Characterization of two novel sialyl N-acetyllactosaminyl nucleotides separated from ovine colostrum

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Abstract

The milk/colostrum of some mammalian species is known to contain sugar nucleotides including uridine diphosphate (UDP) oligosaccharides in addition to lactose and milk oligosaccharides, but the detailed structures of these UDP oligosaccharides have not so far been clarified. In this study we isolated two UDP-sialyl N-acetyllactosamines from ovine colostrum and characterized them using ^{1}H-NMR and MALDI-TOFMS spectroscopies. Their structures were found to be Neu5Gc(α2-3)Gal(β1-4)GlcNAcα1-UDP and Neu5Gc(α2-6)Gal(β1-4)GlcNAcα1-UDP.

Key words: UDP sialyl N-acetyllactosamine, UDP oligosaccharide, milk sugar nucleotide, ovine colostrum, milk oligosaccharide

1. Introduction

Mammalian milk or colostrum usually contains lactose (Gal(β1-4)Glc) as the predominant carbohydrate as well as lower amounts of a variety of oligosaccharides, most of which contain a lactose unit at their reducing ends [1,2]. In the milk of monotremes, marsupials and some species of Carnivora, however, milk oligosaccharides predominate over lactose [2-4].

Lactose is an energy source for neonates of eutherian species, but milk oligosaccharides are not absorbed in their small intestine and thus reach the colon [5], where they act as prebiotics that stimulate the growth of beneficial microorganisms, and act as colonic decoy receptors that inhibit the adhesion of harmful bacteria and viruses [6-8]. In the milk of some species including the dog [9], rat [10], bearded seal [11,12], hamadryas babboon [13], Coquerel's sifaka [14], human [15], red kangaroo [16], brushtail possum[17], wombat [18] and eastern quoll [19], sulphate is bound to lactose or to some milk oligosaccharides present at low concentrations. It has also been found that milk or colostrum of the horse [20], cow [21], goat [22], sheep [22] and camel [22] contains phosphate bound to lactose, N-acetyllactosamine (Gal(β1-4)GlcNAc, LacNAc) or sialyl N-acetyllactosamine at very low concentrations. The biological significance (if any), for the neonates of these species of sulphate or phosphate bound to lactose or milk oligosaccharides, is unknown.

In addition, sugar nucleotides have been identified in the milk or colostrum of several species. For example, it has been reported that human milk contains Gal(β1-4)GlcNAcα1-UDP (uridyl diphosphate) and Fuc(α1-2)Gal(β1-4)GlcNAcα1-UDP [23,24], while caprine colostrum contains Sia(α2-3(6))Gal(β1-4)GlcNAcα1-UDP and Sia(α2-3(6))Gal(β1-6)GlcNAcα1-UDP [25]. Gal(β1-4)GlcNAcα1-UDP has also been found in pig and reindeer milk [26,27]. Usually, sugar nucleotides such as UDP-Gal, UDP-GlcNAc, CMP (cytidyl monophosphate)-Neu5Ac, GDP (guanidyl diphosphate)-Fuc etc function as donors for glycosyltransferases that transfer monosaccharides to acceptor glycoconjugates within the Golgi apparatus of cells. Although the above-mentioned UDP-oligosaccharides could theoretically be utilized as donors for enzymes that transfer LacNAc, fucosyl LacNAc (Fuc(α1-2)Gal(β1-4)GlcNAc) or sialyl LacNAc to suitable

acceptors, such glycosyltransferases have not so far been detected in any natural source, including milk/colostrum.

To date, 13 neutral and 17 sialyl oligosaccharides, as well as lactose phophate and LacNAc phophate, have been found in ovine colostrum [22,28,29], in which oligosaccharides containing N-glycolylneuraminic acid (Neu5Gc) predominate over those containing N-acetylneuraminic acid (Neu5Ac). In this study, we isolated two novel UDP sialyl N-acetyllactosamines, which we characterized using ^{1}H-NMR and MALDI-TOFMS spectroscopies.

2. Materials and methods

2.1. Colostrum samples

The colostrum samples were collected from 8 animals of corriedale ovine breeds at the National Agriculture Research Center for Hokkaido Region, Hitsujigaoka, Sapporo, Japan. The samples (10-50 mL) were collected between Febryary and March 2013, within 24 hours postpartum. They were stored at -20℃ before use.

2.2. Isolation of acidic saccharides from ovine colostrum

The 8 samples of ovine colostrum were combined, yielding a total of 111 mL, which were extracted with four volumes of chloroform/methanol (2:1, v/v), after which the resulting emulsion was centrifuged at 4℃ and 4,000 x g for 30 min, and the lower chloroform layer and the denatured protein were discarded. The upper layer was concentrated to 10 mL by rotary evaporation, which were then treated with 30 mL of ethanol, and allowed to stand overnight at 4℃. The resulting precipitate was removed by centrifugation at 4℃ and 4,000 x g for 30 min, the supernatant was concentrated to dryness by rotary evaporation, and the residue dissolved in 5 mL of water and freeze-dried. The freeze-dried material, designated the carbohydrate fraction, was dissolved in 10 mL of water and passed through a BioGel P-2 column (< 45 μm, 2.5 × 100 cm). Elution was done with distilled water at a flow rate of

15 mL/h, and fractions of 5 mL were collected. Each fraction was analyzed for hexose with phenol-H_2SO_4 [30] and for sialic acid with periodate – resorcinol [31]. Peak fractions were pooled and freeze-dried. The acidic saccharides were contained in fraction SC-2 (see Fig. 1).

The components in SC-2 were separated by high performance liquid chromatography (HPLC) on a TSK gel Amide-80 column (4.6 × 250 mm, pore size 80 Å, particle size 5 μm; Tosoh, Tokyo, Japan) using a LC-10 ATVP pump (Shimadzu, Tokyo, Japan) (Fig. 2). The mobile phase was 50% and 80% (vol/vol) acetonitrile (CH_3CN) in 15 mmol/L potassium phosphate buffer (pH 5.2). Elution was done using a linear gradient of acetonitrile from 80 to 50% at 60℃ at a flow rate of 1 mL/min. The eluates were monitored by measuring the absorbance at 195 nm. The peak fractions of the saccharides were pooled, concentrated by rotary evaporation, and subjected to ^{1}H-NMR and MALDI-TOF MS to determine their structures.

2.3. Proton nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectra were recorded in D_2O (99.96 atom %D, Aldrich, Milwaukee, WI) at 500 or 600 MHz for ^{1}H-NMR with a JEOL ECP-500 Fourier transform-NMR (Jeol, Tokyo, Japan) or a Varian INOVA 600 spectrometer (Varian Inc., Palo Alto, CA) operated at 293.1 K. Chemical shifts are expressed as change relative to internal 3-(trimethylsilyl)-1-propane sulfuric acid, sodium salt, but measured by reference to internal acetone (δ = 2.225).

2.4. Mass spectrometry

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed using an Autoflex Ⅱ TOF/TOF mass spectrometer (Bruker Faltonics, Bremen, Germany). Lyophilized oligosaccharides fractions were dissolved in 5 μL of milli-Q water. The oligosaccharide solution was mixed with an equal volume of 10 mg/mL SDHB (Bruker Daltonics), which is a mixture of 2,5-dihydrobenzoic acid and

2-hydroxy-5-methoxybenzoic acid, ground steel TF (Bruker Daltonics), and dried. Mass spectra were obtained using a pre-installed method. RP__0-2 kDa (a reflector positive ion mode focusing on a mass range of up to 2 kDa). Peptide calibration standard Ⅱ (Bruker Daltonics) was used for external calibration of the mass spectrometer.

3. Results

The carbohydrate fraction from the ovine colostrum separated into four peaks, designated as SC-1 ~ SC-4, during gel filtration on BioGel P-2 (Fig. 1). The components in SC-1 and SC-2 were assumed to be acidic saccharides, because they gave a positive result with periodate – resorcinol. Only the components in SC-2 were further separated by normal phase HPLC with a TSK Amide-80 column, because our focus in this study was to explore sugar nucleotides. It was hypothesized that fraction SC-1 contained large neutral and sialyl oligosaccharides and/or sialyl glycopeptides/glycoproteins, whereas fractions SC-3 and SC-4 contained neutral trisaccharides and lactose, respectively. The HPLC of SC-2 yielded several peaks designated as SC2-1 to SC2-12 (Fig. 2), each of which was purified by HPLC and then characterized by ^{1}H-NMR and MALDI-TOF mass spectrometry.

SC2-7

The ^{1}H-NMR spectrum (chemical shifts in Table 1) of SC2-7 showed that this fraction contained two sialyl oligosaccharides, major and minor. The spectrum had the anomer shifts of α-Glc, β-Glc, and β(1-4) linked Gal at δ 5.223, 4.664 and 4.533, respectively. It had the H-3 axial and equatorial shifts of 2-3) linked sialic acid at δ 1.816 and 2.773, respectively, and H-3 of β(1-4) linked Gal, which was substituted by α(2-3) linked sialic acid, at δ 4.133. The spectrum had the NGc shift at δ 4.118 but no NAc shift, showing the presence of N-glycolyl neuraminic acid (Neu5Gc) and no N-acetylneuraminic acid. From these observations, the major oligosaccharide in this fraction was characterized to be

Neu5Gc(α2-3)Gal(β1-4)Glc (SC2-7-1).

The ^{1}H-NMR spectrum had the other H-1 shifts of α-GlcNAc, β-GlcNAc and β(1-4) linked Gal at δ 5.197, 4.706 and 4.452, respectively. The spectrum had the H-3 axial and equatorial shifts of α(2-6) linked Neu5Gc at δ 1.736 and 2.684, respectively, and NAc shift of GlcNAc at δ 2.067. The minor saccharide in this fraction was therefore characterized to be Neu5Gc(α2-6)Gal(β1-4)GlcNAc (SC2-7-2)

SC2-8

The ^{1}H-NMR spectrum (chemical shifts in Table 1) of SC2-8 had the anomeric shifts of α-Glc, β-Glc and β(1-4) linked Gal at 5.224, 4.671 and 4.431, respectively. The spectrum had the H-3 axial and equatorial, and NGc shifts of α(2-6) linked Neu5Gc at δ 1.763, 2.729 and 4.118, respectively. From these assignments, it was concluded that the fraction contained Neu5Gc(α2-6)Gal(β1-4)Glc.

However, the ^{1}H-NMR spectrum showed that this fraction also contained an unidentified UDP saccharide.

SC2-11

The saccharide in SC2-11 was characterized by ^{1}H-NMR (spectrum in Fig. 3, chemical shifts in Table 2) and comparison with that of UDP-GLcNAc and the published data [32] for UDP-N-acetyllactosamine and other UDP oligosaccharides. The spectrum had the characteristic down field shifts at δ 7.965 and 5.986, which were caused by the uracil of this fraction, and the other characteristic shifts at δ 5.968, 4.370, 4.289 and 4.199, which were caused by ribose; this showed that the saccharide contained uridine diphosphate. The spectrum had the H-1 shifts of α-GlcNAc and β(1-4) linked Gal at δ 5.550 and 4.588, NGc shift of Neu5Gc at δ 4.115, and H-3 axial and equatorial shifts of α(2-3) linked Neu5Gc at δ 1.816 and 2.774, respectively. The spectrum had the NAc shift of GlcNAc at δ 2.058. From these observations, the saccharide in this fraction was characterized to be

Neu5Gc(α2-3)Gal(β1-4)GlcNAcα1-UDP.

This characterization was supported by the MALDI-TOFMS spectrum of this fraction. The MS (Fig. 5a) had the ions at 1175.399, 1191.180, 1197.186, 1213.090 and 1229.074, which corresponded to M+Na+2K-2H, M+3K-2H, M+2Na+2K-3H, M+Na+3K-3H and M+4K-3H, respectively. These results are consistent with a molecular mass of 1076, as calculated from its chemical structure of Neu5Gc(α2-3)Gal(β1-4)GlcNAcα1-UDP.

SC2-12

The ^{1}H-NMR spectrum (Fig. 4, chemical shifts in Table 2) had shifts at δ 7.986 and 5.973 which arose from uracil, and shifts at δ 5.963, 4.289 and 4.199 which arose from ribose, showing the presence of uridine diphospate. The spectrum had the H-1 shifts of α-GlcNAc and β(1-4) linked Gal at δ 5.515 and 4.438, respectively, H-3 axial and equatorial of α(2-6) linked Neu5Gc at δ 1.729 and 2.703, respectively, and NGc of Neu5Gc at δ 4.112. The spectrum had the NAc shift of GlcNAc at δ 2.094. The saccharide in this fraction was therefore characterized to be Neu5Gc(α2-6)Gal(β1-4)GlcNAcα1-UDP.

This characterization was also supported by the MALDI-TOFMS spectrum of this fraction. The MS (Fig. 5b) had the ions at 1137.154, 1153.142, 1175.141, 1191.129, 1197.139, 1213.090 and 1229.073, which corresponded to M+Na+K-H, M+2K-H, M+Na+2K-2H, M+3K-2H, M+2Na+2K-3H, M+Na+3K-3H and M+4K-3H, respectively. These results are consistent with a molecular mass of 1076, as calculated from its chemical structure of Neu5Gc(α2-6)Gal(β1-4)GlcNAcα1-UDP.

SC2-10

The ^{1}H-NMR spectrum showed that this fraction contained UDP-Neu5Gc-LacNAc and Neu5Gc-lactose, but were not characterized in this study because of the weak shift intensities.

SC2-1, SC2-2, SC2-3

These were not characterized because their ^{1}H-NMR spectra could not be obtained.

SC2-4

The ^{1}H-NMR spectrum showed that the saccharide in this fraction contained two phophorylated monosaccharides, because it had the characteristic doublet doublet anomeric shifts at δ 5.460 and 5.427. However, these sugars were not characterized in this study.

SC2-5, SC2-6

The ^{1}H-NMR spectra showed that the components in these fractions were not saccharides.

SC2-9

The component in fraction SC2-9 could not be characterized by its ^{1}H-NMR spectrum in this study because the suitable reference for the assignment could not be obtained.

Discussion

The present is the first study in which the detailed structures of two UDP-sialyl N-acetyllactosamines viz Neu5Gc(α2-3)Gal(β1-4)GlcNAcα1-UDP and Neu5Gc(α2-6)Gal(β1-4)GlcNAcα1-UDP, have been characterized in any milk or colostrum, in this case ovine colostrum. Although Sia(α2-3(6))Gal(β1-4)GlcNAcα1-UDP and Sia(α2-3(6))Gal(β1-6)GlcNAcα1-UDP have been found in caprine colostrum [24], their detailed structures and sialic acid species had not been clarified. In this study Sia of the ovine UDP-sialyl N-acetyllactosamines was shown to be Neu5Gc but not Neu5Ac. In addition, UDP-LacNAc has been identified in

human [23], pig [26] and reindeer milk [27], and UDP-fucosyllactosamine has been found in human milk [24].

There are two possible biosynthetic pathways for the UDP-sialyl N-acetyllactosamines in ovine colostrum. One involves sialyl N-acetyllactosamine-1-monophosphate being condensed with uridine-5'-triphosphate. Since Neu5Ac(α2-6)Gal(β1-4)GlcNAc-1-phosphate and Gal(β1-4)GlcNAc-1-phosphate have been found in bovine and mare colostrum, respectively [20, 21], it is possible that these are the precursors for the synthesis of UDP-N-acetylneuraminyl N-acetyllactosamine and UDP-N-acetyllactosamine, even though these UDP-oligosaccharides have not been found in bovine or mare colostrum.

Another possibility is that galactose and N-glycolylneuraminic acid are transferred from UDP-Gal and CMP-Neu5Gc, respectively, to UDP-GlcNAc, the acceptor for the glycosyltransferases. As UDP-GlcNAc as well as UDP-LacNAc have been found in reindeer milk, it is possible that UDP-LacNAc could be synthesized from UDP-GlcNAc as the acceptor, and UDP-Gal as the donor, by β4galactosyltransferase in the lactating mammary glands.

It can be speculated that the biosynthesis of these UDP-oligosaccharides would most likely take place within the Golgi apparatus or cytosol of the mammary epithelial cells, It is to be hoped that clarification of the biosynthetic pathway as well as the secretion mechanism of the UDP-sialyl N-acetyllactosamine or UDP-N-acetyllactosamine will be subjects for future studies.

So far, UDP oligosaccharides have been found only in milk/colostrum and one may speculate that in ovine milk/colostrum, or in ovine lactating mammary glands, there are oligosaccharide transferases that transfer LacNAc, sialyl LacNAc or fucosyl LacNAc from these UDP oligosaccharides to unknown acceptors. Although this type of glycosyltransferase has not yet been detected, it may in future be possible to identify a sialyllactosaminyltransferase in ovine colostrum or lactating mammary glands. Furthermore UDP oligosaccharides may have other biological functions, considering that their concentrations are similar to those of

sialyllactose or sialyl N-acetyllactosamine as shown by the ratio of the total peak areas of SC2-11 and SC2-12 to SC2-7 and SC2-8 in the HPLC shown in Fig. 2. At this stage, however, it is unclear whether UDP oligosaccharides commonly occur in mammalian milk/colostrum. Future studies may attempt to identify UDP oligosaccharides in the milk/colostrum of other species and also to determine their biological functions.

Although the biological functions of UDP-oligosaccharides have not so far been studied, it can be thought that their biological effects would be similar to those of the free oligosaccharides of milk/colostrum. These effects include action as prebiotics that stimulate the growth of beneficial colonic microorganisms, as decoy receptors that inhibit the adhesion of pathogenic microorganisms to the colonic mucosa, and as modulation for the maturation of colonic epithelial cells and immunomodulation in the colon [6, 7, 8].

It has been shown that *Bifidobacterium longum* subsp. *infantis*, one of the beneficial colonic bacteria, imports milk oligosaccharides through the action of a transporter and utilizes several glycosidases to liberate monosaccharides from the non reducing ends of the oligosaccharides [33]. It is not known, however, whether this bifidobacterium strain can also import UDP-oligosaccharides. Lane et al. (2012) observed that bovine milk oligosaccharides (BMO) inhibited the invasion of HT-29 cells by *Campylobacter jejuni* during the co-culture of HT-29 cells with BMO and *C. jejuni* [34]. Hester et al. (2013) evaluated the in vitro inhibition of the adhesion of rotavirus to MA-104 cells by 3'-sialyllactose and 6'-sialyllactose [35].

Holscher et al. (2014) observed that 2'-fucosyllactose (Fuc(α1-2)Gal(β1-4)Glc), 6'-sialyllactose or lacto-N-neotetraose (Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc) induced the differentiation of epithelial cells such as preconfluent HT-29 cells, preconfluent Caco-2 Bbe cells and postconfluent Caco-2 Bbe cells during *in vitro* growth experiments [36]. Lane et al (2013) observed the transcriptional response of several glycogenes in HT-29 cells after exposure to human and bovine milk oligosaccharides [37].

These observations of the biological effects of free milk oligosaccharides may be indications relating to the possible beneficial effects of

UDP-oligosaccharides found in milk/colostrum. To asses these effects it will first be necessary to develop methods for the large scale preparation of UDP-oligosaccharides.

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Figure legend

Fig. 1 Gel chromatogram of the carbohydrate fraction from ovine colostrum on a BioGel P-2 column (2.5 × 100 cm). Elution was done with distilled water at a flow rate of 15 mL/h and fractions of 5.0 mL were collected. Each fraction was monitored for hexose by the phenol-H_2SO_4 method at 490 nm (solid line) and for sialic acid by the periodate – resorcinol at 630 nm (dotted line).

Fig. 2 High performance liquid chromatogram of fraction SC-2 (see Fig. 1). The HPLC was done using a Shimadzu LC-10 ATVP pump (Shimadz, Tokyo, Japan) on a TSK-gel Amide-80 column (4.6 × 250 mm, pore size 80 Å, particle size 5 µm; Tosoh, Tokyo, Japan). The mobile phase was 50 and 80% (v/v) acetonitrile (CH_3CN) in 15 mmol/L potassium phosphate buffer (pH 5.2). Elution was done using a linear gradient of CH_3CN from 80 to 50% at 60℃ at a flow rate of 1 mL/min. Detection was done by measuring UV absorption at 195 nm.

Fig. 3 ^{1}H-NMR spectra of the UDP sialyl N-acetyllactosamines in fraction SC2-11 (see Fig. 2). The spectrum was obtained in D_2O at 600 MHz with a Varian INOVA 600 spectrometer operated at 283 K. Chemical shifts are expressed relative to internal 3-(trimethylsilyl)-1-propane sulfuric acid, sodium salt, but were actually measured by reference to internal acetone (δ = 2.225)

Fig. 4 ^{1}H-NMR spectra of the UDP sialyl N-acetyllactosamines in fraction SC2-12 (see Fig. 2).

Fig. 5 Matrix-assisted, laser-desorption time-of-flight (MALDI-TOF) mass

spectra of the UDP-sialyl N-acetyllactosamines in fractions (A) SC2-11 and (B) SC2-12 (see Fig. 2). The spectra were performed using an Autoflex Ⅱ TF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The sample solutions (0.5 μL) were mixed on a target plate (MTP 374 target plate ground steel, TF, Bruker), with an equal volume of 10 mg/mL of DHB saturated in distilled water. Mass spectra were obtained using a reflector positive ion mode optimized to the mass rage of 0-3 kDa.

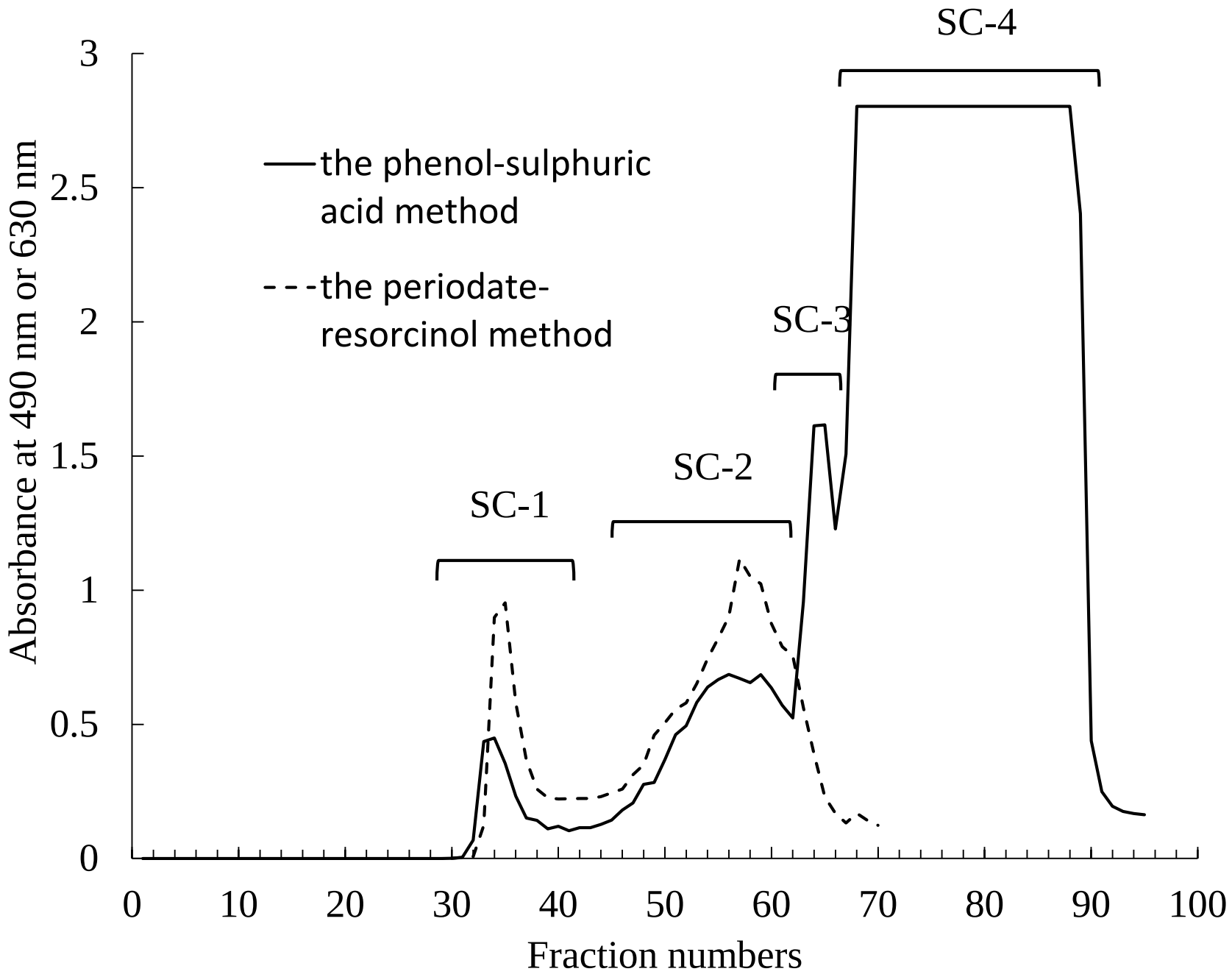
SC-4
SC-3
SC-2
SC-1
the phenol-sulphuric acid method
the periodate-resorcinol method
Absorbance at 490 nm or 630 nm
Fraction numbers
0
0.5
1
1.5
2
2.5
3
0
10
20
30
40
50
60
70
80
90
100

Fig. 1.

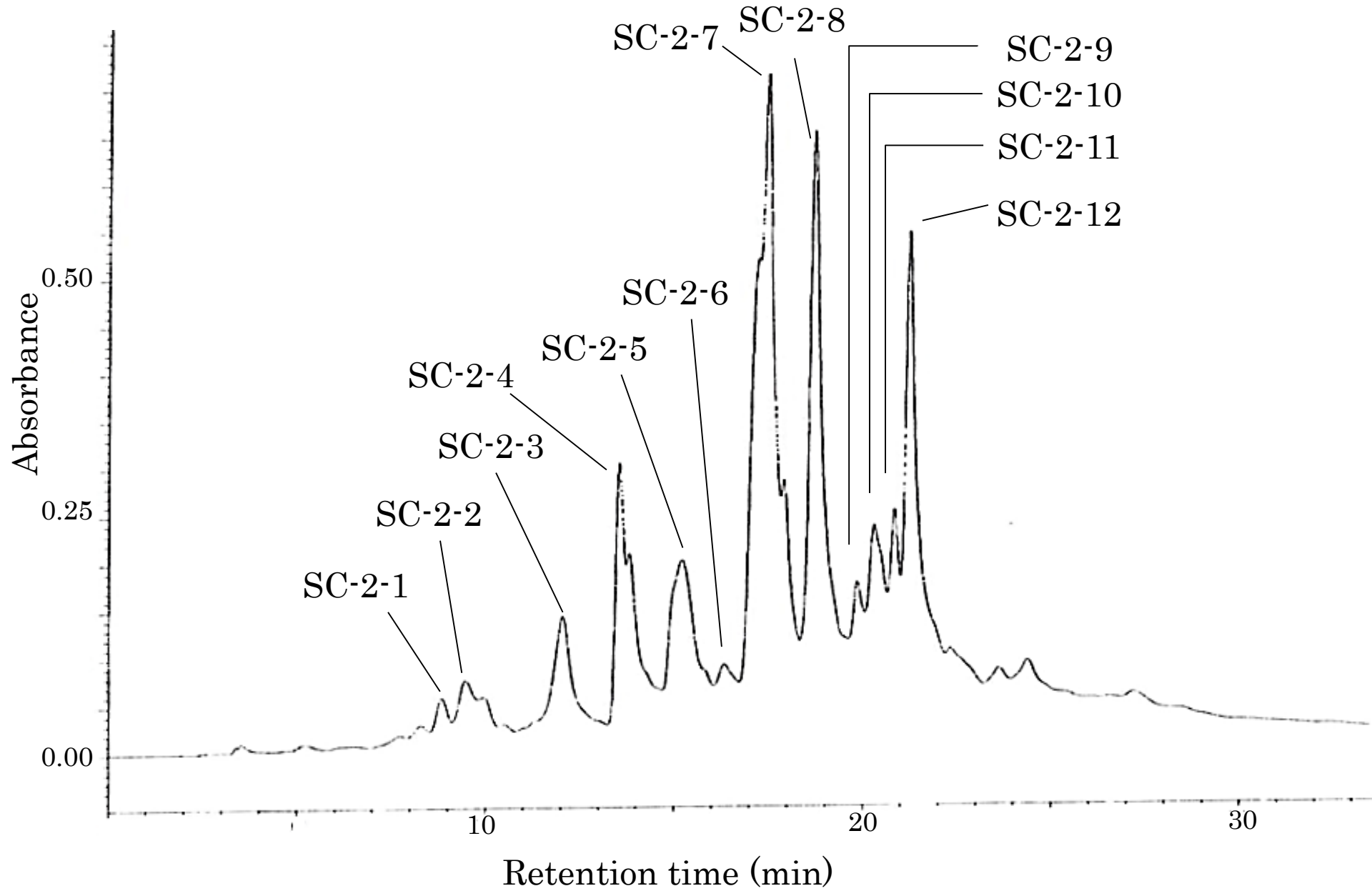
SC-2-7
SC-2-8
SC-2-9
SC-2-10
SC-2-11
SC-2-12
SC-2-6
SC-2-5
SC-2-4
SC-2-3
SC-2-2
SC-2-1
0.50
0.25
0.00
Absorbance
10
20
30
Retention time (min)

Fig. 2.

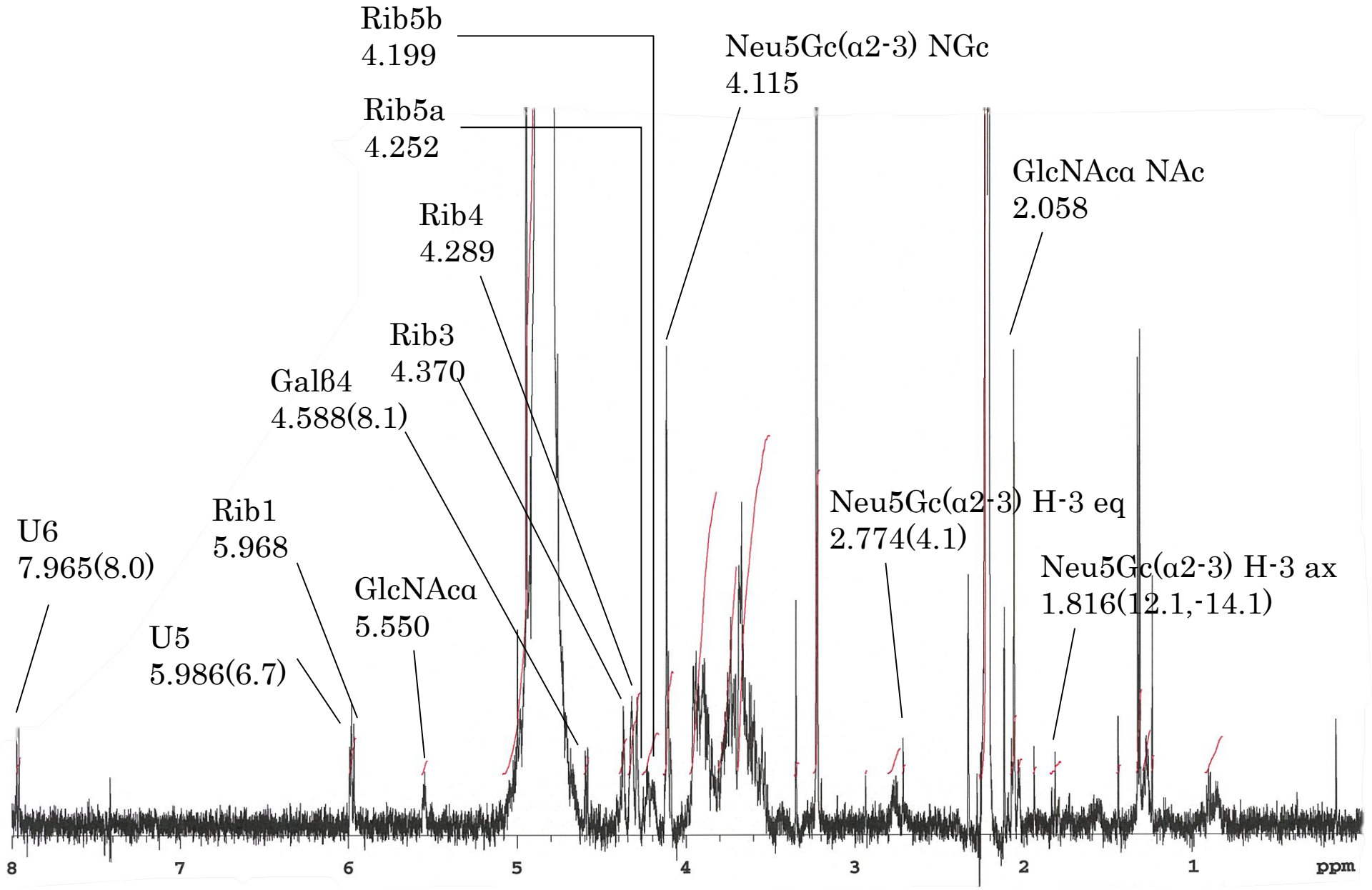

Fig. 3.

Chemical shifts (ppm)

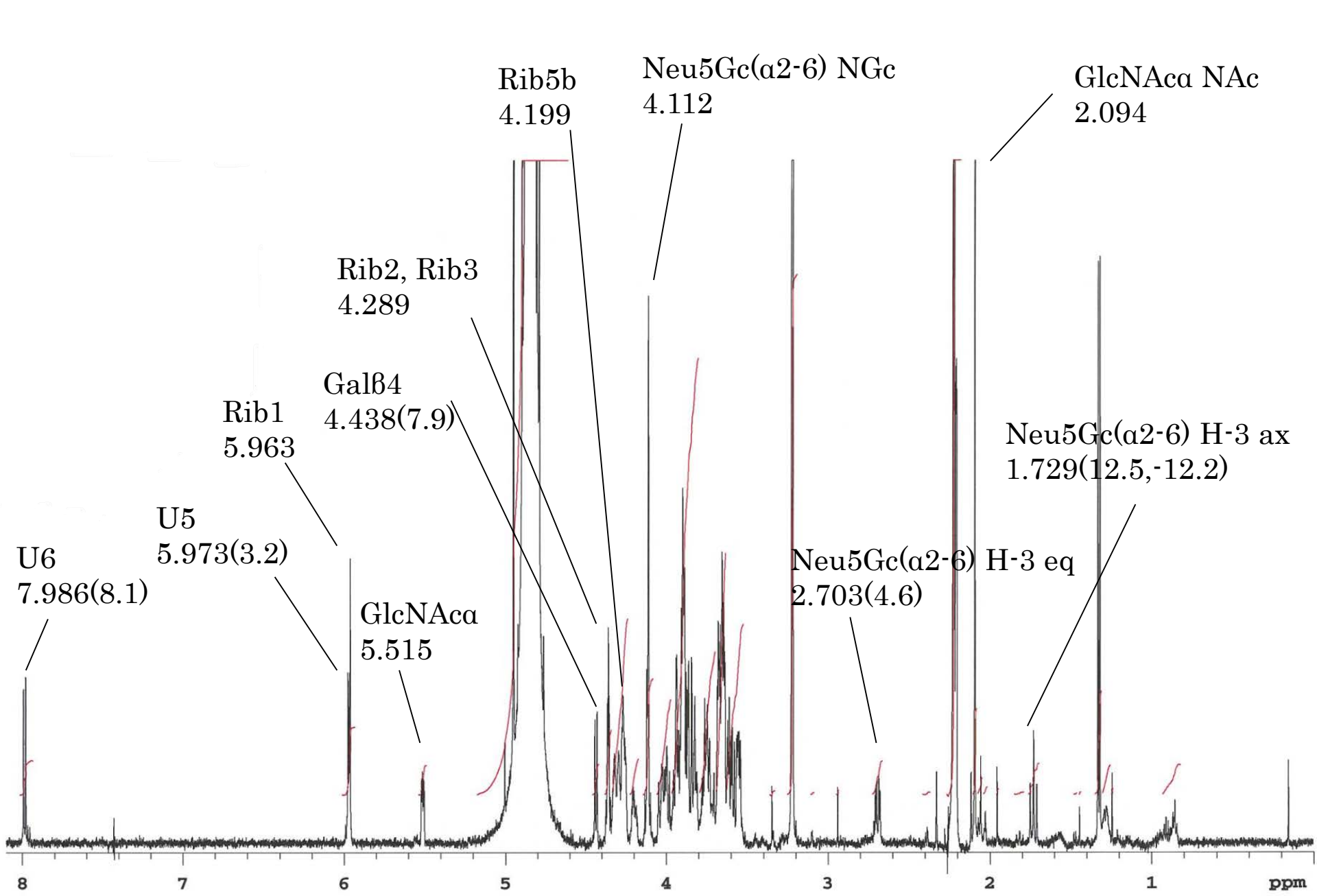

Fig. 4.

Chemical shifts (ppm)

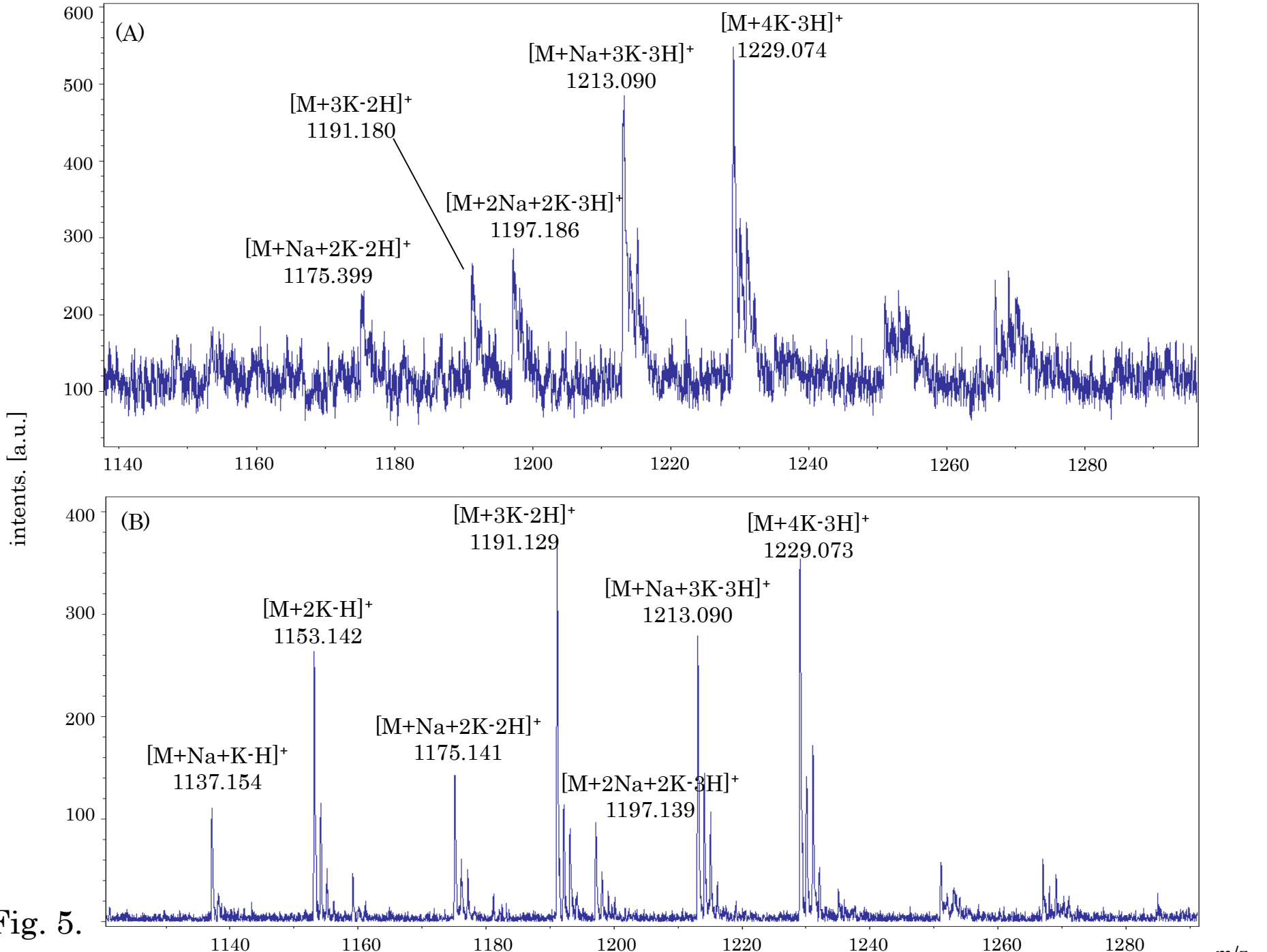
(A)
[M+4K-3H]+
1229.074
[M+Na+3K-3H]+
1213.090
[M+3K-2H]+
1191.180
[M+2Na+2K-3H]+
1197.186
[M+Na+2K-2H]+
1175.399
(B)
[M+3K-2H]+
1191.129
[M+4K-3H]+
1229.073
[M+Na+3K-3H]+
1213.090
[M+2K-H]+
1153.142
[M+Na+2K-2H]+
1175.141
[M+Na+K-H]+
1137.154
[M+2Na+2K-3H]+
1197.139
intents. [a.u.]
m/z

Fig. 5.

Table. 1 Assignment of ^{1}H-NMR chemical shifts of fraction SC2-7 and SC2-8 separated from ovine colostrum.

Reporter Group	Residue	Chemical shifts, δ (coupling constants, Hz)		
		SC2-7-1	SC2-7-2	SC2-8
H-1	Glcα	5.223(3.7)	-	5.224(4.1)
	Glcβ	4.664(7.9)	-	4.671(8.0)
	GlcNAcα	-	5.197	-
	GlcNAcβ	-	4.706	-
	Galβ4	4.533(7.9)	4.452(8.1)	4.431(7.9)
H-4	Galβ4	4.133(2.7[a])	-	-
H-3 ax	Neu5Gc(α2-3)	1.816(12.1[b], -12.1[c])	-	-
	Neu5Gc(α2-6)	-	1.726(10.3[b], -14.1[c])	1.763(12.3[b], -12.1[c])
H-3 eq	Neu5Gc(α2-3)	2.773(7.8[d])	-	-
	Neu5Gc(α2-6)	-	2.684(7.8[d])	2.729(7.9[d])
NGc	Neu5Gc(α2-3)	4.118	-	-
	Neu5Gc(α2-6)	-	4.118	4.118
NAc	GlcNAc	-	2.067	-

[a]$J_{3,\ 4,}$ [b]$J_{3ax,\ 4}$, [c]$J_{3ax,\ 3eq}$, [d]$J_{3eq,\ 4}$

Table. 2 Assignment of ^{1}H-NMR chemical shifts of fraction SC2-11 and SC2-12 separated from ovine colostrum.

Reporter Group	Residue	Chemical shifts, δ (coupling constants, Hz)	
		SC2-11	SC2-12
H-1	GlcNAcα	5.550	5.515
	GlcNAcβ	-	-
	Galβ4	4.588(8.1)	4.438(7.9)
H-4	Galβ4	4.124	-
H-3 ax	Neu5Gc(α2-3)	1.816(12.1[a], -14.1[b])	-
	Neu5Gc(α2-6)	-	1.729(12.5[a], -12.1[c])
H-3 eq	Neu5Gc(α2-3)	2.774(4.1[c])	-
	Neu5Gc(α2-6)	-	2.703(4.6[c])
NGc	Neu5Gc(α2-3)	4.115	-
	Neu5Gc(α2-6)	-	4.112
NAc	GlcNAc	2.058	2.094

[a]$J_{3ax,\ 4}$, [b]$J_{3ax,\ 3eq}$, [c]$J_{3eq,\ 4}$

Table. 3 Chemical structures of sialyllactose, sialyl N-acetyllactosamine and UDP-sialyl N-acetyllactosamine separated from ovine colostrum.

Neu5Gc(α2-3)Gal(β1-4)Glc	(SC2-7-1)
Neu5Gc(α2-6)Gal(β1-4)GlcNAc	(SC2-7-2)
Neu5Gc(α2-6)Gal(β1-4)Glc	(SC2-8)
Neu5Gc(α2-3)Gal(β1-4)GlcNAcα1-UDP	(SC2-11)
Neu5Gc(α2-6)Gal(β1-4)GlcNAcα1-UDP	(SC2-12)