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Synthesis of substrate analogues as potential inhibitors for *Mycobacterium tuberculosis* enzyme MshC

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ABSTRACT

Mycothiol cysteine ligase (MshC) is a key enzyme in the mycothiol (MSH) biosynthesis and a promising target for developing new anti-mycobacterial compounds. Herein, we report on the synthesis of substrate analogues, as potential inhibitors, for the MshC enzyme. The target molecules were synthesized employing a Schmidt glycosylation strategy using an enantiomerically pure inositol acceptor and 2-deoxy trichloroacetimidate glycosyl donors with glycosylation yields greater than 70% and overall yields >5%. The inositol acceptor was obtained *via* chiral resolution of (±)-*myo*-inositol.

1. Introduction

Tuberculosis (TB), an airborne infectious disease caused by bacillus *Mycobacterium tuberculosis*, is an epidemic disease and the second largest cause of human death from an infection only after the human immunodeficiency virus (HIV). As per the *Global Tuberculosis Report 2015* [1] an estimated 10 million new cases of TB were reported, leading to some 1.5 million deaths worldwide, where approximately 30% of those individuals were also infected with HIV. To treat TB, isoniazid (isonicotinylhydrazide [INH]), rifampicin (rifampin), ethambutol (EMB, E), amikacin are commonly used, however, with the emergence of multidrug-resistance (MDR) and *extensively* drug-resistant (XDR) strains, the remedy for tuberculosis has become increasingly more difficult due to compromised immunity and compatibility issues, such as drug-drug interactions, drug toxicities, high pill burden and the immune reconstitution inflammatory syndrome (IRIS) amongst combination drug therapies [2]. The demand for new drug targets is critical due to MDR and XDR TB strains and therefore targeting important cellular processes, such as cell wall synthesis, DNA and RNA replication, detoxification process (removal of antibiotics which are toxic to bacterial cell) etc., are required to avert further complications from TB.

Low molecular weight thiols [3] such as coenzymeA (CoA), glutathione (GSH), bacillithiol (BSH) and mycothiol (MSH) help in regulating redox conditions in variety of eukaryotes, gram-positive and gram-negative bacteria. Among these, mycothiol (MSH, **7**) is a major, low-molecular weight thiol found in most actinomycetes. MSH is the functional equivalent of GSH, which facilitates the survival of the *M. tuberculosis* in an oxygen-rich environment by protecting it from oxidative stress [4,5] and antibiotics [6]. MSH biosynthesis is a complex process involving 5 different enzymes as shown in Fig. 1 [7–9]. In short, conjugation of UDP-GlcNAc (**1**) with Ins-3-P (**2**) is catalyzed by the enzyme MshA [10] to form GlcNAc-Ins-3-P (**3**), which upon dephosphorylation by MshA2 forms GlcNAc-Ins (**4**). Deacetylation of the GlcNAc-Ins by MshB leads to the formation of glucosamine inositol (GlcN-Ins, **5**). The ATP-dependent ligation of cysteine to GlcN-Ins is catalyzed by MshC [11] to form Cys-GlcN-Ins (**6**). Finally, N-acetylation of cysteine is carried out by the enzyme MshD to form AcCys-GlcN-Ins (MSH, **7**). Enzyme MST conjugates MSH with the drug molecules to form drug conjugates (MSH-R, **8**) which are processed by enzyme Mca to eliminate toxins such as alkylating agents, free radicals generated by the biological processes of the cell and administered antibiotics [12]. In the process of toxin elimination (**8** →Cys-R→ elimination), GlcN-Ins is regenerated and incorporated back into the MSH

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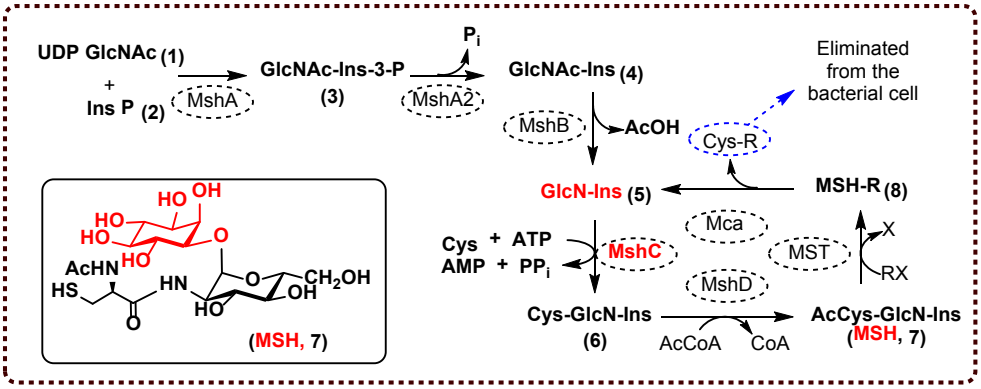


Fig. 1. Mycothiol biosynthesis and metabolic pathway.

biosynthesis cycle (Fig. 1). Cysteine is found to be prone to auto-oxidation. Because of this, high levels of intracellular cysteine are toxic to the cell. MSH acts a cysteine reservoir [13] in order to keep levels in check and important to note is that MSH oxidizes slower than cysteine [14].

Amongst all the enzymatic processes depicted in Fig. 1, the role of the essential enzyme MshC, elucidated by Bornemann and co-workers in 1997, remains to be a critical drug target [15,16]. Based on sequence identification, it has been concluded that MshC shares a close relation to class I cysteinyl-tRNA-synthetases and the mechanism of MshC was proposed as Bi-Uni-Uni-Bi ping pong using steady state kinetics [17] and positional isotope exchange studies [18]. As shown in Fig. 2, in the first phase of the enzymatic reaction, the nucleophilic attack of carboxylate of cysteine onto the phosphate of ATP leads to the formation of the cysteineadenylate intermediate releasing inorganic pyrophosphate (PPi). This cysteineadenylate intermediate is attacked by the amine of GlcN-Ins to form Cys-GlcN-Ins and in the process releases AMP, which completes the second phase of the enzymatic reaction. During this stage, the reacting substrates and coenzymes are held in place by the amino acid residues W227, H55, T46, and D251. Gene knockout studies [16,19] have revealed that MshC plays a pivotal role in MSH biosynthesis in comparison to other enzymes in the mechanistic sequence. Functions of the enzymes in the depicted sequence can be performed by other similar enzymes in the bacterial cell, however and most importantly, the function of MshC is irreplaceable. Therefore, understanding the enzyme structure is critical to developing potential inhibitors. Along those lines, the first partial crystal structure of MshC was solved by Blanchard et al., in 2008, leading to a breakthrough in the organization of the active site [20]. However, in their attempt to obtain the crystal structure, the enzyme was subjected to trypsin digestion which lead to some of the amino acid residues being proteolyzed and hence only a partial crystal structure was obtained. New molecules, such as substrate analogues, are required to obtain a full-length crystal structure and identify the missing amino acids residues in the sequence for a better understanding of enzyme function.

As the mechanism indicates, the enzymatic reaction involves

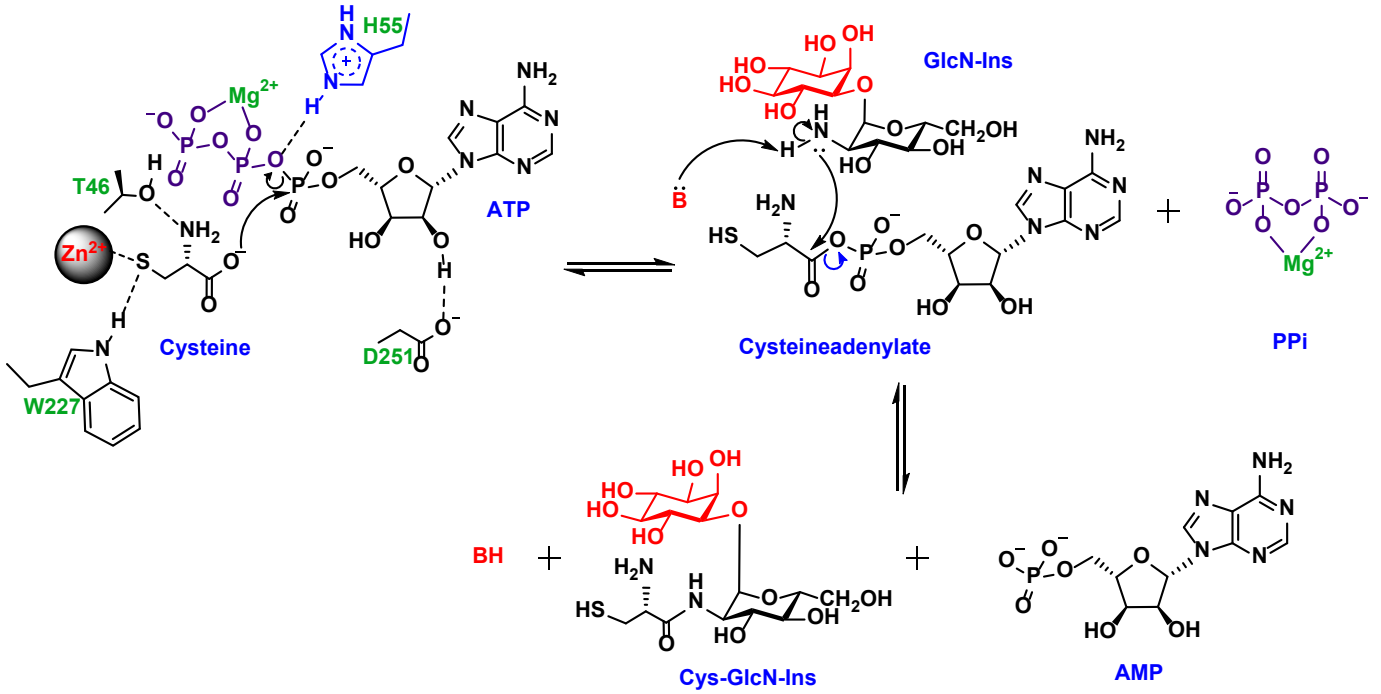


Fig. 2. Proposed enzyme mechanism of MshC.

two different substrates, which gives an extensive range of opportunities to design substrate analogues as potential inhibitors. Screening of chemical libraries led to the identification of some molecules exhibiting notable MshC inhibitory activity, however the antibacterial activities were considered to be caused by their interaction with other biological targets and not specifically against MshC [19,21]. 5′-*O*-[N-(L-cysteinyl)sulfamoyl]adenosine (CSA, **11**) (Fig. 2), an analogue for the cysteineadenylate intermediate of the enzymatic reaction, was found to be an inhibitor of MshC. It was further determined to be a competitive inhibitor when compared to ATP (inhibition constant ~306 nM) and a non-competitive inhibitor when compared to cysteine [17]. Other molecules that showed inhibition were NTF1836 (**12**) [22] and dequalinium chloride (**13**) [23], but they proved to be harmful to mammalian cells and hence were not pursued further. With all considered to date, there has not been a considerable effort towards designing substrate analogues as inhibitors. Therefore, based on this information, we elected to synthesize substrate-based analogues 2-deoxy-azido ($GlcN_3$-Ins, **9**) and 2-deoxy-fluoro sugars (GlcF-Ins, **10**) (Fig. 3). We rationalized our choice of design based on the crystal structure obtained by Blanchard et al. [20]. As per the crystal structure and the modeled proteolyzed loop [20], it is evident that the glucosamine ring of GlcN-Ins is positioned to interact with W227 and D86 as to allow nucleophilic attack of the amine on the electrophilic center of the cysteineadenylate intermediate (Fig. 2). Fluorine, being an electronegative atom, and azide being a bipolar and linear functional group, can both form strong hydrogen bonding interactions with amino acid residues [24–26]. Substituting the amine 2-deoxy position of GlcN-Ins with fluorine and azide is proposed to result in eliminating nucleophilic attack and secondly form tight interactions with histidine (H55) and aspartic acid (D99) residues in the MshC pocket. These interactions may, in turn, allow the enzyme to crystallize and assist in attaining the full-length crystal structure [25]. Fluorine based glycosides have previously been used as inhibitors and transition state analogues for *Mycobacterium tuberculosis* GlmM and GlmU and *Agrobacterium faecalis* β-glucosidase [24,27,28]. Azide based molecules have been widely used in various commercial drugs such as Retrovir® and Azidocillin [29].

Having the target molecule design in mind, we began the syntheses and as expected it proved difficult due to one half of the pseudo-disaccharide being an enantio-pure polyhydroxy-cyclohexane moiety. The reaction sequence began with racemic starting material *myo*-inositol, which was resolved into a pure, single diastereomer before conjugating it with 2-deoxy glycosyl donors *via* Schmidt glycosylation, yielding enzyme substrate GlcN-Ins and proposed inhibitors GlcF-Ins and $GlcN_3$-Ins.

2. Results and discussion

Our strategy to synthesize the desired molecules includes the use of trichloroacetimidate (TCA) donor and an orthogonally protected acceptor to assist in a [1 + 1] Schmidt glycosylation [30]. Since both the donor molecules were 2-deoxy-2-substituted sugars, we expected to obtain a α-selective glycosylation product owing to the anomeric effect in the absence of neighboring group participation (NGP). The target molecules were a set of pseudo-disaccharides, i.e., a combination of sugar and a polyhydroxy-cyclohexane motif [31–34]. Achieving the target molecules was challenging accrediting to the resolution and diastereomeric intermediates.

2.1. Synthesis of glycosyl acceptor (*18*)

The synthesis of *myo*-inositol acceptor commenced by following an established protocol by Schmidt et al. [35] with required modifications to facilitate our coupling strategy. (±)-*Myo*-inositol **(14)** was protected using 1,1-dimethoxy cyclohexane under acidic conditions to yield a mixture of di-*O*-cyclohexylidene derivatives (Scheme 1). The undesired racemates were separated from the mixture by crystallization removing the unwanted, differently protected derivatives of *myo*-inositol and then this was followed by column chromatography to afford the desired molecule **15**. Under refluxing conditions in toluene, **15** was then treated with tributyltin oxide followed by addition of (1*R*)-(-)-menthyl chloroformate in the presence of $NaHCO_3$, resulting in the regioselective protected product **16**. Compound **16** was then subjected to benzylation under unique silver oxide conditions. Since, under basic conditions, the carbonate moieties are prone to migration to the adjacent available hydroxyl groups, in order to prevent this, silver oxide was used instead of sodium hydride [35,36]. Use of freshly prepared silver oxide [37] is highly important in order to obtain complete conversion of the starting material. Separation of the desired diastereomer from the reaction mixture was achieved by precipitation using 100% petroleum ether to afford protected D-*myo*-inositol **17**

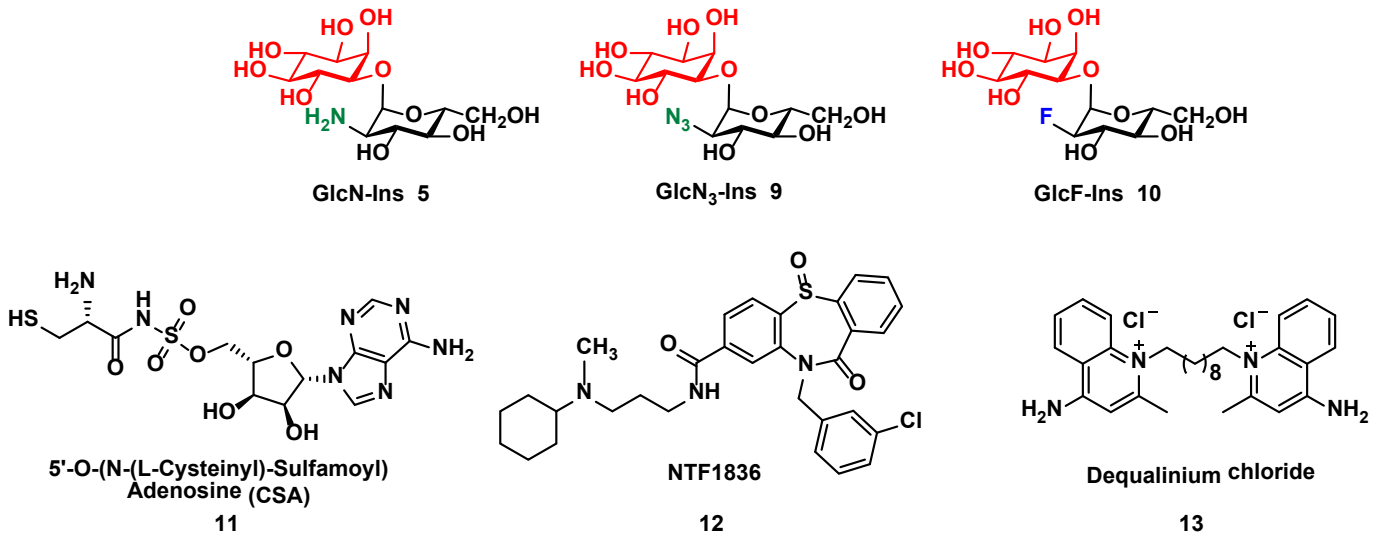
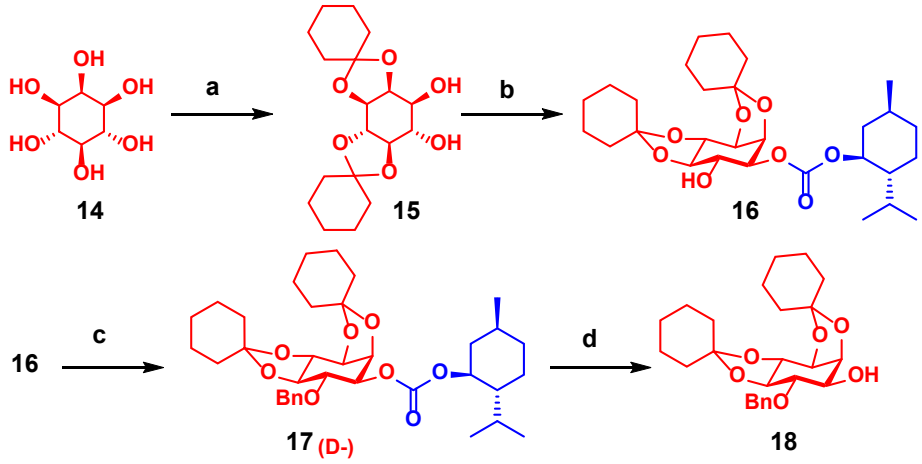


Fig. 3. Substrate for MshC (**5**) and analogues (**9**, **10**). Known inhibitors for MshC (**11**-**13**) [17,22,23].

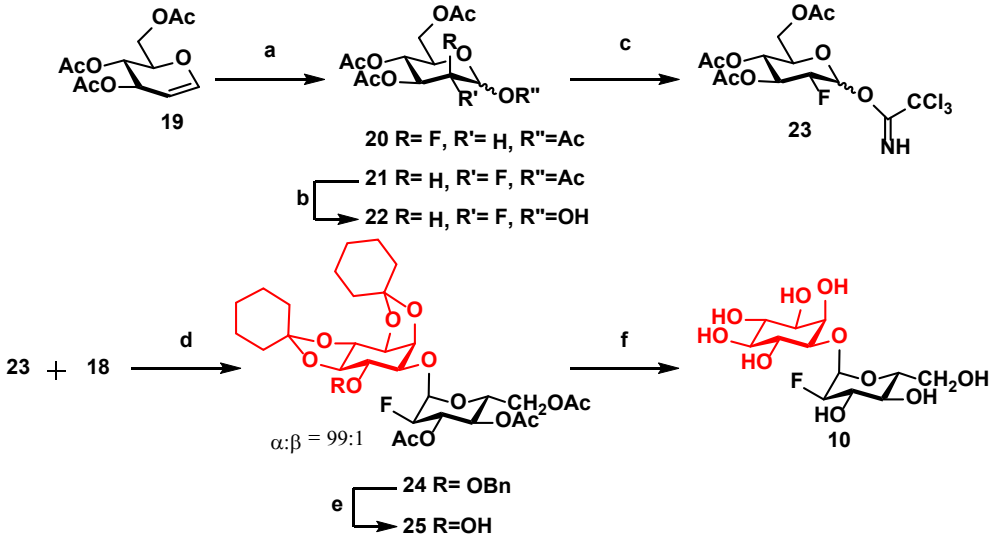


Scheme 1. Reagents and conditions: a) 1,1- dimethoxy cyclohexane, *N,N*-Dimethylformamide(DMF), *p*-TsOH, 95-100 °C, 3 h, (23%); b) (i) $(Bu_3Sn)_2O$, toluene(Tol), reflux, 3 h (ii) $NaHCO_3$, (1*R*)-(-)-menthyl chloroformate, Tol, 0 °C-RT, 24 h, (73%); c) (i) Ag_2O, BnBr, MS-4 Å, anhydrous dichloromethane (DCM), reflux, 6 h, (ii) recrystallization in petroleum ether (45%); d) K_2CO_3, isopropanol:methanol (IPA:MeOH, 1:1), reflux, 6 h, (97%).

(Scheme 1). Enantiopurity confirmation of **17** was achieved employing optical rotation where obtained α values matched quite nicely with those reported in the literature (obs. $[\alpha]_D^{22}$ = -22.2°, $c = 0.5$, $CHCl_3$; lit. $[\alpha]_D^{22}$ = -25.0°, $c = 0.5$, $CHCl_3$). Treatment of the 6-*O*-benzyl derivative **17** with K_2CO_3 to remove the auxillary (1*R*)-(-)-menthyl in isopropanol:methanol (IPA:MeOH, 1:1) furnished desired acceptor **18**.

2.2. Synthesis of GlcF-Ins (*10*)

With our acceptor in hand, the synthesis of the desired glycosyl donor was initiated using commercially available 3,4,6-tri-*O*-acetyl-D-glucal **(19)** (Scheme 2). Compound **19** was subjected to fluorination using Selectfluor® in *N,N*-dimethylformamide:water (DMF:water = 1:1) solvent mixture affording an inseparable mixture of 2-fluoro axial/equatorial epimers. Difficulties in separation were overcome by acetylating the anomeric hydroxyl group upon which molecules **20** and **21** [38] were readily separated *via* silica gel chromatography. The required epimer **21** was fully characterized and confirmed using ^{1}H-NMR. This compound was then subjected to conditions of bromination generating a α-glycosyl bromide donor. Surprisingly, we observed the molecule to be quite unstable so we elected to pursue the trichloroacetimidate donor. Therefore, compound **21** was subjected to conditions for anomeric deacetylation using ammonium acetate [39] in DMF to afford hemiacetal **22**. Under anhydrous conditions and in the presence of K_2CO_3, compound **22** was then treated with trichloroacetonitrile to yield the TCA donor (**23**) in 95% yield [40,41]. Glycosylation was conducted using standard glycosylation procedures which selectively afforded the α-selective product **24** in 78% yield (α:β = 99:1). Initially we observed low yields on the glycosylation step, however, after careful investigation we realized that the cyclohexane ketal protecting groups on the acceptor, which are sensitive to acidic conditions, were being cleaved by trimethylsilyltriflate (TMSOTf), which was used as the promoter in the Schmidt glycosylation reaction. In order to prevent the ketal protecting groups from leaving, the reaction temperature was maintained at -20 °C, lower than the usual temperature for these types



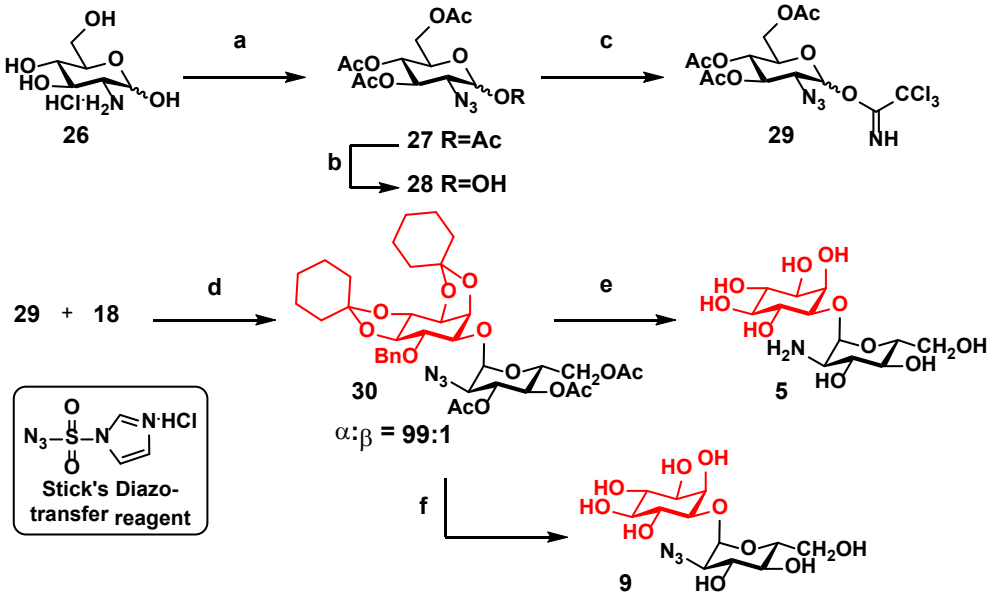
Scheme 2. Reagents and conditions: a) (i) Selectfluor®, DMF:Water, RT, 12 h, (ii) Ac_2O, Pyridine, DMAP, RT, 12 h, (**20** = 33%, **21** = 58%); b) Ammonium acetate, DMF, RT, 12 h, (96%); c) Trichloroacetonitrile, K_2CO_3, anhydrous DCM, 0 °C, 16 h, (93%); d) TMSOTf, anhydrous DCM, MS-4 Å, -20 °C, 1 h, (78%); e) 10% w/w Pd-C, H_2, MeOH, RT, 5 h, (98%); f) (i) NaOMe/MeOH, RT, 1 h, (ii) DOWEX 50WX8-100 ion exchange (H+) resin, MeOH, 35 °C, 1 h, (98%).

of glycosylation reactions and hence we experienced a longer reaction duration. Furthermore, another reason for our initial lower yielding glycosylation step was as a direct result of protection of the acceptor by the trimethylsilyl group from the TMSOTf activator, which was readily confirmed by both ^{1}H-NMR (Compound **18a** in SI) and MS analysis. The silyl protected acceptor was recovered and recycled for use in a simultaneous batch of glycosylation reactions after the removal of the TMS protecting group using tetrabutylammoniumfluoride (TBAF, 1.0 M in THF). Compound **24** was then subjected to palladium catalyzed hydrogenation using 10% w/w Pd-C at 60 psi to remove the benzyl protecting group and furnish **25** [42] (Scheme 2). Compound **25** was further subjected to Zemplén deacetylation conditions [43] followed by gentle heating in the presence of DOWEX 50WX8−100 ion exchange (H^+) resin at 45 °C [44] to assist in a one-pot removal of acetyl and cyclohexylidene protecting groups respectively with a 90% yield over two steps affording **10**.

2.3. Synthesis of GlcN-Ins (5) and GlcN$_3$–Ins (9)

After obtaining GlcF-Ins (**10**), our focus turned towards synthesizing the azido analogue as well as the natural substrate for MshC. We commenced by performing a diazo transfer reaction on glucosamine hydrochloride (**26**) using Stick's diazo transfer reagent [45] (prepared in-house) followed by peracetylation to afford compound **27** in 87% yield over two steps (Scheme 3). Compound **27** was then subjected to anomeric deacetylation using ammonium acetate in DMF to afford hemiacetal **28**. Reaction with trichloroacetonitrile, under anhydrous conditions, afforded TCA donor (**29**) in 90% yield. Following the general Schmidt glycosylation procedure, acceptor **18** and donor **29** were coupled to furnish compound **30** in 81% yield with high α-selectivity (α:β = 99:1) (Scheme 3). The glycosylation reaction presented problems such as low yield due to removal of the ketal groups on acceptor and protection of acceptor by TMSOTf. These issues were also observed in the case of GlcF-Ins and were overcome by carefully monitoring reaction temperature and duration. Compound **30** was then subjected to different deprotection strategies depending on the desired target molecule (Scheme 3). Therefore, in order to obtain GlcN-Ins (**5**), compound **30** was subjected to palladium catalyzed hydrogenation using 10% w/w Pd-C at 60 psi to remove the benzyl protecting group, followed by Zemplén deacetylation conditions with DOWEX 50WX8−100 ion exchange (H^+) resin under heat ultimately giving rise to GlcN-Ins (**5**) in 90% overall yield. In order to obtain GlcN$_3$-Ins (**9**), first the benzyl group was removed selectively *via* oxidative debenzylation [46] as to avoid unwanted azido group reduction. This was accomplished using $NaBrO_3/Na_2S_2O_4$ in a biphasic solvent system. Without purification, the mixture was then subjected to global deprotection with Zemplén deacetylation conditions and finally heating at 45 °C in the presence of DOWEX 50WX8−100 ion exchange (H^+) resin to remove the cyclohexylidene groups respectively to give rise to GlcN$_3$-Ins (**9**).

The desired target molecules described herein, were achieved by overcoming difficulties in separating diastereomeric mixtures of important intermediates. Contrary to utilizing enzymes [47] for resolution, a chiral auxiliary was used to assist in the separation of diastereomers. The glycosylation reaction was optimized to prevent loss of yield and α-selective product formation was confirmed by ^{1}H-NMR analysis. The H-1 proton of the glycosylation products **24** and **30** showed coupling constant values of 4.14 and 2.88 Hz respectively confirming the desired α-configuration at the anomeric position. ^{19}F-NMR was also recorded for all steps in the synthetic route for GlcF-Ins (**10**) in order to confirm the presence of fluorine. GlcF-Ins was obtained from **24** using palladium catalyzed hydrogenation followed by a one-pot acetyl and ketal group deprotection. GlcN$_3$-Ins (**9**) was obtained from **30** following an oxidative debenzylation procedure to avoid the reduction of azide and then a one-pot global deprotection. We were also able to synthesize GlcN-Ins (**5**), the naturally occurring MshC substrate, by subjecting **30** to palladium catalyzed hydrogenation and one-pot global deprotection. As previously mentioned, the fluorine and azide moieties can interact with amino acid residues such as aspartic acid and histidine in the enzyme pocket to induce conformational changes in the enzyme for inhibition purposes as well as aide in obtaining the full crystal of MshC enzyme. The idea of including the azide moiety as a substrate-based analogue can



Scheme 3. Reagents and conditions: a) (i) Stick's diazo transfer reagent, K_2CO_3, $CuSO_4.5H_2O$, MeOH, RT, 3 h, (ii) Ac_2O, Pyridine, DMAP, RT, 12 h (87%); b) Ammonium acetate, DMF, RT, 12 h, (98%); c) Trichloroacetonitrile, K_2CO_3, anhydrous DCM, 0 °C to RT, 16 h, (90%); d) TMSOTf, anhydrous DCM, MS-4 Å, -20 °C, 1 h, (81%); e) (i) 10% w/w Pd-C, H_2, MeOH, RT, 5 h; (ii) NaOMe/MeOH, RT, 1 h, (iii) DOWEX 50WX8-100 ion exchange (H+) resin, MeOH, 35 °C, 1 h, (92%); f) (i) $NaBrO_3$, $Na_2S_2O_4$, EtOAc:H_2O, RT, 6 h, (ii) NaOMe/MeOH, RT, 1 h, (iii) DOWEX 50WX8-100 ion exchange (H+) resin, MeOH, 35 °C, 1 h, (90%).

also be supported by commercial azide-based drug molecules like Retrovir® and Azidocillin.

3. Conclusion

We have synthesized substrate analogues for the *Mycobacterium tuberculosis* enzyme MshC. The synthesis was accomplished employing chiral resolution of the acceptor, stereoselective glycosylation and modified purification procedures leading to overall good yields. Access to the naturally occurring substrate GlcN-Ins is important for probing enzyme function as well as for developing inhibitors. We anticipate that these molecules will be useful in further MshC-based studies leading to the development active molecules against the enzyme and the Tuberculosis disease in a broader spectrum.

4. Experimental

4.1. General methods

All chemicals and solvents were of commercial grade and were used without further purification unless otherwise stated. Chemicals were purchased from Alfa Aesar, Acros Organic, Fisher Scientific, Oakwood Chemicals, Sigma Aldrich and TCI Chemicals. Solvents were purchased from EMD, Fisher Scientific and Sigma Aldrich. Molecular sieves (MS-4 Å) were activated by heating at 150 °C overnight under a vacuum in a high temperature vacuum oven equipped with an inert gas line. All reactions performed were monitored using thin layer chromatography over silica gel-coated TLC plates purchased from Silicycle. TLC plates were visualized under UV light or by charring (5% H_2SO_4-MeOH and Anisladehyde solutions). Flash column chromatography was performed using 200-400 mesh silica gel, Siliaflash®P60, purchased from Silicycle. Size exclusion column was performed using Bio-Rad Bio-Gel® P-2 gel. Optical rotations were measured with Autopol-IV digital polarimeter; concentrations are expressed as g/100 mL. ^{1}H, ^{13}C-APT, NMR spectra were recorded on an Avance 600 MHz NMR spectrometer with $CDCl_3$, MeOD, D_2O as solvents. ^{19}F-NMR was recorded on a GEM 200 MHz spectrometer with $CDCl_3$ and MeOD as the solvents. The residual $CHCl_3$ was referenced to δ 7.27 and δ 77.27 ppm in proton and carbon spectra respectively. The residual MeOH was references to δ 3.31 and δ 49.00 ppm for proton and carbon spectra respectively. The residual HDO was referenced to δ4.9 with spectra taken in D_2O and $CFCl_3$ was used as internal standard for ^{19}F-NMR. Chemical shifts are reported in δ ppm. Data for ^{1}H NMR are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, dt = doublet of triplet, ddd = doublet of doublet of doublet, t = triplet, m = multiplet) and coupling constants in Hertz (Hz). Low resolution mass spectra were obtained using electrospray ionization (ESI) technique. Yields refer to chromatographically and spectroscopically pure material unless otherwise noted.

4.2. General glycosylation procedure

To a solution of acceptor (1 mmol) and trichloroacetimidate donor (1.1 mmol) in anhydrous DCM (30 mL), preactivated molecular sieves (MS-4 Å, 0.25 g) were added and stirred at room temperature for 25 min under argon and then the mixture was cooled to -20 °C. After 5 min, a catalytic amount of the activator, trimethylsilyltriflate (TMSOTf), was added and the reaction was stirred for 1 h until completion as was determined by TLC. Upon completion, the reaction was quenched with triethylamine and then passed through a pad of Celite®-545. The organic layer was concentrated under *vacuo* and the crude mixture was purified using flash column chromatography to furnish highly pure α-selective products.

4.3. Synthesis

4.3.1. 2,3; 4,5-Di-O-cyclohexylidene-D/L-myo-inositol (**15**) [35]

To a solution of (±)-*myo*-inositol (**14**) (50 g, 277 mmol) in *N,N*-dimethylformamide (200 mL), 1,1 dimethoxycyclohexane (108 mL, 721 mmol) and *p*-toluenesulfonic acid (1.36g, 7.2 mmol) were added and the mixture was heated to 95-100 °C for 4 h. After the noted duration, the mixture was cooled to room temperature and diluted with ethyl acetate (450 mL). It was washed with saturated sodium bicarbonate (150 mL) and with water (2 × 100 mL). The organic layer was separated and removed *in vacuo* to give a yellowish syrup. The syrup was dissolved in minimal amount of acetone followed by addition of hexanes until solids began to separate (acetone:hexanes, 1:10). This was left overnight depositing the crystals. The crystals were filtered and washed with hexanes. The remaining mother liquor was purified using silica gel column chromatography with DCM:acetone (24:1 to 9:1) as the eluent to furnish the desired product **15** as a white solid (21.71 g, 63.85 mmol, 23%).

4.3.2. 2,3; 4,5-Di-O-cyclohexylidene- (1R)-menthyloxycarbonyl-D/L-myo-inositol (**16**) [35]

A mixture of **15** (4.36 g, 12.8 mmol) and dibutyltinoxide (3.5 g, 14.08 mmol) in toluene (100 mL) was refluxed employing a Dean-Stark apparatus for 4 h, after observing disappearance of starting material on TLC, the volume of toluene was reduced to 30 mL. The reaction mixture was then cooled to 0 °C followed by addition of dry sodium bicarbonate (0.43 g, 1.28 mmol). (1*R*)-(-)-menthyl chloroformate (3.6 g, 16.64 mmol) was added dropwise. After stirring for 24 h at room temperature the reaction mixture was filtered, concentrated *in vacuo* and purified using silica gel column chromatography with hexanes:ethyl acetate (24:1 to 5:1) as the eluent to furnish the desired product **16** as a white crystalline solid (4.88 g, 9.35 mmol, 73% yield).

4.3.3. 6-O-benzyl-2,3; 4,5-Di-O-cyclohexylidene- (1R)-menthyloxycarbonyl-D-myo-inositol (**17**) [35]

To a solution of **16** (3.2 g, 6.13 mmol) in anhydrous dichloromethane, freshly prepared Ag_2O (3.5 g, 15.3 mmol), molecular sieves (MS 4 Å, 0.5 g) and benzylbromide (1.7 mL, 14.7 mmol) were added and the reaction mixture was heated to 35 °C for 8 h. After allotted time, the reaction mixture was cooled to room temperature and filtered through Celite®-545 and the organic layer was evaporated *in vacuo* to produce a thick syrup. The syrup was purified using silica gel column chromatography to remove excess benzyl bromide to yield a crude diastereomeric-mixture, which was then dissolved in petroleum ether ultimately giving crystals. The crystals were filtered and washed with petroleum ether to give the desired product **17** (1.07 g, 1.7 mmol, 45%).

4.3.4. 6-O-benzyl-2,3; 4,5-Di-O-cyclohexylidene-D-myo-inositol (**18**)

To a solution of **17** (1.07 g, 1.7 mmol) in isopropanol:methanol (IPA:MeOH, 1:1) was added K_2CO_3 (0.35 g, 2.55 mmol) and the reaction mixture was allowed to stir vigorously for 5 h at 60 °C. The reaction was monitored by TLC. After noted spot-to-spot completion, the reaction mixture was cooled to room temperature and filtered through Celite®-545. The solvents were removed *in vacuo* and the mixture was purified employing silica gel column chromatography using hexanes:ethyl acetate (12:1 to 6:1) as the eluent to yield the product **18** as colorless syrup (0.730 g, 1.69 mmol, 97%).

$[\alpha]_D^{22}$ = +4.5° (*c* = 0.5, $CHCl_3$). **^{1}H-NMR (600 MHz, $CDCl_3$)**

δ = 1.65 (m, 20 H, 20-$H_{cyclohex}$), 3.53 (1H, dd, J = 9.27, 7.05 Hz, 4-H), 3.73 (1H, dd, J = 3.12, 9.90 Hz, 6-H), 3.87 (1H, dd, J = 5.79, 6.93 Hz, 3-H), 4.08 (1H, t, J = 9.60 Hz, 5-H), 4.22 (1H, t, J = 5.58 Hz, 2-H), 4.64 (1H, dd, J = 3.09, 5.49 Hz, 1-H), 4.83 (1H, dd, J = 11.70, 158.81 Hz, CH_2Ph), 7.30–7.41 ((m, 5H, Ar-H).). **^{13}C-NMR (150 MHz, $CDCl_3$)** δ = 23.66, 24.03, 24.06, 24.15, 25.21, 25.28, 35.12, 36.12, 36.93, 37.47, 71.58, 71.97, 72.61, 73.41, 75.34, 75.58, 77.43, 80.51, 81.43, 112.74, 112.79, 128.05, 128.38, 128.68, 138.39. **ESI-LRMS [(M + Na)$^+$]** calcd for $C_{25}H_{34}NaO_6$ is 543.23, found 543.67.

4.3.5. 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-fluoro-α-D-mannopyranose (**20**) and 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-fluoro-D-glucopyranose (**21**) [38]

To a solution of 3,4,6-tri-O-acetyl-*D*-glucal (**19**) (5 g, 18.3 mmol) in *N,N*-dimethylformamide:water (1:1, 100 mL) was added Selectfluor® (12.9 g, 36.6 mmol) and the reaction was stirred at room temperature for 12 h until noted TLC completion. The reaction mixture was diluted with ethyl acetate and then washed thrice with water. The organic layer was separated, dried and concentrated under reduced pressure to give an inseparable mixture of α/β 2-fluoro epimers. The crude product was then dissolved in pyridine (30 mL) followed by addition of acetic anhydride (1.72 mL, 18.3 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction mixture was allowed to stir for 8 h. Pyridine was removed *in vacuo* and the remaining syrup was diluted with ethyl acetate and washed with 1N HCl (3 × 50 mL) followed by saturated sodium bicarbonate (2 × 50 mL) and a solution of brine (2 × 25 mL). The organic layer was dried over sodium sulfate and the ethyl acetate evaporated *in vacuo* to afford a peracetylated mixture of **20** and **21** in a 1:2 ratio. The mixture was separated by column chromatography using hexanes:ethyl acetate (9:1 to 3.5:1) as the eluent to furnish the desired product **21** as colorless syrup (3.7 g, 10.5 mmol, 58%) and product **20** as a colorless syrup (2.1 g, 5.96 mmol, 33%).

4.3.6. 3,4,6-Tri-O-acetyl-2-deoxy-2-fluoro-D-glucopyranose (**22**) [41]

Compound **21** (0.510 g, 1.45 mmol) was dissolved in *N,N*-dimethylformamide (5 mL) followed by the addition of ammonium acetate (0.224 g, 2.91 mmol). The reaction mixture was allowed to stir at room temperature for 12 h. The reaction mixture was then diluted with cold water and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated *in vacuo* to yield a thick syrup which was subjected to purification conditions using silica gel column chromatography employing hexanes:ethyl acetate (3:2) as the eluent to afford **22** as a colorless syrup (0.440 g, 1.42 mmol, 98%).

4.3.7. 3,4,6-Tri-O-acetyl-2-deoxy-2-fluoro-D-glucopyranosyl Trichloroacetimidate (**23**) [41]

To a solution of compound **22** (0.300g, 0.97 mmol) in anhydrous dichloromethane, 10 mL of aqueous K_2CO_3 (0.06g, 0.48 mmol) was added. The reaction mixture was maintained at 0 °C until the addition of trichloroacetonitrile (0.07 mL, 0.71 mmol) was completed using a drop-wise technique. The reaction was stirred at room temperature for 16 h. The reaction was then filtered through Celite®-545 and the organic layer was removed *in vacuo*. The residue was purified using silica gel column chromatography employing hexanes:ethyl acetate (3:1) as the eluent to furnish **23** as a colorless syrup (0.408 g, 0.905 mmol, 93%).

4.3.8. 6-O-benzyl-O-(3,4,6-Tri-O-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl)-(1 → 1)-2,3:4,5-di-0-cyclohexylidene-D-myo-inositol (**24**)

The acceptor **18** (323.7 mg, 0.753 mmol) and the trichloroacetimidate donor **23** (0.408 g, 0.904 mmol) were dissolved in anhydrous dichloromethane and the reaction was conducted as per the general glycosylation procedure noted earlier. The crude mixture was purified using flash column chromatography using hexanes:ethyl acetate (9:1 to 3:1) as the eluent to furnish pure α-**24** as a colorless syrup (0.422 g, 0.587 mmol, 78%).

$[\alpha]_D^{22}$ = +82.5°, (c = 1.0, $CHCl_3$). **^{1}H-NMR (600 MHz, $CDCl_3$)** δ = 1.68 (m, 20 H, 20-$H_{cyclohex}$), 2.02 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 3.71 (d, J = 2.82 Hz, 1H, 2-H), 3.71 (d, J = 1.56 Hz, 1H, 4′-H), 3.78 (dd, J = 2.04, 12.06 Hz, 1H, 6b-H), 3.96 (dd, J = 3.78, 12.60 Hz, 1 H, 6a-H), 3.99 (dd, J = 4.92, 6.84 Hz, 1H, 5′-H), 4.11 (t, J = 9.69 Hz, 1H, 3′-H), 4.27 (qd, J = 1.89, 10.41 Hz, 1H, 5-H), 4.35 (t, J = 5.28 Hz, 1 H, 6′-H), 4.48 (ddd, J = 3.84, 9.59, 49.23 Hz, 1H, 2′-H), 4.63 (dd, J = 3.03, 5.67 Hz, 1H, 1′-H), 4.82 (d, J_{gem} = 11.46, 140.51 Hz, 2H, CH_2Ph), 4.99 (t, J = 9.93 Hz, 1H, 4-H), 5.49 (t, J = 9.54, 12.00 Hz, 1H, 3-H), 5.51 (d, J = 41.4 Hz, 1H, 1-H), 7.29–7.41 (m, 5H, Ar-H). **^{13}C-NMR (150 MHz, $CDCl_3$)** δ = 20.64, 20.73, 20.82, 23.48, 23.83, 23.89, 23.96, 24.94, 25.05, 34.79, 35.88, 36.72, 37.08, 61.27, 67.17, 67.68, 67.72, 70.65, 70.78, 71.94, 72.45, 75.15, 75.33, 79.43, 80.42, 81.37, 86.52, 87.81, 94.28, 94.42, 127.84, 128.44, 137.95, 169.61, 170.12, 170.59.**^{19}F-NMR (200 MHz, $CDCl_3$)** δ = -201.51 (1F, dd, J = 12.19, 49.11 Hz). **ESI-LRMS [(M + Na)$^+$]** calcd for $C_{37}H_{49}FNaO_{13}$ is 743.31, found 743.56.

4.3.9. O-(3,4,6-Tri-O-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl)-(1 → 1)-2,3:4,5-di-0-cyclohexylidene-D-myo-inositol (**25**)

Compound **24** (0.200 g, 0.277 mmol) was dissolved in methanol in a Parr hydrogenation flask and a catalytic amount of 10% w/w Pd-C was added. The mixture was stirred under hydrogen at 40 psi on the Parr apparatus until completion of reaction was noted using TLC. The mixture was then filtered through Celite®-545 and the solution was concentrated *in vacuo*. The residue was purified using silica gel column chromatography employing hexanes:ethyl acetate (3:2) as the eluent to furnish **25** as an off-white solid (0.175 g, 0.277 mmol, 99%).

$[\alpha]_D^{22}$ = +50.3° (c = 1.5, $CHCl_3$). **^{1}H-NMR (600 MHz, $CDCl_3$)** δ = 1.68 (m, 20H, 20-$H_{cyclohex}$), 2.05 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 3.71 (dd, J = 2.94, 9.90 Hz, 1H, 2′-H), 3.87 (m, 2 H, 4′,6′-H), 4.00 (t, 1H, 3′-H), 4.10 (dd, J = 2.16, 12.36 Hz, 1H, 6b-H), 4.25 (dd, J = 4.92, 12.46 Hz, 1 H, 6a-H), 4.36 (d, J = 8.94 Hz, 1H, 5-H), 4.37 (d, J = 2.58 Hz, 1H, 5′-H), 4.53 (ddd, J = 3.93, 9.60, 49.14 Hz, 1H, 2-H), 4.64 (dd, J = 3.03, 5.49 Hz, 1H, 1′-H), 5.00 (t, J = 9.90 Hz, 1H, 4-H), 5.49 (d, J = 3.84 Hz, 1H. 1-H), 5.54 (dt, J = 9.59, 11.85 Hz, 1H, 3-H). **^{13}C-NMR (150 MHz $CDCl_3$)** δ = 20.86, 20.97, 20.99, 23.67, 23.91, 23.93, 24.10, 25.13, 25.17, 29.58, 29.81, 29.92, 35.08, 36.19, 36.72, 37.42, 62.13, 67.69, 68.35, 68.40, 70.66, 70.79, 72.31, 73.78, 74.96, 75.17, 80.63, 85.03, 86.61, 87.90, 95.34, 95.48, 113.14, 113.21, 169.93, 170.35, 170.92.**^{19}F-NMR (200 MHz, $CDCl_3$)** δ = -202.34 (1F, dd, J = 12.64, 49.37 Hz). **ESI-LRMS [(M + Na)$^+$]** calcd for $C_{30}H_{43}FNaO_{13}$ is 653.26, found 653.78.

4.3.10. O-(2-deoxy-2-fluoro-α-D-glucopyranosyl)-)-(1 → 1)-D-myo-inositol (GlcF-Ins) (**10**)

Compound **25** (0.175 g, 0.277 mmol) was dissolved in methanol and sodium metal was added until the pH read ~10. The reaction mixture was stirred for 1 h until completion as noted by TLC. Once the reaction was complete, DOWEX 50WX8-100 ion exchange (H+) resin was added until the pH was acidic (pH~1). The reaction mixture was then heated to 45 °C for another 45 min to remove the cyclohexylidene protecting groups and the reaction was monitored *via* TLC until completion. The reaction was filtered through a pad of Celite®-545 and concentrated *in vacuo* to give a precipitate which was purified using Bio-Rad Bio-Gel® P-2 gel size exclusion chromatography to furnish product **10** as yellowish syrup (0.95 g,

0.277 mmol, 99%).

$[\alpha]_D^{22}$ = +36.5°, (*c* = 1.0, MeOH). **^{1}H-NMR (600 MHz, CD_3OD)** δ = 3.22 (t, *J* = 9.27 Hz, 1H, 6′-H), 3.32 (d, *J* = 11.28 Hz, 1H, 2′-H), 3.34 (t, *J* = 9.45 Hz, 1H,4′-H), 3.53 (dd, *J* = 2.79 Hz, 9.63 Hz, 1H, 4-H), 3.60 (t, *J* = 9.54 Hz, 1H, 5′-H), 3.64 (dd, *J* = 5.01,11.79 Hz, 1H, 6b-H), 3.74 (t, *J* = 9.21 Hz, 1H, 5-H), 3.78 (d, *J* = 2.34 Hz, 1H, 6a-H), 3.88 (t, *J* = 5.61Hz, 1H, 3′-H), 3.89 (dd, *J* = 9.54, 21.85 Hz, 1H, 3-H), 4.03 (ddd, *J* = 2.32, 4.93,10.09 Hz, 1H, 1′-H), 4.20 (ddd, *J* = 4.02, 9.51, 49.97 Hz, 1 H, 2-H), 5.46 (d, *J* = 4.02 Hz, 1H, 1-H). **^{13}C-NMR (150 MHz, CD_3OD)** δ = 61.66, 70.66, 70.72, 72.46, 72.58, 72.66, 73.19, 73.92, 73.95, 74.57, 81.19, 90.82, 92.07, 97.56, 97.70.**^{19}F-NMR (200 MHz, CD_3OD)** δ = -201.14 (1F, dd, *J* = 13.10, 49.36 Hz). **ESI-LRMS [(M + Na)$^+$]** calcd for $C_{12}H_{21}FNaO_{10}$ is 367.10, found 367.57.

4.3.11. Stick's diazo transfer reagent [45]

To an ice cold suspension of sodium azide (NaN_3, 10 g, 153.8 mmol) in acetonitrile (175 mL), sulfuryl chloride (12.4 mL, 153.8 mmol) was added and the reaction mixture was stirred overnight at room temperature followed by addition of imidazole (19.89 g, 292.2 mmol) in portions under ice-cold conditions. The resulting mixture was stirred for 4 h at room temperature. The mixture was diluted with ethyl acetate (350 mL), washed with water (2 × 200 mL) and saturated sodium bicarbonate (2 × 200 mL). The organic layer was then dried over Na_2SO_4. To the organic layer, a dropwise solution of HCl in ethanol was added under ice cold conditions and then the mixture was filtered and washed with ethyl acetate to give a solid of Stick's salt.

4.3.12. 1,3,4,6-Tetra-O-acetyl-2-deoxy-2-azido-D-glucopyranose (**27**) [45]

To a mixture of D-glucosamine HCl **26** (9.69 g, 44.81 mmol), K_2CO_3 (16.75 g, 121.19 mmol), $CuSO_4$ H_2O (0.112 g, 0.448 mmol) in methanol (400 mL) and Stick's diazo transfer reagent (imidazole-1-sulfonyl azide hydrochloride), (11.3 g, 53.9 mmol) was added at 0 °C and stirred at room temperature for 3 h until completion as noted by TLC. The reaction mixture was evaporated and then co-evaporated with toluene to remove any moisture. The resulting mass (8.5 g, 414 mmol) was dried on a vacuum pump and then dissolved in 200 mL of pyridine followed by the addition of acetic anhydride (42.44 mL, 448 mmol) and a catalytic amount of DMAP at 0 °C. The reaction mixture was stirred for 12 h at room temperature. It was then concentrated and diluted with ethyl acetate and washed with 1N HCl (4 × 75 mL) followed by saturated sodium bicarbonate (2 × 75 mL) and brine solution (2 × 25 mL). The organic layer was dried over sodium sulfate and evaporated *in vacuo* to give a crude mass of the product which was purified using silica gel chromatography employing hexanes:ethyl acetate (3:1) as the eluent to yield the desired product **27** as yellowish solid (13.5 g, 36.19 mmol, 87%).

4.3.13. 3,4,6-Tri-O-acetyl-2-deoxy-2-azido-D-glucopyranose (**28**) [40]

Compound **27** (0.70 g, 1.87 mmol) was dissolved in *N*,*N*-dimethylformamide followed by the addition of ammonium acetate (0.206 g, 2.68 mmol). The reaction mixture was stirred at room temperature for 12 h. The mixture was diluted with cold water and then extracted with ethyl acetate. The organic layer was dried over sodium sulfate and concentrated *in vacuo* to yield a syrup which was then purified using silica gel chromatography employing hexanes:ethyl acetate (3:2) as the eluent to afford **28** as colorless syrup (0.608 g, 1.83 mmol, 98%).

4.3.14. 3,4,6-Tri-O-acetyl-2-deoxy-2-azido-D-glucopyranosyl trichloroacetimidate (**29**) [40]

To a solution of compound **28** (0.608 g, 1.83 mmol) in anhydrous dichloromethane, 10 mL of aqueous K_2CO_3 (0.155 g, 1.125 mmol) was added. The reaction mixture was maintained at 0 °C until the addition of trichloroacetonitrile (0.165 mL, 1.65 mmol) was completed. The reaction was stirred at room temperature for 16 h. The reaction was filtered through Celite®-545 and the organic layer was removed *in vacuo*. The residue was purified using silica gel column chromatography employing hexanes:ethyl acetate (3:1) as the eluent to furnish **29** as light-yellow colored syrup (0.780 g, 1.64 mmol, 90%).

4.3.15. 6-O-benzyl-O-(3,4,6-Tri-O-acetyl-2-deoxy-2-azido-α-D-glucopyranosyl)-(1 → 1)-2,3:4,5-di-0-cyclohexylidene-D-myo-inositol (**30**)

The acceptor **18** (0.500 g, 1.162 mmol) and trichloroacetimidate donor **29** (0.605 g, 1.278 mmol) were dissolved in anhydrous dichloromethane (30 mL) and then the reaction was conducted as per the general glycosylation procedure explained earlier in the text. The crude was purified *via* flash column chromatography using hexanes:ethyl acetate (9:1 to 3:1) as the eluent to furnish pure α-selective products **30** as off-white solid (0.696 g, 0.936 mmol, 81%).

$[\alpha]_D^{22}$ = +86.5°, (*c* = 1.0, $CHCl_3$). **^{1}H-NMR (600 MHz, $CDCl_3$)** δ = 1.3–1.8 (m, 20 H, 20-$H_{cyclohex}$), 2.02 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 3.36 (dd, *J* = 3.54, 10.56 Hz, 1H, 2-H), 3.67 (dd, *J* = 7.02, 9.30 Hz, 1H, 4′H), 3.71 (dd, *J* = 3.00, 9.96 Hz, 1H, 2′-H), 3.75 (dd, *J* = 1.86, 12.60 Hz, 1H, 6b-H), 3.94 (dd, *J* = 4.53, 13.47 Hz, 1H, 6a-H), 3.97 (dd, *J* = 5.16, 7.02 Hz, 1H, 5′-H), 4.10 (t, *J* = 9.66 Hz, 1H, 3′-H), 4.26 (qd, *J* = 1.81, 10.17 Hz, 1H, 5-H), 4.36 (t, *J* = 5.31 Hz, 1H, 6′-H), 4.64 (dd, *J* = 3.09, 5.55 Hz, 1H, 1′-H), 4.81 (dd, J_{gem} = 11.46, 143.51 Hz, 2H,CH_2Ph), 5.01 (t, *J* = 9.81 Hz, 1H, 4-H), 5.43 (d, *J* = 2.88 Hz, 1H, 1-H), 5.43 (t, *J* = 9.87 Hz, 1H, 3-H), 7.33–7.44 (m, 5H, Ar-H). **^{13}C-NMR (150 MHz $CDCl_3$)** δ = 20.86, 20.93, 20.97, 23.67, 24.01, 24.09, 24.16, 25.13, 25.24, 29.92, 35.02, 36.08, 36.90, 37.32, 61.08, 61.53, 67.69, 68.34, 70.74, 72.17, 75.32, 75.54, 79.49, 80.54, 81.35, 96.28, 112.94, 113.12, 128.07, 128.66, 128.69, 138.08, 169.86, 170.26, 170.80. **ESI-LRMS [(M + Na)$^+$]** calcd for $C_{37}H_{49}FNaO_{13}$ is 743.31, found 743.56.

4.3.16. O-(α-D-glucosaminepyranosyl)-(1 → 1)-D-myo-inositol (GlcN-Ins) (**5**)

To a solution of compound **30** (0.200 g, 0.269 mmol) in methanol (4 mL) was added a catalytic amount of 10% w/w Pd-C. The mixture was stirred under hydrogen at 40 psi until completion of the reaction was noted by TLC. The mixture was then filtered through a pad of Celite®-545 and the organic solvent was removed *in vacuo*. The crude product was then dissolved in methanol and sodium metal was added until the pH reached ~10. The reaction mixture was stirred for 1 h until noted completion of deacetylation. Once the reaction was complete, DOWEX 50WX8-100 ion exchange (H+) resin was added until the pH read acidic (~1) and the reaction mixture was then heated to 45 °C for another 45 min until completion to remove the cyclohexylidene protecting groups. The reaction mixture was then filtered through Celite®-545 and concentrated *in vacuo* to give a precipitate which was purified using Bio-Rad Bio-Gel® P-2 gel size exclusion chromatography to furnish product **5** as an off-white solid (0.085 g, 0.249 mmol, 92%).

$[\alpha]_D^{22}$ = +41.1° (*c* = 1.15, H_2O). **^{1}H-NMR (600 MHz, D_2O)** δ = 3.29 (t, *J* = 9.36 Hz, 1H, 5′-H), 3.36 (dd, *J* = 3.54, 10.68 Hz, 1H, 2-H), 3.49 (t, *J* = 9.39 Hz, 1H, 4-H), 3.53 (dd, *J* = 2.46, 9.90 Hz, 1H, 3′-H), 3.61 (t, *J* = 9.63 Hz, 1H, 4′-H), 3.69 (dd, *J* = 2.70, 10.08 Hz, 1H, 1′-H), 3.77 (m, *J* = 4.83 Hz, 2H, 6b, 6′-H), 3.85 (m, 2H, 5,6a-H), 3.93 (t, *J* = 8.76 Hz, 1H, 3-H), 4.20 (t, *J* = 2.37 Hz, 1H, 2′-H), 5.42 (d, *J* = 3.54 Hz, 1H, 1-H). **^{13}C-NMR (150 MHz D_2O)** δ = 54.17, 60.18, 69.37, 69.46, 70.93, 71.67, 71.86, 71.94, 72.70, 74.11, 78.92, 97.18. **ESI-LRMS [(M + Na)$^+$]** calcd for $C_{12}H_{23}NNaO_{10}$ is 364.12, found 364.42.

*4.3.17. O-(2-deoxy-2-azid-α-D-glucopyranosyl)-(1 → 1)-D-myo-inositol ($GlcN_3$-Ins) (**9**)*

To a solution of **30** (0.100 g, 0.134 mmol) in ethyl acetate (5 mL) was added 3 mL of aqueous sodium bromate ($NaBrO_3$, 0.904 g, 0.603 mmol). A solution of sodium dithionite ($Na_2S_2O_4$, 85%, 0.932 g, 0.536 mmol) in water (6 mL) was added over 20 min and the reaction was vigorously stirred for 6 h at room temperature. The mixture was then diluted with ethyl acetate, quenched with 10% sodium thiosulphite (1 mL) and finally washed with water. The organic layer was separated and dried over sodium sulfate and concentrated *in vacuo*. The crude mixture was then dissolved in methanol and sodium metal was added until the pH read ~10. The reaction mixture was stirred for 1 h until completion. Once the reaction was complete, DOWEX 50WX8-100 ion exchange (H^+) resin was added to until the pH read acidic (~1). The reaction mixture was then heated to 45 °C for another 45 min until completion to remove the cyclohexylidene protecting groups. Finally, the mixture was filtered through Celite®-545 and concentrated *in vacuo* to give a precipitate which was purified using Bio-Rad Bio-Gel® P-2 gel size exclusion chromatography to furnish product **9** as a colorless syrup (0.044 g, 0.119 mmol, 90%).

$[\alpha]_D^{22} = +46.5°$ ($c = 0.35$, H_2O). **^{1}H-NMR (600 MHz, D_2O)** δ = 3.26 (t, J = 9.45 Hz, 1H, 4′-H), 3.36 (dd, J = 3.72, 10.56 Hz, 1H, 2-H), 3.45 (t, J = 9.39 Hz, 1H, 4-H), 3.50 (dd, J = 2.79, 9.99 Hz, 1H, 6′-H), 3.60 (d, J = 19.09 Hz, 1H, 2′-H), 3.61 (d, J = 10.14 Hz, 1H, 5′-H), 3.74 (dd, J = 5.28, 11.88 Hz, 1H, 6b-H), 3.78 (t, J = 9.75 Hz, 1H, 3′-H), 3.82 (dd, J = 2.07, 4.47 Hz, 1H, 5-H), 3.83 (dd, J = 2.13, 29.14 Hz, 1H, 6a-H), 3.92 (dd, J = 9.12, 10.50 Hz, 1H, 3-H), 4.15 (t, J = 2.82 Hz, 1H, 1′-H), 5.29 (d, J = 3.72 Hz, 1H, 1-H). **^{13}C-NMR (150 MHz D_2O)** δ = 60.38, 63.08, 69.78, 70.75, 70.98, 71.68, 71.98, 72.39, 74.27, 79.00, 99.49. **ESI-LRMS [$(M + Na)^+$]** calcd for $C_{12}H_{21}N_3NaO_{10}$ is 390.11, found 390.84.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.carres.2017.10.014.

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