On-Resin Click-Glycoconjugation of Peptoids

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Abstract: Peptoids are unnatural peptide-like oligomers having the side-chain attached to the glycine nitrogen. In order to investigate how such oligomers are affected upon glycoconjugation, a series of glycosylated peptoids has been synthesized. The conjugation between glycosyl residue and peptoid was achieved by azide + alkyne [3+2] cycloaddition (click reaction). The glycosylated peptoids were obtained by either stepwise assembly from sarcosine and glycosylated monomers or global on-resin click glycoconjugation. CD spectroscopic studies were performed on both unglycosylated and glycosylated peptoids with varying chain-length, revealing length and substituent dependences. Additionally, three peptoids were tested for antifreeze activity.

Key words: glycoconjugation, peptoids, click chemistry, triazoles, CD, antifreeze testing

In research areas touching the field of peptide chemistry, problems regarding degradation and low bioavailability are often encountered. Therefore, extensive studies have been made over the years to find ideal unnatural backbone-modified peptides that, for example, possess a defined structure in solution and retain the valuable biological effects of the peptides, but display a higher degree of stability.[1] Peptides composed of N-substituted glycines, so-called peptoids, are examples of such backbone modified peptides. Zuckermann et al. presented in 1992 the first study on this family of oligomers,[2] which have an identical backbone sequence as peptides but differ in the position of the side chains; peptoids have their side chains linked to the backbone nitrogen atoms. Since the side chains are positioned on the nitrogen atom of each monomer, the backbone lacks both stereogenic centers and hydrogen-bond donors. Despite this, peptoids have been shown to fold into stable secondary structures with a preferred handedness due to the attachment of chiral side chains.[3]

Glycosylation of proteins is one of the most frequent post-translational modifications occurring in living cells. Glycoproteins are important in biological recognition processes, such as immuno-differentiation, cell adhesion, cell differentiation, and regulation of cell growth.[4] The carbohydrate residue is often responsible for molecular recognition and biological selectivity.[5] Furthermore, oligosaccharide moieties help to stabilize the conformation of glycoproteins.

Antifreeze glycopeptides and glycoproteins (AFGP) are unique glycoproteins found in the body fluid of polar fish, and are crucial for their survival in subzero environments.[6] Only a single type of AFGP, composed of the repetitive tripeptide sequence $(Ala\text{-}Ala\text{-}Thr)_n$, where the disaccharide β-D-galactosyl-(1→3)-α-*N*-acetyl-D-galactosamine is attached to the hydroxyl group of the threonine residue, has been identified. Their relative molar masses range from 2.6 kDa (n = 4) to 33.7 kDa (n = 50).[6] In order to reveal the contribution of saccharide and peptide moieties to antifreeze properties, a series of glycosylated peptoids was synthesized and investigated with respect to conformation and potential antifreeze properties. Noteworthy, AFGP with monosaccharide moieties also display antifreeze properties.

N-,[7] O-,[8] and C-glycosylation[8b,9] of peptoids have been reported. Herein, we present the synthesis of triazole-linked glycosylated peptoids utilizing the reaction known as 'click chemistry', where azides and alkynes undergo [3+2] cycloaddition in the presence of catalytic amounts of a copper salt. This approach is very efficient to regioselectively obtain 1,4-disubstituted 1,2,3-triazoles.[10] Similarly successful click glycoconjugation strategies for peptides have earlier been reported by the groups of Rutjes[11] and Massi.[12]

Two general strategies were envisaged, stepwise glycopeptoid assembly from glycosylated monomers and sarcosine as well as global click glycoconjugation of alkynyl substituted peptoids. For the synthesis of the glycosylated peptoid building block, Fmoc-protected alkynyl peptoid monomer **3** and glycosyl azides **4**–**6** (Table 1) were used as building blocks in the click reactions. Alkyne **3** was synthesized in 75% overall yield using a two-step strategy developed by Liskamp et al.[13] (Scheme 1). Propargylamine was treated with ethyl bromoacetate under basic conditions to give the ester **2**. Subsequently, ester saponification and Fmoc-protection of the secondary amine was achieved in a one-pot reaction. Ester **2** was stirred under strongly basic conditions (4 M NaOH) for 30 minutes. Then the pH was adjusted to 9–9.5 and *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (Fmoc-OSu) was added and left to react overnight.

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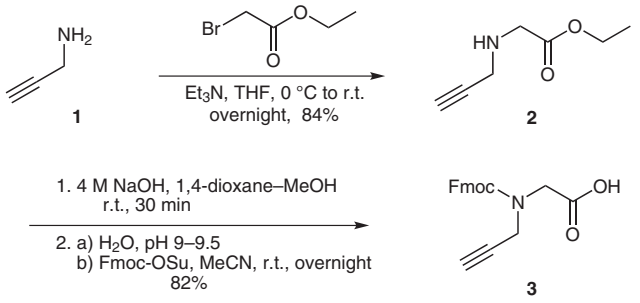
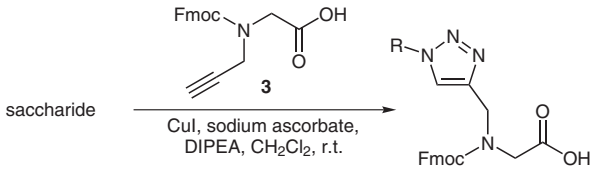
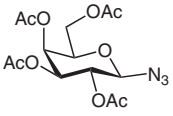
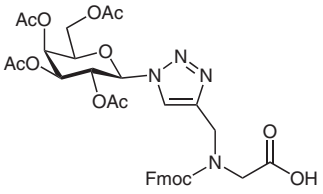
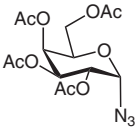
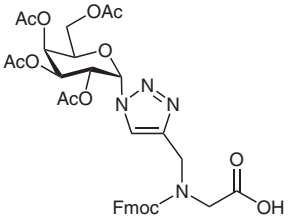
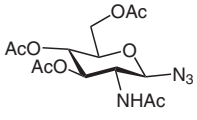
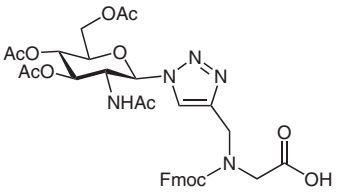


Scheme 1 Synthesis of Fmoc-protected alkynyl building block **3**

Table 1 Variation of the Carbohydrate Moiety



Entry	Saccharide	Glycopeptoid building block (yield, %)[a]
1	 **4**	 **7** (86%)
2	 **5**	 **8** (75%)
3	 **6**	 **9** (75%)

[a] Isolated yield.

The glycosyl azides **4**–**6** were all synthesized according to methods developed by the group of Bernardi.[14] The synthetic strategy for glucose was followed for galactosyl azides **4**[15] and **5**.[16]

The best coupling results for reacting the azides with the alkyne moiety, forming glycosylated peptoid monomers **7**–**9**, were observed under modified Sharpless conditions,[17] involving CuI (1 equiv), sodium ascorbate (1 equiv), and *N*,*N*-diisopropylethylamine (DIPEA, 2 equiv) in dichloromethane. This strategy provided the desired products in high yields.

Fmoc-based solid-phase synthesis was utilized to synthesize oligomers **10**–**20** (Figure 1). Due to the high efficiency by which the glycosyl azides and the alkyne could be reacted, two different routes for obtaining glycosylated peptoid oligomers were viable. In the first case, the glycosylated monomers were used as building blocks in the solid-phase synthesis (Scheme 2, route A), whereas the Fmoc-protected alkyne-substituted peptoid monomer **3** was used in the second case. All carbohydrates were then simultaneously attached to the resin-bound full-length peptoid by on-resin click-glycoconjugation (Scheme 2, route B).

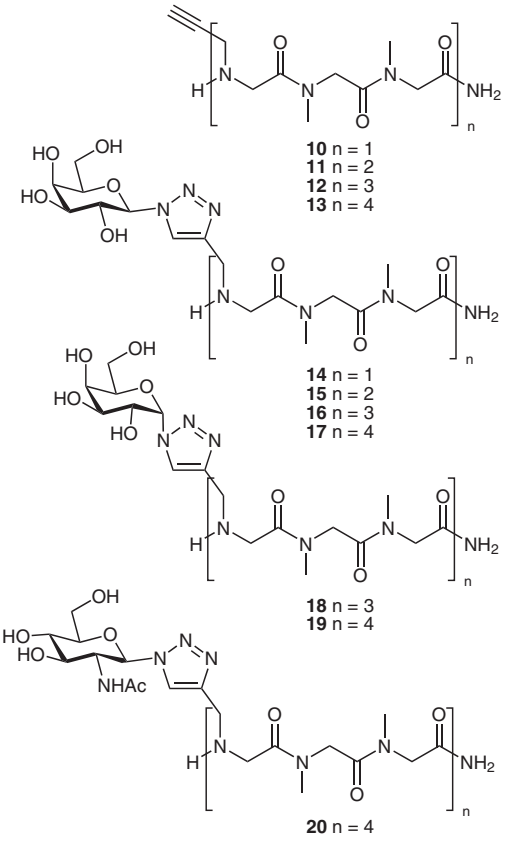


Figure 1 Synthesized peptoid oligomers **10**–**20**

As the second route requires less number of steps, less amount of glycoside building blocks and less purification steps, this pathway is to be preferred. Furthermore, due to the on-resin reaction, the workup is simplified; the salts and unreacted azide are removed by repetitive washing of the resin.

After completion of the synthesis, the peptoid oligomer was cleaved from the resin using a standard cleavage cocktail [trifluoroacetic acid–water–triisopropylsilane (TFA–H_2O–TIPS); 95:2.5:2.5]. The fully deprotected glycosylated peptoids were found to be very polar and, therefore, purification by preparative reversed phase HPLC was performed both before and after the cleavage of the glycoside acetyl protecting groups.

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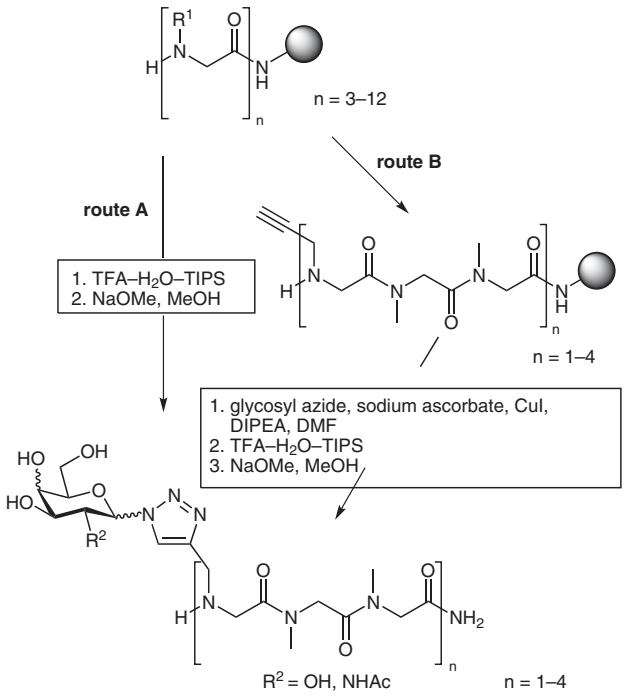

Scheme 2 Routes for obtaining glycosylated peptoid oligomers: direct use of glycosylated monomers and stepwise solid-phase peptoid synthesis (route A, R^1 = glycosylated triazolylmethyl residues or methyl) or global on-resin glycoconjugation (route B, R^1 = propargyl or methyl)

The ability of the peptoid oligomers **10**–**20** to fold into ordered secondary structures was investigated by circular dichroism (CD) spectroscopy in trifluoroethanol (TFE) (c = 0.4 mM, 20 °C) as shown in Figure 2.

As the unglycosylated peptoids completely lack chiral elements, the oligomers **10**–**13** did not show any CD effects. For the glycosylated oligomers **14**–**20**, where chirality resides in the carbohydrate moieties, substitution and length dependent CD effects could be observed. The shortest oligomer, the glycosylated trimer **14**, gave rise to a signature similar to that observed for the unglycosylated peptoids. The glycosylated hexamer **15** showed a CD spectrum implying the existence of a pattern with low molar ellipticity per residue yet with a signature corresponding to a somewhat ordered structure.

Interestingly, the differences between α- and β-anomers were significant. The three longer (9 and 12 residues, respectively) β-glycosides **16**, **17**, and **20** gave rise to CD patterns with rather similar molar ellipticity per residue, which indicates the existence of ordered secondary structures (Figure 2). The α-glycosylated analogues **18** and **19**, however, gave rise to CD patterns, which resemble each other concerning the molar ellipticity per residue, but differ in shape. Whereas the dodecamer **19** has a similar signature as its β-glycosylated analogue **17**, the nonamer **18** has a shifted signature concerning the minima, zero crossing, and maxima.

The CD spectra of **16** and **17** are both characterized by a minimum, zero crossing, and a maximum at 213, 205, and 187 nm, respectively. Glycosylated peptoid **20** gave rise to similar CD spectra, but the zero crossing and minimum were slightly shifted (217 and 207 nm, respectively). The signatures of the glycosylated peptoids resembles the CD shape of a β-sheet like spectrum (negative band at ~215 nm and positive band at ~195 nm), but the lower band does also resemble the signature of a β-turn. The resemblance of β-turn-like spectra is also found in the low

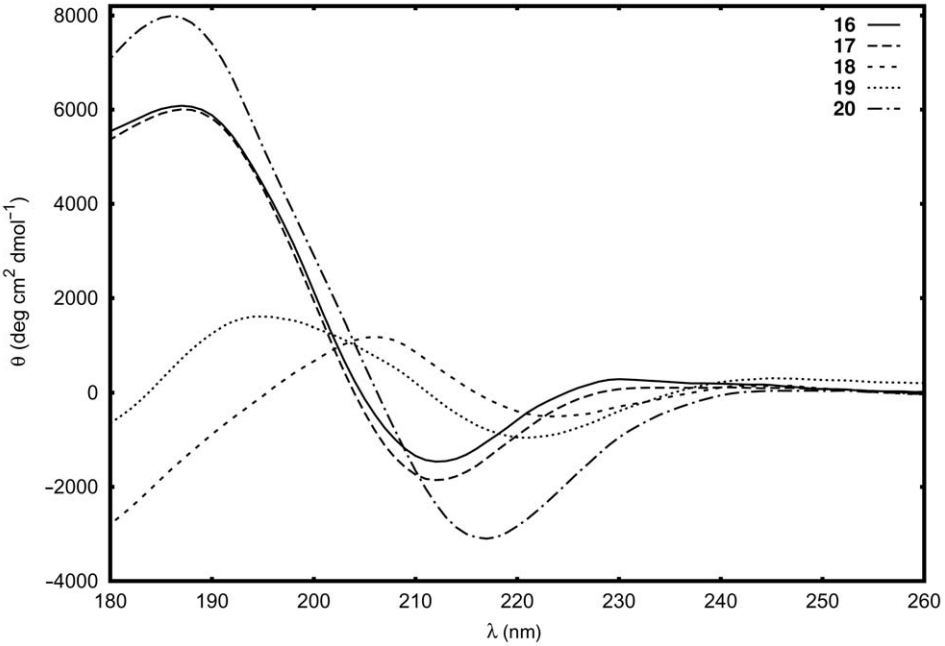

Figure 2 CD spectra of glycosylated peptoids **16**–**20** in TFE, taking into account the number of chromophores; all spectra were acquired at room temperature and at c = 0.4 mM

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intensities of the CD spectra given for the glycosylated peptoids. Moreover, the signatures are also similar to the CD shapes given for Zuckermann's peptoid homopolymer (*S*)-*N*-{1-[(morpholino)carbonyl]ethyl}glycine[3b] as well as the well-established CD signature of 3_{14}-helices of β-peptides.[18]

In addition, dodecamer **20** gave rise to a CD pattern with higher molar ellipticity per residue as compared to the analogue **17**. The observed higher molar ellipticity of **20** was first suspected to be due to its additional amide bond at the C2 of the monosaccharide. This was, however, confirmed not to be the case by measuring CD spectra of Fmoc-deprotected monomers **7**–**9**. Nevertheless, the observed increase of **20** is most likely due to the acetamido group as it provides an additional stabilizing hydrogen bond donor and acceptor.

Taking the number of chromophores into account, the molar ellipticites per residue were similar for the nonamer **16** and the dodecamer **17**. However, as neither the trimer **14** nor the hexamer **15** gave comparable results it can be concluded that the CD pattern arises due to an ordered structure and not due to the monomers themselves.

In addition, the dodecameric glycosylated peptoids **17**, **19**, and **20** were tested for antifreeze activity using the ice recrystallization inhibition technique. The peptoids (c = 2 mg/mL) were dissolved in aqueous solutions containing 45 wt% sucrose. A pure sucrose solution was used as control, and a solution of poly(vinyl alcohol), PVA5.8, was used as reference, since PVA has been shown to be an ice structuring polymer and to readily inhibit ice recrystallization.[19,20] All of the peptoid solutions gave results that were not significantly different from those of the control solution without peptoids. No ice structuring effects were observed in the peptoid solutions. Therefore, we conclude that none of the peptoids **17**, **19**, and **20** exhibit any signs of antifreeze activity (Figure 3).

It has been established previously by Tachibana et al.[21] that the acetamido group plays an essential, but not sufficient role for the antifreeze activity of AFGP. In their studies, antifreeze activity was achieved by replacing the OH group positioned at C2 of the monosaccharide with an acetamido group, as is also the case for the peptoid **20**. However, our results suggest that antifreeze activity relies not only on the carbohydrate moieties but also on other structural requirements. Whether this is due to the lack of NH groups in the backbone, the distance between the carbohydrate moieties, or even other factors remains to be shown in future studies.

In conclusion, we have successfully synthesized glycosylated peptoid monomers by utilizing the click reaction. Furthermore, glycosylated peptoid oligomers with different chain lengths were synthesized by solid-phase chemistry either in a stepwise manner or by global on-resin click glycoconjugation. Structural investigations by CD show that both the carbohydrate itself and the stereochemistry at the anomeric centre had crucial effects on the formation of ordered secondary structures. Length dependence could also be observed.

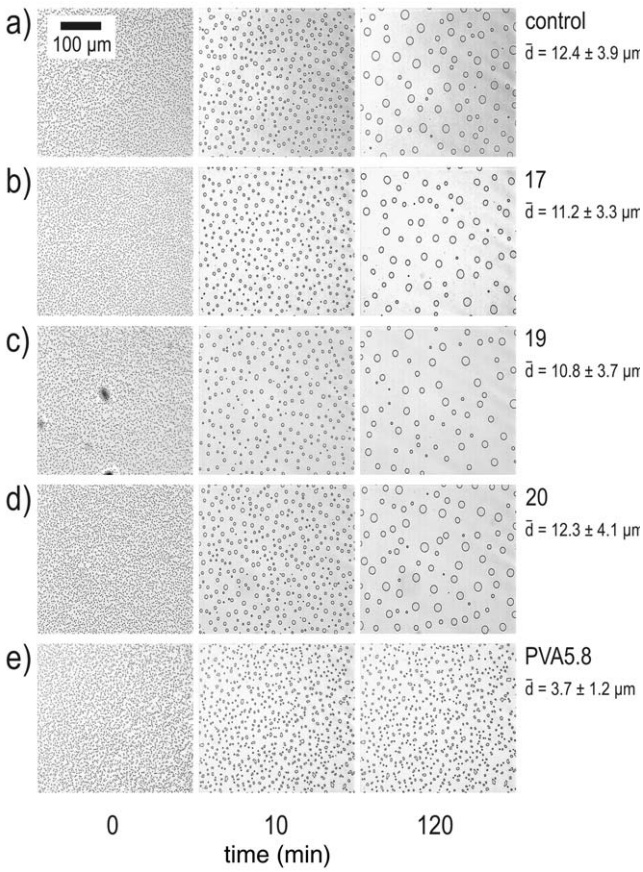

Figure 3 Photographs of ice crystals in aqueous 45 wt% sucrose solutions after 0, 10, and 120 min of annealing time at a temperature of –8 °C: a) control solution without peptoids; b) through d) solutions with peptoids **17** (b) **19** (c) and **20** (d) at a concentration of 2 mg/mL; e) Reference solution with PVA5.8 at a concentration of 2 mg/mL; to the right of each series the average diameter of the crystals after 120 min is given together with its standard deviation

Despite the structural similarities to AFGP and monoglycosylated AFGP analogues, no antifreeze activity was detected for the glycosylated peptoids.

THF was distilled twice; first over CaH_2 followed by a second distillation over Na and benzophenone. CH_2Cl_2 was distilled over K_2CO_3 and Et_2O over KOH. DMF and EtOAc were distilled prior to use. Petroleum ether (PE) used had a boiling range of 30–60 °C. Analytical TLC analyses were conducted using silica gel plates. The compounds were visualized by using either a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, $Ce(SO_4)_2$, and H_2SO_4 in H_2O or a solution of 5% ninhydrin in EtOH (for visualizing free amines). MALDI ToF mass spectra were recorded on a Perseptive Biosystems Voyager DE BioSpectrometry™ Workstation. Optical rotation was measured on a Jasco DIP-360 digital polarimeter, UV measurements were made on a Helios Gamma Spectronic Unicam, and IR spectra were recorded with a Jasco FT/IR-410 spectrometer. CD spectra were recorded on a Jasco J-810 CD spectrometer. Melting points were measured on a Büchi melting point B-540 and are uncorrected. Analytical RP-HPLC was done on a Thermo Separation Products instrument with a P4000 pump, a SN4000 signal converter, a AS100 autosampler and a UV6000LP UV detector, set to detect at

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both 220 nm and 280 nm. The products were separated on a Phenomenex Jupiter C-18 column (250 × 4.60 mm, 5 μm) with a linear gradient of MeCN–H_2O containing a small amount of TFA (either A: H_2O–MeCN–TFA, 95:5:0.1; B: MeCN–H_2O–TFA, 95:5:0.1 or A: H_2O–MeCN–TFA, 98:2:0.05; B: MeCN–H_2O–TFA, 98:2:0.05). Preparative RP-HPLC was also performed on a Thermo Separation Products instrument with a P4000 pump, a SN4000 signal converter, and a UV1000 UV detector set to 220 nm. The products were separated on a Phenomenex Jupiter C-18 column (250 × 21.20 mm, 10 μm) with a linear gradient of MeCN–H_2O containing a small amount of TFA (either A: H_2O–MeCN–TFA, 95:5:0.1; B: MeCN–H_2O–TFA, 95:5:0.1 or A: H_2O–MeCN–TFA, 98:2:0.05; B: MeCN–H_2O–TFA, 98:2:0.05). NMR measurements were done at ambient temperature using the residual solvent signal (DMSO-d_6) or the TMS signal ($CDCl_3$) as internal reference.

H-*N*-(Propargyl)Gly-OEt (2)

Ethyl bromoacetate (100 mmol, 11.1 mL) in THF (30.0 mL) was added dropwise to a cooled (ice-bath) solution of propargylamine (100 mmol, 6.86 mL) and Et_3N (200 mmol, 27.9 mL) in anhyd THF (30.0 mL). The ice-bath was kept for 30 min before the reaction mixture was allowed to attain r.t. After stirring overnight, the mixture was filtered to remove ethylamine hydrobromide. The filtrate was washed repeatedly with Et_2O and the organic phase was removed under reduced pressure. The crude **2** (84.0 mmol, 11.9 g) did not require further purification before the next step.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.20 (q, *J* = 7.1 Hz, 2 H), 3.51 (s, 2 H), 3.49 (d, *J* = 2.4 Hz, 2 H), 2.24 (m, 1 H), 1.29 (t, *J* = 7.1 Hz, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.9, 81.2, 72.0, 60.9, 49.2, 37.7, 14.2.

***N*-(9-Fluorenylmethoxycarbonyl)-*N*-(propargyl)glycine (3)**

Aq 4 M NaOH (20.0 mL) was added to a solution of **2** (84.0 mmol, 11.9 g) in dioxane (100 mL) and MeOH (35.0 mL). After stirring for 40 min, the reaction mixture was concentrated in vacuo. The resulting sodium salt was dissolved in H_2O (80.0 mL) and the pH was adjusted to 9–9.5 with concd HCl. A solution of Fmoc-OSu (84.0 mmol, 28.3 g) in MeCN (130 mL) was subsequently added and the mixture was left to stir overnight at r.t.. The MeCN was removed under reduced pressure and the aqueous phase was acidified (pH 1–2) with concd HCl. The aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL) and the combined organic phases were washed with H_2O (50 mL) and brine (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Recrystallization from EtOAc–hexane gave **3** as white crystals in 82% yield (66.0 mmol, 22.1 g); mp 130–132 °C; R_f = 0.33 (CH_2Cl_2–MeOH–AcOH, 97:2.5:0.5).

In the ^{1}H NMR spectrum of **3** only one major conformer was observed. In the ^{13}C NMR spectra, however, the existence of rotamers was observed as either double sets of peaks or broad signals.

IR (KBr): 3851 (s), 2959 (w), 2893 (w), 1736 (s), 1678 (s), 1541 (w). 1479 (m), 1460 (m), 1403 cm^{-1} (m).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.87 (s, 1 H), 7.89 (m, 2 H), 7.72 (m, 1 H), 7.60 (m, 1 H), 7.35 (m, 4 H), 4.30–4.00 (m, 7 H), 3.30 (m, 1 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 170.99/170.87, 155.48/155.45, 144.1, 141.19/141.15, 128.2, 127.6, 125.75/125.56, 120.6, 79.76/79.57, 68.0 (br), 48.33/47.72, 46.98/46.89, 37.63/37.45.

***N*-(9-Fluorenylmethoxycarbonyl)-*N*-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1*H*-1,2,3-triazol-4-ylmethyl]glycine (7); Typical Procedure**

Azide **4** (4.00 mmol, 1.49 g) and the acetylene **3** (4.00 mmol, 1.34 g) were mixed with CuI (4.00 mmol, 0.76 g) and sodium ascorbate (4.00 mmol, 0.79 g), and dissolved in anhyd CH_2Cl_2 (20 mL). DIPEA (8.00 mmol, 1.40 mL) was added to the mixture. After ca. 5 min, a yellow-green solution was obtained and left to stir overnight at r.t.. The mixture was then diluted with CH_2Cl_2 (30 mL) and washed with dil HCl (pH 1–2). The aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL) and the combined organic phases were dried (Na_2SO_4). Flash column chromatography (CH_2Cl_2–MeOH–AcOH, 97:2.5:0.5) followed by lyophilization to remove traces of AcOH gave **7** as a white fluffy material (3.40 mmol, 2.40 g, 86%); $[\alpha]_D^{21}$ –15.1 (*c* = 1.0, CH_2Cl_2); R_f = 0.40 (CH_2Cl_2–MeOH–AcOH, 95:4:1).

IR (KBr): 3472 (w), 2970 (w), 1757 (s), 1557 (w), 1541 (w), 1454 (m), 1428 cm^{-1} (m).

MS: *m*/*z* calcd for $C_{34}H_{36}N_4O_{13}$: 708.23; found: 709.38 [M + H], 731.33 [M + Na], 747.34 [M + K].

The ^{1}H and ^{13}C NMR spectra of **7** showed the presence of two rotamers, hence the Fmoc group was cleaved in order to assign the structure. The NMR data given below correspond to Fmoc-deprotected **7**.

***N*-[1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-1*H*-1,2,3-triazol-4-ylmethyl]glycine**

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.29 (s, 1 H), 5.90 (d, *J* = 9.4 Hz, 1 H), 5.57 (d, *J* = 3.1 Hz, 1 H), 5.52 (dd, *J* = 9.4, 9.4 Hz, 1 H), 5.31 (dd, *J* = 10.7, 3.1 Hz, 1 H), 4.57 (m, 2 H), 4.31 (dd, *J* = 6.3, 6.3 Hz, 1 H), 4.19 (d, *J* = 6.3 Hz, 2 H), 3.91 (m, 2 H), 2.22 (s, 3 H), 2.05 (s, 3 H), 2.03 (s, 3 H), 1.88 (s, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.5, 170.2, 169.9, 169.6, 169.4, 138.2, 125.0, 86.3, 74.0, 70.5, 68.3, 66.6, 61.0, 46.9, 41.4, 20.6, 20.5, 20.4, 20.0.

***N*-(9-Fluorenylmethoxycarbonyl)-*N*-[1-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-1*H*-1,2,3-triazol-4-ylmethyl]glycine (8)**

Triazole **8** was synthesized according to the procedure and on the same scale (4.00 mmol) as compound **7** using carbohydrate **5** as the azide building block, giving **8** as a white fluffy material (3.00 mmol, 2.12 g, 75%); $[\alpha]_D^{21}$ +58.5 (*c* = 1.0, CH_2Cl_2); R_f = 0.45 (CH_2Cl_2–MeOH–AcOH, 95:4:1).

IR (KBr): 3476 (w), 2974 (w), 1756 (s), 1560 (w), 1538 (w), 1452 (m), 1427 cm^{-1} (m).

MS: *m*/*z* calcd for $C_{34}H_{36}N_4O_{13}$: 708.23; found: 709.63 [M + H], 731.65 [M + Na].

The ^{1}H and ^{13}C NMR spectra of **8** showed the presence of two rotamers, hence the Fmoc group was cleaved in order to assign the structure. The NMR data given below correspond to Fmoc-deprotected **8**.

***N*-[1-(2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl)-1*H*-1,2,3-triazol-4-ylmethyl]glycine**

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.20 (s, 1 H), 6.51 (d, *J* = 6.3 Hz, 1 H), 6.12 (dd, *J* = 10.7, 3.1 Hz, 1 H), 5.69 (d, *J* = 2.5 Hz, 1 H), 5.60 (dd, *J* = 10.7, 5.7 Hz, 1 H), 4.61 (dd, *J* = 6.3, 6.3 Hz, 1 H) 4.58 (m, 2 H), 4.16–4.05 (m, 2 H), 3.99 (br s, 2 H), 2.22 (s, 3 H), 2.02 (s, 3 H), 1.99 (s, 3 H), 1.87 (s, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.6, 170.4, 170.1, 170.0, 169.4, 137.3, 128.0, 82.6, 70.6, 67.5, 67.5, 66.5, 61.1, 47.4, 41.4, 20.6, 20.6, 20.6, 20.1.

***N*-(9-Fluorenylmethoxycarbonyl)-*N*-[1-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-ylmethyl]glycine (9)**

Triazole **9** was synthesized in 2.80 mmol scale according to the same procedure as compound **7** using carbohydrate **6** as azide building block, giving **9** as a white fluffy material (2.10 mmol, 1.49 g,

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75%); $[\alpha]_D^{21}$ –21.0 (c = 1.0, CH_2Cl_2); R_f = 0.38 (CH_2Cl_2–MeOH–AcOH, 90:9:1).

IR (KBr): 3393 (w), 2955 (w), 1749 (s), 1699 (m), 1541 (w), 1507 (w), 1456 (m), 1429 cm^{-1} (m).

MS: m/z calcd for $C_{34}H_{37}N_5O_{12}$: 707.24; found: 730.24 [M + Na], 746.29 [M + K].

The ^1H and ^{13}C NMR spectra of **9** showed the presence of two rotamers, hence the Fmoc group was cleaved in order to assign the structure. The NMR data given below correspond to Fmoc-deprotected **9**.

***N*-[1-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-ylmethyl]glycine**

^1H NMR (500 MHz, DMSO-d_6): δ = 8.41 (s, 1 H), 8.15 (d, J = 9.4 Hz, 1 H), 6.13 (d, J = 10.0 Hz, 1 H), 5.35 (dd, J = 10.0 Hz, 1 H), 5.14 (dd, J = 9.4, 9.4 Hz, 1 H), 4.62 (ddd, J = 10.0, 10.0, 10.0 Hz, 1 H), 4.33–4.27 (m, 3 H), 4.18 (dd, J = 12.6, 5.0 Hz, 1 H), 4.06 (dd, J = 12.6, 1.9 Hz, 1 H), 3.89 (br s, 2 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.96 (s, 3 H), 1.61 (s, 3 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 170.5, 170.1, 170.0, 169.9, 169.4, 138.8, 125.1, 85.5, 73.9, 72.7, 68.4, 62.2, 52.5, 46.7, 41.3, 22.8, 21.0, 20.9, 20.8.

Peptoid Oligomers 10–20

Two synthetic pathways are available for the synthesis of triazole-linked glycosylated peptoid oligomers; either the Fmoc-protected glycosylated peptoid monomers **7–9** were used as building blocks in the on resin synthesis or the acetylene building block **3** was employed. In the latter case, global glycoconjugation was performed after the final Fmoc-deprotection step, but before cleavage from the resin.

Stepwise Solid-Phase Synthesis: For the solid-phase synthesis, Rink amide AM resin (200–400 mesh) was used. The loading of the first peptoid monomer and coupling of the following residues were performed in a similar fashion: Fmoc-deprotection with 20% piperidine in DMF, standard washing (8 × 3 min, 5.00 mL DMF) followed by coupling of the monomer for 1.5–2 h. Before the peptoid was added to the resin, the acid was activated for 3 min using a mixture of HATU and DIPEA in DMF. The synthesis cycle was repeated until the desired peptoid sequence was assembled. Between every step of the synthesis, the resin was washed using the standard washing procedure. After final Fmoc deprotection, the peptoid was cleaved from the resin by treating it with a standard cleavage mixture (TFA–H_2O–TIPS, 95:2.5:2.5) for 1 h. The resin was repeatedly washed with TFA and CH_2Cl_2; the combined washing phases were evaporated and the peptoid was precipitated with cold Et_2O. Oligomers **14–20** were purified both before and after removal of the acetyl protecting groups on the carbohydrate moiety. The deprotection was done by overnight treatment with 1% NaOMe in MeOH (pH 9–10).

Global On-Resin Glycoconjugation: For each acetylene group present, 2 equiv of azide, 3 equiv of sodium ascorbate, and 6 equiv of CuI were used. Additionally, on a 0.10 mmol scale, anhyd DMF (5.00 mL) and DIPEA (2.00 mL) were used in the click reaction, leaving the resin suspension to be shaken overnight. The resin was thereafter washed repeatedly with H_2O and *i*-PrOH in order to remove the copper salts, followed by DMF (3 × 7.00 mL), *i*-PrOH (3 × 7.00 mL) and once again with DMF (7.00 mL). Cleavage from the resin was subsequently done using standard cleavage cocktail. The exact mass data of compounds **10–20** are presented in Table 2.

Table 2 Exact Mass Determination

Peptoid	Calculated mass	Calculated ion mass	Measured ion mass
10	254.13789	255.14502	255.14517
11	491.24923	492.25651 (+ H^+)	492.25623 (+ H^+)
12	728.36057	729.36785 (+ H^+)	729.36721 (+ H^+)
13	965.47191	966.47919 (+ H^+)	966.48075 (+ H^+)
14	459.20776	460.21465	460.21465
15	901.38897	902.39625 (+ H^+)	902.39602 (+ H^+)
16	1343.57018	1344.57746 (+ H^+)	1344.57699 (+ H^+)
17	1785.75140	1786.75867 (+ H^+)	1786.75387 (+ H^+)
18	1343.57018	683.78334 (+ H^+ + Na^+)	683.78210 (+ H^+ + Na^+)
19	1785.75140	893.88297 (+ 2 H^+)	893.88372 (+ 2 H^+)
20	1949.85759	986.92705 (+ H^+ + Na^+)	986.92869 (+ H^+ + Na^+)

Experimental Procedure for Testing Antifreeze Activity

The antifreeze activity experiments were performed with an Olympus BX51 optical microscope, used in transmitted light (bright field) mode and equipped with a Linkam MDBCS 196 cold stage. The samples were located on a temperature-controlled silver block inside the cold stage. Both the interior and the upper window of the cold stage were purged with dry N_2 to avoid H_2O condensation from ambient air during the experiments. The investigated aqueous solutions were prepared with double-distilled H_2O and each contained 45 wt% sucrose and 2.00 mg/mL of either one of the peptoids **17**, **19**, or **20**, or poly(vinyl alcohol), and PVA5.8 (number average molar mass M_n = 3.4 kDa; weight average molar mass M_w = 5.8 kDa). PVA5.8 was used as a reference because previous studies have shown that it exhibits antifreeze activity and ice structuring effects.[19,20] Droplets (2.00 μL) of these solutions were placed between two circular glass cover slips, each 14 mm in diameter. The cover slips were pressed together to produce a sample film with a final thickness of about 10–20 μm. Each sample was cooled rapidly (at 20 °C/min) to –50 °C to produce polycrystalline ice, and was then reheated at 10 °C/min to –8 °C, where it was annealed for 2 h. During this period photographs were taken every min using a digital video camera (image size: 1280 × 1024 pixels corresponding to a field of view of 383 × 307 μm). Figure 3 shows photographs at the beginning, after 10 min, and after 120 min of annealing at –8 °C. The ice recrystallization process is readily observable in the control solution in panel a) where the average ice crystal diameter increases from an initial value of 2.00 μm to about 12.4 μm after 120 min. For comparison, the average ice crystal diameter after 120 min of the solutions containing the peptoids **17**, **19**, or **20** (panels b to d) are not significantly different from that of the control solution without peptoids. In contrast, the ice crystals in the reference solution containing PVA5.8 (panel e) are significantly smaller, in agreement with literature studies ascribing PVA a weak antifreeze activity.[17,18] In addition, none of the peptoid solutions showed any signs of modifications of the ice crystal habits supporting the above conclusions that no specific interactions between ice surfaces and the peptoids **17**, **19**, and **20** do occur. In conclusion, none of the peptoids **17**, **19**, and **20** show any signs of antifreeze activity.

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