# Analytical and Manufacturing Challenges: Preparation of Bacterial Polysaccharide Conjugates

Carl E. Frasch, Ph.D.

Biologics Consultant

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# Conjugate Vaccines: Manufacturing and quality control

**Topics to be discussed:**

- Characteristics of a conjugate vaccine
- Considerations in production of a conjugate
  - -- Polysaccharide activation
  - -- Conjugation
- Quality control and lot release
- Improving conjugation efficiency (yields)

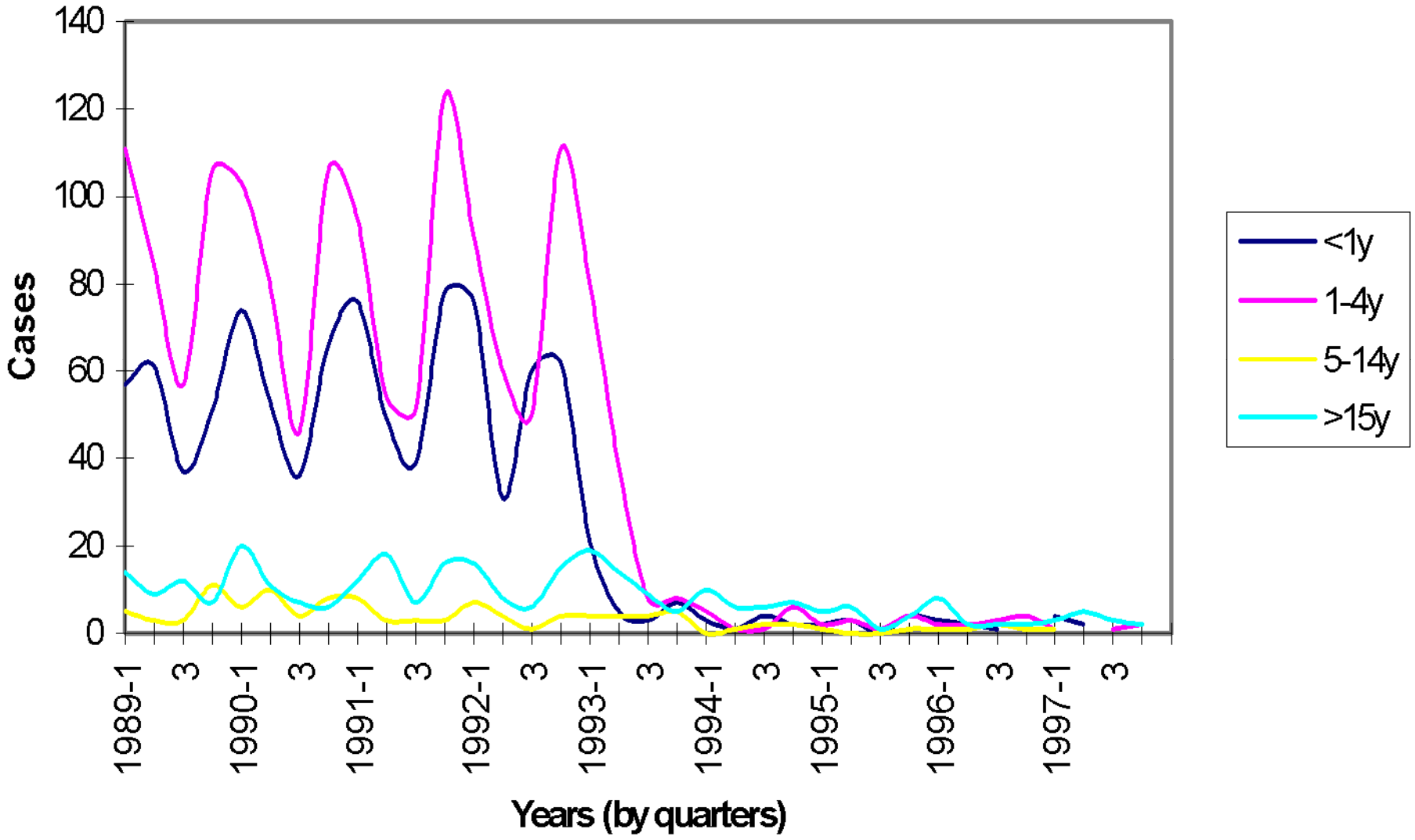
# Properties of a good polysaccharide-protein conjugate vaccine

- Has defined chemical composition and structure
- Can be manufactured with consistent physical and chemical characteristics
- Is safe - has no inherent toxicity
- Induces high avidity bactericidal or opsonic antibodies
- Induces boostable IgG antibody in infants
- Primes for response upon natural exposure to the polysaccharide

# Some currently licensed bacterial polysaccharide protein conjugate vaccines

| Organism | Vaccine manufacturer | First Licensed | Saccharide | Protein carrier | Vaccine dose (μg) |
|---|---|---|---|---|---|
| *Haemophilus influenzae* b | Wyeth | 1988 | Hib oligo | $CRM_{197}$ | 10 |
| | Merck | 1989 | Hib Sz red | OMPC | 7.5 |
| | Sanofi | 1993 | Hib PS | Tet Tox | 10 |
| | GSK | 1996 | Hib PS | Tet Tox | 10 |
| Meningococcal | Wyeth | 1999 | C Sz red | $CRM_{197}$ | 10 |
| | Baxter | 2000 | C De OAc | Tet Tox | 10 |
| | Novartis | 2000 | C oligo | $CRM_{197}$ | 10 |
| | Sanofi | 2005 | A,C,Y,W Sz red | Diph Tox | 4 each |
| Meningo/Hib | GSK | 2005 | Hib, MenC | Tet Tox | 5 each |
| **Pneumococcal** | **Wyeth** | **2000** | **4, 6B, 9V, 14, 18C, 19F 23F** | **$CRM_{197}$** | **2 (4μg 6B)** |

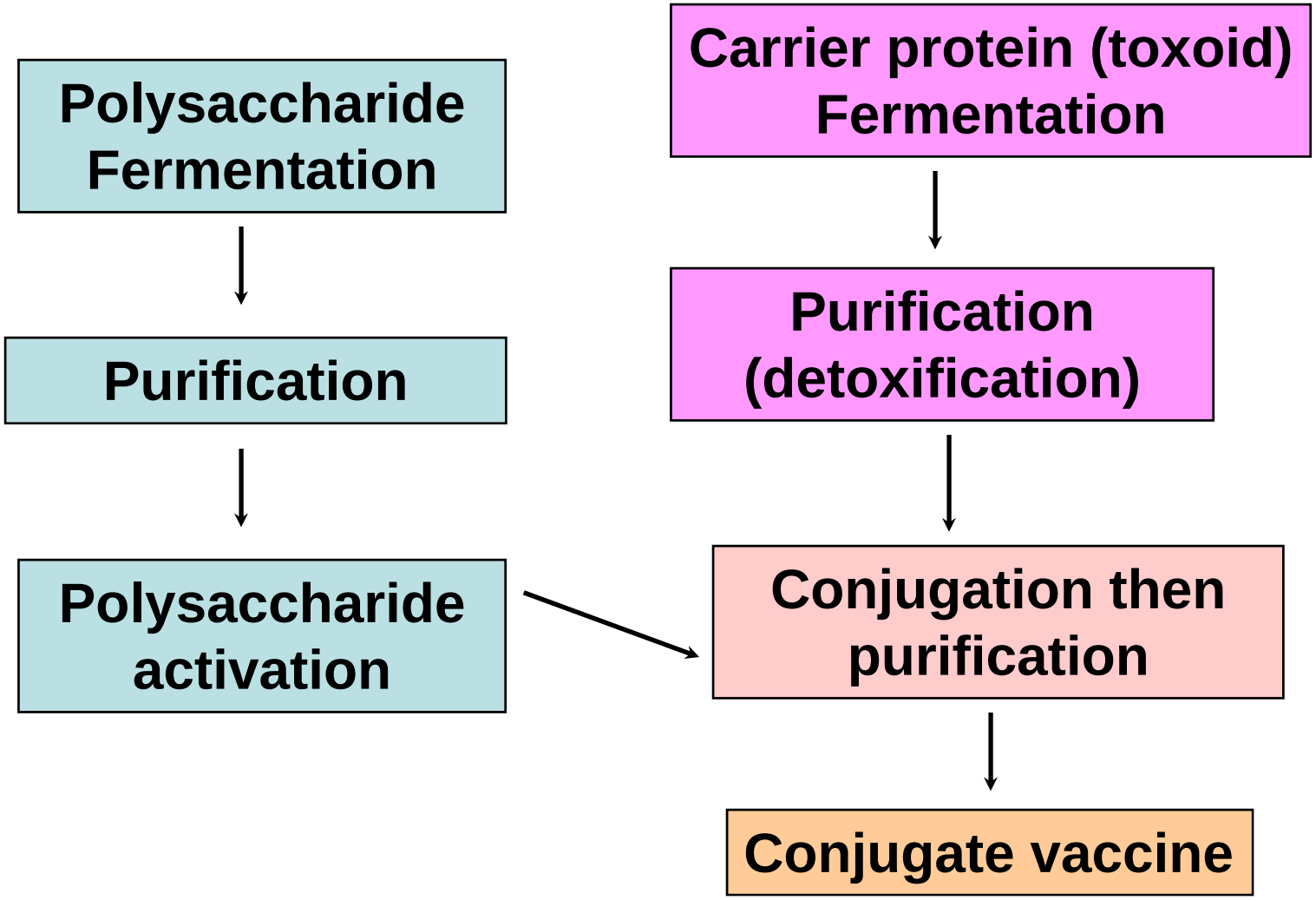
# Laboratory reports of Hib to CDSC.
# England and Wales (bacteraemia + meningitis)



# Major physical and chemical variables in production of glycoconjugate vaccines

1. Size of the polysaccharide or oligosaccharide
2. Chemistry for activation of the polysaccharide
3. Choice of carrier protein
4. Saccharide - protein conjugation chemistry
5. Saccharide loading onto protein carrier, ie, Saccharide to protein ratio

# General process for manufacture of a conjugate vaccine



# Making a conjugate

For a polysaccharide to be chemically linked to a protein, the polysaccharide must be activated, that is, chemically modified.

Methods include:

- Reductive amination
- Cyanylation
- Carbodiimide

# Activation Chemistry

Important difference between methods used to activate the polysaccharide for conjugation

**Periodate activation for reductive amination:**

Activates by cutting carbon-carbon bonds between adjacent carbons having -OH groups to create active aldehyde groups

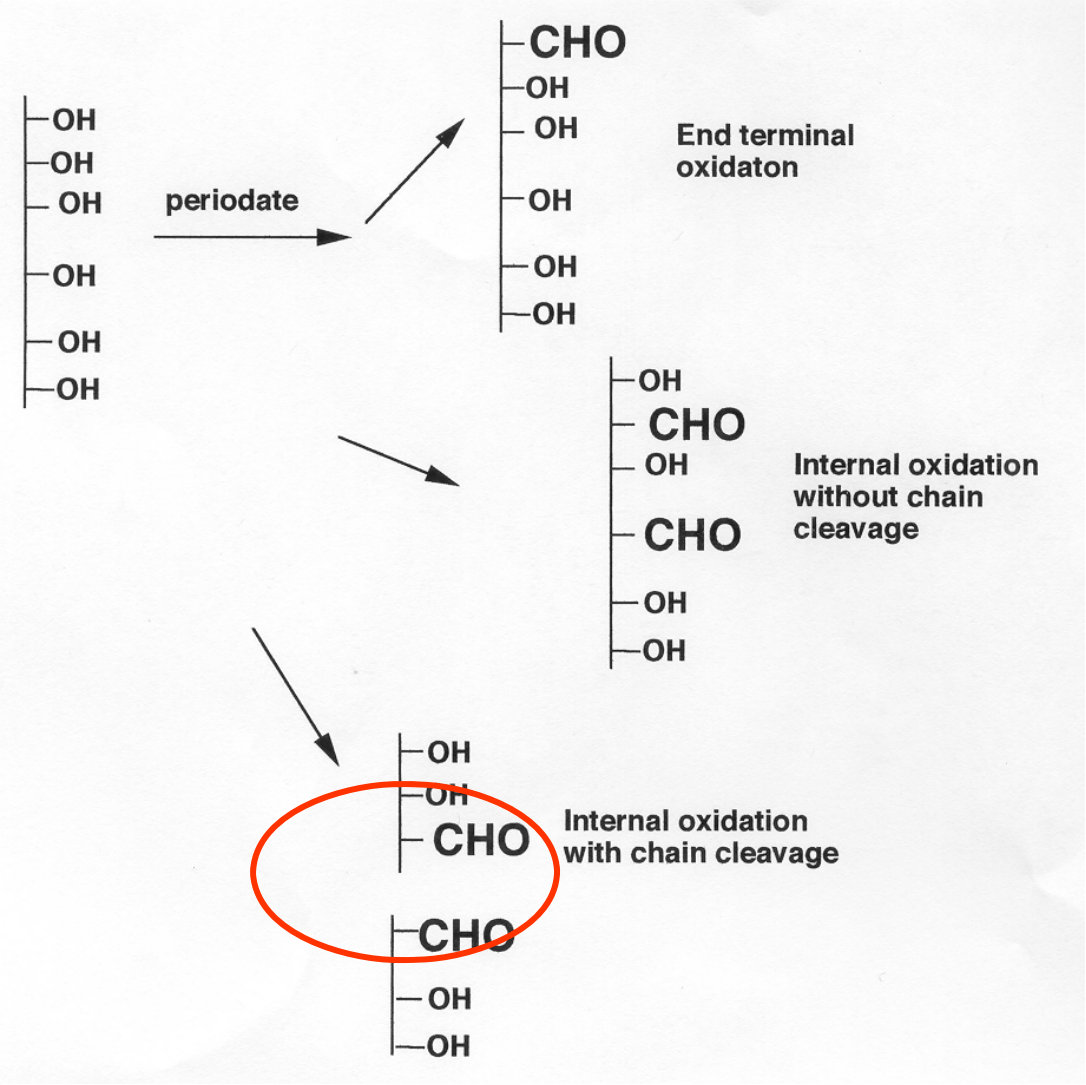
**Cyanylation**

Activtes by randomly changing –OH groups to active –CN groups

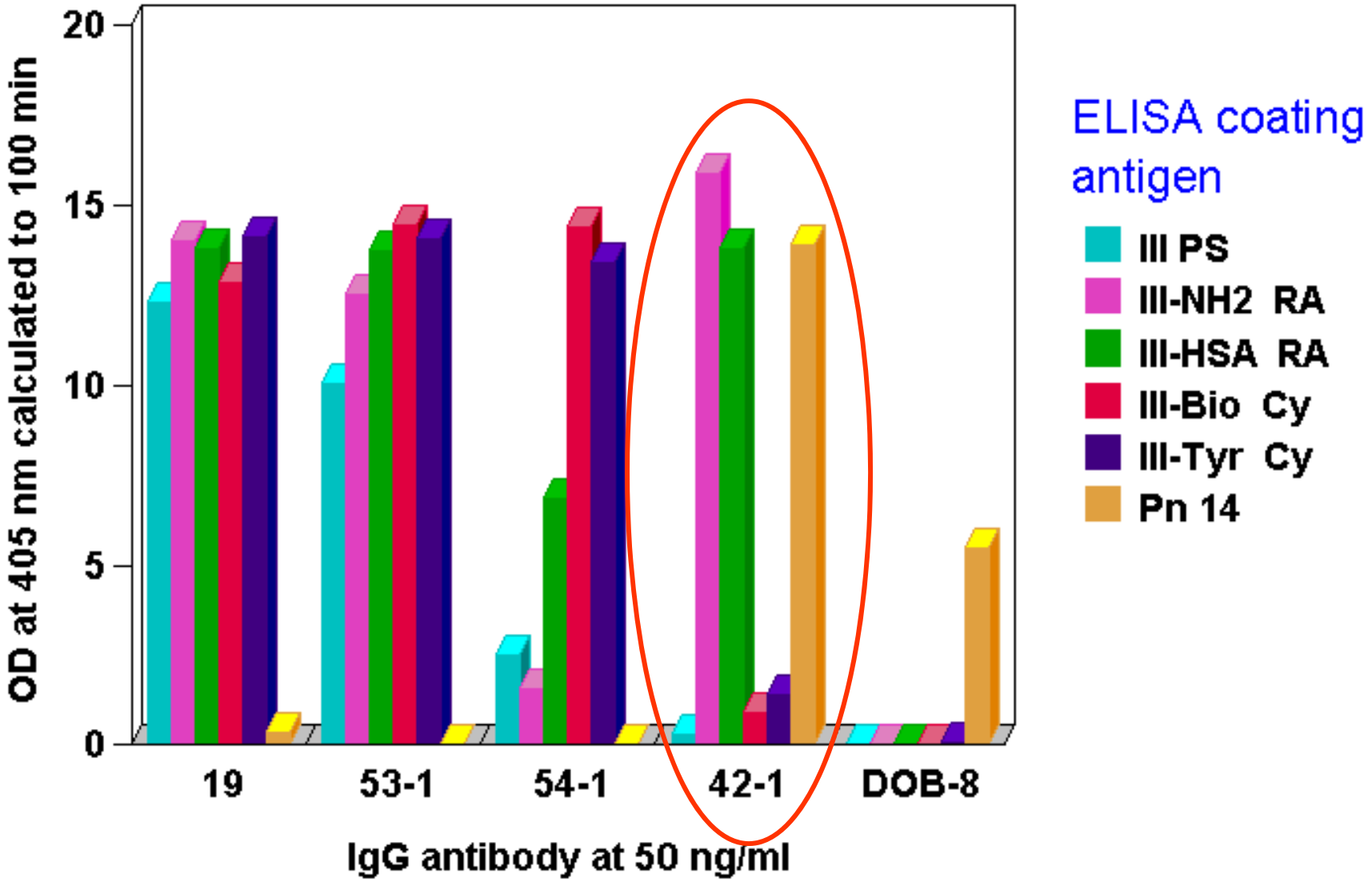
**Carbodiimide**

Activates at carboxyl groups by replacing –OH with carbodiimide

# Periodate oxidation of polysaccharides



# Comparative binding of antibodies at 50 ng/ml to different antigen preparations



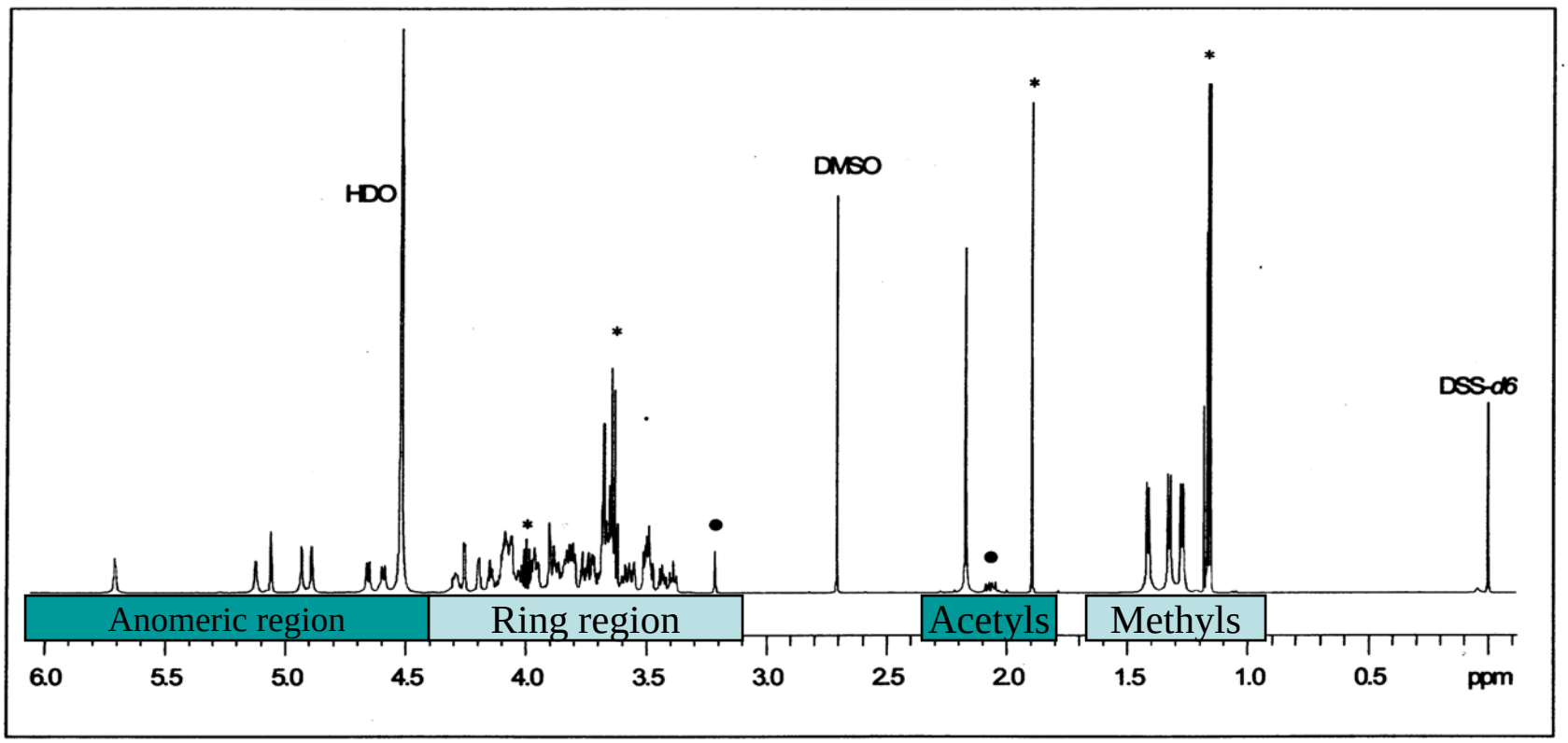
# Important lot release tests for polysaccharide-protein conjugate vaccines

- Purity of polysaccharide and carrier protein
- Saccharide of known molecular size distribution
- Degree of saccharide activation
- Molecular size of conjugate (a stability measure)
- Vaccine Potency:
  - Polysaccharide to protein ratio in conjugate
  - Percent non-conjugated (free) saccharide present in monovalent conjugate bulks

# Newer physical methods to analyze polysaccharide component of a conjugate

| | |
|---|---|
| Identity | NMR |
| Structure | NMR |
| Purity | NMR |
| Quantitation | NMR |
| Stability | SEC-HPLC, NMR |
| Size | SEC-HPLC, MALLS |

# 600-MHz proton spectrum of pneumococcal type 17F polysaccharide



# Stability indicating quality control tests for monovalent bulks and final vaccine

- Change in the molecular size of conjugate during storage
- Increase in free (unbound) saccharide over time
- Change in conjugate solubility during storage
- Change in pH during storage
- Change in degree of adsorption, if vaccine adjuvant adsorbed

# Conjugation efficiency (yields)

- It is very difficult to discern manufacturing yields
- Some manufacturers report high yields based upon recovery of the carrier protein (not relevant)
- Some do not account for losses during activation
- Yields can be much higher when both the polysaccharide and protein are activated before conjugation

**Yields:**

- Utilize native amino groups – about 10 to 30%
- Utilize activated protein -- about 50 %

# New Aldehyde Conjugation Chemistry using **activated proteins** to improve **yields**

1. **Hydrazone formation**

   Aldehyde/hydrazide/reduction

   Benzaldehyde/hydrazide

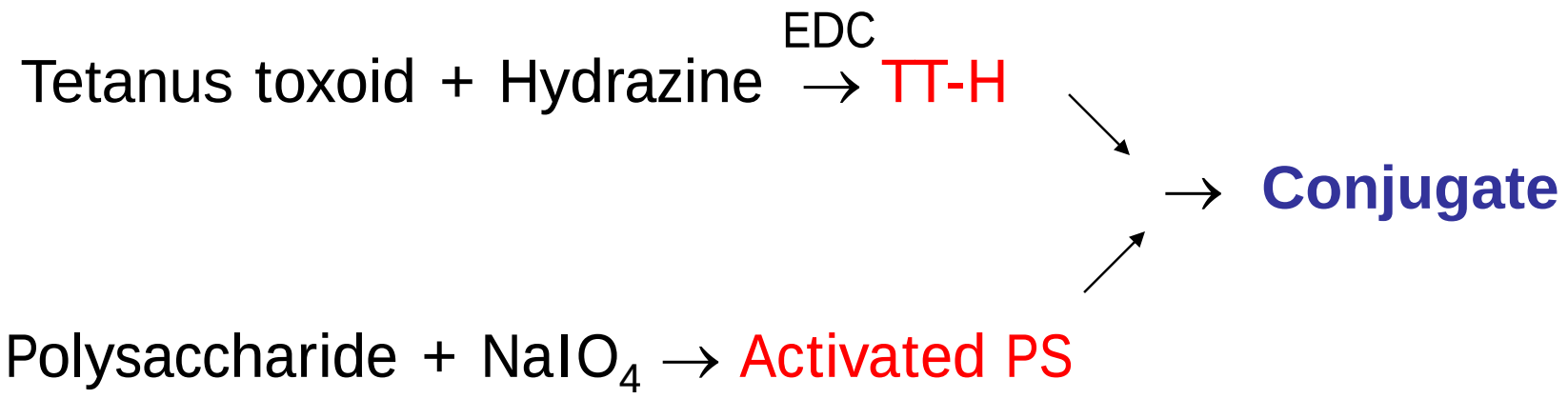
   Activates through the carboxyl groups, not amino

Hydrazones are the condensation of hydrazide or hydrazine with aldehyde

2. **Oxime formation**

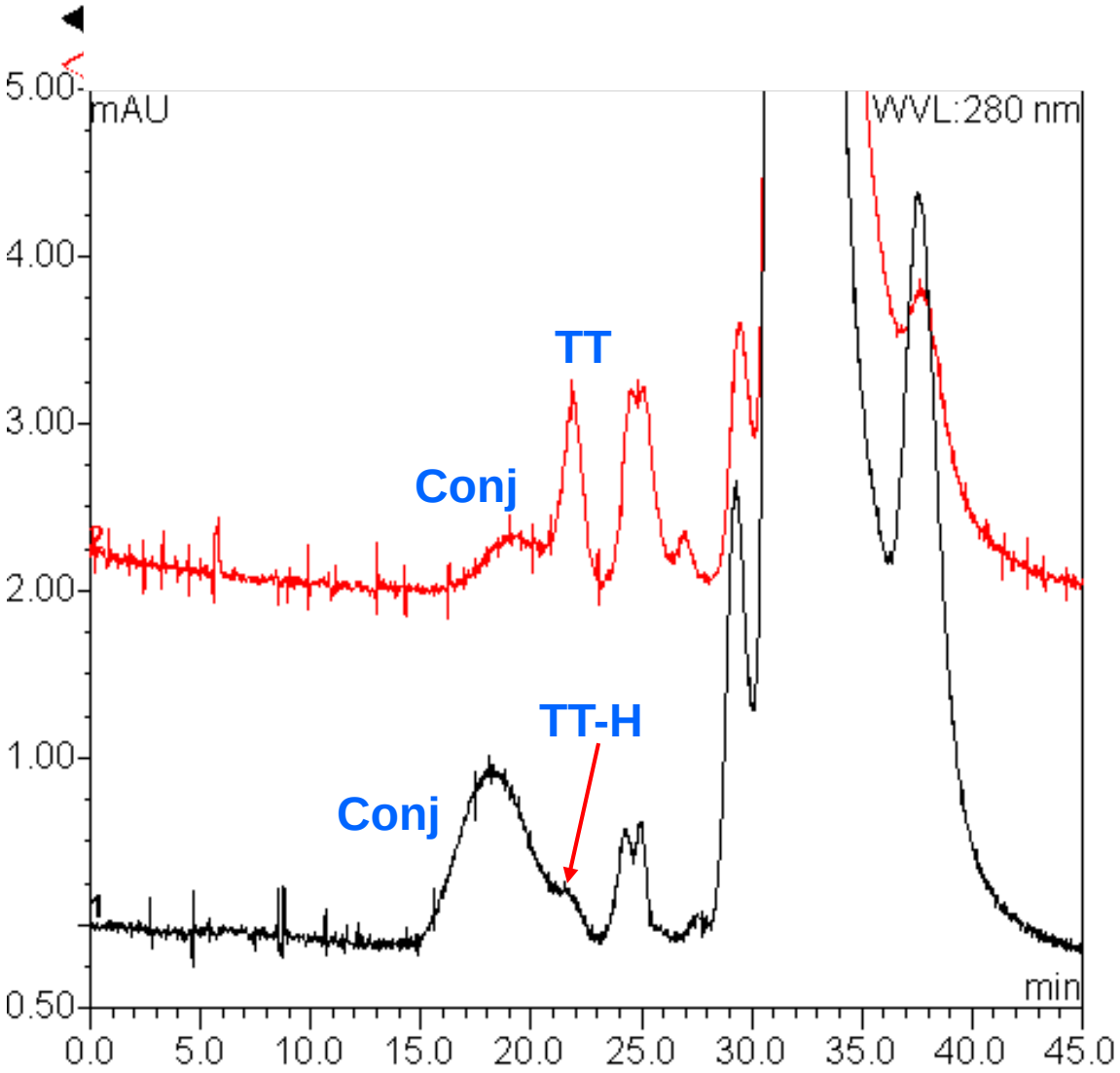
   Creates a highly reactive aminooxy group

# Schematics for Preparation of Polysaccharide-Tetanus Toxoid Conjugate using Hydrazide Chemistry



# Conjugates made with hydrazide activated tetanus toxoid (TT-H) versus unmodified tetanus toxoid (TT)

**SE-HPLC**



# Conclusions

- ✓ There are several methodologies for manufacture of saccharide-protein conjugate vaccines
- ✓ The polysaccharide or oligosaccharide must be chemically activated for conjugation to occur
- ✓ It is important to carefully consider the chemistry used for saccharide acvtivation
- ✓ Activation of both the protein and the polysaccharide will improve conjugation yields
- ✓ Quality control and lot release testing are described in **WHO *Technical Report*** series publications for Hib, meningococcal and pneumococcal conjugate vaccines