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Fucosyltransferases as Synthetic Tools: Glycan Array Based Substrate Selection and Core Fucosylation of Synthetic *N*-Glycans

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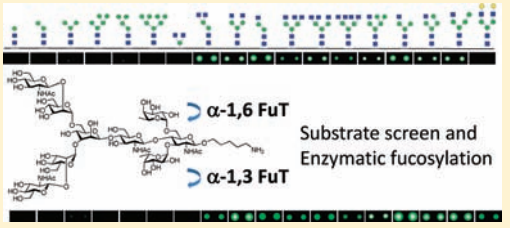
Supporting Information

ABSTRACT: Two recombinant fucosyltransferases were employed as synthetic tools in the chemoenzymatic synthesis of core fucosylated *N*-glycan structures. Enzyme substrates were rapidly identified by incubating a microarray of synthetic *N*-glycans with the transferases and detecting the presence of core fucose with four lectins and one antibody. Selected substrates were then enzymatically fucosylated in solution on a preparative scale and characterized by NMR and MS. With this approach the chemoenzymatic synthesis of a series of α1,3-, α1,6-, and difucosylated structures was accomplished in very short time and with high yields, which otherwise would have required extensive additional synthetic effort and a complete redesign of existing synthetic routes. In addition, valuable information was gathered regarding the specificities of the lectins employed in this study.



INTRODUCTION

Asparagine-linked glycosylation (N-glycosylation) is the most structurally varied form of post-translational modification of eukaryotic proteins. N-glycosylation helps in correct protein folding, increases protein stability against degradation, and has other multiple functions in biological processes involving glycan epitope recognition such as cell adhesion, host−pathogen interactions, and the immune response.[1,2] Many of the interactions of *N*-glycans with proteins are dominated by the terminal sugar moieties or modifications of the glycan core structure with L-fucose or bisecting *N*-acetylglucosamine.

In mammals, core fucose is found exclusively α-1,6-linked to the reducing *N*-acetylglucosamine (GlcNAc) moiety of the chitobiose core,[3] while α-1,3-fucosylation is found in plants, insects, nematodes, trematodes, and slime molds.[4,5] In helminths and insects, both types of core fucosylation and even core bisfucosylations can be observed,[4] and core α1,3-fucosylated *N*-glycan structures could have applications as immunogenic ligands in the design of synthetic conjugate vaccine candidates for the treatment of parasite infections.[6,7] While a comprehensive knowledge of the functions of core fucosylation is still out of reach, the available data already point to a vital role for this type of fucosylation in many biological processes. Core fucosylation of *N*-glycans can have a significant impact on glycan function and conformation,[8] and changes of core fucosylation have been linked to liver and prostate cancer,[9−11] chronic hepatitis,[12] and liver cirrhosis.[13] Core α1,6-fucosylation also has a profound effect on the biological activity (effector functions) of therapeutic antibodies, such as antibody-directed cellular cytotoxicity (ADCC). Depletion of core fucose in the biantennary Fc-glycans improves the binding to Fcγ receptors and increases ADCC activity by up to 50−100-fold.[14−16] Taniguchi et al. have shown that 70% of FUT8 knockout mice lacking an α-1,6 core fucose die after three days and survivors show severe Transforming growth factor beta (TGF-β) growth retardation.[17] The authors also demonstrated that lack of Transforming growth factor beta (TGF-β) core fucosylation led to a significant increase of metalloproteases (MMPs) and a decrease in extracellular matrix proteins (EMPs), both associated with deficient TGF-β signaling in the lung.[17,18] Other effects of core fucosylation on protein function include fucose-dependent Epidermal growth factor/Epidermal growth factor receptor (EGF/EGFR) binding[19] and core fucose regulated expression of E-cadherin and a related increase in cell−cell adhesion.[20]

The ability to efficiently synthesize core fucosylated *N*-glycans in their natural conformation is important for a variety of reasons: (i) well-defined ligands for microarrays are needed to further study the role of core fucosylation in glycan−receptor interactions and (ii) derivatization of isolated glycans by reductive amination with 2-aminopyridine or other UV-active amines leads to an open ring form at the reducing end, changing significantly the natural presentation of the core fucose moiety.[21,22] Furthermore, upregulation of fucosyltransferases (FucT's) in prostate and liver cancers suggests the use of fucosylated glycan structures as antigens for the production of diagnostic antibodies. The identification of abundant antigenic and highly fucosylated *N*-glycan structures in various infective parasites could aid in the design of synthetic conjugate vaccine candidates, as vaccination

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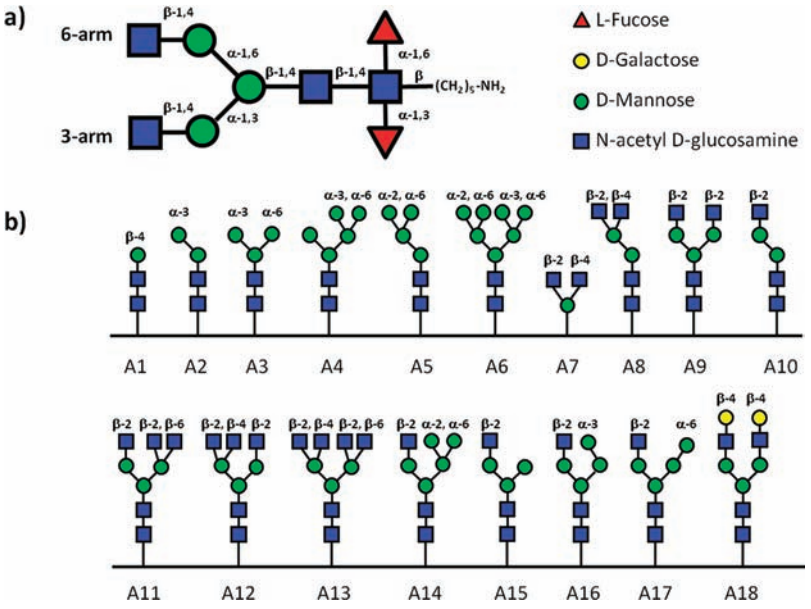


Figure 1. (a) Exemplary bisfucosylated oligosaccharide, pictogram representation according to Consortium of Functional Glycomics guidelines. (b) *N*-Glycan core structures used in this study.

with recombinant proteins can yield disappointing results.[23] Certainly, for various purposes, semisynthetic approaches for the preparation of recombinant glycoproteins could profit from an enzymatic method for the introduction of core fucose.

In this study, we apply for the first time two recombinant fucosyltransferases as synthetic tools for the chemoenzymatic synthesis of *N*-glycan structures on a preparative scale. Although glycan arrays have been used before for the screening of substrate specificity of glycosyltransferases[24−26] and in some cases combined with lectin-based detection of enzyme activity,[27−31] our approach combines the rapid identification of enzyme substrates by microarray analysis using minute amounts of reagents with a direct synthetic scale-up of identified structures for a broad range of array- and non-array-based applications. A *Caenorhabditis elegans* core type α-1,6-fucosyltransferase (CeFUT8; EC 2.4.1.68) and an *Arabidopsis thaliana* core type α-1,3-fucosyltransferase (AtFucTA; E.C. 2.4.1.214) efficiently expressed in a *Pichia pastoris* expression system[32,33] were screened against a panel of synthetic *N*-glycan structures immobilized on a microarray slide to identify candidate structures for enzymatic scale-up in solution. With this approach the chemoenzymatic synthesis of a series of α-1,3-, α-1,6-, and difucosylated structures which otherwise would have required extensive additional synthetic effort and a complete redesign of existing synthetic routes was accomplished in very short time and with high yields.

RESULTS AND DISCUSSION

Compounds **A1**-**A17** used in this study (Figure 1) were prepared by a modular synthetic strategy described previously,[31,34] and details for the synthesis of compounds **A13** and **A18** are given in the Supporting Information. All synthetic ligands were spotted by a robotic noncontact printer at 50 μM concentration on NHS-activated glass slides according to the array design detailed in the Supporting Information and similar to published procedures.[31]

The slides were incubated with increased concentrations (100−150 μg/mL) of both fucosyl transferases, cloned and expressed as previously reported,[32,33] and GDP-fucose. Extended reaction times (72 h) were employed to maximize on-chip enzymatic fucosylation of potential substrates, and therefore, our results most likely do not reflect in vivo substrate specificities.

For the detection of fucose residues on the slides, we used *Lens culinaris* agglutinin (LCA), *Aleuria aurantia* lectin (AAL), *Pisum sativum* agglutinin (PSA), *Aspergillus oryzae* lectin (AOL), and anti-horseradish peroxidase antibody (anti-HRP), an antibody raised against α-1,3 core fucosylated plant glycans.[35,36] This panel of complementary fucose binding proteins with known general specificities and different affinities were used to avoid false negatives resulting from potentially tight lectin binding specificities and to generate a more comprehensive picture of fucosylation on the slides. Background binding to nonfucosylated structures was assessed in control incubations on the glycan array for all probes. AOL and anti-HRP were the only lectins showing complete absence of binding to the nonfucosylated array, while AAL and LCA showed weak binding to **A10** and **A9**, respectively. PSA showed significant binding to nonfucosylated structures **A2**−**A4**, **A6**, **A10**, and **A14**−**17** and was therefore not further used as a probe in the enzyme assay. In any case, due to differing affinities of lectins toward individual compounds, this approach is a qualitative assessment of enzyme activity only. We have therefore confirmed the array data by mass spectrometry.

Applying the above conditions, a slide was first incubated with *Ar. thaliana* core type α-1,3-fucosyltransferase and the presence of core α-1,3-fucosylation analyzed with AAL, anti-HRP, AOL, and LCA (see Figure 2). A first comparison of the four binding profiles revealed compounds **A8**−**A18** as substrates for the enzyme. High mannose glycans **A1**−**A6** and complex **A7** were confirmed as nonbinding nonfucosylated structures by interaction with all fucose binding probes.

While compounds **A9**−**A11** and **A14**−**A17** were recognized as good substrates by all fucose binding probes alike, **A8**, **A12**, and **A13**, having the branched β-1,2-GlcNAc [β-1,4-GlcNAc]-Man trisaccharide on the 3-arm in common, were only picked up by anti-HRP; AAL did not recognize the tetrantennary complex

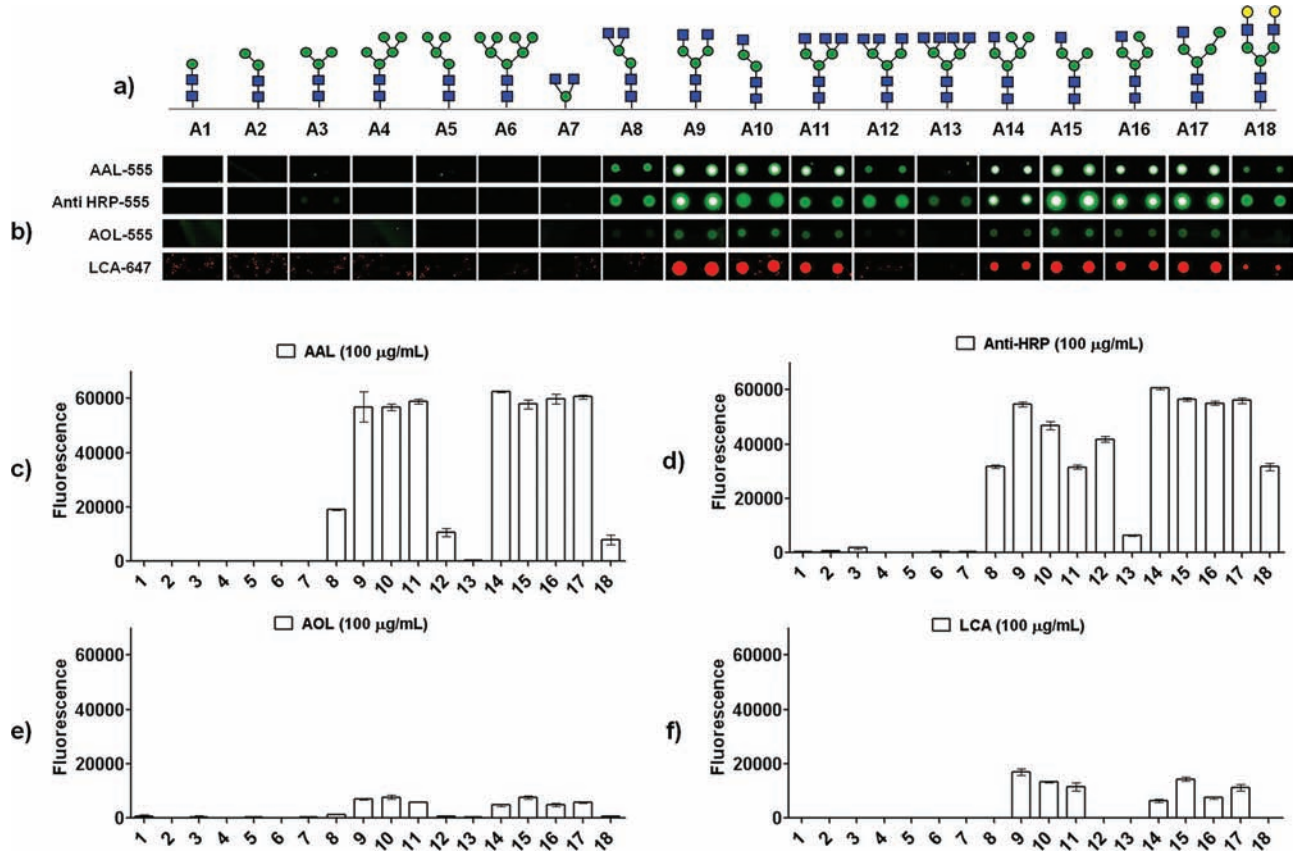


Figure 2. Lectin binding after enzymatic reaction with *Ar. thaliana* core α-1,3-fucosyltransferase. (a) *N*-Glycan structures printed on the array. (b) Microarray images after incubation with fucose-recognizing lectins after enzymatic reaction with AtFucTA. (c) Fluorescence intensities after incubation with AAL-555. (d) Fluorescence intensities after incubation with anti-HRP-555. (e) Fluorescence intensities after incubation with AOL-555. (f) Fluorescence intensities after incubation with LCA-647.

glycan **A13** as a substrate, and AOL and LCA failed to detect **A8** and **A12** as substrates.

Fluorescence intensities for the interaction with AOL were by far the lowest of all lectins tested, but this may be due to a lower (size-dependent) fluorescent tagging of the lectin and does not necessarily reflect a lower binding affinity.[37] The binding pattern however was similar to that of AAL and LCA, suggesting a common mode of binding.[38] While the other lectins showed a higher binding variability, anti-HRP was the only probe used in our assay that bound strongly to all α-1,3-fucosylated structures. The substrate specificity of the enzyme observed by multilectin readout was later confirmed by MALDI-TOF MS analysis of the solution-phase reactions carried out after overnight incubation.

We then applied the same activity screening protocol to the core α-1,6-fucosyltransferase from *C. elegans*. Again an overlay of the binding patterns for the four binding probes showed that compounds **A8**−**A17** were substrates for the enzyme (Figure 3); however, compound **A11** was only picked up as a substrate by AAL and LCA, **A13** only by AAL, and **A8** only by AAL and AOL. Binding to AOL followed the pattern of AAL, while interaction with anti-HRP was close to background values, verifying the high specificity of the antibody for core α-1,3-linkages.[39] The binding selectivity for LCA was essentially the same as for the α-1,3-fucosylated compounds except that the strength was generally higher. MALDI-TOF MS analysis of the solution-phase reaction suggested that compounds **A11** and **A13** bound far weaker after incubation with the enzyme than the other substrates because they were only partially fucosylated. In conclusion and in line with previous findings on the requirement for the prior action of *N*-acetylglucosaminyltransferase I (GlcNAc-TI), which biochemically introduces a β-1,2-GlcNAc residue on the 3-arm of *N*-glycans (see Figure 1; ligands **A1**−**A6** lack this epitope), the substrate specificities for core α-1,6-fucosylation and core α-1,3-fucosylation were similar on our panel of immobilized substrates. Exceptions were the galactosylated substrate **A18**, which was only a substrate for the plant core α-1,3-fucosyltransferase, and **A11**, which is a better substrate for the 1,3-fucosyltransferase. AAL was the only lectin which bound with good selectivity and sensitivity to all core α-1,6-fucosylated structures, while the detection with other lectins was either too weak (AOL) or compromised by higher selectivity (LCA).

Next the substrate specificity for sequential bisfucosylation was explored. Previous reports had shown that core α-1,3-fucosylated structures are not substrates for the α-1,6-fucosyltransferase from *C. elegans*, thereby establishing core α-1,6 before core α-1,3 as the order for bisfucosylation.[36] When the array was incubated, first with *C. elegans* α-1,6-FucT and then with *Ar. thaliana* α-1,3-FucT, we could clearly distinguish by AAL (linkage unspecific) and anti-HRP (specific for α-1,3-fucose) binding the sequential incorporation of both core fucose residues at least to some degree on structures **A8**−**A17**. Again glycans **A13** and **A11** showed considerably weaker binding to the lectin panel after incubation with the enzymes, due to only partial

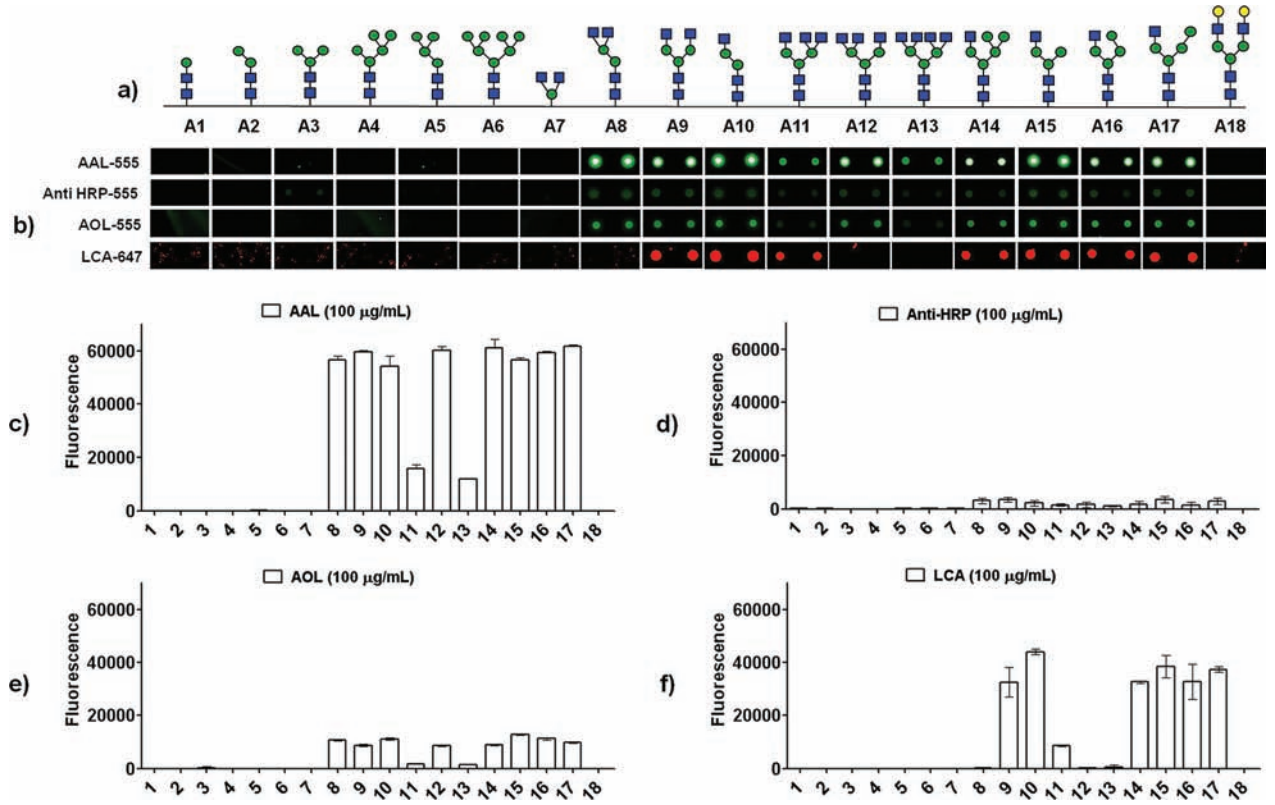


Figure 3. Lectin binding after incubation with *C. elegans* core α-1,6-fucosyltransferase. (a) *N*-Glycan structures printed on the array. (b) Microarray images after incubation with fucose-recognizing lectins after enzymatic reaction with CeFUT8. (c) Fluorescence intensities after incubation with AAL-555. (d) Fluorescence intensities after incubation with anti-HRP-555. (e) Fluorescence intensities after incubation with AOL-555. (f) Fluorescence intensities after incubation with LCA-647.

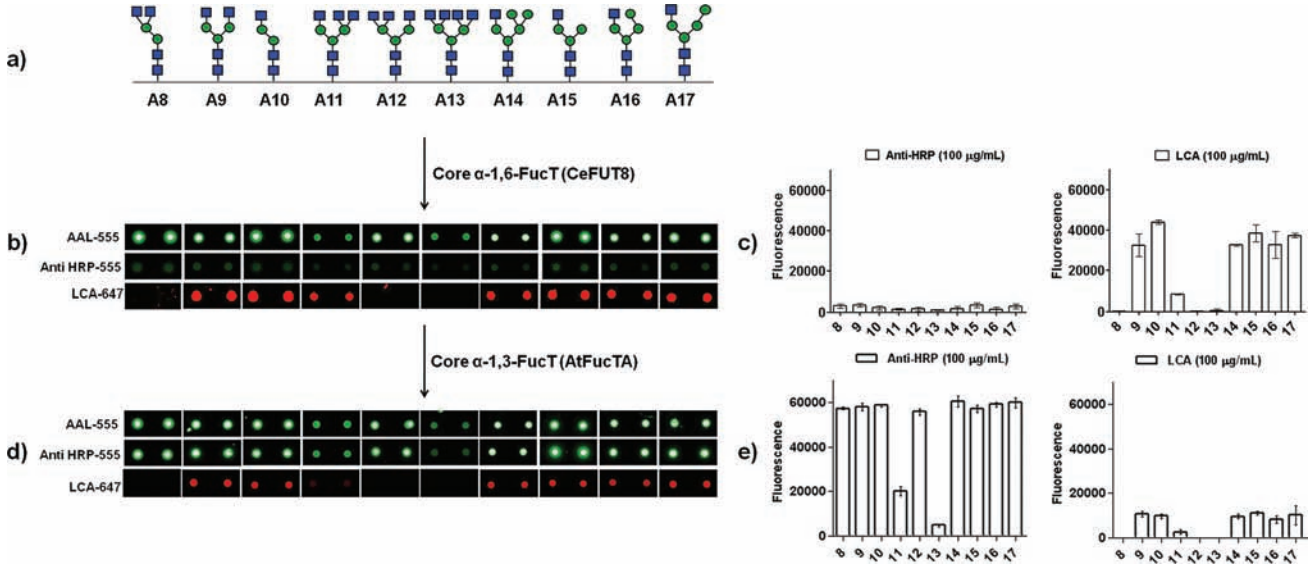
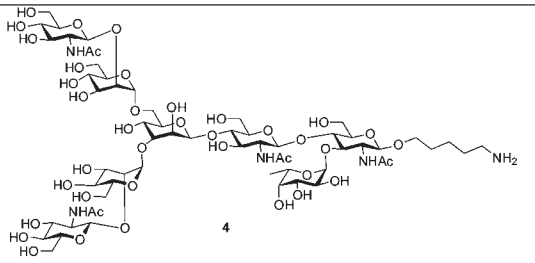
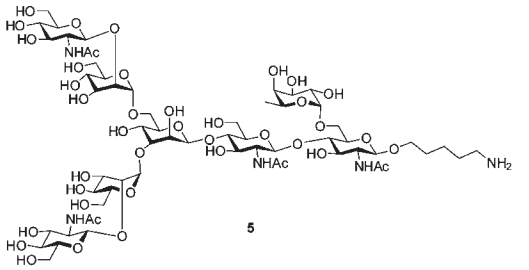
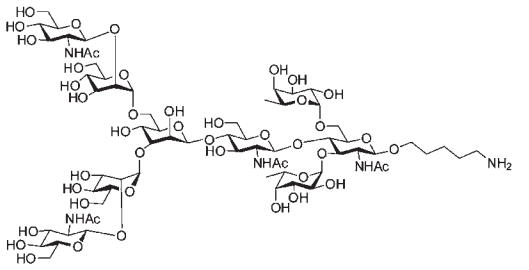
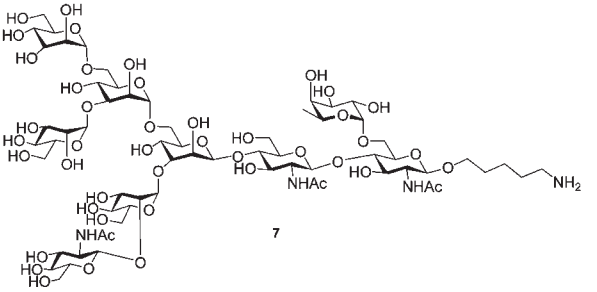


Figure 4. Lectin and antibody binding after enzymatic difucosylation. (a) *N*-Glycan structures printed on the array. (b) Microarray images after enzymatic reaction with *C. elegans* α-1,6-fucosyltransferase incubated with AAL-555, anti-HRP-555, and LCA-647. (c) Fluorescence intensities of α-1,6-fucosylated compounds after incubation with anti-HRP-555 and LCA-647. (d) Microarray images after sequential enzymatic reaction with *C. elegans* α-1,6-fucosyltransferase and *Ar. thaliana* α-1,3-fucosyltransferase incubated with AAL-555, anti-HRP-555, and LCA-647. (e) Fluorescence intensities corresponding to bisfucosylated structures after incubation with anti-HRP-555 and LCA-647.

fucosylation (see the MS data, Supporting Information). Interestingly, the binding of LCA to bisfucosylated structures was decreased nearly 6-fold compared to that to the monofucosylated derivatives, compromising the utility of this lectin for the

Table 1. Fucosylated *N*-Glycans Prepared in Solution

Starting N-glycan	Enzyme[a]	Final compounds
A9	AtFucTA	 **4**
A9	CeFUT8	 **5**
A9	CeFUT8/AtFucTA	 **6**
A14	CeFUT8	 **7**

[a] Reaction conditions: 1.1 equiv of GDP-fucose, 20 mM $MnCl_2$, 80 mM MES, pH 6.5, room temperature.

detection of fucosylated compounds in organisms that present bisfucosylated structures (see Figure 4c,d).

From the pool of substrate ligands, compounds **A9** and **A14** were chosen for the enzymatic core fucosylation on a preparative scale in solution. Incubation with *Ar. thaliana* core α-1,3-fucosyltransferase in the presence of GDP-fucose for 18 h at room temperature completely converted **A9** into the core fucosylated structure **4** (Table 1). Simple purification over graphitized carbon afforded a clean product which was characterized by MS and NMR. A second batch of **A9** was then incubated with the *C. elegans* core α-1,6-fucosyltransferase, affording the 6-fucosylated structure **5** with complete conversion after 18 h. Again NMR and MS analysis confirmed the homogeneity of the compound. The monofucosylated glycan was then incubated with *Ar. thaliana* α-1,3-fucosyltransferase in the same fashion, giving rise to the bisfucosylated structure **6**, which was characterized thoroughly by MS and NMR. Likewise, the incubation with the *C. elegans* α-1,6-fucosyltransferase fully converted **A14** to the 6-fucosylated glycan **7**. The two types of core fucosylations were easily differentiated by characteristic chemical shift values in the ^{1}H NMR spectra. The anomeric proton and the deoxymethyl group were found at 4.80−4.85 and 1.15 ppm, respectively, for the α-1,6-fucose, while the chemical shift values for the same protons in α-1,3-fucosylated compounds were shifted to 5.05 and 1.2 ppm, in agreement with reported NMR data for similar fucosylated *N*-glycans.[40,41]

CONCLUSION

Two recombinant fucosyltransferases have been used for the efficient synthesis of complex mono and bis core fucosylated

N-glycan oligosaccharides on a preparative scale. Using this procedure, common problems associated with the chemical synthesis of fucosylated *N*-glycans, such as the lack of complete stereocontrol in the fucosylation of primary hydroxyl groups,[42] lability of fucosylated intermediates toward acidic reaction conditions, or the increased complexity of the protecting group regime,[43] are avoided and the fucosylated *N*-glycans obtained quantitatively after a simple purification procedure.

An array of 18 *N*-glycans, covering a wide range of structural variation not normally examined in traditional substrate determination studies, has been used to determine the substrate specificity of these recombinant enzymes. Incubation with 3 fucose binding lectins and 1 antibody showed that 10 out of the 18 structures were substrates for both enzymes in vitro, in contrast to the often very narrow substrate specificities of glycosyltransferases in vivo.[44] MALDI-TOF analysis of the solution-phase enzyme reactions confirmed for both enzymes the substrate specificities obtained with the glycan array−lectin platform, demonstrating the suitability of the multilectin detection for the qualitative identification of fucosyltransferase activity. In addition, the overlay of binding profiles provided new insights into the selectivity of lectin binding as well as extending previous fucosyltransferase substrate specificity studies. In our hands, AOL and AAL showed binding patterns very similar to those of multivalently presented α-1,3- and α-1,6-fucosylated structures, which is in contrast to a report which defines AOL as a strong binder to α-1,6-fucosylated structures but not α-1,3 core fucosylated *N*-glycans.[45] For both types of fucosylation, we could only observe weak AOL binding to the presented structures. An explanation for these divergent results could be the altered presentation of core fucose in PA-oligosaccharides with respect to the closed ring form of the reducing end GlcNAc moiety in our study. Our study and the results of others suggest that lectin binding data obtained for fucosylated PA-oligosaccharides or from reverse formats with immobilized lectins such as frontal affinity chromatography (FAC) should be contrasted very carefully with data obtained using immobilized *N*-glycans with more natural fucose presentation.

According to our results, the use of recombinant fucosyltransferases in conjunction with a lectin-based microarray readout system not only overcomes a synthetic problem, but also constitutes a basis for further studies on both carbohydrate binding proteins and on the specificity other glycosyltransferases. An extension of the described methodology to other glycosyltransferases and the preparation of potentially antigenic fucosylated *N*-glycans as ligands for synthetic conjugate vaccine candidates against parasite infections are currently under way in our laboratory.

EXPERIMENTAL SECTION

General Methods. Chemicals were purchased from Sigma-Aldrich or Acros Organics and were used without further purification. THF was freshly distilled from Na/benzophenone, and dichloromethane was freshly distilled from CaH_2. Thin layer chromatography was carried out using Merck aluminum sheets and silica gel 60 F_{254} and visualized by UV irradiation (254 nm) or by staining (15 g of vanillin and 2.5 mL of concentrated H_2SO_4 in 250 mL of EtOH). Microwave irradiation was performed in a Biotage Initiator monomode oven, Biotage AB, Uppsala, Sweden. Purification of compounds was performed by flash chromatography using Merck 62 Å 230−400 mesh silica gel. Size-exclusion chromatography was performed on Biorad P2 gel, Biorad, Hercules, CA. Pooled glycan-containing fractions were lyophilized on an ALPHA-2-4 LSC freeze-dryer from Christ, Osterode, Germany. All organic solvents were concentrated using rotary evaporation. ^{1}H and ^{13}C spectra were acquired on a Bruker 500 MHz spectrometer, and chemical shifts (δ) are given in parts per million relative to the residual signal of the solvent used. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), and m (multiplet). Coupling constants (J) are reported in hertz. The mass spectrometric data were obtained from a Waters LCT Premier XE instrument, Waters, Manchester, U.K., with a standard ESI source by direct injection. The instrument was operated with a capillary voltage of 1.0 kV and a cone voltage of 200 V. The cone and desolvation gas flows were set to 50 and 600 L/h, respectively; the source and desolvation temperatures were 100 °C. MALDI-TOF mass analyses were performed on an Ultraflextreme III time-of-flight mass spectrometer equipped with a pulsed N_2 laser (337 nm) and controlled by FlexControl 3.3 software (Bruker Daltonics). The acquisitions were carried out in positive ion reflectron mode at a laser frequency of 500 Hz. Microarrays were printed on glass slides employing a robotic noncontact spotter Piezorray from Perkin-Elmer, Shelton, CT. NHS-activated glass slides, Nexterion H, were purchased from Schott AG, Mainz, Germany. Lectins were purchased from Vector Laboratories, Burlingame, CA, and labeled with Hilyte Plus 647 and Hilyte Plus 555 protein labeling kits from AnaSpec, Fremont, CA. Enzymatic reactions were performed using hybridization gasket slides from Agilent Technologies, Santa Clara, CA. Lectin incubations were performed using the Fast Frame incubation chambers from Whatman, Kent, U.K. Fluorescence measurements were performed in an Agilent G265BA microarray scanner system, Agilent Technologies. Quantification was performed with ProScanArray Express software, Perkin-Elmer, applying adaptive circle quantitation with a 50−300 μm spot diameter range. Average relative fluorescence units (RFU) values for six replicate spots and the standard deviation of the mean are shown for every lectin/ligand pair. Fluorescence values above a threshold of 5% of the maximum fluorescence were interpreted as positive lectin binding.

Substrate Specificity Assay for *Ar. thaliana* and *C. elegans* Fucosyltransferases. A solution (500 μL) containing *C. elegans* core type α-1,6-FucT (55 μg) or *Ar. thaliana* core type α-1,3-FucT (70 μg), GDP-Fuc (1 mM), and $MnCl_2$ (20 mM) in MES buffer (80 mM, pH 6.5) was added to an incubation chamber from Agilent, and the slide was incubated at room temperature for 72 h. The slide was washed with Phosphate Buffered Saline with 0.5% Tween-20 (PBST, 10 min), Phosphate Buffered Saline (PBS, 10 min), and water (10 min) and dried in a slide spinner. The subarrays were compartimentalized with a 16-well gasket and incubated in the dark for 1 h with different fucose-recognizing lectins: *Al. aurantia* lectin (AAL-555, 100 μg/mL), *L. culinaris* agglutinin (LCA-647, 100 μg/mL), *As. oryzae* lectin (AOL-555, 100 μg/mL), and anti-horseradish peroxidase antibody (anti-HRP-555, 100 μg/mL) in PBST with 1 mM $CaCl_2$. The slide was washed and dried as above.

General Procedure for the Enzymatic Fucosylation in Solution. *5-Aminopentyl Bis[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl]-(1→3),(1→6)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-[α-L-fucopyranosyl]-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside* (***4***). A solution (500 μL) of compound **A9** (1.1 mg, 0.78 μmol), GDP-fucose (507 μg, 0.86 μmol), *Ar. thaliana* core type α-1,3-FucT (AtFucTA) (100 μg), and $MnCl_2$ (20 mM) in MES buffer (80 mM, pH 6.5) was incubated at room temperature for 24 h. The enzyme was precipated by heating the mixture at 95 °C for 5 min and centrifuged, and the supernatant was purified over Biogel P2 with NH_4HCO_3 buffer (40 mM). Fractions were analyzed for product by MALDI-TOF, pooled, and freeze-dried to obtain the title compound as a white powder (1.2 mg, 0.77 μmol, 99%): ^{1}H NMR (500 MHz, D_2O) δ 5.15 (d, J = 3.9 Hz, 1H, H-1$_{\alpha\text{-Fuc}}$), 5.13 (s, 1H, H-1$_{\alpha\text{-Man}}$), 4.93 (s, 1H, H-1$_{\alpha\text{-Man}'}$), 4.76 (br s, 1H, H-1$_{\beta\text{-1,4-Man}}$), 4.60−4.52 (m, 3H, 3 × H-1$_{\text{GlcNAc}}$), 4.50 (d, J = 7.5 Hz, 1H, H-1$_{\text{GlcNAc}}$), 4.26 (s, 1H), 4.20 (s, 1H), 4.12 (s, 1H), 4.02−3.40 (m, 37H), 3.04−2.95 (m, 2H), 2.07 (s, 3H, NHCOCH_3), 2.07 (s, 3H, NHCOCH_3), 2.06

(s, 3H, NHCOCH_3), 2.03 (s, 3H, NHCOCH_3), 1.71−1.56 (m, 4H), 1.48−1.37 (m, 2H), 1.28 (d, J = 6.8 Hz, 3H, CH_3^6 Fuc); ^{13}C NMR (126 MHz, D_2O, selected peaks from HSQC experiment) δ 101.03 (C-1$_{GlcNAc}$), 100.39 (C-1$_{\beta\text{-}1,4\text{-}Man}$), 100.37 (C-1$_{GlcNAc}$), 99.67 (C-1$_{GlcNAc}$), 99.56 (C-1$_{GlcNAc}$), 99.55 (C-1$_{\alpha\text{-}Man}$), 98.23 (C-1$_{\alpha\text{-}Fuc}$), 97.14 (C-1$_{\alpha\text{-}Man}$); HRMS (ESI) m/z calcd for $C_{61}H_{105}N_5NaO_{40}$ $[M + Na]^+$ 1570.6234, found 1570.6320.

5-Aminopentyl Bis[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl]-(1→3),(1→6)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-[α-L-fucopyranosyl]-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (***5***). This compound was prepared according to the general procedure using compound **A9** (1.5 mg, 1.07 μmol) and *C. elegans* core type α-1,6-FucT (CeFUT8) (31 μg). Purification by column chromatography over Biogel P2 with NH_4HCO_3 (40 mM) and lyophilization afforded the title compound as a white powder (1.4 mg, 0.90 μmol, 84%): ^{1}H NMR (500 MHz, D_2O) δ 5.04 (s, 1H, H-1$_{\alpha\text{-}Man}$), 4.84 (s, 1H, H-1$_{\alpha\text{-}Man}$), 4.82 (d, J = 3.9 Hz, 1H, H-1$_{\alpha\text{-}Fuc}$), 4.70 (s, 1H, H-1$_{\beta\text{-}1,4\text{-}Man}$), 4.59 (d, J = 7.7 Hz, 1H, H-1$_{GlcNAc}$), 4.51−4.46 (m, 2H,2x H-1$_{GlcNAc}$), 4.42 (d, J = 7.9 Hz, 1H, H-1$_{GlcNAc}$), 4.18 (s, 1H), 4.10−4.03 (m, 3H), 3.88−3.35 (m, 62H), 2.91 (t, J = 7.8 Hz, 2H), 2.02 (s, 3H, NHCOCH_3), 1.98 (s, 6H, 2 × NHCOCH_3), 1.95 (s, 3H, NHCOCH_3), 1.66−1.46 (m, 4H), 1.39−1.24 (m, 2H), 1.15 (d, J = 6.6 Hz, 3H, CH_3^6 Fuc); ^{13}C NMR (126 MHz, D_2O, selected peaks from HSQC experiment) δ 101.17 (C-1$_{GlcNAc}$), 101.06 (C-1$_{GlcNAc}$), 100.40 (C-1$_{\beta\text{-}1,4\text{-}Man}$), 99.61 (2 × C-1$_{GlcNAc}$), 99.63 (C-1$_{\alpha\text{-}Man}$), 99.19 (C-1$_{\alpha\text{-}Fuc}$), 97.05 (C-1$_{\alpha\text{-}Man}$); HRMS (ESI) m/z calcd for $C_{61}H_{105}N_5NaO_{40}$ $[M + Na]^+$ 1570.6234, found 1570.6252.

5-Aminopentyl Bis[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl]-(1→3),(1→6)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-bis[α-L-fucopyranosyl]-(1→3),(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (***6***). This compound was prepared according to the general procedure using compound **5** (1.4 mg, 0.90 μmol) and AtFucTA (50 μg). Purification by column chromatography over Biogel P2 with NH_4HCO_3 buffer (40 mM) and lyphilization afforded the title compound as a white powder (1.1 mg, 0.65 μmol, 72%): ^{1}H NMR (500 MHz, D_2O) δ 5.07−5.04 (m, 2H, H-1$_{\alpha\text{-}Man}$, H-1$_{\alpha\text{-}Fuc}$), 4.87−4.83 (m, 2H, H-1$_{\alpha\text{-}Man}$, H-1$_{\alpha\text{-}Fuc}$), 4.67 (s, 1H, H-1$_{\beta\text{-}1,4\text{-}Man}$), 4.60 (d, J = 7.9 Hz, 1H, H-1$_{GlcNAc}$), 4.50−4.45 (m, 2H, 2 × H-1$_{GlcNAc}$), 4.41 (d, J = 7.6 Hz, 1H, H-1$_{GlcNAc}$), 4.20−3.31 (m, 21H), 2.95−2.86 (m, 2H), 1.99 (s, 3H, NHCOCH_3), 1.98 (s, 6H, 2 × NHCOCH_3), 1.95 (s, 3H, NHCOCH_3), 1.62−1.54 (m, 2H), 1.51−1.48 (m, 2H), 1.36−1.28 (m, 2H), 1.21 (d, J = 6.6 Hz, 3H, CH_3^6 Fuc), 1.15 (d, J = 6.5 Hz, 3H, CH_3^6 Fuc); ^{13}C NMR (126 MHz, D_2O, selected peaks from HSQC experiment) δ 100.90 (C-1$_{GlcNAc}$), 100.45 (C-1$_{\beta\text{-}1,4\text{-}Man}$), 100.12(C-1$_{GlcNAc}$), 99.58 (C-1$_{\alpha\text{-}Man}$), 99.55 (C-1$_{GlcNAc}$), 99.45 (C-1$_{GlcNAc}$), 98.98 (C-1$_{\alpha1,6\text{-}Fuc}$), 98.24 (C-1$_{\alpha\text{-}1,3\text{-}Fuc}$), 97.03 (C-1$_{\alpha\text{-}Man}$); HRMS (ESI) m/z calcd for $C_{67}H_{115}N_5NaO_{44}$ $[M + Na]^+$ 1716.6813, found 1716.6658.

5-Aminopentyl [2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl]-(1→3)-[bis(α-D-mannopyranosyl)-(1→3),-(1→6)-α-D-mannopyranosyl](1→6)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)- [α-L-fucopyranosyl]-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (***7***). This compound was prepared according to the general procedure using **A14** (2.14 mg, 1.40 μmol) and CeFUT8 (138 μg). Purification by column chromatography over Biogel P2 with NH_4HCO_3 buffer (40 mM) and lyophilization afforded the title compound as a white powder (2.34 mg, 99%): ^{1}H NMR (500 MHz, D_2O) δ 5.04 (s, 1H, H-1$_{\alpha\text{-}Man}$), 5.01 (s, 1H, H-1$_{\alpha\text{-}Man}$), 4.86−4.78 (m, 3H, 2 × H-1$_{\alpha\text{-}Man}$, H-1$_{\alpha\text{-}Fuc}$), 4.68 (s, 1H, H-1$_{\beta\text{-}1,4\text{-}Man}$), 4.58 (d, J = 6.9 Hz, 1H, H-1$_{GlcNAc}$), 4.48 (d, J = 8.4 Hz, 1H, H-1$_{GlcNAc}$), 4.42 (d, J = 7.9 Hz, 1H, H-1$_{GlcNAc}$), 4.15 (s, 1H), 4.10 (s, 1H), 4.06−3.24 (m, 52H), 2.88 (t, J = 7.8 Hz, 2H), 1.98 (s, 3H, NHCOCH_3), 1.96 (s, 3H, NHCOCH_3), 1.93 (s, 3H, NHCOCH_3), 1.62−1.44 (m, 4H), 1.35−1.23 (m, 2H), 1.13 (d, J = 6.4 Hz, 3H, CH_3^6 Fuc); ^{13}C NMR (126 MHz, D_2O, selected peaks from HSQC experiment) δ 102.22 (C-1$_{\alpha\text{-}Man}$), 101.24 (C-1$_{GlcNAc}$), 101.11 (C-1$_{GlcNAc}$), 100.17 (C-1$_{\beta\text{-}1,4\text{-}Man}$), 99.54 (C-1$_{GlcNAc}$), 99.59 (C-1$_{\alpha\text{-}Man}$), 99.56, 99.31 (2 × C-1$_{\alpha\text{-}Man}$, C-1$_{\alpha\text{-}Fuc}$); HRMS (ESI) m/z calcd for $C_{65}H_{112}N_4NaO_{45}$ $[M + Na]^+$ 1691.6497, found 1691.6487.

■ ASSOCIATED CONTENT

Supporting Information. Synthesis of N-glycans **A13** and **A18**, preparation of microarrays, expression of fucosyltransferases, MS analysis of the enzymatic reactions in solution, HRMS and NMR (^{1}H, HSQC) spectra of new compounds, and complete ref 17. This material is available free of charge via the Internet at http://pubs.acs.org/.

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