

# Probing multivalent carbohydrate–lectin interactions by an enzyme-linked lectin assay employing covalently immobilized carbohydrates

Caroline Maierhofer, Katja Rohmer and Valentin Wittmann\*

*Universität Konstanz, Fachbereich Chemie, Fach M 709, 78457 Konstanz, Germany*

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**Abstract**—We report here the synthesis of a series of mono- to trivalent *N*-acetylglucosamine (GlcNAc) derivatives as ligands for the plant lectin wheat germ agglutinin (WGA). Their WGA binding potencies were determined by an established enzyme-linked lectin assay (ELLA) employing microtiter plates with non-covalently immobilized porcine stomach mucin (PSM) as reference ligand and an ELLA with a new GlcNAc derivative covalently immobilized via a thiourea linkage. Comparison of both assays revealed that the type of presentation of GlcNAc residues on the microtiter plates either as part of a glycoprotein or as a covalently immobilized monosaccharide derivative strongly influences the outcome of the assay. Although the apparent dissociation constants  $K_D^{ELLA}$  for the interaction of peroxidase-labeled WGA with the microtiter plates are comparable for both surfaces,  $IC_{50}$  values obtained with the PSM-free ELLA were substantially lower. Even more strikingly, this ELLA displayed a better differentiation between ligands of different valency leading to significantly higher relative inhibitory potencies of multivalent ligands compared to monovalent. Additionally, problems associated with the use of PSM, such as maximum inhibition at considerably less than 100% and poor reproducibility of  $IC_{50}$  values could be overcome with this type of ELLA.

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## 1. Introduction

The specific recognition of carbohydrate structures in biological systems by carbohydrate-binding proteins (lectins)<sup>1–4</sup> is the basis of numerous intra- and intercellular events ranging from the control of protein folding to cell–cell communication during development, inflammation, immune responses, and cancer metastasis.<sup>5–9</sup> High affinity lectin ligands are of great medicinal interest in the diagnosis and inhibition of such carbohydrate-mediated processes.<sup>10–15</sup> The generation of high affinity ligands, however, is not trivial because the interaction of individual carbohydrate epitopes with lectins is in many cases weak and undiscriminating.<sup>16</sup> The binding affinity can be dramatically increased by clustering of lectin binding sites and carbohydrate recognition units.<sup>17–23</sup> The observation that the binding affinity increases exponentially with the number of binding sites has been termed the glycoside cluster effect.<sup>24,25</sup> The po-

tency of a polyvalent inhibitor depends on the spatial presentation of the carbohydrates and the associated possibility of occupying several binding sites simultaneously<sup>26</sup> and is often assessed in terms of a relative inhibitory potency compared to the monovalent interaction. If the 3D structure of a lectin is known, suitable multivalent ligands may be obtained by rational design.<sup>27,28</sup> However, if the three-dimensional orientation of the binding sites of a lectin is not known, many potential ligands have to be synthesized and screened to determine the required orientation of the carbohydrate units.

Enzyme-linked lectin assays (ELLA)<sup>29</sup> provide a fast method to evaluate lectin binding properties of soluble natural or synthetic lectin ligands in solution.<sup>30–34</sup> Microtiter plates are coated with a reference ligand (often a (neo)glycoprotein or polysaccharide) and  $IC_{50}$  values for inhibition of the binding of an enzyme-labeled lectin to the immobilized reference ligand (the matrix) by the soluble ligands to be tested are determined. The advantage of ELLA compared to solid-phase binding assays such as surface plasmon resonance (SPR)<sup>35</sup> is that both, lectin and ligand are in solution. However, the use of glycoproteins from natural sources as reference ligands is limited because their varying complex

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\* Corresponding author. Tel.: +49 7531 88 4572; fax: +49 7531 88 4573; e-mail: [mail@valentin-wittmann.de](mailto:mail@valentin-wittmann.de)

carbohydrate composition due to microheterogeneity<sup>36</sup> leads to reduced reproducibility of  $IC_{50}$  values. Furthermore, incomplete inhibition of lectin binding to the plate surface is often observed, that is, maximal inhibition occurs at considerably less than 100%. To overcome the problem of microheterogeneity, covalent immobilization of defined synthetic carbohydrates and their application in an enzyme-linked immunosorbent assay (ELISA) employing PapG adhesin was reported.<sup>37</sup>

From screening of a 19440-membered split-and-mix library of conformationally restricted spatially diverse glycoclusters, we were able to identify a number of tetra- to hexavalent ligands of the plant lectin wheat germ agglutinin (WGA) with affinities up to 620-fold compared to the binding of the monovalent ligand *N*-acetylglucosamine (GlcNAc).<sup>38</sup> WGA is a 36-kDa lectin composed of two identical glycine- and cysteine-rich subunits.<sup>39</sup> The WGA dimer contains a total of eight binding sites for GlcNAc and oligomers thereof.<sup>40</sup> The results demonstrated that the binding affinity of the identified ligands was not only determined by the number of GlcNAc residues but that ligand architecture is important as well.

Here we present a series of structurally diverse mono- to trivalent GlcNAc derivatives which were synthesized to explore the effects of scaffold structure on the binding affinity toward WGA.  $IC_{50}$  values were determined by a published ELLA<sup>38,41</sup> and an ELLA employing a newly developed GlcNAc derivative which was covalently immobilized to microtiter plates via a thiourea linkage leading to improved properties regarding sensitivity and reproducibility. Comparison of both assays revealed interesting insight into the influence of the type of carbohydrate presentation on the microtiter plates on the results of the assay.

## 2. Results and discussion

### 2.1. Synthesis of WGA ligands

In a previous study we identified several cyclic neoglycopeptides containing four to six GlcNAc residues as ligands for WGA.<sup>38</sup> Interestingly, a pentavalent compound showed a lower  $IC_{50}$  value than a hexavalent, as determined by an ELLA. This indicated that the binding affinity is not only determined by the number of GlcNAc residues but that their spatial presentation is important as well. In order to further investigate the effects of the structure of multivalent GlcNAc derivatives on the binding affinity toward WGA, a series of compounds were prepared. Commercially available amines **1–8** (Fig. 1) were reacted with activated carbonate **9**, which was already used for the preparation of neoglycopeptides,<sup>42</sup> leading to mono- to trivalent glycoconjugates **10–17** in high yields (Table 1). Subsequent O-deacetylation resulted in WGA ligands **18–25** in quantitative yields (Scheme 1). When reacted under Zemplén conditions followed by neutralization using acidic ion exchange resin, some of the products (especially the amines **24** and **25**) adhered to the resin. Therefore,

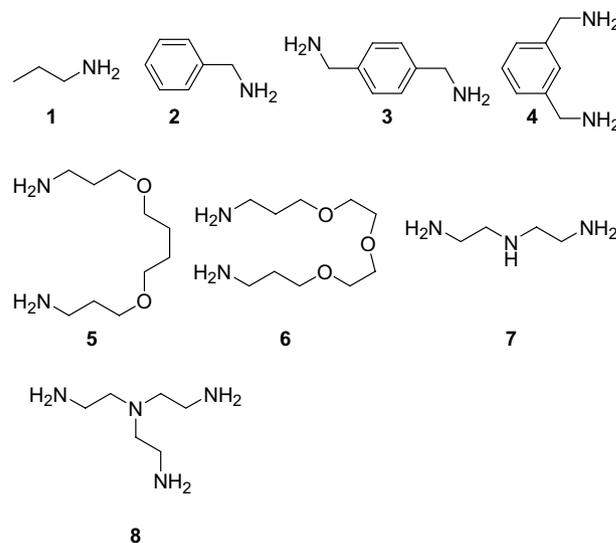


Figure 1. Amines used for the synthesis of WGA ligands.

Table 1. Coupling of activated carbonate **9** to amines **1–8**<sup>a</sup>

Amine	Product	Yield (%)
<b>1</b>	<b>10</b>	85
<b>2</b>	<b>11</b>	94
<b>3</b>	<b>12</b>	97
<b>4</b>	<b>13</b>	96
<b>5</b>	<b>14</b>	83
<b>6</b>	<b>15</b>	92
<b>7</b>	<b>16</b>	94
<b>8</b>	<b>17</b>	99

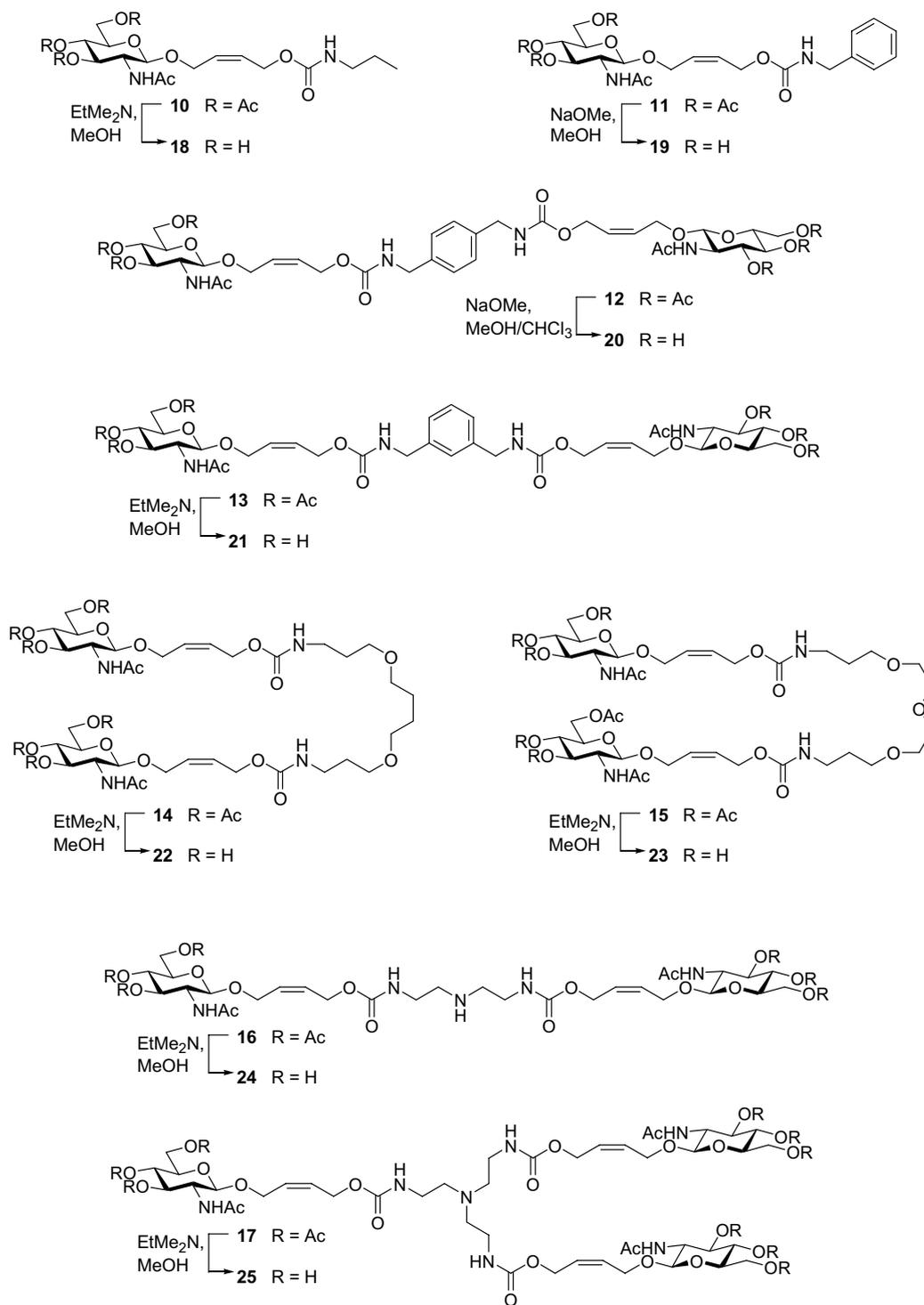
<sup>a</sup> For structures of products, see Scheme 1.

O-deacetylation was conveniently carried out under salt free conditions using *N*-ethylidimethylamine in methanol.

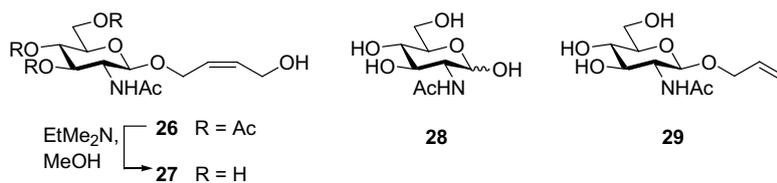
In addition, hydroxybutenyl glycoside **27**, obtained by O-deacetylation of compound **26**,<sup>43</sup> GlcNAc **28**, and allyl 2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside **29** were used as monovalent WGA ligands (Scheme 2).

### 2.2. ELLA employing non-covalently immobilized porcine stomach mucin as reference ligand

Binding potencies of compounds **18–25** and **27–29** were determined by an ELLA employing non-covalently immobilized porcine stomach mucin (PSM) as reference ligand as described earlier.<sup>38,41</sup> PSM-coated microtiter plates were incubated with mixtures of horseradish peroxidase (HRP)-labeled WGA ( $1 \mu\text{g mL}^{-1}$ ) and synthetic WGA ligands in varying concentrations. It is worth mentioning that precipitate formation during incubation



Scheme 1. Deacetylation of glycoconjugates 10–17.

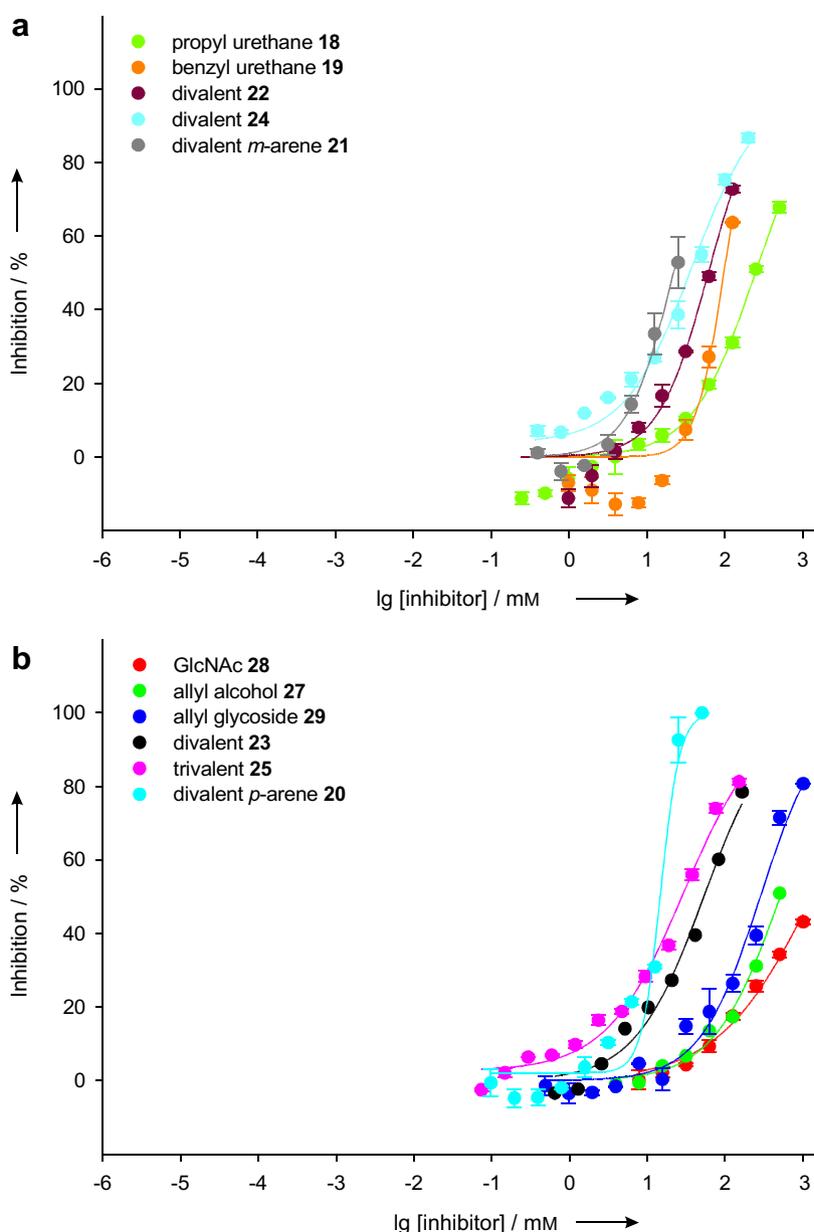


Scheme 2. Additional monovalent WGA ligands 27–29.

under the given experimental conditions was not observed. After incubation, the plates were washed and remaining labeled WGA bound to the reference ligand was quantified by a HRP-catalyzed color reaction using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) as substrate. Dose–response curves for inhibition of the binding of HRP-labeled WGA to immobilized PSM are shown in Figure 2. From these curves the concentrations that reduce the binding of labeled WGA to PSM by 50% ( $IC_{50}$  values) were determined as a measure of potency of the synthesized inhibitors (Table 2, column A). Also shown in Table 2 are relative inhibitory potencies ( $\beta$  values) referenced to GlcNAc **28** ( $\beta = 1$ ).

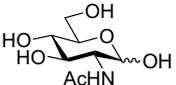
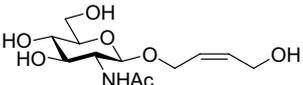
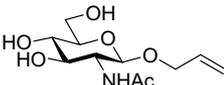
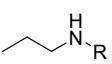
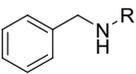
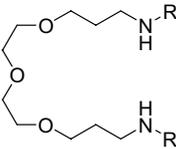
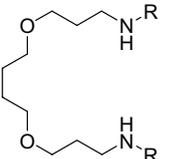
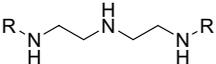
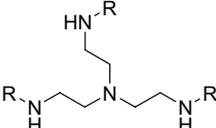
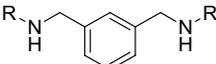
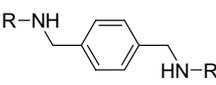
As shown in Table 2, column A, the  $IC_{50}$  value of GlcNAc **28** was determined to be 1000 mM. In order to val-

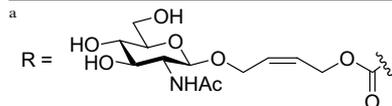
idate the assay, we determined the inhibitory potency of allyl 2-acetamido-2-deoxy- $\alpha$ -D-glucopyranoside at a concentration of 15 mM to be 12% (data not shown). This corresponds well to the value of 15% published by Zanini and Roy.<sup>41</sup> The monovalent ligands **18**, **27**, and **29** are all slightly better inhibitors than GlcNAc **28** with a maximum  $\beta$  value of 4.2 for compound **18**. As expected, a further increase of the inhibitory potency is observed for the multivalent ligands **20–25**. The  $\beta$  values of divalent derivatives **22–24** are in a range of 13–25 and that of trivalent ligand **25** is 40. Remarkably, the best inhibitors are the divalent compounds **21** and **20** ( $\beta = 50$  and 67, respectively). On the first view, this might indicate the influence of the aromatic spacer giving **20** and **21** a lower flexibility than **22–24**. However, a closer examination of the inhibition curves obtained from these ligands containing aromatic spacers reveals



**Figure 2.** Dose–response curves for inhibition of the binding of HRP-labeled WGA to PSM-coated microtiter plates by (a) synthetic ligands **18**, **19**, **21**, **22**, and **24**, (b) **20**, **23**, **25**, and **27–29**.

**Table 2.** Absolute and relative IC<sub>50</sub> values of synthetic ligands **18–25** and **27–29** for inhibition of the binding of HRP-labeled WGA to (A) non-covalently immobilized porcine stomach mucin (PSM) from dose–response curves shown in Figure 2 and (B) covalently immobilized GlcNAc **34** from dose–response curves shown in Figure 4

Compound <sup>a</sup>	(A) Reference ligand: non-covalently immobilized PSM		(B) Reference ligand: covalently immobilized GlcNAc		
	IC <sub>50</sub> (mM)	β <sup>b</sup>	IC <sub>50</sub> (mM)	β <sup>b</sup>	
	<b>28</b>	1000	1	23	1
	<b>27</b>	500	2	12	1.9
	<b>29</b>	280	3.6	10	2.3
	<b>18</b>	240	4.2	—	—
	<b>19</b>	100	10	—	—
	<b>23</b>	75	13	0.288	80
	<b>22</b>	60	17	—	—
	<b>24</b>	40	25	—	—
	<b>25</b>	25	40	0.055	420
	<b>21</b>	20	50	—	—
	<b>20</b>	15	67	0.088	260
<b>38</b>	—	—	0.016	1440	



<sup>b</sup> β = relative inhibitory potency.

that they are exceptionally steep. One possible explanation for this behavior is increasing aggregation of the ligands at higher concentrations, leading to higher valency and, therefore, better inhibition. In this regard, it is conspicuous that the inhibition curve of monovalent derivative **19** containing benzyl groups also displays this behavior.

As shown in Figure 2, the inhibitor concentrations needed for the maximal inhibition of the binding of HRP-labeled WGA to immobilized PSM could not be reached in most cases due to insufficient solubility of the ligands. In addition, we experienced that the reproducibility of the assay is limited when the experimental setup is changed, for example, differences in  $IC_{50}$  values up to one order of magnitude were observed with different sources of PSM and microtiter plates. The order of inhibitory potencies of different ligands, however, is generally robust to these variables. The disadvantages associated with PSM prompted us to develop a new ELLA employing a surface covalently modified with a defined GlcNAc derivative, thus avoiding the use of PSM.

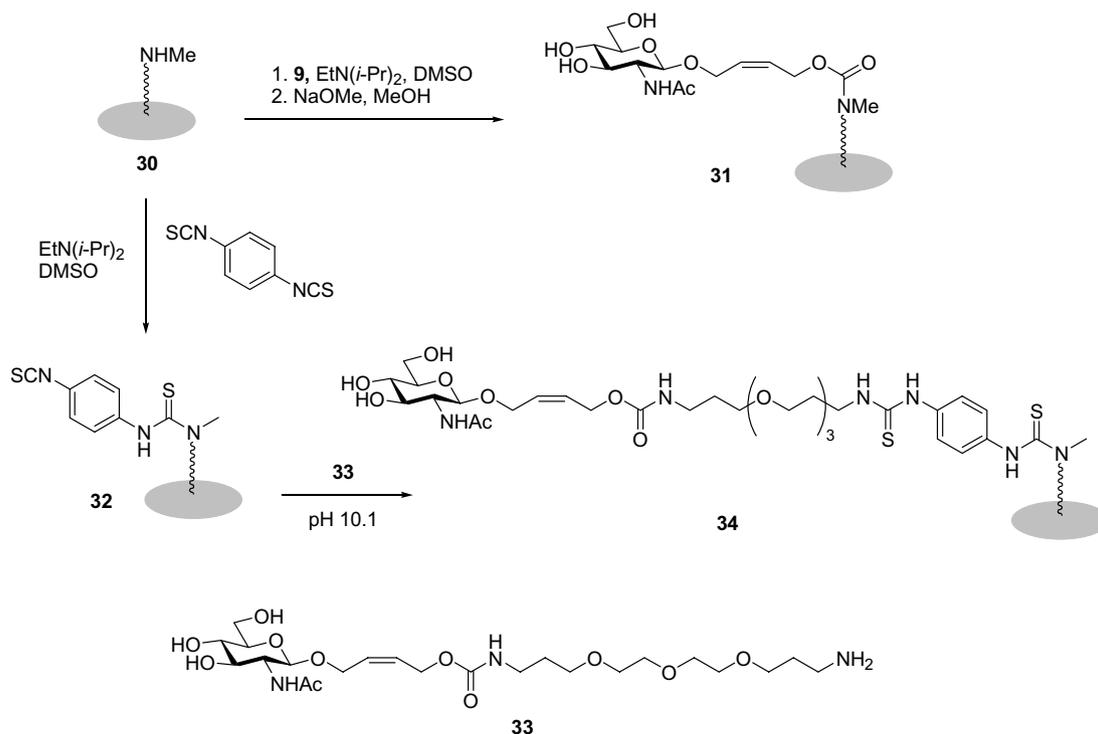
### 2.3. Enzyme-linked lectin assay employing covalently immobilized GlcNAc as reference ligand

**2.3.1. Modification of amino functionalized microtiter plates.** Covalent immobilization of carbohydrates has recently gained immense interest for the preparation of carbohydrate microarrays and several methods for the site-specific, covalent attachment of chemically modified carbohydrates to appropriately derivatized surfaces have been published. The field has been extensively reviewed.<sup>44–49</sup> We compared two methods for chemical

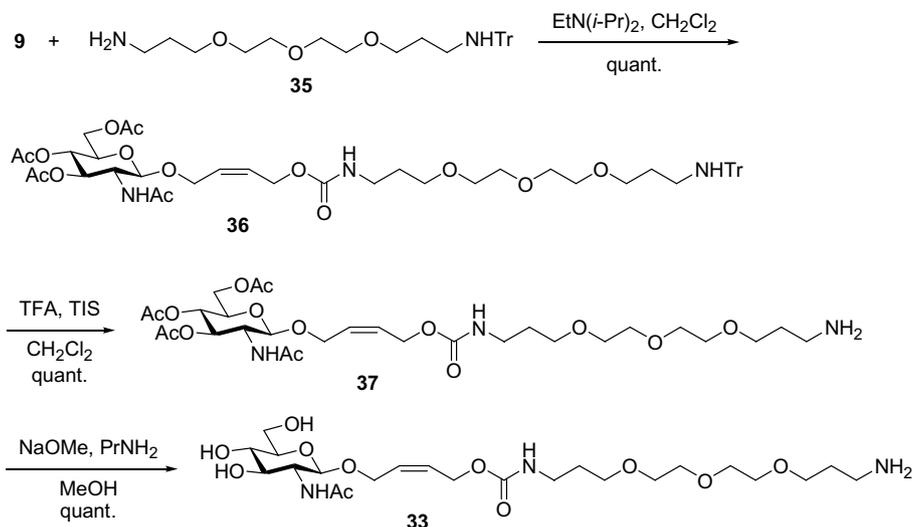
immobilization of GlcNAc leading to constructs with different spacing between the carbohydrate and the surface (Scheme 3). Commercially available amino modified microtiter plates **30** were treated with activated carbonate **9** in the presence of Hünig's base. O-Deacetylation under Zemplén conditions gave GlcNAc-modified microtiter plates **31** containing a short spacer. Alternatively, microtiter plates **30** were reacted with 1,4-phenylene diisothiocyanate according to a modified procedure originally described by Guo et al. for the fabrication of DNA microarrays<sup>50</sup> to generate amine-reactive isothiocyanate surface **32**. Addition of the amino spacer modified GlcNAc derivative **33** gave GlcNAc surface **34** with a longer spacer.

For the synthesis of **33**, activated carbonate **9** was reacted with mono tritylated diamine **35** to give urethane **36** in quantitative yield (Scheme 4). Detritylation using trifluoroacetic acid (TFA) and triisopropyl silane (TIS) and subsequent O-deacetylation of **37** yielded **33**.

To determine the performance of the surfaces **31** and **34**, microtiter plates prepared under various conditions were blocked with bovine serum albumin (BSA) and incubated with a series of differently concentrated WGA-HRP solutions. After washing, HRP substrate ABTS was added and the absorption was measured at 405 nm (Fig. 3). This allowed quantification of HRP-labeled lectin bound to the plates. To generate surface **31**, the concentration of activated carbonate **9** was varied between 0.06 and 100 mM. Incubation with WGA-HRP occurred at concentrations between 0.2 and  $25 \mu\text{g mL}^{-1}$ . As shown in Figure 3a, the amount of bound WGA-HRP is very low ( $A_{405}$  generally below



Scheme 3. Preparation of covalently modified microtiter plates **31** and **34**.



**Scheme 4.** Synthesis of spacer-modified GlcNAc 33.

0.5) and does not correlate with the concentration of activated carbonate **9** used for surface preparation. From these results we conclude that the observed binding of WGA-HRP is not a matter of a specific interaction with the immobilized GlcNAc residues. This may originate from the short distance between the plate surface and the GlcNAc residues reducing the accessibility of the GlcNAc residues to the WGA binding sites.<sup>51</sup>

A completely different behavior was observed with long-spacer surface **34**. A clear relationship between the concentration of **33** used for derivatization of **32** and the amount of WGA-HRP bound to the surface is observed (Fig. 3b). At concentrations of amine **33**  $\geq 1$  mM, the reactive surface of the plate is saturated and binding curves consistent with simple ligand binding are obtained with increasing WGA-HRP concentration. Additionally, the total amount of WGA-HRP bound to surface **34** is much higher compared to **31**. Therefore, all following experiments were carried out with microtiter plates **34** obtained by treatment of **32** with 1 mM solutions of GlcNAc derivative **33**.

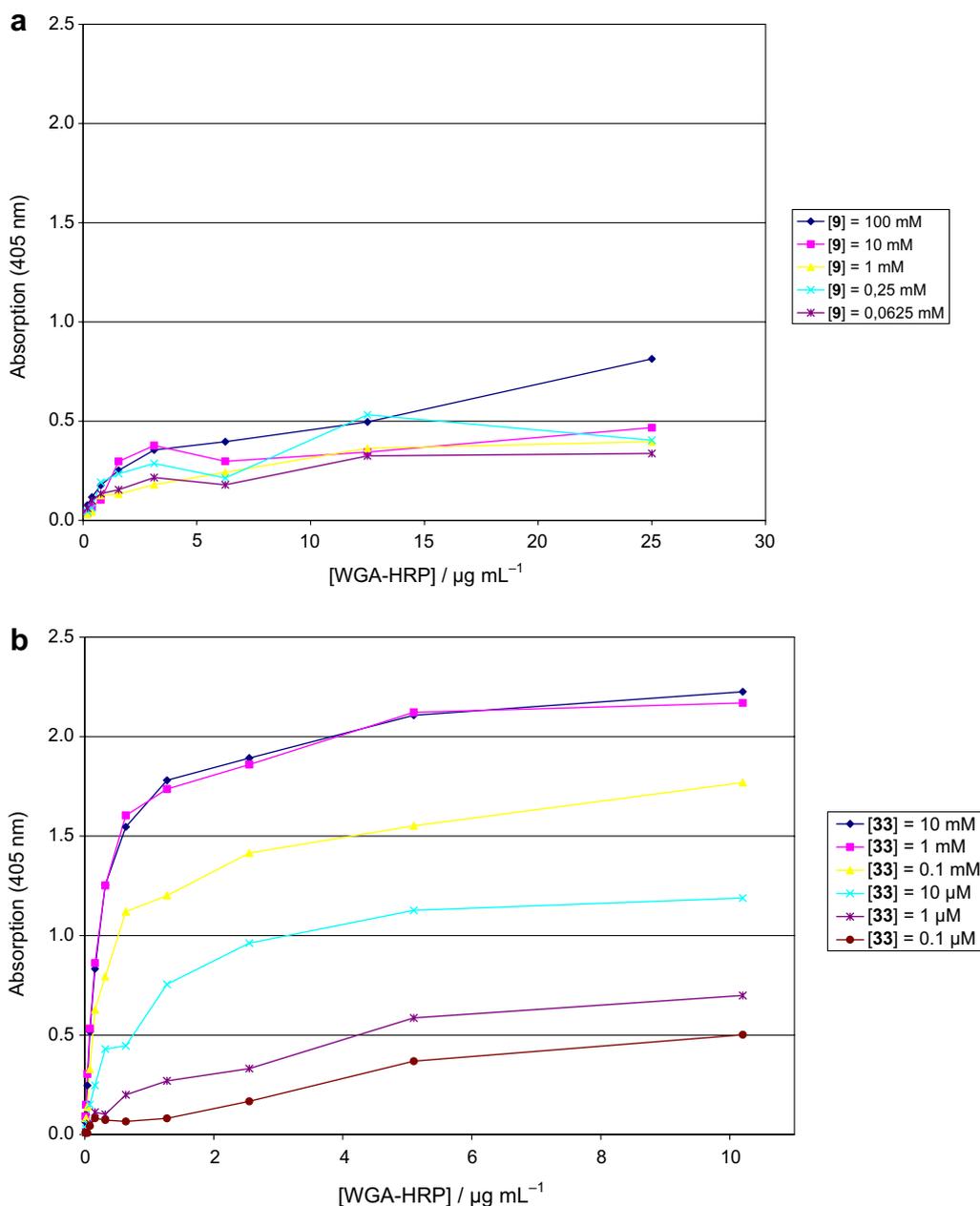
**2.3.2. Determination of inhibitory potencies.** Inhibition experiments with ligands **27–29**, **20**, **23**, and **25** were carried out as described above for PSM-coated microtiter plates at WGA-HRP concentrations of  $1 \mu\text{g mL}^{-1}$ . Dose–response curves for inhibition of the binding of HRP-labeled WGA to covalently immobilized GlcNAc **34** are shown in Figure 4.  $\text{IC}_{50}$  values obtained from these curves are given in Table 2, column B.

As can be seen immediately,  $\text{IC}_{50}$  values obtained with covalently modified microtiter plates were substantially lower than those obtained with PSM-coated plates. The  $\text{IC}_{50}$  value of GlcNAc **28** is 23 mM instead of 1000 mM. Similarly, inhibitory potencies of the other monovalent compounds investigated are lowered by a comparable factor of roughly 30–40, leading not only to the same order of potencies but also to similar relative inhibitory potencies ( $\beta$  values). The  $\text{IC}_{50}$  values of di-

and trivalent ligands **23** and **25** on the other hand are lowered by a much higher factor, resulting in  $\beta$  values of 80 and 420 versus 13 and 40, respectively. The inhibition curve of dimer **20** with the aromatic spacer is not any more as steep as in Figure 2b. The  $\text{IC}_{50}$  value of **20** is still lower than that of divalent **23** but is now higher than the  $\text{IC}_{50}$  of trivalent **25**. If aggregation caused the steep increase of the curves shown in Figure 2b, it is obviously not any more effective at the low concentration needed for inhibition of WGA binding to the covalently modified plates. Additionally, due to the lower  $\text{IC}_{50}$  values observed with this variant of the ELLA, limited solubility did not any more prevent reaching maximal inhibition with all tested ligands.

It is well known that the performance of multivalent carbohydrate ligands is strongly dependent on the assay used to evaluate them because different phenomena may be measured. This does not only apply to absolute  $\text{IC}_{50}$  values but also to observed multivalency effects, that is, relative  $\text{IC}_{50}$  values. Toone et al., for example, compared a range of competitive and non-competitive binding assays, including ELLA, hemagglutination inhibition, and isothermal titration microcalorimetry<sup>32</sup> and Pieters et al. studied multivalent galectin ligands by a solid-phase inhibition assay and fluorescence titrations in solution.<sup>52</sup> There are also few reports that show that the use of ELLA with different matrices can lead to different relative inhibitory potencies.<sup>33,53</sup> This phenomenon, however, is not well understood.

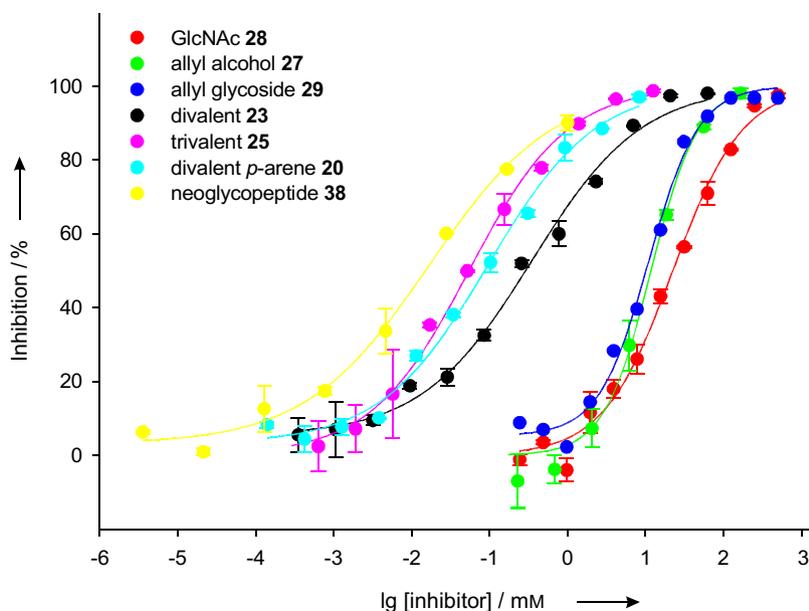
A likely explanation for different absolute  $\text{IC}_{50}$  values found with different matrices could be that the lectins to be investigated have different binding affinities to the immobilized reference ligands and, therefore, varying concentrations of inhibitors are necessary for inhibition of this interaction. To investigate whether this explanation could count for the lower  $\text{IC}_{50}$  values obtained with our PSM-free ELLA, we compared the affinities of WGA-HRP binding to PSM-coated microtiter plates on one hand and covalently modified plates **34**



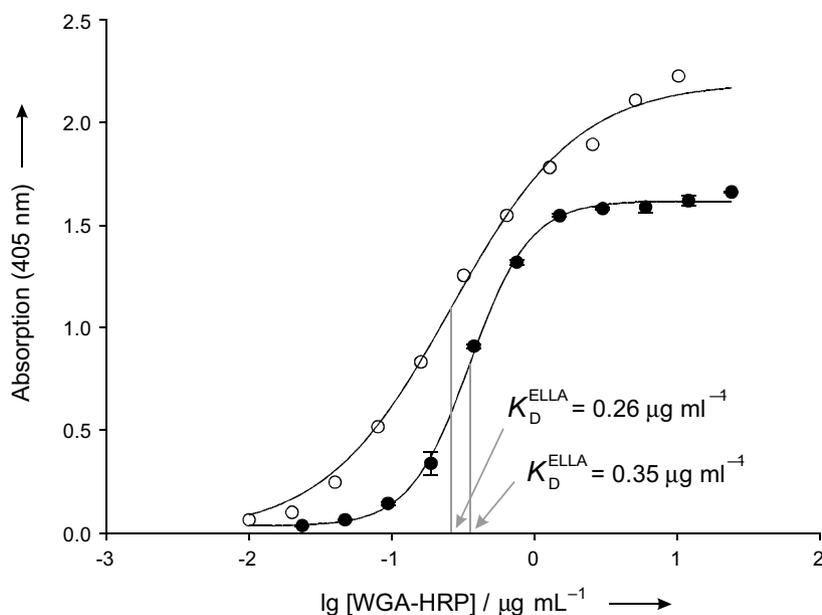
**Figure 3.** Binding of WGA-HRP to covalently modified microtiter plates **31** containing a short linker (a) and **34** containing a long linker (b) prepared with varying concentrations of activated carbonate **9** and amine **33**, respectively. Assays were carried out under identical conditions.

on the other. It was expected that the lower  $\text{IC}_{50}$  values would result from significantly lower binding affinities of the lectin to covalently modified plates **34**. The apparent dissociation constant  $K_D^{\text{ELLA}}$  for the interaction of WGA-HRP with surface **34**, which corresponds to the concentration of WGA-HRP necessary to occupy 50% of the surface binding sites, was readily available from the binding curves shown in Figure 3b. Data for the PSM-coated surface were obtained from an analogous experiment. As shown in the semi-logarithmic plot in Figure 5,  $K_D^{\text{ELLA}}$  of covalently modified plates **34** ( $0.26 \mu\text{g mL}^{-1}$ ) is even slightly lower than  $K_D^{\text{ELLA}}$  of PSM-coated plates ( $0.35 \mu\text{g mL}^{-1}$ ) which corresponds to a *higher* binding affinity and does not explain the observed lower  $\text{IC}_{50}$  values with surface **34**. A closer examination of the bind-

ing curves reveals two differences. Whereas the binding curve for covalently modified plates **34** perfectly fits to a Langmuir isotherm (Hill coefficient = 1), the binding curve for PSM-coated plates is much steeper with a Hill coefficient of 2. At the same time, PSM-coated plates were estimated to bind roughly twice the amount of WGA-HRP per unit surface area compared with surface **34**. These results underline the importance of the topology of carbohydrate presentation on surfaces, such as cell membranes or carbohydrate microarrays, for the affinity and specificity of carbohydrate–lectin interactions. At this point, it is worth mentioning that the  $\text{IC}_{50}$  value of GlcNAc determined by the new assay (23 mM) is in much closer proximity to the  $K_D$  value of GlcNAc which has been determined by several groups



**Figure 4.** Dose–response curves for inhibition of the binding of HRP-labeled WGA to covalently modified microtiter plates **34** by synthetic ligands **20**, **23**, **25**, **27–29**, and **38**.

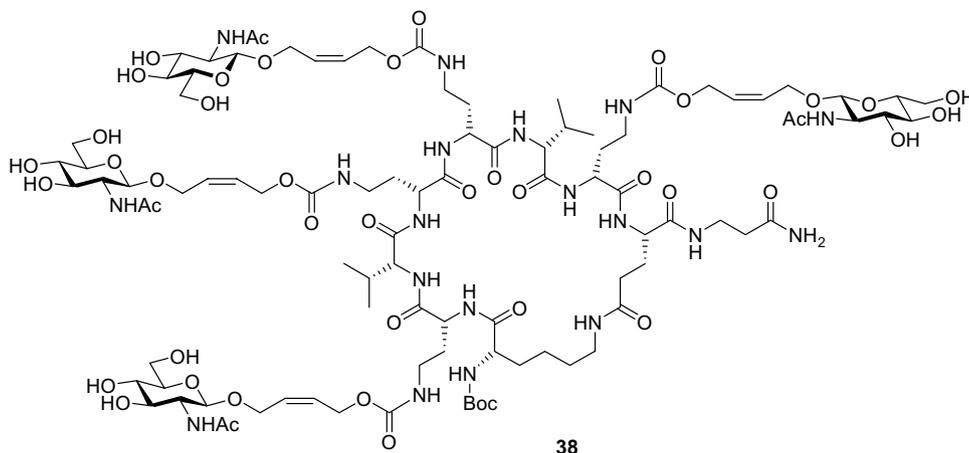


**Figure 5.** Semi-logarithmic plot of the binding of WGA-HRP to (●) PSM-coated microtiter plates and (○) covalently modified microtiter plates **34** produced with a concentration of GlcNAc derivative **33** of 1 mM. Absolute absorption values are not comparable for both experiments due to different assay conditions. PSM-coated plates were estimated to bind roughly twice the amount of WGA-HRP per unit surface area compared with microtiter plates **34**.

employing different methods<sup>54–58</sup> to be in the range of approximately 1–5 mM.

As mentioned in the introduction, a disadvantage of ELLA is that in many cases maximal inhibition occurs at considerably less than 100%. When we, for example, determined the  $IC_{50}$  values of cyclic neoglycopeptides, such as **38**, for inhibition of WGA-HRP binding to PSM-coated microtiter plates, a maximum inhibition of approximately 70% was observed.<sup>38</sup> The reasons for this behavior are obscure but might be rooted in the presence of several different binding sites on PSM which

is a highly glycosylated glycoprotein existing in many glycoforms. It is known that WGA also binds to *N*-acetylneuraminic acid<sup>39</sup> and it may be that this interaction is not inhibited by GlcNAc derivatives. Therefore, we subjected **38** to the ELLA with covalently immobilized GlcNAc. As can be seen in Figure 4, the maximal inhibition of WGA-HRP binding to this surface presenting a homogeneous carbohydrate epitope is now close to 100%. The  $IC_{50}$  value of **38** is 16  $\mu$ M corresponding to a  $\beta$  of 1440 (Table 2). The previously determined  $IC_{50}$  employing PSM-coated microtiter plates was 0.38 mM ( $\beta = 218$ ).<sup>38,59</sup>



### 3. Conclusions

In conclusion, synthetic mono- to tetravalent WGA ligands have been examined for their ability to inhibit binding of WGA-HRP to carbohydrate-coated microtiter plates.  $IC_{50}$  values have been determined by an ELLA with non-covalently immobilized PSM as reference ligand and an ELLA employing covalently immobilized spacer-separated GlcNAc as reference ligand. In general, the inhibitory potency of the ligands increased with the number of GlcNAc residues without a clear preference for a certain spacer. Comparison of the results of both ELLA, however, indicated that the type of presentation of GlcNAc residues on the microtiter plates either as part of a glycoprotein or as a covalently immobilized monosaccharide derivative strongly influences the outcome of the assay. Our investigations revealed two remarkable aspects. Interestingly, although the apparent dissociation constants  $K_D^{ELLA}$  for the interaction of WGA-HRP with the microtiter plates are comparable for both surfaces,  $IC_{50}$  values obtained with the PSM-free ELLA were substantially lower. Furthermore, this ELLA displays a better differentiation between ligands of different valency leading to significantly higher *relative* inhibitory potencies ( $\beta$  values) of multivalent ligands. Covalent immobilization of carbohydrate derivative **33** allows for generation of a chemically defined carbohydrate presentation. Problems associated with the use of PSM, such as maximum inhibition at considerably less than 100%, poor reproducibility of  $IC_{50}$  values, and insufficient solubility of the inhibitors at the concentrations required for maximum inhibition, could be overcome with this ELLA.

### 4. Experimental

#### 4.1. General methods

Solvents were purified by distillation and dried by normal procedures. Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F<sub>254</sub> aluminum sheets. The sheets were visualized by UV light and immersion in the appropriate stain followed by heating.

Preparative flash column chromatography (FC) was carried out on Merck silica gel 60 (40–63  $\mu$ m).  $^1H$  NMR and  $^{13}C$  NMR spectra were recorded on Bruker AC 250, Bruker AMX 400, and Bruker Avance DRX 600 instruments.  $^1H$  chemical shifts are reported in ppm relative to the signal from residual solvent protons ( $CDCl_3$ : 7.26;  $D_2O$ : 4.67 ppm).  $^{13}C$  chemical shifts are referenced to the solvent signal ( $CDCl_3$ : 77.0 ppm) or in case of  $D_2O$  to internal standard  $[D_3]MeCN$  (1.32 ppm). Assignments of proton and carbon signals were carried out with the aid of DQF-COSY, HMQC, and ROESY experiments. MALDI mass spectra were obtained on a Bruker Biflex III instrument with  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) as the matrix (positive mode). High resolution nano-ESI mass spectra were recorded on a Bruker Apex II FT-ICR mass spectrometer equipped with a 7 T actively shielded cryomagnet and an Apollo ESI source. Samples were dissolved in water/MeOH 1:1 (v/v) to a final concentration of 5 pmol  $\mu L^{-1}$ . If required, a solution of 40 mg  $mL^{-1}$  NaI in MeOH was added to the sample solutions in a ratio of 1:200 (v/v). Elemental analyses were performed by the microanalytical facility at the Universität Konstanz.

#### 4.2. Synthesis of the WGA ligands

**4.2.1. General procedure 1 (GP 1): preparation of the acetylated WGA ligands.** [(*Z*)-4-(*p*-Nitrophenoxycarbonyloxy)-but-2-en-1-yl]-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranoside **9**<sup>42</sup> (1.1 equiv per amino group) is dissolved in dry  $CH_2Cl_2$  (3–11 mL per mmol **9**). Addition of the amine and  $EtN(i-Pr)_2$  (1 equiv base per amino group) affords a yellow colored solution. The reaction mixture is stirred at room temperature until the reaction is complete. After evaporation of the solvent, the residue is purified by FC.

**4.2.2. General procedure 2 (GP 2): deprotection of the acetylated WGA ligands.** The acetylated WGA ligand is dissolved in MeOH/ $EtNMe_2$  5:1 (20–30 mL per mmol acetylated compound) and stirred at room temperature until the deacetylation is complete. Evaporation of the solvent gives the product in sufficient purity for further reactions.

**4.2.3. 4-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl-propylamine (10).** According to GP 1, **9** (320 mg, 0.55 mmol), CH<sub>2</sub>Cl<sub>2</sub> (5 mL), propylamine (41  $\mu$ L, 0.5 mmol), and EtN(*i*-Pr)<sub>2</sub> (87  $\mu$ L, 0.5 mmol) were reacted overnight. FC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 30:1) gave **10** (213 mg, 85%) as a white solid. *R*<sub>f</sub> = 0.32 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 15:1); <sup>1</sup>H NMR (600.1 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta$  = 5.84 (d, *J* = 8.2 Hz, 1H; NHAc), 5.73–5.66 (m, 2H; H<sub>vinyl</sub>), 5.27 (‘t’, *J* = 9.8 Hz, 1H; H-3), 5.06 (‘t’, *J* = 9.6 Hz, 1H; H-4), 4.86 (br s, 1H; NH), 4.74 (d, *J* = 8.3 Hz, 1H; H-1), 4.63–4.61 (m, 2H; H<sub>allyl</sub>), 4.36 (dd, *J* = 12.9, 5.1 Hz, 1H; H<sub>allyl</sub>), 4.30 (dd, *J* = 12.9, 6.8 Hz, 1H; H<sub>allyl</sub>), 4.24 (dd, *J* = 12.2, 4.6 Hz, 1H; H-6a), 4.14 (dd, *J* = 12.2, 1.5 Hz, 1H; H-6b), 3.91–3.88 (m, 1H; H-2), 3.72–3.70 (m, 1H; H-5), 3.14–3.13 (m, 2H; CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.08 (s, 3H; C(O)CH<sub>3</sub>), 2.02 (s, 3H; C(O)CH<sub>3</sub>), 2.01 (s, 3H; C(O)CH<sub>3</sub>), 1.93 (s, 3H; C(O)CH<sub>3</sub>), 1.54–1.50 (m, 2H; CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.92 ppm (t, *J* = 7.4 Hz, 3H; CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C NMR (150.9 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta$  = 170.8, 170.8, 170.3, 169.4 (C(O)CH<sub>3</sub>), 156.4 (OC(O)NH), 128.8 (C<sub>olefin</sub>), 128.6 (C<sub>olefin</sub>), 99.5 (C-1), 72.5 (C-3), 71.8 (C-5), 68.6 (C-4), 64.6 (C<sub>allyl</sub>), 62.1 (C-6), 60.4 (C<sub>allyl</sub>), 54.6 (C-2), 42.8 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 23.3 (C(O)CH<sub>3</sub>), 23.2 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 20.7, 20.7, 20.6 (C(O)CH<sub>3</sub>), 11.2 ppm (CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>); elemental analysis calcd (%) for C<sub>22</sub>H<sub>34</sub>N<sub>2</sub>O<sub>11</sub> (502.51): C, 52.58; H, 6.82; N, 5.57; found: C, 52.62; H, 6.75; N, 5.55.

**4.2.4. 4-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl-benzylamine (11).** According to GP 1, **9** (291 mg, 0.5 mmol), CH<sub>2</sub>Cl<sub>2</sub> (1.5 mL), benzylamine (60  $\mu$ L, 0.55 mmol), and EtN(*i*-Pr)<sub>2</sub> (87  $\mu$ L, 0.5 mmol) were reacted overnight. FC (EtOAc) gave **11** (258 mg, 94%) as a slight brown solid. *R*<sub>f</sub> = 0.27 (EtOAc); <sup>1</sup>H NMR (600.1 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta$  = 7.35–7.28 (m, 5H; H<sub>ar</sub>), 5.82 (d, *J* = 8.7 Hz, 1H; NHAc), 5.74–5.66 (m, 2H; H<sub>vinyl</sub>), 5.27 (‘t’, *J* = 10.0 Hz, 1H; H-3), 5.25 (br s, 1H; NH), 5.06 (‘t’, *J* = 9.6 Hz, 1H; H-4), 4.73 (d, *J* = 8.3 Hz, 1H; H-1), 4.70–4.61 (m, 2H; H<sub>allyl</sub>), 4.37–4.29 (m, 4H; H<sub>allyl</sub>, CH<sub>2</sub>N), 4.23 (dd, *J* = 12.2, 4.7 Hz, 1H; H-6a), 4.13 (dd, *J* = 12.2, 1.55 Hz, 1H; H-6b), 3.88 (‘q’, *J* = 9.2 Hz, 1H; H-2), 3.70–3.69 (m, 1H; H-5), 2.07 (s, 3H; C(O)CH<sub>3</sub>), 2.02 (s, 3H; C(O)CH<sub>3</sub>), 2.01 (s, 3H; C(O)CH<sub>3</sub>), 1.90 ppm (s, 3H; C(O)CH<sub>3</sub>); <sup>13</sup>C NMR (150.9 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta$  = 170.8, 170.6, 170.3, 169.4 (C(O)CH<sub>3</sub>), 156.4 (OC(O)NH), 138.3 (C<sub>ar,q</sub>), 128.8 (C<sub>olefin</sub>), 128.7 (C<sub>olefin</sub>), 128.6, 127.5, 127.4 (C<sub>ar</sub>), 99.4 (C-1), 72.5 (C-3), 71.7 (C-5), 68.5 (C-4), 64.5 (C<sub>allyl</sub>), 62.1 (C-6), 60.7 (C<sub>allyl</sub>), 54.6 (C-2), 45.0 (CH<sub>2</sub>N), 23.3, 20.7, 20.7, 20.6 ppm (C(O)CH<sub>3</sub>); elemental analysis calcd (%) for C<sub>26</sub>H<sub>34</sub>N<sub>2</sub>O<sub>11</sub> (550.55): C, 56.72; H, 6.22; N, 5.09; found: C, 56.41; H, 6.08; N, 5.11.

**4.2.5. *N,N'*-Bis-(4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-*p*-xylylenediamine (12).** According to GP 1, **9** (256 mg, 0.44 mmol), CH<sub>2</sub>Cl<sub>2</sub> (4 mL), *p*-xylylenediamine (28 mg, 0.2 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL), and EtN(*i*-Pr)<sub>2</sub> (70  $\mu$ L, 0.4 mmol) were reacted for 4.5 h. FC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 15:1) gave **12** (199 mg, 97%) as a white solid. *R*<sub>f</sub> = 0.18 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 15:1); <sup>1</sup>H NMR (600.1 MHz,

CDCl<sub>3</sub> + 5% CD<sub>3</sub>OD, 20 °C):  $\delta$  = 7.23 (br s, 4H; H<sub>ar</sub>), 5.67–5.66 (m, 4H; H<sub>vinyl</sub>), 5.20 (‘t’, *J* = 9.9 Hz, 2H; H-3), 5.01 (‘t’, *J* = 9.6 Hz, 2H; H-4), 4.67 (d, *J* = 8.2 Hz, 2H; H-1), 4.64–4.63 (m, 2H; H<sub>allyl</sub>), 4.58–4.55 (m, 2H; H<sub>allyl</sub>), 4.33–4.27 (m, 8H; CH<sub>2</sub>N, H<sub>allyl</sub>), 4.19 (dd, *J* = 12.2, 4.4 Hz, 2H; H-6a), 4.11–4.01 (m, 2H; H-6b), 3.86–3.83 (m, 2H; H-2), 3.68 (br s, 2H; H-5), 2.11 (s, 6H; C(O)CH<sub>3</sub>), 2.06 (s, 6H; C(O)CH<sub>3</sub>), 1.99 (s, 6H; C(O)CH<sub>3</sub>), 1.86 ppm (s, 6H; C(O)CH<sub>3</sub>); <sup>13</sup>C NMR (150.9 MHz, CDCl<sub>3</sub> + 5% CD<sub>3</sub>OD, 20 °C):  $\delta$  = 171.1, 171.1 171.1, 169.6 (C(O)CH<sub>3</sub>), 156.7 (OC(O)NH), 137.7 (C-1), 128.6 (C<sub>olefin</sub>), 128.4 (C<sub>olefin</sub>), 127.6 (C<sub>ar</sub>), 99.4 (C-1), 73.7 (C-3), 71.5 (C-5), 68.6 (C-4), 64.5 (C<sub>allyl</sub>), 62.1 (C-6), 60.7 (C<sub>allyl</sub>), 54.1 (C-2), 44.4 (CH<sub>2</sub>N), 22.7, 20.6, 20.6, 20.5 ppm (C(O)CH<sub>3</sub>); elemental analysis calcd (%) for C<sub>46</sub>H<sub>62</sub>N<sub>4</sub>O<sub>22</sub> (1023.00): C, 54.01; H, 6.11; N, 5.48; found: C, 53.60; H, 5.98; N, 5.45.

**4.2.6. *N,N'*-Bis-(4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxy-carbonyl)-*m*-xylylenediamine (13).** According to GP 1, **9** (256 mg, 0.44 mmol), CH<sub>2</sub>Cl<sub>2</sub> (5 mL), *m*-xylylenediamine (26  $\mu$ L, 0.2 mmol), and EtN(*i*-Pr)<sub>2</sub> (70  $\mu$ L, 0.4 mmol) were reacted for 4.5 h. FC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 15:1) gave **13** (197 mg, 96%) as a white solid. *R*<sub>f</sub> = 0.16 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 15:1); <sup>1</sup>H NMR (600.1 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta$  = 7.31–7.18 (m, 4H; H<sub>ar</sub>), 6.10 (d, *J* = 8.5 Hz, 2H; NHAc), 5.74–5.69 (m, 4H; H<sub>vinyl</sub>), 5.49 (br s, 2H; NH), 5.24 (‘t’, *J* = 9.7 Hz, 2H; H-3), 5.04 (‘t’, *J* = 9.4 Hz, 2H; H-4), 4.73–4.59 (m, 6H; H<sub>allyl</sub>, H-1), 4.42–4.33 (m, 8H; H<sub>allyl</sub>, CH<sub>2</sub>N), 4.22 (dd, *J* = 12.1, 4.0 Hz, 2H; H-6a), 4.14–4.12 (m, 2H; H-6b), 3.87 (‘q’, *J* = 9.3 Hz, 2H; H-2), 3.68–3.67 (m, 2H; H-5), 2.07 (s, 6H; C(O)CH<sub>3</sub>), 2.02 (s, 6H; C(O)CH<sub>3</sub>), 2.01 (s, 6H; C(O)CH<sub>3</sub>), 1.90 ppm (s, 6H; C(O)CH<sub>3</sub>); <sup>13</sup>C NMR (150.9 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta$  = 170.9, 170.8, 170.6, 169.4 (C(O)CH<sub>3</sub>), 156.5 (OC(O)NH), 138.0 (C<sub>ar,q</sub>), 129.0 (C<sub>olefin</sub>), 128.4, 126.5, 126.0 (C<sub>ar</sub>), 99.4 (C-1), 72.5 (C-3), 71.6 (C-5), 68.6 (C-4), 64.5 (C<sub>allyl</sub>), 62.1 (C-6), 60.7 (C<sub>allyl</sub>), 54.5 (C-2), 44.8 (CH<sub>2</sub>N), 23.2, 20.8, 20.7, 20.7 ppm (C(O)CH<sub>3</sub>); elemental analysis calcd (%) for C<sub>46</sub>H<sub>62</sub>N<sub>4</sub>O<sub>22</sub> (1023.00): C, 54.01; H, 6.11; N, 5.48; found: C, 53.87; H, 6.29; N, 5.48.

**4.2.7. 1,4-Bis-(3-(4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxy-carbonylamino)-propoxy)-butane (14).** Corresponding to GP 1, **9** (256 mg, 0.44 mmol), CH<sub>2</sub>Cl<sub>2</sub> (5 mL), 1,4-bis-(3-amino-propoxy)-butane (43  $\mu$ L, 0.2 mmol), and EtN(*i*-Pr)<sub>2</sub> (70  $\mu$ L, 0.4 mmol) were reacted for 3 h. FC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 15:1) gave **14** (182 mg, 83%) as a white solid. *R*<sub>f</sub> = 0.18 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 15:1); <sup>1</sup>H NMR (600.1 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta$  = 6.03 (d, *J* = 6.9 Hz, 2H; NHAc), 5.68 (br s, 4H; H<sub>vinyl</sub>), 5.33 (br s, 2H; NH), 5.27 (‘t’, *J* = 9.9 Hz, 2H; H-3), 5.06 (‘t’, *J* = 9.5 Hz, 2H; H-4), 4.74 (d, *J* = 8.3 Hz, 2H; H-1), 4.62–4.60 (m, 4H; H<sub>allyl</sub>), 4.37 (dd, *J* = 12.6, 4.0 Hz, 2H; H<sub>allyl</sub>), 4.30–4.24 (m, 4H; H-6a, H<sub>allyl</sub>), 4.14 (br d, *J* = 12.0 Hz, 2H; H-6b), 3.89 (‘q’, *J* = 9.1 Hz, 2H; H-2), 3.72–3.71 (m, 2H; H-5), 3.49 (br s, 4H; NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 3.43 (br s, 4H; CH<sub>2</sub>), 3.28–3.27 (m, 4H; NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 2.08 (s, 6H; C(O)CH<sub>3</sub>), 2.02 (s, 6H; C(O)CH<sub>3</sub>), 2.01 (s, 6H; C(O)CH<sub>3</sub>), 1.93 (s, 6H; C(O)CH<sub>3</sub>), 1.77 (br s, 4H; NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 1.62 ppm (br s, 4H; CH<sub>2</sub>); <sup>13</sup>C NMR (150.9 MHz,

CDCl<sub>3</sub>):  $\delta$  = 170.8, 170.4, 169.4 (C(O)CH<sub>3</sub>), 156.4 (OC(O)NH), 128.8 (C<sub>olefin</sub>), 128.5 (C<sub>olefin</sub>), 99.6 (C-1), 72.6 (C-3), 71.8 (C-5), 70.8 (CH<sub>2</sub>), 69.2 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 68.6 (C-4), 64.6 (C<sub>allyl</sub>), 62.1 (C-6), 60.4 (C<sub>allyl</sub>), 54.6 (C-2), 39.4 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 29.5 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 26.4 (CH<sub>2</sub>), 23.2, 20.7, 20.7, 20.6 ppm (C(O)CH<sub>3</sub>); elemental analysis calcd (%) for C<sub>48</sub>H<sub>74</sub>N<sub>4</sub>O<sub>24</sub> (1091.11): C, 52.84; H, 6.84; N, 5.13; found: C, 52.53; H, 6.81; N, 5.06.

**4.2.8. N,N'-Bis-(4-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-4,7,10-trioxa-1,13-tridecanediamine (15).** According to GP 1, **9** (256 mg, 0.44 mmol), CH<sub>2</sub>Cl<sub>2</sub> (5 mL), 4,7,10-trioxa-1,13-tridecanediamine (44  $\mu$ L, 0.2 mmol), and EtN(*i*-Pr)<sub>2</sub> (70  $\mu$ L, 0.4 mmol) were reacted for 3 h. FC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1) gave **15** (204 mg, 92%) as a white solid. *R*<sub>f</sub> = 0.34 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1); <sup>1</sup>H NMR (600.1 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta$  = 6.28 (d, *J* = 8.6 Hz, 2H; NHAc), 5.67 (m, 4H; H<sub>vinyl</sub>), 5.54 (br s, 2H; NH), 5.26 (‘t’, *J* = 9.9 Hz, 2H; H-3), 5.06 (‘t’, *J* = 9.6 Hz, 2H; H-4), 4.73 (d, *J* = 8.4 Hz, 2H; H-1), 4.63–4.55 (m, 4H; H<sub>allyl</sub>), 4.36 (dd, *J* = 12.8, 4.0 Hz, 2H; H<sub>allyl</sub>), 4.29–4.23 (m, 4H; H<sub>allyl</sub>, H-6a), 4.13 (dd, *J* = 12.2, 1.4 Hz, 2H; H-6b), 3.91 (‘q’, *J* = 9.3 Hz, 2H; H-2), 3.72–3.71 (m, 2H; H-5), 3.63–3.62 (m, 4H; CH<sub>2</sub>CH<sub>2</sub>), 3.59–3.58 (m, 4H; CH<sub>2</sub>CH<sub>2</sub>), 3.54 (t, *J* = 5.4 Hz, 4H; NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 3.27 (‘q’, *J* = 5.8 Hz, 4H; NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 2.07 (s, 6H; C(O)CH<sub>3</sub>), 2.01 (s, 6H; C(O)CH<sub>3</sub>), 2.01 (s, 6H; C(O)CH<sub>3</sub>), 1.93 (s, 6H; C(O)CH<sub>3</sub>), 1.76 ppm (‘t’, *J* = 5.9 Hz, 4H; NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O); <sup>13</sup>C NMR (150.9 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta$  = 170.8, 170.6, 169.4, 163.2 (C(O)CH<sub>3</sub>), 156.4 (OC(O)NH), 128.7 (C<sub>olefin</sub>), 128.5 (C<sub>olefin</sub>), 99.6 (C-1), 72.5 (C-3), 71.7 (C-5), 70.4 (CH<sub>2</sub>CH<sub>2</sub>), 70.1 (CH<sub>2</sub>CH<sub>2</sub>), 69.6 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 68.6 (C-4), 64.6 (C<sub>allyl</sub>), 62.1 (C-6), 60.4 (C<sub>allyl</sub>), 54.5 (C-2), 39.2 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 29.3 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O), 23.2, 20.7, 20.7, 20.6 ppm (C(O)CH<sub>3</sub>); elemental analysis calcd (%) for C<sub>48</sub>H<sub>74</sub>N<sub>4</sub>O<sub>25</sub> (1107.11): C, 52.07; H, 6.74; N, 5.06; found: C, 51.53; H, 6.69; N, 4.94.

**4.2.9. 1,7-Bis-(4-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-diethylenetriamine (16).** According to GP 1, **9** (384 mg, 0.66 mmol), CH<sub>2</sub>Cl<sub>2</sub> (2 mL), diethylenetriamine (32  $\mu$ L, 0.3 mmol), and EtN(*i*-Pr)<sub>2</sub> (105  $\mu$ L, 0.6 mmol) were reacted overnight. FC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1) gave **16** (280 mg, 94%) as a white solid. *R*<sub>f</sub> = 0.15 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 6:1); <sup>1</sup>H NMR (600.1 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta$  = 6.35 (br s, 2H; NHAc), 5.67 (br s, 4H; H<sub>vinyl</sub>), 5.58 (br s, 2H; NH), 5.25 (‘t’, *J* = 9.9 Hz, 2H; H-3), 5.03 (‘t’, *J* = 9.5 Hz, 2H; H-4), 4.73 (d, *J* = 8.2 Hz, 2H; H-1), 4.62–4.54 (m, 4H; H<sub>allyl</sub>), 4.22 (dd, *J* = 12.2, 4.3 Hz, 2H; H-6a), 4.11–4.07 (m, 2H; H-6b), 3.89 (m, 2H; H-2), 3.73–3.72 (m, 2H; H-5), 3.26–3.25 (m, 4H; CH<sub>2</sub>CH<sub>2</sub>), 2.75 (br s, 4H; CH<sub>2</sub>CH<sub>2</sub>), 2.06 (s, 6H; C(O)CH<sub>3</sub>), 2.00 (s, 6H; C(O)CH<sub>3</sub>), 1.99 (s, 6H; C(O)CH<sub>3</sub>), 1.91 ppm (s, 6H; C(O)CH<sub>3</sub>); <sup>13</sup>C NMR (150.9 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta$  = 171.1, 170.7, 170.5, 169.4 (C(O)CH<sub>3</sub>), 156.6 (OC(O)NH), 128.9, 128.4 (C<sub>olefin</sub>), 99.5 (C-1), 72.5 (C-3), 71.6 (C-5), 68.6 (C-4), 64.5 (C<sub>allyl</sub>), 62.1 (C-6), 60.3 (C<sub>allyl</sub>), 54.4 (C-2), 48.4 (CH<sub>2</sub>CH<sub>2</sub>), 40.5 (CH<sub>2</sub>CH<sub>2</sub>), 23.2, 21.0, 20.7, 20.6 ppm (C(O)CH<sub>3</sub>); elemental analysis calcd (%) for C<sub>42</sub>H<sub>63</sub>N<sub>5</sub>O<sub>22</sub> (989.97): C, 50.96; H, 6.41; N, 7.07; found: C, 51.11; H, 6.46; N, 6.67.

**4.2.10. Tris-(2-(4-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-aminoethyl)-amine (17).** Corresponding to GP 1, compound **9** (384 mg, 0.66 mmol), CH<sub>2</sub>Cl<sub>2</sub> (5 mL), tris-(2-aminoethyl)-amine (30  $\mu$ L, 0.2 mmol), and EtN(*i*-Pr)<sub>2</sub> (105  $\mu$ L, 0.6 mmol) were reacted for 3.5 h. FC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1) gave **17** (292 mg, 99%) as a white solid. *R*<sub>f</sub> = 0.31 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1); <sup>1</sup>H NMR (600.1 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta$  = 6.67 (br s, 3H; NHAc), 5.90 (br s, 3H; NH), 5.68 (br s, 6H; H<sub>vinyl</sub>), 5.25 (‘t’, *J* = 9.6 Hz, 3H; H-3), 5.05 (‘t’, *J* = 9.0 Hz, 3H; H-4), 4.72 (d, *J* = 8.1 Hz, 3H; H-1), 4.63–4.55 (m, 6H; H<sub>allyl</sub>), 4.36–4.23 (m, 9H; H<sub>allyl</sub>, H-6a), 4.14–4.12 (m, 3H; H-6b), 3.96 (‘q’, *J* = 9.0 Hz, 3H; H-2), 3.75–3.73 (m, 3H; H-5), 3.21 (br s, 6H; CH<sub>2</sub>CH<sub>2</sub>), 2.55 (br s, 6H; CH<sub>2</sub>CH<sub>2</sub>), 2.07 (s, 9H; C(O)CH<sub>3</sub>), 2.00 (s, 9H; C(O)CH<sub>3</sub>), 1.92 ppm (s, 9H; C(O)CH<sub>3</sub>); <sup>13</sup>C NMR (150.9 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta$  = 170.9, 170.8, 170.6, 169.4 (C(O)CH<sub>3</sub>), 156.9 (OC(O)NH), 129.1, 128.3 (C<sub>olefin</sub>), 99.8 (C-1), 72.7 (C-3), 71.6 (C-5), 68.7 (C-4), 64.6 (C<sub>allyl</sub>), 62.1 (C-6), 60.4 (C<sub>allyl</sub>), 54.2 (C-2), 53.7 (CH<sub>2</sub>CH<sub>2</sub>), 38.8 (CH<sub>2</sub>CH<sub>2</sub>), 23.2, 20.7, 20.7, 20.6 ppm (C(O)CH<sub>3</sub>); elemental analysis calcd (%) for C<sub>63</sub>H<sub>93</sub>N<sub>7</sub>O<sub>33</sub> (1476.44): C, 51.25; H, 6.35; N, 6.64; found: C, 50.85; H, 6.44; N, 6.67.

**4.2.11. 4-(2-Acetamido-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl-propylamine (18).** Corresponding to GP 2, **10** (190 mg, 378  $\mu$ mol) was dissolved in MeOH/EtMe<sub>2</sub>N 5:1 and reacted for 48 h. Compound **18** was obtained in quantitative yield as a white solid. *R*<sub>f</sub> = 0.54 (MeCN/H<sub>2</sub>O 4:1); <sup>1</sup>H NMR (600.1 MHz, D<sub>2</sub>O, 20 °C):  $\delta$  = 5.73–5.68 (m, 2H; H<sub>vinyl</sub>), 4.55 (br s, 2H; H<sub>allyl</sub>), 4.47 (d, *J* = 8.4 Hz, 1H; H-1), 4.33–4.25 (m, 2H; H<sub>allyl</sub>), 3.84 (br d, *J* = 12.3 Hz, 1H; H-6a), 3.68 (br d, *J* = 10.0 Hz, 1H; H-6b), 3.62 (‘t’, *J* = 9.3 Hz, 1H; H-2), 3.46 (‘t’, *J* = 8.2 Hz, 1H; H-3), 3.39–3.37 (m, 2H; H-4, H-5), 3.00 (br s, 2H; NCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.96 (s, 3H; C(O)CH<sub>3</sub>), 1.43–1.39 (m, 2H; NCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.80 ppm (t, *J* = 7.2 Hz, 3H; NCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C NMR (150.9 MHz, D<sub>2</sub>O, 20 °C):  $\delta$  = 175.4 (C(O)CH<sub>3</sub>), 158.0 (OC(O)NH), 129.8 (C<sub>olefin</sub>), 129.4 (C<sub>olefin</sub>), 100.8 (C-1), 76.8 (C-5 or C-4), 74.8 (C-3), 70.7 (C-4 or C-3), 65.5 (C<sub>allyl</sub>), 61.7 (C<sub>allyl</sub>), 61.6 (C-6), 56.4 (C-2), 43.1 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 23.2 (C(O)CH<sub>3</sub>), 23.0 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 11.3 ppm (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>); HRMS (nano ESI, pos. mode, H<sub>2</sub>O/MeOH) for C<sub>16</sub>H<sub>28</sub>N<sub>2</sub>O<sub>8</sub>: [M+H]<sup>+</sup> calcd 377.1918; found: 377.1914, [M+Na]<sup>+</sup> calcd 399.1738; found: 399.1737.

**4.2.12. 4-(2-Acetamido-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl-benzylamine (19).** **11** (214 mg, 389  $\mu$ mol) was suspended in dry MeOH (3 mL). After addition of a 5.4 M methanolic NaOMe solution (10  $\mu$ L, 54  $\mu$ mol) and stirring for 2.5 h, the reaction mixture was neutralized using strong acidic ion exchanger (DOWEX 50 WX 8, H<sup>+</sup> form). Filtration and crystallization gave **19** (148 mg, 90%) as a slight yellow solid. *R*<sub>f</sub> = 0.67 (MeCN/H<sub>2</sub>O 4:1); <sup>1</sup>H NMR (600.1 MHz, D<sub>2</sub>O, 20 °C):  $\delta$  = 7.35–7.34 (m, 2H; H<sub>ar</sub>), 7.28–7.27 (m, 3H; H<sub>ar</sub>), 5.74–5.68 (m, 2H; H<sub>vinyl</sub>), 4.57 (br s, 2H; H<sub>allyl</sub>), 4.46 (d, *J* = 6.7 Hz, 1H; H-1), 4.30–4.24 (m, 4H; H<sub>allyl</sub>, CH<sub>2</sub>N), 3.83 (br d, *J* = 12.2 Hz, 1H; H-6a),

3.67–3.60 (m, 2H, H-6b, H-2), 3.46 (‘t’,  $J = 8.6$  Hz, 1H; H-3), 3.39–3.36 (m, 2H; H-4, H-5), 1.95 ppm (s, 3H; C(O)CH<sub>3</sub>); <sup>13</sup>C NMR (150.9 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 175.3$  (C(O)CH<sub>3</sub>), 159.2 (OC(O)NH), 138.0 (C<sub>ar,q</sub>), 129.6 (C<sub>olefin</sub>), 129.5 (C<sub>olefin</sub>), 128.3, 127.9 (C<sub>ar</sub>), 100.7 (C-1), 76.8 (C-5), 74.8 (C-3), 70.7 (C-4), 65.4 (C<sub>allyl</sub>), 61.9 (C<sub>allyl</sub>), 61.5 (C-6), 56.4 (C-2), 44.9 (CH<sub>2</sub>N), 23.0 ppm (C(O)CH<sub>3</sub>); HRMS (nano ESI, pos. mode, H<sub>2</sub>O/MeOH) for C<sub>20</sub>H<sub>28</sub>N<sub>2</sub>O<sub>8</sub>: [M+Na]<sup>+</sup> calcd 447.1738; found: 447.1743.

**4.2.13. *N,N'*-Bis-(4-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-*p*-xylylenediamine (20).**

Compound **12** (190 mg, 186  $\mu$ mol) was dissolved in MeOH/CHCl<sub>3</sub> 1:1 (5 mL) and a 5.4 M methanolic NaOMe solution (5  $\mu$ L, 27  $\mu$ mol) was added upon which a white precipitate formed. After stirring for 2.5 h, water was added until the precipitate disappeared. The reaction mixture was neutralized using strong acidic ion exchanger (DOWEX 50 WX 8, H<sup>+</sup> form). Filtration and evaporation gave **20** (142 mg, 99%) as a white solid.  $R_f = 0.35$  (MeCN/H<sub>2</sub>O 4:1); <sup>1</sup>H NMR (600.1 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 7.21$  (br s, 4H; H<sub>ar</sub>), 5.71–5.66 (m, 4H; H<sub>vinyl</sub>), 4.55 (br s, 4H; H<sub>allyl</sub>), 4.45 (d,  $J = 6.7$  Hz, 2H; H-1), 4.29–4.25 (m, 4H; H<sub>allyl</sub>), 4.20 (br s, 4H; CH<sub>2</sub>N), 3.81 (br d,  $J = 12.2$  Hz, 2H; H-6a), 3.65–3.58 (m, 4H; H-6b, H-2), 3.44 (‘t’,  $J = 9.0$  Hz, 1H; H-3), 3.37–3.34 (m, 4H; H-4, H-5), 1.93 ppm (s, 6H; C(O)CH<sub>3</sub>); <sup>13</sup>C NMR (150.9 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 175.4$  (C(O)CH<sub>3</sub>), 159.2 (OC(O)NH), 138.6 (C<sub>ar,q</sub>), 129.7 (C<sub>olefin</sub>), 129.5 (C<sub>olefin</sub>), 128.2 (C<sub>ar</sub>), 100.7 (C-1), 76.8 (C-5), 74.8 (C-3), 70.7 (C-4), 65.4 (C<sub>allyl</sub>), 61.9 (C<sub>allyl</sub>), 61.5 (C-6), 56.4 (C-2), 44.6 (CH<sub>2</sub>N), 23.0 ppm (C(O)CH<sub>3</sub>); HRMS (nano ESI, pos. mode, H<sub>2</sub>O/MeOH) for C<sub>34</sub>H<sub>50</sub>N<sub>4</sub>O<sub>16</sub>: [M+H]<sup>+</sup> calcd 771.3295; found: 771.3281, [M+Na]<sup>+</sup> calcd 793.3114; found: 793.3115.

**4.2.14. *N,N'*-Bis-(4-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxy-carbonyl)-*m*-xylylenediamine (21).**

According to GP 2, **13** (182 mg, 178  $\mu$ mol) was dissolved in MeOH/EtMe<sub>2</sub>N 5:1 and reacted for 9 days. **21** was obtained in quantitative yield as a white solid.  $R_f = 0.51$  (MeCN/H<sub>2</sub>O 4:1); <sup>1</sup>H NMR (600.1 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 7.32$ –7.29 (m, 2H; H<sub>ar</sub>), 7.17–7.15 (m, 2H; H<sub>ar</sub>), 5.73–5.67 (m, 4H; H<sub>vinyl</sub>), 4.56 (br s, 4H; H<sub>allyl</sub>), 4.46 (d,  $J = 7.6$  Hz, 2H; H-1), 4.29–4.24 (m, 4H; H<sub>allyl</sub>), 4.22 (br s, 4H; CH<sub>2</sub>N), 3.81 (br d,  $J = 12.0$  Hz, 2H; H-6a), 3.66–3.59 (m, 4H; H-6b, H-2), 3.45 (‘t’,  $J = 9.2$  Hz, 2H; H-3), 3.38–3.35 (m, 4H; H-4, H-5), 1.94 ppm (s, 6H; C(O)CH<sub>3</sub>); <sup>13</sup>C NMR (150.9 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 175.4$  (C(O)CH<sub>3</sub>), 159.2 (OC(O)NH), 140.0 (C<sub>ar,q</sub>), 129.9 (C<sub>ar</sub>), 129.7 (C<sub>olefin</sub>), 129.5 (C<sub>olefin</sub>), 126.9 (C<sub>ar</sub>), 126.3 (C<sub>ar</sub>), 100.8 (C-1), 76.8 (C-5), 74.8 (C-3), 70.7 (C-4), 65.5 (C<sub>allyl</sub>), 61.9 (C<sub>allyl</sub>), 61.6 (C-6), 56.4 (C-2), 44.8 (CH<sub>2</sub>N), 23.0 ppm (C(O)CH<sub>3</sub>); HRMS (nano ESI, pos. mode, H<sub>2</sub>O/MeOH) for C<sub>34</sub>H<sub>50</sub>N<sub>4</sub>O<sub>16</sub>: [M+H]<sup>+</sup> calcd 771.3295; found: 771.3306, [M+Na]<sup>+</sup> calcd 793.3114; found: 793.3125.

**4.2.15. 1,4-Bis-(3-(4-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxy-carbonylamino)-propoxy)-butane (22).** According to GP 2, **14** (150 mg, 137  $\mu$ mol) was dissolved in MeOH/EtMe<sub>2</sub>N 5:1 and reacted for 5 days. Compound **22** was obtained in quantitative yield as a white solid.  $R_f = 0.33$  (MeCN/H<sub>2</sub>O 4:1); <sup>1</sup>H NMR

(600.1 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 5.73$ –5.69 (m, 4H; H<sub>vinyl</sub>-H), 4.55 (br s, 4H; H<sub>allyl</sub>), 4.48 (d,  $J = 8.2$  Hz, 2H; H-1), 4.31–4.28 (m, 4H; H<sub>allyl</sub>), 3.85 (br d,  $J = 12.1$  Hz, 2H; H-6a), 3.68 (br d,  $J = 11.5$  Hz, 2H; H-6b), 3.62 (‘t’, 2H; H-2), 3.47–3.45 (m, 10H; CH<sub>2</sub>, CH<sub>2</sub>, H-3), 3.37 (br s, 4H; H-5, H-4), 3.12 (br s, 4H; CH<sub>2</sub>), 1.97 (s, 6H; C(O)CH<sub>3</sub>), 1.69 (br s, 4H; CH<sub>2</sub>), 1.55 ppm (br s, 4H; CH<sub>2</sub>); <sup>13</sup>C NMR (150.9 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 175.3$  (C(O)CH<sub>3</sub>), 159.1 (OC(O)NH), 129.8 (C<sub>olefin</sub>), 129.5 (C<sub>olefin</sub>), 100.8 (C-1), 76.8 (C-4 or C-5), 74.8 (C-3), 71.2 (CH<sub>2</sub>), 70.8 (C-5 or C-4), 68.7 (CH<sub>2</sub>), 65.5 (C<sub>allyl</sub>), 61.7 (C<sub>allyl</sub>), 61.6 (C-6), 56.4 (C-2), 38.5 (CH<sub>2</sub>), 29.7 (CH<sub>2</sub>), 26.2 (CH<sub>2</sub>), 23.0 ppm (C(O)CH<sub>3</sub>); HRMS (nano ESI, pos. mode, H<sub>2</sub>O/MeOH) for C<sub>36</sub>H<sub>62</sub>N<sub>4</sub>O<sub>18</sub>: [M+H]<sup>+</sup> calcd 839.4132; found: 839.4124, [M+Na]<sup>+</sup> calcd 861.3951; found: 861.3957.

**4.2.16. *N,N'*-Bis-(4-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-4,7,10-trioxa-1,13-tridecanediamine (23).**

According to GP 2, **15** (144 mg, 130  $\mu$ mol) was dissolved in MeOH/EtMe<sub>2</sub>N 5:1 and reacted for 5 days. Compound **23** was obtained in quantitative yield as a white solid.  $R_f = 0.32$  (MeCN/H<sub>2</sub>O 4:1); <sup>1</sup>H NMR (600.1 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 5.95$ –5.92 (m, 4H; H<sub>vinyl</sub>), 4.78 (br s, 4H; H<sub>allyl</sub>), 4.70 (d,  $J = 8.4$  Hz, 2H; H-1), 4.54–4.50 (m, 4H; H<sub>allyl</sub>), 4.07 (br d,  $J = 11.9$  Hz, 2H; H-6a), 3.90 (br d,  $J = 12.0$  Hz, 2H; H-6b), 3.86–3.82 (m, 10H; CH<sub>2</sub>, CH<sub>2</sub>, H-2), 3.73 (br s, 4H; CH<sub>2</sub>), 3.69 (br s, 2H; H-3), 3.60 (br s, 4H; H-4, H-5), 3.35 (br s, 4H; CH<sub>2</sub>), 2.19 (s, 6H; C(O)CH<sub>3</sub>), 1.93 ppm (‘t’,  $J = 5.8$  Hz, 4H; CH<sub>2</sub>); <sup>13</sup>C NMR (150.9 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 175.3$  (C(O)CH<sub>3</sub>), 159.1 (OC(O)NH), 129.8 (C<sub>olefin</sub>), 129.5 (C<sub>olefin</sub>), 100.8 (C-1), 76.8 (C-5 or C-4), 74.8 (C-3), 70.7 (C-4 or C-5), 70.5 (CH<sub>2</sub>), 70.2 (CH<sub>2</sub>), 69.3 (CH<sub>2</sub>), 65.5 (C<sub>allyl</sub>), 61.7 (C<sub>allyl</sub>), 61.6 (C-6), 56.4 (C-2), 38.5 (CH<sub>2</sub>), 29.6 (CH<sub>2</sub>), 23.0 ppm (C(O)CH<sub>3</sub>); HRMS (nano ESI, pos. mode, H<sub>2</sub>O/MeOH) for C<sub>36</sub>H<sub>62</sub>N<sub>4</sub>O<sub>19</sub>: [M+H]<sup>+</sup> calcd 855.4081; found: 855.4080, [M+Na]<sup>+</sup> calcd 877.3900; found: 877.3907.

**4.2.17. 1,7-Bis-(4-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-diethylenetriamine (24).**

Corresponding to GP 2, **16** (270 mg, 274  $\mu$ mol) was dissolved in MeOH/EtMe<sub>2</sub>N 5:1 and reacted for 4 days. Compound **24** was obtained in quantitative yield as a slight yellow solid.  $R_f = 0.08$  (MeCN/H<sub>2</sub>O 2:1); <sup>1</sup>H NMR (600.1 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 5.72$ –5.68 (m, 4H; H<sub>vinyl</sub>), 4.56 (br s, 4H; H<sub>allyl</sub>), 4.47 (d,  $J = 8.4$  Hz, 2H; H-1), 4.30–4.26 (m, 4H; H<sub>allyl</sub>), 3.84 (br d,  $J = 12.1$  Hz, 2H; H-6a), 3.67 (dd,  $J = 11.1$ , 1.7 Hz, 2H; H-6b), 3.61 (‘t’,  $J = 8.1$  Hz, 2H; H-2), 3.46 (br s, 2H; H-3), 3.37 (br s, 4H; H-4, H-5), 3.18 (br s, 4H; CH<sub>2</sub>), 2.65 (br s, 4H; CH<sub>2</sub>), 1.96 ppm (s, 6H; C(O)CH<sub>3</sub>); <sup>13</sup>C NMR (150.9 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 175.4$  (C(O)CH<sub>3</sub>), 159.3 (OC(O)NH), 129.8 (C<sub>olefin</sub>), 129.5 (C<sub>olefin</sub>), 100.8 (C-1), 76.8 (C-4), 74.8 (C-3), 70.8 (C-5), 65.5 (C<sub>allyl</sub>), 61.9 (C<sub>allyl</sub>), 61.6 (C-6), 56.4 (C-2), 48.3 (CH<sub>2</sub>), 40.3 (CH<sub>2</sub>), 23.0 ppm (C(O)CH<sub>3</sub>); HRMS (nano ESI, pos. mode, H<sub>2</sub>O/MeOH) for C<sub>30</sub>H<sub>51</sub>N<sub>5</sub>O<sub>16</sub>: [M+H]<sup>+</sup> calcd 738.3404; found: 738.3389.

**4.2.18. Tris-(2-(4-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl)-aminoethyl)-amine (25).** Corresponding to GP 2, **17** (293 mg, 198  $\mu$ mol) was dis-

solved in MeOH/EtMe<sub>2</sub>N 5:1 and reacted for 7 days. Compound **25** was obtained as a white solid in quantitative yield.  $R_f = 0.35$  (MeCN/H<sub>2</sub>O 2:1); <sup>1</sup>H NMR (600.1 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 5.71$ – $5.70$  (m, 6H; H<sub>vinyl</sub>), 4.57 (br s, 6H; H<sub>allyl</sub>), 4.48 (d,  $J = 8.4$  Hz, 3H; H-1), 4.34–4.26 (m, 6H; H<sub>allyl</sub>), 3.86 (br d,  $J = 12.0$  Hz, 3H; H-6a), 3.69 (br d,  $J = 11.8$  Hz, 3H; H-6b), 3.63 (t,  $J = 9.3$  Hz, 3H; H-2), 3.48 (t,  $J = 8.4$  Hz, 3H; H-3), 3.40–3.38 (m, 6H; H-4, H-5), 3.15 (br s, 6H; CH<sub>2</sub>), 2.60 (br s, 6H; CH<sub>2</sub>), 1.98 ppm (s, 9H; C(O)CH<sub>3</sub>); <sup>13</sup>C NMR (150.9 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 175.2$  (C(O)CH<sub>3</sub>), 159.0 (OC(O)NH), 129.6 (C<sub>olefin</sub>), 100.8 (C-1), 76.8 (C-5 or C-4), 74.8 (C-3), 70.7 (C-4 or C-5), 65.5 (C<sub>allyl</sub>), 61.8 (C<sub>allyl</sub>), 61.6 (C-6), 56.4 (C-2), 53.5 (CH<sub>2</sub>), 38.9 (CH<sub>2</sub>), 23.1 ppm (C(O)CH<sub>3</sub>); HRMS (nano ESI, pos. mode, H<sub>2</sub>O/MeOH) for C<sub>45</sub>H<sub>75</sub>N<sub>7</sub>O<sub>24</sub>: [M+H]<sup>+</sup> calcd 1098.4936; found: 1098.4938, [M+Na]<sup>+</sup> calcd 1120.4736; found: 1120.4803.

**4.2.19. 4-Hydroxy-(Z)-but-2-enyl-2-acetamido-2-deoxy-β-D-glucopyranoside (27).** According to GP 2, 4-hydroxy-(Z)-but-2-enyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside **26**<sup>43</sup> (280 mg, 0.671 mmol) was dissolved in MeOH/EtMe<sub>2</sub>N 5:1 and reacted for 24 h. Compound **27** was obtained as a white solid in quantitative yield.  $R_f = 0.38$  (MeCN/H<sub>2</sub>O 4:1); <sup>1</sup>H NMR (400.1 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 5.78$ – $5.71$  (m, 1H; H<sub>vinyl</sub>), 5.64–5.53 (m, 1H; H<sub>vinyl</sub>), 4.44 (d,  $J = 8.5$  Hz, 1H; H-1), 4.28–4.18 (m, 2H; H<sub>allyl</sub>), 4.09–4.07 (m, 2H; H<sub>allyl</sub>), 3.83 (dd, 1H,  $J = 12.3, 1.2$  Hz; H-6b), 3.73–3.63 (m, 1H; H-6a), 3.59 (dd, 1H,  $J = 10.2, 8.6$  Hz; H-2), 3.46–3.43 (m, 1H; H-5), 3.42–3.38 (m, 2H; H-3, H-4), 1.95 ppm (s, 3H; C(O)CH<sub>3</sub>); <sup>13</sup>C NMR (100.6 MHz, D<sub>2</sub>O, 20 °C):  $\delta = 174.4$  (C(O)CH<sub>3</sub>), 132.7 (C<sub>olefin</sub>), 126.5 (C<sub>olefin</sub>), 99.6 (C-1), 75.7 (C-4 or C-3), 69.7 (C-3 or C-4), 73.7 (C-5), 64.3 (C<sub>allyl</sub>), 60.5 (C-6), 56.9 (C<sub>allyl</sub>), 55.3 (C-2), 21.9 ppm (C(O)CH<sub>3</sub>); elemental analysis calcd (%) for C<sub>12</sub>H<sub>21</sub>NO<sub>7</sub> (291.30): C, 49.48; H, 7.27; N, 4.81; found: C, 49.22; H, 7.44; N, 4.64.

**4.2.20. 4-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl-(3-(2-(2-(3-(trityl-amino)-propoxy)-ethoxy)-ethoxy)propyl)-amine (36).** Compound **9** (1.36 g, 2.335 mmol) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (20 mL). Addition of 3-(2-(2-(3-(trityl-amino)-propoxy)-ethoxy)-ethoxy)-propylamine (900 mg, 1.946 mmol) dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and EtN(*i*-Pr)<sub>2</sub> (340 μL, 1.946 mmol) afforded a yellow colored solution. After stirring overnight at room temperature, removal of the solvent and FC (EtOAc) gave **36** as a white foam in quantitative yield.  $R_f = 0.18$  (EtOAc); <sup>1</sup>H NMR (600.1 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta = 7.38$ – $7.36$  (m, 6H; H<sub>ar</sub>), 7.15–7.12 (m, 6H; H<sub>ar</sub>), 7.06–7.03 (m, 3H; H<sub>ar</sub>), 5.65 (br s, 1H; NH), 5.59 (br s, 2H, H<sub>vinyl</sub>), 5.26 (t,  $J = 9.9$  Hz, 1H; H-3), 4.98 (t,  $J = 9.6$  Hz, 1H; H-4), 4.75 (d,  $J = 7.8$  Hz, 1H; H-1), 4.54–4.47 (m, 2H; H<sub>allyl</sub>), 4.29–4.18 (m, 3H; H<sub>allyl</sub>, H-6a), 4.05 (d,  $J = 10.8$  Hz; H-6b), 3.84 (q,  $J = 9.2$  Hz, 1H; H-2), 3.72–3.70 (m, 1H; H-5), 3.50–3.48 (m, 2H; CH<sub>2</sub>), 3.45–3.43 (m, 6H; CH<sub>2</sub>), 3.39–3.38 (m, 4H; CH<sub>2</sub>), 3.15 (d,  $J = 6.0$  Hz, 2H; CH<sub>2</sub>), 2.14 (br s, 2H; CH<sub>2</sub>), 1.95 (s, 3H; C(O)CH<sub>3</sub>), 1.90 (s, 3H; C(O)CH<sub>3</sub>), 1.89 (s, 3H; C(O)CH<sub>3</sub>), 1.83 (s, 3H; C(O)CH<sub>3</sub>), 1.66–1.63 ppm (m, 4H; CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR (150.9 MHz, CDCl<sub>3</sub>,

20 °C):  $\delta = 170.8, 170.7, 170.5, 169.5$  (C(O)CH<sub>3</sub>), 156.4 (OC(O)NH), 129.0, 128.7, 128.6, 128.1, 127.7, 126.2 (C<sub>ar</sub>, C<sub>vinyl</sub>), 99.5 (C-1), 72.7 (C-3), 71.6 (C-5), 70.9, 70.5, 70.4, 70.2, 70.0, 69.9, 69.4 (CH<sub>2</sub>), 69.0 (C-4), 64.5 (C<sub>allyl</sub>), 62.2 (C-6), 60.3 (C<sub>allyl</sub>), 54.4 (C-2), 41.1, 39.03 (CH<sub>2</sub>), 30.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 29.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 23.1, 21.0, 20.9, 20.6 ppm (C(O)CH<sub>3</sub>); MALDI-MS (pos. mode, CHCA) for C<sub>48</sub>H<sub>63</sub>N<sub>3</sub>O<sub>14</sub>: [M+Na]<sup>+</sup> calcd 928.4; found: 928.5, [M+K]<sup>+</sup> calcd 944.4; found: 944.4.

**4.2.21. 4-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl-(3-(2-(2-(3-amino-propoxy)-ethoxy)-ethoxy)propyl)-amine (37).** Compound **36** (358 mg, 0.395 mmol) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (6 mL). Trifluoroacetic acid (487 μL, 6.32 mmol) and tri-*iso*-propylsilane (243 μL, 1.185 mmol) were added. After stirring for 1.5 h, the solvent was removed. FC (CHCl<sub>3</sub>/MeOH 6:1) gave **37** in quantitative yield as colorless oil.  $R_f = 0.06$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 9:1); <sup>1</sup>H NMR (600.1 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta = 6.89$  (br s, 1H; NHAc), 5.80 (br s, 1H; NH), 5.60 (br s, 2H, H<sub>vinyl</sub>), 5.19 (t,  $J = 9.9$  Hz, 1H; H-3), 4.96 (t,  $J = 9.0$  Hz, 1H; H-4), 4.68 (d,  $J = 8.4$  Hz, 1H; H-1), 4.58–4.50 (m, 2H; H<sub>allyl</sub>), 4.32–4.16 (m, 3H; H<sub>allyl</sub>, H-6a), 4.04 (d,  $J = 12.6$  Hz, 1H; H-6b), 3.93 (q,  $J = 9.2$  Hz, 1H; H-2), 3.76 (br s, 1H; H-5), 3.55–3.46 (m, 12H; OCH<sub>2</sub>), 3.25–3.18 (m, 2H; CH<sub>2</sub>N), 2.80 (br s, 2H; CH<sub>2</sub>N), 1.99 (s, 3H; C(O)CH<sub>3</sub>), 1.93 (s, 6H; C(O)CH<sub>3</sub>), 1.85 (s, 3H; C(O)CH<sub>3</sub>), 1.69 ppm (br s, 4H; CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR (150.9 MHz, CDCl<sub>3</sub>, 20 °C):  $\delta = 170.7, 170.6, 170.3, 169.4$  (C(O)CH<sub>3</sub>), 156.6 (OC(O)NH), 128.7 (C<sub>olefin</sub>), 128.6 (C<sub>olefin</sub>), 99.6 (C-1), 72.7 (C-3), 71.6 (C-5), 70.4, 70.3, 70.0, 69.9, 69.6, 69.3 (OCH<sub>2</sub>), 68.8 (C-4), 64.6 (C<sub>allyl</sub>), 62.2 (C-6), 60.3 (C<sub>allyl</sub>), 54.3 (C-2), 39.6 (CH<sub>2</sub>N), 38.9 (CH<sub>2</sub>N), 31.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 29.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 23.2, 23.1, 21.0, 20.9 ppm (C(O)CH<sub>3</sub>); HRMS (nano ESI, pos. mode, H<sub>2</sub>O/MeOH) for C<sub>29</sub>H<sub>49</sub>N<sub>3</sub>O<sub>14</sub>: [M+H]<sup>+</sup> calcd 664.3287; found: 664.3261.

**4.2.22. 4-(2-Acetamido-2-deoxy-β-D-glucopyranosyloxy)-(Z)-but-2-enyloxycarbonyl-(3-(2-(2-(3-amino-propoxy)-ethoxy)-ethoxy)propyl)-amine (33).** Compound **37** (259 mg, 0.39 mmol) was dissolved in dry MeOH (8 mL). After addition of propylamine (192 μL, 2.34 mmol) and 0.5 M methanolic NaOMe solution (450 μL, 225 μmol) and stirring for 2.5 h, the solvent was evaporated. Compound **33** was obtained in quantitative yield as colorless oil that was directly used for covalent immobilization. <sup>1</sup>H NMR (600.1 MHz, D<sub>2</sub>O, 20 °C)  $\delta = 5.70$ – $5.58$  (m, 2H; H<sub>vinyl</sub>), 4.49–4.48 (m, 2H; H<sub>allyl</sub>), 4.41 (d,  $J = 8.4$  Hz, 1H; H-1), 4.25–4.21 (m, 2H; H<sub>allyl</sub>), 3.78 (d,  $J = 12.0$  Hz, 1H; H-6a), 3.62 (dd,  $J = 12.0, 4.8$  Hz, 1H; H-6b), 3.57–3.40 (m, 14H; H-2, H-5, OCH<sub>2</sub>), 3.31–3.30 (m, 2H; H-4, H-5), 3.10–3.05 (m, 2H; CH<sub>2</sub>N), 2.64 (t,  $J = 6.9$  Hz, 2H; CH<sub>2</sub>N), 1.90 (s, 3H; C(O)CH<sub>3</sub>), 1.65–1.62 ppm (m, 4H; CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>); <sup>13</sup>C NMR (150.9 MHz, D<sub>2</sub>O, 20 °C)  $\delta = 175.3$  (C(O)CH<sub>3</sub>), 159.2 (OC(O)NH), 129.9 (C<sub>olefin</sub>), 129.5 (C<sub>olefin</sub>), 100.9 (C-1), 76.9 (C-4 or C-5), 74.9 (C-3), 70.8 (C-5 or C-4), 70.6, 70.3, 69.8, 69.4 (CH<sub>2</sub>), 65.5 (C<sub>allyl</sub>), 61.8 (C<sub>allyl</sub>), 61.7 (C-6), 56.5 (C-2), 38.7, 38.5, 37.4 (CH<sub>2</sub>), 31.3, 29.7 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 23.1 ppm (C(O)CH<sub>3</sub>); HRMS (nano ESI, pos. mode, H<sub>2</sub>O/MeOH) for C<sub>23</sub>H<sub>43</sub>N<sub>3</sub>O<sub>11</sub>: [M+H]<sup>+</sup> calcd 538.2970; found: 538.2965.

### 4.3. Screening of WGA ligands

**4.3.1. Protocol for non-covalent immobilization of porcine stomach mucin.** Microtiter plates (Becton–Dickinson, Falcon ProBind, Cat. No. 353915) were incubated with a solution of porcine stomach mucin (Serva, fraction 5, pH 7.0) ( $4.12 \mu\text{g mL}^{-1}$ ) in PBS buffer (10 mM sodium phosphate, 150 mM NaCl, pH 7.3) overnight at room temperature (100  $\mu\text{L}$  per well). The plates were then washed five times with PBST buffer (PBS buffer containing 0.05% Tween 20).

**4.3.2. Protocol for the ELLA employing microtiter plates with non-covalently immobilized porcine stomach mucin.**

The coated microtiter plates were blocked with 1% bovine serum albumin (BSA) in PBS buffer for 90 min at 37 °C (150  $\mu\text{L}$  per well). The plates were then washed five times with PBST buffer. Solutions of WGA-HRP (final concentration of  $1 \mu\text{g mL}^{-1}$ ) and any tested inhibitor in serial dilutions in PBS buffer were pre-incubated for 60 min at 37 °C. Then the WGA-HRP/inhibitor solutions were transferred to the blocked microtiter plates (100  $\mu\text{L}$  per well) and incubated at 37 °C for 90 min. After having washed the plates five times with PBST buffer, a solution of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (12.5 mg per 50 mL) in citrate/phosphate buffer (200 mM, pH 4.0) containing 0.015% of  $\text{H}_2\text{O}_2$  was added (50  $\mu\text{L}$  per well). The plates were incubated for 30 min at room temperature in the dark. The reaction then was stopped by addition of 1 M  $\text{H}_2\text{SO}_4$  (50  $\mu\text{L}$  per well). After 5 min in darkness, the absorption was measured at 405 nm using a plate reader (BMG Labtech, FLUOstar OPTIMA). Percent inhibition was calculated with the following equation:

$$\% \text{ inhibition} = \frac{[A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})}]/A_{(\text{no inhibitor})}}{\times 100}$$

$\text{IC}_{50}$  values were reported as the concentration of soluble ligand required for inhibition of 50% of the binding of WGA-HRP to coated microtiter plates. All tests were performed in duplicate.

**4.3.3. Preparation of microtiter plates 32 with amino-reactive isothiocyanate surface.** Microtiter plates (Nunc, CovaLink NH modules, Cat. No. 478042) **30** were treated with a 0.5% solution of 1,4-phenylene diisothiocyanate (PDC) in  $\text{DMSO}^{50,60}$  containing 0.4%  $\text{EtN}(i\text{-Pr})_2$  for 3 h at room temperature (100  $\mu\text{L}$  per well). Then the plates were emptied and rinsed twice with isopropanol, twice with water, and twice with sodium carbonate/bicarbonate buffer (100 mM, pH 10.1) to give modified microtiter plates (**32**).

**4.3.4. Protocol for covalent immobilization of compound 33 to the modified microtiter plates (32).** The modified microtiter plates **32** were incubated with a 1 mM solution of compound **33** in sodium carbonate/bicarbonate buffer (100 mM, pH 10.1) overnight at room temperature (100  $\mu\text{L}$  per well). The wells were then washed once with isopropanol and twice with PBST buffer (pH 7.3) to give microtiter plates **34**.

**4.3.5. Protocol for the ELLA employing covalently modified microtiter plates (34).** This assay was carried out as described above for microtiter plates with non-covalently immobilized porcine stomach mucin except that 100  $\mu\text{L}$  per well of each ABTS solution and  $\text{H}_2\text{SO}_4$  were used. All tests were performed in duplicate.

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