# 5.3: Q-NMR for Analysis and Characterization in Vaccine Preparations

PedvaxHIB is a vaccine that is made by chemically conjugating the capsular polysaccharide of *Haemophilius influenzae type b* (Hib) to an outer membrane protein from *Neisseria meningitidis* to form a protein-conjugated vaccine that is very effective in preventing invasive Hib infection in infants and young children. This example shows the utility of NMR for both the characterization of the derivatized polysaccharide and its quantitative analysis. The advantages of NMR include its nondestructive nature and its ability to detect molecules that do not contain a UV-visible chromophore.

To obtain an accurate determination of the solution temperature, a linear calibration of the HDO chemical shift was carried out. Shimming of the magnet was performed using the DMSO peak and the proton 90° pulse was calibrated for each solution to compensate for differences in solution ionic strength. The DMSO was also used as the internal reference. The spectral width was 10.5 ppm with a digital resolution of 0.3 Hz. Spectra were measured with 16 transients with a total recycle time of 60 s. The total data acquisition time was 20 min.

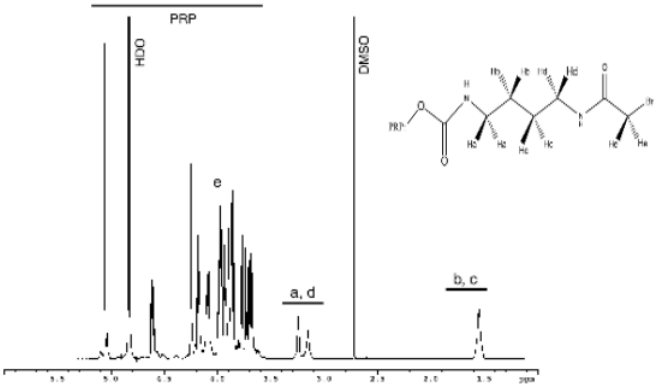


**Figure 1.** $^{1}$H NMR spectrum of the derivatized capsular polysaccharide. Inset is the sidechain structure of the PRP dervativzed with butanediamine bromoacetyl chloride (PRPBuA2). Reprinted from Anal. Biochem. 337, Q. Xu, J. Klees, J. Teyral, R. Capen, M. Huang, A. W. Sturgess, J. P. Hennessey, Jr., M. Washabaugh, R. Sitrin, C. Abegunawardana, Quantitative nuclear magnetic resonance analysis and characterization of the derivatized *Haemophilus influenzae* type b polysaccharide intermediate for PedvaxHIB, 234-245, Copyright (2005), with permission from Elsevier.

Figure 1 shows the $^{1}$H NMR spectrum of an intermediate in the synthesis of the capsular polyribosylribitol phosphate (PRP) – outer membrane protein complex. PRP is first activated with 1,1'=carbonyldiimidazole and then reacted with an excess of butanediamine. The resonances of the derivatized PRP are well resolved in this spectrum. Quantitation was performed using an internal reference because it alleviates the need for a standard calibration curve. In determining the percentage of the various forms, neither the molecular weight of the polysaccharide nor the degree of polymerization was needed for calculation. The Q-NMR assay developed in this paper can find application in product release or process monitoring in the pharmaceutical industry and can potentially replace tedious chromatographic and colorimetric methods.

## Reference

Xu, Q.; Klees, J.; Teyral, J.; Capen, R.; Huang, M.; Sturgess, A. W.; Hennessy, J.P.; Washabaugh, M.; Sitrin, R.; Abeygunawardana, C. *Anal. Biochem.* **2005**, *337*, 235-245

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