CHEMICAL SYNTHESIS OF HOMOGENEOUS GLYCOPROTEINS

Reported by Bradley Nakamura April 7th, 2015

INTRODUCTION

Glycoproteins have been considered impractical targets for total synthesis due to their complexity and the belief that other recombinant protein expression systems have greater synthetic potential.[1] However, many therapeutically relevant targets including Erythropoietin (EPO) are naturally expressed as a mixture of isomers with varying glycosylation patterns which have been shown to affect biologic activity (Figure 1).[2] Attempts to express and purify these mixtures have proven in many cases impossible with conventional techniques, and as a result glycoproteins are most often sold and studied as heterogeneous mixtures. Techniques for the selective production of homogenous glycoforms are important not only for determining structure-function relationships, but also for tuning activity in a way analogous to small molecule therapeutics. Recent advances have positioned total synthesis as a potent method for accessing and studying homogenous wild type and mutant versions of these valuable therapeutic targets including the total synthesis of homogeneous EPO by the Danishefsky group.[3]

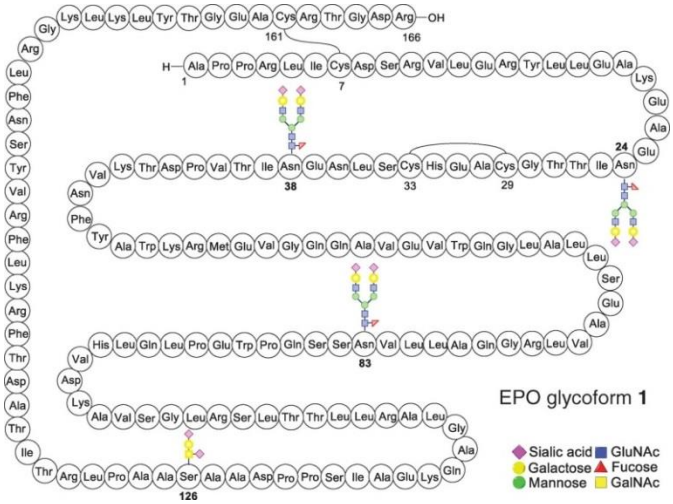
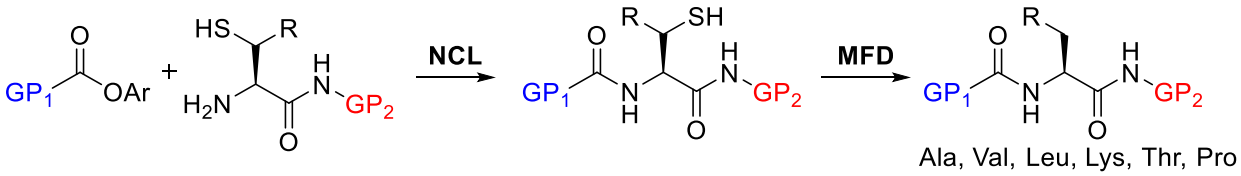


Figure 1. Erythropoietin glycoform[3]

NEW STRATEGIES FOR LINKING PEPTIDE UNITS

Since the advent of Solid Phase Peptide Synthesis (SPPS) it has been possible to rapidly synthesize peptides up to about 50 amino acids in an iterative manner, but longer chains lose efficiency in elongation.[4] The development of native chemical ligation (NCL) allowed linkage of polypeptides at cysteine residues, and extended the size of synthetic peptides achievable to around 150 residues (Scheme 1).[5] Unfortunately not all polypeptides feature appropriately placed cysteines for linking together subunits, but new methods allow the conversion of cysteine-like residues to valine, leucine, alanine and other residues using metal free desulfurization (MFD) after NCL has taken place.

These advances set the stage for preparing selectively glycosylated fragments by SPPS and linking them wherever necessary without dependence on the presence of certain amino acid sequences.



Scheme 1. Native chemical ligation and metal free desulfurization allows the linkage of glycoproteins without natural cysteins.[6]

CONSTRUCTION AND ATTACHMENT OF GLYCANS

Even with access to many polypeptide targets enabled by MFD methods, synthesizing and appending the correct glycans site selectively remains an important challenge. More than 40 different sugar amino acid linkages have been observed and maintaining the correct stereochemistry and linkage of these glycans is critical to studying and tuning their function. Previously glycosylated amino acids were incorporated into larger chains in a sequential pathway, but this route can greatly decrease yields in further elongation, and lead to issues of insolubility. The development of the Lansbury Aspartylation allowed the ability to selectively aspartylate within an elongated chain leading to a convergent sequence.[7] However, in cases with multiple peptide chains this becomes more difficult and side reactions involving aspartimide formation and decomposition frustrate attempts to generate longer chains. Unverzagt and coworkers demonstrated that appending a temporary pseudo-proline dipeptide at the glycosylation consensus sequence dramatically cut down on the formation of unwanted side products enabling the practical synthesis of glycoproteins from glycosylated fragments.

SUMMARY

Glycoproteins represent a valuable therapeutic area where total synthetic efforts are in a unique position to provide insight into the structure activity relationship behind many post translation glycosylations. The chemical synthesis of EPO demonstrates that such challenging targets are within the reach of modern techniques including new ligation strategies and selective glyosylation.

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