

Figure 1

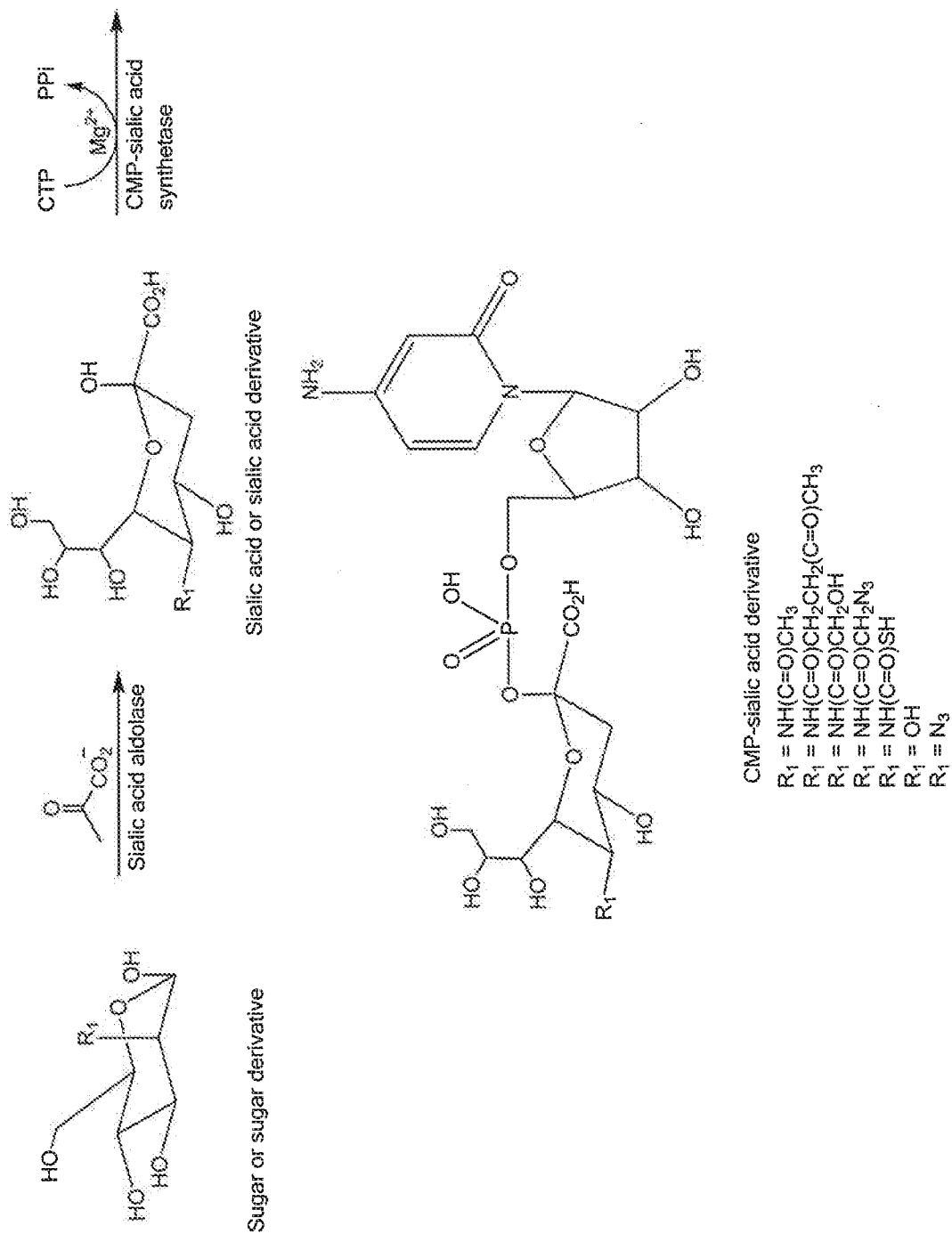
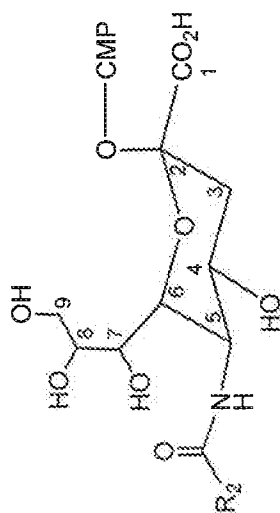
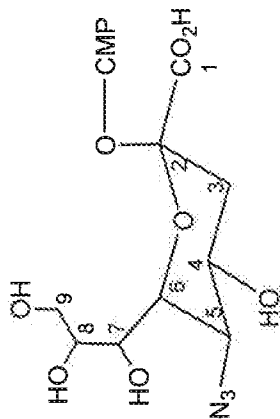


Figure 2

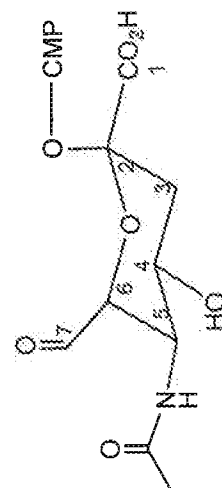


CMP-sialic acid C5 N-acetyl derivative

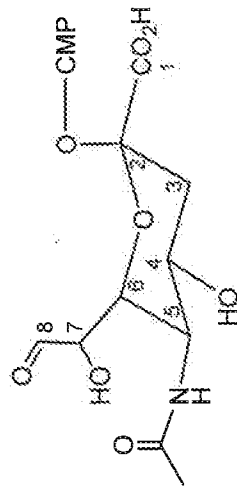
$R_2 = \text{CH}_3$
 $R_2 = \text{CH}_2\text{CH}_2(\text{C}=\text{O})\text{CH}_3$
 $R_2 = \text{CH}_2\text{OH}$
 $R_2 = \text{CH}_2\text{N}_3$
 $R_2 = \text{SH}$



CMP-sialic acid C5 azide derivative



CMP-sialic acid C7 aldehyde derivative



CMP-sialic acid C8 aldehyde derivative

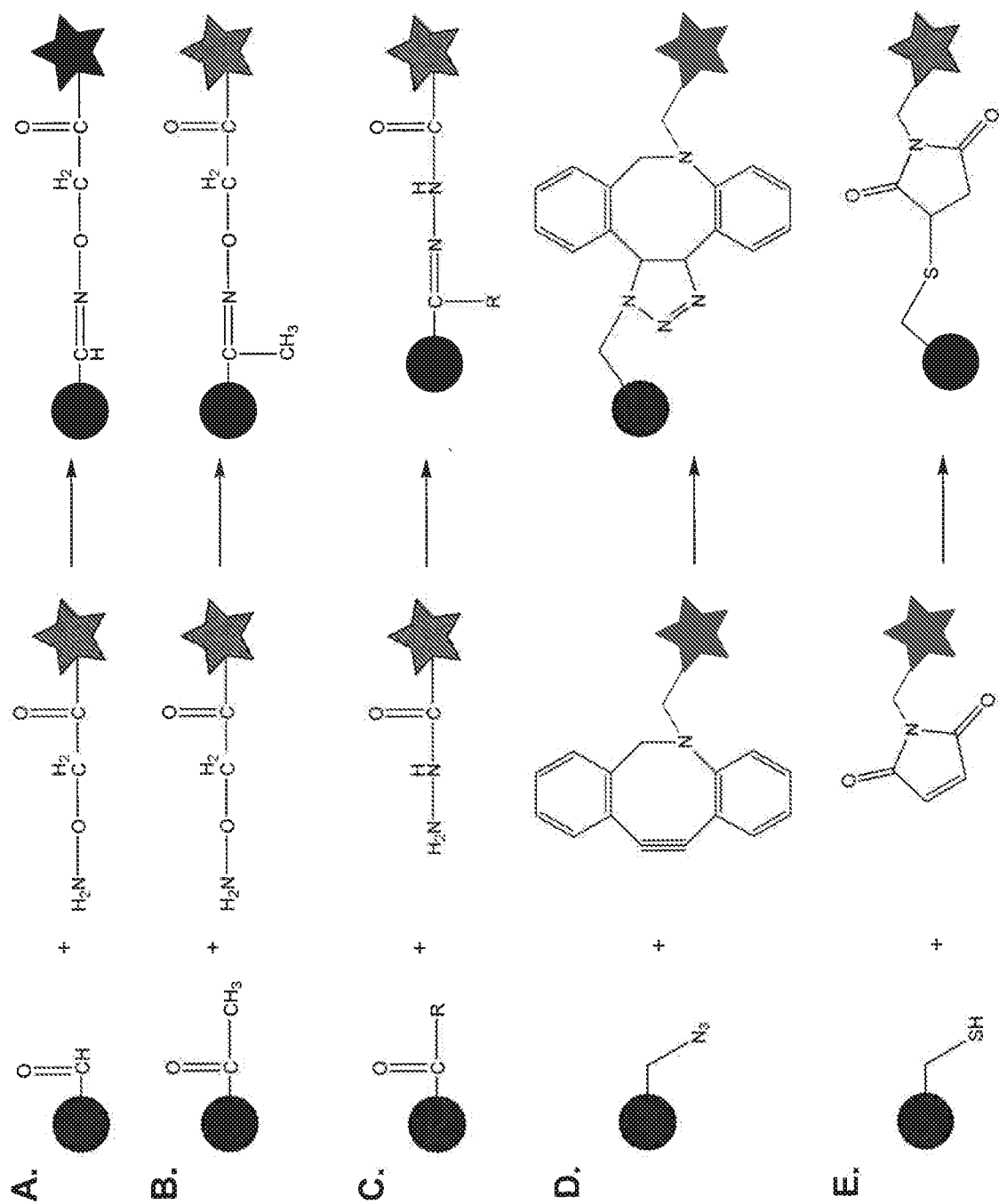


Figure 3

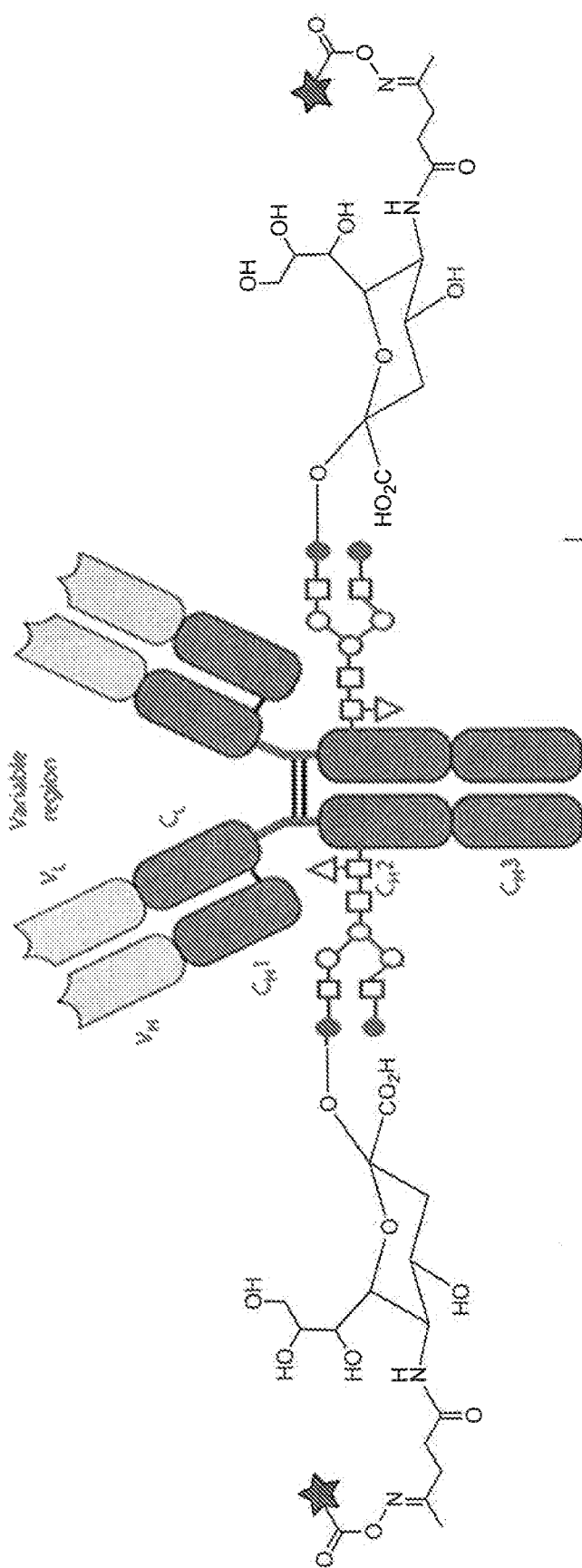


Figure 4

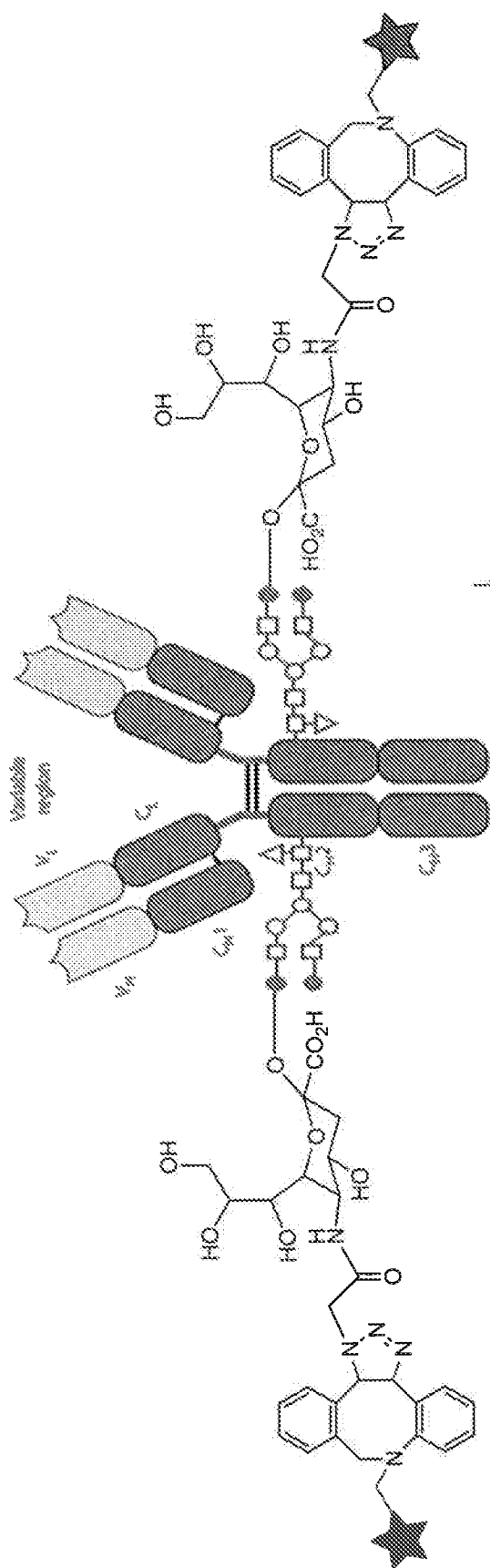


Figure 5

Figure 6

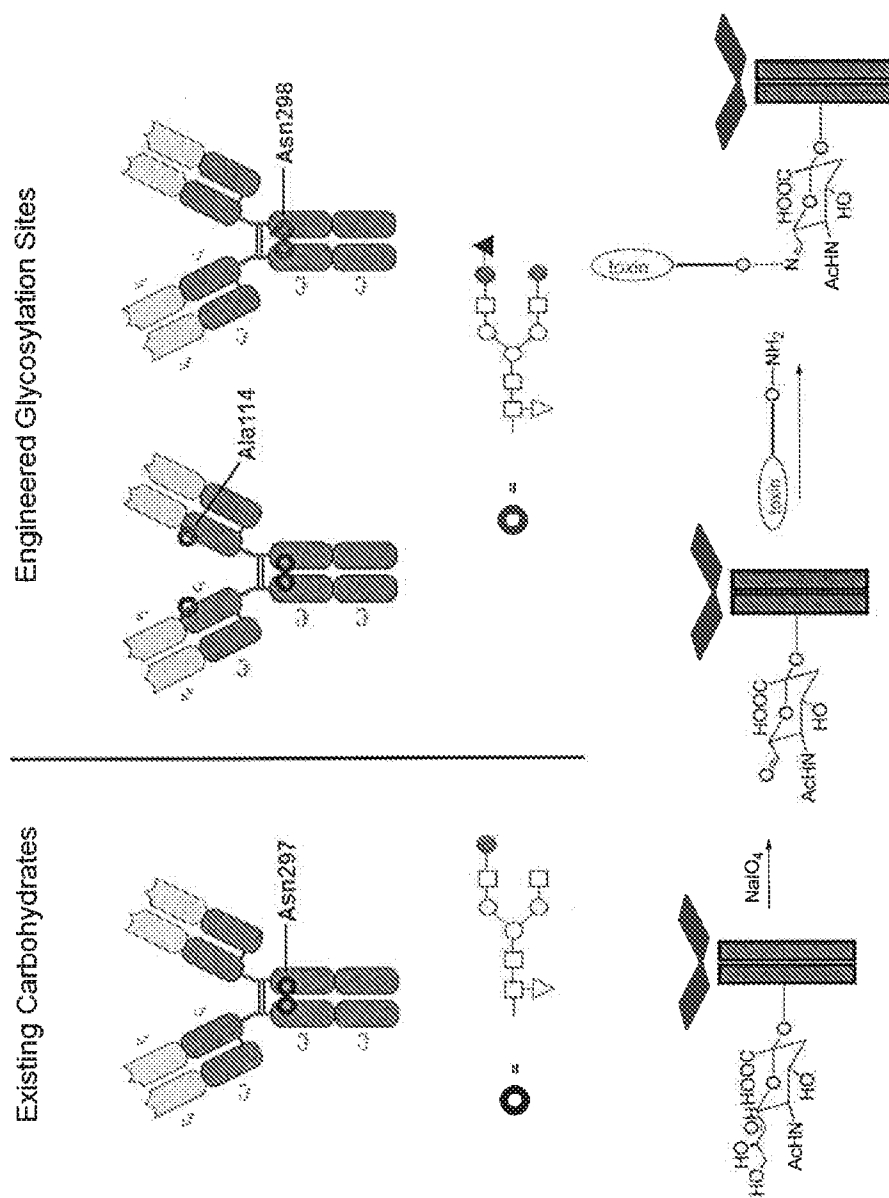


Figure 7

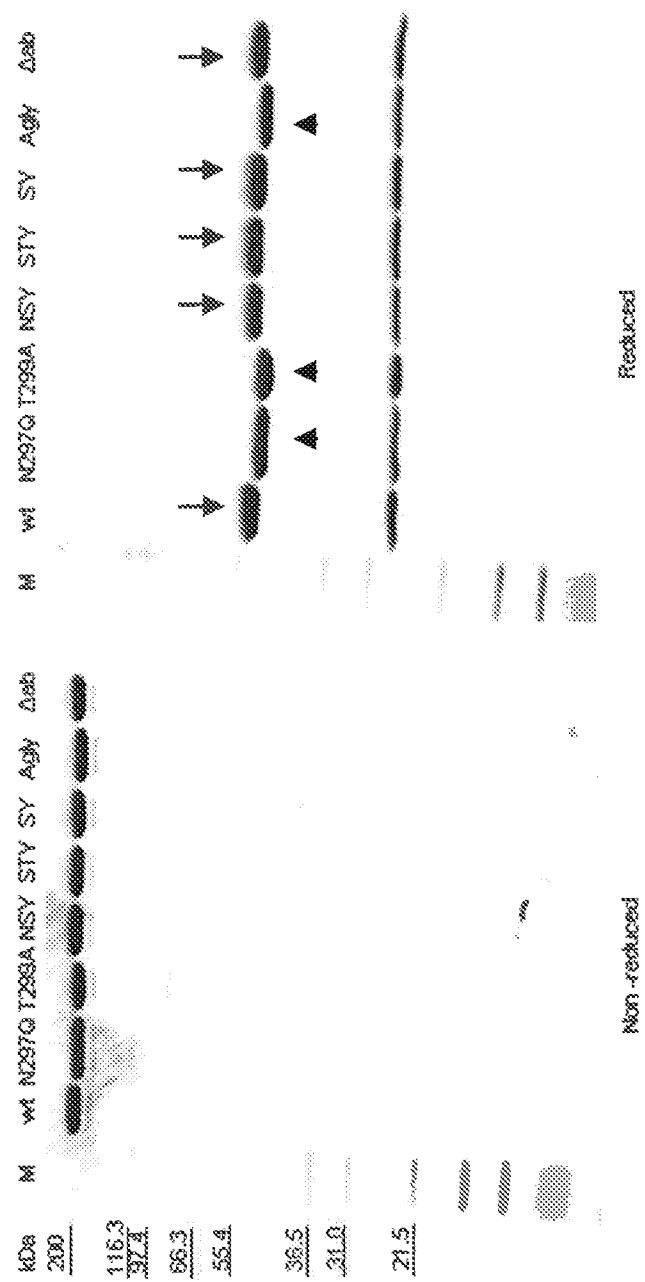
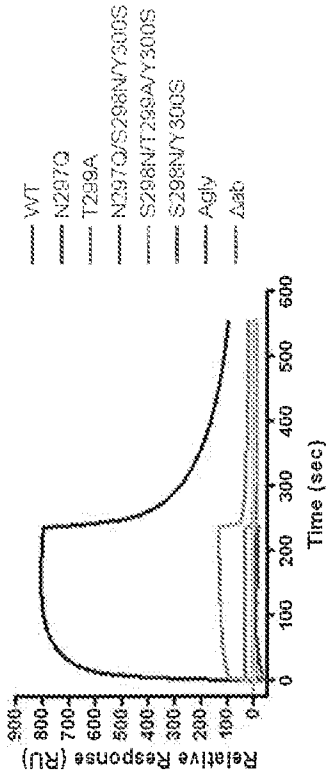


Figure 8

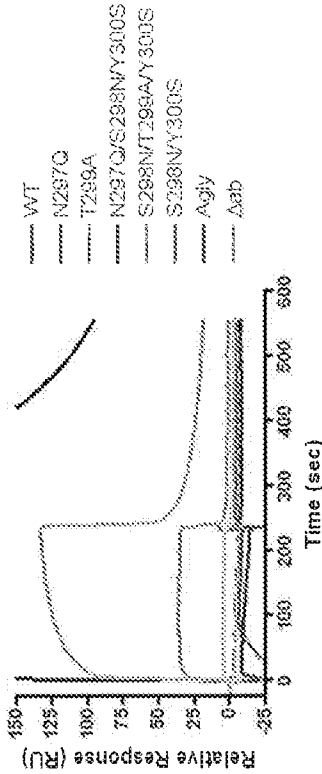
Fullscale: CD16a-Val158

Human FcγRIIIa-Val158 Binding of αβTCR HEBE1 IgG mutants



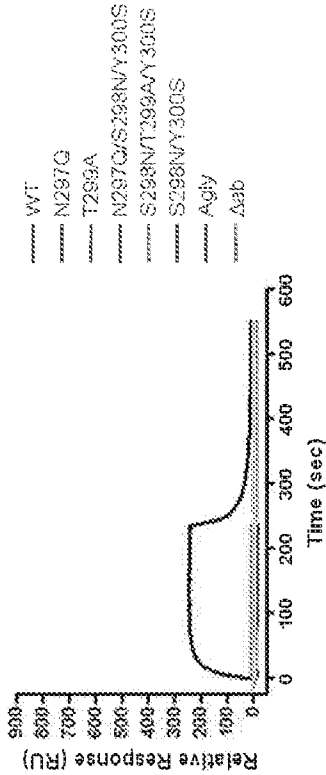
zoomed:

Human FcγRIIIa-Val158 Binding of αβTCR HEBE1 IgG mutants



Fullscale: CD16a-Phe158

Human FcγRIIIa-Phe158 Binding of αβTCR HEBE1 IgG mutants



zoomed:

Human FcγRIIIa-Phe158 Binding of αβTCR HEBE1 IgG mutants

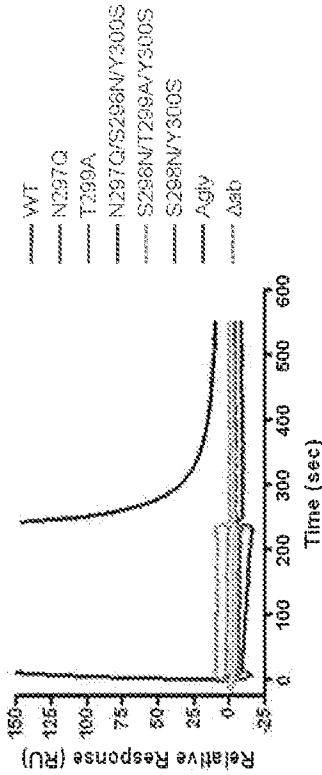


Figure 9

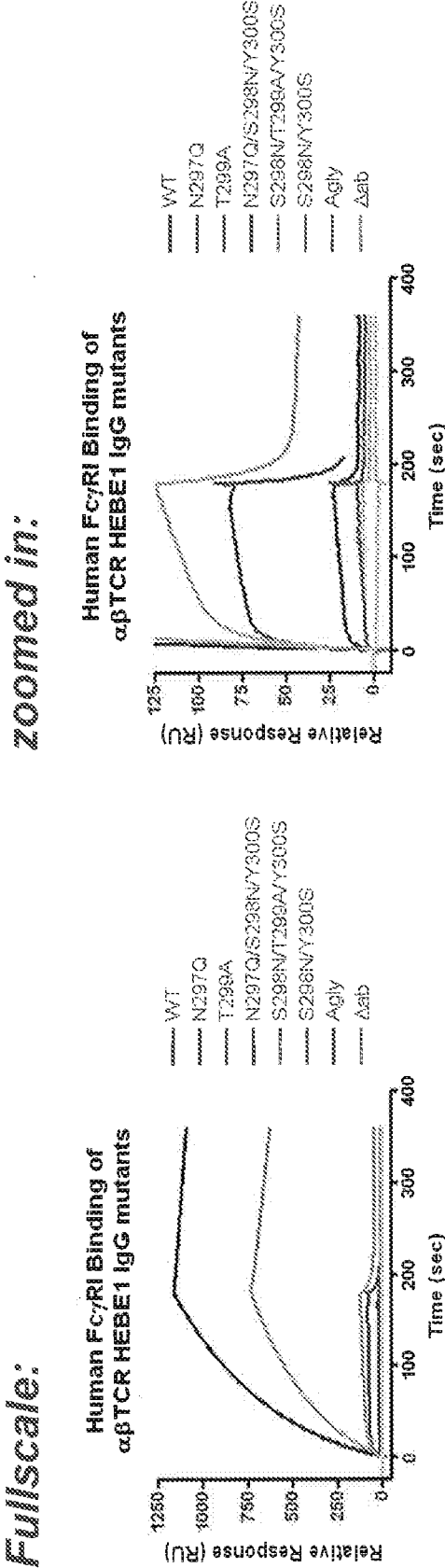


Figure 10

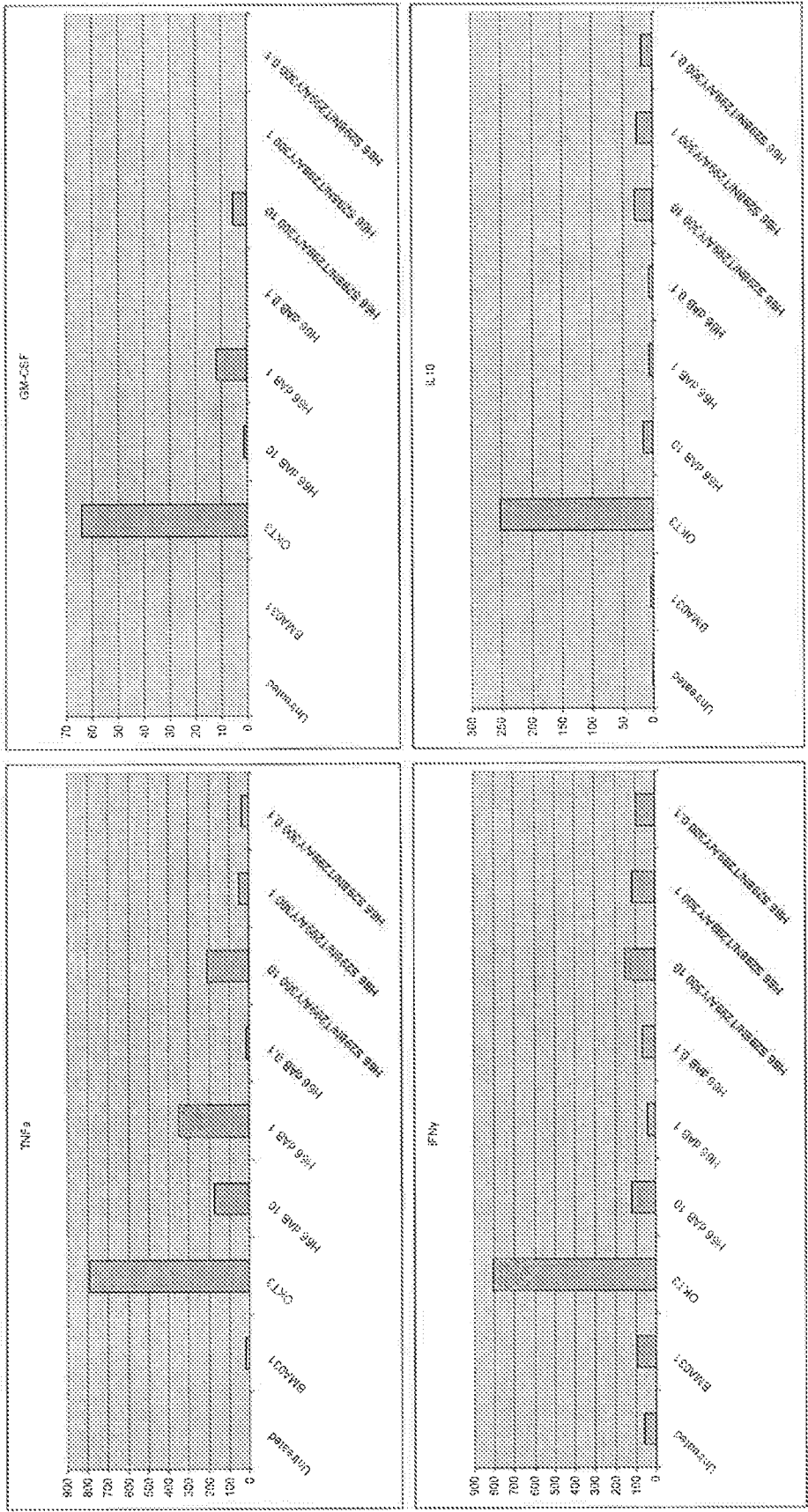
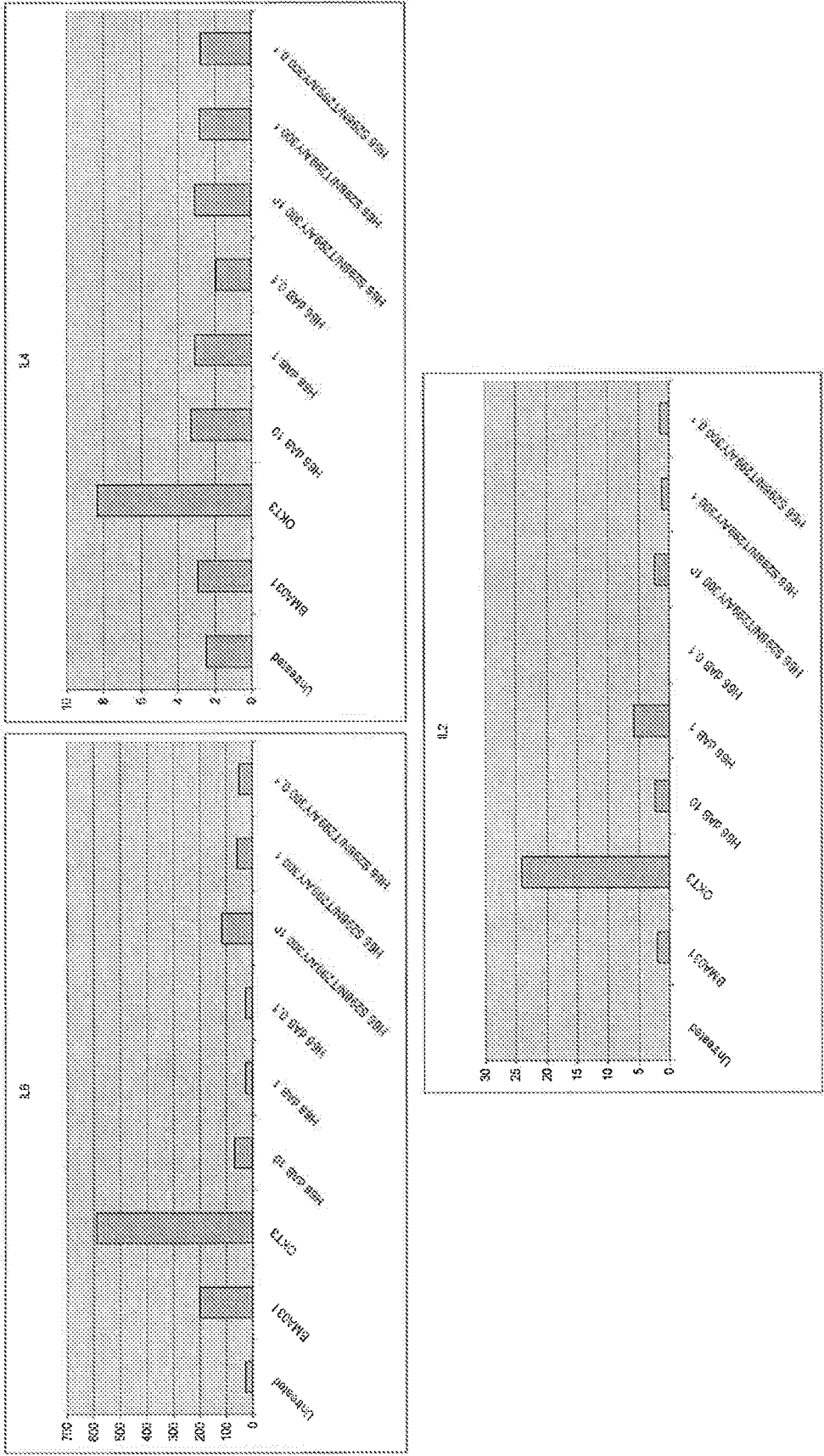


Figure 11



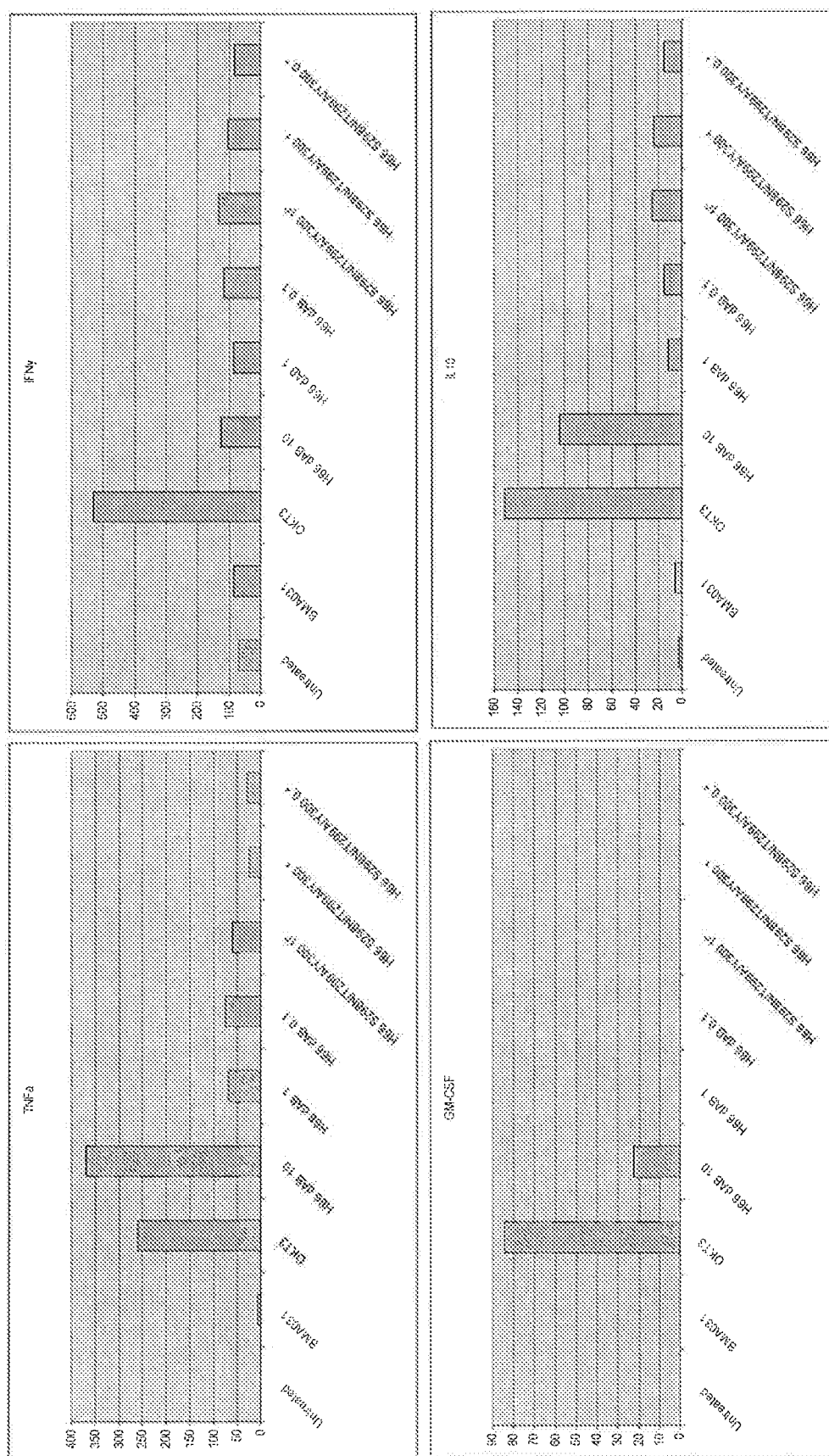


Figure 12

Figure 13

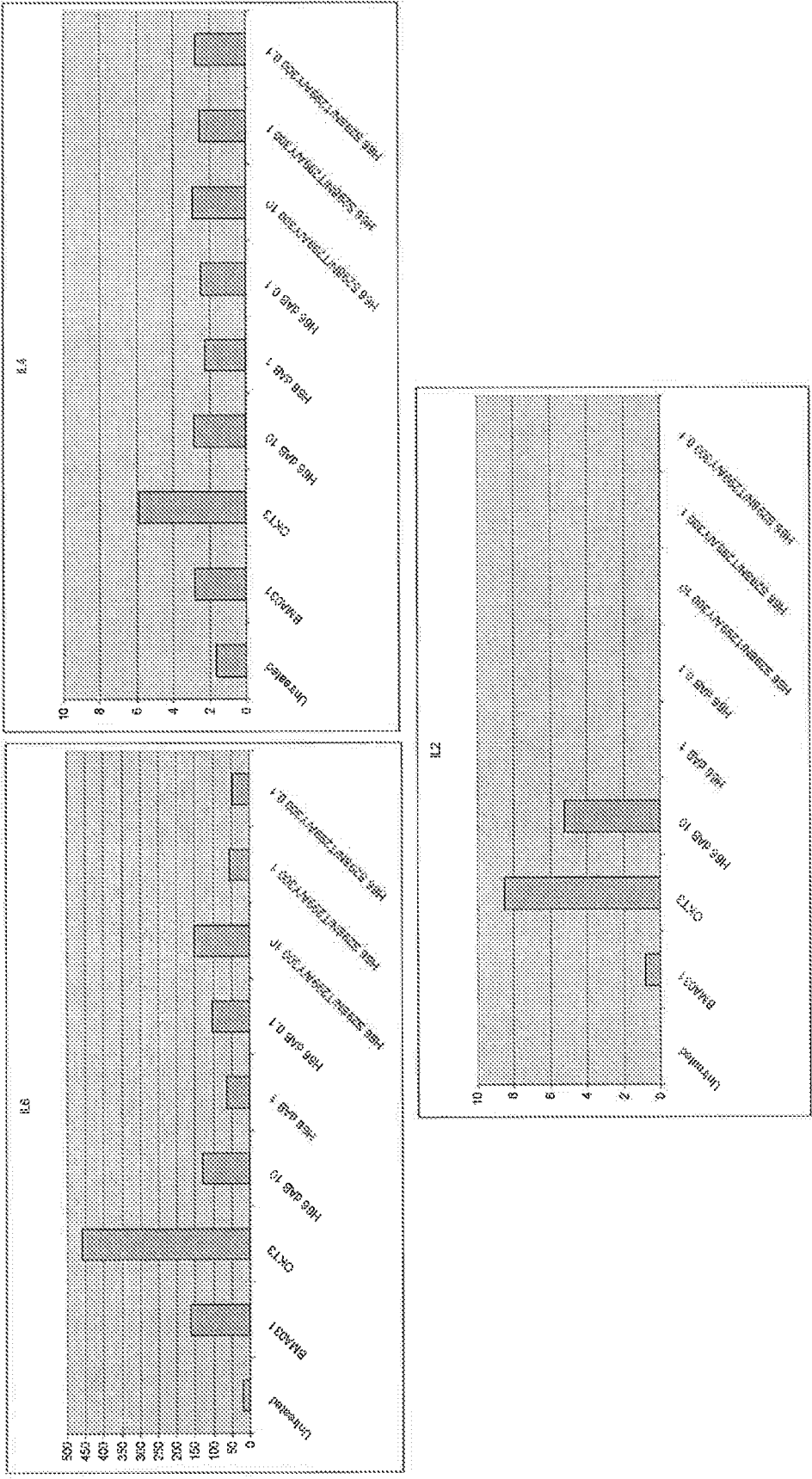
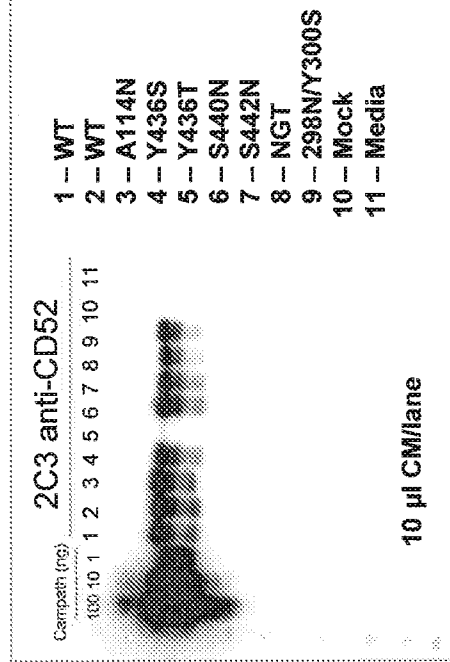


Figure 14

A.

Western Blot



B.

Biacore

Unknown	Conc (µg/mL)	Relative Response (RU)	Calc Conc (M)	Calc Conc (µg/mL)
2C3 in media	3.00	855.75	2.45E-08	3.689
2C3 in media	3.00	860.96	2.47E-08	3.710
2C3 in media	3.00	866.09	2.51E-08	3.758
A114N		146.5	1.00E-09	0.240
Mock		97.84	8.62E-10	0.129
NGT		124.52	1.30E-09	0.196
S298N/Y300S		112.14	1.15E-09	0.172
S440N		146.36	1.61E-09	0.240
S442N		121.21	1.28E-09	0.189
WT (Katya)		148.41	1.63E-09	0.244
WT (Tim)		148.08	1.62E-09	0.244
Y436S		158.72	1.79E-09	0.267
Y436T		84.27	8.22E-10	0.123
incubated media		87.36	8.52E-10	0.129

Figure 15

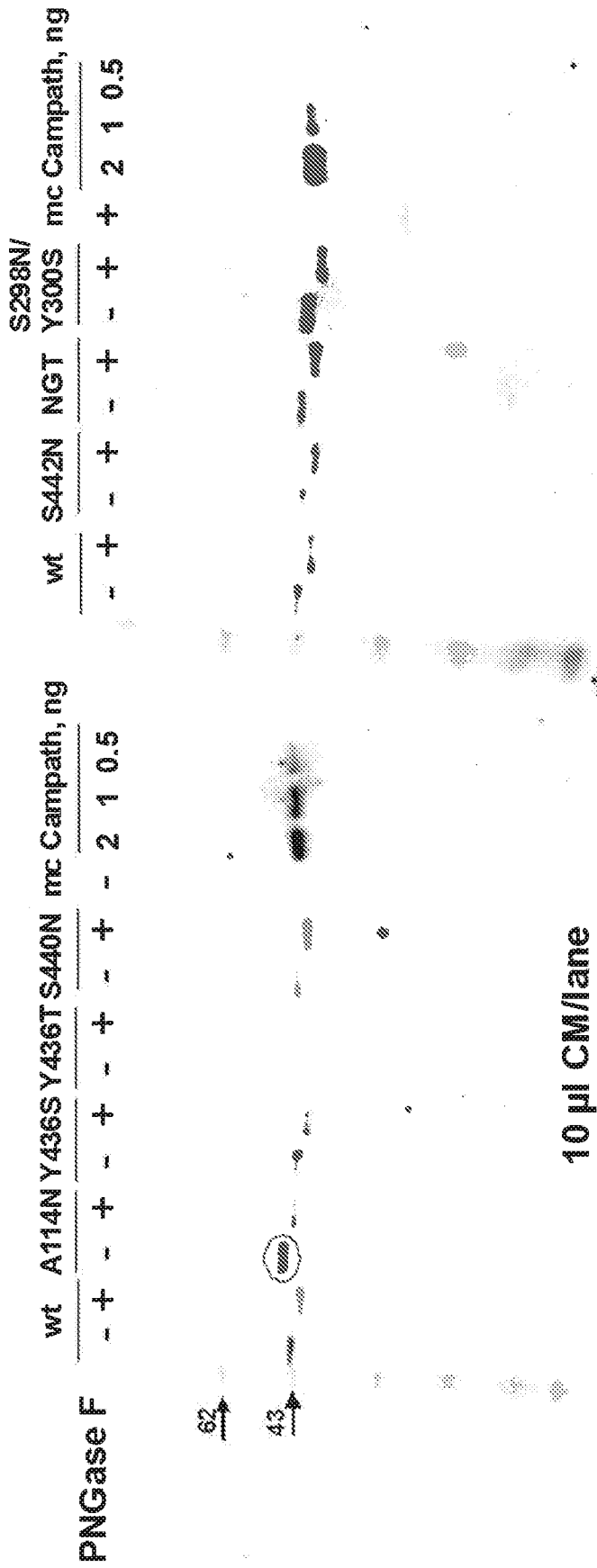
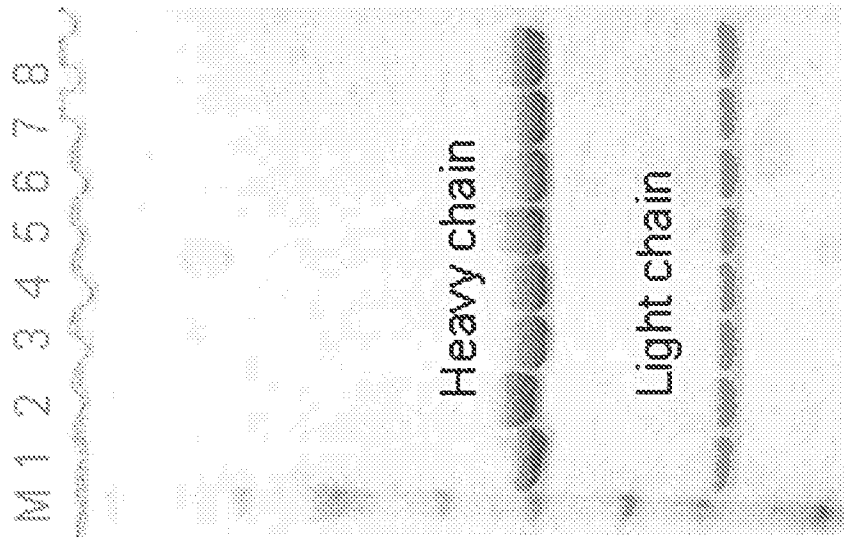


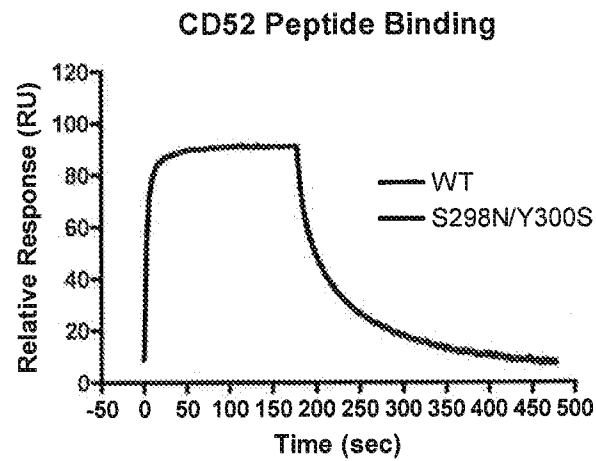
Figure 16



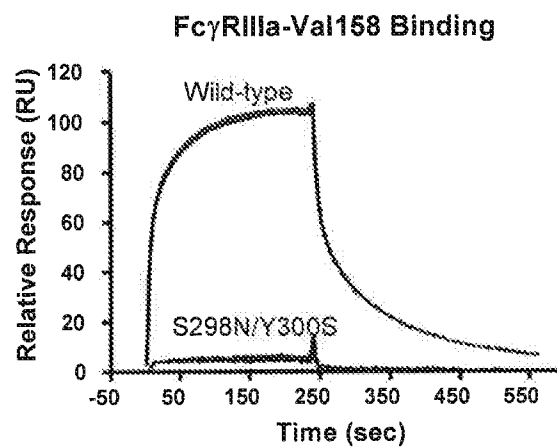
Lane	Mutant	mg/ml
1	wt	2.66
2	A114N	2.99
3	Y436S	1.61
4	S298N/Y300S	0.99
5	Y436T	0.41
6	S440N	1.21
7	S442N	1.62
8	NGT	2.21

Figure 17

A.



B.



C.

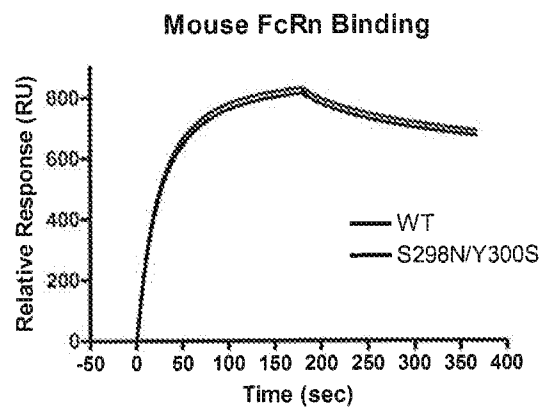


Figure 18

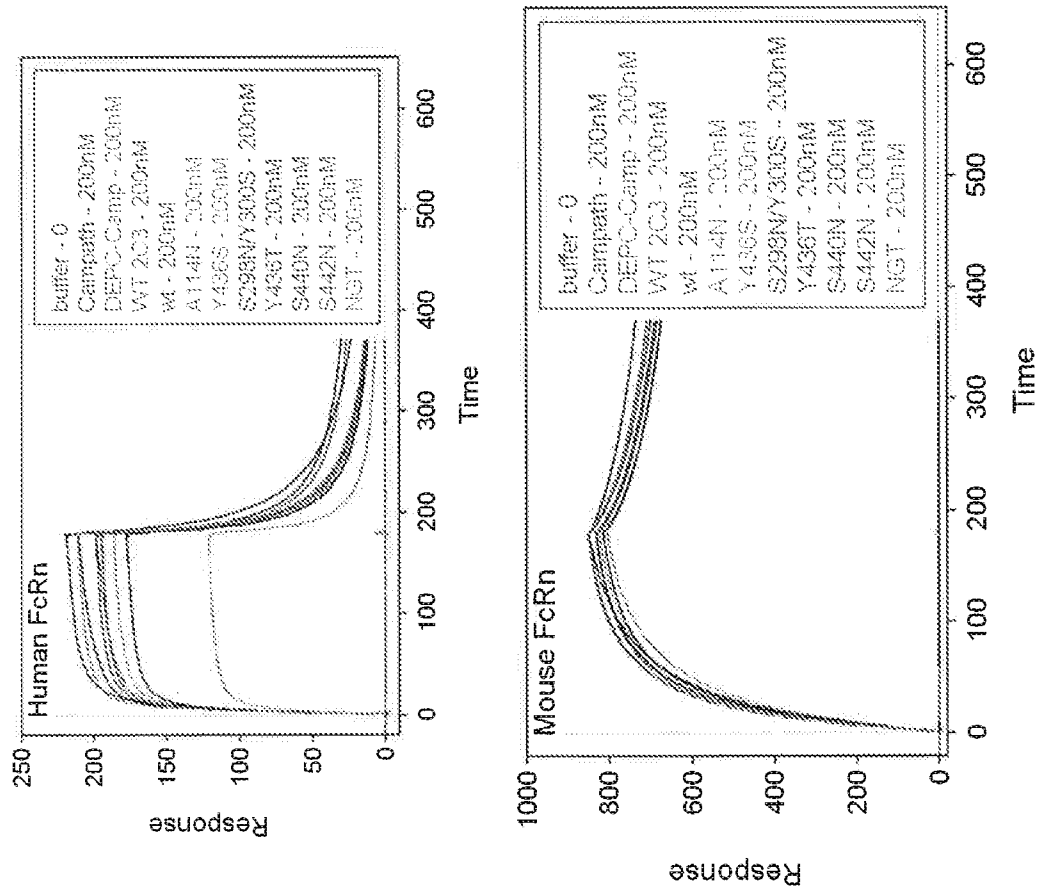
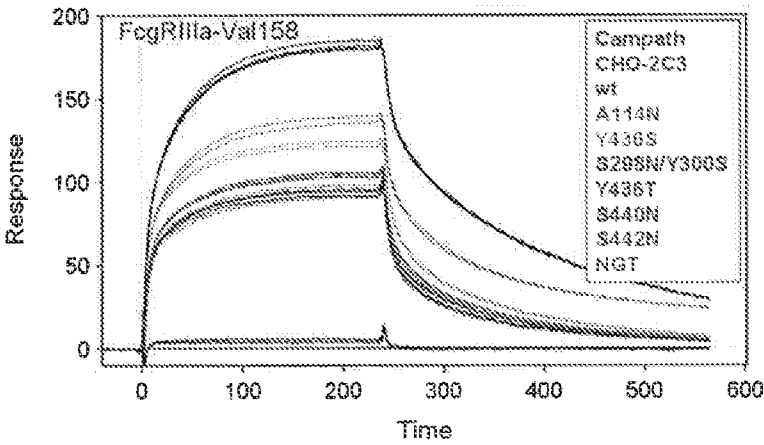


Figure 19

A.



B.

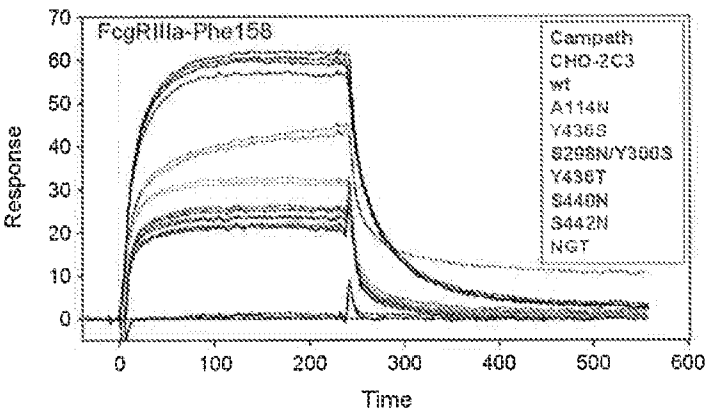


Figure 20

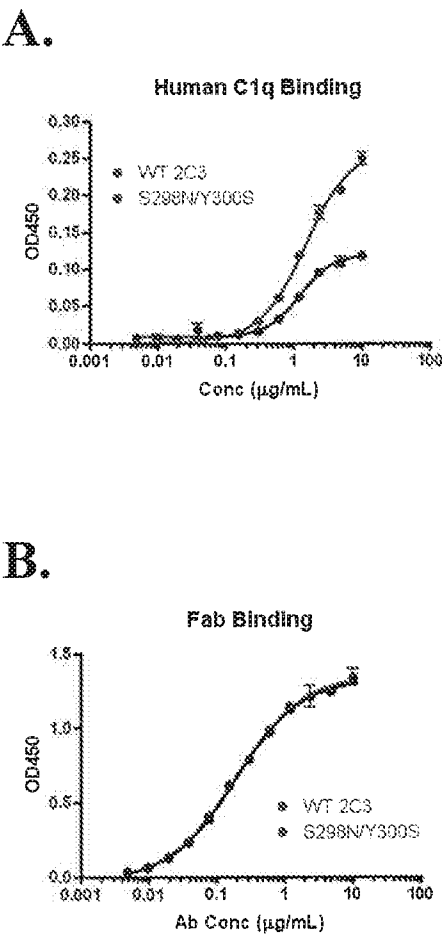


Figure 21

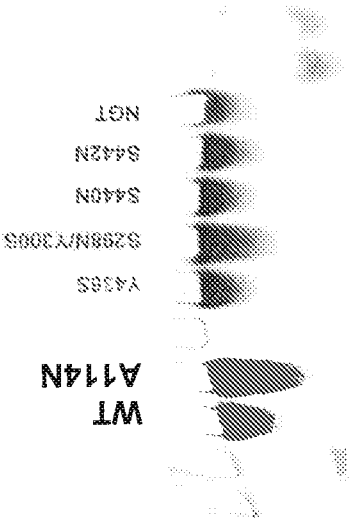
Sample	k_a ($\times 10^6$ /Ms)	k_d ($\times 10^{-2}$ /s)	R_{max} (RU)	K_D (nM)
GLD52	7.0	1.7	67.0	2.44
WT 2C3	6.0	1.1	64.2	1.75
A114N	4.7	1.1	59.5	2.45
Y436S	5.9	1.0	66.9	1.73
S298N/Y300S	5.7	1.0	63.3	1.80
Y436T	4.8	0.9	65.7	1.95
S440N	5.8	1.1	66.8	1.84
S442N	5.7	1.1	66.2	1.85
NGT	7.9	1.1	70.2	1.35

Figure 22

Sample	Kon (x10 ⁶ M ⁻¹ s ⁻¹)	Koff (x10 ⁻² s ⁻¹)	KD (nM)
WT 2C3	5.2	1.1	2.1
A114N	5.3	1.3	2.4

Figure 23

A.



B.

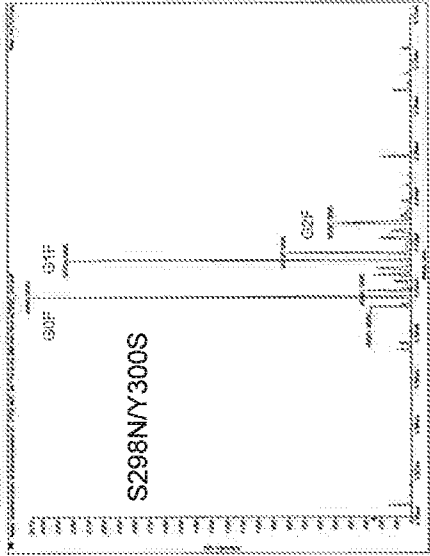


Figure 23

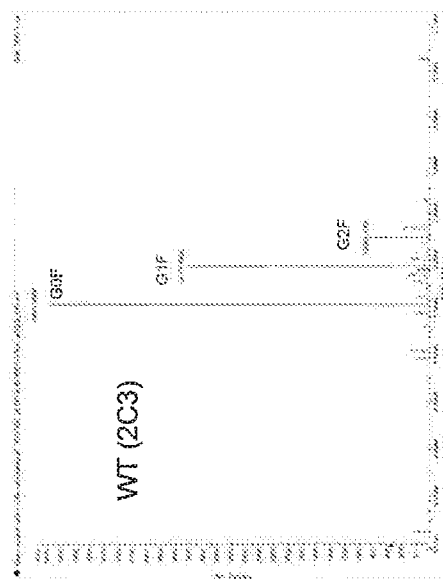
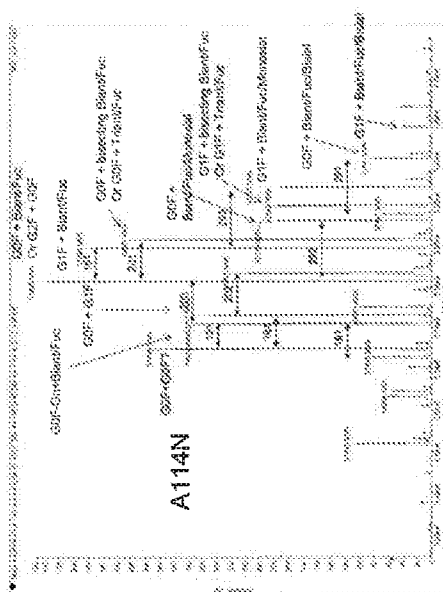


Figure 24

A.

Sample	lot #	Octet Conc (µg/mL)
Mock media	11/23/2009	too low
wt 2C3	11/23/2009	2.54
A114N	11/23/2009	2.83
S298N/Y300S	11/23/2009	1.36
S440N	11/23/2009	1.32
S442N	11/23/2009	1.21
Y436S	11/23/2009	1.92
Y436T	11/23/2009	0.34
NGT	11/23/2009	1.90

B.

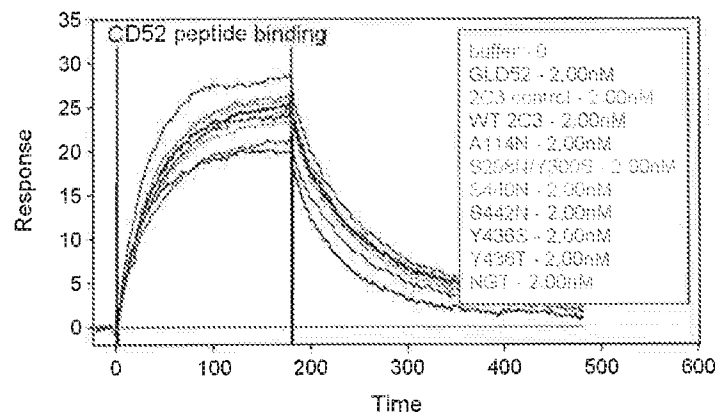


Figure 25

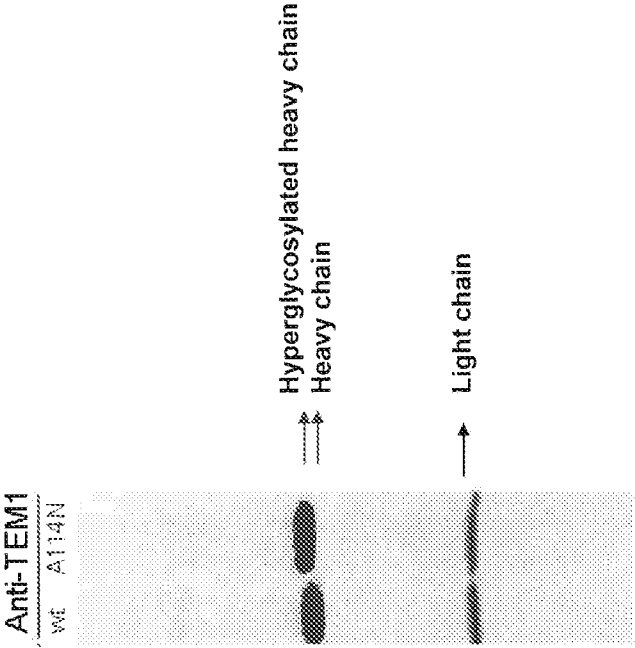


Figure 26

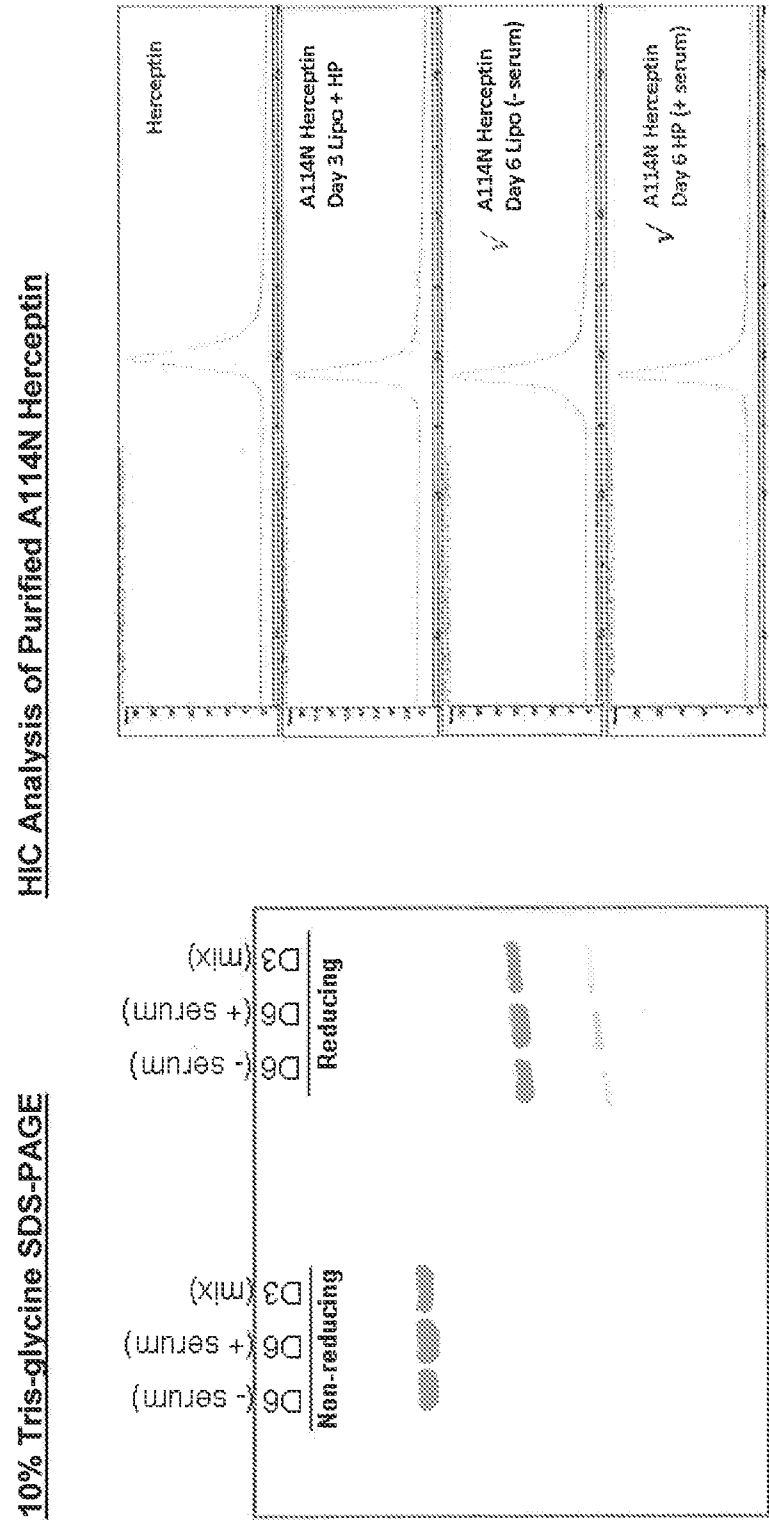


Figure 27

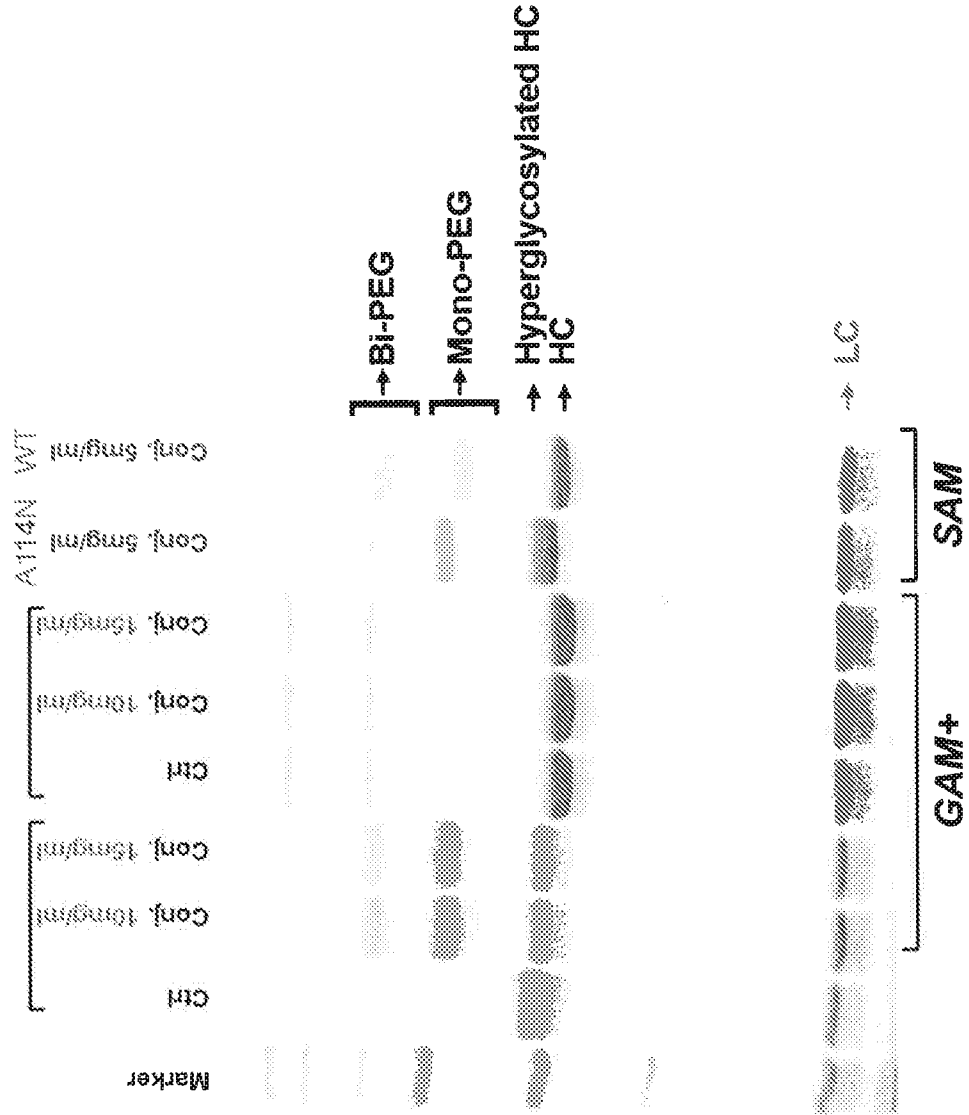
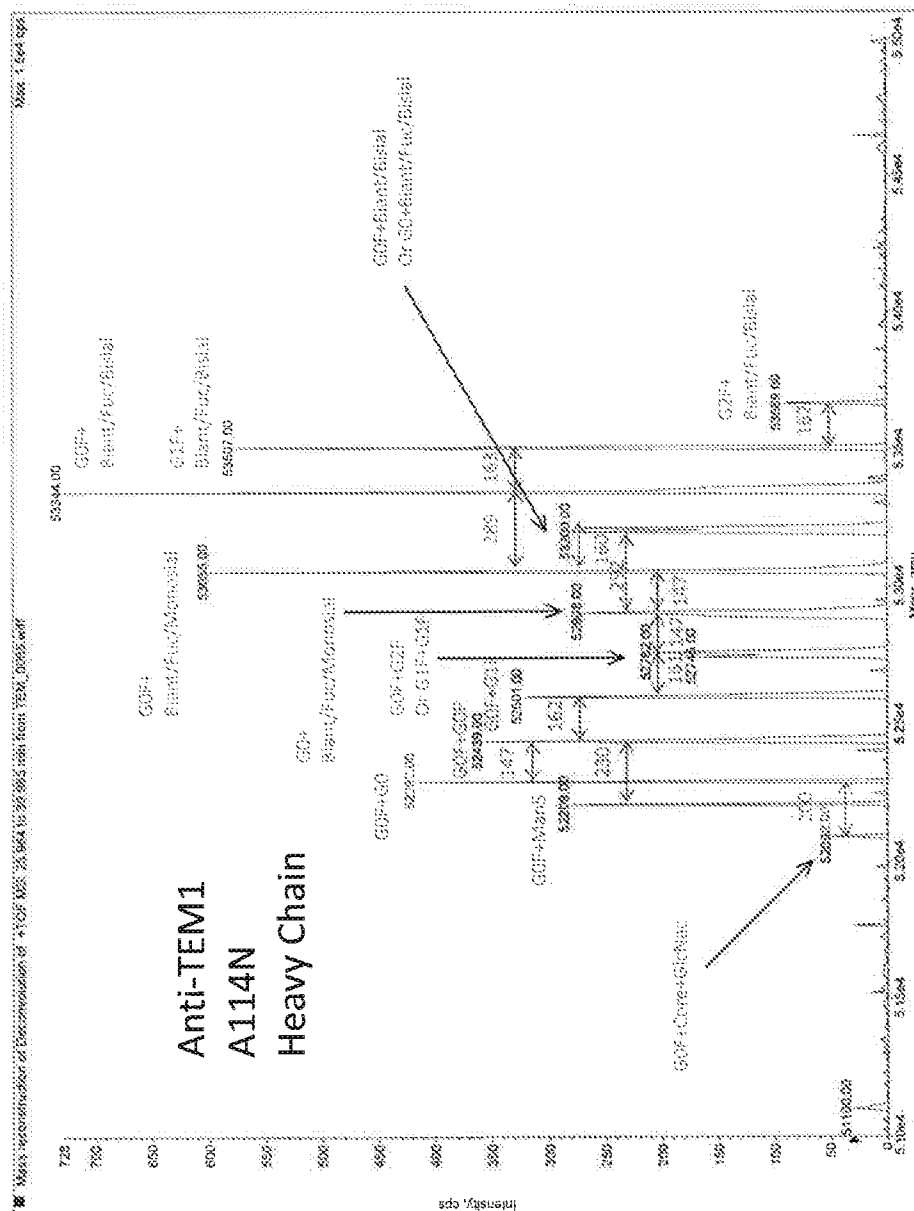


Figure 28



30/96

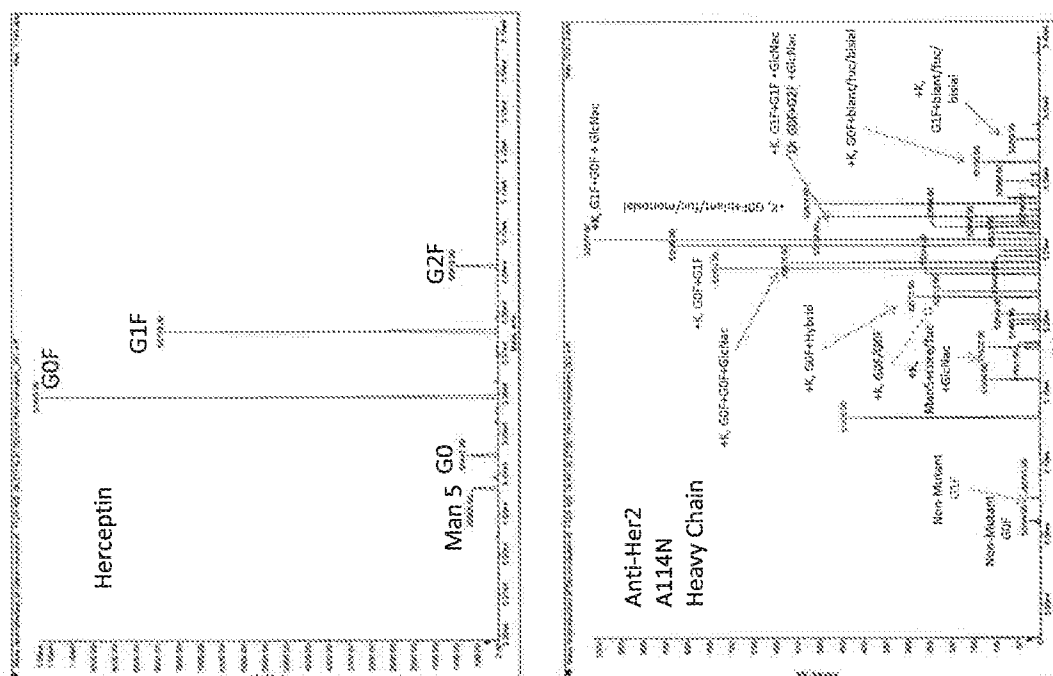


Figure 30

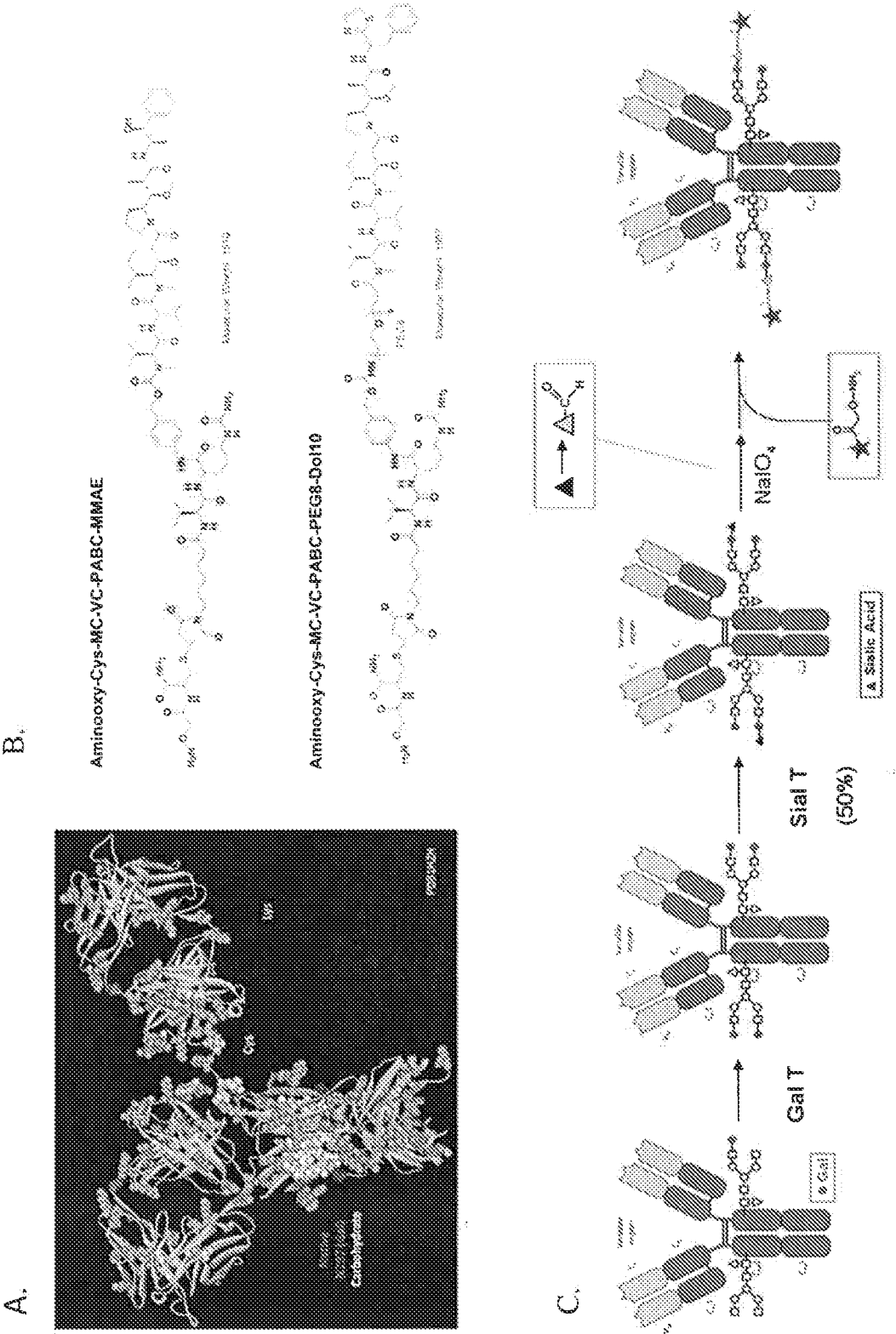


Figure 31

Synthesis of aminoxy-Cys-MC-VC-PABC-MMAE and aminoxy-Cys-MC-VC-PABC-PEG8-Dol10

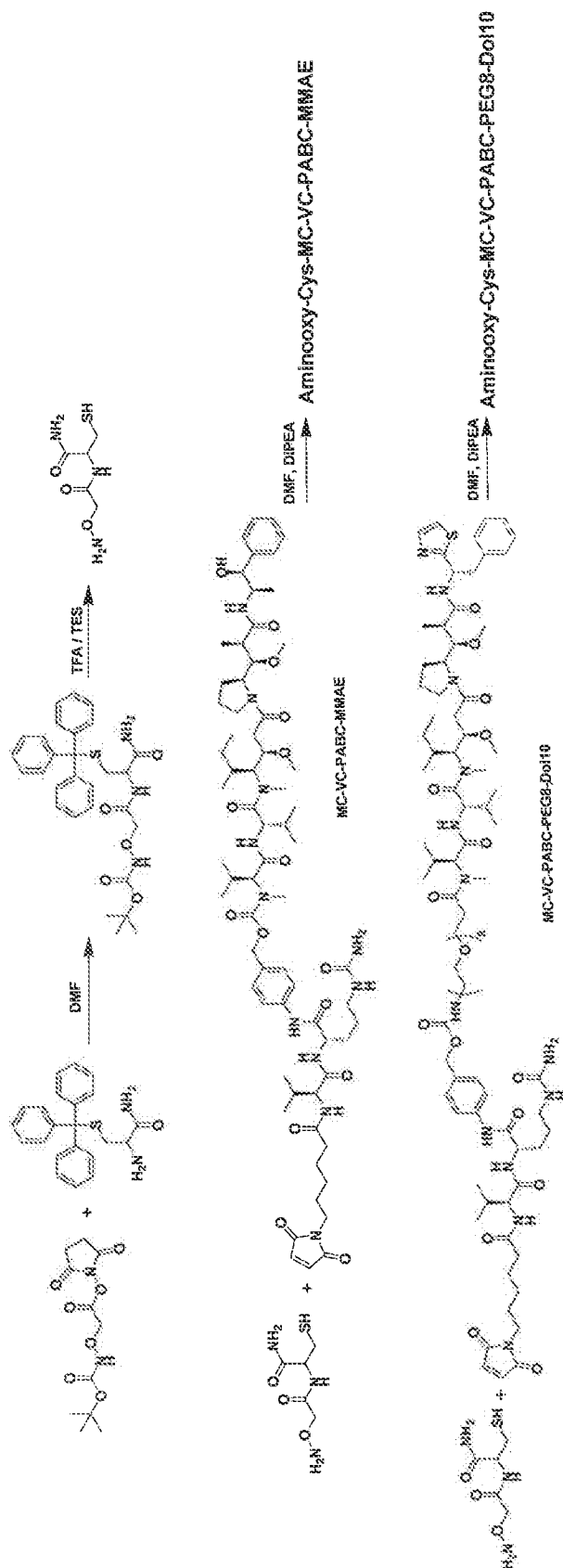


Figure 32

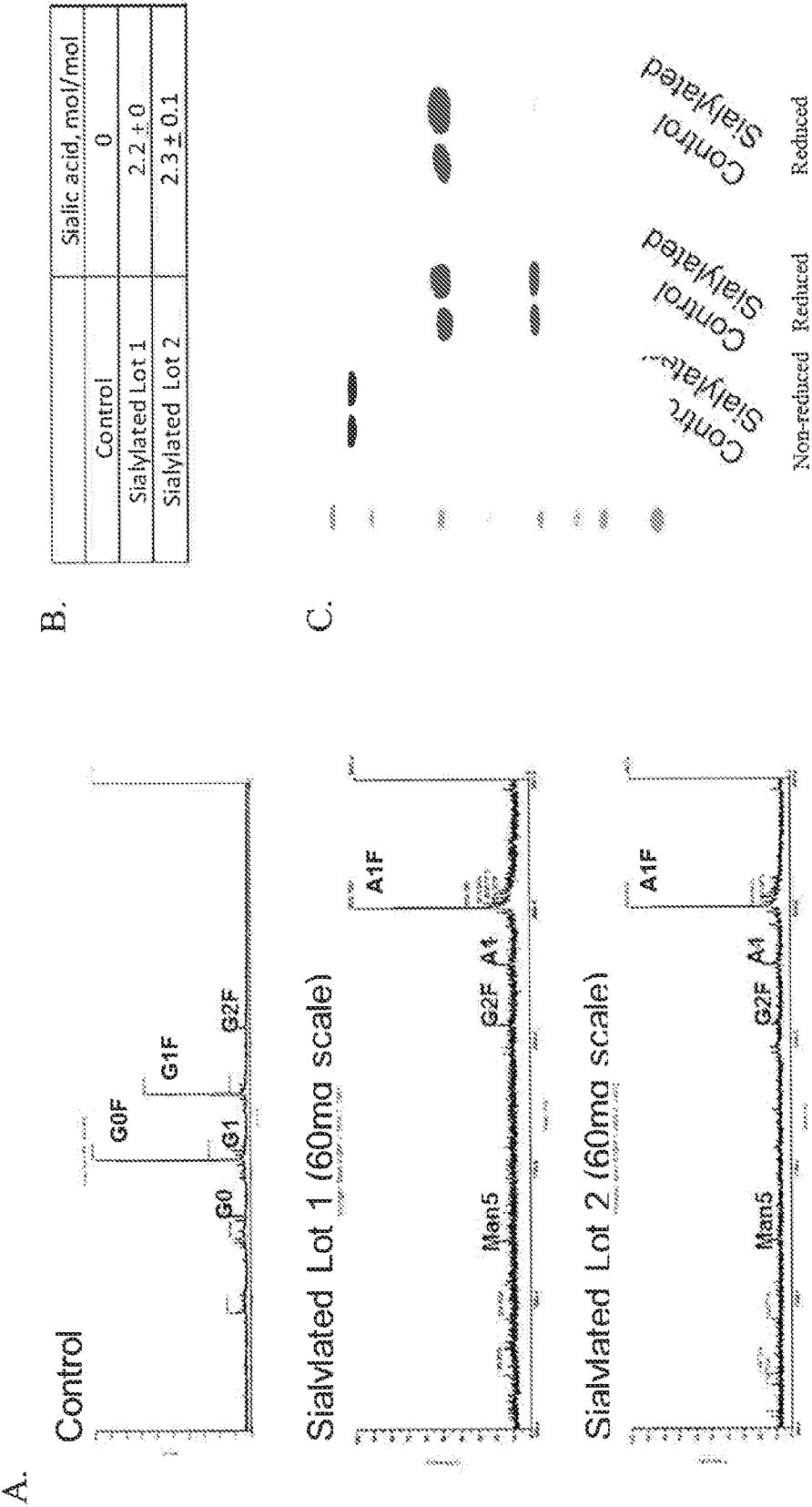


Figure 33

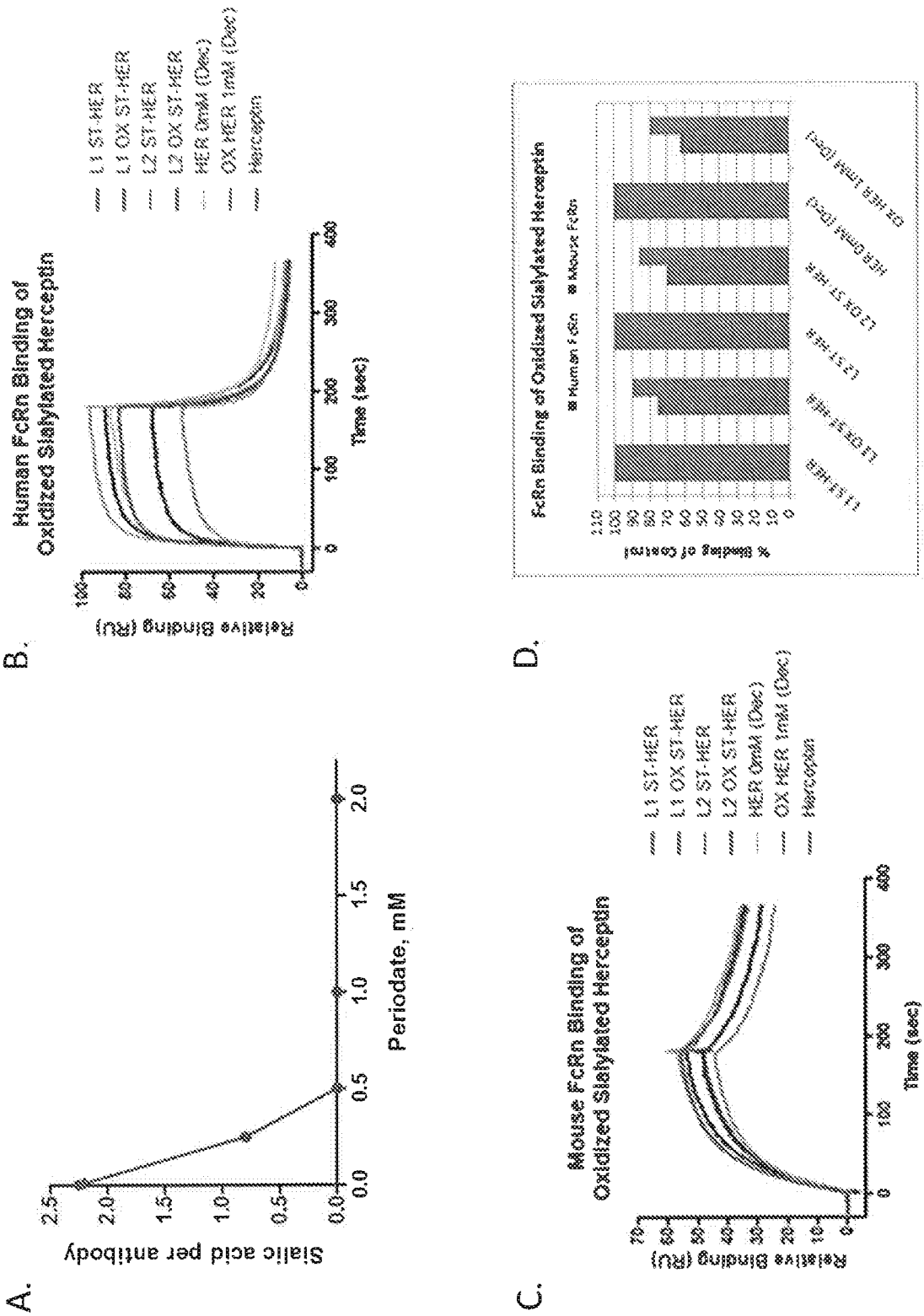


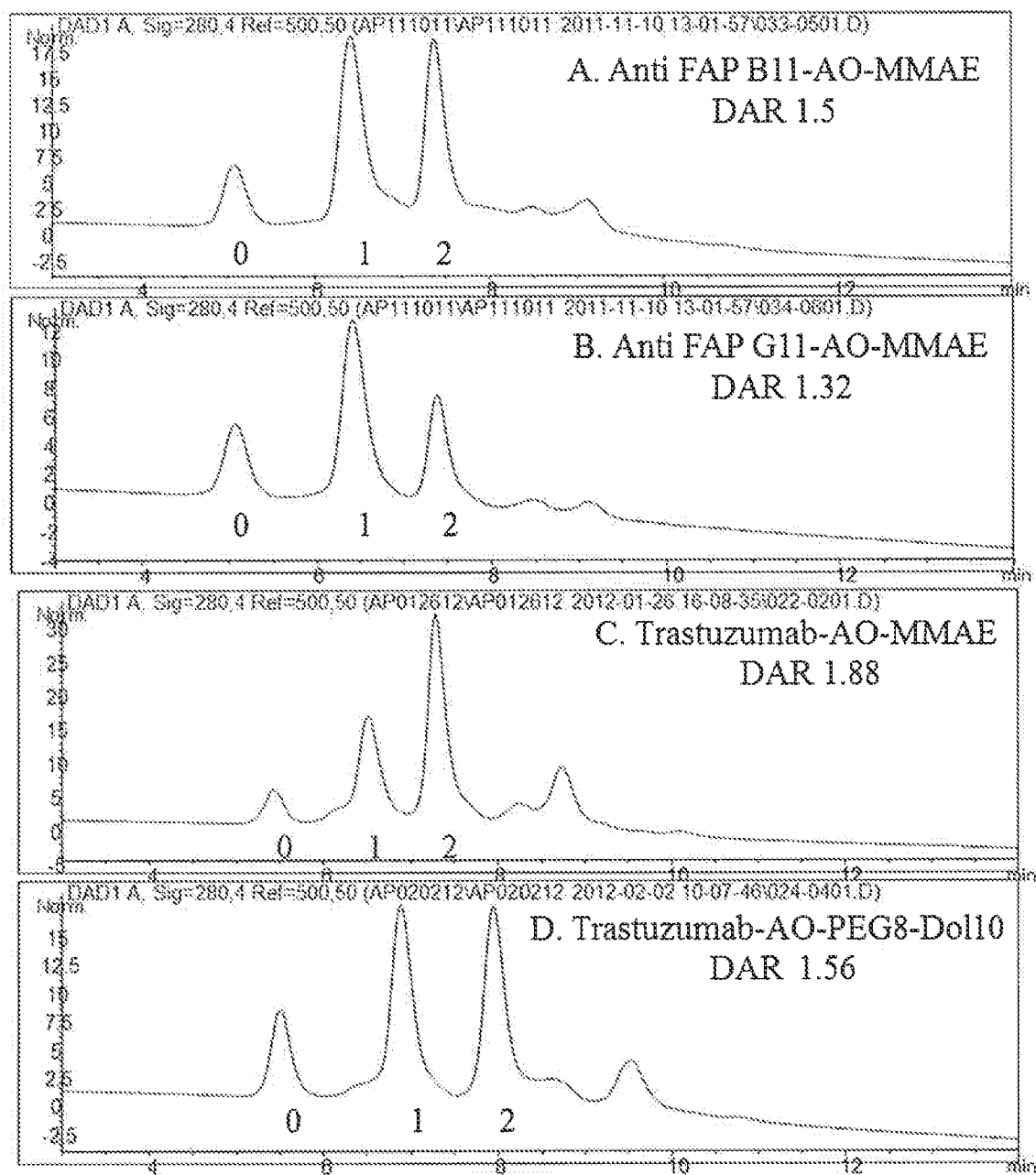
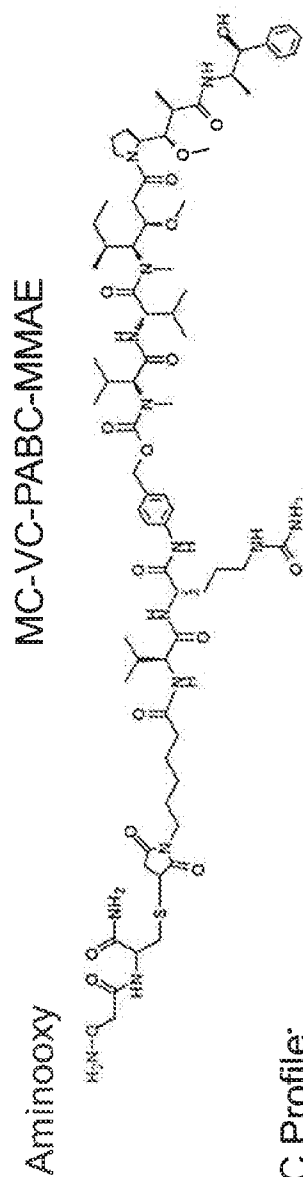
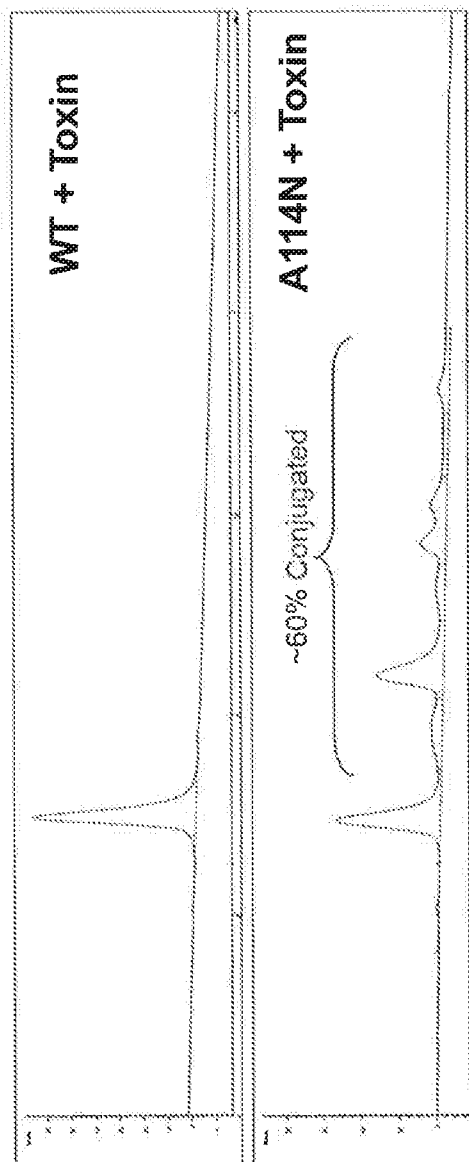
Figure 34

Figure 35



HIC Profile:



A. Park, L. Hou, B. Chen, J. Stefano

Figure 36

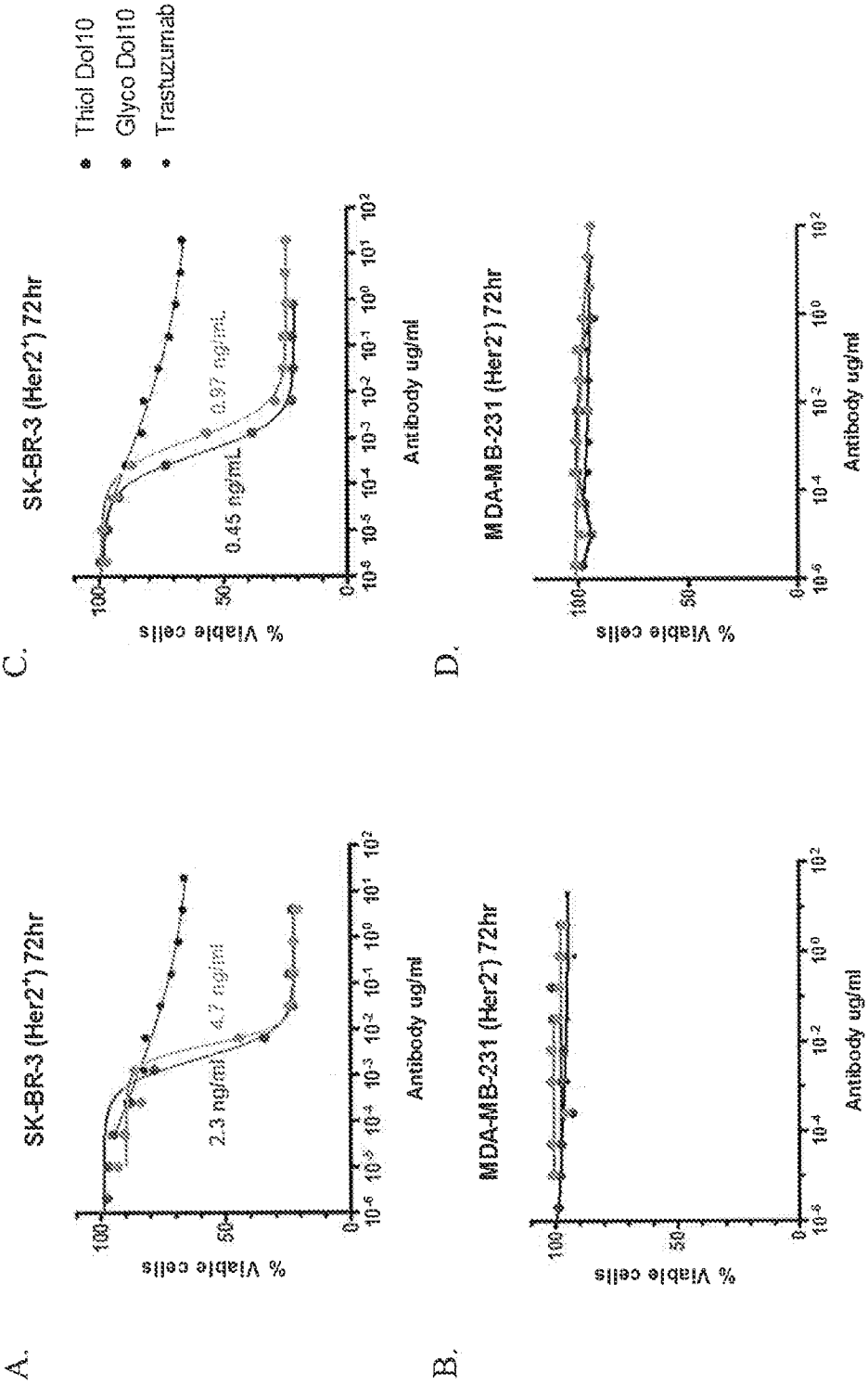


Figure 37

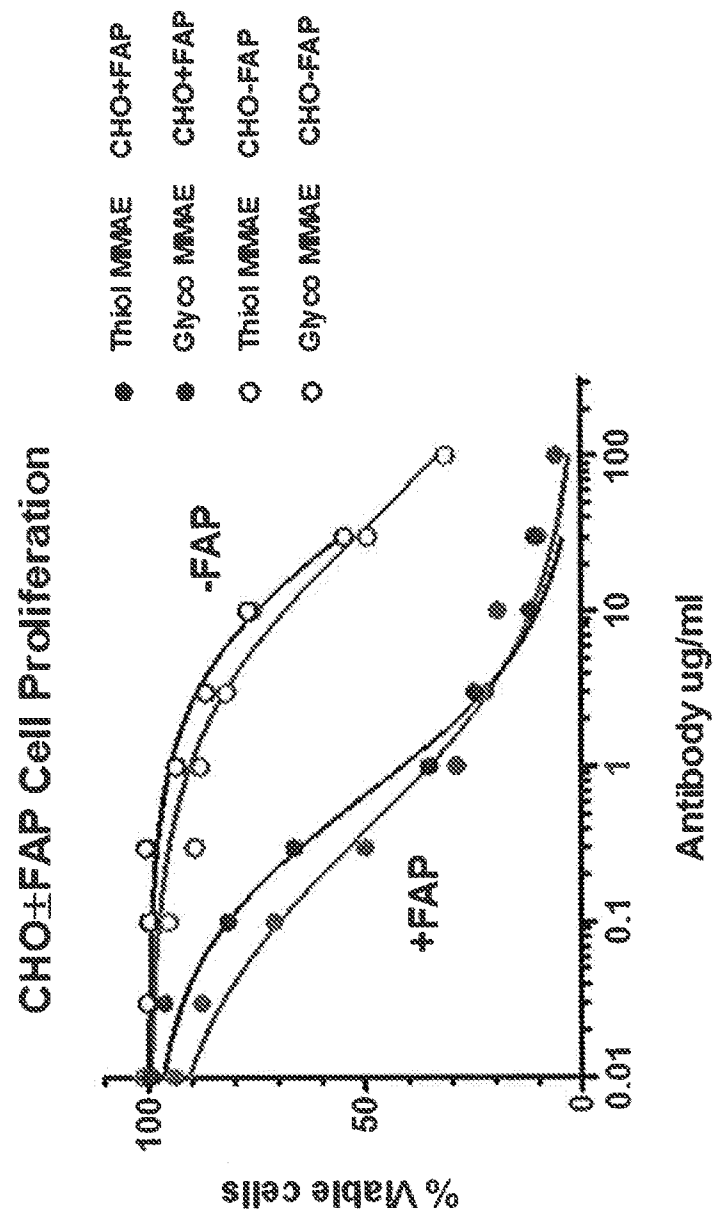


Figure 38

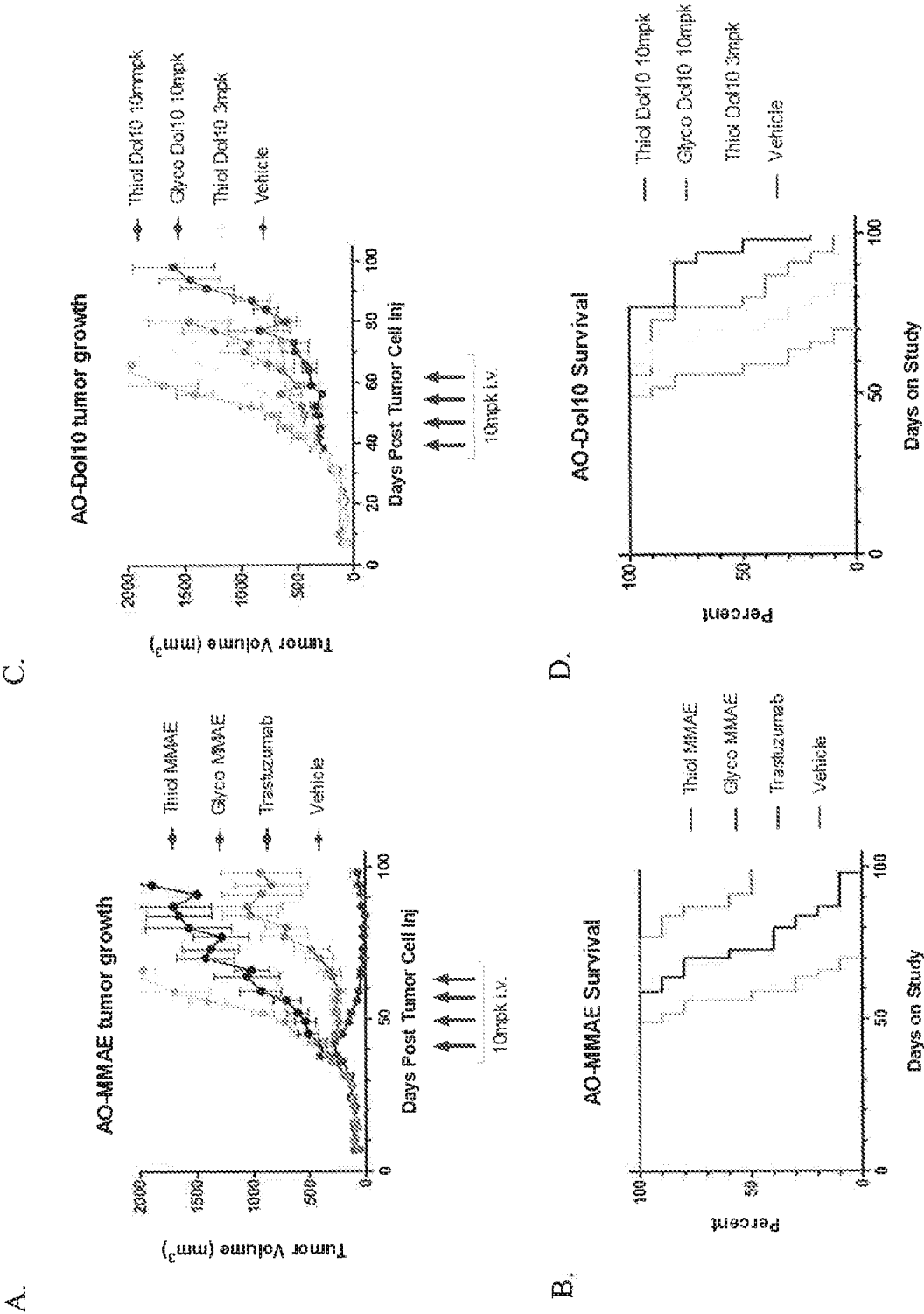


Figure 39

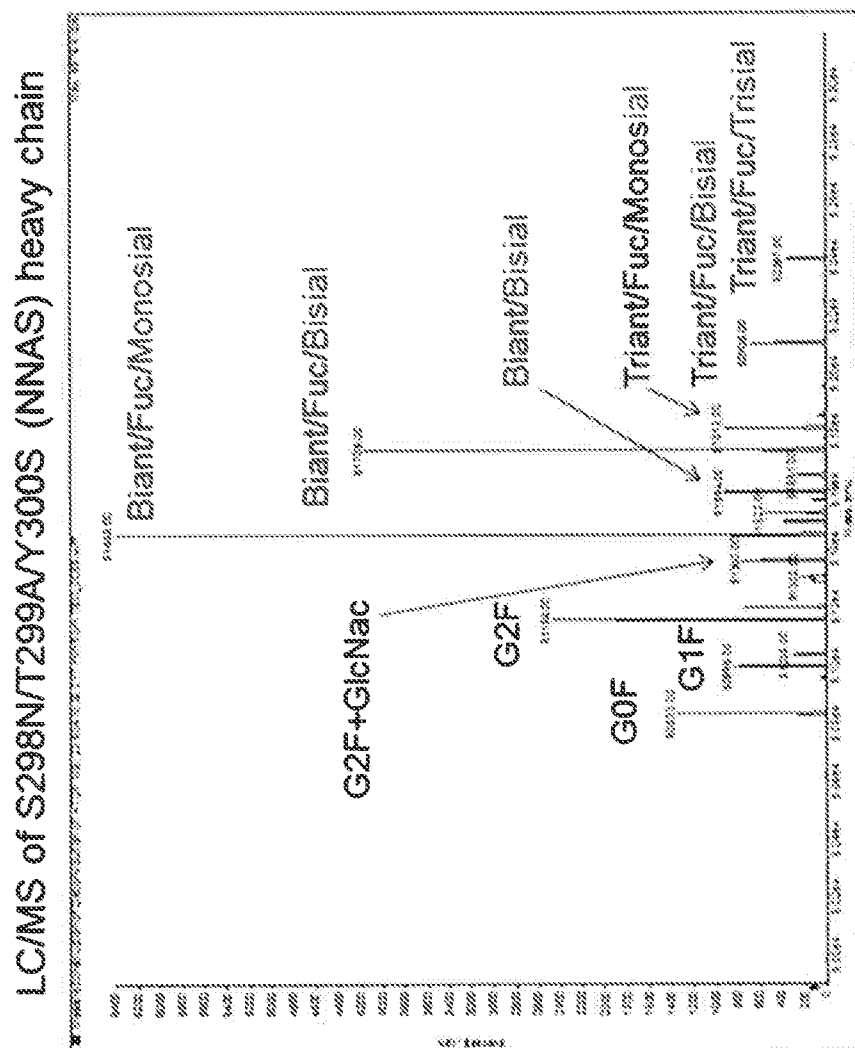


Figure 40

Δ ab control S298N/T299A/Y300S

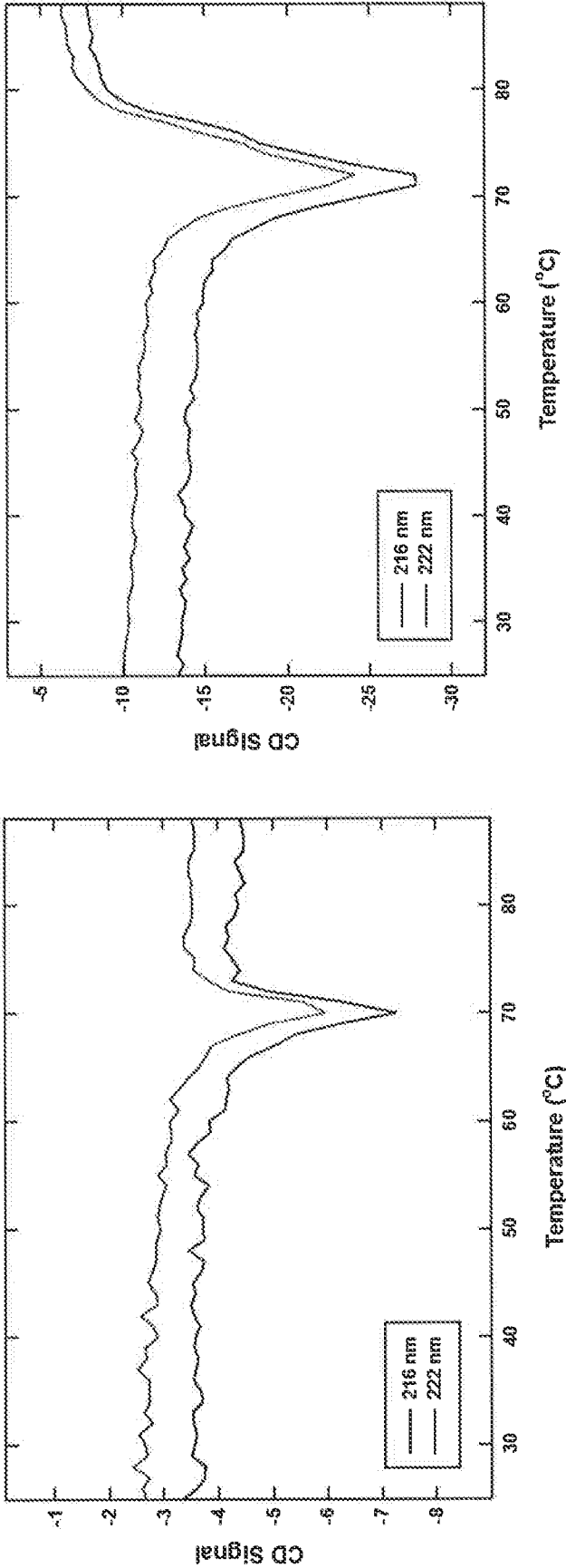


Figure 41

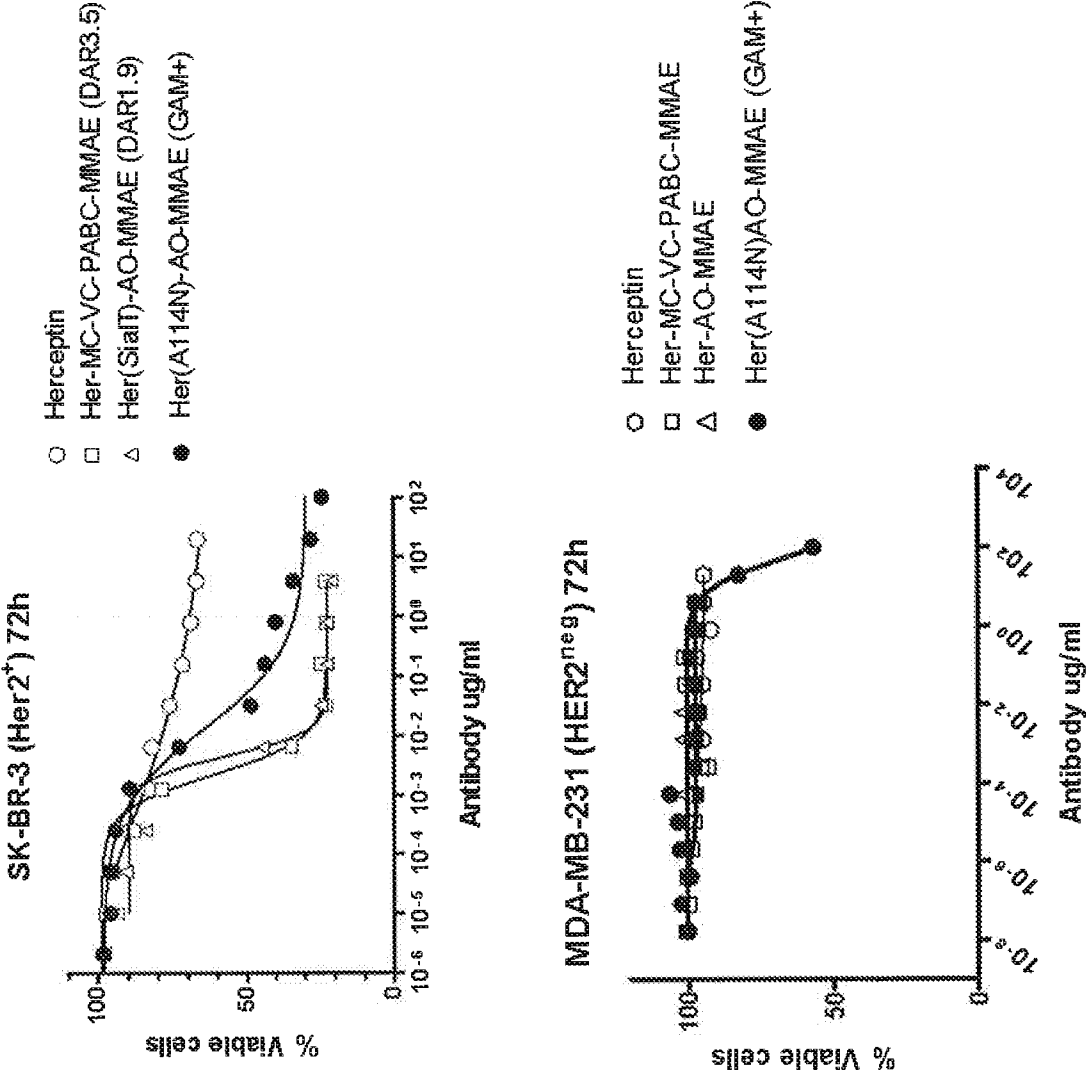


Figure 42

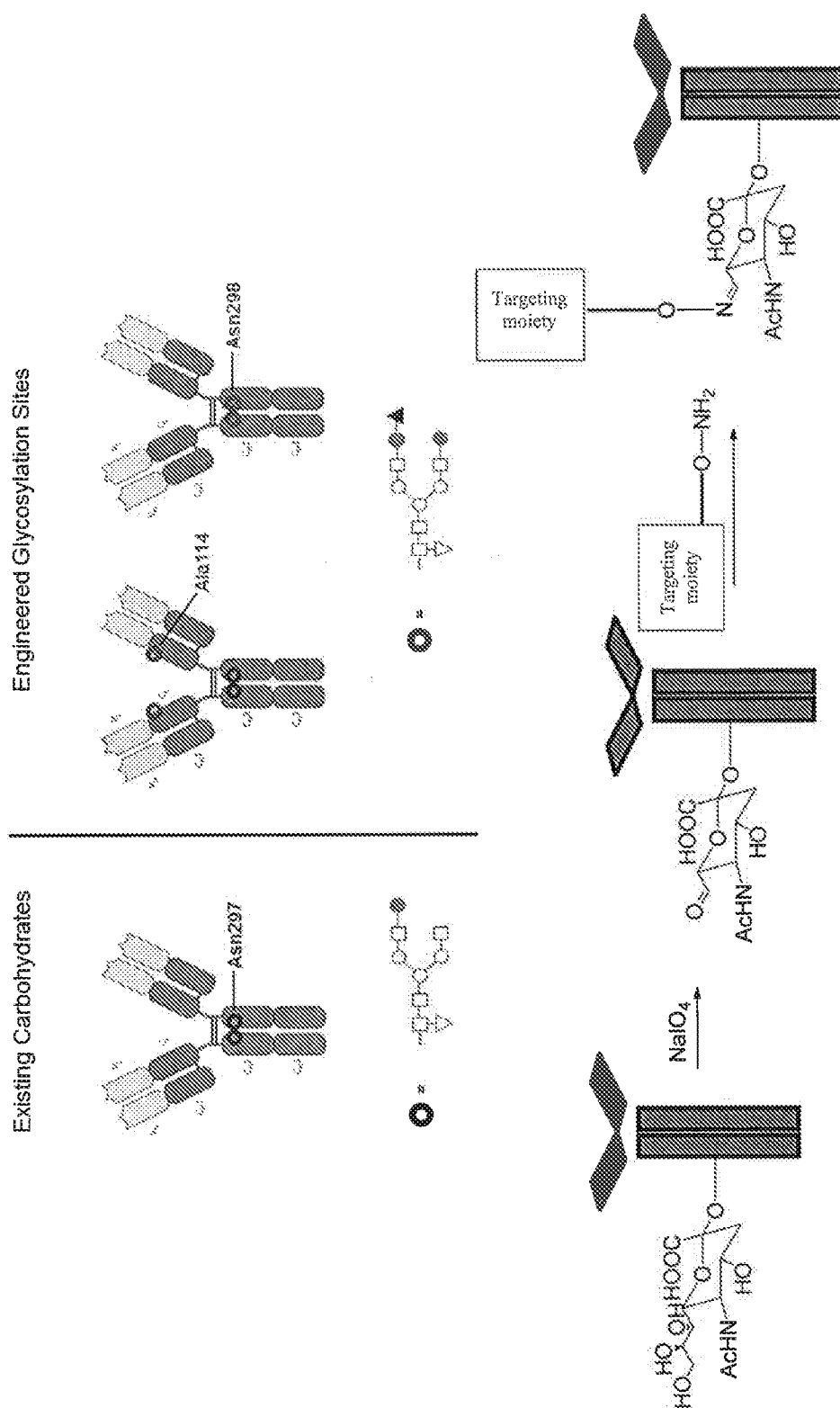


Figure 43

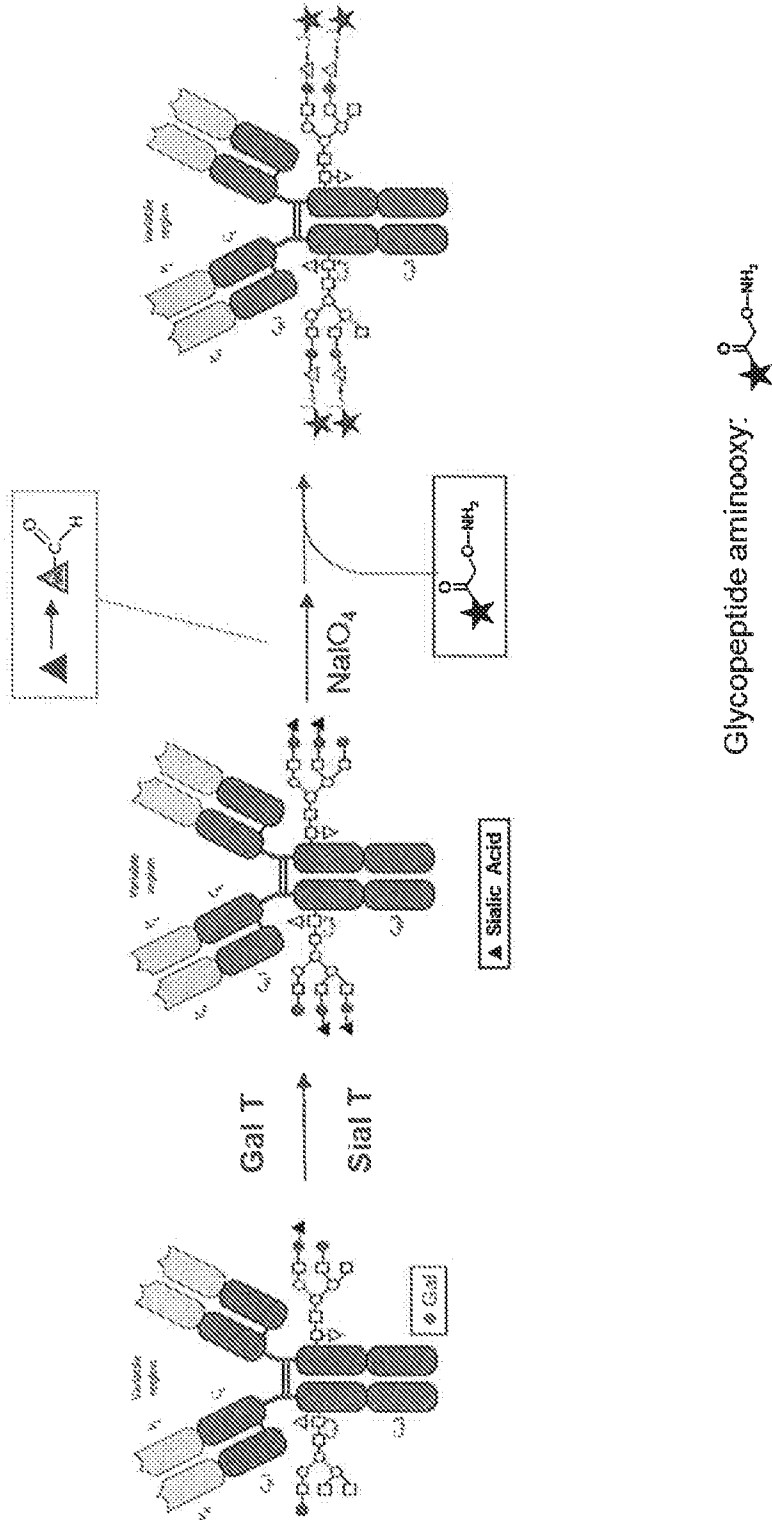


Figure 44

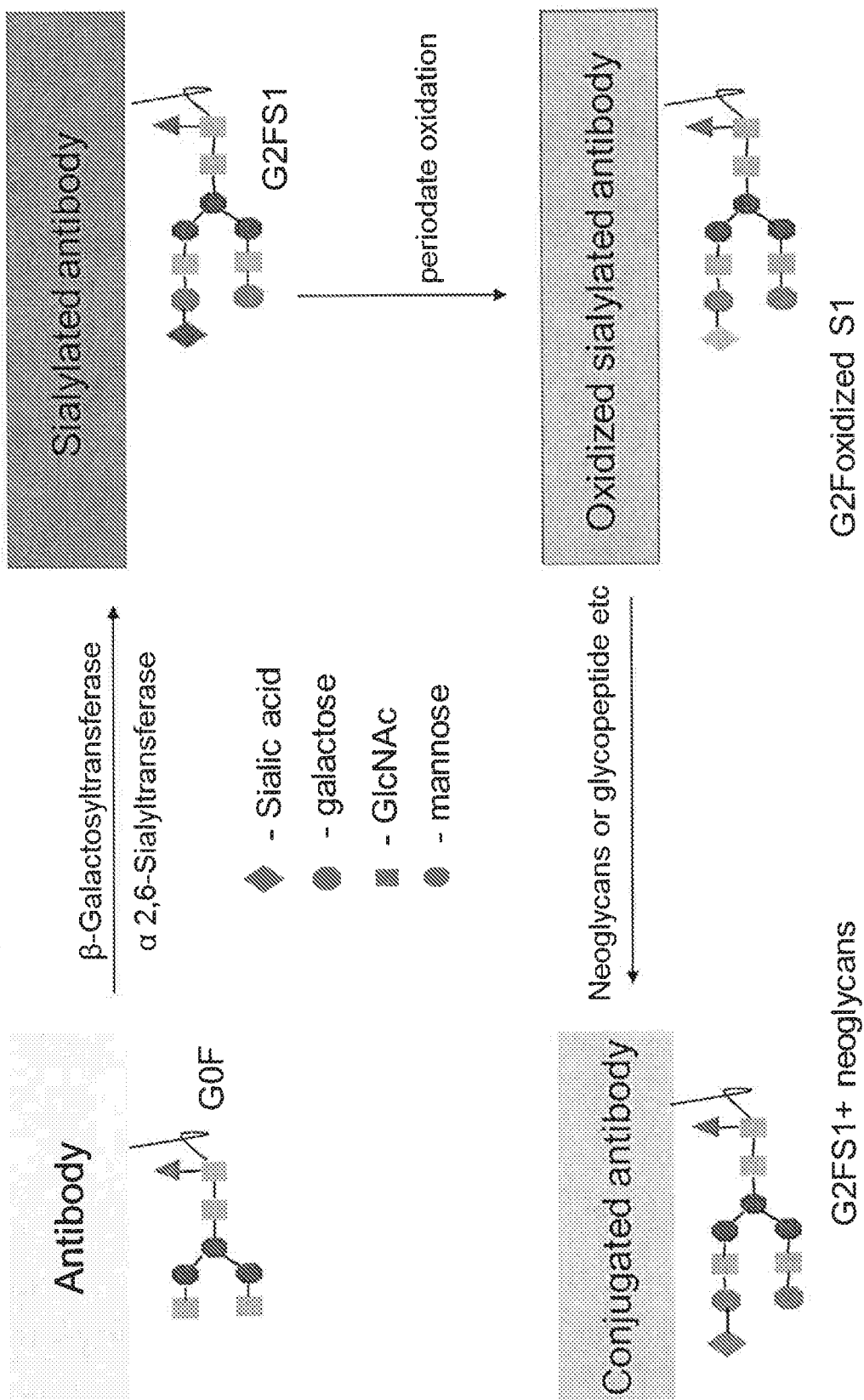
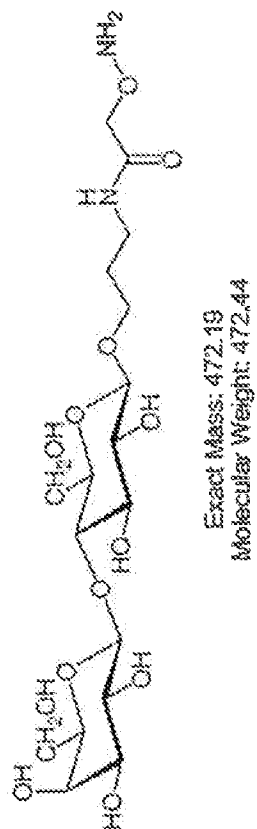


Figure 45

Lactose aminoxy for native glycans (SAM or GAM):



bisM6P hexamannose aminoxy
(SAM or GAM):

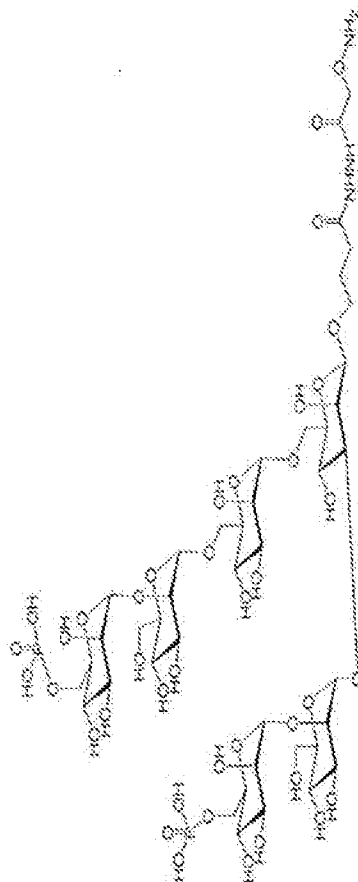
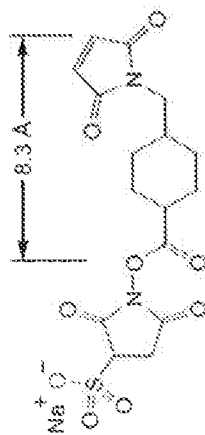
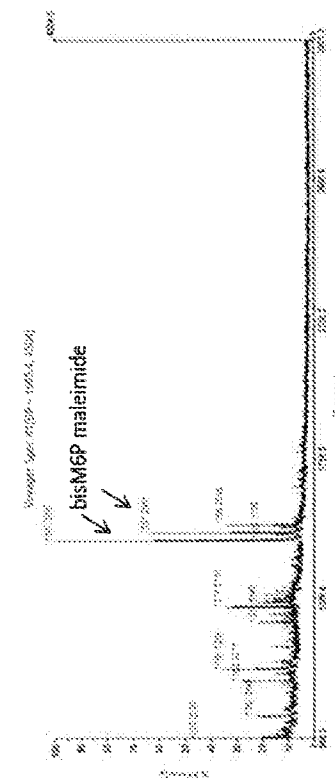


Figure 46

bisM6P hexamannose aminoxy
(SAM on Fc glycans):



Sulfo SMCC



bisM6P hexamannose maleimide
(Targeting hinge cysteines):

- maleimide

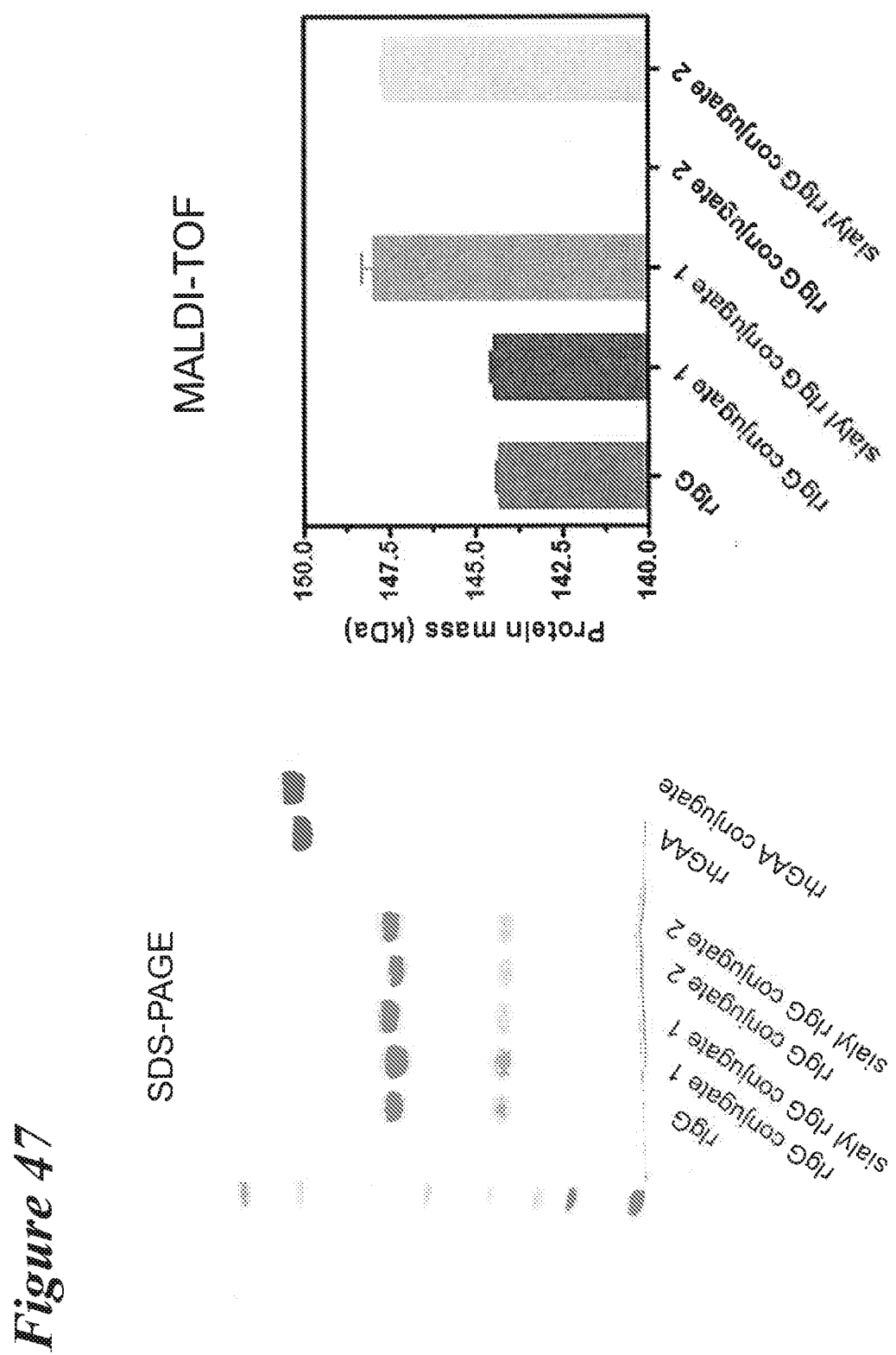


Figure 48

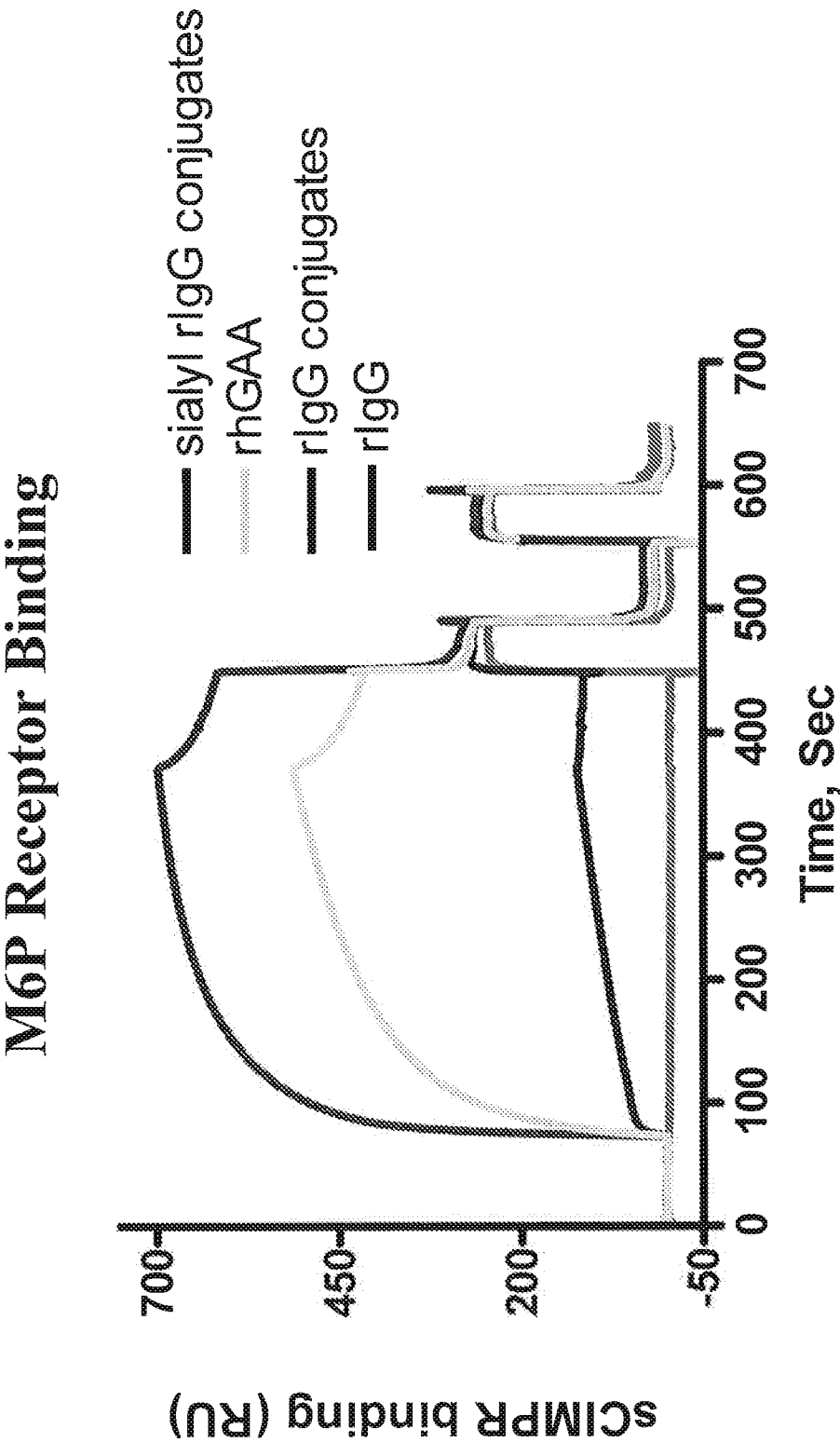


Figure 49

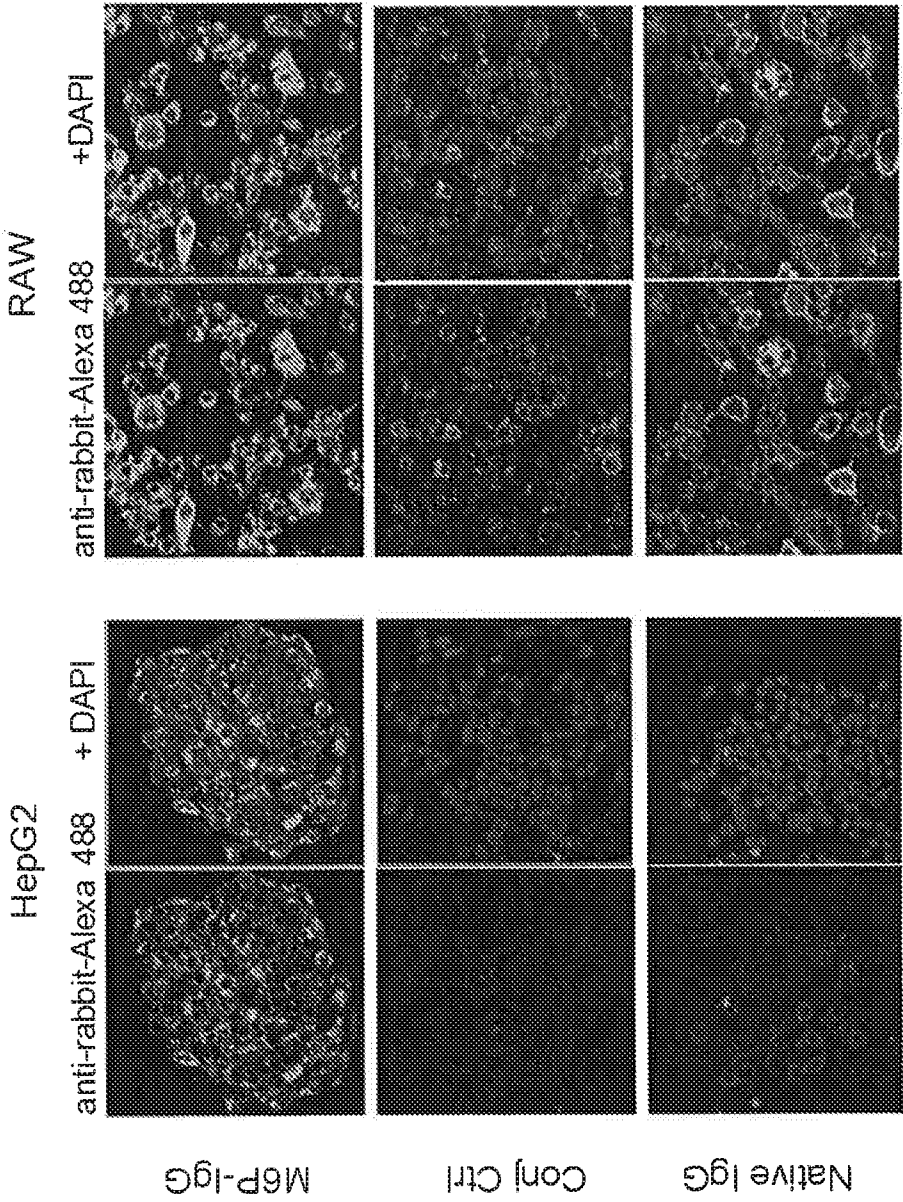


Figure 50

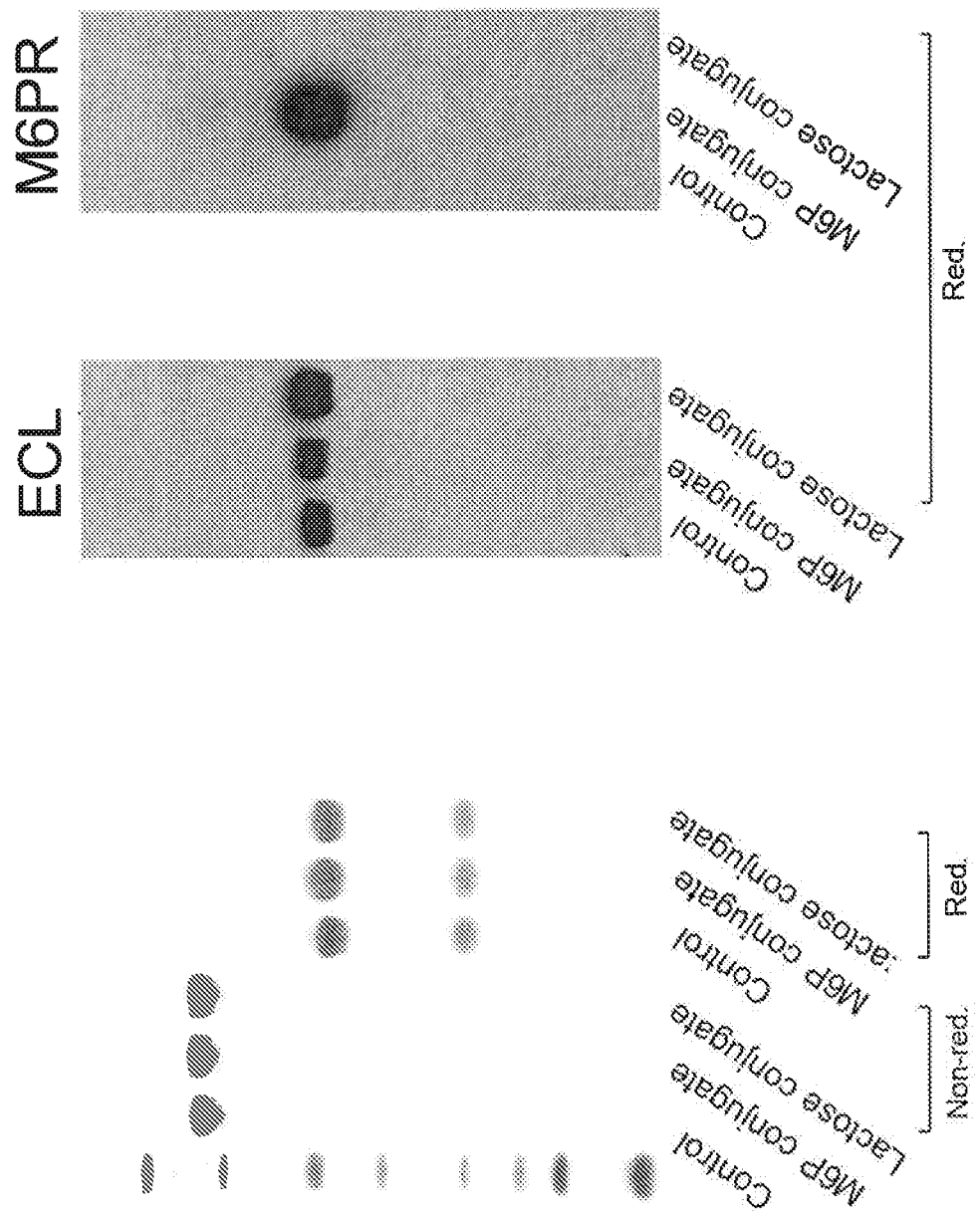


Figure 51

Samples	MW (kDa)	# Glycan
Control	145.42 ± 0.24	0.00
M6P conjugate	147.95 ± 0.09	1.92
Lactose conjugate	146.35 ± 0.36	1.96

Figure 52

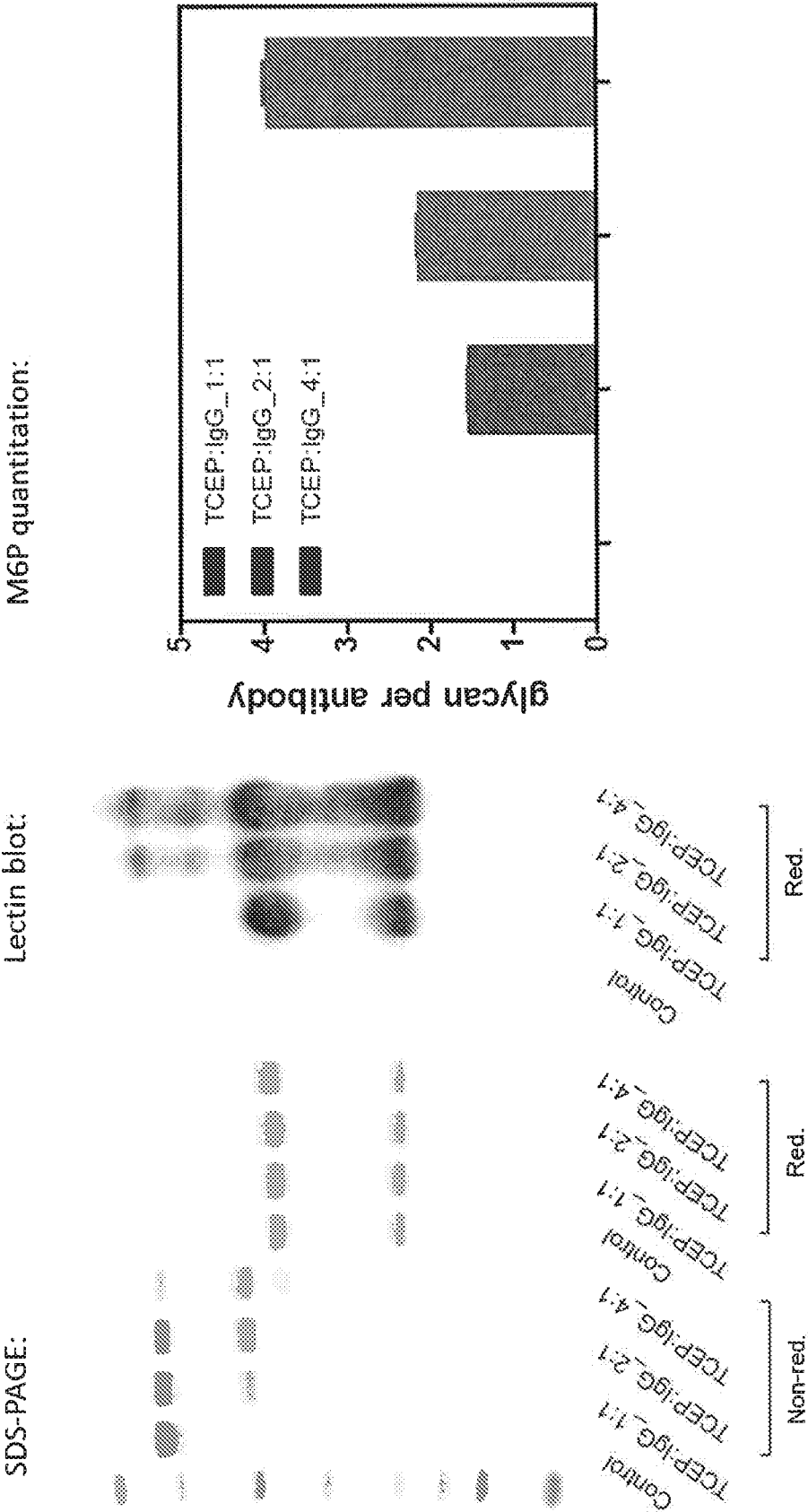
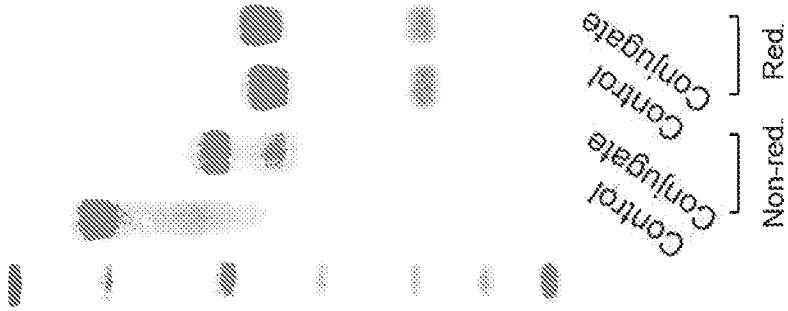


Figure 53

SDS-PAGE:



Galactose quantitation:

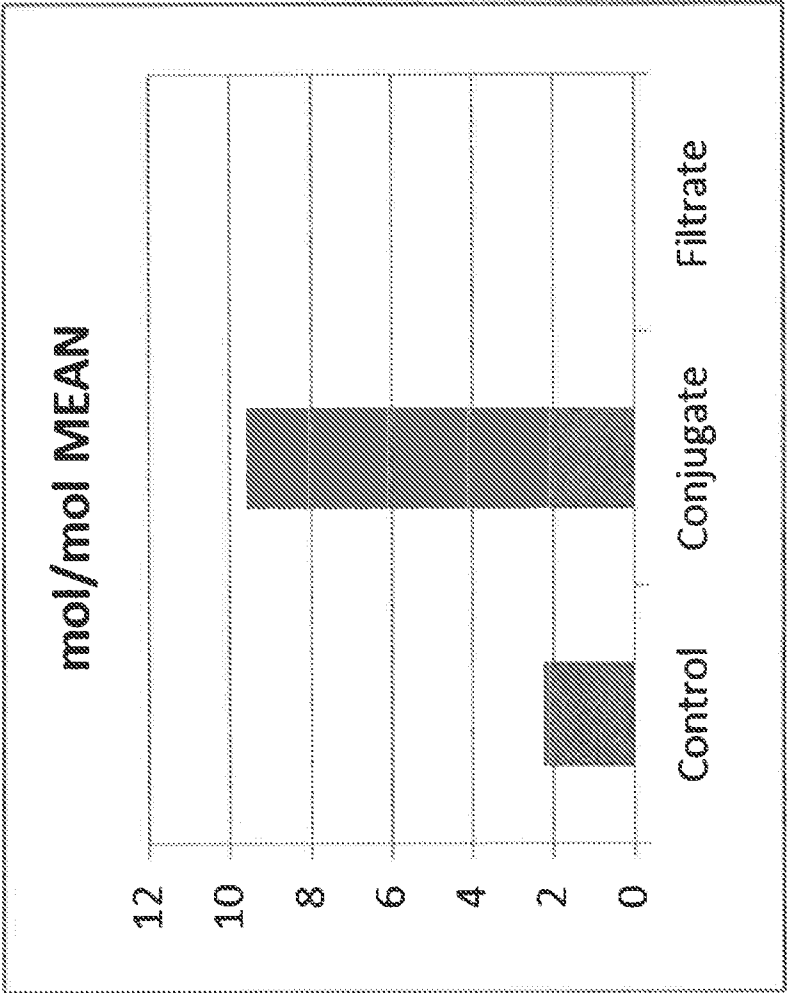


Figure 54

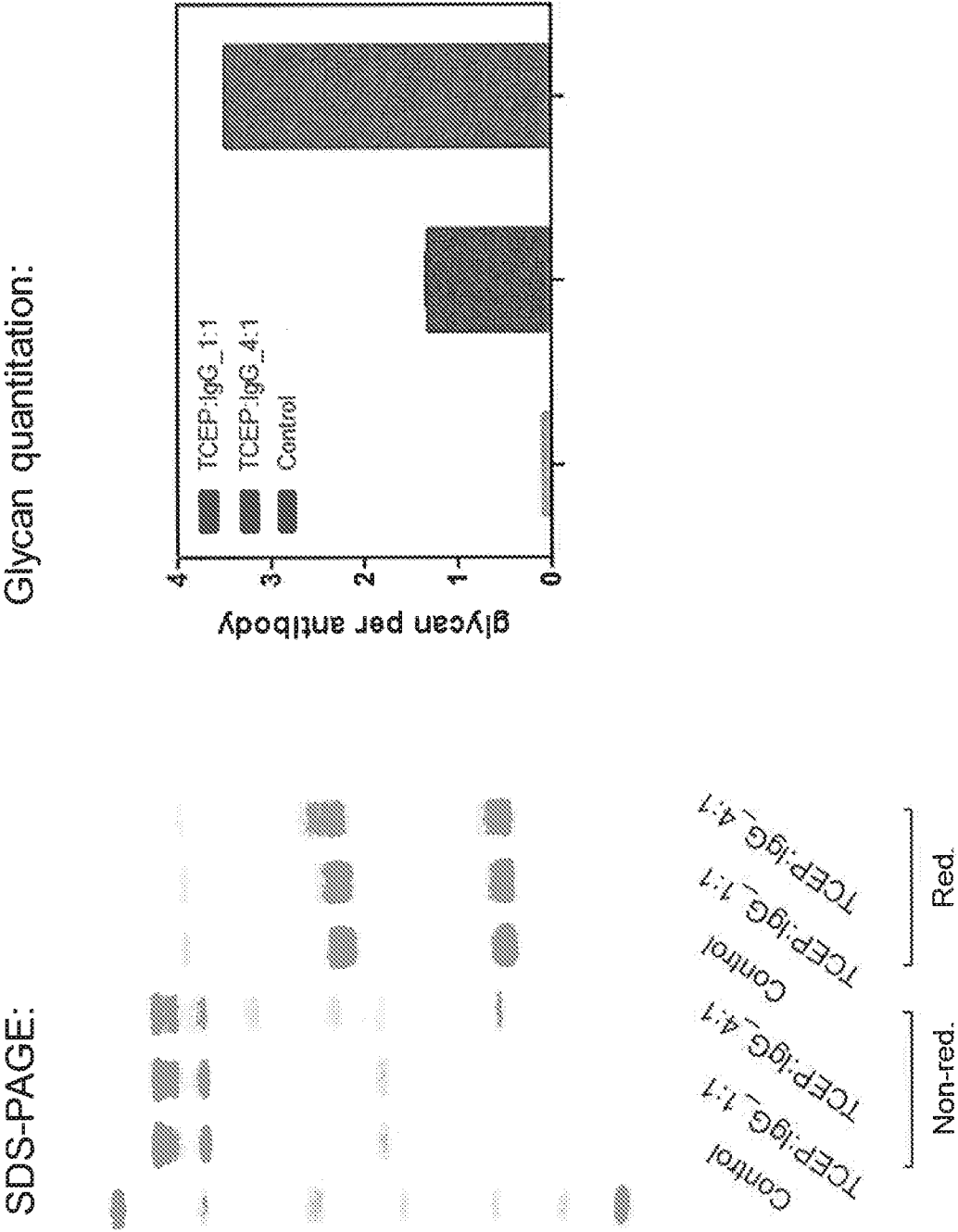


Figure 55

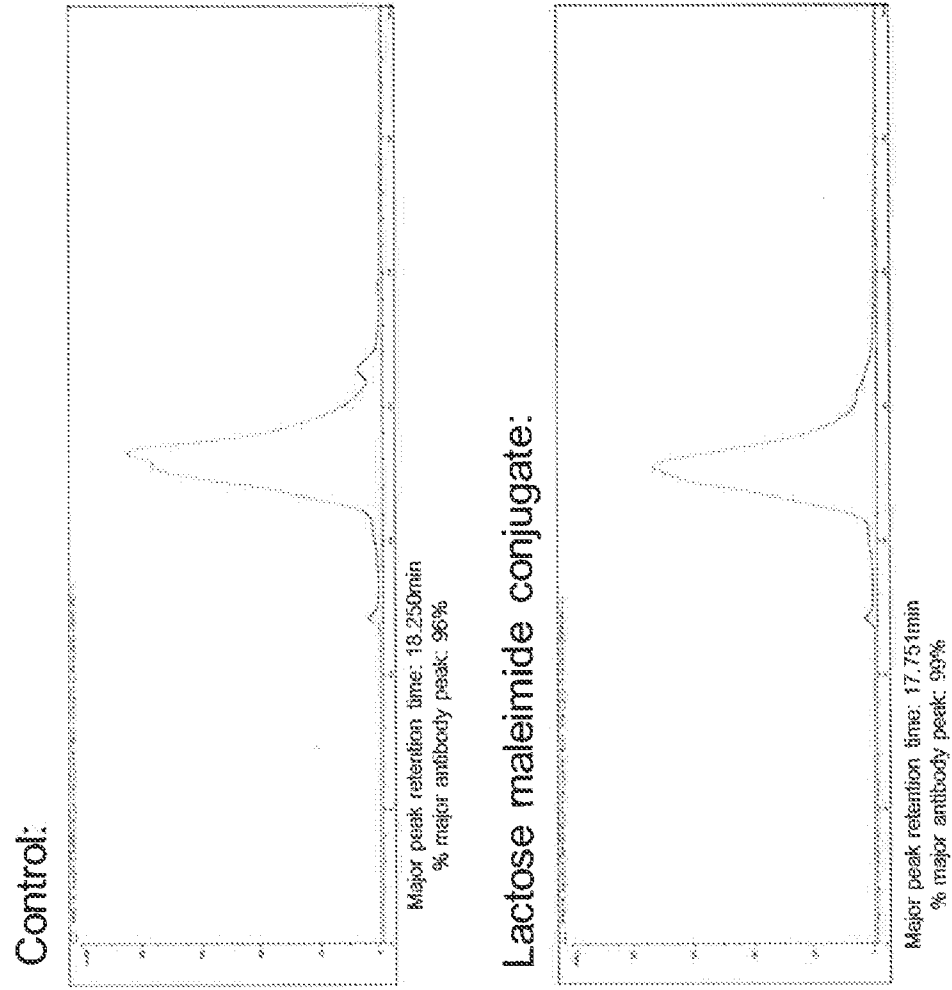


Figure 56

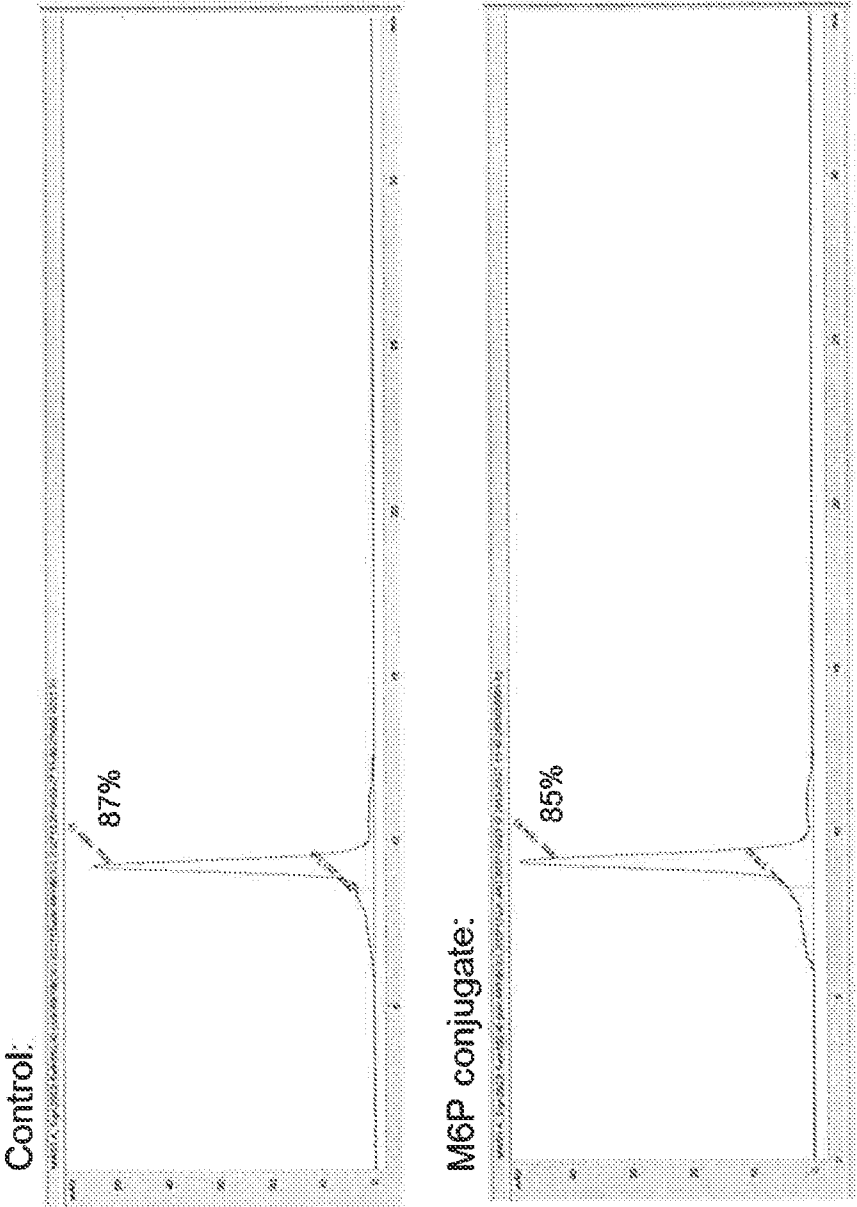
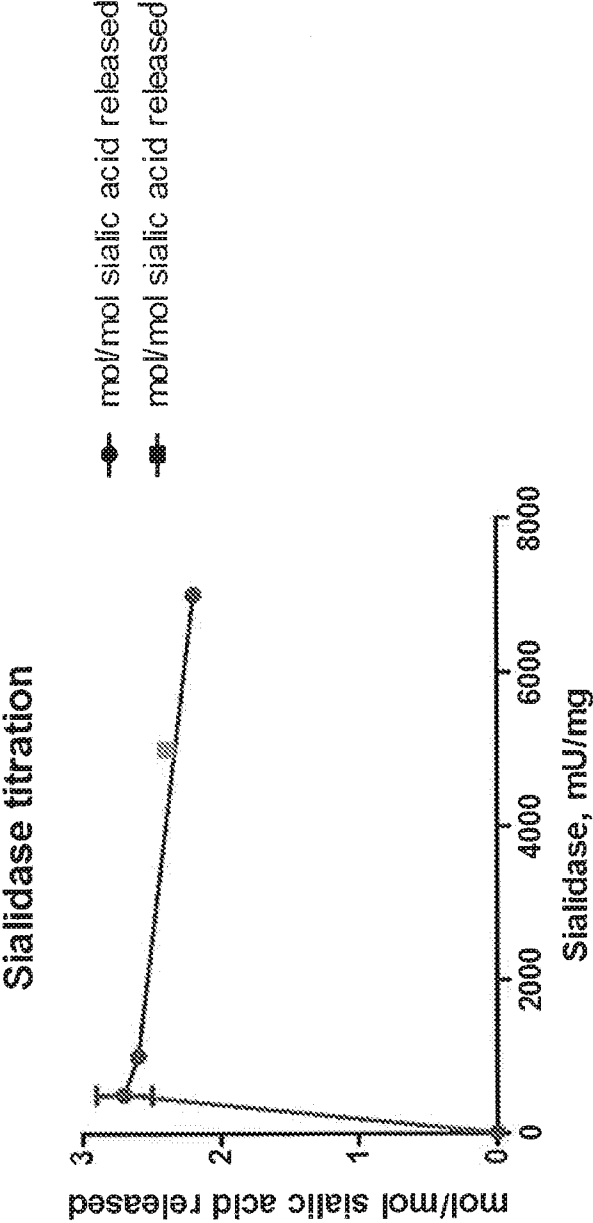


Figure 57



	mol per mol sialic acid
NNAS	2.4 ± 0
Sialylated NNAS	3.7 ± 0.2
Desialylated and galactosylated NNAS	0 ± 0

Figure 58

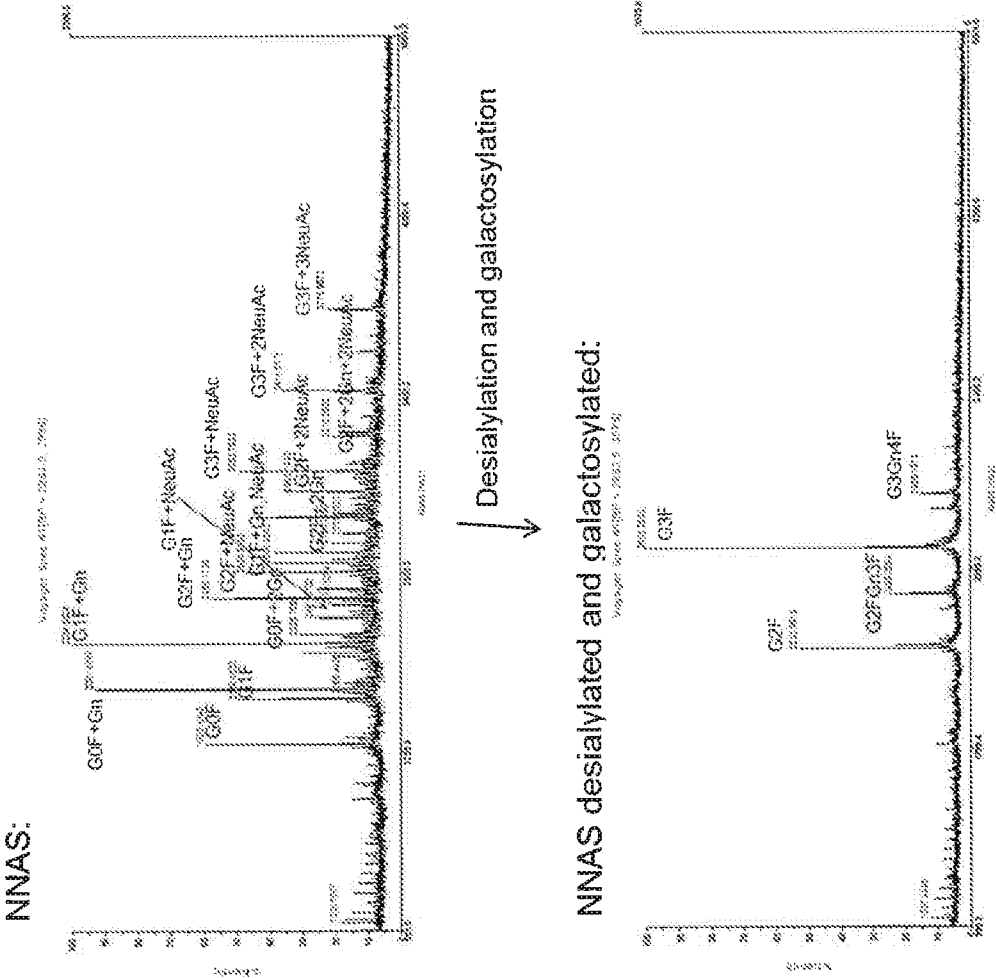


Figure 59

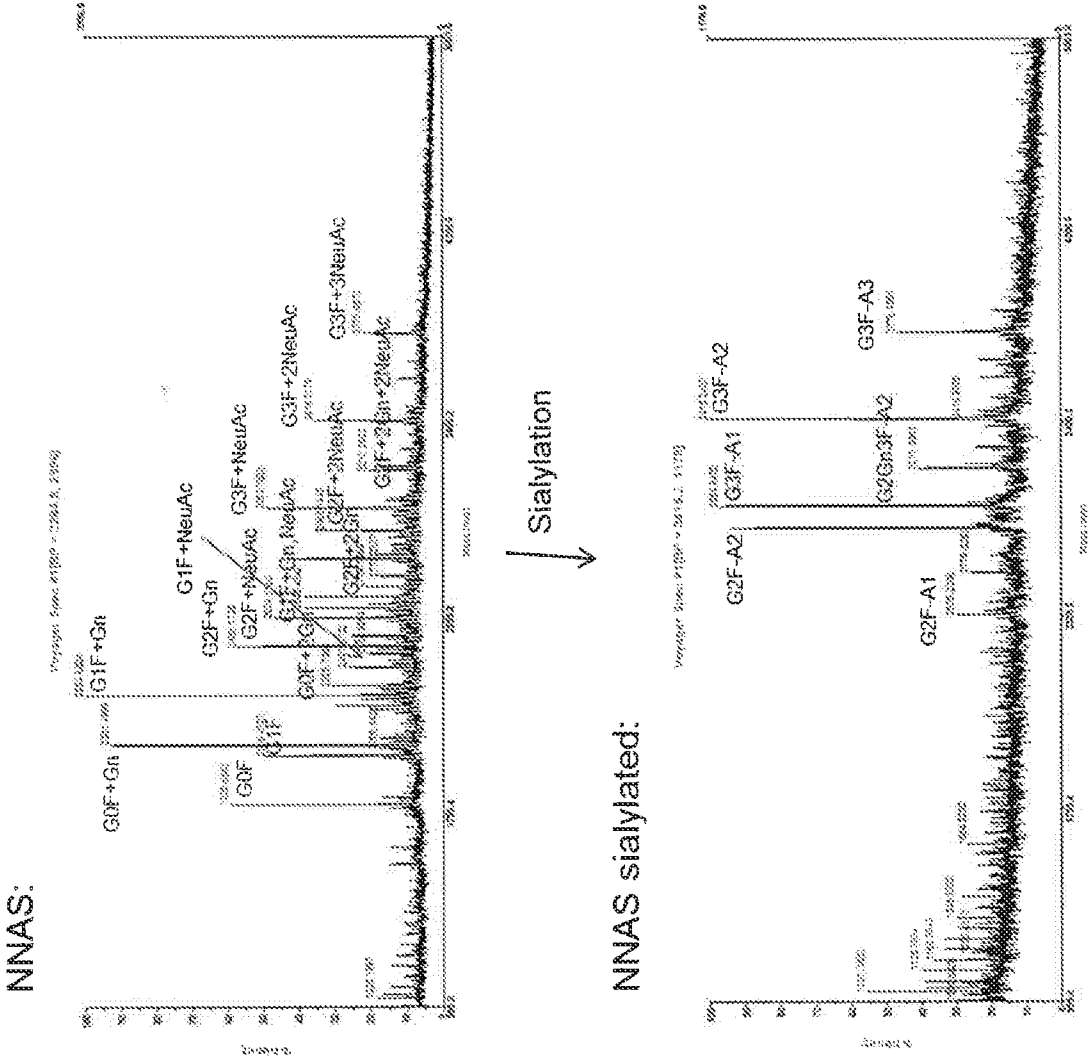


Figure 60

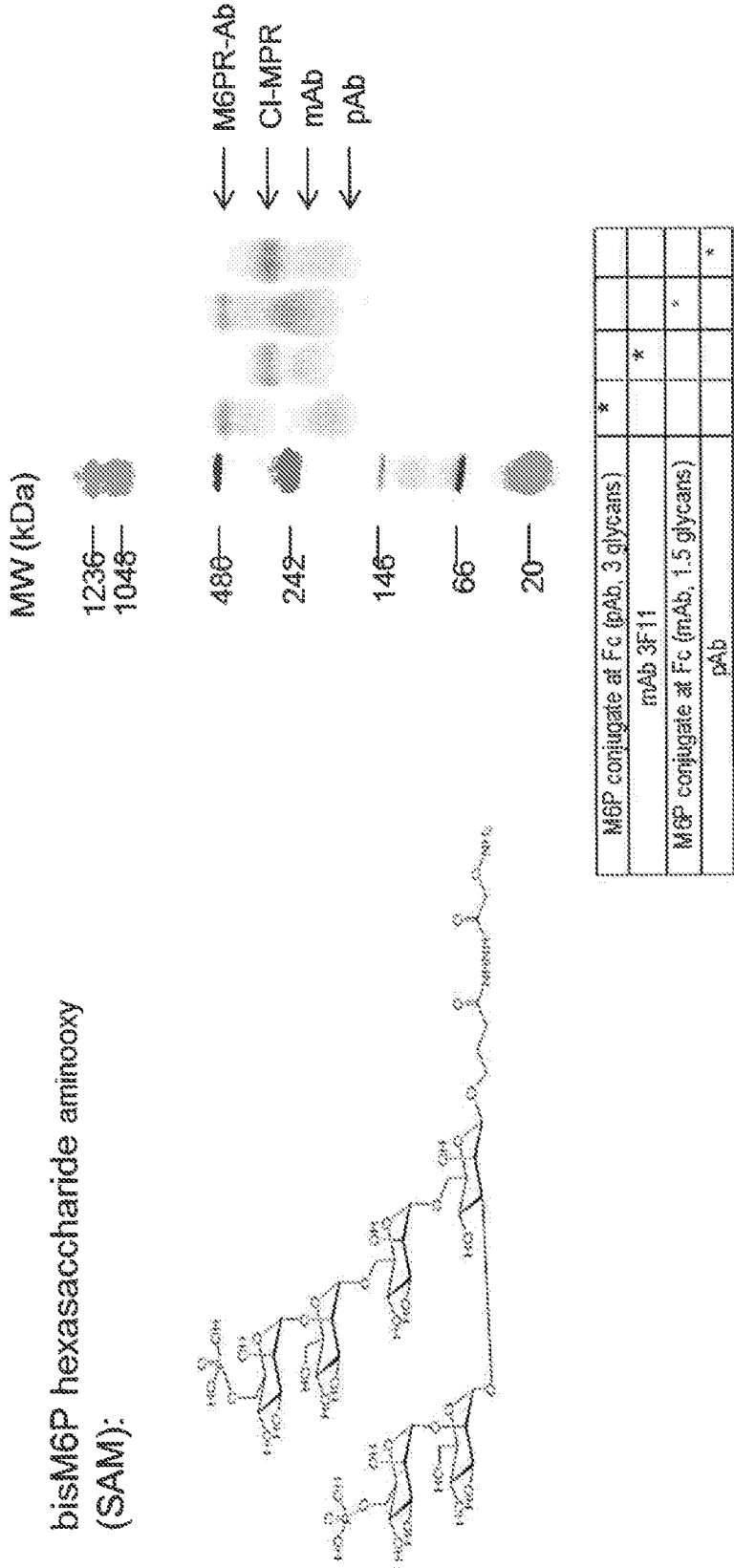


Figure 61

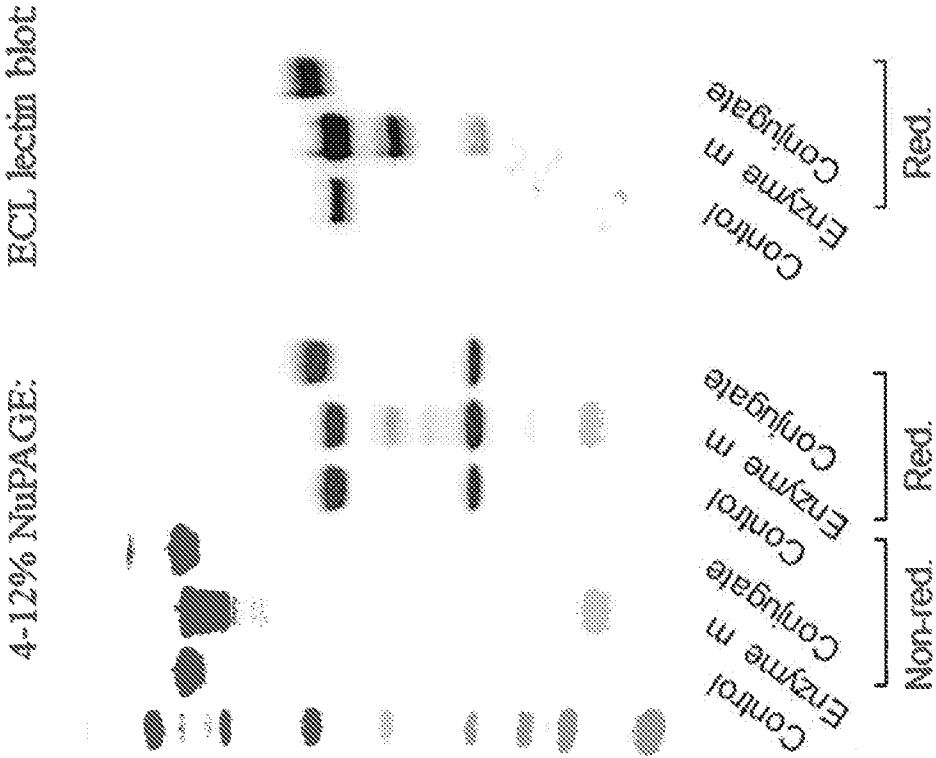


Figure 62

	mol galactose per mol Ab	mol glycopeptide per mol Ab
NNAS	1.4 + 0	0
NNAS desialylated/galactosylated	7.4 + 0.3	0
NNAS conjugate	9.4 + 0	3.1

Figure 63

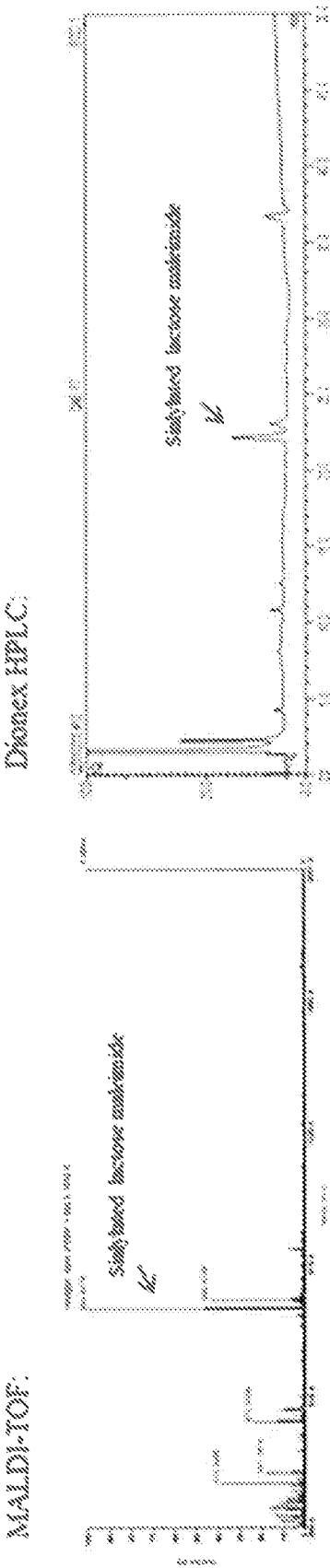


Figure 64

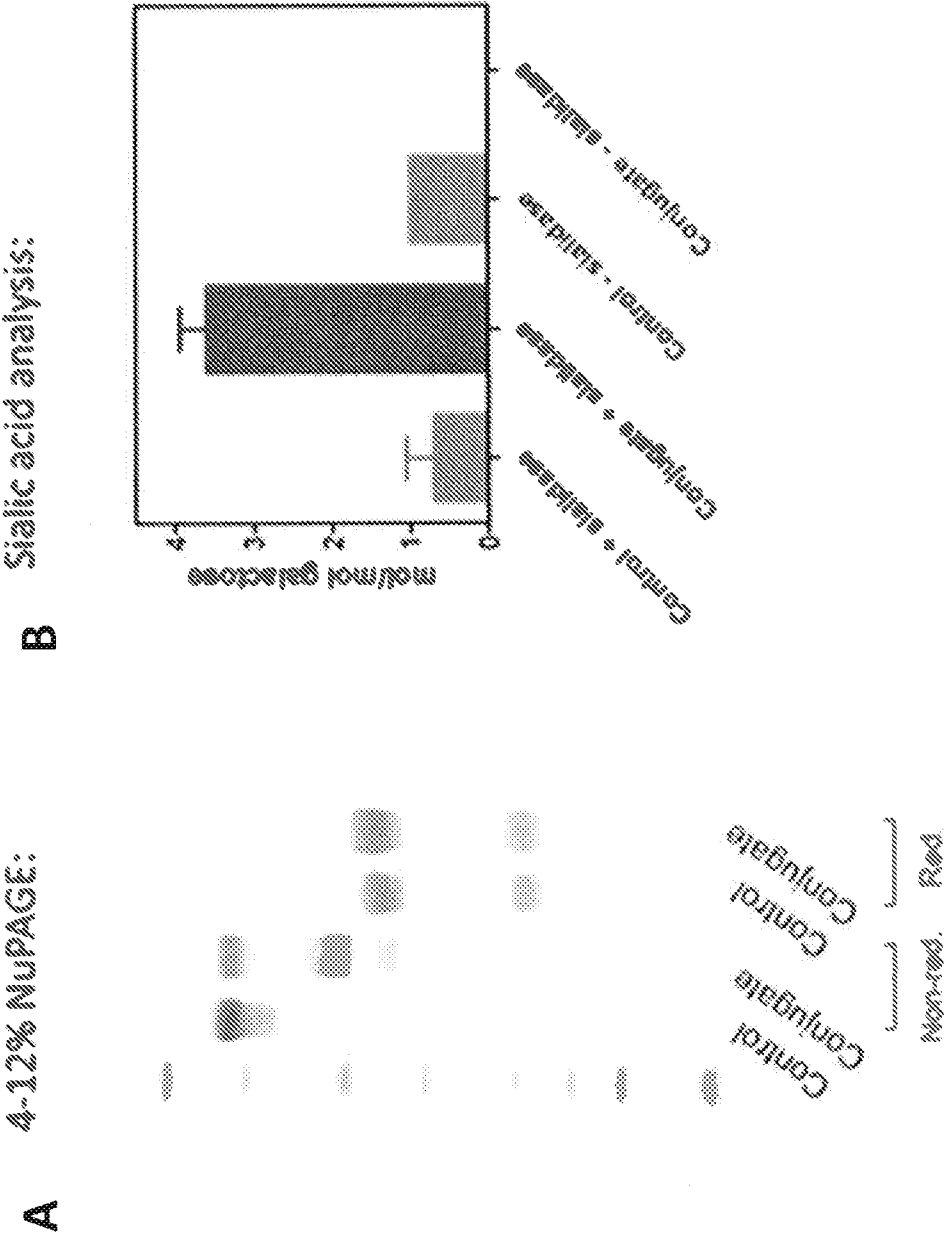


Figure 65

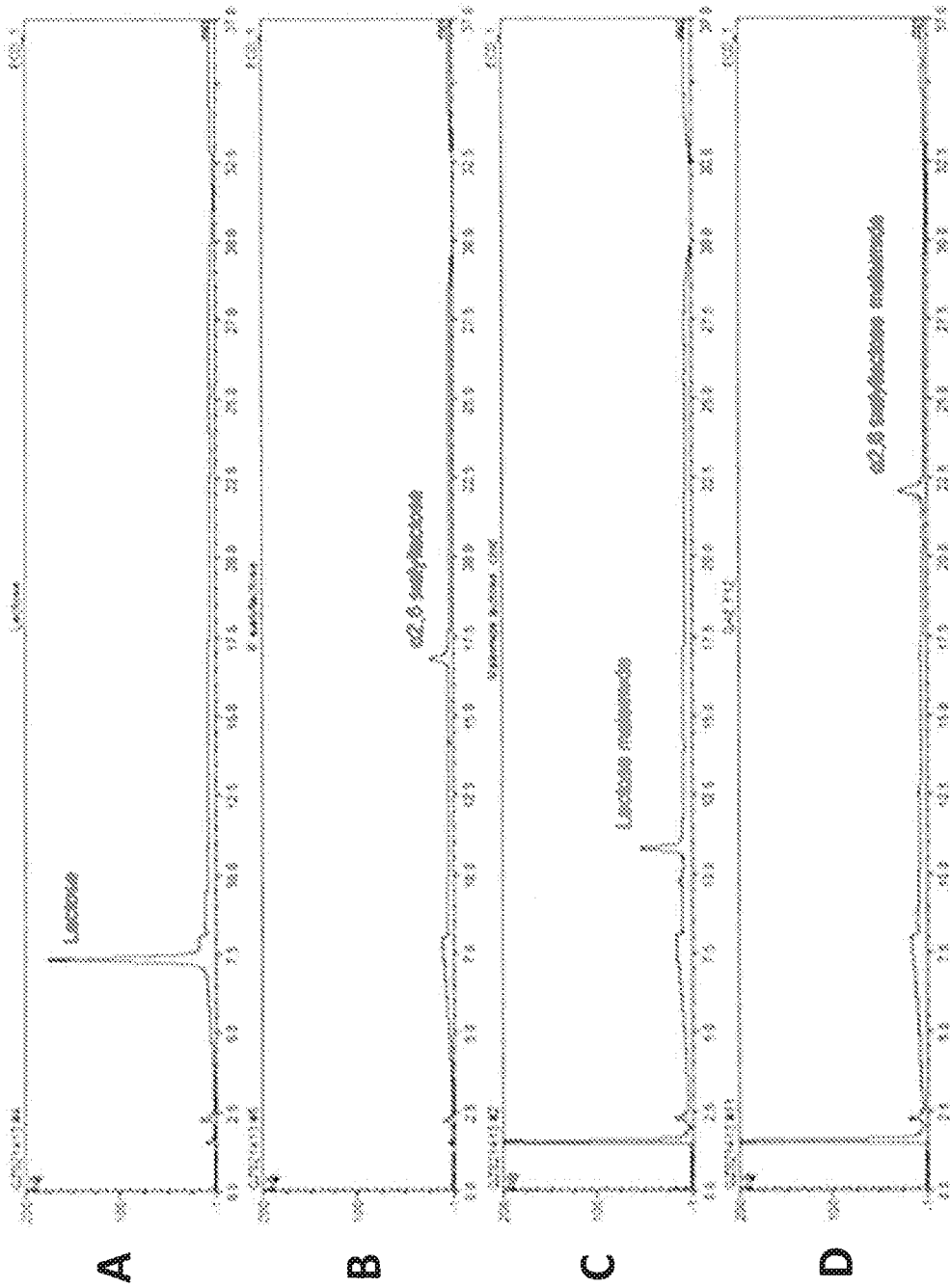


Figure 66

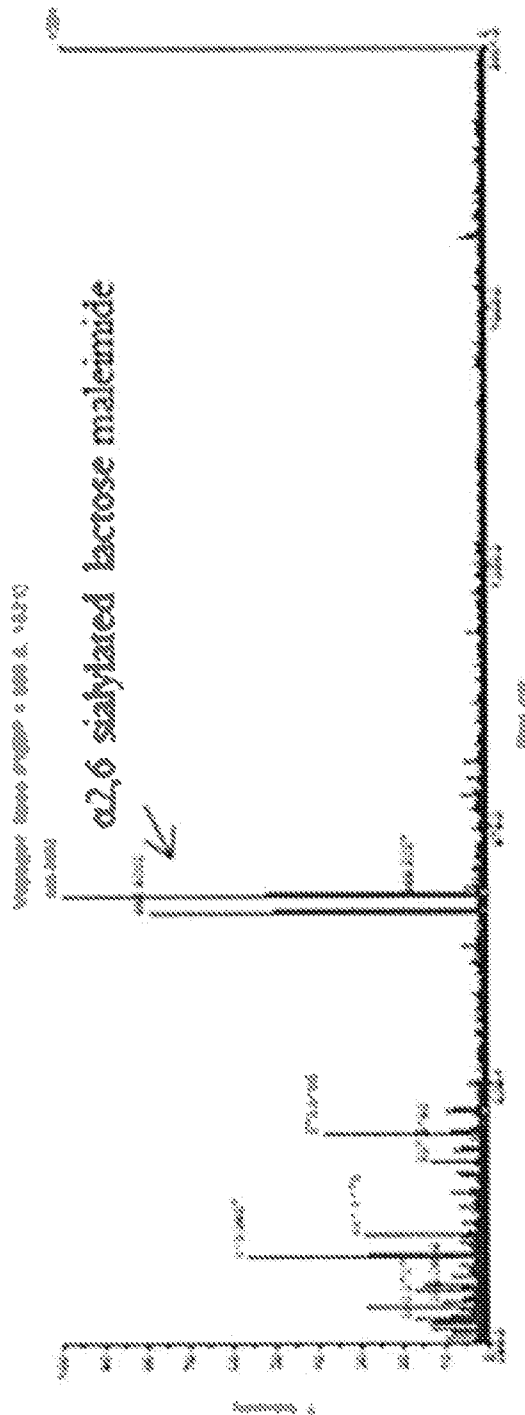


Figure 67

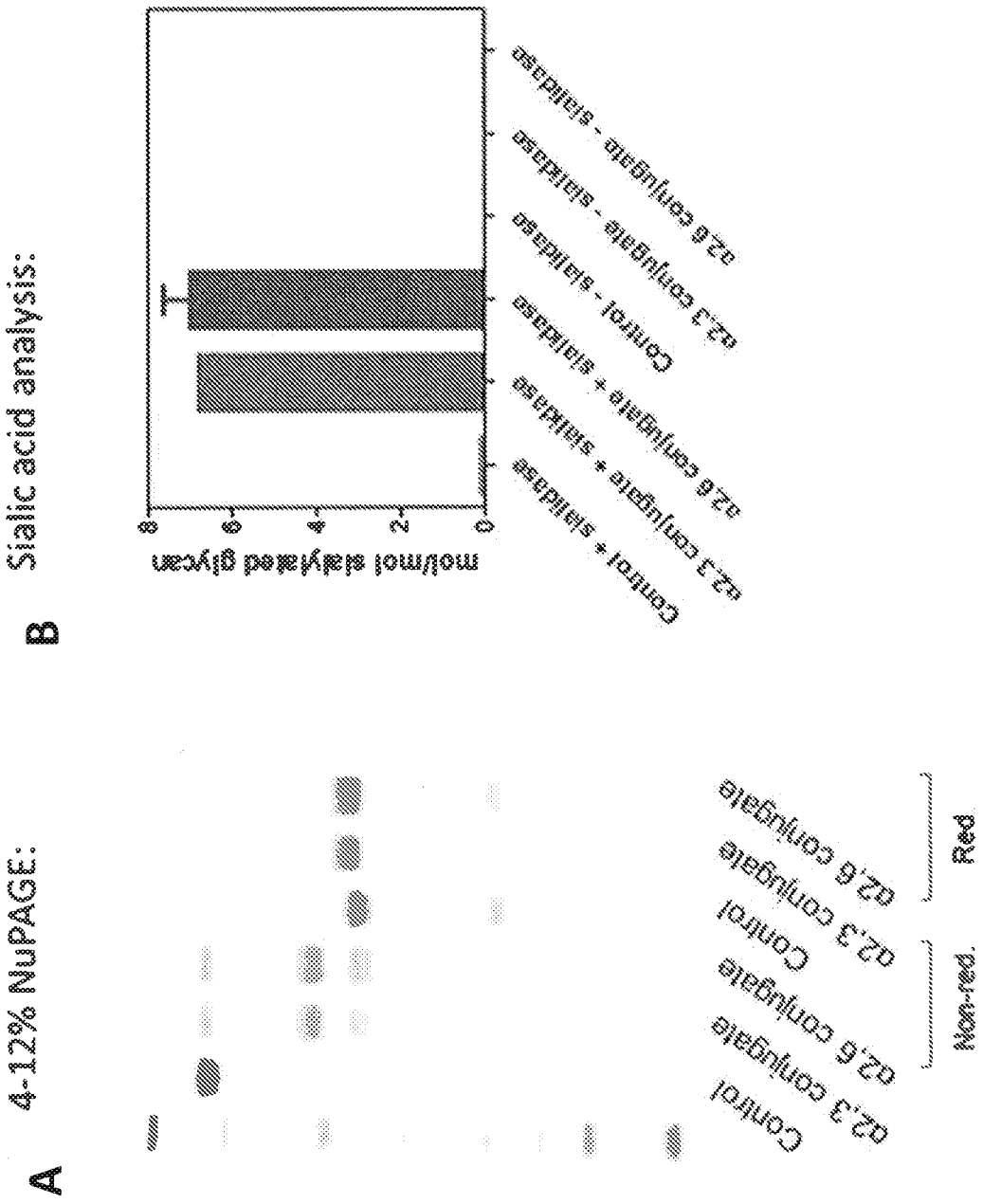


Figure 68



Figure 69

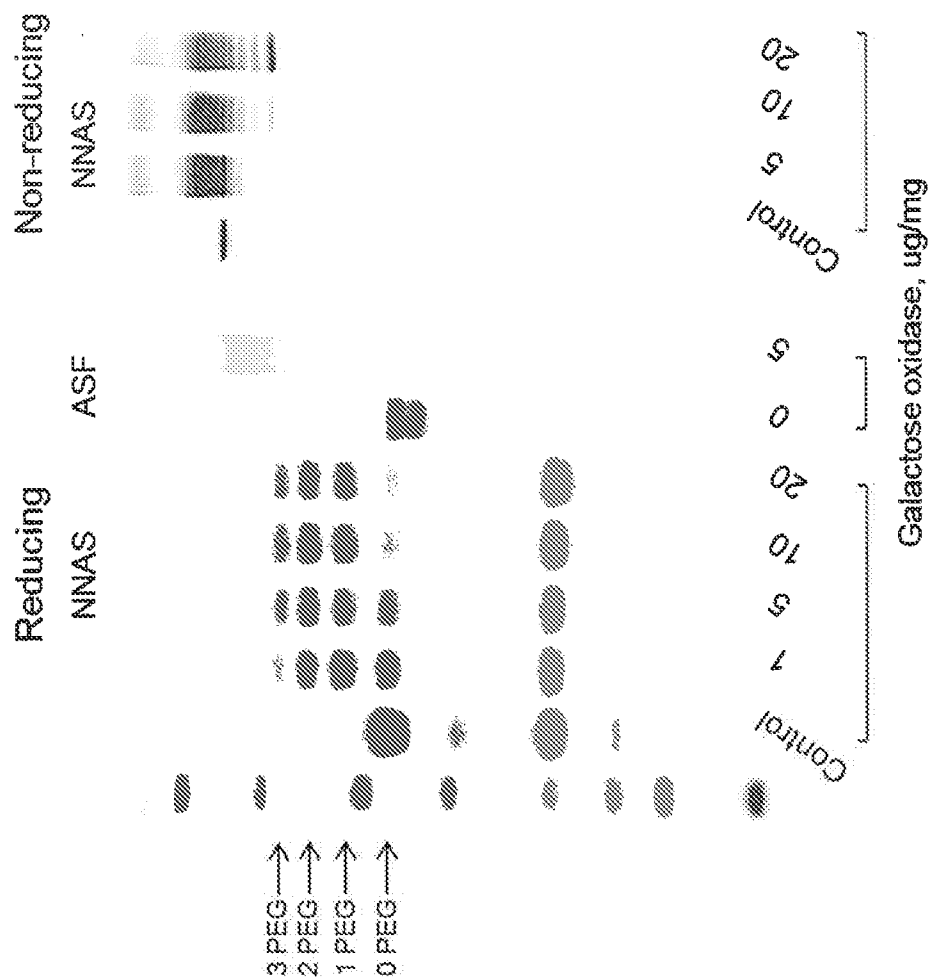


Figure 70

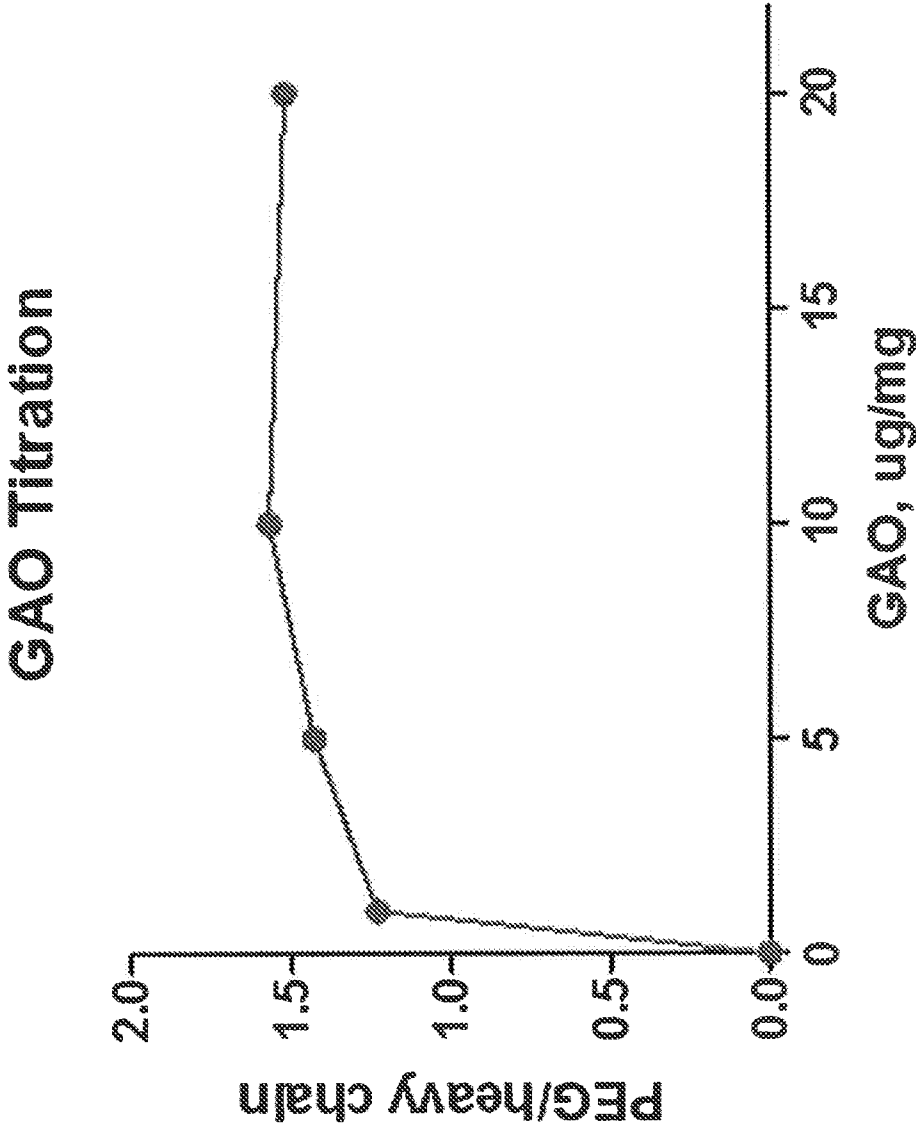
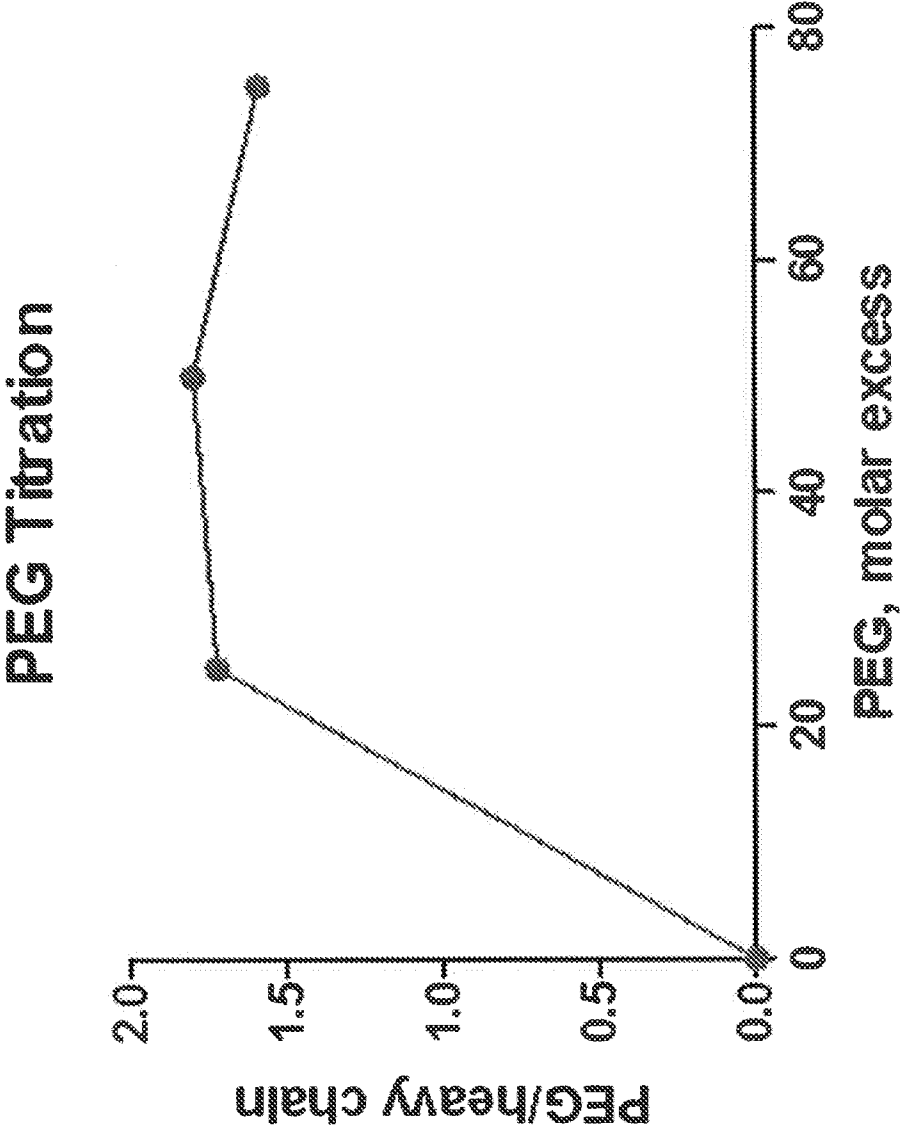


Figure 72



Glycopeptide aminoxy: lactose₃-Cys₃-Gly₄

Figure 74

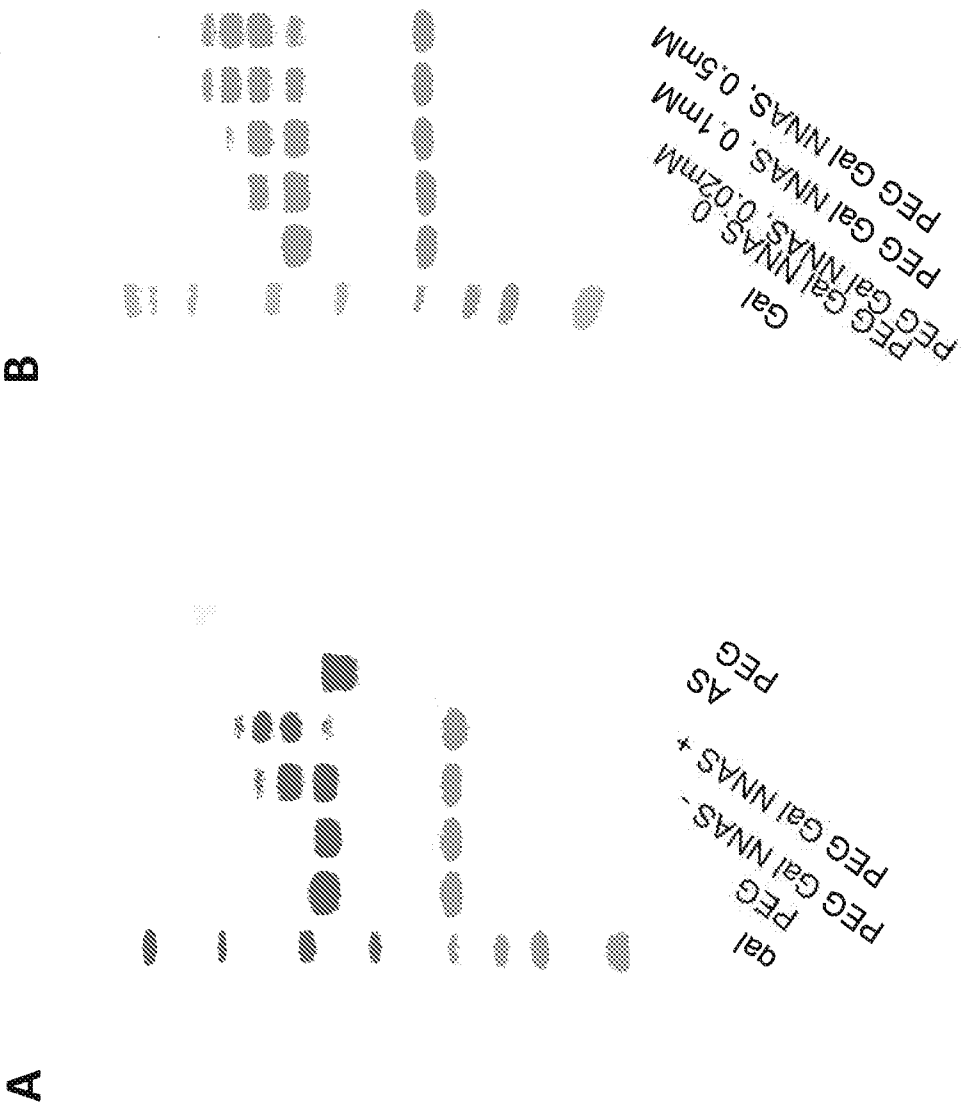


Figure 75

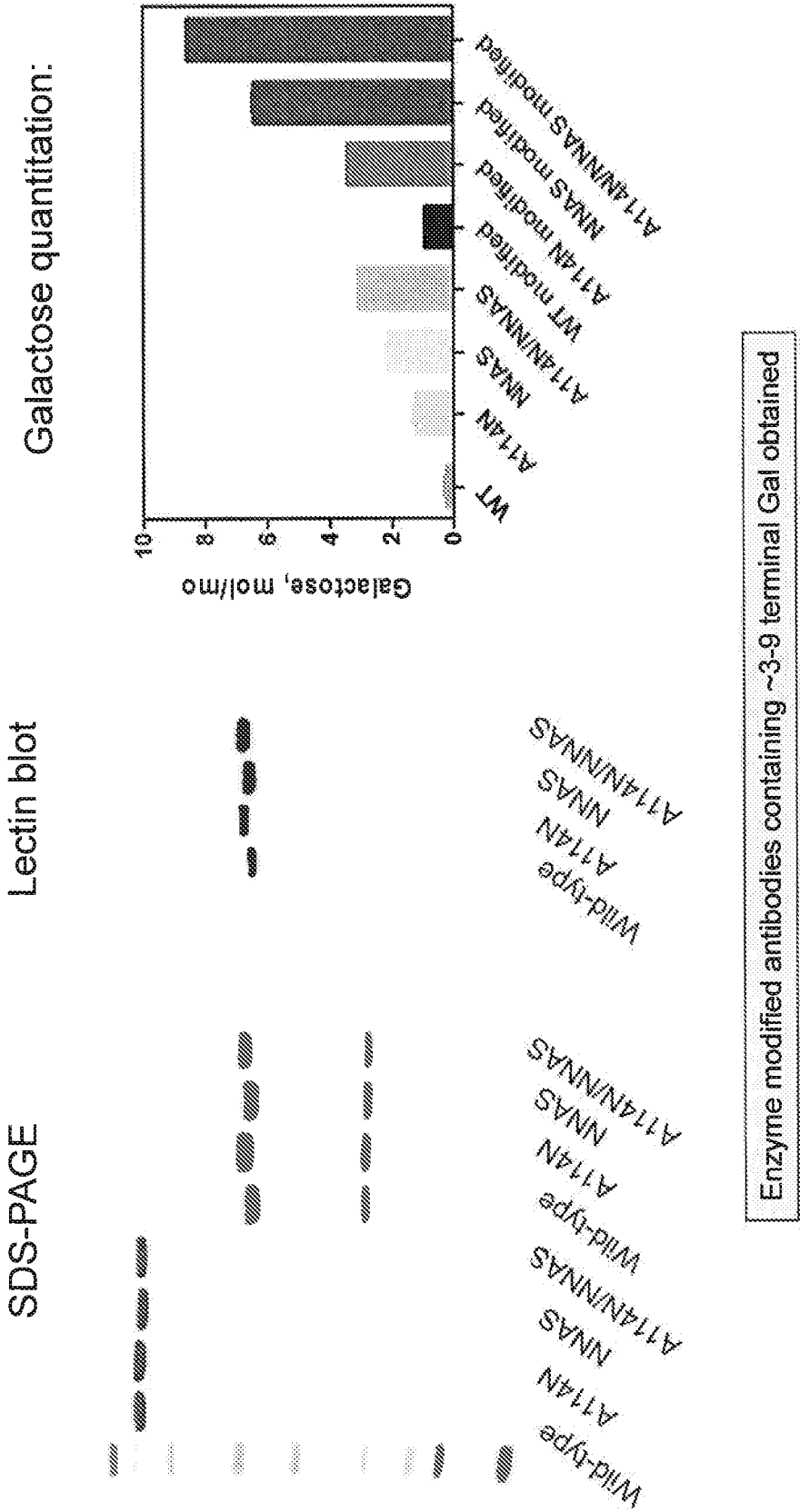


Figure 76

	Mol per mol, Mean $\pm$ SD
Wild-type	0.1 $\pm$ 0
A114N	0.5 $\pm$ 0
NNAS	2.3 $\pm$ 0
A114N/NNAS	2.7 $\pm$ 0

Figure 77

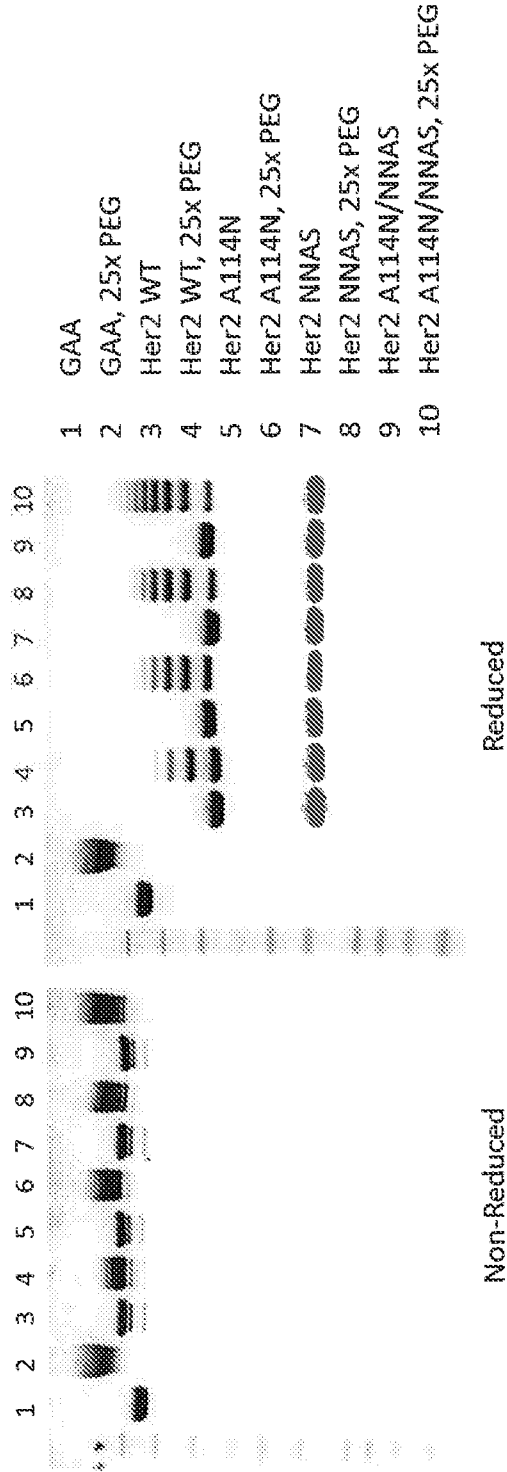


Figure 78

	Mol per mol, Mean + SD
Wild-type	1.4
A114N	2.7
NNAS	3.4
A114N/NNAS	4.6

Figure 79

Polyclonal antibody uptake into HepG2 cells

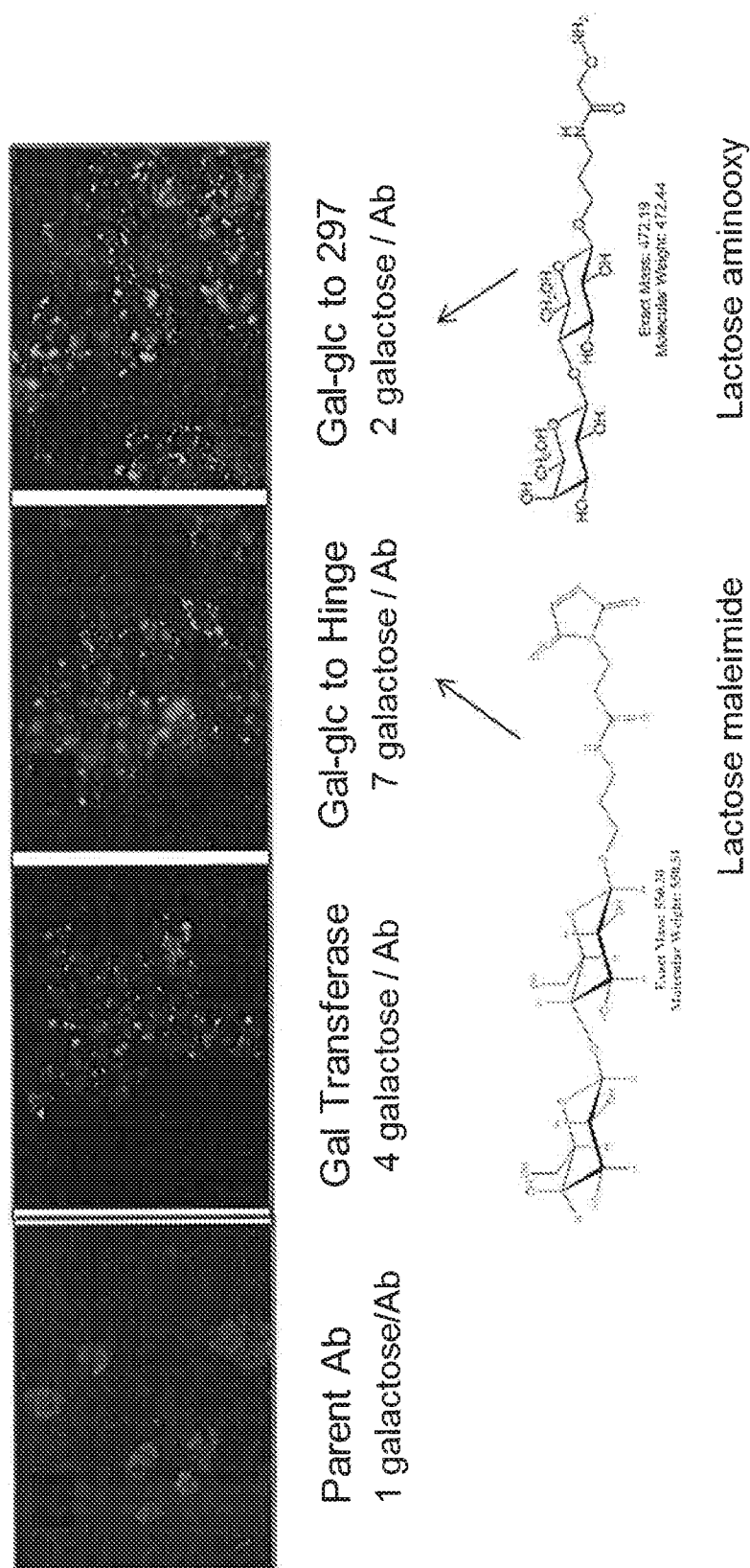


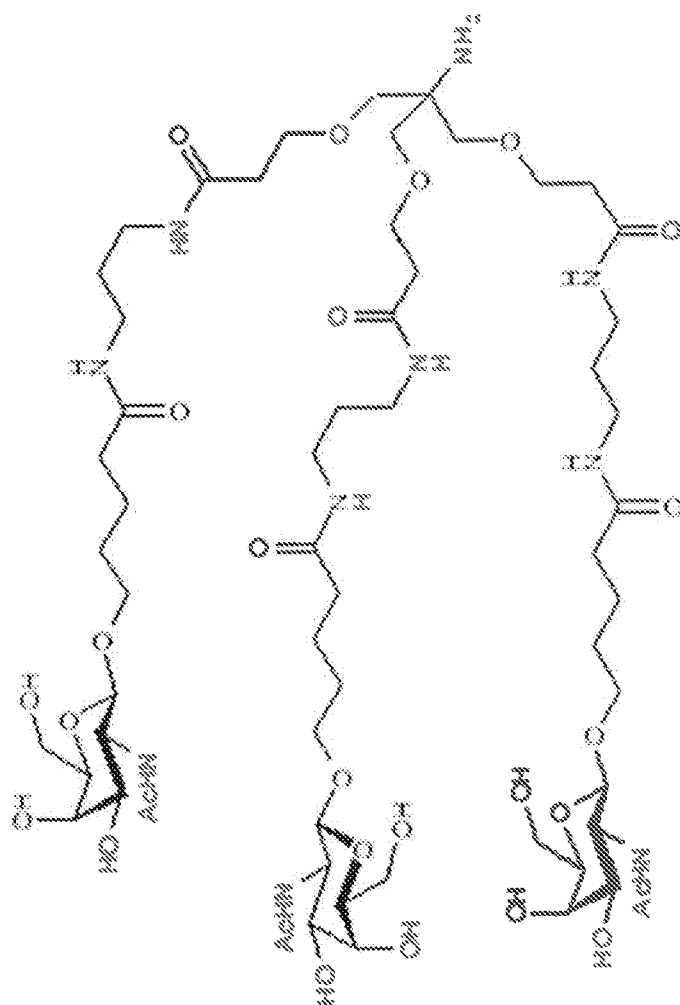
Figure 80

Figure 81

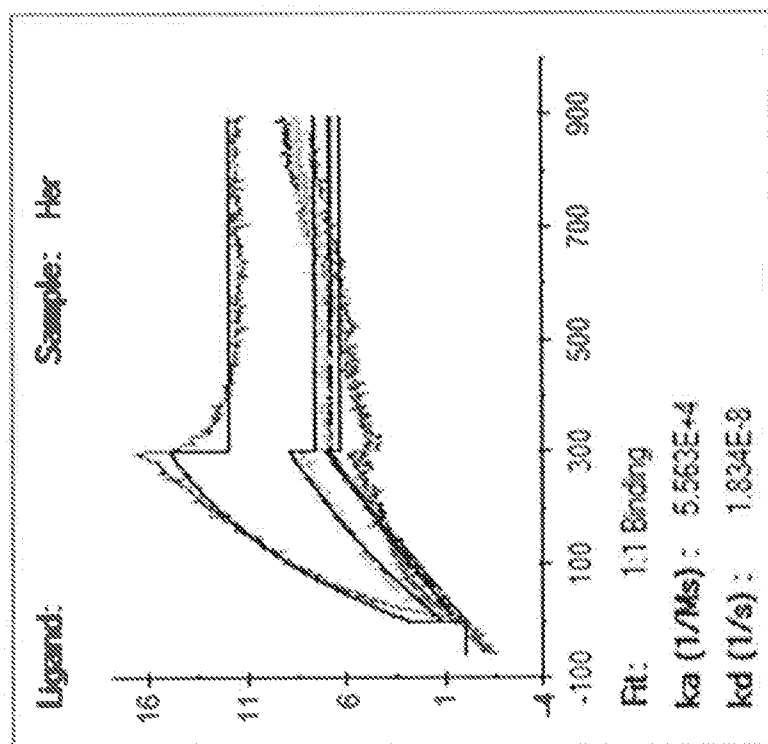
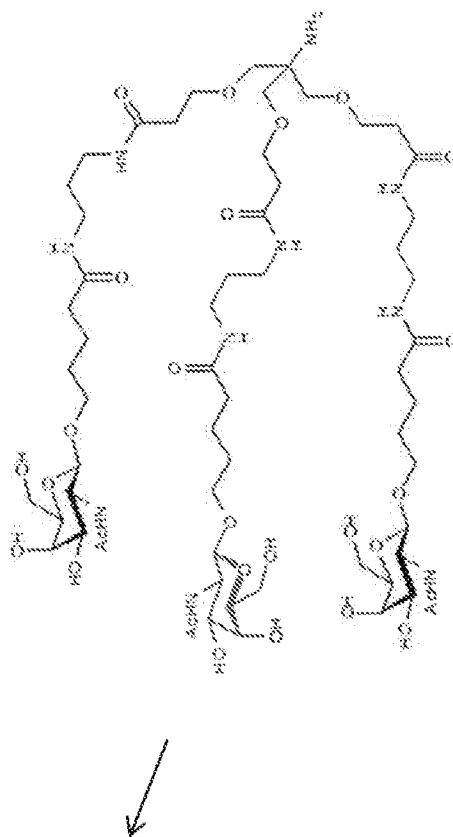


Figure 82

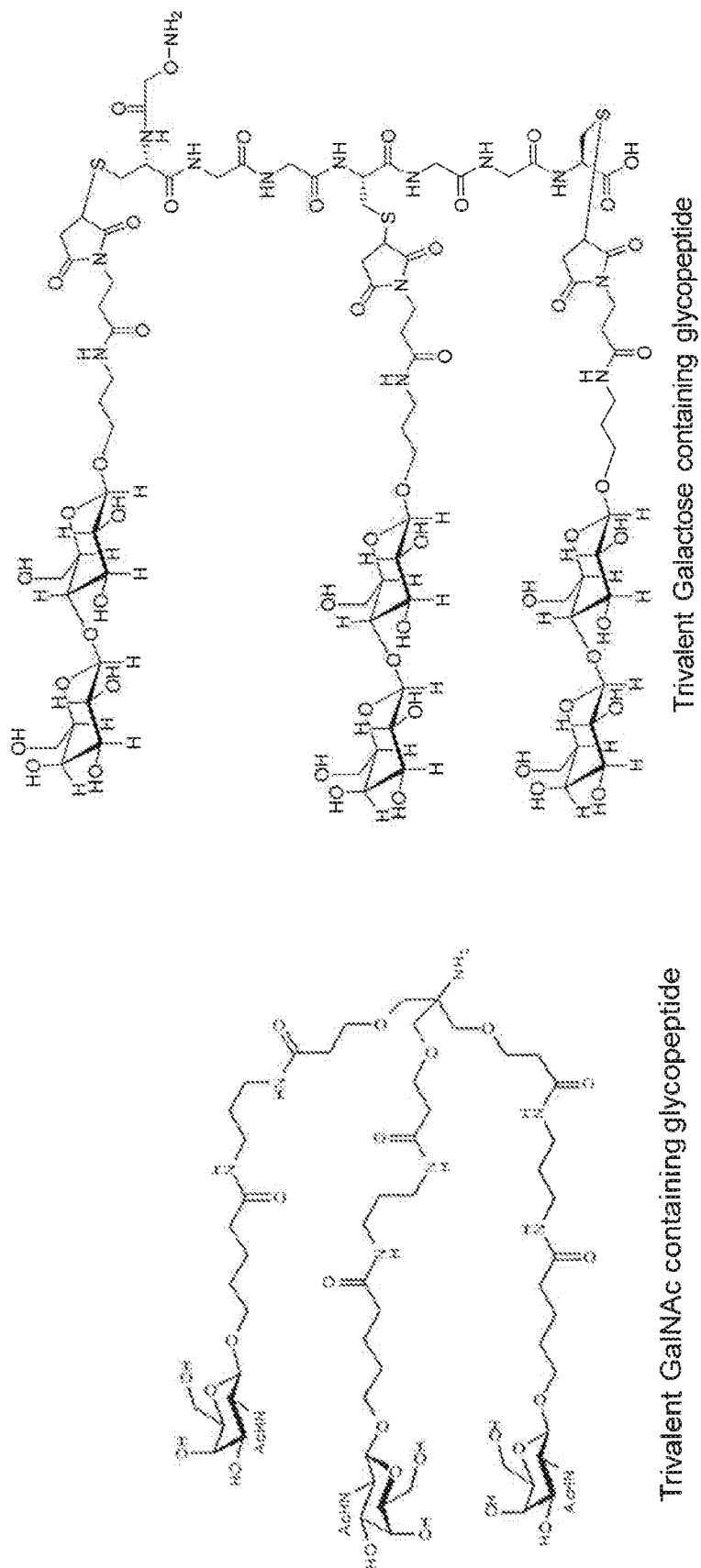


Figure 83

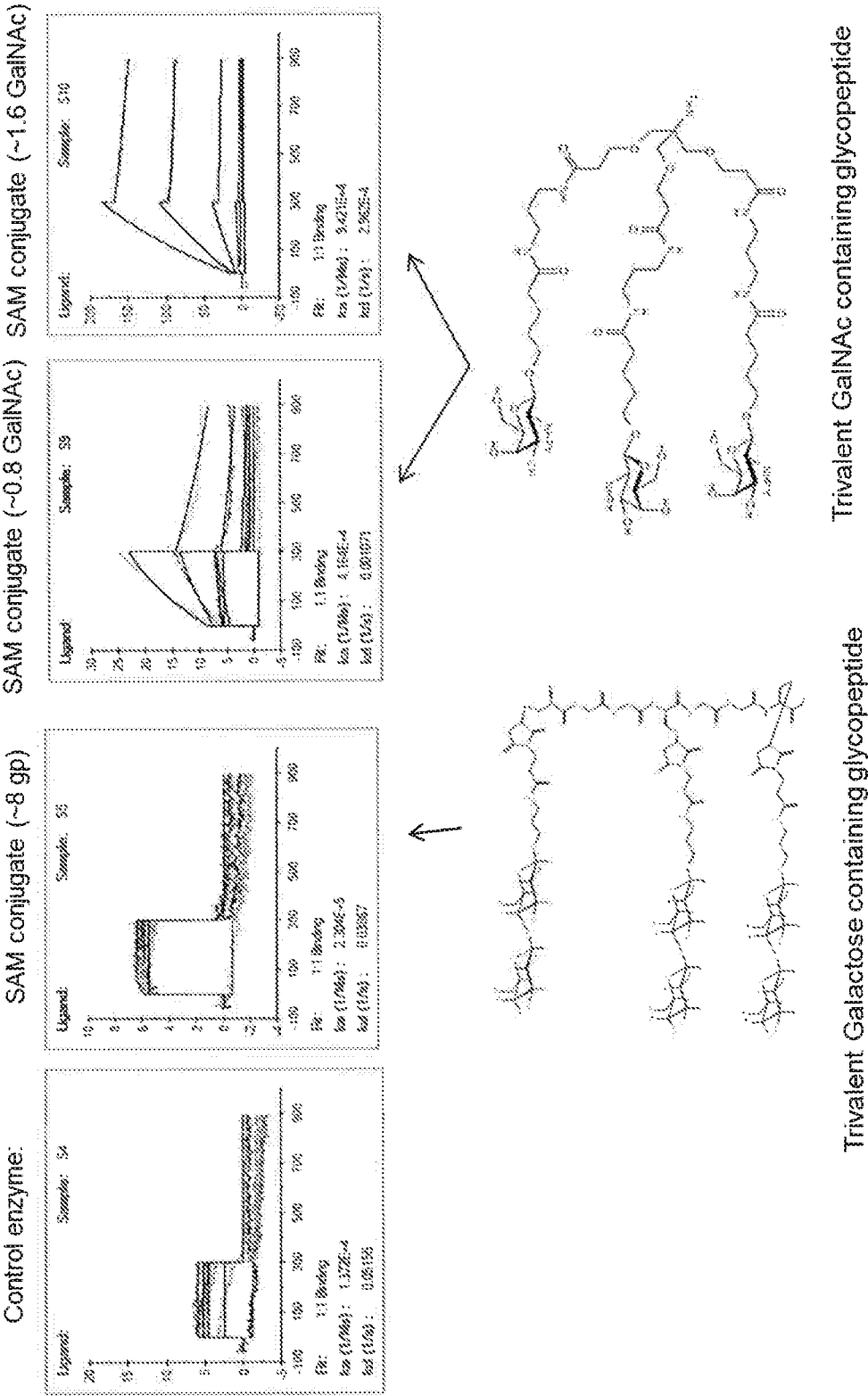


Figure 84

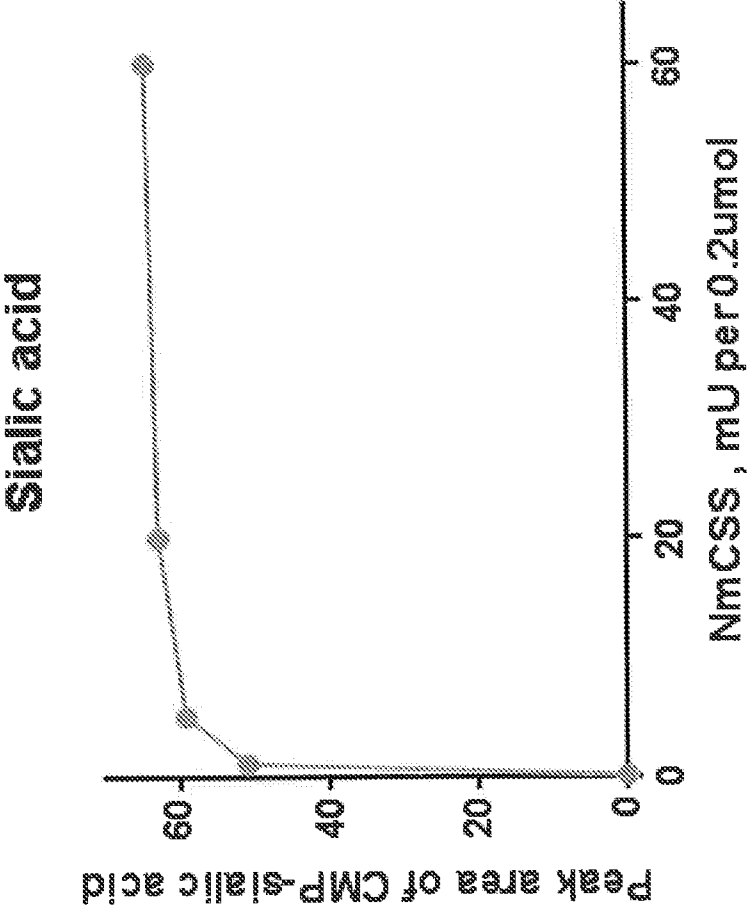


Figure 85

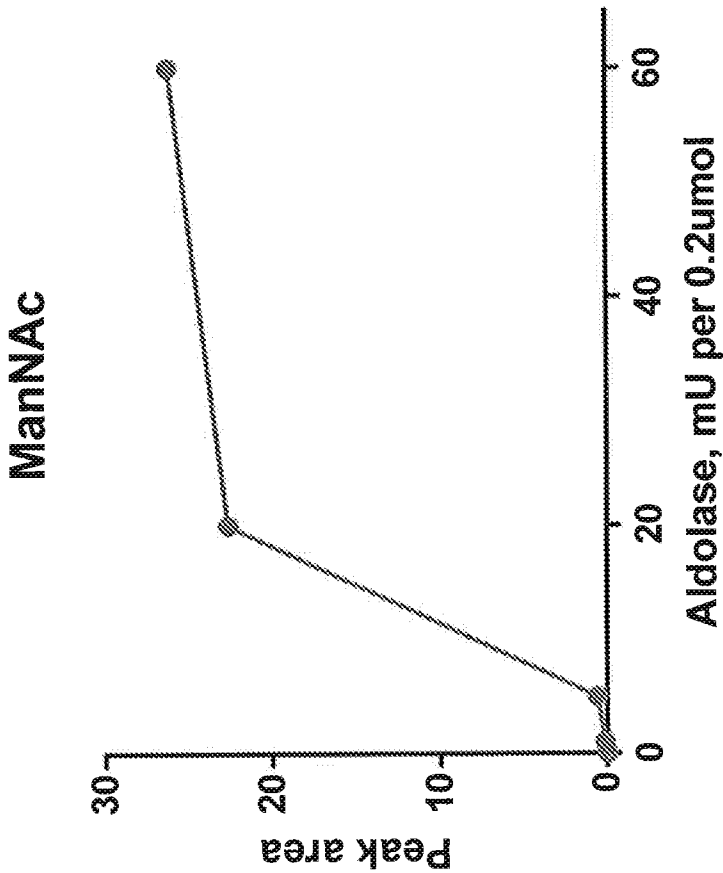


Figure 86

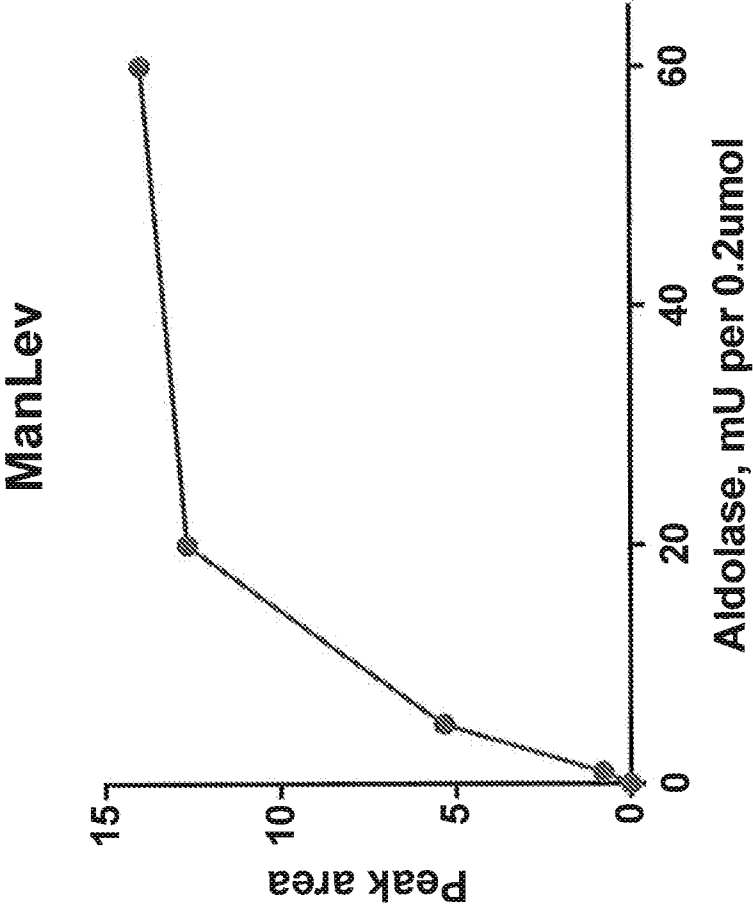


Figure 87

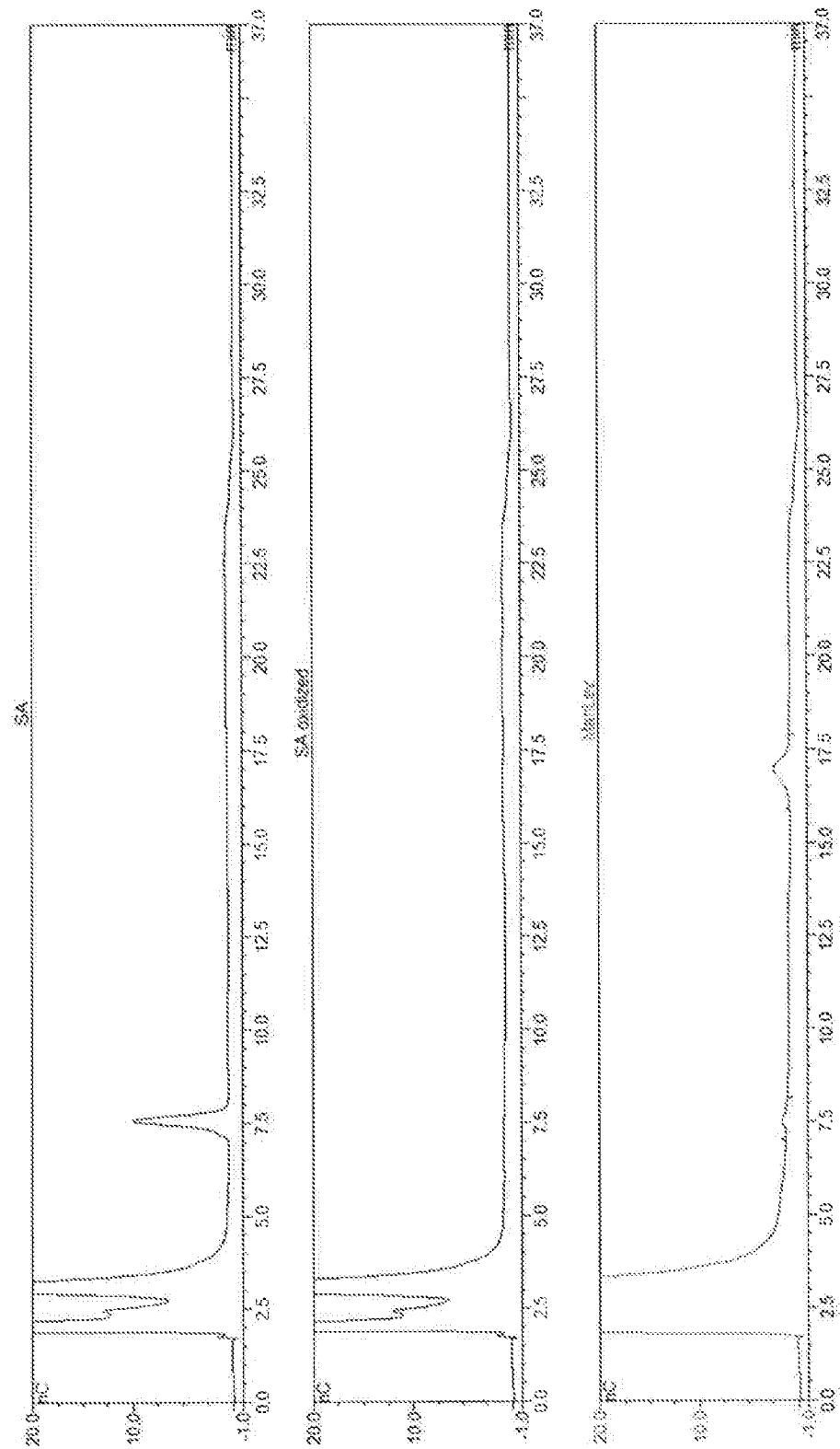


Figure 88

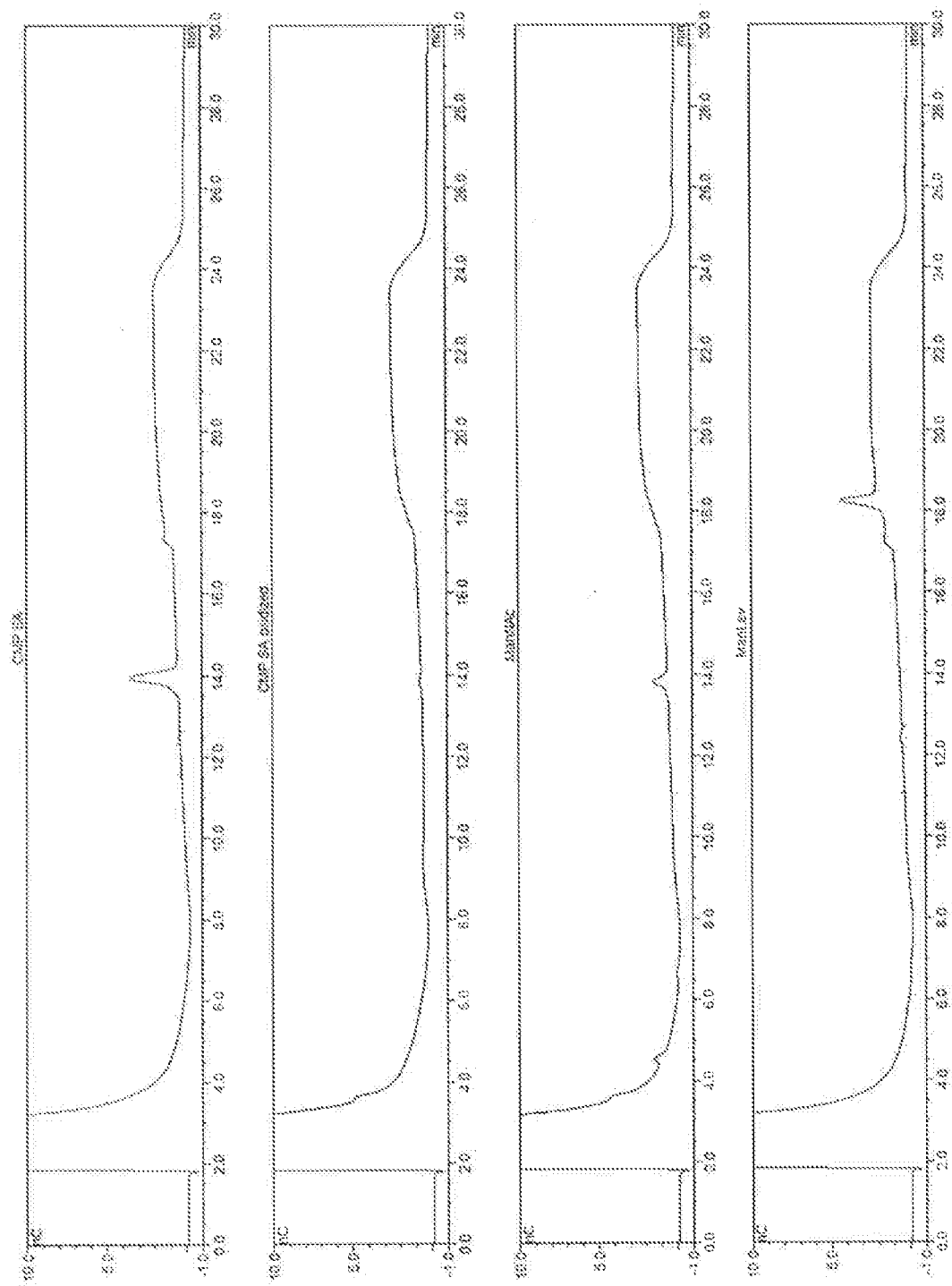


Figure 89

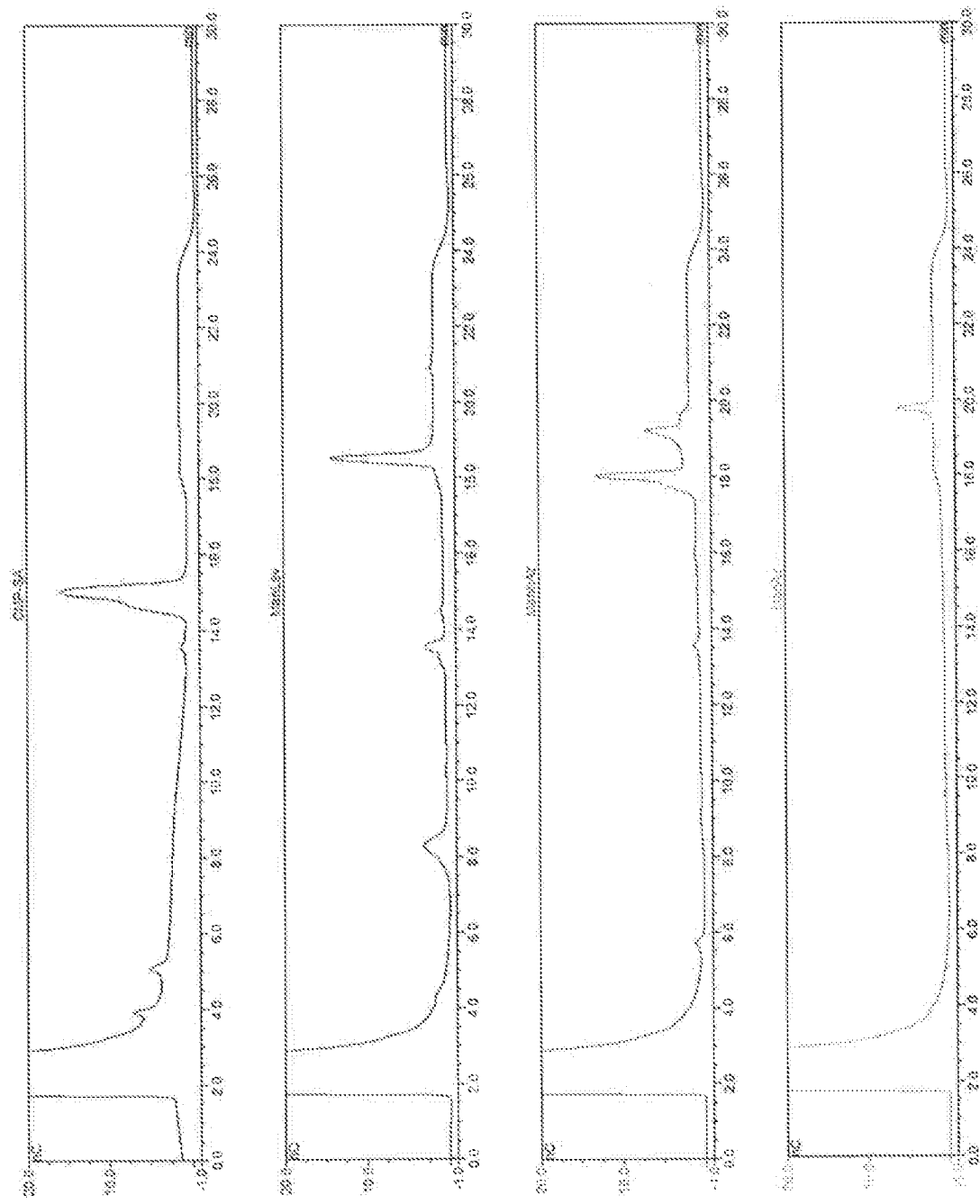


Figure 90

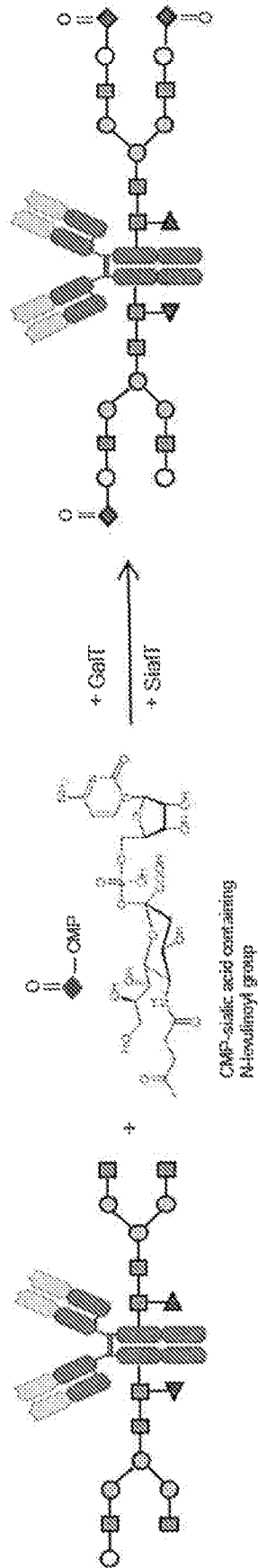


Figure 91

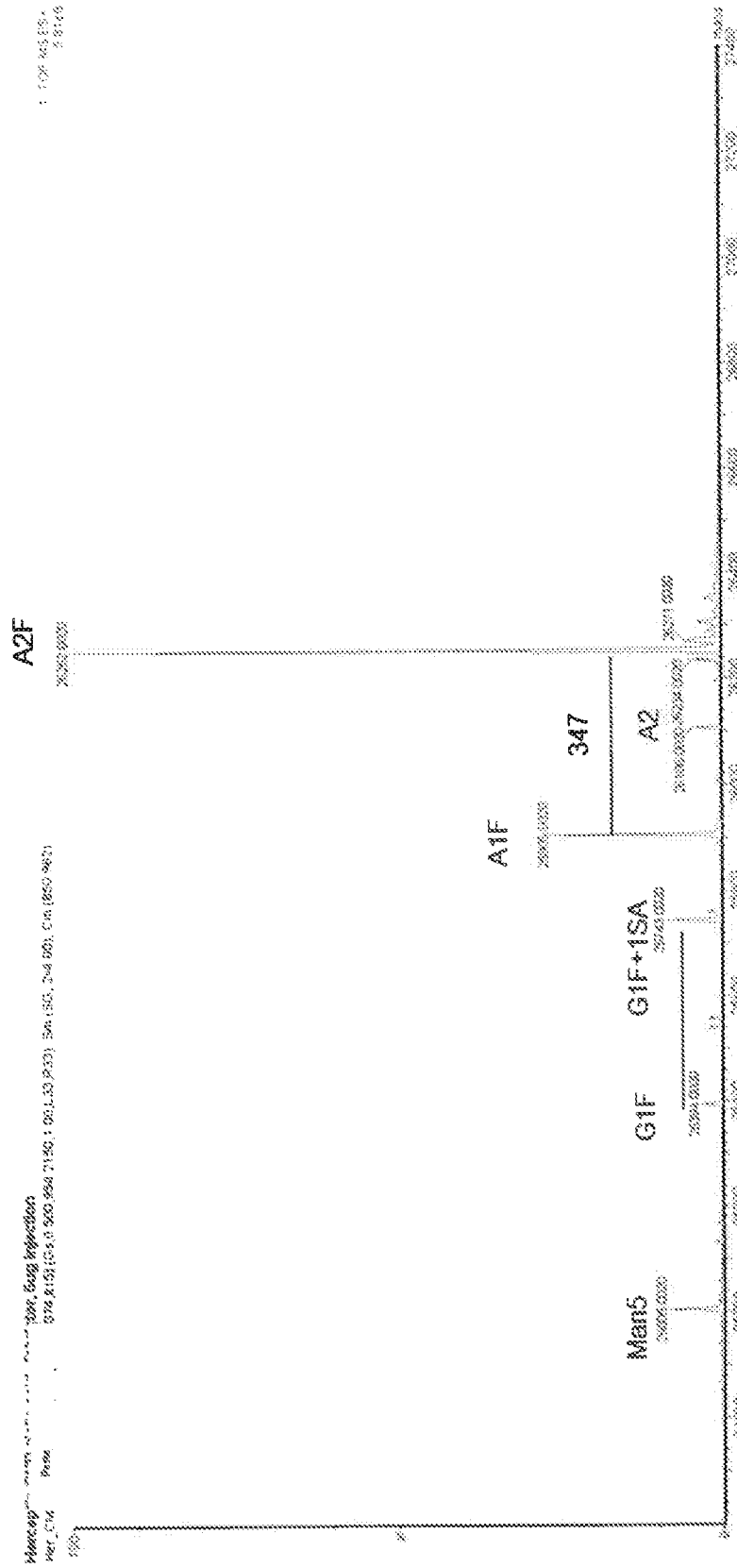


Figure 92

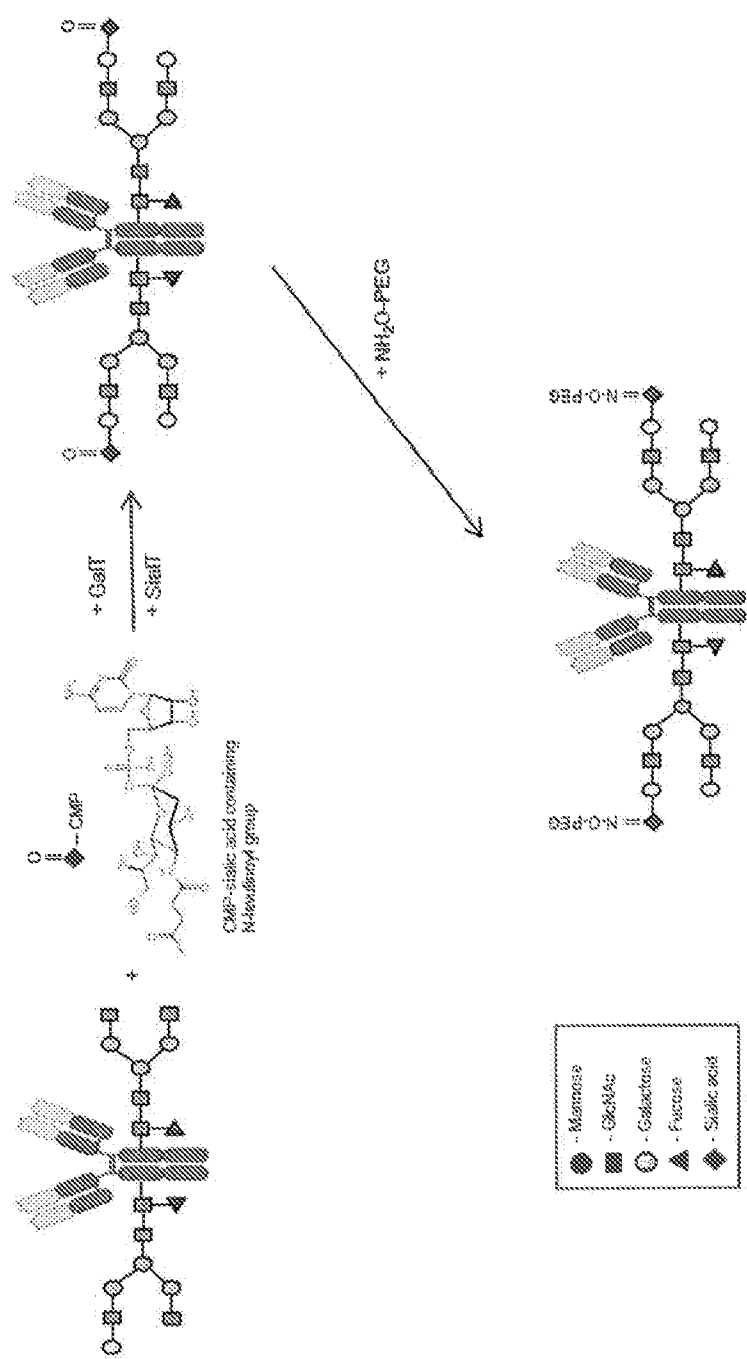


Figure 93

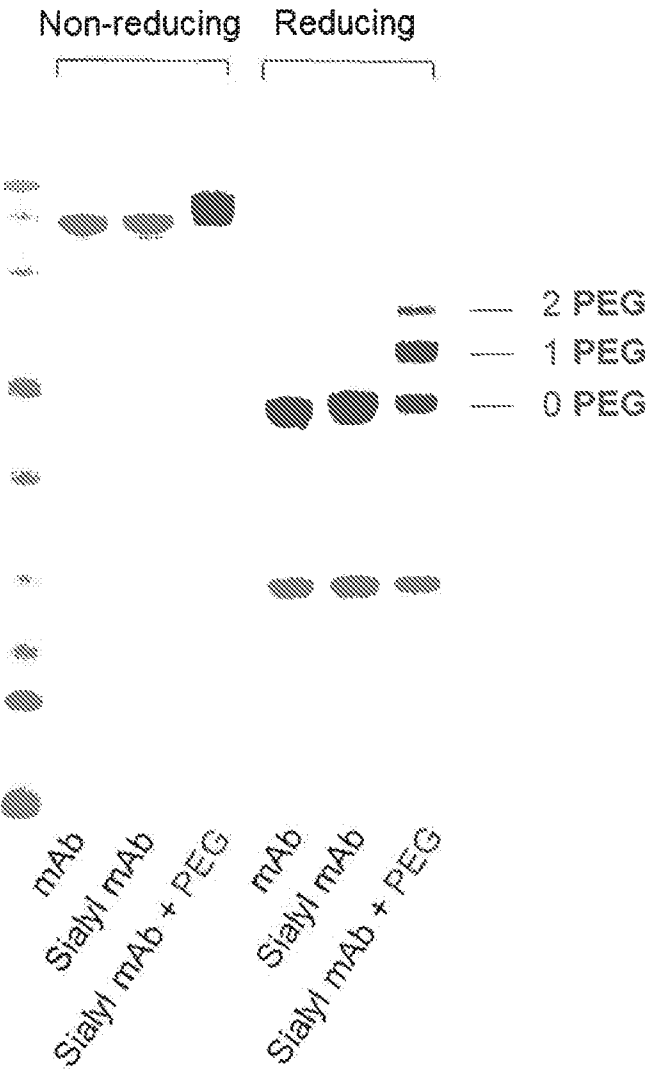


Figure 94

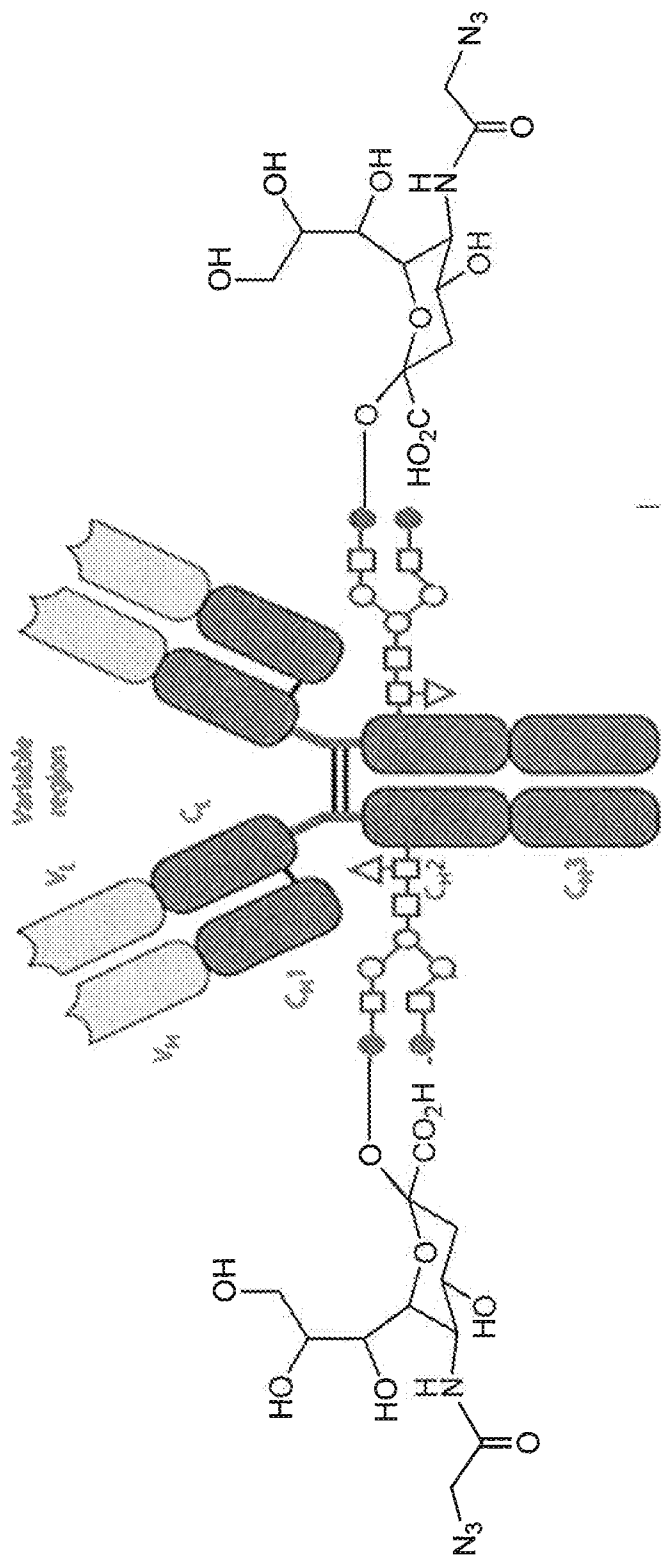


Figure 95

