Sequential Analysis of α-Glucooligosaccharides with α-(1→4) and α-(1→6) Linkages by Negative Ion Q-TOF MS/MS Spectrometry

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Negative ion Q-TOF MS/MS spectra are shown to be very useful for sequential analysis of the glycosidic linkage in the α-gluco-oligosaccharides (DP 3-6) derived from an amylopectin molecule. The composition of the fragmentation ions generated from these compounds enabled us to distinguish two kinds of glycosidic linkage, α-(1→4) and α-(1→6), at same time to determine the glucose sequence from the reducing end of the oligosaccharide.

Keywords α-Gluco-oligosaccharide; Sequential analysis; Glycosidic linkage; Q-TOF MS/MS spectrometry

INTRODUCTION

Over the past few decades, a combination of soft ionization methods in mass spectrometry, such as FABMS, MALDI-TOFMS, and ESIMS, has been developed as a powerful technique to analyze the sequence and linkage positions

of oligosaccharides.[1–8] Mainly, oligosaccharides from natural sources such as glycoproteins and glycolipids have been studied by these techniques because these products are often available in only small amounts and therefore require a very sensitive analytical approach.[6,9,10] Analyzing the sequence and linkage positions of the homo-oligosaccharide chains such as glucose oligomers remains a great challenge, however, because isomers of such homo-oligosaccharides have the same mass number as one another. The aim of our research is to elucidate the fine structure of starch and amylopectin. There is no universal method for the sugar sequence of neutral sugars such as gluco-oligosaccharides derived from amylopectin and glycogen. Traditional methods for sequential analysis of α-gluco-oligosaccharides with α-(1→4) and α-(1→6) linkages produced by the partial degradation of amylopectin are time and cost consuming. [11] In this aspect, some attempts for distinguishing two kinds of glycosidic linkages, α-(1→4) and α-(1→6), in α-gluco-oligosaccharides have been made based on the relative intensities of the fragment ions using MALDI/PSD analysis.[12,13] However, fragmentation is poorly controllable, but some progress has been reported by the choice of the matrix.[14] The capability of a quadrupole ion-trap mass spectrometer (QIT MS/MS) to carry out consecutive fragmentations of molecules has been applied to structural elucidation of neutral human milk oligosaccharides in the negative ion mode when using deprotonated molecules as precursor ions.[5,6]

In this study, sequential analysis of underivatized α-gluco-oligosaccharides with α-(1→4) and α-(1→6) linkages has been performed by using negative ion Q-TOF MS/MS spectrometry (Fig. 1). Under these conditions, neutral oligosaccharides can be investigated as deprotonated molecules with high sensitivity; their fragmentation behavior has been elucidated based on certain isomeric glycoforms yielding both complete sequence and linkage information, allowing us to obtain structural information.

RESULTS AND DISCUSSION

Garozzo et al. have reported that discrimination of (1→4), (1→6), (1→3), and (1→2) linkages of the four isomeric glucobioses, regardless of anomeric configuration, has been made possible by using negative mode FAB to produce deprotonated molecules from the carbohydrate and recording high-energy sector mass.[15] Thus, α-(1→6)-linked reducing terminal residue loses 90 Da, while the α-(1→4)-linked reducing terminal residue loses 78 Da (Sch. 1).[15] In addition, Suzuki et al. have reported fragmentation of neutral oligosaccharides in negative ion MALDI MS spectrometry.[16] The principle of this method was introduced to analysis of the model trisaccharides panose [α-D-Glc-(1→6)-α-D-Glc-(1→4)-D-Glc] and isopanose [α-D-Glc-(1→4)-α-D-Glc-(1→6)-D-Glc] containing α-(1→4) and α-(1→6) linkages, which are present at the branch point of an amylopectin molecule. The negative ion mode Q-TOF MS/MS spectra of

panose

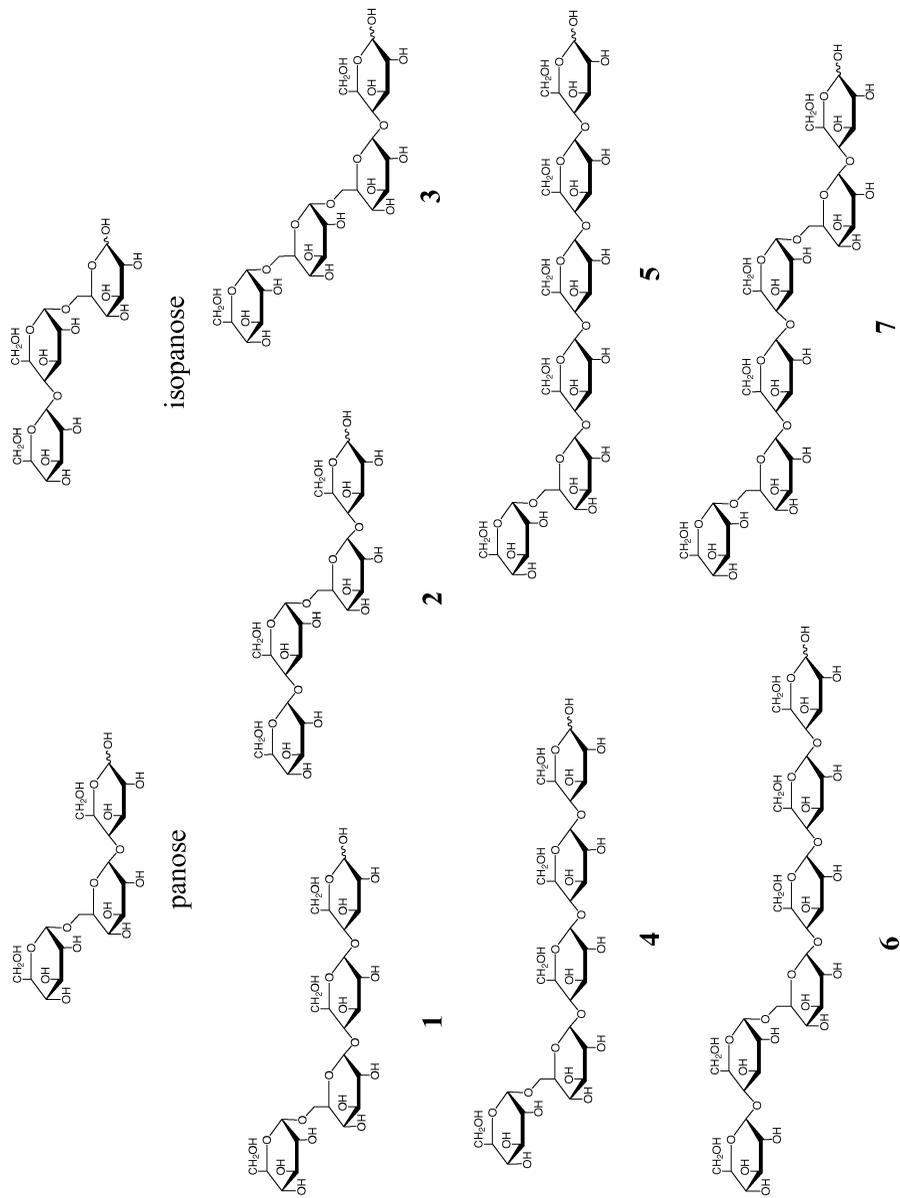isopanose

1

2

3

4

5

6

7

Figure 1: Structures of α-gluco-oligosaccharides (DP 3–6) used.

the deprotonated ions of panose and isopanose are shown in Figure 2. The two isomers show in common a series of three peaks at m/z 503, which is the deprotonated molecular ion [M-H]$^-$; m/z 341 [M-Glc (G: glycosyl residue)]$^-$; and m/z 179 [M-G2]$^-$, the interval of which (162 Da) corresponds to one glycosyl residue. Furthermore, by analyzing the fragment ions of the peaks corresponding to these progressively degraded oligosaccharides, it is possible to observe diagnostic mass losses that characterize the glycosidic linkage sequence along the oligosaccharide chain. In panose, the primary loss of 78 Da (peak at m/z 425) from the peak at m/z 503 appears to be characteristic of the α-(1→4) structure of the reducing unit, and the loss of 90 Da (peak at m/z 251) from the peak at m/z 341 appears to be characteristic of the α-(1→6) linkage type between rings 2 and 3. In isopanose, by contrast, the presence of a peak at m/z 413 due to a loss of 90 Da, coupled with an absence of the peak at m/z 425, is characteristic of the α-(1→6) linkage of the reducing unit; and the loss of 78 Da from the peak at m/z 341, coupled with an absence of the peak at m/z 263, seems to be characteristic of the α-(1→4) linkage between rings 2 and 3. Therefore, the losses of 78 and 90 Da were confirmed to be diagnostic for the respective α-(1→4) and α-(1→6) linkages between adjacent glucose
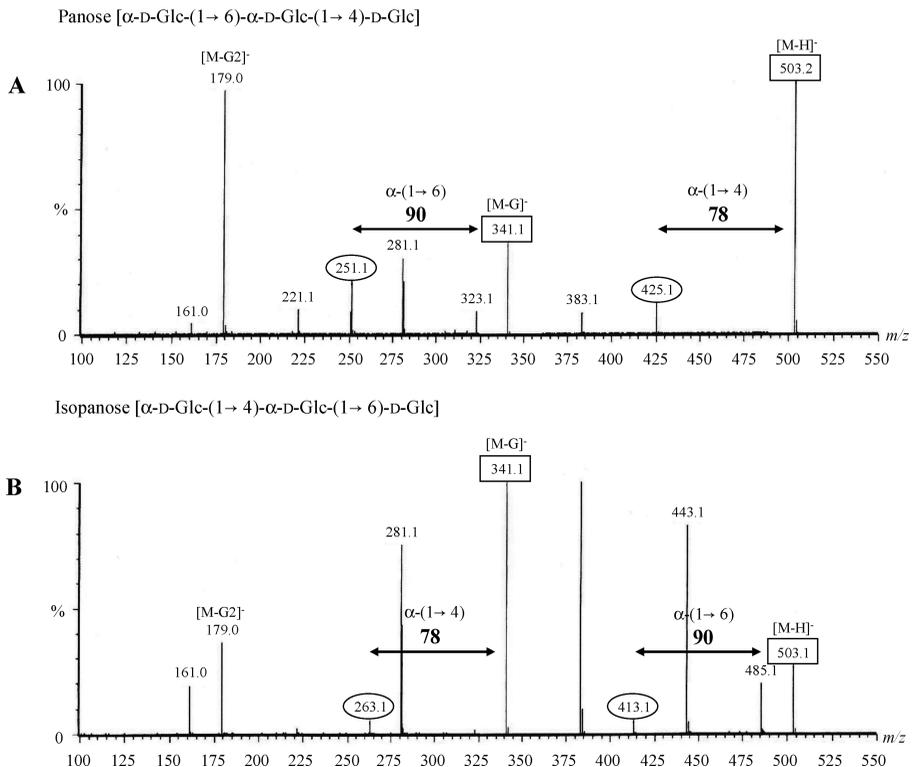
Figure 2: Negative ion Q-TOF MS/MS spectra of panose (A) and isopanose (B).

α-(1→4) linkage   **α-(1→6) linkage**
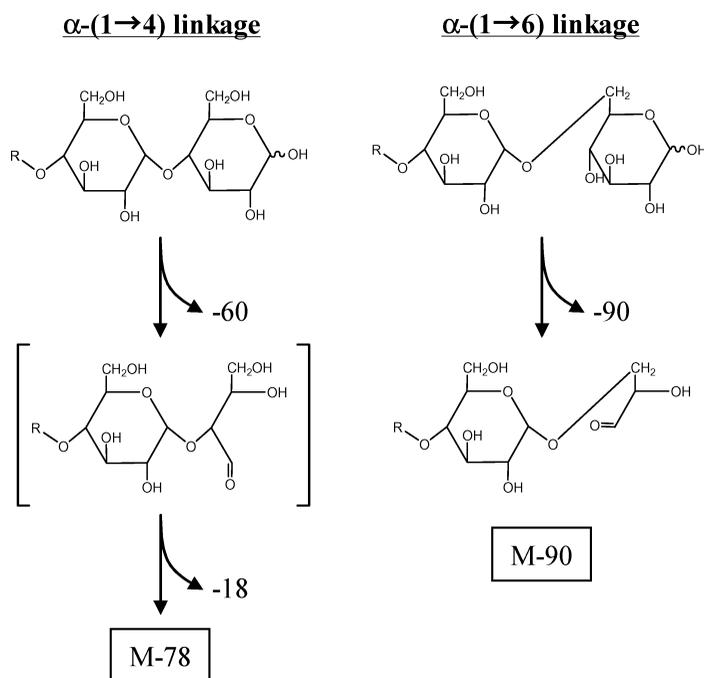
Scheme 1: Fragmentation of α-oligosaccharides with α-(1→4) and α-(1→6) linkages.

units. This is the first report on the fragmentations for α-(1→4) and α-(1→6) gluco-oligosaccharides using ESI.

The method was applied to the following larger glucotetrasaccharides: 4-*O*-α-isomaltosylmaltose [**1**, α-D-Glc-(1→6)-α-D-Glc-(1→4)-α-D-Glc-(1→4)-D-Glc], 6-*O*-α-maltosylmaltose [**2**, α-D-Glc-(1→4)-α-D-Glc-(1→6)-α-D-Glc-(1→4)-D-Glc], and 6-*O*-α-isomaltosylmaltose [**3**, α-D-Glc-(1→6)-α-D-Glc-(1→6)-α-D-Glc-(1→4)-D-Glc]. These three isomers showed in common a series of four peaks at m/z 665, which is the deprotonated precursor ion; m/z 503 [M-G–; m/z 341 [M-G2]⁻, and m/z 179 [M-G3]⁻, with a successive loss of 162 Da as shown in Figure 3. The primary loss of 78 Da (peak at m/z 587) from the molecular ion at m/z 665, together with an absence of a peak corresponding to loss of 90 Da, which is common to all three spectra, reveals the α-(1→4) linkage of the reducing unit in the tetrasaccharides. In the spectrum of **1**, by analogy with panose, the respective losses of 78 and 90 Da from [M-G]⁻ and [M-G2]⁻ identify the α-(1→4) linkage between rings 2 and 3 and the α-(1→6) linkage between rings 3 and 4, respectively. The reverse is seen in the spectrum of **2**: that is, by analogy with isopanose, the respective α-(1→6) and α-(1→4) linkage types between rings 2 and 3 and rings 3 and 4 can be ascertained. In **3**, on the other hand, the loss of 90 Da from both [M-G]⁻ and [M-G2]⁻ identifies the α-(1→6) type of linkage between rings 2 and 3 and rings 3 and 4. The negative ion spectrum of 4-*O*-α-isomaltosylmaltotriose [**4**, α-D-Glc-(1→6)-α-D-

Glc-(1→4)-α-D-Glc-(1→4)-α-D-Glc-(1→4)-D-Glc] showed a series of five peaks at m/z 827, which is the deprotonated precursor ion; m/z 665 [M-G]⁻; m/z 503 [M-G2]⁻; m/z 341 [M-G3]⁻; and m/z 179 [M-G4]⁻ with a successive loss of 162 Da. The loss of 78 Da from [M-H]⁻, [M-G]⁻, and [M-G2]⁻ was clearly observed, identifying the respective α-(1→4) type of linkage between rings 1 and 2, rings 2 and 3, and rings 3 and 4. The loss of 90 Da from [M-G3]⁻ reveals the α-(1→6) linkage of the nonreducing unit in compound **4** (data not shown). The negative ion spectra of two higher oligosaccharide isomers, 4-*O*-α-isomaltosylmaltotetraose [**5**, α-D-Glc-(1→6)-α-D-Glc-(1→4)-α-D-Glc-(1→4)-α-D-Glc-(1→4)-α-D-Glc-(1→4)-D-Glc] and 6-*O*-α-maltosylmaltotetraose [**6**, α-D-Glc-(1→4)-α-D-Glc-(1→6)-α-D-Glc-(1→4)-α-D-Glc-(1→4)-α-D-Glc-(1→4)-D-Glc], are depicted in Figure 4. These two isomers differ from one another in the form of α-(1→6) linkage along the hexasaccharide chains. In both spectra, the first three ions, [M-H]⁻, [M-G]⁻, and [M-G2]⁻, show the same loss of 78 Da, thereby identifying the respective α-(1→4) type of linkage between rings 1 and 2, rings 2 and 3, and rings 3 and 4. The next two ions, [M-G3]⁻ and [M-G4]⁻, show a different loss to one another. In compound **5**, the loss of 78 Da from [M-G3]⁻ identifies the α-(1→4) linkage between rings 4 and 5, and that of 90 Da from [M-G4]⁻ identifies the α-(1→6) linkage of the nonreducing end unit. The reverse relationship for [M-G3]⁻ and [M-G4]⁻ was observed for compound **6**, thereby ascertaining the α-(1→6) type of linkage between rings 4 and 5 and the α-(1→4) linkage of the nonreducing end unit. The negative ion spectrum of 4-*O*-α-panosylpanose [**7**, α-D-Glc-(1→6)-α-D-Glc-(1→4)-α-D-Glc-(1→4)-α-D-Glc-(1→6)-α-D-Glc-(1→4)-D-Glc], which has two panosyl repeating units, was similarly analyzed. Successive losses from the [M-H]⁻, [M-G]⁻, [M-G2]⁻, [M-G3]⁻, and [M-G4]⁻ ions of 78, 90, 78, 78, and 90 Da, respectively, were observed, thereby identifying the respective α-(1→4) type of linkage between rings 1 and 2, rings 3 and 4, and rings 4 and 5 and the respective α-(1→6) type of linkage between rings 3 and 4 and rings 5 and 6 (data not shown). Thus, these results indicate that the glucohexasaccharide has a sequential order of α-(1→4), α-(1→6), α-(1→4), α-(1→4), and α-(1→6) glycosidic linkages from the reducing end unit. In conclusion, the overall negative ion fragmentation process described above facilitates sequential analysis of the glycosidic linkage along the α-gluco-oligosaccharide found in amylopectin.

EXPERIMENTAL

Samples

Panose,[17] isopanose,[17] **1**,[18] **2**,[18] **3**,[19] and **7**[20] were prepared according to previously described methods. Compounds **4**, **5**, and **6** were kindly donated by Hayashibara Biochemical Lab. Inc. (Okayama, Japan).[21] These purities
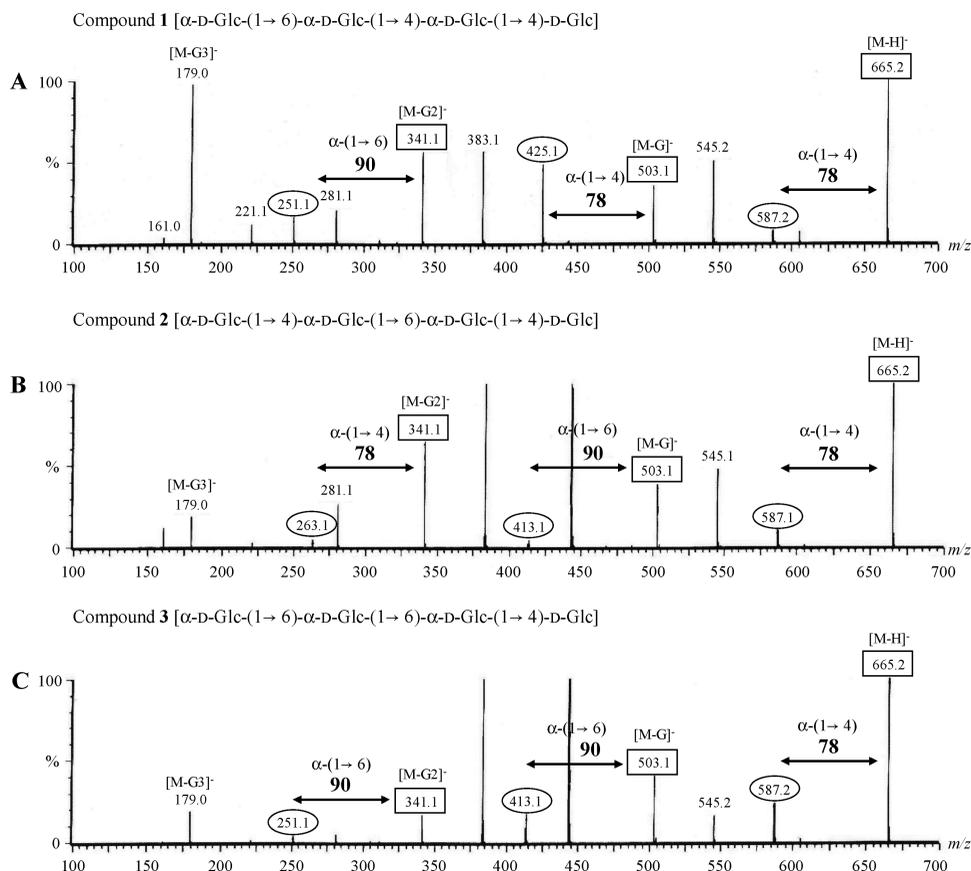
Figure 3: Negative ion Q-TOF MS/MS spectra of three glucotetrasaccharides: **1** (A), **2** (B), and **3** (C).

of panose, isopanose, **1**, **2**, **3**, **4**, **5**, **6**, and **7** were 99%, 99%, 95%, 98%, 96%, 98%, 98%, 93%, and 99%, respectively, based on HPLC analysis (see Analytical Methods). The structural features of these oligosaccharides have been confirmed by ^{1}H and ^{13}C NMR spectra. The major assignments are as follows. ^{1}H NMR data (D$_2$O, 500 MHz) of **1**: δ 5.38 (d, J = 4.0 Hz, H-1′), 5.37 (d, J = 3.9 Hz, H-1′), 5.21 (d, J = 3.6 Hz, H-1α), 4.94 (d, J = 3.4 Hz, H-1‴), 4.63 (d, J = 7.7 Hz, H-1b). ^{13}C NMR (D$_2$O, 500 MHz): δ 100.8 (C-1″), 100.4 (C-1′α), 100.3 (C-1′β), 99.0 (C-1‴), 96.6 (C-1β), 92.8 (C-1α). ^{1}H NMR data (D$_2$O, 500 MHz) of **2**: δ 5.37 (d, J = 4.0 Hz, H-1‴), 5.36 (d, J = 4.0 Hz, H-1′), 5.22 (d, J = 3.6 Hz, H-1α), 4.94 (d, J = 3.7 Hz, H-1″), 4.63 (d, J = 8.0 Hz, H-1β). ^{13}C NMR (D$_2$O, 500 MHz): δ 100.8 (C-1″), 100.4 (C-1′α), 100.3 (C-1′β), 99.0 (C-1‴), 96.6 (C-1β), 92.8 (C-1α). ^{1}H NMR data (D$_2$O, 500 MHz) of **3**: δ 5.34 (d, J = 3.7 Hz, H-1′), 5.23 (d, J = 3.7 Hz, H-1α), 4.97 (d, J = 3.1 Hz, 1″), 4.97 (d, J = 3.3 Hz, H-1″), 4.66 (d, J = 7.9 Hz, H-1β). ^{13}C NMR (D$_2$O, 500 MHz): δ 100.7 (C-1′α), 100.6 (C-1′β), 98.9

Compound **5** [α-D-Glc-(1→ 6)-α-D-Glc-(1→ 4)-α-D-Glc-(1→ 4)-α-D-Glc-(1→ 4)-α-D-Glc-(1→ 4)-D-Glc]

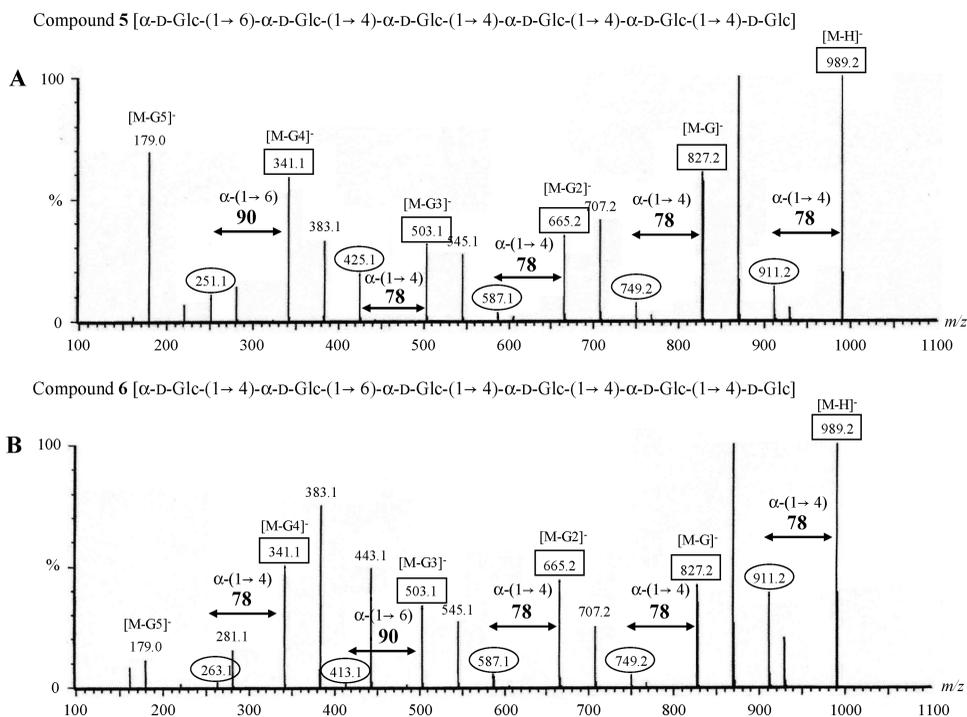Compound **6** [α-D-Glc-(1→ 4)-α-D-Glc-(1→ 6)-α-D-Glc-(1→ 4)-α-D-Glc-(1→ 4)-α-D-Glc-(1→ 4)-D-Glc]

Figure 4: Negative ion Q-TOF MS/MS spectra of two gluco-oligosaccharide isomers: **5** (A) and **6** (B).

(C-1″), 98.6 (C-1‴), 96.6 (C-1β), 92.8 (C-1α). ^{1}H NMR data (D$_2$O, 500 MHz) of **4**: δ 5.40–5.38 (3H, H-1′, 1″, 1‴), 5.21 (d, $J = 3.7$ Hz, H-1α), 4.94 (d, $J = 3.4$ Hz, H-1⁗), 4.64 (d, $J = 7.7$ Hz, H-1β). ^{13}C NMR (D$_2$O, 500 MHz): δ 100.6 (C-1‴), 100.5 (C-1″), 100.4 (C-1′β), 100.2 (C-1′α), 99.0 (C-1⁗), 96.7 (C-1β), 92.8 (C-1α). ^{1}H NMR data (D$_2$O, 500 MHz) of **5**: δ 5.41–5.39 (4H, H-1′, 1″, 1‴, 1⁗), 5.23 (d, $J = 4.0$ Hz, H-1α), 4.96 (d, $J = 3.7$ Hz, H-1⁗), 4.66 (d, $J = 7.9$ Hz, H-1β). ^{13}C NMR (D$_2$O, 500 MHz): δ 100.6 (C-1⁗), 100.4 (C-1′, 1″), 100.3 (C-1′β), 100.2 (C-1′α), 98.9 (C-1‴), 96.6 (C-1β), 92.7 (C-1α). ^{1}H NMR data (D$_2$O, 500 MHz) of **6**: δ 5.40–5.37 (4H, H-1′, 1″, 1‴, 1⁗), 5.22 (d, $J = 3.9$ Hz, H-1α), 4.94 (d, $J = 3.7$ Hz, H-1⁗), 4.64 (d, $J = 7.9$ Hz, H-1β). ^{13}C NMR (D$_2$O, 500 MHz): δ 101.0 (C-1⁗), 100.8 (C-1‴), 100.6 (C-1″), 100.5 (C-1′β), 100.4 (C-1′α), 98.9 (C-1⁗), 96.8 (C-1β), 92.9 (C-1α). ^{1}H NMR data (D$_2$O, 500 MHz) of **7**: δ 5.39 (3H, H-1′, 1‴, 1⁗), 5.24 (d, $J = 3.7$ Hz, H-1α), 4.96 (d, 2H, $J = 3.1$ Hz, H-1″, 1⁗), 4.66 (d, $J = 7.8$ Hz, H-1β). ^{13}C NMR (D$_2$O, 500 MHz): δ 101.2 (C-1⁗), 101.1 (C-1‴), 100.9 (C-1′β), 100.8 (C-1′α), 99.3(C-1⁗), 99.1 (C-1″), 97.0 (C-1β), 93.1 (C-1α).

Analytical Methods

For mass analysis, a Micromass Q-TOF Ultima API spectrometer (Waters) equipped with a nano-ESI source was used. The spray needles used were glass capillaries with an orifice diameter in the range of 1 to 2 μm. For negative ESI analysis, oligosaccharides were dissolved in MeOH-water (1:1, v/v) and introduced directly via a syringe. The instrument was scanned from m/z 2000 to m/z 50. The accelerating voltage was 1 kV. MS/MS spectra of molecular ions were obtained by collision-induced dissociation (CID), using argon as the collision gas and varying the collision energy between ~10 and ~60 eV. HPLC analysis was carried out using a Unison UK-Amino column (4.6 × 250 mm, Imtakt) with a JASCO Intelligent system liquid chromatograph and detection at ELSD. The bound material was eluted with 75% CH_3CN at a flow rate of 0.7 mL/min at 60°C. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA 500 spectrometer at 25°C. Chemical shifts are expressed in δ relative to sodium 4,4-dimethyl-4-silapentanoate (TPS) as the external standard in D_2O.

ACKNOWLEDGMENT

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