Glycoconjugate vaccine stability and formulation

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Medicines and Healthcare Products Regulatory Agency

What are glycoconjugate vaccines?

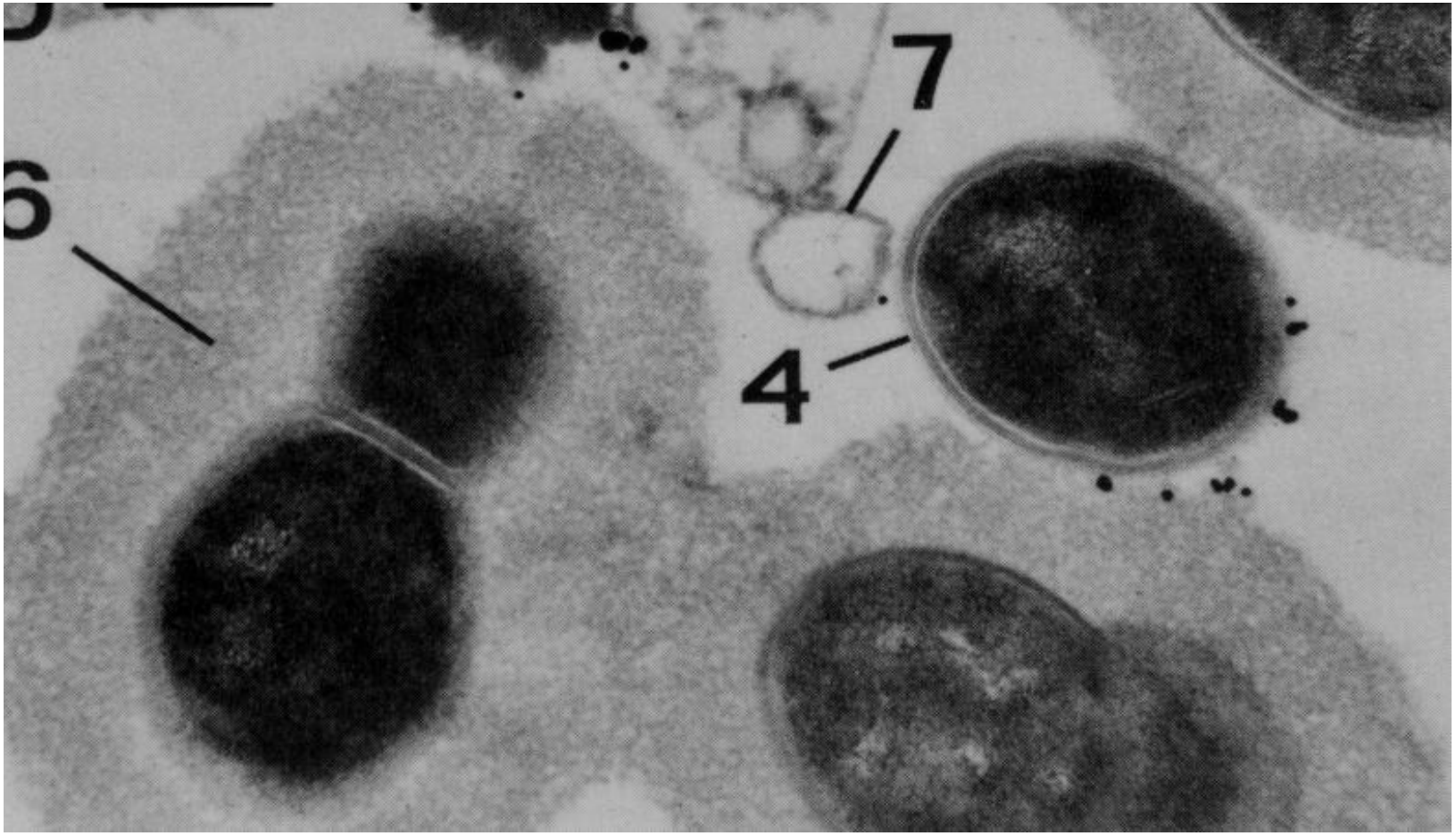
- Cell surface polysaccharides are bacterial virulence factors
- Antibodies against cell surface polysaccharides may be protective against disease
- Purified polysaccharides are used as vaccines
- (Poly)saccharides conjugated to carrier proteins induce a superior immunological response
- Typical doses:
  - 25 - 50 μg per serotype per s.h.d. for polysaccharide vaccines
  - Up to 23 components in each vaccine
  - 2 - 10 μg per serotype per s.h.d. for conjugate vaccines
  - Up to 13 glycoconjugate components per vaccine (so far…….)

Vaccines

- *Haemophilus influenzae* Type b (Hib)
- *Neisseria meningitidis* Groups A, C, W135 and Y
- *Streptococcus pneumoniae* (7-, 10- or 13- serotypes)
- *Salmonella enterica* serovar Typhi

- Group B Streptococcus
- *Salmonella enterica* serovar Paratyphi A
- *Staphylococcus aureus* Types 5 and 8
- *Pseudomonas aeruginosa*

Capsular polysaccharides



Capsular polysaccharide from *Streptococcus pneumoniae.*
Reproduced from Skov Sørensen *et al.*, *Infect. Immun.* 1988, 56, 1890-1896 *with permission*

Capsular polysaccharide structures

- Repeating polysaccharides with (normally) a strict repeat unit
  - Repeat unit contains between 1 and 8 sugars.
  - A wide range of different monosaccharide residues may be present
  - Linear or branched chains
  - May contain alditols and phosphodiester links
  - May contain O-acetyl groups, phosphoglycerol or pyruvyl ketals as substituents.
  - Molecular weights between ca. 100kDa and 2MDa
  - Stability dependent on structure of the repeat unit.

Polysaccharide structures

- *Haemophilus influenzae* Type b (6 serotypes)

  →3DRib*f*β1→1DRib'ol5→OPO_3→

- Meningococcals (12 serogroups)

  →6DManNAc(3OAc)α1→OPO_3→ (Group A)

  →6DGlcα1→4DNeu5Ac(9OAc)α2→ (Group Y)

  →6DGalα1→4DNeu5Ac(9OAc)α2→ (Group W135)

- *S.* Typhi Vi (only one)

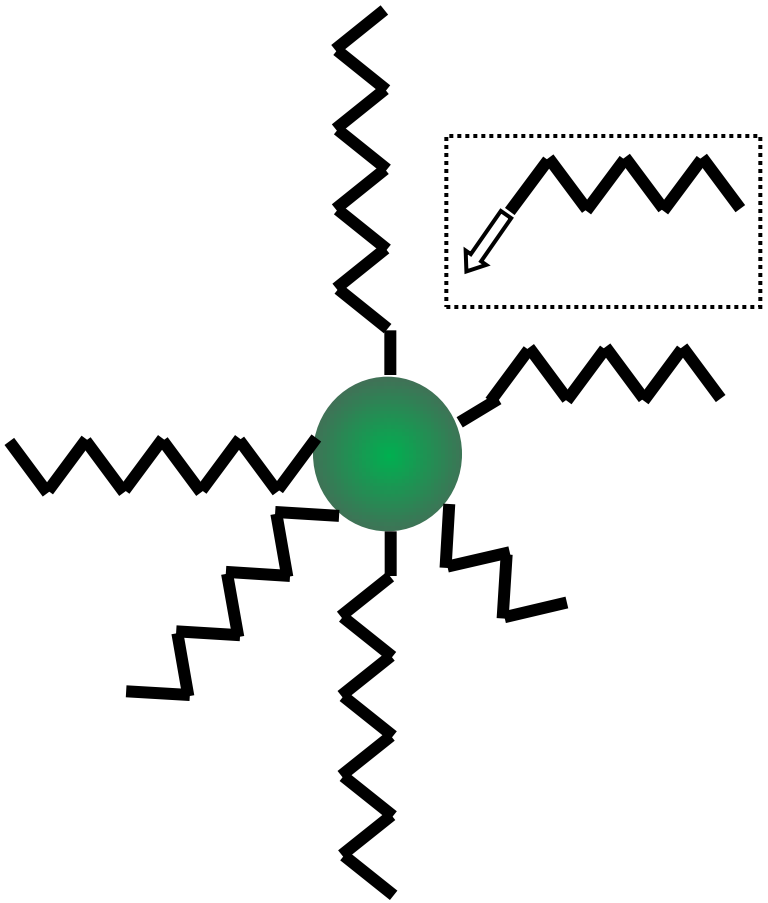
  →4DGalNAcA(3OAc)α1→

- Pneumococcal (92+ serotypes)

  →3DManNAcβ1→3LFucNAcα1→3DGalNAcα1→4DGal(2,3(*S*)Pyr)α1→

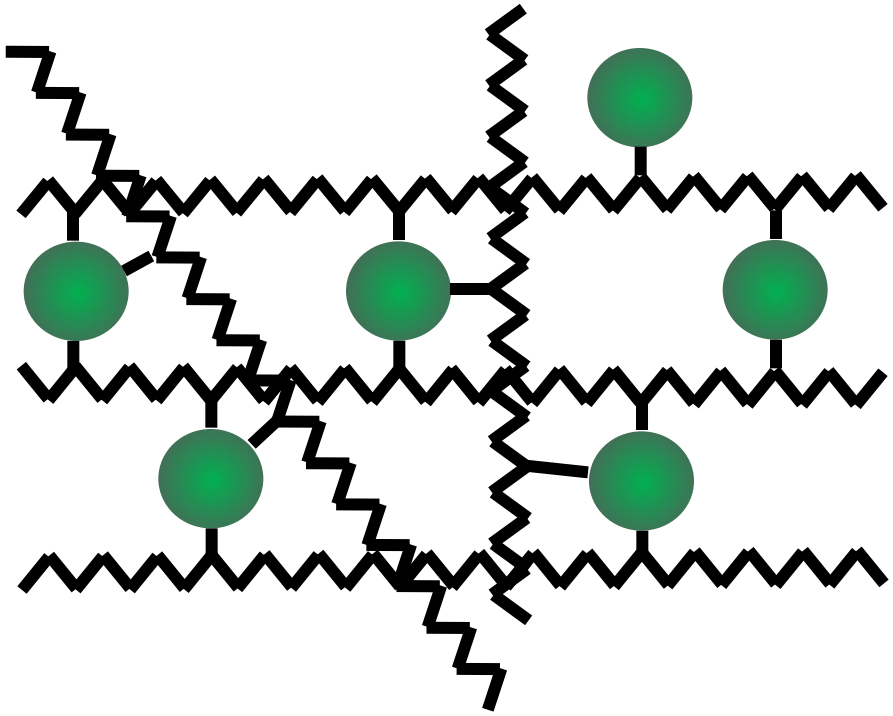
  →6DGlcNAcβ1→3DGalβ1→4DGlcβ1→
  4
  |
  βDGal

Glycoconjugate vaccines – 1



- Produced by coupling monofunctional oligosaccharides
- Produced by coupling bifunctional oligosaccharide at low coupling efficiencies.
- Either direct or indirect attachment to carrier protein (ie. through linker)
- Most often used with CRM197 as carrier, producing a conjugate with MW ca. 90kDa and 30% w/w carbohydrate
- Similar to a typical plasma protein
- Also called fuzzy balls

Glycoconjugate vaccines – 2



- Produced by random activation of high mass polysaccharides, with multiple activations per chain.
- Coupled to carrier protein through non-specific chemistry
- Each polysaccharide chain attached to multiple carrier proteins
- Each carrier protein coupled to multiple polysaccharide chains
- Often used with tetanus toxoid as carrier protein
- Network of high mass (typically 5MDa for a Hib conjugate)

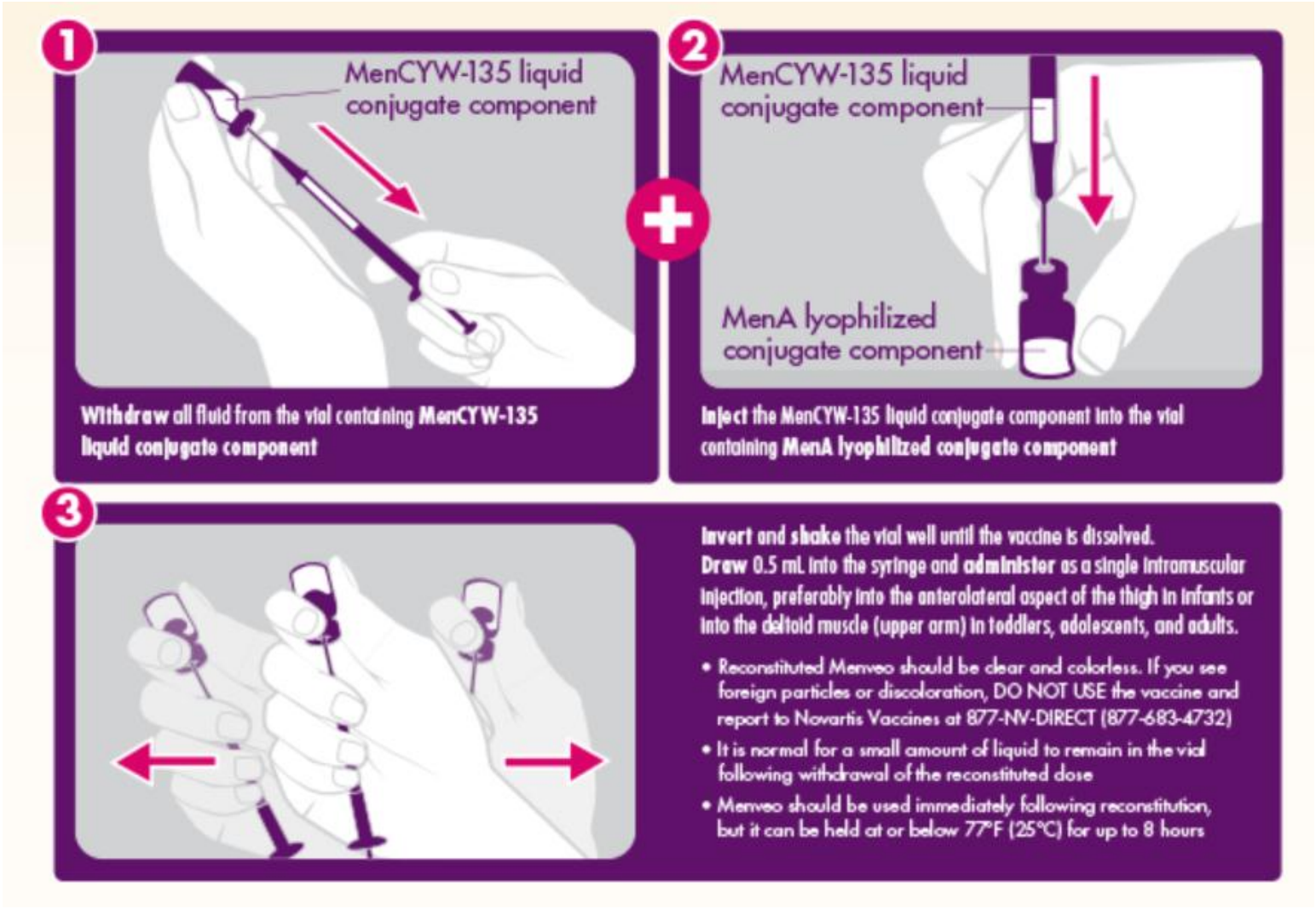
Formulation and presentation options

- Vial or prefilled syringe
- Single dose or multi-dose vials
  - Inclusion of preservative for multidose vials
- Lyophilised or liquid
  - Lyophilised samples will need a sterile diluent
  - Cost implications for price-sensitive products
- Adjuvanted or not adjuvanted
  - Which adjuvants?
  - Adjuvanted diluents? Proposed for some other vaccines
- Combining options in combination vaccines
  - Reconstitution of lyophilised components with adjuvanted liquid fills containing other components

General requirements for final fills

- Sterile
- pH and isotonicity ranges to minimise local pains
- Endotoxin/pyrogenicity limits
- Aluminium specification (if adjuvanted)
- Preservative (in multidose vials)
- Limits on process residuals (eg. bacterial protein, bacterial DNA, formaldehyde, CTAB)
- Limits on residual reagents (eg. coupling reagents)
- See USP <1234> *Vaccines for Human Use – Polysaccharide and Glycoconjugate Vaccines*

Mixed Lyo and liquid formulations



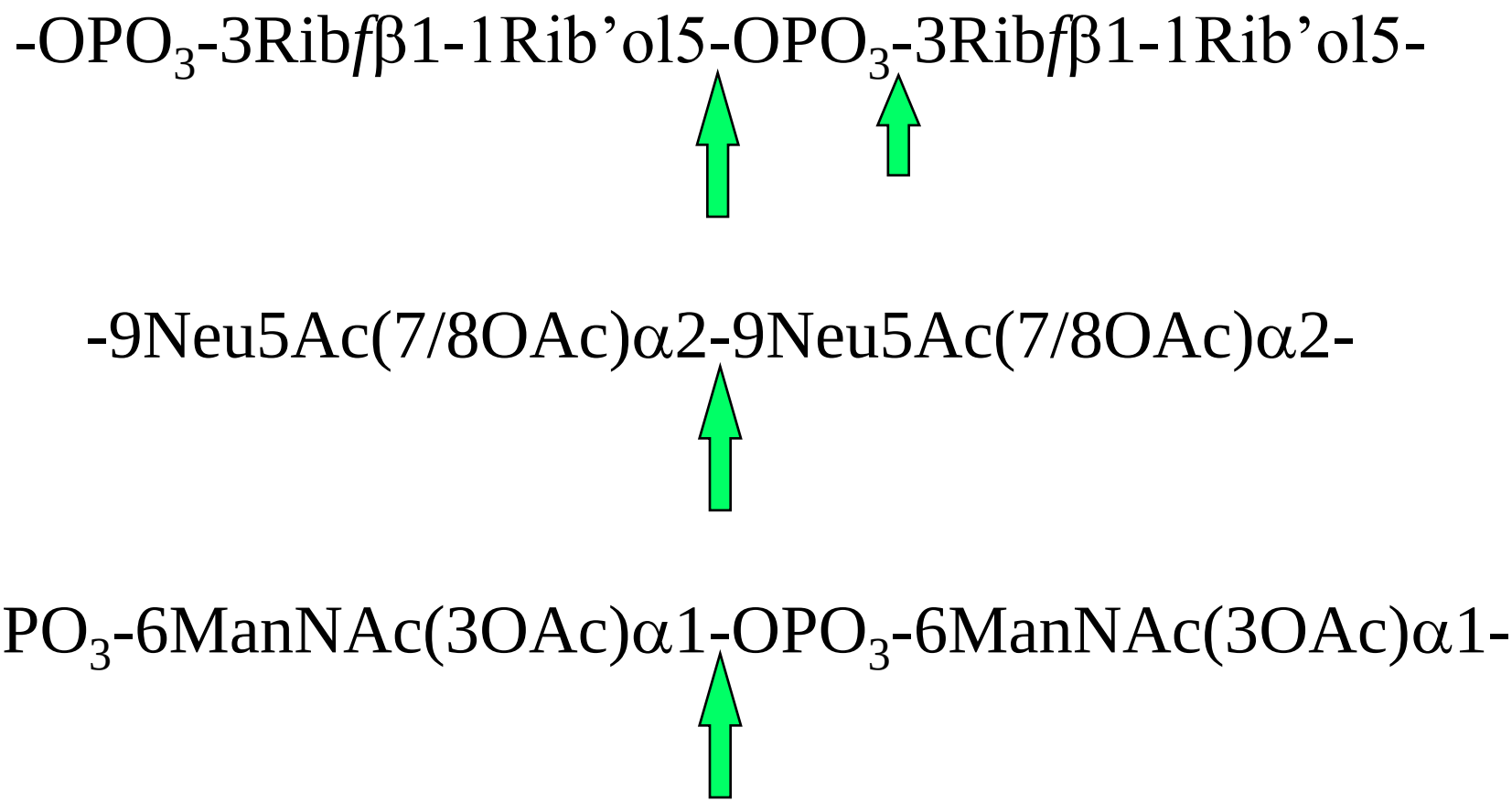
From the Novartis website

Potential sources of instability

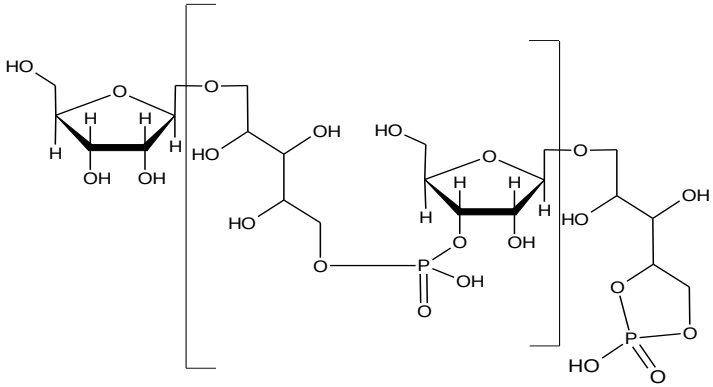
- Depolymerisation of the saccharide chain
  - Hydrolysis
  - Non hydrolytic degradation
- Changes in the glycan structure
  - O-acetyl group migration
  - De-*O*-acetylation
- Cleavage of glycan from the carrier protein at linker region
- Carrier protein denaturation
  - Monitor carrier protein integrity by circular dichroism

- Intention: Plan for a two year shelf life

Low stability glycans in conjugate vaccines



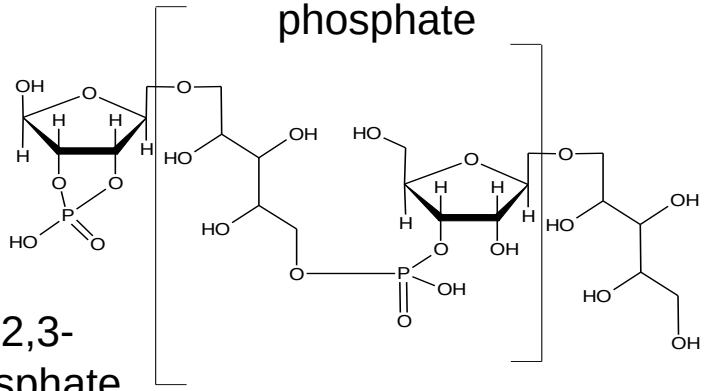
Endgroups formed by Hib PRP degradation



Ribitol-4,5-cyclophosphate

H_2O/OH^-

Ribitol-4-phosphate
or
Ribitol-5-phosphate

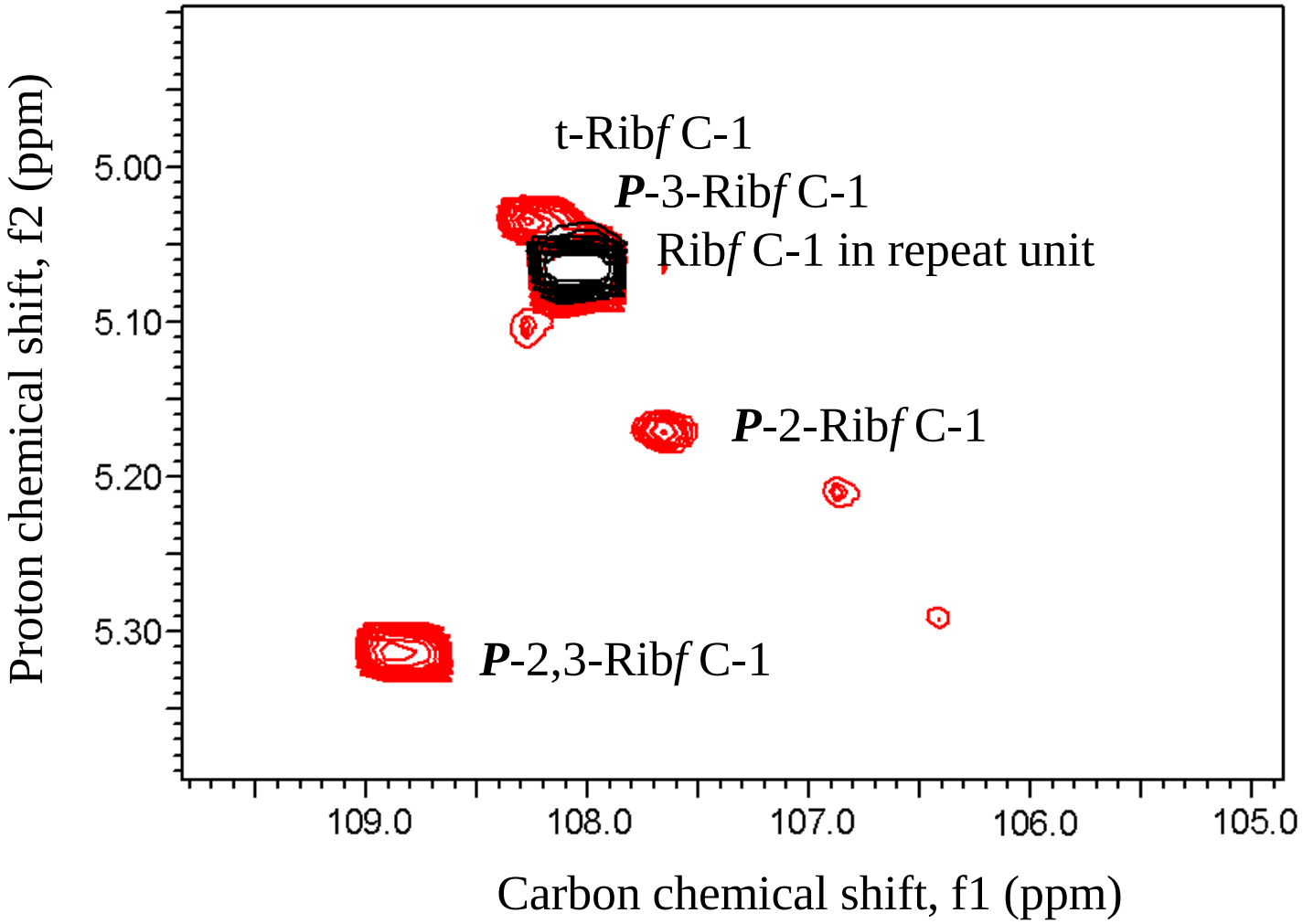


β-Rib*f*-2,3-cyclophosphate

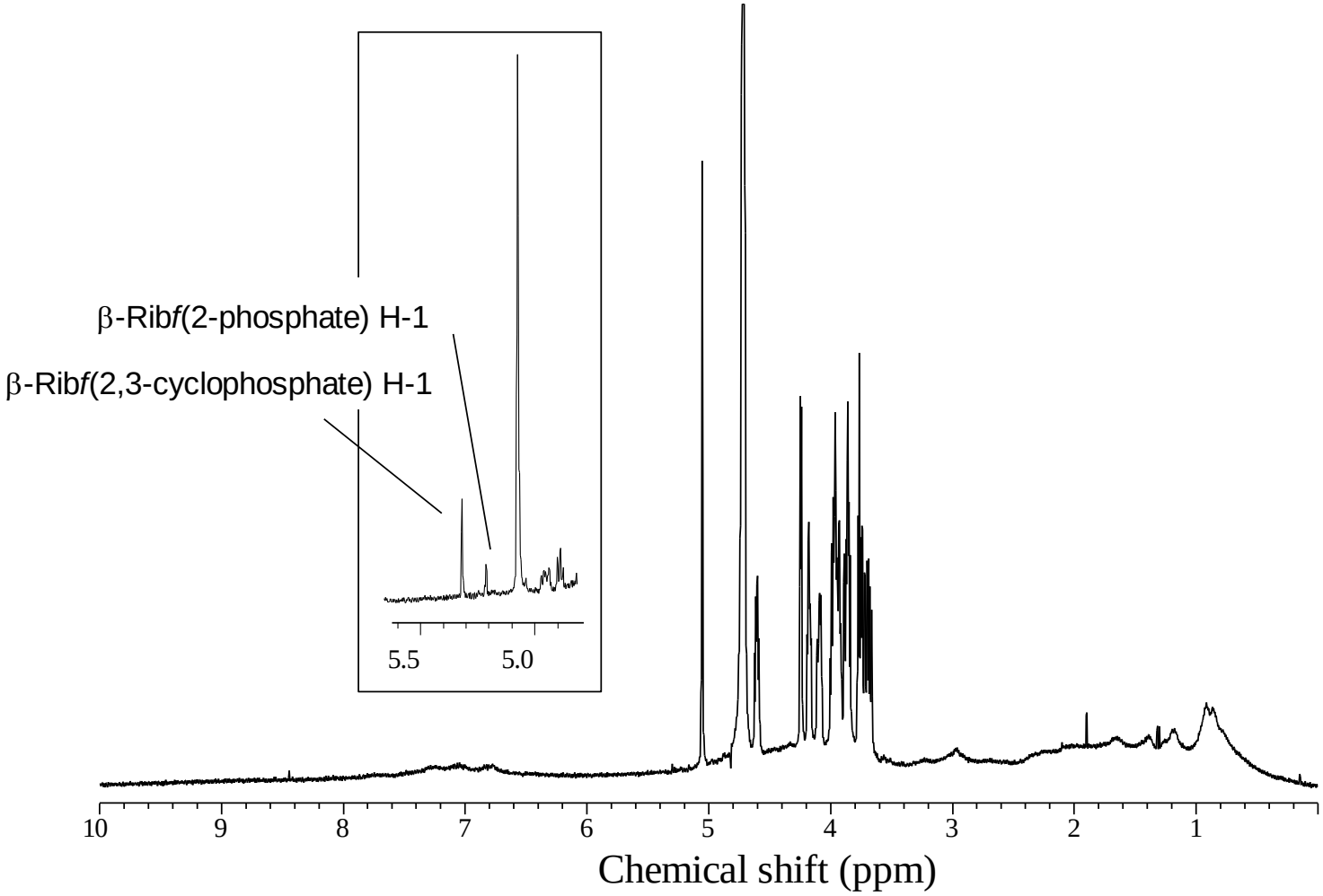
H_2O/OH^-

β-Rib*f*-2-phosphate
or
β-Rib*f*-3-phosphate

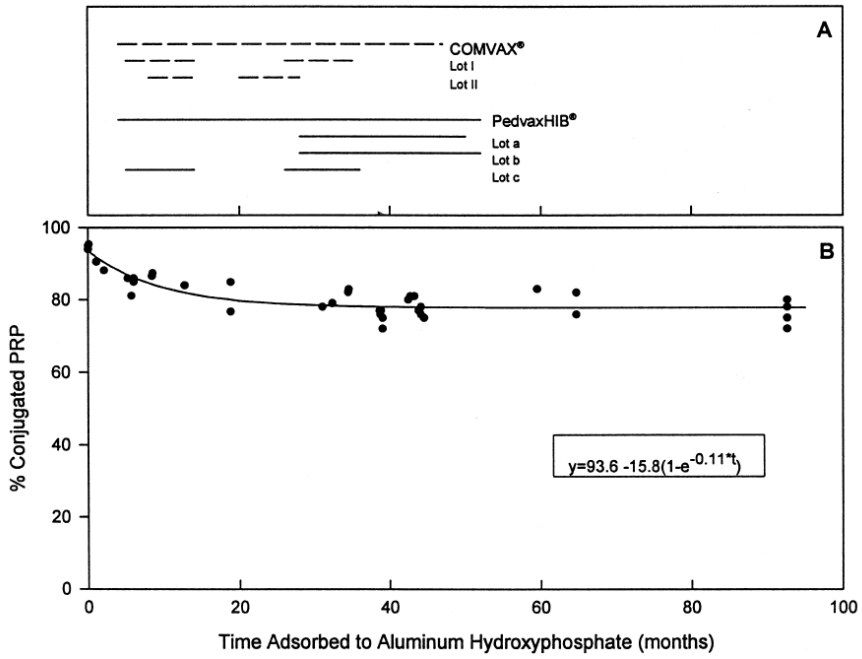
Degradation of Hib PRP, by NMR



Hib conjugate vaccine degrd'n



Merck studies with $Al(OH)_3$ adjuvant



Haemophilus influenzae type b conjugate vaccine stability: catalytic depolymerization of PRP in the presence of aluminum hydroxide

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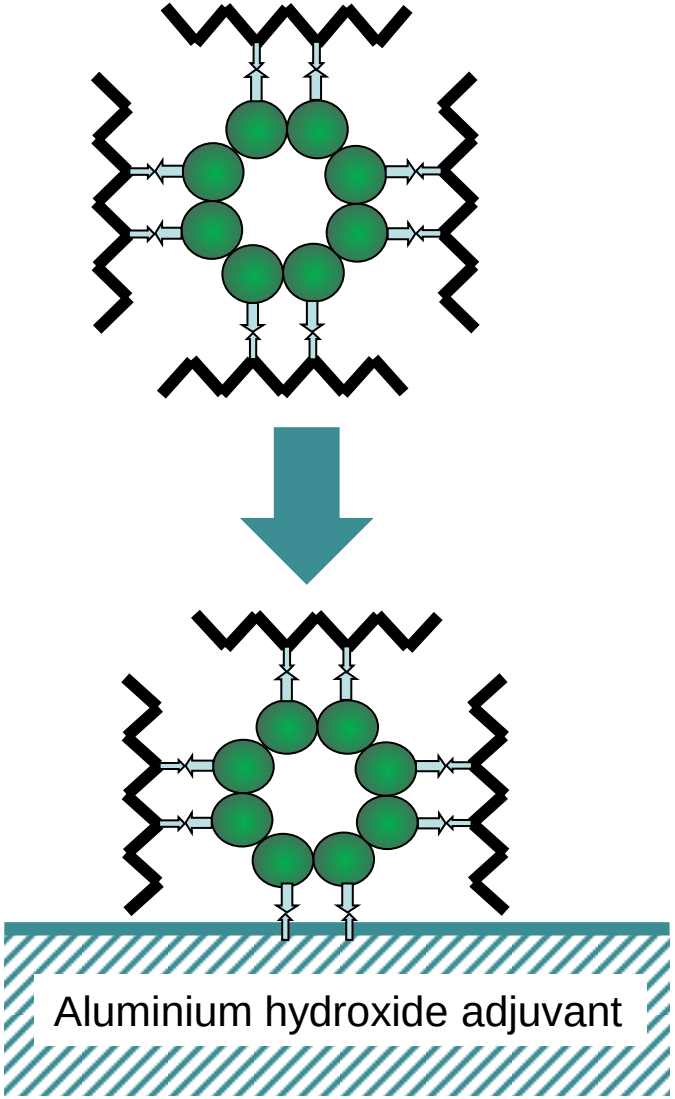
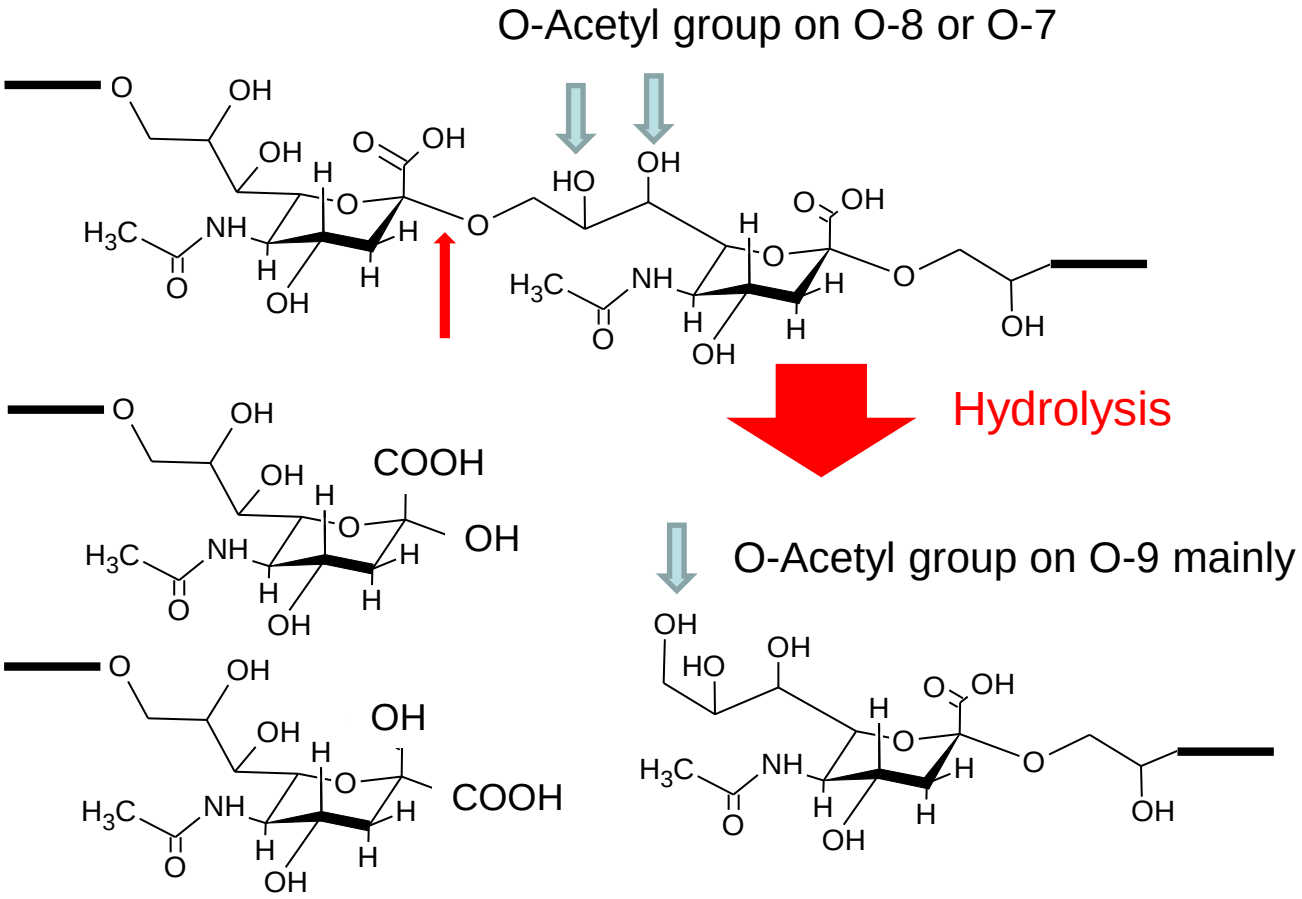
Vaccine, 1999, 17, 1169-1178



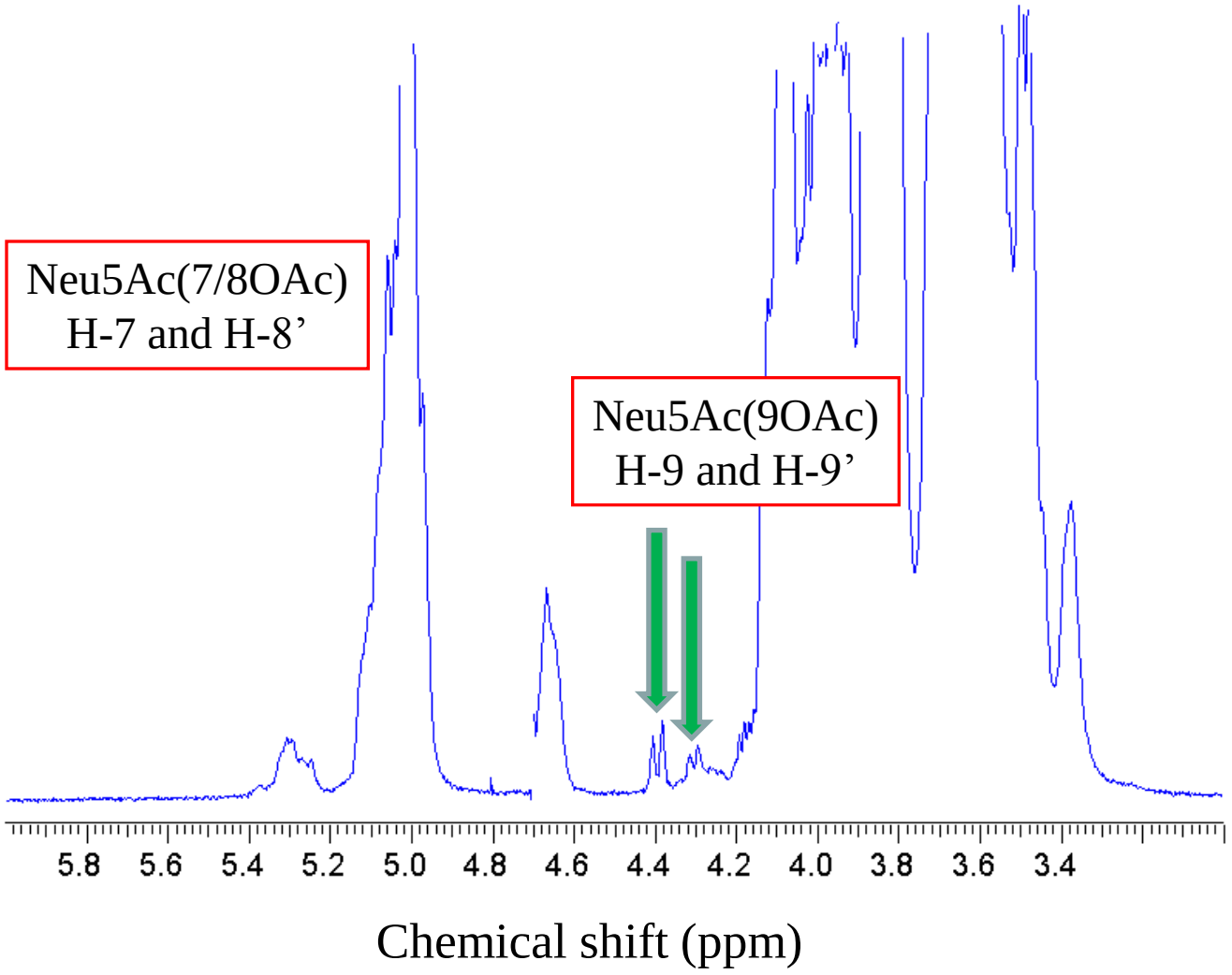
Depolymerisation of Men C CPS



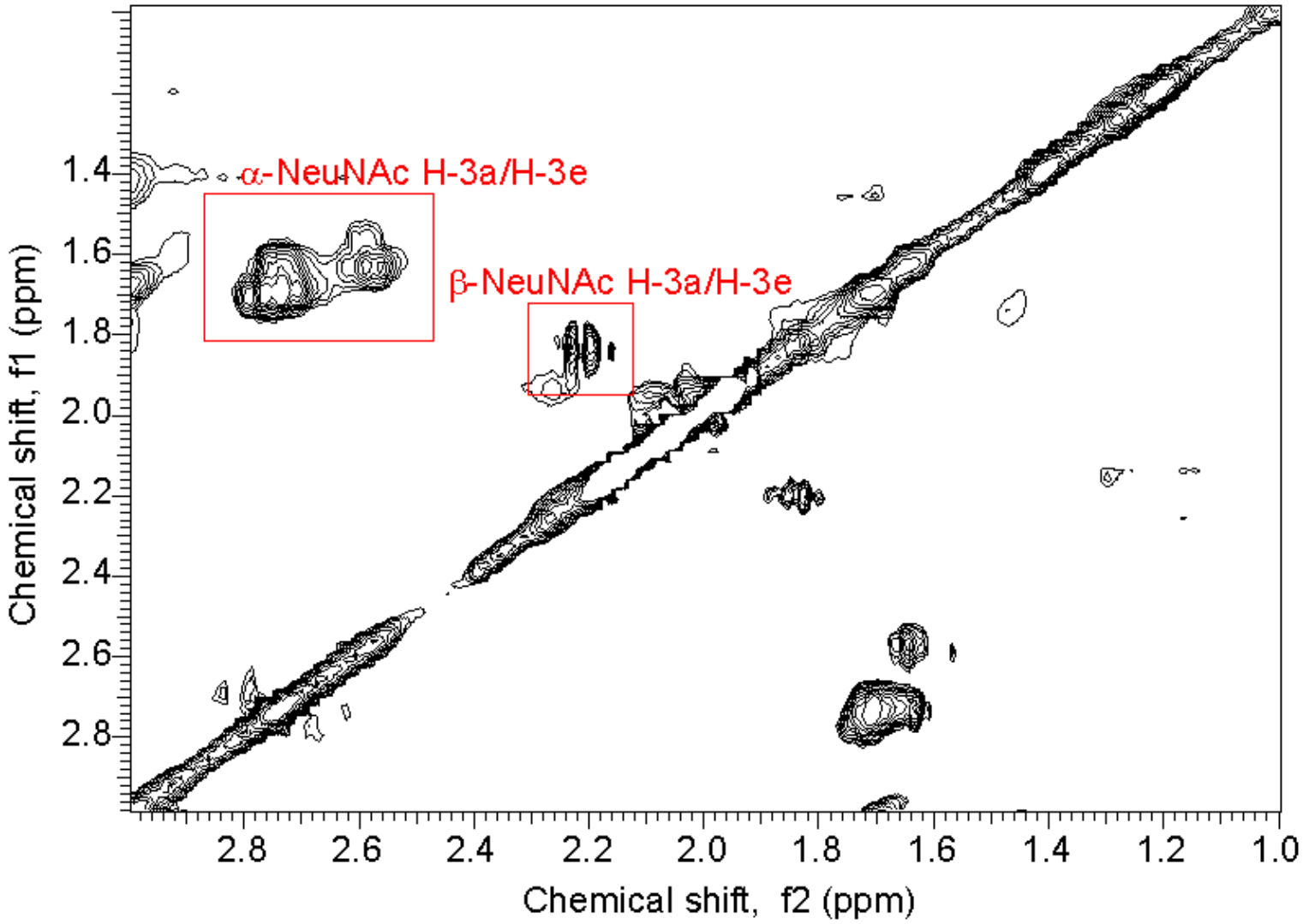
Two anomeric forms

Migration of O-acetyl group

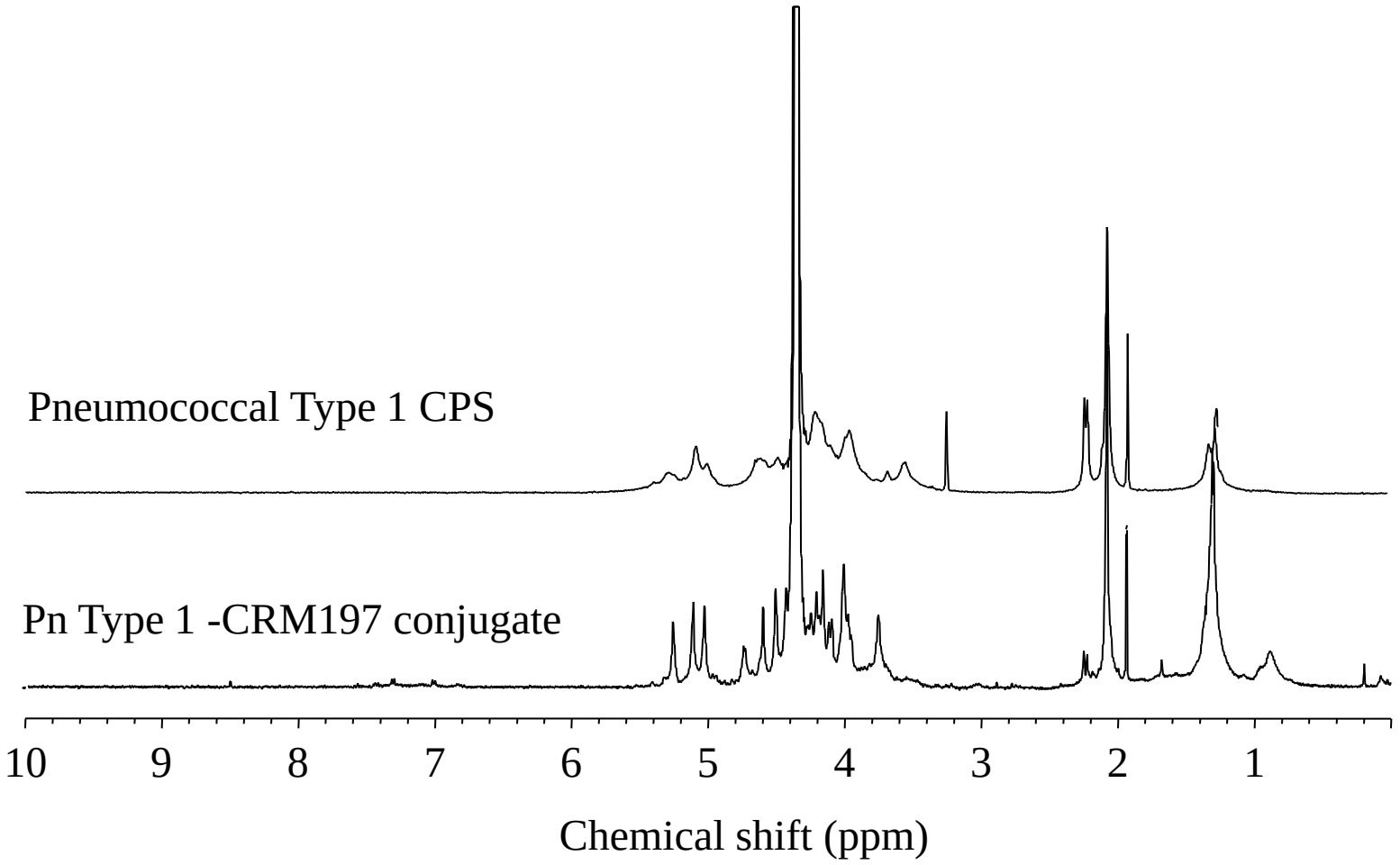
NMR of Men C conjugate vaccine degradation



Degradation of MenC-CRM conjugate, by NMR



De-O-acetylation during conjugation



Measuring degradation

Polysaccharide depolymerisation

- Reduction in conjugate molecular size
  - Monitor by gel permeation chromatography or HPSEC or HPSEC-MALLS
  - Easier on bulk formulated conjugate (“drug substance”) than on final fills (“drug product”)
- Increase in unconjugated (“free”) polysaccharide
  - Separate free PS from conjugate
  - Quantify saccharide in both
  - Workable with final fills

Stability measurement

- Physicochemical methods
  - Measure consistency, not biological effect
- Immunogenicity in (small) animal models
  - Not clear that animal models accurately reflect responses in human
  - Species, often in-bred
  - Obtaining statistically reliable data
- Immunogenicity data in human
  - Not ethical to give a child a degraded vaccine
  - Would need very large numbers to gain statistically relevant results
  - Immunogenicity may not be the same as protection
- We may be guessing when trying to assess how much degradation is permissible

Cold chain and enhanced stability

- Vaccines delivered to ALL the population
- Low stability products usually need a 2 – 8°C cold chain.
- WHO developed a modified protocol allowing short periods outside the cold-chain

The pre-qualified Men A conjugate vaccine can be used in a Controlled Temperature Chain (CTC) system. The label draws attention to

“**Stable up to 40°C for 4 days prior to reconstitution. Use within 6 hours of reconstitution”.**

Expect something similar for typhoid conjugate vaccines