4 CONCLUSION

This Ph. D. thesis is a systematic study of the substrate specificity and the synthetic potential of β-*N*-acetylhexosaminidases (EC 3.2.1.52) with structurally modified substrates. It comprises four publications in international journals, one review and 17 oral and poster contributions.

The following parts of the substrate molecule were modified: 2-acetamido moiety, the C-6 hydroxyl (oxidations, introduction of a cyano group) and the aglycon part (glycosyl azides – C-N bond hydrolysis). Thirteen modified substrates were synthesized, seven of them were described for the first time. They were tested for hydrolysis and transglycosylation by over thirty fungal β-*N*-acetylhexosaminidases (culture collections at Charles University and at the Institute of Microbiology, Academy of Sciences of the Czech Republic) and the results were discussed in relation to the conclusions of molecular modeling (β-*N*-acetylhexosaminidase from *Aspergillus oryzae* CCF 1066). Eight oligosaccharidic structures (six of them novel) were prepared by semi-preparative transglycosylation reactions (tens of miligrams), isolated (mostly 16–37% yields, even 78% yield) and fully characterized. Noteworthy properties like immunoactivity (binding to natural killer cell activation receptors) and inhibitory potential were disclosed in four new compounds prepared. An original synthetic methodology with glycosyl azides as donors in β-*N*-acetylhexosaminidase-catalyzed transglycosylations was introduced. β-*N*-Acetylhexosaminidase from *Talaromyces flavus* CCF 2686 was identified as a prospective candidate for cloning and directed mutagenesis due to its exceptional substrate specificity.

The findings of this work will be further applied, *e. g.*, in the synthesis with glycosidases and glycosyl azide donors, the development of glycosidase inhibitors, and the design of multivalent immunomodulatory glycomimetics.