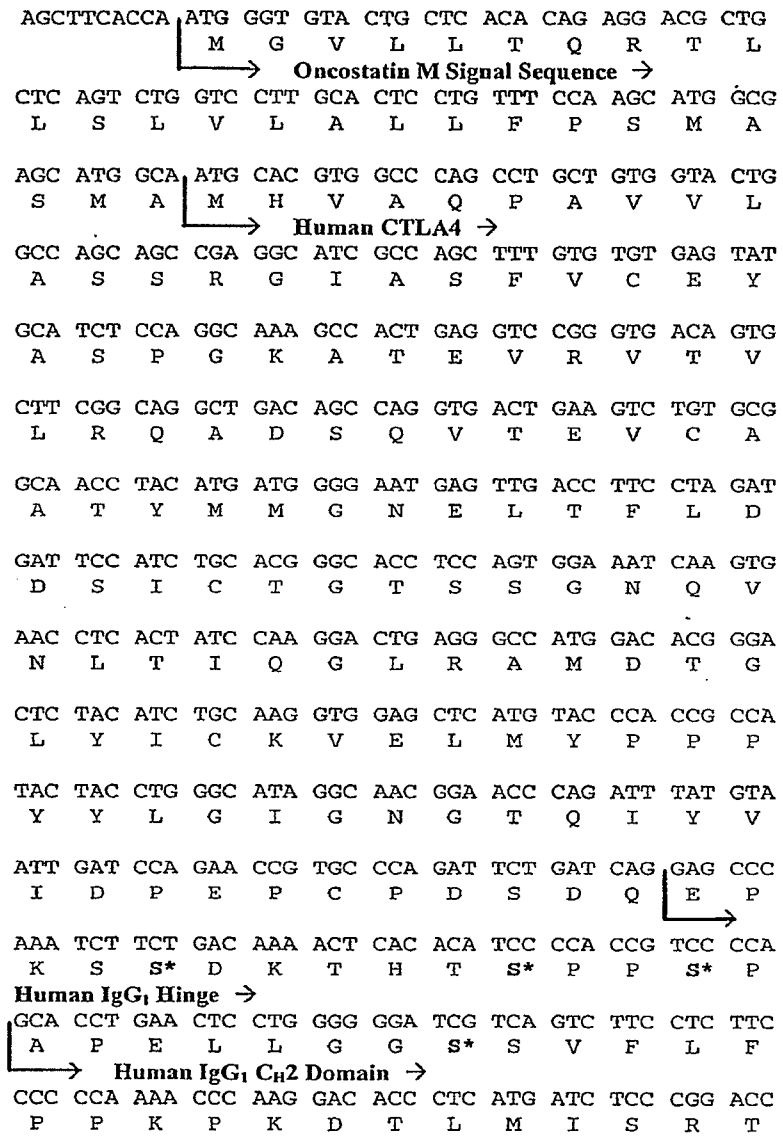

1 AGCTTCACCA ATG GGT GTA CTG CTC ACA CAG AGG ACG CTG
M G V L L T Q R T L
Oncostatin M Signal Sequence →
41 CTC AGT CTG GTC CTT GCA CTC CTG TTT CCA AGC ATG GCG
L S L V L A L L F P S M A
80 AGC ATG GCA ATG CAC GTG GCC CAG CCT GCT GTG GTA CTG
S M A M H V A Q P A V V L
Human CTLA4 →
119 GCC AGC AGC CGA GGC ATC GCC AGC TTT GTG TGT GAG TAT
A S S R G I A S F V C E Y
158 GCA TCT CCA GGC AAA GCC ACT GAG GTC CGG GTG ACA GTG
A S P G K A T E V R V T V
197 CTT CGG CAG GCT GAC AGC CAG GTG ACT GAA GTC TGT GCG
L R Q A D S Q V T E V C A
236 GCA ACC TAC ATG ATG GGG AAT GAG TTG ACC TTC CTA GAT
A T Y M M G N E L T F L D
275 GAT TCC ATC TGC ACG GGC ACC TCC AGT GGA AAT CAA GTG
D S I C T G T S S G N Q V
314 AAC CTC ACT ATC CAA GGA CTG AGG GCC ATG GAC ACG GGA
N L T I Q G L R A M D T G
353 CTC TAC ATC TGC AAG GTG GAG CTC ATG TAC CCA CCG CCA
L Y I C K V E L M Y P P P
392 TAC TAC CTG GGC ATA GGC AAC GGA ACC CAG ATT TAT GTA
Y Y L G I G N G T Q I Y V
431 ATT GAT CCA GAA CCG TGC CCA GAT TCT GAT CAG GAG CCC
I D P E P C P D S D Q E P
470 AAA TCT TCT GAC AAA ACT CAC ACA TCC CCA CCG TCC CCA
K S S* D K T H T S* P P S* P
Human IgG1 Hinge →
509 GCA CCT GAA CTC CTG GGG GGA TCG TCA GTC TTC CTC TTC
A P E L L G G S* S V F L F
Human IgG1 CH2 Domain →
548 CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC
P P K P K D T L M I S R T

FIG. 1A

```
587  CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA
       P   E   V   T   C   V   V   V   D   V   S   H   E

626  GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG
       D   P   E   V   K   F   N   W   Y   V   D   G   V

665  GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG
       E   V   H   N   A   K   T   K   P   R   E   E   Q

704  TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC
       Y   N   S   T   Y   R   V   V   S   V   L   T   V

743  CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC
       L   H   Q   D   W   L   N   G   K   E   Y   K   C

782  AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA
       K   V   S   N   K   A   L   P   A   P   I   E   K

821  ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG
       T   I   S   K   A   K   G   Q   P   R   E   P   Q
                               ⟶ Human IgG1 CH3 Domain →
860  GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG
       V   Y   T   L   P   P   S   R   D   E   L   T   K

899  AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT
       N   Q   V   S   L   T   C   L   V   K   G   F   Y

938  CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG
       P   S   D   I   A   V   E   W   E   S   N   G   Q

977  CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC
       P   E   N   N   Y   K   T   T   P   P   V   L   D

1016 TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG
       S   D   G   S   F   F   L   Y   S   K   L   T   V

1055 GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC
       D   K   S   R   W   Q   Q   G   N   V   F   S   C

1094 TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG
       S   V   M   H   E   A   L   H   N   H   Y   T   Q

1133 AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA GTGCGACG
       K   S   L   S   L   S   P   G   K   -

1172 GCCGGCAAGC CCCGCTCCCC GGGCTCTCGC GGTCGCAC GAGGATGCTT
1222 CTAGA
```

FIG. 1B

```
ATGGGTGTACTGCTCACACAGAGGACGCTGCTCAGTCTGGTCCTTGCACTCCTGTTTCCA      -19
M--G--V--L--L--T--Q--R--T--L--L--S--L--V--L--A--L--L--F--P--      -7

AGCATGGCGAGCATGGCAATGCACGTGGCCCAGCCTGCTGTGGTACTGGCCAGCAGCCGA      +42
S--M--A--S--M--A--M--H--V--A--Q--P--A--V--V--L--A--S--S--R--      +14
          +1

GGCATCGCTAGCTTTGTGTGTGAGTATGCATCTCCAGGCAAATATACTGAGGTCCGGGTG      +102
G--I--A--S--F--V--C--E--Y--A--S--P--G--K--Y--T--E--V--R--V--      +34

ACAGTGCTTCGGCAGGCTGACAGCCAGGTGACTGAAGTCTGTGCGGCAACCTACATGATG      +162
T--V--L--R--Q--A--D--S--Q--V--T--E--V--C--A--A--T--Y--M--M--      +54

GGGAATGAGTTGACCTTCCTAGATGATTCCATCTGCACGGGCACCTCCAGTGGAAATCAA      +222
G--N--E--L--T--F--L--D--D--S--I--C--T--G--T--S--S--G--N--Q--      +74

GTGAACCTCACTATCCAAGGACTGAGGGCCATGGACACGGGACTCTACATCTGCAAGGTG      +282
V--N--L--T--I--Q--G--L--R--A--M--D--T--G--L--Y--I--C--K--V--      +94

GAGCTCATGTACCCACCGCCATACTACGAGGGCATAGGCAACGGAACCCAGATTTATGTA      +342
E--L--M--Y--P--P--P--Y--Y--E--G--I--G--N--G--T--Q--I--Y--V--      +114

ATTGATCCAGAACCGTGCCCAGATTCTGATCAGGAGCCCAAATCTTCTGACAAAACTCAC      +402
I--D--P--E--P--C--P--D--S--D--Q--E--P--K--S--S--D--K--T--H--      +134

ACATCCCCACCGTCCCCAGCACCTGAACTCCTGGGGGGGATCGTCAGTGTTCCTCTTCCCC      +462
T--S--P--P--S--P--A--P--E--L--L--G--G--S--S--V--F--L--F--P--      +154

CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTG      +522
P--K--P--K--D--T--L--M--I--S--R--T--P--E--V--T--C--V--V--V--      +174

GACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG      +582
D--V--S--H--E--D--P--E--V--K--F--N--W--Y--V--D--G--V--E--V--      +194

CATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGC      +642
H--N--A--K--T--K--P--R--E--E--Q--Y--N--S--T--Y--R--V--V--S--      +214

GTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC      +702
V--L--T--V--L--H--Q--D--W--L--N--G--K--E--Y--K--C--K--V--S--      +234

AACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA      +762
N--K--A--L--P--A--P--I--E--K--T--I--S--K--A--K--G--Q--P--R--      +254

GAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGC      +822
E--P--Q--V--Y--T--L--P--P--S--R--D--E--L--T--K--N--Q--V--S--      +274

CTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT      +882
L--T--C--L--V--K--G--F--Y--P--S--D--I--A--V--E--W--E--S--N--      +294

GGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTC      +942
G--Q--P--E--N--N--Y--K--T--T--P--P--V--L--D--S--D--G--S--F--      +314

TTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCA      +1002
F--L--Y--S--K--L--T--V--D--K--S--R--W--Q--Q--G--N--V--F--S--      +334

TGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCT      +1062
C--S--V--M--H--E--A--L--H--N--H--Y--T--Q--K--S--L--S--L--S--      +354

CCGGGTAAATGA
P--G--K--*
```

FIG. 2

MGVLLTQRTLLSLVLALLFPSMASMA

MHVAQPAVVLASSRGIASFVCEYASPGKYTEVRVTVLRQADSQVTEVCAA 50

TYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPP 100

PYYEGIGNGTQIYVIDPEPCCPDSDQEPKSSDKTHTSPPSPAPELLGGSSV 150

FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK 200

PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK 250

GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN 300

YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS 350

LSLSPGK

Pro-sequence

FIG. 3

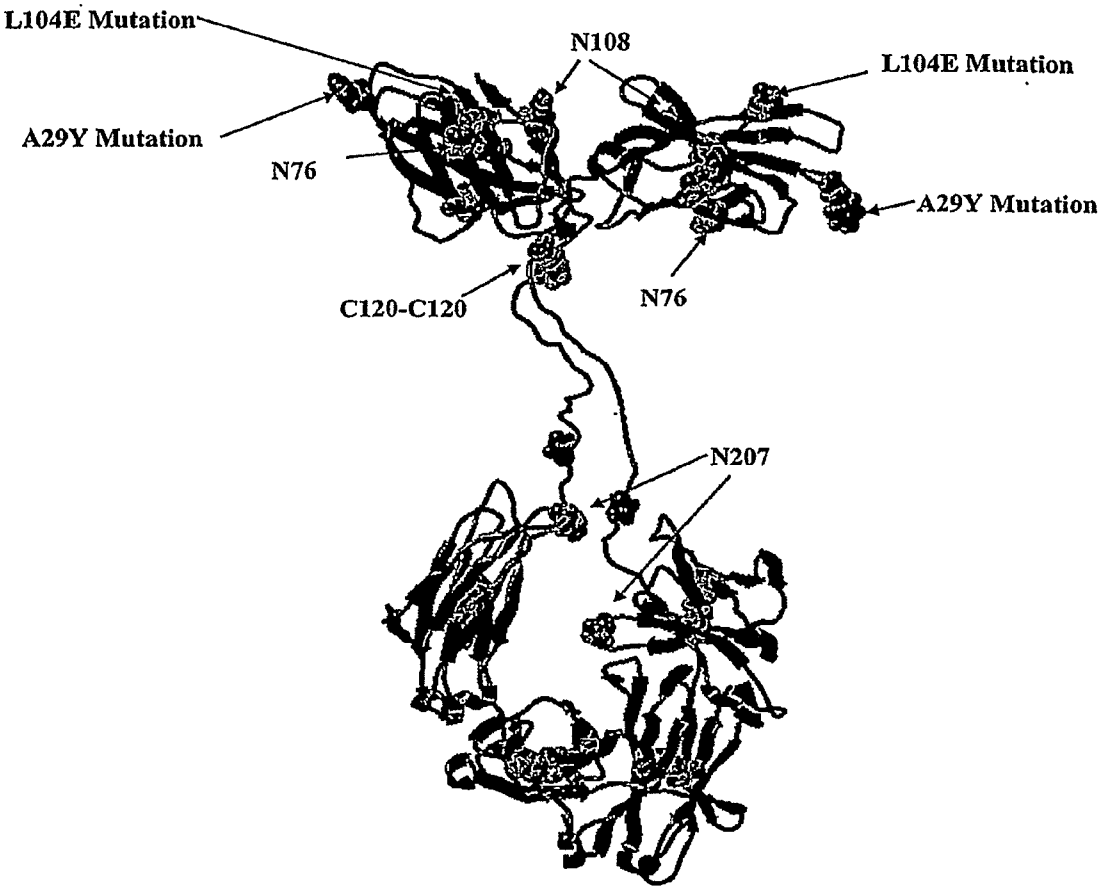

L104E Mutation
N108
L104E Mutation
A29Y Mutation
N76
A29Y Mutation
C120-C120
N76
N207

FIG. 4

MGVLLTQRTLLSLVLALLFPSMASMA[*]

M[**]**HVAQPAVVLASSRGIASFVCEYASPGKYTEVRVTVLRQADSQVTEVCAA**[50]

TYMMGNELTFLDDSICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYPP[100]

PYYEGIGNGTQIYVIDPEPCPDSDQEPKSSDKTHTSPPSPAPELLGGSSV[150]

FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK[200]

PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK[250]

GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN[300]

YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS[350]

LSLSPGK[***]

G[****]

Key:
Pro-sequence
CTLA-4 domain with two amino acid substitutions (L104E and A29Y)
Human IgG Fc fragment
Amino Acid Substitution
N-Linked Glycosylation Sites
C-120, Inter-chain disulfide bond

FIG. 5

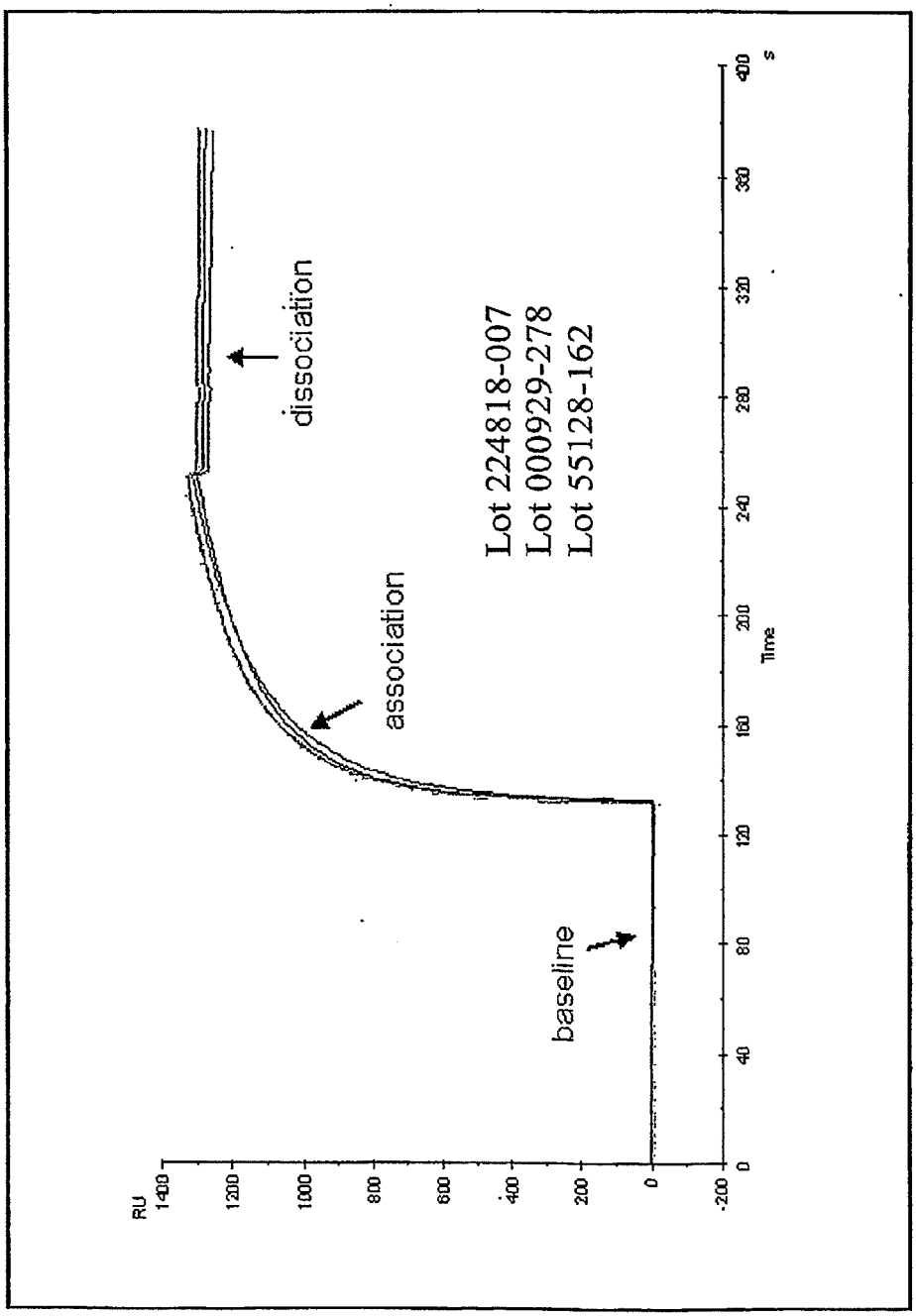

RU
1400
1200
1000
800
600
400
200
0
-200
0
40
80
120
160
200
240
280
320
360
400
s
Time
dissociation
association
baseline
Lot 224818-007
Lot 000929-278
Lot 55128-162

FIG. 6

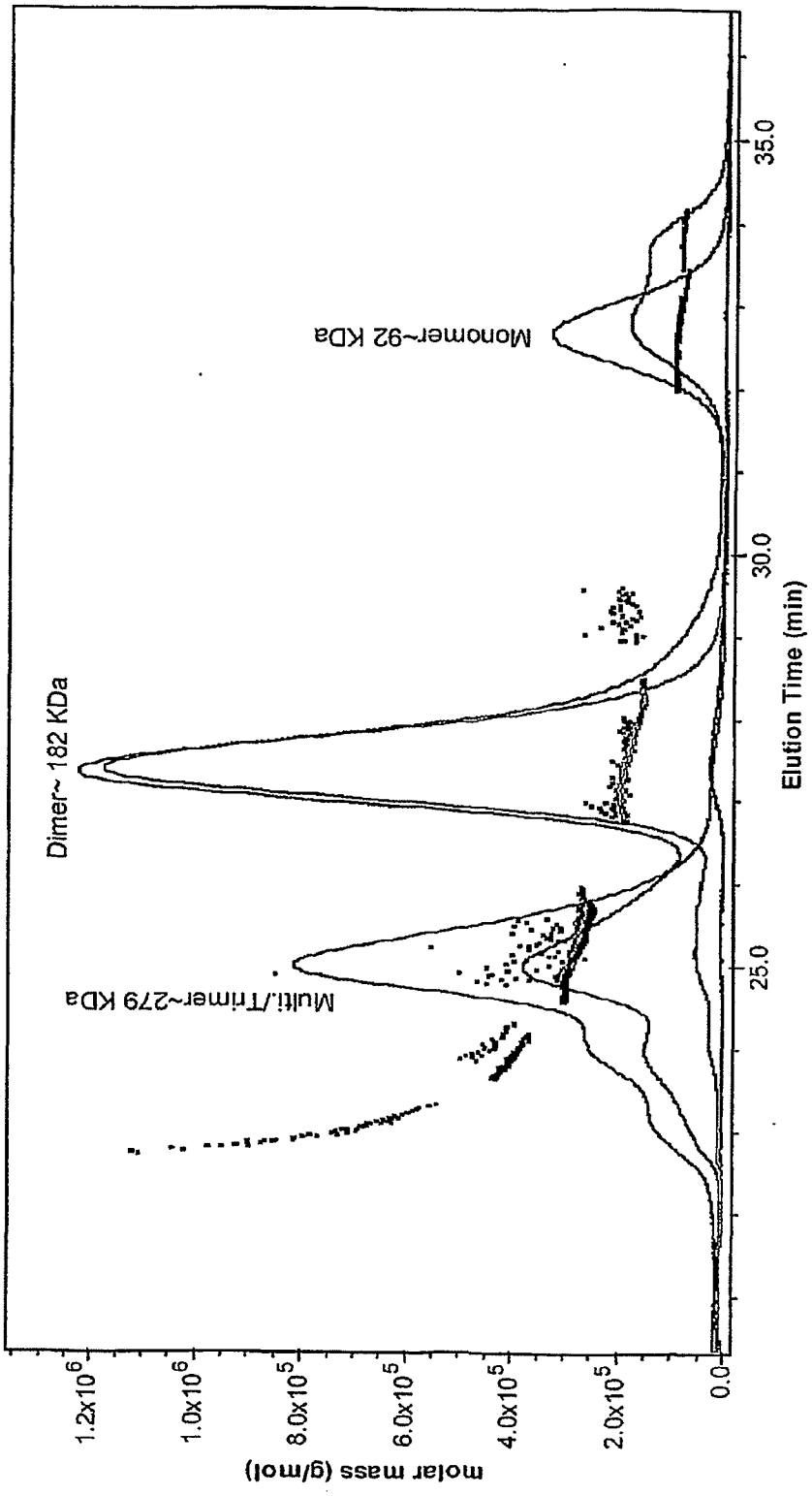

Monomer~92 KDa
Dimer~ 182 KDa
Multi./Trimer~279 KDa
molar mass (g/mol)
1.2x10^6
1.0x10^6
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0.0
25.0
30.0
35.0
Elution Time (min)

FIG. 7

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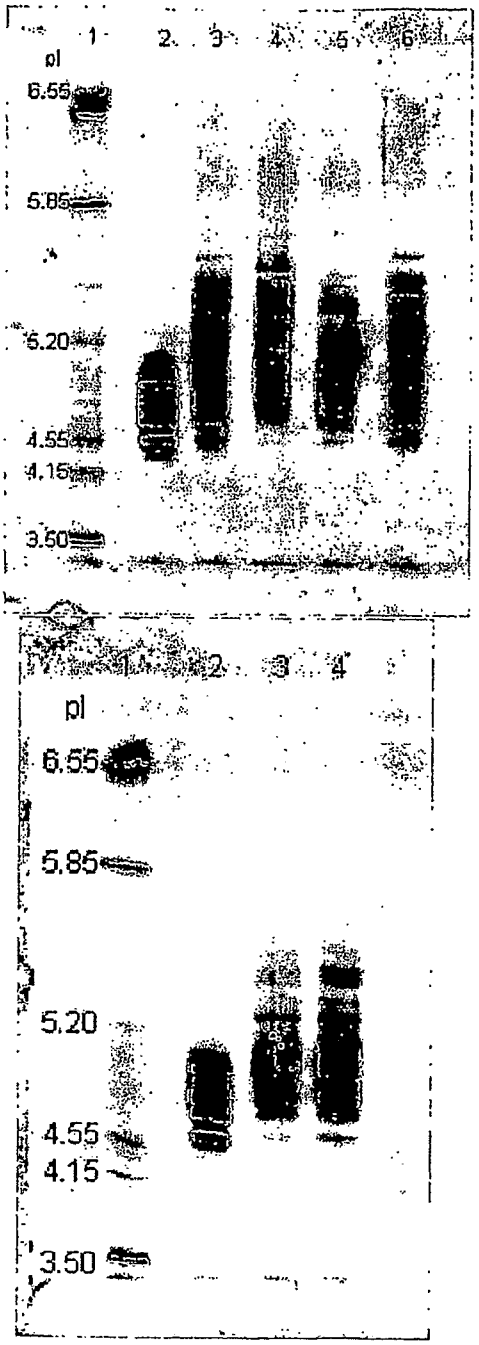

1
2
3
4
5
6
pI
6.55
5.85
5.20
4.55
4.15
3.50
1
2
3
4
pI
6.55
5.85
5.20
4.55
4.15
3.50

FIG. 8A and 8B

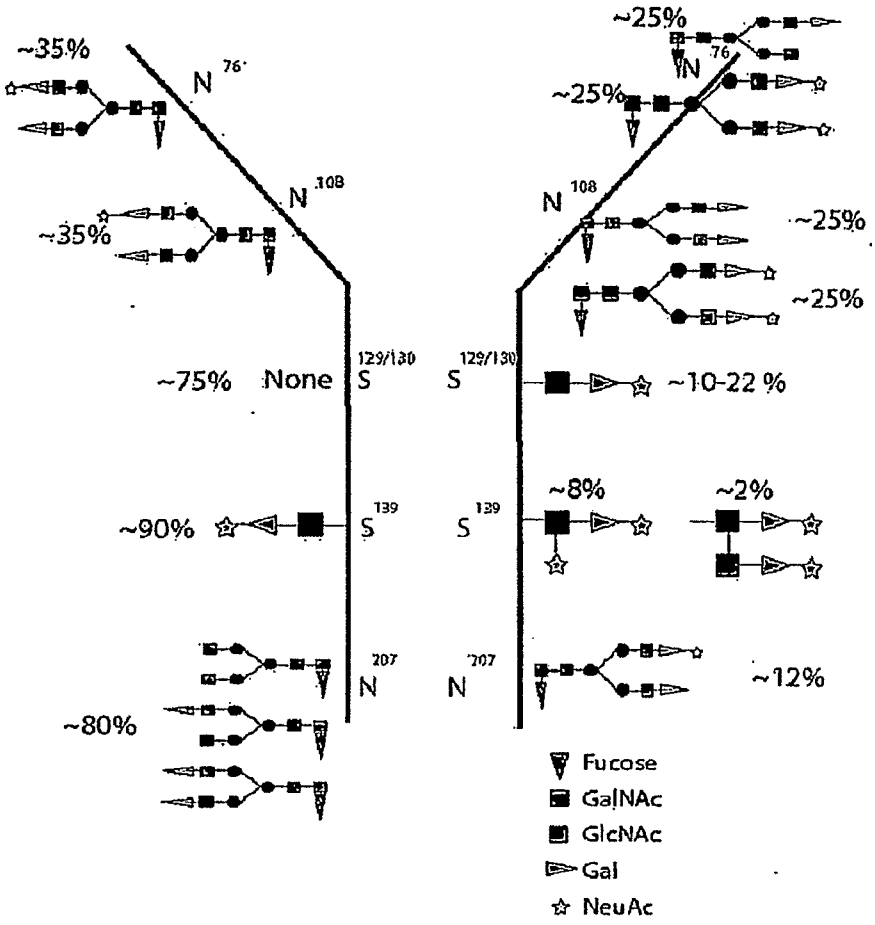

~35%
N 76
N 108
~35%
~75% None
S 129/130
~90%
S 139
~80%
N 207
~25%
N 76
~25%
N 108
~25%
~25%
S 129/130
~10-22 %
~8%
S 139
~2%
N 207
~12%
Fucose
GalNAc
GlcNAc
Gal
NeuAc

FIG. 9

PAGE INTENTIONALLY LEFT BLANK

FIG. 10

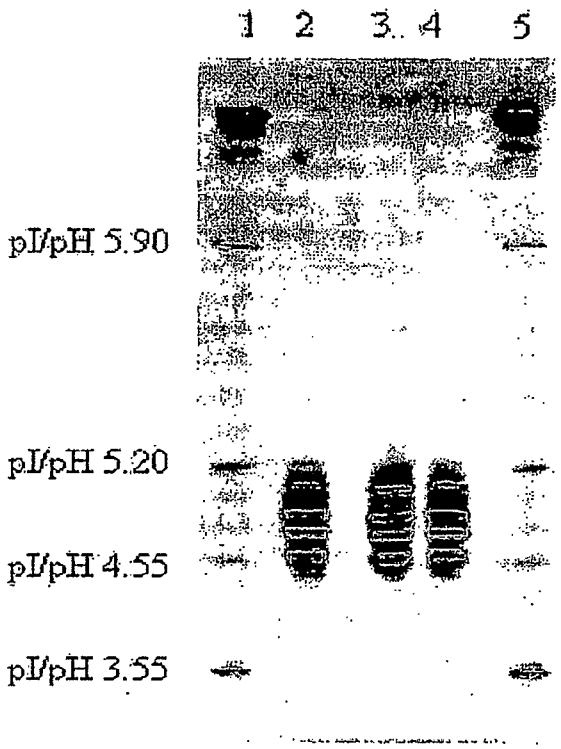


FIG. 11

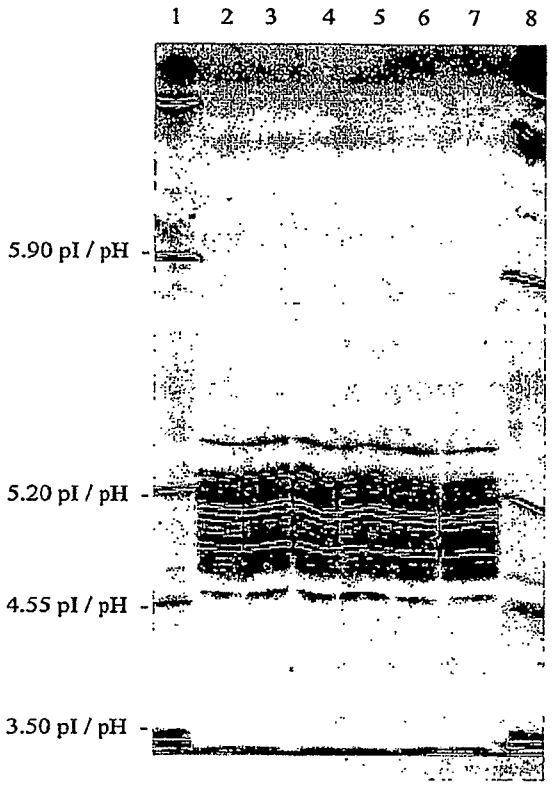


FIG. 12

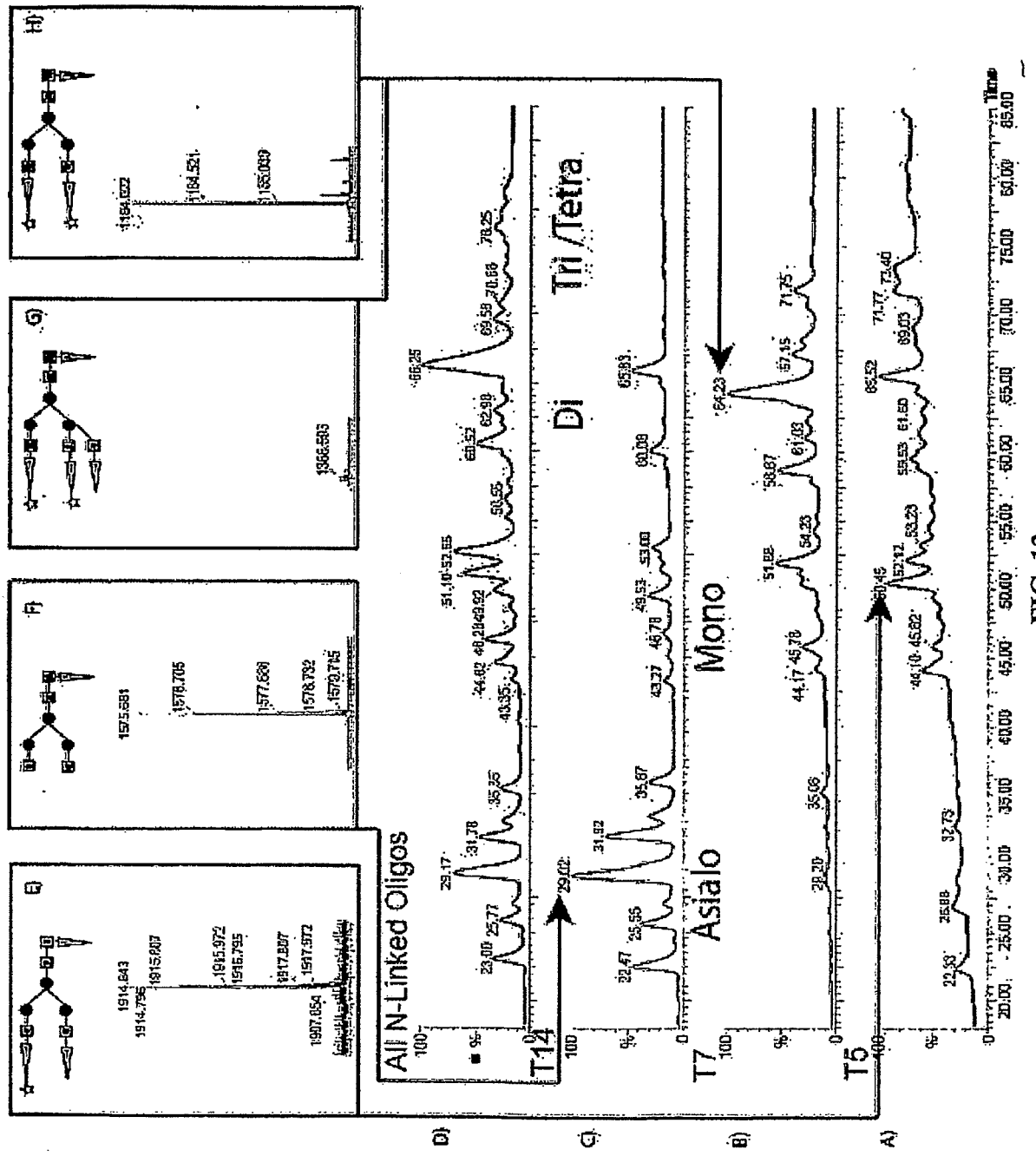

All N-Linked Oligos
Di Tri /Tetra
Asialo Mono
T12
T7
T5
A)
B)
C)
D)
E)
F)
G)
H)

FIG. 13

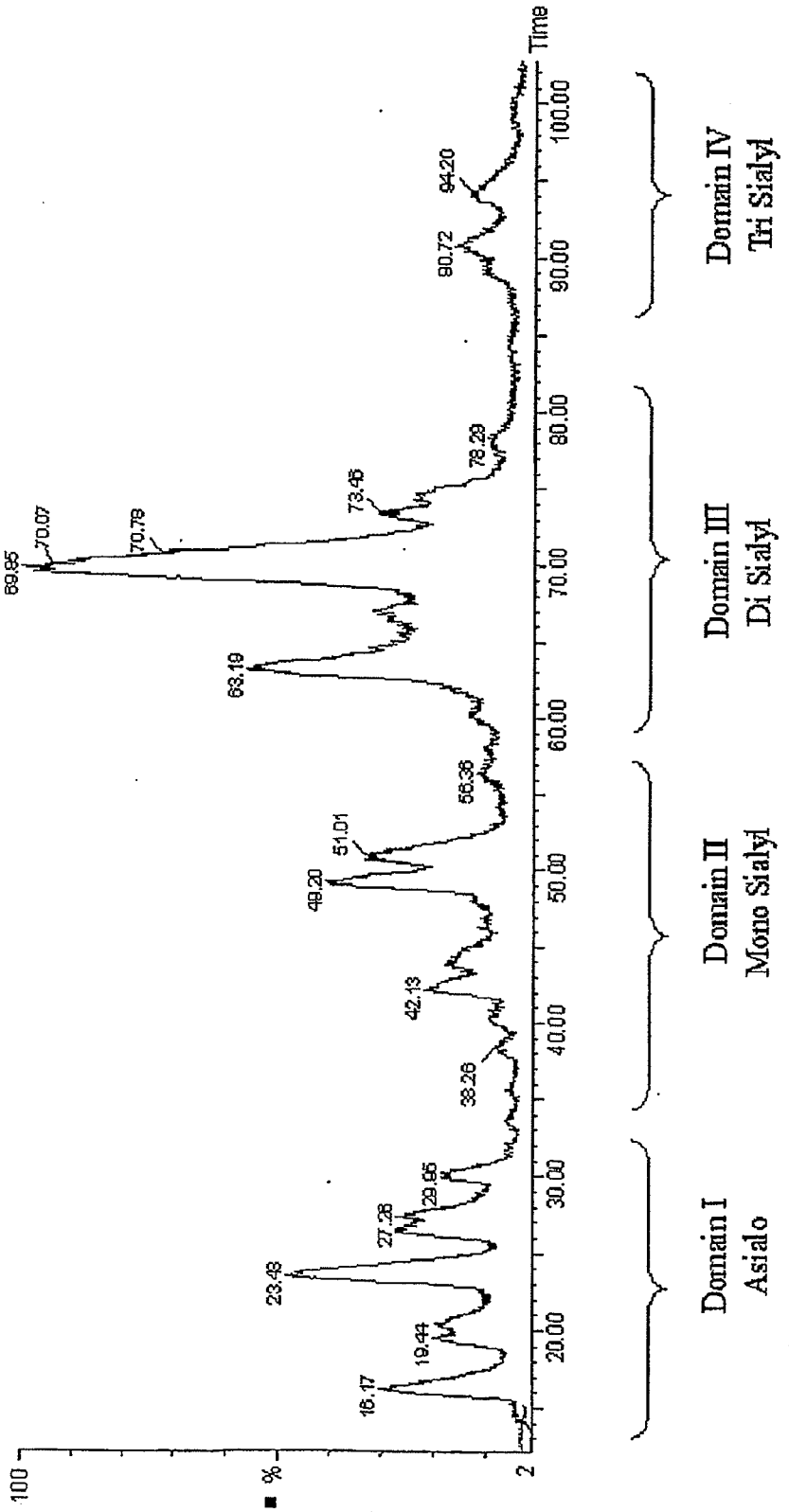

100
%
2
Time
16.17
19.44
23.48
27.28
29.65
38.26
42.13
49.20
51.01
56.36
63.19
69.85
70.07
70.78
73.45
78.29
90.72
94.20
20.00
30.00
40.00
50.00
60.00
70.00
80.00
90.00
100.00
Domain I
Asialo
Domain II
Mono Sialyl
Domain III
Di Sialyl
Domain IV
Tri Sialyl

FIG. 14A

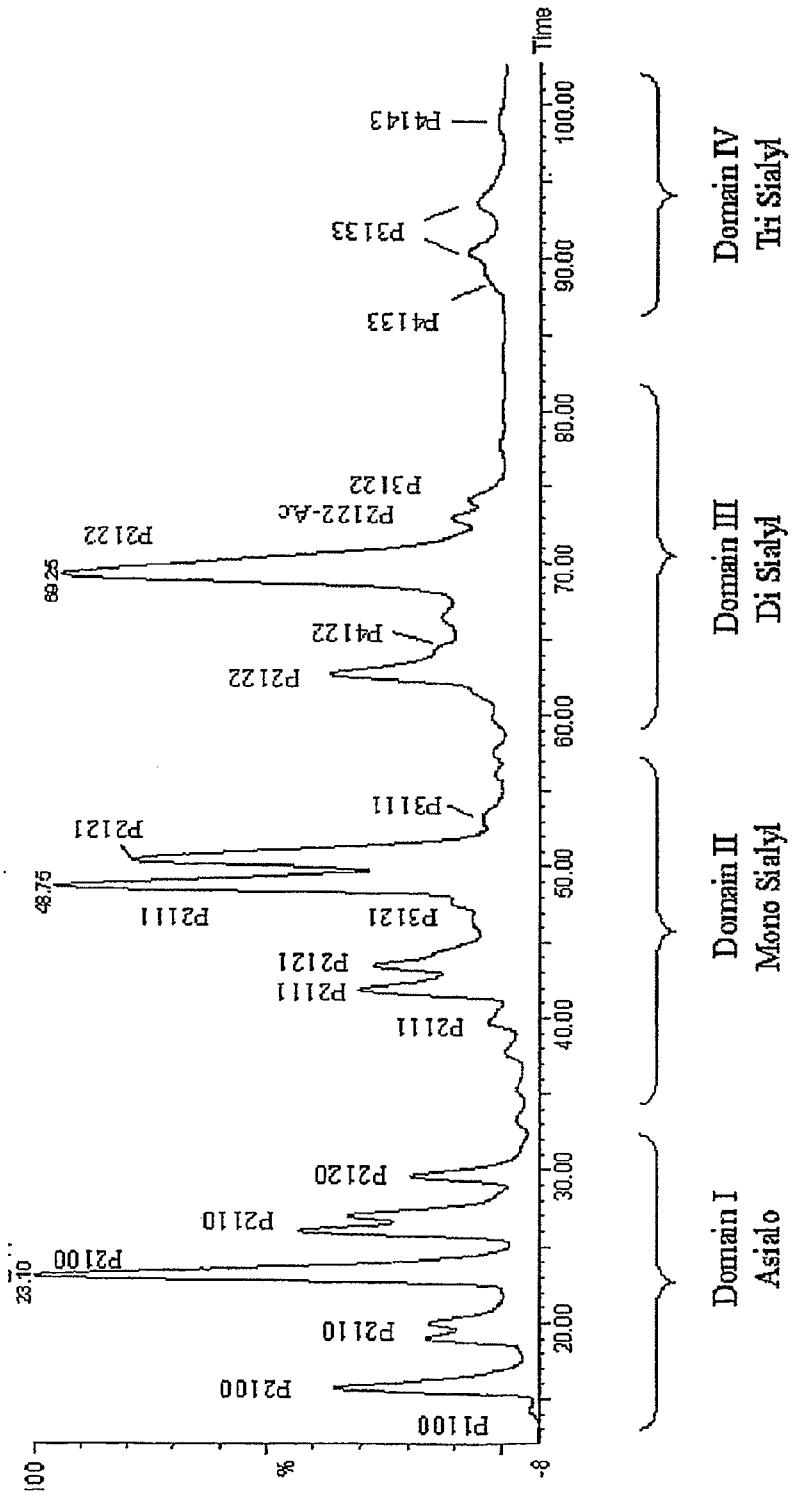

Time
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
%
100
0
P1100
P2100
P2110
P2100
P2110
P2120
P2111
P2121
P2111
P3121
P2111
48.75
P3111
P2121
P2122
P4122
89.25
P2122
P3122
P2122-Ac
P4133
P3133
P4143
Domain I
Asialo
Domain II
Mono Sialyl
Domain III
Di Sialyl
Domain IV
Tri Sialyl

FIG. 14B

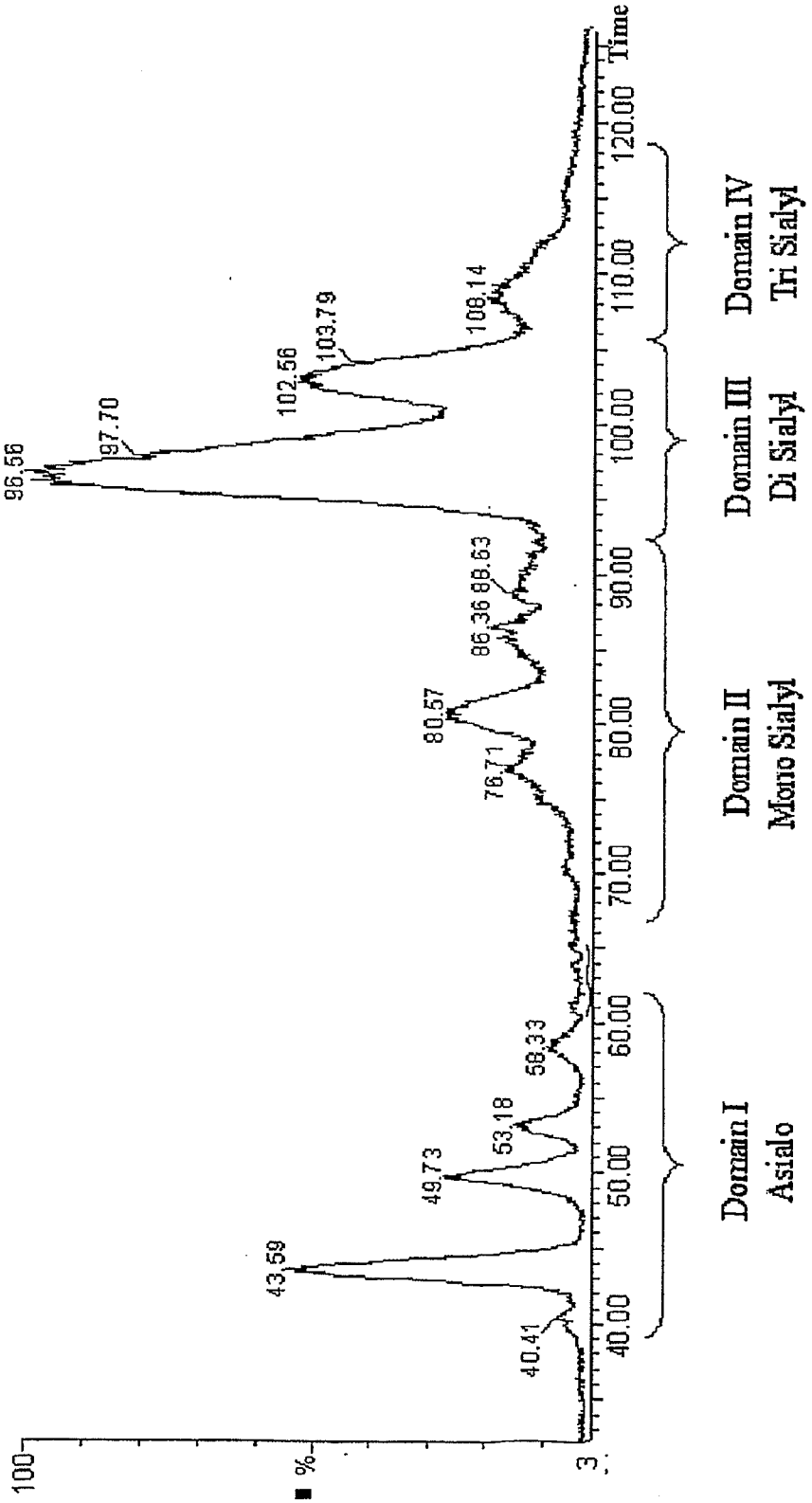

100
%
3
40.00
50.00
60.00
70.00
80.00
90.00
100.00
110.00
120.00
Time
43.59
40.41
49.73
53.18
58.33
76.71
80.57
86.36
88.63
96.56
97.70
102.56
103.79
108.14
Domain I
Asialo
Domain II
Mono Sialyl
Domain III
Di Sialyl
Domain IV
Tri Sialyl

FIG. 15A

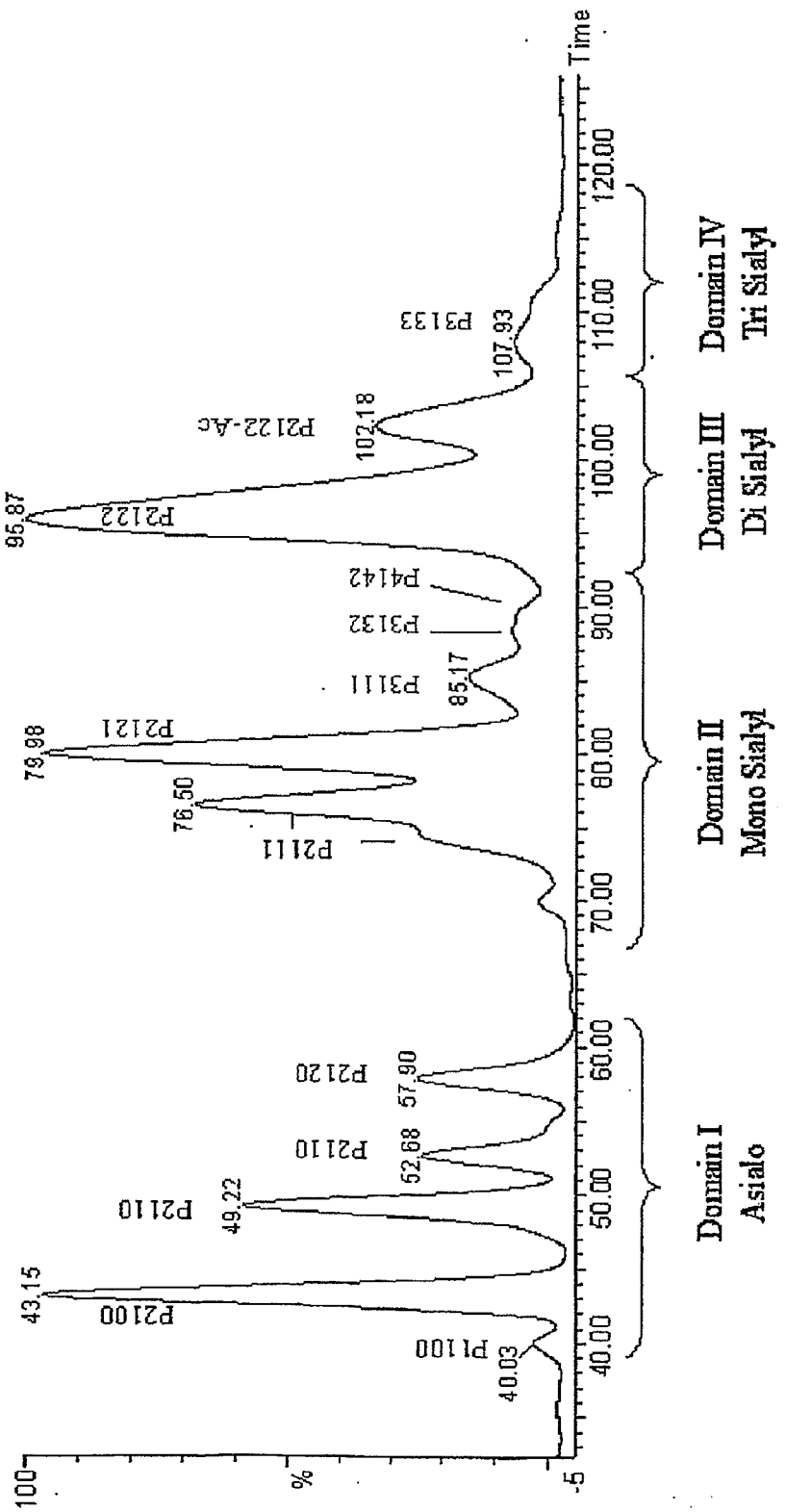
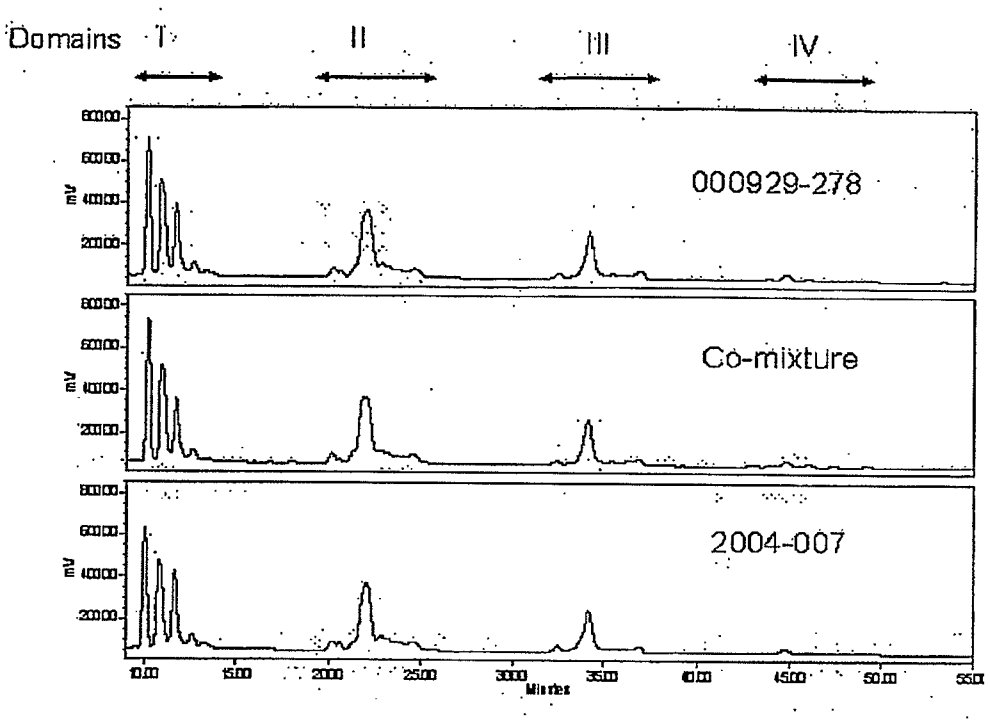

Time
120.00
110.00
100.00
90.00
80.00
70.00
60.00
50.00
40.00
100
%
-5
P3133
107.93
P2122-Ac
102.18
P2122
95.87
P4142
P3132
P3111
85.17
P2121
79.98
76.50
P2111
P2120
57.90
P2110
52.68
P2110
49.22
P2100
43.15
P1100
40.03
Domain I
Asialo
Domain II
Mono Sialyl
Domain III
Di Sialyl
Domain IV
Tri Sialyl

FIG. 15B



Lot	Area % Domain I	Area % Domain II	Area % Domain III	Area % Domain IV
Process B Lot 000929-278	45.8	34.1	17.8	2.4
Co-mixture Lot 55128-162	46.7	33.6	17.5	2.2
Process C Lot 224818-2004-007	47.5	33.4	17.4	1.8

FIG. 16

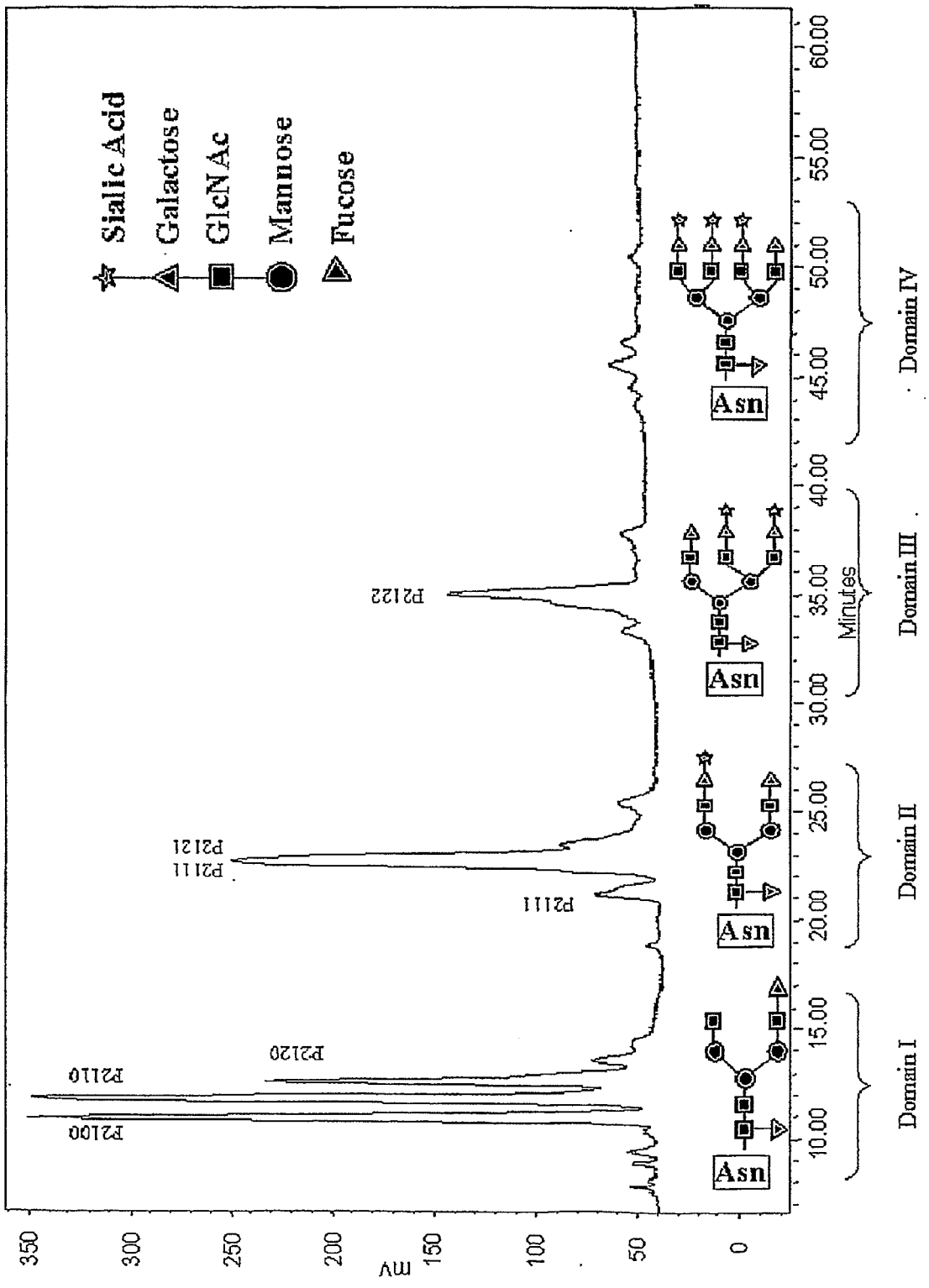

Sialic Acid
Galactose
GlcNAc
Mannose
Fucose
P2100
P2110
P2120
P2111
P2121
P2111
P2122
mV
Minutes
Asn
Asn
Asn
Asn
Domain I
Domain II
Domain III
Domain IV

FIG. 17

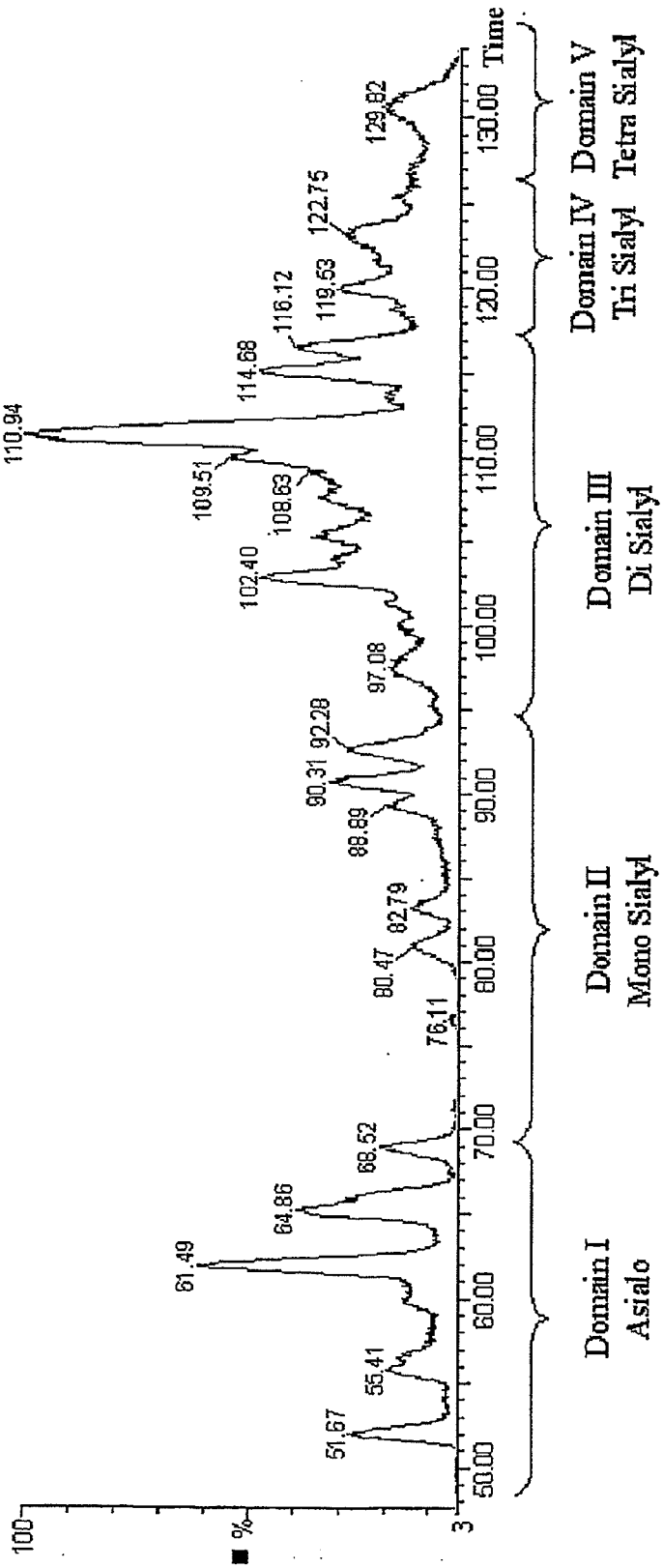

100
%
3
50.00
60.00
70.00
80.00
90.00
100.00
110.00
120.00
130.00
Time
51.67
55.41
61.49
64.86
68.52
76.11
80.47
82.79
88.89
90.31
92.28
97.08
102.40
108.63
109.51
110.94
114.68
116.12
119.53
122.75
129.82
Domain I
Asialo
Domain II
Mono Sialyl
Domain III
Di Sialyl
Domain IV
Tri Sialyl
Domain V
Tetra Sialyl

FIG. 18A

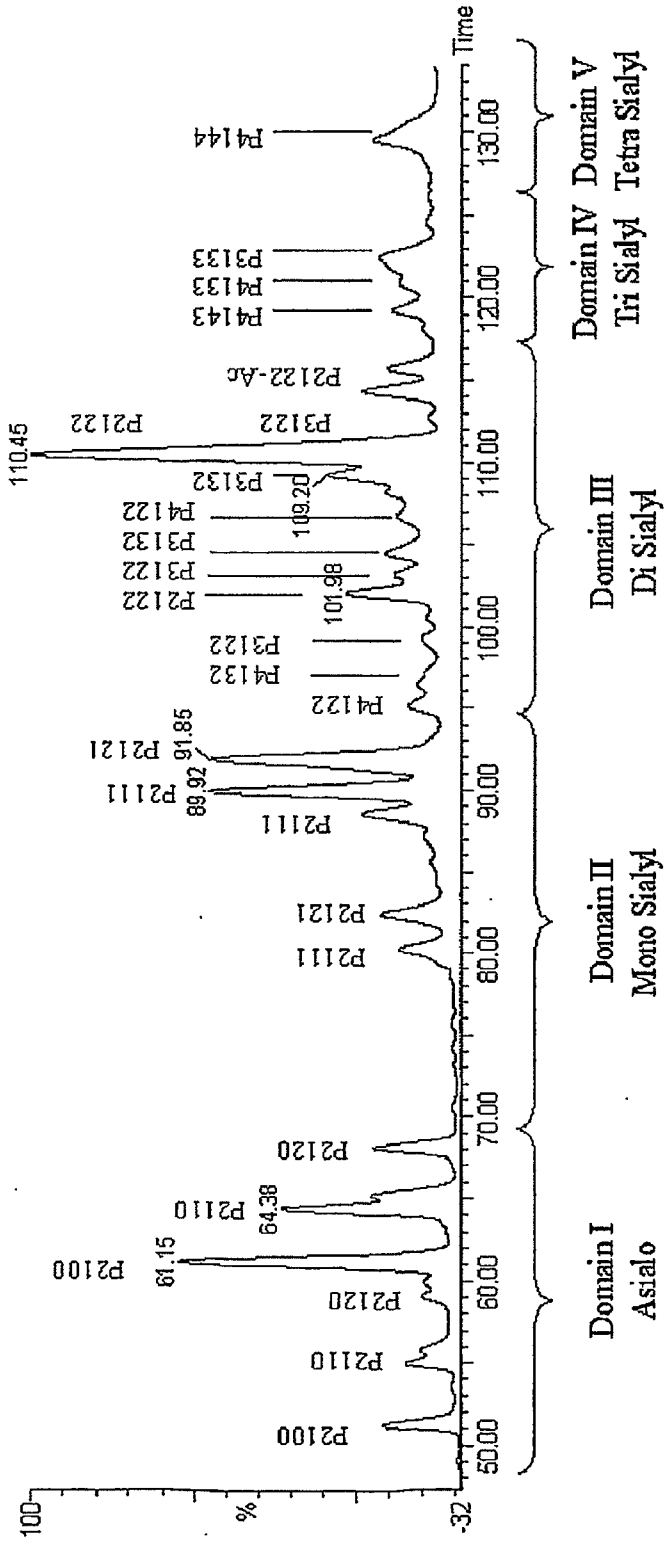

Time
50.00
60.00
70.00
80.00
90.00
100.00
110.00
120.00
130.00
100
%
-32
Domain I
Asialo
Domain II
Mono Sialyl
Domain III
Di Sialyl
Domain IV
Tri Sialyl
Domain V
Tetra Sialyl
P2100
P2110
P2120
61.15
64.38
P2120
P2110
P2100
P2111
P2121
P2111
89.92
91.85
P2121
P4122
P4132
P3122
101.98
P2122
P3122
P3132
P4122
109.20
P3132
110.45
P2122
P3122
P2122-Ac
P4143
P4133
P3133
P4144

FIG. 18B

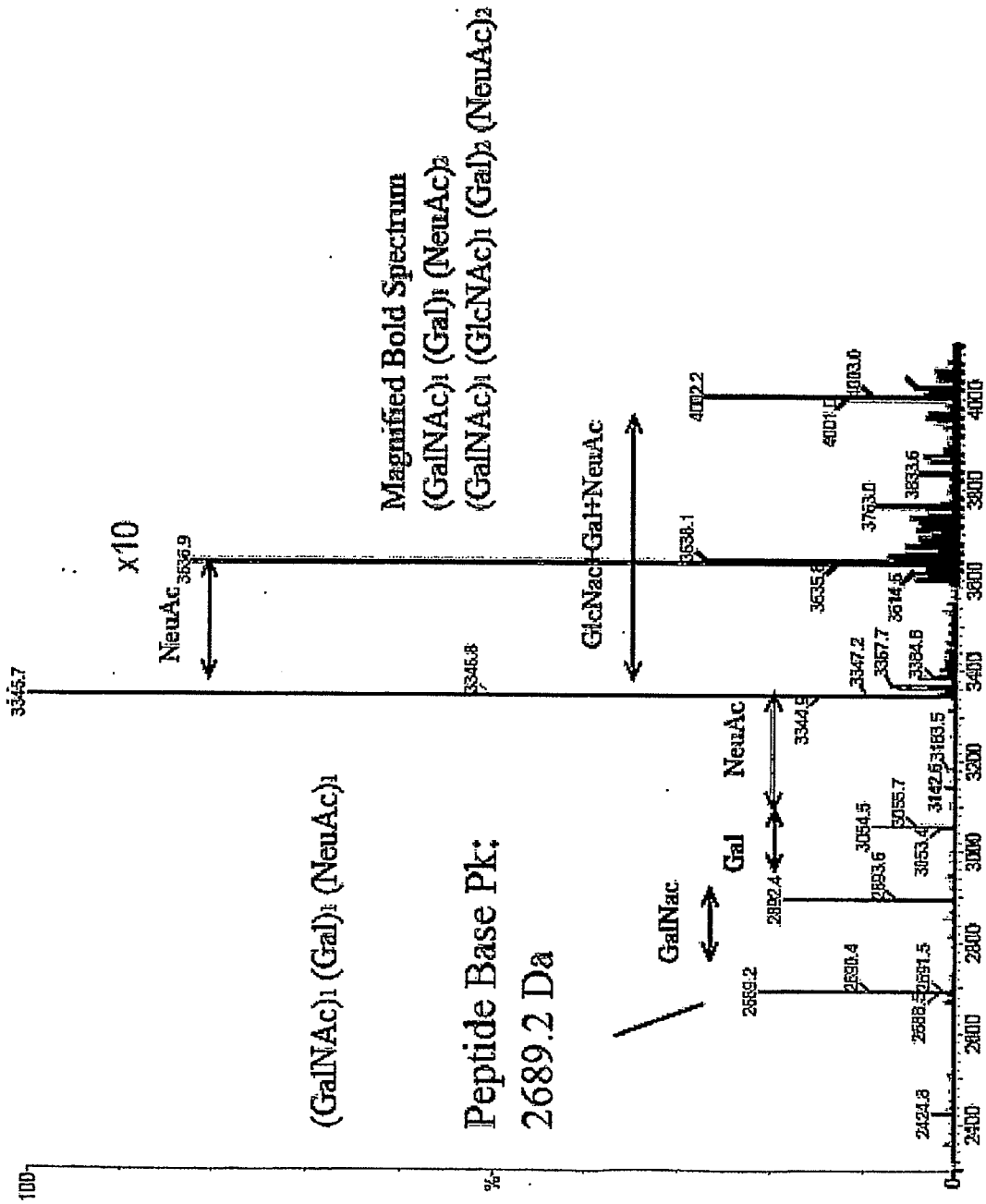

Magnified Bold Spectrum
(GalNAc)1 (Gal)1 (NeuAc)2
(GalNAc)1 (GlcNAc)1 (Gal)2 (NeuAc)2
x10
Peptide Base Pk:
2689.2 Da
(GalNAc)1 (Gal)1 (NeuAc)1
NeuAc
GlcNac-Gal+NeuAc
GalNac
Gal
NeuAc
100
%
0

FIG. 19

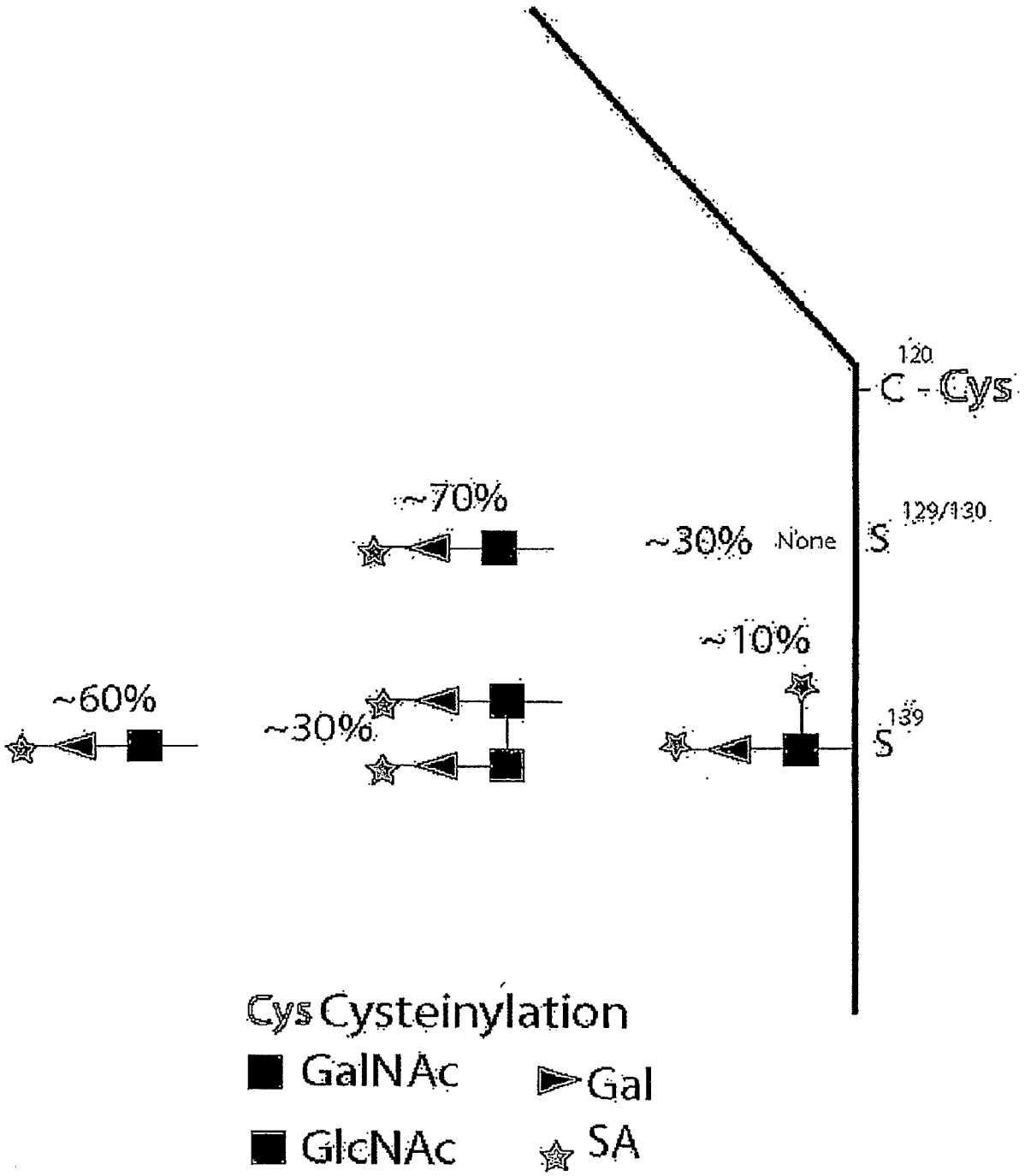

120
C - Cys
~70%
~30%
None
S
129/130
~10%
~60%
~30%
S
139
Cys Cysteinylation
GalNAc
Gal
GlcNAc
SA

FIG. 20

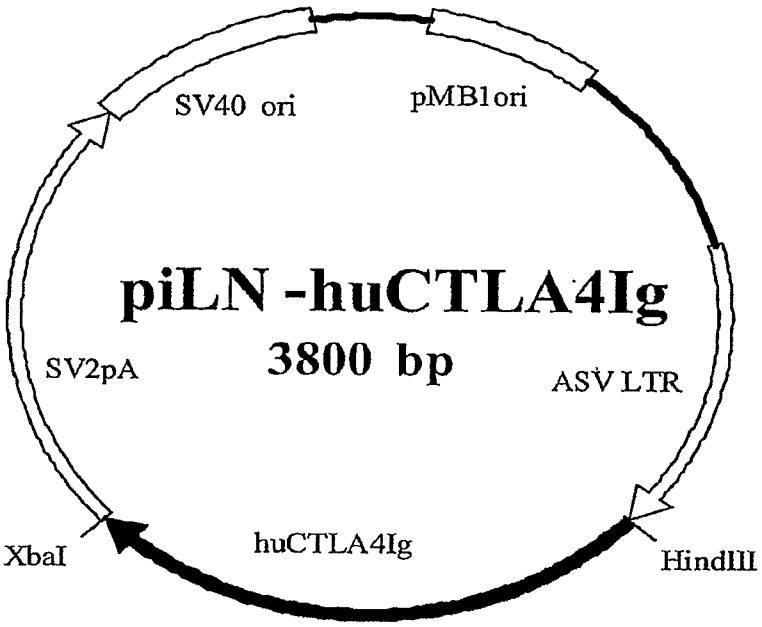

SV40 ori
pMB1ori
piLN -huCTLA4Ig
3800 bp
SV2pA
ASV LTR
XbaI
huCTLA4Ig
HindIII

FIG. 21

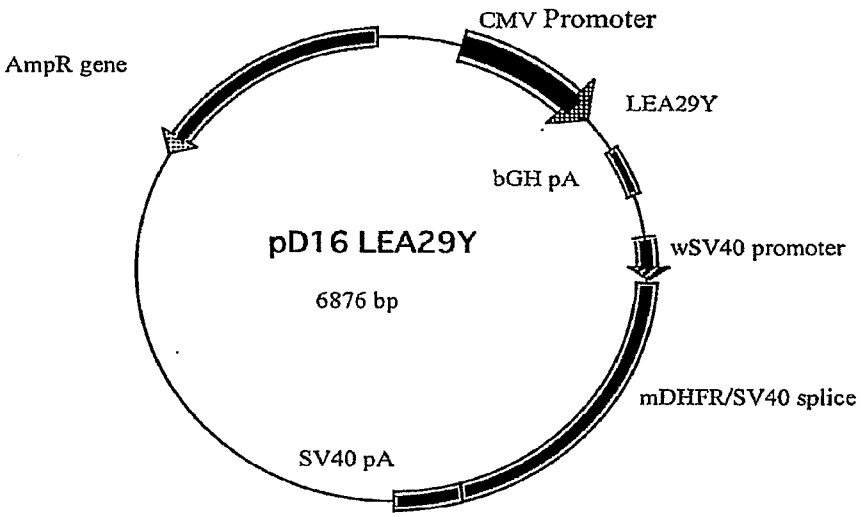

CMV Promoter
AmpR gene
LEA29Y
bGH pA
pD16 LEA29Y
6876 bp
wSV40 promoter
mDHFR/SV40 splice
SV40 pA

FIG. 22

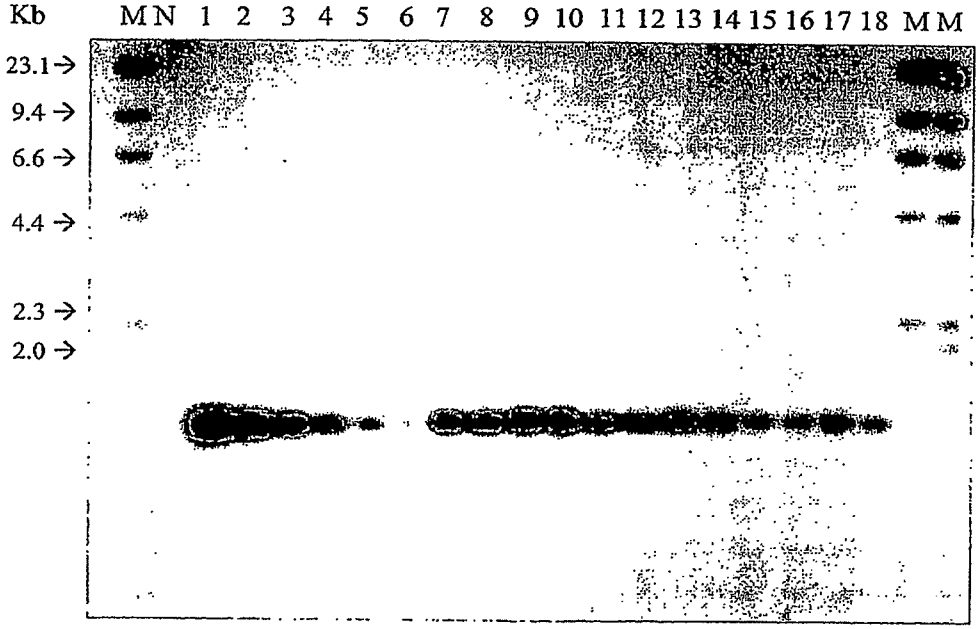


FIG. 23

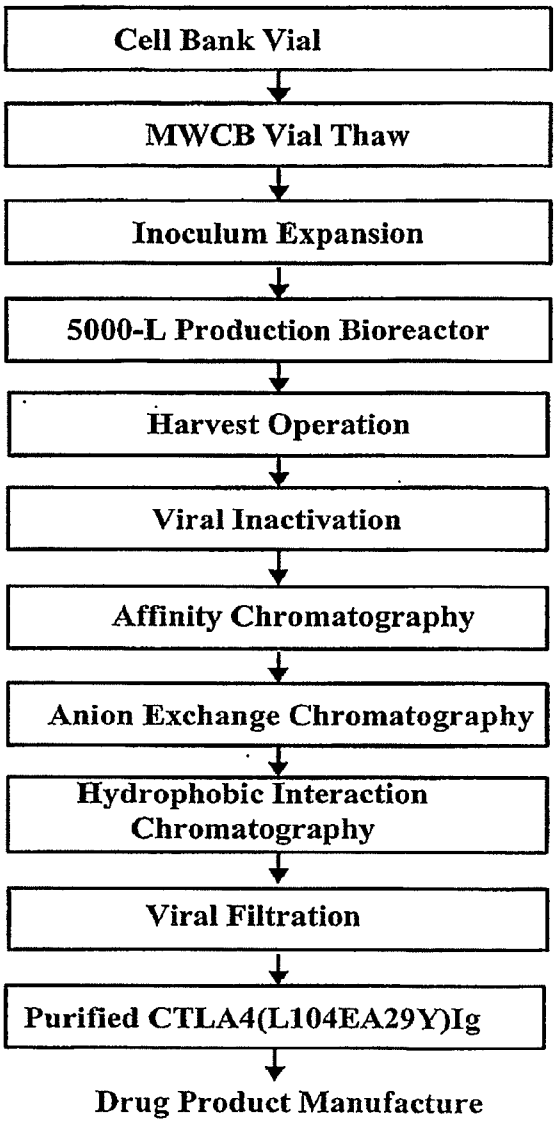

Cell Bank Vial
MWCB Vial Thaw
Inoculum Expansion
5000-L Production Bioreactor
Harvest Operation
Viral Inactivation
Affinity Chromatography
Anion Exchange Chromatography
Hydrophobic Interaction Chromatography
Viral Filtration
Purified CTLA4(L104EA29Y)Ig
Drug Product Manufacture

FIG. 24

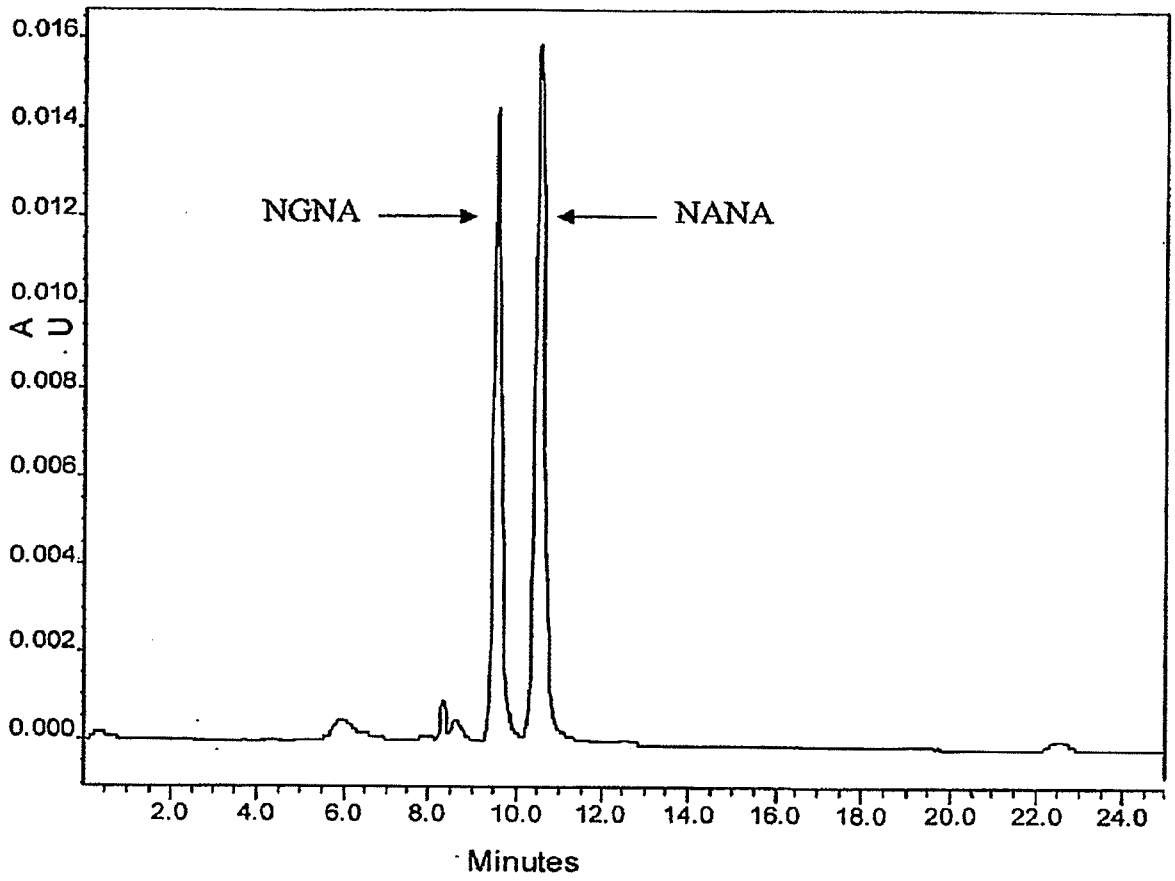

0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
A U
NGNA
NANA
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
24.0
Minutes

FIG. 25

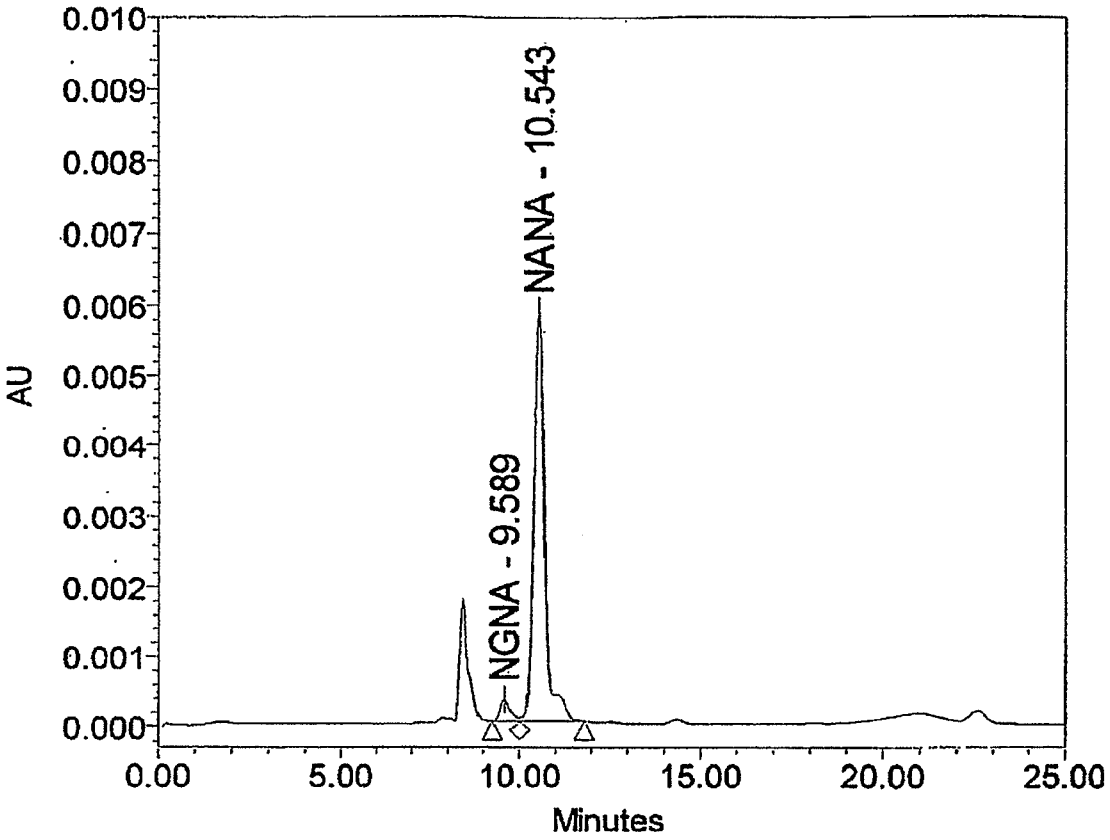

0.010
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
AU
NANA - 10.543
NGNA - 9.589
0.00
5.00
10.00
15.00
20.00
25.00
Minutes

FIG. 26

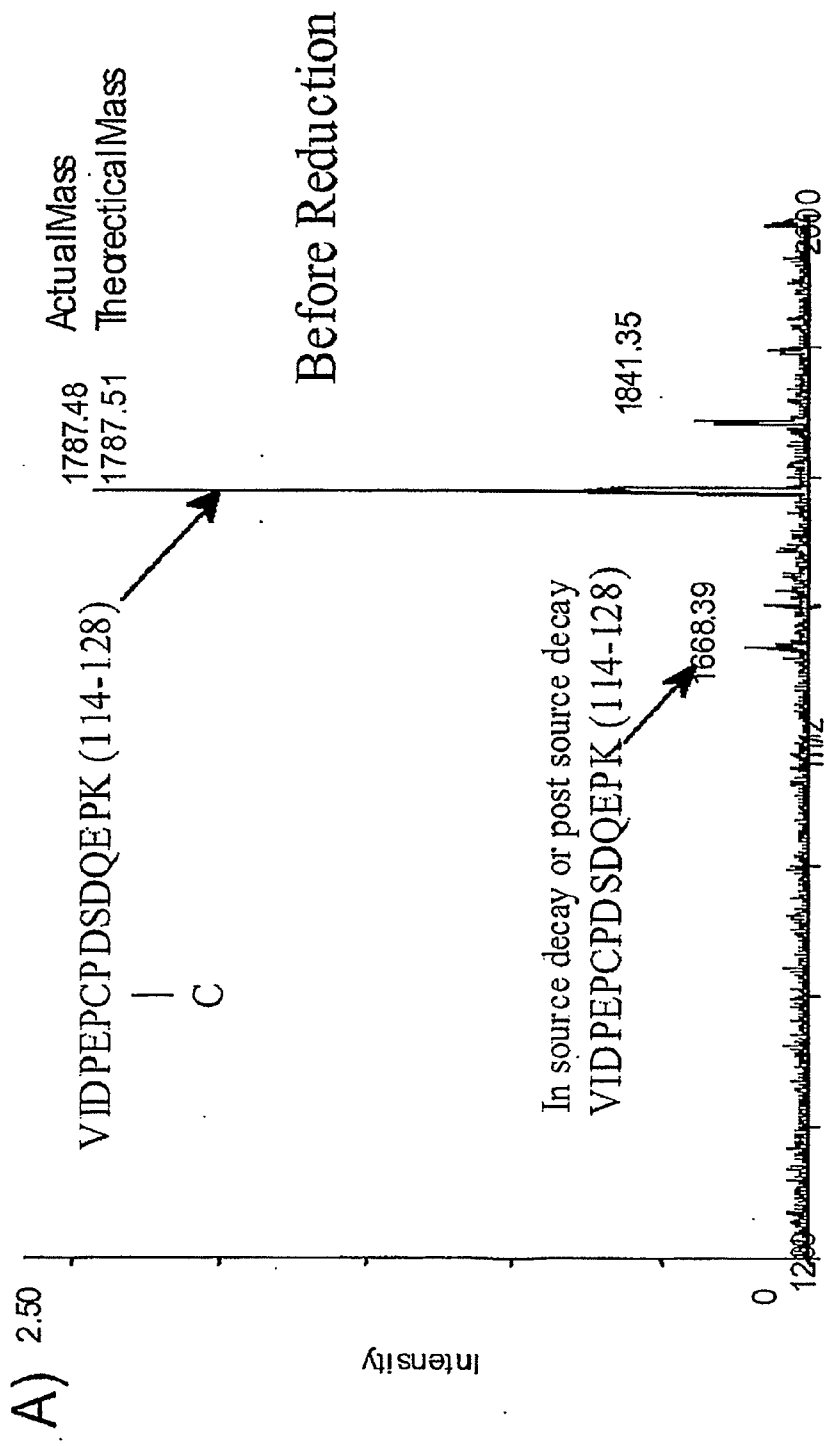

A)
2.50
Intensity
0
1787.48
1787.51
ActualMass
TheoreticalMass
Before Reduction
VIDPEPCPDSDQEPK (114-128)
C
1841.35
In source decay or post source decay
VIDPEPCPDSDQEPK (114-128)
1668.39

FIG. 27A

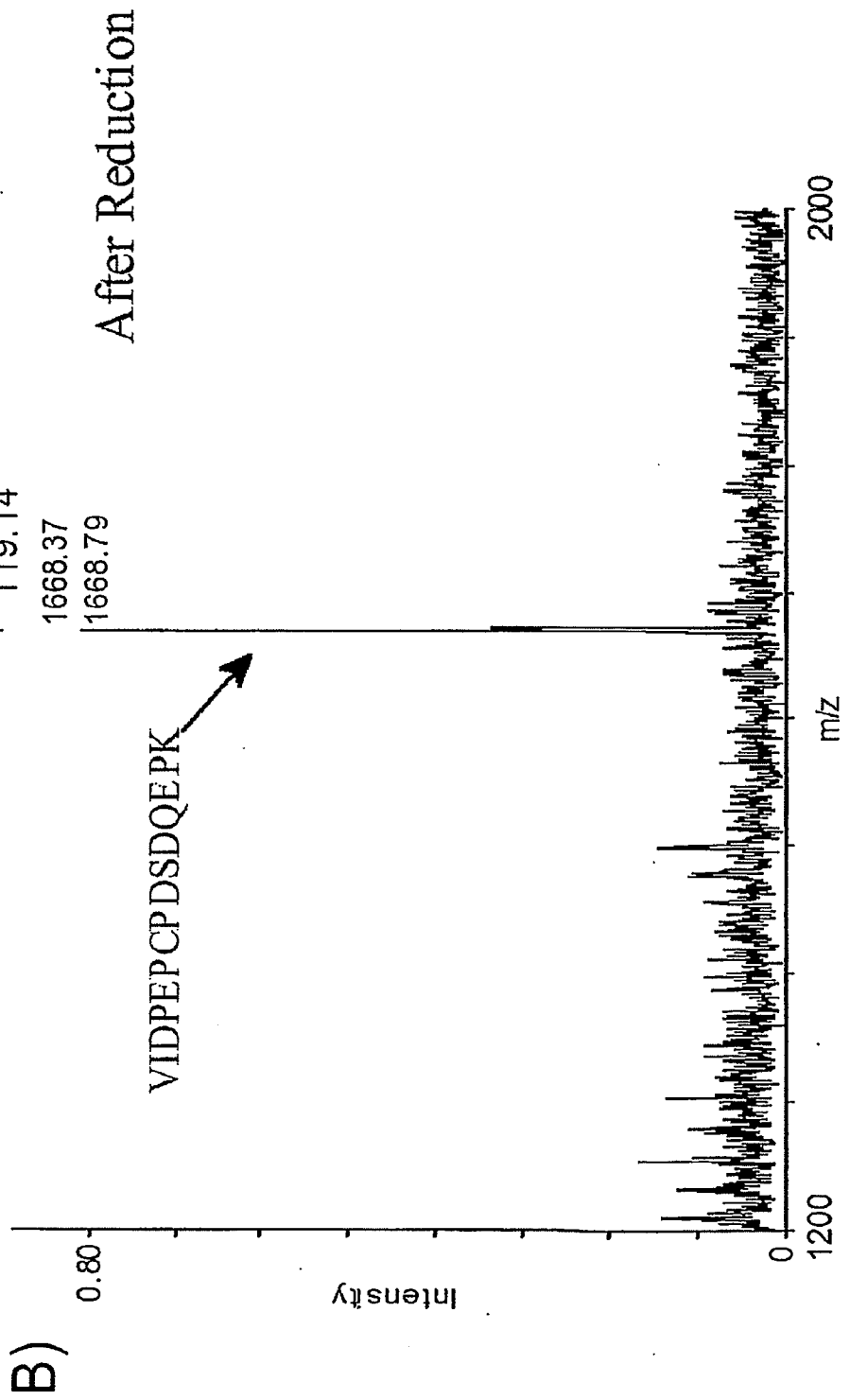

B)
After Reduction
119.11
119.14
1668.37
1668.79
VIDPEPCPDSDQEPK
Intensity
0.80
0
1200
m/z
2000

FIG. 27B

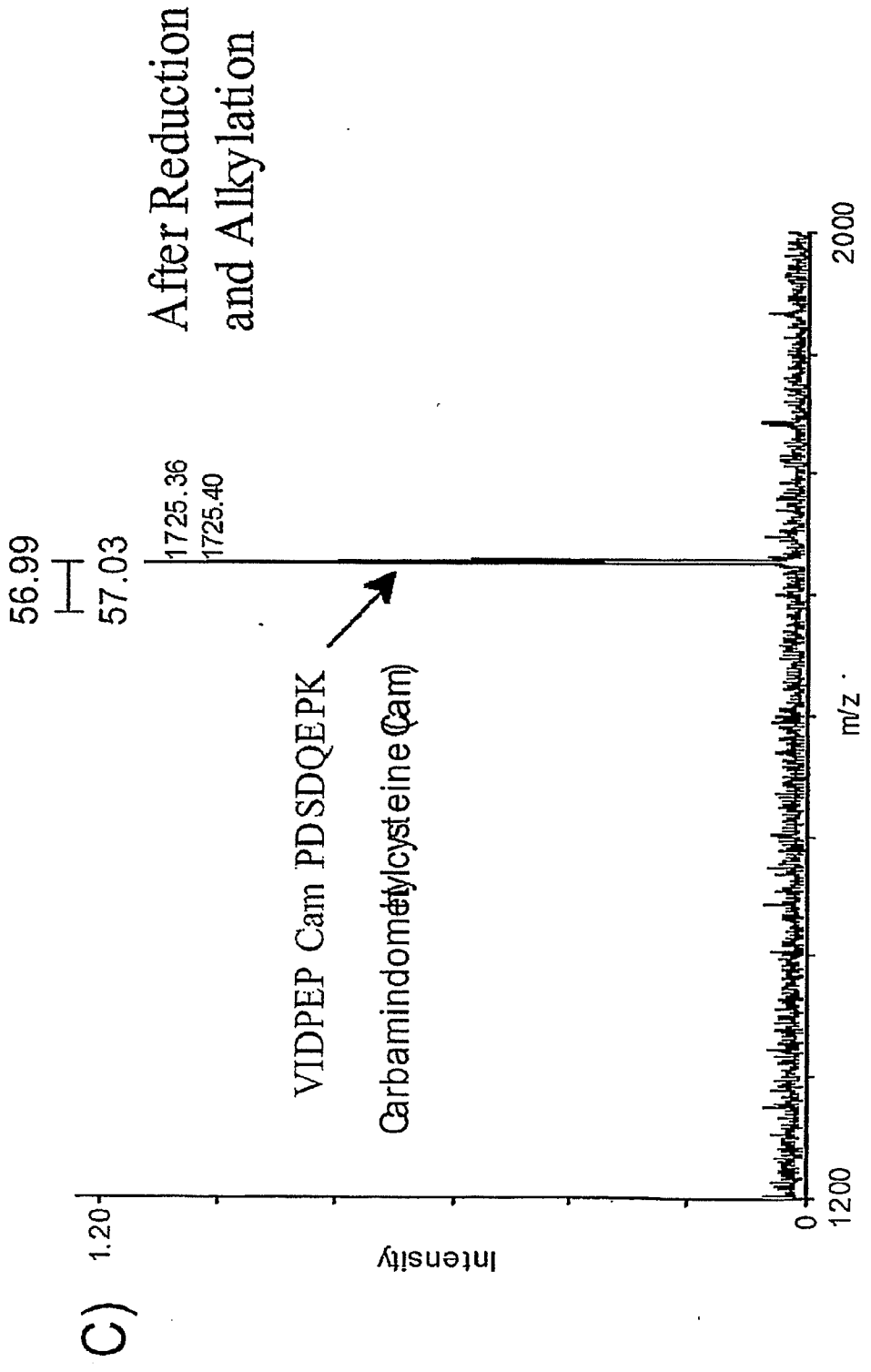

C)
1.20
0
Intensity
1200
2000
m/z
After Reduction
and Alkylation
1725.36
1725.40
56.99
57.03
VIDPEP Cam PDSDQEPK
Carbamindomethylcysteine (Cam)

FIG. 27C

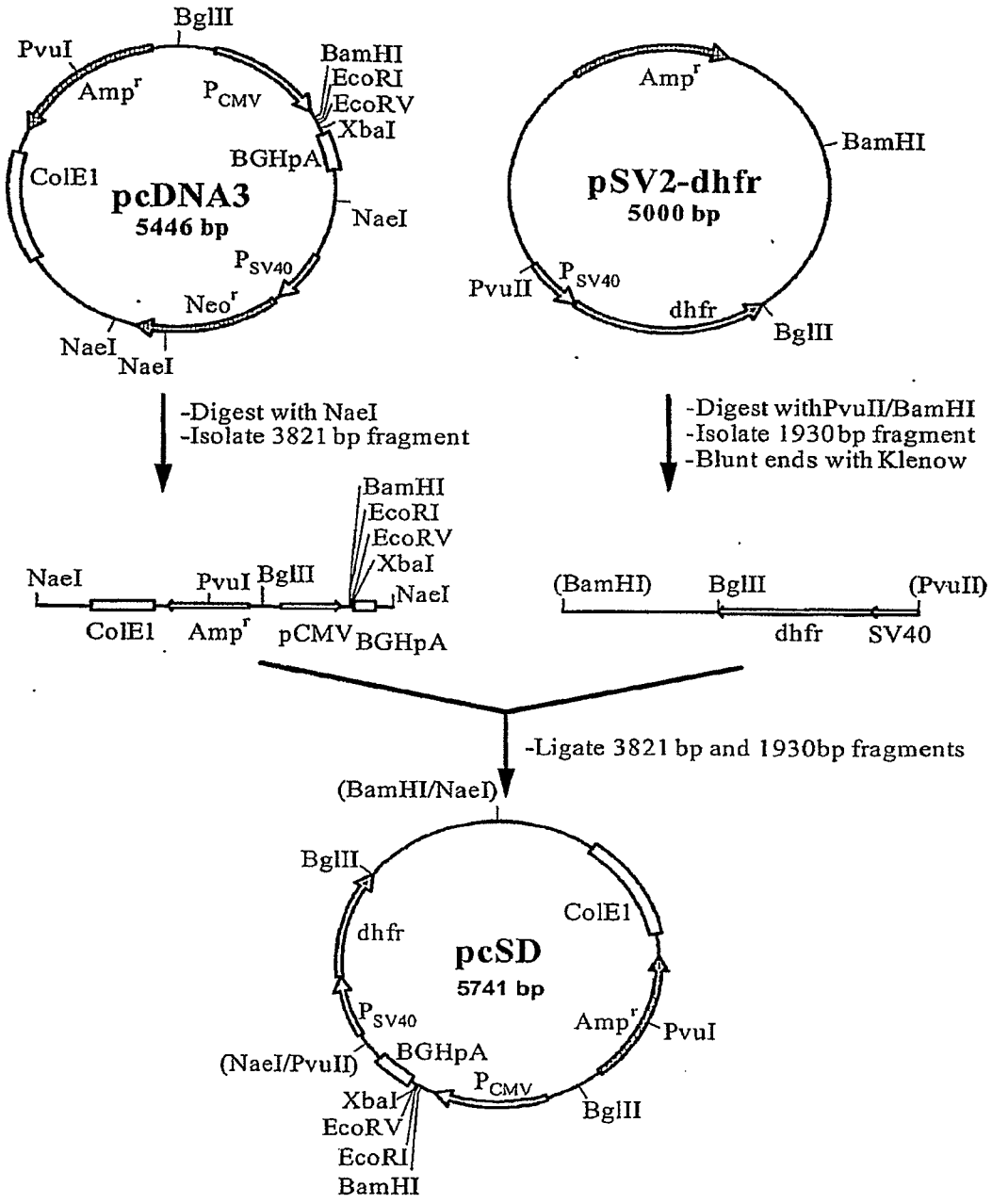

BglII
PvuI
BamHI
EcoRI
EcoRV
XbaI
Amp^r
P_CMV
BGHpA
ColE1
pcDNA3
5446 bp
NaeI
P_SV40
Neo^r
NaeI
NaeI
Amp^r
BamHI
pSV2-dhfr
5000 bp
PvuII
P_SV40
dhfr
BglII
-Digest with NaeI
-Isolate 3821 bp fragment
-Digest withPvuII/BamHI
-Isolate 1930bp fragment
-Blunt ends with Klenow
BamHI
EcoRI
EcoRV
XbaI
NaeI
NaeI
PvuI
BglII
ColE1
Amp^r
pCMV
BGHpA
(BamHI)
BglII
(PvuII)
dhfr
SV40
-Ligate 3821 bp and 1930bp fragments
(BamHI/NaeI)
BglII
dhfr
ColE1
pcSD
5741 bp
P_SV40
Amp^r
PvuI
(NaeI/PvuII)
BGHpA
P_CMV
XbaI
BglII
EcoRV
EcoRI
BamHI

FIG. 28

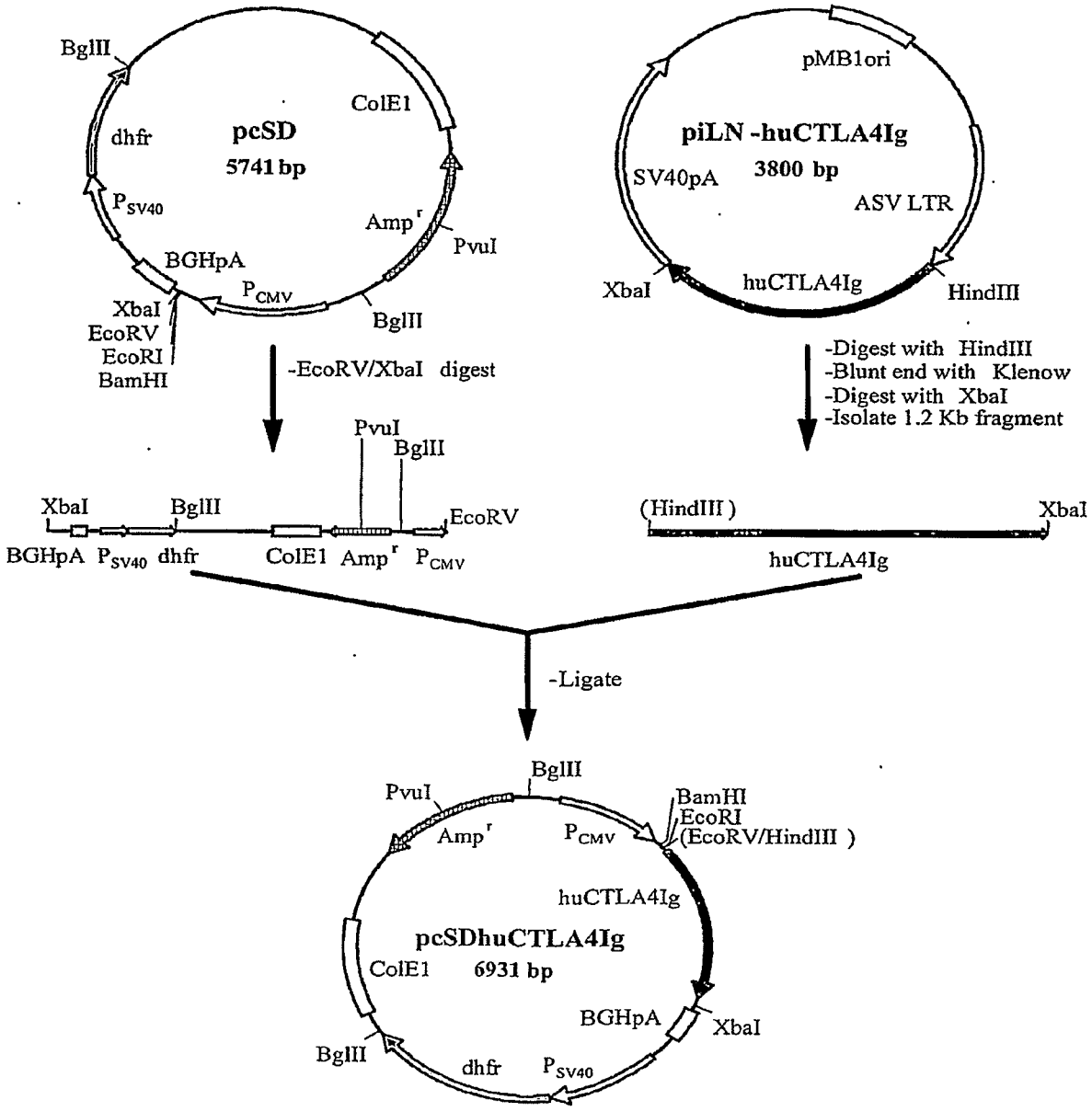

BglII
ColE1
dhfr
pcSD
5741 bp
P SV40
Amp r
PvuI
BGHpA
P CMV
XbaI
EcoRV
EcoRI
BamHI
BglII
pMB1ori
piLN -huCTLA4Ig
SV40pA
3800 bp
ASV LTR
XbaI
huCTLA4Ig
HindIII
-EcoRV/XbaI digest
-Digest with HindIII
-Blunt end with Klenow
-Digest with XbaI
-Isolate 1.2 Kb fragment
PvuI
BglII
XbaI
BglII
EcoRV
BGHpA P SV40 dhfr
ColE1 Amp r P CMV
(HindIII)
XbaI
huCTLA4Ig
-Ligate
BglII
PvuI
BamHI
EcoRI
Amp r
P CMV
(EcoRV/HindIII)
huCTLA4Ig
pcSDhuCTLA4Ig
ColE1
6931 bp
BGHpA
XbaI
BglII
dhfr
P SV40

FIG. 29

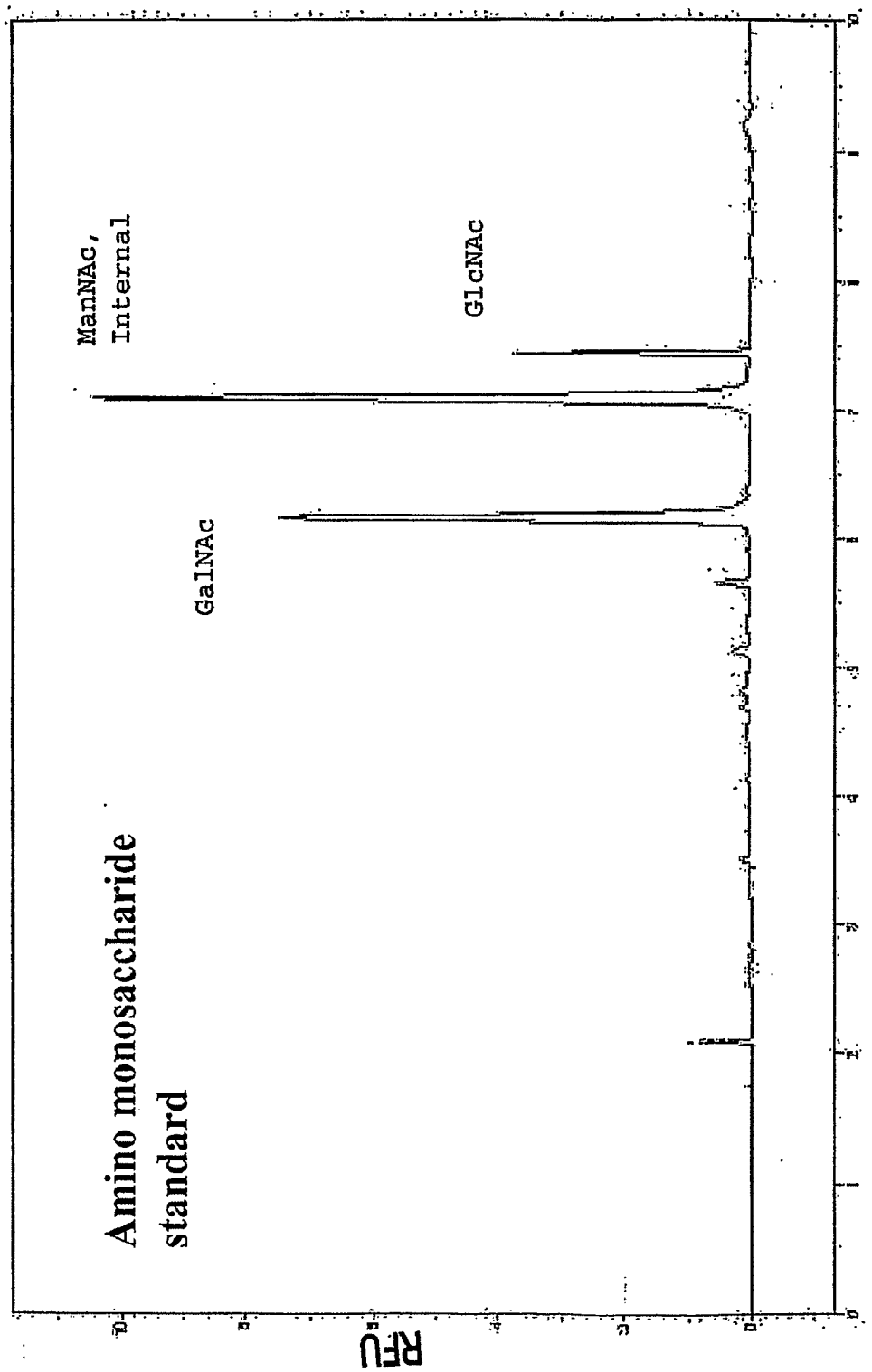

Amino monosaccharide standard
ManNAc, Internal
GlcNAc
GalNAc
RFU
Minutes

FIG. 30

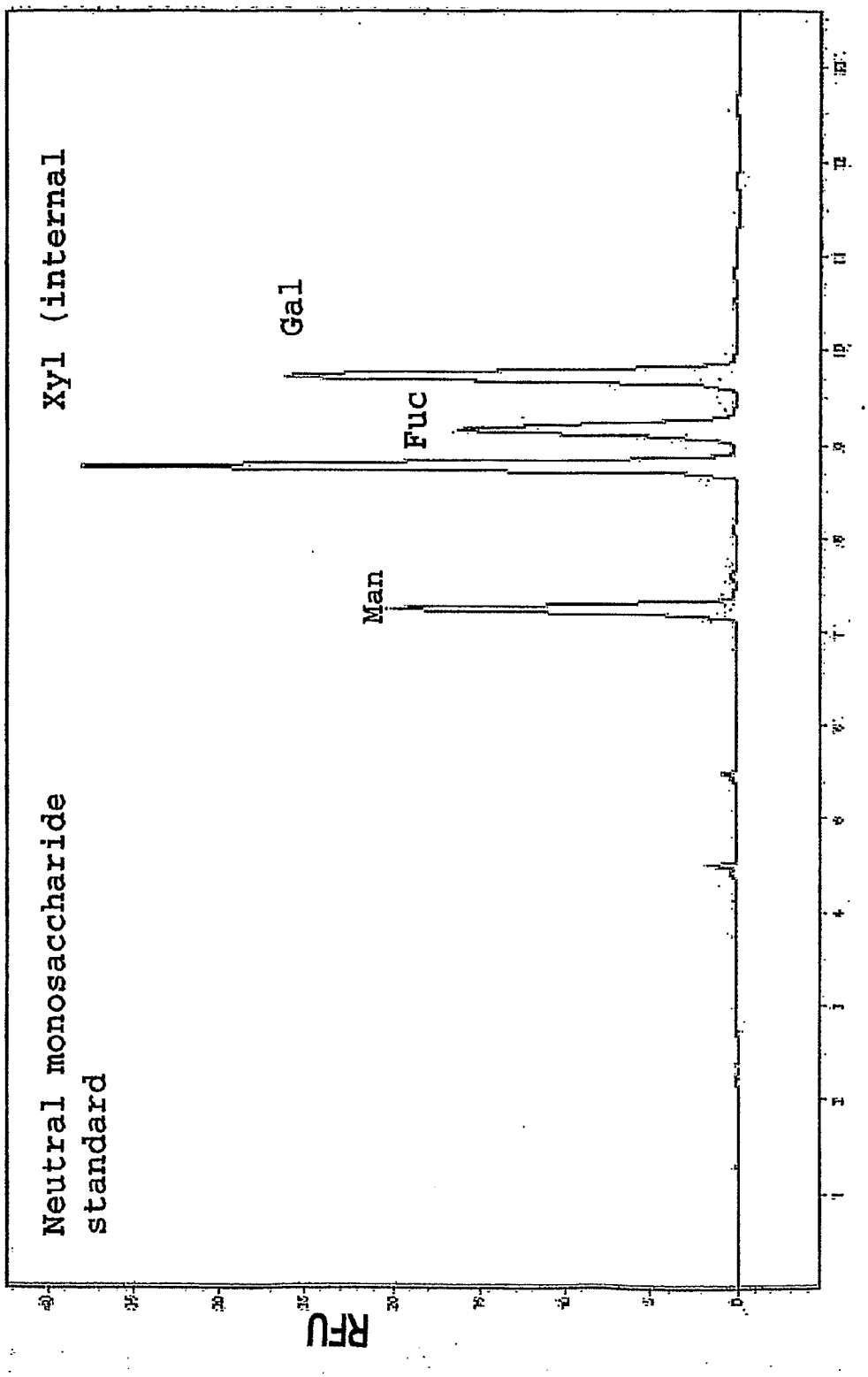

Neutral monosaccharide
standard
Xyl (internal
Man
Fuc
Gal
RFU
Minutes

FIG. 31

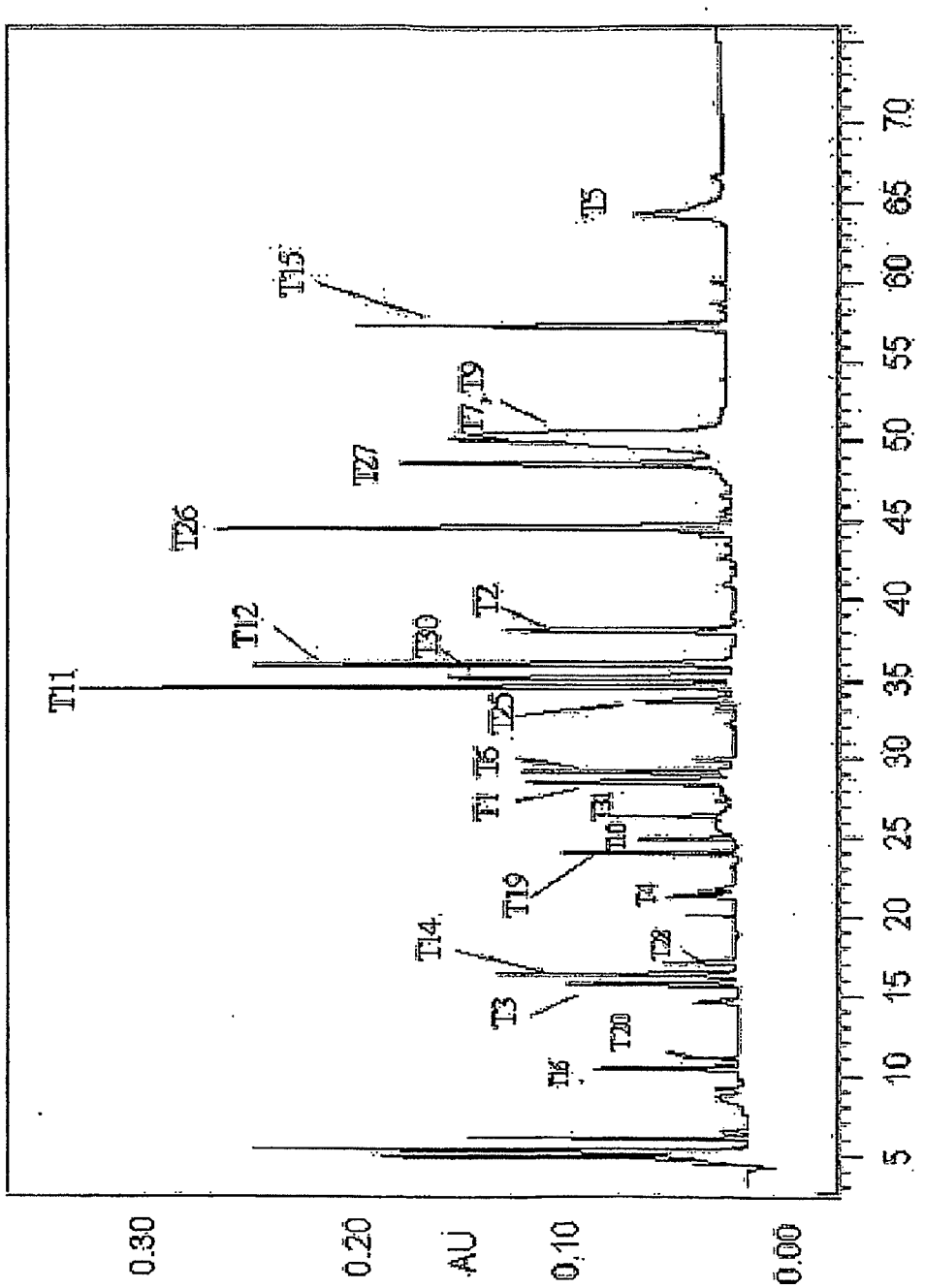

0.30
0.20
AU
0.10
0.00
5
10
15
20
25
30
35
40
45
50
55
60
65
70
Time (min)
T11
T12
T30
T2
T26
T27
T7, T9
T15
T5
T25
T6
T1
T31
T10
T19
T4
T28
T14
T3
T20
T16

FIG. 32

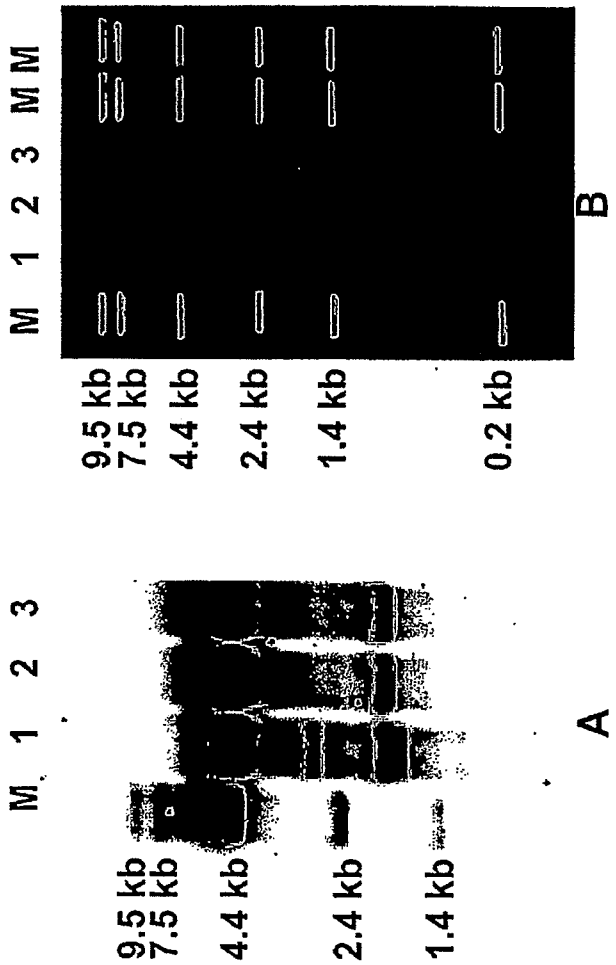

M 1 2 3 M M
9.5 kb
7.5 kb
4.4 kb
2.4 kb
1.4 kb
0.2 kb
B
M 1 2 3
9.5 kb
7.5 kb
4.4 kb
2.4 kb
1.4 kb
A

FIG. 33

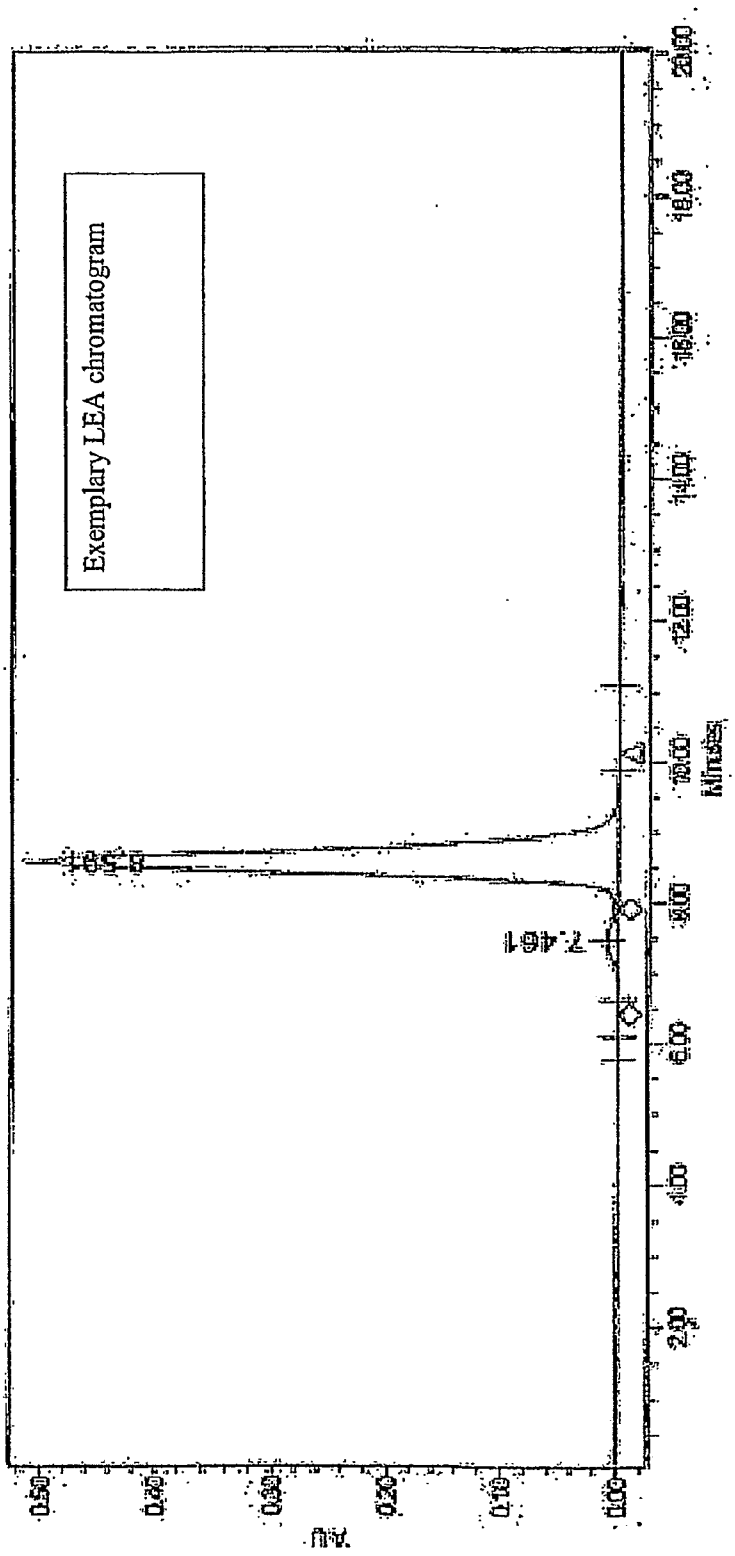

Exemplary LEA chromatogram
7.481
Minutes
AU
0.50
0.40
0.30
0.20
0.10
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00

FIG. 34A

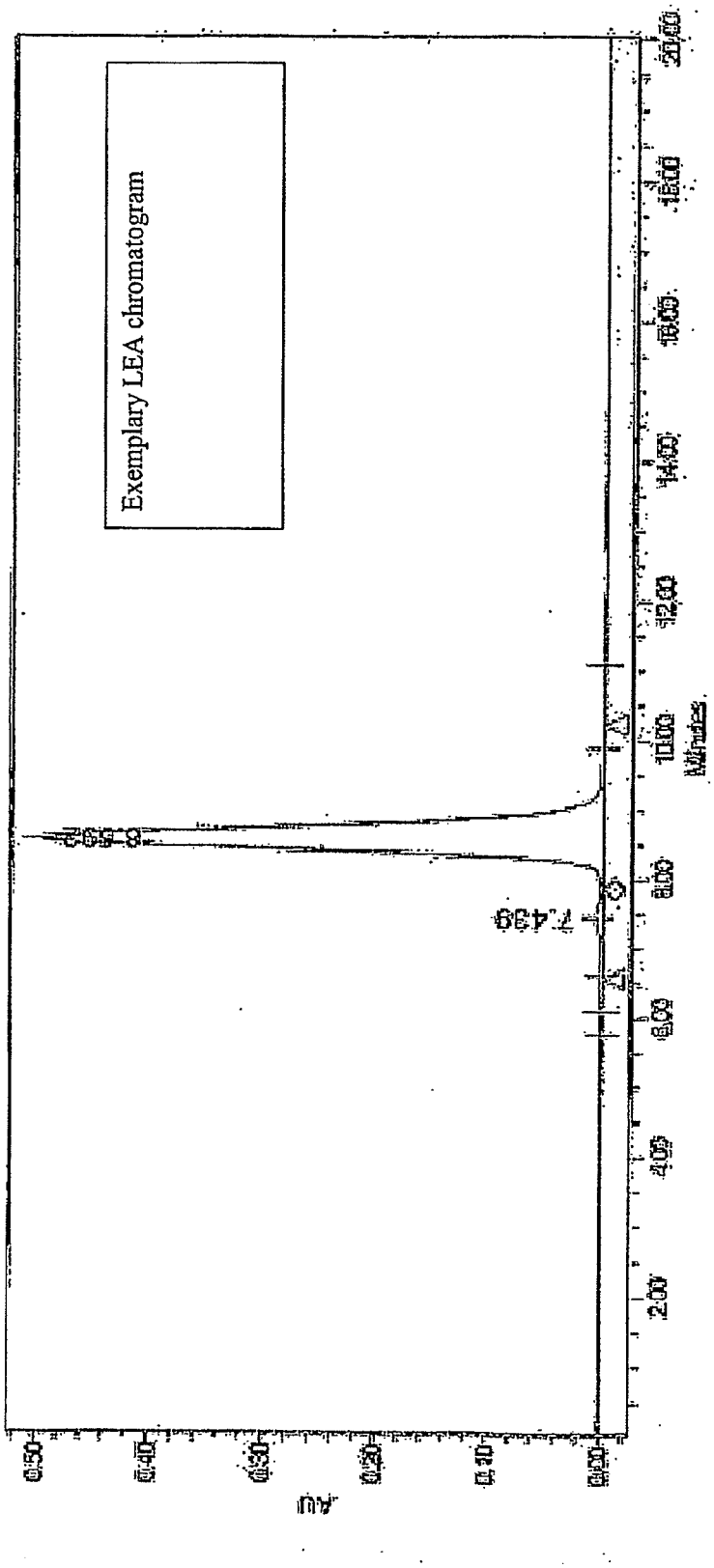


FIG. 34B

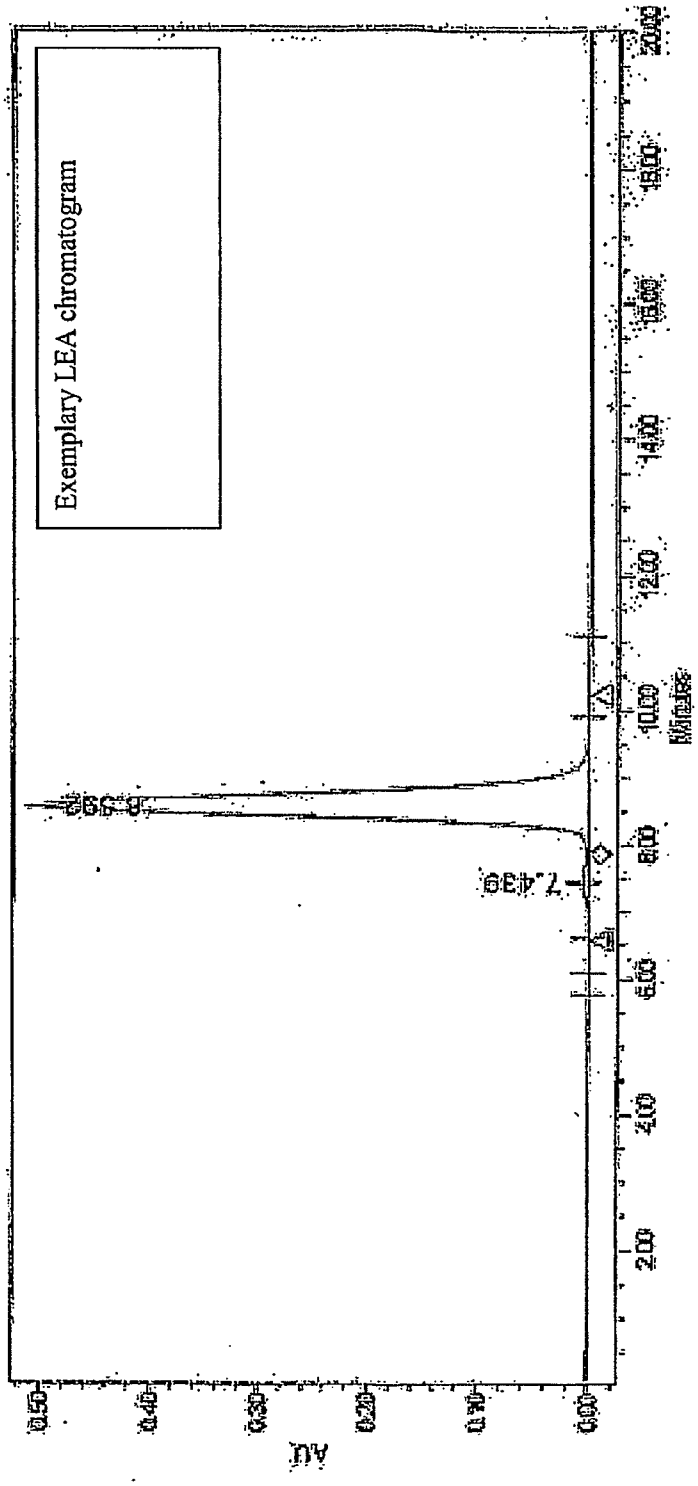


FIG. 34C

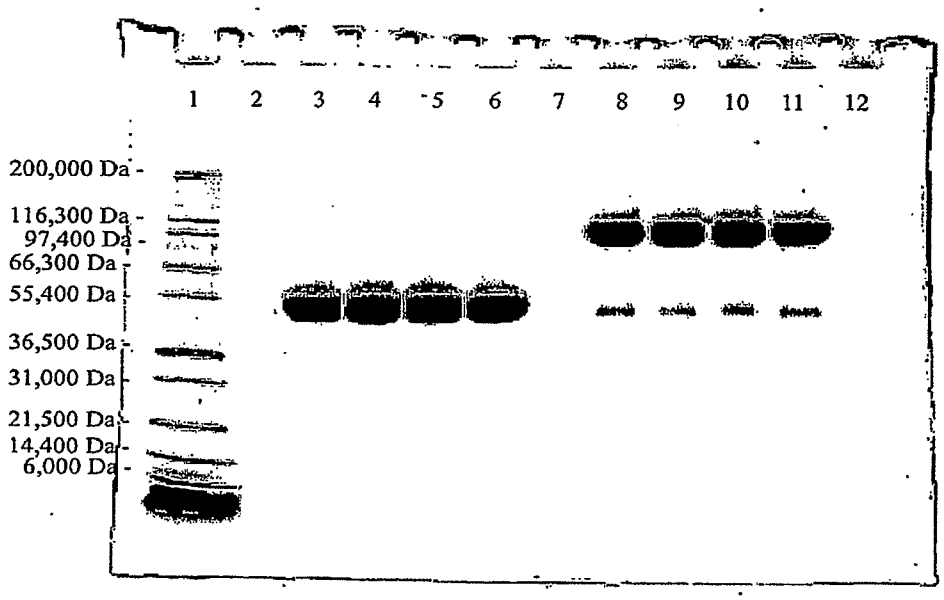

1
2
3
4
5
6
7
8
9
10
11
12
200,000 Da
116,300 Da
97,400 Da
66,300 Da
55,400 Da
36,500 Da
31,000 Da
21,500 Da
14,400 Da
6,000 Da

FIG. 35

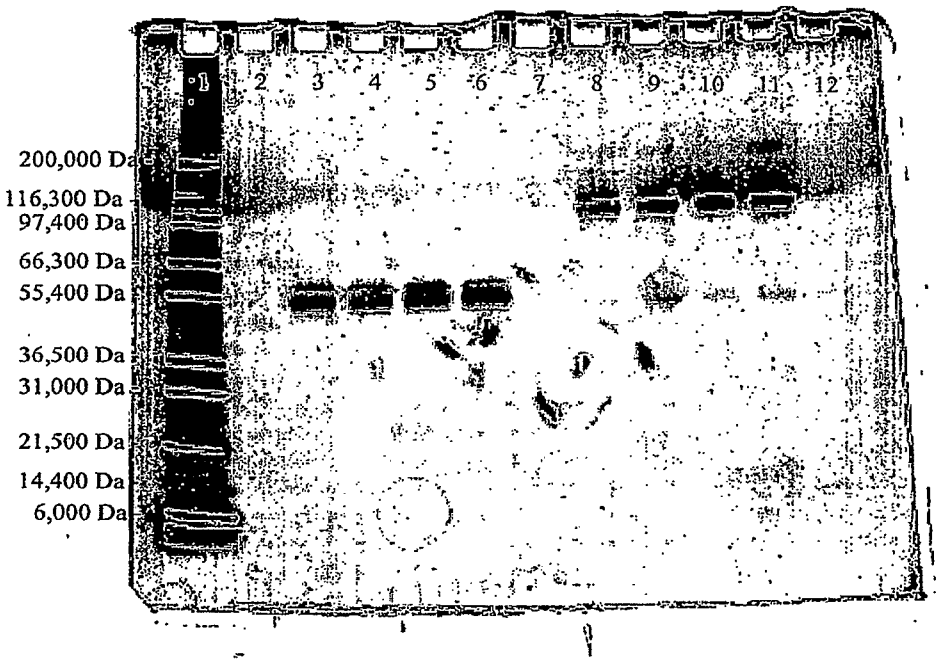

1
2
3
4
5
6
7
8
9
10
11
12
200,000 Da
116,300 Da
97,400 Da
66,300 Da
55,400 Da
36,500 Da
31,000 Da
21,500 Da
14,400 Da
6,000 Da

FIG. 36

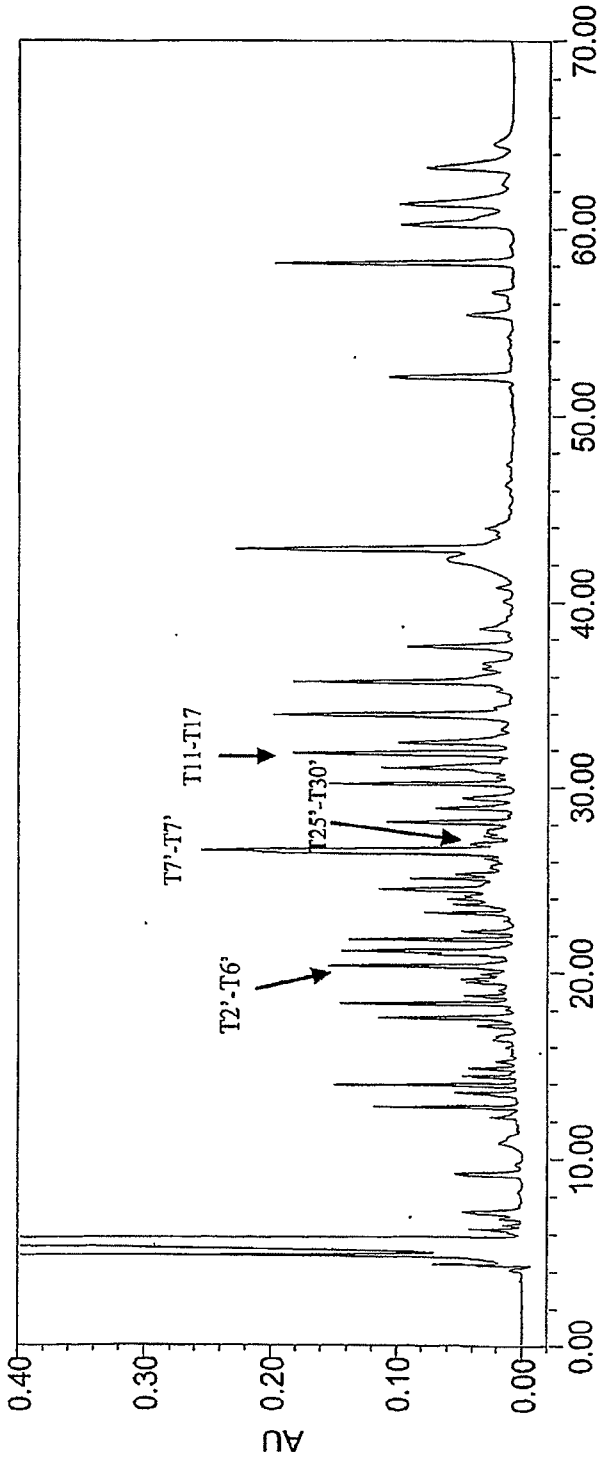

AU
0.40
0.30
0.20
0.10
0.00
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
Minutes
T2'-T6'
T7'-T7'
T11-T17
T25'-T30'

FIG. 37

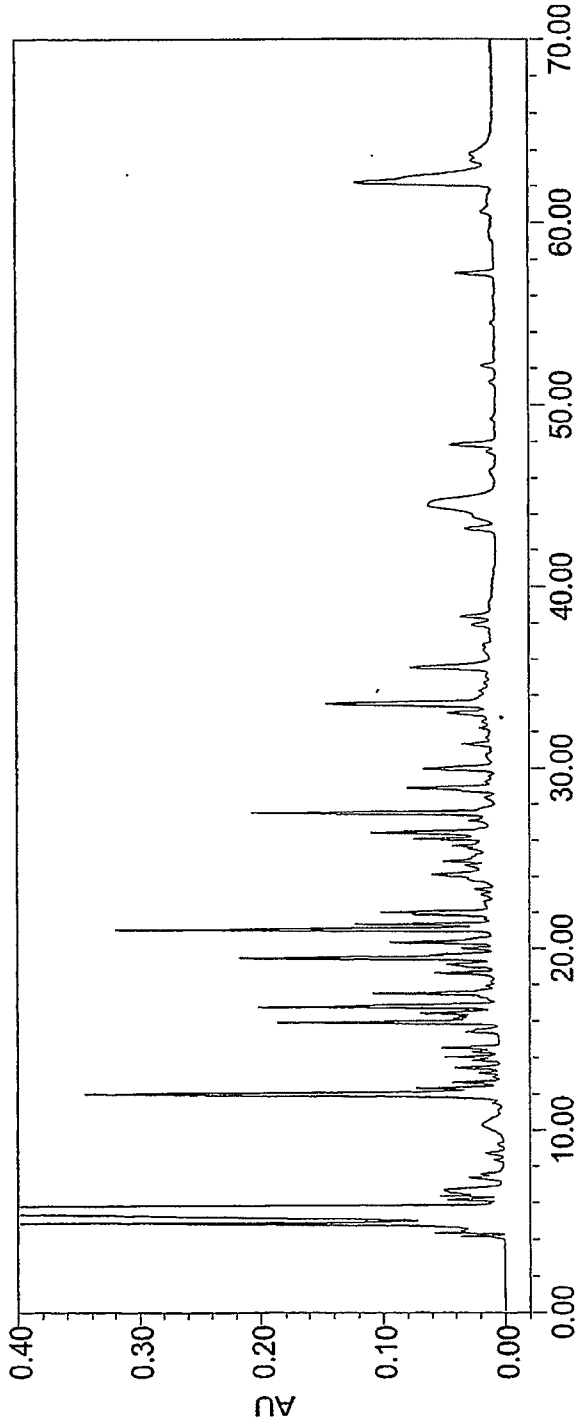

0.40
0.30
0.20
0.10
0.00
AU
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
Minutes

FIG. 38

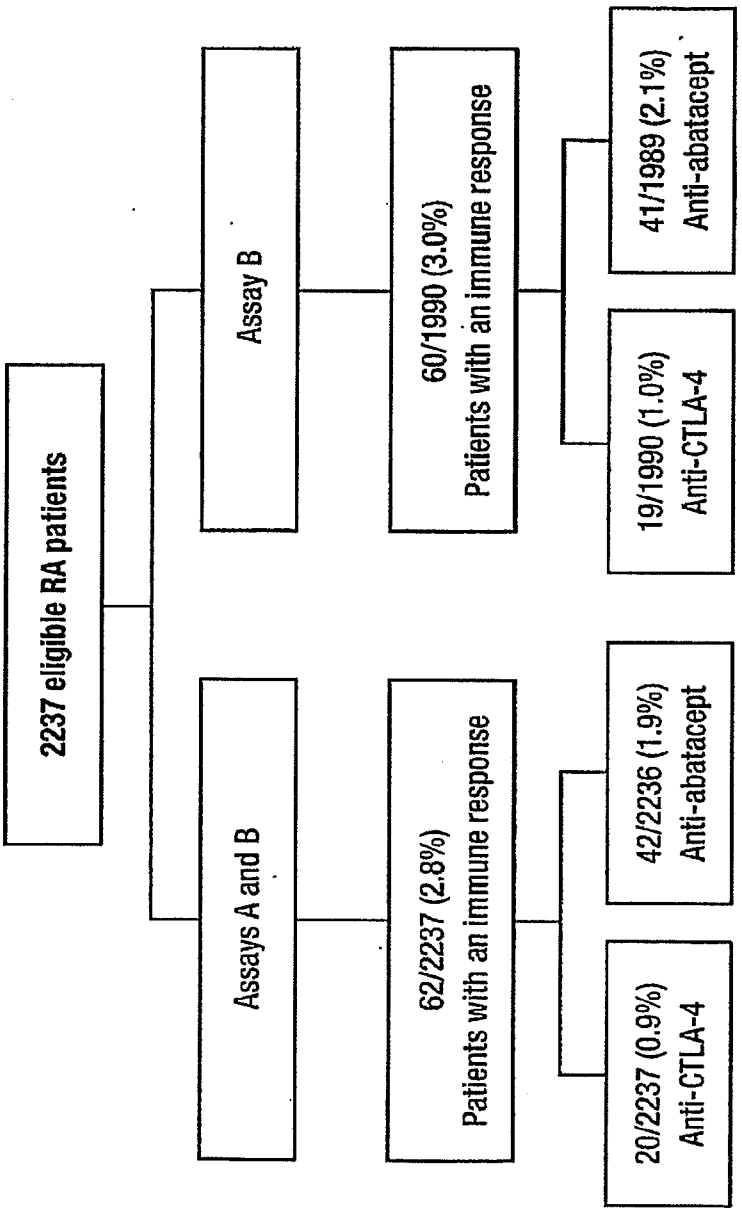

2237 eligible RA patients
Assays A and B
Assay B
62/2237 (2.8%)
Patients with an immune response
60/1990 (3.0%)
Patients with an immune response
20/2237 (0.9%)
Anti-CTLA-4
42/2236 (1.9%)
Anti-abatacept
19/1990 (1.0%)
Anti-CTLA-4
41/1989 (2.1%)
Anti-abatacept

FIG. 39

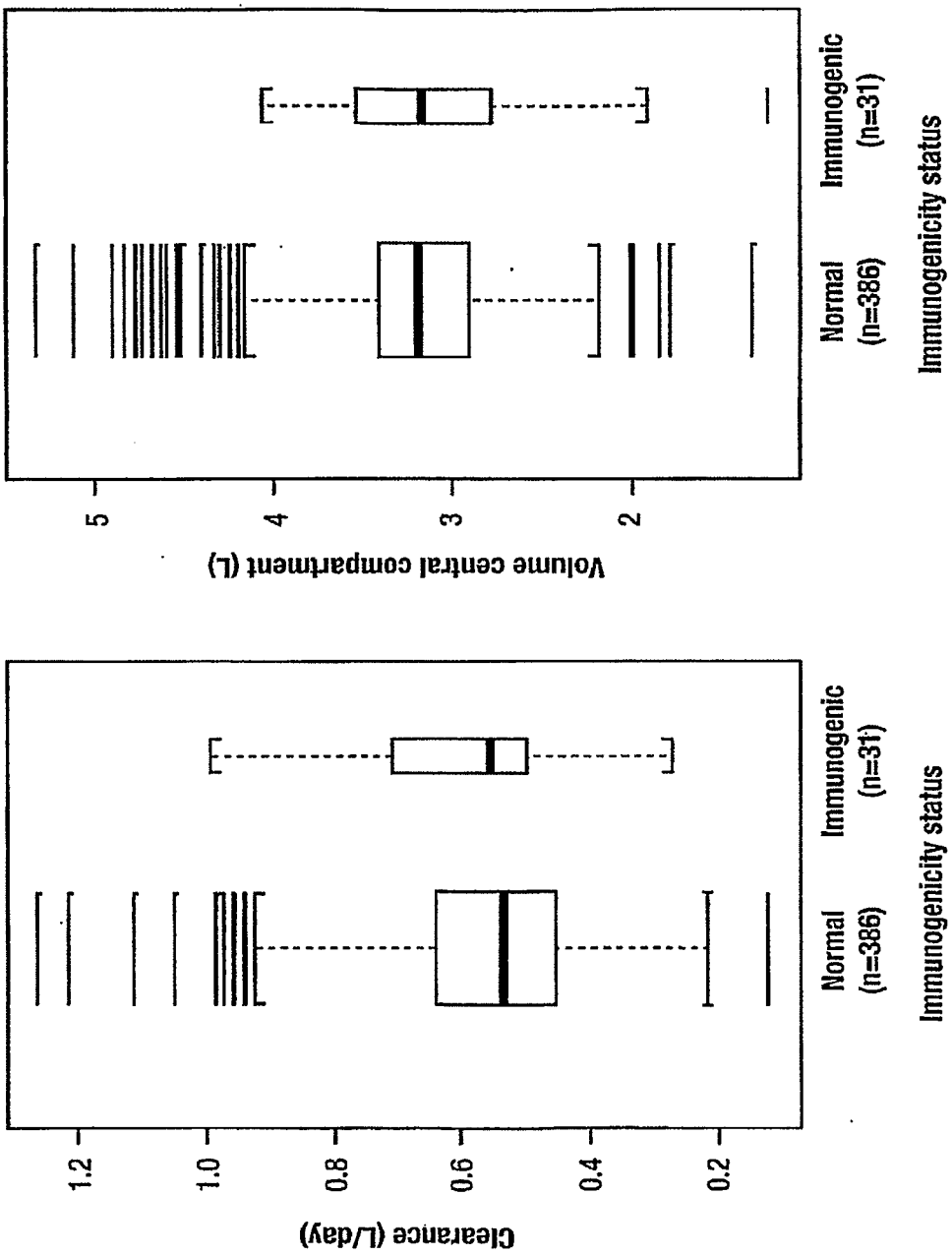

Volume central compartment (L)
5
4
3
2
Normal (n=386)
Immunogenic (n=31)
Immunogenicity status
Clearance (L/day)
1.2
1.0
0.8
0.6
0.4
0.2
Normal (n=386)
Immunogenic (n=31)
Immunogenicity status

FIG. 40

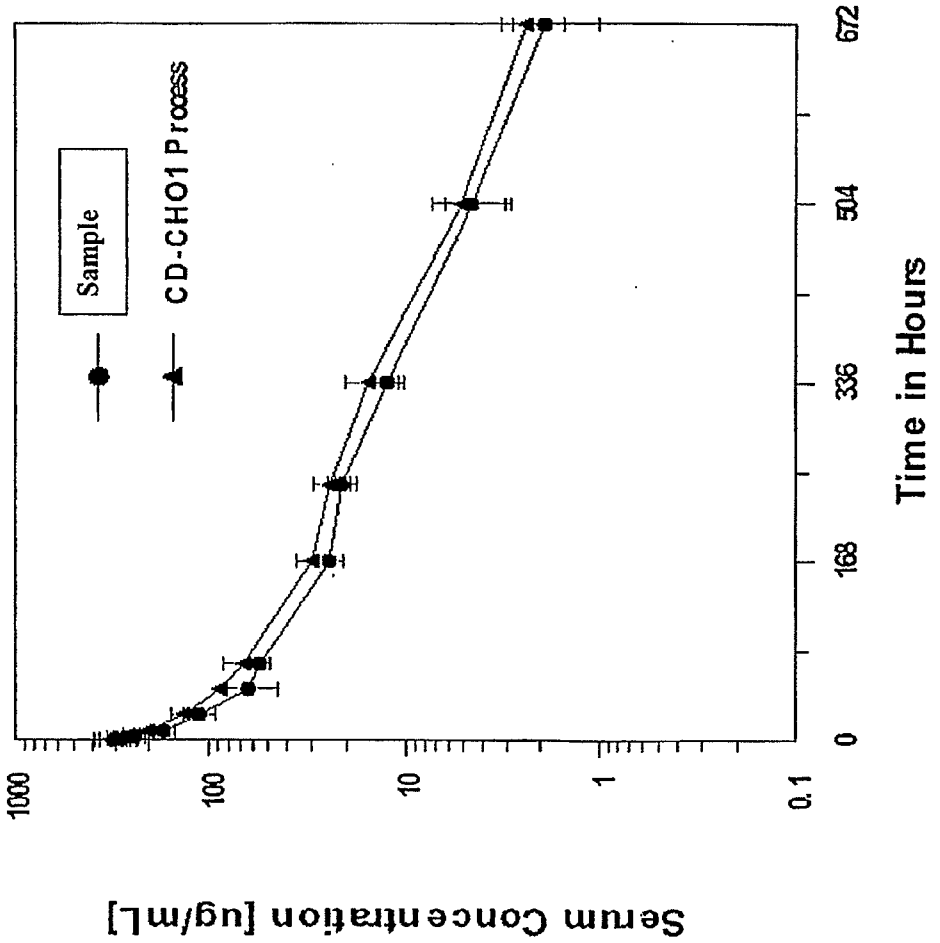

Sample
CD-CHO1 Process
Serum Concentration [ug/mL]
1000
100
10
1
0.1
0
168
336
504
672
Time in Hours

FIG. 41

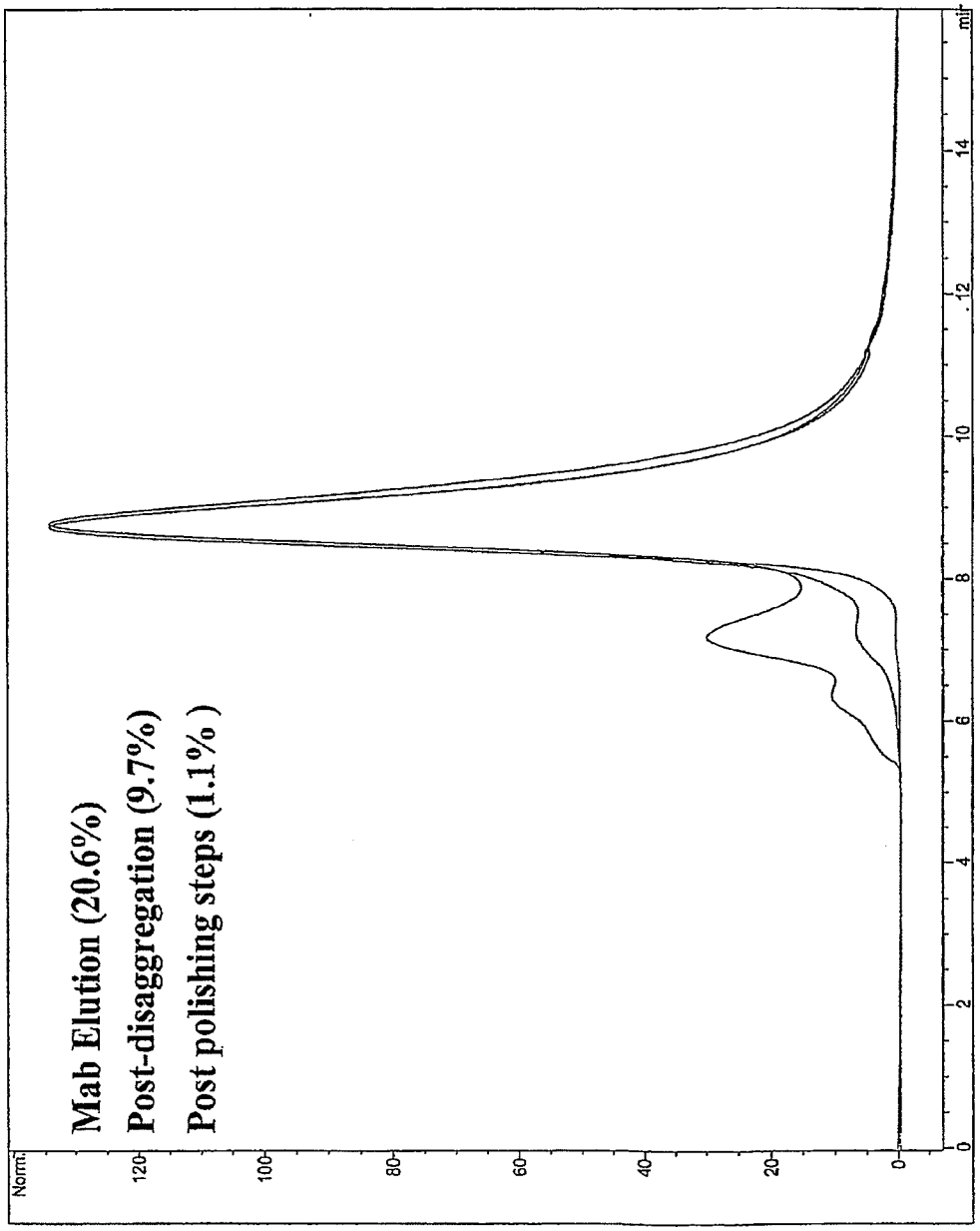

Mab Elution (20.6%)
Post-disaggregation (9.7%)
Post polishing steps (1.1%)
Norm.
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
min

FIG. 42

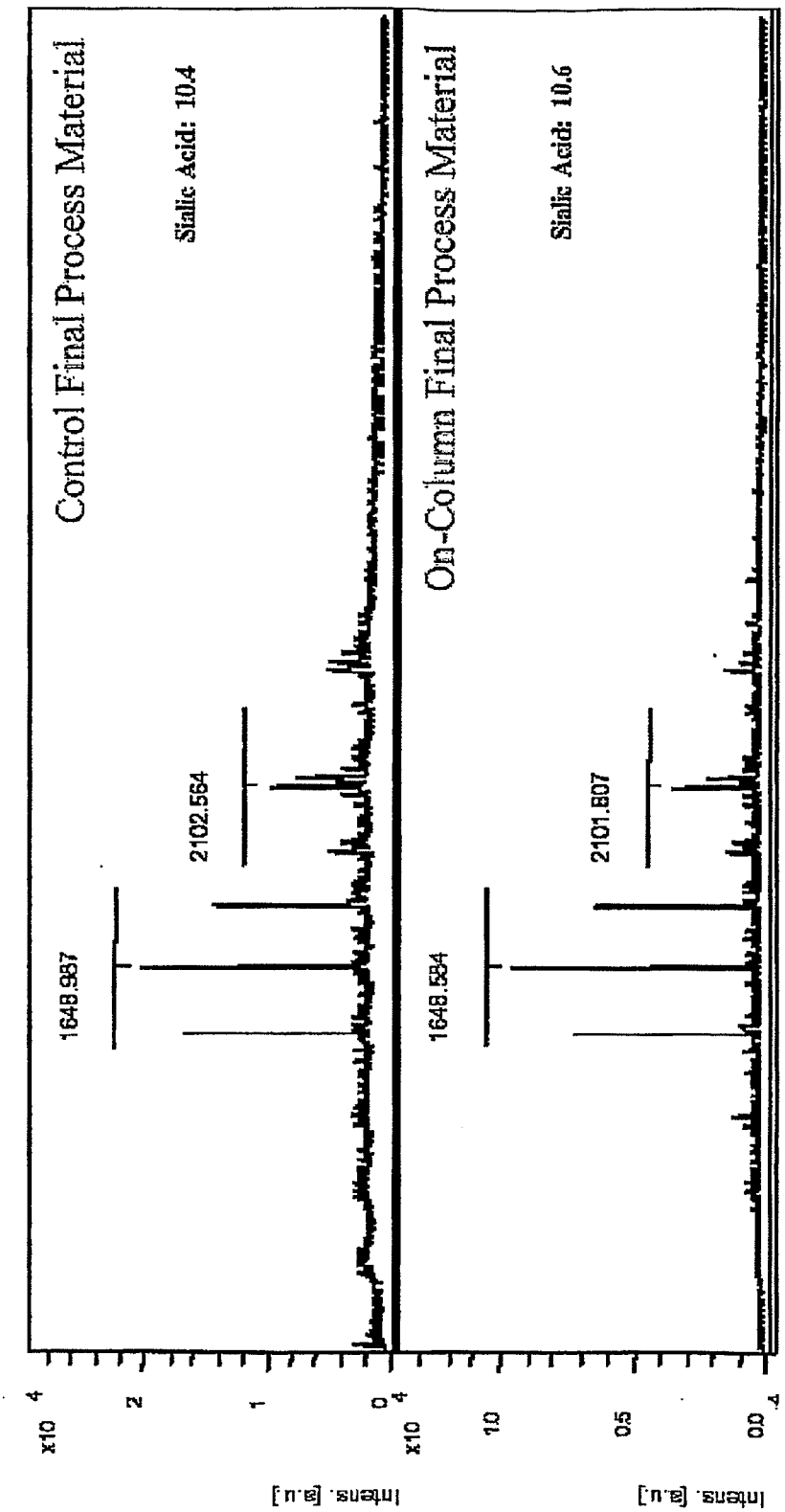

Control Final Process Material
Sialic Acid: 10.4
1648.987
2102.564
On-Column Final Process Material
Sialic Acid: 10.6
1648.584
2101.807
Intens. [a.u.]
Intens. [a.u.]
x10 4
x10 4

FIG. 43

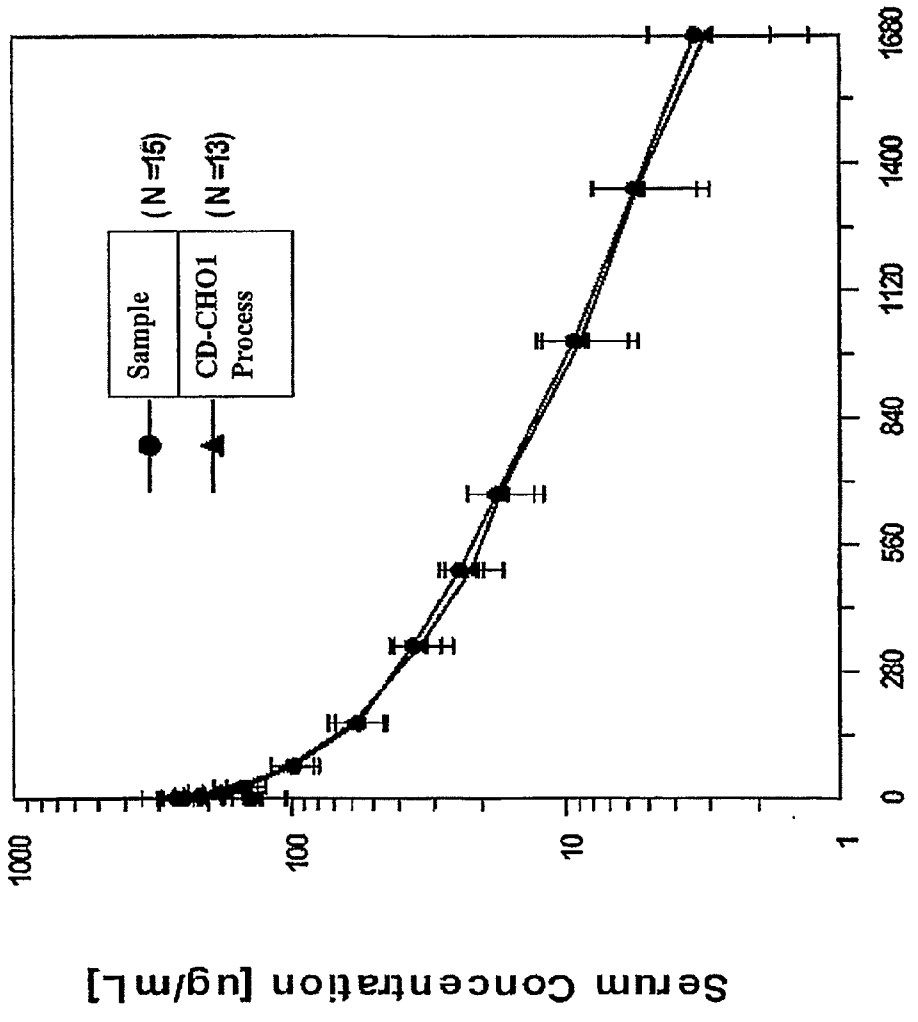

Serum Concentration [ug/mL]
1000
100
10
1
0
280
560
840
1120
1400
1680
Time in Hours
Sample
CD-CHO1 Process
(N =15)
(N =13)

FIG. 44

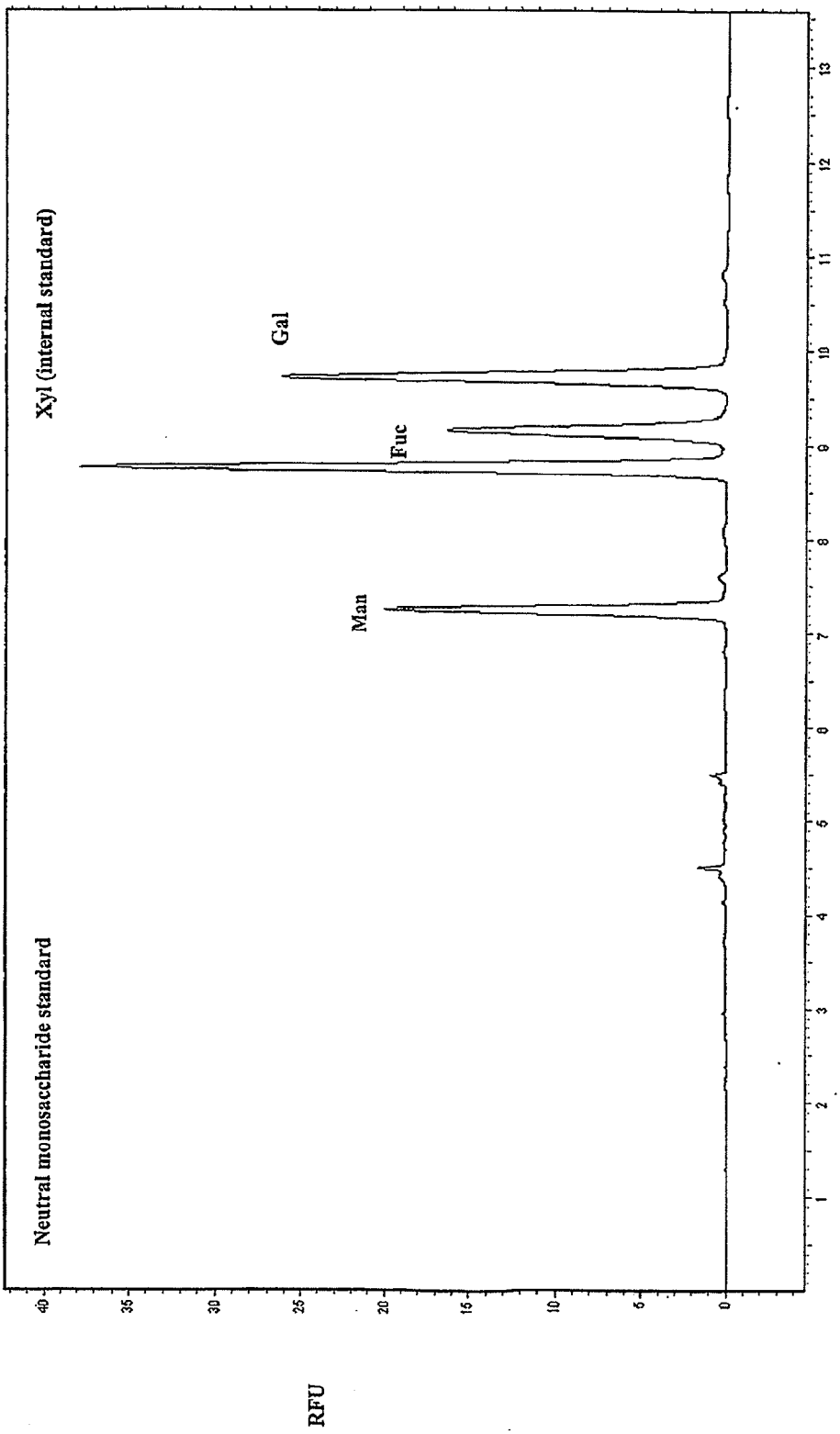

Neutral monosaccharide standard
Xyl (internal standard)
Gal
Fuc
Man
RFU
Minutes

FIG. 45

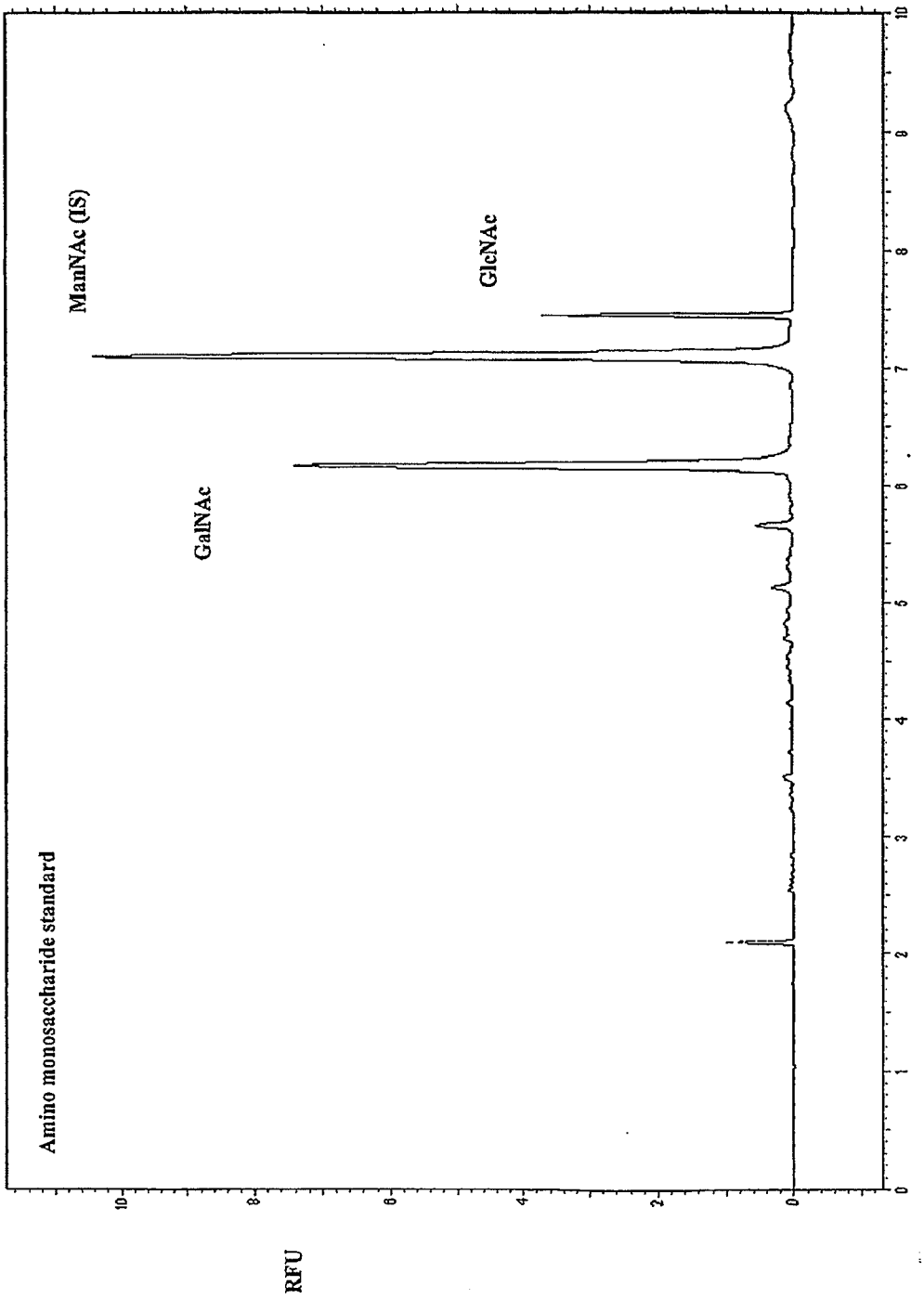

Amino monosaccharide standard
ManNAc (IS)
GlcNAc
GalNAc
RFU
Minutes
0
2
4
6
8
10
0
1
2
3
4
5
6
7
8
9
10

FIG. 46

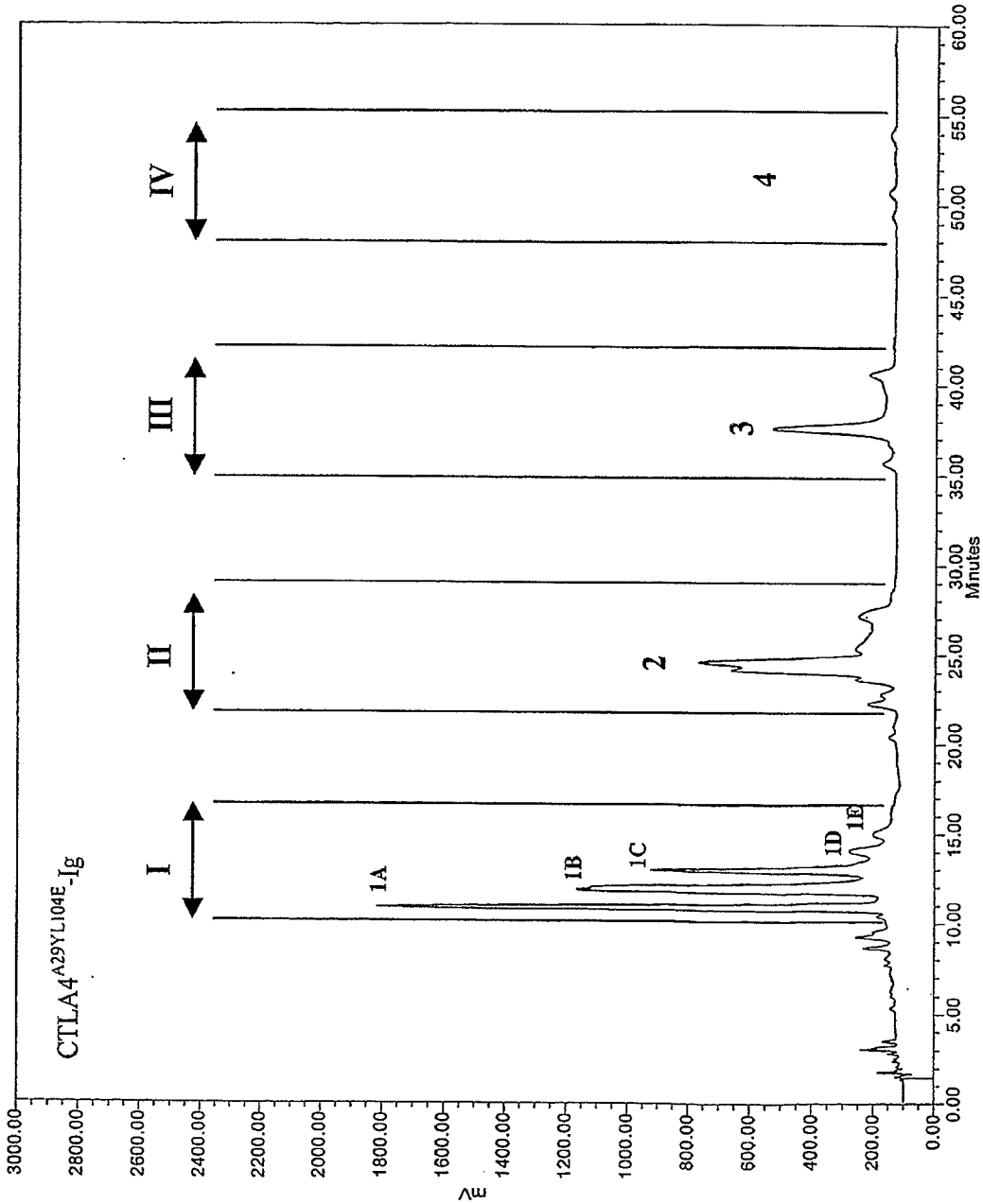

CTLA4A29YL104E-Ig
I
II
III
IV
1A
1B
1C
1D
1E
2
3
4
mV
Minutes
3000.00
2800.00
2600.00
2400.00
2200.00
2000.00
1800.00
1600.00
1400.00
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00

FIG. 47

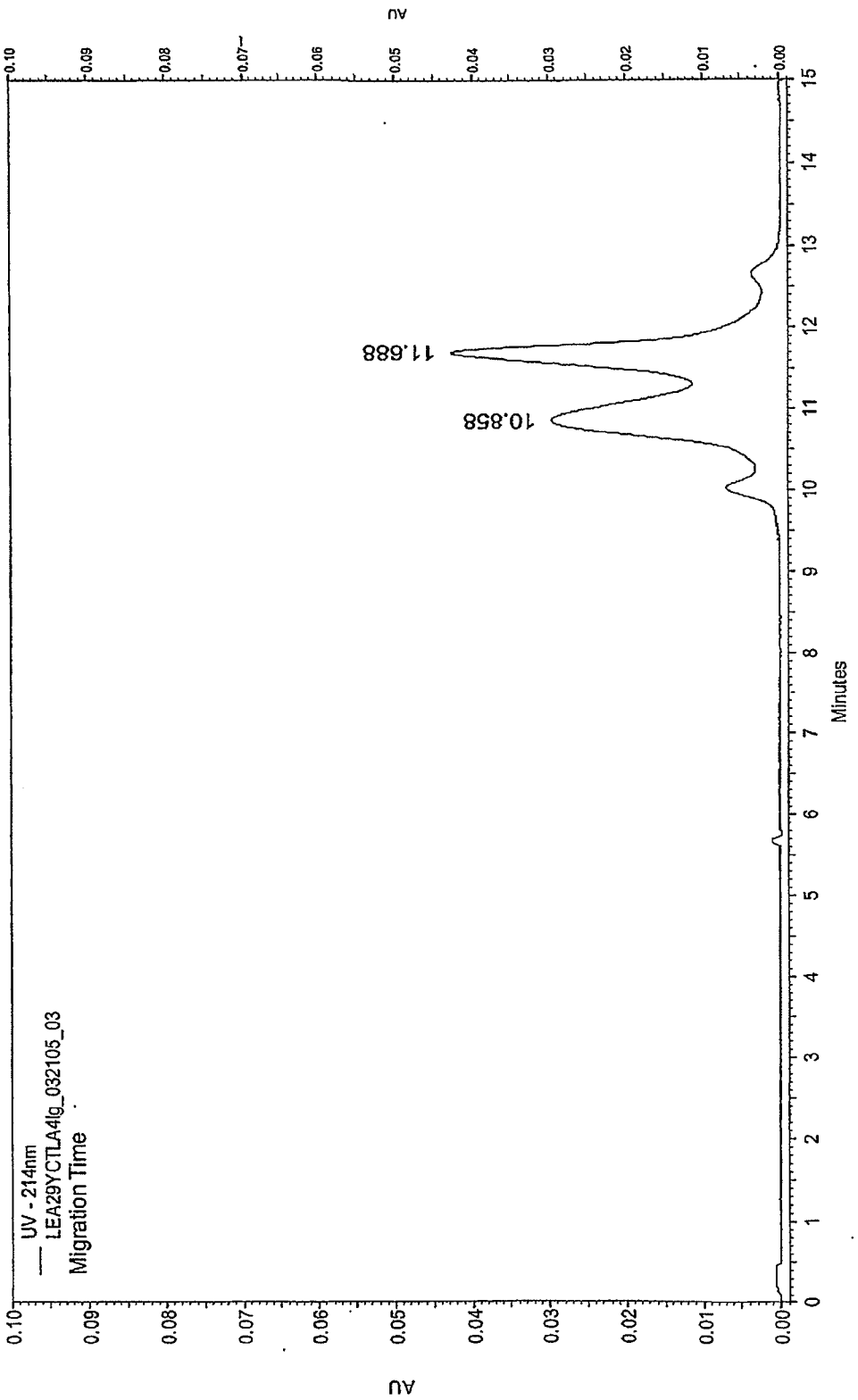

AU
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Minutes
11.688
10.858
UV - 214nm
LEA29YCTLA4Ig_032105_03
Migration Time

FIG. 48

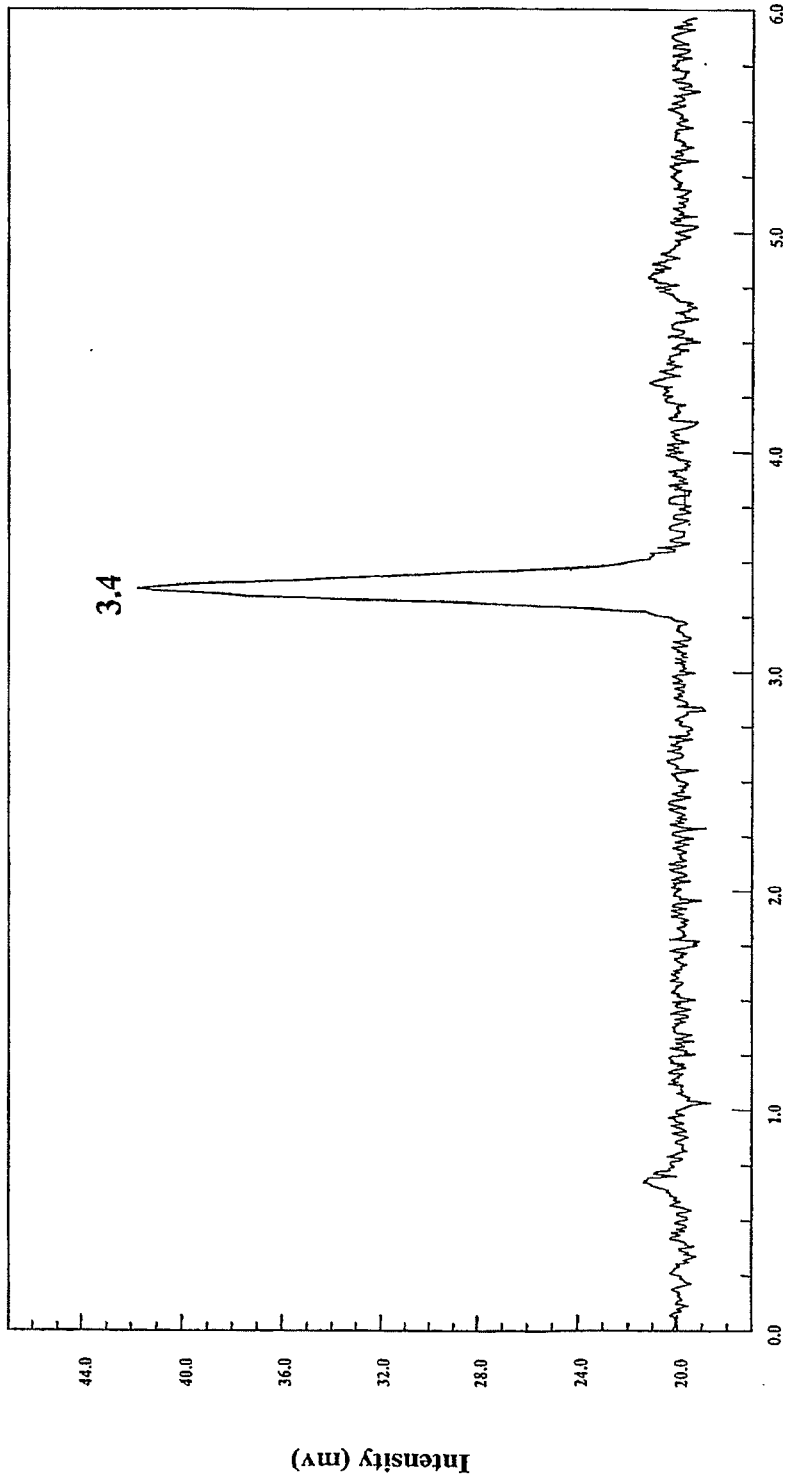

3.4
Intensity (mv)
44.0
40.0
36.0
32.0
28.0
24.0
20.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
Time (minutes)

FIG. 49

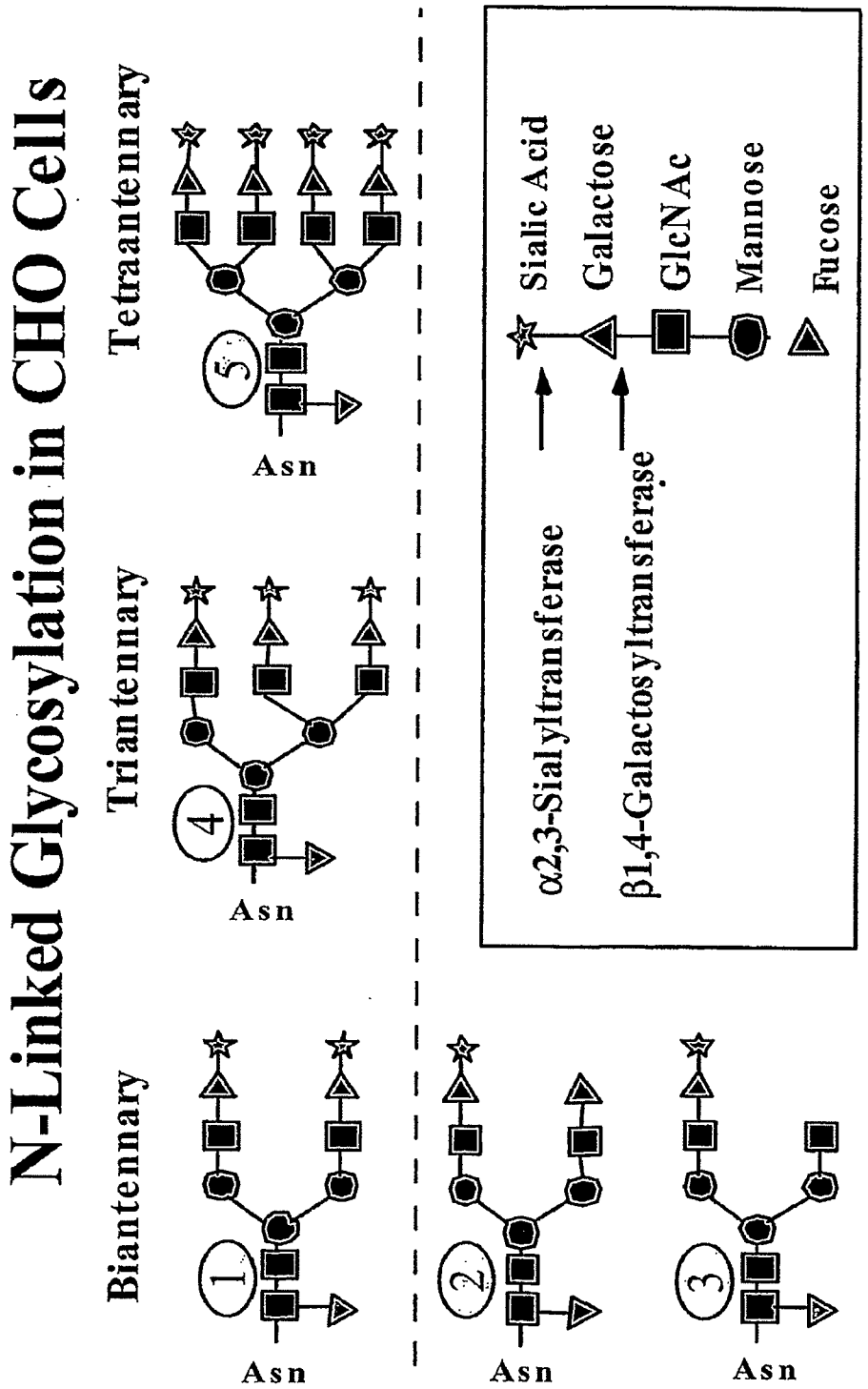

N-Linked Glycosylation in CHO Cells
Biantennary
Triantennary
Tetraantennary
Asn
Asn
Asn
Asn
Asn
1
2
3
4
5
α2,3-Sialyltransferase
β1,4-Galactosyltransferase
Sialic Acid
Galactose
GlcNAc
Mannose
Fucose

FIG. 50

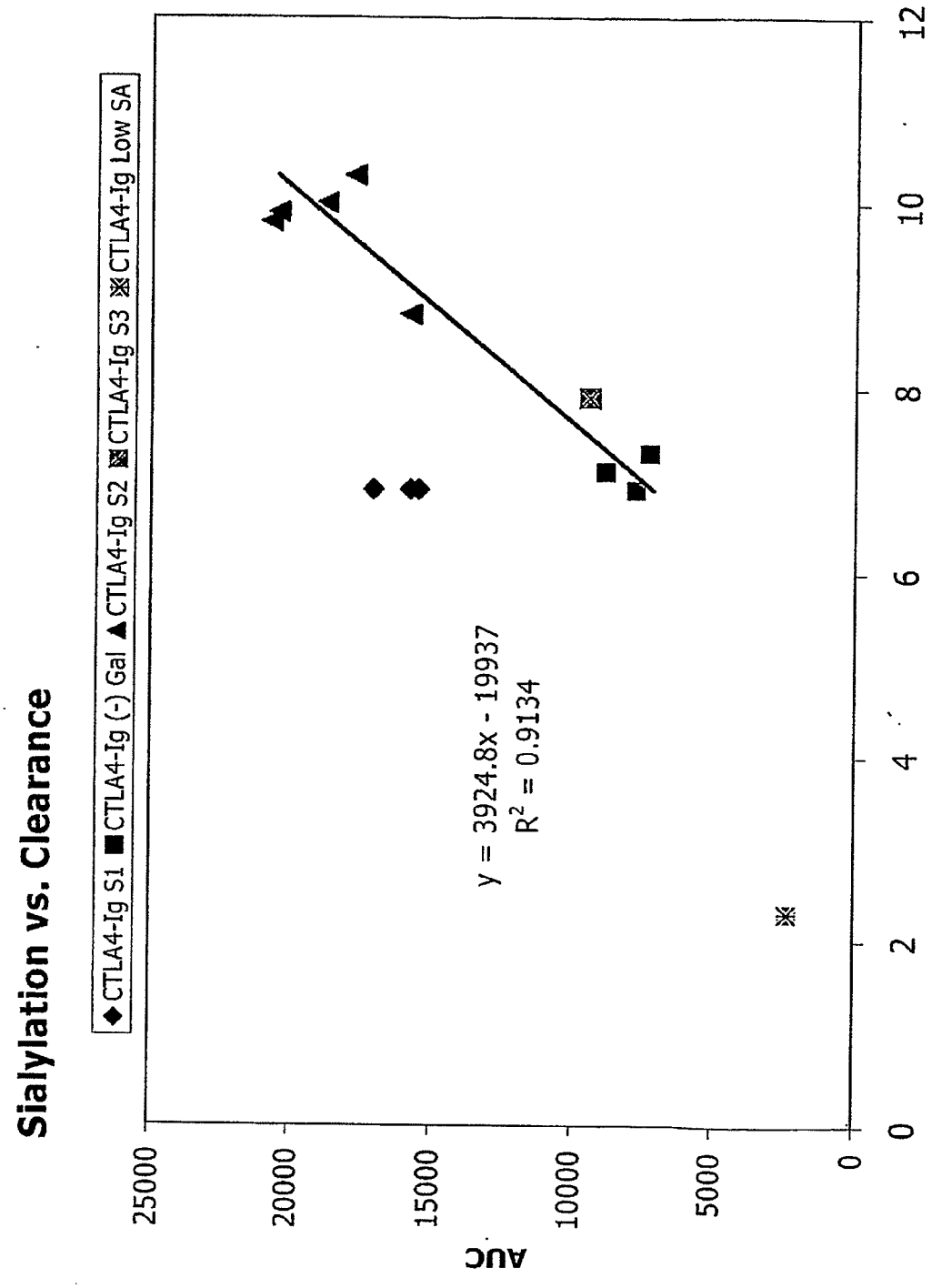

Sialylation vs. Clearance
◆CTLA4-Ig S1 ■CTLA4-Ig (-) Gal ▲CTLA4-Ig S2 ⊠CTLA4-Ig S3 ⊠CTLA4-Ig Low SA
y = 3924.8x - 19937
R² = 0.9134
AUC
NANA Ratio
0
5000
10000
15000
20000
25000
0
2
4
6
8
10
12

FIG. 51

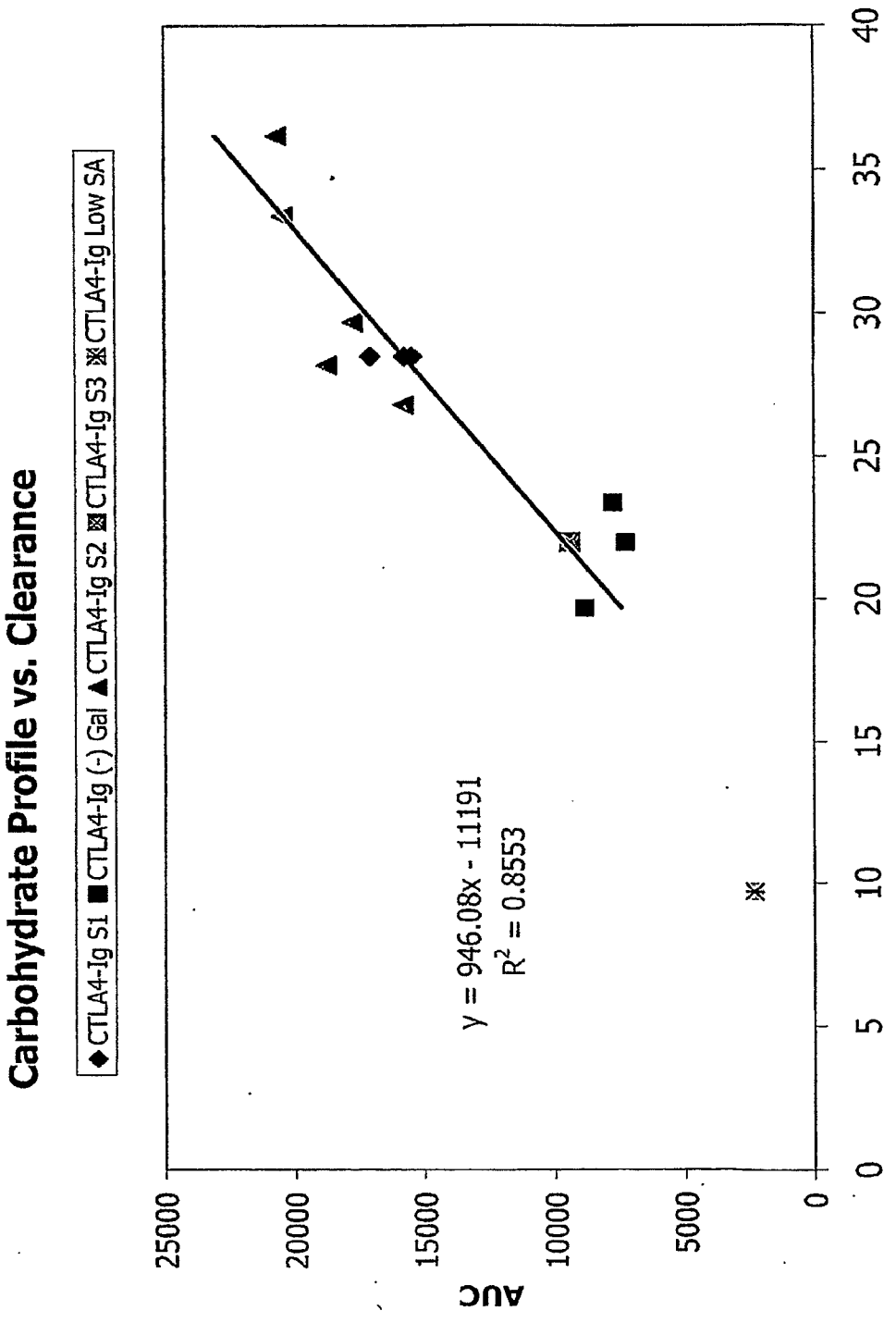

Carbohydrate Profile vs. Clearance
◆CTLA4-Ig S1 ■CTLA4-Ig (-) Gal ▲CTLA4-Ig S2 ⊠CTLA4-Ig S3 ⊠CTLA4-Ig Low SA
y = 946.08x - 11191
R2 = 0.8553
AUC
25000
20000
15000
10000
5000
0
0
5
10
15
20
25
30
35
40
%.Domain III and IV

FIG. 52

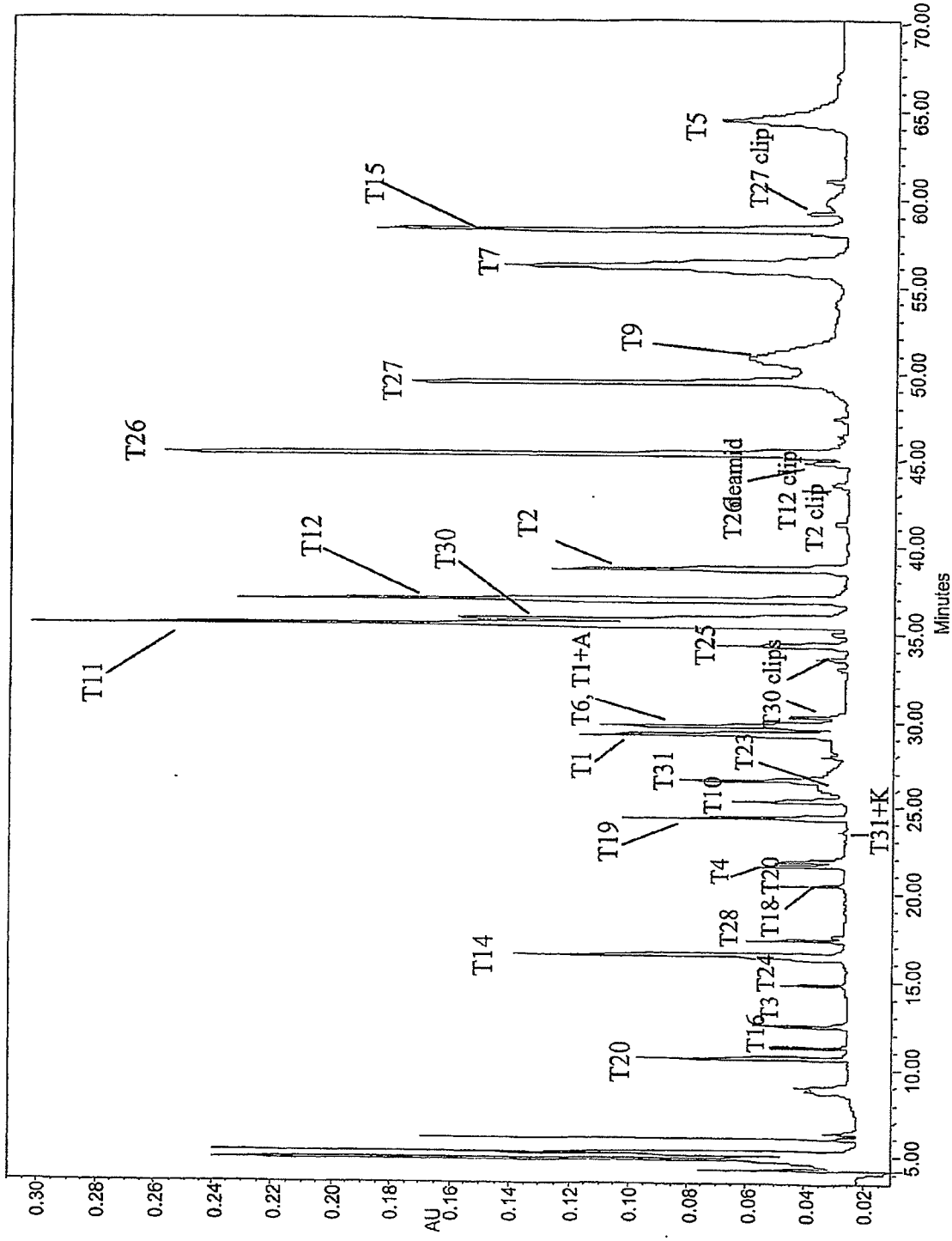

T11
T12
T30
T26
T2
T27
T9
T7
T15
T5
T27 clip
T12 clip
T2 clip
T26deamid
T25
T30 clips
T6, T1+A
T1
T31
T10
T23
T19
T4
T20
T18
T28
T14
T24
T3
T16
T20
T31+K
AU
Minutes
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00
65.00
70.00

FIG. 53

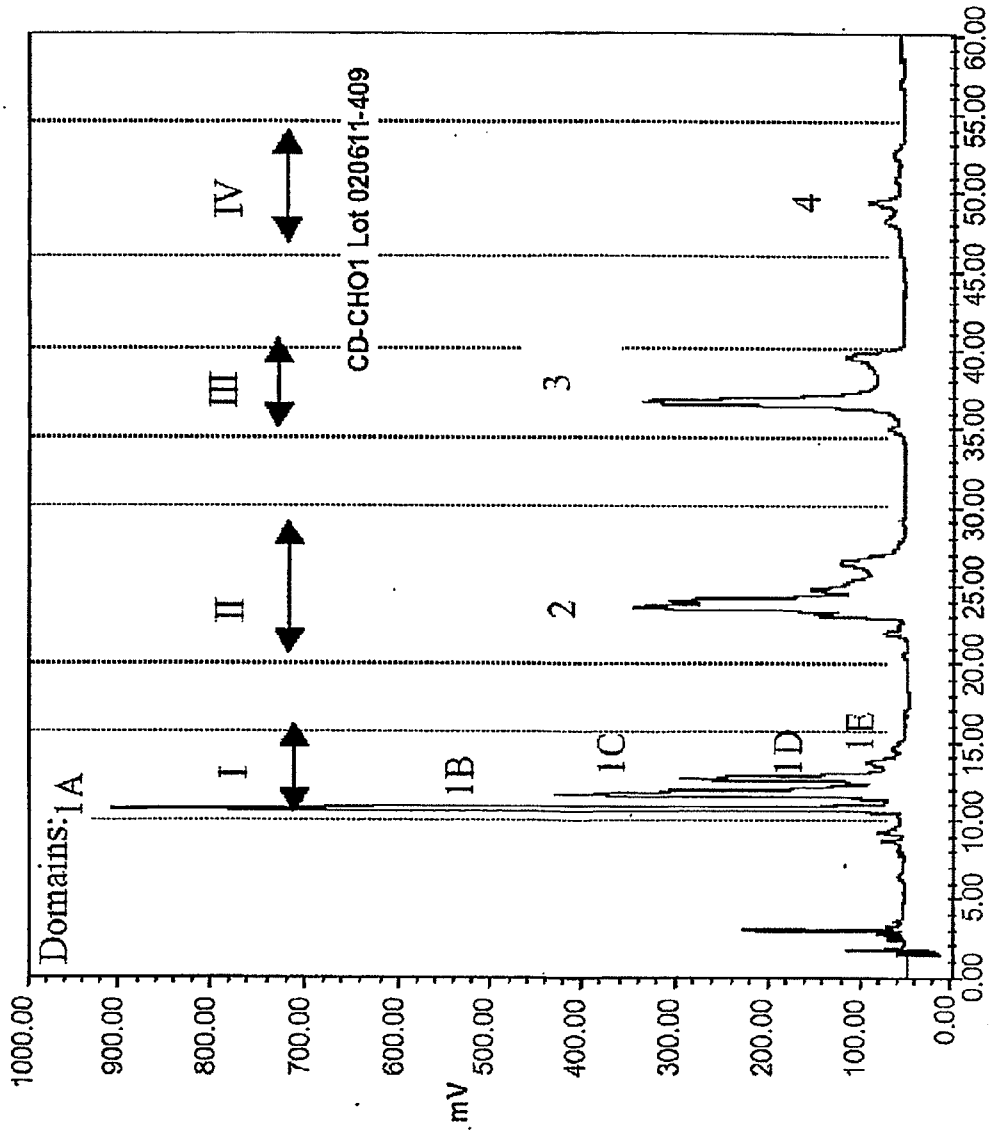

Domains:
1A
1B
1C
1D
1E
2
3
4
I
II
III
IV
CD-CHO1 Lot 020611-409
mV
1000.00
900.00
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00
Minutes

FIG. 54

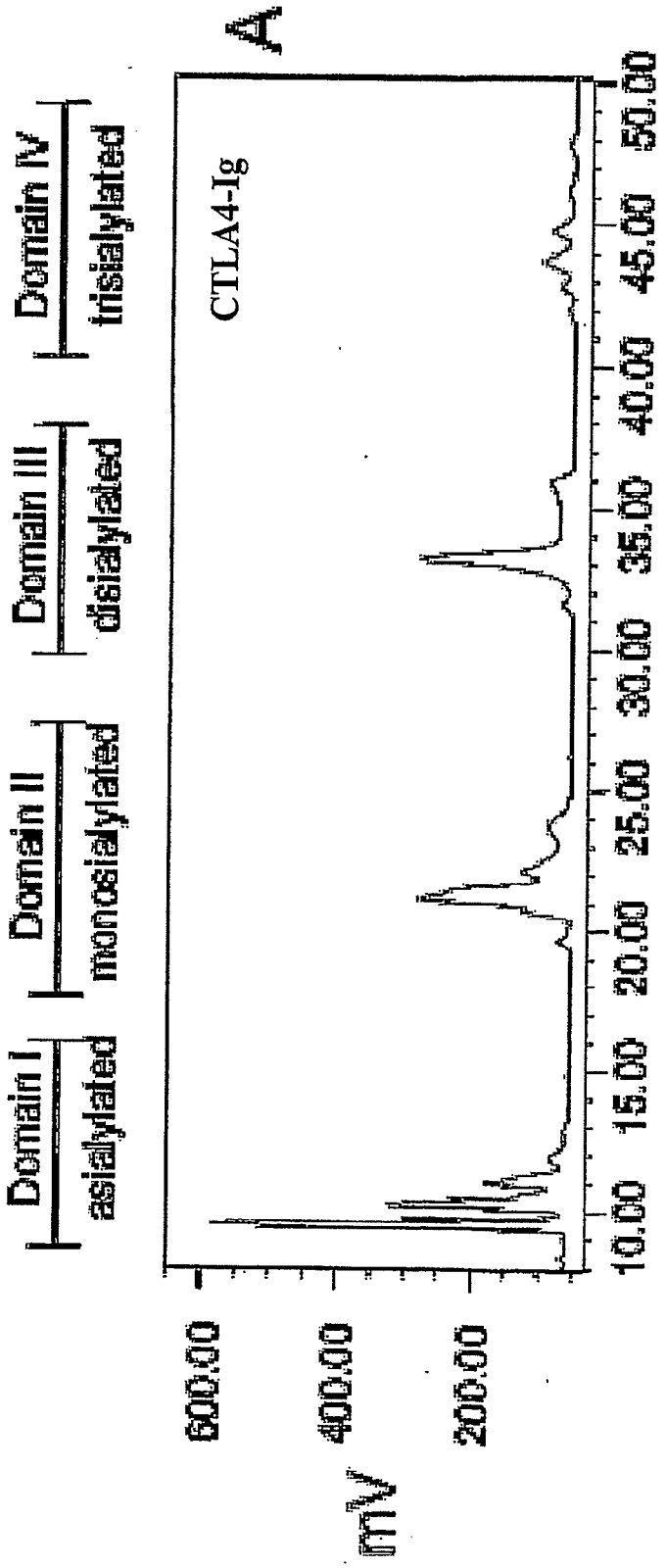

A
CTLA4-Ig
Domain I
asialylated
Domain II
monosialylated
Domain III
disialylated
Domain IV
trisialylated
mV
600.00
400.00
200.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
Minutes

FIG. 55A

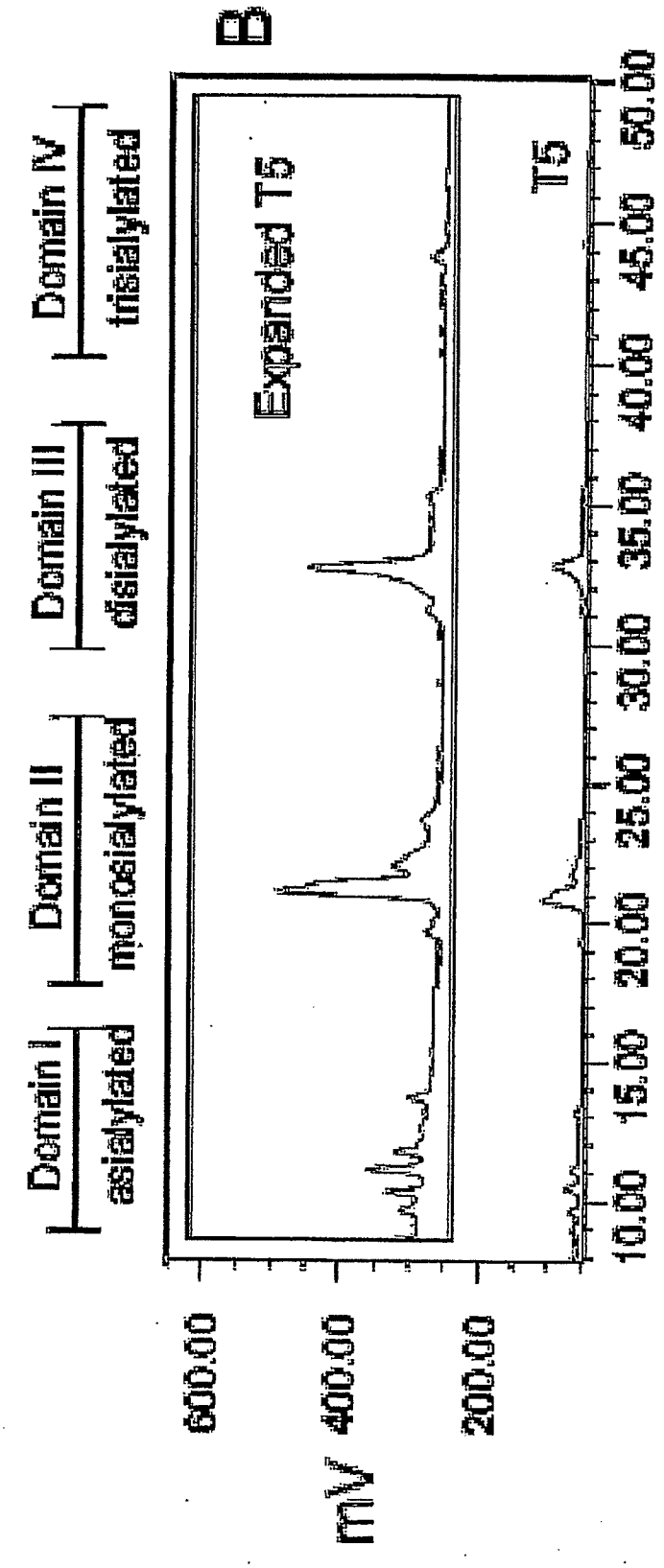

B
Domain I
asialylated
Domain II
monosialylated
Domain III
disialylated
Domain IV
trisialylated
Expanded T5
T5
mV
600.00
400.00
200.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
Minutes

FIG. 55B

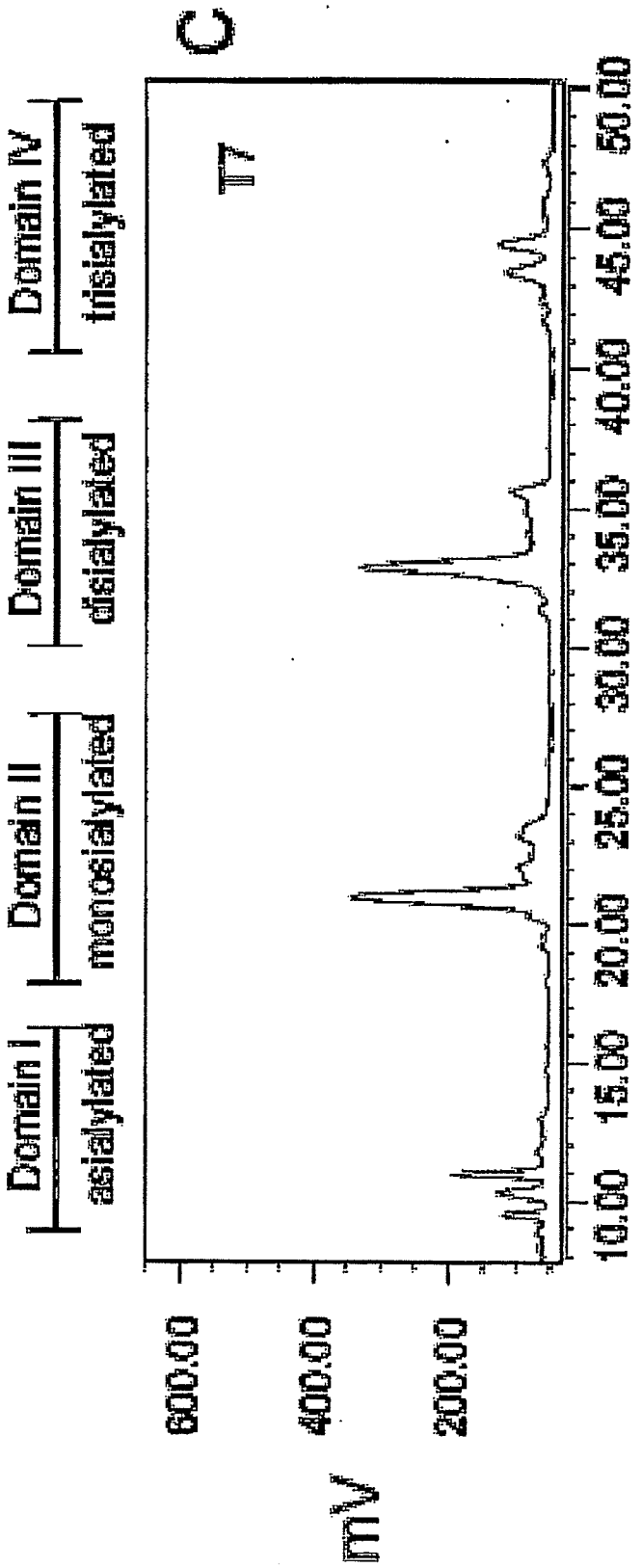
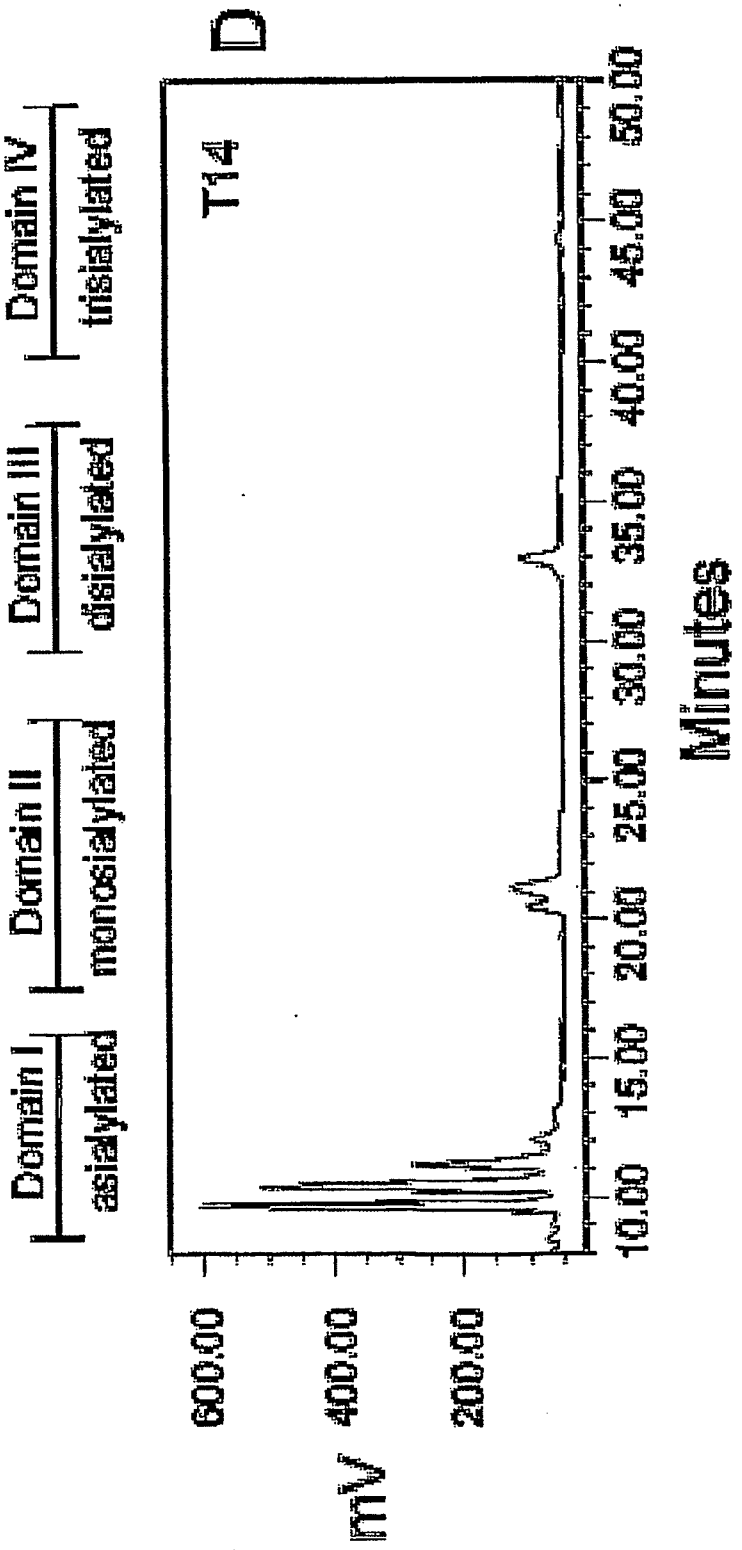

C
T7
Domain I
asialylated
Domain II
monosialylated
Domain III
disialylated
Domain IV
trisialylated
mV
600.00
400.00
200.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
Minutes

FIG. 55C


D
T14
Domain I
asialylated
Domain II
monosialylated
Domain III
disialylated
Domain IV
trisialylated
600.00
400.00
200.00
mV
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
Minutes

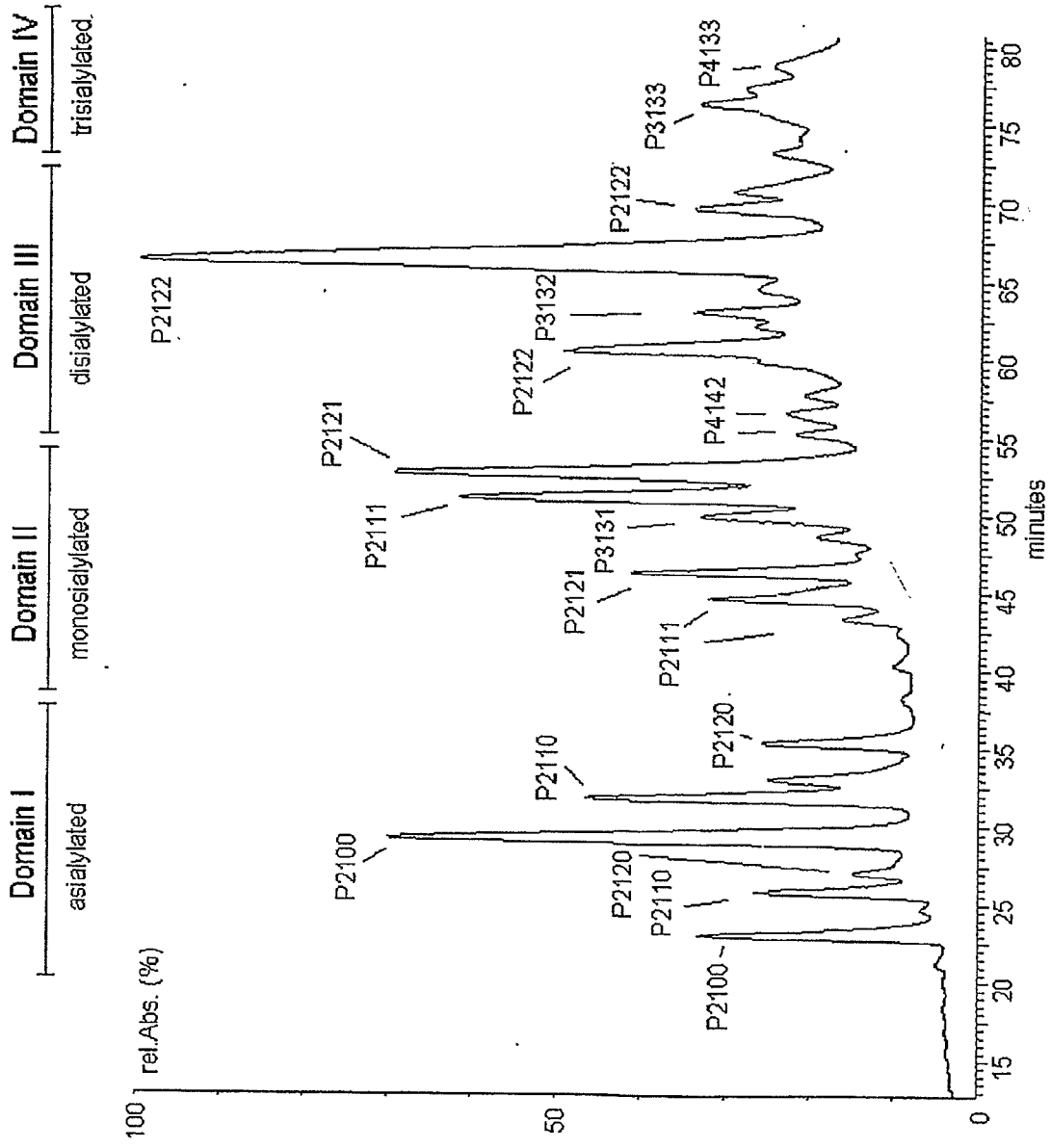

Domain I
asialylated
Domain II
monosialylated
Domain III
disialylated
Domain IV
trisialylated
rel.Abs. (%)
100
50
0
minutes
15
20
25
30
35
40
45
50
55
60
65
70
75
80
P2100
P2120
P2110
P2100
P2110
P2120
P2111
P2121
P3131
P2121
P2111
P2122
P3132
P2122
P4142
P2122
P3133
P4133

FIG. 56

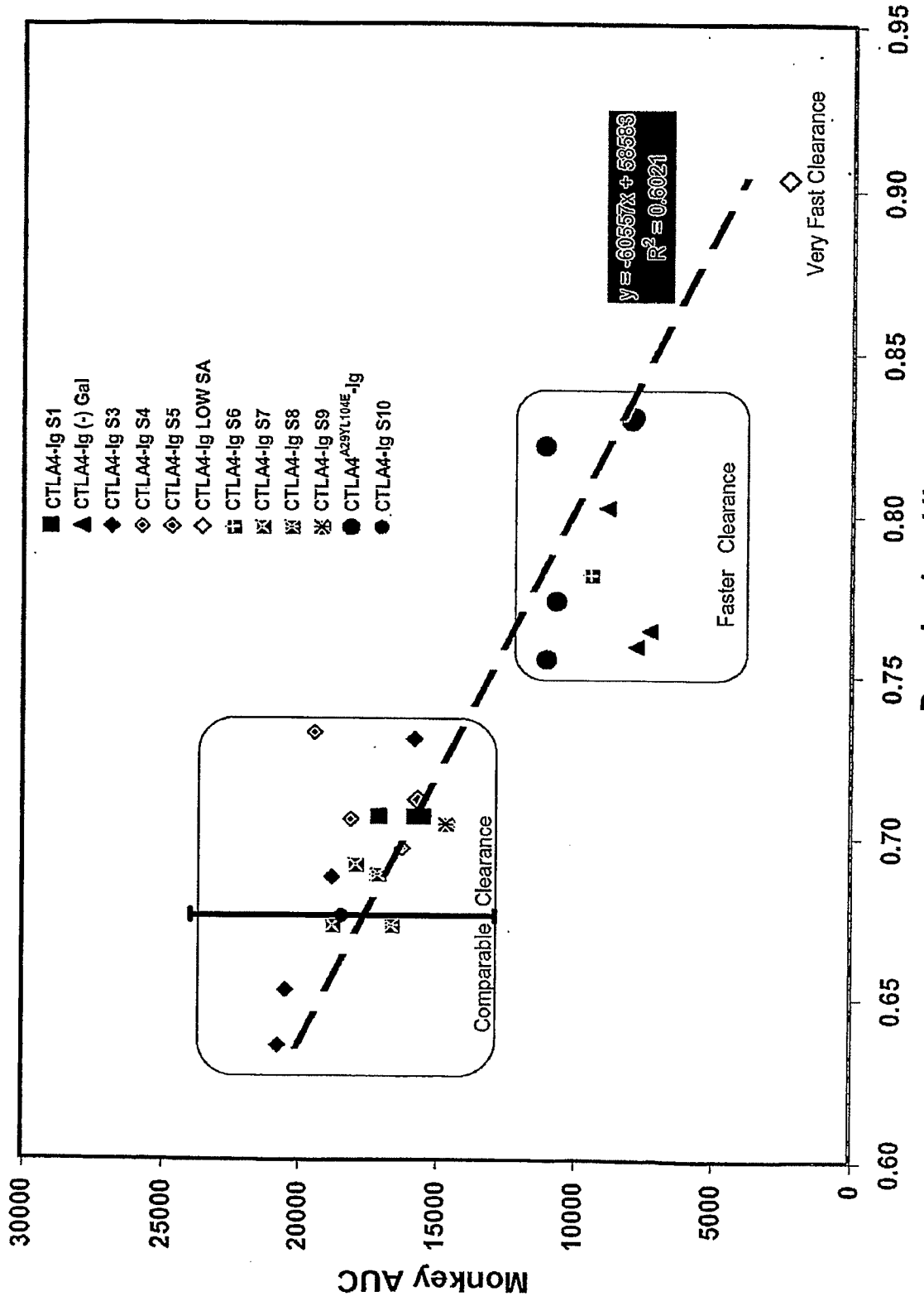

Monkey AUC
Domains I and II
CTLA4-Ig S1
CTLA4-Ig (-) Gal
CTLA4-Ig S3
CTLA4-Ig S4
CTLA4-Ig S5
CTLA4-Ig LOW SA
CTLA4-Ig S6
CTLA4-Ig S7
CTLA4-Ig S8
CTLA4-Ig S9
CTLA4-Ig S10
y = -60557x + 58583
R² = 0.6021
Comparable Clearance
Faster Clearance
Very Fast Clearance
30000
25000
20000
15000
10000
5000
0
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95

FIG. 57

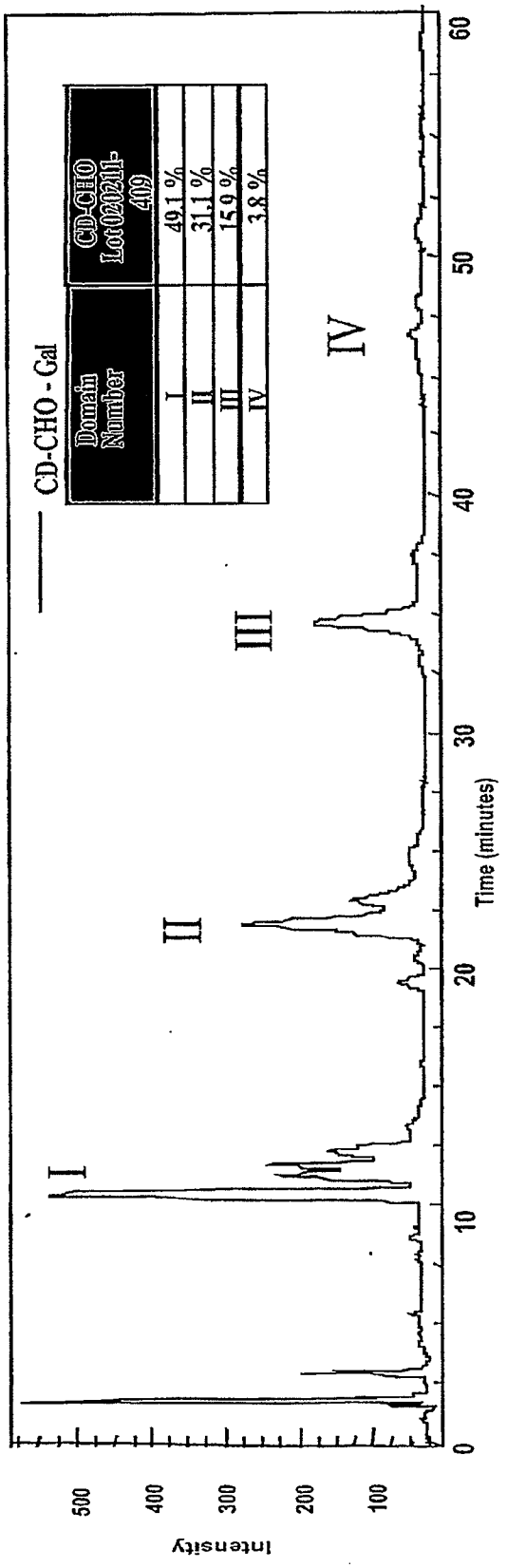

CD-CHO - Gal
Domain Number
CD-CHO Lot020211-409
I
49.1 %
II
31.1 %
III
15.9 %
IV
3.8 %
I
II
III
IV
Intensity
500
400
300
200
100
0
10
20
30
40
50
60
Time (minutes)

FIG. 58A

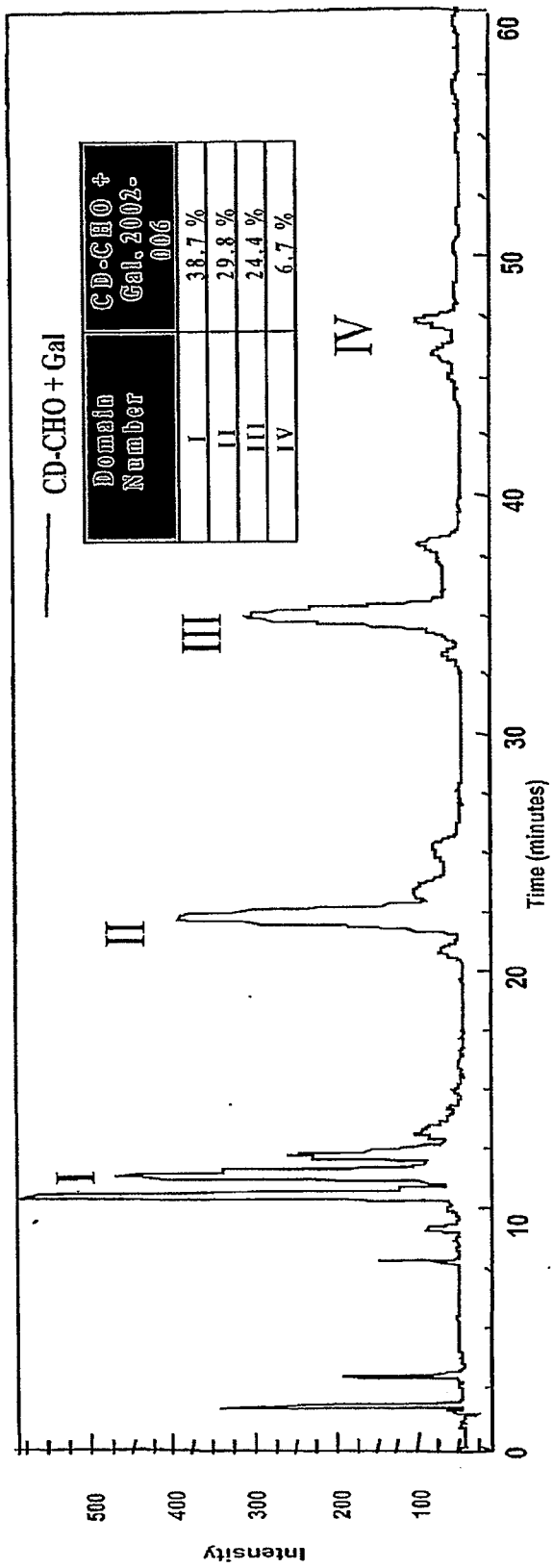

CD-CHO + Gal
Domain Number
CD-CHO + Gal, 2002-006
I
38.7 %
II
29.8 %
III
24.4 %
IV
6.7 %
I
II
III
IV
Intensity
500
400
300
200
100
0
10
20
30
40
50
60
Time (minutes)

FIG. 58B

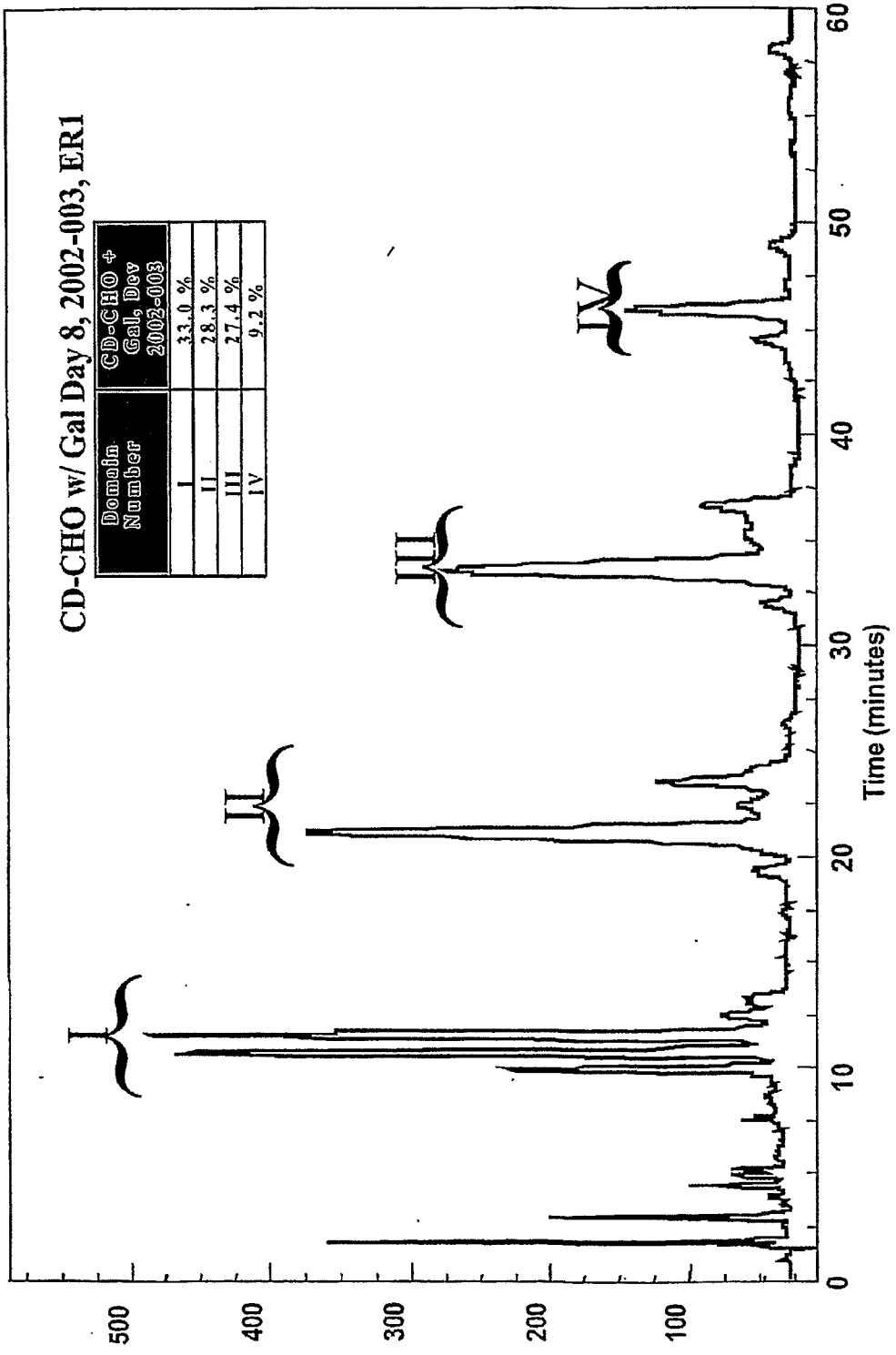

CD-CHO w/ Gal Day 8, 2002-003, ER1
Domain Number
CD-CHO + Gal, Dev 2002-003
I
33.0 %
II
28.3 %
III
27.4 %
IV
9.2 %
I
II
III
IV
500
400
300
200
100
0
10
20
30
40
50
60
Time (minutes)

FIG. 59

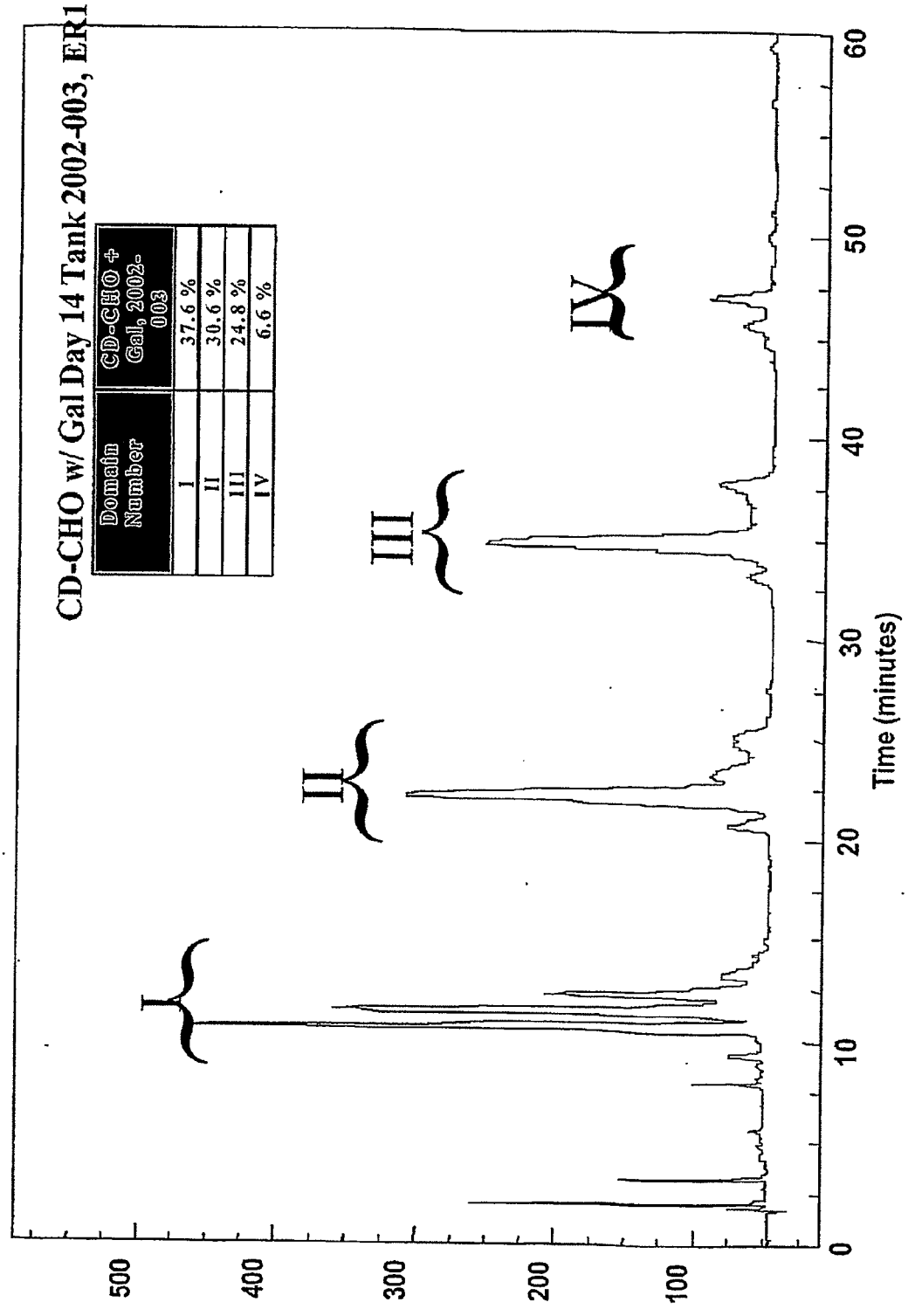

CD-CHO w/ Gal Day 14 Tank 2002-003, ER1
Domain Number
CD-CHO + Gal, 2002-003
I
37.6 %
II
30.6 %
III
24.8 %
IV
6.6 %
I
II
III
IV
500
400
300
200
100
0
10
20
30
40
50
60
Time (minutes)

FIG. 60

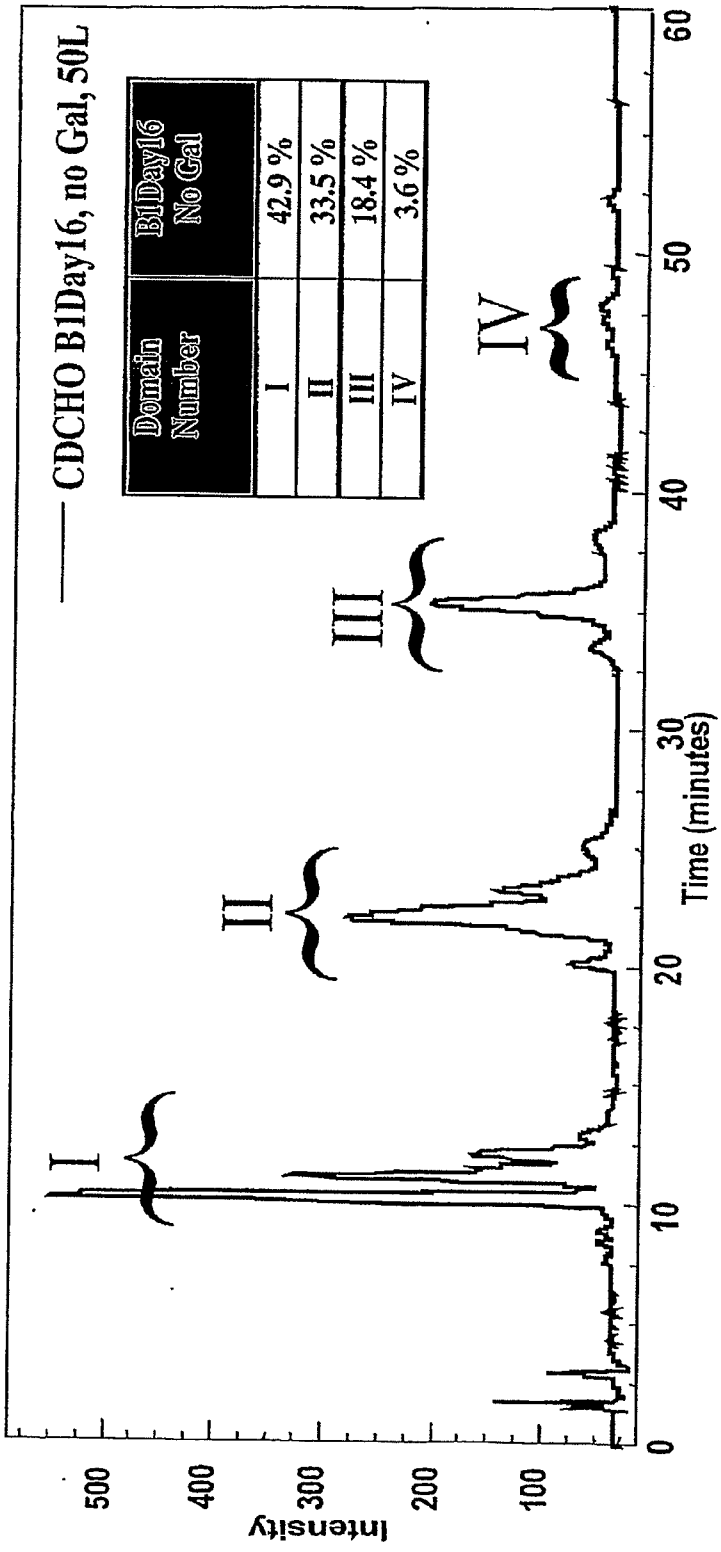

CDCHO B1Day16, no Gal, 50L
Domain Number
B1Day16 No Gal
I
42.9 %
II
33.5 %
III
18.4 %
IV
3.6 %
I
II
III
IV
Intensity
Time (minutes)
0
10
20
30
40
50
60
100
200
300
400
500

FIG. 61A

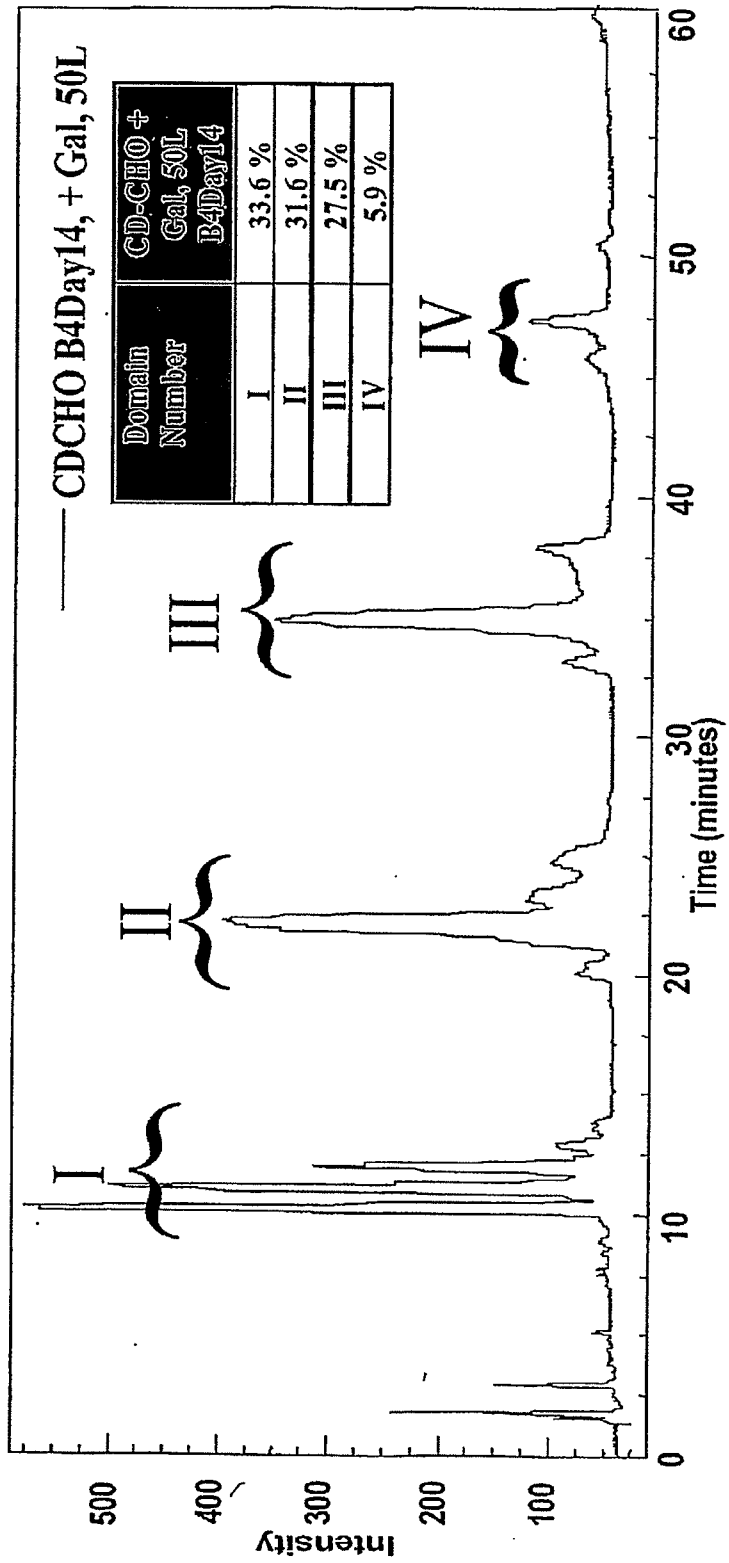

CDCHO B4Day14, + Gal, 50L
Domain Number
CD-CHO + Gal, 50L B4Day14
I
33.6 %
II
31.6 %
III
27.5 %
IV
5.9 %
I
II
III
IV
Intensity
Time (minutes)
0
10
20
30
40
50
60
100
200
300
400
500

FIG. 61B

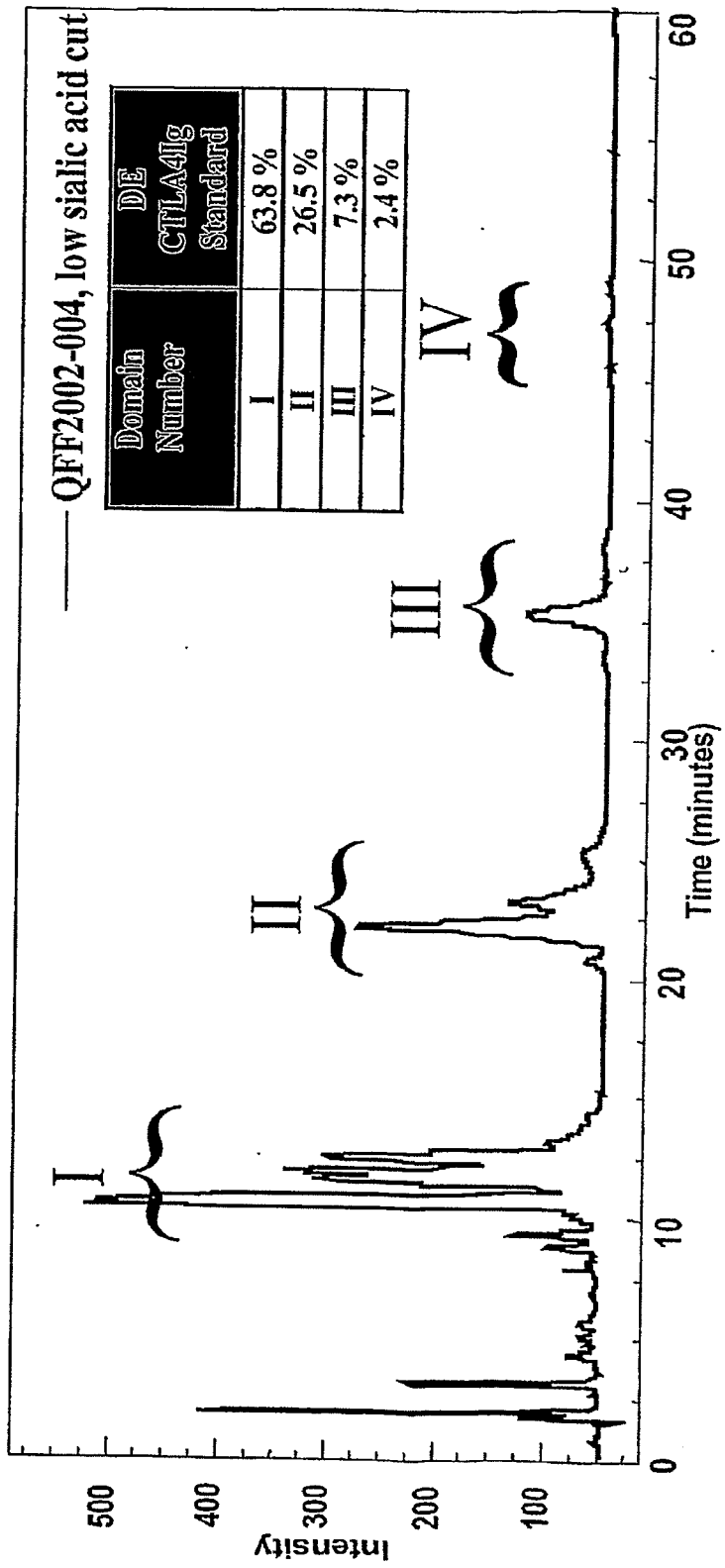

QFF2002-004, low sialic acid cut
Domain Number
DE CTLA4Ig Standard
I
63.8 %
II
26.5 %
III
7.3 %
IV
2.4 %
I
II
III
IV
Intensity
500
400
300
200
100
0
10
20
30
40
50
60
Time (minutes)

FIG. 62

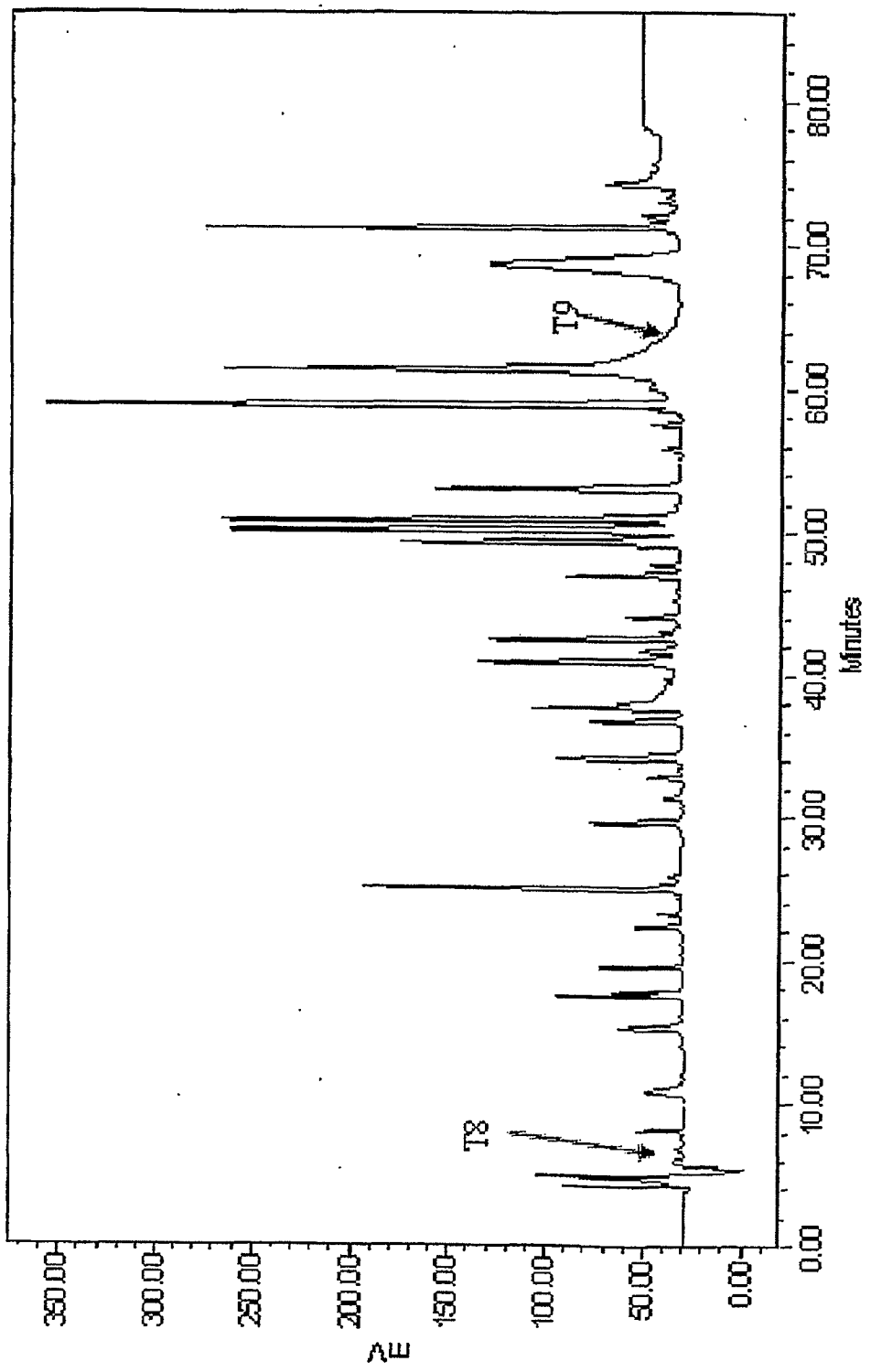

mV
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
Minutes
T8
T9

FIG. 63

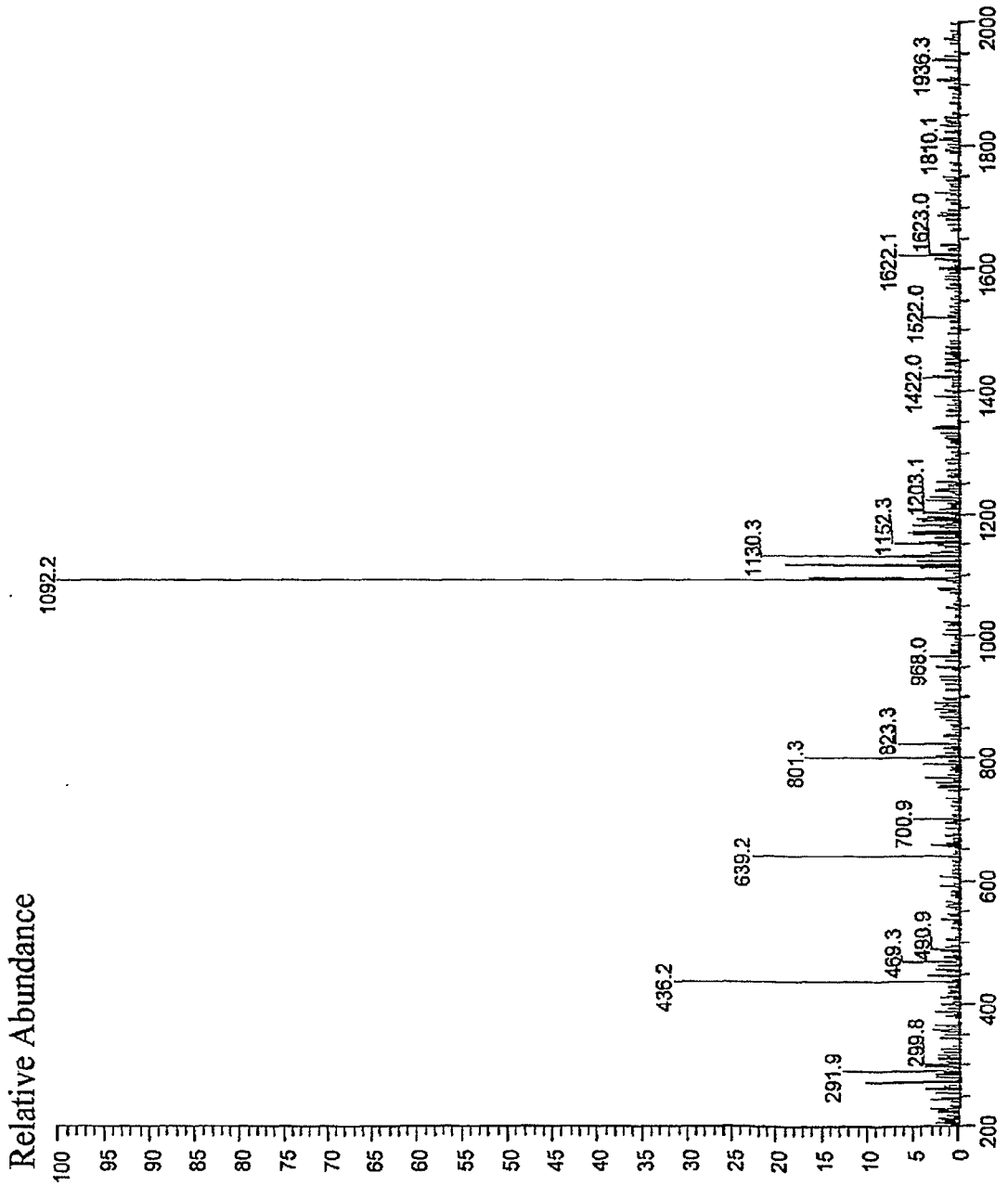

Relative Abundance
m/z
1092.2
1130.3
1152.3
1203.1
1422.0
1522.0
1622.1
1623.0
1810.1
1936.3
968.0
823.3
801.3
700.9
639.2
436.2
469.3
490.9
299.8
291.9

FIG. 64

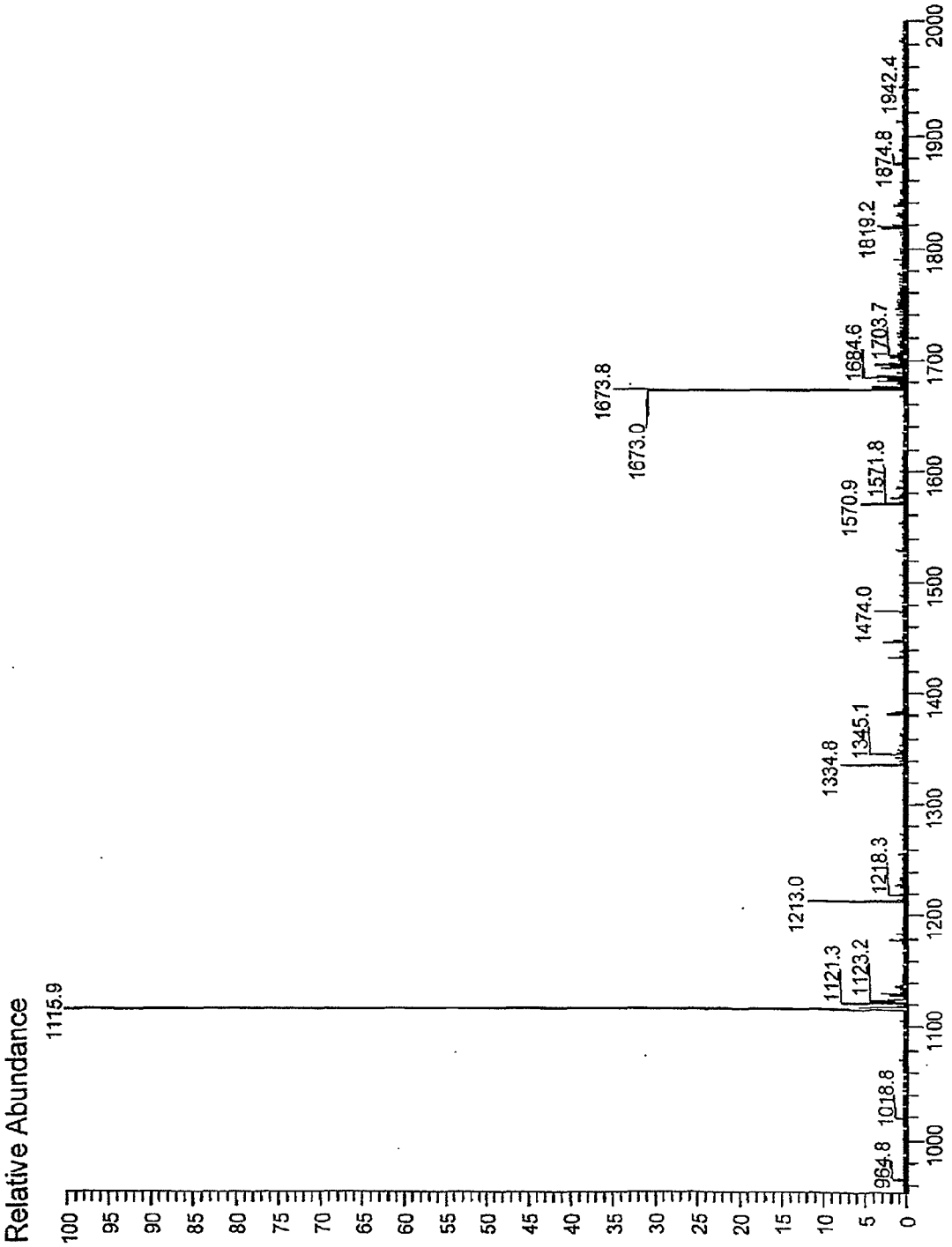

Relative Abundance
m/z
1115.9
1673.8
1673.0
1213.0
1334.8
1345.1
1474.0
1570.9
1571.8
1684.6
1703.7
1819.2
1874.8
1942.4
964.8
1018.8
1121.3
1123.2
1218.3

FIG. 65

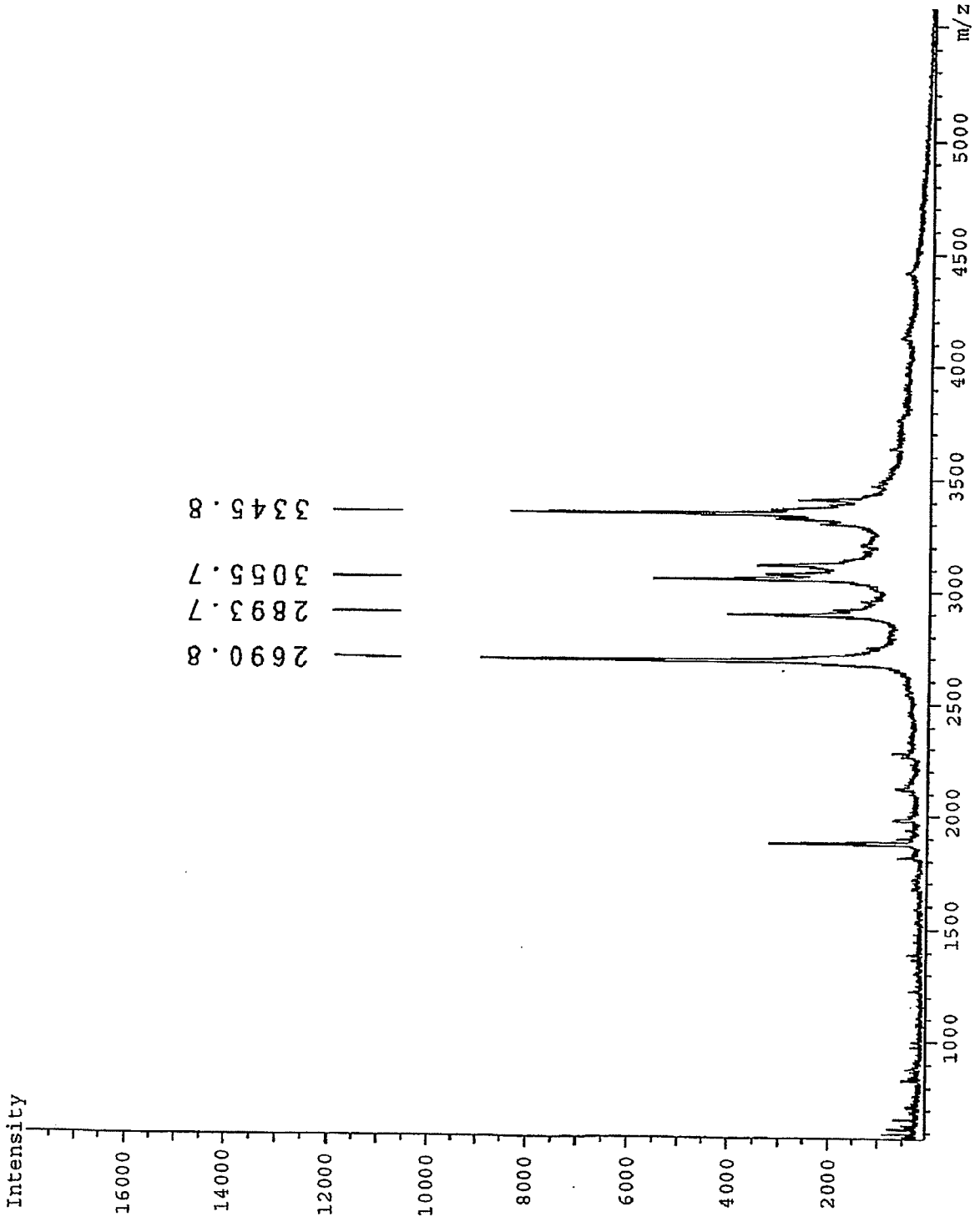

Intensity
16000
14000
12000
10000
8000
6000
4000
2000
1000
1500
2000
2500
3000
3500
4000
4500
5000
m/z
2690.8
2893.7
3055.7
3345.8

FIG. 66

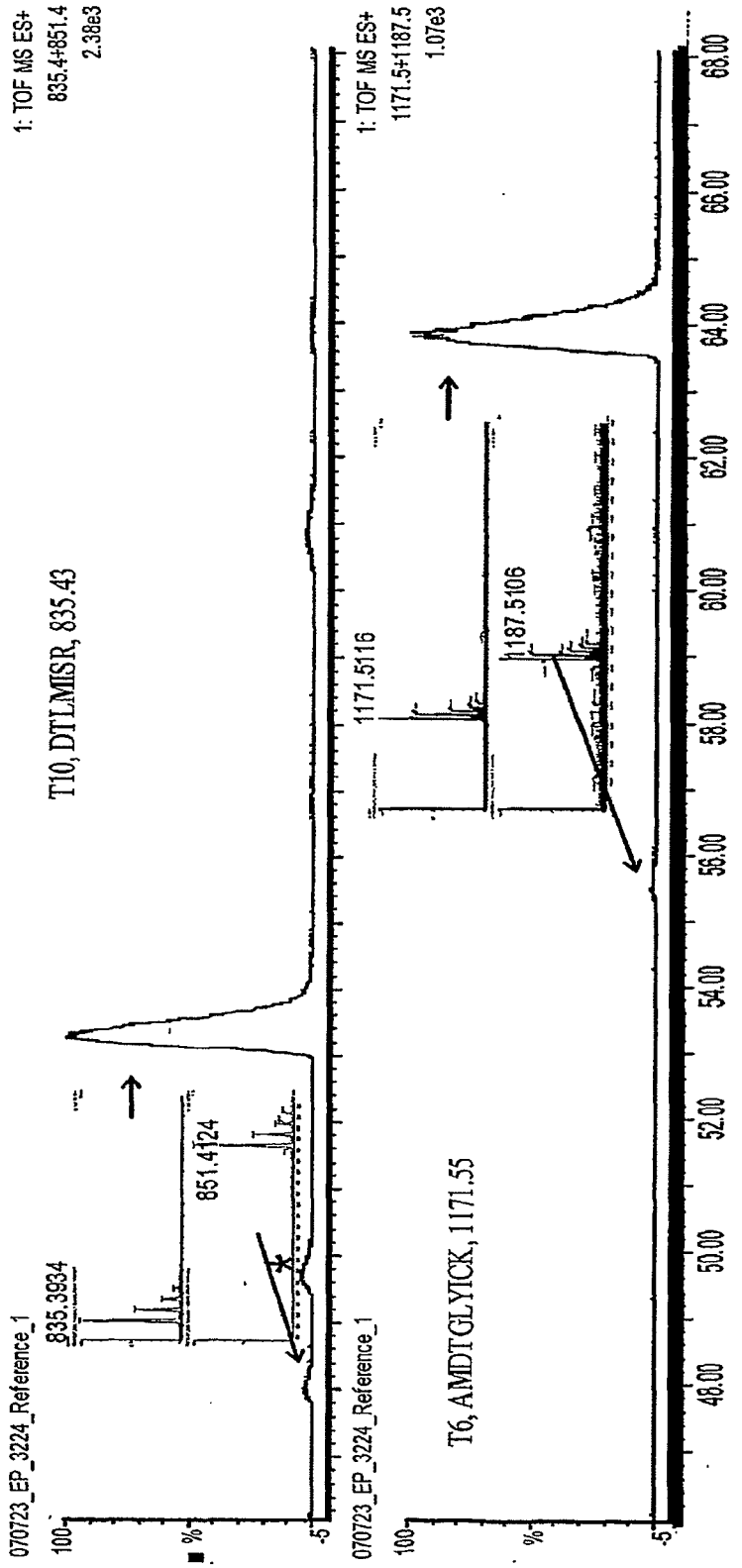

070723_EP_3224_Reference_1
100
%
-5
835.3934
851.4124
1: TOF MS ES+
835.4+851.4
2.38e3
T10, DTLMISR, 835.43
070723_EP_3224_Reference_1
100
%
-5
1171.5116
1187.5106
1: TOF MS ES+
1171.5+1187.5
1.07e3
T6, AMDTGLYICK, 1171.55
46.00
50.00
52.00
54.00
56.00
58.00
60.00
62.00
64.00
66.00
68.00
Time

FIG. 67A

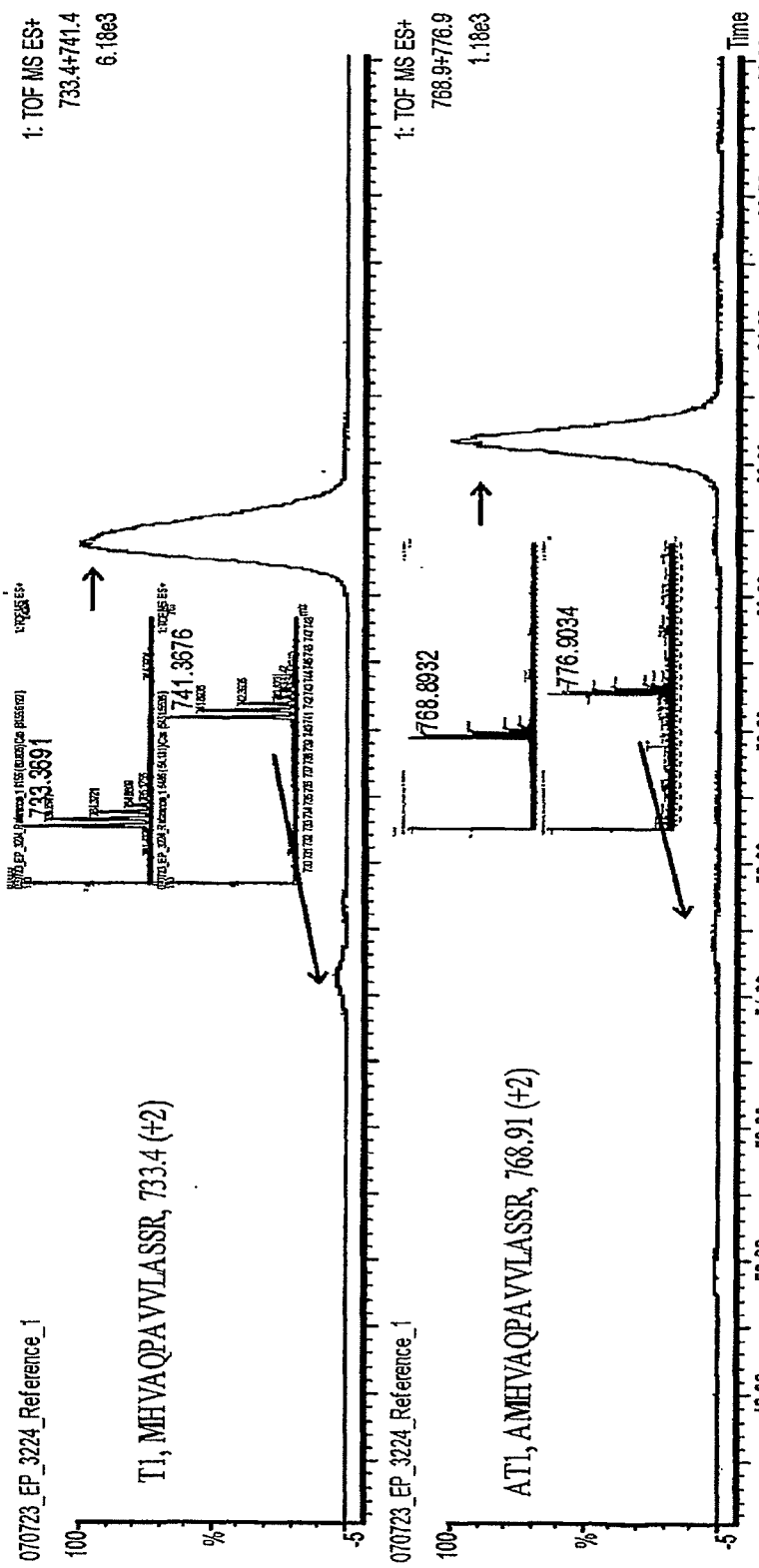

070723_EP_3224_Reference_1
100
%
-5
T1, MHVAQPAVVLASSR, 733.4 (+2)
1: TOF MS ES+
733.4+741.4
6.18e3
733.3691
741.3676
070723_EP_3224_Reference_1
100
%
-5
AT1, AMHVAQPAVVLASSR, 768.91 (+2)
1: TOF MS ES+
768.9+776.9
1.18e3
768.8932
776.9034
48.00
50.00
52.00
54.00
56.00
58.00
60.00
62.00
64.00
66.00
68.00
Time

FIG. 68

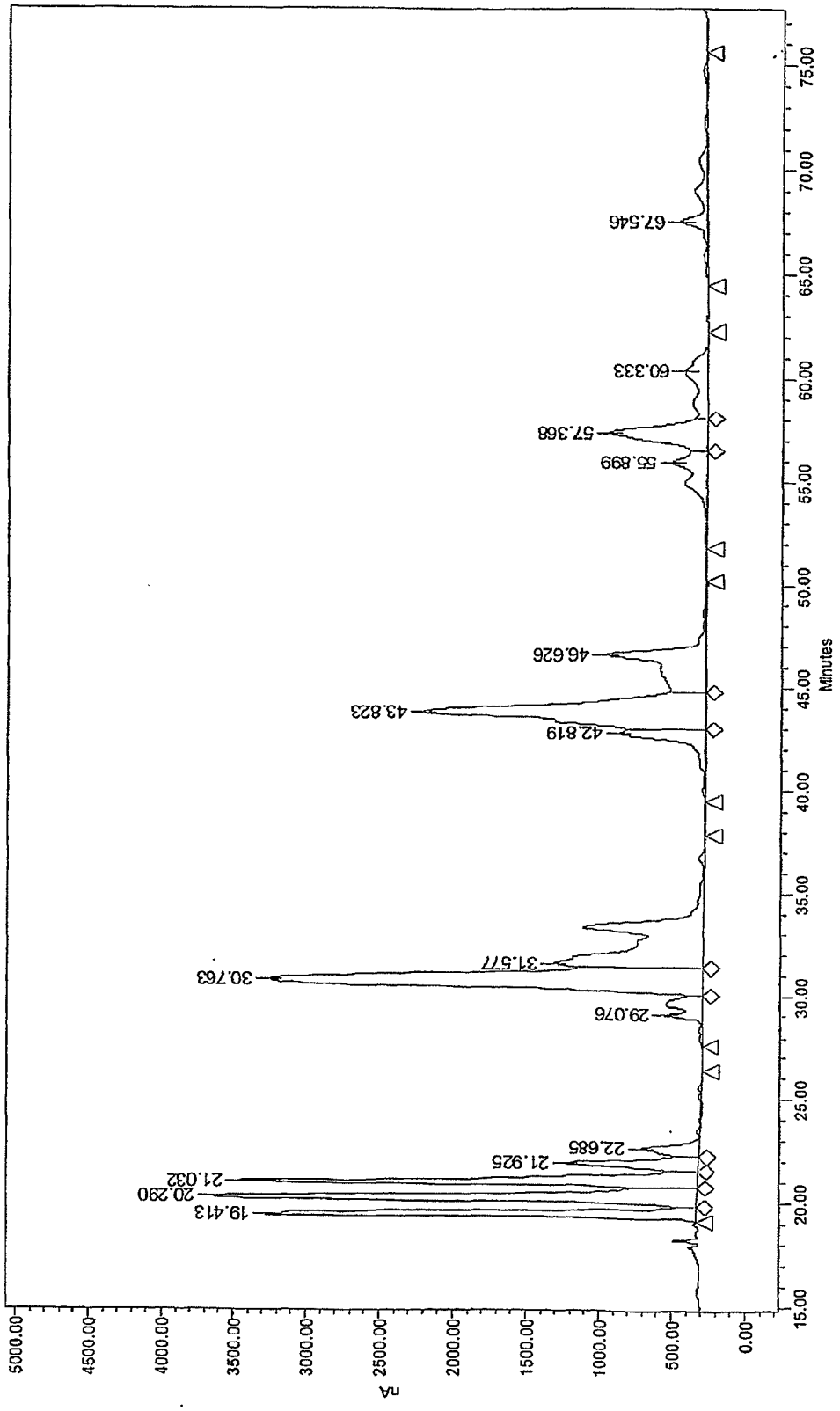
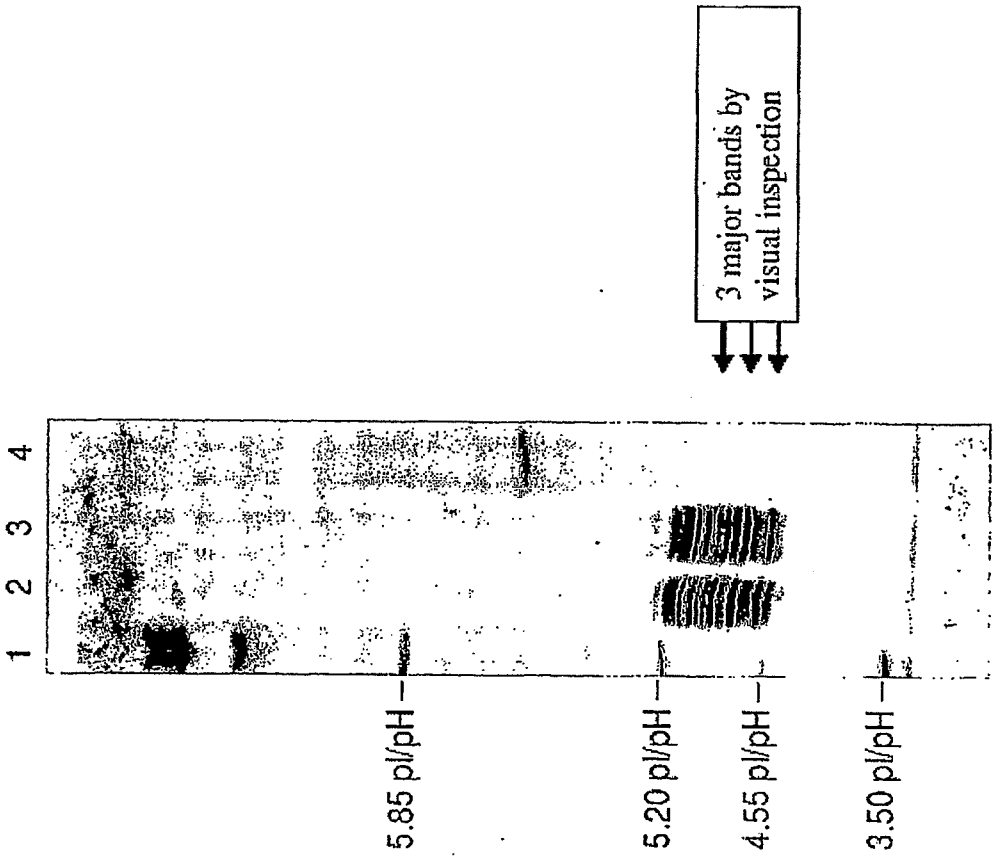

nA
5000.00
4500.00
4000.00
3500.00
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00
65.00
70.00
75.00
Minutes
19.413
20.290
21.032
21.925
22.685
29.076
30.763
31.577
42.819
43.823
46.626
55.899
57.368
60.333
67.546

FIG. 69


3 major bands by visual inspection
1 2 3 4
5.85 pI/pH
5.20 pI/pH
4.55 pI/pH
3.50 pI/pH

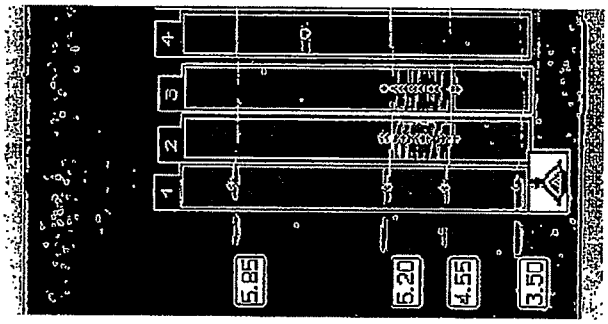


FIG. 70

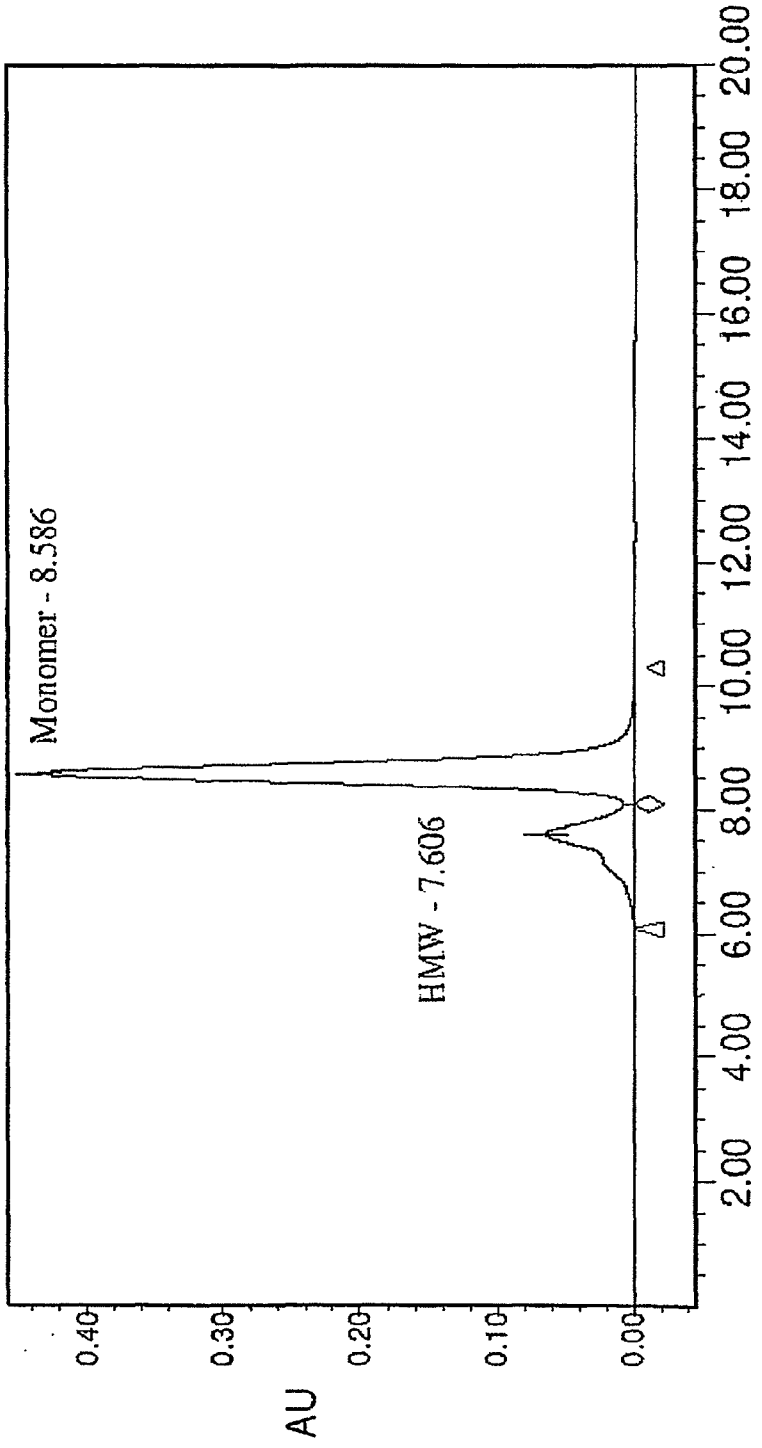

Monomer - 8.586
HMW - 7.606
AU
0.40
0.30
0.20
0.10
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Minutes

FIG. 71A

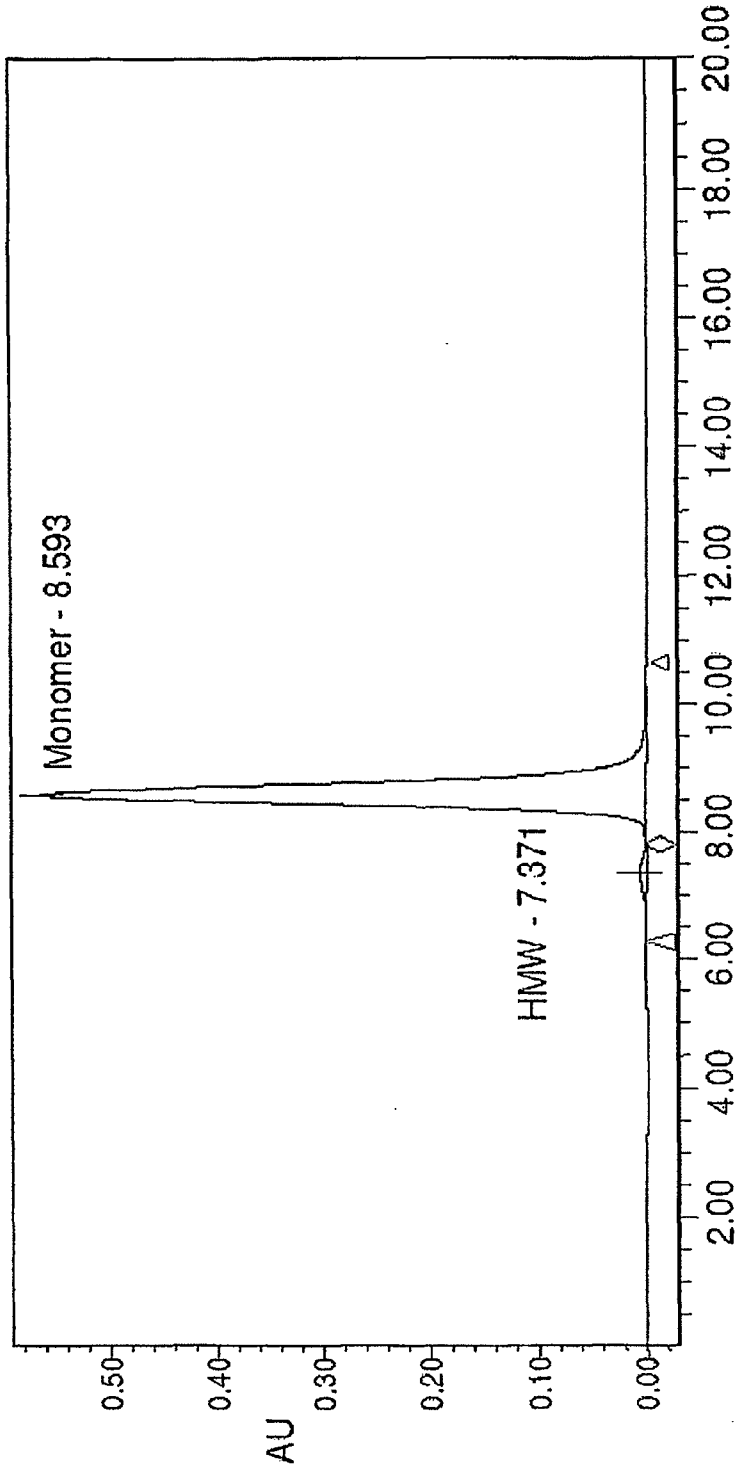

Monomer - 8.593
HMW - 7.371
AU
0.50
0.40
0.30
0.20
0.10
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Minutes

FIG. 71B

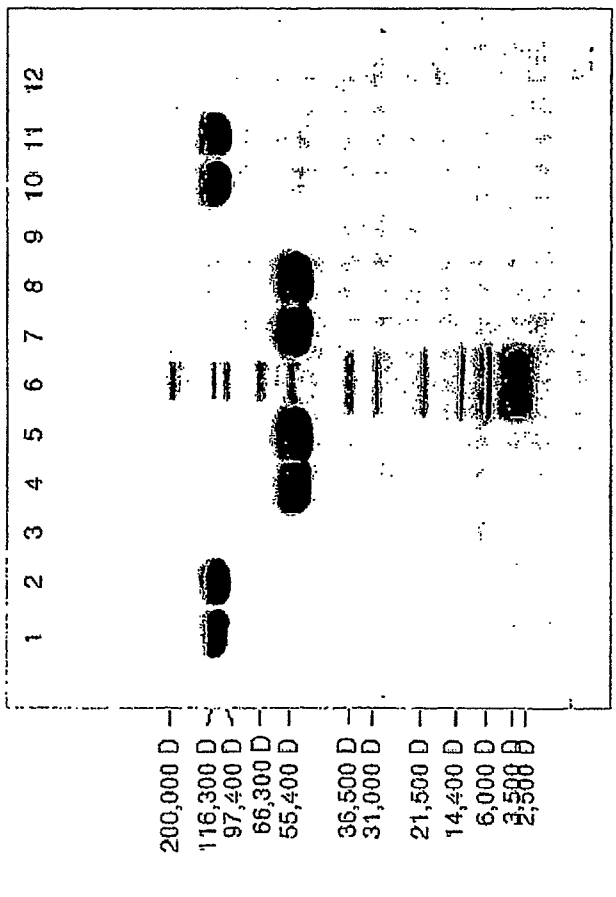

1 2 3 4 5 6 7 8 9 10 11 12
200,000 D
116,300 D
97,400 D
66,300 D
55,400 D
36,500 D
31,000 D
21,500 D
14,400 D
6,000 D
3,500 D
2,500 D

FIG. 72

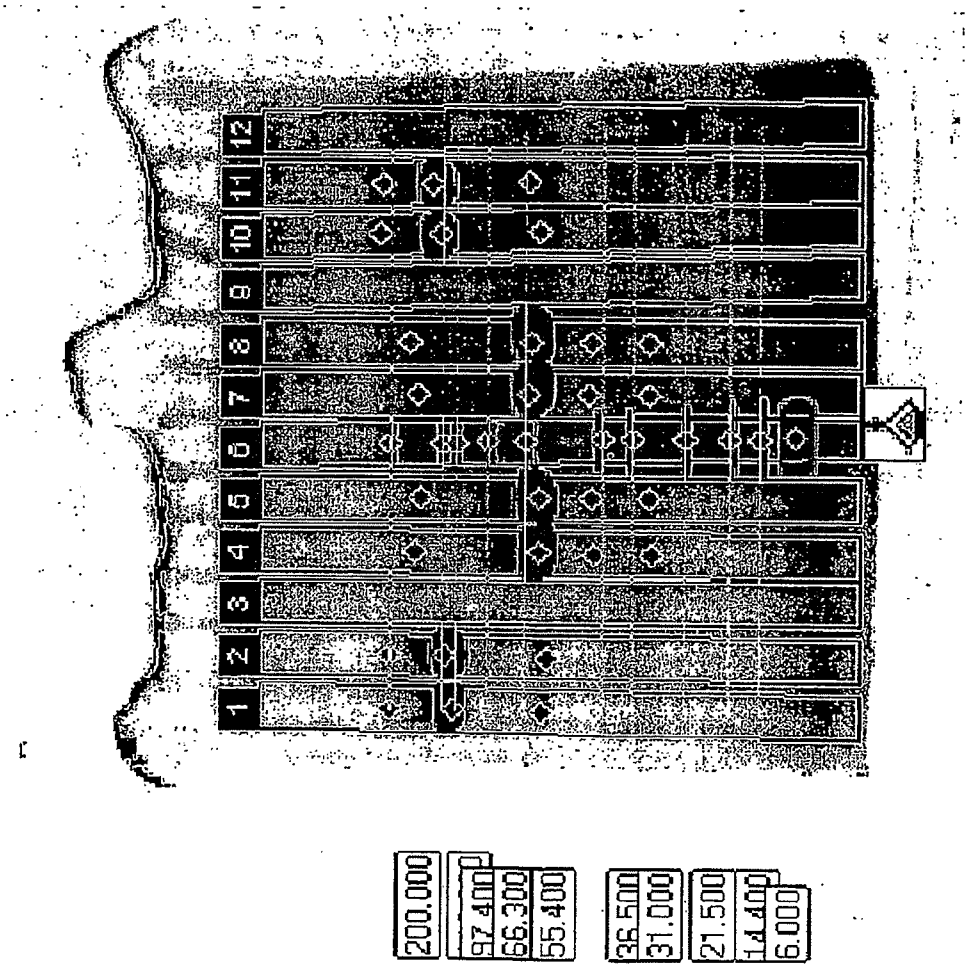

1
2
3
4
5
6
7
8
9
10
11
12
200.000
97.400
66.300
55.400
36.500
31.000
21.500
14.400
6.000

FIG. 73

H0406006/ABC04014 (NR)	Lane 1	Band	1	2	3								
	Lane 1	Band %	0	99.54	0.46								
	Lane 1	MW (kd)	200	101.62	51.99								
Ref. 188667-2003-015 (NR)	Lane 2	Band	1	2	3								
	Lane 2	Band %	0.13	98.92	0.96								
	Lane 2	MW (kd)	200	112.8	50.59								
H0406006/ABC04014 (R)	Lane 4	Band	1	2	3	4							
	Lane 4	Band %	0.02	99.9	0.01	0.07							
	Lane 4	MW (kd)	159.23	52.56	38.34	27.51							
Ref. 188667-2003-015 (R)	Lane 5	Band	1	2	3	4							
	Lane 5	Band %	0.13	99.71	0.11	0.05							
	Lane 5	MW (kd)	150	52.56	38.93	27.98							
MWM Mark 12	Lane 6	Band	1	2	3	4	5	6	7	8	9	10	11
	Lane 6	Band %	3.01	2.7	3.88	4.69	4.53	6.36	5.21	6.27	7.14	11.96	44.25
	Lane 6	MW (kd)	200	116.3	97.4	66.3	55.4	36.5	31	21.5	14.4	6	0
Ref. 188667-2003-015 (R)	Lane 7	Band	1	2	3	4							
	Lane 7	Band %	0.13	99.43	0.39	0.04							
	Lane 7	MW (kd)	153.94	54.95	39.74	27.98							
H0504028/4J88292 (R)	Lane 8	Band	1	2	3	4							
	Lane 8	Band %	0.11	99.86	0.01	0.02							
	Lane 8	MW (kd)	167.26	54.32	38.93	27.98							
Ref. 188667-2003-015 (NR)	Lane 10	Band	1	2	3								
	Lane 10	Band %	0.14	98.78	1.09								
	Lane 10	MW (kd)	217	119.86	52.56								
H0504028/4J88292 (NR)	Lane 11	Band	1	2	3								
	Lane 11	Band %	0.04	99.25	0.72								
	Lane 11	MW (kd)	213.08	133.33	54.95								

FIG. 74

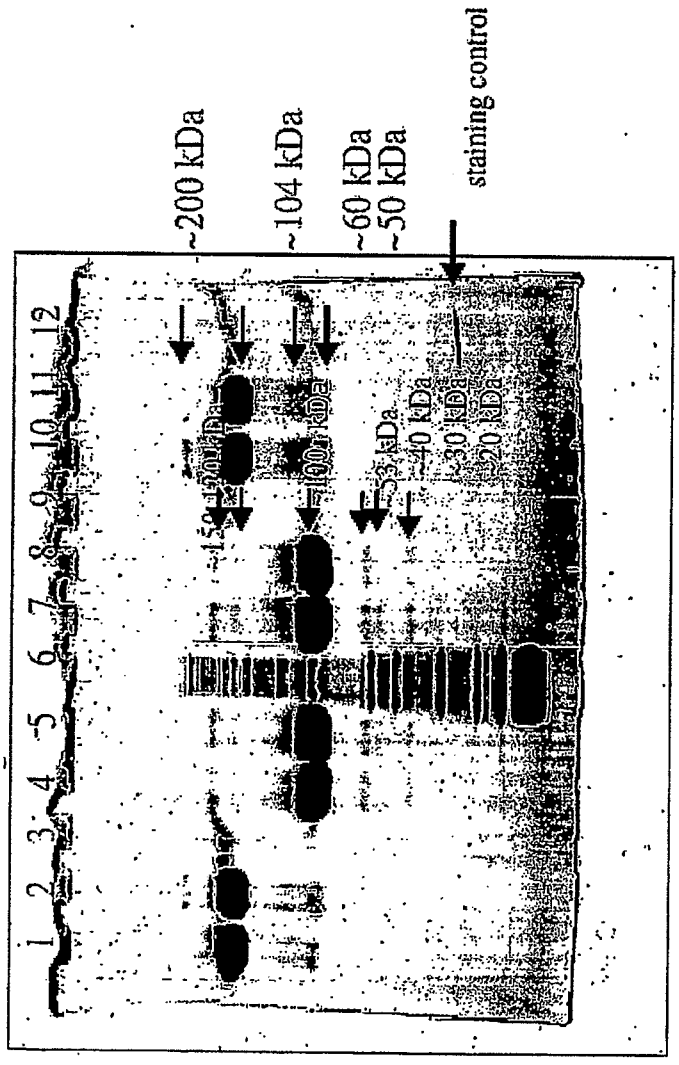

~200 kDa
~104 kDa
~60 kDa
~50 kDa
staining control
1 2 3 4 5 6 7 8 9 10 11 12
~150 kDa
~100 kDa
~53 kDa
~40 kDa
~30 kDa
~20 kDa

FIG. 75

FIG. 76

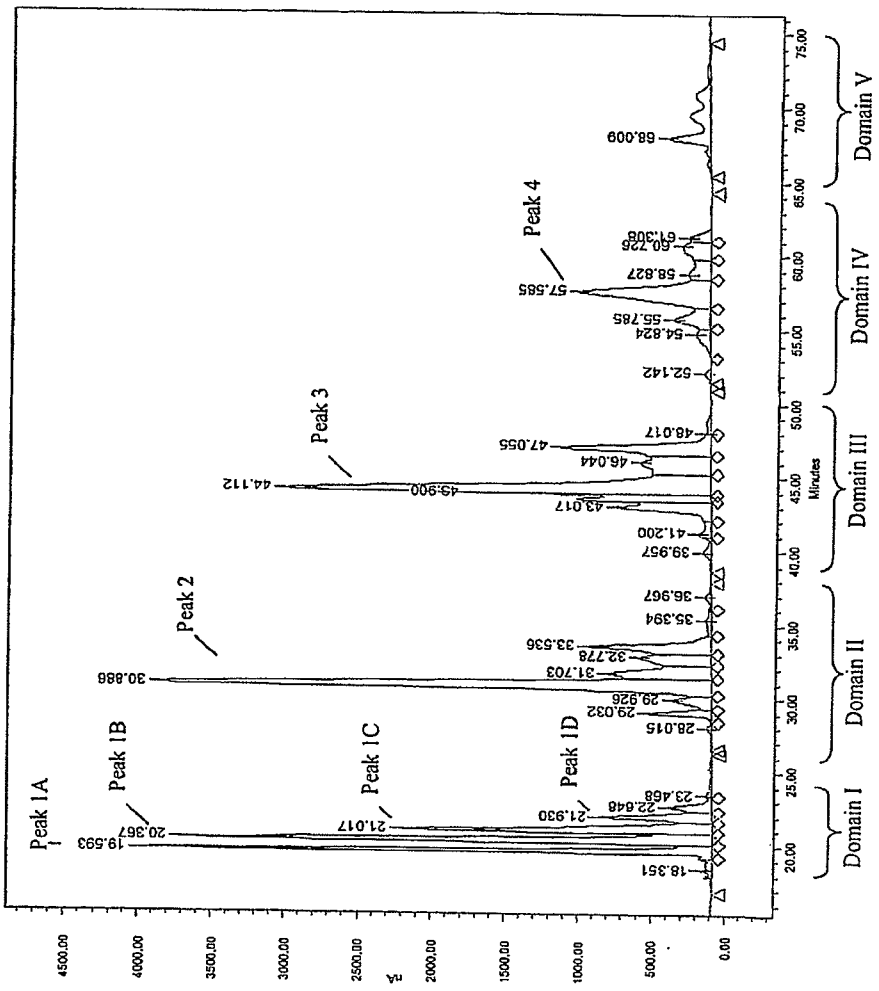
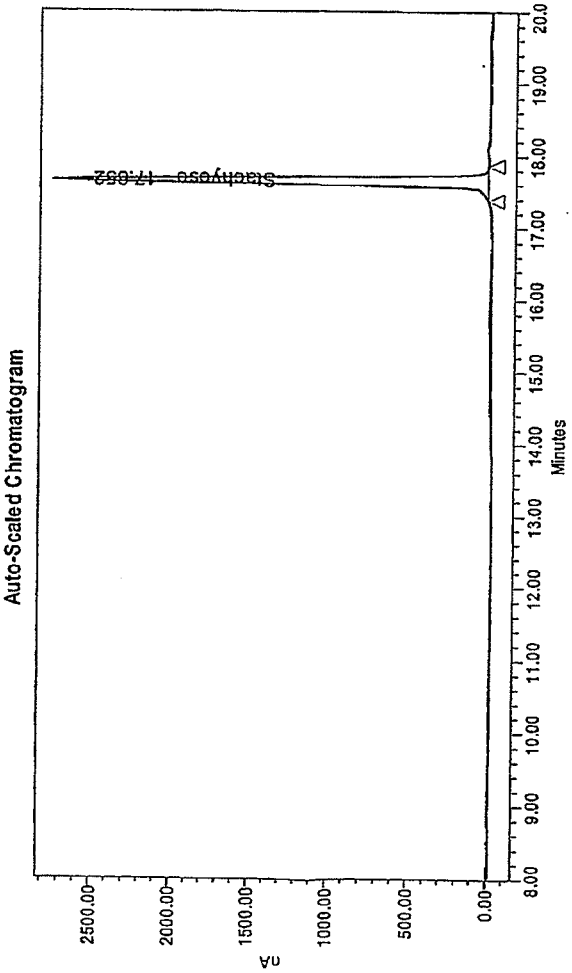

Peak 1A
Peak 1B
Peak 1C
Peak 1D
Peak 2
Peak 3
Peak 4
Domain I
Domain II
Domain III
Domain IV
Domain V
Minutes
nA
18.351
19.593
20.367
21.017
21.930
22.648
23.468
28.015
29.032
29.926
30.888
31.703
32.778
33.536
35.394
36.967
39.957
41.200
43.017
44.112
45.900
46.044
47.055
48.017
52.142
54.824
55.785
57.585
58.827
60.726
61.308
68.009

Fig. 77



Peak Results

	Name	RT	Area	Height	USP Plate Count	USP Tailing
1	Stachyose	17.652	3250362	534326	189613	1.01

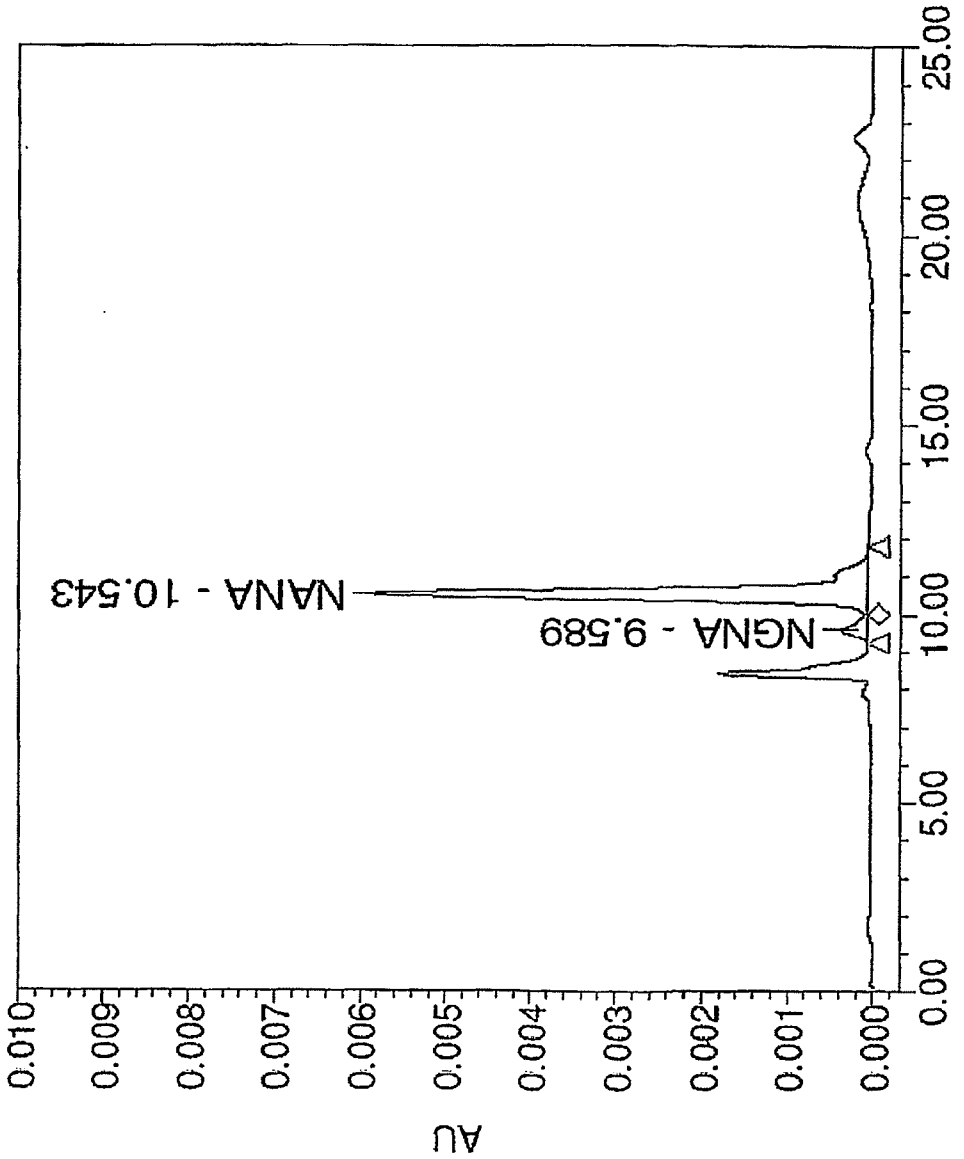

AU
0.010
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
0.00
5.00
10.00
15.00
20.00
25.00
Minutes
NGNA - 9.589
NANA - 10.543

FIG. 78

Fig. 79

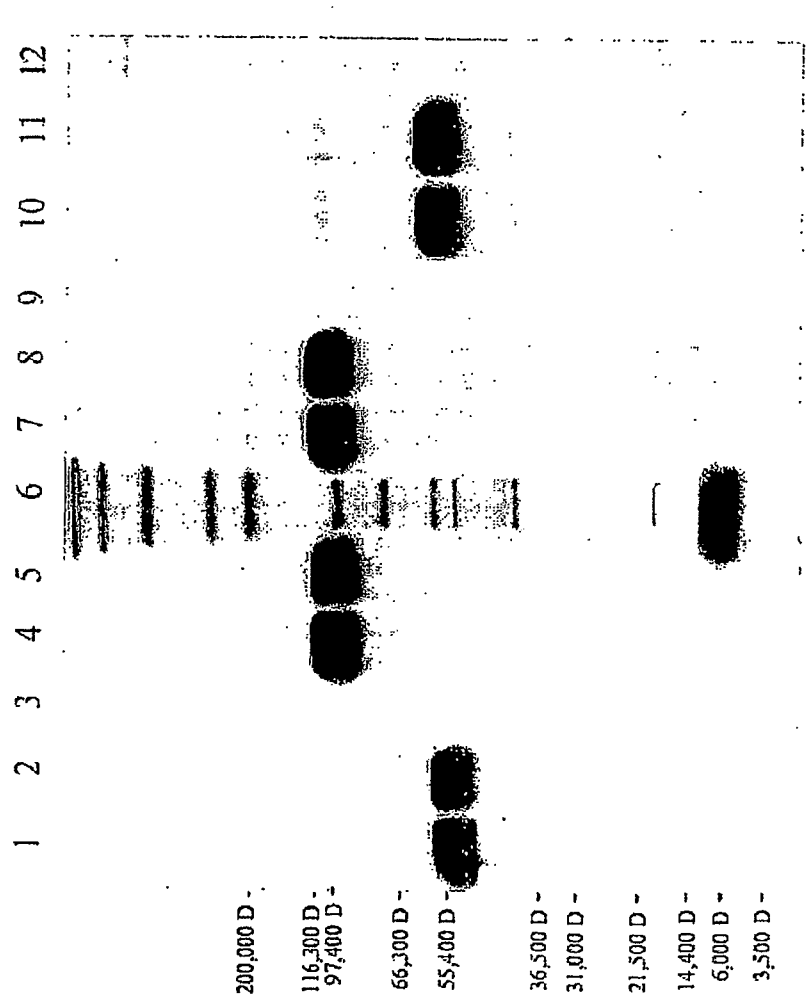

1 2 3 4 5 6 7 8 9 10 11 12
200,000 D -
116,300 D -
97,400 D -
66,300 D -
55,400 D -
36,500 D -
31,000 D -
21,500 D -
14,400 D -
6,000 D -
3,500 D -

Fig. 80

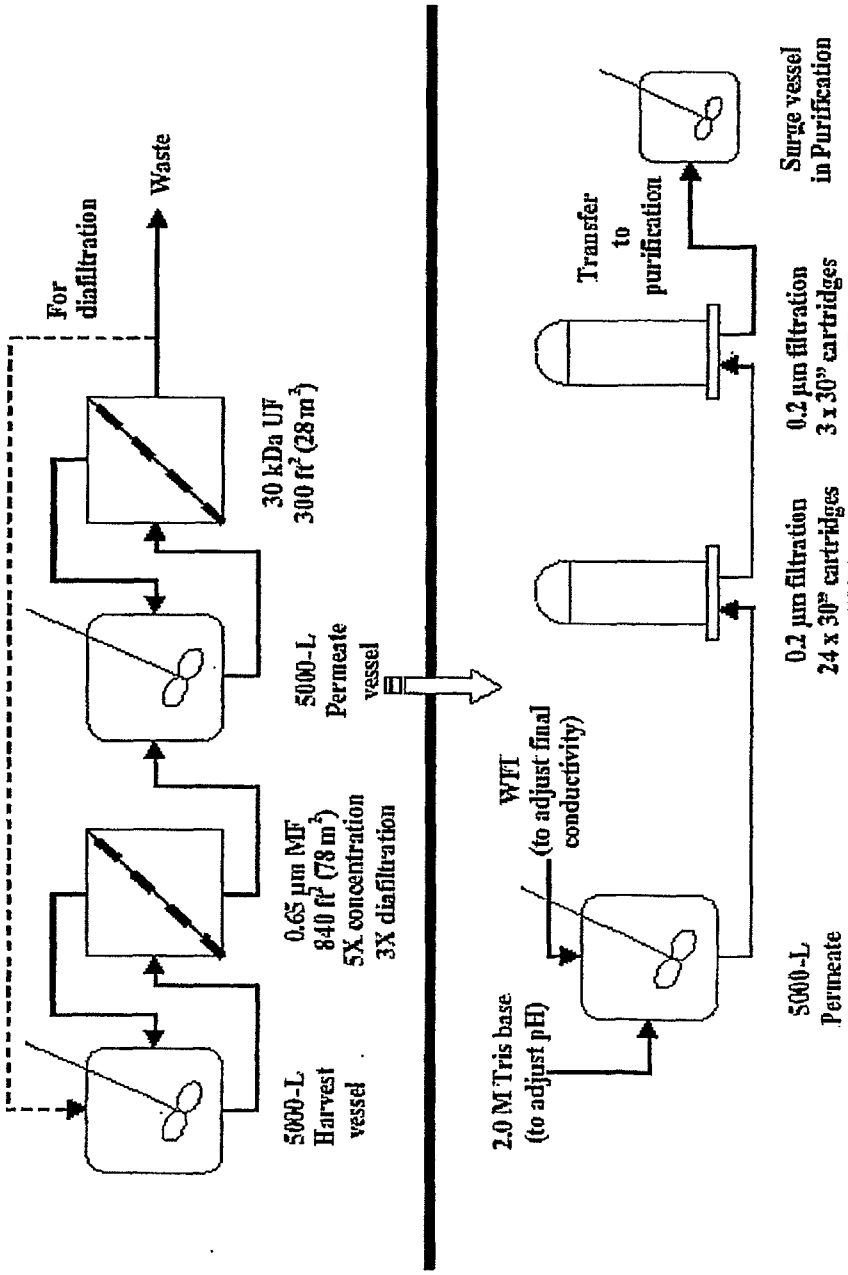
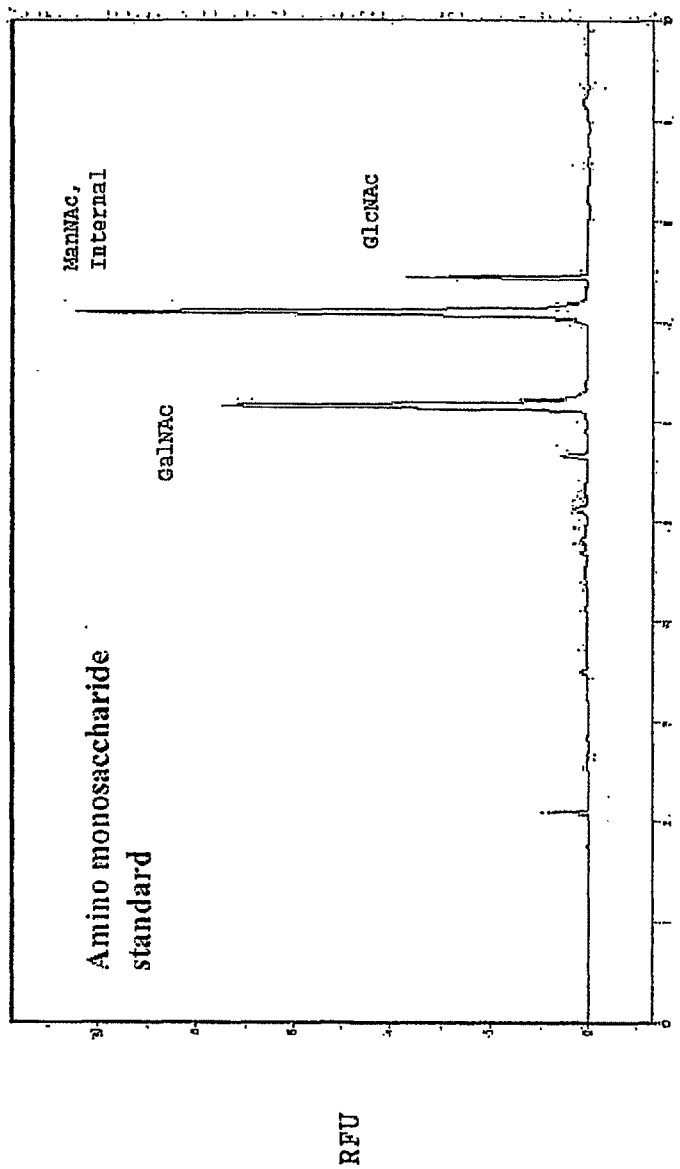
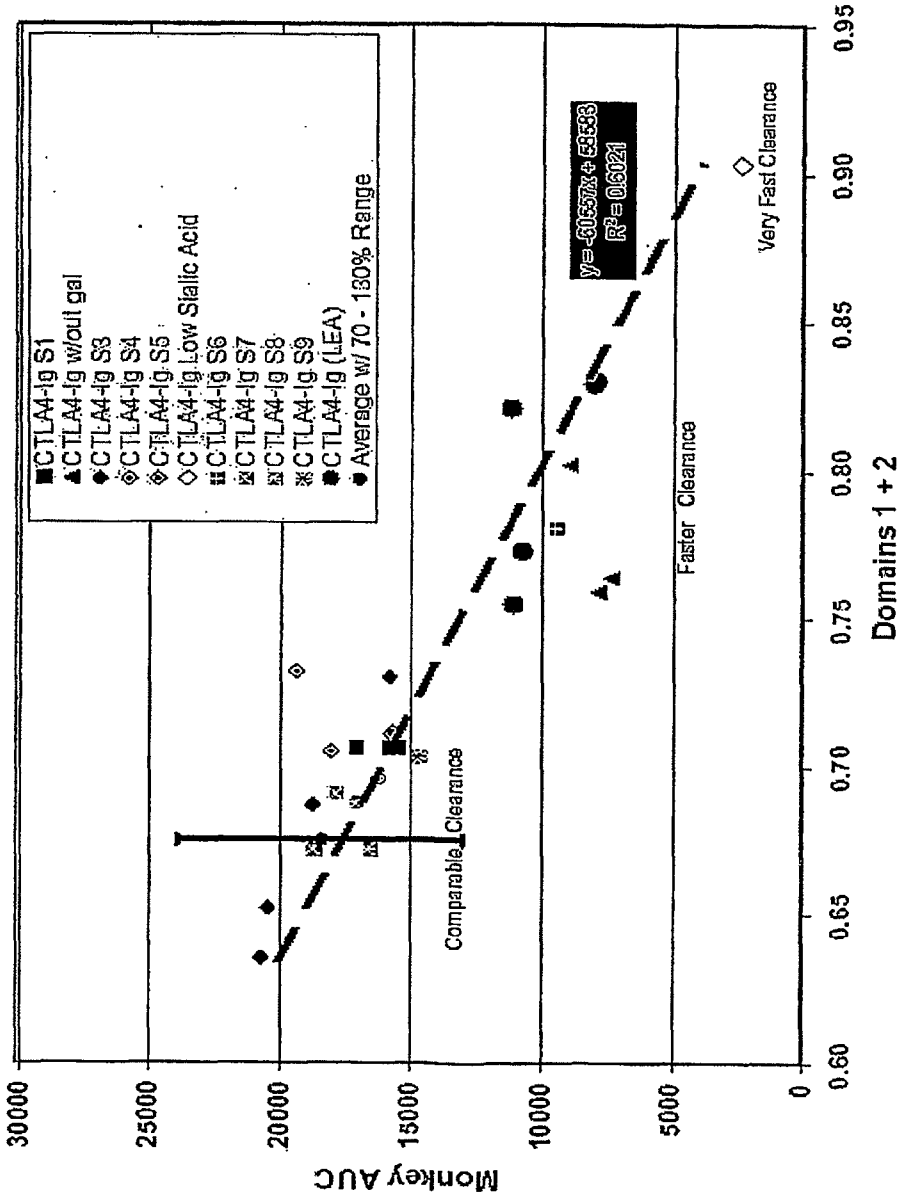

5000-L Harvest vessel
0.65 µm MF 840 ft² (78 m²) 5X concentration 3X diafiltration
5000-L Permeate vessel
30 kDa UF 300 ft² (28 m²)
For diafiltration
Waste
2.0 M Tris base (to adjust pH)
WFI (to adjust final conductivity)
5000-L Permeate vessel
0.2 µm filtration 24 x 30" cartridges PVDF
0.2 µm filtration 3 x 30" cartridges PVDF
Transfer to purification
Surge vessel in Purification

FIG. 81



Note: Relative Fluoresence Units (RFU)

FIG. 82


CTLA4-Ig S1
CTLA4-Ig w/out gal
CTLA4-Ig S3
CTLA4-Ig S4
CTLA4-Ig S5
CTLA4-Ig Low Sialic Acid
CTLA4-Ig S6
CTLA4-Ig S7
CTLA4-Ig S8
CTLA4-Ig S9
CTLA4-Ig (LEA)
Average w/ 70 - 130% Range
y = -60657x + 58583
R² = 0.6021
Comparable Clearance
Faster Clearance
Very Fast Clearance
Monkey AUC
Domains 1 + 2
30000
25000
20000
15000
10000
5000
0
0.60
0.65
0.70
0.75
0.80
0.85
0.90
0.95

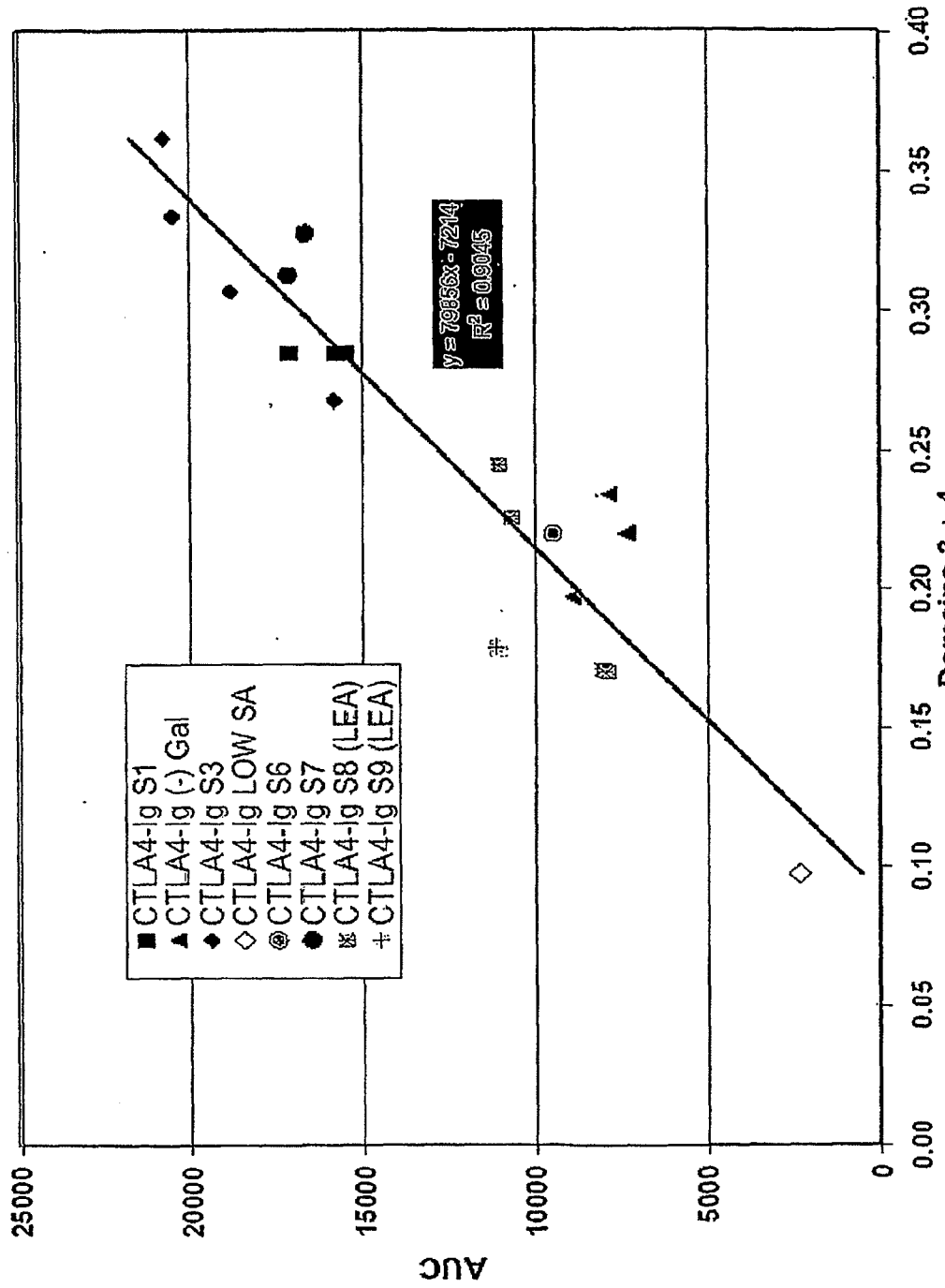

AUC
Domains 3 + 4
y = 79856x - 7214
R² = 0.9045
CTLA4-Ig S1
CTLA4-Ig (-) Gal
CTLA4-Ig S3
CTLA4-Ig LOW SA
CTLA4-Ig S6
CTLA4-Ig S7
CTLA4-Ig S8 (LEA)
CTLA4-Ig S9 (LEA)
25000
20000
15000
10000
5000
0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40

Fig. 83

Fig. 84

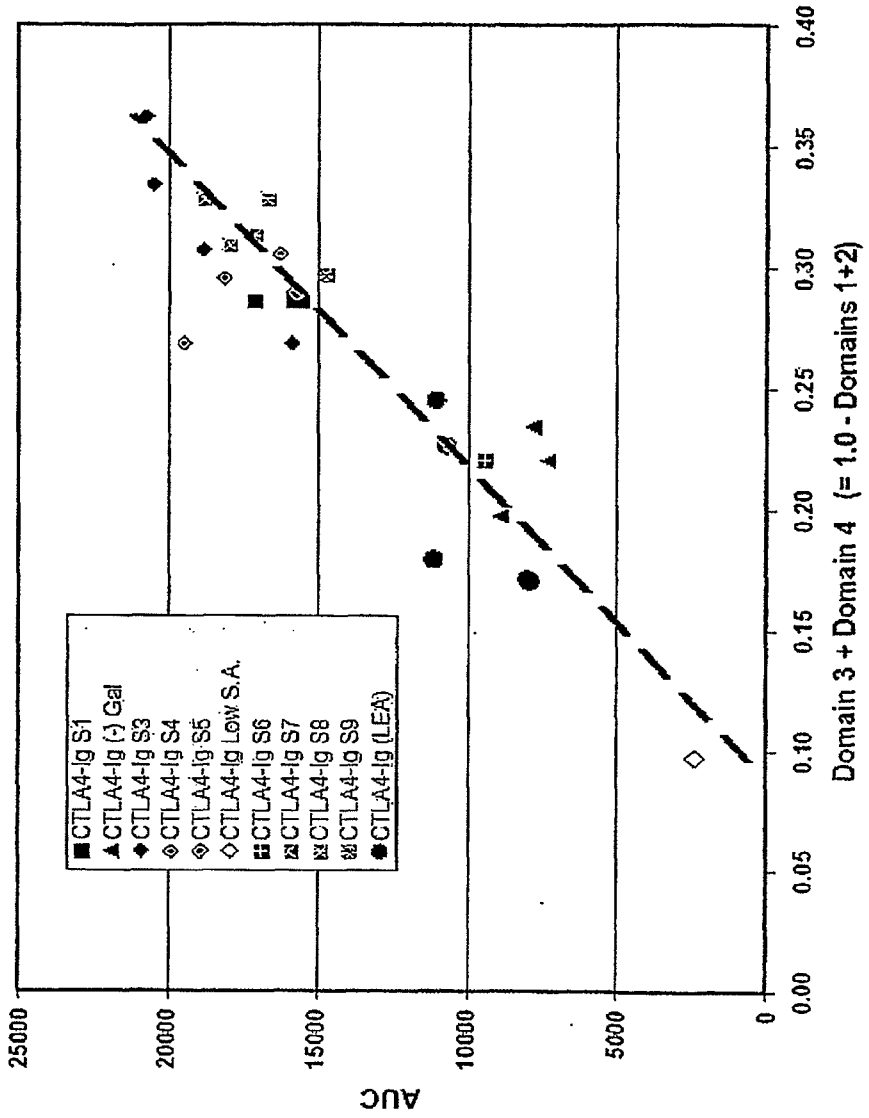

AUC
25000
20000
15000
10000
5000
0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
Domain 3 + Domain 4 (= 1.0 - Domains 1+2)
CTLA4-Ig S1
CTLA4-Ig (-) Gal
CTLA4-Ig S3
CTLA4-Ig S4
CTLA4-Ig S5
CTLA4-Ig Low S.A.
CTLA4-Ig S6
CTLA4-Ig S7
CTLA4-Ig S8
CTLA4-Ig S9
CTLA4-Ig (LEA)

Fig. 85

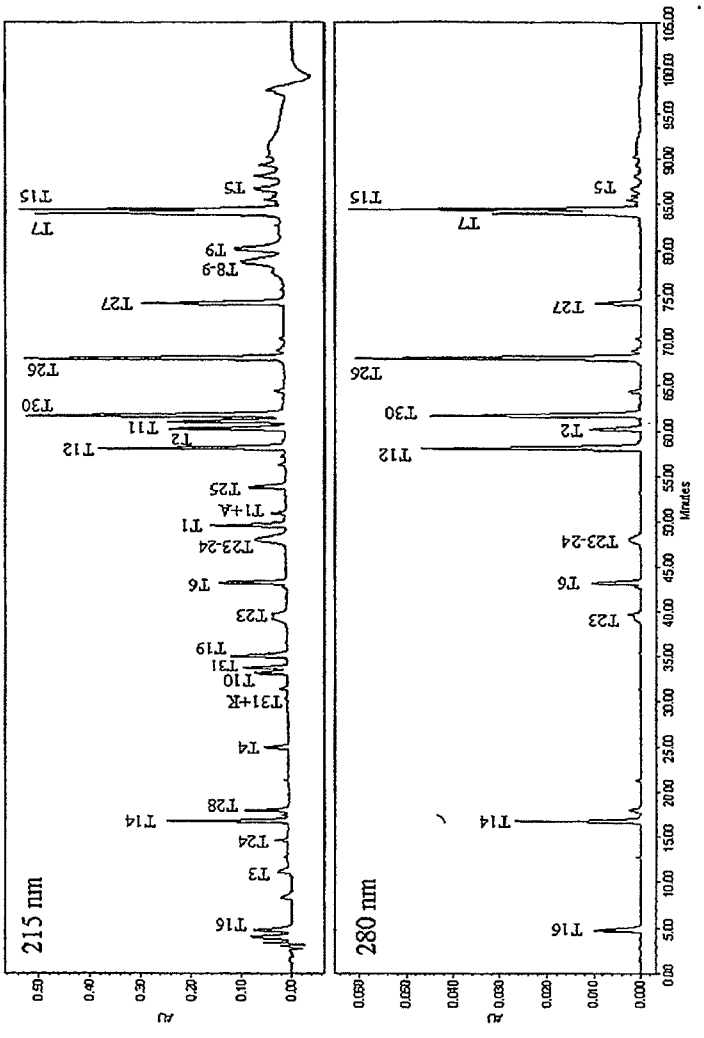

215 nm
280 nm
AU
Minutes
T16
T3
T24
T14
T28
T4
T31+K
T10
T31
T19
T23
T6
T23-24
T1
T1+A
T25
T12
T2
T11
T30
T26
T27
T8-9
T9
T7
T15
T5

Fig. 86

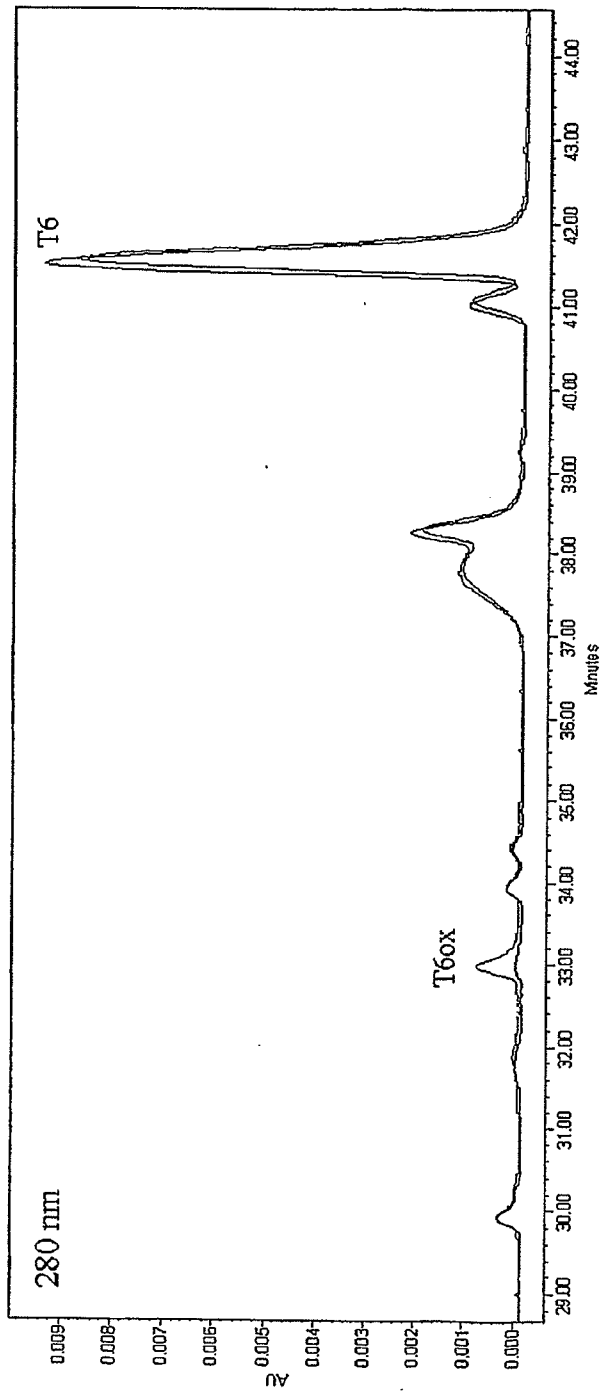
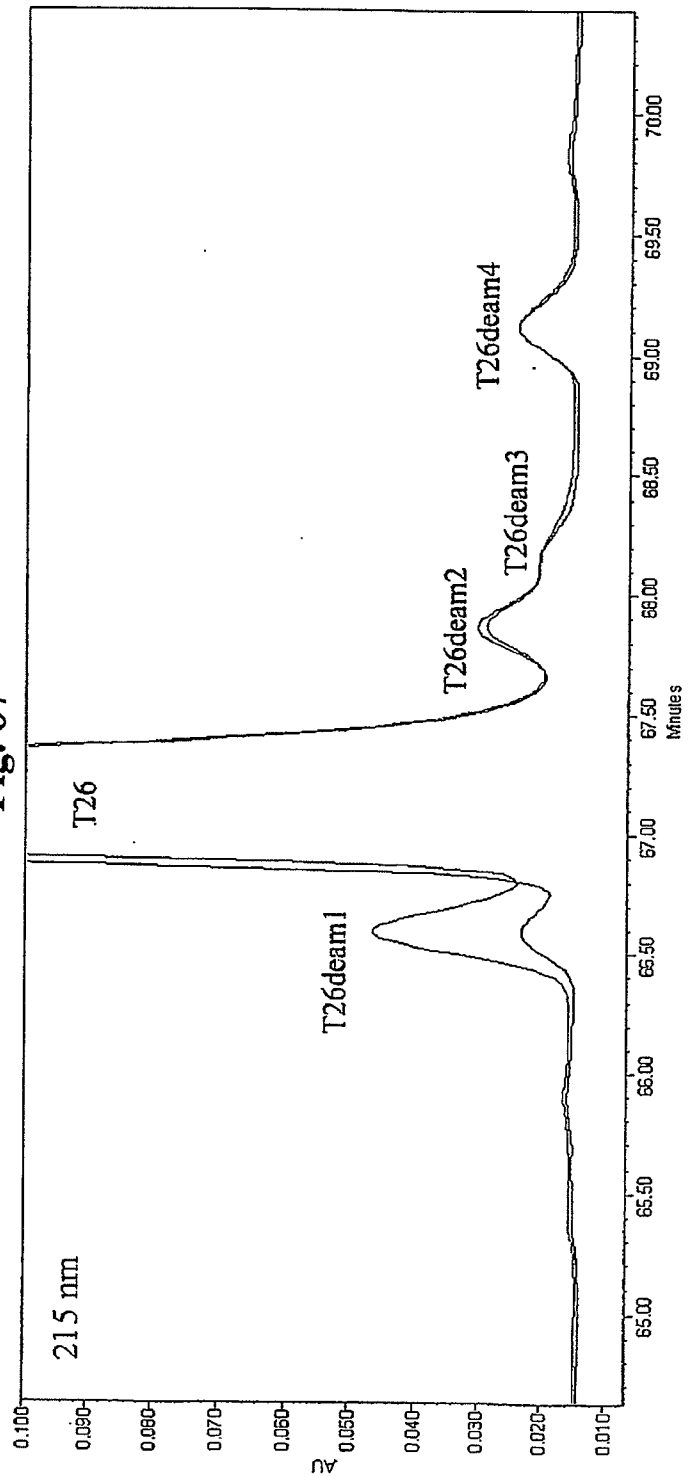

280 nm
T6
T6ox
AU
Minutes
0.000
0.001
0.002
0.003
0.004
0.005
0.006
0.007
0.008
0.009
29.00
30.00
31.00
32.00
33.00
34.00
35.00
36.00
37.00
38.00
39.00
40.00
41.00
42.00
43.00
44.00

Fig. 87


215 nm
T26
T26deam1
T26deam2
T26deam3
T26deam4
AU
Minutes
0.100
0.090
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
65.00
65.50
66.00
66.50
67.00
67.50
68.00
68.50
69.00
69.50
70.00