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# Synthesis and *In-Vitro* Biological Studies of 1-Hepta-*O*-Benzoyl-β-D-Lactosyl-3-Alkyl Carbamates

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## ABSTRACT

*Several 1-Hepta-O-benzoyl-β-D-lactosyl-3-alkyl carbamates have been synthesized by the interaction of Hepta-O-benzoyl-β-D-lactosyl isocyanate with various alcohols. The identities of these newly synthesised N-lactosyl carbamates have been established on the basis of usual chemical transformations and IR, $^1H$ NMR and Mass spectral studies. These compounds were screened for their antibacterial activity and antifungal activity against some selected pathogenic organisms to get potent bioactive molecule.*

**Keywords:** *N*-lactosyl isocyanate, alcohols, *N*-lactosyl carbamates, antimicrobial screening.

## INTRODUCTION

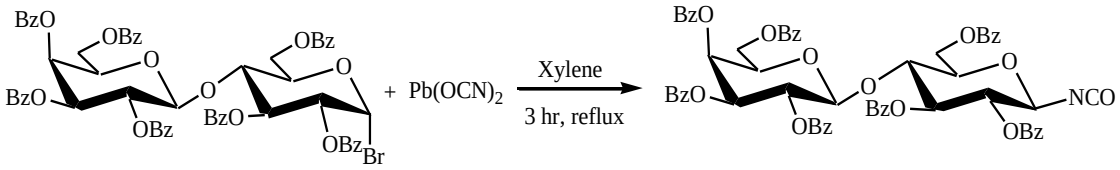
The area of sugar urea derivatives has received considerable attention in recent years because of the unique structural properties and activities that these compounds display. Glycosyl carbamates are important because of their stability to alkaline conditions [1] and some of them are reported to act as glycosyl donors [2,3]. In addition, glycosyl carbamates are studied as dopamine pro drugs [4,5] and surfactants [6]. Recently, carbamate linkages were explored for studying carbohydrate–protein interactions [7] and also for ligation [8]. *N*-lactosyl carbamates can be prepared by a reaction of corresponding *N*-lactosyl isocyanate [9] and alcohols. This gives easy access but in moderate to good yields depending on the type of substituent. Thus, methods that enable easy synthesis of *N*-lactosyl carbamates without the use of toxic isocyanates from stable lactosyl donors are invaluable. The foregoing discussion clearly highlights the merit of synthesis of *N*-lactosyl carbamate as a glycosyl donor.

## MATERIALS AND METHODS

All the chemicals and solvents were obtained from commercial and purified using standard procedure wherever required. Melting points were taken by the open capillary method and were uncorrected. The reactions were monitored by thin layer chromatography on silica gel G plates (Merck silica- 60 $F_{258}$). Optical rotations $[\alpha]_D^{31}$ were measured on the Equip-Tronics EQ-800 Digital Polarimeter at 31$^oC$ in $CHCl_3$. The structures of all the newly synthesized compounds were confirmed by IR Spectra which recorded on Perkin-Elmer spectrum RXI FTIR Spectrometer (Range: 4000-450 $cm^{-1}$). $^1H$ NMR was obtained on Bruker DRX-300 NMR spectrometer operating at 300 MHz Samples were prepared in $CDCl_3$

with TMS as an internal reference. Mass spectra were obtained on Thermo Finnegan LCQ Advantage max ion trap mass spectrometer.

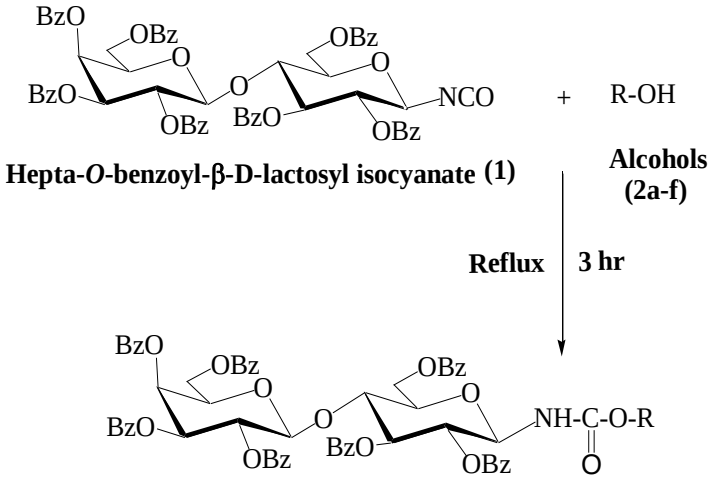
**Synthesis of Hepta-*O*-benzoyl-β-D-lactosyl isocyanate (1):** To the suspension of Hepta-*O*-benzoyl-α-D-lactosyl bromide (0.039 M, 15 g) in sodium dried 60 mL xylene was added lead cyanate (0.039 M, 4.5 g). The mixture was refluxed gently for 3 h. The xylene filtrate was then treated with petroleum ether (60-80$^{o}$C) to afford a solid. It was purified by dissolving it in minimum quantity of chloroform and reprecipitating with petroleum ether **(Scheme-1).**




**Hepta-*O*-benzoyl-α-D-lactosyl bromide** **Hepta-*O*-benzoyl-β-D-lactosyl isocyanate (1)**

**Scheme-1**

**Synthesis of 1-Hepta-*O*-benzoyl-β-D-lactosyl-3-methyl carbamates (3a):** 1-Hepta-*O*-benzoyl-β-D-lactosyl isocyanate (0.001 M, 1.0 g) was added to 20 mL dry methanol and the reaction mixture was refluxed over boiling water bath for 3 h. It was then allowed to cool and poured in ice cold water with vigorous stirring. A whiter granular solid was separated out. It was crystallized from ethanol **(Scheme-2).** This reaction of 1-Hepta-*O*-benzoyl-β-D-lactosyl isocyanate was also extended to several other alcohols and the corresponding 1-Hepta-*O*-benzoyl-β-D-lactosyl-3-alkyl carbamates **(3b-f)** have been isolated.




**Hepta-*O*-benzoyl-β-D-lactosyl isocyanate (1)**

**1-Hepta-*O*-benzoyl-β-D-lactosyl-3-alkyl carbamates (3a-f)**

**Scheme 2**

Where, OBz = $OCOC_6H_5$, R = a) methyl, b) ethyl, c) *n*-propyl, d) iso-propyl, e) *n*-butyl, f) iso-butyl.

## RESULTS AND DISCUSSION

We report here the synthesis of various 1-Hepta-*O*-benzoyl-β-D-lactosyl-3-alkyl carbamates **(3a-f)** by inteaction of Hepta-*O*-benzoyl-β-D-lactosyl isocyanate (**1**) and various alkyl alcohols **(2)**. All the products were crystallized from ethanol before recording the physical data (Table-1). The purity of compound was checked by TLC. The spectral analysis [10-12] IR, $^{1}$H NMR and Mass spectra of the product were observed. Optical rotation of the product was also recorded.

**Table 1:** Physical characterization of 1-Hepta-*O*-benzoyl-β-D-lactosyl-3-alkyl carbamates (3a-f)

| Sr. No. | Product (3a-f) | Reactants (2a-f) | Yield (%) | m. p. ($^oC$) | $[\alpha]_D^{31}$ (c, $CHCl_3$) | Elemental Analysis Found (Required) | $R_f$ (3:2, $CHCl_3$:EtOAc) |
|---|---|---|---|---|---|---|---|
| | | | | | | N | |
| 1 | 3a | -3-methyl- | 73.92 | 125 | +61.54$^o$ (0.96) | 1.20 (1.24) | 0.59 |
| 2 | 3b | -3-ethyl- | 46.30 | 122 | +49.71$^o$ (0.96) | 1.19 (1.22) | 0.61 |
| 3 | 3c | -3-*n*-propyl- | 62.26 | 115 | +82.01$^o$ (0.98) | 1.15 (1.21) | 0.72 |
| 4 | 3d | -3-iso-propyl- | 78.11 | 118 | +76.52$^o$ (0.97) | 1.17 (1.21) | 0.69 |
| 5 | 3e | -3-*n*-butyl- | 59.25 | 110 | +59.76$^o$ (0.96) | 1.16 (1.19) | 0.5 |
| 6 | 3f | -3-iso-butyl- | 67.21 | 129 | +87.22$^o$ (0.99) | 1.14 (1.19) | 0.56 |

Satisfactory C and H analysis were found in all cases.

**Spectral Data**

**3a: IR (KBr, $cm^{-1}$) :** υ, 3443 (-NH), 3010 (Ar C-H), 2965 (Ali C-H), 1730 (C=O), 1269(C-N), 1175 (C-O), 1069 & 1026 $cm^{-1}$ (Characteristic of Lactose); **$^1$H NMR ($CDCl_3$, ppm):** δ 7.98-7.10 (m, 35H, Ar-H), 5.431 (s, 1H, NH), 5.87-4.26 (m, 14H, lactosyl protons), 1.60 (s, 3H, methyl protons); **Mass (m/z):** 1143 ($M^+$), 1053, 947, 931, 917, 135, 121, 105. (Anal. Calcd. for $C_{63}H_{53}O_{19}N$, Requires: C, 67.08; H, 4.70; N, 1.24; Found: C, 66.92; H, 4.62; N, 1.20%.).

**3b: IR (KBr, $cm^{-1}$) :** υ, 3321 (-NH), 3015 (Ar C-H), 2970 (Ali C-H), 1730 (C=O), 1268 (C-N), 1175 (C-O), 1069 & 1026 $cm^{-1}$ (Characteristic of Lactose); **$^1$H NMR ($CDCl_3$, ppm):** δ 7.82-7.07 (m, 35H, Ar-H), 5.638 (s, 1H, NH), 5.74-3.88 (m, 14H, lactosyl protons), 1.25-1.21 (m, 5H, ethyl protons); **Mass (m/z):** 1157 ($M^+$), 1053, 975, 135, 123. (Anal. Calcd. for $C_{64}H_{55}O_{19}N$, Requires: C, 67.30; H, 4.82; N, 1.22; Found: C, 67.24; H, 4.76; N, 1.19%.).

**3e: IR (KBr, $cm^{-1}$) :** υ, 3378 (-NH), 3010 (Ar C-H), 2962 (Ali C-H), 1729 (C=O), 1269 (C-N), 1175 (C-O), 1069 & 1026 $cm^{-1}$ (Characteristic of Lactose); **$^1$H NMR ($CDCl_3$, ppm):** δ 7.43-7.10 (m, 35H, Ar-H), 5.445 (s, 1H, NH), 6.14-5.22 (m, 14H, lactosyl protons), 1.88-1.42 (m, 6H, methylene protons), 1.65 (t, 3H, methyl protons); **Mass (m/z):** 1185 ($M^+$), 1053, 931, 135, 121, 105. (Anal. Calcd. for $C_{66}H_{59}O_{19}N$, Requires: C, 67.75; H, 5.04; N, 1.19; Found: C, 67.69; H, 4.92; N, 1.16%.).

**Antimicrobial Studies:** All the compounds have been screened for both antibacterial and antifungal activities using cup plate agar diffusion method [13-15] by measuring the inhibition zone in mm. The compounds were taken at a concentration of 1 mg/ml using dimethyl sulphoxide as solvent. Amikacin (100µg $mL^{-1}$) was used as a standard for antibacterial and antifungal activity and Fluconazole (100µg $mL^{-1}$) as a standard for antifungal activity. The compounds were screened for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella typhi*, *Klebsiella Pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis* in nutrient agar medium and for antifungal activity against *Candida albicancs* and *Aspergillus niger* in potato dextrose agar medium. These sterilized agar media were poured into Petri dishes and allowed to solidify on the surface of the media, microbial suspensions

were spread with the help of sterilized triangular loop. A stainless steel cylinder of 8 mm diameter (pre-sterilized) was used to bore the cavities. 0.1 mL portions of the test compounds in solvent were added into these wells. The drug solution was allowed to diffuse for about an hour into the medium. The plates were incubated at 37°C for 24 h and 30°C for 48 h for antibacterial and antifungal activities respectively. The zone of inhibition observed around the cups after respective incubation was measured. The results are presented in table 2. It was observed that some of these compounds exhibit interesting microbial activities. 3b, 3d and 3f exhibit most significant activity against all *E.coli, S. typhi, P. aeruginosa, C. albicance* and *A. niger*. All other compounds exhibited low to moderate activity (Table 2).

**Table 2:** Results of antimicrobial activity tests of the synthesized compounds (3a-f)

| Compd. | Antibacterial** | | | | | | | Antifungal** | |
|---|---|---|---|---|---|---|---|---|---|
| | *E. Coli* | *S. aureus* | *P. vulgaris* | *S. typhi* | *K. Pneumoniae* | *P. aeruginosa* | *B. Subtilis* | *C. albicance* | *A. niger* |
| **3a** | 18 | 16 | 19 | 18 | 17 | 21 | 19 | 21 | 20 |
| **3b** | 22 | 19 | 21 | 22 | 19 | 23 | 18 | 23 | 22 |
| **3c** | 19 | 17 | 20 | 21 | 15 | 12 | 13 | 19 | 21 |
| **3d** | 17 | 20 | 18 | 20 | 21 | 20 | 22 | 22 | 23 |
| **3e** | 16 | 17 | 17 | 18 | 13 | 16 | 12 | 18 | 20 |
| **3f** | 19 | 21 | 20 | 23 | 17 | 18 | 21 | 22 | 24 |
| **Amikacin** | 25 | 22 | 25 | 28 | 23 | 26 | 24 | - | |
| **Fluconazole** | - | - | - | - | - | - | - | 28 | 27 |

**zone of inhibition in mm (15 or less) resistance, (16-20 mm) moderate and (more than 20mm) sensitive.
*Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Proteus vulgaris (P. vulgaris), Salmonella typhi (S. typhi), Klebsialla Pneumoniae (K. Pneumoniae), Pseudomonas auriginosa (P. auriginosa), Bacillus subtilis (B. subtlis), Candida albicancs (C.albicancs) and Aspergillus niger (A. niger).*

## APPLICATIONS

The synthesized *N*-lactosyl carbamates showed significant antimicrobial activities and lead for the development of new drugs due to the nature of presence of oxygen and nitrogen present in it. The applicability of synthesized compounds is also supported by the various references quoted in the script.

## CONCLUSIONS

The new *N*-lactosyl carbamates 3b, 3d and 3f exhibits promising antibacterial and antifungal activities against the organism tested. The method adopted in this investigation is simple efficient inexpensive and is useful in synthesizing pharmacologically important molecules.

## ACKNOWLEDGEMENT

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