

Figure 1

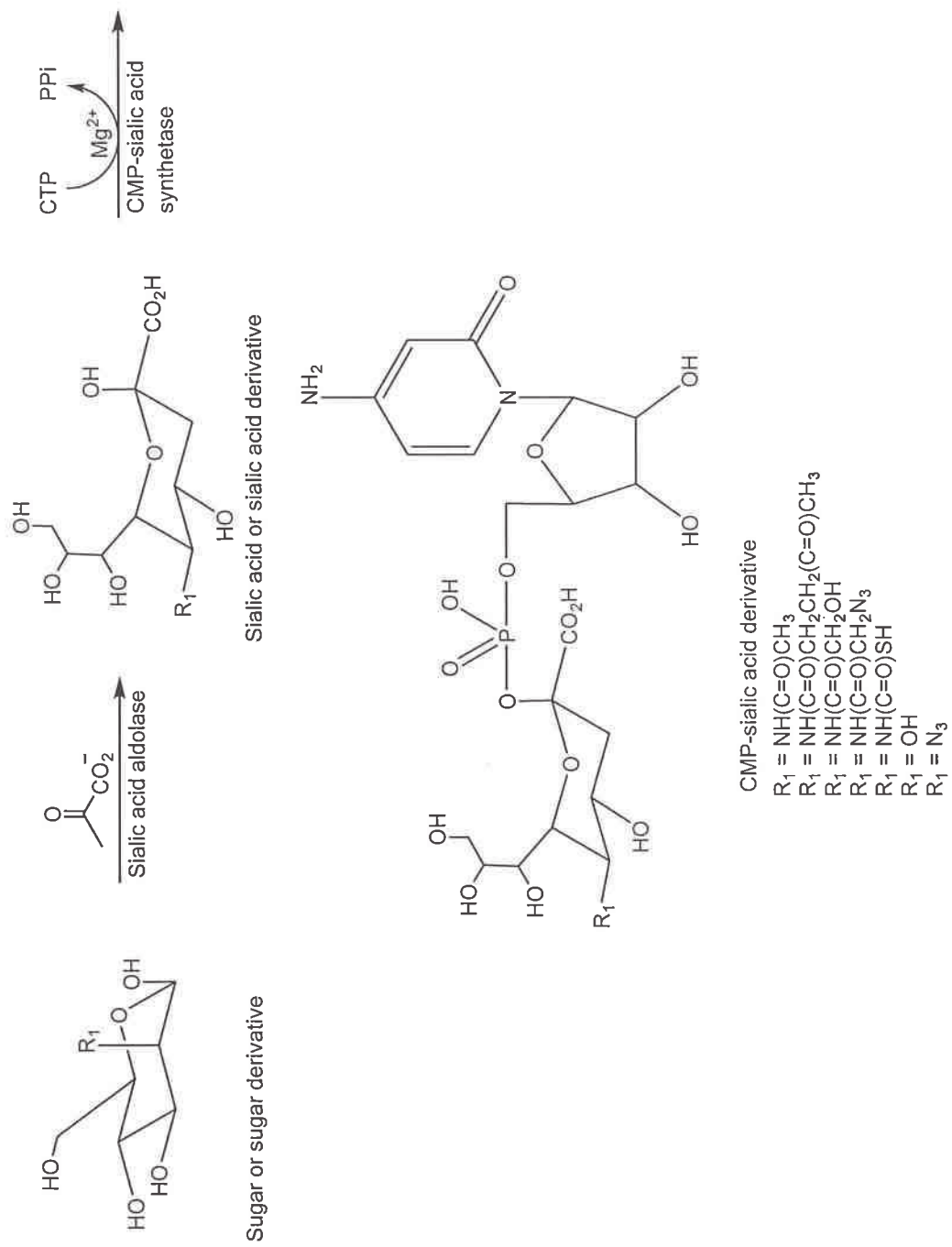
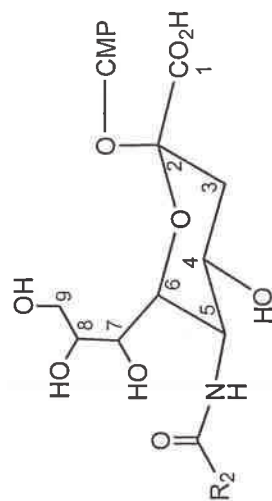
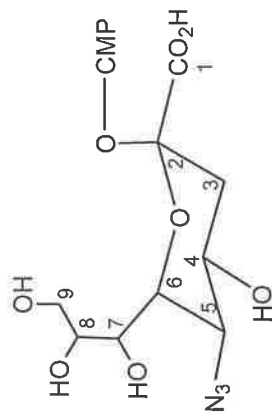


Figure 2

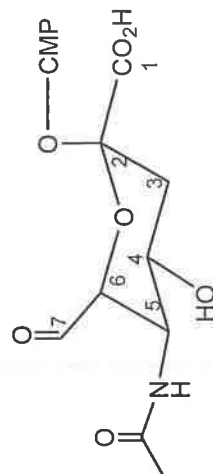


CMP-sialic acid C5 N-acetyl derivative

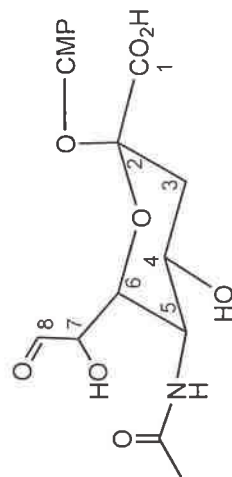
$R_2 = \text{CH}_3$
 $R_2 = \text{CH}_2\text{CH}_2(\text{C}=\text{O})\text{CH}_3$
 $R_2 = \text{CH}_2\text{OH}$
 $R_2 = \text{CH}_2\text{N}_3$
 $R_2 = \text{SH}$



CMP-sialic acid C5 azide derivative



CMP-sialic acid C7 aldehyde derivative



CMP-sialic acid C8 aldehyde derivative

Figure 3

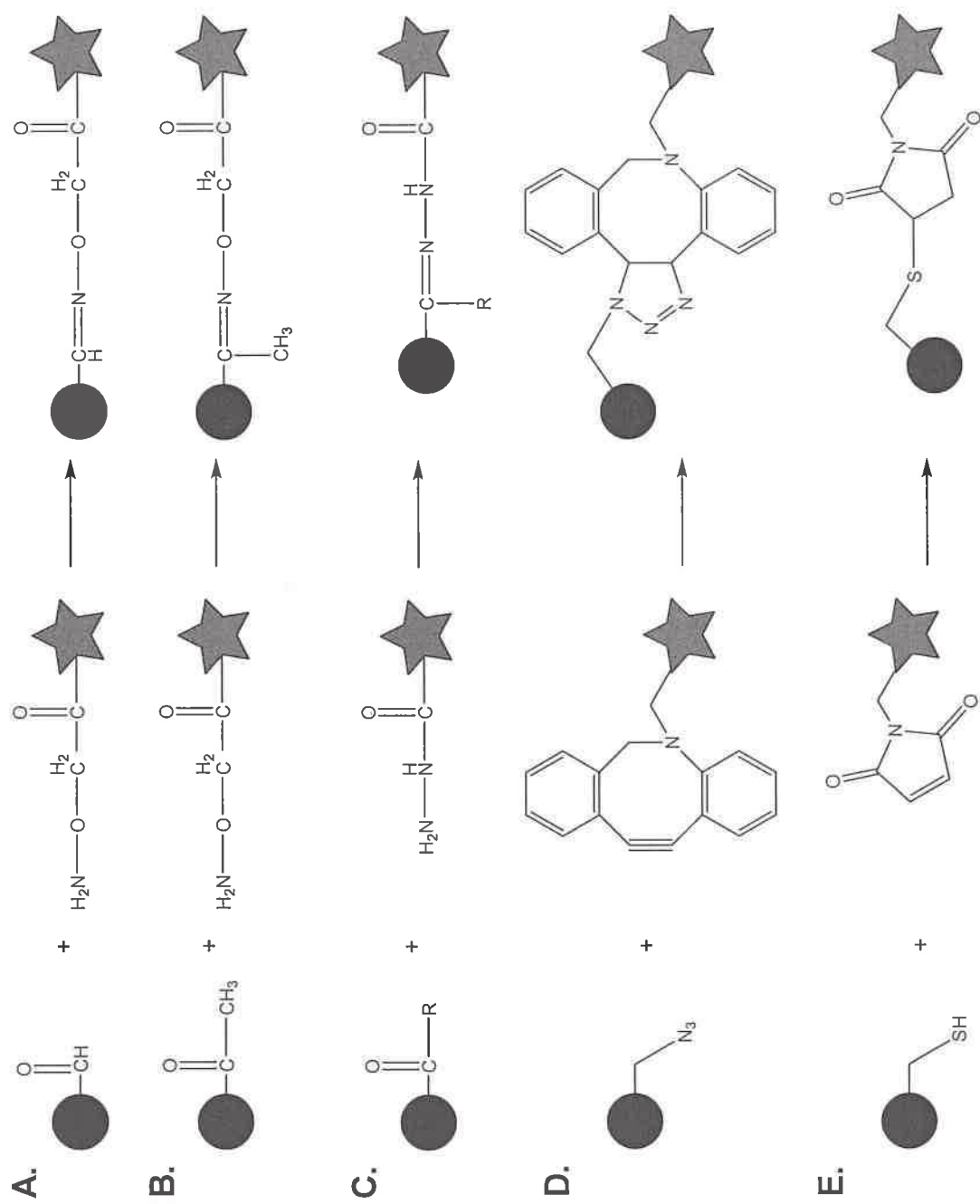


Figure 4

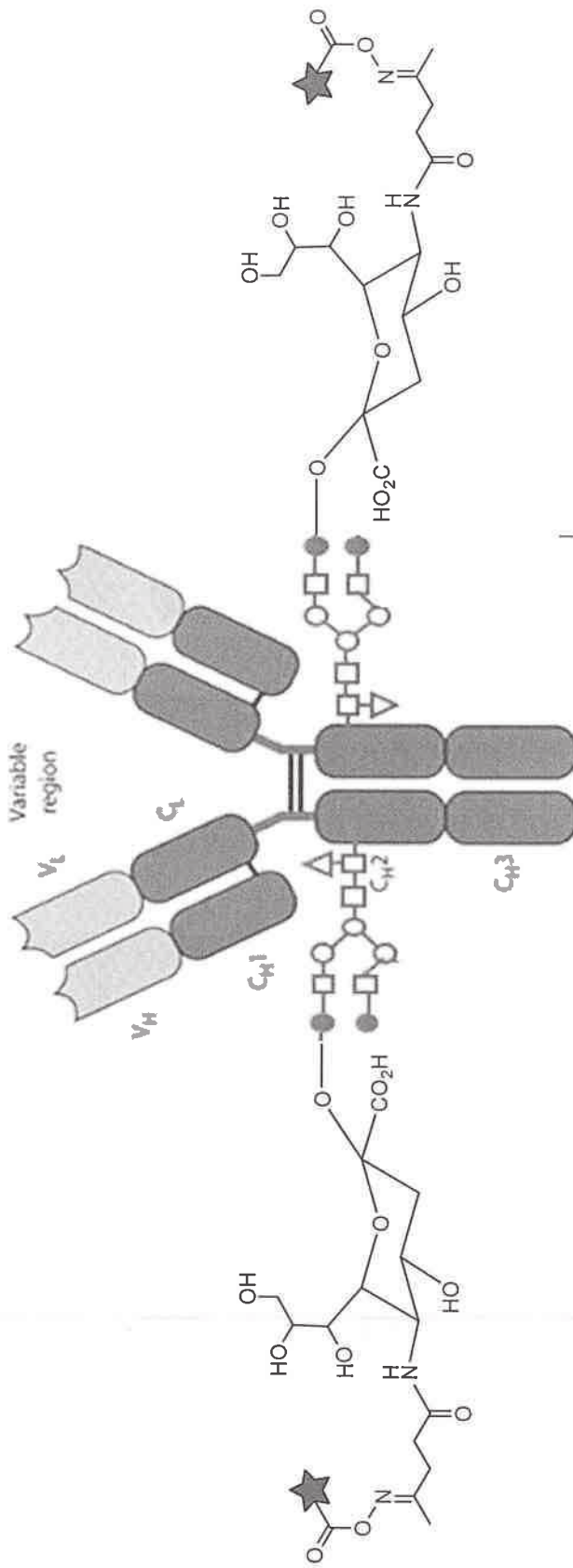
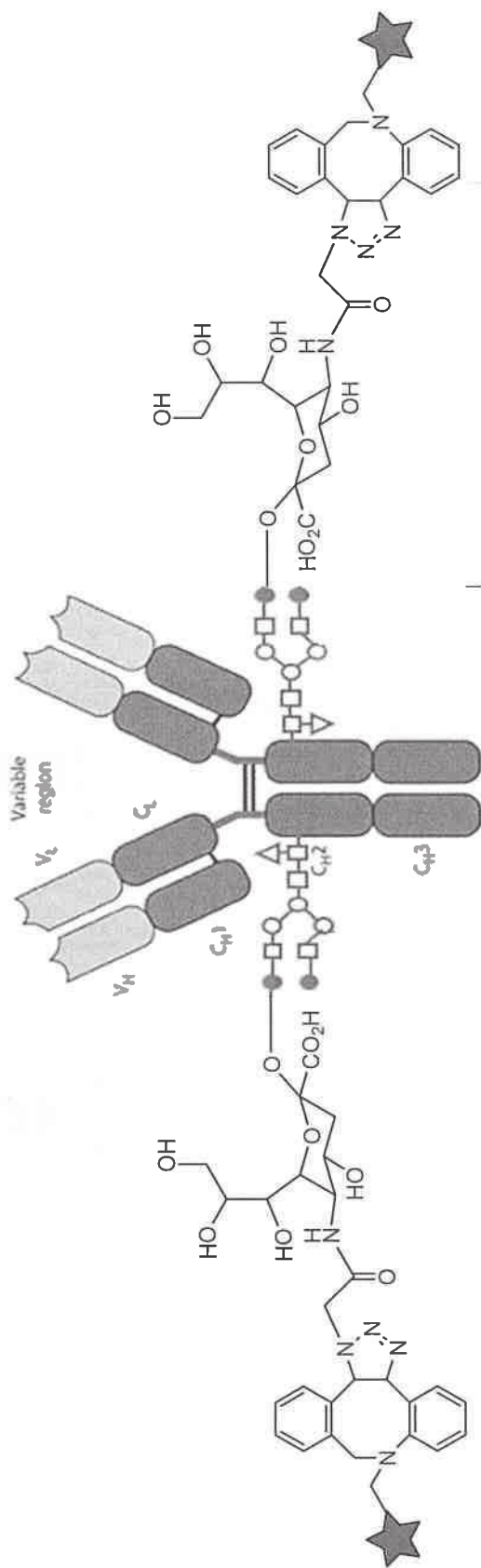


Figure 5



6/96

Figure 7

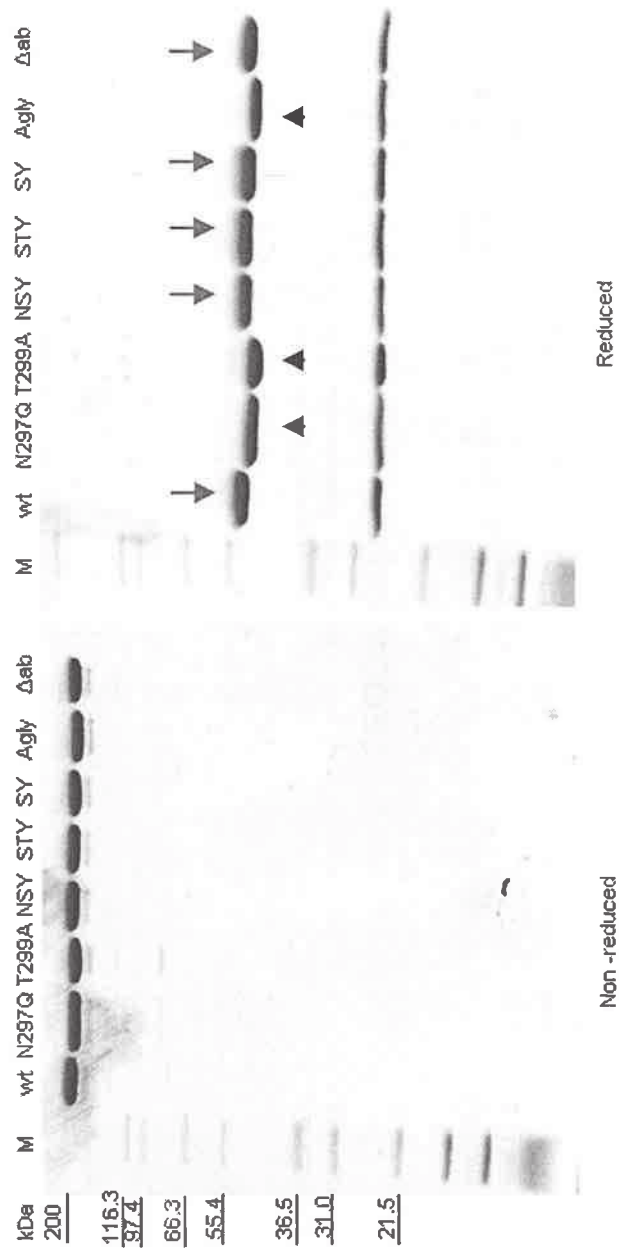
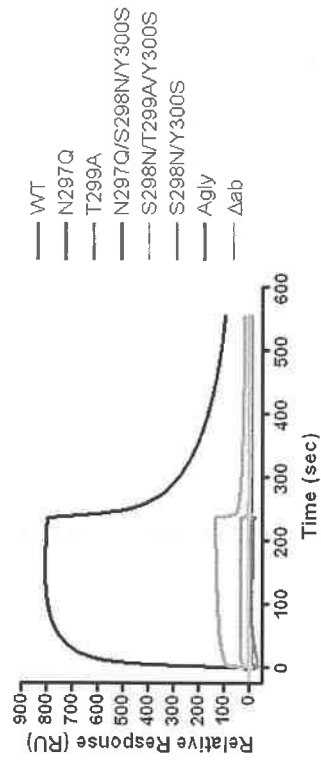


Figure 8

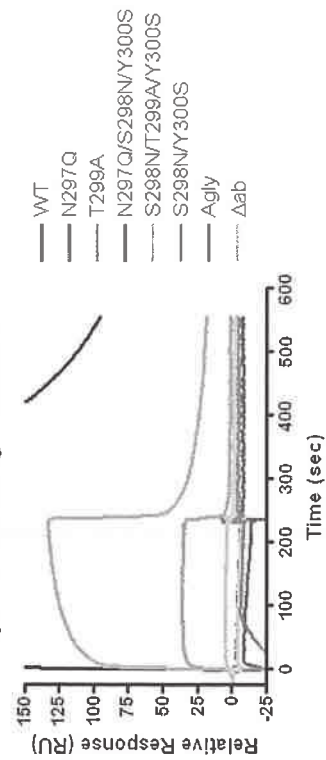
Fullscale: CD16a-Val158

Human FcγRIIIa-Val158 Binding of αβTCR HEBE1 IgG mutants



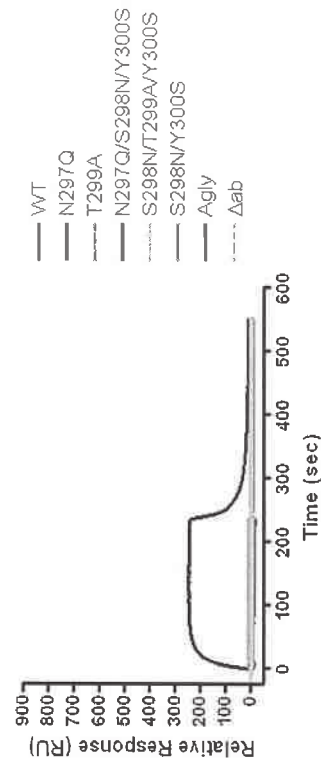
zoomed:

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

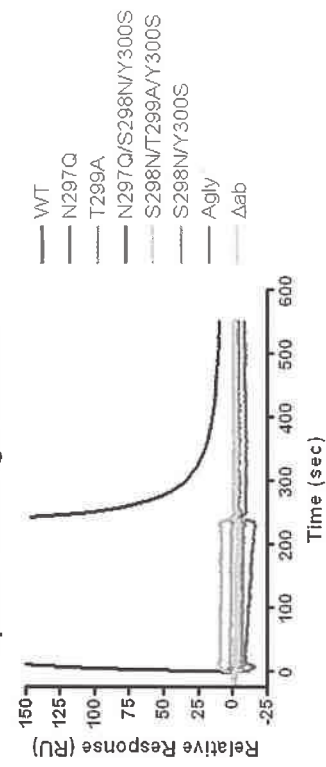
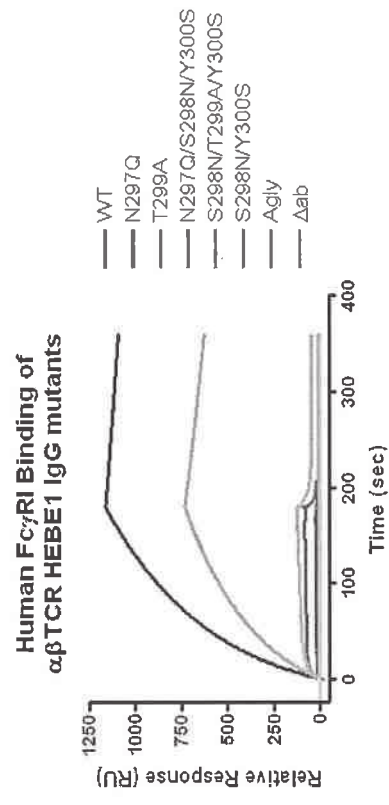


Figure 9

Fullscale:



zoomed in:

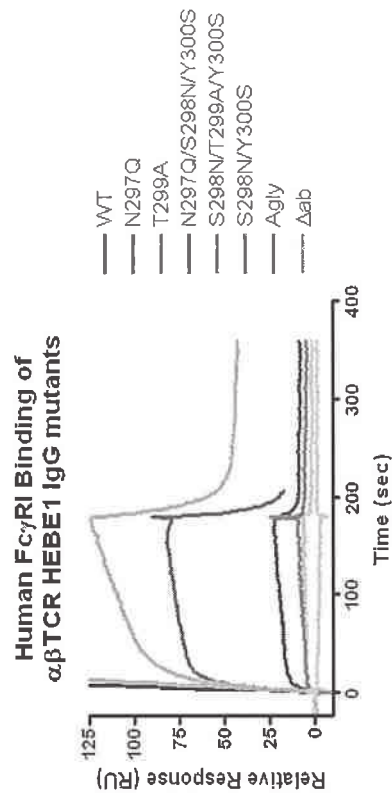


Figure 10

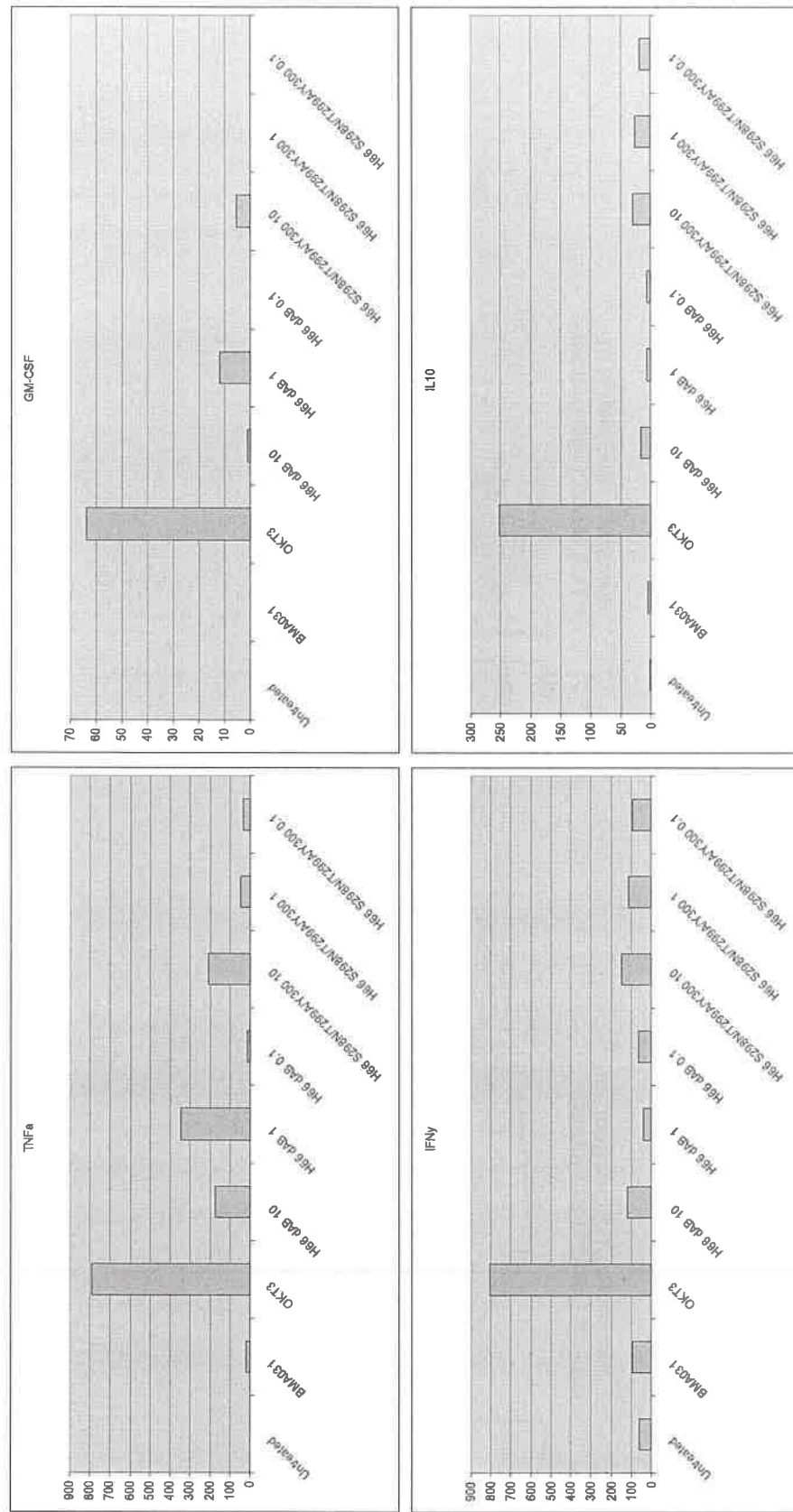


Figure 11

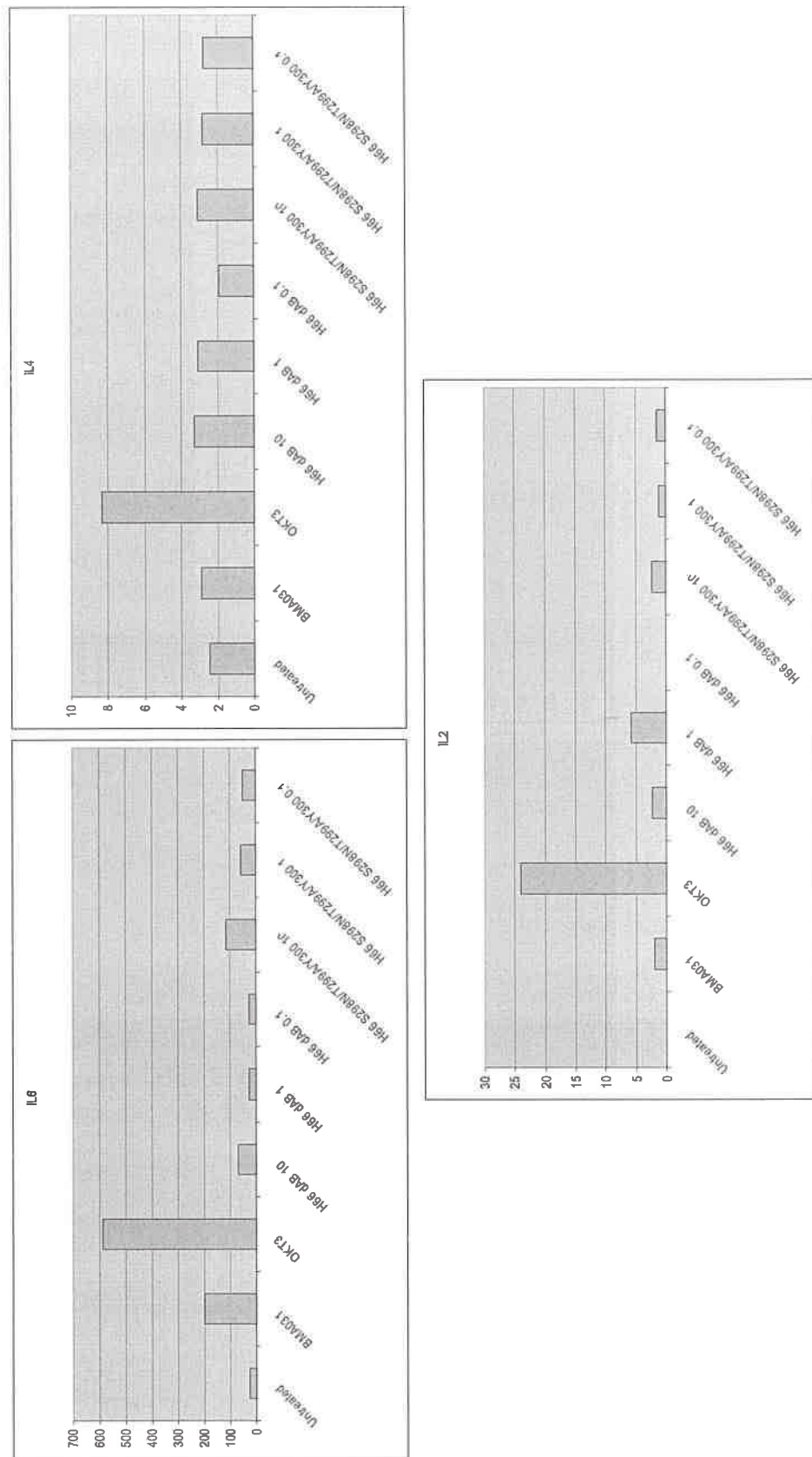


Figure 12

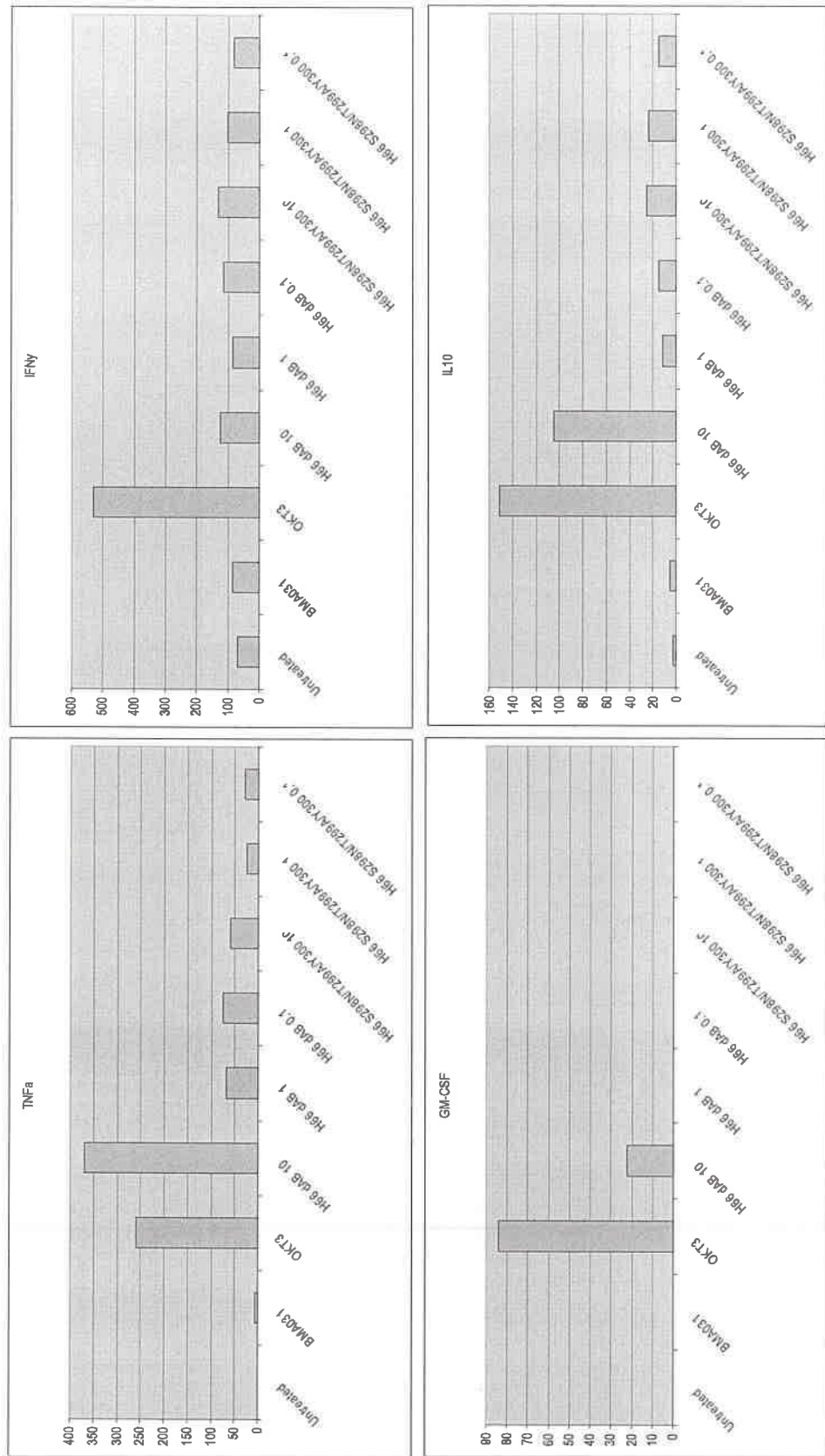


Figure 13

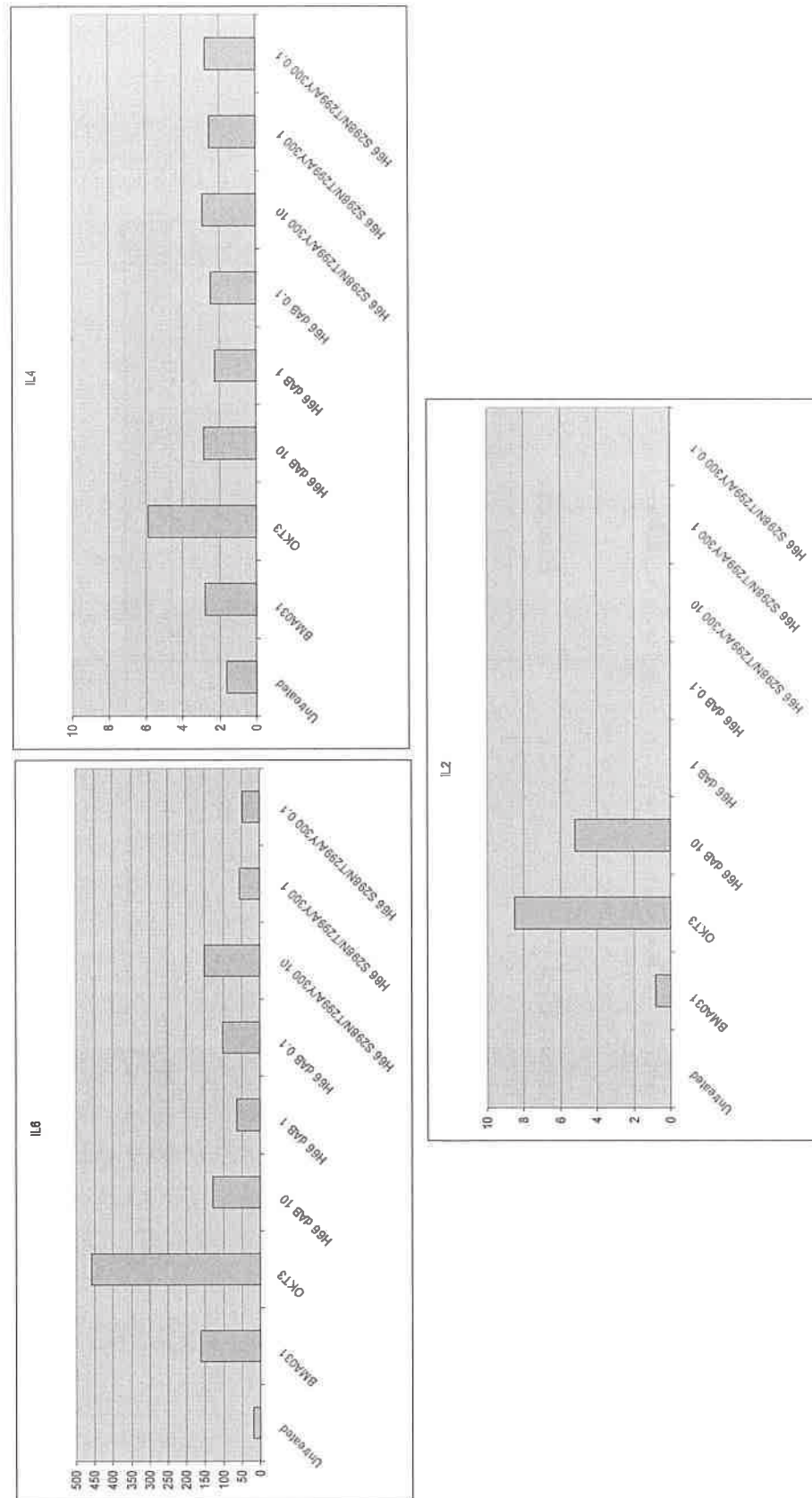
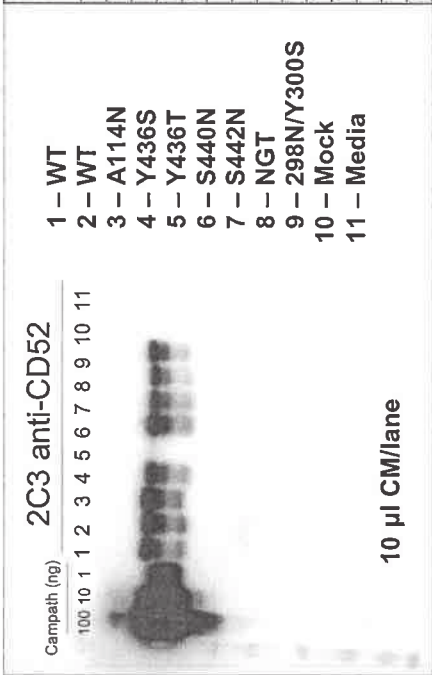


Figure 14

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	3.669
2C3 in media	3.00	860.96	2.47E-08	3.710
2C3 in media	3.00	866.89	2.51E-08	3.758
A114N		146.5	1.60E-09	0.240
Mock		87.84	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	1.60E-09	0.240
S442N		121.21	1.26E-09	0.189
WT (Katya)		148.41	1.63E-09	0.244
WT (Tim)		148.08	1.62E-09	0.244
Y436S		156.72	1.78E-09	0.267
Y436T		84.27	8.23E-10	0.123
incubated media		87.36	8.57E-10	0.129

Figure 15

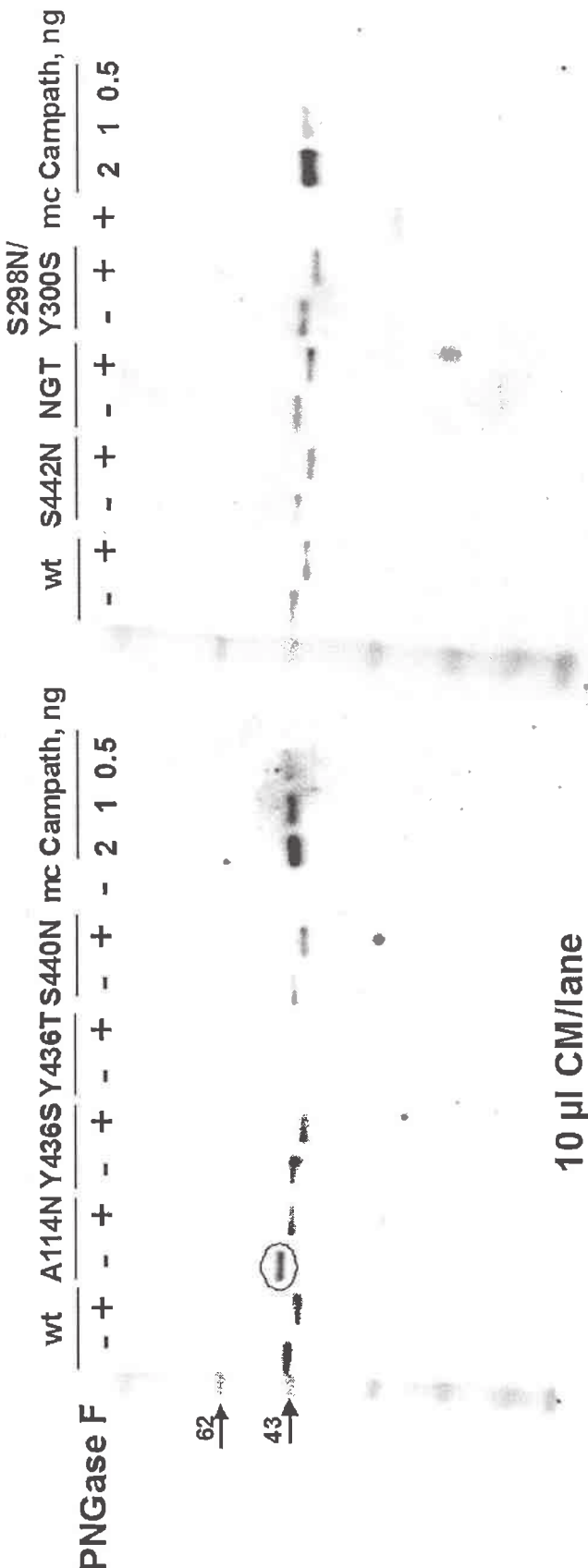
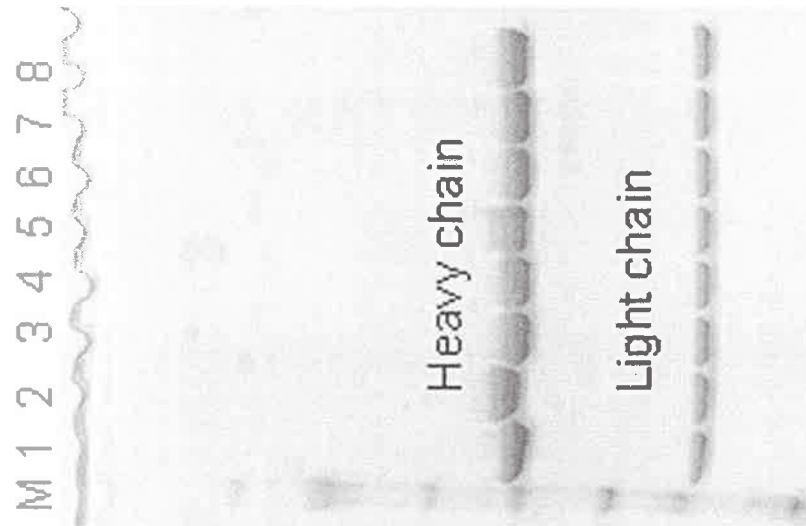


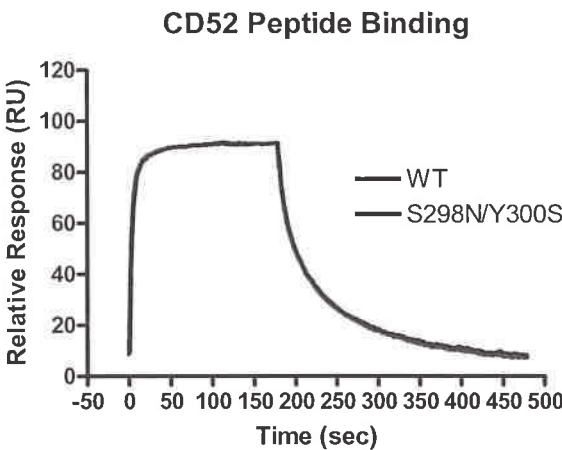
Figure 16



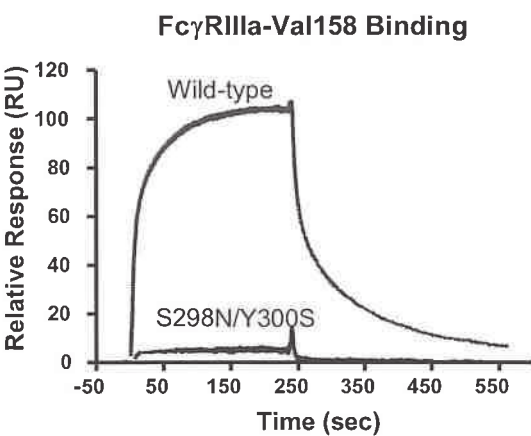
Lane	Mutant	mg/ml
1	wt	2.66
2	A114N	2.99
3	Y436S	1.61
4	S298N/Y300S	0.99
5	Y436T	0.41
6	S440N	1.21
7	S442N	1.62
8	NGT	2.21

Figure 17

A.



B.



C.

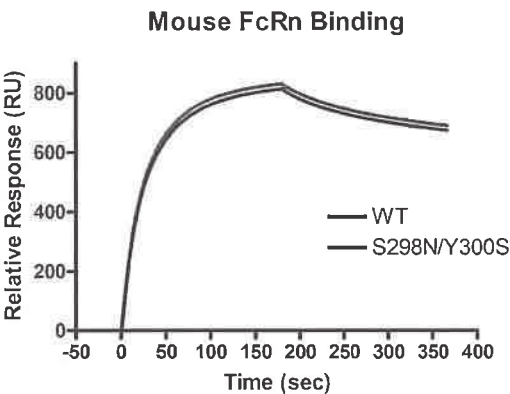


Figure 18

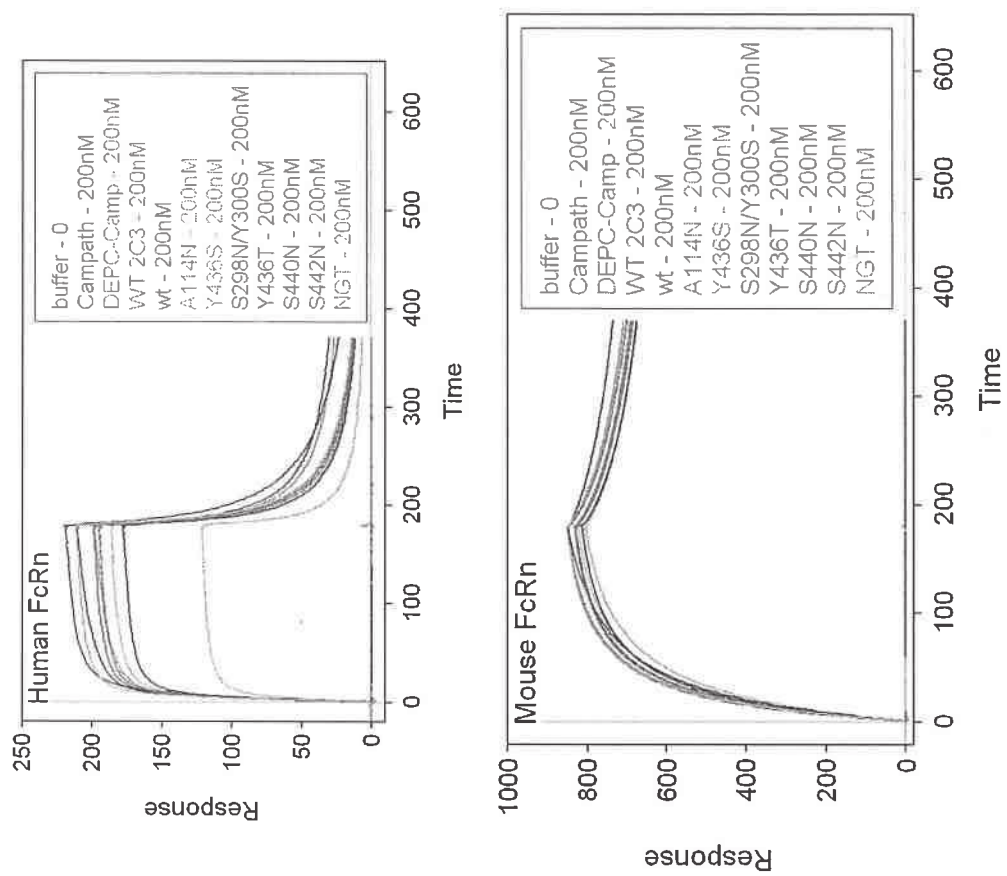
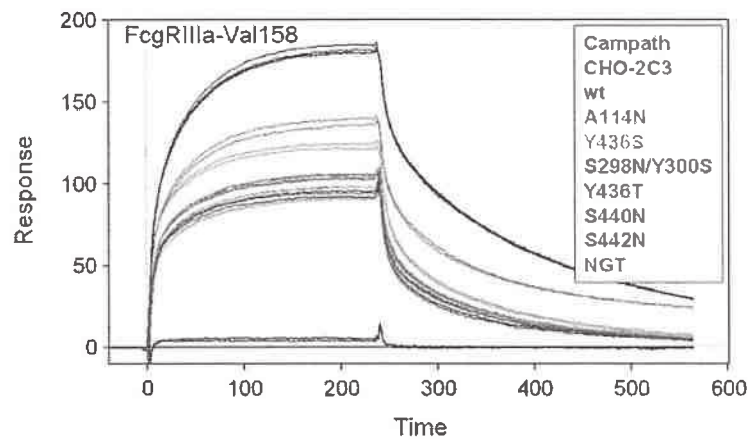


Figure 19

A.



B.

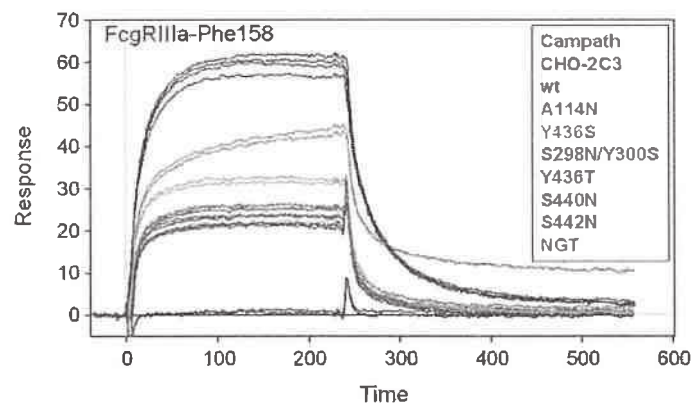
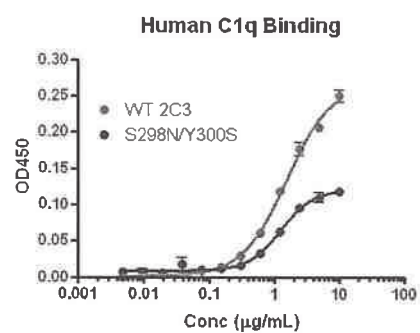


Figure 20

A.



B.

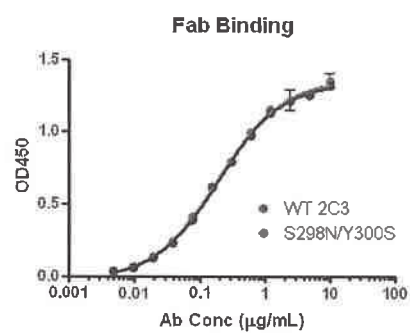


Figure 21

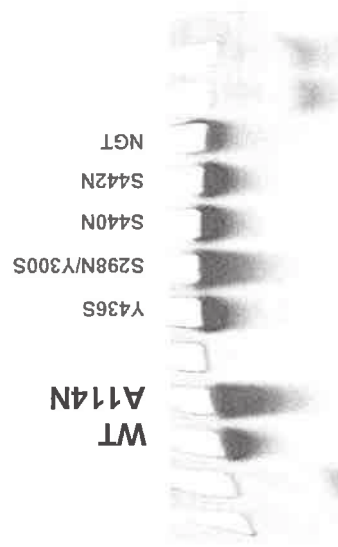
Sample	k_a ($\times 10^6/\text{M}\cdot\text{s}$)	k_d ($\times 10^{-2}/\text{s}$)	R_{max} (RU)	K_D (nM)
GLD52	7.0	1.7	67.0	2.44
WT 2C3	6.0	1.1	64.2	1.75
A114N	4.7	1.1	59.5	2.45
Y436S	5.9	1.0	66.9	1.73
S298N/Y300S	5.7	1.0	63.3	1.80
Y436T	4.8	0.9	65.7	1.95
S440N	5.8	1.1	66.8	1.84
S442N	5.7	1.1	66.2	1.85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

Figure 22

Sample	Kon ($\times 10^6 \text{M}^{-1} \text{s}^{-1}$)	Koff ($\times 10^{-2} \text{s}^{-1}$)	KD (nM)
WT 2C3	5.2	1.1	2.1
A114N	5.3	1.3	2.4

Figure 23

A.



B.

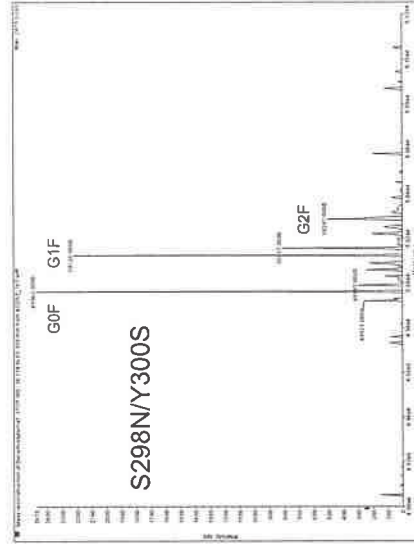


Figure 23

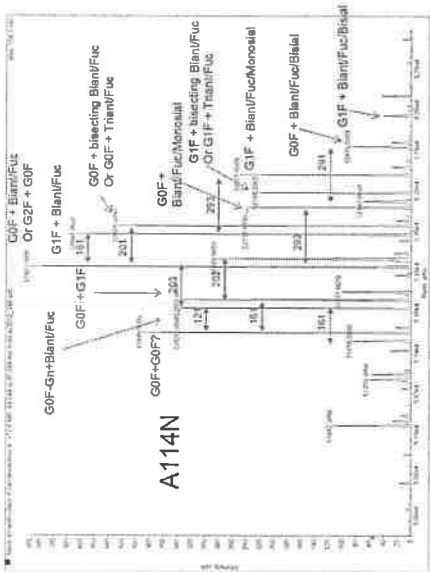
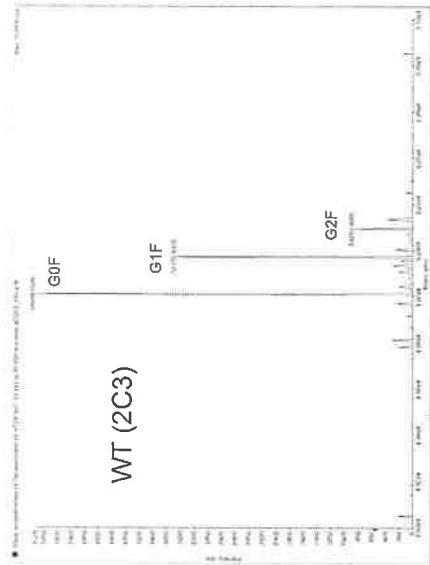


Figure 24

A.

Sample	lot #	Octet Conc ($\mu\text{g/mL}$)
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.

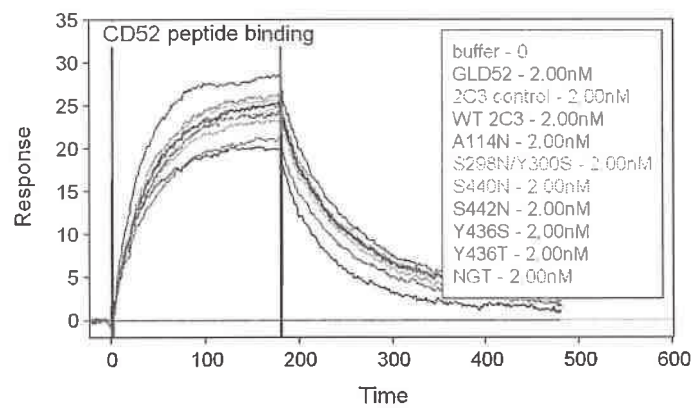


Figure 25

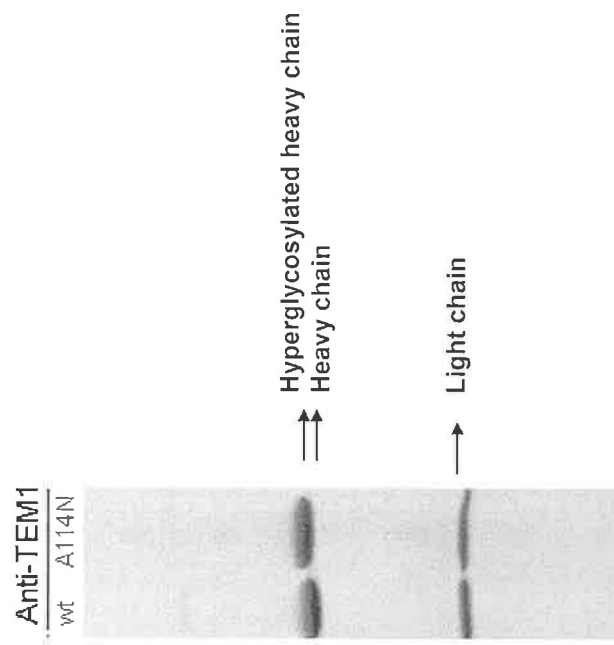


Figure 26

10% Tris-glycine SDS-PAGE

HIC Analysis of Purified A114N Herceptin

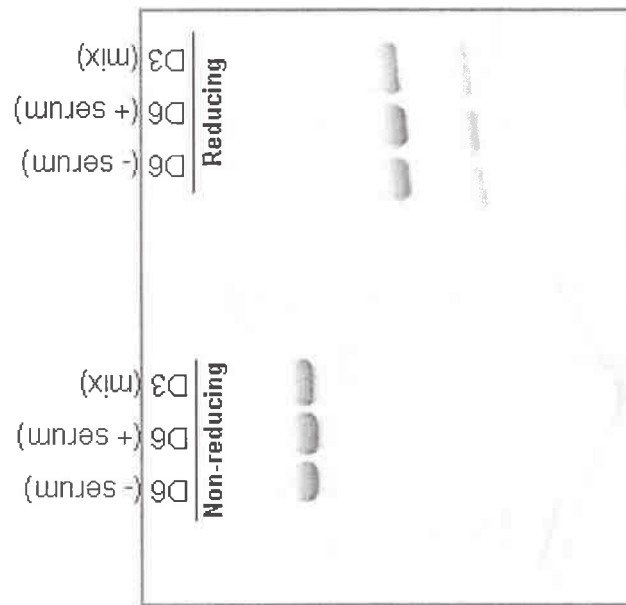
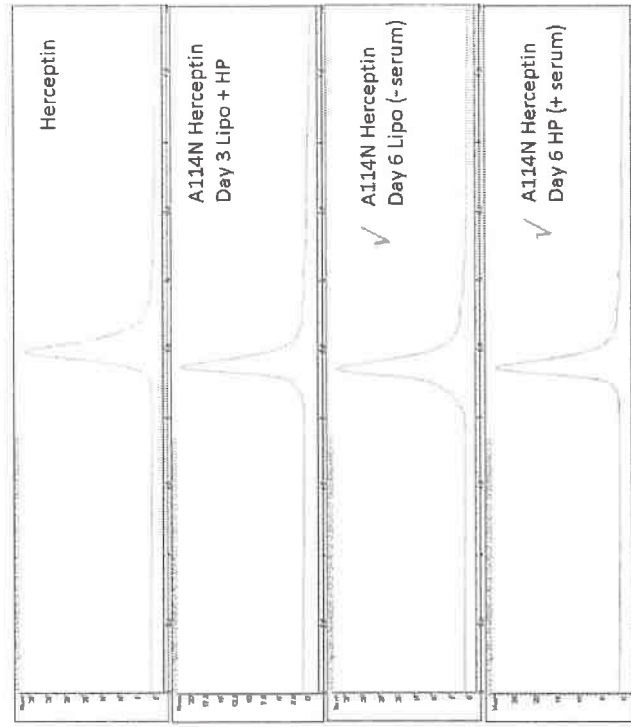


Figure 27

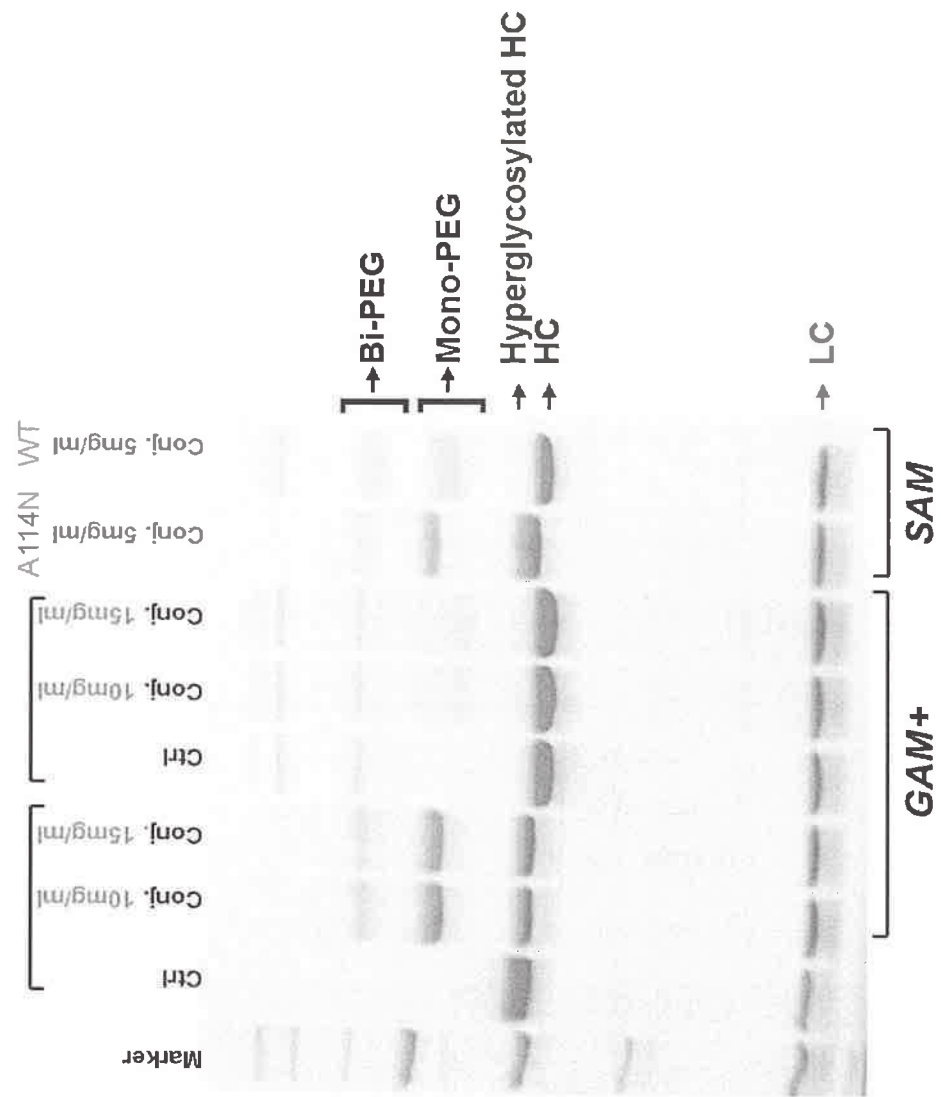


Figure 28

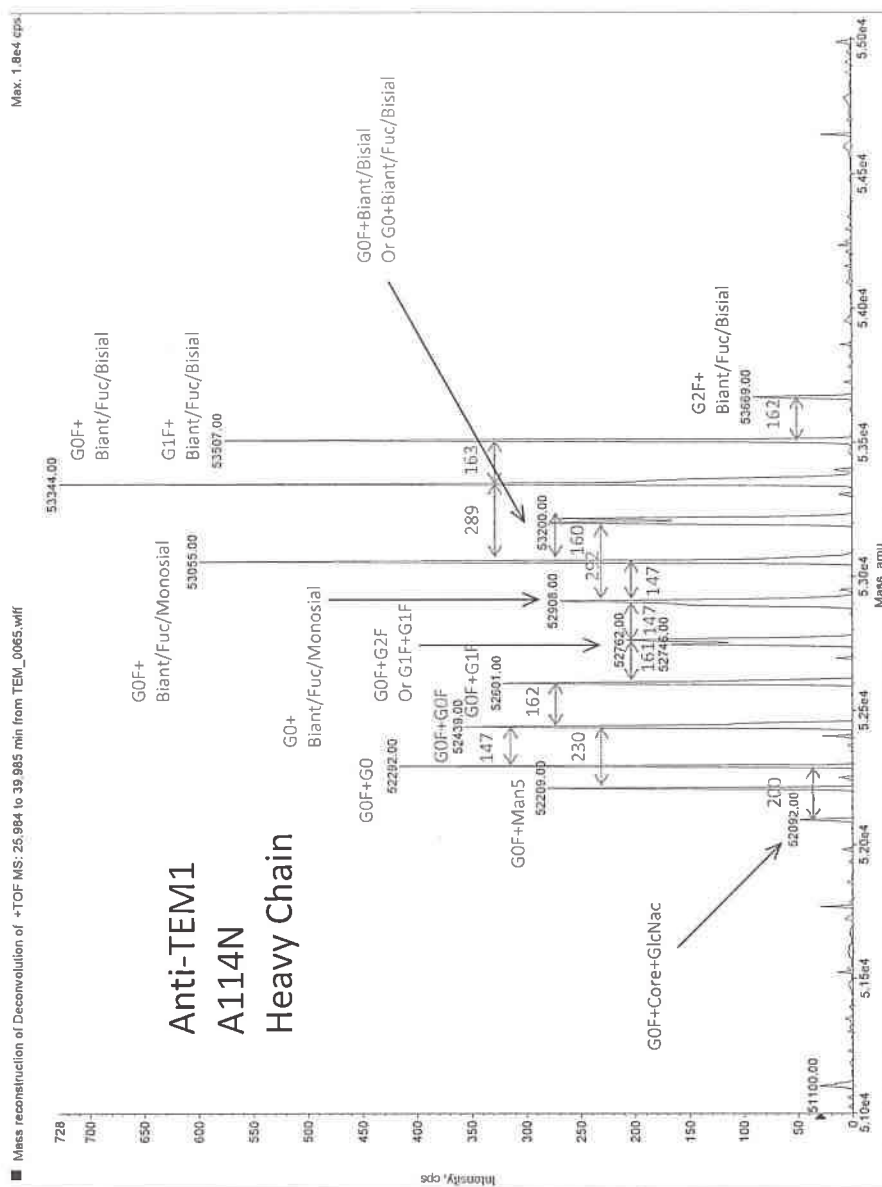


Figure 29

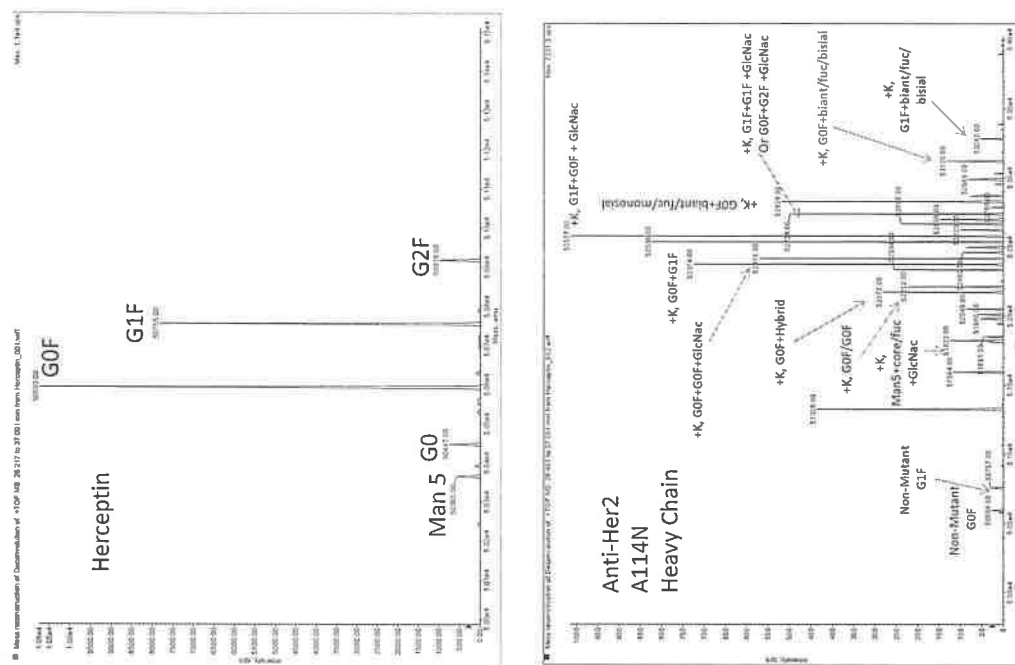


Figure 30

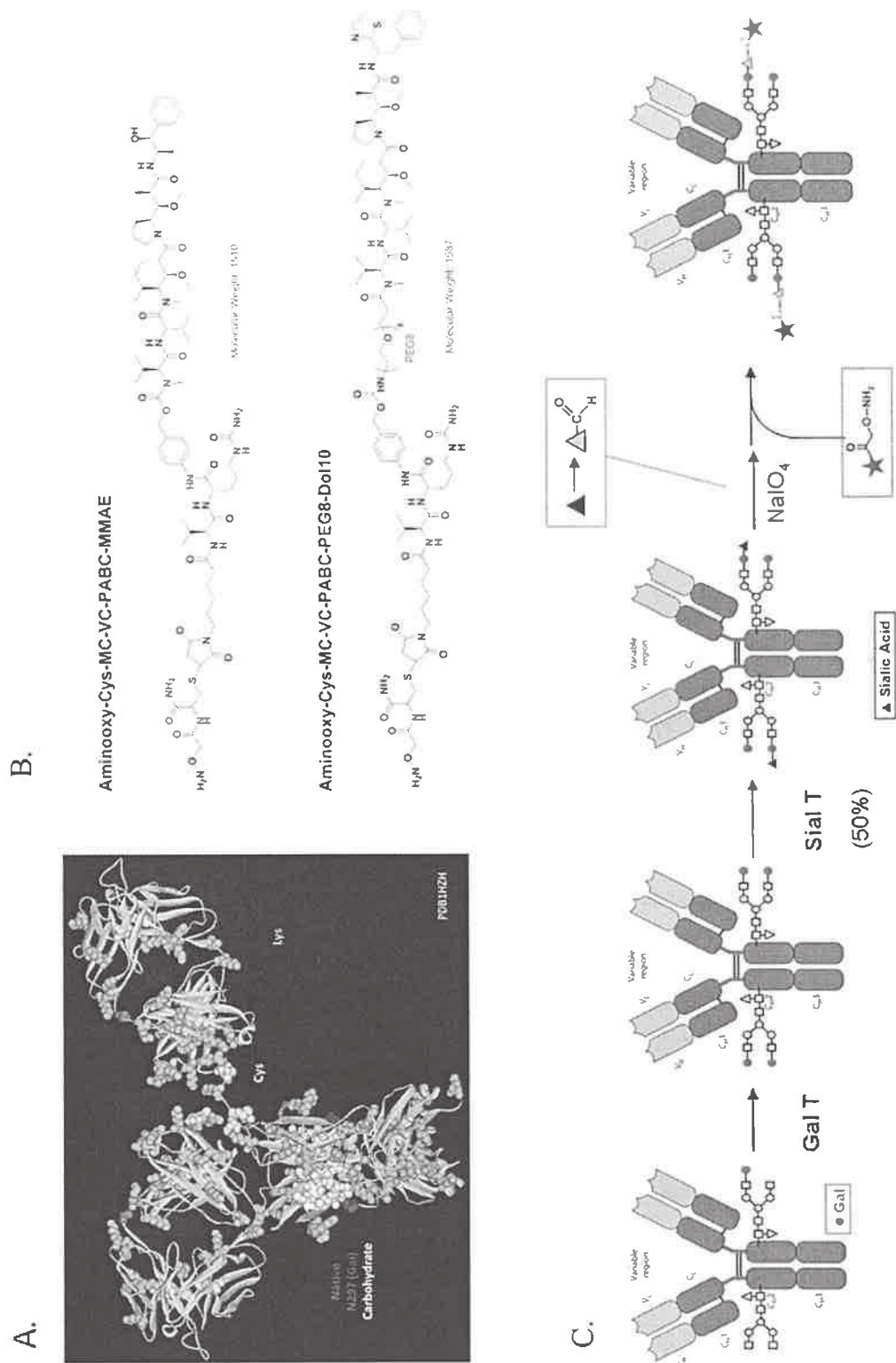


Figure 31

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

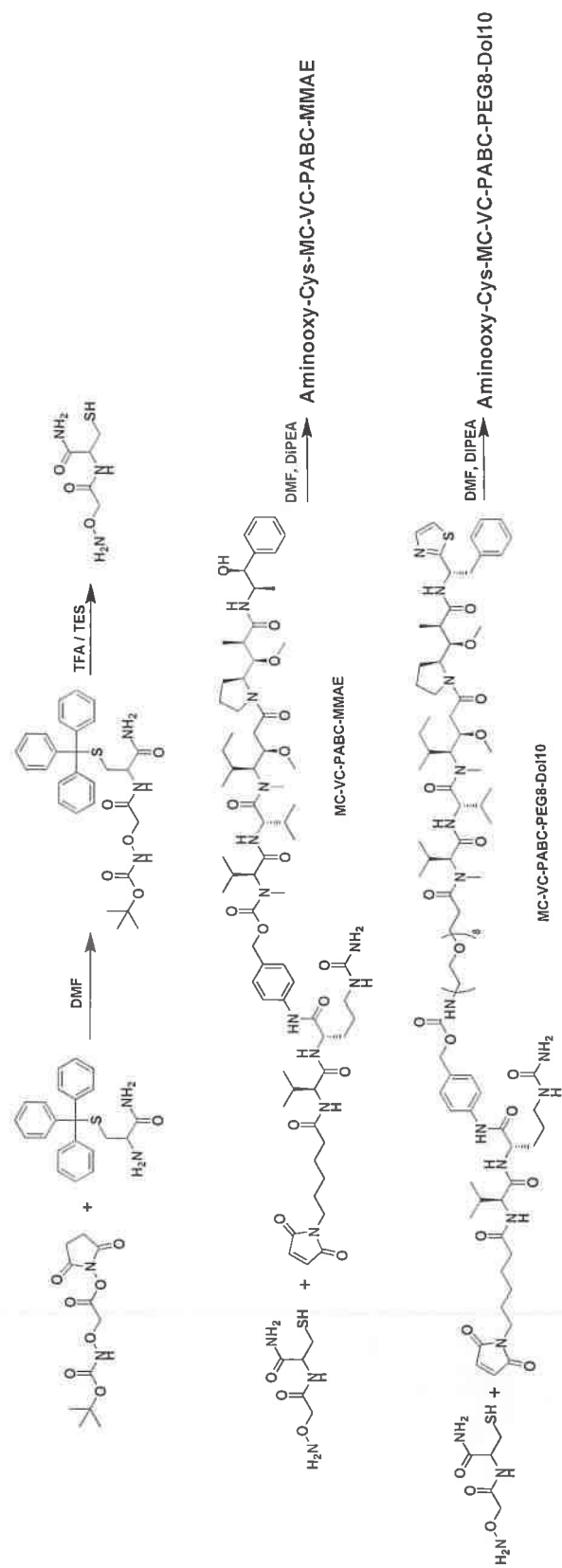


Figure 32

A.



B.

	Sialic acid, mol/mol
Control	0
Sialylated Lot 1	2.2 ± 0
Sialylated Lot 2	2.3 ± 0.1

C.

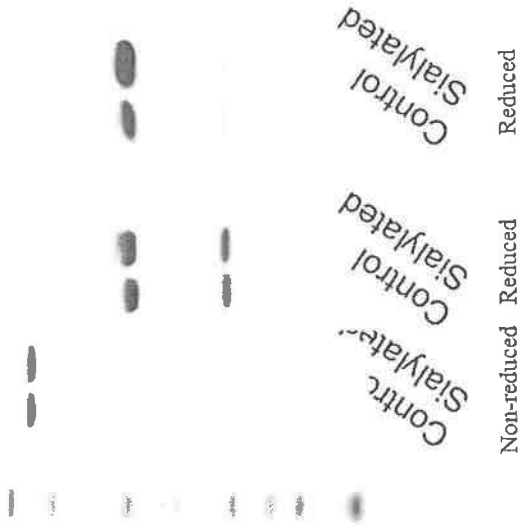


Figure 33

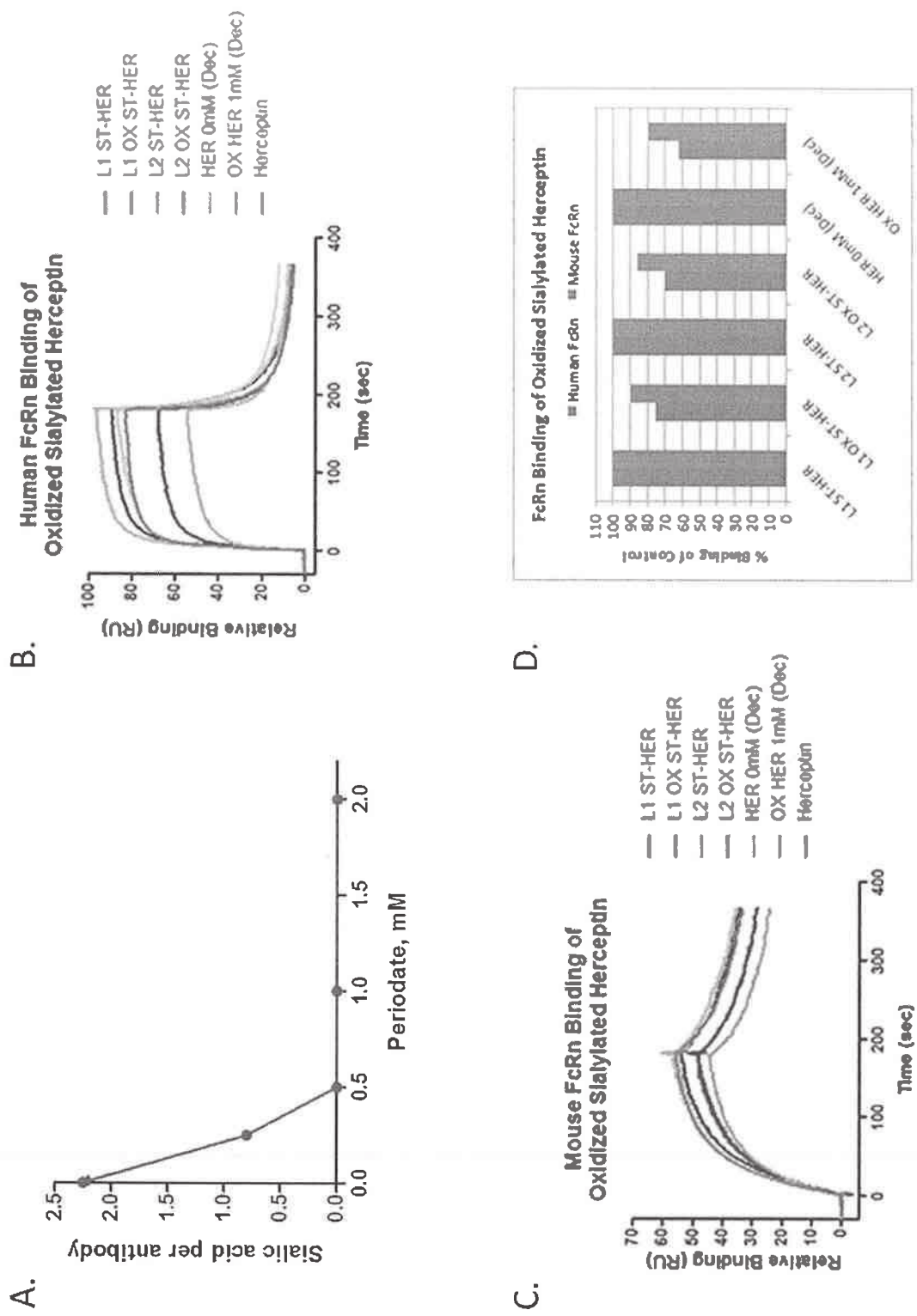


Figure 34

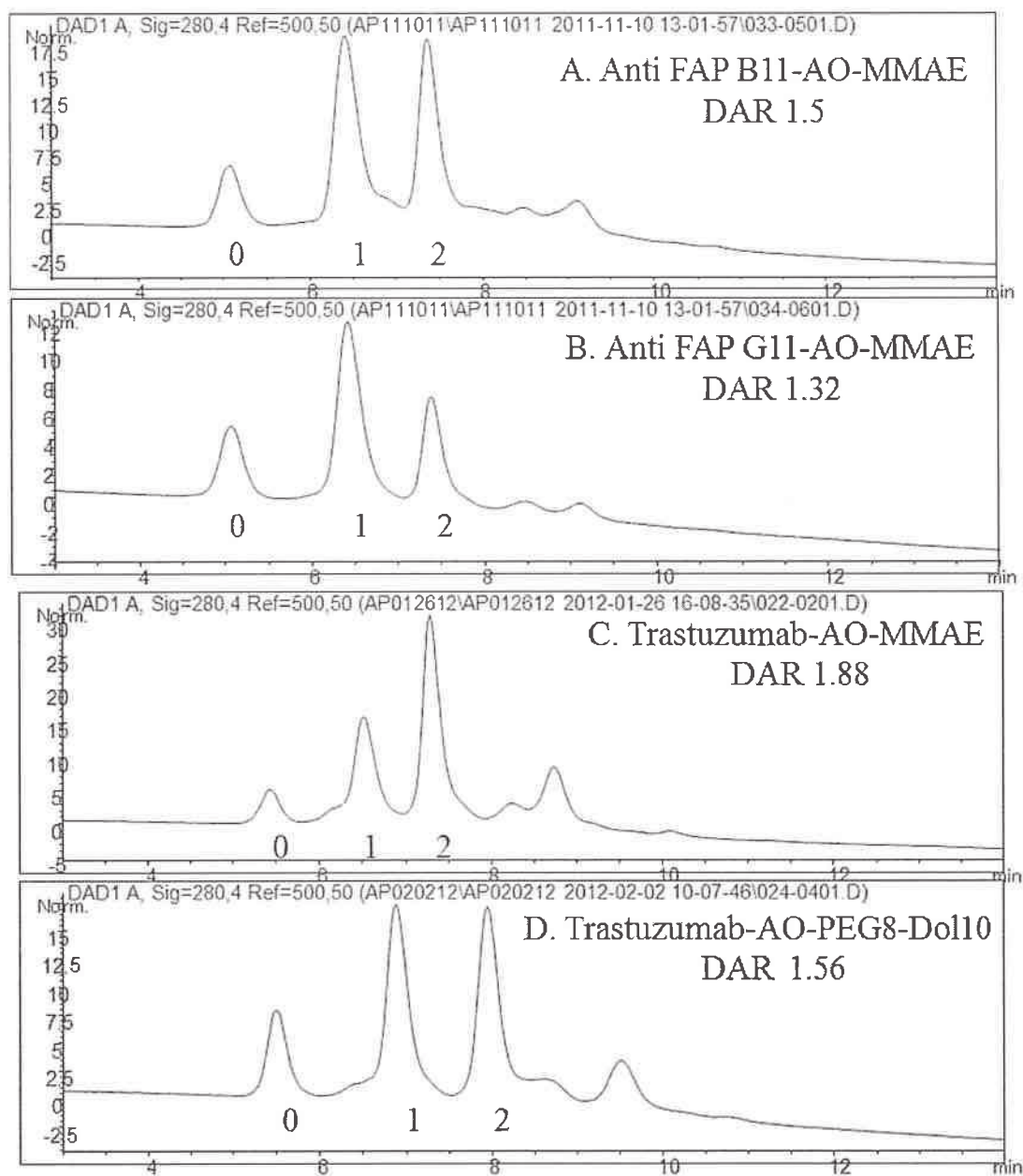
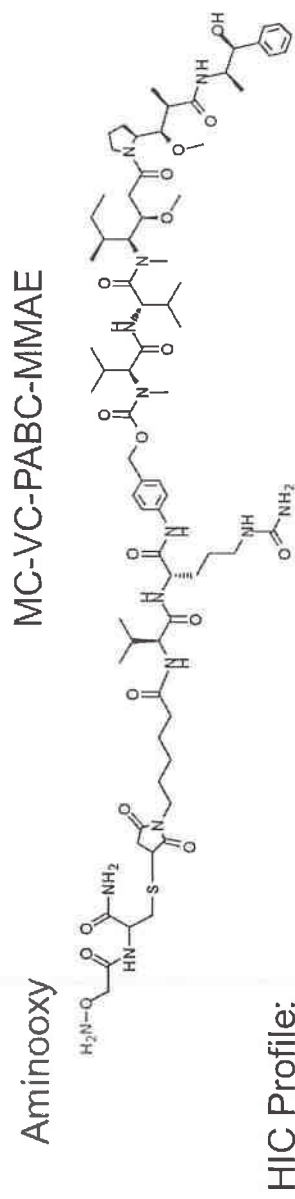
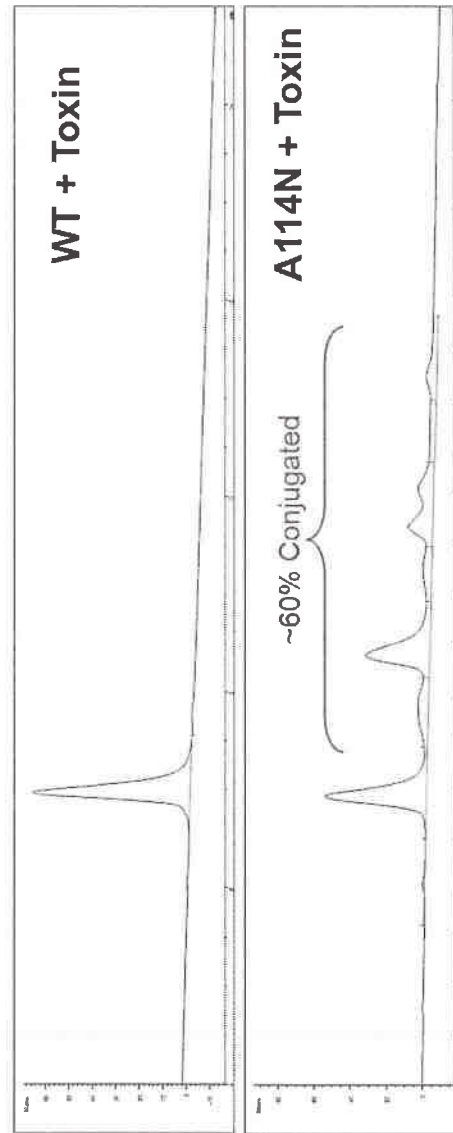


Figure 35



HIC Profile:



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

Figure 36

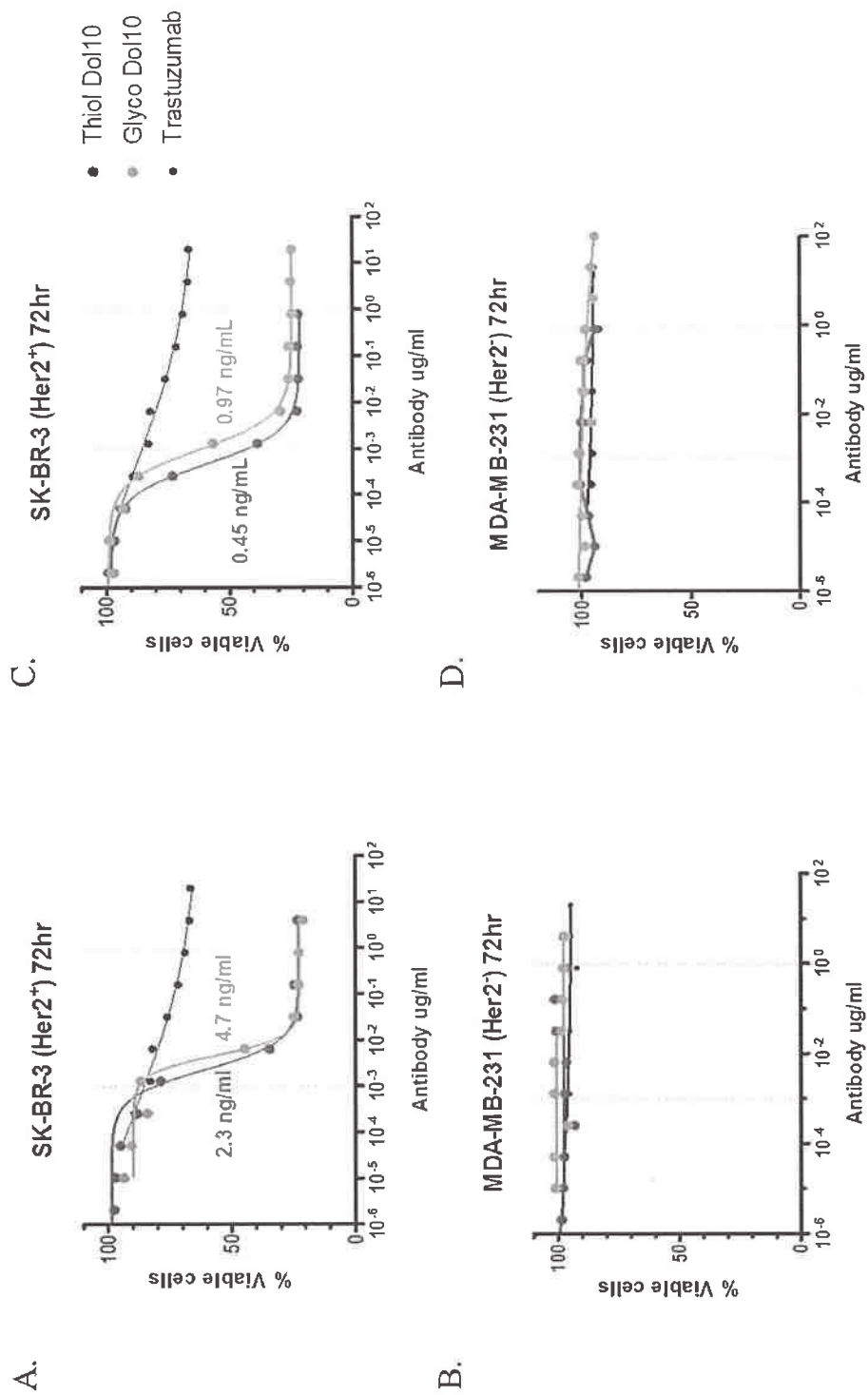


Figure 37

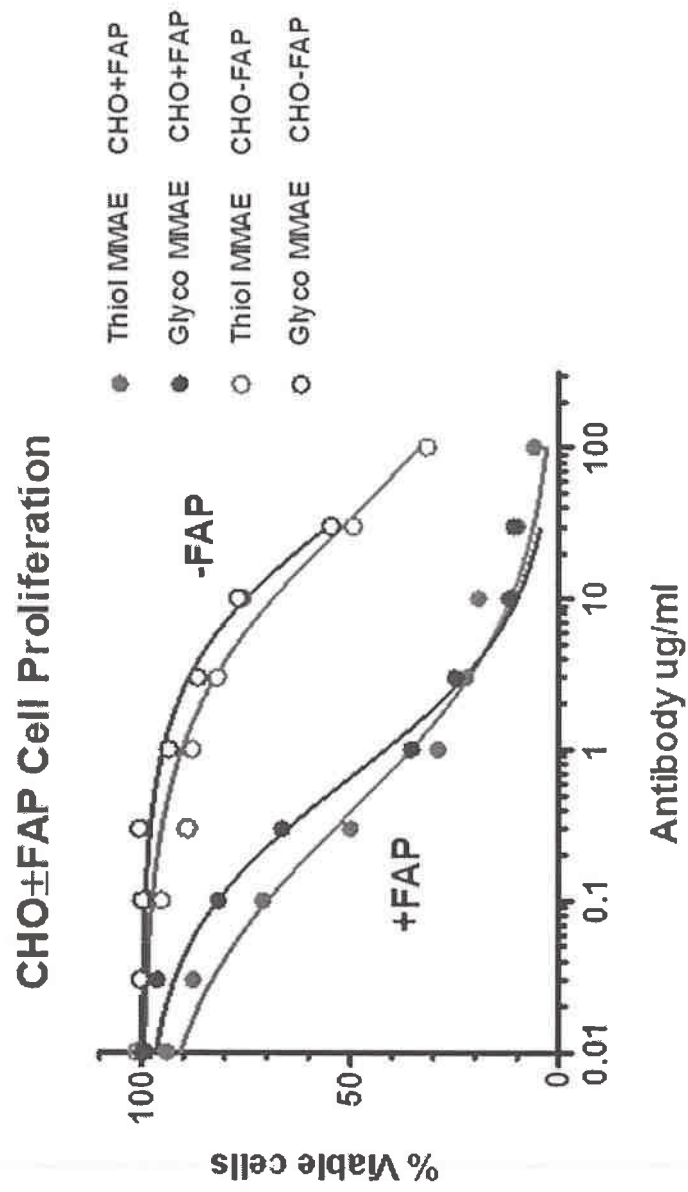
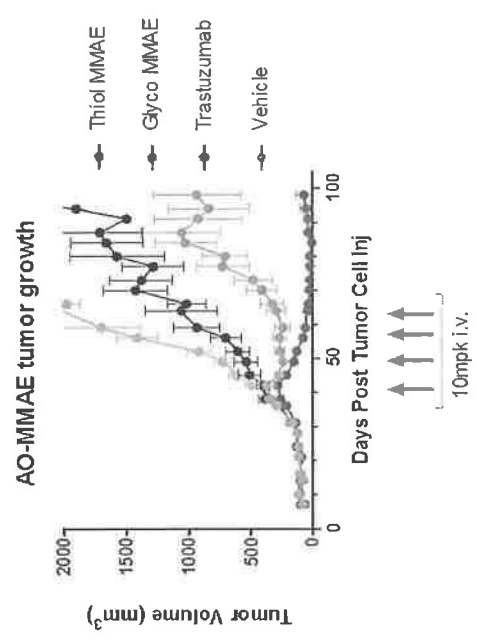
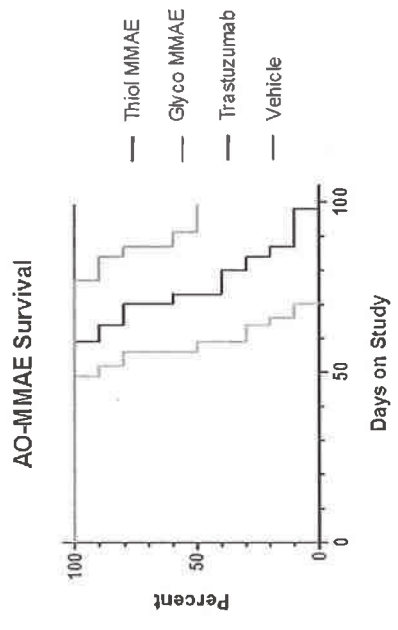


Figure 38

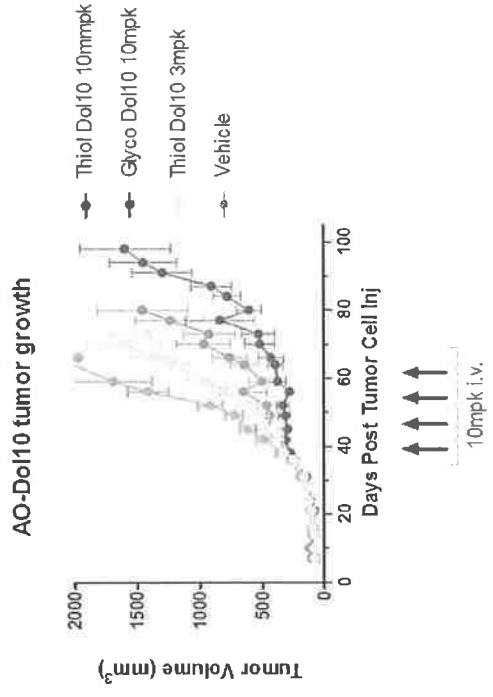
A.



B.



C.



D.

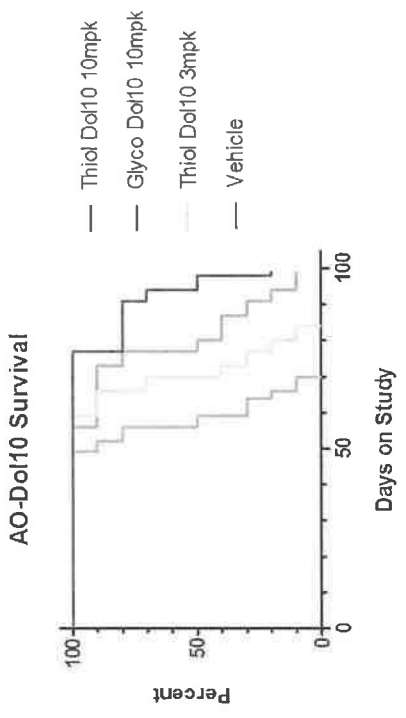


Figure 39

LC/MS of S298N/T299A/Y300S (NNAS) heavy chain

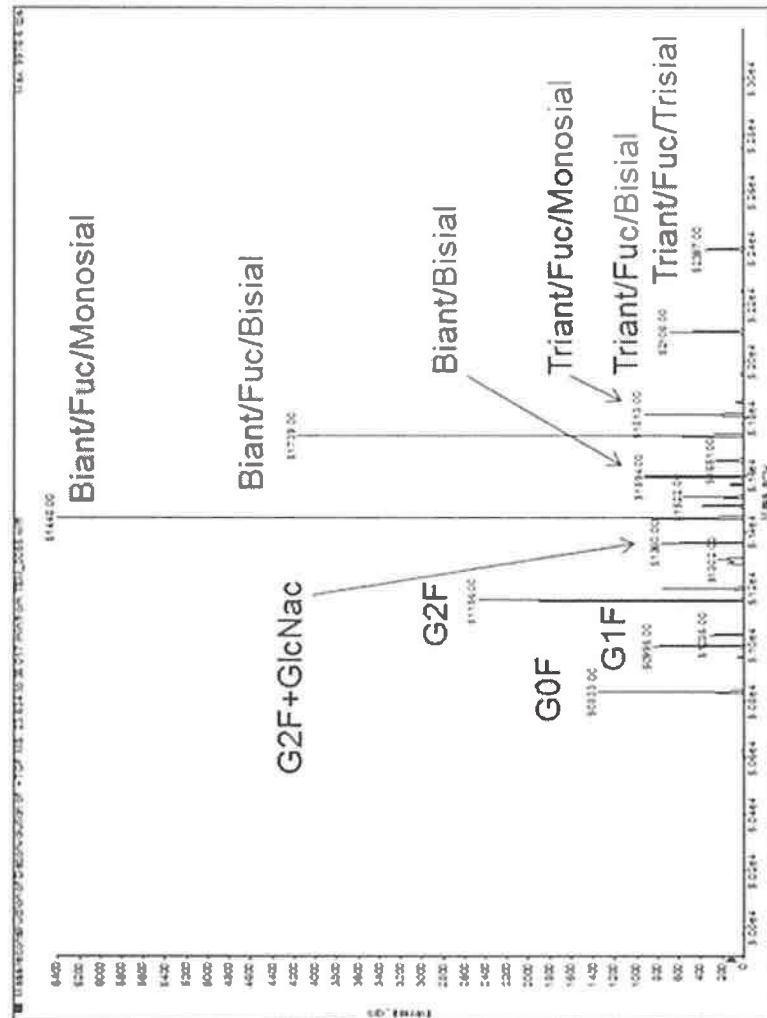


Figure 40

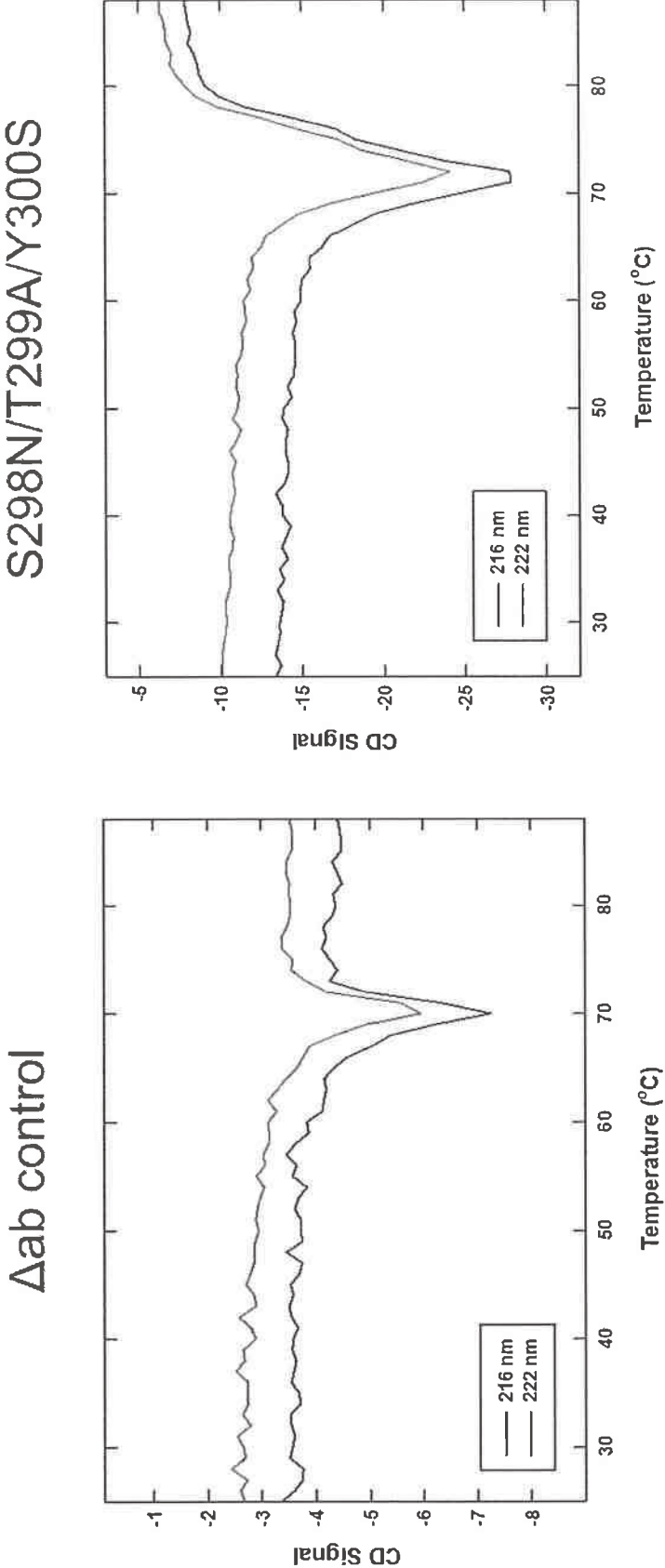


Figure 41

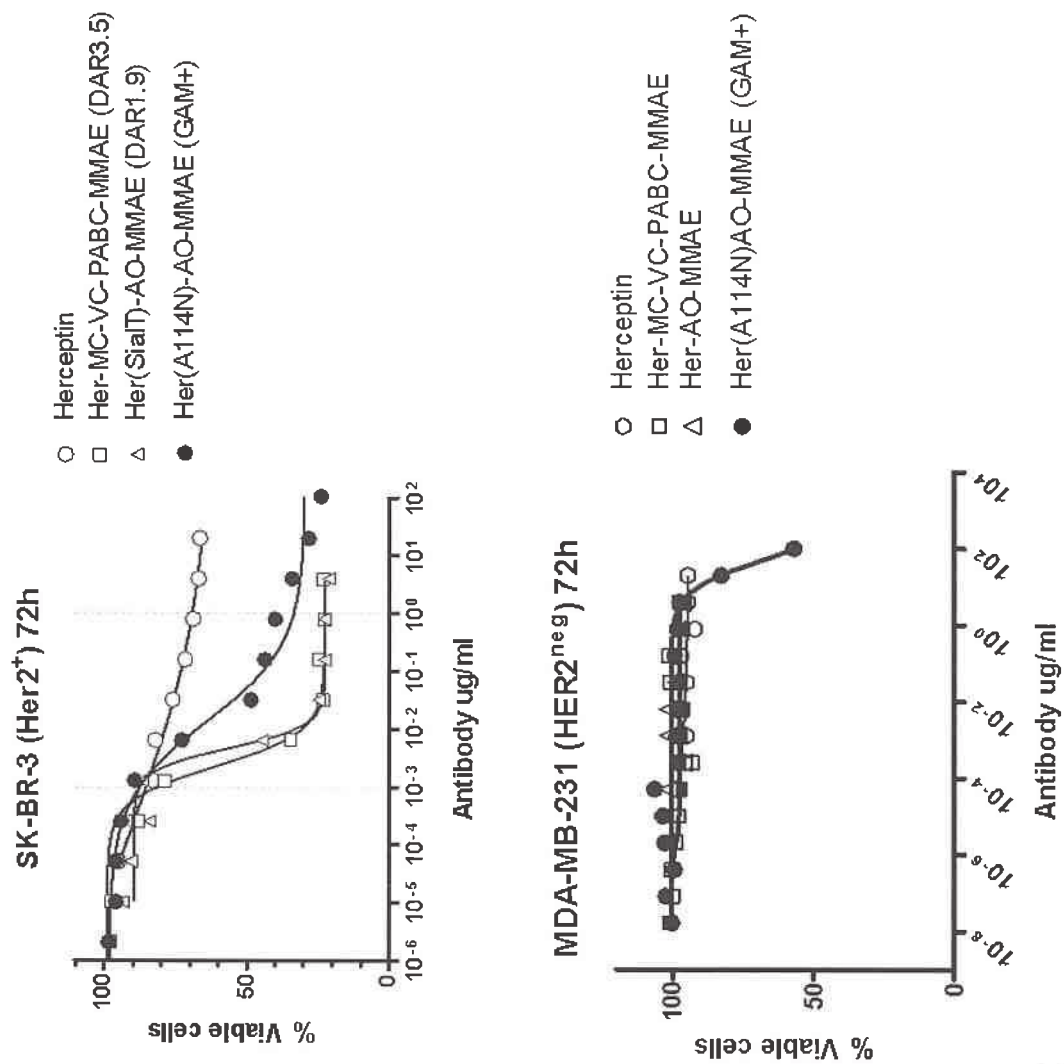


Figure 42

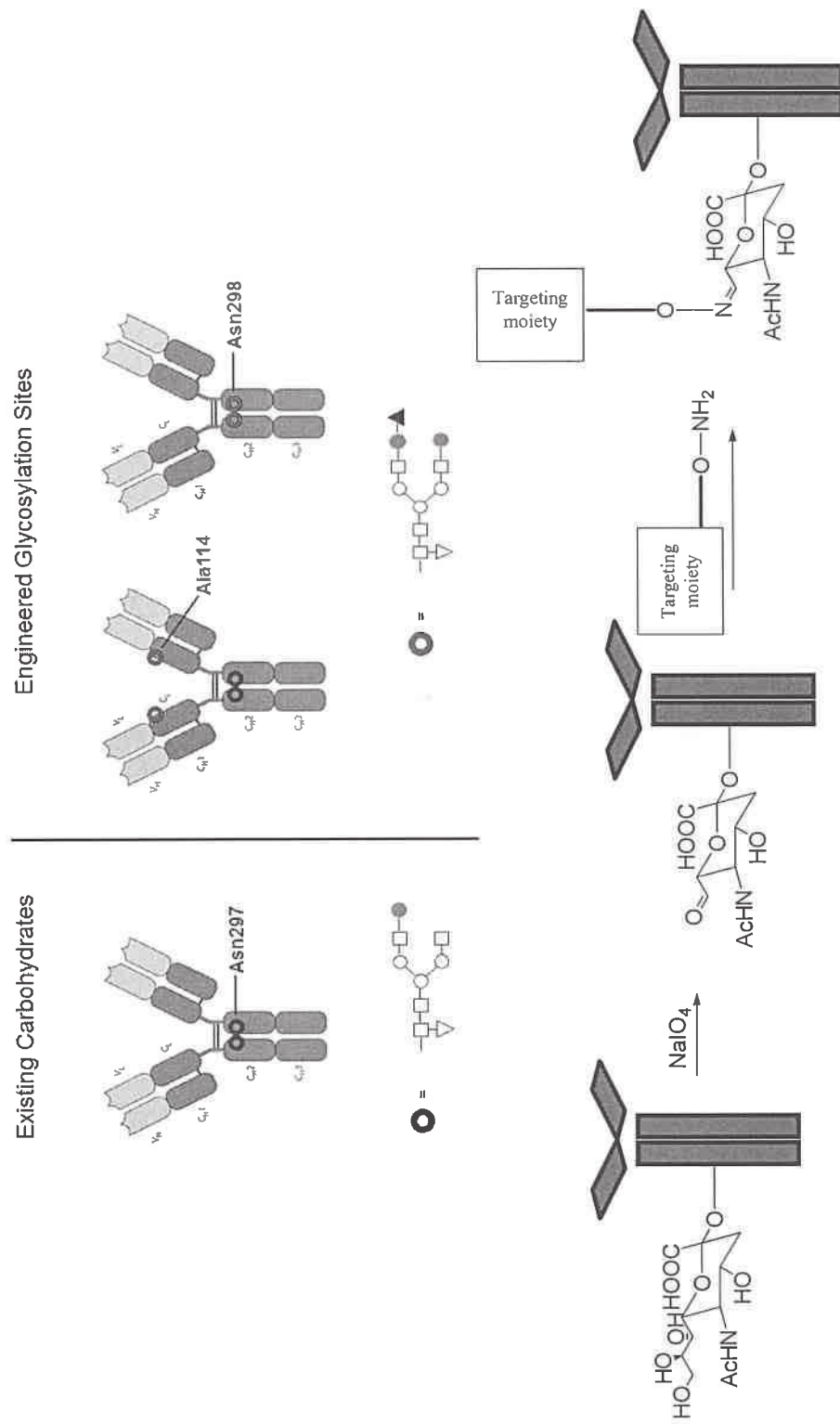


Figure 43

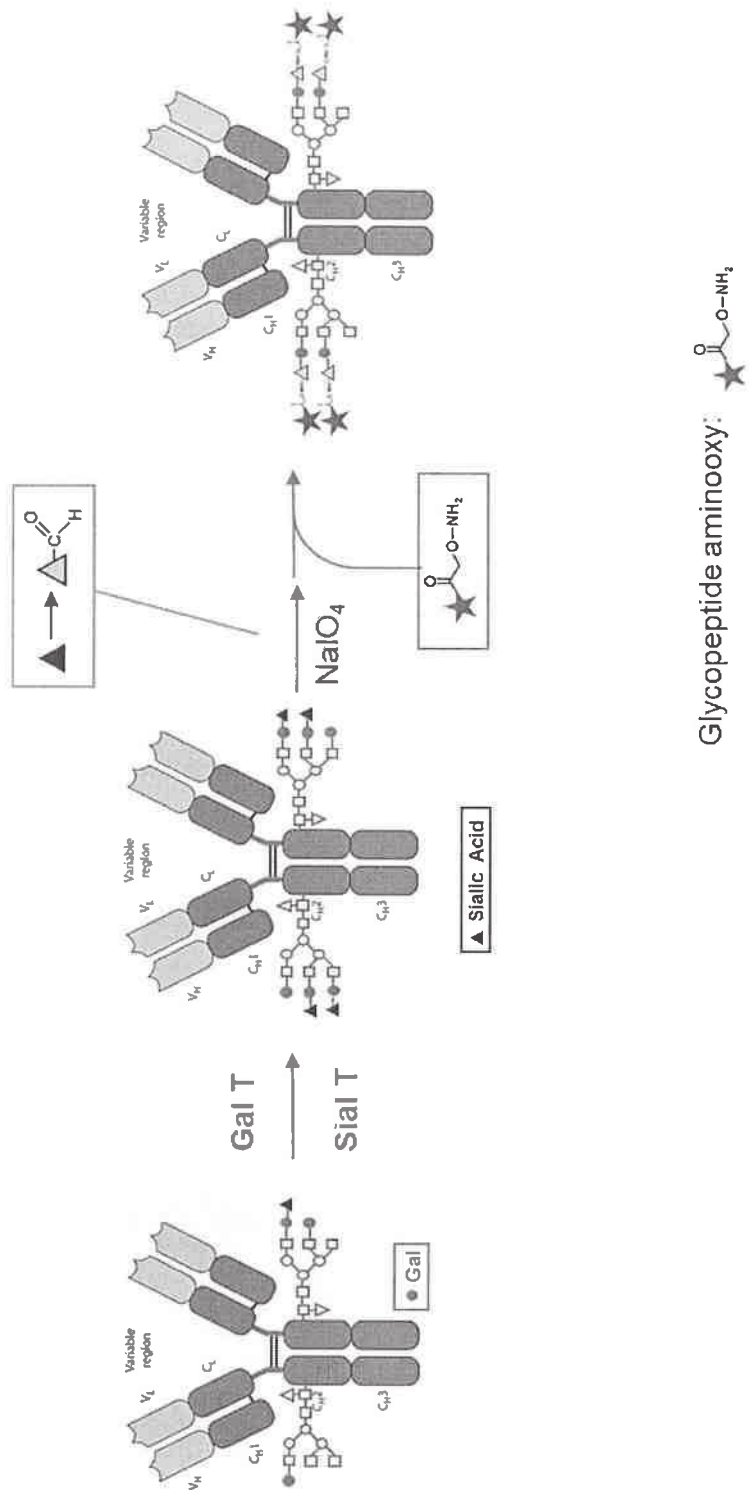


Figure 44

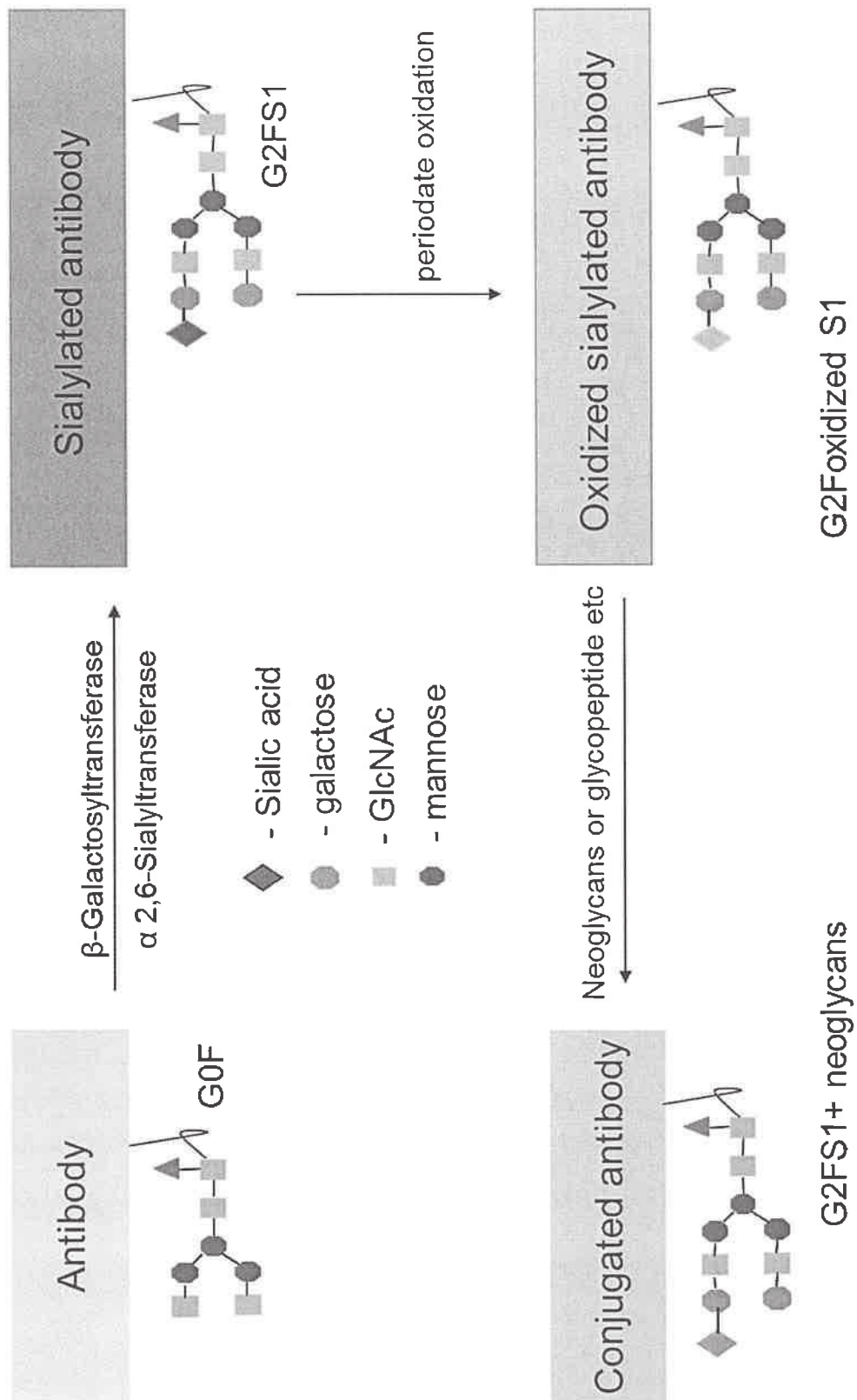
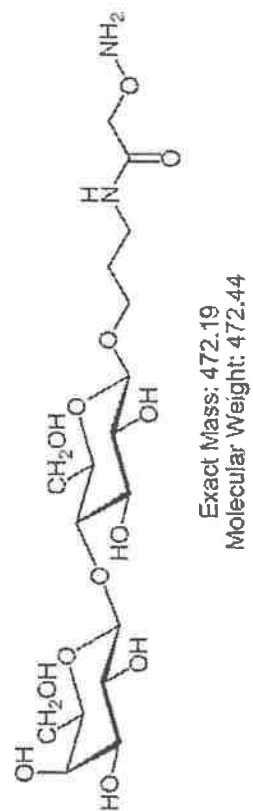


Figure 45

Lactose aminoxy for native glycans (SAM or GAM):



bisM6P hexamannose aminoxy
(SAM or GAM):

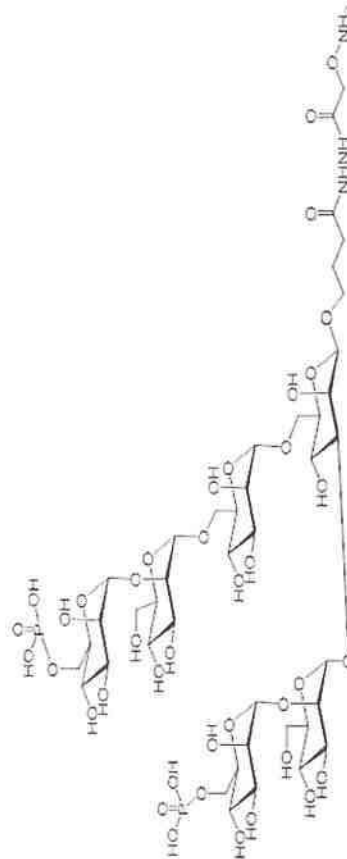
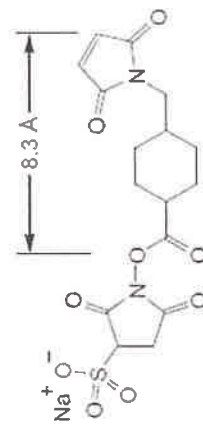
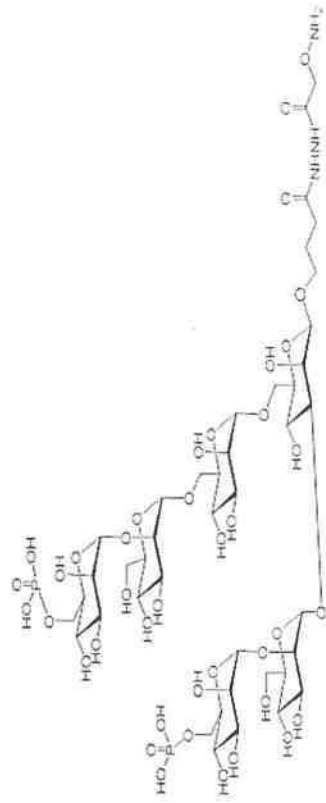
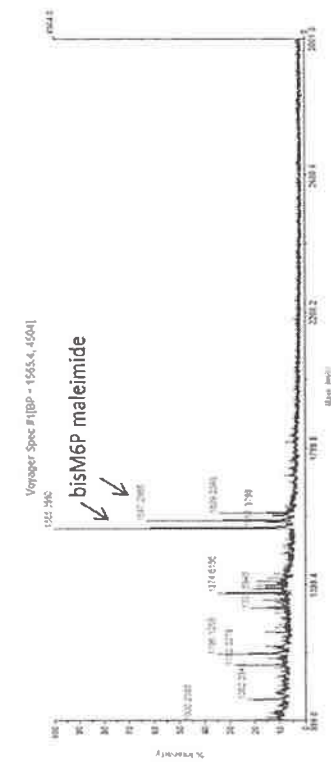


Figure 46

bisM6P hexamannose aminoxy
(SAM on Fc glycans):



Sulfo SMCC



bisM6P hexamannose maleimide
(Targeting hinge cysteines):

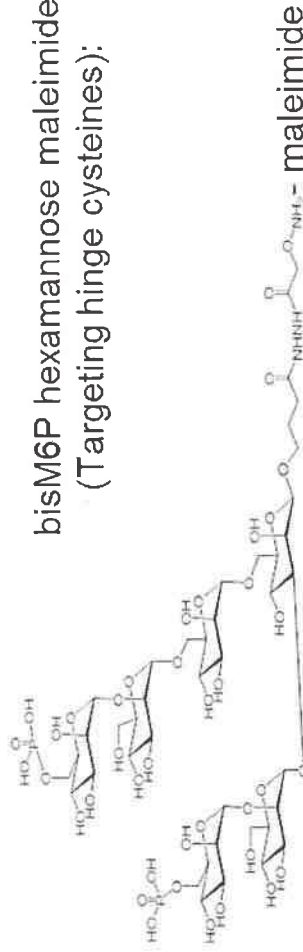


Figure 47

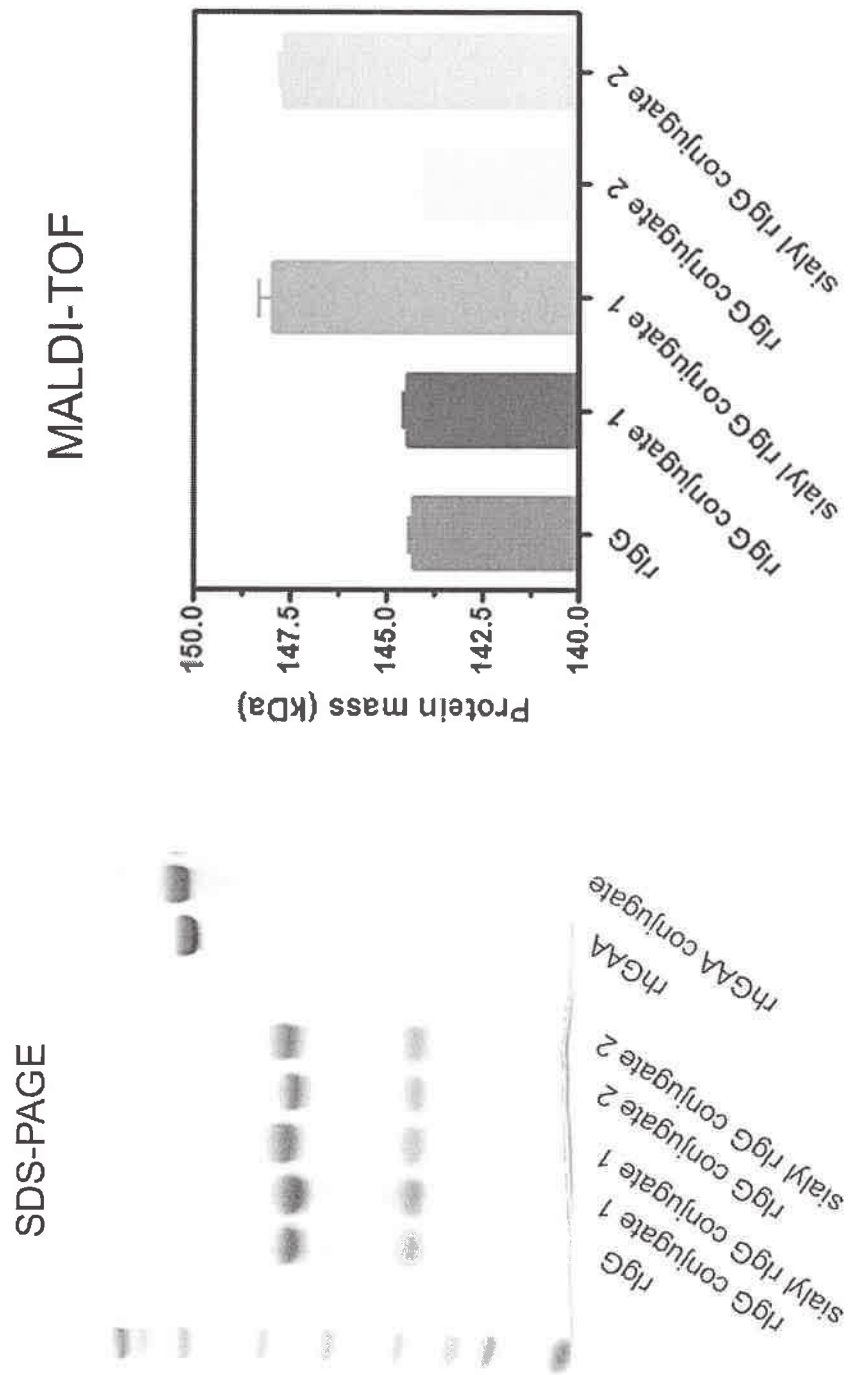


Figure 48

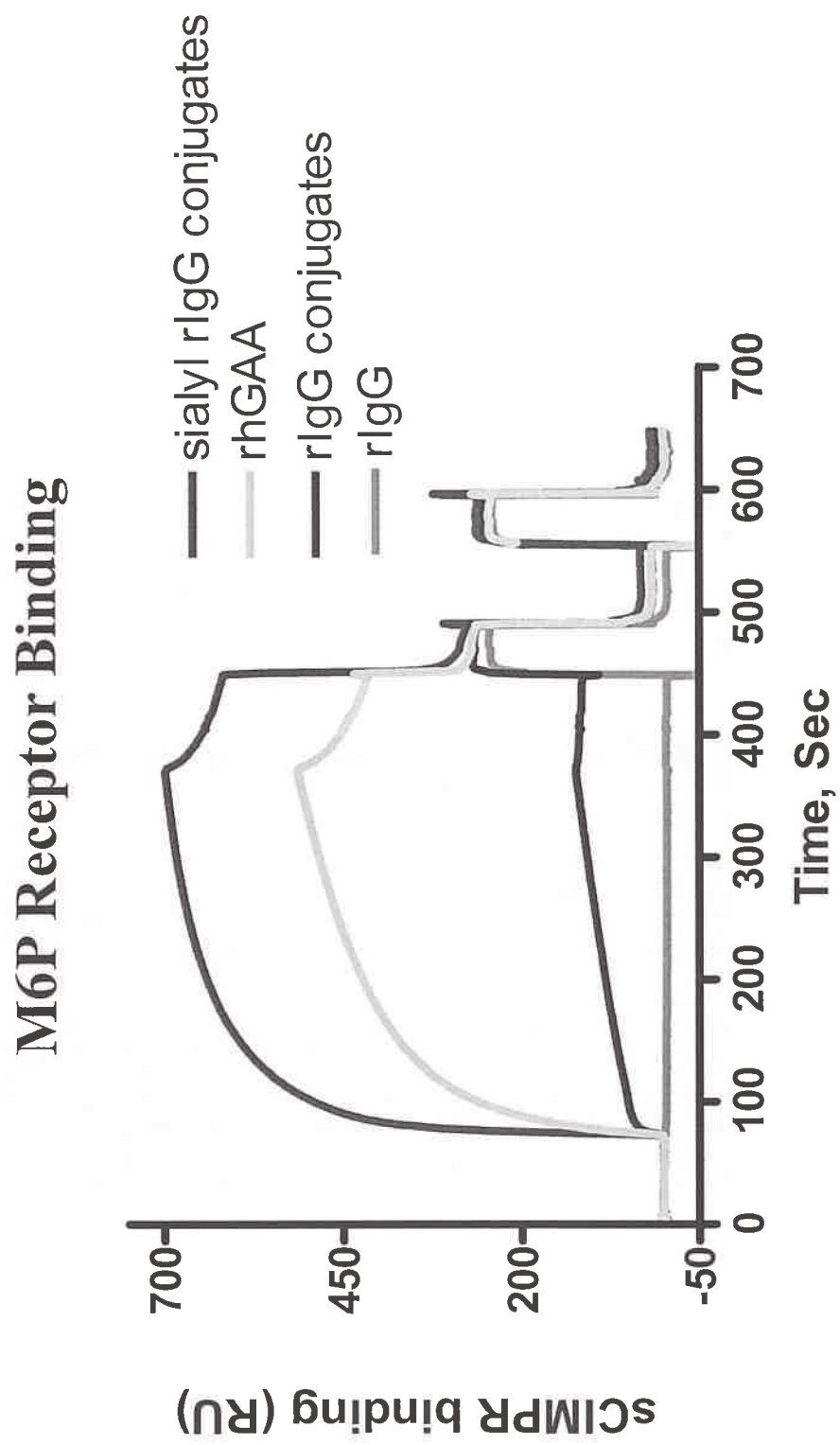


Figure 49

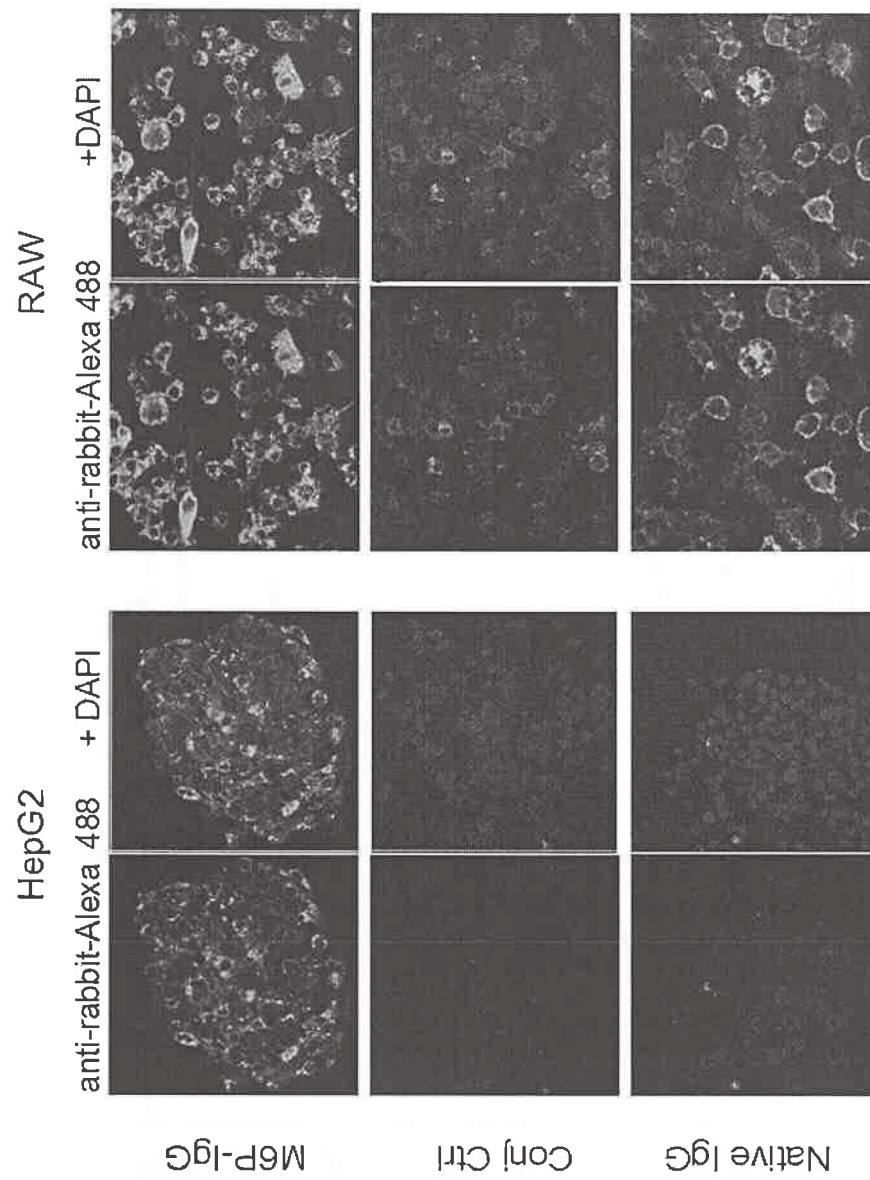


Figure 50

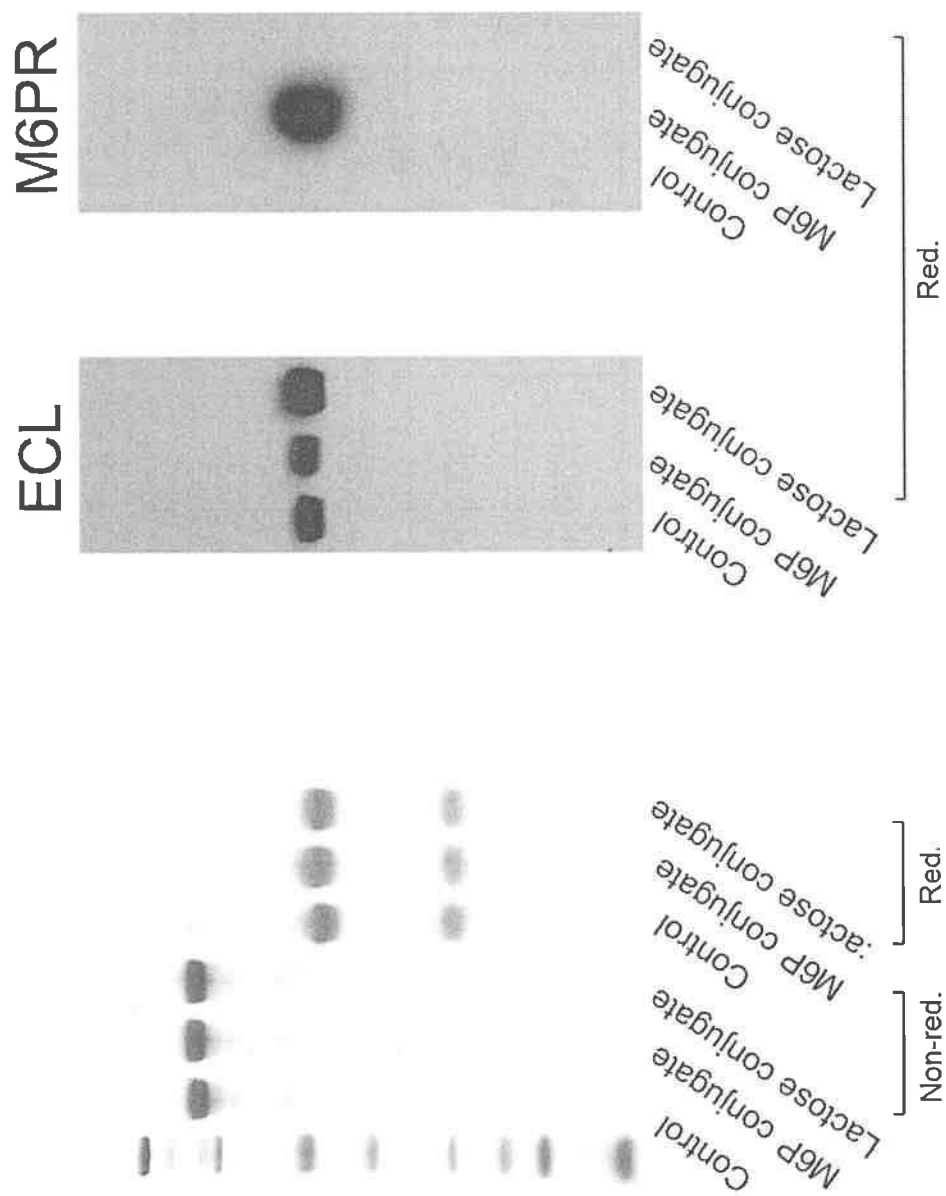


Figure 51

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

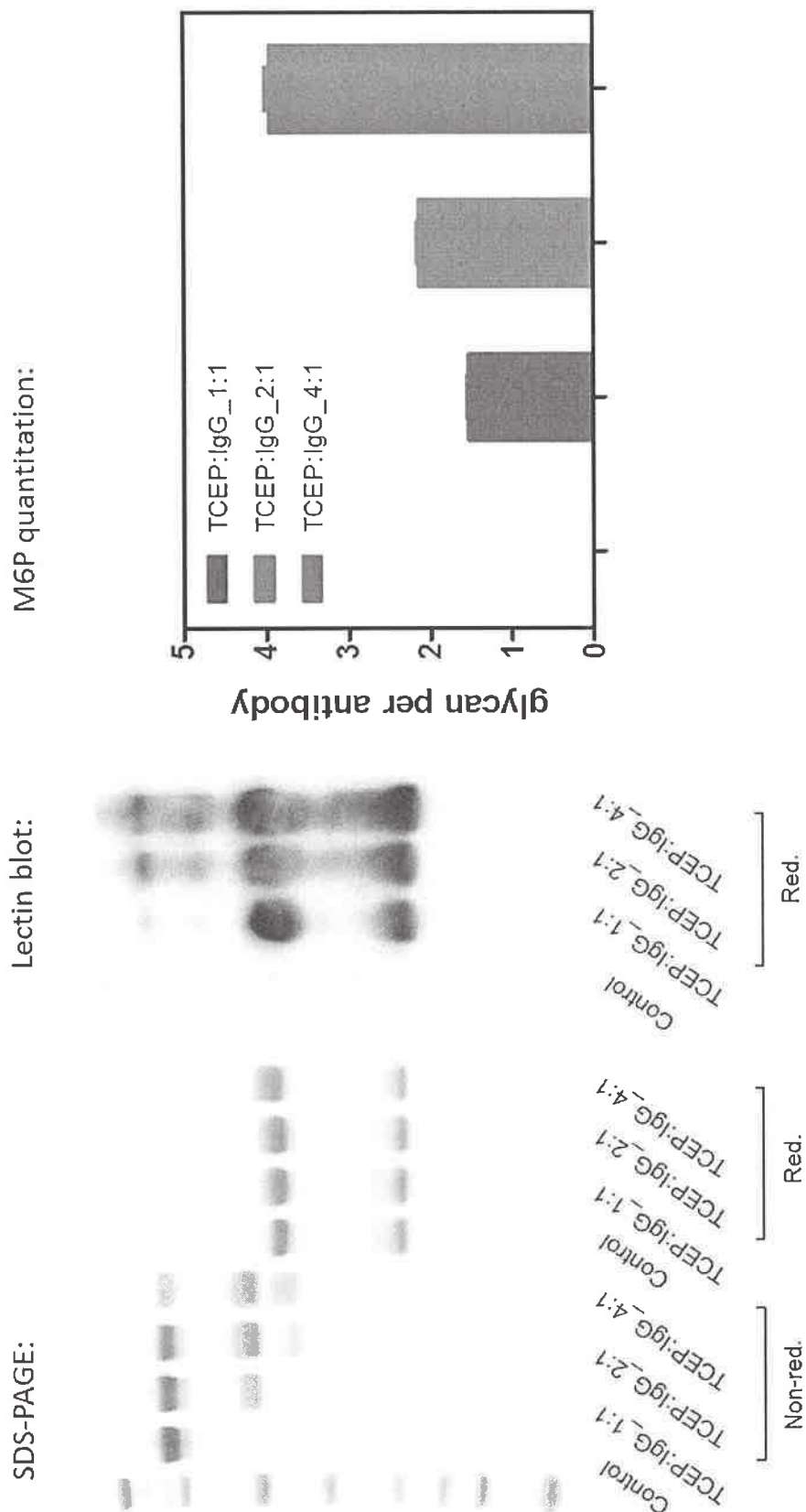


Figure 53

SDS-PAGE:



Galactose quantitation:

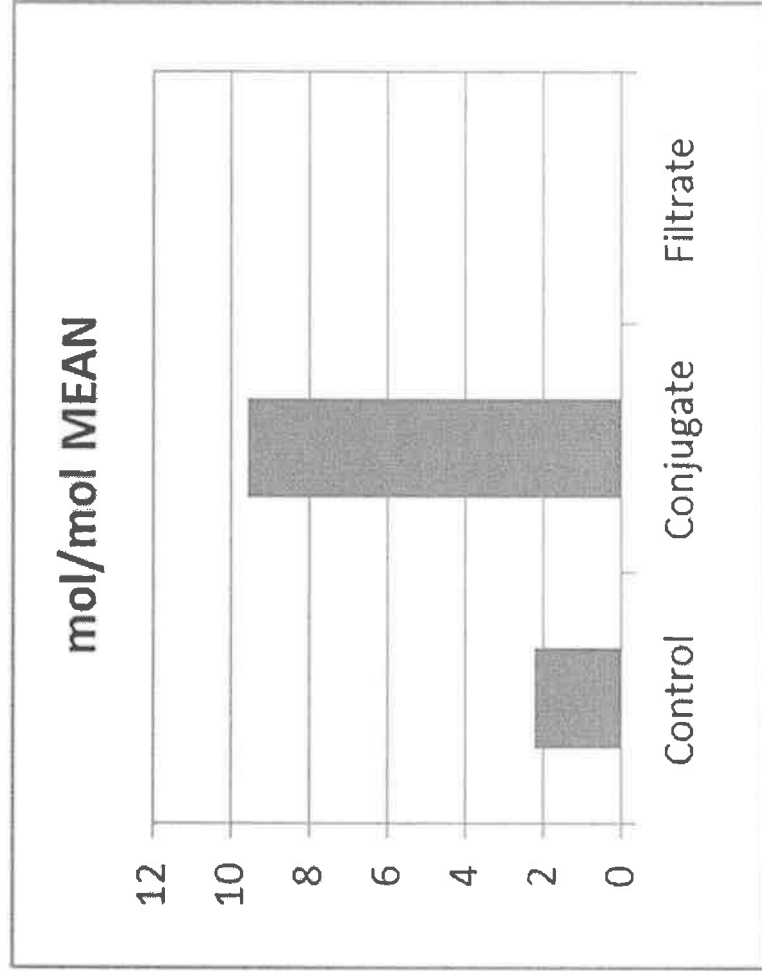


Figure 54

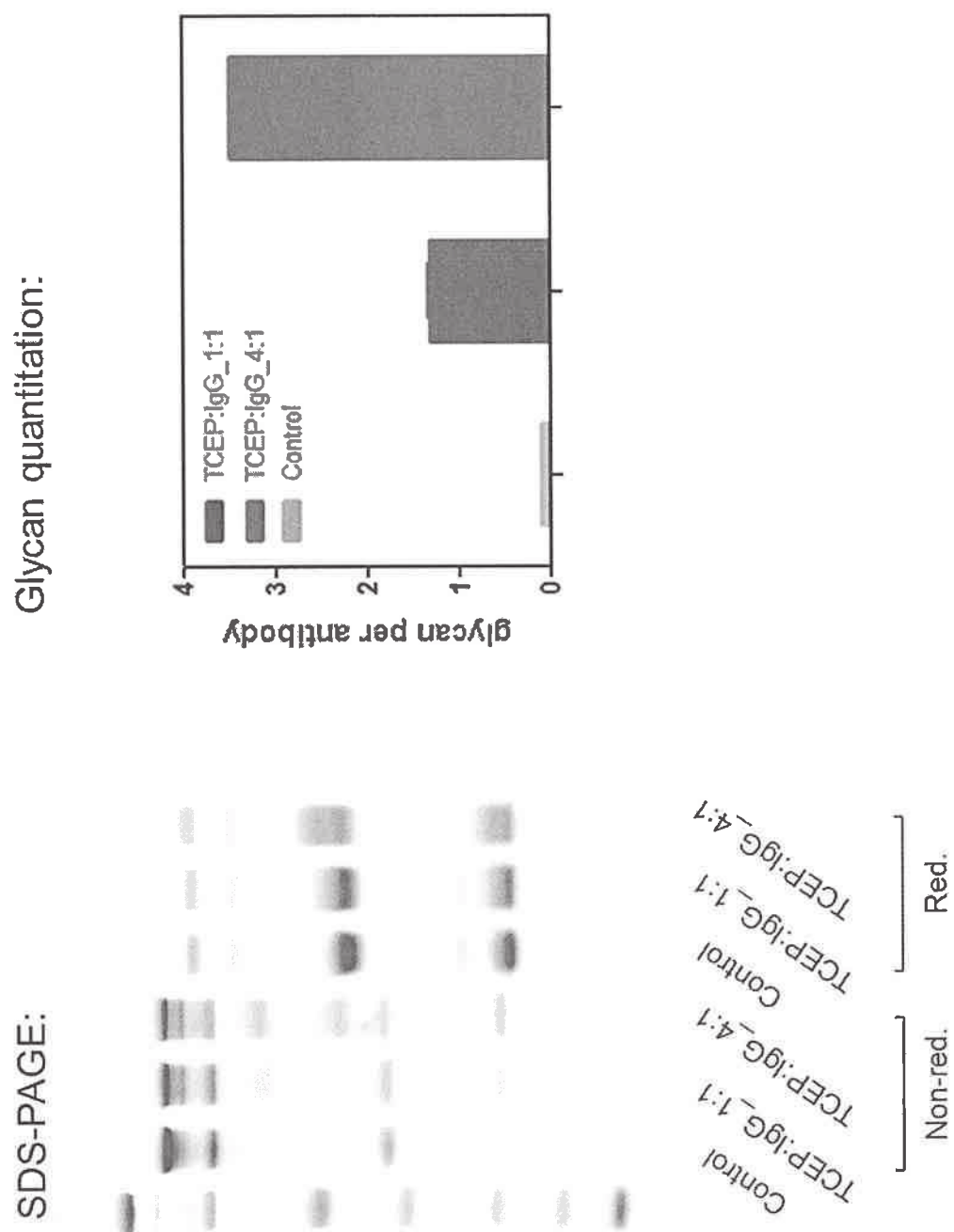
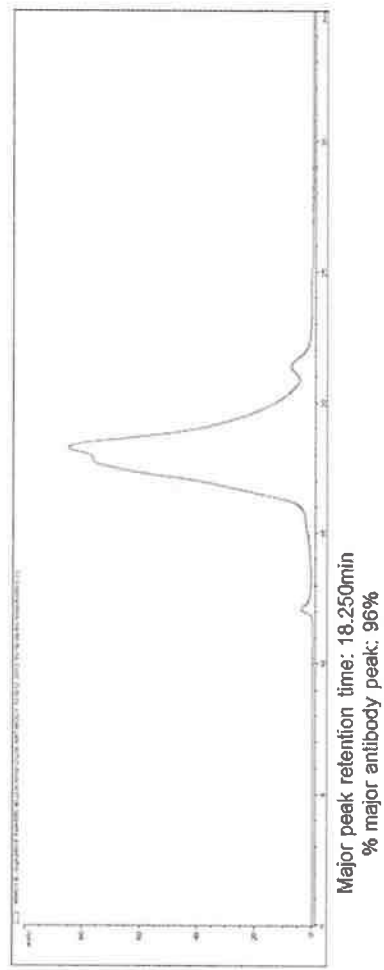


Figure 55

Control:



Lactose maleimide conjugate:

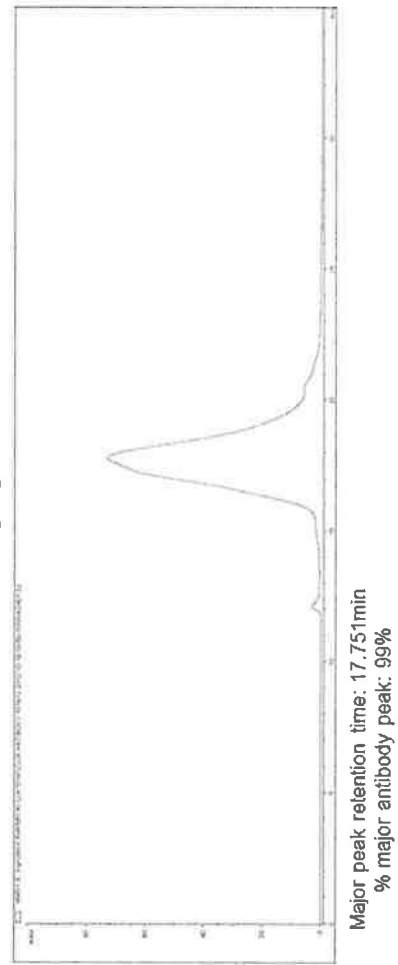


Figure 56

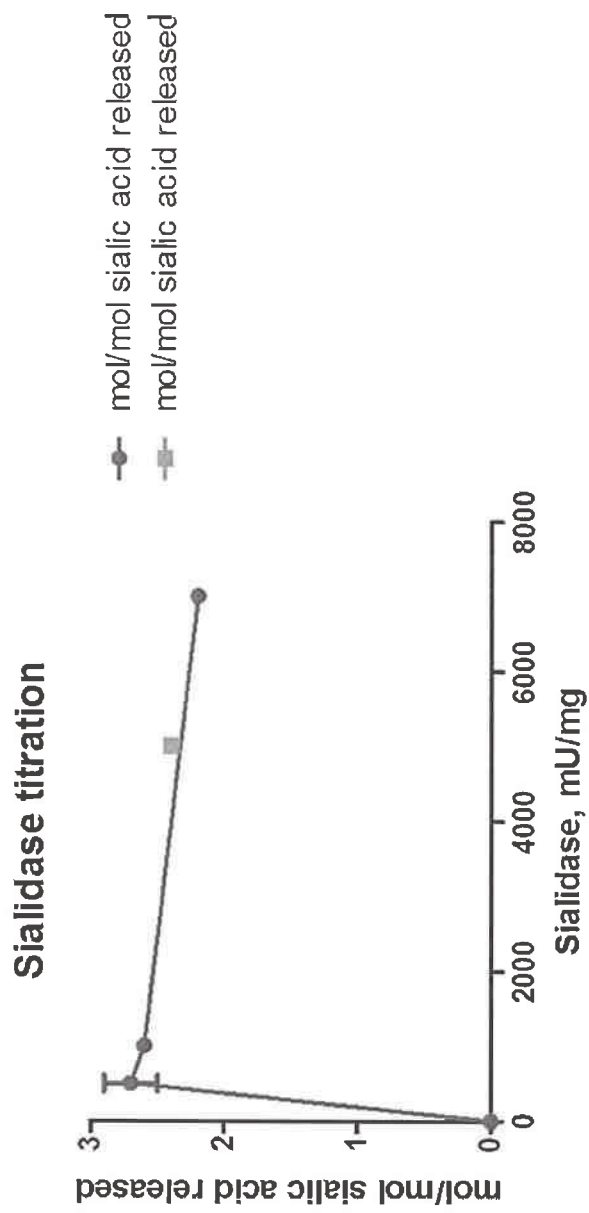
Control:



M6P conjugate:



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

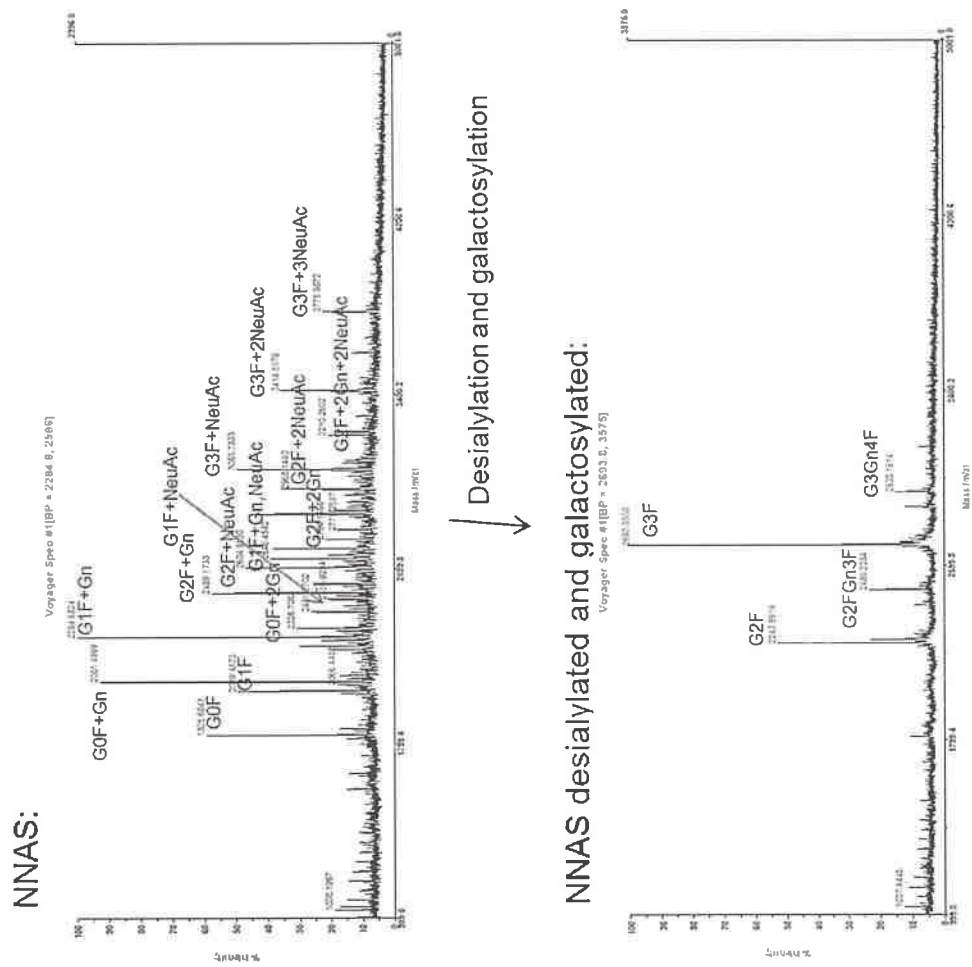


Figure 59

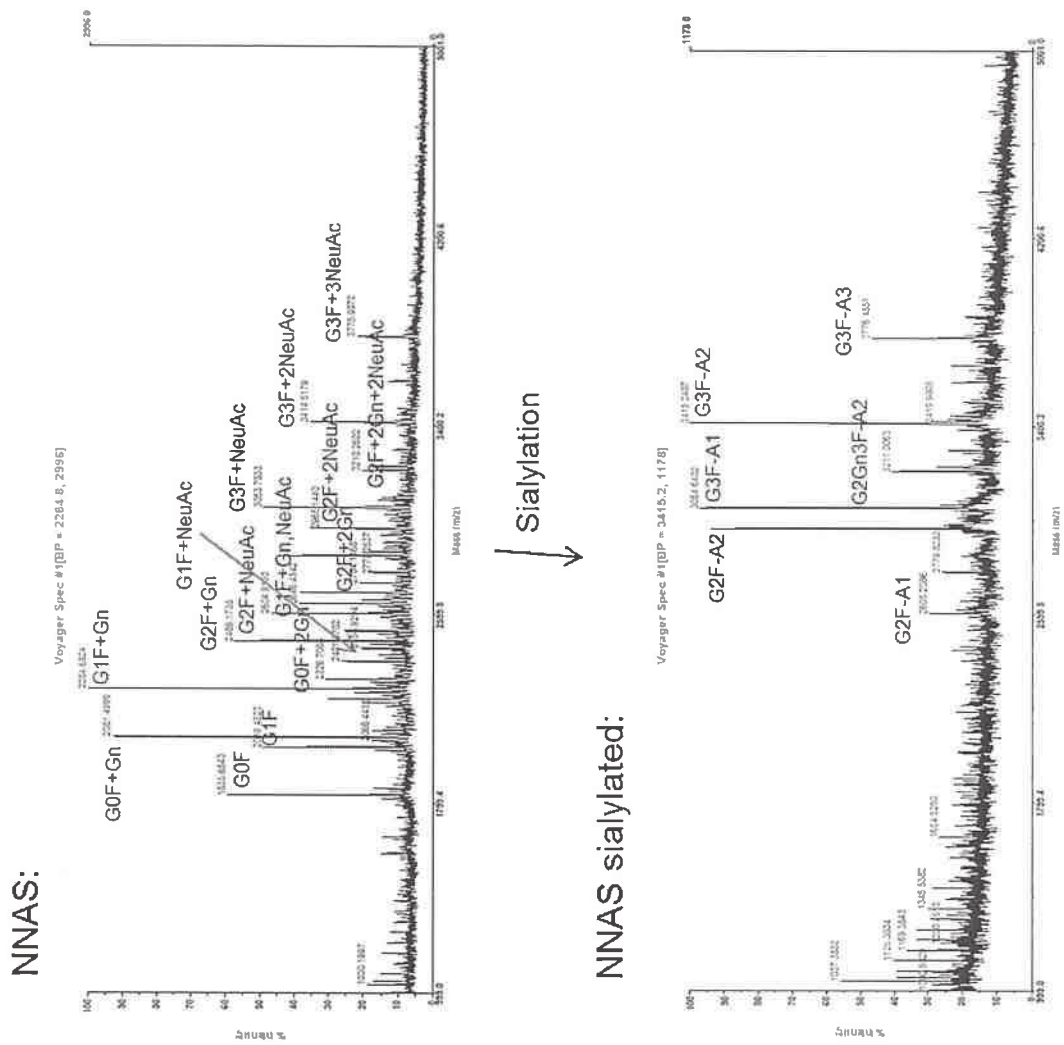
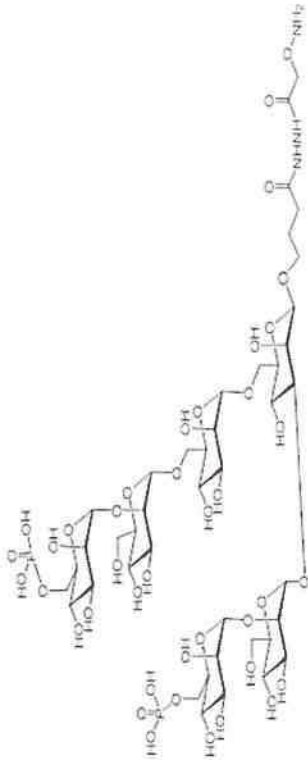
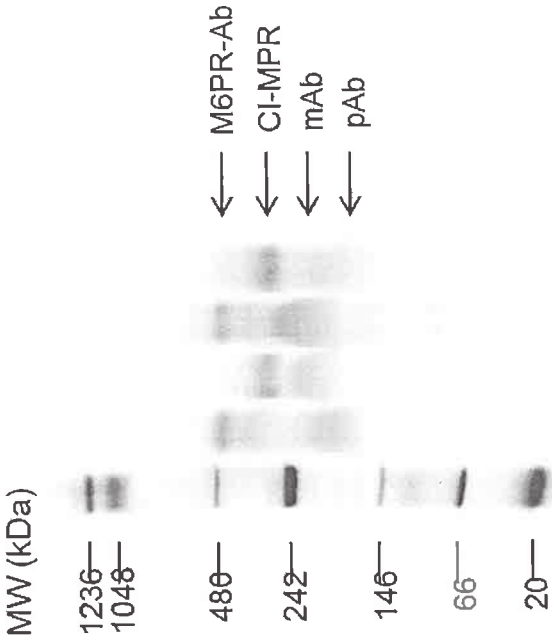


Figure 60

bisM6P hexasaccharide aminoxy
(SAM):



M6P conjugate at Fc (pAb, 3 glycans)	*		
mAb 3F11		*	
M6P conjugate at Fc (mAb, 1.5 glycans)			*
pAb			*

Figure 61

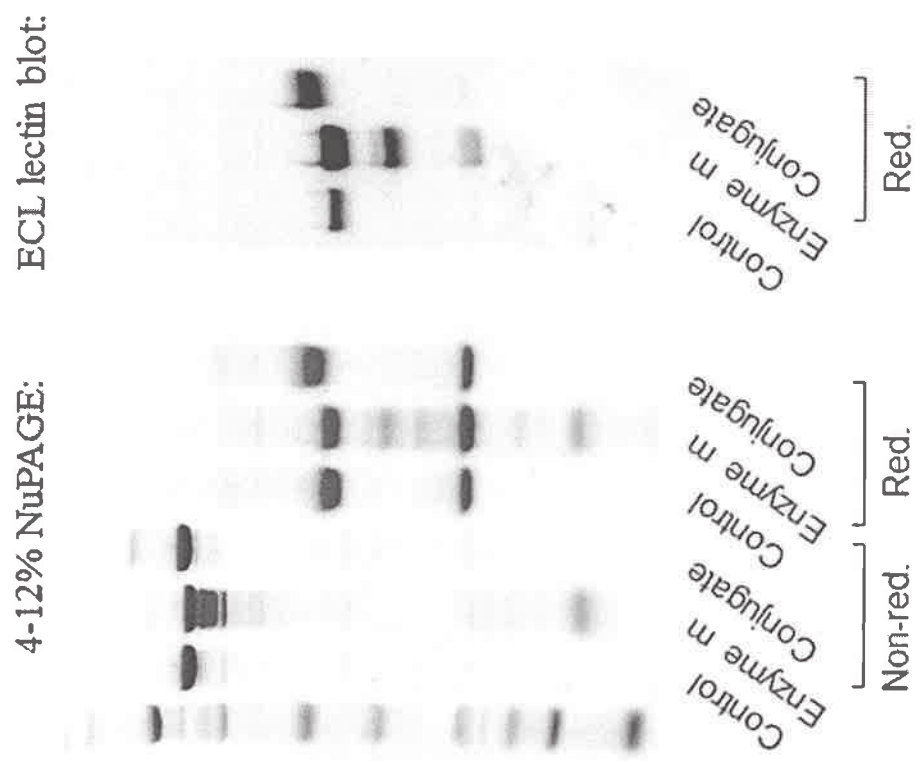


Figure 62

	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

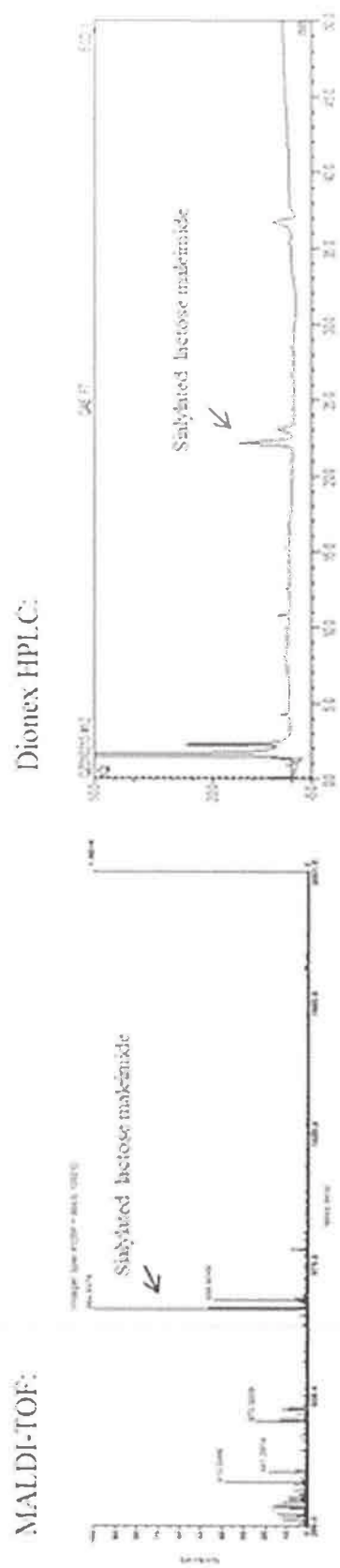


Figure 64

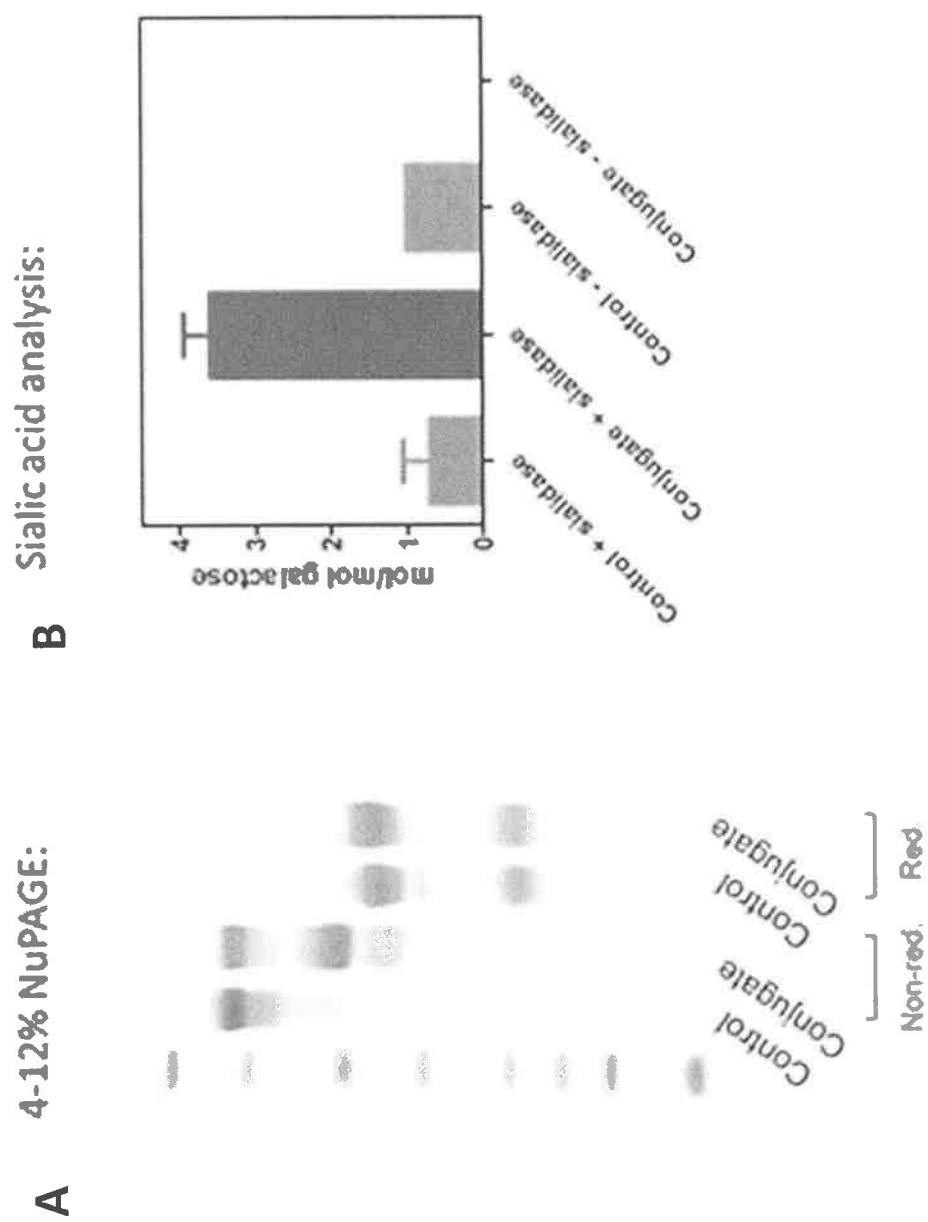


Figure 65

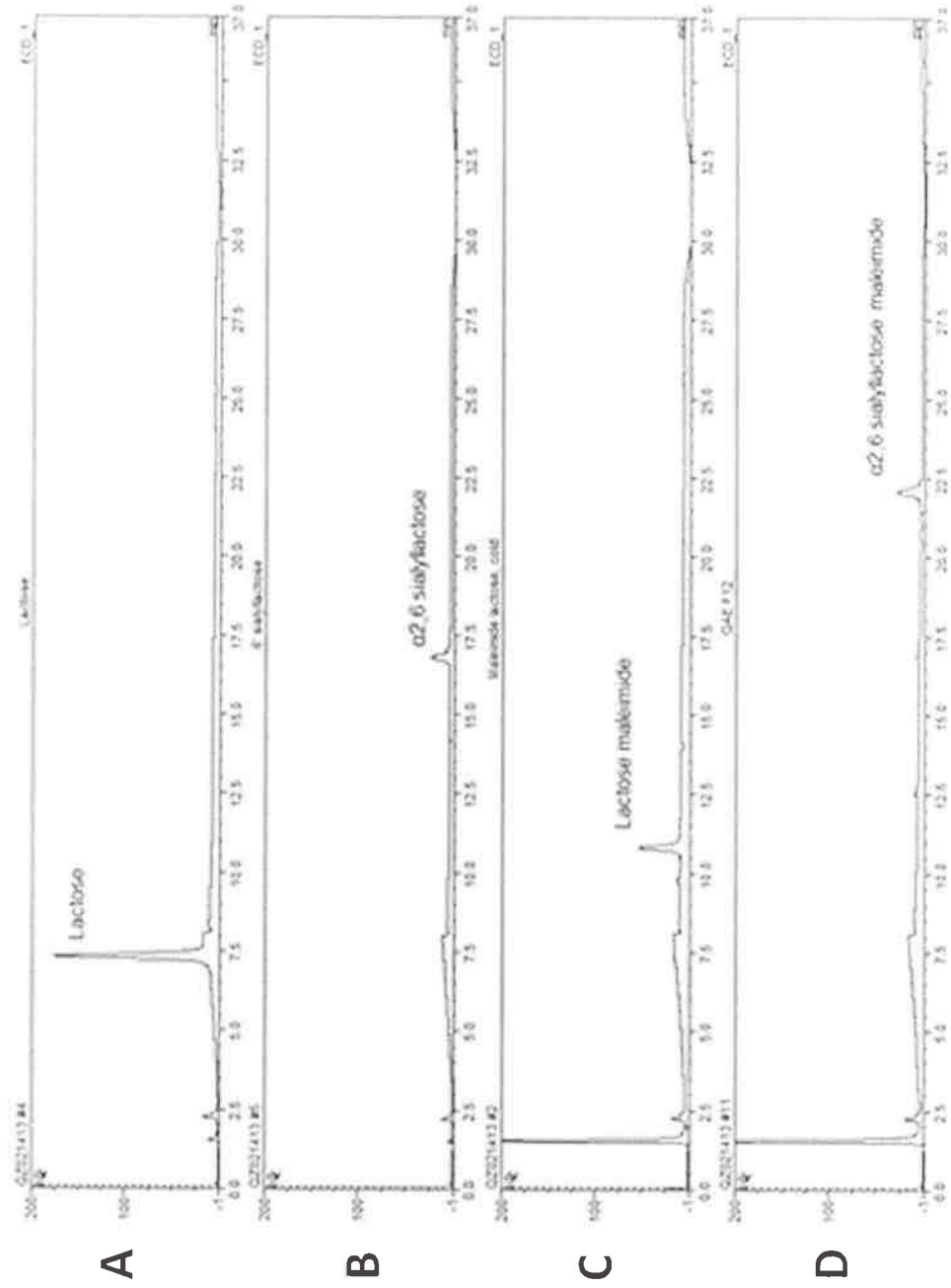


Figure 66

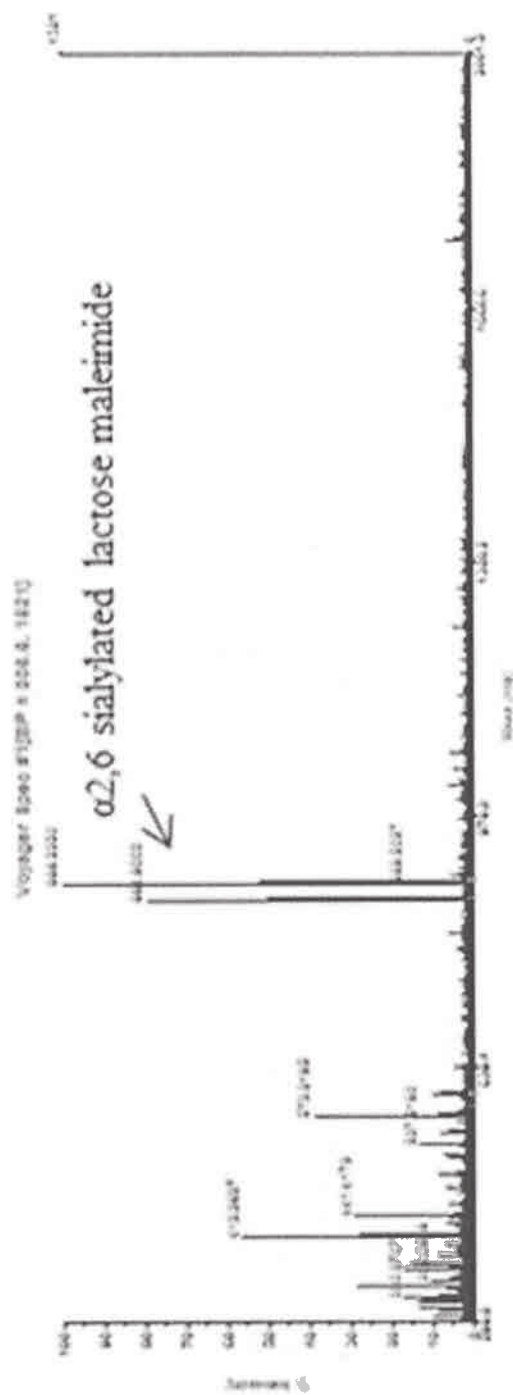


Figure 67

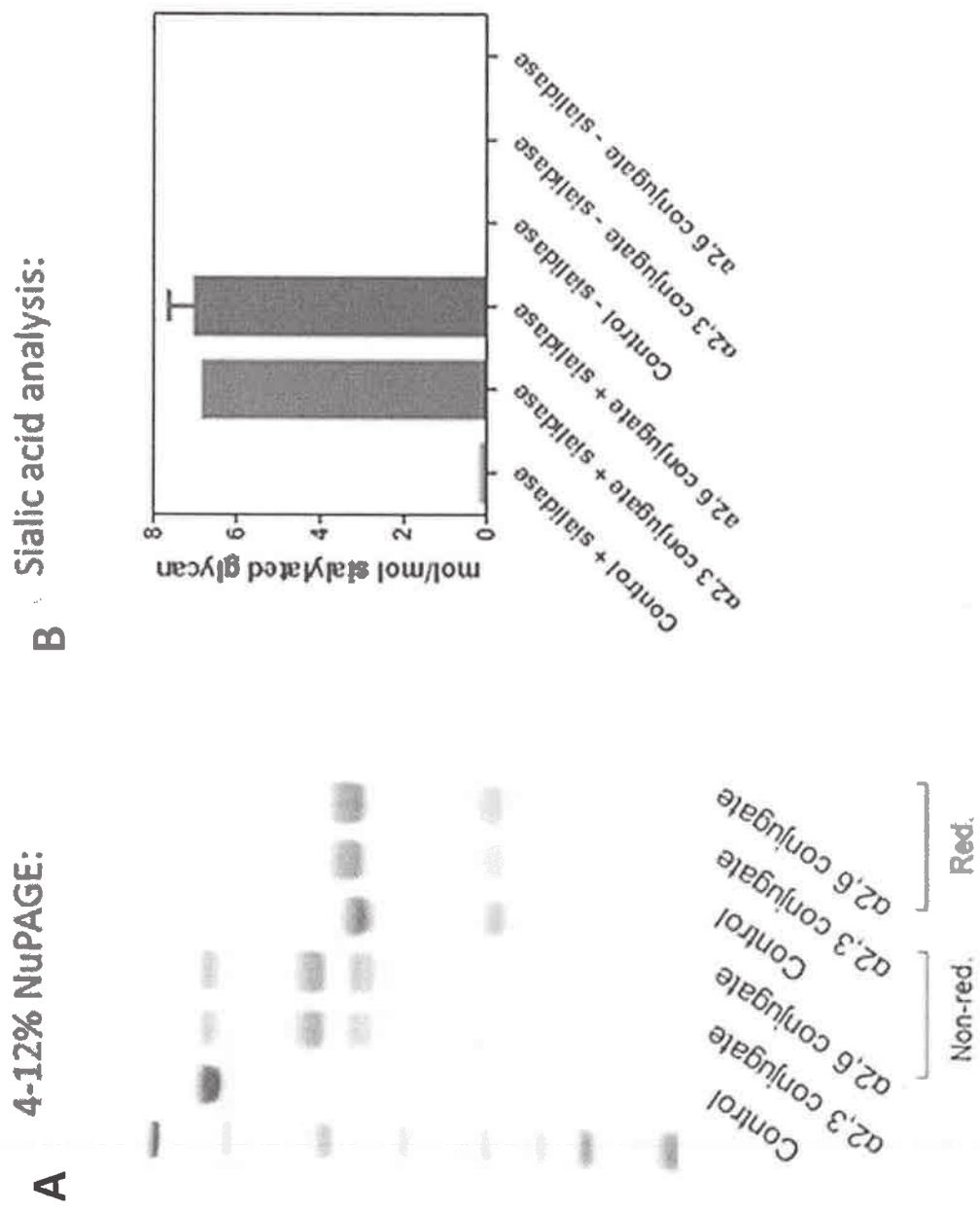


Figure 68



Figure 69

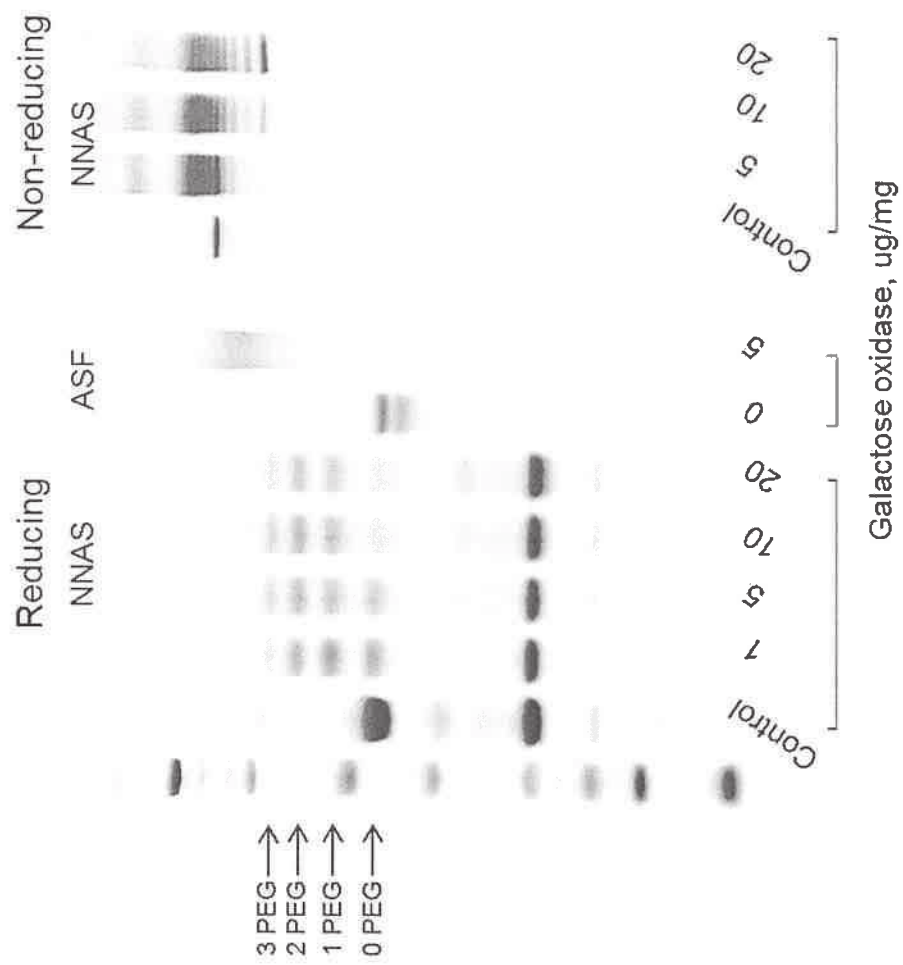


Figure 70

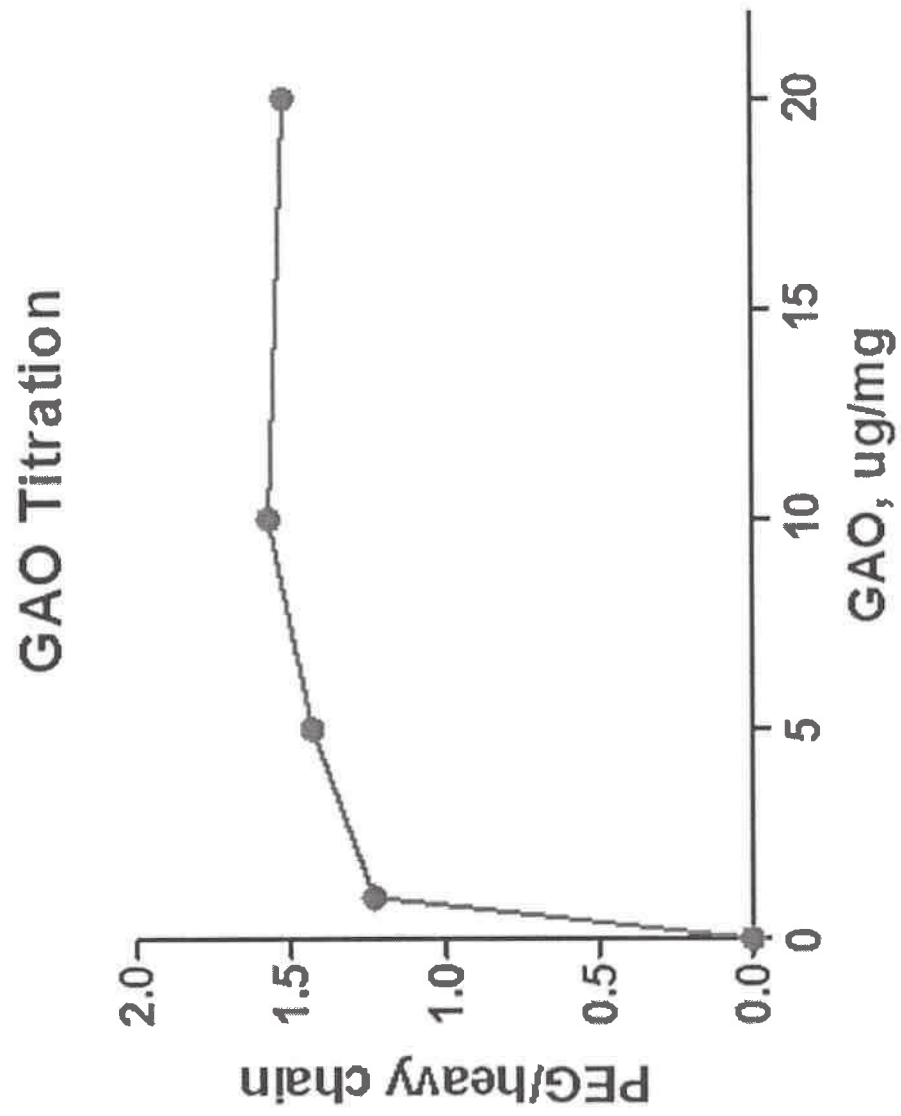


Figure 71

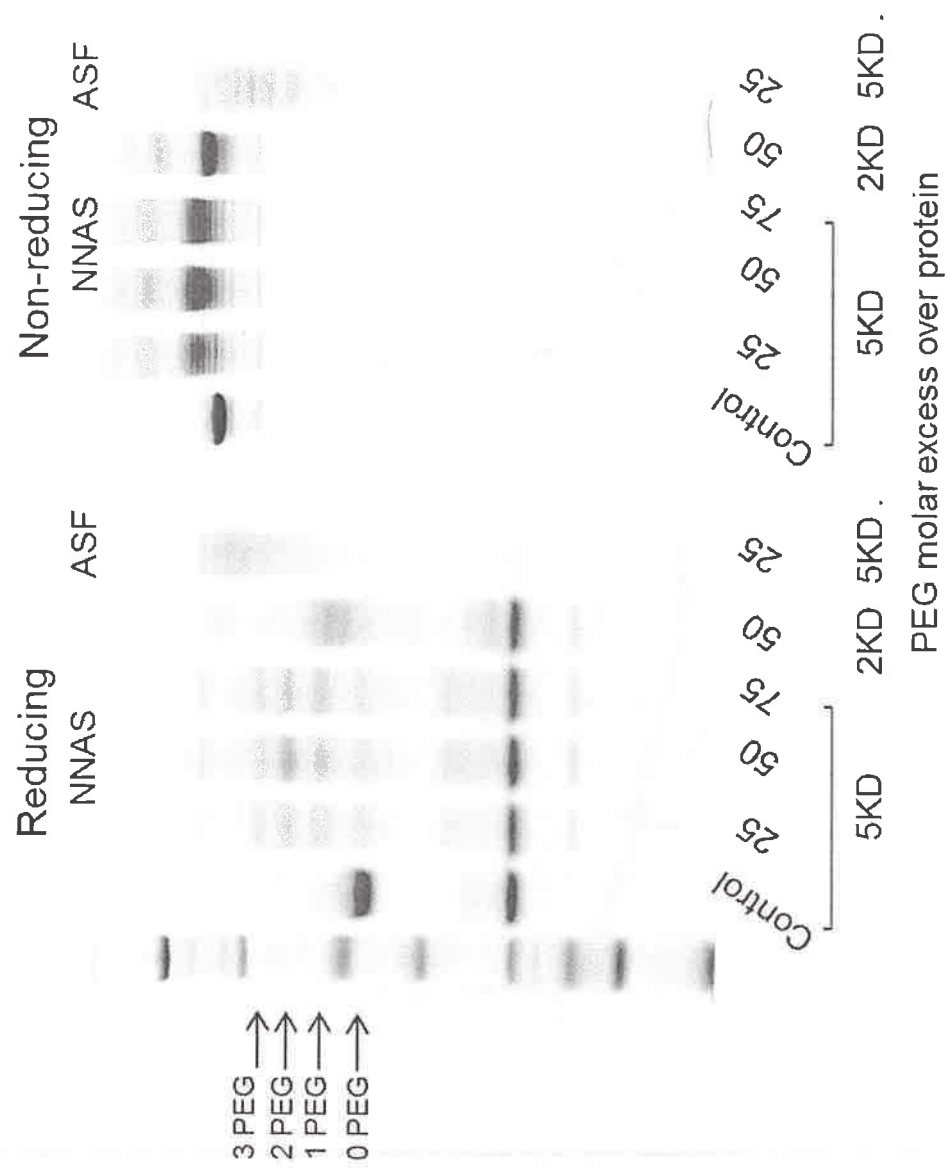
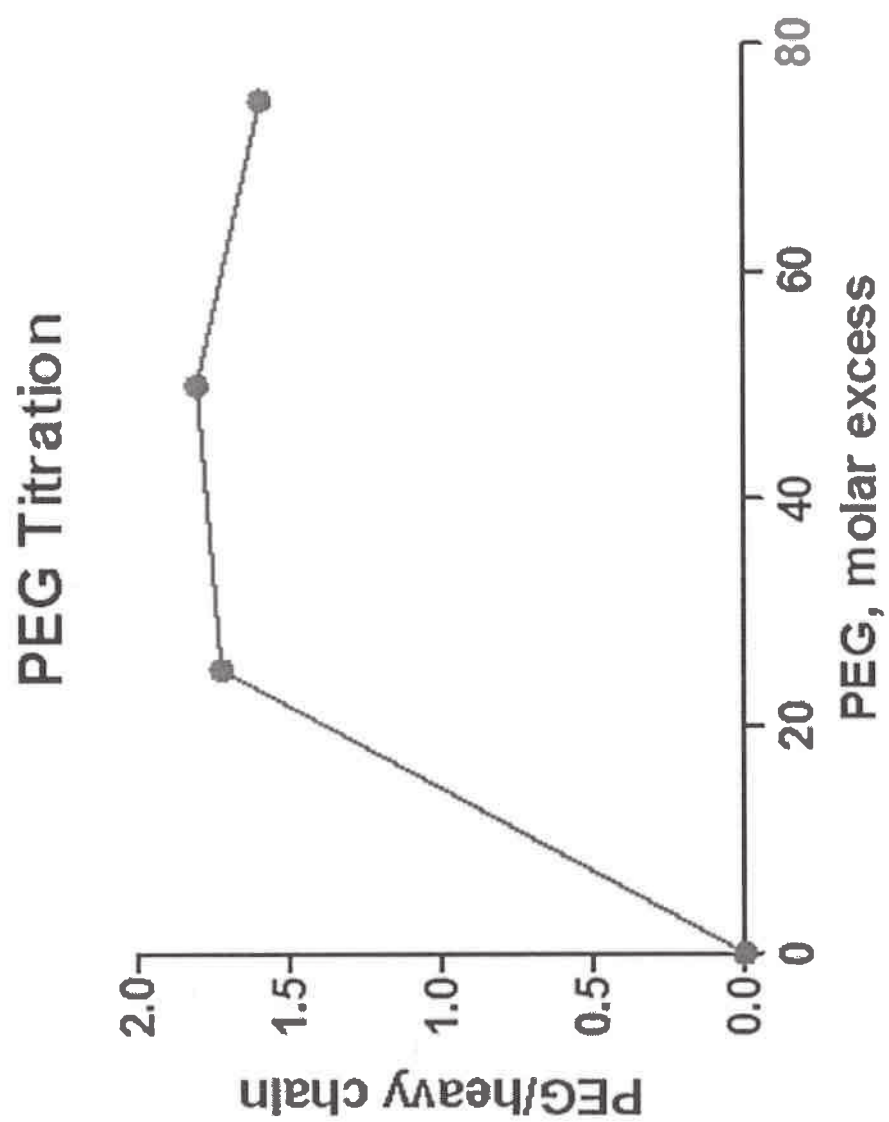


Figure 72



[illegible]

Glycopeptide aminoxy: lactose₃-Cys₃-Gly₄

Figure 74

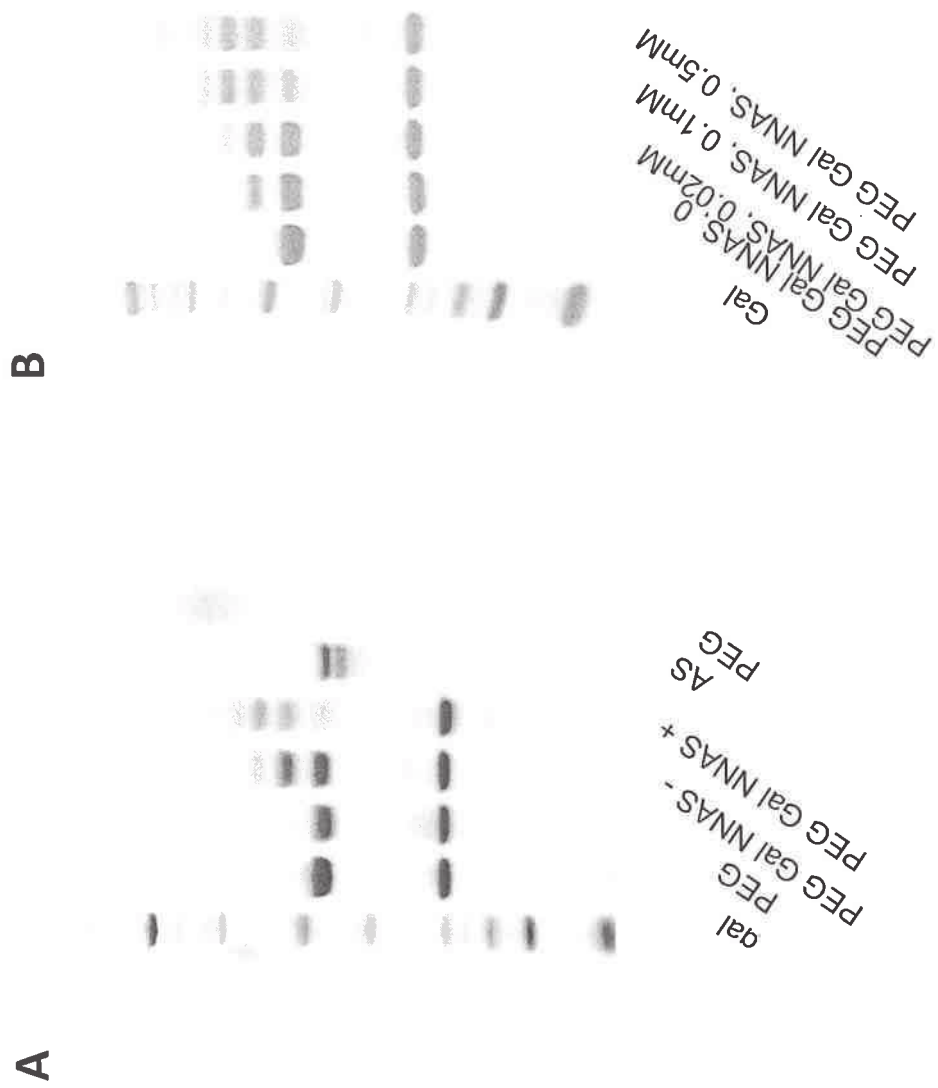


Figure 75

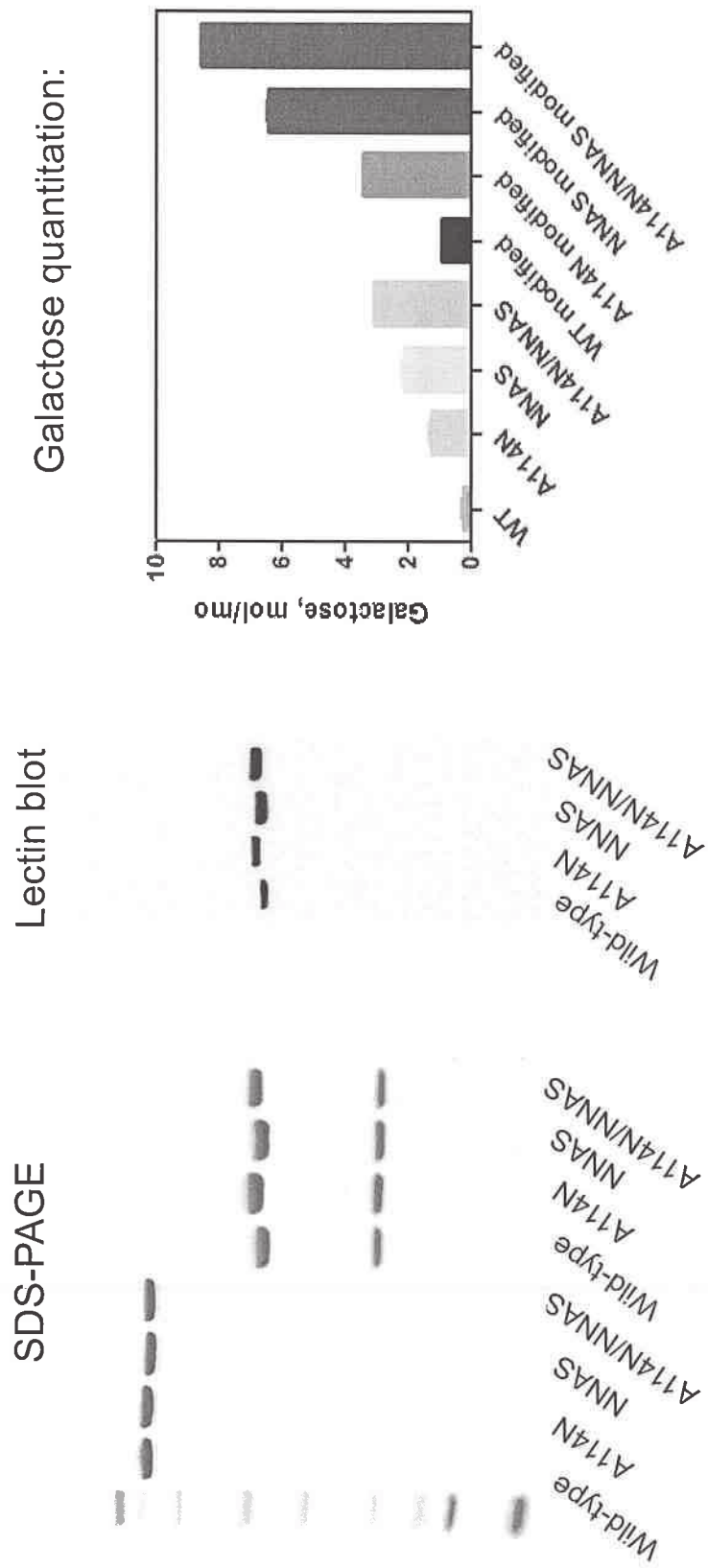


Figure 76

	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

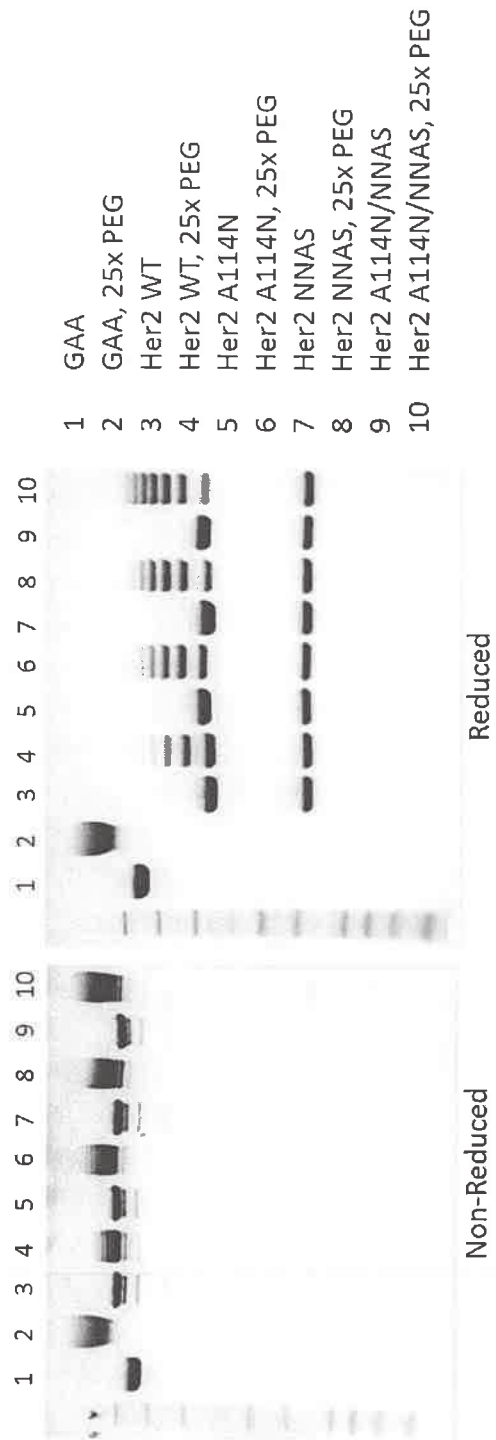


Figure 78

	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

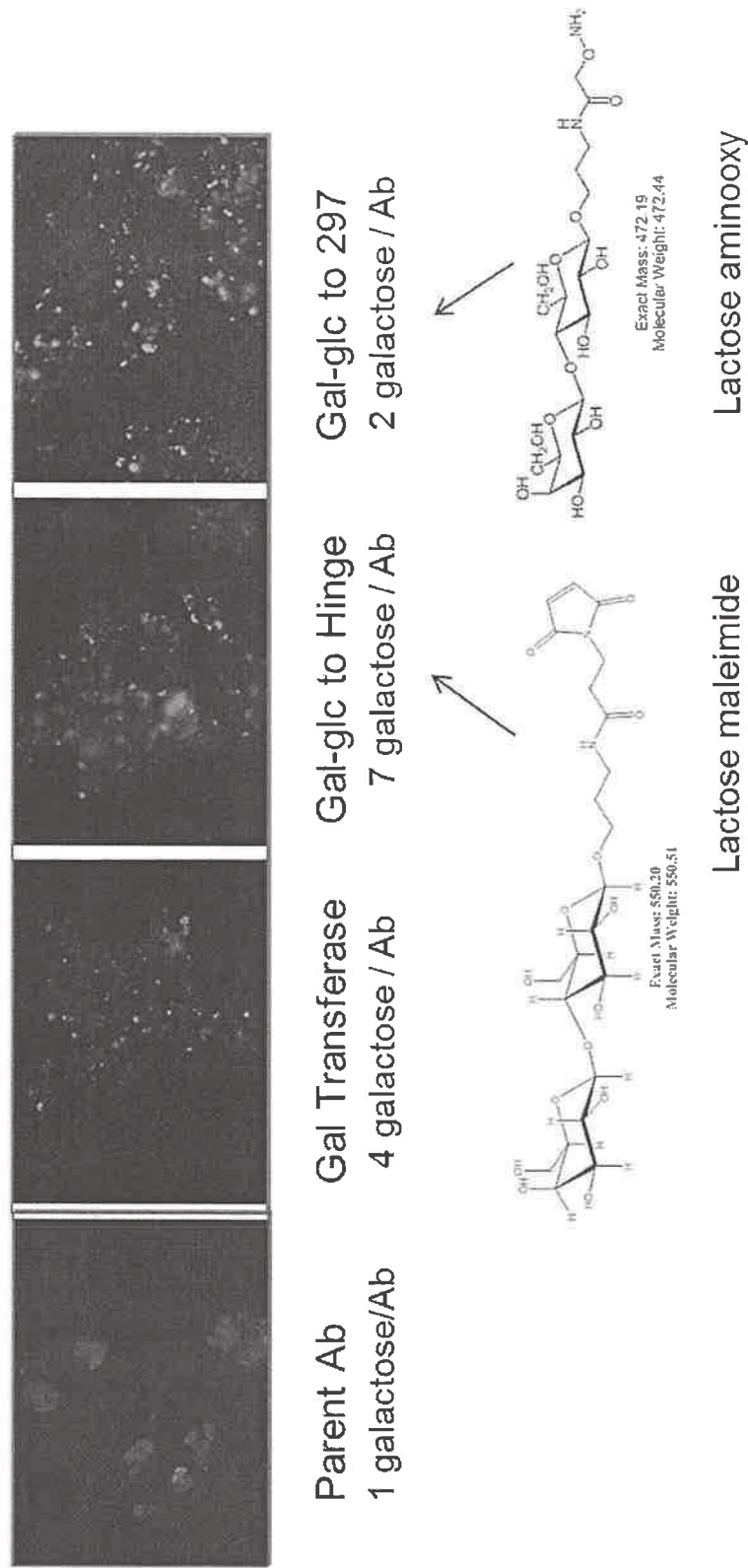


Figure 80

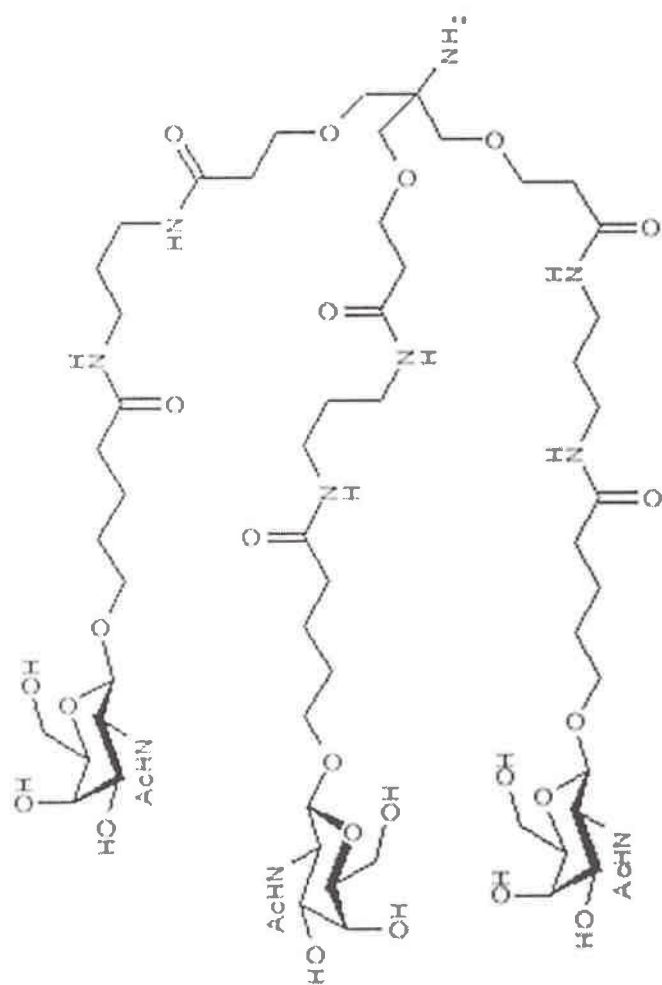


Figure 81

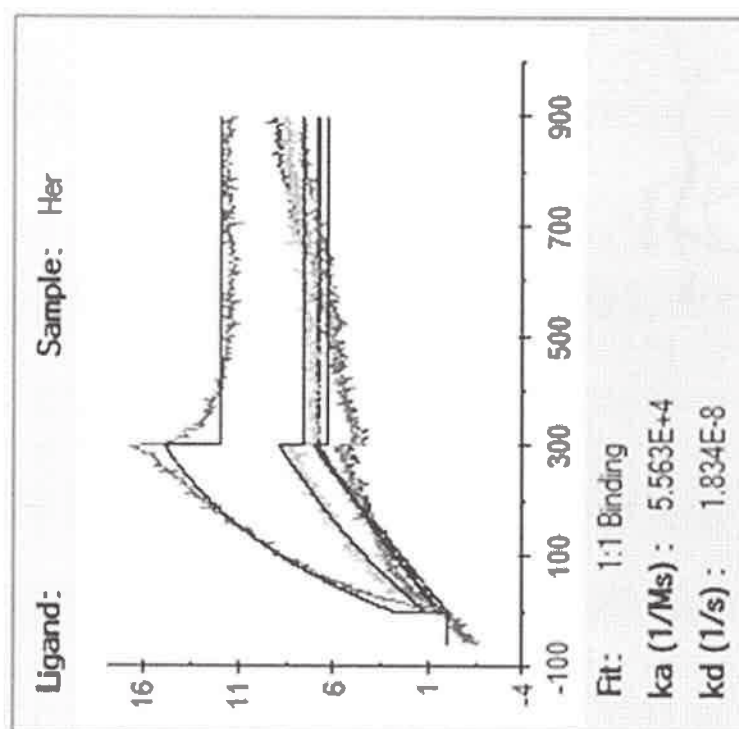
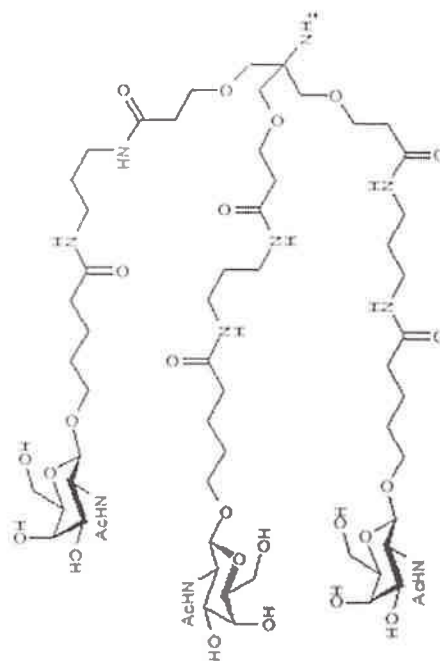


Figure 82

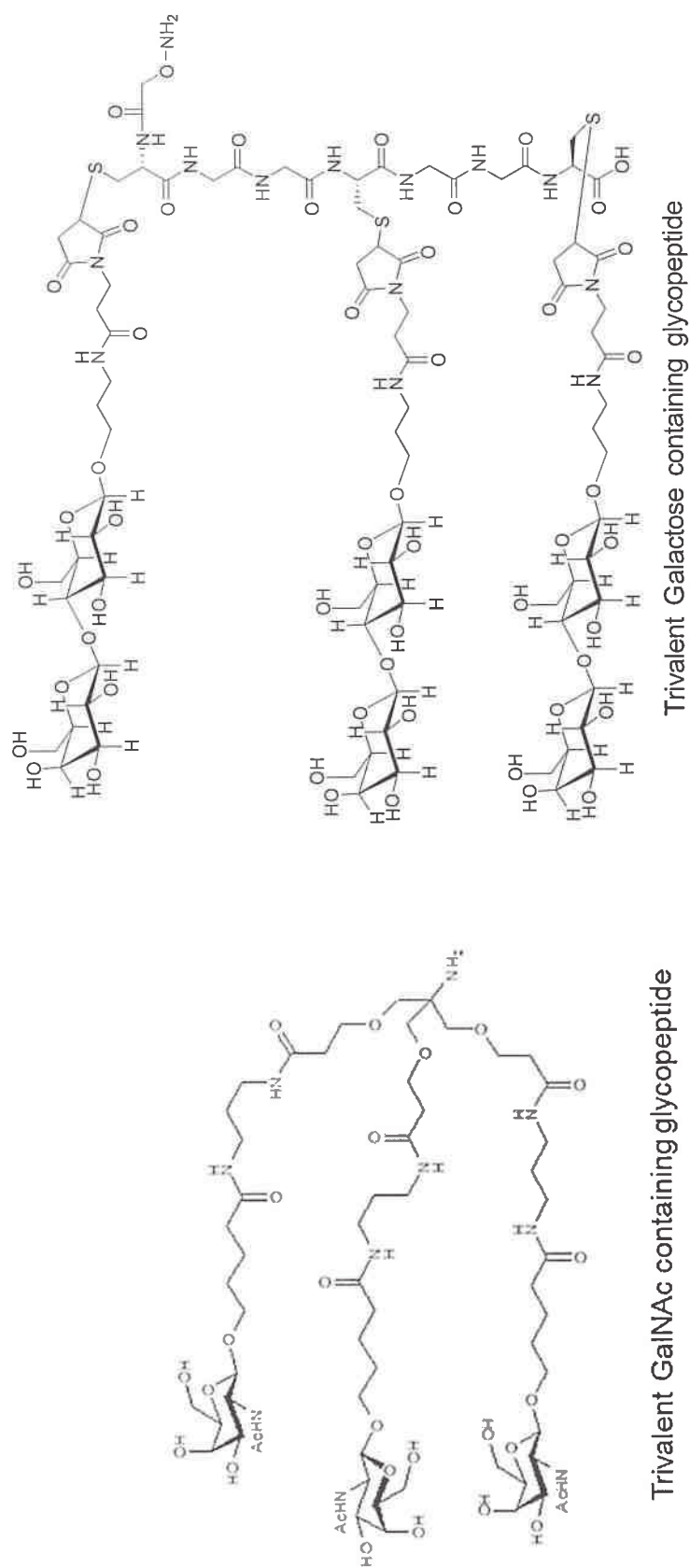


Figure 83

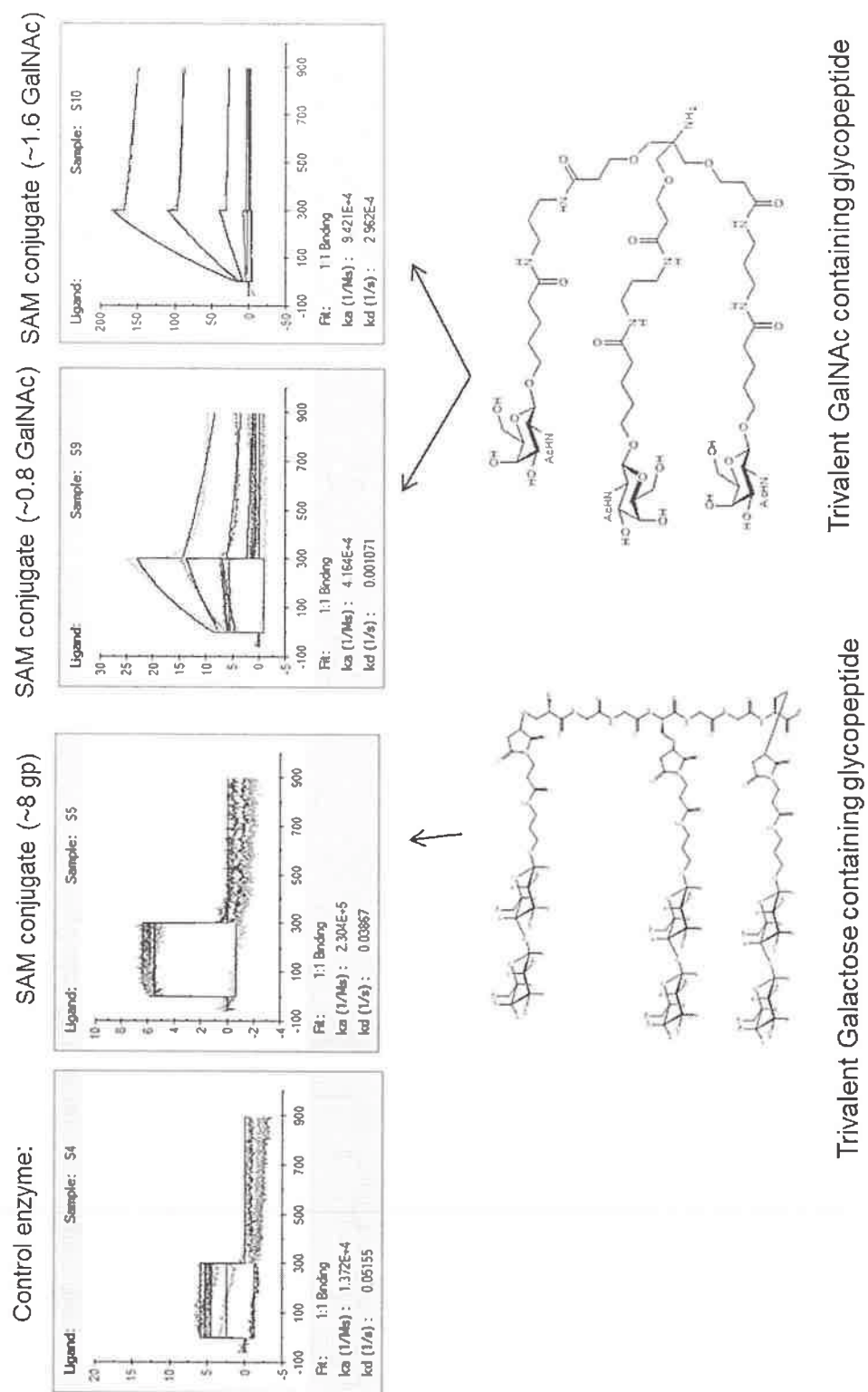


Figure 84

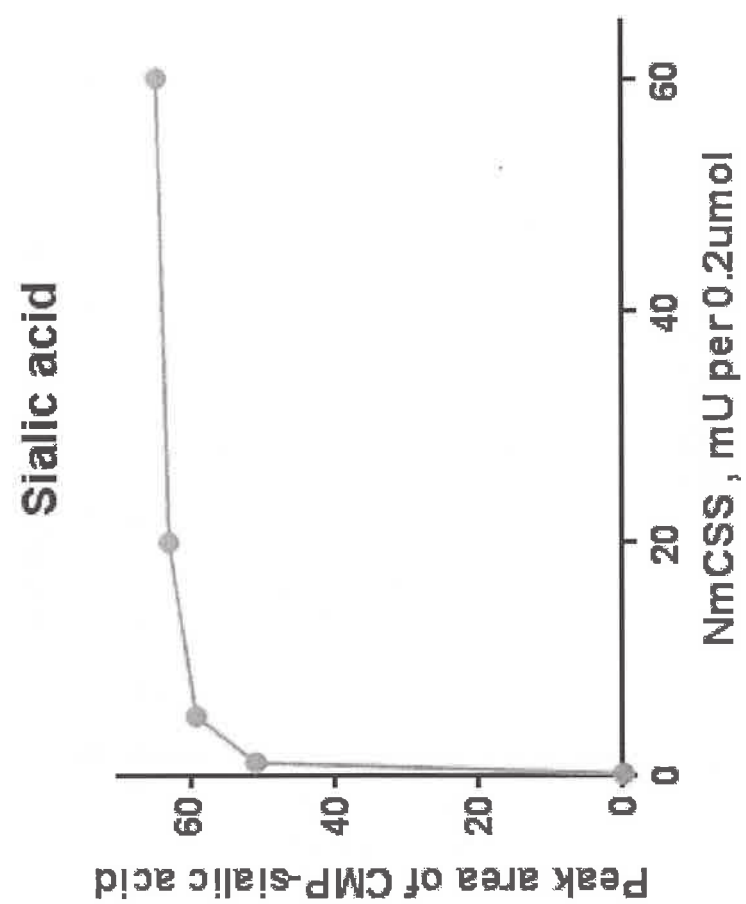


Figure 85

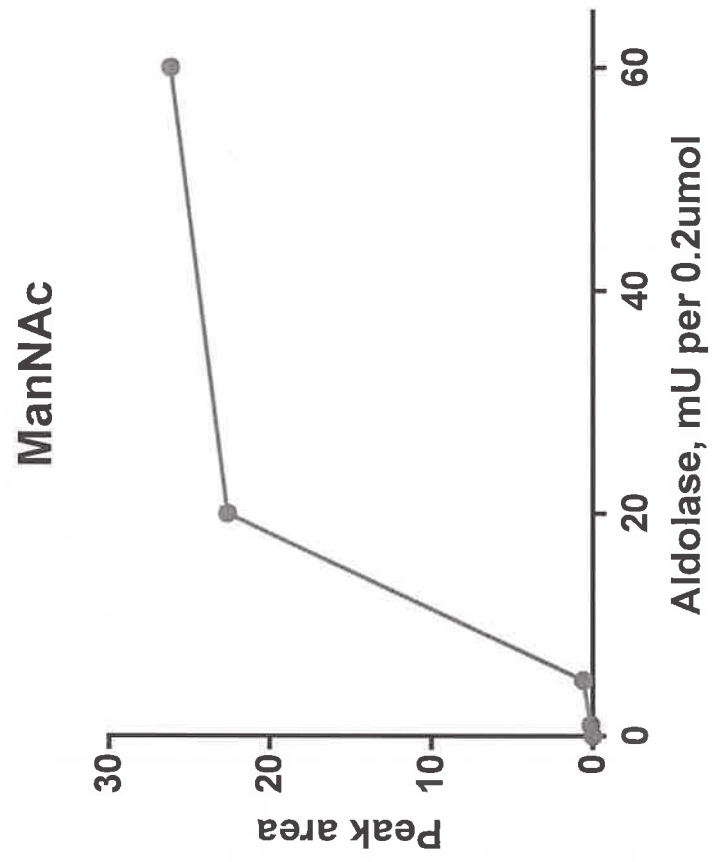


Figure 86

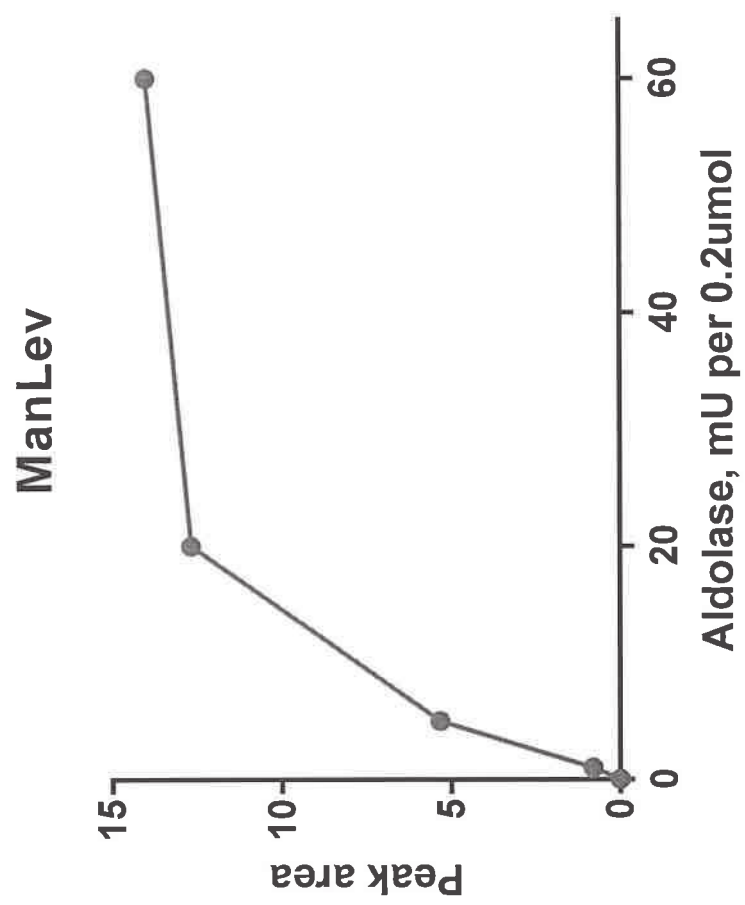


Figure 87



Figure 88

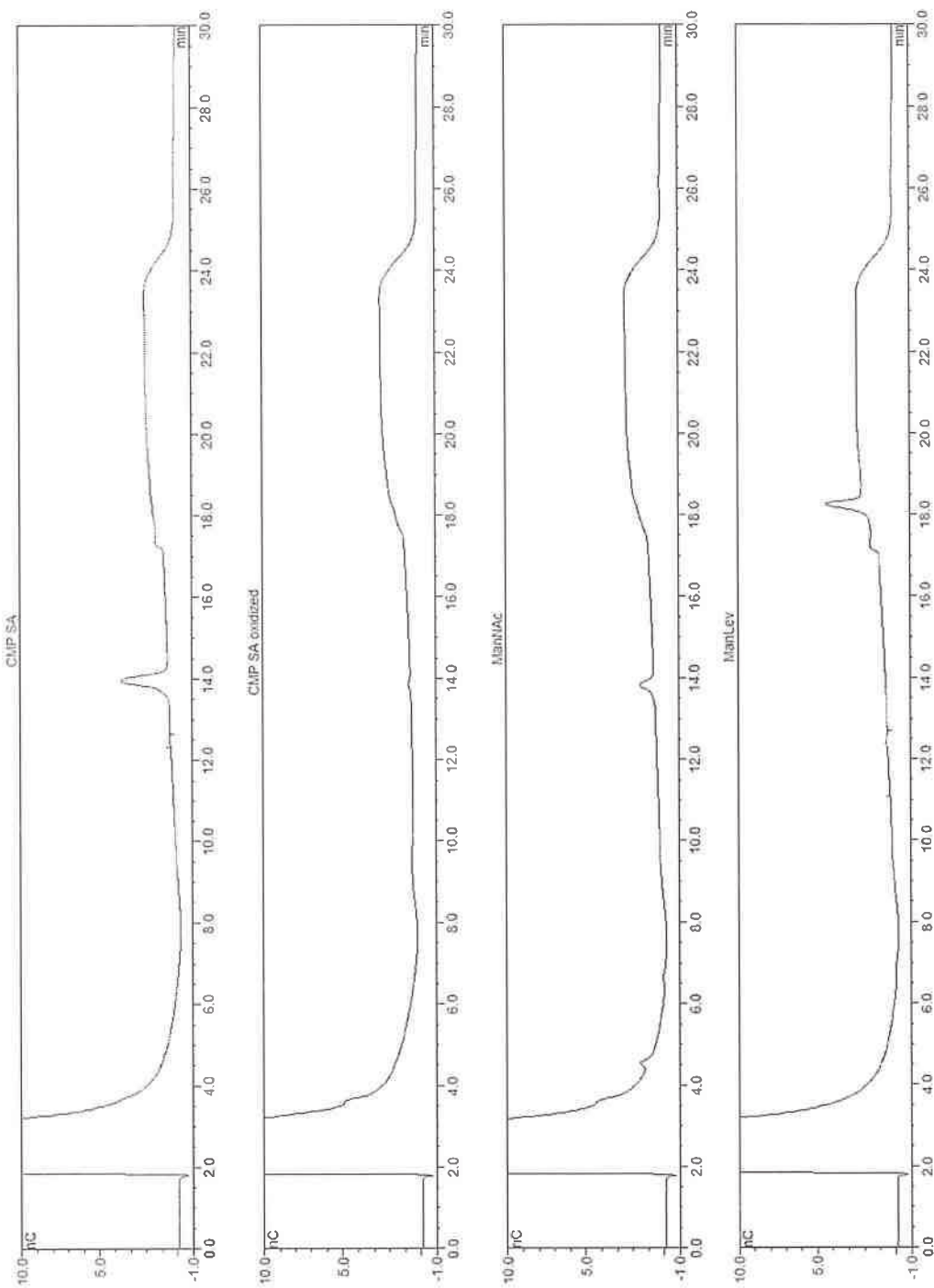


Figure 89

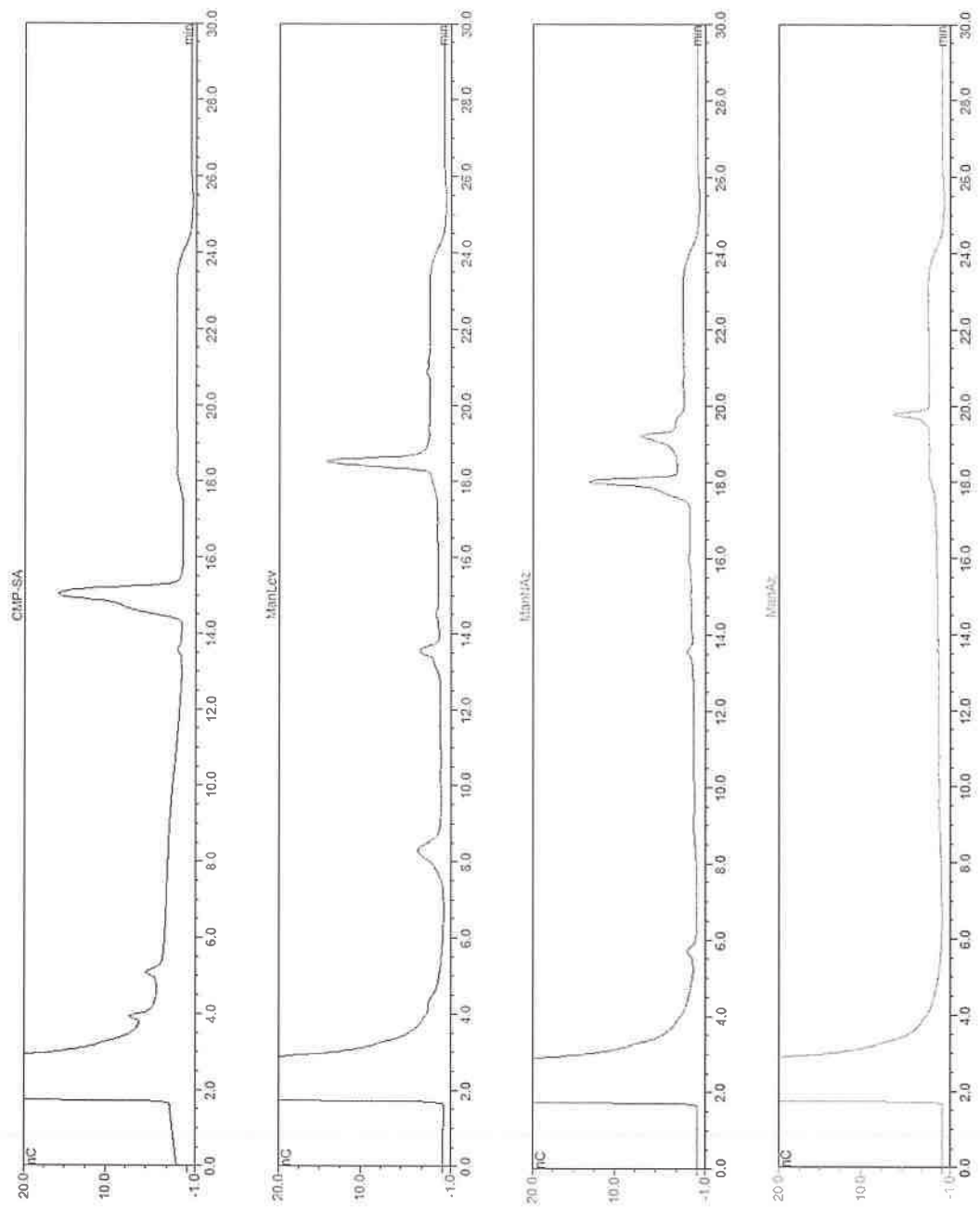


Figure 90

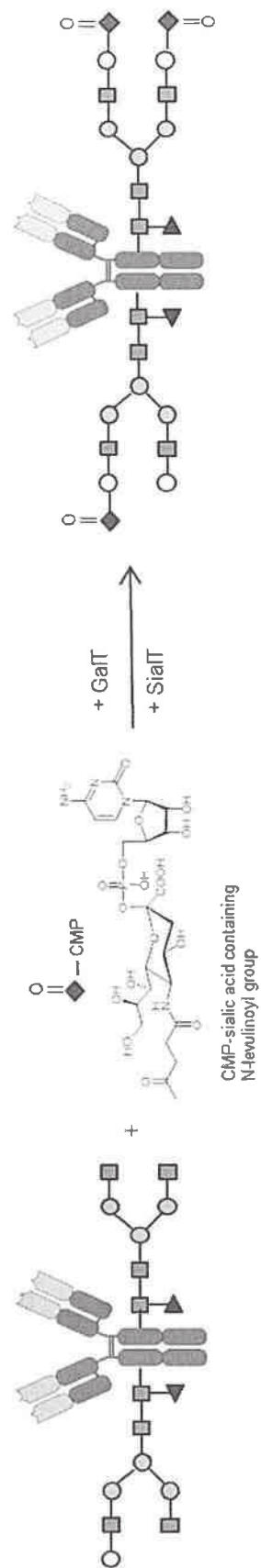


Figure 91

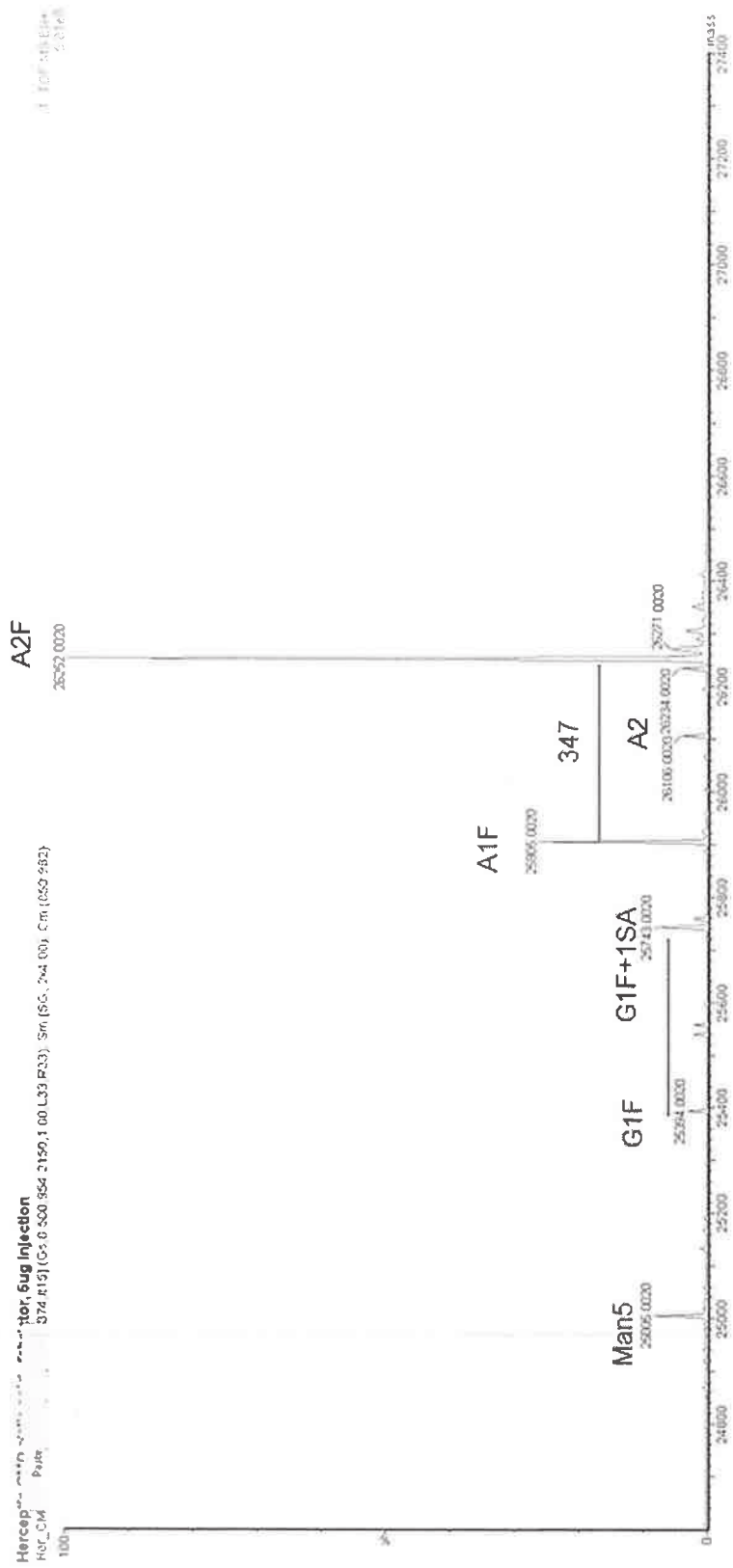


Figure 92

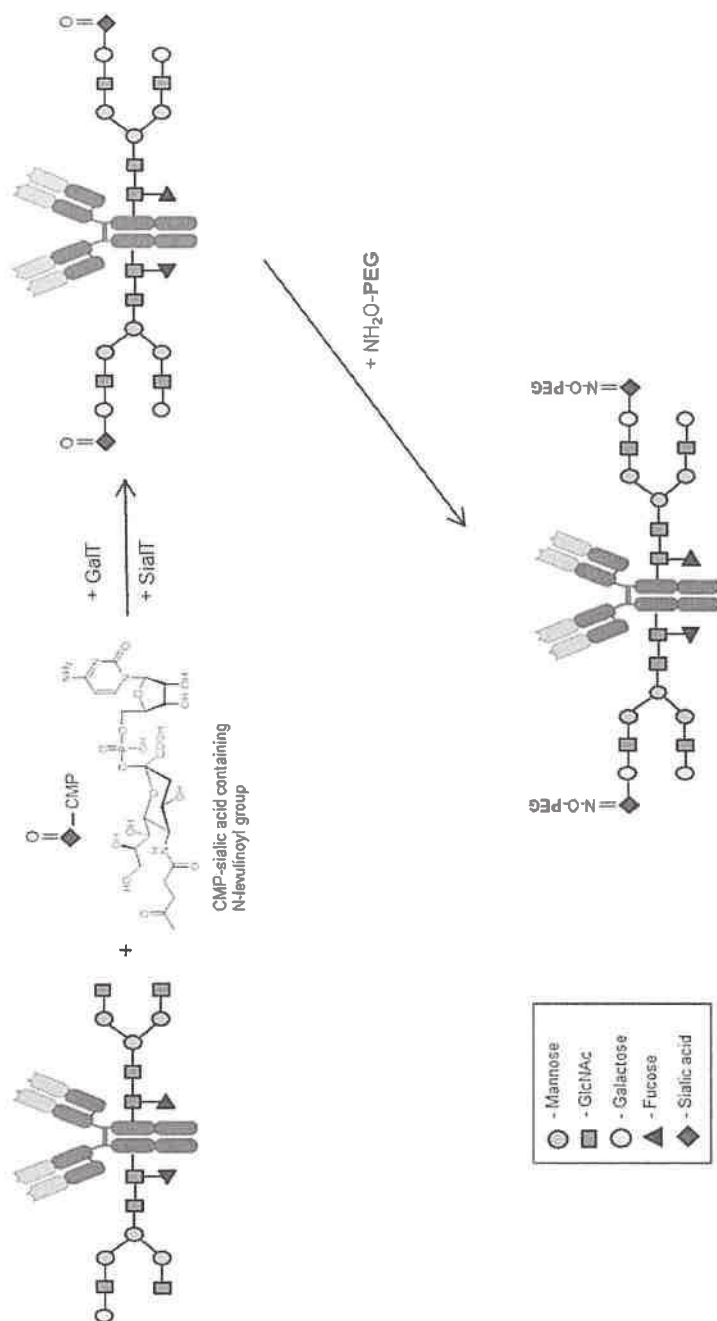


Figure 93

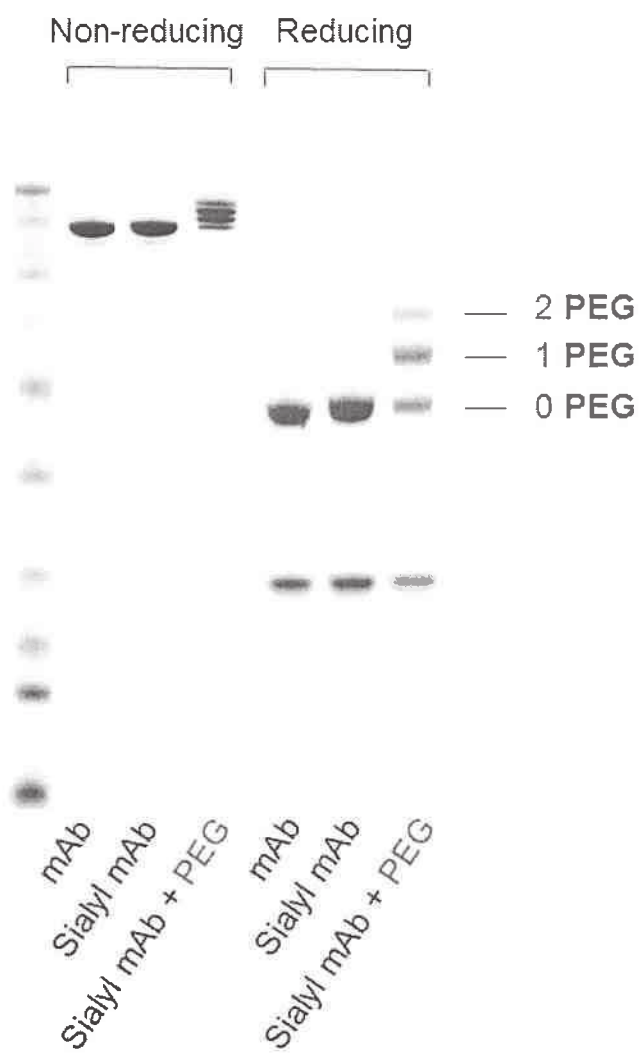


Figure 94

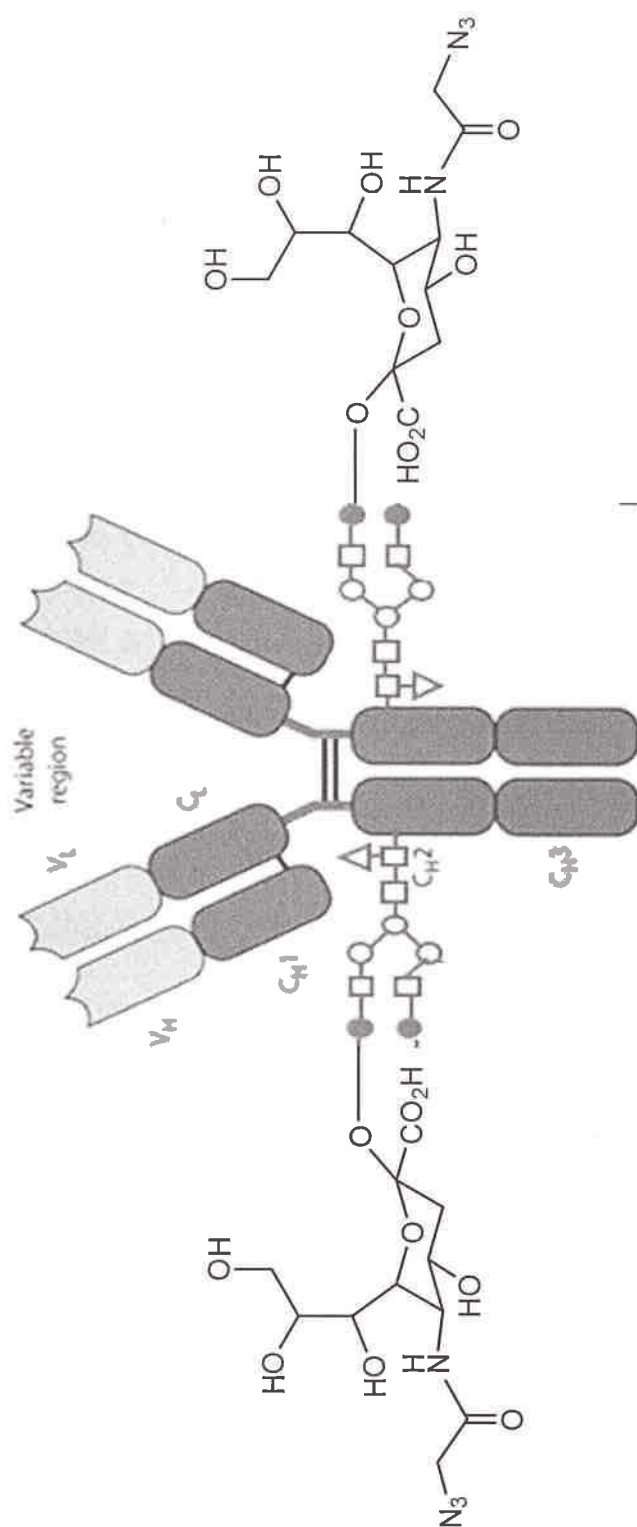


Figure 95

