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Stereochemical Studies of Sialic Acid Derivatives by Vibrational Circular Dichroism

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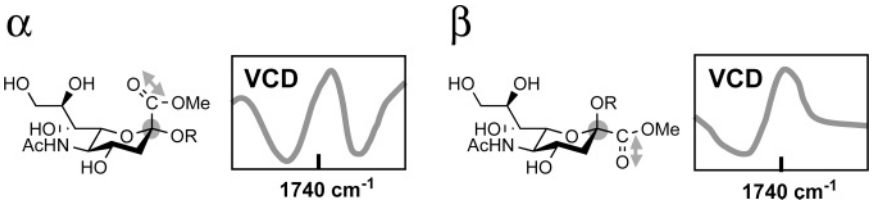
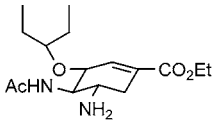
ABSTRACT



Systematic VCD studies of *N*-acetylneuraminic acid (Neu5Ac), a recognition-related unique carbohydrate, were performed for the first time. Two pairs of anomeric isomers regarding a quaternary C2 asymmetric carbon of Neu5Ac derivatives were synthesized. VCD spectral patterns around the ester carbonyl region, as well as other Mid-IR regions, would be practical markers to distinguish the C2 stereochemistry.

Sialic acids are a diverse family of naturally occurring 2-keto-3-deoxynononic acids that are typically found attached to the terminal positions of several classes of cell-surface and secreted glycan molecules in vertebrates.[1] Proteins that recognize sialic acid are involved in a broad range of biological processes, including intercellular adhesion, signaling, and microbial attachment.[1] Utilizing the fact that an influenza virus recognizes this unique nine-carbon backbone monosaccharide, successful drug discovery of inhibitors of sialidases have been developed, such as the Tamiflu (**1**).[2] Although it appears that sialic acids work as bio-markers of living species, there are more than 50 structural variations in nature. Also, the diversity in sialic acids was generated by various substitution patterns at the C4, C5, C7, C8, and C9 positions associated with linkage variation. *N*-Acetylneuraminic acid (Neu5Ac, **2**), *N*-glycolyl neuraminic acid (Neu5Gc, **3**), and 3-deoxy-L-glycero-D-galacto-nonulo-pyranosonic acid (KDN, **4**) are representative structures of sialic acids. In particular, Neu5Ac, which is the most prevalent



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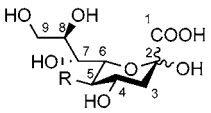

2 Neu5Ac: R=NHAc
3 Neu5Gc: R=NHCOCH$_2$OH
4 KDN: R=OH

sialic acid, occurs often at the non-reducing ends of glycoproteins and glycolipids in mammalian cellular systems frequently via α-glycosidic linkages to galactose and *N*-acetylgalactosamine.[1]

To understand the structure and functional aspects, synthetic studies of sialic acid derivatives have been widely investigated during the past half century by utilizing chemical and/or enzymatical methods.[3] Stereoselective glycosidation reactions at the C2 carbon to a galactose derivative were

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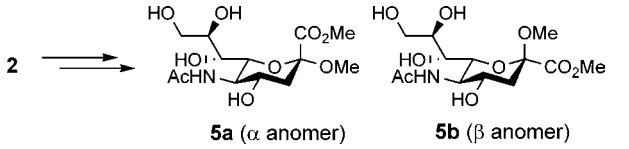
especially focused on,[3b,c] since the resulting α or β stereochemistry at the C2 quaternary carbon affects significant aspects of the entire carbohydrate's conformation, thus leading to its biological activities. Although only α glycosides are believed to occur in nature, determination of this stereochemistry of the quaternary carbon is still complicated. The commonly used empirical ^{1}H NMR rules are based on differences among H3, H4, H9 chemical shifts, as well as coupling constants between H7 and H8: (1) δ αH3eq > δ β H3eq,[4a] (2) δ αH4 < δ βH4,[4b] (3) $|\delta$ αH9 − δ αH9′| < $|\delta$ βH9 − δ βH9′|,[4c] (4) $\alpha J_{7,8} > \beta J_{7,8}$.[4c] However, they include many exceptions and frequently cannot be applied to protected derivatives.

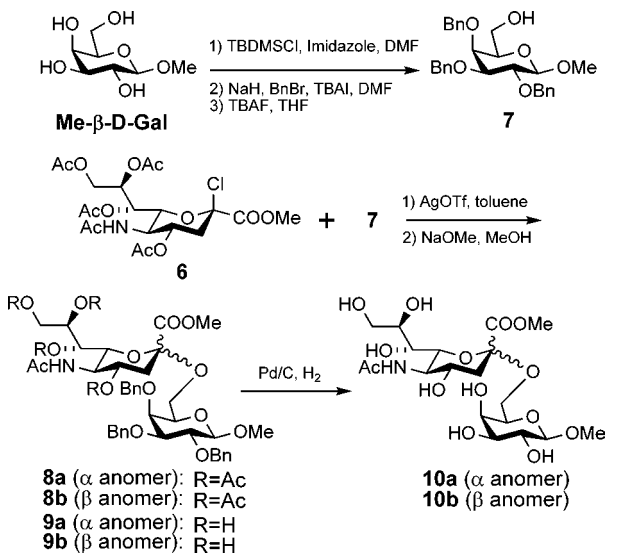
We recently reported that the vibrational circular dichroism (VCD) technique is a powerful tool for stereochemical analysis of glycoconjugates, including carbohydrates and glycopeptides.[5,6] VCD measures the differential absorption of left versus right circularly polarized IR radiation by molecular vibrational transitions, having the advantage of both CD and IR features. VCD is an emerging tool for stereochemical analyses in the field of life sciences as well as material sciences.[7] Herein, we report the first application of VCD to the stereochemical analysis of sialic acid derivatives. This technique would be an especially powerful tool for determination of stereochemistry at a quaternary asymmetric carbon of the *N*-acetylneuraminic acid derivative. To obtain a clear VCD signal of a carbonyl group and to consider their synthetic advantages, a methoxy carbonyl group was selected as the sensitive VCD probe. Simple methyl glycosides **5a** (α anomer) and **5b** (β anomer) were prepared by well confirmed methods[8] to ensure their stereochemistries at each C2 carbon. In a Koenigs−Knorr reaction, a full esterfied Neu5Ac chloride (**6**)[8b] was treated with Ag_2CO_3 and methanol to afford a full esterfied α-methyl glycoside. The following deacetylation gave an α anomer (**5a**) as a single stereoisomer. The β anomer was prepared by the acid-catalyzed Fisher method to give **5b** (Scheme 1).[8]

Scheme 1



Naturally occurring Neu5Ac(2−6)Gal analogs were synthesized via the Koenigs−Knorr glycosylation[3c,8] (Scheme 2). A primary alcohol and three secondary hydroxyl groups

Scheme 2



of a galactose methyl glycoside were protected as *tert*-butyldimethyl silyl and benzyl ethers, respectively. Selective deprotection of the C6 primary alcohol with a tetrabutylammonium fluoride produced the glycosyl acceptor (**7**)[9] in moderate yield. The fully esterified Neu5Ac chloride (**6**) as a glycosyl donor and the glycosyl acceptor (**7**) were treated with a promoter AgOTf (1.5 equiv) and MS 4Å in toluene to give a diastereomeric mixture (α:β = 1:1) of the disaccharides (**8a**, **8b**) in 40% yield, which were separated by simple flash column chromatography. A base-catalyzed mild transesterification and subsequent hydrogenolysis reactions of each anomer gave a free α glycoside (**10a**) and a β glycoside (**10b**) of sialyl methyl esters, respectively, in good yield (Scheme 2).[10] Stereochemistry of **10a** was confirmed

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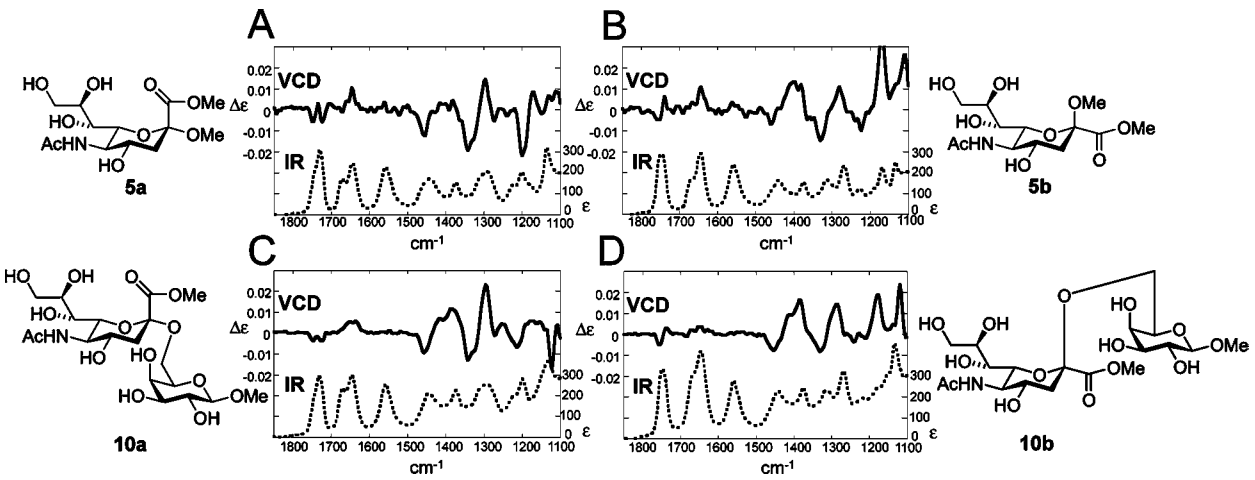

Figure 1. IR (- - -) and VCD (—) spectra in DMSO-d_6 (c = 0.16 M, l = 71 μm for spectra in A and B, and c = 0.18 M, l =100 μm for others) of (A) **5a**, (B) **5b**, (C) **10a**, and (D) **10b**. Data collection time and resolution were 3 h and 8 cm^{-1}, respectively.

by a comparison with previously reported ^{1}H NMR data[11] as its free acid, after alkali hydrolysis of **10a**. Also, the empirical ^{1}H NMR rule comparing chemical shifts of α H3 (eq) and β H3 (eq) between **5a** and **5b** and **10a** and **10b** supported these reliable assignments.[4a,10]

To obtain basic chiral VCD information of the sialyl glycoside part, VCD studies of the simplest sialyl methyl glycosides were first performed. IR and VCD spectra were measured at 8 cm^{-1} resolution with a Bomem/BioTools ChiralIR spectrometer equipped with the dual PEM system.[12] To avoid overlapping sample signals with strong water absorptions at 1650 cm^{-1}, all of the spectra were obtained in DMSO-d_6 with a BaF_2 or CaF_2 cell of 71 or 100 μm path length, at a concentration of approximately 0.16−0.18 M. The IR and VCD spectra of sialyl methyl glycosides **5a** (α anomer) and **5b** (β anomer) are shown in Figure 1A,B. When compared to IR and VCD spectra of common monosaccharides such as glucose, galactose, etc,[5f,7e] they show more complex spectra, exhibiting characteristic absorption bands at around 1750, 1730, 1670, 1645, 1560, and 1440 cm^{-1}. These bands are most likely due to two ester carbonyl stretches, two amidic carbonyl stretches (amide I), one *trans*-amide NH bending (amide II), and one *cis*-amide NH bending (amide II) vibration, respectively.[13] These vibrational assignments agree with the previously reported IR spectra of 2-acetamide-2-deoxy-D-glucose (GlcNAc), 2-acetamide-2-deoxy-D-galactose (GalNAc), and 2-acetamide-2-deoxy-D-mannose (ManNAc).[5f,7e] The IR spectra of the α and β anomers (**5a**, **5b**) are quite similar, except for the 1300−1150 cm^{-1} region; however, their VCD spectra are unusually dissimilar. These different VCD patterns obviously show their potential for an efficient, novel differentiation methodology toward α or β anomers.

The VCD spectrum of the β anomer (**5b**) shows a characteristic strong positive band around 1170 cm^{-1} ($g = \Delta A/A = 1.7 \times 10^{-4}$). This positive band agrees well with the previously reported glycoside band occurring predominately with the $^2C_5$ ring conformation ($^1C_4$ for other common pyranoses) with the axial β methyl glycoside bond.[5a,f,g,14] On the other hand, the α anomer shows a relatively flat VCD pattern around 1150 cm^{-1}, with an ambiguous neighboring negative peak, probably due to other complex coupled vibrational modes. Focusing on the carbonyl region utilizes the great advantage of the sialic acid VCD analysis, because of the absence of the carboxyl group on the other common sugars, and also the completely different VCD pattern around 1740 cm^{-1}. The α anomer **5a** shows negative (1747 cm^{-1}, $\Delta\epsilon = -0.0057$), positive (1736 cm^{-1}, $\Delta\epsilon = 0.0029$), and negative (1724 cm^{-1}, $\Delta\epsilon = -0.0058$) signals, whereas β anomer **5b** shows characteristic negative (1755 cm^{-1}, $\Delta\epsilon = -0.0049$) and positive (1740 cm^{-1}, $\Delta\epsilon = 0.0064$) signals around 1750 cm^{-1}. These results encourage us to conduct VCD studies of more complicated sialyl disaccharides to ensure generality in these VCD patterns.

Figure 1C,D shows VCD spectra of 2−6 linkages sialyl glycosides **10a** and **10b**. The VCD spectral patterns are very similar to those of methyl glycosides, respectively (**10a**, 1458 cm^{-1}, $\Delta\epsilon = -0.0095$; 1342 cm^{-1}, $\Delta\epsilon = -0.013$; 1296 cm^{-1}, $\Delta\epsilon = 0.023$; 1273 cm^{-1}, $\Delta\epsilon = -0.0012$; 1254 cm^{-1}, $\Delta\epsilon = 0.0045$; **5a**, 1454 cm^{-1}, $\Delta\epsilon = -0.012$; 1342 cm^{-1}, $\Delta\epsilon = -0.019$; 1304 cm^{-1}, $\Delta\epsilon = 0.0097$; 1273 cm^{-1}, $\Delta\epsilon = -0.0054$; 1257 cm^{-1}, $\Delta\epsilon = 0.0006$). Since the VCD spectrum of the galactose possessing a β-methyl glycoside as the second saccharide is generally silent,[5a,f] the entire VCD spectrum could be dominated by sialyl moiety features. As

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was expected, the VCD spectrum around the carbonyl stretching region shows different patterns between the α and β anomers. Figure 2 shows VCD spectra of **10a** and **10b**

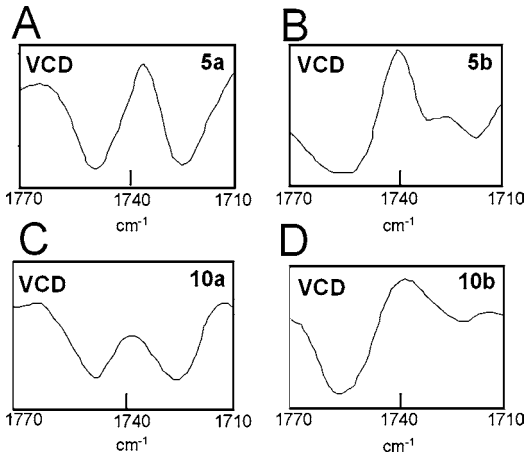

Figure 2. VCD spectra of the carbonyl regions of (A) **5a**, (B) **5b**, (C) **10a**, and (D) **10b**.

around 1750 cm^{-1}. From the higher wavenumber side, VCD showed negative, positive, and negative signs in α anomers and negative and positive signs in β anomers, as in the same case of methyl glycosides. The VCD technique has an advantage in resolving multiple conformational states that are interchanging on a subpicosecond time scale.[5d,7] Since these compounds show bisignate or more complicated carbonyl VCD spectra, two or more conformers concerning the single carboxyl group could be expected in solution. A previous X-ray crystallographic study[15] revealed that **5a** (α anomer) exhibits an intramolecular hydrogen bond between OH groups of the C8 and C1 carbonyl oxygen. It was also mentioned that such a hydrogen bond cannot occur in the β form because of the equatorial position of the carboxyl group. These conformational differences around the carboxyl group at C1 between α and β anomers could be ascribed to their carbonyl VCD patterns.

In conclusion, we applied VCD to the stereochemical analysis of sialic acid derivatives for the first time, and found that the VCD patterns derived from the C═O stretch as well as other mid-IR regions could be practical markers to distinguish between α and β anomers. To date, the α,β discrimination of sialic acid derivatives has been mainly performed by the NMR rules including a $^3J_{C1,H3ax}$ method.[16] Occasionally, the difference in the hydrolysis rate between α- and β-glycosidic bonds with α sialidase[17] and electronic circular dichroism (ECD)[18] have also been used for the same purpose, but the former method requires both α and β anomers, and the latter can only be used for certain cases. These preliminary VCD studies of the sialic acid derivatives suggest that VCD could be a powerful tool for the discrimination of the anomeric configuration of sialic acids, although further investigation is required for application of this method to more complex glycoconjugates such as glycoproteins and glycolipids.

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Supporting Information Available: Synthetic procedures and spectral data of new compounds **8a**, **8b**, **9a**, **9b**, **10a**, and **10b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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