Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Automated glycan assembly as an enabling technology

Alonso Pardo-Vargas[1], Martina Delbianco[1] and Peter H Seeberger[1,2]

Access to complex carbohydrates remains a limiting factor for the development of the glycosciences. Automated glycan assembly (AGA) has accelerated and simplified the synthetic process and, with the first commercially available instrument and building blocks, glycan synthesis can now be practiced by any chemist. All classes of glycans, including sulfated or sialylated carbohydrates and polysaccharides as long as 50mers are now accessible owing to optimized reaction conditions and new methodologies. These synthetic glycans have helped to understand many biological functions and to advance diagnostic and vaccine development. Establishing detailed structure–function relationships will eventually enable the production of unnatural materials with tuned properties.

Addresses
[1] Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, Am Mühlenberg 1, 14476 Potsdam, Germany
[2] Freie Universität Berlin, Institute of Chemistry and Biochemistry, Arnimallee 22, 14195 Berlin, Germany

Corresponding author: Seeberger, Peter H (peter.seeberger@mpikg.mpg.de)

Current Opinion in Chemical Biology 2018, **46**:48–55

This review comes from a themed issue on **Synthetic biomolecules**

Edited by **Richard J. Payne** and **Nicolas Winssinger**

For a complete overview see the Issue and the Editorial

Available online 30th April 2018

https://doi.org/10.1016/j.cbpa.2018.04.007

Introduction

Carbohydrates are the most abundant biopolymers on earth [1], and their complexity is evident in the variety of important roles they play, which include structural definition, energy storage, and regulation of host–pathogen interactions. Nevertheless many of these biological functions remain largely descriptive or unknown. In contrast, other biopolymers such as oligonucleotides (DNA and RNA) and proteins are well understood at the molecular level. For these biopolymers, automated sequencing and synthesis fuel rapid progress in the development of molecular biology tools. The easy manipulation of polynucleotides in concert with gene expression technologies, made pure proteins accessible, thereby advancing proteomics and genomics [2].

Molecular glycobiology is still in its infancy. Access to structurally diverse and complex carbohydrate structures is either achieved by extraction from natural sources or by chemical synthesis. Several monosaccharides serve as building blocks, forming branched structures, with each glycosidic linkage creating a new stereogenic center. Glycan diversity renders purification from natural sources very challenging and not always possible. Chemical synthesis is frequently the only way to obtain pure glycans. The total synthesis of glycans is based on the stereoselective installation of glycosidic linkages and protecting group manipulations [3–5]. These protocols have improved to the point where complex structures, such as a 92 unit arabinogalactan, can be prepared on a milligram scale [6]. Total synthesis is labor intensive, and often requires months or even years to complete [7]. For the development of molecular glycobiology and material science, pure natural and non-natural structures are required before structure–function relationships can be established. The development of reliable automated glycan assembly (AGA) technologies summarized here forms the basis for a better understanding of glycans and for the development of molecular glycobiology.

Solution-phase methods

Most solution-based methods, aiming to accelerate the synthetic process by reducing the number of purification steps in between, are not yet fully automated and still remain labor intensive. The semi-automated computer-based one-pot synthesis is based on the sequential addition of building blocks (BBs) according to the difference in their reactivity calculated by the OptiMer software [8]. This conceptually attractive methodology requires many different monosaccharide, disaccharide and trisaccharide BBs. Another one-pot method is based on the anodic oxidation of the glycosyl donor in the presence of tetrabutylammonium triflate (Bu_4NOTf) in an electrolysis cell to generate the corresponding triflate donor. The thioglycoside acceptor is then added and, upon glycosylation, the oxidation process is repeated [9•]. An instrument was developed to control temperature, delivery of reagents, and the electrical potential of the reaction cell. DFT calculations are performed prior synthesis to estimate the oxidation potentials of the building blocks. Recently, the synthesis of the GPI anchor core trisaccharide [10] and a TMG-chitotriomycin precursor [11] were reported.

Another method to simplify purification uses a fluorous tag linker that is coupled to the desired glycan. After every step, deprotection and chain elongation, the tagged

compound is separated from the reaction mixture by fluorous solid-phase extraction (FSPE). A robot can handle the solutions autonomously [12]. Linear and branched β-oligomannosides were synthesized using a β-directing C5 participation strategy [13,14]. Recently, automated fluorous-assisted synthesis using hypervalent iodonium as glycosylation promoter at ambient temperatures permitted the synthesis of a β(1,6)-glucan tetramer [15]. A renewable benzyl-type fluorous tag was prepared to reduce costs [16]. To date, FSPE techniques was mainly applied to the synthesis of relatively short oligomers rather than long polysaccharides. Similar to fluorous tag methodology, the hydrophobically assisted switching phase method (HASP) uses a hydrophobic tag to simplify the separation of the desired oligosaccharide from the reaction mixture. This strategy was illustrated in the context of a nonamannoside synthesis [17].

Solid-phase methods

Solid-phase oligosaccharide synthesis has been fully automated. The Glyconeer 2.1^{TM} was the result of a development process starting from an adapted peptide synthesizer via multiple home-built systems [18,19•,20] (Figure 1a and 1b). With this Glyconeer 2.1^{TM}, the desired oligosaccharide is assembled using repeating cycles of glycosylation, capping and deprotection steps on a polystyrene Merrifield resin (Figure 1c). Excess reagents are removed by simple filtration.

Linker

The glycan is attached to the solid support via a linker that is cleaved upon completion of the assembly, affording the target compound. The linker has to tolerate the reaction conditions during the assembly process and permit the easy liberation of the final product. Following metathesis labile linkers [21,22] and several base-labile linkers [23–25], the photocleaveble linkers were developed for their orthogonality to a wide range of reaction conditions (Figure 1d). Photocleavable linkers are stable in both acidic and basic conditions and compatible with wide variety of protecting groups including Nap, Fmoc, Lev, Bn and Bz. Linker **3** affords conjugation-ready glycans [26]. Traceless linker **4** affords a free reducing end after the final deprotection [27]. The current challenge involves further development of the photocleavage efficiency, currently around 60–70%, to improve overall yields for AGA.

AGA coupling cycle

The glycosylation reaction is the key step during AGA. A set of 'approved' building blocks was developed and many BBs are now commercially available [20]. For each 'approved' BB, key reaction parameters, such as activator, reaction temperature, and equivalents of BB per cycle were optimized and reported. In order to minimize the formation of deletion sequences, a large excess of sugar donor (ten equiv. per coupling step over two glycosylation cycles) was traditionally used to drive the reaction to completion [20]. Optimized reaction temperature and concentration permit the completion of the reaction with only one glycosylation cycle (five to eight equiv. of BB) [28••].

To further improve the coupling cycle, a capping step was introduced to block any unreacted acceptor in less than 30 min by acetylation using methanesulfonic acid and acetic anhydride [29••]. Capping is compatible with all the protecting groups used to date, increases the isolated yield of the desired compound and facilitates the purification of the reaction mixture by reducing the amount of side-products. The capping step was tested for the assembly of a polymannoside (50mer) resulting in a four-fold yield increase (20% yield) while reducing the amount of building block used by 33% [29••]. Capping is now incorporated in the standard coupling cycle.

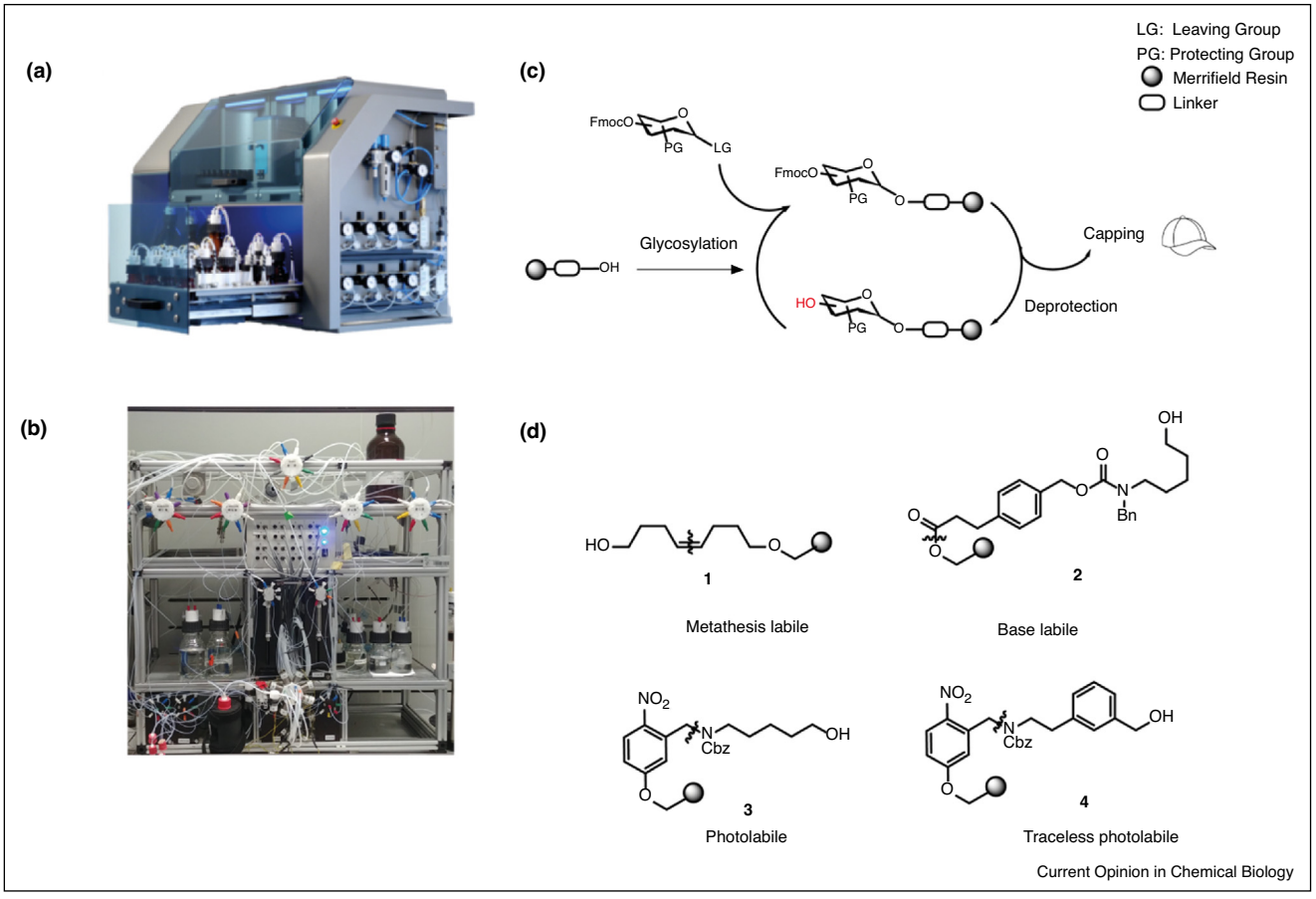
Synthetic improvements

AGA had been mainly used to synthesize *trans*-glycosidic linkages, where the C2-participating protecting group ensures stereoselective couplings. Since stereocontrol during *cis*-glycosylations cannot rely on C2-participation, anomeric mixtures are normally observed. The use of a particular mannuronic acid donor, together with activation at low temperature, has permitted the challenging synthesis of mannuronic acid alginate oligomers, with up to twelve *cis*-linkages [30]. More recently, oligosaccharides containing multiple *cis*-glycosidic linkages were prepared efficiently by AGA using monosaccharide BBs equipped with acetyl or benzoyl esters as remote participating protecting groups [31••] (Figure 2). Nine biologically important structures containing *cis*-galactosidic and *cis*-glucosidic linkages were assembled.

Access to sialylated glycans remains challenging. In particular, glycosylation with *N*-acetyl-neuraminic acid (Neu5Ac) results in low yields and anomeric mixtures due to low reactivity and lack of stereo control. The attempt to produce α(2−6) and α(2−3) sialylated glycans by AGA using sialyl-monomer was successful, albeit low yielding [32]. A combination where the glycan backbone is obtained by AGA and further functionalized with sialyltransferase (PmST1) permitted easy access to five (2,3)-sialylated glycans [33] (Figure 2).

Glycosaminoglycans (GAGs) are an important class of negatively charged glycans and one of the most challenging targets for carbohydrate chemists. The first successful AGA of hyaluronic acid (HA) oligomers was reported using a disaccharide BB [34]. In addition, most GAGs structures are highly sulfated, posing additional challenges to the synthesis of such compounds. Chondroitin sulfate GAGs with different sulfation patterns were prepared using a glucuronic acid BB and two *N*-acetylgalactosamine BBs equipped with two temporary

Figure 1



(a) The commercial Glyconeer 2.1™ AGA synthesizer, **(b)** home-built AGA synthesizer, **(c)** overview of the coupling cycle, **(d)** examples of the different linkers used for AGA.

protecting groups. The Fmoc group was used for chain elongation and the orthogonal levulinoyl (Lev) esters marked the hydroxyl groups for sulfation. Fully protected chondroitin-6-sulfate and chondroitin-4-sulfate hexasaccharides were synthesized [26,35]. Keratan sulfates (KS) containing β(1-4) galactose, β(1-3) *N*-acetyl-galactosamine and different sulfation patterns were obtained following a similar approach. Three different temporary protecting groups were exploited: Nap and Lev esters for subsequent sulfation and Fmoc for chain elongation. The orthogonality of the protecting groups permitted the synthesis of four differently sulfated KS tetrasaccharides from a common tetrasaccharide precursor [36•] (Figure 2).

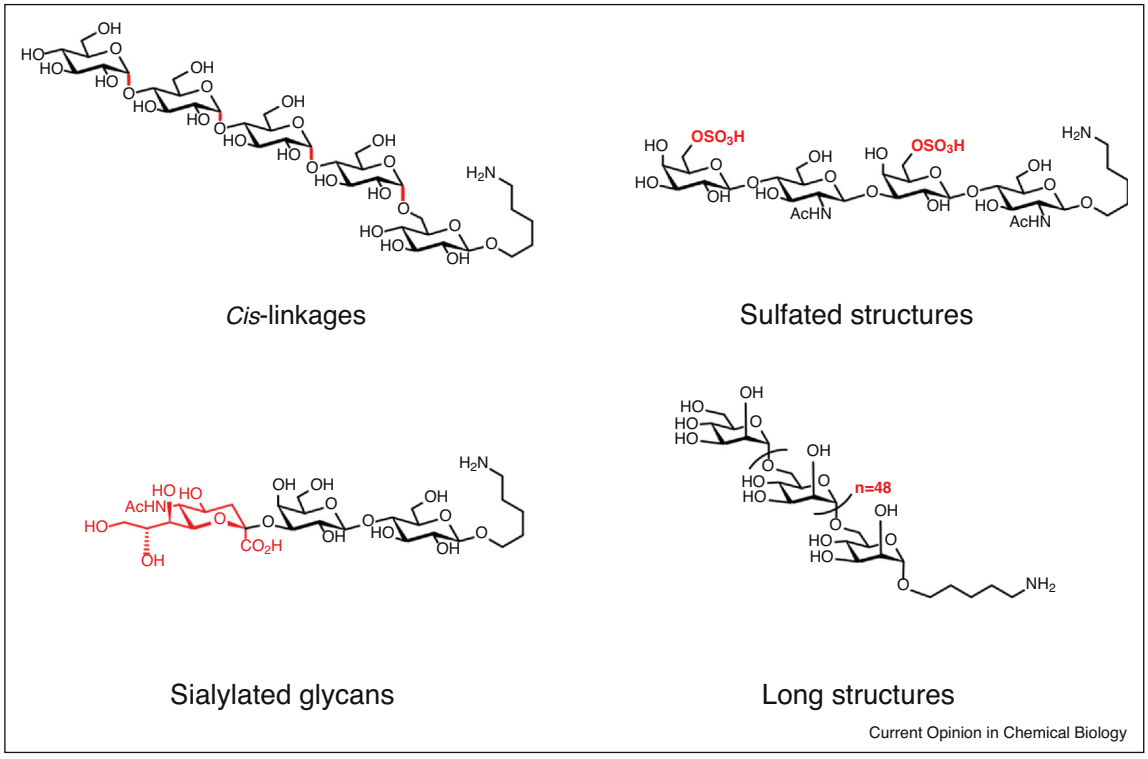
Most of the previously described advancements were applied to the synthesis of oligosaccharides associated with blood group determinants [37]. Those are tumor-associated carbohydrate antigens (TACAs) and cancer vaccine candidates, also associated with immunodeficiency disorders, atherosclerosis, and Guillain–Barré syndrome. *cis*-Linkage formation, sulfation as well as the use of orthogonal temporary protective groups permitted the fast assembly of biologically relevant glycans including H-type I (3) and II (4) glycans.

Instrumentation

Fifteen years since the first home-built synthesizer, finally, the commercial Glyconeer 2.1™ has been placed in several laboratories [19•]. The combination of this instrument with standardized purifications and quality-control techniques for the easy access to synthetic glycans has fueled the development of glycoscience [19•,38,39]. The introduction of a novel cyanopivaloyl (Piv-CN) directing/protecting group into a rhamnose BB was key to the assembly of 16mer oligorhamnans on the Glyconeer 2.1™ [40].

Efforts to develop an inexpensive synthesis instrument employed a modified HPLC [41,42]. A column packed with the solid support is used as a reactor. A JandaJel resin functionalized with a base labile linker serves as the glycosyl acceptor. HPLC pumps are used to deliver the glycosyl donor (glucosyl imidate BB, five-ten equivalents) and the deprotection solution. The promotor is

Figure 2



Recent AGA milestones.

delivered through the autosampler. The synthesis is monitored using the integrated UV detector set at 254 nm. This simple set up has been limited to the production of a pentaglucoside so far. Temperature control throughout the synthesis and delivery lines for reagents will be key points to be address for further development of this system.

Applications of glycans assembled by AGA

Glycan arrays

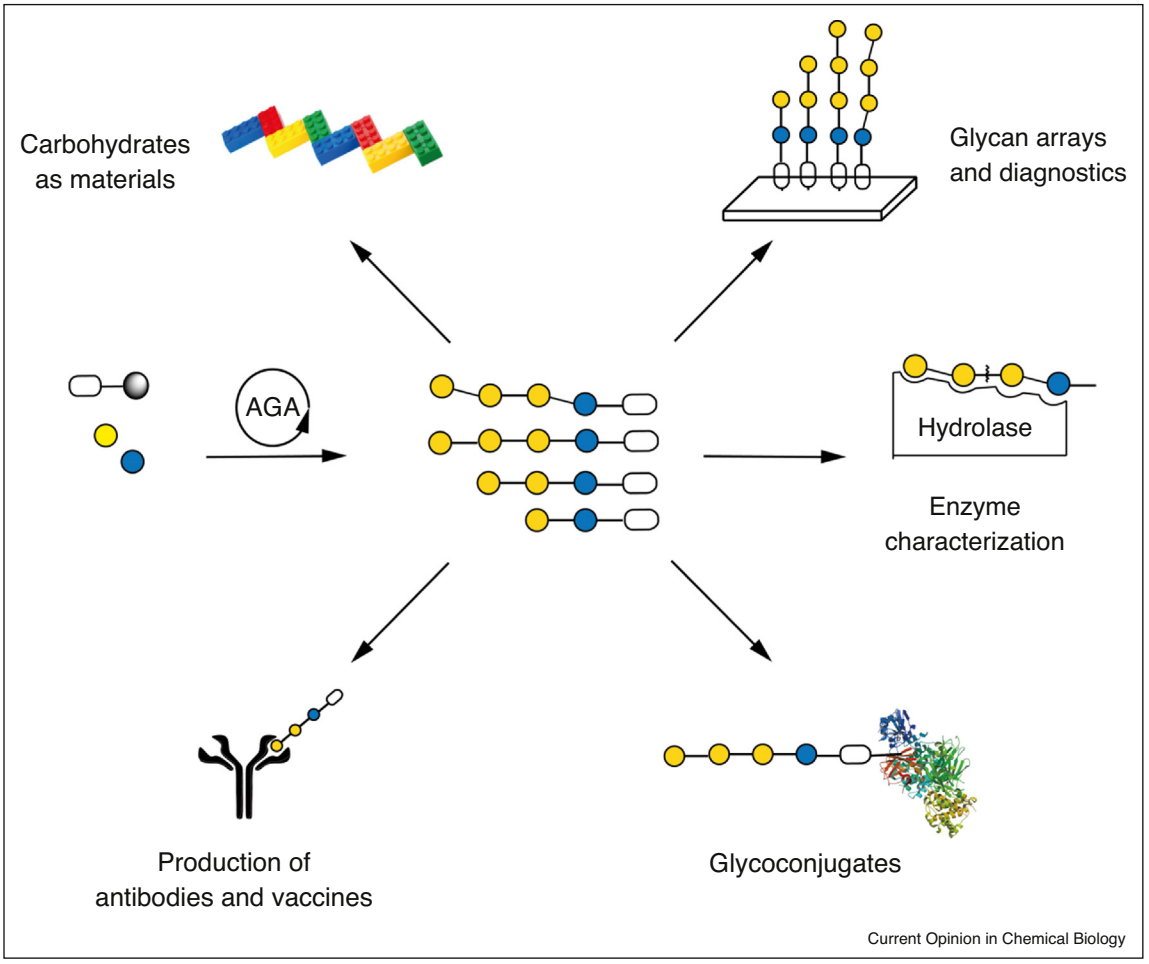
Glycan arrays are standard tools for the high throughput analysis of protein-carbohydrate interactions [43,44]. Antibodies, associated with diseases, can be detected in biological samples on glycan microarrays [43,45]. Access to pure glycans for covalent attachment to a surface of the array remains as limiting factor. AGA is the ideal tool for the creation of large collections of related compounds, permitting the study of glycans through a medicinal chemistry approach (Figure 3). Conjugation-ready linear β(1,3)-glucan and branched β(1,3)β(1,6)-glucan oligosaccharides were assembled and immobilized on microarrays. Incubation with human sera revealed that most individuals create antibodies that bind to protective linear glucan, but not to the non-protective branched analogues [46]. Synthetic keratan sulfate analogues with different sulfation patterns obtained with AGA were printed on microarrays. Specific interaction between the disulfated KS tetrasaccharide and the adeno-associated virus AAVrh10 gene-therapy vector were observed [36•].

AGA was used to create glycans for determining the binding epitopes of a large number of plant cell-wall glycan-directed mAbs using a microarray [47–49]. This information provided a tool for *in situ* cell wall labeling studies to gain detailed information on cell wall polysaccharides in the model organism *Brachypodium distachyon* [50]. The binding specificities for 25 mAbs that recognize galactosylated XG structures were determined using the same approach [51].

Enzyme substrate determination

The oligosaccharide fragments of plant glycans assembled by AGA were used to characterize the enzymes involved in biosynthesis (Figure 3). Plant arabinogalactan, glucan and arabinoxylan oligosaccharides were assembled to study the substrate specificity of β(1,4)-endogalactanases [52], lichenase [53], and to map the active site of GH10 and GH11 xylanase [54]. These findings have implications for the structural analyses of pectic polysaccharides. This plants oligosaccharide toolbox is currently applied to the characterization of biosynthetic enzymes and arabinogalactan-directed and arabinan-directed antibodies [55].

Figure 3



Applications of glycans synthesized using AGA.

Vaccine development

Glycoconjugates containing synthetic oligosaccharides attached to a carrier protein are attractive candidates as semisynthetic vaccines against infectious diseases (Figure 3). Semisynthetic oligosaccharides, resembling the capsular polysaccharides from *Streptococcus Pneumonia* ST8, assembled with AGA, were combined with the marketed polysaccharide-based pneumococcal vaccine Prevnar 13® while retaining the immunogenicity of both components [56]. Synthetic antigens from ST3, prepared by AGA, are tested as vaccine candidates [57].

Materials chemistry

The synthesis of 50mer oligomannosides (Figure 2), the longest polysaccharides assembled from monomers, set the stage to use AGA for the investigations of carbohydrates as materials [29••,58]. In the attempt to expand the use of AGA to the synthesis of different polysaccharides, well-defined oligosaccharides and polysaccharides, resembling natural as well as unnatural structures, were prepared. Those are ideal probes for the fundamental study of glycans [28••]. Molecular dynamics simulations and NMR analysis suggested that different classes of polysaccharides adopt fundamentally different conformations. Single-site substitutions alter such conformations, granting the ability to tune the shape and properties (i.e. solubility) of such compounds (Figure 3).

Perspectives

Over the past two decades AGA has evolved from humble beginnings to a reliable synthesizer, with optimized reaction conditions, and commercially available building blocks that have made the rapid synthesis of complex oligosaccharides a reality [19•,20]. Several orthogonal protecting groups are routinely used, permitting the fast formation of linear, as well as branched, structures. Even though challenging *cis*-linkages and structures such as charged polysaccharides are now accessible, some linkages (e.g. β-mannose) as well as the stability of sulfates during the deprotection process remain synthetic hurdles.

So far, these recent developments (*cis*-linkages and sulfated structures) remained confined to short oligomers, with only few examples extending past hexamers. The next important step will be the implementation of such methodologies to access long polysaccharides, expanding the complexity of synthetic glycans. Additionally, as longer polysaccharides can be assembled, new challenges arise, such as the formation of insoluble compounds and/or aggregates after global deprotection [28••]. The detailed structural analysis of defined oligosaccharides and polysaccharides will advance the synthesis. Specific substitution aiming to alter the structure and properties of these compounds will be systematically studied using AGA. These achievements, together with improvements in quality control and purifications techniques, will have an enormous impact in glycoscience [38].

With an expanding collection of pure and well-defined glycans it will be possible to shed light on new aspects of glycobiology such as carbohydrate–protein interactions as well as to aid the development and characterization of new enzymes and antibodies. The development of new diagnostics and vaccines is being enabled by AGA. Multivalent systems, so far limited to simple monosaccharides or disaccharides [59], can now benefit from AGA to encompass more complex structures to better resemble the biological environment. AGA will grant access to new carbohydrate-based materials dissecting fundamental interactions in polysaccharides. In the future, we envision the development of unnatural compounds with tuned properties. With the availability of AGA technology to synthesize glycans, analogous to that for DNA and peptides, we expect the opening up of new possibilities and applications for carbohydrates.

Conflict of interest statement

P.H.S. declares a significant financial interest in GlycoUniverse GmbH & Co KGaA the company that commercializes the synthesis instrument, building blocks and other reagents.

Acknowledgements

We thank the Max Planck Society and EU ITN Marie-Curie program (IMMUNOSHAPE – Grant No. 642870) for generous financial support.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Varki A, Cummings R, Esko J, Freeze H, Stanley P, Hart G, Seeberger PH: *Essentials of Glycobiology*. edn 3rd. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2017.

2. Tyers M, Mann M: **From genomics to proteomics**. *Nature* 2003, **422**:193-197.

3. Werz DB: **Chemical synthesis of carbohydrates and their surface immobilization: a brief introduction**. In *Carbohydrate Microarrays: Methods and Protocols.* Edited by Chevolot Y. Humana Press; 2012:13-29.

4. Crich D: **Mechanism of a chemical glycosylation reaction**. *Acc Chem Res* 2010, **43**:1144-1153.

5. Mydock LK, Demchenko AV: **Mechanism of chemical O-glycosylation: from early studies to recent discoveries**. *Org Biomol Chem* 2010, **8**:497-510.

6. Wu Y, Xiong DC, Chen SC, Wang YS, Ye XS: **Total synthesis of mycobacterial arabinogalactan containing 92 monosaccharide units**. *Nat Commun* 2017, **8**:14851.

7. Zhu X, Schmidt RR: **New principles for glycoside-bond formation**. *Angew Chem Int Ed Engl* 2009, **48**:1900-1934.

8. Wu C-Y, Wong C-H: **Automated programmable one-pot synthesis of glycans**. In *Glycoscience: Biology and Medicine.* Edited by Taniguchi N, Endo T, Hart GW, Seeberger PH, Wong C-H. Springer Japan; 2015:45-52.

9. • Nokami T, Hayashi R, Saigusa Y, Shimizu A, Liu CY, Mong KK, Yoshida J: **Automated solution-phase synthesis of oligosaccharides via iterative electrochemical assembly of thioglycosides**. *Org Lett* 2013, **15**:4520-4523.
Automated one pot solution phase synthesis based on the anodic oxidation.

10. Manmode S, Sato T, Sasaki N, Notsu I, Hayase S, Nokami T, Itoh T: **Rational optimization of the mannoside building block for automated electrochemical assembly of the core trisaccharide of GPI anchor oligosaccharides**. *Carbohydr Res* 2017, **450**:44-48.

11. Nokami T, Isoda Y, Sasaki N, Takaiso A, Hayase S, Itoh T, Hayashi R, Shimizu A, Yoshida J: **Automated electrochemical assembly of the protected potential TMG-chitotriomycin precursor based on rational optimization of the carbohydrate building block**. *Org Lett* 2015, **17**:1525-1528.

12. Tang SL, Pohl NL: **Automated solution-phase synthesis of beta-1,4-mannuronate and beta-1,4-mannan**. *Org Lett* 2015, **17**:2642-2645.

13. Tang SL, Pohl NLB: **Automated fluorous-assisted solution-phase synthesis of beta-1,2-, 1,3-, and 1,6-mannan oligomers**. *Carbohydr Res* 2016, **430**:8-15.

14. Tang SL, Linz LB, Bonning BC, Pohl NL: **Automated solution-phase synthesis of insect glycans to probe the binding affinity of pea enation mosaic virus**. *J Org Chem* 2015, **80**:10482-10489.

15. Saliba RC, Wooke ZJ, Nieves GA, Chu A-HA, Bennett CS, Pohl NLB: **Challenges in the conversion of manual processes to machine-assisted syntheses: activation of thioglycoside donors with aryl(trifluoroethyl)iodonium triflimide**. *Org Lett* 2018, **20**:800-803.

16. Chai YH, Feng YL, Wu JJ, Deng CQ, Liu AY, Zhang Q: **Recyclable benzyl-type fluorous tags: preparation and application in oligosaccharide synthesis**. *Chin Chem Lett* 2017, **28**:1693-1700.

17. Meng S, Tian T, Wang YH, Meng XB, Li ZJ: **Convergent synthesis of oligosaccharides on the gram-scale using cetyl thioglycoside based on a hydrophobically assisted switching phase method**. *Org Biomol Chem* 2016, **14**:7722-7730.

18. Plante OJ, Palmacci ER, Seeberger PH: **Automated solid-phase synthesis of oligosaccharides**. *Science* 2001, **291**:1523-1527.

19. • Hahm HS, Schlegel MK, Hurevich M, Eller S, Schuhmacher F, Hofmann J, Pagel K, Seeberger PH: **Automated glycan assembly using the Glyconeer 2.1 synthesizer**. *Proc Natl Acad Sci U S A* 2017, **114**:E3385-E3389.
First application of a commercially available synthesiser for AGA, in combination with standardized purification and quality control methods.

20. Seeberger PH: **The logic of automated glycan assembly**. *Acc Chem Res* 2015, **48**:1450-1463.

21. Routenberg Love K, Seeberger PH: **Automated solid-phase synthesis of protected tumor-associated antigen and blood group determinant oligosaccharides**. *Angew Chem Int Ed Engl* 2004, **43**:602-605.

22. de Jong AR, Volbeda AG, Hagen B, van den Elst H, Overkleeft HS, van der Marel GA, Codée JDC: **A second-generation tandem ring-closing metathesis cleavable linker for solid-phase oligosaccharide synthesis**. *Eur J Org Chem* 2013, **2013**:6644-6655.

23. Krock L, Esposito D, Castagner B, Wang CC, Bindschadler P, Seeberger PH: **Streamlined access to conjugation-ready glycans by automated synthesis**. *Chem Sci* 2012, **3**:1617-1622.

24. Wu X, Grathwohl M, Schmidt RR: **A new phenoxyacetate-based linker system for the solid-phase synthesis of oligosaccharides**. *Org Lett* 2001, **3**:747-750.

25. Czechura P, Guedes N, Kopitzki S, Vazquez N, Martin-Lomas M, Reichardt N-C: **A new linker for solid-phase synthesis of heparan sulfate precursors by sequential assembly of monosaccharide building blocks**. *Chem Commun* 2011, **47**:2390-2392.

26. Eller S, Collot M, Yin J, Hahm HS, Seeberger PH: **Automated solid-phase synthesis of chondroitin sulfate glycosaminoglycans**. *Angew Chem Int Ed Engl* 2013, **52**:5858-5861.

27. Wilsdorf M, Schmidt D, Bartetzko MP, Dallabernardina P, Schuhmacher F, Seeberger PH, Pfrengle F: **A traceless photocleavable linker for the automated glycan assembly of carbohydrates with free reducing ends**. *Chem Commun* 2016, **52**:10187-10189.

28. •• Delbianco M, Kononov A, Poveda A, Yu Y, Diercks T, Jiménez-Barbero J, Seeberger PH: **Well-defined oligo- and polysaccharides as ideal probes for structural studies**. *J Am Chem Soc* 2018 http://dx.doi.org/10.1021/jacs.8b00254.

AGA is used to produce well-defined oligosaccharides for structural investigation of glycans, towards the production of carbohydrate-based materials.

29. •• Yu Y, Kononov A, Delbianco M, Seeberger PH: **A capping step during automated glycan assembly enables access to complex glycans in high yield**. *Chem Eur J* 2018, **24**:6075-6078.

The introduction of a new capping step in the AGA cycle permitted the synthesis of a 50mer in 22% yield, while decreasing the amount of building block and easing purification.

30. Walvoort MTC, Elst Hvd, Plante OJ, Kröck L, Seeberger PH, Overkleeft HS, Marel GAvd, Codée JDC: **Automated solid-phase synthesis of β-mannuronic acid alginates**. *Angew Chem Int Ed* 2012, **51**:4393-4396.

31. •• Hahm HS, Hurevich M, Seeberger PH: **Automated assembly of oligosaccharides containing multiple cis-glycosidic linkages**. *Nat Commun* 2016, **7**:12482.

Synthesis of *cis*-glycosidic linkages using building blocks equipped with remote participating protecting groups enables access to new compounds by AGA.

32. Lai CH, Hahm HS, Liang CF, Seeberger PH: **Automated solid-phase synthesis of oligosaccharides containing sialic acids**. *Beilstein J Org Chem* 2015, **11**:617-621.

33. Fair RJ, Hahm HS, Seeberger PH: **Combination of automated solid-phase and enzymatic oligosaccharide synthesis provides access to alpha(2,3)-sialylated glycans**. *Chem Commun* 2015, **51**:6183-6185.

34. Walvoort MTC, Volbeda AG, Reintjens NRM, van den Elst H, Plante OJ, Overkleeft HS, van der Marel GA, Codée JDC: **Automated solid-phase synthesis of hyaluronan oligosaccharides**. *Org Lett* 2012, **14**:3776-3779.

35. Liang C-F, Hahm HS, Seeberger PH: **Automated synthesis of chondroitin sulfate oligosaccharides**. In *Glycosaminoglycans: Chemistry and Biology*. Edited by Balagurunathan K, Nakato H, Desai UR. New York: Springer; 2015:3-10.

36. • Hahm HS, Broecker F, Kawasaki F, Mietzsch M, Heilbronn R, Fukuda M, Seeberger PH: **Automated glycan assembly of oligo-N-acetyllactosamine and keratan sulfate probes to study virus-glycan interactions**. *Chemistry* 2017, **2**:114-124.

The use of multiple orthogonal protecting groups permitted the synthesis of a collection of glycans with different sulfation patterns.

37. Hahm HS, Liang CF, Lai CH, Fair RJ, Schuhmacher F, Seeberger PH: **Automated glycan assembly of complex oligosaccharides related to blood group determinants**. *J Org Chem* 2016, **81**:5866-5877.

38. Hofmann J, Hahm HS, Seeberger PH, Pagel K: **Identification of carbohydrate anomers using ion mobility-mass spectrometry**. *Nature* 2015, **526**:241-244.

39. Nagy G, Peng T, Pohl NLB: **Recent liquid chromatographic approaches and developments for the separation and purification of carbohydrates**. *Anal Methods* 2017, **9**:3579-3593.

40. Geert Volbeda A, van Mechelen J, Meeuwenoord N, Overkleeft HS, van der Marel GA, Codee JDC: **Cyanopivaloyl ester in the automated solid-phase synthesis of oligorhamnans**. *J Org Chem* 2017, **82**:12992-13002.

41. Pistorio SG, Nigudkar SS, Stine KJ, Demchenko AV: **HPLC-assisted automated oligosaccharide synthesis: implementation of the autosampler as a mode of the reagent delivery**. *J Org Chem* 2016, **81**:8796-8805.

42. Ganesh NV, Fujikawa K, Tan YH, Stine KJ, Demchenko AV: **HPLC-assisted automated oligosaccharide synthesis**. *Org Lett* 2012, **14**:3036-3039.

43. Broecker F, Seeberger PH: **Synthetic glycan microarrays**. *Methods Mol Biol* 2017, **1518**:227-240.

44. Park S, Gildersleeve JC, Blixt O, Shin I: **Carbohydrate microarrays**. *Chem Soc Rev* 2013, **42**:4310-4326.

45. Seeberger PH: **Glycan arrays and other tools produced by automated glycan assembly**. *Perspect Sci* 2017, **11**:11-17.

46. Weishaupt MW, Hahm HS, Geissner A, Seeberger PH: **Automated glycan assembly of branched beta-(1,3)-glucans to identify antibody epitopes**. *Chem Commun* 2017, **53**:3591-3594.

47. Schmidt D, Schuhmacher F, Geissner A, Seeberger PH, Pfrengle F: **Automated synthesis of arabinoxylan-oligosaccharides enables characterization of antibodies that recognize plant cell wall glycans**. *Chem Eur J* 2015, **21**:5709-5713.

48. Dallabernardina P, Schuhmacher F, Seeberger PH, Pfrengle F: **Automated glycan assembly of xyloglucan oligosaccharides**. *Org Biomol Chem* 2016, **14**:309-313.

49. Bartetzko MP, Schuhmacher F, Hahm HS, Seeberger PH, Pfrengle F: **Automated glycan assembly of oligosaccharides related to arabinogalactan proteins**. *Org Lett* 2015, **17**:4344-4347.

50. Ruprecht C, Bartetzko MP, Senf D, Dallabernadina P, Boos I, Andersen MCF, Kotake T, Knox JP, Hahn MG, Clausen MH *et al.*: **A synthetic glycan microarray enables epitope mapping of plant cell wall glycan-directed antibodies**. *Plant Physiol* 2017, **175**:1094-1104.

51. Dallabernardina P, Ruprecht C, Smith PJ, Hahn MG, Urbanowicz BR, Pfrengle F: **Automated glycan assembly of galactosylated xyloglucan oligosaccharides and their recognition by plant cell wall glycan-directed antibodies**. *Org Biomol Chem* 2017, **15**:9996-10000.

52. Bartetzko MP, Schuhmacher F, Seeberger PH, Pfrengle F: **Determining substrate specificities of beta1,4-endogalactanases using plant arabinogalactan oligosaccharides synthesized by automated glycan assembly**. *J Org Chem* 2017, **82**:1842-1850.

53. Dallabernardina P, Schuhmacher F, Seeberger PH, Pfrengle F: **Mixed-linkage glucan oligosaccharides produced by automated glycan assembly serve as tools to determine the substrate specificity of lichenase**. *Chem Eur J* 2017, **23**:3191-3196.

54. Senf D, Ruprecht C, de Kruijff GH, Simonetti SO, Schuhmacher F, Seeberger PH, Pfrengle F: **Active site mapping of xylan-deconstructing enzymes with arabinoxylan oligosaccharides produced by automated glycan assembly**. *Chem Eur J* 2017, **23**:3197-3205.

55. Pfrengle F: **Synthetic plant glycans**. *Curr Opin Chem Biol* 2017, **40**:145-151.

56. Schumann B, Hahm HS, Parameswarappa SG, Reppe K, Wahlbrink A, Govindan S, Kaplonek P, Pirofski LA, Witzenrath M, Anish C *et al.*: **A semisynthetic *Streptococcus pneumoniae* serotype 8 glycoconjugate vaccine**. *Sci Transl Med* 2017, **9**.

57. Weishaupt MW, Matthies S, Hurevich M, Pereira CL, Hahm HS, Seeberger PH: **Automated glycan assembly of a *S. pneumoniae* serotype 3 CPS antigen**. *Beilstein J Org Chem* 2016, **12**:1440-1446.

58. Naresh K, Schumacher F, Hahm HS, Seeberger PH: **Pushing the limits of automated glycan assembly: synthesis of a 50mer polymannoside**. *Chem Commun* 2017, **53**:9085-9088.

59. Delbianco M, Bharate P, Varela-Aramburu S, Seeberger PH: **Carbohydrates in supramolecular chemistry**. *Chem Rev* 2016, **116**:1693-1752.