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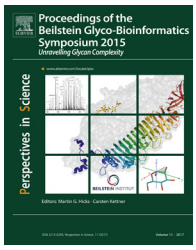

Glycan arrays and other tools produced by automated glycan assembly☆

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Summary Carbohydrates are the dominant biopolymer on earth and play important roles ranging from building material for plants to function in many biological systems. Glycans remain poorly studied due to a lack of synthetic tools. The goal of my laboratory has been to develop a general method for the automated assembly of glycans. The general protocols we developed resulted in the commercialisation of the Glyconeer 2.1™ synthesizer as well as the building blocks and all reagents. Oligosaccharides as long as 50-mers are now accessible within days. Rapid access to defined oligosaccharides has been the foundation to many applications including synthetic tools such as glycan microarrays, glycan nanoparticles and anti-glycan antibodies. The platform technology is helping to address real-life problems by the creation of new vaccines and diagnostics. After addressing mainly mammalian glycobiology earlier, material science and plant biology are benefitting increasingly from synthetic glycans.

Automated glycan assembly from concept to commercialisation

Automated access to peptides and oligonucleotides fundamentally changed the way structure-function studies could be addressed. While the synthesis of oligopeptides (Merrifield and Stewart, 1965) or oligonucleotides (Caruthers, 1985) was a tremendous challenge until the 1970s, solid-phase synthesis methods in concert with improved methods of separation provided access to defined oligomers. Synthetic oligonucleotides were the basis for amplification by polymerase chain reaction (PCR). Analogs of

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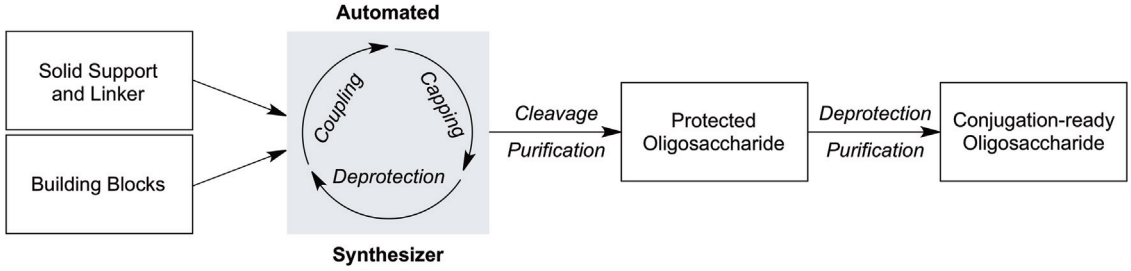

Fig. 1 The automated glycan assembly process. (Reprinted from Seeberger, 2015).

oligopeptides and oligonucleotides proved useful for applications in the medical chemistry and materials applications.

Establishing structure—function relationships in the glycosciences is virtually impossible without pure glycans. Access to pure glycans has been extremely difficult since no amplification methods exist while purification is always challenging and sometimes impossible. Molecular tools are required to advance the fundamental glycosciences and milligram quantities of such glycans have to be accessed by chemical synthesis.

Inspired by the concepts of solid-phase peptide and oligonucleotide synthesis, the automated glycan assembly process relies on a solid support equipped with a linker that is used to install one building block after another using an automated synthesizer (Fig. 1) (Plante et al., 2001; Seeberger 2015). Under the solid-phase synthesis paradigm excess building block is used for mass action to drive reactions to completion. Excess reagents can be readily removed by washing the resin with solvent. High coupling yields and stereoselectivity are important to be able to access pure oligosaccharides using only one purifucation step at the end of the synthesis.

The center-piece of automated glycan assembly is the instrument where the entire assembly process is performed under computer control. Since 2014, a commercial system, the Glyconeer 2.1™ is available and has been installed in several locations world-wide (Fig. 2) (www.glycouniverse.de).

Controlled by a computer program that executes modules of commands the liquid handling in the Glyconeer 2.1™ is managed by valves through which the reagents and solvents flow driven by inert gas pressure. The building blocks are stored in a carousel whereas other reagents reside in reservoirs. The jacketed glass reaction vessel contains the polymeric resin on top of a glass frit. Reaction temperatures can be adjusted from −50 to 50 °C. The effluent following glycosylations can be collected to recycle unreacted building blocks. The reaction efficiency of glycosylations is monitored after removal of Fmoc protecting groups using a UV sensor that is also used in peptide synthesizers. One monosaccharide building block after another is added to the polymer-bound chain using coupling cycles that consist of glycosylation, capping and removal of the temporary protecting group steps.

Automated glycan assembly can provide access to long carbohydrate chains as demonstrated for a 30mer α-(1,6)-oligomannoside as a proof-of-principle (Calin et al., 2013). Mannosyl phosphate building block **1** carries permanent

Fig. 2 The Glyconeer 2.1™—the first dedicated automated oligosaccharide synthesizer.

benzoyl protecting groups that ensure the formation of trans-glycosidic linkages and can be readily removed with base. The temporary C6 fluorenylmethoxycarbonyl protection of the hydroxyl group is readily removed by piperidine. Merrifield resin equipped with photolabile *o*-nitrobenzyl alcohol linker served as solid support for the automated syntheses. α-(1,6)-Oligomannosides ranging in length from disaccharide **3** to 30mer **8** were prepared using this automated method (Scheme 1).

Glycosaminoglycans (GAGs) are structurally diverse macromolecules that are usually located in the extracellular matrix and are essential for many fundamental cellular processes. These acidic, negatively charged polysaccharides transduce extracellular signals to the interior of the cell. GAGs are highly variable in size, ranging from 20 to 200 disaccharide repeating units, backbone composition, and the degree and pattern of sulfation. Chondroitin sulfate contains *N*-acetyl-β-D-galactosamine and β-D-glucuronic acid and sulfation and acetylation of particular hydroxyl and amino groups vary. Two chondroitin sulfate hexasaccharides served as targets to illustrate that automated glycan assembly can be used to procure this class of molecules quickly. Two differentially-protected galactosamine (GalNAc) and