Carbohydrate Research 402 (2015) 124–132

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Minireview

Recent progress in the field of glycoconjugates

Carina I. C. Crucho [a,*], Paula Correia-da-Silva [b], Krasimira T. Petrova [a], M. Teresa Barros [a,*]

[a] REQUIMTE, CQFB, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[b] Instituto Superior de Ciências da Saúde Egas Moniz, 2829-511 Caparica, Portugal

ARTICLE INFO

Article history:
Received 21 April 2014
Received in revised form 7 August 2014
Accepted 10 October 2014
Available online 20 October 2014

Keywords:
Glycoconjugates
Glycobiology
Carbohydrates

ABSTRACT

The ubiquity of glycoconjugates in nature and their role in different biological processes, has led to the development of several methodologies to synthesize these molecules. Synthetic glycoconjugates are now used to answer a variety of glycoconjugate-related biological questions and have provided new potential vaccines against cancer, viral, and bacterial infections and new biotechnological tools. This review aims to collect and compile the recent advances in the field of glycopeptides, glycoproteins, and glycolipid synthesis and also to update the previous reviews made on this subject. Finally, by highlighting the successes and failures of past research, we hope that this review will inspire fruitful research in this important medicinal chemistry field.

1. Introduction

Glycoconjugates are very important compounds in biology. They consist of carbohydrates of varying size and complexity, covalently linked to non-sugar moieties such as proteins, peptides, and lipids. Advances in glycobiology highlight the sophisticated functions served by naturally occurring glycoconjugates in different biological processes.[1] Especially the sugar portions have been found to play an integral role in specific recognition events between cells and as factors controlling the generation of biological phenomena.[2] Glycoconjugate structures are often very complex and many specific biological messages can be encoded on a single saccharide. Therefore, synthetic methods for the preparation of well-defined scaffolds are excellent tools to probe the natural glycoconjugate roles in different biological processes.[2] Understanding the influence of structural features is relevant not only to glycobiology, but also to molecular and cellular biology, proteomics, and medicine, because disorders in these processes lead to severe diseases.[1] On the other hand, biomimetic approaches for the molecular design and synthesis of glycoconjugates are based on the expectation of generating new polymeric materials that possess unique properties with similar functions or even superior to those encountered in natural glycoconjugates.

2. Glycoconjugate synthesis

Due to the presence of multiple functional groups, carbohydrates can assemble into both linear and branch polymer structures. The monomeric units can be coupled by two stereochemical linkages (α and β) and each monosaccharide can exist in two tautomeric forms (pyranose and furanose) (Fig. 1). In addition, the complexity can be further compounded by functionalization on any of the hydroxyl groups (methylation, sulfation, phosphorylation, or acylation). Traditional synthesis of saccharides requires tedious and time-consuming protection/deprotection steps and stereocontrol in each glycosylation reaction.[3] Masking of the hydroxyl groups thus needs to ensure not only the chemoselectivity of the reactions, but also the stereoselectivity in the formation of glycosides.

Typically, two major strategies can be envisioned for the synthesis of glycoconjugates.[4] The first is the formation of the glycan–aglycone link early by assembly of protected or partially protected glycosylated building blocks (Fig. 2A). The second is a convergent approach in which the required fragments or building blocks are each built independently and the link is created later on in the synthesis (Fig. 2B). Given the instability of the glycosidic bond and the need for protected building blocks, the latter approach is the more attractive one.

Here we present the last developments on glycoconjugate synthesis, which have provided access to highly complex polyglycosylated structures in the last decade.

* Corresponding authors. Tel.: +351 212948355; fax: +351 2948550.
E-mail addresses: cic17468@campus.fct.unl.pt (C.I.C. Crucho), mtb@fct.unl.pt (M.T. Barros).

http://dx.doi.org/10.1016/j.carres.2014.10.005
0008-6215/

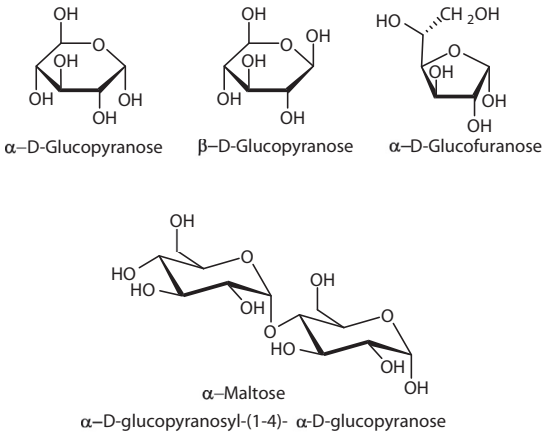

Figure 1. Combinatorial possibilities of carbohydrates. Three isomers of D-glucose and one of the 73 possible disaccharides that can be formed from two D-glucose units.

2.1. Glycopeptides and glycoproteins

Broadly speaking, carbohydrates are linked to polypeptide chains either by an amide linkage on asparagine (N-linked), or, the more diverse O-linked glycopeptides whereby a saccharide is linked to the hydroxyl of serine, threonine, or tyrosine by an ether linkage (Fig. 3). Central to any glycopeptide/glycoprotein synthetic strategy is the formation of a glycosidic bond, to covalently connect a sugar molecule to another group, which may be another sugar, an oligopeptide or a protein. A variety of methods have been developed for the preparation of glycan–peptide linkages as well as several assembly strategies, which has been comprehensively reviewed.[5]

Glycopeptides and glycoproteins with well-defined carbohydrate moieties (glycans) have been prepared by several strategies. The first is the linear assembly of glycosylated amino acids by conventional solid-phase peptide synthesis (SPPS).[6,7] However, peptides larger than 50 residues are difficult to obtain due to poor yields and accumulating by-products from incomplete couplings and epimerization.[7] Clearly this size limitation has hampered the development of SPPS for the total synthesis of long glycopeptides and glycoproteins. To overcome these difficulties in linear SPPS, the convergent condensation of unprotected or partially protected peptide/glycopeptide building blocks have been applied as an alternative strategy. However, this strategy suffers occasionally from the acid and base lability of the glycosidic bond, particularly in the conditions required for final deprotection of individual amino acid protecting groups and release of peptide from the resin.[8] On the other hand, O-glycosylation is often plagued by

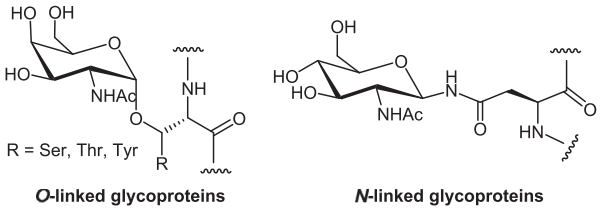

Figure 3. Examples of O-linked and N-linked glycosides.

low yields due to bulky glycans and low solubility of the peptides under chemical glycosylation conditions.[7] Another strategy is the direct coupling between a glycosylamine and an aspartic acid containing peptide.[9,10] The major advantages of this approach are its convergence and that the protecting group manipulations are kept to a minimum since they are performed on smaller fragments. Nevertheless, potential problems of this approach are the efficiency of the key coupling step due to steric hindrance between the large oligosaccharide and peptide components and for *N*-glycopeptides an unwanted side reaction occurs due to formation of intramolecular aspartimides.[10] The first problem has been solved by applying more potent coupling reagents, and the second problem by fine-tuning of the coupling conditions.[11]

Nowadays, chemical synthesis seems to be a very reliable way to obtain homogeneous glycopeptides or glycoproteins.[12,13] Chemoselective ligation strategies, as well as the use of 'click' conjugation techniques, emerged as an alternative to 'classical' glycosylation approaches.[14] These required protected sugar units, strictly anhydrous conditions, and the presence of promoters or metal-based catalysts. In sharp contrast, chemoselective glycosylation techniques allow the efficient conjugation of sugars with unprotected aglycons in aqueous media. The success of this method is attributed to the glycosyl donor, which has a potential leaving group that also temporarily protects the anomeric center, avoiding protecting group manipulations. However, the major concern of this approach is the number of available protecting groups suitable for the chemoselective sequential glycosylation strategy. By far, the most efficient means of constructing glycopeptides and glycoproteins by chemical synthesis is native chemical ligation (NCL).[5,12] In NCL methodology, a chemoselective reaction between N-terminal cysteine residue on one peptide and a C-terminal thioester of another, yields a thioester intermediate that undergoes spontaneous irreversible N → S acyl shift to form the thermodynamically favored native amide bond. NCL is carried out in mild reaction conditions, in aqueous media, and in the absence of protecting groups. One limitation of NCL is the requirement for the N-terminal cysteine residue. However, to overcome this drawback, removable auxiliaries which can act as cysteine surrogates have been used.[12] A second limitation is the inherent instability of

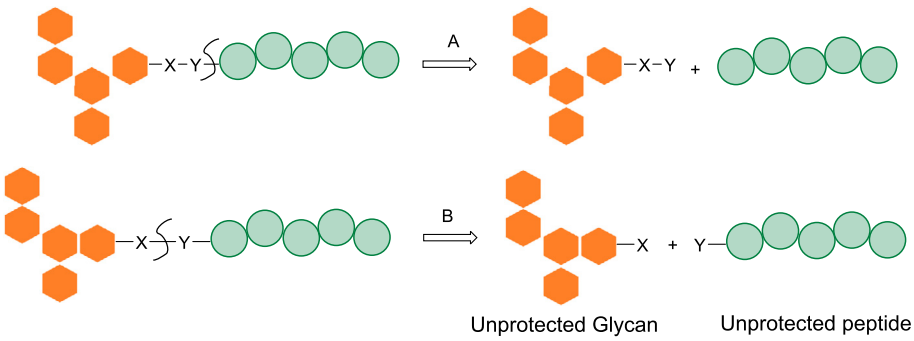

Figure 2. Disconnective analysis for glycopeptide/glycoprotein synthesis.