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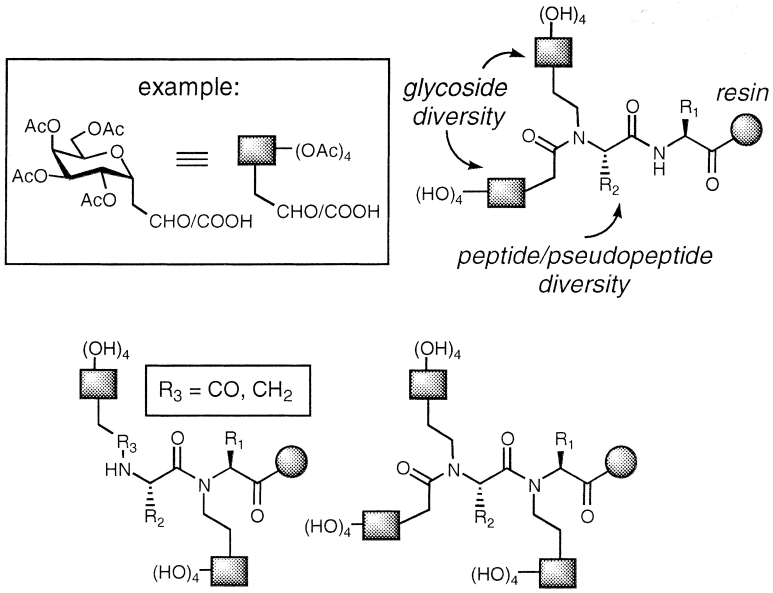

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Glycomimetics: A Programmed Approach toward Neoglycopeptide Libraries[1a]

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A programmed synthesis of neoglycopeptides has been developed in which two, similar or different, glycoside moieties could be attached either (i) at the N-terminal of short peptides or (ii) one at the N-internal and the other(s) at the N-terminal site, in a highly flexible and controlled manner. A stepwise branching of N-terminal peptides has been achieved by glycoside aldehyde reductive amination followed by the glycoside carboxylic acid coupling (model 1). In another approach, after N-alkylation with glycoside aldehyde, the N-glycosylated derivative is subjected to peptide synthesis. This is then followed by the attachment of the second glycoside moiety at the N-terminal using either glycoside aldehyde or glycoside carboxylic acid derivative (model 2). Alternatively, the attachment of second and third glycoside derivatives could be achieved simultaneously, by reductive amination/carboxylic acid couplings (model 3). The methodologies presented here are highly versatile and combine diversity in both peptides/pseudopeptides and glycoside moieties.

Introduction

There is a growing interest in understanding the role of cell-surface glycoconjugates that are involved in various biological (i.e., fertilization) and pathological (i.e., bacterial, viral, metastasis) processes.[2,3] Unlike oligonucleotides and polypeptides, oligosaccharides are complex, branched molecules and present tremendous challenges in obtaining synthetic samples in sufficient quantities.[4] Simple compounds that could function like complex carbohydrates allow for an understanding of the structure and function of complex carbohydrates and serve as lead compounds for developing new therapeutics. Due to the flexible and branched nature of oligosaccharides, rational-based design strategies to obtain structural and functional mimics of carbohydrates are difficult to apply and have met with little success. In addition, the lack of a molecular-based understanding and the inherent individual weak carbohydrate−protein interactions (i.e., millimolar region) further limit the application of rational designs.[2h] A combinatorial approach to obtain diverse compounds in a rapid manner seems to be an appropriate choice.[5] By applying combinatorial strategies, the ultimate challenge is to obtain compounds that are simple in nature and possess similar or even better binding potential with the target receptors.[6]

Background

Over the years, tremendous progress has been made toward the synthesis of glycopeptides and glycopeptide mimics.[7] These compounds serve as useful probes for understanding carbohydrate ligand−carbohydrate binding protein receptor mediated biorecognition processes. The building block approach is commonly utilized for the synthesis of glycopeptides, although solid-phase glycosylation of PEG resin-bound peptides has been achieved in some cases.[8] In search of glycopeptides as mimics of oligosaccharides, St. Hilaire and Meldal reported the synthesis of encoded-glycopeptide libraries using a building block approach.[9] Several glycosylated-based amino acid derivatives were synthesized by solution phase and then subjected to solid-phase synthesis. Encoded, resin-bound glycopeptide libraries were screened against the labeled lectin *Lathyrus odoratus*, and several glycopeptide-based ligands having Man- and Glc-NAc as glycoside moieties were identified in solid-phase hemagglutination assay.[9]

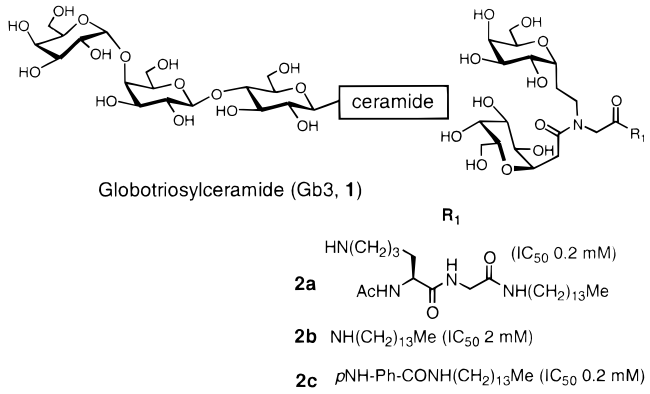

Figure 1. α-Galactoside-based C-linked neoglycopeptides.

Recently, we reported an automated, solid-phase synthesis of carbon-linked neoglycopeptides in which glycoside-based carboxylic acids were coupled onto the side chain, ϵ-amino group(s) of resin-bound peptides.[10] For example, C-linked α-galactosyl carboxylic acid and C-linked *N*-di-α-galactosylglycine[10b] were coupled to resin-bound peptides, and fully protected neoglycopeptides were obtained in 50−65% isolated yields. During the course of our study, three neoglycopeptide derivatives (Figure 1, **2a**−**c**) were identified using ELISA, as inhibitors of verotoxin binding to globotriosyl-

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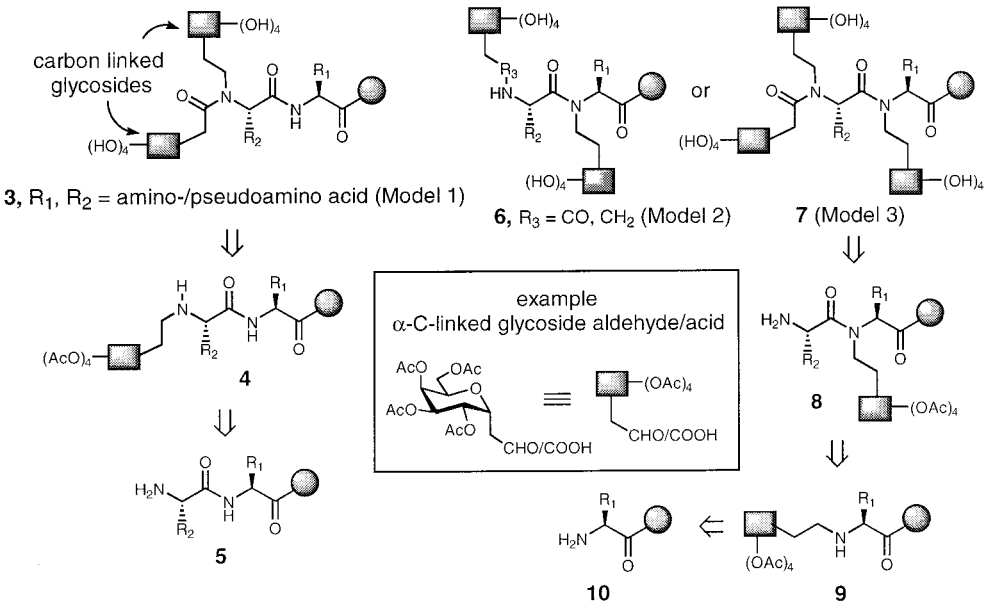


Figure 2. Retrosynthetic analysis−solid-phase approach to neoglycopeptide libraries.

ceramide, Gb3 (**1**).[11] Gb3 is present on human epithelial cells and is involved in the binding of verotoxin receptors. Verotoxin and structurally homologous Shiga and Shiga-like toxins are produced by *Shigella* bacterium or by certain enterohemorrhagic *Escherichia coli*. The binding subunits of verotoxin are lectins, arranged as a symmetrical pentamer, that recognize the galabiose moiety (Galα1-4Gal) of glycosphingolipid derivatives.[12] To our surprise, neoglycopeptide derivatives **2a**−**c** contain only a portion of the recognition element of the trisaccharide portion of the Gb3 ligand and yet exhibit inhibitory effects at submillimolar concentrations (i.e., $IC_{50} = 2.0-0.2$ mM).[12f] The appropriate presentation of the two α-*C*-galactose moieties at the N-terminal of the short peptides/pseudopeptides seems to play a crucial role in generating this effect. In addition, the secondary groups (i.e., groups other than the carbohydrates) may either contribute in subsite-oriented interactions with the protein receptors or mimic the internal sugars of the cell-surface ligand, Gb3.

Synthetic Strategy and Rationale

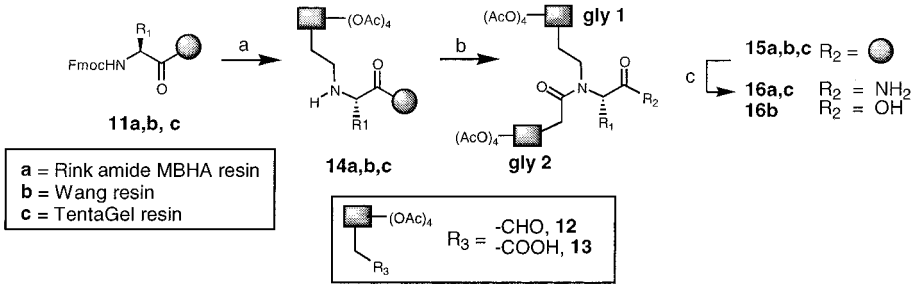
Herein, we present an automated, solid-phase strategy to obtain libraries of neoglycopeptides (for retrosynthetic analysis, see Figure 2). In our approach, different α- or *β*-carbon-linked carbohydrate based aldehyde and carboxylic acid derivatives can be incorporated either at the N-terminal moiety or at the internal amide nitrogen of short peptides/pseudopeptides in a highly flexible and control-oriented manner. Using neoglycopeptide derivatives, the contribution of the secondary groups (i.e., peptide/pseudopeptide backbone) to overall binding, through additional subsite-oriented interactions with protein receptors or by mimicking portions of the complex carbohydrate, could also be explored. To facilitate this study, we developed a programmed multistep synthesis on solid phase, where two carbon-linked moieties (homo- or hetero- derivatives) could be connected at the N-terminal site of amino acids or short peptides/pseudopeptides. This approach allows for the investigation of the diversity of both glycoside and the peptide/pseudopeptide moiety. For example, it is possible to obtain 1600 neoglycopeptide derivatives of compound **3** (Figure 2, model 1) from 4 glycoside aldehydes, 4 glycoside carboxylic acids, and 10 amino acids. A library of short peptides (such as dipeptide **5**) could react with a glycoside aldehyde via reductive amination to produce compound **4**. Neoglycopeptide **3** can be obtained from the coupling of compound **4** with a glycoside carboxylic acid.

In another approach, a plan would be to incorporate the glycoside moiety at the internal amide nitrogen sites, followed by N-terminal branching as discussed above (for retrosynthetic analysis, see Figure 2, models 2 and 3). In this strategy, the first step is reductive amination, which is performed using glycoside aldehydes to obtain glycosylated derivatives of amino acids, **9**. This is followed by peptide coupling leading to internal *N*-glycosyl dipeptide derivatives, **8**. Compound **8** could be subjected to various reactions, (i) coupling with glycosyl carboxylic acid to obtain compound **6** (R_3 = CO, model 2), (ii) reductive amination with glycosyl aldehyde to obtain diglycosyl derivatives of dipeptide **6** (R_3 = CH_2, model 2), and (iii) stepwise reaction with the glycosyl aldehydes followed by coupling of glycosyl carboxylic acids to obtain the triglycosyl derivative, **7** (model 3). Like in the previous case, this approach represents a highly controlled and flexible manner whereby one can anchor similar or different glycoside moieties at various positions of the peptide/pseudopeptide backbone to generate neoglycopeptide libraries.

Results and Discussion

Programmed Synthesis of C-Linked Diglycosyl Branched Amino Acid Amides (16a−c, Scheme 1). To develop a multistep solid-phase methodology, in an automated manner, initial studies of the coupling of glycoside moieties were carried out with a single amino acid attached to Rink amide MBHA and TentaGel resin. Reaction conditions for each step were optimized, initially, by manual solid-phase synthesis. Using Rink amide MBHA and TentaGel resin, reductive

Scheme 1.[a] Programmed Synthesis of C-Linked, Branched Glycosyl Amino Acids



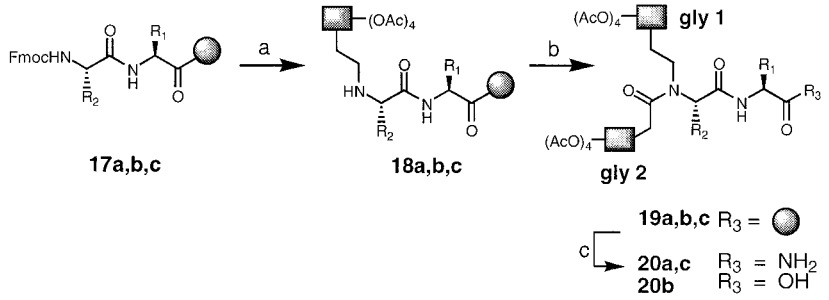
Entry	Resin	gly 1	gly 2	R_1	R_2
1	a	α-gal	α-gal	H	NH_2
2	a	β-gal	α-gal	H	NH_2
3	a	α-glc	α-gal	H	NH_2
4	a	α-gal	α-gal	Me	NH_2
5	a	β-gal	α-gal	Me	NH_2
6	a	α-glc	α-gal	Me	NH_2
7	a	α-gal	α-gal	Bn	NH_2
8	a	β-gal	α-gal	Bn	NH_2
9	a	α-glu	α-gal	Bn	NH_2
10	a	β-gal	β-gal	H	NH_2
11	b	α-gal	α-gal	H	OH
12	b	α-gal	α-gal	Me	OH
13	b	α-gal	α-gal	Bn	OH
14	c	α-glc	α-man	Me	NH_2
15	c	β-glc	α-glc	Me	NH_2
16	c	α-man	α-glc	Me	NH_2
17	c	α-man	α-man	Me	NH_2
18	c	α-glc	α-man	Bn	NH_2
19	c	β-glc	α-glc	Bn	NH_2
20	c	α-man	α-glc	Bn	NH_2
21	c	α-man	α-man	Bn	NH_2
22	c	α-glc	α-man	$CH_2CH(Me)_2$	NH_2
23	c	β-glc	α-glc	$CH_2CH(Me)_2$	NH_2
24	c	α-man	α-glc	$CH_2CH(Me)_2$	NH_2
25	c	α-man	α-man	$CH_2CH(Me)_2$	NH_2

[a] For a 0.03 mmol scale synthesis: (a) (i) 20% piperidine/DMF (2 × 1.5 mL), (ii) **12** (2.5 equiv), $NaCNBH_3$ (2.5 equiv), TMOF, repeat; (b) **13** (4.0 equiv), HATU (4.0 equiv), DIEPA (8.0 equiv), DMF, repeat; (c) 95% TFA/2.5 TIPS/2.5% H_2O (1.5 mL).

amination of Gly, Ala, Phe, and Leu was tried with several carbon-linked glycoside aldehydes (i.e., α-gal, *β*-gal, α-man, α-glc), and monoglycosylamino acids were obtained in 50−85% isolated yields.[13] For the fully programmed automated solid-phase synthesis of branched glycosides on a single amino acid, we commenced with the removal of the Fmoc protecting group from Rink amide MBHA and TentaGel resin using Advanced ChemTech 496 multiple organic synthesizer (MOS). This was performed twice, for a half hour duration each time, utilizing 20% piperidine in DMF. After a DMF and methanol washing protocol, the amino acid (Fmoc-Gly-OH, Fmoc-Ala-OH, Fmoc-Phe-OH, Fmoc-Leu-OH) was coupled to the resin to give compounds **11a**,**c**. Double coupling of the amino acid was performed in DMF using the HATU or HBTU coupling reagent, with the addition of DIPEA, for 5−6 h. This was followed by another wash cycle and a Fmoc protecting group removal cycle. Next, a C-linked α- or *β*-glycoside aldehyde (**12**, α-galactose, *β*-galactose, α-glucose, or α-mannose) was added to the free amino group on the resin via reductive amination to obtain compounds **14a**,**c**. This was performed in 3−5 h for imine formation and 1−4 h for reduction, using TMOF as solvent and $NaCNBH_3$ as the reducing agent, and with a second repetition of the cycle. After another washing cycle, C-linked α- or *β*-glycoside carboxylic acid (**13**) was coupled to the terminal secondary amine to produce branched diglycosylated amino acids on resin, **15a**,**c**. The coupling was accomplished in a double cycle of 12−15 h duration, once again using the HATU protocol mentioned earlier. A final wash cycle was then completed, and the products were cleaved from the resin employing 95% TFA/2.5% TIPS/2.5% H_2O, followed by a methanol rinse. The products, **16a**,**c**, obtained were then evaporated to dryness, and their purity was determined by reverse-phase HPLC (30−75% purified isolated yields).[14]

Three examples of the automated solid-phase synthesis of C-linked branched glycosides on a single amino acid using Wang resin (**b** series: **11b** to **16b**) preloaded with the amino acid were also carried out. In the use of Wang resin we

Scheme 2.[a] Programmed Synthesis of C-Linked Neoglycopeptides (Model 1)



Entry	Resin	gly 1	gly 2	R_1	R_2	R_3
1	a	α-gal	α-gal	Me	H	NH_2
2	a	α-gal	α-gal	H	Me	NH_2
3	a	α-glc	α-gal	H	Me	NH_2
4	b	α-gal	α-gal	H	Bn	OH
5	b	α-gal	α-gal	H	Me	OH
6	b	α-glc	α-gal	H	Me	OH
7	c	α-glc	α-man	Me	Bn	NH_2
8	c	α-glc	α-glc	Me	Bn	NH_2
9	c	α-man	α-glc	Me	Bn	NH_2
10	c	α-man	α-man	Me	Bn	NH_2
11	c	α-glc	α-man	$CH_2CH(Me)_2$	Me	NH_2
12	c	α-glc	α-glc	$CH_2CH(Me)_2$	Me	NH_2
13	c	α-man	α-glc	$CH_2CH(Me)_2$	Me	NH_2
14	c	α-man	α-man	$CH_2CH(Me)_2$	Me	NH_2

[a] For a 0.03 mmol scale synthesis, standard peptide synthesis: 20% piperidine/DMF (2 × 1.5 mL); a.a. (4.0 equiv), HATU or HBTU (4.0 equiv), DIPEA (8.0 equiv), DMF, repeat; (a) (i) 20% piperidine/ DMF (2 × 1.5 mL), (ii) **12** (3.0 equiv), $NaCNBH_3$ (3.0 equiv), TMOF, repeat; (b) **13** (4.0 equiv), HATU (4.0 equiv), DIPEA (8.0 equiv), DMF, repeat; (c) 95% TFA/2.5% TIPS/2.5% H_2O (1.5 mL).

generate C-terminal acids rather than C-terminal amides, as one generates with the Rink amide MBHA resin. The procedure used for the synthesis is identical to that outlined for the Rink amide MBHA resin, with the exception that the first two steps are not required.

Programmed Synthesis of N-Terminal, C-Linked Diglycosyl Branched Dipeptides (20a−c, Scheme 2, Model 1). In a similar manner, N-terminal, C-linked diglycoside branched dipeptides were also generated using Rink amide MBHA resin, preloaded Wang and TentaGel resin. This was performed in the same manner as for the branched diglycoside synthesis on a single amino acid with just the addition of extra Fmoc removal and amino acid coupling steps (30−60% purified isolated yields).[14]

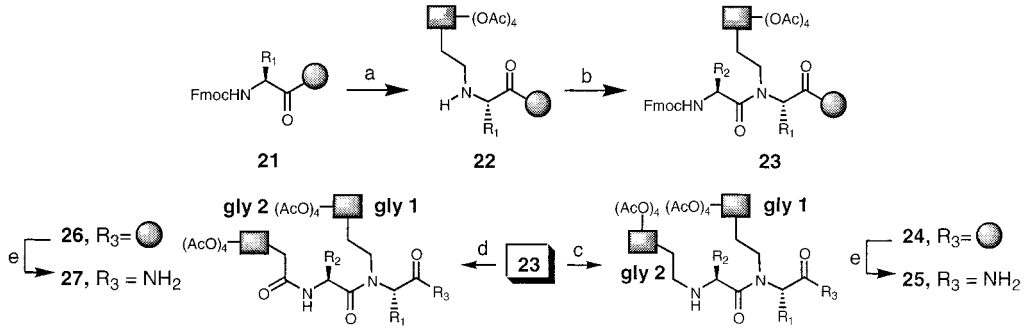
Programmed Synthesis of C-Linked Neoglycopeptides (25 and 27, Scheme 3, Model 2). Using model 2, the plan is to develop a synthetic methodology in which it is possible to incorporate the glycosyl moiety at the N-internal site of the peptide backbone and at the N-terminal site in a stepwise manner. The internal glycoside moiety was added to dipeptides as a branched glycoside through reductive amination. After coupling of the glycoside aldehyde to the first amino acid via reductive amination (**22**), it was coupled with the Fmoc protected amino acid to generate a dipeptide derivative with C-linked glycoside residue at the internal nitrogen of the amide bond (**23**). Following Fmoc removal, it was then subjected to two independent set of reaction conditions, (i.e., reductive amination with C-glycoside aldehyde, and coupling with C-glycoside carboxylic acid). The reductive amination approach led to the synthesis of neoglycopeptide derivatives **25** (35−45% purified isolated yields).[14] Neoglycopeptides **27** were obtained (40−55% purified isolated yields) from coupling with C-glycoside carboxylic acid.

Programmed Synthesis of C-Linked Neoglycopeptides (29, Scheme 4, Model 3). In yet another diverse class of related neoglycopeptides having three glycosides, an internal glycoside moiety was added to the dipeptide branched glycosides through reductive amination. After the Fmoc removal from compound **23**, it was subjected to reductive amination with C-glycoside aldehyde followed by coupling with the C-glycoside carboxyl acid as discussed earlier. Three products, **25** (10−25% purified isolated yields), **27** (15−25% purified isolated yields), and **29** (15−20% purified isolated yields), were isolated from each reaction, after the cleavage from the resin. The ratio of the products is dependent upon the nature of the glycoside moiety. Further work is in progress to maximize the yields of neoglycopeptide derivatives based upon model 3.

Conclusion

We have developed a new methodology to obtain diverse neoglycopeptides using multistep solid-phase synthesis. The total synthesis was carried out in an automated manner using a robotic Advanced ChemTech 496 multiple organic synthesizer. The approaches presented are highly versatile and can be used to exploit the diversity of the glycoside and of

Scheme 3.[a] Programmed Synthesis of C-Linked Neoglycopeptides (Model 2)



Neoglycopeptide 25

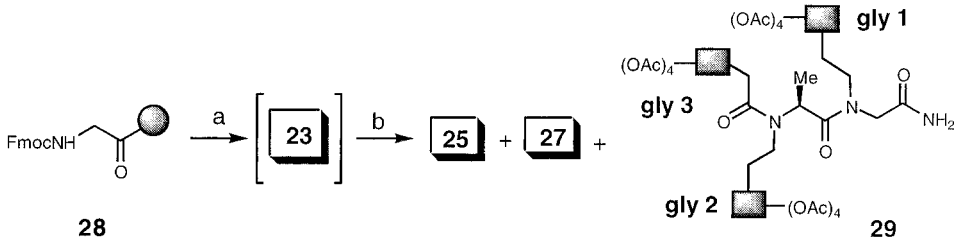
Entry	gly 1	gly 2	R_1	R_2	R_3
1	α-glc	α-glc	H	Bn	NH_2
2	α-man	α-man	H	Bn	NH_2
3	α-glc	α-glc	Me	Bn	NH_2
4	α-man	α-man	Me	Bn	NH_2
5	α-glc	α-glc	H	Me	NH_2
6	α-man	α-man	H	Me	NH_2

Neoglycopeptide 27

Entry	gly 1	gly 2	R_1	R_2	R_3
1	α-glc	α-glc	H	Bn	NH_2
2	α-man	α-man	H	Bn	NH_2
3	α-man	α-glc	H	Bn	NH_2
4	α-glc	α-glc	Me	Bn	NH_2
5	α-man	α-man	Me	Bn	NH_2
6	α-man	α-glc	Me	Bn	NH_2
7	α-glc	α-glc	H	Me	NH_2
8	α-man	α-man	H	Me	NH_2
9	α-man	α-glc	H	Me	NH_2

[a] For a 0.03 mmol scale synthesis: (a) (i) 20% piperidine/DMF (2 × 1.5 mL), (ii) **12** (2.5 equiv), $NaCNBH_3$ (2.5 equiv), TMOF, repeat; (b) a.a. (4.0 equiv), HATU (4.0 equiv), DIPEA (8.0 equiv), DMF, repeat; (c) repeat step (a); (d) (i) repeat (a) (i), (ii) **13** (4.0 equiv), HATU (4.0 equiv), DIPEA (8.0 equiv), DMF, repeat; (e) 95% TFA/2.5% TIPS/2.5% H_2O (1.5 mL).

Scheme 4.[a] Programmed Synthesis of C-Linked Neoglycopeptides (Model 3)



Neoglycopeptide 29

Entry	gly 1	gly 2	gly 3
1	α-gal	α-glc	α-gal
2	α-gal	α-gal	α-gal
3	α-glc	α-glc	α-gal
4	α-glc	α-gal	α-gal

[a] For a 0.03 mmol scale synthesis: (a) (i) 20% piperidine/DMF (2 × 1.5 mL), (ii) **12** (3.0 equiv), $NaCNBH_3$ (3.0 equiv), TMOF, repeat, (iii) (a) (i), (iv) a.a. (4.0 equiv), HATU (4.0 equiv), DIPEA (8.0 equiv), DMF, repeat; (b) (i) (a) (i), (ii) **12** (3.0 equiv), $NaCNBH_3$ (3.0 equiv), TMOF, repeat, (iii) **13** (4.0 equiv), HATU (4.0 equiv), DIPEA (8.0 equiv), DMF, repeat, (iv) 95% TFA/2.5% TIPS/2.5% H_2O (1.5 mL).

the peptide/pseudopeptide moiety. To demonstrate the potential of this approach, several neoglycopeptides containing either two or three, similar or different, glycosides were synthesized in a parallel manner. Using parallel or split-mix synthesis, further work is in progress to generate complex libraries with different glycoside derivatives.

Experimental Section

Analytical Data for Entries 1−25 (Scheme 1). 1: ^{1}H NMR (400 MHz, CD_3OD): δ 1.63−1.88 (m, 2H), 1.95−2.18 (m, 24H), 2.63−2.88 (m, 2H), 3.32−3.76 (m, 2H), 3.93−4.35 (m, 9H), 4.40−4.43 (m, 1H), 4.81−5.13 (m, 2H), 5.26−5.36 (m, 2H), 5.40−5.45 (m, 2H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{34}H_{48}N_2O_{20}$: 805.3 (MH^+), 827.3 (MNa^+). **2:** ^{1}H NMR (400 MHz, CD_3OD): δ 1.63−1.92 (m, 2H), 1.95−2.18 (m, 24H), 2.57−2.86 (m, 2H), 3.44−3.72 (m, 3H), 3.83−4.36 (m, 8H), 4.79−4.83 (m, 1H), 5.10−5.47 (m, 6H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{34}H_{48}N_2O_{20}$: 805.3 (MH^+), 827.3 (MNa^+). **3:** ^{1}H NMR (400 MHz, CD_3OD): δ 1.92−2.21 (m, 26H), 2.60−2.78 (m, 1H), 3.32−3.38 (m, 1H), 3.51−3.72 (m, 1H), 3.78−3.88 (m, 1H), 3.92−4.39 (m, 8H), 4.40−4.46 (m, 1H), 4.81−4.98 (m, 1H), 5.01−5.58 (m, 6H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{34}H_{48}N_2O_{20}$: 805.3 (MH^+), 827.3 ($M + Na^+$). **4:** ^{1}H NMR (400 MHz, CD_3OD): δ 1.44−1.57 (m, 4H), 1.96−2.20 (m, 25H), 2.58−2.89 (m, 1H), 3.32−3.77 (m, 3H), 3.95−4.45 (m, 8H), 4.84−4.88 (m, 1H), 5.12−5.37 (m, 4H), 5.42−5.47 (m, 2H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{35}H_{50}N_2O_{20}$: 820.3 ($M + 2H^+$), 842.2 ($MHNa^+$). **5:** ^{1}H NMR (400 MHz, CD_3OD): δ 1.43−1.53 (m, 3H), 1.69−1.82 (m, 1H), 1.95−2.20 (m, 25H), 2.62−2.84 (m, 2H), 3.53−3.77 (m, 3H), 4.00−4.36 (m, 7H), 4.81−4.82 (m, 1H), 5.10−5.15 (m, 2H), 5.26−5.33 (m, 2H), 5.42−5.47 (m, 2H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{35}H_{50}N_2O_{20}$: 820.3 ($M + 2H^+$), 842.2 ($MHNa^+$). **6:** ^{1}H NMR (400 MHz, CD_3OD): δ 1.33−1.46 (m, 5H), 1.96−2.20 (m, 24H), 2.52−2.88 (m, 1H), 3.22−4.44 (m, 11H), 4.66−4.95 (m, 1H), 5.05−5.55 (m, 6H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{35}H_{50}N_2O_{20}$: 820.3 ($M + 2H^+$), 842.2 ($MHNa^+$). **7:** ^{1}H NMR (400 MHz, CD_3OD): δ 1.71−1.82 (m, 2H), 1.96−2.13 (m, 24H), 2.54−3.10 (m, 3H), 3.25−3.42 (m, 2H), 3.66−3.79 (m, 1H), 4.00−4.31 (m, 7H), 4.41−4.46 (m, 1H), 4.64−4.98 (m, 1H), 5.05−5.43 (m, 6H), 7.24−7.37 (m, 5H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{41}H_{54}N_2O_{20}$: 895.3 (MH^+), 917.3 (MNa^+). **8:** ^{1}H NMR (400 MHz, CD_3OD): δ 1.48−1.85 (m, 2H), 1.96−2.19 (m, 24H), 2.59−2.78 (m, 2H), 3.03−3.18 (m, 1H), 3.29−4.30 (m, 10H), 4.43−4.67 (m, 1H), 4.88−5.43 (m, 7H), 7.25−7.31 (m, 5H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{41}H_{54}N_2O_{20}$: 895.3 (MH^+), 917.3 ($M + Na^+$). **9:** ^{1}H NMR (400 MHz, CD_3OD): δ 1.59−1.87 (m, 2H), 1.96−2.14 (m, 24H), 2.30−3.15 (m, 3H), 3.32−4.32 (m, 11H), 4.61−5.42 (m, 7H), 7.24−7.31 (m, 5H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{41}H_{54}N_2O_{20}$: 895.3 (MH^+), 917.3 (MNa^+). **11:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{34}H_{47}NO_{21}$: 806.5 (MH^+), 828.5 (MNa^+). **12:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{35}H_{49}NO_{21}$: 820.2 (MH^+), 842.1 (MNa^+). **13:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{41}H_{53}NO_{21}$: 896.6 (MH^+), 918.6 (MNa^+). **14:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{35}H_{50}N_2O_{20}$: 820.2 ($M + 2H^+$). **15:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{35}H_{50}N_2O_{20}$: 820.2 ($M + 2H^+$). **16:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{35}H_{50}N_2O_{20}$: 820.2 (MH^+). **17:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{35}H_{50}N_2O_{20}$: 820.2 (MH^+). **18**: LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{41}H_{54}N_2O_{20}$: 896.2 (MH^+). **19**: LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{41}H_{54}N_2O_{20}$: 896.2 (MH^+). **20**: LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{41}H_{54}N_2O_{20}$: 896.2 (MH^+). **21**: LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{41}H_{54}N_2O_{20}$: 896.2 (MH^+). **22:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{38}H_{56}N_2O_{20}$: 862.2 (MH^+). **23:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{38}H_{56}N_2O_{20}$: 862.2 (MH^+). **24:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{38}H_{56}N_2O_{20}$: 862.2 (MH^+). **25:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{38}H_{56}N_2O_{20}$: 862.2 (MH^+).

In a similar manner, N-terminal, C-linked diglycosides branched dipeptides, shown in Scheme **2**, were also generated using Rink amide MBHA resin and preloaded Wang and TentaGel resins. This was performed in the same manner as for the branched diglycoside synthesis on a single amino acid with just the addition of extra initial Fmoc removal and amino acid coupling steps.

Analytical Data for Entries 1−14, Model 1 (Scheme 2). 1: ^{1}H NMR (400 MHz, CD_3OD): δ 1.37−1.44 (m, 3H), 1.96−2.20 (m, 26H), 2.65−2.92 (m, 2H), 3.32−4.63 (m, 15H), 5.09−5.46 (m, 4H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{53}N_3O_{21}$: 876.4 (MH^+), 898.3 ($M + Na^+$). **2:** ^{1}H NMR (400 MHz, CD_3OD): δ 1.50−1.64 (m, 4H), 1.96−2.18 (m, 25H), 2.62−2.86 (m, 2H), 3.41−4.45 (m, 14H), 4.76−4.94 (m, 1H), 5.11−5.44 (m, 4H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{53}N_3O_{21}$: 876.4 (MH^+), 898.3 (MNa^+). **3:** ^{1}H NMR (400 MHz, CD_3OD): δ 1.39−1.67 (m, 5H), 1.96−2.14 (m, 24H), 2.59−3.50 (m, 4H), 3.66−4.42 (m, 12H), 4.67−4.88 (m, 1H), 5.06−5.49 (m, 4H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{53}N_3O_{21}$: 876.4 (MH^+), 898.3 (MNa^+). **4:** ^{1}H NMR (400 MHz, CD_3OD): δ 1.76−1.91 (m, 2H), 1.96−2.22 (m, 24H), 2.30−2.92 (m, 3H), 3.02−4.44 (m, 13H), 4.55−4.994 (m, 1H), 5.06−5.42 (m, 6H), 7.25−7.37 (m, 5H); LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{43}H_{56}N_2O_{22}$: 953.4 (MH^+), 975.3 ($M + Na^+$). **5:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{52}N_2O_{22}$: 877.4 (MH^+). **6:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{52}N_2O_{22}$: 877.3 (MH^+), 899.3 (MNa^+). **7**: LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{44}H_{59}N_3O_{21}$: 966.3 (MH^+). **8**: LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{44}H_{59}N_3O_{21}$: 966.2 (MH^+). **9**: LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{44}H_{59}N_3O_{21}$: 966.2 (MH^+), 967.2 ($M + 2H^+$). **10**: LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{44}H_{59}N_3O_{21}$: 966.3 (MH^+), 967.2 ($M + 2H^+$). **11**: LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{41}H_{61}N_3O_{21}$: 932.3 (M^+), 933.3 (MH^+). **12**: LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{41}H_{61}N_3O_{21}$: 932.3 (M^+), 933.3 (MH^+). **13**: LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{41}H_{61}N_3O_{21}$: 932.3 (M^+), 933.3 (MH^+). **14**: LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{41}H_{61}N_3O_{21}$: 932.3 (M^+), 933.3 (MH^+).

Analytical Data for Entries 1−6, Neoglycopeptide 25, Model 2 (Scheme 3). 1: LRMS (electrospray, H_2O, positive

ion mode, *m*/*z*) for $C_{43}H_{59}N_3O_{20}$: 938.3 (MH^+), 939.3 (M + 2H). **2:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{43}H_{59}N_3O_{20}$: 938.3 (MH^+), 939.3 ($M + 2H^+$). **3:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{44}H_{61}N_3O_{20}$: 952.3 (MH^+), 953.3 ($M + 2H^+$). **4:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{44}H_{61}N_3O_{20}$: 952.3 (MH^+), 953.3 ($M + 2H^+$). **5:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{55}N_3O_{20}$: 862.3 (MH^+), 863.3 ($M + 2H^+$). **6:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{55}N_3O_{20}$: 862.3 (MH^+), 863.3 ($M + 2H^+$).

Analytical Data for Entries 1−9, Neoglycopeptide 27, Model 2 (Scheme 3). 1: LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{43}H_{57}N_3O_{21}$: 952.3 (MH^+), 953.3 (M + 2H). **2:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{43}H_{57}N_3O_{21}$: 952.3 (MH^+), 953.3 (M + 2H). **3:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{43}H_{57}N_3O_{21}$: 952.3 (MH^+), 953.3 (M + 2H). **5:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{44}H_{59}N_3O_{21}$: 966.3 (MH^+), 967.3 (M + 2H). **6:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{44}H_{59}N_3O_{21}$: 966.3 (MH^+), 967.3 (M + 2H). **7:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{53}N_3O_{21}$: 876.3 (MH^+), 877.3 ($M + 2H^+$). **8:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{55}N_3O_{20}$: 876.3 (MH^+), 877.3 ($M + 2H^+$). **9:** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{55}N_3O_{20}$: 876.3 (MH^+), 877.2 ($M + 2H^+$).

Analytical Data for Entries 1−4, Neoglycopeptides 25, 27, and 29, Model 3 (Scheme 4). 1: (27) LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{53}N_3O_{21}$: 876.4 (MH^+), 898.3 (MNa^+); **(25):** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{55}N_3O_{20}$: 820.3 ($MH^+ -$ Ac), 842.3 ($MNa^+ -$ Ac). **(29):** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{53}H_{75}N_3O_{30}$: 834.4 ($MH^+ - C_{18}H_{24}O_{10}$), 856.3 ($MNa^+ - C_{18}H_{24}O_{10}$), 1220.4 ($M + 2H^+ - CH_3$). **2: (25):** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{55}N_3O_{20}$: 820.3 ($MH^+ -$ Ac), 842.3 ($MNa^+ -$ Ac). **(29):** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{53}H_{75}N_3O_{30}$: 834.4 ($MH^+ - C_{18}H_{24}O_{10}$), 856.3 ($MNa^+ - C_{18}H_{24}O_{10}$), 1220.4 ($M + 2H^+ - CH_3$). **3: (27)** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{53}N_3O_{21}$: 876.4 (MH^+), 898.3 (MNa^+); **(25):** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{55}N_3O_{20}$: 820.3 ($MH^+ -$ Ac), 842.3 ($MNa^+ -$ Ac). **(29):** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{53}H_{75}N_3O_{30}$: 834.4 ($MH^+ - C_{18}H_{24}O_{10}$), 856.3 ($MNa^+ - C_{18}H_{24}O_{10}$), 1220.4 ($M + 2H^+ - CH_3$). **4: (27)** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{53}N_3O_{21}$: 876.4 (MH^+), 898.3 ($M + Na^+$); **(25):** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{37}H_{55}N_3O_{20}$: 820.3 ($MH^+ -$ Ac), 842.3 ($MNa^+ -$ Ac). **(29):** LRMS (electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{53}H_{75}N_3O_{30}$: 834.4 ($MH^+ - C_{18}H_{24}O_{10}$), 856.3 ($MNa^+ - C_{18}H_{24}O_{10}$), 1220.3 ($M + 2H^+ - CH_3$).

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(13) Unlike with simple aldehydes, reductive amination reaction with carbon-linked glycoside aldehydes is not trivial. The reaction is dependent upon the nature of the amino acid/peptide and of the glycoside moiety. Several attempts have been made to optimize reaction conditions for all the steps before the methodology was transferred to a programmed synthesizer.

(14) In our study, no significant difference in reaction yields was noticed between Rink amide MBHA and TentaGel resin. Neoglycopeptides **24** and **25** were synthesized using TentaGel resin only.

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