Biomacromolecules

Perspective

Cite This: Biomacromolecules 2020, 21, 18−29

pubs.acs.org/Biomac

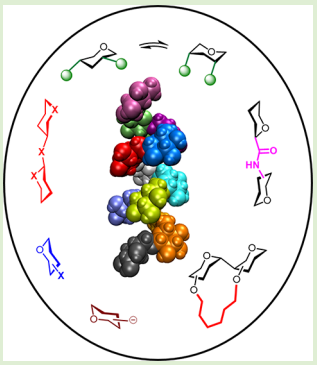
Structural Studies Using Unnatural Oligosaccharides: Toward Sugar Foldamers

Theodore Tyrikos-Ergas,[†,‡,§] Giulio Fittolani,[†,‡,§] Peter H. Seeberger,[*,†,‡] and Martina Delbianco[*,†]

[†]Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, Am Mühlenberg 1, 14476 Potsdam, Germany

[‡]Department of Chemistry and Biochemistry, Freie Universität Berlin, Arnimallee 22, 14195 Berlin, Germany

ABSTRACT: Biopolymers, like DNA and proteins, fold in specific conformations in order to exert complex biological functions. Synthetic modifications are commonly used to alter those conformations and create engineered biomaterials. In stark contrast, the chemical complexity and dynamic nature of polysaccharides have hampered a detailed structural characterization and structure−function correlations are still incomplete. Many synthetic strategies have been developed to access complex unnatural oligosaccharides, capable of mimicking or even improving the properties of the natural counterpart. However, the structural features behind these results are often neglected. This perspective highlights the approaches adopted to develop unnatural glycans, with a particular focus on how the insertion of specific modifications results in more flexible or more constrained structures. Synthetic analogues of natural oligosaccharides could shine light on fundamental structural features. The combination of modern synthetic, computational, and analytical methods will result in novel carbohydrate based foldamers, with defined shape and aggregation behavior. Multiple applications in biology, material science, and nanotechnology can be envisioned.



1. INTRODUCTION

The folding of biomacromolecules, like proteins or nucleic acids, is responsible for most biological functions, such as molecular recognition, information storage, and catalysis. Recurrent three-dimensional features, such as the helical motive,[1,2] have fascinated chemists for many years, and much effort has been paid to understand and reproduce such conformations with synthetic analogues. A multitude of chemical modifications has been employed to tune the 3D structure and the function of biological entities. Unnatural oligomers that display biopolymer-like folding behavior (i.e., foldamers) were designed and permitted to tune several biological processes.[3,4] These structural features form the bases of engineered materials like DNA[5,6] and protein[1,7] origami, with implication in the medical and energy field.

In contrast, glycan folding is often neglected and polysaccharides have always been considered highly flexible molecules with ill-defined conformations.[8,9] However, carbohydrates offer the possibility to form several directional hydrogen bonds[10] and polysaccharide materials, such as cellulose, often show high crystallinity.[11] The challenging synthesis and 3D analysis of carbohydrates hampered the development of reliable methods to predict glycan folding.[9] X-ray analysis of polysaccharide fibers gave valuable information on how the monosaccharide type and linkage affects the 3D structure.[12] In addition, electron microscopy (EM) permitted the biophysical characterization of natural polysaccharides' gel microstructure and their mechanical properties.[13] However, these studies are limited to heterogeneous polysaccharide samples and their solid phase (or gel) structure. Little effort has been directed to understand the folding of simpler oligosaccharides, at the molecular level. Thus, the development of glycomimetics, aiming for an increased biological activity or resistance to enzymatic degradation, often lacks a precise description of the effect of the modification on the glycan 3D structure. This lack of research is surprising if we consider how subtle differences in the sugar backbone of DNA and RNA (i.e., deoxyribose vs ribose) have a major effect on the overall conformation and consequently function.[14]

Recent advances in synthesis,[15] characterization techniques,[16−18] and modeling[8] allowed for new insights into carbohydrate chemistry. It was recently shown that simple oligosaccharides can adopt fundamentally different shapes depending on the monosaccharide composition and their conformation can be tuned with single-site substitution.[19] These modifications have major effects on the single-chain conformation but also on the aggregation and properties of such compounds.[19−21] It became obvious that molecular details are highly important for the study of glycan−protein interactions. Molecular glycobiology shifted its attention toward single-molecule characterization, and glycan conformation, previously neglected, is now considered important.[22] Indeed, glycans are still regarded as highly flexible molecules, but the description shifted from an ill-defined conformation to

Special Issue: Future of Biomacromolecules at a Crossroads of Polymer Science and Biology

Received: August 7, 2019
Revised: September 11, 2019
Published: September 13, 2019

DOI: 10.1021/acs.biomac.9b01090
Biomacromolecules 2020, 21, 18−29

an ensemble of conformations. It was suggested that lectins, glycan binding proteins, are able to discriminate between those conformers and select one for binding.[23]

Oligosaccharide-based drugs should be designed with careful consideration of folding and supported by molecular models and prediction tools. Rational considerations on the type and position of the chemical modifications need to be implemented, as they could play a crucial role in the bioactivity of glycomimetic drugs.[24]

In this perspective, we analyze how chemical modifications affect the conformation of natural oligosaccharides, resulting in more flexible (section 2) or more rigid (section 3) structures. We demonstrate that unnatural analogues offer an attractive option to better understand fundamental aspects of glycan conformation. The precise manipulation of the chemical structure can shine light on the structural features essential for the activity of such compounds. As a result, new sugar-based foldamers with different conformations and geometries could be created. Those structures may well become powerful building blocks for the creation of supramolecular architectures with applications in materials science. Analogues to peptides and DNA, novel well-defined materials based on unnatural oligosaccharides, could fuel the development of glyco-nanotechnology.

For the description of oligosaccharide conformation, standard definitions should be remembered. The main variable implicated in polysaccharide conformation is the geometry of the glycosidic linkage, with the monosaccharide units generally considered rigid. The torsion angles Φ ($H_1-C_1-O_x-C_x$) and Ψ ($C_1-O_x-C_x-H_x$) define the relative orientation of two monosaccharides involved in a glycosidic bond. For 1,6-linkages, the ω torsion angle ($O_6-C_6-C_5-O_5$) provides additional flexibility. The most populated conformation is generally the *exo-syn*(Φ),[25] due to hyperconjugation between the exocyclic oxygen lone electron pair and the antibonding orbital (σ^*) of the endocyclic C−O bond (*exo*-anomeric effect) (Figure 1).[26] The Ψ dihedral is more sensitive to sterics, favoring the *anti*(Ψ) conformer.[27] The addition of water to the system, which can disrupt intermolecular hydrogen bonds, further complicates the description. As a result, glycosidic linkages possess a significant degree of freedom, resulting in quite flexible oligosaccharides.[28]

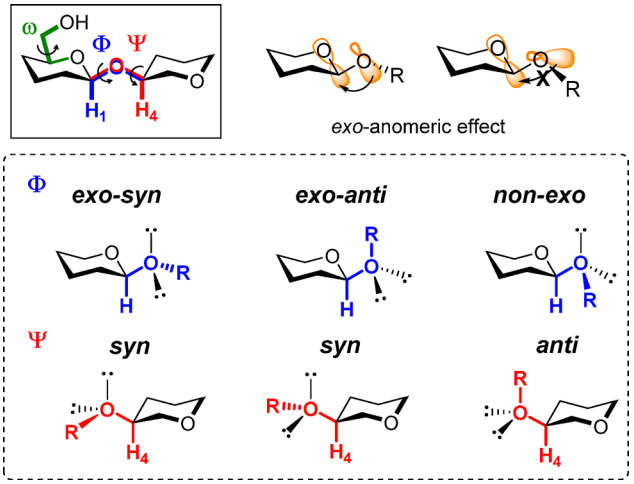


Figure 1. Standard definition of dihedral angles used for the description of a glycosidic bond exemplified for a β-glycosidic linkage.

2. CHEMICALLY MODIFIED OLIGOSACCHARIDES

A large variety of oligosaccharide mimetics was developed to improve on the performance of the natural glycans.[29] The synthesis was rarely based on rational design, and very little attention was paid to the effect of the modification on the overall conformation. Still, in the rare occasions when detailed structural considerations were applied, glycomimetics with remarkable target affinity were identified.[30,31] Herein, we will demonstrate how chemical modifications alter the oligosaccharide conformation, with the vast majority of modifications resulting in glycomimetics with increased flexibility. Thorough structural considerations are essential for the development of better oligosaccharide mimetics.

2.1. Functional Groups Modifications. Carbohydrates bear functional groups such as hydroxyls, amines, or sulfate, which play a fundamental role in the properties and functions of oligo- and polysaccharides. A vast number of cellulose derivatives (i.e., cellulose esters, ethers) have been obtained and today produced in industrial scale, with dramatic changes in the material properties.[32] A wide range of chemical methodologies has been developed in order to achieve a certain degree of regioselectivity;[33] however, this "post-functionalization" approach has some drawbacks. Limited control over the regiochemistry and degree of modification hampered detailed structure−property correlations and generate unreproducible results. In contrast, a total synthesis approach can selectively introduce functional groups in specific positions to obtain well-defined oligomers but is applicable only for simpler structures. Thereby, several unnatural oligosaccharide mimics were created. However, the laborious synthetic approach has limited systematic structural studies. Automated glycan assembly (AGA) has allowed for the fast synthesis of well-defined oligomers, by iteratively combining monosaccharide units on a solid support. Unnatural building blocks bearing specific modifications have been employed to synthesize a collection of methylated, deoxygenated, deoxyfluorinated, as well as carboxymethylated cellulose analogues (Figure 2). Each modification was designed to manipulate specific intramolecular H-bonds, introduce charge, and tune dipole orientation, to alter the overall conformation. All modified compounds are more water-soluble and less

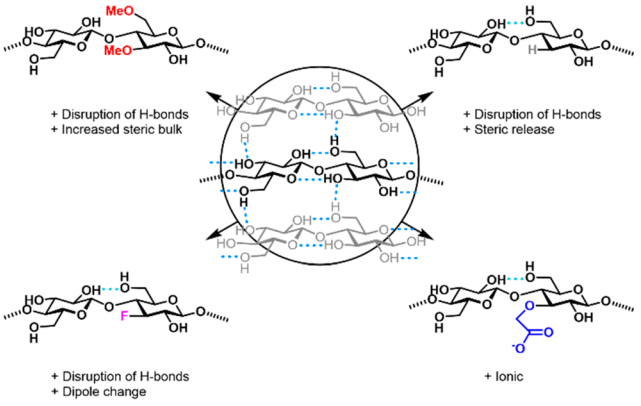


Figure 2. Systematic approach to study structure−property correlations in carbohydrate materials by single-site modifications. Reproduced from ref 21 under Creative Commons Attribution http://creativecommons.org/licenses/by/4.0/. Copyright 2019 Y. Yu et al.

DOI: 10.1021/acs.biomac.9b01090
Biomacromolecules 2020, 21, 18−29

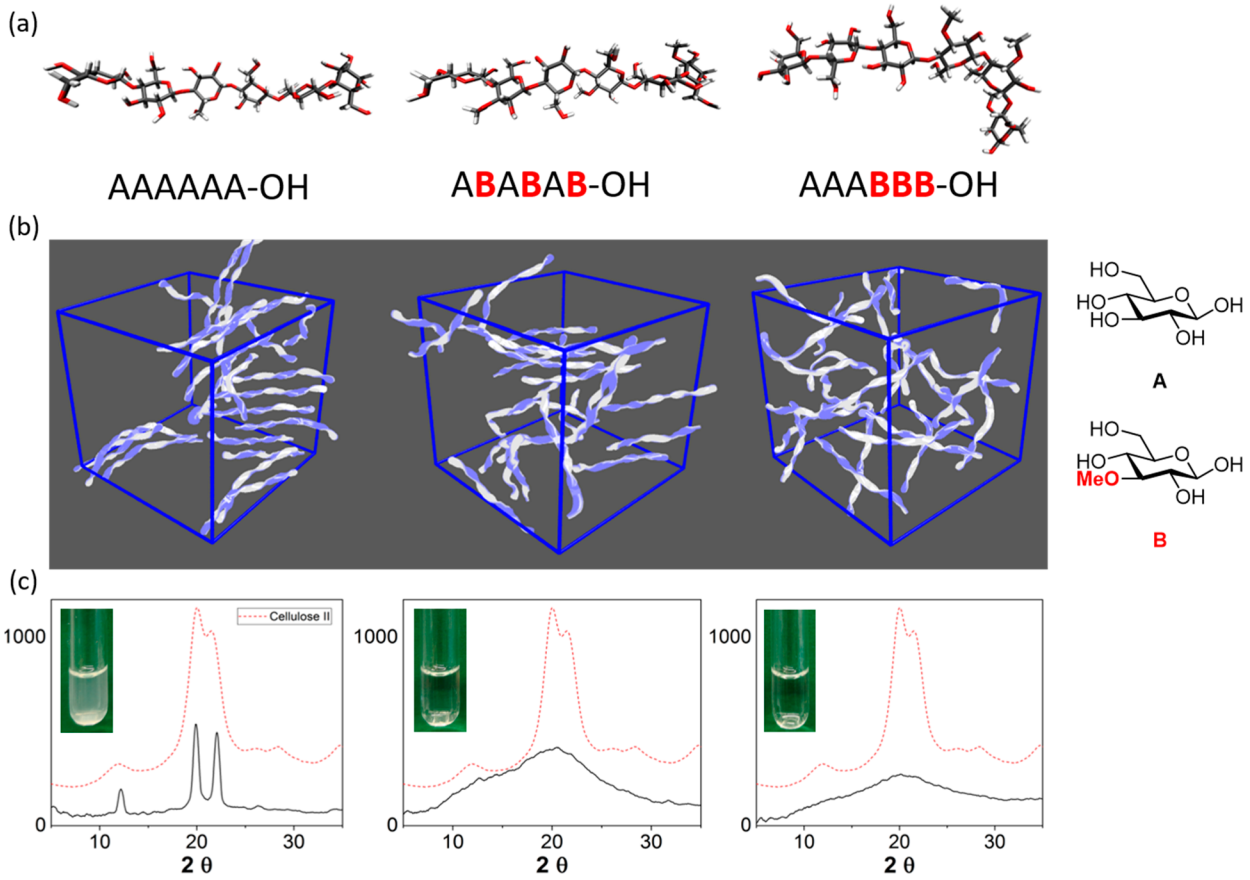


Figure 3. Representative snapshots of MD simulations for methylated analogues at the single-chain level (a) and in concentrated solutions (b). (c) Powder XRD diffraction and solubility test (inset). Reproduced from ref 21 under the terms of the Creative Commons Attribution License (CC BY). Copyright 2019 Y. Yu et al. Published by Wiley-VCH Verlag GmbH & Co. KGaA.

crystalline than the natural cellulose oligomers, as a result of the increased flexibility of the single chains.

Importantly, MD simulations revealed that compounds with the same degree of methylation but different substitution pattern behave drastically different, highlighting the importance of rational design compared to the traditional random functionalization.[21] Alternated substitution patterns result in quasi-linear structures, whereas more bent geometries are observed with a block arrangement (Figure 3a). The single-chain conformation affects the overall material properties, with high crystallinity observed for the natural analogue, a completely amorphous behavior for the block substituted compound, and an intermediate behavior for the compounds with an alternated methylation pattern (Figure 3).

The importance of the substitution pattern was recently shown for glycosaminoglycans (GAGs), a major component of mammalian cell surfaces and extracellular matrix. GAG analogues interacted with heavy metal ions depending on the sulfation pattern, rather than the sulfation degree. This effect is attributed to the conformations adopted by the differently sulfated oligosaccharide.[34] This preliminary study could be implemented in the development of unnatural sulfated oligosaccharides for sensing and devices.

Introduction of Fluorine. The specific installation of fluorine into an organic backbone has been widely applied in medicinal chemistry, preparation of agrochemicals, and material chemistry.[35,36] New chemical entities with unique chemical, physical, and biological properties arose due to the chemical inertness of the C−F bond, increased hydrophobicity, small size, and strong inductive effect of fluorine. Additionally, a wide range of functional groups can be replaced by fluorine with considerable effects on biological activity.[36]

Fluorination can tune hydrogen bonding and interactions with water molecules, suggesting that OH/F substitutions in oligosaccharides can have a profound effect on the glycan conformation. Limited studies have been carried out on fluorinated glycans, since the installation of fluorine into an oligosaccharide backbone can be extremely challenging. In addition, the conformational changes are not always well understood at the molecular level and their effect is often underestimated. In contrast, fluorinated peptides are much better understood and exploited. Fluorination of alkyl and aromatic side chains is commonly used to provide stabilization or guide protein folding to improve denaturation resistance and tune biological activity.[37,38]

To date, the installation of specific fluorine atoms proved to be a successful strategy to increase the *in vivo* stability of glycoproteins. Indeed, fluorinated oligosaccharides have proven more resistant to enzymatic hydrolysis.[39] However, conformational aspects have never been taken into consideration to explain enzymatic inhibition, generally attributed to the reduced reactivity of the fluorinated analogues. To enhance the immunogenicity and resistance to enzymatic degradation, a collection of deoxyfluorinated disaccharides was synthesized.[40] Similarly, a significant increase in immunogenicity was observed for fluorinated analogues of a disaccharidic tumor-

DOI: 10.1021/acs.biomac.9b01090
Biomacromolecules 2020, 21, 18−29

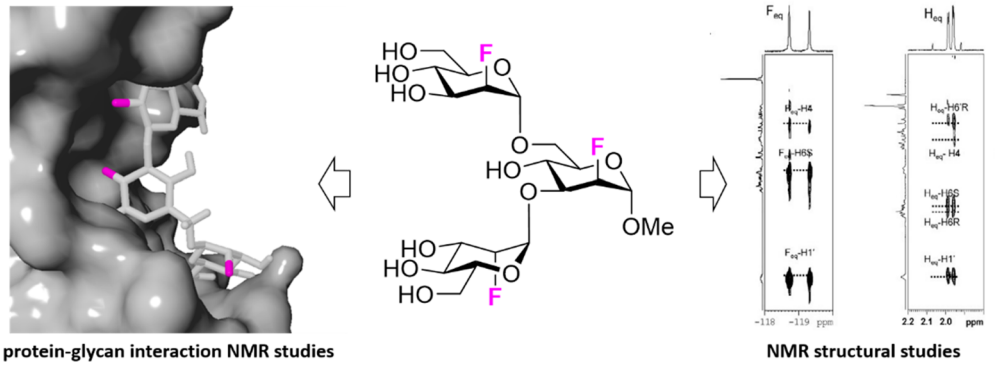


Figure 4. Fluorinated glycans can give an insight into protein binding events (left) and provide a tool for detailed structural NMR analysis (right). Adapted from ref 55 with permission from John Wiley and Sons. Copyright 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

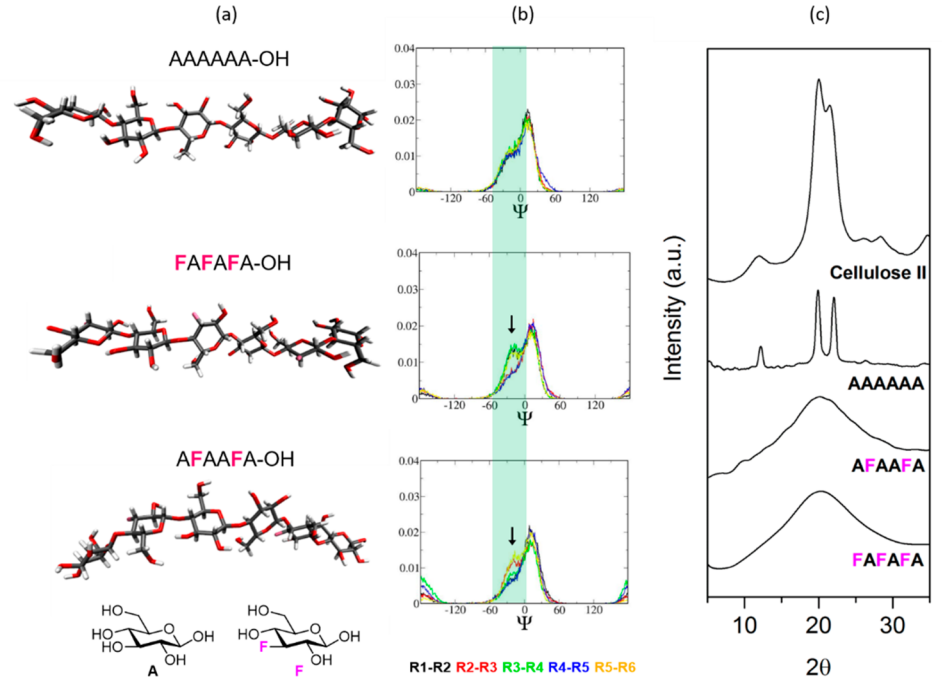


Figure 5. (a) Representative snapshots of fluorinated analogues from MD simulations. (b) Ψ dihedral analysis showing a visible change in the population distribution for the fluorinated analogues. (c) Powder XRD illustrating dramatic changes in crystallinity.

associated carbohydrate antigen (TACA). The causes of the increased immunogenicity are not fully understood, with no insight on how fluorination affects the disaccharide 3D structure nor its interaction with the protein.[41]

Surprisingly, ^{19}F NMR has barely been used for the structural analysis of glycans. However, recent examples suggested that ^{19}F NMR could offer a unique solution to simplify the, otherwise extremely complex, NMR analysis of carbohydrates.[42,43] The simplification of NMR spectra provides access to unique techniques (e.g., residual dipolar couplings, H-bond analysis) that can lead to excellent 3D characterization.[8,44] The replacement of specific hydroxyls of the sugar ring with fluorine was employed to study protein−carbohydrate interactions (Figure 4). For this approach, a preliminary structural analysis remains necessary, since fluorination should not affect the overall oligosaccharide conformation.[42] For instance, a fluorinated sialyl-lactosamine trisaccharide showed excellent binding to the protein of interest and, importantly, X-ray crystallography proved that the fluorination of position 2 of the central galactose unit provides excellent mimicry of the natural trisaccharide.[45]

The OH/F substitution was also used to selectively block intramolecular hydrogen bonds. In this case, the overall conformation of the modified oligosaccharide was affected with dramatic effects over the macroscopic properties (Figure 5). A collection of deoxyfluorinated cellulose analogues with specific fluorination patterns was prepared by AGA.[15] A detailed MD analysis of the torsional angles (i.e., θ and Ψ) predicted the high flexibility of such systems, with partial disruption of the rigid cellulose conformation. Higher solubility and lower tendency to arrange in crystalline domains was verified by powder XRD.[21] This systematic approach can be applied to several glycans to shine light on important three-dimensional features required for a particular activity.

We strongly believe that fluorinated oligosaccharides will play a key role in gaining a fundamental understanding of glycans. The specific placement of the F substituent can either disrupt hydrogen bonds or provide a perfect mimic of the hydroxyl groups. Furthermore, fluorine can affect glycan−

DOI: 10.1021/acs.biomac.9b01090
Biomacromolecules 2020, 21, 18−29

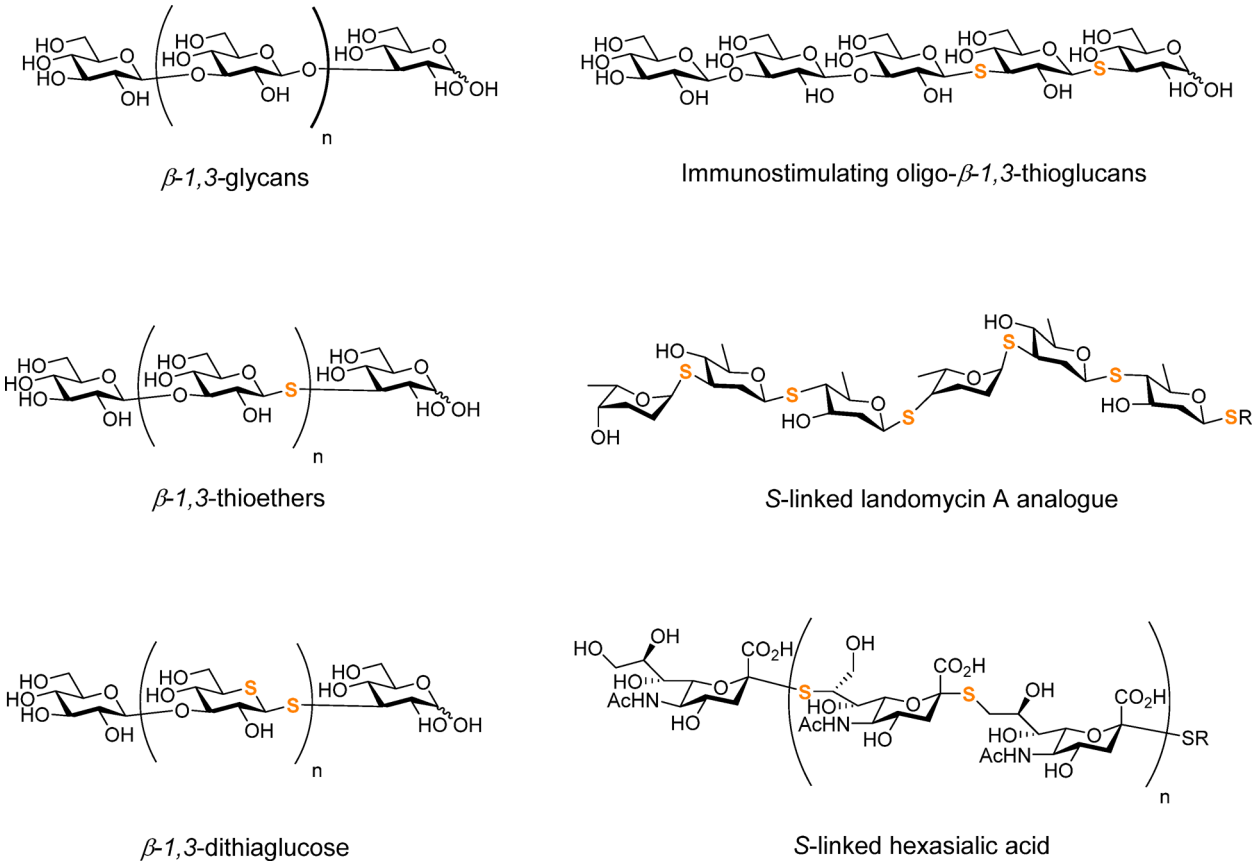


Figure 6. Thioglycoside derivatives of β-1,3-glycans and *S*-linked analogues.

water interactions, crucial for the conformation of glycans in solution.[8] New methods for the selective installation of fluorine atoms are needed to explore the wide variety of glycan conformations. To date, only heavily fluorinated monosaccharides have been prepared and their use in oligosaccharide synthesis remains challenging. In the future, highly fluorinated analogues could result in completely new conformations and macroscopic properties.[46,47]

2.2. Endocyclic Oxygen Replacement. The replacement of the endocyclic oxygen with unnatural functionalities is a common strategy to increase oligosaccharide stability toward enzymatic degradation. Carbasugars are a class of oligosaccharide mimics in which the endocyclic oxygen has been replaced with a methylene unit (CH_2).[48] The methylene group has an important effect on oligosaccharide conformation. While the pyranose chair conformation remains substantially unaffected,[49] the electronic properties of the glycosidic linkage are drastically affected. The absence of the *exo*-anomeric effect permits high flexibility, and the glycosidic linkage can explore a wide range of conformations, such as the generally not populated *non-exo*(Φ).[50] The flexibility of these compounds results in an entropic cost during binding to proteins, even though the *non-exo*(Φ) conformation remains in most cases less stable, due to steric clash.[51,52]

Thiasugars bear an endocyclic sulfur atom in place of the oxygen that makes them more flexible at the glycosidic linkages, due to stereoelectronic properties (weaker *exo*-anomeric effect). In contrast to carbasugars, they retain the same overall conformation of the natural counterparts (*exo-syn*(Φ) and *exo-anti*(Φ) are the most abundant conformations).[53,54]

The replacement of the endocyclic oxygen with CH_2 or S increases the flexibility of the oligomer, and "unnatural" conformations are observed. Since high flexibility could mean a high entropic cost during binding, a CF_2 moiety has been installed in place of the endocyclic oxygen to restore the *exo*-anomeric effect in a maltose mimic. A favored overlap between the oxygen lone pair and the antibonding orbital of the C−CF_2 bond (σ^*_{C1-CF2}) mimicked the interactions present in natural glycosidic linkages and favored the *exo*(Φ) conformations.[55]

2.3. Unnatural Glycosidic Linkages. The substitution of the oxygen of the glycosidic linkage with other moieties has been widely explored for the production of new molecular architectures. These unnatural analogues are capable of mimicking the functions as well as, in some cases, improving the bioactivities of natural oligosaccharides. The most common modifications, developed to resist hydrolysis by glycosidases, are *S*-glycosides and *C*-glycosides. The long *in vivo* lifetime[56−58] made these analogues useful for therapeutic applications,[59,60] as shown in the case of the *S*-linked antitumor agent Landomycin A (Figure 6).[61] In addition to *in vivo* stability, a collection of short β-1,3-glycan thioglycoside derivatives showed an enhanced affinity for hydrophobic binding pockets of various lectins, dectins, and receptors such as the complement receptor 3 (CR3).[62−65] Following a similar idea, the synthesis of *S*-linked oligosialic acid antigens was reported for the development of carbohydrate-based vaccines (Figure 6).[66]

To date, the attention has been directed mainly on the bioactivity of such compounds, neglecting the effect of the linkage modification on the overall oligosaccharide conformation. Little effort has been devoted to the structural features of

DOI: 10.1021/acs.biomac.9b01090
Biomacromolecules 2020, 21, 18−29

these analogues.[51,67,68] Increased flexibility was observed for simple *S*-linked dimers (Figure 7). Molecular mechanics

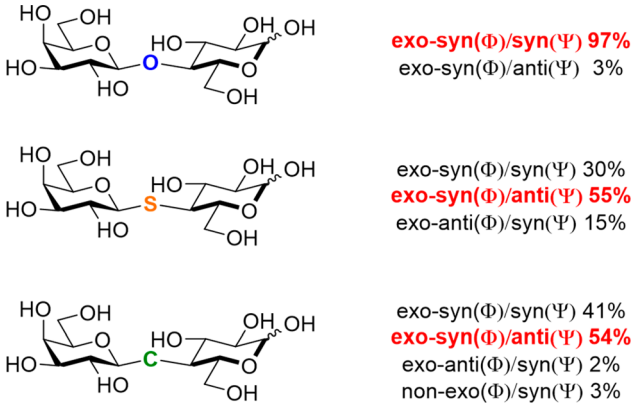


Figure 7. Conformational distribution of *S*- and *C*-glycosides compared to the natural disaccharide with the most populated conformation highlighted in red.

calculations showed that the "standard" *exo*−*syn*(Φ) conformation of α-*S*-glycosides is significantly less rigid than the corresponding *O*-linked analogue.[69] Moreover, two additional conformations (*exo*−*syn*(Φ)/*anti*(ψ) and *non-exo*(Φ)/*syn*(ψ)) become accessible, due to the weaker *exo*-anomeric effect. As expected from the absence of the stabilizing *exo*-anomeric effect, the *C*-linked analogues show high conformational flexibility with a highly populated *non-exo* conformation (Figure 7).[70,71]

The conformational diversity and flexibility of these systems may play a major role in the molecular recognition and biological activity of such mimetics. Even though flexible systems could suffer from an entropic loss during binding to proteins, these systems could be important with lectins that preferentially bind to specific low populated conformations.[72] Thus, a deeper structural analysis of such oligomers will allow for fundamental structure−activity correlations, toward the design of more potent drugs. Moreover, other functionalities have been used to replace the conventional *O*-glycosidic bond. *N*- and *Se*-Glycosides[54,73] as well as carbohydrate sequences linked through a phosphate[74] (i.e., carbonucleotoids) or a triazole[75] moiety have been reported and could offer interesting alternatives for the formation of completely new geometries.

3. TOWARD SUGAR FOLDAMERS

Most of the previous examples employed chemical manipulations to improve bioactivities and/or stability, but very little structural design was employed. Nevertheless, as a result of the modifications, conformational changes occurred and a more flexible system was generated. In this section, we discuss the use of modified carbohydrate backbones to reduce the flexibility of the system. Tricks to stabilize particular 3D structures as well as methods to switch between conformations will be analyzed.

3.1. Peptide Mimics. In the last decades, compounds with defined folding have been designed to mimic polypeptide secondary structure. Foldamers, short oligomers able to adopt stable and defined secondary structures, are now important for nanotechnology and biology.[3,4] Following the same idea, a functionalized pentasaccharide was designed to bind to the minor groove of DNA. The design was inspired by a DNA-binding protein with α-helical structural motifs that interact with DNA in a specific manner. While the backbone plays a minor role, the presentation of the side chains is crucial for DNA binding. The rod-like pentasaccharide, constituted of 2-deoxyfucose linked with α-1,4 linkages, permitted a close resemblance to the helical region of a DNA-binding protein. 2-Deoxyfucose was chosen because it is considerably more hydrophobic than other monosaccharides and expected to facilitate binding to DNA grooves. Guanidinium groups mimic the arginine side chain. NMR allowed the structure of the pentasaccharide in solution to be determined, which clarified the selectivity toward DNA over RNA.[76] Along the same lines, a trisaccharide scaffold bearing amino acid side chains was designed to bind to a tumor protein (i.e., MDM2). The trisaccharide was based on the critical structural elements of a peptide (i.e., p53 cellular tumor antigen) that binds to the protein MDM2 (Figure 8). The oligogalactose showed a rod-

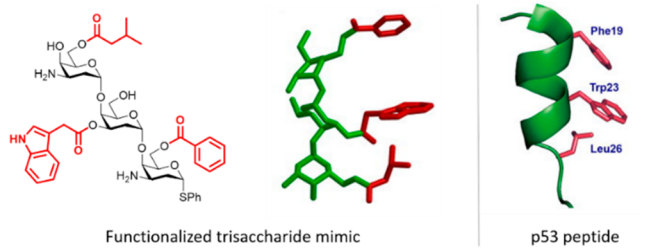


Figure 8. Functionalized trisaccharide mimic designed to resemble a p53 peptide that binds to the MDM2 protein. Adapted with permission from ref 77. Copyright 2010 Elsevier.

like shape with the amino acid side chains (i.e., Phe, Trp, and Leu) oriented on the same side of the molecule, providing a mimic of the alpha helical peptide domain. Furthermore, the amphiphilic character of the peptide was reproduced replacing some hydroxyl moieties with amines in the pyranose rings. However, despite the rational design, only a weak inhibitory activity of p53 was found, suggesting that the side chain position and conformation were still not optimal.[77] Much work remains to predict the conformation of oligosaccharides and produce mimetics with a rational structural design. However, these studies suggest that oligosaccharides could offer a valid platform for the synthesis of a tunable peptide mimetic to target specific binding sites.

Aiming to obtain stable secondary structures, a new class of peptidomimetics was developed. Carbopeptoids are oligosaccharides in which the glycosidic oxygen atom is replaced with an amido group.[78] Analogous to *S*- and *C*-glycosides, carbopeptoids are more stable toward proteolytic cleavage than their natural counterparts. These amido-linked hybrids fold into architecturally ordered geometries,[79,80] generally helical structures. Long-range NOEs in NMR and circular dichroism analysis permitted the folding process to be followed, showing that a dense intramolecular hydrogen bonding network stabilizes these structures. Molecular dynamics (MD) simulations have been intensively utilized to predict the folding process.[81−83]

The replacement of the regular glycosidic bond by an amidic linkage simplifies the synthetic strategy, eliminating the issue of stereocontrol of "standard" glycosylations. A cationic ring-opening polymerization has been used to obtain enantiopure poly-amido-saccharides (PASs).[84] Molecular models were

DOI: 10.1021/acs.biomac.9b01090
Biomacromolecules 2020, 21, 18−29

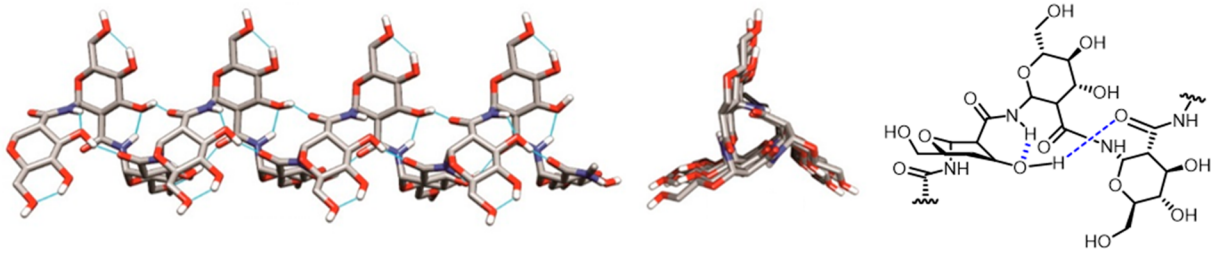


Figure 9. Predicted structure of poly-amido-saccharides based on gas phase minimization with MMFF94s (molecular models side and front views). A dense network of H-bonds stabilizes the helix. Adapted with permission from ref 84. Copyright 2012 American Chemical Society.

constructed to predict the helical structure, stabilized by several internal hydrogen bonds and by the rigidity of the pyranose-polyamide backbone (Figure 9).

A transition-metal-mediated radical reaction led to well-defined carbopeptoids with different lengths.[85] Circular dichroism revealed that these structures adopt well-extended left- or right-handed conformations, depending on the size of the chain.

Despite the quite straightforward synthesis,[85−87] carbopeptoids have not yet been extensively exploited as structural building blocks. In contrast, peptides with defined secondary structures as well as unnatural foldamers have raised considerable attention in biology and materials science.[3,4,88] We believe that a similar approach applied to carbopeptoids could fuel the formation of novel materials. In particular, the methodical insertion of sugar appendages or single-site substitutions would be a pioneering approach for structural manipulation. A systematic study has shown that the conformation of sugar-amino-acid derived foldamers could be tuned upon introduction of sugar appendages.[89] The structural changes obtained by this specific modification revealed new interactions with several biological targets.

3.2. Conformational Locks. To lower the entropy of the unfolded state, Nature has devised some tricks to stabilize protein folding (e.g., disulfide bridges). Likewise, chemists developed some techniques to stabilize synthetic molecules, such as short peptides, into bioactive structural motifs. Macrocyclization is a well-established strategy to access efficient biomimetic drugs that has been accomplished in a wide variety of manners using, for instance, disulfide-, lactam ring-, or metal-mediated bridges, hydrocarbon stapling, or hydrogen bond surrogate (HBS).[90] The "reinforced" alpha helices aim for higher biological activity and proteolytic resistance.[91,92] Successful examples of stapled peptides for *in vivo* applications as drugs have been reported.[93]

Intrinsic glycan flexibility limited the use of such strategies. However, recent works showed that short oligosaccharides can adopt stable structures in solution, influencing their binding.[16−19,94,95] Moreover, especially in plants, many examples of macrocyclic tethered oligosaccharides (e.g., tricolorin and calonyctin A) with interesting biological activities were identified.[96,97] As a consequence, artificial constrained oligosaccharides gained popularity (Figure 10). Preorganized glycans pay a lower flexibility-induced entropic cost upon binding, resulting in higher affinity constants. However, the prediction of the conformation adopted by the stapled structure is not trivial. In addition, the structural complexity of glycans posed a bottleneck in the design of stapled-glycomimetics and conformational control is hardly achieved. The first example employed a methylene bridge to mimic an intramolecular hydrogen bond present in the lectin−glycan

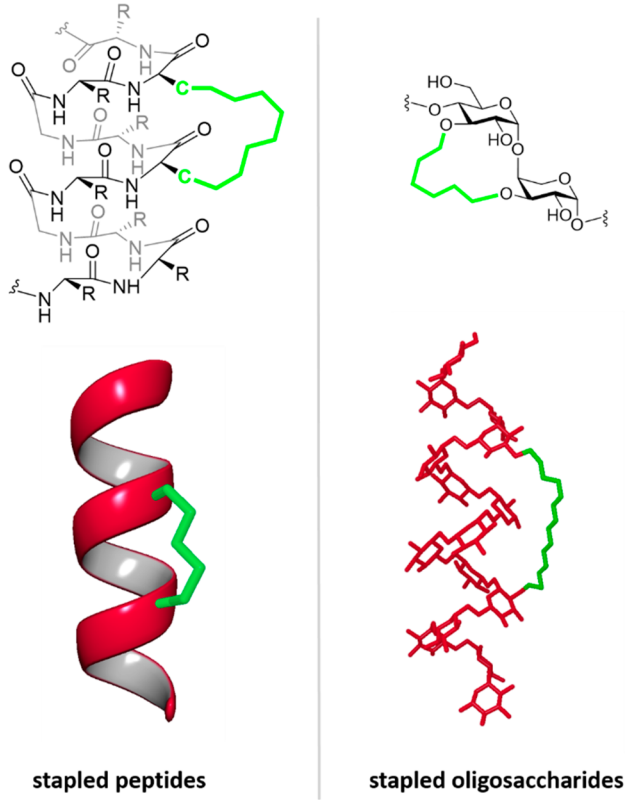


Figure 10. Hydrocarbon stapling is a common method to stabilize helical peptides (left). The same approach has been adopted to stabilize certain conformations of glycans (right).

complex. Still, the resulting locked structure showed no binding improvement.[98] Hydrocarbon stapling has been applied to oligosaccharides to lock the bioactive conformation, but moderate success has been achieved up to now.[96,99] Recent advances in MD[8] and NMR studies[44] will be important tools for the future development of conformationally constrained saccharides. Interestingly, such an approach can be used to "freeze" oligosaccharides into unnatural conformations or low populated conformational states, that can shine light on the actual receptor/bound conformation.[100]

3.3. Conformational Switches. Conformational switches are used by Nature to control DNA binding, where a ring flip of deoxyribose can induce the formation of a kink, favoring binding to proteins.[101] Similarly, modern synthetic molecular machines make use of conformational switches to perform their function.[102] In the field of oligosaccharides, this approach is reported mainly for cyclodextrins (CDs). Several synthetic methodologies have been developed, and structural features of such systems are well characterized.[96] For instance, the size

DOI: 10.1021/acs.biomac.9b01090
Biomacromolecules 2020, 21, 18−29

tuning of the cavity has been accomplished, inserting more flexible linkages between monosaccharide units in a CD mimic.[103] Photoswitchable systems have been designed where an azobenzene moiety allows for the control of the shape, cavity size, and solubility of unnatural CDs (Figure 11).[104,105]

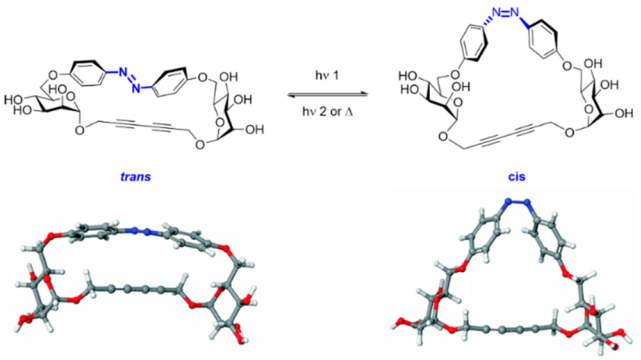


Figure 11. Photoswitchable system to mimic a CD and its energy minimized 3D model. Adapted with permission of The Royal Society of Chemistry from ref 105. Copyright 2018; permission conveyed through Copyright Clearance Center, Inc.

Translating a similar concept to oligosaccharides can be potentially used to control the conformation at the molecular level and alter binding events. So far, metal promoted chair flipping was applied to an unnatural xylopyranose unit. The installation of two amino moieties in positions 2 and 4 allowed switching between the $^4C_1$ and $^1C_4$ chair conformation, upon an external stimulus (Figure 12). The addition of a Pt(II) salt

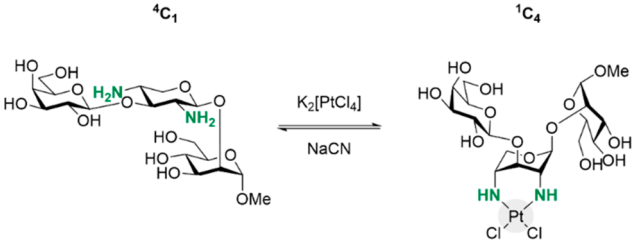


Figure 12. A trisaccharide molecular hinge triggered by addition of Pt salt.

promoted the reversible bending of a trisaccharide model.[101,106−108] Chair flipping can lead to interesting applications, in particular where the ring conformation is key for a certain biological activity (e.g., L-iduronate during binding to antithrombin).[109] Furthermore, applications in the field of carbohydrate materials can be envisioned, since the conformational switch can trigger different aggregation behaviors.

4. CONCLUSIONS AND FUTURE PERSPECTIVE

Recent advances in synthetic methodologies enabled access to novel carbohydrate analogues. Unnatural oligosaccharides show interesting properties, like resistance to enzymatic degradation and improved activity. However, the structural features that could be responsible for such behavior are often neglected. This prevents use of this knowledge for the rational design of new and more potent drugs. It became clear that a better understanding of the 3D structure could enormously help the development of new sugar drugs. To do so, three main aspects should be considered: synthesis, modeling, and analysis.

From the synthetic point of view, much effort has been put into automating oligosaccharide synthesis, with several machines now available.[110−112] Access to well-defined compounds is fundamental for structural elucidations; however, up to now, natural glycans have been the main target of synthetic chemists. We believe that unnatural modifications should receive more attention, since it was shown that collections of unnatural analogues, produced by AGA, permitted elucidation of the 3D structures of some oligosaccharides.[19] A systematic approach was followed to disrupt specific H-bonds to tune the properties of some polysaccharide materials.[21] This powerful method could shine light on the structural features that are essential for the activity of biologically relevant compounds. In this regard, deoxygenation and methylation of specific positions offer a valuable strategy to manipulate H-bond networks. Fluorinated analogues also gained popularity, since they allow for the use of NMR techniques.[42] Increasing attention is given to the synthesis of fluorinated compounds, and we envision the creation of highly fluorinated collections with completely novel geometries and activities.[47,113] Additionally, the possibility to block particular conformations (stapling) or to switch between conformations will offer a different approach to gain structure−function information.[92,114] New synthetic strategies should be developed, since only limited examples are available.

The prediction of the effect of a modification on the overall conformation remains a major challenge. New force fields, specifically optimized for carbohydrates, are now available, and software packages to perform MD simulations have become more user-friendly.[8,115] Still, predicting oligosaccharide conformations in a solvated system remains a major challenge and novel and more accurate water models are currently investigated.[116] In the past decade, the physical models used for the MD simulations have become substantially more accurate;[117] however, the lack of standards to validate the computational predictions poses a severe bottleneck. In particular, most force fields do not have parameters for modified structures and a massive effort is still needed to reach the same level of modeling available for peptides and DNA. Simple prediction tools, such as CarbBuilder, are appearing as a resource for organic chemists to guide oligosaccharide synthesis.[118,119] The impressive advancement of computational predictions for protein or DNA structures opened up new areas such as *de novo* protein design[120] and DNA[121] and protein[1,122] nanotechnology. We believe that an improvement in the computational methods, supported by the synthesis of structural probes, will fuel the field of sugar foldamers. New oligomers with known shape or folding behavior are expected, thanks to a more rational approach during the design of such molecules.

From the analytical standpoint, advanced NMR studies opened up new avenues. Labeled structures and the use of lanthanide complexes permitted breaking of the chemical shift degeneracy of carbohydrates and simplified the analysis.[123−125] Other analytical techniques, like atomic force miscroscopy (AFM)[126−129] and ion-mobility mass spectrometry (IM-MS),[130] could also provide structural information; however, their use in glyco-analysis is still in its infancy. Still, the perfect analytical technique for the visualization of structural detailed at the molecular level was not identified. A major breakthrough will be single-molecule visualization. As happened for peptides

DOI: 10.1021/acs.biomac.9b01090
Biomacromolecules 2020, 21, 18−29

and DNA, imaging techniques like AFM and scanning tunneling microscopy (STM) could offer the desired resolution to explore glycans at the molecular level. The combination of new analytical techniques with spectroscopic methods (e.g., ion-mobility IR spectroscopy[131−133] or ESI-STM[134]) could also offer interesting insight into polysaccharide aggregations, as recently reported for peptides and proteins.

The most important and difficult challenge is to combine these three aspects toward a common goal. We strongly believe that such fundamental studies will fuel the formation of tailor-made glycans with defined shape and tuned properties. We have demonstrated that modified structures are perfect substrates for 3D investigations. In analogy with peptides, we envision a future where carbohydrate foldamers will find applications in biology as well as in materials science. Tuned aggregation and self-assembling could be achieved.[20] The natural abundance of carbohydrates will offer an advantage compared to other biomolecules, and industrial applications are plenty.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: peter.seeberger@mpikg.mpg.de.
*E-mail: martina.delbianco@mpikg.mpg.de.

ORCID

Martina Delbianco: 0000-0002-4580-9597

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Author Contributions

§T.T.-E., G.F.: These authors contributed equally.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Max-Planck Society, the Minerva Fast Track Program, the MPG-FhG Cooperation Project Glyco3Dysplay, and the DFG (SFB1340 "Matrix-in Vision") for generous financial support.

REFERENCES

(1) Lapenta, F.; Aupič, J.; Strmšek, Ž.; Jerala, R. Coiled Coil Protein Origami: From Modular Design Principles towards Biotechnological Applications. *Chem. Soc. Rev.* **2018**, *47* (10), 3530−3542.

(2) Fittolani, G.; Seeberger, P. H.; Delbianco, M. Helical Polysaccharides. *Pept. Sci.* **2019**, No. e24124.

(3) Gellman, S. H. Foldamers: A Manifesto. *Acc. Chem. Res.* **1998**, *31* (4), 173−180.

(4) Guichard, G.; Huc, I. Synthetic Foldamers. *Chem. Commun.* **2011**, *47* (21), 5933.

(5) Hong, F.; Zhang, F.; Liu, Y.; Yan, H. DNA Origami: Scaffolds for Creating Higher Order Structures. *Chem. Rev.* **2017**, *117* (20), 12584−12640.

(6) McLaughlin, C. K.; Hamblin, G. D.; Sleiman, H. F. Supramolecular DNA Assembly. *Chem. Soc. Rev.* **2011**, *40* (12), 5647−5656.

(7) Luo, Q.; Hou, C.; Bai, Y.; Wang, R.; Liu, J. Protein Assembly: Versatile Approaches to Construct Highly Ordered Nanostructures. *Chem. Rev.* **2016**, *116* (22), 13571−13632.

(8) Woods, R. J. Predicting the Structures of Glycans, Glycoproteins, and Their Complexes. *Chem. Rev.* **2018**, *118* (17), 8005−8024.

(9) Wormald, M. R.; Petrescu, A. J.; Pao, Y. L.; Glithero, A.; Elliott, T.; Dwek, R. A. Conformational Studies of Oligosaccharides and Glycopeptides: Complementarity of NMR, X-Ray Crystallography, and Molecular Modelling. *Chem. Rev.* **2002**, *102* (2), 371−386.

(10) Delbianco, M.; Bharate, P.; Varela-Aramburu, S.; Seeberger, P. H. Carbohydrates in Supramolecular Chemistry. *Chem. Rev.* **2016**, *116* (4), 1693−1752.

(11) Nishiyama, Y.; Sugiyama, J.; Chanzy, H.; Langan, P. Crystal Structure and Hydrogen Bonding System in Cellulose Iα from Synchrotron X-Ray and Neutron Fiber Diffraction. *J. Am. Chem. Soc.* **2003**, *125* (47), 14300−14306.

(12) Rao, V. S.; Balaji, P. V.; Chandrasekaran, R. *Conformation of Carbohydrates*; Harwood: Amsterdam, The Netherlands, 1998.

(13) Hernandez-Cerdan, P.; Mansel, B. W.; Leis, A.; Lundin, L.; Williams, M. A. K. Structural Analysis of Polysaccharide Networks by Transmission Electron Microscopy: Comparison with Small-Angle X-Ray Scattering. *Biomacromolecules* **2018**, *19* (3), 989−995.

(14) Rohs, R.; Jin, X.; West, S. M.; Joshi, R.; Honig, B.; Mann, R. S. Origins of Specificity in Protein-DNA Recognition. *Annu. Rev. Biochem.* **2010**, *79* (1), 233−269.

(15) Guberman, M.; Seeberger, P. H. Automated Glycan Assembly: A Perspective. *J. Am. Chem. Soc.* **2019**, *141* (14), 5581−5592.

(16) Canales, A.; Mallagaray, A.; Pérez-Castells, J.; Boos, I.; Unverzagt, C.; André, S.; Gabius, H.-J.; Cañada, F. J.; Jiménez-Barbero, J. Breaking Pseudo-Symmetry in Multiantennary Complex N-Glycans Using Lanthanide-Binding Tags and NMR Pseudo-Contact Shifts. *Angew. Chem., Int. Ed.* **2013**, *52* (51), 13789−13793.

(17) Canales, A.; Boos, I.; Perkams, L.; Karst, L.; Luber, T.; Karagiannis, T.; Domínguez, G.; Cañada, F. J.; Pérez-Castells, J.; Häussinger, D.; Unverzagt, C.; Jiménez-Barbero, J. Breaking the Limits in Analyzing Carbohydrate Recognition by NMR Spectroscopy: Resolving Branch-Selective Interaction of a Tetra-Antennary N-Glycan with Lectins. *Angew. Chem., Int. Ed.* **2017**, *56* (47), 14987−14991.

(18) Fernández de Toro, B.; Peng, W.; Thompson, A. J.; Domínguez, G.; Cañada, F. J.; Pérez-Castells, J.; Paulson, J. C.; Jiménez-Barbero, J.; Canales, Á. Avenues to Characterize the Interactions of Extended N-Glycans with Proteins by NMR Spectroscopy: The Influenza Hemagglutinin Case. *Angew. Chem.* **2018**, *130* (46), 15271−15275.

(19) Delbianco, M.; Kononov, A.; Poveda, A.; Yu, Y.; Diercks, T.; Jiménez-Barbero, J.; Seeberger, P. H. Well-Defined Oligo- and Polysaccharides as Ideal Probes for Structural Studies. *J. Am. Chem. Soc.* **2018**, *140* (16), 5421−5426.

(20) Yu, Y.; Gim, S.; Kim, D.; Arnon, Z. A.; Gazit, E.; Seeberger, P. H.; Delbianco, M. Oligosaccharides Self-Assemble and Show Intrinsic Optical Properties. *J. Am. Chem. Soc.* **2019**, *141* (12), 4833−4838.

(21) Yu, Y.; Tyrikos-Ergas, T.; Zhu, Y.; Fittolani, G.; Bordoni, V.; Singhal, A.; Fair, R. J.; Grafmüller, A.; Seeberger, P. H.; Delbianco, M. Systematic Hydrogen-Bond Manipulations To Establish Polysaccharide Structure-Property Correlations. *Angew. Chem., Int. Ed.* **2019**, *58* (37), 13127−13132.

(22) Lakshminarayanan, A.; Richard, M.; Davis, B. G. Studying Glycobiology at the Single-Molecule Level. *Nat. Rev. Chem.* **2018**, *2* (8), 148−159.

(23) Nagae, M.; Kanagawa, M.; Morita-Matsumoto, K.; Hanashima, S.; Kizuka, Y.; Taniguchi, N.; Yamaguchi, Y. Atomic Visualization of a Flipped-Back Conformation of Bisected Glycans Bound to Specific Lectins. *Sci. Rep.* **2016**, *6* (1), 22973.

(24) Ernst, B.; Magnani, J. L. From Carbohydrate Leads to Glycomimetic Drugs. *Nat. Rev. Drug Discovery* **2009**, *8* (8), 661−677.

(25) Imberty, a; Delage, M. M.; Bourne, Y.; Cambillau, C.; Pérez, S. Data Bank of Three-Dimensional Structures of Disaccharides: Part II, N-Acetyllactosaminic Type N-Glycans. Comparison with the Crystal Structure of a Biantennary Octasaccharide. *Glycoconjugate J.* **1991**, *8* (6), 456−483.

(26) Booth, H.; Khedhair, K. A. Endo-Anomeric and Exo-Anomeric Effects in 2-Substituted Tetrahydropyrans. *J. Chem. Soc., Chem. Commun.* **1985**, *8* (8), 467.

(27) Perić-Hassler, L.; Hansen, H. S.; Baron, R.; Hünenberger, P. H. Conformational Properties of Glucose-Based Disaccharides Inves-

DOI: 10.1021/acs.biomac.9b01090
Biomacromolecules 2020, 21, 18−29

tigated Using Molecular Dynamics Simulations with Local Elevation Umbrella Sampling. *Carbohydr. Res.* **2010**, *345* (12), 1781−1801.
(28) Woods, R. J.; Pathiaseril, A.; Wormald, M. R.; Edge, C. J.; Dwek, R. A. The High Degree of Internal Flexibility Observed for an Oligomannose Oligosaccharide Does Not Alter the Overall Topology of the Molecule. *Eur. J. Biochem.* **1998**, *258* (2), 372−386.
(29) Tamburrini, A.; Colombo, C.; Bernardi, A. Design and Synthesis of Glycomimetics: Recent Advances. *Med. Res. Rev.* **2019**, No. med.21625, DOI: 10.1002/med.21625.
(30) Kolb, H. C.; Ernst, B. Development of Tools for the Design of Selectin Antagonists. *Chem. - Eur. J.* **1997**, *3* (10), 1571−1578.
(31) Bernardi, A.; Checchia, A.; Brocca, P.; Sonnino, S.; Zuccotto, F. Sugar Mimics: An Artificial Receptor for Cholera Toxin. *J. Am. Chem. Soc.* **1999**, *121* (10), 2032−2036.
(32) Klemm, D.; Heublein, B.; Fink, H. P.; Bohn, A. Cellulose: Fascinating Biopolymer and Sustainable Raw Material. *Angew. Chem., Int. Ed.* **2005**, *44* (22), 3358−3393.
(33) Fox, S. C.; Li, B.; Xu, D.; Edgar, K. J. Regioselective Esterification and Etherification of Cellulose: A Review. *Biomacromolecules* **2011**, *12* (6), 1956−1972.
(34) Alshanski, I.; Blaszkiewicz, J.; Mervinetsky, E.; Rademann, J.; Yitzchaik, S.; Hurevich, M. Sulfation Patterns of Saccharides and Heavy Metal Ions Binding. *Chem. - Eur. J.* **2019**, *25* (52), 12083−12090.
(35) Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. Fluorine in Medicinal Chemistry. *Chem. Soc. Rev.* **2008**, *37* (2), 320−330.
(36) Biffinger, J. C.; Kim, H. W.; DiMagno, S. G. The Polar Hydrophobicity of Fluorinated Compounds. *ChemBioChem* **2004**, *5* (5), 622−627.
(37) Salwiczek, M.; Nyakatura, E. K.; Gerling, U. I. M.; Ye, S.; Koksch, B. Fluorinated Amino Acids: Compatibility with Native Protein Structures and Effects on Protein−Protein Interactions. *Chem. Soc. Rev.* **2012**, *41* (6), 2135−2171.
(38) Woll, M. G.; Hadley, E. B.; Mecozzi, S.; Gellman, S. H. Stabilizing and Destabilizing Effects of Phenylalanine → F_5-Phenylalanine Mutations on the Folding of a Small Protein. *J. Am. Chem. Soc.* **2006**, *128* (50), 15932−15933.
(39) Lo, H.-J.; Krasnova, L.; Dey, S.; Cheng, T.; Liu, H.; Tsai, T.-I.; Wu, K. B.; Wu, C.-Y.; Wong, C.-H. Synthesis of Sialidase-Resistant Oligosaccharide and Antibody Glycoform Containing α2,6-Linked $3F^{Ax}$-Neu5Ac. *J. Am. Chem. Soc.* **2019**, *141* (16), 6484.
(40) Baumann, A.; Marchner, S.; Daum, M.; Hoffmann-Röder, A. Synthesis of Fluorinated Leishmania Cap Trisaccharides for Diagnostic Tool and Vaccine Development. *Eur. J. Org. Chem.* **2018**, *2018* (27−28), 3803−3815.
(41) Yang, F.; Zheng, X.-J.; Huo, C.-X.; Wang, Y.; Zhang, Y.; Ye, X.-S. Enhancement of the Immunogenicity of Synthetic Carbohydrate Vaccines by Chemical Modifications of STn Antigen. *ACS Chem. Biol.* **2011**, *6* (3), 252−259.
(42) Diercks, T.; Infantino, A. S.; Unione, L.; Jiménez-Barbero, J.; Oscarson, S.; Gabius, H. J. Fluorinated Carbohydrates as Lectin Ligands: Synthesis of OH/F-Substituted N-Glycan Core Trimannoside and Epitope Mapping by 2D STD-TOCSYreF NMR Spectroscopy. *Chem. - Eur. J.* **2018**, *24* (59), 15761−15765.
(43) Denavit, V.; Lainé, D.; Bouzriba, C.; Shanina, E.; Gillon, É.; Fortin, S.; Rademacher, C.; Imberty, A.; Giguère, D. Stereoselective Synthesis of Fluorinated Galactopyranosides as Potential Molecular Probes for Galactophilic Proteins: Assessment of Monofluorogalactoside−LecA Interactions. *Chem. - Eur. J.* **2019**, *25* (17), 4478−4490.
(44) Battistel, M. D.; Azurmendi, H. F.; Yu, B.; Freedberg, D. I. NMR of Glycans: Shedding New Light on Old Problems. *Prog. Nucl. Magn. Reson. Spectrosc.* **2014**, *79*, 48−68.
(45) Allman, S. A.; Jensen, H. H.; Vijayakrishnan, B.; Garnett, J. A.; Leon, E.; Liu, Y.; Anthony, D. C.; Sibson, N. R.; Feizi, T.; Matthews, S.; Davis, B. G. Potent Fluoro-Oligosaccharide Probes of Adhesion in Toxoplasmosis. *ChemBioChem* **2009**, *10* (15), 2522−2529.
(46) Denavit, V.; St-Gelais, J.; Tremblay, T.; Giguére, D. Exploring the Chemistry of Non-sticky Sugars: Synthesis of Polyfluorinated Carbohydrate Analogs of D-allopyranose. *Chem. - Eur. J.* **2019**, *25* (39), 9272−9279.
(47) Denavit, V.; Lainé, D.; St-Gelais, J.; Johnson, P. A.; Giguère, D. A Chiron Approach towards the Stereoselective Synthesis of Polyfluorinated Carbohydrates. *Nat. Commun.* **2018**, *9* (1), 4721.
(48) Ren, W.; Pengelly, R.; Farren-Dai, M.; Shamsi Kazem Abadi, S.; Oehler, V.; Akintola, O.; Draper, J.; Meanwell, M.; Chakladar, S.; Świderek, K.; Moliner, V.; Britton, R.; Gloster, T. M.; Bennet, A. J. Revealing the Mechanism for Covalent Inhibition of Glycoside Hydrolases by Carbasugars at an Atomic Level. *Nat. Commun.* **2018**, *9* (1), 3243.
(49) Bock, K.; Fernandez Bolanos Guzman, J.; Ogawa, S. A ^{1}H- and ^{13}C-n.m.r. Spectroscopic Analysis of Six Pseudohexoses. *Carbohydr. Res.* **1988**, *174*, 354−359.
(50) Arjona, O.; Gómez, A. M.; López, J. C.; Plumet, J. Synthesis and Conformational and Biological Aspects of Carbasugars. *Chem. Rev.* **2007**, *107* (5), 1919−2036.
(51) Asensio, J. L.; Cañada, F. J.; García-Herrero, A.; Murillo, M. T.; Fernández-Mayoralas, A.; Johns, B. A.; Kozak, J.; Zhu, Z.; Johnson, C. R.; Jiménez-Barbero, J. Conformational Behavior of Aza-C-Glycosides: Experimental Demonstration of the Relative Role of the Exo-Anomeric Effect and 1,3-Type Interactions in Controlling the Conformation of Regular Glycosides. *J. Am. Chem. Soc.* **1999**, *121* (49), 11318−11329.
(52) Montero, E.; García-Herrero, A.; Asensio, J. L.; Hirai, K.; Ogawa, S.; Santoyo-González, F.; Cañada, F. J.; Jiménez-Barbero, J. The Conformational Behaviour of Non-Hydrolizable Lactose Analogues: The Thioglycoside, Carbaglycoside, and Carba-Iminoglycoside Cases. *Eur. J. Org. Chem.* **2000**, *2000* (10), 1945−1952.
(53) Yuasa, H.; Hashimoto, H. Chemical Synthesis of Bioactive Oligosaccharides. Recent Advances in the Development of Unnatural Oligosaccharides. Confornration and Bioactivity. *Trends Glycosci. Glycotechnol.* **2001**, *13* (69), 31−55.
(54) Weimar, T.; Kreis, U. C.; Andrews, J. S.; Pinto, B. M. Conformational Analysis of Maltoside Heteroanalogues Using High-Quality NOE Data and Molecular Mechanics Calculations. Flexibility as a Function of the Interglycosidic Chalcogen Atom. *Carbohydr. Res.* **1999**, *315* (3−4), 222−233.
(55) Xu, B.; Unione, L.; Sardinha, J.; Wu, S.; Ethève-Quelquejeu, M.; Pilar Rauter, A.; Blériot, Y.; Zhang, Y.; Martín-Santamaría, S.; Díaz, D.; Jiménez-Barbero, J.; Sollogoub, M. Gem-Difluorocarbadisaccharides: Restoring the Exo -Anomeric Effect. *Angew. Chem., Int. Ed.* **2014**, *53* (36), 9597−9602.
(56) Koffi Teki, D. S.-E.; Bil, A.; Moreau, V.; Chagnault, V.; Fanté, B.; Adjou, A.; Kovensky, J. Synthesis of Multivalent S-Glycoside Analogs of a Heparan Sulfate Sequence. *Org. Chem. Front.* **2019**, *6* (15), 2718−2725.
(57) Baryal, K. N.; Zhu, J. Stereoselective Synthesis of S-Linked Hexasaccharide of Landomycin A via Umpolung S-Glycosylation. *Org. Lett.* **2015**, *17* (18), 4530−4533.
(58) Zou, W. C-Glycosides and Aza-C-Glycosides as Potential Glycosidase and Glycosyltransferase Inhibitors. *Curr. Top. Med. Chem.* **2005**, *5* (14), 1363−1391.
(59) Leclerc, E.; Pannecoucke, X.; Ethève-Quelquejeu, M.; Sollogoub, M. Fluoro-C-Glycosides and Fluoro-Carbasugars, Hydrolytically Stable and Synthetically Challenging Glycomimetics. *Chem. Soc. Rev.* **2013**, *42* (10), 4270−4283.
(60) Koester, D.; Holkenbrink, A.; Werz, D. Recent Advances in the Synthesis of Carbohydrate Mimetics. *Synthesis* **2010**, *2010* (19), 3217−3242.
(61) Baryal, K. N.; Zhu, J. Stereoselective Synthesis of S-Linked Hexasaccharide of Landomycin A via Umpolung S-Glycosylation. *Org. Lett.* **2015**, *17* (18), 4530−4533.
(62) Wen, P.; Větvička, V.; Crich, D. Synthesis and Evaluation of Oligomeric Thioether-Linked Carbacyclic β-(1→3)-Glucan Mimetics. *J. Org. Chem.* **2019**, *84* (9), 5554−5563.
(63) Liao, X.; Větvička, V.; Crich, D. Synthesis and Evaluation of 1,5-Dithia-D-Laminaribiose, Triose, and Tetraose as Truncated β-(1→3)-Glucan Mimetics. *J. Org. Chem.* **2018**, *83* (24), 14894−14904.

DOI: 10.1021/acs.biomac.9b01090
Biomacromolecules 2020, 21, 18−29

(64) Ferry, A.; Malik, G.; Guinchard, X.; Větvička, V.; Crich, D. Synthesis and Evaluation of Di- and Trimeric Hydroxylamine-Based β-(1→3)-Glucan Mimetics. *J. Am. Chem. Soc.* **2014**, *136* (42), 14852−14857.
(65) Sylla, B.; Legentil, L.; Saraswat-Ohri, S.; Vashishta, A.; Daniellou, R.; Wang, H. W.; Vetvicka, V.; Ferrières, V. Oligo-β-(1 → 3)-Glucans: Impact of Thio-Bridges on Immunostimulating Activities and the Development of Cancer Stem Cells. *J. Med. Chem.* **2014**, *57* (20), 8280−8292.
(66) Liang, C. F.; Kuan, T. C.; Chang, T. C.; Lin, C. C. Stereoselective Synthesis of S-Linked α(2→8) and α(2→8)/α(2→9) Hexasialic Acids. *J. Am. Chem. Soc.* **2012**, *134* (38), 16074−16079.
(67) Montero, E.; García-Herrero, A.; Asensio, J. L.; Hirai, K.; Ogawa, S.; Santoyo-González, F.; Cañada, F. J.; Jiménez-Barbero, J. The Conformational Behaviour of Non-Hydrolizable Lactose Analogues: The Thioglycoside, Carbaglycoside, and Carba-Iminoglycoside Cases. *Eur. J. Org. Chem.* **2000**, *2000* (10), 1945−1952.
(68) Espinosa, J.-F.; Cañada, F. J.; Asensio, J. L.; Martín-Pastor, M.; Dietrich, H.; Martín-Lomas, M.; Schmidt, R. R.; Jiménez-Barbero, J. Experimental Evidence of Conformational Differences between C-Glycosides and O-Glycosides in Solution and in the Protein-Bound State: The C-Lactose/O-Lactose Case. *J. Am. Chem. Soc.* **1996**, *118* (44), 10862−10871.
(69) Nilsson, U.; Johansson, R.; Magnusson, G. Synthesis, Conformational Analysis and Comparative Protein Binding of a Galabioside and Its Thioglycoside Analogues. *Chem. - Eur. J.* **1996**, *2* (3), 295−302.
(70) Koester, D. C.; Kriemen, E.; Werz, D. B. Flexible Synthesis of 2-Deoxy-C-Glycosides and (1→2)-, (1→3)-, and (1→4)-Linked C-Glycosides. *Angew. Chem., Int. Ed.* **2013**, *52* (10), 2985−2989.
(71) Espinosa, J.-F.; Bruix, M.; Jarreton, O.; Skrydstrup, T.; Beau, J.-M.; Jiménez-Barbero, J. Conformational Differences Between C- and O-Glycosides: The -C-Mannobiose/-O-Mannobiose Case. *Chem. - Eur. J.* **1999**, *5* (2), 442−448.
(72) Jiménez-Barbero, J.; Asensio, J. L.; Cañada, F. J.; Poveda, A. Free and Protein-Bound Carbohydrate Structures. *Curr. Opin. Struct. Biol.* **1999**, *9* (5), 549−555.
(73) Sánchez-Fernández, E. M.; Rísquez-Cuadro, R.; Ortiz Mellet, C.; García Fernández, J. M.; Nieto, P. M.; Angulo, J. Sp2-Iminosugar O-, S-, and N-Glycosides as Conformational Mimics of α-Linked Disaccharides; Implications for Glycosidase Inhibition. *Chem. - Eur. J.* **2012**, *18* (27), 8527−8539.
(74) Nicolaou, K. C.; Flörke, H.; Egan, M. G.; Barth, T.; Estevez, V. A. Carbonucleotoids and Carbopeptoids: New Carbohydrate Oligomers. *Tetrahedron Lett.* **1995**, *36* (11), 1775−1778.
(75) Tiwari, V. K.; Mishra, B. B.; Mishra, K. B.; Mishra, N.; Singh, A. S.; Chen, X. Cu-Catalyzed Click Reaction in Carbohydrate Chemistry. *Chem. Rev.* **2016**, *116* (5), 3086−3240.
(76) Xuereb, H.; Maletic, M.; Gildersleeve, J.; Pelczer, I.; Kahne, D. Design of an Oligosaccharide Scaffold That Binds in the Minor Groove of DNA. *J. Am. Chem. Soc.* **2000**, *122* (9), 1883−1890.
(77) Sakurai, K.; Kahne, D. Design and Synthesis of Functionalized Trisaccharides as P53-Peptide Mimics. *Tetrahedron Lett.* **2010**, *51* (29), 3724−3727.
(78) Jiménez Blanco, J. L.; Ortega-Caballero, F.; Ortiz Mellet, C.; García Fernández, J. M. (Pseudo)Amide-Linked Oligosaccharide Mimetics: Molecular Recognition and Supramolecular Properties. *Beilstein J. Org. Chem.* **2010**, *6*, 1−18.
(79) Jockusch, R. A.; Talbot, F. O.; Rogers, P. S.; Simone, M. I.; Fleet, G. W. J.; Simons, J. P. Carbohydrate Amino Acids: The Intrinsic Conformational Preference for a β-Turn-Type Structure in a Carbopeptoid Building Block. *J. Am. Chem. Soc.* **2006**, *128* (51), 16771−16777.
(80) Claridge, T. D. W.; Long, D. D.; Baker, C. M.; Odell, B.; Grant, G. H.; Edwards, A. A.; Tranter, G. E.; Fleet, G. W. J.; Smith, M. D. Helix-Forming Carbohydrate Amino Acids. *J. Org. Chem.* **2005**, *70* (6), 2082−2090.
(81) Sunkari, Y. K.; Pulukuri, K. K.; Kandiyal, P. S.; Vaishnav, J.; Ampapathi, R. S.; Chakraborty, T. K. Conformation Analysis of GalNAc-Appended Sugar Amino Acid Foldamers as Glycopeptide Mimics. *ChemBioChem* **2018**, *19* (14), 1507−1513.
(82) Chakraborty, T. K.; Srinivasu, P.; Tapadar, S.; Mohan, B. K. Sugar Amino Acids and Related Molecules: Some Recent Developments. *Proc. - Indian Acad. Sci., Chem. Sci.* **2004**, *116* (4), 187−207.
(83) Baron, R.; Bakowies, D.; van Gunsteren, W. F. Principles of Carbopeptoid Folding: A Molecular Dynamics Simulation Study. *J. Pept. Sci.* **2005**, *11* (2), 74−84.
(84) Dane, E. L.; Grinstaff, M. W. Poly-Amido-Saccharides: Synthesis via Anionic Polymerization of a β-Lactam Sugar Monomer. *J. Am. Chem. Soc.* **2012**, *134* (39), 16255−16264.
(85) Tian, G. Z.; Hu, J.; Zhang, H. X.; Rademacher, C.; Zou, X. P.; Zheng, H. N.; Xu, F.; Wang, X. L.; Linker, T.; Yin, J. Synthesis and Conformational Analysis of Linear Homo- and Heterooligomers from Novel 2-C-Branched Sugar Amino Acids (SAAs). *Sci. Rep.* **2018**, *8* (1), 4−11.
(86) Tian, G.-Z.; Wang, X.-L.; Hu, J.; Wang, X.-B.; Guo, X.-Q.; Yin, J. Recent Progress of Sugar Amino Acids: Synthetic Strategies and Applications as Glycomimetics and Peptidomimetics. *Chin. Chem. Lett.* **2015**, *26* (8), 922−930.
(87) Xiao, R.; Grinstaff, M. W. Chemical Synthesis of Polysaccharides and Polysaccharide Mimetics. *Prog. Polym. Sci.* **2017**, *74*, 78−116.
(88) Bonduelle, C. Secondary Structures of Synthetic Polypeptide Polymers. *Polym. Chem.* **2018**, *9* (13), 1517−1529.
(89) Siriwardena, A.; Pulukuri, K. K.; Kandiyal, P. S.; Roy, S.; Bande, O.; Ghosh, S.; Garcia Fernández, J. M.; Ariel Martin, F.; Ghigo, J.-M.; Beloin, C.; Ito, K.; Woods, R. J.; Ampapathi, R. S.; Chakraborty, T. K. Sugar-Modified Foldamers as Conformationally Defined and Biologically Distinct Glycopeptide Mimics. *Angew. Chem., Int. Ed.* **2013**, *52* (39), 10221−10226.
(90) Guarracino, D. A.; Riordan, J. A.; Barreto, G. M.; Oldfield, A. L.; Kouba, C. M.; Agrinsoni, D. Macrocyclic Control in Helix Mimetics. *Chem. Rev.* **2019**, *119* (17), 9915−9949.
(91) Walensky, L. D.; Bird, G. H. Hydrocarbon-Stapled Peptides: Principles, Practice, and Progress. *J. Med. Chem.* **2014**, *57* (15), 6275−6288.
(92) Lau, Y. H.; de Andrade, P.; Wu, Y.; Spring, D. R. Peptide Stapling Techniques Based on Different Macrocyclisation Chemistries. *Chem. Soc. Rev.* **2015**, *44* (1), 91−102.
(93) Walensky, L. D.; Kung, A. L.; Escher, I.; Malia, T. J.; Barbuto, S.; Wright, R. D.; Wagner, G.; Verdine, G. L.; Korsmeyer, S. J. Activation of Apoptosis in Vivo by a Hydrocarbon-Stapled BH3 Helix. *Science* **2004**, *305* (5689), 1466−1470.
(94) Zierke, M.; Smieško, M.; Rabbani, S.; Aeschbacher, T.; Cutting, B.; Allain, F. H.-T.; Schubert, M.; Ernst, B. Stabilization of Branched Oligosaccharides: Lewis x Benefits from a Nonconventional C−H···O Hydrogen Bond. *J. Am. Chem. Soc.* **2013**, *135* (36), 13464−13472.
(95) Aeschbacher, T.; Zierke, M.; Smieško, M.; Collot, M.; Mallet, J.; Ernst, B.; Allain, F. H.-T.; Schubert, M. A Secondary Structural Element in a Wide Range of Fucosylated Glycoepitopes. *Chem. - Eur. J.* **2017**, *23* (48), 11598−11610.
(96) Xie, J.; Bogliotti, N. Synthesis and Applications of Carbohydrate-Derived Macrocyclic Compounds. *Chem. Rev.* **2014**, *114* (15), 7678−7739.
(97) Rencurosi, A.; Mitchell, E. P.; Cioci, G.; Pérez, S.; Pereda-Miranda, R.; Imberty, A. Crystal Structure of Tricolorin A: Molecular Rationale for the Biological Properties of Resin Glycosides Found in Some Mexican Herbal Remedies. *Angew. Chem.* **2004**, *116* (44), 6044−6048.
(98) Navarre, N.; van Oijen, A. H.; Boons, G. J. The Design and Synthesis of a Conformationally Constrained Trisaccharide for Probing Carbohydrate-Protein Interactions. *Tetrahedron Lett.* **1997**, *38* (11), 2023−2026.
(99) Rodriguez, J.; O'Neill, S.; Walczak, M. A. Constrained Saccharides: A Review of Structure, Biology, and Synthesis. *Nat. Prod. Rep.* **2018**, *35* (3), 220−229.

DOI: 10.1021/acs.biomac.9b01090
Biomacromolecules 2020, 21, 18−29

(100) Geyer, A.; Müller, M.; Schmidt, R. R. A Glycosidic Linkage Constrained to the "Anti" Conformation. *J. Am. Chem. Soc.* **1999**, *121* (26), 6312−6313.
(101) Yuasa, H. Ring Flip of Carbohydrates: Functions and Applications. *Trends Glycosci. Glycotechnol.* **2006**, *18* (104), 353−370.
(102) Erbas-Cakmak, S.; Leigh, D. A.; McTernan, C. T.; Nussbaumer, A. L. Artificial Molecular Machines. *Chem. Rev.* **2015**, *115* (18), 10081−10206.
(103) Pathigoolla, A.; Sureshan, K. M. Reverse-CD Mimics with Flexible Linkages Offer Adaptable Cavity Sizes for Guest Encapsulation. *Chem. Commun.* **2014**, *50* (3), 317−319.
(104) Despras, G.; Hain, J.; Jaeschke, S. O. Photocontrol over Molecular Shape: Synthesis and Photochemical Evaluation of Glycoazobenzene Macrocycles. *Chem. - Eur. J.* **2017**, *23* (45), 10838−10847.
(105) Hain, J.; Despras, G. A Two-Step Approach to a Glycoazobenzene Macrocycle with Remarkable Photoswitchable Features. *Chem. Commun.* **2018**, *54* (62), 8563−8566.
(106) Yuasa, H.; Hashimoto, H. Bending Trisaccharides by a Chelation-Induced Ring Flip of a Hinge-Like Monosaccharide Unit. *J. Am. Chem. Soc.* **1999**, *121* (21), 5089−5090.
(107) Yuasa, H.; Miyagawa, N.; Izumi, T.; Nakatani, M.; Izumi, M.; Hashimoto, H. Hinge Sugar as a Movable Component of an Excimer Fluorescence Sensor. *Org. Lett.* **2004**, *6* (9), 1489−1492.
(108) Izumi, T.; Hashimoto, H.; Yuasa, H. Switching Extended 1,3-Diequatorial and Bent 1,3-Diaxial States of a Disubstituted Hinge Sugar by Ligand Exchange Reactions on Pt(Ii)Electronic Supplementary Information (ESI) Available: Experimental Procedures and Full Characterization of the Reported Co. *Chem. Commun.* **2004**, *0* (1), 94.
(109) Das, S. K.; Mallet, J.-M.; Esnault, J.; Driguez, P.-A.; Duchaussoy, P.; Sizun, P.; Hérault, J.-P.; Herbert, J.-M.; Petitou, M.; Sinaÿ, P. Synthesis of Conformationally Locked Carbohydrates: A Skew-Boat Conformation Of L-Iduronic Acid Governs the Antithrombotic Activity of Heparin. *Angew. Chem., Int. Ed.* **2001**, *40* (9), 1670−1673.
(110) Panza, M.; Pistorio, S. G.; Stine, K. J.; Demchenko, A. V. Automated Chemical Oligosaccharide Synthesis: Novel Approach to Traditional Challenges. *Chem. Rev.* **2018**, *118* (17), 8105−8150.
(111) Li, T.; Liu, L.; Wei, N.; Yang, J.-Y.; Chapla, D. G.; Moremen, K. W.; Boons, G.-J. An Automated Platform for the Enzyme-Mediated Assembly of Complex Oligosaccharides. *Nat. Chem.* **2019**, *11* (3), 229−236.
(112) Krasnova, L.; Wong, C.-H. Oligosaccharide Synthesis and Translational Innovation. *J. Am. Chem. Soc.* **2019**, *141* (9), 3735−3754.
(113) Uhrig, M. L.; Lantaño, B.; Postigo, A. Synthetic Strategies for Fluorination of Carbohydrates. *Org. Biomol. Chem.* **2019**, *17* (21), 5173−5189.
(114) Beharry, A. A.; Woolley, G. A. Azobenzene Photoswitches for Biomolecules. *Chem. Soc. Rev.* **2011**, *40* (8), 4422.
(115) Woods, R. J.; Dwek, R. A.; Edge, C. J.; Fraser-Reid, B. Molecular Mechanical and Molecular Dynamic Simulations of Glycoproteins and Oligosaccharides. 1. GLYCAM_93 Parameter Development. *J. Phys. Chem.* **1995**, *99* (11), 3832−3846.
(116) Mahoney, M. W.; Jorgensen, W. L. Diffusion Constant of the TIP5P Model of Liquid Water. *J. Chem. Phys.* **2001**, *114* (1), 363−366.
(117) Dirks, R. M.; Shaw, D. E.; Dror, R. O.; Grossman, J. P.; Xu, H. Biomolecular Simulation: A Computational Microscope for Molecular Biology. *Annu. Rev. Biophys.* **2012**, *41* (1), 429−452.
(118) Cheng, C. W.; Zhou, Y.; Pan, W. H.; Dey, S.; Wu, C. Y.; Hsu, W. L.; Wong, C. H. Hierarchical and Programmable One-Pot Synthesis of Oligosaccharides. *Nat. Commun.* **2018**, *9* (1), 1−9.
(119) Kuttel, M. M.; Ståhle, J.; Widmalm, G. CarbBuilder: Software for Building Molecular Models of Complex Oligo- and Polysaccharide Structures. *J. Comput. Chem.* **2016**, *37*, 2098−2105.
(120) Huang, P.-S.; Boyken, S. E.; Baker, D. The Coming of Age of de Novo Protein Design. *Nature* **2016**, *537* (7620), 320−327.
(121) Veneziano, R.; Ratanalert, S.; Zhang, K.; Zhang, F.; Yan, H.; Chiu, W.; Bathe, M. Designer Nanoscale DNA Assemblies Programmed from the Top Down. *Science* **2016**, *352* (6293), 1534.
(122) Luo, Q.; Hou, C.; Bai, Y.; Wang, R.; Liu, J. Protein Assembly: Versatile Approaches to Construct Highly Ordered Nanostructures. *Chem. Rev.* **2016**, *116* (22), 13571−13632.
(123) Canales, Á.; Mallagaray, Á.; Berbís, M. Á.; Navarro-Vázquez, A.; Domínguez, G.; Cañada, F. J.; André, S.; Gabius, H.-J.; Pérez-Castells, J.; Jiménez-Barbero, J. Lanthanide-Chelating Carbohydrate Conjugates Are Useful Tools To Characterize Carbohydrate Conformation in Solution and Sensitive Sensors to Detect Carbohydrate−Protein Interactions. *J. Am. Chem. Soc.* **2014**, *136* (22), 8011−8017.
(124) Canales, A.; Mallagaray, A.; Pérez-Castells, J.; Boos, I.; Unverzagt, C.; André, S.; Gabius, H.-J.; Cañada, F. J.; Jiménez-Barbero, J. Breaking Pseudo-Symmetry in Multiantennary Complex N-Glycans Using Lanthanide-Binding Tags and NMR Pseudo-Contact Shifts. *Angew. Chem., Int. Ed.* **2013**, *52* (51), 13789−13793.
(125) Fernández de Toro, B.; Peng, W.; Thompson, A. J.; Domínguez, G.; Cañada, F. J.; Pérez-Castells, J.; Paulson, J. C.; Jiménez-Barbero, J.; Canales, Á. Avenues to Characterize the Interactions of Extended N-Glycans with Proteins by NMR Spectroscopy: The Influenza Hemagglutinin Case. *Angew. Chem.* **2018**, *130* (46), 15271−15275.
(126) Schefer, L.; Adamcik, J.; Mezzenga, R. Unravelling Secondary Structure Changes on Individual Anionic Polysaccharide Chains by Atomic Force Microscopy. *Angew. Chem., Int. Ed.* **2014**, *53* (21), 5376−5379.
(127) Schefer, L.; Adamcik, J.; Diener, M.; Mezzenga, R. Supramolecular Chiral Self-Assembly and Supercoiling Behavior of Carrageenans at Varying Salt Conditions. *Nanoscale* **2015**, *7* (39), 16182−16188.
(128) Schefer, L.; Usov, I.; Mezzenga, R. Anomalous Stiffening and Ion-Induced Coil-Helix Transition of Carrageenans under Monovalent Salt Conditions. *Biomacromolecules* **2015**, *16* (3), 985−991.
(129) Diener, M.; Adamcik, J.; Sánchez-Ferrer, A.; Jaedig, F.; Schefer, L.; Mezzenga, R. Primary, Secondary, Tertiary and Quaternary Structure Levels in Linear Polysaccharides: From Random Coil, to Single Helix to Supramolecular Assembly. *Biomacromolecules* **2019**, *20* (4), 1731−1739.
(130) 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.
(131) Seo, J.; Hoffmann, W.; Warnke, S.; Huang, X.; Gewinner, S.; Schöllkopf, W.; Bowers, M. T.; von Helden, G.; Pagel, K. An Infrared Spectroscopy Approach to Follow β-Sheet Formation in Peptide Amyloid Assemblies. *Nat. Chem.* **2017**, *9* (1), 39−44.
(132) Seo, J.; Hoffmann, W.; Warnke, S.; Bowers, M. T.; Pagel, K.; von Helden, G. Retention of Native Protein Structures in the Absence of Solvent: A Coupled Ion Mobility and Spectroscopic Study. *Angew. Chem., Int. Ed.* **2016**, *55* (45), 14173−14176.
(133) Seo, J.; Jang, J.; Warnke, S.; Gewinner, S.; Schöllkopf, W.; von Helden, G. Stacking Geometries of Early Protoporphyrin IX Aggregates Revealed by Gas-Phase Infrared Spectroscopy. *J. Am. Chem. Soc.* **2016**, *138* (50), 16315−16321.
(134) Abb, S.; Tarrat, N.; Cortés, J.; Andriyevsky, B.; Harnau, L.; Schön, J. C.; Rauschenbach, S.; Kern, K. Carbohydrate Self-Assembly at Surfaces: STM Imaging of Sucrose Conformation and Ordering on Cu(100). *Angew. Chem.* **2019**, *131* (25), 8424−8428.

DOI: 10.1021/acs.biomac.9b01090
Biomacromolecules 2020, 21, 18−29