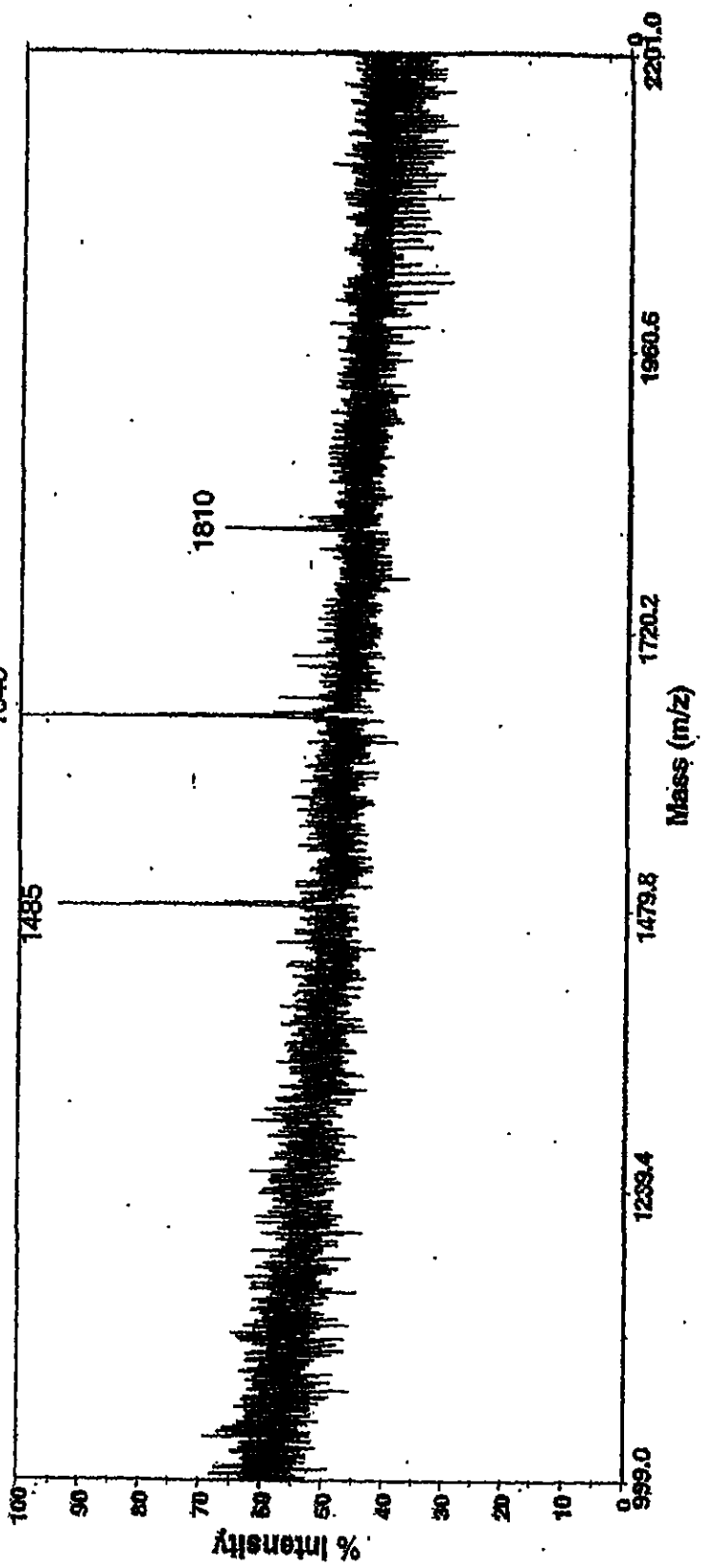

1810
1648
1485
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
999.0
1239.4
1479.8
1720.2
1960.6
2201.0

Figure 1

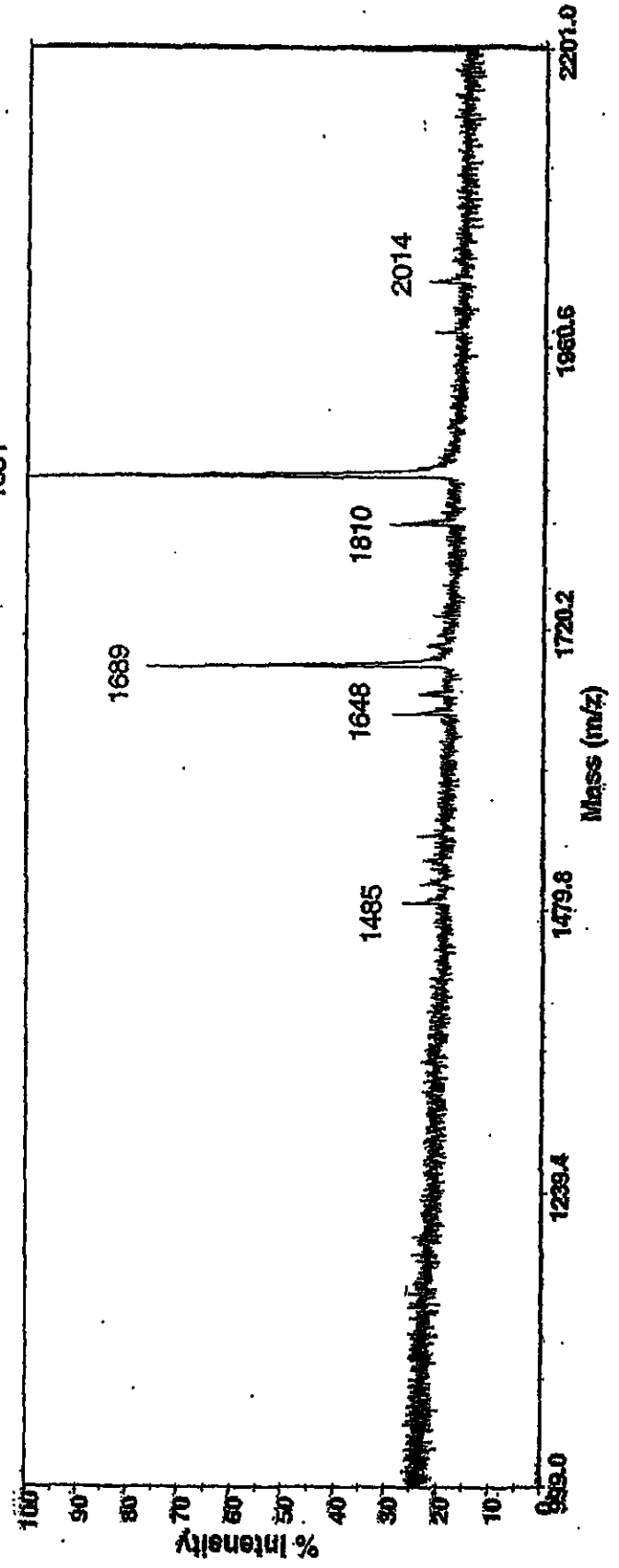

1851
2014
1810
1689
1648
1485
% Intenelty
100
90
80
70
60
50
40
30
20
10
989.0
1239.4
1479.8
1720.2
1960.6
2201.0
Mass (m/z)

Figure 2

Figure 3

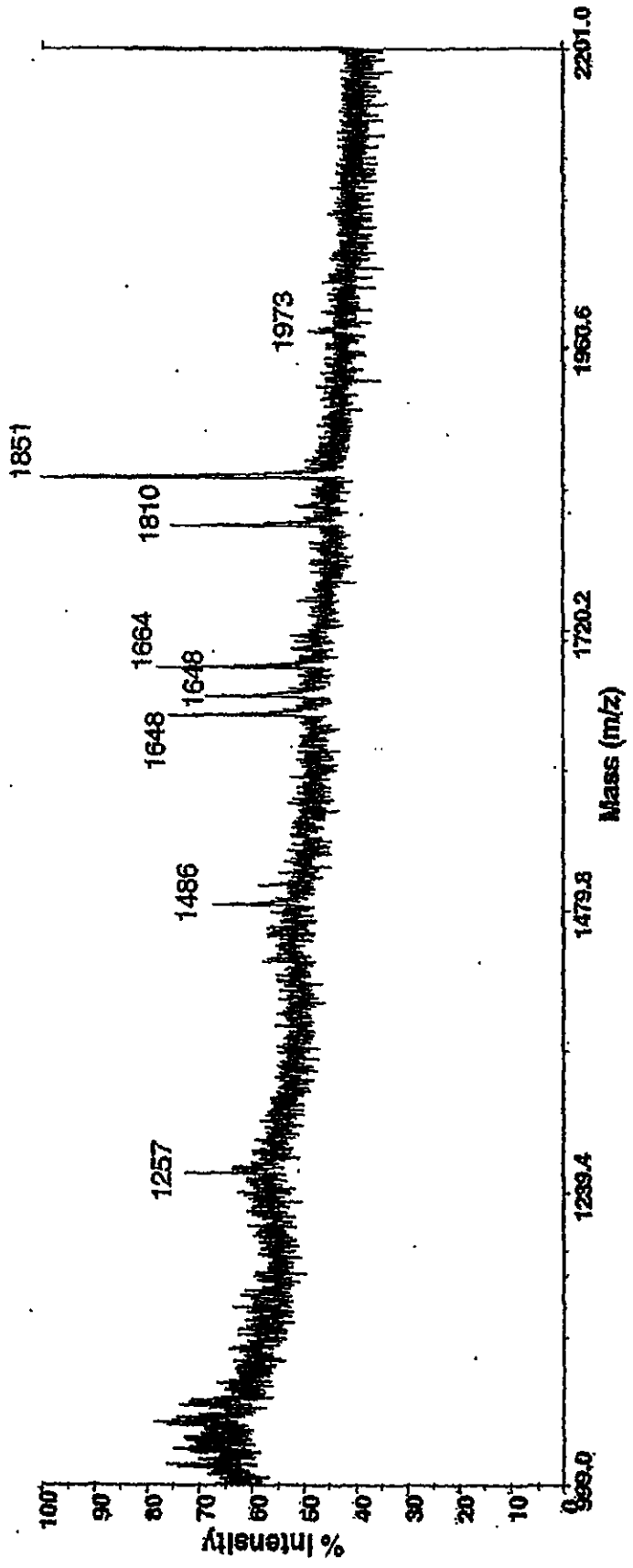

1851
1810
1973
1664
1648
1648
1486
1257
% Intenelty
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
2201.0
1960.6
1720.2
1479.8
1239.4

Figure 4

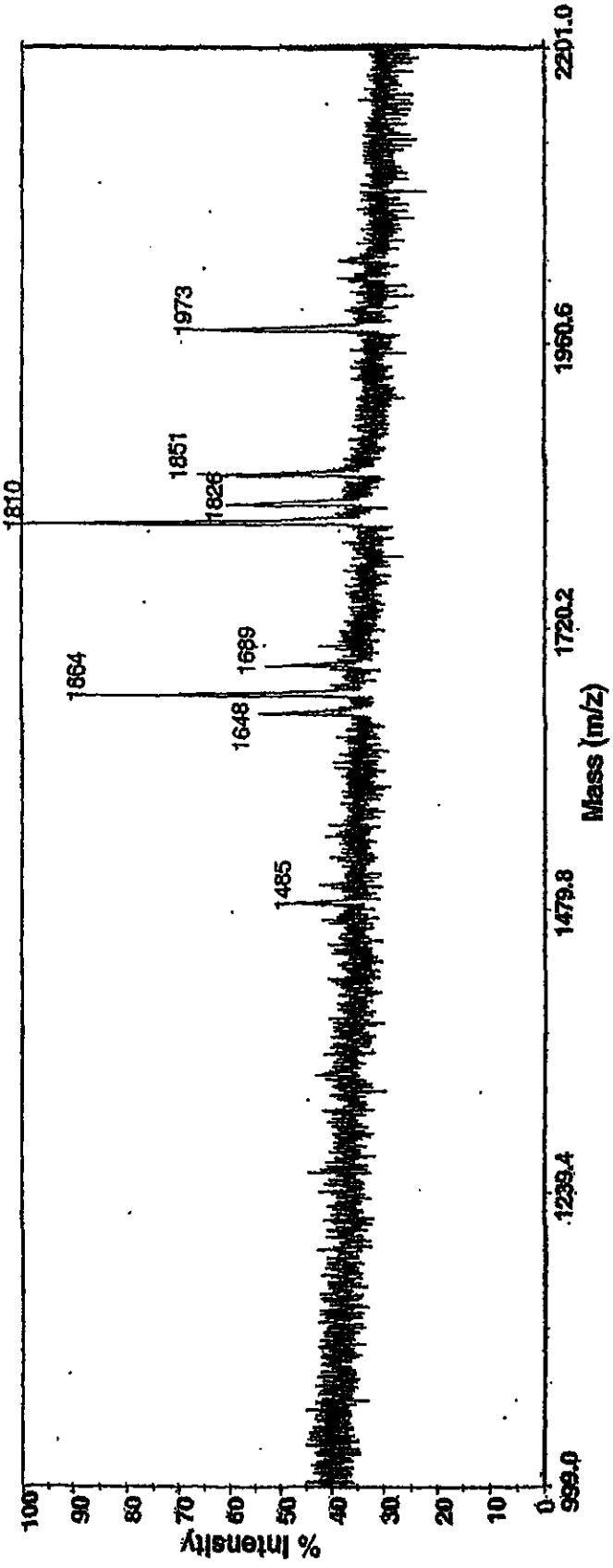

% Intensity
100
90
80
70
60
50
40
30
20
10
0
999.0
1239.4
1479.8
1720.2
1960.6
2201.0
Mass (m/z)
1810
1864
1973
1851
1826
1699
1648
1485

Figure 5

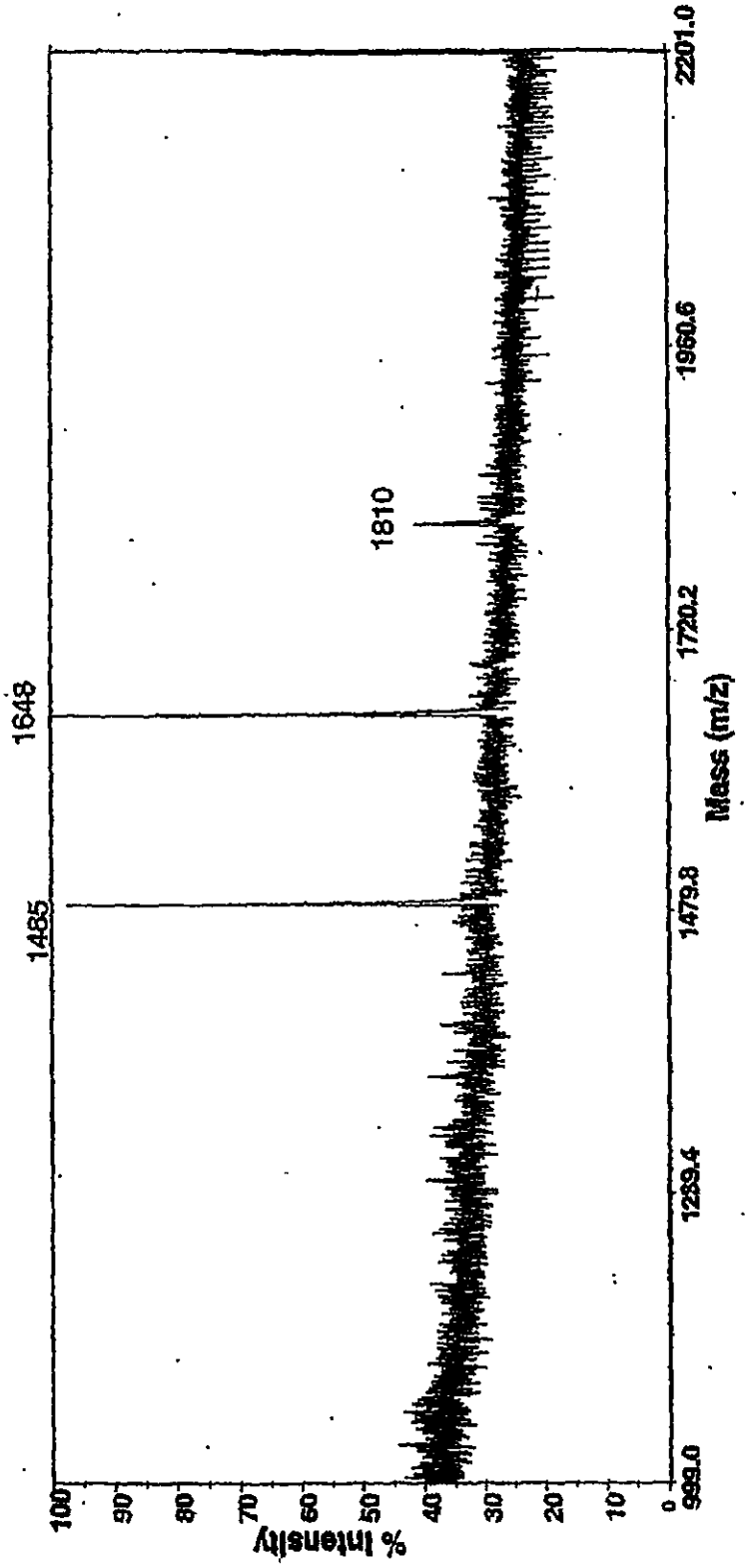

1648
1485
1810
100
90
80
70
60
50
40
30
20
10
0
% Intensity
999.0
1239.4
1479.8
1720.2
1960.6
2201.0
Mass (m/z)

Figure 6

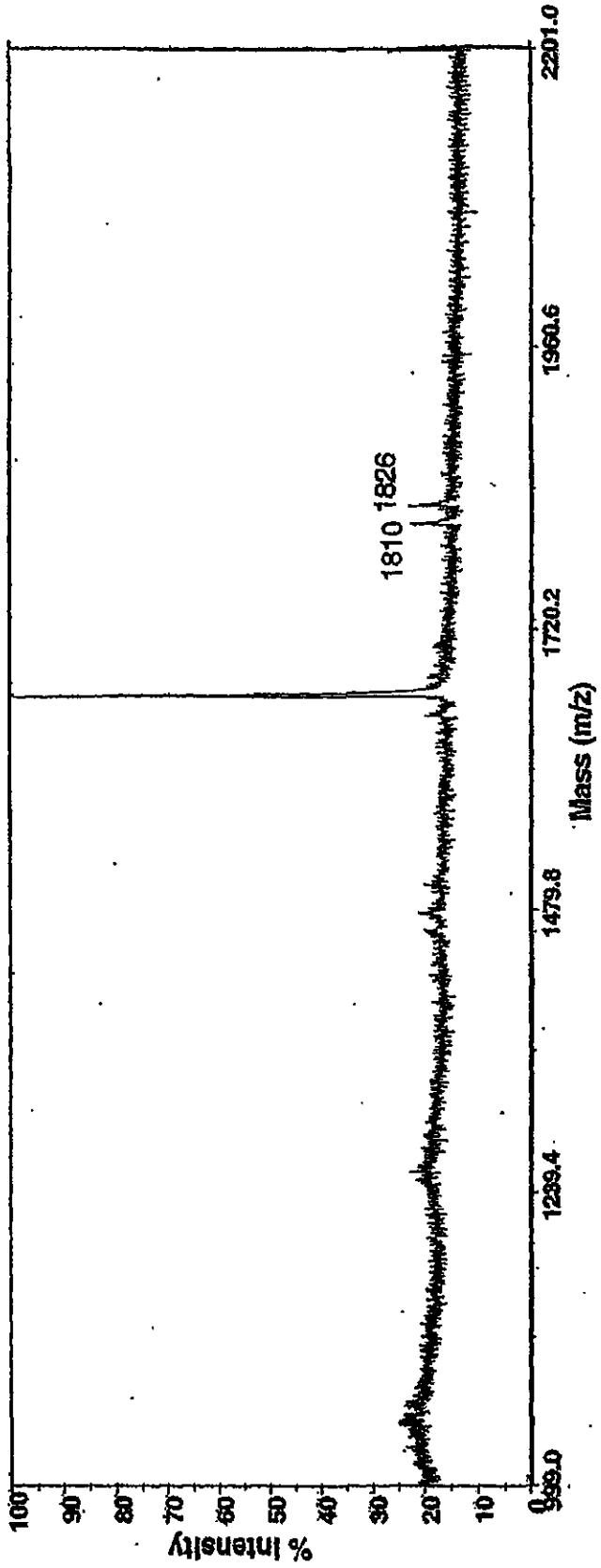
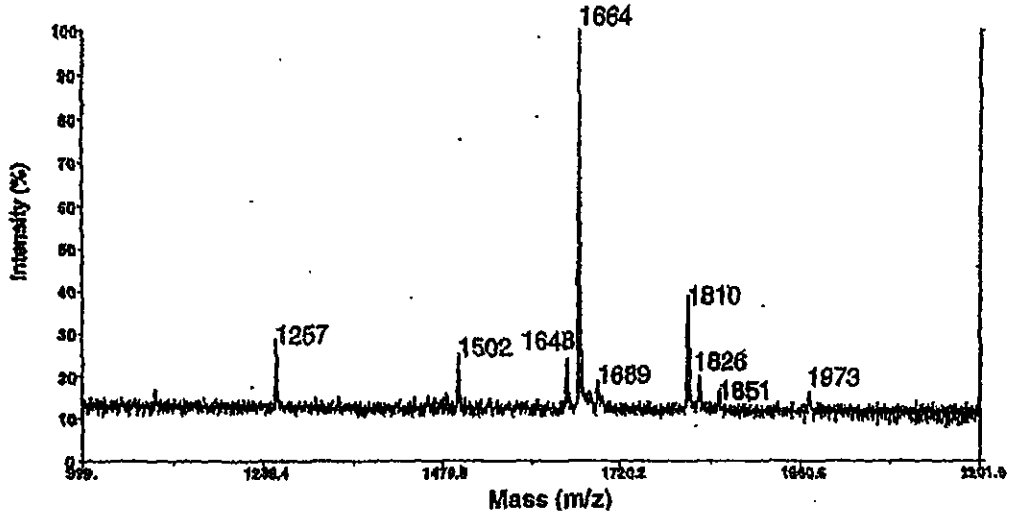

1664
1810
1826
100
90
80
70
60
50
40
30
20
10
0
% Intensity
999.0
1239.4
1479.8
1720.2
1960.6
2201.0
Mass (m/z)

Figure 7

(a)


1664
1810
1257
1502
1648
1689
1826
1851
1973
Intensity (%)
Mass (m/z)

(b)

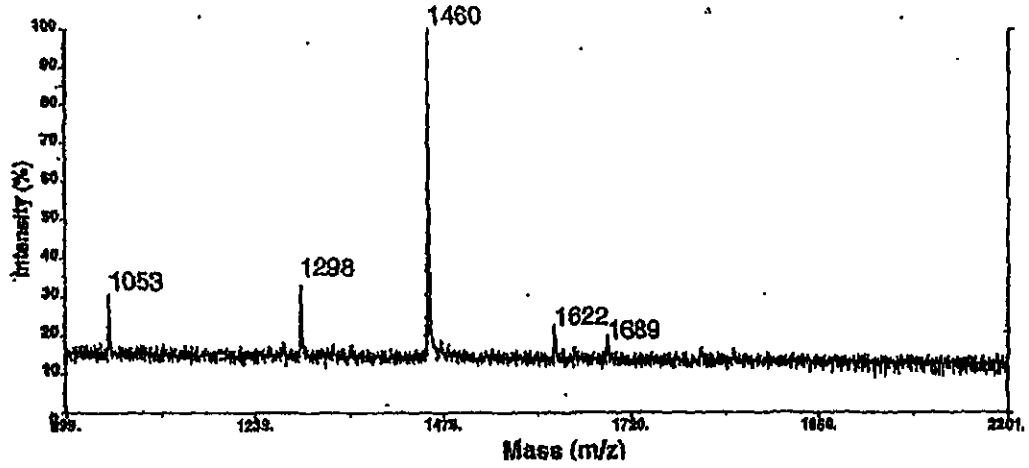
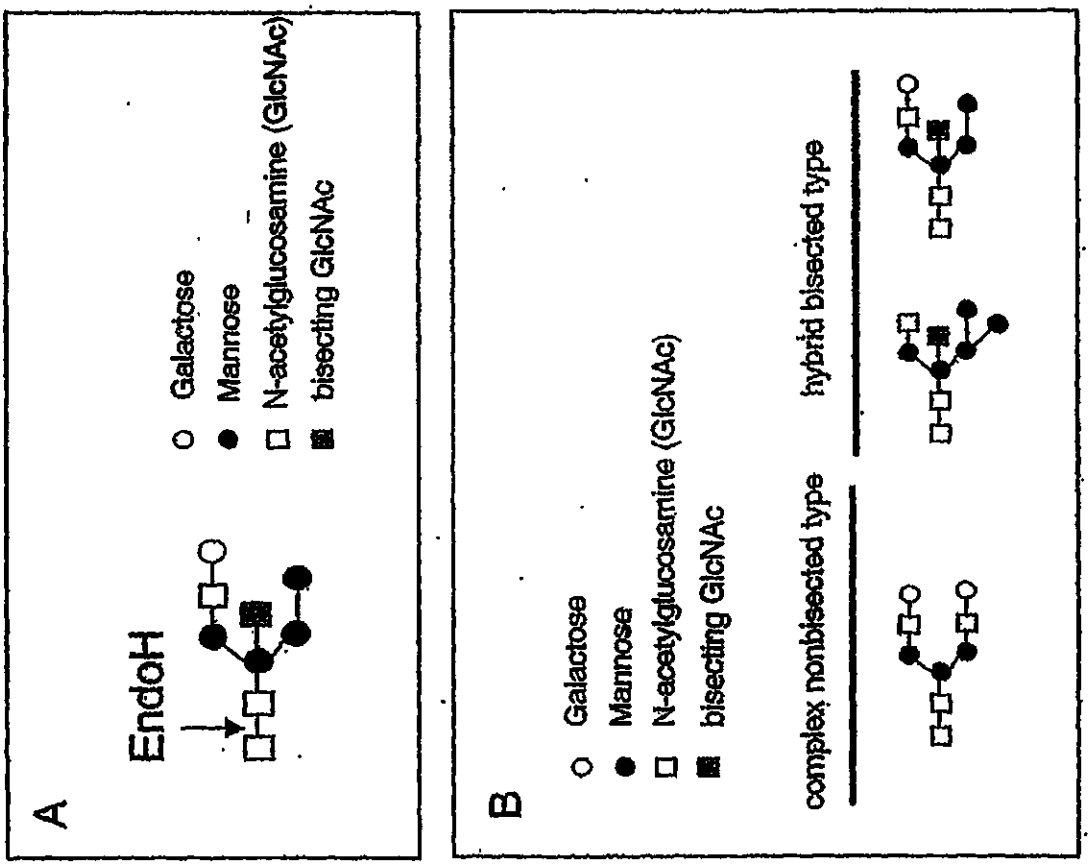

1460
1053
1298
1622
1689
Intensity (%)
Mass (m/z)

Figure 8


A
EndoH
Galactose
Mannose
N-acetylglucosamine (GlcNAc)
bisecting GlcNAc
B
Galactose
Mannose
N-acetylglucosamine (GlcNAc)
bisecting GlcNAc
complex nonbisected type
hybrid bisected type

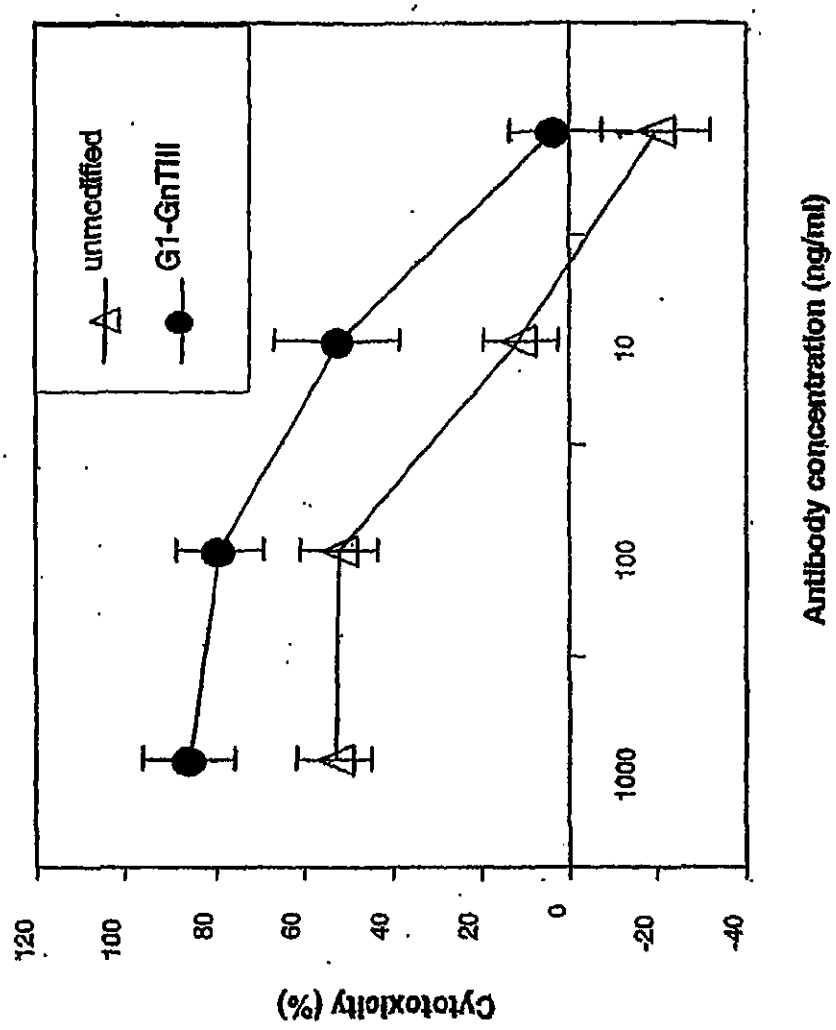

unmodified
G1-GnTIII
120
100
80
60
40
20
0
-20
-40
Cytotoxicity (%)
1000
100
10
1
Antibody concentration (ng/ml)

Figure 9

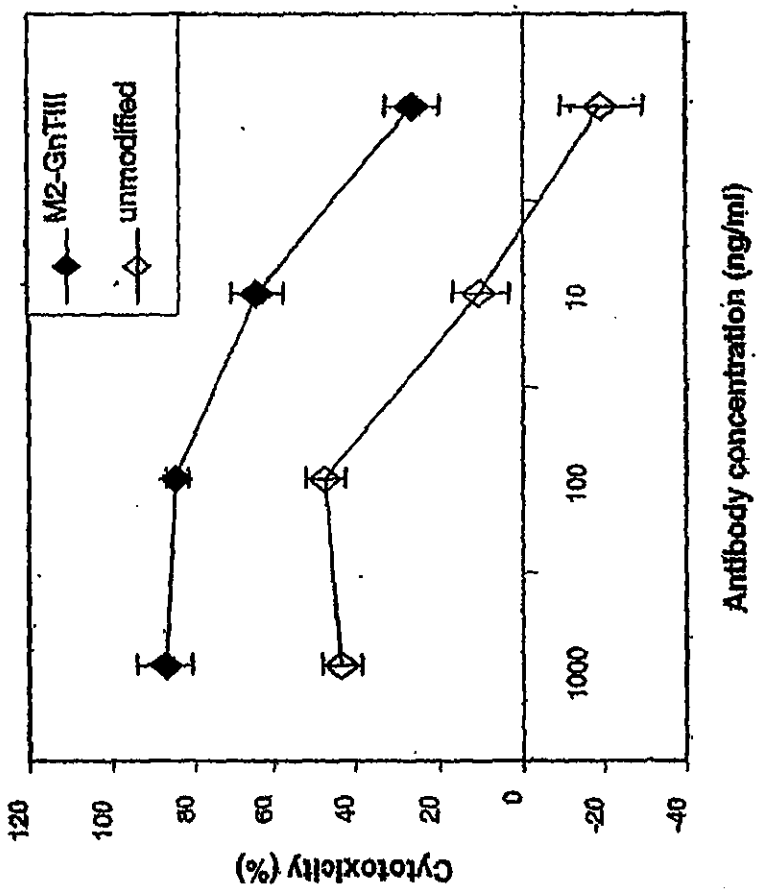

M2-GnTIII
unmodified
Cytotoxicity (%)
120
100
80
60
40
20
0
-20
-40
1000
100
10
Antibody concentration (ng/ml)

Figure 10

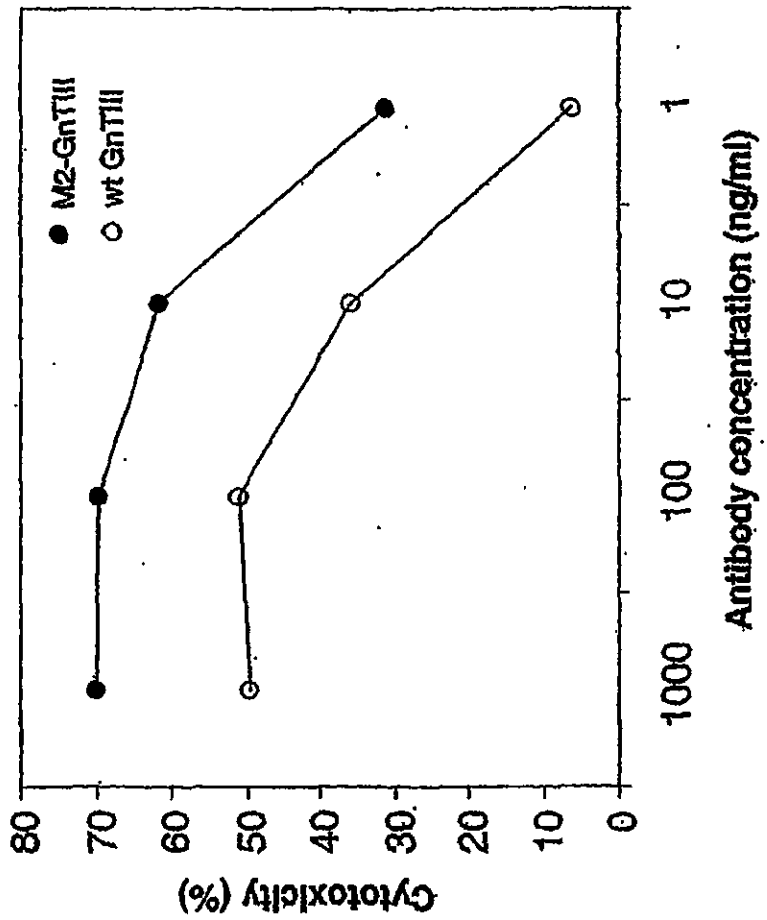

M2-GnTIII
wt GnTIII
Cytotoxicity (%)
0
10
20
30
40
50
60
70
80
1000
100
10
1
Antibody concentration (ng/ml)

Figure 11

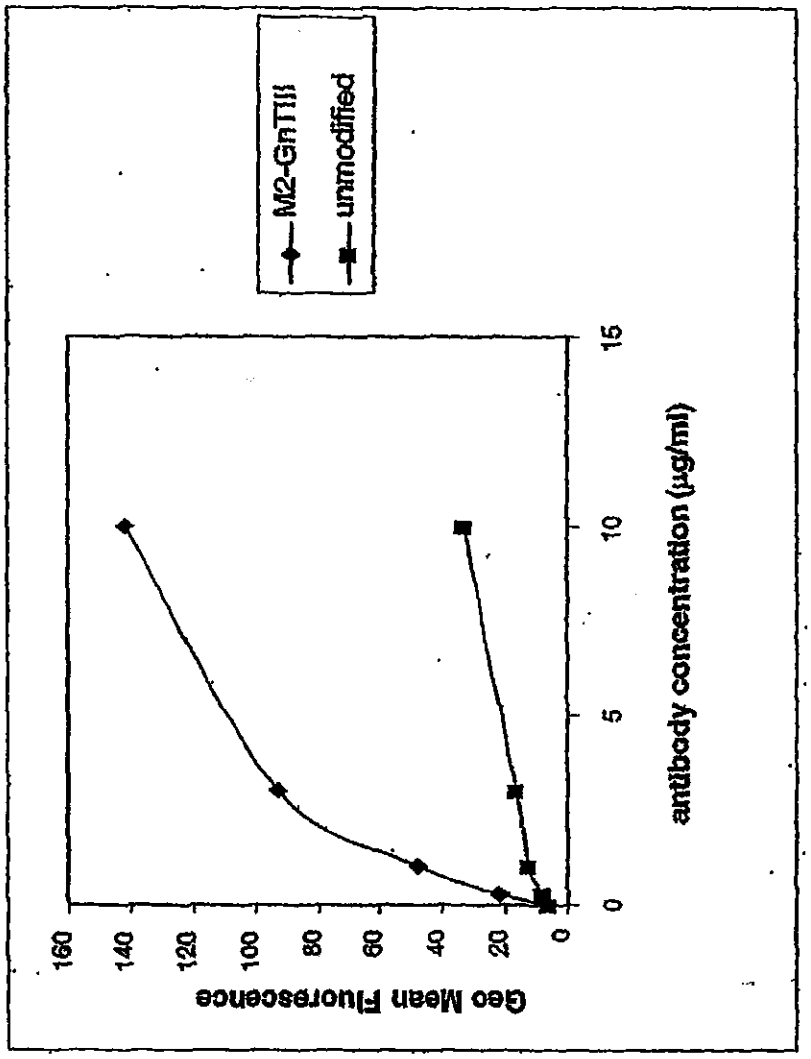

M2-GnTIII
unmodified
Geo Mean Fluorescence
0
20
40
60
80
100
120
140
160
0
5
10
15
antibody concentration (µg/ml)

Figure 12

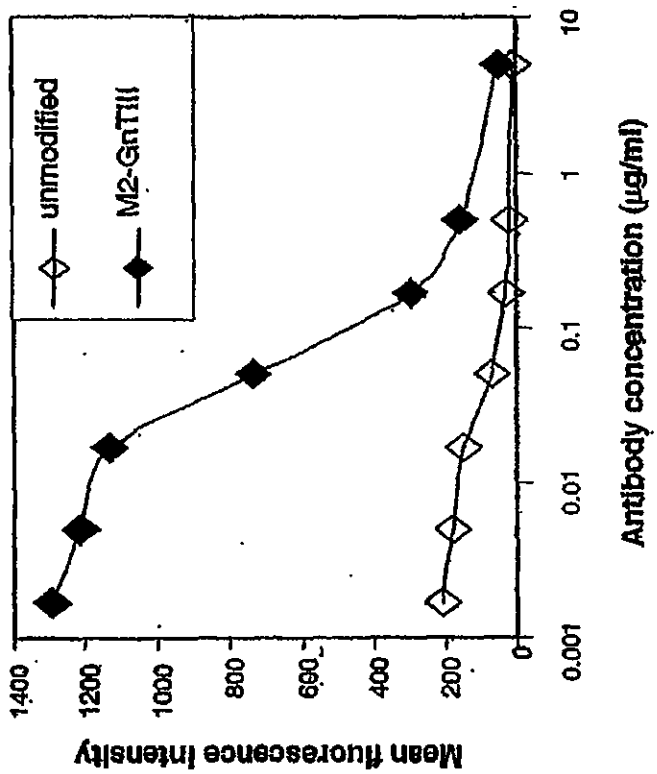
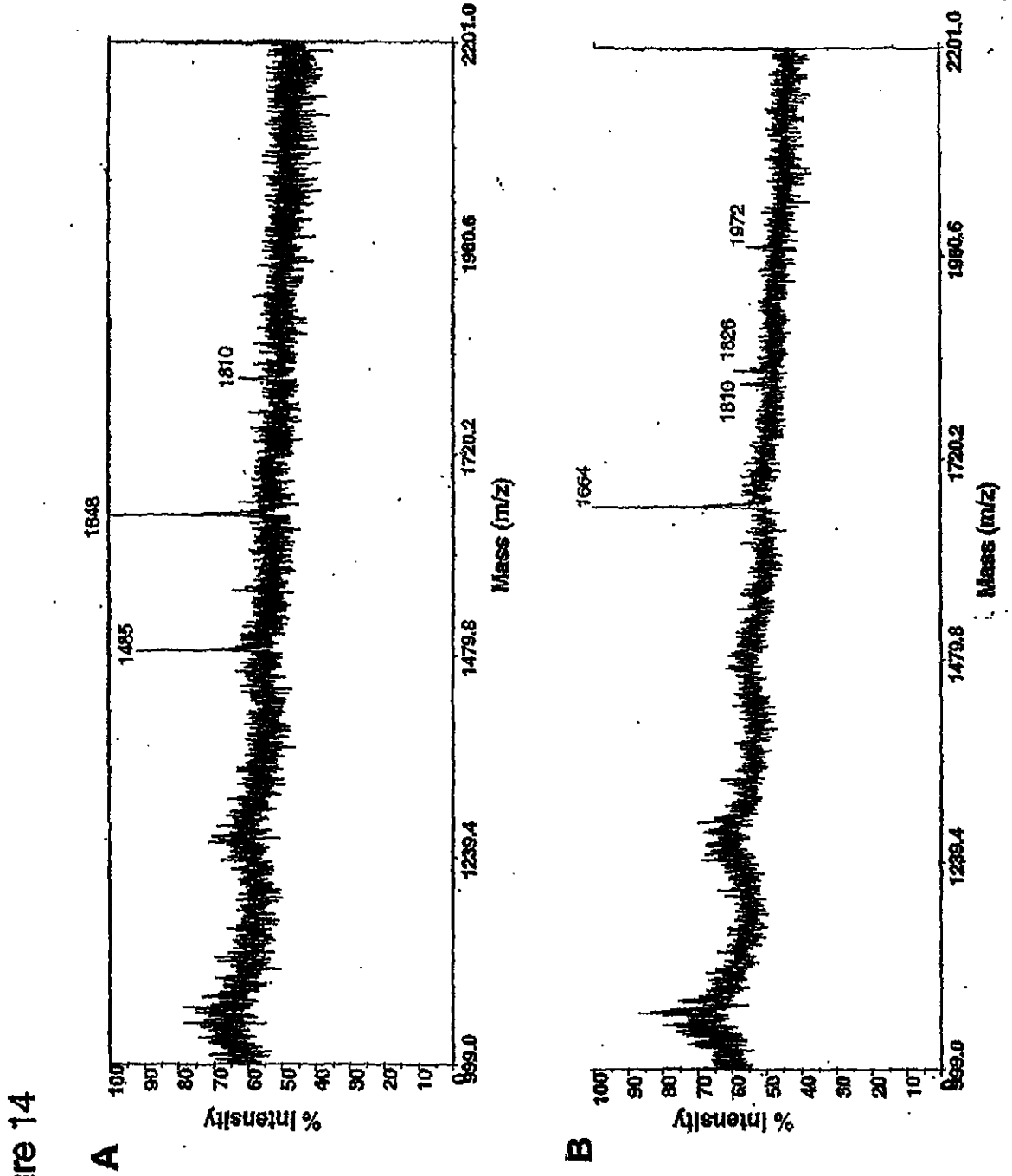

unmodified
M2-GnTIII
Mean fluorescence intensity
1400
1200
1000
800
600
400
200
0
0.001
0.01
0.1
1
10
Antibody concentration (µg/ml)

Figure 13

Figure 14


A
% Intensity
100
90
80
70
60
50
40
30
20
10
999.0
1239.4
1479.8
1720.2
1960.6
2201.0
Mass (m/z)
1485
1648
1810
B
% Intensity
1664
1810
1826
1972
Mass (m/z)

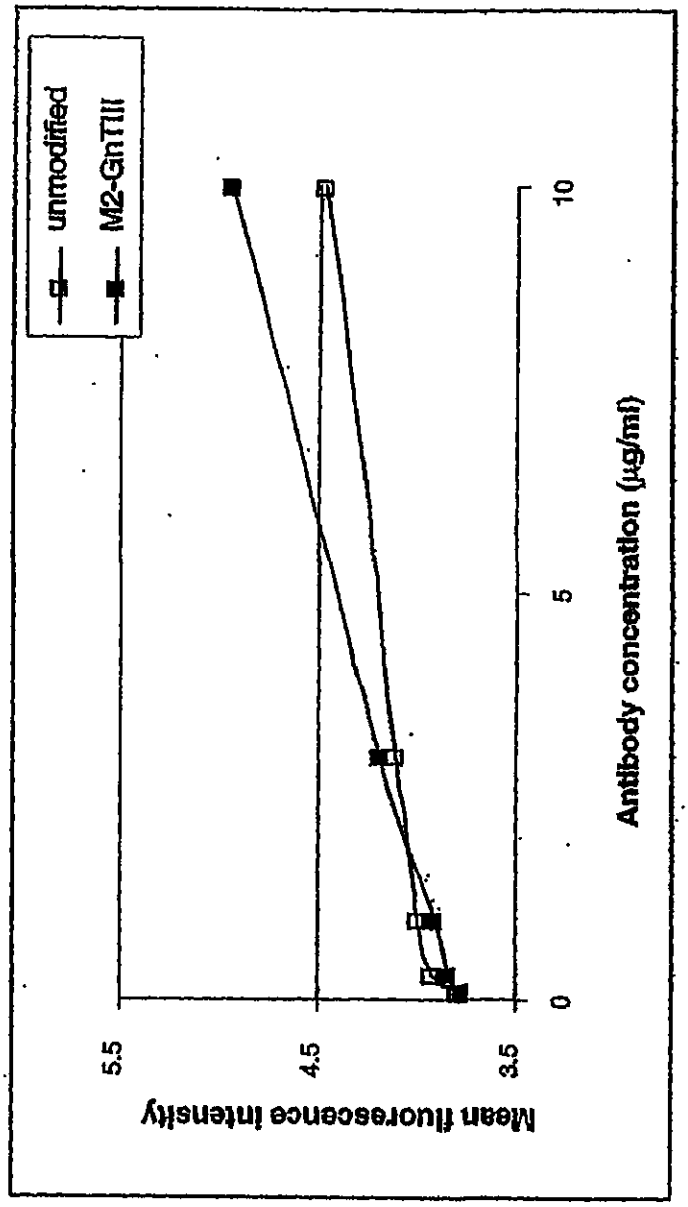

unmodified
M2-GnTIII
Mean fluorescence intensity
5.5
4.5
3.5
0
5
10
Antibody concentration (µg/ml)

Figure 15

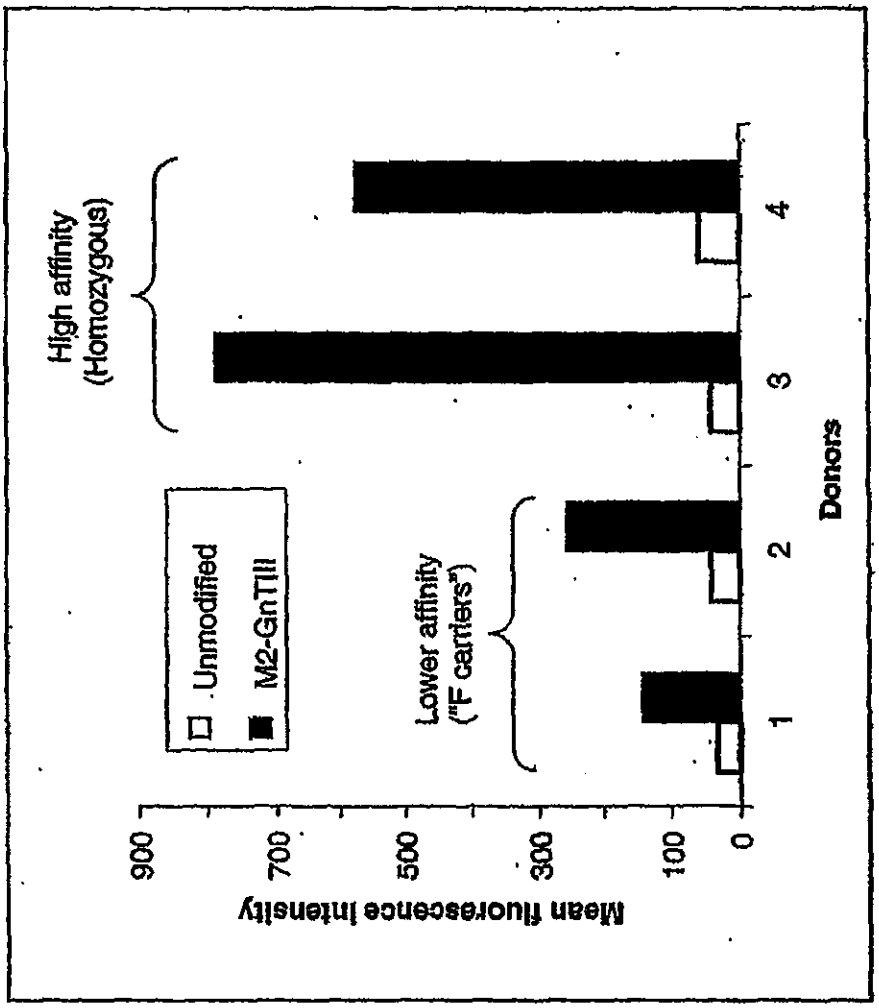

Mean fluorescence intensity
900
700
500
300
100
0
1
2
3
4
Donors
Unmodified
M2-GnTIII
High affinity
(Homozygous)
Lower affinity
("F carriers")

Figure 16

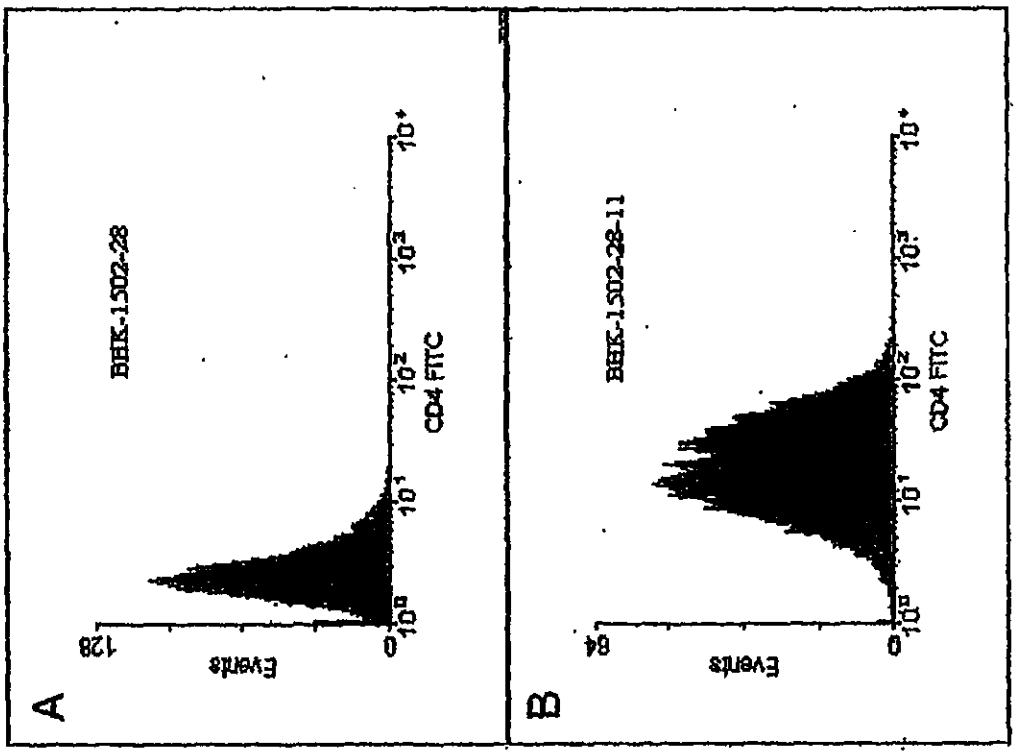
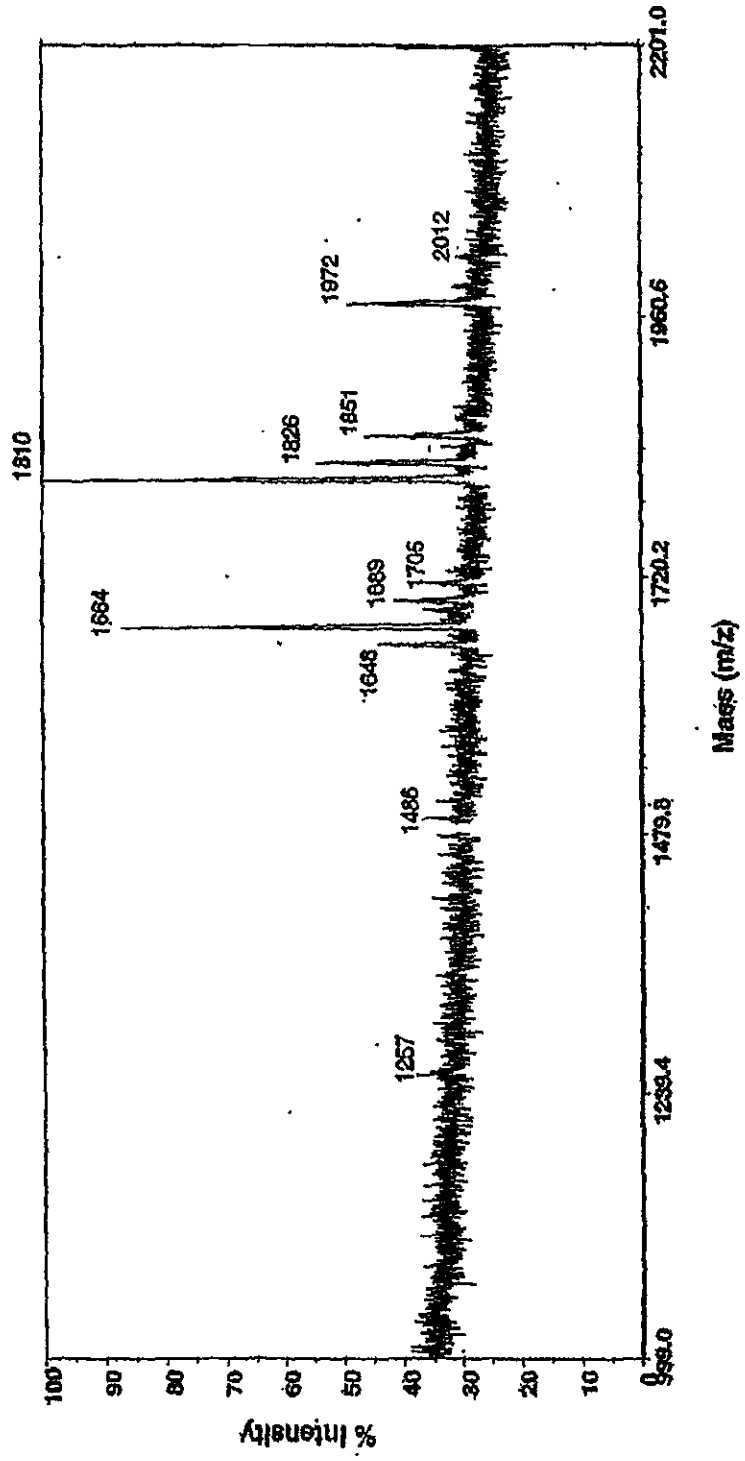

A
BEK-1502-28
Events
128
0
10^0
10^1
10^2
10^3
10^4
CD4 FITC
B
BEK-1502-28-11
Events
84
0
10^0
10^1
10^2
10^3
10^4
CD4 FITC

Figure 17

Figure 18


% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
998.0
1239.4
1479.8
1720.2
1960.6
2201.0
1810
1826
1851
1972
2012
1664
1689
1705
1648
1486
1257

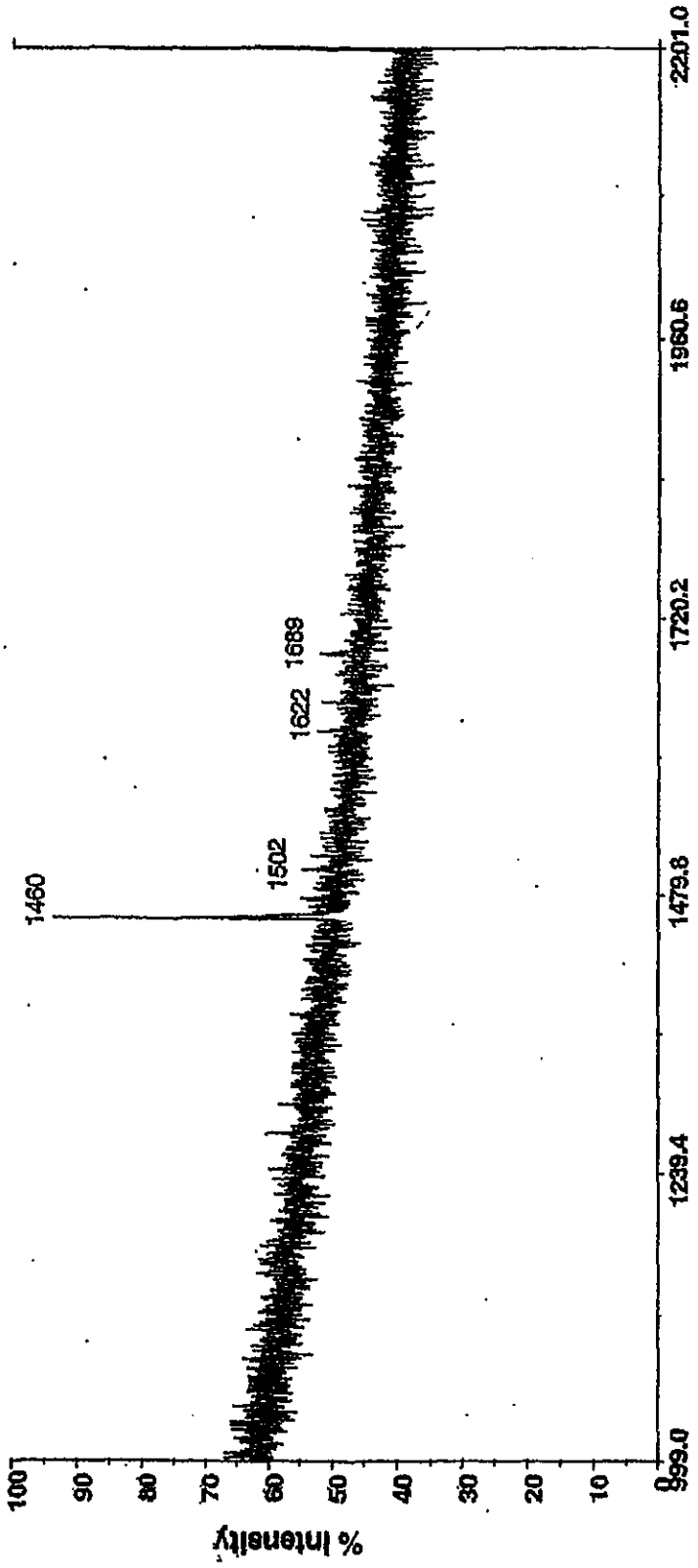

% Intensity
100
90
80
70
60
50
40
30
20
10
0
999.0
1239.4
1479.8
1720.2
1960.6
2201.0
Mass (m/z)
1460
1502
1622
1689

Figure 19

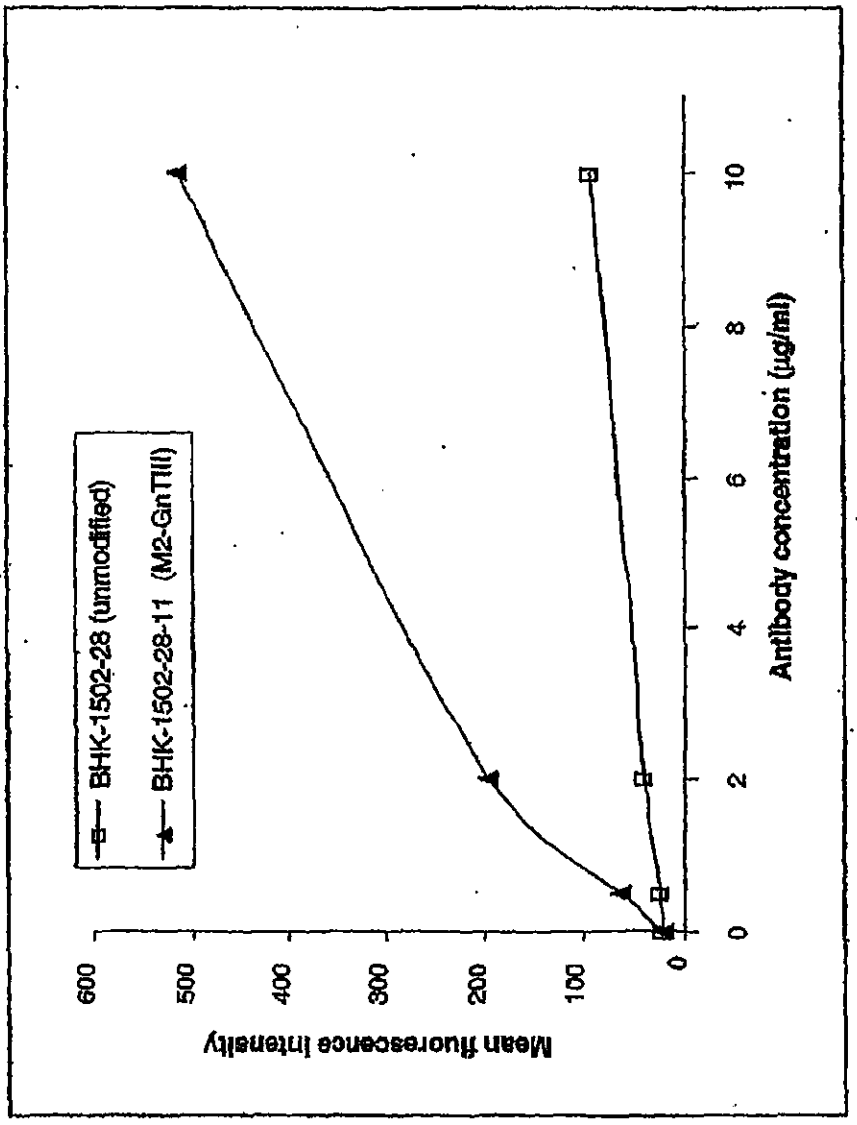

BHK-1502-28 (unmodified)
BHK-1502-28-11 (M2-GnTIII)
Mean fluorescence intensity
600
500
400
300
200
100
0
0
2
4
6
8
10
Antibody concentration (µg/ml)

Figure 20

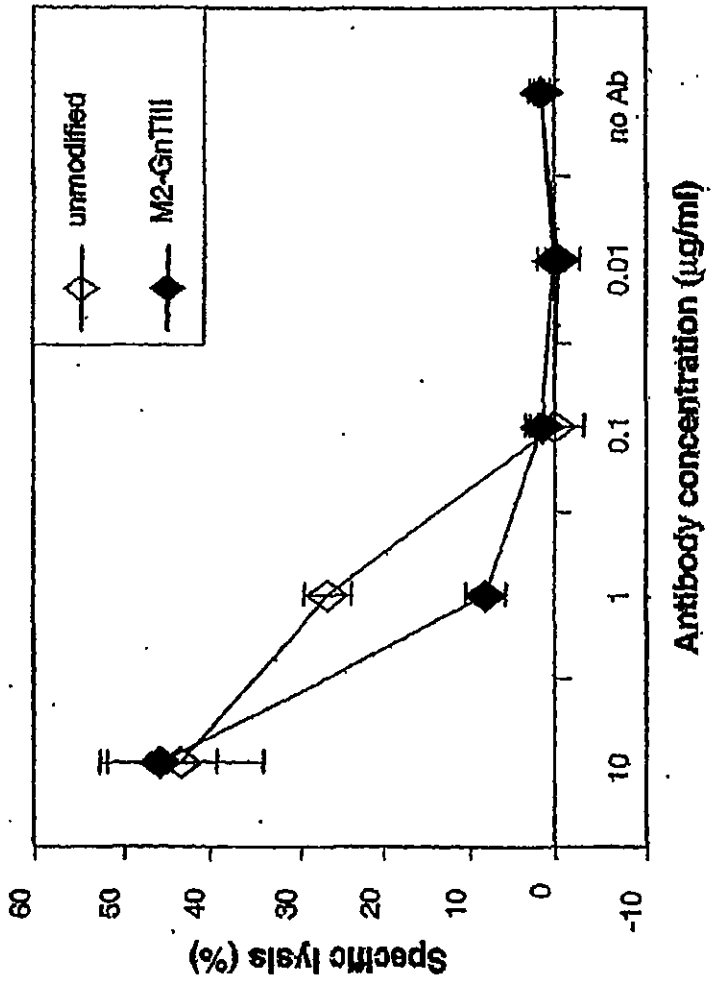
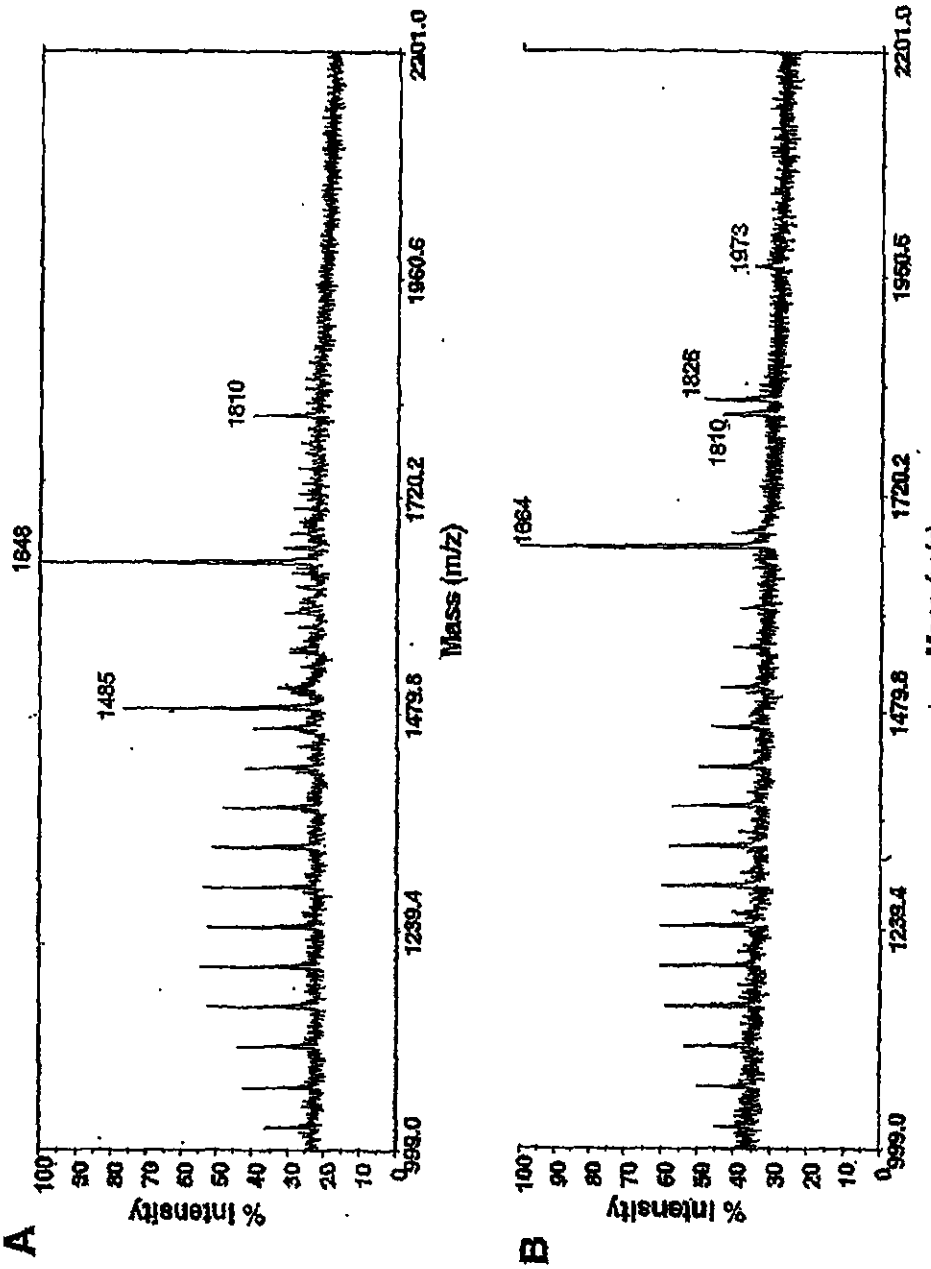

unmodified
M2-GnTIII
Specific lysis (%)
60
50
40
30
20
10
0
-10
10
1
0.1
0.01
no Ab
Antibody concentration (µg/ml)

Figure 21

Figure 22


A
% Intensity
100
90
80
70
60
50
40
30
20
10
0
999.0
1239.4
1479.8
1720.2
1960.6
2201.0
Mass (m/z)
1848
1485
1810
B
% Intensity
100
90
80
70
60
50
40
30
20
10
0
999.0
1239.4
1479.8
1720.2
1960.6
2201.0
Mass (m/z)
1964
1826
1810
1973

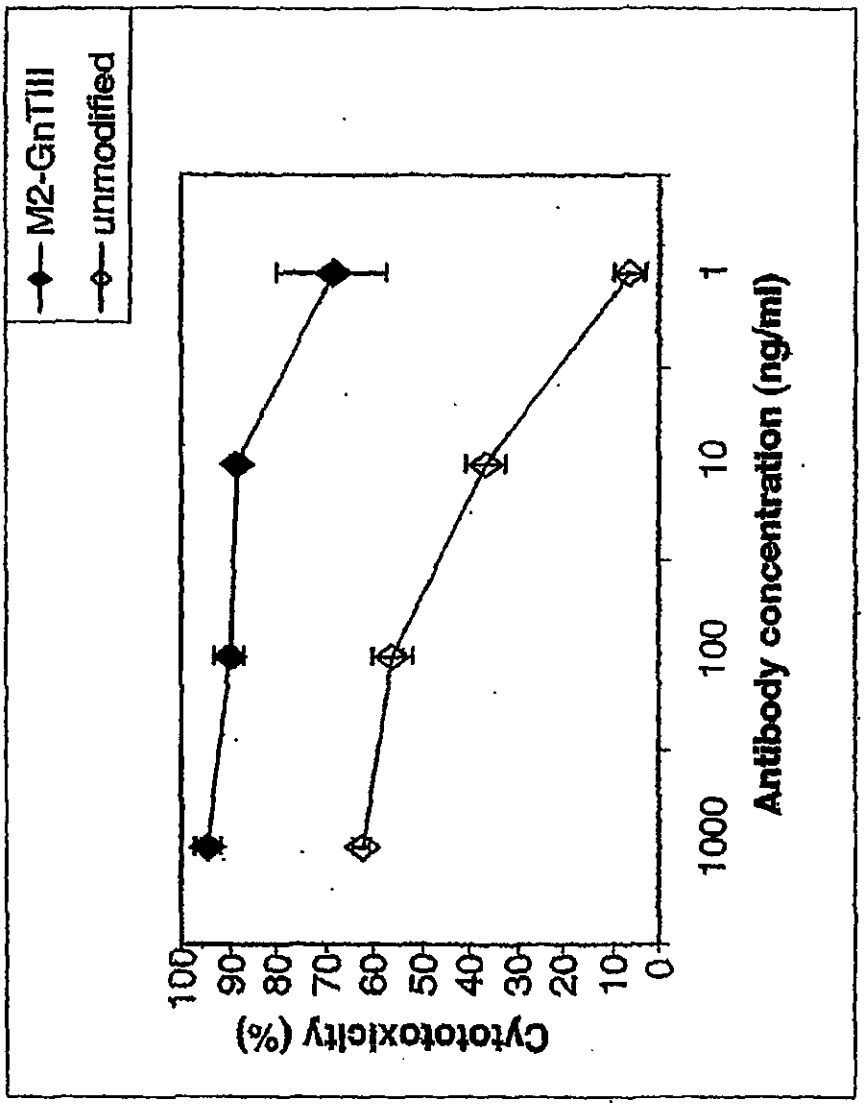

M2-GnTIII
unmodified
Cytotoxicity (%)
100
90
80
70
60
50
40
30
20
10
0
1000
100
10
1
Antibody concentration (ng/ml)

Figure 23

ManII-GnTIII:

Bold: ManII localization domain (cytoplasmic tail + transmembrane region + stem region)

ATGAAGTTAAGCCGCCAGTTCACCGTGTTCGGCAGTGCGATCTTCTGTGTGGTGATTTTC
TCGCTCTACCTGATGCTGGACCGGGGTCACTTAGACTACCCCAGGAACCCGCGCCGCGAG
GGCTCCTTCCCTCAGGGCCAGCTCTCAATGTTGCAAGAAAAAATAGACCATTTGGAGCGT
TTGCTAGCTGAGAATAATGAGATCATCTCAAATATTAGAGACTCAGTCATCAATTTGAGT
GAGTCTGTGGAGGATGGTCCGAAAAGTTCACAAAGCAATTTCAGCCAAGGTGCTGGCTCA
CCCCTGCTCCAGCCACTGTCCCCTAGCAAGGCCACCGAAGAACTGCACCGGGTGGACTTC
GTGTTGCCGGAGGACACCACAGAGTATTTTGTGCGCACCAAAGCTGGCGGTGTGTGCTTC
AAACCAGGTACCAGGATGCTGGAGAAACCTTCTCCAGGGCGGACAGAGGAGAAGACCAAG
GTGGCTGAGGGGTCCTCGGTCCGGGGTCCTGCTCGGAGGCCTATGCGGCATGTGTTGAGT
GCACGGGAGCGCCTGGGAGGCCGGGGCACTAGGCGCAAGTGGGTTGAGTGTGTGTGCCTG
CCAGGCTGGCACGGGCCCAGCTGCGGGGTGCCCACTGTGGTCCAGTATTCCAACCTGCCC
ACCAAGGAGCGCCTGGTACCCAGGGAGGTGCCGAGGCGGGTTATCAACGCCATCAACATC
AACCATGAGTTCGACCTGCTGGATGTGCGCTTCCATGAGCTGGGCGATGTTGTGGACGCC
TTTGTGGTCTGCGAATCCAATTTCACCGCCTACGGGGAGCCTCGGCCGCTCAAGTTCCGA
GAGATGCTGACCAATGGCACCTTCGAGTACATCCGCCACAAGGTGCTCTACGTCTTCCTG
GACCACTTCCCACCTGGTGGCCGTCAGGACGGCTGGATTGCAGACGACTACCTGCGTACC
TTCCTCACCCAGGATGGTGTCTCCCGCCTGCGCAACCTGCGACCTGATGACGTCTTTATC
ATCGACGACGCGGACGAGATCCCTGCGCGTGATGGTGTGCTGTTCCTCAAGCTCTACGAT
GGCTGGACAGAGCCCTTCGCCTTCCATATGCGCAAGTCCCTGTATGGTTTCTTTTGGAAG
CAACCAGGCACACTGGAGGTGGTGTCAGGCTGCACCATTGACATGCTGCAGGCTGTGTAT
GGGCTGGACGGCATCCGCCTGCGCCGCCGTCAGTACTACACCATGCCCAACTTTCGACAG
TATGAGAACCGCACCGGCCACATCCTAGTGCAGTGGTCTCTCGGCAGCCCCCTGCACTTC
GCGGGCTGGCACTGCTCCTGGTGCTTCACACCCGAGGGCATCTACTTCAAACTCGTGTCG
GCCCAGAATGGTGACTTCCCCCGCTGGGGTGACTACGAGGACAAGAGGGACCTCAATTAC
ATCCGAAGCTTGATTCGCACTGGGGGATGGTTCGACGGCACGCAGCAGGAGTACCCTCCT
GCAGACCCCAGTGAACACATGTATGCTCCTAAGTACCTGCTCAAGAACTATGACCAGTTC
CGCTACTTGCTCGAAAATCCCTACCGGGAGCCCAAGAGCACTGTAGAGGGTGGGCGCCGG
AACCAGGGCTCAGACGGAAGGTCATCTGCTGTCAGGGGCAAGTTGGATACAACGGAGGGC
CCGGAACAGAAACTGATCTCTGAAGAGGACCTGTAG

MKLSRQFTVFGSAIFCVVIFSLYLMLDRGHLDYPRNPRREGSFFQGQLSMLQEKIDHLER
LLAENNEILSNIRDSVINLSNSVEDGFKSSQSNFSQGAGSPLLQFLSPSKATEELHRVDF
VLPEDTPEYFVRTKAGGVCFKPGTRMLEKPSPGRTEEKTKVAEGSSVRGPARRPMRHVLS
ARERLGGRGTRRKWVECVCLPGWHGPSCGVPTVVQYSNLPTKERLVPREVPRRVINAINI
NHEFDLLDVRFHELGDVVDAFVVCESNFTAYGEPRPLKFREMLTNGTFEYIRHKVLYVFL
DHFPPGGRQDGWIADDYLRTFLTQDGVSRLRNLRPDDVFIIDDADEIPARDGVLFLKLYD
GWTEPFAFHMRKSLYGFFWKQPGTLEVVSGCTIDMLQAVYGLDGIRLRRRQYYTMPNFRQ
YENRTGHILVQWSLGSPLHFAGWHCSWCFTPEGIYFKLVSAQNGDFPRWGDYEDKRDLNY
IRSLIRTGGWFDGTQQEYPPADPSEHMYAPKYLLKNYDQFRYLLENPYREPKSTVEGGRR
NQGSDGRSSAVRGKLDTTEGPEQKLISEEDL

Figure 24

GnTI-GnTIII

Bold: GnTI ManII localization domain (cytoplasmic tail + transmembrane region + stem region)

ATGCTGAAGAAGCAGTCTGCAGGGCTTGTGCTGTGGGGCGCTATCCTCTTTGTGGCCTGG
AATGCCCTGCTGCTCCTCTTCTTCTGGACGCGCCCAGCACCTGGCAGGCCACCCTCAGTC
AGCGCTCTCGATGGCGACCCCGCCAGCCTCACCCGGGAAGTGATTCGCCTGGCCCAAGAC
GCCGAGGTGGAGCTGGAGCGGCAGCGTGGGCTGCTGCAGCAGATCGGGGATGCCCTGTCG
AGCCAGCGGGGGAGGGTGCCCACCGCGGCCCCTCCCGCCCAGCCGCGTGTGCCTGTGACC
CCCGCGCCCCTGCTCCAGCCACTGTCCCCTAGCAAGGCCACCGAAGAACTGCACCGGGTG
GACTTCGTGTTGCCGGAGGACACCACAGAGTATTTTGTGCGCACCAAAGCTGGCGGTGTG
TGCTTCAAACCAGGTACCAGGATGCTGGAGAAACCTTCTCCAGGGCGGACAGAGGAGAAG
ACCAAGGTGGCTGAGGGGTCCTCGGTCCGGGGTCCTGCTCGGAGGCCTATGCGGCATGTG
TTGAGTGCACGGGAGCGCCTGGGAGGCCGGGGCACTAGGCGCAAGTGGGTTGAGTGTGTG
TGCCTGCCAGGCTGGCACGGGCCCAGCTGCGGGGTGCCCACTGTGGTCCAGTATTCCAAC
CTGCCCACCAAGGAGCGCCTGGTACCCAGGGAGGTGCCGAGGCGGGTTATCAACGCCATC
AACATCAACCATGAGTTCGACCTGCTGGATGTGCGCTTCCATGAGCTGGGCGATGTTGTG
GACGCCTTTGTGGTCTGCGAATCCAATTTCACCGCCTACGGGGAGCCTCGGCCGCTCAAG
TTCCGAGAGATGCTGACCAATGGCACCTTCGAGTACATCCGCCACAAGGTGCTCTACGTC
TTCCTGGACCACTTCCCACCTGGTGGCCGTCAGGACGGCTGGATTGCAGACGACTACCTG
CGTACCTTCCTCACCCAGGATGGTGTCTCCCGCCTGCGCAACCTGCGACCTGATGACGTC
TTTATCATCGACGACGCGGACGAGATCCCTGCGCGTGATGGTGTGCTGTTCCTCAAGCTC
TACGATGGCTGGACAGAGCCCTTCGCCTTCCATATGCGCAAGTCCCTGTATGGTTTCTTT
TGGAAGCAACCAGGCACACTGGAGGTGGTGTCAGGCTGCACCATTGACATGCTGCAGGCT
GTGTATGGGCTGGACGGCATCCGCCTGCGCCGCCGTCAGTACTACACCATGCCCAACTTT
CGACAGTATGAGAACCGCACCGGCCACATCCTAGTGCAGTGGTCTCTCGGCAGCCCCCTG
CACTTCGCGGGCTGGCACTGCTCCTGGTGCTTCACACCCGAGGGCATCTACTTCAAACTC
GTGTCGGCCCAGAATGGTGACTTCCCCCGCTGGGGTGACTACGAGGACAAGAGGGACCTC
AATTACATCCGAAGCTTGATTCGCACTGGGGGATGGTTCGACGGCACGCAGCAGGAGTAC
CCTCCTGCAGACCCCAGTGAACACATGTATGCTCCTAAGTACCTGCTCAAGAACTATGAC
CAGTTCCGCTACTTGCTCGAAAATCCCTACCGGGAGCCCAAGAGCACTGTAGAGGGTGGG
CGCCGGAACCAGGGCTCAGACGGAAGGTCATCTGCTGTCAGGGGCAAGTTGGATACAACG
GAGGGCCCGGAACAGAAACTGATCTCTGAAGAGGACCTGTAG

MLKKQSAGLVLWGALLFVAWNALLLLFFWTRPAPGRPPSVSALDGDPASLTREVIRLAQD
AEVELERQRGLLQQIGDALSSQRGRVPTAAPPAQPRVPVTPAPLLQPLSPSKATEELHRV
DFVLPEDTTEYFVRTKAGGVCFKPGTRMLEKPSPGRTEEKTKVAEGSSVRGPARRPMRHV
LSARERLGGRGTRRKNVECVCLPGWHGPSCGVPTVVQYSNLPTKERLVPREVPRRVINAI
NINHEFDLLDVRFHELGDVVDAFVVCESNFTAYGEPRPLKFREMLTNGTFEYIRHKVLYV
FLDHFPPGGRQDGWIADDYLRTFLTQDGVSRLRNLRPDDVFIIDDADEIPARDGVLFLKL
YDGWTEPFAFHMRKSLYGFFWKQPGTLEVVSGCTIDMLQAVYGLDGIRLRRRQYYTMPNF
RQYENRTGHILVQWSLGSPLHFAGWHCSWCFTPEGIYFKLVSAQNGDFPRWGDYEDKRDL
NYIRSLIRTGGWFDGTQQEYPPADPSEHMYAPKYLLKNYDQFRYLLENPYREPKSTVEGG
RRNQGSDGRSSAVRGKLDTTEGPEQKLISEEDL

Figure 25.

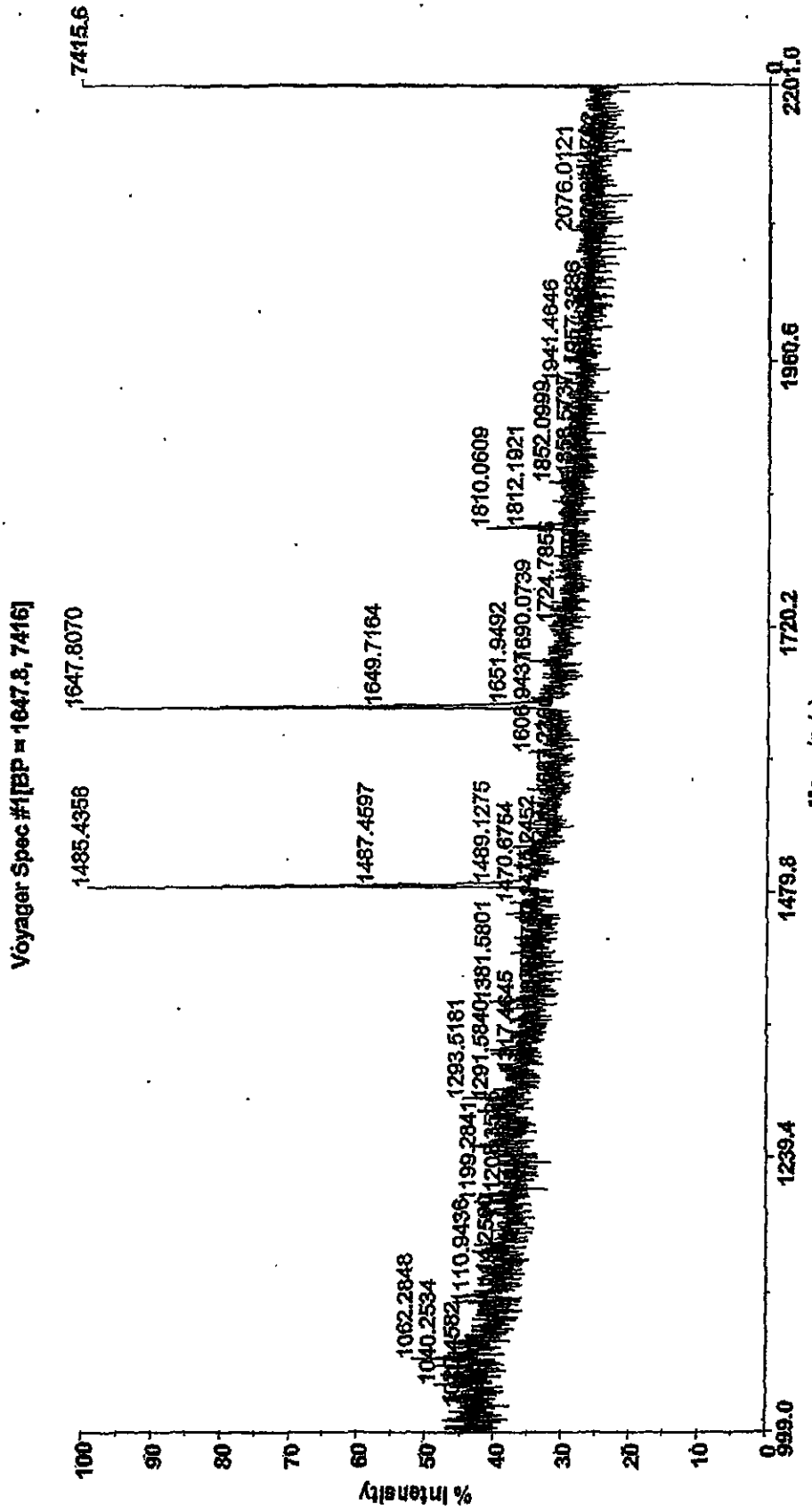

Voyager Spec #1[BP = 1647.8, 7416]
% Intenalty
Mass (m/z)
7415.6
1647.8070
1649.7164
1485.4356
1487.4597
1810.0609
1812.1921
1852.0999
1941.4846
2076.0121
1651.9492
1690.0739
1724.7855
1489.1275
1470.6754
1606
1293.5181
1291.5840
1381.5801
1199.2841
1110.9436
1062.2848
1040.2534
999.0
1239.4
1479.8
1720.2
1960.6
2201.0

FIG. 26

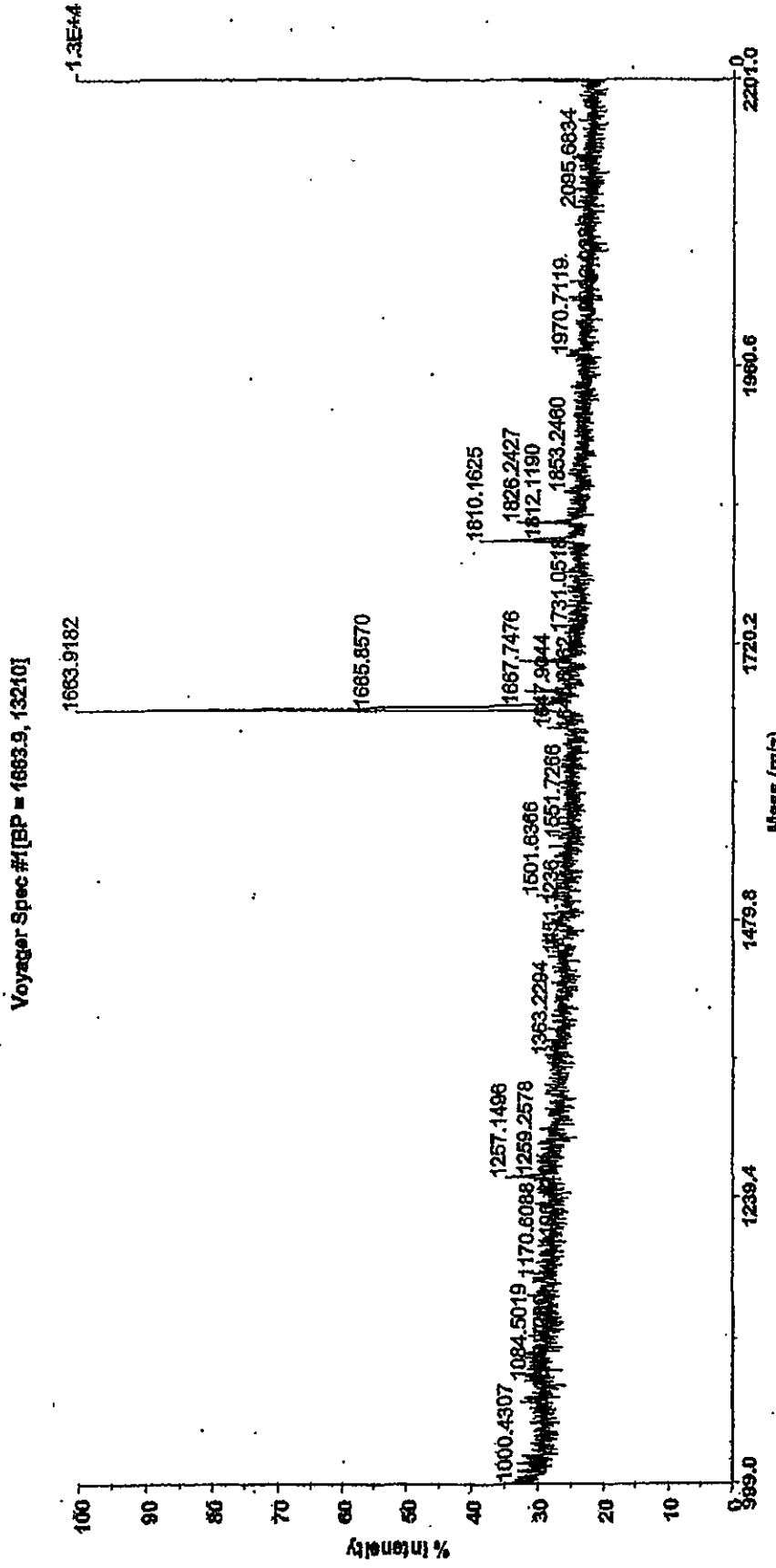

Voyager Spec #1[BP = 1663.9, 13210]
1.3E+4
0
1663.9182
1685.8570
1667.7476
1810.1625
1826.2427
1612.1190
1853.2460
1970.7119
2095.6834
1731.0518
1501.6366
1551.7266
1363.2294
1257.1496
1259.2578
1170.6088
1084.5019
1000.4307
% Intensity
100
90
80
70
60
50
40
30
20
10
0
989.0
1239.4
1479.8
1720.2
1960.6
2201.0
Mass (m/z)

FIG. 27A

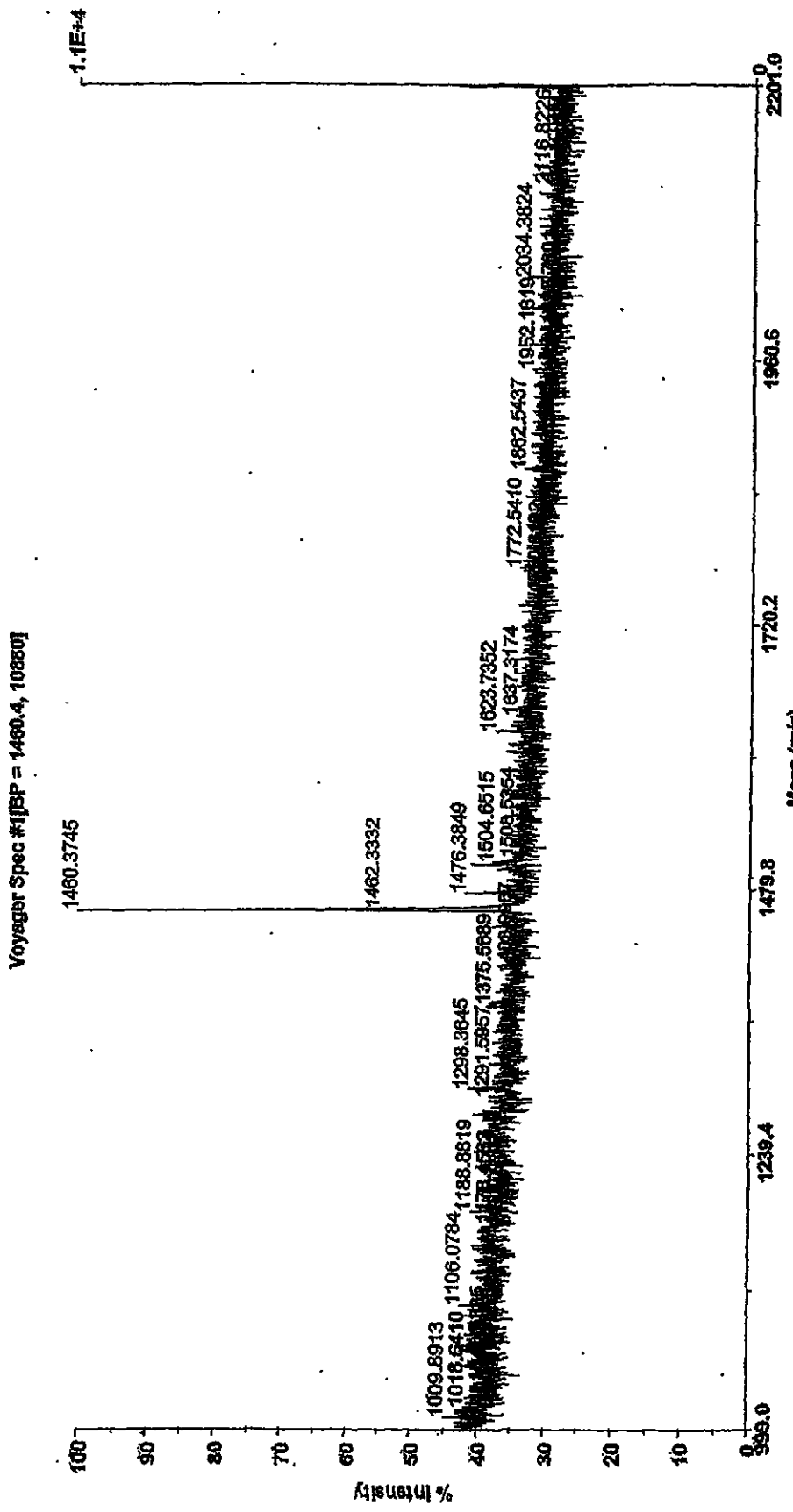

Voyager Spec #1[BP = 1460.4, 10880]
1.1E+4
% Intensity
100
90
80
70
60
50
40
30
20
10
0
999.0
1239.4
1479.8
1720.2
1960.6
2201.0
Mass (m/z)
1460.3745
1462.3332
1476.3849
1504.6515
1623.7352
1637.3174
1772.5410
1862.5437
1952.1819
2034.3824
1298.3645
1291.5957
1375.5689
1188.8819
1106.0784
1009.8913
1018.6410

FIG. 27B

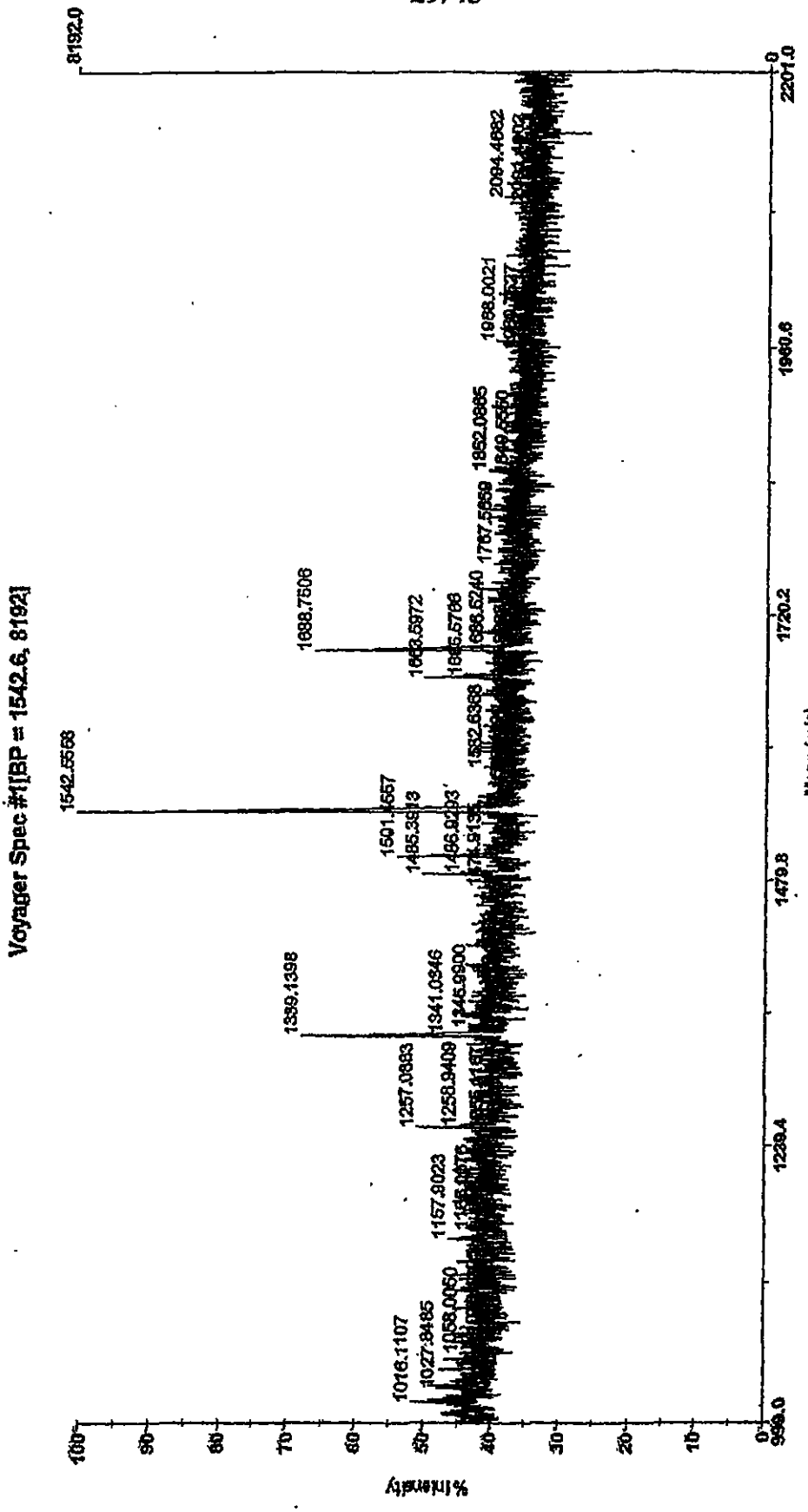

Voyager Spec #1[BP = 1542.6, 8192]
%Intensity
Mass (m/z)
8192.0
0
2201.0
1960.6
1720.2
1479.8
1239.4
999.0
1542.5568
1698.7506
1339.1398
1501.1667
1485.3813
1486.9263
1532.6388
1668.5972
1665.5766
1686.6240
1767.5859
1852.0865
1988.0021
2094.4662
1341.0346
1345.9900
1257.0693
1258.9409
1157.9023
1058.0050
1027.9485
1016.1107

FIG. 28A

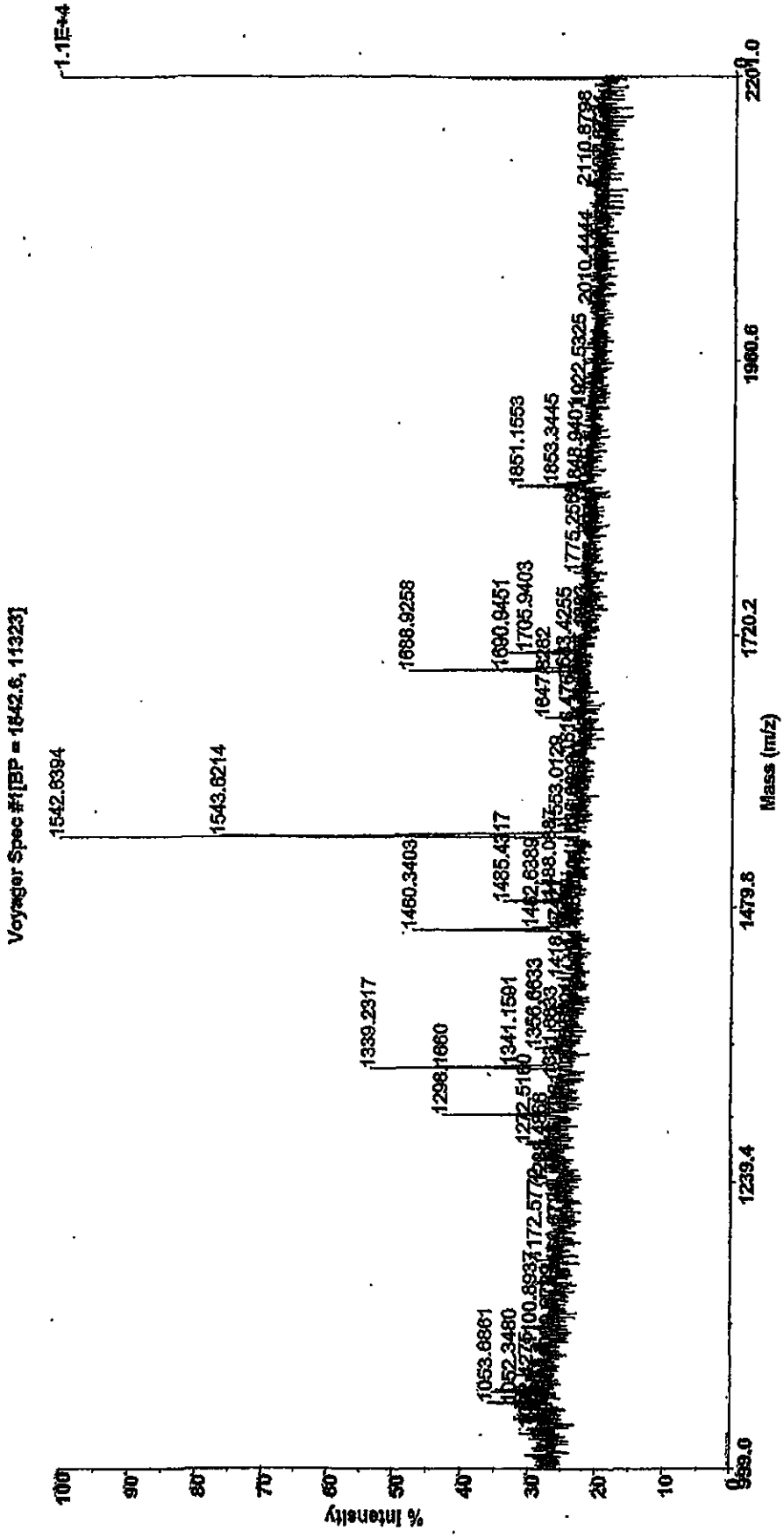

Voyager Spec #1[BP = 1542.6, 113231]
1.1E+4
% Intensity
Mass (m/z)
1542.6394
1543.6214
1688.9258
1690.9451
1705.9403
1851.1553
1853.3445
1480.3403
1485.4317
1339.2317
1660
1341.1591
1298
1053.6861
1052.3480
1239.4
1479.8
1720.2
1960.6
2201.0
989.0

FIG. 28B

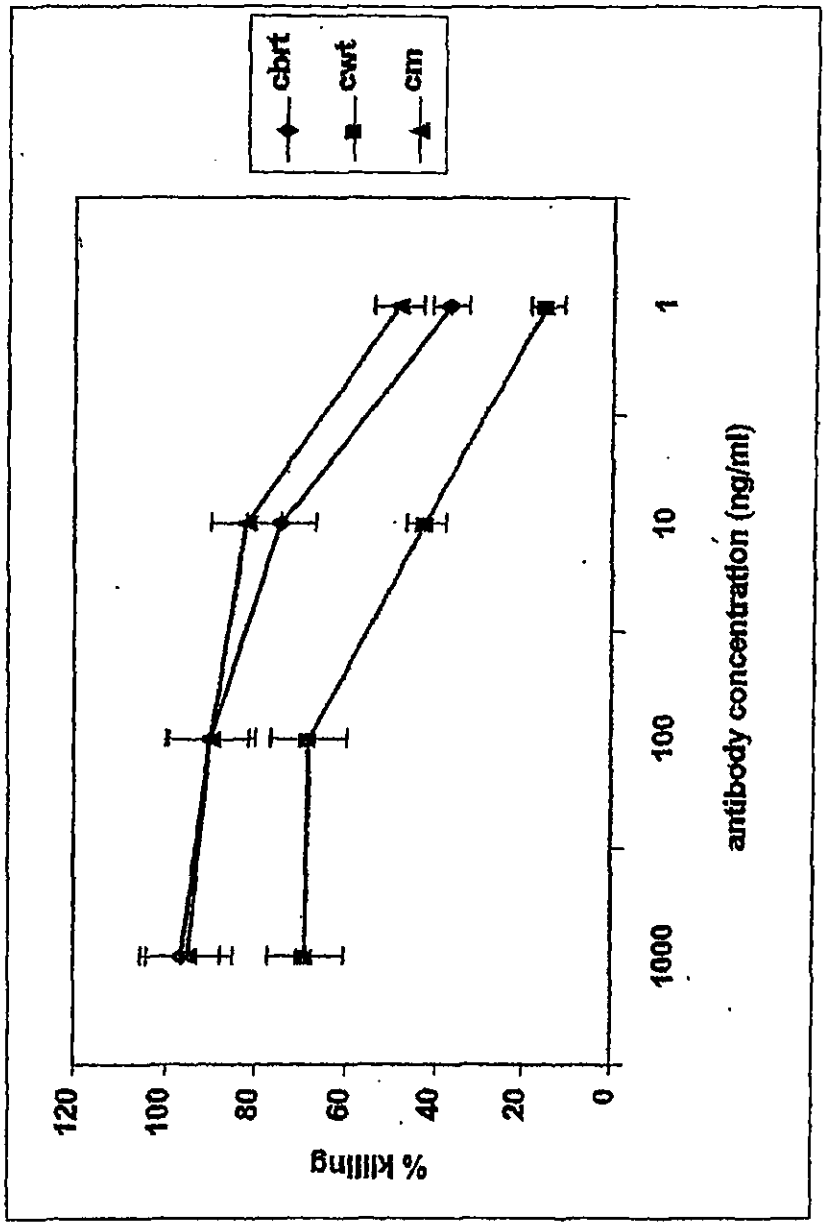

cbrt
cwt
cm
% killing
120
100
80
60
40
20
0
1000
100
10
1
antibody concentration (ng/ml)

FIG. 29

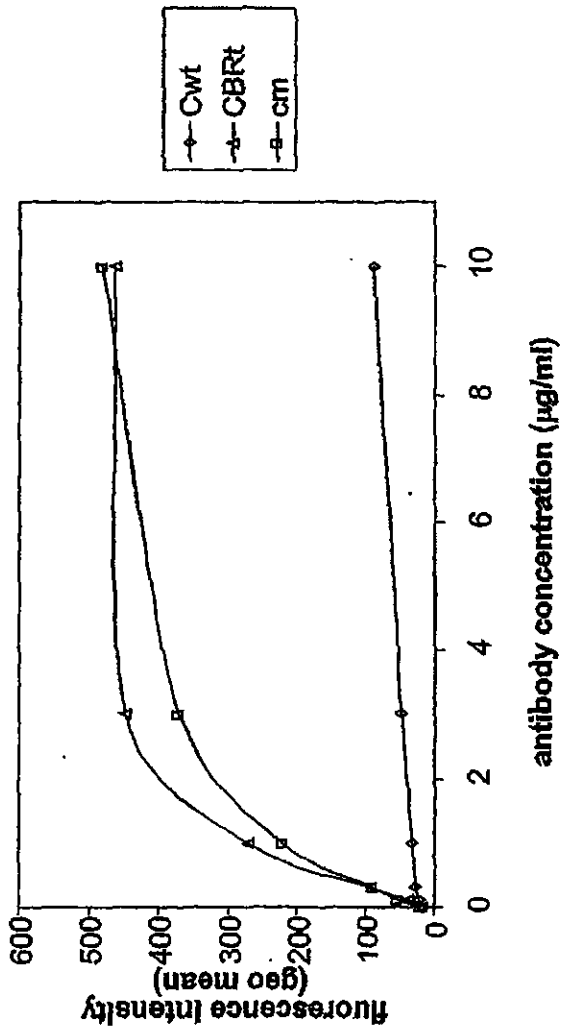

Cwt
CBRt
cm
fluorescence intensity
(geo mean)
600
500
400
300
200
100
0
0
2
4
6
8
10
antibody concentration (µg/ml)

FIG. 30

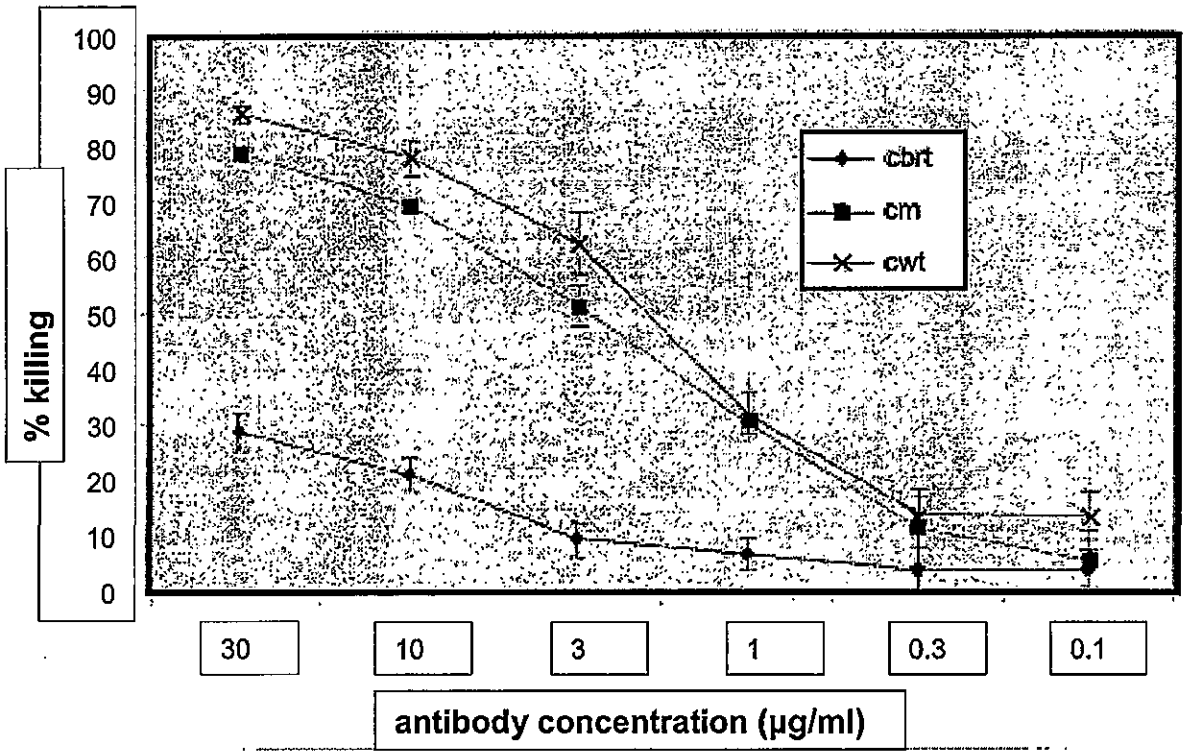

100
90
80
70
60
50
40
30
20
10
0
% killing
cbrt
cm
cwt
30
10
3
1
0.3
0.1
antibody concentration (µg/ml)

FIG. 31

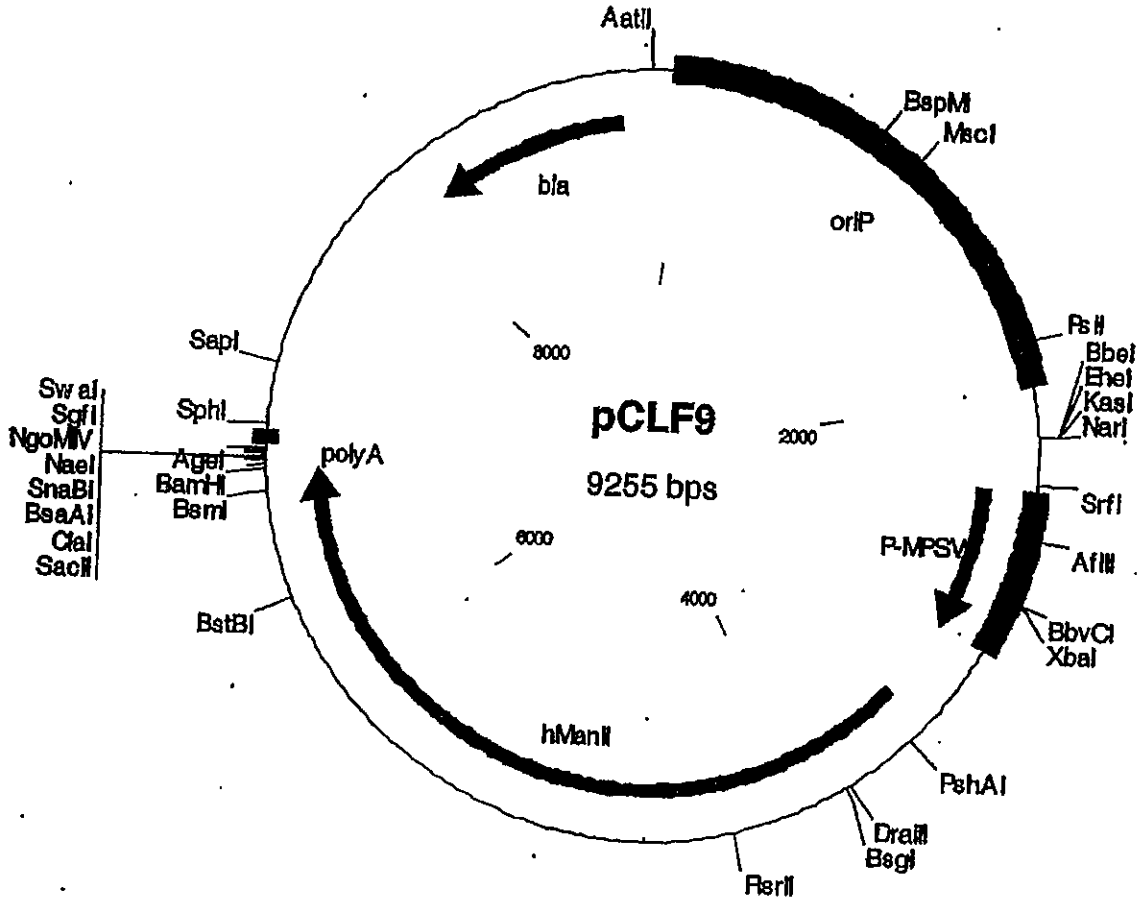

AatII
BspMI
MscI
bla
oriP
PsiI
BbeI
EheI
KasI
NarI
SrfI
AflII
BbvCI
XbaI
PshAI
DraIII
BsgI
RsrII
hManII
P-MPSV
BstBI
polyA
SapI
SphI
AgeI
BamHI
BsmI
SwaI
SgfI
NgoMIV
NaeI
SnaBI
BsaAI
ClaI
SacII
pCLF9
9255 bps
2000
4000
6000
8000

FIG. 32A

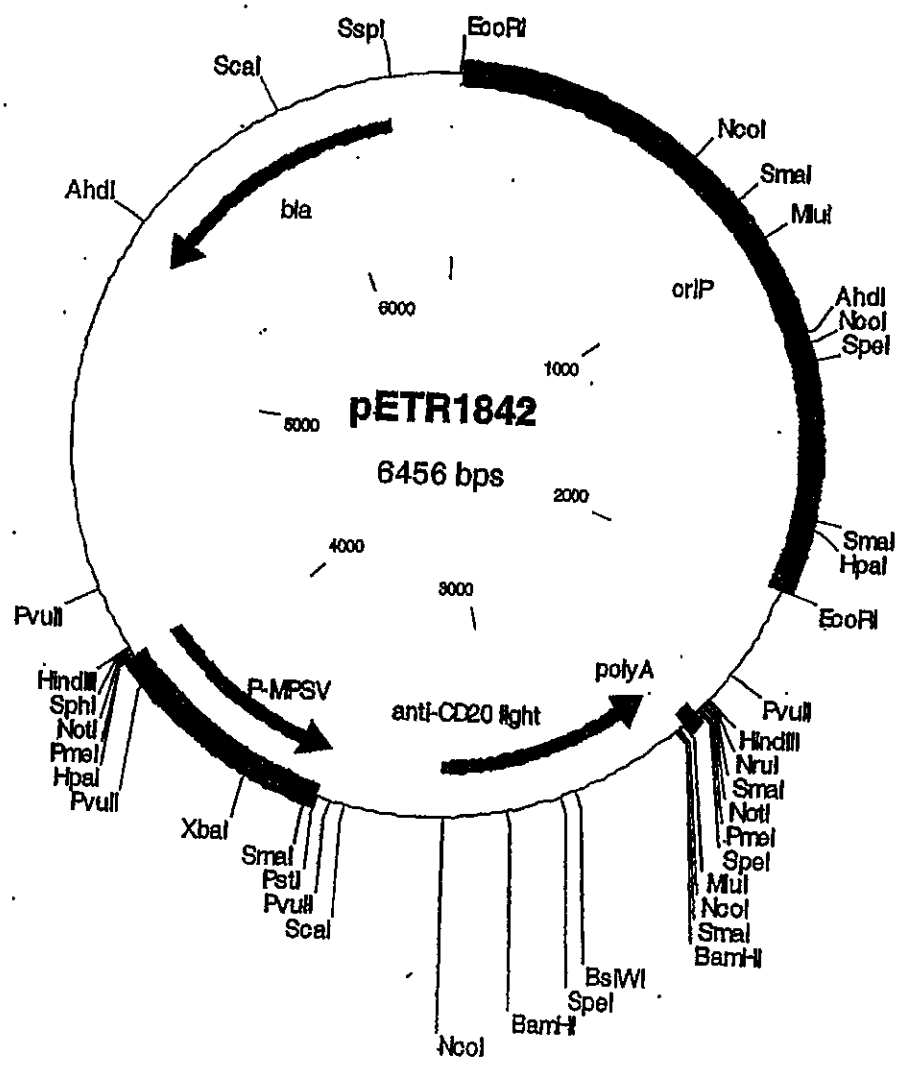

pETR1842
6456 bps
1000
2000
3000
4000
5000
6000
bla
oriP
P-MPSV
anti-CD20 light
polyA
SspI
EcoRI
ScaI
NcoI
SmaI
MluI
AhdI
NcoI
SpeI
SmaI
HpaI
EcoRI
PvuII
HindIII
SphI
NotI
PmeI
HpaI
PvuII
XbaI
SmaI
PstI
PvuII
ScaI
NcoI
BamHI
BsiWI
SpeI
PvuII
HindIII
NruI
SmaI
NotI
PmeI
SpeI
MluI
NcoI
SmaI
BamHI

FIG. 32B

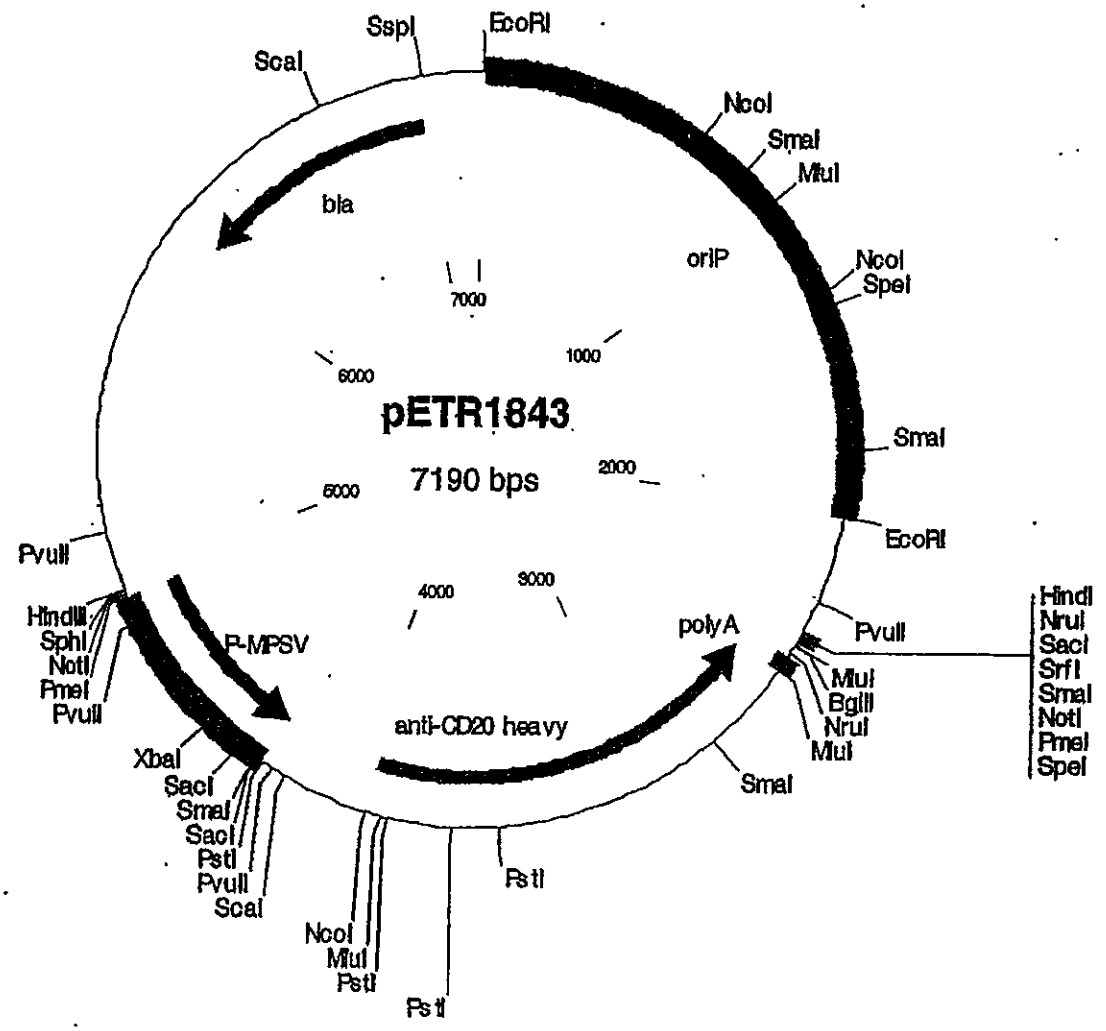

pETR1843
7190 bps
bla
oriP
P-MPSV
anti-CD20 heavy
polyA
1000
2000
3000
4000
5000
6000
7000
SspI
EcoRI
ScaI
NcoI
SmaI
MluI
NcoI
SpeI
SmaI
EcoRI
PvuII
HindIII
NruI
SacI
SrfI
SmaI
NotI
PmeI
SpeI
PvuII
MluI
BglII
NruI
MluI
SmaI
PstI
PstI
NcoI
MluI
PstI
XbaI
SacI
SmaI
SacI
PstI
PvuII
ScaI
HindIII
SphI
NotI
PmeI
PvuII

FIG. 32C

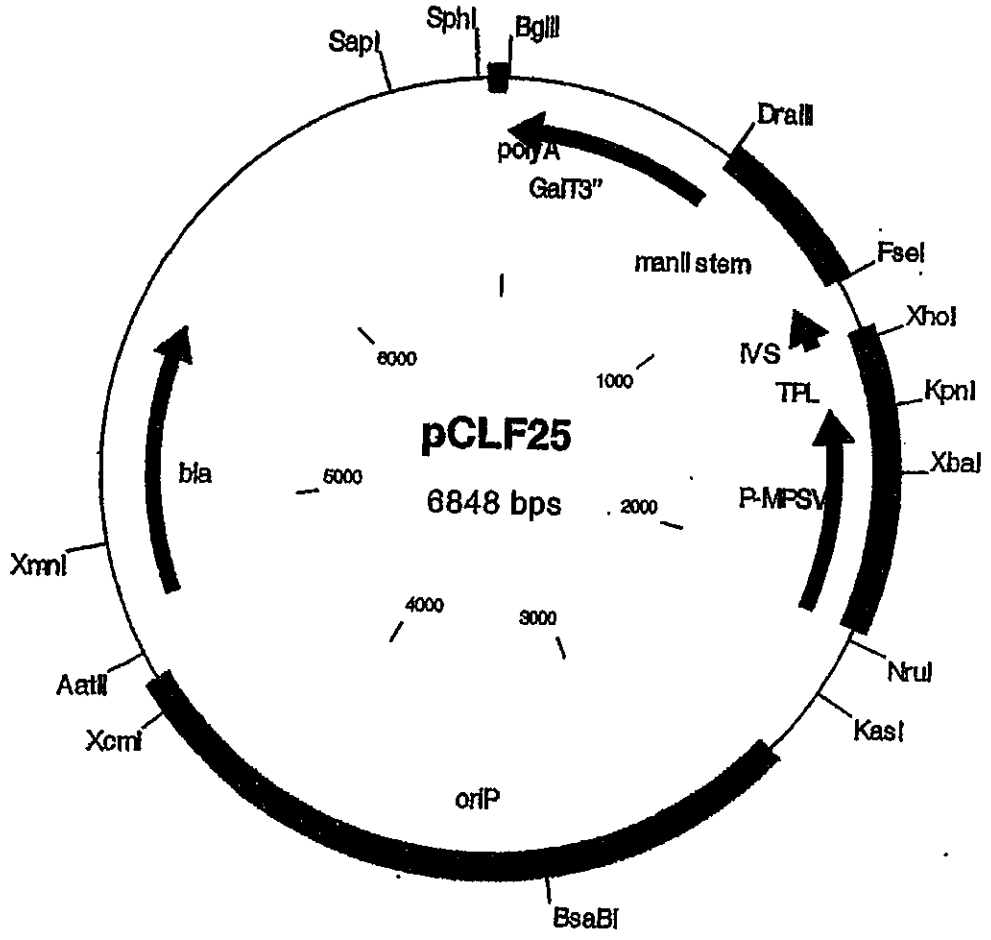

SapI
SphI
BglII
DraIII
polyA
GalT3"
manII stem
FseI
XhoI
IVS
TPL
KpnI
XbaI
P-MPSV
NruI
KasI
BsaBI
oriP
XcmI
AatII
XmnI
bla
pCLF25
6848 bps
1000
2000
3000
4000
5000
6000

FIG. 33A

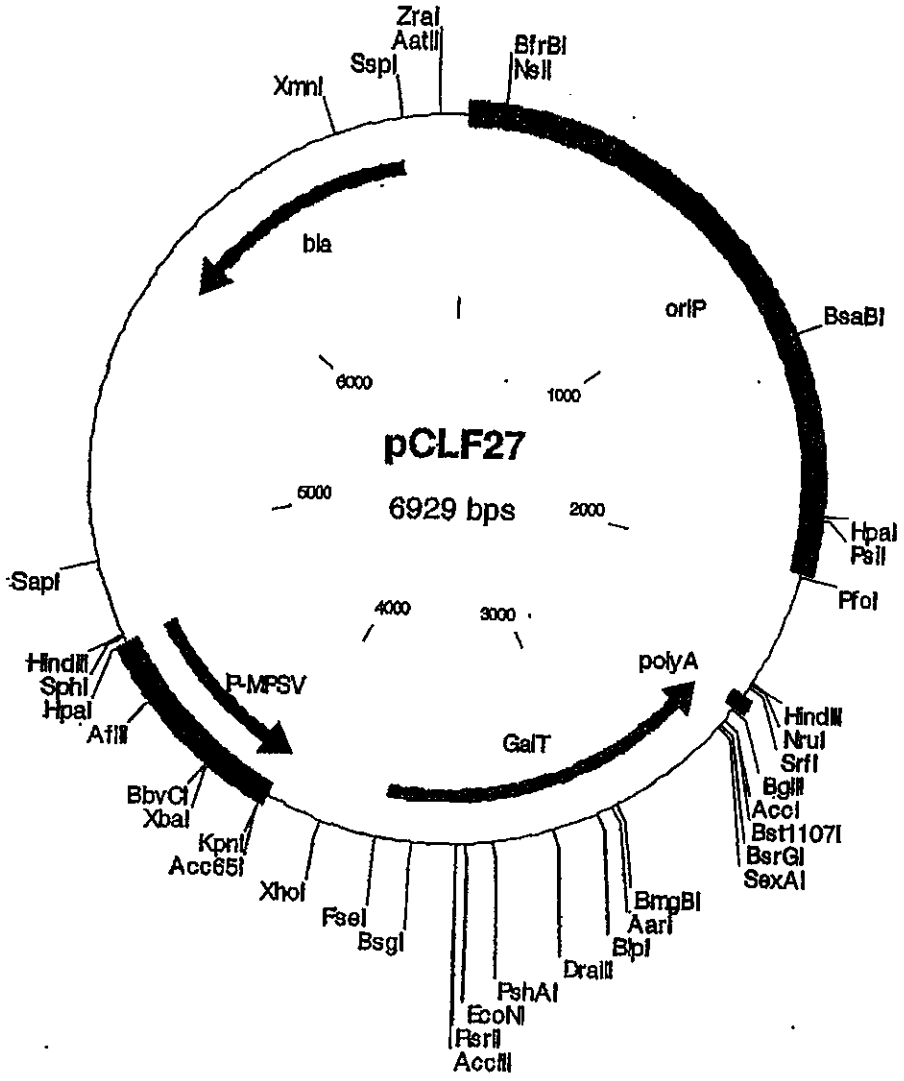
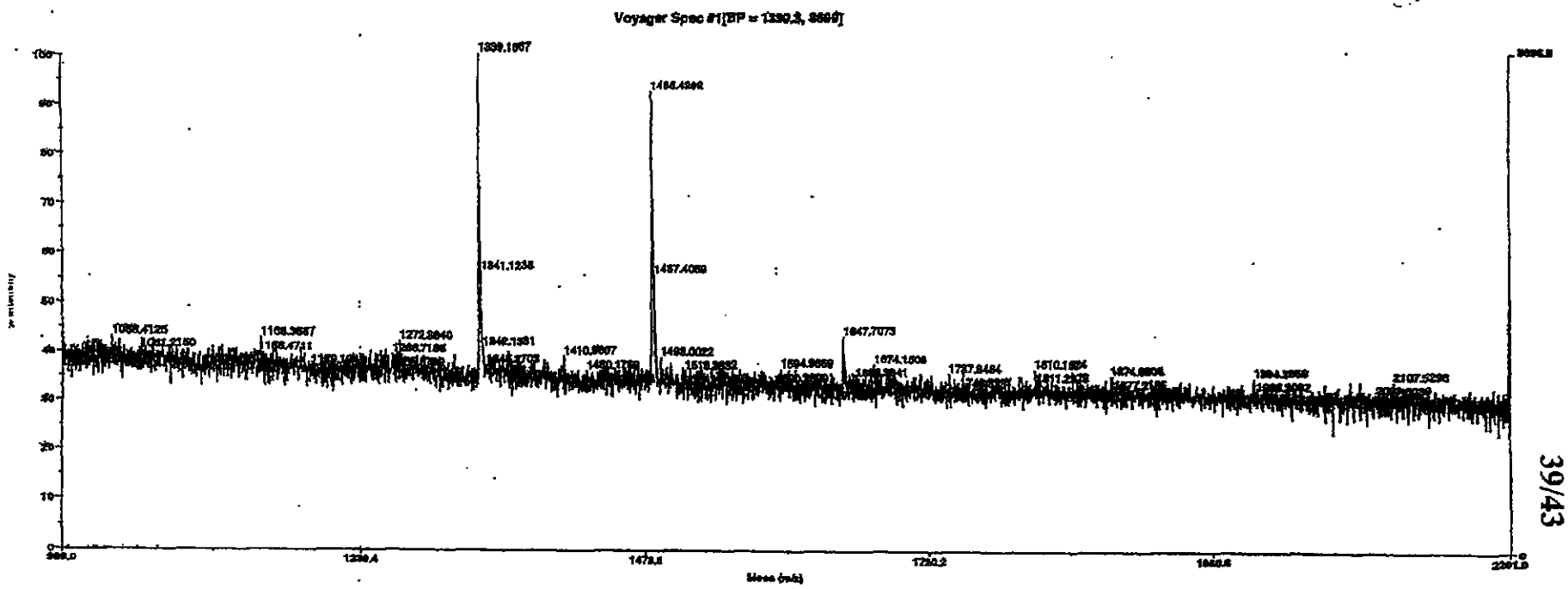

ZraI
AatII
BfrBI
NsiI
SspI
XmnI
bla
oriP
BsaBI
6000
1000
pCLF27
6929 bps
5000
2000
HpaI
PsiI
PfoI
SapI
4000
3000
HindIII
SphI
HpaI
AflII
P-MPSV
polyA
GalT
HindIII
NruI
SrfI
BglII
AccI
Bst1107I
BsrGI
SexAI
BbvCI
XbaI
KpnI
Acc65I
XhoI
FseI
BsgI
BmgBI
AarI
BpiI
DraIII
PshAI
EcoNI
PsrII
AccIII

FIG. 33B



	Manll
1339	48.40%
1486	43.30%
1647	8.30%
tot complex	100.00%
non-fuc, complex	48.40%

FIG. 34

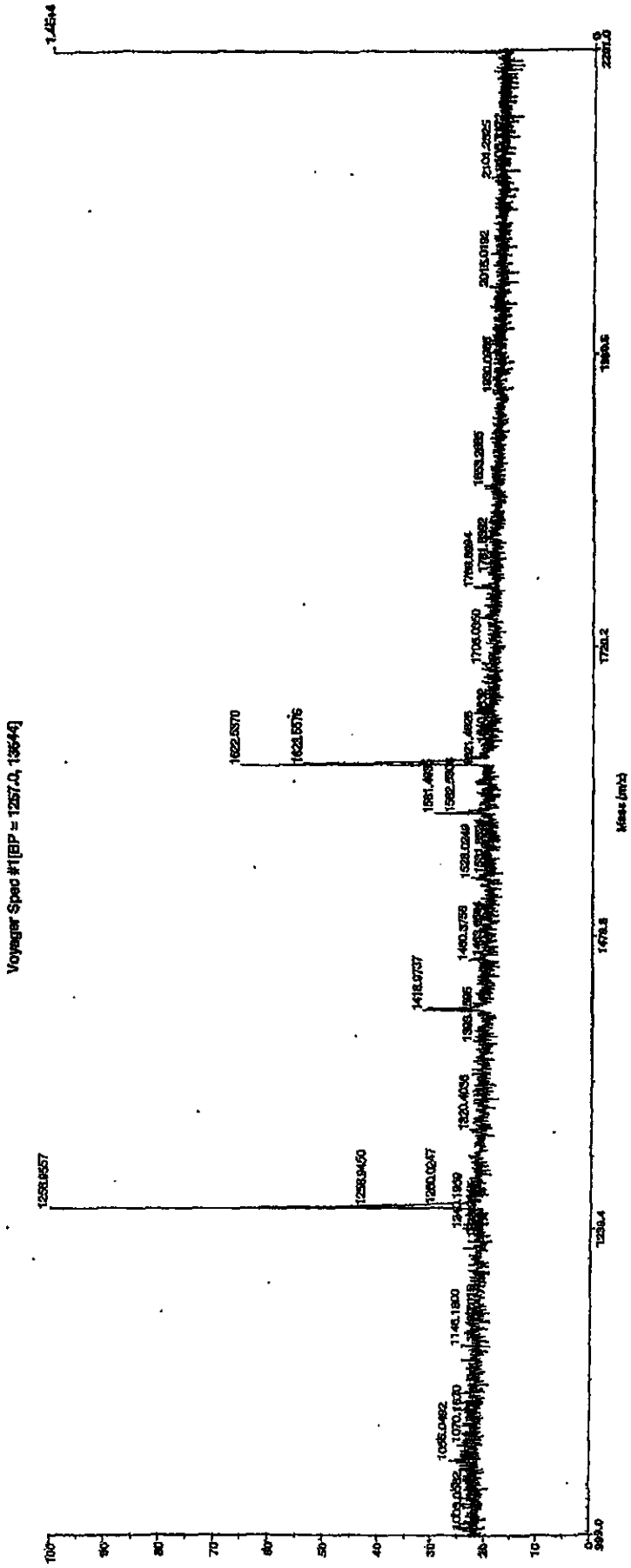

Voyager Spec #1[BP = 1257.0, 13644]
1622.8370
1622.8376
1258.8557
1258.9450
1418.0737
1148.1930
1055.0492
1070.1570
Mass (m/z)

FIG. 35A

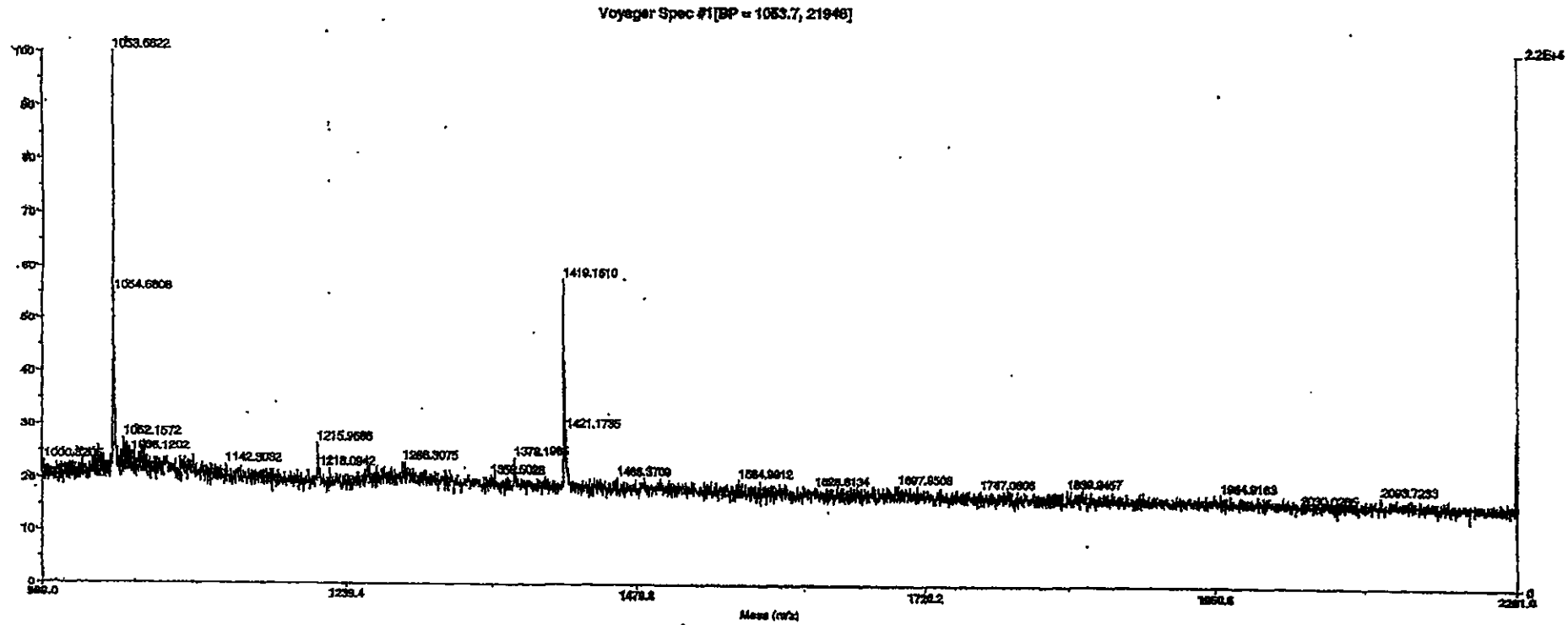


FIG. 35B

	pManII-GalT	+EndoH
1053		66.50%
1256	51.90%	
1419	8.20%	33.50%
1581	7.00%	
1622	30.00%	
1769	2.90%	
	100.00%	100.00%

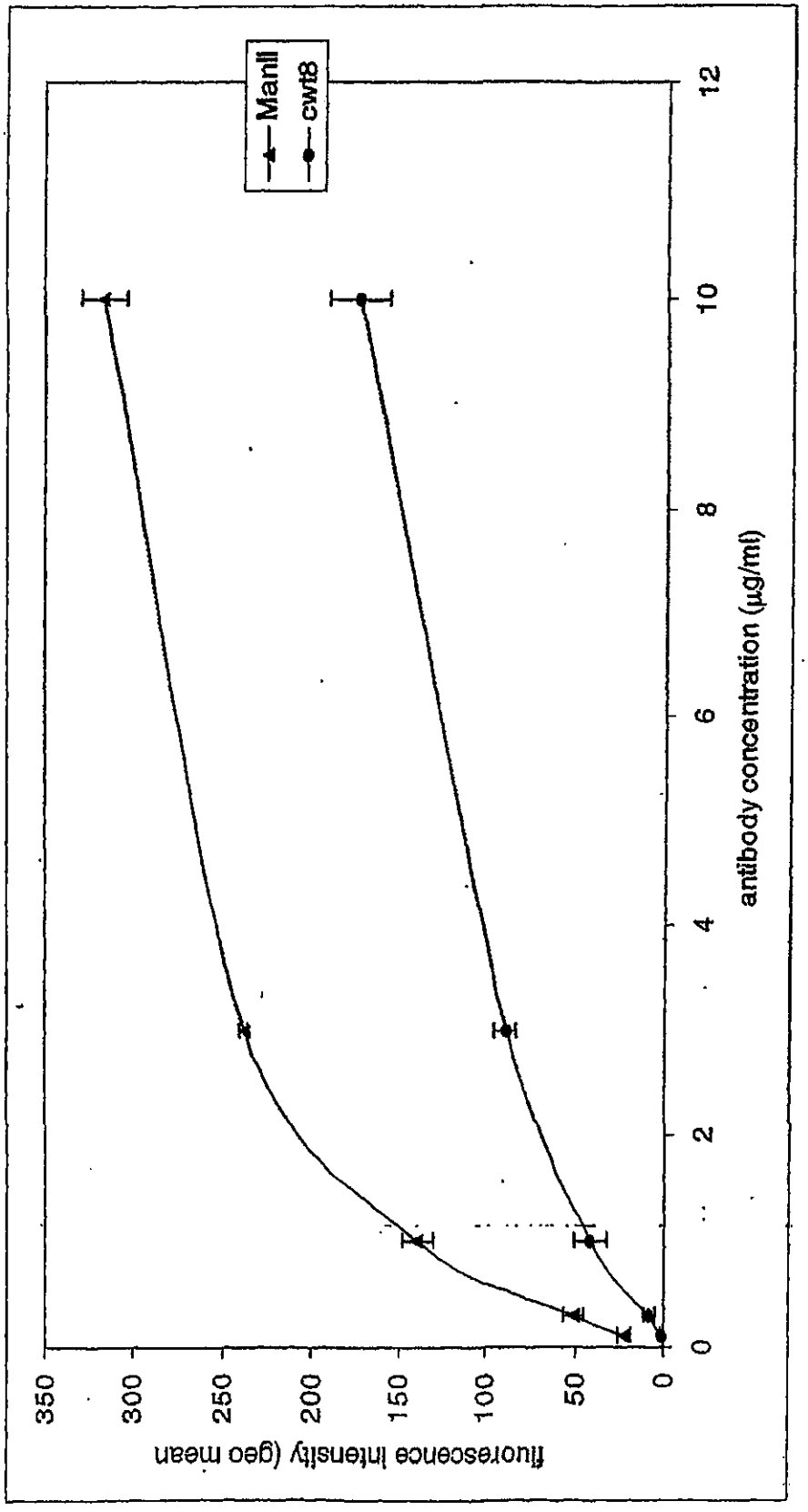

ManII
cwt8
fluorescence intensity (geo mean
0
50
100
150
200
250
300
350
0
2
4
6
8
10
12
antibody concentration (µg/ml)

FIG. 36

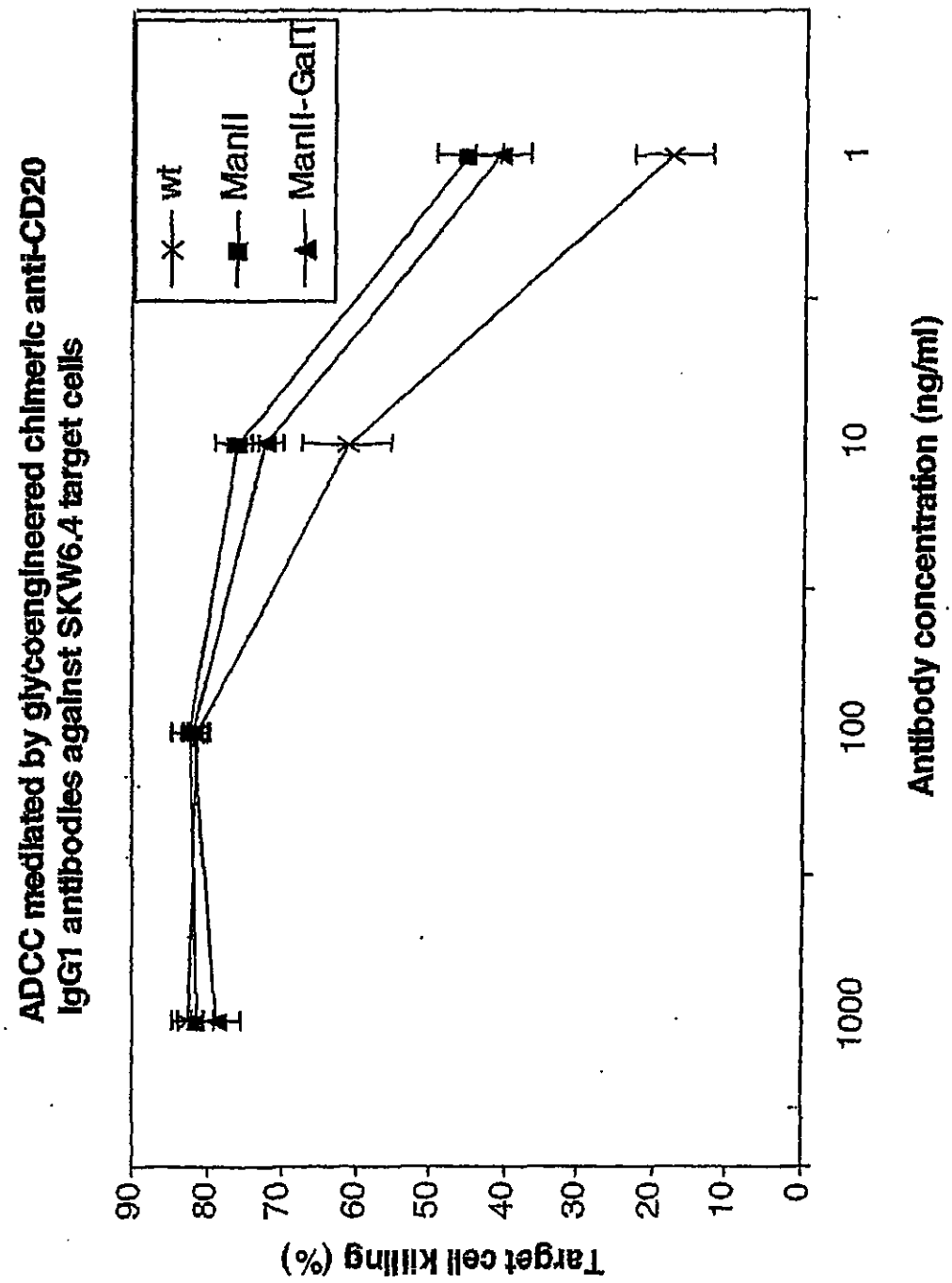

ADCC mediated by glycoengineered chimeric anti-CD20
IgG1 antibodies against SKW6.4 target cells
wt
ManII
ManII-GalT
Target cell killing (%)
0
10
20
30
40
50
60
70
80
90
1
10
100
1000
Antibody concentration (ng/ml)

FIG. 37