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**

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