was washed with DMF, MeOH (repeat two times), and then DMF. The sugar acid was coupled using acetylated galactose acid (α-Gal 2.0 equiv, β-Gal 1.5 equiv in 700 μL of DMF), HATU (2.0 equiv in 700 μL of DMF), DIPEA (4.0 equiv in 600 uL of DMF), mixed for 40 h, and washed with DMF, MeOH, DMF, and the cycle was repeated. Washings were done with additional DMF, DCM, and MeOH and were repeated. Deprotection of the acetyl groups was carried out using hydrazine hydrate (2 mL, 1:11 in DMF), mixed for 4.5 h, emptied, and washed with DMF, MeOH, 30% AcOH, MeOH, 30% AcOH, and finally MeOH. The resin was dried thoroughly by emptying the rack 30 min × 8 times. The compounds were cleaved using 2 mL of 95% TFA (2.5% H_2O, 2.5% TIS), mixed for 2 h, emptied, and washed with TFA and then MeOH. Wash cycles consisted of adding solvent (2 mL) to the each well, mixing for 3 min at 500 rpm, emptying the wells, and repeating.

2. Automated Synthesis of Artificial Glycopeptide Library by Multiple Organic Synthesizer (Figure 3). 2.a. Automated Synthesis of α-Glucoside and α-Mannoside Based Artificial Glycopeptide Libraries. TentaGel S RAM resin was purchased from Rapp Polymere GmbH. HBTU was purchased from Advanced ChemTech. Rink amide MBHA and all Fmoc amino acids with side chain protection compatible with Fmoc chemistry were obtained from Nova-Biochem. DIPEA, TFA, hydrazine hydrate, triisopropylsilane, sodium cyanoborohydride, and trimethyl orthoformate were purchased from Aldrich. All solvents (highest quality grade) were obtained from commercial suppliers and used without further purification. Reverse-phase HPLC was carried out on a Vydac C-18 reverse-phase column.

2.b. First Amino Acid Loading. TentaGel SRAM resin (200 mg, loading 0.24 mmol/g) was placed in each well of the 96-well block of the Advanced ChemTech 496 MOS. The resin was allowed to swell in DMF (2.0 mL) for 30 min and then was drained. The Fmoc group was removed with 20% piperidine/DMF (2.0 mL) for 40 min (2×). The resin was washed with DMF (2×), MeOH (2×), DMF (2×), MeOH (2×), DMF (2×). The C-terminal Fmoc amino acid (4 equiv) was coupled using HBTU (4 equiv) and DIPEA (8 equiv) for 25 h with one to two repeats based on MS analysis of representative samples. The resin was then washed with DMF (2×), MeOH (2×), DMF (2×), methanol (2×), and DMF (2×). The Fmoc group was removed as described, and the resin was washed as above using an additional wash with TMOf (2×) to prepare the resin for the next step.

2.c. Reductive Amination. The following reagents were added successively to the amino acid resin: protected C-linked sugar aldehyde (1.5−2.5 equiv) dissolved in TMOf, $NaBH_3CN$ (1.5−2.5 equiv dissolved in a minimum of dry MeOH and diluted to volume with TMOf), and glacial acetic acid in TMOf (0.5% final concentration calculated on the 2.0 mL volume of solvent in the well). The reagents were mixed for 4.5 h. The concentration of the sugar aldehyde must be optimized for the different sugars. For example, 2-(tetra-*O*-acetyl-D-mannopyranosyl)ethanal required 1.5 equiv, and the same 1.5 equiv was used for $NaBH_3CN$. However, 2-(tetra-*O*-acetly-D-glucopyrannosyl)ethanal required 2.0 equiv, and the corresponding 2.0 equiv of $NaBH_3CN$ was used. After this reaction the resin was washed successively with TMOf (2×), DCM (2×), MeOH (2×), DCM (2×), MeOH (2×), and DMF (2×).

2.d. Amino Acid Coupling. Fmoc amino acid (4 equiv) was coupled with HATU (4 equiv) and DIPEA (8 equiv) for 40 h with one repeat, and samples were checked by ES-MS to ascertain if further couplings are required. After completion, the resin was washed successively with DMF (2×), MeOH (2×), DMF (2×), MeOH (2×), and DMF (2×). The Fmoc group was removed, and the resin was washed as described above. The next reaction was either a second reductive amination reaction (same procedure as the first reductive amination reaction as described above) or the coupling of the sugar acid.

2.e. Sugar Acid Coupling. The protected C-linked sugar derivative [2-(tetra-*O*-acetyl-D-manno- or glucopyranosyl)-ethanoic acid] (4 equiv) was coupled using HATU (4 equiv) and DIPEA (8 equiv) for 30 h with one repeat. Upon completion, the neoglycopeptide resin was washed with DMF

(2×), MeOH (2×), DCM (2×), MeOH (2×), DCM (2×), MeOH (2×), and DMF (2×).

2.f. Deacetylation. The acetyl groups were then removed with $H_2NNH_2 \cdot H_2O$/DMF (1:11 v/v), 2 mL for 4.5 h. After deacetylation, the artificial glycopeptide resin was washed with the following: DMF (2×), MeOH (2×), 30% acetic acid/MeOH, MeOH, 30% acetic acid/MeOH, MeOH.

2.g. Cleavage of Artificial Glycopeptide from the Resin. The resin was prepared for cleavage, dried for 1 h, and cleaved from the resin with TFA/TIPS/H_2O (95:2.5:2.5 v/v/v) for 2 h.

2.h. Isolation of Artificial Glycopeptides. The cleaved neoglycopeptides were concentrated on the GeneVac and dried overnight under high vacuum. ES-MS was determined on the samples, and representative samples were purified by RP-HPLC on a Gilson HPLC equipped with a model 215 liquid handler.

Acknowledgment. The authors thank Michael Barnes, Don Leek, and Malgosia Darosewska for technical support. Financial support from an NRC-SIMS postdoctoral fellowship was given to K.D.R. and A.B. Dr. Brady Clark is thanked for providing critical comments on the manuscript.

Supporting Information Available. Additional synthesis procedures for glycopeptides and HPLC and MS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357.

(2) (a) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683. (b) Varki, A. *Glycobiology* **1993**, *3*, 97. Branza-Nichita, N.; Petrescu, A. J.; Negroiu, G.; Dwek, R. A.; Petrescu, S. M. *Chem. Rev.* **2000**, *100*, 4697. Butters, T. D.; Dwek, R. A.; Platt, F. M. *Chem. Rev.* **2000**, *100*, 4683. Duus, J. Ø.; St. Hilaire, P. M.; Meldal, M.; Bock, K. *Pure Appl. Chem.* **1999**, *71*, 755.

(3) (a) Danishefsky, S. J.; Allen, J. A. *Angew. Chem., Int. Ed.* **2000**, *39*, 836 and references therein. (b) Gege, C.; Vogel, J.; Bendas, G.; Rothe, U.; Schmidt, R. R. *Chem.—Eur. J.* **2000**, *6*, 111. (c) Seeberger, P. H.; Danishefsky, S. J. *Acc. Chem. Res.* **1998**, *31*, 685. (d) Meinjohanns, E.; Meldal, M.; Paulsen, H.; Dwek, R. A.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1998**, 549. (e) Sames, D.; Chen, X.-T.; Danishefsky, S. J. *Nature* **1997**, *389*, 587. (f) Seitz, O.; Wong, C.-H. *J. Am. Chem. Soc.* **1997**, *119*, 8766.

(4) For recent articles on automated synthesis of complex carbohydrates and carbohydrate conjugates, see the following. (a) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523. (b) Sears, P.; Wong, C.-H. *Science* **2001**, *291*, 2344. (c) Seeberger, P. H.; Haase, W.-C. *Chem. Rev.* **2000**, *100*, 4349. (d) Koeller, K. M.; Wong, C.-H. *Chem. Rev.* **2000**, *100*, 4465. (e) Herzner, H.; Reipen, T.; Schultz, M.; Kunz, H. *Chem. Rev.* **2000**, *100*, 4495.

(5) (a) *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1997. (b) Benjamin, G.; Davis, J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2137. (c) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503. Hanessian, S.; Lou, B. *Chem. Rev.* **2000**, *100*, 4443. (d) Boons, G.-J.; Demchenko, A. V. *Chem. Rev.* **2000**, *100*, 4539. (e) Jung, K.-H.; Muller, M.; Schmidt, R. R. *Chem. Rev.* **2000**, *100*, 4423.

(6) (a) Balkenhohol, F.; von dem Bussche-Hunnefeld, C.; Lansky, A.; Zechel, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2288. (b) *Combinatorial Chemistry and Molecular Diversity in Drug Discovery*; Gordon, E. M., Kerwin, J. F., Jr., Eds.; Wiley: New York, 1998. (c) Obrecht, D.; Villalgordo, J. M. *Solid-Supported Combinatorial and Parallel Synthesis of Small-Molecular-Weight Compound Libraries*; Pergamon: New York, 1998. (d) *Solid Phase Organic Synthesis*; Burgess, K., Ed.; Wiley-Interscience: New York, 2000.

(7) (a) Marcaurelle, L. A.; Bertozzi, C. R. *Chem.—Eur. J.* **1999**, *5*, 1384. (b) Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833. (c) Sears, P.; Wong, C.-H. *Angew. Chem., Int. Ed.* **1999**, *38,* 2300. (d) St. Hilaire, P. M.; Meldal, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 1162.

(8) For complex carbohydrates, carbohydrate conjugates, and cabohydrate mimics related combinatorial chemistry, see the following. (a) Arya, P.; Ben, R. N. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1280. (b) Arya, P.; Ben, R. N.; Kutterer, K. M. K. In *Organic Synthesis Highlights*; Schmalz, H.-G., Ed.; Wiley-VCH: Weinheim, Germany, 2000; vol. 4, p 337. (c) Barkley, A.; Arya, P. *Chem.—Eur. J.* **2001**, *7*, 555. (d) Randell, K. D.; Barkley, A.; Arya. P. *Comb. Chem. High Throughput Screening*, in press. (e) Sofia, M. J. *Mol. Diversity* **1998**, *3,* 75. Wang, Z.-G.; Hindsgaul, O. *Glycoimmunology* **1998**, *2,* 219. (f) Schweizer, F.; Hindsgaul, O. *Curr. Opin. Chem. Biol.* **1999**, *3*, 291.

(9) Arya, P.; Kutterer, K. M. K.; Barkley, A. *J. Comb. Chem.* **2000**, *2*, 120−126.

(10) For a recent review on carbon-linked glycosyl amino acids, see the following. Dondoni, A.; Marra, A. *Chem. Rev.* **2000**, *100,* 4395.

(11) (a) Helenius, A.; Aebi, M. *Science* **2001**, *291*, 2364. (b) Rudd, P. M.; Elliot, T.; Cresswell, P.; Wilson, I. A.; Dwek, R. A. *Science* **2001**, *291*, 2370. (c) Ellgaard, L.; Molinari, M.; Helenius, A. *Science* **1999**, *286*, 1882. (d) Zapun, A.; Jakab, C. A.; Thomas, D. Y.; Bergeron, J. M. *Structure* **1999**, *7*, R173. (e) Chevet, E.; Jakob, C. A.; Thomas, D. Y.; Bergeron, J. J. M. *Semin. Cell Dev. Biol.* **1999**, *10*, 473.

(12) Barkley, A.; Randell, K. D.; Arya, P.; Tessier, D.; Thomas, D. Y. Unpublished results.

(13) (a) McNeil, M. R. In *Genetics of Bacterial Polysaccharides*; Goldberg, J. B., Ed.; CRC Press: New York, 1999. (b) Ma, Y.; Stern, R. J.; Scherman, M. S.; Vissa, V. D.; Yan, W.; Cox Jones, V.; Zhang, F.; Franzblau, S. G.; Lewis, W. H.; McNeil, M. R. *Antimicrob. Agents Chemother.* **2001**, *45*, 1407.

(14) Barkley, A.; Randell, K. D., Arya, P.; McNeil, M. R. *unpublished results*.

CC020001I