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The Quest for Anticancer Vaccines: Deciphering the Fine-Epitope Specificity of Cancer-Related Monoclonal Antibodies by Combining Microarray Screening and Saturation Transfer Difference NMR

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Supporting Information

ABSTRACT: The identification of MUC1 tumor-associated Tn antigen (αGalpNAc1-*O*-Ser/Thr) has boosted the development of anticancer vaccines. Combining microarrays and saturation transfer difference NMR, we have characterized the fine-epitope mapping of a MUC1 chemical library (naked and Tn-glycosylated) toward two families of cancer-related monoclonal antibodies (anti-MUC1 and anti-Tn mAbs). Anti-MUC1 mAbs clone VU-3C6 and VU-11E2 recognize naked MUC1-derived peptides and bind GalNAc in a peptide-sequence-dependent manner. In contrast, anti-Tn mAbs clone 8D4 and 14D6 mostly recognize the GalNAc and do not bind naked MUC1-derived peptides. These anti-Tn mAbs show a clear preference for glycopeptides containing the Tn-Ser antigen rather than the Tn-Thr analogue, stressing the role of the underlying amino acid (serine or threonine) in the binding process. The reported strategy can be employed, in general, to unveil the key minimal structural features that modulate antigen−antibody recognition, with particular relevance for the development of Tn-MUC1-based anticancer vaccines.

MUC1 is a glycoprotein that shows a tandem repeating domain, with five possible *O*-glycosylation sites, of conserved 20 amino acids (HGVT*S*APDT*RPAPGS*T*APPA, where asterisk shows a potential *O*-glycosylation site).[1] In normal tissues, the protein backbone carries complex oligosaccharides, with an α-*O*-GalNAc unit directly linked to the hydroxyl group of serine (Ser) or threonine (Thr). In tumor cells, the expression of MUC1 is usually increased with aberrant glycosylation, as the carbohydrate side chains are incomplete.[2] As a result, different epitopes, such as the Tn antigen (α-GalNAc-1-*O*-Ser/Thr), are exposed to the immune system and can be used to design synthetic MUC1-based antitumor vaccines.[3] Efforts to overcome α-Tn's low immunogenicity have been addressed on the basis of clustered Tn-antigen mimetics.[4] As well, a therapeutic vaccine that encompasses Tn-antigen clusters and peptidic CD4+ T-cell epitopes (MAG-Tn3) recently entered into clinical trial.[5] On this basis, elucidation of the key MUC1 antigenic elements is a matter of high interest.[6] On the one hand, the specificity of anti-MUC1 monoclonal antibodies (mAbs) has been attributed to the chemical nature of the glycans attached to their peptide epitopes.[7] On the other hand, density of the Tn motif and the involvement of additional amino acids in the antigenic determinant, namely the aglyconic part of the Tn structure (Ser vs Thr), are critical for anti-Tn mAbs' specificity.[8,9] Despite these advances, the precise chemical epitopes of most anticancer mAbs remain uncertain.

The microarray (MA) technique has arisen in recent years as a versatile platform for accomplishing massive parallel screening and processing of a ligand−protein comparative profile in a compact format.[10] MAs are commonly used for epitope mapping analysis of potentially therapeutic antibodies,[11,12] but the limitation of the MA technique to providing deep knowledge of the binding mode makes to necessary to pair it with other experimental approaches. Furthermore, the design of accurate anticancer vaccines requires a full understanding of the interactions, at the atomic level, between tumor-associated motifs and their specific antibodies. X-ray crystallography and NMR spectroscopy have definitely become the main sources for structural information on ligand−receptor complexes.[13] However, the intrinsic flexibility of carbohydrate antigens may

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hamper a detailed X-ray analysis.[14] Thus, NMR methods assisted by computational calculations may give access to key insights into the structure and dynamics of ligand−antibody complexes.[15] Saturation transfer difference (STD) NMR is very sensitive for weak to medium binders, like most glycan−receptor systems, and highly accurate for identifing the atoms of a ligand in close contact with a receptor.[16]

STD-NMR has been employed to determine the epitope of short MUC1 glycopeptides for the breast-cancer-selective SM3 mAb and for endogenous macrophage galactose-type lectin.[17] In this work, we combine MA and STD-NMR to unveil, for the first time, the structural elements required for recognition of MUC1 tumor-associated peptides by two groups of cancer-related mAbs (Methods in Supporting Information (SI)). The first group comprises a peptide-specific mAb family, the anti-MUC1 VU-3C6 and VU-11E2 mAbs, that recognize the 12-mer GVTSAPDTRPAP of the MUC1 tandem repeat.[6] In contrast, the second family consists of anti-Tn-specific mAbs, 14D6 and 8D4, generated using a synthetic Tn-based vaccine (MAG-Tn3) with a demonstrated affinity toward non-related MUC1 multi-Tn peptide structures and a positive reaction toward human cancer cell lines.[9] Therefore, a rather distinct recognition profile should be expected for each group of mAbs, allowing us to explore our integrated strategy as a new method to unveil the minimal key interactions, with atomic resolution, of antigen−antibody complexes.

A proper MUC1 chemical library has been designed for STD and MA assays (Table 1) containing naked peptides (**1** and **2**/**2′**) and those Tn-glycosylated in all Ser/Thr positions at the three MUC1 regions, GVTSA, PDTRP, and GSTAP (**3**/**3′**−**7**/**7′**). To expedite the synthesis of the (glyco)peptides, we employed microwave-assisted solid-phase synthesis and a "double-activation" approach (Methods in SI).[18] For MA, the glycan array slide was selected due to the non-fouling surface and covalent immobilization through an oxime bond (Methods in SI).[11,19] The mAb concentration was adjusted to facilitate optimal detection and to get comparable relative fluorescence unit values among the mAbs. STD-NMR experiments were performed on 1:40 molar ratio mixtures of the mAbs in the presence of the individual compounds **1**−**8** (Methods in SI). The combined MA and STD-NMR data point out that VU-3C6 (Figures 1A, SI3, SI4, SI9−SI16) and VU-11E2 (Figures SI2A and SI5) mAbs specifically bind to the non-glycosylated MUC1-derived peptides. Accordingly, the STD analysis identified the TR peptide moiety of the PDTRP region as the main structural motif for the recognition of VU-3C6 mAb (Figures 1A, SI9−SI12), whereas VU-11E2 needs a more extended epitope involving all of the PDTRP sequence (Figures SI2A, SI38−SI41).The STD-derived epitope (**1** vs **2**) seems to be independent of the peptide length. For glycopeptide **5** (PDT*RP), the STD-NMR results disclosed that GalNAc recognition is established through the H2 proton and the *N*-acetyl moieties (Figures 1B and SI2B). Detailed analyses of the STD-NMR results for the MUC1 glycopeptides **3**−**7** in the presence of VU-3C6 (Figure 1B) or VU-11E2 (Figure SI2B) mAbs show the remarkable binding selectivity for the PDTRP peptide region. For the Tn-bearing structures at the GVTSA (**3** and **4**) and GSTAP (**6** and **7**) regions of MUC1, only those amino acids present in the PDTRP fragment received saturation from the VU-3C6 mAb, and the GalNAc residue does not show any contact (Figure 1B). Therefore, STD-NMR seems to show a direct correlation with MA data, in which all glycopeptides manifest binding to VU-3C6 mAb, as they include the Arg residue of the region PDTR. In the case of VU-11E2, the extended PDTRP epitope region is required for a stable binding event (Figure SI2B). Fittingly, no STD response is observed for Tn-glycopeptides at GSTAP (**6** and **7**). MA results show that the introduction of GalNAc at PDTRP region (**5′** vs **2′**) improved binding affinity, highlighting the influence of glycosylation on the tumor-specific epitope for anti-MUC1 antibodies, in accordance with the significant STD signals observed in the sugar residue (Figure 1A,B) and the higher relative fluorescence response observed by MA (Figures SI4 and SI5).[20] Therefore, both anti-MUC1 mAbs bind GalNAc in a strict peptide-sequence-dependent manner, with a fairly specific binding profile with respect to the glycosylation position. The GalNAc residue at PDTRP does not impede the binding, and the peptide contact epitope deduced by STD is the same as that observed for the naked peptides, indicating that glycosylation at that position must not significantly modify the peptide conformations bound by these antibodies. (Figures 1A,B and SI2A,B).

Table 1. Synthetic (Glyco)peptides Used for Antibody Mapping by STD-NMR Analysis and MA Screening

entry	(glyco)peptide[a]	entry	(glyco)peptide[b]
1	APDTRPAPGS		
2	HGVTSAPDTRPAPGSTAPPA	**2’**	GVTSAPDTRPAPGSTAPPAHGVT
3	PPAHGV**T**SAPDTR	**3’**	GV**T**SAPDTRPAPGSTAPPAHGVT
4	PAHGVT**S**APDTRP	**4’**	GVT**S**APDTRPAPGSTAPPAHGVT
5	VTSAPD**T**RPAPGS	**5’**	GVTSAPD**T**RPAPGSTAPPAHGVT
6	TRPAPG**S**TAPPAH	**6’**	GVTSAPDTRPAPG**S**TAPPAHGVT
7	RPAPGS**T**APPAHG	**7’**	GVTSAPDTRPAPGS**T**APPAHGVT

The white letters highlighted in black are the amino acids bearing Tn. [a]For STD-NMR, each compound has an acetyl group at the *N*-terminus and an amide group at the *C*-terminus. [b]For MA assay, each (glyco)peptide has a 5-oxo-hexanoyl group and a PEG linker at the *N*-terminus and an amide group at the *C*-terminus.

Binding epitopes of the non-glycosylated PDTRP pentapeptide and the Tn-glycopentapeptide in the presence of SM3 breast-cancer-related mAb were previously determined, highlighting a peptide epitope concentrated at the PDT segment in the naked peptide and a more extended epitope map, whereas the PDTRP sequence is interacting in the case of the Tn-glycopentapeptide.[17a] The 14D6 and 8D4 mAbs recognize multiple Tn-based non-correlated with MUC1 peptide fragments.[9] Accordingly, the combined MA and STD-NMR approach unequivocally demonstrates that the Tn motif in the MUC1 sequence is required for binding (Figures 1C and SI2C). In addition, MA data clearly indicate that 14D6 and 8D4 display higher affinity to glycopeptides containing the Tn-Ser antigen (**4′** and **6′**) rather than the Tn-Thr alternative (**3′**, **5′**, and **7′**) (Figures 1D, SI2D, SI6, and SI8). By MA alone, these mAbs did not recognize the Tn-Thr glycopeptide **3′** at detectable levels, and high concentrations of mAbs were required to detect binding of **5′** and **7′** (Figure SI7). In contrast, specific STD signals were observed for all Tn-bearing glycopeptides **3**−**7**, highlighting that both mAbs mostly recognize the GalNAc residue, with clear participation of the acetamide moiety in the binding. The peptide backbone is marginally involved in intermolecular contacts (Figures 1D and SI2D). In addition, STD-NMR unequivocally demonstrated that the GalNAc monosaccharide specifically binds to 8D4 and 14D6 mAbs with a similar epitope as glycopeptides **3**−**7** (Figures SI29 and SI30). Similar behavior was found for the glycopeptide-specific 237 mAb.[21] STD competition experiments (see SI) allowed us to deduce that glycopeptides **3**−**7**

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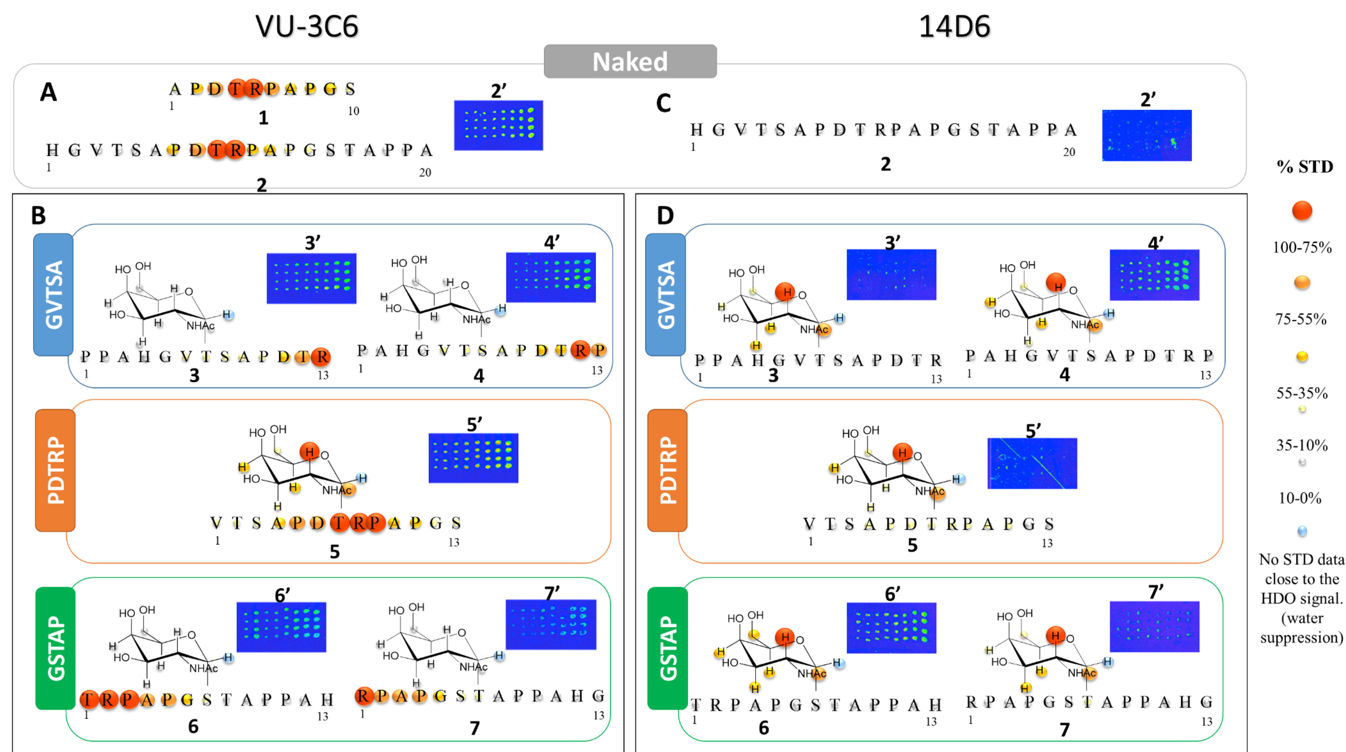

Figure 1. STD-derived epitope and MA fluorescent scan for MUC1-derived (glyco)peptides with VU-3C6 and 14D6 mAbs (see Methods in SI). (A) Naked peptides **1**, **2**, and **2′** with VU-3C6. (B) Glycopeptides **3**–**7** and **3′**–**7′** with VU-3C6. (C) Naked peptides **1**, **2**, and **2′** with 14D6. (D) Glycopeptides **3**–**7** and **3′**–**7′** with 14D6. Figures SI9–SI28 show the ^{1}H STD spectra and additional STD-epitope representations. For MA analysis, each peptide was printed on a slide at eight different concentrations and incubated with VU-3C6 (10 μg/mL) or 14D6 (200 μg/mL). Figures SI3, SI4, SI6, and SI7 show MA fluorescent response graphs.

displace GalNAc from the binding site (Figures 2A and SI50) and that none of the glycopeptides bind with higher affinity than GalNAc (glycopeptide/GalNAc ratios >1 do not produce reduction in GalNAc STD intensity by >50%), in very good agreement with the STD-derived epitope. Fittingly, analysis of

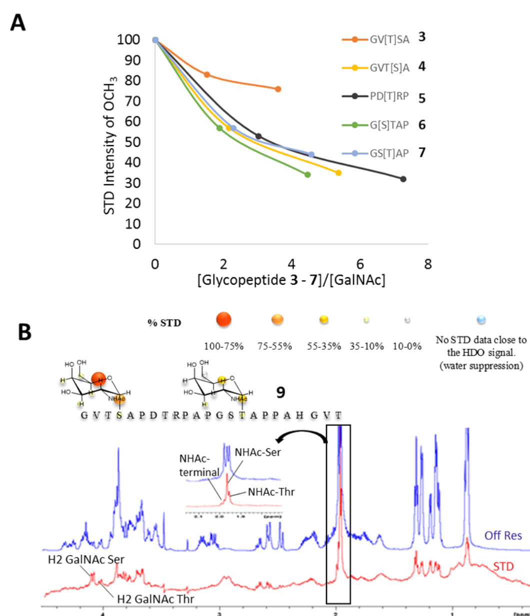

Figure 2. (A) STD intensity of the OCH_3 group of **8** as a function of [**3**–**7**]/[GalNAc]. (B) STD epitope mapping and ^{1}H STD-NMR spectrum of **9** in the presence of 14D6.

the STD competition data indicated that Tn-Thr glycopeptide **3** was the weaker binder toward 14D6, probably in the low mM range. The data indicate that 14D6 presents a typical lower affinity of anti-carbohydrate antibodies that could be improved, in principle, by the multivalence effects of a dense MUC1 Tn-antigen presence in tumor cells.[13] The preference of this mAbs family toward Tn-Ser glycopeptides was also corroborated by STD-NMR. The STD data for **9**, displaying two simultaneous glycosylations within the MUC1 sequence (Ser at GVTSA, as in **4**, and Thr at GSTAP, as in **7**), also reflect the selectivity of 14D6 mAb toward glycopeptides carrying Tn-Ser, in agreement with MA data. The H2 and NHAc resonance signals of GalNAc at the Ser glycosylation site received much more saturation from 14D6 than the corresponding signals at the GalNAc-Thr fragment (Figure 2B). Mazal et al. demonstrated that Ser/Thr's selectivity plays a key role for anti-Tn antibodies' expression and specificity for breast and colon cancer detection.[9] Accordingly, the data presented herein strongly suggest that the chemical nature of the amino acid carrier (Ser vs Thr) plays a key role for anti-Tn antibodies' recognition. Differences in the molecular recognition features between Ser- and Thr-containing Tn antigens have been previously reported for lectins and SM3 antibody.[22]

The conformational behavior of ligands **3**–**7** was deduced by NOE studies (Figures SI51–SI56), supported by molecular dynamics simulations with time-averaged restraints, highlighting that the side chains of GalNAc-Ser peptides are significantly more flexible than those containing GalNAc-Thr fragments (Figures SI57–SI61). The additional flexibility of Tn-Ser glycopeptides may allow them to adopt the proper complementary conformation in the bound state, without a

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major entropy penalty. The relevance of the flanking amino acids around Ser/Thr glycosylation cannot be ignored, and the presentation mode of the sugar epitope is rather distinct in the Ser and Thr glycosylated peptides.

There is an interest in understanding how molecules are displayed on MAs and the contribution of the solid support.[23] By comparing MA results with STD-NMR data, we can hypothesize that the presentation of glycopeptides on glycan array slides through an oxime linker was successful and can contribute to identifying specific epitopes.

A combined multidisciplinary approach—integrating synthetic chemistry methods, mAb generation, MA, NMR, and computational methods—has been applied to identify the molecular elements of the recognition region of the antigens for two different families of cancer-related monoclonal antibodies. The combination of MA and STD-NMR provides a unique opportunity to investigate the functional significance of glycosylated peptides as antigens, giving detailed information for the design of tailored Tn-based vaccines like MAG-Tn3. Our results highlight that, for anti-MUC1 mAbs, the amino acids sequence modulates the affinity of the mAb, while for anti-Tn mAbs, it is the type of residue that modulates the binding. The integrated methodology reported herein can be employed, in general, to study antigen−antibody interactions and will be of paramount importance to designing a potent multivalent Tn-MUC1-based anticancer vaccine that raises functional antibodies against tumor-associated antigens.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b06787.

Methods and additional figures (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Hattrup, C. L.; Gendler, S. J. *Annu. Rev. Physiol.* **2008**, *70*, 431.

(2) (a) Hollingsworth, M. A.; Swanson, B. J. *Nat. Rev. Cancer* **2004**, *4*, 45. (b) Dube, D. H.; Bertozzi, C. R. *Nat. Rev. Drug Discovery* **2005**, *4*, 477.

(3) Gaidzik, N.; Westerlind, U.; Kunz, H. *Chem. Soc. Rev.* **2013**, *42*, 4421.

(4) Richichi, B.; Thomas, B.; Fiore, M.; Bosco, R.; Qureshi, H.; Nativi, C.; Renaudet, O.; BenMohamed, L. *Angew. Chem., Int. Ed.* **2014**, *53*, 11917.

(5) Lo-Man, R.; Vichier-Guerre, S.; Perraut, R.; Deriaud, E.; Huteau, V.; BenMohamed, L.; Diop, O. M.; Livingston, P. O.; Bay, S.; Leclerc, C. *Cancer Res.* **2004**, *64*, 4987.

(6) (a) Schol, D. J.; Meulenbroek, M. F.; Snijdewint, F. G.; von Mensdorff-Pouilly, S.; Verstraeten, R. A.; Murakami, F.; Kenemans, P.; Hilgers, J. *Tumor Biol.* **1998**, *19* (Suppl 1), 35. (b) Blixt, O.; Lavrova, O. I.; Mazurov, D. V.; Clo, E.; Kracun, S. K.; Bovin, N. V.; Filatov, A. V. *Glycobiology* **2012**, *22*, 529.

(7) (a) Karsten, U.; Diotel, C.; Klich, G.; Paulsen, H.; Goletz, S.; Müller, S.; Hanisch, F. G. *Cancer Res.* **1998**, *58*, 2541. (b) von Mensdorff-Pouilly, S.; Petrakou, E.; Kenemans, P.; et al. *Int. J. Cancer* **2000**, *86*, 702.

(8) Grinstead, J. S.; Koganty, R. R.; Krantz, M. J.; Longenecker, B. M.; Campbell, A. P. *Biochemistry* **2002**, *41*, 9946.

(9) Mazal, D.; Lo-Man, R.; Bay, S.; et al. *Cancer Immunol. Immunother.* **2013**, *62*, 1107.

(10) (a) Fukui, S.; Feizi, T.; Galustian, C.; Lawson, A. M.; Chai, W. *Nat. Biotechnol.* **2002**, *20*, 1011. (b) Smith, D. F.; Song, X.; Cummings, R. D. Use of Glycan Microarrays to Explore Specificity of Glycan-Binding Proteins. In *Methods in Enzymology*; Fukuda, M., Ed.; Elsevier: Amsterdam, 2010; Vol. *480*, pp 417−444. (c) Foong, Y. M.; Fu, J.; Yao, S. Q.; Uttamchandani, M. *Curr. Opin. Chem. Biol.* **2012**, *16*, 234.

(11) Matsushita, T.; Takada, W.; Igarashi, K.; Naruchi, K.; Miyoshi, R.; García-Martín, F.; Amano, M.; Hinou, H.; Nishimura, S.-I. *Biochim. Biophys. Acta, Gen. Subj.* **2014**, *1840*, 1105.

(12) (a) Oyelaran, O.; Li, Q.; Farnsworth, D.; Gildersleeve, J. C. *J. Proteome Res.* **2009**, *8*, 3529. (b) Borgert, A.; Heimburg-Molinaro, J.; Song, X.; et al. *ACS Chem. Biol.* **2012**, *7*, 1031. (c) Cai, H.; Palitzsch, B.; Hartmann, S.; Stergiou, N.; Kunz, H.; Schmitt, E.; Westerlind, U. *ChemBioChem* **2015**, *16*, 959.

(13) Haji-Ghassemi, O.; Blackler, R. J.; Young, N. M.; Evans, S. V. *Glycobiology* **2015**, *225*, 920.

(14) Agirre, J.; Davies, G.; Wilson, K.; Cowtan, K. *Nat. Chem. Biol.* **2015**, *11*, 303.

(15) Marcelo, F.; Cañada, F. J.; Jimenez-Barbero, J. In *Anticarbohydrate Antibodies: from molecular basis to clinical application*; Kosma, P., Müller-Leonnies, S., Eds.; Springer-Verlag: Vienna, 2012; pp 385−402 and references cited therein.

(16) (a) Mayer, M.; Meyer, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1784; *Angew. Chem.* **1999**, *111*, 1902. (b) Mayer, M.; Meyer, B. *J. Am. Chem. Soc.* **2001**, *123*, 6108. (c) Meyer, B.; Peters, T. *Angew. Chem., Int. Ed.* **2003**, *42*, 864; *Angew. Chem.* **2003**, *115*, 890.

(17) (a) Möller, H.; Serttas, N.; Paulsen, H.; Burchell, J. M.; Taylor-Papadimitriou, J.; Meyer, B. *Eur. J. Biochem.* **2002**, *269*, 1444. (b) Marcelo, F.; García-Martín, F.; Matsushita, T.; et al. *Chem.-Eur. J.* **2014**, *20*, 16147.

(18) (a) Matsushita, T.; Hinou, H.; Kurogochi, M.; Shimizu, H.; Nishimura, S.-I. *Org. Lett.* **2005**, *7*, 877. (b) García-Martín, F.; Hinou, H.; Matsushita, T.; Hayakawa, S.; Nishimura, S.-I. *Org. Biomol. Chem.* **2012**, *10*, 1612.

(19) García-Martín, F.; Matsushita, T.; Hinou, H.; Nishimura, S.-I. *Chem.-Eur. J.* **2014**, *20*, 15891.

(20) Karsten, U.; Serttas, N.; Paulsen, H.; Danielczyk, A.; Goletz, S. *Glycobiology* **2004**, *14*, 681.

(21) Brooks, C. L.; Schietinger, A.; Borisova, S. N.; Kufer, P.; Okon, M.; Hirama, T.; Mackenzie, C. R.; Wang, L.-X.; Schreiber, H.; Evans, S. V. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 10056.

(22) (a) Madariaga, D.; Martínez-Sáez, N.; Somovilla, V. J.; et al. *Chem.-Eur. J.* **2014**, *20*, 12616. (b) Martínez-Sáez, N.; Castro-López, J.; Valero-González, J.; et al. *Angew. Chem., Int. Ed.* **2015**, *54*, 9830.

(23) Wang, L.; Cummings, R. D.; Smith, D. F.; et al. *Glycobiology* **2014**, *24*, 507.