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An improved procedure for the synthesis of fully acetylated *N*-(β-glycosyl)azidoacetamides and *N*-(β-glycosyl)iodoacetamides useful as chemical ligating agents

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Abstract

A simpler, convenient and efficient four-step procedure was developed for the synthesis of two classes of chemical ligating agents viz., fully acetylated *N*-(β-glycosyl)azidoacetamides and *N*-(β-glycosyl)iodoacetamides both obtained in 65% overall yield. The procedure involves an efficient conversion of fully acetylated α-glycosyl chlorides in aqueous acetone to the corresponding β-glycosyl azides, hydrogenolysis of these azides to the corresponding glycosylamines in dry methanol followed by chloroacetylation of the resultant glycosylamines in the same pot and the final divergent transformation of the fully acetylated *N*-(β-glycosyl)chloroacetamides thus obtained to the corresponding *N*-(β-glycosyl)azidoacetamides and *N*-(β-glycosyl) iodoacetamides.

Keywords. Synthesis, *N*-(Glycosyl)azidoacetamide, *N*-(Glycosyl)iodoacetamide, Carbohydrate, *N*-Chloroacetylation, Chemical ligation

Introduction

Cell surface glycoprotein glycans play key roles in many important biological processes that include inflammation, bacterial and viral infection, metastasis, cellular differentiation and development.[1] Many of these processes are mediated by specific carbohydrate-protein interactions. *N*-Glycosylation is the most common type of eukaryotic glycosylations, wherein amide nitrogen of asparagine is covalently attached to the anomeric carbon of 2-acetamido-2-deoxy-β-D-glucopyranosyl moiety (GlcNAcβ) (Figure 1) (Spiro, 2002).[2] The structural diversity, complexity and microheterogeneity of glycan chains of glycoproteins and their low occurrence in nature pose a major challenge to study the structure-function correlations.

This challenge can be overcome to a large extent by the synthesis of structurally well-defined homogeneous glycopeptides. Selectively functionalized saccharides, called as chemical ligating agents, are very valuable for preparing such glycopeptides as well as other glycoconjugates. Among the several chemical ligating agents reported earlier in literature,[3] the azido derivatives are uniquely attractive in view of their a) stability of the azide functionality under a range of chemical as well as enzymatic conditions[4] b) ready conversion under mild conditions to more reactive and rather difficult to handle amino compounds and c) ability to undergo regioselective cycloaddition with an alkyne in the presence of Cu-(I).[5] The Cu-(I) catalyzed azide-alkyne [3+2] cycloaddition is termed as C*lick Reaction.*[6] Several reports have appeared in literature on the Cu-(I) catalyzed azide-alkyne [3+2] cycloaddition. 2-*O*-Azidoethyl glycosides, which are mimetics of the linkage region in *O*-glycoproteins, have often been utilized as chemical ligating agents to prepare a variety of neoglycoconjugates.[7] Azidomethyl thioglycosides[8] that were introduced later, have the advantage over 2-*O*-Azidoethyl glycosides, of being relatively resistant to hydrolysis by glycosidases and aqueous acid.

The first report on selectively functionalized *N*-linked azido derivatives that mimic *N*-glycoprotein linkage region as chemical ligating agent appeared from our laboratory.[9] The reported methodology for the synthesis of these fully acetylated *N*-(β-glycosyl)azidoacetamides involves selective *N*-chloroacetylation of free β-glycosylamines, complete acetylation of the resultant *N*-(β-glycosyl)chloroacetamides catalyzed by sodium form of β -zeolite and displacement of the chloride with azide using NaN_3 in aqueous acetone. The free glycosylamines employed here as starting materials are relatively tedious to prepare and require either several weeks of crystallization at low temperature to obtain

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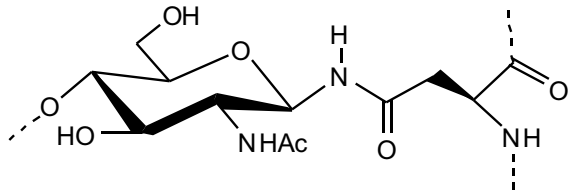
them in pure form or freeze drying of aqueous solution of the crude product, depending on their method of preparation. Thus this reaction can not be readily scaled up. There is, therefore, a need to develop simpler and more efficient procedure for the synthesis of fully acetylated *N*-(β -glycosyl)azidoacetamides.

Being a very good leaving group, iodide can be easily displaced by heteroatoms such as O, N, S and carbon nucleophiles. So, iodoacetamides can be used for coupling of glycans to peptides and proteins. In literature, *N*-(β-glycosyl)iodoacetamide has been used as chemical ligating agent where *N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)iodoacetamide was coupled with peptides and proteins containing cysteine residues such as glutathione and bovin serum albumin.[10] The iodoacetamide derivative was also employed in the



GlcNAcβ-Asn

Figure 1: Linkage region of *N*-glycoproteins

solid phase synthesis of thioether-linked glycopeptide mimics.[11] The *N*-(β-glycosyl)iodoacetamides have recently been used by our group for the asymmetric alkylation of ethyl nitroacetate leading to the synthesis of *N*-(β-glycosyl)asparagine precursors.[12] In this present work, an efficient procedure has been developed for the synthesis of several N-(β-glycosyl)chloroacetamides which were converted to the corresponding iodoacetamides and azidoacetamides by replacing the chloride by iodide and azide, respectively.

Results and Discussion

Fully acetylated β-glycosyl azides are chosen as synthons for the synthesis of fully acetylated ***N*-(**β-glycosyl)azidoacetamides and *N*-(β-glycosyl)iodoacetamides in the present work as these can be prepared in large scale from fully acetylated α-glycosyl halides. Among the various methods reported for the preparation of fully acetylated β-glycosyl azides, the procedure[13] involving the displacement of α-glycosyl bromides with NaN_3 in aqueous acetone medium was chosen to be adopted here in view of its simplicity and excellent yield. Replacement of fully acetylated α -glycosyl bromides with the corresponding chlorides[14] represents a simple modification of the above procedure brought about in the present work that obviates the difficulty in (a) handling liquid bromine and (b) handling the relatively unstable α-glycosyl bromides.

Treatment of fully acetylated sugars derived from Glc, Gal, Man, GlcNAc, Xyl, L-Rha, Lac and Cell (Scheme 1) with 10 mol% BiOCl along with 2 equiv of thionyl chloride furnished, after simple work-up, the corresponding α-glycosyl chlorides as syrups. A solution of the syrup in aqueous acetone was reacted with NaN_3 and the crude product obtained was recrystallised from a mixture of ethyl acetate-hexane to furnish fully acetylated β-glycosyl azides in 90 - 95% yield. All the sugar azides (**1** - **6**) thus prepared were characterized based on the comparison of their physical and spectral data with those reported in literature.[15]

Fully acetylated α-D-mannopyranosyl chloride and α-L-rhamnopyranosyl chloride, both prepared using the BiOCl / $SOCl_2$ method,[14] were treated in separate reactions with NaN_3 in acetone / H_2O in an effort to obtain the corresponding azides (**7 and 8**). Column purification of the product obtained above afforded 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose (85.5 %) and 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranose (80%), respectively, as major products along with 2,3,4,6-tetra-*O*-acetyl-α/β-D-mannopyranosyl azides (4.5%) and 2,3,4-tri-*O*-acetyl-α/β-L-rhamnopyranosyl azides (4%) as minor products (Scheme 2). These products were also characterized based on the comparison of their physical and spectral data with those reported earlier.[15] The predominant hydrolysis of the 1,2-*trans* glycosyl chlorides derived from Man and Rha under the above mentioned reaction conditions could be rationalized based on the well known neighboring group participation of C-2 acetoxyl moiety that seems to occur exceedingly faster than the substitution of chloride by azide ion.

On the other hand, reaction of fully acetylated α-D-mannopyranosyl chloride and fully acetylated α-L-rhamnopyranosyl chloride in separate experiments with

NaN_3 in dry DMF as solvent at 80 °C (Scheme 3) gave an anomeric mixture of 2,3,4,6-tetra-*O*-acetyl-α/β-mannopyranosyl azides and 2,3,4-tri-*O*-acetyl-α/β-rhamnopyranosyl azides, respectively, in excellent yield. Flash column chromatography of the mixture over silica gel (230 - 400 mesh) afforded, in each case, both the anomeric azides in pure form and these have been characterized based on the comparison of their physical and spectral data with those reported in literature.[15]

Catalytic reduction of all the fully acetylated glycosyl azides, prepared as described above, was performed under hydrogenolysis conditions (Pd/C) in dry methanol to obtain the corresponding glycosylamines. *N*-Chloroacetylation of these glycosyl amines furnished fully acetylated *N*-(β-glycosyl)chloroacetamides in 90 - 95% yield. Treatment of the resultant chloroacetamides with NaN_3 in aqueous acetone under reflux condition gave the desired fully acetylated *N*-(β-glycosyl)azidoacetamides (**9** - **15**) in 90 - 95% yield (Table 1, Scheme 4).

All these azidoacetamides **9 - 15** exhibited the characteristic azide group stretching in their IR spectra around 2100 cm^{-1} and the signal due to the methylene carbon in the ^{13}C NMR (100 MHz) spectrum typically around δ 52.5 ppm, downfield shifted from a value of about 42.0 ppm observed for that of the chloroacetamido derivatives. All the synthesized fully acetylated *N*-(β-glycosyl)azidoacetamides excepting lactose and cellobiose have been characterized by comparison of their physical and spectral data with those reported in literature.[9] Compound **10** has earlier been obtained by reaction of relatively expensive lithium azide with the corresponding chloroacetamido derivative in dry acetone at 60 - 65 °C for 16 h. However, excepting GlcNAc, no other azidoacetamido derivatives have been prepared using this method. Although Staudinger reaction of glycosyl azides with chloroacetyl chloride serves as alternative method for the preparation of chloroacetamido derivatives, the phosphinimine intermediate formed shows poor reactivity in some cases resulting in low yield or formation of an inseparable mixture of anomeric chloroacetamido derivative.[16] Starting from free sugar

Table 1 Synthesis of fully acetylated *N*-(β-D-glycosyl)azidoacetamides (**9 - 15**)

Entry	Fully acetylated glycosyl azide	Fully acetylated *N*-(β-D-glycosyl)azidoacetamide	Yield (%)[a]
1	GlcβN_3 (**1**)	GlcβNHCOCH_2N_3 (**9**)	95
2	GlcNAcβN_3 (**2**)	GlcNAcβNHCOCH_2N_3 (**10**)	82
3	GalβN_3 (**3**)	GalβNHCOCH_2N_3 (**11**)	96
4	XylβN_3 (**4**)	XylβNHCOCH_2N_3 (**12**)	75
5	LacβN_3 (**5**)	LacβNHCOCH_2N_3 (**13**)	78
6	CellN_3 (**6**)	CellβNHCOCH_2N_3 (**14**)	91
7	ManβN_3 (**7**)	ManβNHCOCH_2N_3 (**15**)	96

[a]Yield of isolated pure product

Table 2 Synthesis fully acetylated *N*-(β-D-glycosyl)iodoacetamides (**16 - 21**)

Entry	Fully acetylated glycosyl azide	Fully acetylated *N*-(β-glycosyl)iodoacetamide	Yield (%)[a]
1	GlcβN_3 (**1**)	GlcβNHCOCH_2I (**16**)	94
2	GlcNAcβN_3 (**2**)	GlcNAcβNHCOCH_2I (**17**)	94
3	GalβN_3 (**3**)	GalβNHCOCH_2I (**18**)	95
4	CellN_3 (**6**)	CellβNHCOCH_2I (**19**)	65
5	ManβN_3 (**7**)	ManβNHCOCH_2I (**20**)	92
6	L-RhaN_3 (**8**)	L-RhaβNHCOCH_2I (**21**)	91

[a]Yield of isolated pure product

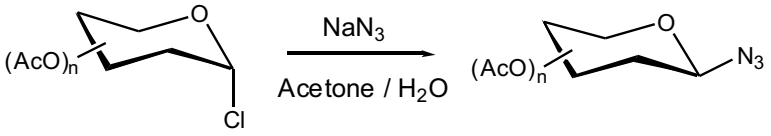
the overall yield of fully acetylated *N*-(β-D-glycosyl)azidoacetamides obtained by the previous method [9] was only 35% whereas that of the present procedure is substantially higher (65%).

Displacement of chloro group in fully acetylated *N*-(β-glycosyl)chloroacetamides by iodo group was carried out using KI in aqueous acetone at 60 - 65 °C to obtain the iodoacetamido derivatives **16 - 21** in 90-95% yield (Scheme 5, Table 2). All the synthesized fully acetylated *N*-(β-glycosyl)iodoacetamides excepting the known GlcNAc derivateive[10] have been fully characterized based on their physical and spectral data. The ^{1}H NMR (400 MHz) spectrum displayed the signal due to the methylene protons of CH_2I group at 3.69 - 3.61 ppm as an ABq. In the ^{13}C NMR (100 MHz) spectrum, the methylene carbon of CH_2I appeared at -2.3 ppm. Comparison of the chemical shift (42.2 ppm) of the methylene carbon (-CH_2Cl) of the corresponding chloroacetamido compound with a value of -2.3 ppm mentioned above is consistent with the well known heavy atom effect of iodine.

In conclusion, the title compounds have been synthesized starting from free sugar using a simple and convenient set of reactions including the transformation of fully acetylated glycosyl chlorides to the corresponding azides in aqueous acetone for the first time with an overall yield of 65%. Both the fully acetylated *N*-(β-glycosyl)azidoacetamides and fully acetylated *N*-(β-glycosyl)iodoacetamides, mimetics of the *N*-glycoprotein linkage region, would prove to be very valuable for the creation of carbohydrate microarrays[17] and structurally well-defined synthetic glycoconjugates greatly facilitating the study of protein-carbohydrate interactions.

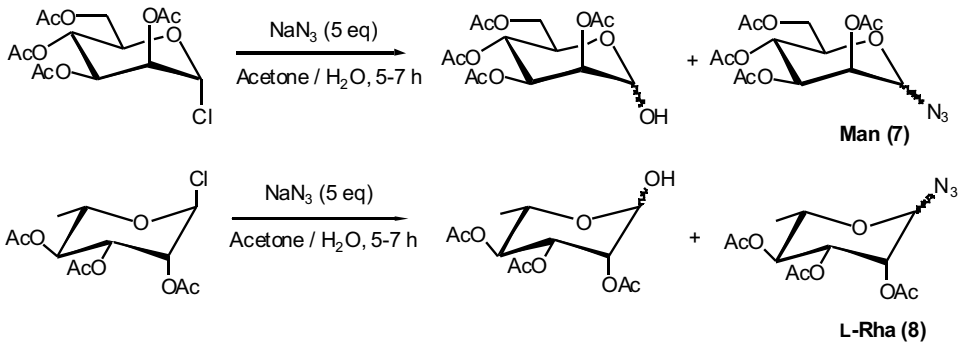
Experimental Section

All sugars were purchased from Sigma-Aldrich, USA or carbosynth, UK and used as such. Chloroacetic anhydride, NaN_3 and KI were purchased from Across Organics, Belgium. Column chromatography was done using silica gel (100 - 200 mesh) using a mixture of ethyl acetate and hexane. Optical rotation was measured at 30 °C on a JASCO-DIP 200 digital polarimeter using a cell of 10 mm length. IR spectra were recorded using Nicolet 6700 FT-IR, spectrometer. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker spectrometer at 400 MHz and 100 MHz, respectively using TMS as an



Glc (**1**), GlcNAc (**2**), Gal (**3**), Xyl (**4**), Lac (**5**), Cell (**6**)

Scheme 1 Preparation of fully acetylated β-D-glycosyl azides (**1**-**6**)



Scheme 2 Attempted preparation of fully acetylated α/β-mannopyranosyl azides (**7**) and α/β-rhamnopyranosyl azides (**8**) using aqueous acetone as the medium

internal standard. The chemical shift values are on a δ scale and the coupling constants (*J*) are in Hz. Electrospray ionization (ESI) Mass spectral data were recorded on a Micromass Q-Tof mass spectrometer.

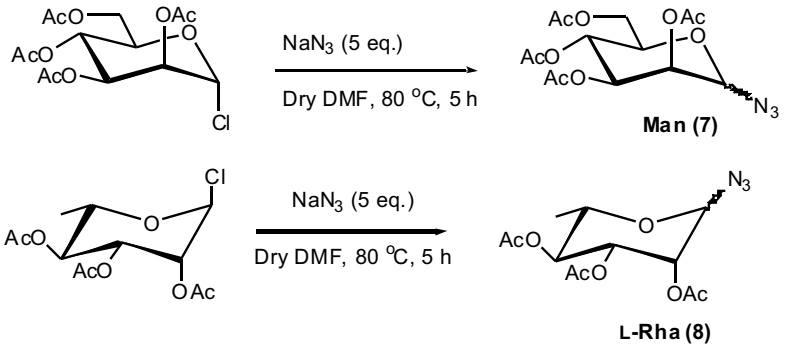
General procedure for the preparation of fully acetylated β-glycosyl azides (1 - 8)

In a 100 mL RB flask, fully acetylated glycosyl chloride[14] (derived from Glc, Gal, GlcNAc, Xyl, Lac or Cell) (10 mmol), was taken and dissolved in acetone (60 mL). To this reaction mixture, an aqueous solution (30 mL) of NaN_3 (3.25 g, 50 mmol) was added and the reaction mixture was stirred for 12 h at room temperature. After removal of acetone on a rotoevaporator, ethyl acetate (60 mL) was added to the reaction mixture. The contents were transferred to a separating funnel, shaken well and the aqueous layer was separated. The organic layer was twice washed with water (30 mL x 2), then with brine solution (30 mL) and was concentrated to dryness. The crude product obtained was recrystallized from a mixture of ethyl acetate-hexane to furnish fully acetylated β-glycosyl azides in 90-95 % yield.

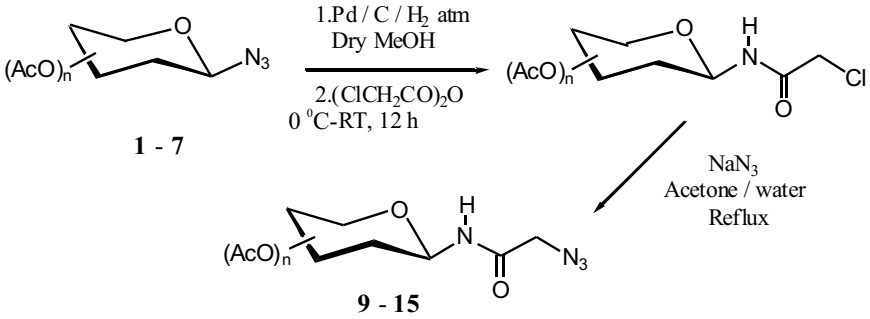
Reaction of fully acetylated α-D-mannopyranosyl chloride[14] and α-L-rhmnopyranosyl chloride[14] in separate experiments with NaN_3 in dry DMF medium at 80 °C furnished an anomeric mixture of 2,3,4,6-tetra-*O*-acetyl-D-mannopyranosyl azide (80 % yield) and 2,3,4-tri-*O*-acetyl-L-rhamnopyranosyl azide (80 % yield). The composition of α- and β-anomers present in both these mixtures was determined by ^{1}H NMR spectroscopy to be 66 % and 34 %, respectively. Flash column chromatography of these two mixtures over silica gel (230 - 400 mesh) using a mixture of ethyl acetate and hexane as eluent afforded each of the anomeric azides in analytically pure form.

General procedure for the preparation of fully acetylated *N*-(β-glycosyl)azidoacetamides (9 - 15)

Fully acetylated β-glycopyranosyl azide (1 mmol) and Pd/C (10 wt %) were taken in a 100 mL two necked RB flask. One neck of the flask was fitted with a rubber septum and the other neck with an adapter connected to a nitrogen balloon. Dry methanol (10 mL) was added



Scheme 3 Preparation of fully acetylated α/β-mannopyranosyl azides (**7**) and α/β-rhamnopyranosyl azides (**8**) in dry DMF medium



Glc (**9**), GlcNAc (**10**), Gal (**11**), Xyl (**12**), Lac (**13**), Cell (**14**), Man (**15**)

Scheme 4 Synthesis of fully acetylated *N*-(β-glycosyl)azidoacetamides

through a syringe to the reaction mixture under gentle stirring followed by the replacement of nitrogen balloon with the one containing hydrogen gas. The contents of the flask were stirred vigorously. The progress of the reaction was monitored by TLC. After the disappearance of the glycosyl azide, chloroacetic anhydride was added (255 mg, 1.5 mmol) at 0 °C and the reaction mixture was stirred for about 12 h at room temperature. Then, it was filtered through a pad of celite and concentrated to dryness to furnish a syrup (90 - 95 % yield). Crystallization of the crude product derived from Glc, GlcNAc and Gal from a mixture of ethyl acetate and hexane afforded the corresponding chloroacetamides (9 - 11) in analytically pure form. The crude products derived from the rest of the azides (4 - 7), on the other hand, required to be purified by column chromatography using silica gel (100 - 200 mesh) to get the corresponding chloroacetamides in analytically pure form.

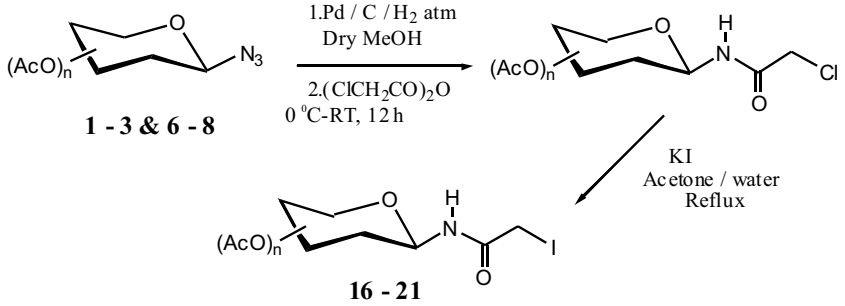
Fully acetylated *N*-(β-glycosyl)chloroacetamide (1 mmol) obtained as described above was taken in a 100 mL RB flask and dissolved in acetone (10 mL). An aqueous solution (5 mL) of sodium azide (325 mg, 5 mmol) was then added to the flask. The reaction mixture was stirred for 6 h at reflux condition. The reaction mixture was then cooled to room temperature. Acetone was removed on a rotoevaporator and the reaction mixture was extracted into ethyl acetate (60 mL). The ethyl acetate solution was washed with water (30 mL x 2) and finally with brine solution (30 mL). The organic layer was concentrated to dryness to give the analytically pure per-*O*-acetylated *N*-(β-glycosyl)azidoacetamides (**9 - 15**) in 90-95% yield.

***N*-[4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl]azidoacetamide (13)**

$[\alpha]_D$ = 3.7° (c = 1, $CHCl_3$); IR (ν, cm^{-1}): 3320, 2943, 2251, 2110, 1748, 1556, 1371, 1232, 1048, 954, 915; ^{1}H NMR (400 MHz, $CDCl_3$): 7.10 (d, 1H, *J* = 9.2 Hz, -NH), 5.36 (d, 1H, H-4'), 5.31 (m, 1H, H-3), 5.18 (t, 1H, *J* = 9.3 Hz, H-1), 5.11 (m, 1H, H-2'), 4.95 (dd, 1H, *J* = 3.2 & 10.4 Hz, H-3'), 4.89 (t, 1H, H-2), 4.48-4.35 (m, 1H, H-1'& H-6a'), 4.15-4.09 (m, 3H, H-6b', H-6a & H-6b), 4.01-3.94 (ABq, 2H, -CH_2N_3), 3.88 (m, 1H, H-4), 3.80-3.71 (m, 2H, H-5 & H-5'), 2.16, 2.13, 2.08, 2.07, 2.06, 2.05, 1.99 (7 x -$COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 171.1, 170.4, 170.3, 170.1, 169.9, 169.4, 169.0 (7 x -$COCH_3$), 167.8 (-NHCO-), 100.7 (C-1'), 77.8 (C-1), 77.5, 75.7, 74.5, 72.5, 70.9, 70.6, 69.0, 66.6, 61.9, 60.8, 52.1 (-CH_2N_3), 20.7, 20.6, 20.5 (2 x C), 20.4 (2 x C), 20.3 (7 x $COCH_3$); ESI-MS: Calcd for $C_{28}H_{38}N_4O_{18}Na$ ($[M+Na]^+$): 741.2082. Found 741.2079.

***N*-[4-*O*-(2',3',4',6'-Tetra-*O*-acetyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl]azidoacetamide (14)**

M. p: 174 °C; $[\alpha]_D$ = -6.6° (c = 1, $CHCl_3$); IR (ν, cm^{-1}): 2923, 2852, 2108, 1740, 1530, 1367, 1213, 1170, 1036, 907; ^{1}H NMR (400 MHz, $CDCl_3$): 7.02 (d, 1H, *J* = 9.1 Hz, -NH), 5.29 (m, 1H, H-3), 5.20-5.11 (m, 2H, H-1 & H-4'), 5.07 (t, 1H, *J* = 9.6 Hz, H-3'), 4.96-4.85 (m, 2H, H-2' & H-2), 4.50 (d, 1H, *J* = 7.2 Hz, H-1'), 4.48 (m, 1H, H-6a), 4.37 (dd, 1H, J = 4.4 & 12.8, H-6a'), 4.12 (dd, 1H, *J* = 4.2 & 12.4 Hz, H-6b), 4.04 (m, 1H, H-6b'), 4.10-3.99 (ABq, 2H, -CH_2N_3), 3.80-3.70 (m, 2H, H-4 & H-5), 3.66 (m, 1H, H-5'), 2.13, 2.10, 2.05, 2.04, 2.03, 2.01, 1.98 (7 x $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 171.2, 170.6, 170.4 (2 x C), 169.5, 169.4, 169.1 (7 x -$COCH_3$), 167.4 (NHCO-), 100.8 (C-1'), 78.1 (C-1), 76.8 , 76.3, 74.8,



Glc (**16**), GlcNAc (**17**), Gal (**18**),Cell (**19**), Man (**20**), L-Rha (**21**)

Scheme 5 Synthesis of fully acetylated *N*-(β-glycosyl)iodoacetamides

73.0, 72.1, 71.7, 70.8, 68.0, 61.9, 61.8, 52.7 (-$\underline{C}H_2N_3$), 22.8 (2 x C), 21.0, 20.8, 20.7, 20.6 (2 x C) (7 x -CO$\underline{C}H_3$); ESI-MS: Calcd for C_{28} H_{38} N_4 O_{18} Na ([M+Na]$^+$): 741.2089. Found 741.2079.

General procedure for the preparation of fully acetylated *N*-(β-glycosyl)iodoacetamides (16 - 21)

To a solution of fully acetylated *N*-(β-glycosyl)chloroacetamide (1 mmol) in acetone (10 mL) taken in a 100 mL RB flask, was added an aqueous solution (5 mL) of KI (830 mg, 5 mmol). The reaction mixture was refluxed for 6 h. After cooling to room temperature, the reaction mixture was concentrated on a rotoevaporator to remove acetone. The reaction mixture was extracted into ethyl acetate (60 mL). The ethyl acetate solution was washed with water (30 mL x 2) and finally with brine solution (30 mL).The organic layer was separated, dried over anhydrous sodium sulfate and concentrated to dryness to obtain a syrupy or a solid product.

***N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)iodoacetamide (16)**

M. p: 110-112 °C; $[\alpha]_D$ = 4.5° (c = 1, acetone); IR (ν, cm^{-1}): 3372, 2943, 2103, 1750, 1692, 1525, 1375, 1218, 1097, 1033, 906; ^{1}H NMR (400 MHz, $CDCl_3$): 6.76 (d, 1H, *J* = 9.1 Hz, -N$\underline{H}$), 5.31 (t, 1H, *J* = 9.6 Hz, H-3), 5.20 (t, 1H, *J* = 9.2 Hz, H-1), 5.08 (t, 1H, *J* = 9.6 Hz, H-4), 4.97 (t, 1H, H-2), 4.31 (dd, 1H, *J* = 4.4 & 12.6 Hz, H-6a), 4.09 (dd, 1H, *J* = 2.0 & 12.5 Hz, H-6b), 3.84 (m, 1H, H-5), 3.69-3.61 (ABq, 2H, -C$\underline{H}_2$I), 2.09, 2.08, 2.05, 2.03 (4s, 12H, 4 x -COC$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 170.7, 170.6, 169.8, 169.6 (4 x -$\underline{C}$OCH$_3$), 168.5 (-NH$\underline{C}$OCH$_2$-), 78.5 (C-1), 73.7, 72.7, 70.3, 68.2, 61.8, 20.8-20.6 (4 x -CO$\underline{C}$H$_3$), -2.3 (-$\underline{C}$H$_2$I); ESI-MS: Calcd for C_{16} H_{22} N O_{10} I Na ([M+Na]$^+$): 538.0193. Found 538.0186.

***N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)iodoacetamide (18)**

M. p: 165-167 °C; $[\alpha]_D$ = -7.0° (c = 1, $CHCl_3$); IR (ν, cm^{-1}): 3475, 3323, 3056, 1750, 1712, 1691, 1546, 1425, 1372, 1228, 1082, 1064, 960, 904, 706; ^{1}H NMR (400 MHz, $CDCl_3$): 6.97 (d, 1H, *J* = 8.6 Hz, -N$\underline{H}$), 5.46 (s, 1H, H-4), 5.26-5.13 (m, 3H, H-1, H-2 & H-3), 4.19-4.06 (m, 3H, H-6a, H-6b & H-5), 3.71-3.66 (ABq, -C$\underline{H}_2$I), 2.16, 2.10, 2.05, 2.00 (4s, 12H, 4 x -COC$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 171.1, 170.4, 170.0, 169.8 (4 x -$\underline{C}$OCH$_3$), 168.3 (-NH$\underline{C}$OCH$_2$-), 79.0 (C-1), 72.6, 70.8, 68.0, 67.3, 61.2, 20.9, 20.7, 20.6, 20.5 (4 x -CO$\underline{C}$H$_3$), -2.3 (-$\underline{C}$H$_2$I); ESI-MS: Calcd for C_{16} H_{22} N O_{10} I Na ([M+Na]$^+$): 538.0181. Found 538.0186.

***N*-[4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl]iodoacetamide (19)**

M. p: 180-182 °C; $[\alpha]_D$ = -10.5° (c = 0.5, $CHCl_3$); IR (ν, cm^{-1}): 3435, 2957, 1759, 1675, 1591, 1385, 1353, 1224, 1069, 1040, 995, 880; ^{1}H NMR (400 MHz, $CDCl_3$): 6.67 (d, 1H, *J* = 9.0 Hz, -N$\underline{H}$), 5.29 (t, 1H, *J* = 8.8 Hz, H-4'), 5.19-5.01 (m, 3H, H-3, H-3' & H-1), 4.97-4.85 (m, 2H, H-2 & H-2'), 4.51 (d, 1H, *J* = 7.6 Hz, H-1'), 4.36 (m, 1H, H-6a'), 4.37 (dd, 1H, *J* = 4.4 & 12.8, H-6a), 4.14 (m, 1H, H-6b'), 4.04 (m, 1H, H-6b), 3.82-3.58 (m, 5H, H-4, H-5, H-5' & -C$\underline{H}_2$I), 2.13, 2.10, 2.08, 2.04 (2 x C), 2.01, 2.00 (7 x-COC$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 171.3, 170.6, 170.3, 170.2, 169.2, 169.4, 169.1 (7 x -$\underline{C}$OCH$_3$), 168.0 (-NH$\underline{C}$OCH$_2$-), 100.7 (C-1'), 78.7 (C-1), 77.4, 74.7, 72.9, 72.0, 71.6, 70.5, 67.9, 59.1, 20.9-20.6 (7 x -CO$\underline{C}$H$_3$), -2.3 (-$\underline{C}$H$_2$I); ESI-MS: Calcd for C_{28} H_{38} N O_{18} I Na ([M+Na]$^+$): 826.1025. Found 826.1031.

***N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranosyl)iodoacetamide (20)**

$[\alpha]_D$ = -50.0° (c = 0.1, $CHCl_3$); IR (*ν*, cm^{-1}): 3392, 2925, 1754, 1692, 1532, 1370, 1258, 1223, 1059, 977, 850; ^{1}H NMR (400 MHz, $CDCl_3$): 6.86 (d, 1H, *J* = 9.2 Hz, -N$\underline{H}$), 5.50 (d, 1H, *J* = 9.2 Hz, H-1), 5.40 (m, 1H, H-2), 5.23 (t, 1H, *J* = 10.0 Hz, H-4), 5.13 (dd, 1H, *J* = 3.2 & 10.1 Hz, H-3), 4.31 (dd, 1H, *J* = 5.2 & 12.4 Hz, H-6a), 4.09 (dd, 1H, *J* = 1.6 & 12.4 Hz, H-6b), 3.79 (m, 1H, H-5), 3.75-3.67 (ABq, 2H, -C$\underline{H}_2$I), 2.25, 2.10, 2.06, 1.99 (4s, 12H, 4 x COC$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 170.7, 170.2, 169.9, 169.7 (4 x -$\underline{C}$OCH$_3$), 166.5 (-NH$\underline{C}$OCH$_2$-), 76.8 (C-1), 74.5, 71.4, 69.7, 65.3, 62.3, 20.9, 20.8, 20.6 (4 x -CO$\underline{C}$H$_3$), -1.5 (-$\underline{C}$H$_2$I); ESI-MS: Calcd for C_{16} H_{22} N O_{10} I Na ([M+Na]$^+$): 538.0181. Found 538.0186.

***N*-(2,3,4-Tri-*O*-acetyl-β-L-rhamnopyranosyl)iodoacetamide (21)**

M. p: 190-192 °C; $[\alpha]_D$ = 3.0° (c = 0.2, $CHCl_3$); IR (ν, cm^{-1}): 3392, 2925, 1754, 1692, 1532, 1370, 1258, 1223, 1059, 977, 850; ^{1}H NMR (400 MHz, $CDCl_3$): 6.66 (d, 1H, *J* = 9.1 Hz, -N$\underline{H}$), 5.43 (d, 1H, *J* = 9.2 Hz, H-1), 5.37 (m, 1H, H-2), 5.10-4.90 (m, 2H, H-3 & H-4), 3.75-3.66 (ABq, 2H, -C$\underline{H}_2$I-), 3.63 (m, 1H, H-5), 2.25, 2.06, 1.95 (3s, 9H, 3 x COC$\underline{H}_3$), 1.25 (m, 3H, -C$\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 170.4, 170.1, 170.0 (3 x -$\underline{C}$OCH$_3$), 166.5 (-NH$\underline{C}$OCH$_2$-), 76.5 (C-1), 72.6, 71.4, 70.1, 70.0, 21.0, 20.9, 20.7 (3 x-CO$\underline{C}$H$_3$), 17.6 (-C$\underline{H}_3$), -1.3 (-$\underline{C}$H$_2$I); ESI-MS: Calcd for C_{14} H_{21} N O_8 I ([M+H]$^+$): 458.0315.

Found 458.0312.

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