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Isolation and Structure elucidation of novel Dodecasaccharide Uruose from Lal-Muha Cow milk

Muzeeb Khan, Anil Mishra and Desh Deepak*

Department of Chemistry, University of Lucknow, Lucknow-226007 India

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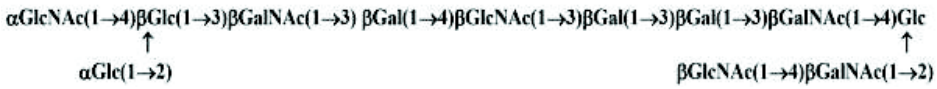
Abstract

Oligosaccharides which have been isolated from different bovine milk exhibit high potent biological activities like antiviral, anticancer, antitumor, antioxidant, antibacterial, antifungal, antimicrobial, antihepatitis, anti-inflammatory, anticoagulant, immunostimulant, antituberculosis. Oligosaccharides of cow milk have shown structural resemblance with human milk oligosaccharides; therefore cow milk is currently being used in a variety of health- promoting supplements worldwide. Several scriptures and studies supported the constructive effects of supplementation of cow milk in diarrhea in human with immune-deficiency syndrome, NSAID-induced gastrointestinal disturbances. With a view to isolate biologically active oligosaccharide from Lal- Muha cow (a rare species of cow found in north eastern part of India at high altitude) milk was taken and processed by the modified method of Kobata and Ginsburg followed by gel filtration, HPLC and column chromatography which resulted in the isolation of novel oligosaccharide namely uruose. The structure of isolated oligosaccharide, uruose was elucidated by chemical transformations, chemical degradation, NMR (^{1}H, ^{13}C 2D COSY, TOCSY, HSQC and HMBC) and mass spectrometry.



Uruose

Keywords: Milk Oligosaccharide; Isolation and structure elucidation**;** Dodecasaccharide; 2D NMR and Uruose.

1. Introduction

Carbohydrates are immediate source of energy and play important roles in cellular recognition, structural material in cell walls, insect and shell fish exoskeletons.[1] Carbohydrates are present in free monosaccharides, oligosaccharides, polysaccharides and with vital component of glycoconjugates such as glycolipids, glycoproteins, glycopeptides & glycosylated natural products. Oligosaccharide is the most important carbohydrate. Earlier the oligosaccharides has been defined as carbohydrates with a maximum number of monosaccharides[2] 3 up to 10 and as there is no physiological or chemical reason to set the limit of milk oligosaccharide, it has been seen that oligosaccharides may contain of 3 up to 19 units of monosaccharides.[3] Milk oligosaccharides consist of six monosaccharides namely, glucose, galactose, *N*-acetyl-glucosamine, *N*-acetyl-galactosamine, fucose, and *N*-acetyl-neuraminic acid. These components combine in different ways to form a large chain of oligosaccharides carrying varied glycosidic linkages at different position in milk oligosaccharide. Milk oligosaccharides have potential to produce prebiotics,[4] anti adhesion,[5,6] anti-inflammation,[7,8] brain development,[9,10] mineral absorption[11] and immuno-modulation effects[12]. The composition of milk is influenced by a range of different factors, like diet[13], genetics,[14] number and stage of lactation,[15] and, seasonal variation,[16] somatic cell count,[17] and milk processing.[18] The components found in milk play a significant role in preventing various disorders such as hypertension and cardiovascular diseases, obesity, osteoporosis, gastrointestinal health, colorectal cancer, ageing[19-25] etc. Milk oligosaccharides confer unique health benefits to the neonate as selectively promoting the growth of beneficial bifidobacteria in the colon, preventing infection by inhibiting the adhesion of pathogenic bacteria to the intestinal mucosal surface, enhancing brain development and cognitive function of neonates.[26-29] We may classify various milk oligosaccharides on the basis of structure of monosaccharides present these in (1) Acidic Oligosaccharides contain neuraminic acid or sialic acid (2) Fucosylated oligosaccharides containing fucose at their non reducing end and neutral oligosaccharides which are made up of Glc (glucose), Gal (galactoase), GlcNHAc (*N*-acetyl-glucosamine) and GalNHAc (*N*-acetyl-galactosamine). The

* Corresponding author : Dr. Desh Deepak
Email: deshdeepakraju@rediffmail.com

fucosylated and sialylated oligosaccharides reduce the adhesion of bacteria and viruses to intestinal cells. Fucosylated and sialylated glycoconjugates of human milk can incorporate as inhibitors in blocking the interactions between lectin receptors of the pathogen and host epithelial cells.[30,31] The ability of pathogens to bind to specific oligosaccharides is found to be intrinsically correlated with their structure. A number of milk oligosaccharides have been isolated from different milk sources (cow, buffalo, human, mare, goat, yalk, donkey etc.) which contain high concentration of bioactive oligosaccharides and show a number of biological activities like antitumor, anticancer, antigenic, immunostimulant etc. Cow milk oligosaccharides reduce the adhesion of enterotoxic *E. Coli* strains of the calf.[32] Buffalo milk oligosaccharides have also been evaluated for their ability to stimulate non-specific immunological resistance of the host against parasitic infections.[33] Mare's milk has shown anti oxidant, lipid lowering and post heparin lipolytic activity[34] as well as they promote cellular immune response as observed in vitro both in terms of cellular proliferation and reactive oxidative burst and also interpreted as an activation of innate immune defense mechanism.[35] Mare's milk is plentiful of the fat-resembling substances that contribute in the transfer of certain nerve impulses and the regulation of blood pressure. Camel milk oligosaccharides, which contain sialyl oligosaccharides and this sialic acid exhibit a number of health benefits for human infants, including the promotion of infant brain development.[36,37] The elephant milk oligosaccharide fraction containing a high ratio of sialyl oligosaccharides was significant with respect to the formation of brain components, such as gangliosides of the suckling calves.[38] The dog milk has a dominant *N*-acetylneuraminlactose sulphate oligosaccharide which plays an important role in the nutrition of the rat pups.[39] Sheep milk is a rich source of fucosylated oligosaccharides which has definite biological effects like α-1,2-linked fucosylated oligosaccharides, in conjugation with other families of oligosaccharide, constitute a powerful innate immune system of human milk.[40] It also aggravates hiccup and dyspnoea and eliminates pitta, kapha and fat.[41] Sheep milk is an important source of bioactive inhibitory and hypertensive defence and control of microbial infection. Bioactive component present in sheep milk have their affect in cardiovascular, nervous and immune system. Donkey milk oligosaccharides have ability to stimulate non-specific and specific immunological resistance[42]. Goat milk oligosaccharides have anti-inflammatory effects in rats with trinitrobenzenesulfonic (T) acid induced colitis and may be useful in the management of inflammatory bowel disease.[43] Its oligosaccharides play important roles in intestinal protection and repair after damage caused by DSS (dextron sodium sulphate) induced colitis and their implication in human intestinal inflammation.[44] Human milk oligosaccharides are metabolized by specific strains of bifidobacteria and thereby contribute to the establishment of a unique beneficial gut microbiota in infants during breast-feeding, so can be interpreted as prebiotics.[45] The cow milk is important for human life which is written in ancient literature. Indian ancient Physician Dhanvantri confirmed that it protects the human from heart diseases and leucoderma. The Ayurveda has described the medicinal importance of black cow milk and Rigveda says that Cow milk is Amrita, protects human being from diseases, its milk have the curative and prophylactic effects. Keeping in mind the biological activities of oligosaccharide Lal-Muha cow (a rare species of cow found in north eastern part of India at high altitude) milk was taken and processed by the modified method of Kobata and Ginsburg followed by gel filtration, HPLC and column chromatography which resulted in the isolation of novel oligosaccharide namely uruose. The structure of isolated oligosaccharides was elucidated by chemical transformations, chemical degradation, NMR (^{1}H, ^{13}C and 2D COSY, TOCSY, HSQC and HMBC) and mass spectrometry.

2.1 Experimental:

General procedures and the methods used for the isolation of Lal-Muha cow's milk oligosaccharides and the acetylation of oligosaccharide mixture were same as described in our previous article.[46]

2.2. Purification of acetylated milk oligosaccharide

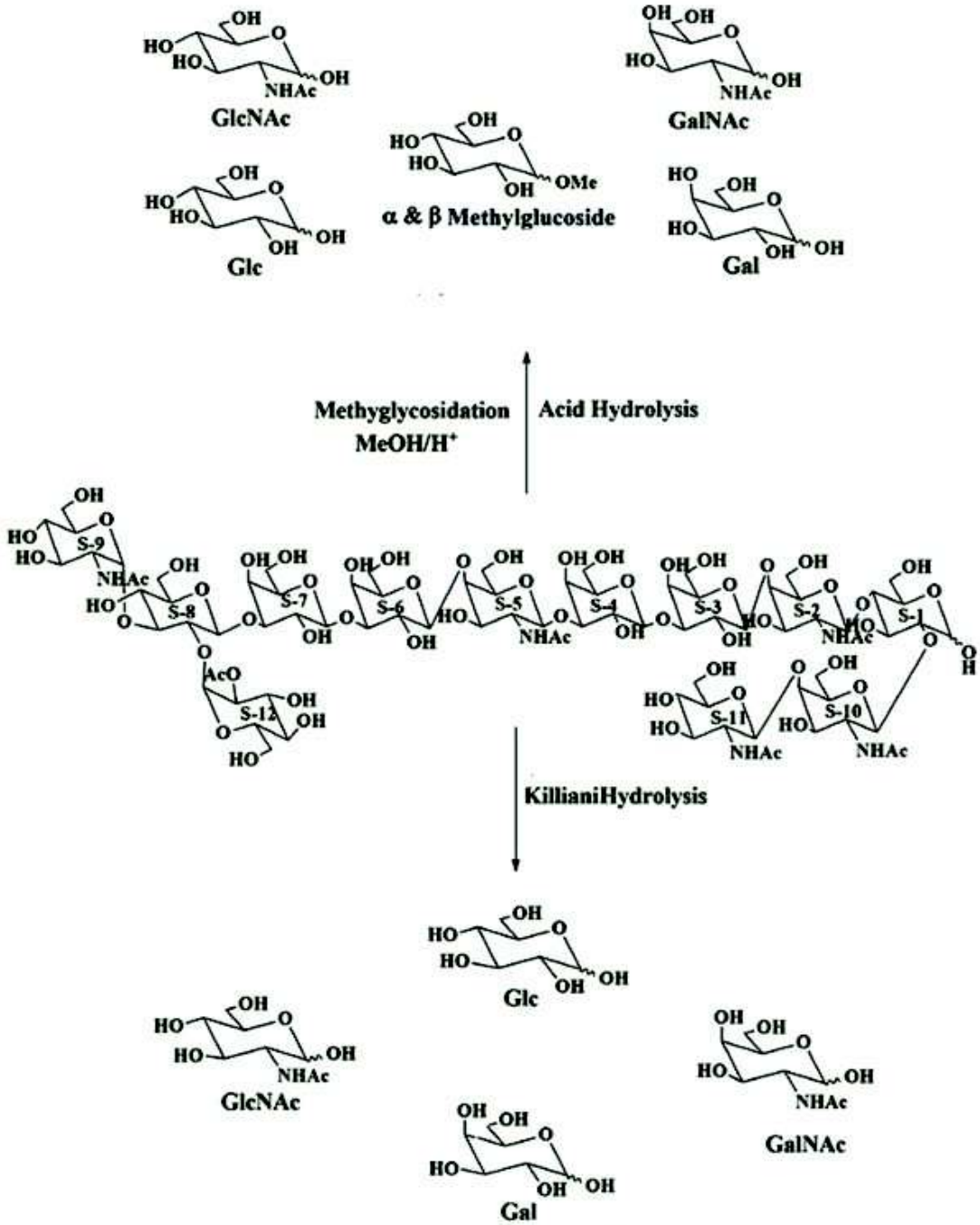
Separation (purification) of the acetylated products (11.0 g) was done by column chromatography. The silica was used in the ratio of 1:100 using various proportions of hexane: $CHCl_3$, $CHCl_3$ and $CHCl_3$: MeOH mixture which resolved into eight fractions namely I (1.45 g), II (1.10 g), III (2.15 g), IV (1.20 g), V (2.30 g), VI (2.10 g), VII (1.40 g) and VIII (800 mg) respectively. These fractions were containing a mixture of three to four compounds. Repeated column chromatography of fraction VI, led to the isolation of chromatographically pure uruose acetate (84 mg).

2.3. Deacetylation of Uruose acetate

84 mg Compound e were obtained from column chromatography-4 of acetylated oligosaccharide mixture. 45 mg Compound e were dissolved in acetone (3 mL) and 4 mL of NH_3 and left overnight in a

stoppered hydrolysis flask. After 24h ammonia was removed under reduced pressure and the compound was washed with (3 x 5 mL) $CHCl_3$ (to remove acetamide) and the water layer was finally freeze dried giving the deacetylated oligosaccharide E (37.0mg).

2.4. Methyl glycosidation/ Acid Hydrolysis of Uruose



Scheme 1: Methylglycosidation/Acid Hydrolysis and Killiani Hydrolysis of Uruose

6 mg Compound E were ref1uxed with absolute MeOH (2 mL) for 18 h in the presence of cation exchange IR-120 (H) resin. The reaction mixture was filtered while hot and filtrate was concentrated. To a solution of methyl glycoside of E in 1,4-dioxane (1 mL), 0.1 N H_2SO_4 (1 mL) was added and the solution was warmed for 30 min at 50 °C and solution was left over night. The hydrolysis was complete after 24 h. The hydrolysate was neutralized with freshly prepared $BaCO_3$, filtered and concentrated under reduced pressure to afford α-and β-methylglucosides along with the Glc, Gal, GalNAc and GlcNAc. Their identification was confirmed by comparison with authentic samples (TLC, PC).

2.5. Killiani Hydrolysis:

4 mg Compound E were dissolved in 2 mL Kiliani mixture (AcOH-H_2O-HCI, 7:11:2) and heated at 100 °C for 1 h followed by evaporation under reduced pressure. It was dissolved in 2 mL of H_2O and extracted twice with 3 mL $CHCl_3$. The aqueous residual solution was made neutral by addition of 1-2 drops of 2N NaOH, to it and was evaporated under reduced pressure to afford glucose, galactose, GalNAc and GlcNAc on comparison with authentic samples of glucose, galactose, GalNAc and GlcNAc, given in **Scheme-1** (Supplementary File).

2.6. Description of compound

2.7. Compound, Uruose

84 mg Compound e were obtained from fraction 84-117 of chromatography-4 from acetylated oligosaccharide mixture. On deacetylation of 45 mg of compound c with NH_3/ acetone it afforded substance E (37 mg) as a viscous syrup, = 28 (c, 4, H_2O). For experimental analysis, this compound was dried over P_2O_5 at 100 ºC and 0.1 mm pressure for 8 h. Compound uruose gave positive Phenol-sulphuric acid test,[47] Feigl test[48] and Morgan-Elson test.[49]

$C_{82}H_{137}O_{61}N_5$	%C	%H	%N
Calcd.	45.42	6.32	3.24
Found	45.40	6.32	3.23

^{1}H NMR of Uruose acetate in $CDCl_3$ at 300 MHz

δ 6.26 [d, 1 H, *J* = 3.9 Hz, -Glc (S-1) H-1], δ 5.37 [d, 1 H, *J* = 3.9 Hz, -Glc (S-12) H-1], δ 5.34 [d, 1 H, *J* = 8.0 Hz, β-Glc (S-1) H-1], δ 5.26 [d, 1H, *J* =3.9 Hz, -GlcNAc (S-9) H-1], δ 4.74 [d, 1 H, *J* = 7.8 Hz, β-GlcNAc (S-11) H-1], δ 4.67 [d, 1 H, *J* = 8.4 Hz, β-Glc (S-8) H-1], δ 4.55 [d, 1 H, *J* = 7.9 Hz, β-Gal (S-4) H-1], δ 4.51 [d, 1 H, *J* = 7.6 Hz, β-GalNAc (S-5) H-1], δ 4.46 [d, 2 H, *J* = 8.1 Hz, β-Gal (S-3 & S-6) H-1], δ 4.45 [d, 1 H, *J* = 8.0 Hz, β-Gal (S-7) H-1], δ 4.37 [d, 2 H, *J* = 8.1 Hz, β-GalNAc (S-2 & S-10) H-1], δ 4.05 [m, 2 H, β-GalNAc (S-2 & S-5) H-4], δ 3.90 [m, 1 H, β-Glc (S-1) H-2], δ 3.78 [m, 1H, β-Glc (S-1) H-4], δ 3.75 [m, 1H, β-Gal (S-4) H-3], δ 3.70 [m, 1H, β-Gal (S-3 & S-6) H-3],

^{13}C NMR of Uruose acetate in $CDCl_3$ at 75 MHz

δ 90.12 [1 C, -Glc (S-1) C-1], δ 90.28 [1 C, β-Glc (S-1) C-1], δ 92.36 [1 C, -GlcNAc (S-9) C-1], δ 92.98 [1 C, -Glc (S-12) C-1], δ 95.21 [1 C, β-GlcNAc (S-11) C-1], δ 95.28 [1 C, β-Glc (S-8) C-1], δ 100.83 [1 C, β-GalNAc (S-5) C-1], δ 101.05 [1 C, β-Gal (S-7) C-1], δ 101.27 [1 C, β-Gal (S-4) C-1], δ 103.49 [1 C, β-GalNAc (S-10) C-1], δ 103.67 [1 C, β-GalNAc (S-2) C-1], δ 104.31 [1C, β-Gal(S-3) C-1] and δ104.43 [1C, β-Gal(S-6) C-1].

^{1}H NMR of Uruose in D_2O at 300 MHz

δ 5.35 [d, 1 H, *J* = 4.1 Hz, -Glc (S-1) H-1], δ 5.23 [d, 2 H, *J* = 3.3 Hz, -GlcNAc (S-9) & -Glc (S-12) H-1], δ 4.67 [d, 3 H, *J* = 7.6 Hz, β-Glc (S-1 & S-8) & β-Gal (S-7) H-1], δ 4.58 [d, 1 H, *J* = 8.0 Hz, β-Gal (S-3) H-1], δ 4.55 [d, 1 H, *J* = 7.8 Hz, β-Gal (S-4) H-1], δ 4.52 [d, 1 H, *J* = 8.0 Hz, β-Gal (S-6) H-1], δ 4.45 [d, 4 H, *J* = 7.5 Hz, β-GalNAc (S-2, S-5 & S-10) & β-GlcNAc (S-11) H-1], δ 3.28 [m, β-Glc (S-1), H-2], δ 2.09 [s, 3 H, NHCO$\underline{CH_3}$, -GlcNAc (S-9)], δ 2.00 [s, 6 H, NHCO$\underline{CH_3}$, β-GalNAc (S-2 & S-10)], δ 1.99 [s, 6 H, NHCO$\underline{CH_3}$, β-GalNAc (S-5) & β-GlcNAc (S-11)].

ES Mass of Uruose

2167 [M^+], 2089 [2167-CH_2OHCHO, -H_2O], 1908 [2005-CH_2OCHO, -$2H_3O^+$], 1856 [2005-$NHCOCH_3$,-HOCHCHOH,-HCHO,-H^+], 1784 [1802-H_2O], 1666 [1784 -$NHCOCH_3$,-CH_2OHCHO], 1617 [1666-CH_3OH,-OH], 1576 [1666 ,-HOCHCHOH,-HCHO],1419 [1599-$NHCOCH_3$,-$CH_2OHCOCO$, -H_2O,-OH], 1366 [1396-HCHO], 1261 [1366-HOCHCHOH,-CH_3CHO,-H^+], 1218 [1396-$CH_2OHCOCO$, -HOCHCHOH, -HCHO, H^+], 1070 [1234-2CH_2OHCHO,-CH_3CHO], 1028 [1070-CH_3CHO,], 906 [1028,-HOCHCHOH,-CH_3CHO, -H_2O], 820 [910-$NHCOCH_3$, -CH_3OH], 685 [820-CH_2OCHO,-CH_3CHO, -CH_3OH], 637 [707-CH_3OH ,-H_2O], 583 [637 -3H_2O], 483 [545-CH_3CHO,-H_2O], 466 [483-OH], 465 [466-H^+], 365 [383-H_2O], 319 [365-CHO,-OH], 318 [319-H^+], 303 [383-2H_2O,-CH_3CHO], 244 [303-CH_2OCHO] and 274 [303-$NHCOCH_3$,-CH_2CO,-CHO]and 180 [342-S_2].

3. Result and discussion

3.1. Structure elucidations of Uruose

3.2. NMR spectroscopy

Compound E, Uruose $C_{82}H_{137}O_{61}N_5$ $[\alpha]_D^{25}$ -28º gave positive Phenol-sulphuric acid test,[47] Fiegl test[48] and Morgan-Elson test[49] showing the presence of normal and amino sugars in uruose. The ^{1}H NMR spectrum Fig-4 (s. f.) of uruose showed seven doublets for thirteen anomeric proton signals at δ 5.35 (1H), δ 5.23 (2H), δ 4.67 (3H), δ 4.58 (1H), δ 4.55 (1H), δ 4.52 (1H) and δ 4.45 (4H) in ^{1}H NMR spectrum of uruose in D_2O at 300 MHz. The ^{1}H NMR of uruose had two doublets at δ 5.35 (J = 4.1 Hz) and δ 4.67 (J = 7.6 Hz) for its α and β anomers suggesting that uruose was a dodecasaccharide in its reducing form. The chemical shifts of α and β anomeric protons signals at δ 5.35 and δ 4.67 suggested that the reducing[50] monosaccharide may be glucose. Besides the anomeric proton signal of glucose in ^{1}H NMR spectrum of uruose in D_2O at 300 MHz it also contain another anomeric doublet at δ 4.45 (J = 7.5 Hz) along with a singlet at 2.00 suggested the presence of β-GalNAc (S-2) residue as the next monosaccharide unit. In addition to signals of β-Glc and β-GalNAc, presence of a triplet at δ 3.28 which was due to H-2 of β-Glc(S-1) suggested the presence of Lactose type of structure containing a NHAc group i.e. β-GalNAc(1-4)→Glc (structure reporter group)[51,52] at the reducing end of uruose. Further the HSQC spectrum Fig 1 of acetylated uruose at 300 MHz exhibited thirteen cross peaks for thirteen anomeric protons and carbons at δ 6.26 x 90.12, δ 5.37 x 92.98, δ 5.34 x 90.28, δ 5.26 x 92.36, δ 4.74 x

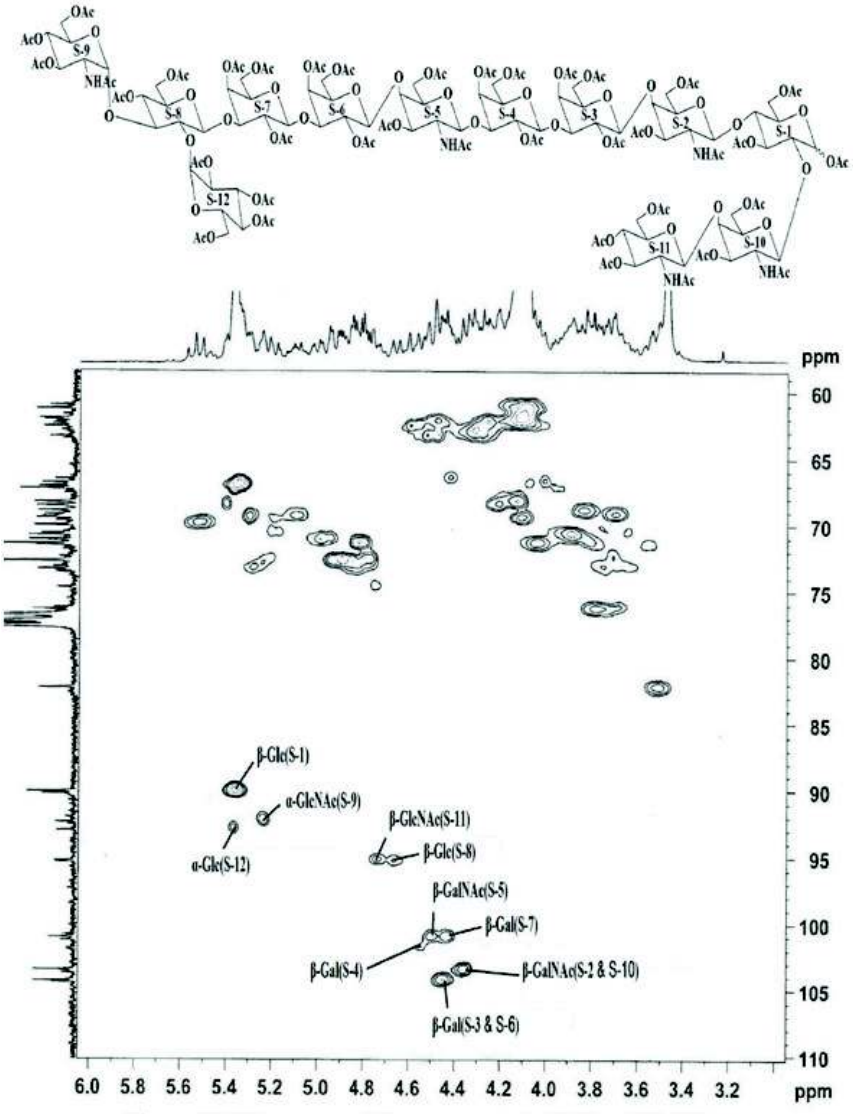


Figure 1: HSQC Spectrum of Uruose Acetate in $CDCl_3$ at 300 MHz.

95.21, δ 4.67 x 95.28, δ 4.55 x 101.27, δ 4.51 x 100.83, δ 4.46 x 104.31, δ 4.46 x 104.43, δ 4.45 x 101.05, δ 4.37 x 103.49 and δ 4.37 x 103.67. These cross peaks suggested that uruose may be a dodecasaccharide in its reducing form. The chemical shift values of cross peaks at δ 6.26 x 90.12 and δ 5.34 x 90.28 suggested presence of and anomers of glucose at its reducing end of oligosaccharide. The reducing nature of uruose was further confirmed by its methylglycosylation MeOH/H^+ followed by its acid hydrolysis Scheme 1. Which led to the isolation of α and β-methyl glucosides, along with Glc, Gal, GlcNAc and GalNAc suggesting the presence of glucose at the reducing end and presence of Glc, Gal, GlcNAc and GalNAc moieties in uruose. The twelve monosaccharides present in compound have been designated as S-1, S-2, S-3, S-4, S-5, S-6, S-7, S-8, S-9, S-10, S-11 and S-12 for convenience starting from the reducing end. The dodecasaccharide nature of the acetylated uruose was also confirmed by the presence of eleven doublets for thirteen anomeric proton and thirteen anomeric carbon at δ 6.26 (1H), δ 5.37 (1H), δ 5.34 (1H), δ 5.26 (1H), δ 4.74 (1H), δ 4.67 (1H), δ 4.55 (1H), δ 4.51 (1H), δ 4.46 (2H), δ 4.45 (1H), and δ 4.37 (2H) in ^{1}H NMR at 300 MHz Fig 2 and δ 90.12, δ 90.28, δ 92.36, δ 92.98, δ 95.21, δ 95.28, δ 100.83, δ 101.05, δ 101.27, δ 103.49, δ 103.67, δ 104.31 and δ 104.43 in its

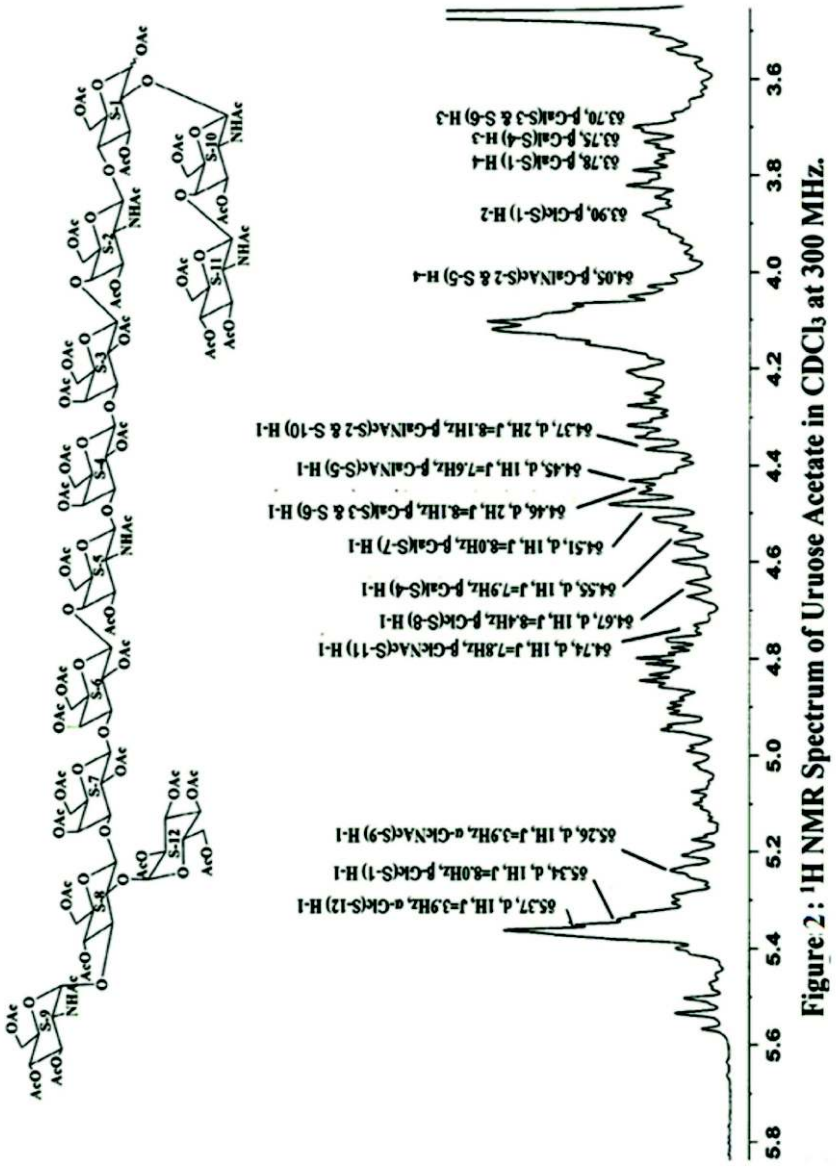


Figure 2: ^{1}H NMR Spectrum of Uruose Acetate in $CDCl_3$ at 300 MHz.

^{13}C NMR at 75 MHz Fig 3 respectively. The monosaccharides constituents in uruose were also confirmed by its Killiani hydrolysis[53] Scheme 1 under strong acidic condition, followed by paper chromatography and TLC. In this hydrolysis four spots were found identical with the authentic samples of Glc, Gal, GlcNAc and GalNAc by co-chromatography (PC, TLC), which confirmed that uruose contained four types of monosaccharides units i.e. Glc, Gal, GlcNAc and GalNAc. The ^{1}H and ^{13}C NMR spectra of uruose justify the thirteen anomeric signals for dodecasaccharide with total integral intensity of twelve anomeric protons and carbons. Further the mass ion peak at 2167[M]$^{+}$ present in ES-MS of uruose was in agreement with molecular formula $C_{82}H_{137}O_{61}N_{5.}$

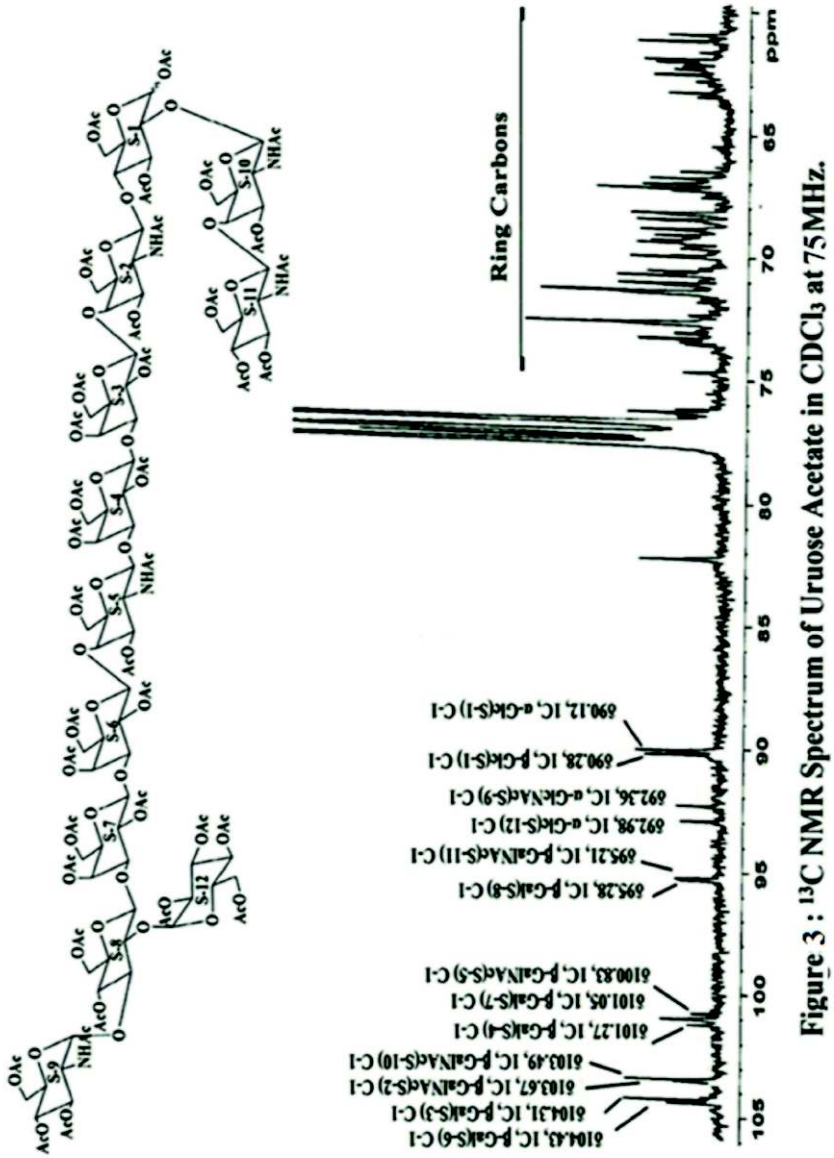


Figure 3 : ^{13}C NMR Spectrum of Uruose Acetate in $CDCl_3$ at 75 MHz.

Table 1: ^{1}H NMR: Anomeric proton chemical shift values of uruose in D_2O and $CDCl_3$ at 300 MHz

Moieties	In D_2O		In $CDCl_3$	
	^{1}H NMR (δ)	Coupling constant (J)	^{1}HNMR (δ)	Coupling constant (J)
α-Glc (S-1)	5.35	4.1 Hz	6.26	4.5 Hz
β-Glc (S-1)	4.67	7.6 Hz	5.34	8.0 Hz
β-GalNAc (S-2)	4.45	7.5 Hz	4.37	7.8 Hz
β-Gal (S-3)	4.58	8.0 Hz	4.46	8.1 Hz
β-Gal (S-4)	4.55	7.8 Hz	4.55	7.9 Hz
β-GalNAc (S-5)	4.45	7.5 Hz	4.45	7.6 Hz
β-Gal (S-6)	4.52	8.0 Hz	4.46	8.1 Hz
β-Gal (S-7)	4.67	7.6 Hz	4.51	8.0 Hz
β-Glc (S-8)	4.67	7.6 Hz	4.67	8.4 Hz
α-GlcNAc (S-9)	5.23	3.3 Hz	5.26	3.9 Hz
β-GalNAc (S10)	4.45	7.5 Hz	4.37	7.8 Hz
β-GlcNAc (S11)	4.45	7.5 Hz	4.74	7.8 Hz
α-Glc (S-12)	5.23	3.3 Hz	5.37	3.9 Hz

Table 2: ^{1}H NMR chemical shift values of acetylated uruose in $CDCl_3$ at 300 MHz

Moieties	H-1	H-2	H-3	H-4	H-5	H-6	NHCO$\underline{CH_3}$
S-1	5.34	3.90	4.81	3.78	-	-	-
S-2	4.37	3.81	4.88	4.05	-	-	2.00
S-3	4.46	4.27	3.70	4.95	-	-	-
S-4	4.55	4.25	3.75	4.90	-	-	-
S-5	4.45	3.85	5.20	4.05	-	-	1.99
S-6	4.46	4.27	3.70	4.95	-	-	-
S-7	4.51	4.30	3.76	5.10	-	-	-
S-8	4.67	3.55	3.80	4.95	-	-	-
S-9	5.26	3.91	4.21	4.80	-	-	2.09
S-10	4.37	3.81	4.88	4.05	-	-	2.00
S-11	4.74	3.80	5.20	5.40	-	-	1.99
S-12	5.37	4.93	4.45	5.25	-	-	-

Simultaneously ^{1}H NMR **Fig 2** and ^{13}C NMR spectrum **Fig 3** of uruose acetate also showed downfield shifted α and β anomeric proton and carbon of reducing monosaccharides (S-1) i.e. Glc (S-1) at δ 6.26 (J = 4.5 Hz), δ 5.34 (J = 8.0 Hz) and δ 90.12, δ 90.28 respectively.[54] The anomeric proton signal present at δ 5.34 in ^{1}H NMR Spectrum of uruose acetate assigned to β-Glc (S-1) gave three cross peaks at δ 5.34 x 3.78, δ 5.34 x 3.90 and δ 5.34 x 4.81 in TOCSY Spectrum **Fig 5** of uruose acetate which was later identified as H-4, H-2 and H-3 of reducing Glc respectively by COSY spectrum **Fig 6** of uruose acetate. The chemical shift of the cross peak at δ 5.34 x 3.78 and δ 5.34 x 3.90 suggested that in glucose S-1, two positions were available for glycosidic linkage by next monosaccharide unit. The earlier suggested (1→4) linkage between β-Glc (S-1) and β-GalNAc (S-2) was further confirmed by HMBC spectrum **Fig 7** of uruose acetate at 300 MHz which contain a cross peak at δ 3.78 x 103.67 which was between H-4 of β-Glc (S-1) and anomeric carbon of next monosaccharide (C-1) i.e. β-GalNAc (S-2). The anomeric carbon signal at δ 103.67 showed its complimentary signal at δ 4.37 in HSQC spectrum of uruose acetate in $CDCl_3$ at 300 MHz. The chemical shift of δ 103.67 and δ 4.37 of anomeric carbon and anomeric proton were having resemblance with literature value of anomeric chemical shift value of β-GalNAc (S-2), hence S-2 monosaccharide was confirmed as β-GalNAc (S-2).[55] The coupling constant of anomeric proton signal present at δ 4.37 (S-2) had a J value of 7.8 Hz in $CDCl_3$ suggesting a β glycosidic linkage between S-2→S-1. Simultaneously the ^{1}H NMR spectrum **Fig 4** of uruose in D_2O at 300 MHz contain anomeric proton signal at δ 4.45 (J = 7.5 Hz) along with a singlet of three proton of amide methyl at δ 2.00 suggested that S-2 was GalNAc. Further signal of anomeric proton at δ 4.45 with J value 7.5 Hz in the ^{1}H NMR of uruose in D_2O at 300 MHz also confirmed a β glycosidically linked GalNAc (S-2). The (1→4) linkage between β-Glc (S-1) and β-GalNAc (S-2) was also supported by the presence of H-4 signal of S-1 at δ3.78 in upfield region of ^{1}H NMR spectrum and a cross peak at δ 3.78 x 76.20 in glycosidic region of HSQC spectrum of uruose acetate in $CDCl_3$. Since it was ascertained by the COSY and TOCSY spectrum of uruose acetate that the β-Glc (S-1) has two vacant positions i.e. H-2 and H-4 and it was already confirmed that H-4 of S-1 was linked with β-GalNAc (S-2) whereas H-2 position of β-Glc (S-1) was lying vacant for glycosidic linkage by next monosaccharide unit. The ^{1}H NMR signal of H-2 (S-1) at δ 3.90 gave a cross peak at δ 3.90 x δ103.49 in HMBC spectrum of uruose acetate confirming a (1→2) glycosidic linkage between S-10 and S-1. The anomeric carbon signal present at δ 103.49 showed its complimentary signal at δ4.37 in HSQC spectrum of uruose acetate in $CDCl_3$ at 300 MHz. Simultaneously the ^{1}H NMR spectrum of uruose in D_2O at 300 MHz contain anomeric proton signal at δ 4.45 (J = 7.5 Hz) along with a singlet of three proton of amide methyl at δ 2.00 confirming that S-10 was GalNAc.[55] The coupling constant of anomeric signal β-GalNAc (S-10) with larger value of 7.8 Hz in $CDCl_3$ suggested a β glycosidic linkage between S-10→S-1. Further signal of anomeric proton at δ 4.45 with J value 7.5 Hz in the ^{1}H NMR of uruose in D_2O at 300 MHz also confirmed β glycosidically linked GalNAc (S-10). The (1→2) linkage between β-Glc (S-1) and β-GalNAc (S-10) was also supported by the presence of H-2 signal of S-1 at δ 3.90 in upfield region of ^{1}H NMR spectrum and a cross peak at δ 3.90 x 70.82 in glycosidic region of HSQC spectrum **Fig 1** of uruose acetate in $CDCl_3$. The anomeric proton signal present at δ 4.37 in TOCSY Spectrum of uruose acetate assigned to β-GalNAc (S-10) gave three cross peaks at δ 4.37 x 3.81, δ 4.37 x 4.05 and δ 4.37 x 4.88 which were later identified as H-2 (containing NHAc group), H-4 and H-3 respectively by COSY spectrum of uruose acetate. Out of these signals one proton signal present at δ 3.70 corresponded to H-2 position of β-GalNAc (S-10) while the other proton signal H-4 of β-GalNAc (S-10) which was observed at δ 4.05 was available for (1→4) glycosidic linkages by the next monosaccharide unit. The H-4 position of β-GalNAc (S-10) at δ 4.05 showed its C-4 position at δ 71.25 in HSQC spectra of uruose acetate which showed a long range coupling with anomeric proton of next monosaccharide S-11 i.e. H-1 of S-11 and C-4 of S-10 at δ 4.74 x 71.25 in Reverse HMBC of acetylated uruose in $CDCl_3$ at 300 MHz confirming a (1→4) linkage between S-11 and S-10. The anomeric proton signal at δ 4.74 showed its complimentary carbon signal at δ 95.21 in HSQC spectrum of uruose acetate in $CDCl_3$ at 300 MHz. Simultaneously the ^{1}H NMR spectrum of uruose in D_2O at 300 MHz contain another anomeric proton signal at δ 4.45 (J = 7.5 Hz) along with a singlet of three proton of amide methyl at δ 1.99 suggesting that S-11 was GlcNAc.[57,58,59] The coupling constant of anomeric proton signal present at δ 4.74 (S-11) had a J value of 7.8 Hz in $CDCl_3$ suggesting a β glycosidic linkage between S-11→S-10. Further signal of anomeric proton at δ 4.45 with J value 7.5 Hz in the ^{1}H NMR of uruose in D_2O at 300 MHz also confirmed a β glycosidically linked GlcNAc (S-11). The (1→4) linkage between β-GalNAc (S-10) and β-GlcNAc[56] (S-11) was supported by the presence of H-4 signal of S-10 at δ 4.05 in upfield region of ^{1}H NMR spectrum and a cross peak at δ 4.05 x 71.25 in glycosidic region of HSQC spectrum of uruose

acetate in $CDCl_3$. The anomeric protons signal present at δ 4.74 in TOCSY Spectrum **Fig 5** of uruose acetate assigned to β-GlcNAc (S-11) gave three cross peaks at δ 4.74 x 3.80, δ 4.74 x 5.20 and δ 4.74 x 5.40 which were later identified as H-2 (containing NHAc group), H-3 and H-4 respectively by COSY spectrum **Fig 6** of uruose acetate. This anomeric proton did not show any cross peak in the linkage region hence confirming that β-GlcNAc (S-11) was present at non-reducing end of uruose and none of its hydroxyl group were available for glycosidic linkage.

Further the anomeric protons signal present at δ 4.37 in

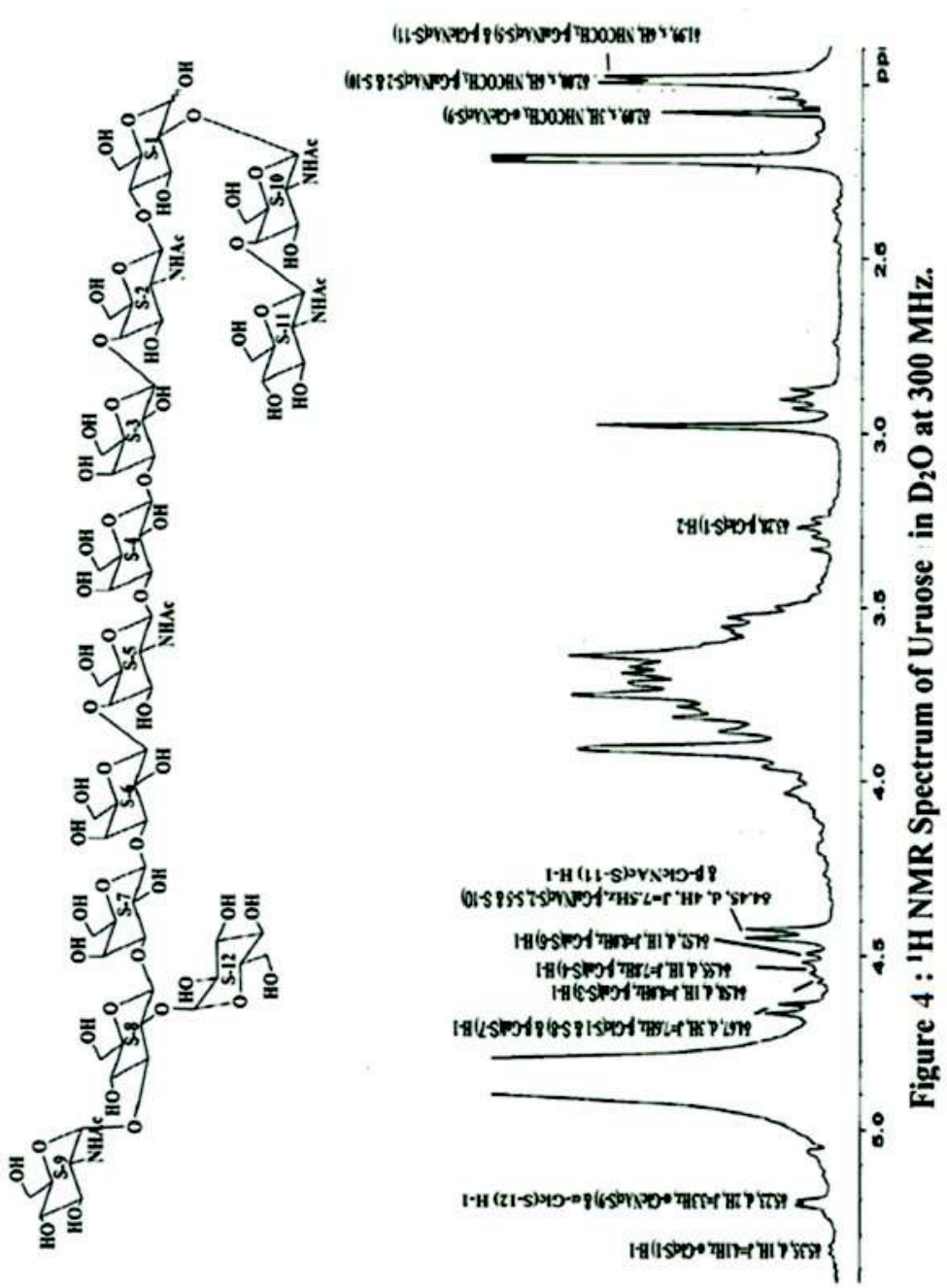


Figure 4 : ^{1}H NMR Spectrum of Uruose in D_2O at 300 MHz.

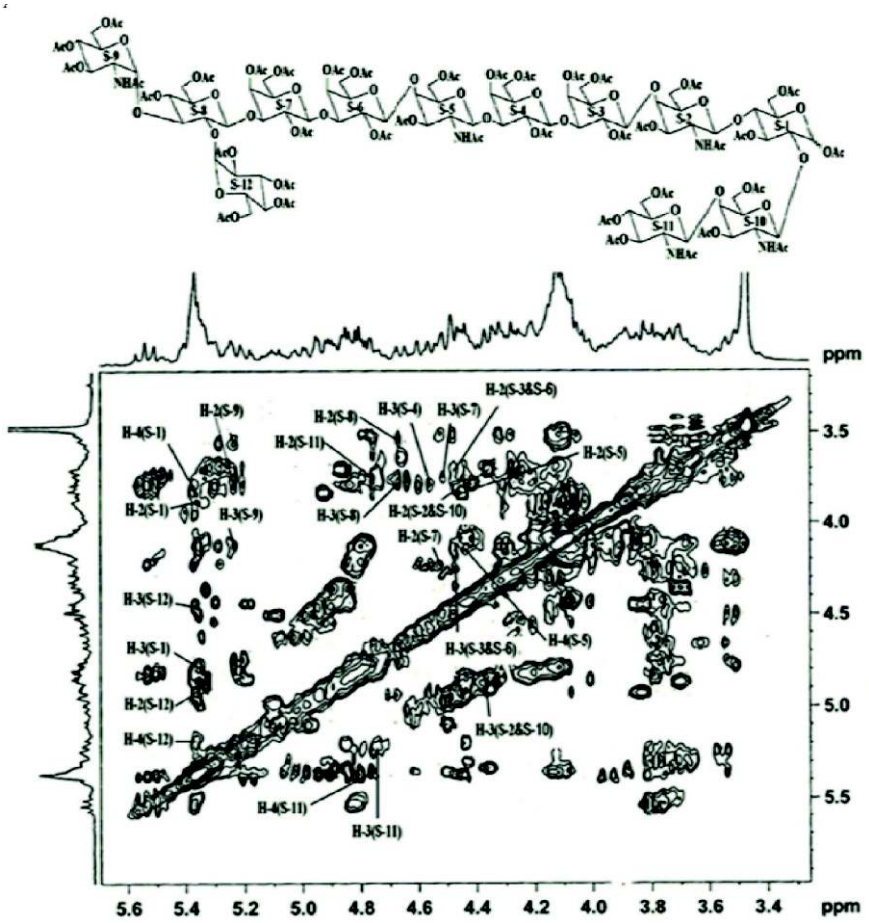


Figure 5 : TOCSY Spectrum of Uruose Acetate in $CDCl_3$ at 300 MHz.

^{1}H NMR Spectrum of uruose acetate assigned to β-GalNAc (S-2) gave three cross peaks at δ 4.37 x 3.81, δ 4.37 x 4.05 and δ 4.37 x 4.88 in TOCSY Spectrum **Fig 5** of uruose acetate, which were later identified as H-2, H-4 and H-3 respectively with COSY spectrum **Fig 6** of uruose acetate. Out of these signals one proton signal at δ 3.70 corresponded to H-2 position of β-GalNAc (S-2) where the other proton signal H-4 of β-GalNAc (S-2) observed at δ 4.05 was available for (1→4) glycosidic linkages by the next monosaccharide unit. The ^{1}H NMR signal of H-4 (S-2) at δ 4.05 gave a cross peak at δ 4.05 x 104.31 in HMBC spectrum **Fig 7** of uruose acetate confirming a (1→4) linkage between S-3 and S-2. The anomeric carbon signal present at δ 104.31 gave its complimentary signal at δ 4.46 in HSQC spectrum of uruose acetate in $CDCl_3$ at 300 MHz. The coupling constant of anomeric signal β-Gal[60,61] (S-3) with *J* value of 8.1Hz suggesting a *β* glycosidic linkage between S-3 and S-2. Further the signal of anomeric proton at δ 4.58 with J value 8.0 Hz in the ^{1}H NMR of uruose in D_2O at 300 MHz also confirmed a *β* glycosidically linked Gal (S-3). This (1→4) linkage between S-3 and S-2 was supported by the presence of H-4 signal of β-GalNAc (S-2) at δ 4.05 in upfield region of ^{1}H NMR spectrum and a cross peak at δ 4.05 x 71.25 in glycosidic region of HSQC spectrum of uruose acetate in $CDCl_3$. The anomeric protons signal present at δ 4.46 in ^{1}H NMR spectrum of uruose acetate assigned to β-Gal (S-3) gave three cross peaks at δ 4.46 x 3.70, δ 4.46 x 4.27 and δ 4.46 x 4.95 in TOCSY Spectrum of uruose acetate, which were later identified as H-3, H-2 and H-4 respectively by COSY spectrum of uruose acetate. The chemical shift of the cross peak at δ 4.46 x 3.70 suggested that in sugar S-3, one position was available for glycosidic linkage by next monosaccharide unit. The ^{1}H NMR signal of H-3 (S-3) at δ 3.70 gave a cross peak at δ 3.70 x 101.27 in HMBC spectrum of uruose acetate confirmed a (1→3) glycosidic linkage between

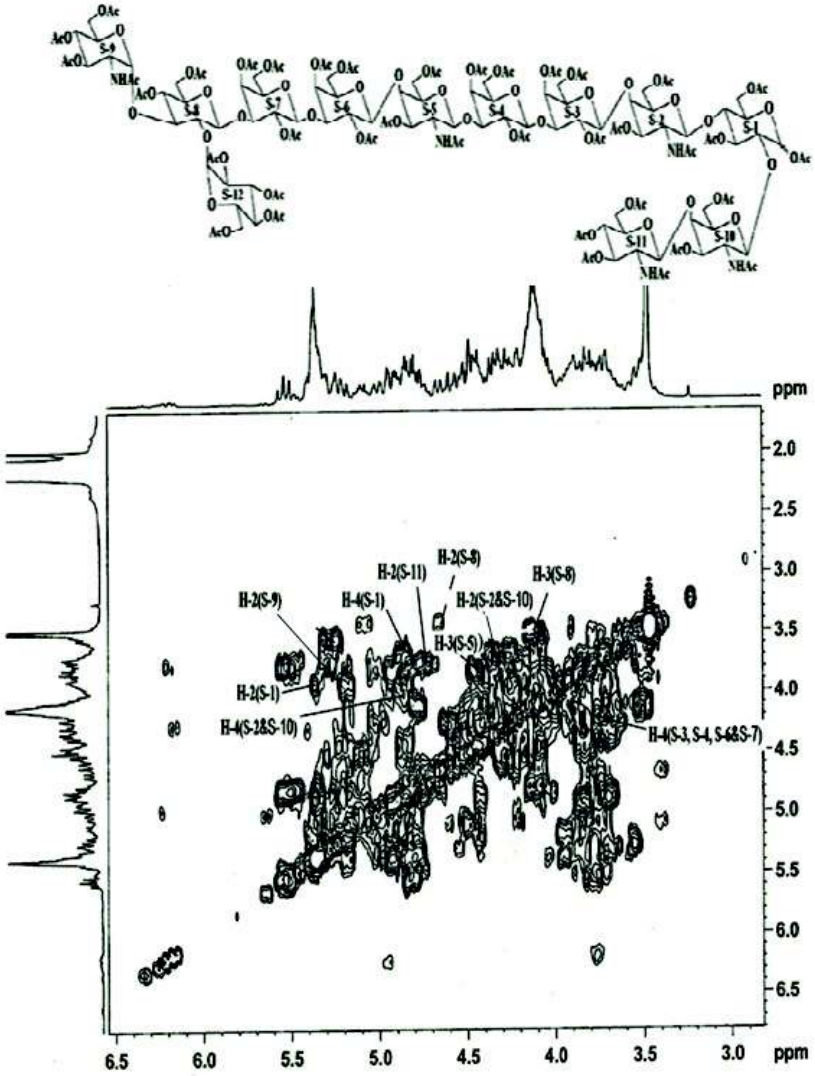


Figure 6: COSY Spectrum of Uruose Acetate in $CDCl_3$ at 300 MHz.

S-4 and S-3. The anomeric carbon signal at δ 101.27 gave its complimentary anomeric proton signal at δ 4.55 in the HSQC spectrum of uruose acetate. The anomeric proton signal present at δ 4.55 (S-4) had a *J* value of 7.9 Hz suggesting a *β* glycosidic linkage between S-4→S-3. Further signal of anomeric proton at δ 4.58 with *J* value of 8.0 Hz in the ^{1}H NMR of uruose in D_2O at 300 MHz also confirmed a β glycosidically linked Gal (S-4). The (1→3) linkage between β-Gal (S-3) and β-Gal (S-4)[60,61] was supported by the presence of H-3 signal of S-3 at δ 3.70 in upfield region of ^{1}H NMR spectrum and a cross peak at δ 3.70 x 69.11 in glycosidic region of HSQC spectrum of uruose acetate in $CDCl_3$. The anomeric proton signal present at δ 4.55 in ^{1}H NMR spectrum of uruose acetate assigned to β-Gal (S-4) gave three cross peaks at δ 4.55 x 3.75, δ 4.55 x 4.25 and δ 4.55 x 4.90 in TOCSY Spectrum of uruose acetate, which was later identified as H-3, H-2 and H-4 respectively by COSY spectrum of uruose acetate. The chemical shift of the cross peak at δ 4.55 x 3.75 suggested that in sugar S-4, one position was available for glycosidic linkage by next monosaccharide unit. The ^{1}H NMR signal of H-3 (S-4) at δ 3.75 gave a cross peak at δ 3.75 x 100.83 in HMBC spectrum of uruose acetate which confirmed a (1→3) linkage between (S-5) and (S-4). The anomeric carbon of (S-5) at δ 100.83

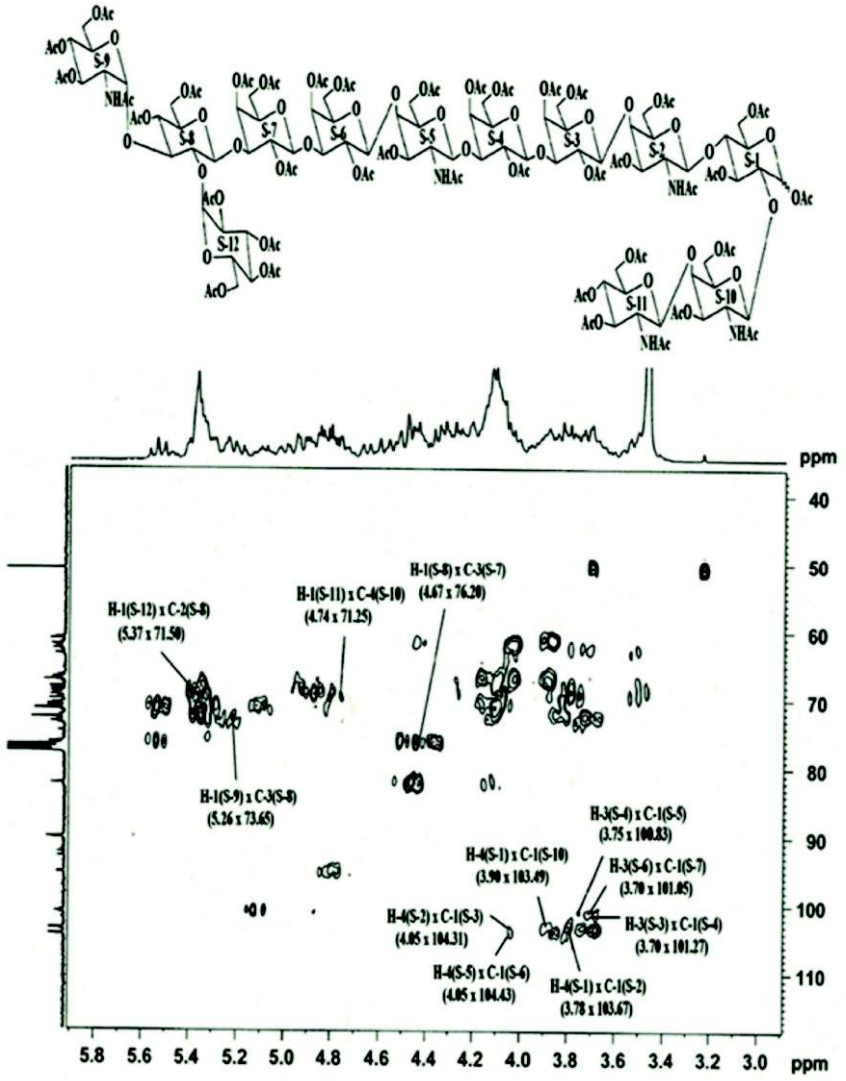
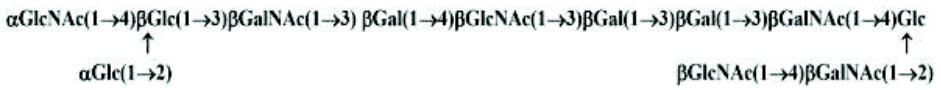


Figure 7: HMBC Spectrum of Uruose Acetate in $CDCl_3$ at 300 MHz.

gave its complimentary anomeric proton signal at δ 4.45 in the HSQC spectrum of uruose acetate. Simultaneously the ^{1}H NMR spectrum of uruose in D_2O at 300 MHz contain anomeric proton signal at δ 4.45 (*J* = 7.5 Hz) along with a singlet of three proton of amide methyl at δ 1.99 suggested that S-5 was GalNAc.[62] The anomeric proton signal present at δ 4.45 (S-5) had a *J* value of 7.6 Hz confirmed a *β* glycosidic linkage between S-5→S-4. Further signal of anomeric proton at δ 4.45 with *J* value of 7.5 Hz in the ^{1}H NMR of uruose in D_2O at 300 MHz also confirmed a β glycosidically linked GalNAc (S-5). The (1→3) linkage between β-Gal (S-4) and β-GalNAc (S-5)[55] was supported by the presence of H-3 signal of S-4 at δ 3.75 in upfield region of ^{1}H NMR spectrum and a cross peak at δ 3.75 x 72.82 in glycosidic region of HSQC spectrum **Fig 1** of uruose acetate in $CDCl_3$. The anomeric protons signal present at δ 4.45 in ^{1}H NMR spectrum of uruose acetate assigned to β-GalNAc (S-5) gave three cross peaks at δ 4.45 x 3.85, δ 4.45 x 4.05 and δ 4.45 x 5.20 in TOCSY spectrum **Fig 5** of uruose acetate, which were later identified as H-2 (containing NHAc group), H-4 and H-3 respectively by COSY spectrum **Fig 6** of uruose acetate. Out of these signals one proton signal at δ 3.85

corresponded to H-2 position of β-GalNAc (S-5) where as the other proton signal H-4 of β-GalNAc (S-5) observed at δ 4.05 was available for (1→4) glycosidic linkages by the next monosaccharide unit. The ^{1}H NMR signal of H-4 (S-5) at δ4.05 gave a cross peak at δ 4.05 x 104.43 in HMBC spectrum of uruose acetate confirming a (1→4) linkage between S-6 and S-5. The anomeric carbon signal present at δ 104.43 gave its complimentary signal at δ 4.46 in HSQC spectrum of uruose acetate in $CDCl_3$ at 300 MHz. The coupling constant of anomeric proton signal β-Gal (S-6) with *J* value of 8.1 Hz confirmed a *β* glycosidic linkage between S-6 and S-5. Further signal of anomeric proton at δ 4.52 with *J* value 8.0 Hz in the ^{1}H NMR of uruose in D_2O at 300 MHz also confirmed *β* glycosidically linked Gal (S-6).This (1→4) linkage between S-6 and S-5 was supported by the presence of H-4 signal of β-GalNAc (S-5) at δ 4.05 in upfield region of ^{1}H NMR spectrum and cross peak at δ 4.05 x 66.92 in glycosidic region of HSQC spectrum of uruose acetate in $CDCl_3$. The anomeric protons signal present at δ 4.46 in ^{1}H NMR Spectrum of uruose acetate assigned to β-Gal (S-6)[60,61] gave three cross peaks at δ 4.46 x 3.70, δ 4.46 x 4.27 and δ 4.46 x 4.95 in TOCSY Spectrum **Fig 5** of uruose acetate, which was later identified as H-3, H-2 and H-4 respectively by COSY spectrum **Fig 6** of uruose acetate. The chemical shift of the cross peak at δ 4.46 x 3.70 suggested that in sugar S-6, one position was available for glycosidic linkage by next monosaccharide unit. The ^{1}H NMR signal of H-3 (S-6) at δ 3.70 gave a cross peak at δ 3.70 x 101.05 in HMBC spectrum **Fig 7** of uruose acetate confirming a (1→3) linkage between (S-7) and (S-6). The anomeric carbon of β-Gal (S-7) at δ 101.05 gave its complimentary anomeric proton signal at δ4.51 in the HSQC spectrum of uruose acetate. The anomeric proton signal present at δ 4.51 (S-7) had a *J* value of 8.0 Hz confirmed a β glycosidic linkage between S-7→S-6. Further signal of anomeric proton at δ 4.67 with *J* value 7.6 Hz in the ^{1}H NMR of uruose in D_2O at 300 MHz also confirmed β glycosidically linked Gal (S-7). The (1→3) linkage between β-Gal (S-6) and β-Gal (S-7) was supported by the presence of H-3 signal of S-6 at δ 3.70 in upfield region of ^{1}H NMR spectrum The anomeric protons signal present at δ 4.51 in ^{1}H NMR Spectrum of uruose acetate assigned to β-Gal (S-7) gave three cross peaks at δ 4.51 x 3.76, δ 4.51 x 4.30 and δ 4.51 x 5.10 in TOCSY Spectrum **Fig 5** of uruose acetate which were later identified as H-3, H-2 and H-4 respectively by COSY spectrum **Fig 6** of uruose acetate. The chemical shift of the cross peak at δ 4.51x3.76 suggested that in sugar S-7, one position was available for glycosidic linkage by next monosaccharide unit. The H-3 position of β-Gal (S-7) at 3.76 showed its C-3 position at δ 76.20 in HSQC spectra of uruose acetate which showed a long range coupling with anomeric proton of next monosaccharide S-8 i.e. H-1 of S-8 and C-3 of S-7 at δ 4.67 x 76.20 in HMBC spectrum **Fig 7** of acetylated uruose in $CDCl_3$ at 300 MHz confirming a (1→3) linkage between S-8 and S-7. The anomeric proton of β-Glc (S-8) at δ 4.67 gave its complimentary anomeric carbon signal at δ95.28 in the HSQC spectrum of uruose acetate. The anomeric proton signal present at δ 4.67 (S-8) had a *J* value of 8.4 Hz suggesting a *β* glycosidic linkage between S-8→S-7. Further signal of anomeric proton at δ4.67 with J value 7.6 Hz in the ^{1}H NMR of uruose in D_2O at 300 MHz also confirmed *β* glycosidically linked Glc (S-8). This (1→3) linkage between S-8 and S-7 was supported by the presence of H-3 signal of β-Glc (S-8)[55,63] at δ 3.76 in upfield region of ^{1}H NMR spectrum and a cross peak at δ 3.76 x 76.20 in glycosidic region of HSQC spectrum of uruose acetate in $CDCl_3$. The anomeric protons signal present at δ 4.67 in ^{1}H NMR spectrum of uruose acetate assigned to β-Glc (S-8) gave three cross peaks at δ 4.67 x 3.55, δ 4.67 x 3.80 and δ 4.67 x 4.95 in TOCSY Spectrum of uruose acetate which were later identified as H-2, H-3 and H-4 respectively by COSY spectrum of uruose acetate. The chemical shift of the cross peak at δ 4.67 x 3.55 and δ 4.67 x 3.80 suggested that in sugar S-8, two positions were available for glycosidic linkage by next monosaccharide units. The H-3 position of β-Glc (S-8) at 3.80 showed its C-3 position at δ 73.65 in HSQC spectra of uruose acetate which showed a long range coupling with anomeric proton of next monosaccharide S-9 i.e. H-1 of S-9 and C-3 of S-8 at δ 5.23 x 73.65 in Reverse HMBC of acetylated uruose in $CDCl_3$ at 300 MHz suggesting the (1→3) linkage between S-9 and S-8. The anomeric proton at δ 5.26 gave its complimentary anomeric proton signal at δ 92.36 in the HSQC spectrum of uruose acetate. The anomeric proton signal present at δ 5.26 had a *J* value of 3.9 Hz confirmed *α* glycosidic linkage between S-9→S-8. Simultaneously the ^{1}H NMR spectrum of Uruose in D_2O at 300 MHz contain anomeric proton signal at δ 5.26 (*J* = 3.3Hz) along with a singlet of three proton of amide methyl at δ 2.09 suggesting that S-11 was GlcNAc. Further signal of anomeric proton at δ 5.23 with *J* value 3.3 Hz in the ^{1}H NMR of Uruose in D_2O at 300 MHz also confirmed α glycosidically linked GlcNAc (S-9). The (1→3) linkage between β-Glc (S-8) and α-GlcNAc (S-9)[56] was supported by the presence of H-3 signal of S-8 at δ 3.80 in upfield region of ^{1}H NMR spectrum and a cross peak at δ 3.76 x 76.20 in glycosidic region of HSQC spectrum of uruose acetate in $CDCl_3$. The anomeric proton signal

at δ 5.26 assigned to α-GlcNAc (S-9) gave three cross peaks at δ 5.26 x 3.91, δ 5.26 x 4.21 and δ 5.26 x 4.80 in the TOCSY spectrum, were later identified as H-2 (containing NHAc group), H-3 and H-4 respectively by COSY spectrum of uruose acetate. Therefore this anomeric proton did not show any cross peak in the linkage region hence confirming that α-GlcNAc (S-9) was present at non-reducing end and none of its hydroxyl group were available for glycosidic linkage. Since it was ascertained by the COSY and TOCSY spectrum of uruose acetate that the β-Glc (S-8) had two vacant positions i.e. H-2 and H-3 and it was already confirmed that H-3 of S-8 was linked with α-GlcNAc (S-9) whereas H-2 position of β-Glc (S-8) was lying vacant for glycosidic linkage by next monosaccharide unit. The H-2 position of β-Glc (S-8) at 3.55 showed its C-2 position at δ 71.50 in HSQC spectra of uruose acetate which showed a long range coupling with anomeric proton of next monosaccharide S-12 i.e. H-1 of S-12 and C-2 of S-8 at δ 5.37 x 71.50 in Reverse HMBC of acetylated uruose in $CDCl_3$ at 300 MHz suggesting a (1→2) linkage between S-12 and S-8. The anomeric proton signal at δ 5.37 gave its complimentary anomeric carbon signals at δ 92.98 in the HSQC spectrum of uruose acetate. The anomeric proton signal present at δ 5.37 for α-Glc (S-12)[63] had a *J* value of 3.9 Hz confirmed α glycosidic linkage between S-12→S-8. Further signal of anomeric proton at δ 5.23 with *J* value 3.3 Hz in the ^{1}H NMR of uruose in D_2O at 300 MHz also confirmed α glycosidically linked Glc (S-8). The (1→2) linkage between β-Glc(S-8) and α-Glc (S-12) was supported by the presence of H-2 signal of S-8 at δ3.55 in upfield region of ^{1}H NMR spectrum and a cross peak at δ3.55x71.50 in glycosidic region of HSQC spectrum of uruose acetate in $CDCl_3$. Further the anomeric proton signal at δ5.37 in ^{1}H NMR assigned to α-Glc (S-12) gave three cross peaks at δ 5.37x4.45, δ5.37x4.93 and δ5.37x5.25 in the TOCSY spectrum **Fig 5** of uruose acetate since this anomeric proton did not show any cross peak in the linkage region confirming that α-Glc (S-12) was present at non-reducing end and no hydroxyl group were available for glycosidic linkage. All signals obtained in ^{1}H and ^{13}C NMR of Uruose were in conformity with the assigned structure and their position which were confirmed by 2D NMR viz. COSY, TOCSY, HSQC and HMBC experiments of uruose acetate. Thus based on the pattern of chemical shifts of ^{1}H NMR, ^{13}C NMR, COSY, TOCSY, HSQC and HMBC



Uruose

experiments, it was interpreted that the compound E, uruose, was a dodecasaccharide having structure as-

ES MASS

The result obtained from the ES mass spectrum **Fig 8** further substantiated the structure of uruose which was derived by its ^{1}H and ^{13}C NMR spectra. The electron spray mass spectrometry data of uruose confirmed the derived structure and supported the sequence of monosaccharide in uruose. The highest mass ion peak was recorded at m/z 2167 which was due to $[M^+]$ confirming the molecular weight of uruose was 2167 and in agreement with its molecular formula $C_{82}H_{137}O_{61}N_5$. Further the mass fragments were formed by repeated H^+ transfer in the oligosaccharide and was accompanied by the elimination of terminal sugar less water. The fragmentation pathway confirmed the sequence of monosaccharide units in the dodecasaccharide. The dodecasaccharide uruose m/z 2167(I) fragmented to give mass ion peak at m/z 2005(II) [2167-S_{12}], this fragment was arisen due to the loss of terminal α-Glc (S_{12}) moiety from dodecasaccharide indicating the presence of α-Glc (S_{12}) at the non-reducing end. It further fragmented to give mass ion peak at m/z 1802(III) [2005-S_{11}] which was due to loss of β-GlcNAc (S_{11}) moiety from undecasaccharide.

This fragment of 1802 (III) further fragmented to give mass ion peak at m/z 1599(IV) [1802-S_{10}] which was due to loss of β-GalNAc (S_{10}) moiety from the

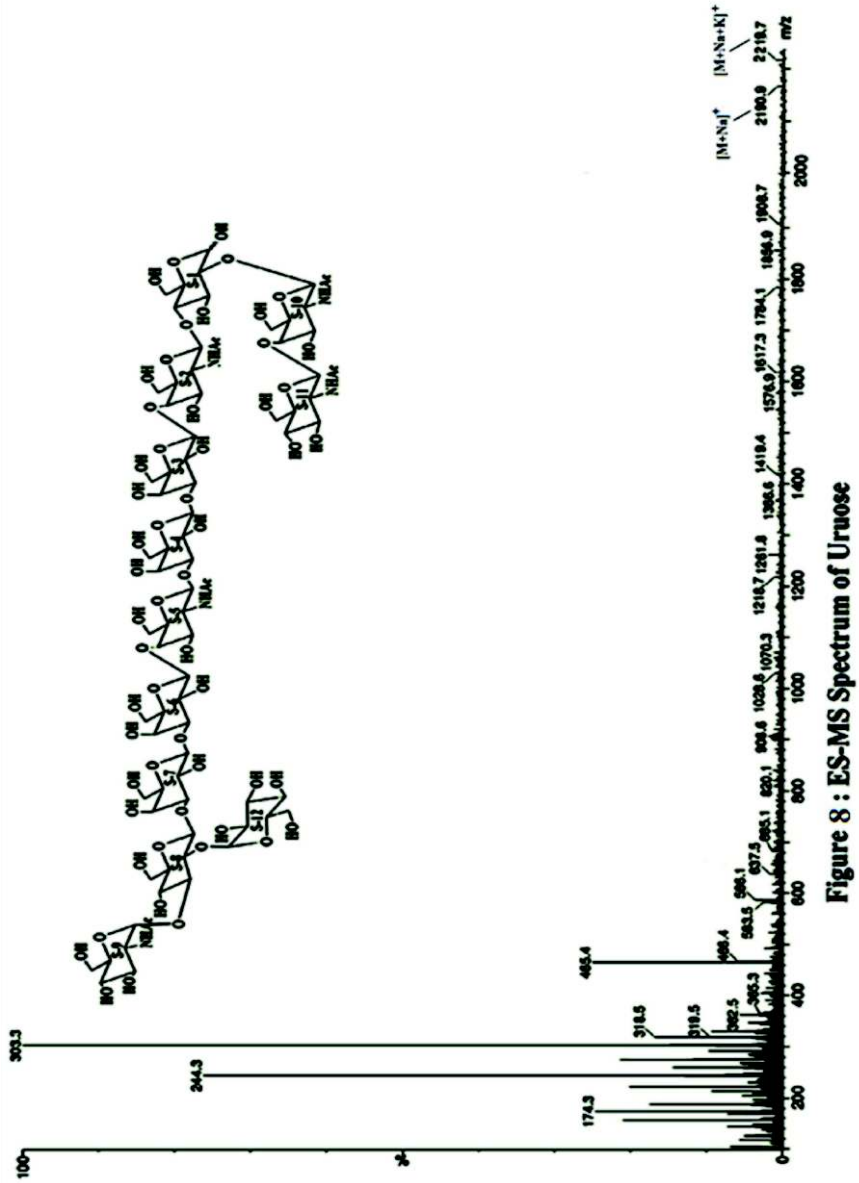
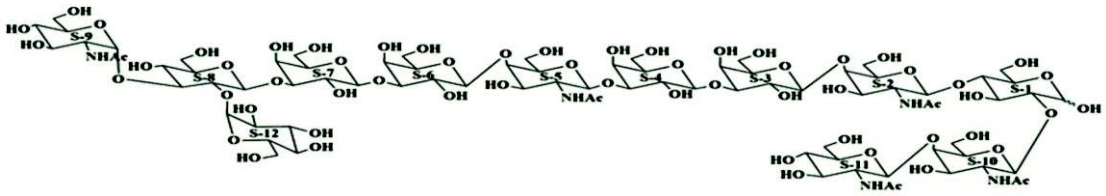


Figure 8 : ES-MS Spectrum of Uruose

decasaccharide. This nonasaccharide unit fragmented to give mass ion peak at m/z 1396(V) [1599-S_9] was due to loss of α-GlcNAc (S_9) moiety from nonasaccharide. The nonasaccharide m/z 1396(V) fragmented to give mass ion at m/z 1234(VI) [1396-S_8], this fragment was arisen due to the loss of α-Glc (S_8) moiety from octasaccharide indicating the presence of α-Glc (S_8). This fragment of 1234(VI) further to give mass ion peak at m/z 1072(VII) [1234-S_7] which was due to loss of β-Gal (S_7) moiety. This hexasaccharide unit fragmented to give mass ion peak at m/z 910(VIII) [1072-S_6], which was due to loss of β-Gal (S_6) moiety. The pentasaccharide m/z 910(S_5) fragmented to give mass ion at m/z 707 (IX) [910-S_5], this fragment was arisen due to the loss of β-GalNAc (S_5) moiety from pentasaccharide indicating the presence of β-GalNAc (S_5). This tetrasaccharide unit fragmented to give mass ion peak at m/z 545(X) [707-S_3], which was due to loss of β-Gal (S_4) moiety from trisaccharide. The trisaccharide m/z 545(S_3) fragmented to give mass ion at m/z 383(XI) [545-S_3], this fragment was arisen due to the loss of β-Gal (S_3) moiety from tetrasaccharide. Further peak at fragment of 383(XI) was fragmented to give mass ion peak at m/z 180(XII) [383-S_2] which was

due to loss of β-GalNAc (S_2) moiety from the disaccharide. The other fragmentation pathway in ES Mass spectrum of E, uruose, m/z 2167 shows the mass ion peak at 2089[2167-CH_2OHCHO, -H_2O], 1908[2005-CH_2OCHO, -$2H_3O^+$], 1856[2005-$NHCOCH_3$,-HOCHCHOH,-HCHO,-H^+], 1784[1802-H_2O], 1666[1784 -$NHCOCH_3$,-CH_2OHCHO], 1617[1666-CH_3OH,-OH], 1576[1666 ,-HOCHCHOH,-HCHO], 1419[1599-$NHCOCH_3$,-$CH_2OHCOCO$, -H_2O,-OH], 1366[1396-HCHO], 1261[1366-HOCHCHOH,-CH_3CHO,-H^+], 1218[1396-$CH_2OHCOCO$, -HOCHCHOH, -HCHO, H^+], 1070[1234-2CH_2OHCHO,-CH_3CHO], 1028[1070-CH_3CHO,], 906[1028,-HOCHCHOH,-CH_3CHO, -H_2O], 820[910-$NHCOCH_3$, -CH_3OH], 685[820-CH_2OCHO,-CH_3CHO, -CH_3OH], 637[707-CH_3OH ,-H_2O], 583[637 -3H_2O], 483[545-CH_3CHO,-H_2O], 466[483-OH], 465[466-H^+], 365[383-H_2O], 319[365-CHO,-OH], 318[319-H^+], 303[383-2H_2O,-CH_3CHO], 244[303-CH_2OCHO] and 274[303-$NHCOCH_3$,-CH_2CO,-CHO]. Based on result obtained from chemical degradation/acid hydrolysis, Chemical transformation, Electro spray mass spectrometry, structure reporter group and 1D-NMR viz. ^{1}H NMR, ^{13}C NMR, and 2D-NMR viz. COSY, TOCSY, HMBC and HSQC spectra of uruose acetate and uruose, the structure and sequence of isolated novel oligosaccharide uruose was deduced as-



URUOSE

4. Conclusion

In summary, the novel milk oligosaccharide, namely, uruose has been isolated from Lal-Muha cow milk and its structure has been elucidated with the help of ^{1}H and ^{13}C 2D NMR spectroscopy and mass spectrometry.

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