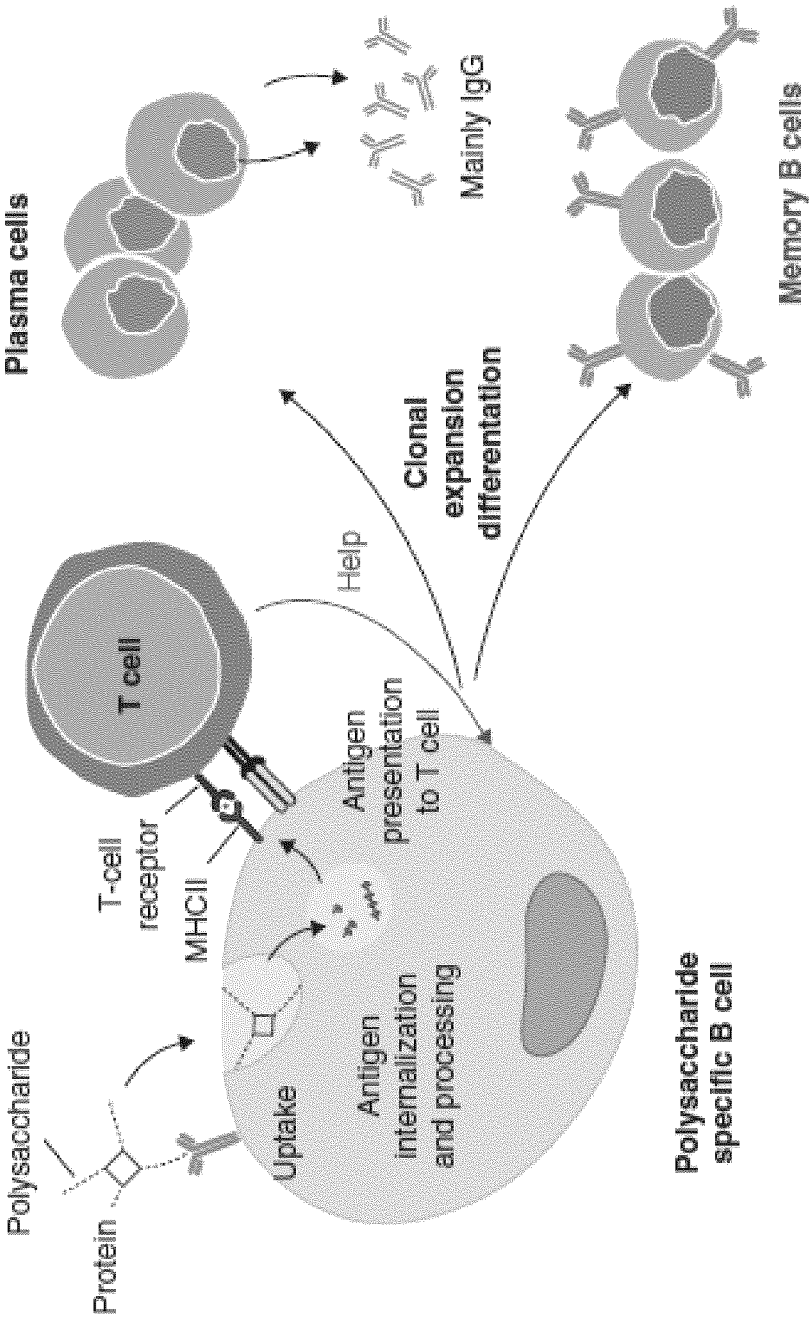

Plasma cells
Mainly IgG
Memory B cells
Clonal expansion differentiation
Help
T cell
T-cell receptor
MHCII
Antigen presentation to T cell
Uptake
Antigen internalization and processing
Polysaccharide
Protein
Polysaccharide specific B cell

Figure 1

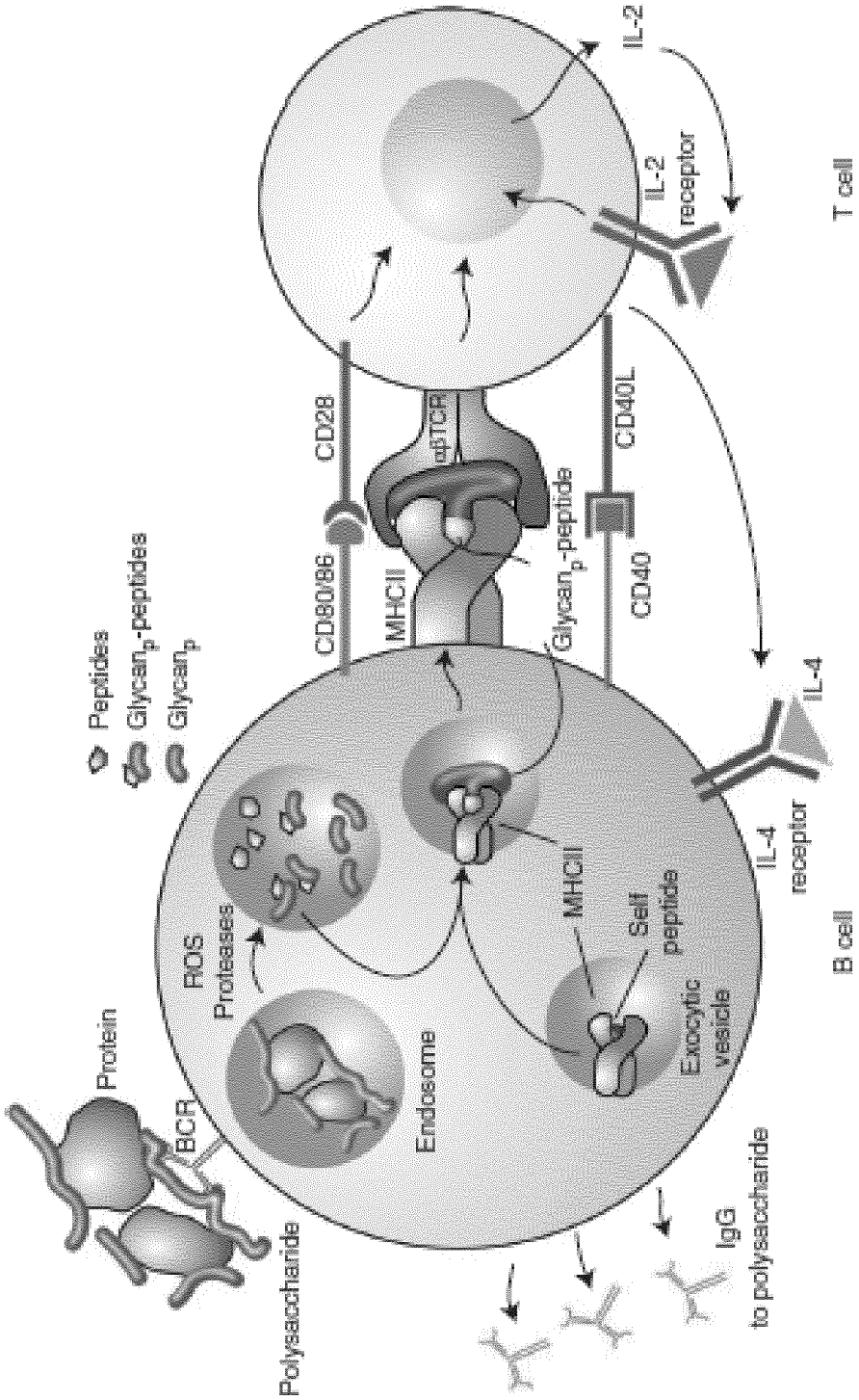

Peptides
Glycan$_p$-peptides
Glycan$_p$
Protein
BCR
Polysaccharide
ROS
Proteases
Endosome
MHCII
Self peptide
Exocytic vesicle
B cell
CD80/86
CD28
MHCII
αβTCR
Glycan$_p$-peptide
CD40
CD40L
IL-2
IL-2 receptor
T cell
IL-4
IL-4 receptor
IgG
to polysaccharide

Figure 2

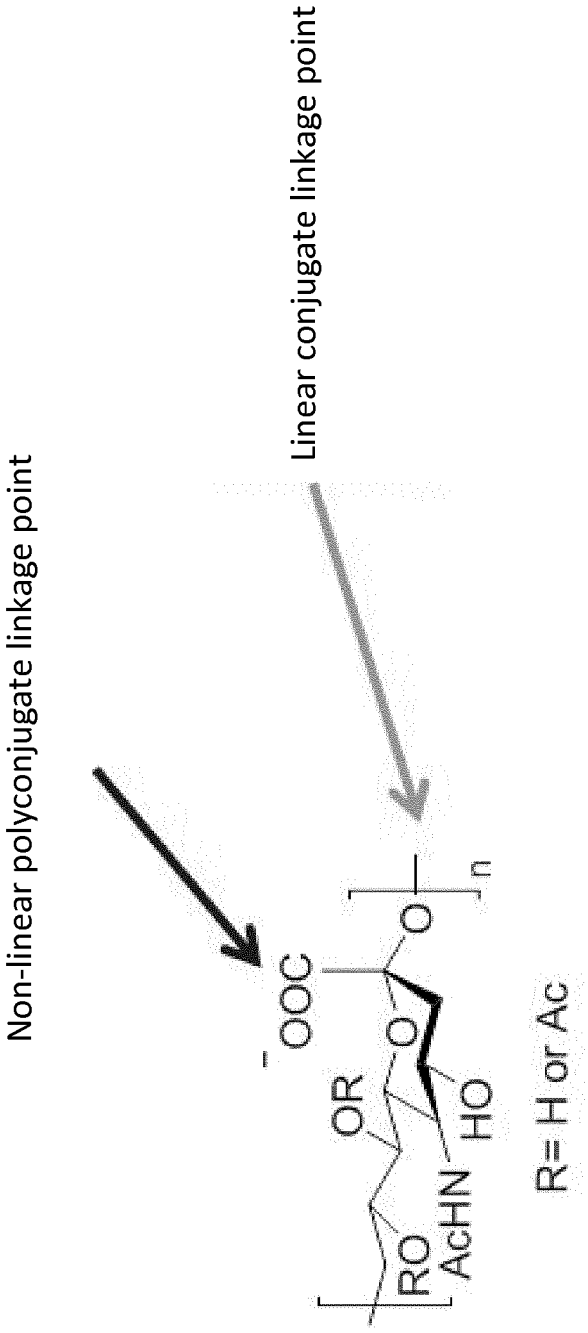

Non-linear polyconjugate linkage point
Linear conjugate linkage point
- OOC
OR
RO
AcHN
HO
O
O
n
R= H or Ac

Figure 3

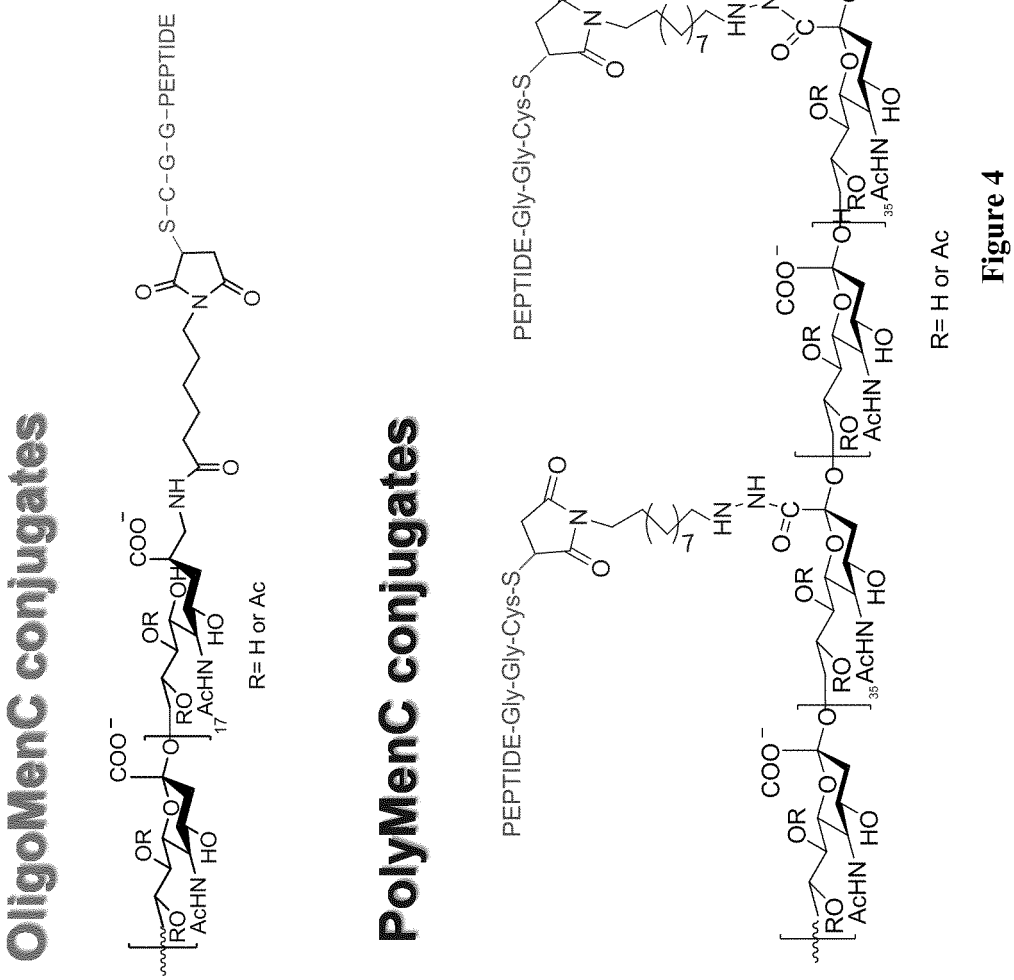

OligoMenC conjugates
S-C-G-G-PEPTIDE
R= H or Ac
PolyMenC conjugates
PEPTIDE-Gly-Gly-Cys-S
R= H or Ac

Figure 4

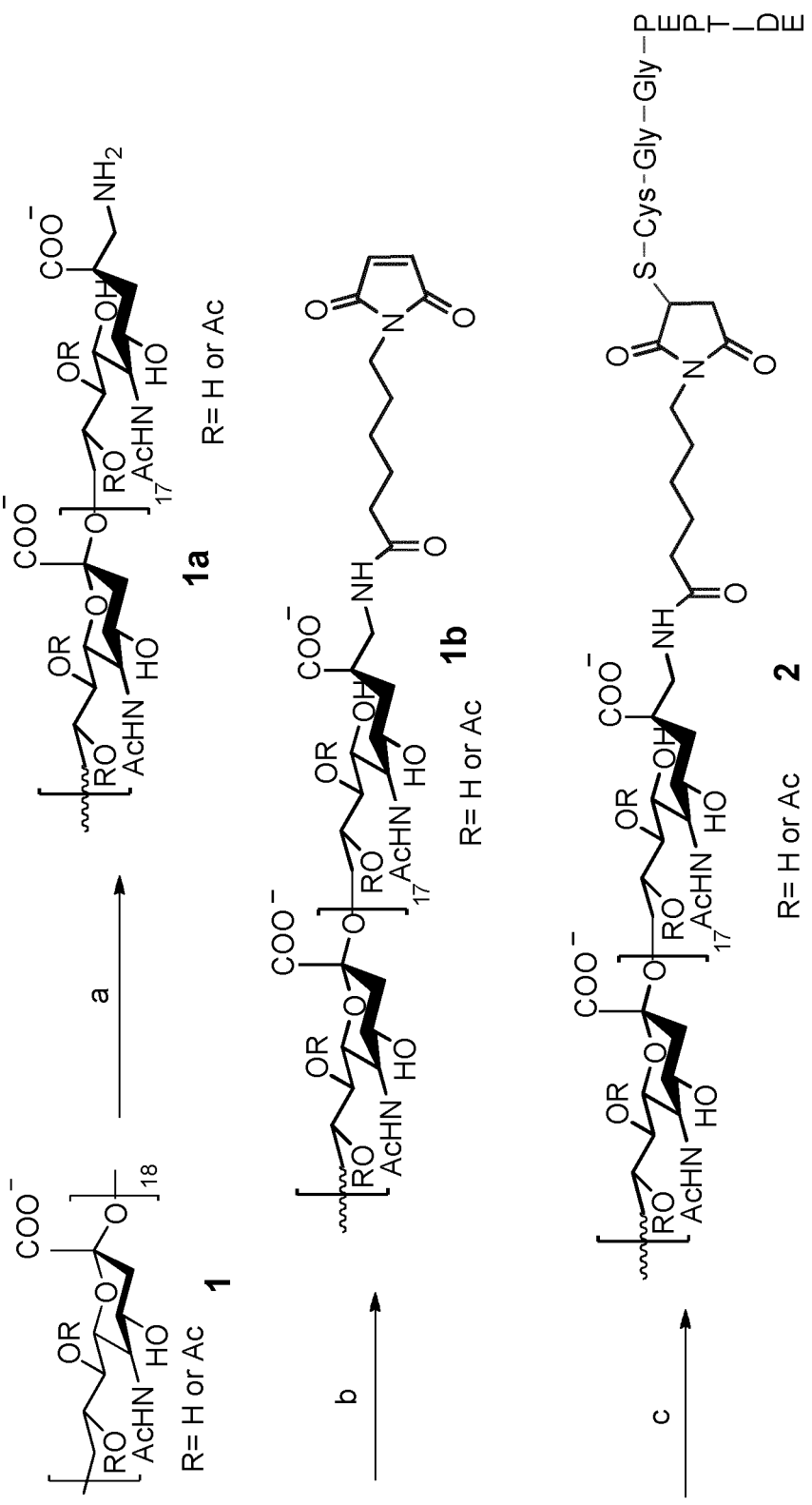


Figure 5

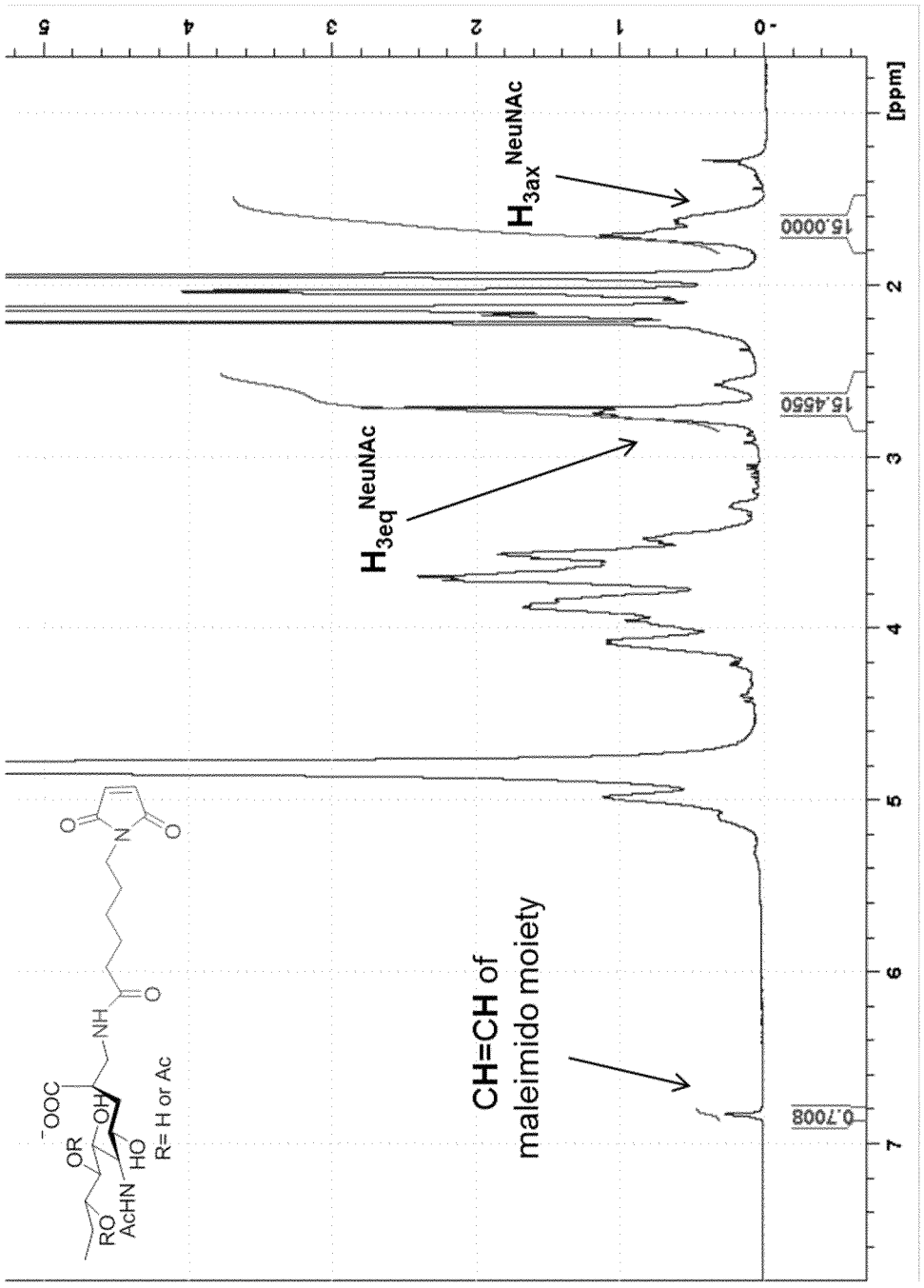

[ppm]
15.0000
15.4550
0.7008
H_{3ax}^{NeuNAc}
H_{3eq}^{NeuNAc}
CH=CH of
maleimido moiety
R= H or Ac

Figure 6

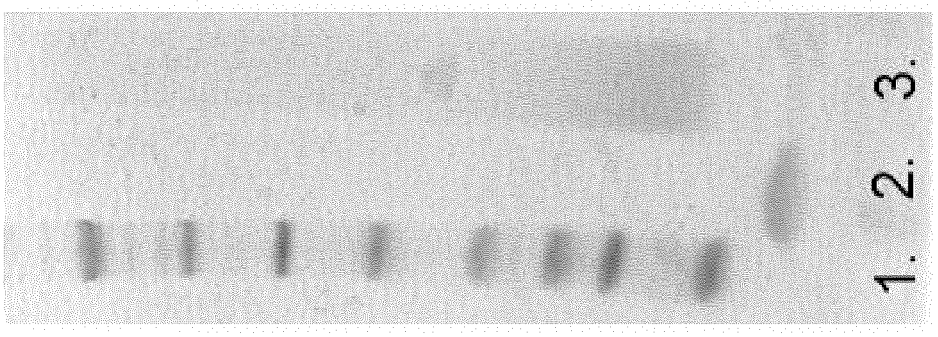


Figure 7

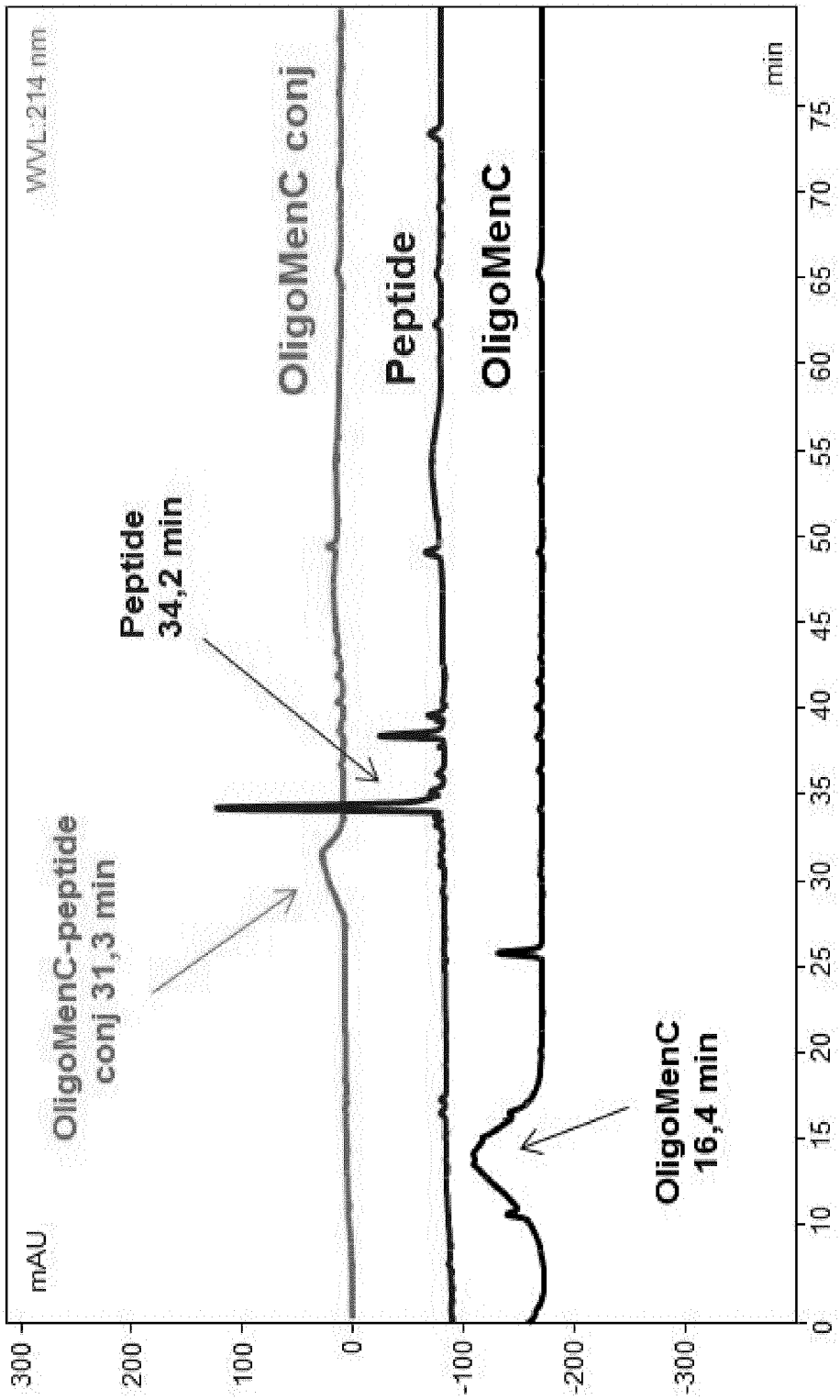

WVL:214 nm
mAU
min
OligoMenC conj
Peptide
OligoMenC
Peptide
34,2 min
OligoMenC-peptide
conj 31,3 min
OligoMenC
16,4 min
300
200
100
0
-100
-200
-300
0
10
15
20
25
30
35
40
45
50
55
60
65
70
75

Figure 8

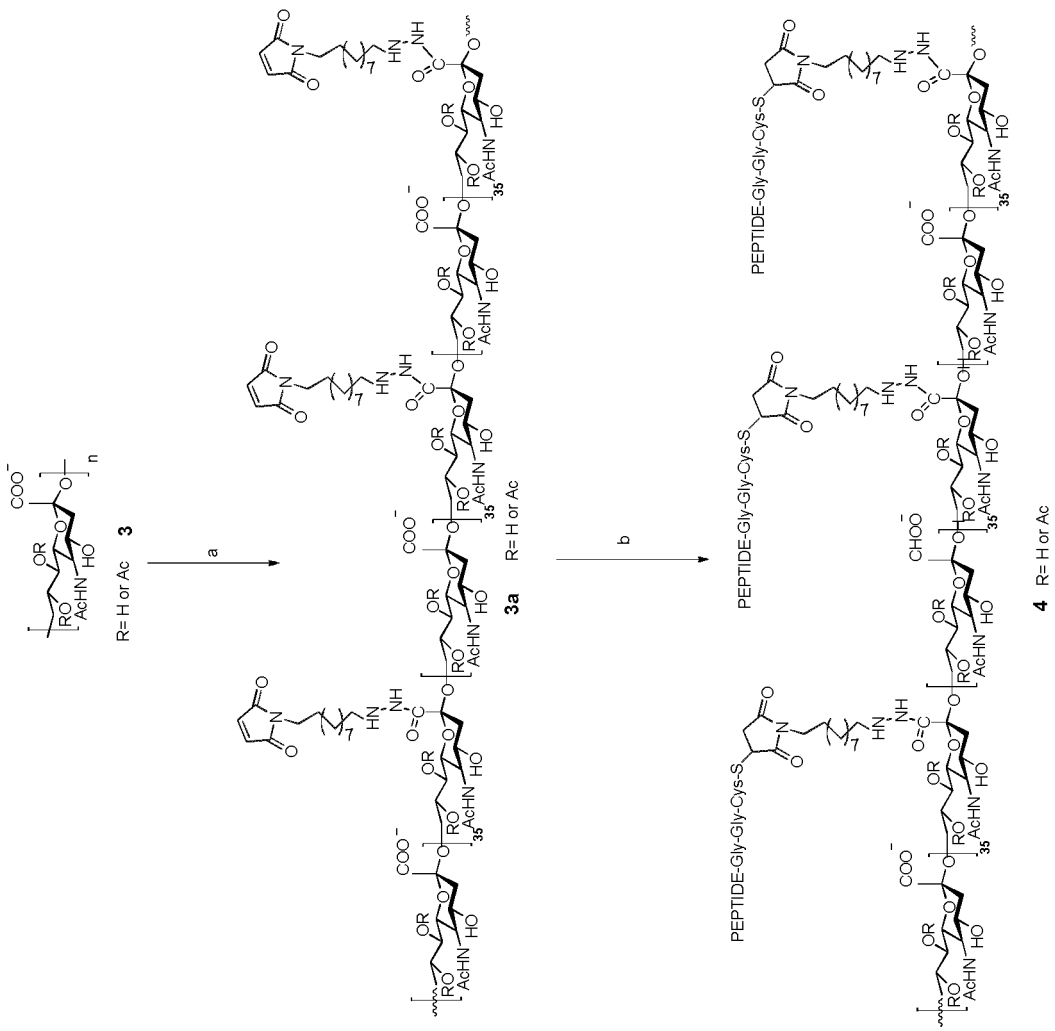


Figure 9

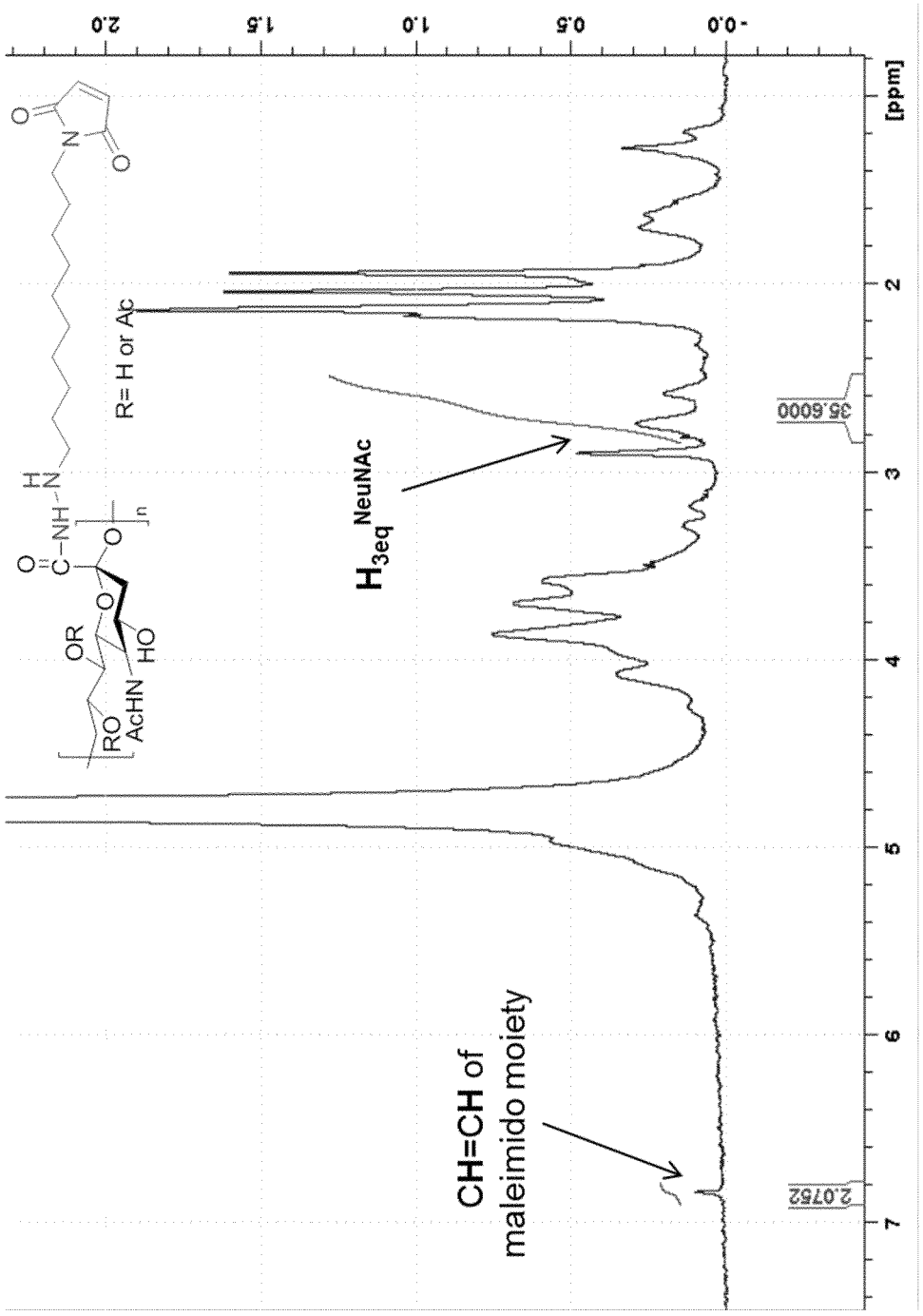

CH=CH of
maleimido moiety
H_{3eq}^{NeuNAc}
R= H or Ac
2.0752
36.6000
[ppm]

Figure 10

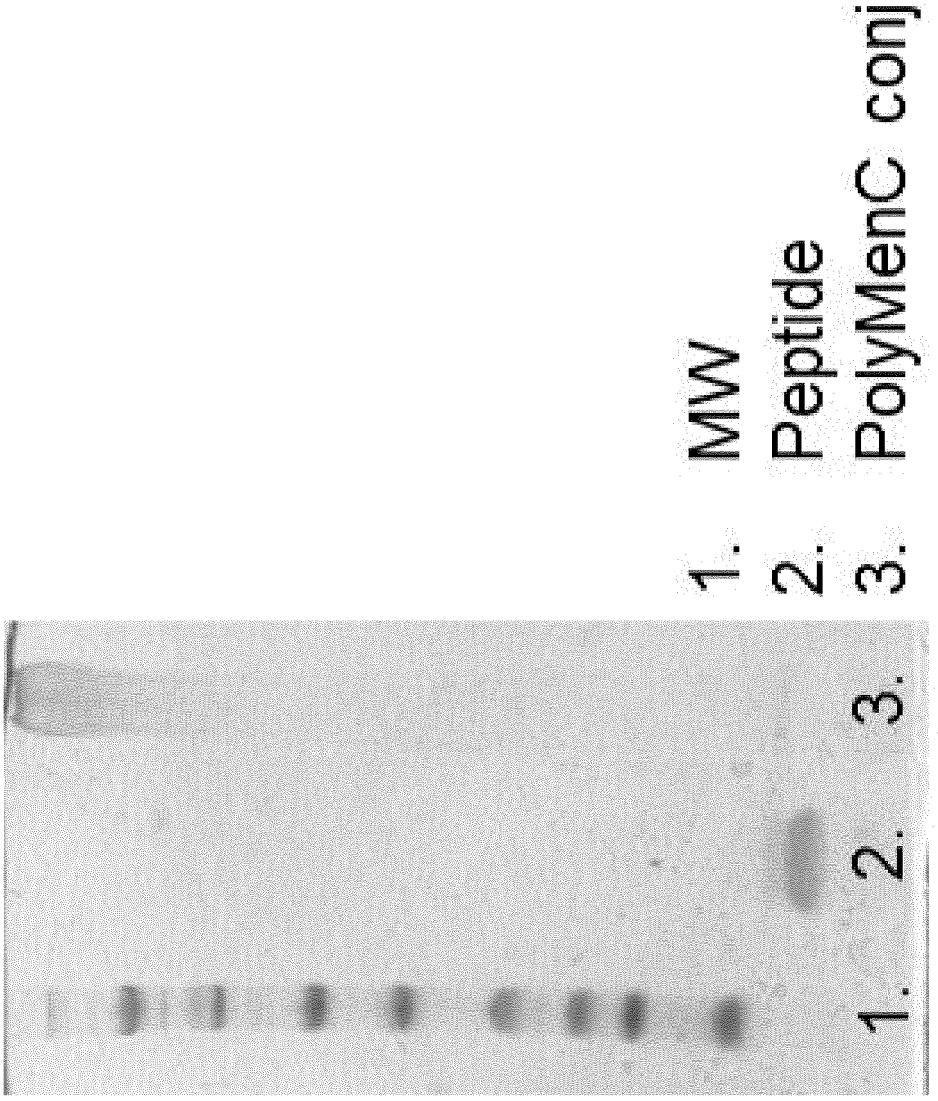


Figure 11

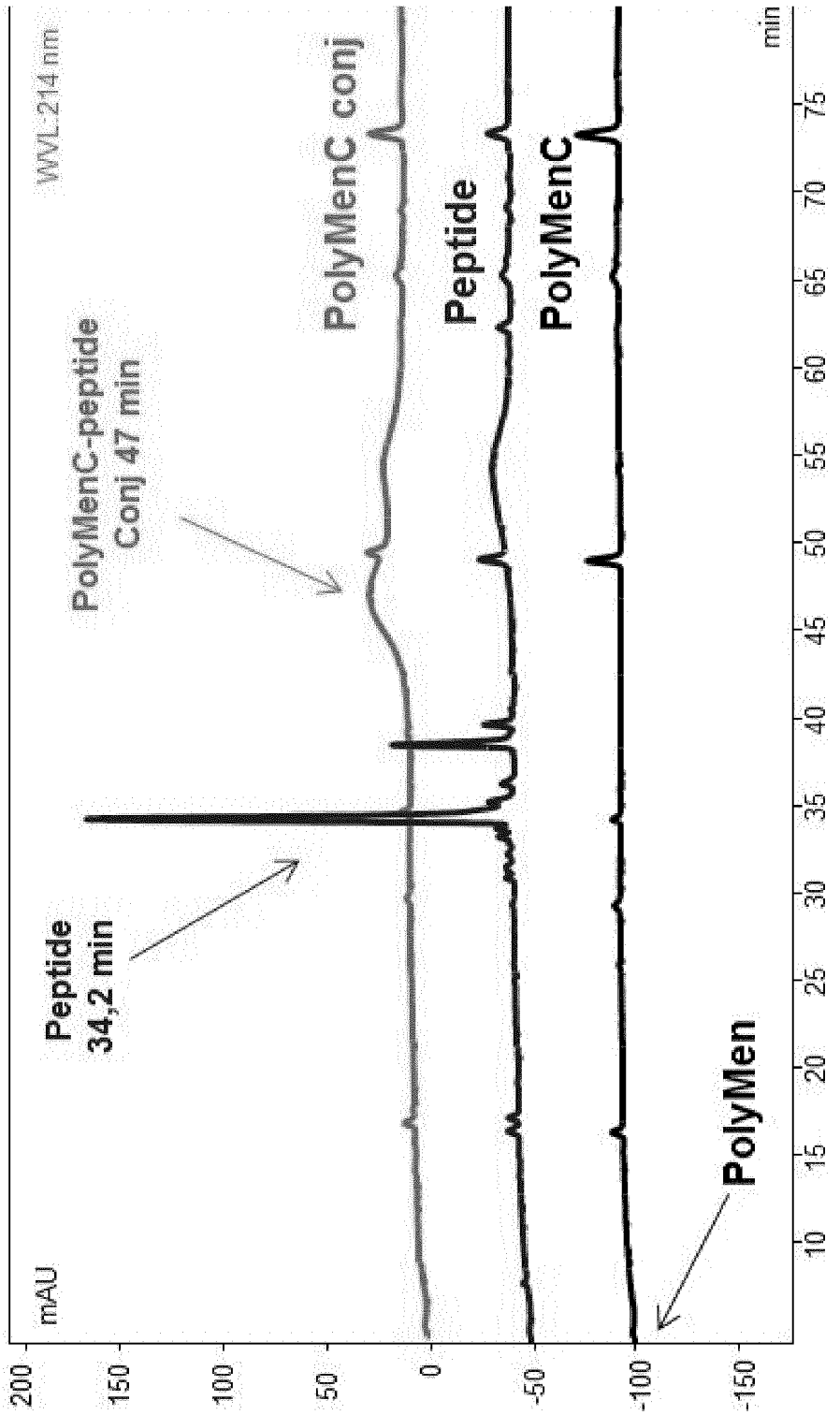

WVL:214 nm
mAU
min
PolyMenC-peptide
Conj 47 min
Peptide
34,2 min
PolyMenC conj
Peptide
PolyMenC
PolyMen
200
150
100
50
0
-50
-100
-150
10
15
20
25
30
35
40
45
50
55
60
65
70
75

Figure 12

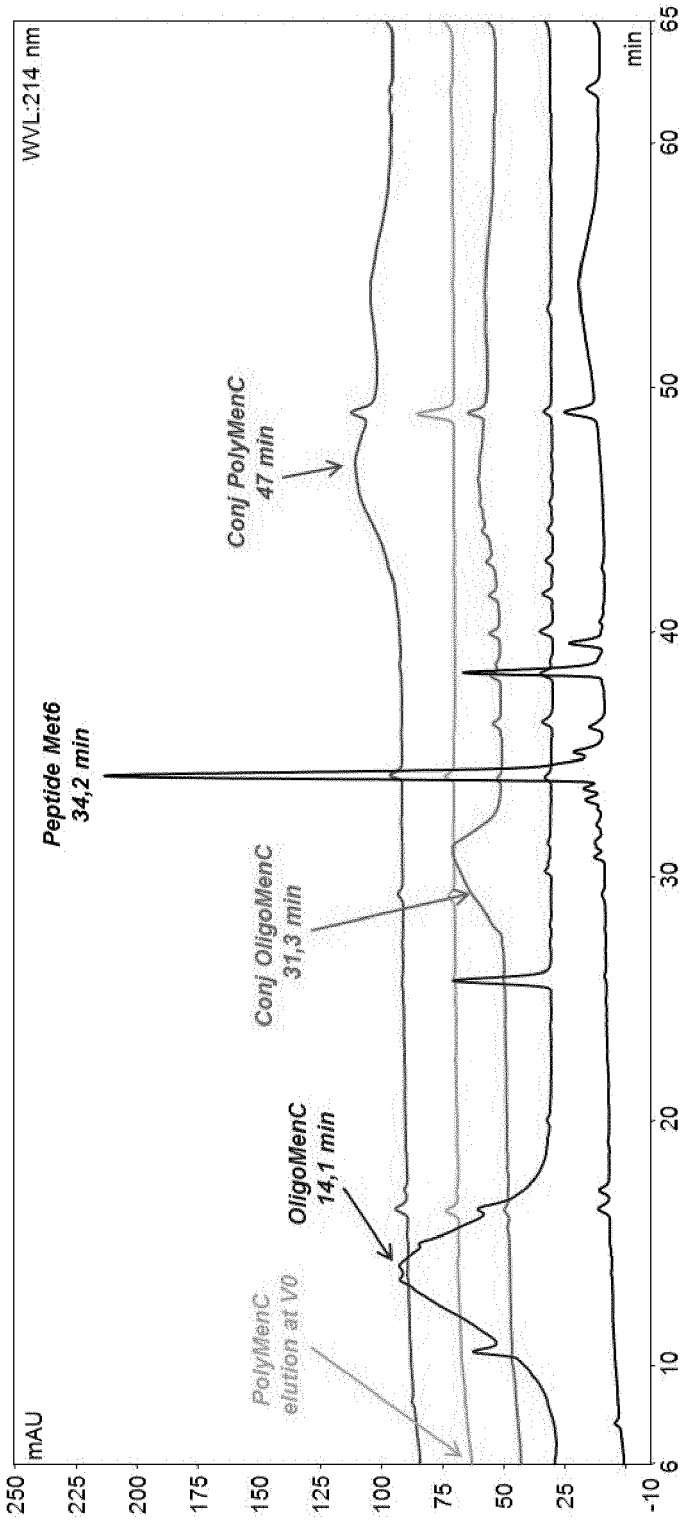

WVL:214 nm
mAU
min
Peptide Met6
34,2 min
Conj PolyMenC
47 min
Conj OligoMenC
31,3 min
OligoMenC
14,1 min
PolyMenC
elution at V0
250
225
200
175
150
125
100
75
50
25
-10
6
10
20
30
40
50
60
65

Figure 13

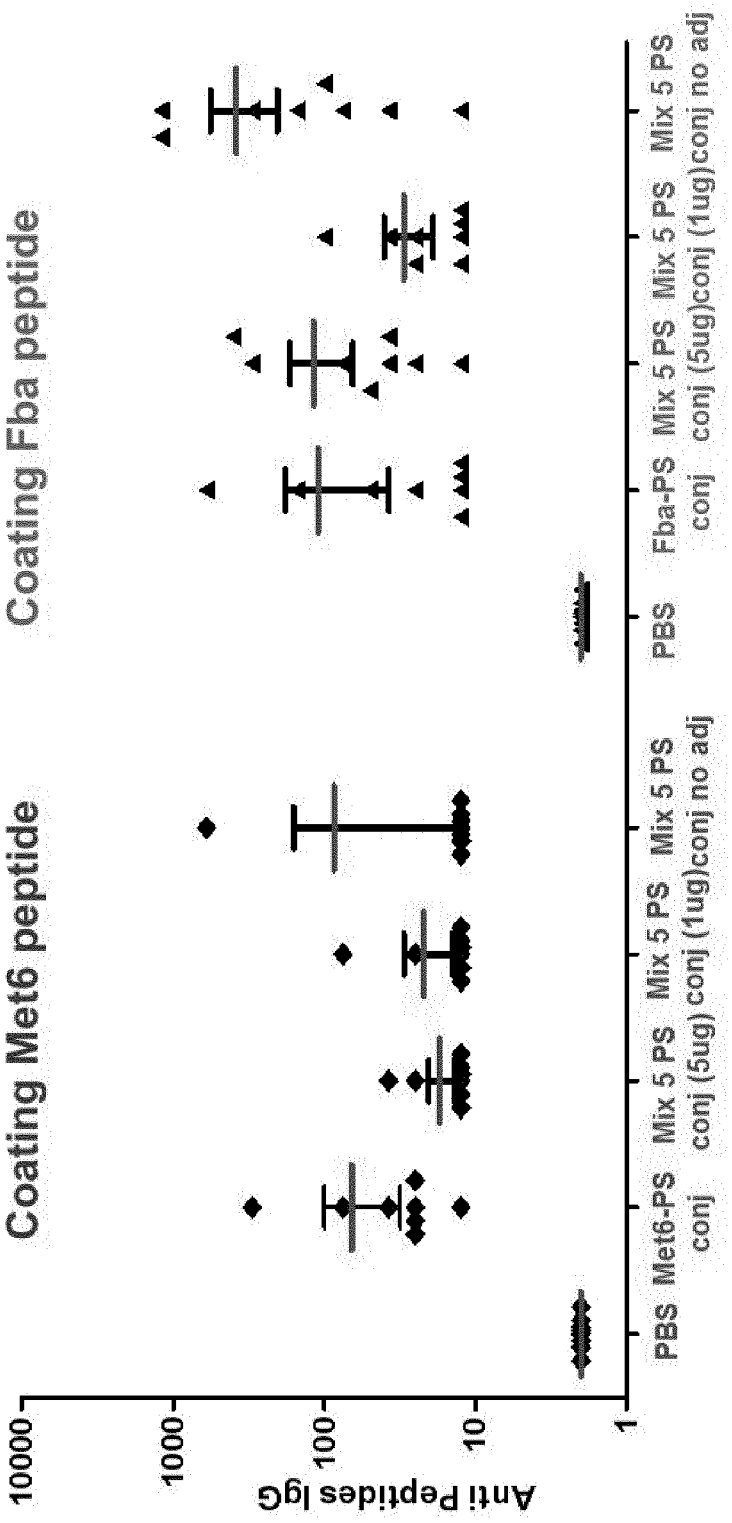

Coating Met6 peptide
Coating Fba peptide
Anti Peptides IgG
10000
1000
100
10
1
PBS
Met6-PS conj
Mix 5 PS conj (5ug)
Mix 5 PS conj (1ug)
Mix 5 PS conj no adj
PBS
Fba-PS conj
Mix 5 PS conj (5ug)
Mix 5 PS conj (1ug)
Mix 5 PS conj no adj

Figure 14

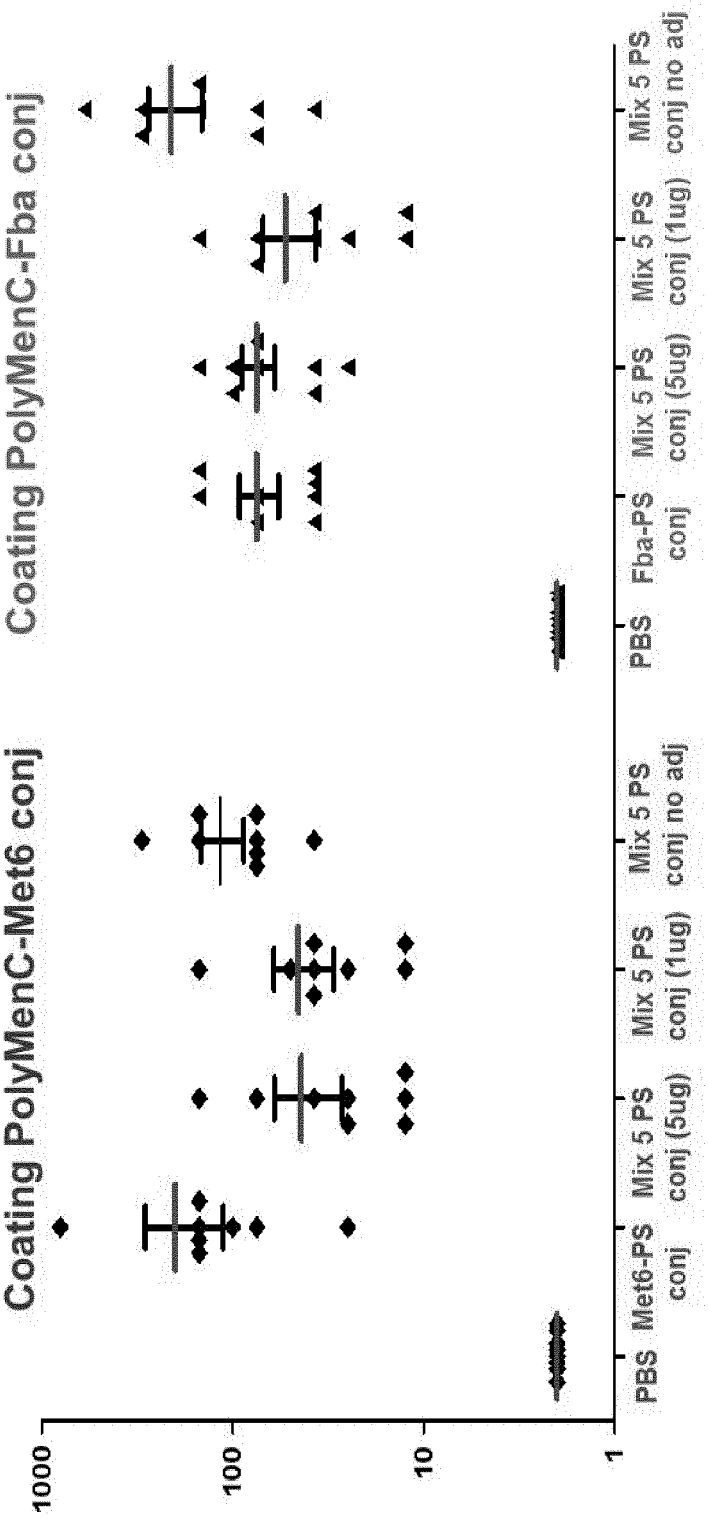

Coating PolyMenC-Met6 conj
Coating PolyMenC-Fba conj
1000
100
10
1
PBS
Met6-PS conj
Mix 5 PS conj (5ug)
Mix 5 PS conj (1ug)
Mix 5 PS conj no adj
PBS
Fba-PS conj
Mix 5 PS conj (5ug)
Mix 5 PS conj (1ug)
Mix 5 PS conj no adj

Figure 15

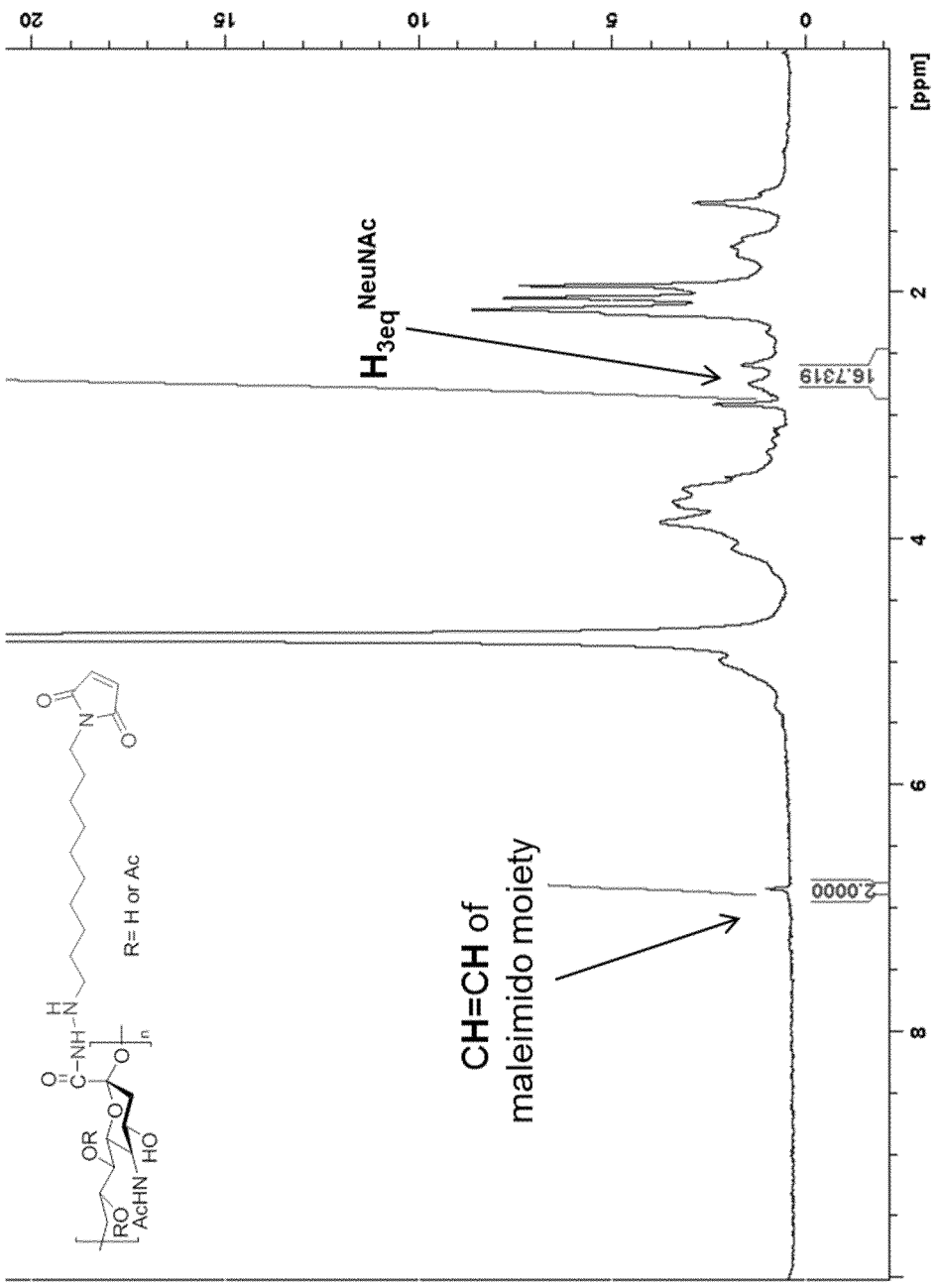

20
15
10
5
0
[ppm]
2
4
6
8
16.7319
2.0000
H_{3eq}^{NeuNAc}
CH=CH of
maleimido moiety
R= H or Ac

Figure 16

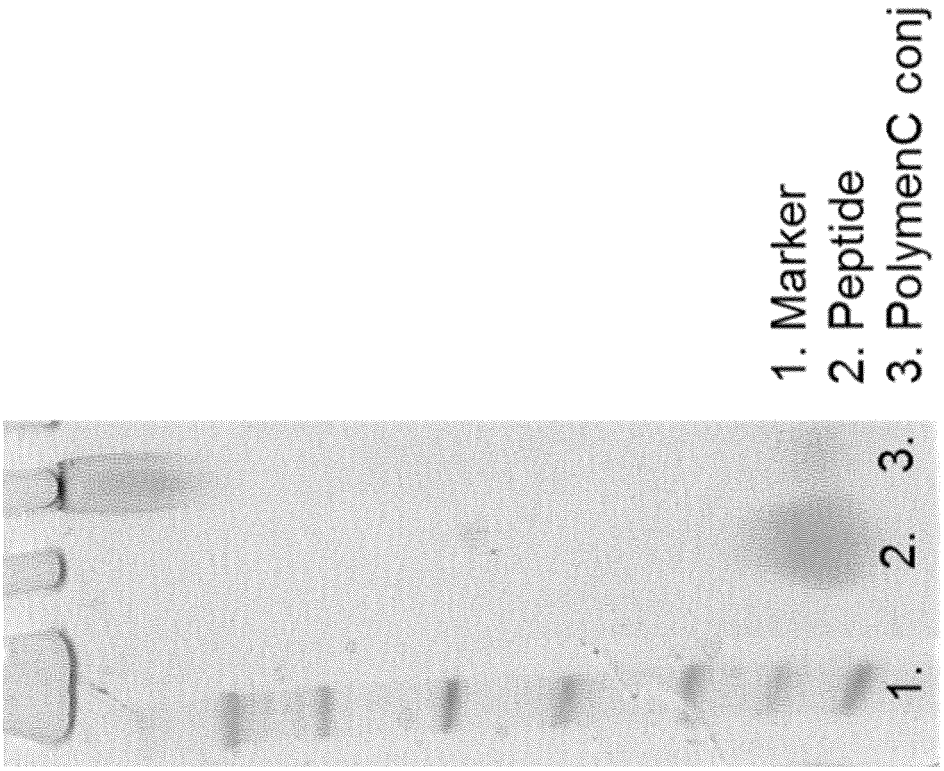


Figure 17

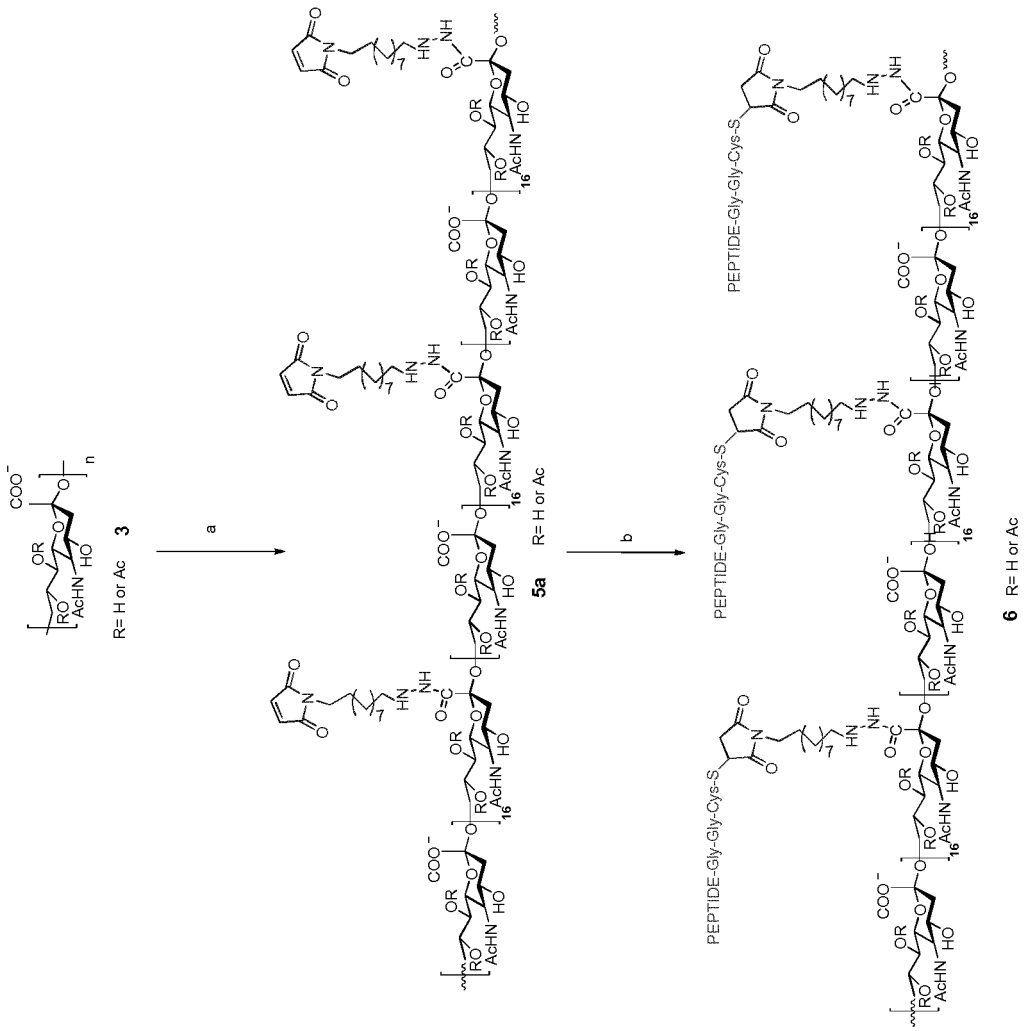


Figure 18

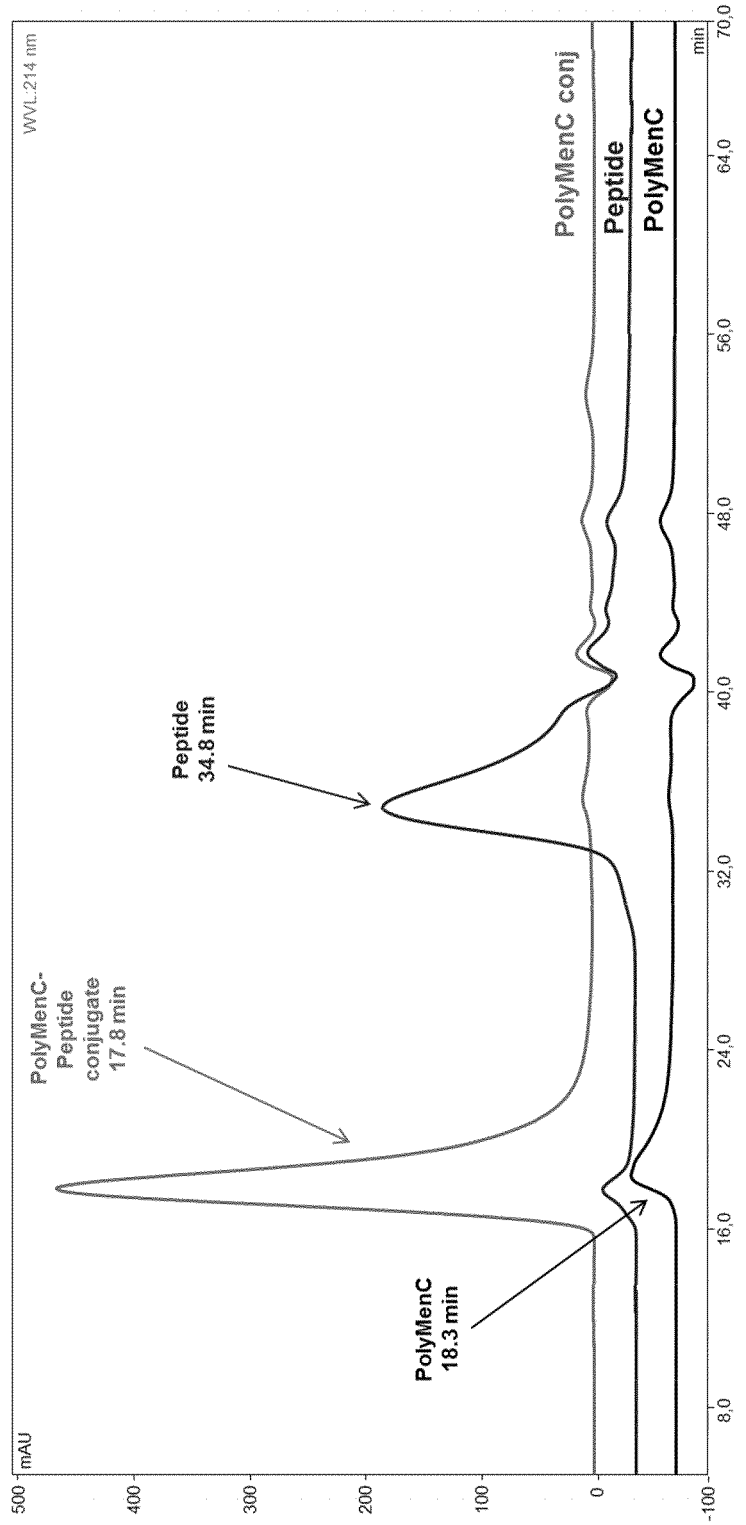

WVL:214 nm
mAU
500
400
300
200
100
0
-100
8,0
16,0
24,0
32,0
40,0
48,0
56,0
64,0
70,0
min
PolyMenC conj
Peptide
PolyMenC
Peptide
34.8 min
PolyMenC-
Peptide
conjugate
17.8 min
PolyMenC
18.3 min

Figure 19

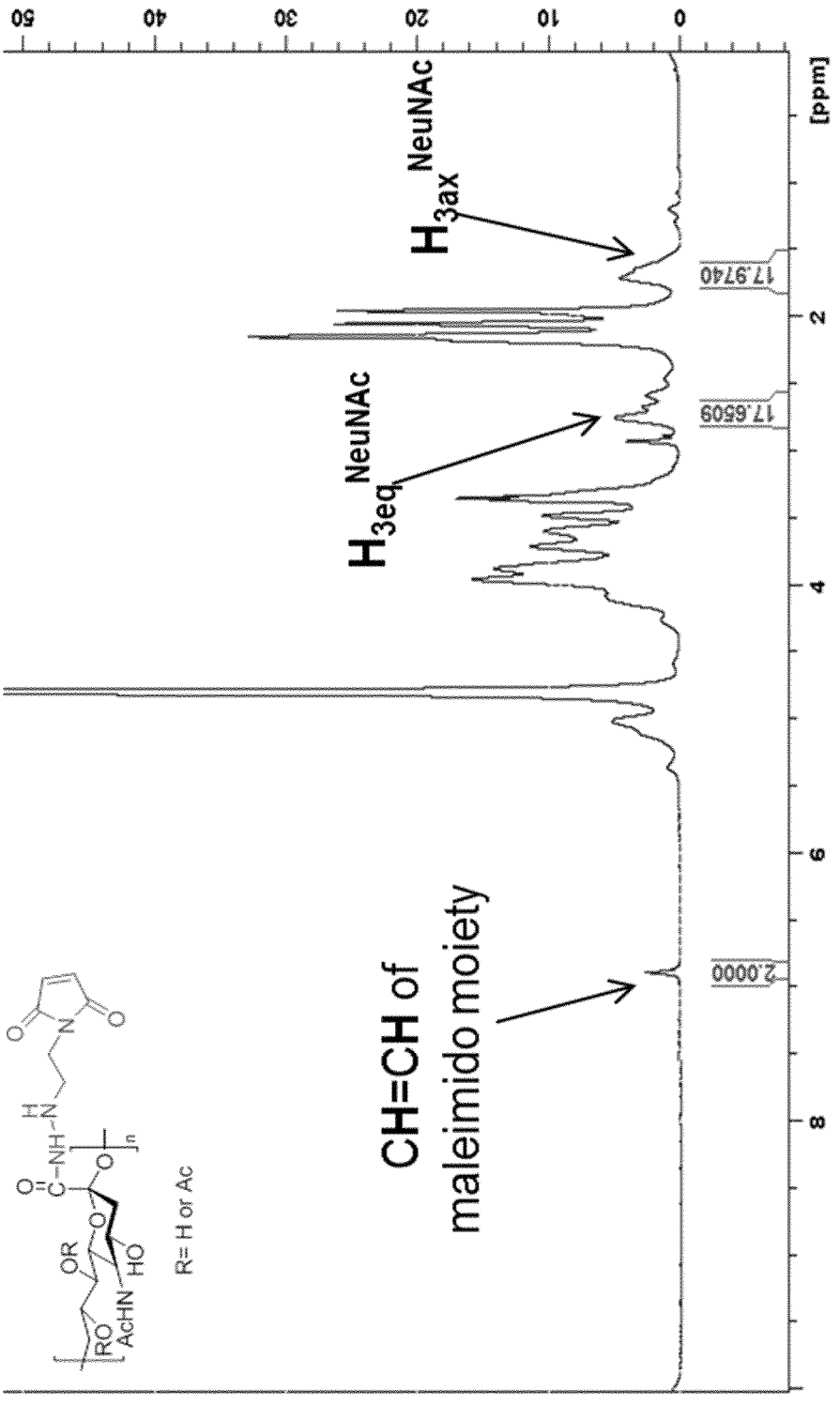

50
40
30
20
10
0
[ppm]
2
4
6
8
17.9740
17.6509
2.0000
H_{3ax}^{NeuNAc}
H_{3eq}^{NeuNAc}
CH=CH of
maleimido moiety
R= H or Ac

Figure 20

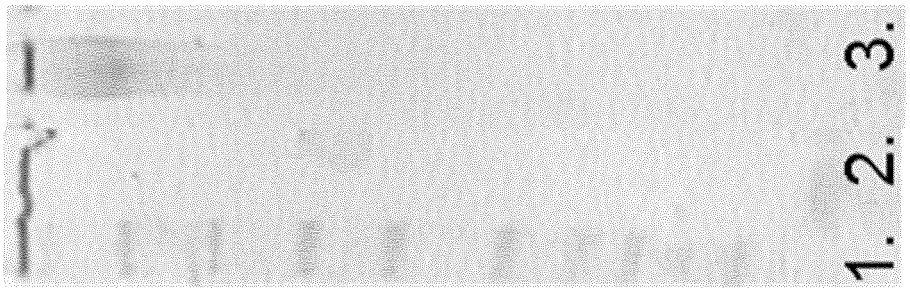


Figure 21

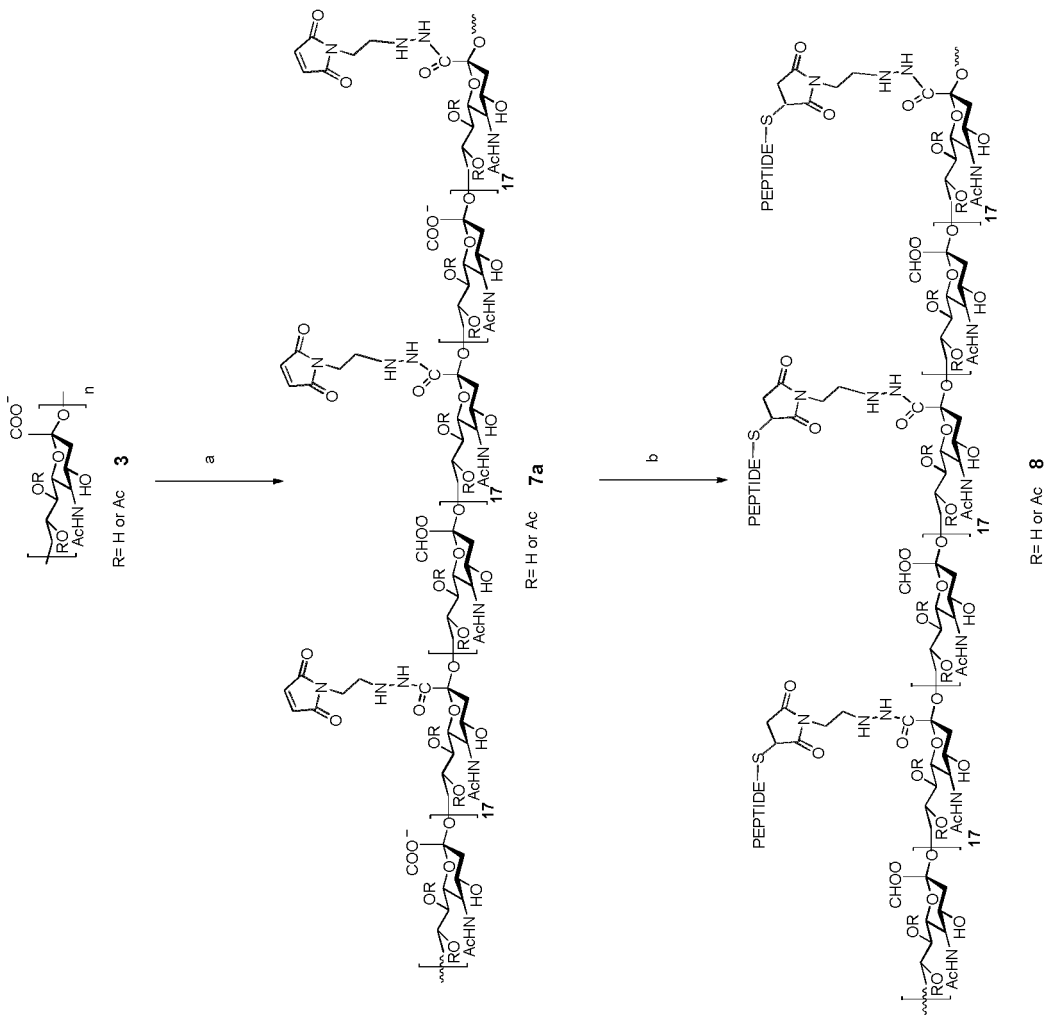


Figure 22

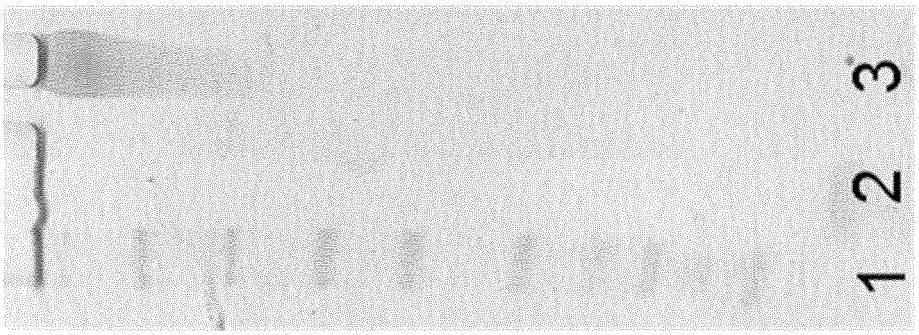


Figure 23

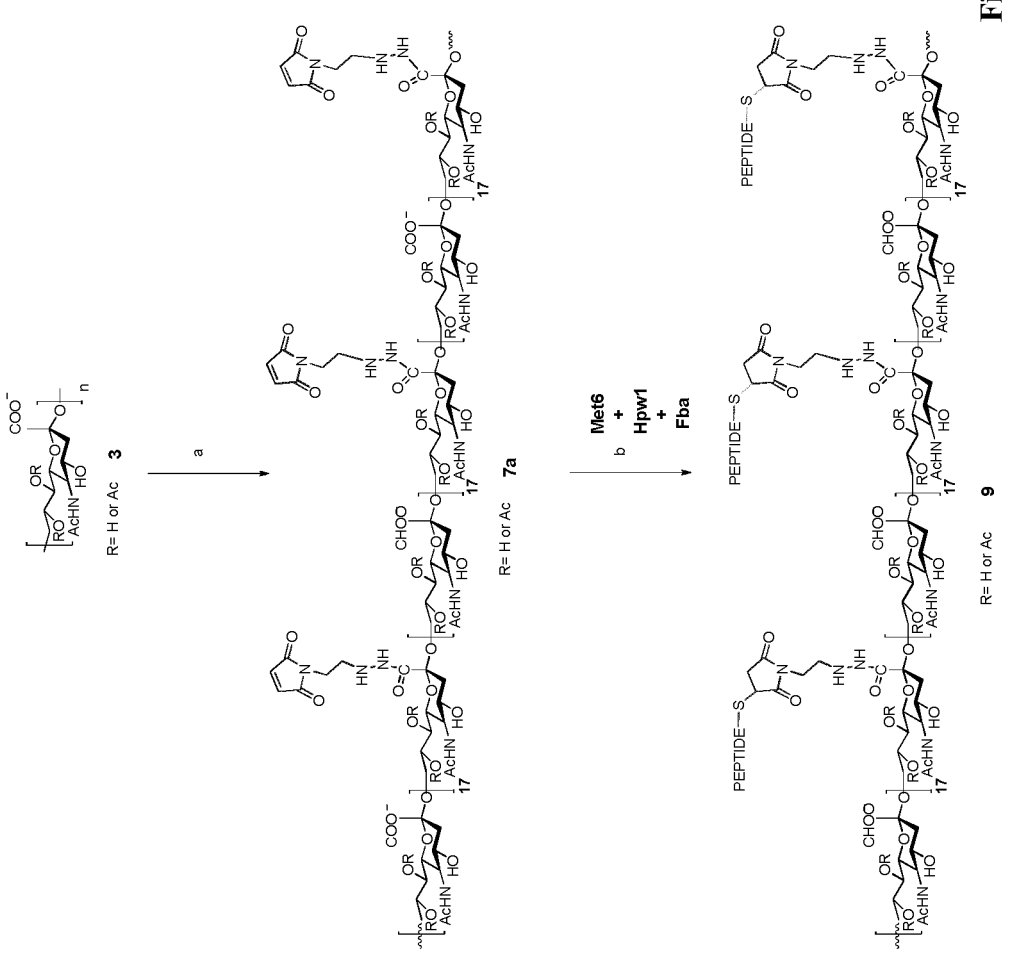


Figure 24

Scheme 5:

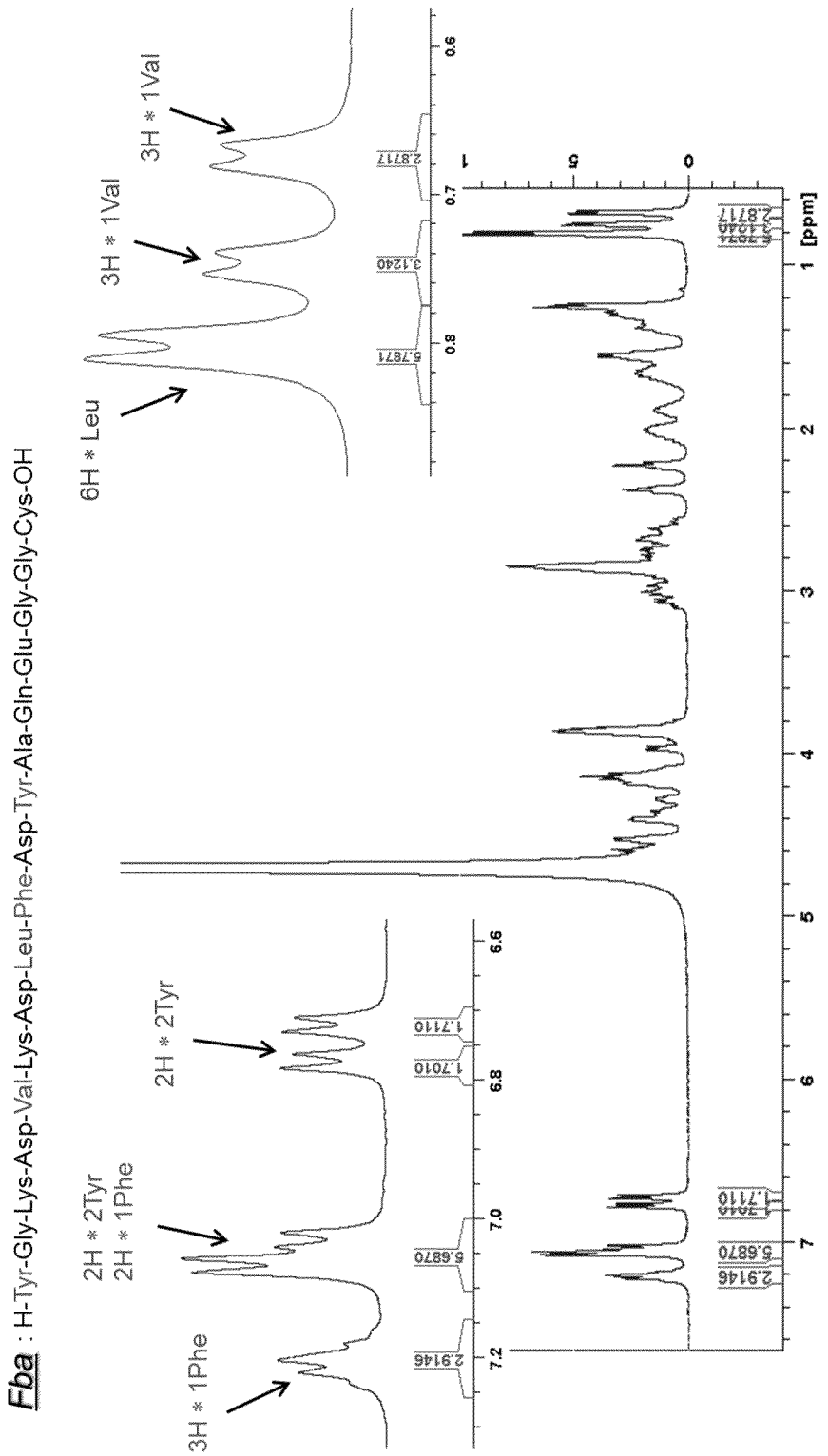

Fba : H-Tyr-Gly-Lys-Asp-Val-Lys-Asp-Leu-Phe-Asp-Tyr-Ala-Gln-Glu-Gly-Gly-Cys-OH
6H * Leu
3H * 1Val
3H * 1Val
2H * 2Tyr
2H * 2Tyr
2H * 1Phe
3H * 1Phe
2.8717
3.1240
5.7871
1.7110
1.7010
5.6870
2.9146
[ppm]

Figure 25

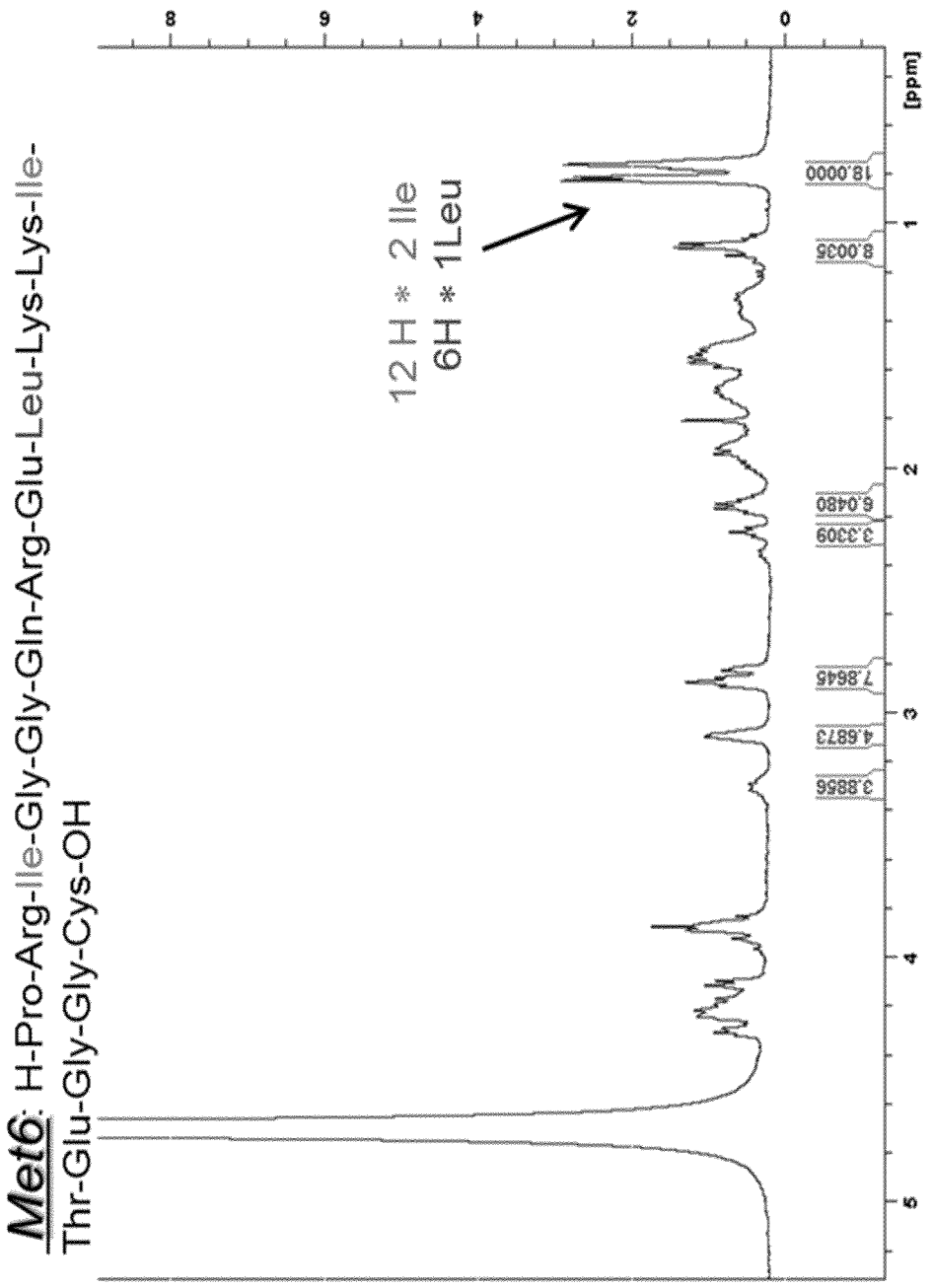

Met6: H-Pro-Arg-Ile-Gly-Gly-Gln-Arg-Glu-Leu-Lys-Lys-Ile-Thr-Glu-Gly-Gly-Cys-OH
12 H * 2 Ile
6H * 1Leu
18.0000
8.0036
6.0480
3.3309
7.8645
4.6873
3.8856
[ppm]

Figure 26

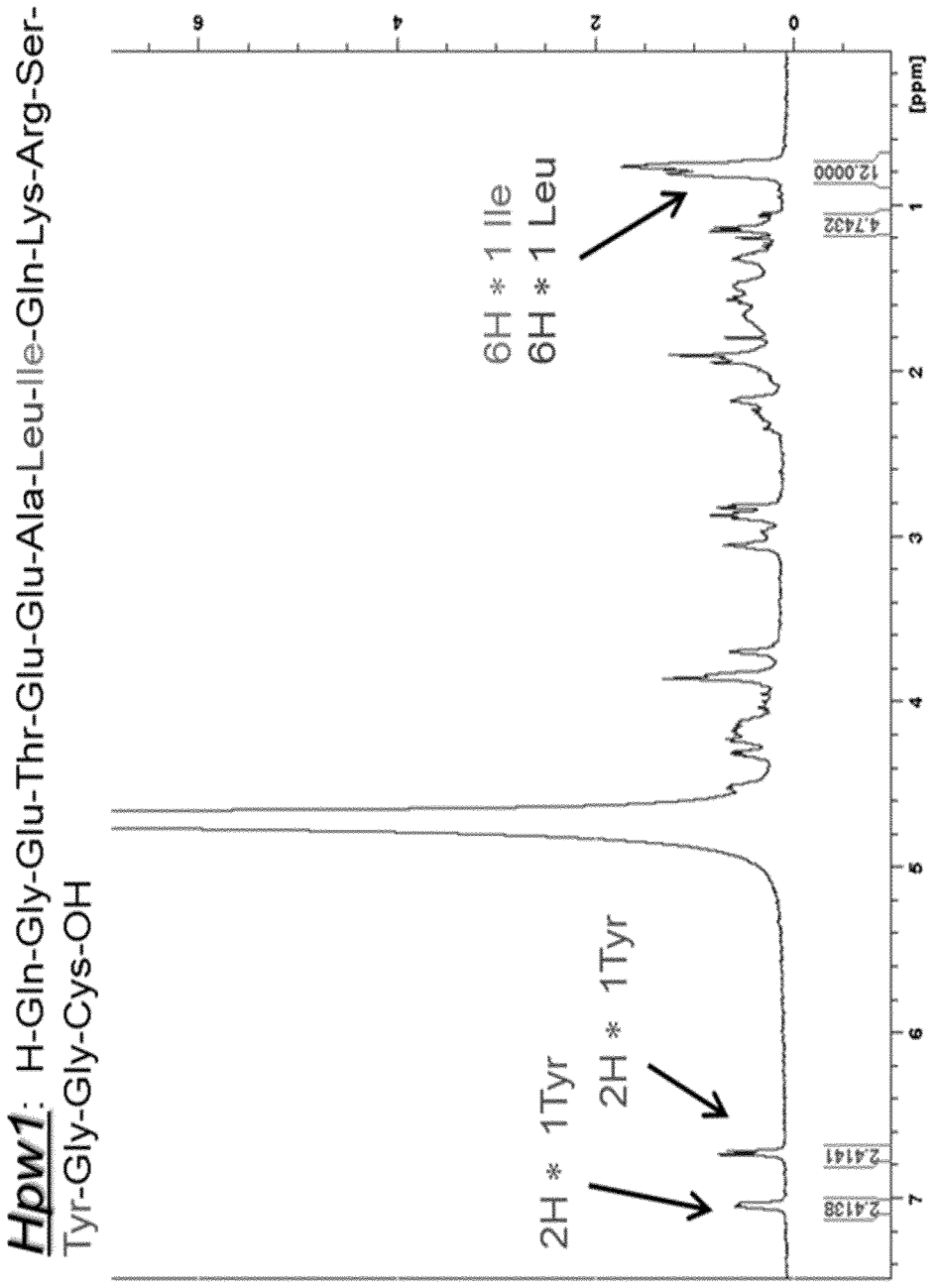

Hpw1: H-Gln-Gly-Glu-Thr-Glu-Glu-Ala-Leu-Ile-Gln-Lys-Arg-Ser-Tyr-Gly-Gly-Cys-OH
6H * 1 Ile
6H * 1 Leu
2H * 1Tyr
2H * 1Tyr
12.0000
4.7432
2.4141
2.4138
[ppm]

Figure 27

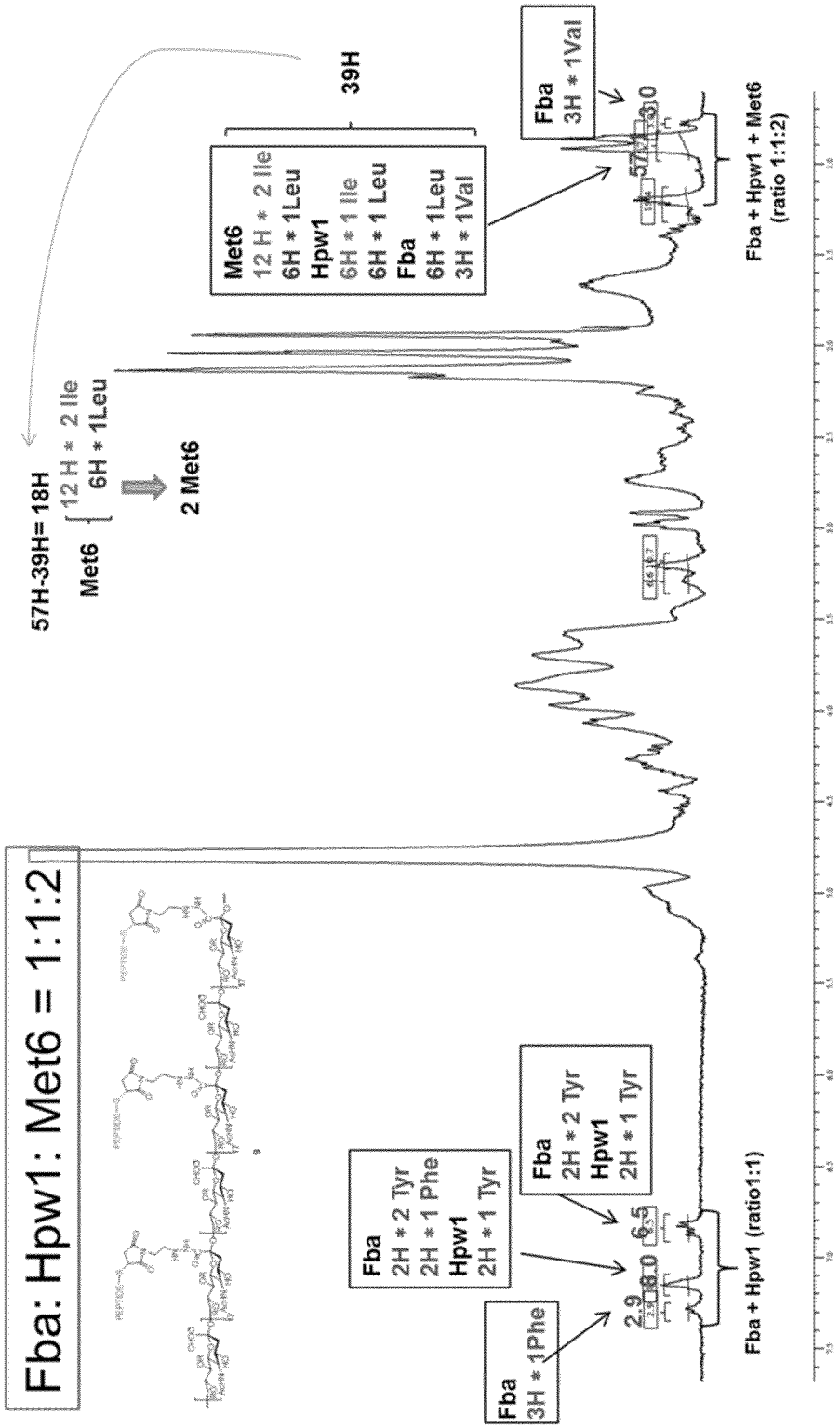
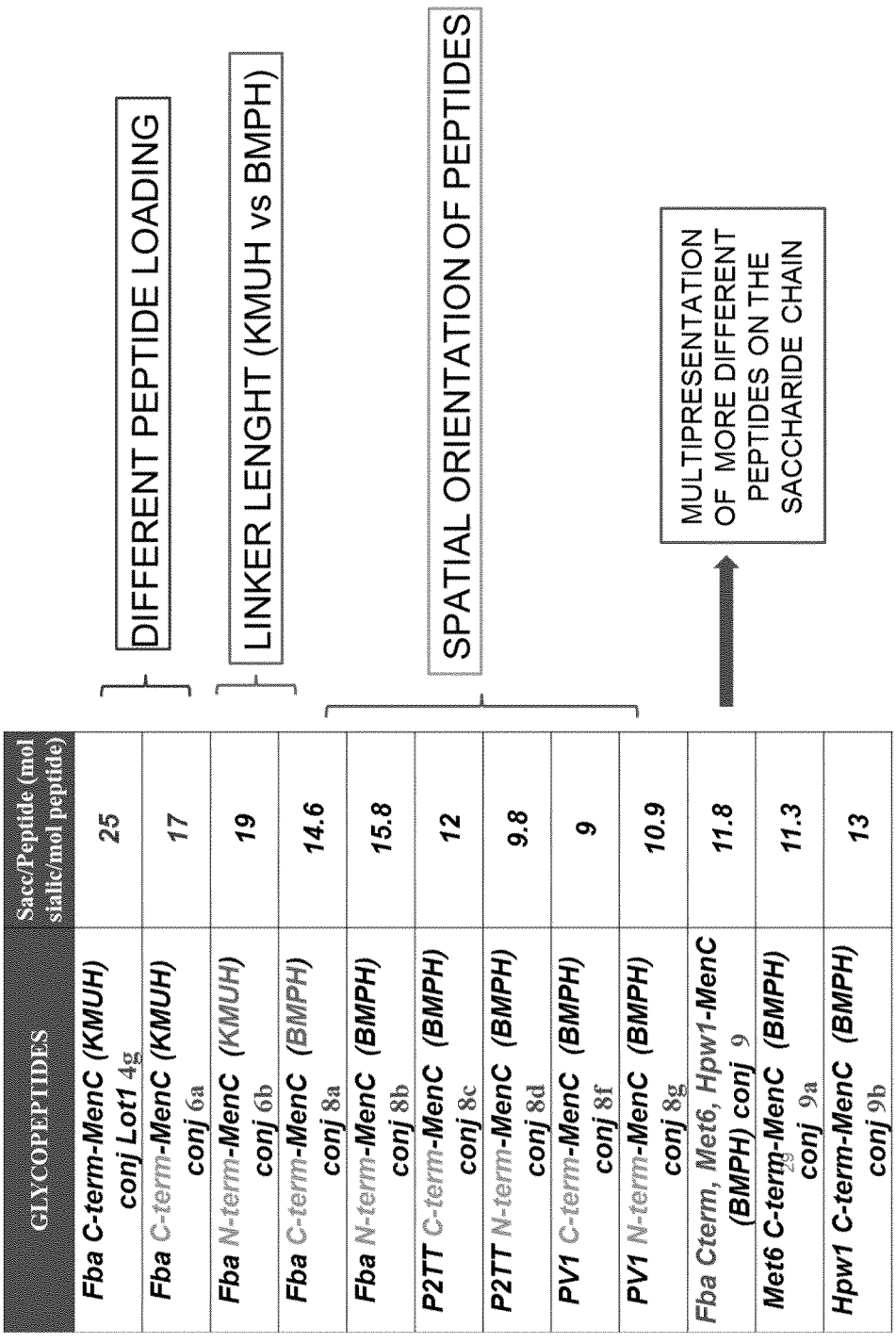

Fba: Hpw1: Met6 = 1:1:2
57H-39H= 18H
Met6
12 H * 2 Ile
6H * 1Leu
2 Met6
39H
Met6
12 H * 2 Ile
6H * 1Leu
Hpw1
6H * 1 Ile
6H * 1 Leu
Fba
6H * 1Leu
3H * 1Val
Fba
3H * 1Val
Fba + Hpw1 + Met6
(ratio 1:1:2)
Fba
2H * 2 Tyr
2H * 1 Phe
Hpw1
2H * 1 Tyr
Fba
2H * 2 Tyr
Hpw1
2H * 1 Tyr
Fba
3H * 1Phe
Fba + Hpw1 (ratio1:1)

Figure 28



GLYCOPEPTIDES	Sacc/Peptide (mol sialic/mol peptide)
Fba C-term-MenC (KMUH) conj Lot1 4g	***25***
Fba C-term-MenC (KMUH) conj 6a	***17***
Fba N-term-MenC (KMUH) conj 6b	***19***
Fba C-term-MenC (BMPH) conj 8a	***14.6***
Fba N-term-MenC (BMPH) conj 8b	***15.8***
P2TT C-term-MenC (BMPH) conj 8c	***12***
P2TT N-term-MenC (BMPH) conj 8d	***9.8***
PV1 C-term-MenC (BMPH) conj 8f	***9***
PV1 N-term-MenC (BMPH) conj 8g	***10.9***
Fba Cterm, Met6, Hpw1-MenC (BMPH) conj 9	***11.8***
Met6 C-term-MenC (BMPH) conj 9a	***11.3***
Hpw1 C-term-MenC (BMPH) conj 9b	***13***

Figure 29

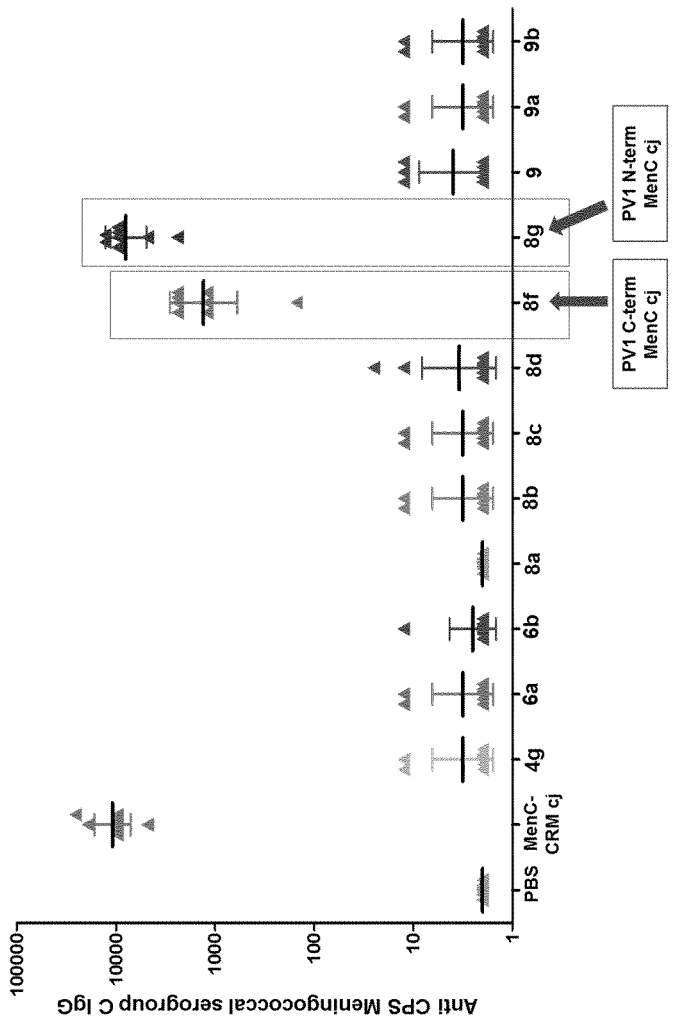

Anti CPS Meningococcal serogroup C IgG
100000
10000
1000
100
10
1
PBS
MenC-CRM cj
4g
6a
6b
8a
8b
8c
8d
8f
8g
9
9a
9b
PV1 C-term MenC cj
PV1 N-term MenC cj

Figure 30

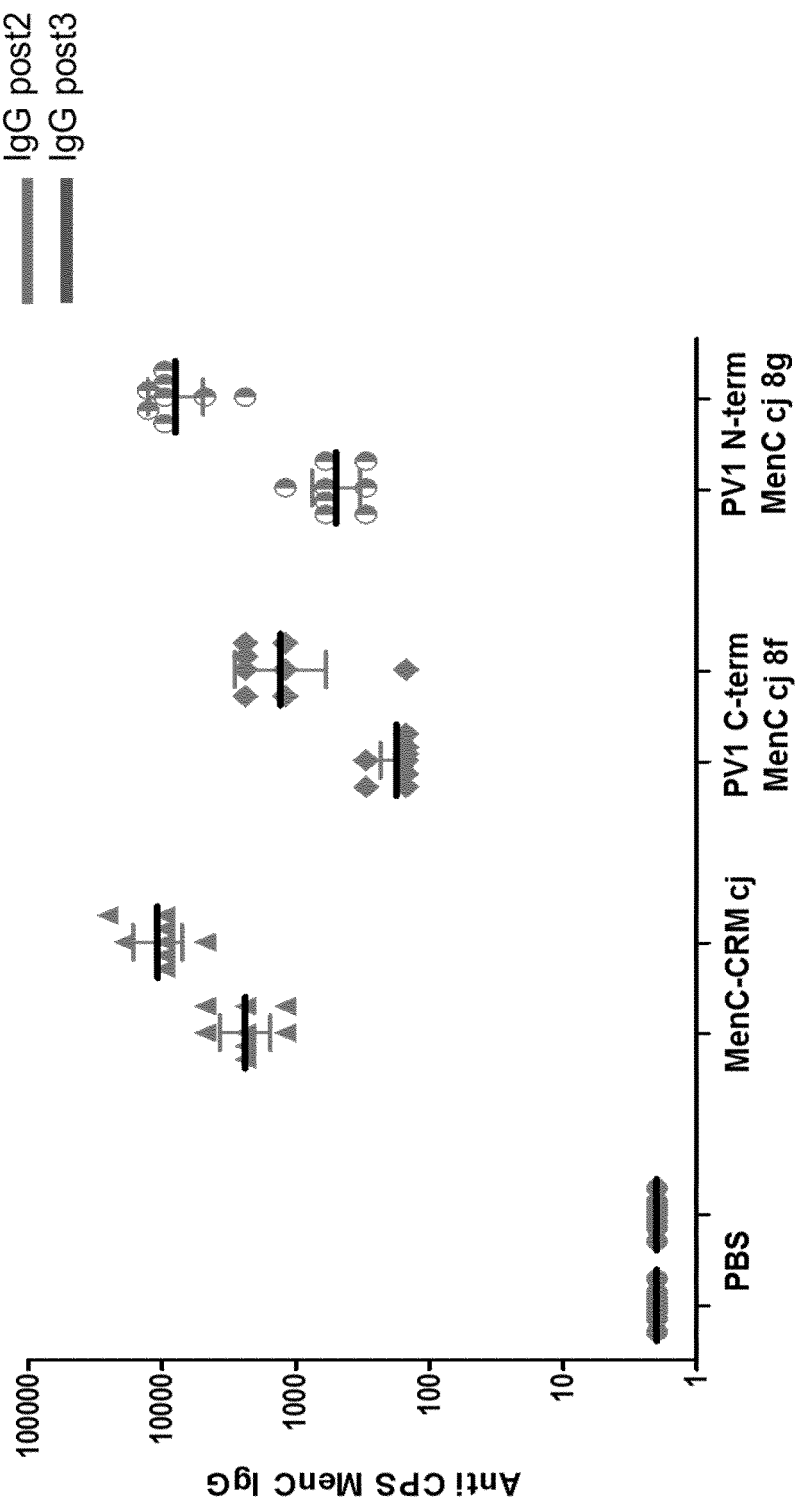

IgG post2
IgG post3
Anti CPS MenC IgG
100000
10000
1000
100
10
1
PBS
MenC-CRM cj
PV1 C-term MenC cj 8f
PV1 N-term MenC cj 8g

Figure 31

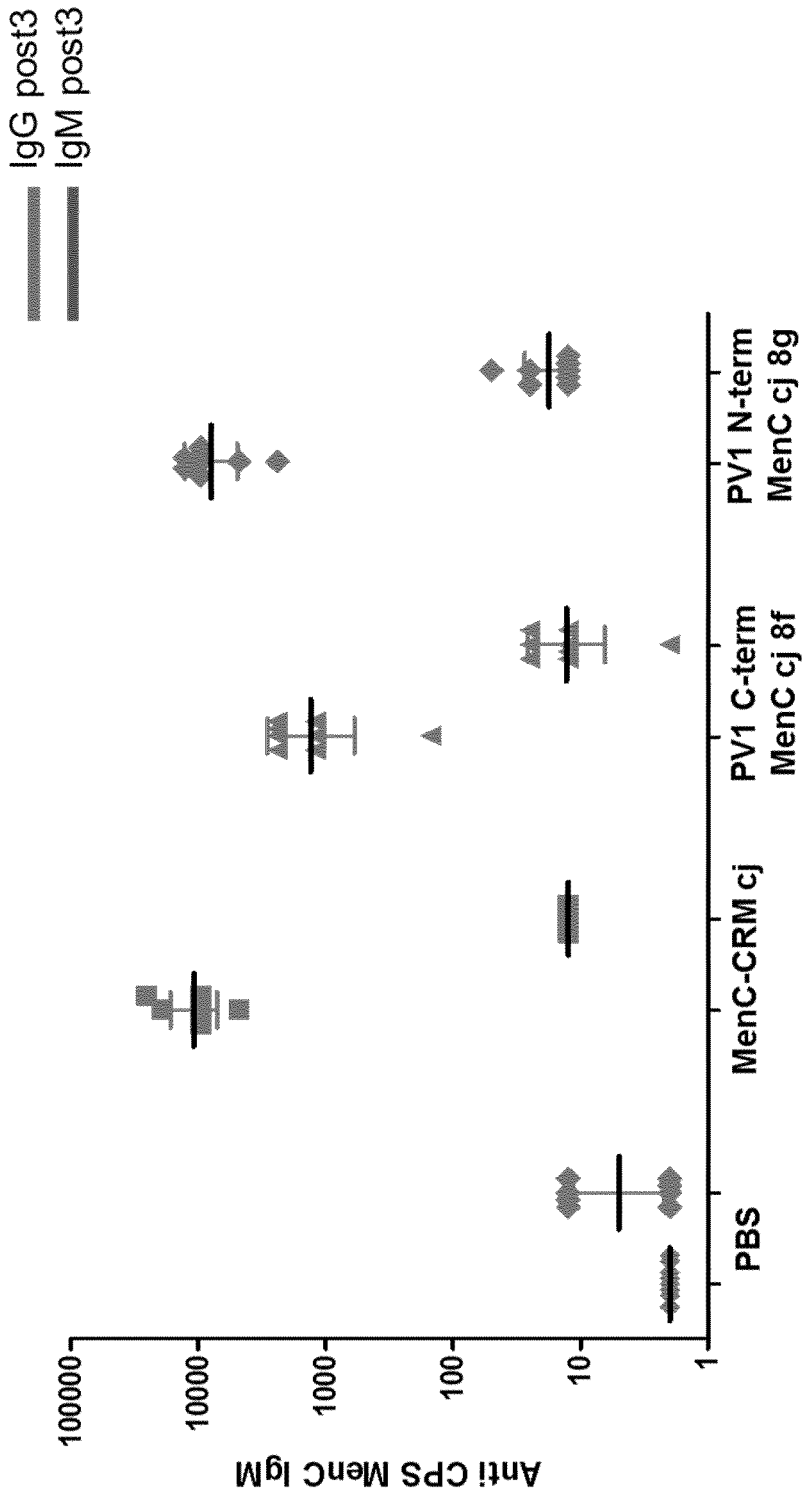

IgG post3
IgM post3
Anti CPS MenC IgM
100000
10000
1000
100
10
1
PBS
MenC-CRM cj
PV1 C-term MenC cj 8f
PV1 N-term MenC cj 8g

Figure 32

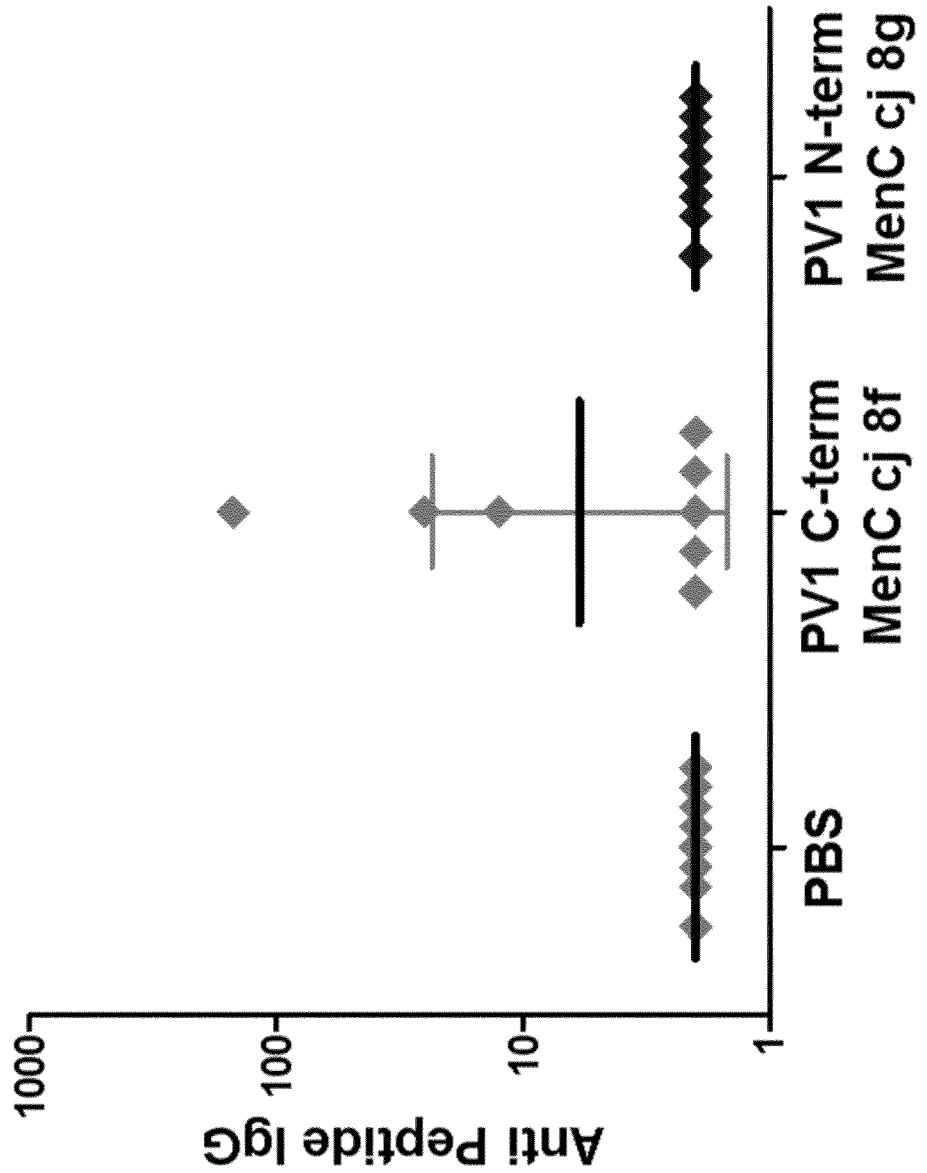
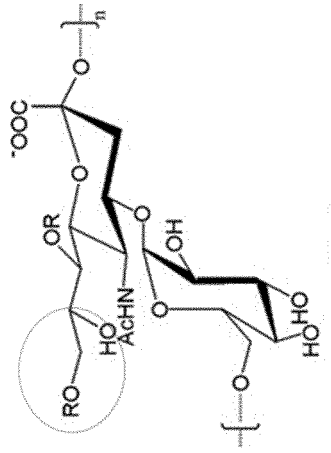
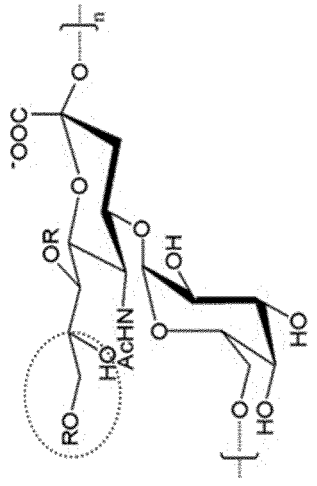

Anti Peptide IgG
1000
100
10
1
PBS
PV1 C-term
MenC cj 8f
PV1 N-term
MenC cj 8g

Figure 33



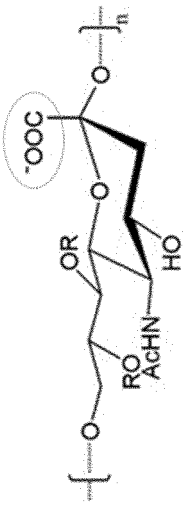
MenY

→6)-α-D-Glcp-(1→4)-α-D-Neu5Ac(7/9OAc)-(2→



MenW-135

→6)-α-D-Gal*p*-(1→4)-α-D-Neu5Ac(7/9OAc)-(2→



MenC

→9)-α-D-Neu5Ac(7/9OAc)-(2→

Figure 34

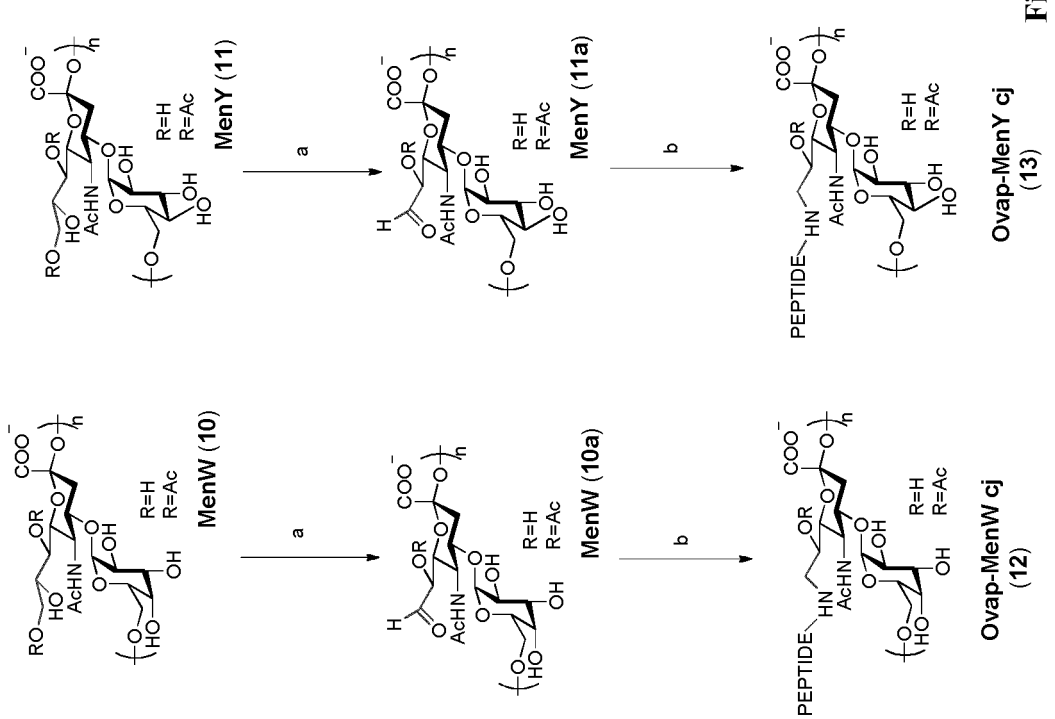

MenW (10)
a
MenW (10a)
b
Ovap-MenW cj
(12)
MenY (11)
a
MenY (11a)
b
Ovap-MenY cj
(13)
R=H
R=Ac
PEPTIDE

Figure 35

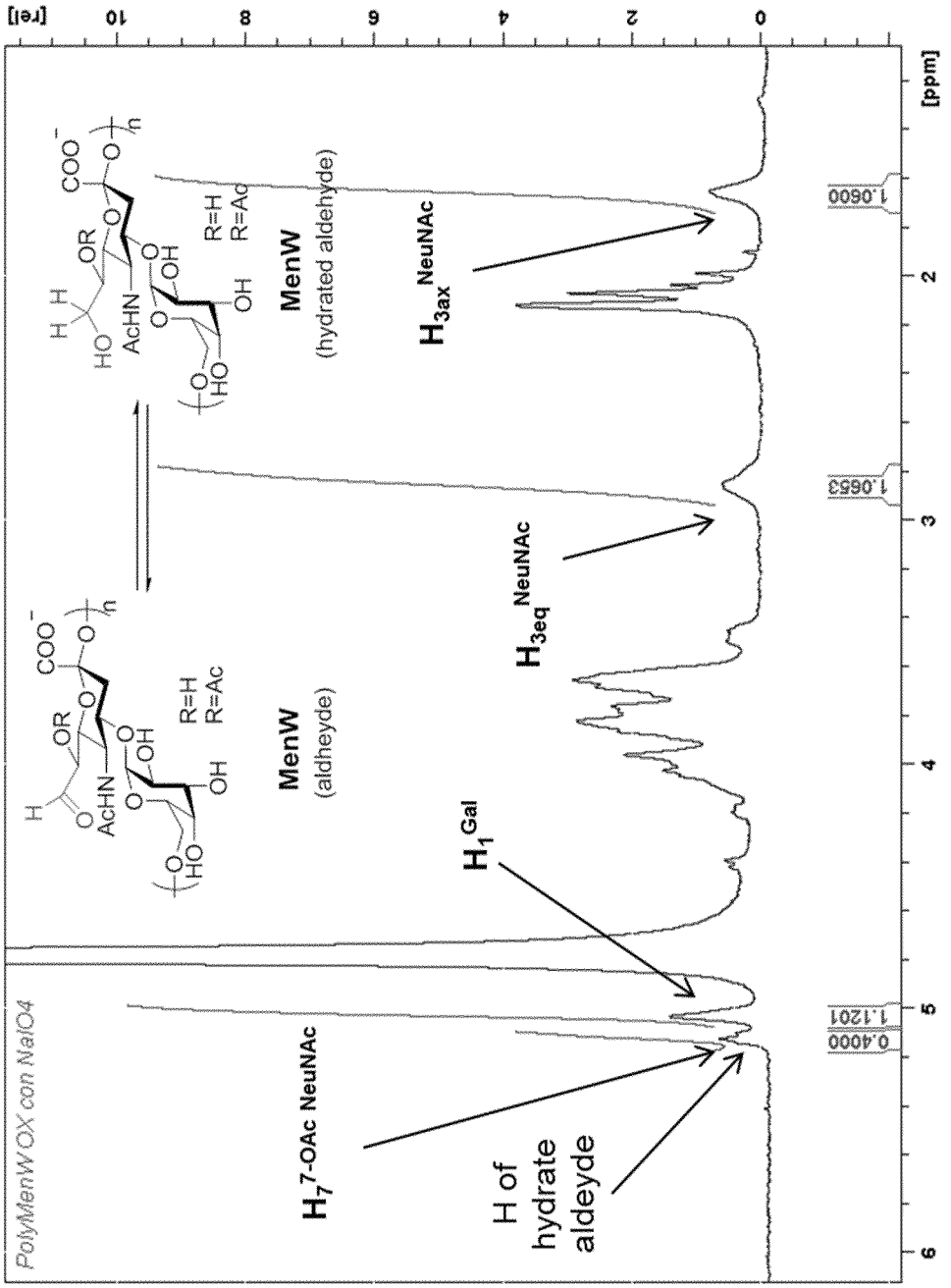

PolyMenW OX con NaIO4
H7 7-OAc NeuNAc
H of hydrate aldeyde
H1 Gal
H3eq NeuNAc
H3ax NeuNAc
MenW (aldheyde)
MenW (hydrated aldehyde)
R=H R=Ac
R=H R=Ac
0.4000
1.1201
1.0653
1.0600
[ppm]
[rel]

Figure 36

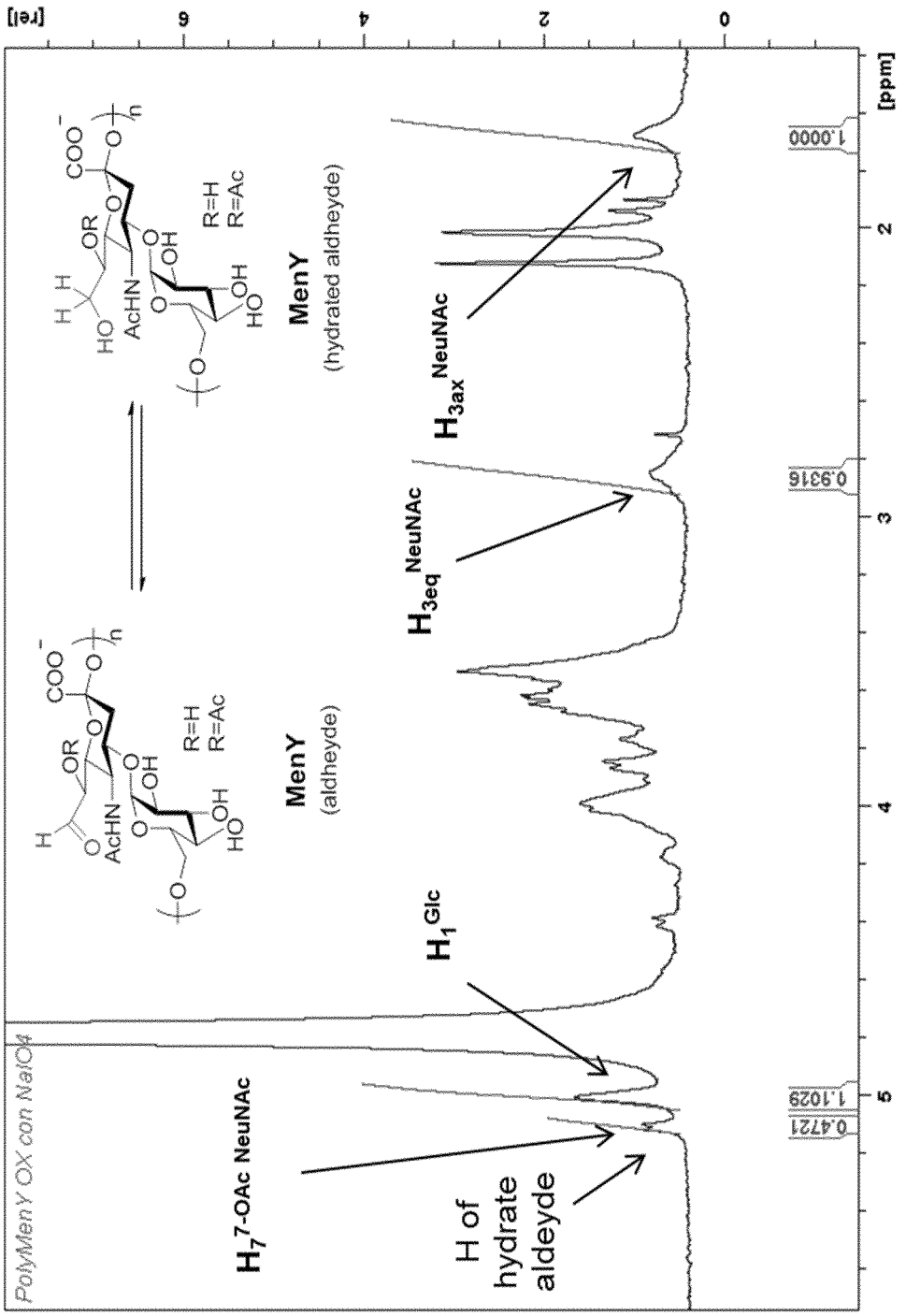

PolyMenY OX con NaIO4
H7 7-OAc NeuNAc
H of hydrate aldeyde
H1 Glc
H3eq NeuNAc
H3ax NeuNAc
MenY
(aldheyde)
MenY
(hydrated aldheyde)
R=H
R=Ac
[rel]
[ppm]
1.0000
0.9316
1.1029
0.4721

Figure 37

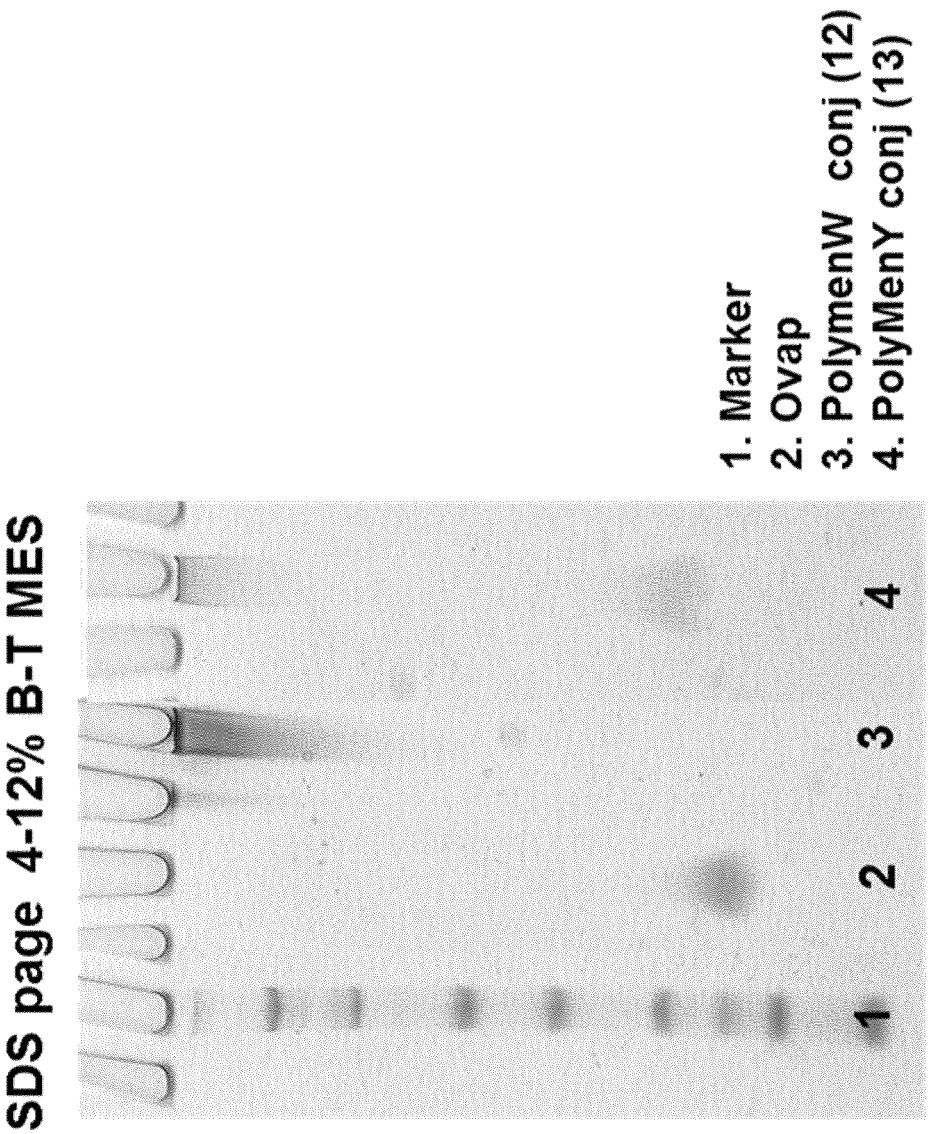

SDS page 4-12% B-T MES
1. Marker
2. Ovap
3. PolymenW conj (12)
4. PolyMenY conj (13)
1
2
3
4

Figure 38

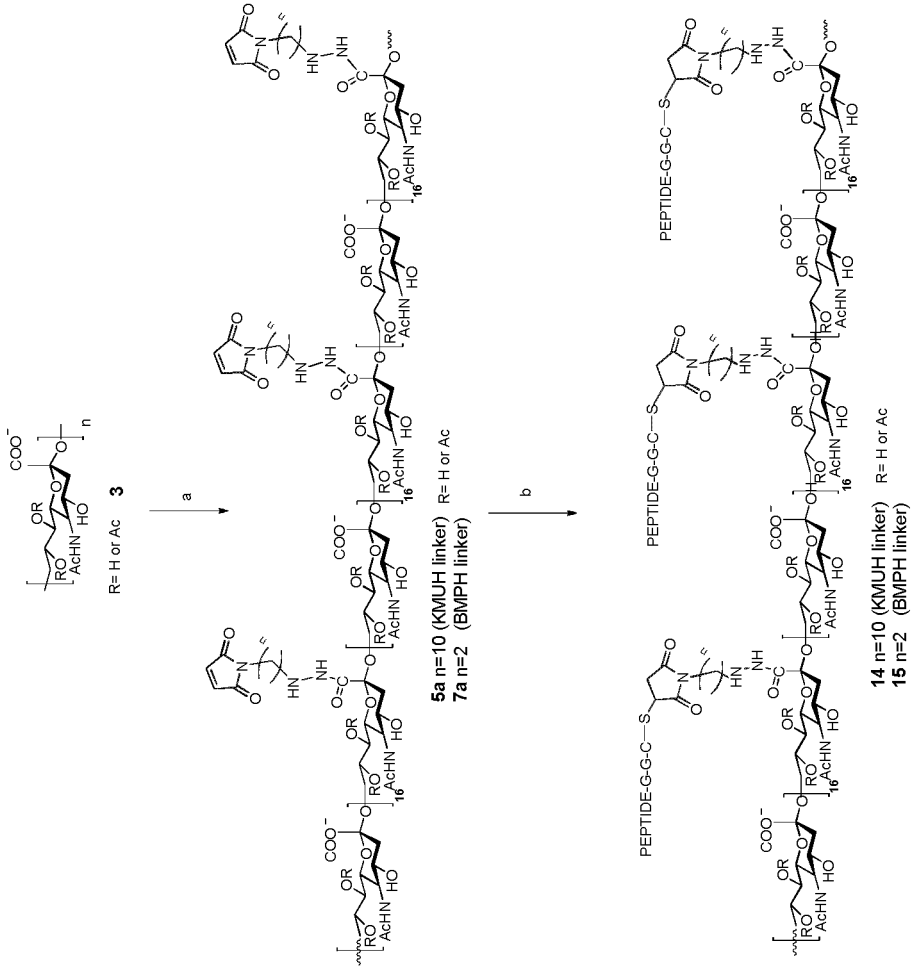

R= H or Ac 3
a
5a n=10 (KMUH linker) R= H or Ac
7a n=2 (BMPH linker)
b
PEPTIDE-G-G-C—S
14 n=10 (KMUH linker) R= H or Ac
15 n=2 (BMPH linker)

Figure 39

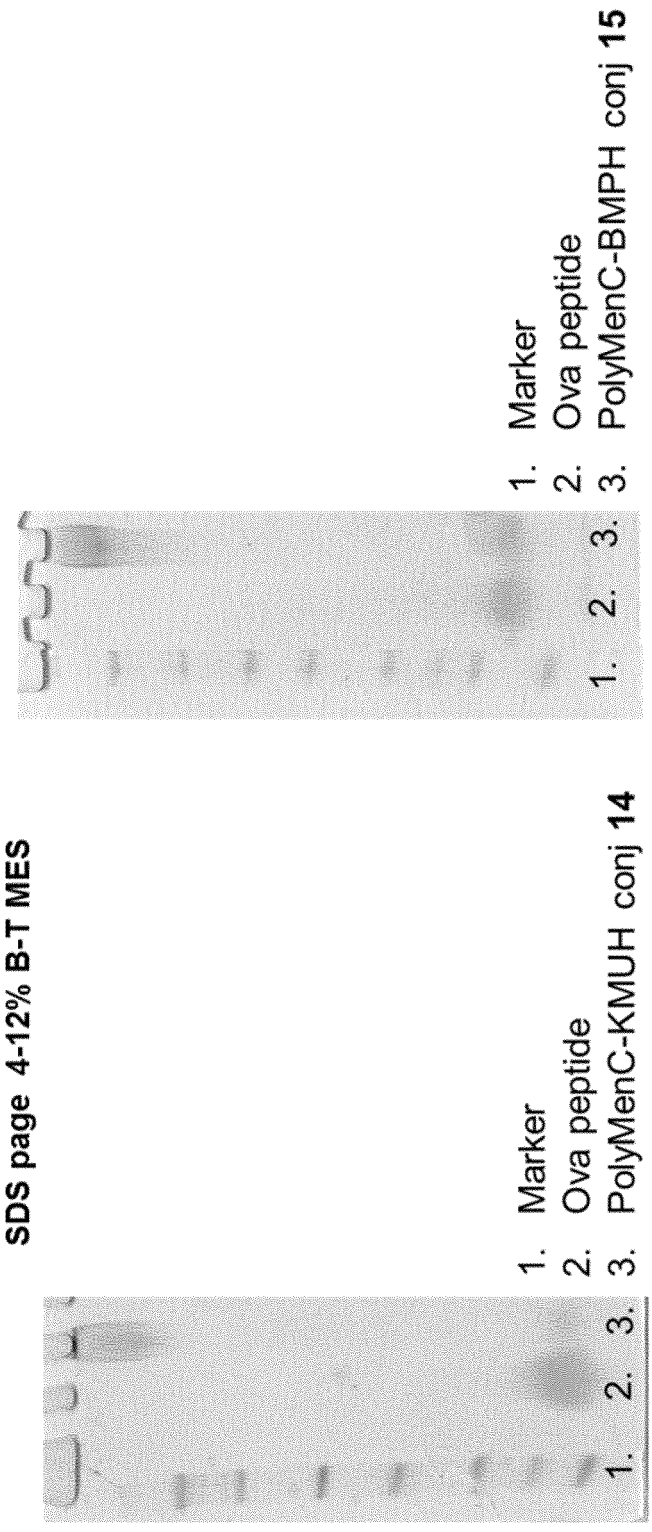

SDS page 4-12% B-T MES
1. Marker
2. Ova peptide
3. PolyMenC-KMUH conj 14
1. 2. 3.
1. Marker
2. Ova peptide
3. PolyMenC-BMPH conj 15
1. 2. 3.

Figure 40

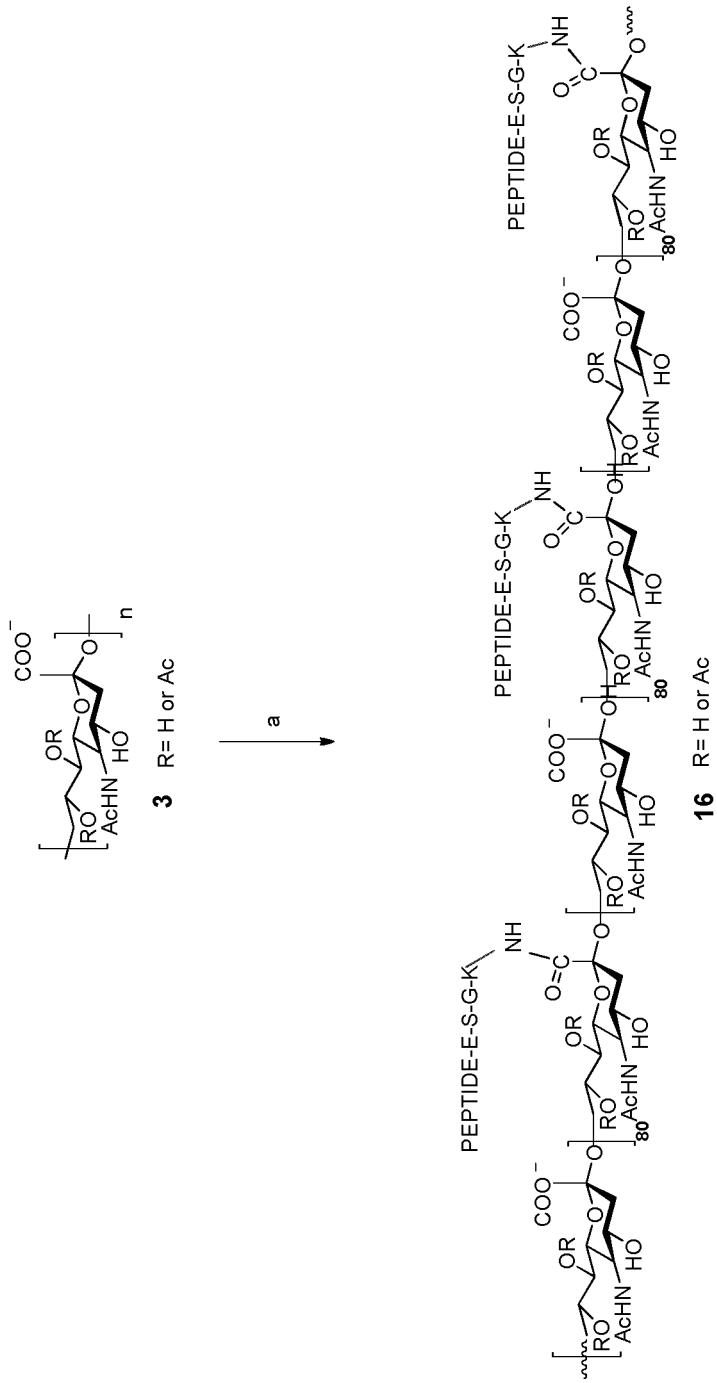


Figure 41

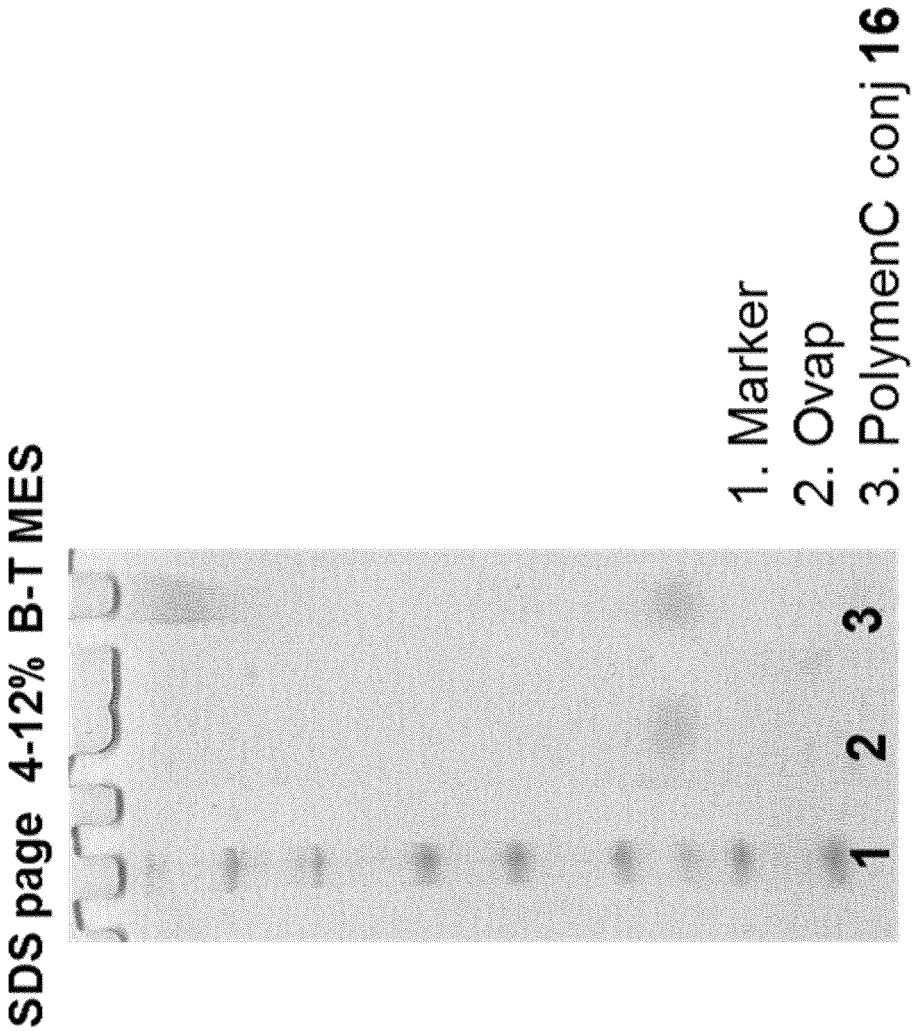


Figure 42

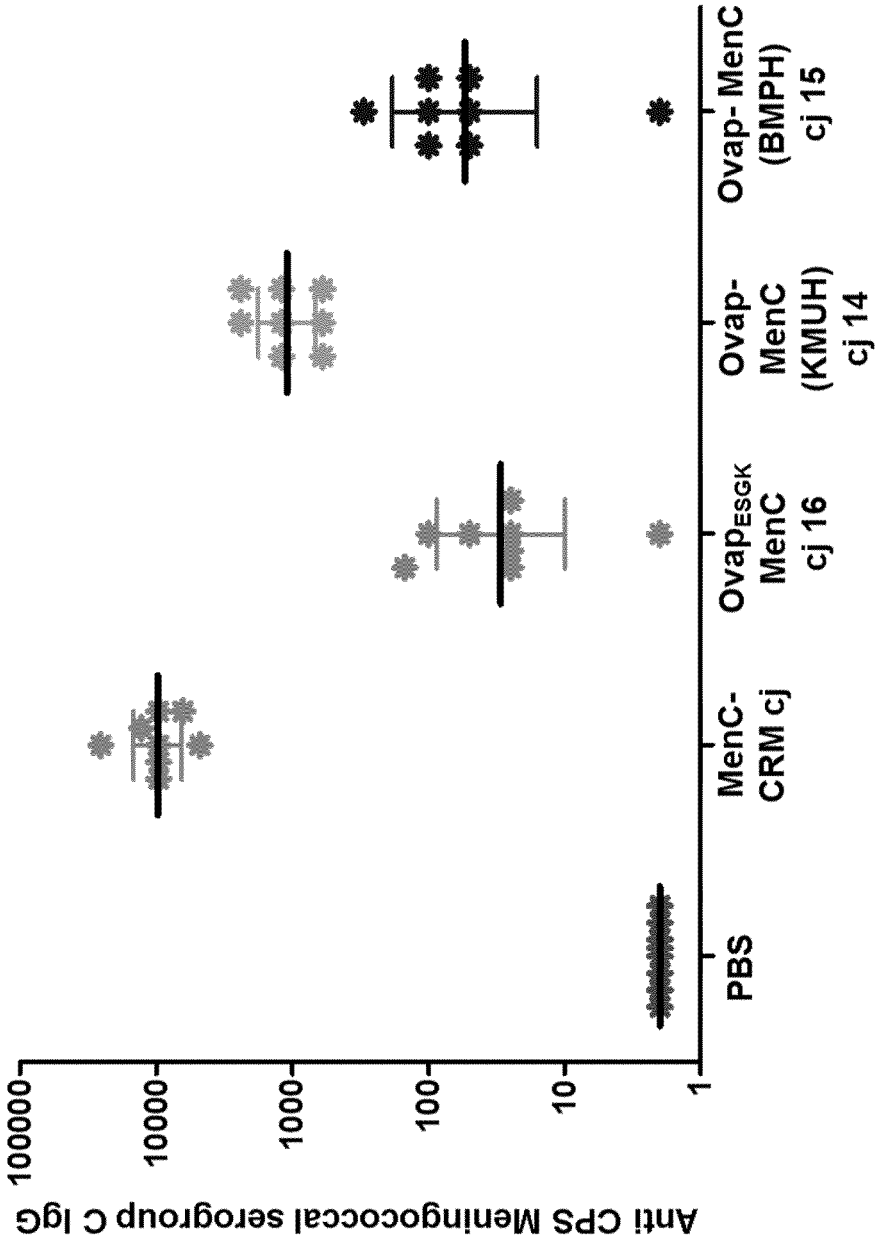

Anti CPS Meningococcal serogroup C IgG
100000
10000
1000
100
10
1
PBS
MenC-CRM cj
OvapESGK MenC cj 16
Ovap-MenC (KMUH) cj 14
Ovap- MenC (BMPH) cj 15

Figure 43

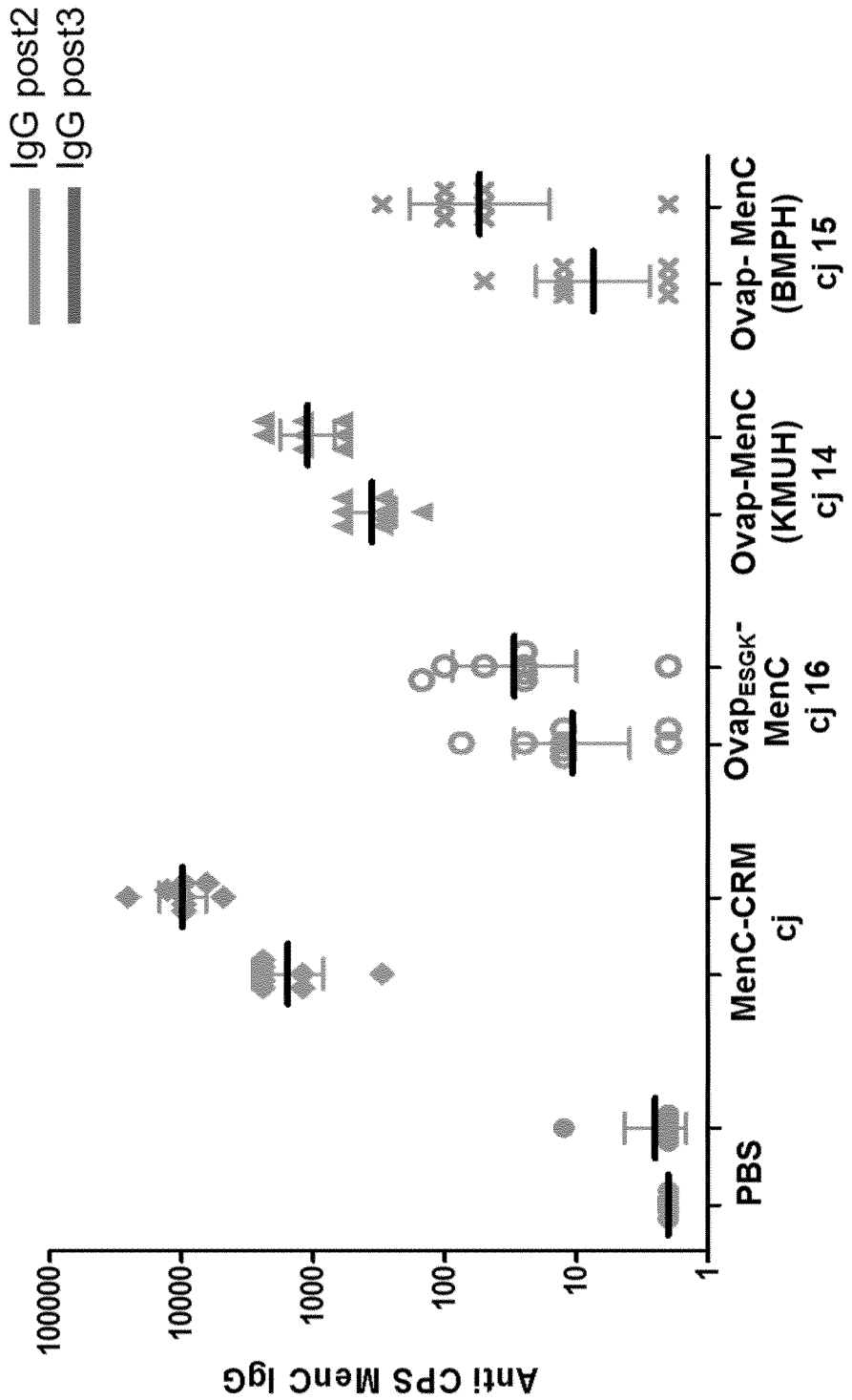

IgG post2
IgG post3
Anti CPS MenC IgG
100000
10000
1000
100
10
1
PBS
MenC-CRM cj
OvapESGK- MenC cj 16
Ovap-MenC (KMUH) cj 14
Ovap- MenC (BMPH) cj 15

Figure 44

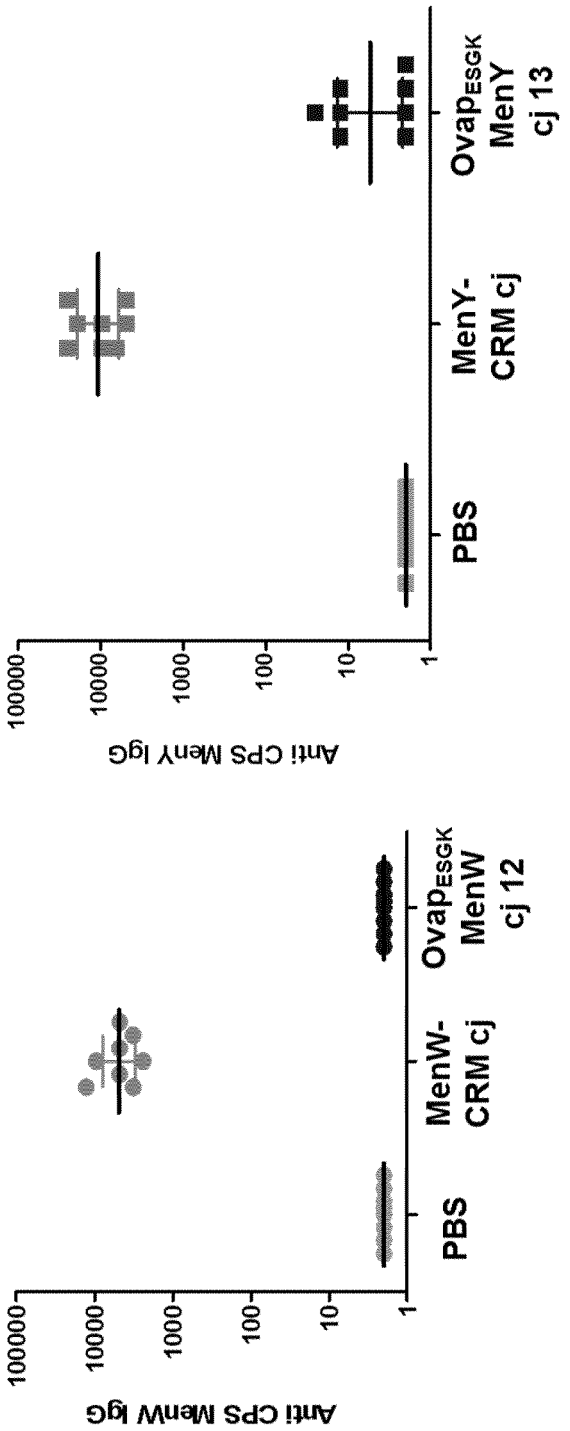

Anti CPS MenW IgG
100000
10000
1000
100
10
1
PBS
MenW-CRM cj
OvapESGK MenW cj 12
Anti CPS MenY IgG
100000
10000
1000
100
10
1
PBS
MenY-CRM cj
OvapESGK MenY cj 13

Figure 45

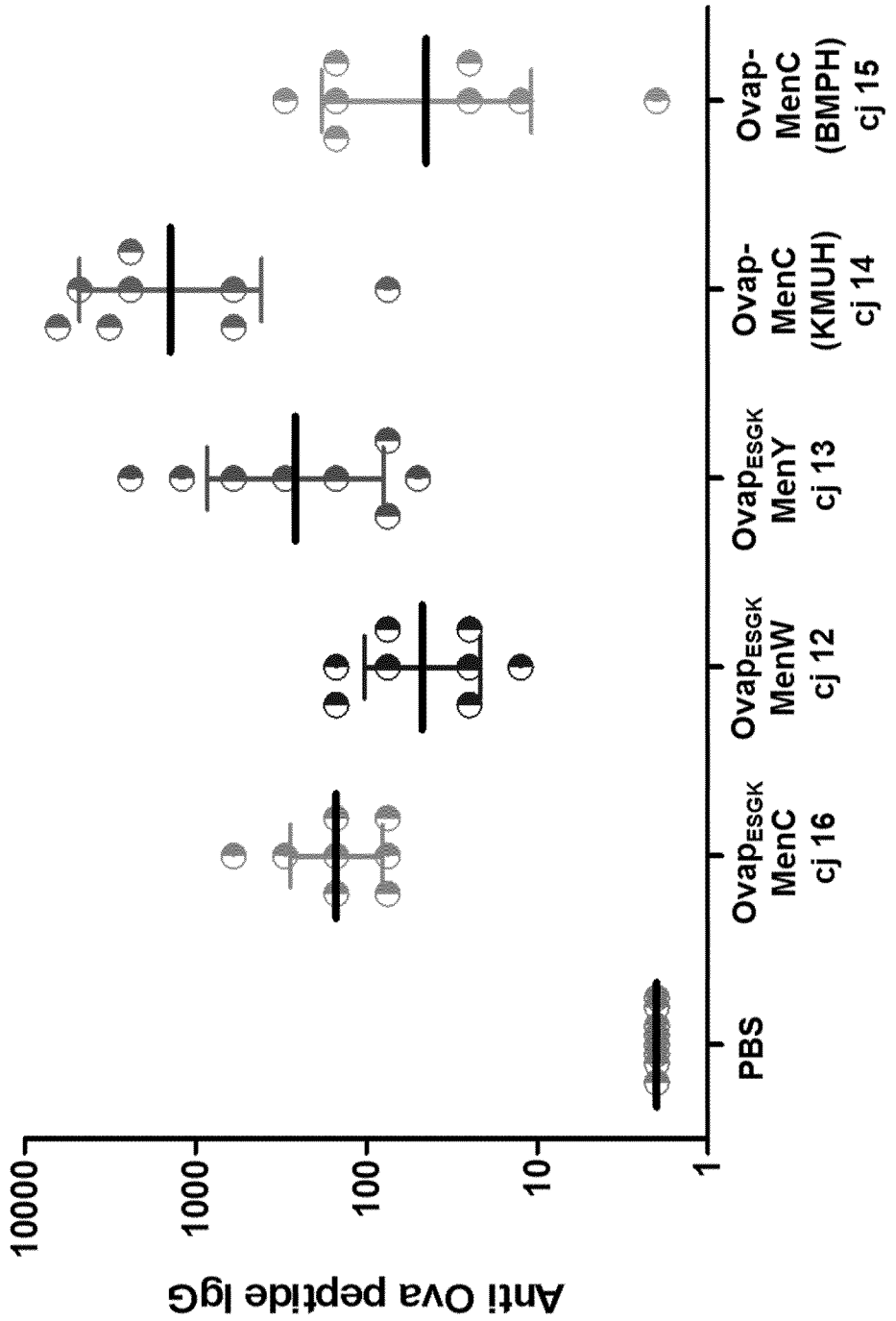

Anti Ova peptide IgG
10000
1000
100
10
1
PBS
OvapESGK MenC cj 16
OvapESGK MenW cj 12
OvapESGK MenY cj 13
Ovap-MenC (KMUH) cj 14
Ovap-MenC (BMPH) cj 15

Figure 46

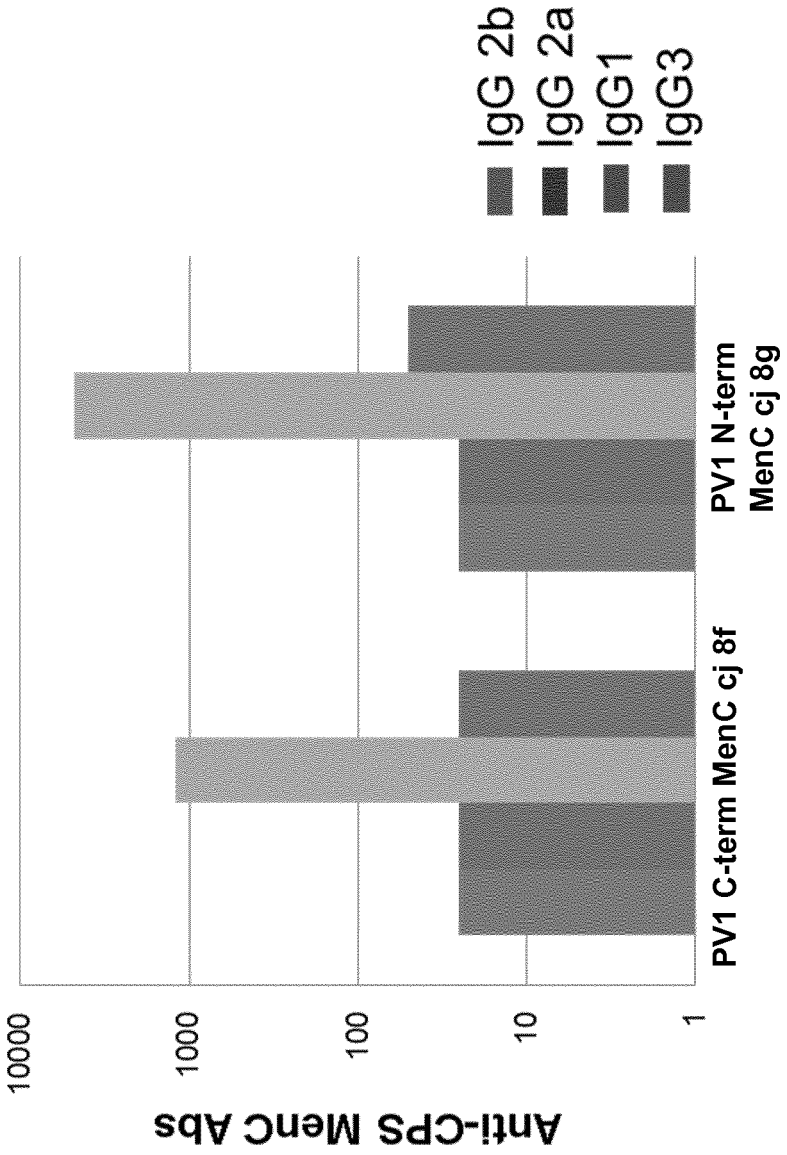

IgG 2b
IgG 2a
IgG1
IgG3
PV1 N-term MenC cj 8g
PV1 C-term MenC cj 8f
Anti-CPS MenC Abs
10000
1000
100
10
1

Figure 47

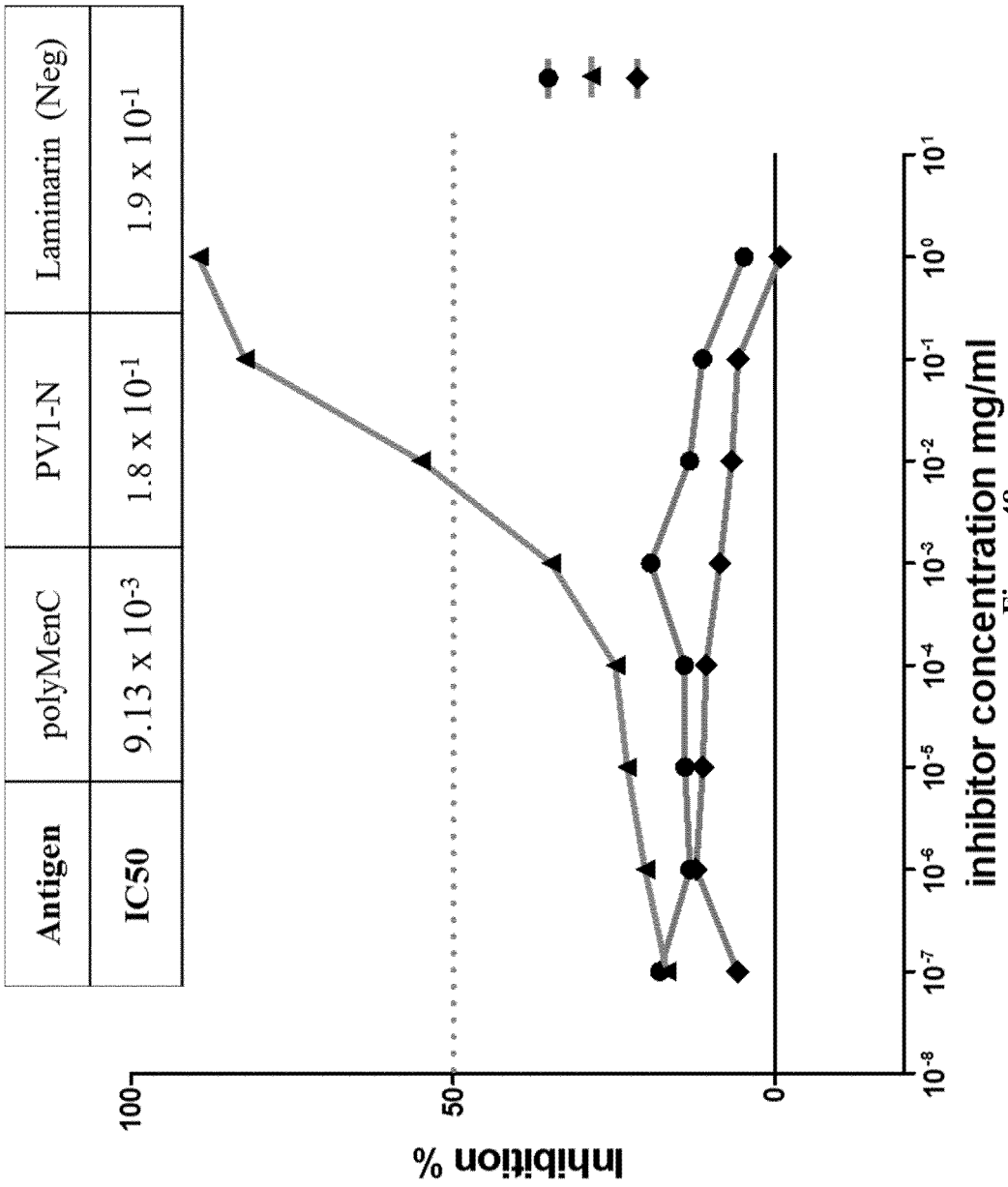

Antigen
IC50
polyMenC
9.13 x 10-3
PV1-N
1.8 x 10-1
Laminarin (Neg)
1.9 x 10-1
100
50
0
Inhibition %
10-8
10-7
10-6
10-5
10-4
10-3
10-2
10-1
100
101
inhibitor concentration mg/ml

Figure 48