A Data Set of Ion Mobility Collision Cross Sections and Liquid Chromatography Retention Times from 71 Pyridylaminated N-Linked Oligosaccharides

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ABSTRACT: Determination of the glycan structure is an essential step in understanding structure–function relationships of glycans and glycoconjugates including biopharmaceuticals. Mass spectrometry, because of its high sensitivity and mass resolution, is an excellent means of analyzing glycan structures. We previously proposed a method for rapid and precise identification of N-glycan structures by ultraperformance liquid chromatography-connected ion mobility mass spectrometry (UPLC/IM-MS). To substantiate this methodology, we here examine 71 pyridylaminated (PA-) N-linked oligosaccharides including isomeric pairs. A data set on collision drift times, retention times, and molecular mass was collected for these PA-oligosaccharides. For standardization of the observables, LC retention times were normalized into glucose units (GU) using pyridylaminated α-1,6-linked glucose oligomers as reference, and drift times in IM-MS were converted into collision cross sections (CCS). To evaluate the CCS value of each PA-oligosaccharide, we introduced a CCS index which is defined as a CCS ratio of a target PA-glycan to the putative standard PA-glucose oligomer of the same m/z. We propose a strategy for practical structural analysis of N-linked glycans based on the database of m/z, CCS index, and normalized retention time (GU).

KEYWORDS: ion mobility mass spectrometry, N-glycan, ultraperformance liquid chromatography, collision cross section, retention time, glucose unit, CCS index

INTRODUCTION

Glycans are attached onto proteins and lipids and are involved in many biological phenomena. Determination of glycan structures is key in gaining an understanding of glycan function. Mass spectrometry (MS) plays a major role in analyzing glycan structures owing to its high sensitivity and mass resolution.

A central issue in glycan mass analysis is the ambiguity of structural assignments due to the heterogeneity and complexity of glycan structures. Although tandem mass analysis can potentially provide information on glycan structure, the analysis is often time consuming and unsuitable for high-throughput analysis of glycan mixtures in glycomics studies. The complexity of glycan mass analysis is mainly related to structural isomerism: anomericity (α/β), linkage pattern (1→2/1→3/1→4/1→6 etc.), and composition (Glc/Gal/Man and GlcNAc/GalNAc, etc.). In most MS-based glycomics studies, each mass peak is not assigned a unique isomeric structure but the most probable structure(s) based on existing knowledge.

Currently, the demand for definitive and accurate glycan structures is increasing. It has been established that effector functions of IgG antibodies are dependent on the structure of N-glycans attached onto Fc. As a result, the properties of biopharmaceuticals may well differ according to differences in their glycan structures. More specifically, recent studies indicate the importance of taking into account the asymmetry of glycans with multiple branches. There is experimental evidence to suggest that some lectins will bind preferentially with a specific branch. This highlights the importance of distinguishing each glycan isomer of multiple branches.

Separation of isomeric glycans is expedited by liquid chromatography (LC), and the LC method has been used to identify N-glycan structures often in combination with NMR analysis. Recently, ion mobility mass spectrometry (IM-MS) has emerged as a complementary technique for discriminating between isomers. Previously we proposed a method for rapid and confident identification of N-glycan structures by ultraperformance liquid chromatography-connected ion mobility mass spectrometry (UPLC/IM-MS). To
correlate the experimentally obtained collision cross section (CCS) with 3D structure of glycans, research is now in progress to theoretically calculate a CCS.8

There is a practical need for the rapid and accurate identification of glycan structures from accumulated IM-MS data. This includes both intact and fragmented ions,9 and a high priority would be the development of a public IM-MS database.10 It has been established that a traveling wave CCS (\(T^w\)CCS) can be converted from an absolute drift tube CCS (\(DT\) CCS) of a dextran ladder,11 which means a CCS database can be widely utilized without machine bias.

The GlycoMob database has been developed containing CCS data of released glycans without fluorescent tagging. To boost the structural analysis of fluorescently tagged N-glycans by UPLC/IM-MS, we here collected CCS, mass and retention time data of 71 pyridylaminated N-linked oligosaccharides including complex-type, high-mannose-type, and hybrid-type glycans. The data set can become a prototype for a CCS database and can, in the future, be extended and improved upon.

## MATERIALS AND METHODS

**Materials.** Pyridylaminated oligosaccharide derivatives (PA-glycans) were purchased from TaKaRa Bio, Inc. (Shiga, Japan), Masuda Chemical Industries Co., Ltd. (Kagawa, Japan), and GLYENCE Co. (Nagoya, Japan). Dextran (\(\alpha\)-1,6-linked glucose oligomer) from *Leuconostoc mesenteroides* (\(M_m = 1,000\) and \(5,000\)) were obtained from Merck (Darmstadt, Germany). Pyridylaminated \(\alpha\)-1,6-linked glucose oligomers (DP = \(3\)–\(22\), PA-dextran ladder) were obtained from TaKaRa Bio Inc. (Shiga, Japan).

**UPLC/IM-MS Measurement.** Mass measurements and UPLC separation in HILIC mode were performed as reported previously.7 The LC system is equipped with an Acquity UPLC H-Class PLUS Bio binary pump and a fluorescence detector (Waters Corp., Milford, MA). An Acquity UPLC BEH Glycan column (2.1 × 150 mm, 1.7 mm), which has an amide stationary phase, was used for the separation of PA-glycans. The column temperature was set to \(60 ^\circ\)C, and the flow rate of the mobile phase was 0.4 mL/min. The glycans were eluted with a linear gradient (solvent A: 50 mM formic acid (pH 4.4) and solvent B: acetonitrile) starting from 73% to 40% of solvent B for 46.5 min. The injection needle was washed with milli Q water and 20% (v/v) methanol. Fluorescent excitation and emission wavelengths were set at 320 and 400 nm, respectively, and the fluorescent signal was detected at a rate of 1 Hz. All mass measurements were performed on a SYNAPT G2 HDMS (separated mode), SYNAPT G2-S HDMS, or SYNAPT XS (tandemly combined with UPLC) (Waters Corp., Milford, MA), an electrospray ionization quadrupole-time-of-flight mass spectrometer with ion mobility phase. The ion source conditions were as follows: capillary voltage, 3.0 kV; sampling cone voltage, 10 V; temperature of ion source, 120 °C; desolvation gas temperature, 350 °C, and desolvation gas flow, 1000 L/h. For ion mobility measurements, helium gas was introduced into the entrance of the mobility cell and nitrogen gas used as a drift gas. The pressures of the helium and nitrogen gas were kept at 4.26 mbar and 2.76 mbar, respectively. For ion mobility separation, the IMS wave velocity and pulse height were set at 600 m/s and 40.0 V, respectively. A peak intensity of 1000 or more in mobiligram was treated as a peak.

**Conversion of UPLC Retention Time into Glucose Unit.** Retention times of PA-oligosaccharides in UPLC were normalized to glucose units (GU) using pyridylaminated \(\alpha\)-1,6-linked glucose oligomers (DP = \(3\)–\(22\)) as a reference standard.

**Calculation of CCS and CCS Index.** In our previous work, we calibrated CCS values of doubly protonated PA-glycans using the absolute CCS values of polyalanine with the same protonation state in \(N_2\) gas12; e.g., CCS values of doubly protonated PA-glycans were calibrated using the absolute CCS values of doubly protonated polyalanine. In this study, a dextran ladder was used as reference instead of polyalanine because absolute CCS values (sodium ion-adducted) have now been published.13

To obtain the IM data of a sodium ion-adducted dextran ladder, we dissolved it in 1 mM NaHPO\(_4\) and applied it to the ion mobility phase. In this paper, \(T^w\)CCS was calculated from the reported absolute \(DT\) CCS of the dextran ladder by

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**Figure 1.** Plots of \(T^w\)CCS against \(m/z\) for glucose oligomer and PA-glucose oligomer. Data of singly charged ions (a) and doubly charged ions (b) are shown as black dots (glucose oligomer) and red dots (PA-glucose oligomer). [M+Na]+ and [M+2Na]+ ions were used for plotting data of glucose oligomer and [M+H]+ and [M+H+NH\(_4\)]\(^+\) ions for PA-glucose oligomer.
the method of Harvey et al. Furthermore, by calculating the \( TW_{\text{CCS}} \) of the PA-dextran ladder, a linear correlation was established between \( m/z \) and \( TW_{\text{CCS}} \) of PA-glucose oligomers. Using this correlation, a CCS index was determined for each PA-glycan from the ratio:

\[
\text{CCS index} = \frac{TW_{\text{CCS}}(\text{PA-glycan})}{TW_{\text{CCS}}(\text{putative PA-glucose oligomer of the same } m/z)}
\]

### RESULTS AND DISCUSSION

**Preparation of a Standard CCS Data Set Using PA-glucose Oligomer.** Since the absolute \( DT_{\text{CCS}} \)s of glucose oligomers (dextran ladder) have already been reported by Hofmann et al., we first collected UPLC/IM-MS data of glucose oligomers to correlate the observed drift time with the reported absolute \( DT_{\text{CCS}} \)s. In addition, we collected UPLC/IM-MS data of pyridylaminated glucose oligomers (PA-glucose oligomers), which was used as reference for normalization of the LC retention times. According to the method of Harvey et al., we calculated the \( TW_{\text{CCS}} \)s from the drift time of the glucose oligomers and PA-glucose oligomers (Supplementary Tables S1 and S2). We here selected the Na\(^+\)-adducted ions of the glucose oligomers \((z = 1 \text{ and } z = 2)\) since the absolute \( DT_{\text{CCS}} \)s are reported for sodium-adducted ions. For PA-glucose oligomers, H\(^+\)-adducted \((z = 1)\) and H\(^+\)- and NH\(_4\)^+-adducted \((z = 2)\) ions were used. Plots of \( TW_{\text{CCS}} \) against \( m/z \) \((z = 1 \text{ and } z = 2)\) show good linearity for both glucose oligomers and PA-glucose oligomers (Figure 1). This linearity for both \( z = 1 \text{ and } z = 2 \) substantiates the suitability of these materials as references. \( TW_{\text{CCS}} \) of the glucose oligomer is not significantly affected by modification of its reducing end with 2-aminopyridine. It is also evident from the linearity that the glucose oligomer does not assume any DP-dependent conformational preference in the gas phase. Since the glucose oligomer is a linear \( \alpha-1,6 \)-linked chain, we hypothesized that the degree of branching (conformation) of the specimen could be evaluated by comparing the \( TW_{\text{CCS}} \) with that of the linear glucose oligomer. Here, we use the \( TW_{\text{CCS}} \) of the PA-glucose oligomer as a reference, which is used to compare the \( TW_{\text{CCS}} \) of each PA-glycan with the reference value.

**Collection of CCS Data Set of 71 PA-glycans.** UPLC/IM-MS measurements were performed for 71 PA-glycans including isomeric pairs. The \( TW_{\text{CCS}} \) value of each PA-glycan was then calculated from the drift time and \( m/z \) (Table 1). Figure 2 shows a plot of the \( TW_{\text{CCS}} \) values against \( m/z \) for the singly and doubly charged PA-glycans. The plot shows approximate linearity for both singly and doubly charged ions. Some PA-glycan isomers (same \( m/z \) but different chemical structure) exhibit different CCSs (indicated in box), suggesting that IM-MS could differentiate these isomers by comparing with the CCS data.

**Calculation of CCS Index for 71 PA-glycans.** To compare the CCS of PA-glycan with that of PA-glucose oligomer, a CCS index was introduced and defined as CCS (PA-glycan)/CCS (PA-glucose oligomer) (Table 1). For reference, CCS values of PA-glucose oligomers are also plotted against \( m/z \) in Figure 2. The results show that PA-glycans have a CCS index greater than 1 except for one PA-glycan \((m47, z = 1)\) with CCS index of 0.994. This observation indicates that most PA-glycans have a different 3D structure compared with the linear glucose oligomer of the same \( m/z \).

We assume that a CCS index greater than 1 originates at least partially from the branching structure of each glycan, e.g., Man\(_{10}\)l\(_3\)/a1-6 branching. We then plotted the CCS index of each PA-glycan against the number of branching points (Figure 3). Although the CCS index shows considerable variation, there is a weak linear correlation between the CCS index and the number of branch points for the data set of singly charged ions \((z = 1)\) with a coefficient of determination \( R^2 = 0.1047 \). However, there is no significant correlation for doubly charged ions \((z = 2)\). For singly charged ions, the single positive H\(^+\) may attach to the PA tag. The CCS value of a singly charged ion will reflect the 3D structure of the glycan without being influenced by the charge on the PA. It is likely that the CCSs of doubly charged ions are affected by the position of the second charge, which may be different for each PA-glycan.

**Isomer Separation by CCS Difference.** Identification of glycosidic linkage is a central issue in MS-based structural analysis. Methods to identify sialic acid linkages have been extensively explored, particularly in the glycomics arena. To demonstrate the usefulness of isomer separation by IM-MS, driftgrams of four disialylated PA-glycan isomers were chosen and overlaid (Figure 4a). These isomers are difficult to identify by mass spectrometry even with the aid of linkage-specific derivatization. Although these peaks are not completely resolved in the driftgram, confident identification of each isomer can be done by UPLC separation and the glucose unit deduced from the elution time. Figure 4b shows the plot of linkage isomers, including Neu5Ac \( a2-3/2-6 \) and Gal \( b1-3/1-4 \). The utilization of IM has enabled the separation of such glycan isomers, this separation having been difficult by LC or MS. From these results, we suggest that IM-MS has great potential to distinguish a variety of PA-glycan isomers provided that the CCSs are different.

**CCS Data Set As Reference for Practical N-Glycan Analysis.** In this study, a data set of retention time (Glucose unit), \( m/z \), and CSS of 71 PA-glycans has been constructed. The idea behind the CCS index is to provide a measure of the 3D shape of a PA-glycan compared with the equivalent linear glucose oligomer. Referring to this data set, will enable typical N-glycans to be identified rapidly (Figure 5). Furthermore, with the aid of linkage-specific exoglycosidase treatments such as with sialidase/galactosidase/hexosaminidase/fucosidase, a glycan structure will be determined with a high degree of confidence. At present, the number of PA-glycans examined here is limited to 71, which certainly is not enough to cover all possible N-glycans (>1,000) found in glycoproteins, but it represents a beginning, and the database can be expanded and improved as additional data on glycans becomes available.

In addition to 2-aminopyridine, other fluorescent tags are widely used such as 2-aminobenzamide (2-AB). It is worth analyzing the CCSs of 2AB-labeled glycans and sharing the database. We found that the addition of the PA tag to glucose oligomers did not significantly affect the CCSs (Figure 1). Hopefully 2AB-labeled glycans and tag-free glycans show the same trend with similar CCS variations.

We found a weak linear correlation between the CCS index and the number of branching points for \( z = 1 \) ions (Figure 3a). This suggests that branching affects the 3D structure of glycans in vacuo. Furthermore, singly charged PA-glycans could be a suitable target of MD simulation for analyzing the
Table 1. UPLC/IM-MS Data of Each PA-glycan

| No  | Structure | linkage | Mass    | HILIC R.T. (min) | G.U. | [M+H]⁺ m/z | TW CCS (Å²) | CCS index | [M+2H]²⁺ m/z | TW CCS (Å²) | CCS index | [M+3H]³⁺ m/z | TW CCS (Å²) | CCS index |
|-----|-----------|---------|---------|-----------------|------|------------|-------------|-----------|--------------|-------------|-----------|--------------|-------------|-----------|
| m001| ![Diagram](image1.png) |         | 2026.687 | 23.85           | 10.00| -          | -           | -         | 1070.899     | 480.3       | 1.047     | -            | -           | -         |
| m002| ![Diagram](image2.png) |         | 1825.661 | 15.03           | 7.14 | 1922.738   | 459.3       | 1.043     | 961.873      | 454.0       | 1.045     | -            | -           | -         |
| m003| ![Diagram](image3.png) |         | 1971.719 | 16.01           | 7.42 | -          | -           | -         | 1034.902     | 478.4       | 1.081     | -            | -           | -         |
| m004| ![Diagram](image4.png) |         | 1768.640 | 15.37           | 7.23 | -          | -           | -         | 933.361      | 441.1       | 1.030     | -            | -           | -         |
| m005| ![Diagram](image5.png) |         | 1095.397 | 7.00            | 4.74 | 1192.473   | 342.4       | 1.035     | -            | -          | -         | -            | -           | -         |
| m006| ![Diagram](image6.png) |         | 1501.555 | 9.79            | 5.68 | -          | -           | -         | -            | -          | -         | -            | -           | -         |
| m008| ![Diagram](image7.png) |         | 1606.587 | 12.41           | 6.40 | 1703.664   | 426.3       | 1.046     | 852.335      | 437.6       | 1.066     | -            | -           | -         |
| m009| ![Diagram](image8.png) |         | 1403.507 | 11.16           | 6.04 | 1500.584   | 397.7       | 1.055     | 750.796      | 410.5       | 1.058     | -            | -           | -         |
| m010| ![Diagram](image9.png) |         | 1444.534 | 9.91            | 5.66 | 1541.610   | 403.9       | 1.054     | 771.309      | 425.8       | 1.084     | -            | -           | -         |
| m011| ![Diagram](image10.png) |         | 1606.587 | 12.87           | 6.53 | 1703.664   | 415.9       | 1.020     | 852.335      | 427.1       | 1.040     | -            | -           | -         |
| m012| ![Diagram](image11.png) |         | 1622.582 | 14.22           | 6.91 | 1719.660   | 412.2       | 1.005     | 860.333      | 421.4       | 1.022     | -            | -           | -         |
| m013| ![Diagram](image12.png) | β 1→4   | 1987.714 | 18.14           | 8.04 | -          | -           | -         | 1042.899     | 483.5       | 1.069     | -            | -           | -         |
| m014| ![Diagram](image13.png) | β 1→4  | 1887.714 | 17.80           | 7.94 | -          | -           | -         | 1042.900     | 482.0       | 1.065     | -            | -           | -         |
| No  | Structure | linkage | Mass   | HILIC R.T. (min) | G.U.  | \([\text{M+H}]^+\) m/z | \([\text{M+H}]^+\) CCS index | \([\text{M+2H}]^{2+}\) m/z | \([\text{M+2H}]^{2+}\) CCS index | \([\text{M+3H}]^{3+}\) m/z | \([\text{M+3H}]^{3+}\) CCS index |
|-----|-----------|---------|--------|----------------|-------|----------------|------------------|----------------|------------------|----------------|----------------|
| m015 |           |         | 2352.846 | 22.24 | 9.34 | - | - | - | 1225.466 | 507.0 | 1.029 | - | - | - |
| m020 |           |         | 1298.476 | 8.78 | 5.30 | 1395.553 | 3722 | 1.030 | 698.280 | 405.7 | 1.077 | - | - | - |
| m024 | α 2–6     |         | 1913.677 | 18.39 | 8.11 | - | - | - | 1005.881 | 452.1 | 1.018 | - | - | - |
| m025 | α 2–6     |         | 1913.677 | 18.48 | 8.14 | - | - | - | 1005.881 | 459.6 | 1.035 | - | - | - |
| m026 | α 2–6     | α 2–6   | 2204.772 | 22.37 | 9.39 | - | - | - | 1151.429 | 491.7 | 1.032 | - | - | - |
| m029 |           |         | 892.317  | 5.69 | 4.17 | 989.396 | 3176 | 1.057 | - | - | - | - | - |
| m030 |           |         | 1216.423 | 10.91 | 6.03 | 1313.499 | 3712 | 1.063 | - | - | - | - | - |
| m031 |           |         | 1378.476 | 13.85 | 6.88 | 1475.552 | 3998 | 1.071 | 746.794 | 416.9 | 1.076 | - | - | - |
| m032 |           |         | 1702.581 | 19.72 | 8.60 | - | - | - | - | - | - | - | - | - |
| m033 |           |         | 1864.634 | 21.83 | 9.29 | - | - | - | *989.87273 | *471.8352 | *1.070 | - | - | - |
| m035 |           |         | 1540.529 | 17.00 | 7.78 | 1637.607 | 4173 | 1.049 | 827.820 | 437.2 | 1.079 | - | - | - |
| m036 |           |         | 1540.529 | 16.85 | 7.73 | 1637.605 | 4259 | 1.071 | 819.306 | 418.7 | 1.038 | - | - | - |

※[M+H+NH₃]²⁺ form
Table 1. continued

| No | Structure | linkage | Mass (m/z) | HILIC R.T. (min) | G.U. | [M+H]^+ m/z | CCS index | [M+2H]^2+ m/z | CCS index | [M+3H]^3+ m/z | CCS index |
|----|-----------|---------|------------|-----------------|------|-------------|-----------|-------------|-----------|-------------|-----------|
| m041 |           |         | 730.264 | 3.90            | 3.36 | 827.341     | 285.9     | 1.035       |           |           |           |
| m042 |           |         | 1038.375 | 6.59            | 4.58 | 1135.453    | 333.6     | 1.035       |           |           |           |
| m043 |           |         | 1257.449 | 9.75            | 5.67 | 1354.5268   | 383.9     | 1.025       | 1.024     |           |           |
| m044 |           |         | 1647.613 | 11.11           | 6.02 | -           | -         | -           | 872.849   | 443.0      | 1.067     |
| m045 |           |         | 1257.449 | 9.67            | 5.59 | 1354.526   | 369.1     | 1.039       |           |           |           |
| m046 |           |         | 1460.529 | 11.30           | 6.08 | 1557.804    | 392.5     | 1.018       |           |           |           |
| m047 |           |         | 1460.529 | 11.64           | 6.18 | 1557.806    | 383.4     | 0.994       |           |           |           |
| m048 |           |         | 1403.507 | 10.79           | 5.93 | 1500.583    | 395.4     | 1.048       |           |           |           |
| m049 |           |         | 730.264  | 3.65            | 3.23 | 827.341     | 287.7     | 1.042       |           |           |           |
| m050 |           |         | 1095.397 | 7.24            | 4.77 | 1192.474    | 355.3     | 1.074       |           |           |           |
| m051 |           |         | 1241.454 | 8.31            | 5.15 | 1338.531    | 393.5     | 1.115       |           |           |           |
| m055 |           |         | 1622.582 | 14.41           | 6.96 | 1719.660    | 434.8     | 1.061       |           |           |           |
| m056 |           |         | 1704.635 | 11.03           | 6.00 | -           | -         | -           | 901.360   | 446.7      | 1.060     |
Table 1. continued

| No  | Structure | linkage | Mass    | HILIC | [M+H]^+ | [M+2H]^{2+} | [M+3H]^{3+} |
|-----|-----------|---------|---------|-------|---------|-------------|-------------|
|     |           |         |         | R.T.  | m/z     | TWCCS (Å²) | m/z         | TWCCS (Å²) | m/z         | TWCCS (Å²) |
|     |           |         |         | G.U.  |         | CCS index  |             |             | CCS index   |             |
| m057|           | α 2–6   | 2204.772| 21.06  | 8.95   | -          | 1151.428    | 506.2       | 1.063       | -           |
| m058|           | α 2–3   | 2204.772| 21.16  | 8.99   | -          | 1151.429    | 499.8       | 1.049       | -           |
| m059|           | α 2–3   | 2204.772| 19.84  | 8.56   | -          | 1151.429    | 514.1       | 1.079       | -           |
| m060|           | α 2–3   | 1913.677| 17.03  | 7.71   | -          | 1005.881    | 463.9       | 1.044       | -           |
| m061|           | α 2–3   | 1913.677| 17.03  | 7.71   | -          | 1005.880    | 464.9       | 1.046       | -           |
| m062|           | α 2–3   | 2059.735| 18.02  | 8.00   | -          | 1078.910    | 487.4       | 1.059       | -           |
| m063|           | α 2–3   | 2059.735| 17.90  | 7.98   | -          | 1078.911    | 481.8       | 1.047       | -           |
| m066|           |         | 1581.555| 15.62  | 7.32   | 1678.632   | 422.9       | 1.047       | 839.819     | 429.9       | 1.054       | -           |
| m067|           | α 2–6   | 1872.650| 19.89  | 8.59   | 1969.731   | 447.4       | 1.000       | 985.368     | 461.3       | 1.049       | -           |
| m068|           |         | 1460.529| 11.20  | 6.07   | 1557.607   | 402.1       | 1.043       | 779.397     | 420.7       | 1.067       | -           |
| m075|           |         | 1241.454| 8.26   | 5.15   | 1338.531   | 371.5       | 1.053       | 669.769     | 386.8       | 1.045       | -           |
| m076|           |         | 1241.454| 8.45   | 5.21   | 1338.532   | 364.3       | 1.033       | 669.769     | 395.8538    | 433.8881    | 1.069       | 1.172       |
Table 1. continued

| No  | Structure | linkage | Mass   | HILIC R.T. (min) | HILIC G.U. | [M+H]+ TWCCS (Å²) | CCS index | [M+2H]2+ TWCCS (Å²) | CCS index | [M+3H]3+ TWCCS (Å²) | CCS index |
|-----|-----------|---------|--------|-----------------|------------|-------------------|-----------|-------------------|-----------|-------------------|-----------|
| m077|           |         | 1079.402 | 5.98            | 4.31       | 1176.479 | 345.3 | 1.051                         | -          | -                 | -          |
| m078|           |         | 876.322  | 4.65            | 3.74       | 973.399 | 316.1 | 1.061                         | -          | -                 | -          |
| m083| α2−3      |         | 2350.830  | 20.55           | 8.81       | -         | -     | -                             | 1224.458   | 525.0 | 1.066                         | -          | -                 | -          |
| m084|           |         | 1095.397  | 7.29            | 4.81       | 1192.474 | 343.0 | 1.037                         | 596.740    | 421.6 | 1.190                         | -          | -                 | -          |
| m085|           |         | 1095.397  | 6.78            | 4.62       | 1192.474 | 336.5 | 1.017                         | -          | -                 | -          |
| m505| α2−3      |         | 1694.603  | 13.73           | 6.79       | 1791.680 | 455.9 | 1.083                         | 896.344    | 447.3 | 1.064                         | -          | -                 | -          |
| m507| α2−3      |         | 1694.603  | 14.24           | 6.93       | 1791.682 | 436.1 | 1.037                         | 896.344    | 441.3 | 1.050                         | -          | -                 | -          |
| m508| α2−3      |         | 1897.682  | 15.64           | 7.33       | -         | -     | -                             | 997.883    | 470.8 | 1.064                         | -          | -                 | -          |
| m514| α2−3      |         | 1897.682  | 15.18           | 7.20       | -         | -     | -                             | 997.884    | 473.4 | 1.070                         | -          | -                 | -          |
| m541|           |         | 2350.793  | 27.81           | 11.56      | -         | -     | -                             | 1232.952   | 514.8 | 1.042                         | -          | -                 | -          |
| t005|           |         | 2133.772  | 19.97           | 8.62       | -         | -     | -                             | 1115.928   | 491.4 | 1.049                         | -          | -                 | -          |
Table 1. continued

| No  | Structure | linkage | Mass   | HILIC | [M+H]^+ | [M+2H]^{2+} | [M+3H]^{3+} |
|-----|-----------|---------|--------|-------|---------|-------------|-------------|
|     |           |         |        |       | m/z     | m/z         | m/z         |
|     |           |         |        |       | TWCCS (Å²) | TWCCS (Å²) | TWCCS (Å²) |
|     |           |         |        |       | CCS index | CCS index | CCS index |
| t006|           |         | 2498.904 | 23.78 | 9.91    | 1298.495   | 533.4       | 1.049       |
|     |           |         | 2133.772 | 18.95 | 8.30    | 1115.927   | 492.9       | 1.052       |
| t011|           |         | 2498.904 | 22.76 | 9.55    | 1298.495   | 525.3       | 1.033       |
|     |           |         | 1501.555 | 10.11 | 5.74    | 799.820    | 442.9       | 1.110       |
| t015|           |         | 2110.783 | 14.50 | 7.01    | 1104.439   | 495.9       | 1.064       |
| t024|          α 2-6|       | 2861.000 | 28.15 | 11.64   | 1479.543   | 568.3       | 1.036       |
| t025|          α 2-6|       | 3152.095 | 28.89 | 11.97   | 1633.804   | 610.8       | 1.049       |
| t054|           |         | 1540.529 | 16.50 | 7.63    | 827.819    | 433.9       | 1.071       |
| t056|           |         | 1702.581 | 19.17 | 8.43    | 908.846    | 448.8       | 1.061       |
| t058|           |         | 1054.370 | 7.90  | 5.06    | 1151.447   | 340.1       | 1.048       |
correlation between 3D structure and CCS. We tried to find another type of correlation between CCS and glycan modification, such as core fucosylation (α1-6Fuc) at the innermost GlcNAc or addition of bisecting GlcNAc to β1-4Man, which are known to attenuate the population of each conformation.

However, clear relationships were not established, probably due to the limited data set. Investigation into the correlation between CCS and glycan structures by MD simulations is warranted. In particular, simulation may shed light on the 3D structure of doubly charged PA-glycan m084 which showed an irregularly large CCS index (Figure 5, Table 1).

Currently, information is still lacking on how glycan structure affects biological function of proteins and lipids. Rapid identification of glycan structures by using the CCS database will open a path to better understand the functional aspects of glycans. It will also aid in the quality assessment of biopharmaceuticals, including therapeutic antibodies.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jasms.2c00165.

Table S1, raw data of glucose oligomer; Table S2, raw data of PA-glucose oligomer (PDF)
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Notes
The authors declare no competing financial interest.

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REFERENCES

(1) Varki, A. Biological roles of glycans. Glycobiology 2017, 27 (1), 3—49.
(2) Yamaguchi, Y.; Barb, A. W. A synopsis of recent developments defining how N-glycosylation impacts immunoglobulin G structure and function. Glycobiology 2020, 30 (4), 214—225. Yamaguchi, Y.; Nishimura, M.; Nagano, M.; Yagi, H.; Sasakawa, H.; Uchida, K.; Shiitara, K.; Kato, K. Glycoform-dependent conformational alteration of the Fc region of human immunoglobulin G1 as revealed by NMR spectroscopy. Biochim. Biophys. Acta, Gen. Subj. 2006, 1760 (4), 693—700.
(3) Wang, Z.; Chinoy, Z. S.; Ambro, S. G.; Peng, W.; McBride, R.; de Vries, R. P.; Glushka, J.; Paulson, J. C.; Boons, G. J. A general strategy for the chemoenzymatic synthesis of asymmetrically branched N-glycans. Science 2013, 341 (6144), 379—383. Nagae, M.; Yanamaka, K.; Hanashima, S.; Ikeda, A.; Morita-Matsumoto, K.; Satoh, T.; Matsumoto, N.; Yamamoto, K.; Yamaguchi, Y. Recognition of bisecting N-acetylglucosamine: structural basis for asymmetric interaction with the mouse lectin dendritic cell inhibitory receptor 2. J. Biol. Chem. 2013, 288 (47), 33598—33610. Benevides, R. G.; Ganne, G.; Simoes Rda, C.; Schubert, V.; Niemietz, M.; Unverzagt, C.; Chazalet, V.; Breton, C.; Varrot, A.; Cavada, B. S.; Imberty, A. A lectin from Platypodium elegans with unusual specificity.
and affinity for asymmetric complex N-glycans. J. Biol. Chem. 2012, 287 (31), 26352–26364.

(4) Tomiya, N.; Aways, J.; Kurono, M.; Endo, S.; Arata, Y.; Takahashi, N. Analyses of N-linked oligosaccharides using a two-dimensional mapping technique. Anal. Biochem. 1988, 171 (1), 73–90.

(5) Takahashi, N.; Tsukamoto, Y.; Shiosaka, S.; Kishi, T.; Hakoshima, T.; Arata, Y.; Yamaguchi, Y.; Kato, K.; Shimada, I. N-glycan structures of murine hippocampus serine protease, neuropsyn, produced in Trichoplusia ni cells. Glycoconj. J. 1999, 16 (8), 405–414. Masuda, K.; Yamaguchi, Y.; Kato, K.; Takahashi, N.; Shimada, I.; Arata, Y. Pairing of oligosaccharides in the Fc region of immunoglobulin G. F.E.B.S. Lett. 2000, 473 (3), 349–357. Kanagawa, M.; Matsumoto, K.; Iwasaki, N.; Hayashi, Y.; Yamaguchi, Y. Structural analysis of N-glycans attached to pig kidney Na+/K+-ATPase. J. Glycomics Lipidomics 2013, 5, 005.

(6) Manz, C.; Pagel, K. Glycan analysis by ion mobility-mass spectrometry and gas-phase spectroscopy. Curr. Opin. Chem. Biol. 2018, 42, 16–24. Hofmann, J.; Pagel, K. Glycan analysis by ion mobility-mass spectrometry. Angew. Chem., Int. Ed. Engl. 2017, 56 (29), 8342–8349. Hofmann, J.; Hahm, H. S.; Seeberger, P. H.; Pagel, K. Identification of carbohydrate anomers using ion mobility-mass spectrometry. Nature 2015, 526 (7572), 241–244.

(7) Yamaguchi, Y.; Nishima, W.; Re, S.; Sugita, Y. Confident identification of isomeric N-glycan structures by combined ion mobility mass spectrometry and hydrophilic interaction liquid chromatography. Rapid Commun. Mass Spectrom. 2012, 26 (24), 2877–2884.

(8) Re, S.; Watabe, S.; Nishima, W.; Muneyuki, E.; Yamaguchi, Y.; MacKerell, A. D., Jr.; Sugita, Y. Characterization of conformational ensembles of protonated N-glycans in the gas-phase. Sci. Rep. 2018, 8 (1), 1644.

(9) Hinneburg, H.; Hofmann, J.; Struwe, W. B.; Thader, A.; Altmann, F.; Varon Silva, D.; Seeberger, P. H.; Pagel, K.; Kolarich, D. Distinguishing N-acetylneuraminic acid linkage isomers on glycopeptides by ion mobility-mass spectrometry. Chem. Commun. (Camb) 2016, 52 (23), 4381–4384.

(10) Struwe, W. B.; Pagel, K.; Benesch, J. L.; Harvey, D. J.; Campbell, M. P. GlycoMob: an ion mobility-mass spectrometry collision cross section database for glycomics. Glycoconj. J. 2016, 33 (3), 399–404.

(11) Harvey, D. J.; Watanabe, Y.; Allen, J. D.; Rudd, P.; Pagel, K.; Crispin, M.; Struwe, W. B. Collision cross sections and ion mobility separation of fragment ions from complex N-glycans. J. Am. Soc. Mass Spectrom. 2018, 29 (6), 1250–1261.

(12) Bush, M. F.; Campuzano, I. D.; Robinson, C. V. Ion mobility mass spectrometry of peptide ions: effects of drift gas and calibration strategies. Anal. Chem. 2012, 84 (16), 7124–7130.

(13) Hofmann, J.; Struwe, W. B.; Scarff, C. A.; Scrivens, J. H.; Harvey, D. J.; Pagel, K. Estimating collision cross sections of negatively charged N-glycans using traveling wave ion mobility-mass spectrometry. Anal. Chem. 2014, 86 (21), 10789–10795.

(14) Nishikaze, T. Sialic acid derivatization for glycan analysis by mass spectrometry. Proc. Ipn. Acad., Ser. B 2019, 95 (9), 523–537.

(15) Nishima, W.; Miyashita, N.; Yamaguchi, Y.; Sugita, Y.; Re, S. Effect of Bisecting GlcNAc and Core Fucosylation on Conformational Properties of Bi-antennary Complex-Type N-Glycans in Solution. J. Phys. Chem. B 2012, 116 (29), 8504–8512.