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PAPER

Multifunctional multivalency: a focused library of polymeric cholera toxin antagonists†

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Structural pre-organization of the multivalent ligands is important for successful interaction with multimeric proteins. Polymer-based heterobifunctional ligands that contain pendant groups prearranged into heterodimers can be used to probe the active site and surrounding area of the receptor. Here we describe the synthesis and activities of a series of galactose conjugates on polyacrylamide and dextran. Conjugation of a second fragment resulted in nanomolar inhibitors of cholera toxin, while the galactose-only progenitors showed no detectable activity.

Introduction

Discovery of novel ligands that can disrupt the binding of proteins to their target receptors is an important branch of medicinal chemistry. Rational design is based on structural information previously obtained for known complexes, whereas, activity screening of available compound libraries or ligands obtained by combinatorial chemistry relies on chance and high throughput techniques. A fragment-based approach attempts to capitalize on serendipity and design by incremental improvement of the lead inhibitor *via* attachment of moieties that, individually, bind only weakly to adjacent areas on the protein surface but, together, substantially enhance the potency of composite ligands.[1] The fragment-based approach seems particularly appropriate for carbohydrate-binding proteins,[2–4] where low-molecular-weight inhibitors have traditionally met with limited success. Weak intrinsic binding constants for native ligands often necessitates aggregation of lectins into multimeric assemblies in order to perform their function. Functional multivalency is usually expected from the carbohydrate ligands to achieve biologically relevant strength of interaction. New methods that allow facile identification of non-competitive weak binding moieties are required. We propose here a fragment-based ligand discovery approach that takes advantage of multivalency in the early phase of the drug development process.

Cholera is a severe diarrheal disease that is caused by consumption of food and water contaminated with the bacterium *Vibrio cholerae*. Treatment for *Vibrio cholerae* consists of fluid replacement therapy to restore electrolyte balance[5] and, in severe cases, administration of antibiotics. Concern with growing antibiotic resistance[6–8] encourages the investigation of alternative therapies that exert less evolutionary pressure, for example, by targeting bacterial adhesion and colonization or deactivation of bacterial toxins. Cholera toxin (CT), an AB_5 toxin, is the most important virulence factor responsible for the disease. The A subunit is an enzyme that activates adenylate cyclase and elevates the level of cyclic AMP, which in turn affects ion transport across the cell membrane and upsets osmotic balance within the intestine leading to diarrhea.[9,10] The B subunit (CTB) binds to the surface of the apical side of epithelial cells in the host intestines and facilitates endocytosis and intracellular transport of the A subunit. The principle receptor of CT is the complex glycolipid GM_1 (Gal-β(1-3)-GalNAc-β(1-4)-[NeuNAc-α(2-3)]-Gal-β(1-4)-Glc-β(1-3)-ceramide) but other gangliosides (GM_2, GD_{1b}) have also been shown to bind CT.[11]

A variety of approaches have been pursued in the search for potent inhibitors of CT. The highest activities were attained *via* multivalent presentation of various carbohydrate ligands. A decameric Starfish-type inhibitor[12] applied to cholera toxin was reported by Zhang *et al.*[13] Octavalent GM_1 dendrimer derivatives exhibited exceptionally high activity[14] but more surprisingly a simple β-galactopyranoside dendrimer constructed by similar chemistry showed activity comparable to GM_1.[15] Several reviews have described progress in this area.[16–20]

Our research is aimed at development of effective ligands that would bind CT and could be orally administered to patients to reduce toxin burden during *Vibrio cholerae* infection. Here we report a novel approach for the design and synthesis of a small library of heterobifunctional ligands conjugated to a polymer carrier. A polymer carrier provides a framework that facilitates synthesis, purification and biological assay of the library members. This polymer scaffold is also necessary for multivalent presentation of the ligands to achieve high avidity for the homopentameric protein, CTB. In the case of gastrointestinal infections when a drug does not need to cross biological barriers in order to engage its target, the high molecular weight of the polymeric ligand might be beneficial to ensure noninvasiveness of anti-toxin

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† Electronic supplementary information (ESI) available: NMR and IR spectra, solid-phase assay data for inhibition of heat-labile enterotoxin (LT). See DOI: 10.1039/c0ob01089h

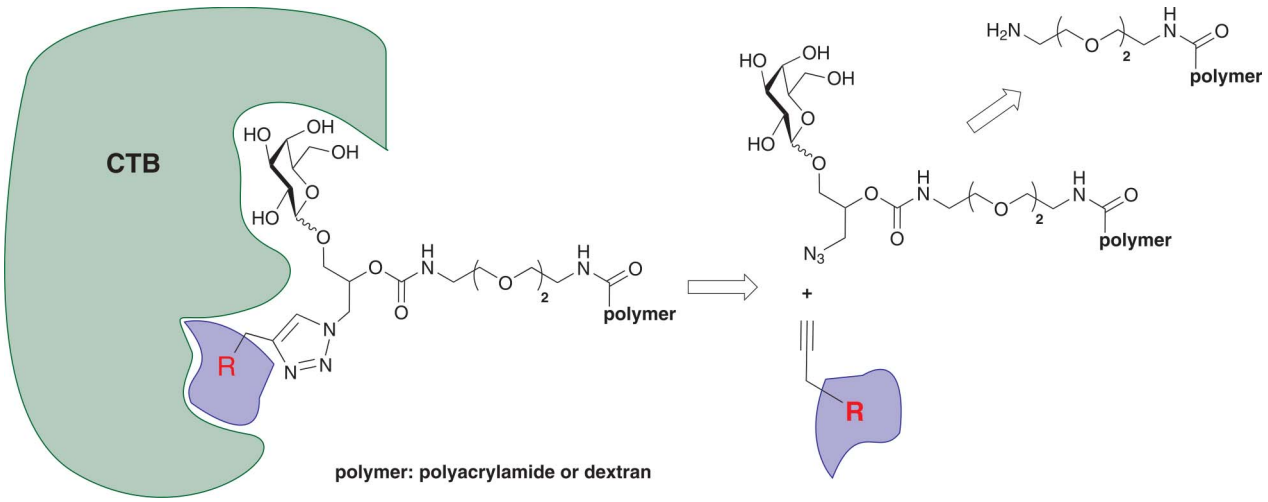


Fig. 1 General design of a focused library of heterobifunctional polymers.

therapy. In this study we report the generation of a polymer-based diversity-oriented compound library, in which the pendant ligands contain an invariable anchor fragment, galactose, and variable non-galactose fragments, which probe the second part of the GM_1 binding site that normally accommodates neuraminic acid (Fig. 1).

Results

Synthesis of aminated polymers

Two polymers were used in this study: polyacrylamide was employed for facile synthesis and screening for activity of the library compounds and dextran was used in combination with select derivatives as a prototype of a clinically acceptable polymer for *in vivo* trials and future drug development (Scheme 1).

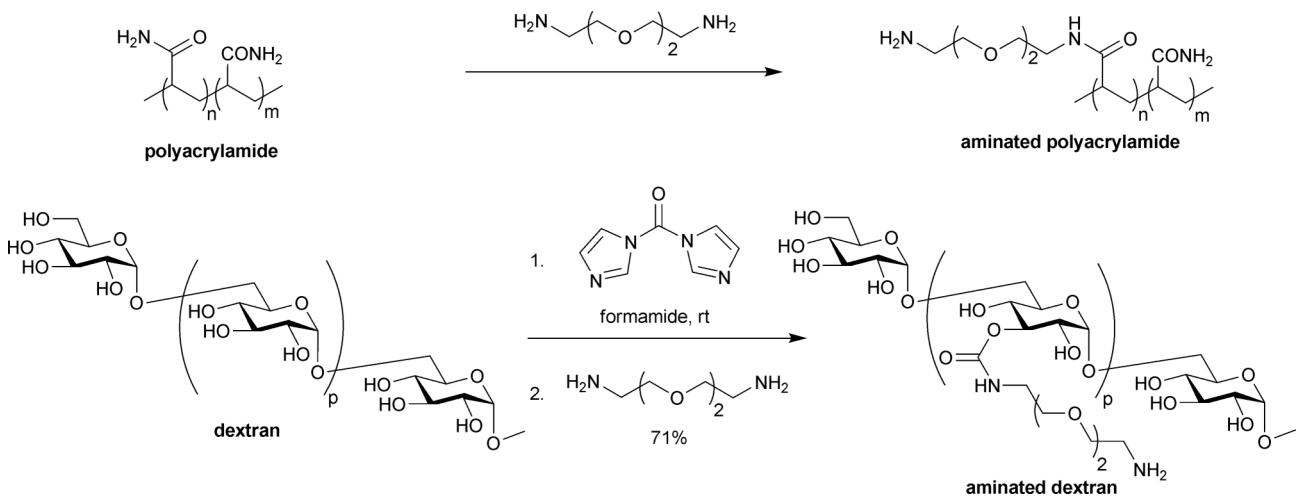
Polyacrylamide was rendered amenable for conjugation by partial *trans*-amination of amide side chains at 80 °C, the procedure is analogous to that previously described for *trans*-amination of cross-linked polyacrylamide.[21] Increased incubation times led to increasing content of amino groups in the polymer. Up to 10% NH_2 per repeat unit can be achieved without significant loss due to cross-linking and gel formation. Degree of incorporation was deduced from integration of appropriate signals in NMR spectra.

For functionalization of dextran we followed a two-step procedure described by Lukyanov *et al*[22] involving first activation of the polysaccharide with carbonyl diimidazole then introduction of the linker in the form of a diamine derivative.

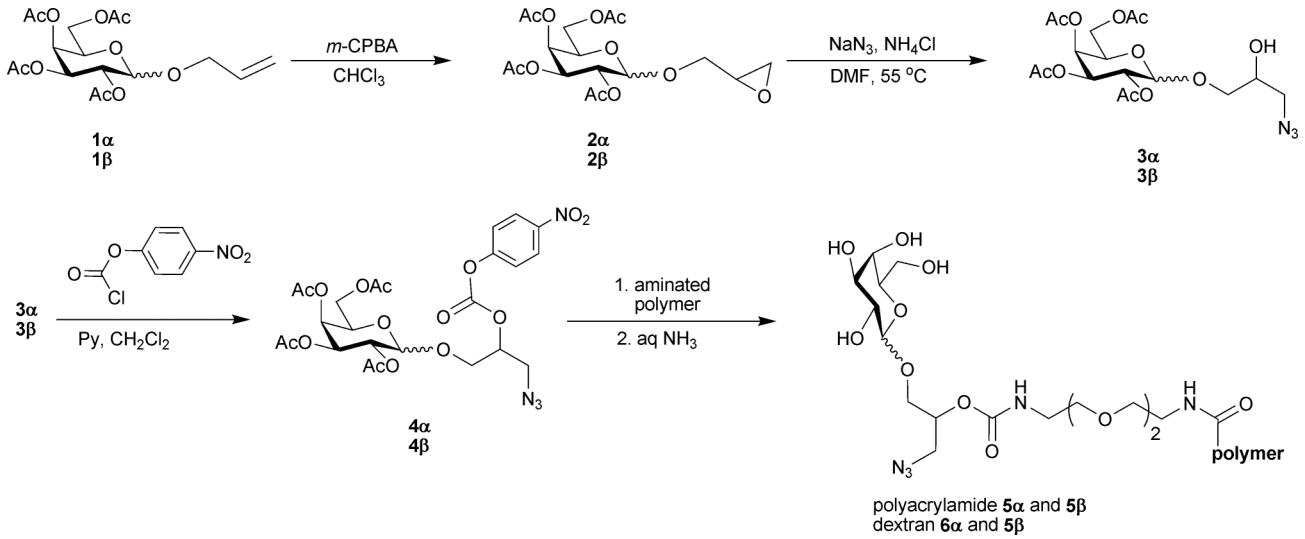
Synthesis of heterobifunctional polymers containing α-Gal and β-Gal moieties

Two series of galactopyranose derivatives were synthesized in uniform fashion starting from the corresponding peracetylated allyl glycosides (Scheme 2). Treatment of **1α** and **1β** with m-CPBA gave the corresponding epoxides **2α** and **2β**, the oxirane ring of which was opened with azide to provide 3-azido-3-deoxy-glycerol derivatives **3α** and **3β**. Activation of the hydroxyl group as a 4-nitrophenyl carbonate gave **4α** and **4β**, which were conjugated to a polymer scaffold to afford key library precursors **5α**, **5β**, **6α**, and **6β**.

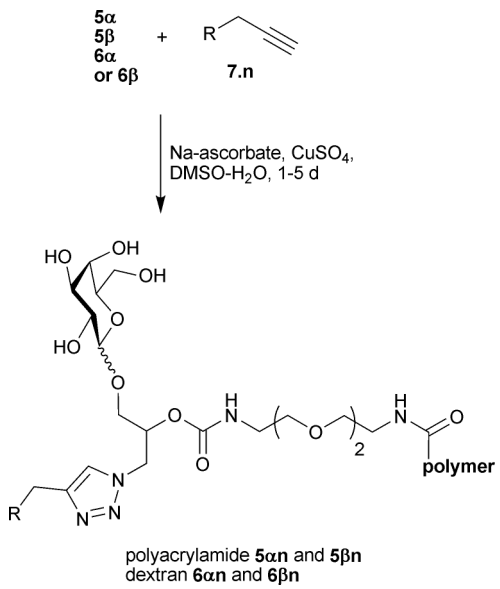
Introduction of the second component was achieved by conjugation of various propargyl derivatives under conditions of the copper-catalyzed cycloaddition "click chemistry" reaction[23]



Scheme 1 Preparation of aminated polymeric scaffolds, polyacrylamide and dextran.

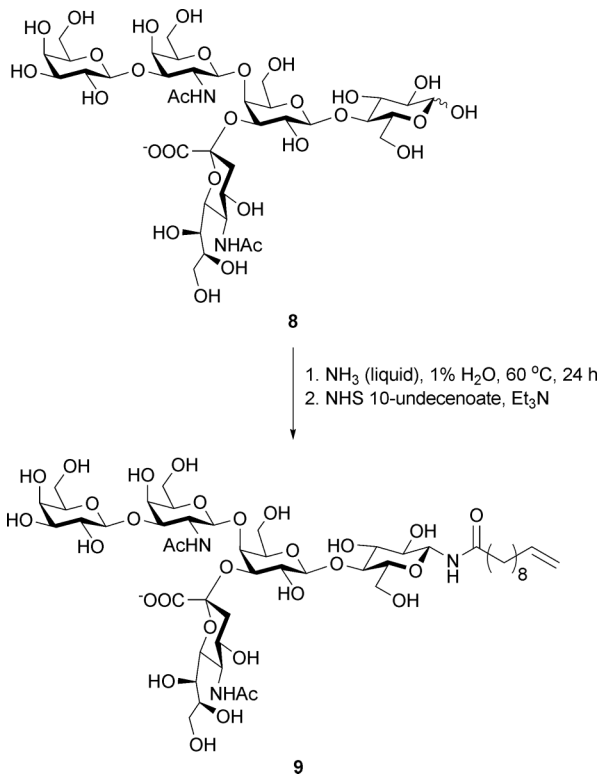


Scheme 2 Synthesis of galactoside containing polymeric precursors of heterobifunctional ligands. Identical procedures were implemented separately for α- and β-galactoside series.



Scheme 3 Conjugation of the second moiety by "click chemistry" reaction.

(Scheme 3). Completion of the reaction was confirmed by NMR spectra and the disappearance in the IR spectra of the characteristic band at 2100 cm^{-1} corresponding to the azide stretching frequency.



Scheme 4 Preparation of coating reagent **9** for solid-phase assay.

Evaluation of inhibitory activity

Enzyme-linked immunoassay (ELISA) was performed in 96-well plates coated with a GM_1 *N*-acylated glycoside **9**, which was synthesized from GM_1-oligosaccharide (GM_1OS) **8**[24] *via* amination with liquid ammonia followed by acylation with the NHS ester of 10-undecenoic acid (Scheme 4). We found that during amination of a reducing sugar in liquid ammonia addition of ~1% water improved the yield and accelerated the reaction approximately 10 fold.

CTB (Sigma) conjugated to horseradish peroxidase (HRP) was incubated in the wells in the presence of a serially diluted inhibitor. After washing with buffer the presence of bound CTB was detected by color reaction with 3,3′,5,5′-tetramethylbenzidine (TMB). As positive control, serially diluted **8** was included on every plate to ensure consistent assay conditions. For routine screening, one well per data point was used while assays for select compounds were performed in triplicates. The results are presented in Table 1.

Table 1 Inhibitory activities of heterobifunctional polymers against CT

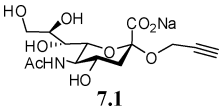
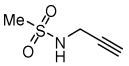
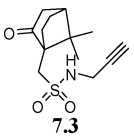
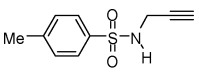
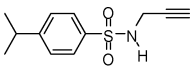
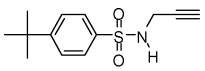
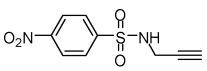
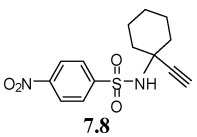
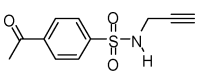
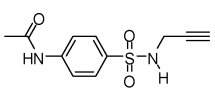
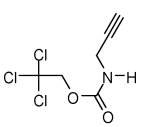
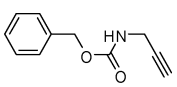
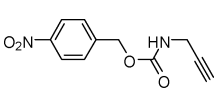
Alkyne derivative **7.n**	α-Gal polymer	IC_{50}, μM[a]	β-Gal polymer	IC_{50}, μM[a]
None	**5α**	Inactive	**5β**	Inactive
None	**6α**	Inactive	**6β**	Inactive
 **7.1**	**5α1**	828 (h^b = 1.0)	**5β1**	584 (h^b = 0.9)
 **7.2**	**5α2**	832 (h = 1.3)	**5β2**	1246 (h = 1.0)
 **7.3**	**5α3**	623 (h = 1.1)	**5β3**	630 (h = 0.8)
 **7.4**	**5α4**	1286 (h = 0.85)	**5β4**	629 (h = 0.9)
 **7.5**	**5α5**	Inactive	**5β5**	1934
 **7.6**	**5α6**	153 (h = 4.3)	**5β6**	122 (h = 3.3)
 **7.7**	**5α7**	339 (h = 1.5)	**5β7**	325 (h = 1.6)
 **7.8**	**5α8**	389 (h = 2.6)	**5β8**	230 (h = 2.1)
 **7.9**	**5α9**	200 (h = 3.8)	**5β9**	156 (h = 2.6)
 **7.10**	**5α10**	243 (h = 3.0)	**5β10**	225 (h = 3.0)
 **7.11**	**5α11**	80 (h = 1.8)	**5β11**	104 (h = 3.0)
 **7.12**	**5α12**	108 (h = 1.9)	**5β12**	124 (h = 3.1)
 **7.13**	**5α13**	100 (h = 2.7)	**5β12**	58 (h = 2.5)

Table 1 *(Contd.)*

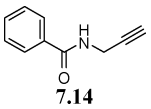
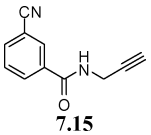
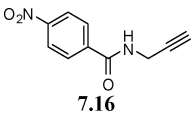
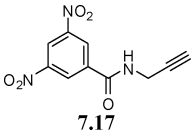
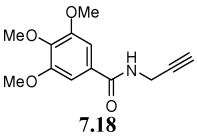
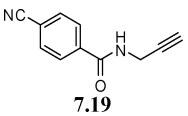
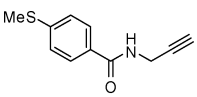
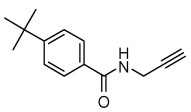
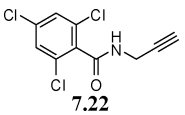
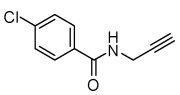
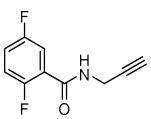
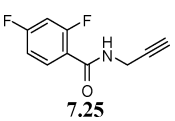
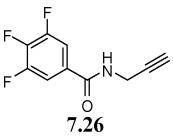
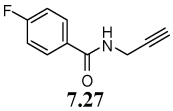
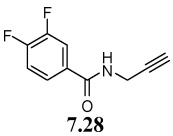
Alkyne derivative **7.n**	α-Gal polymer	IC_{50}, μM[a]	β-Gal polymer	IC_{50}, μM[a]
 **7.14**	**5α14**	0.24 (h = 0.4)	**5β14**	1.8 (h = 0.6)
 **7.15**	**5α15**	5.9 (h = 0.6)	**5β15**	5.2 (h = 0.5)
 **7.16**	**5α16**	45 (h = 1.4)	**5β16**	23 (h = 1.3)
 **7.17**	**5α17**	61 (h = 3.0)	**5β17**	53 (h = 2.2)
 **7.18**	**5α18**	103 (h = 3.5)	**5β18**	109 (h = 3.3)
 **7.19**	**5α19**	0.9 (h = 0.2)	**5β19**	0.023 (h = 0.4)
 **7.20**	**5α20**	0.3 (h = 0.3)	**5β20**	0.19 (h = 0.3)
 **7.21**	**5α21**	Inactive	**5β21**	1934
 **7.22**	**5α22**	209 (h = 2.5)	**5β22**	169 (h = 3.5)
 **7.23**	**5α23**	0.17 (h = 0.3)	**5β23**	0.02 (h = 0.3)
 **7.24**	**5α24**	31 (h = 2.3)	**5β24**	39 (h = 2.6)
 **7.25**	**5α25**	3.2 (h = 0.8)	**5β25**	9.0 (h = 0.45)

Table 1 *(Contd.)*

Alkyne derivative **7.n**	α-Gal polymer	IC_{50}, μM[a]	β-Gal polymer	IC_{50}, μM[a]
 **7.26**	**5α26**	8.2 (h = 0.7)	**5β26**	0.06 (h = 0.25)
 **7.27**	**5α27** (3%)	0.0055 (h = 0.3)	**5β27** (3%)	0.014 (h = 0.4)
	5α27 (5%)	0.0012 (h = 0.4)	**5β27** (5%)	0.013 (h = 0.2)
	5α27 (9%)	0.0014 (h = 0.7)	**5β27** (9%)	0.056 (h = 0.3)
	6α27 (6.6%)	0.005 (h = 0.3)	**6β27** (6.6%)	0.018 (h = 0.2)
 **7.28**	**5α28** (3%)	0.07 (h = 0.3)	**5β28** (3%)	0.02 (h = 0.3)
	5α28 (9%)	0.008 (h = 0.3)	**5β28** (9%)	0.01 (h = 0.3)
	6α28 (6.6%)	0.004 (h = 0.2)	**6β28** (6.6%)	0.024 (h = 0.3)

[a] activities per pendant ligand. [b] Hill coefficient.

Discussion

Due to the synthetic complexity of the native ligand, GM_1 oligosaccharide, several attempts to simplify the structure have been reported. Bernardi and co-workers have designed a number of conformationally restricted GM_1 mimetics, in which the core lactose moiety is replaced by a conformationally constrained *cis*-cyclohexanediol-1,2, thus, reducing synthetic complexity somewhat while relying on both Gal and Neu for the interaction.[25] Recently, they evaluated a large set of non-hydrolysable Gal-Neu dimers.[26] Hol and Fan have discovered *m*-nitrophenyl galactoside (MNPG) to be about 50–100 fold more potent than galactose.[27]

However, since none of the unimeric mimics approached the activity level of GM_1OS (100–200 nM) most researchers resort to multivalency for the construction of viable inhibitors.[13–20] The crystal structure of the CTB-GM_1OS complex shows that the nearest bound ligands are separated by ~31 Å as measured between the anomeric oxygen atoms of the terminal galactose. Therefore, spanning this distance requires long linker-arms making multivalent display rather challenging though rewarding considering the substantial activity enhancements that could be attained. In recent years, a variety of dendrimers and polymers bearing ligands as simple as galactose monosaccharide[15] to ligands as complex as the GM_1 pentasaccharide[14,28] were employed to achieve up to subnanomolar inhibitors; albeit very high activities were only observed with pendant GM_1 pentasaccharide ligands. Tailored multivalent ligands possessing the same 5-fold symmetry as CTB were shown to attain activities that approach that of GM_1OS even though just a simple monosaccharide, meta-nitrophenyl α-galactopyranoside (MNPG), was used as specific ligand.[13]

In our study, we needed a multivalent platform for generation and screening of focused compound libraries. Polymers provide convenient albeit less structurally defined scaffolds for multimeric display of a ligand. The separation distance between pendant ligands is tunable by controlling the degree of incorporation of side chains. Polymer-supported synthesis is often used for generation of compound libraries.[29–32] We propose to employ a polymeric support both to facilitate synthesis and biological assays of novel ligands. Since the basic anchoring ligand was only a monosaccharide a multivalent scaffold was also necessary to permit detection of weak activities.

High solubility in water and ease of modifications established polyacrylamide as a standard polymeric carrier used in studies on multivalency.[33–35] However, limited solubility of polyacrylamide in organic solvents imposes substantial limitations to the scope of chemical modifications. Since dipolar cycloaddition of alkynes to azides known as "click chemistry" is one of a few reactions suitable for efficient conjugation in aqueous solutions, we utilized it to generate a library of pendant ligands. Both α-Gal and β-Gal glycosides **3α** and **3β** were used as an invariant pendant ligand in the corresponding series of heterobifunctional inhibitors. The mixture of water and DMSO was found to be an appropriate media for performing conjugation of a large variety of alkyne derivatives under homogeneous conditions.

The original polymers **5α** and **5β** that are devoid of the variable fragment failed to inhibit cholera toxin at 10 mg mL^{-1} and were considered inactive under the assay conditions. Surprisingly, both hetero-bifunctional ligands **5α1** and **5β1** containing neuraminic acid as a complementary ligand have shown only partial inhibition at the same high concentration. The length of the linker is sufficient for both Gal and Neu to reach their respective positions in the GM_1 binding site on the surface of cholera toxin; however, entropy loss due to linker flexibility offsets the contribution from this additional interaction.

The galactose moiety occupies the deepest indentation in the GM_1 binding pocket on the surface of cholera toxin.[36] Thermodynamic analysis of the CTB-GM_1 interaction[37] identified galactose as the keystone for affinity and selectivity albeit with intrinsic dissociation constant of ~50 mM. The other important fragment, neuraminic acid, which binds with K_d 210 mM, would

have never been selected as a lead ligand in fragment-based design of inhibitors. A large negative enthalpy of neuraminic acid binding to CTB is largely offset by an almost equal and unfavourable entropy term (negative entropy). Nevertheless, essentially only these two monosaccharides, galactose and neuraminic acid, contribute substantially to formation of the GM_1-CTB complex, a lectin-carbohydrate complex with one of the highest known intrinsic affinity constants. The rest of the GM_1 pentasaccharide forms a scaffold that severely restricts the conformational space sampled by Gal and Neu in solution[37] and, thus, greatly reduces the entropic penalties that GM_1 has to pay upon binding to the protein. Recently reported CTB inhibitors containing Gal and Neu connected *via* flexible linkers show modest activities in mM range,[26] again underscoring the importance of optimal rigid scaffold.

Most of the sulfonamide derivatives (**5α2–5α10** and **5β2–5β10**) that were generated from arbitrarily chosen, commercially available sulfonyl chlorides had a modest contribution to binding, while some carbamate analogs (*i.e.* **5β12**) exceeded the activity of neuraminic acid derivative by 10 fold. Further activity improvements were observed when substituted benzamides **5α14–5α28** and **5β14–5β28** were tested. For several compounds, IC_{50} values in the low nanomolar region were attained.

While activities of most compounds in two parallel series were not affected by the anomeric form of the galactopyranose residue, some analogs showed a substantial difference. Of these, the β-analogs had substantially higher activity. An important exception, the α-Gal analog of *p*-fluorobenzamide derivative, had superior inhibitory power not only with respect to the β-counterpart but generally to all tested compounds. This, in some cases dramatic, difference between α- and β-series is indicative of specific interactions of the variable ligand with the protein surface.

In an attempt to further increase activities of the multivalent ligands presenting mono- and difluorobenzamide moieties we explored the influence of pendant ligand density. Generally, no significant avidity gains were observed for polymers with a higher payload of heterobifunctional head groups (Table 1, entries **5.27** and **5.28**). This modification caused the solubility of the polymers to be substantially decreased compared to the standard 3% payload on polyacrylamide. Activities of dextran-based polymers were generally similar to polyacrylamide analogs (Table 1, entries **6.27** and **6.28**). The greater water solubility of dextran over polyacrylamide and its biodegradability renders dextran-based heterobifunctional ligands promising drug candidates in future biological evaluation.

The multivalent format for measuring activities permits higher discrimination between pendant ligands with similar affinity due to amplification of small differences in binding energies. What is rarely discussed though is that the multistage binding of multivalent ligands may result in a staggered shape of the inhibition curve and apparent decrease in Hill coefficients. Curiously, when data from the Table 1 are plotted as a scatter graph, a bell shaped correlation between Hill coefficients and avidity of the polymeric inhibitors could be observed (Fig. 2). It appears that the shape of the binding curve depends not on the nature of the polymeric scaffold or the pendant ligand but solely on the range of IC_{50} value. Very sharp inhibition curves are observed for midrange

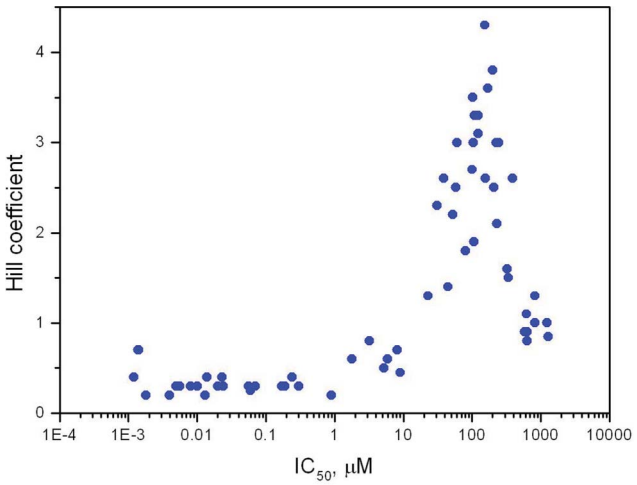


Fig. 2 Bell-shape relationship between avidities and Hill coefficients.

activities (Fig. 3, **5β12**), while high and low avidity ligands show weak concentration dependence. Moreover, the highest avidity ligands present even more complex shape of binding curves: part of the curve corresponding to low concentration of inhibitor can be fitted to near perfect log function with a Hill coefficient close to unity and higher. However, when approximately 80–90% inhibition is attained the second, extremely shallow part of the curve begins and complete inhibition is finally attained at high μM concentrations. Several derivatives of **7.27** and **7.28** have also been tested for inhibitory activity against heat labile toxin (LT), a toxin homologous to CT and produced by enteropathogenic *E. coli* (ESI,† Table 1S). Activities in the picomolar range were observed for some compounds with Hill coefficients close to unity. However, although less pronounced, an inhibitory profile (resistance to complete inhibition after reaching a certain limit) similar to that with CT was observed.

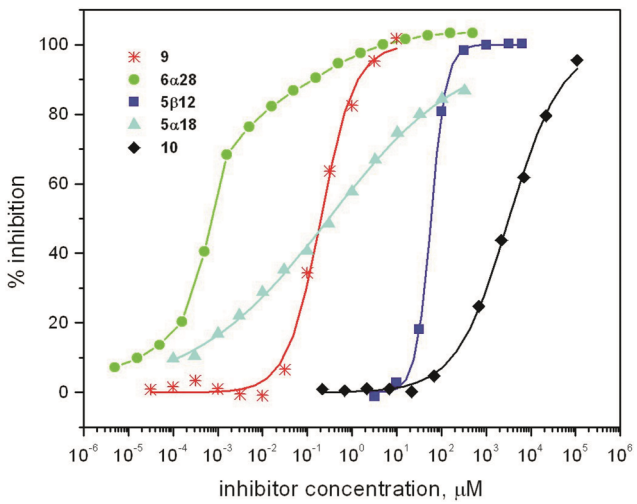


Fig. 3 Representative examples of solid-phase inhibition data.

Increase in Hill coefficients with increase in avidity followed by some reduction of their value upon further avidity increase

was previously observed for dendrimer-based ligands for Cholera toxin on a limited set of inhibitors,[14] the present work may permit generalization of this phenomenon. As a tentative explanation for the observed tendency we propose to rationalize reduction of Hill's coefficients in terms of a multistep binding of CTB to the plate due to incomplete saturation of multiple binding sites by the flexible polymeric ligand. At low concentrations of the polymeric ligand, only one polymer chain binds to the pentameric toxin. Since the intrinsic binding constant for the pendant ligands is low, the intramolecular binding results in only partial saturation of the binding sites. Therefore, the initial part of the inhibition curve appears as a normal log curve but reaches a limit corresponding to 1 : 1 complex formation. At this point no free toxin is left. The second phase requires inhibition of the binding of 1 : 1 complex between toxin and the single chain of a multivalent inhibitor. The binding of the second chain of the inhibitor to this complex is less efficient since fewer binding sites are available for this interaction. As a result, the complete inhibition of toxin occurs at substantially higher concentration of the inhibitor. The partially inhibited species of CTB are able to bind to the plate *via* binding sites that remain free and, after washing off the ligand, a fraction of these weakly bound toxin species rebinds *via* the liberated binding sites and remains on the plate. This process may be facilitated by essentially irreversible deposition of the toxin to a GM_1 coated plate as revealed by detailed investigation of ELISA conditions.[38]

The idealized mechanism, a process involving only two steps, is illustrated in Fig. 4; the higher heterogeneity of the real polymeric ligand would result in more staggered binding phases and less distinct transitions than shown in Fig. 3. Two step inhibition due to significant interaction of toxin species with partially saturated binding sites on a GM_1-coated plate results in the staggered shape of the inhibition curve. With these caveats, the ELISA assay is clearly suitable for ranking multivalent inhibitors according to their relative potencies. The uniform preparation enables direct comparison of affinities in a multivalent format instead of measuring low intrinsic binding constants of unimeric ligands.

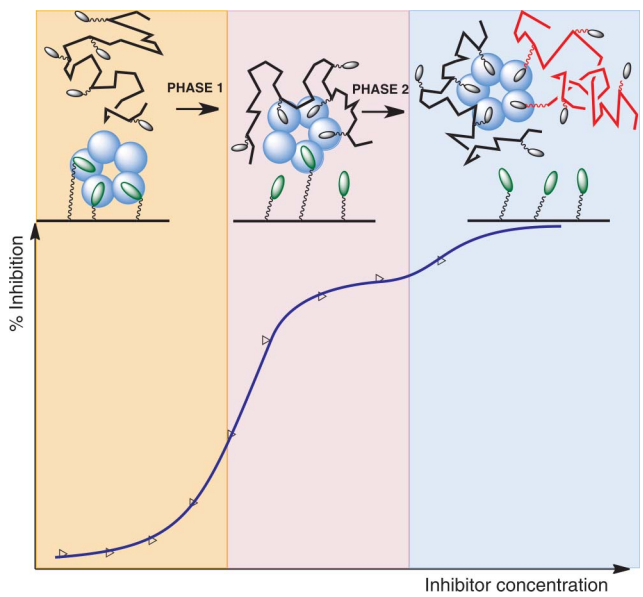


Fig. 4 Proposed mechanism of sequential inhibition of multivalent proteins by polymeric inhibitors.

Two pendant hetero-bifunctional ligands, which possessed the higher inhibitory power when presented on polyacrylamide, were evaluated as univalent analogs **10** and **11**. They show approximately 100-fold higher activities than galactose measured in a similar solid phase assay and compare well with a benchmark univalent ligand, MNPG, which also contains only a monosaccharide binding fragment (Fig. 5). The apparent 10-fold discrepancy in the activities of MNPG as reported in literature[39] and obtained in this work could be explained by different solid-phase assay conditions, particularly, the different ganglioside, GD_{1b}, previously employed as the solid phase capture reagent for cholera toxin. This ligand is known to have substantially weaker affinity for the toxin than GM_1.[11]

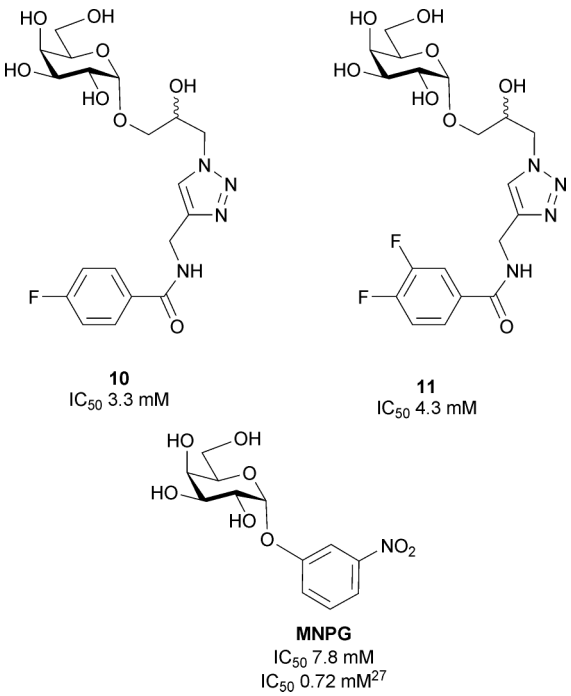


Fig. 5 Inhibitory activities of unimeric analogs.

Conclusions

A polymer-supported focused library of hetero-bifunctional ligands assisted in identifying weak ligands that have the potential to complement the affinity of galactose for cholera toxin by binding to a complementary binding site on the protein surface. The choice of the linker separating Gal from the second fragment was dictated by synthetic expediency and was not intended to provide a comparable level of structural pre-organization to that seen in GM_1. The relatively high activities were achieved here *via* multivalent presentation rather than as the result of high intrinsic binding constants, which explains the shallow shape of the inhibition curves and difficulty to attain complete inhibition of CTB binding to GM_1-coated plates. Optimization of the heterobivalent scaffold should be the next step in search for potent cholera toxin antagonists.

Experimental

General methods

Commercially available reagents were used as supplied without further purification. Evaporation and concentration *in vacuo* was conducted under water-aspirator pressure. All solution-phase reactions were carried out under a nitrogen atmosphere. Reactions were monitored by analytical thin-layer chromatography (TLC) with pre-coated silica gel 60 F_{254} glass plate (Merck). Plates were visualized under UV light or stained by treatment with either cerium ammonium molybdate solution or 5% sulfuric acid in ethanol followed by heating at 180 °C. Purification of products was conducted by column chromatography using *silica gel* SiliaFlashF60 (40–63 μm, 60 Å) from *SiliCycle® Inc*. Optical rotations were measured with a Perkin Elmer 241 polarimeter at 22 °C. Melting points (mp) were determined on a Gallenkamp melting point apparatus and uncorrected. IR data were recorded on *Nic-Plan IR Microscope* (solid film); only signals corresponding to functional groups indicative to the structure are reported. NMR spectra were recorded at 400, 500 or 600 MHz, at 27 °C in $CDCl_3$ or D_2O. Coupling constants are first order and chemical shifts are referenced to residual solvent ($CDCl_3$) at 7.24 ppm for ^1H and 77.0 ppm for ^{13}C and relative to 0.1% external acetone at 2.225 ppm for ^1H for solution in D_2O. Electrospray ionization mass spectra were recorded on a Micromass Zabspec TOF-mass spectrometer.

(*R*,*S*)-3-azido-2-hydroxypropyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (3α). A suspension of (*R*,*S*)-2,3-epoxypropyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside[40] (**2α**, 3.41 g, 8.43 mmol), NaN_3 (1.64 g, 25.3 mmol), NH_4Cl (1.35 g, 25.3 mmol) in dry DMF (20 mL) was stirred at 55 °C for 7.5 h then cooled and diluted with water (150 mL). The aqueous solution was extracted with EtOAc (4 × 50 mL). The combined organic layers were washed with saturated aqueous NH_4Cl (2 × 100 mL), brine (1 × 100 mL), dried over anhydrous $mgSO_4$ and filtered. After the removal of solvent, the resulting residue was purified by flash chromatography, eluting with EtOAc–CH_2Cl_2 (35 : 65), to yield **3α** (2.67 g, 71%) as a colourless, oily mixture of diastereomers: $[\alpha]_D$ +118.05 (c 1.04, $CHCl_3$); IR (KRS-5 disk) ν 2098 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ 5.46 (dd, 1 H, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ 1.2 Hz, H-4), 5.33 (dd, 1 H, $J_{3,4}$ 3.4 Hz, $J_{2,3}$ 10.6 Hz, H-3), 5.20–5.13 (m, 2 H, H-1, H-2), 4.30–4.24 (m, 1 H, H-5), 4.12–3.34 (m, 7 H, H-6a, H-6b, CH_2O, CH_2N, C*H*OH), 2.52 and 2.45 (2d, 1 H total, OH), 2.15 (s, 3 H, OAc), 2.08 (2 s, 3 H total, OAc), 2.06 (2 s, 3 H total, OAc), 2.00 (s, 3 H, OAc), ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.49, 170.48, 170.26, 170.24, 170.18, 170.06, 97.15, 96.92, 70.42, 70.36, 69.46, 69.45, 68.01, 68.00, 67.98, 67.96, 67.47, 67.42, 66.78, 66.69, 61.83, 61.79, 53.36, 53.08, 20.75, 20.74, 20.69, 20.65, 20.63; ES HRMS: calcd for $C_{17}H_{25}N_3O_{11}Na$ ([M + Na]$^+$) 470.13813, found 470.13795.

(*R*,*S*)-3-azido-2-hydroxypropyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (3β). The title compound **3β** (58%) was obtained from **2β**[40] as described for **3α**. $[\alpha]_D$ +7.76 (c 1.02, $CHCl_3$); IR (KRS-5 disk) ν 2103 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ 5.40 (dd, 1 H, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 1.0 Hz, H-4), 5.21 (dd, 1 H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 10.5 Hz, H-2), 5.03 (dd, 1 H, H-3), 4.51 (2 : 1 mixture of d, 1 H total, H-1), 4.16 (m, 2 H, CH_2), 3.97 (m, 1 H, H-5), 3.93–3.72 (m, 3 H, CH_2O, C*H*OH), 3.40–3.30 (m, 2 H, CH_2N), 2.98 and 2.81 (2 : 1 mixture of d, 1 H total, OH), 2.17 (2 s, 3 H total, OAc), 2.08 (m, 6 H, OAc), 2.00 (s, 3 H total, OAc); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.46, 170.43, 170.16, 170.07, 170.06, 169.61, 169.57, 101.97, 101.82, 72.83, 72.20, 71.04, 71.02, 70.71, 70.70, 69.75, 69.60, 68.80, 68.77, 66.98, 66.95, 61.49, 61.43, 53.10, 53.0, 20.77, 20.644, 20.640, 20.63, 20.62, 20.55; ES HRMS: calcd for $C_{17}H_{25}N_3O_{11}Na$ ([M + Na]$^+$) 470.13813, found 470.13824.

(*R*,*S*)-3-azido-2-(4-nitrophenyloxycarbonyl)oxypropyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (4α). To a stirred solution of alcohol **3α** (2.53 g, 5.66 mmol) and *p*-nitrophenyl chloroformate (1.37 g, 6.79 mmol) in dry CH_2Cl_2 (35 mL) pyridine (0.69 mL, 8.5 mmol) was added dropwise at room temperature. The reaction mixture was stirred at room temperature for 1.5 h. After the removal of solvent, the resulting residue was purified by flash chromatography, eluting with EtOAc–hexane (1 : 1) to yield **4α** (3.28 g, 97%) as a colourless solid: mp 42–44 °C; IR (KRS-5 disk) ν 2109 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.34–8.27 (m, 2 H, arom.), 7.47–7.40 (m, 2 H, arom.), 5.47 (m, 1 H, H-4), 5.37–5.31 (m, 1 H, H-3), 5.22–5.01 (m, 3 H, H-1, H-2, CH), 4.29–4.20 (m, 1 H, H-5), 4.13–3.59 (m, 6 H, H-6a, H-6b, CH_2O, CH_2N), 2.15–2.01 (m, 12 H, OAc); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.40, 170.37, 170.35, 170.31, 170.13, 170.03, 169.99, 155.22, 155.19, 151.87, 151.84, 145.70, 145.64, 125.42, 125.40, 121.88, 121.77, 96.88, 96.65, 76.08, 76.05, 67.99, 67.96, 67.86, 67.84, 67.32, 67.30, 66.84, 66.72, 66.29, 61.62, 61.60, 50.45, 50.19, 20.71, 20.70, 20.67, 20.65, 20.63; ES HRMS: calcd for $C_{24}H_{28}N_4O_{15}Na$ ([M + Na]$^+$): 635.14434, found: 635.14460.

(*R*,*S*)-3-azido-2-(4-nitrophenyloxycarbonyl)oxypropyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (4β). The title compound **4β** (96%) was obtained from **3β** as described for **4α** as a colourless solid: mp 50–52 °C; IR (KRS-5 disk) ν 2107 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.34–8.28 (m, 2 H, arom.), 7.49–7.41 (m, 2 H, arom.), 5.42 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 5.27–5.22 (m, 1 H, H-2), 5.09–5.04 (m, 2 H, H-3, CH), 4.57–4.54 (m, 1 H, H-1), 4.20–4.09 (m, 3 H), 3.97–3.94 (m, 1 H, H-5), 3.86–3.82 (m, 1 H), 3.67–3.58 (m, 2 H), 2.18 (s, 3H, OAc), 2.09–2.04 (m, 6 H, OAc), 2.01 (s, 3H, OAc); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.38, 170.14, 170.07, 169.54, 169.47, 155.29, 155.24, 151.81, 145.62, 125.37, 121.86, 121.79, 101.73, 101.14, 76.25, 76.09, 71.01, 70.99, 70.65, 70.59, 68.55, 68.41, 67.41, 67.08, 66.90, 61.20, 50.47, 50.42, 20.73, 20.65, 20.56; ESI HRMS: calcd for $C_{24}H_{28}N_4O_{15}Na$ ([M + Na]$^+$): 635.14434, found: 635.14472.

Synthesis of *trans*-aminated polyacrylamide

To a solution of polyacrylamide (10 g for payload 3% and 5%, 5 g for payload 9%) in water (50 mL) 2,2′-(ethylenedioxy)bis(ethylamine) (50 mL) was added and the reaction mixture was stirred at 80 °C for 20 min (for payload 3%), 40 min (for payload 5%), or 90 min (for payload 9%) then heat was removed and ice was added. The mixture was extensively dialyzed against distilled water then freeze-dried to give colourless powder.

Synthesis of galactoside azidopolyacrylamide 5α and 5β

To a viscous solution of *trans*-aminated polyacrylamide (1.00 g) in water (13 mL) a solution of carbonates **4α** or **4β** (0.60 g, 1.0 mmol) in DMSO (8 mL) was added dropwise at room temperature followed by Et_3N (0.15 mL). The reaction mixture was tumbled at

room temperature for 3 days. Aqueous NH_4OH (3 mL) was added and the reaction mixture was tumbled for 1 day then extensively dialyzed against water and lyophilized to yield colourless foam. IR confirmed incorporation of azido groups, and ^1H NMR showed clear peaks of the galactose moiety on the polymer. The payload was determined by integration of appropriate galactose and polymer signals.

α-Galactoside azidopolyacrylamide 5α: colourless foam (71%). IR (KRS-5 disk) ν 2106 cm^{-1}.

β-Galactoside azidopolyacrylamide 5β: colourless foam (79%). IR (KRS-5 disk) ν 2106 cm^{-1}.

Synthesis of aminated dextran

To a stirred, clear solution of dextran (M_n ~100–200 kDa, 2.00 g, 12.3 mmol of glucose) in formamide (50 mL) a solution of 1,1′-carbonyldiimidazole (0.50 g, 3.1 mmol) in formamide (15 mL) was added at room temperature. The reaction mixture was stirred at room temperature for 6 h then it was added dropwise 2,2′-(ethylenedioxy)bis(ethylamine) (135 mL). The reaction mixture was stirred at room temperature overnight, and extensively dialyzed using dialysis tube (MWCO 12 000–14 000) for 3 d, and lyophilized to yield aminated dextran (1.52 g, 72%) as a colourless foam. ^1H NMR is consistent with the conjugate structure.

Synthesis of galactoside azidodextran 6α and 6β

To a solution of aminated dextran (200 mg, 70 μmol) in water (2 mL) a solution of carbonate **4α** or **4β** (85 mg, 140 μmol) in DMSO (8 mL) was added at room temperature followed by Et_3N (0.15 mL). The reaction mixture was tumbled at room temperature for 3 d then aqueous NH_4OH (3 mL) was added and the mixture was tumbled for 24 h then dialyzed extensively against water using dialysis tube (MWCO 12 000–14 000) for 3 d, and lyophilized to yield colourless foam. IR confirmed incorporation of azido groups, and ^1H NMR showed clear peaks of the galactose moiety on the polymer.

α-Galactoside azidodextran 6α: colourless foam (95%). IR (KRS-5 disk) ν 2105 cm^{-1}.

β-Galactoside azidodextran 6β: colourless foam (100%). IR (KRS-5 disk) ν 2107 cm^{-1}.

(Prop-2-ynyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid)onic acid (7.1). To a solution of methyl (prop-2-ynyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosid)onate[41] (31.8 mg, 60.0 μmol) in MeOH (1 mL) aqueous 1.5 M NaOH (0.3 mL) was added. The mixture was stirred at room temperature for 24 h, DOWEX (H^+) was added and the mixture was concentrated, taken up in water and freeze-dried to give the title compound (9.8 mg, 95%) as a colourless foam; mp > 250 °C (dec. upon heating); IR (solid) ν 2126 cm^{-1}; ^1H NMR (400 MHz, D_2O): δ 4.37 (d, 1 H, J_{gem} 16 Hz CH_2), 4.23 (d, 1 H, J_{gem} 16 Hz, CH_2), 3.93–3.55 (m, 7 H, H-4, H-5, H-6, H-7, H-8, H-9a, H-9b), 2.73 (dd, 1 H, $J_{3a,4}$ 4.6 Hz, $J_{3a,3b}$ 12.5 Hz, H-3a), 2.02 (s, 3 H, NHAc), 1.67 (t, 1 H, $J_{3b,4}$ 12.5 Hz, H-3b). ^{13}C NMR (125 MHz, D_2O): δ 176.05, 173.77, 101.58, 80.07, 73.80, 72.63, 69.17, 69.12, 63.61, 53.49, 52.76, 41.24, 23.00; ES HRMS calcd for $C_{14}H_{20}NO_9$ ($[M-H]^-$) 346.1144, found 346.1137.

General synthesis of propargylsulfonamides

To a stirred solution of propargylamine (200 μL, 3.12 mmol) and Et_3N (0.65 mL, 4.7 mmol) in dichloromethane (10 mL) the corresponding sulfonyl chloride (3.75 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 2–16 h. After removal of the solvent, the resulting residue was purified by flash chromatography, eluting with EtOAc–hexane to yield sulfonamides in moderate to high yields.

***N*-(Prop-2-ynyl)methanesulfonamid (7.2).**[42]

***N*-(Prop-2-ynyl)-(*S*)-(+)-10-camphorsulfonyl-methanesulfonamide (7.3)** as a colourless solid (70%): mp 96–97 °C; IR (solid) ν 2115 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.09 (s, br, 1 H, NH), 4.1 (ddd, 1 H, *J* 2.5 Hz, *J* 8.5 Hz, *J* 18.2 Hz, propargyl CH_2), 3.94 (ddd, 1 H, *J* 1.2 Hz, *J* 2.5 Hz, *J* 18.2 Hz, propargyl CH_2), 3.73 (d, 1 H, *J* 15.2 Hz, CH_2S), 3.06 (d, 1 H, *J* 15.2 Hz, CH_2S), 2.43 (ddd, 1 H, *J* 2.4 Hz, *J* 4.9 Hz, *J* 18.6 Hz, CH_2), 2.32 (t, 1 H, propargyl CH), 2.16–1.92 (m, 5 H, CH_2), 1.51–1.42 (m, 1 H, CH), 1.01 (s, 3 H, CH_3), 0.94 (s, 3 H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 217.85, 80.08, 74.02, 60.38, 52.55, 49.80, 43.79, 43.50, 33.80, 28.35, 27.82, 20.74, 20.09; ES HRMS calcd for $C_{13}H_{19}NO_3SNa$ ($[M + Na]^+$) 292.0978, found 292.0977.

4-Methyl-*N*-prop-2-ynylbenzenesulfonamide (7.4).[43]

4-Isopropyl-*N*-prop-2-ynylbenzenesulfonamide (7.5) as a colourless solid (59%): mp 51–52 °C; IR (KRS-5 disk) ν 2120 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.81 (m, 2 H), 7.36 (m, 2 H), 4.84 (s, 1 H, NH), 3.83 (dd, 2 H, *J* 2.5 Hz, *J* 6.1 Hz, NCH_2), 2.98 (m, 1 H, C*H*Me_2), 2.09 (t, 1 H, *J* 2.5 Hz, CH), 1.27 (d, 6 H, *J* 7.0, CH_3).^{13}C NMR (100 MHz, $CDCl_3$): δ 154.54, 136.71, 127.51, 127.16, 78.05, 72.87, 34.17, 32.84, 23.65; ES HRMS calcd for $C_{12}H_{15}NO_2SNa$ ($[M + Na]^+$) 260.07157, found 260.07125.

4-*tert*-Butyl-*N*-prop-2-ynylbenzenesulfonamide (7.6) as a colourless solid (84%): mp 61–62 °C; IR (solid) ν 2112 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 7.82 (m, 2 H, arom.), 7.53 (m, 2 H, arom.), 4.78 (s, 1 H, NH), 3.83 (dd, 2 H, *J* 2.7 Hz, *J* 6.0 Hz, CH_2), 2.10 (t, 1 H, *J* 2.5 Hz, CH), 1.35 (s, 9 H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.83, 136.35, 127.22, 126.06, 78.05, 72.86, 35.15, 32.86, 31.07; ES HRMS calcd for $C_{13}H_{17}NO_2SNa$ ($[M + Na]^+$) 274.0872, found 274.0872.

4-Nitro-*N*-prop-2-ynylbenzenesulfonamide (7.7).[44]

4-Nitro-*N*-(1-ethynylcyclohexyl)benzenesulfonamide (7.8) as a colourless solid (35%): mp 144–145 °C; IR (KRS-5 disk) ν 2117 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.33 (m, 2 H, arom.), 8.11 (m, 2 H, arom.), 5.00 (s, 1 H, NH), 2.15 (s, 1 H, CH), 2.1–2.0 (m, 2 H, CH_2), 1.72–1.50 (m, 7 H, CH_2), 1.30–1.20 (m, 1 H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.89, 147.62, 128.92, 123.83, 83.01, 74.60, 54.80, 38.93, 24.70, 22.22; ES HRMS calcd for $C_{14}H_{16}N_2O_4SNa$ ($[M + Na]^+$) 331.07230, found 331.07214.

4-Acetyl-*N*-prop-2-ynylbenzenesulfonamide (7.9) as a colourless solid (94%): mp 110–111 °C; IR (KRS-5 disk) ν 2129 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.11 (d, 2 H, *J* 8.4 Hz, arom.), 7.92 (d, 2 H, *J* 8.4 Hz, arom.), 4.93 (t, 1 H, *J* 5.8 Hz, NH), 3.93 (dd, 2 H, *J* 2.4 Hz, *J* 6.0 Hz, CH_2), 2.68 (s, 3 H, CH_3), 2.10 (t, 1 H, *J* 2.4 Hz, CH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.83, 143.71, 140.25, 128.92, 128.85, 127.70, 77.60, 73.36, 32.93, 26.91; ES HRMS calcd for $C_{11}H_{11}NO_3SNa$ ($[M + Na]^+$) 260.03519, found 260.03505.

***N*-Acetylsulfanilylamide (7.10)** as a colourless solid (80%): mp 178–179 °C (dec.); IR (KRS-5 disk) ν 2121 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.29 (s, 1 H, NH), 7.96 (t, 1 H, *J* 6

Hz, NH), 7.72 (m, 4 H, arom.), 3.64 (dd, 2 H, *J* 2.5 Hz, *J* 6.0 Hz, CH_2), 3.03 (t, *J* 2.5 Hz, 1 H, CH), 2.0 (s, 3 H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.95, 142.83, 133.98, 127.82, 118.39, 79.40, 74.50, 31.84, 24.09; ES HRMS calcd for $C_{11}H_{12}N_2O_3SNa$ ([M + Na]$^+$) 275.04609, found 275.04602.

General synthesis of *N*-propargyl carbamates

To a stirred solution of propargylamine (100 μL, 1.56 mmol) and pyridine (189 μL, 2.34 mmol) in dry CH_2Cl_2 (5 mL) the corresponding chloroformate (1.87 mmol) was added at 0 °C. The reaction mixture was stirred overnight at room temperature. After the removal of solvent, the resulting residue was purified by flash chromatography to yield respective carbamates.

***N*-(2,2,2-Trichloroethyloxycarbonyl)propargylamine (7.11)** as a colourless oil (93%) which crystallized upon standing: mp 48–49 °C; IR (KRS-5 disk) ν 2128 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.23 (s, 1 H, NH), 4.75 (s, 2 H, CH_2), 4.04 (dd, 2 H, *J* 2.5 Hz, *J* 5.6 Hz, CH_2), 2.29 (t, 1 H, *J* 2.5 Hz, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.09, 95.24, 78.87, 74.75, 72.16, 31.08; ES HRMS calcd for $C_6H_6NO_2Cl_3Na$ ([M + Na]$^+$) 251.93563, found 251.93570.

***N*-(Benzyloxycarbonyl)propargylamine (7.12).**[45]

***N*-(4-Nitrobenzyloxycarbonyl)propargylamine (7.13)** as a colourless solid (70%): mp 125–126 °C; IR (KRS-5 disk) ν 2116 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.21 (d, 2 H, *J* 8.1 Hz, arom.), 7.51 (d, 2 H, *J* 8.1 Hz, arom.), 5.22 (s, 2 H, CH_2), 5.09 (s, 1 H, NH), 4.01 (dd, 2 H, *J* 2.3 Hz, *J* 5.4 Hz, CH_2), 2.27 (t, 1 H, *J* 2.4 Hz, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.35, 147.65, 143.61, 128.17, 123.75, 79.29, 71.88, 65.51, 30.95; ES HRMS calcd for $C_{11}H_{10}N_2O_4Na$ ([M + Na]$^+$) 257.05339, found 257.05328.

General synthesis of *N*-propargyl amides

To a stirred solution of proparylamine (150 μL, 2.34 mmol) and pyridine (284 μL, 3.51 mmol) in dry CH_2Cl_2 (7.5 mL) the corresponding acyl chloride (2.81 mmol) was added at 0 °C. The reaction mixture was stirred overnight at room temperature. After the removal of solvent, the resulting residue was purified by flash chromatography to yield *N*-(prop-2-ynyl)amide derivatives.

***N*-(prop-2-ynyl)benzamide (7.14).**[46]

3-Cyano-*N*-(prop-2-ynyl)benzamide (7.15) as a colourless solid (92%): mp 132–133 °C; IR (KRS-5 disk) ν 2234 cm^{-1};^{1}H NMR (300 MHz, DMSO-d_6): δ 9.14 (t, 1 H, *J* 5.4 Hz, NH), 8.26 (t, 1 H, *J* 1.7 Hz, arom.), 8.15 (dt, 1 H, *J* 1.3 Hz, *J* 8.0 Hz, arom.), 8.01 (dt, 1 H, *J* 1.4 Hz, *J* 7.7 Hz, arom.), 7.69 (t, 1 H, *J* 7.8 Hz, arom.), 4.07 (dd, 2 H, *J* 2.5 Hz, *J* 5.5 Hz, CH_2), 3.14 (t, 1 H, *J* 2.5 Hz, CH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.12, 134.88, 134.71, 132.02, 130.89, 129.82, 118.22, 111.53, 80.76, 73.20, 28.66; ES HRMS calcd for $C_{11}H_8N_2ONa$ ([M + Na]$^+$) 207.05288, found 207.05275.

4-Nitro-*N*-(prop-2-ynyl)benzamide (7.16).[47]

3,5-Dinitro-*N*-(prop-2-ynyl)benzamide (7.17) as a colourless solid (91%): mp 132–133 °C; IR (KRS-5 disk) ν 2127 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.19 (m, 1 H, arom.); 8.98 (m, 2 H, arom.), 6.6 (broad s, 1 H, NH), 4.34 (dd, 2 H, *J* 2.5 Hz, *J* 5.2 Hz, CH_2), 2.37 (t, 1 H, *J* 2.5 Hz, CH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.47, 148.76, 136.79, 128.15, 121.58, 80.87, 74.15, 29.57; ES HRMS calcd for $C_{10}H_7N_3O_5Na$ ([M + Na]$^+$) 272.02779, found 272.02772.

3,4,5-Trimethoxy-*N*-(prop-2-ynyl)benzamide (7.18) as a colourless solid (87%): mp 56–57 °C; IR (solid) ν 2116 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.02 (s, 2 H, arom.), 6.57 (t, 1 H, *J* 4.9 Hz, NH), 4.22 (dd, 2 H, *J* 2.5 Hz, *J* 5.3 Hz, CH_2), 3.86 (s, 9 H, OMe), 2.26 (t, 1 H, *J* 2.5 Hz, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.94, 153.16, 141.11, 129.09, 104.51, 79.52, 71.74, 60.87, 56.28, 29.82. ES HRMS calcd for $C_{13}H_{15}NO_4Na$ ([M + Na]$^+$) 272.0893, found 272.0895.

4-Cyano-*N*-(prop-2-ynyl)benzamide (7.19) as a colourless solid (98%): mp 174–175 °C; IR (KRS-5 disk) ν 2235, 2120 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (d, 2 H, *J* 8.2 Hz, arom.), 7.76 (d, 2 H, *J* 8.2 Hz, arom.), 6.33 (s, 1 H, NH), 4.28 (dd, 2 H, *J* 2.5 Hz, *J* 5.1 Hz, CH_2), 2.32 (t, 1 H, *J* 2.5 Hz, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.53, 137.68, 132.45, 128.08, 118.22, 113.81, 80.80, 73.13, 28.65; ES HRMS calcd for $C_{11}H_8N_2ONa$ ([M + Na]$^+$) 207.05288, found 207.05277.

4-(Methylthio)-*N*-(prop-2-ynyl)benzamide (7.20) as a colourless solid (87% yield): mp 136–137 °C; IR (KRS-5 disk) ν 2113 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (m, 2 H, arom.), 7.26 (m, 2 H, arom.), 6.26 (s, 1 H, NH), 4.25 (dd, 2 H, *J* 2.5 Hz, *J* 5.2 Hz, CH_2), 2.51 (s, 3 H, CH_3), 2.28 (t, 1 H, *J* 2.5 Hz, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.53, 143.91, 129.77, 127.39, 125.39, 79.51, 71.86, 29.76, 14.99; ES HRMS calcd for $C_{11}H_{11}NOSNa$ ([M + Na]$^+$) 228.04536, found 228.04518.

4-*tert*-Butyl-*N*-(prop-2-ynyl)benzamide (7.21) as a colourless solid (90%): mp 116–117 °C; IR (KRS-5 disk) ν 2117 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (m, 2 H, arom.), 7.45 (m, 2 H, arom.), 6.39 (s, 1 H, NH), 4.25 (dd, 2 H, *J* 2.6 Hz, *J* 5.2 Hz, CH_2), 2.27 (t, 1 H, *J* 2.6 Hz, CH), 1.33 (s, 9 H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.06, 155.28, 130.86, 126.88, 125.51, 79.67, 71.70, 34.93, 31.13, 29.69; ES HRMS calcd for $C_{14}H_{17}NONa$ ([M + Na]$^+$) 238.12024, found 238.11994.

2,4,6-Trichloro-*N*-(prop-2-ynyl)benzamide (7.22) as a colourless solid (72%): mp 165–166 °C; IR (KRS-5 disk) ν 2101 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33 (s, 2 H, arom.), 6.08 (broad s, 1 H, NH), 4.26 (dd, 2 H, *J* 2.6 Hz, *J* 5.3 Hz, CH_2), 2.29 (t, 1 H, *J* 2.6 Hz, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.35, 136.03, 133.77, 133.03, 128.11, 78.27, 72.35, 29.73; ES HRMS calcd for $C_{10}H_6NOCl_3Na$ ([M + Na]$^+$) 283.94072, found 283.94079.

4-Chloro-*N*-(prop-2-ynyl)benzamide (7.23) as a colourless solid (92%): mp 145–146 °C; IR (solid) ν 2120 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (m, 2 H, arom.), 7.42 (m, 2 H, arom.), 6.33 (s, 1 H, NH), 4.25 (q, 2 H, *J* 2.5 Hz, CH_2), 2.29 (t, 1 H, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.04, 138.10, 132.07, 128.89, 128.45, 79.23, 72.05, 29.86; ES HRMS calcd for $C_{10}H_8NClONa$ ([M + Na]$^+$) 216.0187, found 216.0187.

2,5-Difluoro-*N*-(prop-2-ynyl)benzamide (7.24) as a colourless solid (84%): mp 73–74 °C; IR (KRS-5 disk) ν 2009 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.83–780 (m, 1 H, arom.), 7.19–7.10 (m, 2 H, arom.), 6.94 (s, 1 H, NH), 4.28 (m, 2 H, CH_2), 2.30 (t, 1 H, *J* 2.5 Hz, CH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.5 (d, J_{CF} 332 Hz), 155.2 (d, J_{CF} 246 Hz), 124.7 (dd, J_{CF} 7.5 Hz, J_{CF} 17.5 Hz), 119.1 (dd, J_{CF} 9.0 Hz, J_{CF} 24.0 Hz), 118 (dd, J_{CF} 8 Hz, J_{CF} 25.5 Hz), 116.2 (d, J_{CF} 25 Hz), 80.63, 73.08, 28.59; ES HRMS calcd for $C_{10}H_7NOF_2Na$ ([M + Na]$^+$) 218.03879, found 218.03875.

2,4-Difluoro-*N*-(prop-2-ynyl)benzamide (7.25) as a colourless solid (93%): mp 70–71 °C; IR (solid) ν 2116 cm^{-1}; ^{1}H NMR

(300 MHz, $CDCl_3$): δ 8.20–8.11 (m, 1 H, arom.), 7.01 (td, 1 H, *J* 6.6 Hz, *J* 8.9 Hz, arom.), 6.88 (ddd, 1 H, *J* 2.4 Hz, *J* 8.4 Hz, *J* 12.0 Hz), 6.83 (s, br, 1 H, NH), 4.27 (m, 2 H, CH_2), 2.29 (t, 1 H, *J* 2.6 Hz, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.95 (dd, J_{CF} 13.4 Hz, J_{CF} 256.0 Hz), 162.00, 161.00 (dd, J_{CF} 12.6 Hz, J_{CF} 250.4 Hz), 133.92 (d, J_{CF} 10.5 Hz), 112.47 (d, J_{CF} 21.0 Hz), 104.27 (t, J_{CF} 107.2 Hz), 79.13, 71.87, 29.71; ES HRMS calcd for $C_{10}H_7NOF_2Na$ ([M + Na]$^+$) 218.03879, found 218.03869.

3,4,5-Trifluoro-*N*-(prop-2-ynyl)benzamide (7.26) as a colourless solid (93%): mp 122–123 °C; IR (solid) ν 2130 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.46 (m, 2 H, arom.), 6.37 (s, 1 H, NH), 4.24 (dd, 2 H, *J* 2.5 Hz, *J* 5.2 Hz, CH_2), 2.31 (t, 1 H, *J* 2.5 Hz, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.04, 151.15 (dd, J_{CF} 7.7 Hz, J_{CF} 252.25 Hz), 142.16 (dt, 14.6 Hz, J_{CF} 257.81 Hz), 111.86 (m), 78.72, 72.35, 30.07; ES HRMS calcd for $C_{10}H_6NOF_3Na$ ([M + Na]$^+$) 236.02937, found 236.02955.

4-Fluoro-*N*-(prop-2-ynyl)benzamide (7.27).[48]

3,4-Difluoro-*N*-(prop-2-ynyl)benzamide (7.28) as a colourless solid (92%): mp 53–54 °C; IR (solid) ν 2124 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67 (ddd, 1 H, *J* 2.2 Hz, *J* 7.5 Hz, *J* 10.5 Hz, arom.), 7.53 (m, 1 H, arom.), 7.23 (ddd, 1 H, *J* 7.6 Hz, *J* 8.6 Hz, *J* 9.7 Hz, arom.), 6.26 (s, 1 H, NH), 4.25 (dd, 2 H, *J* 2.6 Hz, *J* 5.2 Hz, CH_2), 2.30 (t, 1 H, *J* 2.6 Hz, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.04, 151.15 (dd, J_{CF} 9.9 Hz, J_{CF} 252.9 Hz), 142.16 (dt, J_{CF} 14.4 Hz, J_{CF} 257.8 Hz), 129.55 (d, J_{CF} 3.7 Hz), 111.87 (m), 78.72, 72.35, 30.07; ES HRMS calcd for $C_{10}H_7NOF_2Na$ ([M + Na]$^+$) 218.03879, found 218.03866.

General procedure for synthesis of polymeric heterobifunctional compounds 5αn, 5βn 6αn, and 6βn

To a stirred solution of an azido-polymer **5α**, **5β**, **6α**, or **6β** (20 mg, 7.0 μmol) and an alkyne derivative **7.n** (14 μmol) in DMSO–H_2O (3 : 2 by volume, 2.5 mL) aqueous sodium ascorbate (1 M, 84 μL, 84 μmol) was added followed by aqueous $CuSO_4 \cdot 0.5H_2O$ (0.05 M, 168 μL, 8.4 μmol) at room temperature. The progress of the reaction was monitored during 3–5 d by disapperance of the characteristic N_3 band in IR spectra. When completed the reaction was quenched by addition of aqueous EDTA (0.5 M, 1 mL), and the mixture was dialyzed extensively against deionized water using dialysis tube with MWCO 12 000–14 000. Lyophilization of the residue yielded the corresponding conjugate.

3-(4-((4-Fluorobenzamido)methyl)-1H-1,2,3-triazol-1-yl)-2-(R,S)-hydroxypropyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (12). To a clear, stirred solution of **3α** (120 mg, 268 μmol) and 4-fluoro-*N*-(prop-2-ynyl)benzamide (**7.27**, 95 mg, 536 μmol) in H_2O–MeOH (1 : 10, 11 mL) aqueous 1 M sodium ascorbate (80.4 μL, 80.4 μmol) and aqueous $CuSO_4 \cdot 0.5H_2O$ (0.05 M, 268 μL, 13.4 μmol) were added at room temperature. The reaction mixture was stirred at room temperature for 72 h. After removal of the solvent, the resulting residue was purified by flash chromatography (EtOAc) to yield a diastereoisomeric mixture **12** (60%) as a colourless foam: IR (KRS-5 disk) ν 3367, 3058, 2934, 1748, 1650, 1605, 1541, 1503, 1373, 1233, 1055, 855, 768 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.85–7.75 (m, 3 H, triazole, arom.), 7.15–7.10 (m, 3 H, NH, arom.), 5.48–5.46 (m, 1 H, H-4), 5.35 and 5.30 (2dd, total 1 H, $J_{3,2}$ 10.6 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.20–5.14 (m, 2 H, H-1, H-2), 4.70 (m, 2 H, CH_2), 4.58–4.52 (m, 1 H, H-5), 4.47–4.40 (m, 1 H), 4.33–4.26 (m, 2 H), 4.10 (dd, 2 H, *J* 4.3 Hz, *J* 6.4 Hz, CH_2), 3.81 and 3.72 (2dd, total 1 H, *J* 4.5 Hz, *J* 10.5 Hz, CH_2), 3.58 and 3.48 (2dd, total 1 H, *J* 5.8 Hz, *J* 10.5 Hz, CH_2), 2.147 and 2.145 (2 s, total 3 H, OAc), 2.10 and 2.09 (2 s, total 3 H, OAc), 2.05 and 2.03 (2 s, total 3 H, OAc), 2.01 (s, 3 H, OAc), 1.75 (s, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.59, 170.52, 170.26, 170.18, 170.17, 170.15, 170.10, 166.44, 165.87, 163.86, 144.59, 144.51, 130.11, 130.08, 129.47, 129.40, 123.89, 123.87, 115.73, 115.56, 97.11, 97.02, 70.15, 70.10, 69.01, 68.99, 68.02, 67.95, 67.92, 67.90, 67.47, 67.42, 66.74, 66.65, 61.71, 52.92, 52.68, 35.42, 20.79, 20.78, 20.73, 20.68, 20.64; ES HRMS calcd. for $C_{27}H_{33}FN_4O_{12}Na$ ([M + Na]$^+$) 647.19712, found 647.19642.

3-(4-((3,4-Difluorobenzamido)methyl)-1H-1,2,3-triazol-1-yl)-2-(R,S)-hydroxypropyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (13). To a clear, stirred solution of **3α** (120 mg, 268 μmol) and 3,4-difluoro-*N*-(prop-2-ynyl)benzamide (**7.28**) (105 mg, 536 μmol) in H_2O–MeOH (1 : 10, 11 mL) aqueous 1 M sodium ascorbate (80.4 μL, 80.4 μmol) and aqueous $CuSO_4 \cdot 0.5H_2O$ (0.05 M, 268 μL, 13.4 μmol) were added then stirred for 22 h at room temperature. After removal of the solvent, the resulting residue was purified by flash chromatography (EtOAc) to yield a diastereoisomeric mixture **13** (154 mg, 89%) as a colourless foam: IR (KRS-5 disk) ν 3354, 3078, 2939, 1746, 1651, 1606, 1510, 1227, 1051, 775, 760; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.81 (d, 1 H, *J* 2.4 H, triazole), 7.74–7.70 (m, 2 H, arom.), 7.61–7.60 (m, 1 H, NH), 7.21 (q, 1 H, *J* 8.5 Hz, arom.) 5.46 (m, 1 H, H-4), 5.34 and 5.30 (2dd, total 1 H, $J_{3,2}$ 10.4 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.18–5.14 (m, 2 H, H-1, H-2), 4.67–4.65 (m, 2 H, CH_2), 4.59–4.530 (m, 1 H), 4.47–4.41 (m, 1 H), 4.34–4.22 (m, 2 H), 4.10 (dd, 2 H, *J* 2.9 Hz, 6.5 Hz, CH_2), 3.82 and 3.74 (2dd, total 1 H, *J* 4.6 Hz, *J* 10.6 Hz, CH_2), 3.56 and 3.50 (2dd, total 1 H, *J* 4.9 Hz, *J* 10.6 Hz, CH_2), 2.16 (s, 3 H, OAc), 2.09 (s, 3 H, OAc), 2.04 and 2.02 (2 s, total 3 H, OAc), 2.01 (s, 3 H, OAc); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.64, 170.57, 170.31, 170.30, 170.22, 170.20, 170.16, 165.39, 153.65, 153.54, 151.62, 151.52, 151.22, 151.12, 149.25, 149.15, 144.31, 144.27, 144.24, 144.21, 130.89, 123.77, 117.52, 117.38, 117.07, 116.92, 97.05, 96.93, 70.06, 70.02, 68.94, 68.91, 68.05, 67.98, 67.94, 67.91, 67.48, 67.43, 66.70, 66.61, 61.71, 53.13, 52.93, 35.24, 20.77, 20.67; ES HRMS calcd. for $C_{27}H_{32}F_2N_4O_{12}Na$ ([M + Na]$^+$) 665.18770, found 665.18676.

3-(4-((4-Fluorobenzamido)methyl)-1H-1,2,3-triazol-1-yl)-2-(*R*,*S*)-hydroxypropyl α-D-galactopyranoside (10). To a stirred solution of **12** (120 mg, 1.9 mmol) in MeOH (10 mL) at room temperature was added aqueous NH_4OH (1 mL). The reaction mixture was stirred overnight at room temperature. After the removal of solvent, the resulting residue was purified by column chromatography on silica gel (EtOAc–acetone) to yield a mixture of diastereoisomers **10** (68 mg, 78%) as a colourless foam: IR (KRS-5 disk) ν 3358 (br, s), 2931, 1640, 1604, 1559, 1505, 1456, 1340, 1233, 1059, 853, 767 cm^{-1}; ^{1}H NMR (500 MHz, D_2O): δ 7.96 (s, 1 H, triazole), 7.81–7.75 (m, 2 H, arom.), 7.20 (t, 2 H, *J* 8.5 Hz, arom.), 4.94–4.92 (m, 1 H, H-1), 4.65–4.48 (m, 4 H, CH_2), 4.33–4.28 (m, 1 H, CH), 3.96–3.94 (m, 1 H, H-4), 3.92–3.68 (m, 6 H, H-2, H-3, H-5, H-6a, H-6b, CH_2), 3.55 (dd, 0.5 H, *J* 4.2 Hz, *J* 10.6 Hz, CH_2), 3.46 (dd, 0.5 H, *J* 6.0 Hz, *J* 10.7 Hz, CH_2); ^{13}C NMR (125 MHz, D_2O): δ 170.59, 166.70, 166.09, 164.71, 156.92, 152.10, 145.56, 130.59, 130.52, 130.36, 125.58, 125.52, 116.64, 116.46, 99.72, 99.55, 72.01, 71.95, 70.29, 70.27, 70.12, 69.64, 69.61, 69.59, 69.52, 69.28, 62.04, 53.64, 53.49, 35.87; ES HRMS calcd. for $C_{19}H_{25}FN_4O_8Na$ ([M + Na]$^+$) 479.15486, found 479.15450.

3-(4-((3,4-Difluorobenzamido)methyl)-1H-1,2,3-triazol-1-yl)-2-(*R*,*S*)-hydroxypropyl α-D-galactopyranoside (11). The title compound (78 mg, 90%, colourless foam) was obtained as a mixture of diastereoisomers as described for **10** by deacetylation of **13**: IR (KRS-5 disk) *ν* 3350 (br, s), 2931, 1648, 1606, 1558, 1512, 1427, 1300, 1108, 1060, 777 cm^{-1}; ^1H NMR (500 MHz, D_2O): *δ* 8.05 (s, 1 H, triazole), 7.74–7.71 (m, 1 H, arom.), 7.65–7.63 (m, 1 H, arom.), 7.44–7.39 (m, 1 H, arom.), 5.02 (m, 1 H, H-1), 4.73–4.57 (m, 4 H, CH_2), 4.40–4.37 (m, 1 H, CH), 4.04–4.03 (m, 1 H, H-4), 4.00–3.76 (m, 6 H, H-2, H-3, H-5, H-6a, H-6b, CH_2), 3.64 (dd, 0.5 H, *J* 4.3 Hz, *J* 10.7 Hz, CH_2), 3.54 (dd, 0.5 H, *J* 6.0 Hz, *J* 10.6 Hz, CH_2); ^{13}C NMR (125 MHz, D_2O): *δ* 169.42, 154.48, 154.38, 152.47, 152.37, 151.82, 151.72, 149.85, 149.75, 145.48, 131.30, 125.71, 125.66, 125.26, 125.23, 125.20, 125.17, 118.74, 118.60, 117.75, 117.60, 99.82, 99.65, 72.11, 72.05, 70.39, 70.36, 70.22, 69.75, 69.71, 69.69, 69.61, 69.37, 69.14, 53.74, 53.59, 35.99; ES HRMS calcd. for $C_{19}H_{24}F_2N_4O_8Na$ ([M + Na]$^+$) 497.1454, found 497.1451.

***N*-β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-[(5-amino-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulosonic acid)-yl-(1→3)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-undecyl-10-enylamide (9).** A tightly enclosed vial containing solution of GM_1OS **8** obtained as previously described[24] (28 mg 0.028 mmol) in wet liquid ammonia (~2 mL, ~20 μL water added) was incubated at 60 °C in oil bath (caution: possible explosion, use protective gear!) for 24 h. The vial was cooled in dry ice-acetone and opened. Ammonia was allowed to escape; then the mixture was taken up in water and freeze-dried. To a solution of the residue in methanol (2 mL) and Et_3N (12 μL) 10-undecenoic acid NHS ester[49] (3 eq., 24 mg) in DCM (0.5 mL) was added slowly at room temperature. After 16 h the mixture was concentrated and purified by HPLC on C18 column in gradient water–MeOH to give the title compound (20.6 mg, 63%), $[\alpha]_D$ +9.5 (c 1.05, water); IR (solid) *ν* 3351, 2927, 2856, 1639, 1411, 1076 cm^{-1}; ^1H NMR (600 MHz, D_2O, Gal1 and Gal2 assignments belong to the middle and the terminal galactose moieties correspondingly): *δ* 5.94–5.88 (m, 1 H, CH═CH_2), 5.06–5.02 (m, 1 H, CH═CH_2), 4.98–4.95 (m, 2 H, *J* 9.1 Hz, H-1_{Glc}, CH═CH_2), 4.78 (d, 1 H, *J* 8.4 Hz, H-1_{GalNAc}), 4.52 (d, 2 H, *J* 7.8 Hz, H-1_{Gal1}, H-1_{Gal2}), 4.16–4.11 (m, 3 H, H-3_{Gal1}, H-3_{Gal1}, H-4_{GalNAc}), 4.03 (dd, 1 H, *J* 11.0 Hz, H-2_{GalNAc}), 3.94–3.57 (m, 23 H), 3.52–3.47 (m, 2 H, H-2_{Gal2}, H-6_{Neu}), 3.41 (dd, 1 H, *J* 9.2 Hz, H-2_{Glc}), 3.36 (dd, 1 H, *J* 8.4 Hz, H-2_{Gal1}), 2.65 (dd, 1 H, *J* 4.3 Hz, *J* 12.6 Hz, H-$3e_{Neu}$), 2.31–2.28 (m, 2 H, CH_2CO), 2.04 (dd, 2 H, *J* 6.8 Hz, *J* 13.6 Hz, CH_2), 2.02 (s, 3 H, NAc), 1.99 (s, 3 H, NAc), 1.92 (dd, 1 H, *J* 11.6 Hz, H-$3a_{Neu}$), 1.62–1.57 (m, 2 H, CH_2), 1.39–1.34 (m, 2 H, CH_2),1.32–1.26 (m, 8 H, CH_2). ^{13}C NMR (150 MHz, D_2O): *δ* 179.65, 175.91, 175.65, 174.98, 141.38, 114.82, 105.62, 103.42, 103.38, 102.51, 81.22, 79.99, 78.89, 78.02, 77.24, 75.99, 75.77, 75.24, 75.22, 74.96, 73.97, 73.39, 73.15, 72.28, 71.58, 70.87, 69.58, 69.48, 68.92, 68.79, 63.72, 61.99, 61.83, 61.52, 60.83, 52.50, 52.08, 37.87, 36.70, 33.99, 29.38, 29.23, 29.16, 29.08, 29.04, 28.99, 25.93, 23.49, 22.95; ES HRMS calcd. for $C_{48}H_{80}N_3O_{29}$ ([M – H]$^-$) 1162.4883, found 1162.4885.

Inhibition ELISA assay

To PVC microtiter plates (Gibco BRL Inc.) a solution of coating compound **9** (1 μg mL^{-1}, 100 μL $well^{-1}$) in PBS buffer was added and incubated at room temperature overnight, then washed (6×) with PBST (0.05% Tween 20 in phosphate buffer saline, PBS). Inhibitor at decreasing concentrations (starting at 20 mg mL^{-1}, dilution factor 3.16; 50 μL $well^{-1}$) was mixed with cholera toxin B-subunit conjugated to horseradish peroxidase (Sigma, 20 ng mL^{-1}; 50 μL well) and the mixtures were applied to the plates. After incubation at room temperature for 2 h the plates were washed (6×) with PBST. 3,3:5,5-Tetramethylbenzidine solution (TMB purchased from KPL, 100 μL $well^{-1}$) was added. After 15 min incubation the reaction was stopped by addition of H_3PO_4 (100 μL $well^{-1}$) and absorbance was measured at 450 nm.

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