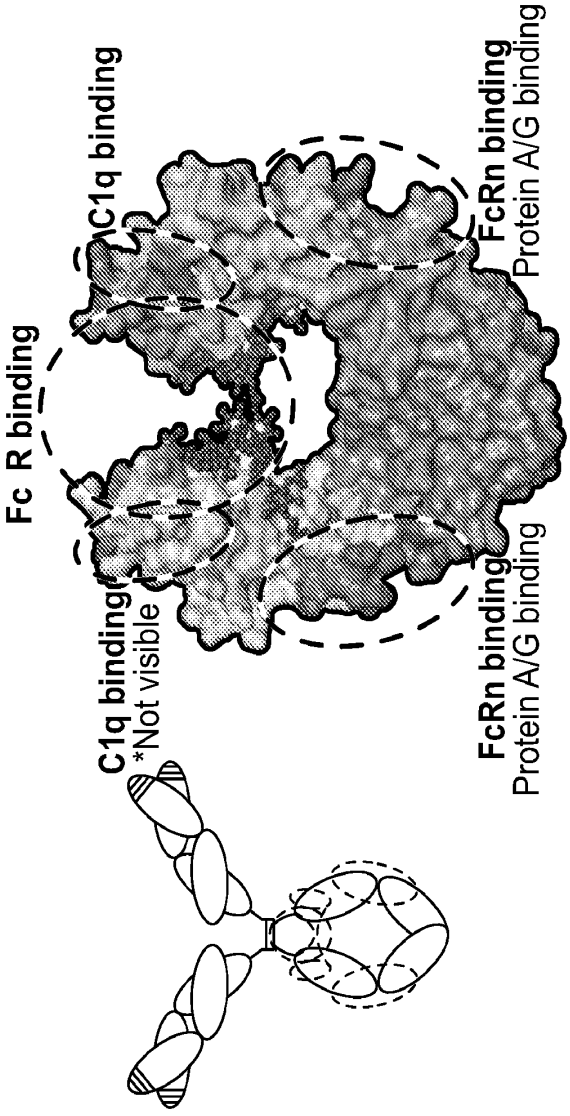


Binding affinity values of the Fc Rs for various IgG subclasses depicted in M^{-1} unit

Structure	Name	CD	Gene	Alleles*	IgG1	IgG2	IgG3	IgG4	Major function
	Fc RIIIA	CD16A	*FCGR3A*	Val158 Phe158	$2x10^5$ $1x10^5$	$7x10^4$ $3x10^4$	$10x10^{6¶}$ $8x10^{6¶}$	$2x10^5$ $2x10^5$	Activation

FIG. 1A

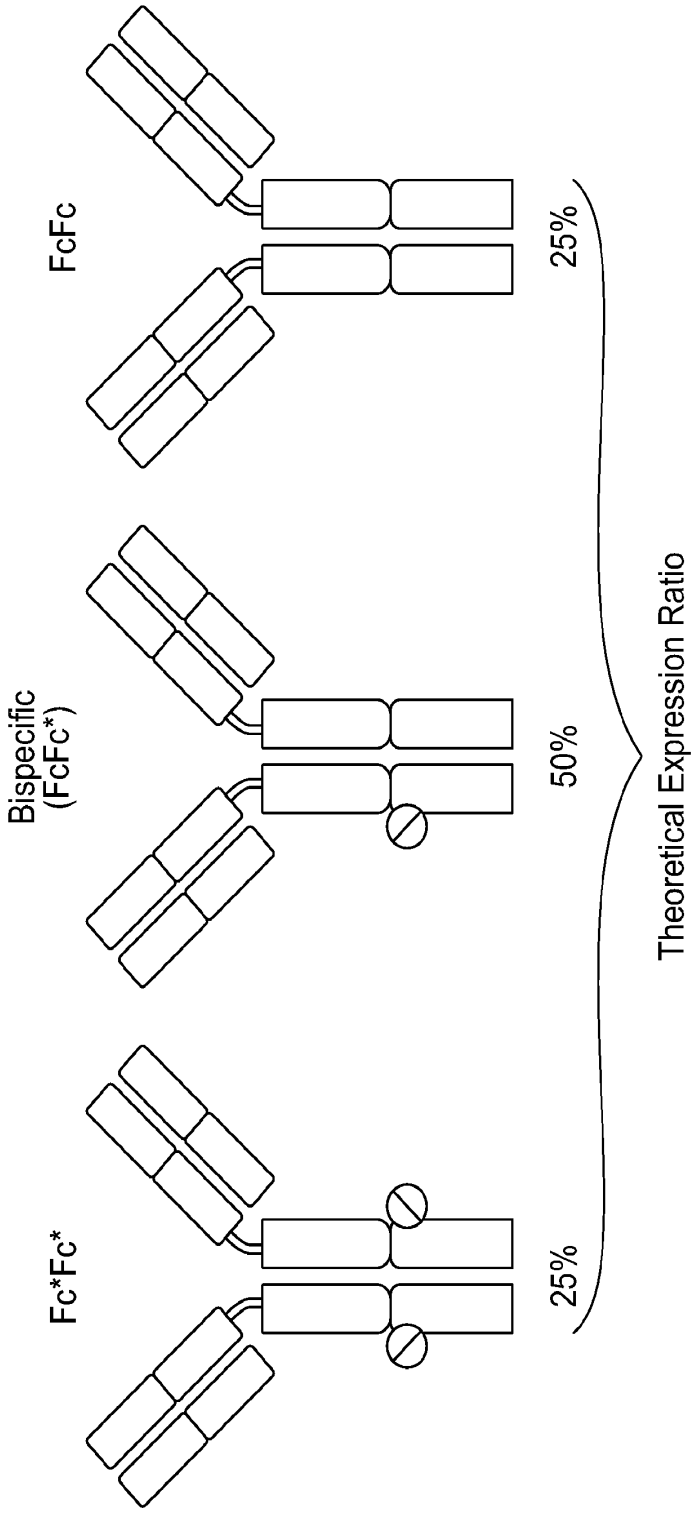

FcFc
Bispecific
(FcFc*)
Fc*Fc*
25%
50%
25%
Theoretical Expression Ratio

FIG. 1B

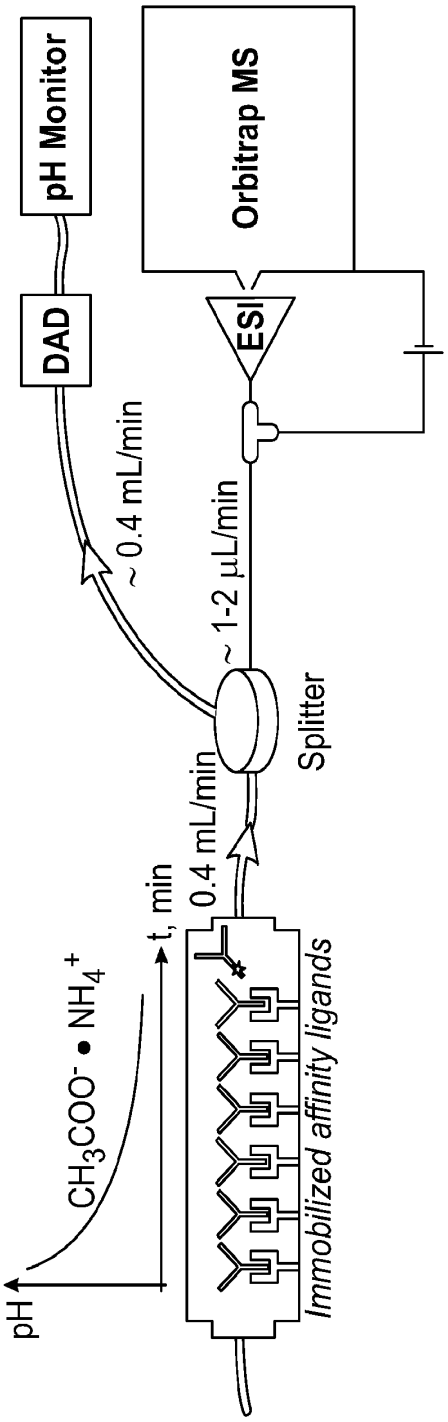

pH Monitor
DAD
Orbitrap MS
ESI
~ 0.4 mL/min
~ 1-2 µL/min
Splitter
0.4 mL/min
t, min
pH
$CH_3COO^- \bullet NH_4^+$
Immobilized affinity ligands

FIG. 2A

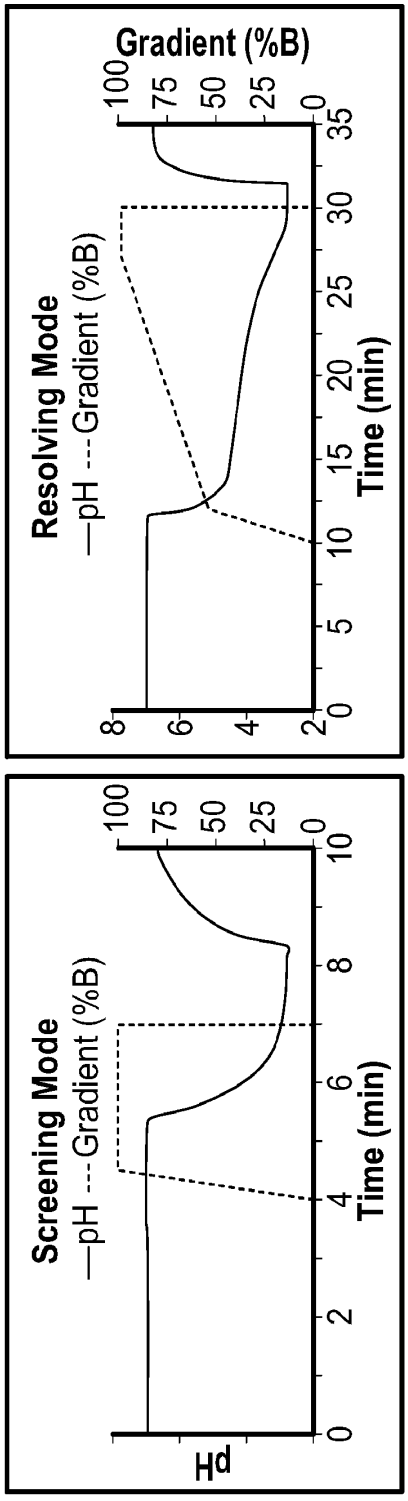

Gradient (%B)
Resolving Mode
—pH ---Gradient (%B)
Time (min)
Screening Mode
—pH ---Gradient (%B)
Time (min)
pH

FIG. 2B

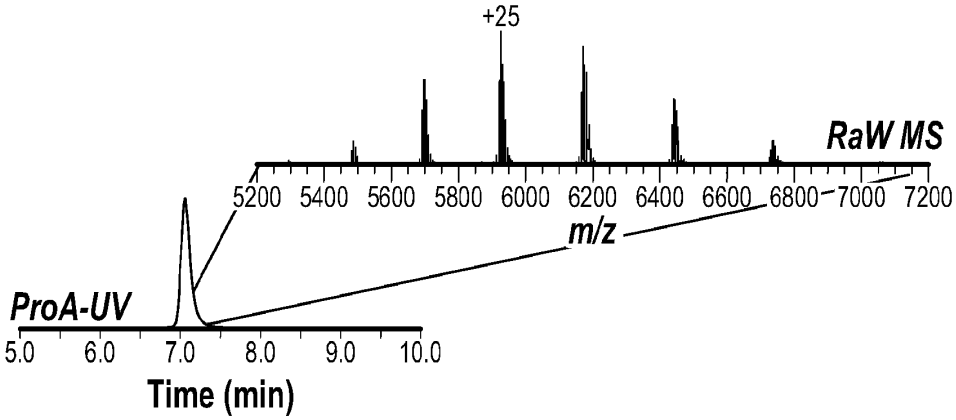
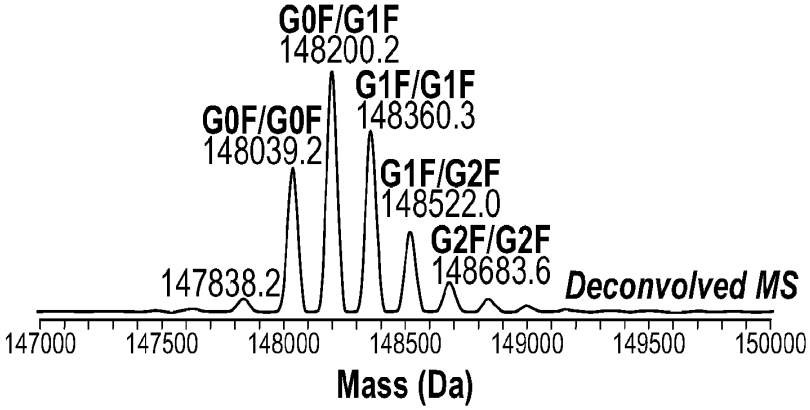


FIG. 3A



	M_{theo} (Da)	M_{exp} (Da)
G0F/G0F	148,036.4	148,039.2
G0F/G1F	148,198.6	148,200.2
G1F/G1F	148,360.7	148,360.3

FIG. 3B

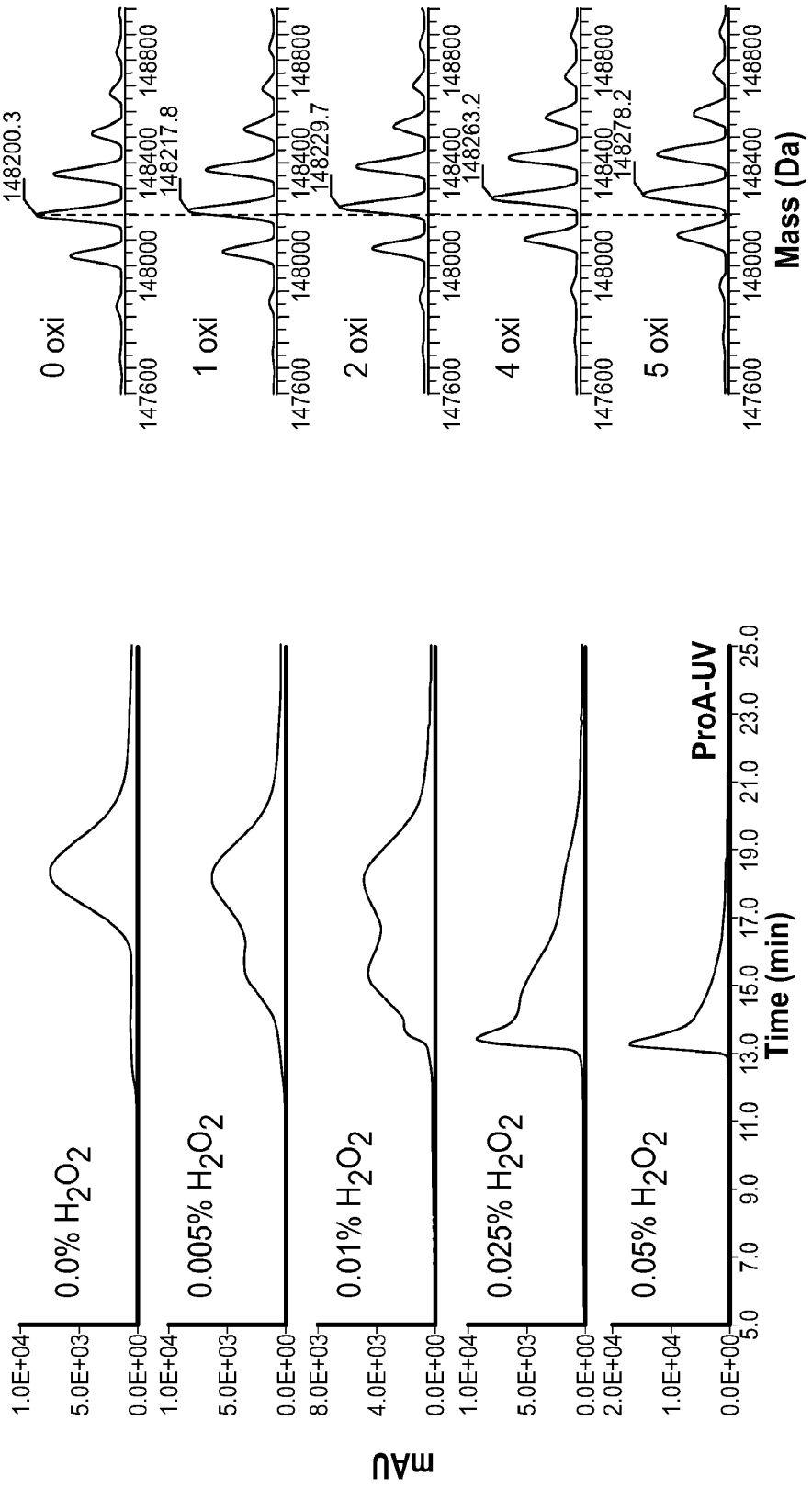

0.0% H_2O_2
0.005% H_2O_2
0.01% H_2O_2
0.025% H_2O_2
0.05% H_2O_2
mAU
ProA-UV
Time (min)
0 oxi
1 oxi
2 oxi
4 oxi
5 oxi
148200.3
148217.8
148229.7
148263.2
148278.2
Mass (Da)

FIG. 4

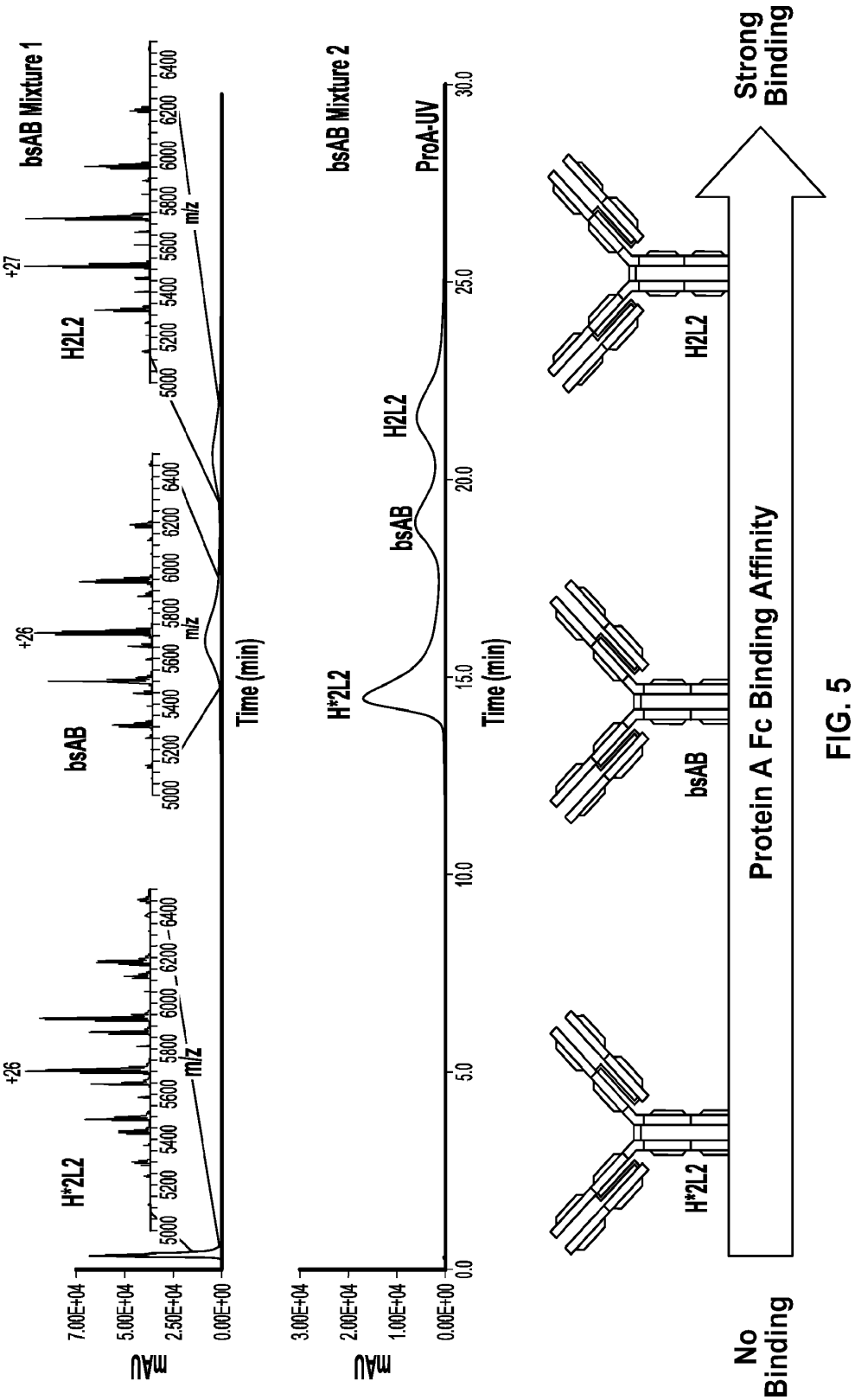

bsAB Mixture 1
H*2L2
bsAB
H2L2
+26
+27
m/z
Time (min)
mAU
7.00E+04
5.00E+04
2.50E+04
0.00E+00
5000
5200
5400
5600
5800
6000
6200
6400
bsAB Mixture 2
ProA-UV
H*2L2
bsAB
H2L2
0.0
5.0
10.0
15.0
20.0
25.0
30.0
3.00E+04
2.00E+04
1.00E+04
0.00E+00
Strong Binding
No Binding
Protein A Fc Binding Affinity
H*2L2
bsAB
H2L2

FIG. 5

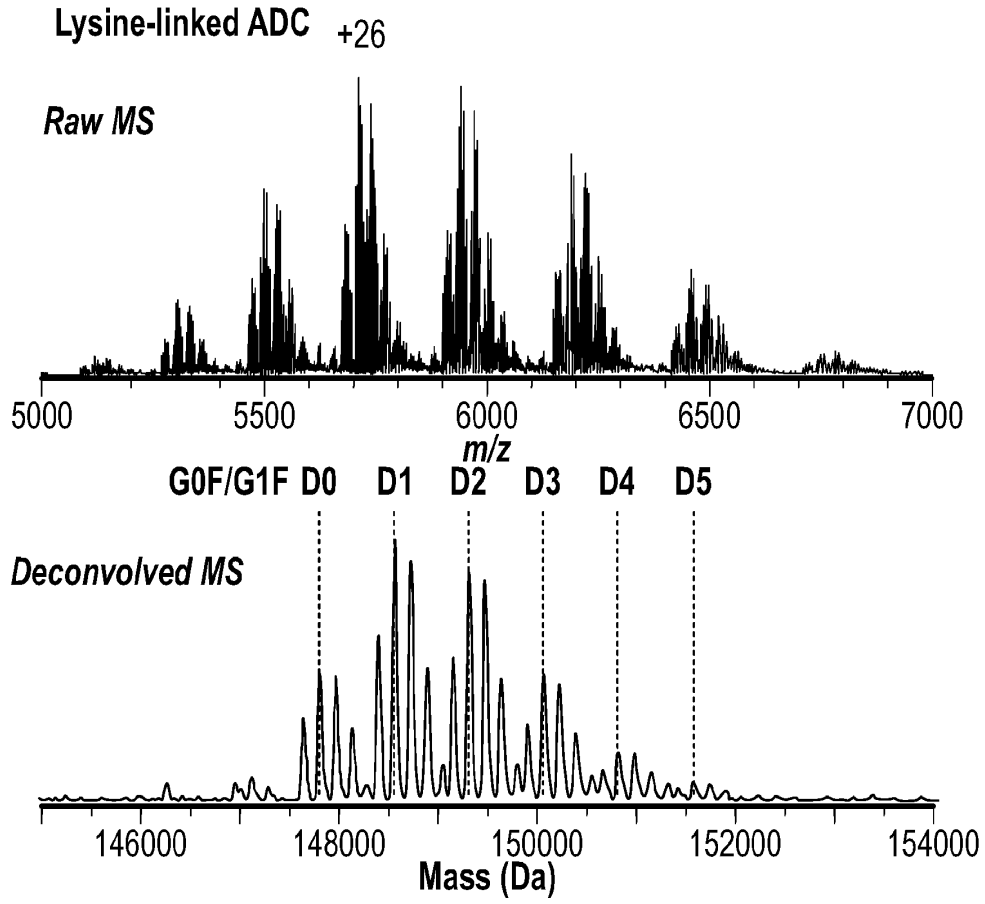

Lysine-linked ADC +26
Raw MS
5000
5500
6000
6500
7000
m/z
G0F/G1F D0 D1 D2 D3 D4 D5
Deconvolved MS
146000
148000
150000
152000
154000
Mass (Da)

FIG. 6

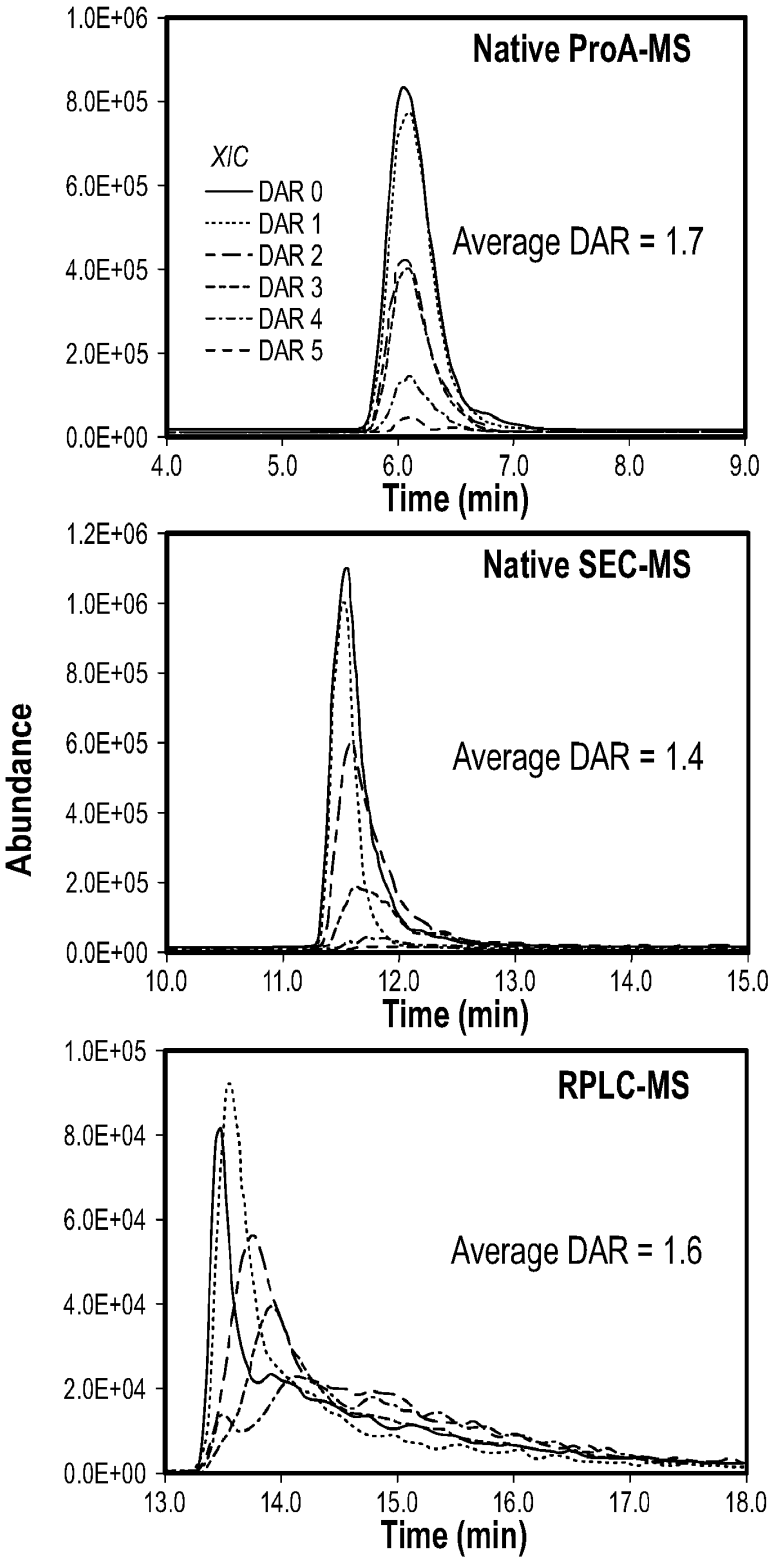

Native ProA-MS
XIC
DAR 0
DAR 1
DAR 2
DAR 3
DAR 4
DAR 5
Average DAR = 1.7
1.0E+06
8.0E+05
6.0E+05
4.0E+05
2.0E+05
0.0E+00
4.0
5.0
6.0
7.0
8.0
9.0
Time (min)
Native SEC-MS
Average DAR = 1.4
Abundance
1.2E+06
1.0E+06
8.0E+05
6.0E+05
4.0E+05
2.0E+05
0.0E+00
10.0
11.0
12.0
13.0
14.0
15.0
Time (min)
RPLC-MS
Average DAR = 1.6
1.0E+05
8.0E+04
6.0E+04
4.0E+04
2.0E+04
0.0E+00
13.0
14.0
15.0
16.0
17.0
18.0
Time (min)

FIG. 7

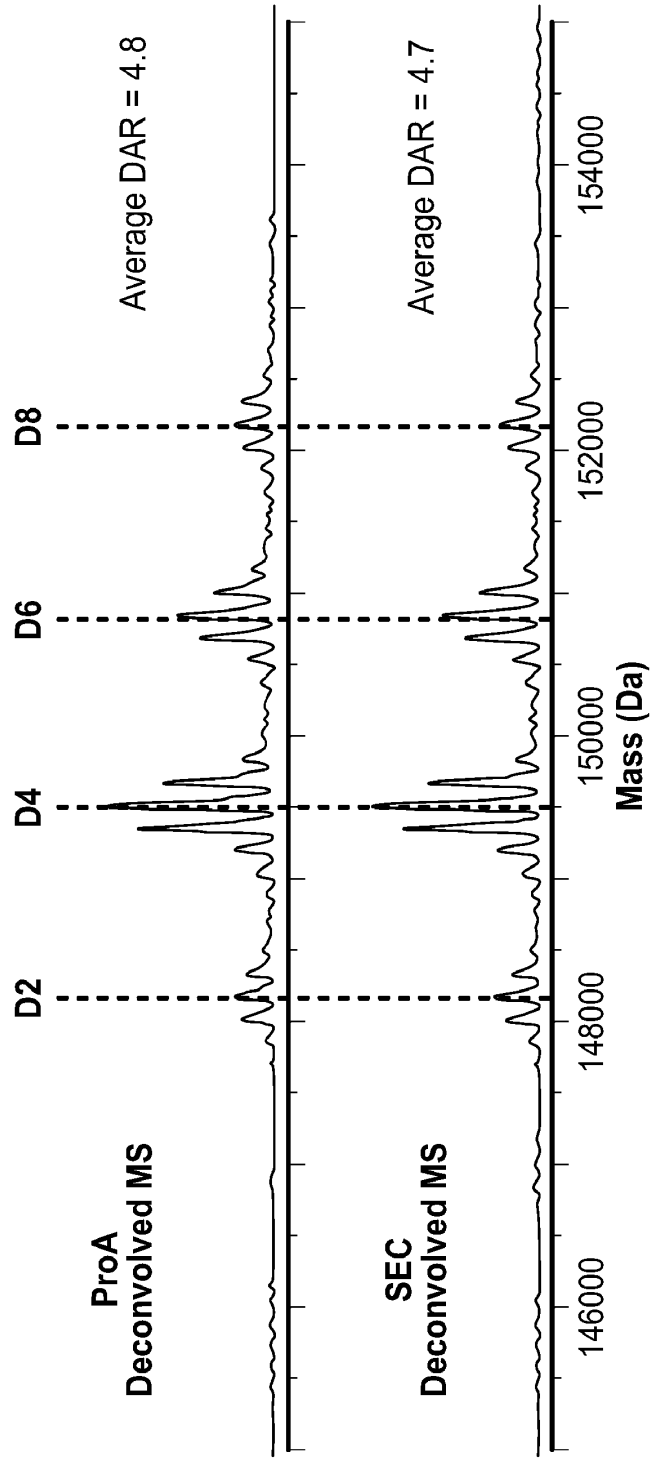

ProA
Deconvolved MS
SEC
Deconvolved MS
Average DAR = 4.8
Average DAR = 4.7
D2
D4
D6
D8
146000
148000
150000
152000
154000
Mass (Da)

FIG. 8

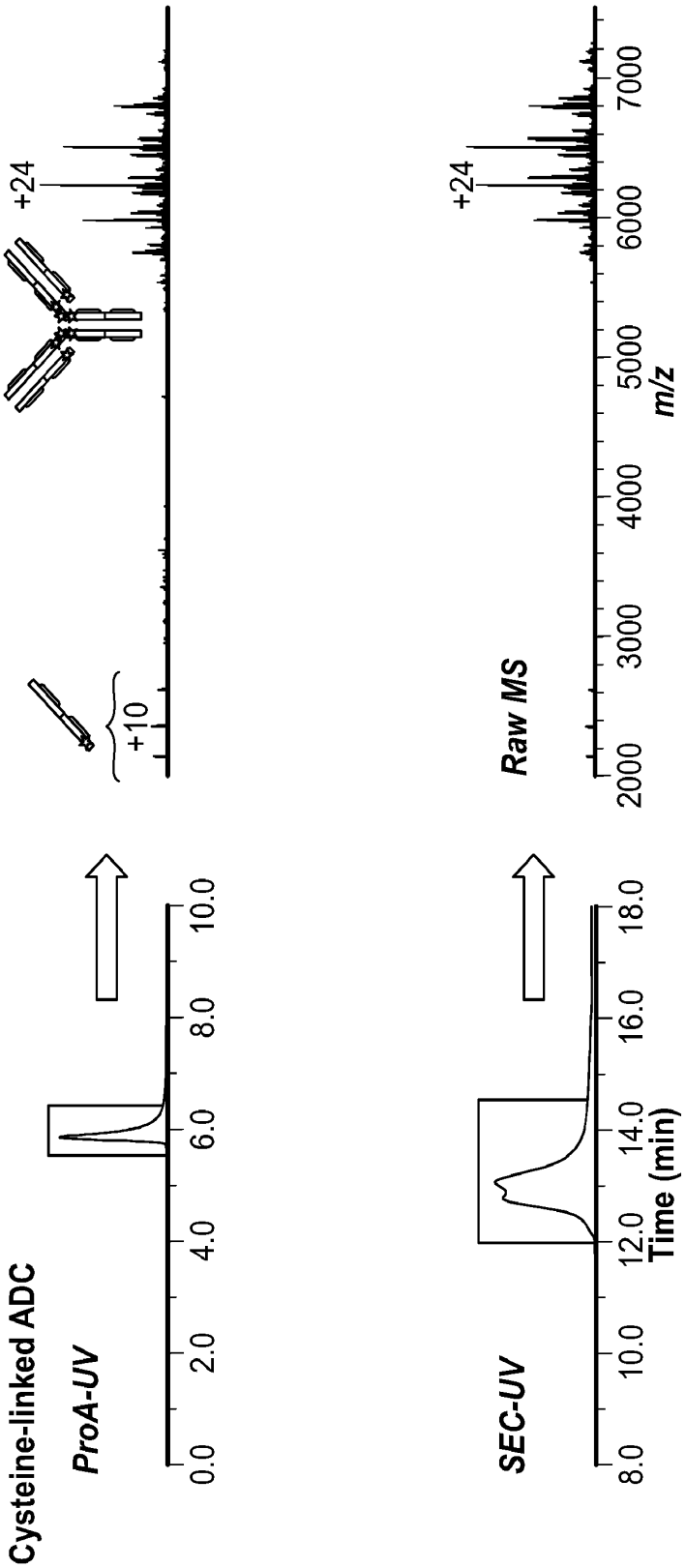

Cysteine-linked ADC
ProA-UV
0.0
2.0
4.0
6.0
8.0
10.0
+10
+24
SEC-UV
8.0
10.0
12.0
14.0
16.0
18.0
Time (min)
Raw MS
2000
3000
4000
5000
6000
7000
m/z
+24

FIG. 9

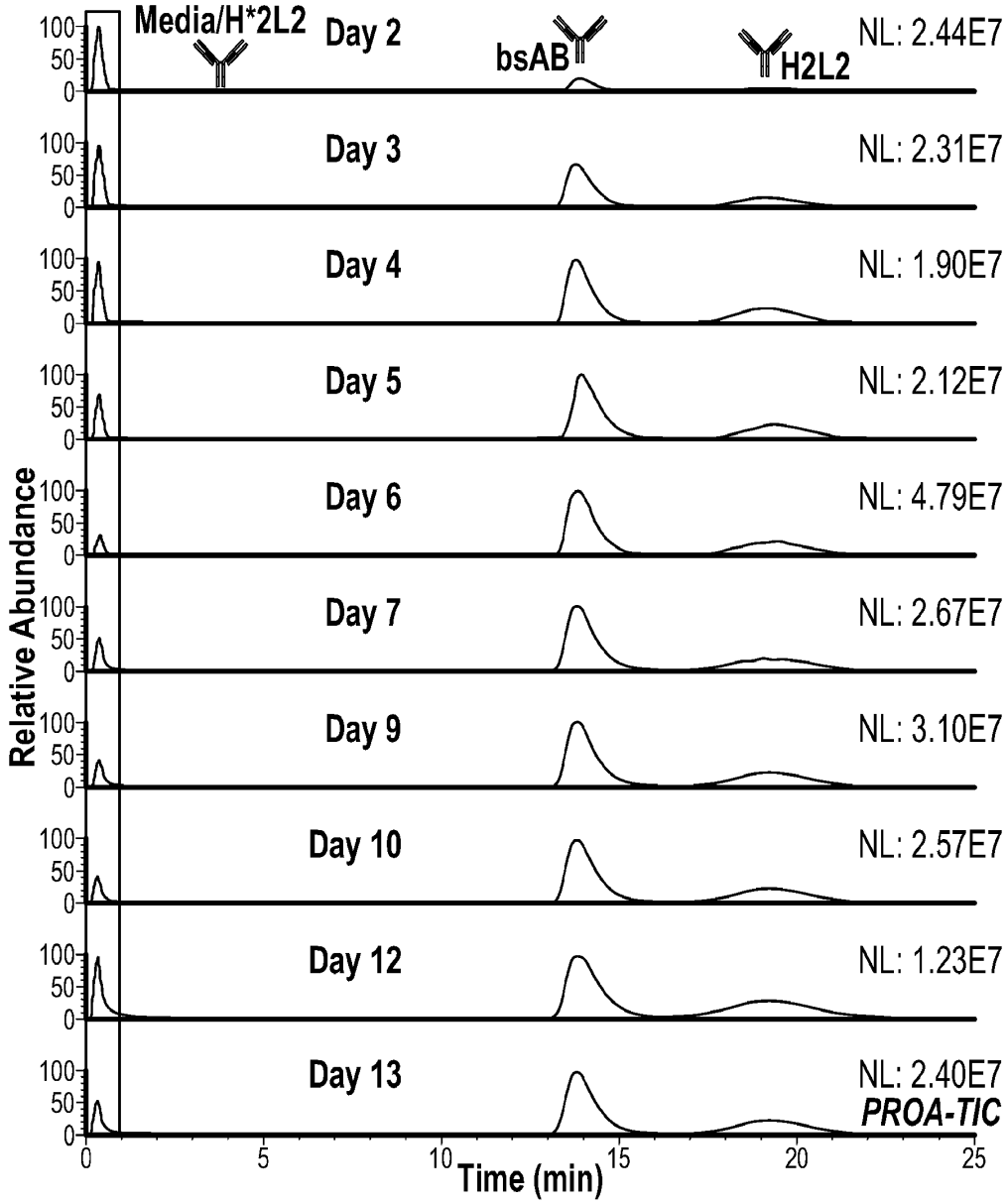
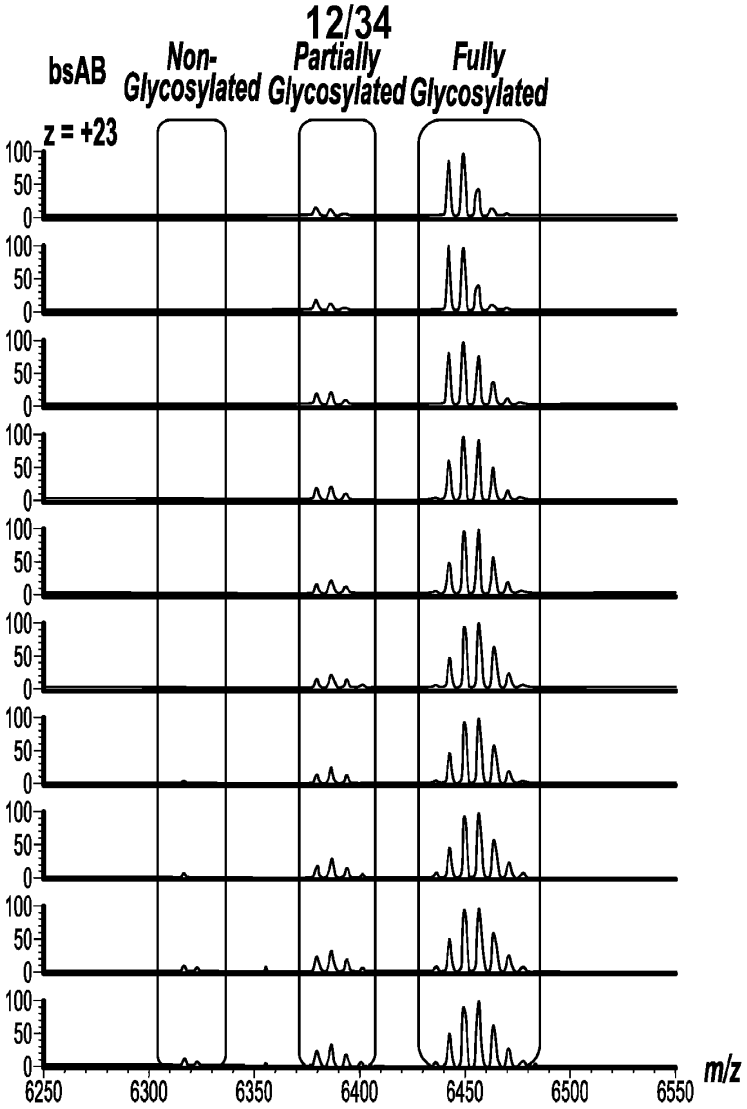

Media/H*2L2
bsAB
H2L2
Day 2
NL: 2.44E7
Day 3
NL: 2.31E7
Day 4
NL: 1.90E7
Day 5
NL: 2.12E7
Day 6
NL: 4.79E7
Day 7
NL: 2.67E7
Day 9
NL: 3.10E7
Day 10
NL: 2.57E7
Day 12
NL: 1.23E7
Day 13
NL: 2.40E7
PROA-TIC
Relative Abundance
100
50
0
0
5
10
15
20
25
Time (min)

FIG. 10



Titers of MAB4 in cell culture time course samples

Day	Fc*/Fc Titer (mg/mL)
1	0.002842505
2	
3	
4	
5	0.270078506
6	0.544304624
7	
9	
10	1.224500254
11	1.412629501
12	1.423253484

FIG. 10 (Continued)

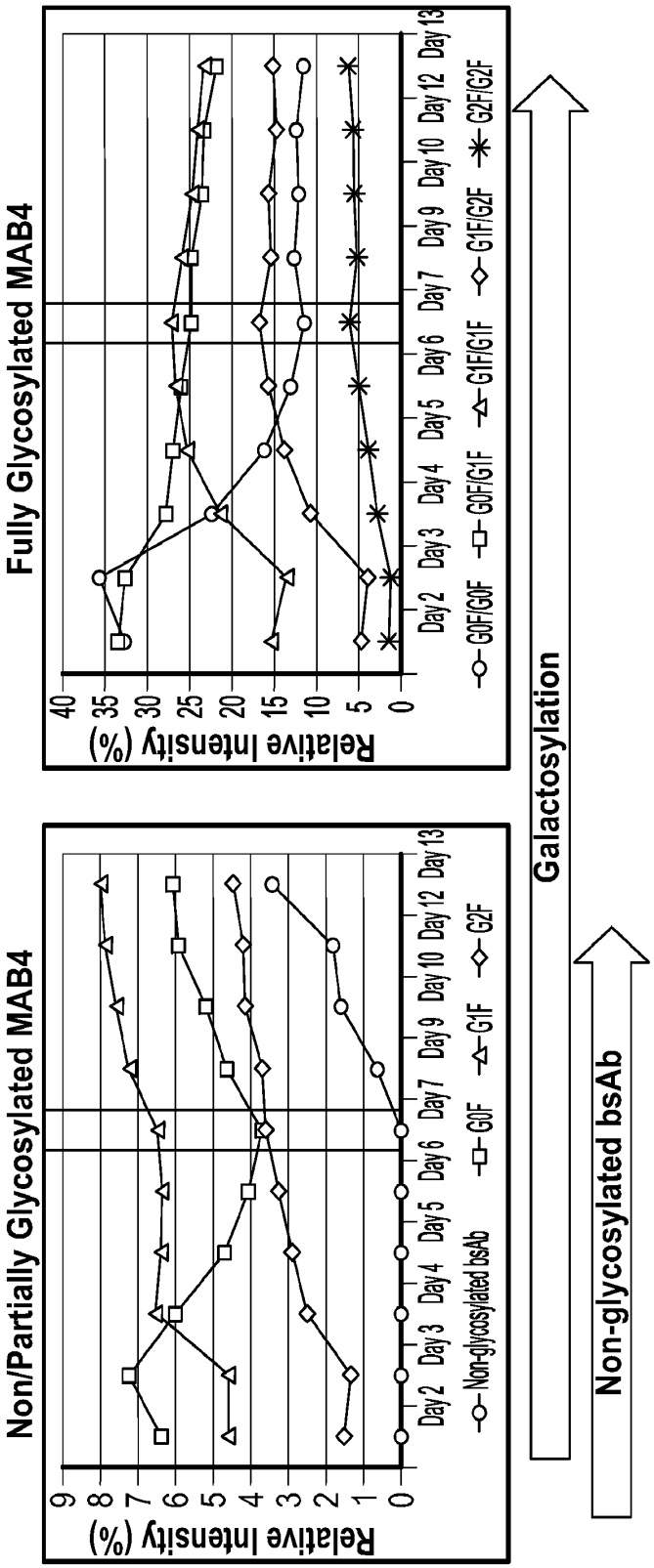

Fully Glycosylated MAB4
Relative Intensity (%)
0
5
10
15
20
25
30
35
40
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 9
Day 10
Day 12
Day 13
G0F/G0F
G0F/G1F
G1F/G1F
G1F/G2F
G2F/G2F
Galactosylation
Non/Partially Glycosylated MAB4
Relative Intensity (%)
0
1
2
3
4
5
6
7
8
9
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 9
Day 10
Day 12
Day 13
Non-glycosylated bsAb
G0F
G1F
G2F
Non-glycosylated bsAb

FIG. 11

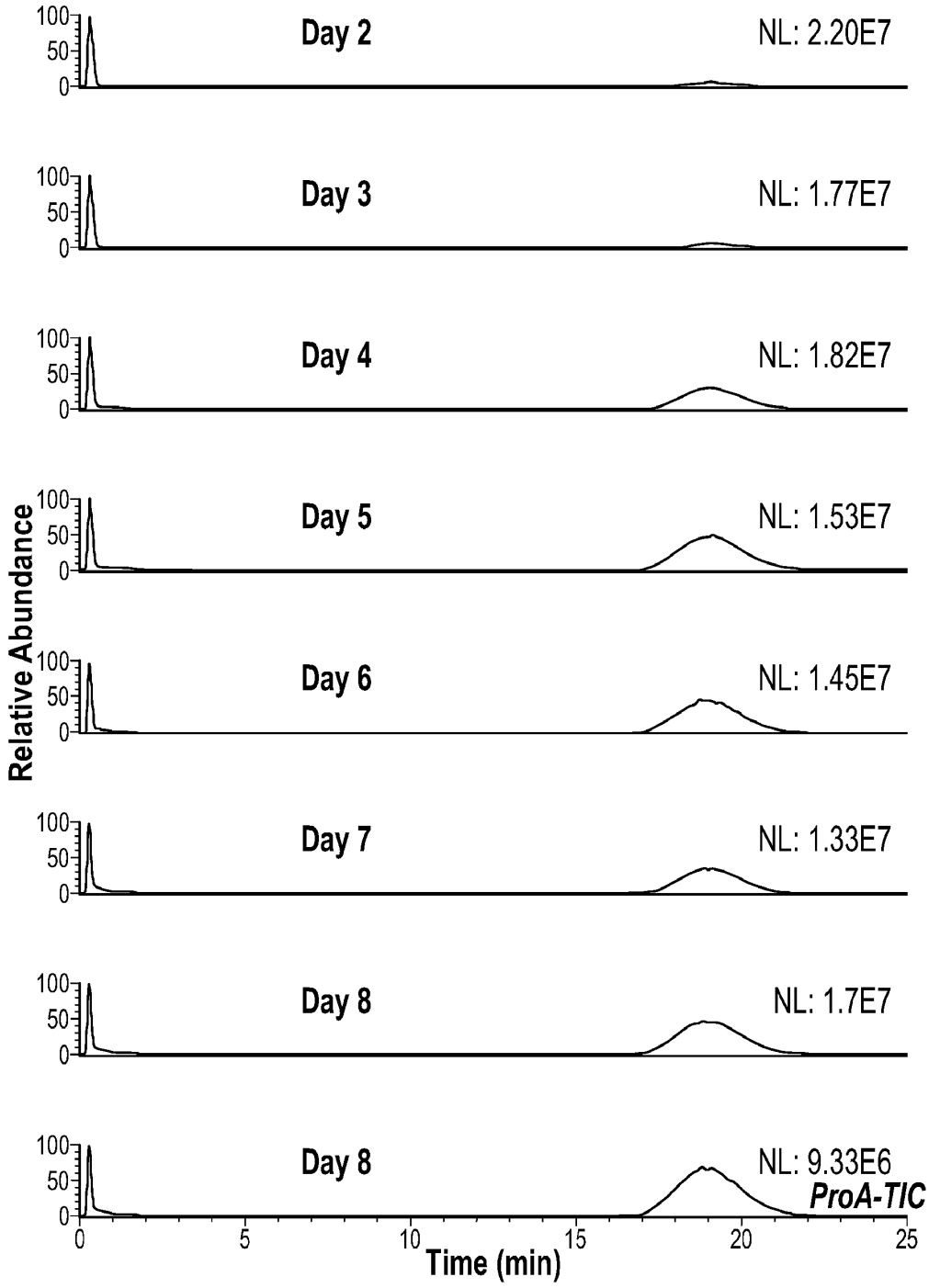
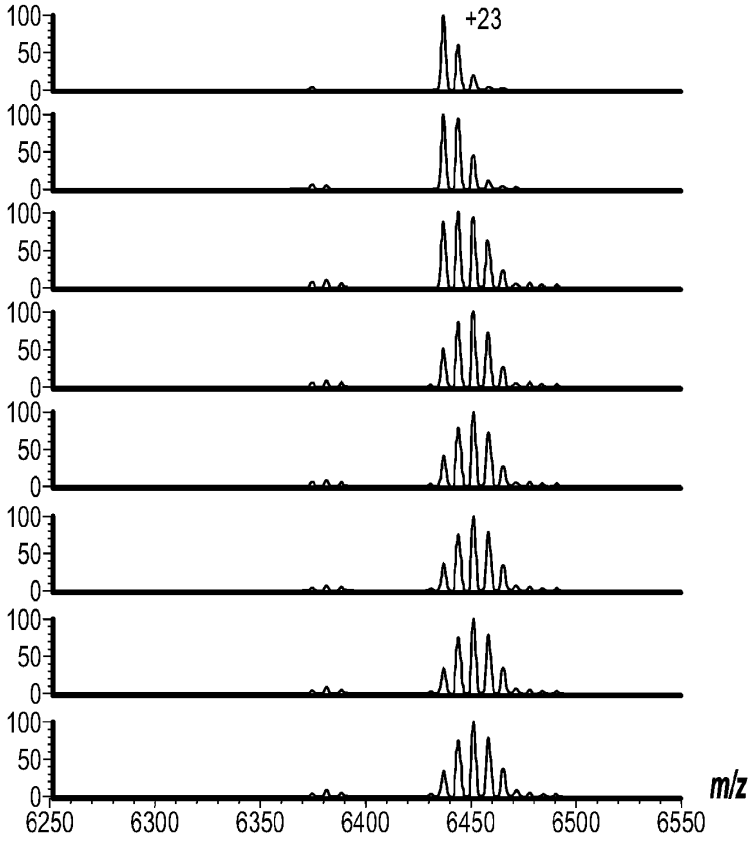

Day 2
NL: 2.20E7
Day 3
NL: 1.77E7
Day 4
NL: 1.82E7
Day 5
NL: 1.53E7
Day 6
NL: 1.45E7
Day 7
NL: 1.33E7
Day 8
NL: 1.7E7
Day 8
NL: 9.33E6
ProA-TIC
Relative Abundance
100
50
0
0
5
10
15
20
25
Time (min)

FIG. 12



Titers of MAB5 in cell Culture time course samples

Day	Titer (mg/mL)
2	0.03377006
3	0.043047705
4	0.148567665
5	0.272146707
6	0.481532535
7	0.677272655
8	0.80840479
9	0.865176048

FIG. 12 (Continued)

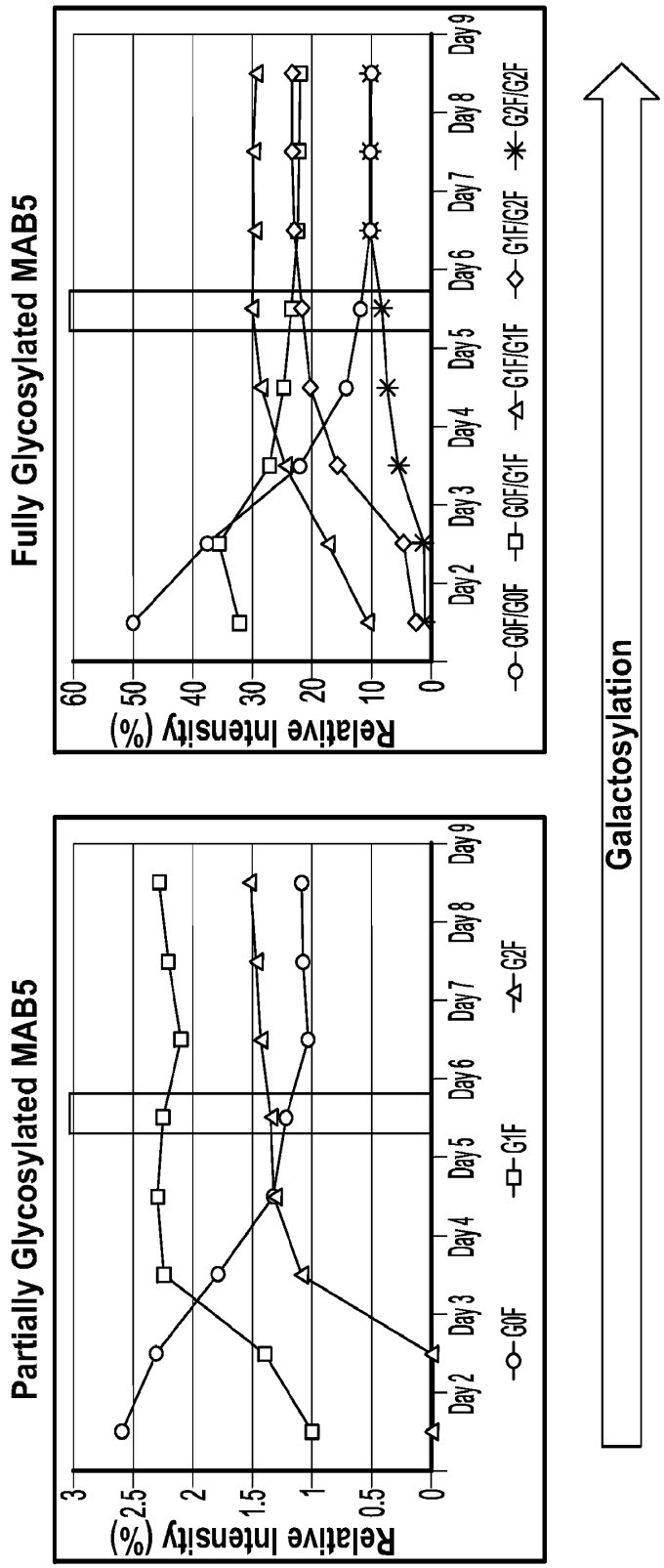

Fully Glycosylated MAB5
Relative Intensity (%)
0
10
20
30
40
50
60
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
G0F/G0F
G0F/G1F
G1F/G1F
G1F/G2F
G2F/G2F
Partially Glycosylated MAB5
Relative Intensity (%)
0
0.5
1
1.5
2
2.5
3
G0F
G1F
G2F
Galactosylation

FIG. 13

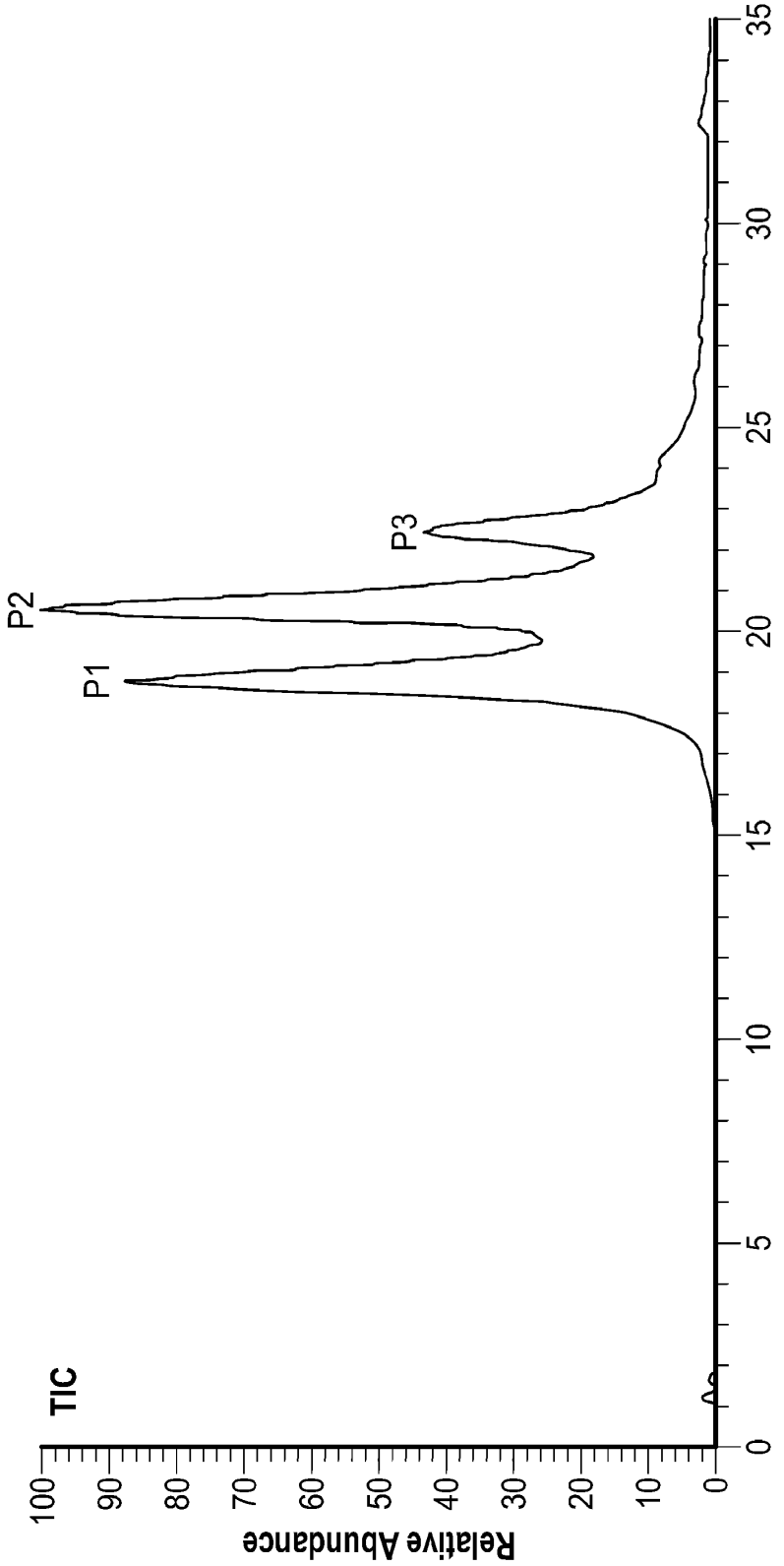

TIC
Relative Abundance
Time (min)
P1
P2
P3
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30
35

FIG. 14

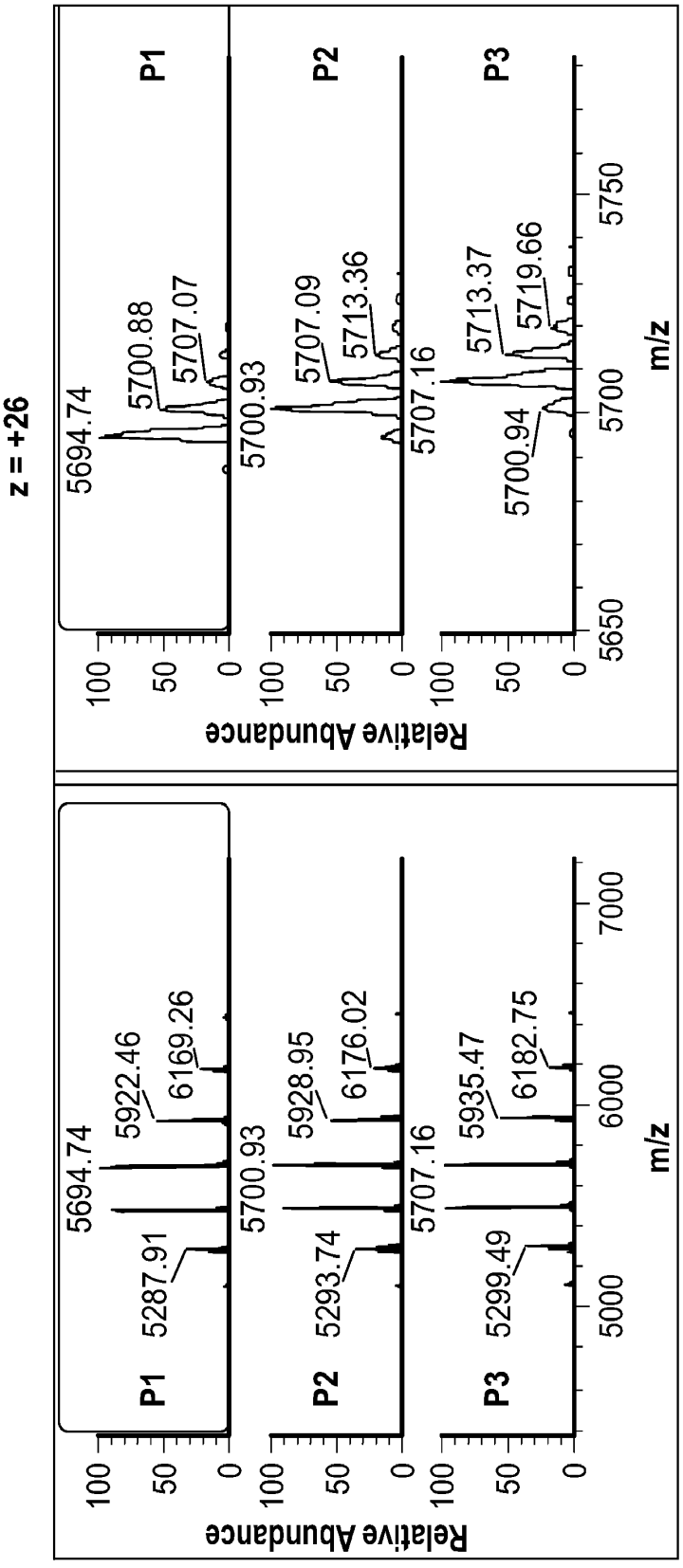


FIG. 14 (Continued)

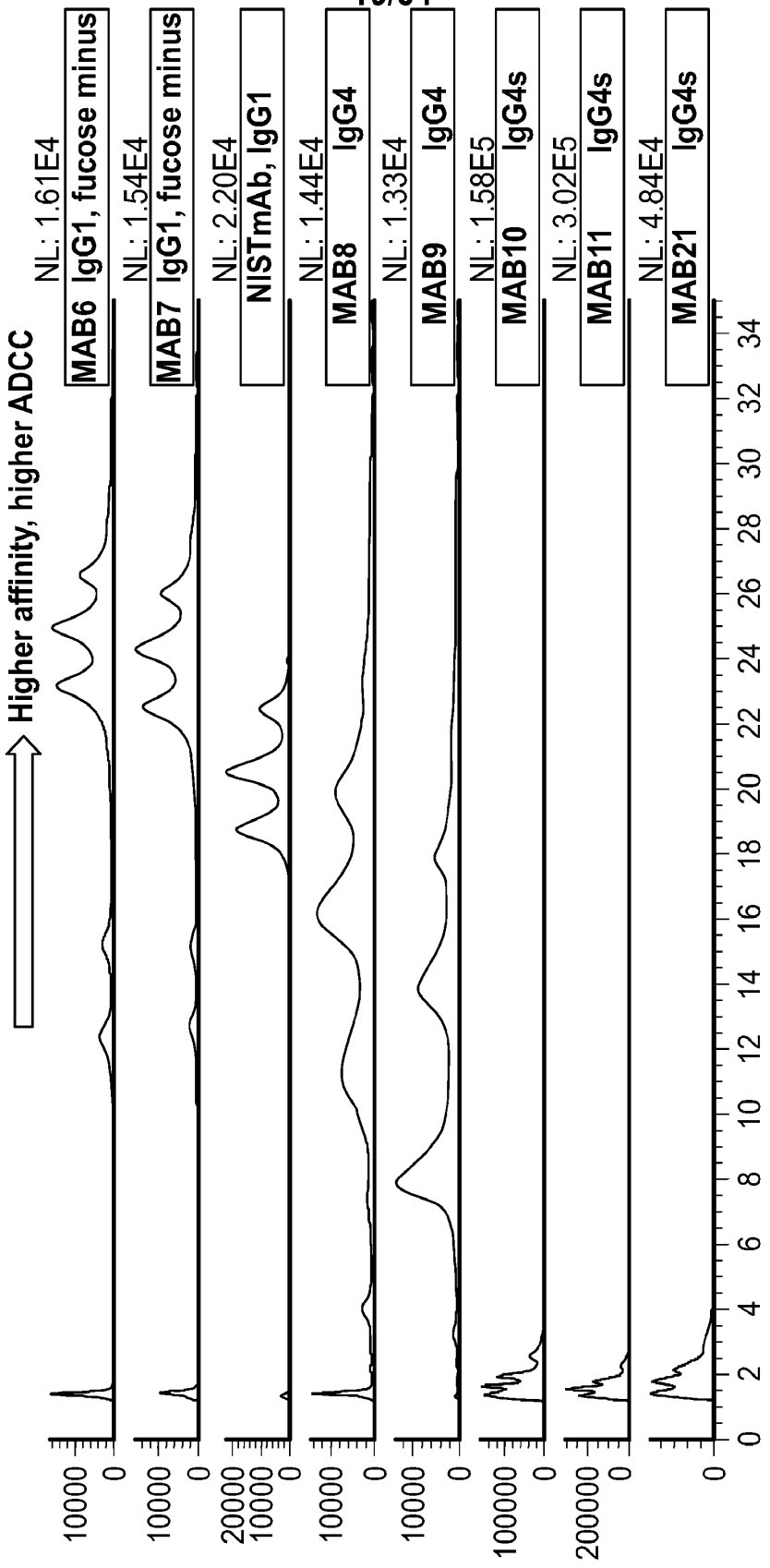
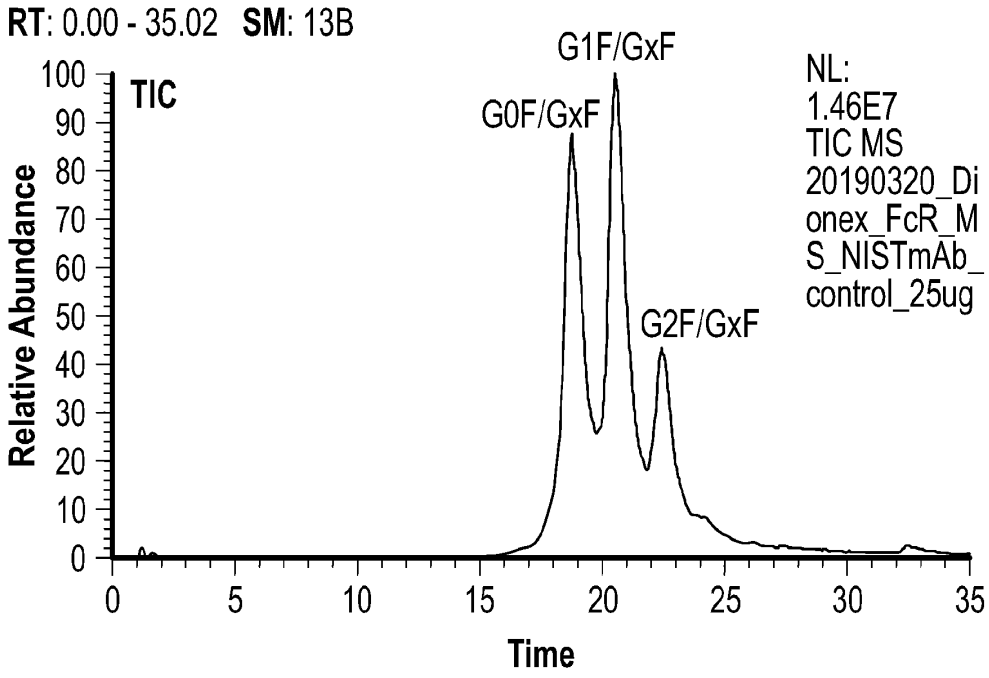

Higher affinity, higher ADCC
NL: 1.61E4
MAB6 IgG1, fucose minus
NL: 1.54E4
MAB7 IgG1, fucose minus
NL: 2.20E4
NISTmAb, IgG1
NL: 1.44E4
MAB8 IgG4
NL: 1.33E4
MAB9 IgG4
NL: 1.58E5
MAB10 IgG4s
NL: 3.02E5
MAB11 IgG4s
NL: 4.84E4
MAB21 IgG4s
Time (min)

FIG. 15


RT: 0.00 - 35.02 SM: 13B
TIC
G1F/GxF
G0F/GxF
G2F/GxF
NL:
1.46E7
TIC MS
20190320_Di
onex_FcR_M
S_NISTmAb_
control_25ug
Relative Abundance
Time

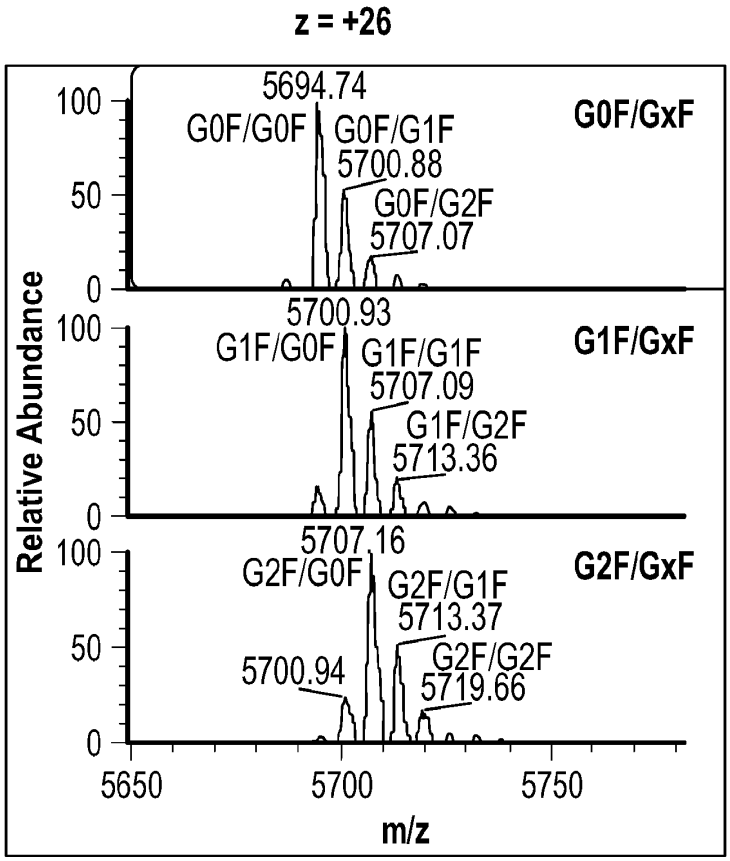

z = +26
5694.74
G0F/G0F
G0F/G1F
5700.88
G0F/G2F
5707.07
G0F/GxF
5700.93
G1F/G0F
G1F/G1F
5707.09
G1F/G2F
5713.36
G1F/GxF
5707.16
G2F/G0F
G2F/G1F
5713.37
G2F/G2F
5700.94
5719.66
G2F/GxF
Relative Abundance
m/z

FIG. 16

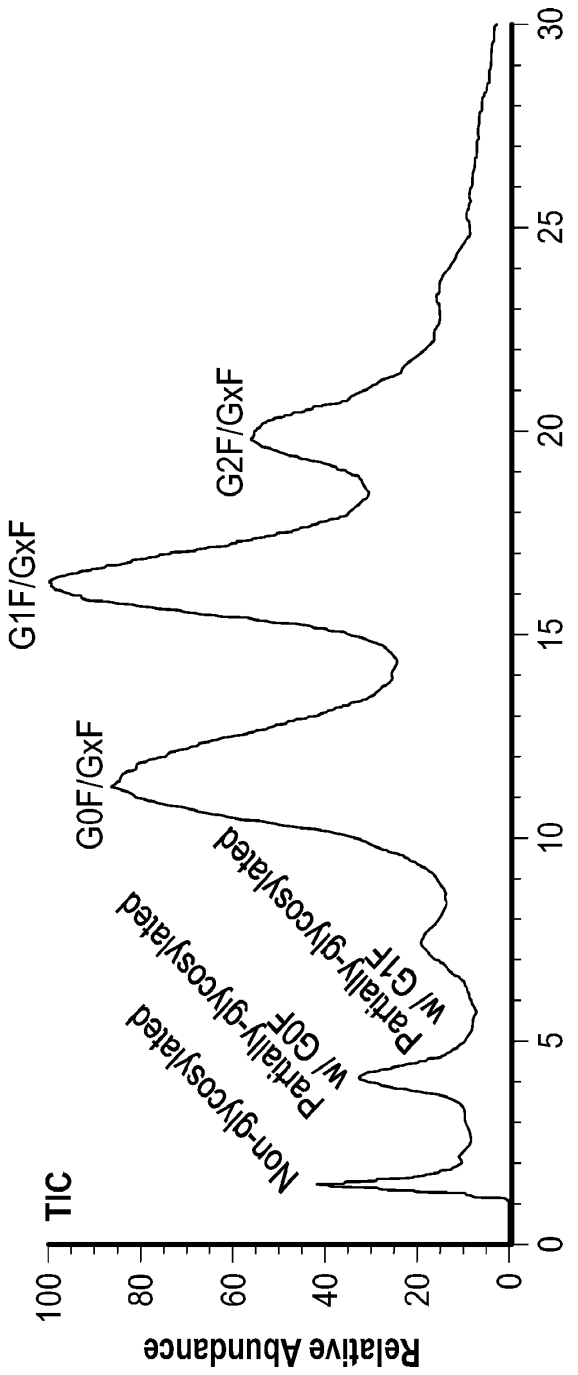

TIC
Relative Abundance
Time (min)
Non-glycosylated
Partially-glycosylated w/ G0F
Partially-glycosylated w/ G1F
G0F/GxF
G1F/GxF
G2F/GxF
0
20
40
60
80
100
0
5
10
15
20
25
30

FIG. 17

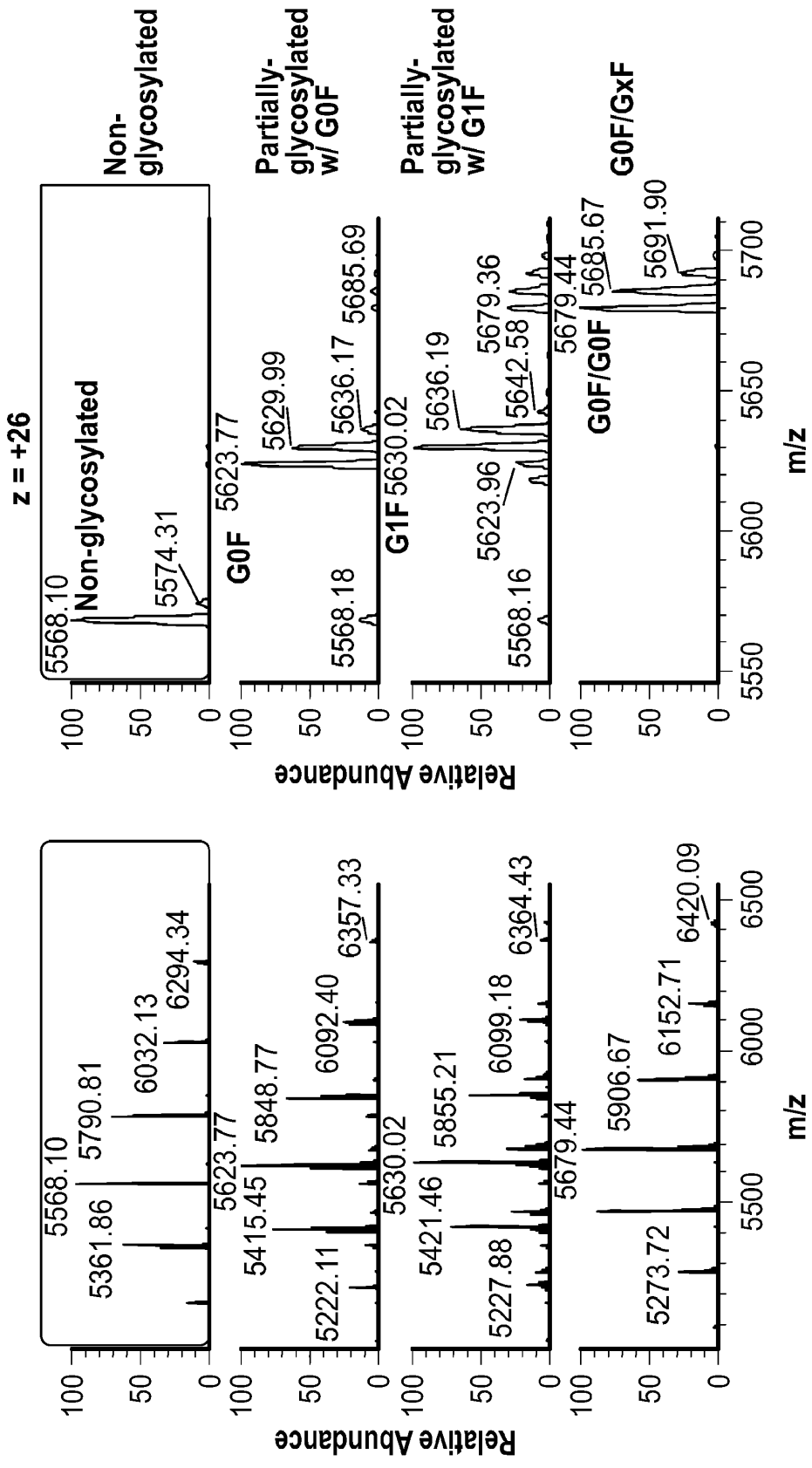


FIG. 17 (Continued)

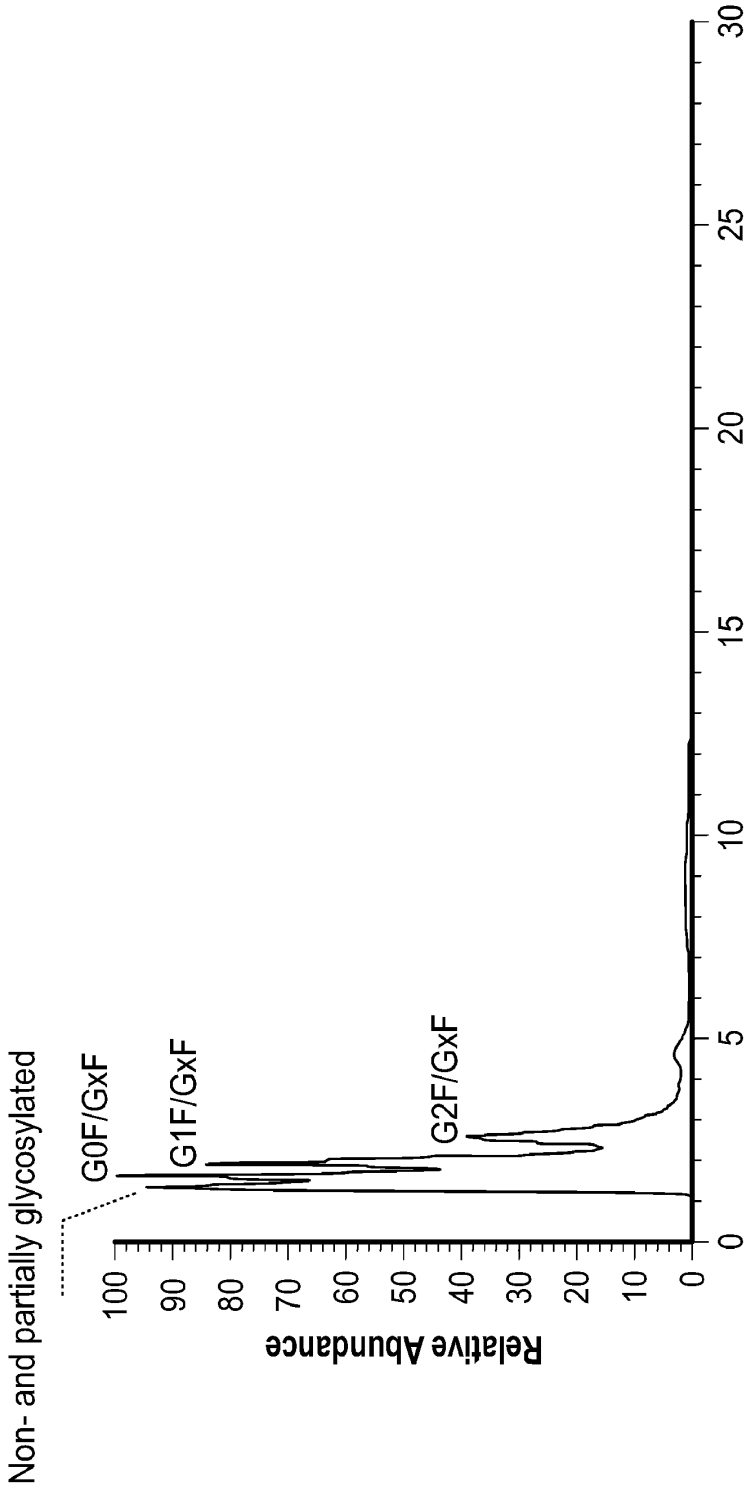

Non- and partially glycosylated
G0F/GxF
G1F/GxF
G2F/GxF
Relative Abundance
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
Time (min)

FIG. 18

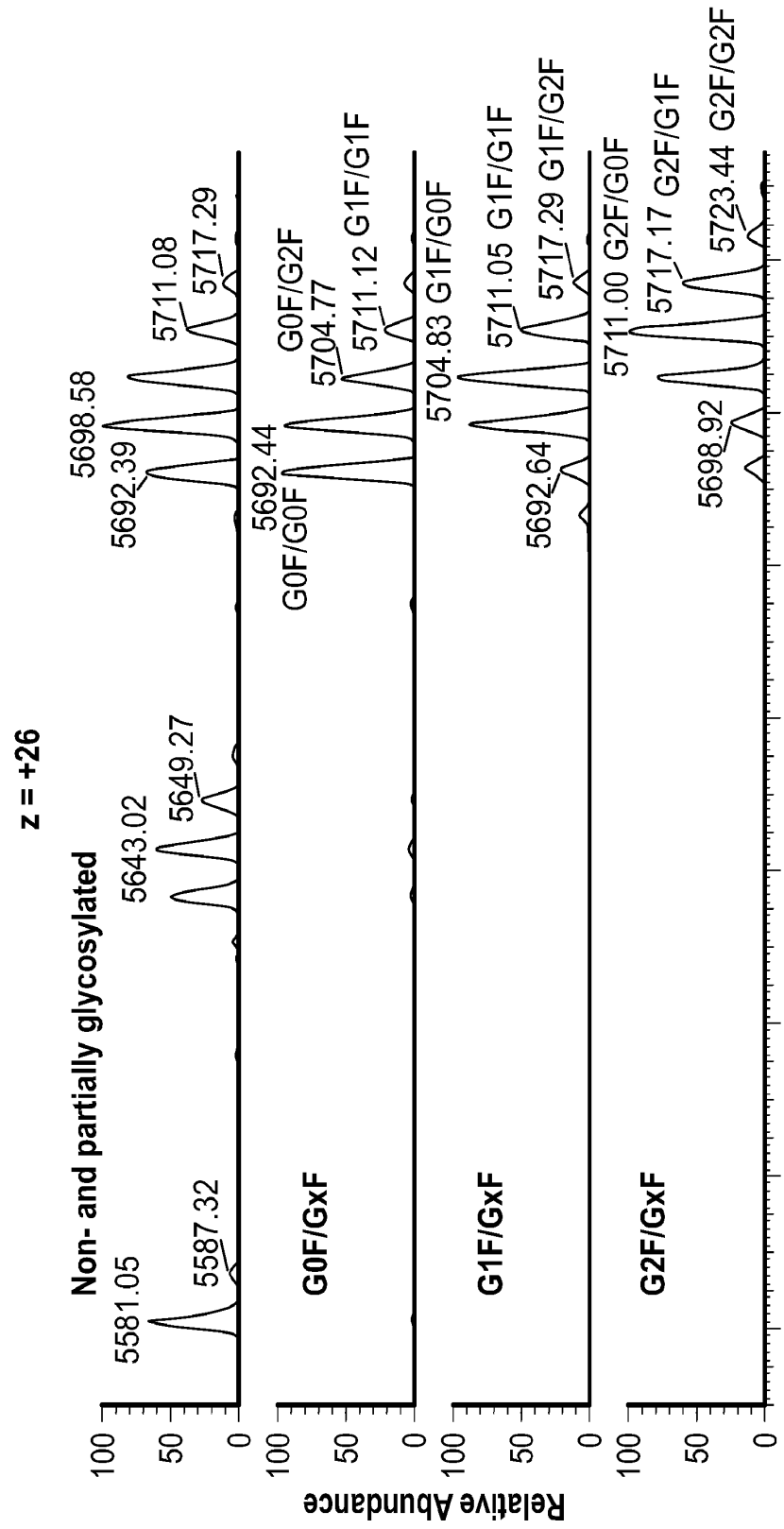


FIG. 18 (Continued)

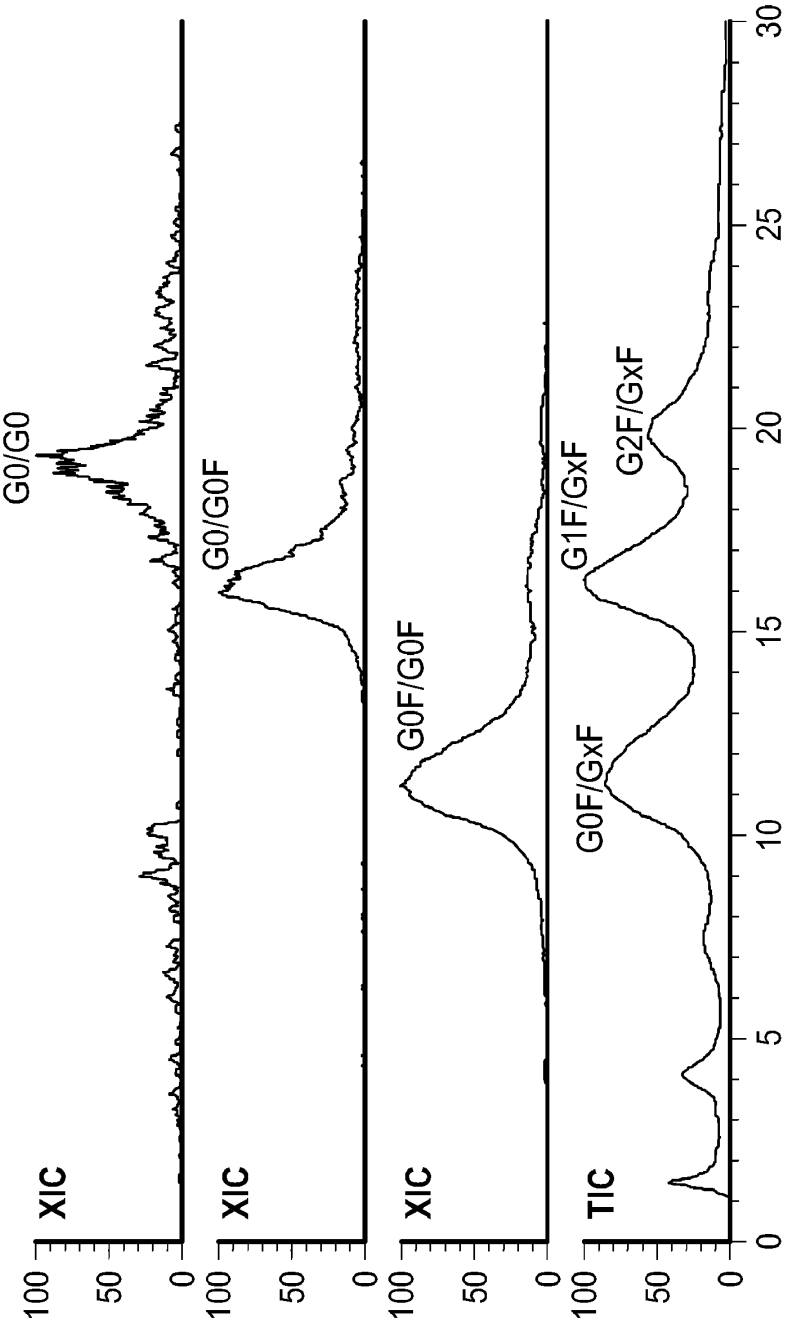

G0/G0
G0/G0F
G0F/G0F
G0F/GxF
G1F/GxF
G2F/GxF
XIC
XIC
XIC
TIC
100
50
0
0
5
10
15
20
25
30
Time (min)

FIG. 19

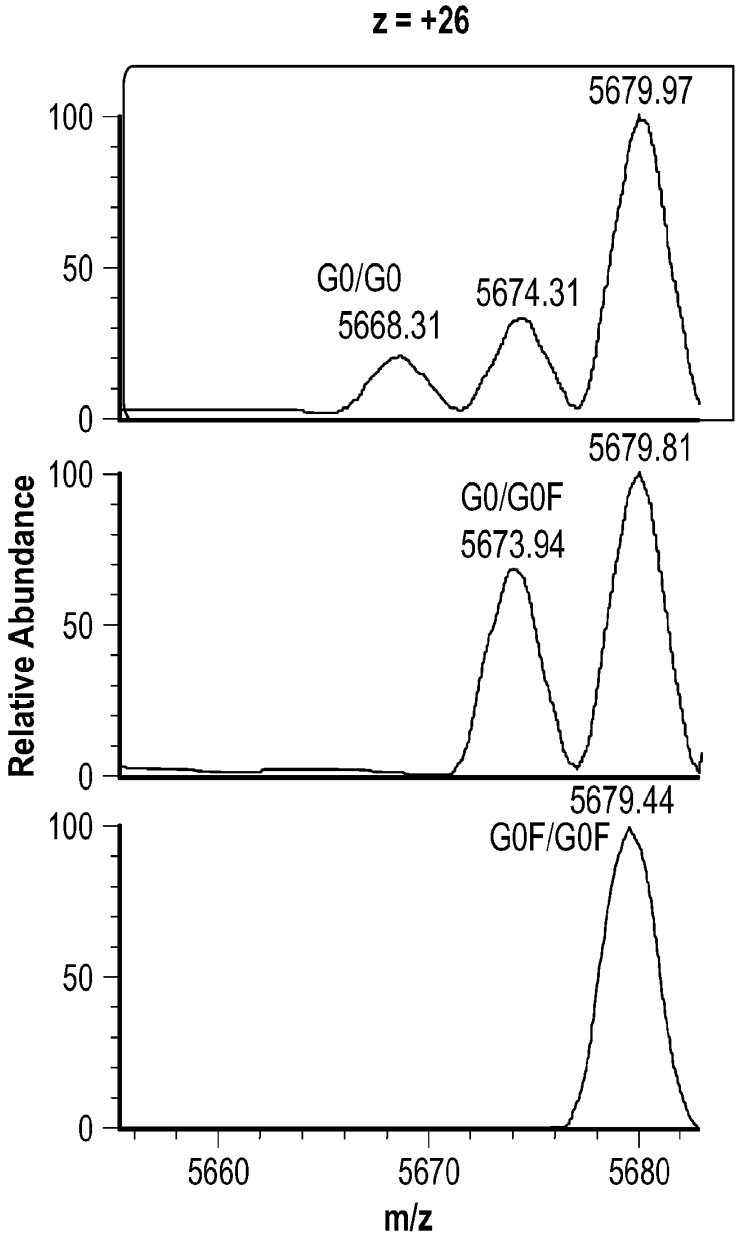


FIG. 19 (Continued)

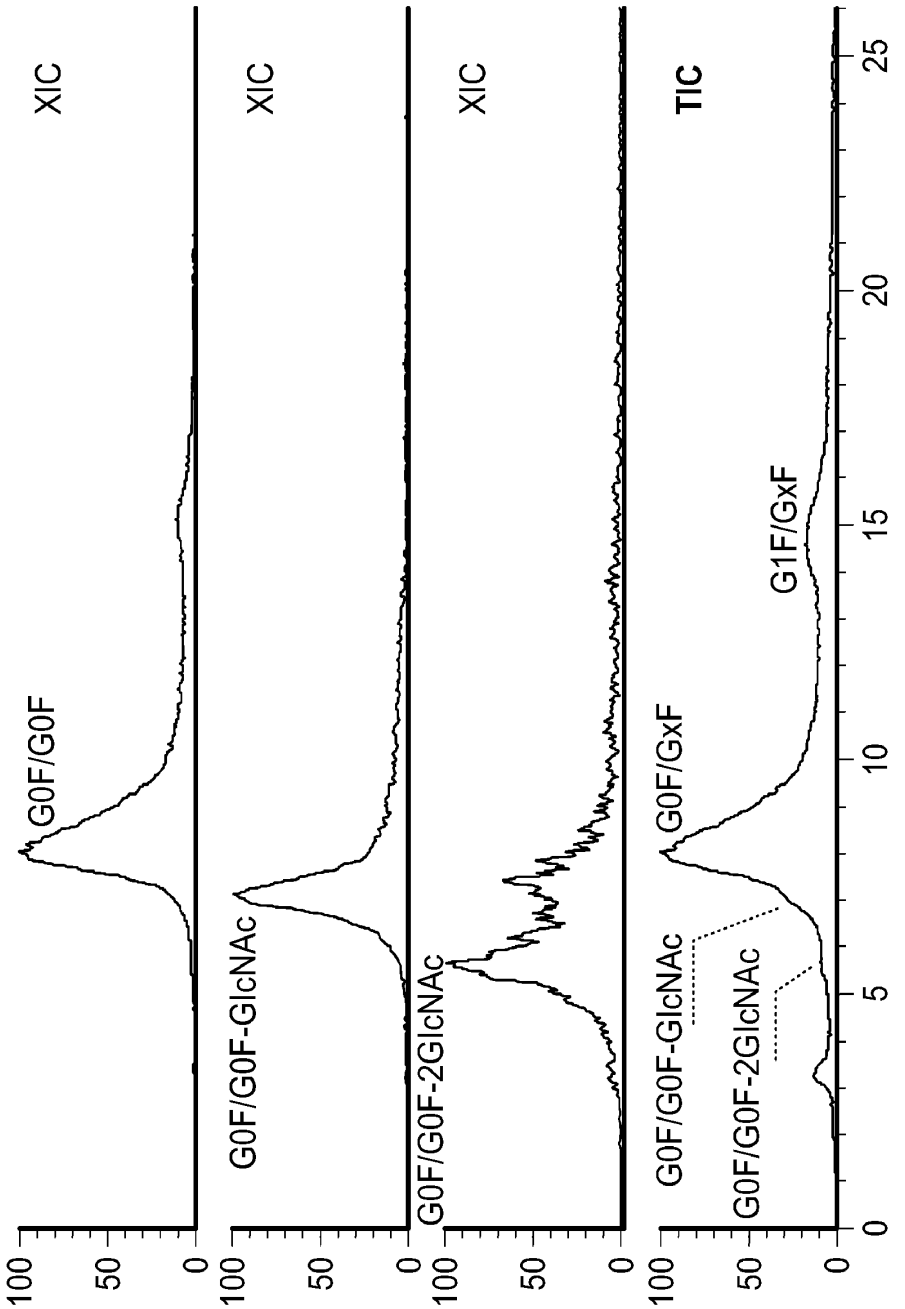

XIC
G0F/G0F
XIC
G0F/G0F-GlcNAc
XIC
G0F/G0F-2GlcNAc
TIC
G0F/G0F-GlcNAc
G0F/G0F-2GlcNAc
G0F/GxF
G1F/GxF
100
50
0
0
5
10
15
20
25
Time (min)

FIG. 20

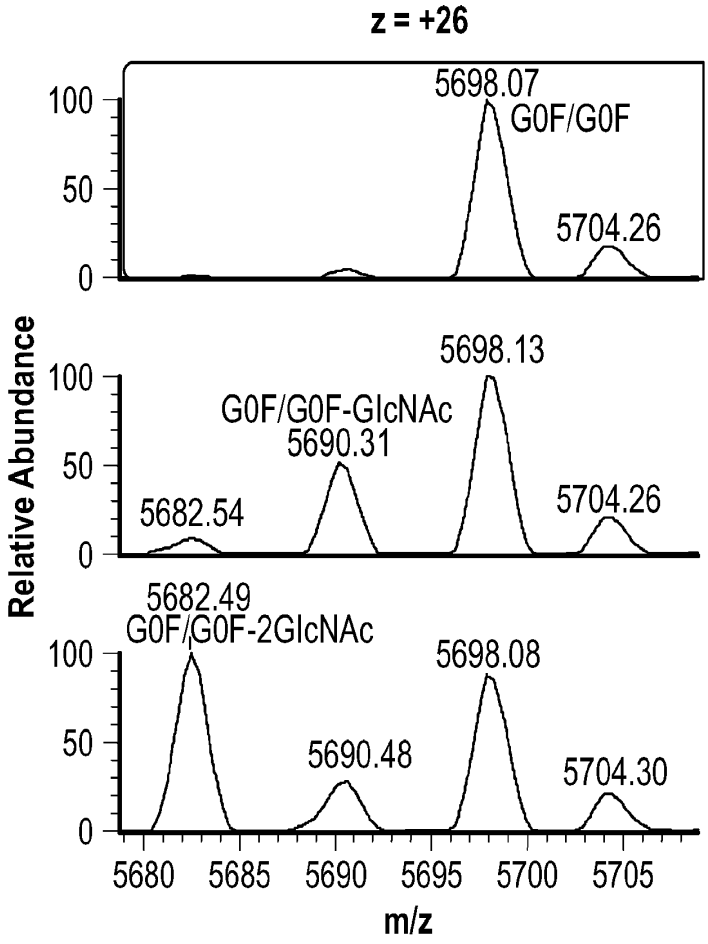
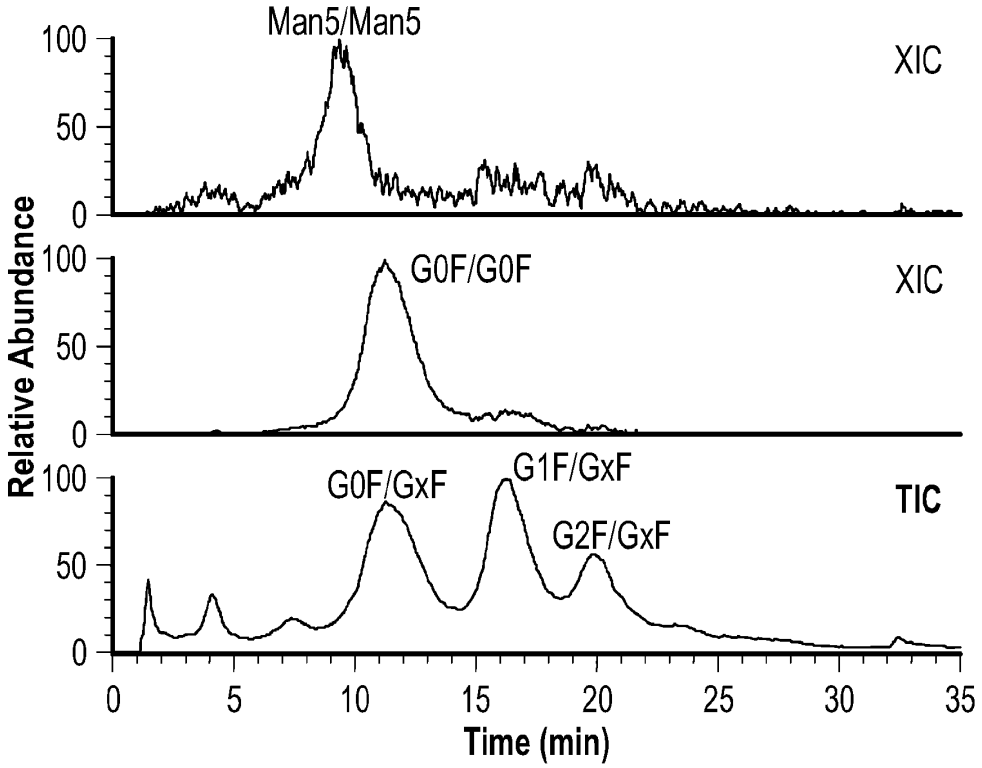


FIG. 20 (Continued)


Man5/Man5
XIC
G0F/G0F
XIC
G0F/GxF
G1F/GxF
G2F/GxF
TIC
Relative Abundance
Time (min)

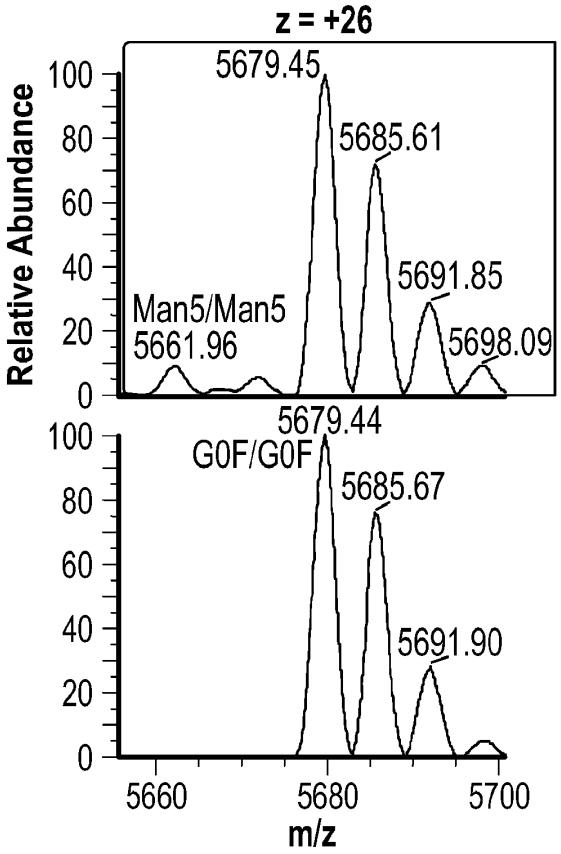

z = +26
5679.45
5685.61
5691.85
Man5/Man5
5661.96
5698.09
5679.44
G0F/G0F
5685.67
5691.90
Relative Abundance
m/z

FIG. 21

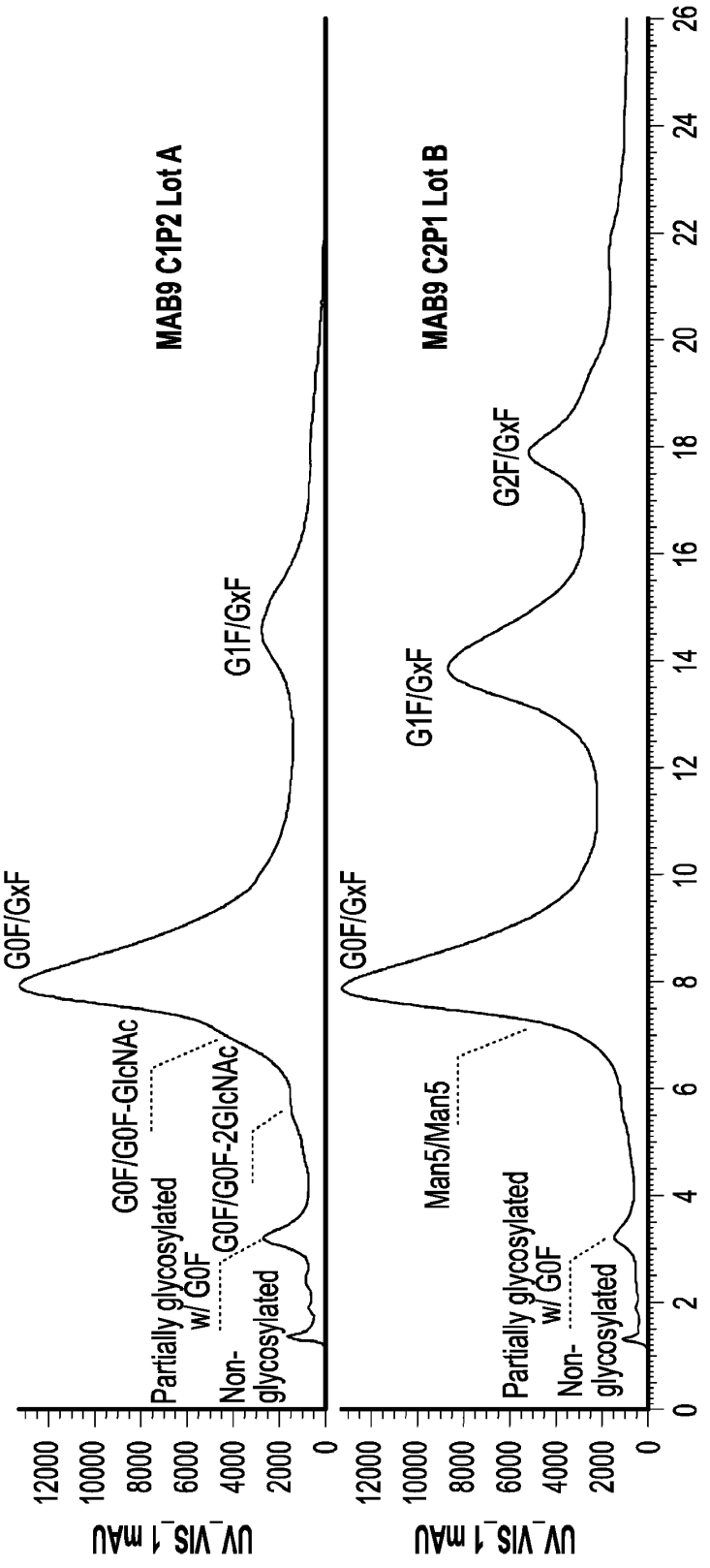

MAB9 C1P2 Lot A
G0F/GxF
G1F/GxF
G0F/G0F-GlcNAc
G0F/G0F-2GlcNAc
Partially glycosylated w/ G0F
Non-glycosylated
MAB9 C2P1 Lot B
G0F/GxF
G1F/GxF
G2F/GxF
Man5/Man5
Partially glycosylated w/ G0F
Non-glycosylated
UV_VIS_1 mAU
Time (min)

FIG. 22

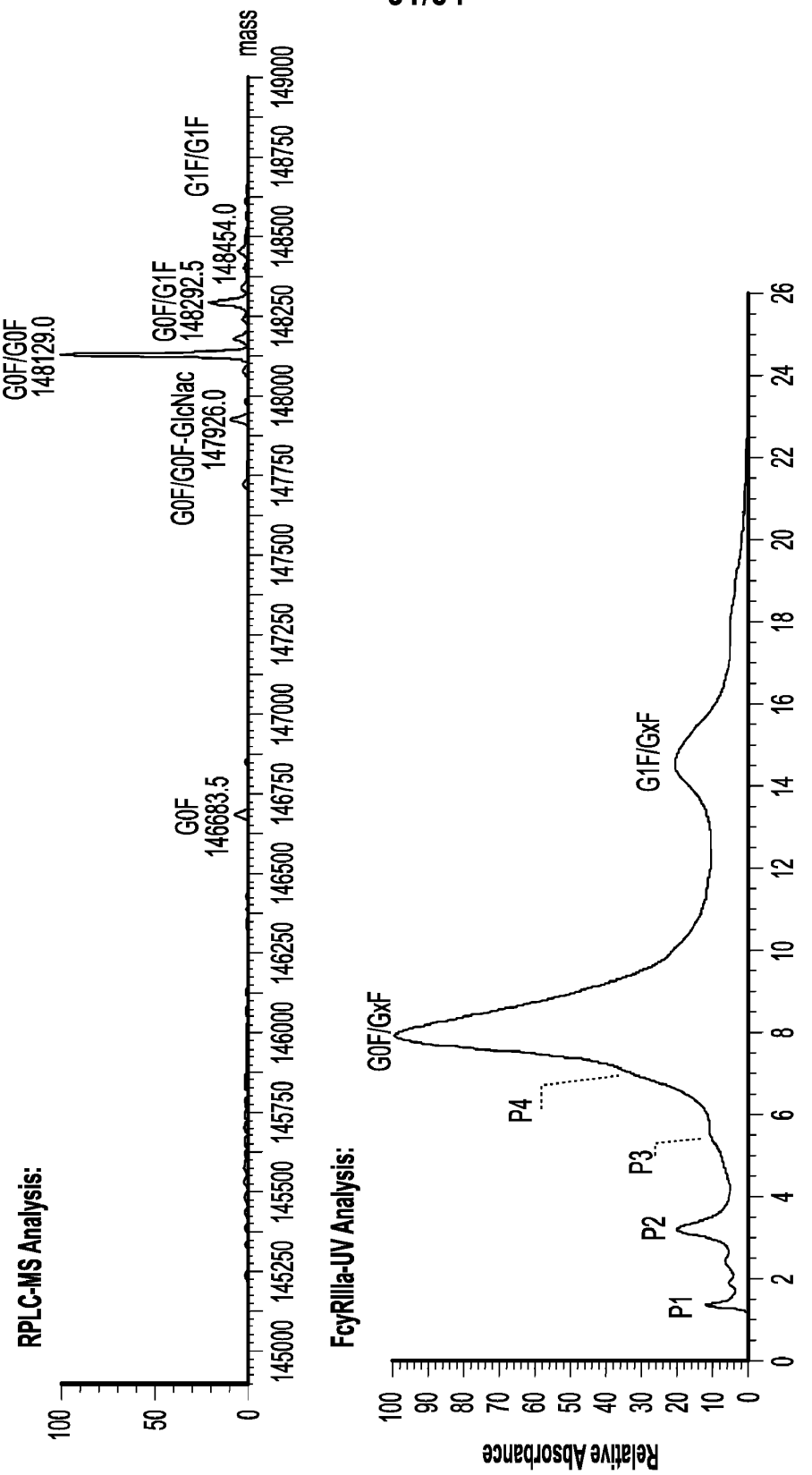

RPLC-MS Analysis:
G0F/G0F
148129.0
G0F/G1F
148292.5
G1F/G1F
148454.0
G0F/G0F-GlcNac
147926.0
G0F
146683.5
mass
FcγRIIIa-UV Analysis:
G0F/GxF
P4
P3
P2
P1
G1F/GxF
Relative Absorbance
Time (min)

FIG. 23A

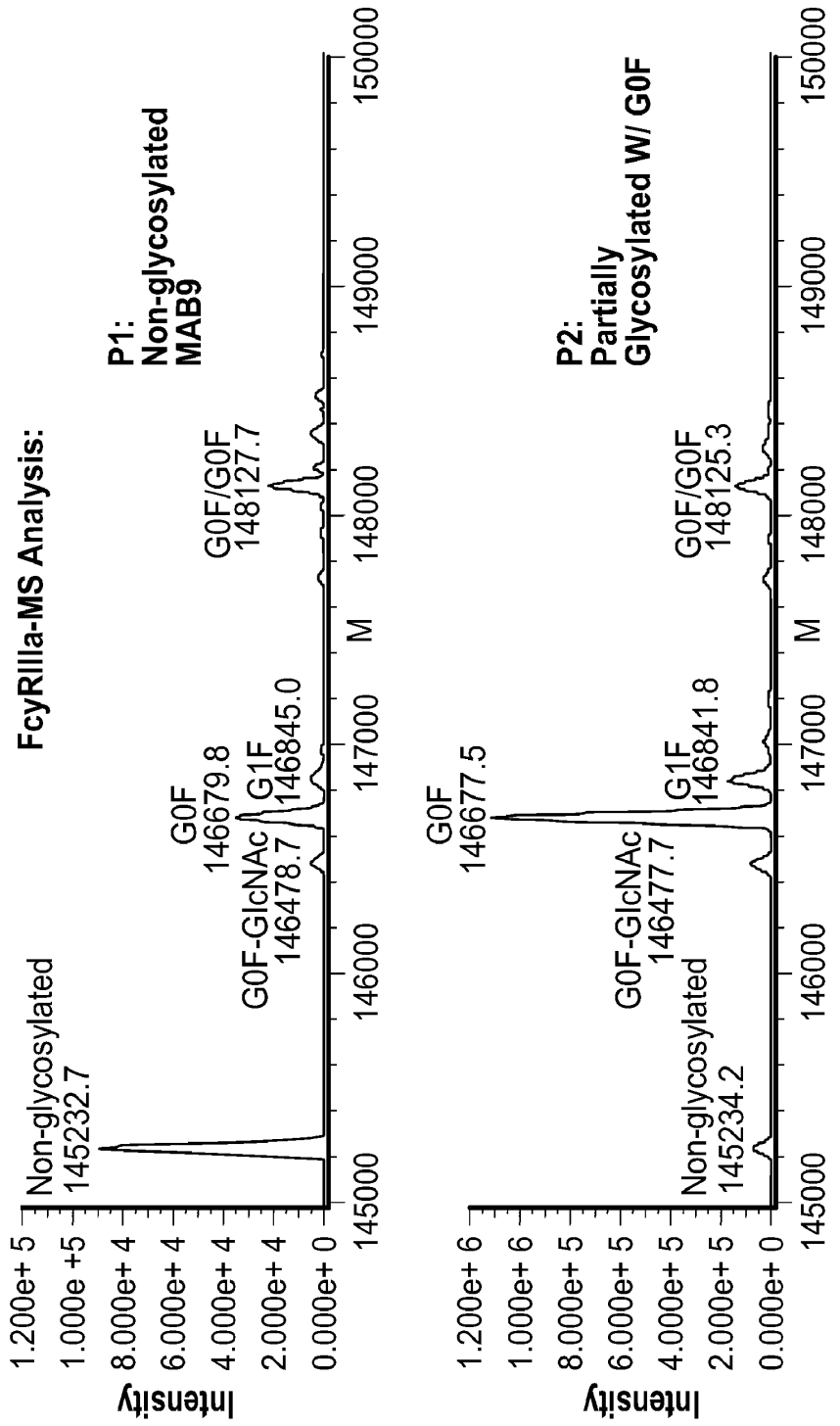

FcyRIIIa-MS Analysis:
P1:
Non-glycosylated
MAB9
Non-glycosylated
145232.7
G0F-GlcNAc
146478.7
G0F
146679.8
G1F
146845.0
G0F/G0F
148127.7
Intensity
1.200e+ 5
1.000e +5
8.000e+ 4
6.000e+ 4
4.000e+ 4
2.000e+ 4
0.000e+ 0
145000
146000
147000
M
148000
149000
150000
P2:
Partially
Glycosylated W/ G0F
Non-glycosylated
145234.2
G0F-GlcNAc
146477.7
G0F
146677.5
G1F
146841.8
G0F/G0F
148125.3
Intensity
1.200e+ 6
1.000e+ 6
8.000e+ 5
6.000e+ 5
4.000e+ 5
2.000e+ 5
0.000e+ 0
145000
146000
147000
M
148000
149000
150000

FIG. 23B

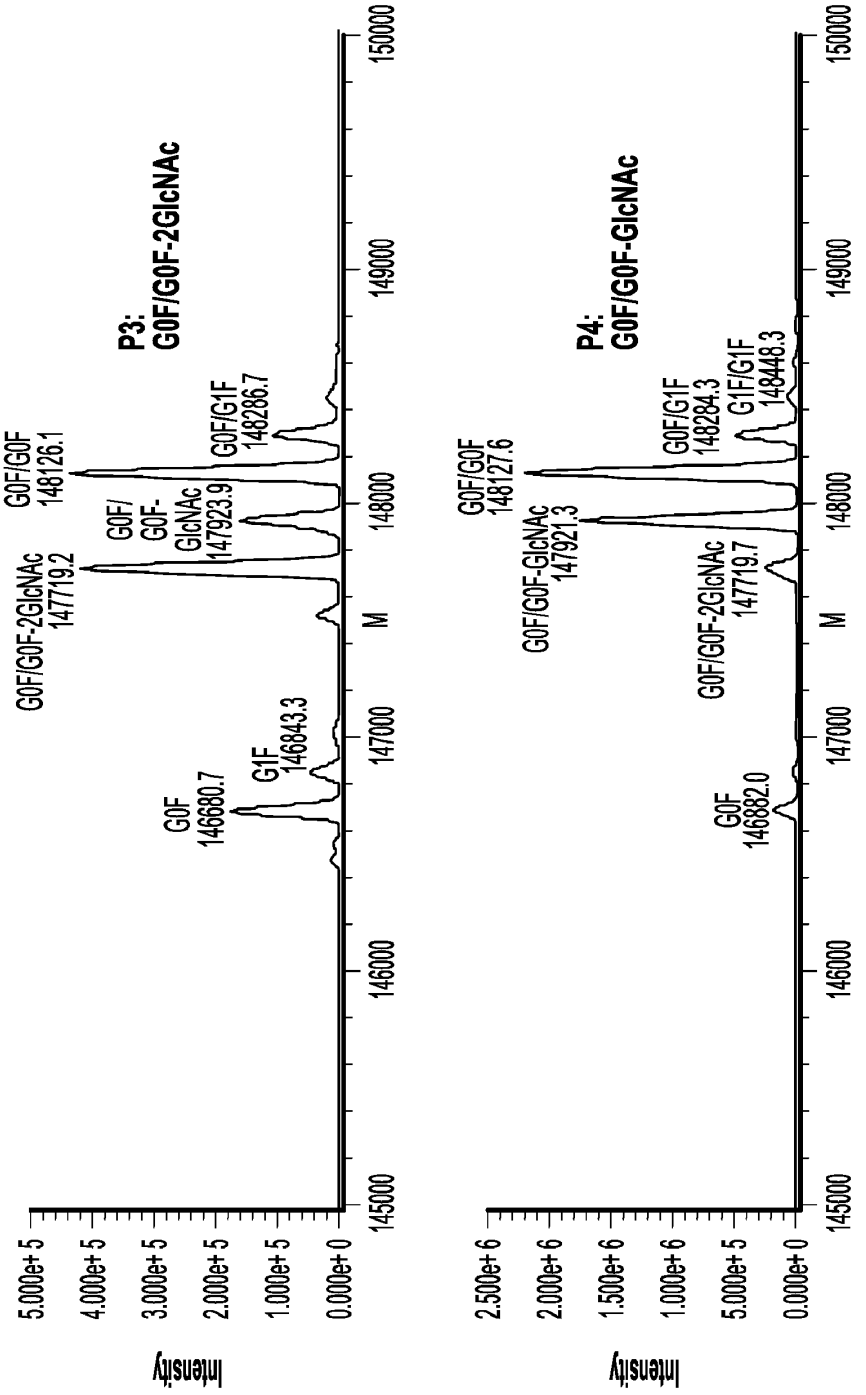


FIG. 23B (Continued)

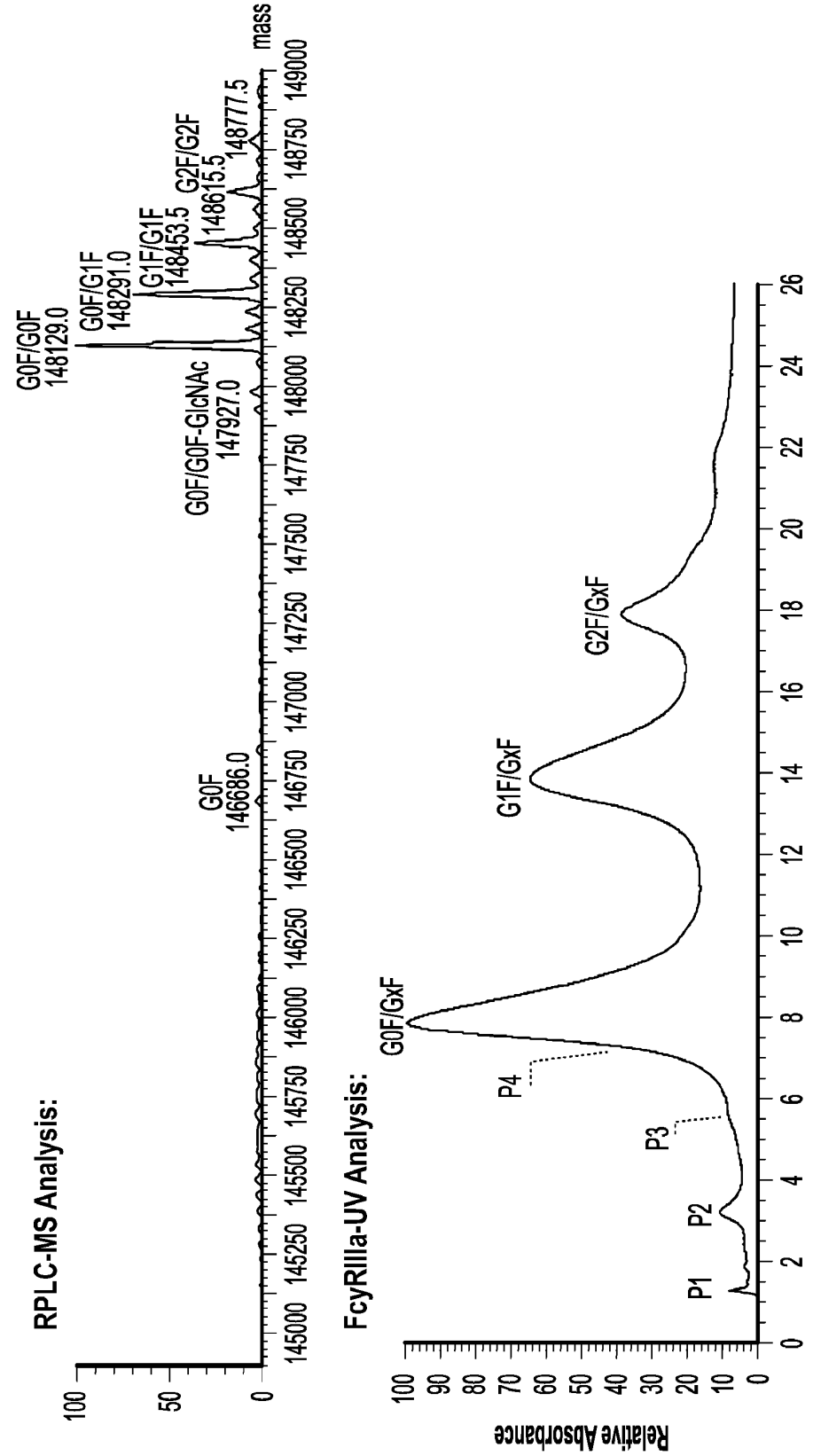

RPLC-MS Analysis:
G0F/G0F
148129.0
G0F/G1F
148291.0
G1F/G1F
148453.5
G2F/G2F
148615.5
148777.5
G0F/G0F-GlcNAc
147927.0
G0F
146686.0
mass
FcγRIIIa-UV Analysis:
G0F/GxF
G1F/GxF
G2F/GxF
P1
P2
P3
P4
Relative Absorbance

FIG. 24A