Principal Investigator: Geert-Jan Boons
Grant Title: Glycan Complexity and Biological Recognition

Abstract

objectives: Almost all cell surface and secreted proteins are modified by covalently-linked carbohydrate moieties and the glycan structures on these so-called glycoproteins are essential mediators of biological processes such as protein folding, cell signaling, fertilization, embryogenesis, neuronal development, hormone activity, and the proliferation of cells and their organization into specific tissues. In addition, overwhelming data supports the relevance of glycosylation in pathogen recognition, inflammation, innate immune responses, and the development of autoimmune diseases and cancer. Advances in understanding the biological roles played by glycans, along with the factors that influence or alter their functions, will be central to understanding biology and will provide important avenues for the development of therapeutics, diagnostics, and nutraceuticals of the future. Progress in glycoscience is greatly hampered by a lack of well-defined complex oligosaccharide standards that are need for the fabrication of the next generation of microarray, for the development of analytical protocols to determine exact structures of isolated glycans and glycoconjugates, and for the elucidation of pathways of glycoconjugate biosynthesis.

Methods used. We have developed a chemoenzymatic approach that can provide oligomeric *N*-acetyllactosamine derivatives that are modified by different patterns of fucosides and sialosides. It is based on the chemical synthesis of trimeric LacNAc derivatives in which amino groups are functionalized to block fucosylation by Fut-V. After enzymatic modification, the protecting groups can be removed to install natural GlcNAc. The final compounds could be further derivatized using sialyl transferases.

Results. It has been found that LacNAc derivatives having Boc at C-2 amine cannot be modified by fucosyl transferases. An efficient approach has been developed for the synthesis of a trimeric LacNAc derivative in which amines are modified by TFA, Boc and CBz. This compound could be deprotected and converted into various hexasaccharides having different patterns of Boc protecting groups. These compounds could be selectively fucosylated to give different patterns of mono- and bis-fucosylation. The Boc protecting group of the resulting derivatives could be removed and the resulting free amines acetylated to give differently fucosylated tri-LacNAc derivatives. These compounds could be sialylated to give a panel of valuable glycans. The collection of compounds will be used to determine ligand requirements of various glycan-binding proteins including hemagglutinins (HAs) and selectins. In addition, the compounds will employed as standards to develop advanced separation and mass spectrometric methods to identify exact structures of complex glycans in biological samples. Knowledge about exact structures of glycan in biological samples will be critical to establish in which way glycan complexity contributes to binding and biological activity.