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

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Natural and synthetic polyvalent ligands can act as inhibitors of biological processes or as effectors of signal-transduction pathways.[1,2] In the case of carbohydrate–lectin interactions, polyvalency is of particular importance because individual carbohydrate epitopes are usually bound with only weak affinity and often broad specificity.[3,4] The potency of a polyvalent inhibitor depends on the spatial presentation of the carbohydrates and the associated possibility of occupying several binding sites simultaneously. If the 3D structure of the protein is known, suitable polyvalent ligands may be obtained by rational design. This has been impressively demonstrated for the bacterial AB_5 toxins[5] as well as for non-carbohydrate systems[6] ("directed" polyvalency). However, if the three-dimensional orientation of the binding sites of a polyvalent lectin is not known, many potential ligands have to be synthesized and screened to determine the required orientation of the carbohydrate units.[4,7] This holds true particularly for conformationally restricted glycoclusters which may lead in such cases to ligands with especially strong affinities.[1,6,8] Screening of a library of spatially diverse glycoclusters could accelerate this process significantly. St. Hilaire, Meldal, et al. reported on split-and-mix libraries of linear glycopeptides.[9] First studies on the behavior of dynamic combinatorial carbohydrate libraries of four[10] and 21[11] compounds in the presence of lectins have been published as well.

Here we report the first example of a split-and-mix library of conformationally restricted, spatially diverse glycoclusters, and their screening for binding to the *N*-acetylglucosamine-(GlcNAc)-binding plant lectin wheat-germ agglutinin (WGA). As scaffold molecules (templates) for the presentation of the carbohydrate residues we chose side-chain-cyclized pep-

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Supporting information for this article (syntheses of **1**, **3**, **5**, and **9**, and the procedure for the solid-phase enzyme-immuno lectin-binding assay) is available on the WWW under http://www.angewandte.org or from the author.

tides.[12] Figure 1 shows the composition of library **1**, which was obtained by convergent solid-phase synthesis on TentaGel without employing a linker according to a synthetic strategy[13] described by us. At the combinatorially varied positions marked in gray, L- and D-amino acids without further side-chain functionality as well as L- and D-diamino acids, such as L-2,3-diaminopropionic acid (Dpr), D-2,4-diaminobutyric acid (D-Dab), and D-lysine, were used. The latter served as attachment points for GlcNAc residues by means of urethane formation with a linker especially developed for this purpose based on the allyloxycarbonyl (Aloc) protecting group. With this library design, spatial diversity may be generated in two "dimensions". Positional diversity leads to different carbohydrate patterns on the peptide scaffolds. Variation of the stereochemistry of the amino acids (stereodiversity) increases spatial diversity by modulating the folding of the peptide backbones.[14] In addition, this library design allows for the analysis of the neoglycopeptides bound to single beads.[15] The carbohydrate contents of all 19440 library members ranges from 0 (2.6% of all compounds) through 1 (14.5%), 2 (30.3%), 3 (30.9%), 4 (16.6%), 5 (4.5%), and 6 (0.5%).

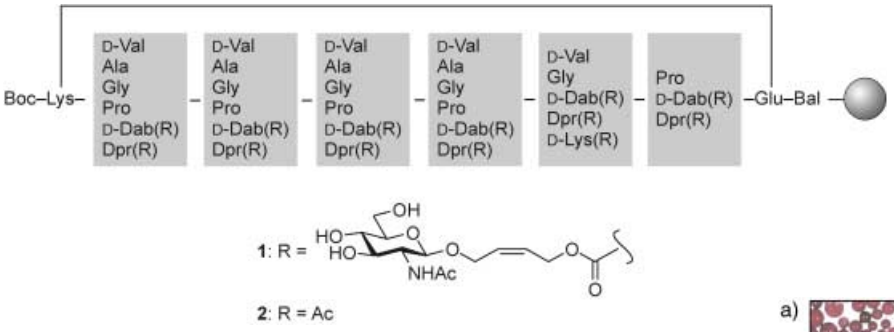


Figure 1. Neoglycopeptide library **1** consisting of 19440 compounds and control library **2** for the spatial screening of WGA ligands.

WGA is a 36-kDa lectin composed of two identical glycine- and cysteine-rich subunits. The WGA dimer contains a total of eight binding sites for GlcNAc and oligomers thereof.[16] Therefore, WGA was a promising candidate for a polyvalent interaction. To screen for binding properties to WGA, we developed the on-bead enzyme-immuno lectin-binding assay shown in Figure 2. Approximately 100 mg of library **1** (corresponding almost 100000 resin beads or five copies of every library member) were incubated with biotinylated WGA, and after washing, a conjugate of a monoclonal anti-biotin antibody and alkaline phosphatase (AP) was added. After further washing the resin beads bearing WGA ligands were visualized by an alkaline phosphatase (AP)-catalyzed reaction delivering a water-insoluble dye.

As shown in Figure 3a, initially approximately 97–98% of all the beads were colored. This result was not unexpected, since this corresponds to the proportion of compounds within **1** with at least one GlcNAc residue. To select for the best WGA ligands in **1**, we added competing monomeric GlcNAc in varying concentrations during incubation with WGA and all subsequent washing steps (Figure 3b and c).[17] Thus it was possible to reduce the number of colored beads to approximately 0.1% (Figure 3c) or in the extreme case even completely. Screening of the non-glycosylated library **2** and a control experiment omitting the addition of

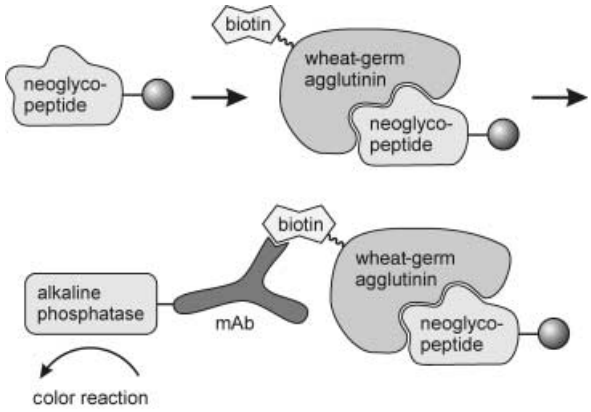


Figure 2. Solid-phase enzyme-immuno lectin-binding assay for screening of **1** for WGA ligands. mAb = monoclonal antibody.

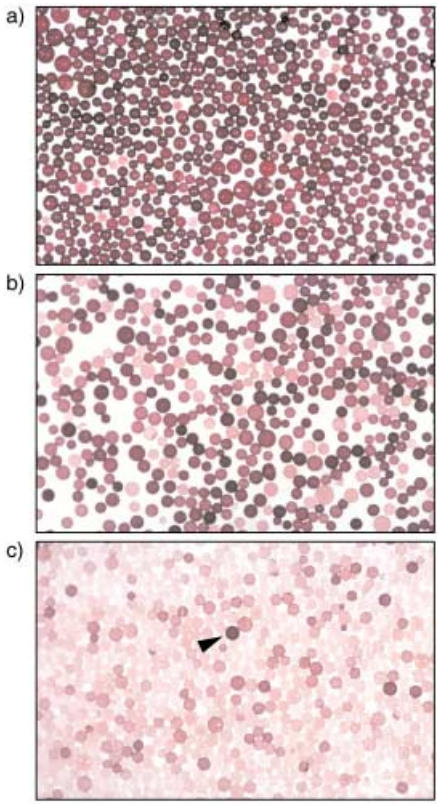


Figure 3. Resin beads of library **1** stained by the WGA binding assay shown in Figure 2. a) Assay carried out without addition of GlcNAc; b) WGA incubation with 225 mM GlcNAc, all further steps with 16 mM GlcNAc; c) WGA incubation with 680 mM GlcNAc, all further steps with 167 mM GlcNAc. The marked bead was identified as a hit. Average diameter of the beads is 130 μm.

biotinylated WGA led to colorless beads as well. From this we conclude that the result of the binding assay is indeed evoked by a carbohydrate–lectin interaction. In contrast, the initially used binding assay developed by Kahne et al.[18] to screen an oligosaccharide library, in which an AP–streptavidin conjugate is used instead of the AP–antibody conjugate, was not suitable since library **1** apparently contains a small proportion of streptavidin ligands.

The most darkly stained beads were manually selected under a low-power stereo microscope and analyzed by microsequencing (Edman degradation) after palladium-catalyzed detachment of the sugars according to a strategy described by us.[15] Two tetravalent (**3**, **4**), one pentavalent (**5**), and four hexavalent (**6–9**) neoglycopeptides could be identified. If the performed binding assay responds to polyvalency, we expected to find many compounds with a high GlcNAc content. For **3–9** this is clearly the case. Interestingly, however, not all hexavalent neoglycopeptides in library **1** (0.5 %) lead to comparable staining. Instead, one

3: Boc-Lys-D-Dab(R)-D-Val-D-Dab(R)-D-Dab(R)-D-Val-D-Dab(R)-Glu-Bal-NH_2

4: Boc-Lys-Ala-Ala-Dpr(R)-D-Dab(R)-D-Dab(R)-Dpr(R)-Glu-Bal-NH_2

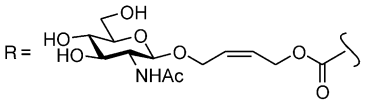
5: Boc-Lys-D-Dab(R)-Pro-Dpr(R)-D-Dab(R)-D-Lys(R)-Dpr(R)-Glu-Bal-NH_2

6: Boc-Lys-Dpr(R)-D-Dab(R)-D-Dab(R)-D-Dab(R)-D-Lys(R)-Dpr(R)-Glu-Bal-NH_2

7: Boc-Lys-D-Dab(R)-Dpr(R)-D-Dab(R)-D-Dab(R)-D-Dab(R)-D-Dab(R)-Glu-Bal-NH_2

8: Boc-Lys-D-Dab(R)-D-Dab(R)-D-Dab(R)-D-Dab(R)-D-Dab(R)-Dpr(R)-Glu-Bal-NH_2

9: Boc-Lys-D-Dab(R)-D-Dab(R)-D-Dab(R)-D-Dab(R)-D-Lys(R)-D-Dab(R)-Glu-Bal-NH_2



pentavalent and two tetravalent compounds are among the ligands on the most intensively stained resin beads.

To verify that the result of the binding assay is reproducible in solution, neoglycopeptides **3**, **5**, and **9** were resynthesized as single compounds. IC_{50} values for inhibition of the binding of porcine stomach mucin to peroxidase-labeled WGA (Figure 4, Table 1) were determined by an enzyme-linked lectin assay (ELLA) as described by Zanini and Roy.[19] According to this assay the affinities of the tetra- (**3**), penta- (**5**), and hexavalent (**9**) neoglycopeptides are 218-, 619-, and 568-fold higher, respectively, than that of the monomeric GlcNAc reference (β values[1]). This shows that the result of the solid-phase lectin-binding assay correlates with the binding properties of **3**, **5**, and **9** in solution and is not dominated by a surface effect on the resin beads. This is remarkable in light of observations made by Kahne et al. upon screening a library of immobilized monovalent oligosaccharides against the lectin from *Bauhinia purpurea*. They did not find a

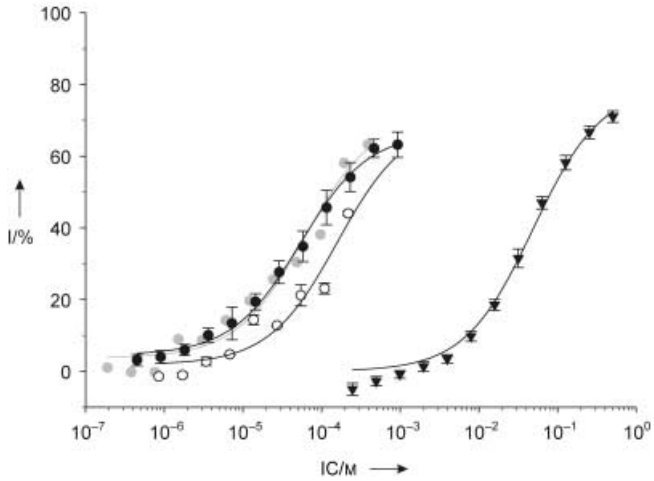


Figure 4. Dose–response curves for inhibition of the binding of porcine stomach mucin to peroxidase-labeled WGA obtained from an enzyme-linked lectin-binding assay (ELLA). Triangles: GlcNAc, white circles: **3**, gray circles: **5**, black circles: **9**. I = inhibition, IC = inhibitor concentration.

Table 1: IC_{50} values for inhibition of the binding of porcine stomach mucin to peroxidase-labeled WGA by **3**, **5**, and **9** obtained from the dose–response curves shown in Figure 4.

Compound	IC_{50} [mM]	β
GlcNAc	83	1
3	0.381	218
5	0.134	619
9	0.146	568

correlation between the solution affinities of monovalent ligands and the corresponding avidities on the surface of resin beads.[18] The fact that **5** and **9** have similar IC_{50} values illustrates that the binding affinity is not only determined by the number of GlcNAc residues but that ligand architecture is important as well. This coincides with observations on other systems.[20]

In summary, we have presented the first example of a split-and-mix library of conformationally restricted, spatially diverse glycoclusters and their screening by a novel solid-phase lectin-binding assay which does not produce false-positive results due to an interaction with streptavidin. The binding enhancement of the identified polyvalent ligands **3**, **5**, and **9** compared to monovalent GlcNAc is the highest ever found for GlcNAc clusters of that size binding to WGA.[19,21] Since the non-glycosylated cyclopeptide library **2** does not contain any WGA ligands, we assume the spatial presentation of the GlcNAc residues on the cyclopeptide templates to be responsible for the high affinity to WGA. The presented procedure of a spatial screening[22] of polyvalent lectin ligands can easily be applied to other lectins because the carbohydrates are coupled to the neoglycopeptide library in the last synthetic step and, therefore, can be replaced easily.

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[1] M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908 – 2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754 – 2794.
[2] L. L. Kiessling, J. E. Gestwicki, L. E. Strong, *Curr. Opin. Chem. Biol.* **2000**, *4*, 696 – 703.
[3] a) T. K. Dam, C. F. Brewer, *Chem. Rev.* **2002**, *102*, 387 – 429; b) J. J. Lundquist, E. J. Toone, *Chem. Rev.* **2002**, *102*, 555 – 578; c) B. T. Houseman, M. Mrksich, *Top. Curr. Chem.* **2002**, *218*, 1 – 44; d) T. K. Lindhorst, *Top. Curr. Chem.* **2002**, *218*, 201 – 235; e) L. L. Kiessling, T. Young, K. H. Mortell in *Glycoscience: Chemistry and Chemical Biology, Vol. II* (Eds.: B. Fraser-Reid, K. Tatsuta, J. Thiem), Springer, Heidelberg, **2001**, pp. 1817 – 1861; f) R. Roy, *Top. Curr. Chem.* **1997**, *187*, 241 – 274.
[4] R. T. Lee, Y. C. Lee, *Glycoconjugate J.* **2000**, *17*, 543 – 551.
[5] a) P. I. Kitov, J. M. Sadowska, G. Mulvery, G. D. Armstrong, H. Ling, N. S. Pannu, R. J. Read, D. R. Bundle, *Nature* **2000**, *403*, 669 – 672; b) E. Fan, Z. Zhang, W. E. Minke, Z. Hou, C. L. M. J. Verlinde, W. G. J. Hol, *J. Am. Chem. Soc.* **2000**, *122*, 2663 – 2664.
[6] N. Schaschke, G. Matschiner, F. Zettl, U. Marquardt, A. Bergner, W. Bode, C. P. Sommerhoff, L. Moroder, *Chem. Biol.* **2001**, *8*, 313 – 327.
[7] a) Y. C. Lee, R. T. Lee, *Acc. Chem. Res.* **1995**, *28*, 321 – 327; b) *Neoglycoconjugates. Preparation and Applications* (Eds.: Y. C. Lee, R. T. Lee), Academic Press, San Diego, **1994**.
[8] J. Rao, J. Lahiri, R. M. Weis, G. M. Whitesides, *J. Am. Chem. Soc.* **2000**, *122*, 2698 – 2710.
[9] a) P. M. St. Hilaire, T. L. Lowary, M. Meldal, K. Bock, *J. Am. Chem. Soc.* **1998**, *120*, 13312 – 13320; b) K. M. Halkes, P. M. St. Hilaire, P. R. Crocker, M. Meldal, *J. Comb. Chem.* **2003**, *5*, 18 – 27.
[10] S. Sakai, Y. Shigemasa, T. Sasaki, *Tetrahedron Lett.* **1997**, *38*, 8145 – 8148.
[11] O. Ramström, J.-M. Lehn, *ChemBioChem* **2000**, *1*, 41 – 48.
[12] For the use of cyclic peptides as templates see: a) U. Sprengard, M. Schudok, W. Schmidt, G. Kretzschmar, H. Kunz, *Angew. Chem.* **1996**, *108*, 359 – 362; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 321 – 324; b) H. Franzyk, M. K. Christensen, R. M. Jørgensen, M. Meldal, H. Cordes, S. Mouritsen, K. Bock, *Bioorg. Med. Chem.* **1997**, *5*, 21 – 40; c) G. Tuchscherer, M. Mutter, *Pure Appl. Chem.* **1996**, *68*, 2153 – 2162; d) J. Eichler, A. W. Lucka, R. A. Houghten, *Peptides* **1994**, *7*, 300 – 307.
[13] V. Wittmann, S. Seeberger, *Angew. Chem.* **2000**, *112*, 4508 – 4512; *Angew. Chem. Int. Ed.* **2000**, *39*, 4348 – 4352.
[14] R. Haubner, D. Finsinger, H. Kessler, *Angew. Chem.* **1997**, *109*, 1440 – 1456; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1374 – 1389.
[15] V. Wittmann, S. Seeberger, H. Schägger, *Tetrahedron Lett.* **2003**, *44*, 8243 – 9246.
[16] C. S. Wright, G. E. Kellogg, *Protein Sci.* **1996**, *5*, 1466 – 1476.
[17] Other attempts to increase the selectivity of the assay, such as lowering the lectin concentration, were not successful.
[18] a) R. Liang, L. Yan, J. Loebach, M. Ge, Y. Uozumi, K. Sekanina, N. Horan, J. Gildersleeve, C. Thompson, A. Smith, K. Biswas, W. C. Still, D. Kahne, *Science* **1996**, *274*, 1520 – 1522; b) R. Liang, J. Loebach, N. Horan, M. Ge, C. Thompson, L. Yan, D. Kahne, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 10554 – 10559.
[19] D. Zanini, R. Roy, *Bioconjugate Chem.* **1997**, *8*, 187 – 192.
[20] a) J. E. Gestwicki, C. W. Cairo, L. E. Strong, K. A. Oetjen, L. L. Kiessling, *J. Am. Chem. Soc.* **2002**, *124*, 14922 – 14933; b) S. André, R. J. Pieters, I. Vrasidas, H. Kaltner, I. Kuwabara, F.-T. Liu, R. M. J. Liskamp, H.-J. Gabius, *ChemBioChem* **2001**, *2*, 822 – 830.
[21] T. Furuike, S. Aiba, S.-I. Nishimura, *Tetrahedron* **2000**, *56*, 9909 – 9915.
[22] The term "spatial screening" was first used by Kessler et al. in connection with the screening of libraries of stereoisomeric peptides: H. Kessler, B. Diefenbach, D. Finsinger, A. Geyer, M. Gurrath, S. L. Goodman, G. Hoelzemann, R. Haubner, A. Jonczyk, G. Müller, E. Graf von Roedern, J. Wermuth, *Lett. Pept. Sci.* **1995**, *2*, 155 – 160.