



STUDIES ON SYNTHESIS OF SOME BIOLOGICALLY IMPORTANT *N*-GLYCOSYLATED-3-AMIDINO CARBAMIDES AS ANTIBACTERIAL AND ANTIFUNGAL AGENTS

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ABSTRACT

Condensation of several acetyl/benzoyl glycosyl isocyanates and guanidine resulting in the formation of *N*-glycosylated-3-amidino carbamides has been studied. The required acetyl/benzoyl glycosyl bromides were prepared by the processes of acetylation, benzylation followed by bromination. This was converted in to acetyl/benzoyl glycosyl isocyanates by the reaction respective glycosyl bromides with lead cyanate. The identities of these newly synthesized compounds have been established on the basis of usual chemical transformations, elemental analysis and IR, ¹H NMR and Mass spectral studies. The compounds have been screened for their antibacterial activity against *E.coli*, *S. aureus*, *P. vulgaris*, *S. typhi* and *K. pneumoniae* and for antifungal activity against *P. species* and *A. niger*. The study reveals that most of the compounds show promising activities.

Key words: Guanidine, Glycosyl isocyanates, Amidino carbamides, Antibacterial and Antifungal activity.

INTRODUCTION

The sugar science has been extensively studied and many excellent reviews are available. The synthesis of new compounds incorporating the guanidine unit and glycosyl isocyanates resulting a group of very reactive chemical substances and acts as precursor of various heterocyclic compounds. Recent example of synthetic biologically active¹ guanidines include antimicrobial activity², thrombin inhibitors³, Na⁺/H⁺ exchanger (NHE) inhibitors⁴, transport for delivery of anticancer agents⁵ and anti-influenza agents⁶. Their wide importance has promoted the study of new approaches to guanidine derivatives. In this overview, we summarized the synthesis of *N*-glycosylated-3-amidino carbamides (**2a-f**) (**Scheme 2**) by the condensation of several acetyl/benzoyl glycosyl isocyanates (**1a-f**) and guanidine.

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EXPERIMENTAL

All the chemicals used were of research grade. The melting points of synthesized compounds were determined with the help of thermonic melting point apparatus and uncorrected. The structures of newly synthesized compounds were elucidated on the basis of elemental and IR, ^1H NMR and Mass spectral analysis. IR spectra were recorded in KBr disks on Shimadzu IR Affinity-1 FTIR spectrometer. ^1H NMR spectra were obtained on Bruker Avance II 400 NMR spectrometer, sample were prepared in CDCl_3 with TMS as an internal reference. The Mass spectra were obtained on Water, Q-T FO Micromass (LC-MS) spectrometer. Optical rotation $[\alpha]_D^{31}$ were measured on the Equip-Tronics EQ-800 Digital polarimeter at 31°C in CHCl_3 . Thin layer chromatography (TLC) was performed on silica gel G for TLC (Merk) and spot were visualized by iodine vapor.

On the basis of above facts the product was assigned a structure as *N*-glycosylated-3-amidno carbamides (**2a-f**).

Material and method

The reagents required for the given synthesis are prepared as follows:

Synthesis of tetra-*O*-acetyl- β -D-glucosyl isocyanate (**1a**)

The tetra-*O*-acetyl- β -D-glucosyl isocyanate (**1a**) was synthesized by the condensation of tetra-*O*-acetyl- α -D-glucosyl bromide and lead cyanate in boiling sodium dried xylene (25 mL) for 3 hr with frequent shaking. Solution then filtered and the filtrate was concentrated to get the syrupy mass. It was triturated with petroleum ether ($60-80^\circ$) and purified by dissolving it in minimum quantity of chloroform and reprecipitating with petroleum ether.

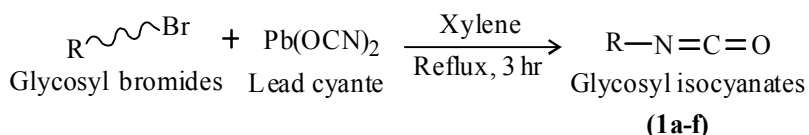
Silmilarly, the hepta-*O*-acetyl- β -D-lactosyl isocyanate (**1b**), hepta-*O*-acetyl- β -D-maltosyl isocyanate (**1c**), tetra-*O*-benzoyl- β -D-glucosyl isocyanate (**1d**), hepta-*O*-benzoyl- β -D-lactosyl isocyanate (**1e**), hepta-*O*-benzoyl- β -D-maltosyl isocyanate (**1f**) were synthesized by interaction of respective glycosyl bromides with lead cyanate in xylene medium.

Synthesis of 1-tetra-*O*-acetyl- β -D-glucosyl-3-amidino carbamide (**2a**)

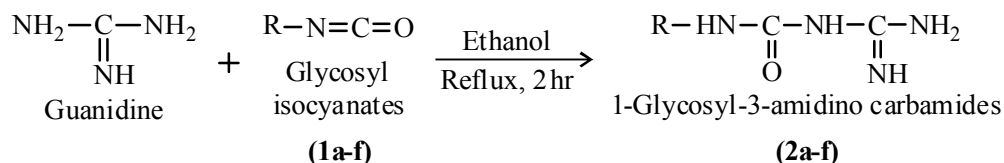
1-tetra-*O*-acetyl- β -D-glucosyl-3-amidino carbamide (**2a**) was synthesized by refluxing the mixture of guanidine (0.005 M, 0.61 g) and tetra-*O*-acetyl- β -D-glucosyl isothiocyanate (**1a**) (0.005 M, 1.94 g) in ethanolic medium (20 mL) for 2:30 hr on water bath. After completion of reaction, the solvent was removed by vacuum distillation. The sticky mass was titurated several times with petroleum ether ($60-80^\circ$) afford the light colour solid, with m.p. 103°C . Crystallized from ethanol-water system.

Similarly, when the reaction of guanidine was extended to other glycosyl isocyanates **1(b-f)** the corresponding 1-glycosyl-3-amidino carbamides **2(b-f)** were isolated.

It was soluble in ethanol, acetone, chloroform, carbon tetrachloride and benzene while insoluble in water and petroleum ether. It charred when boiled with conc. sulfuric acid. It was desulfurisable with alkaline plumbite solution. The purity of the product was checked by TLC. The product was optically active and gives positive Molish's test.

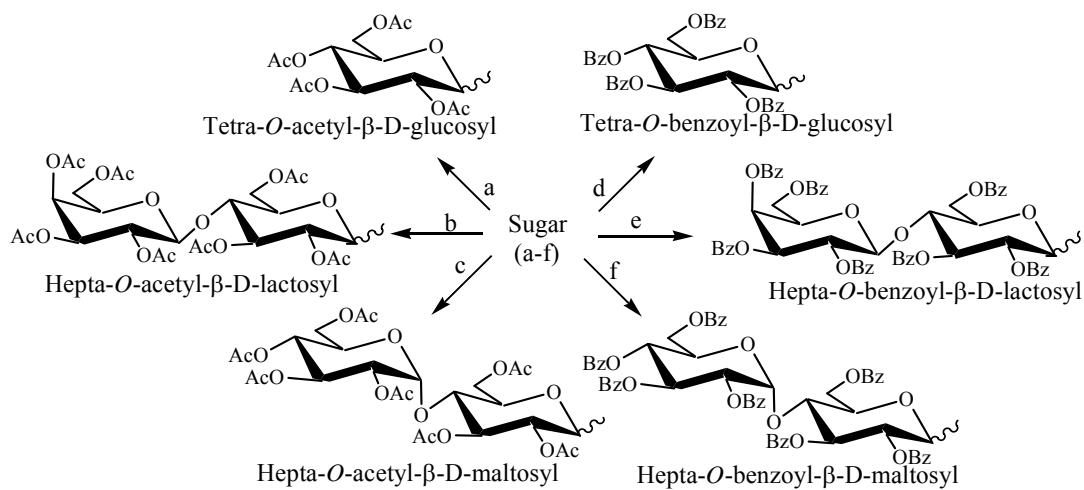


Scheme 1



Scheme 2

Where, R = Glycosyl =



Ac = COCH₃, Bz = COC₆H₅.

RESULTS AND DISCUSSION

Several *N*-glycosylated-3-amidino carbamides (**2a-f**) were prepared by the condensation of various acetyl/benzoyl glycosyl isocyanates (**1a-f**) and guanidine in ethanol medium. After complete reaction, the solvent was distilled off and the resultant sticky residue was triturated with petroleum ether (60-80°C) to afford the products.

Similarly, when the reaction of guanidine was extended to other glycosyl isocyanates **1(b-f)**, the corresponding 1-glycosyl-3-amidino thiocarbamides **2(b-f)** were isolated.

All the products were crystallized from ethanol-water system before recording the physical data. The purity of compounds were checked by TLC. The spectral analysis IR⁷⁻¹⁰, ¹H NMR and Mass spectra of the product were observed. Optical rotation of the product was also recorded¹¹. The compounds gives positive Molish's test. The characteristics of the newly synthesized compounds are mentioned in Table 1.

Table 1: Characterization data of *N*-glycosylated-3-amidino carbamides (2a-f)

Reactants: (1) Glycosyl isocyanates (1a-f) (2) Guanidine

Compds.	Yield (%)	m.p. (°C)	$[\alpha]_D^{31}$ (CHCl ₃)	N Found (Required) (%)	R _f
2a	68.65	103	+40 (c,0.998)	2.94 (2.99)	0.78
2b	76.48	126	+92.22 (c,0.998)	7.71 (7.77)	0.59
2c	64.96	121	+87 (c,0.998)	7.74 (7.77)	0.75
2d	81.48	188	+70.12 (c,0.998)	8.20 (8.23)	0.57
2e	80.65	178	+ 35 (c,0.998)	4.79 (4.84)	0.67
2f	78.48	172	+ 78.3 (c,0.998)	4.81 (4.84)	0.76

C and H analysis were found satisfactory in all cases

Spectral data

1-Tetra-*O*-acetyl-β-D-glucosyl-3-amidino carbamide (2a)

IR (KBr, cm⁻¹): ν 2962 (Alk C-H), 1743 (C=O), 1689 (C=N), 1629 (N-H bending),

1367 (C-N), 1236 (C-O), 983, 947 and 916 (Characteristic of glucose unit). ^1H NMR (CDCl_3) ppm: δ 6.33-4.08 ppm (7H, m, glucosyl protons), δ 2.18-2.02 ppm (12H, m, acetyl protons), δ 2.18 ppm (1H, s, NH), δ 2.09 ppm (1H, s, NH), δ 2.06 ppm (1H, s, NH), δ 1.67 ppm (1H, s, NH), δ 1.25 ppm (1H, s, NH). Mass (m/z): 431 (M^+), 331, 271, 211, 169, 109. Anal. calcd. for $\text{C}_{16}\text{H}_{23}\text{O}_{10}\text{N}_4$, Required: C, 44.54; H, 7.42; N, 2.99; Found: C, 44.50; H, 7.39; N, 2.94%.

1-Hepta-O-acetyl- β -D-maltosyl-3-amidino carbamide (2c)

IR (KBr, cm^{-1}): ν 3502 (N-H), 2956 (Ali C-H), 1751 (C=O), 1602 (C=N), 1379 (C-N), 1244 (C-O), 1128, 1051, 991 and 941 (Characteristic of maltose unit). ^1H NMR (CDCl_3) ppm: δ 5.58-3.73 ppm (14H, m, maltosyl protons), δ 2.15-2.00 ppm (21H, m, acetyl protons), δ 4.74 ppm (1H, s, NH), δ 2.15 ppm (1H, s, NH), δ 2.10 ppm (1H, s, NH), δ 2.03 ppm (1H, s, NH), δ 2.00 ppm (1H, s, NH). Mass (m/z): 720 (M^+ , Protonated), 617, 559, 331, 271, 211, 169, 109. Anal. calcd. for $\text{C}_{28}\text{H}_{40}\text{O}_{10}\text{N}_4$, Required: C, 46.66; H, 5.57; N, 7.77; Found: C, 46.59; H, 5.49; N, 7.71%.

1-Hepta-O-benzoyl- β -D-lactosyl-3-amidino carbamide (2e)

IR (KBr, cm^{-1}): ν 3250 (N-H), 3062 (Ar C-H), 2962 (Ali C-H), 1732 (C=O), 1651 (C=N), 1487 (C=C), 1315 (C-N), 1271 (C-O), 1070, 1026, 1001 and 981 (Characteristic of lactosyl unit). ^1H NMR (CDCl_3) ppm: δ 8.20-7.18 ppm (35H, m, Ar-H), δ 6.14-3.70 ppm (14H, m, lactosyl protons), δ 3.77 ppm (1H, s, NH), δ 3.18 ppm (1H, s, NH), δ 1.68 ppm (1H, hump, NH), δ 1.25 ppm (2H, s, NH). Mass (m/z): 1154 (M^+), 1119, 1070, 1053, 949, 931, 579, 475. Anal. calcd. for $\text{C}_{63}\text{H}_{54}\text{O}_{10}\text{N}_4$, Required: C, 65.51; H, 4.67; N, 4.84; Found: C, 65.48; H, 4.65; N, 4.79%.

Antimicrobial activities

All the titled compounds were screened for both; antibacterial and antifungal activity using cup plate agar diffusion method^{12,13} by measuring the inhibition zone in mm. The compound were taken at a concentration of 1 mg/mL using dimethyl sulphoxide (DMSO) as solvent. The compounds were screened for antibacterial activity against *E. coli*, *S. aureus*, *P. vulgaris* and *K. pneumoniae* in nutrient agar medium. Amikacin (100 ug/mL) was used as a standard for antibacterial activity. The compounds were screened for antifungal activity against *P. species* and *A. niger* in potato dextrose agar and fluconazole (100 ug/mL) as a standard for antifungal activity. The results are presented in Table 2.

Table 2: Result of antimicrobial activity of *N*-glycosylated-3-amidino carbamides (2a-f)

Compd.	Antibacterial**			Antifungal**		
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. vulgaris</i>	<i>K. pneumoniae</i>	<i>P. species</i>	<i>A. niger</i>
2a	07	10	10	10	06	10
2b	06	12	15	07	07	06
2c	07	09	11	09	07	06
2d	10	12	17	12	08	07
2e	12	10	12	10	09	06
2f	10	13	15	12	07	08
Amikacin	09	13	12	11	-	-
Fluconazole	-	-	-	-	09	11

** zone of inhibition in mm (15 or less) resistance, (16-20mm) moderate and (more than 20 mm) sensitive. *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Proteus vulgaris* (*P. vulgaris*), *Salmonella typhi* (*S. typhi*) and *Klebsiella pneumonia* (*K. pneumonia*), *Penicilium species* (*P. species*) and *Aspergillus niger* (*A. niger*).

The result showed that among the tested some of these compounds exhibited interesting antibacterial activities. The compound **2e** exhibit most significant activity against *Escherichia coli*, **2b** and **2f** found active against *Staphylococcus aureus*, **2d** and **2f** exhibit most significant activity against *Proteus vulgaris*, **2d** and **2f** also exhibit most significant activity against *Klebsiella pneumonia*. All the other compounds exhibited low to moderate activity.

The result showed that among the tested some of these compounds exhibited interesting antifungal activities. The compound **2e** was effective towards *Penicilium notatum* and **2a** inhibited *Aspergillus niger*. While all other compounds showed low to moderate activities are tabulated in Table 2.

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