FIGURE 1

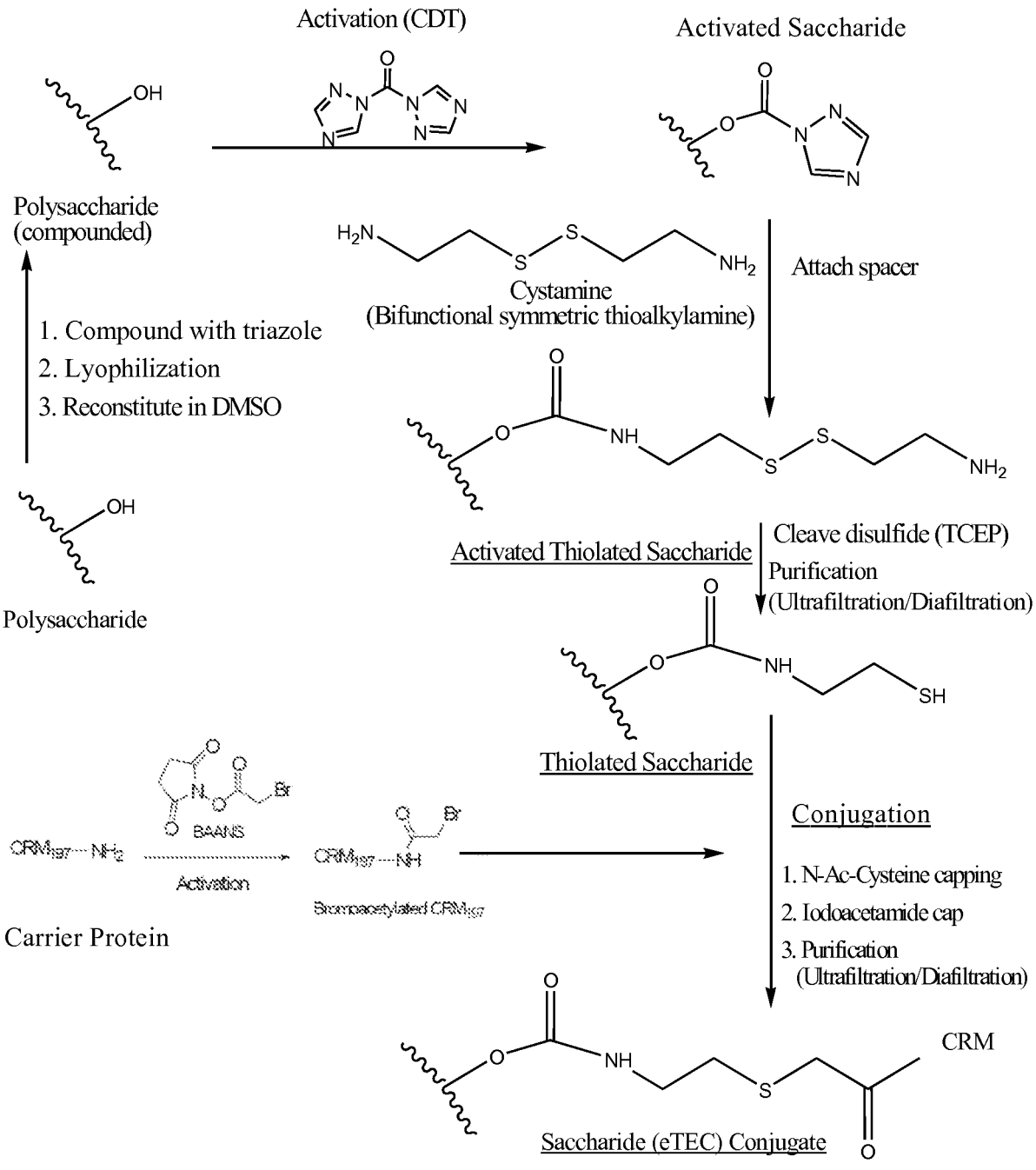

Activation (CDT)
Activated Saccharide
Polysaccharide (compounded)
Cystamine
(Bifunctional symmetric thioalkylamine)
Attach spacer
1. Compound with triazole
2. Lyophilization
3. Reconstitute in DMSO
Activated Thiolated Saccharide
Cleave disulfide (TCEP)
Purification
(Ultrafiltration/Diafiltration)
Polysaccharide
Thiolated Saccharide
BAANS
Activation
Bromoacetylated CRM197
Carrier Protein
Conjugation
1. N-Ac-Cysteine capping
2. Iodoacetamide cap
3. Purification
(Ultrafiltration/Diafiltration)
CRM
Saccharide (eTEC) Conjugate

FIGURE 2

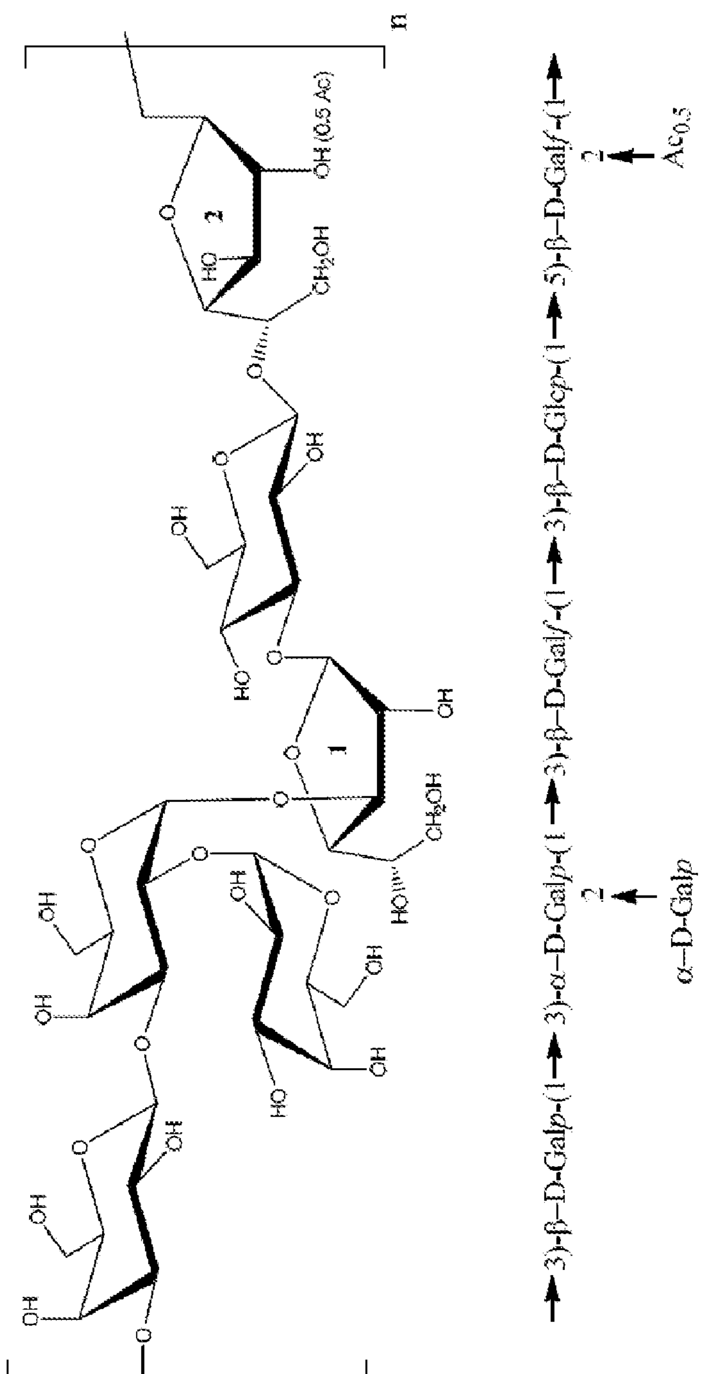

n
1
2
OH (0.5 Ac)
CH2OH
→3)-β-D-Galp-(1→3)-α-D-Galp-(1→3)-β-D-Galf-(1→3)-β-D-Glcp-(1→5)-β-D-Galf-(1→
α-D-Galp
Ac0.5

FIGURE 3

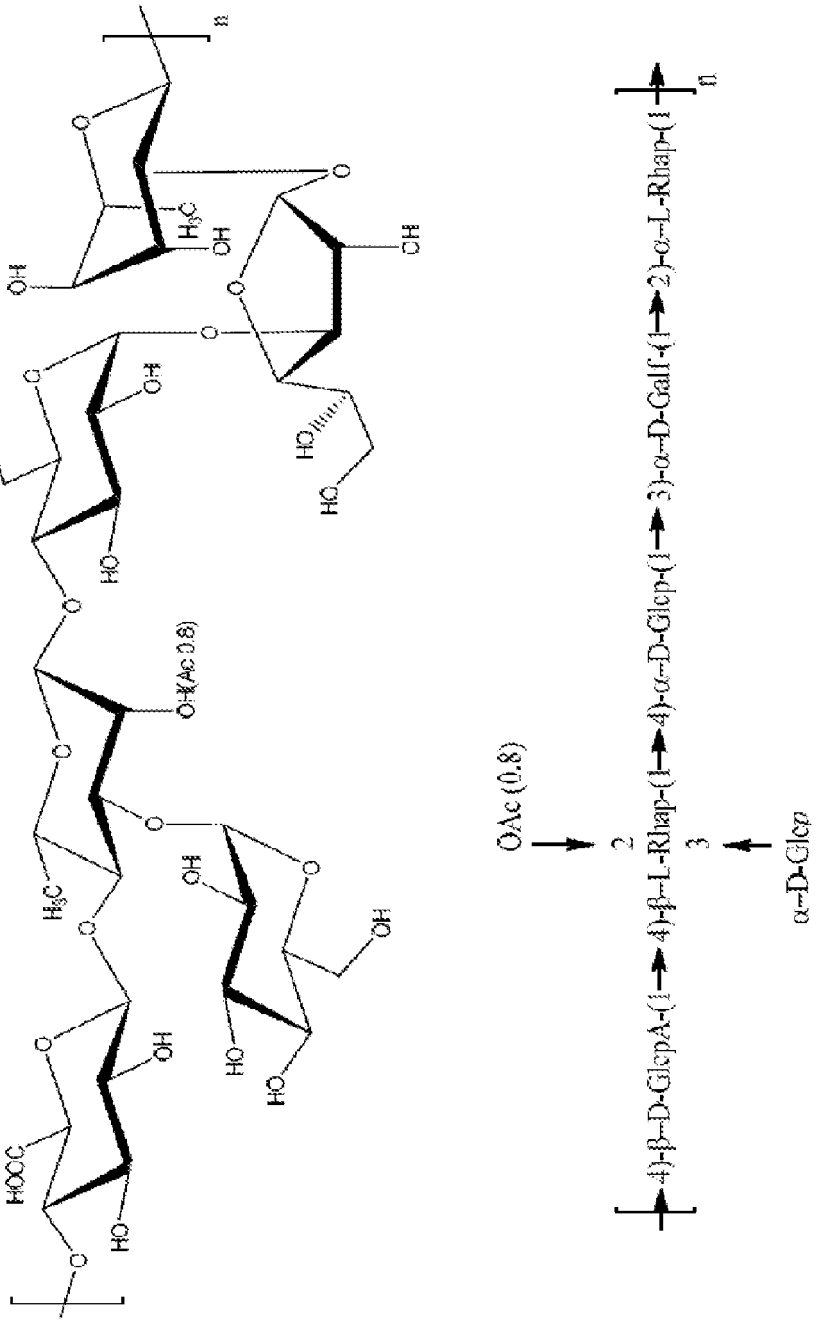


FIGURE 4

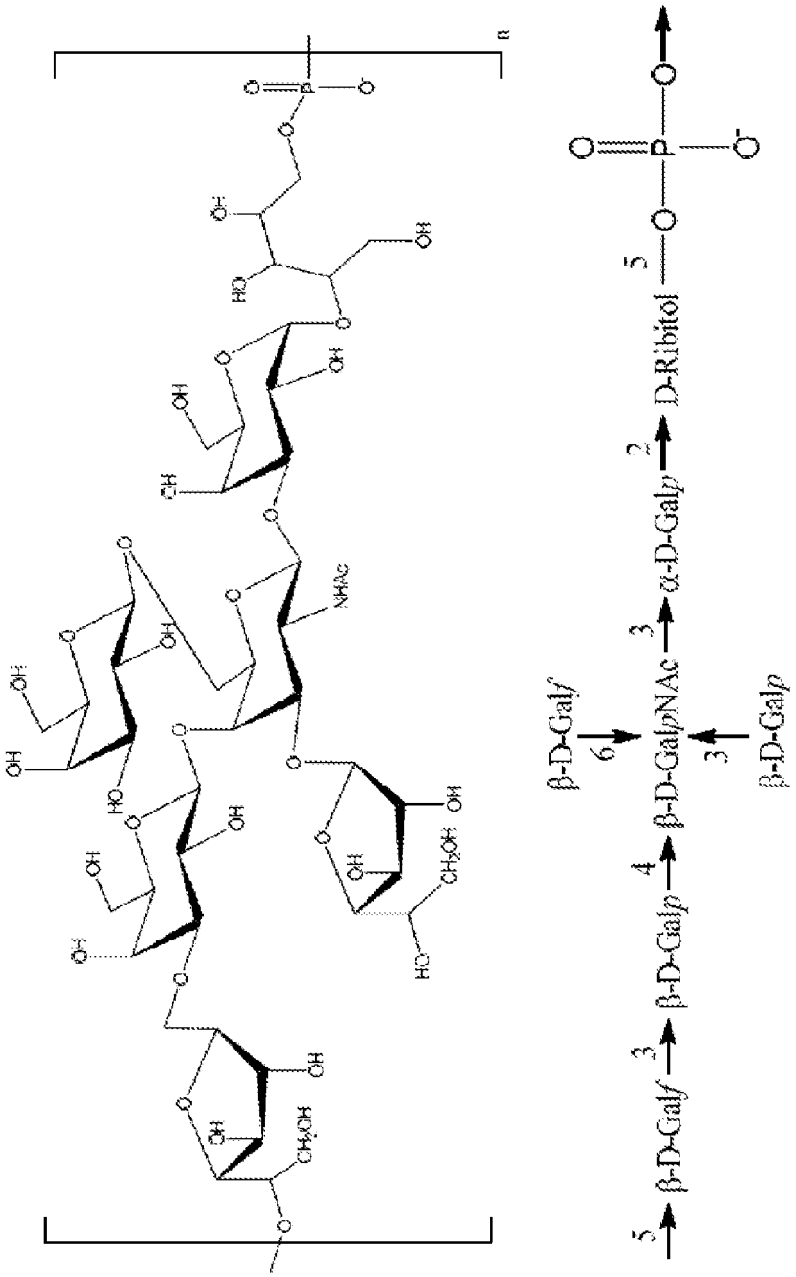


FIGURE 5

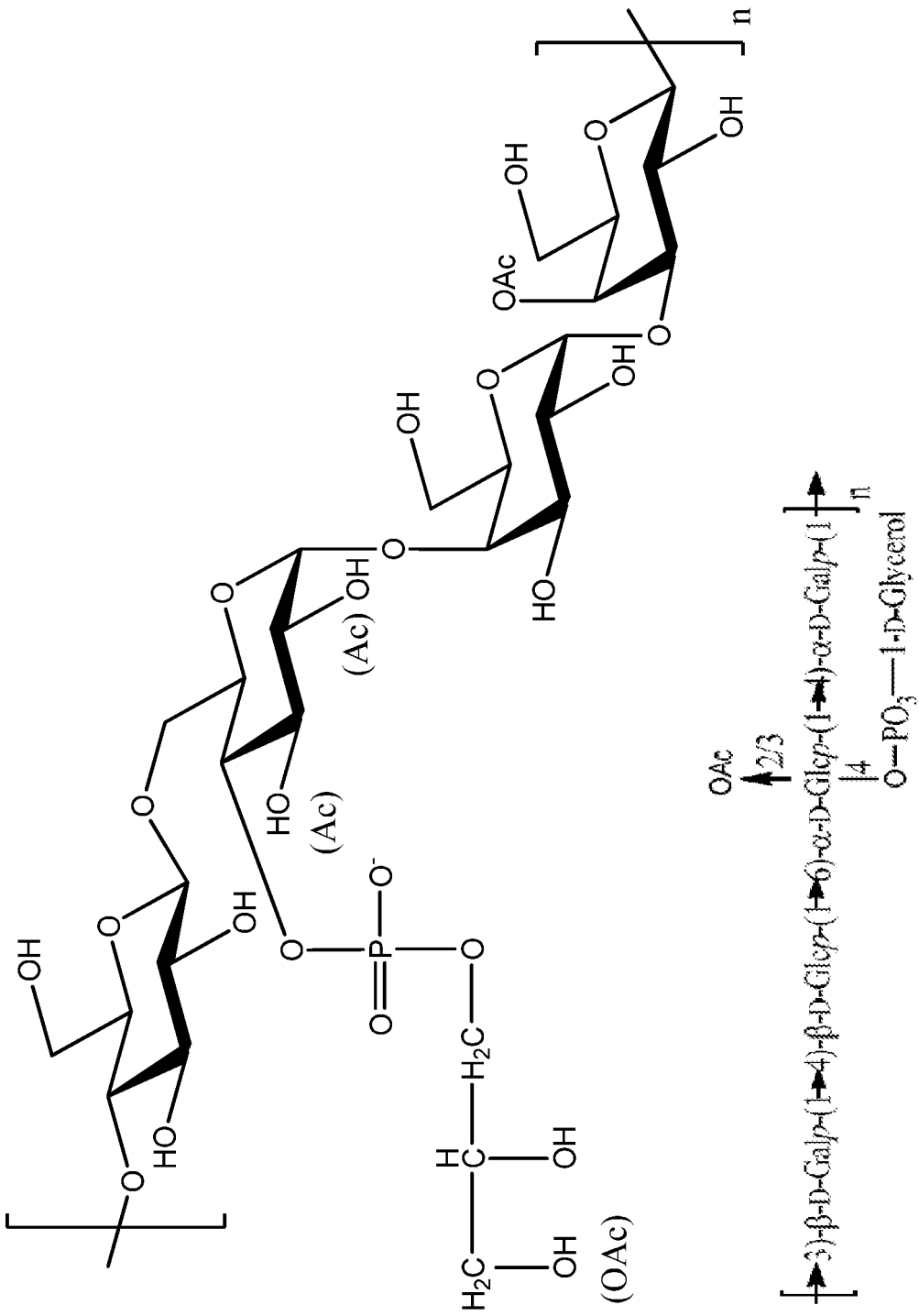

OAc
2/3
4
n
3)-β-D-Galp-(1→4)-β-D-Glcp-(1→6)-α-D-Glcp-(1→4)-α-D-Galp-(1
O—PO3—1-D-Glycerol

FIGURE 6A

A)

Pneumo Serotype 33F-CRM_{197} Conjugate (eTEC Chemistry)

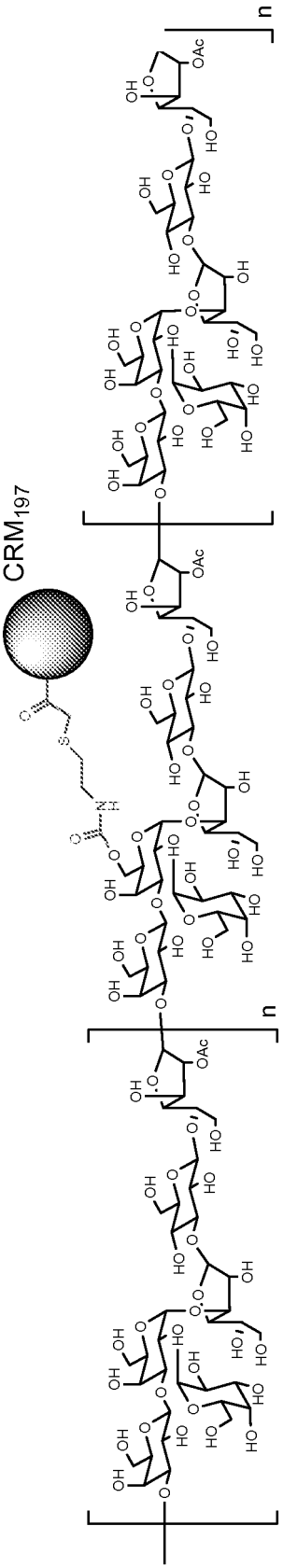


FIGURE 6B

B)

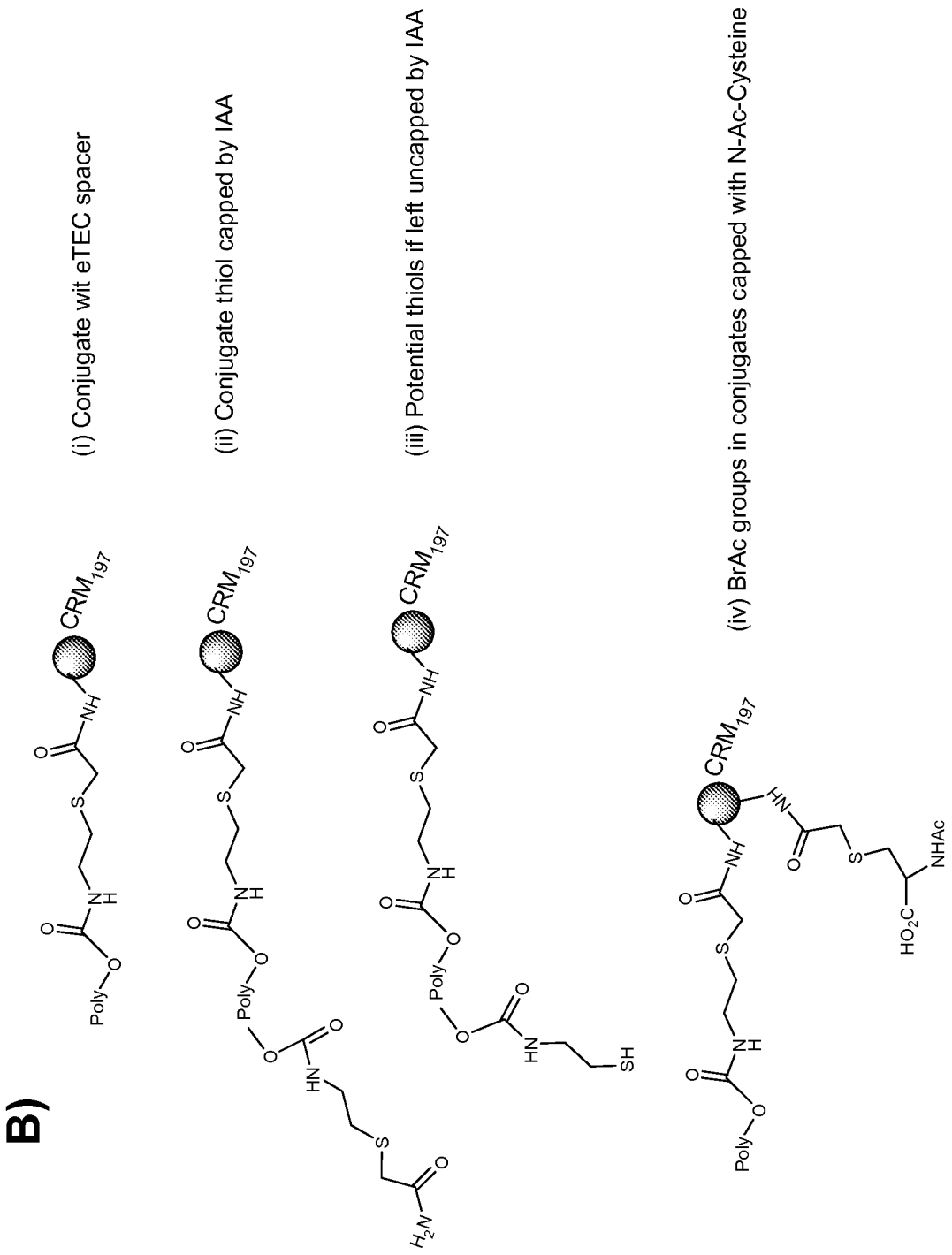

(i) Conjugate wit eTEC spacer
(ii) Conjugate thiol capped by IAA
(iii) Potential thiols if left uncapped by IAA
(iv) BrAc groups in conjugates capped with N-Ac-Cysteine
CRM197
Poly
NHAc

FIGURE 7

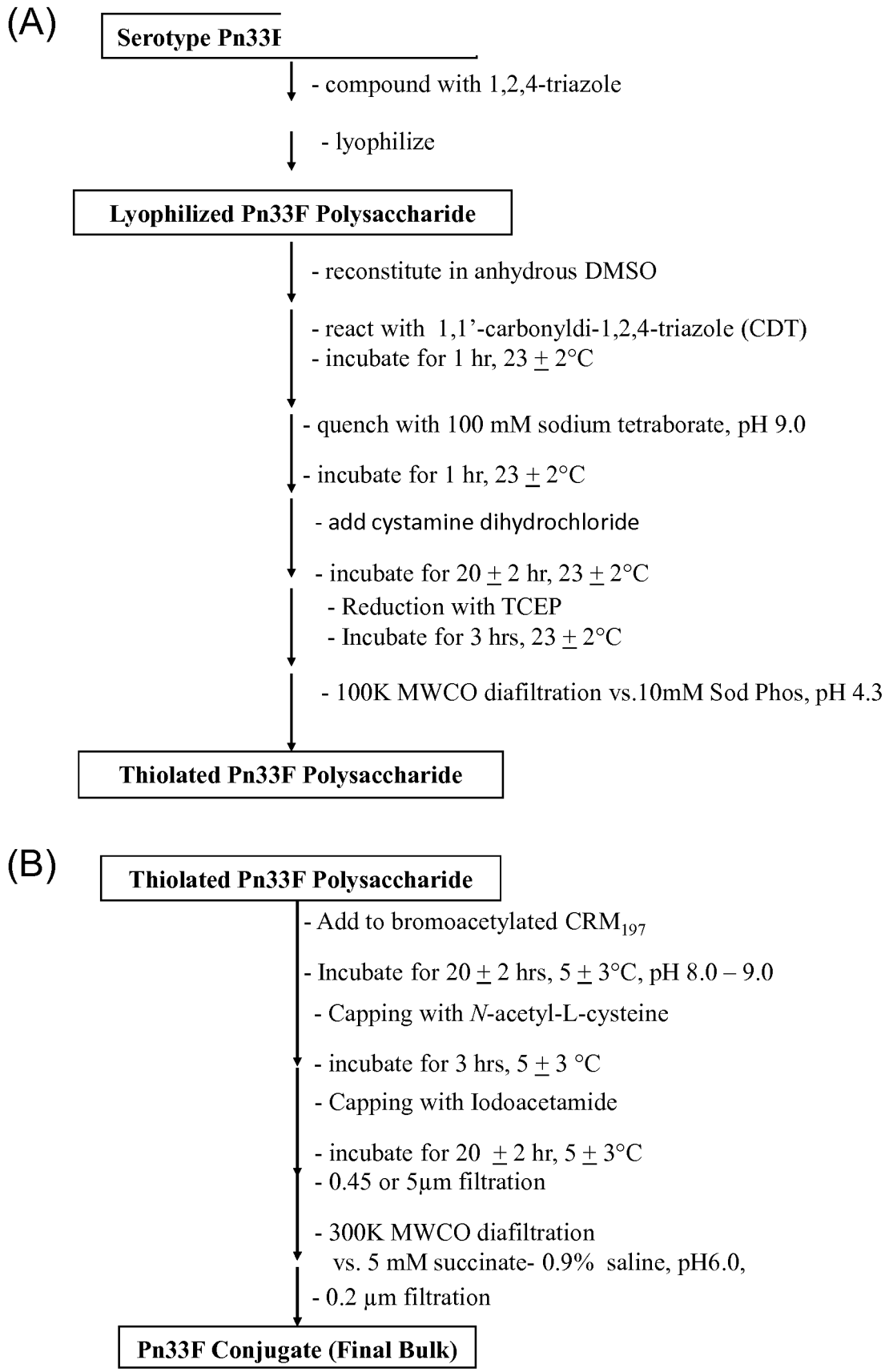
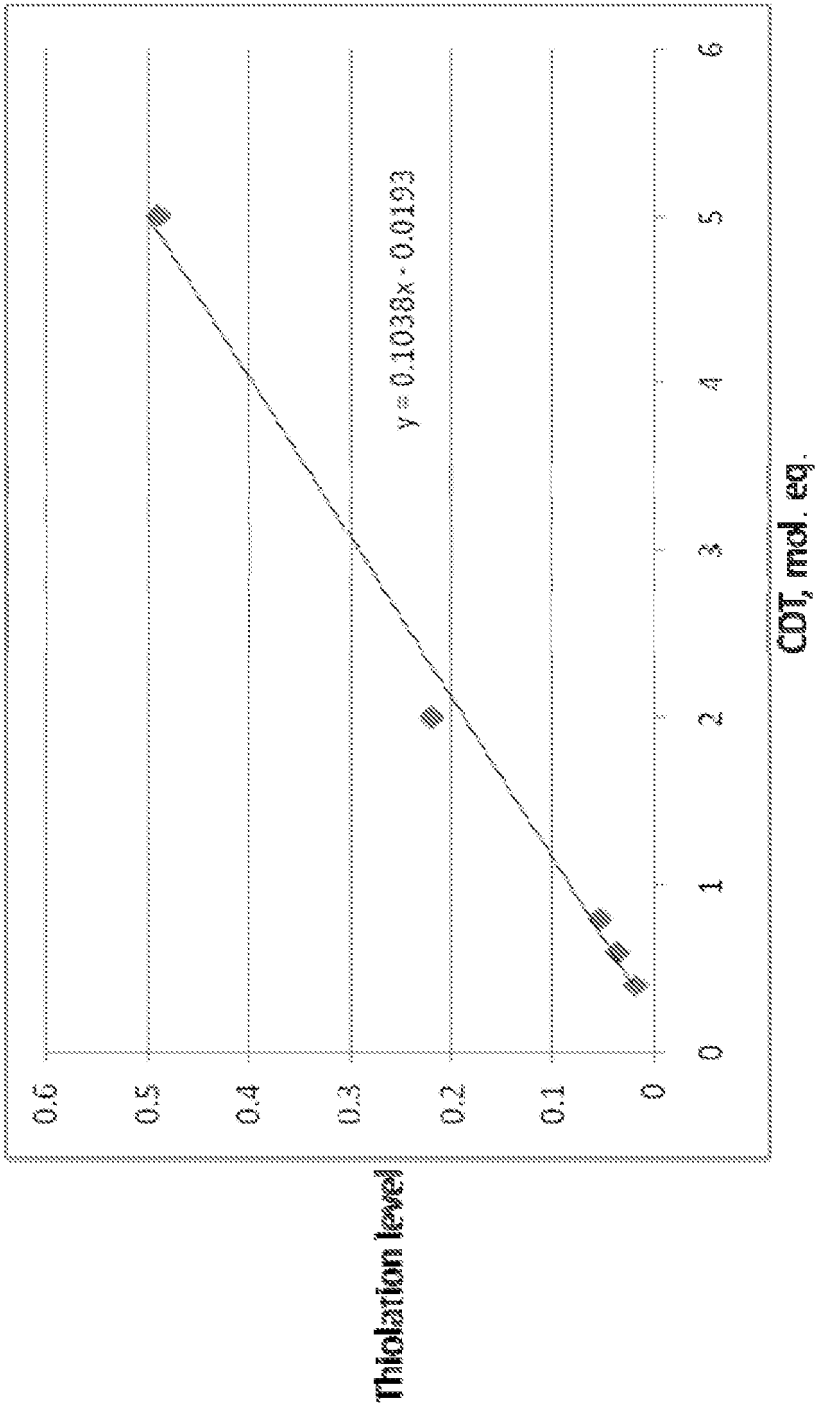

(A)
Serotype Pn33F
- compound with 1,2,4-triazole
- lyophilize
Lyophilized Pn33F Polysaccharide
- reconstitute in anhydrous DMSO
- react with 1,1'-carbonyldi-1,2,4-triazole (CDT)
- incubate for 1 hr, 23 ± 2°C
- quench with 100 mM sodium tetraborate, pH 9.0
- incubate for 1 hr, 23 ± 2°C
- add cystamine dihydrochloride
- incubate for 20 ± 2 hr, 23 ± 2°C
- Reduction with TCEP
- Incubate for 3 hrs, 23 ± 2°C
- 100K MWCO diafiltration vs.10mM Sod Phos, pH 4.3
Thiolated Pn33F Polysaccharide
(B)
Thiolated Pn33F Polysaccharide
- Add to bromoacetylated CRM197
- Incubate for 20 ± 2 hrs, 5 ± 3°C, pH 8.0 – 9.0
- Capping with N-acetyl-L-cysteine
- incubate for 3 hrs, 5 ± 3 °C
- Capping with Iodoacetamide
- incubate for 20 ± 2 hr, 5 ± 3°C
- 0.45 or 5µm filtration
- 300K MWCO diafiltration
vs. 5 mM succinate- 0.9% saline, pH6.0,
- 0.2 µm filtration
Pn33F Conjugate (Final Bulk)

FIGURE 8


Thiolation level
0.6
0.5
0.4
0.3
0.2
0.1
0
y = 0.1038x - 0.0193
0
1
2
3
4
5
6
CDT, mol. eq.