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Design and synthesis of hyaluronan-mimetic gemini disaccharides

Suri S. Iyer,[a,b] Shyam M. Rele,[a,b] S. Baskaran[a,b] and Elliot L. Chaikof[a,b,*]

[a]*Departments of Surgery and Biomedical Engineering, Emory University, Atlanta, GA 30332, USA*
[b]*School of Chemical Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA*

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Abstract—An efficient strategy has been designed for the preparation of synthetic mimics of hyaluronan (HA, **1**) and its dimerized (Gemini) disaccharides (**2a**,**b**) via *n*-pentenyl glycoside formation. Construction of the target molecules was achieved through a combination of protection/deprotection protocols, imidate glycosylation methodology followed by ozonolysis, and reductive amination. These tailored synthetic mimics could act as versatile building blocks with therapeutic applications in tissue engineering, treatment of cancer and as drug delivery agents.

1. Introduction

Glycosoaminoglycans (GAG) are complex biomolecules which mediate a variety of events, including cell growth, inflammatory and immunological responses, tumor metastasis, as well as bacterial and viral recognition processes.[1] Of particular importance is hyaluronan[2] (HA, **1**) which is a high molecular weight, non-sulfated and linear polysaccharide consisting of alternating D-glucuronic acid (D-GlcA) and D-*N*-acetyl glucosamine (D-GlcNAc) moieties linked by β-(1→3) and β-(1→4) linkages (Fig. 1). Both high molecular weight and short oligomeric chains of hyaluronan are important constituents of the extracellular matrix, the synovial fluid of joints, and the scaffolding of cartilage. In this context, HA acts as a signaling molecule via cellular receptors, CD44 and RHAMM, to modulate inflammatory and mesenchymal cell adhesion, migration, and phenotypic differentiation.[2–5] Thus, controlling and modulating HA mediated biological events with selective and specific small carbohydrate molecules will likely have an important impact in the control of a number of pathological processes.

In this report, we describe a linear strategy for the development of HA-based oligomers consisting of (GlcA-β-(1→3)-GlcNAc) separated by a flexible spacer ((Fig. 2) **2a** and **2b**). Previously reported syntheses of di-, tri- and tetrasaccharides of HA afforded methyl glycosides or 4-methoxyphenyl glycosides with D-GlcA or D-GlcNAc moieties at the reducing end.[6–8] However, in all of these reports, the glucuronic acid residue was generated after the construction of the oligosaccharide backbone by selective oxidation of the $-CH_2OH$ group (C-6) of a D-glucose residue. This invariably led to low yields of the final oxidized product. Consequently, in this series of investigations we elected to use readily available acetobromo D-glucuronic acid methyl ester as a precursor for the synthesis of the trichloroacetimidate glycosyl donor. Significantly, an *n*-pentenyl glycoside was designed as the acceptor. Following the work of Fraser-Reid,[9,10] we envisioned that incorporating an *n*-pentenyl group at the anomeric position would offer a number of potential advantages. For example, apart from serving as a glycosidic donor,[9] using oxidation or radical chemistry,[10] the olefinic unit contained in the *n*-pentenyl glycoside could be readily

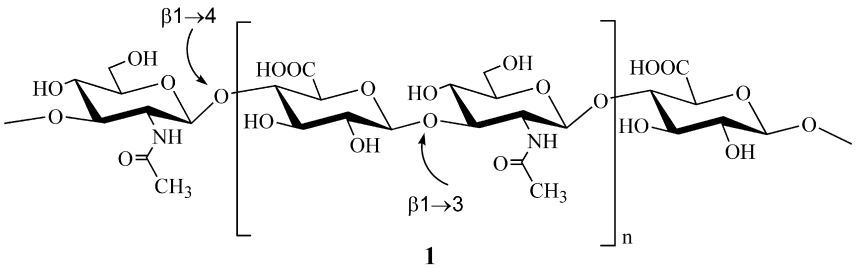

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Figure 1. Structure of natural hyaluronan (**1**) illustrating β-(1→3) and β-(1→4) linkages.

Keywords: hyaluronan; glycosoaminoglycans; carbohydrate–protein interactions; biomimetics; *n*-pentenyl glycoside.

* Corresponding author. Address: 5105 Woodruff Memorial Research Building, 1639 Pierce Drive, Atlanta, GA 30322, USA. Tel.: +1-404-727-8413; fax: +1-404-727-3660; e-mail: echaiko@emory.edu

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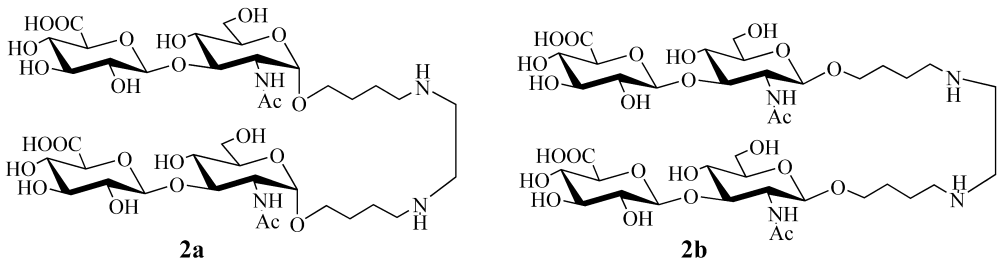


Figure 2. Gemini disaccharides of HA (**1**).

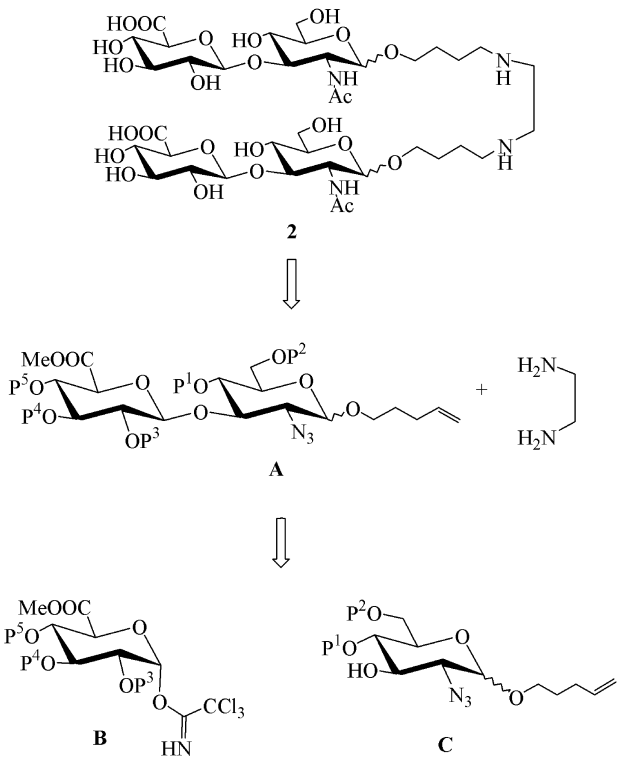


Figure 3. General retrosynthetic strategy for synthesis of dimers possessing β-(1→3) HA dissacharides (P^1–P^5=protecting groups).

attached to spacer functionalities, a carrier protein[11] or a polymer backbone providing direct access to glycodendrimers and/or glycopolymers.[12]

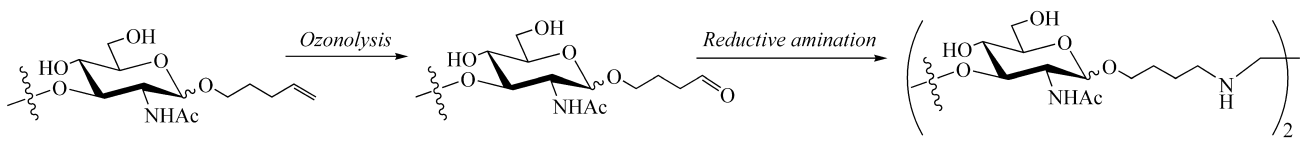
2. Results and discussion

The success of a synthetic strategy involving GAGs depends on a number of factors, including the preparation of a suitable glycosyl acceptor and donor as building blocks, use of selective protecting/deprotecting strategies, appropriate glycosylation or glycosidic bond formation, as well as facile removal of protecting groups with appropriate incorporation of acetamido or sulfate groups. On the basis of a retrosynthetic analysis of **2** (Fig. 3), an efficient strategy was devised to obtain the desired monomeric building blocks, i.e. the imidate donor (**B**) and the *n*-pentenyl glycosyl acceptor (**C**) via a series of key intermediates on a multigram scale.

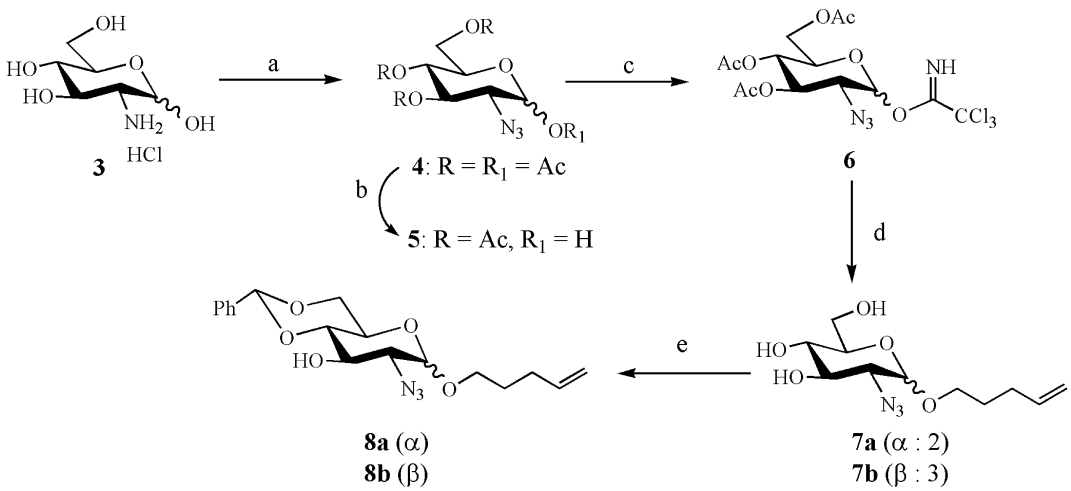
We envisioned that incorporation of a versatile spacer arm like *n*-pentenyl would facilitate direct access to well-defined higher ordered glycoconjugates. As an initial assessment of this approach, Gemini disaccharides were produced via reductive amination with ethylenediamine, after conversion of the terminal olefinic bond to an aldehyde (Scheme 1). Our rationale for the selection of the ethylenediamine linker in the glycomimetic design was based on the consideration that it would provide a sufficiently flexible chain possessing head-to-head HA-based disaccharides. Since, the ligand affinity and specificity is often dependant upon the proper spacing and orientation of the carbohydrate residues, we anticipate that such compounds would then be able to preorganize and fold into a conformation suitable for fitting and complexing with the specific receptor binding site—a fundamental requirement for bioactivity. Nonetheless, generation of such flexible tethered compounds with distinct carbohydrate recognition domains can easily be screened, and the resulting bound antagonists/inhibitors identified.

The methodology for the synthesis of *n*-pentenyl terminated glycoside acceptor **8** is summarized in Scheme 2. 2-Azido-2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranosyl acetate **4** was synthesized from TfN_3 and D-glucosamine hydrochloride.[13] Although the synthesis of an *n*-pentenyl glycoside from the mannose analog, 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-D-mannosepyranosyl acetate,[14] using 4-penten-1-ol and $BF_3{\cdot}Et_2O$ were successful, similar attempts using compound **4** in presence of $BF_3{\cdot}Et_2O$, $SnCl_4$, or TMSOTf as promoters did not succeed. As an alternate route, selective hydrolysis of the anomeric acetate to the hydroxyl group **5** was performed using hydrazine acetate. The hydroxyl group was then converted to the imidate derivative **6** in 74% yield. The *n*-pentenyl group at the anomeric position was produced using a catalytic amount of TMSOTf at 0°C.

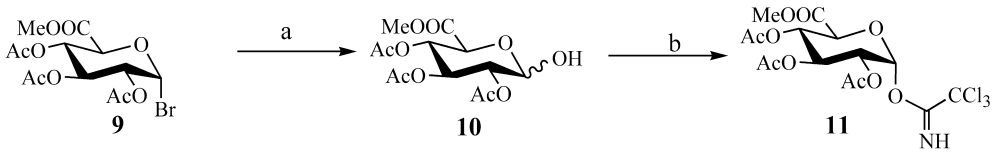
Despite variations in temperature, promoter, and solvents to increase the selectivity ratio, α/β isomers were obtained in a 2:3 ratio. Zemplen conditions[15] performed on the mixture



Scheme 1. Conceived strategy for further manipulation of alkene functionality in the *n*-pentenyl glycoside to obtain hyaluronan mimetic dimers.



Scheme 2. *Reagents and conditions*: (a) (i) TfN_3, MeOH, DMAP, 25°C, 18 h, (ii) Ac_2O, pyridine, 0°C, 10 h, yield=75%; (b) $H_2NNH_2{\cdot}AcOH$, DMF, 0–25°C, 45 min, yield=70%; (c) anhydrous K_2CO_3, CCl_3CN, dry CH_2Cl_2, 25°C, 48 h, yield=74%; (d) (i) 4 Å molecular sieves, TMSOTf, 4-pentene-1-ol, dry CH_2Cl_2, 0°C, 1 h, (ii) MeONa, MeOH, 0–25°C, 6.0 h, yield=80% over two steps; (e) camphorsulfonic acid, dry THF, $C_6H_5CH(OMe)_2$, reflux, 6 h, yield=75%.



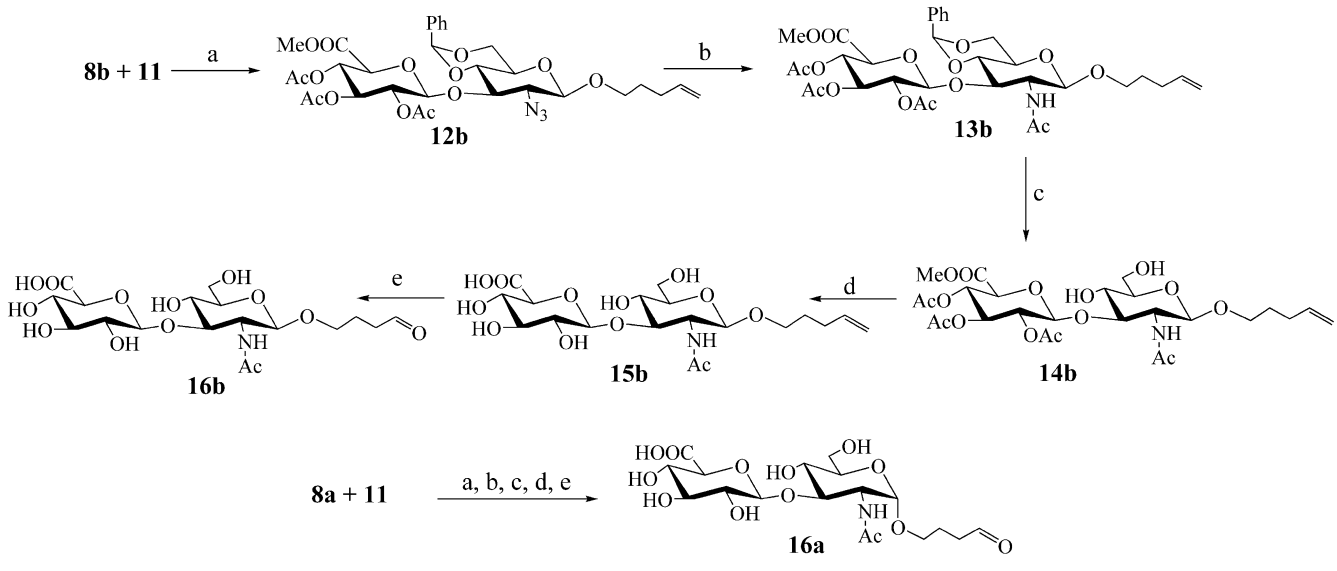
Scheme 3. (a) $CdCO_3$, H_2O (2 equiv.), CH_3CN, 75°C, 4 h, yield=75%; (b) CCl_3CN, 1,2-dichloroethane, 1,8-DBU, −10 to 0°C, 1 h, yield=60%.

subsequently realized the triol in 80% yield, which was easily converted to the acceptor **8** in 75% yield over two steps. The α- and β- isomers were separated using silica column chromatography using hexane/ethylacetate (70:30) as the eluant.

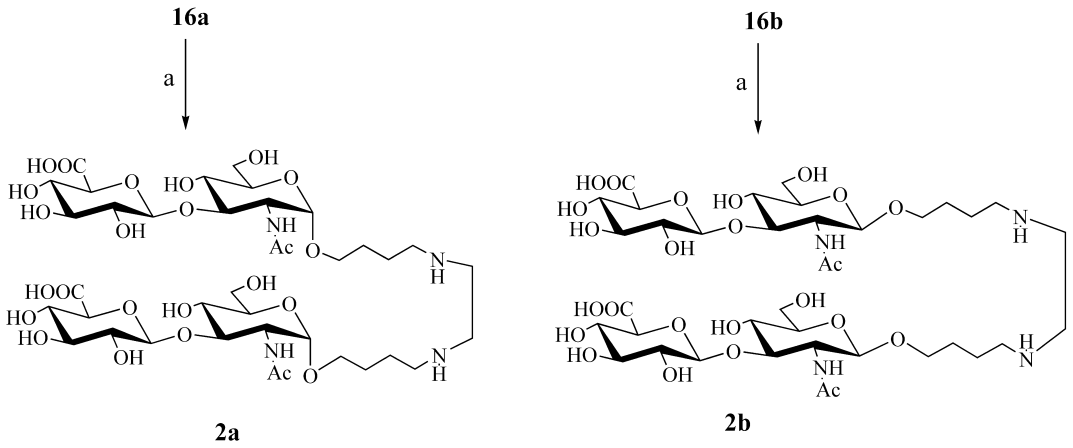
The glucuronic acid imidate donor was synthesized in 50% yield in two steps from the commercially available acetobromo-α-D-glucuronic acid methyl ester **9** (Scheme 3). In turn, **9** was converted to its corresponding free hemiacetal **10** using $CdCO_3/H_2O$, which was subsequently converted to the α-imidate derivative **11** using trichloroacetonitrile and 1,8-diazabicyclo [5,4,0] undec-7-ene.

Glycosylation of the *n*-pentenyl glycoside acceptor **8b**, carried out separately for both α- and β- isomers, with the trichloroacetimidate donor **11** in the presence of a Lewis catalyst TMSOTf afforded the β-(1→3) linked disaccharide **12b** in 78% yield (Scheme 4).

Characterization of compound **12b** by NMR and FAB-MS spectrum was in agreement with the expected structure.



Scheme 4. *Reagents and conditions*: (a) TMSOTf, CH_2Cl_2, 0–25°C, 3.5 h, yield=78%; (b) CH_3COSH, 25°C, 24 h, yield=70%; (c) 3 ml of TFA/H_2O (2:1), CH_2Cl_2, 0°C, 1 h, yield=86%; (d) 3 M NaOH, 9:1 MeOH/H_2O, 25°C, 2 h, yield=86%; (e) O_3 (−78°C), then add Me_2S, −78°C to 25°C, 24 h, yield=85%.



Scheme 5. *Reagents and conditions*: (a) MeOH, ethylenediamine (0.5 equiv.), stir for 3 h, add $NaCNBH_3$ (2 equiv.), 25°C, 3 h, yield=80%.

Thus, for the β-isomer **12b**, the anomeric protons (H-1 and H-1′) were observed as a doublet at δ 4.78 (J=8.0 Hz) and 4.35 (J=8.0 Hz) ppm characteristic of the 1,2-*trans* system. Reduction of the azido group to the acetamido functionality using standard Staudinger reaction[16] conditions (PPh_3/CH_2Cl_2/Ac_2O) led to very low yields. Alternatively, conversion of the azido functionality to the acetamido (–NHAc) group was performed using thioacetic acid,[17] which furnished **13b** in appreciable yield (70%). The ^1H NMR spectrum of **13b** showed a variable doublet between δ 5.8–6.3 (J=6.8–9.2 Hz) ppm attributed to the –NH proton of the acetamido group in addition to the $-NHCOCH_3$ peak at 1.94 ppm. The benzylidene protecting group was then removed using aqueous TFA to give **14b**, which was subsequently saponified using 3 M NaOH in MeOH/water (9:1) mixture to yield the completely deprotected disaccharide **15b** in 86% yield. The crude product was purified using Sephadex LH-20 with MeOH as the eluant. The synthesized disaccharide fragment obtained is similar to the native β-(1→3) linked region of hyaluronan (**1**) with a pendant *n*-pentenyl functionality as a spacer arm at the anomeric position of the GlcNAc moiety. The presence of characteristic signals in the ^{13}C NMR spectrum at δ 173.0 (–COOH), 171.0 (–NHCO–) along with resonance signals at δ 138.2 (=CH), 114.1 ($=CH_2$), 103.8 and 101.1 (C-1 and C-1′) and 22.0 ($-COCH_3$) ppm further corroborated the formation of compound **15b**.

The presence of an *n*-pentenyl group in compound **15b** potentially serves as a versatile handle by transformation of the terminal olefin to aldehyde, carboxylic acid, ester, thioether, thioester or hydroxyl group.[10] To this end, the *n*-pentenyl disaccharide **15b** was subjected to reductive ozonolysis which afforded a terminal aldehyde glycoside intermediate **16b** in 85% yield (Scheme 4). The appearance of the aldehyde signal at δ 9.63 and 203.8 ppm in the ^1H and ^{13}C NMR spectrum, respectively, along with the disappearance of the olefinic double bond peaks at δ 5.81 (δ_c 138.2) and 5.1–4.9 (δ_c 114.1) from the parent compound confirmed the formation of **16b**. Further extension of the aliphatic glycosyl aldehyde by reductive amination of **16b** with the aglycone linker ethylenediamine resulted in the formation of a homodimerized β-(1→3) linked HA neoglycoconjugate analogue **2b** (yield 80%). The crude product was purified using Sephadex G-10 gel filtration column using water as the eluant and was lyophilized to yield **2b** as white foam (Scheme 5).

Computational modeling procedures[18] have suggested that the intrinsic binding affinity of oligosaccharide mimetics to target proteins depends upon the distance and appropriate orientation of the sugar epitopes, as well as the carbohydrate conformational flexibility.[19] Moreover, small and subtle differences in configuration, conformation and functionality can have a profound influence on the biological activity of glycoconjugates. To this end, while the β-linked neoglycoconjugate **2b** resembles the native β-(1→3) linked HA structure, compounds based on α-linked disaccharides may exhibit different receptor binding affinities. With this background, the reductive amination procedure was repeated with α-acceptor (**8a**) to obtain the homodimeric α-product **2a**. Thus, starting with the acceptor **8a/8b** the final gemini homodimers **2a/2b** were obtained in overall yield of 23% over six steps. Complete spectral characterization of the two target compounds using NMR (^1H,^{13}C) and mass spectral (FAB-HRMS) analysis is provided in Section 4.

3. Conclusions

In summary, an efficient strategy that provides well-defined HA disaccharide analogues with a β-(1→3) linkage between D-GlcA and D-GlcNAc units is described. Further, step-wise transformations afforded dimerized HA oligomers via an *n*-pentenyl disaccharide intermediate. Investigations of carbohydrate–protein binding interactions are ongoing and will be reported in due course. Addressing these challenges will ultimately provide a foundation for the design and synthesis of small molecule glycomics for modulating cellular interactions relevant to the design of novel biomaterials, therapeutics and diagnostic agents.

4. Experimental

4.1. General methods

All chemicals were reagent grade and used as supplied unless otherwise noted. Solvents were purchased from

Aldrich and dried and distilled before use. Molecular sieves were activated at 350°C for 3 h in vacuo. Dichloromethane was distilled from CaH_2 and stored over 4 Å molecular sieves. All of the reactions were performed with oven-dried glassware under argon atmosphere and monitored by TLC on 250-μm Whatmann aluminum pre-coated silica gel plates. Detection was performed by examination under UV light (254 nm) and by charring with 10% sulfuric acid in water. Flash chromatography was performed on silica gel (Fischer, mesh 200–425) with the appropriate solvents. Size exclusion chromatography was performed on Sephadex G-10 and LH-20 (Sigma) with water or methanol, respectively, as eluant. Extracts were concentrated under reduced pressure at <45°C (water bath). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 300 MHz or INOVA 400 MHz or on 600 MHz NMR spectrometer. For ^{1}H NMR and ^{13}C NMR spectra recorded in $CDCl_3$, CD_3OD, D_2O and DMSO chemical shifts (δ) are given in ppm relative to solvent peaks (for $CDCl_3$, ^{1}H δ=7.26, ^{13}C δ=77.3; for CD_3OD ^{1}H δ=4.87 and 3.32, ^{13}C δ=47.8; for CD_3SOCD_3 ^{1}H δ=2.5, ^{13}C δ=39.8; for D_2O ^{1}H δ=4.85) as internal standard. Coupling constants (J) are reported in Hertz (Hz). Hetero-nuclear multiple quantum coherence (HMQC) experiments were carried out on compounds **15a/b**, **16a/b** and **2a/b**. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter using a 1 cm cell, and $[\alpha]_D$ values are given in units of 10^{-1}° cm^3 g^{-1} at 25°C. High-resolution fast atom bombardment mass spectrometry (FAB-HRMS) was recorded on JEOL SX102 at 6kV-XE using 3-nitrobenzylalcohol, in some cases with addition of LiI as a matrix.

4.1.1. 2-Azido 2-deoxy-3,4,6 tri-*O*-acetyl α,β-D-glucopyranosyl acetate (4). To a round-bottomed flask containing NaN_3 (0.43 mol, 28 g) and equipped with an argon balloon, water (80 ml) and CH_2Cl_2 (75 ml) was added to it. The emulsion was stirred and cooled to 0°C followed by dropwise addition of dry Tf_2O (0.088 mol, 15 ml) via syringe. The reaction mixture was stirred at 0°C for 2 h at 25°C for 15 min. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2×25 ml). The combined organic layers were neutralized with a saturated solution of $NaHCO_3$, washed with water, dried over $MgSO_4$ and filtered. This stock solution contains ca. 0.1 mol of TfN_3.

In a separate 3-necked flask equipped with an argon inlet containing D-glucosamine hydrochloride (**3**, 0.042 mol, 9 g) dissolved in methanol (250 ml), NaOMe (80 ml of 0.5 M solution in MeOH) was added and stirred for 30 min. DMAP (5 g) was added to it and the reaction mixture was diluted with additional 100 ml of MeOH. The freshly prepared solution of TfN_3 was added to this reaction mixture via cannula under positive pressure of argon and left to stir at room temperature for 18 h. The solvent was removed in vacuo to give a sticky yellow paste. The residue obtained was then acetylated at 0–4°C for 10 h using anhydrous pyridine (250 ml) and dry acetic anhydride (120 ml). On completion of the reaction, the reaction mixture was co-evaporated with toluene (3×50 ml) to give a light brown colored sticky residue. Flash chromatography (EtOAc/hexane 60:40) of the crude reaction mixture using silica gel gave the desired product **4**. Yield=11.5 g (75%). ^{1}H NMR ($CDCl_3$) δ 6.30 (d, 1H, H-1α, J=3.6 Hz), 5.55 (d, 1H, H-1β, J=8.7 Hz), 5.43 (t, 1H, J=8.0 Hz), 5.18–5.04 (m, 3H), 4.34–4.27 (m, 2H), 4.11–4.05 (m, 4H), 3.68–3.64 (m, 2H), 2.19 (s, 6H) 2.11 (s, 3H), 2.10 (s, 3H), 2.08 (s, 6H), 2.05 (s, 3H), 2.02 (s, 3H).

4.1.2. 2-Azido-2-deoxy-3,4,6 tri-*O*-acetyl α,β D-glucopyranoside (5). To an ice cooled solution of **4** (0.0241 mol, 9.0 g) dissolved in 100 ml of dry DMF, solid hydrazine acetate (2.5 equiv., 0.06 mol, 5.5 g) was added under argon atmosphere. The ice-bath was then removed and the reaction mixture was allowed to stir for 45 min. On completion (TLC), the reaction mixture was quenched using ethyl acetate (50 ml) and water (50 ml). The organic layer was the separated and the aqueous layer extracted with EtOAc (3×25 ml). Finally, the combined organic layers were washed with saturated $NaHCO_3$, brine and dried over $MgSO_4$. Concentration of the solvent under vacuo gave a light yellow colored gel which on flash column chromatography (EtOAc/hexane 80:20) furnished **5** as a white solid. Yield=5.9 g (70%). ^{1}H NMR ($CDCl_3$) δ 5.35 (t, 2H, J=10.4, 9.6 Hz), 5.23 (d, 1H, H-1α, J=3.2 Hz), 4.88 (m, 2H), 4.58 (d, 1H, H-1β, J=8.0 Hz), 4.12 (m, 2H), 3.94 (m, 2H), 3.80 (br s, 2H, OH), 3.35–3.28 (m, 2H), 3.20 (s, 2H), 2.09 (s, 6H), 2.08 (s, 6H), 2.08 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 171.3, 171.2, 170.5, 170.4, 170.2, 170.0, 96.1, 91.9, 72.5, 71.7, 70.5, 68.7, 68.5, 67.2, 64.8, 62.2, 61.4, 20.7, 20.6.

4.1.3. 2-Azido 2-deoxy-3,4,6 tri-*O*-acetyl α,β D-glucopyranosyl trichloroacetimidate (6). To the reaction flask containing compound **5** (0.024 mol, 8 g) dissolved in dry CH_2Cl_2 (35 ml), anhydrous K_2CO_3 (0.06 mol, 8.33 g) was added. This was followed by the dropwise addition of Cl_3CCN (0.144 mol, 14.5 ml) after which the reaction mixture was stirred for 48 h under argon atmosphere. On completion (TLC), the reaction mixture was cooled to 0°C, diluted with 25 ml of CH_2Cl_2 and quenched with 20 ml of ice cold water. The organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (2×20 ml). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated to yield a light yellow colored solid. Purification of the crude mixture by column chromatography (EtOAc/hexane 60:40) gave compound **6** (off-white solid). Yield=8.5 g (74 %). ^{1}H NMR ($CDCl_3$) δ 8.81 (s, 1H, NH), 8.79 (s, 1H, NH), 6.43 (d, 1H, H-1α, J=3.6 Hz), 5.68 (d, 1H, H-1β, J=7.8 Hz), 5.55 (dd, 2H, J=9.6, 9.6 Hz), 5.11 (t, 1H, J=10.0 Hz), 5.09 (m, 1H), 4.23 (m, 4H), 4.05 (m, 2H), 3.76 (dd, 2H, J=3.6 Hz), 2.05 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 2.0 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H); ^{13}C ($CDCl_3$) δ 170.7, 170.6, 170.0, 169.8, 169.7, 160.6, 96.5, 94.1, 90.6, 72.8, 72.7, 70.8, 70.2, 68.1, 63.4, 61.5, 60.7, 20.8, 20.7.

4.1.4. Pent-4-enyl 2-azido 2-deoxy- α,β D-glucopyranoside (7). To a oven-dried flask containing activated 4 Å sieves and **6** (0.016 mol, 7.5 g) dissolved in anhydrous CH_2Cl_2 (25 ml), 4-penten-1-ol (0.0195 mol, 2.0 ml) was added via syringe. The reaction mixture and was initially stirred for 10 min at room temperature and then cooled to 0°C under argon atmosphere. Freshly prepared solution of TMSOTf (1.6 mmol, 7.4 ml of 0.22 M solution in anhydrous CH_2Cl_2) was added dropwise through a syringe to the reaction mixture and stirred at 0°C. On completion

(TLC), the reaction mixture was quenched with Et_3N, filtered through a pad of celite and concentrated under reduced pressure to give a semicrystalline material. Flash chromatography (EtOAc/hexane 50:50) afforded a light yellow semi-crystalline material, which was directly used for the next step. (The compound obtained is contaminated with trace amounts of 4-penten-1-ol.) The above mixture was dissolved in anhydrous MeOH followed by addition of NaOMe (10 ml of 0.5 M in MeOH, 0.05 mol) at 0°C under argon atmosphere. The reaction mixture was stirred at room temperature for 6 h (monitored by TLC), and quenched with DOWEX 50W X 8-200 (H^+) ion-exchange resin at 0°C. The resin was filtered and on evaporation of the solvent gave a light brown colored syrup, which on flash chromatography (EtOAc/hexane 50:50 to MeOH/$CHCl_3$ 10:90) furnished **7** as a light yellow colored compound. Yield over two steps: 3.56 g (80%). ^{1}H NMR ($CDCl_3$) δ 5.81 (m, 1H, CH═CH_2), 5.02–4.92 (m, 2H, CH═CH_2), 4.87 (d, 0.3H, H-1α, J=3.2 Hz), 4.6 (br s, 3H, OH), 4.34 (d, 0.7H, H-1β, J=7.6 Hz), 3.98–3.74 (m, 3H), 3.68–3.31 (m, 4H), 3.28–3.20 (m, 1H), 2.11–2.05 (m, 2H, CH_2–CH═CH_2), 1.80–1.65 (m, 2H, –CH_2); ^{13}C NMR ($CDCl_3$) δ 138.7, 115.1, 102.2, 98.1, 75.2, 74.6, 71.4, 70.1, 70.05, 69.5, 67.7, 66.0, 62.6, 61.4, 61.1, 30.3, 30.1, 28.8, 28.6.

4.1.5. Pent-4-enyl 2-azido 2-deoxy-4,6-*O*-benzylidene α,β D-glucopyranoside (8). To a solution of compound **7** (0.011 mol, 3.2 g) dissolved in anhydrous THF (25 ml), 200 mg of (+) camphorsulfonic acid as a catalyst was added followed by dropwise addition of benzaldehyde dimethyl acetal (0.0197 mol, 2.95 ml). The reaction mixture was refluxed under argon atmosphere for 6 h (monitored by the complete disappearance of starting material), cooled to room temperature, diluted with 50 ml of EtOAc and quenched with 10 ml of saturated solution of $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted with 3×25 ml of EtOAc. The organic layers were combined and washed with brine, dried over Na_2SO_4 and concentrated to yield a light yellow colored solid. Purification of the crude product by flash chromatography (EtOAc/hexane 5:95 to EtOAc/hexane 30:70) gave **8** as a mixture of α and β isomers. Yield=3.17 g (75 %). The α (**8a**) and β (**8b**) isomers were separated by silica gel column chromatography using hexane/ethylacetate mixture (70:30).

4.1.6. Pent-4-enyl 2-azido 2-deoxy-4,6-*O*-benzylidene α-D-glucopyranoside (8a). $[\alpha]_D^{25}$=−37.31 (c=0.98, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.50–7.45 (m, 2H, C_6H_5), 7.39–7.27 (m, 3H, C_6H_5), 5.81 (m, 1H, CH═CH_2), 5.59 (s, 1H, CHPh), 5.08–4.99 (m, 2H, CH═CH_2), 4.89 (d, 1H, H-1α, J=3.6 Hz), 4.28–4.21 (m, 2H), 3.87–3.83 (m, 1H), 3.75–3.70 (m, 2H), 3.53–3.47 (m, 2H), 3.24 (dd, 1H, J=4, 3.6 Hz), 2.80 (br s, 1H, OH), 2.19–2.16 (m, 2H), 1.77–1.73 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 138.1, 137.1, 129.6, 128.6, 126.5, 115.3, 102.3, 98.6, 82.1, 69.0, 68.8, 68.1, 63.2, 62.6, 30.4, 28.5.

4.1.7. Pent-4-enyl 2-azido 2-deoxy-4,6-*O*-benzylidene β-D-glucopyranoside (8b). $[\alpha]_D^{25}$=+33.6 (c=1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.48–7.45 (m, 2H, Ph), 7.38–7.26 (m, 3H, Ph), 5.83–5.79 (m, 1H, CH═CH_2), 5.56 (s, 1H, CHPh), 5.06–4.97 (m, 2H, CH═CH_2), 4.33 (d, 1H, H-1β, J=7.8 Hz), 4.29 (m, 1H), 3.89 (m, 1H), 3.73 (t, 1H, J=10.0 Hz), 3.56–3.48 (m, 3H), 3.45–3.38 (m, 2H), 2.65 (br s, 1H, OH) 2.17–2.15 (m, 2H), 1.76–1.73 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 138.1, 137.1, 129.6, 128.6, 126.5, 115.4, 102.7, 102.1, 80.8, 72.0, 70.1, 68.7, 66.7, 66.3, 30.22, 28.94.

4.1.8. Synthesis of imidate donor methyl-(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl trichloacetimidate) glucoronate (11). Acetobromo-α-D-glucuronic acid methyl ester (**9**, 0.025 mol, 10 g) was added to a flame dried three-necked flask containing $CdCO_3$ (0.026 mol, 4.5 g), CH_3CN (150 ml) and 0.8–1.0 ml of degassed H_2O. The reaction mixture was stirred and heated to 70°C for a period of 2 h under argon, filtered through celite, washed with 50 ml of CH_3CN and concentrated in vacuo. Purification of the crude residue by flash chromatography (EtOAc/hexane 50:50) afforded the hydrolyzed product **10** (white solid), which was subsequently dissolved in 200 ml of $(ClCH_2)_2$, Cl_3CCN (10 equiv., 0.142 mol, 13.6 ml) was added to it under argon. After cooling to 0°C, 1,8-diazabicyclo[5.4.0]-undec-7-ene (1,8-DBU) (0.28 equiv., 3.96 mmol, 595 μl) was added in a dropwise manner. The reaction mixture was allowed to stir for 1 h and the mixture was concentrated to afford a sticky dark brown residue. Subsequent flash column chromatography of the crude extract (EtOAc/hexane 40:60, 1% Et_3N) furnished an off-white compound **11** (60%). ^{1}H NMR ($CDCl_3$, 400 MHz) 8.71 (s, 1H, NH), 6.58 (d, 1H, H-1, J=3.6 Hz), 5.62 (t, 1H, H-4, J=10 Hz), 5.26 (t, 1H, H-?, J=10 Hz), 5.11 (dd, 1H, H-2, J=3.2 Hz), 4.43 (d, 1H, H-5, J=10.2 Hz), 3.74 (s, 3H, COOMe), 2.05 (s, 6H, OAc), 2.02 (s, 3H, OAc).

4.1.9. Pent-4-enyl 2-azido 4,6-*O*-benzylidene 2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl uronate)-α/β-D-glucopyranoside (12a/12b). The acceptor **8a/8b** (1.8 mmol, 0.65 g) was dissolved in dry CH_2Cl_2 and stirred at −15°C under argon atmosphere. To this freshly prepared solution of TMSOTf (0.9 μmol, 0.41 ml of 0.22 M) was added dropwise over a period of 5 min via syringe. The imidate donor **11** (2.3 mmol, 1.12 g) dissolved in dry CH_2Cl_2 was then added to the reaction mixture via cannula and the reaction mixture was stirred for 2 h at 0°C and warmed to room temperature over a period of 30 min. On completion (TLC), the reaction mixture was quenched with *N*,*N*-diisopropylamine, concentrated and subjected to column chromatography (EtOAc/hexane 30:70) to give the desired disaccharide **12a/12b** as an off-white solid. Yield=1.2 g (78 %). For **12a**: $[\alpha]_D^{25}$=+42.9 (c=2.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.45–7.30 (m, 5H, C_6H_5), 5.81 (m, 1H, CH═CH_2), 5.51 (s, 1H, CHPh), 5.25–5.08 (m, 3H), 5.06–4.96 (m, 2H), 4.88 (d, 1H, H-1, J=3.6 Hz), 4.80 (d, 1H, H-1′, J=7.8 Hz), 4.25–4.18 (m, 2H), 3.86–3.70 (m, 5H), 3.55 (s, 3H), 3.49 (m, 1H), 3.26 (dd, 1H, J=3.6, 3.6 Hz), 2.22–2.12 (m, 2H), 2.08 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.82–1.76 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.4, 170.0, 169.6, 167.1, 137.9, 137.4, 129.1, 128.4, 126.2, 115.5, 101.8, 101.1, 98.6, 80.4, 72.7, 72.3, 72.0, 69.5, 69.1, 67.8, 63.1, 52.8, 30.4, 28.7, 20.8; HRMS (FAB) calcd for $C_{31}H_{39}O_{14}N_3$ (M^++Li) 684.2592, found 684.2597. For **12b**: $[\alpha]_D^{25}$=−29.7 (c=2.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.42–7.28 (m, 5H, C_6H_5), 5.82 (m, 1H, CH═CH_2), 5.49 (s, 1H, CHPh), 5.25–5.10 (m, 2H), 5.07–4.97 (m, 3H), 4.78 (d, 1H, H-1′, J=8.0 Hz), 4.35 (d, 1H, H-1, J=8.0 Hz), 4.31–4.28 (dd, 1H, J=3.6 Hz), 3.92 (m, 1H), 3.82–3.60 (m, 5H),

3.58 (s, 3H), 3.42–3.34 (m, 2H) 2.09–2.07 (m, 2H), 2.06 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.78–1.74 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.4, 169.10, 169.06, 166.55, 137.49, 136.64, 129.1, 128.77, 125.64, 114.90, 102.34, 101.03, 100.71, 79.62, 79.15, 72.4, 72.1, 71.4, 69.8, 69.3, 68.4, 66.2, 65.9, 52.7, 29.9, 28.6, 20.5; HRMS (FAB) calcd for $C_{31}H_{39}O_{14}N_3$ (M^++Li) 684.2592, found 684.2567.

4.1.10. Pent-4-enyl 4,6-*O*-benzylidene 2-deoxy 2-*N*-acetyl 3-*O*-(methyl 2,3,4-tri-*O*-acetyl β-D-gluco pyranosyluronate)-α/β-D-glucopyranoside (13a/13b). In a dry flame dried flask compound **12a/12b** (1.5 mmol, 1.0 g) was dissolved in 5 ml of thioacetic acid (CH_3COSH). The reaction mixture was then allowed to stir for 24 h under argon atmosphere. On completion of the reaction, the excess thioacetic acid was removed in vacuo and the reaction mixture was subjected to column chromatography ($CHCl_3$/CH_3OH 100:0 to $CHCl_3$/CH_3OH 2.5:95) to give the *N*-acetylated product **13a/13b** as a white crystalline material. Yield=0.71 g (70%). For **13a**: $[\alpha]_D^{25}$=+19.5 (c=3.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.45–7.30 (m, 5H, C_6H_5), 6.20 (d, 1H, J=9.0 Hz, NH), 5.70 (m, 1H, CH=CH_2), 5.51 (1H, s, CHPh), 5.17 (m, 2H), 5.01–4.92 (m, 3H), 4.88 (d, 1H, H-1′, J=7.8 Hz), 4.78 (d, 1H, H-1, J=3.6 Hz), 4.32–4.19 (m, 2H), 4.02–3.96 (m, 1H), 3.79–3.58 (m, 5H), 3.55 (s, 3H), 3.45–3.34 (m, 1H), 2.18–2.10 (m, 2H), 2.01 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.84–1.78 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.4, 170.0, 169.8, 169.7, 167.2, 137.9, 137.4, 129.2, 128.5, 126.3, 115.4, 101.5, 100.3, 98.1, 80.5, 72.7, 72.4, 72.0, 69.6, 69.1, 67.8, 63.1, 52.8, 30.5, 28.7, 23.4, 20.8; HRMS (FAB) calcd for $C_{33}H_{43}O_{15}N$ (M^++Li) 700.2793, found 700.2795. For **13b**: $[\alpha]_D^{25}$=−9.8 (c=0.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.40–7.32 (m, 5H, C_6H_5), 5.91 (d, 1H, J=6.8 Hz, NH), 5.80 (m, 1H, CH=CH_2), 5.46 (s, 1H, CHPh), 5.16–5.08 (m, 2H), 5.03–4.91 (m, 3H), 4.79 (d, 1H, H-1′, J=7.6 Hz), 4.70 (t, 1H, J=9.6, 9.6 Hz), 4.29 (dd, 1H, J=5.1, 4.8 Hz), 3.85–3.49 (m, 6H), 3.60 (s, 3H), 3.03 (m, 1H), 2.09–2.06 (m, 2H), 1.97 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 1.62 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.2, 169.6, 138.0, 137.3, 129.2, 128.4, 128.3, 126.0, 115.0, 101.6, 99.3, 80.9, 72.1, 71.7, 71.6, 69.5, 69.1, 68.8, 65.8, 58.4, 52.8, 30.1, 28.9, 23.6, 20.7; HRMS (FAB) calcd for $C_{33}H_{43}O_{15}N$ (M^++Li) 700.2793, found 700.2787.

4.1.11. Pent-4-enyl 2-deoxy 2-*N*-acetyl 3-*O*-(methyl 2,3,4 tri-*O*-acetylβ-D-glucopyranosyluronate)-α/β-D-glucopyranoside (14a/14b). Compound **13a/13b** (0.014 mmol, 1.0 g) was dissolved in 10 ml of anhydrous CH_2Cl_2 and cooled to 0°C. 3.6 ml of TFA/H_2O (2.5:1.1) was then added to the reaction mixture in a dropwise manner. The reaction mixture was stirred at room temperature for 5 h and on completion of the reaction (TLC) was cooled to 0°C and quenched with a saturated solution of $NaHCO_3$. The organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (3×10 ml). The combined organic layers were washed with brine and the organic layer was separated, dried over Na_2SO_4. Evaporation of the solvent gave a light yellow colored gel which when subjected to column chromatography (EtOAc/hexane 20:80 to $CHCl_3$/MeOH 95:5) afforded the debenzylidenated product **14a/14b**. Yield=0.85 g, 86%. For **14a**: $[\alpha]_D^{25}$=+17.3 (c=2.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.81 (m, 1H, CH=CH_2), 5.61 (d, 1H, J=9.0 Hz, NH), 5.25–5.21 (m, 2H), 5.08–4.94 (m, 3H), 4.72 (d, 1H, H-1, J=3.6 Hz), 4.69 (d, 1H, H-1′, J=7.8 Hz), 4.28–4.23 (m, 1H), 4.13–4.09 (m, 1H), 3.92–3.79 (m, 1H), 3.75 (s, 3H), 3.73–3.56 (m, 5H), 3.42–3.36 (m, 1H), 2.18–2.09 (m, 2H), 2.08 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.76–1.64 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.4, 169.6, 167.2, 138.8, 115.4, 100.8, 97.4, 84.3, 71.8, 71.4, 69.9, 69.1, 67.4, 63.1, 53.5, 51.6, 30.5, 28.4, 23.6, 20.8; HRMS (FAB) calcd for $C_{26}H_{39}O_{15}N$ (M^++Li) 612.2480, found 612.2490. For **14b**: $[\alpha]_D^{25}$=−3.0 (c=0.51, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.76 (m, 1H, CH=CH_2), 5.64 (d, 1H, J=7.2 Hz, NH), 5.29–5.16 (m, 2H, CH=CH_2), 5.03–4.97 (m, 3H), 4.86 (d, 1H, H-1′, J=8.4 Hz), 4.66 (d, 1H, H-1, J=7.6 Hz), 4.41 (dd, 1H, J=8.4, 8.4 Hz), 4.11–4.05 (m, 2H), 3.95–3.78 (m, 3H), 3.75 (s, 3H), 3.52–3.42 (m, 3H), 3.03 (m, 1H), 2.09–2.06 (m, 2H), 2.05 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.62 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 170.1, 169.9, 167.2, 138.7, 115.0, 100.3, 99.0, 83.1, 75.0, 72.1, 71.7, 71.5, 70.6, 69.2, 68.6, 63.1, 57.5, 53.2, 30.2, 29.9, 28.9, 23.9, 20.7; HRMS (FAB) calcd for $C_{26}H_{39}O_{15}N$ (M^++Li) 612.2480, found 612.2493.

4.1.12. Pent-4-enyl 2-deoxy 2-*N*-acetyl 3-*O*-(β-D-glucopyranosyluronate)-α/β-D-glucopyranoside (15a/15b). Compound **14a/14b** (1.3 mmol, 0.8 g) was dissolved in 9:1 MeOH/H_2O mixture (3 ml) and stirred at 0°C. 3 M NaOH solution was added to it and the reaction mixture was allowed to stir at room temperature for 5 h (monitor by TLC). The reaction was then quenched using strongly acidic Dowex H^+ ion exchange resin. The resin was filtered, filtrate concentrated and the product was then purified using gel filtration Sephadex LH-20 column with methanol as eluant. Removal of solvent followed by subsequent lyophilization gave the saponified product as a white solid **15a/15b**. Yield=86%. For **15a**: $[\alpha]_D^{25}$=+30.6 (c=2.7, CH_3OH); ^{1}H NMR (D_2O) δ 5.71–5.68 (m, 1H, CH=CH_2), 4.90–4.79 (m, 2H, CH=CH_2), 4.34 (d, 1H, H-1′, J=8.0 Hz), 3.90 (dd, 1H, J=3.3, 3.6 Hz), 3.77 (d, 1H, J=7.2 Hz), 3.68–3.60 (m, 3H), 3.55–3.49 (m, 2H), 3.42–3.25 (m, 2H), 3.15 (t, 1H, J=9.0, 8.1 Hz), 2.01–1.93 (m, 2H), 1.88 (s, 3H), 1.53–1.48 (m, 2H); ^{13}C NMR (DMSO) δ 170.8, 170.4, 138.9, 115.7, 103.2, 97.4, 80.2, 76.6, 76.1, 73.5, 73.3, 72.0, 69.4, 66.9, 61.2, 53.2, 30.4, 28.7, 23.2; HRMS (FAB) calcd for $C_{19}H_{30}O_{12}N$ (M^++2Li−H) 478.2088, found 478.2083. For **15b**: $[\alpha]_D^{25}$=−54.4 (c=0.98, CH_3OH); ^{1}H NMR (CD_3OD) δ 5.81 (m, 1H, CH=CH_2), 5.1–4.91 (m, 2H, CH=CH_2), 4.45 (d, 1H, H-1′, J=8.0 Hz), 4.37 (d, 1H, H-1, J=8.0 Hz), 3.88 (m, 3H), 3.72–3.63 (m, 3H), 3.62–3.42 (m, 2H), 3.41–3.38 (m, 2H), 3.29 (br s, 2H), 2.09–2.01 (m, 2H), 1.95 (s, 3H), 1.62–1.49 (m, 2H); ^{13}C NMR (CD_3OD) δ 173.0, 171.0, 138.20, 114.1, 103.8, 101.1, 84.3, 76.2, 75.9, 74.8, 73.0, 71.7, 69.2, 68.7, 61.4, 55.0, 30.0, 28.7, 22.0; HRMS (FAB) calcd for $C_{19}H_{30}O_{12}N$ (M^++2Li−H) 478.2088, found 478.2068.

4.1.13. 3-Formylpropyl-2-deoxy-2-*N*-acetyl-*O*-(β-D-glucopyranosyluronate)-α/β-D-glucopyranoside (16a/16b). Compound **15a/15b** (10 mmol, 500 mg) was dissolved in 3 ml of dry methanol and cooled to −78°C. Ozone was bubbled through the light yellow solution for 1.5 h. The pale yellow solution appeared to turn pale blue. To this, 3 ml of dimethyl sulfide was added at −78°C and the reaction was stirred and allowed to warm up to room temperature

overnight. The reaction mixture was concentrated and the crude product was purified using Sephadex LH-20 with methanol as eluant. The pure compound obtained was then lyophilized to give an off white solid. Yield=85%. For **16a**: ^{1}H NMR (DMSO) δ 9.67 (1H, CHO), 7.58 (d, 1H, NH, J=7.8 Hz), 4.67 (d, 1H, H-1, J=3.3 Hz), 4.43 (d, 1H, H-1′, J=7.8 Hz), 3.74–3.58 (m, 2H), 3.61–3.47 (m, 7H), 3.38–3.14 (m, 8H), 3.03 (m, 1H), 1.79 (s, 3H, $NHCOCH_3$), 1.70–1.40 (br m, 4H); ^{13}C NMR (DMSO) δ 204.1, 171.0, 170.5, 103.2, 97.3, 80.5, 76.7, 75.4, 73.4, 72.2, 69.5, 66.9, 61.2, 58.3, 53.0, 23.2, 22.4; HRMS (FAB) calcd for $C_{18}H_{29}O_{13}N$ (M^++Li) 474.2556, found 474.2552. For **16b**: ^{1}H NMR (DMSO) δ 9.63 (s, 1H, CHO), 7.78 (d, 1H, NH, J=8.1 Hz), 5.15 (br s, 1H), 4.64–4.57 (m, 2H), 4.35 (d, 1H, H-1, J=7.8 Hz), 4.27 (d, 1H, H-1′, J=7.8 Hz), 3.72–3.64 (m, 4H), 3.63–3.40 (br m, 6H), 3.32–3.16 (m, 4H), 3.10–3.07 (m, 1H), 1.77 (s, 3H, $NHCOCH_3$), 1.70–1.46 (m, 4H); ^{13}C NMR (CD_3OD) δ 203.8, 173.1, 170.9, 104.5, 103.8, 101.1, 84.4, 76.2, 75.9, 74.8, 73.1, 71.7, 69.3, 69.1, 61.5, 52.3, 29.1, 24.5, 22.6; HRMS (FAB) calcd for $C_{18}H_{29}O_{13}N$ (M^++Li) 474.2556, found 474.2550.

4.1.14. Synthesis of 2a/2b. Compound **16a/16b** (0.043 mmol, 20 mg) was dissolved in 0.5 ml dry methanol and ethylenediamine (0.021 mmol, 1.4 μl) was added to it in a dropwise manner. There was an immediate precipitation of a white solid. The reaction mixture was stirred for 1 h at room temperature and $NaBH_3CN$ (0.11 mmol, 6.9 mg) was then added to it and the reaction mixture was allowed to stir overnight. Removal of the solvent gave a residue, which was purified using Sephadex G-25 with water as eluant and subsequently lyophilized to give a white powder (**2a/2b**). Yield=16 mg (80%). For **2a**: ^{1}H NMR (DMSO) δ 7.95 (br, 1H, NH), 7.84 (d, 1H, J=8 Hz, NH), 4.74 (d, 1H, H-1′, J=2.7 Hz), 4.70 (d, 1H, H-1′, J=2.7 Hz), 4.46 (d, 1H, H-1, J=7.8 Hz), 4.32 (br m, 2H), 3.9–3.55 (m, 13H, ring protons), 3.48–3.18 (m, 8H, ring protons), 3.12–2.85 (m, 10H), 1.81 (s, 6H, $COCH_3$), 1.60–1.40 (m, 8H); ^{13}C NMR (DMSO) δ 170.8, 170.7, 104.5, 103.0, 97.7, 97.0, 80.5, 77.1, 74.0, 73.5, 73.3, 72.9, 72.7, 69.8, 69.4, 67.2, 61.4, 61.2, 53.2, 53.1, 29.4, 27.2, 24.8, 23.3; HRMS (FAB) calcd for $C_{38}H_{67}O_{24}N_4$ (M^++H) 963.3493, found 963.4137. For **2b**: ^{1}H NMR (DMSO) δ 7.70 (br, 2H, NH), 4.36 (d, 1H, J=7.8 Hz), 4.31 (br m, 2H), 4.18 (d, 1H, J=7.2 Hz), 4.10 (m, 2H), 3.88–3.28 (m, 14H), 3.22–3.08 (br m, 10H), 2.98–2.82 (m, 8H), 1.75 (s, 6H, $COCH_3$), 1.60–1.20 (br m, 8H); ^{13}C NMR (DMSO) δ 170.5, 167.7, 132.3, 129.4, 104.3, 104.0, 101.2, 101.1, 85.0, 77.2, 76.6, 74.5, 73.7, 72.3, 69.5, 68.8, 68.1, 61.5, 54.8, 52.9, 37.4, 36.7, 34.6, 32.2, 30.5, 29.2, 29.0, 24.9, 23.9, 23.1; HRMS (FAB) calcd for $C_{38}H_{67}O_{24}N_4$ (M^++H) 963.3493, found 963.4139.

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