V22. SYNTHETIC INTERMEDIATE FOR MENINGOCCOCAL SEROGROUP C CONJUGATE VACCINE PRODUCTION. QUANTIFICATION AND STRUCTURAL ASPECTS.

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INTRODUCTION Capsular polysaccharide from *Neisseria meningitidis* serogroup C (PSC), is a homopolymer of partially O-acetylated α-(2→9) 5-N-acetylneuraminic acid; that is considered a very important raw material in glycoconjugate synthesis, especially in reductive-animation conjugation approach. A step of PSC mild oxidation by sodium periodate is required, generating oligomer with terminal aldehydes. Although N-acetyl-neuraminic acid oxidation has been well described in literature, especially for (2 → 8) Nacetylneuraminic acid; slightest difference in structure, may occur depending of sialic acid glycosidic bond pattern. To solve this question a structural characterization study is required. In addition, aldehyde quantification, generally performed by colorimetric analysis, is important for process control.

OBJECTIVE The main goal of this study is to investigate the structure of oxidized polysaccharide *Neisseria meningitidis* serogroup C by NMR and computational methods to evaluate and to quantify the relative amount of aldehyde by qNMR.

METHODOLOGY The oxidized PSC was O-deacylated in alkaline medium and the product was purified by sizeexclusion chromatography. The samples were analyzed by 2D NMR experiments (gCOSY, TOCSY, HSQC and HMBC). Computational calculations were performed on Spartan'10 (Wavefunction, Inc.) using the MMFF94 force field. Dimeric structural models was based on X-ray crystallography data from the Protein Data Bank (PDB ID: 1KQR). Quantitative NMR were performed in Bruker Advance 400MHz, NS 64, D1 25, temperature of 313K and using D_2O as solvent.

RESULTS In NMR spectra, it was possible to verify the aldehyde-hydrates formation in the range of 5.0-5.1 ppm; and the oxidative cleavage between C7—C8.

In computation studies, an equilibrium between opened and cyclic hemicetal forms of (2→8) dimer, may exist through chair-chair interconversion. However, the cyclic form of (2→9) dimer, a twisted-boat conformation is required. The qNMR analysis showed that 9.0% of the oxidized PSC, which is in accordance with the chemical analysis 9.3%.

CONCLUSION NMR data of the oxidized (2→9) Neu5Ac structure is compatible with the hydrate form and computation method demonstrated that its cyclization involves the twisted-boat interconversion; while the hemiacetal was not verified. Computational data shows that cyclization involves as interconversion from chair to twistedboat conformations. By qNMR, it was possible to determinate that only 9,0% of native PSC was converted to aldehyde. Together, these data will contribute to better process control.

KEYWORDS capsular polysaccharide, qNMR, computational studies.