Synthesis of fluorescently labelled nucleoside diphosphate 6-amino-6-deoxy-β-L-galactoses

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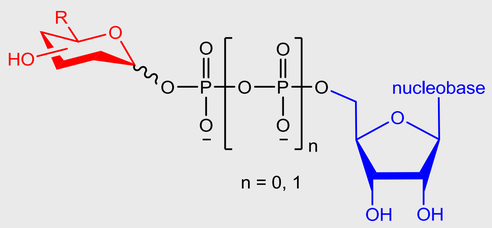
Introduction

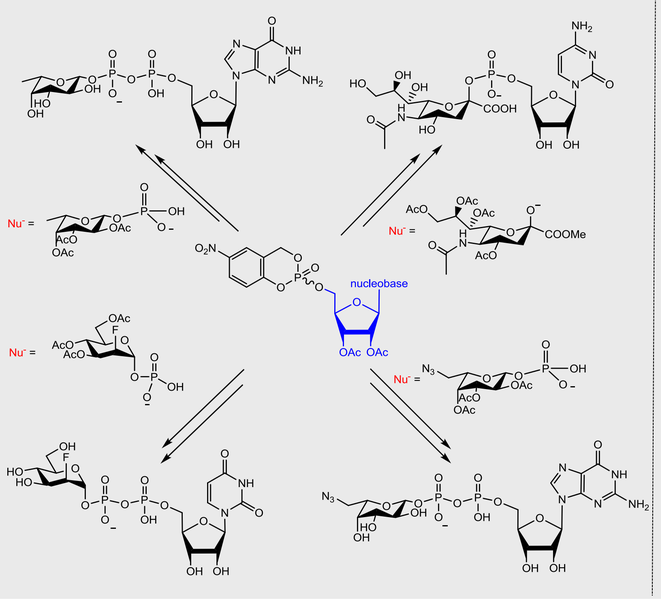
Glycosylation is one of the most complex posttranslational modifications of proteins and shows an enormous structural diversity. Furthermore, it influences several essential biological processes from storage to signaling.[1] Nucleoside monophosphate (NMP) and nucleoside diphosphate (NDP) sugars are glycosyldonors and serve therefore as substrates in various biochemical processes. The sugar transfer to specific acceptor molecules is catalysed by enzymes, generally glycosyltransferases.[2] Fucosylation, mediated by fucosyltransferases (FucT) from GDP-L-fucose, is often the final glycosylation step in the biosynthesis of biologically significant glycans. It is important for basic physiological and pathological procedures such as cell-cell-communication, immune response or tumor metastasis. Since fucosyltransferases show a great tolerance to modification in position C6 of the substrate[3], it is possible to label a NDP fucose derivative with a dye in this position.

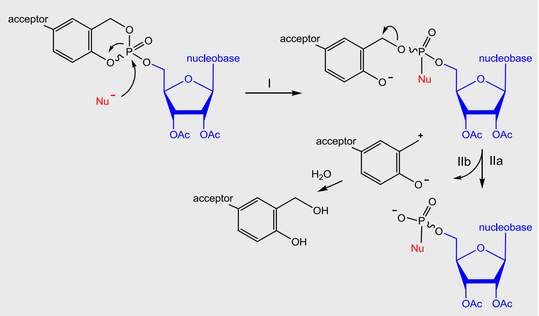


The *cyclo*Sal-method

The *cyclo*Sal-approach has been proven to be a suitable method for the synthesis of a great number of biological significant biomolecules, including the formation of natural and modified nucleoside diphosphate sugars.[4,5] The *cyclo*Sal-triesters serve here as activated phosphate donors and can be attacked by a vast number of nucleophiles, for example sugar phosphates.[5] By using 5-acceptor substituted *cyclo*Sal-nucleotides the electrophilicity of the phosphorous atom can be increased.[6]

Examples of previously prepared NM(D)P-conjugates





I: The nucleophile attacks the *cyclo*Sal-triester. Since the phenolic ester bond is the most labile one, it breaks at first.

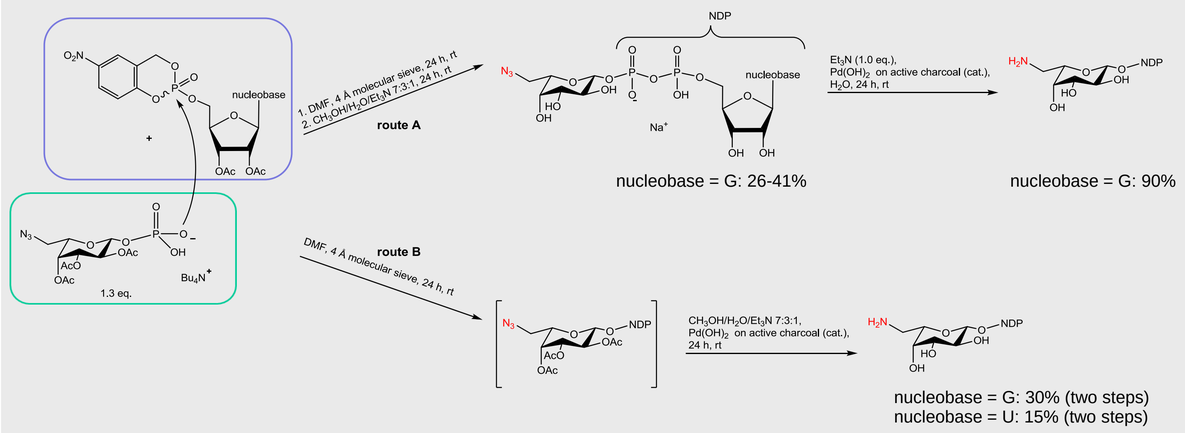
IIa: The second step is spontaneous, since the phenolate destabilises the benzylic ester bond. The product is released.

IIb: After hydrolysation, the released *cyclo*Sal-mask is converted to the corresponding salicyl alcohol.

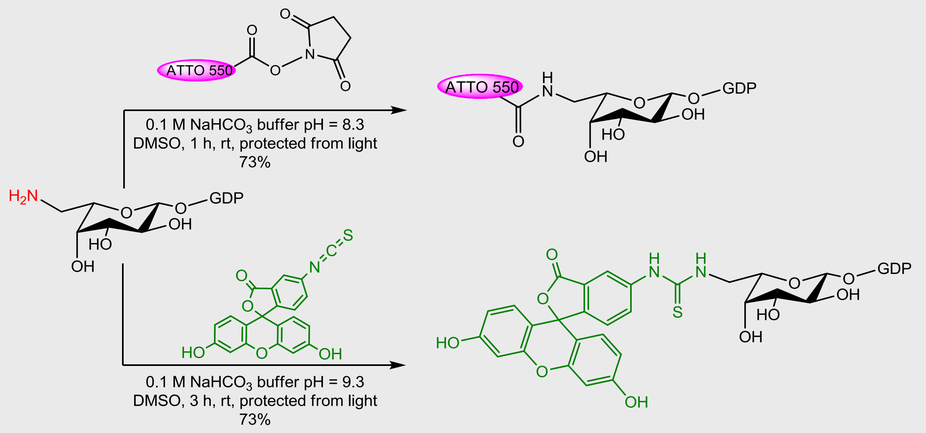
Objectives

Since detailed information about the reaction processes for most fucosyltransferases are still uncertain, the aim of this project was to synthesise fluorescently labelled NDP fucose derivatives and using them as substrates in different biochemical assays, e.g. monitoring fucosyltransferase activity. Furthermore, the protein fetuin ought to be labelled with a dye to visualise fucosylated glycons later.

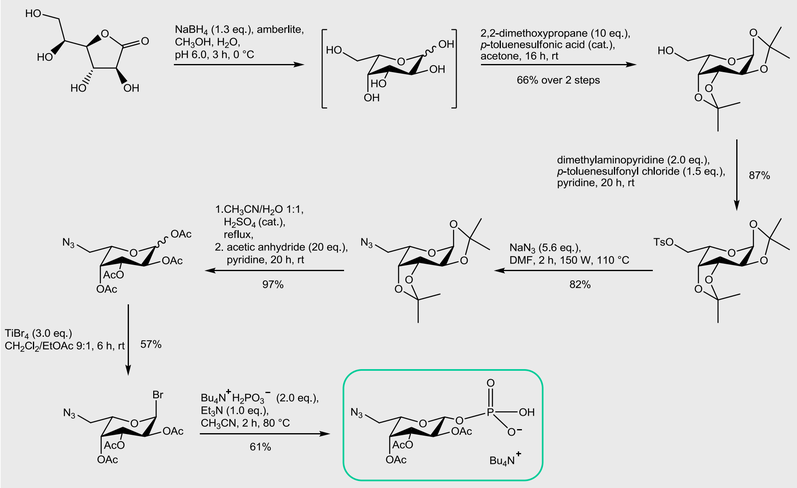
Synthesis of the modified nucleoside diphosphate sugars



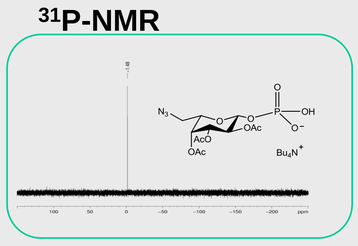
Synthesis of the fluorescently labelled nucleoside diphosphate sugars



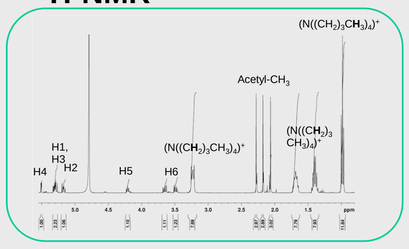
Synthesis of the sugar phosphate



^{31}P-NMR



^{1}H-NMR



Synthesis of the *cyclo*Sal-triesters

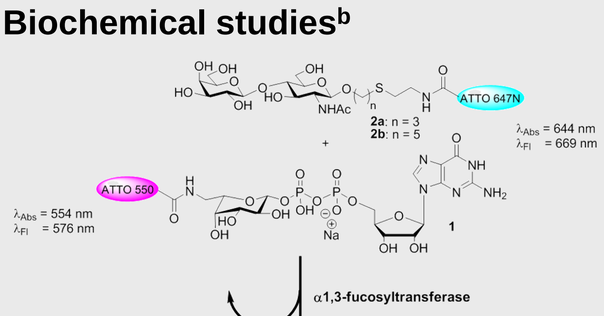
5-Nitro-*cyclo*Sal-2',3'-di-*O*-acetyluridine monophosphate

^{31}P-NMR

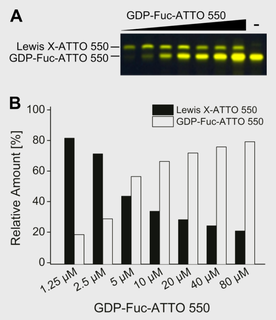
5-Nitro-*cyclo*Sal-N^2-acetyl-2',3'-di-*O*-acetylguanosine monophosphate

^{31}P-NMR

Biochemical studies[b]



Formation of fluorescently labelled Lewis X **3a,b** catalyzed by α1,3-fucosyltransferases.[7]



Assay for measurement of fucosyltransferase activity by HPTLC using ATTO-labelled donor.

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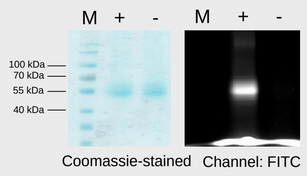
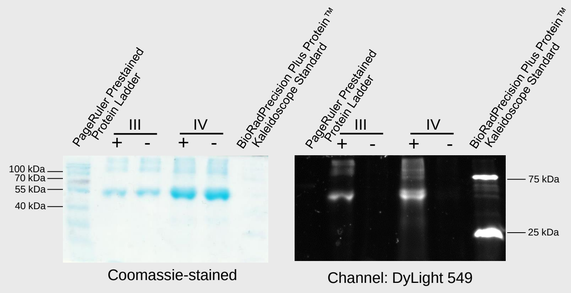
reaction rate: 15 pmol/h[7]

Protein labelling

FITC labelled GDP-6-amino-6-deoxy-β-L-galactose

ATTO 550 labelled GDP-6-amino-6-deoxy-β-L-galactose





- 9% SDS-PAGE
- +: Reaction mixture with *Helicobacter pylori* FucT
- - : Reaction mixture without *Helicobacter pylori* FucT
- III - fetuin typ III, glycoprotein from foetal bovine serum
- IV - s. typ III, further purified with gel filtration, to reduce the free amount of *N*-acetylneuraminic acid

Conclusion

We successfully synthesised two different fluorescently labelled nucleoside diphosphate 6-amino-6-deoxy-β-L-galactoses. For the coupling to give the NDP sugars, the *cyclo*Sal-procedure was the method of choice. With the fluorescently labelled GDP sugars we were able to prove the tolerance of hFucT-IX towards substratemodifications with a dye. Moreover, the formation of fluorescently labelled Lewis X and the measurement of fucosyltransferase activity was successful.[7] Finally, we succeeded in labelling the protein fetuin with ATTO 550 as well as with fluorescein isothiocyanate (FITC) using helicobacter pylori α1,3-FucT. These results open access to further biochemical assays like a high-throughput screening of fucosyltransferase IX variants using fluorescence polarisation.

References

[1] C. Haase, O. Seitz, *Top. Curr. Chem.* **2007**, *267*, 1-36. **[2]** G. K. Wagner, T. Pesnot, R. A. Field, *Nat. Prod. Rep.* **2009**, *26*, 1172-1194. **[3]** T. Maeda, S.-I. Nishimura, *Chem. Eur. J.* **2008**, *14*, 478-487. **[4]** S. Wolf, R. M. Berrio, C. Meier, *Eur. J. Org. Chem*. **2011**, 6304-6313. **[5]** S. Wolf, T. Zismann, N. Lunau, S. Warnecke, S. Wendicke, C. Meier, *Eur. J. Cell Biol.* **2010**, *89*, 63-75; S. Wolf, T. Zismann, N. Lunau, C. Meier, *Chem. Eur. J.* **2009**, *15*, 7656-7664. **[6]** S. Wendicke, S. Warnecke, C. Meier, *Angew. Chem. Int. Ed.* **2008**, *47*, 1500-1502. **[7]** N. Lunau, K. Seelhorst, S. Kahl, K. Tscherch, C. Stacke, S. Rohn, J. Thiem, U. Hahn, C. Meier, *Chem. Eur. J.*, submitted.

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