Asian Journal of Chemistry; Vol. 30, No. 10 (2018), 2310-2316

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2018.21472

Synthesis and Schematic Mechanism of 3-Phenylamino-4-phenyl-5-tetra-O-acetyl-β-D-glucopyranosylimino-1,2,4-dithiazolidines and Its De-acetylated Nucleoside

ARUNA HARDAS[1] and PRITI TAYADE (GOSAVI)[2,*]

[1]Department of Chemistry, S.F.S. College, Seminary Hills, Nagpur-440 006, India
[2]Innovation Hub, Raman Science Centre, Nagpur-440 018, India

*Corresponding author: E-mail: gosavipriti@gmail.com

Received: 24 May 2018; *Accepted*: 3 July 2018; *Published online*: 31 August 2018; AJC-19064

A systematic synthesis of 3-phenylimino-4-phenyl-5-tetra-O-acetyl-β-D-glucopyranosylimino-1,2,4-dithiazolidine (acetylated glucopyranosylimino nucleoside) from glucose as starting material. The steps included acetylating glucose to glucose penta-acetate (II). Step 2 involves the bromination of glucose penta-acetate (II) to 2,3,4,6 tetra-O-acetyl-α-D-glucopyranosyl bromide (III). In step 3 compound (III) reacted with lead thiocyanate to give 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (IV). In the step 4 N-phenyl-3-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (V) was prepared. In the last step on refluxing compound (V) with N-phenyl S-chloro isothiocarbamoyl chloride to yield acetylated glucopyranosyl nucleoside. Furthermore de-acetylating of acetylated glucopyranosyl nucleoside was carried out to obtain 3-phenylimino-4-phenyl-5-β-D-gluopyranosyl imino 1,2,4-dithiazolidine (de-acetylated glucopyranosylimino nucleoside). The synthesized acetylated glucopyranosylimino nucleoside and deacetylated glucopyranosylimono nucleoside were structurally confirmed by elemental analysis, ultraviolet spectral analysis, infrared spectroscopy, nuclear magnetic resonance spectroscopy and mass spectroscopy.

Keywords: Glucopyranosylimino nucleoside, Mass spectroscopy, IR spectroscopy.

INTRODUCTION

Broadly speaking nucleosides are glycosylamine which can be converted to nucleotides *i.e*. having phosphate group [1]. The major constituents of nucleoside are nitrogenous base and ribose or de-oxyribose. The base in nucleoside is connected to ribose or de-oxyribose through β-glycosidic linkage [2]. Some naturally occurring nucleosides are cytidine, uridine, adenosine, guanosine, thymidine and inosine *etc*. [3,4]. However, there is need of synthetic nucleoside analogues in order to arrest viral growth, cancer cell growth and for some other related medicinal issues. The benefit of using synthetic nucleoside is that, these compounds after administration get activated in cells and get converted into its active nucleotides. Usually nucleotides are charged and don't cross the cell membrane easily, which can be easily done by using nucleoside [5]. These synthetic nucleosides also work as sugar backbone and does not hydrolyse as easy as RNA, hence, provides the stability in such cases [6].

Various synthetic nucleosides such as 2-tetra-*O*-benzoyl-β-D-glucopyranosyl-4-phenyl-5-arylimino-1,3,4-thiadiazolidines [7], substituted 2-amino-(6-substituted)benzo-1,3-thiazoles [8] and benzoylated glucopyranosyl disubstituted thiocarbamide [9], *etc*. have been reported. However, open access literature is lacking in detail synthesis route.

Other than this reported route, synthetic nucleosides can be prepared by interaction of S-chloro-N-phenyl isothiocarbamoyl chloride and 1-phenyl-3-tetra-O-acetyl-β-D-glucopyranosyl thiocarbamides. Further de-acetylating process results into the corresponding hydroxylated nucleoside.

Present paper describes the stepwise synthesis of 3-phenylimino-4-phenyl-5-tetra-O-acetyl-β-D-glucopyranosylimino-1,3,4-dithiazolidine from glucose as starting material. Furthermore, de-acetylating process result into actual required glucospyranosylimino nucleoside. 3-Phenylimino-4-phenyl-5-tetra-O-acetyl-β-D-glucopyranosylimino-1,3,4-dithiazolidine is broadly referred as acetylated glucopyranosylimino nucleoside. Similarly, 3-phenylimino-4-phenyl-5-tetra-

hydroxyl-β-D-glucopyranosylimino-1,3,4-dithiazolidine is broadly referred as de-acetylated glucopyranosylimino nucleoside.

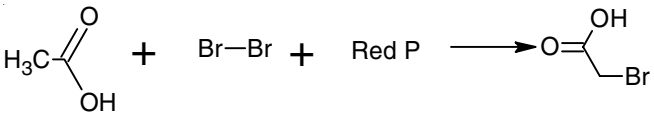
The work also elucidates the mechanistic scheme for both acetylated and de-acetylated glucopyranosylimino nucleosides by using mass spectrometry.

EXPERIMENTAL

All the precursor chemicals namely, glucose, ethanol, methanol, acetic anhydride, acetic acid, benzene, chloroform, xylene, carbon tetrachloride, red phosphorus were obtained from make E-Merck.

Synthesis of acetylated nucleoside *i.e.* 3-phenylimino-4-phenyl-5-tetra-oacetyl β-D-glucopyaranosyl imino-1,2,4-dithiazolidine was carried out from glucose as starting material. Furthermore acetylated nucleoside is de-acetylated to the actual nucleoside.

Synthesis of brominating reagent: The brominating reagent was prepared by using Hell-Volhard-Zelinsky process. In the particular process, 30 mL of glacial acetic acid was taken in conical flask and 3 g of red phosphorous was added. To this mixture 7 mL of molecular bromine was added gradually with constant shaking and cooling. The resultant mixture was allowed to stand for 15 min at room temperature [10,11]. The solution was then filtered through cotton filter in order to remove suspended impurities (**Scheme-I**).



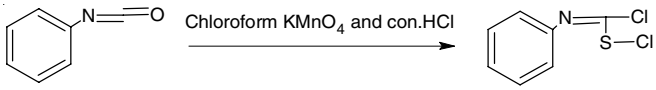
Scheme-I: Synthesis of reagent-I

Synthesis of lead isothiocyanate: Lead nitrate (33 g) solution was mixed with 15 g of ammonium thiocyanate. The white coloured granular lead thiocyanate was obtained. The granules were filtered and washed with distilled water and dried in an oven at 50 °C [12] (**Scheme-II**).

$$Pb(NO_3)_2 + 2(NH_4SCN) \longrightarrow Pb(SCN)_2 + 2NH_4NO_3$$

Scheme-II: Synthesis of reagent-II

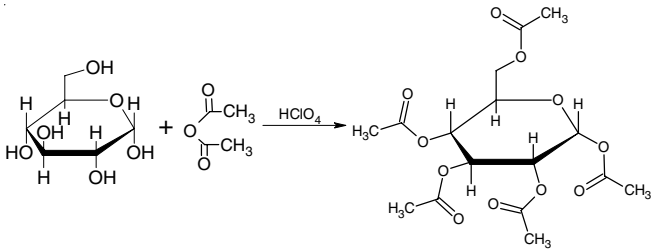
Synthesis of N-pheyl-S-chloro isothiocarbamoyl chloride: A solution of 7 mL phenyl isocyanate in 15 mL of chloroform was prepared. Chlorine gas generated from 3.2 g of potassium permangante and 20 conc. HCl was bubbled while maintaining temperature below 10 °C [13]. After addition of chlorine the reaction mixture turned to yellow colour. This yellow reaction mixture was diluted with 40 mL dry petroleum ether. The solvent was removed by dry distillation under vacuum. The whole process of distillation is repeated till we obtained pale yellow oil of N-phenyl-S-chloro isothiocarbamoyl chloride (**Scheme-III**) [14].



Scheme-III: Synthesis of reagent-III

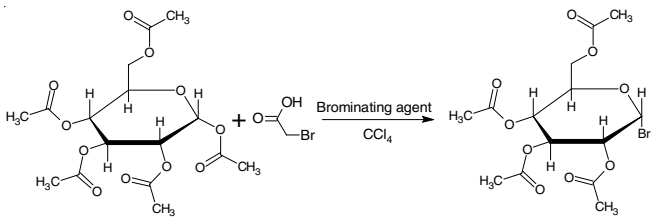
Synthesis of acetylated nucleoside from glucose

Preparation of glucose pentaacetate: The synthesis method opted for synthesis of glucose pentaacetate is reported by Huang *et al*. [15]. In the particular process 40 mL of an ice cold acetic anhydride was added to 0.25 mL perchloric acid. The resultant pale yellow coloured solution was added gradually to 10 g of D glucose with constant shaking. The reaction mixture was kept without disturbing for 15 min at room temperature. The solution was precipitated out in ice cold water. The precipitate is of glucose pentaacetate. Glucose pentaacetate was crystallized in methanol. Presence of glucose pentaacetate was confirmed by observing melting point 110 °C (**Scheme-IV**).



Scheme-IV: Reaction step-I

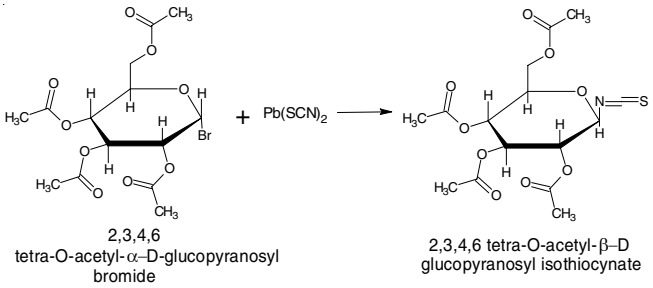
Synthesis of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide: 21.6 g of glucose pentaacetate was added to the brominating reagent. The resultant solution was kept undisturbed at room temperature for 2 h. The reaction mixture was mixed with 30 mL of carbon tetrachloride with vigorous shaking for 15 min. The resultant mixture was poured in to the ice-cold water. The carbon tetrachloride is separated out from product. The product was washed several times with sodium bicarbonate to remove excess of glacial acetic acid. Similarly, product was also washed by sodium meta-bisulphide to remove excess of bromide. Finally product was washed with water several times. The layer of carbon tetrachloride was then dried over anhydrous calcium chloride. The solvent was removed through vacuum distillation to obtain the solid tetra-o-acetyl-α-D-glucopyranosyl bromide. The product is crystallized from petroleum ether and confirmed by melting point as 88 °C [16] (**Scheme-V**).



Scheme-V: Reaction step-II

Synthesis of 2,3,4,6-tetra-o-acetyl β-D-glucopyranosyl-isothiocyanate: A solution of 20 g of tetra-O-acetyl-α-D-glucopyranosyl bromide in 150 mL xylene was prepared. To this solution 16 g of lead isothiocyanate was added. The reaction mixture was refluxed for 3 h with frequent shaking. The solution was allowed to cool and separated out by filtration from lead bromide [9,17]. The xylene filtrate was added to

petroleum ether result into solid product with yield of 14 g. The product was purified by dissolving it in minimum quantity of ethanol and re-precipitated by water. The product was confirmed by melting point (112-114 °C) (**Scheme-VI**).



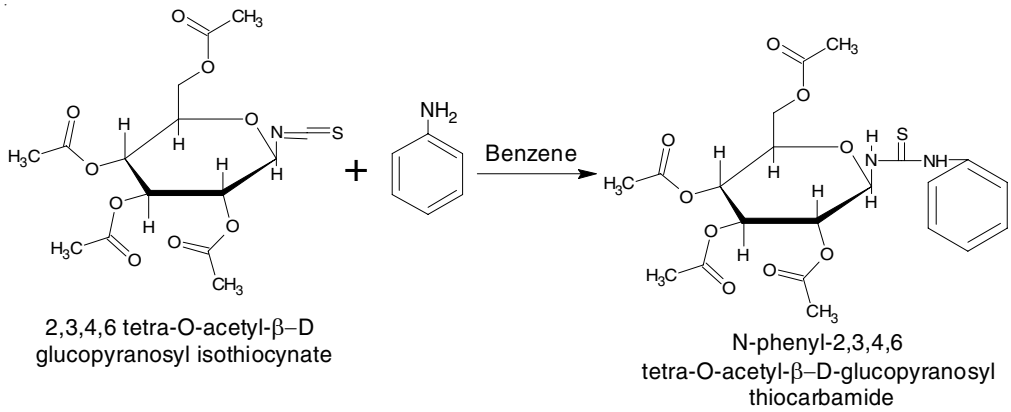
Scheme-VI: Reaction step-III

Synthesis of N-phenyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl thiocarbamide: 0.02 M *i.e.* 7.8 g of 2,3,4,6-tetra-O-acetyl-β-D-glucopyronosyl isothiocyanate was dissolved in 50 mL benzene. In another flask solution of 1.8 g of aniline in 10 g of benzene was prepared. Both the solutions were mixed and allowed to reflux over boiling water bath for 3 h. After refluxing with said conditions benzene was removed by distillation. A sticky mass was obtained as residue, which was triturated several times with petroleum ether. A granular solid with yield of 6.5 g was obtained. The product was crystallized from aq. ethanol (m.p. 162 °C) (**Scheme-VII**).

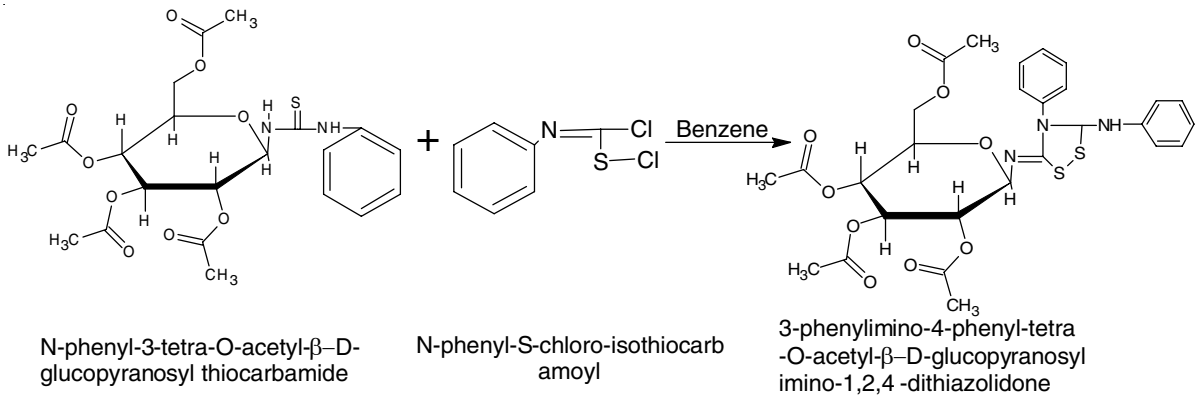
Synthesis of 3-phenylimino-4-phenyl-5-tetra-O-acetyl-β-D-glucupyranosyl imino-1,2,4-dithizolidine: A suspension by adding 9.6 g of powdered N-phenyl-2,3,4,6-tetra-O-acetyl-β-D-glucupyranosyl thiocarbamide into 40 mL benzene was prepared. To another flask, solution of 4.12 g of N-phenyl-S-chloro isothiocarbamoyl chloride was added to 20 mL benzene to form solution. The prepared suspension and solution were mixed and refluxed over boiling water bath for 4 h. While reflux brisk reaction takes place with the evolution of HCl and gradual conversion of solid into solution. After refluxing the solution was filtered to remove suspended particle. Afterwards solvent was distilled off. An oily residue was obtained, which was triturated with petroleum ether followed by ethanol. A pale yellow solid was obtained which was crystallized in aqueous ethanol (m.p. 199 °C) (**Scheme-VIII**).

De-acetylating 3-phenylimino-4-phenyl-5-tetra-o-acetyl-β-D-glucopyranosyl imino-1,2,4-dithiazolidine: 2 g of 3-phenylimino-4-phenyl-5-tetra-O-acetyl-β-D-glucopyranoyl imino-1,2,4-dithiazolidine was suspended in 35 mL pure methanol. To this suspension 4 mL of liquor ammonia was added. The reaction mixture was kept in an ice bath and stirred magnetically for 6 h. Gradually solid dissolved to form solution. The reaction solution was allowed to left overnight. The solvent residue was removed by distillation. The obtained product was allowed to treat with methanol to obtain final product [18]. The product was crystallized by using ethanol. The yield of product was 1.2 g *i.e.* 82 % (**Scheme-IX**).

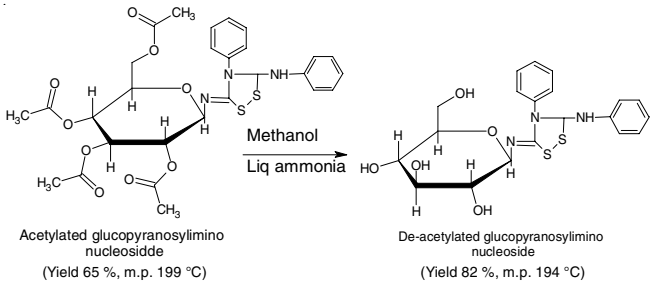
The product was confirmed by several methods such as melting point, elemental analysis, UV, IR, NMR and mass spectra.



Scheme-VII: Reaction step-IV



Scheme-VIII: Reaction step-V



Scheme-IX: Reaction step-VI

RESULTS AND DISCUSSION

The specific rotation $[\alpha]_D^{30}$ of acetylated glucopyranosylimino nucleoside was found to be +199 (0, 0.94) in chloroform. The specific rotation $[\alpha]_D^{30}$ of de-acetylated product in methanol was recorded to be -96.53 (0, 0.13).

The acetylated glucopyranosylimino nucleoside was insoluble in water. However, it was soluble in organic solvents such as benzene, ethanol, chloroform and acetone. The de-acetylated glucopyranosylimino nucleoside was soluble in methanol and ethanol.

Effect of conc. H_2SO_4: The obtained solid product was placed in a China dish and concentrated H_2SO_4 was dropped onto it. In the next turn same procedure was followed for de-acetylated product. According to the observation, acetylated and de-acetylated both the products got charred when treated with conc. H_2SO_4.

Effect of alkaline plumbite solution: Alkaline plumbite solution was added to the both acetylated nucleoside and de-acetylated nucleoside separately and boiled to observe release of sulphur. According to the observations, both the products *i.e*. acetylated and de-acetylated glucopyranosylimino nucleoside was found to be non-de-sulphurizable when boiled with alkaline plumbite solution. Which implies sulphur is present in the ring, which could not be released by boiling with alkaline plumbite solution.

Acetylated and de-acetylated glucopyranosylimino nucleosides were allowed to decompose thermally. Perceptible odour of phenylisocyanate was observed.

Elemental analysis of acetylated glucopyronosylimino nucleoside was found to be C = 55.49, H = 4.81, N = 6.87, S = 10.10 corresponding to the m.f. $C_{28}H_{29}N_3O_9S_2$. The elemental compositions (%) were anticipated to be, C = 55.63, H = 4.71, N = 6.82, S = 10.24.

For de-acetylated glucopyranosylimino nucleoside was found to be; C = 53.79, H = 4.76, N = 9.35, S = 13.47. The molecular formula estimated from this was $C_{20}H_{21}O_5N_3S_2$. The elemental analysis was found to be very close to expected numbers *i.e.* C = 53.69, H = 4.69, N = 9.39, S = 13.21 by percentage.

The UV spectrum of the acetylated glucopyranosylimino nucleoside was observed at λ_{max} of 240 nm in chloroform shown in Fig. 1. UV spectrum of de-acetylated glucopyranosylimino nucleoside was recorded at λ max of 210 nm is shown in Fig. 2.

IR spectral peaks for acetylated and de-acetylated glucopyranosylimino nucleoside are listed in Table-1.

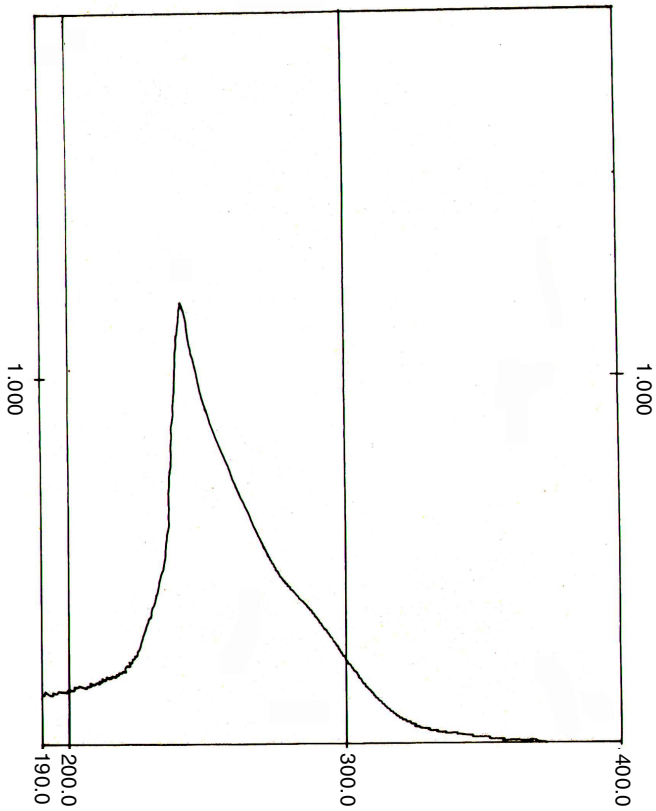


Fig. 1. UV spectra for 3-phenylimino-4-phenyl-5-tetra-O-acetyl-β-D-glucopyranosylimino-1,2,4-dithiazolidone

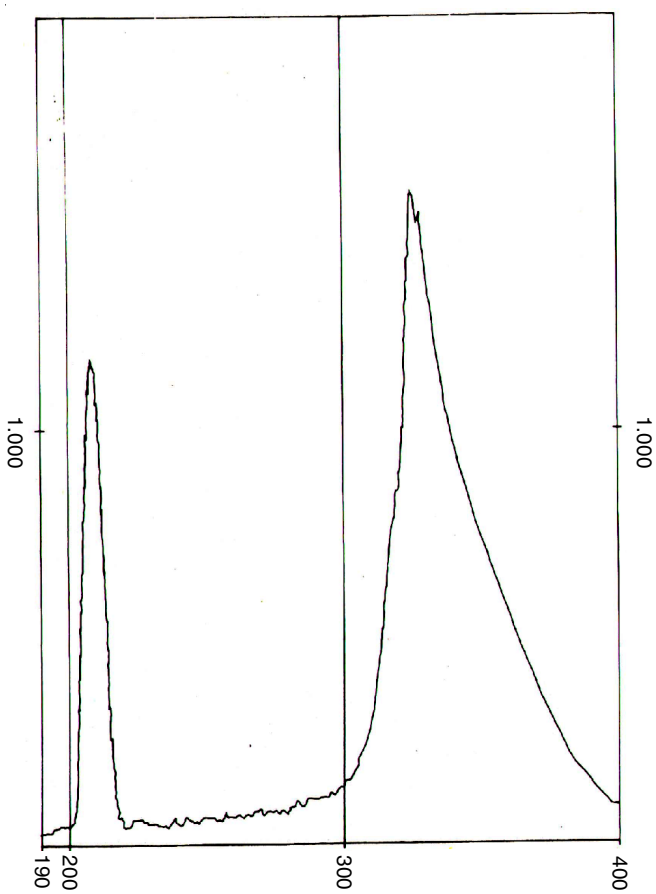


Fig. 2. UV spectra for 3-phenylimino-4-phenyl-5-tetra-hydroxy-β-D-glucopyranosylimino-1,2,4-dithiazolidone

TABLE-1
IR ADSORPTION PEAK FOR ACETYLATED GLUCOPYRANOSYLIMINO NUCLEOSIDE AND DE-ACETYLATED GLUCOPYRANOSYLIMINO NUCLEOSIDE

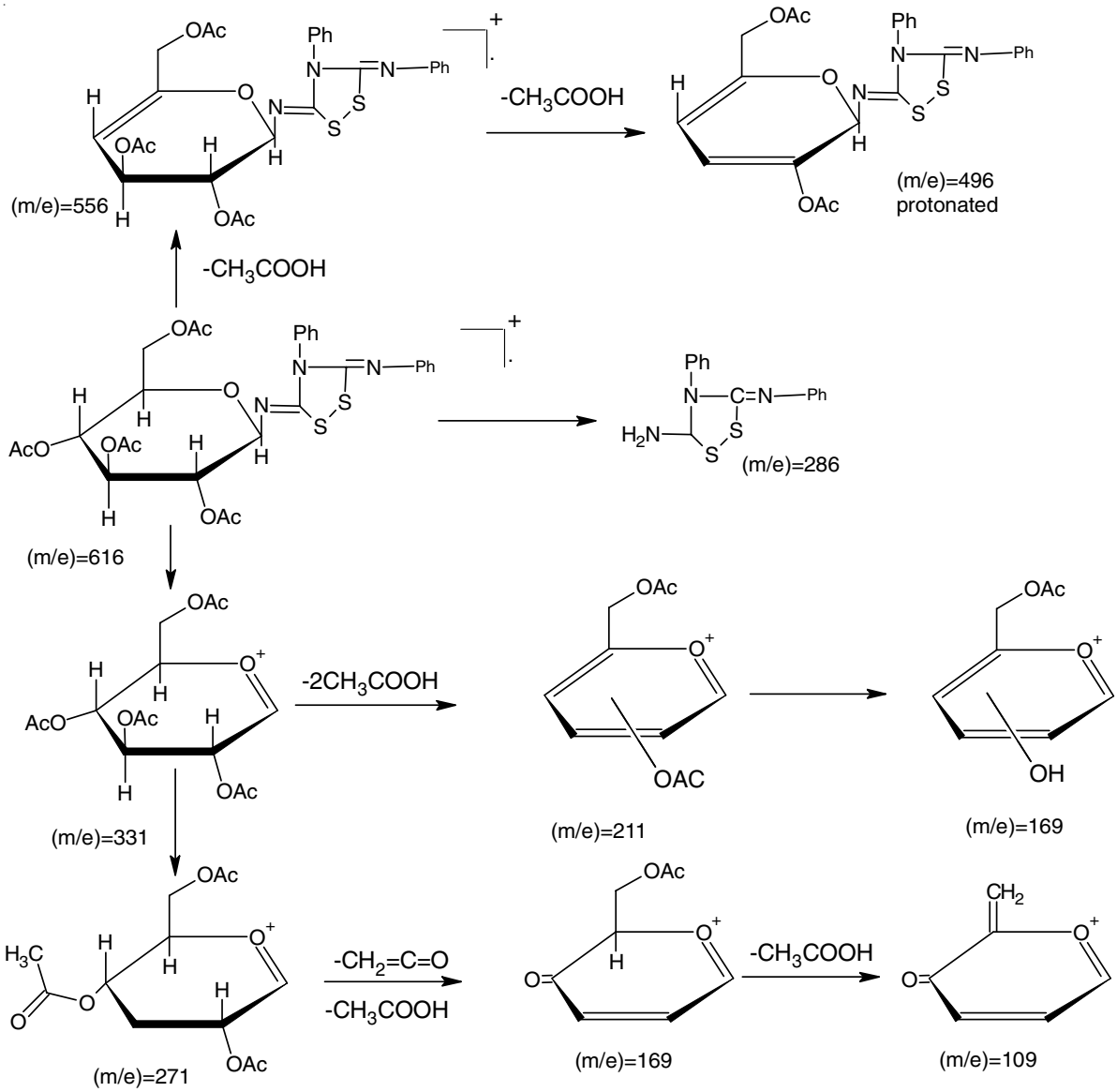
Adsorption (cm^{-1})	Adsorption corresponding group
IR adsorption bands for acetylated glucopyranosylimino nucleoside)	
1750	C=O stretching
1610	C=N stretching
1330	C-N stretching
690	C-S stretching
480	S-S stretching
898	Glycosidic C-H deformation-β-anomer
Adsorption bands for de-acetylated glucopyranosylimino nucleoside)	
3520	O-H stretching
1580	C=N stretching
1280	C-N Stretching
690	C-S stretching
500	S-S stretching
890	Glycosidic C-H deformation-β-anomer

The NMR spectrum of acetylated glucopyranosylimino nucleoside shows the signals due to aromatic proton were observed at δ 6.80 to δ 7.30 ppm. The signal due to acetyl proton was observed at δ 2.05 ppm. Signals due to pyranosyl ring was observed at δ 3.5-4.2 ppm and 4.75-5.25 pmm.

The NMR spectrum of de-acetylated glucopyranosylimino nucleoside shows a signal at 6.5-7.3 ppm due to aromatic protons. β-D-glucopyranosyl ring protons at δ of 2.5-3.2 ppm. Signal at δ of 4-5 ppm was observed which are responsible for OH protons.

Mass spectra: The mass spectra of acetylated and de-acetylated glucopyranosyl nucleosides were recorded by using 'Thermo LCQ Advantage Max Ion Trap Mass spectrometer' (ESI-MS, APCI-MS and LC-MS/MS).

The spectra of acetylated glucopyranosylimino nucleoside shows the protonated molecular ion peak along with other important fragment peaks with their relative abundances are listed in Table-2. The probable fragmentation pattern of molecular ion are shown in **Scheme-X** of mechanism.



Scheme-X: Schematic mechanism of 3-phenylimino-4-phenyl-5-tetra-O-acetyl-β-D-glucopyranosylimino-1,2,4-dithiazolidone through mass spectral fragments

TABLE-2
MASS SPECTRAL DATA OF ACETYLATED AND DE-ACETYLATED GLUCOPYRANOSYLIMINO NUCLEOSIDES

S. No.	Ion	*m/e*	Relative abundance (%)
	Acetylated glucopyranosylimino nucleoside		
1	M^+	615	Not located
2	Protonated	616	100
3	$(M\text{-}CH_2{=}C{=}O)$	574	3
4	$(M\text{-}CH_3COOH)$	556	17
5	$(M\text{-}CH_3COOH\ CH_2CO)^+$	496	1
6	$(MC_{12}H_{10}N_3S_2)^{++}$ *i.e.* TAG	331	65
7	$(M\text{-}C_{16}H_{17}O_9)^+$	286	5
8	$(TAG\text{-}3CH_3COOH)^+$	271	14
9	$(TAG\text{-}AcOCH{=}O)^+$	243	0.02
10	$(TAG\text{-}2CH_3COOH)$	211	4.5
11	$(TAG\text{-}CH_3COOH,\ CH_2{=}O)$	229	7.5
12	$(TAG\text{-}2CH_3COOH,\ CH_2{=}O)^+$	169	33
13	$(TAG\text{-}3CH_3COOH,\ CH_2CO)^+$	109	6
	De-acetylated glucopyranosylimino nucleoside		
1	M^+	447	7.4
2	$M\text{-}C_6H_{11}O5$	285	10
3	$C_{13}H_{10}N_2S$	225	45.4
4	$C_6H_{12}O_6$	180	16.7
5	$C_7H_5NS_2$	167	42.3
6	C_7H_5NS	135	57.5
7	C_6H_5N	93	
8	C_6H_5	77	100

The mass spectrum for de-acetylated glucopyranosyl nucleoside shows the molecular ion peak as well as other fragmented peaks with their relative abundance are listed in Table-2. The probable fragmentation pattern of molecular ion is shown in **Scheme-XI**.

Conclusion

3-Phenylimino-4-phenyl-5-tetra-O-acetyl-β-D-glucopyranosylimino-1,2,4-ditiazolidine (*i.e.* acetylated glucopyranosylimino nucleoside) have been synthesized from glucose.

De-acetylating of product have been carried out to give 4-phenyl-5-tetra-hydroxy-β-D-glucopyranosyl imino-1,2,4-dithiazolidine and broadly called as de-acetylated glucopyranosylimino nucleoside throughout the paper.

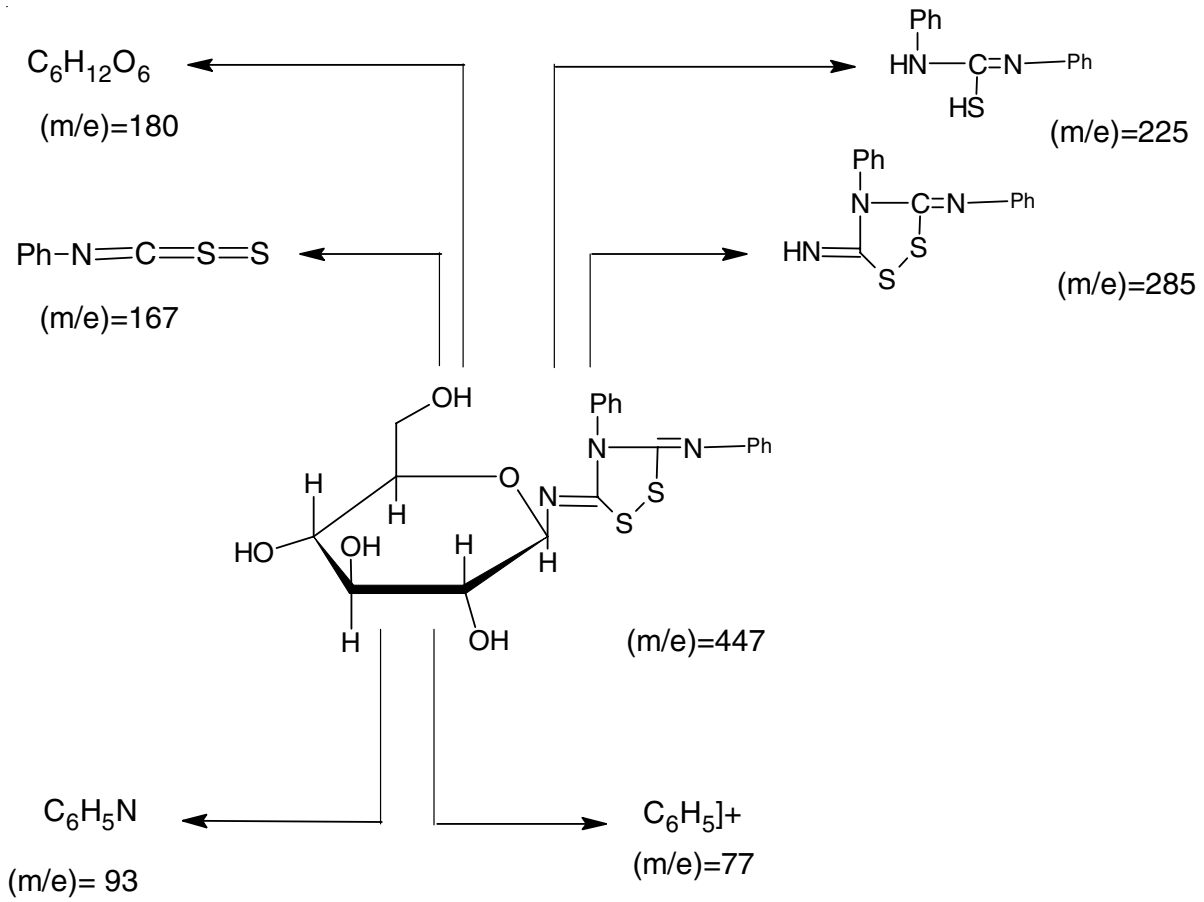
Both the acetylated and de-acetylated glucopyranosylimino nucleoside have been structurally confirmed by spectral analysis which includes UV, IR, NMR and mass spectroscopy. Schematic fragmentation pattern of both acetylated and de-acetylated glucopyranosylimino nucleoside have been studied.

ACKNOWLEDGEMENTS

The authors acknowledge CDRI, Lucknow, India for spectral analyses of samples. The authors acknowledge to Department of Chemistry, RTM Nagpur University, Nagpur, India for providing the necessary research facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.



Scheme-XI: Schematic mechanism of 3-phenylimino-4-phenyl-5-tetra-hydroxy-β-D-glucopyranosylimino-1,2,4-dithiazolidone through mass spectral fragments

REFERENCES

1. G.P. Moss, P.A.S. Smith and D. Tavernier, *Pure Appl. Chem.*, **67**, 1307 (1995); https://doi.org/10.1351/pac199567081307.
2. T. Lindahl, *Nature*, **362**, 709 (1993); https://doi.org/10.1038/362709a0.
3. L. Stryer, Biochemistry, W. H. Freeman & Co: New York, edn 4 (1988).
4. J.D. Watson and F.H.C. Crick, *Nature*, **171**, 737 (1953); https://doi.org/10.1038/171737a0.
5. S. Wurtzer, S. Compain, H. Benech, A.J. Hance and F. Clavel, *J. Virol.*, **79**, 14815 (2005); https://doi.org/10.1128/JVI.79.23.14815-14821.2005.
6. E. Zielonacka-lis, *Nucleos. Nucleot. J.*, **8**, 383 (1989); https://doi.org/10.1080/07328318908054183.
7. K.N. Puri and G.V. Korpe, *Rasayan J. Chem.*, **9**, 401 (2016).
8. N.D. Thanh, *E-J. Chem.*, **9**, 55 (2012); https://doi.org/10.1155/2012/615601.
9. K.N. Puri and G.V. Kopre, *Int. J. Chem. Sci.*, **13**, 1787 (2015).
10. J.F.J. Dippy, S.R.C. Hughes and A. Rozanski, *J. Chem. Soc.*, 2492 (1959); https://doi.org/10.1039/jr9590002492.
11. S. Natelson and S. Gottfried, *Org. Synth. Coll*., **23**, 37 (1943); https://doi.org/10.15227/orgsyn.023.0037.
12. T. Urbanski, Chemistry and Technology of Explosives, Pergamon Press, Oxford and Polish Scientific Publishers, Warsaw, Vol. L (1964).
13. H.N. Alyea, *J. Chem. Educ.*, **46**, A218 (1969); https://doi.org/10.1021/ed046pA310.5.
14. P.P. Deohate, *Der Pharm. Chem.*, **4**, 2368 (2012).
15. G. Huang, Q. Tang, D. Li, Y. Huang, D. Zhang, *Curr. Org. Synth.*, **13**, 82 (2016); https://doi.org/10.2174/1570179412999150703112245.
16. A. Mohammed and R.S. Jwad, *Scient. J. Kerbala Univ.*, **9**, 42 (2011).
17. Z. Tian, T. Hrinyo-Pavlina, R.W. Roeske and P.N. Rao, *J. Chromatogr. A*, **541**, 297 (1991); https://doi.org/10.1016/S0021-9673(01)96001-7.
18. Z.D. Wang, Y. Mo, C.-L. Chiou and M.A. Liu, *Molecules*, **15**, 374 (2010); https://doi.org/10.3390/molecules15010374.