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Research Article

Synthesis and Antitubercular Evaluation of Diverse Glycosylated Ureas from D-Glucose

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N, *N*-disubstituted glycosylated ureas have been synthesized in good-to-excellent yields by 1,4-conjugate addition of amines to glycosylated olefinic esters followed by reaction of resulting glycosyl *β*-amino esters with diisocyanates. The developed sugar-based molecules were well characterized by extensive standard spectroscopic analysis including NMR (^{1}H, ^{13}C, and DEPT), IR, MS, and elemental analysis and were screened for their biological activity against *Mycobacterium tuberculosis* (*M. Tb.*), where some of them displayed potent antitubercular activity. The molecules have been designed keeping in view the well-known inhibitors of the enzymes involved in the biosynthesis of mycobacterial cell wall polymers and may be further explored as lead molecules for the successful development of a potential antitubercular drug candidate.

Dedicated to Dr. Rama P. Tripathi, Former Chief Scientist at CSIR-Central Drug Research Institute, Lucknow, India, for his extraordinary contribution to "Drug development against Tuberculosis"

1. Introduction

Mycobacterium tuberculosis (*M.* TB) is a widespread infectious disease in developing countries like India. It is a very old disease, and it needs prolonged treatment, but because of the appearance of multidrug resistance (MDR) and extensively drug resistance (XDR), tuberculosis is the current major problems that affect people to fight this disease and it kills approximately two million people globally [1]. Tuberculosis needs early and accurate detection and continuous treatment to combat latent infection. Many conventional first-line and second-line drugs are facing a serious problem mainly due to the emergence of resistance to treatment, and the combination of tuberculosis and HIV infection with multidrug resistance (MDR) further worsened the situation [2, 3]. Therefore, there is a vital need to design a novel class of antitubercular agents that can minimize the period of its treatment and also reduce the factors of resistance development. The cell wall of mycobacterium has a mAGP complex in which carbohydrate-derived cell wall polymers are present [4, 5]. Sugars are thus essential compounds for the development of a new class of anti-TB drugs as possible inhibitors of the enzymes involved in the biosynthesis of cell wall polymers such as arabinogalactan and arabinomannan [5]. Many amino alcohols, ureas, and other compounds were reported as potent inhibitors of aspartic proteases [6–8]. The latter has implications in many diseases [9, 10], for example, AIDS, hypertension, Alzheimer disease, malaria, fungal infections, and many other infectious diseases. Disubstituted urea and aryl ureides have notable biological importance in the treatment of other diseases [11, 12]. Compounds linked with urea skeleton are known to display a wide range of pharmacological activities, particularly for the effective treatment of parasitic and infectious diseases [13]. They are

good for hydrogen bonding ability, and therefore many *N, N*-disubstituted ureas have been prepared as isostere of peptides to study molecular interactions as chemical models of the protein β-sheet. Furthermore, many glycohybrid compounds derived from readily available carbohydrates are known to display potential antitubercular activity [14]. The phenyl group as a substituent on the nitrogen atom in the ureas provides conformational control during molecular interactions [15]. It was reported that the phenyl ureas have antimicrobacterial activity [16], which attracted the attention of the scientific community to explore this class of molecules.

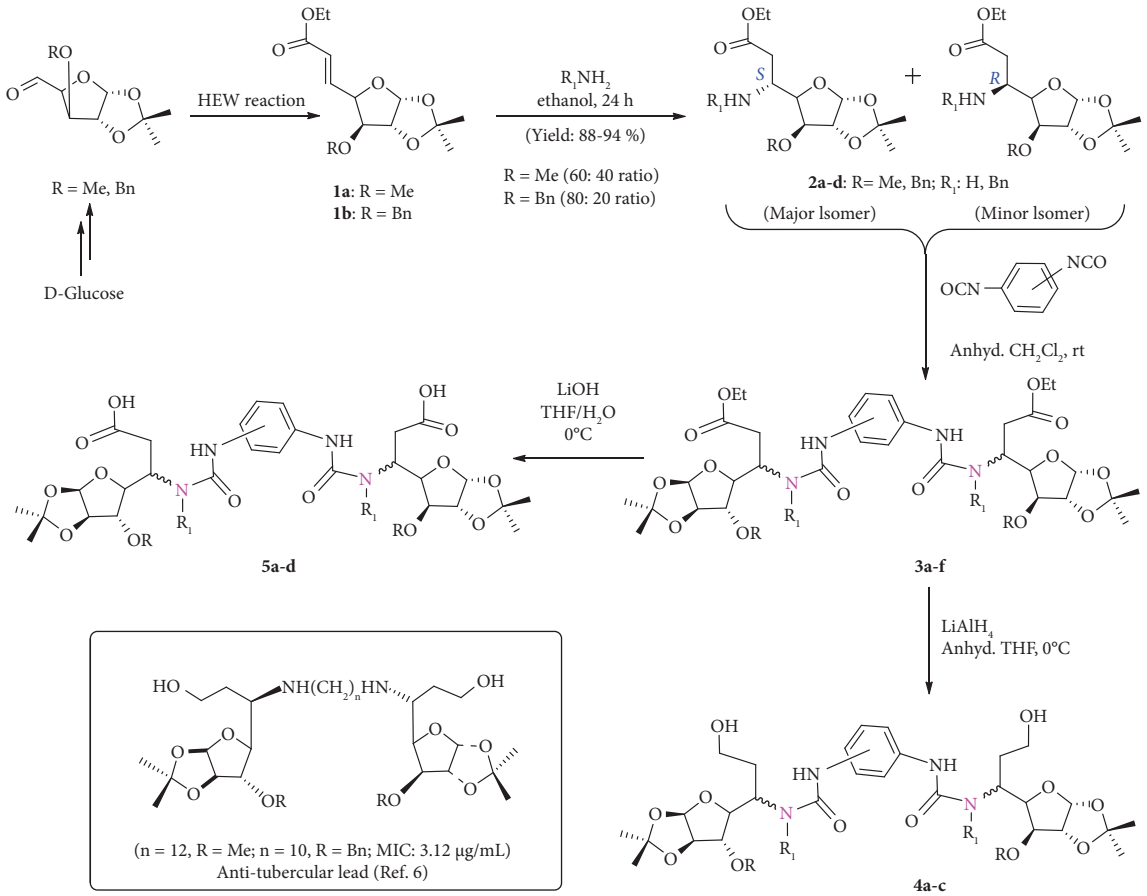
Carbohydrates are an essential class of biomolecules due to their multifunctional groups, less toxicity, and better solubility. Many glycosyl thioureas flanked by phenyl rings have been used to study the multivalent carbohydrate interactions with other molecules [17]. *Mycobacterium tuberculosis* resides in macrophages, the primary defense system of the host, and it was envisioned to synthesize some potential glycohybrid compounds which can penetrate the macrophages. Carbohydrates as earlier discussed have the potential for structural diversity [18–25] and have some crucial roles in the diverse biological process, notably host-pathogen interactions [26], signal transduction [27], and also inflammations [28, 29]. Because of the abovementioned reasons, we have synthesized substituted glycosyl ureas and their derivatives to see the structure-activity relationship (SAR) against *M. tuberculosis* and, at the same time, possessed *in vitro* activity as it would lead to a good candidate for the successful development of a mechanism-based new class of antituberculosis drug candidates. Therefore, a high-yielding convenient synthesis of several phenylene-bridged glycosyl ureas, ester, carboxy, and alcohol functionalities has been undertaken, and the compounds have been evaluated against *M. tuberculosis*.

2. Results and Discussion

2.1. Chemistry. Our synthesis commenced with the synthesis of glycosyl β-amino ester **2a** starting from readily available D-glucose in six high-yielding standard synthetic steps including 1,2; 5,6-di-*O*-isopropylidene protection followed by 3-*O*-methylation, selective 5,6-isopropylidene deprotection, $NaIO_4$ oxidation, and HEW olefination reaction and at the end 1,4-conjugate addition of amine to the resulting glycosyl olefinic ester **1a** [30]. The synthetic procedure for the required β-amino ester **2b** from olefinic ester **1b** was followed exactly as the method employed for β-amino ester **2a** as shown in Scheme 1. Compounds **2a–d** were obtained in good yields by 1,4-conjugate addition of different primary amines (R_1NH_2, for example, ammonia and benzyl amine) separately to the glycosylated olefinic esters **1a** and **1b** and have been then used as the carbohydrate scaffold for this investigation. Thus, the reaction of two equivalents of glycosyl β-amino ester **2a** with one equivalent of 1,4-phenylene diisocyanate in anhydrous dichloromethane at room temperature led to the formation of the respective phenylene-bridged glycoside **3a** as a mixture of diastereoisomers in the 95% yield (Scheme 1).

Similarly, compounds **3b–3f** have been synthesized in good-to-excellent yields using similar optimized conditions. The ester functionality of synthesized diureides **3a**, **3c**, and **3d** was then reduced by using lithium aluminium hydride in anhydrous THF to give a good yield of the respective phenylene-bridged glycosylated β-amino alcohols **4a**, **4b**, and **4c**. Later on, the resulting ester functionality of *bis*-ureides with 1,4-phenylene substituent (**3a** and **3c**) and *bis*-ureides with 1,3-phenylene substituent (**3b** and **3d**) was hydrolyzed by reacting it with $LiOH.H_2O$ in THF/water and afforded high yields of respective glycosylated acid analogs **5a**, **5b**, **5c**, and **5d** (Scheme 1).

The reaction yields of the desired glycosyl ureidyl esters (**3a–f**), glycosyl ureidyl amino alcohols (**4a–c**), and glycosyl ureidyl amino acids (**5a–d**) are depicted in Table 1. The structures of all the developed molecules have been ascertained by its detailed spectroscopic analysis including NMR (^1H, ^{13}C NMR, and DEPT), IR, and FAB-MS data. The purity of these molecules has been determined by C, H, and N elemental analysis, and the observed were found in close agreement with their calculated (C, H, and N analysis) values. The structure of compound **3a** could be ascertained based on its spectroscopic data and C, H, and N analysis. In the IR spectrum, the absorption frequencies for the characteristic ester and amide functional groups have been observed at 1734 and 1656 cm^{-1}, respectively. The MS-FAB spectrum of this compound showed $[M + Na]^+$ at *m/z* 761 and $[M + H]^+$ at *m/z* 739 corresponding to its molecular weight. In ^1H and ^{13}C NMR spectra of this C_2 symmetric compound, the signals for only half of the molecule have been observed as the second half is identical in chemical structure. In ^1H NMR of the ureidyl ester **3a,** signals for Ar-H exhibited as a multiplet at δ 7.01–7.26 ppm, while the characteristic anomeric sugar proton (H-1) appeared as doublet ($J = 3.7$ Hz) at δ 5.94 ppm and H-2 and H-3 protons appeared as doublet at δ 4.59 ($J = 3.7$ Hz) and 3.79 ($J = 2.9$ Hz) ppm, respectively. The H-4 and CH_2 of the carbethoxymethyl group attached at C-5 of the xylofuranose ring appeared as a multiplet at δ 4.38–4.44 and 2.66–2.71 ppm, respectively; while the signals for corresponding methylene and methyl protons of OCH_2CH_3 appeared as *q* and *t* at δ 4.13 and 1.24 ppm, respectively, with *J* values of 7.0 Hz. Four singlets at δ 3.69, 1.73, 1.47, and 1.34 ppm correspond to 3-OCH_3, exchangeable NH, and two isopropylidene methyl ($2 \times >C(CH_3)_2$), respectively. In ^{13}C NMR of **3a**, signals for three quaternary carbons of ester O-C=O (ester), N-C=O (amide) functional groups, and phenyl ring have been observed at δ 172.6, 156.3, and 134.5 ppm, respectively. While aromatic sp^2 carbons appeared at δ 122.2 and 121.6 ppm, the anomeric C-1 carbon of the xylofuranose sugar moiety appeared at δ 105.8 ppm. Other tertiary carbons of the xylofuranose sugar have been observed at δ 84.2 (C-2), 81.0 (C-4), 80.6 (C-3), and 47.3 (C-5). The two methylene carbons belonging to OCH_2CH_3 and CH_2COOEt appeared at δ 61.1 and 37.4 ppm, respectively, whereas the four methyl carbons such as isopropylidene ($2 \times >C(CH_3)_2$), 3-OCH_3, and OCH_2CH_3 were appeared at δ 27.1, 26.6, 57.9, and 14.9 ppm, respectively.



Scheme 1: Synthesis of diverse *bis*-glycosylated ureas and their analogs from D-glucose.

Table 1: Synthesis of glycosyl ureidyl esters (**3a–f**), glycosyl ureidyl amino alcohols (**4a–c**), and glycosyl ureidyl amino acids (**5a–d**).

Compounds	R	R1	Phenylene ring	Yield (in %)
Glycosyl ureide ester **3a**	CH_3	H	1,4-substitution	95
Glycosyl ureide ester **3b**	CH_3	H	1,3-substitution	97
Glycosyl ureide ester **3c**	CH_2Ph	H	1,4-substitution	95
Glycosyl ureide ester **3d**	CH_2Ph	H	1,3-substitution	95
Glycosyl ureide ester **3e**	CH_3	CH_2Ph	1,4-substitution	90
Glycosyl ureide ester **3f**	CH_2Ph	CH_2Ph	1,4-substitution	92
Glycosyl ureide alcohol **4a**	CH_3	H	1.4-substitution	85
Glycosyl ureide alcohol **4b**	CH_2Ph	H	1,4-substitution	82
Glycosyl ureide alcohol **4c**	CH_2Ph	H	1,3-substitution	85
Glycosyl ureide acid **5a**	CH_3	H	1,4-substitution	80
Glycosyl ureide acid **5b**	CH_2Ph	H	1,4-substitution	80
Glycosyl ureide acid **5c**	CH_2Ph	H	1,3-substitution	75
Glycosyl ureide acid **5d**	CH_3	H	1,3-substitution	78

For the structure-activity relationship of the glycosylated urea analog, we vary the substituent position in the phenyl bridge of the glycosyl urea by reacting two equivalents of the glycosylated β-amino ester **2a** to one equivalent of 1,3-phenylene isocyanate to give the required glycosylated *bis* ureide **3b** as a diastereomeric mixture. The structure of compound **3b** has also been established based on its spectral data. The characteristic signal at δ 114.6 ppm in its ^{13}C NMR was observed as an upfield shift of the aromatic carbon in between the two ureidyl functionalities of the 1,3-substituted phenyl ring. Similarly, other related developed compounds have been also analyzed for their complete structural elucidation.

2.2. Antitubercular Activity. All the developed carbohydrate-containing urea and their analogs have been evaluated for their identification of microbial strains, such as *Mycobacterium tuberculosis* H37Ra (ATCC 25177), and the result of biological activity is depicted in Table 2. The screening methods used for the bioevaluation of the abovementioned

Table 2: Bioevaluation of phenylene-bridged substituted glycosyl ureides (**3a–3f**), glycosylated alcohols (**4a–4c**), and glycosylated acids (**5a, 5b,** and **5c**) against *M. tuberculosis* H37Ra and H37Rv strains.

Glycohybrid ureides (**3–5**)	MIC (μg/mL) against H37Ra	MIC (μg/mL) against H37Rv
Glycosyl ureide ester **3a**	>50	12.5
Glycosyl ureide ester **3b**	>25	>25
Glycosyl ureide ester **3c**	>50	>50
Glycosyl ureide ester **3d**	>50	>25
Glycosyl ureide ester **3e**	>25	>50
Glycosyl ureide ester **3f**	>25	>50
Glycosyl ureide alcohol **4a**	>25	>50
Glycosyl ureide alcohol **4b**	>25	12.5
Glycosyl ureide alcohol **4c**	>25	12.5
Glycosyl ureide acid **5a**	>100	>100
Glycosyl ureide acid **5b**	>50	>100
Glycosyl ureide acid **5c**	>100	>100
Ethambutol (reference)	Not done	3.25

compounds against *M. tuberculosis* are micro Alamar blue assay (MABA) and agar microdilution techniques followed as the standard reported in the literature [31, 32].

Although most of the compounds have not exhibited any significant activity against the tubercular strains since the MIC values for them are either >25 or >50 μg/mL, however, it is very interesting to report that compounds **3a**, **4b**, and **4c** show remarkable biological activities as an antimicrobial activity with MIC values of 12.5 μg/mL considered significant for developing new antitubercular agents (Table 2).

It is shown in the study of the structure-activity relationship (SAR) that the optimum hydrophobicity and hydrophilicity are required for better inhibition and is evidenced by a closure look into the structures of compounds **4b** and **4c**, displaying a better antitubercular profile having a hydrophobic 3-*O*-benzyl substituent as well as a hydrophilic, hydroxylethyl substituent at C-5 of the xylofuranose moiety. However, in compound **3a**, it is balanced by a less hydrophobic 3-*O*-methyl substituent and a hydrophobic carbethoxy methyl substituent at C-5. We compared these results with our sugar-derived diamino alcohol compounds [12] having a chain of twelve carbons, as shown in Scheme 1, because it was also very effective with MIC 3.12 μg/mL, but further optimization is required due to some toxicity. It is clear from the abovementioned data that the compounds belonging to this series have the potential to penetrate macrophages and may further be optimized to get better analogs to develop new drugs.

3. Conclusions

The glycosyl diureides were synthesized by using glycosyl amino esters with phenylene diisocyanate in anhydrous dichloromethane at room temperature and led to the formation of the corresponding phenylene-bridged glycosides in quantitative yields, and furthermore, they were reduced and hydrolyzed to give respective phenylene-bridged alcohols and carboxylic acids. The synthesized carbohydrate derivatives having phenylene ureidyl moiety with C_2 symmetry are a new class of glycohybrid compounds having the potential to derive antimicrobial agents. The study can certainly help for further optimization to get a better antitubercular candidate.

4. Experimental Section

4.1. General. The synthetic procedure involved in reactions was carried out under an inert (argon) atmosphere, and all reagents are of analytical grade. The solvents used were anhydrous, and all glasswares were dried in an oven at 100°C for 2 hour and cooled to room temperature in desiccators just before it was used. 60 F254 silica gel precoated aluminium plates were used for thin layer chromatography (TLC), and the spots were located by an iodine chamber and further confirmed by charing with ethanolic sulphuric acid at 60°C. NMR (^{1}H, ^{13}C) was recorded at 200 and 50 MHz, respectively. Chemical shifts were measured in parts per million (ppm) and trimethyl silane (TMS) as an internal standard. The coupling constant (*J*) values are given in Hertz. Nujol mulls in KBr pellets were used for recording of IR spectra of the developed compounds. For the determination of mass spectra of the compounds, electron spray ionization mass spectrometry (ESI-MS) was used.

4.2. Typical Experimental Procedure and Physical Data of N, N-Disubstituted Glycosylated Ureas [12]

*4.2.1. 1,4-Phenylene-[3,3′-{bis-(5R/S)-carbethoxymethyl-5-deoxy-1,2-O-isopropylidene-3-O-methyl-α-D-xylofuranos-5-yl}]-diureide (**3a**).* 1,4-Phenylene diisocyanate (405 mg, 2.60 mmol) was added to the stirring solution of glycosyl amino ester **2a** (1.5 g, 5.20 mmol) in anhydrous dichloromethane (10 mL), and the reaction was continued for the overnight at room temperature. The progress of the reaction was monitored by TLC (ethyl acetate in *n*-hexane as solvent system). The solvent was evaporated (below 55°C) and the resulting residue was then subjected to purification by flash column chromatography over SiO_2 using ethyl acetate/*n*-hexane (2 : 3) as eluent to give the desired compound **3a** as a colorless solid. m.p. = 114°C; yield (1.8 g, 94%); [α]

$_D^{25}$ = −33.8 (c, 0.18, CH_3OH); MS-FAB m/z = 761 $[M + Na]^+$; IR (film) ν_{max} cm^{-1} 3345 (NH), 1734 (OC=O), and 1656 (NC=O); ^1H NMR ($CDCl_3$, 200 MHz): δ = 7.26 (m, 4H, Ar-H), 5.94 and 5.88 (each *d*, each J = 3.7 Hz, 1 H, H-1 and H-1′), 4.59 and 4.55 (each *d*, each J = 3.7 Hz, 1 H, H-2 and H-2′), 4.46–4.43 (m, 2 H, H-4 and H-5), 4.13 (q, J = 7.1 Hz, 2 H, OCH_2CH_3), 3.79 (d, J = 2.9 Hz, 1 H, H-3), 3.69 and 3.36 (each *s*, each 3 H, 2 × OCH_3), 2.71 (m, 2 H, for CH_2COOEt), 1.73 (bs, 1 H, NH, D_2O exchangeable-H), 1.47, 1.34 (each *s*, each 3 H, 2 × >$C(CH_3)_2$), 1.24 (t, J = 7.1 Hz, 3 H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 172.6, 172.1 (C=O, ester), 156.3 (C=O, amide), 134.5 (Ar-qC), 122.2, 121.6, and 112.1 (Ar-CH), 105.8, 105.3 (C-1), 84.2 (C-2), 80.6 (C-3), 71.0 (C-4), 61.1 (OCH_2CH_3), 57.9 (OCH_3), 47.3 (C-5), 37.4 (CH_2COOEt), 27.1 and 26.6 (2 × >$C(CH_3)_2$), 14.9, and 14.8 (2 × OCH_2CH_3); Anal. Calcd for $C_{34}H_{50}N_4O_{14}.H_2O$: C, 53.96; H, 6.87; N, 7.40; Found C, 53.47; H, 6.65; N, 7.12%.

*4.2.2. 1,3-Phenylene-[3,3′-{bis-(5R/S)-carbethoxymethyl-5-deoxy-1,2-O-isopropylidene-3-O-methyl-α-D-xylofuranos-5-yl}]-diureide (**3b**).* The compound **3b** was synthesized and isolated as colorless solid in 78% yield by the reaction of compound **2a** (1.50 g, 5.19 mmol) and 1,3-phenylene diisocyanate (0.41 g, 2.59 mmol) in anhydrous DCM (10 mL) for overnight at room temperature and purified as the procedure described above. Yield (1.1 g, 87%); m.p. = 78°C; $[\alpha]_D^{25}$ = −40.0 (c, 0.14, CH_3OH); MS-FAB m/z = 761 $[M + Na]^+$; IR (film) ν_{max} cm^{-1} 3365 (NH), 1722 (C=O, ester), and 1666 (C=O, amide); ^1H NMR ($CDCl_3$, 200 MHz): δ = 7.26–7.20 (m, 4 H, Ar-H), 5.92 and 5.88 (each *d*, J = 3.8 Hz, 1 H, H-1 and H-1′), 4.58 (*d*, J = 3.8 Hz, 1 H, H-2), 4.59–4.37 (m, 2 H, H-4 and H-5), 4.16 (q, J = 7.0 Hz, 2 H, OCH_2CH_3), 3.78 and 3.69 (each *d*, each J = 2.8 Hz, each 1 H, H-3 and H-3′), 3.36 and 3.35 (each *s*, each 3 H, OCH_3), 2.69–2.67 (m, 2H, CH_2COOEt), 1.83 (bs, 1H, NH, D_2O exchangeable-H), 1.47 and 1.31 (each *s*, each 3H, 2 × >$C(CH_3)_2$), 1.24 (t, J = 7.2 Hz, 3 H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 172.2 (C=O ester), 155.9 (C=O, amide), 141.5 and 140.0 (Ar-qC), 129.7, 123.6, and 114.6 (Ar-CH), 112.1 and 111.4 (2 × >$C(CH_3)_2$), 105.3 and 105.1 (C-1 and C-1′), 86.0 (C-2), 84.6 and 84.2 (C-4 and C-4′), 81.8 and 80.9 (C-3 and C-3′), 61.1 (OCH_2CH_3), 58.6 and 57.9 (diastereomeric OCH_3), 46.6 (C-5), 37.5 (CH_2COOEt), 27.1 and 26.6 (2 × >$C(CH_3)_2$), 14.5 (OCH_2CH_3) ppm; Anal. Calcd for $C_{34}H_{50}N_4O_{14}.H_2O$: C, 53.96; H, 6.87; N, 7.40; Found C, 53.75; H, 6.63; N, 7.22%.

*4.2.3. 1,4-Phenylene-[3,3′-{bis-3-O-benzyl-5(R/S)-carbethoxymethyl-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranos-5-yl}]-diureide (**3c**).* This compound **3c** was obtained by the reaction of glycosyl β-amino ester **2b** (3.0 g, 8.21 mmol) and 1,4-phenylene diisocyanate (0.65 g, 4.10 mmol) in anhydrous DCM. The workup of the reaction was carried out after 12 h. The crude product obtained was subjected to purification by flash column chromatography over SiO_2 using methanol/chloroform (3 : 97) as an eluent to give the title compound **3c** as a mixture of diastereomers as a colorless solid. Yield (2.8 g, 77%), m.p. = 96°C; $[\alpha]_D^{25}$ = −29.6 (c, 0.3, CH_3OH); MS-FAB: m/z = 891 $[M + H]^+$; IR (film) ν_{max} cm^{-1} 3365 (NH), 1729 (OC=O, ester), and 1572 (NC=O, amide); ^1H NMR ($CDCl_3$, 200 MHz): δ = 7.20 (m, 7 H, Ar-H), 5.94 (d, J = 3.0 Hz, 1H, H-1), 5.75 (m, 1 H, NH, D_2O exchangeable-H), 4.69 (d, 1H, J = 11.6 Hz, OC-CH_APh), 4.61 (d, J = 3.4 Hz, 1H, H-2), 4.54 (m, 4 H, H-2, OCH_2Ph, and H-4), 4.09 (m, 3 H, H-3 and OCH_2CH_3), 2.64 (m, 2H, H-6 as CH_2COOEt), 1.87 (bs, 1 H, NH, D_2O exchangeable-H), 1.45 and 1.27 (each *s*, each 3 H, 2 × >$C(CH_3)_2$), 1.22 (t, J = 7.0 Hz, 3H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 172.2 (C=O, ester), 155.8 (CONH), 137.4, 137.2, and 134.4 (Ar-C), 128.9, 128.5, 121.7, 121.5, and 120.9 (Ar-CH), 112.3 and 112.2 (2 × >$C(CH_3)_2$, 105.0 (C-1), 82.5 (C-2), 82.1 (C-4), 80.9, 80.5 (C-3), 72.5 and 72.2 (OCH_2Ph), 61.1 (OCH_2Me), 47.6 (C-5), 37.4 and 37.2 (CH_2COOEt), 27.2 and 26.6 (2 × >$C(CH_3)_2$), 14.5 (OCH_2CH_3) ppm; Anal. Calcd for $C_{46}H_{58}N_4O_{14}.H_2O$: C, 60.79; H, 6.60; N, 6.16; Found C, 61.09; H, 6.85; N, 6.11%.

*4.2.4. 1,3-Phenylene-[3,3′-{bis-3-O-benzyl-5(S)-carbethoxymethyl-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranos-5-yl}]-diureide (**3d**).* This was obtained by the reaction of compound **2b** (3.2 g, 8.76 mmol) and 1,3-phenylene diisocyanate (0.70 g, 4.38 mmol) as described above and isolated in pure form as colorless solid by flash column chromatography using methanol:chloroform (2 : 98). Yield (3.1 g, 79%), m.p. = 76°C, $[\alpha]_D^{25}$ = −22.8 (c, 0.17, CH_3OH); MS-FAB m/z = 913 [M + Na]; IR (film) ν_{max} cm^{-1} 3360 (NH), 1729 (OC=O, ester), and 1666 (N-C=O, amide); ^1H NMR ($CDCl_3$, 200 MHz): δ = 7.26 (m, 7 H, Ar-H), 5.95 (d, J = 3.7 Hz, 1H, H-1), 5.56 (m, 1 H, NH, D_2O exchangeable-H), 4.69 (d, 1 H, J = 11.6 Hz, OCH_APh), 4.61 (d, J = 3.7 Hz, 1 H, H-2), 4.54–4.51 (m, 1 H, H-4), 4.43 (d, J = 11.6 Hz, 1 H, OCH_BPh), 4.11 (q, J = 7.1 Hz, 2 H, OCH_2Me), 3.90 (d, J = 2.70 Hz, 1 H, H-3), 3.75–3.73 (m, 1 H, H-5), 2.57–3.55 (m, 2 H, CH_2COOEt), 1.77 (m, 1 H, NH, D_2O exchangeable-H), 1.45 and 1.29 (each *s*, each 3 H, 2 × >$C(CH_3)_2$), 1.22 (t, J = 7.1 Hz, 3 H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 172.1 (COO, ester), 155.6 (CONH, amide), 139.5 and 137.2 (Ar-C), 129.6, 128.9, 128.5, and 114.6 (Ar-CH), 112.2 ($C(CH_3)_2$), 105.1 (C-1), 82.4 (C-2), 82.0 (C-4), 80.6 (C-3), 72.1 (OCH_2Ph), 61.1 (OCH_2CH_3), 47.2 (C-5), 37.2 (CH_2COOEt), 27.1 and 26.6 (2 × >$C(CH_3)_2$), 14.5 (OCH_2CH_3) ppm; Anal. Calcd for $C_{46}H_{58}N_4O_{14}.H_2O$: C, 60.79; H, 6.60; N, 6.16; Found C, 60.82; H, 6.29; N, 5.87%.

*4.2.5. 1,4-Phenylene-[bis{3-benzyl,3′-(5S)-carbethoxymethyl-5-deoxy-1,2-O-isopropylidene-3-O-methyl-α-D-xylofuranos-5-yl}]-diureide (**3e**).* To the stirring solution of glycosyl β-amino ester **2c** (2.0 g, 5.27 mmol) in anhydrous dichloromethane (15 mL), 1, 4-phenylene diisocyanate (0.42 g, 2.63 mmol) was added, and the reaction was continued for the overnight. The solvent was evaporated and the resulting crude product was subjected to purification by flash column chromatography using the solvent gradient of methanol/chloroform (1 : 24) to give the title compound **3e** as a colorless solid. Yield (1.5 g, 62%), m.p. = 141°C; $[\alpha]_D^{25}$ = −75.4 (c, 0.11 CH_3OH); MS-FAB m/z = 941 $[M + Na]^+$; IR (film) ν_{max} cm^{-1} 3423 (NH), 1723 (OC=O, ester), and

1652 (NC=O, amide); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.28$ (m, 7 H, Ar-H), 5.93 (d, $J = 3.6$ Hz, 1H, H-1), 4.62–4.59 (m, 3 H, NCH_2Ph and H-2), 4.46 (m, 1H, H-4), 4.11 (q, $J = 7.0$ Hz, 2H, OCH_2Me), 3.59 (d, $J = 2.6$ Hz, 1 H, H-3), 3.37 (s, 3 H, OCH_3), 2.81–2.79 (m, 1 H, CH_ACOOEt), 2.41–2.39 (m, 1 H, CH_BCOOEt), 1.57 (s, 1 H, NH, D_2O exchangeable-H), 1.39 and 1.31 (each *s*, each 3H, $2 \times >C(CH_3)_2$), 1.21 (t, $J = 7.0$ Hz, 3H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 171.7$ (COO), 157.3 (CONH), 139.2, 134.9, 133.6, and 132.0 (Ar-C), 129.0, 127.5, 121.3, 120.4, and 116.6 (Ar-CH), 112.2 and 109.5 ($2 \times >C(CH_3)_2$), 105.0 (C-1), 84.0 (C-2), 81.3 (C-4), 79.9 (C-3), 61.4 (OCH_2Me), 57.6 (OCH_3), 53.9 (NCH_2Ph), 35.8 (CH_2COOEt), 27.0 and 26.6 ($2 \times >C(CH_3)_2$), 14.4 (OCH_2CH_3) ppm; Anal. Calcd for $C_{48}H_{62}N_4O_{14}.H_2O$: C, 61.53; H, 6.83; N, 5.98; Found C, 61.58; H, 6.53; N, 6.14%.

*4.2.6. (1R, 2R, 3S, 4R) Ethyl (3-O-benzyl-5-benzylamino-5,6-dideoxy-1,2-O-isopropylidene)-β-L-Ido-Heptofuranuronate (**2d**).* The reaction of glycosyl olefinic ester **1b** (3.0 g, 8.62 mmol) with benzylamine (0.93 g, 8.69 mmol) gave compound **2d** as light-yellow foam (60%). IR (KBr): ν_{max} cm^{-1} 3350 (–NH), 3020, 2980 and 2920 (1710 (>C=O); MS-FAB: 456 $[M + H]^+$.

*4.2.7. 1,4-Phenylene-[bis{3-benzyl,3′-(3-O-benzyl-5(S)-carbethoxymethyl-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranos-5-yl)}]-diureide (**3f**).* The compound **3f** was prepared by the reaction of glycosylated amino β-ester **2d** (1.50 g, 3.29 mmol) and 1,4-phenylene diisocyanate (0.26 g, 1.64 mmol) in anhydrous DCM (10 mL) as described above and isolated in pure form as a colorless foam using the solvent gradient of $CHCl_3/CH_3OH$ (97 : 3). Yield (1.21 g, 69%); m.p. = 78°C; $[\alpha]_D^{25} = -9.0$ (c, 0.1, CH_3OH); MS-FAB $m/z = 1071$ $[M + H]^+$; IR (film) ν_{max} cm^{-1} 3357 (NH), 1729 (OC=O, ester), and 1668 (NC=O, amide); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.40–7.09$ (m, 12 H, Ar-H), 5.96 (d, $J = 3.6$ Hz, 1H, H-1), 4.70 (d, $J = 11.9$ Hz, 1 H, OCH_APh), 4.55–4.74 (m, 3 H, NCH_2Ph and H-2), 4.41–4.37 (m, 3 H, OCH_BPh merged with H-4), 4.04 (q, $J = 7.0$ Hz, 2 H, OCH_2Me), 3.77 (d, $J = 2.8$ Hz, 1 H, H-3), 2.64–2.62 (m, 1 H, CH_ACOOEt), 2.11–2.09 (m, 1H, CH_BCOOEt), 1.58 (bs, 1 H, NH, D_2O exchangeable-H), 1.38 and 1.31 (each *s*, each H, $2 \times >C(CH_3)_2$), 1.21 (t, $J = 7.0$ Hz, 3 H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 171.6$ (OC=O, ester), 157.4 (CONH, amide), 139.3, 137.0, and 134.9 (Ar-C), 129.0, 128.9, 128.6, 128.5, 127.5, and 120.4 (Ar-CH), 112.2 (>$C(CH_3)_2$), 105.1 (C-1), 82.1 (C-2), 81.2 (C-4), 79.7 (C-3), 71.9 and 71.8 (OCH_2Ph and OCH_2CH_3), 61.3 (NCH_2Ph), 35.2 (CH_2COOEt), 27.1 and 26.7 ($2 \times >C(CH_3)_2$), 14.5 (OCH_2CH_3) ppm; Anal. Calcd for $C_{60}H_{70}N_4O_{14}.H_2O$: C, 66.17; H, 6.61; N, 5.14; Found C, 66.37; H, 6.44; N, 4.88%.

*4.2.8. 1,4-Phenylene-[3,3′-{bis-(5S)-hydroxyethyl-5-deoxy-1,2-O-isopropylidene-3-O-methyl-α-D-xylofuranos-5-yl}]-diureide (**4a**).* To the slurry of $LiAlH_4$ (41 mg, 1.08 mmol) in anhydrous tetrahydrofuran (THF) (8 mL), solution of compound **3a** (800 mg, 1.08 mmol) in THF (5 mL) was added dropwise at ice cooled temperature. The stirring was continued for 20 min at 0°C and then the reaction was continued for additional 4 h at rt. The progress of reaction was monitored by TLC, and after completion, it was quenched with Na_2SO_4 saturated solution (in H_2O). The reaction mixture was filtered over celite and the solid cake was washed with THF (5 mL) and the solvent was evaporated under vaccum (>50°C), and the resulting crude mass was subjected to flash column chromatography using gradient of $CH_3OH/CHCl_3$ (4 : 96) to provide the title compound **4a** as a colorless foam. Yield (680 mg, 93%); $[\alpha]_D^{25} = -52.8$ (c, 0.07, CH_3OH); FAB-MS $m/z = 677$ $[M + Na]^+$; IR (film) ν_{max} cm^{-1} 3387 (NH) and 1671 (NC=O, amide); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.04$ (m, 2 H, Ar-H), 5.93 (d, $J = 3.6$ Hz, 1 H, H-1), 4.60 (d, $J = 3.6$ Hz, 1 H, H-2), 4.55–4.52 (m, 1 H, H-4), 4.16–4.13 (m, 1 H, H-5), 3.76 (m, 3 H, H-3 and CH_2OH), 3.30 (s, 3 H, OCH_3), 1.79 (m, 3 H, CH_2CH_2OH merged with NH), 1.49 and 1.31 (each *s*, each 3H, $2 \times >C(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 157.8$ (NC=O, amide), 134.5 (Ar-C), 121.4 (Ar-CH), 111.9 (>$C(CH_3)_2$), 105.0 (C-1), 84.4 (C-2), 82.6 (C-4), 81.8 (C-3), 58.6 (CH_2OH), 58.1 (OCH_3), 35.8 (CH_2CH_2OH), 27.0 and 26.4 ($2 \times >C(CH_3)_2$) ppm; Anal. Calcd for $C_{30}H_{46}N_4O_{12}.H_2O$: C, 53.57; H, 7.14; N, 8.33; Found C, 53.25; H, 6.73; N, 8.01%.

*4.2.9. 1,4-Phenylene-[3,3′-{bis-3-O-benzyl-5(S)-hydroxyethyl-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranos-5-yl}]-diureide (**4b**).* The title compound **4b** was obtained as a colorless foam by the reduction of compound **3c** (1.2 g, 1.34 mmol) with $LiAlH_4$ (50 mg, 1.34 mmol) in anhydrous THF and workup as the procedure described above. Yield (0.98 g, 91%), $[\alpha]_D^{25} = -33.07$ (c, 0.13, CH_3OH); MS-FAB *m/z*: 829 $[M + Na]^+$; IR (film): ν_{max} cm^{-1} 3391 (NH) and 1670 (NC=O, amide); ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 7.28$ (m, 5H, Ar-H), 6.99 (m, 2H, Ar-H), 5.93 (d, $J = 3.7$ Hz, 1 H, H-1), 5.38 (s, 1H, NH). 4.54–4.50 (m, 4 H, H-2 was merged with H-4 and OCH_2Ph), 4.14–4.12 (m, 1 H, H-5), 3.91 (d, $J = 2.8$ Hz, 1 H, H-3), 3.69–3.67 (m, 2 H, CH_2OH), 1.85 (m, 3 H, CH_2CH_2OH and D_2O exchangeable NH), 1.46 and 1.30 (each *s*, each 3H, $2 \times >C(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 157.6$ (NC=O, amide), 137.2 and 134.5 (Ar-C), 128.9, 128.5, and 121.6 (Ar-CH), 112.0 (>$C(CH_3)_2$), 105.0 (C-1), 82.4 (C-2), 82.2 (C-4, C-3), 58.5 (CH_2OH), 46.3 (C-5), 36.6 (CH_2CH_2OH), 27.1 and 26.4 ($2 \times >C(CH_3)_2$) ppm; Anal. Calcd for $C_{42}H_{54}N_4O_{12}.H_2O$: C, 61.16; H, 6.79; N, 6.79; Found C, 61.14; H, 6.56; N, 6.39%.

*4.2.10. 1,3-Phenylene-[3,3′-{bis-3-O-benzyl-5(S)-hydroxyethyl-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranos-5-yl}]-diureide (**4c**).* The compound **4c** was obtained by the reduction of compound **3d** (1.0 g, 1.12 mmol) with $LiAlH_4$ (42 mg, 1.12 mmol) in anhydrous THF (10 mL) as the procedure described above and after purification by flash column chromatography using $CHCl_3$/MeOH (24 : 1) isolated in pure form as a colorless solid. Yield (0.85 g, 94%); m.p. = 139°C; $[\alpha]_D = -23.1$ (c, 0.1, CH_3OH); MS-FAB *m/z* = 829 [M + Na]; IR (film): ν_{max} cm^{-1} 3387 (NH) and 1668

(NC=O, amide); ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.35–7.31 (m, 7 H, Ar-H), 5.94 (d, J = 3.7 Hz, 1 H, H-1), 4.67–4.65 (m, 4 H, H-2, H-4, and OCH_2Ph), 4.14–4.12 (m, 1 H, H-5), 3.91 (d, J = 2.8 Hz, 1 H, H-3), 3.75–3.73 (m, 2 H, CH_2OH), 1.86–1.83 (m, 3 H, D_2O exchangeable NH and CH_2CH_2OH), 1.46 and 1.30 (each *s*, each 3 H, 2 × >$C(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 156.8 (NC=O, amide), 136.7 and 136.5 (Ar-C), 129.0, 128.5, 128.3, 128.0, 127.9, and 114.5 (Ar-CH), 112.0 (>$C(CH_3)_2$), 104.8 (C-1), 82.3 (C-2), 81.9 (C-4), 81.2 (C-3), 71.8 (OCH_2Ph), 58.0 (CH_2OH), 45.9 (C-5), 35.4 (CH_2CH_2OH), 27.6 and 26.6 (2 × >$C(CH_3)_2$) ppm. Anal. Calcd for $C_{42}H_{54}N_4O_{12}.H_2O$: C, 61.16; H, 6.79; N, 6.79; Found C, 60.80; H, 6.52; N, 6.53%.

*4.2.11. 1,4-Phenylene-[3,3′-{bis-(5S)-carboxymethyl-5-deoxy-1,2-O-isopropylidene-3-O-methyl-α-D-xylofuranos-5-yl}]-diureide (**5a**).* This compound was obtained by the basic hydrolysis of glycosyl ureide **3a** (0.80 g, 1.08 mmol) with $LiOH.H_2O$ (91 mg, 2.16 mmol) in distilled water (10 mL) at 0°C to room temperature for 3 hours. The reaction mixture was neutralized with 2N HCl solution at 0°C followed by evaporation of solvent under rotatory evaporator (>55°C), and the resulting crude mass was then subjected to flash column chromatography using gradient of $CHCl_3$/CH_3OH (24 : 1) as an eluent to give the desired title compound **5a** as a colorless foam. Yield (0.64 g, 86%); $[\alpha]_D$ = –40.5 (c, 0.08, CH_3OH); MS-FAB: m/z = 699 $[M + Li]^+$; IR (film) ν_{max} cm^{-1} 3412 (NH), 1710 (-O-C=O, acid) and 1575 (N-C=O, amide); ^{1}H NMR (D_6-DMSO, 200 MHz): δ = 10.1 (bs, 1H, D_2O exchangeable COOH), 8.98 (bs, 1H, NH, D_2O exchangeable-H), 7.27–7.25 (m, 2 H, Ar-H), 5.80 (d, J = 3.8 Hz, 1H, H-1), 4.62–4.59 (m, 2 H, H-2 and H-4), 4.29–4.27 (m, 1 H, H-4), 3.79–3.77 (m, 1 H, H-5), 3.37 (s, 3 H, OCH_3), 2.86–3.84 (m, 1 H, H-3), 2.41–4.39 (m, 2 H, CH_2COOH), 1.80 (bs, 1 H, NH, D_2O exchangeable-H), 1.47 and 1.31 (each *s*, each 3 H, 2 × >$C(CH_3)_2$); ^{13}C NMR (D_6-DMSO, 50 MHz): δ = 174.1 (-OC=O, acid), 155.0 (N-C=O, amide), 134.7 (Ar-C), 129.2, 128.5, 118.4 (Ar-CH), 110.7 (>$C(CH_3)_2$), 104.5 (C-1), 83.5 (C-2), 81.0 (C-4), 80.8 (C-3), 57.1 (OCH_3), 45.5 (CH_2COOH), 27.8 and 26.4 (2 × >$C(CH_3)_2$) ppm; Anal. Calcd for $C_{30}H_{42}N_4O_{14}.H_2O$: C, 51.42; H, 6.28; N, 8.00; Found C, 51.78; H, 6.53; N, 8.38%.

*4.2.12. 1,4-Phenylene-[3,3′-{bis-3-O-benzyl-5-carboxymethyl-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranos-5-yl}]-diureide (**5b**).* This compound was obtained by the hydrolysis of glycosyl ureide **3c** (0.80 g, 0.89 mmol) by $LiOH.H_2O$ (70 mg, 1.79 mmol) following the procedure described above and isolated as a colorless foam. Yield (0.59 g, 80%) $[\alpha]_D$ = –65.5 (c, 0.1, CH_3OH), MS-FAB: m/z = 841 $[M + Li]^+$; IR (film): ν_{max} cm^{-1} 3395 (NH), 1594 (NC=O, amide), ^{1}H NMR (D_6-DMSO, 200 MHz): δ = 8.59 (br *s*, 1 H, COOH as D_2O exchangeable-H), 6.40 (bs, 1 H, NH, D_2O exchangeable-H), 7.37–7.33 (m, 7 H, Ar-H), 6.87 (bs, 1H, NH, D_2O exchangeable-H), 5.87 (d, J = 3.3 Hz, 1 H, H-1), 4.78 (d, J = 3.3 Hz, 1 H, H-2), 4.66 (d, J = 11.3 Hz, 1H, OCH_APh), 4.48 (d, J = 11.3 Hz, 1 H, OCH_BPh), 4.29–4.27 (m, 1 H, H-4), 3.88 (d, J = 3.0 Hz, 1 H, H-3), 3.67–3.65 (m, 1 H, H-5), 2.44–2.42 (m, 2 H, CH_2COOH), 1.80 (m, 1 H, NH, D_2O exchangeable-H), 1.43 and 1.37 (each *s*, each 3 H, 2 × >$C(CH_3)_2$); ^{13}C NMR (D_6-DMSO, 50 MHz): δ = 175.1 (O-C=O, acid), 155.6 (N-C=O, amide), 137.8, 137.6, 134.6, and 134.5 (Ar-C), 128.28, 128.21, and 128.0, 127.7, 127.5, 127.0, 118.4, and 118.1 (Ar-CH), 110.5 and 110.3 (2 × > $C(CH_3)_2$, 104.3 and 104.1 (C-1, C-1′), 81.6 (C-2), 81.3 (C-4), 80.8, 80.5 (C-3), 71.2 (OCH_2Ph), 48.5 (C-5), 40.6 (CH_2CO_2H), 26.5 and 26.1 (2 × >$C(CH_3)_2$) ppm; Anal. Calcd for $C_{42}H_{50}N_4O_{14}.H_2O$: C, 59.15; H, 6.10; N, 6.57; Found C, 59.39; H, 6.48; N, 6.78%.

*4.2.13. 1,3-Phenylene-[3,3′-{bis-3-O-benzyl-5-carboxymethyl-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranos-5-yl}]-diureide (**5c**).* The title compound **5c** was obtained by the basic hydrolysis of glycosyl ureide **3d** (1.0 g, 1.12 mmol) by $LiOH.H_2O$ (94 mg, 2.24 mmol) in THF/H_2O (7 : 3) as a solvent system as the procedure described above. The compound **5c** was isolated in the pure form as colorless foam by after flash column chromatography using a gradient of $CHCl_3$: CH_3OH (95 : 5). Yield: 0.75 g (80%); $[\alpha]_D^{25}$ = –20.4 (c, 0.18, CH_3OH); MS-FAB: m/z = 841 $[M + Li]^+$; IR (film): ν_{max} cm^{-1} 3401 (NH) and 1603 (NC=O, amide); ^{1}H NMR (D_6-DMSO, 300 MHz): δ = 9.00 (bs, 1 H, COOH, D_2O exchangeable-H), 7.29–7.26 (m, 7 H, Ar-H), 6.94 (bs, 1 H, NH, D_2O exchangeable-H), 5.75 (d, J = 3.6 Hz, 1 H, H-1), 4.69(d, J = 3.6 Hz, 1 H, H-2), 4.60 (d, J = 11.4 Hz, 1 H, OCH_APh), 4.45 (d, J = 11.4 Hz, 1 H, OCH_BPh), 4.23–4.21 (m, 1 H, H-4), 3.77–3.75 (m, 1 H, H-5), 3.08 (d, J = 3.0 Hz, 1H, H-3), 2.48 and 2.18 (m, each 1H, CH_ACOOH and CH_BCOOH), 1.80 (bs, 1 H, NH, D_2O exchangeable-H), 1.33 and 1.18 (each *s*, each 3H, 2 × >$C(CH_3)_2$); ^{13}C NMR (D_6-DMSO, 75 MHz): δ = 175.5 (O-C=O, acid), 155.5 (N-C=O, amide), 141.0 and 137.9 (Ar-C), 128.6, 128.4, 128.2, 127.9, and 127.7 (Ar-CH), 111.1 and 110.6 (2 × >$C(CH_3)_2$, 104.3 (C-1), 81.6 (C-2), 81.4 (C-4), 80.8 (C-3), 73.9 (OCH_2Ph), 48.5 (C-5), 46.4 (CH_2COOH), 26.8 and 26.3 (2 × >$C(CH_3)_2$ ppm; Anal. Calcd for $C_{42}H_{52}N_4O_{15}$: C, 59.15; H, 6.10; N, 6.57; Found C, 59.40; H, 5.99; N, 6.40%.

4.3. Bioevaluation of Developed N, N-Disubstituted Glycosylated Ureas [31, 32]

4.3.1. Micro Almar Blue Assay

M. tuberculosis H37Ra strain was used to screen the developed *N, N*-disubstituted glycosylated urea and their analogs by the procedure reported in the literature for the micro Almar blue assay (MABA) [31, 32]. It is a suitable surrogate for the virulent H37Rv strain. Almar blue dye is (Reaszurin dye) stable at 4°C for several months, and it is a common indicator of cellular growth or viability. The blue color oxidized from the redox indicator is nonfluorescent and becomes pink and fluorescent upon reduction. In bioevaluation, ethambutol (EMB), a standard first-line antitubercular drug was used as a positive control. Young cultures (7-8 days) were diluted in a liquid medium to give an optimal density of 0.02 at 550 nm spectrophotometrically,

which further resulted to change in color of Almar blue (blue to pink) in around a week. The *M. tuberculosis* H37Ra was taken in log phase which was diluted to give a final $OD_{550\ nm}$ of 0.05 in Sauton's medium. In different 96-well white plates, an amount of 190 μL of culture was dispensed in each well.

A dimethylsulphoxide (DMSO) solution of the test drug candidate was added to 96-well plates to make a final test concentration of 25 μg/ml (5 μg test compound is dispensed in 10 μL of DMSO). After that, the plate was incubated at 37°C/5% CO_2 for 5 d. An amount of 15 μL Alamar blue solution was added to each well of the plate on 5th day. The plate was then again incubated for 12 hours at 37°C/5% CO_2 incubator. The fluorescence was observed on BMG Polarstar with an emission frequency of 590 nm and an extinction frequency of 544 nm.

4.3.2. Agar Microdilution Method. The determination of minimum inhibition concentration (MIC) of test compounds or drugs and its susceptibility against *M.* tuberculosis H37Rv was done by using the agar microdilution method. In this method, two-fold dilutions of each test compound were added into 7H10 agar and *M. tuberculosis* H37Rv was taken as the test organism. The MIC of the test compounds was determined by incorporating a reducing concentration of the test compounds in the middle brook 7H10 agar medium which was supplemented with ODAC. A culture of *M. tuberculosis* H37Rv growing on the L-J medium was harvested in 85% saline along with 0.05% Tween-80. Further suspension of 1 μg/mL concentration of compounds was prepared in DMSO. This prepared suspension was added into tubes of 7H10 middle brook's medium (having 1.7 mL medium, 0.2 mL OADC supplement) at various concentrations of the test compounds with fixed volume, i.e., 0.1 mL. The medium was allowed to cool, maintaining the tube in a slanting position. Furthermore, tubes were incubated at 37°C for one day followed by streaking of *M. tuberculosis* H37Rv (5×104 bacilli/tube). Subsequently, these tubes were incubated at 37°C. Growth of bacilli was observed after 21 days of incubation. The control tubes and the tubes having the compounds were compared where the medium alone was incubated with H37Rv. The active concentration of the test compound was taken at which the complete inhibition of colonies was observed.

Data Availability

Physical data of the developed molecules are included in manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

NT and VKT were involved in the synthesis of glycosylated ureas and their analogs starting from readily available monosaccharide, D-glucose. They analyzed the data and established the structures of the synthesized compounds given in this scheme. Both the authors prepared figures and tables and wrote the manuscript.

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