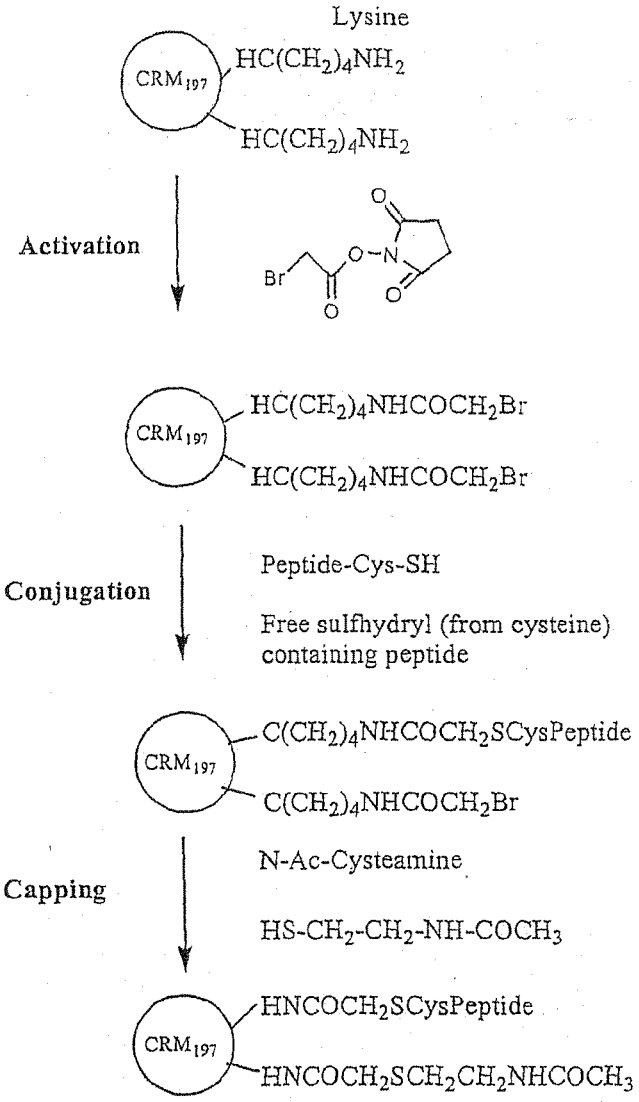


Capped CRM Conjugate

Figure 1

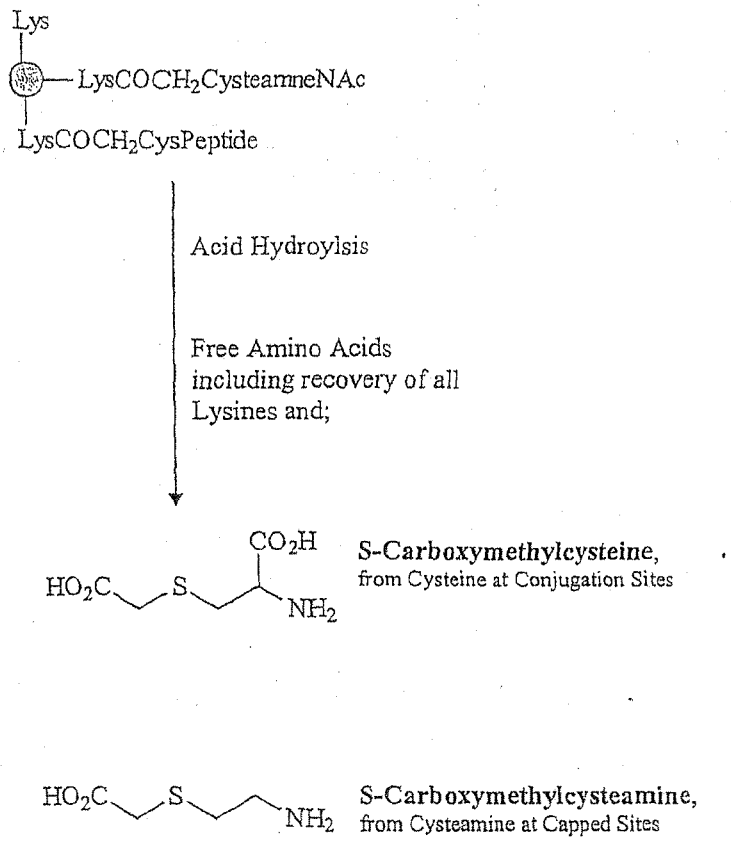

Lys
LysCOCH2CysteamneNAc
LysCOCH2CysPeptide
Acid Hydroylsis
Free Amino Acids
including recovery of all
Lysines and;
CO2H
HO2C
S
NH2
S-Carboxymethylcysteine,
from Cysteine at Conjugation Sites
HO2C
S
NH2
S-Carboxymethylcysteamine,
from Cysteamine at Capped Sites

Figure 2

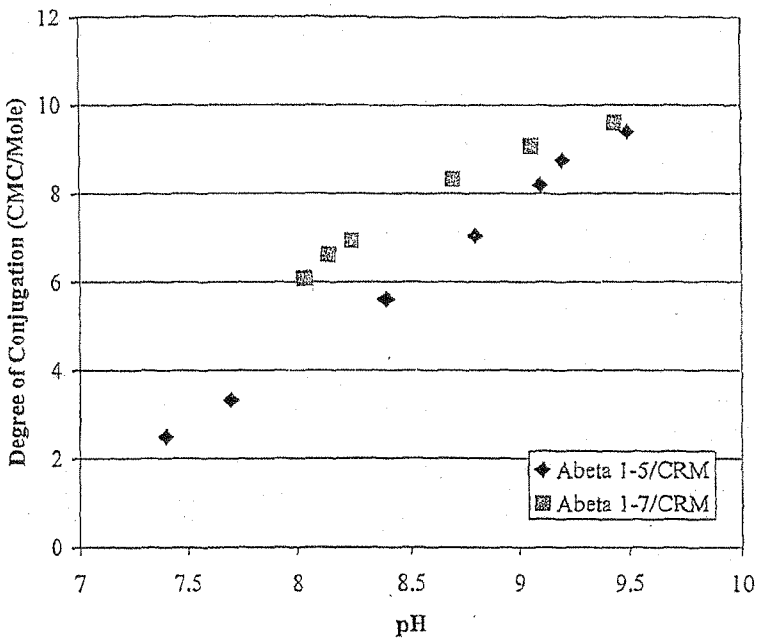

12
10
8
6
4
2
0
Degree of Conjugation (CMC/Mole)
7
7.5
8
8.5
9
9.5
10
pH
Abeta 1-5/CRM
Abeta 1-7/CRM

Figure 3

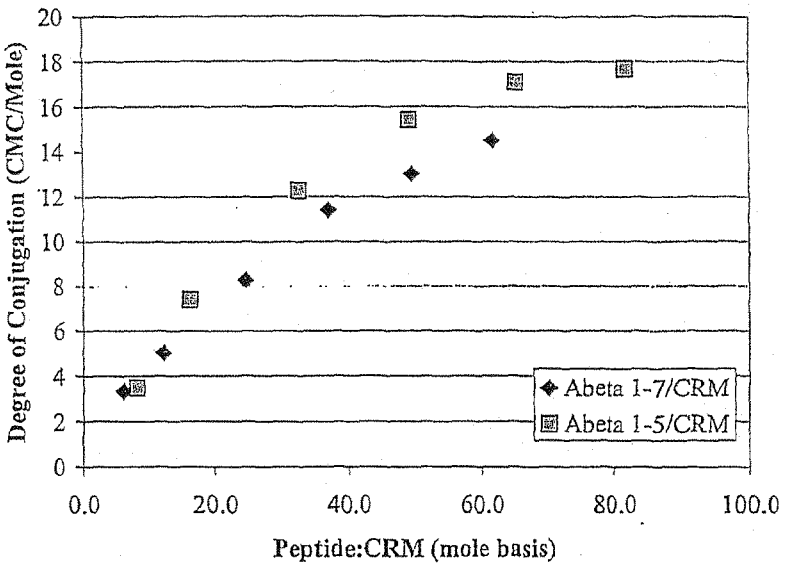

Degree of Conjugation (CMC/Mole)
20
18
16
14
12
10
8
6
4
2
0
0.0
20.0
40.0
60.0
80.0
100.0
Peptide:CRM (mole basis)
Abeta 1-7/CRM
Abeta 1-5/CRM

Figure 4

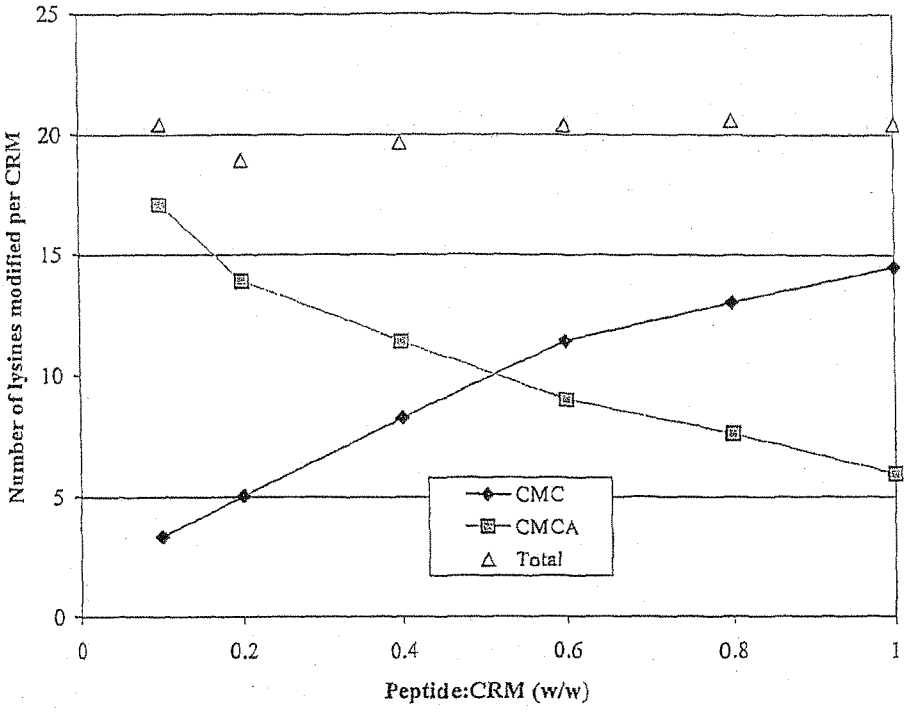

25
20
15
10
5
0
Number of lysines modified per CRM
0
0.2
0.4
0.6
0.8
1
Peptide:CRM (w/w)
CMC
CMCA
Total

Figure 5

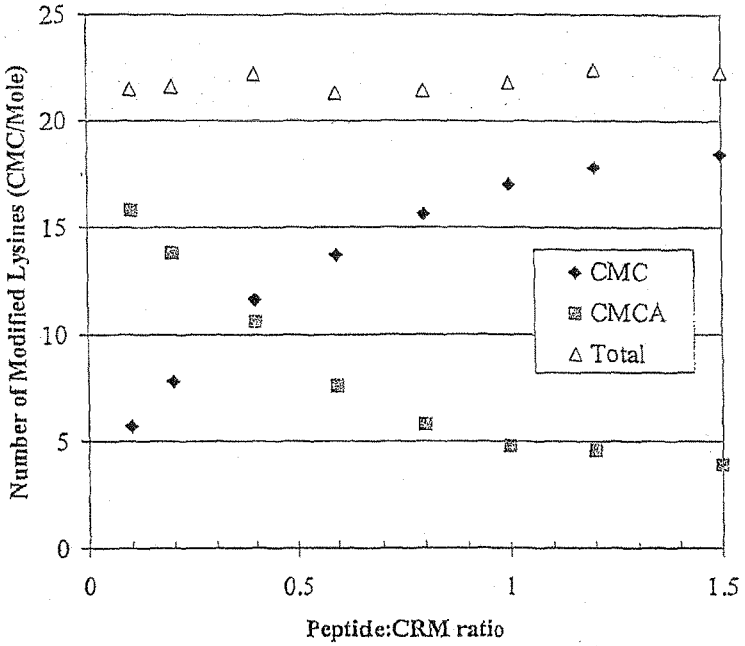

25
20
15
10
5
0
0
0.5
1
1.5
Number of Modified Lysines (CMC/Mole)
Peptide:CRM ratio
CMC
CMCA
Total

Figure 6

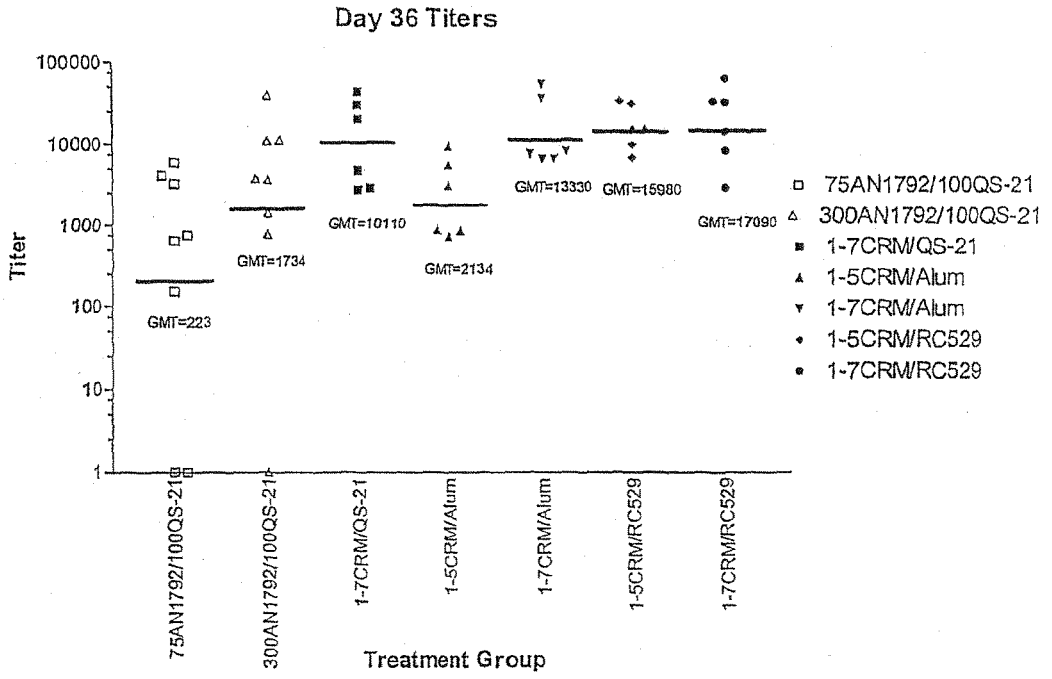

Day 36 Titers
Titer
100000
10000
1000
100
10
1
GMT=223
GMT=1734
GMT=10110
GMT=2134
GMT=13330
GMT=15980
GMT=17090
75AN1792/100QS-21
300AN1792/100QS-21
1-7CRM/QS-21
1-5CRM/Alum
1-7CRM/Alum
1-5CRM/RC529
1-7CRM/RC529
Treatment Group

Figure 7

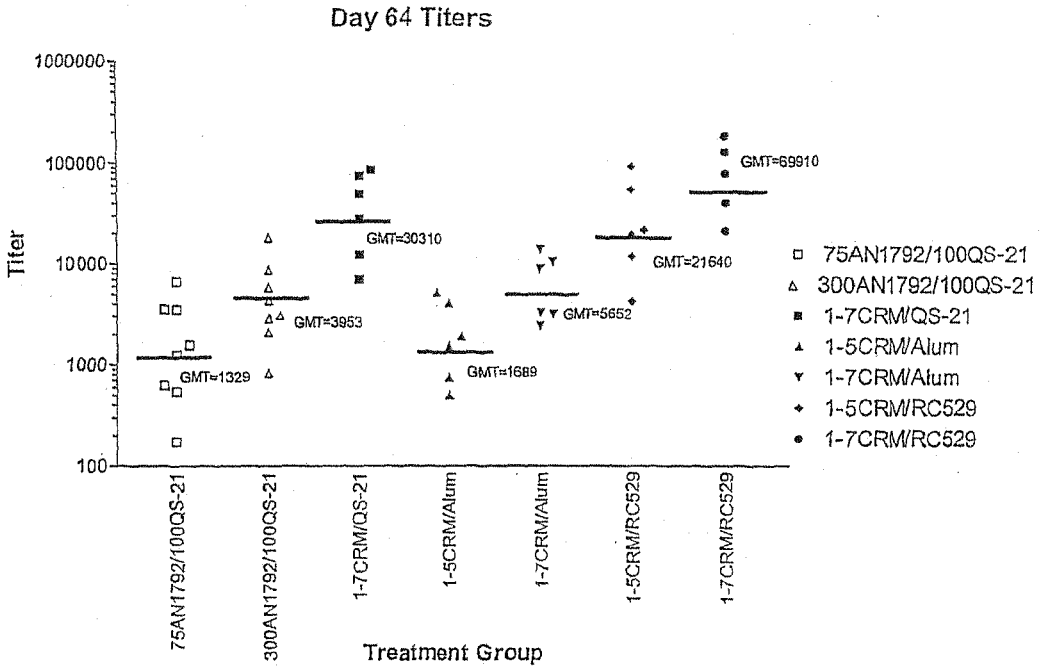

Day 64 Titers
Titer
1000000
100000
10000
1000
100
GMT=1329
GMT=3953
GMT=30310
GMT=1689
GMT=5652
GMT=21640
GMT=69910
75AN1792/100QS-21
300AN1792/100QS-21
1-7CRM/QS-21
1-5CRM/Alum
1-7CRM/Alum
1-5CRM/RC529
1-7CRM/RC529
Treatment Group

Figure 8

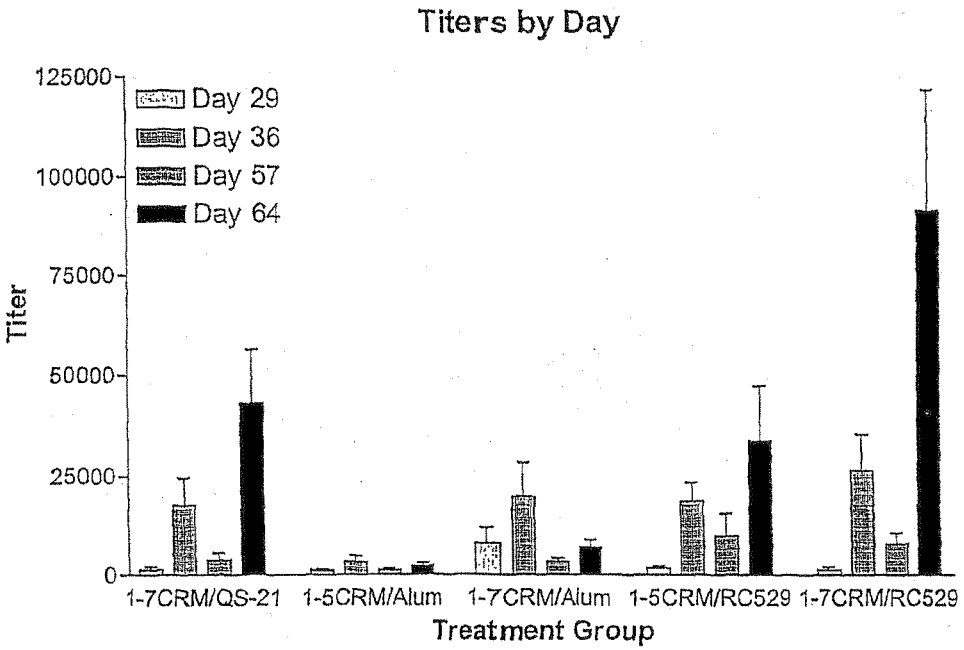

Titers by Day
125000
100000
75000
50000
25000
0
Titer
Day 29
Day 36
Day 57
Day 64
1-7CRM/QS-21
1-5CRM/Alum
1-7CRM/Alum
1-5CRM/RC529
1-7CRM/RC529
Treatment Group

Figure 9

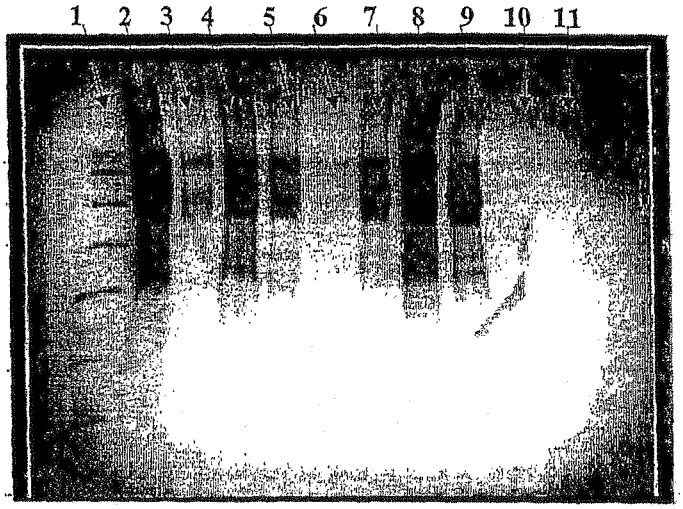


Figure 10

SEQUENCE LISTING

<110> Elan Pharma International Limited
Wyeth LLC

<120> Aß IMMUNOGENIC PEPTIDE CARRIER CONJUGATES AND METHODS OF PRODUCING SAME

<130> SP7775B

<140>

<141>

<150> US 60/530,480

<151> 2003-12-17

<160> 54

<170> PatentIn version 3.3

<210> 1

<211> 6

<212> PRT

<213> Homo sapiens

<400> 1

Asp Ala Glu Phe Arg Cys
1 5

<210> 2

<211> 8

<212> PRT

<213> Homo sapiens

<400> 2

Asp Ala Glu Phe Arg His Asp Cys
1 5

<210> 3
<211> 10
<212> PRT
<213> Homo sapiens

<400> 3

Asp Ala Glu Phe Arg His Asp Ser Gly Cys
1 5 10

<210> 4
<211> 13
<212> PRT
<213> Homo sapiens

<400> 4

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val Cys
1 5 10

<210> 5
<211> 10
<212> PRT
<213> Homo sapiens

<400> 5

Asp Ala Glu Phe Arg Gly Ala Gly Ala Cys
1 5 10

<210> 6
<211> 12
<212> PRT
<213> Homo sapiens

<400> 6

Asp Ala Glu Phe Arg His Asp Gly Ala Gly Ala Cys

1 5 10

<210> 7
<211> 14
<212> PRT
<213> Homo sapiens

<400> 7

Asp Ala Glu Phe Arg His Asp Ser Gly Gly Ala Gly Ala Cys
1 5 10

<210> 8
<211> 17
<212> PRT
<213> Homo sapiens

<400> 8

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val Gly Ala Gly Ala
1 5 10 15

Cys

<210> 9
<211> 13
<212> PRT
<213> Homo sapiens

<400> 9

Val Glu Tyr Gly Ser Asp His Arg Phe Glu Ala Asp Cys
1 5 10

<210> 10

<211> 4
<212> PRT
<213> Homo sapiens

<400> 10

Gly Ala Gly Ala
1

<210> 11
<211> 13
<212> PRT
<213> Homo sapiens

<400> 11

Pro Lys Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr
1 5 10

<210> 12
<211> 13
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<222> (3)..(3)
<223> Xaa can be any naturally occurring amino acid

<400> 12

Ala Lys Xaa Val Ala Ala Trp Thr Leu Lys Ala Ala Ala
1 5 10

<210> 13
<211> 16
<212> PRT

<213> Homo sapiens

<400> 13

Glu Lys Lys Ile Ala Lys Met Glu Lys Ala Ser Ser Val Phe Asn Val
1 5 10 15

<210> 14
<211> 10
<212> PRT
<213> Homo sapiens

<400> 14

Phe Glu Leu Leu Thr Arg Ile Leu Thr Ile
1 5 10

<210> 15
<211> 19
<212> PRT
<213> Homo sapiens

<400> 15

Asp Gln Ser Ile Gly Asp Leu Ile Ala Glu Ala Met Asp Lys Val Gly
1 5 10 15

Asn Glu Gly

<210> 16
<211> 14
<212> PRT
<213> Homo sapiens

<400> 16

Gln Val His Phe Gln Pro Leu Pro Pro Ala Val Val Lys Leu
1 5 10

<210> 17
<211> 15
<212> PRT
<213> Homo sapiens

<400> 17

Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu
1 5 10 15

<210> 18
<211> 21
<212> PRT
<213> Homo sapiens

<400> 18

Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys Val Ser
1 5 10 15

Ala Ser His Leu Glu
20

<210> 19
<211> 15
<212> PRT
<213> Homo sapiens

<400> 19

Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr
1 5 10 15

<210> 20
<211> 51
<212> PRT
<213> Homo sapiens

<400> 20

Asp Ala Glu Phe Arg His Asp Gln Tyr Ile Lys Ala Asn Ser Lys Phe
1 5 10 15

Ile Gly Ile Thr Glu Leu Cys Phe Asn Asn Phe Thr Val Ser Phe Trp
20 25 30

Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu Asp Ala Glu Phe
35 40 45

Arg His Asp
50

<210> 21
<211> 42
<212> PRT
<213> Homo sapiens

<400> 21

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30

Gly Leu Met Val Gly Gly Val Val Ile Ala
35 40

<210> 22
<211> 22
<212> PRT
<213> Homo sapiens

<400> 22

Asp Ala Glu Phe Arg His Asp Gln Tyr Ile Lys Ala Asn Ser Lys Phe
1 5 10 15

Ile Gly Ile Thr Glu Leu
20

<210> 23
<211> 28
<212> PRT
<213> Homo sapiens

<400> 23

Asp Ala Glu Phe Arg His Asp Phe Asn Asn Phe Thr Val Ser Phe Trp
1 5 10 15

Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu
20 25

<210> 24
<211> 43
<212> PRT
<213> Homo sapiens

<400> 24

Asp Ala Glu Phe Arg His Asp Gln Tyr Ile Lys Ala Asn Ser Lys Phe
1 5 10 15

Ile Gly Ile Thr Glu Leu Phe Asn Asn Phe Thr Val Ser Phe Trp Leu
20 25 30

Arg Val Pro Lys Val Ser Ala Ser His Leu Glu
35 40

<210> 25
<211> 22
<212> PRT
<213> Homo sapiens

<400> 25

Glu Phe Arg His Asp Ser Gly Gln Tyr Ile Lys Ala Asn Ser Lys Phe
1 5 10 15

Ile Gly Ile Thr Glu Leu
20

<210> 26
<211> 20
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<222> (3)..(3)
<223> Xaa can be any naturally occurring amino acid

<400> 26

Ala Lys Xaa Val Ala Ala Trp Thr Leu Lys Ala Ala Ala Asp Ala Glu
1 5 10 15

Phe Arg His Asp
20

<210> 27
<211> 34
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<222> (24)..(24)
<223> Xaa can be any naturally occurring amino acid

<400> 27

Asp Ala Glu Phe Arg His Asp Asp Ala Glu Phe Arg His Asp Asp Ala
1 5 10 15

Glu Phe Arg His Asp Ala Lys Xaa Val Ala Ala Trp Thr Leu Lys Ala
20 25 30

Ala Ala

<210> 28
<211> 34
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<222> (3)..(3)
<223> Xaa can be any naturally occurring amino acid

<400> 28

Ala Lys Xaa Val Ala Ala Trp Thr Leu Lys Ala Ala Ala Asp Ala Glu
1 5 10 15

Phe Arg His Asp Asp Ala Glu Phe Arg His Asp Asp Ala Glu Phe Arg
20 25 30

His Asp

<210> 29
<211> 20
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<222> (10)..(10)
<223> Xaa can be any naturally occurring amino acid

<400> 29

Asp Ala Glu Phe Arg His Asp Ala Lys Xaa Val Ala Ala Trp Thr Leu
1 5 10 15

Lys Ala Ala Ala
20

<210> 30
<211> 24
<212> PRT
<213> Homo sapiens

<400> 30

Asp Ala Glu Phe Arg His Asp Ile Ser Gln Ala Val His Ala Ala His
1 5 10 15

Ala Glu Ile Asn Glu Ala Gly Arg
20

<210> 31
<211> 24
<212> PRT
<213> Homo sapiens

<400> 31

Phe Arg His Asp Ser Gly Tyr Ile Ser Gln Ala Val His Ala Ala His
1 5 10 15

Ala Glu Ile Asn Glu Ala Gly Arg
20

<210> 32
<211> 24
<212> PRT
<213> Homo sapiens

<400> 32

Glu Phe Arg His Asp Ser Gly Ile Ser Gln Ala Val His Ala Ala His
1 5 10 15

Ala Glu Ile Asn Glu Ala Gly Arg
20

<210> 33
<211> 34
<212> PRT

<213> Homo sapiens

<400> 33

Pro Lys Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr Asp Ala Glu
1 5 10 15

Phe Arg His Asp Asp Ala Glu Phe Arg His Asp Asp Ala Glu Phe Arg
20 25 30

His Asp

<210> 34
<211> 27
<212> PRT
<213> Homo sapiens

<400> 34

Asp Ala Glu Phe Arg His Asp Pro Lys Tyr Val Lys Gln Asn Thr Leu
1 5 10 15

Lys Leu Ala Thr Asp Ala Glu Phe Arg His Asp
20 25

<210> 35
<211> 34
<212> PRT
<213> Homo sapiens

<400> 35

Asp Ala Glu Phe Arg His Asp Asp Ala Glu Phe Arg His Asp Asp Ala
1 5 10 15

Glu Phe Arg His Asp Pro Lys Tyr Val Lys Gln Asn Thr Leu Lys Leu
20 25 30

Ala Thr

<210> 36
<211> 27
<212> PRT
<213> Homo sapiens

<400> 36

Asp Ala Glu Phe Arg His Asp Asp Ala Glu Phe Arg His Asp Pro Lys
1 5 10 15

Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr
20 25

<210> 37
<211> 79
<212> PRT
<213> Homo sapiens

<400> 37

Asp Ala Glu Phe Arg His Asp Pro Lys Tyr Val Lys Gln Asn Thr Leu
1 5 10 15

Lys Leu Ala Thr Glu Lys Lys Ile Ala Lys Met Glu Lys Ala Ser Ser
20 25 30

Val Phe Asn Val Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly Ile
35 40 45

Thr Glu Leu Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro
50 55 60

Lys Val Ser Ala Ser His Leu Glu Asp Ala Glu Phe Arg His Asp
65 70 75

<210> 38
<211> 58
<212> PRT
<213> Homo sapiens

<400> 38

Asp Ala Glu Phe Arg His Asp Asp Ala Glu Phe Arg His Asp Asp Ala
1 5 10 15

Glu Phe Arg His Asp Gln Tyr Ile Lys Ala Asn Ser Lys Phe Ile Gly
20 25 30

Ile Thr Glu Leu Cys Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg
35 40 45

Val Pro Lys Val Ser Ala Ser His Leu Glu
50 55

<210> 39
<211> 44
<212> PRT
<213> Homo sapiens

<400> 39

Asp Ala Glu Phe Arg His Asp Gln Tyr Ile Lys Ala Asn Ser Lys Phe

1 5 10 15

Ile Gly Ile Thr Glu Leu Cys Phe Asn Asn Phe Thr Val Ser Phe Trp
20 25 30

Leu Arg Val Pro Lys Val Ser Ala Ser His Leu Glu
35 40

<210> 40
<211> 535
<212> PRT
<213> Homo sapiens

<400> 40

Gly Ala Asp Asp Val Val Asp Ser Ser Lys Ser Phe Val Met Glu Asn
1 5 10 15

Phe Ser Ser Tyr His Gly Thr Lys Pro Gly Tyr Val Asp Ser Ile Gln
20 25 30

Lys Gly Ile Gln Lys Pro Lys Ser Gly Thr Gln Gly Asn Tyr Asp Asp
35 40 45

Asp Trp Lys Glu Phe Tyr Ser Thr Asp Asn Lys Tyr Asp Ala Ala Gly
50 55 60

Tyr Ser Val Asp Asn Glu Asn Pro Leu Ser Gly Lys Ala Gly Gly Val
65 70 75 80

Val Lys Val Thr Tyr Pro Gly Leu Thr Lys Val Leu Ala Leu Lys Val
85 90 95

```
Asp Asn Ala Glu Thr Ile Lys Lys Glu Leu Gly Leu Ser Leu Thr Glu
            100                 105                 110

Pro Leu Met Glu Gln Val Gly Thr Glu Glu Phe Ile Lys Arg Phe Gly
        115                 120                 125

Asp Gly Ala Ser Arg Val Val Leu Ser Leu Pro Phe Ala Glu Gly Ser
    130                 135                 140

Ser Ser Val Glu Tyr Ile Asn Asn Trp Glu Gln Ala Lys Ala Leu Ser
145                 150                 155                 160

Val Glu Leu Glu Ile Asn Phe Glu Thr Arg Gly Lys Arg Gly Gln Asp
                165                 170                 175

Ala Met Tyr Glu Tyr Met Ala Gln Ala Cys Ala Gly Asn Arg Val Arg
            180                 185                 190

Arg Ser Val Gly Ser Ser Leu Ser Cys Ile Asn Leu Asp Trp Asp Val
        195                 200                 205

Ile Arg Asp Lys Thr Lys Thr Lys Ile Glu Ser Leu Lys Glu His Gly
    210                 215                 220

Pro Ile Lys Asn Lys Met Ser Glu Ser Pro Asn Lys Thr Val Ser Glu
225                 230                 235                 240

Glu Lys Ala Lys Gln Tyr Leu Glu Glu Phe His Gln Thr Ala Leu Glu
                245                 250                 255
```

```
His Pro Glu Leu Ser Glu Leu Lys Thr Val Thr Gly Thr Asn Pro Val
            260                 265                 270

Phe Ala Gly Ala Asn Tyr Ala Ala Trp Ala Val Asn Val Ala Gln Val
        275                 280                 285

Ile Asp Ser Glu Thr Ala Asp Asn Leu Glu Lys Thr Thr Ala Ala Leu
    290                 295                 300

Ser Ile Leu Pro Gly Ile Gly Ser Val Met Gly Ile Ala Asp Gly Ala
305                 310                 315                 320

Val His His Asn Thr Glu Glu Ile Val Ala Gln Ser Ile Ala Leu Ser
                325                 330                 335

Ser Leu Met Val Ala Gln Ala Ile Pro Leu Val Gly Glu Leu Val Asp
            340                 345                 350

Ile Gly Phe Ala Ala Tyr Asn Phe Val Glu Ser Ile Ile Asn Leu Phe
        355                 360                 365

Gln Val Val His Asn Ser Tyr Asn Arg Pro Ala Tyr Ser Pro Gly His
    370                 375                 380

Lys Thr Gln Pro Phe Leu His Asp Gly Tyr Ala Val Ser Trp Asn Thr
385                 390                 395                 400

Val Glu Asp Ser Ile Ile Arg Thr Gly Phe Gln Gly Glu Ser Gly His
                405                 410                 415

Asp Ile Lys Ile Thr Ala Glu Asn Thr Pro Leu Pro Ile Ala Gly Val
```

420 425 430

Leu Leu Pro Thr Ile Pro Gly Lys Leu Asp Val Asn Lys Ser Lys Thr
435 440 445

His Ile Ser Val Asn Gly Arg Lys Ile Arg Met Arg Cys Arg Ala Ile
450 455 460

Asp Gly Asp Val Thr Phe Cys Arg Pro Lys Ser Pro Val Tyr Val Gly
465 470 475 480

Asn Gly Val His Ala Asn Leu His Val Ala Phe His Arg Ser Ser Ser
485 490 495

Glu Lys Ile His Ser Asn Glu Ile Ser Ser Asp Ser Ile Gly Val Leu
500 505 510

Gly Tyr Gln Lys Thr Val Asp His Thr Lys Val Asn Ser Lys Leu Ser
515 520 525

Leu Phe Phe Glu Ile Lys Ser
530 535

<210> 41
<211> 17
<212> PRT
<213> Homo sapiens

<400> 41

Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
1 5 10 15

Arg

<210> 42
<211> 42
<212> PRT
<213> Mus musculus

<400> 42

Asp Ala Glu Phe Gly His Asp Ser Gly Phe Glu Val Arg His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30

Gly Leu Met Val Gly Gly Val Val Ile Ala
35 40

<210> 43
<211> 9
<212> PRT
<213> Artificial

<220>
<223> A-beta 18-25 + C

<400> 43

Val Phe Phe Ala Glu Asp Val Gly Cys
1 5

<210> 44
<211> 9
<212> PRT

<213> Artificial

<220>
<223> A-beta 17-24 + C

<400> 44

Leu Val Phe Phe Ala Glu Asp Val Cys
1 5

<210> 45
<211> 9
<212> PRT
<213> Artificial

<220>
<223> A-beta 16-23 + C

<400> 45

Lys Leu Val Phe Phe Ala Glu Asp Cys
1 5

<210> 46
<211> 9
<212> PRT
<213> Artificial

<220>
<223> A-beta 18-25 + C

<400> 46

Cys Val Phe Phe Ala Glu Asp Val Gly
1 5

<210> 47
<211> 9

<212> PRT
<213> Artificial

<220>
<223> C + A-beta 18-25

<400> 47

Cys Leu Val Phe Phe Ala Glu Asp Val
1 5

<210> 48
<211> 9
<212> PRT
<213> Artificial

<220>
<223> C + A-beta 16-23

<400> 48

Cys Lys Leu Val Phe Phe Ala Glu Asp
1 5

<210> 49
<211> 8
<212> PRT
<213> Artificial

<220>
<223> A-beta 18-24 + C

<400> 49

Val Phe Phe Ala Glu Asp Val Cys
1 5

<210> 50

<211> 8
<212> PRT
<213> Artificial

<220>
<223> A-beta 17-23 + C

<400> 50

Leu Val Phe Phe Ala Glu Asp Cys
1 5

<210> 51
<211> 8
<212> PRT
<213> Artificial

<220>
<223> A-beta 16-22 + C

<220>
<221> misc_feature
<222> (8)..(8)
<223> CRM 197 added via terminal cysteine

<400> 51

Lys Leu Val Phe Phe Ala Glu Cys
1 5

<210> 52
<211> 8
<212> PRT
<213> Artificial

<220>
<223> C + A-beta 18-24

<220>
<221> misc_feature
<222> (1)..(1)
<223> CRM 197 added via N-terminal cysteine

<400> 52

Cys Val Phe Phe Ala Glu Asp Val
1 5

<210> 53
<211> 8
<212> PRT
<213> Artificial

<220>
<223> C + A-beta 17-23

<400> 53

Cys Leu Val Phe Phe Ala Glu Asp
1 5

<210> 54
<211> 8
<212> PRT
<213> Artificial

<220>
<223> C + A-beta 16-22 + C

<220>
<221> misc_feature
<222> (1)..(1)
<223> CRM 197 added via N-terminal cysteine

<400> 54

Cys Lys Leu Val Phe Phe Ala Glu
1 5