Synthesis and application of sialic acid-containing building blocks for glycopeptide libraries.[1] Establishing glycosylation conditions †

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Three different sialic acid-containing building blocks (**6**–**8**) were synthesized for use in solid-phase glycopeptide libraries. Investigation of the conditions for glycosylation of threonine (Thr) with various sialic acid donors revealed that the best results were obtained by coupling glycosyl xanthate **2** to the acceptors Fmoc-Thr-OH (**5**) or the α-azido acid analogue of Thr, **4**. Among several catalysts employed, phenylsulfanyl triflate (PST) afforded the best yields. Both the *N*-Fmoc and α-azido analogues of Thr allowed glycosylation with good stereoselectivity in 80% (⟶ **8**) and 84% (⟶ **6**) yield, respectively. Introduction of a phenylthio group in the 3 position of the sialic acid donor **3**, to assist the stereoselective outcome of the glycosylation reaction, gave good results; however difficulties in the removal of the phenylthio auxiliary group made this route less attractive.

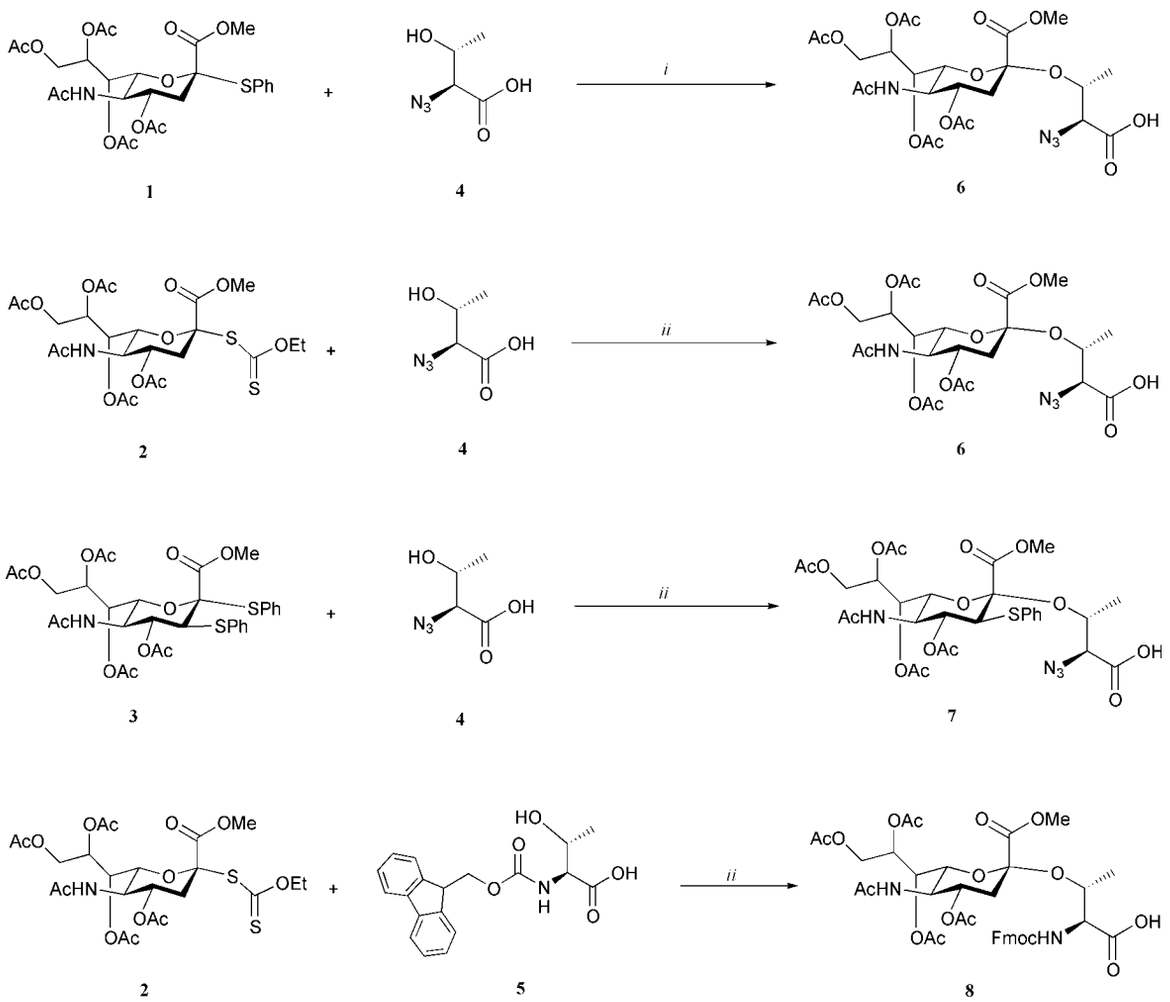
Both building blocks **6** and **8** were successfully introduced in solid-phase glycopeptide synthesis. Interestingly, alkaline deprotection of the Fmoc group of **8**, necessary for subsequent introduction of amino acids, resulted in an immediate attack of the α-amino group on the sialic acid methyl ester to form the lactam **14**. This side reaction was also observed during reduction of the azido acid building block **6** under alkaline conditions, but could be suppressed by performing the reduction under acidic conditions. Lactam formation was completely avoided by hydrolysis of the methyl ester prior to reduction of the azide.

Introduction

Sialic acid located at the periphery of glycolipids or glycoproteins is involved in a variety of biological phenomena, such as cell differentiation, inflammation, or tumor progression and metastasis.[1] The role of sialic acid in these interactions can, in general terms, be considered two-fold. First, it makes a major contribution to the net negative charge, which through electrostatic repulsion can be important in modulating cell–cell interactions mediated by specific cell-adhesion molecules.[2] Second, sialic acid can function as a specific recognition determinant at the cell surface, *e.g.* leucocyte/E-selectin interaction[3] or the macrophage sialic acid receptor.[4]

The mechanisms of the majority of the above mentioned interactions are not yet well elucidated due to the difficulty in obtaining the glycoconjugates in a pure form and sufficient quantities. The synthesis of the individual, complex oligosaccharide structures required to study the ligand–receptor interactions is cumbersome and time consuming.[5] Often, only a few residues at the non-reducing end of a complex glycan are necessary for tight interaction with the receptor.[6] Therefore, the use of simplified synthetic molecules that can be rapidly generated and can mimic the natural ligand can give important information about the nature and topology of the ligand–receptor interaction.[7] In fairly recent work it has been shown that complex oligosaccharide structures can be mimicked by glycopeptides.[8,9] The peptide-scaffold may even actively participate in the ligand–receptor interaction, thus enhancing the overall affinity of the ligand to the receptor, and furthermore, the ease of glycopeptide assembly has allowed large and diverse libraries[10] to be synthesized by the split-and-combine method. In order to synthesize sialic acid-containing glycopeptides for binding to sialic acid-recognizing receptors like the sialoadhesin lectin, amino acids glycosylated with sialic acid were required. Glycosylation of the hydroxy groups of amino acids is considered to be more difficult than glycosylation of carbohydrates, presumably due to their poor reactivity arising from the unfavorable H-bonding with the commonly used urethane N^{α}-protecting groups of amino acids.[11] In addition, the formation of the glycosidic bond of sialic acid is more difficult compared with that of other carbohydrates[12] due to three inherent factors. First, the presence of the carboxylic acid function at the anomeric center (C-2) electronically disfavors oxonium ion formation. Secondly, the carboxylate group interferes sterically with the glycoside formation, and finally the lack of a functional group at C-3 excludes the use of neighboring-group participation for stereochemical control. Some of the above mentioned problems could be circumvented by attaching sialic acid to an amino acid *via* a linker molecule. While the use of a linker molecule to couple sialic acid to amino acids will simplify the synthesis, it will also reduce the proximity of carbohydrate and peptide and introduce additional degrees of rotational freedom that may result in a greater entropic penalty and reduced binding. Therefore, direct *O*-sialylation of an amino acid was preferred in order to retain conformational rigidity in the glycopeptide through carbohydrate–peptide interactions. While many examples of glycosylated amino acids[13–16] have been described in the literature, to our knowledge no sialylated amino acids O-linked *via* the glycosidic bond have been previously reported. In the present paper we describe the synthesis of three sialylated threonine building blocks, and their application in solid-phase glycopeptide synthesis.

† Mass spectra of peptides released as a single bead are available as supplementary data. For direct electronic access see http://www.rsc.org/suppdata/p1/a9/a908321i



Scheme 1 *Reagents*: *i*, DMTST, CH_2Cl_2; *ii*, PST, CH_3CN.

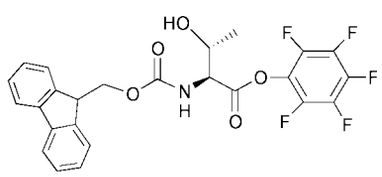
Results and discussion

Building block synthesis

Three versatile sialic acid donors, methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-non-2-ulopyranosid)onate[17] **1**, *O*-ethyl *S*-[methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-non-2-ulopyranosid)onate] dithiocarbonate[17] (**2**), and methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-5-deoxy-3-*S*-phenyl-2,3-dithio-D-*erythro*-L-*gluco*-non-2-ulopyranosid)-onate[12,18,19] **3** were prepared (Scheme 1). Synthesis of the donors **1** and **2** proceeded as described in the literature with comparable yields. Synthesis of the sialic acid donor **3**,[12] which has a C-3 auxiliary phenylthio group for stereocontrol in the glycosylation reaction, proved to be cumbersome. Yields of 57–77% are reported for the crucial reaction step, in which benzenesulfenyl chloride[20] is added to the double bond of sialic acid glycal. However, in our hands, a yield of only 30% was obtained, partly due to difficulties in the purification of the complex reaction mixture.

Initially Fmoc-Thr-OPfp **9** was selected as the acceptor molecule, since glycosylation with other carbohydrates has previously been successful,[21,22] and, furthermore, the Fmoc/OPfp building block strategy for the synthesis of O- and N-linked glycopeptides has been proven to be very versatile.[23] Unfortunately, attempted glycosylation of acceptor **9** with the sialic acid donors **1** or **2** was unsuccessful, and resulted primarily in hydrolysis or elimination of the activating group. Typically yields of approximately 10% were obtained, with no stereoselectivity.

It was initially thought that the low yields were due to steric hindrance caused by the bulky Fmoc N^{α}-protecting group. It has been shown[24] that sialylation reactions progress in higher yields when less sterically hindered and more nucleophilic acceptors are used. The use of an acceptor molecule containing an α-azido group as the masked α-amine,[25–27] and no carboxylic acid protecting group,[28] satisfies both the steric and electronic requirements. Consequently, the acceptor, 2-(*S*)-azido-3-(*R*)-hydroxybutanoic acid (N_3^{α}-Thr-OH) was synthesized in two steps starting from commercially available H-Thr(*t*Bu)-OH. The amine was first converted to the azide[26,29] by treatment with freshly prepared triflic azide,[30] followed by subsequent removal of the *t*Bu group, with 95% trifluoroacetic acid (TFA). The desired acceptor (**4**, Scheme 1) was obtained in 93% yield for the two steps.



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Glycosylation of **4** with phenylthio donor **1**, using *N*-iodosuccinimide (NIS)–triflic acid (TfOH) as promoter, was unsuccessful and yielded mainly the elimination product of the donor. Glycosylation using the same donor–acceptor pair under dimethyl(methylthio)sulfonium triflate (DMTST) activation[31] yielded the desired compound **6** (Scheme 1) in 59% yield and an α : β ratio of 1 : 1. Attempts to increase the α-selectivity during the glycosylation reaction by decreasing the reaction temperature were unsuccessful and led to formation of an increasing amount of glycal and a reduced glycosylation yield.

Table 1 Glycosylation conditions investigated[a]

Donor	Acceptor	Product	Reaction conditions	Yield (%) (α : β ratio)
1 or **2**	**9**		DMTST, 0 °C	≈10[b]
1	**9**		NIS–TfOH, −30 °C	≈10[b]
1	**4**		NIS–TfOH, −30 °C	
1	**4**	**6**	DMTST, 0 °C	59 (1 : 1)
2	**4**	**6**	MeSBr, −30 ⟶ 0 °C	19 (1 : 1)
2	**4**	**6**	PST, −30 °C	83 (3 : 1)
2	**4**	**6**	PST, −60 °C, CH_3CN–CH_2Cl_2 (4 : 1)	84 (5 : 1)
3	**4**	**7**	PST, −30 °C	93
2	**5**	**8**	PST, −40 °C	80 (9 : 1)

[a] All reactions were performed in CH_3CN, unless otherwise indicated, in the presence of powdered 3 Å molecular sieves. [b] Estimated from TLC.

Glycosylation of **4** with the xanthate donor **2**, using methanesulfenyl bromide (MeSBr) as a promoter, afforded the desired compound **6** in 19% yield, with no stereoselectivity. However, when phenylsulfenyl triflate (PST)[19] was used as the catalyst, **6** was formed in 83% yield and an α : β ratio of 3 : 1 (determined by comparison of the 3_{eq}-H signals in ^{1}H NMR). Changing the solvent system from acetonitrile to an acetonitrile–dichloromethane mixture allowed the lowering of the reaction temperature to −60 °C, leading to formation of the product in the same yield (84%), but with an increased stereoselectivity (α : β ratio of 5 : 1). From this mixture the α-anomer could be isolated in 56% yield.

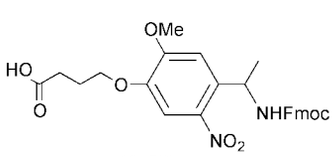
Since the separation of the α- and β-anomers of **6** by vacuum liquid chromatography (VLC) was difficult, the glycosylation of **4** with donor **3** having a C-3 auxiliary phenylthio group that directs the formation of an α-linkage through neighboring-group participation was investigated. This glycosylation afforded exclusively the α-anomer of **7** (Scheme 1), in an excellent yield of 93%. Unfortunately, removal of the C-3 auxiliary group using either tributyltin hydride or triphenyltin hydride and 2,2′-azoisobutyronitrile (AIBN) in refluxing toluene, or Raney Nickel gave a complex reaction mixture from which no product could be isolated.

To determine whether the improvement in glycosylation yield and stereoselectivity between acceptor **9** and **4** could be solely attributed to steric hindrance or to the electron-donating properties of the azide compared with Fmoc, glycosylation of commercially available Fmoc-Thr-OH (**5**, Scheme 1) was attempted.

The coupling of donor **2** to acceptor **5**, at −40 °C in acetonitrile using PST as catalyst, surprisingly gave 80% of a 9 : 1 mixture of the anomers of **8** (Scheme 1), from which the α-anomer could be isolated in a 62% yield. The low yield obtained with **9** as an acceptor is therefore most likely due to the combined steric effect of the pentafluorophenyl group and the Fmoc group as previously suggested.[22] Results of the glycosylation reaction are compiled in Table 1.

Glycopeptide synthesis

As a preface to the synthesis of sialic acid-containing glycopeptide libraries, the conditions of successful incorporation of building blocks **6** and **8** on a solid phase were investigated. The photolabile amide linker **10**[32] was used to facilitate



10 (PII)

mild cleavage of the glycopeptide from the solid support [acryloylated bis(2-aminopropyl)poly(ethylene glycol)/acrylamide copolymer ($PEGA_{1900}$)] and to permit expedient analysis *via* matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) mass spectrometry. Peptide **11** (Scheme 2) was synthesized using the syringe technology[33] and Fmoc/OPfp-derivatized amino acids which were activated with 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-OH).

Coupling of **8** with model peptide **11**, using *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU)/*N*-ethylmorpholine (NEM) activation, gave the desired glycopeptide **12** (Scheme 2) as shown by MALDI-TOF mass spectrometry. Coupling of **6** with model peptide **11**, using TBTU/NEM activation, afforded glycopeptide **13** (Scheme 2) as shown by MALDI-TOF mass spectrometry and IR spectroscopy.

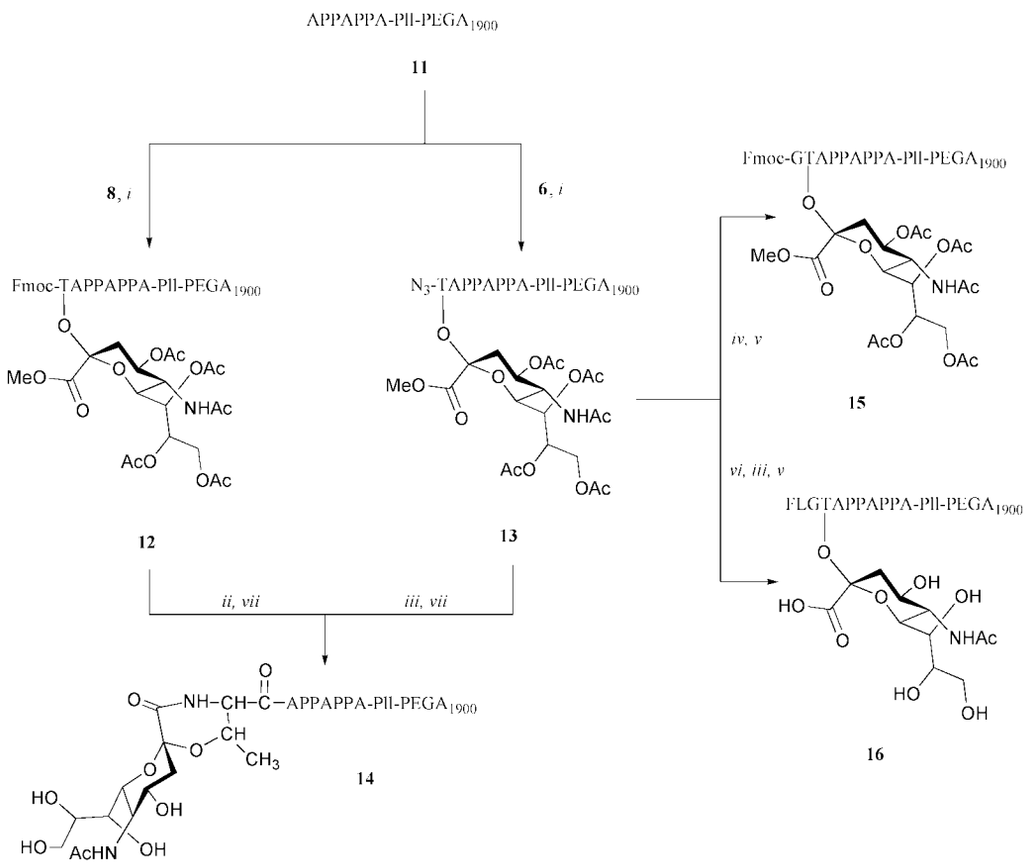
Coupling of amino acids subsequent to the incorporation of sialylated building blocks proved challenging. Removal of the Fmoc protecting group of **12** under alkaline conditions [20% piperidine in dimethylformamide (DMF)] resulted in formation of the sialic acid-Thr lactam and deacetylation gave **14** (Scheme 2). Compound **14** was isolated, upon photolytic cleavage, by HPLC in 52% overall yield starting from the introduction of **10** to the solid phase. Compound **14** was characterized by NMR spectroscopy (see Table 2) and ES mass spectroscopy.

Additionally, reduction of the azide of **13** under alkaline conditions {dithiothreitol (DTT) and 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) in DMF} and removal of *O*-acetyl groups gave also the sialic acid-Thr lactam **14**.[34] However, protonation of the amino function by performing the reduction under acidic conditions (TMSCl and NaI in acetonitrile) decreased the formation of lactam, as could be shown by subsequently incorporation of Fmoc-Gly-OPfp (to give **15**, Scheme 2). Despite the successful coupling of amino acids, the concomitant loss of sialic acid from the peptide [masses at 1005.21 ($[M + Na]^+$) and 1021.21 ($[M + K]^+$), mass spectra are given in supplementary material] under the acidic reduction conditions made this route less attractive. An alternative route would be to remove the protecting groups from sialic acid, prior to reduction. In this way, no lactam will be formed during alkaline azide reduction and no loss of sialic acid, due to acidic azide reduction conditions, will occur. Removal of the protecting groups, especially the methyl ester, proved to be difficult on the solid phase. Normal deprotection strategies for solution-phase deprotection (LiOH or NaOH[35] in water–alcohol mixtures or LiI in pyridine[36]) were not successful, as indicated by the formation of the cyclized side product during azide reduction. However, addition of calcium chloride[37,38] to a 1 M solution of LiOH in 70% propan-2-ol in water in conjunction with sonication of the mixture afforded smoothly the desired deprotected sialic acid derivative. It is assumed that the Ca^{2+} stabilizes electrostatically the transition state during the hydrolysis process. Azide reduction on the deprotected glycopeptide (DTT and DBU in DMF) followed by incorporation of three more amino acids afforded the desired compound **16** (Scheme 2). Compound **16** was isolated, upon photolytic cleavage, by HPLC in 38% overall yield starting from incorporation of **10** on the solid phase. Compound **16** was characterized by NMR spectroscopy (see Table 2) and ES mass spectrometry.

Table 2 Chemical-shift table for compounds **14**[a] and **16**[a]

	14					**16**					
	NH	Hα	Hβ	Hγ	Hδ	NH	Hα	Hβ	Hγ	Hδ	2,6-H, 3,5-H, 4-H
Phe						7.12, 6.88	4.11	3.21, 3.02			7.32, 7.24
Leu							4.42	1.69, 1.53	1.59	0.87, 0.90	
Gly						8.40	3.84, 3.57				
Thr	8.32	3.82	4.27	1.21		7.45	4.05	4.17	1.17		
Ala[b]	8.32	4.52	1.22			8.23	4.35	1.11			
Pro[b]		4.57	2.16, 1.83	1.91	3.64, 3.48		4.45	1.92, 1.85	1.69	3.52, 3.37	
Pro[b]		4.31	1.98, 1.85	[c]	3.64, 3.52		4.29	1.97, 1.79	1.85	3.63, 3.46	
Ala[b]	7.82	4.45	1.15			7.81	4.44	1.14			
Pro[b]		4.53	2.13, 1.86	1.93	3.63, 3.46		4.53	2.12, 1.83	1.95	3.61, 3.46	
Pro[b]		4.27	2.02, 1.87	[c]	3.64, 3.57		4.27	2.02, 1.84	1.89	3.64, 3.55	
Ala[b]	7.69	4.11	1.20			7.69	4.11	1.19			
	$H3_{ax}$	$H3_{eq}$	H4	H5	H6	NH	Ac				
14-OSA	1.35	2.13	4.35	3.52	3.75						
16-OSA	1.31	2.63	4.68	3.39	3.54	7.93	1.81				

[a] All spectra were acquired at 25 °C in DMSO-d_6 and referenced to DMSO at δ_H 2.49. [b] The assignments of these resonances are based on connectivities from $H^{\alpha}(i)$–$H^{\alpha}(i+1)$, $H^{\alpha}(i)$–NH$(i+1)$, $H^{\alpha}(i)$–$H^{\delta}(i)$–$H^{\delta}(i+1)$ and Ala $H^{\beta}(i)$–$H^{\delta}(i+1)$. If no fully unambiguous sequential assignment could be made among these the order can be interchanged. [c] These resonances are most likely totally overlapped with the upfield resonance of Hβ.



Scheme 2 *Reagents*: *i*, TBTU, NEM, DMF; *ii*, 20% piperidine–DMF; *iii*, DTT, DBU, DMF; *iv*, TMSCl, NaI, CH_3CN; *v*, Fmoc-Aa-OPfp, Dhbt-OH, DMF; *vi*, LiOH, $CaCl_2$, 70% Pr^iOH–H_2O; *vii*, 80% aqueous hydrazine in MeOH.

In summary, we have synthesized three sialic acid-containing building blocks, **6**, **7** and **8**, in high yield and good stereoselectivity. Building block **7** proved unsuitable for further use since the C-3 auxilary phenylthio group could not be removed from the glycosyl amino acid. Building blocks **6** and **8** were smoothly introduced during solid-phase glycopeptide synthesis. The Fmoc building block can be used for synthesizing a library that gives rise to lactamized products that cannot be further extended, while the azide building block can be used for the synthesis of a sialylated glycopeptide library, provided the methyl ester is hydrolyzed prior to azide reduction.

Experimental

Materials and general methods

All solvents were of p.a. quality, and were distilled from appropriate drying agents when necessary. DMF was stored over 3 Å molecular sieves. Reaction mixtures were dried with Na_2SO_4 and concentrated under reduced pressure at temperatures less than 30 °C (water-bath). VLC was performed on Merck Silica Gel 60 H, and chromatography under dry conditions was performed on dried silica gel (120 °C; 24 h), eluting with dry solvents. Solid-phase peptide-coupling reactions were

monitored using the Kaiser test,[39] and solution-phase reactions were monitored by thin-layer chromatography (TLC) performed on Merck Silica Gel 60 F_{254} aluminium-backed sheets with detection by charring with sulfuric acid, or by UV light when applicable. $PEGA_{1900}$ resin (300–500 μm) was obtained from Polymer Laboratories (Amherst, MA). Suitably protected N^{α}-Fmoc amino acids were purchased from NovaBiochem (Switzerland), TBTU and Dhbt-OH from Fluka (Switzerland), NEM from Merck (Germany), DBU, silver triflate (AgOTf, recrystallized from toluene) and DTT from Aldrich (USA). Optical rotations were measured on a Perkin-Elmer 241 polarimeter and $[a]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. Preparative HPLC of compounds **14** and **16** was performed over a 25 × 200 mm semipreparative RP-18 column (Millipore Delta Pak 15 μ). Eluents A (1% TFA in water) and B (10% of A with 1% TFA in acetonitrile) were used in a linear gradient, starting with 85% A and 15% B, a slope of 0.5% min^{-1}, and a flow rate of 10 cm^3 min^{-1}. ES-MS spectra were recorded in the positive mode on a Fisons VG Quattro Instrument. NMR spectra were recorded on a Bruker AMX-250 or a Bruker DRX-600 MHz spectrometer. The ^{1}H and ^{13}C resonances were assigned by ^{1}H, ^{13}C, ^{1}H–^{1}H COSY, and HSQC experiments. NMR spectra were recorded in $CDCl_3$, D_2O or $CDCl_3$–CD_3OD mixtures. Chemical shifts are given in ppm and referenced to $CDCl_3$ (δ_H 7.29 and δ_C 77.0); *J* values are given in Hz.

MALDI-TOF mass spectrometry

Beads were irradiated on stainless steel targets with a strong UV lamp for 30 min. The analyte was extracted on the target from the beads using 0.5 mm^3 of 70% acetonitrile and then dried at room temperature (RT). The appropriate matrix was added, the sample dried at 40 °C, and the spectrum recorded on a Bruker Reflex™ III MALDI-TOF mass spectrometer. Spectra were obtained (1–100 pulses) using the lowest power required to facilitate desorption and ionization. Ions were accelerated toward the discrete dynode multiplier detector with an acceleration voltage of 20 kV. The matrix α-cyano-4-hydroxycinnamic acid (CHC, 10 mg in 1 cm^3 of 70% acetonitrile) was used to analyze both peptides and protected glycopeptides. Unprotected glycopeptides were analyzed using the matrix 2,6-dihydroxyacetophenone (DHAP) to which was added pyridine (DHAP, 10 mg in 1 cm^3 of 70% acetonitrile and 50 mm^3 of pyridine). Bradykin (1060.2 mu), renin (1759.0 mu), and mellitin (2846.5 mu) were used as the standards for internal calibration of the mass spectra.

N_3^{α}-Thr-OH 4

H-Thr(*t*Bu)-OH (1.00 g, 5.71 mmol) and $CuSO_4 \cdot 5H_2O$ were dissolved in a mixture of water (18 cm^3) and methanol (36 cm^3), then K_2CO_3 was added until pH 10 (pH paper) was obtained. Under vigorous stirring, a solution of triflic azide (12 mmol) in dichloromethane (30 cm^3) was added, and the pH of the solution was re-adjusted to 10 by addition of K_2CO_3. The reaction mixture was stirred overnight at RT, then diluted with dichloromethane (50 cm^3). The layers were separated and the organic phase was twice extracted with water. The combined aqueous layers were acidified using 2 M H_2SO_4 and extracted with dichloromethane (3 × 50 cm^3). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated to dryness. To the crude mixture was added 95% aq. TFA. The solution was stirred for 30 min and then diluted with toluene and concentrated to dryness. The product was applied to a VLC column and eluted with chloroform–methanol (6:1) to yield compound **4** (0.77 g, 93%), $[a]_D^{21}$ −50 (*c* 1.0, MeOH) [Found: (ES-MS negative-ion mode) *m*/*z* 144.8. $C_4H_7N_3O_3$ requires *M*, 145.1]; δ_H(250 MHz; $CDCl_3$) 4.31 (1 H, dq, CH$^{\beta}$), 3.87 (1 H, d, $J_{CH\alpha,CH\beta}$ 3.2, CH$^{\alpha}$), 1.42 (3 H, d, $J_{CH\gamma,CH\beta}$ 5.9, CH$^{\gamma}$); δ_C(75 MHz; $CDCl_3$) 173.1 (COOH), 76.1 (C$^{\beta}$), 69.0 (C$^{\alpha}$), 20.2 (C$^{\gamma}$).

N_3^{α}-Thr[methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid)onate]-OH 6

***via* Donor 1.** Donor **1** (0.31 g, 0.54 mmol), acceptor **4** (26 mg, 0.18 mmol), molecular sieves 3 Å, and a magnet were placed in a predried 25 cm^3 flask. The air in the flask was evacuated and replaced by an atmosphere of argon. Dry acetonitrile (4 cm^3) was added. The suspension was cooled to 0 °C, DMTST[31] was added (0.137 g, 0.54 mmol) and the suspension was stirred for 12 h at RT. The reaction mixture was filtered over Celite, then directly applied to a VLC column and eluted with chloroform–methanol (9:1) to afford **6** (65 mg, 59%) as a 1:1 mixture of anomers.

***via* Donor 2.** Donor **2** (2.23 g, 3.75 mmol), acceptor **4** (0.328 g, 1.87 mmol), molecular sieves 3 Å, and magnet were placed in a predried 50 cm^3 flask. The air in the flask was evacuated and replaced by an atmosphere of argon. Acetonitrile (12 cm^3) and dichloromethane (3 cm^3) were added and the suspension was cooled to −60 °C. AgOTf (1.10 g, 4.31 mmol) and benzenesulfenyl chloride (0.54 cm^3, 3.75 mmol) were added and the reaction mixture stirred for 3 h. The suspension was neutralized with *N*,*N*-diisopropylethylamine (DIPEA) (0.75 cm^3, 4.31 mmol), filtered over Celite, and concentrated to dryness. The product was applied to a VLC column and eluted with chloroform–methanol (9:1) to yield **6** (845 mg, 84%) as a 5:1 mixture of anomers. Separation of the anomers on a VLC column eluted with toluene–acetone (5:1) afforded the pure α-anomer of **6** (640 mg, 56%), $[a]_D$ −93 (*c* 1.0, $CHCl_3$) [Found: (ES-MS positive-ion mode) *m*/*z* 619.2. $C_{24}H_{34}N_4O_{15}$ requires *M*, 618.5]; δ_H(600 MHz; $CDCl_3$) 5.44–5.41 (1 H, m, 8-H), 5.38 (1 H, br d, $J_{NH,H5}$ 9.8, N*H*COCH$_3$), 5.34 (1 H, br d, $J_{7,8}$ 8.5, $J_{6,7}$ <1, 7-H), 4.97–4.94 (1 H, m, 4-H), 4.59–4.57 (1 H, m, CH$^{\beta}$), 4.39 (1 H, dd, $J_{8,9b}$ 2.6, 9a-H), 4.12 (1 H, dd, $J_{8,9a}$ 6.0, $J_{9a,9b}$ 12.4, 9b-H), 4.05 (1 H, dd, $J_{4,5}$ 10.1, 5-H), 3.98 (1 H, br d, $J_{5,6}$ 10.6, 6-H), 3.83 (3 H, s, CO_2CH_3), 3.63 (1 H, d, $J_{CH\alpha,CH\beta}$ 3.4, CH$^{\alpha}$), 2.69 (1 H, dd, $J_{3eq,4}$ 4.5, $J_{3eq,3ax}$ 12.4, 3eq-H), 2.19, 2.09 and 2.07 (3 H, 3 H, 6 H, 3 s, 4 × O_2CCH_3), 1.95 (3 H, s, NHCOC*H*$_3$), 1.896 (1 H, t, $J_{3ax,4}$ 12.4, 3ax-H), 1.49 (3 H, d, $J_{CH\gamma,CH\beta}$ 6.2, CH$^{\gamma}$); δ_C(75 MHz; $CDCl_3$) 171.9, 171.7 (2), 171.2 (2) and 170.8, (4 × O_2CCH_3, NH*C*OCH$_3$, and *C*O$_2$CH$_3$), 167.9 (CO_2H), 100.2 (C-2), 73.4 (C$^{\beta}$), 73.0 (C-6), 69.5 (C-4), 69.4 (C-8), 67.3 (C-7), 66.7 (C$^{\alpha}$), 62.9 (C-9), 53.5 (CO_2CH_3), 50.1 (C-5), 38.6 (C-3), 23.54 (NHCO*C*H$_3$), 21.7 and 21.3 (3) (4 × O_2CCH_3), 21.0 (C$^{\gamma}$).

N_3^{α}-Thr[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-5-deoxy-3-*S*-phenyl-3-thio-D-*erythro*-β-L-*gluco*-non-2-ulopyranosid)onate]-OH 7

Donor **3** (145 mg, 209 μmol), acceptor **4** (33 mg, 189 μmol), molecular sieves 3 Å, and magnet were placed in a predried 10 cm^3 flask. The air in the flask was evacuated and replaced by an atmosphere of argon. Acetonitrile (5 cm^3) was added and the suspension cooled to −40 °C. AgOTf (124 mg, 0.48 mmol) and benzenesulfenyl chloride (30 mm^3, 209 μmol) were added and the reaction mixture was stirred for 1 h. The suspension was neutralized with DIPEA (82 mm^3, 0.48 mmol), filtered over Celite, and concentrated to dryness. The product was applied to a VLC column and eluted with chloroform–methanol (30:1 → 9:1) to yield **7** (148 mg, 93%), $[a]_D$ +99 (*c* 1.0, $CHCl_3$) [Found: (ES-MS positive-ion mode) *m*/*z* 727.2. $C_{30}H_{38}N_4O_{17}S$ requires *M*, 726.7]; δ_H(250 MHz; $CDCl_3$) 7.45–7.22 (5 H, m, SPh), 5.35 (1 H, t, $J_{3,4=4,5}$ 10.9, 4-H), 5.34 (1 H, d, *J* 10.7, N*H*COCH$_3$), 5.26–5.21 (1 H, m, 8-H), 5.16 (1 H, dd, $J_{6,7}$ 1.56, $J_{7,8}$ 9.25, 7-H), 4.47 (1 H, m, CH$^{\beta}$), 4.18 (1 H, dd, $J_{8,9b}$ 2.0, $J_{9a,9b}$ 12.2, 9b-H), 4.16 (1 H, t, $J_{5,6}$ 9.92, 5-H), 4.07 (1 H, dd, 6-H), 3.94 (1 H, d, $J_{CH\alpha,CH\beta}$ 3.4, CH$^{\alpha}$), 3.90 (1 H, dd, $J_{8,9a}$ 6.4, 9a-H), 3.82 (3 H, s, CO_2CH_3), 3.14 (1 H, d, $J_{3eq,4}$ 10.9, 3-H), 2.04, 2.03, 1.97 and 1.88 (each 3 H, 4 s, 4 × O_2CCH_3), 1.75 (3 H, s, NHCOC*H*$_3$), 1.20 (3 H, d, $J_{CH\gamma,CH\beta}$ 6.3, CH$^{\gamma}$).

N^{α}-Fmoc-Thr[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid)onate]-OH 8

Donor **2** (1.10 g, 1.85 mmol), acceptor **5** (0.315 g, 0.92 mmol), molecular sieves 3 Å, and magnet were placed in a predried 50 cm^3 flask. The air in the flask was evacuated and replaced by an atmosphere of argon. Acetonitrile (8 cm^3) was added and the suspension cooled to −35 °C. AgOTf (0.95 g, 3.69 mmol) and benzenesulfenyl chloride (133 mm^3, 0.92 mmol) were added and the reaction mixture was stirred for 3 h. The suspension was neutralized with DIPEA (0.64 cm^3, 3.69 mmol), filtered over Celite, and concentrated to dryness. The product was applied to a VLC column and eluted with chloroform–methanol (9 : 1) to yield **8** (599 mg, 80%) as a 9 : 1 mixture of anomers. Separation of the anomers on a VLC column eluted with toluene–acetone (4 : 1) afforded the pure α-anomer of **8** (464 mg, 62%), $[\alpha]_D$ +8 (*c* 1.0, $CHCl_3$) [Found: (ES-MS positive-ion mode) *m*/*z* 814.6. $C_{39}H_{46}N_2O_{17}$ requires *M*, 814.8]; δ_H(600 MHz; $CDCl_3$) 7.98–7.22 (8 H, m, Fmoc ArH), 5.51 (1 H, br d, $J_{NH,H5}$ 9.3, N*H*COCH$_3$), 5.37–5.31 (1 H, m, 8-H), 5.21 (1 H, br d, $J_{7,8}$ 8.7, $J_{6,7}$ <1, 7-H), 4.85–4.77 (1 H, m, 4-H), 4.49–4.47 (1 H, m, CH^{β}), 4.35–4.29 (3 H, m, Fmoc CH_2 and 9a-H), 4.29 (1 H, d, $J_{CH\alpha,CH\beta}$ 2.3, CH^{α}), 4.17 [1 H, t, *J*(CH, Fmoc CH_2) 7.0, Fmoc CH], 4.01 (1 H, dd, $J_{8,9a}$ 5.9, $J_{9a,9b}$ 12.4, 9b-H), 3.93–3.89 (2 H, m, 5- and 6-H), 3.71 (3 H, s, CO_2CH_3), 2.54 (1 H, dd, $J_{3eq,4}$ 4.4, $J_{3eq,3ax}$ 12.4, 3eq-H), 2.08, 2.07, 1.97 and 1.96 (each 3 H, 4 s, 4 × O_2CCH_3), 1.90 (1 H, t, $J_{3ax,4}$ 12.4, 3ax-H), 1.84 (3 H, s, NHCOCH_3), 1.31 (3 H, d, $J_{CH\gamma,CH\beta}$ 6.2, CH^{γ}); δ_C(75 MHz; $CDCl_3$) 172.8, 171.8, 171.6, 171.3, 171.1 and 170.8 (4 × O_2CCH_3, NH*C*OCH$_3$, and *C*O_2CH$_3$), 168.2 (CO_2H), 157.3 (Fmoc CO), 144.4 (2), 141.9 (2), 128.3 (2), 127.7 (2), 125.7 (2) and 120.6 (2) (12 × Fmoc ArC), 100.1 (C-2), 73.1 (C-6), 72.2 (C^{β}), 69.6 (C-4), 69.3 (C-8), 67.9 (C^{α}), 67.8 (C-7), 63.1 (C-9), 59.4 (Fmoc CH_2), 53.6 (CO_2CH_3), 50.0 (C-5), 47.7 (Fmoc CH), 38.4 (C-3), 23.7 (NHCO*C*H$_3$), 21.7 and 21.4 (3) (4 × O_2CO*C*H$_3$), 20.7 (C^{γ}).

Synthesis of model peptide 11

$PEGA_{1900}$ resin was washed with dichloromethane (6×) in a syringe fitted with a Teflon filter, then dried under vacuum (lyophilizer) for at least 24 h before use. Fmoc-Lys(Fmoc)-OH (3 equiv.) was coupled to the resin, using TBTU (2.9 equiv.)–NEM (5 equiv.) activation, in order to double the capacity of the resin (final loading 0.24 mmol g^{-1}). The Fmoc protecting groups were removed by treatment with 20% piperidine in DMF and the photolabile linker **10** (1.5 equiv.) was attached using TBTU (1.4 equiv.)–NEM (3 equiv.) activation.

All remaining peptide couplings were performed with the Fmoc amino acid OPfp ester (3 equiv.), which were activated with Dhbt-OH (1 equiv.). The progress of each coupling was followed by the Kaiser test. The Fmoc group was removed using 20% piperidine in DMF solution. After each coupling or deprotection step the resin was washed with DMF (8×). Photolytic release of the compound, followed by MALDI-TOF mass spectrometry, established the identity of the product. Mass calc.: 619.74 $[M + H]^+$, 641.73 $[M + Na]^+$, 657.84 $[M + K]^+$; mass found: 619.31 $[M + H]^+$, 641.29 $[M + Na]^+$, 657.25 $[M + K]^+$.

Synthesis of 12

To resin-bound peptide **11** (38 mg resin, 7.9 μmol) was added a solution of **8** (19 mg, 23.8 μmol), TBTU (6.9 mg, 21.5 μmol), and NEM (6 mm^3) in 400 mm^3 dry DMF. After 3 h, the reagents were removed by suction and the resin was washed with DMF (8×). Photolytic release of the compound, followed by MALDI-TOF mass spectrometry, established the identity of the product. Mass calc.: 1437.54 $[M + Na]^+$, 1453.53 $[M + K]^+$; mass found: 1437.39 $[M + Na]^+$, 1453.39 $[M + K]^+$.

Synthesis of 13

To resin bound peptide **11** (42 mg resin, 8.09 μmol NH_2 functions) was added a solution of **7** (15 mg, 24.3 μmol), TBTU (6.9 mg, 23.5 μmol), and NEM (6 mm^3) in 400 mm^3 of dry DMF. After 3 h, the reagents were removed by suction and the resin was washed with DMF (8×). Photolytic release of the compound, followed by MALDI-TOF mass spectrometry, established the identity of the product. Mass calc.: 1241.24 $[M + Na]^+$, 1257.15 $[M + K]^+$; mass found: 1241.28 $[M + Na]^+$, 1257.24 $[M + K]^+$.

Synthesis of 14

***via* Compound 12.** Compound **12** (10 mg resin) was treated with 20% piperidine in DMF solution. After 20 min, suction was applied and the resin was washed with DMF (8×). The Kaiser test showed that no amine functions were present. The Fmoc protecting group was removed, then the acetyl protecting groups were removed by overnight treatment of the resin with 80% aq. hydrazine (56 mm^3) in methanol (1 cm^3). The product was photolytically released of the resin, and purified by HPLC to yield **14** (1.9 mg, 52% overall yield starting from the introduction of **10** on resin). The identity of the product was established by NMR spectroscopy (see Table 2) and ES-MS. Mass calc.: 953.24 $[M + H]^+$, 975.25 $[M + Na]^+$; mass found: 953.5 $[M + H]^+$, 975.6 $[M + Na]^+$

***via* Compound 13.** Compound **13** (15 mg resin) was treated with 300 mm^3 of a 0.1 M DTT solution in DMF to which was added DBU (1 equiv. compared with **13**). After 1 h, suction was applied and the resin was washed with DMF (8×). The Kaiser test showed that no amine functions were present. The acetyl protecting groups were removed by overnight treatment of the resin with 80% aq. hydrazine (56 mm^3) solution in methanol (1 cm^3).

Synthesis of 15

A solution of sodium iodide (3.6 mg, 24 μmol) in dry acetonitrile (100 mm^3) was added to resin-bound compound **13** (11.6 mg resin, 2.4 μmol) and, after 5 min, chlorotrimethylsilane (3.6 mm^3, 38 μmol) was injected. After 30 min, suction was applied and the resin was washed successively with 10% aq. $Na_2S_2O_3$ (3×), water (2×), and acetonitrile (6×). To the resin was added a solution of Fmoc-Gly-OPfp (11.1 mg, 24 μmol) and Dhbt-OH (2.5 mg, 8 μmol) in 200 mm^3 of dry DMF. After 6 h, the reagents were removed by suction and the resin was washed with DMF (8×). Photolytic release of the compound, followed by MALDI-TOF mass spectrometry, established the identity of the products. Mass calc.: 1494.59 $[M + Na]^+$, 1510.53 $[M + K]^+$; mass found: 1494.30 $[M + Na]^+$ (35%), 1510.26 $[M + K]^+$ (30%), 1199.32 $[M_{Lact} + K]^+$ (7.5%), 1183.27 $[M_{Lact} + Na]^+$ (2.5%), 1021.21 $[M - SA + K]^+$ (12.5%), 1005.23 $[M - SA + Na]^+$ (12.5%).

Synthesis of 16

A solution of 50 mm^3 of 1 M aq. LiOH in 1.45 cm^3 of 0.8 M $CaCl_2$ in 70% Pr^iOH–H_2O was added to resin-bound compound **13** (13 mg resin). The mixture was sonicated for 3 h at 0 °C, then the reagents were removed by suction and the resin was washed successively with water (3×), 95% acetic acid (2×), water (3×), 5% DIPEA in DMF (3×), and DMF (6×).

The resin was treated with 300 mm^3 of a 0.1 M DTT solution in DMF to which was added DBU (1 equiv. with respect to **13**). After 1 h, suction was applied and the resin was washed with DMF (8×). The Kaiser test established the presence of amine functions. All remaining peptide couplings were performed with the Fmoc amino acid OPfp esters (3 equiv.), which were activated with Dhbt-OH (1 equiv.). The progress of each coupling was followed by the Kaiser test. The Fmoc group was

removed using 20% piperidine in DMF. After each coupling or deprotection step the resin was washed with DMF (8×).

The product was photolytically released of the resin, and purified by HPLC to yield **16** (1.6 mg, 38% overall yield starting from the introduction of **10** on resin). The identity of the product was established by NMR spectroscopy (see Table 2) and ES-MS. Mass calc.: 1328.54 $[M + H]^+$, 1350.60 $[M + Na]^+$; mass found: 1328.7 $[M + H]^+$, 1350.7 $[M + Na]^+$, 1372.7 $[M - H + 2Na]^+$

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References

1 R. Schauer, *Trends Glycosci. Glycotechnol.*, 1997, **9**, 315.
2 R. Schauer, A. K. Shukla, C. Schröder and E. Müller, *Pure Appl. Chem.*, 1984, **56**, 907.
3 M. Vinson, S. Mucklow, A. P. May, E. Y. Jones, S. Kelm and P. R. Crocker, *Trends Glucosci. Glycotechnol.*, 1997, **9**, 283.
4 D. Nath, P. A. van der Merwe, S. Kelm, P. Bradfield and P. R. Crocker, *J. Biol. Chem.*, 1995, **270**, 26184.
5 H. Ishida, Y. Ohta, Y. Tsukada, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, 1993, **246**, 75.
6 Y. C. Lee, *Binding Modes of Mammalian Hepatic Gal/GalNAc Receptors*, in *Carbohydrate Recognition in Cellular Function*, eds. G. Bock and S. Harnett, John Wiley & Sons, Chichester, England, 1st edn., 1989, pp. 80–95.
7 M. Meldal, I. Christiansen-Brams, M. K. Christensen, S. Mouritsen and K. Bock, *Synthesis and Biological Application of Glycosylated Peptide Templates*, in *Complex Carbohydrates in Drug Research. Structural and Functional Aspects*, eds. K. Bock and H. Clausen, Munksgaard, Copenhagen, 1st edn., 1994, pp. 153–165.
8 M. K. Christensen, M. Meldal, K. Bock, H. Cordes, S. Mouritsen and H. Elsner, *J. Chem. Soc., Perkin Trans. 1*, 1994, 1299.
9 S.-H. Wu, M. Shimazaki, C.-C. Lin, L. Qiao, W. J. Moree, G. Weitz-Schmidt and C.-H. Wong, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 88.
10 P. M. St. Hilaire, T. L. Lowary, M. Meldal and K. Bock, *J. Am. Chem. Soc.*, 1998, **120**, 13312.
11 R. Polt, L. Szabo, J. Treiberg, Y. Li and V. J. Hruby, *J. Am. Chem. Soc.*, 1992, **114**, 10249.
12 T. Ercegovic and G. Magnusson, *J. Org. Chem.*, 1995, **60**, 3378.
13 M. Meldal, *Glycopeptide Synthesis*, in *Neoglycoconjugates: Preparation and Application*, eds. Y. C. Lee and R. T. Lee, Academic Press, San Diego, 1994, ch. 4, pp. 145–198.
14 H. Paulsen, S. Peters and T. Bielfeldt, *Chemical Synthesis of Glycopeptides*, in *Glycoproteins*, Elsevier, Amsterdam, 1st edn., 1995, pp. 87–121.
15 C. M. Taylor, *Tetrahedron*, 1998, **54**, 11317.
16 G. Arsequell and G. Valencia, *Tetrahedron: Asymmetry*, 1997, **8**, 2839.
17 A. Marra and P. Sina, *Carbohydr. Res.*, 1989, **187**, 35.
18 Y. Ito and T. Ogawa, *Tetrahedron*, 1990, **46**, 89.
19 V. Martichonok and G. M. Whitesides, *Carbohydr. Res.*, 1997, **302**, 123.
20 S. Thea and G. Cevasco, *Tetrahedron Lett.*, 1988, **29**, 2865.
21 A. M. Jansson, M. Meldal and K. Bock, *Tetrahedron Lett.*, 1990, **31**, 6991.
22 M. Meldal and K. J. Jensen, *J. Chem. Soc., Chem. Commun.*, 1990, 483.
23 E. Meinjohanns, M. Meldal, H. Paulsen and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1995, 405.
24 K. Okamoto and T. Goto, *Tetrahedron*, 1990, **46**, 5835.
25 R. U. Lemieux and R. M. Ratcliffe, *Can. J. Chem.*, 1979, **57**, 1244.
26 J. Zaloom and D. C. Roberta, *J. Org. Chem.*, 1981, **46**, 5173.
27 P. Zimmermann and R. R. Schmidt, *Liebigs Ann. Chem.*, 1988, 663.
28 M. Elofsson, S. Roy, L. A. Salvador and J. Kihlberg, *Tetrahedron Lett.*, 1996, **37**, 7645.
29 P. B. Alper, S.-C. Hung and C.-H. Wong, *Tetrahedron Lett.*, 1996, **37**, 6029.
30 C. J. Cavender and V. J. Shiner, *J. Org. Chem.*, 1972, **37**, 3567.
31 P. Fugedi and P. J. Garegg, *Carbohydr. Res.*, 1986, **149**, C9.
32 C. P. Holmes and D. G. Jones, *J. Org. Chem.*, 1995, **60**, 2318.
33 M. K. Christensen, M. Meldal and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1993, 1453.
34 E. Meinjohanns, M. Meldal, T. Jensen, O. Werdelin, L. Galli-Stampino, S. Mouritsen and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1997, 871.
35 T. W. Greene and P. G. M. Wuts, in *Protective Groups in Organic Synthesis*, John Wiley & Sons, New York, 2nd edn., 1991.
36 F. Elsinger, J. Schreiber and A. Eschenmoser, *Helv. Chim. Acta*, 1960, **43**, 113.
37 R. Pascal and R. Sola, *Tetrahedron Lett.*, 1997, **38**, 4549.
38 R. Pascal and R. Sola, *Tetrahedron Lett.*, 1998, **39**, 5031.
39 E. Kaiser, R. L. Colescott, C. D. Bossinger and P. I. Cook, *Anal. Biochem.*, 1970, **34**, 595.