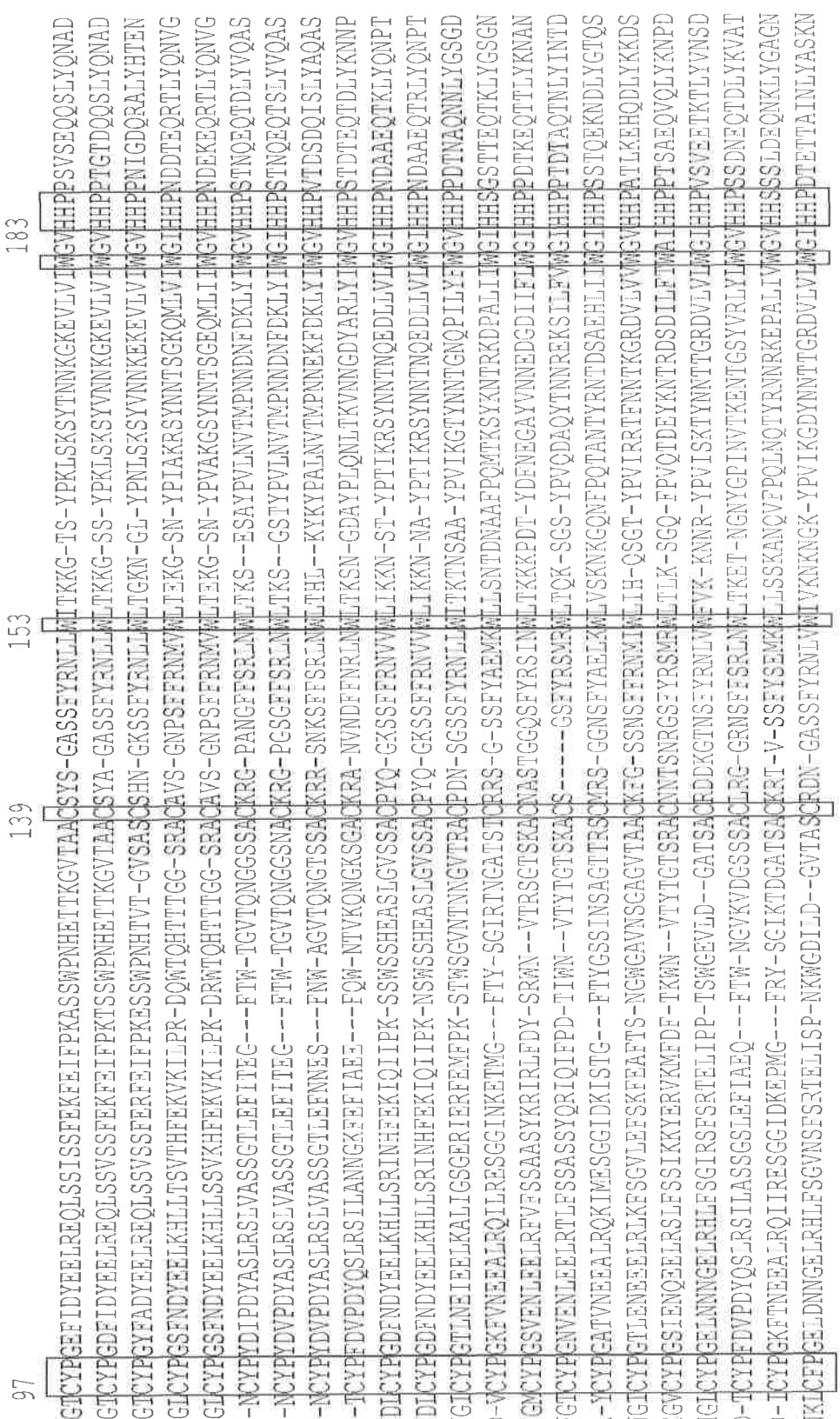

97
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153
183
H1_Av
H1_Hu1
H1_Hu2
H2_Av
H2_Hu
H3_Av
H3_Hu1
H3_Hu2
H4_Av
H5_Av1
H5_Av2
H6_Av
H7_Av
H8_Av
H9_Av
H10_Av
H11_Av
H12_Av
H13_Av
H14_Av
H15_Av
H16_Av

FIG. 1A

```
H1_Av    AYVSVGSSKYNRRFAPEIAARPEVRGQAGRMNYYWTLLDQGDTITFEATGNLIAPWYAFALNKGSD--------SGIITS-DAPVH-NCDTRCQTPHGALNSSLPFQNVHPIT
H1_Hu1   AYVSVGSSKYNRRFTPEIAARPKVRDQAGRMNYYWTLLEPGDTITFEATGNLIAPWYAFALNRGSG--------SGIITS-DAPVH-DCNTKCQTPHGAINSSLPFQNIHPVT
H1_Hu2   AYVSVVSSHYSRRFTPEIAKRPKVRDQEGRINYYWTLLEPGDTIIFEANGNLIAPWYAFALSRGFG--------SGIITS-NAPMD-ECDAKCQTPQGAINSSLPFQNVHPVT
H2_Av    TYVSVGTSTLNKRSIPEIATRPKVNGQGGRMEFSWTLLETWDVINFESTGNLIAPEYGFKISKRGS--------SGIMKT-EXTLE-NCETKCQTPLGAINTTLPFHNIHPLT
H2_Hu    TYVSVGTSTLNKRSTPDIATRPKVNGLGSRMEFSWTLLDMWDTINFESTGNLIAPEYGFKISKRGS--------SGIMKT-EGTLE-NCETKCQTPLGAINTTLPFHNVHPLT
H3_Av    GRVTVSTRRSQQTIIPNIGSRPWVRGQPGRISIYWTIVKPGDVLVINSNGNLIAPRGYFKMRTG--------KSSIMRS-DAPID-TCISECITPNGSIPNDKPFQNVNKIT
H3_Hu1   GRVTVSTRRSQQTIIPNIGSRPWVRGLSSRISIYWTIVKPGDVLVINSNGNLIAPRGYFKMRTG--------KSSIMRS-DAPID-TCISECITPNGSIPNDKPFQNVNKIT
H3_Hu2   GRITVSTKRSQQTVIPNIGYRPRVRDISSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSG--------KSSIMRS-DAPIG-KCNSECITPNGSIPNDKPFQNVNRIT
H4_Av    GRVTVSTKTSQTSVVPNIGSRPWVRGQSGRISIYWTIVDPGDIIVINTIGNLIAPRGHYKLNSQK--------KSTILNT-AVPIG-SCVSKCHTDRGSITTTKPFQNISRIS
H5_Av1   TYISVGTSTLNQRLVPRIATRSKVNGQSGRMEFTWTILKPNDAINFESNGNFIAPEYAYKIVKKGD--------STIMKS-ELEYG-NCNTKCQTPMGAINSSMPFHNIHPLT
H5_Av2   TYISVGTSTLNQRLVPKIATRSKVNGQNGRMEFTWTILKPNDAINFESNGNFIAPEYAYKIVKKGD--------SAIMKS-ELEYG-NCNTKCQTPMGAINSSMPFHNIHPLT
H6_Av    RYVRMGTESMNFAKGPEISARPVVNGQRGRIDYYWSVLKPGETLNVESNGNLIAPWYAYKFVSTNSK--------GAVFKS-NLPIE-NCDATCQTIAGVLRTNKTFQNVSPLW
H7_Av    KLITVESSNYQQSFVPSPGARPKVDGQSGRIDFHWLMLNPNDTITFSFNGAFIAPDRASFLR--------GKSMGIQS-GVQVDDNCEGDCYHSGGTIISNLPFQNINSRA
H8_Av    TLSSVTNTINRSFQPNIGPRPLVRGQQGRMDYYWGILKRGETLKIRTNGNLIAPEFGYLLKGESY--------GRIIQNEDIPIG-NCNTKCQTYACAINSSKPFQNASRHY
H9_Av    TTTSVTTEDLNRIFKPVIGPRPLVNGLQGRINYYWSLVKPGQTLRVRSNGNLIAPWYGHVLSGGSH--------GRILKT-DLNSG-NCVVQCQTEKGGLMSTLPFHNISKYA
H10_Av   LSISVGSSTYQNNFVPVVGARPQVNGQSGRIDFEWTMVQPGDNITFSHNGGLIAPSRVSKLK--------CRGLGIQS-GASVDNDCESKCFWKGGSINTKLPFQNLSPRT
H11_Av   SYVAVDSESYNRRFTPEISTRPKVNGQAGRMTFYWTIVKPGEAITFESNGAFLAPRYAFELVSLGN--------GKLFRS-DLNIE-SCSTKCQSEIGGINTNRSFHNVHRNT
H12_Av   TLSSVTDEINRSFKPNIGPRPLVRGQQGRMDYYWAVLKPGQTVKIQTNGNLIAPEYGHLITGKSH--------GRILKN-NLPVG-QCVTECQLNEGVMNTSKPFQNTSKHY
H13_Av   PYTLVSTKSWSEKYKLETGVRPGYNGQRSWMKIYWSLLHPGEMITFESNGGLLAPRYGYIIEEYGK--------GRIFQS-RIRMS-KCNTKCQTSVGGINTNRTFQNIDKNA
H14_Av   GRVTVSTRSDQISIVPNIGSRPRVRNQSGRISIYWTLVNPGDSIIFNSIGNLIAPRGHYKISKST--------KSTVLKS-DKRIG-SCTSPCLTDKGSIQSDKPFQNVSRIA
H15_Av   KLITVGSSKYQQSFSPSPGDRPKVNGQAGRIDFHWMLLDPGDTVTFTFNGAFIAPDRAIFLRSNAPSGVEYNGKSLGIQS-DAQIDESCEGECFYSGGTINSPLPFQNIDSWA
H16_Av   PYTLVSTKEWSKRYELEIGTRIC-DGQRSWMKLYWHLMRPGERIMFESNGGLIAPRYGYIIEKYGT--------GRIFQS-GVRMA-KCNTKCQTSLGGINTNKTFQNIERNA
```

FIG. 1B

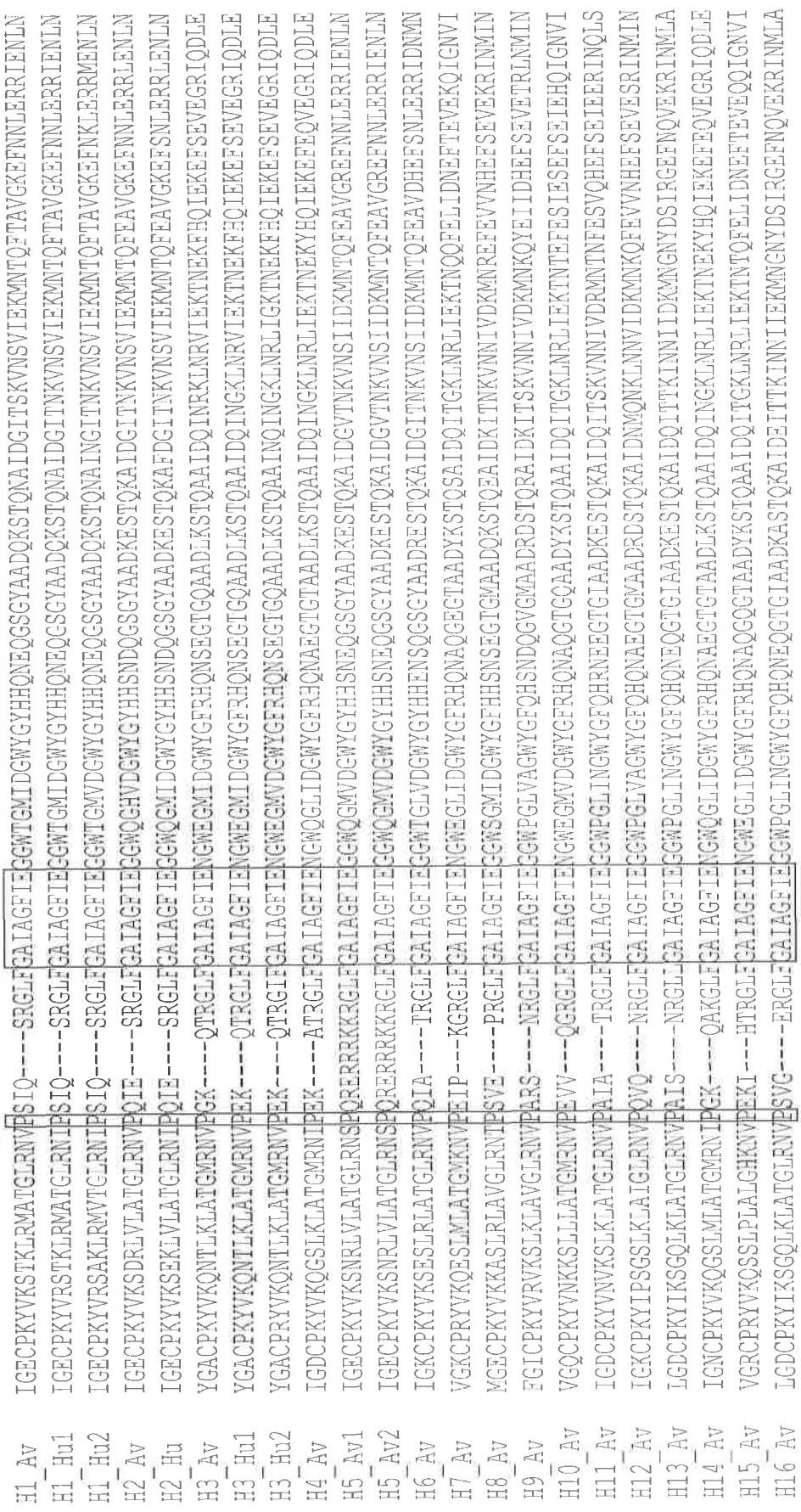

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H1_Hu1
H1_Hu2
H2_Av
H2_Hu
H3_Av
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H3_Hu2
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H5_Av1
H5_Av2
H6_Av
H7_Av
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H15_Av
H16_Av

FIG. 1C

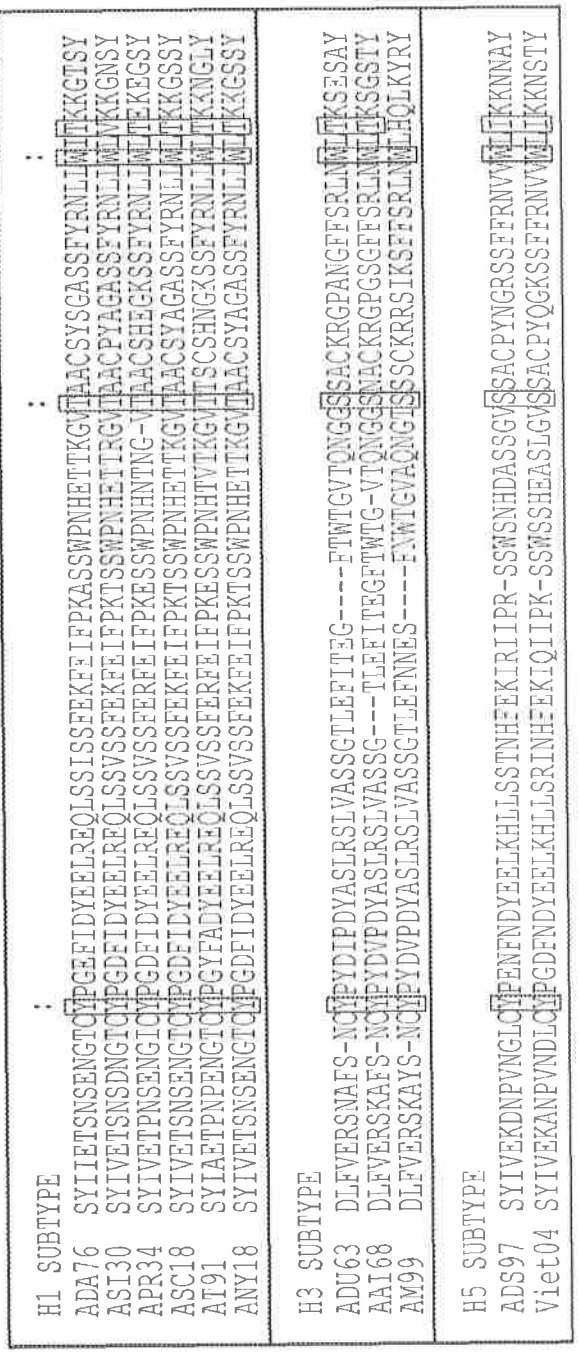

98
136
153
H1 SUBTYPE
ADA76 SYIIETSNSENGTCYPGEFIDYEELREQLSSISSFEKFEIFPKASSWPNHETTKGVTAACSYSGASSFYRNLLWLTKKGTSY
AST30 SYIVETSNSDNGTCYPGDFIDYEELREQLSSVSSFEKFEIFPKTSSWPNHETTRGVTAACPYAGASSFYRNLLWLVKKGNSY
APR34 SYIVETPNSENGICYPGDFIDYEELREQLSSVSSFERFEIFPKESSWPNHNTNG-VTAACSHEGKSSFYRNLLWLTEKEGSY
ASC18 SYIVETSNSENGTCYPGDFIDYEELREQLSSVSSFEKFEIFPKTSSWPNHETKGVTAACSYAGASSFYRNLLWLTKKGSSY
AT91 SYIAETPNPENGTCYPGYFADYEELREQLSSVSSFERFEIFPKESSWPNHTVTKGVTTSCSHNGKSSFYRNLLWLTKKNGLY
ANY18 SYIVETSNSENGTCYPGDFIDYEELREQLSSVSSFEKFEIFPKTSSWPNHETTKGVTAACSYAGASSFYRNLLWLTKKGSSY
H3 SUBTYPE
ADU63 DLFVERSNAFS-NCYPYDIPDYASLRSLVASSGTLEFITEG----FTWTGVTQNGGSSACKRGPANGFFSRLNWLTKSESAY
AAI68 DLFVERSKAFS-NCYPYDVPDYASLRSLVASSG---TLEFITEGFTWTG-VTQNGGSNACKRGPGSGFFSRLNWLTKSGSTY
AM99 DLFVERSKAYS-NCYPYDVPDVASLRSLVASSGTLEFNNES----FNWTGVAQNGTSSSCKRRSIKSFFSRLNWLHQLKYRY
H5 SUBTYPE
ADS97 SYIVEKDNPVNGLCYPFENFNDVEELKHLLSSTNHFEKIRIIPR-SSWSNHDASSGVSSACPYNGRSSFFRNVVWLIKKNNAY
Viet04 SYIVEKANPVNDLCYPGDFNDYEELKHLLSRINHFEKIQIIPK-SSWSSHEASLGVSSACPYQGKSSFFRNVVWLIKKNSTY

FIG. 2A

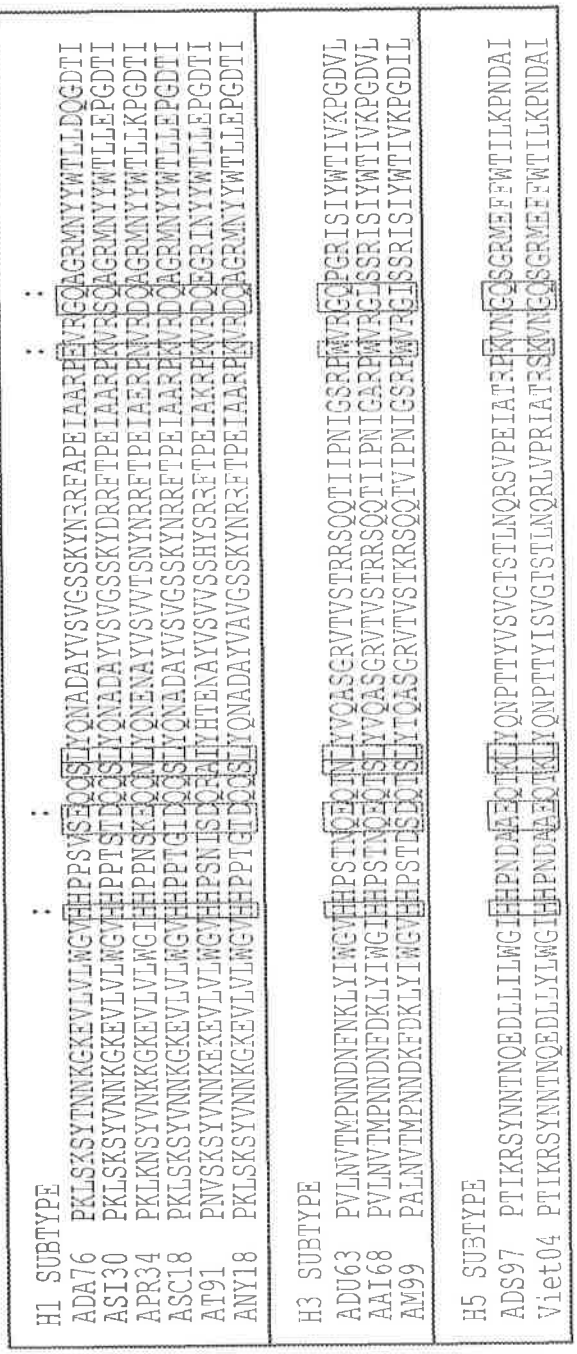

183 190 222 226
H1 SUBTYPE
ADA76 PKLSKSYTNNKGKEVLVLWGVHHPSVEIQDQKSLYQNADAYVSVGSSKYNRRFAPEIAARPKVRDQAGRMNYYWTLLDQGDTI
ASI30 PKLSKSYVNNKGKEVLVLWGVHHPPTSTDQQSLYQNADAYVSVGSSKYDRRFTPEIAARPKVRDQAGRMNYYWTLLEPGDTI
APR34 PKLKNSYVNKKGKEVLVLWGIHHPPNSKEQQNLYQNENAYVSVVTSNYNRRFTPEIAERPKVRDQAGRMNYYWTLLKPGDTI
ASC18 PKLSKSYVNNKGKEVLVLWGVHHPPTGTDQQSLYQNADAYVSVGSSKYNRRFTPEIAARPKVRDQAGRMNYYWTLLEPGDTI
AT91 PNVSKSYVNNKEKEVLVLWGVHHPSNISDQEALYHTENAYVSVVSSHYSRRFTPEIAKRPKVRDQEGRINYYWTLLEPGDTI
ANY18 PKLSKSYVNNKGKEVLVLWGVHHPPTGTDQQSLYQNADAYVAVGSSKYNRRFTPEIAARPKVRDQAGRMNYYWTLLEPGDTI
H3 SUBTYPE
ADU63 PVLNVTMPNNDNFNKLYIWGVHHPSTNQEQTNLYVQASGRVTVSTRRSQQTIIPNIGSRPWVRGQSGRISIYWTIVKPGDVL
AAI68 PVLNVTMPNNDNFDKLYIWGIHHPSTNQEQTSLYVQASGRVTVSTRRSQQTIIPNIGARPWVRGLSSRISIYWTIVKPGDVL
AM99 PALNVTMPNNDKFDKLYIWGVHHPSTDSDQTSLYTQASGRVTVSTKRSQQTVIPNIGSRPWVRGISSRISIYWTIVKPGDIL
H5 SUBTYPE
ADS97 PTIKRSYNNTNQEDLLILWGIHHPNDAAEQTKLYQNPTTYVSVGTSTLNQRSVPEIATRPKVNGQSGRMEFFWTILKPNDAI
Viet04 PTIKRSYNNTNQEDLLYLWGIHHPNDAAEQTKLYQNPTTYISVGTSTLNQRLVPRIATRSKVNGQSGRMEFFWTILKPNDAI

FIG. 2B

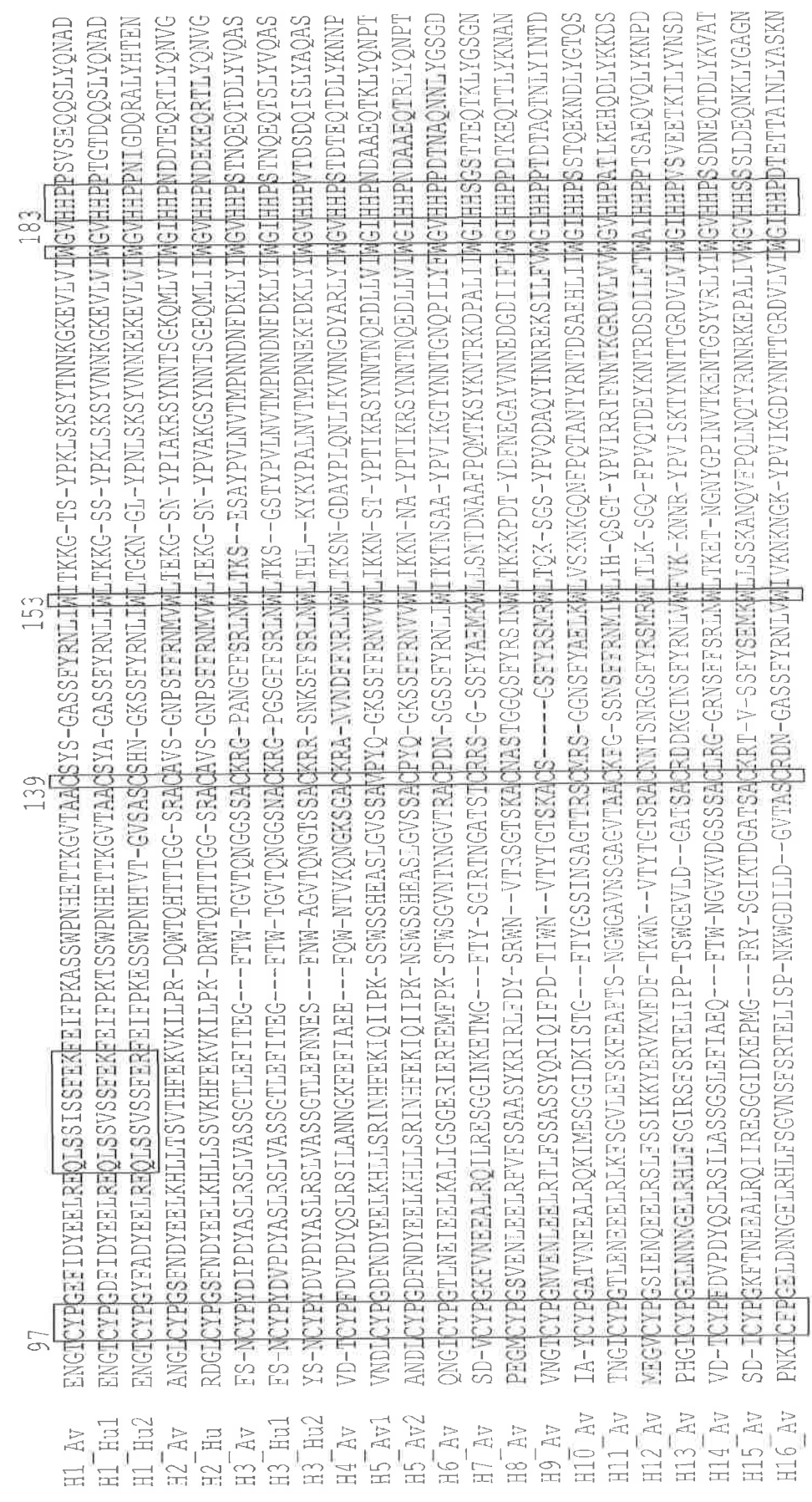

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H1_Hu2
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H2_Hu
H3_Av
H3_Hu1
H3_Hu2
H4_Av
H5_Av1
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H6_Av
H7_Av
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H13_Av
H14_Av
H15_Av
H16_Av

FIG. 3A

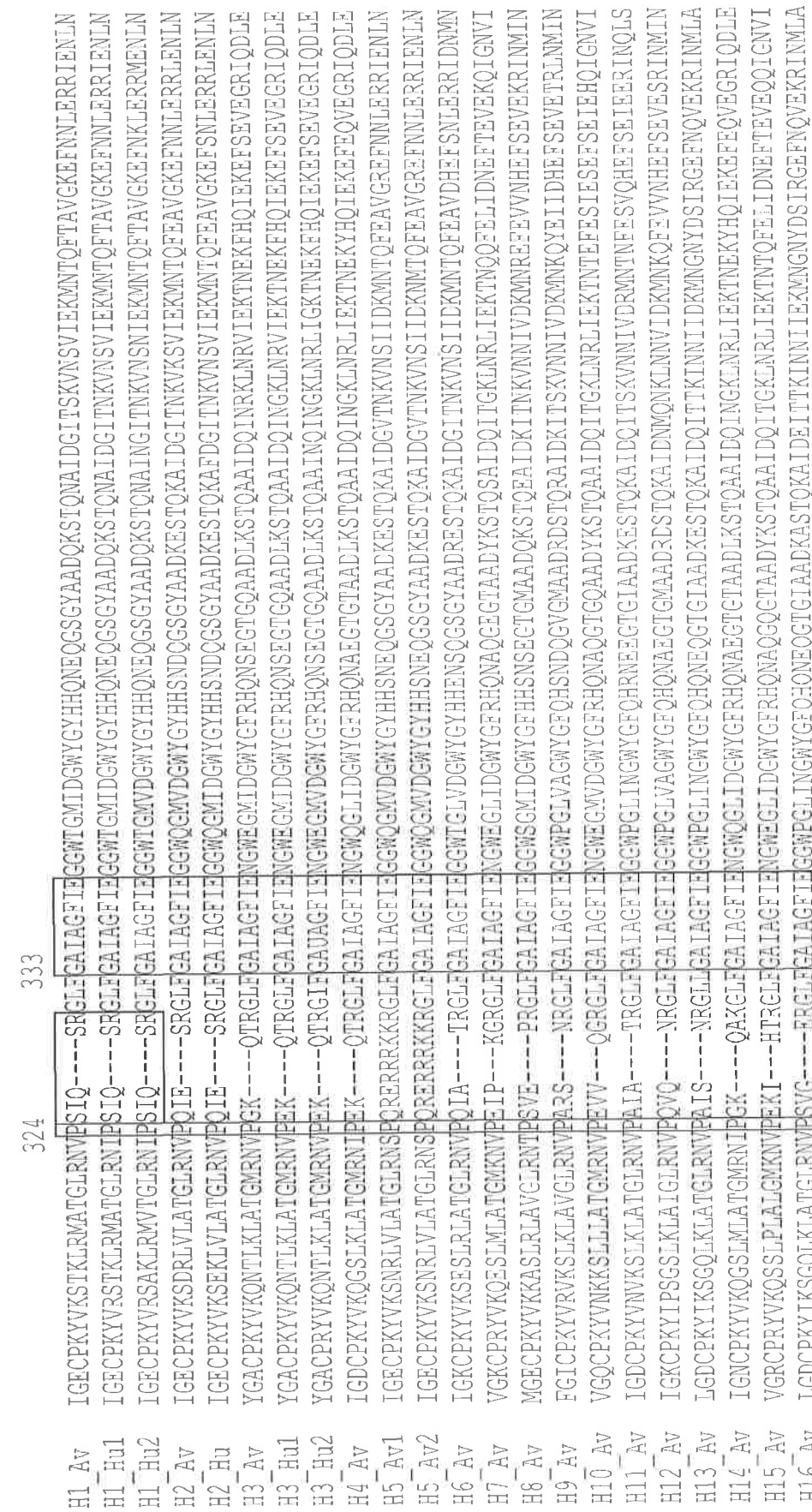

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H1_Hu1
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H2_Hu
H3_Av
H3_Hu1
H3_Hu2
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H5_Av1
H5_Av2
H6_Av
H7_Av
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H14_Av
H15_Av
H16_Av

FIG. 3B

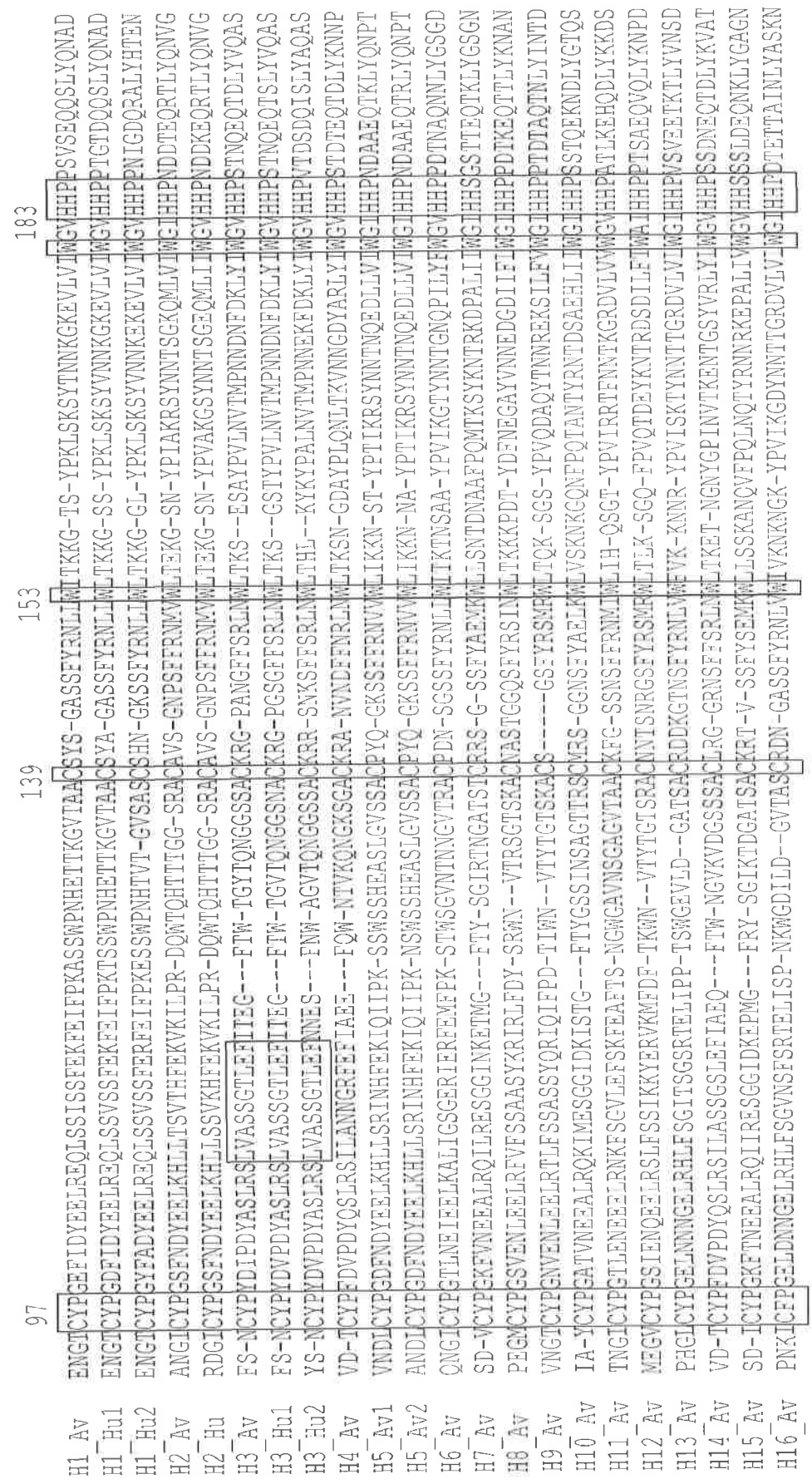

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139
153
183
H1_Av
H1_Hu1
H1_Hu2
H2_Av
H2_Hu
H3_Av
H3_Hu1
H3_Hu2
H4_Av
H5_Av1
H5_Av2
H6_Av
H7_Av
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H13_Av
H14_Av
H15_Av
H16_Av

FIG. 4A

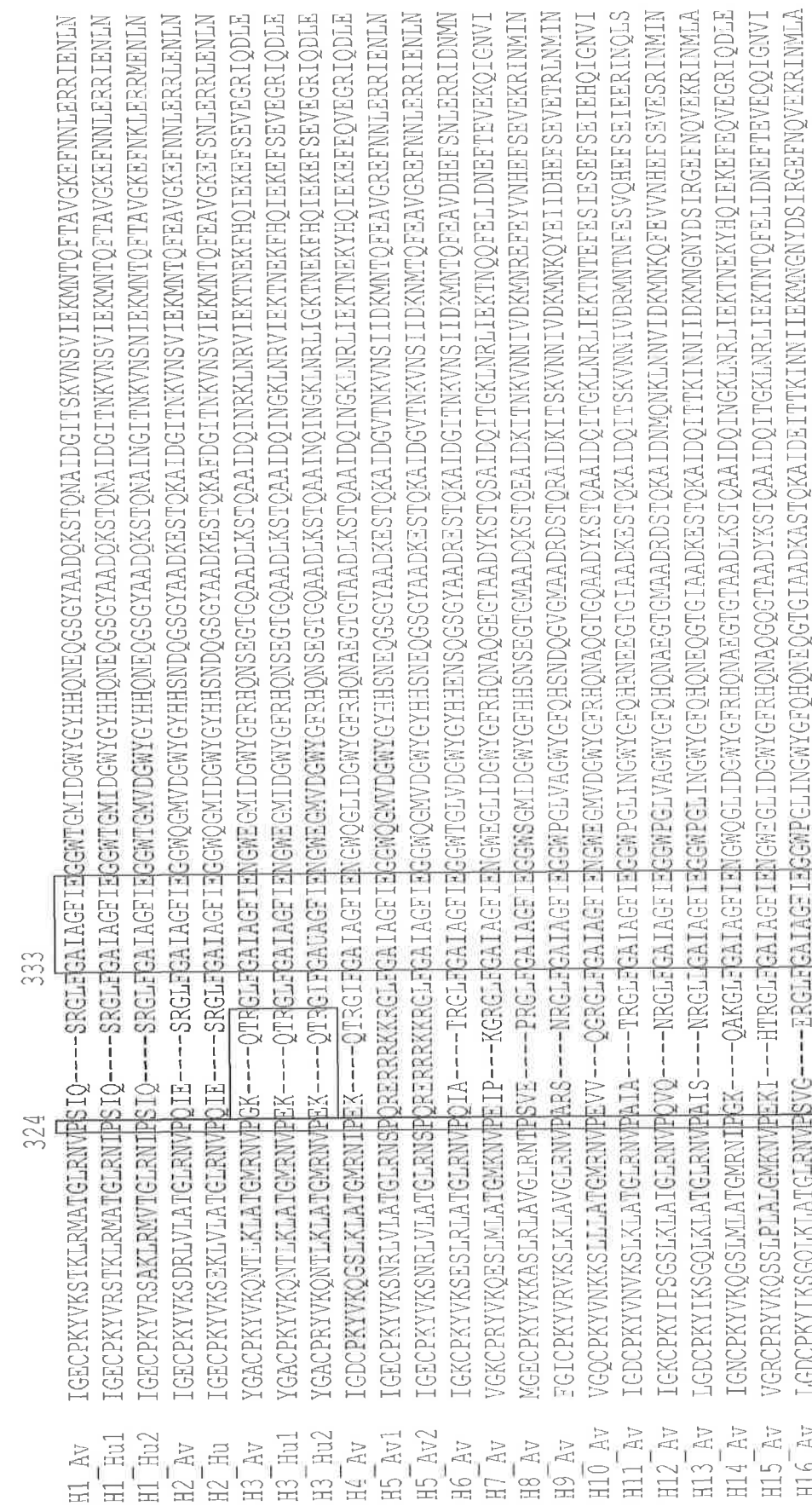

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333
H1_Av
H1_Hu1
H1_Hu2
H2_Av
H2_Hu
H3_Av
H3_Hu1
H3_Hu2
H4_Av
H5_Av1
H5_Av2
H6_Av
H7_Av
H8_Av
H9_Av
H10_Av
H11_Av
H12_Av
H13_Av
H14_Av
H15_Av
H16_Av

FIG. 4B

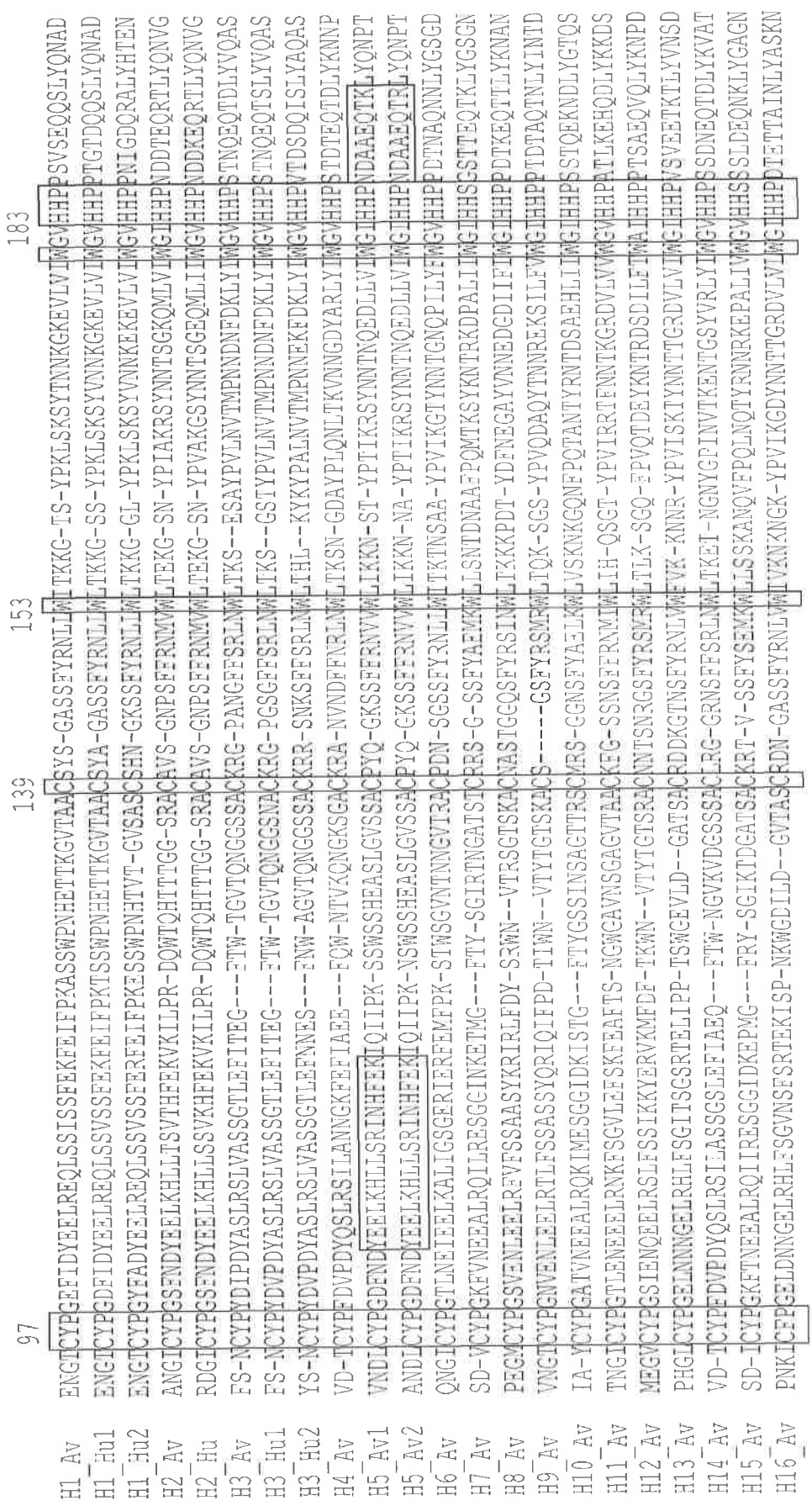

97
139
153
183
H1_Av
H1_Hu1
H1_Hu2
H2_Av
H2_Hu
H3_Av
H3_Hu1
H3_Hu2
H4_Av
H5_Av1
H5_Av2
H6_Av
H7_Av
H8_Av
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H11_Av
H12_Av
H13_Av
H14_Av
H15_Av
H16_Av

FIG. 5A-1

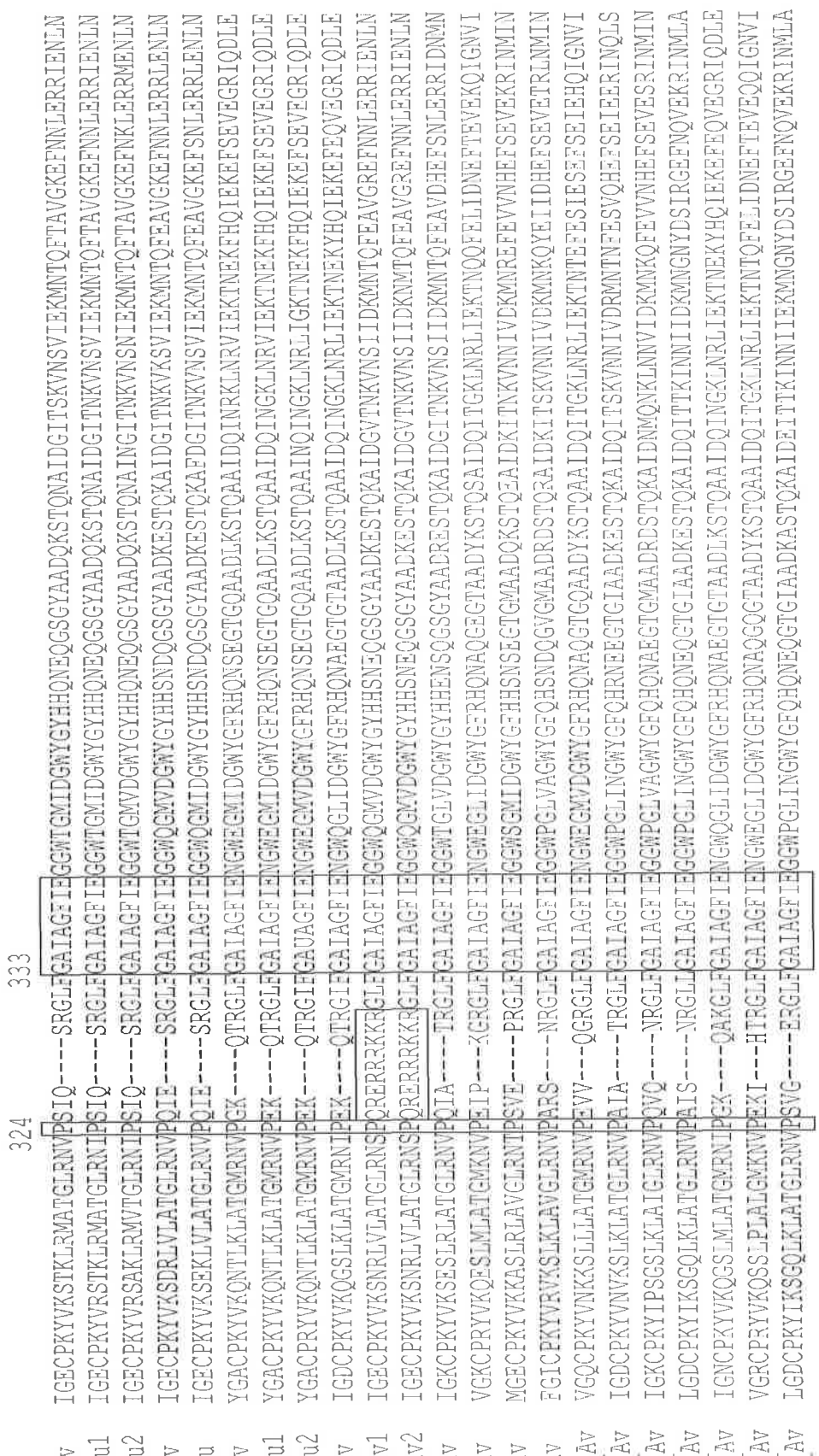

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333
H1_Av
H1_Hu1
H1_Hu2
H2_Av
H2_Hu
H3_Av
H3_Hu1
H3_Hu2
H4_Av
H5_Av1
H5_Av2
H6_Av
H7_Av
H8_Av
H9_Av
H10_Av
H11_Av
H12_Av
H13_Av
H14_Av
H15_Av
H16_Av

FIG. 5A-2

```
POSITIONS FROM 1 TILL 60
CONSENSUS       MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDL
AAL59142        ..............................................................
AAZ29963        .......E......................................................
ABA70758        .......F......................................................
ABB87042        ..R..IA...I.I..G..............K...............E.....S.
ABD14810        ..............................................................
ABD46740        ---...........................................................
ABD85144        -------F......................................................
ABE97569        ..............................................................

POSITIONS FROM 61 TILL 120
CONSENSUS       DGVKPLILRDCSVAGWLLGNPMCDEFINVPEWSYIVEKANPANDLCYPGDFNDYEELKEL
AAL59142        ..............................S.D.............................
AAZ29963        ..............................V...............................
ABA70758        ..............................V...............................
ABB87042        K..R...K.....................L.....D..I.G....................
ABD14810        .....K........................................I.............
ABD46740        ..............................L.....I.........N.............
ABD85144        ..............................L.....I.........N.............
ABE97569        ..............................................N.............
```

FIG. 5B-1

```
POSITIONS FROM 121 TILL 180
CONSENSUS    LSRINHFEKIQIIPKSSWSDHEASSGVSSACPYQGKSSFFRNVVWLIKKNSAYPTIKRSY
AAL59142     ....................N.....................H.....................
AAZ29963     ....................S.....L.....................T.....
ABA70758     ....................S.....L.....................P.....T.....
ABB87042     ....................R.....N.D.....................N.R.....N.....T.
ABD14810     ....................................................I..
ABD46740     ....................................R.....................DN.....
ABD85144     ....................................R.....................DN.....
ABE97569     ....................................L.....................

POSITIONS FROM 181 TILL 240
CONSENSUS    NNTNQEDLLVLWGIHHPNDAAEQTKLYQNPTTYISVGTSTLNQRLVPKIATRSKVNGQSG
AAL59142     ....................................................
AAZ29963     ....................R.....
ABA70758     .....V.....R.....
ABB87042     ....I.....I.....SN..V.....SI.E.....P.....
ABD14810     ....................R.....
ABD46740     ....................R.....
ABD85144     ....................R.....
ABE97569     ....................R.....
```

FIG. 5B-2

```
POSITIONS FROM 241 TILL 300
CONSENSUS       RMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSTIMKSELEYGNCNTKCQTPMGA
AAL59142        ..............................A.............................
AAZ29963        ..............................................................
ABA70758        ..............................................................
ABB87042        ..............S...............A.............D.....V..
ABD14810        ..............V...............................D..............
ABD46740        ..............................N...............................I..
ABD85144        ..............S...............N...............................I..
ABE97569        ..............................A...............................

POSITIONS FROM 301 TILL 360
CONSENSUS       INSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRNSPQRERRKKRGLFGAIAGFIEGGW
AAL59142        ..............................T....G.........................
AAZ29963        ..............................................K...............
ABA70758        ..............................................................
ABB87042        ..............V...............DK.....V....----T..............
ABD14810        ..............................................-...............
ABD46740        ..............................................G...............
ABD85144        ..............................................G...............
ABE97569        ..............................................................
```

FIG. 5B-3

```
POSITIONS FROM 361 TILL 420
CONSENSUS       QGMVDGWYGYHHSNEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLE
AAL59142        ..................................................K..
AAZ29963        ..................................................
ABA70758        ..........K.......................................
ABB87042        ..................I...............T..K.....
ABD14810        ..................................................
ABD46740        ..................................................
ABD85144        ..................................................
ABE97569        ..................................................

POSITIONS FROM 421 TILL 480
CONSENSUS       RRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELG
AAL59142        ..................................................
AAZ29963        ..................................................
ABA70758        ..................................................
ABB87042        ..................................................
ABD14810        ..................................................
ABD46740        ..................................................
ABD85144        ..................................................
ABE97569        ..................................................
```

FIG. 5B-4

```
POSITIONS FROM 481 TILL 540
CONSENSUS       NGCTEEYHKCDNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGTYQILSIYSTVA
AAL59142        .....K.....N.....M.....
AAZ29963        .....I.....
ABA70758        .....I.....
ABB87042        .....S..N...D.....M.....
ABD14810        ..........
ABD46740        .....R.....
ABD85144        .....R.....
ABE97569        .....I.....N.....I.....

POSITIONS FROM 541 TILL 568
CONSENSUS       SSLALAIMVAGLSLWMCSNGSLQCRICI
AAL59142        ..........
AAZ29963        ..........----------
ABA70758        ..........
ABB87042        .....F.....
ABD14810        ..........
ABD46740        ..........----------
ABD85144        .....F.....VTM---
ABE97569        .....M....----------
```

FIG. 5B-5

AAL59142: 568 Avian 4 (HA) H5N1 Hong Kong 2000 Influenza A virus (A/Goose/Hong Kong/385.3.2000(H5N1))

AAZ29963: 556 Avian 4 (HA) H5N1 Thailand 2004 Influenza A virus (A/Ostrich/Samut Prakan/Thailand/CU-19/04(H5N1))

ABA70758: 568 Avian 4 (HA) H5N1 Belgium 2004 Influenza A virus (A/crested eagle/Belgium/01/2004(H5N1))

ABB87042: 564 Avian 4 (HA) H5N2 Canada 1976/08/12 Influenza A virus (A/mallard duck/ALB/57/1976(H5N2))

ABD14810: 567 Avian 4 (HA) H5N1 China 2004 Influenza A virus (A/duck/Guangxi/13/2004(H5N1))

ABD46740: 556 Avian 4 (HA) H5N1 Nigeria 2006/01/17 Influenza A virus (A/chicken/Nigeria/641/2006(H5N1))

ABD85144: 557 Avian 4 (HA) H5N1 Egypt 2006 Influenza A virus (A/chicken/Egypt/960N3-004/2006(H5N1))

ABE97569: 553 Avian 4 (HA) H5N1 Indonesia 2004 Influenza A virus (A/turkey/Kedaton/BPPV3/2004(H5N1))

FIG. 5B-6

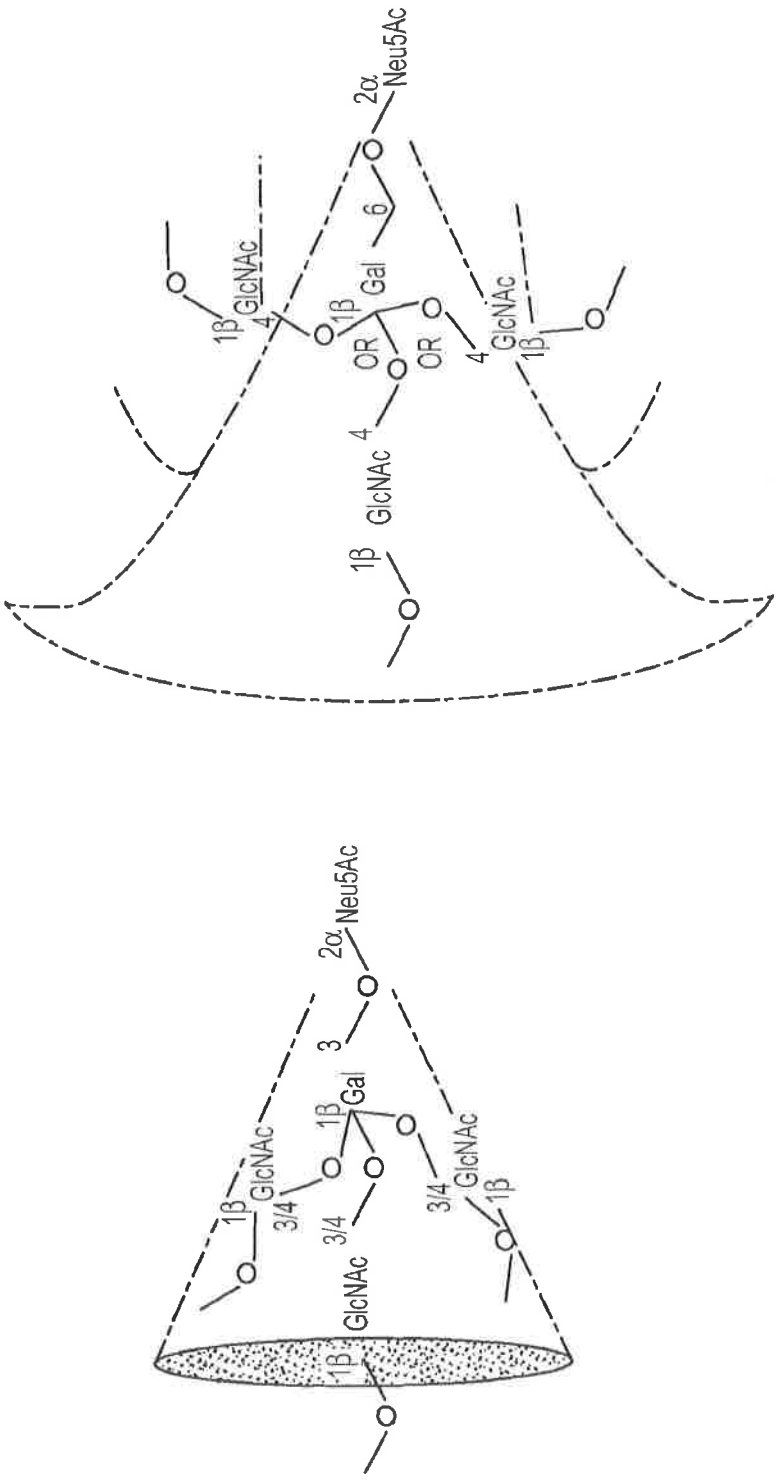

2α Neu5Ac
6
1β GlcNAc 4
1β Gal
OR
OR
4 GlcNAc 1β
4 GlcNAc 1β
2α Neu5Ac
3
1β GlcNAc 3/4
1β Gal
3/4
3/4
3/4 GlcNAc 1β
GlcNAc 1β

FIG. 6A

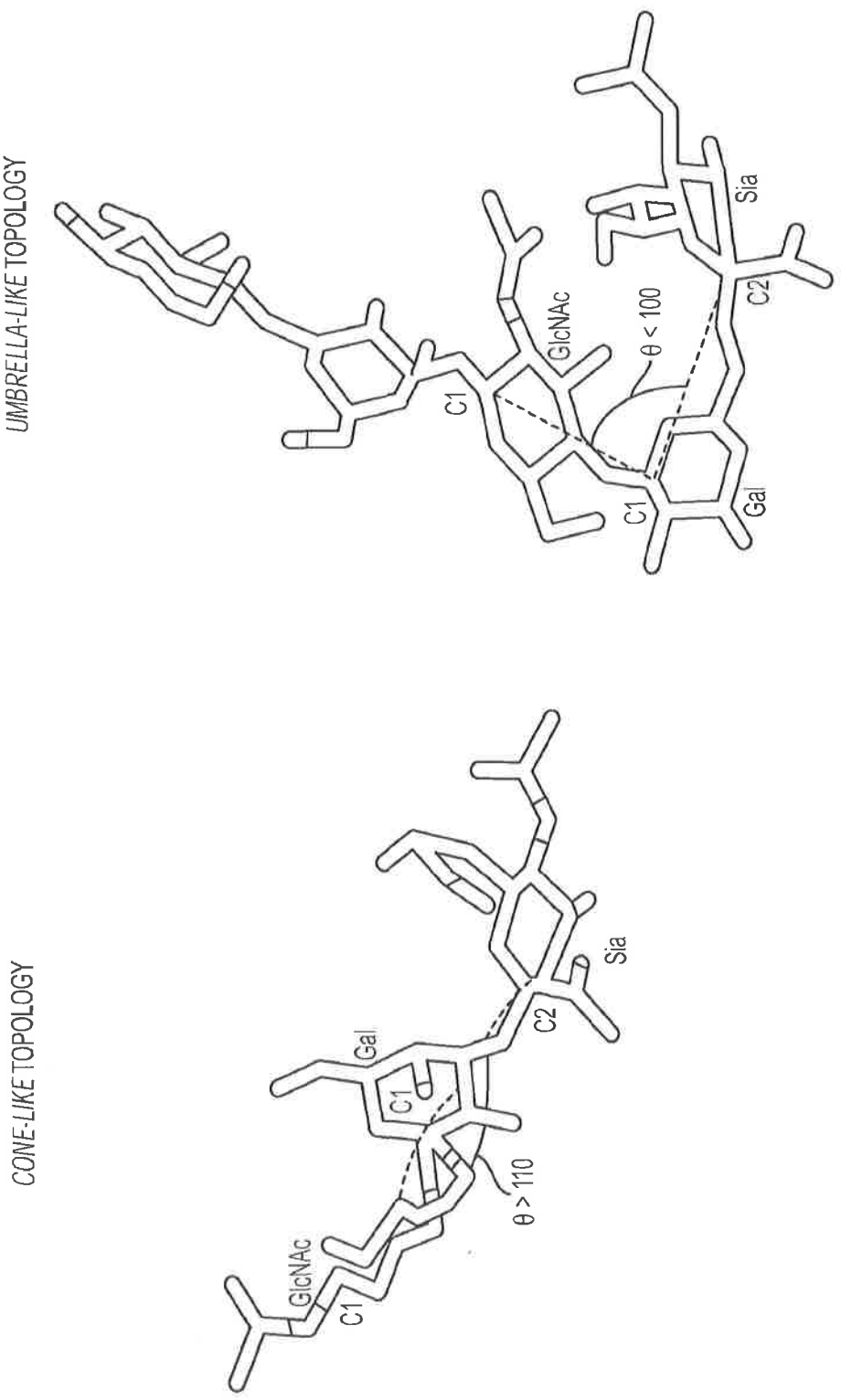

UMBRELLA-LIKE TOPOLOGY
C1
GlcNAc
θ < 100
C2
Sia
C1
Gal
CONE-LIKE TOPOLOGY
GlcNAc
C1
θ > 110
C1
Gal
C2
Sia

FIG. 6B-1

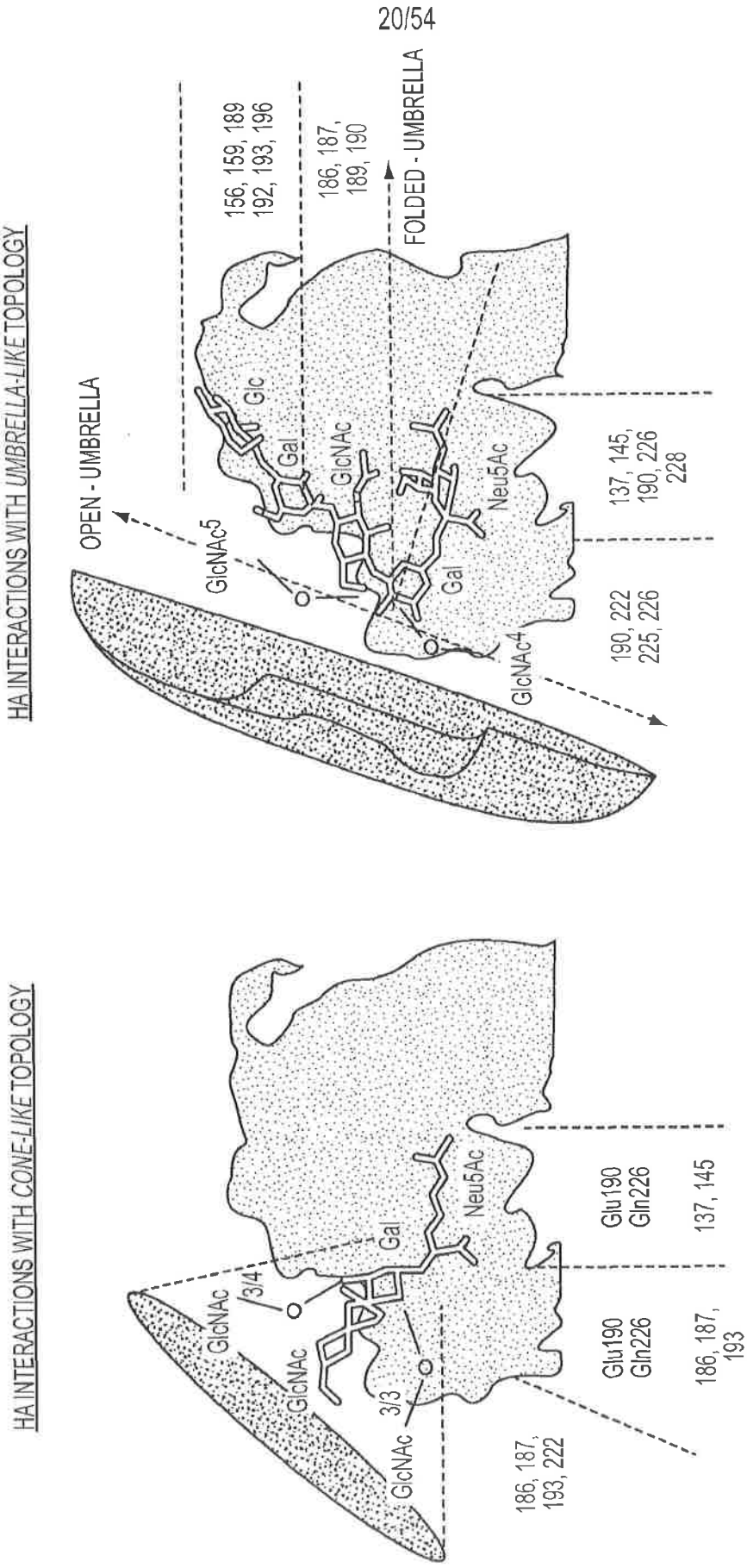

HA INTERACTIONS WITH UMBRELLA-LIKE TOPOLOGY
OPEN - UMBRELLA
FOLDED - UMBRELLA
156, 159, 189
192, 193, 196
186, 187,
189, 190
Glc
Gal
GlcNAc
Neu5Ac
GlcNAc5
GlcNAc4
137, 145,
190, 226
228
190, 222
225, 226
HA INTERACTIONS WITH CONE-LIKE TOPOLOGY
GlcNAc
3/4
GlcNAc
3/3
GlcNAc
Gal
Neu5Ac
Glu190
Gln226
137, 145
Glu190
Gln226
186, 187,
193
186, 187,
193, 222

FIG. 6B-2

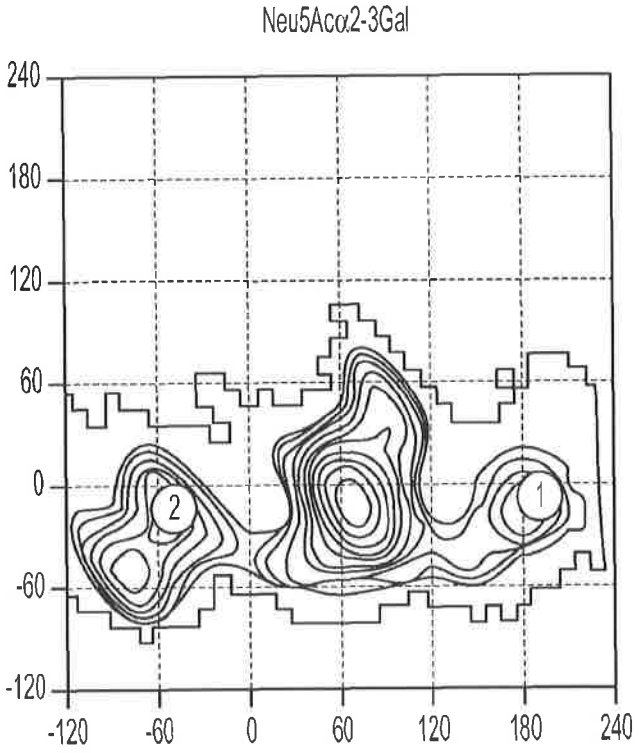


FIG. 6C-A

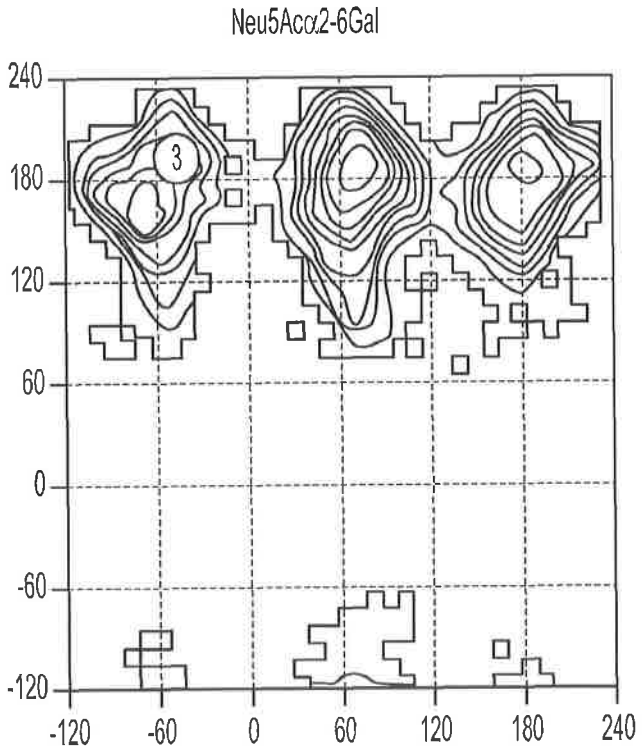


FIG. 6C-B

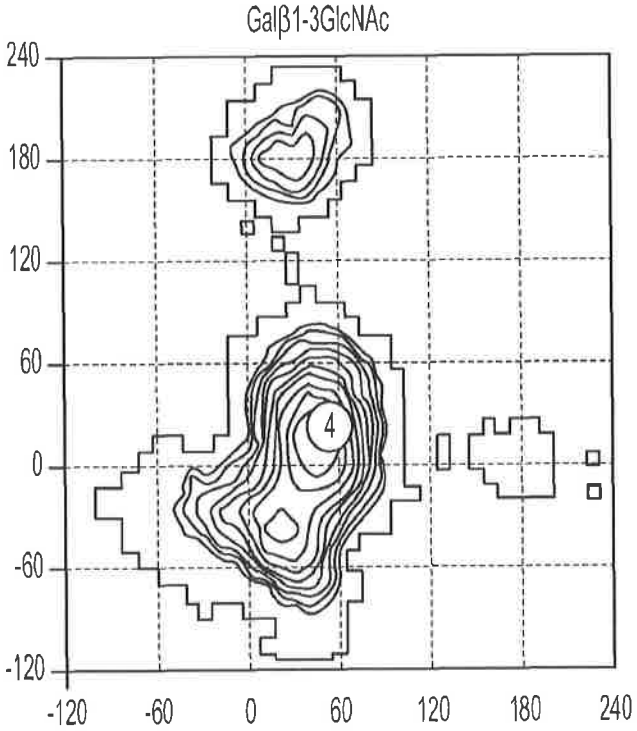


FIG. 6C-C

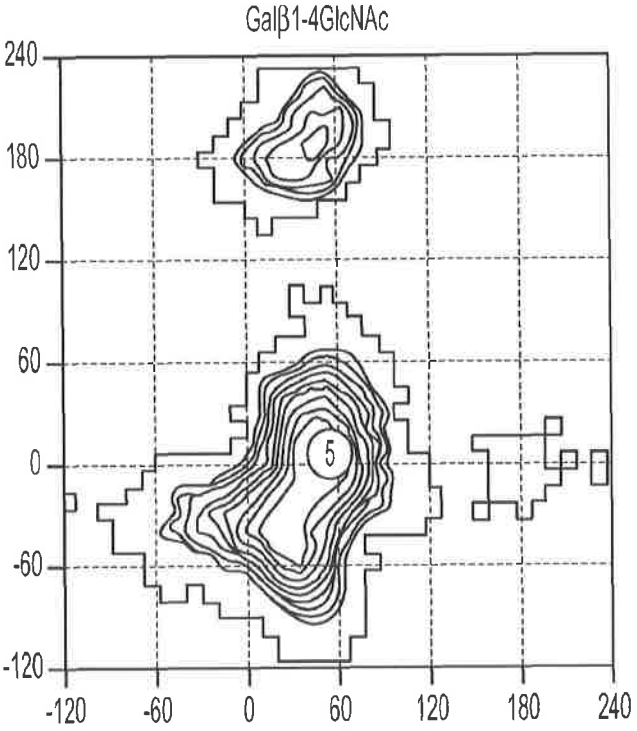


FIG. 6C-D

CONFORMATIONAL SAMPLING OF α2-3 LINKAGE

CONE-LIKE	100%
UMBRELLA-LIKE	0%

FIG. 6C-E

CONFORMATIONAL SAMPLING OF α2-6 LINKAGE

	UMBRELLA-LIKE (%) : *CONE*-LIKE (%)
ω = - 60°	60 : 20
ω = + 60°	10 : 40
ω = 180°	30 : 30

FIG. 6C-F

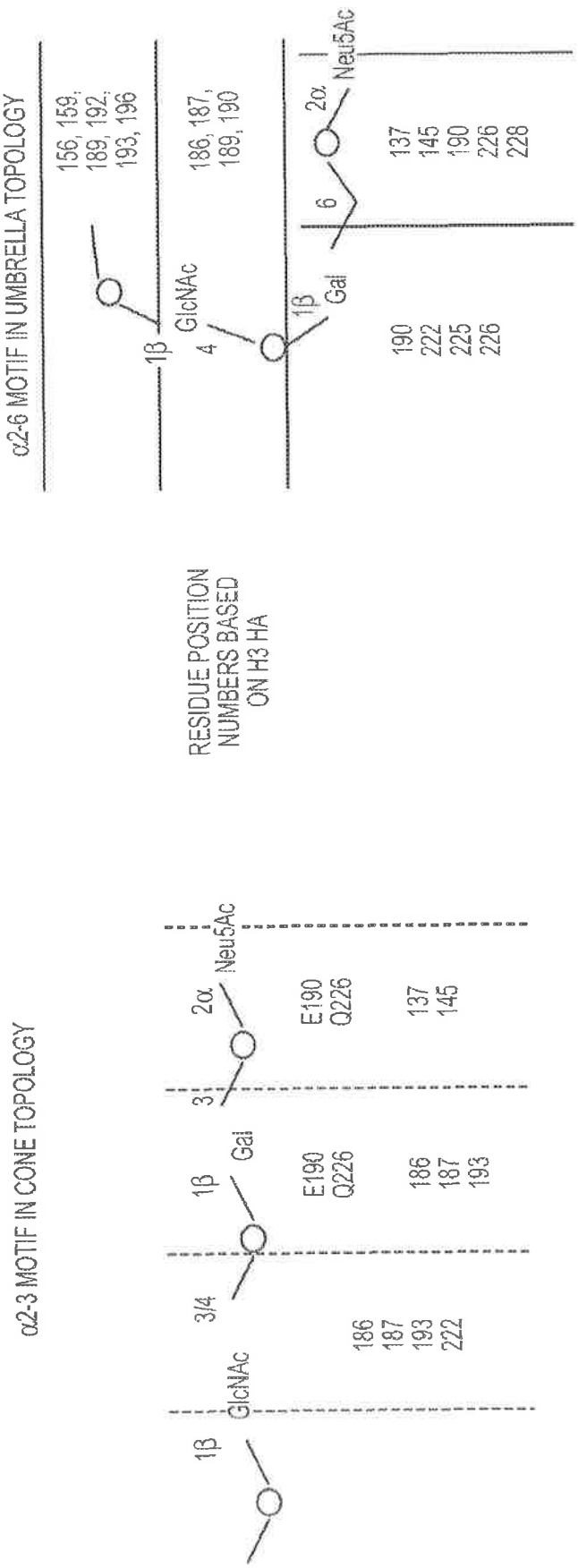

α2-6 MOTIF IN UMBRELLA TOPOLOGY
156, 159, 189, 192, 193, 196
186, 187, 189, 190
137 145 190 226 228
190 222 225 226
1β GlcNAc
4
1β Gal
6
2α Neu5Ac
RESIDUE POSITION NUMBERS BASED ON H3 HA
α2-3 MOTIF IN CONE TOPOLOGY
1β GlcNAc
3/4
186 187 193 222
1β Gal
E190 Q226
186 187 193
3
2α Neu5Ac
E190 Q226
137 145

FIG. 7

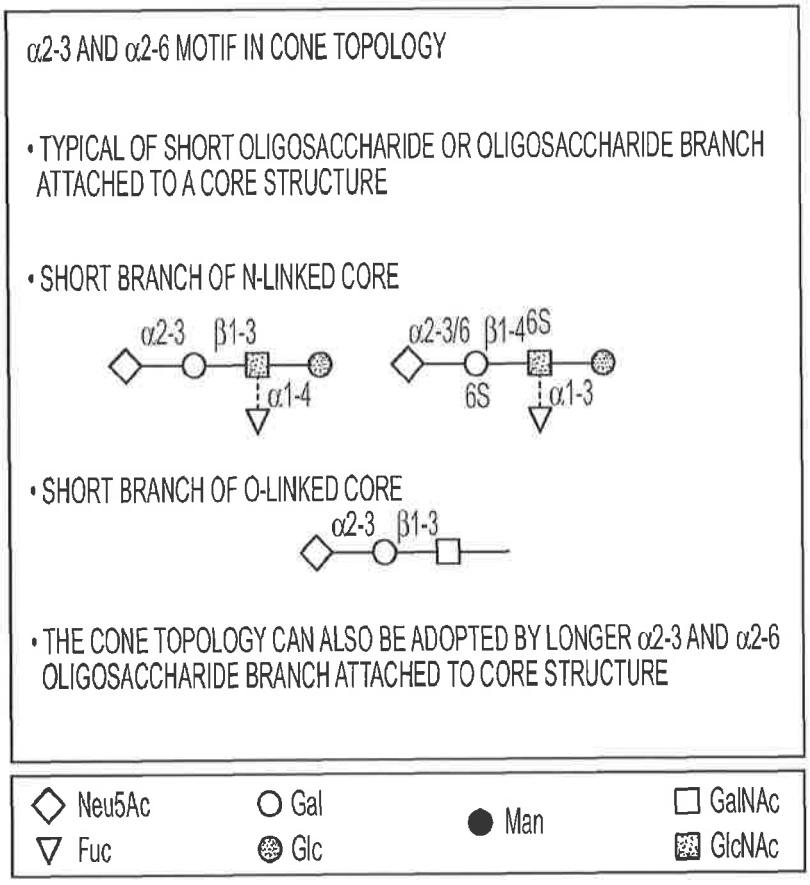

α2-3 AND α2-6 MOTIF IN CONE TOPOLOGY
• TYPICAL OF SHORT OLIGOSACCHARIDE OR OLIGOSACCHARIDE BRANCH ATTACHED TO A CORE STRUCTURE
• SHORT BRANCH OF N-LINKED CORE
α2-3 β1-3 α1-4
α2-3/6 β1-4 6S 6S α1-3
• SHORT BRANCH OF O-LINKED CORE
α2-3 β1-3
• THE CONE TOPOLOGY CAN ALSO BE ADOPTED BY LONGER α2-3 AND α2-6 OLIGOSACCHARIDE BRANCH ATTACHED TO CORE STRUCTURE
Neu5Ac
Gal
Man
GalNAc
Fuc
Glc
GlcNAc
DOTTED GRAY LINES, 4S AND 6S INDICATE POTENTIAL SITES FOR FUCOSYLATION AND SULFATION MODIFICATIONS

FIG. 8

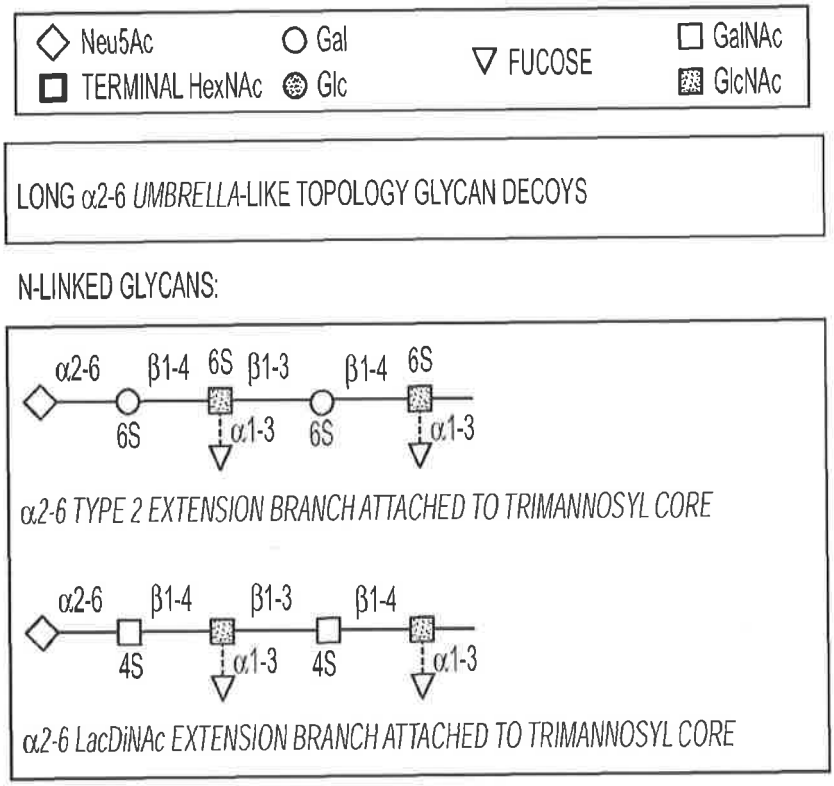
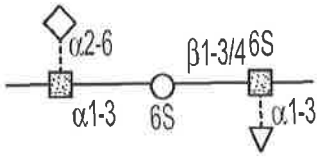

Neu5Ac
Gal
FUCOSE
GalNAc
TERMINAL HexNAc
Glc
GlcNAc
LONG α2-6 UMBRELLA-LIKE TOPOLOGY GLYCAN DECOYS
N-LINKED GLYCANS:
α2-6
β1-4
6S
β1-3
β1-4
6S
6S
α1-3
6S
α1-3
α2-6 TYPE 2 EXTENSION BRANCH ATTACHED TO TRIMANNOSYL CORE
α2-6
β1-4
β1-3
β1-4
4S
α1-3
4S
α1-3
α2-6 LacDiNAc EXTENSION BRANCH ATTACHED TO TRIMANNOSYL CORE

FIG. 9A-1

LONG α2-6 *UMBRELLA*-LIKE TOPOLOGY GLYCANS THAT ARE NOT DECOYS

N-LINKED GLYCANS:



α2-6 LINKAGE ON GlcNAc OF TYPE 1/TYPE 2 EXTENSION

FIG. 9A-2

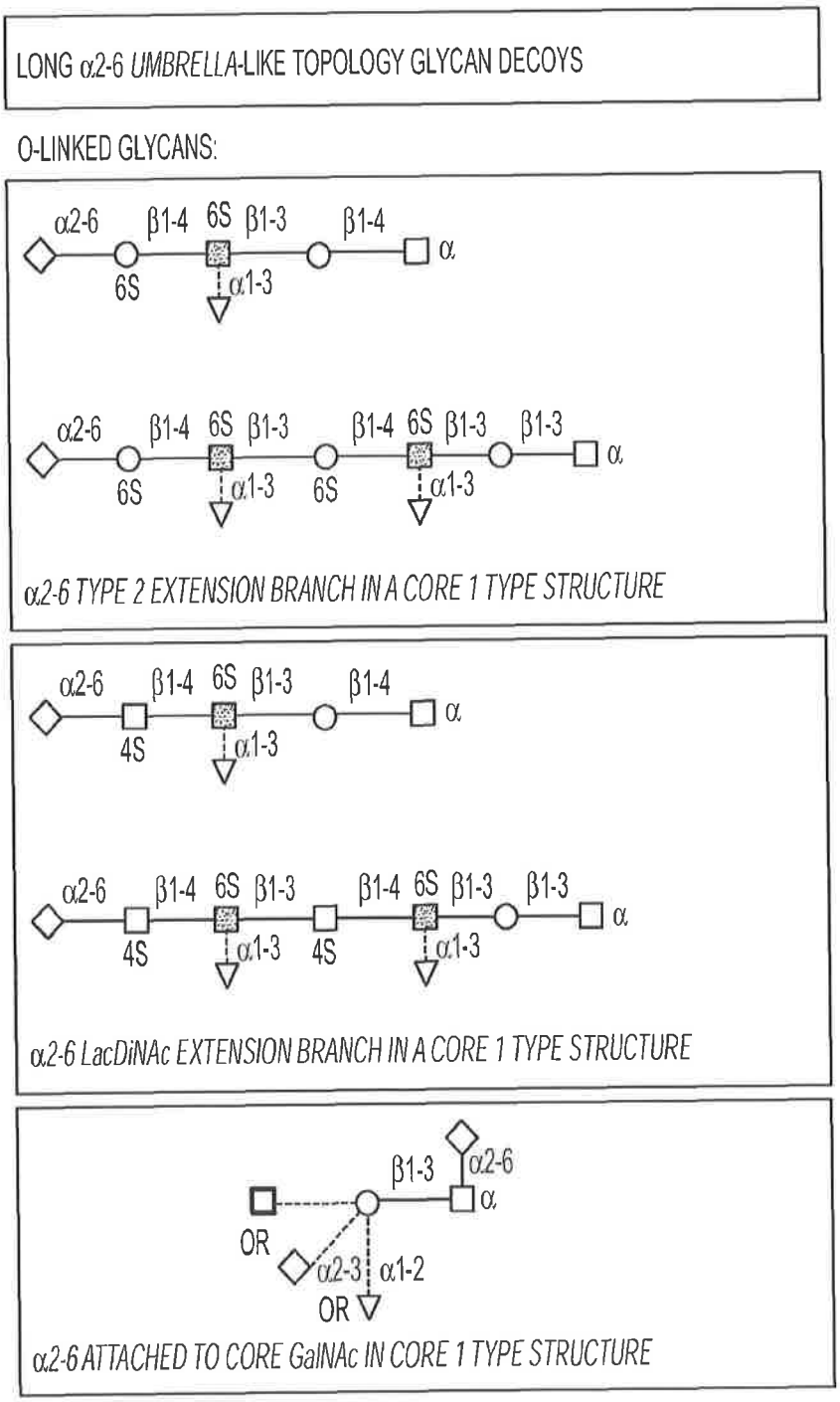

LONG α2-6 UMBRELLA-LIKE TOPOLOGY GLYCAN DECOYS
O-LINKED GLYCANS:
α2-6 β1-4 6S β1-3 β1-4 α
6S α1-3
α2-6 β1-4 6S β1-3 β1-4 6S β1-3 β1-3 α
6S α1-3 6S α1-3
α2-6 TYPE 2 EXTENSION BRANCH IN A CORE 1 TYPE STRUCTURE
α2-6 β1-4 6S β1-3 β1-4 α
4S α1-3
α2-6 β1-4 6S β1-3 β1-4 6S β1-3 β1-3 α
4S α1-3 4S α1-3
α2-6 LacDiNAc EXTENSION BRANCH IN A CORE 1 TYPE STRUCTURE
β1-3 α2-6 α
OR
α2-3 α1-2
OR
α2-6 ATTACHED TO CORE GalNAc IN CORE 1 TYPE STRUCTURE

FIG. 9A-3

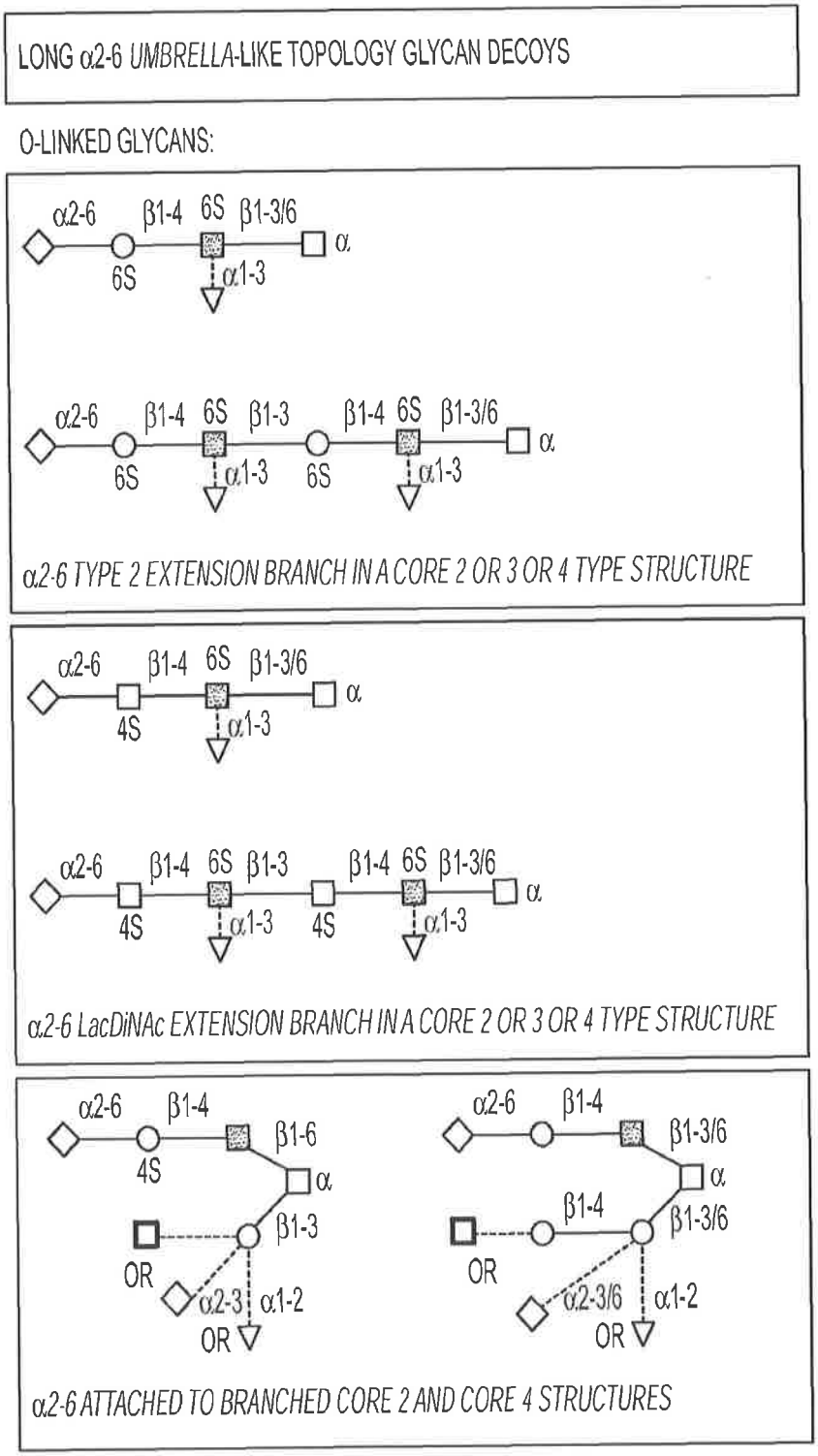
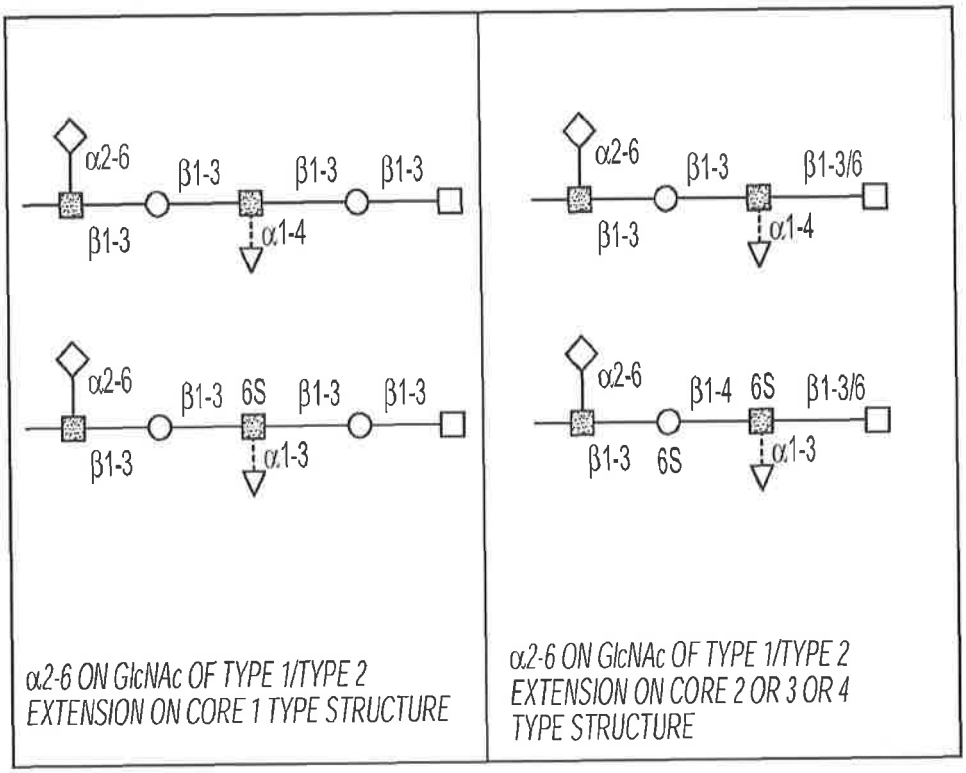

LONG α2-6 UMBRELLA-LIKE TOPOLOGY GLYCAN DECOYS
O-LINKED GLYCANS:
α2-6 β1-4 6S β1-3/6 α
6S α1-3
α2-6 β1-4 6S β1-3 β1-4 6S β1-3/6 α
6S α1-3 6S α1-3
α2-6 TYPE 2 EXTENSION BRANCH IN A CORE 2 OR 3 OR 4 TYPE STRUCTURE
α2-6 β1-4 6S β1-3/6 α
4S α1-3
α2-6 β1-4 6S β1-3 β1-4 6S β1-3/6 α
4S α1-3 4S α1-3
α2-6 LacDiNAc EXTENSION BRANCH IN A CORE 2 OR 3 OR 4 TYPE STRUCTURE
α2-6 β1-4 β1-6 α
4S
β1-3
OR
α2-3 α1-2
OR
α2-6 β1-4 β1-3/6 α
β1-4 β1-3/6
OR
α2-3/6 α1-2
OR
α2-6 ATTACHED TO BRANCHED CORE 2 AND CORE 4 STRUCTURES

FIG. 9A-4

LONG α2-6 *UMBRELLA*-LIKE TOPOLOGY GLYCAN THAT ARE NOT DECOYS

O-LINKED GLYCANS:



α2-6 ON GlcNAc OF TYPE 1/TYPE 2 EXTENSION ON CORE 1 TYPE STRUCTURE

α2-6 ON GlcNAc OF TYPE 1/TYPE 2 EXTENSION ON CORE 2 OR 3 OR 4 TYPE STRUCTURE

FIG. 9A-5

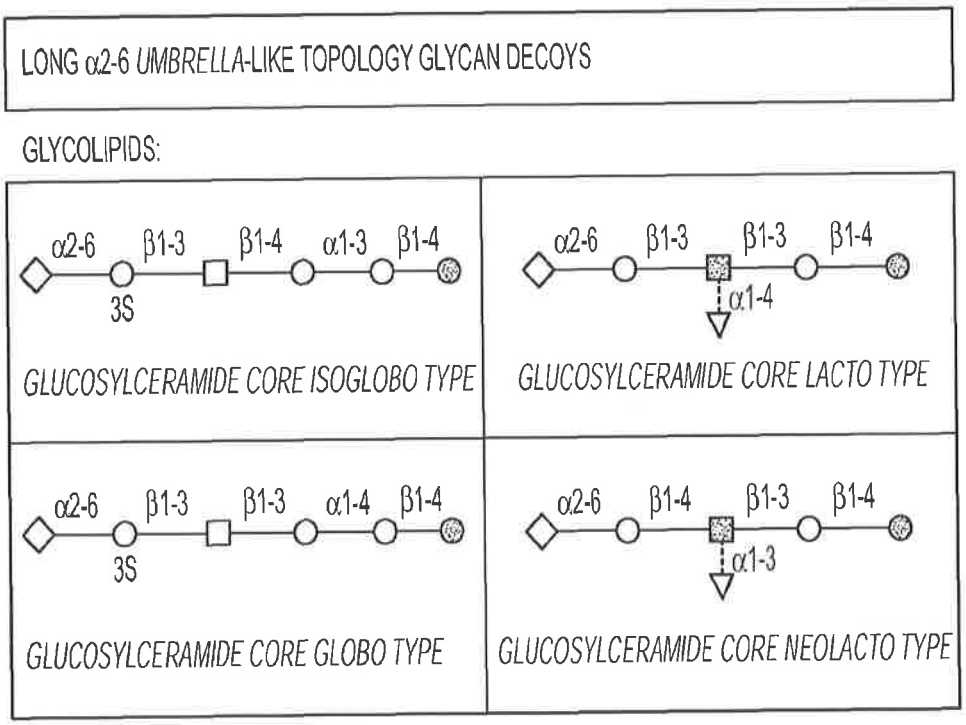
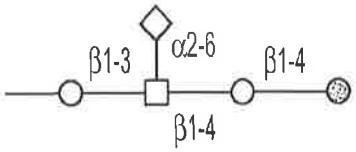

LONG α2-6 UMBRELLA-LIKE TOPOLOGY GLYCAN DECOYS
GLYCOLIPIDS:
α2-6
β1-3
β1-4
α1-3
β1-4
3S
GLUCOSYLCERAMIDE CORE ISOGLOBO TYPE
α2-6
β1-3
β1-3
β1-4
α1-4
GLUCOSYLCERAMIDE CORE LACTO TYPE
α2-6
β1-3
β1-3
α1-4
β1-4
3S
GLUCOSYLCERAMIDE CORE GLOBO TYPE
α2-6
β1-4
β1-3
β1-4
α1-3
GLUCOSYLCERAMIDE CORE NEOLACTO TYPE

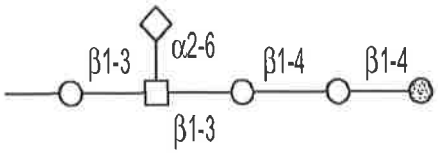
FIG. 9A-6

LONG α2-6 *UMBRELLA*-LIKE TOPOLOGY GLYCAN THAT ARE NOT DECOYS

GLYCOLIPIDS:



GLUCOSYLCERAMIDE CORE GANGLIO TYPE



GLUCOSYLCERAMIDE CORE GLOBO TYPE

FIG. 9A-7

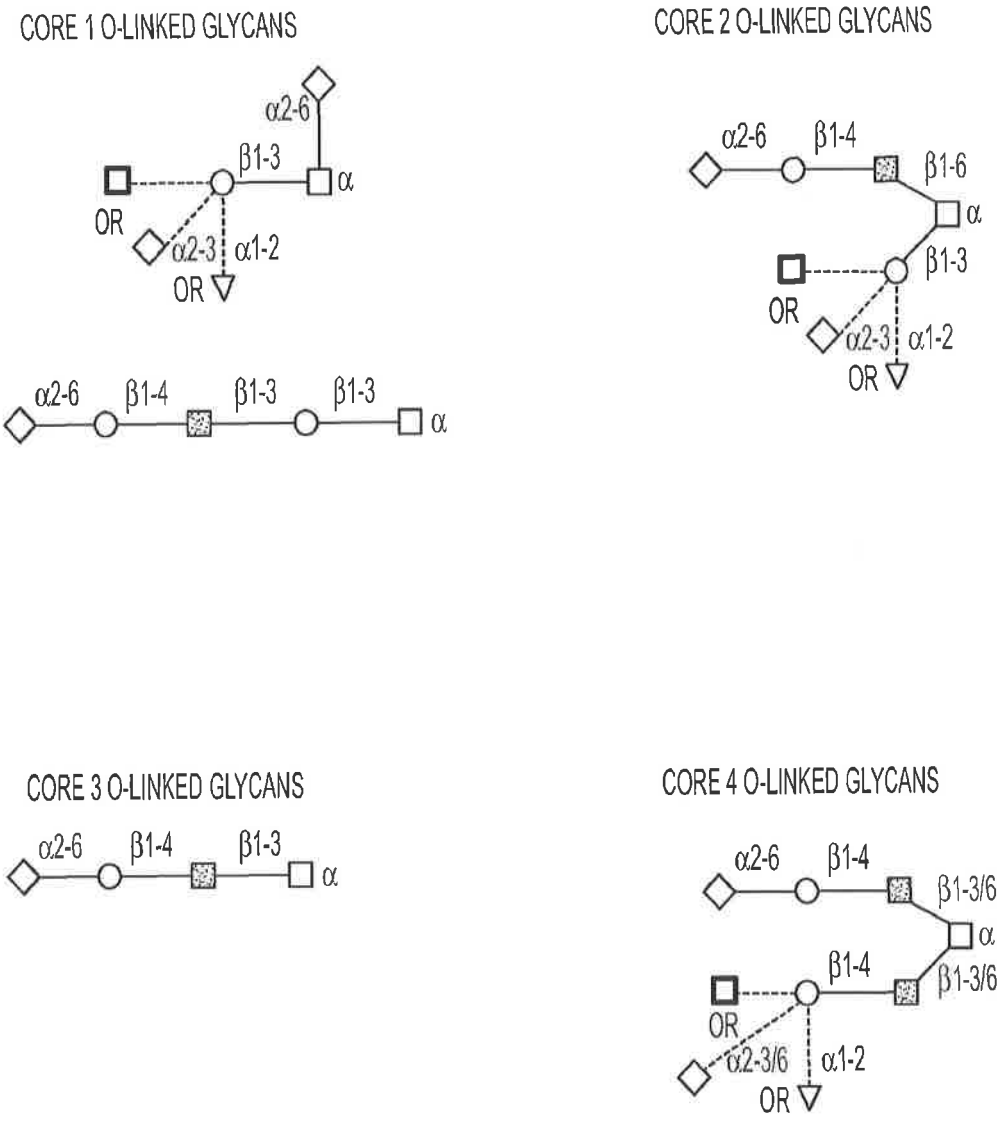

CORE 1 O-LINKED GLYCANS
α2-6
β1-3
α
OR
α2-3
α1-2
OR
α2-6
β1-4
β1-3
β1-3
α
CORE 2 O-LINKED GLYCANS
α2-6
β1-4
β1-6
α
β1-3
OR
α2-3
α1-2
OR
CORE 3 O-LINKED GLYCANS
α2-6
β1-4
β1-3
α
CORE 4 O-LINKED GLYCANS
α2-6
β1-4
β1-3/6
α
β1-4
β1-3/6
OR
α2-3/6
α1-2
OR
GlcNAc
α GalNAc O-LINKED TO PROTEIN
Neu5Ac
FUCOSE
Gal
TERMINAL HexNAc

FIG. 9B

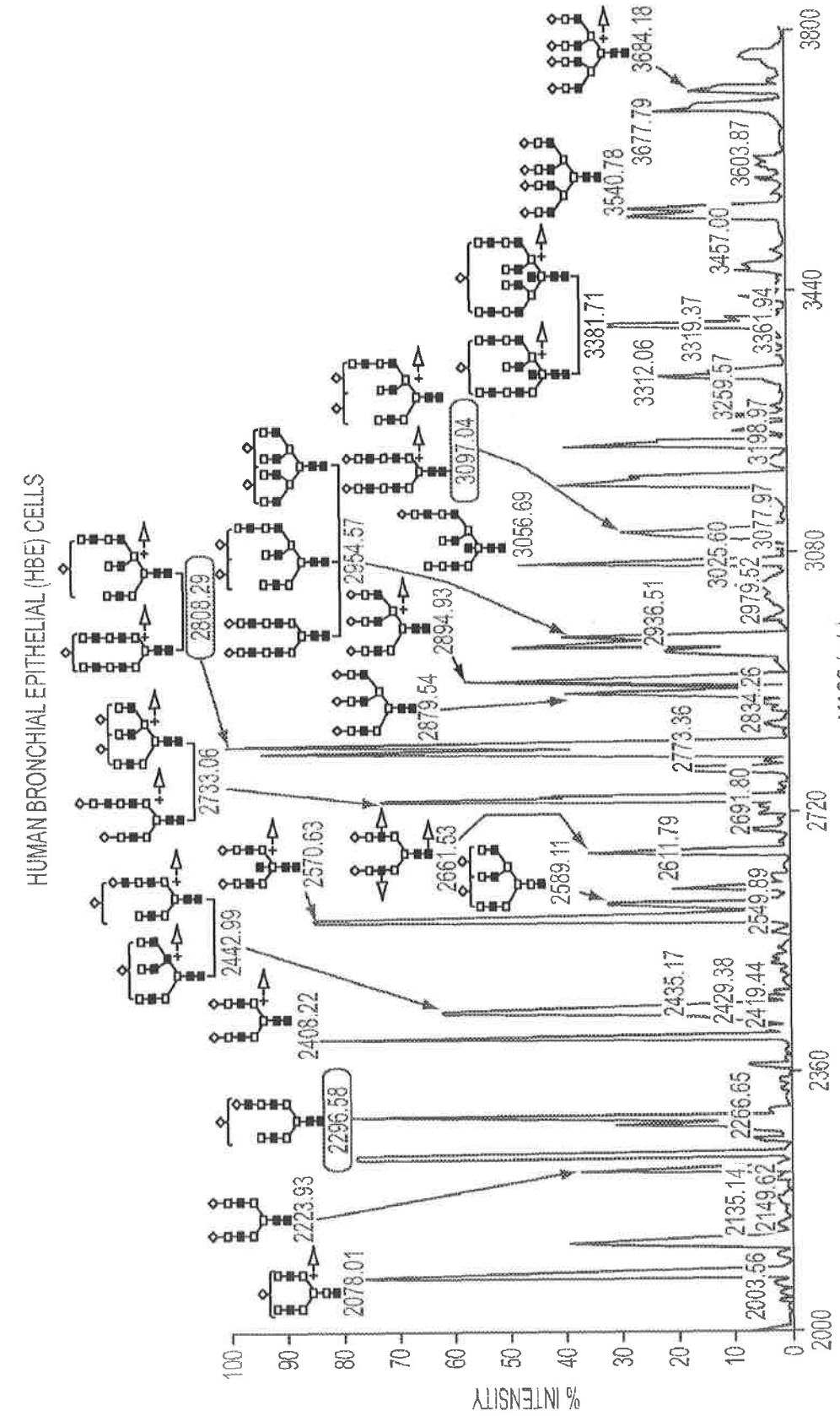

HUMAN BRONCHIAL EPITHELIAL (HBE) CELLS
% INTENSITY
MASS (m/z)
2000
2360
2720
3080
3440
3800
2078.01
2223.93
2296.58
2408.22
2442.99
2570.63
2733.06
2808.29
2879.54
2894.93
2954.57
3056.69
3097.04
3381.71
3684.18

FIG. 10A

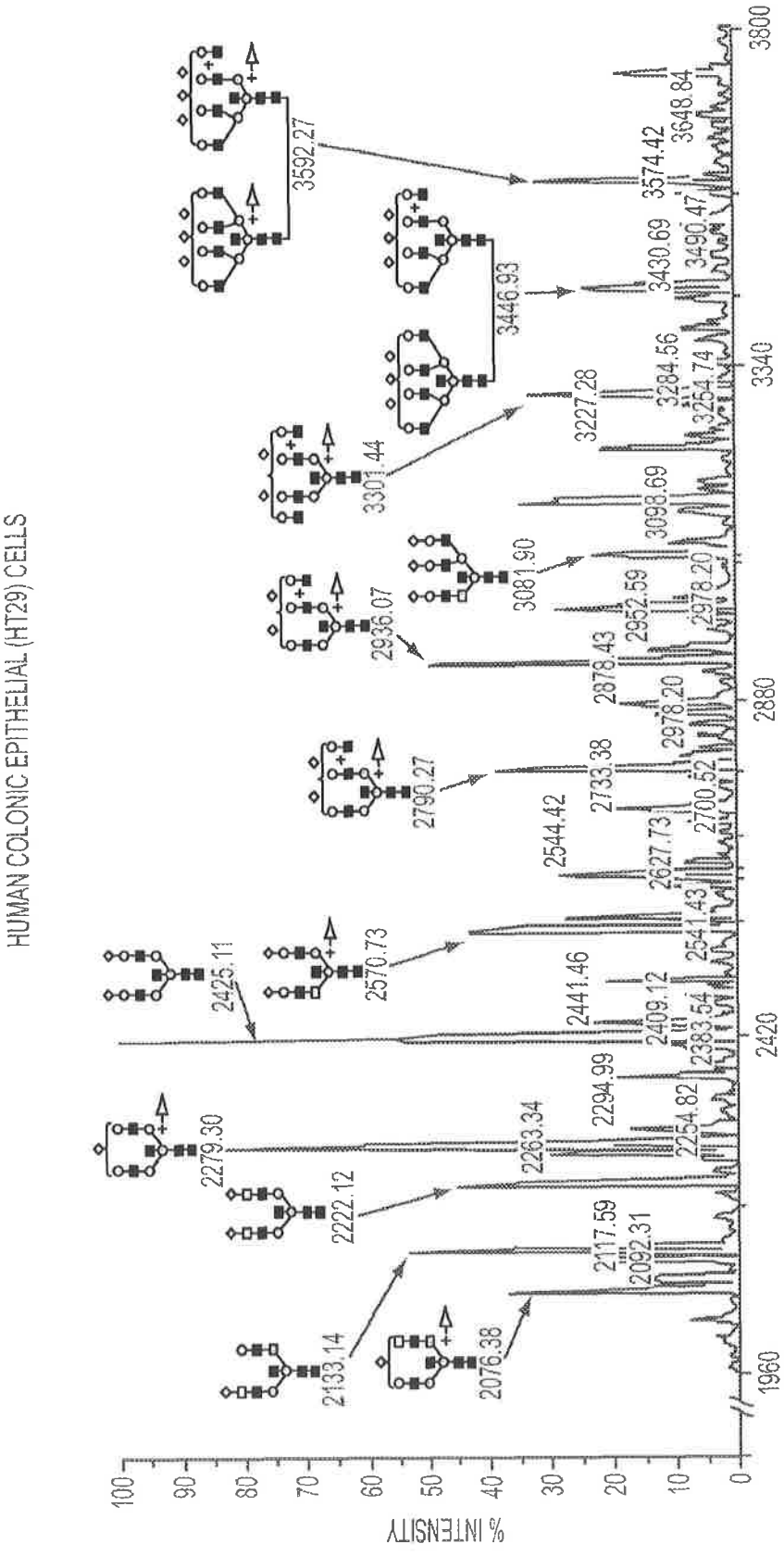

HUMAN COLONIC EPITHELIAL (HT29) CELLS
% INTENSITY
MASS (m/z)
100
90
80
70
60
50
40
30
20
10
0
1960
2420
2880
3340
3800
2133.14
2076.38
2222.12
2279.30
2263.34
2117.59
2092.31
2294.99
2254.82
2425.11
2570.73
2441.46
2409.12
2383.54
2541.43
2544.42
2733.38
2627.73
2700.52
2790.27
2978.20
2878.43
2936.07
2952.59
3081.90
3098.69
3301.44
3227.28
3284.56
3254.74
3446.93
3430.69
3490.47
3574.42
3592.27
3648.84

FIG. 10B

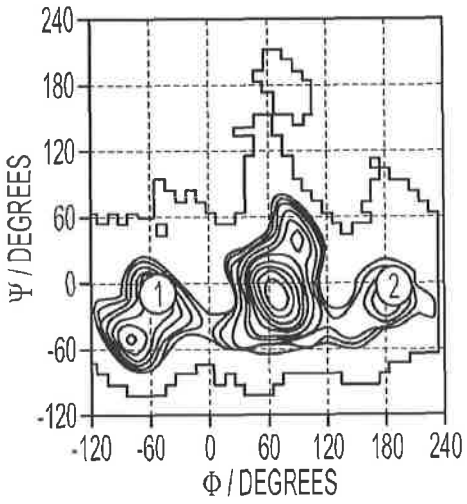


FIG. 11A

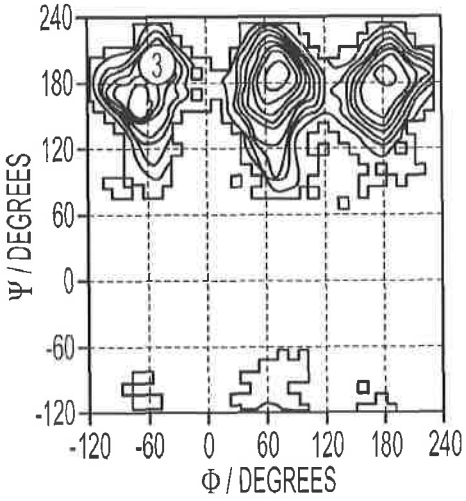


FIG. 11B

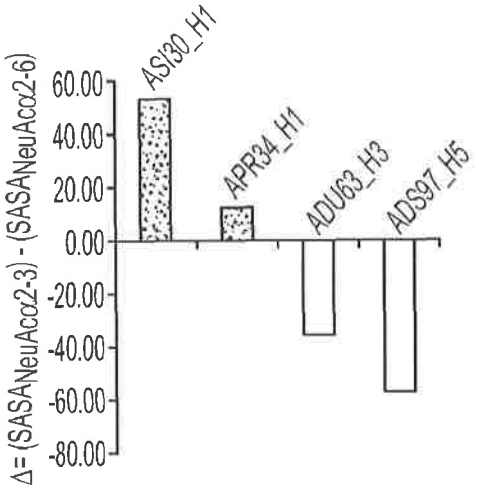


FIG. 11C

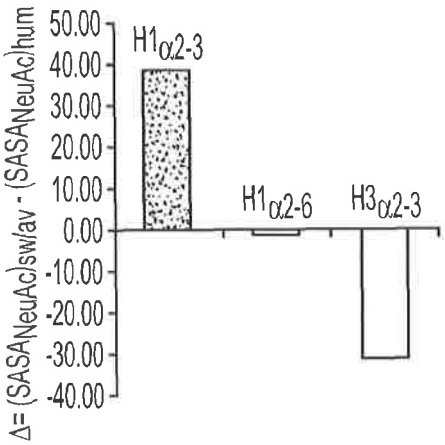
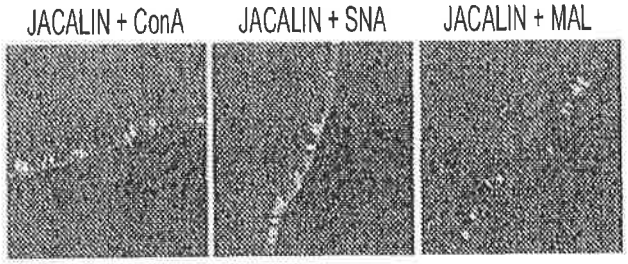


FIG. 11D



LECTIN STAINING OF UPPER RESPIRATORY TISSUE SECTIONS

FIG. 12A

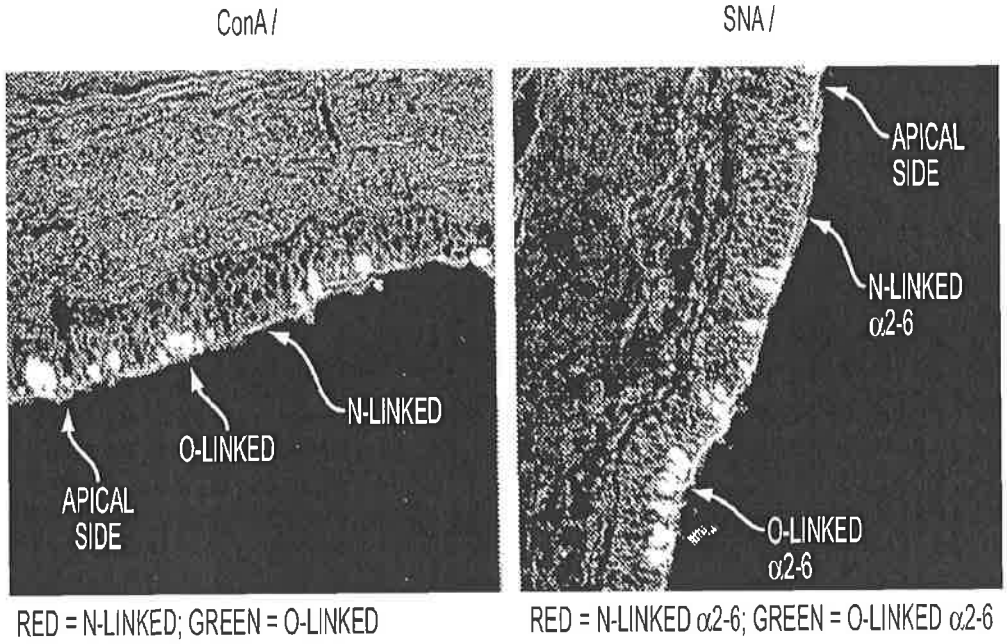

ConA /
SNA /
APICAL SIDE
O-LINKED
N-LINKED
APICAL SIDE
N-LINKED α2-6
O-LINKED α2-6
RED = N-LINKED; GREEN = O-LINKED
RED = N-LINKED α2-6; GREEN = O-LINKED α2-6

FIG. 12B

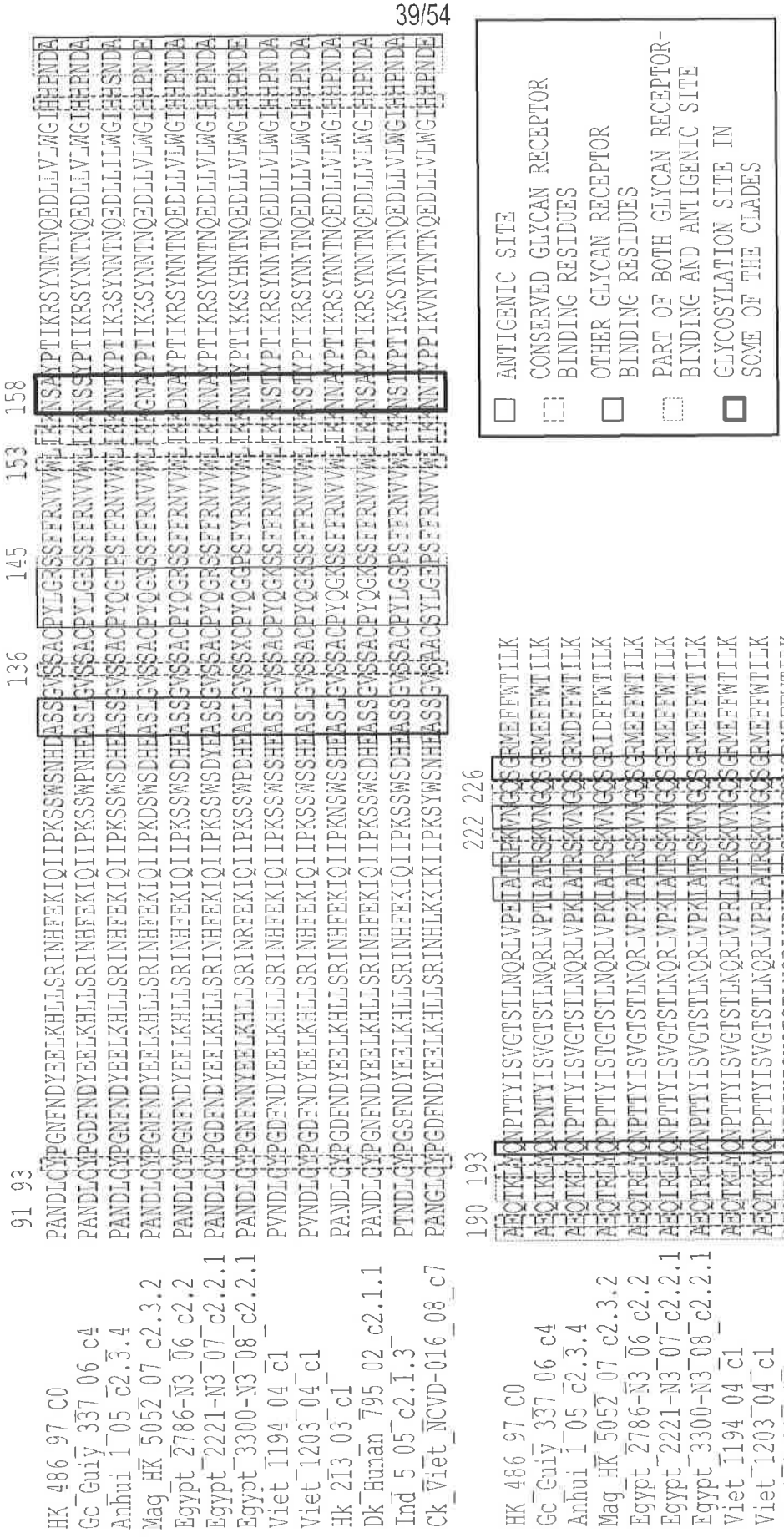

91 93
136 145 153 158
222 226
ANTIGENIC SITE
CONSERVED GLYCAN RECEPTOR BINDING RESIDUES
OTHER GLYCAN RECEPTOR BINDING RESIDUES
PART OF BOTH GLYCAN RECEPTOR-BINDING AND ANTIGENIC SITE
GLYCOSYLATION SITE IN SOME OF THE CLADES

FIG. 13

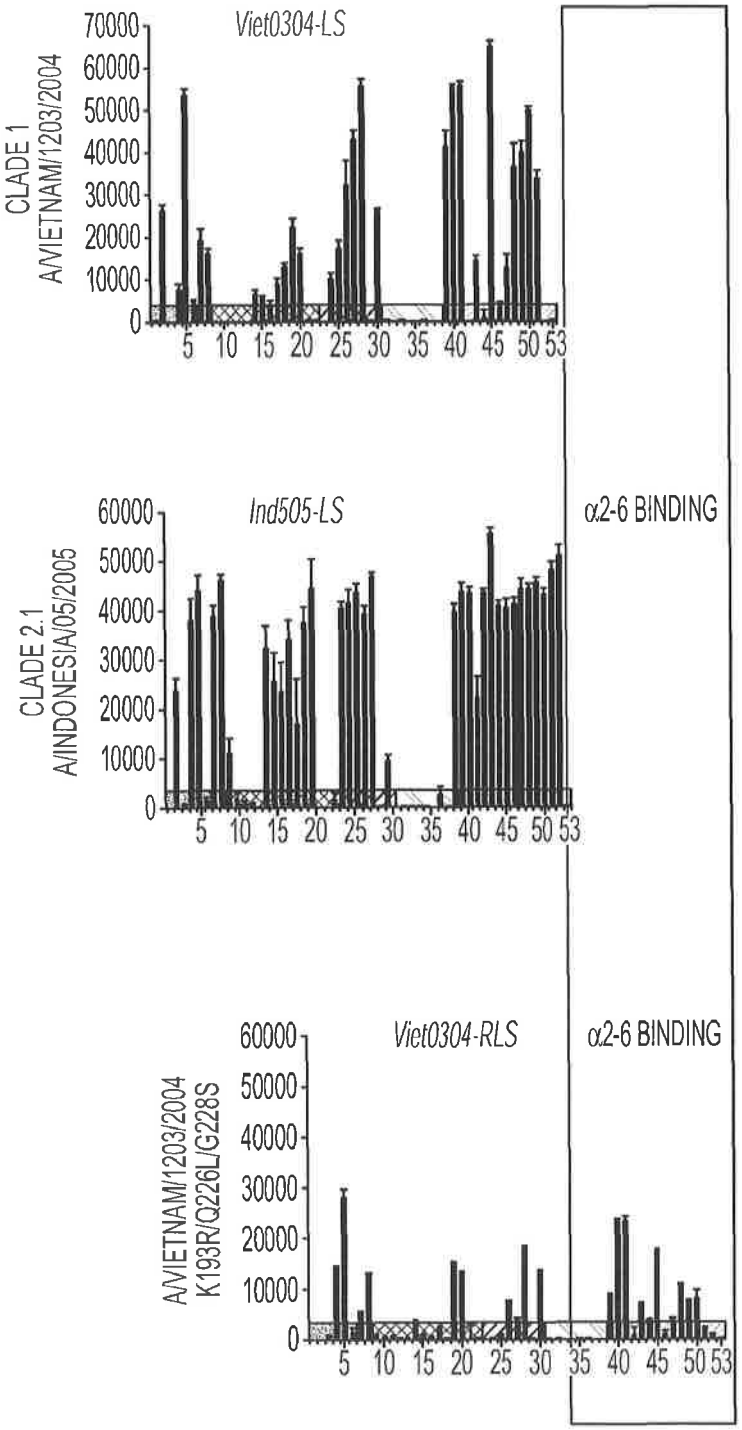

Viet0304-LS
CLADE 1
A/VIETNAM/1203/2004
70000
60000
50000
40000
30000
20000
10000
0
5 10 15 20 25 30 35 40 45 50 53
Ind505-LS
CLADE 2.1
A/INDONESIA/05/2005
α2-6 BINDING
Viet0304-RLS
A/VIETNAM/1203/2004
K193R/Q226L/G228S

FIG. 14

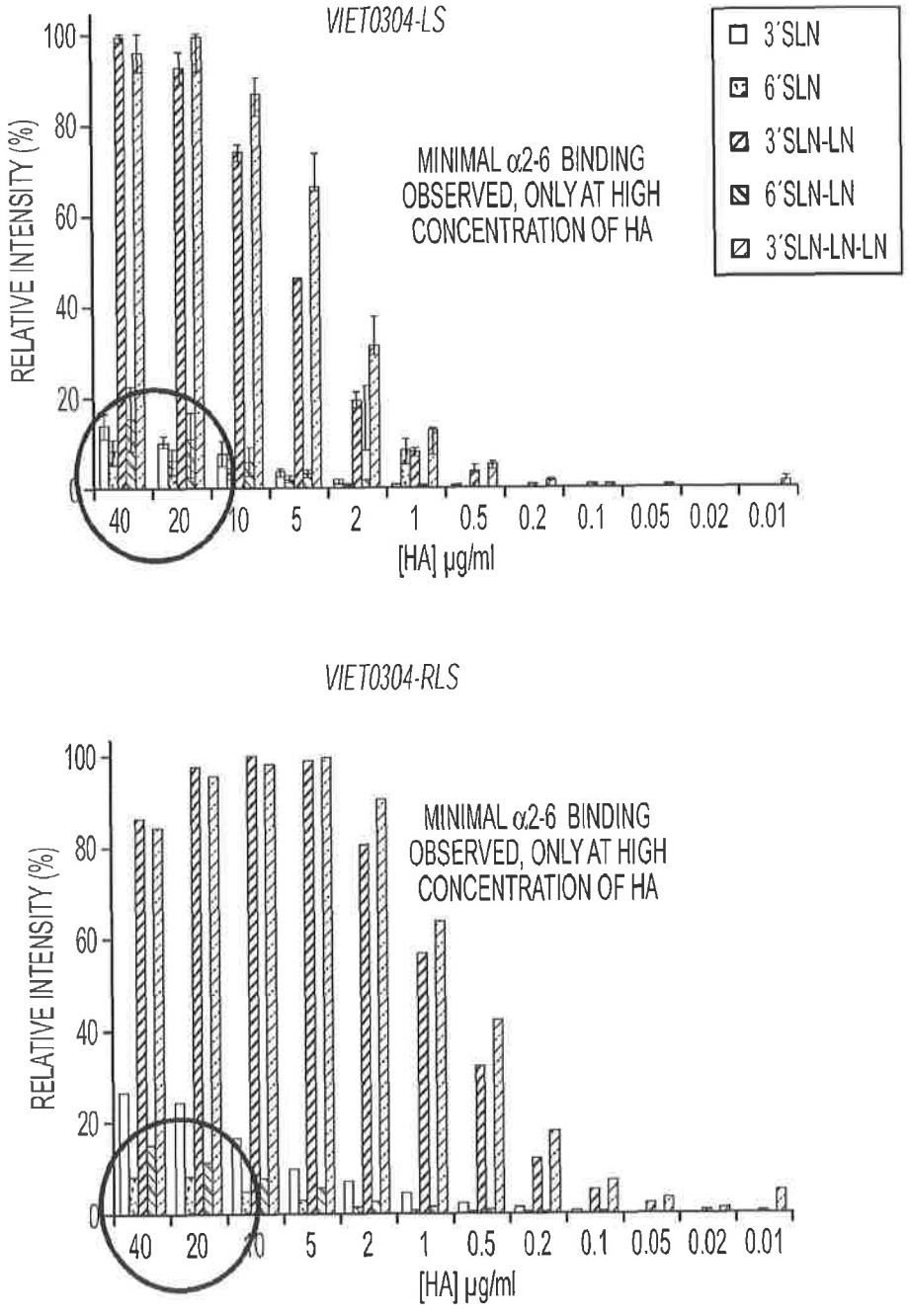

VIET0304-LS
3'SLN
6'SLN
3'SLN-LN
6'SLN-LN
3'SLN-LN-LN
MINIMAL α2-6 BINDING OBSERVED, ONLY AT HIGH CONCENTRATION OF HA
RELATIVE INTENSITY (%)
100
80
60
40
20
0
40 20 10 5 2 1 0.5 0.2 0.1 0.05 0.02 0.01
[HA] µg/ml
VIET0304-RLS
MINIMAL α2-6 BINDING OBSERVED, ONLY AT HIGH CONCENTRATION OF HA
RELATIVE INTENSITY (%)
100
80
60
40
20
0
40 20 10 5 2 1 0.5 0.2 0.1 0.05 0.02 0.01
[HA] µg/ml

FIG. 15

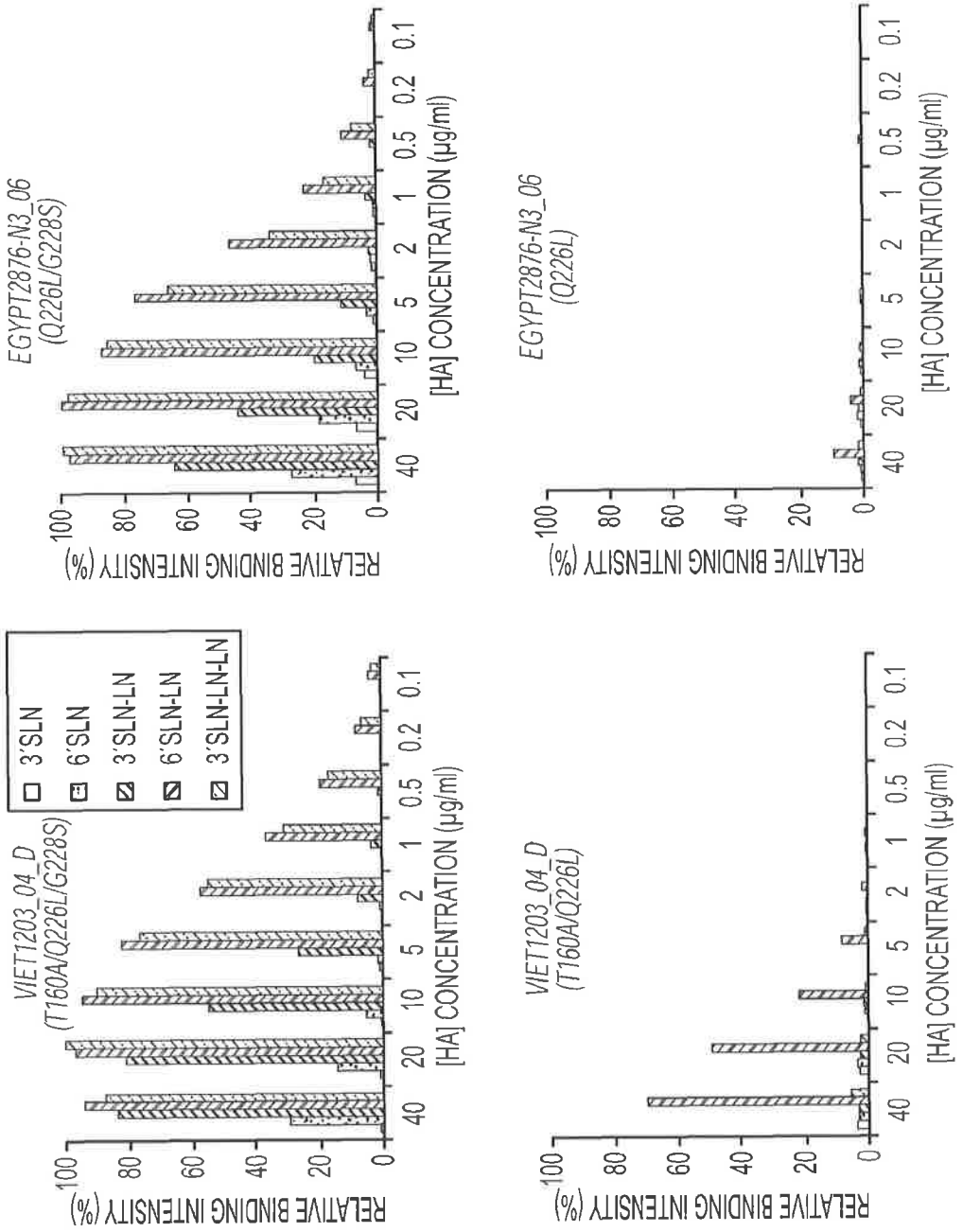

EGYPT2876-N3_06
(Q226L/G228S)
RELATIVE BINDING INTENSITY (%)
[HA] CONCENTRATION (µg/ml)
EGYPT2876-N3_06
(Q226L)
VIET1203_04_D
(T160A/Q226L/G228S)
3'SLN
6'SLN
3'SLN-LN
6'SLN-LN
3'SLN-LN-LN
VIET1203_04_D
(T160A/Q226L)

FIG. 16

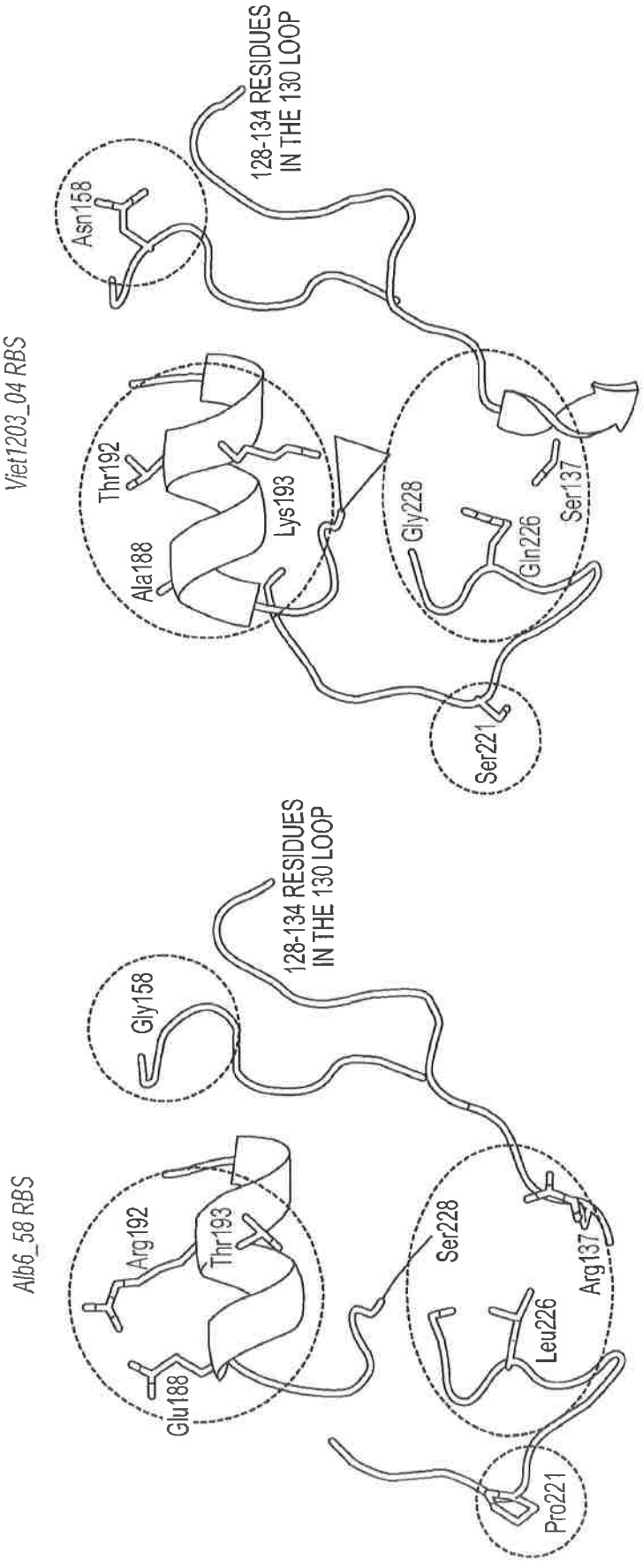

Viet1203_04 RBS
128-134 RESIDUES IN THE 130 LOOP
Asn158
Thr192
Ala188
Lys193
Gly228
Gln226
Ser137
Ser221
Alb6_58 RBS
128-134 RESIDUES IN THE 130 LOOP
Gly158
Arg192
Thr193
Glu188
Ser228
Leu226
Arg137
Pro221

FIG. 17

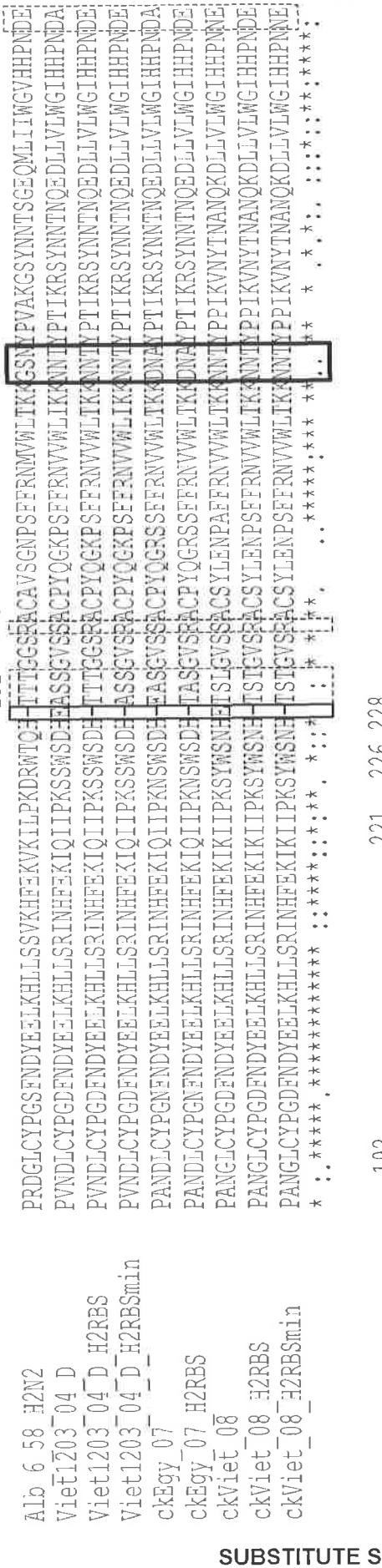


FIG. 18

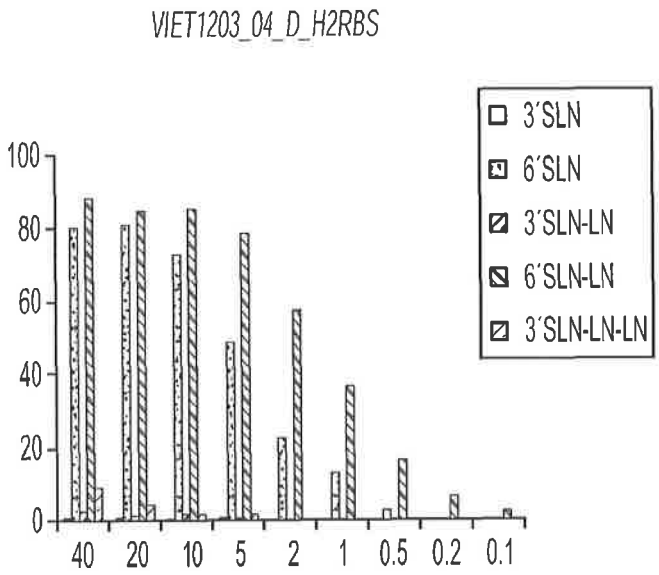

VIET1203_04_D_H2RBS
3'SLN
6'SLN
3'SLN-LN
6'SLN-LN
3'SLN-LN-LN
100
80
60
40
20
0
40
20
10
5
2
1
0.5
0.2
0.1

FIG. 19

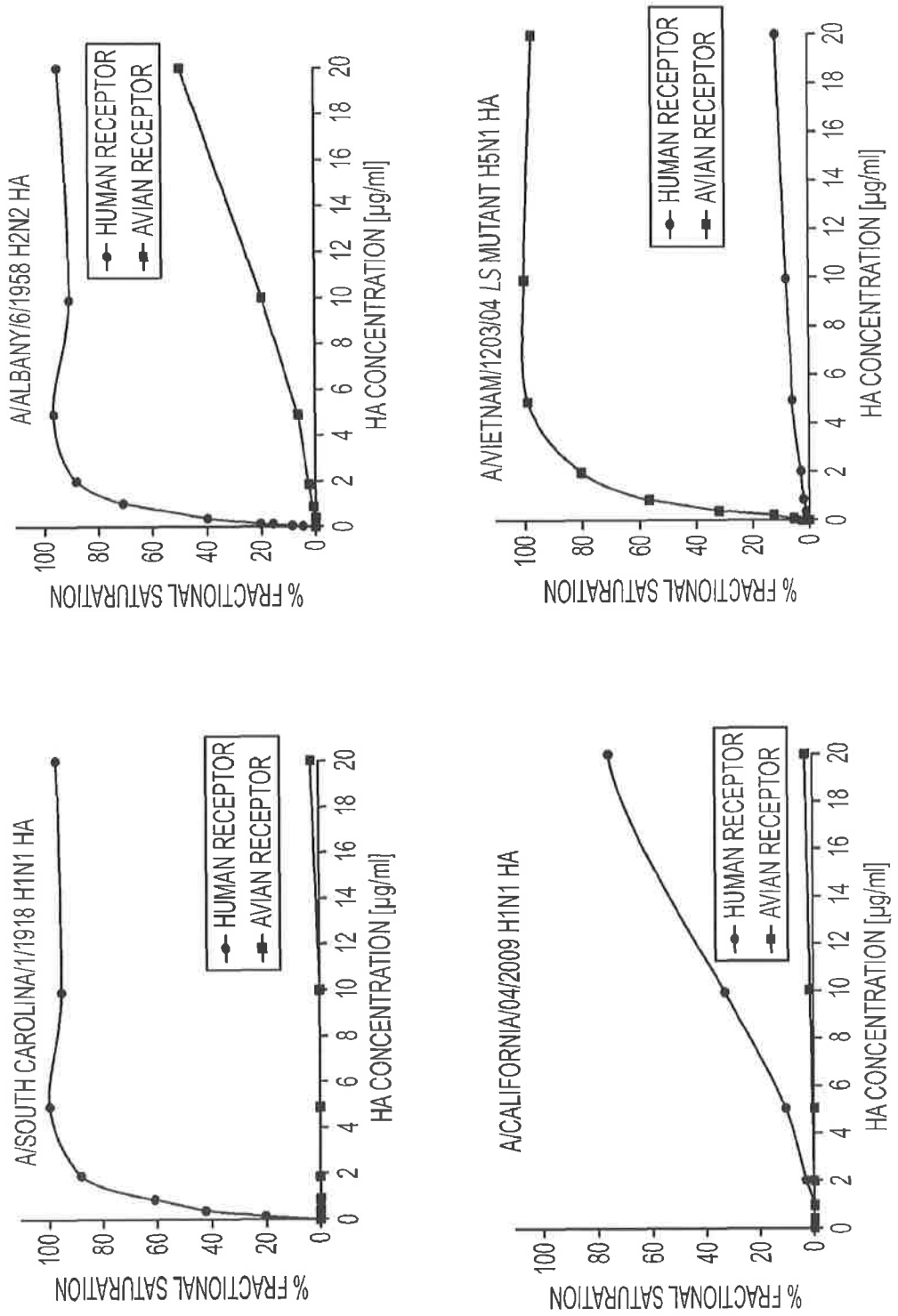

A/ALBANY/6/1958 H2N2 HA
A/VIETNAM/1203/04 LS MUTANT H5N1 HA
A/SOUTH CAROLINA/1/1918 H1N1 HA
A/CALIFORNIA/04/2009 H1N1 HA
HUMAN RECEPTOR
AVIAN RECEPTOR
% FRACTIONAL SATURATION
HA CONCENTRATION [µg/ml]
0
20
40
60
80
100
0
2
4
6
8
10
12
14
16
18
20

FIG. 20

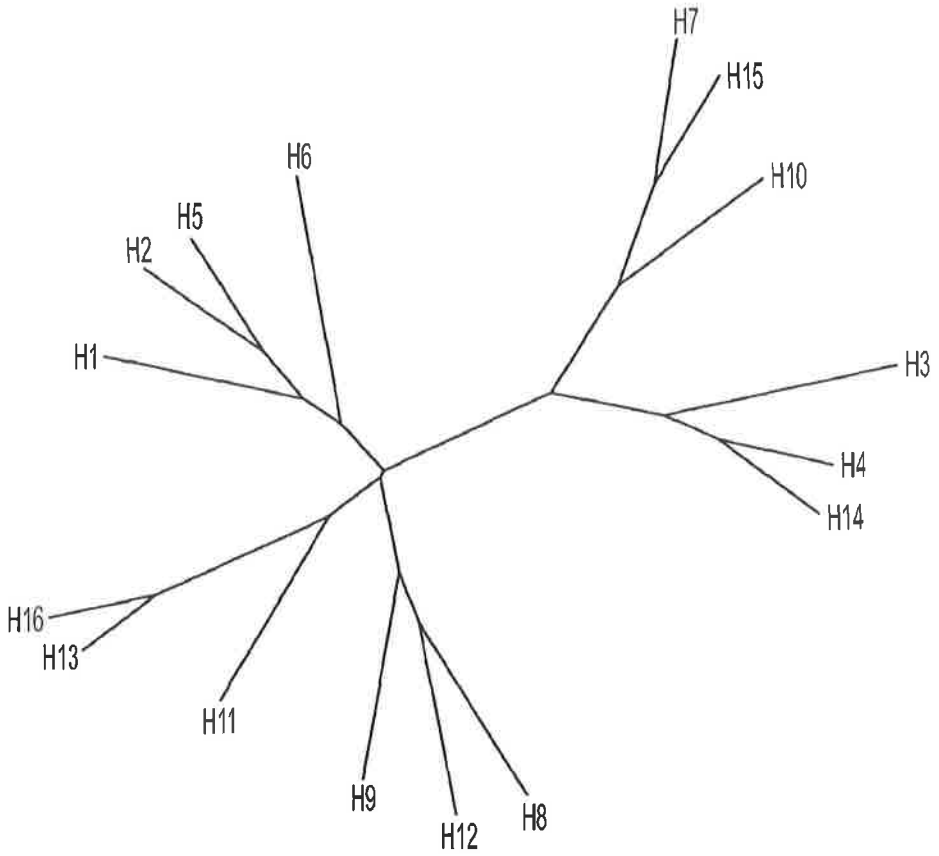


FIG. 21

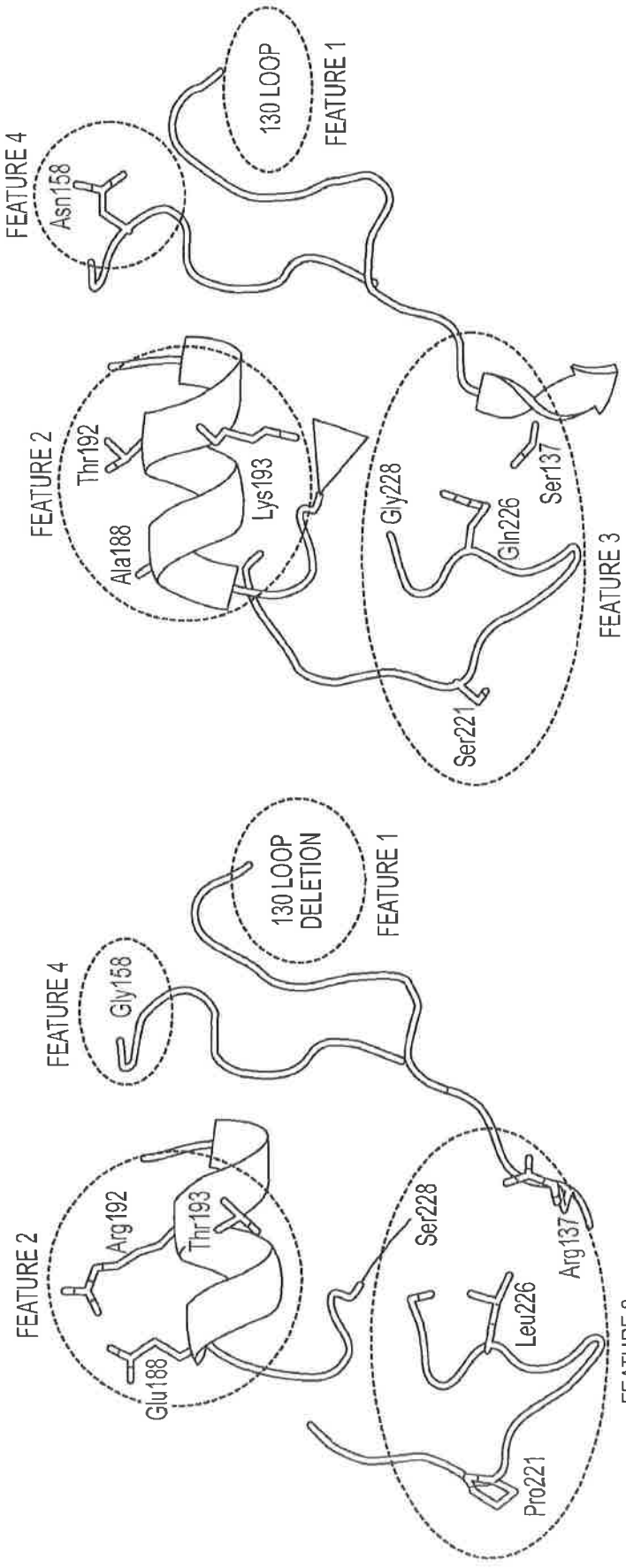

130 LOOP
FEATURE 1
FEATURE 4
Asn158
FEATURE 2
Thr192
Ala188
Lys193
Gly228
Gln226
Ser137
Ser221
FEATURE 3
Viet1203_04 RBS
130 LOOP DELETION
FEATURE 1
FEATURE 4
Gly158
FEATURE 2
Arg192
Thr193
Glu188
Ser228
Leu226
Arg137
Pro221
FEATURE 3
Alb58 RBS

FIG. 22

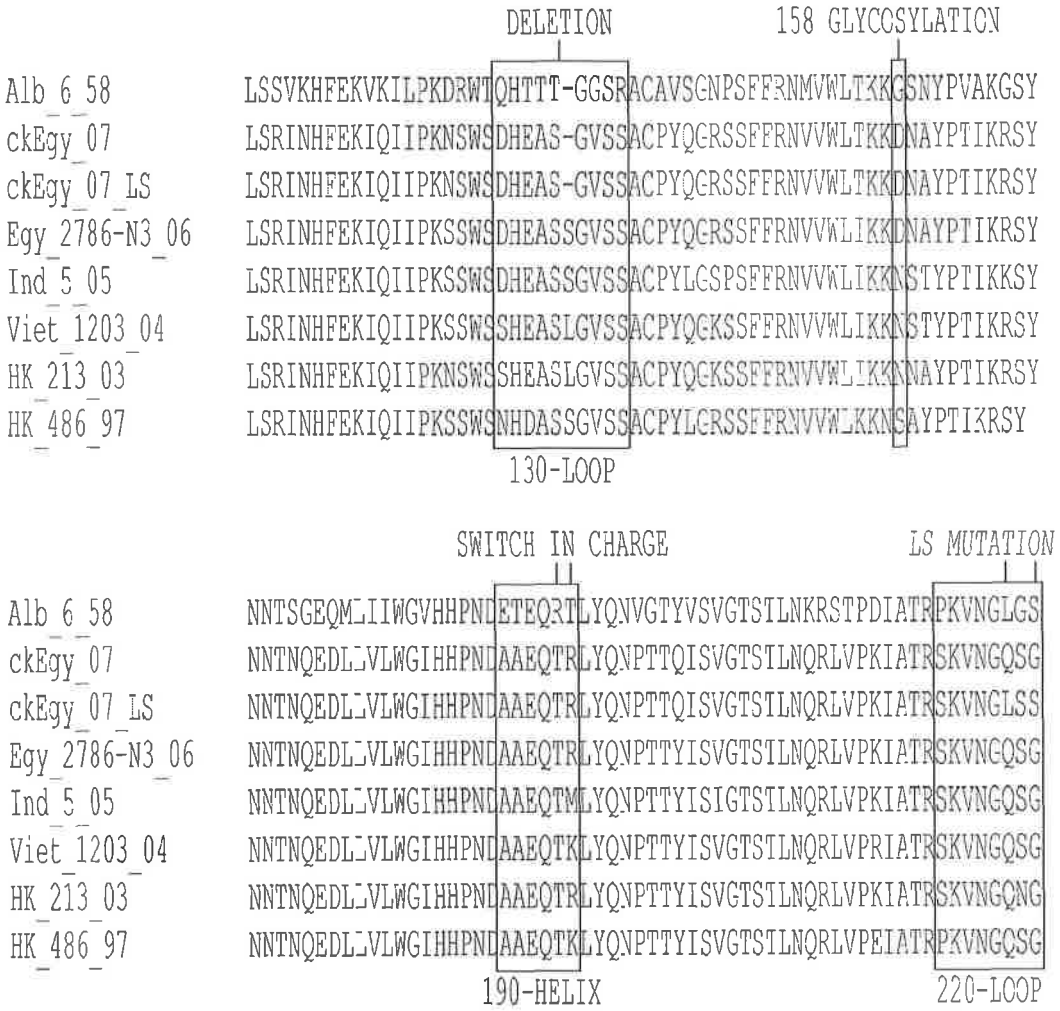

DELETION
158 GLYCOSYLATION
Alb_6_58 LSSVKHFEKVKILPKDRWTQHTTT-GGSRACAVSGNPSFFRNMVWLTKKGSNYPVAKGSY
ckEgy_07 LSRINHFEKIQIIPKNSWSDHEAS-GVSSACPYQGRSSFFRNVVWLTKKDNAYPTIKRSY
ckEgy_07_LS LSRINHFEKIQIIPKNSWSDHEAS-GVSSACPYQGRSSFFRNVVWLTKKDNAYPTIKRSY
Egy_2786-N3_06 LSRINHFEKIQIIPKSSWSDHEASSGVSSACPYQGRSSFFRNVVWLIKKDNAYPTIKRSY
Ind_5_05 LSRINHFEKIQIIPKSSWSDHEASSGVSSACPYLGSPSFFRNVVWLIKKNSTYPTIKKSY
Viet_1203_04 LSRINHFEKIQIIPKSSWSSHEASLGVSSACPYQGKSSFFRNVVWLIKKNSTYPTIKRSY
HK_213_03 LSRINHFEKIQIIPKNSWSSHEASLGVSSACPYQGKSSFFRNVVWLIKKNAYPTIKRSY
HK_486_97 LSRINHFEKIQIIPKSSWSNHDASSGVSSACPYLGRSSFFRNVVWLKKNSAYPTIKRSY
130-LOOP
SWITCH IN CHARGE
LS MUTATION
Alb_6_58 NNTSGEQMLIIWGVHHPNDETEQRTLYQNVGTYVSVGTSTLNKRSTPDIATRPKVNGLGS
ckEgy_07 NNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTQISVGTSTLNQRLVPKIATRSKVNGQSG
ckEgy_07_LS NNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTQISVGTSTLNQRLVPKIATRSKVNGLSS
Egy_2786-N3_06 NNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTYISVGTSTLNQRLVPKIATRSKVNGQSG
Ind_5_05 NNTNQEDLLVLWGIHHPNDAAEQTMLYQNPTTYISIGTSTLNQRLVPKIATRSKVNGQSG
Viet_1203_04 NNTNQEDLLVLWGIHHPNDAAEQTKLYQNPTTYISVGTSTLNQRLVPRIATRSKVNGQSG
HK_213_03 NNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTYISVGTSTLNQRLVPKIATRSKVNGQNG
HK_486_97 NNTNQEDLLVLWGIHHPNDAAEQTKLYQNPTTYISVGTSTLNQRLVPEIATRPKVNGQSG
190-HELIX
220-LOOP

FIG. 23

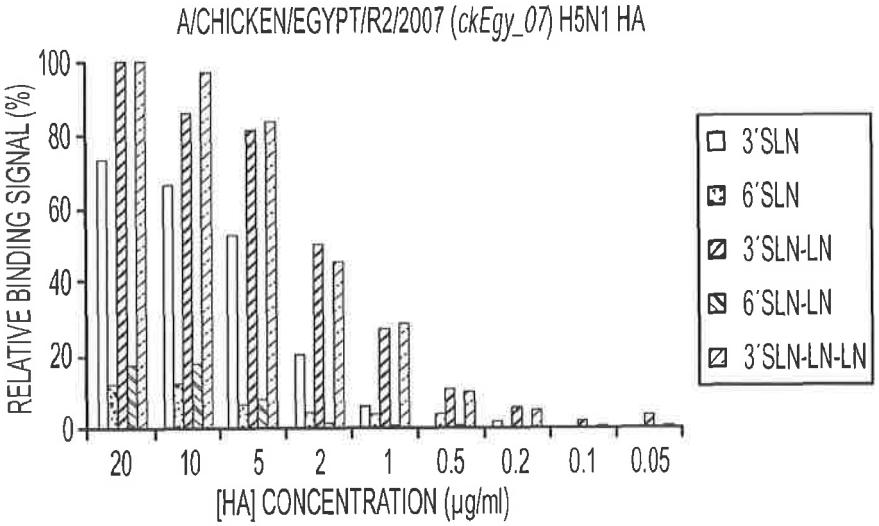


FIG. 24A

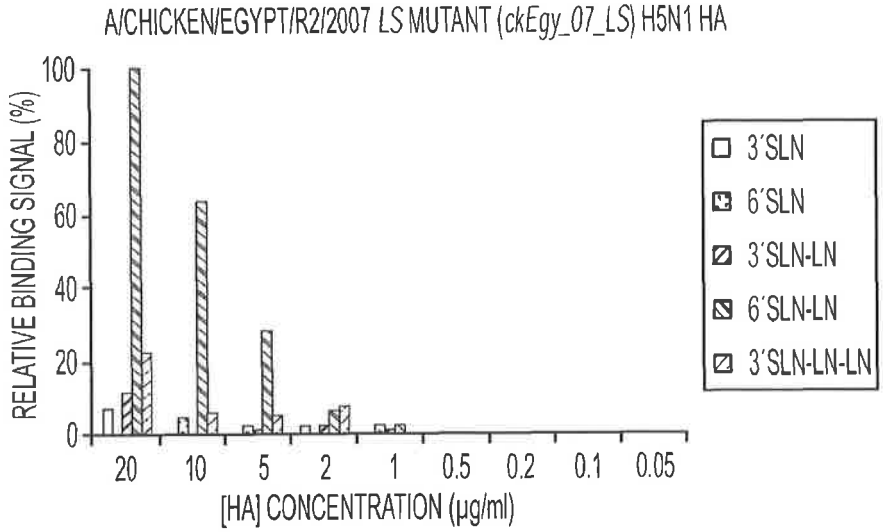
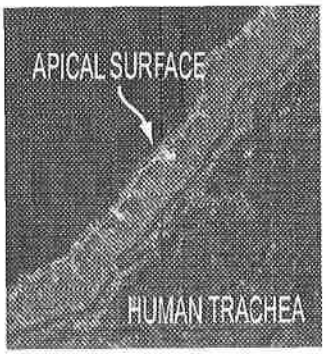


FIG. 24B



ckEgy_07_LS MUTANT HA/PI

FIG. 24C

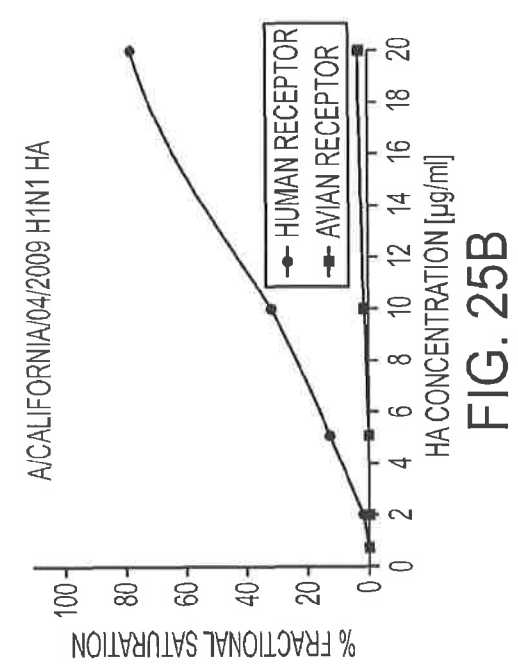


FIG. 25B

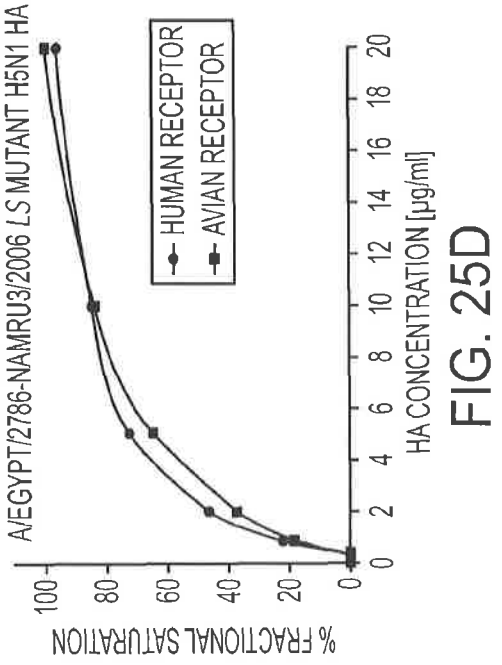


FIG. 25D

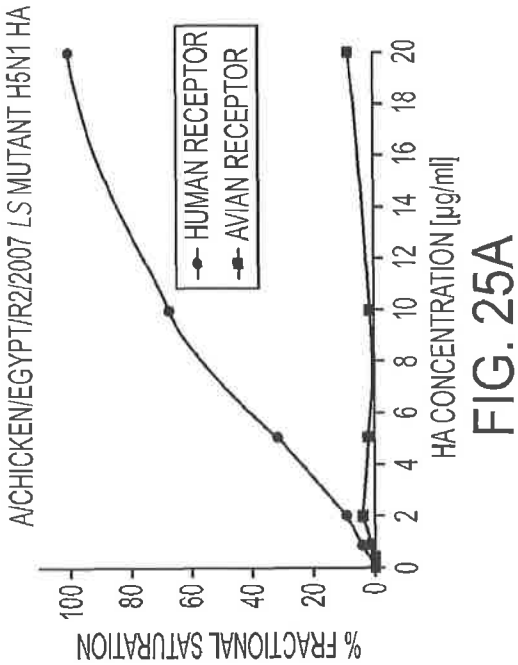


FIG. 25A

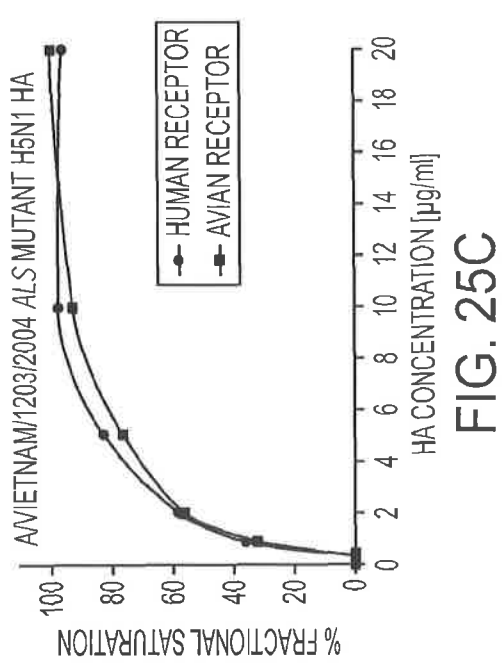


FIG. 25C

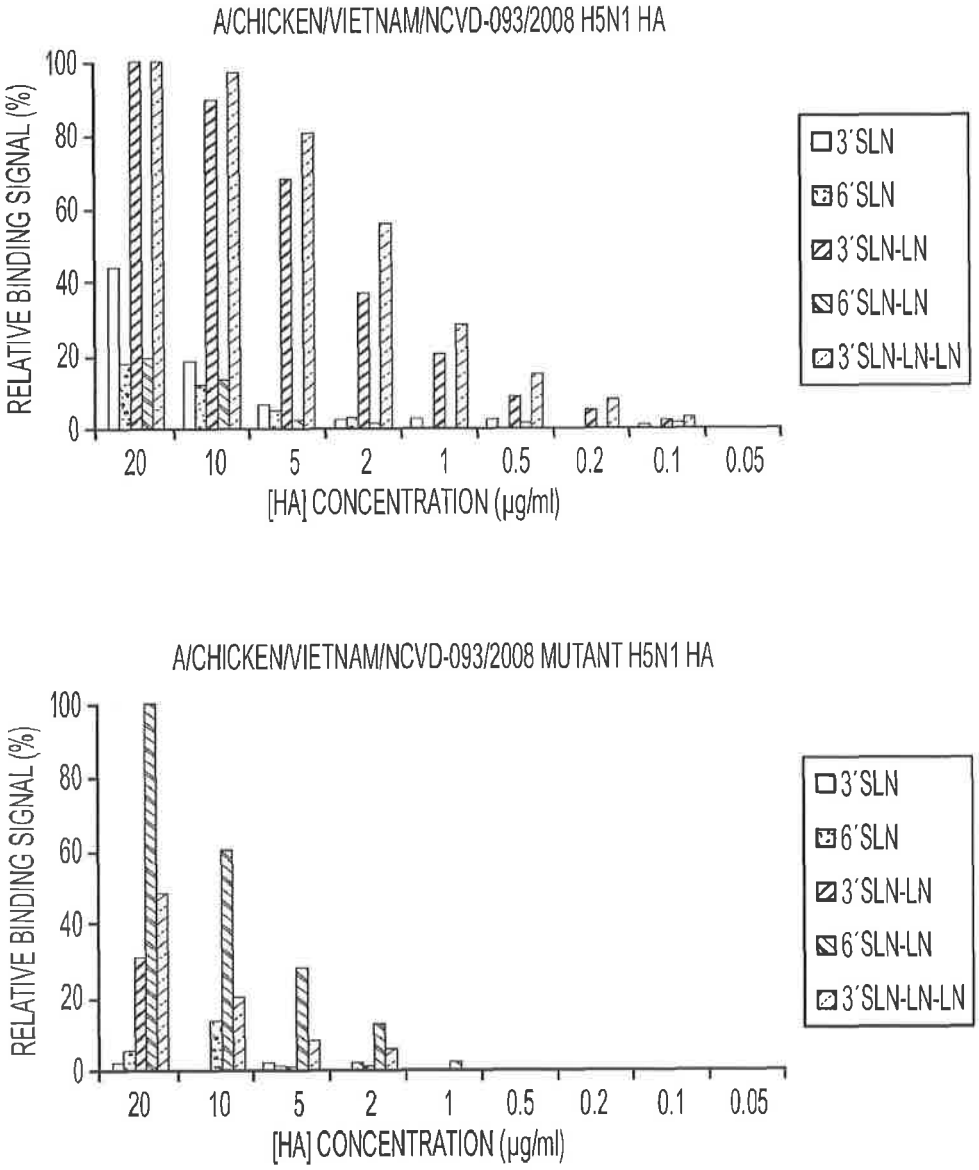

A/CHICKEN/VIETNAM/NCVD-093/2008 H5N1 HA
RELATIVE BINDING SIGNAL (%)
100
80
60
40
20
0
20
10
5
2
1
0.5
0.2
0.1
0.05
[HA] CONCENTRATION (µg/ml)
3´SLN
6´SLN
3´SLN-LN
6´SLN-LN
3´SLN-LN-LN
A/CHICKEN/VIETNAM/NCVD-093/2008 MUTANT H5N1 HA
RELATIVE BINDING SIGNAL (%)
100
80
60
40
20
0
20
10
5
2
1
0.5
0.2
0.1
0.05
[HA] CONCENTRATION (µg/ml)
3´SLN
6´SLN
3´SLN-LN
6´SLN-LN
3´SLN-LN-LN

FIG. 26

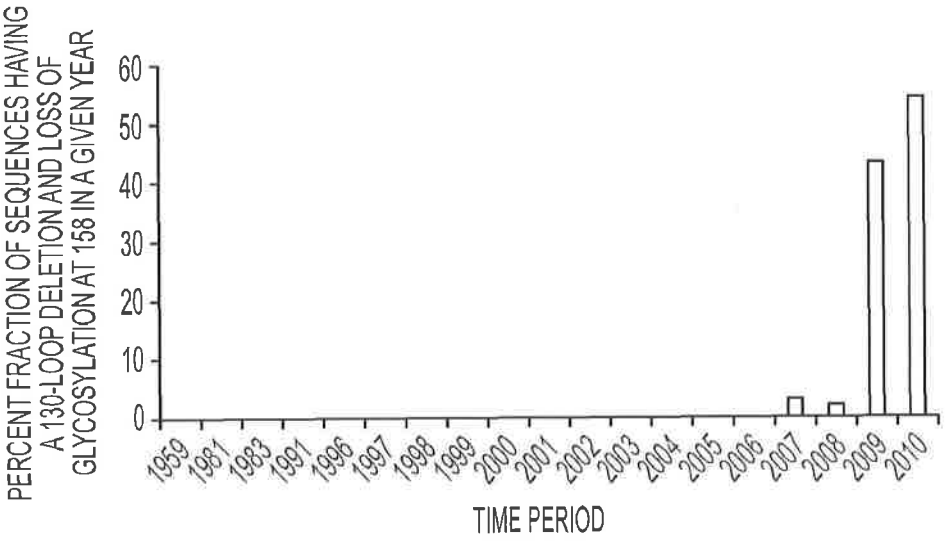


FIG. 27A

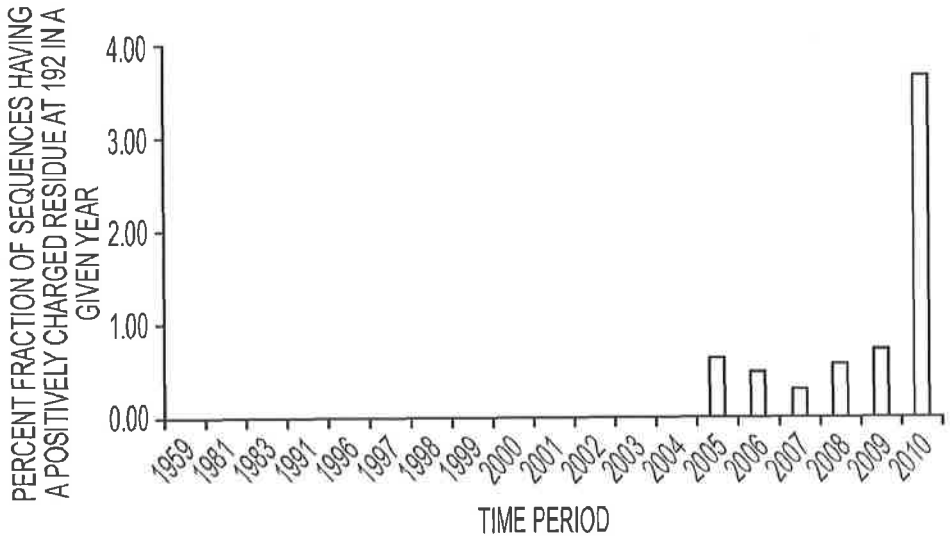


FIG. 27B

EXPANDED NOMENCLATURE OF GLYCANS USED IN THE GLYCAN ARRAY

GLYCAN	EXPANDED NOMENCLATURE
3´SLN	Neu5Acα2-3Galβ1-4GlcNAcβ1-
6´SLN	Neu5Acα2-6Galβ1-4GlcNAcβ1-
3´SLN-LN	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-
6´SLN-LN	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-
3´SLN-LN-LN	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1

KEY: Neu5Ac: N-ACETYL D-NEURAMINIC ACID; Gal: D-GALACTOSE; GlcNAc: N-ACETYL D-GLUCOSAMINE. α / β: ANOMERIC CONFIGURATION OF THE PYRANOSE SUGARS. ALL THE SUGARS ARE LINKED VIA A SPACER TO BIOTIN (-Sp-LC-LC-BIOTIN AS DESCRIBED IN http://www.functionalglycomics.org/static/consortium/resources/resourcecored5.shtml)

FIG. 28