

Per-*O*-acetyl Lactosyl Isothiourea Derivatives: Synthesis and Antimicrobial Activities

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Abstract: Glycosyl (iso) thioureas being an important intermediate in the synthesis of thioureido carbohydrates herein we reported the synthesis of per-*O*-acetyl lactosyl aryl isodithiobiuret derivatives. These compounds were synthesized by nucleophilic addition of per-*O*-acetyl lactosyl aryl isothiourea to tolyl isothiocyanate. Structures of these compounds were confirmed on the basis of IR, HNMR, Mass spectra and elemental analysis. These compounds were also screened for their *in vitro* antimicrobial activities against bacteria *S. aureus*, *E. coli*, *P. vulgaris*, *P. aeruginosa* and fungi *A. niger*, *Penicillium*.

Keywords: Lactosyl isothioureas, isodithiobiurets, Synthesis, Antibacterial, Antifungal activity

INTRODUCTION

A number of thiourea derivatives have been reported to exhibit marked antibacterial¹, herbicidal and fungicidal² activities. Sugar thioureas³ has synthetic applications in neoglycoconjugate synthetic strategies⁴, including neoglycoproteins⁵, glycodendrimers⁶, glycoclusters⁷ and pseudooligosaccharides⁸.

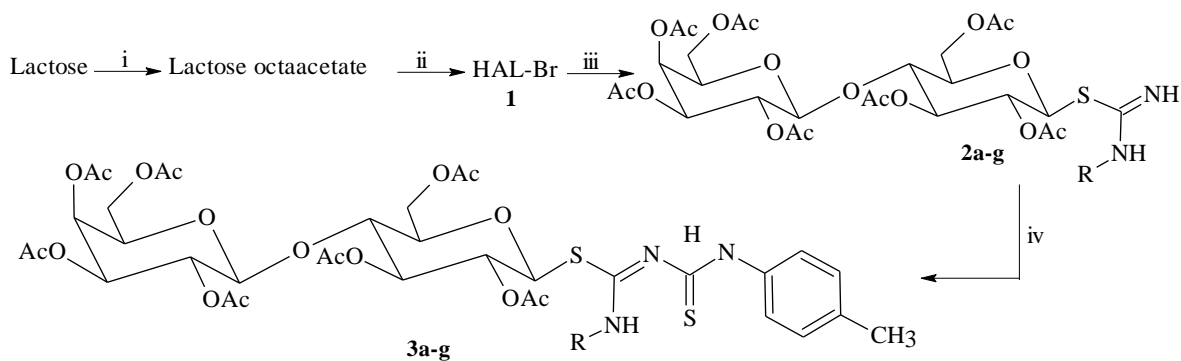
Thiobiurets (mono and di) are also important derivatives of (thio) urea which may increase the biological activity of (thio)ureas. 1-Allyl-2-thiobiuret was shown to regulate the growth of germinating wheat and cucumber seeds⁹. Oliver and coworkers¹⁰ reported chemosterilising action of some dithiobiuret derivatives in male house flies. The mono and dithiobiuret derivative are effective fungicides, bactericides, herbicides and also have demonstrated effective growth regulating activity¹¹. Some thiobiuret derivatives also showed analgesic¹², anticonvulsant and hypnotic activity¹³. Glycosyl urea and their biuret derivatives are reported as potential glycoenzyme inhibitors¹⁴. Mangte and Deshmukh¹⁵ reported antibacterial and antifungal activities of per-*O*-acetylated lactosyl iso(di)thiobiurets.

In view of the advantage conferred by glycosyl thiourea synthesis that allows rapid, convenient access to a wide array of thioureido carbohydrates¹⁶, together with the notable biological activities of nucleoside analogues, a great deal of work has focused on the development of novel glycosyl thiourea derivatives. However, there are little reports on the synthesis and bioactivity of lactosyl thiourea derivatives. In this paper, a very efficient synthetic route to novel lactosyl isothiurea derivatives and their antimicrobial activities are reported.

RESULTS AND DISCUSSION

The key intermediate per-*O*-acetyl lactosyl arylisothiureas¹⁷ (**2a-g**) were synthesised in three steps. First, the acetylation of lactose with acetic anhydride and catalytic amount of perchloric acid, it was then brominated by P/Br₂/AcOH. This per-*O*-acetyl lactosyl bromide underwent nucleophilic substitution reaction with various aryl thiureas in propan-2-ol to yield (**2a-g**) **Scheme 1**. IR spectrum of these compounds possessed a characteristic band at 3348–3458 cm⁻¹ (N–H) and at 1053, 905 cm⁻¹ for lactosyl protons. FAB–MS of (**2a-g**) showed protonated molecular ion peaks at *m/z* 771, 805, 785 for phenyl, chlorophenyl and tolyl substituents respectively with other characteristic fragment peaks at 619, 559, 331, 169 and 109. The ¹HNMR spectrum of these compounds distinctly displayed signals due to aromatic protons at δ 7.6–6.8 ppm, protons of lactosyl ring at δ 5.3–3.4 ppm and acetyl protons at δ 2.1–1.9 ppm.

Scheme 1. Synthesis of protected lactosyl thiourea derivatives **3a-g**: (i) Ac₂O, HClO₄, 5–10°C; (ii) AcOH, P, Br₂; (iii) R-NH-C(S)-NH₂, propan-2-ol, 70°C; (iv) *p*-tolyl isothiocyanate, benzene, reflux.



R = a) phenyl, b) *o*-Cl-phenyl, c) *m*-Cl-phenyl, d) *p*-Cl-phenyl, e) *o*-tolyl, f) *m*-tolyl, g) *p*-tolyl

Per-*O*-acetyl lactosyl aryl isothiourea (**2a-g**) was reacted with *p*-tolyl isothiocyanate in boiling benzene to give title compounds (**3a-g**). The syrupy mass obtained on solvent evaporation was triturated with petroleum ether. The off white solid obtained was crystallised with EtOH-water. The yields of **3** vary from 67–85%. The melting points, yields, R_f values, specific rotations and elemental analysis of synthesised compounds **3** are tabulated in Table 1. The structures of the compounds **3a-g** were confirmed by elemental analysis and IR, HNMR, Mass spectra. The IR spectra¹⁸⁻¹⁹ of the compounds showed strong characteristic absorption of lactosyl protons in the range of 900–910, 1000–1100 cm^{-1} for stretching vibration of C–H bond. The stretching band for acetyl C=O has appeared in the region 1749–1750 cm^{-1} . The absorption band for N–H was appeared at 3455–3468 cm^{-1} .

¹HNMR spectrum¹⁸⁻²⁰ of the products shows characteristic of lactosyl protons at δ 5.6–3.7 ppm, resonance signals for aromatic protons at δ 7.5–7.1 ppm and acetyl protons were appeared at δ 2.1–1.9 ppm.

Mass spectra²¹⁻²² exhibited molecular ion peak along with characteristic fragments of lactose unit at m/z , 619, 559, 331, 169 and 109. Mass fragmentation was depicted in **Figure 1** with its characteristic fragments.

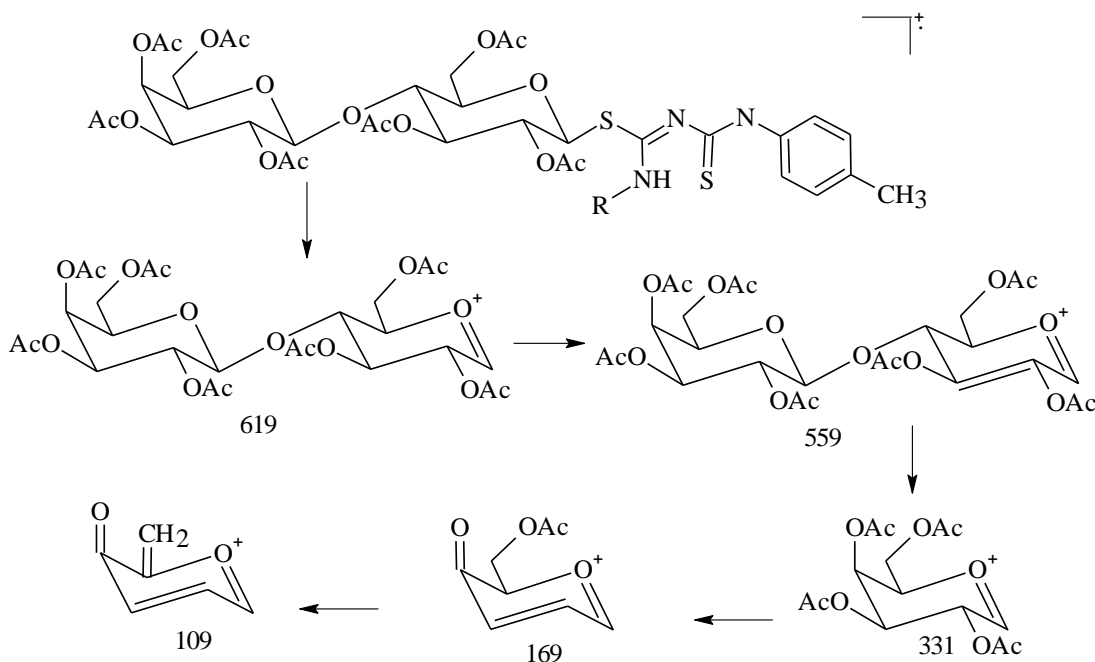


Figure 1 Mass fragmentation of Lactosyl isothiourea derivatives **3a-g**

Antibacterial activity These compounds were screened for their *in vitro* antimicrobial activities against gram positive bacteria viz. *S. aureus* and gram negative bacteria viz. *E. coli*, *P. vulgaris*, *P. aeruginosa*. Amikacin was used as a positive control for bacteria. Compounds **3a**, **3d** exhibited comparable inhibitory activity as compared to the control against *S. aureus* whereas others showed moderate to less activity. **3c** and **3f** showed inhibition as good as control drug against *P. aeruginosa* and **3a** was inactive. Other compounds showed moderate to less activity while some were inactive Table 2.

Antifungal Activity The synthesized compounds have also been evaluated for their antifungal activity against two representative fungi viz., *Penicillium* and *A. niger* by cup plate agar diffusion method using Fluconazole as standard drug. Compounds **3g** exhibited comparable activity against *A. niger* and others were moderately active against fungi. Although the rest of the compounds showed varying degree of inhibition, none were as effective as Fluconazole Table 2.

Table 1 Physical characterisation data of Lactosyl isothiurea derivatives **3a–g**

| Entry | M. p. °C | % Yield | [α] _D ³¹ (c, 1.0 in CHCl ₃) | R _f (CHCl ₃ : EtOAc,3:1) | Found (Calculated) | |
|-----------|-------------|------------|---|---|--------------------|------------|
| | | | | | N | S |
| 3a | 160–163 | 78 | 80.00 | 0.64 | 4.28(4.57) | 6.78(6.96) |
| 3b | 149–150 | 69 | 142.8 | 0.82 | 4.21(4.40) | 6.52(6.71) |
| 3c | 128–130 | 67 | –30.00 | 0.72 | 4.30(4.40) | 6.60(6.71) |
| 3d | 132–133 | 77 | 70.00 | 0.56 | 4.25(4.40) | 6.49(6.71) |
| 3e | 121–123 | 86 | 99.16 | 0.85 | 4.28(4.50) | 6.64(6.85) |
| 3f | 157–159 | 81 | 122.4 | 0.79 | 4.34(4.50) | 6.58(6.85) |
| 3g | 153–154 | 85 | –40.00 | 0.80 | 4.39(4.50) | 6.72(6.85) |

EXPERIMENTAL

Melting points were recorded on electro thermal melting point apparatus without correction. Specific rotations [α]_D were measured on an Equip–Tronics digital polarimeter model no. EQ 800 at 31°C in CHCl₃. IR spectra were recorded on a Perkin – Elmer spectrum RXI (4000–450cm⁻¹) FTIR spectrometer. ¹HNMR spectrum were obtained on a Bruker DRX-300 (300 MHz FT NMR) NMR spectrometer in CDCl₃ solution with TMS as an internal reference. The mass spectra were recorded on a Jeol SX–102 mass spectrometer.

The antimicrobial activity of synthesized compounds were tested *in vitro* against bacteria *S. aureus*, *E. coli*, *P. vulgaris*, *P. aeruginosa* and fungi *A. niger*, *Penicillium* by cup plate agar diffusion method²³.

Table 2 Antimicrobial activities of Lactosyl isothiurea derivatives **3a–g**

| Entry | Bacteria | | | | Fungi | |
|--------------------|----------------|--------------------|------------------|----------------------|----------------|--------------------|
| | <i>E. coli</i> | <i>P. vulgaris</i> | <i>S. aureus</i> | <i>P. aeruginosa</i> | <i>A.niger</i> | <i>Penicillium</i> |
| 3a | ++ | +++ | ++++ | – | ++ | +++ |
| 3b | +++ | ++ | +++ | ++ | +++ | ++ |
| 3c | ++ | +++ | ++ | ++++ | – | ++ |
| 3d | +++ | ++ | ++++ | +++ | ++ | – |
| 3e | +++ | – | +++ | ++ | ++ | +++ |
| 3f | ++ | ++ | +++ | ++++ | +++ | ++ |
| 3g | +++ | +++ | – | ++ | ++++ | – |
| Amikacin | ++++ | ++++ | ++++ | ++++ | --- | --- |
| Fluconazole | --- | --- | --- | --- | ++++ | ++++ |

++++ Strongly active (Above 20mm), +++ moderately active (15mm–20mm), ++ weakly active (8mm–14mm), – inactive (below 8mm)

The compounds were taken at a concentration of 1mg/ml and compared with Amikacin and Fluconazole as a positive control for different strains of bacteria and fungi for antibacterial and antifungal activity respectively (Table2).

Reaction of *p*-tolyl isothiocyanate with per-*O*-acetyl lactosyl aryl isothiureas (2a–g) A solution of Hepta-*O*-acetyl lactosyl aryl isothiureas (**2a–g**, 5 mmol) and *p*-tolyl isothiocyanate (5mmol) in benzene (20ml) was refluxed for 8–9 h. Progress of reaction was monitored by TLC. Then solvent was distilled off, sticky mass obtained was triturated with petroleum ether (60–80°). The granular solid obtained was crystallized from ethanol–water.

***S*-hepta-*O*-acetyl lactosyl-1-phenyl-5-*p*-tolyl-2,4-isodithiobiuret (3a)** ¹H-NMR (CDCl₃) δ: 7.44–7.12 (m, 9H, Ar-H), 5.11–3.77 (m, 14H, lactose unit), 2.35 (s, 3H, CH₃), 52.18–1.96 (m, 21H, 7COCH₃). IR (KBr)cm⁻¹: 3468(N-H),1752(C=O), 1600(C=N), 1441(C-N), 1229(C-O), 1049 & 906 (Lactose unit). FAB-MS *m/z*: 919 (M⁺), 619, 559, 331, 169, 109.

3b ¹H-NMR (CDCl₃) δ: 7.53–7.28 (m, 8H, Ar-H), 5.18–3.64 (m, 14H, lactose unit), 2.34 (s, 3H, CH₃), 2.18–1.95 (m, 21H, 7COCH₃). IR (KBr)cm⁻¹: 3467(N-H), 1750(C=O), 1624(C=N), 1372(C-N), 1229(C-O), 1044 & 905 (Lactose unit). FAB-MS *m/z*: 953 (M⁺), 619, 559, 331, 169, 109.

3e ¹H-NMR (CDCl₃) δ: 7.26–7.11 (m, 8H, Ar-H), 5.11–3.77 (m, 14H, lactose unit), 2.36 (s, 3H, CH₃), 2.15–1.96 (m, 21H, 7COCH₃). IR (KBr)cm⁻¹: 3455(N-H), 1751(C=O), 1624(C=N), 1431(C-N), 1229(C-O), 1052 & 906 (Lactose unit). FAB-MS *m/z*: 933 (M⁺), 619, 559, 331, 169, 109.

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