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Regioselective opening of unsymmetrical cyclic anhydrides: synthesis of *N*-glycosylated isoasparagine and isoglutamine conjugates†

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N-Glycopeptide mimetic with *N*-glycosylated isoasparagine and isoglutamine conjugates were synthesized by regioselective opening of unsymmetrical cyclic anhydride derivatives of L-aspartic acid and L-glutamic acid, using per-*O*-acetylated β-D-glycopyranosyl amine. The α-chloro derivative gave a mixture of asparagine and isoasparagine linked glycoconjugates, whereas the trifluoroacetamide derivatives gave predominantly the isoasparagine and isoglutamine linked glycoconjugates as the product. The X-ray crystal structure of the α-chloro isoasparagine linked glycoconjugate showed unique pattern of hydrogen bonding.

Introduction

Regioselective chemical ligation of biomolecules is regularly used for the synthesis of bioconjugates with complex structures useful for targeted biomedical applications. This method also has the scope for synthesis of other possible isomers of the bioconjugates potentially useful as a mimic of the natural isomers with improved pharmacokinetic properties. *N*-Glycosylation is a major post-translational or co-translational modification of proteins. The glycan part influences the biological significance of the protein, in addition to controlling their folding and solubility. The linkage region in *N*-linked glycoproteins is conserved as GlcNAc-β-Asn-Xaa-Ser/Thr, where D-GlcNAc is covalently attached to the amide side chain of asparagine by β-linkage, followed by any amino acid other than proline (Xaa) and serine or threonine.[1–4] Naturally occurring *N*-linked glycoproteins are susceptible to hydrolysis by enzymes such as the *N*-glycanase family, thus decreasing their bioavailability and reducing their therapeutic application. In recent years there have been several approaches to improve the proteolytic stability and bioavailability of *N*-linked glycoproteins and utilize their wide range of bioactivity in drug design. With small modification in structure of the natural glycoproteins, these *N*-linked glycoprotein mimics are facilitated with better proteolytic stability and increased bioavailability required for their therapeutic application. Though the naturally occurring glycosyl asparagine and to some extent its one carbon higher homolog glutamine conjugate are synthesized and studied, their regioisomers *i.e.* *N*-glycosylated isoasparagine and isoglutamine have not been explored in the literature of glycopeptide mimetics (Fig. 1).

Asparagine to isoasparagine conversion is not a rare phenomenon in naturally occurring peptides and other bioconjugates. The formation of an isoasparagine linkage inserts an extra methylene group in the peptide backbone resulting in changes in the conformation of the peptide as well as their biological activities. Since some of the asparagine to isoasparagine conversions are related to the immunological disorder in living organism there are reports on the use of isoasparagine in peptide mimetics and evaluation of their therapeutic importance.[5–17] But in the literature of *N*-linked glycoprotein mimetics there are few reports on the synthesis of glycosyl isoasparagine conjugates where a mixture of both glycosyl asparagine and isoasparagine was obtained in almost 1 : 1 ratio.[18,19] The substituent on the amine part of asparagine has less significance in controlling the conformation of the linkage region.[20] In this present work we have synthesized

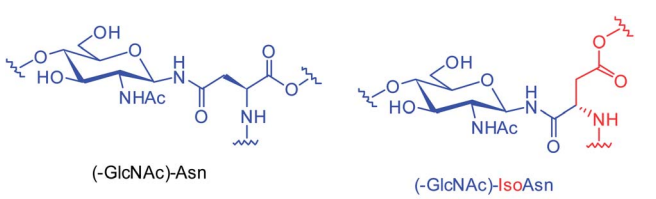

Fig. 1 *N*-Glycoprotein with GlcNAc–asparagine (natural) and GlcNAc–isoasparagine (unnatural) linkage.

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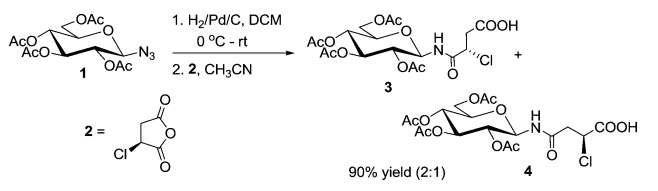
N-glycosylated isoasparagine and isoglutamine conjugates by regioselective opening of cyclic aspartic or glutamic acid anhydrides using per-*O*-acetylated glycopyranosyl amine.

Results and discussion

In addition to the importance of halogenated bio-molecules in medicinal chemistry, considering the higher electronegativity of halogens regioselective reaction of cyclic anhydrides of aspartic or glutamic acid were planned to be achieved using halogenated derivatives of the amino acids. The reaction of per-*O*-acetylated β-D-glucopyranosyl amine (derived from the reduction of the corresponding azide) with 2-chlorosuccinic anhydride gave mixture of two regioisomers (Scheme 1). Both the isoasparagine (**3**) and asparagine (**4**) derivatives were obtained in 90% overall yield with 2 : 1 ratio of the two regioisomers. Purification of the crude reaction mixture by column chromatography followed by recrystallization gave the chlorine substituted isoasparagine precursor **3** in 50% yield. The rest of the product was obtained as a mixture of compounds **3** and **4**.

In the ^{1}H NMR spectrum of compound **3** the anomeric proton appeared as a doublet at 5.22 ppm with coupling constant of 9.2 Hz, whereas the amido proton (N*H*) appeared at 7.45 ppm with a coupling constant of 8.8 Hz. The alpha proton of the chlorine functionalized glycoamino acid (–C*H*Cl) appeared as a multiplet at 4.64–4.61 ppm which was correlated to the methylene protons which appeared as a multiplet in the range of 3.22–3.04 ppm. In the ^{13}C NMR spectrum (100 MHz, $CDCl_3$), the anomeric carbon appeared at 78.6 ppm. The alpha carbon of the chlorine functionalized glycoamino acid (–*C*HCl) appeared at 53.1 ppm and the methylene carbon of the chloroamino acid appeared at 38.8 ppm. The molecular ion peak $[M + H]^+$ of compound appeared at 482.1049 in the ESI-MS HRMS spectrum.

The chlorinated *N*-linked glycoamino acid **3** was recrystallized from a mixture of ethyl acetate and hexane at room temperature by slow evaporation method and the structure was solved in the *monoclinic* space group *P*21. The X-ray structure confirmed the isoaspargine linkage in the molecule. But there are two different conformations of the molecule in the crystal structure, depicted as **A** and **B** in Fig. 2. The conformation of the chlorinated aglycon part of the molecule differs in two different conformers (**A** and **B**) of the molecule in the crystal structure. The torsion angles in both the conformers of the molecule are listed in Table 1. The amide bond has a torsion angle of −125.06° (O5A–C1A–N1A–C1′) and 114.38° (C2A–C1A–N1A–C1′) with the ring oxygen and C-2 carbon of glucose in conformer **A**.



Scheme 1 Synthesis of α-chloro *N*-glycosylated derivative of aspartic acid.

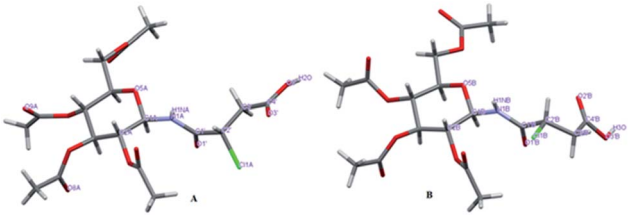


Fig. 2 Conformers **A** and **B** in the crystal structure of molecule **3**.

In conformer **B** the ring oxygen and the C-2 carbon have a torsion angle of −134.57° (O5B–C1B–N1B–C1′B) and 106.19° (C2B–C1B–N1B–C1′B) with the amide bond, respectively. The *N*-glycosylated amide bond and the free acid group in compound **3** has a torsion angle of 173.42° (C1′–C2′–C3′–C4′) and 62.59° (C1′B–C2′B–C3′B–C4′B) in conformer **A** and **B**, respectively. The chlorine has a torsion angle of 143.55° (N1A–C1′–C2′–Cl1A) and 72.69° (N1B–C1′B–C2′B–Cl1B) with the amide bond for conformer **A** and **B**, respectively; whereas the angle between the chlorine and the free acid is −88.82° (Cl1A–C2′–C3′–C4′) and −178.11° (Cl1B–C2′B–C3′B–C4′B) in **A** and **B**, respectively.

The two conformers (**A** and **B**) of molecule **3** not only differ in their conformation, but also in their participation in hydrogen bonding in the crystal structure. The bond parameters of three different types of hydrogen bonds in molecule **3** are listed in Table 2.

All the molecules with conformation **A** are connected to each other by regular H-bonds O2′–H2O···O8A in zigzag style, where the acid groups connected to anomeric positions are H-bonded to C-3 acetate carbonyls (Fig. 3).

On the other hand, all the molecules with conformation **B** are connected to each other by an infinite chain of H-bonds N1B–H1NB···O1′B, where the N–H protons of the anomeric amide groups are connected to CO oxygen of anomeric amide of the next molecule. This infinite chain of hydrogen bonding arranges the molecules (conformer **B**) in anti-parallel orientation (Fig. 4).

The molecules with conformation **A** are connected to molecules with conformation **B** by regular H-bonds O3′B–H3O··· O9A, where the C-4 acetate CO group in conformer **A** are H-bonded to an acid functionality at the anomeric position of conformer **B**. These three different types of regular H-bonds

Table 1 Selected torsion angles in molecule **3**

Conformer **A**		Conformer **B**	
Selected atoms	Angle (°)	Selected atoms	Angle (°)
O5A–C1A–N1A–C1′	−125.06	O5B–C1B–N1B–C1′B	−134.57
C2A–C1A–N1A–C1′	114.38	C2B–C1B–N1B–C1′B	106.19
N1A–C1′–C2′–Cl1A	143.55	N1B–C1′B–C2′B–Cl1B	72.69
O1′–C1′–C2′–Cl1A	−37.75	O1′B–C1′B–C2′B–Cl1B	−106.60
C1′–C2′–C3′–C4′	173.42	C1′B–C2′B–C3′B–C4′B	62.59
Cl1A–C2′–C3′–C4′	−88.82	Cl1B–C2′B–C3′B–C4′B	−178.11
C2′–C3′–C4′–O2′	−8.41	C2′B–C3′B–C4′B–O2′B	20.04
C2′–C3′–C4′–O3′	174.58	C2′B–C3′B–C4′B–O3′B	−160.05

Table 2 H-bond parameters

D–H···A	D–A (Å)	Angle D–H···A (°)
N1B–H1NB···O1′B	2.861	165.11
O2′–H2O···O8A	2.685	165.66
O3′B–H3O···O9A	2.763	175.81

arranged the molecules in a three-pillared structure, where two pillars of conformer **B** arranged in an anti-parallel way are connected to molecules with conformation **A** (Fig. 5 and 6).

Since the reaction with chlorinated cyclic anhydride gave a mixture of asparagine and isoasparagine derivatives we concentrated on improving the regioselectivity of the reaction. Compared to literature reported methods when the amino group of the aspartic acid was replaced by a more electronegative group such as chlorine, the unnatural glycosyl asparagine precursor was obtained as the major product. Reports on the use of a larger group such as –cbz in place of chlorine showed lack of regioselectivity in the reaction (1 : 1).[19] These results suggested that electronic factors, rather than steric factors, play a major role in controlling the regioselectivity of the reaction. Keeping this in mind, the amino group of aspartic acid was converted to its trifluoroacetamido derivative by reacting aspartic acid with trifluoroacetic anhydride to give 2-trifluoroacetamido succinic anhydride (**6**). 2-Deoxy-2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl amine synthesized by catalytic hydrogenation of 2-deoxy-2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl azide (**5**) was reacted with 2-trifluoroacetamido succinic anhydride in dry acetonitrile (Scheme 2) to give the unnatural glycosyl isoasparagine (**7**) as the major product along with a small amount of compound **8** (ratio 9 : 1). The crude reaction mixture was purified by column chromatography to give the unnatural glycosyl isoasparagine derivative **7** in 80% overall yield.

The anomeric proton of compound **7** appeared as multiplet in the range of 5.08–5.04 ppm with H-4 in the ^{1}H NMR spectrum of compound. The alpha proton of the trifluoroacetamido functionalized glycoamino acid (–C*H*NHCOCF$_3$) appeared as a triplet at 4.71 ppm which was correlated to the methylene protons which appeared in the range of 2.98–2.79 ppm. In the ^{13}C NMR spectrum the anomeric carbon appeared at 78.4 ppm. The formation of the product **7** was further confirmed by appearance of the molecular ion peak $[M + Na]^+$ at 580.1379 in the high resolution ESI-MS spectrum.

After synthesis of the glycosyl isoasparagine conjugate by reaction of per-*O*-acetylated glycopyranosyl amine and 2-substituted succinic anhydride, the methodology was extended to the synthesis of glycosyl glutamine conjugates. The synthesis of glycosyl glutamine conjugate was carried out under identical reaction conditions involving conversion of L-glutamic acid to anhydride (**9**) by reaction with trifluoroacetic anhydride followed by reaction of the vacuum dried anhydride with 2-deoxy-2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl amine (Scheme 3). The ^{1}H NMR and ^{13}C NMR spectra of the column purified product showed formation of single product. The ^{1}H NMR spectrum of compound **11** showed the anomeric proton appearing as multiplet along with H-3 in the range of 5.27–5.22 ppm. The alpha proton of the glycoamino acid appeared along with H-6a as a multiplet in the range of 4.30–4.22 ppm, which was correlated to the methylene protons, which appeared as three different multiplets in the range of 2.50–1.90 ppm. The absence of peaks corresponding to the trifluoroacetamido group (NHCO*C*F$_3$) in the ^{13}C NMR spectrum suggested the formation of cyclic glycoamino acid **11** which was further confirmed by the appearance of a molecular ion peak at 480.1587 in the ESI-MS HRMS spectrum.

The mechanism of formation of the unnatural cyclic glutamine conjugate can be explained as follows. When glutamine is treated with trifluoroacetic anhydride, the two acid groups form a mixed anhydride with trifluoroacetic anhydride. The amine reacts with the γ-amino acid anhydride to get the five membered ring containing cyclic amide compound (**9**) whereas the α-acid group remains as the mixed anhydride which upon reaction with 2-deoxy-2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl amine forms the single cyclic glutamine conjugate (**11**).

After synthesis of the GlcNAc–IsoGln derivative (**11**), the methodology was further verified with D-glucose and the corresponding cyclic glutamine conjugate (**10**) was found to be the only product based on their NMR and ESI-MS HRMS

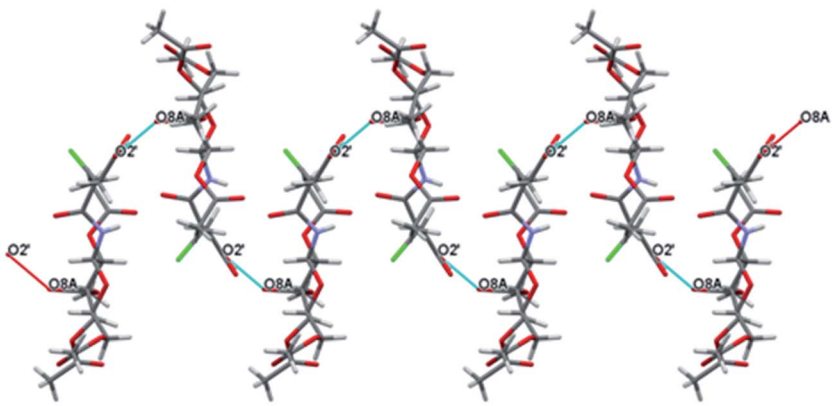


Fig. 3 Infinite chain of H-bonding in compound **3** (conformer **A**).

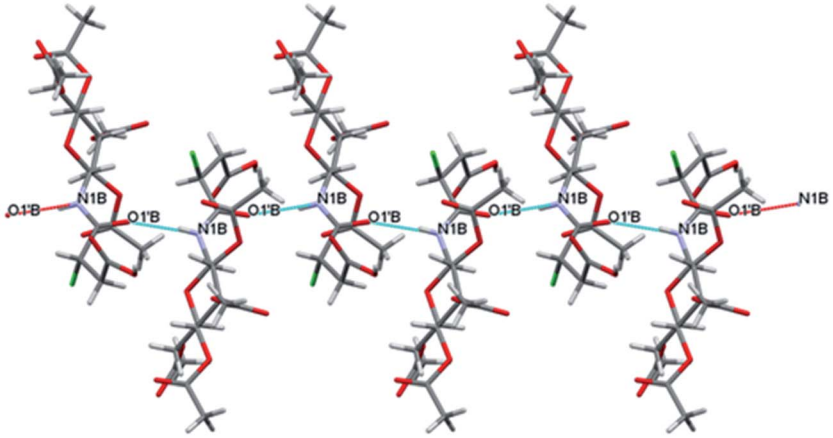


Fig. 4 Infinite chain of hydrogen bonding in compound 3 (conformer B).

spectroscopic analysis after column purification. Compared to aspartic acid in the case of glutamic acid the presence of an additional methylene group in the side chain facilitates the formation of *N*-glycosylated cyclic amide derivatives of glutamine (**10** and **11**).

Summary and conclusion

In conclusion, chlorinated as well as trifluoroacetamido derivative of *N*-glycosylated isoasparagine and cyclic isoglutamine conjugates were synthesized from the reaction of sugar amine and corresponding cyclic anhydrides. The synthesis of cyclic amides from amino acid using simple methodology done in this present work is not known in the literature and can be used in the area of peptidomimetic and glycopeptide synthesis. Although the chlorinated derivative gave a mixture of asparagine and isoasparagine linked products, the X-ray structural study of the isoaspargine derivative (**4**) showed a unique pattern of hydrogen bonding. The regioselective reaction of trifluoroacetamide functionalized cyclic anhydrides gave the corresponding isoasparagine derivative as the major product and the cyclic isoglutamine derivatives as the only product. The replacement of the methylene group from the *N*-glycosylated side chain in natural GlcNAc–Asn linkage to the peptide backbone in isoasparagine will increase the conformational flexibility and proteolytic stability. The application of these

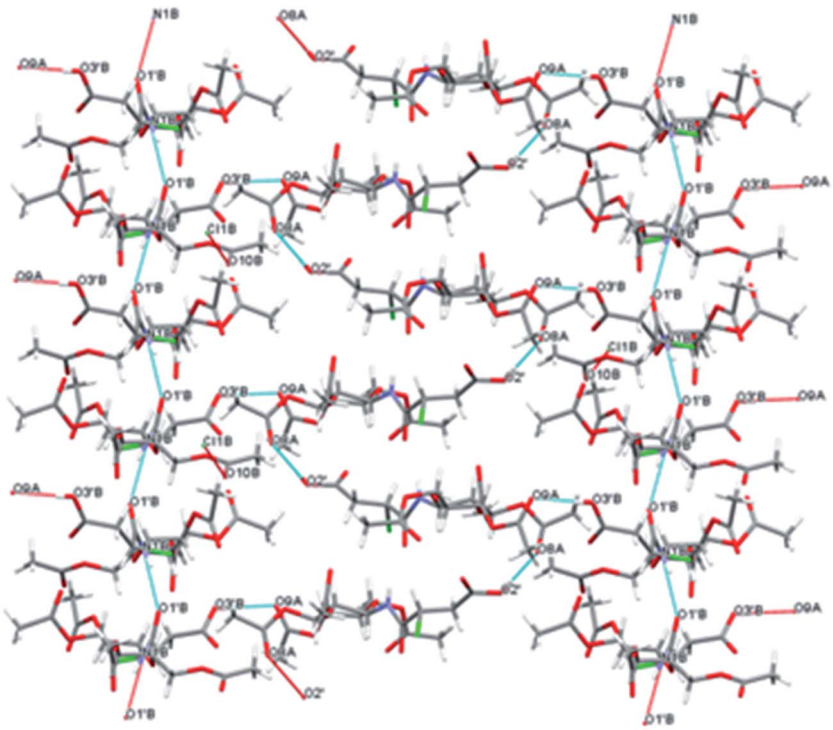


Fig. 5 Three-pillared hydrogen bonding in compound 3 (conformer A and B).

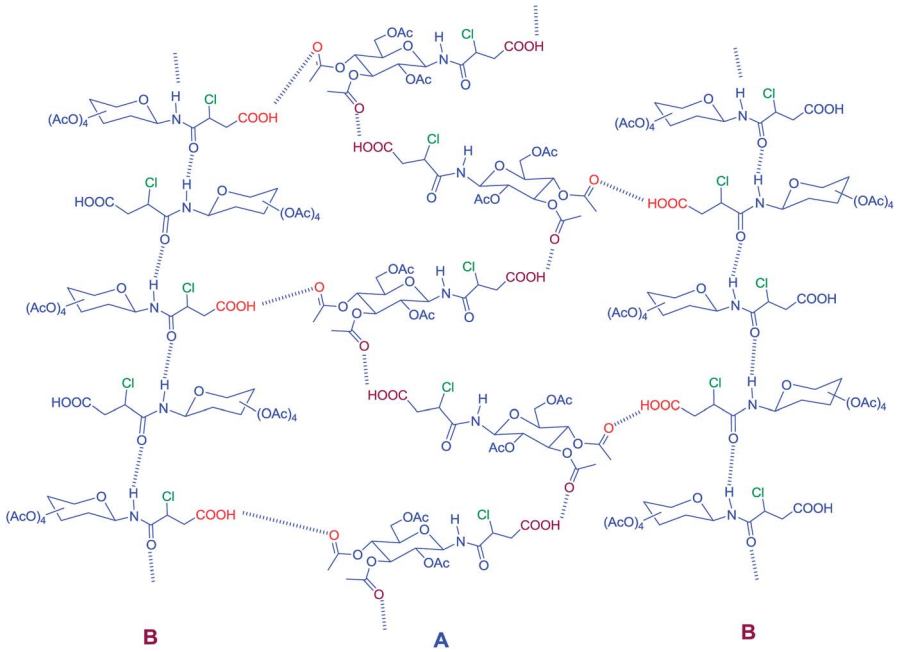
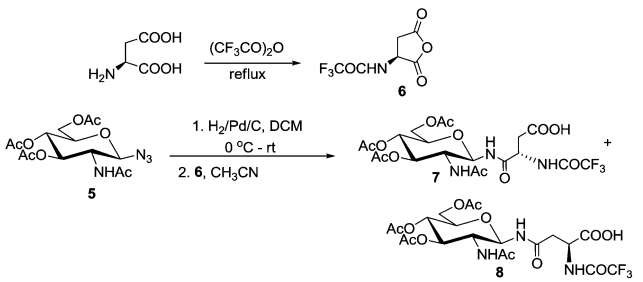
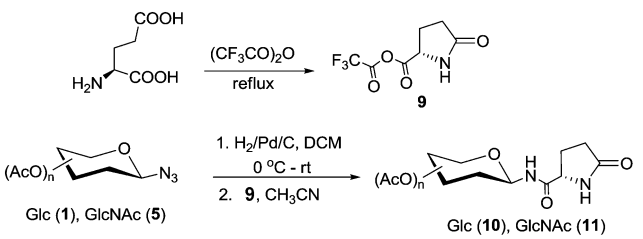


Fig. 6 Hydrogen bonding pattern in the crystal structure of compound **3** (conformer **A** and **B**).



Scheme 2 Synthesis of α-*N*-trifluroacetamido *N*-glycosylated derivative of aspartic acid.



Scheme 3 Synthesis of *N*-glycosylated lactam derivative of L-glutamic acid.

isoaspargine and isoglutamine conjugates in the synthesis of glycopeptide mimic has enormous scope in biomedical research yet to be explored.

Experimental section

General methods

All the solvents were used after distillation and dry solvents were prepared using standard methods. All reagents purchased from commercial sources were used without any purification. NMR spectra were recorded on 400 MHz NMR spectrometer. The assignment of ^{1}H NMR spectra was done with the help of ^{1}H–^{1}H COSY spectra. All mass spectra were recorded in Q-TOF electrospray ionization spectrometer. Column chromatography was performed over 100–200 mesh silica with ethyl acetate and hexane as the eluent.

1. Synthesis of glycopyranosyl asparagine analogs by reaction of per-*O*-acetylated glycopyranosyl amine and 2-substituted succinic anhydride (3 and 7). Per-*O*-acetylated glycopyranosyl azide (**1** or **5**; 1 mmol) was reduced to the corresponding amine using Pd/C (10 wt%) in dry dichloromethane (5 mL) under hydrogen atmosphere. After the reduction was over, indicated by TLC, the solution was cooled to 0 °C. To this a solution 2-substituted succinic anhydride (**2** or **6**, 1 mmol in 5 mL dry acetonitrile) was added dropwise. The reaction was continued for 24 h at room temperature. Acetonitrile (50 mL) was added to the reaction mixture and the contents were filtered through a filter paper. The filtrate was concentrated to dryness and the crude product was purified by column chromatography.

Compound 3. Yield 60%, m.p.: 155–159 °C, $[\alpha]_D$: −18.8° (c = 0.5, MeOH, 25 °C), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.45 (d, 1H, J = 8.8 Hz, N*H*), 5.33 (t, 1H, J = 9.2 Hz, H-3), 5.22 (t, 1H, J = 9.2 Hz, H-1), 5.09 (t, 1H, J = 9.6 Hz, H-4), 5.02 (t, 1H, J = 9.2 Hz, H-2), 4.64–4.61 (m, 1H, Cl–C*H*–CH_2), 4.31–4.28 (dd, 1H, H-6a), 4.11–4.08 (dd, 1H, H-6b), 3.85–3.83 (m, 1H, H-5), 3.22–3.04 (dq, 2H, Cl–CH–CH_2), 2.09, 2.06, 2.03 (×2) (4s, 12H, $COCH_3$) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.1, 170.7, 170.0, 169.5, 168.5 (–CO–CH_3), 78.6, 73.7, 72.6, 70.2, 68.1, 61.5, 53.1, 38.8, 20.7, 20.5 ppm; ESI-MS HRMS: observed 482.1049 for $[M + H]^+$ calculated 482.1065 for $C_{18}H_{25}NO_{12}Cl$.

Compound 7. Yield 80%, ^{1}H NMR (400 MHz, MeOD + $CDCl_3$): δ 5.14 (t, 1H, J = 9.2 Hz, H-3), 5.08–5.04 (m, 2H, H-1 & H-4), 4.71

(t, 1H, $J = 9.6$ Hz, NH–C*H*–CH_2), 4.29–4.24 (dd, 1H, H-6a), 4.13–4.09 (dd, 2H, H-2 & H-6b), 3.81–3.78 (m, 1H, H-5), 2.98–2.79 (dq, 2H, NH–CH–CH_2), 2.08, 2.04, 2.03, 1.93 (4s, 12H, COCH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 170.0, 169.6, 169.5, 169.3, 156.3, 156.0, 117.0 ($J_{C-F} = 291.9$ Hz, CF_3), 78.4, 73.0, 72.3, 68.3, 61.6, 51.8, 50.1, 34.9, 22.3, 20.4, 20.2 ppm; ESI-MS HRMS: observed 580.1379 for [M^+ + Na] calculated 580.1366 for $C_{20}H_{26}N_3O_{12}NaF_3$.

2. Synthesis of glycopyranosyl glutamine analogs (10, 11). Per-*O*-acetylated glycopyranosyl azide (**1** or **5**; 1 mmol) was reduced to the corresponding amine using Pd/C (10 wt%) in dry dichloromethane (5 mL) under hydrogen atmosphere. After the reduction was over, indicated by TLC, the solution was cooled to 0 °C. Glutamic acid (1 mmol) was taken in a 100 mL two necked RB flask fitted to a reflux condenser. To this trifluoroacetic anhydride (5 mL) was added and the reaction mixture was stirred at 40 °C for 30 minutes. After that, the condenser was removed and excess trifluoroacetic anhydride was removed under vacuum. To the syrupy material (**9**) obtained, dry acetonitrile (5 mL) was added and the total content was added to the cooled solution of per-*O*-acetylated glycopyranosyl amine synthesized following procedure described in **1**. The reaction was continued for 24 h at room temperature. Acetonitrile (50 mL) was added to the reaction mixture and the contents were filtered through a filter paper. The filtrate was concentrated to dryness and purified by column chromatography.

*Compound **10**.* Yield 60%, $[\alpha]_D$: +14.3° ($c = 0.7$, MeOH, 25 °C), ^{1}H NMR (400 MHz, DMSO-d6): δ 8.78 (d, 1H, $J = 9.2$ Hz, N*H*), 7.83 (s, 1H, N*H*), 5.40–5.30 (m, 2H, H-1 & H-3), 4.94–4.87 (m, 2H, H-2 & H-4), 4.19–3.95 (3m, 4H, H-6a, H-6b, H-5 & C*H*–CH_2–CH_2), 2.37–2.05 (2m, 3H, CH–CH_2–CH_2), 1.99, 1.98, 1.94, 1.92 (4s, 12H, COCH_3), 1.85–1.75 (m, 1H, CH–CH_2–CH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d6): δ 177.4, 173.1, 170.0, 169.5, 169.3, 169.1, 76.9, 72.7, 72.1, 70.5, 67.7, 61.6, 55.7, 28.9, 25.1, 20.4 ppm; ESI-MS HRMS: observed 481.1430 for [M^+ + Na] calculated 481.1434 for $C_{19}H_{26}N_2O_{11}Na$.

*Compound **11**.* Yield 60%, m.p.: 175–178 °C; $[\alpha]_D$: −35.8° ($c = 0.2$, MeOH, 25 °C), ^{1}H NMR (400 MHz, D_2O): δ 5.27–5.22 (m, 2H, H-1 & H-3), 5.00 (t, 1H, $J = 9.6$ Hz, H-4), 4.30–4.22 (m, 2H, –C*H*–CH_2–CH_2– & H-6a), 4.10–4.05 (m, 2H, H-2 & H-6b), 4.01–3.98 (m, 1H, H-5), 2.50–2.45 (m, 1H, CH–CH_2–CH_2), 2.31 (t, 2H, $J = 8.0$ Hz, CH–CH_2–CH_2), 2.02, 2.00, 1.97, 1.85 (4s, 13H, CH–CH_2–CH_2 & COCH_3) ppm; ^{13}C NMR (100 MHz, D_2O): δ 175.8, 174.8, 173.3, 172.8 (×2), 78.0, 73.7, 73.0, 68.4, 62.0, 56.9, 52.2, 29.0, 25.3, 21.8, 20.1, 20.0 ppm; ESI-MS HRMS: observed 480.1587 for [M^+ + Na] calculated 480.1594 for $C_{19}H_{27}N_3O_{10}Na$.

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