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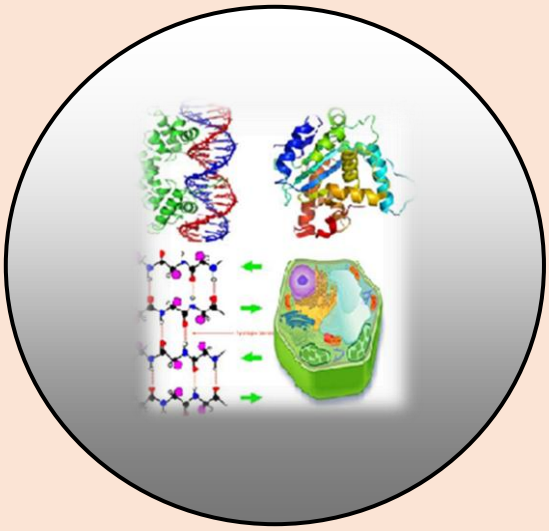

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# Isolation and Structure Elucidation of Novel Oligosaccharide Vediose from *Bubalus bubalis* Colostrum

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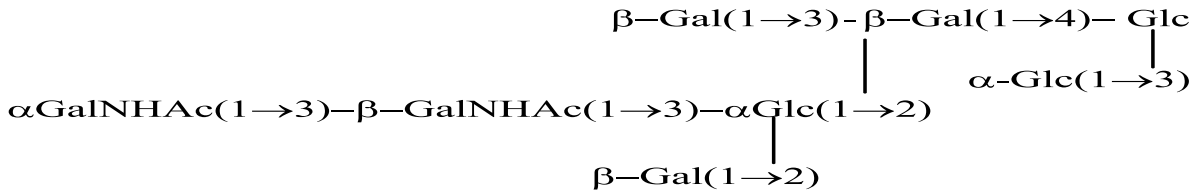
## ABSTRACT

***Milk is a rich source of carbohydrate which includes oligosaccharides and glyconjugates. Oligosaccharides are natural constituents of all bacteria, fungi, plants, and milk. Milk oligosaccharides stimulate the growth of beneficial microorganisms in the infant colon. They also inhibit the adhesion of pathogenic microorganisms to the intestinal and urinary tract by acting as receptor analogues, thus preventing gastric and urinary infections. The milk oligosaccharides recognize cancer associated antigens, used as antimicrobial agents; tumor associated antigens and has physiological significance in infants. It protects any new born against infections and provides nutrients. Milk obtained from different types of animal origin is of two types. One is "colostrum" means early milk and another is "mature milk" that is late or normal milk. The colostrum contains significant quantities of complement components that act as natural antimicrobial agent which actively stimulate the maturation of an infant's immune system. Buffalo milk have ability to stimulate non-specific immunological resistance of the host against parasitic infections. Buffalo colostrum intake influences metabolism, endocrine systems and the nutritional state of neonate. In continuation to our previous studies and keeping above mentioned biological activities of Buffalo milk oligosaccharides in mind, we have isolated buffalo colostrum oligosaccharide mixture by modified method of Kobata and Ginsburg. This oligosaccharide mixture on acetylation followed by silica gel column chromatography resulted into isolation of a novel oligosaccharide namely Vediose. The structure of isolated compound was elucidated by chemical transformation, chemical degradation, $^{1}H$ NMR, 2-D NMR (COSY, TOCSY, HSQC) and mass spectrometry.***




***VEDIOSE***

***Keywords: Carbohydrate, Bubalus bubalis, Colostrum, Oligosaccharide and Vediose.***

## INTRODUCTION

Milk is a natural source for the physiological and biological development of any neonate provided to him by his mother in any mammal. It is a biological fluid of unique complexity and richness. Oligosaccharides are found as natural constituents of fruits, vegetables, milk, blood, bacteria and fungus etc. and have various physiological functions (Rawle, et al., 2000, Kanemitsu and Kanie, 1999, Imberty and Perez, 2000). Oligosaccharides play an essential role in many molecular processes impacting eukaryotic biology and diseases and exhibit varied biological activity such as immunostimulant, hypoglycemic, antithrombotic, antitumor (Lee et al. 1987), antiviral, anticancer, anticoagulant, anticomplementary, immunological and anti-inflammatory activities (Ehresmann et al., 1979). A large number of biologically active oligosaccharides have been isolated from human, buffalo (Gangwar et al., 2017), donkey, cow (Gunjan et al., 2016), mare (Maurya R.K. et al., 2017), sheep (Ranjan et al., 2015) and goat milk (Kumar et al., 2016). Buffalo milk is a rich source of riboflavin, vitamin $B_{12}$, vitamin A, and thiamine. Buffalo milk oligosaccharides have ability to stimulate non-specific immunological resistance (Deepak et al., 1998) of the host against parasitic infections (Saxena, et al., 1999). Keeping in mind the immunostimulant activity of buffalo milk it was thought to isolate some more novel biologically active oligosaccharide from colostrum of buffalo. Present research work includes isolation of a novel oligosaccharide from colostrum of buffalo and its three dimensional structure was elucidated by chemical degradation, chemical transformation and spectroscopic techniques like NMR including $^{1}H$, $^{13}C$, and 2-D NMR (COSY, TOCSY, HSQC) and Mass Spectrum.

## EXPERIMENTAL PROCEDURE

### General procedure

General procedure was same as described in our previous articles (Gunjan et al. 2016).

### Isolation of milk oligosaccharides from Buffalo Colostrum by the modified method of Kobata and Ginsburg

Isolation of buffalo colostrum milk oligosaccharide was done by the modified method of Kobata and Ginsburg (Kumar et al., 2016) which was described in our previous communication (Khan et al., 2018). The isolation was done from 10 litre of buffalo colostrum. The yield of oligosaccharide mixture was 443 gm.

### Acetylation of Buffalo colostrum oligosaccharide mixture

10 gm of crude oligosaccharide mixture was acetylated by adding pyridine (10 ml) and acetic anhydride (10 ml) at 60$^{o}$C with constant stirring and kept overnight. The mixture was evaporated under reduced pressure and the viscous residue was taken in $CHCl_3$ (250 ml) and washed with ice cold water. The organic layer was dried over anhydrous $Na_2SO_4$, filtered and evaporated to dryness yielding the acetylated mixture (9.60 gm). The acetylation converted the free sugars into their nonpolar acetyl derivatives which were resolved nicely on TLC, giving eight spots on TLC i.e., a, b, c, d, e, f, g and h.

### Purification of acetylated oligosaccharide mixture by column chromatography

Acetylated buffalo colostrum oligosaccharide mixture (9.60gm) gave eight spots a,b,c,d,e,f,g and h on TLC which on column chromatography over silica gel by various proportion of $CHCl_3$ and $CHCl_3$: MeOH resulted into isolation of compound Vediose in pure form (72mg).

**Deacetylation of compound c (Vediose acetate)**

Compound 'Vediose' (72 mg) was obtained from fifth column chromatography of acetylated oligosaccharide mixture. 72 mg of compound Vediose was dissolved in acetone (2 ml) and 3 ml of $NH_3$ was added into it and was left overnight in a stoppered hydrolysis flask. After 24 hrs ammonia was removed under reduced pressure and the compound was washed thrice with $CHCl_3$ (5 ml) (to remove acetamide) and water layer was finally freeze dried giving the deacetylated oligosaccharide Vediose C (26 mg).

**Methyl glycosidation/Acid hydrolysis of compound C Vediose**

Compound Vediose (8 mg) was ref1uxed with absolute MeOH (2 ml) at 70°C for 18 h in the presence of cation exchange IR-l20 (H) resin. The reaction mixture was filtered while hot and filtrate was concentrated. To this solution 1, 4-dioxane (1 ml), and 0.1N $H_2SO_4$ (1 ml) was added and the solution was warmed for 30 minute at 50˚C. The hydrolysis was complete after 24 hours. The hydrolysate was neutralized with freshly prepared $BaCO_3$ and concentrated under reduced pressure to afford α-and β-methyl glucosides along with the Glc, Gal and GlcNAc. Their identification was confirmed by comparison with authentic samples on TLC, and PC.

**Kiliani hydrolysis of compound C (Vediose)**

Compound Vediose (5 mg) was dissolved in 2 ml Kiliani mixture (AcOH-$H_2O$-HCl, 7:11:2) and heated at 100$^o$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 and was evaporated under reduced pressure to afford glucose, Gal and GlcNAc on comparison with authentic samples of glucose, Gal and GlcNAc.

**Description of compound Vediose**

**Compound (MSBC-2) Vediose:**

Compound Vediose (34mg) was obtained from fraction 41-59 of fifth column chromatography. On deacetylation of 34 mg of substance with $NH_3$ / acetone, it afforded compound C (31mg), for experimental analysis, this compound was dried over $P_2O_5$ at 100$^0$ C and 0.1 mm pressure for 8 hr**.**

| $C_{52}H_{88}O_{14}N_2$ | %C | %H | %N |
|---|---|---|---|
| Calculated | 44.69 | 6.30 | 2.00 |
| Found | 44.70 | 6.35 | 2.00 |

It gave positive Phenol-Sulphuric acid test, Feigl test and Morgon-Elson test.

**$^1$H NMR Vediose: δ in $D_2O$ at 300 MHz**

δ 5.20 [d, 1H, J = 3.2, α- Glc(S-1), H-1], 4.62[ d, 1H, J = 7.8, β Glc(S-1), H-1], 5.70 [d,1H, J= 2.0 Hz, α Glc(S-3), H-1], 5.54 [d, 1H, J = 3.0, α -Glc (S-6), H-1], 4.52 [d, 1H, J = 7.4, β-Gal (S-7), H-1], 4.51 [d, 1H, J = 7.8, β-Gal (S-8), H-1], 4.43 [d, 1H, J = 7.8, β-GalNAc (S-4),H-1], 1.96 [S,3H, β-GalNAc (S-4)NHCOCH$_3$], 1.92 [S,3H, α-GalNAc (S-5)NHCOCH$_3$].

**$^1$H NMR of Vediose acetate: δ in $CDCl_3$ at 400 MHz**

6.22 [d, 1H, J = 3.2 Hz, α -Glc (S-1) H-1], 5.65 [d, 1H, J = 8.4 Hz, β-Glc (S-1) H-1], 5.57 [d,1H, J = 2.0 Hz, α -Glc (S-3) H-1], 5.34 [d, 1H, J = 3.0 Hz, α -Glc (S-6) H-1], 5.27[d, 1H, J =1.6 Hz, α -GalNAc (S-5) H-1], 4.67 [d, 1H, J = 6.0Hz, β-Gal (S-2) H-1], 4.60 [d, 1H, J =7.2, β-Gal (S-7) H-1], 4.54 [d, 1H, J = 8.4, β-Gal (S-8) H-1], 4.46 [d, 1H, J = 7.8, β-GalNAc (S-4) H-1], 3.52 [m, 1H, β-Glc (S-1) H-4], 3.73 [m, 2H, β-Glc (S-1) H-3& β-Gal(S-2) H-3], 3.70 [m, 2H, α -Glc (S-3) H-3& β-GalNAc (S-4) H-3], 3.53 [m, 1H, α -Glc (S-3) H-2, 3.40 [m, 1H, β-Gal (S-2)H-2].

**$^{13}$C of Vediose acetate**: δ **in $CDCl_3$ at 400 MHz**

89.00[1C,-Glc (S-1) C-1], 91.08[1C, β -Glc (S-1) C-1], 93.06[1C, α -Glc (S-3) C-1], 90.00 [1C, α -Glc (S-6) C-1], 92.01[1C, α -GalNAc(S-5) C-1], 96.01[1C, β-Gal (S-2) C-1], 102.02 [3C, β-Gal(S-7, S-8, S-4), C-1]

**ES Mass of compound C Vediose**

1458[M+Na+K], 1435$[M+K]^+$, 1396[M] +, 1297, 1277, 1234 ,1217, 1205, 1072, 1041,1013, 910, 861, 835, 852, 707, 545, 504, 486, 428, 473, 342, 310, 283, 291, 180

## RESULT AND DISCUSSION

### Structure elucidations of Vediose

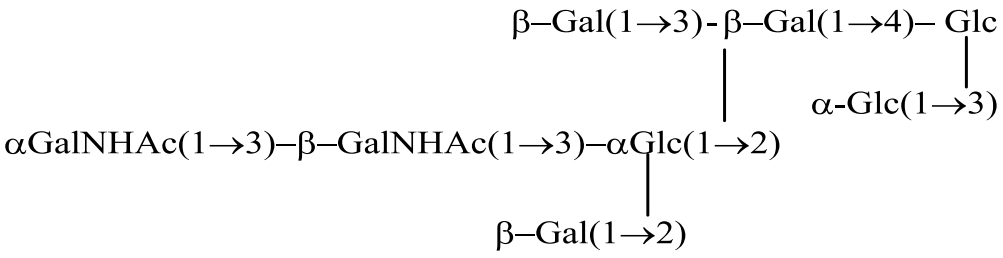
### NMR spectroscopy

Compound C, $C_{52}H_{88}O_{41}N_2$, gave positive phenol-sulphuric acid test (Dubois et al., 1956), Fiegl test (Fiegl et al., 1975) and Morgon-Elson test (Gey et al., 1996) showing the presence of normal and amino sugars moiety in the compound Vediose. The HSQC spectrum of acetylated Vediose showed the presence of nine cross peaks of anomeric protons and carbons in the anomeric region at δ 6.22 x 89.00, 5.65 x 91.08, 5.57 x 93.06, 5.34 x 90.00, 5.27 x 92.01, 4.67 x 96.01, 4.60 x 102.02, 4.54 x 102.02 and 4.46 x 102.02 suggesting the presence of nine anomeric protons and carbons in it. The presence of nine anomeric protons were confirmed by the presence of nine anomeric proton doublets at δ 6.2 δ 2 (J = 3.2 Hz), 5.65 (J = 8.4 Hz), 5.57 (J = 2.0 Hz), 5.34 (J = 3.0 Hz), 5.27 (J = 1.6 Hz), 4.67 (J = 6.0 Hz), 4.60 (J= 7.2 Hz), 4.54 (J = 8.4 Hz) and 4.46 (J = 7.8 Hz) in the $^1$H NMR of acetylated compound C (Fig. 3.25). The presence of nine anomeric carbons were confirmed by the presence of nine anomeric carbon signals at δ 102.02 (3C), 96.01 (1C), 93.06(1C), 92.01 (1C), 91.08 (1C), 90.00 (1C), 89.00 (1C), in the $^{13}$C NMR spectrum of acetylated Vediose. These data suggested that compound Vediose may be an octasaccharide in its reducing form. The $^1$H NMR of Vediose in $D_2O$ at 300 MHz shows doublet for anomeric protons at δ 5.70 (1H), 5.54 (1H), 5.20 (1H), 4.62 (1H), 4.52 (1H), 4.51 (1H), 4.43(1H). Further the ES Mass spectrum of Vediose showed the highest mass ion peaks at m/z 1458 assigned to $[M+Na+K]^+$ and m/z 1435 assigned to $[M+K]^+$, it also contain the molecular ion peak at m/z 1396 confirming the molecular weight as 1396 which was in agreement of derived composition $C_{52}H_{88}O_{41}N_2$. The reducing nature of compound Vediose was confirmed by methylglycosylation of compound Vediose by $MeOH/H^+$ followed by its acid hydrolysis which led to the isolation of α and methyl glucosides, suggesting the presence of glucose at the reducing end. For convenience the eight monosaccharides present in compound Vediose have been designated as S-1, S-2, S-3, S-4, S-5, S-6, S-7 and S-8 respectively starting from the reducing end. The monosaccharide constituents in compound Vediose were confirmed by Killiani hydrolysis (Killiani, 1930) under strong acidic conditions, followed by PC and TLC. In this hydrolysis four spots were found on PC and TLC which were found identical in glucose (Glc), galactose (Gal), N-acetylglucosamine (GlcNAc) and N- acetylgalactosamine (GalNAc) and by co-chromatography with authentic samples. Thus the octasaccharide contained four types of monosaccharide units i.e. Glc, Gal, GalNAc and GlcNAc in it. The presence of two anomeric proton signals at δ 5.20 (J =3.2 Hz) and δ 4.62 (J = 7.8 Hz) in the $^1$H NMR spectrum of Vediose in $D_2O$ at 300 MHz were assigned for α

and β anomers of glucose (S-1) confirming the presence of Glc (S-1) at the reducing end in compound Vediose. The $^{1}$H NMR of Vediose also contain a triplet δ 3.26 (Stucture Reporter Groups) (Dua, and Bush, 1983) suggesting for presence of lactose type of structure at reducing end confirming the 1→4 linkage between β-Gal (S-2) and β-Glc (S-1), hence suggesting the presence of lactosyl moiety at the reducing end. Since the HSQC spectrum of Vediose acetate showed nine anomeric protons and carbon signal, confirming Vediose has octasaccharide in its reducing form. The anomeric signal for α and β anomers were detected at δ 6.22 (J =3.2 Hz) and δ 5.65 (J = 8.4 Hz), respectively, which were assigned for reducing glucose. In the TOCSY spectrum of Vediose acetate the anomeric signal of β-Glc (S-1) at δ 5.65 (J = 8.4 Hz) gave cross peak at δ 5.65 x 3.52, 5.65 x 3.73, 5.65 x 5.00, out of which two peaks at δ 5.65 x 3.52 & 5.65 x δ 3.73, in the TOCSY spectrum of Vediose acetate in $CDCl_3$ showed that the two position in S-1 were available for glycosidic linkages, showing that the two hydroxyl groups of β-Glc (S-1) were involved in glycosidic linkages by other monosaccharide moieties (Fig. 3.26). These signals at δ 3.52 and δ 3.73 were identified for H-4 and H-3 of β-Glc (S-1) by the COSY spectrum of Vediose acetate suggesting that H-4 and H-3 of β-Glc (S-1) were available for glycosidic linkages by the next monosaccharide units. In the$^{1}$H NMR presence of another anomeric proton doublet at δ 4.67 (J = 6.0 Hz) of Vediose acetate in CDCl3, confirming the presence of β-Gal (S-2) residue as the next monosaccharide. Since it was explained earlier by $^{1}$H NMR of Vediose in $D_2O$ at 300 MHz that due to the presence of anomeric proton signals at δ 5.20 (J = 3.2 Hz) and 4.62 (J = 7.8 Hz) for reducing glucose along with a triplet at δ 3.26 suggesting a lactose type structure at the reducing end, hence further the $^{1}$H NMR signal for H-4 of S-1 at δ 3.52 in the $^{1}$H NMR of Vediose acetate confirmed H-4 of Glc (S-1) was glycosidically linked to Gal (S-2), concluded that Gal (S-2) must be glycosidically linked to H-4 of Glc (S-1). The coupling constant of anomeric proton signal at δ 4.67 β-Gal (S-2) with J value of 7.2 Hz confirmed the β-configuration of the β-Gal (S-2) moiety and hence β 1→4 glycosidic linkage between S-2 and S-1 was confirmed. The next anomeric proton signal appeared as doublet at δ 5.34 (J = 3 Hz) in the $^{1}$H NMR spectrum of Vediose acetate in CDCl3 at 400 MHz was due to the presence of α-Glc (S-6) moiety, since the Glc (S-1) possess H-3 and H-4 position for glycosidic linkage and position H-4 was already occupied by Gal (S-2). Hence, the leftover H-3 of β-Glc(S-1) was glycosically linkaged by the α-Glc (S-6) moiety. The anomeric position at δ 5.34 have a small coupling constant (J = 3.0 Hz) and it was confirmed that α-1→3 glycosidic linkage between S-6 and S-1. Since, none of methine proton of α-Glc (S-6) gave its cross peak in the linkage region i.e. δ 3-4 in the TOCSY spectrum of Vediose acetate confirms that α-Glc (S-6) was linked at the non-reducing end. Further in the $^{1}$H NMR of Vediose acetate presence of anomeric proton doublet at δ 4.67 (J =6.0 Hz), assigned to β-Gal (S-2), in the TOCSY spectrum of Vediose acetate anomeric signal at δ 4.67 (J = 6.0 Hz) showed cross peak at δ 4.67 x 3.40, 4.67 x 3.50, 4.67 x 3.64, 4.67 x 3.73, out of which two cross peaks at δ 4.67 x 3.40 & 4.67 x 3.73 in the $^{1}$H NMR of Vediose acetate in $CDCl_3$ showed their two position in S-2 were available for glycosidic linkages, showing that the two hydroxyl groups of β-Gal (S-2) were involved in glycosidic linkages by other monosaccharide moieties. These position of δ 3.40 and δ 3.73 were later confirmed as H-2 and H-3 of β-Gal (S-2) confirming that H-2 and H-3 of β-Gal (S-2) were available for glycosidic linkage by next monosaccharides. The next anomeric proton signal appeared as doublet at δ4.6(J = 7.2 Hz) in the $^{1}$H NMR spectrum of

Vediose acetate in $CDCl_3$ at 400 MHz was due to the presence of β Gal (S-7) moiety. The $^1H$ NMR signals at δ 4.60 of Vediose acetate gave its complementary signal at δ 102.2 in the HSQC spectrum of Vediose acetate. Further the anomeric signal of 102.2 shows its cross peak with the chemical shift of $^1H$ NMR signal present at δ 3.73 and gave cross peak at 102.2 x 3.73 in the HMBC spectrum of Vediose acetate confirming that the Gal (S-7) was linked glycosidically with H-3 of S-2 confirming the 1→3 glycosidic linkage between S-7 and S-2. The anomeric proton signal of S-7 present at δ 4.60 (J = 7.2 Hz) showed a large coupling constant suggesting a β-glycosidic linkage between S-7 and S-2 confirming the 1→3 glycosidic linkage between S-7 and S-2 (Fig. 3.28). Since, none of methine proton of β-Gal (S-7) came in the linkage region in the TOCSY spectrum of Vediose acetate it confirmed that β-Gal (S-7) was linked at the non-reducing end. The anomeric position at δ 4.60 have a large coupling constant (J = 7.2 Hz) and it was confirmed that β-(1→3) glycosidic linkage between S-7 and S-2. Since, none of methine proton of β-Gal (S-7) gave its cross peak in the linkage region i.e. δ 3-4 in the TOCSY spectrum of Vediose acetate confirms that β-Gal (S-7) was linked at the non-reducing end. The next anomeric proton signal at δ 5.57 (J = 2.0 Hz) in the $^1H$ NMR spectrum of Vediose acetate was assigned to α-Glc (S-3), since it was already established earlier that H-3 of S-2 was glycosidically linked by Gal (S-7) and H-2 of S-2 was vacant therefore α-Glc (S-3) must be attached to H-2 of S-2. The anomeric proton signal of S-3 present at δ 5.57 (J = 2.0 Hz) showed a small coupling constant suggesting the α -glycosidic linkage between S-3 and S-2 confirming the α-1→3 glycosidic linkage between S-3 and S-2. The anomeric position at δ 5.57 have a small coupling constant (J = 2.0 Hz) and it was confirmed that α-1→2 glycosidic linkage between S-3 and S-2. Further the anomeric proton signal of α-Glc (S-3) at δ 5.57 (J =2.0 Hz) in the TOCSY spectrum of Vediose acetate showed cross peak at 5.57 x 3.53, 5.57 x 3.70 in $CDCl_3$ at 400 MHz. These signals showed their two positions in S-3 were available for glycosidic linkages, showing that the two hydroxyl groups of α-Glc (S-3) were involved in glycosidic linkages by other monosaccharide moieties. These position of δ 3.53 and δ 3.70 were later confirmed as H-2 and H-3 of α-Glc (S-3) confirming that H-2 and H-3 of α-Glc (S-3) were available for glycosidic linkage by next monosaccharides. The next anomeric proton signal appeared as doublet at δ 4.54(J = 8.4 Hz) in the $^1H$ NMR spectrum of Vediose acetate in $CDCl_3$ at 400 MHz was due to the presence of β Gal (S-8) moiety. The $^1H$ NMR signals at δ 4.54 in the $^1H$ NMR of Vediose acetate gave its complementary signal at δ 102.2 in the HSQC spectrum. Further the anomeric signal of 102.2 shows its cross peak with the chemical shift of $^1H$ NMR signal present at δ 3.53, gave cross peak at 102.2 x 3.53 in the HMBC spectrum confirming that the Gal (S-8) was linked glycosidically with H-2 of S-3 confirming the β-1→2 glycosidic linkage between S-8 and S-3 (Fig. 3.28). The anomeric proton signal of S-8 present at δ 4.54 (J = 8.4 Hz) showed a large coupling constant suggesting a β-glycosidic linkage between S-8 and S-3 confirming β-1→2 glycosidic linkage between S-8 and S-3. Since, none of methine proton of β-Gal (S-8) came in the linkage region which also confirmed that β-Gal (S-8) was linked at the non-reducing end. The next anomeric proton signal at δ 4.46 (J = 8.4 Hz) along with a singlet of amide methyl (-NHCOCH3) at δ 1.96 in the $^1H$ NMR spectrum of Vediose acetate was assigned to β- GalNAc (S-4). It was already established earlier that H-2 of S-3 was already glycosidically linked by Gal (S-8) and H-3 of S-3 was vacant therefore β-GalNAc (S-4) must be attached to H-3 of S-3 confirming the 1→3 glycosidic linkage between S-4 and S-3.

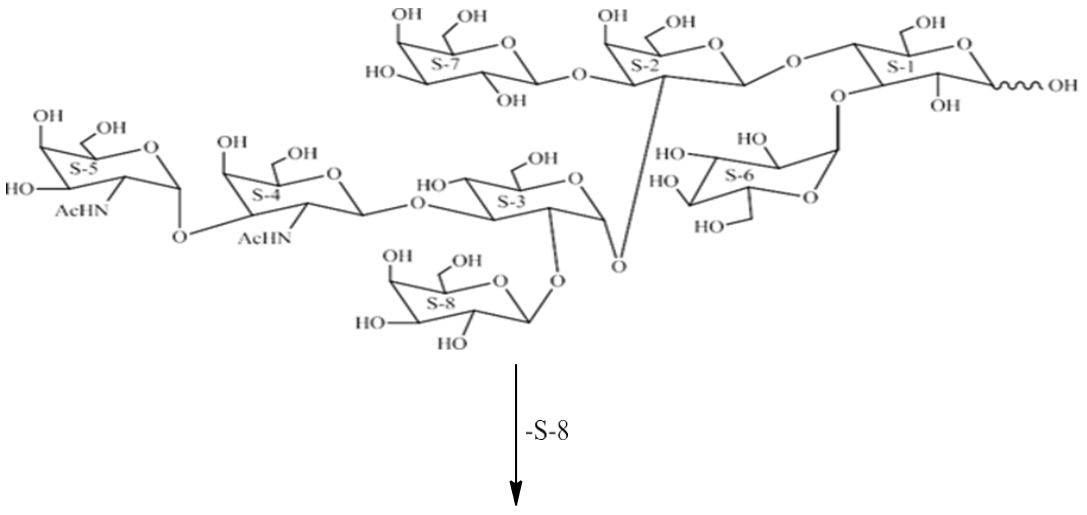
The anomeric proton signal of S-4 present at δ 4.46 (J = 8.4 Hz) showed a large coupling constant suggesting a β-glycosidic linkage between S-4 and S-3 confirming the β-1→3 glycosidic linkage between S-4 and S-3. Further the anomeric proton signal of β-GalNAc (S-4) at δ 4.46 (J = 8.4 Hz) in the TOCSY spectrum of Vediose acetate showed cross peak at 4.46 x 3.70, 4.46 x 4.13, 4.46 x 4.90, in $CDCl_3$ at 400 MHz, out of which one signals δ 4.46 x 3.70, showed in the linkage region, indicate their one position in S-4 were available for glycosidic linkages, which showed that the one hydroxyl groups of β-GalNAc (S-4) were involved in glycosidic linkages by other monosaccharide moieties. Other signal at δ 4.46 x 4.13 confirms the position of NAc group at H-2 position of S-4 moiety at δ4.13. The position of δ 3.70 was later confirmed at H-3 of β-GalNAc (S-4) confirming that H-3 of β-GalNAc (S-4) was available for glycosidic linkage by next monosaccharides. The next anomeric proton signal at δ 5.27 (J = 1.6 Hz) in the $^1$H NMR spectrum of Vediose acetate was assigned to α-GalNAc (S-5) and it was already established earlier that H-3 of S-4 was vacant, therefore, α-GalNAc (S-5) must be attached to H-3 of S-4. The next anomeric proton signal appeared as doublet at δ 5.27(J = 1.6 Hz) along with a singlet of amide methyl (-NHCOCH3) at δ 1.92 in the $^1$H NMR spectrum of Vediose in CDCl3 at 400 MHz was due to the presence of α- GalNAc (S-5) moiety. Hence the H-3 of β GalNAc (S-4) was glycosically linkaged by the α-GalNAc (S-5) moiety and hence α–(1→3) glycosidic linkage between S-5 and S-4 were confirmed. The small coupling constant of α-GalNAc (S-5) (J = 1.6 Hz) confirmed the α-glycosidic linkage between α-GalNAc (S-5) and β-GalNAc (S-4) and none of methine proton of α-GalNAc (S-5) came in the linkage region which also confirms that α-GalNAc (S-5) was linked at the non-reducing end. The absence of methine protons in linkage region of α –GalNAc (S-5) confirm that α-GalNAc (S-5) was also present at non reducing end and also confirmed by the TOCSY and COSY spectrum. All the $^1$H NMR assignments for ring protons of monosaccharide units of Vediose were confirmed by COSY and TOCSY spectrum. The positions of glycosidation in the oligosaccharide were confirmed by position of anomeric signals, and comparing the signals in $^1$H and $^{13}$C NMR of acetylated oligosaccharide. The glycosidic linkages in Vediose were assigned by the cross peaks for glycosidically linked carbons with their protons in the HSQC spectrum of acetylated Vediose. The values of cross peaks appeared as- β -Glc (S-1) H-4 and C-4 at δ 3.52 x 70.0 showed (1→4) linkage between S-2 and S-1, β -Glc (S-1) H-3 and C-3 at δ 3.73 x 71.0 showed (1→3) linkage between S-6 and S-1, β -Gal (S-2) H-3 and C-3 at δ 3.73 x 71.0 showed (1→3) linkage between S-7 and S-2, β -Gal (S-2) H-2 and C-2 at δ 3.40 x 73.0 showed (1→2) linkage between S-3 and S-2, α-Glc (S-3) H-3 and C-3 at 3.70 x 72.0 showed (1→3) linkage between S-4 and S-3, α-Glc (S-3) H-2 and C-3 at δ 3.53 x 70.0 showed (1→2) linkage between S-8 and S-3, β -Gal (S-4) H-3 and C-3 at δ 3.64 x 80 showed (1→3) linkage between S-5 and S-4. All signals obtained in $^1$H and $^{13}$C NMR of compound Vediose were in conformity with the assigned structure and their position were confirmed by 2D NMR $^1$H-$^1$H COSY, TOCSY and HSQC spectra. Thus based on the pattern of chemical shifts of $^1$H, $^{13}$C, COSY, TOCSY and HSQC NMR spectra it is interpreted that the compound has octasaccharide structure as-

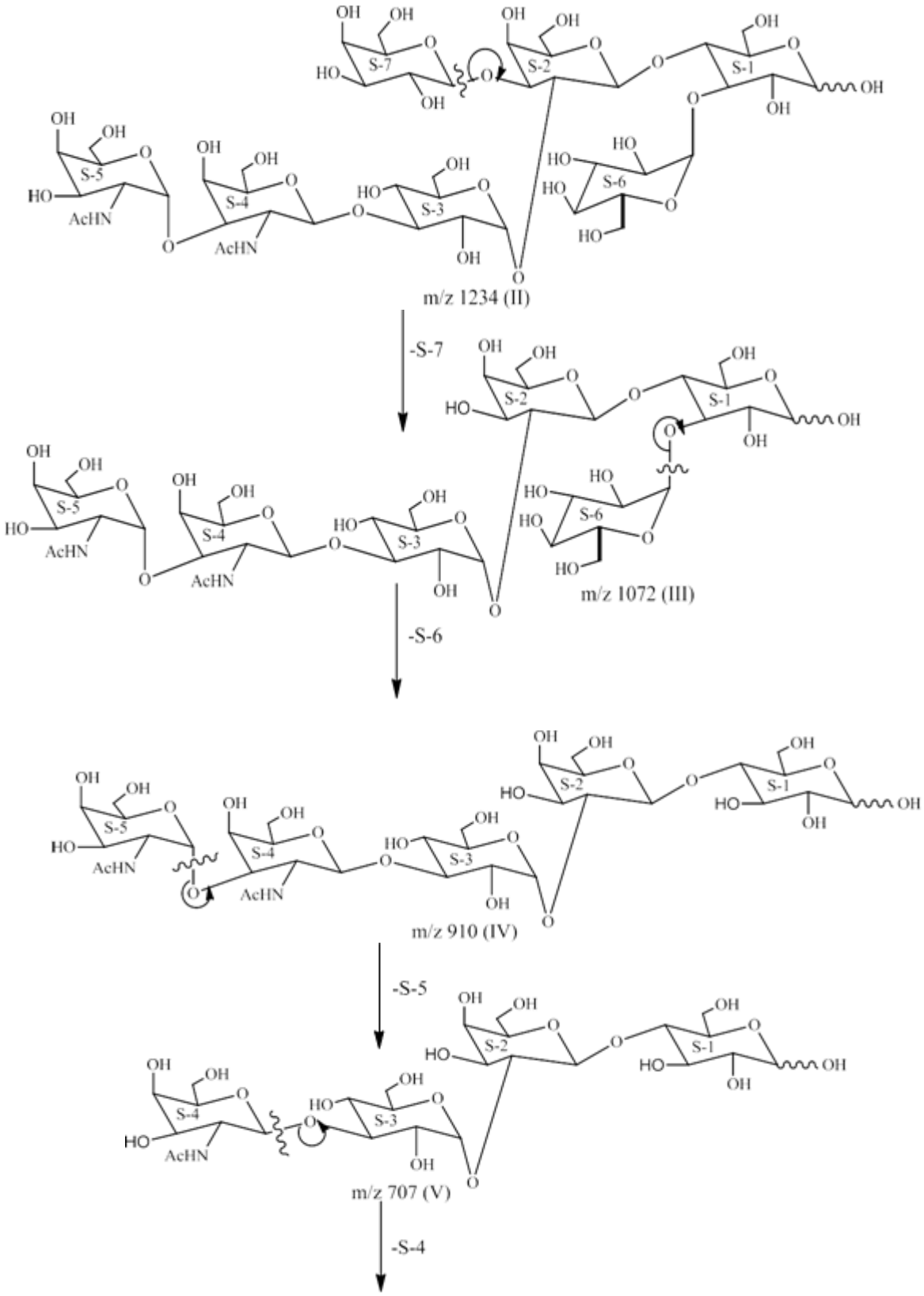


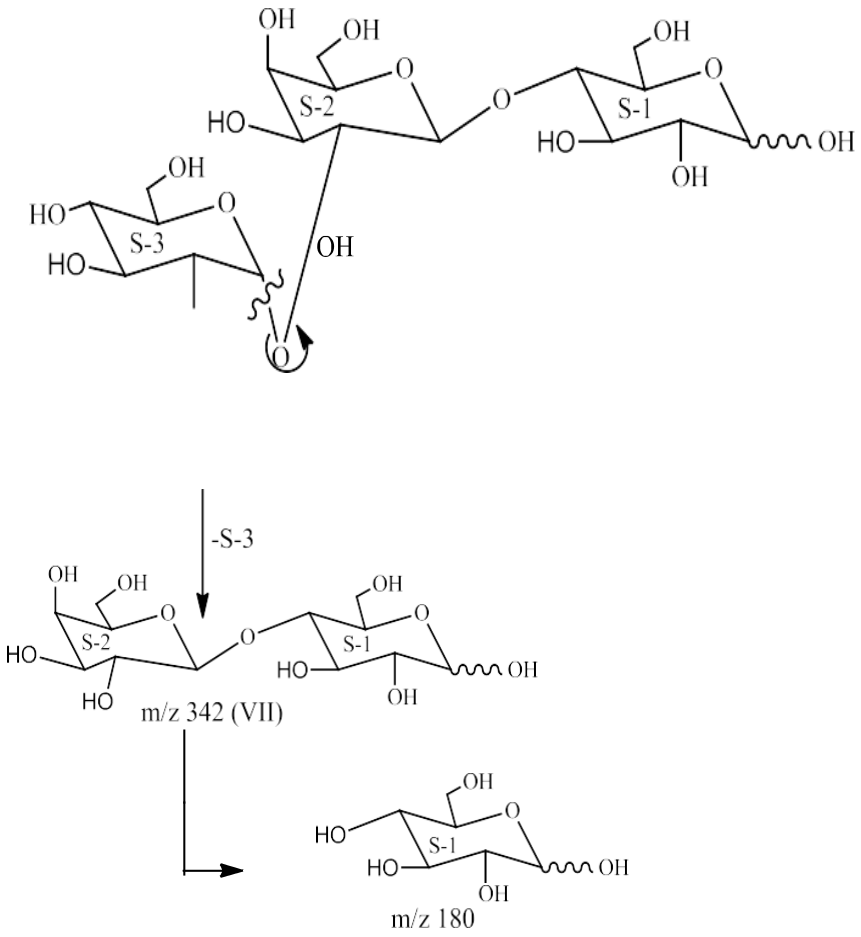

**VEDIOSE**

**Mass Fragmentation of Compound Vediose**

The electronspray Mass spectrometry data of Vediose not only confirmed the derived structure but also supported the sequence of monosaccharide in Vediose. The highest mass ion peaks were recorded at m/z 1458 and 1435 which weredue to [M+Na+K] and [M+K+], respectively. It also contains the molecular ion peak at m/z 1396 confirming the molecular weight of Vediose as 1396 and was in agreement with its molecular formula $C_{52}H_{88}O_{41}N_2$. 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 octasaccharide m/z 1396 (I) fragmented to give mass ion at m/z 1234(II) [1396-S-8], this fragment was arised due to the loss of terminal b-Gal (S-8) moiety from octasaccharide. It further fragmented from octasacharide to give mass ion peak at m/z 1072 (III) [1234-S-7] which was due toloss of Gal S-7 moiety from heptasaccharide. This fragment of [1072-S-6] further fragmented to give mass ion peak at m/z 910 (IV) hexasaccharide. It further fragmented from pentasaccharide [910-S-5] to give mass ion peak at m/z 707 (V) tetrasaccharide which was due to loss of GalNAc (S-5) moiety from the pentasaccharide. Further, fragmentation gives mass ion peak at m/z 504 [707-S-4](VI) which was due to loss of GalNAc moiety from tetrasaccharide. This fragment of trisacharide at 504 further fragmented to give mass ion peak at m/z 342 (VII) [504-S3], which was due to loss of Glc (S-3) moiety from trisaccharide. This disaccharideunit fragmented to give mass ion peak at m/z 180(VIII) [342-S-1], which was due toloss of Gal (S-1) moiety from disaccharide. These eight mass ion peak II, III, IV, V, VI, VII and VIII, were appeared due to the consequent loss of S-8, S-7, S6, S-5, S-4, S-3 and S-2 from original molecule. The mass spectrum also contain the mass ion peak at m/z 707, 586, 545, corresponding to the mass ion fragment A, B and C, which confirm the position of S-1, S-2, S-3, S-4, S-5, S-6, S-7, S-8. The other fragmentation pathway in ES Mass spectrum of compound Vediose m/z 1396 shows the mass ion peak at 1297 [1396 -$CH_2OHCHO$, -$CH_2OCHO$], 127[1396 -CHO], 1234 [1396 -S-8], 1217 [1234-OH], 1205 [1234 -CHO], 1072 [1234 -S-7], 1041 [1072 -$CH_2OH$], 1013 [1072 -$CH_2OCHO$], 910 [1072 -S-6], 852 [910 -$NHCOCH_3$], 861 [910 -$CH_2OH$, $H_2O$], 835 [910 -$CH_3$, -$CH_2OHCHO$], 707 [910 -S5], 504 [707 -S-4], 428 [504 -$NHCOCH_3$, H2O], 473 [504 -$CH_2OH$], 486 [504 -$H_2O$],342 [504 -S-3], 310 [342 -$CH_2OH$, -H+], 283 [342 -$CH_2OCHO$ ], 180 [342 -S-2] .Based on the above results obtained from chemical degradation/ acid hydrolysis, chemical transformation, electrospray mass spectrometry, $^1H$, $^{13}C$ NMR and 2D NMR- COSY, TOCSY and HSQC spectra.




m/z 1234 (II)
-S-7
m/z 1072 (III)
-S-6
m/z 910 (IV)
-S-5
m/z 707 (V)
-S-4




**Mass fragmentations of compound Vediose**

## CONCLUSION

In summary, the novel milk oligosaccharides namely as (Vediose) has been isolated from Bubalus bubalis (buffalo) colostrum and its structure was elucidated with the help of $^{1}H$, $^{13}C$, 2D NMR spectroscopy and mass spectrometry.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