Synthetic Lipid-Linked Oligosaccharide for Structural Studies of Alg6

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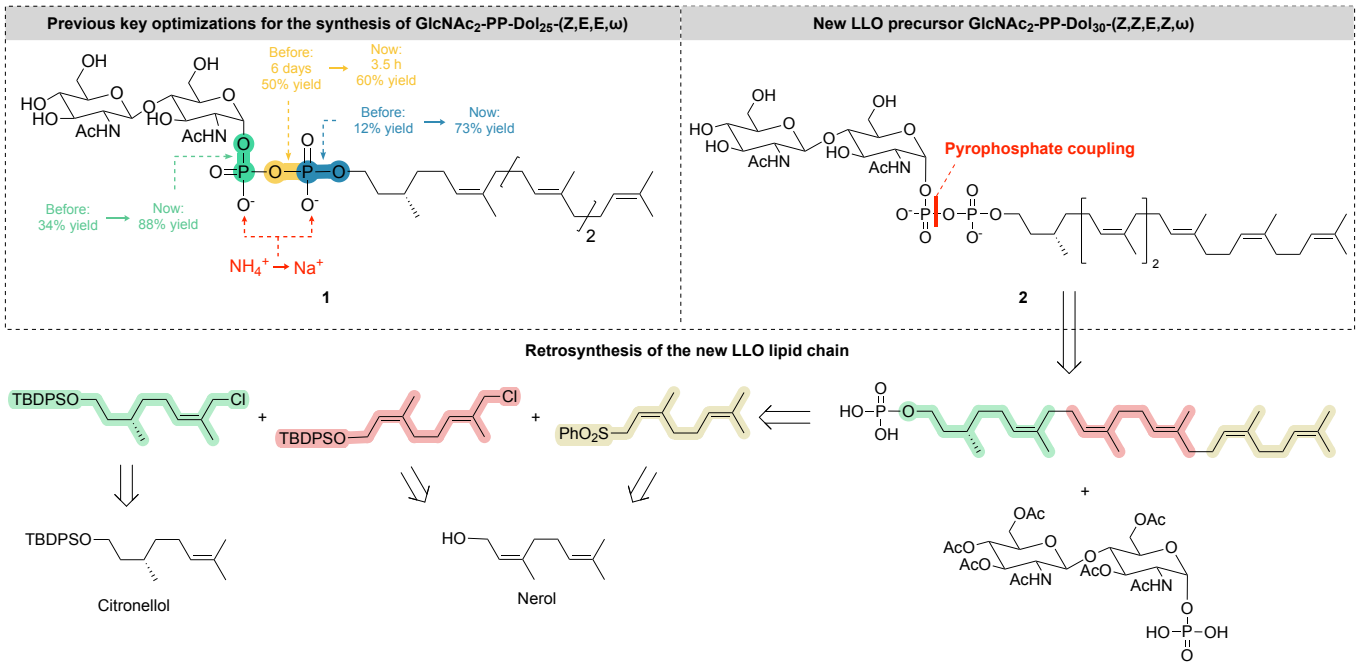
N-linked glycosylation is a crucial post-translational modification that drives the formation of structurally diverse N-glycans.[1] Central to this pathway are lipid-linked oligosaccharides (LLOs), which serve as key substrates for glycosylation enzymes such as oligosaccharyltransferase (OST) and members of the ALG (asparagine-linked glycosylation) family.[2,3] To facilitate the study and application of this biosynthetic route, our laboratory has developed simplified LLO precursors. Among the substrates evaluated, $GlcNAc_2$-PP-Dol_{25} (**1**) has proven to be the most efficient for enzymatic synthesis of LLO analogues.

The synthesis of compound **1** involves a lengthy sequence and challenging purification steps, due to its sensitivity to acidic and basic conditions. We have previously optimized this synthetic route, ensuring a reliable supply for biochemical investigations.[4] While highly efficient for most enzymatic studies, **1** does not provide sufficient resolution in cryo-EM studies of ALG6 ternary complex. To overcome this limitation, we are currently developing a lipid analogue that more closely mimics the native dolichol backbone. $GlcNAc_2$-PP-Dol_{30} (**2**) incorporates an additional isoprenyl unit and features predominantly *Z* double bonds, consistent with the structure of the native dolichol. For this, different strategies are being explored for the construction of this lipid chain. These structural refinements are expected to enhance the stability of the enzyme–substrate complex, thereby enabling higher-resolution cryo-EM studies of ALG6.



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[2] A. S. Ramírez, M. de Capitani, G. Pesciullesi, J. Kowal, J. S. Bloch, R. N. Irobalieva, J.-L. Reymond, M. Aebi, K. P. Locher. *Nat Commun* **2022**, *13*, 7296.
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