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FULL PAPER

Heteromultivalent glycooligomers as mimetics of blood group antigens

Katharina Susanne Bücher[a], Patrick Benjamin Konietzny[a], Nicole L. Snyder[b], Laura Hartmann*[a]

Abstract: Precision glycomacromolecules have proven to be important tools for the investigation of multivalent carbohydrate-lectin interactions by presenting multiple glycan epitopes on a highly-defined synthetic scaffold. Here we present a new strategy for the versatile assembly of heteromultivalent glycomacromolecules presenting different carbohydrate motifs in close proximity within the side chains. A new building block suitable for the solid phase polymer synthesis (SPPoS) of precision glycomacromolecules was developed exhibiting a branching point in the side chain that bears a free alkyne and a TIPS-protected alkyne moiety enabling the subsequent attachment of different carbohydrate motifs by on-resin copper mediated azide alkyne cycloaddition reactions (CuAAC). Applying this synthetic strategy, heteromultivalent glycooligomers presenting fragments of histo-blood group antigens (HBGA) and human milk oligosaccharides (HMO) were synthesized and tested for their binding behavior towards bacterial lectin LecB.

Introduction

Glycoconjugates are ubiquitous in nature and are important components of the extracellular matrix and glycocalyx, a dense layer of carbohydrate-based molecules on the cell surface. Their specific interactions with carbohydrate recognition receptors such as lectins, play important roles in many biochemical processes,[1] including cell-cell communication, immune response, fertilization, cell migration,[2,3] or cancer metastasis.[3,4] Furthermore they are known to mediate interactions with pathogens[5,6] such as viruses and bacteria that engage specific carbohydrates within the glycocalyx to attach, enter and infect these cells. In particular, histo-blood group antigens (HBGAs) including AB0 blood groups and Lewis antigens, represent important target structures for many pathogenic lectins.[6,7] While there are still many open questions concerning the biological function and role of HBGAs *e.g.* their specificity towards selected pathogens, it has already been shown that glycomimetic structures[8] can be used as suitable model compounds to study the role of HBGAs, which in turn may be used to develop new inhibitors *e.g.* in antibacterial or antiviral treatment.[9]

In principle, glycomimetic structures of HBGAs are built from a specific epitope fragment of the HBGA, in the simplest case a fucose unit is presented in a multivalent fashion on a synthetic scaffold.[10] There are numerous reports of glycosylated macromolecular scaffolds and their binding interactions with pathogen-related lectins such as LecB from *Pseudomonas aeruginosa*.[11,12] However, the question remains as to how the affinity and selectivity of HBGAs mimetics might be altered by including not only one but different glycan fragments imitating more closely the natural ligand's heterofunctional structure.[13,14,15] Indeed, it has been shown that the combination of different carbohydrate moieties in heterofunctional glycoconjugates and glycomimetic structures strongly affects their recognition process.[16] Therefore, here we introduce a new strategy towards obtaining glycomimetic ligands using an oligo(amidoamine) scaffold with sequence-controlled divalent heterofunctional glycan side chains based on different fragments of HBGA and human milk oligosaccharides HMO ligands.

Previously we reported the synthesis of sequence-controlled glycooligo(amidoamines), so-called precision glycomacromolecules, and their use as multivalent glycomimetic ligands.[17] In short, tailor-made building blocks are assembled in a stepwise fashion on solid support introducing functional moieties in the side chains at defined positions within the scaffold that provide for the attachment of different carbohydrate ligands. Through the straightforward exchange of building blocks during synthesis, we have been able to access a library of glycomacromolecules varying for example the number and position of carbohydrates, the overall length or architecture of the scaffold, as well as different linker or main chain motifs.[18,19,20] Furthermore, we have developed different methods to obtain heteromultivalent glycomacromolecules *e.g.* by using orthogonal coupling strategies or sequential introduction of ligands during scaffold assembly.[21] CuAAC has proven to be an important tool for the introduction of carbohydrate ligands both for homo- and heteromultivalent glycomacromolecules yielding highly efficient coupling directly on solid support. The required alkyne and azido functionalities can be either placed on the building block, *e.g.* TDS (triple bond diethylenetriamine succinyl)[17] or BADS (*p*-(azidomethyl)benzoyl diethylene-triamine succinyl)[19], or on the carbohydrate ligand. In this study we extend this approach by introducing a new building block carrying two alkyne groups (one free and one protected) that allows for the controlled introduction of different carbohydrates using CuAAC. After coupling of a first carbohydrate ligand using the free alkyne, the second, protected alkyne moiety is deprotected and can be conjugated with a

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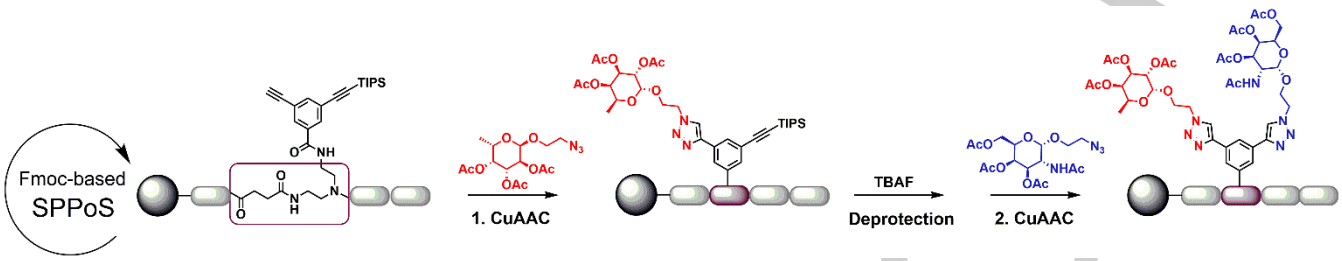
second carbohydrate ligand using the same reaction conditions (Scheme 1).

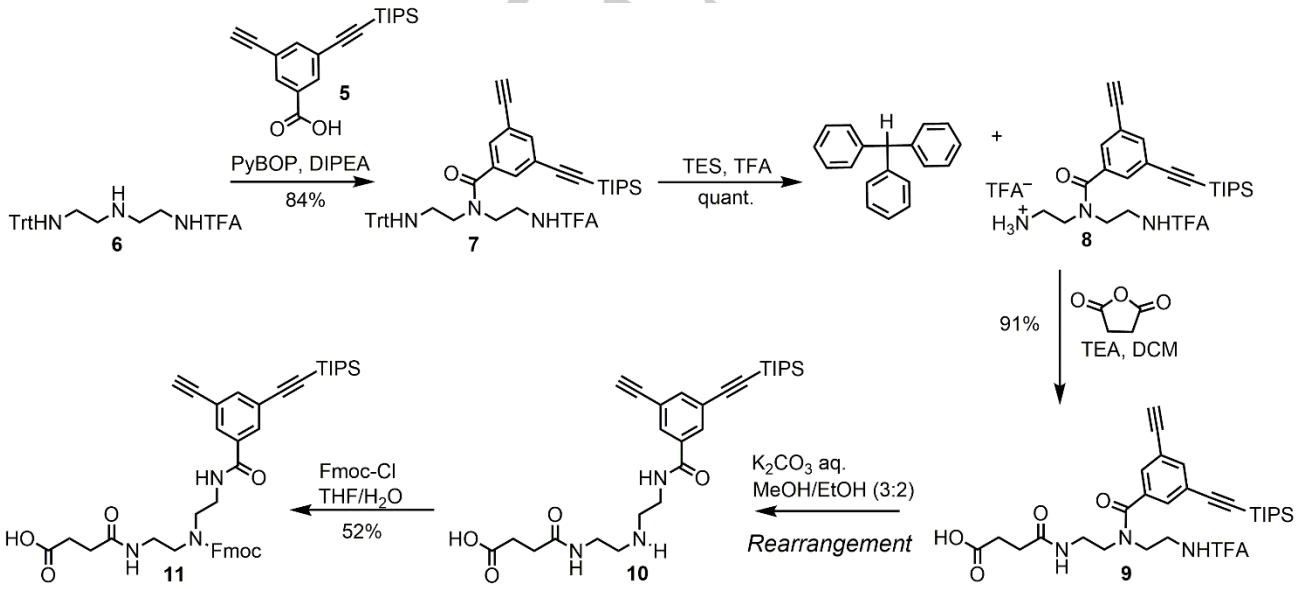


Scheme 1. Introduction of new building block during SPPoS provides for the asymmetrical conjugation of ligands by first coupling a carbohydrate ligand to the free alkyne using CuAAC (*e.g.* fucose in red), followed by deprotection of the second alkyne moiety and coupling of a second carbohydrate ligand (*e.g.* GalNAc in blue).

Results and Discussion

Building Block Synthesis – *iso*DTDS (11)

In order to mimic the heteromultivalent presentation of neighboring monosaccharide motifs in branched oligosaccharide structures more closely, a new building block, *iso*-DTDS (*iso*-Di-Triple-bond Diethylenetriamine Succinic acid) (**11**) was developed. *iso*-DTDS is based on a previously established key intermediate,[17,20] a diethylenetriamine with asymmetrically protected primary amine groups (Scheme 2) that allows for introduction of functional side chains at the central secondary amine position. For *iso*-DTDS, the functional side chain exhibits a rigid phenylene-linker as a branching point with two acetylene units, one of which is protected with a TIPS (triisopropylsilyl) group (Scheme 1). TIPS was selected because it is a well-established alkyne protecting group that can be selectively cleaved on solid support[22] to provide for subsequent CuAAC-based functionalization in the presence of another alkyne, in this case with azido functionalized carbohydrates.



Scheme 2. Synthetic route for new building block *iso*-DTDS **11**, combining precursor **5**[23] and key intermediate **6**[17,20].

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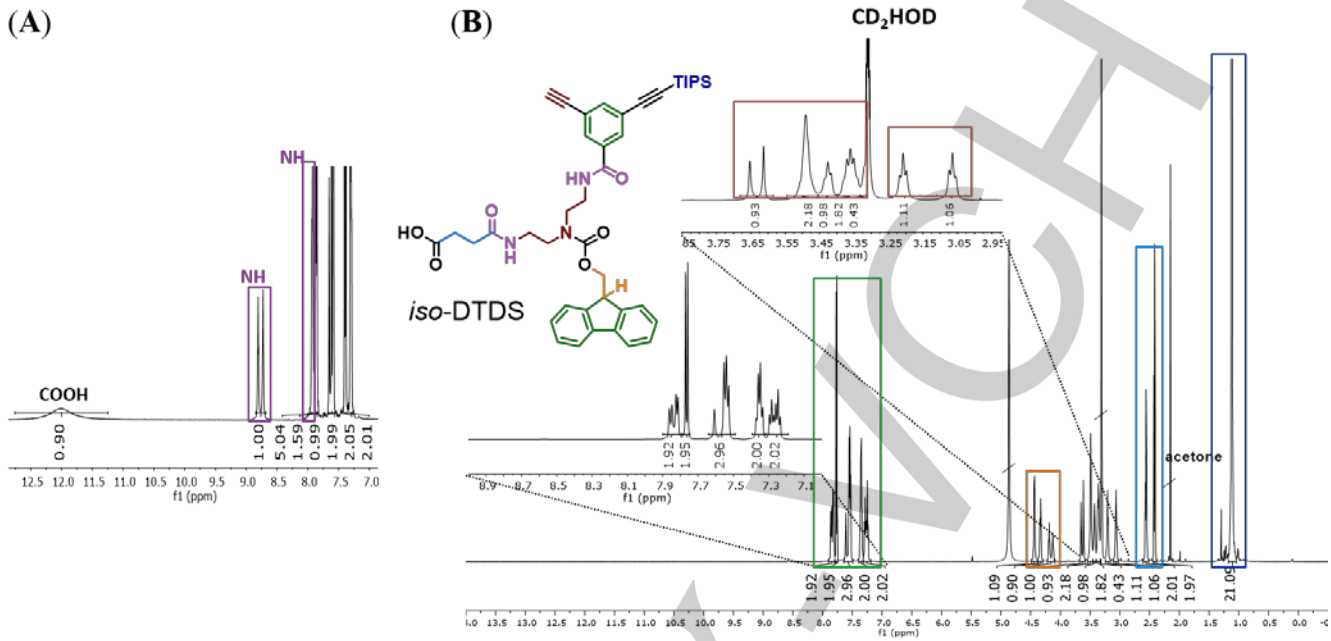

Figure 1. ^{1}H NMR spectra of *iso*-DTDS: (**A**) excerpt of spectrum in DMSO-d_6; (**B**) full spectrum in methanol-d_4.

Scheme 2 depicts the synthesis of *iso*-DTDS. First, compound **5** was synthesized according to literature protocols[23] starting from asymmetrically meta-halogenized benzoic acid **1**. After methyl protection of the acid group (**2**), thermo-selective double Sonogashira reactions were conducted using a Pd[P(Ph_3)]$_4$/CuI catalytic system, first substituting TIPS-acetylene at C3 on the benzene ring (**3**) followed by trimethylsilyl(TMS)-acetylene substitution at the C5 position. Compound **4** was then treated with KOH in THF to remove the TMS and the methyl-protecting groups simultaneously to give **5** in 71% overall yield (see SI).

The synthesis of *iso*-DTDS is based on previous reports for key intermediate **6** (trityl- and TFA-protected diethylenetriamine).[17,20] Compound **5** was coupled to the free secondary amine of **6** using PyBOP and DIPEA. The resulting intermediate **7** was treated with TFA and triethylsilane (TES) as a scavenger to cleave the trityl protecting group giving a mixture of **8** and triphenylmethane. Crude **8** was used in the next reaction step without further purification. The *C*-terminal carboxylic acid group was introduced using succinic anhydride. After precipitation into aqueous citric acid solution, compound **9** was isolated. In the next step, the TFA-protecting group was removed. As already described, a rearrangement can occur during this step.[19] The formation of primary amine and rearrangement product was monitored by LC/MS (see SI). Running the reaction at 60 °C for 8 h followed by stirring at room temperature for 16 h afforded only isomerized product **10** with minor impurities from the TIPS-cleaved side product. After removal of solvent, crude product **10** was subjected to Fmoc-chloride yielding the final *iso*-DTDS building block **11** after purification. *Iso*-DTDS was obtained in 40% overall yield starting from **6** and 98% purity as determined by integration of UV signals in RP-HPLC (see SI).

^{1}H NMR spectra of *iso*-DTDS **11** were recorded in DMSO-d_6 and methanol-d_4 (see Figure 1 and SI). In DMSO-d_6, the characteristic amide protons of isomerized building block **11** at 8.77 ppm and 7.90 ppm can be observed as well as the carboxylic acid proton at 12.01 ppm as shown in figure 1**A**. Unfortunately, the broad solvent peak at 3.38 ppm complicates the analysis of the methylene protons of *iso*-DTDS (see SI). Therefore, additional analysis was performed in methanol-d_4 (Figure 1, **B**). Signals of TIPS protecting group at 1.12 ppm and Fmoc protecting group between 7.0-8.0 ppm for aromatic protons as well as 4.39 ppm and 4.16 ppm for aliphatic protons are clearly visible in ^{1}H NMR (for detailed analysis see SI).

Synthesis of glycooligomers

Syntheses of precision glycooligo(amidoamines) were performed by applying previously reported procedures of Fmoc-based SPPoS.[17] *iso*-DTDS was combined with the previously established ethylenedioxy-bis(ethylamine) succinyl building block (EDS)[17] using PyBOP as coupling reagent (Scheme 3). Coupling efficiency of *iso*-DTDS was evaluated by Fmoc quantification based on UV/Vis measurements of the cleavage solution for test sequence (EDS-DTDS-EDS) for single coupling with 5 eq. as well as double coupling with 3 eq. of building block, respectively. Coupling efficiencies for the introduction of *iso*-DTDS were about 86% for the single coupling and 95% for the double coupling. The use of alternative coupling reagents did not improve the coupling

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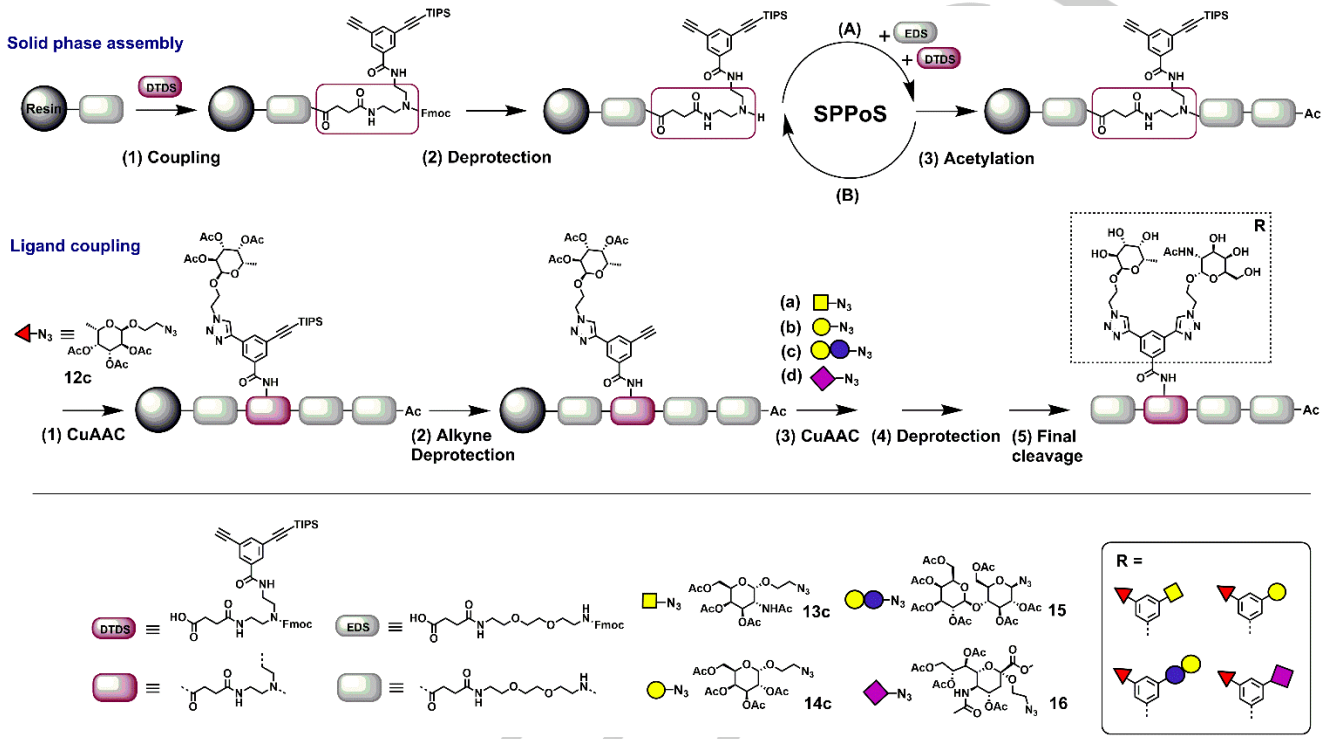
efficiency, with DIC showing 21% and HATU 80% product formation for a single coupling (compared to PyBOP: 86%). EDS building block coupling onto the *iso*-DTDS chain end yielded 96% efficiency under standard coupling conditions (see SI) showing that chain elongation is successful after introduction of *iso*-DTDS.

Based on these results, further glycooligo(amidoamines) were synthesized using standard coupling conditions for EDS (see SI) and double coupling for *iso*-DTDS (3 eq. of building block and PyBOP with 30 eq. of DIPEA in DMF for 1.5 h).



Scheme 3. Synthetic strategy for the synthesis of heteromultivalent glycooligomers applying *iso*-DTDS; introduction of different carbohydrate units by consecutive CuAAC on solid support.

Scheme 3 shows the solid phase synthesis of heteromultivalent glycooligomers using *iso*-DTDS. After assembly of the oligomer backbone and acetylation of the final amine group, carbohydrate ligands are conjugated to the *iso*-DTDS side chain. As a first step for all glycooligomers, acetylated α-L-fucopyranosylazide **12c** (Fuc) was coupled using previously established conditions for CuAAC reaction. After removing excess reagents through successive washing, the TIPS-protecting group was cleaved using TBAF in DMF.[24] The corresponding glycooligomer could then be subjected to a second CuAAC reaction with another azido functionalized carbohydrate derivative (*e.g.* GalNAc (**13c**), Gal (**14c**), Lac (**15**) or Sia (**16**)). In the final step, carbohydrate side chains were deacetylated on resin and the crude final glycooligomers were cleaved off the resin under acidic conditions and isolated by precipitation and lyophilization.

Following this protocol, a first generation of homo- and heteromultivalent glycooligomers introducing two *iso*-DTDS building blocks and thereby four carbohydrate ligands were synthesized (Figure 2). In total, six glycooligomers were synthesized using a scaffold with the sequence EDS-DTDS-EDS-EDS-DTDS-EDS. As first carbohydrate we introduced Fuc, a common monosaccharide motif of different HBGAs. This glycooligomer was then split into four batches and further functionalized with either a GalNAc, Gal, Lac or Sia generating glycooligomers **17**-**20** (Figure 2). In addition, a homomultivalent all-Fuc glycooligomer **21** and an all-Gal glycooligomer **22** were synthesized for comparison in later binding studies. The aromatic unit in the glycomimetic structures was installed with the goal of mimicking the branching sugar unit in the natural trisaccharide, with an additional ethyl-linker introduced via the functionalized monosaccharides in an effort to balance the rigidity of the aromatic branching unit.

Nomenclature of glycooligomers follows previously introduced systematics: the carbohydrates attached to a single branching unit (*iso*-DTDS) are written in brackets (*e.g.* (Fuc,Gal)) followed by the position of *iso*-DTDS within the oligomer chain and the overall chain length as given by the total number of building blocks, *e.g.* (Fuc,Gal)[2,5]-6 for glycooligomer **18**.

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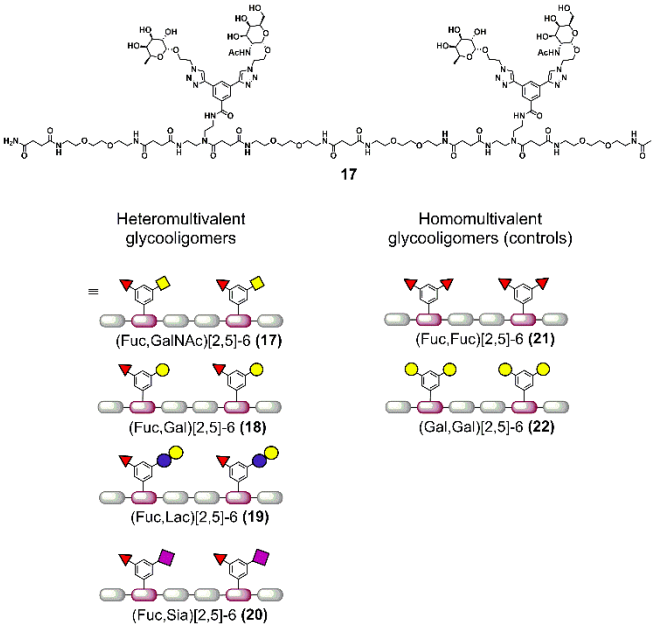

Figure 2. Hetero- and homomultivalent glycooligomer structures as HBGA and HMO mimicry.

Crude glycooligomers were directly analyzed by RP-HPLC/MS (see SI) after cleavage off the resin. The results demonstrate that corresponding glycooligomers are synthesized in good purity (72-85%). Nevertheless, all structures were further purified by using an ion exchange resin and semi-preparative RP-HPLC giving the final structures in high purities (> 99%) (see SI). Glycooligomer **20** carrying two Sia ligands was isolated carrying a methyl protecting group (**20-Me**). Only after purification, glycooligomer **20-Me** was subjected to cleavage of the methyl group giving glycooligomer **20** (see Table 1). Final analysis of glycooligomer structures was performed by ^{1}H-NMR, RP-HPLC/MS, HR-ESI-MS and MALDI-TOF-MS (see SI).

Lectin binding studies of heteromultivalent glycooligomers towards LecB

After the successful synthesis of the first generation of heteromultivalent glycooligomers imitating fragments of HBGAs, we investigated their potential to act as HBGA mimetics by studying their binding behavior towards LecB from *Pseudomonas aeruginosa.* It is well known that LecB binds to different HBGAs and HMOs of which Le^{a} shows the highest affinity (K_D = 210 nM) compared to monovalent ligand L-fucose (K_D = 2.9 µM).[25,12] We have previously reported a surface plasmon resonance (SPR) inhibition competition assay that allows for the measurement of half maximum inhibitory concentration (IC_{50}) values of glycooligomers binding to LecB.[26] In short, a commercially available streptavidin-coated sensor chip was functionalized with commercially available biotinylated polyacrylamide bearing either fucose (PAA-Fuc) as positive control or galactose (PAA-Gal) as negative control at the reference cell. Glycooligomers were preincubated for 1 h at different concentrations with LecB (200 nM) in TRIS-buffer and the ligand-LecB-complex was injected to the SPR sensor chip. Inhibitory potencies of glycooligomers were measured as the reduction of LecB adhesion to the fucosylated sensor chip surface with increasing amount of glycooligomer. α-L-Methylfucose (MeFuc) was measured as reference compound. Galactosylated oligomer **22** was used as negative control whereas homomultivalent fucosylated oligomer **21** served as a positive control as comparison for the effect of heteromultivalency in the HBGA mimetics **17**-**20**. Negative control glycooligomer **22** showed no binding to LecB (see SI). Table 2 shows the results of the inhibition-competition assay of glycooligomers **17**-**21** and α-L-methylfucose.

Table 1. Analytical data of glycooligomers **17**-**22**.

Entry	Type of sugar	Natural model fragment	MW [g/mol]	Yield[a] [%]
17	Fuc/GalNAc	HBGA A	2701.88	40
18	Fuc/Gal	Lewis^{a}	2619.78	49
19	Fuc/Lac	2`Fucosyllactose (2`FL)	2855.95	46
20-Me	Fuc/Sia(Me)	-	2906.06	40
20	Fuc/Sia	Sialyl Lewis^{a}	2878.01	36
21	Fuc/Fuc	Control	2587.78	26
22	Gal/Gal[b]	Negative control	2651.77	36

[a] Overall yield after purification by ion exchange resin and semi-preparative HPLC with a gradient of water/acetonitrile. [b] Alpha/beta mixture of galactose.

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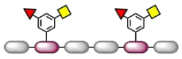
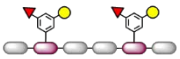
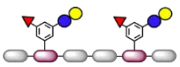
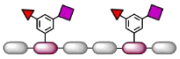
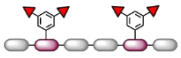
Table 2. Results from SPR inhibition competition assay of glycooligomers **17-21** and LecB (200 nM).

Ligand	n[a]	IC_{50} [nM][b]	RIP[c]	RIP/n[c,d]
MeFuc	1	300 ± 28	1 ± 0.1	1
	2	61 ± 16	4.9 ± 0.3	2.5
	2	35 ± 9	8.6 ± 0.3	4.3
	2	60 ± 11	5.0 ± 0.2	2.5
	2	76 ± 8	3.9 ± 0.1	2.0
	4	54 ± 8	5.6 ± 0.2	1.4

[a] Number of fucose units within the ligand. [b] IC_{50} values determined by two independent measurements with standard error of the mean (SEM). [c] Relative inhibitory potencies (RIP) based on α-L-methylfucose (MeFuc), RIP = IC_{50}(MeFuc) / IC_{50}(glycooligomer). [d] Relative inhibitory potency normalized on fucose units per oligomer (RIP/n).

Compared to α-L-methylfucose, all glycooligomers showed increased inhibition of LecB (Table 2). Previously we could show that binding to LecB increases with an increasing number of fucose units on the oligomeric backbone.[26] Surprisingly, positive control **21** exhibiting a total of four fucose side chains does not show an increase in binding in comparison to the heteromultivalent glycooligomers presenting only two fucose ligands. For further comparison we normalized the IC_{50} values on the IC_{50} value of α-L-methylfucose to provide relative inhibitory potencies (RIP) that can be further normalized to the number of fucose ligands (RIP/n) (Table 2). Indeed, RIP values fit well with previously investigated homomultivalent glycooligomers binding to LecB, where an RIP/n of about 2-3 was observed.[26] Similar values for heteromultivalent glycooligomers **17**, **19** and **20** indicate that the second carbohydrate motif seems to play no role in improving the overall binding to LecB. Only glycooligomer **18** with an additional galactose ligand in close proximity to the fucose ligand shows a lower IC_{50} value and thereby increased binding. One possible explanation could be a partial mimicking of the Le^a ligand. Interestingly, sLe^a mimetic **20** did not show increased binding although sLe^a is also known as a potent binder of LecB.[12] Thus further studies are required to investigate participation of the different carbohydrate motifs in LecB binding in more detail *e.g.* by STD-NMR or crystallography. Ongoing studies also include the analysis of the glycooligomer conformation and thereby distancing between carbohydrate side chains attached via the DTDS building block *e.g.* by means of molecular modelling and light scattering.

Conclusions

A new building block, *iso*-DTDS, suitable for solid phase polymer synthesis was introduced providing for the introduction of closely neighboring carbohydrate ligands in the side chains of precision glycomacromolecules. *iso*-DTDS can be used to create heteromultivalent glycooligomeric constructs combining different carbohydrate motifs and thereby more closely mimicking complex oligosaccharide ligands. In this report, *iso*-DTDS was applied to the synthesis of glycooligomers presenting fragments of HBGAs. Inhibitory potencies of these glycomimetic oligomers towards LecB were investigated. Interestingly, a glycooligomer presenting only Fuc-ligands showed similar inhibitory effects as glycooligomers combining Fuc with GalNAc, Lac or Sia. Only the glycooligomer combining Fuc and Gal showed a slight increase in the inhibitory potential indicating additional binding of the Gal ligands within the structure based on fragments of the natural Le^a ligand. Overall this strategy gives straightforward access to a variety of heteromultivalent glycooligomers and extends our platform of precision glycomacromolecules. Following the presented concept, macromolecular mimetics of other oligosaccharides or combinations of carbohydrates with additional non-carbohydrate binding motifs are now accessible.

Experimental Section

Synthesis of *iso*-DTDS

Key intermediate **6** was synthesized according literature.[17,20] Synthesis of precursor intermediate **5** was performed according to literature.[23]

3-Ethynyl-5-((triisopropylsilyl)ethynyl)benzoic acid (**5**): To a solution of methyl-3-((triisopropylsilyl)ethynyl)-5-((trimethylsilyl)ethynyl)benzoate (**4**) (5.98 g, 14.5 mmol, 1 eq.) in THF (15 mL) was added 15 mL aq. KOH solution (0.2 g/mL, 3 g, 53 mmol, 3.7 eq.) and the reaction mixture was stirred for 24 h at room temperature. Upon completion, 15 mL of water were added and a solid precipitated. The THF was evaporated, and the remaining aqueous suspension was cooled with an ice bath. Aqueous HCl (58 mL, 174 mmol, 3 M, 12 eq.) was added, and the mixture was stirred for at least one hour. The obtained solid precipitate was filtered in vacuum and dried under high vacuum overnight to afford 3-ethynyl-5-((triisopropylsilyl)ethynyl)benzoic acid (**5**) as colorless solid (4.35 g, 13.3 mmol, 92 %). ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 8.15 (p, ^4J = 1.6 Hz, 2H, H_{Ar}-2, H_{Ar}-6), 7.8 (t, ^4J = 1.6 Hz, 1H, H_{Ar}-4), 3.15 (s, 1H, CΞC-*H*), 1.15-1.13 (m, 21H, -C*H*-(CH_3)$_2$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 170.7 (-COOH), 140.3 (C_{Ar}-4), 133.8 (C_{Ar}-2), 133.4 (C_{Ar}-6), 129.9 (C_{Ar}-1), 124.8 (C_{Ar}-5), 123.2 (C_{Ar}-3), 104.7 (CΞC-TIPS), 93.6 (CΞC-TIPS), 81.7 (CΞC-H), 79.1 (CΞC-H), 18.8 (CH_3), 11.4 (CH(CH_3)$_2$). R_f = 0.46 (DCM/MeOH (10:1)). ESI-MS: *m/z* calcd. for $C_{20}H_{26}O_2Si$ $[M+H]^+$ 327.2, found 327.2. HR-ESI-MS: *m/z* calcd. for $C_{20}H_{26}O_2Si$ $[M+H]^+$ 327.1775, found 327.1772. RP-HPLC: (gradient from 80 % to 100 % eluent **B** in 10 min, proceeding at 100 % **B**, 17 min, 25 °C) t_R = 5.8 min, determined purity 98 %.

3-Ethynyl-*N*-(2-(2,2,2-trifluoroacetamido)ethyl)-5-((triisopropylsilyl)-ethynyl)-*N*-(2-(tritylamino)ethyl)benzamide (**7**): To a solution of 2,2,2-trifluoro-*N*-(2-((2-(tritylamino)ethyl)amino)ethyl)acetamide (key intermediate, **6**) (2.58 g, 5.84 mmol, 1 eq.) in DMF (40 mL) were added dialkyne acid **5** (2 g, 6.13 mmol, 1.05 eq.), PyBOP (3.19 g, 6.13 mmol, 1.05 eq.), HOBt (0.89 g, 5.84 mmol, 1 eq.) and DIPEA (1.66 mL, 17.52 mmol, 3 eq.). The mixture was stirred for 16 h at room temperature. The resulting yellowish solution was poured into 400 mL of water and left overnight. The suspension was centrifuged and the water was decanted. The solid product was redissolved in ethyl acetate and extracted three times with water, dried over $MgSO_4$, filtered and concentrated in vacuum. Column chromatography (gradient of hexane/ethyl acetate (3:1-2:1)) afforded the product as colorless crystals (3.68 g, 4.9 mmol, 84%). ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.85 (s, 1H, NH), 7.66 (s, 1H, H_{Bz}), 7.53 (s, 1H, H_{Bz}), 7.46 (s, 1H, H_{Bz}), 7.37 (d, ^3J = 7.8 Hz, 6H, $C(C_6H_5)_3$), 7.28 (t, ^3J = 7.7 Hz, 6H, $C(C_6H_5)_3$), 7.19 (t, ^3J = 7.3 Hz, 3H, $C(C_6H_5)_3$), 3.63-3.61 (m, 2H, (C=O)NH-CH_2-CH_2), 3.51-3.48 (m, 2H, (C=O)NH-CH_2-CH_2), 3.43 (t, ^3J = 5.9 Hz, 2H, $C(Ph)_3$-NH-CH_2-CH_2), 3.13 (s, 1H, CΞC-H), 2.25 (t, ^3J = 5.9 Hz, 2H, $C(Ph)_3$-NH-CH_2), 1.13-1.12 (m, 21H, -CH-(CH_3)$_2$). ^{13}C NMR (151 MHz, $CDCl_3$): δ [ppm] = 172.6 ((C=O)Ph), 157.8 ((C=O)CF_3), 157.6, 145.3 (C-1$_{Phenyl}$), 136.8 (C-4$_{Bz}$), 136.0 (C-1$_{Bz}$), 130.4 (C-2$_{Bz}$, C-6$_{Bz}$), 129.8 (C-2$_{Bz}$, C-6$_{Bz}$), 128.4 (*o*-C$_{Phenyl}$, *m*-C$_{Phenyl}$), 128.1 (*o*-C$_{Phenyl}$, *m*-C$_{Phenyl}$), 126.6 (*p*-C$_{Phenyl}$), 124.8 (C-5$_{Bz}$), 123.0 (C-3$_{Bz}$), 116.7 (CF_3), 104.7 (C-TIPS), 93.5 (CΞC-TIPS), 81.8 (CΞC-H), 79.2 (CΞC-H), 71.0 (C-Ph_3), 60.4 (*residual ethyl acetate*), 50.4 ($N(CH_2)_2$), 44.2 ($N(CH_2)_2$), 42.2 (NH-CH_2), 39.5 (NH-CH_2), 21.1 (*residual ethyl acetate*), 18.6 (CH_3), 14.2 (*residual ethyl acetate*), 11.3 (CH(CH_3)$_2$). HR-ESI-MS: *m/z* calcd. for $C_{45}H_{50}F_3N_3O_2Si$ (monoisotopic mass 749.3624) $[M+H]^+$ 750.3697, found 750.3699. RP-HPLC: (gradient from 80% to 100% eluent **B** in 10 min, proceeding at 100% **B**, 17 min, 25 °C) t_R = 11.2 min, determined purity 99%. R_f = 0.79 (Hex/EE (1:1)).

2,2,2-trifluoroacetaldehyde,2-(3-ethynyl-N-(2-(2,2,2-trifluoroacetamido)-ethyl)-5-((triisopropylsilyl)ethynyl)benzamido)ethan-1-aminium salt (**8**): To a solution of **7** (18.09 g, 24.1 mmol, 1 eq.) **i**n DCM (240 mL) were added TES (10.8 mL, 68 mmol, 2.8 eq.) and TFA (26 mL, 337 mmol, 10% v/v). The colorless solution was stirred 1 h at room temperature and the reaction progress was determined by TLC (hexane/ethyl acetate (1:1)). Then TFA was co-evaporated with toluene. The crude product containing triphenylmethane as a side product (1:1 mixture) was obtained as white solid (20.86 g crude mixture: calc. containing product: 14.9 g, 24 mmol, quant.). Remaining triphenylmethane was not successfully separated but could be removed in the next reaction step. The obtained crude product was used without further purification. ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 8.18 (s, 3H, NH), 7.96 (s, 1H, (CO)NH), 7.63 (s, 1H, H_{Bz}), 7.43 (s, 1H, H_{Bz}), 7.38 (s, 1H, H_{Bz}), 7.30-7.27 (m, 6H, C_5H_6), 7.25 (*residual toluene*), 7.23-7.20 (m, 3H, C_5H_6), 7.17 (*residual toluene*), 7.13-7.11 (m, 6H, C_5H_6), 5.56 (s, 1H, CH_3-Ph_3), 3.81 (s, 2H, CH_2) 3.72 (s, 2H, NH), 3.55 (s, 2H, CH_2), 3.36-3.31 (m, 4H, CH_2), 3.15 (s, 1H, CΞC-H), 2.36 (*residual toluene*), 1.14-1.09 (m, 21H, CH(CH_3)$_2$). ESI-MS: *m/z* calcd. for $C_{26}H_{36}F_3N_3O_2Si$ $[M+H]^+$ 508.3, found 508.2. HR-ESI-MS: *m/z* calcd. for $C_{26}H_{36}F_3N_3O_2Si$ $[M+H]^+$ 508.2602, found 508.2607. R_f = 0.72 (DCM/MeOH (5:1)).

4-((2-(3-ethynyl-N-(2-(2,2,2-trifluoroacetamido)ethyl)-5-((triisopropylsilyl)-ethynyl)benzamido) ethyl)amino)-4-oxobutanoic acid (**9**): To a solution of 20.86 g of crude product **8** (about 14.97 g, 24 mmol, 1 eq. of product **8** respectively) in 240 mL DCM (0.1 M) were added succinic anhydride (2.411 g, 24 mmol, 1 eq.) and NEt_3 (10 mL, 72 mmol, 3 eq.). The reaction mixture was stirred for 2 h at room temperature. The reaction progress was determined by TLC (DCM/MeOH (10:1) v/v, acetic acid 1 droplet). After complete consumption the reaction mixture was concentrated to a total volume of 100 mL under reduced pressure. The mixture was precipitated in 2 L of aq. citric acid (10%) and stirred for 1 h. The precipitate was filtered in vacuum and the solid was washed intensively with water to remove excess citric acid followed by washing with cold DCM. The product was obtained as white solid (13.30 g, 22 mmol, 91%). ^{1}H NMR (300 MHz, CD_3OD, $CDCl_3$): δ [ppm] = 7.57 (t, ^4J = 1.5 Hz, 1H, *p*-H_{Bz}), 7.37 (s, 2H, *o*-H_{Bz}), 3.72-3.67 (m, 1H, NCH_2CH_2), 3.63-3.56 (m, 2H, NCH_2CH_2), 3.52-3.44 (m, 2H, NCH_2CH_2), 3.40-3.33 (m, 3H, NCH_2CH_2, CΞCH, *overlapping with signal from CD_2HOD signal*), 3.25-3.20 (m, 1H, N-CH_2CH_2), 2.63-2.53 (m, 2H, $(CO)CH_2$), 2.48-2.37 (m, 2H, $(CO)CH_2$), 1.09 (s, 21H, $CH(CH_3)_2$). ESI-MS: *m/z* calcd. for $C_{30}H_{40}F_3N_3O_5Si$ $[M+H]^+$ 608.3, found 608.3; $[M+Na]^+$ 630.3, found 630.2. HR-ESI-MS: *m/z* calcd. for $C_{30}H_{40}F_3N_3O_5Si$ $[M+H]^+$ 608.2762, found 608.2753. RP-HPLC: (gradient from 40% to 100% eluent **B** in 30 min, 25 °C) t_R = 15.6 min, determined purity 87%. R_f = 0.52 (DCM/MeOH (9:1) + one droplet AcOH).

4-((2-((2-(3-ethynyl-5-((triisopropylsilyl)ethynyl)benzamido)ethyl)amino)-ethyl)amino)-4-oxobutanoic acid (**10**): Compound **9** (10.0 g, 16.5 mmol, 1 eq.) was suspended in a mixture of MeOH (250 mL) and EtOH (180 mL) and stirred at 40 °C until the starting material was dissolved completely. K_2CO_3 (16 g, 116 mmol, 7 eq.) in water (100 mL) was added and the mixture was heated to 60 °C and allowed to stir at 60 °C for 8 h, and then at room temperature overnight. Remaining solvents were removed under reduced pressure at 40 °C. The crude product was analyzed by RP-HPLC and used without further purification. ESI-MS: *m/z* calcd. for $C_{28}H_{41}N_3O_4Si$ $[M+H]^+$ 512.29, found 512.04. RP-HPLC: (gradient from 100% to 50% eluent **A** in 5 min, decreasing to 0% **A** until 12 min, proceeding at 0% **A**, 17 min, 25 °C) t_R = 8.8 min, determined purity 92%.

4-((2-((((9H-fluoren-9-yl)methoxy)carbonyl)(2-(3-ethynyl-5-((triisopropyl-silyl)ethynyl)benzamido)ethyl)amino)ethyl)amino)-4-oxobutanoic acid (**11**): The slurry of **10** was redissolved in THF (200 mL) and water (200 mL). Fmoc-Cl (4.67 g, 18 mmol, 1.1 eq.) was added and the reaction mixture was stirred for 18 h at room temperature. The emulsion was evaporated under reduced pressure. The pH was tested and needs to be pH > 7. The gel-like residue was redissolved in ethyl acetate (50 mL), water (300 mL) and brine (200 mL). The aqueous layer was washed two times with ethyl acetate to remove remaining Fmoc-based byproducts. Citric acid (1L, 10% solution in water) was added to the aqueous layer to adjust the pH < 4. The product was then extracted three times extensively from the aqueous solution with ethyl acetate. The collected organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Crude product (11.69 g, 15.9 mmol, 96%) was further purified by silica gel column chromatography with DCM/MeOH (20:1) to afford 6.3 g (8.5 mmol, 52%) of pure product **11**. ^{1}H NMR (600 MHz, CD_3OD): δ [ppm] = 7.84 (dd, $^{4,4}J$ = 21.4, 8.0 Hz, 2H, *o*-H_{Bz}), 7.76 (d, ^3J = 7.6 Hz, 2H, H_{Ar} (Fmoc)), 7.64-7.49 (m, 3H, *p*-H_{Bz}, H_{Ar} (Fmoc)), 7.35 (m, 2H, H_{Ar} (Fmoc)), 7.27 (dt, $^{3,4}J$ = 21.5, 7.3 Hz, 2H, H_{Ar} (Fmoc)), 4.44 (d, ^3J = 5.8 Hz, 1H, OCH_2CH), 4.34 (d, ^3J = 6.1 Hz, 1H, OCH_2CH), 4.16 (dt, $^{3,4}J$ = 36.4, 5.7 Hz, 1H, OCH$_2$CH), 3.63 (d, ^5J = 23.9 Hz, 1H, CΞC-H), 3.49 (s, br, 2H, NHCH_2CH_2), 3.45-3.40 (m, 1H, NHCH_2CH_2), 3.39-3.34 (m, 2H, NHCH_2CH_2), 3.34-3.30 (m, *overlapping with methanol-d_4*, NHCH_2CH_2), 3.21 (t, ^3J = 5.9 Hz, 1H, NHCH_2CH_2), 3.06 (t, ^3J = 5.8 Hz, 1H, NHCH_2CH_2), 2.56 (t, ^3J = 6.9 Hz, 2H, $(CO)CH_2$), 2.42 (t, ^3J = 7.0 Hz, 2H, $(CO)CH_2$), 1.15-1.08 (m, 21H, *CH*(*C*H_3)$_2$). ^{13}C NMR (151 MHz, DMSO-d_6): δ [ppm] = 173.8 (CO_2H), 172.0 (CH_2(CO)NH), 171.1 (CH_2(CO)NH), 164.5 (PhCO), 155.6 (O(CO)NH), 155.5 (O(CO)NH), 143.8 (C_{Ar} (Fmoc)), 143.8 (C_{Ar} (Fmoc)), 140.7 (C_{Ar} (Fmoc)), 136.7, 136.6 (*o*-C$_{Bz}$), 135.5, 135.4 (*o*-C$_{Bz}$), 130.7, 130.5 (C-1$_{Bz}$), 127.6 (C_{Ar} (Fmoc)), 127.6 (C_{Ar} (Fmoc)), 127.1 (C_{Ar} (Fmoc)), 125.1 (C_{Ar} (Fmoc)), 125.0 (C_{Ar} (Fmoc)), 123.1 (C-5$_{Bz}$), 123.0 (C-5$_{Bz}$), 122.6 (C-3$_{Bz}$), 122.5 (C-3$_{Bz}$), 120.1 (C_{Ar} (Fmoc)), 120.1 (C_{Ar} (Fmoc)), 105.1 (CΞC-TIPS), 92.0 (CΞC-TIPS), 91.9 (CΞC-TIPS), 82.5 (CΞC-H), 82.3 (CΞC-H), 81.7 (CΞC-H), 67.9 (Fmoc-CH-CH_2), 66.9 (Fmoc-CH-CH_2), 46.9, 46.7, 46.6, 46.5, 38.1, 37.5, 36.8 (all HNCH_2CH_2), 30.1 (succinyl-CH_2), 30.0 (succinyl-CH_2), 29.1 (succinyl-CH_2), 21.1 (CH(CH_3)$_2$), 18.5 (CH_3), 10.6. ESI-MS: *m/z* calcd. for $C_{43}H_{51}N_3O_6Si$ $[M+H]^+$ 734.4, found 734.3; $[M+Na]^+$ 756.3, found 756.2. HR-ESI-MS: *m/z* calcd. for $C_{43}H_{51}N_3O_6Si$ (monoisotopic mass

733.3547) $[M+H]^+$ 734.3620, found 734.3623. RP-HPLC: (gradient from 80% to 100% eluent **B** in 10 min, proceeding at 100% **B**, 17 min, 25 °C) t_R = 6.1 min, determined purity 98%. R_f = 0.71 (DCM/MeOH (10:1) + droplet AcOH).

Glycooligomers analysis

(Fuc,GalNAc)[2,5]-6 (**17**): ^{1}H NMR (600 MHz, D_2O): δ [ppm] = 8.50-8.48 (m, 2H, N=N-N-C*H*), 8.46-8.44 (m, 2H, N=N-N-C*H*), 8.12-8.09 (m, 2H, *p*-H_{Ph}), 7.94-7.90 (m, 4H, *o*-H_{Ph}), 4.88 (d, ^3J = 3.6 Hz, 2H, Fuc-H1*α*), 4.84-4.82 (m, 2H, GalNAc-H1*α*), 4.75-4.69 (m, 8H, N=N-N-CH_2), 4.16 (dt, $^{2,3}J$ = 10.1, 4.5 Hz, 2H, N=N-N-CH$_2$-CH_2), 4.10-4.00 (m, 6H, N=N-N-CH$_2$-CH_2 GalNAc-H3), 3.95 (dt, $^{2,3}J$ = 10.5, 4.8 Hz, 2H, N=N-N-CH$_2$-CH_2), 3.87 (d, ^3J = 2.9 Hz, 2H, GalNAc-H2), 3.82-3.79 (m, 2H, GalNAc-H4), 3.74-3.44 (m, 62H, CH_2-O-(CH_2)$_2$-O-CH_2, N-CH_2-CH_2-NH, Fuc-H2, GalNAc-H6, Fuc-H3, Fuc-H4), 3.40-3.26 (m, 16H, O=C-NH-CH_2-CH$_2$-O), 3.22 (dt, $^{3,3}J$ = 17.7, 5.4 Hz, 2H, Fuc-H5), 3.14-3.09 (m, 2H, GalNAc-H5), 2.76-2.69 (m, 4H, O=C-CH_2-CH_2-C=O), 2.55-2.47 (m, 20H, O=C-CH_2-CH_2-C=O), 1.96, 1.95 (s, s, 3H, (CH$_2$-NH)-(O=)C-CH_3), 1.78-1.77 (m, 6H, NH-(O=)C-CH_3) (*GalNAc*)), 0.93-0.91 (m, 6H, Fuc-H6). MALDI-TOF-MS: *m/z* calcd. for $C_{116}H_{181}N_{29}O_{45}$ $[M+Na]^+$ 2723.28, found 2723.34. HR-ESI-MS: *m/z* calcd. for $C_{116}H_{181}N_{29}O_{45}$ (monoisotopic mass 2700.2766): $[M+3H]^{3+}$ 901.0995, found 901.0988. RP-HPLC: (gradient from 100% to 50% eluent **A** in 30 min, 25 °C): t_R = 14.0 min, determined purity 99%.

(Fuc,Gal)[2,5]-6 (**18**): ^{1}H NMR (600 MHz, D_2O): δ [ppm] = 8.47-8.44 (m, 4H, N=N-N-C*H*), 8.05-8.03 (m, 2H, *p*-H_{Ph}), 7.89-7.86 (m, 4H, *o*-H_{Ph}), 4.98 (d, ^3J = 3.7 Hz, 2H, Gal-H1*α*), 4.88 (d, ^3J = 3.5 Hz, 2H, Fuc-H1*α*), 4.75-4.68 (m, 8H, N=N-N-CH_2), 4.17-4.15 (m, 2H, N=N-N-CH$_2$-CH_2), 4.10-4.07 (m, 2H, N=N-N-CH$_2$-CH_2), 4.02-3.98 (m, 4H, N=N-N-CH$_2$-CH_2), 3.83-3.79 (m, 4H, Gal-H2, Gal-H4), 3.76-3.69 (m, 6H, Fuc-H2, Gal-H3, Gal-H6), 3.67-3.43 (m, 55H, CH_2-O-(CH_2)$_2$-O-CH_2, N-CH_2-CH_2-NH, Fuc-H3, Fuc-H4), 3.41-3.24 (m, 18H, O=C-NH-CH_2-CH$_2$-O, Gal-H6), 3.20 (dt, $^{3,3}J$ = 19.6, 5.1 Hz, 2H, Fuc-H5), 3.16-3.13 (m, 2H, Gal-H5), 2.75-2.69 (m, 4H, O=C-CH_2-CH_2-C=O), 2.54-2.48 (m, 20H, O=C-CH_2-CH_2-C=O), 1.95, 1.94 (s, s, 3H, O=C-CH_3), 0.94-0.92 (m, 6H, Fuc-H6). MALDI-TOF-MS: *m/z* calcd. for $C_{112}H_{175}N_{27}O_{45}$ $[M+Na]^+$ 2641.2, found 2641.3. HR-ESI-MS: *m/z* calcd. for $C_{112}H_{175}N_{27}O_{45}$ (monoisotopic mass 2618.2235): $[M+3H]^{3+}$ 873.7485, found 873.7479. RP-HPLC: (gradient from 100% to 50% eluent **A** in 30 min, 25 °C): t_R = 13.7 min, determined purity 99%.

(Fuc,Lac)[2,5]-6 (**19**): ^{1}H NMR (600 MHz, D_2O): δ [ppm] = 8.58 (s, 2H, N=N-N-C*H*), 8.40-8.39 (m, 2H, N=N-N-C*H*), 8.01-7.09 (m, 2H, *p*-H_{Ph}), 7.88-7.79 (m, 4H, *o*-H_{Ph}), 5.87 (dd, $^{3,4}J$ = 9.1, 1.7 Hz, 2H, Glc-H1β), 4.87 (d, ^3J = 3.4 Hz, 2H, Fuc-H1*α*), 4.73-4.64 (m, 4H, N=N-N-CH_2), 4.57 (d, ^3J = 7.7 Hz, 2H, Gal-H1β), 4.15 (dt, $^{2,3}J$ = 8.9, 2.0 Hz, 2H, N=N-N-CH$_2$-CH_2), 4.08-4.05 (m, 4H, N=N-N-CH$_2$-CH_2, Glc-H), 4.00-3.94 (m, 12H, Glc-H, Gal-H), 3.88-3.84 (m, 2H, Glc-H), 3.82-3.79 (m, 4H, Gal-H2, Gal-H4), 3.74-3.69 (m, 6H, Fuc-H2, Gal-H3, Gal-H6), 3.64-3.44 (m, 51H, CH_2-O-(CH_2)$_2$-O-CH_2, N-CH_2-CH_2-NH, Fuc-H3, Fuc-H4), 3.42-3.27 (m, 17H, O=C-NH-CH_2-CH$_2$-O), 3.24-3.12 (m, 6H, Gal-H6, Fuc-H5, Gal-H5), 2.73-2.68 (m, 4H, O=C-CH_2-CH_2-C=O), 2.55-2.45 (m, 20H, O=C-CH_2-CH_2-C=O), 1.94, 1.93 (s, s, 3H, O=C-CH_3), 0.95-0.93 (m, 6H, Fuc-H6). MALDI-TOF-MS: *m/z* calcd. for $C_{120}H_{187}N_{27}O_{53}$ $[M+Na]^+$ 2877.3, found 2877.3. HR-ESI-MS: *m/z* calcd. for $C_{120}H_{187}N_{27}O_{53}$ (monoisotopic mass 2854.2768): $[M+3H]^{3+}$ 952.4329, found 952.4325. RP-HPLC: (gradient from 100% to 50% eluent **A** in 30 min, 25 °C): t_R = 12.8 min, determined purity ≥99%.

(Fuc,Sia(methyl-protected))[2,5]-6 (**20-Me**): ^{1}H NMR (600 MHz, D_2O): δ [ppm] = 8.48-8.46 (m, 2H, N=N-N-C*H*), 8.37-8.36 (m, 2H, N=N-N-C*H*), 8.08-8.06 (m, 2H, *p*-H_{Ph}), 7.92-7.88 (m, 4H, *o*-H_{Ph}), 4.88 (d, ^3J = 3.5 Hz, 2H, Fuc-H1*α*), 4.76-4.62 (m, 11H, COO-CH$_3$, N=N-N-CH_2, *overlapping with HDO-signal*), 4.24-4.22 (m, 2H, N=N-N-CH$_2$-CH_2), 4.10-4.07 (m, 2H, N=N-N-CH$_2$-CH_2), 4.02-3.98 (m, 4H, N=N-N-CH$_2$-CH_2), 3.83 (t, ^3J = 10.2 Hz, 2H, Sia-H8), 3.76-3.43 (m, 73H, CH_2-O-(CH_2)$_2$-O-CH_2, N-CH_2-CH_2-NH, Fuc-H2, Fuc-H3, Fuc-H4, Sia-H4-H7, Sia-H9), 3.41-3.25 (m, 17H, O=C-NH-CH_2-CH$_2$-O), 3.20 (dt, $^{3,3}J$ = 18.7, 5.2 Hz, 2H, Fuc-5), 3.16-3.11 (m, 2H, Sia-H), 2.76-2.70 (m, 4H, O=C-CH_2-CH_2-C=O), 2.62 (dd, $^{2,3}J$ = 12.9, 4.6 Hz, 2H, Sia-H$_e$3), 2.55-2.47 (m, 21H, O=C-CH_2-CH_2-C=O), 2.00 (s, 6H, (NH)CO-CH_3 (*Sia*)), 1.95, 1.95 (s, s, 3H, O=C-CH_3)), 1.79 (t, $^{2,3}J$ = 12.4 Hz, 2H, Sia-H$_a$3), 0.94-0.93 (m, 6H, Fuc-H6). MALDI-TOF-MS: *m/z* calcd for $C_{124}H_{193}N_{29}O_{51}$ $[M+Na]^+$ 2927.34, found 2927.47. HR-ESI-MS: *m/z* calcd for $C_{124}H_{193}N_{29}O_{51}$ (monoisotopic mass 2904.3400): $[M+3H]^{3+}$ 969.1206, found 969.1191. RP-HPLC: (gradient from 100% to 50% eluent **A** in 30 min, 25 °C): t_R = 14.6 min, determined purity 99%.

(Fuc,Sia)[2,5]-6 (**20**): ^{1}H NMR (300 MHz, D_2O): δ [ppm] = 8.46-8.43 (m, 2H, N=N-N-C*H*), 8.38-8.34 (m, 2H, N=N-N-C*H*), 8.05-8.01 (m, 2H, *p*-H_{Ph}), 7.89-7.82 (m, 4H, *o*-H_{Ph}), 4.88 (d, ^3J = 3.0 Hz, 2H, Fuc-H1*α*), 4.74-4.60 (m, 8H, N=N-N-CH_2), 4.25-4.20 (m, 2H, N=N-N-CH$_2$-CH_2), 4.13-3.95 (m, 8H, N=N-N-CH$_2$-CH_2, Sia-H8), 3.85-3.11 (m, 94H, CH_2-O-(CH_2)$_2$-O-CH_2, N-CH_2-CH_2-NH, Fuc-H2, Fuc-H3, Fuc-H4, Sia-H4-H7, Sia-H9, O=C-NH-CH_2-CH$_2$-O, Fuc-H-5), 2.77-2.49 (m, 28H, O=C-CH_2-CH_2-C=O, Sia-H3$_{eq}$), 2.00 (s, 6H, NC(=O)CH_3 (*Sia*)), 1.95, 1.94 (s, s, 3H, O=C-CH_3), 1.75 (t, $^{2,3}J$ = 12.1 Hz, 2H, Sia-H3$_{ax}$), 0.93 (d, ^3J = 6.6 Hz, 6H, Fuc-H6). HR-ESI-MS: *m/z* calcd. for $C_{122}H_{189}N_{29}O_{51}$ (monoisotopic mass 2878.3087): $[M+3H]^{3+}$ 959.7769, found 959.7761. RP-HPLC: (gradient from 100% to 50% eluent **A** in 30 min, 25 °C): t_R = 13.6 min, 13.8 min, determined purity 96%.

(Fuc,Fuc)[2,5]-6 (**21**): ^{1}H NMR (600 MHz, D_2O): δ [ppm] = 8.39 (s, 4H, N=N-N-C*H*), 7.99-7.97 (m, 2H, *p*-H_{Ph}), 7.82-7.79 (m, 4H, *o*-H_{Ph}), 4.78 (d, ^3J = 3.1 Hz, 4H, Fuc-H1*α*), 4.67-4.56 (m, 8H, N=N-N-CH_2), 4.04-3.89 (m, 8H, N=N-N-CH$_2$-CH_2), 3.63-3.09 (m, 76H, Fuc-H2, Fuc-H3, Fuc-H4, CH_2-O-(CH_2)$_2$-O-CH_2, N-CH_2-CH_2-NH, O=C-NH-CH_2-CH$_2$-O), 3.06-2.99 (m, 4H, Fuc-H5), 2.68-2.59 (m, 4H, O=C-CH_2-CH_2-C=O), 2.47-2.36 (m, 20H, O=C-CH_2-CH_2-C=O), 1.86, 1.86 (s, s, 3H, O=C-CH_3), 0.82 (d, ^3J = 6.5 Hz, 12H, Fuc-H6). MALDI-TOF-MS: *m/z* calcd. for $C_{112}H_{175}N_{27}O_{43}$ $[M+Na]^+$ 2609.2, found 2609.3. HR-ESI-MS: *m/z* calcd. for $C_{112}H_{175}N_{27}O_{43}$ (monoisotopic mass 2586.2337): $[M+3H]^{3+}$ 863.0852, found 863.0851. RP-HPLC: (gradient from 100% to 50% eluent **A** in 30 min, 25 °C): t_R = 14.3 min, determined purity 99%.

(Gal,Gal)[2,5]-6 (**22**): Negative control **22** exhibits galactose units of an α/β-mixture (3:1). ^{1}H NMR (600 MHz, D_2O): δ = 8.38-8.34 (m, 4H, N=N-N-C*H*), 7.97-7.91 (m, 2H, *p*-H_{Ph}), 7.81-7.75 (m, 4H, *o*-H_{Ph}), 5.00 (d, ^3J = 1.4 Hz, 1H, Gal-H1*α*), 4.74-4.65 (m, 8H, N=N-N-CH_2), 4.45 (d, ^3J = 7.4 Hz, Gal-H1*β*), 4.39-4.32 (m, 3H, N=N-N-CH$_2$-CH_2), 4.19-4.12 (m, 4H, N=N-N-CH$_2$-CH_2), 4.08-4.01 (m, 3H, N=N-N-CH$_2$-CH_2, Gal-H2*α*), 3.92 (d, *J* = 3.2 Hz, 4H, Gal-H2*β*), 3.79-3.12 (m, 89H, Gal-H2, Gal-H4, Gal-H5, Gal-H3, Gal-H6, CH_2-O-(CH_2)$_2$-O-CH_2, N-CH_2-CH_2-NH, O=C-NH-CH_2-CH$_2$-O), 2.76-2.68 (m, 4H, O=C-CH_2-CH_2-C=O), 2.52-2.47 (m, 20H, O=C-CH_2-CH_2-C=O), 1.94-1.92 (m, 3H, O=C-CH_3). MALDI-TOF-MS: *m/z* calcd. for $C_{112}H_{175}N_{27}O_{47}$ $[M+Na]^+$ 2673.2134, found 2673.294. HR-ESI-MS: *m/z* calcd for $C_{112}H_{175}N_{27}O_{47}$ (monoisotopic mass 2650.2134): $[M+3H]^{3+}$ 884.4117, found 884.4114. RP-HPLC: (gradient from 100% to 50% eluent **A** in 30 min, 25 °C): t_R = 12.9 min, 13.1 min, 13.3 min, determined purity 99%.

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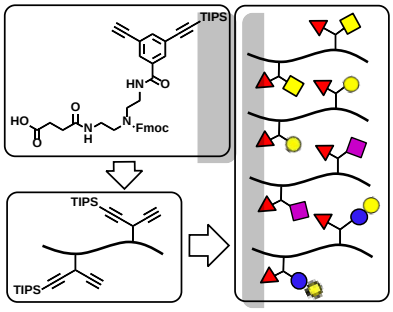

Heteromultivalent glycooligomers are synthesized by solid phase assembly using a building block with a free alkyne and a TIPS-protected alkyne moiety enabling the subsequent attachment of different carbohydrate motifs by on-resin copper mediated azide alkyne cycloaddition reactions. Different fragments of blood group antigens are thereby conjugated onto a macromolecular scaffold and investigated for their binding to bacterial lectin LecB.

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