Supplementary Material

Biocompatible glyconanomaterials based on HPMA-copolymer for specific targeting of galectin-3

P. Bojarová1*, M. R. Tavares2, D. Laaf3, L. Bumba1, L. Petrásková1, R. Konefal2, M. Bláhová2, H. Pelantová1, L. Elling3, T. Etrych2, P. Chytil2* and V. Křen1

1 Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, Prague 4, CZ-14220, Czech Republic.
2 Institute of Macromolecular Chemistry, Czech Academy of Sciences, Heyrovský Sq. 2, Prague 6, CZ-16206, Czech Republic.
3 Laboratory for Biomaterials, Institute for Biotechnology and Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University, Pauwelsstraße 20, Aachen, D-52074, Germany.

* Correspondence: bojarova@biomed.cas.cz; chytil@imc.cas.cz.
These authors contributed equally to this work.

Abstract

Background: Galectin-3 (Gal-3) is a promising target in cancer therapy with a high therapeutic potential due to its abundant localization within the tumor tissue and its involvement in tumor development and proliferation. Potential clinical application of Gal-3-targeted inhibitors is often complicated by their insufficient selectivity or low biocompatibility. Nanomaterials based on N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer are attractive for in vivo application due to their good water solubility and lack of toxicity and immunogenicity. Their conjugation with tailored carbohydrate ligands can yield specific glyconanomaterials applicable for targeting biomedically relevant lectins like Gal-3.

Results: In the present study we describe the synthesis and the structure-affinity relationship study of novel Gal-3-targeted glyconanomaterials, based on hydrophilic N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers. HPMA copolymers decorated with varying amounts of Gal-3 specific epitope GalNAcβ1,4GlcNAc (LacdiNAc) were analyzed in a competitive ELISA-type assay and their binding kinetics was described by surface plasmon resonance. We showed the impact of various linker types and epitope distribution on the binding affinity to Gal-3. The synthesis of specific functionalized LacdiNAc epitopes was accomplished under the catalysis by mutant β-N-acetylhexosaminidases. The glycans were conjugated to statistic HPMA copolymer precursors through diverse linkers in a defined pattern and density using Cu(I)-catalyzed azide–alkyne cycloaddition. The resulting water-soluble and structurally flexible synthetic glyconanomaterials exhibited affinity to Gal-3 in low µM range.

Conclusions: The results of this study reveal the relation between the linker structure, glycan distribution and the affinity of the glycopolymers nanomaterial to Gal-3. They pave the way to specific biomedical glyconanomaterials that target Gal-3 as a therapeutic goal in cancerogenesis and other disorders.

Keywords: carbohydrate: galectin-3; glyconanomaterial; HPMA copolymer, surface plasmon resonance
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1. LacdiNAc disaccharides – structural characterization

1.1. NMR data and spectra

Table S1

**Part A, β-1-4-1-α-D-GlcNAc**

| Atom | δc | m. | δ2H | n2H | m. | J[Hz] |
|------|----|----|-----|-----|----|-------|
| 1    | 90.69 | D   | 4.976 | 1 | d | 3.3 |
| 2    | 53.80 | D   | 3.658 | 1 | dd | 10.7, 3.3 |
| 3    | 69.59 | D   | 3.687 | 1 | dd | 10.7, 7.8 |
| 4    | 79.83 | D   | 3.422 | 1 | dd | 10.0, 7.8 |
| 5    | 70.22 | D   | 3.67 | 1 | m | |
| 6    | 60.32 | T   | 3.572 | 1 | dd | 12.1, 2.3 |
|      |      |     |      | 1 | dd | 12.1, 4.8 |

2-CO 174.70  S   -   0
2-Ac 22.13  Q   1.820  3   s

**Part B, β-1-4-1-β-D-GlcNAc**

| Atom | δc | m. | δ2H | n2H | m. | J[Hz] |
|------|----|----|-----|-----|----|-------|
| 1    | 95.10 | D   | 4.485 | 1 | m | ΣJ = 8.3 |
| 2    | 56.25 | D   | 3.484 | 1 | m | |
| 3    | 72.83 | D   | 3.484 | 1 | m | |
| 4    | 79.38 | D   | 3.412 | 1 | m | |
| 5    | 74.84 | D   | 3.307 | 1 | ddd | 9.8, 5.5, 2.1 |
| 6    | 60.43 | T   | 3.614 | 1 | dd | 12.3, 2.1 |
|      |      |     |      | 3 | dd | 12.3, 5.5 |

2-CO 174.97  S   -   0
2-Ac 22.42  Q   1.818  3   s

**GalNAc**

| Atom | δc | m. | δ2H | n2H | m. | J[Hz] |
|------|----|----|-----|-----|----|-------|
| 1    | 101.97 | D   | 4.305 | 1 | d | 8.4 |
| 2    | 52.80 | D   | 3.710 | 1 | dd | 10.8, 8.4 |
| 3    | 70.93 | D   | 3.531 | 1 | dd | 10.8, 3.3 |
| 4    | 67.87 | D   | 3.727 | 1 | d | 3.3 |
| 5    | 75.58 | D   | 3.51 | 1 | m | |
| 6    | 61.19 | T   | 3.56 | 2 | m | |
2-CO 175.00  S   -   0
2-Ac 22.43  Q   1.848  3   s

a... might be interchanged; h... HSQC readout
Fig. S1 (a) $^1$H and (b) $^{13}$C NMR spectra of disaccharide 5 (700.13 MHz for $^1$H, 176.05 MHz for $^{13}$C, D$_2$O, 30 °C).
Table S2 $^1$H and $^{13}$C NMR data of disaccharide 6 (600.23 MHz for $^1$H, 150.94 MHz for $^{13}$C, D$_2$O, 25 °C).

| Atom       | $\delta$ C | m. | $\delta$ H | n H | m.   | $J$[Hz] |
|------------|-------------|----|-------------|-----|------|---------|
| spacer     |             |    |             |     |      |         |
| 1'         | 68.96       | T  | 3.768       | 1   | ddd  | 11.4, 5.6, 3.0 |
|            | 3.489       |    |             |     |      |         |
| 2'         | 50.55       | T  | 3.207       | 1   | ddd  | 13.8, 7.6, 3.0 |
|            | 3.144       |    |             |     |      |         |
| GlcNAc$^b$ |             |    |             |     |      |         |
| 1          | 101.16      | D  | 4.307       | 1   | d    | 8.3     |
| 2          | 54.97       | D  | 3.490       | 1   | dd   | 10.4, 8.3 |
| 3          | 72.78       | D  | 3.439       | 1   | dd   | 10.4, 8.3 |
| 4          | 79.31       | D  | 3.355       | 1   | dd   | 9.8, 8.3 |
| 5          | 74.75       | D  | 3.254       | 1   | ddd  | 9.8, 5.5, 2.2 |
| 6          | 60.31       | T  | 3.584       | 1   | dd   | 12.2, 2.2 |
|            |             |    |             |     |      |         |
| 2-CO       | 174.87      | S  | -           | 0   |      |         |
| 2-Ac       | 22.44       | Q  | 1.764       | 3   | s    |         |
| GalNAc$^b$ |             |    |             |     |      |         |
| 1          | 101.92      | D  | 4.248       | 1   | d    | 8.5     |
| 2          | 52.73       | D  | 3.657       | 1   | dd   | 10.9, 8.5 |
| 3          | 70.86       | D  | 3.475       | 1   | dd   | 10.9, 3.3 |
| 4          | 67.79       | D  | 3.669       | 1   | dd   | 3.3, 0.9 |
| 5          | 75.52       | D  | 3.450       | 1   | ddd  | 8.2, 4.0, 0.9 |
| 6          | 61.14       | T  | 3.527       | 1   | dd   | 11.8, 8.2 |
|            |             |    |             |     |      |         |
| 2-CO       | 174.97      | S  | -           | 0   |      |         |
| 2-Ac       | 22.37       | Q  | 1.796       | 3   | s    |         |
Fig. S2 (a) $^1$H and (b) $^{13}$C NMR spectra of disaccharide 6 (600.23 MHz for $^1$H, 150.94 MHz for $^{13}$C, D$_2$O, 25 °C).

Table S3 $^1$H and $^{13}$C NMR data of disaccharide 7 (700.13 MHz for $^1$H, 176.05 MHz for $^{13}$C, D$_2$O, 30 °C)

| Atom  | $\delta$ | m. | $\delta$ | n$_d$ | m. | $J$[Hz] |
|-------|----------|----|----------|-------|----|---------|
| GlcNAc$^a$ |          |    |          |       |    |         |
| 1     | 88.80    | D  | 4.519    | 1     | m  |         |
| 2     | 54.63    | D  | 3.509    | 1     | m  |         |
| 3     | 72.64    | D  | 3.509    | 1     | m  |         |
| 4     | 78.88    | D  | 3.431    | 1     | m  |         |
| 5     | 76.74    | D  | 3.365    | 1     | ddd| 9.8, 5.1, 2.1 |
| 6     | 60.25    | T  | 3.631    | 1     | dd | 12.3, 2.1 |
|       |          |    |          |       |    |         |
| 2-CO  | 175.06   | S  | -        | 0     |    |         |
| 2-Ac  | 22.37    | Q  | 1.812    | 3     | s  |         |
| GalNAc$^a$ |        |    |          |       |    |         |
| 1     | 101.95   | D  | 4.286    | 1     | d  | 8.4     |
| 2     | 52.80    | D  | 3.692    | 1     | dd | 10.9, 8.4 |
| 3     | 70.93    | D  | 3.512    | 1     | m  |         |
| 4     | 67.87    | D  | 3.704    | 1     | dd | 3.0, 0.7 |
| 5     | 75.59    | D  | 3.485    | 1     | ddd| 8.2, 4.2, 0.7 |
| 6     | 61.22    | T  | 3.562    | 1     | dd | 11.8, 8.2 |
|       |          |    |          |       |    |         |
| 2-CO  | 175.04   | S  | -        | 0     |    |         |
| 2-Ac  | 22.44    | Q  | 1.830    | 3     | s  |         |
Fig. S3 (a) $^1$H and (b) $^{13}$C NMR spectra of disaccharide 7 (700.13 MHz for $^1$H, 176.05 MHz for $^{13}$C, D$_2$O, 30 °C)
Fig 54 Structure of 2-acetamido-2-deoxy-β-β-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-β-glucopyranosyl azide. The measured 1H and 13C NMR data and MS data were in a good agreement with the previously published results [1].

1.2. MS spectra

Fig. 5S MS spectrum (ESI+) of compound 5 ([M + K]+, m/z 461.3; [M + Na]+, m/z 447.2)
**Fig S6** MS spectrum (ESI-) of compound 6 ([M-H], m/z 492.2; [M+HCOO], m/z 538.2; [M+CH₃COO], m/z 552.2)

**Fig. S7** MS spectrum (ESI-) of compound 7 ([M-H], m/z 448.2; [M + HCOO], m/z 494.2; [M-H-NH₃], m/z 405.2)
1.3. HPLC chromatograms

Fig S8 HPLC chromatogram of 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranose (LacdiNAc; 5).

Fig S9 HPLC chromatogram of 2-azidoethyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (6).
2. Glyconanomaterials – synthesis and characterization

2.1. Synthesis of polymer precursors

The syntheses are shown in Scheme 2 of the main text.

**General procedure for the synthesis of polymer precursors 10-12.** The polymer precursors bearing thiazolidine-2-thione (TT) groups poly(HPMA-co-MA-AP-TT) (10-12) were prepared by reversible addition-fragmentation chain transfer (RAFT) polymerization of N-(2-hydroxypropyl)methacrylamide (HPMA; 8) and 3-(3-methacrylamidopropanoyl)thiazolidine-2-thione (MA-AP-TT; 9) using the chain transfer agent 2-cyanopropan-2-yl dithiobenzoate (CTA) and the initiator 2,2‘-azobisisobutyronitrile (AIBN), followed by dithiobenzoate end group removal. The reaction conditions were adapted from our previous work [2]. The molar ratio of monomer/CTA/AIBN was 350/2/1 (copolymers 10, 11) or 450/2/1 (copolymer 12) and the molar ratio of reactants 8/9 was 9/1 (copolymer 10), 8/2 (copolymer 11) or 7/3 (copolymer 12).

**Example of the synthesis of polymer precursor 10.** HPMA (1.5 g, 10.5 mmol) was dissolved in tert-butyl alcohol (10.35 mL) and mixed with a solution of MA-AP-TT (300.7 mg, 1.16 mmol), AIBN (9.32 mg, 33.2 µmol), and CTA (14.7 mg, 67 µmol) in dimethylsulfoxide (DMSO) (2.60 mL, resulting in 0.9 M solution of monomers). The reaction mixture was poured into a glass ampoule, bubbled with argon and sealed. After 16 h in a thermostat controlled water bath at 70°C, the ampoule was cooled, and the reaction mixture was poured into an excess of a mixture of dry acetone and diethyl ether (2/1; 300 mL). After centrifugation at 7800 rpm for 5 min, followed by decantation of the precipitate, the copolymer was purified by precipitation from methanol (13 mL) into the mixture of dry acetone and diethyl ether (2/1; 300 mL). The copolymer was filtered off and
dried under vacuum (1.29 g, 72 % yield). For the removal of the dithiobenzoate end group, the intermediate copolymer and AIBN (193 mg) were dissolved in N,N-dimethylacetamide (DMA) (9 mL), poured into a glass ampoule, bubbled with argon and sealed. After 2 h in a thermostat controlled water bath at 80 °C, the ampoule was cooled, and the copolymer was isolated by precipitation into the mixture of dry acetone and diethyl ether (2/1; 200 mL). The precipitate was filtered off and purified by precipitation from methanol (9 mL) into the same mixture. The copolymer was filtered off and dried under vacuum to yield the title compound 10 (1.09 g).

**General procedure for the synthesis of polymer precursors 13-15.** The polymer precursors bearing propargyl groups poly[(HPMA-co-MA-AP-propargyl] (13-15) were synthesized by aminolysis of TT groups of respective copolymers poly[(HPMA-co-MA-AP-TT) (10-12)] with propargylamine in DMA using N,N-disopropylethylamine (DIPEA) as a base under the reaction conditions described previously [2].

**Example of the synthesis of polymer precursor 13.** Propargylamine (60 µL, 0.93 mmol) and DIPEA (162 µL, 0.93 mmol) were added to the stirred solution of copolymer 10 (1.09 g, 0.62 mmol of TT groups) dissolved in DMA (7 mL). The reaction was carried out at 24 °C for 40 min. The polymer was purified using Sephadex LH-20 column with methanol elution and UV detection. The polymer-containing fraction was concentrated in vacuo to 6 mL, and the polymer precursor was isolated by precipitation into an excess of the mixture of dry acetone and diethyl ether (2/1; 200 mL) followed by filtration and drying under vacuum to yield the title compound 13 (0.70 g).

**Synthesis of polymer precursor 16.** The polymer precursor bearing dibenzocyclooctyne (DBCO) groups poly[(HPMA-co-MA-AP-DBCO)] (16) was synthesized by aminolysis of TT groups of the copolymer poly[(HPMA-co-MA-AP-TT)] (10) with DBCOamine in DMA using DIPEA as a base by the similar procedure as described above. A solution of DBCO-amine (13.9 mg, 50 µmol) in DMA (300 µL) was added to the stirred solution of the copolymer 10 (150 mg, 86 µmol of TT groups) in DMA (3 mL). The reaction was carried out at 24 °C for 40 min. The unreacted TT groups were quenched with 1-aminopropan-2-ol (7.4 µL, 97 µmol) and the polymer was purified using Sephadex LH-20 column with methanol elution and UV detection. The polymer-containing fraction was concentrated in vacuo to 3 mL, and the polymer precursor was isolated by precipitation into an excess of acetone and diethyl ether (2/1; 100 mL) followed by filtration and drying under vacuum to yield the title compound 16 (127 mg).

**General procedure for the synthesis of polymer precursors 17 and 18.** Polymer precursors with different contents of ethynylphenyl groups poly[(HPMA-co-MA-AP-ethynylphenyl); 17 and 18] were synthesized via aminolysis of TT groups of respective copolymers poly[(HPMA-co-MA-AP-TT)] (10, 11) with (4-ethynylphenyl)methanamine in DMA using DIPEA as a base by the similar procedure as described above.

**Example of the synthesis of polymer precursor 17.** A solution of copolymer 10 (150 mg, 86 µmol of TT groups) in DMA (2 mL) was added to the stirred solution of (4-ethynylphenyl)methanamine hydrochloride (21.8 mg, 0.13 mmol) and DIPEA (23 µL, 0.13 mmol) in DMA (1 mL). The reaction was carried out at 24 °C for 20 h. The polymer was purified using Sephadex LH-20 column with methanol elution and UV detection. The polymer-containing fraction was concentrated in vacuo to 2 mL, and the polymer precursor was isolated by precipitation into an excess of dry acetone and diethyl ether (2/1; 100 mL) followed by filtration and drying in vacuo to yield the title compound 17 (89 mg).

**Synthesis of polymer precursor 20.** The polymer precursor poly[(HPMA-co-APMA)] (20) was prepared by RAFT polymerization of HPMA (8) and N-[3-tert-butoxycarbonylaminoaryl)methacrylamide (APMA-Boc; 19), followed by the removal of dithiobenzoate end group and Boc group. HPMA (0.5 mg, 3.49 mmol) was dissolved in deionized water (2.74 mL) and mixed with a solution of APMA-Boc (142 mg, 0.62 mmol), 4-cyano-4-[(thiobenzoylthio)pentanoic acid (6.9 mg, 24 µmol), and 4,4’-azobis(4-cyanopentanoic acid) (ACVA; 3.3 mg, 12 µmol) in 1,4-dioxane (1.37 mL, resulting in 1 M solution of monomers). The reaction mixture was poured into a glass ampoule, bubbled with argon and sealed. After 7 h in a thermostat controlled water bath at 70 °C, the ampoule was cooled, and the reaction mixture was poured into an excess of acetone (100 mL). After centrifugation at 7800 rpm for 5 min, followed by decantation of the precipitate, the copolymer was purified by precipitation from methanol (5 mL) into the mixture of acetone and diethyl ether (2/1; 100 mL). The copolymer was filtered off and dried under vacuum (0.36 g, 57 % yield). The removal of the dithiobenzoate end group was done as described above for polymer 10. Afterwards, the intermediate copolymer was dissolved in the mixture TFA/triisopropylsilane/ water 95/2.5/2.5 (vol. %) at a concentration of 10 wt. %, precipitated into excess of diethyl ether followed by filtration. The product was dissolved in water and pH of the solution was adjusted with NaOH to ca 7-8. The copolymer was isolated by gel filtration (Sephadex G25, eluent water) and freeze-dried (0.27 g).

**Synthesis of compound 22.** 3,5-Diethynylbenzyl thiazolidine-2-thione (22) was prepared by the reaction of 2-thiazoline-2-thiol and 3,5-diethynylbenzoic acid using carbodiimide. 3,5-Diethynylbenzonic acid (21; 150 mg, 0.88 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl; 404 mg, 2.11 mmol) were dissolved in dichloromethane (8 mL) and cooled to -18 °C. 2-Thiazoline-2-thiol (210 mg, 1.76 mmol) and a catalytic amount of 4-dimethylaminopyridine were dissolved in dichloromethane (5 mL) and cooled to -18 °C. Both solutions were combined and
the reaction mixture was cooled for another 2 h at -18 °C and then left overnight at 4 °C. The dichloromethane solution was washed with brine (3 × 100 mL) and with water (1 × 100 mL) and the organic layer was dried over anhydrous MgSO₄. After MgSO₄ was filtered off, the dichloromethane solution was concentrated under reduced pressure and the title compound 22 (185 mg, 77 % yield) was isolated by crystallization from ethyl acetate. The purity of the product was checked by a Shimadzu HPLC system equipped with a C18 reversed-phase Chromolith Performance RP-18e column, showing a single peak with a retention time of 3.1 min. TLC: ethyl acetate/hexane (1/1, v/v), RI = 0.75.

Synthesis of polymer precursor 23. The polymer precursor poly(HPMA-co-APMA-diethynylphenyl) (23) was prepared by the aminolytic reaction of precursor 20 with 3,5-diethynylbenzyl thiazolidine-2-thione (22) in DMA using DIPEA as a base by the similar procedure as described above. A solution of the copolymer 20 (150 mg, 89 µmol of NH₂ groups) in DMA (2 mL) was added to the stirred solution of (4-ethynylphenyl)methanamine hydrochloride (21.83 mg, 130 µmol) and DIPEA (23 µL, 130 µmol) in DMA (1 mL). The reaction was carried out at 24 °C for 20 h. The polymer was purified using Sephadex LH-20 column with methanol elution and UV detection. The polymer-containing fraction was concentrated in vacuum to 2 mL, and the polymer precursor was isolated by precipitation into an excess of dry acetone and diethyl ether (2/1; 100 mL) followed by filtration and drying in vacuo to yield the title compound 23 (89 mg).

2.2. Synthesis of glycanonamaterials

The syntheses are shown in Scheme 2 and all structures are shown in Scheme 3 of the main text.

General procedure for the synthesis of glycomaterials 24-32. Glycomaterials 24-32 were prepared by the reaction of propargyl groups in polymer precursors 13-15 with the respective azido-functionalized carbohydrates 6 or 7 by Cu⁺-catalyzed azide–alkyne cycloaddition (CuAAC) in water. The first example represents the synthesis of all conjugates except for 28 and 32 containing the highest disaccharide content. Due to their limited solubility in methanol, the purification was modified; the explicit procedure is described below from compound 28.

Example of the synthesis of glycomaterial 25. A solution of CuSO₄·5H₂O (2.0 mg, 16 µmol) in water (23 µL) was added to a mixture of the copolymer 10 (25 mg, 17.4 µmol of propargyl groups), sodium ascorbate (1.60 mg, 16.2 µmol), and disaccharide 6 (8.0 mg, 16.2 µmol) dissolved in water (230 µL). The reaction mixture was bubbled with argon before and after the addition of CuSO₄·5H₂O. The reaction was carried out under magnetic stirring at 24 °C for 1 h. Afterwards, PBS buffer containing EDTA disodium salt (5 wt. %; 1 mL) was added and the title compound was purified by gel filtration using Sephadex G-25 (PD10 column). The polymer fraction was freeze-dried and diluted in methanol (2 mL). An excess of 8-quinolinol was added and after 20 min the sample was purified from the remaining copper using Sephadex LH-20 column with methanol as a mobile phase. The polymer fraction was concentrated under vacuum, diluted in water and freeze-dried to yield the title compound (18.4 mg).

Example of the synthesis of glycomaterial 28. A solution of CuSO₄·5H₂O (5.3 mg, 42.2 µmol) in water (60 µL) was added to a mixture of copolymer 12 (25 mg, 46.7 µmol of propargyl groups), sodium ascorbate (4.2 mg, 42.2 µmol), and disaccharide 6 (20.82 mg, 42.2 µmol) dissolved in water (190 µL). The reaction mixture was bubbled with argon before and after the addition of CuSO₄·5H₂O. The reaction was carried out under magnetic stirring at 24 °C for 1 h. After this, PBS buffer with EDTA disodium salt (5 wt. %; 1 mL) was added and the polymer was purified by gel filtration by Sephadex G-25 using water as a mobile phase. The polymer fraction was freeze-dried and the sample was diluted in 2 mL of DMSO. The 8-quinolinol (42 µmol) was added and after 20 minutes the reaction mixture was poured into an excess of ethyl acetate (100 mL). After centrifugation at 7800 rpm during 5 min, the precipitate was filtered and purified by precipitation from DMSO solution (1 mL) into ethyl acetate (50 mL). The title compound was filtered off, diluted in water and freeze-dried (27 mg).

General procedure for the synthesis of glycomaterials 33-34. Glycomaterials 33-34 were prepared by the reaction of DBCO groups in polymer precursor 16 with the respective azido-functionalized carbohydrates 6 or 7 by Cu(I)-free azide–alkyne cycloaddition in methanol.

Example of the synthesis of glycomaterial 33. A solution of disaccharide 6 (4.7 mg, 9.6 µmol) in methanol (350 µL) was added into the stirred solution of the copolymer 16 (14 mg, 4.6 µmol of DBCO) in methanol (400 µL). The reaction mixture was bubbled with argon and carried out under magnetic stirring at 24 °C for 20 h. The polymer product was purified using Sephadex LH-20 column with methanol elution. The polymer fraction was concentrated under vacuum to approximately 1 mL and isolated by precipitation into an excess of ethyl acetate (50 mL) followed by filtration and drying under vacuum (27.5 mg).

General procedure for the synthesis of glycomaterials 35-37. Glycomaterials 35-37 were prepared by the reaction of ethynylphenyl groups in polymer precursors 17 or 18 with the respective azido-functionalized carbohydrates 6 or 7 by CuAAC in the mixture of water and DMF.

Example of the synthesis of glycomaterial 35. A solution of CuSO₄·5H₂O (2 mg, 16.0 µmol) in water (20 µL) was added to a mixture of copolymer 17 (25 mg, 14.3 µmol of ethynylphenyl groups), sodium ascorbate (1.6 mg, 16.0 µmol), and disaccharide 7 (7.2 mg, 16.0 µmol) dissolved in the mixture of water and DMF (1/1, v/v; 270 µL). The reaction mixture was
bubbled with argon before and after the addition of CuSO₄·5H₂O. The reaction was carried out under magnetic stirring at 24 °C for 20 h. After this, PBS buffer with EDTA disodium salt (5 wt. %, 1 mL) was added and the polymer was purified by gel filtration by Sephadex G-25 using water as a mobile phase. The polymer fraction was freeze-dried and the sample was diluted in methanol (1 mL). An excess of 8-quinolinol was added and after 20 min the sample was purified from the remaining copper using Sephadex LH-20 column with methanol elution. The solvent was evaporated under vacuum, the title compound was diluted in water and freeze-dried (16 mg).

**General procedure for the synthesis of glycomaterials 38-39.** Glycomaterials 38-39 were prepared by the reaction of diethynylphenyl groups in polymer precursor 23 with the excess of the respective azido-functionalized carbohydrates 6 or 7 by CuAAC in the mixture of water and DMF.

**Example of the synthesis of glycomaterial 39.** A solution of CuSO₄·5H₂O (3.74 mg, 15 µmol) in water (45 µL) was added to a mixture of copolymer 23 (19 mg, 12 µmol of ethynyl groups), sodium ascorbate (3 mg, 15 µmol), and disaccharide 7 (6.74 mg, 15 µmol) dissolved in a mixture of water and DMF (1/1, v/v; 210 µL). The reaction mixture was bubbled with argon before and after the addition of CuSO₄·5H₂O. The reaction was carried out under magnetic stirring at 24 °C for 20 h. After this, PBS buffer with EDTA disodium salt (5 wt. %; 1 mL) was added and the polymer was purified by gel filtration using Sephadex G-25 and water as the eluent and the polymer fraction was freeze-dried. The powder was diluted in methanol (2 mL) and an excess of 8-quinolinol was added. After 20 min the title compound was purified from the remaining copper using Sephadex LH-20 column with methanol elution. The solvent was evaporated under vacuum, the title compound 39 was diluted in water and freeze-dried (14 mg).

### 2.3. NMR spectra

The structures of glycomaterials and their precursors were analyzed using NMR Bruker Avance III 600 spectrometer operating at 600.2 MHz using DMSO-d₆ or D₂O as solvents. A representative sample of each group of compounds was chosen to illustrate. In the case of compound 22, the sample was analyzed using ¹H-NMR (300 MHz).

![Figure S12](image-url) 

**Fig S12** ¹H NMR spectrum of copolymer precursor 14 in D₂O.
Fig S13 $^1$H NMR spectrum of copolymer precursor 17 (containing 9.9 mol. % ethynylphenyl linker) in D$_2$O.

Fig S14 $^1$H NMR spectrum of copolymer precursor 18 (containing 20.2 mol. % ethynylphenyl linker) in DMSO-$d_6$. 
Fig S15 $^1$H NMR spectrum of compound 22 in CDCl$_3$.

Fig S16 $^1$H NMR spectrum of copolymer precursor 23 in DMSO-$d_6$. 
Fig S17 $^1$H NMR spectrum of glycopolymer 27 in D$_2$O.

Fig S18 $^1$H NMR spectrum of glycopolymer 31 in D$_2$O.
Fig S19: $^1$H NMR spectrum of glycopolymer 33 in D$_2$O.

Fig S20: $^1$H NMR spectrum of glycopolymer 34 in D$_2$O.
Fig S21 $^1$H NMR spectrum of glycopolymer 35 in D$_2$O. Hydrogen no. 14 is related to the remaining ethynyl groups.

Fig S22 $^1$H NMR spectrum of glycopolymer 37 in D$_2$O.
Fig S23 ¹H NMR spectrum of glycopolymer 38 in D₂O.

Fig S24 ¹H NMR spectrum of glycopolymer 39 in D₂O.
2.4. Hydrodynamic radii

**Table S4** Hydrodynamic radius of copolymers 13, 29-32, 38 and 39

| Compound | Sugar motif with spacer | Content of saccharide (mol. %) | \(R_H\) (nm)\(^a\) |
|----------|-------------------------|-------------------------------|-------------------|
| 13       | -                       | 0                             | 3.5 ± 0.1         |
| 29       | LacdiNAc-N-triazole     | 2.7                           | 3.4 ± 0.1         |
| 30       | LacdiNAc-N-triazole     | 8.1                           | 3.7 ± 0.2         |
| 31       | LacdiNAc-N-triazole     | 12.0                          | 4.6 ± 0.1         |
| 32       | LacdiNAc-N-triazole     | 24.8                          | 3.3 ± 0.2         |
| 38       | (LacdiNAc-OEt-triazole)\(_2\)Ph | 12.3                        | 4.4 ± 0.3         |
| 39       | (LacdiNAc-N-triazole)\(_2\)Ph | 14.0                        | 4.8 ± 0.3         |

\(^a\) Hydrodynamic radii (\(R_H\)) were determined in water by dynamic light scattering at a concentration of 5 mg mL\(^{-1}\). They were not extrapolated to zero concentration.

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