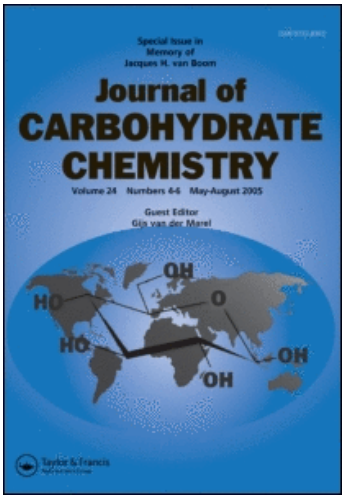

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An Efficient Structural Analysis of *N*-Methyl-1-deoxynojirimycin-Containing Sialo-oligosaccharides by Ion-Spray Mass Spectrometry and the Mechanism of Palladium Catalyzed *N*-Methylation in Methanol[1]

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AN EFFICIENT STRUCTURAL ANALYSIS OF *N*-METHYL-1-DEOXYNOJIRIMYCIN-CONTAINING SIALO-OLIGOSACCHARIDES BY ION-SPRAY MASS SPECTROMETRY AND THE MECHANISM OF PALLADIUM CATALYZED *N*-METHYLATION IN METHANOL[1]

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ABSTRACT

The structures of a series of sialo-oligosaccharides containing *N*-methyl-1-deoxynojirimycin (*N*-methyl-DNJ) were effectively analyzed by ion-spray mass and tandem mass (MS/MS) spectrometry. Based on those analytical results, the mechanism of the palladium catalyzed *N*-methylation of DNJ in methanol was investigated.

INTRODUCTION

In the course of a synthetic approach[2] designed to develop a novel series of biologically active sialo-oligosaccharides containing 1-deoxynojirimycin (DNJ), we have found[2b, 3] that the *N*-benzyloxycarbonyl-DNJ derivatives were readily hydrogenolyzed over palladium catalyst in methanol to give the *N*-methyl-DNJ derivatives with a dramatic conformational change of the piperidine ring (FIG.1). However, unambiguous assignment of such unusual *N*-methylation products was difficult to make from usual ^{1}H NMR analysis only, because of the irregularity of the spectrum resulting from a diastereomerization on nitrogen atom.[4]

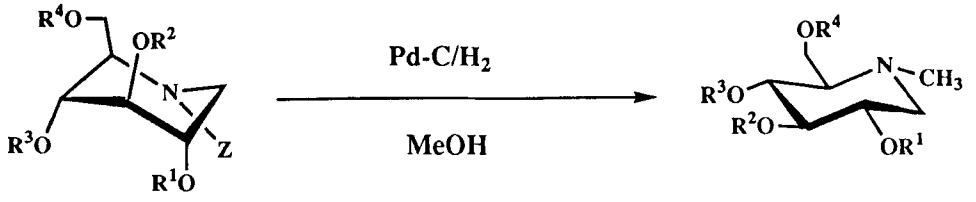


FIG.1. R^{1-4}=H, acetyl, benzyl, glycosyl etc.

Recently ion-spray mass spectrometry[5] (ion-spray MS), which permits the formation of gas phase ions directly from solution at atmospheric pressure, has become very useful for analyzing the structures of a variety of biopolymers,[6] providing a simple and highly sensitive method to determine the precise molecular weight even in mixtures. In this connection, a new analytical procedure of pyridylamino (PA-)oligosaccharides using an on-line system combining HPLC and ion-spray MS has also been reported.[7]

We describe here an efficient structural analysis of some synthetic sialo-oligosaccharides containing *N*-methyl-DNJ by ion-spray MS and tandem MS (MS/MS) spectrometry, and discuss a possible mechanism of the palladium catalyzed *N*-methylation of DNJ in methanol.

MATERIALS AND METHODS

Materials. The sialo-oligosaccharides containing DNJ or *N*-methyl-DNJ employed in this study were synthesized according to the procedure described in refs. 2 and 3.

General methods. Electrospray mass spectra were recorded on an API-III triple-quadrupole mass spectrometer (Perkin-Elmer Sciex Instruments, Thornhill, Canada) fitted with an atmospheric pressure ionization source. The mass spectrometer was operated in the positive mode; the ion-spray voltage was set to 4500 V and the interface plate voltage was 650 V. The orifice voltage was 70-110 V. For the negative mass spectra, the ion-spray voltage was set to -3500 V and the interface plate voltage was -650 V. The orifice voltage was -90 V. The pressure of the nebulizing gas was 30 psi and the flow rate was 0.8 L/min. The collisionally activated dissociated (CAD) spectrum was measured with argon as the collision gas, and the collision gas pressure was set at 300 x 10^{12} atoms/cm^2. The collision energy was 120 eV.

RESULTS AND DISCUSSION

To elucidate the mechanism of the palladium catalyzed *N*-methylation of DNJ in methanol, we undertook the following experiments (FIG. 2). The protected α-Neu5Ac-

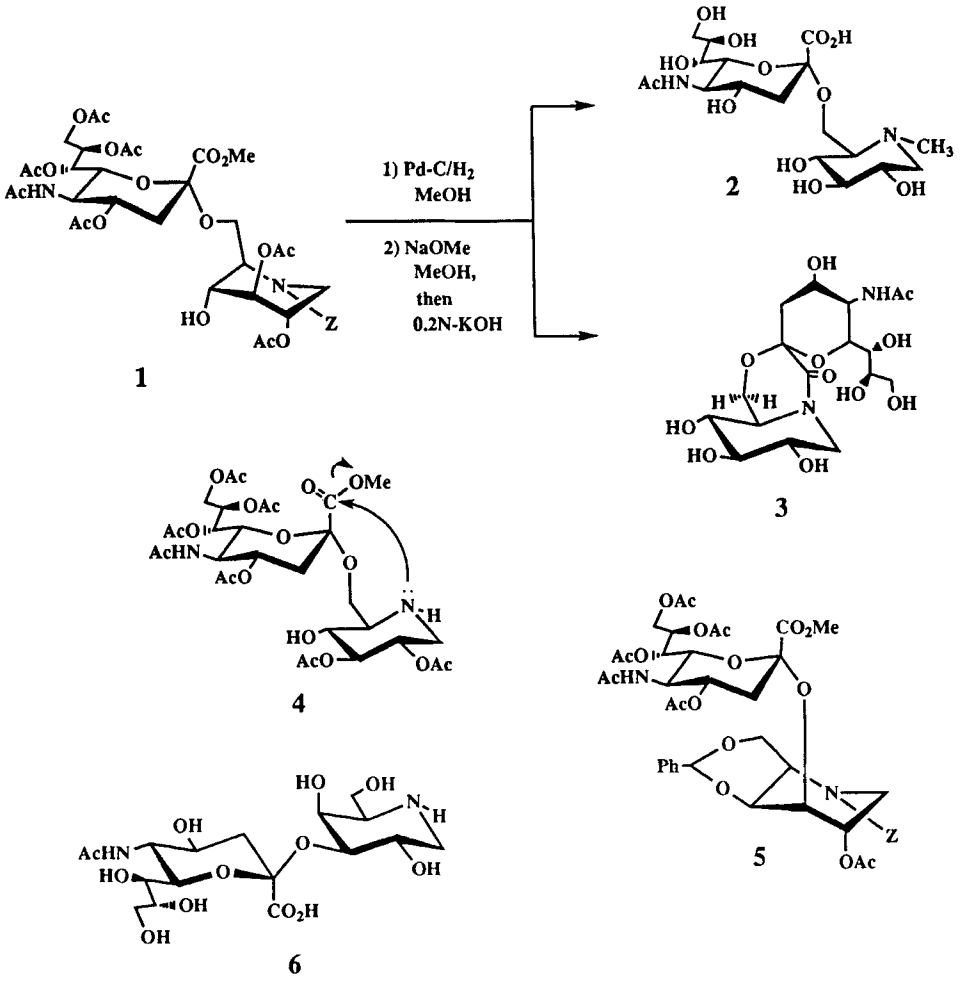


FIG. 2

(2→6)-DNJ derivative[3a] **1** was hydrogenolyzed for 30 min in the presence of 10% palladium-on-carbon in methanol, followed by *O*-deacetylation and saponification of the methoxycarbonyl group, to give the *N*-methyl derivative **2** in 19% yield together with the lactam derivative **3** (81%).

The ion-spray MS spectra of **2** in both positive and negative ion modes are shown in FIG.3, (A) and (B), respectively. In both spectra, a significant base peak (100% relative intensity) was clearly detected at m/z 469.2 ($[M + H]^+$) or 466.8 ($[M - H]^-$), respectively, showing the average molecular weight of $C_{18}H_{32}N_2O_{12}$ (468.46) that corresponds to the expected *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-1,5-dideoxy-1,5-imino-*N*-methyl-D-glucitol (**2**).

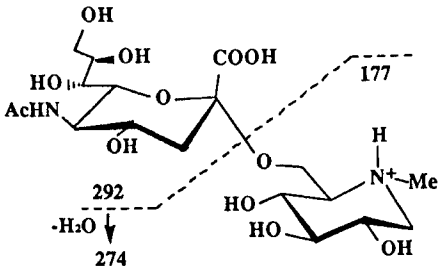


FIG. 3, (C)

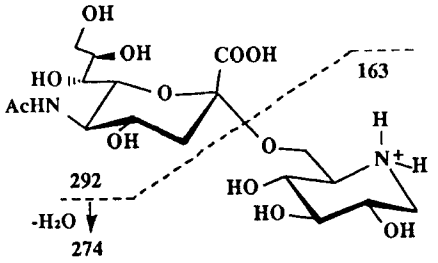


FIG. 3, (D)

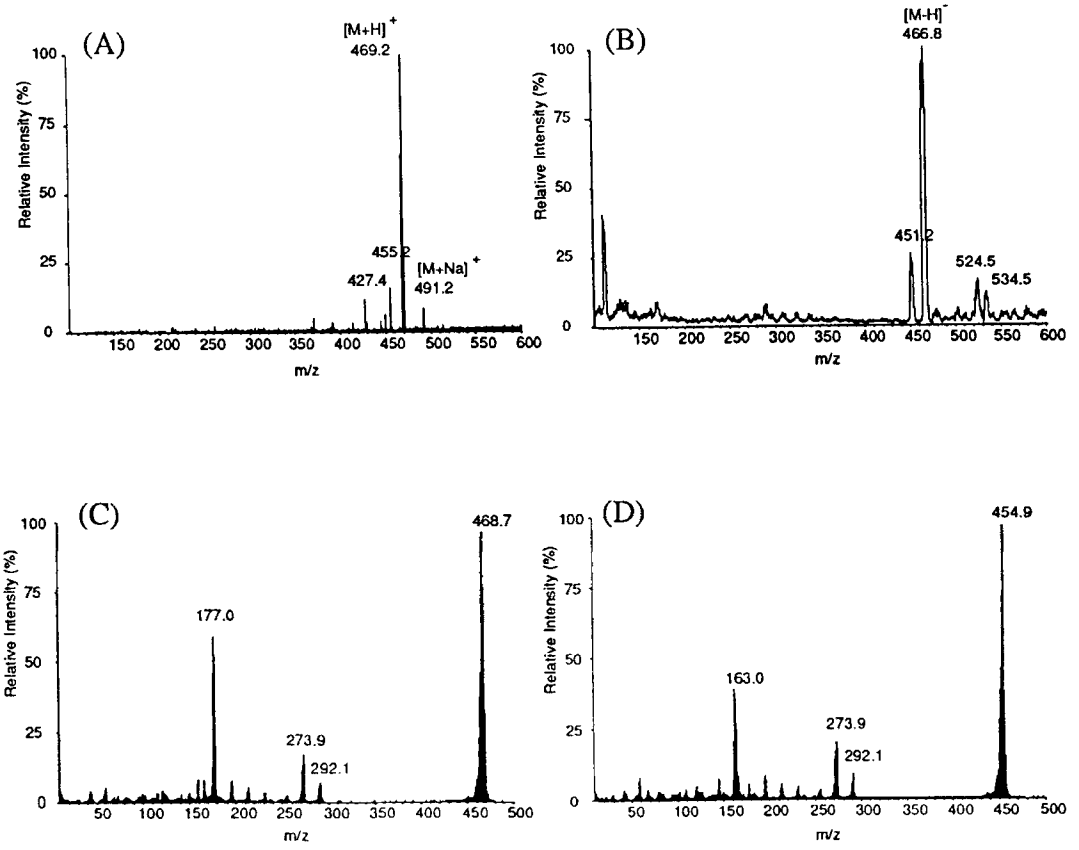


FIG. 3. Ion-spray MS and tandem MS (MS/MS) spectra for ca. 50 pmol of compound **2**; infusion rate 3μL/min in 1:1 CH_3CN-H_2O with 0.05% TFA. (A) Positive-ion spectrum; sum of 10 scans (m/z 100-600, step 0.2, 2.7 s/scan), orifice voltage = 80 V. (B) Negative-ion spectrum; sum of 10 scans (m/z 100-600, step 0.2, 2.7 s/scan), orifice voltage = -90 V. (C) MS/MS spectrum from CAD of the precursor-ion (m/z 469.2); sum of 10 scans (m/z 10-500, step 0.2, 2.6 s/scan), orifice voltage = 90 V. (D) MS/MS spectrum from CAD of the precursor-ion (m/z 455.2); sum of 30 scans (m/z 10-500, step 0.2, 2.6 s/scan), orifice voltage = 90 V.

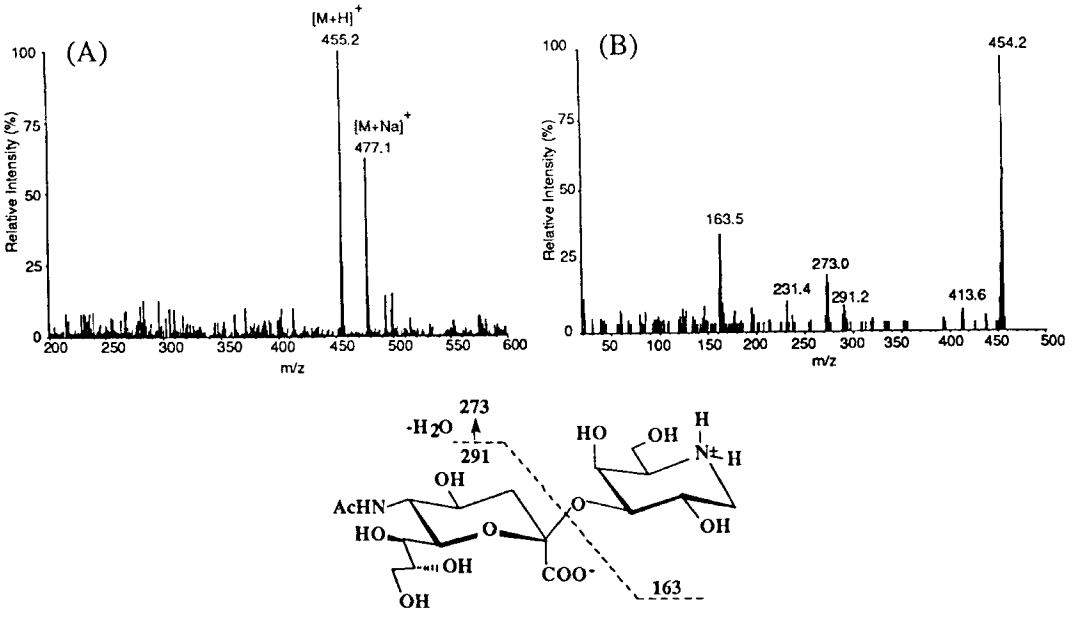


FIG. 4, (B)

Fig. 4. Ion-spray MS and tandem MS (MS/MS) spectra for ca. 25 pmol of compound **6**, infusion rate 5 μL/min in 1:1 CH_3CN-NH_4OAc (1 mM, pH 4.0). (A) Positive-ion spectrum; sum of 6 scans (m/z 200-600, step 0.1, 4.5 s/scan), orifice voltage = 80 V. (B) MS/MS spectrum from CAD of the precursor-ion (m/z 455.2); sum of 21 scans (m/z 20-500, step 0.1, 5.2 s/scan), orifice voltage = 70 V.

Comparing the simplicity of spectra, the positive ion mode seems to be superior to the negative ion mode for this series of compounds.

In the MS/MS spectrum of P = 469.2 ($[M + H]^+$) in positive ion mode, four significant daughter ions were detected at m/z 177 ($C_7H_{15}NO_4$; protonated *N*-methyl-DNJ part), 292 ($C_{11}H_{18}NO_8$; Neu5Ac part) and 274 (Neu5Ac - H_2O), providing the unambiguous evidence for the structure **2** [FIG.3, (C)]. A small peak at m/z 455.2 ($[M - Me + H]^+$) gave a significant, stable daughter ion at m/z 163 (protonated DNJ part) together with two common ion peaks at m/z 292 and 274 derived from Neu5Ac part [FIG.3, (D)]. On the other hand, formation of **3** as the major product strongly suggests the initial formation of free amine **4** as the precursor. In other words, the free amine **4** formed in the initial stage was efficiently trapped as the lactam **3** indicating that the major part of *N*-Z was once cleaved before *N*-methylation.

For further investigation of the palladium catalyzed *N*-methylation mechanism, we undertook the hydrogenation of **5** in acetic acid. *O*-Deacetylation of the products and saponification of the methoxycarbonyl group as just described gave a novel α-Neu5Ac-

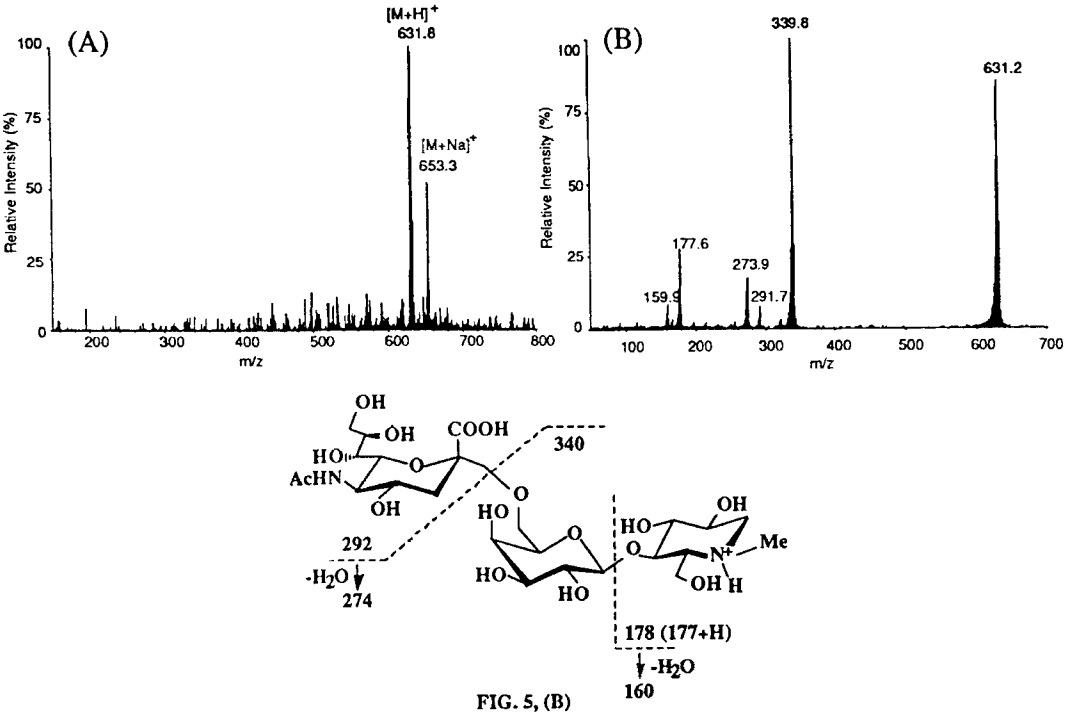
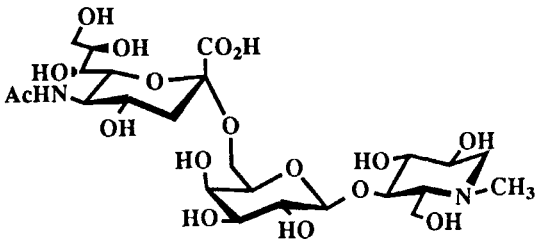


Fig. 5. Ion-spray MS and tandem MS (MS/MS) spectra for ca. 50 pmol of compound **7**, infusion rate 3 μL/min in 1:1 CH3CN-H2O with 0.05% TFA. (A) Positive-ion spectrum; sum of 10 scans (m/z 150-800, step 0.1, 7 s/scan), orifice voltage = 70 V. (B) MS/MS spectrum from CAD of the precursor-ion (m/z 631.8); sum of 20 scans (m/z 50-700, step 0.1, 6.95 s/scan), olifice voltage = 100 V.

(2→3)-DNJ (galactose type) derivative **6** in high yield. The ion-spray MS of this product [FIG.4, (A)] showed two significant ion peaks at m/z 455.2 ([M + H]+, base peak) and 477.1 ([M + Na]+). The MS/MS spectrum of P = 455.2 [FIG.4, (B)] gave the characteristic daughter ions at m/z 163.5 (protonated DNJ part), 291.2 (Neu5Ac part) and 273 (Neu5Ac - H_2O) as described for **5** in FIG.3, (D), indicating the structure of free amine **6** in which the lactam formation does not take place. These experimental results show that the presence of methanol may be critical for the *N*-methylation of DNJ.

In 1955, Rice and Kohn reported[8] the Raney nickel catalyzed *N*-alkylation of aromatic amines with alcohols. A similar reaction was also reported[9] by Ainsworth in 1956. In both reports, it was concluded that the possible mechanism of the over-all reaction involved the dehydrogenation of the alcohol by Raney nickel to give aldehyde, followed by Schiff base formation and subsequent reduction to alkylated amine. However, no *N*-methylated amine was obtained by this method.

In the immediately preceding paper,[3b] we have reported the synthesis of novel *N*-methyl-1-deoxynojirimycin-containing sialo-oligosaccharides related to ganglioside GM_3. The ion-spray MS and MS/MS spectra of *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-(β-D-galactopyranosyl)-(1→4)-1,5-dideoxy-1,5-imino-*N*-methyl-D-glucitol (**7**) are shown in FIG.5. The molecular ion peaks were identified as $[M + H]^+$ (base peak) and $[M + Na]^+$ at m/z 631.8 and 653.3, respectively [FIG.5, (A)]. In the MS/MS spectrum of P = 631.8 [FIG.5, (B)], five characteristic daughter ions were detected at m/z 339.8 ($[M - Neu5Ac + H]^+$), 291.7 (Neu5Ac part), 273.9 (Neu5Ac - H_2O), 177.6 (protonated *N*-methyl-DNJ part), and 159.9 (protonated *N*-methyl-DNJ - H_2O), respectively, showing the *N*-methylated



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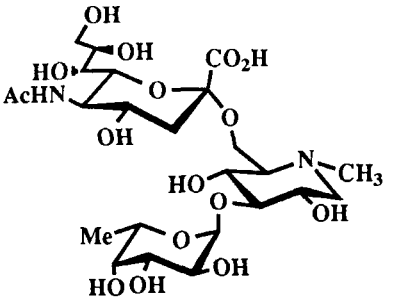

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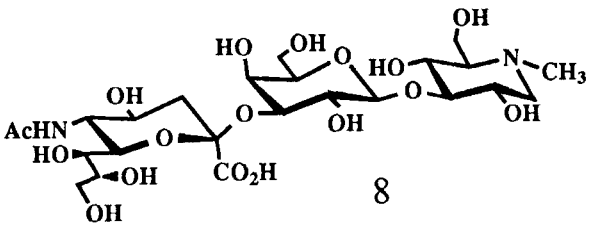

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trisaccharide structure assigned **7**. The α-Neu5Ac-(2→6)-Gal linkage seems to readily be cleaved to give a stable daughter ion at m/z 339.8.

The efficient and precise method to determine the α-Neu5Ac-(2→6)- and α-Neu5Ac-(2→3)-Gal glycoside linkages is particularly interesting, because of their critical importance in cell recognition, proliferation, differentiation, oncogenesis and so on.[10] The MS/MS spectrum of *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-1,5-dideoxy-1,5-imino-*N*-methyl-D-glucitol (**8**), which corresponds to the α-Neu5Ac-(2→3)-isomer of **7**, is shown in FIG.6. Among the detected five daughter ion peaks, the intensity of $[M-Neu5Ac + H]^+$ at m/z 339.8 is relatively small compared with that detected for **7** under the similar analytical condition [see, FIG.5, (B)]. If this difference is attributed to the critical difference of the glycoside linkages of Neu5Ac, the MS/MS approach may be

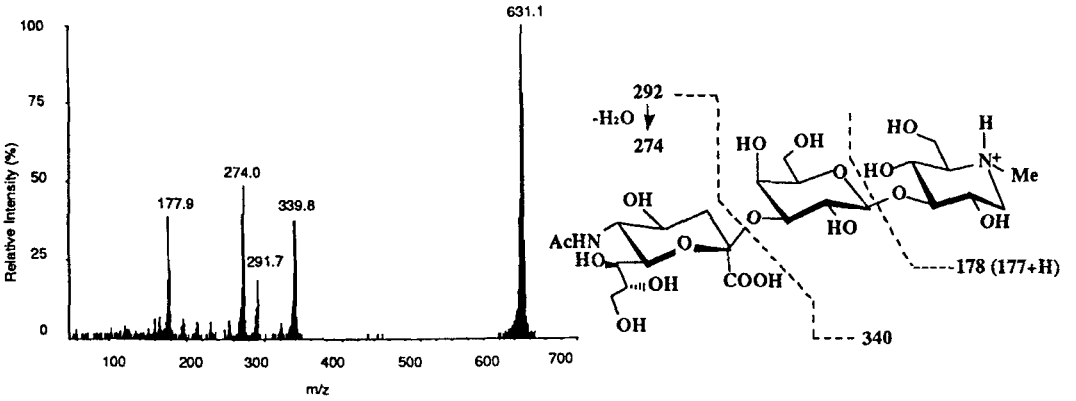

Fig. 6. Tandem MS (MS/MS) spectra for ca. 50 pmol of compound **8**, infusion rate 3 μL/min in 1:1 CH_3CN-H_2O with 0.05% TFA. MS/MS spectrum from CAD of the precursor-ion (m/z 631.1); sum of 20 scans (m/z 50-700, step 0.1, 7s/scan), olifice voltage = 40 V.

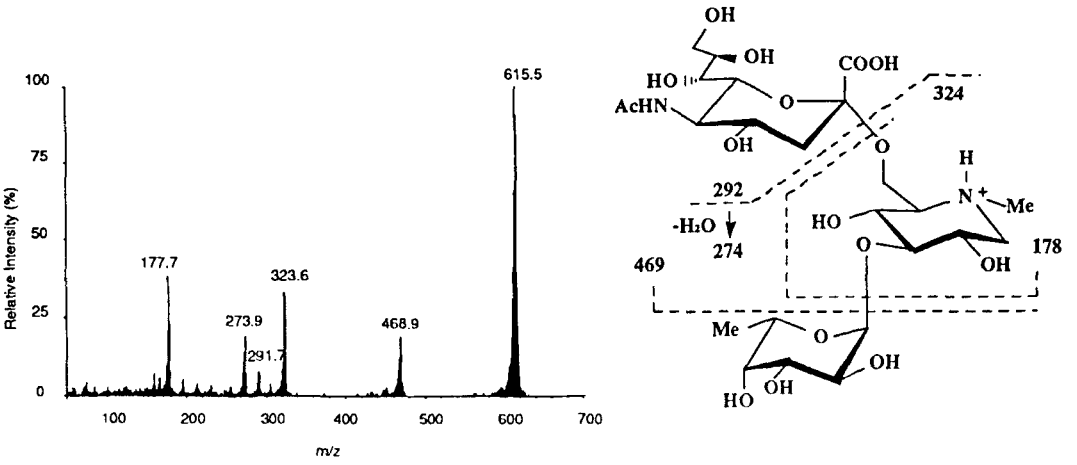

Fig. 7. Tandem MS (MS/MS) spectra for ca. 50 pmol of compound **9**, infusion rate 3 μL/min in 1:1 CH_3CN-H_2O with 0.05% TFA. MS/MS spectrum from CAD of the precursor-ion (m/z 615.5); sum of 20 scans (m/z 50-700, step 0/1, 7s/scan), olifice voltage = 110 V .

highly useful for the analysis of the glycosylation site of Neu5Ac in sialo-oligosaccharides.

FIG.7 shows the MS/MS spectrum (P = 615.5) of a novel trisaccharide (**9**) containing both sialic acid and L-fucose. Two characteristic daughter ions at m/z 469 and 324 are assigned to the $[M - Fuc + H]^+$ and $[M - Neu5Ac + H]^+$ ions, respectively, and the other three daughter ions at m/z 292, 274 and 178 indicate the structure of α-Neu5Ac-(2→6)-*N*-methyl-DNJ disaccharide [see, FIG.3, (C)].

In conclusion, the ion-spray MS and tandem MS (MS/MS) are highly effective for analyzing the structure of sialo-oligosaccharides containing DNJ or *N*-methyl-DNJ at the reducing end. Both the Neu5Ac and DNJ parts gave the characteristic stable daughter ions, providing unambiguous evidences for the structure assigned. This method may also be useful for elucidation of the mode of glycoside linkage of sialic acid. Based on these analytical results, the mechanism of the palladium catalyzed *N*-methylation of DNJ in methanol is believed to involve the dehydrogenation of methanol by palladium to form formaldehyde, which then reacts with the free amine derived from the *N*-benzyloxycarbonyl derivative of DNJ. The resulting Schiff base is finally reduced to yield the corresponding *N*-methyl-DNJ derivative. Therefore, the palladium catalyzed *N*-alkylation of amine may be most effectively achieved under the coexistence of the corresponding aldehyde in the individual alcohols.

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