G00002

163163

FIG. 1

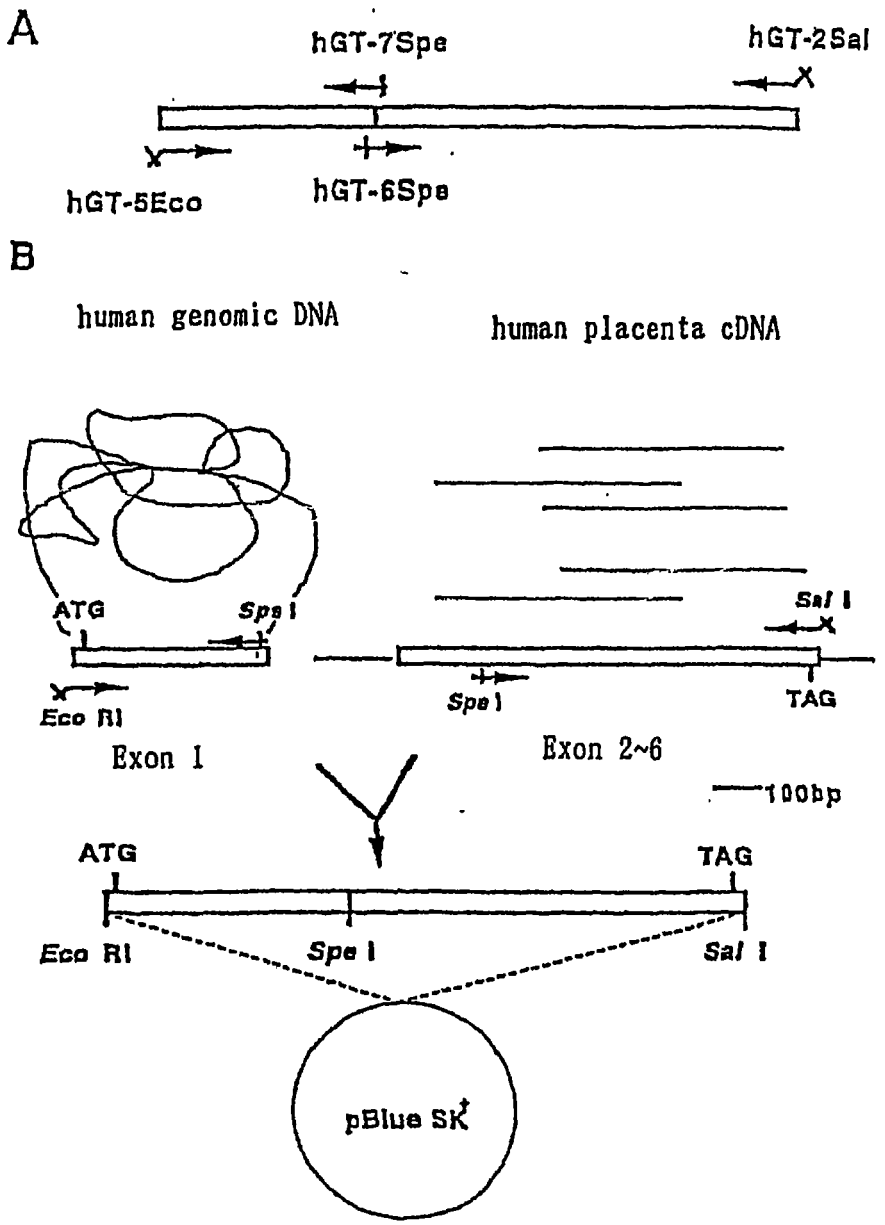


FIG. 2

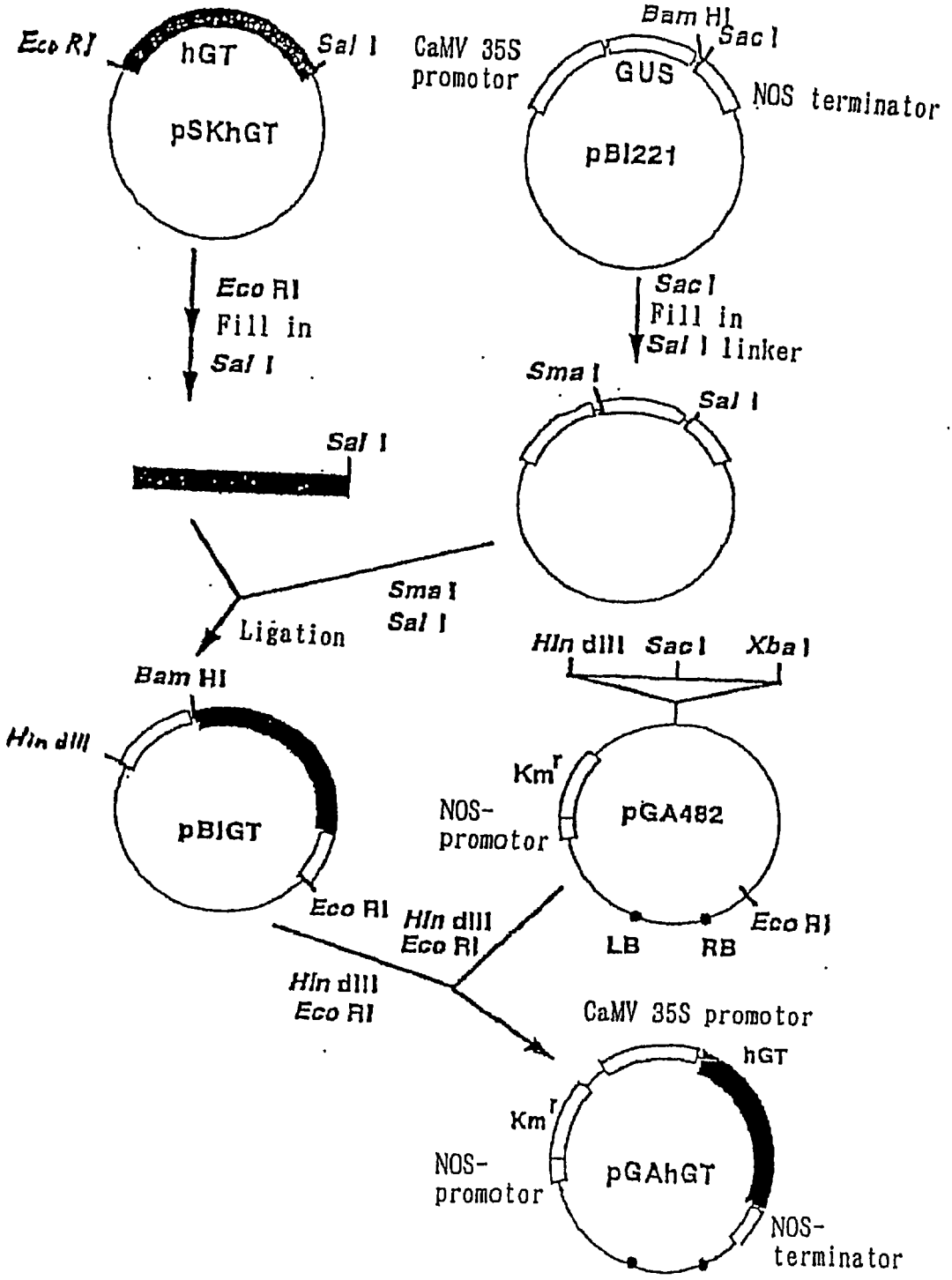

Eco RI
hGT
Sal I
pSKhGT
CaMV 35S promotor
Bam HI
Sac I
GUS
NOS terminator
pBI221
Eco RI
Fill in
Sal I
Sac I
Fill in
Sal I linker
Sma I
Sal I
Sal I
Sma I
Sal I
Ligation
Bam HI
Hin dIII
pBIGT
Eco RI
Hin dIII
Sac I
Xba I
Km r
NOS-
promotor
pGA482
Eco RI
LB
RB
Hin dIII
Eco RI
Hin dIII
Eco RI
CaMV 35S promotor
hGT
Km r
NOS-
promotor
pGAhGT
NOS-
terminator

FIG. 4A

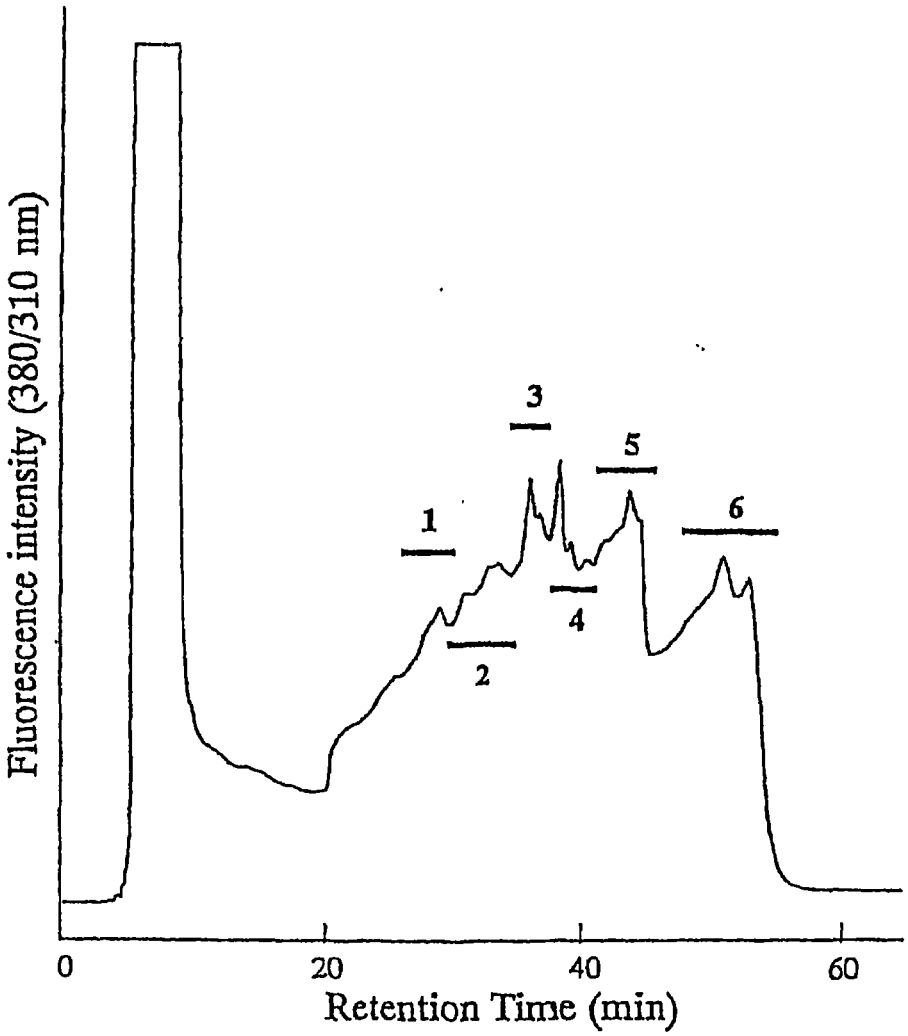

Fluorescence intensity (380/310 nm)
1
2
3
4
5
6
0
20
40
60
Retention Time (min)

FIG. 4B

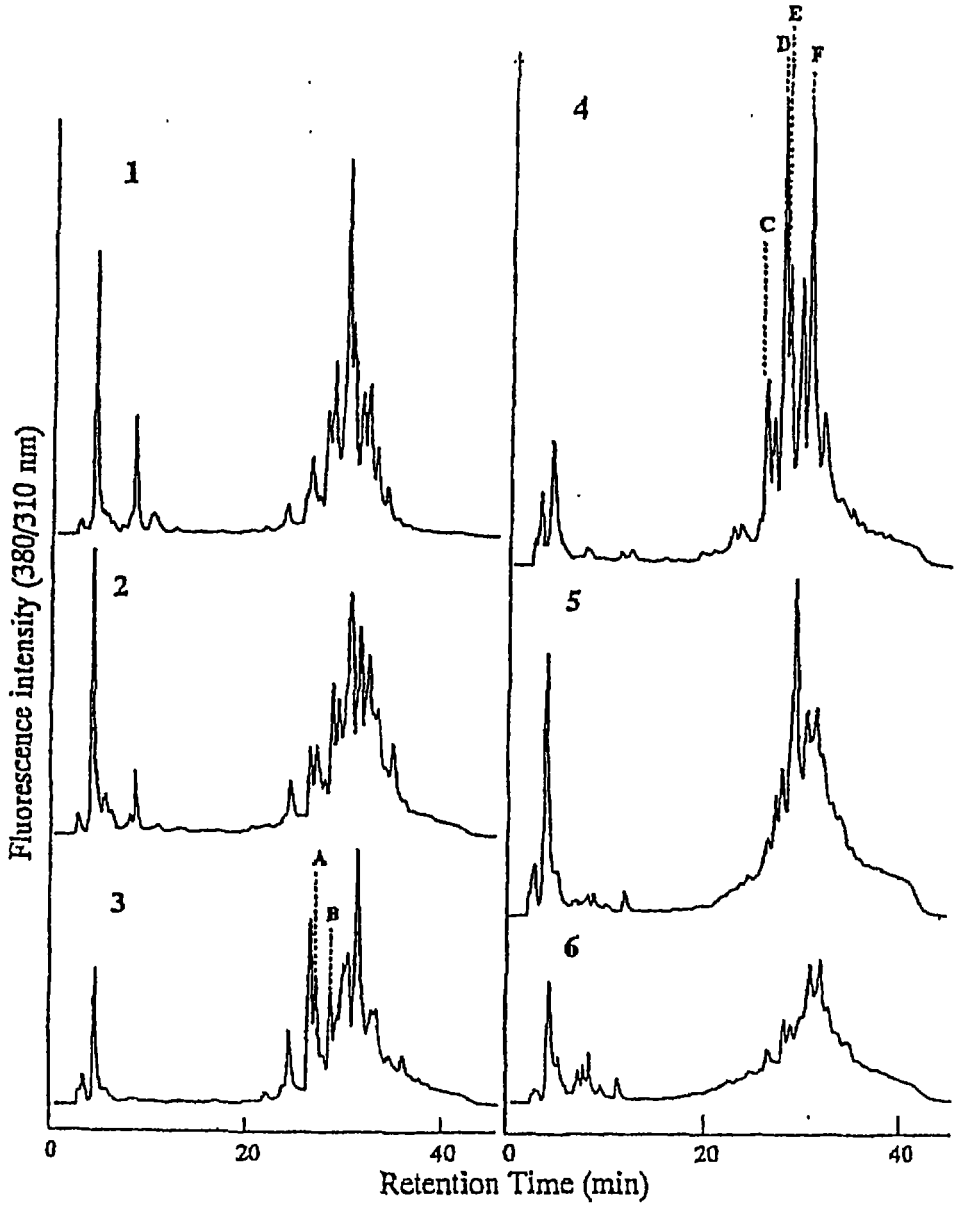

Fluorescence intensity (380/310 nm)
1
2
3
4
5
6
A
B
C
D
E
F
0
20
40
0
20
40
Retention Time (min)

FIG. 5

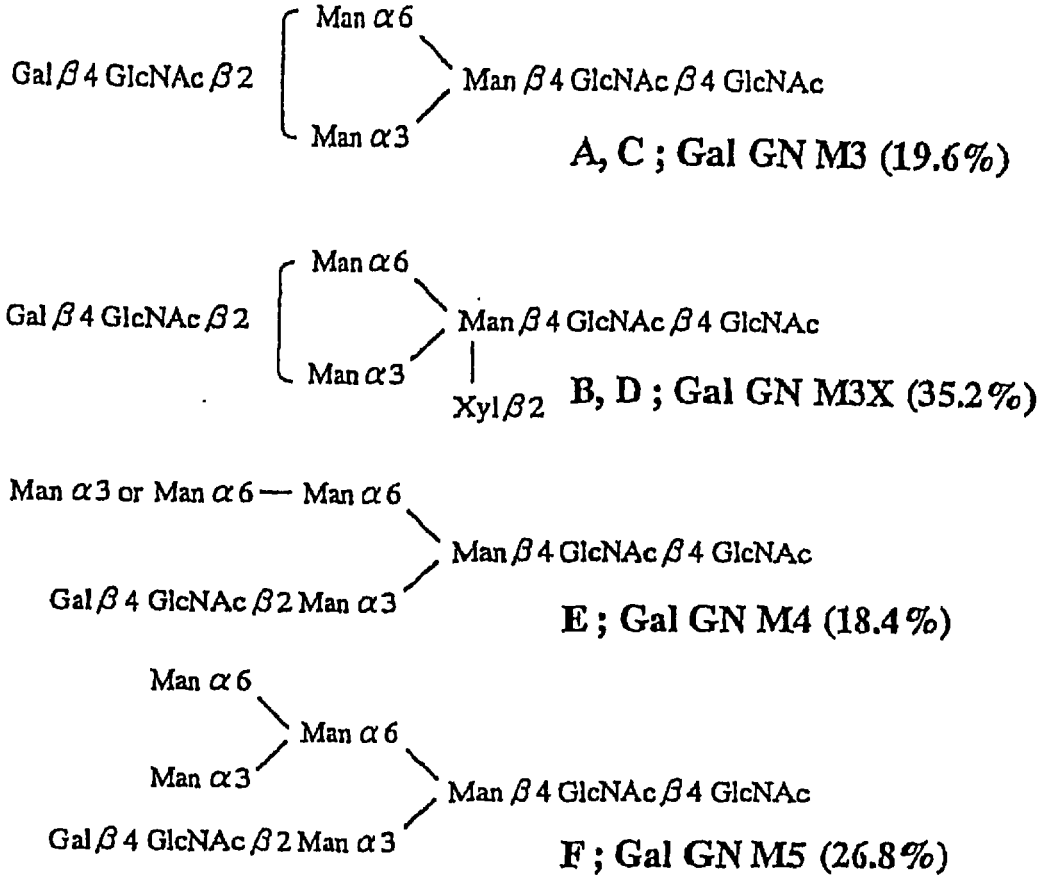

Gal β 4 GlcNAc β 2
Man α 6
Man α 3
Man β 4 GlcNAc β 4 GlcNAc
A, C ; Gal GN M3 (19.6%)
Gal β 4 GlcNAc β 2
Man α 6
Man α 3
Man β 4 GlcNAc β 4 GlcNAc
Xyl β 2
B, D ; Gal GN M3X (35.2%)
Man α 3 or Man α 6 — Man α 6
Gal β 4 GlcNAc β 2 Man α 3
Man β 4 GlcNAc β 4 GlcNAc
E ; Gal GN M4 (18.4%)
Man α 6
Man α 6
Man α 3
Gal β 4 GlcNAc β 2 Man α 3
Man β 4 GlcNAc β 4 GlcNAc
F ; Gal GN M5 (26.8%)

FIG. 6

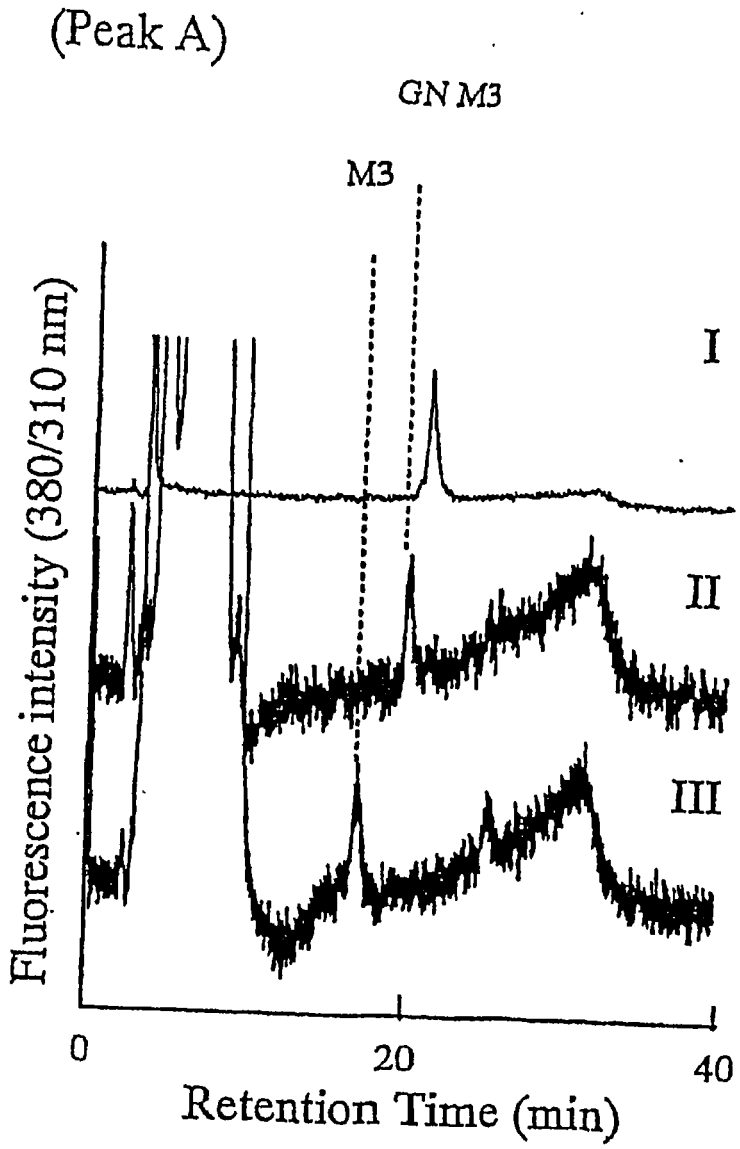

(Peak A)
GN M3
M3
I
II
III
Fluorescence intensity (380/310 nm)
0
20
40
Retention Time (min)

FIG. 7

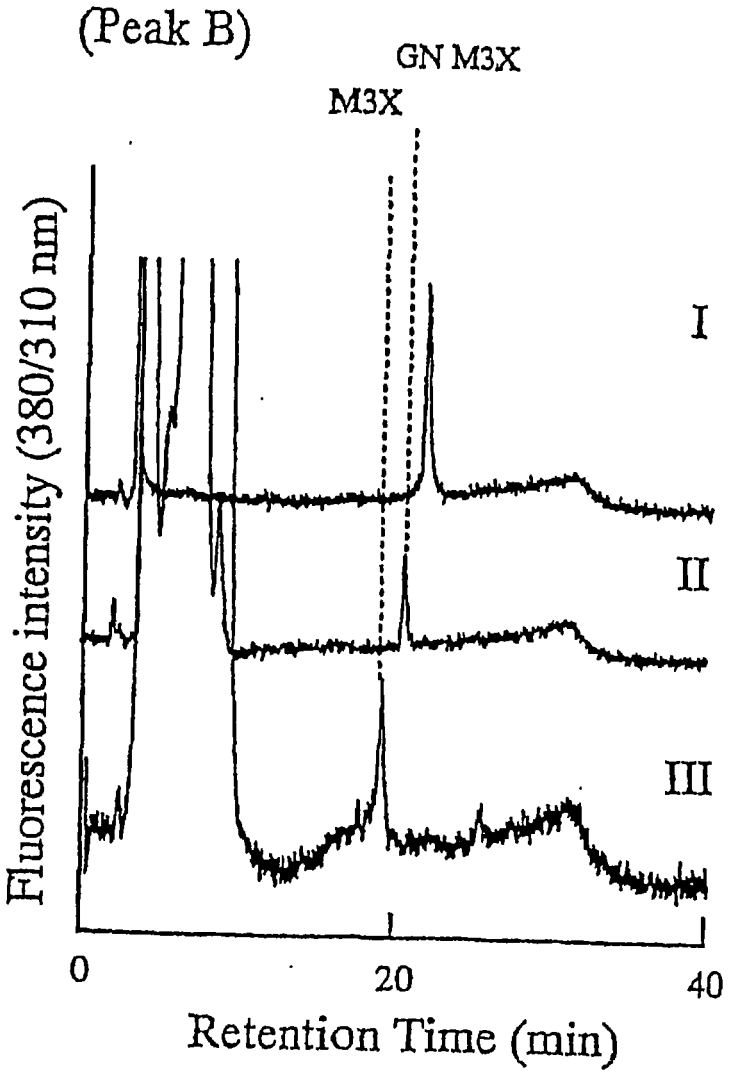

(Peak B)
GN M3X
M3X
Fluorescence intensity (380/310 nm)
I
II
III
0
20
40
Retention Time (min)

FIG.8

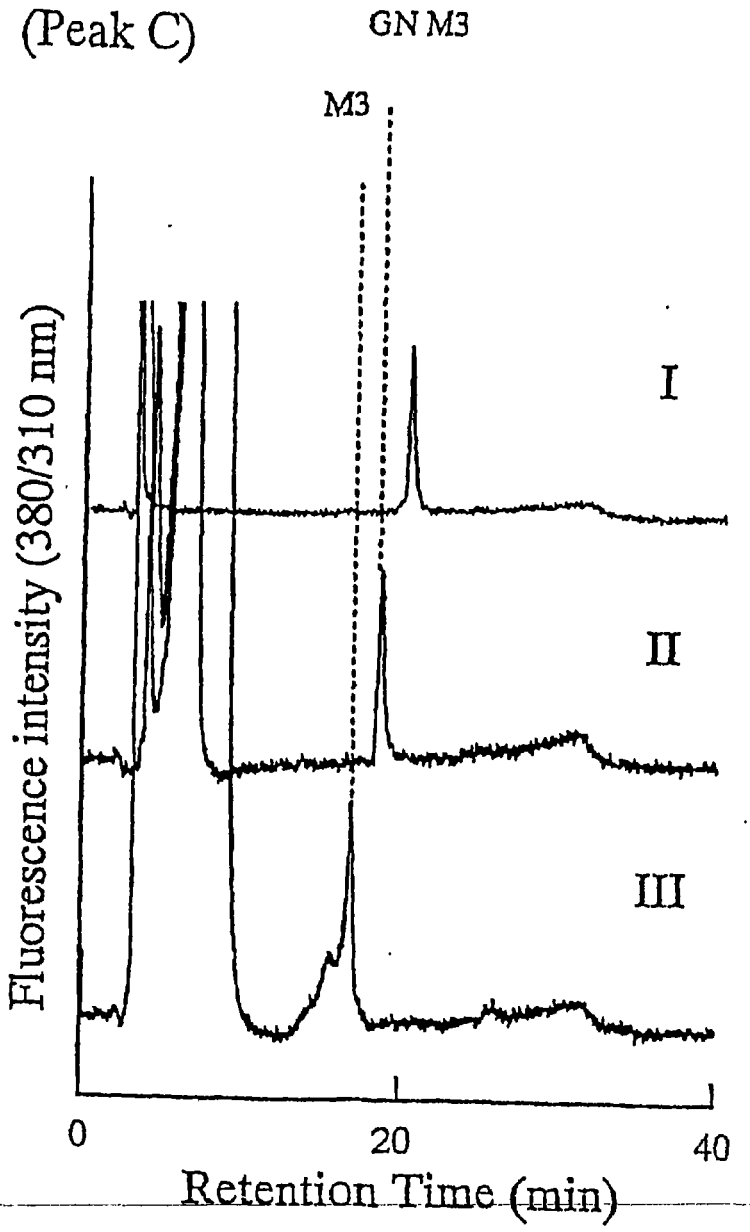

(Peak C)
GN M3
M3
I
II
III
Fluorescence intensity (380/310 nm)
0
20
40
Retention Time (min)

FIG. 9

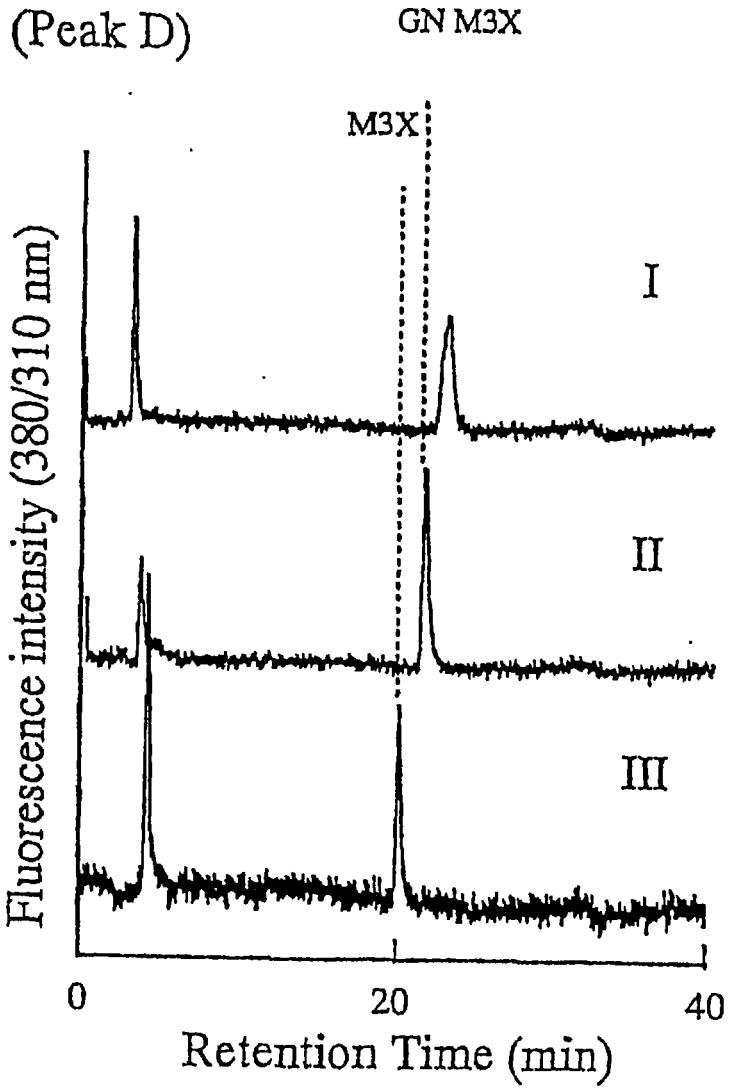

(Peak D)
GN M3X
M3X
I
II
III
Fluorescence intensity (380/310 nm)
0
20
40
Retention Time (min)

FIG. 10

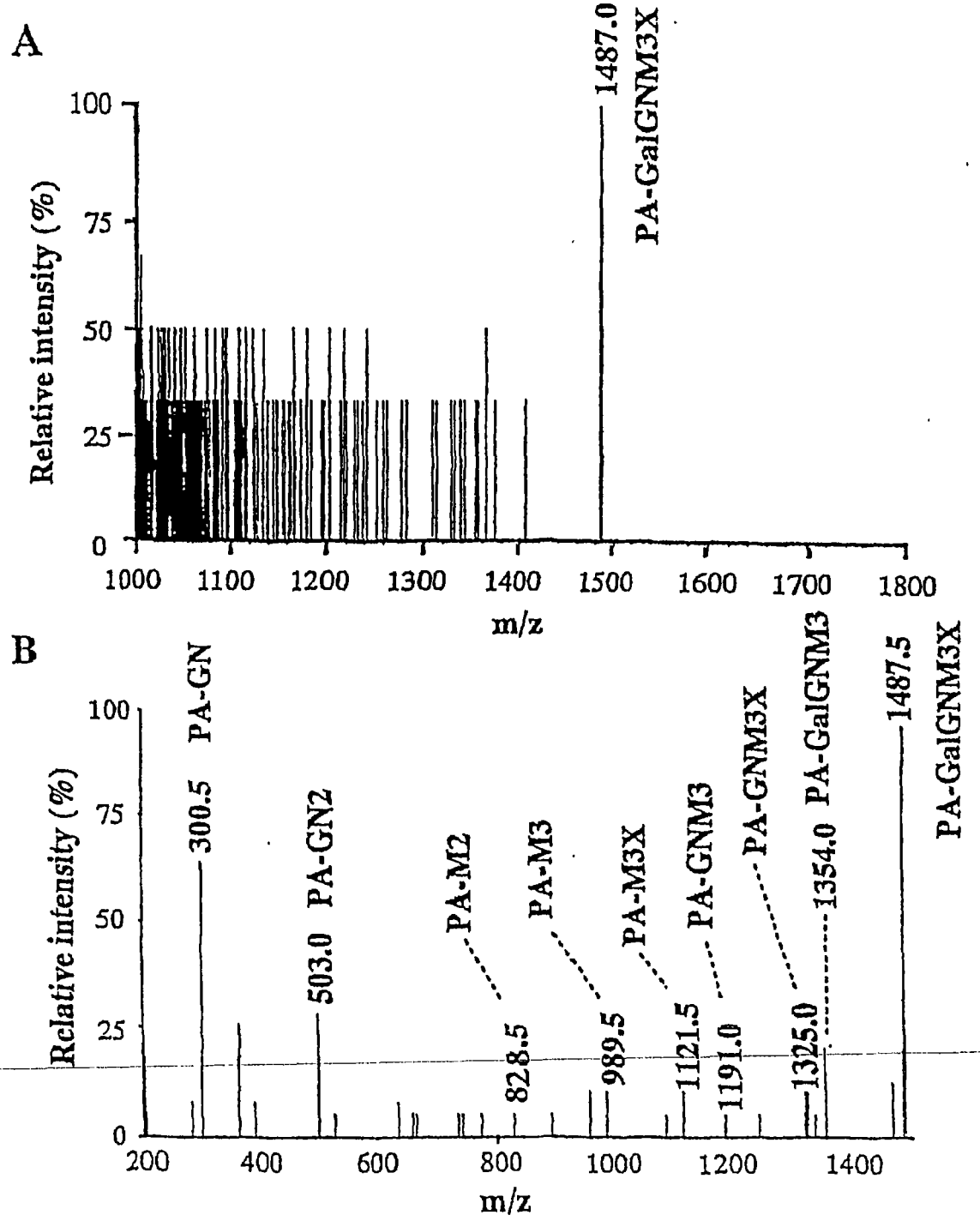

A
Relative intensity (%)
100
75
50
25
0
1000
1100
1200
1300
1400
1500
1600
1700
1800
m/z
1487.0
PA-GalGNM3X
B
Relative intensity (%)
100
75
50
25
0
200
400
600
800
1000
1200
1400
m/z
300.5
PA-GN
503.0
PA-GN2
828.5
PA-M2
989.5
PA-M3
1121.5
PA-M3X
1191.0
PA-GNM3
1325.0
PA-GNM3X
1354.0
PA-GalGNM3
1487.5
PA-GalGNM3X

FIG. 11

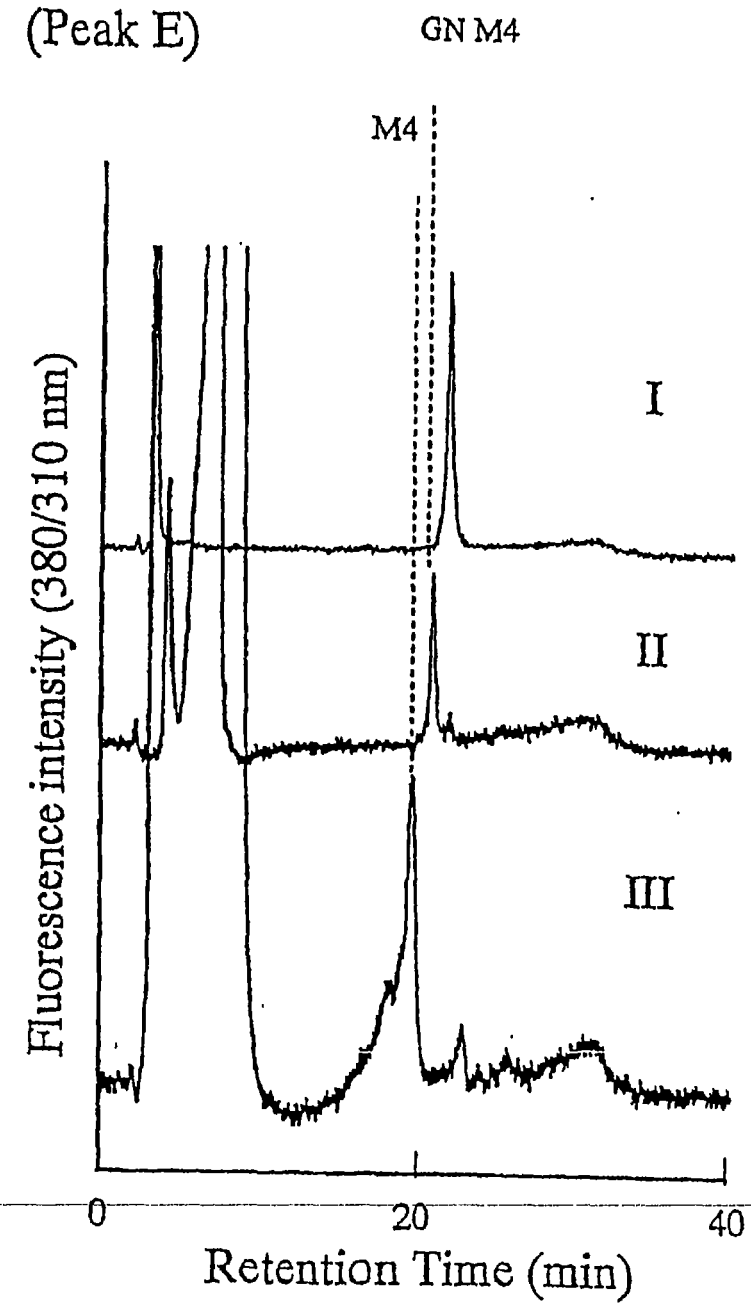

(Peak E)
GN M4
M4
I
II
III
Fluorescence intensity (380/310 nm)
0
20
40
Retention Time (min)

FIG. 12

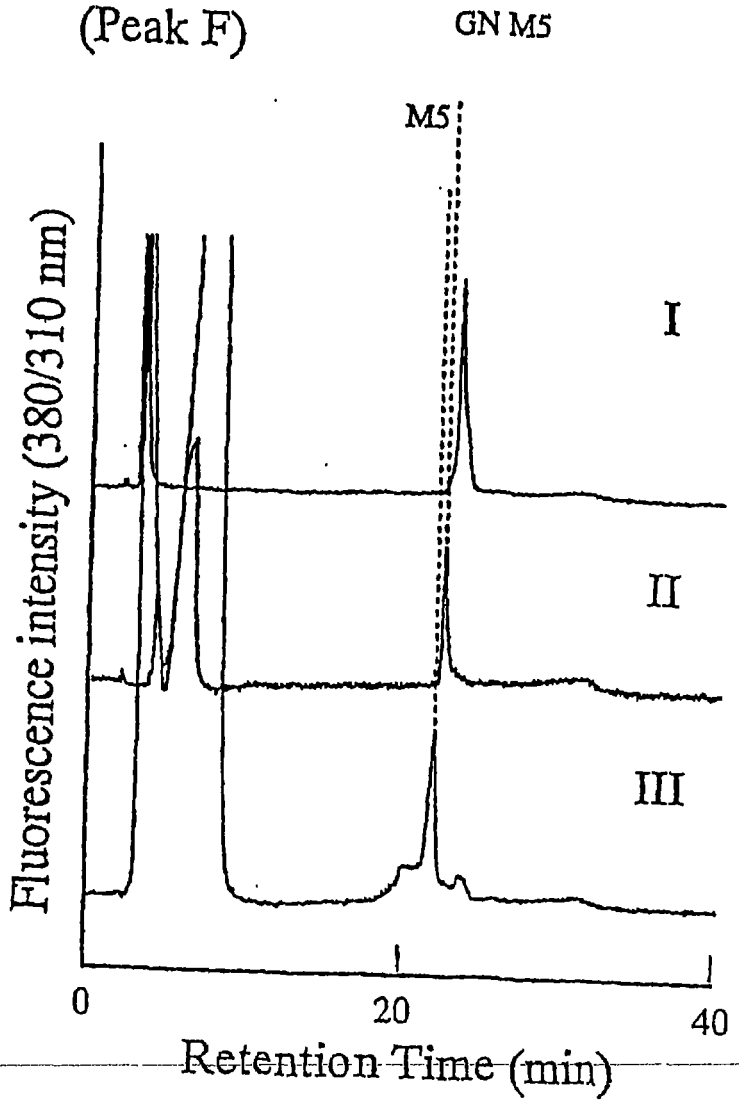
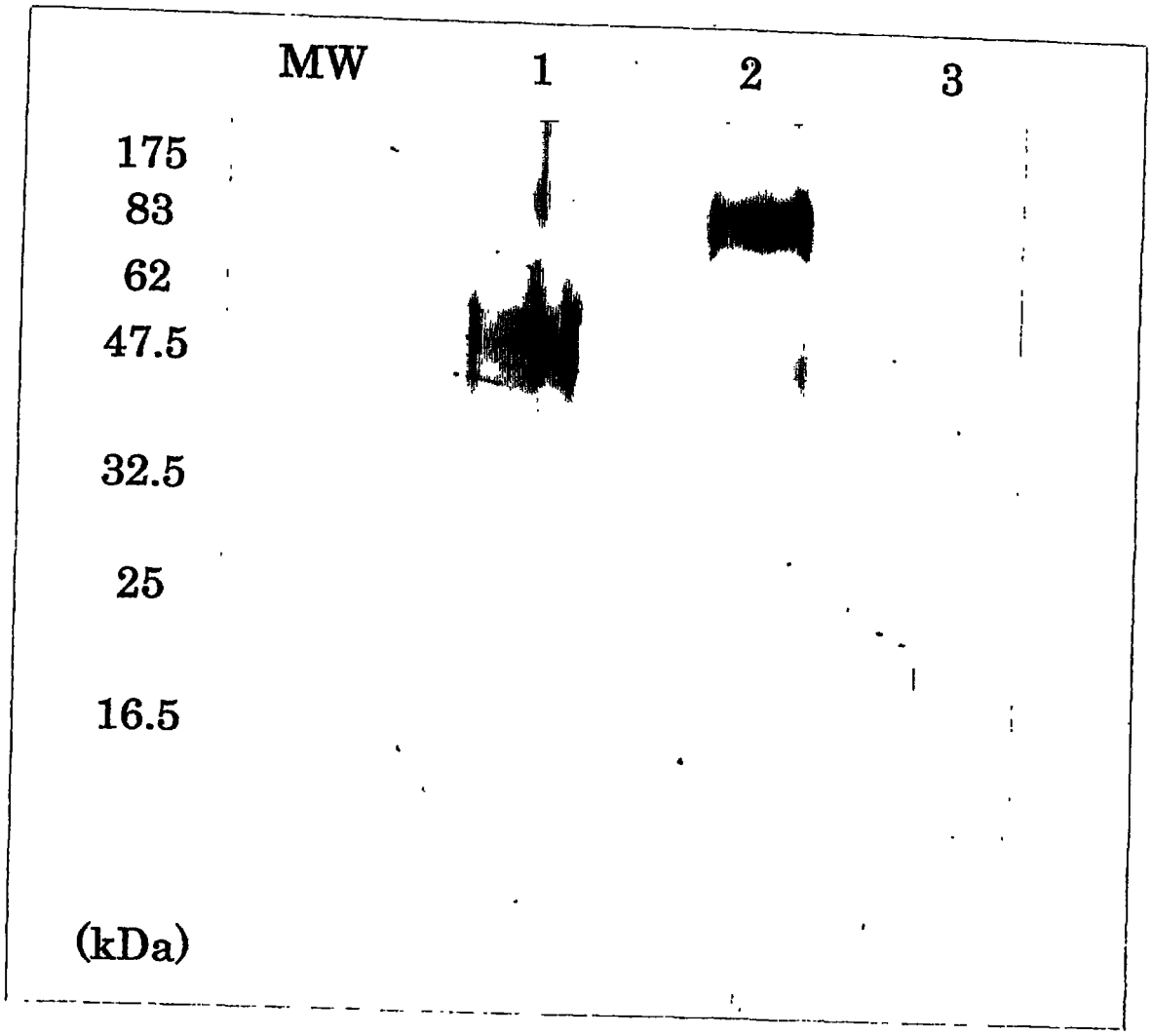

(Peak F)
GN M5
M5
I
II
III
Fluorescence intensity (380/310 nm)
0
20
40
Retention Time (min)

FIG. 13



MW; Protein Molecular Weight Marker (Daiichi Kagaku yakuhin)

FIG. 14A

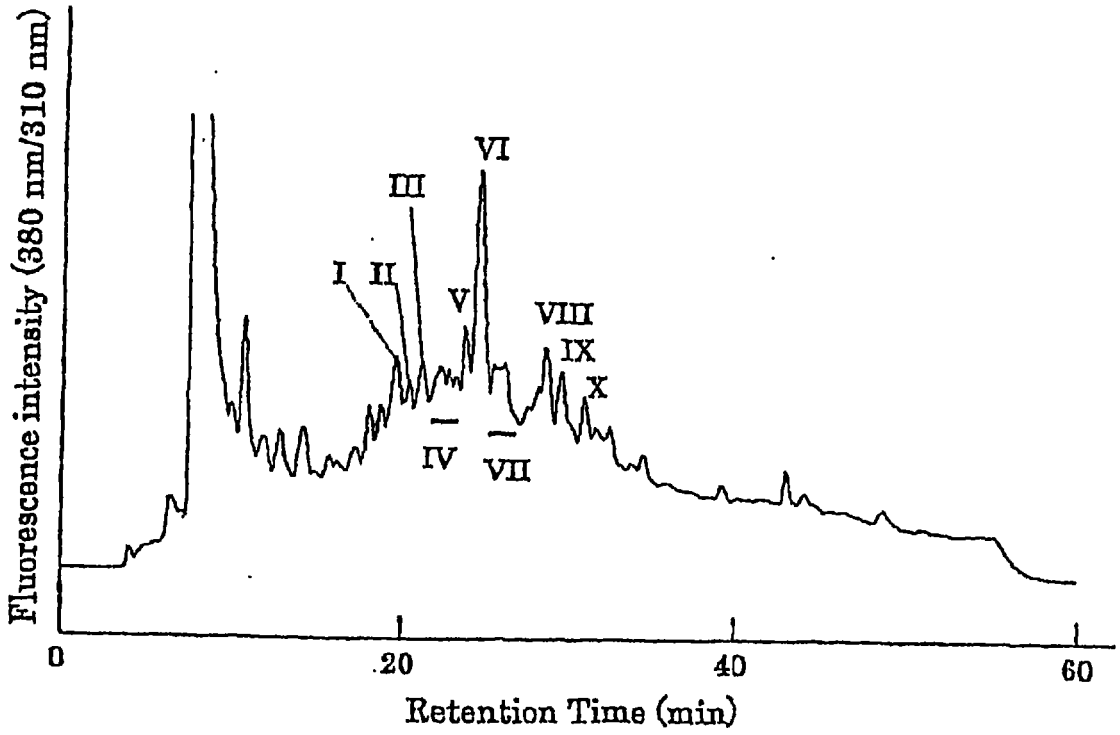

Fluorescence intensity (380 nm/310 nm)
I
II
III
IV
V
VI
VII
VIII
IX
X
0
20
40
60
Retention Time (min)

FIG. 14B

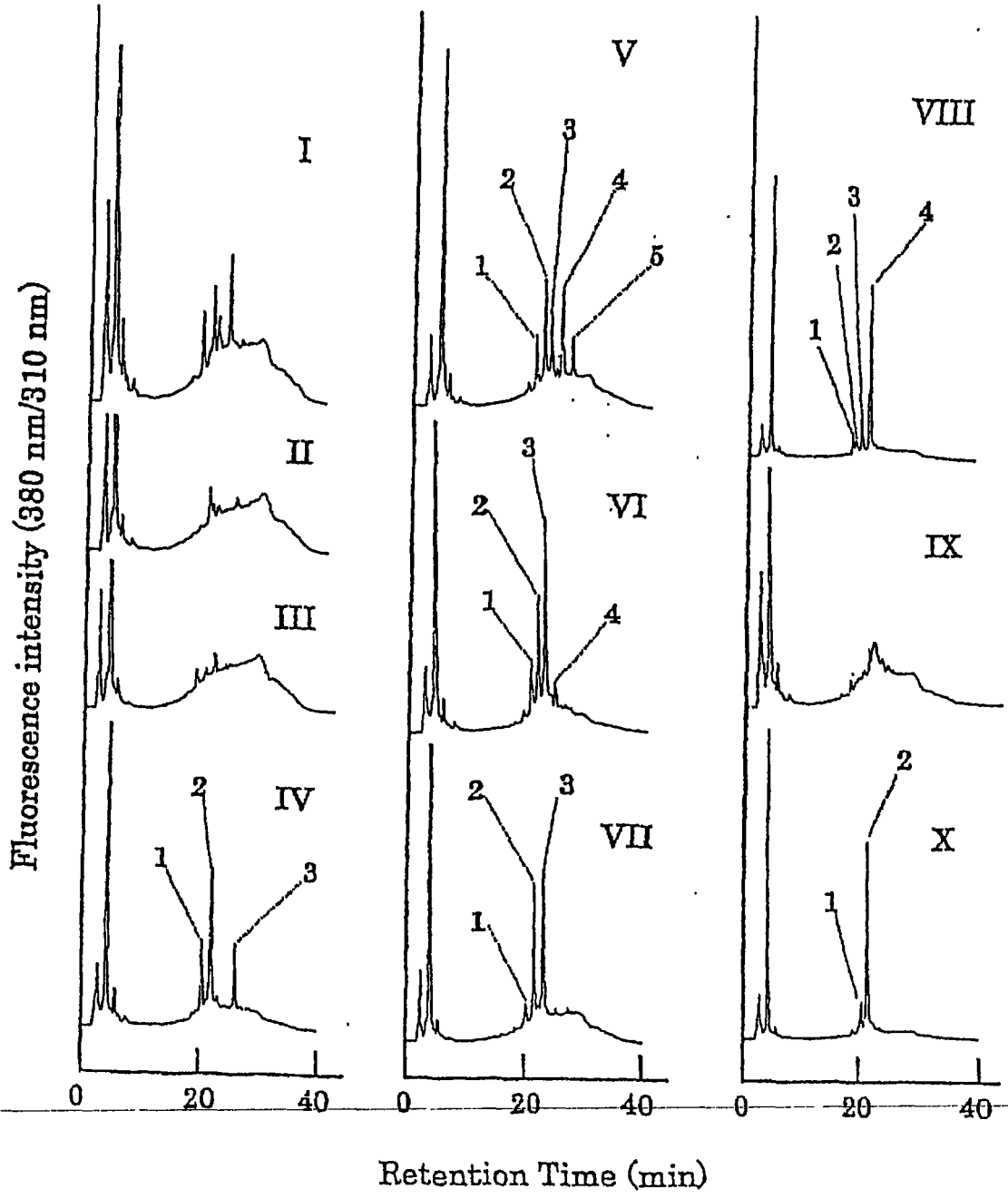

Fluorescence intensity (380 nm/310 nm)
I
II
III
IV
V
VI
VII
VIII
IX
X
1
2
3
4
5
0
20
40
Retention Time (min)

FIG. 15A

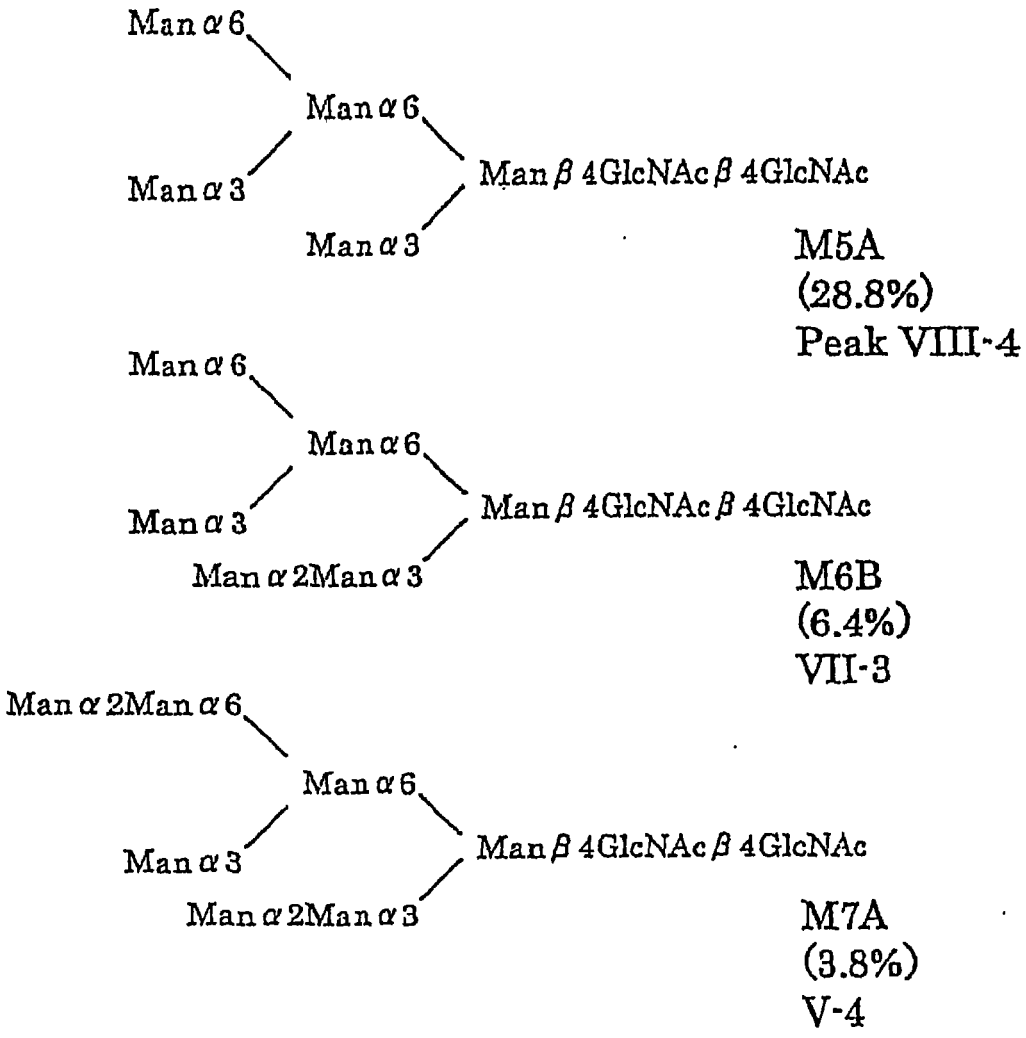

Man α 6
Man α 6
Man α 3
Man β 4GlcNAc β 4GlcNAc
Man α 3
M5A
(28.8%)
Peak VIII-4
Man α 6
Man α 6
Man α 3
Man β 4GlcNAc β 4GlcNAc
Man α 2Man α 3
M6B
(6.4%)
VII-3
Man α 2Man α 6
Man α 6
Man α 3
Man β 4GlcNAc β 4GlcNAc
Man α 2Man α 3
M7A
(3.8%)
V-4

FIG. 15B


GN2M3
(4.9%)
X-1
GlcNAcβ2Manα6
Manβ4GlcNAcβ4GlcNAc
GlcNAcβ2Manα3
GN2M3X
(32.1%)
X-2
GlcNAcβ2Manα6
Manβ4GlcNAcβ4GlcNAc
Xylβ2
GlcNAcβ2Manα3
M5A
(28.8%)
VII-4
Manα6
Manα6
Manβ4GlcNAcβ4GlcNAc
Manα3
Manα3
M6B
(6.4%)
VII-3
Manα6
Manα6
Manβ4GlcNAcβ4GlcNAc
Manα3
Manα2Manα3
M7A
(3.8%)
V-4
Manα2Manα6
Manα6
Manβ4GlcNAcβ4GlcNAc
Manα3
Manα2Manα3
M3X
(3.7%)
VIII-1
Manα6
Manβ4GlcNAcβ4GlcNAc
Manα3
Xylβ2
M3FX
(8.3%)
IV-1, V-1
Manα6
Manβ4GlcNAcβ4GlcNAc
Manα3
Xylβ2
Fucα3
GN1M3X
(0.9%)
VII-1
Manα6
Manβ4GlcNAcβ4GlcNAc
GlcNAcβ2Manα3
Xylβ2
GN1M3X
(5.3%)
VIII-3
GlcNAcβ2Manα6
Manβ4GlcNAcβ4GlcNAc
Manα3
Xylβ2
GN1M3FX
(5.8%)
VII-2
Manα6
Manβ4GlcNAcβ4GlcNAc
GlcNAcβ2Manα3
Xylβ2
Fucα3

FIG. 16

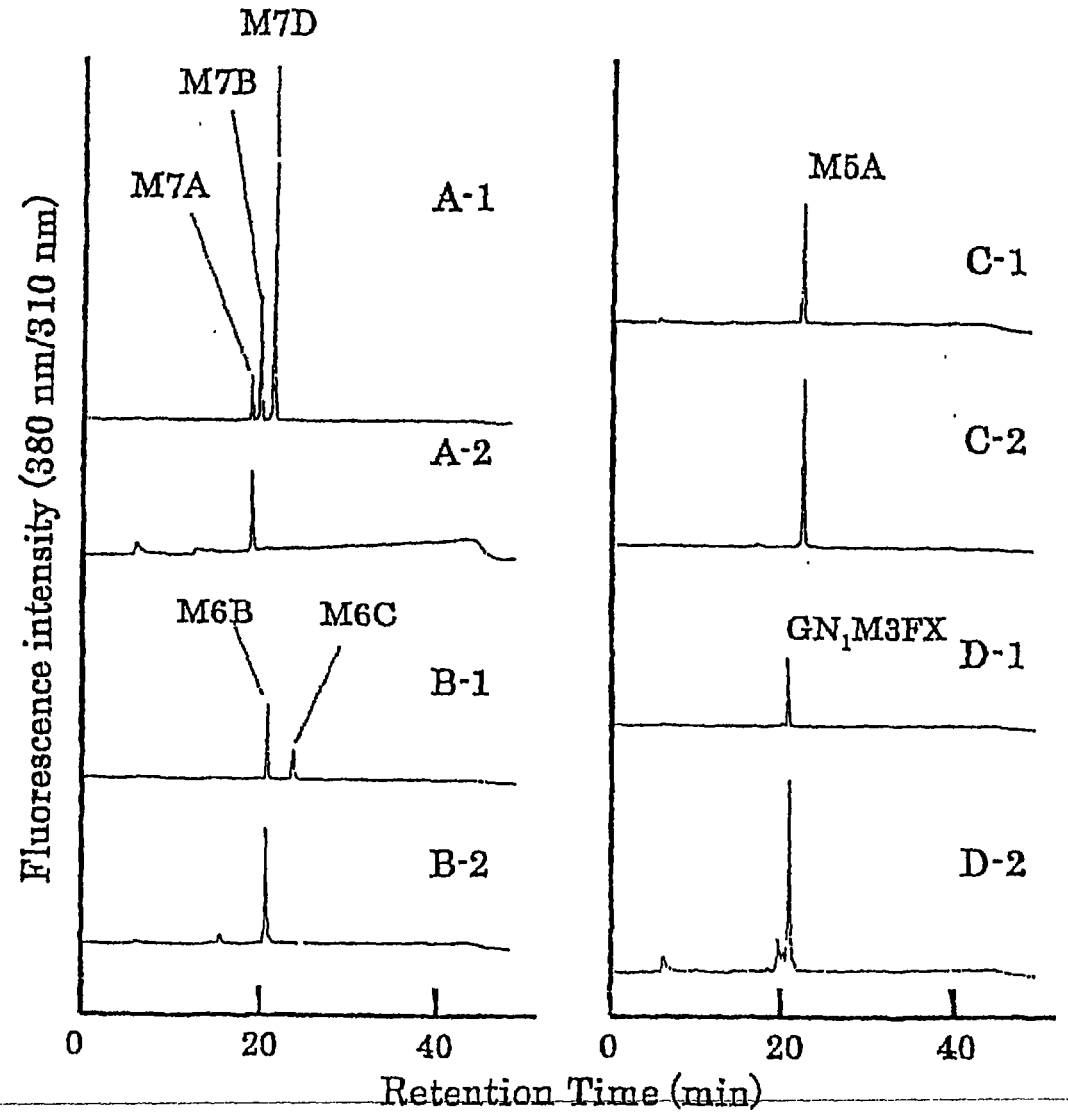

M7D
M7B
M7A
A-1
A-2
M6B
M6C
B-1
B-2
M5A
C-1
C-2
GN1M3FX
D-1
D-2
Fluorescence intensity (380 nm/310 nm)
0
20
40
0
20
40
Retention Time (min)

FIG. 17

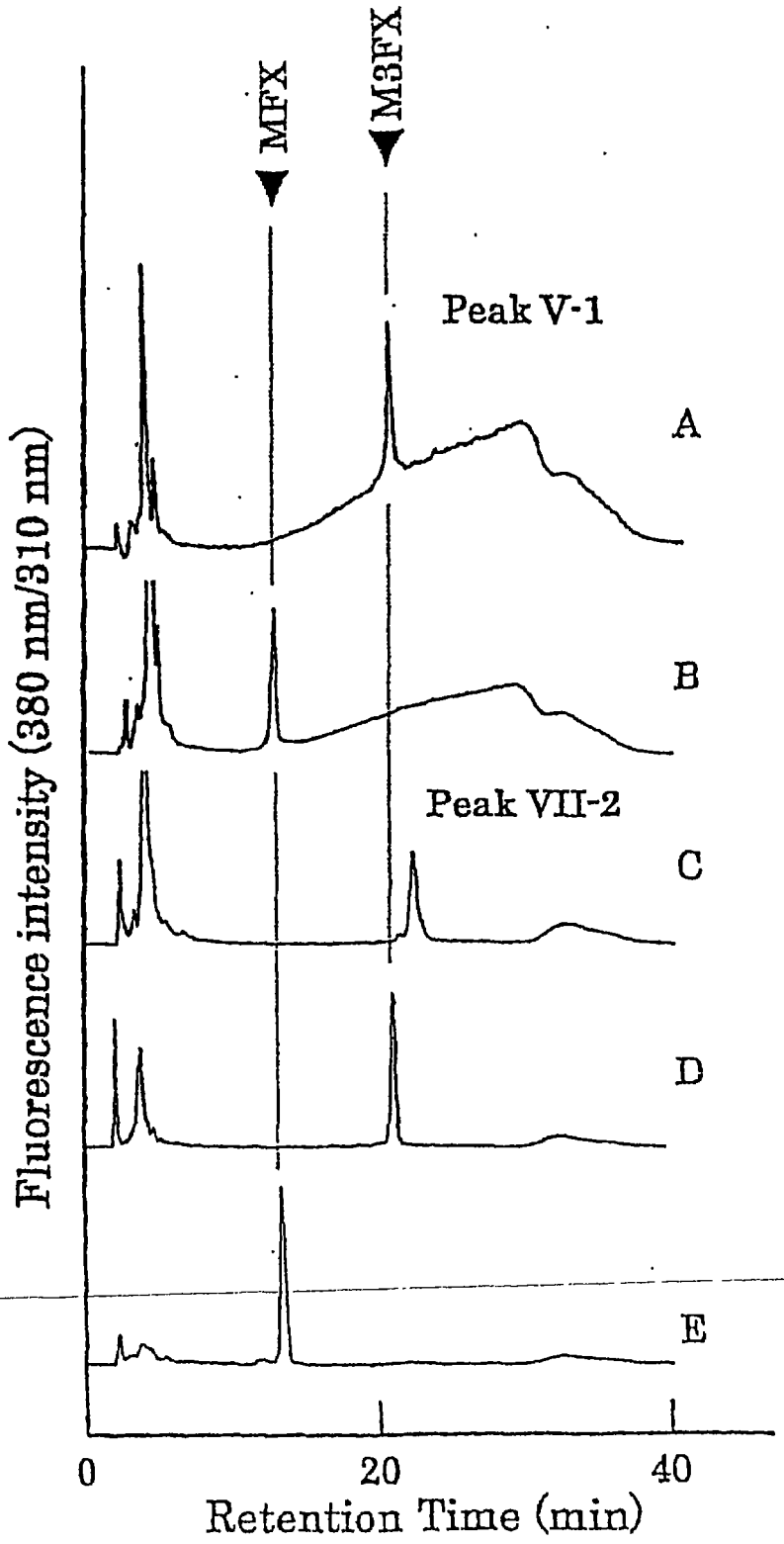

MFX
M3FX
Peak V-1
A
B
Peak VII-2
C
D
E
Fluorescence intensity (380 nm/310 nm)
0
20
40
Retention Time (min)

FIG. 18A

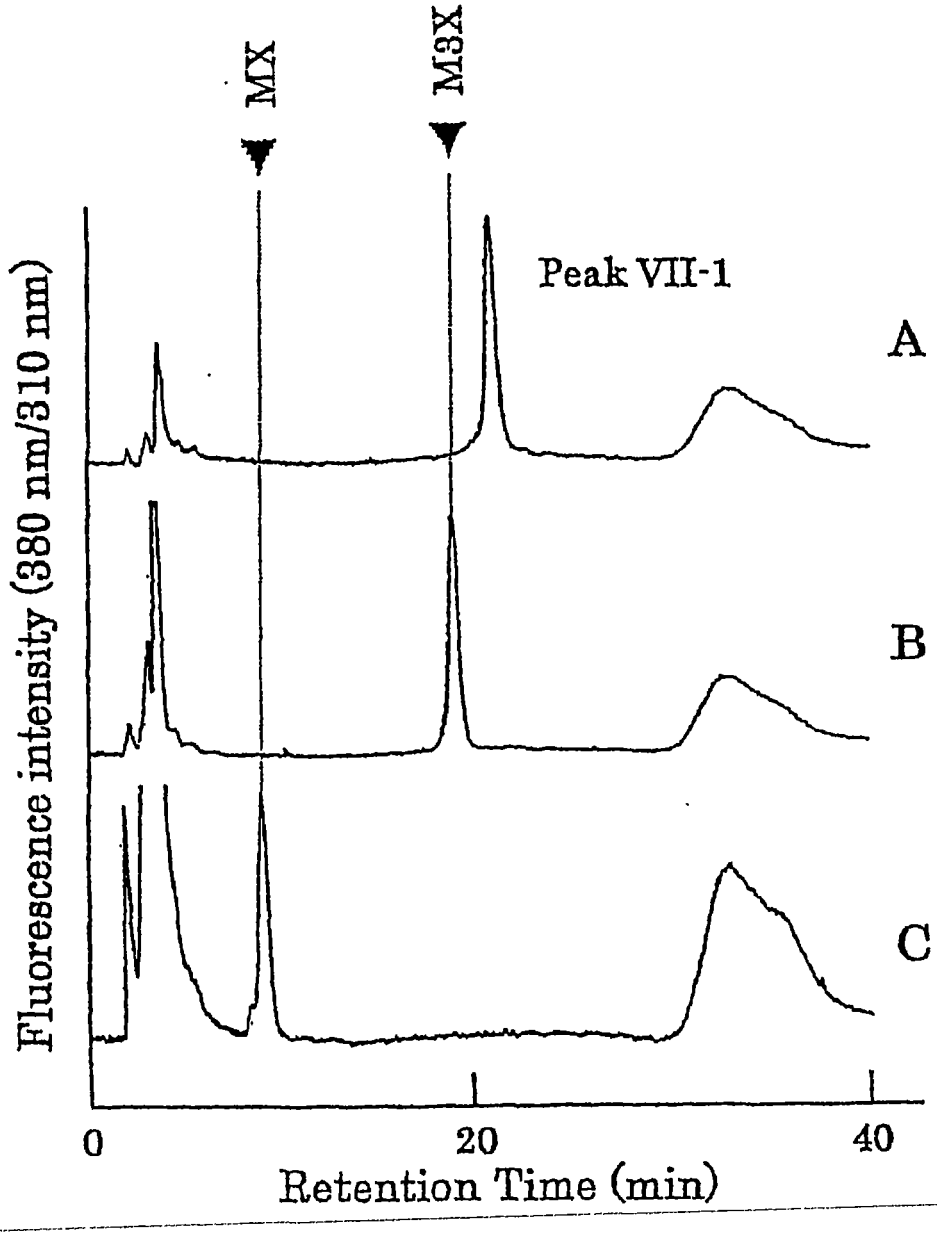

MX
M3X
Peak VII-1
A
B
C
Fluorescence intensity (380 nm/310 nm)
0
20
40
Retention Time (min)

FIG. 18B

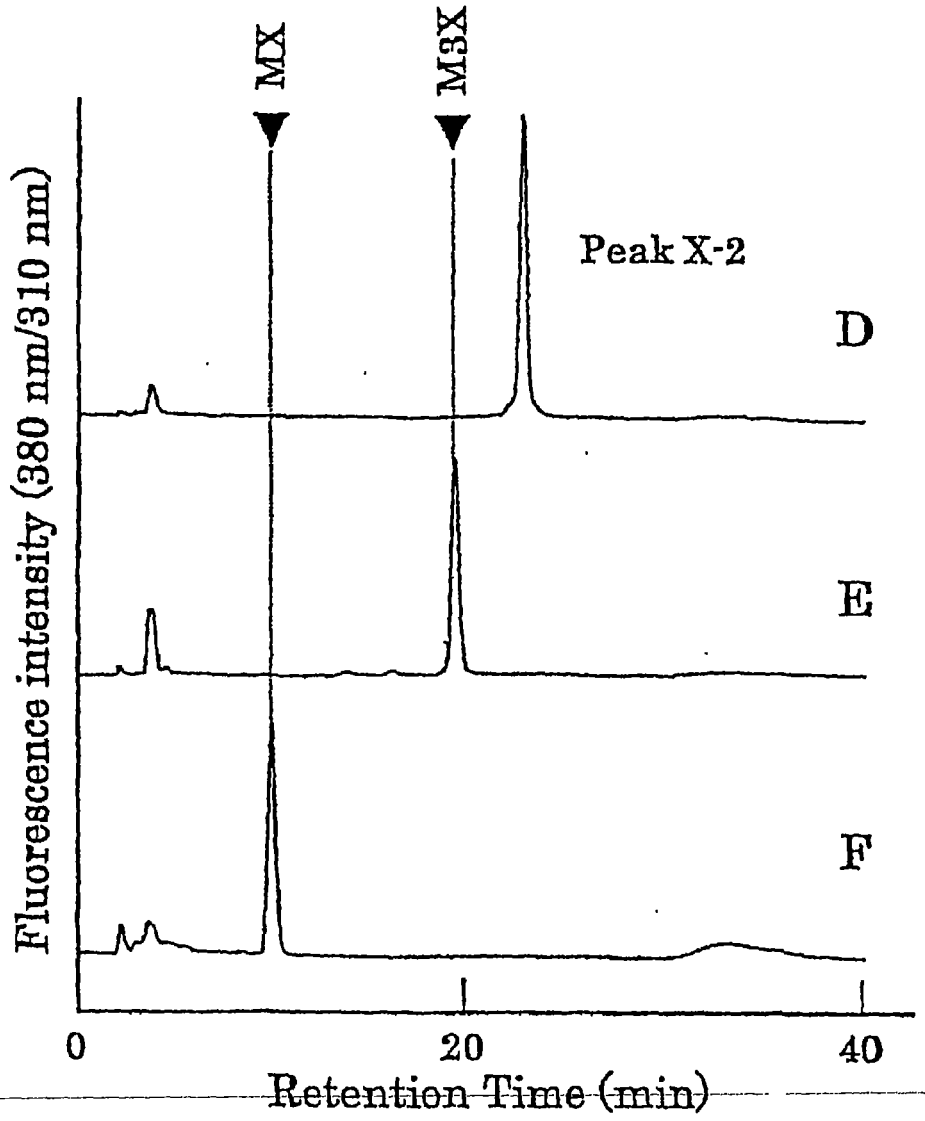

MX
M3X
Peak X-2
D
E
F
Fluorescence intensity (380 nm/310 nm)
0
20
40
Retention Time (min)

FIG. 19

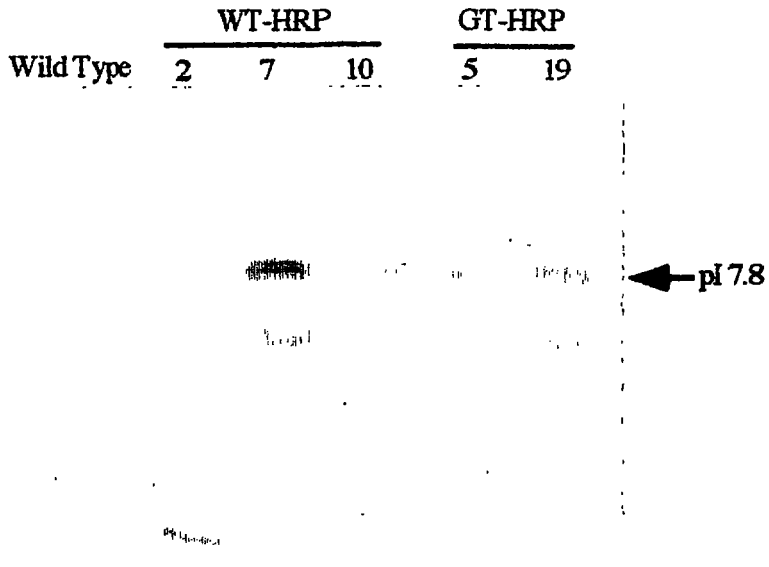
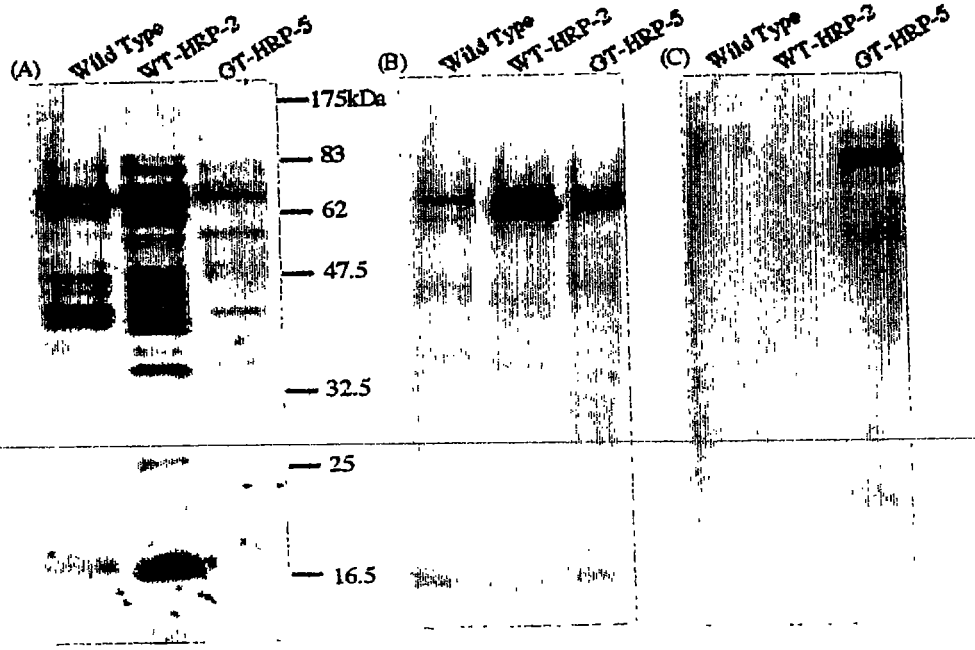

WT-HRP
GT-HRP
Wild Type
2
7
10
5
19
pI 7.8

FIG. 20


(A)
Wild Type
WT-HRP-2
GT-HRP-5
(B)
Wild Type
WT-HRP-2
GT-HRP-5
(C)
Wild Type
WT-HRP-2
GT-HRP-5
175kDa
83
62
47.5
32.5
25
16.5

SEQUENCE LISTING

<110> Tatsuji, Seki and Kazuhito, Fujiyama

<120> Methods for excretory production of glycoproteins having human-type sugar chains

<130> J198020401

<160> 6

<170> PatentIn Ver. 2.0

<210> 1

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer hGT-5Eco

<400> 1

aaagaattcg cgatgccagg cgcgcgtccc t 31

<210> 2

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer hGT-2Sal

<400> 2

tcgatcgcaa aaccatgtgc agctgatg 28

<210> 3

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer hGT-7Spe

<400> 3
acgggactcc tcaggggcga tgatcataa 29
<210> 4
<211> 27
<212> DNA
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:primer hGT-6Spe
<400> 4
aagactagtg ggccccatgc tgattga 27
<210> 5
<211> 1158
<212> DNA
<213> Homo sapiens
<220>
<221> CDS
<222> (1)..(1155)
<400> 5
atg cca ggc gcg tcc cta cag cgg gcc tgc cgc ctg ctc gtg gcc gtc 48
Met Pro Gly Ala Ser Leu Gln Arg Ala Cys Arg Leu Leu Val Ala Val
1 5 10 15
tgc gct ctg cac ctt ggc gtc acc ctc gtt tac tac ctg gct ggc cgc 96
Cys Ala Leu His Leu Gly Val Thr Leu Val Tyr Tyr Leu Ala Gly Arg
20 25 30
gac ctg agc cgc ctg ccc caa ctg gtc gga gtc tcc aca ccg ctg cag 144
Asp Leu Ser Arg Leu Pro Gln Leu Val Gly Val Ser Thr Pro Leu Gln
35 40 45
ggc ggc tcg aac agt gcc gcc gcc atc ggg cag tcc tcc ggg gag ctc 192
Gly Gly Ser Asn Ser Ala Ala Ala Ile Gly Gln Ser Ser Gly Glu Leu

```
     50                  55                  60
cgg acc gga ggg gcc cgg ccg ccg cct cct cta ggc gcc tcc tcc cag   240
Arg Thr Gly Gly Ala Arg Pro Pro Pro Pro Leu Gly Ala Ser Ser Gln
 65                  70                  75                  80
ccg cgc ccg ggt ggc gac tcc agc cca gtc gtg gat tct ggc cct ggc   288
Pro Arg Pro Gly Gly Asp Ser Ser Pro Val Val Asp Ser Gly Pro Gly
                 85                  90                  95
ccc gct agc aac ttg acc tcg gtc cca gtg ccc cac acc acc gca ctg   336
Pro Ala Ser Asn Leu Thr Ser Val Pro Val Pro His Thr Thr Ala Leu
            100                 105                 110
tcg ctg ccc gcc tgc cct gag gag tcc ccg cta cta gtg ggc ccc atg   384
Ser Leu Pro Ala Cys Pro Glu Glu Ser Pro Leu Leu Val Gly Pro Met
        115                 120                 125
ctg att gag ttt aac atg cct gtg gac ctg gag ctc gtg gca aag cag   432
Leu Ile Glu Phe Asn Met Pro Val Asp Leu Glu Leu Val Ala Lys Gln
    130                 135                 140
aac cca aat gtg aag atg ggc ggc cgc tat gcc ccc agg gac tgc gtc   480
Asn Pro Asn Val Lys Met Gly Gly Arg Tyr Ala Pro Arg Asp Cys Val
145                 150                 155                 160
tct cct cac aag gtg gcc atc atc att cca ttc cgc aac cgg cag gag   528
Ser Pro His Lys Val Ala Ile Ile Ile Pro Phe Arg Asn Arg Gln Glu
                165                 170                 175
cac ctc aag tac tgg cta tat tat ttg cac cca gtc ctg cag cgc cag   576
His Leu Lys Tyr Trp Leu Tyr Tyr Leu His Pro Val Leu Gln Arg Gln
            180                 185                 190
cag ctg gac tat ggc atc tat gtt atc aac cag gcg gga gac act ata   624
Gln Leu Asp Tyr Gly Ile Tyr Val Ile Asn Gln Ala Gly Asp Thr Ile
        195                 200                 205
ttc aat cgt gct aag ctc ctc aat gtt ggc ttt caa gaa gcc ttg aag   672
```

Phe Asn Arg Ala Lys Leu Leu Asn Val Gly Phe Gln Glu Ala Leu Lys
210 215 220

gac tat gac tac acc tgc ttt gtg ttt agt gac gtg gac ctc att cca 720
Asp Tyr Asp Tyr Thr Cys Phe Val Phe Ser Asp Val Asp Leu Ile Pro
225 230 235 240

atg aat gac cat aat gcg tac agg tgt ttt tca cag cca cgg cac att 768
Met Asn Asp His Asn Ala Tyr Arg Cys Phe Ser Gln Pro Arg His Ile
245 250 255

tcc gtt gca atg gat aag ttt gga ttc agc cta cct tat gtt cag tat 816
Ser Val Ala Met Asp Lys Phe Gly Phe Ser Leu Pro Tyr Val Gln Tyr
260 265 270

ttt gga ggt gtc tct gct cta agt aaa caa cag ttt cta acc atc aat 864
Phe Gly Gly Val Ser Ala Leu Ser Lys Gln Gln Phe Leu Thr Ile Asn
275 280 285

gga ttt cct aat aat tat tgg ggc tgg gga gga gaa gat gat gac att 912
Gly Phe Pro Asn Asn Tyr Trp Gly Trp Gly Gly Glu Asp Asp Asp Ile
290 295 300

ttt aac aga tta gtt ttt aga ggc atg tct ata tct cgc cca aat gct 960
Phe Asn Arg Leu Val Phe Arg Gly Met Ser Ile Ser Arg Pro Asn Ala
305 310 315 320

gtg gtc ggg agg tgt cgc atg atc cgc cac tca aga gac aag aaa aat 1008
Val Val Gly Arg Cys Arg Met Ile Arg His Ser Arg Asp Lys Lys Asn
325 330 335

gaa ccc aat cct cag agg ttt gac cga att gca cac aca aag gag aca 1056
Glu Pro Asn Pro Gln Arg Phe Asp Arg Ile Ala His Thr Lys Glu Thr
340 345 350

atg ctc tct gat ggt ttg aac tca ctc acc tac cag gtg ctg gat gta 1104
Met Leu Ser Asp Gly Leu Asn Ser Leu Thr Tyr Gln Val Leu Asp Val
355 360 365

cag aga tac cca ttg tat acc caa atc aca gtg gac atc ggg aca ccg 1152
Gln Arg Tyr Pro Leu Tyr Thr Gln Ile Thr Val Asp Ile Gly Thr Pro
370 375 380

agc tag 1158
Ser
385

<210> 6
<211> 385
<212> PRT
<213> Homo sapiens
<400> 6

Met Pro Gly Ala Ser Leu Gln Arg Ala Cys Arg Leu Leu Val Ala Val
1 5 10 15
Cys Ala Leu His Leu Gly Val Thr Leu Val Tyr Tyr Leu Ala Gly Arg
20 25 30
Asp Leu Ser Arg Leu Pro Gln Leu Val Gly Val Ser Thr Pro Leu Gln
35 40 45
Gly Gly Ser Asn Ser Ala Ala Ala Ile Gly Gln Ser Ser Gly Glu Leu
50 55 60
Arg Thr Gly Gly Ala Arg Pro Pro Pro Pro Leu Gly Ala Ser Ser Gln
65 70 75 80
Pro Arg Pro Gly Gly Asp Ser Ser Pro Val Val Asp Ser Gly Pro Gly
85 90 95
Pro Ala Ser Asn Leu Thr Ser Val Pro Val Pro His Thr Thr Ala Leu
100 105 110
Ser Leu Pro Ala Cys Pro Glu Glu Ser Pro Leu Leu Val Gly Pro Met
115 120 125
Leu Ile Glu Phe Asn Met Pro Val Asp Leu Glu Leu Val Ala Lys Gln
130 135 140

Asn Pro Asn Val Lys Met Gly Gly Arg Tyr Ala Pro Arg Asp Cys Val
145 150 155 160
Ser Pro His Lys Val Ala Ile Ile Ile Pro Phe Arg Asn Arg Gln Glu
165 170 175
His Leu Lys Tyr Trp Leu Tyr Tyr Leu His Pro Val Leu Gln Arg Gln
180 185 190
Gln Leu Asp Tyr Gly Ile Tyr Val Ile Asn Gln Ala Gly Asp Thr Ile
195 200 205
Phe Asn Arg Ala Lys Leu Leu Asn Val Gly Phe Gln Glu Ala Leu Lys
210 215 220
Asp Tyr Asp Tyr Thr Cys Phe Val Phe Ser Asp Val Asp Leu Ile Pro
225 230 235 240
Met Asn Asp His Asn Ala Tyr Arg Cys Phe Ser Gln Pro Arg His Ile
245 250 255
Ser Val Ala Met Asp Lys Phe Gly Phe Ser Leu Pro Tyr Val Gln Tyr
260 265 270
Phe Gly Gly Val Ser Ala Leu Ser Lys Gln Gln Phe Leu Thr Ile Asn
275 280 285
Gly Phe Pro Asn Asn Tyr Trp Gly Trp Gly Gly Glu Asp Asp Asp Ile
290 295 300
Phe Asn Arg Leu Val Phe Arg Gly Met Ser Ile Ser Arg Pro Asn Ala
305 310 315 320
Val Val Gly Arg Cys Arg Met Ile Arg His Ser Arg Asp Lys Lys Asn
325 330 335
Glu Pro Asn Pro Gln Arg Phe Asp Arg Ile Ala His Thr Lys Glu Thr
340 345 350
Met Leu Ser Asp Gly Leu Asn Ser Leu Thr Tyr Gln Val Leu Asp Val
355 360 365
Gln Arg Tyr Pro Leu Tyr Thr Gln Ile Thr Val Asp Ile Gly Thr Pro

370 375 380

Ser

385