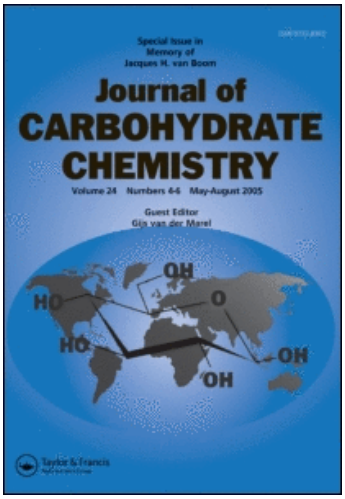

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Preparation of Some Glycosyl Amino Acid Building Blocks via Click Reaction and Construction of a Glycotetrapeptide Library Using Spot Synthesis

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Institute of Organic Chemistry, University of Tuebingen, Germany

The copper-catalyzed 1,3-dipolar cycloaddition reaction between ethyl 2,3,4-tri-*O*-actetyl-6-azido-6-deoxy-1-thio-β-D-glucopyranoside (**2**), ethyl 2,3,4-tri-*O*-actetyl-6-azido-6-deoxy-1-thio-β-D-galactopyranoside (**4**), methyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranoside (**7**), and methyl 2,3,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranoside (**9**), and *tert*-butyl-protected Fmoc-asparaginic acid propargylamide (**10**) gave the corresponding protected glycosyl amino acid building blocks **11**, **13**, **15**, and **17** in 67% to 95% yield. The latter were converted into the corresponding pentafluorophenyl esters **12**, **14**, **16**, and **18**, which were used for a spot synthesis of a combinatorial library containing 256 glycotetrapeptides. The library was screened for lectin-binding affinity with the lectins Concanavalin A (Con A), *phaseolus vulgaris* (PHA-E), and *galantus nivalis* (GNA).

Keywords Glycosyl amino acids, Click reaction, Glycopeptides, Combinatorial spot synthesis, Oligosaccharide mimics

INTRODUCTION

Specific interactions of proteins with complex carbohydrate structures associated with cell surfaces play a major role in many biologically important mechanisms such as, for example, cell–cell recognition, signal transduction,

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infection and inflammation mechanisms, and immunological processes. Different glycosylation patterns and posttranslational modifications of carbohydrate structures of glycoproteins are also responsible for their heterogeneity and biological properties.[1] Cells can be physically and biologically distinguished through their surface carbohydrate patterns. This is an important medicinal aspect with regard to specific tumor markers on cell surfaces, which often consist of distinct complex oligosaccharide structures.[2] Therefore, studying carbohydrate–protein interactions at a molecular level provides a deeper understanding of fundamental biological regulation mechanisms and opens the gate for novel analytical tools or to manipulate such specific processes for therapeutic purposes. Unfortunately, isolation of pure complex oligosaccharides from natural sources in order to study carbohydrate–protein interaction in detail is a rather difficult venture owing to the micro-heterogeneity of naturally occurring saccharides. Synthetic oligosaccharides, on the other hand, provide for sufficient amounts of pure material for this purpose. However, the chemical synthesis of complex oligosaccharides is still a laborious, sometimes even tedious, and often difficult task, although significant achievements in this field had been accomplished in the past decades. Thus, novel approaches for the efficient preparation of well-defined saccharide-containing structures that can mimic the interaction between a specific protein and its natural saccharide ligand are highly desirable.

For the construction of mimics for complex oligosaccharides, we follow a concept in which simple glycosyl amino acid building blocks are used for the efficient combinatorial preparation of fully glycosylated peptides (glycopeptoids), which, in turn, can bind to carbohydrate-recognizing proteins.[3] Recently, we prepared a series of glycosyl amino acid building blocks via click reaction (Cu-catalyzed 1,3-dipolar cycloaddition between azides and alkynes)[4] of 6-azido-6-deoxy-glycosides with suitably protected asparaginic acid propargylamide.[5] Click reactions have been widely applied in carbohydrate chemistry already, and allow for a highly efficient synthesis of glycosyl amino acid building blocks.[6] The 1,2,3-triazole moiety also mimics amino acids and, thus, is well suited for the construction of glycosylated peptides.[7]

Here, we extended our previous approach[5] to the highly efficient synthesis of glycosylated amino acid building blocks to other glycosides. Furthermore, we converted the latter into the corresponding pentafluorophenyl esters and used these active esters for the construction of a combinatorial library consisting of 256 glycotetrapeptides.

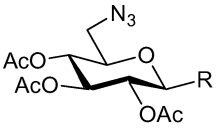
RESULTS AND DISCUSSION

Previously, ethyl 1-thio-glucoside (**2**) was prepared from ethyl 2,3,4-tri-*O*-acetyl-1-thio-6-*O*-tosyl-β-D-glucopyranoside by nucleophilic substitution of the

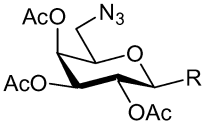
tosyl group with azide. However, the reaction resulted in anomerization and yielded **2** in 41% yield only.[5] In analogy to the known procedure for the synthesis of this compound, we now prepared **2** from easily available 1,2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranose **1**.[8,9] Reaction of the latter with $Me_3SiSEt/ZnCl_2$afforded **2** in 84% yield.[8] However, using $EtSH/BF_3$-etherate instead of $Me_3SiSEt/ZnCl_2$ resulted in a significantly lower yield, giving **2** in 37% yield. Similarly, treatment of 1,2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-D-galactopyranose **3**[10] with $EtSH/BF_3$-etherate afforded 1-thio-galactoside **4** in 35% yield. Methyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranoside (**7**) was prepared from methyl α-D-mannopyranoside **5** via its 6-*O*-tosyl derivative **6** by a slight modification of the previously described procedure[11] (see the experimental section for details). Methyl 2,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranoside **9** was prepared in 46% yield from methyl β-D-glucosamine (**8**)[12] by a catalytic diazotransfer from triflyl azide (Sch. 1).[13]

Next, azides **2**, **4**, **7**, and **9** were coupled with *N*-fluorenylmethoxycarbonyl-aspartic-1-propargylamide-4-*t*-butyl ester (**10**)[5] under catalysis with $(EtO)_3PCuI$[14] in toluene and irradiation with microwave[10] to afford the corresponding 1,2,3-triazole-linked glycosyl asparaginic acid derivatives **11**, **13**, **15**, and **17** in 67% to 95% yield. In general, the glucoside **2** and the mannoside **7** reacted faster and gave higher yields of the coupled products **12** and **16**, respectively, than the corresponding galactoside **4** and glucosamine **9**. Without microwave irradiation of the reaction mixture, only a very slow cycloaddition reaction was observed. Subsequent deprotection of compounds **11**, **13**, **15**, and **17** with trifluoroacetic acid in dichloromethane, followed by esterification of the intermediate carboxylic acids with pentafluorophenol and dicyclohexyl carbodiimide (DCC), gave the pentafluorophenyl esters **12**, **14**, **16**, and **18** in 51% to 74% overall yield. The latter were used as active ester building blocks for the preparation of β-peptides via spot synthesis because they can be stored without decomposition for an extended period of time.

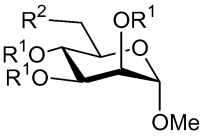
For the preparation of a combinatorial peptide library consisting of 256 tetra-glycopeptides derived from the four building blocks **12**, **14**, **16**, and **18**, an automated spot synthesis on cellulose paper sheets was applied.[15] The building blocks were denominated as building blocks **R** (Glc), **N** (Gal), **A** (Man), and **D** (GlcN) (Sch. 1). A format of a 16 $\times$ 16 spot array on 10 $\times$ 14 cm paper sheets was chosen. The cellulose paper sheets were derivatized by a slight modification of Wenschuh's procedure[16] (Sch. 2). First, the paper sheet was treated with epibromohydrin and a catalytic amount of $HClO_4$ in dioxane, followed by 4,7,10-trioxa-1,13-tridecanediamine in DMF and methanolic sodium methanolate solution to give the "safety catch" modified cellulose sheet **I**. The density of free amino groups was determined to be in the range of 500 to 600 nmol/cm^2 by reacting the paper sheet with Fmoc-protected pentafluorophenyl glycinate **20**, cleaving off the Fmoc groups of the bound glycine and determining the amount of cleaved Fmoc groups photospectrometrically.[17] Next,



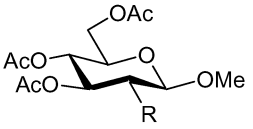
1 R = OAc
2 R = SEt



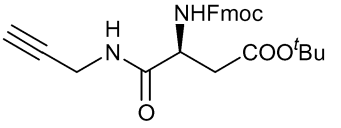
3 R = α,β-OAc
4 R = SEt



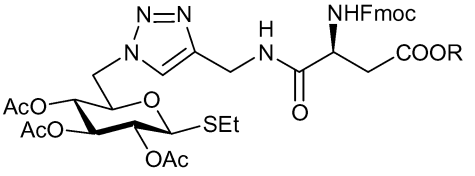
5 R^1, R^2 = H
6 R^1 = Ac, R^2 = TsO
7 R^1 = Ac, R^2 = N_3



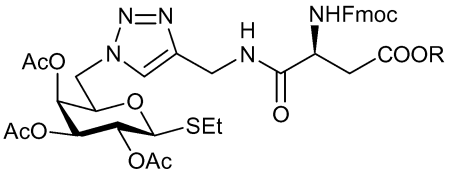
8 R = NH_2
9 R = N_3



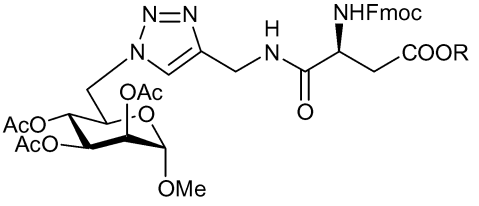
10



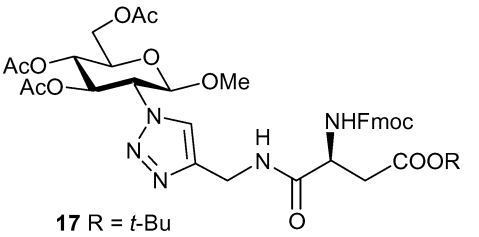
11 R = *t*-Bu
12 R = C_6F_5 Building Block **R**



13 R = *t*-Bu
14 R = C_6F_5 Building Block **N**



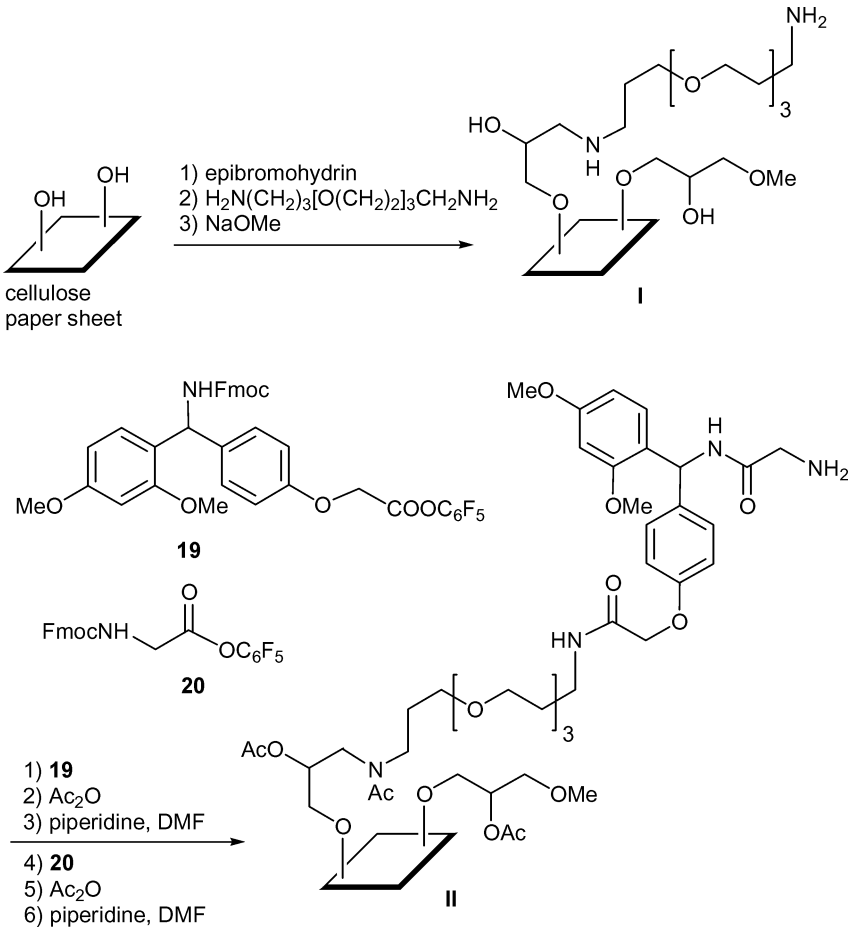
15 R = *t*-Bu
16 R = C_6F_5 Building Block **A**



17 R = *t*-Bu
18 R = C_6F_5 Building Block **D**

Scheme 1.

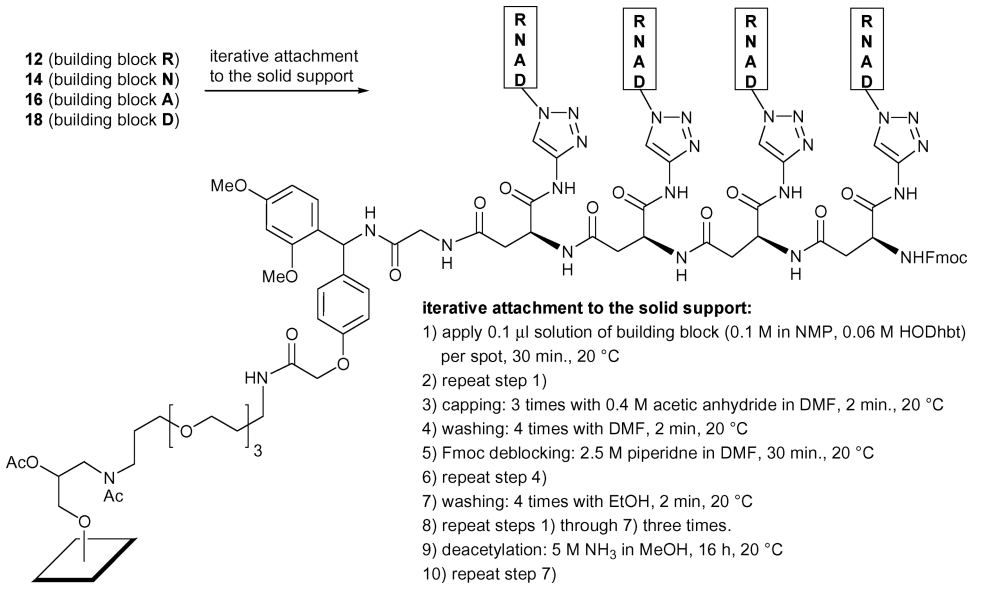
Fmoc-protected Rink linker **19** was coupled onto the modified cellulose sheet (*N*,*N*′-diisopropyl carbodiimide, pentafluorophenol in *N*-methyl pyrrolidone), followed by capping of the unreacted amino groups with acetic anhydride and cleavage of the Fmoc groups with piperidine in DMF. Finally, glycinate **20** was coupled to the Rink linker and deprotected to give the paper sheet **II**, ready for attaching the building blocks (Sch. 2). The terminal glycine moiety was introduced in order to avoid steric hindrance for the attachment of the first glycosyl amino acid building block since the primary amino group of the glycine moiety is better accessible than the secondary amino group of the Rink linker. Rink



Scheme 2.

linker **19** and glycine derivative **20** were not coupled to the entire paper sheet but, instead, only applied to the 256 spots where the glycopeptides were to be synthesized. This strategy diminished the unwanted unspecific binding of protein to the entire paper sheet via interaction with the hydrophobic Rink linker in the final lectin screening tests (see below). It also enabled the easy visualization of the progress of the final cleavage of the glycopeptides from the paper sheet with trifluoroacetic acid vapor, which gave dark red spots produced by the cations formed by the remaining Rink linker moiety.[3d]

Next, the building blocks were spotted onto the paper sheet in a 16 × 16 matrix using an autospot robot (Sch. 3). On each of the 256 spots, 2 × 10 nmol of building blocks **R**, **N**, **A**, and **D** were applied, resulting in spots of approximately 0.13 cm^2. The building blocks were applied to the individual spots on the paper sheet as a 0.1-M solution in *N*-methyl pyrrolidone (NMP) containing



Scheme 3.

0.06 M 3-hydroxy-1,2,4-benzotriazine-4-(3*H*)-one (HODhbt) for enhancing the efficiency of the coupling steps.[16b] An overall amount of approximately 20.5 μmol of building blocks **R**, **N**, **A**, and **D** were needed for the synthesis of the entire library of 256 individual glycotetrapeptides. A critical and time-consuming operation in the spot synthesis of the glycotetrapeptide library was the washing cycles after the coupling and Fmoc deprotection steps, which had to be performed thoroughly. The completeness of the coupling, Fmoc deprotection, and washing steps was best controlled by staining the paper sheet with bromophenol blue solution in DMF.[15a] The final deacetylation of the bound glycotetrapeptides was effected by rinsing the cellulose membrane with a methanolic solution of NH_3 overnight followed by washing with ethanol.[3d] Detachment of the glycotetrapeptides containing a *N*-terminal glycineamide from the membrane for analytical purposes can be achieved by exposing the membrane to an atmosphere of trifluroacetic acid in a desiccator as described previously.[3d]

The glycotetrapeptide library was screened for lectin-binding affinities with horseradish peroxidise conjugates of Concanavalin A (Con A, *canavalis ensiformis* lectin), phytohaemagglutinine (PHA-E, phaseolus vulgaris lectin), and galantus nivalis lectin (GNA) as described previously.[3d] Figure 1 shows the typical pattern of red spots for the binding of GNA after the peroxidase-catalyzed formation of Wuster's Red from 3-amino-9-ethyl-carbazole and H_2O_2.[18a] The latter staining procedure was superior to other commonly applied staining methods using lectin-peroxidase conjugates[18b–d] because the background staining of the cellulose membrane was kept to a minimum.

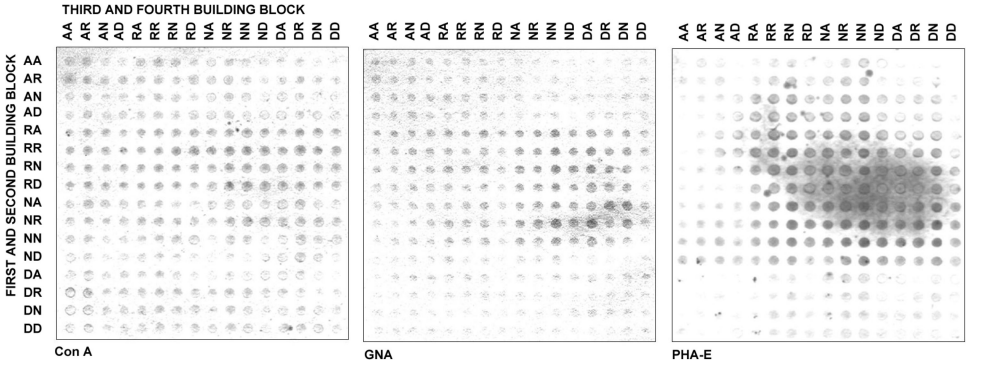


Figure 1. Screening Results.

Compared to the binding of lectins to previous glycotetrapeptide libraries obtained from similar building blocks in which the sugar moieties were glycosidically linked to the β-peptide backbone via alkyl spacers,[3a,d] the tested lectins showed a different binding affinity here. Specifically, Con A bound to all spots with rather low selectivity. This can be attributed to the relatively low selectivity of Con A for binding to mannose and glucose. Nevertheless, Con A bound slightly stronger to those glycotetrapeptides that contained block **R** (glucose) as the last attached building block. No significantly enhanced binding to peptides rich in block **A** (mannose) could be observed though. GNA, which displays a selectivity for mannose-rich oligosaccharides, did not bind any stronger to glycotetrapeptides rich in block **A** (mannose) here. Instead, GNA preferentially bound to those peptides that contained blocks **R** (glucose) and **N** (galactose) as the last attached blocks. Furthermore, the sequence **RXNN**, where **X** is any other sugar moiety, preferentially bound GNA. Similarly, PHA-E lectin, which selectively binds to the trisaccharide sequence Gal-β1,4-GlcNAc-β1,2-Man, also preferentially bound to those glycotetrapeptides with blocks **R** (glucose) or **N** (galactose) as the last attached block. Here, an enhanced affinity of PHA-E for the sequences **RXNN**, **RXNR**, and **RXRN** was observed. In general, those peptides that contain ethyl 1-thio-glycosides bound the lectins better than those containing methyl glycosides.

CONCLUSION

It could be shown that glycotetrapeptides constructed out of β-peptidically linked asparaginic acid having glycosyl 1,2,3-triazole moieties attached to each α-carboxylic group of the peptide backbone can indeed mimic the binding of lectins to their natural ligands. Thus, glycopeptoids of the type described here may be suitable devices for studying protein–carbohydrate interactions on a molecular level in order to gain a better insight in the various factors

determining the binding of proteins to carbohydrates. Further binding studies with additional lectins and with glycopeptide libraries constructed out of building blocks containing glycosidically linked sugar moieties,[5] as well as quantitative binding studies of lectins to such glycopeptides, are under way now.

EXPERIMENTAL

All solvents were dried and distilled prior to their use. Reactions were performed under Ar and monitored by TLC on Polygram Sil G/UV silica gel plates from Machery & Nagel. Detection was affected by charring with H_2SO_4 (5% in EtOH) or by inspection of the TLC plates under UV light. NMR spectra were recorded on a Bruker ARX 250 spectrometer at 100 MHz for proton spectra and 62.9 MHz for carbon spectra, and on a Bruker Avance 400 spectrometer at 400 MHz for proton spectra and 100 MHz for carbon spectra. Tetramethylsilane was used at the internal standard. FAB-MS was performed on a Finnigan MAT TSQ 70 spectrometer. HRFD-MS was performed on a Bruker FT-ICR spectrometer. Elemental analyses were performed on a Hekatech CHN analyzer. Optical rotations were measured with a Perkin-Elmer Polarimeter 341. Preparative chromatography was performed on silica gel (0.032–0.063 mm) from Machery & Nagel using different mixtures of solvents as eluents. Microwave-assisted reactions were performed with a CEM-focused microwave synthesis system, type Discover. Spot syntheses were performed with an Intavis AG Bioanalysis Instruments AutoSpot Robot ASP 222.

Ethyl 2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-1-thio-*β*-D-glucopyranoside (2)

BF_3 etherate (1.08 mL, 8.60 mmol) was added at 0°C to a solution of compound **1**[8,9] (2.00 g, 5.36 mmol) and ethyl mercaptane (0.79 mL, 9.4 mmol) in dichloromethane (100 mL). The mixture was stirred for 2 h at 0°C, warmed to rt, and stirred for additional 8 h. The mixture was washed with saturated aqueous $NaHCO_3$ solution, dried with Na_2SO_4, and filtered. Concentration of the filtrate in vacuo and chromatography of the residue (toluene, ethyl acetate 3:1) afforded **2** (0.74 g, 37%) as a white amorphous foam. The compound was identical to the one previously prepared.[5,8]

Ethyl 2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-1-thio-*β*-D-galactopyranoside (4)

Treatment of compound **3**[10] (2.00 g, 5.36 mmol), ethyl mercaptane (0.79 mL, 9.4 mmol), and BF_3 etherate (1.08 mL, 8.60 mmol) in dichloromethane (100 mL) as described for the preparation of compound **2** gave **4** (0.71 g, 35%)

as a white amorphous foam. $[\alpha]_D^{20} = -13.8$ (c 1.00, $CHCl_3$). FAB MS m/z: found 376.0, calcd. 376.1 $[M+H]^+$. ^1H NMR ($CDCl_3$): δ (ppm) = 5.39 (d, 1 H, $J_{1,2}$ = 3.5 Hz , H-1), 5.25 (t, 1 H, $J_{3,4} = 9.9$ Hz, H-3), 5.08 (dd, 1 H, $J_{2,3} = 3.3$ Hz, H-2), 4.56 (d, 1 H, $J = 10.2$ Hz, H-4), 3.84 (dd, 1 H, $J = 3.8$ Hz , H-5), 3.53 (m, 1 H, H-6a), 3.14–3.19 (m, 1 H, H-6b), 2.71–2.82 (m, 2 H, SCH_2), 2.18, 2.08, 1.99 (3s, 9 H, $COCH_3$), 1.30 (t, 3 H, J = 7.4 Hz, SCH_2CH_3). ^{13}C NMR ($CDCl_3$): δ (ppm) = 170.2, 169.9, 169.5 (C=O), 83.8 (C-1), 76.2 (C-5), 71.8 (C-3), 68.1 (C-2), 67.2 (C-4), 50.7 (C-6), 24.3 (SCH_2), 20.7, 20.6, 20.5, ($COCH_3$), 14.7 (SCH_2CH_3).

Methyl 2,3,4-Tri-*O*-acetyl-6-*O*-*p*-toluolsulfonyl-α-D-mannopyranoside (6)

p-Toluenesulfonyl chloride (5.90 g, 31.0 mmol) was added with stirring at 0°C to a solution of methyl α-D-mannopyranoside **5** (5.00 g, 25.8 mmol) in pyridine (50 mL), and stirring was continued for 2 h. Acetic anhydride (50 mL, 0.53 mol) was added and stirring at 0°C was continued for 3 h. The solution was poured onto crushed ice, and the mixture was extracted with chloroform (3 × 100 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$,and filtered. Concentration of the filtrate in vacuo and filtration of the residue over a short column of silica gel (toluene/ethyl acetate 3:1) gave crude **6** (7.01 g, 79%), which was sufficiently pure for the next step. ^1H NMR ($CDCl_3$): δ (ppm) = 7.77 (d, 2 H, $J = 8.1$ Hz, Tos-Ar-H), 7.31 (d, 2 H, $J = 8.1$ Hz, Tos-Ar-H), 4.93–5.25 (m, 3 H, H-1,2,3), 4.65 (s, 1 H, H-5), 3.80–3.86 (m, 1 H, H-4), 3.33 (s, 3 H, OCH_3), 3.15–3.32 (m, 2 H, H-6a,b), 2.01, 2.00, 1.93 (3s, 9 H, $COCH_3$).^{13}C NMR ($CDCl_3$): δ (ppm) = 169.3, 169.3, 169.2 (C=O),145.0, 132.4, 129.7, 128.3 (Tos-Ar-C), 97.8 (C-1), 69.2 (C-2), 68.8 (C-4), 68.2 (C-3), 66.5 (C-5), 54.6 (OCH_3), 67.3 (C-6), 21.8 (Tos-CH_3), 20.2, 20.1, 19.9, ($COCH_3$).

Methyl 2,3,4-Tri-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranoside (7)

A solution of compound **6** (7.00 g, 20.09 mmol) and NaN_3(10.0 g, 0.154 mol) in dry DMSO (100 mL) was stirred at 50°C for 4 h followed by stirring at rt for 16 h. The resulting slurry was poured onto crushed ice, and the mixture was extracted with dichloromethane (4 × 50 mL). The combined extracts were washed two times with water, dried over $MgSO_4$, and filtered. Concentration of the filtrate in vacuo and filtration of the residue over a short column of silica gel (n-hexane/ethyl acetate 5:1) followed by crystallization from ethanol gave **7** (5.62 g, 81%). mp. 100°C (mp.[11] 99–100°C). $[\alpha]_D^{20} = +65.7$ (c 1.0, $CHCl_3$). All NMR data are identical to those reported in the literature.[11]

Methyl 2,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-*β*-D-glucopyranoside (9)

According to reference [13a], a 9-M solution of triflyl azide in pyridine was freshly prepared by dissolving NaN_3 (0.59 g, 9.06 mmol) in pyridine (10 mL), adding trifluorometanesulfonic anhydride (1.24 mL, 7.2 mmol) at 0°C, stirring the mixture at 0°C for 2 h, and filtering off all precipitated salts. The solution of triflyl azide was added dropwise at 0°C to a solution of compound **8**[12] (2.00 g, 6.26 mmol), triethylamine (1.75 mL, 12.52 mmol), and $CuSO_4 \times 5\ H_2O$ (16 mg, 0.0626 mmol) in pyridine (10 mL). After the addition of the triflyl azide solution was completed, and the mixture was stirred at rt for 16 h and concentrated in vacuo. Chromatography (toluene/ethyl acetate 3:1) of the residue afforded **9** (1.0 g, 46%). $[\alpha]_D^{20} = +5.0$ (*c* 1.0, $CHCl_3$). FAB MS m/z: found 346.0, calcd. 346.31 $[M+H]^+$. ^1H NMR ($CDCl_3$): δ (ppm) = 4.90–4.97 (m, 2 H, H-1,5), 4.18–4.25 (m, 2 H, H-3,4), 4.02–4.05 (m, 1 H, H-6a), 3.59–3.62 (m, 1 H, H-6b), 3.56 (s, 3 H, OCH_3), 3.38–3.42 (m, 1 H, H-2), 2.00, 2.00, 1.93 (3s, 9 H, $COCH_3$). ^{13}C NMR ($CDCl_3$): δ (ppm) = 170.6, 170.0, 169.5 (C=O), 102.9 (C-1), 72.5 (C-3), 71.7 (C-5), 68.4 (C-4), 63.7 (C-6), 61.8 (OCH_3), 57.4 (C-2), 21.2, 21.1, 20.9 ($COCH_3$).

t-Butyl *N*2-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-{[1-(2,3,4-tri-*O*-acetyl-6-deoxy-1-ethylthio-*β*-D-glucopyranos-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl}-L-*α*-asparaginate (11)

A solution of compound **2** (225 mg, 0.6 mmol), compound **10**[5] (261 mg, 0.6 mmol), diisopropyl ethylamine (0.31 mL, 1.8 mmol), and $(EtO)_3PCuI$ (24 mg, 0.06 mmol) in toluene (5 mL) was stirred 80°C under microwave irradiation (30 W) for 30 min. Concentration of the solution in vacuo and chromatography of the residue (ethyl acetate) afforded **11** (450 mg, 91%). HRFD MS m/z: found 846.29859, calcd. 846.29906 $[M+Na]^+$. The compound was identical to the one previously obtained.[5]

Pentafluorophenyl *N*2-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-{[1-(2,3,4-tri-*O*-acetyl-6-deoxy-1-ethylthio-*β*-D-glucopyranos-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl}-L-*α*-asparaginate (12)

A solution of compound **11** (397 mg, 0.5 mmol) and trifluoroacetic acid (1.0 mL, 13.1 mmol) in dichloromethane (3 mL) was stirred at rt for 3 h, concentrated in vacuo, coevaporated with toluene (5 × 10 mL), and redissolved in ethyl acetate (5 mL). Pentafluorophenol (92 mg, 0.5 mmol) and dicyclohexyl carbodiimide (103 mg, 0.7 mmol) were added to the solution at 0°C and the mixture was stirred for 14 h at 0°C. Filtration of the mixture, concentration of the filtrate in vacuo, and chromatography of the residue (n-hexane/ethyl acetate 1:5) afforded **12** (337 mg, 72%). $[\alpha]_D^{20} = +2.5$ (*c* 1.0, $CHCl_3$). FAB MS m/z: found

956.22218, calcd. 956.22065 $[M+Na]^+$. ^1H NMR ($CDCl_3$): δ (ppm) = 7.71 (d, 2 H, J = 7.6 Hz, Ar-H), 7.60 (s , 1 H, triazole-H), 7.49 (d, 2 H, J = 7.4 Hz, Ar-H), 7.35 (t, 2 H, J = 7.6 Hz, Ar-H), 7.18–7.26 (m, 3 H, Ar-H, NH), 5.87 (s, 1 H, NH), 5.15 (t, 1 H, $J_{1,2}$ = 9.4 Hz, H-1), 4.92 (t, 1 H, $J_{3,4}$ = 9.9 Hz, H-3), 4.77 (t, 1 H, $J_{2,3}$ = 9.7 Hz, H-2), 4.69 (s, 1 H, 3′) 4.36–4.56 (m, 9 H, Fmoc, H-2, H-4, H-6a, H-5, CH_2), 3.79 (s, 1 H, H-6b), 3.08–3.26 (m, 2 H, H-2′a,b), 2.48 (q, 2 H, J = 7.1 Hz, SCH_2) 2.00, 1.99, 1.95 (3s, 9 H, $COCH_3$), 1.18 (t, 3 H, J = 7.1 Hz, SCH_2CH_3).^{13}C NMR ($CDCl_3$): δ (ppm) = 170.0, 169.8, 169.7, 169.3, 167.5, 156.1 (C=O), 144.2 (triazole C-4), 143.4, 141.3 (Fmoc), 136.6–139.5 (Pfp), 127.8, 127.0 124.9, 120.0, (Fmoc), 124.2 (triazole C-5), 83.5 (C-1), 76.0 (C-3), 73.4 (C-2), 69.6 (C-4), 69.5 (C-5), 67.4 (Fmoc-CH_2), 51.0 (C-6), 49.3 (Asp-CH), 47.0 (Fmoc-CH), 35.3 (Asp-CH_2), 35.0 (CH_2), 24.3 (SCH_2), 20.6, 20.5, 20.5, ($COCH_3$), 14.8 (SCH_2CH_3).

t-Butyl *N*2-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-{[1-(2,3,4-tri-*O*-acetyl-6-deoxy-1-ethylthio-β-D-galactopyranos-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl}-L-α-asparaginate (13)

A solution of compound **4** (225 mg, 0.6 mmol), compound **10**[5] (261 mg, 0.6 mmol), diisopropyl ethylamine (0.31 mL, 1.8 mmol), and $(EtO)_3PCuI$ (24 mg, 0.06 mmol) in toluene (5 mL) was stirred 80°C under microwave irradiation (30 W) for 1 h. Concentration of the solution in vacuo and chromatography of the residue (ethyl acetate) afforded **13** (331 mg, 67%). $[\alpha]_D^{20}$ = +23.4 (*c* 1.0, $CHCl_3$). HRFD MS m/z: found 846. 29917, calcd. 846.29906 $[M+Na]^+$. ^1H NMR ($CDCl_3$): δ (ppm) = 7.70 (d, 2 H, J = 7.4 Hz, Ar-H), 7.58 (s, 1 H, triazole- H), 7.50 (d, 2 H, J = 7.4 Hz, Ar-H), 7.42 (s, 1 H, NH), 7.33 (t, 2 H, J = 5.8 Hz, Ar-H), 7.22 (t, 2 H, J = 7.4 Hz, Ar-H), 6.16 (d, 1 H, J = 8.4 Hz, NH), 5.38 (s, 1 H, H-1), 5.16 (t, 1 H, $J_{3,4}$ = 9.7 Hz, H-3), 5.03 (s, 1 H, H-3′), 4.25–4.52 (m, 8 H, Fmoc, H-2, H-4, H-5, CH_2), 3.97–4.11 (m, 2 H, H-6a,b), 2.80 (d, 1 H, J = 15.5 Hz, H-2′a), 2.63 (dd, 1 H, J = 6.1, H-2′b), 2.50 (t, 2 H, J = 6.1 Hz, SCH_2) 2.13, 1.98, 1.92 (3s, 9 H, $COCH_3$), 1.38 (s, 9 H, CH_3), 1.08 (t, 3 H, J = 7.4 Hz, SCH_2CH_3). ^{13}C NMR ($CDCl_3$): δ (ppm) = 170.9, 170.6, 170.0, 169.7, 169.3, 155.9 (C=O), 144.5 (triazole C-4), 143.4, 141.1 (Fmoc), 127.5, 126.9, 124.9, 119.8, (Fmoc), 123.6 (triazole C-5), 83.7 (C-1), 81.4 (t-Bu C), 76.7 (C-3), 75.0 (C-2), 71.5 (C-4), 67.9 (C-5), 67.0 (Fmoc CH_2), 51.2 (Asp CH), 51.1 (C-6), 46.9 (Fmoc-CH), 37.4 (Asp-CH_2), 34.8 (CH_2), 27.8 (CH_3), 24.4 (SCH_2), 20.5, 20.5, 20.3 ($COCH_3$), 14.7 (SCH_2CH_3).

Pentafluorophenyl *N*2-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-[1-(2,3,4-tri-*O*-acetyl-6-deoxy-1-ethylthio-β-D-galactopyranos-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl-L-α-asparaginate (14)

A solution of compound **13** (397 mg, 0.5 mmol) and trifluoroacetic acid (1.0 mL, 13.1 mmol) in dichloromethane (3 mL) was stirred at rt for 3 h,

concentrated in vacuo, coevaporated with toluene (5 × 10 mL), and redissolved in ethyl acetate (5 mL). Pentafluorophenol (92 mg, 0.5 mmol) and dicyclohexyl carbodiimide (103 mg, 0.7 mmol) were added to the solution at 0°C, and the mixture was stirred for 14 h at 0°C. Filtration of the mixture, concentration of the filtrate in vacuo, and chromatography of the residue (n-hexane/ethyl acetate 1:5) afforded **14** (238 mg, 51%). $[\alpha]_D^{20} = +14.6$ (*c* 1.0, $CHCl_3$). HRFD MS m/z: found 956.21171, calcd. 956.22065 $[M+Na]^+$. ^1H NMR ($CDCl_3$): δ (ppm) = 7.71 (d, 2 H, $J = 7.4$ Hz, Ar-H), 7.59 (s, 2 H, tziazole-H, NH), 7.50 (d, 2 H, $J = 7.6$ Hz, Ar-H), 7.35 (t, 2 H, $J = 7.1$ Hz, Ar-H), 7.23 (t, 2 H, $J = 7.4$ Hz, Ar-H), 6.22 (d, 1 H, $J = 8.4$ Hz, NH), 5.36 (s, 1 H, H-1), 5.16 (t, 1 H, $J_{3,4} = 9.9$ Hz, H-3), 5.00 (dd, 1 H, $J = 2.8$ Hz, H-3′), 4.25–4.56 (m, 9 H, Fmoc, H-2, H-4, H-6a, H-5, CH_2), 4.07–4.13 (m, 1 H, H-6b), 3.13–3.26 (m, 2 H, H-2′a,b), 2.53 (t, 2 H, $J = 7.4$ Hz, SCH_2) 2.00, 2.00, 1.93 (3s, 9 H, $COCH_3$), 1.11 (t, 3 H, $J = 7.4$ Hz, SCH_2CH_3). ^{13}C NMR ($CDCl_3$): δ (ppm) = 170.3, 169.7, 169.6, 169.5, 167.2, 156.1 (C=O), 144.2 (triazole C-4), 143.4, 141.1 (Fmoc), 135.8–139.5 (Pfp), 127.7, 127.0 124.9, 119.9, (Fmoc), 123.8 (triazole C-5), 83.9 (C-1), 75.1 (C-3), 71.6 (C-2), 67.9 (C-4), 67.3 (C-5), 66.9 (Fmoc-CH_2), 50.2 (C-6), 49.5 (Asp-CH), 46.9 (Fmoc-CH), 35.6 (Asp-CH_2), 34.9 (CH_2), 24.4 (SCH_2), 20.6, 20.5, 20.4, ($COCH_3$), 14.7 (SCH_2CH_3).

t-Butyl *N*2-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-{[1-(2,3,4-tri-*O*-acetyl-6-deoxy-1-methoxy-α-D-mannopyranos-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl}-L-α-asparaginate (15)

A solution of compound **7** (207 mg, 0.6 mmol), compound **10**[5] (261 mg, 0.6 mmol), diisopropyl ethylamine (0.31 mL, 1.8 mmol), and $(EtO)_3PCuI$ (24 mg, 0.06 mmol) in toluene (5 mL) was stirred 80°C under microwave irradiation (30 W) for 30 min. Concentration of the solution in vacuo and chromatography of the residue (ethyl acetate) afforded **15** (462 mg, 95%). $[\alpha]_D^{20} = +24.2$ (*c* 1.0, $CHCl_3$). HRFD MS m/z: found 816.62200, calcd. 816.30626 $[M+Na]^+$. ^1H NMR ($CDCl_3$): δ (ppm) = 7.75 (d, 2 H, $J = 7.6$ Hz, Ar-H), 7.66 (s , 1 H, triazole-H), 7.39 (d, 2 H, $J = 7.4$ Hz, Ar-H), 7.39 (t, 2 H, $J = 7.4$ Hz, Ar-H, 7.30 (t, 2 H, $J = 7.4$ Hz, Ar-H), 7.04 (s, 1 H, NH), 5.89 (d, 1 H, $J = 8.1$ Hz, NH), 5.30 (dd, 1 H, $J_{1,2} = 3.6$, H-2), 5.20 (s, 1 H, H-1), 5.15 (t, 1 H, $J = 9.9$ Hz, H-3′), 4.60 (s, 1 H, H-3), 4.41–4.55 (m, 6 H, Fmoc, H-4, CH_2), 4.32 (dd, 1 H, $J_{5,6a} = 8.9$ Hz, H-5), 4.18 (t, 1 H, $J = 6.6$ Hz, H-6a), 4.07–4.13 (m, 1 H, H-6b), 3.05 (s, 3 H, CH_3), 2.88 (dd, 1 H, $J = 3.8$ Hz, H-2′a), 2.59 (dd, 1 H, $J = 5.3$ Hz, H-2′b), 2.13, 2.09, 1.86 (3s, 9 H, $COCH_3$), 1.42 (s, 9 H, CH_3). ^{13}C NMR ($CDCl_3$): δ (ppm) = 170.9, 170.5, 170.1, 169.8, 169.7, 156.0 (C=O), 144.6 (triazole C-4), 143.6, 141.3 (Fmoc), 127.7, 127.1, 124.9, 120.0 (Fmoc), 123.9 (triazole C-5), (98.3 (C-1), 81.8 (t-Bu C), 69.3(C-2), 69.0 (C-4), 68.6 (C-3), 67.3 (C-5), 67.1 (Fmoc-CH_2), 55.4 (CH_3), 51.2 (Asp-CH), 50.5 (C-6), 47.1 (Fmoc-CH), 37.2 (Asp-CH_2), 35.1 (CH_2), 28.0 (CH_3), 20.8, 20.7, 20.6 ($COCH_3$).

Pentafluorophenyl *N*2-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-{[1-(2,3,4-tri-*O*-acetyl-6-deoxy-1-methoxy-α-D-mannopyranos-6-yl)-1*H*-1,2,3-triazol-4-yl]methyl}-L-α-asparaginate (16)

A solution of compound **13** (397 mg, 0.5 mmol) and trifluoroacetic acid (1.0 mL, 13.1 mmol) in dichloromethane (3 mL) was stirred at rt for 3 h, concentrated in vacuo, coevaporated with toluene (5 × 10 mL), and redissolved in ethyl acetate (5 mL). Pentafluorophenol (92 mg, 0.5 mmol) and dicyclohexyl carbodiimide (103 mg, 0.7 mmol) were added to the solution at 0°C, and the mixture was stirred for 14 h at 0°C. Filtration of the mixture, concentration of the filtrate in vacuo, and chromatography of the residue (n-hexane/ethyl acetate 1:5) afforded **14** (298 mg, 66%). $[\alpha]_D^{20} = +15.5$ (*c* 1.0, $CHCl_3$). HRFD MS m/z: found 926.22785, calcd. 926.22785 $[M+Na]^+$. ^1H NMR ($CDCl_3$): δ (ppm) = 7.71–7.74 (m, 3 H, $J = 7.6$ Hz, Ar-H, triazole-H), 7.53 (d, 2 H, $J = 7.4$ Hz, Ar-H), 7.26–7.38 (m, 5 H, Ar-H, NH), 6.07–6.12 (m, 1 H, NH), 5.29 (d, 1 H, $J_{1,2} = 3.6$, H-2), 5.21 (s, 1 H, H-1), 5.10 (t, 1 H, $J = 9.9$ Hz, H-3′), 4.72 (s, 1 H, H-3), 4.30–4.60 (m, 7 H, Fmoc, H-4, H-5, CH_2), 4.08–4.15 (m, 2 H, H-6a,b), 3.13–2.27 (m, 2 H, H-2′a,b), 3.06 (s, 3 H, $COCH_3$), 2.08, 2.03, 1.98 (3s, 9 H, Acetyl-CH_3). ^{13}C NMR ($CDCl_3$): δ (ppm) = 171.3, 170.0, 169.8, 169.7, 167.3, 156.1 (C=O), 144.0 (triazole C-4), 143.4, 141.2 (Fmoc), 136.6–139.5 (Pfp), 128.6, 127.7, 124.8, 119.9, (Fmoc), 124.0 (triazole C-5), 98.3 (C-1), 70.8 (C-2), 69.2 (C-4), 68.9 (C-3), 68.6 (C-5), 67.3 (Fmoc-CH_2), 55.1 (CH_3), 50.9 (C-6), 49.1 (Asp-CH), 46.9 (Fmoc-CH), 35.3 (Asp-CH_2), 34.9 (CH_2), 20.6, 20.5, 20.5 ($COCH_3$).

t-Butyl *N*2-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-{[1-(3,4,6-tri-*O*-acetyl-2-deoxy-1-methoxy-β-D-glucopyranos-2-yl)-1*H*-1,2,3-triazol-4-yl]methyl}-L-α-asparaginate (17)

A solution of compound **9** (207 mg, 0.6 mmol), compound **10**[5] (261 mg, 0.6 mmol), diisopropyl ethylamine (0.31 mL, 1.8 mmol), and $(EtO)_3PCuI$ (24 mg, 0.06 mmol) in toluene (5 mL) was stirred 80°C under microwave irradiation (30 W) for 1 h. Concentration of the solution in vacuo and chromatography of the residue (ethyl acetate) afforded **15** (400 mg, 84%). $[\alpha]_D^{20} = +38.7$ (*c* 1.0, $CHCl_3$). HRFD MS m/z: found 816.30618, calcd. 816.30626 $[M+Na]^+$. ^1H NMR ($CDCl_3$): δ (ppm) = 7.69 (d, 2 H, $J = 7.6$ Hz, Ar-H), 7.50–7.53 (m , 3 H, triazole-H, Ar-H), 7.33 (t, 2 H, $J = 7.4$ Hz, Ar-H), 7.21–7.26 (m, 3 H, Ar-H, NH), 6.07 (d, 1 H, $J = 6.9$ Hz, NH), 5.72 (t, 1 H, $J = 9.9$ Hz, H-3′), 5.09 (t, 1 H, $J_{1,2} = 9.7$ Hz, H-1), 4.92 (d, 1 H, $J_{2,3} = 8.4$ Hz, H-2), 4.36–4.51 (m, 4 H, Fmoc, H-5,), 4.42–4.36 (m, 4 H, CH_2, H-3), 4.10–4.17 (m, 2 H, H-4,6a), 3.83–3.86 (m, 1 H, H-6b), 3.32 (s, 3 H, CH_3), 2.81 (d, 1 H, $J = 12.2$ Hz, H-2′a), 2.62 (dd, 1 H, $J = 5.3$ Hz, H-2′b), 2.04, 1.98, 1.95 (3s, 9 H, $COCH_3$), 1.39 (s, 9 H, CH_3). ^{13}C NMR ($CDCl_3$): δ (ppm) = 170.9, 170.6, 170.3, 169.4, 168.9, 155.9 (C=O), 144.2 (triazole C-4), 143.5, 141.1 (Fmoc), 127.6, 126.9, 124.8, 119.8, (Fmoc), 123.7 (triazole C-5), 101.1

(C-1), 81.5 (t-Bu C), 72.0 (C-3), 71.6 (C-5), 68.5 (C-4), 66.9 (Fmoc-CH_2), 63.8 (C-6), 61.6 (CH_3), 57.2 (C-2), 51.2 (Asp-CH), 46.9 (Fmoc-CH), 37.4 (Asp-CH_2), 34.9 (CH_2), 27.8 (CH_3), 20.5, 20.3, 20.0, (CO*C*H_3).

Pentafluorophenyl *N*2-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-*N*-{[1-(3,4,6-tri-*O*-acetyl-2-deoxy-1-methoxy-β-D-glucopyranos-2-yl)-1*H*-1,2,3-triazol-4-yl]methyl}-L-α-asparaginate (18)

A solution of compound **13** (397 mg, 0.5 mmol) and trifluoroacetic acid (1.0 mL, 13.1 mmol) in dichloromethane (3 mL) was stirred at rt for 3 h, concentrated in vacuo, coevaporated with toluene (5 × 10 mL), and redissolved in ethyl acetate (5 mL). Pentafluorophenol (92 mg, 0.5 mmol) and dicyclohexyl carbodiimide (103 mg, 0.7 mmol) were added to the solution at 0°C, and the mixture was stirred for 14 h at 0°C. Filtration of the mixture, concentration of the filtrate in vacuo, and chromatography of the residue (n-hexane/ethyl acetate 1:5) afforded **18** (336 mg, 74%). $[\alpha]_D^{20} = +19.2$ (*c* 1.0, $CHCl_3$). HRFD MS m/z: found 926.22858, calcd. 926.22785 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.69 (d, 2 H, $J = 7.4$ Hz, Ar C*H*), 7.57–7.61 (m , 2 H, triazole-H, NH), 7.50 (t, 2 H, $J = 7.1$ Hz, Ar-H), 7.33 (t, 2 H, $J = 7.4$ Hz, Ar-H), 7.21 (t, 2 H, $J = 7.4$ Hz, Ar-H), 6.26 (d, 1 H, $J = 8.9$ Hz, NH), 5.70 (t, 1 H, $J = 9.9$ Hz, H-3′), 5.10 (t, 1 H, $J_{1,2} = 9.7$ Hz, H-1), 4.90 (d, 1 H, $J_{2,3} = 8.1$ Hz, H-2), 4.76 (d, 1 H, $J_{3,4} = 6.6$ Hz, H-3), 4.27–4.47 (m, 6 H, Fmoc, H-5, CH_2), 4.04–4.16 (m, 2 H, H-4,6a), 3.82 (d, 1 H, $J_{5,6b} = 8.4$ Hz, H-6b), 3.28 (s, 3 H, CH_3), 2.19 (s, 2 H, H-2′a,b), 2.01, 1.99, 1.95 (3s, 9 H, $COCH_3$). ^{13}C NMR ($CDCl_3$): δ (ppm) = 170.5, 169.8, 164.6, 169.1, 167.1, 156.0 (C=O), 144.1 (triazole C-4), 143.8, 141.1 (Fmoc), 136.4–139.5 (Pfp), 127.6, 126.9 124.8, 119.8, (Fmoc), 124.0 (triazole C-5), 101.0 (C-1), 72.0 (C-3), 71.6 (C-5), 68.4 (C-4), 67.1 (Fmoc-CH_2), 63.9 (C-6), 61.6 (CH_3), 57.0 (C-2), 51.0 (Asp -H), 46.9 (Fmoc-CH), 35.5 (Asp-CH_2), 34.8 (CH_2), 20.4, 20.2, 19.8, (CO*C*H_3).

Derivatized Cellulose Membrane I

Rectangular cellulose paper sheets (10 × 14 cm) were rinsed in dioxane (3 × 20 mL) and methanol (3 × 20 mL) for 10 min each and dried in a desiccator in vacuo. Each membrane was soaked with dioxane (3 mL) containing 10 vol-% epibromohydrin (0.3 mL) and 1 vol-% perchloric acid (30 μL) at rt for 2 h followed by rinsing with dioxane and methanol and drying the membrane as described above. Each membrane was then rinsed in DMF (20 mL) containing 20 vol-% 4,7,10-trioxa-1,13-tridecanediamine (4 mL) at rt for 2 h followed by rinsing in a 5-M solution of NaOMe in methanol (20 mL) for 30 min, washing the membrane with methanol (7 × 20 mL) for 2 min each, and drying it in vacuo in a desiccator.

Pentafluorophenyl 4′-{(R,S)-α-[1-(9-Fluorenyl) methoxycarbonylamino]-2,4-dimethoxybenzyl}-phenoxyacetate (19)

A solution of 4′-{(R,S)-α-[1-(9-fluorenyl)methoxycarbonylamino]-2,4-dimethoxybenzyl}-phenoxyacetatic acid (Rink amide linker) (135 mg, 0.25 mmol), pentafluorophenol (46 mg, 0.25 mmol), and diisopropyl carbodiimide (40 μL, 0.26 mmol) in *N*-methylpyrrolidone (NMP) (1 mL) was stirred at rt for 30 min to give a ca. 0.25-M solution of **19** in NMP, ready for spotting the Rink Amide Liner onto the derivatized cellulose membrane I.

Derivatized Cellulose Membrane II

A. Derivatization with Rink amide linker. A derivatized cellulose membrane I was placed in the autospot robot and 256 spots in a 16 × 16 matrix with a distance of 4.8 mm between the spots were created on the membrane by automated pipetting 0.1 μL of the above solution of **19** in NMP onto each spot, and repeating the spotting cycle for all spots after 30 min. The membrane was then rinsed two times for 2 min with a solution of acetic anhydride (2 mL) and diisopropyl ethylamine (4 mL) in DMF (20 mL), and washed with DMF (2 × 20 mL) for 2 min each. Next, the membrane was rinsed with a solution of piperidine (5 mL) in DMF (20 mL) for 30 min at rt, rinsed with DMF (4 × 20 mL) and ethanol (4 × 20 mL) for 2 min each, and dried in vacuo in a desiccator.

B. Derivatization with Glycine. The membrane was placed back in the autospot robot and 0.1 μL of a 0.25-M solution of pentafluorphenyl (9-fluorenyl)methoxycarbonylaminoacetate (Fmoc-Gly-OPfp) **20** in NMP was applied on each spot. After 20 min at rt the cycle was repeated and after an additional 20 min, the membrane was rinsed, and the Fmoc group was cleaved off as described above under A.

Glycotetrapeptide Library

A derivatized cellulose sheet II was placed in the autospot robot for the successive coupling of building blocks **A** (**16**), **R** (**12**), **N** (**14**), and **D** (**18**). For each building block the following sequence was applied. A: 0.1 μL of a solution of each building block (0.1 mmol) and HODhbt (60 μmol) in NMP (1 mL) was pipetted on the Rink Amid Linker/glycine derivatized spots. After 30 min the entire cycle was repeated. B: The membrane was rinsed two times for 2 min at rt with a solution of acetic anhydride (0.8 mL) in DMF (20 mL). C: The membrane was rinsed four times for 2 min at rt with DMF (20 mL). D: The membrane was rinsed for 30 min at rt with a solution of piperidine (5 mL) in DMF (20 mL). E: The membrane was rinsed as described under C followed by rinsing four times at rt with ethanol (20 mL). F: The cycles A through E were repeated three times until all 256 combinations of building blocks **A**, **R**, **N**, and

D were spotted onto the paper sheet. G: The membrane was dried in vacuo in a desiccator after termination of the final step E.

Deblocking of the Glycotetrapeptide Library on the Cellulose Sheet

The cellulose membrane containing the 256 glycotetrapeptides was rinsed with a 5-M solution of NH_3 in methanol (50 mL) in a Petri dish and shaking the dish at rt for 16 h, followed by rinsing the membrane with ethanol (2 × 20 mL) for 2 min each and drying the membrane in vacuo.

Lectin Screening

A. Binding of lectins. The membrane was rinsed with 20 mM tris-hydroxymethyl aminoethane (TRIS) buffer pH 7.5 (3 × 25 mL) for 5 min each, followed by rinsing with 20 mM TRIS buffer pH 7.5 containing 50 mg TWEEN per liter for 5 min. The wet membrane was then rinsed under shaking in a Petri dish for 1 h at rt with a solution of the respective peroxidase-lectin conjugate of Con A, PHA-E, and GNA (5 nmol) in 20 mM tris-hydroxymethyl aminoethane buffer pH 7.5 (100 mL) containing 10 μL of a 0.1-M aqueous solution of $CaCl_2$ and 10μL of a 0.1-M aqueous solution of $MnCl_2$, followed by rinsing the membrane successively with 20 mM TRIS buffer pH 7.5 (3 × 25 mL) for 5 min each and 20 mM TRIS/TWEEN buffer pH 7.5 (25 mL) for 5 min. B. Staining of bound lectins. The membrane containing the bound peroxidase-lectin conjugate was rinsed in staining buffer, freshly prepared by mixing 20 mM acetate buffer pH 5.0 (47.5 mL), a solution of 3-amino-9-ethylcarbazole (20 mg, 90 mmol) in DMF (2.5 mL), and 30% aqueous H_2O_2 solution (25 μL) until a visible red color of the spots developed (5–15 min). The membrane is then rinsed with water and scanned for documentary purposes.

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