Mass Spectrometry

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# Ion-Mobility Spectrometry Can Assign Exact Fucosyl Positions in Glycans and Prevent Misinterpretation of Mass-Spectrometry Data After Gas-Phase Rearrangement

*Javier Sastre Toraño, Ivan A. Gagarinov, Gaël M. Vos, Frederik Broszeit, Apoorva D. Srivastava, Martin Palmer, James I. Langridge, Oier Aizpurua-Olaizola, Victor J. Somovilla, and Geert-Jan Boons**

***Abstract:*** *The fucosylation of glycans leads to diverse structures and is associated with many biological and disease processes. The exact determination of fucoside positions by tandem mass spectrometry (MS/MS) is complicated because rearrangements in the gas phase lead to erroneous structural assignments. Here, we demonstrate that the combined use of ion-mobility MS and well-defined synthetic glycan standards can prevent misinterpretation of MS/MS spectra and incorrect structural assignments of fucosylated glycans. We show that fucosyl residues do not migrate to hydroxyl groups but to acetamido moieties of N-acetylneuraminic acid as well as N-acetylglucosamine residues and nucleophilic sites of an anomeric tag, yielding specific isomeric fragment ions. This mechanistic insight enables the characterization of unique IMS arrival-time distributions of the isomers which can be used to accurately determine fucosyl positions in glycans.*

Glycosylation is the structurally most complex post-translational modification of proteins, and it plays key roles in many biological and disease processes.[1] To understand these processes on a molecular level, an accurate analysis of glycan structures is essential.[2] Current analytical methods generally entail glycan release, derivatization, and purification, followed by chromatographic separation and matrix-assisted laser desorption/ionization (MALDI), or electrospray ionization (ESI) mass spectrometry (MS).[3] Accurate mass measurements can provide glycan compositions, whereas sequence and linkage positions, which are more difficult to elucidate given the complexity and diversity of glycans, can be probed through tandem mass spectrometry (MS/MS) experiments.[4]

Most MS experiments are performed in positive-ion mode, which leads to glycosidic bond cleavage and yields MS/MS spectra with B- and Y-type fragment ions (Figure 1),

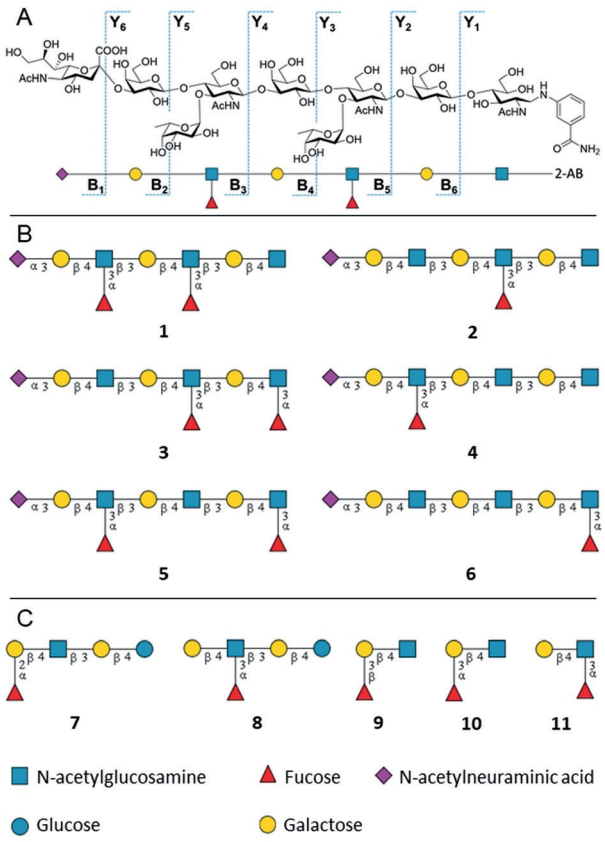


***Figure 1.*** Library of oligosaccharide standards used for establishing fucosyl rearrangements in glycans with IMS-MS. A) Structure of sialosyl-fucosyl oligo-*N*-acetyllactosamine with α(1,3)-linked fucose on the central and terminal *N*-acetylglucosamines. The compound is derivatized with 2-aminobenzamide (2-AB) at the reducing end. Symbols of the carbohydrate units are explained at the bottom. Annotation of fragment ions (B- and Y-type) according to the commonly used nomenclature, with the exception that only fragments of the sialosyl oligo-LacNac chain are annotated without taking into account fucosyl residues in order to have a uniform annotation between the various sialosyl-fucosyl oligo-*N*-acetyllactosamine compounds. B) Sialosyl-fucosyl oligo-*N*-acetyllactosamine standards. C) Fragment standards of sialosyl-fucosyl oligo-*N*-acetyllactosamines.

[*] J. Sastre Toraño, I. A. Gagarinov, G. M. Vos, F. Broszeit, A. D. Srivastava, Dr. O. Aizpurua-Olaizola, Dr. V. J. Somovilla, Prof. Dr. G.-J. Boons
Department of Chemical Biology and Drug Discovery
Utrecht University
Universiteitsweg 99, 3584 CG Utrecht (The Netherlands)
E-mail: g.j.p.h.boons@uu.nl
Dr. M. Palmer, Dr. J. I. Langridge
Waters Corporation
Stamford Avenue, Altrincham Road, SK9 4AX Wilmslow (UK)
Prof. Dr. G.-J. Boons
Complex Carbohydrate Research Center and Department of Chemistry, University of Georgia
315 Riverbend Road, Athens, GA 30602 (USA)
Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/anie.201909623.

facilitating structural assignment.[5] Structure determination can, however, be erroneous due to molecular rearrangements of intact[6] and fragmented[7] protonated glycans. Usually, such rearrangements involve residues with rather labile glycosidic linkages such as fucosides[8] that can migrate to an anomeric tag,[9] sialic acid residues,[10] and between *N*-glycan antennae.[11] The mechanism of fucosyl rearrangement is difficult to clarify using MS/MS, and proposed fucose locations are mainly based on hypothetical models and theoretical calculations.[9,12]

In humans, the structural diversity of fucosylated glycans arises from thirteen fucosyl transferases that, in concert with other glycosyl transferases, create a wide variety of glycan epitopes. Fucosylated epitopes mediate many physiological and disease processes such as leukocyte adhesion during inflammation,[13] fertilization,[14] tissue development,[15] and tumor metastasis.[16] Therefore, the determination of exact fucoside positions and the affirmation of rearrangements are critical for the application of glycans as clinical biomarkers and to understand their biological roles at a molecular level.

Here, we report a general approach to determine the exact positions of fucosyl residues in structurally complex glycans based on the combined use of ion-mobility spectrometry (IMS)-MS and well-defined oligosaccharide standards. IMS can separate gas-phase ions based on their mobility through a buffer gas under the influence of a weak electric field, resulting in different ion-arrival times at the end of the mobility cell. IMS arrival-time distributions (ATD), which can be used to derive collision-cross-section (CCS) values of ions, depend on the charge, size, and shape of the ions, making IMS very suitable for the separation of different ion isomers and conformers.[17] We anticipated that fucosyl migration would yield isomeric fragment ions that can be distinguished by their ATD in IMS.[18] Furthermore, we expected that MS/MS analysis of well-defined oligosaccharides with fucosyl residues at different but clearly defined sites (**1**–**11**, Figure 1 B–D) would reveal positions to which fucose can migrate (Figure 1 C). This study provides mechanistic insight into fucosyl rearrangement and it is shown that migration occurs only to nucleophilic amides and amines of GlcNAc, Neu5Ac, and anomeric tags. This information will make it possible to determine ATDs of fragments that have native and rearranged fucosyl residues.

To identify products formed during gas-phase fucosyl rearrangement, oligosaccharide standards **1**–**6**[19] (Figure 1 B) were labeled with 2-aminobenzamide (2-AB) and fragmented by MS/MS using low-energy collision-induced dissociation (CID)/MS. What makes these standards attractive is that they have the same linear tri-LacNAc backbone, modified by different but well-defined patterns of fucosylation, making it possible to unambiguously assign fragment ions arising from the parent compound and fucosyl migration. Furthermore, compounds **1**–**6** are important constituents of *N*- and *O*-glycans as well as glycolipids, and contain *N*-acetyl neuraminic acid (Neu5Ac) residues to which fucose can migrate.

The fragmentation spectra of the protonated standards, $[M+H]^+$ **1**–**6** showed several B- and Y-ions derived from the parent compound, but also fragment ions arising from intramolecular fucosyl migration (Supporting Information, Figures S1–S6). For example, the spectrum of compound **1**, which has fucosides at the middle and terminal *N*-acetylglucosamine (GlcNAc) residue (Figure 1 A,B), showed fucosyl residues on $Y_1$, $Y_2$, and $Y_4$ fragment ions (Figure 2) and even two fucosyl residues on an $Y_2$ ion (Figure 2). However, no more fucosyl residues than were originally present were observed in the MS/MS spectra, excluding intermolecular migrations.

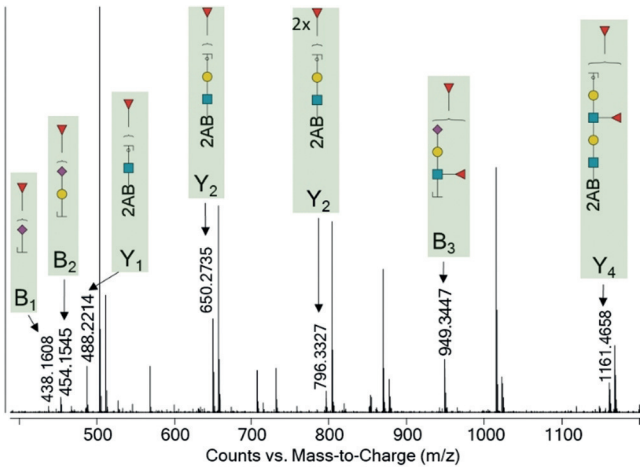


***Figure 2.*** MS/MS of singly charged $[M+H]^+$ 2-AB-labeled sialosyl-difucosyl oligo-*N*-acetyl lactosamine with fucosides on the middle and terminal *N*-acetylglucosamine (compound **1**, Figure 1 B) at $m/z =$ 1817.69. Several fragment ions originating from fucosyl rearrangements (highlighted in green) were detected.

Fucosides were also observed on $B_1$, $B_2$ and $B_3$ ions (Figure 2) demonstrating migration to Neu5Ac, which previously was proposed to involve an anhydrofucosyl intermediate migrating to the acetamido moiety of sialic acid.[10] This functional group is also present on GlcNAc, and therefore we expect a similar migration will occur to this residue. To confirm this mode of rearrangement, $Y_4$-like standards **7** and **8** (Figure 1 C; Figure S13), which have a benzyl group at the anomeric center, were analyzed by CID/MS. Upon fragmentation of compound **8**, which has a lactose moiety at the reducing end, no fucosyl rearrangement was observed towards the reducing end or other carbohydrate moieties, even at a high activation energy (Figure S7), indicating that fucosides do not migrate to hydroxyl groups, although this was previously proposed as a possible mode of rearrangement.[12b] On the contrary, when a similar experiment was performed with compound **7**, which has a GlcNAc group without a native fucosyl residue (Figure 1 C), a fucosyl residue was observed on the $B_2/Y_3$ ion (GlcNAc; Figure S8), supporting migration to acetamido residues. Furthermore, when the anomeric benzyl tag of compound **7** was replaced by 2-AB, additional $Y_1$, $Y_2$, and $Y_3$ fragment ions were observed, which could only be assigned to migration to this tag.[5] Based on these observations, we conclude that proton-mediated fucosyl rearrangement results in compounds that are modified at one of the somewhat nucleophilic amide moieties (Figure 3). Additionally, migra-

  

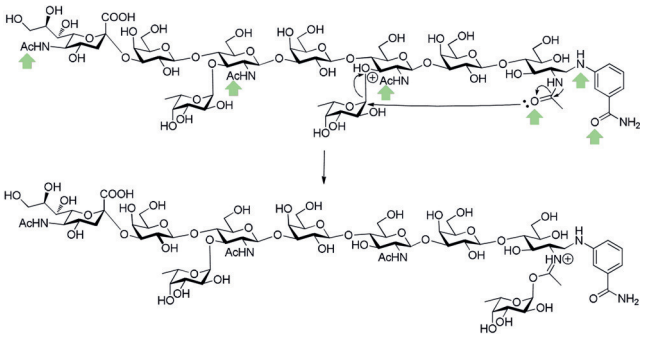

***Figure 3.*** Proposed mechanism for proton-mediated fucose migration to the 2-AB tag as well as Neu5Ac and GlcNAc residues in sialosyl-fucosyl oligo-*N*-acetyl lactosamine. Fucosyl residues may rearrange to both oxygen as well as nitrogen of acetamido moieties although oxygen is more nucleophilic.[21] Furthermore, the rearrangement as shown for the first GlcNAc residue can also occur at other migration locations (marked with green arrows).

tion to the secondary amine of reductive aminated glycans is possible,[9] as demonstrated by the analysis of standard **7** modified by an aniline tag (Figure S10). These intramolecular rearrangements can occur stepwise, which provides a rationale for two migrated fucosyl residues on some of the $Y_2$ fragment ions.

Fucosyl migration to different positions of an oligo-LacNAc backbone will yield several positional-isomeric fragment ions, and we anticipated that these can be resolved by IMS.[20] Thus, fragments arising from glycans **1**–**6**, which have a native 1,3-linked as well as migrated fucosyl residues, were examined with drift tube IMS (DTIMS, resolution: $\approx 60\ \Omega/\Delta\Omega$) after in-source fragmentation to obtain linkage-specific ATDs. The ATDs of $Y_2$ ions, which have native linked fucoside on GlcNAc, showed a single peak (Figures 4B and S11), while the ATDs of the isomeric $Y_2$ ions with rearranged fucose exhibited several peaks (Figures 4C and S11) that belong to isomers with this residue on either the acetamido moiety of a GlcNAc moiety or the 2-AB tag. Differences in ATDs between fragments having native and rearranged fucosyl residues were observed on Y-type ions as well as on other fragments, such as $B_3$ ions (Figure 5), allowing the discrimination of these fragment ions by DTIMS.

To assign the peaks in the ATDs to specific glycan structures and further evaluate previously reported rearrangement mechanisms, three fucosylated trisaccharide standards (compounds **9**–**11**, Figures 1, S13, S14, and S16) were analyzed by DTIMS. The ATD of **11**, which has an α(1,3)-linked fucoside at the GlcNAc moiety (Figure 4A, **11**), exhibited an identical profile as those of an $Y_2$ fragment ion having native linked fucoside (Figure 4B, **6**), confirming the $Y_2$ fragment-ion identity. The ATDs of standards **9** and **10**, which have fucoside linked α- or β(1,3) to Gal, showed a major peak (Figure 4A, **9** and **10**) which is absent in the ATD of the oligo-LacNAc standards with a rearranged fucose residue (Figure 4C), excluding fucosyl rearrangement to the cleaved glycosidic linkage side (C-3 on Gal), which was previously proposed as a mode of migration.[12a] Standards **9** and **10** (Figure 1), however, exhibited two additional signals

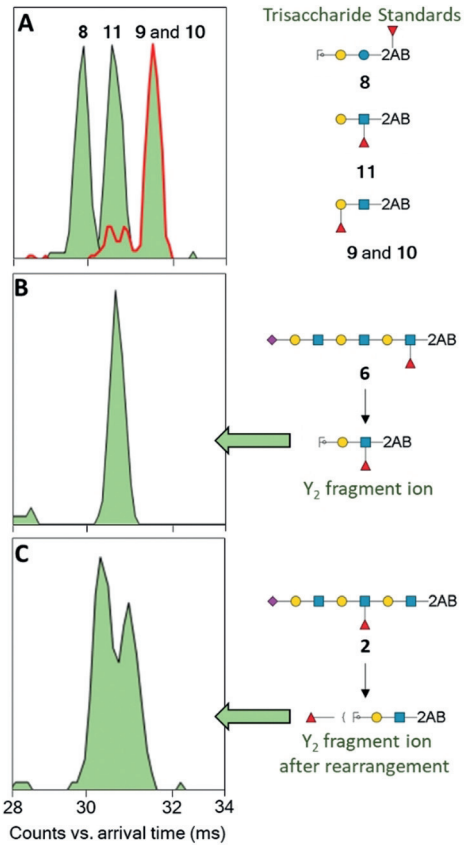


***Figure 4.*** Intensity-normalized DTIMS ATDs of $Y_2$ fragment ions with native or rearranged fucosides, and $Y_2$-like standards with differently linked fucosides. A) ATDs with separated peaks of 2-AB fucosylated lactose (obtained from **8** at $m/z$ 609.248) and acetyllactosamines with differently linked fucosides (standards **9**, **10** and **11** at $m/z = 650.279$); B) ATD of a $Y_2$ fragment ion (at $m/z = 650.277$) from in-source fragmentation of standard **6**, showing one peak for a fragment with a native fucoside. C) $Y_2$ fragment ion (at $m/z = 650.2788$) after in-source fragmentation of standard **2**, showing two peaks for a fragment with rearranged fucoside. ATDs from standards in panel A were used to identify the peaks in panels B and C.

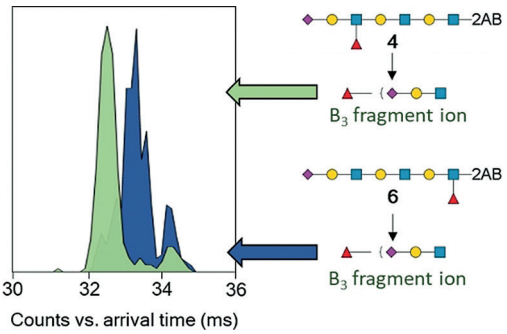


***Figure 5.*** Intensity-normalized DTIMS ATDs of $B_3$ fragment ions from standard **6** (at $m/z = 803.2909$) and **4** (at $m/z = 803.2890$), which contains a sialyl Lewis$^x$ epitope. The ATD of an ion with native fucoside on *N*-acetylglucosamine (green signal) is easily distinguishable from an ion with a rearranged fucosyl residue on *N*-acetylglucosamine and *N*-acetylneuraminic acid residues (blue signal).

that match the arrival times of fragments having rearranged fucose residues (Figure 4C), confirming that rearrangements

  

not only occur in MS upon fragmentation but also in intact glycans.[6] The analysis of a fragment derived from compound **8** (Figure 1) in which fucose had migrated to the 2-AB tag showed a single peak with a short arrival time (Figure 4A, **8**), indicating that the fragment peak derived from the oligo-LacNAc standards with the shortest arrival time (Figure 4C) corresponds to a fragment with a fucosyl residue on the 2-AB tag. The other major peak in the ATD should then correspond to a fragment with a fucosyl residue on the acetamido moiety of GlcNAc. These results highlight that ATDs can be assigned to fragment ions with native and migrated fucosyl residues. Importantly, once the ATDs of such fragments are known, the structure of similar unknown compounds can be elucidated without a need for further synthetic standards.

To demonstrate the applicability of IMS-MS to determine the exact positions of fucosides in glycans, two biantennary *N*-glycans containing either $Le^x$ epitopes or modified by core fucosylation (compounds **12** and **13**,[22] respectively; Figures 6 and S15) were analyzed with IMS-MS. Since in-source fragmentation of larger glycans is more complicated, the standards were analyzed with travelling-wave IMS (TWIMS)-MS on a linear and on a cyclic IMS (cIMS) instrument, which has the geometry ESI-Q-cIMS-TOF and contains both a pre-IMS and post-IMS CID cell. The pre-IMS allows for the ATD measurement of fragment ions from glycans.[23] The *N*-glycans were observed in the MS spectrum as double-charged sodium adducts ($[M+2Na]^{2+}$ and $[M+H+Na]^{2+}$). No fucosyl migration was observed in adducts containing two sodium ions, whereas the presence of a proton facilitated migration, supporting a rearrangement mechanism mediated by a mobile proton with poor charge fixation.[9,12] Furthermore, no fucosyl migration was observed in protonated glycans from the core to an antenna, although this rearrangement cannot be excluded in other glycan structures.

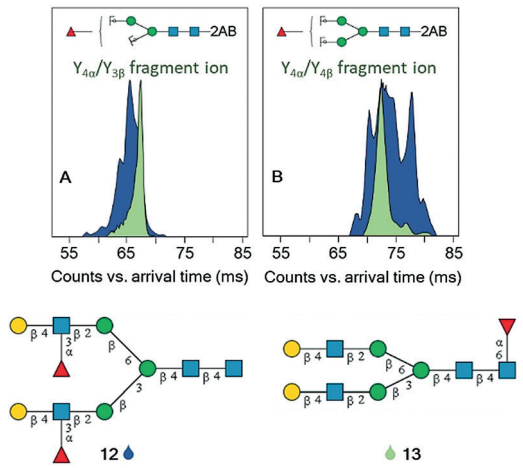


***Figure 6.*** Intensity-normalized TWIMS ATDs of sodium-adducted fragment ions from 2-AB-labeled *N*-glycans with core fucosylation (**13**, $[M+H+Na]^{2+}$ at $m/z=965.3577$) or antennae fucosylation (**12**, $[M+H+Na]^{2+}$ at $m/z=1038.3866$). Precursor ions were selected in the quadrupole, fragmented in the trap-collision cell, the resulting fragment ions were subjected to three passes of the cIM (which corresponds to an IMS resolution of $\approx 110\ \Omega/\Delta\Omega$) with mass measurement in the TOF. ATDs of $Y_{4\alpha}/Y_{4\beta}$ (A) and $Y_{4\alpha}/Y_{3\beta}$ (B) fragment ions show that sodium-adducted fragment ions with native $\alpha(1,6)$-linked core fucose (green signal) can be discriminated from fragment ions originating from rearranged fucosides on the *N*-acetylglucosamine and 2-AB tag (blue signal).

The fragmentation spectra of protonated *N*-glycans $[M+H+Na]^{2+}$ showed sodium adducts of $Y_{4\alpha}/Y_{3\beta}$ and $Y_{4\alpha}/Y_{4\beta}$ fragments containing fucose (Figure S12) for both *N*-glycans (**12** and **13**), which leads to the correct identification of core fucosylation in **13** but to an erroneous structural assignment of the antennae-fucosylated glycan **12**.

Here, TWIMS can provide a definitive answer about the exact fucoside position. ATDs obtained by linear and cyclic TWIMS instruments with one pass were similar and showed the ATD reproducibility, with an increase in cIM passes resulting in an improved ATD resolution. The cIM ATDs at three passes of fragments with native fucoside (from standard **13**) are dominated by one major signal that originates from the native fucosylated fragment, with additional minor peaks (Figure 6A,B, green signals), originating from fucosyl rearrangements to the acetamido moieties of GlcNAc residues and the 2-AB tag. On the contrary, the ATDs of fragments that can only arise from rearranged fucoside residues (from standard **12**) show multiple, broad, and partially resolved peaks, revealing the presence of different positional isomers (Figure 6A,B, blue signal) that match arrival times of the minor peaks of the native fucosylated fragment (green signal). The presence of relatively strong signals of several isomers allowed to discriminate between *N*-glycans with native core fucosylation and those with rearranged fucosides on the core structure.

In summary, IMS-MS of well-defined oligosaccharides has demonstrated that proton-mediated fucosyl migration occurs intramolecularly to acetamido moieties of Neu5Ac and GlcNAc residues as well as nucleophilic sites of the 2-AB tag. Contrary to previous reports, no rearrangement to hydroxyl groups was observed, which limits the number of positions for fucosyl migration. This new mechanistic insight enables the straightforward characterization of diagnostic Y- and B-type fragment ions arising from native and rearranged fucosyl residues with IMS. MS/MS fragments that have rearranged fucosides can be identified by the presence of multiple peaks in the ATD or by a shift in the arrival time and CCS value with respect to well-defined fragment-like standards. The described strategy can now be applied broadly, and we anticipate that a comprehensive database of ATDs and CCS values of fragment ions derived from native and rearranged fucosides can be generated by analyzing a limited set of well-defined oligosaccharides with branched structures modified by various types of Lewis- and blood-group antigens. Such a database will facilitate the fast and unambiguous assignment of fucosylation sites of unknown glycans without the need for additional synthetic standards. The set of compounds described here has already provided diagnostic ions that can confirm the presence of a sialyl $Lewis^x$ motif and identify core fucosylation. It is expected that a database of ATDs and CCS values combined with multi-stage IMS and MS instrumentation will also enable de-novo sequencing of glycans.[24] Similar strategies can be applied for the identification of sites of sulfation in oligosaccharides[25] and phos-

  

phorylation in peptides[26] to prevent erroneous structure elucidation due to rearrangement.

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## Conflict of interest

The authors declare no conflict of interest.

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[1] A. Varki, *Glycobiology* **2017**, *27*, 3–49.
[2] S. Gaunitz, G. Nagy, N. L. B. Pohl, M. V. Noyotny, *Anal. Chem.* **2017**, *89*, 389–413.
[3] O. Aizpurua-Olaizola, J. Sastre Toraño, J. M. Falcon-Perez, C. Williams, N. Reichardt, G. J. Boons, *TrAC Trends Anal. Chem.* **2018**, *100*, 7–14.
[4] L. Han, C. E. Costello, *Biochemistry* **2013**, *78*, 710–720.
[5] B. Küster, T. J. Naven, D. J. Harvey, *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1645–1651.
[6] E. Mucha, M. Lettow, M. Marianski, D. A. Thomas, W. B. Struwe, D. J. Harvey, G. Meijer, P. H. Seeberger, G. von Helden, K. Pagel, *Angew. Chem. Int. Ed.* **2018**, *57*, 7440–7443; *Angew. Chem.* **2018**, *130*, 7562–7565.
[7] M. Wuhrer, A. M. Deelder, Y. E. van der Burgt, *Mass Spectrom. Rev.* **2011**, *30*, 664–680.
[8] J. N. BeMiller, *Adv. Carbohydr. Chem.* **1967**, *22*, 25–108.
[9] D. J. Harvey, T. S. Mattu, M. R. Wormald, L. Royle, R. A. Dwek, P. M. Rudd, *Anal. Chem.* **2002**, *74*, 734–740.
[10] B. Ernst, D. R. Muller, W. J. Richter, *Int. J. Mass Spectrom. Ion Processes* **1997**, *160*, 283–290.
[11] M. Wuhrer, C. A. Koeleman, C. H. Hokke, A. M. Deelder, *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1747–1754.
[12] a) Y. L. Ma, I. Vedernikova, H. Van den Heuvel, M. Claeys, *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 136–144; b) A. H. Franz, C. B. Lebrilla, *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 325–337.
[13] J. Li, H.-C. Hsu, J. D. Mountz, J. G. Allen, *Cell Chem. Biol.* **2018**, *25*, 499–512.
[14] P.-C. Pang, P. C. N. Chiu, C.-L. Lee, L.-Y. Chang, M. Panico, H. R. Morris, S. M. Haslam, K.-H. Khoo, G. F. Clark, W. S. B. Yeung, A. Dell, *Science* **2011**, *333*, 1761–1764.
[15] a) D. J. Becker, J. B. Lowe, *Glycobiology* **2003**, *13*, 41R–53R; b) B. Ma, J. L. Simala-Grant, D. E. Taylor, *Glycobiology* **2006**, *16*, 158R–184R; c) D. Solter, B. B. Knowles, *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 5565–5569; d) T. Muramatsu, H. Muramatsu, *Glycoconjugate J.* **2004**, *21*, 41–45.
[16] a) E. Staudacher, F. Altmann, I. B. H. Wilson, L. März, *Biochim. Biophys. Acta Gen. Subj.* **1999**, *1473*, 216–236; b) M. Fukuda, *Cancer Res.* **1996**, *56*, 2237–2244.
[17] J. Hofmann, K. Pagel, *Angew. Chem. Int. Ed.* **2017**, *56*, 8342–8349; *Angew. Chem.* **2017**, *129*, 8458–8466.
[18] a) D. J. Harvey, Y. Watanabe, J. D. Allen, P. Rudd, K. Pagel, M. Crispin, W. B. Struwe, *J. Am. Soc. Mass Spectrom.* **2018**, *29*, 1250–1261; b) C. J. Gray, B. Thomas, R. Upton, L. G. Migas, C. E. Eyers, P. E. Barran, S. L. Flitsch, *Biochim. Biophys. Acta Gen. Subj.* **2016**, *1860*, 1688–1709.
[19] I. A. Gagarinov, T. Li, N. Wei, J. Sastre Torano, R. P. de Vries, M. A. Wolfert, G. J. Boons, *Angew. Chem. Int. Ed.* **2019**, *58*, 10547–10552; *Angew. Chem.* **2019**, *131*, 10657–10662.
[20] a) C. Manz, K. Pagel, *Curr. Opin. Chem. Biol.* **2018**, *42*, 16–24; b) D. J. Harvey, W. B. Struwe, *J. Am. Soc. Mass Spectrom.* **2018**, *29*, 1179–1193.
[21] G. Fraenkel, C. Franconi, *J. Am. Chem. Soc.* **1960**, *82*, 4478–4483.
[22] L. Liu, A. R. Prudden, C. J. Capicciotti, G. P. Bosman, J. Y. Yang, D. G. Chapla, K. W. Moremen, G. J. Boons, *Nat. Chem.* **2019**, *11*, 161–169.
[23] K. Giles, J. Ujma, J. Wildgoose, S. Pringle, K. Richardson, D. Langridge, M. Green, *Anal. Chem.* **2019**, *91*, 8564–8573.
[24] P. Both, A. P. Green, C. J. Gray, R. Sardzik, J. Voglmeir, C. Fontana, M. Austeri, M. Rejzek, D. Richardson, R. A. Field, G. Widmalm, S. L. Flitsch, C. E. Eyers, *Nat. Chem.* **2014**, *6*, 65–74.
[25] D. T. Kenny, S. M. Issa, N. G. Karlsson, *Rapid Commun. Mass Spectrom.* **2011**, *25*, 2611–2618.
[26] C. M. Potel, S. Lemeer, A. J. R. Heck, *Anal. Chem.* **2019**, *91*, 126–141.

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