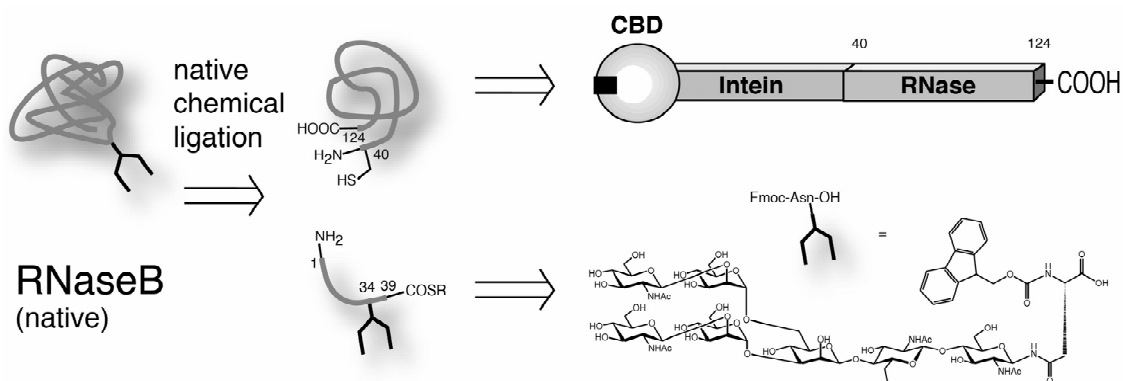


CHEMOENZYMATIC SYNTHESIS & MOLECULAR BIOLOGY: SEARCHING AN ACCESS TO SINGLE FORM GLYCOPROTEINS

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Recent efforts to obtain functional glycoproteins as a single glycoform are an ongoing challenge for interdisciplinary chemical research. Approaches to reduce the diversity of natural glycoproteins by glycan remodeling, transglycosylation, reverse proteolysis or native chemical ligation have been investigated [1]. The most promising approach for the synthesis of authentic complex type N-glycoproteins appears the native chemical ligation of glycoprotein fragments. As a model glycoprotein we chose RNase because structure-activity studies indicated that the enzymatic activity is dependent on the glycosylation pattern. Glycosylated RNase B was retrosynthetically disconnected into two fragments ranging from amino acids 1-39 and 40-124 with the N-glycosylation site at Asn 34. Using an intein-based fusion protein approach the fragment 40-124 was expressed in *E. coli* and cleaved after refolding.



In parallel the synthesis of the glycosylated fragment 1-39 as an activated thioester was investigated using Ellman's variant [2] of a safety-catch linker. A newly developed dual-linker resin has allowed the efficient incorporation of synthetic N-glycans [3] into the RNase fragment 30-39 as a thioester [4]. This thioester containing a complex-type heptasaccharide N-glycan was ligated with synthetic RNase 40-68 and recombinant RNase 40-124 yielding the fully glycosylated RNase B fragments 30-68 and 30-124.

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[2] Backes, B. J.; Ellman, J. A. (1999) *J. Org. Chem.*, 64, 2322-2330.

[3] Weiss, H.; Unverzagt, C. (2003) *Angew. Chem. Int. Ed.*, 42, 4261-4263.

[4] Mezzato, S.; Schaffrath, M; Unverzagt C. *Angew. Chem. Int. Ed.*, 2005, 44, 1650-1654.