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Chemo- and Stereoselective Glycosylation of Hydroxylamino Derivatives : A Versatile Approach to Glycoconjugates

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Abstract: A general method for the stereoselective coupling of unprotected oligosaccharides with any substrate containing a N,O-disubstituted hydroxylamine group is described. The cyclic nature of the oligosaccharide reducing unit is preserved and the substrate glycosylated with high diastereoselectivity to sugar through an amino ($N[OR_2]$-) or an aminoxy ($N[R_1]$-O-) linkage. Due to the uniquely high chemical reactivity and specificity of disubstituted hydroxylamine toward the sugar reducing end, neither protecting groups nor activation methods are required to perform the reaction in aqueous solution. The characteristic features and the scope of this new type of glycosylation reaction are exemplified for the chemoselective synthesis of model glycopeptides. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Glycopeptides; Glycosylation; Stereocontrol.

INTRODUCTION

The critical role of specific oligosaccharide structures in the biological function of many glycoproteins is now well appreciated[1]. The importance of protein-bound oligosaccharides in cell-cell recognition events and in modulating protein folding and stability has been highlighted in a number of recent landmark studies, inspiring the development of elegant chemical[2] and enzymatic[3] approaches for the construction of glycoproteins with defined, homogeneous glycoforms. However, the regiospecific chemical approach to glycoconjugates remains a challenging task due to the requirement for extensive protecting group manipulations and the chemical sensitivity of glycosidic linkages. Convergent methods exploiting available sources of carbohydrates appear to give flexible and rapid entry to the synthesis and functional screening of carbohydrates-based-antigens or conjugates. In this context, methods employing the chemoselective oxime bond formation have been introduced recently for rapid access to neoglycopeptides. Chemoselective ligation methodologies[4] are based on the introduction of mutually and uniquely reactive moieties (e.g. a hydroxylamino group and an aldehyde group) on to fragments that makes coupling of these fragments possible in aqueous solution without protecting groups or activation procedures. Oxime-bound carbohydrate molecules have been readily obtained from ligation of unprotected oligosaccharides to free-hydroxylamino-peptide fragments[5,6] and α-O-glycosylated peptide-aldehydes to hydroxylamino-galactose derivatives[7]. However, the presence of the E/Z oxime isomers or equilibrium between cyclic and linear oxime-sugar forms has led to conformational heterogeneity of the glycoconjugate that may significantly hamper the general scope and potentiality of these convenient approaches.

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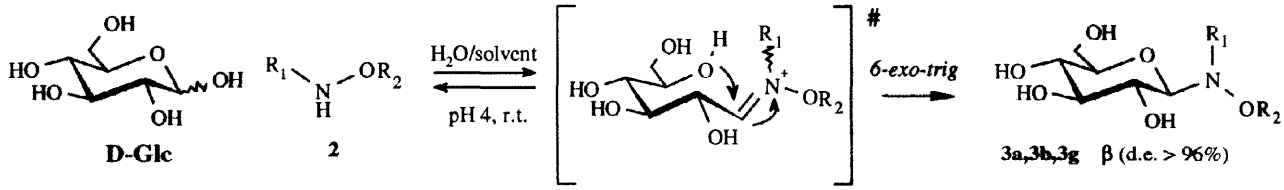
We study here the glycosylation of *N,O*-substituted hydroxylamines with reducing sugars (Scheme 1). We report that, in contrast to oxime formation[5] or reductive amination[8] methods, the cyclic nature of the saccharide reducing unit is completely preserved. We also demonstrate that the reaction proceeds chemoselectively and with high anomeric stereoselection without requirement of activation at the anomeric center and protection of functional groups. We illustrate some possible applications conjugating a model tripeptide with natural mono- di- and trisaccharides.

RESULTS AND DISCUSSION

In order to assess the potential of this approach, alkyl *N,O*-substituted hydroxylamines **2** were reacted with available carbohydrate molecules **1a-g**. We were pleased to find that the reaction effectively provides the expected glycosylated compounds **3a-g**[9] in good and reproducible yields (Table 1). The reaction proceeded under mild conditions in aqueous buffer (pH 4) or in polar organic solvents (generally a mixture of acetic acid and DMF) and was generally complete within 20 hours for all carbohydrates but mannose and lactose.



Scheme 1. Principle of the chemoselective glycosylation of *N,O*-substituted hydroxylamine **2** with D-Glucose.

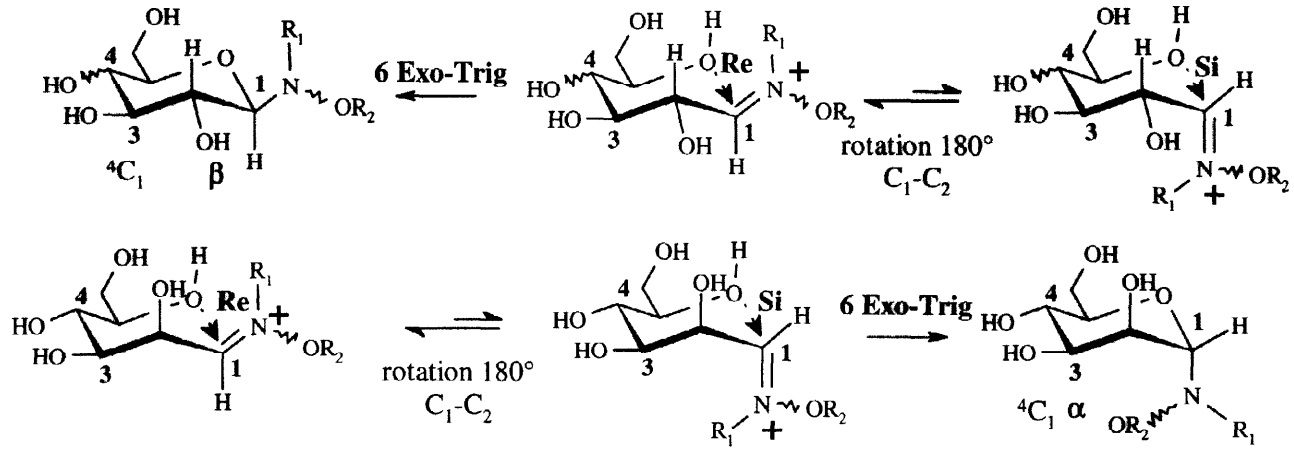
Compounds **3a-g** were obtained exclusively in the pyranose form with a typical $^4C_1$ conformation as ascertained by NMR investigations[10,11] in solution except for galactose and mannose derivatives **3d** and **3f**, where 20% and 13% of the furanose form was also detected respectively[12,13].

Table 1. Yields and Reaction Times of Glycosylation of *N,O*-substituted Hydroxylamines.

Product	Sugar 1a-g	R_1	R_2	Times (h)	Yield (%)
3a	D-Glc	Et	Bn	20	90
3b	D-Glc	Me	Me	20	92
3c	D-GlcNAc	Me	Me	20	40
3d	D-Gal	Et	Bn	20	80
3e	Lactose	Me	Me	60	95
3f	D-Man	Me	Me	144	60
3g	D-Glc	Octyl	Me	20	30

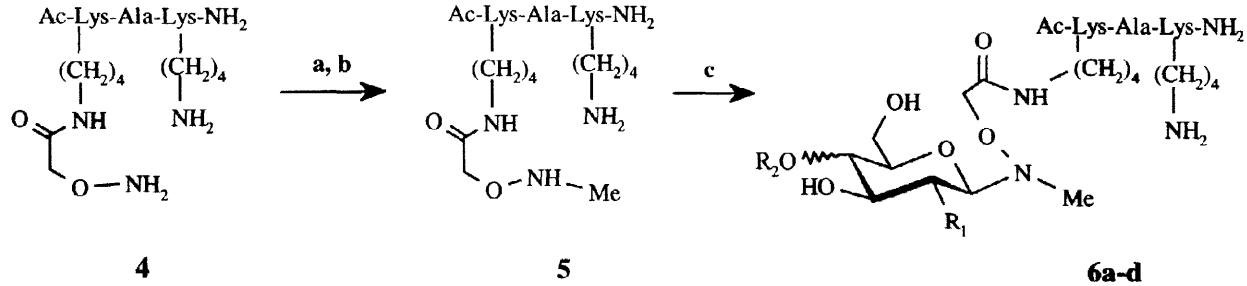
Noteworthy is the analysis of $^3J_{H1\text{-}H2}$ and $^1J_{^{13}C1\text{-}H1}$ coupling constant values which indicates that the reaction proceeds with high diastereoselectivity (d.e. > 96%)[10,11]. Only the β-pyranose forms were observed for the glucose and galactose derivatives (**3a-e** and **3g**), whereas for the mannose derivative **3f** the α anomeric configuration was preferred. These high diastereoselectivities are indicative of a thermodynamic control of the

intermediate during the ring closure reaction. The presence of an oxy-imminium intermediate (Scheme 1) is supported by *in situ* $NaBH_3CN$ quenching of the reaction which afforded the corresponding open chain hydroxylamine derivative. The β or α pyranose forms obtained here can be rationalised by a preferential 6-exo-trig ring closure with OH-5 attacking the Re or Si oxy-iminium face respectively (Scheme 2) while the furanoside forms originate from 5-exo-trig ring closure with OH-4.



Scheme 2. 6-exo-trig ring closure of the oxy-iminium intermediate with OH-5 attacking preferentially the Re or Si face to afford diastereoselectively β-galacto or β-gluco (top) and α-manno (bottom) derivatives **3a-g**.

With these results in hand, we exploited the method to the β-*N*-glycosylation of a model peptide (Scheme 3). N[Me]-O-peptide **5** was obtained simply by reduction of the formaldehyde-oxime of the corresponding primary aminooxy peptide **4** (Scheme 3, steps a and b). As exemplified in Table 2 for D-glucose derivatives, the desired peptides **6a-d** were obtained in moderate to good yields with the β-pyranose configuration as ascertained by NMR spectroscopy ($^3J_{H1-H2} > 8$ Hz).



Scheme 3. Preparation and chemoselective glycosylation of aminooxy-peptide **5** with D-Glucose derivatives. a) CH_2O, Aqueous sodium acetate buffer, pH 4. b) $NaBH_3CN$, AcOH. c) Aqueous sodium acetate buffer, pH 4, 60°C, sugar.

Analysis of the reaction course by HPLC indicates that a single product was formed corresponding after NMR analysis to the expected glycoconjugates **6a-d**. In particular, in the ROESY spectra the presence of a ROE cross peak between the H-1 of the sugar unit and the N-Me hydrogens of the hydroxylamino group is of diagnostic value for the regioselectivity toward the hydroxylamino moiety and thus rules out a possible parallel

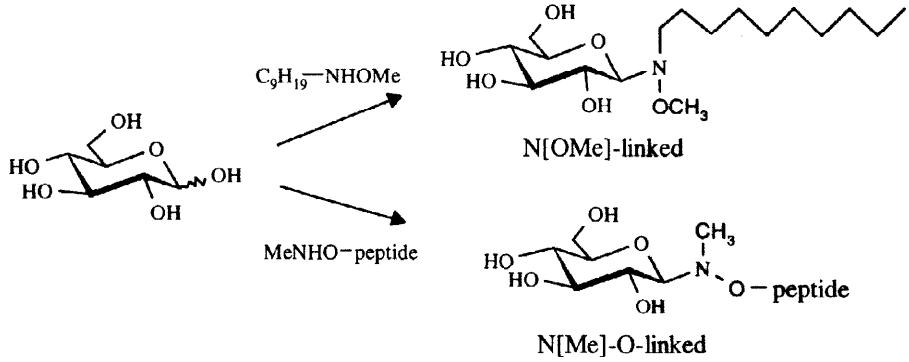
amino glycosylation with the free lysine amino group. This demonstrates the chemoselectivity of our hydroxylamino glycosylation even under long reaction times and harsh conditions (60°C). Consequently, neither protecting groups nor activation methods are required to perform the glycosylation step which represents an interesting alternative to existing chemical approaches to glycopeptides[2].

Table 2. Yields and Reaction Times of Glycosylation of Peptide **5**.

Product	Sugar	Time (h)	Yield (%)
6a	D-Glc	48	62
6b	D-Lactose	144	57
6c	Maltotriose	144	35
6d	GalNAc	48	70

These reaction conditions and the very good stereoselectivity observed open up the possibility to glycosylate unprotected peptides directly on a solid support. This may then allow the use of an excess of sugar to improve the yields of glycosylation as well as the recycling of that unreacted material by filtration.

Although the products we obtained here do not have a natural aglycon linkage, the conformation of the first sugar unit which is thought to play a dominant role in biological activity[14,15] is preserved. Therefore glycoconjugates of this type may prove useful in glycobiology as mimic of natural occurring glycosylated molecules. Another advantage of using *N,O* substituted hydroxylamine is that the oligosaccharide chain can be conjugated either to R_2 through an aminooxy (N[R_1]-O-, **6a-d**) linkage or to R_1 through an amino (N[OR_2]-, **3g**) linkage depending on how the substrate is tethered to the hydroxylamine (Scheme 4). Compounds **3g** is an illustrative example of a N[OR_2]- conjugation of a lipid moiety (octyl) to glucose which after reduction of the N-O bond (Zn / AcOH, 30 min 85%) provides an efficient synthetic entry to the corresponding N-Octyl-(β-D-glucopyranosyl)amine known for immunostimulation properties[16].



Scheme 4. N[OMe]- and N[Me]-O-linked glycoconjugates.

CONCLUSION

The results obtained herein demonstrate that the reaction of an *N,O*-substituted hydroxylamine with carbohydrates provides a rapid synthetic entry to stereoselectively glycosylated molecules such as peptides or lipids with naturally or chemically accessible oligosaccharides. The reaction makes use of oxime bond chemistry but without its usual inconveniences. This should provide a very efficient method for the rapid synthesis of more complex targets such as carbohydrate-based antigens for immunological studies.

EXPERIMENTAL SECTION

Materials and Methods. Reagents and solvents were purchased from Fluka (Buchs, CH) and used without further purification. All protected amino acids were purchased from Calbiochem-Novabiochem (Laufelfingen, CH). HPLC was performed on Waters equipment using column packed with Vydac Nucleosil 300 Å 5 μm C_{18} particles unless otherwise stated. The analytical column (250 x 4.6 mm) was operated at 1 mL/min and the preparative column (250 x 21 mm) at 18 mL/min, monitoring at 214 nm. Solvent A consisted of 0.09% TFA and solvent B of 0.09% TFA in 90% acetonitrile unless otherwise stated. Mass spectra were obtained by electron spray ionisation (ESI-MS) on a Finningan MAT SSQ 710C. NMR spectra were recorded in D_2O at 400 MHz (Bruker ARX spectrometer) or at 600MHz (Bruker AMX-2 spectrometer) at 300 K. 2D experiments were typically acquired using 2K x 512 matrices over a 2000 Hz sweep width in both dimensions. Quadrature detection in the indirect dimension was achieved by using the TPPI procedure[17]. Scalar connectivities were recovered from 2D double quantum filtration (DQF) COSY experiments[18]. Dipolar connectivities were obtained either through the conventional NOESY sequence[19] or the ROESY sequence[20] with mixing times from 150 to 200 ms. A randomisation of the mixing length (± 5%) was introduced in the NOESY experiments in order to minimise coherence transfer. The spin lock mixing interval of the ROESY sequence was applied by coherent CW irradiation at $\gamma B_2/2\pi$ = 1 KHz. Experimental data processing was performed using the Felix software package. The standard sinebell squared routine was employed for apodization with a shift range of 60-90° and zero filling in both dimensions before 2D transformations were applied to end up with square matrices of 2K x 2K real point data. Proton resonance assignments were made using (DQF) COSY experiments, HMQC was used to assign unambiguously the carbon resonances. J coupling constants were directly measured from high-resolution ^{1}H-1D spectra (±0.2 Hz).

N-Ethyl-O-benzylhydroxylamine (2a). A solution of *O*-benzylhydroxylamine hydrochloride (1g, 6.3 mmol), AcONa (1g) and acetaldehyde (12.6 mmol, 720 μl) in water/methanol (100 mL, 5:1 mixture), was stirred at room temperature for 10 min. The solvent was concentrated *in vacuum* and the residue was taken up in ethyl acetate (200 mL), washed with aqueous citric acid (200 mL, 10% w/v), dried over sodium sulphate and evaporated to dryness to afford the oxime as a colourless oil (0.87 g, 92%) which was used subsequently without further purification. A solution of the *O*-benzyl oxime of acetaldehyde (0.87 g, 5.8 mmol) in acetic acid (3mL) was treated with $NaCNBH_3$ (1.46 g, 4 equiv.) for 1 h at room temperature. The reaction mixture was concentrated, the residue taken up in diethyl ether (200 mL), washed with brine, dried on sodium sulphate, filtered, and concentrated. The product was purified by flash chromatography on silica-gel (25% ethyl acetate/hexane) and recovered as a colourless oil (0.7 g, 80% yield). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.13 (t,

3H, CH_3, ^3J = 7.0 Hz), 3.01 (q, 2H, CH_2-N, ^3J = 7.0 Hz), 4.74 (s, 2H, CH_2-OPh), 5.39 (bs, 1H, NH), 7.26-7.41 (m, 5H, H_{arom}); ^{13}C-NMR (400 MHz, $CDCl_3$): δ = 14.8 (CH_3), 48.8 (CH_2-N), 78.6 (CH_2-O), 130.2,130.7, 140.2 (C_{arom}); MS: m/z = 152 (M + H^+), 91; Anal. Calcd. for $C_9H_{13}NO$ (151.20): C 71.49, H 8.67, N 9.26. Found: C 71.51, H 8.71, N 9.34.

N-Ethyl-O-benzyl-N-(β-D-glucopyranosyl)hydroxylamine (3a). A solution of D-glucose (50 mg, 0.28 mmol) in 4 mL of DMF/AcOH 50% and *N*-Ethyl-*O*-benzylhydroxylamine (63 mg, 0.42 mmol), was stirred for 20 h at room temperature. After removal of the volatile, the product was purified by flash chromatography on silica gel (10% methanol/ethyl acetate) and recovered as a colourless oil which precipitated from diethyl ether as a white solid (78 mg, 90%). $[\alpha]^{25}_D$ = -4.6 (*c* = 0.4, MeOH). ^{1}H NMR (400 MHz, D_2O): δ = 1.12 (t, 3 H, $\underline{CH_3}CH_2N$, ^3J = 7.0 Hz), 2.91 (dq, 1H, $CH_3\underline{CH_2}N$, ^2J =12.0 Hz, ^3J =7.0 Hz,), 3.11 (dq, 1H, $CH_3\underline{CH_2}N$, ^2J = 12.0 Hz, ^3J = 7.0 Hz), 3.26 (m, 1H, H4), 3.27 (m, 1H, H5), 3.43 (dd, 1H, H3, $^3J_{2\text{-}3}$= $^3J_{3\text{-}4}$ = 9.0 Hz), 3.58 (dd, 1H, H2, $^3J_{2\text{-}3}$= $^3J_{1\text{-}2}$ = 9.0 Hz), 3.60 (dd, 1H, H6, $^2J_{6\text{-}6'}$ = 12.0 Hz, $^3J_{6\text{-}5}$ = 1.6 Hz), 3.76 (dd, 1H, H6', $^2J_{6\text{-}6'}$ = 12.0 Hz, $^3J_{6'\text{-}5}$ = 1.6 Hz), 4.12 (d, 1H, H1, $^3J_{1\text{-}2}$ = 9.0 Hz), 4.75 (2H, CH_2Ph), 7.3 (m, 5H, H_{arom});^{13}C NMR (400 MHz, D_2O): δ =13.9 ($\underline{C}H_3CH_2N$), 49.7 ($CH_3\underline{C}H_2N$), 63.0 (C6), 71.6 (C4), 72.2 (C2), 79.3 ($\underline{C}H_2$Ph), 79.5 (C3), 79.5 (C5), 93.9 (C1), 131.0, 131.1, 131.7, 138.0 (C_{arom}); MS (ESI): m/z = 314.3 (M + H^+). Anal. Calcd. for $C_{15}H_{23}NO_6$ (313.3): C 57.50, H 7.40, N 4.47. Found: C 57.39, H 7.28, N 4.32.

N,O-Dimethyl-N-(β-D-glucopyranosyl)hydroxylamine (3b). A solution of D-glucose (90 mg, 0.5 mmol) and *N,O*-dimethyl-hydroxylamine hydrochloride (195 mg, 2 mmol) in 5 mL of aqueous buffer (sodium acetate/acetic acid 0.1 M, pH 4), was stirred for 20 h at room temperature. The solvent was removed and flash chromatography (water/methanol/ethyl acetate = 1:24:75) afforded the pure product as a colourless oil (102 mg, 0.46 mmol, 92%). $[\alpha]^{25}_D$ = -9.5 (*c* = 1.2, MeOH). ^{1}H NMR (400 MHz, D_2O): δ = 2.66 (s, 3 H, CH_3N), 3.24-3.34 (m, 2H, H3 and H5), 3.38-3.47 (m, 2H, H2 and H4), 3.49 (s, 3H, CH_3O), 3.64 (dd, 1H, H6, $^3J_{5\text{-}6}$ = 5.4 Hz, $^2J_{6\text{-}6'}$ = 12.1 Hz), 3.83 (dd, 1H, H6', $^3J_{5\text{-}6}$ = 1.8 Hz, $^2J_{6\text{-}6'}$ = 12.1 Hz), 4.02 (d, 1H, H1, $^3J_{1\text{-}2}$ = 8.5 Hz);^{13}C NMR (400 MHz, D_2O): δ = 40.35 (CH_3N), 62.28 (CH_3O), 63.23 (C6), 71.82 (C3), 72.10 (C4), 79.42,(C2), 79.71 (C5), 95.19 (C1); MS: m/z = 224 (M+H^+), 192 (M-OMe); Anal. Calcd. for $C_8H_{17}NO_6$ (223.2): C 43.05, H 7.68, N 6.28; found. C 43.25, H 7.76, N 6.22.

N,O-Dimethyl-N-(2-aminoacetyl-2-deoxy-β-D-glucopyranosyl)hydroxylamine (3c). *N*-Acetyl-glucosylamine (100 mg, 0.45 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (176 mg, 1.8 mmol) were reacted using the same procedure described for **3b**. The crude material was purified by chromatography (water/methanol/ethyl acetate = 1:24:75) affording, after precipitation from diethyl ether, 47 mg of **3c**, (40%). $[\alpha]^{25}_D$ = +3.1 (*c* = 2.2, MeOH). ^{1}H NMR (400 MHz, D_2O): δ = 1.90 (s, 3H, CH_3CO), 2.58 (s, 3 H, CH_3N), 3.25-3.30 (m, 1H, H4), 3.30 (s, 3H, CH_3O), 3.30-3.45 (m, 1H, H3), 3.55-3.65 (m, 1H, H5), 3.65-3.80 (m, 2H, H6 and H6'), 3.84 (dd, 1H, H2, $^3J_{2\text{-}3}$ = $^3J_{1\text{-}2}$ = 9.8 Hz), 4.06 (d, 1H, H1);^{13}C NMR (400 MHz, D_2O): 17.8 ($\underline{C}H_3CO$), 35.4 (CH_3N), 56.5 (CH_3O), 58.0 (C5), 58.1 (C2), 66.6 (C6), 72.6 (C3), 74.4 (C4), 88.3 (C1), 171.5 ($CH_3\underline{C}O$); MS: m/z = 265 (M+H^+), 234 (M- OMe); $C_{10}H_{20}N_2O_6$ (264.3) calcd. C 45.44, H 7.63, N 10.60; found C 45.47, H 7.71, N 10.57.

N-ethyl-O-benzyl-N-(β-D-galactosyl)hydroxylamine. D-galactose (50 mg, 0.28 mmol) and *N*-ethyl-*O*-benzylhydroxylamine (63 mg, 0.42 mmol) were reacted using the same protocol as for **3a**. The product was purified to afford 52 mg of a colourless oil (60%). The product consisted of a mixture of the β-pyranose (80%) and the β-furanose (20%) forms. MS (ESI): m/z = 314.3 (M + H^+); Anal. Calcd. for $C_{15}H_{23}NO_6$ (313.3): C

57.50, H 7.40, N 4.47; found: C 57.42, H 7.37, N 4.49. As it was not possible to separate the two forms by chromatographic methods the two compounds were characterised as a mixture: *N-Ethyl-O-benzyl-N-(β-D-galactopyranosyl)hydroxylamine (3d).* ^{1}H NMR (400 MHz, D_2O): δ = 1.09 (t, 3 H, $\underline{CH_3}CH_2N$, ^3J = 7.2 Hz), 2.91 (dq, 1H, $CH_3\underline{CH_2}N$, ^2J=12.7 Hz, ^3J=7.2 Hz,), 3.07 (dq, 1H, $CH_3\underline{CH_2}N$, ^2J = 12.7 Hz, ^3J = 7.2 Hz), 3.49-3.68 (m, 4H, H3-H5-H6-H6'), 3.73 (dd, 1H, H2, $^3J_{1\text{-}2}$= $^3J_{2\text{-}3}$= 9.1 Hz), 3.80 (d, 1H, H4, $^3J_{3\text{-}4}$ = 3.3 Hz), 4.08 (d, 1H, H1, $^3J_{1\text{-}2}$ = 9.1 Hz), 4.77 (2H, CH_2Ph), 7.3 (m, 5H, H_{arom}); ^{13}C NMR (400 MHz, D_2O): δ = 14.0 ($\underline{CH_3}CH_2N$), 48.9 ($CH_3\underline{CH_2}N$), 63.5 (C6), 69.7 (C2), 71.4 (C4), 76.5 (C3), 78.9 (C5), 79.1 ($\underline{CH_2}Ph$), 94.7 (C1), 131.0, 131.7, 138.0 (C_{arom}). *N-Ethyl-O-benzyl-N-(β-D-galactofuranosyl)hydroxylamine.* ^{1}H NMR (400 MHz, D_2O): δ =1.05-1.15 (m, 3 H, $\underline{CH_3}CH_2N$), 2.8–3.1 (m, 2H, $CH_3\underline{CH_2}N$), 3.5-3.8 (m, 4H, H4,H5, 2H6), 4.04 (dd, 1H, H3, $^3J_{2\text{-}3}$= $^3J_{3\text{-}4}$= 7.0 Hz), 4.24 (dd, 1H, H2), 4.47 (d, 1H, H1, $^3J_{1\text{-}2}$= 7.0 Hz), 4.7-4.8 (m, 2H, CH_2Ph).

N,O-Dimethyl-N-(β-D-lactosyl)hydroxylamine (3e). D-Lactose monohydrate (100 mg, 0.28 mmol) was reacted with *N,O*-dimethylhydroxylamine hydrochloride (110 mg, 1.12 mmol) in water (acetate buffer, pH 4) for 20 h at room temperature. The solvent was removed, and flash chromatography (water/methanol/chloroform = 0.5:50:50) afforded pure **3e** which was finally precipitated in diethyl ether as a white solid (102 mg, 0.27 mmol, 95% yield). $[\alpha]^{25}_D$ = -3.0 (*c* = 0.3, MeOH). ^{1}H NMR (400 MHz, D_2O): δ = 2.64 (s, 3H, CH_3N), 3.47 (s, 3H, CH_3O), 3.4-3.8 (m, 11H, H2, H2', H3, H3', H4, H4', H5, H5'and 3 protons among H6 and H6'), 3.87 (dd, 1H, H6 or H6', $^3J_{5\text{-}6}$ = 2.1 Hz, $^2J_{6\text{-}6'}$ = 12.3 Hz), 4.03 (d, 1H, H1, $^3J_{1\text{-}2}$ = 9.0 Hz), 4.34 (d, 1H, H1', $^3J_{1'\text{-}2'}$ = 7.8 Hz); ^{13}C NMR (400 MHz, D_2O): δ = 40.4 (C7), 62.4 (C8), 62.6, 63.4 (C6-C6'), 70.9, 71.8, 73.3, 74.8, 77.7, 78.0, 78.6, 80.5 (C2-C2'-C3-C3'-C4-C4'-C5-C5'), 95.0 (C1), 105.2 (C1'); MS: m/z = 386 ($M + H^+$); $C_{14}H_{27}NO_{11}$ (385.4) calcd. C 43.63, H 7.06, N 3.63, found C 43.44, H 7.04, N 3.69.

N,O-Dimethyl-N-(α-D-mannosyl)hydroxylamine. A solution of D-mannose (100 mg, 0.55 mmol) and *N,O*-dimethyl-hydroxylamine hydrochloride (270 mg, 2.2 mmol) in 5 mL of aqueous buffer (sodium acetate/acetic acid 0.1 M, pH 4) was stirred at room temperature. After one week the solvent was evaporated and the product purified by flash chromatography on silica gel (water/methanol/ethyl acetate = 1:24:75) to afford 30 mg of **3f** (24% yield). The final product contained 77% of the α-pyranose form and 13% of the α–furanose form that were not isolated but characterised in the mixture. The analysis of the mixture gave the following values: MS: m/z = 224 ($M+H^+$), 192 (M-OMe); Anal. Calcd. for $C_8H_{17}NO_6$ (223.2): C 43.05, H 7.68, N 6.28; found: C 42.98, H 7.73, N 6.31. *N,O-Dimethyl-N-(α-D-mannopyranosyl)hydroxylamine (3f).* ^{1}H NMR (400 MHz, D_2O): δ = 2.54 (s, 3 H, CH_3N), 3.46 (s, 3H, CH_3O), 3.50 (dd, 1H, H4, $^3J_{3\text{-}4}$= $^3J_{4\text{-}5}$= 9.0 Hz), 3.62 (dd, 1H, H6, $^3J_{5\text{-}6}$= 6.0 Hz, $^2J_{6\text{-}6'}$= 12 Hz), 3.70 (m, 1H, H5), 3.74 (dd, 1H, H6'), 3.77 (dd, 1H, H3, $^3J_{2\text{-}3}$= 3.4 Hz, $^3J_{3\text{-}4}$= 9.0 Hz), 4.05 (d, 1H, H1, $^3J_{1\text{-}2}$ = 2.1 Hz), 4.08 (dd, 1H, H2, $^3J_{1\text{-}2}$= 2.1 Hz, $^3J_{2\text{-}3}$= 3.4 Hz);^{13}C NMR (400 MHz, D_2O): 36.80 (CH_3N), 57.07 (CH_3O), 58.25 (C6), 64.03 (C4), 65.52 (C2), 68.04 (C3), 72.71 (C5), 90.90 (C1). *N,O-Dimethyl-N-(α-D-mannofuranosyl)hydroxylamine.* ^{1}H NMR (400 MHz, D_2O): δ = 2.59 (s, 3 H, CH_3N), 3.48 (s, 3H, CH_3O), 3.5-3.8 (m, 3H, H5, H6, H6'), 3.93 (d, 1H, H4, $^3J_{4\text{-}5}$= 9.0 Hz), 4.23 (dd, 1H, H3, $^3J_{3\text{-}4}$= 2.5 Hz), 4.33 (dd, 1H, H2, $J_{2\text{-}3}$= 4.3 Hz), 4.47 (d, 1H, H1, $^3J_{1\text{-}2}$= 7.0 Hz).

N-Octyl-O-methyl-N-(β-D-glucopyranosyl)hydroxylamine (3g). *N*-Octyl-*O*-methyl hydroxylamine was synthesised from caprylic aldehyde through oxime formation and subsequent reduction with $NaCNBH_3$ as described for compound **2a**. A solution of D-glucose (90 mg, 0.5 mmol) and *N*-octyl-*O*-methylhydroxylamine (80mg, 0.5 mmol) in 5 mL of DMF/water (9:1, v/v), was stirred for 20 h at room temperature. The solvent was evaporated and the crude purified by flash chromatography (methanol/ethyl acetate = 1:9) to afford 49 mg of a

colourless oil (30% yield). ^{1}H NMR (400 MHz, D_2O): δ = 0.73 (bt, 3 H, CH_3octyl), 1-1.5 (m, 12H, CH_2octyl), 2.73 (m, 1H, N-CH_A), 2.94 (m, 1H, N-CH_B), 3.21-3.26 (m, 2H, H4 and H5), 3.37 (dd, 1H, H3, $^3J_{2\text{-}3}$= $^3J_{3\text{-}4}$ = 8.9 Hz), 3.44 (dd, 1H, H2, $^3J_{1\text{-}2}$= $^3J_{2\text{-}3}$ = 8.9 Hz), 3.48 (s, 3H, OCH_3), 3.58 (dd, 1H, H6, $^3J_{5\text{-}6}$= 4.0 Hz, $^2J_{6\text{-}6'}$= 11.9 Hz), 3.76 (bd, 1H, H6'), 4.02 (d, 1H, H1, $^3J_{1\text{-}2}$ = 8.9 Hz);^{13}C NMR (400 MHz, $CDCl_3$): δ = 16.6 (CH_3octyl), 25.2, 29.9, 30.0, 31.8, 32.0, 34.3 (CH_2octyl), 56.0 (N-CH_2), 63.9 (C6), 65.5 (OCH_3), 71.8 (C3), 72.4 (C4), 79.7 (C2), 80.1 (C5), 95.2 (C1). MS: m/z = 322 (M+ H^+). Anal. Calcd. for $C_{15}H_{31}NO_6$ (321.2): C 56.08, H 9.66, N 4.36. Found: C 56.32, H 9.88, N 4.54.

N-Methyl-hydroxylaminopeptide (5). The tripeptide Ac-Lys(Dde)-Ala-Lys(Boc)-NH_2 was synthesised using Nα-Fmoc chemistry according to the typical procedure for solid phase synthesis. The Nα-Fmoc amino acids side chains were protected as follows: 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) for N-terminal Lys, *tert*-butoxycarbonyl (Boc) for the other Lys. The Nα-Fmoc amino acids were assembled on Rink amide MBHA resin (2g, 0.82 mmol) using the following coupling conditions: Fmoc-amino acid (1.5 equiv.), benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) (640 mg, 1.5 equiv.) in presence of *N,N*-diisopropylethylamine (DIPEA) (420 µl, 3 equiv.) in 20 mL of dimethylformamide (DMF) for 30 min. Removal of Fmoc protecting groups was achieved by subsequent piperidine cycles (20% v/v in DMF, 3x10 min). N-acetylation of the N-terminal lysine α-amino group was performed with 20 mL of a solution of Ac_2O (10%) and pyridine (10%) in DMF (30 min, room temperature) affording the protected peptide Ac-Lys(Dde)-A-Lys(Boc)-resin. The ε-lysine Dde protecting group was then removed with hydrazine (2%, v/v) in DMF and the succinimide ester of the *N*-Boc,*O*-carboxymethylhydroxylamine (M_r = 288 , 520 mg, 2.2 equiv.) was directly coupled to the peptide-resin in the presence of DIPEA (560 µl, 4 equiv.). The peptide was then cleaved from the resin by treatment with a mixture 2.5% (v/v) of tris-isopropylsilane (TIS), 2.5% water and 85% trifluoroacetic acid (TFA) at room temperature (3 x 1 hour). In these conditions the Boc groups were also cleaved. The combined filtrates were precipitated with diethyl ether, filtered and washed three times with cold diethyl ether. The crude peptide was purified by preparative HPLC (gradient 0% to 50% solvent B in 30 min) to afford **4** as a white powder (R_t= 9.3 min., M_r = 460, 360 mg, 95% yield). ESI-MS: *m/z* = 460.4 (M^+). Peptide **4** (200 mg, 0.43 mmol) was reacted with aqueous formaldehyde (37% solution in water, 50 µl, 1.1 equiv.) in 10 mL of 0.1 M acetate buffer (pH 4.0) and the corresponding *N*-methyloxime-peptide was purified by semipreparative HPLC (gradient of 0% to 50% solvent B in 30 min). After lyophilisation, the pure oxime-peptide was recovered as a white powder (92% yield). The oxime-peptide (M_r = 472, 100 mg, 0.21mmol) was finally reduced with $NaCNBH_3$ (54 mg, 4 equiv.) in 10 mL of acetic acid (10 min, room temperature). The crude peptide **5** was purified by semipreparative HPLC (gradient of 0% to 50% solvent B in 30 min) affording peptide **5** (180 mg, 89%) (R_t= 13.1 min, ESI-MS: *m/z* = 474.7 (M+H).

^{1}H-NMR (400 MHz, D_2O): δ = 1.27 (d, 3H, CH_3 Ala , J = 7.1 Hz), 1.2-1.8 (m, 12 H, $CH_{2\beta}$, $CH_{2\gamma}$, $CH_{2\delta}$ of Lys1 and Lys 3), 1.90 (s, 3H, CH_3CO), 2.72 (bd, 3H, CH_3N), 2.88 (bt, 2H, $CH_{2\varepsilon}$ Lys 3), 3.14 (bt, 2H, $CH_{2\varepsilon}$ Lys 1), 3.22 (s, 2H, CH_2-O-N), 4.05-4.20 (m, 3H, H_α Ala, Lys 1,Lys 3); MS (ESI): m/z = 474.1 (M+H).

General Procedure for Sugar Conjugation to peptide 5 (6a-d). Peptide **5** (20 mg, 0.042 mmol) dissolved in 2 mL of 0.1 M acetate buffer (pH 4) was reacted with the sugar (0.05 mmol). After completion of the reaction and lyophilization, the crude product was purified by preparative HPLC. After 48 h of reaction and HPLC purification, glycopeptide **6a** was obtained as a white powder (16 mg, 62%).

^{1}H-NMR (600 MHz, D_2O): δ = 1.26 (d, 3H, CH_3 Ala, J = 7.2 Hz), 1.2-1.8 (m, 12 H, $CH_{2\beta}$, $CH_{2\gamma}$, $CH_{2\delta}$ of Lys 1 and Lys 3), 1.90 (s, 3H, CH_3CO), 2.67 (s, 3H, CH_3N), 2.89 (dd, 2H, $CH_{2\varepsilon}$ Lys 3, $^3J_{\varepsilon-\delta}$ = 7.5 Hz, $^2J_{\varepsilon-\varepsilon'}$ = 4.0 Hz), 3.12 (bt, 2H, $CH_{2\varepsilon}$ Lys 1, $^3J_{\varepsilon-\delta}$ = 8.4), 3.22 (s, 2H, CH_2-O-N), 3.32 (m, 1H, H4 Glc), 3.47 (m, 1H, H5, $^3J_{4-5}$ = 8 Hz Glc), 3.76 (m, 1H, H3 and H2 Glc),3.62 (dd, 1H, H6, $^2J_{6-6'}$ = 12.5 Hz, $^3J_{5-6}$ = 5.9 Hz Glc), 3.80 (dd, 1H, H6', $^2J_{6-6'}$ = 12.5 Hz, $^3J_{5-6'}$ = 1.9 Hz Glc), 4.13 (d, 1H, H1, $^3J_{1-2}$ = 8.7 Hz Glc), 4.15 (m, 1H, H_α Lys 1), 4.21 (m, 1H, H_α Lys 3), 4.25 (m, 1H, H_α Ala 1),MS (ESI): m/z = 635.9 (M+H).

Glycopeptide **6b** was obtained after 144 h at 60 °C (19 mg, 57% yield). R_t 11.1 min (gradient of 0% to 50% solvent B in 30 min), ESI-MS: *m/z* = 798.2 (M+H). Glycopeptide **6c** was obtained after 144 h at 60 °C (14 mg, 35% yield). R_t 10.3 min (gradient of 0% to 50% solvent B in 30 min), ESI-MS: *m/z* = 959.8 (M+H). Glycopeptide **6d** was obtained after 48 h at room temperature (20 mg, 70% yield). R_t 13.6 min (gradient of 0% to 50% solvent B in 60 min), ESI-MS: *m/z* = 677.7 (M+H).

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REFERENCES AND NOTES

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constants $^3J_{2H-3H}$ and /or $^3J_{3H-4H}$ and/or $^3J_{4H-5H}$ are indicative of a $^4C_1$ conformation. In the case of the D-mannose derivative **3f**, the α form is attributed from a $^1J_{13C1-H1}$ value of about 160Hz[11].

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