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Supporting Information

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Spatial Screening of Cyclic Neoglycopeptides: Identification of Multivalent Wheat Germ Agglutinin Ligands**

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Experimental Section

General. Solid-phase peptide synthesis was carried out in plastic syringe reactors. Fmoc-protected amino acids were obtained from Novabiochem (Läufelfingen, Switzerland) or prepared according to standard procedures. Bovine serum albumin (A7638), biotinylated WGA (L5142), alkaline phosphatase-coupled monoclonal anti biotin antibody (A6561), BCIP/NBT tablets (B5655), peroxidase-labeled WGA (L7017), and porcine stomach mucin type III (M1778) were obtained from Sigma. Compound numbers used within this supporting information are the same as in the printed article.

Preparation of Neoglycopeptide Library 1.

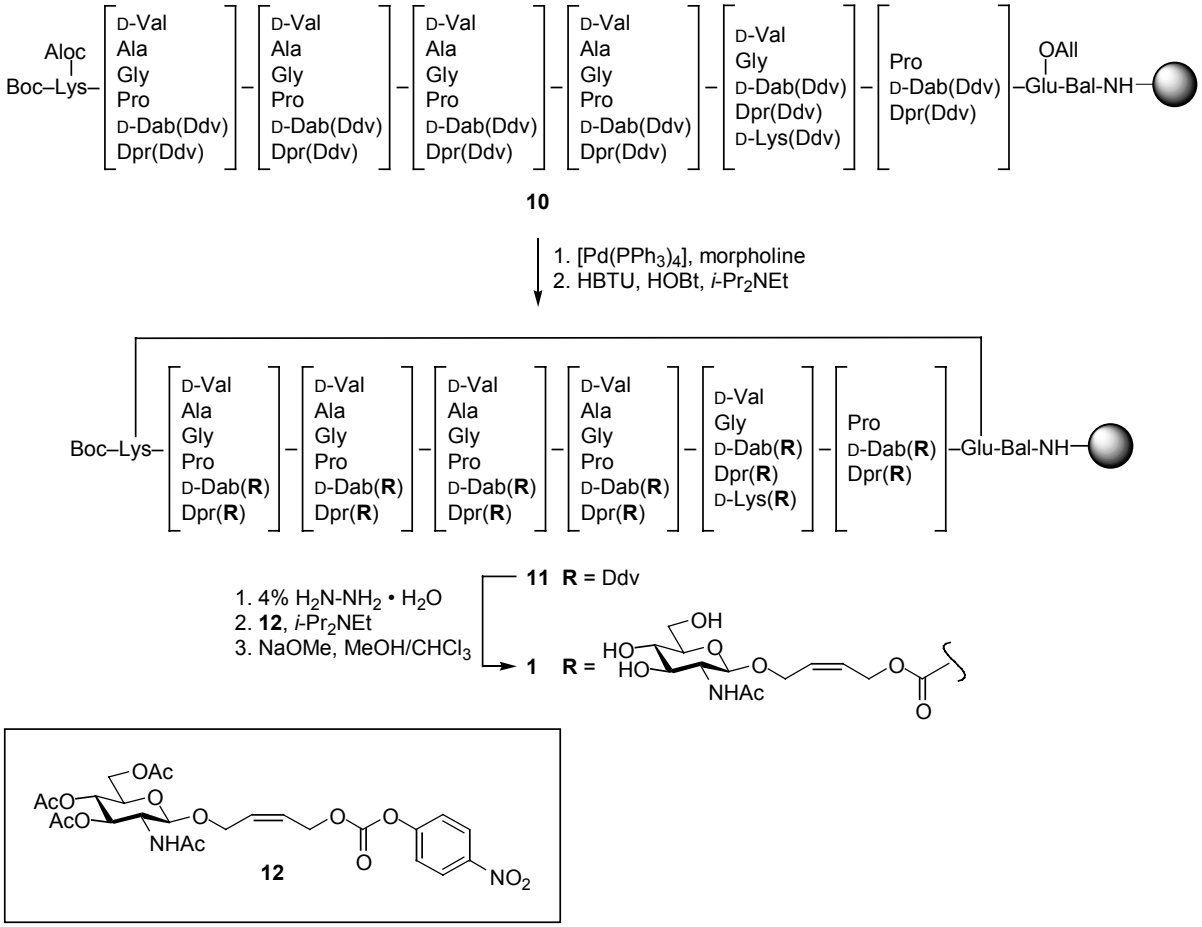
Library 10. Solid-phase peptide synthesis of library **10** (Scheme S-1) was carried out on TentaGel S-NH_2 (5g, 130 μm beads, initial loading: 0.31 mmol g^{-1}; Rapp-Polymere, Tübingen) following the standard Fmoc protocol[1] employing amino acid derivatives Fmoc-Bal-OH, Fmoc-Glu(OAll)-OH, Fmoc-Pro-OH, Fmoc-D-Dab(Ddv)-OH, Fmoc-Dpr(Ddv)-OH, Fmoc-D-Val-OH, Fmoc-Gly-OH, Fmoc-D-Lys(Ddv)-OH, Fmoc-Ala-OH, and in the last step Boc-Lys(Aloc)-OH. Combinatorially varied positions were generated using the split-mix synthesis protocol.[2] Couplings were performed with HATU/HOAt and HBTU/HOBt in 1-methyl-2-pyrrolidone (NMP).

Library 11. Library **10** (3.5 g) was shaken for 15 h under argon with morpholine (880 μl, 10 mmol) and [$Pd(PPh_3)_4$] (197 mg, 164 μmol) in DMF/DMSO (1:1) (35 mL) and subsequently washed with DMF (5 × 1 min), sodium diethyldithiocarbamate (0.5 % in DMF) (4 × 1 min) and DMF (10 × 1 min). The resin was treated with a 5 % solution of HOBt in DMF (3 × 2 min) and subsequently washed with DMF (5 × 1 min) and then NMP (2 × 2

[**] The abbreviations used are given in reference [8].

min). A mixture of HOBt (780 mg, 5.08 mmol), HBTU (1.29 g, 3.38 mmol), NMP (20 mL), and *i*-Pr_2NEt (1.19 ml, 6.83 mmol) was added and after having been shaken for 24 h at room temperature, the resin was washed with DMF.

Library 1. Library **11** (700 mg) was deprotected by treatment with hydrazine hydrate/DMF (4 : 96) (8 × 5 min) and washed with DMF and NMP. After addition of NMP (4.15 mL), *i*-Pr_2NEt (270 μL, 1.55 mmol), and **12** (902 mg, 1.55 mmol), the resin was shaken overnight at room temperature and subsequently washed with DMF, CH_2Cl_2 and dried. The absence of free amino groups was verified by Kaiser test[3] and by addition of bromophenol blue[4] (monitoring of single beads under a microscope). Deacetylation of the carbohydrates was achieved by treatment of the resin with MeOH (20 mL) containing a solution of NaOMe in MeOH (5.4 M) (100 μL, 540 μmol) for 4 h at room temperature. Finally, the resin was washed with MeOH, DMF, and CH_2Cl_2 and dried under vacuum.



Scheme S-1. Split-mix synthesis of neoglycopeptide library **1**. At the positions denoted by square brackets the resin was distributed at three to six reaction vessels and each coupled with one of the given amino acids.

On-Bead Immunosorbent Lectin Binding Assay for Screening of Library 1 Against WGA

Library **1** (100 mg of derivatized TentaGel beads) was washed three times with 1 mL of PBST buffer (10 mM sodium phosphate, 150 mM NaCl, 0.05% Tween-20, pH 7.2) and then suspended in 1 mL of PBST buffer containing 3% bovine serum albumin (BSA). After having been shaken for 30 min, the beads were washed three times with 1 mL of PBST buffer containing 1% BSA and subsequently incubated for 3 h at room temperature with 1 mL of a solution of biotinylated WGA (5 μg mL^{-1} in PBST buffer) containing 680 mM GlcNAc (the lectin/GlcNAc solution was prepared 30 min before being used). The beads were washed three times with 1 mL of TBST buffer [20 mM tri(hydroxymethyl)aminomethane hydrochloride (Tris • HCl), 500 mM NaCl, 0.05% Tween-20, pH 7.5] containing 0.5% BSA and 167 mM GlcNAc, incubated for 1 h at 37 °C with 1 mL of an alkaline phosphatase-coupled monoclonal anti biotin antibody solution (1:10000 in TBST buffer with 0.5% BSA and 167 mM GlcNAc) and washed three times with 1 mL of TBST buffer containing 0.5% BSA and 167 mM GlcNAc and three times with 1 mL of alkaline phosphatase buffer (100 mM Tris • HCl, 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5) containing 167 mM GlcNAc. Then the beads were incubated for 10 min with 4 mL of a solution of 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT) (prepared by dissolving one BCIP/NBT tablet in 10 mL of water). The staining was monitored under a low power stereo microscope (Olympus SZ4045TR) and terminated by washing the beads twice with 1 mL of water and twice with 1 mL of EDTA solution (20 mM, pH 7.4).

Darkly stained beads were manually selected under the stereo microscope and washed with 8 M urea containing 100 M acetic acid (10 × 1 mL), water (10 ×), MeOH (2 ×), CH_2Cl_2 (5 ×), MeOH (2 ×), water (5 ×), urea/acetic acid (10 ×), and water (10 ×) in order to remove bound WGA as well as water-insoluble dye. To restore Boc groups which might have been partly deprotected during the treatment with acetic acid, the resin was shaken for 1 h at 40 °C with a solution of di-*tert*-butyldicarbonate (21 mg, 96 μmol) and Et_3N (28 μL, 0.2 mmol) in 1 mL water/dioxane (1:1), washed with DMF and CH_2Cl_2 and dried in vacuum. Sequence determination was carried out after removal of the GlcNAc residues as described previously.[5]

Control experiments. (a) The lectin binding assay was repeated with an increased GlcNAc concentration (680 mM during incubation with biotinylated WGA and 452 mM during the following steps). (b) The lectin binding assay was carried out as described but without addition of biotinylated WGA and GlcNAc. The dilution of the monoclonal anti biotin

antibody was 1:1000. (c) The lectin binding assay was repeated as described employing the non-glycosylated, acetylated library **2**. All control experiments resulted in colorless beads.

Solid-Phase Synthesis of Neoglycopeptides 3, 5, and 9. Synthesis was carried out on polystyrene resin derivatized with the Sieber linker[6] (Novabiochem) following the same protecting group strategy employed for the preparation of library **1**. Final purification was achieved by RP-HPLC on C18 reversed-phase columns (250 × 4 mm, 0–45 % acetonitrile in water/0.1 % TFA over 30 min, flow = 1 mL min^{-1}).

3: HPLC: retention time t_R = 14.3 min. MALDI-MS: ($M + Na^+$): calcd. 2317.1, found 2316.9.

5: HPLC: t_R = 12.8 min. MALDI-MS: ($M + Na^+$): calcd. 2633.2, found 2633.2.

9: HPLC: t_R = 12.5 min. MALDI-MS: ($M + Na^+$): calcd. 2981.3, found 2980.0.

Enzyme-Linked Lectin Assay. This assay was carried out as described before by Zanini and Roy[7] employing Falcon ProBind microtiter plates.

References

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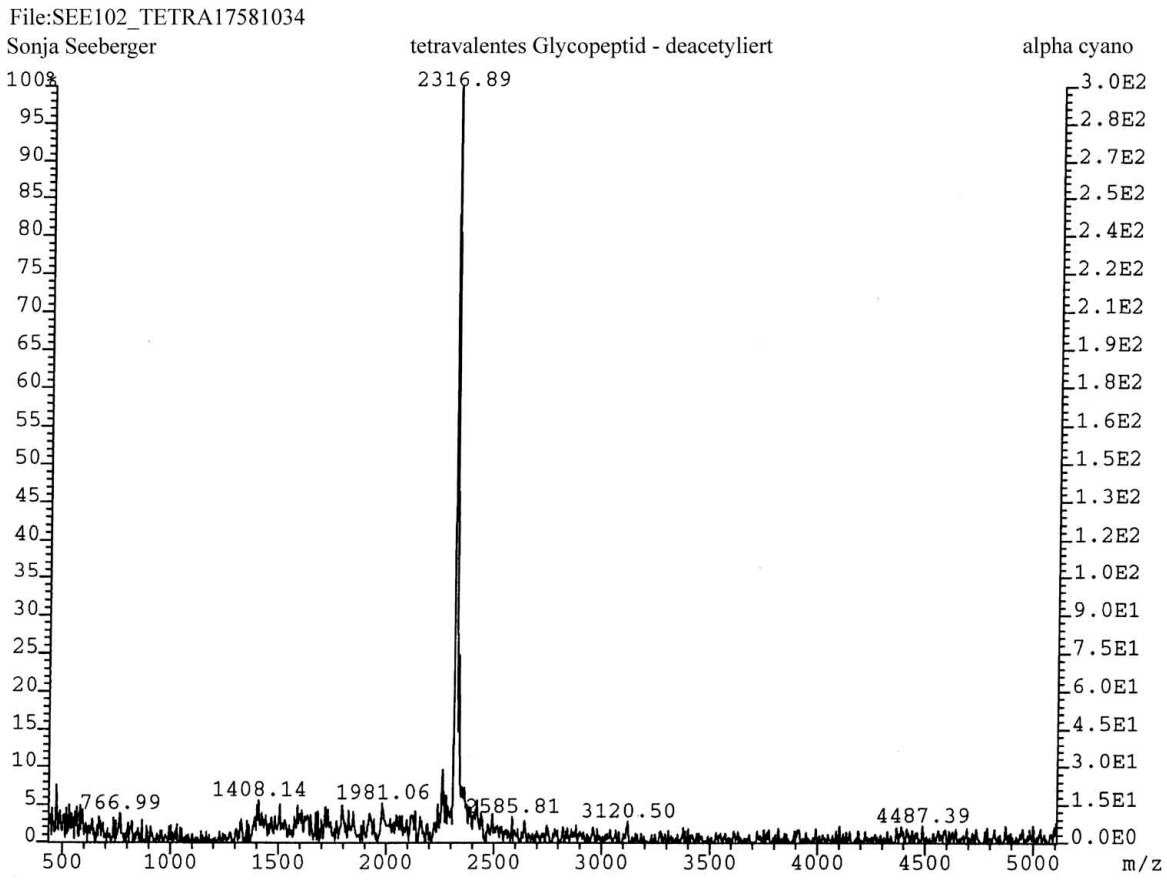
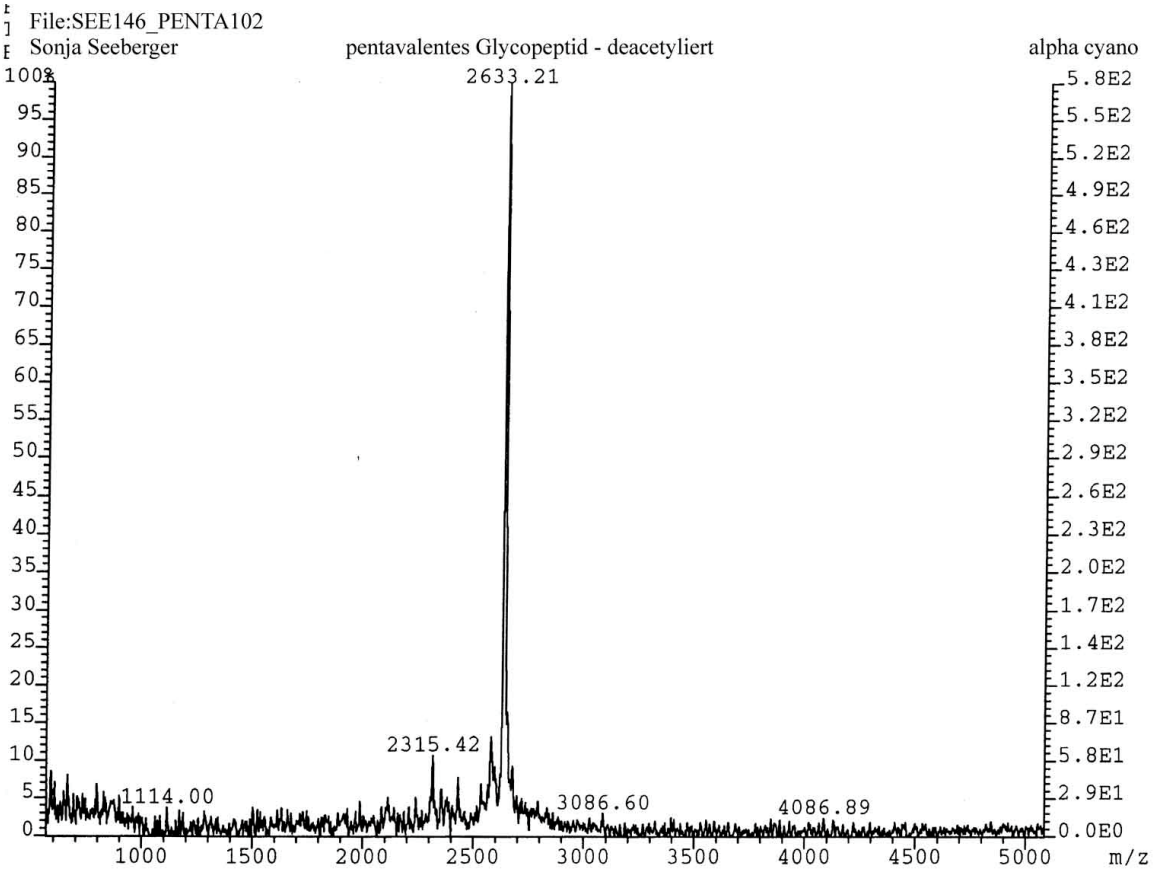
[8] Abbreviations used: All = allyl, Aloc = allyloxycarbonyl, Bal = *β*-alanine, Ddv = 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)isovaleryl, HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate, HBTU = *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate, HOAt = 1-hydroxy-7-aza-1*H*-benzotriazole, HOBt = 1-hydroxy-1*H*-benzotriazole, NMP = 1-methyl-2-pyrrolidone, WGA = wheat germ agglutinin.

MALDI-MS of tetravalent neoglycopeptide **3**:



MALDI-MS of pentavalent neoglycopeptide **5**:



MALDI-MS of hexavalent neoglycopeptide **9**: