FIG. 1A

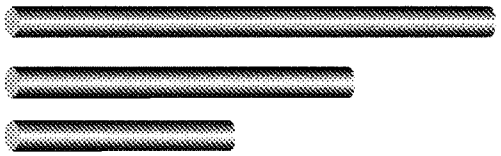


FIG. 1B

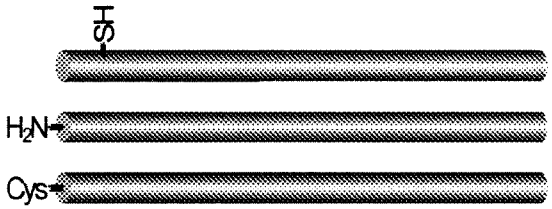


FIG. 1C

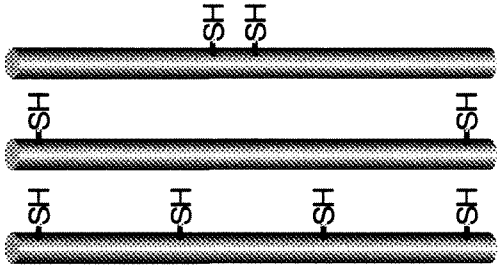


FIG. 1D

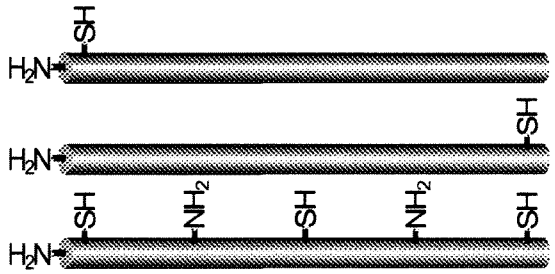


FIG. 1E

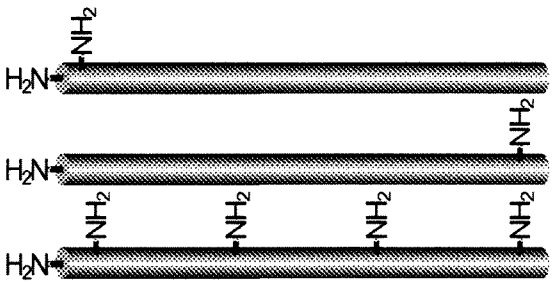


FIG. 1

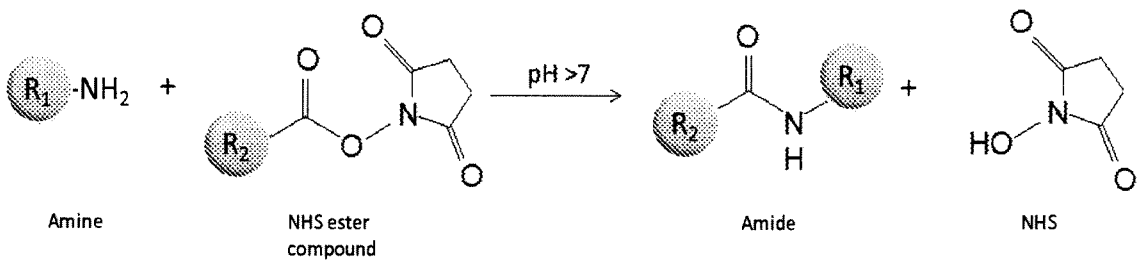

R1-NH2
+
R2
O
N
O
pH >7
R2
N
H
R1
+
HO
N
O
O
Amine
NHS ester compound
Amide
NHS

FIG. 2

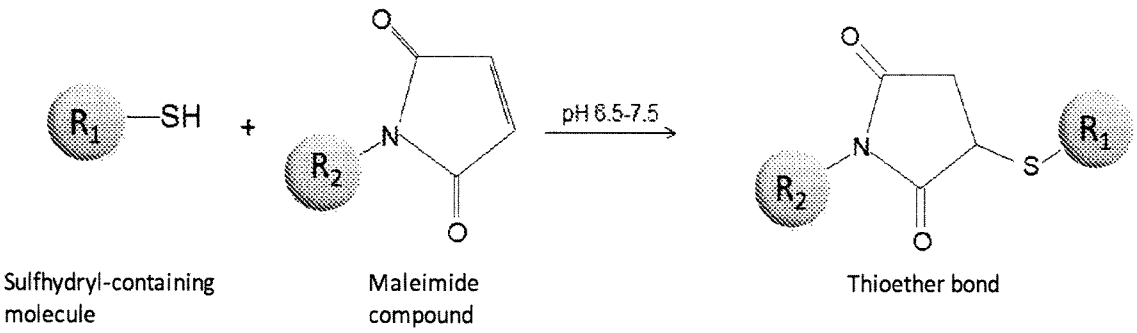

R1—SH
+
R2
N
O
O
pH 6.5-7.5
R2
N
O
O
S
R1
Sulfhydryl-containing molecule
Maleimide compound
Thioether bond

FIG. 3

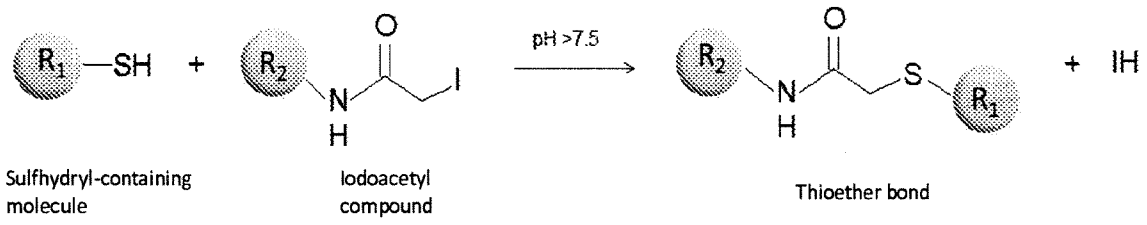

R1—SH
+
R2
O
N
H
I
pH >7.5
R2
O
N
H
S
R1
+ IH
Sulfhydryl-containing molecule
Iodoacetyl compound
Thioether bond

FIG. 4

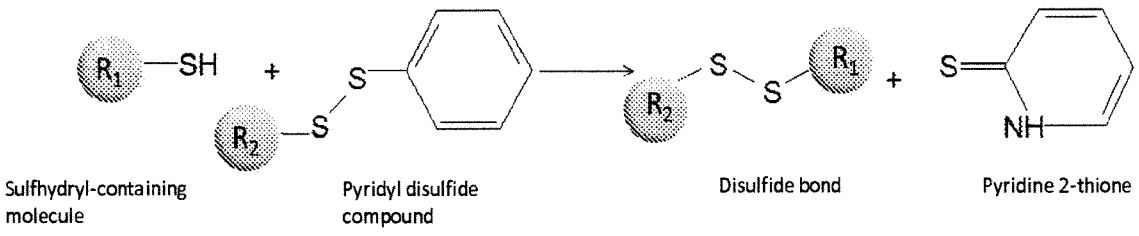

R1—SH
+
S
R2—S
R2
S
S
R1
+
S
NH
Sulfhydryl-containing molecule
Pyridyl disulfide compound
Disulfide bond
Pyridine 2-thione

FIG. 5

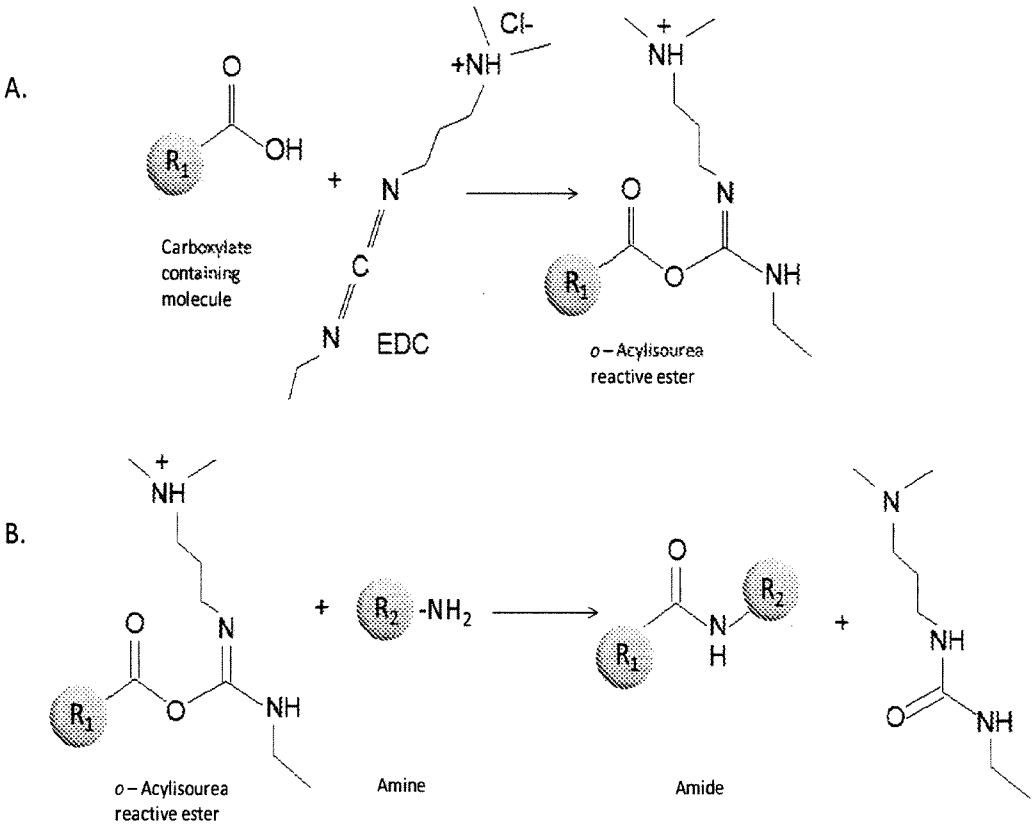

A.
Cl-
+NH
O
OH
R1
+
N
C
N
EDC
Carboxylate containing molecule
+
NH
O
N
R1
O
NH
o – Acylisourea reactive ester
B.
+
NH
O
N
R1
O
NH
o – Acylisourea reactive ester
+
R2 -NH2
Amine
O
R2
N
H
R1
Amide
+
N
NH
O
NH

FIG. 6

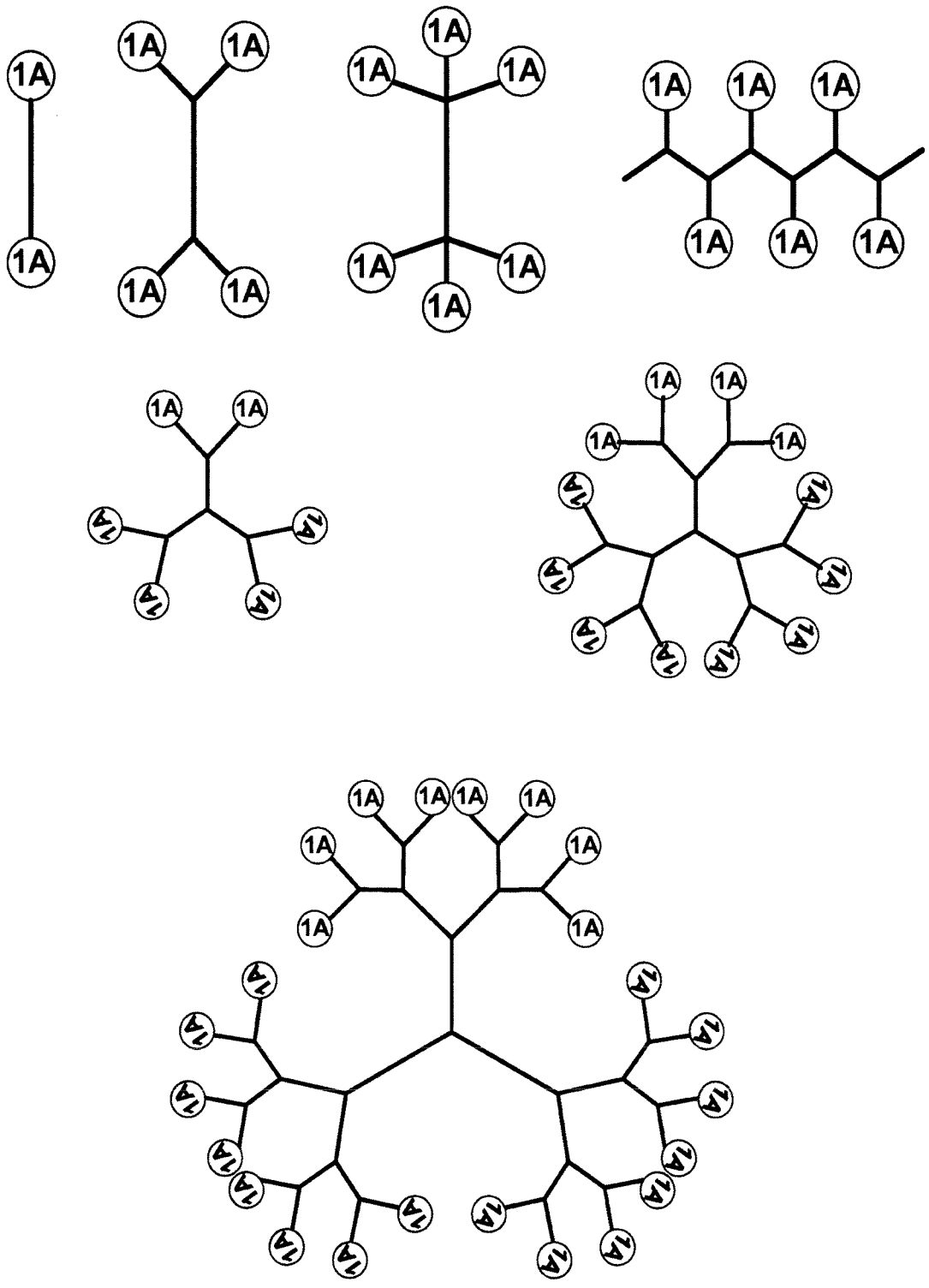


FIG. 7

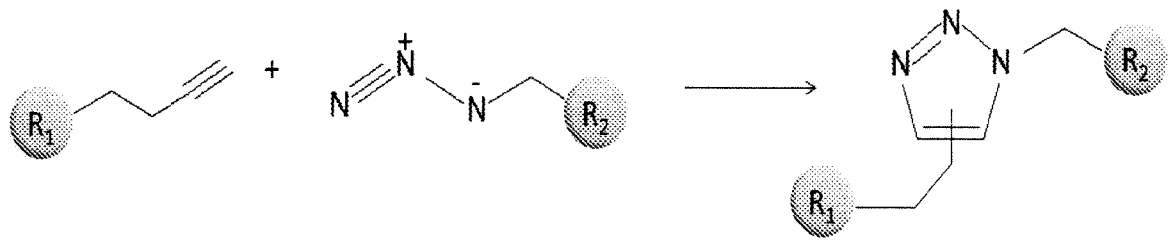


FIG. 8

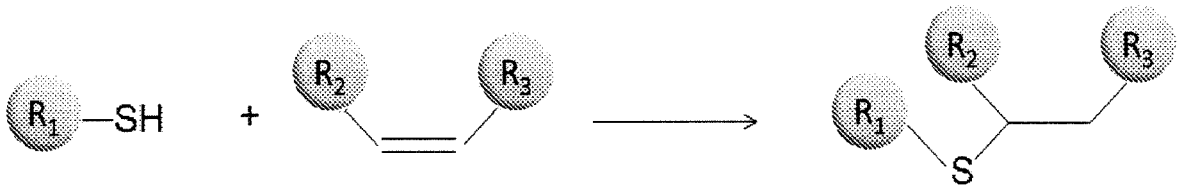


FIG. 9

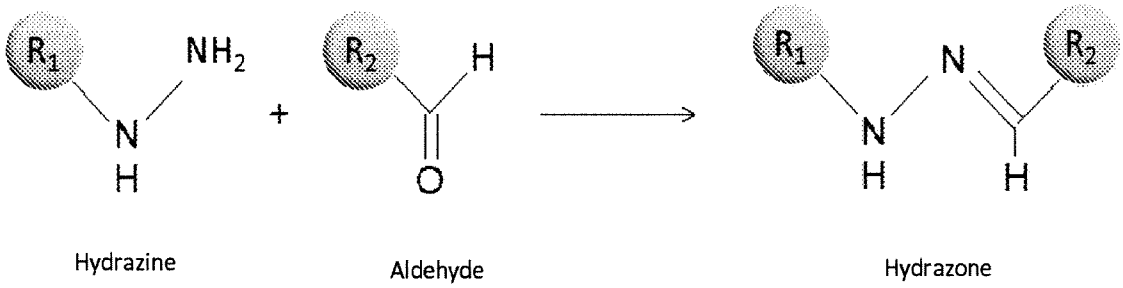


FIG. 10

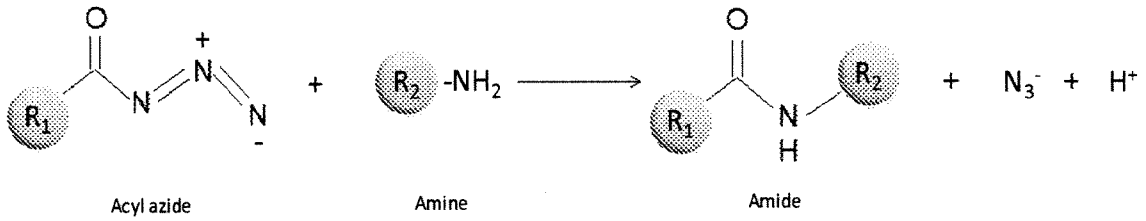


FIG. 11

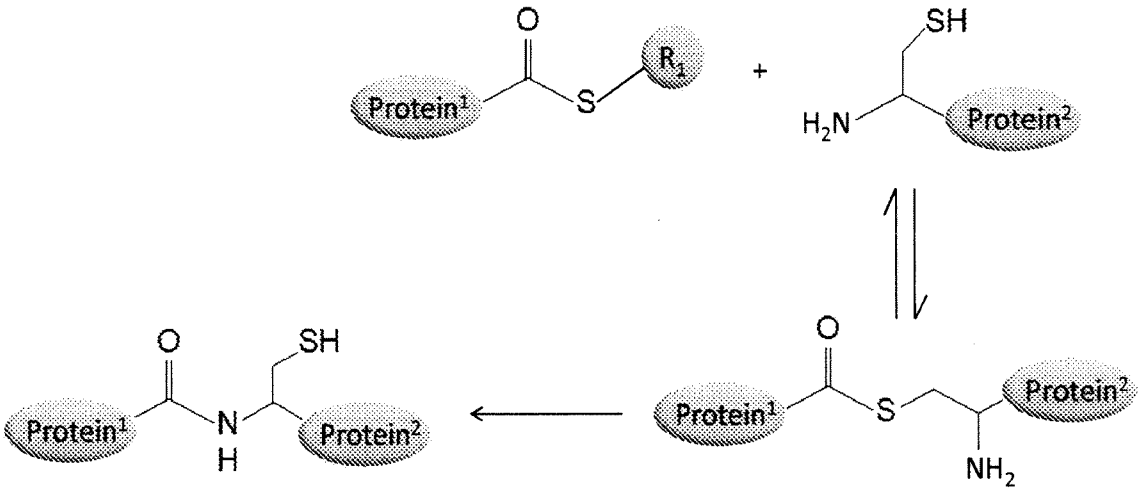

O
R1
Protein1
S
+
SH
H2N
Protein2
O
SH
Protein1
N
H
Protein2
O
Protein1
S
Protein2
NH2

FIG. 12

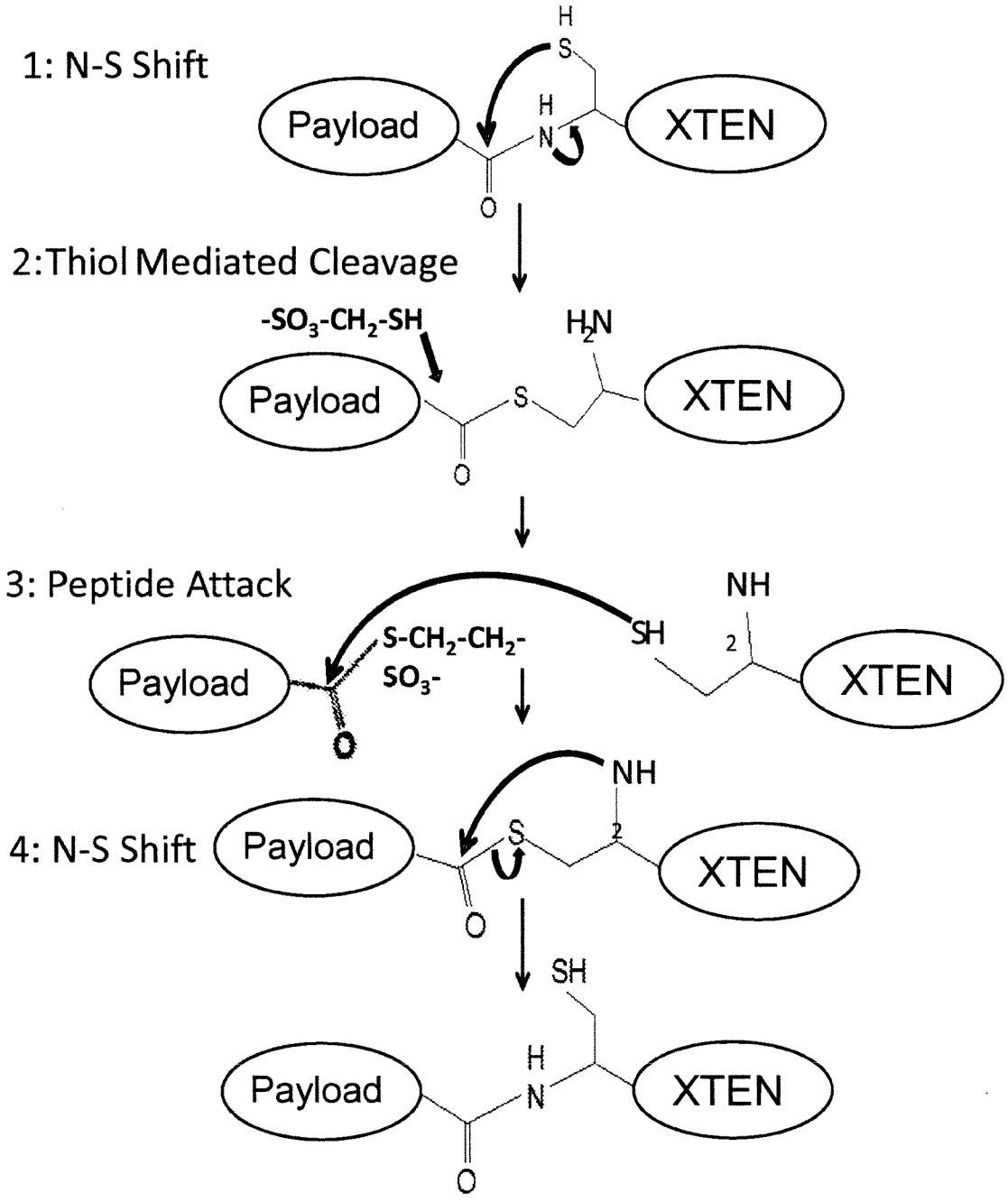

1: N-S Shift
Payload
XTEN
2:Thiol Mediated Cleavage
-SO3-CH2-SH
H2N
3: Peptide Attack
S-CH2-CH2-
SO3-
NH2
4: N-S Shift

FIG. 13

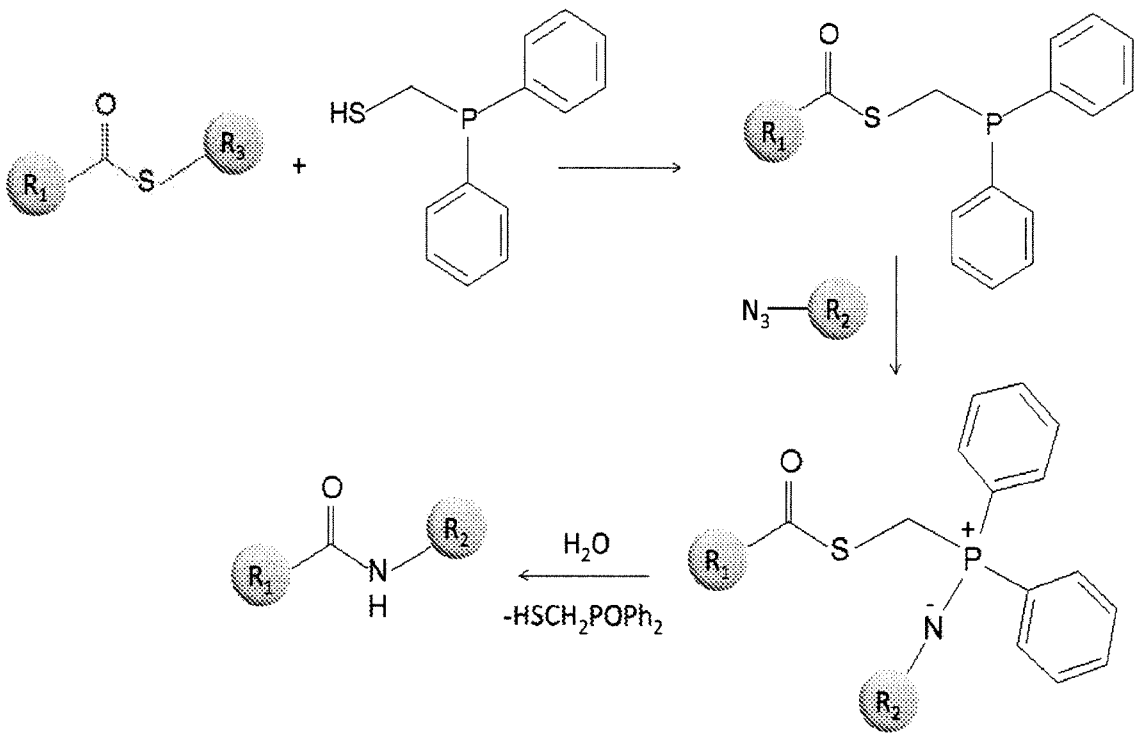


FIG. 14

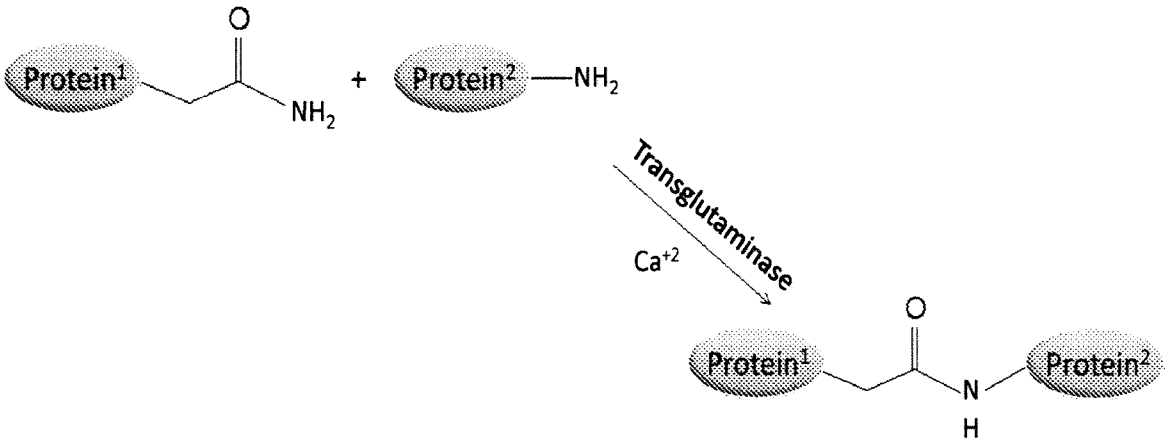

Protein1
O
NH2
+
Protein2
NH2
Transglutaminase
Ca+2
Protein1
O
N
H
Protein2

FIG. 15

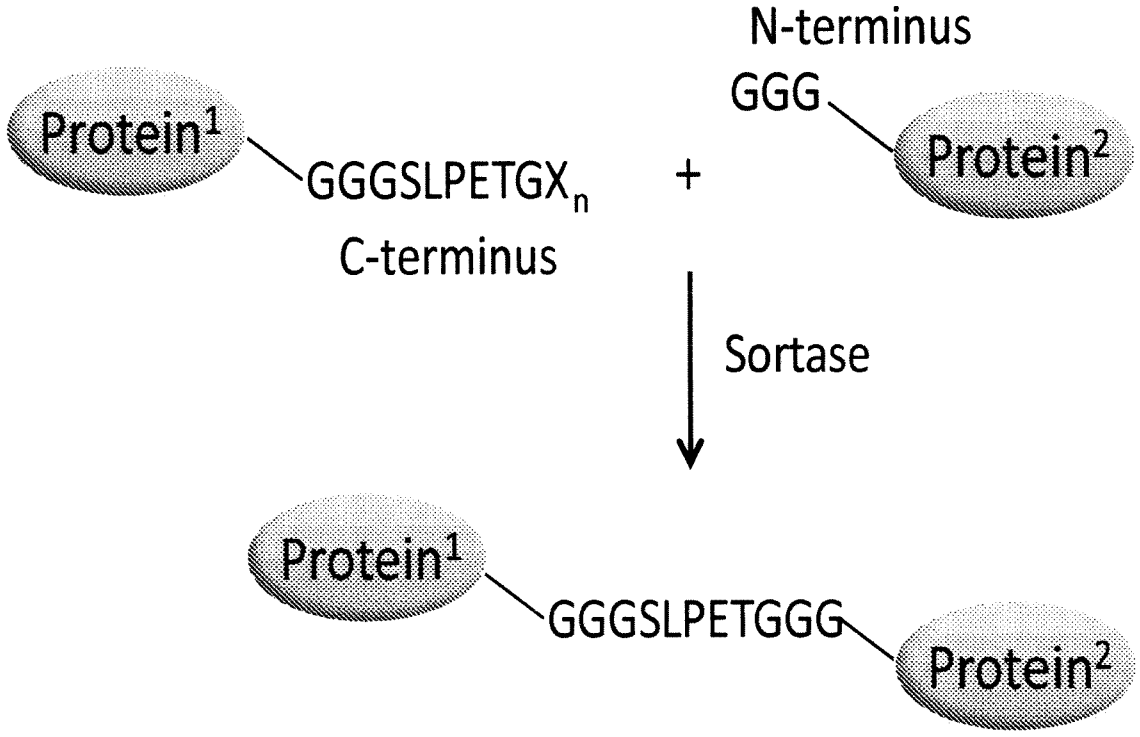

N-terminus
GGG
Protein1
GGGSLPETGXn
C-terminus
+
Protein2
Sortase
Protein1
GGGSLPETGGG
Protein2

FIG. 16

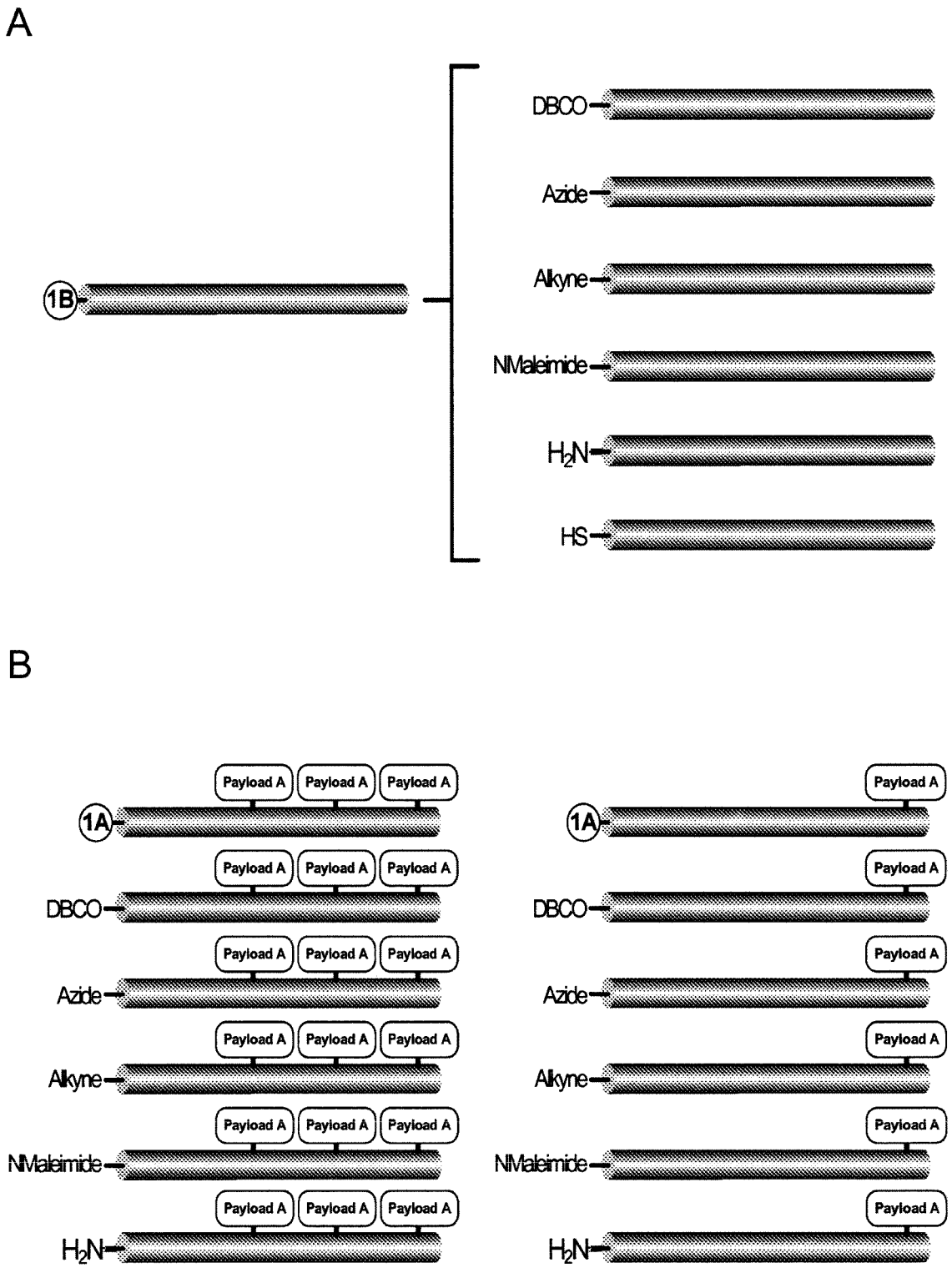

A
1B
DBCO
Azide
Alkyne
NMaleimide
H2N
HS
B
1A
Payload A
DBCO
Azide
Alkyne
NMaleimide
H2N

FIG. 17

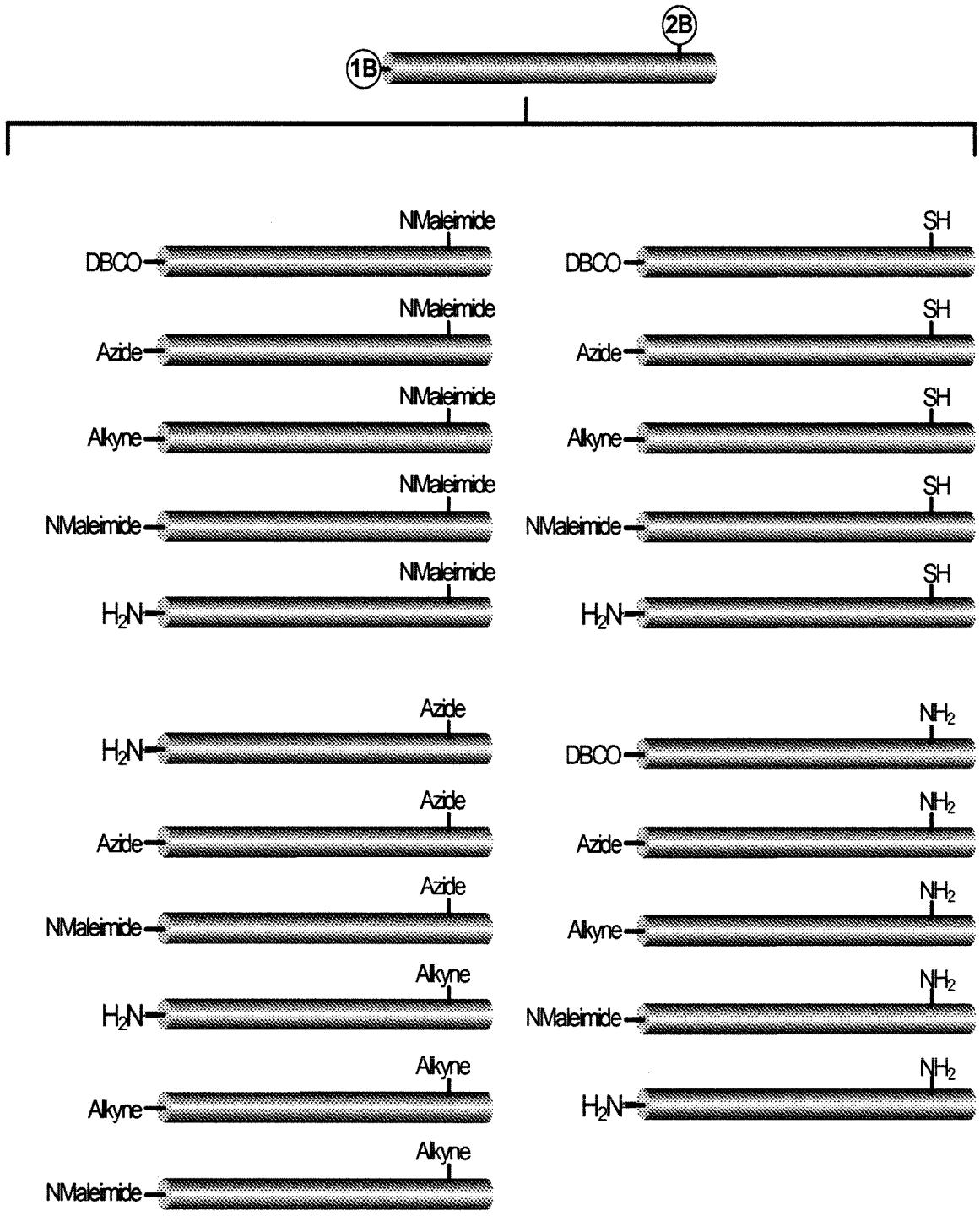

2B
1B
NMaleimide
DBCO
NMaleimide
Azide
NMaleimide
Alkyne
NMaleimide
NMaleimide
NMaleimide
H2N
SH
DBCO
SH
Azide
SH
Alkyne
SH
NMaleimide
SH
H2N
Azide
H2N
Azide
Azide
Azide
NMaleimide
Alkyne
H2N
Alkyne
Alkyne
Alkyne
NMaleimide
NH2
DBCO
NH2
Azide
NH2
Alkyne
NH2
NMaleimide
NH2
H2N

FIG. 18

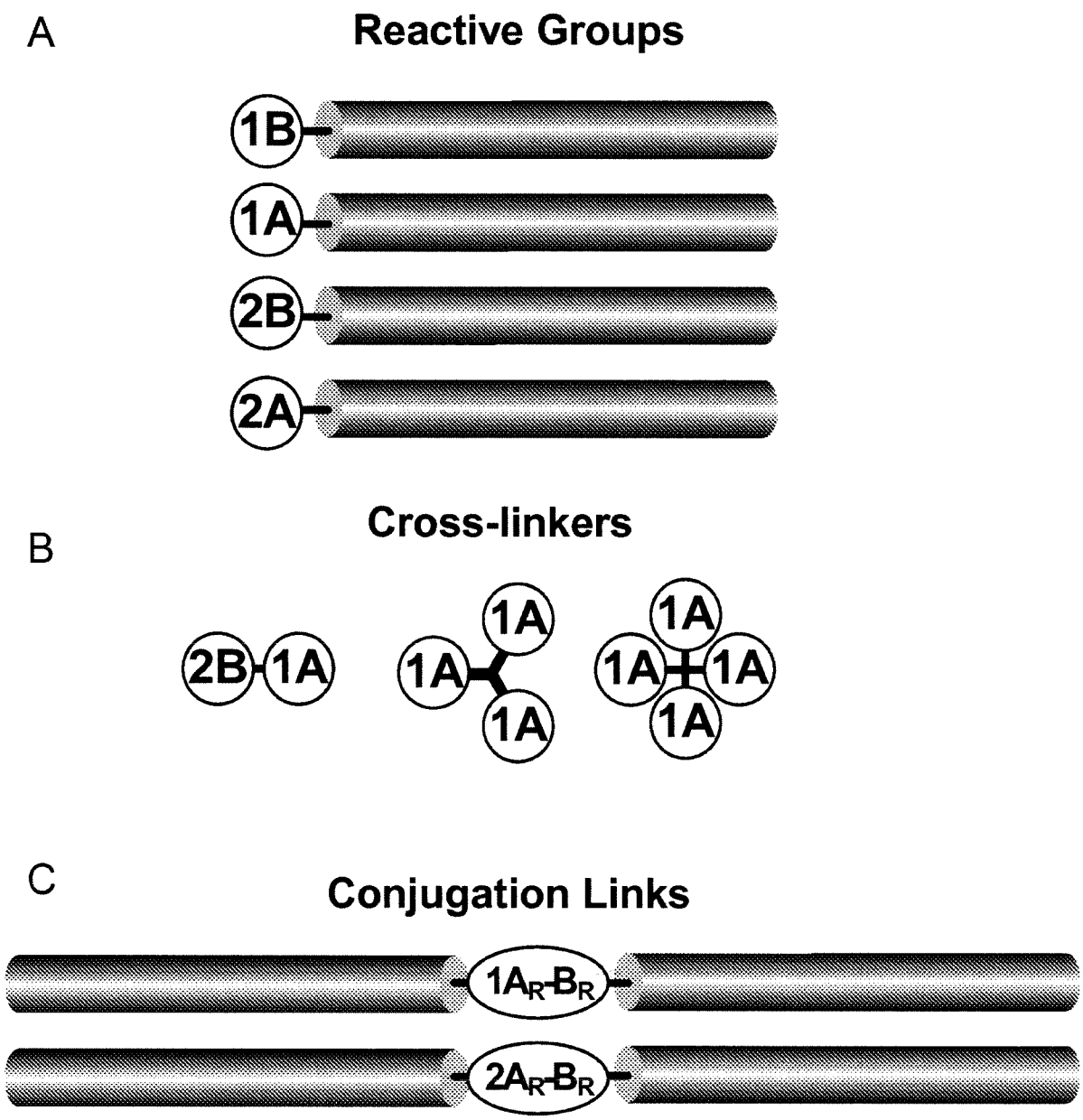

A
Reactive Groups
1B
1A
2B
2A
B
Cross-linkers
2B
1A
1A
1A
1A
1A
1A
1A
C
Conjugation Links
1A$_R$-B$_R$
2A$_R$-B$_R$

FIG. 19

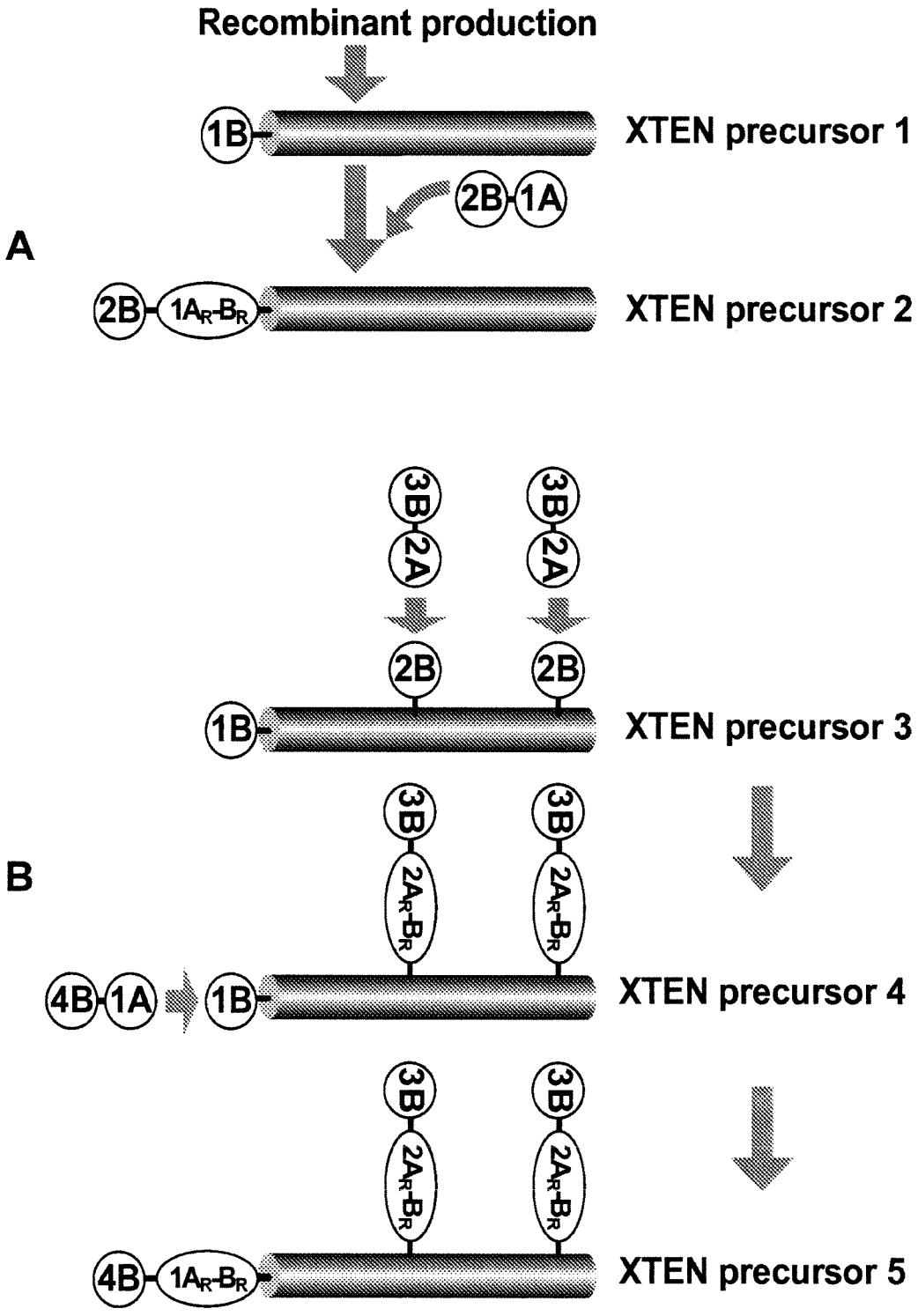

Recombinant production
1B
XTEN precursor 1
2B
1A
A
2B
1A$_R$-B$_R$
XTEN precursor 2
3B
2A
2B
1B
XTEN precursor 3
B
2A$_R$-B$_R$
4B
1A
XTEN precursor 4
4B
1A$_R$-B$_R$
XTEN precursor 5

FIG. 20

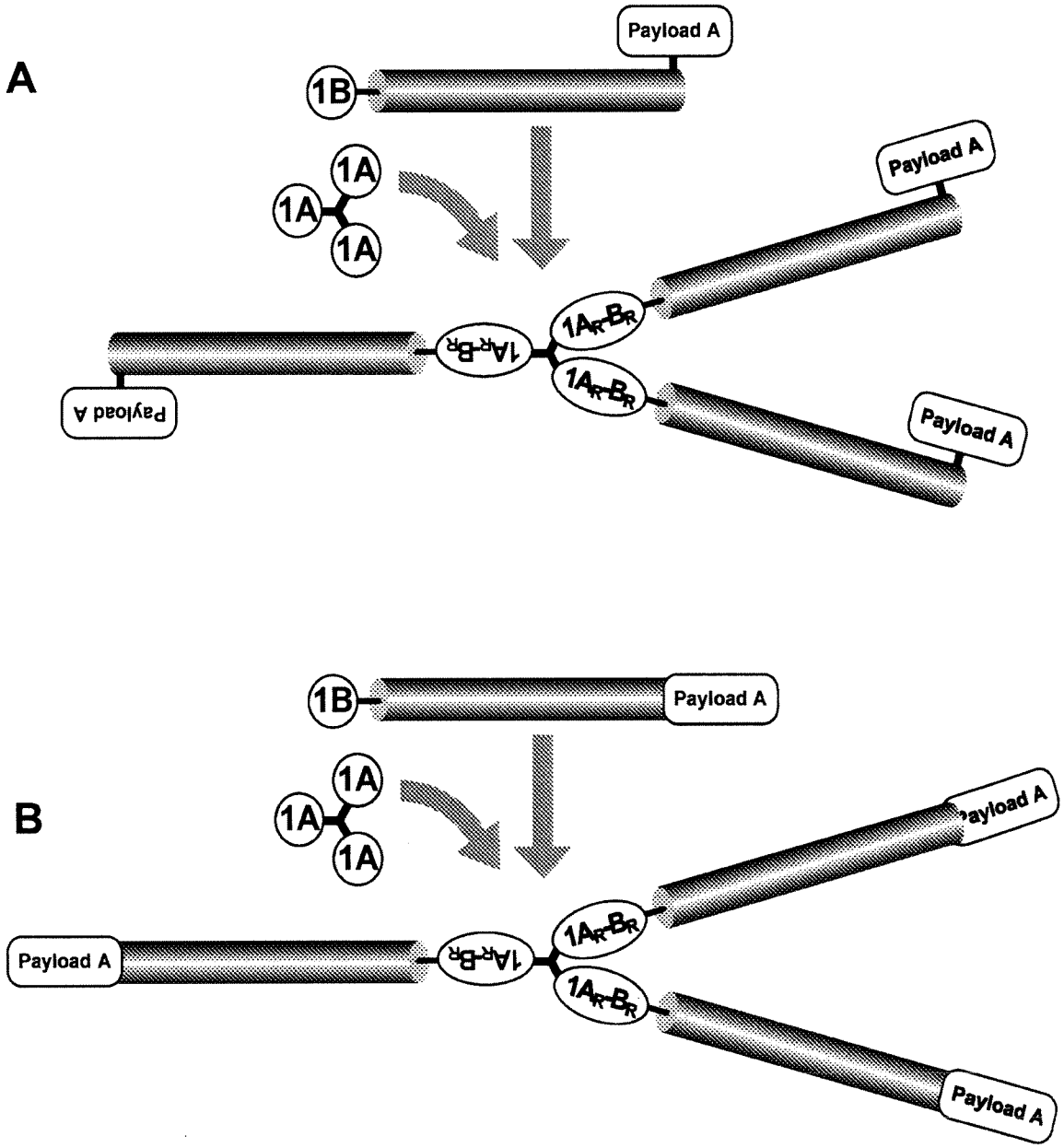

A
Payload A
1B
1A
1A
1A
Payload A
1A$_R$-B$_R$
1A$_R$-B$_R$
1A$_R$-B$_R$
Payload A
Payload A
B
1B
Payload A
1A
1A
1A
Payload A
1A$_R$-B$_R$
1A$_R$-B$_R$
1A$_R$-B$_R$
Payload A

FIG. 21

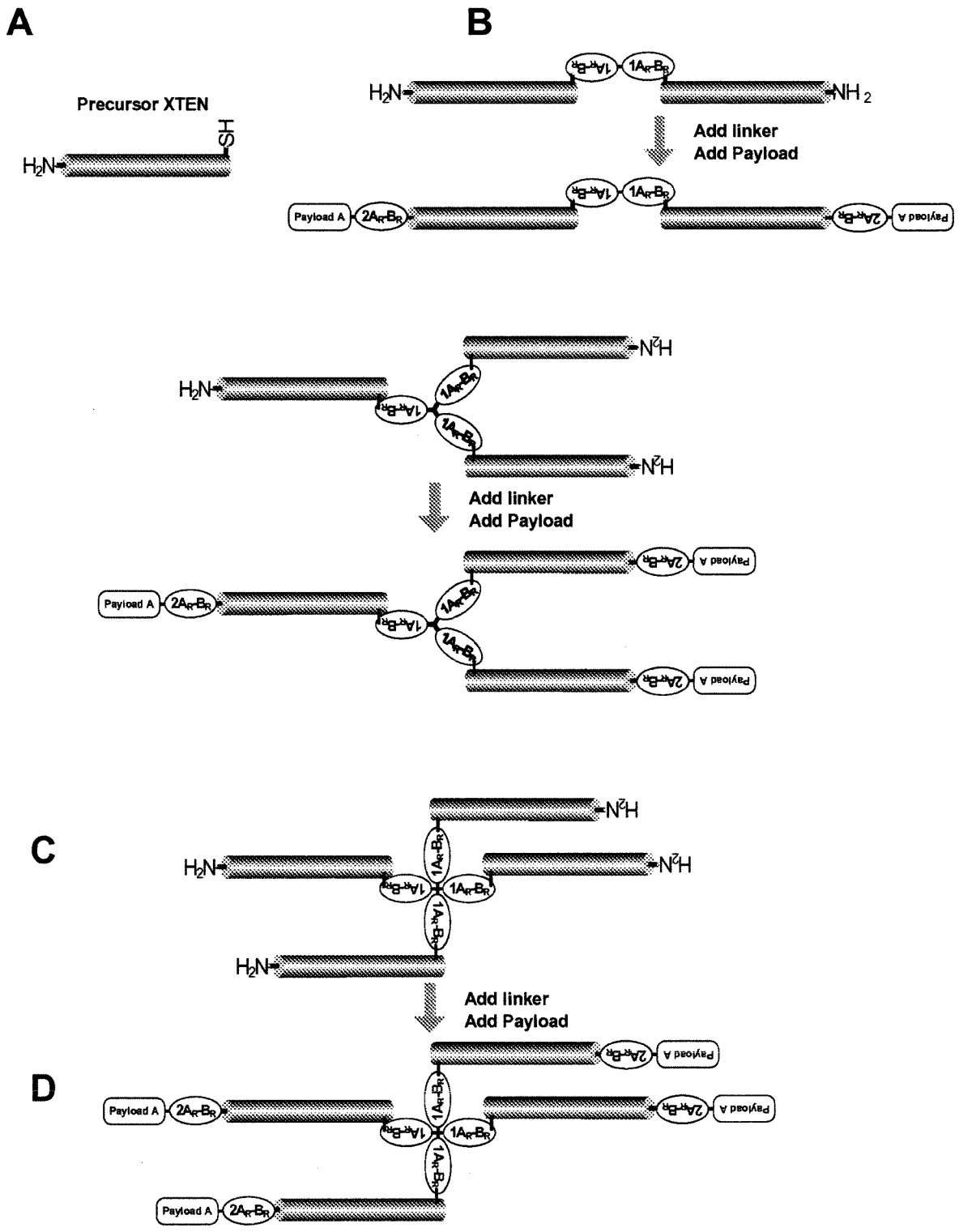

A
B
Precursor XTEN
SH
H_2N
NH_2
Add linker
Add Payload
Payload A
$2A_RB_R$
$1A_RB_R$
C
D

FIG. 22

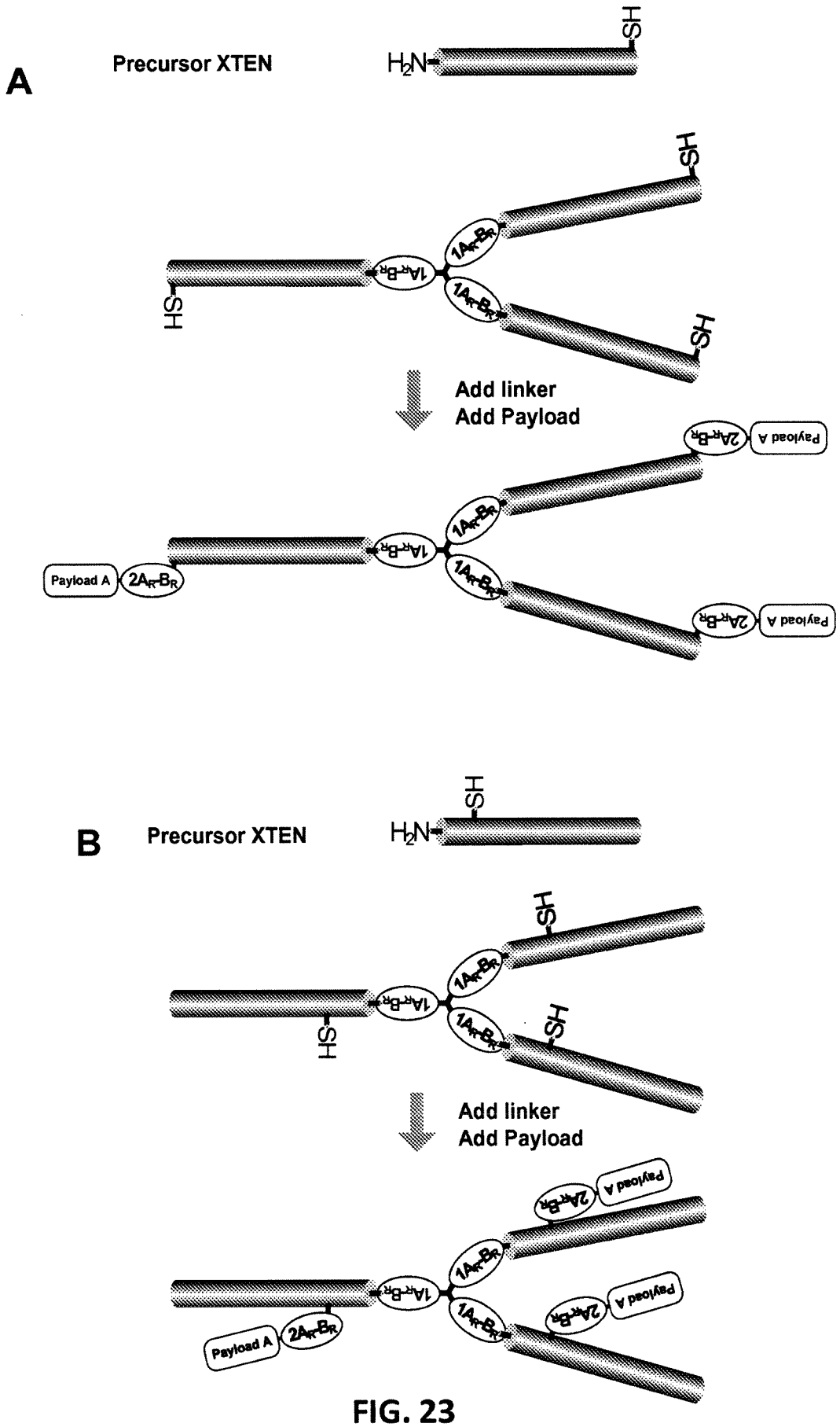

A
Precursor XTEN
H_2N
SH
Add linker
Add Payload
Payload A
$2A_R$-B_R
$1A_R$-B_R
B
Precursor XTEN
H_2N
SH
Add linker
Add Payload
Payload A
$2A_R$-B_R
$1A_R$-B_R

FIG. 23

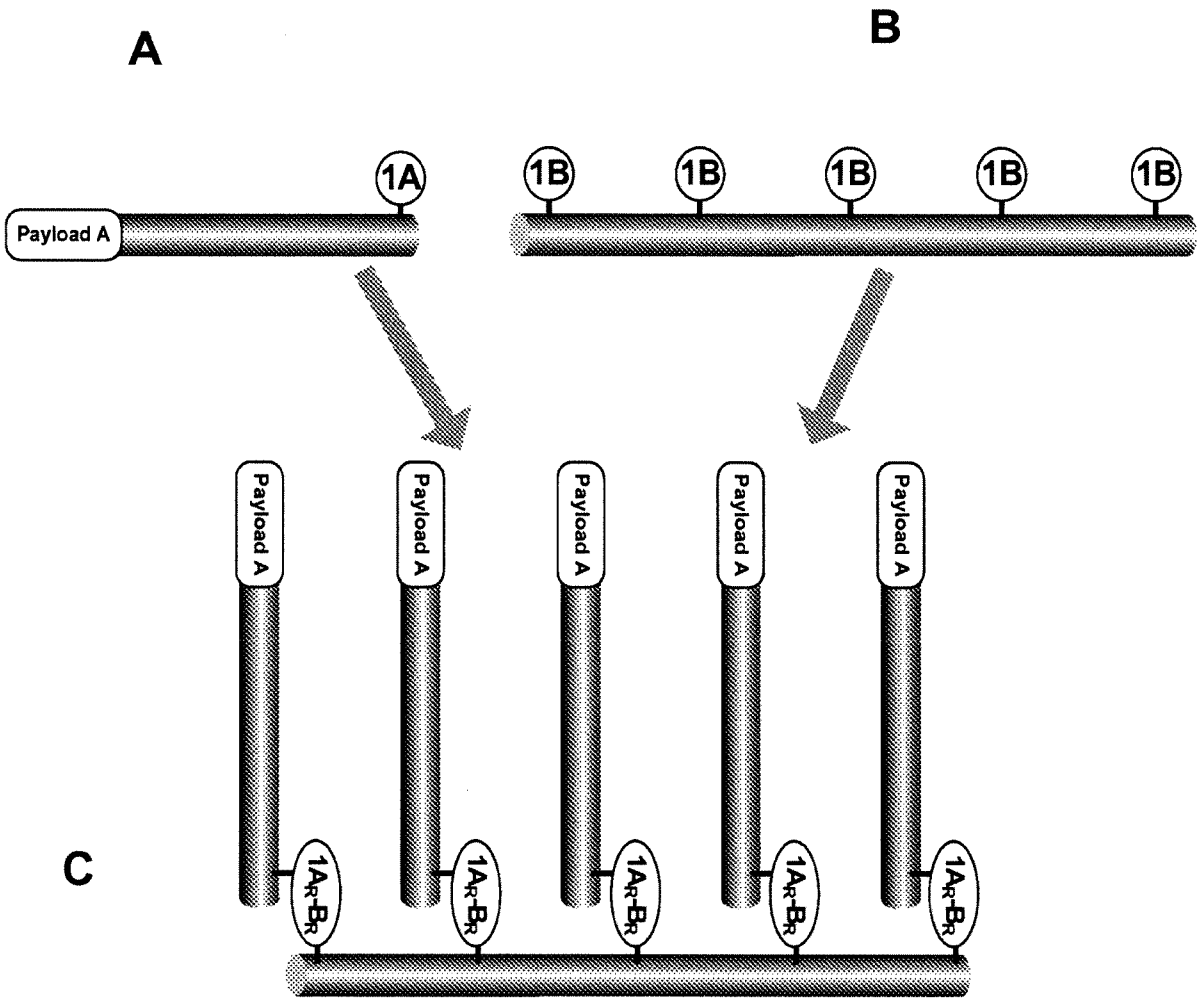

A
B
C
Payload A
1A
1B
1B
1B
1B
1B
Payload A
Payload A
Payload A
Payload A
Payload A
1AR-BR
1AR-BR
1AR-BR
1AR-BR
1AR-BR

FIG. 24

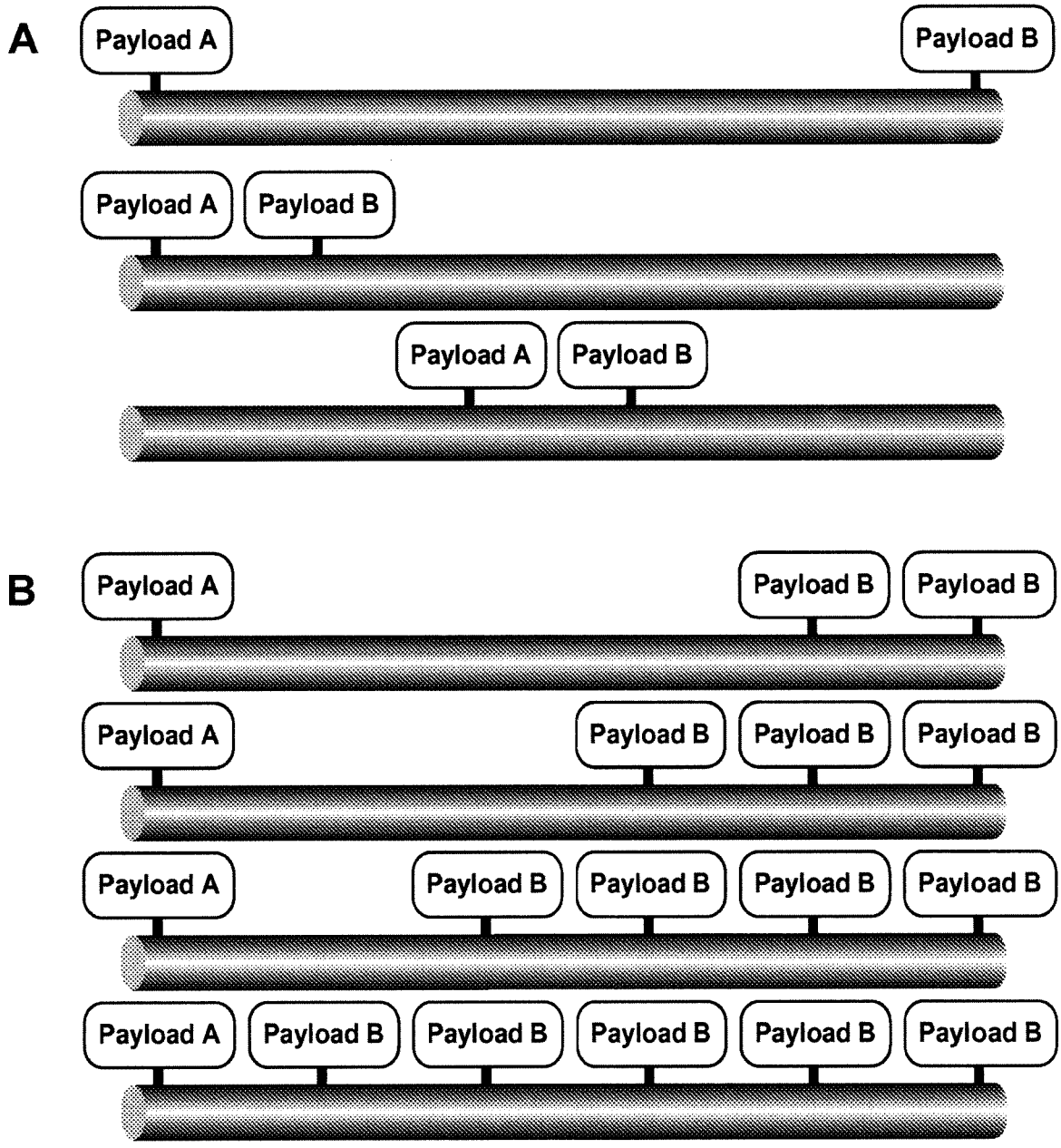

A
Payload A
Payload B
Payload A
Payload B
Payload A
Payload B
B
Payload A
Payload B
Payload B
Payload A
Payload B
Payload B
Payload B
Payload A
Payload B
Payload B
Payload B
Payload B
Payload A
Payload B
Payload B
Payload B
Payload B
Payload B

FIG. 25

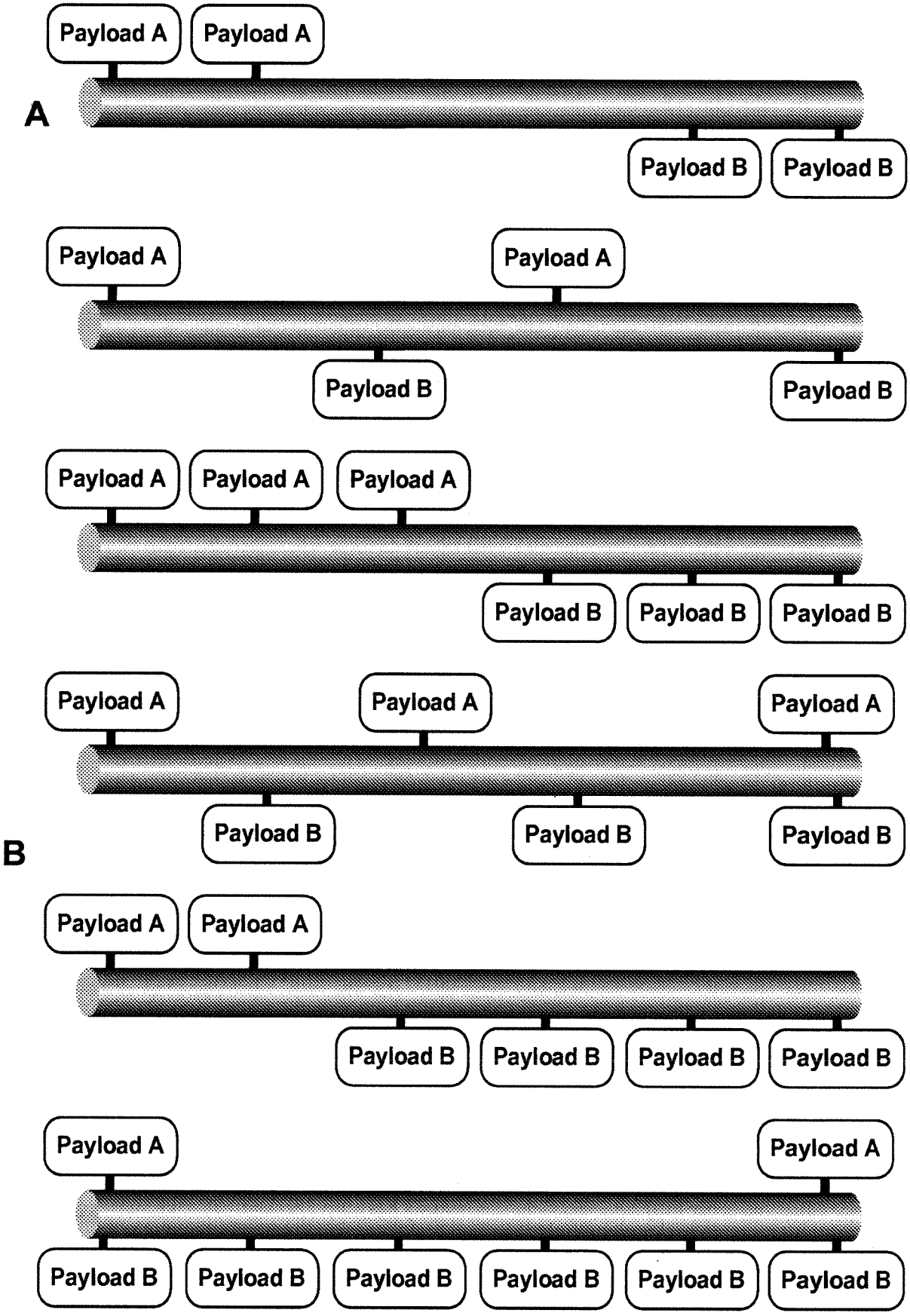

A
Payload A
Payload A
Payload B
Payload B
Payload A
Payload A
Payload B
Payload B
Payload A
Payload A
Payload A
Payload B
Payload B
Payload B
B
Payload A
Payload A
Payload A
Payload B
Payload B
Payload B
Payload A
Payload A
Payload B
Payload B
Payload B
Payload B
Payload A
Payload A
Payload B
Payload B
Payload B
Payload B
Payload B
Payload B

FIG. 26

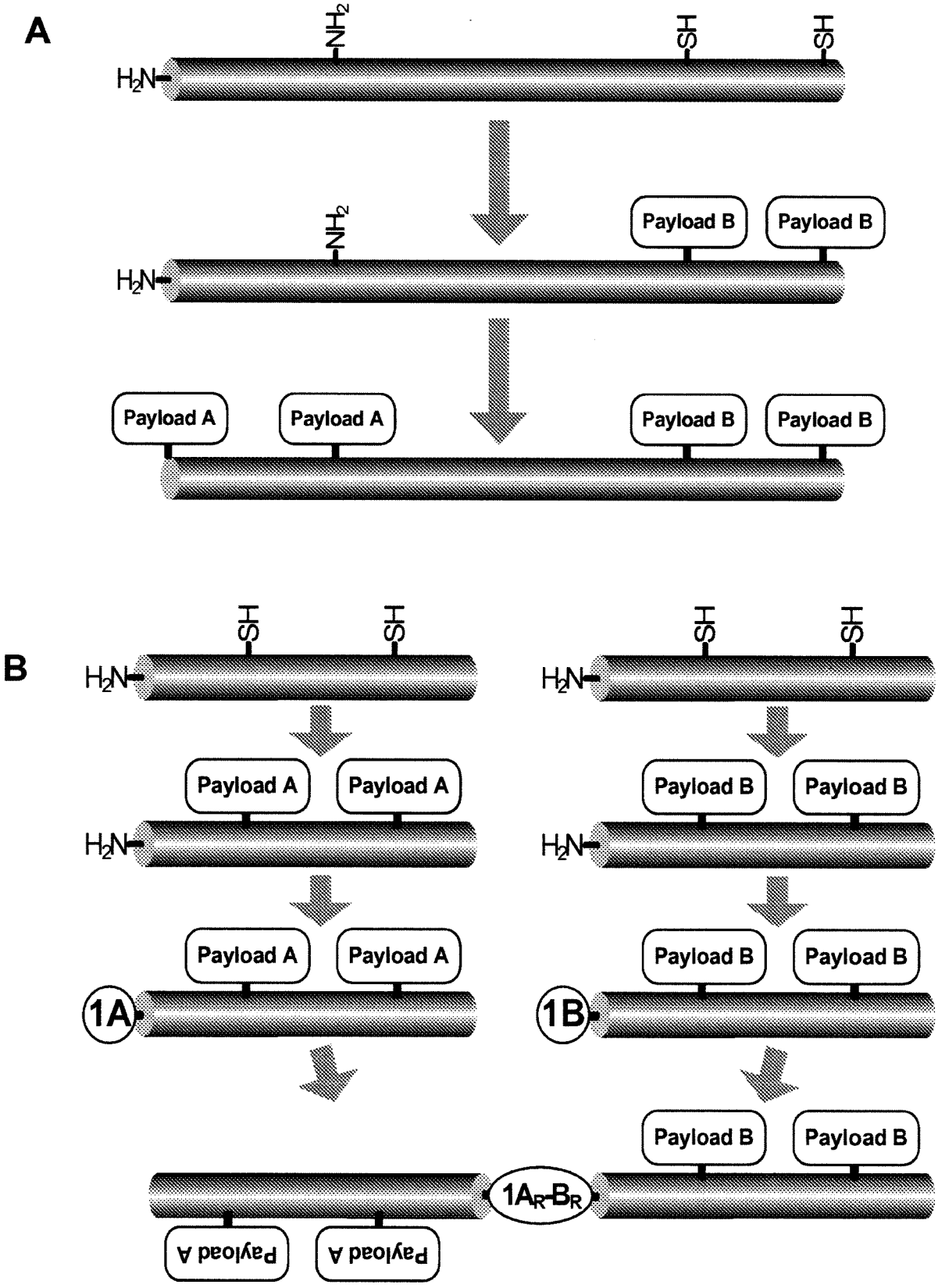

A
NH_2
SH
SH
H_2N
Payload B
Payload B
NH_2
H_2N
Payload A
Payload A
Payload B
Payload B
B
SH
SH
H_2N
Payload A
Payload A
H_2N
Payload A
Payload A
1A
SH
SH
H_2N
Payload B
Payload B
H_2N
Payload B
Payload B
1B
Payload B
Payload B
$1A_R$-B_R
Payload A
Payload A

FIG. 27

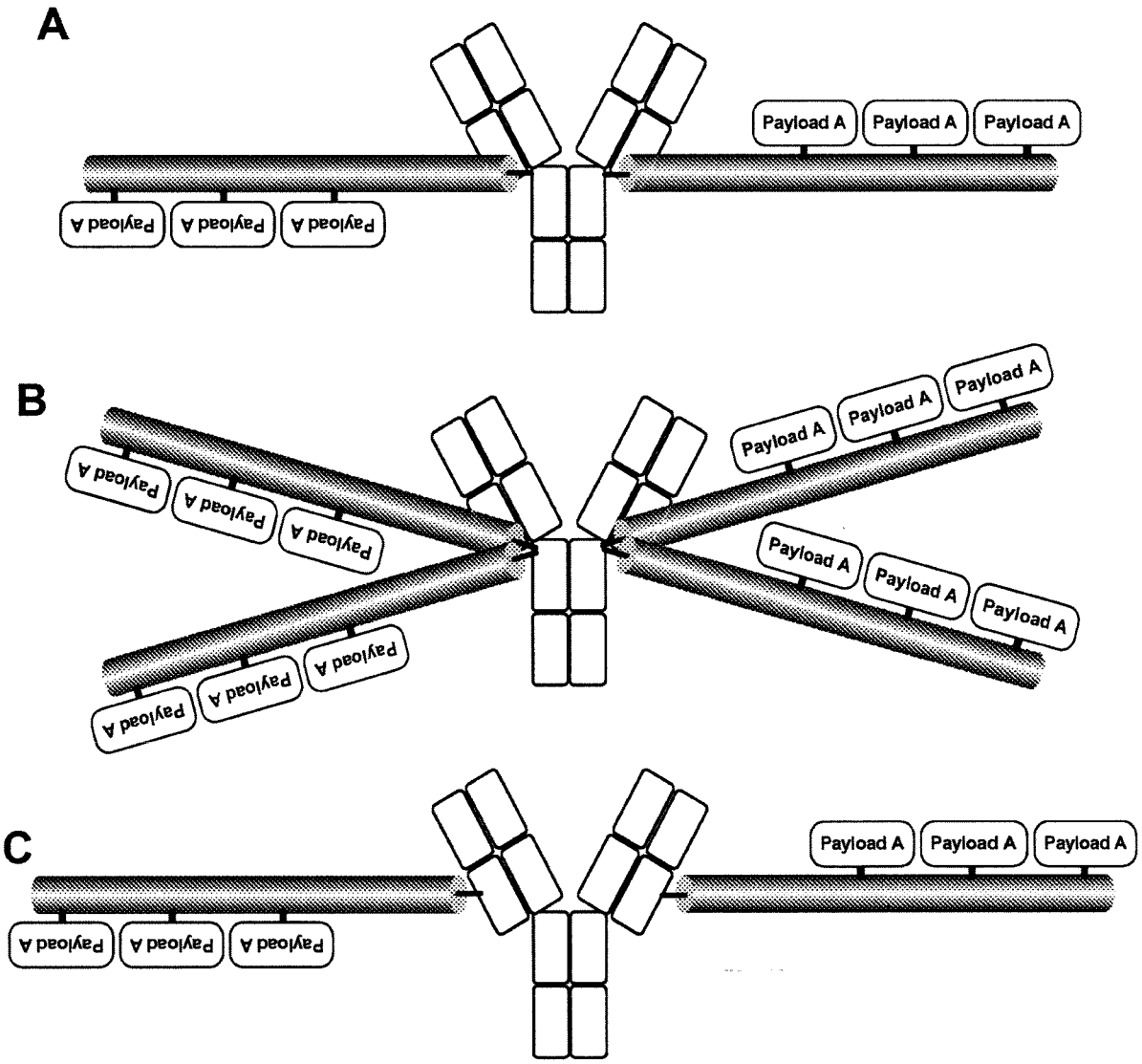

A
Payload A
Payload A
Payload A
Payload A
Payload A
Payload A
B
Payload A
Payload A
Payload A
Payload A
Payload A
Payload A
Payload A
Payload A
Payload A
Payload A
Payload A
Payload A
C
Payload A
Payload A
Payload A
Payload A
Payload A
Payload A

FIG. 28

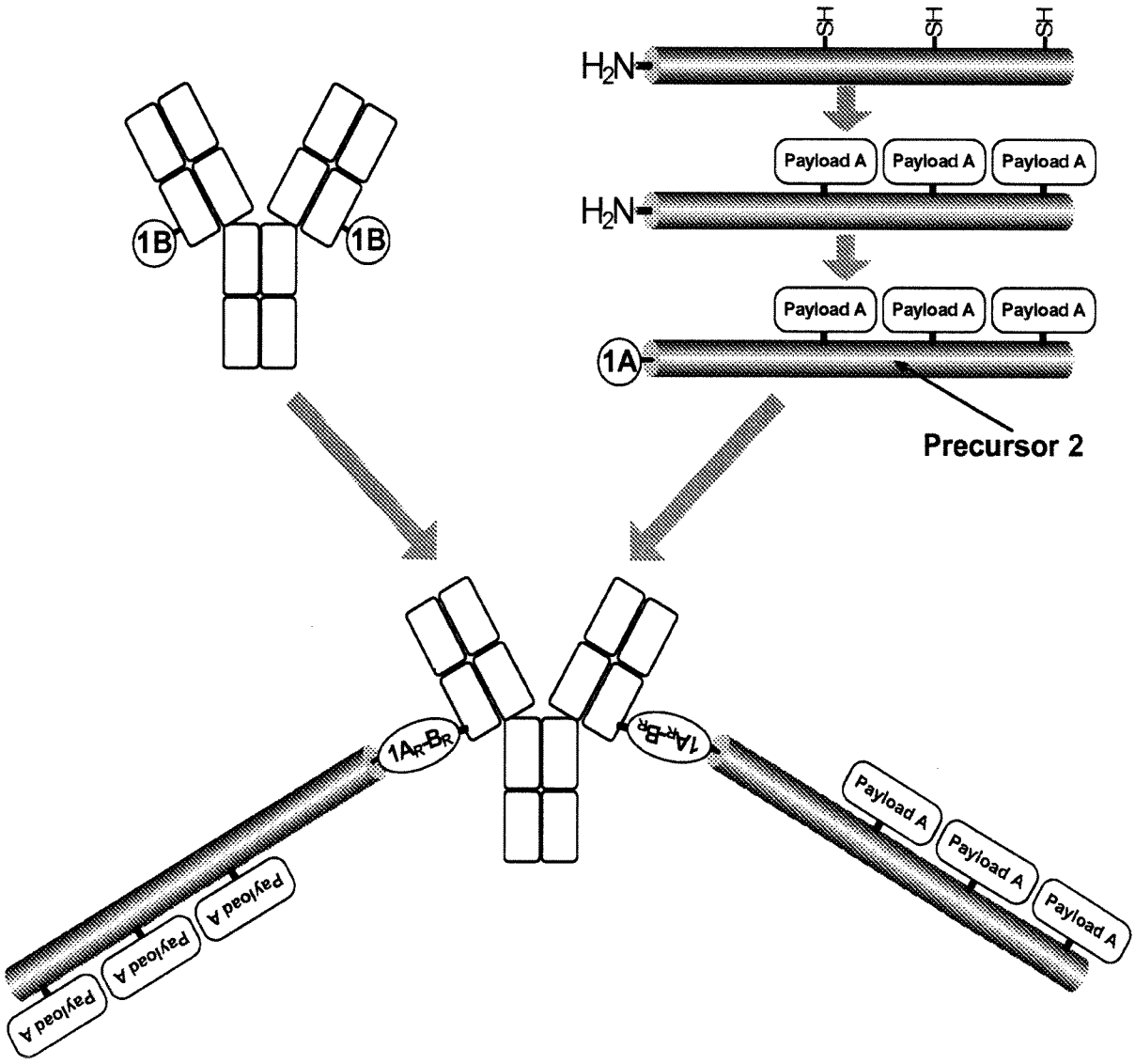

SH
SH
SH
H_2N
Payload A
Payload A
Payload A
H_2N
Payload A
Payload A
Payload A
1A
Precursor 2
1B
1B
$1A_R$-B_R
$1A_R$-B_R
Payload A
Payload A
Payload A
Payload A
Payload A
Payload A

FIG. 29

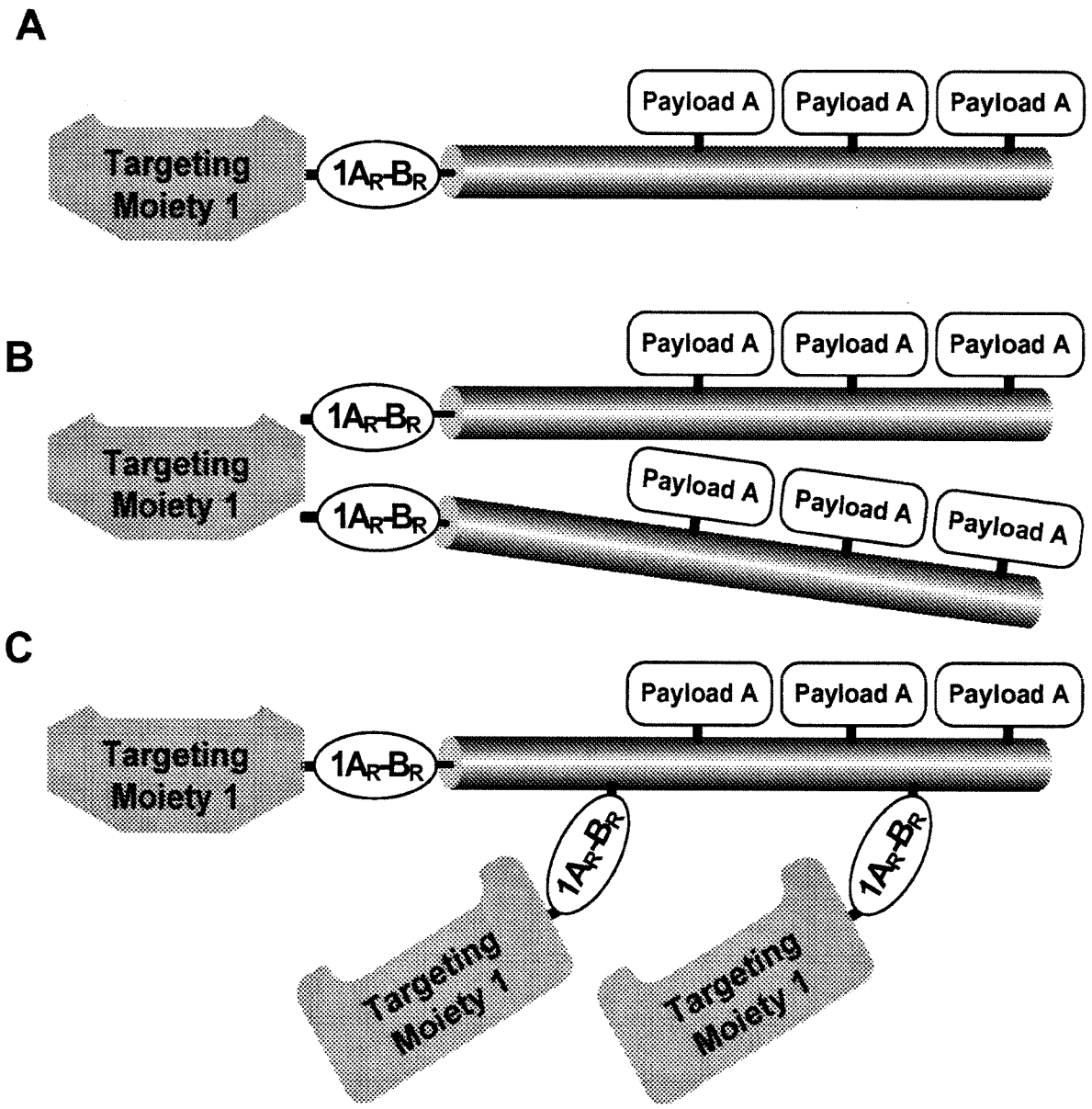

A
Payload A
Payload A
Payload A
Targeting Moiety 1
1AR-BR
B
Payload A
Payload A
Payload A
1AR-BR
Targeting Moiety 1
Payload A
Payload A
Payload A
1AR-BR
C
Payload A
Payload A
Payload A
Targeting Moiety 1
1AR-BR
1AR-BR
Targeting Moiety 1
Targeting Moiety 1

FIG. 30

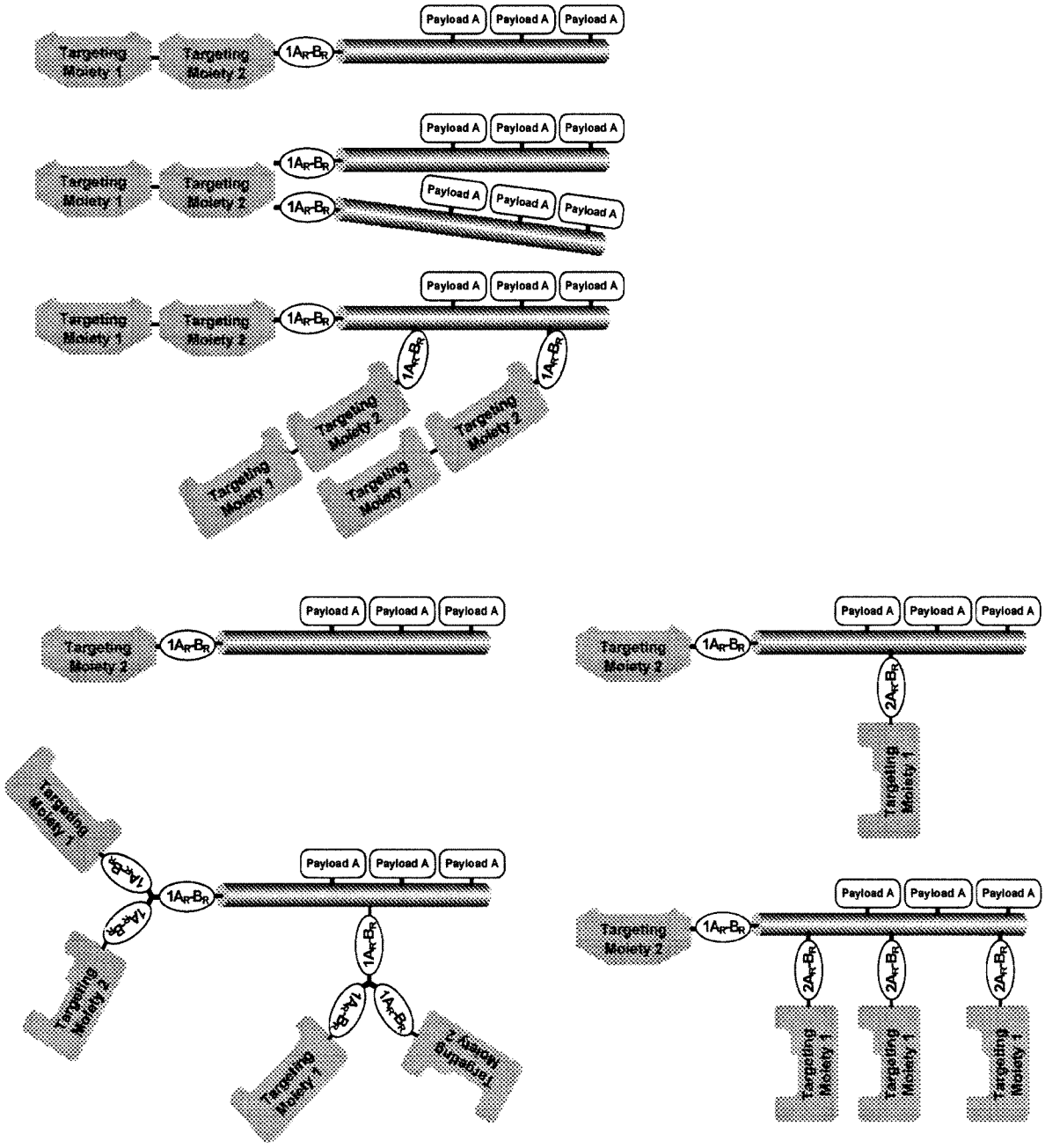

Payload A
Payload A
Payload A
Targeting Moiety 1
Targeting Moiety 2
1AR-BR
2AR-BR

FIG. 31

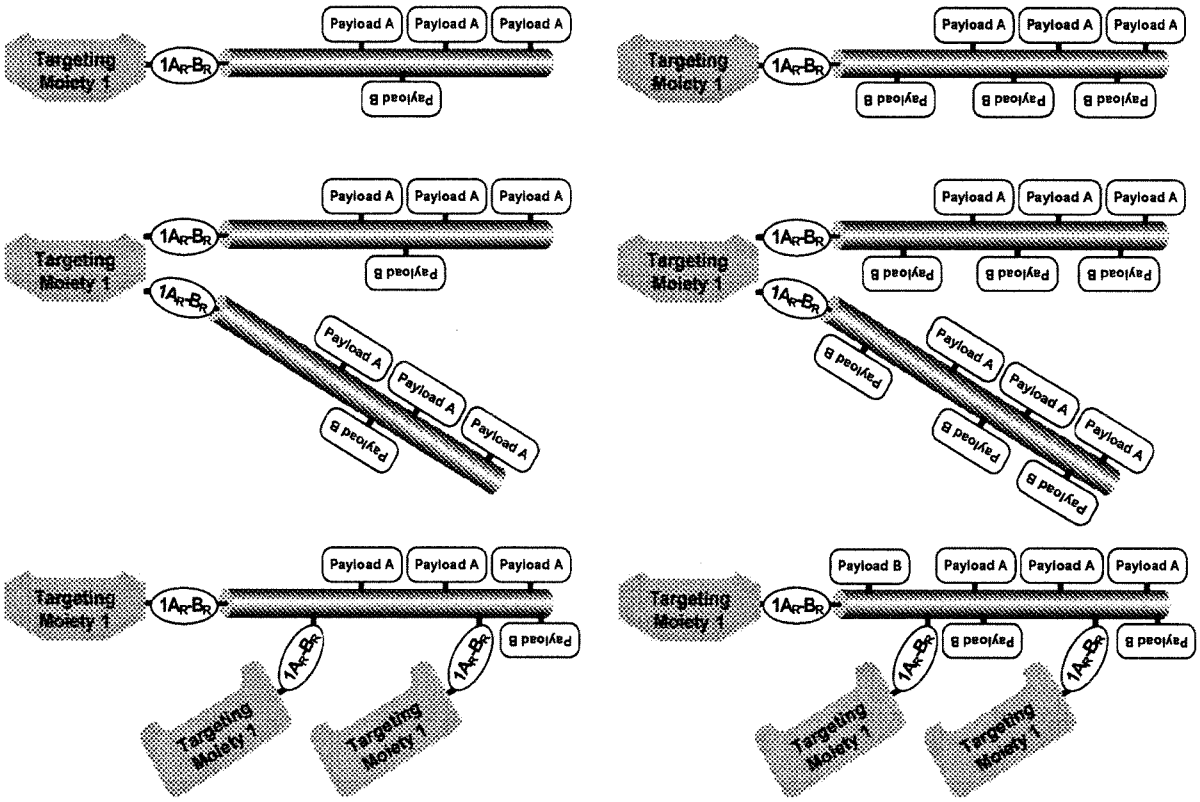


FIG. 32

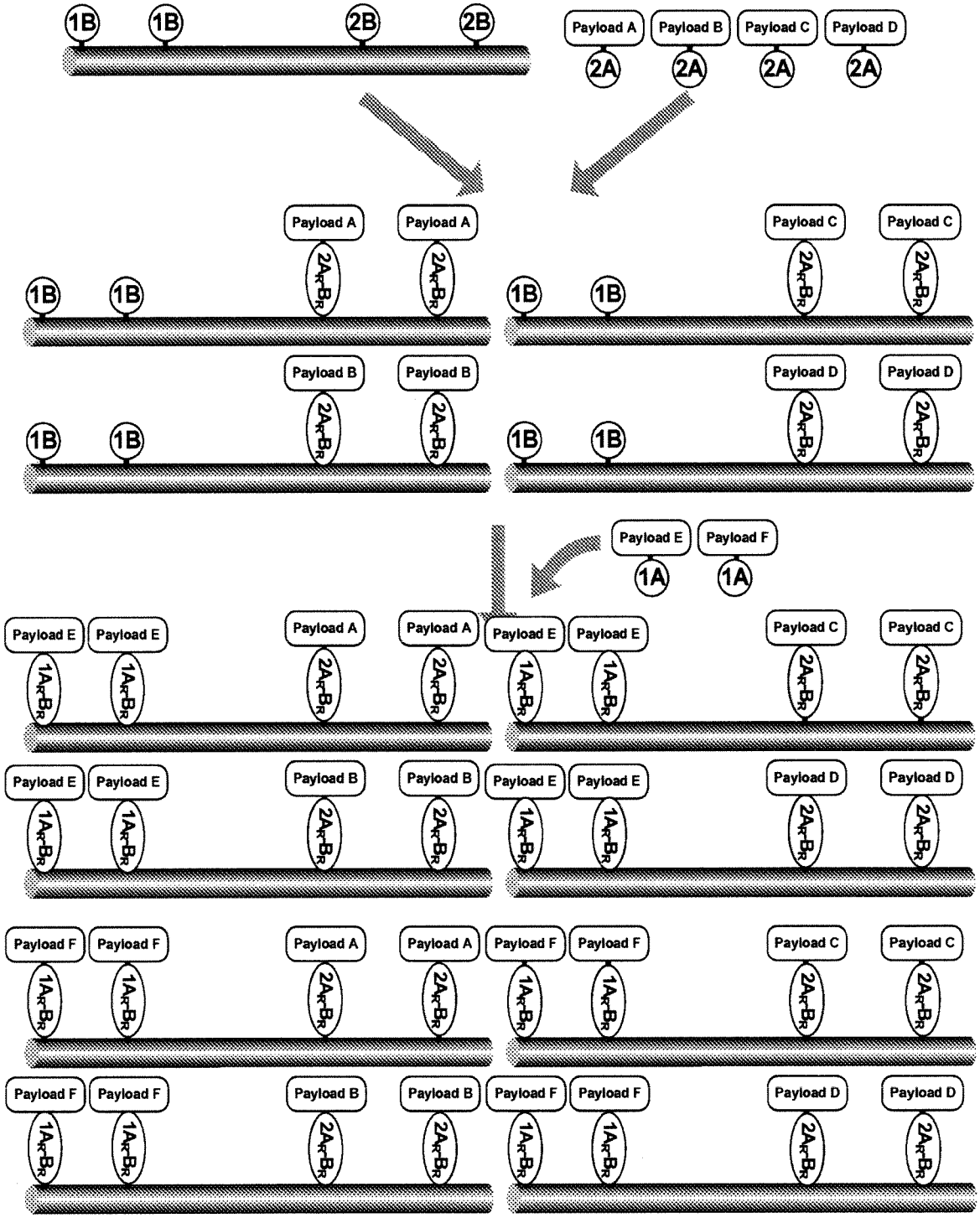

1B
1B
2B
2B
Payload A
Payload B
Payload C
Payload D
2A
2A
2A
2A
Payload A
Payload A
Payload C
Payload C
Payload B
Payload B
Payload D
Payload D
Payload E
Payload F
1A
1A
Payload E
Payload E
Payload F
Payload F

FIG. 33

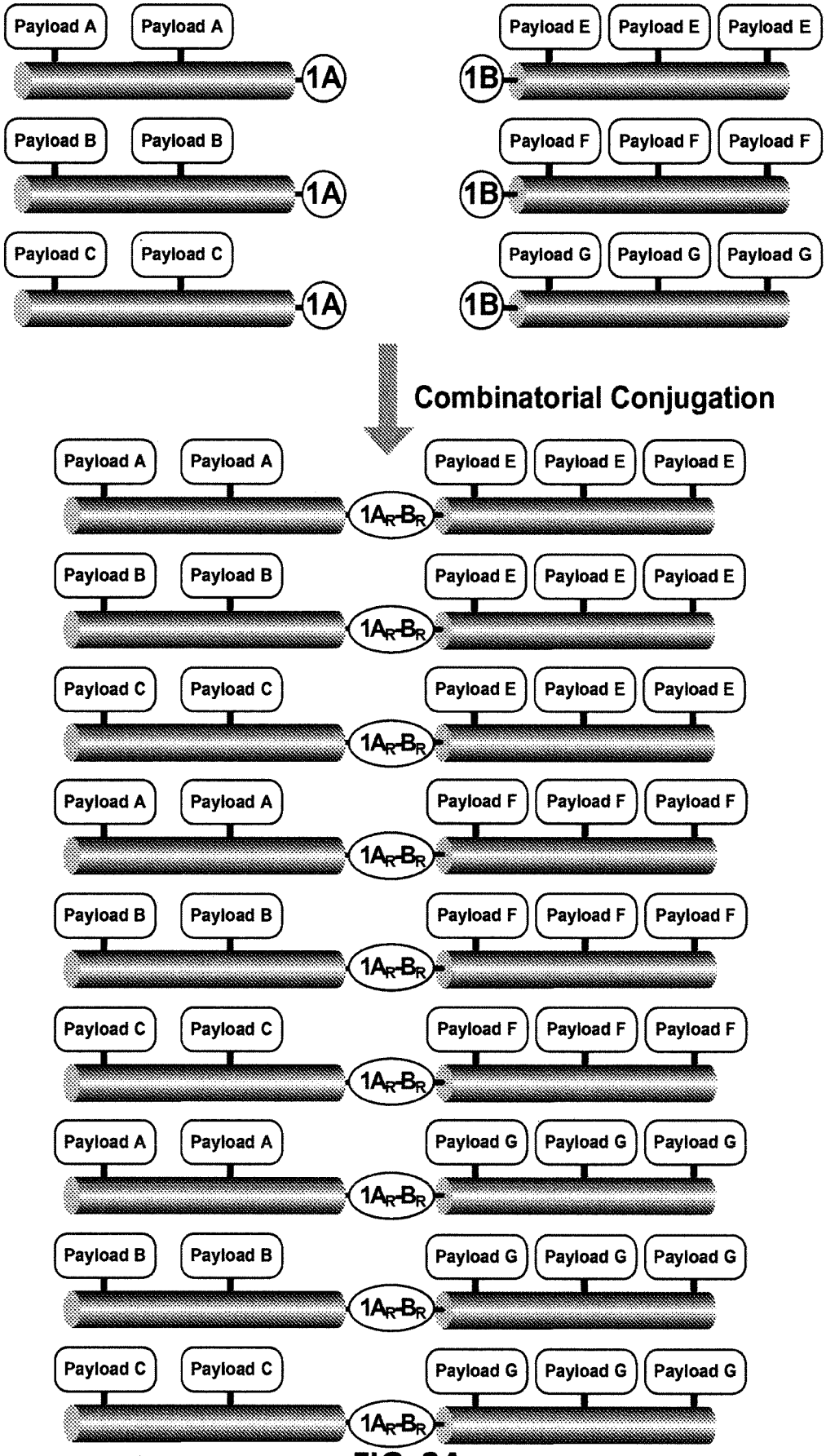

Payload A
Payload A
1A
Payload B
Payload B
1A
Payload C
Payload C
1A
Payload E
Payload E
Payload E
1B
Payload F
Payload F
Payload F
1B
Payload G
Payload G
Payload G
1B
Combinatorial Conjugation
1A_R-B_R
Payload A
Payload A
Payload E
Payload B
Payload B
Payload C
Payload C
Payload F
Payload G

FIG. 34

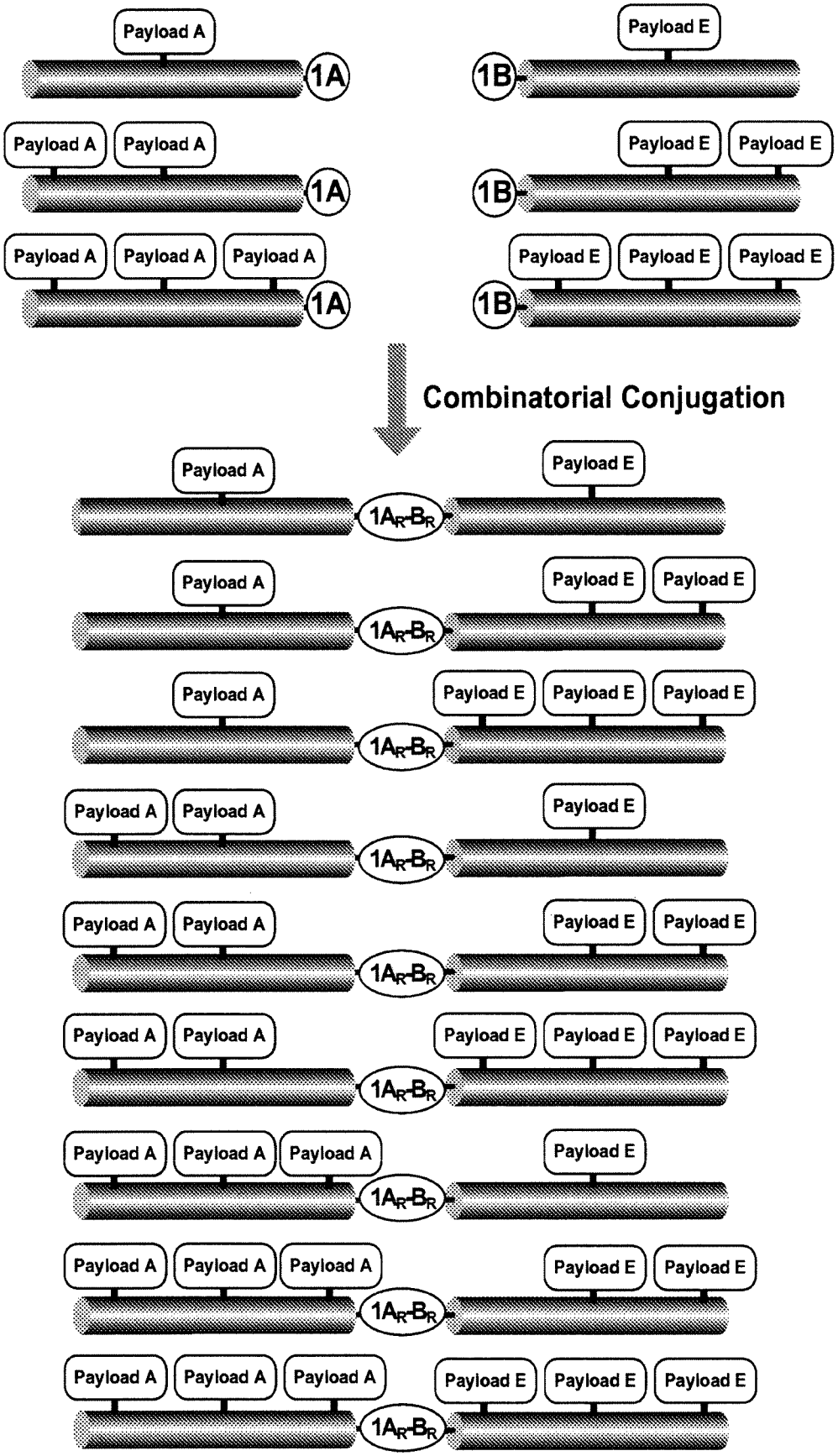

Payload A
1A
Payload E
1B
Payload A
Payload A
Payload E
Payload E
Payload A
Payload A
Payload A
Payload E
Payload E
Payload E
Combinatorial Conjugation
1AR-BR

FIG. 35

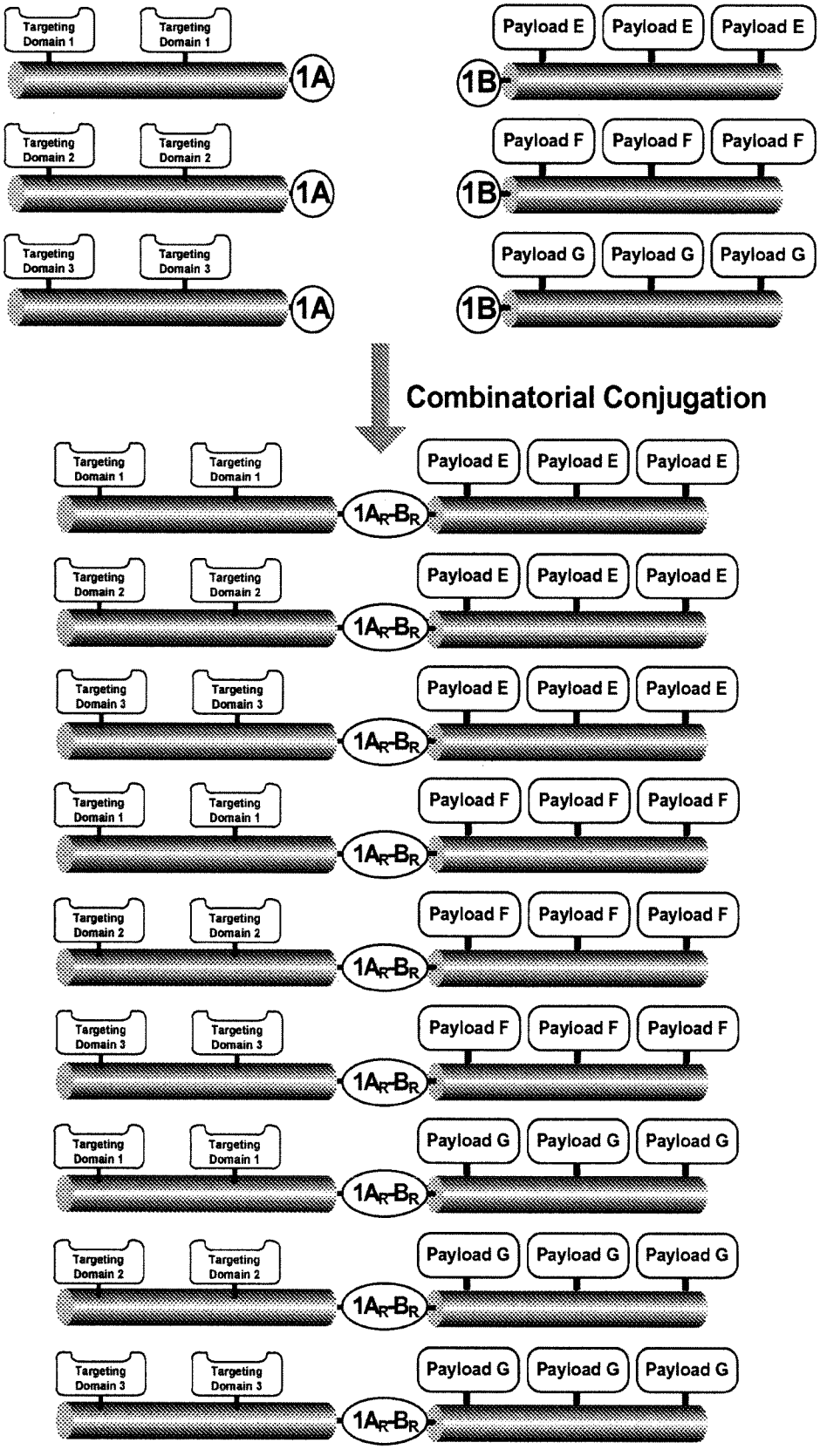

Targeting Domain 1
Targeting Domain 1
1A
Payload E
Payload E
Payload E
1B
Targeting Domain 2
Targeting Domain 2
1A
Payload F
Payload F
Payload F
1B
Targeting Domain 3
Targeting Domain 3
1A
Payload G
Payload G
Payload G
1B
Combinatorial Conjugation
1A$_R$-B$_R$

FIG. 36

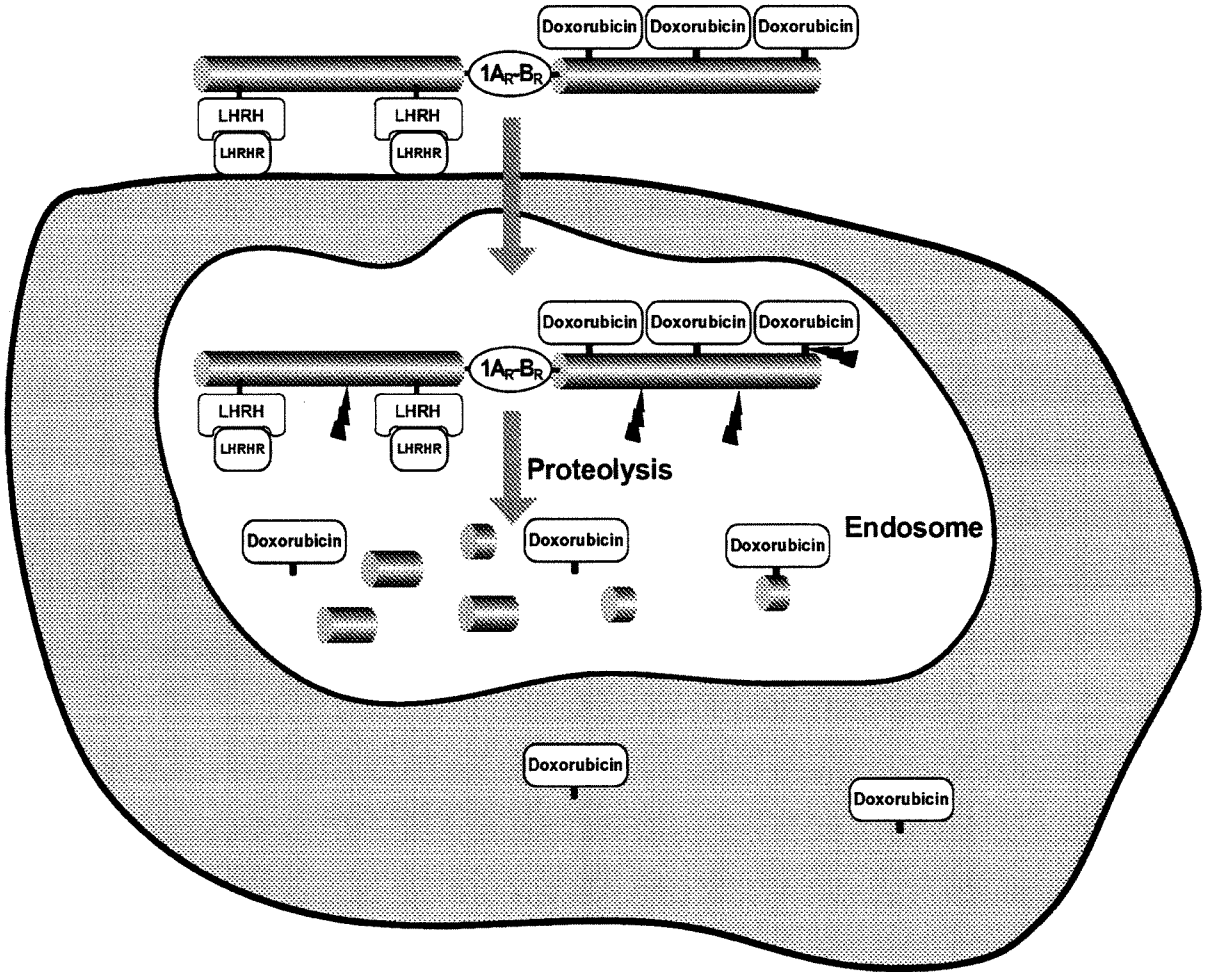

Doxorubicin
Doxorubicin
Doxorubicin
1A$_R$-B$_R$
LHRH
LHRH
LHRHR
LHRHR
Doxorubicin
Doxorubicin
Doxorubicin
1A$_R$-B$_R$
LHRH
LHRH
LHRHR
LHRHR
Proteolysis
Endosome
Doxorubicin
Doxorubicin
Doxorubicin
Doxorubicin
Doxorubicin

FIG. 37

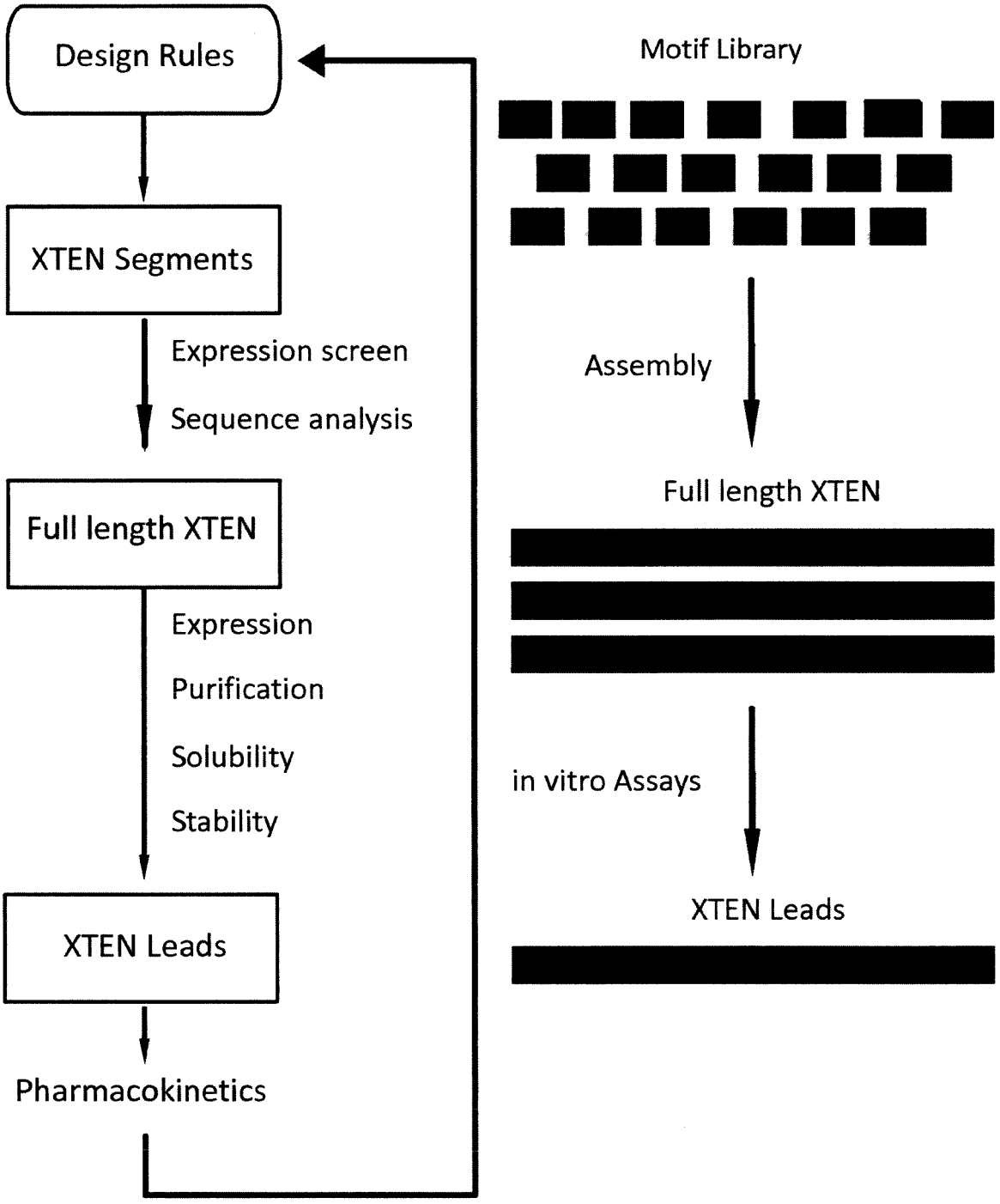

Design Rules
XTEN Segments
Expression screen
Sequence analysis
Full length XTEN
Expression
Purification
Solubility
Stability
XTEN Leads
Pharmacokinetics
Motif Library
Assembly
Full length XTEN
in vitro Assays
XTEN Leads

FIG. 38

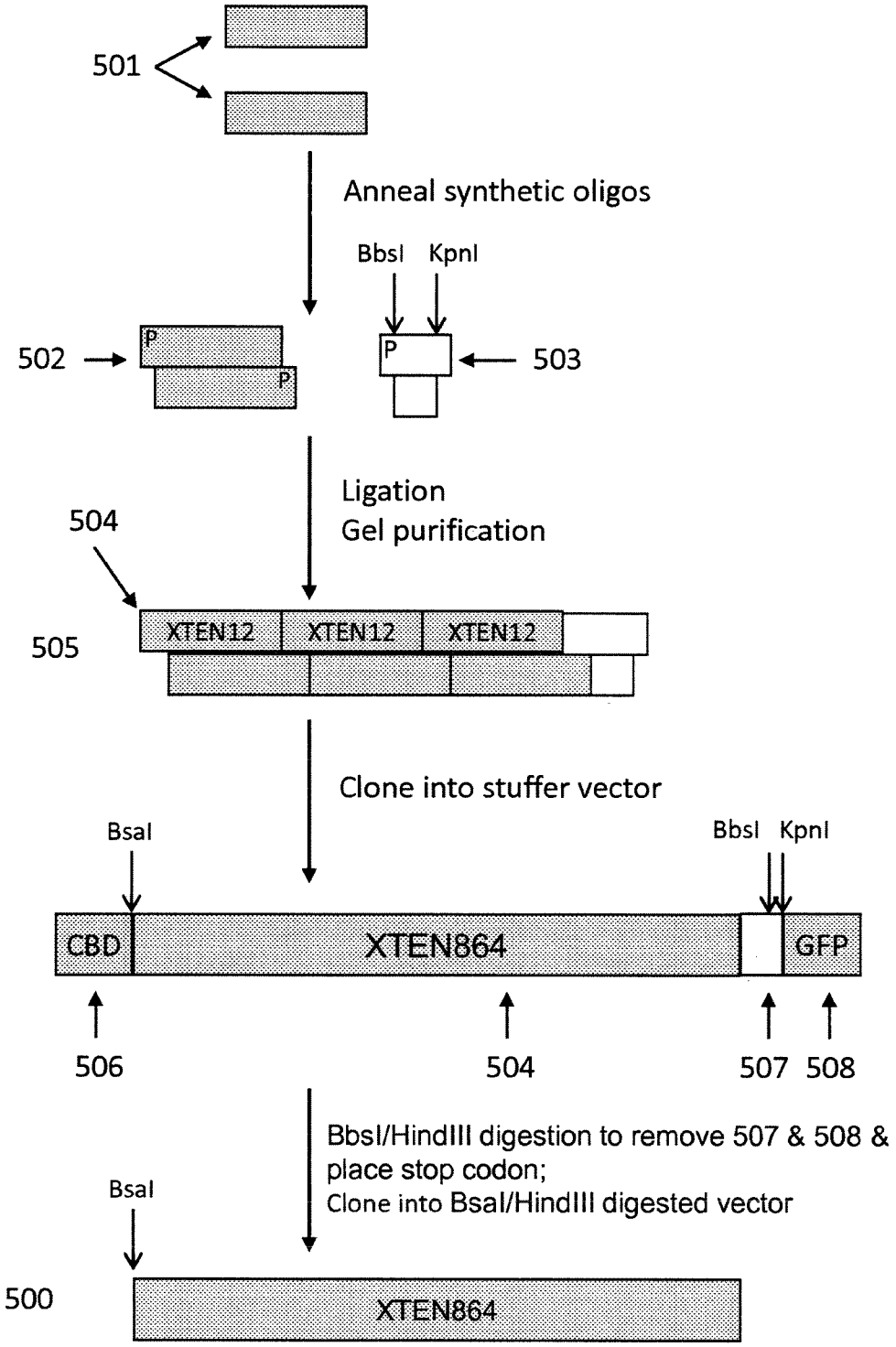

501
Anneal synthetic oligos
BbsI
KpnI
502
P
P
P
503
Ligation
Gel purification
504
505
XTEN12
XTEN12
XTEN12
Clone into stuffer vector
BsaI
BbsI
KpnI
CBD
XTEN864
GFP
506
504
507
508
BbsI/HindIII digestion to remove 507 & 508 &
place stop codon;
Clone into BsaI/HindIII digested vector
BsaI
500
XTEN864

FIG. 39

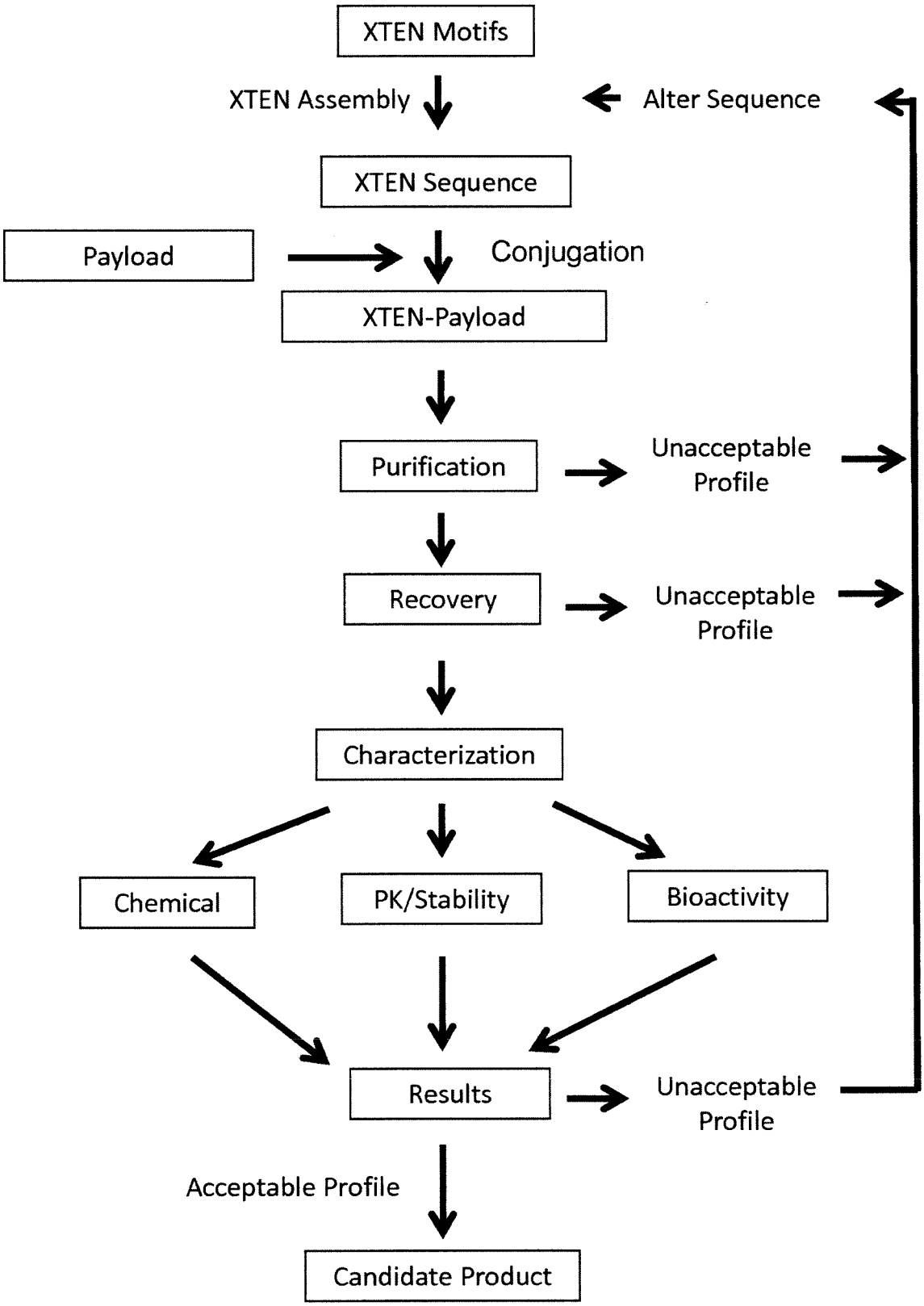

XTEN Motifs
XTEN Assembly
Alter Sequence
XTEN Sequence
Payload
Conjugation
XTEN-Payload
Purification
Unacceptable Profile
Recovery
Unacceptable Profile
Characterization
Chemical
PK/Stability
Bioactivity
Results
Unacceptable Profile
Acceptable Profile
Candidate Product

FIG. 40

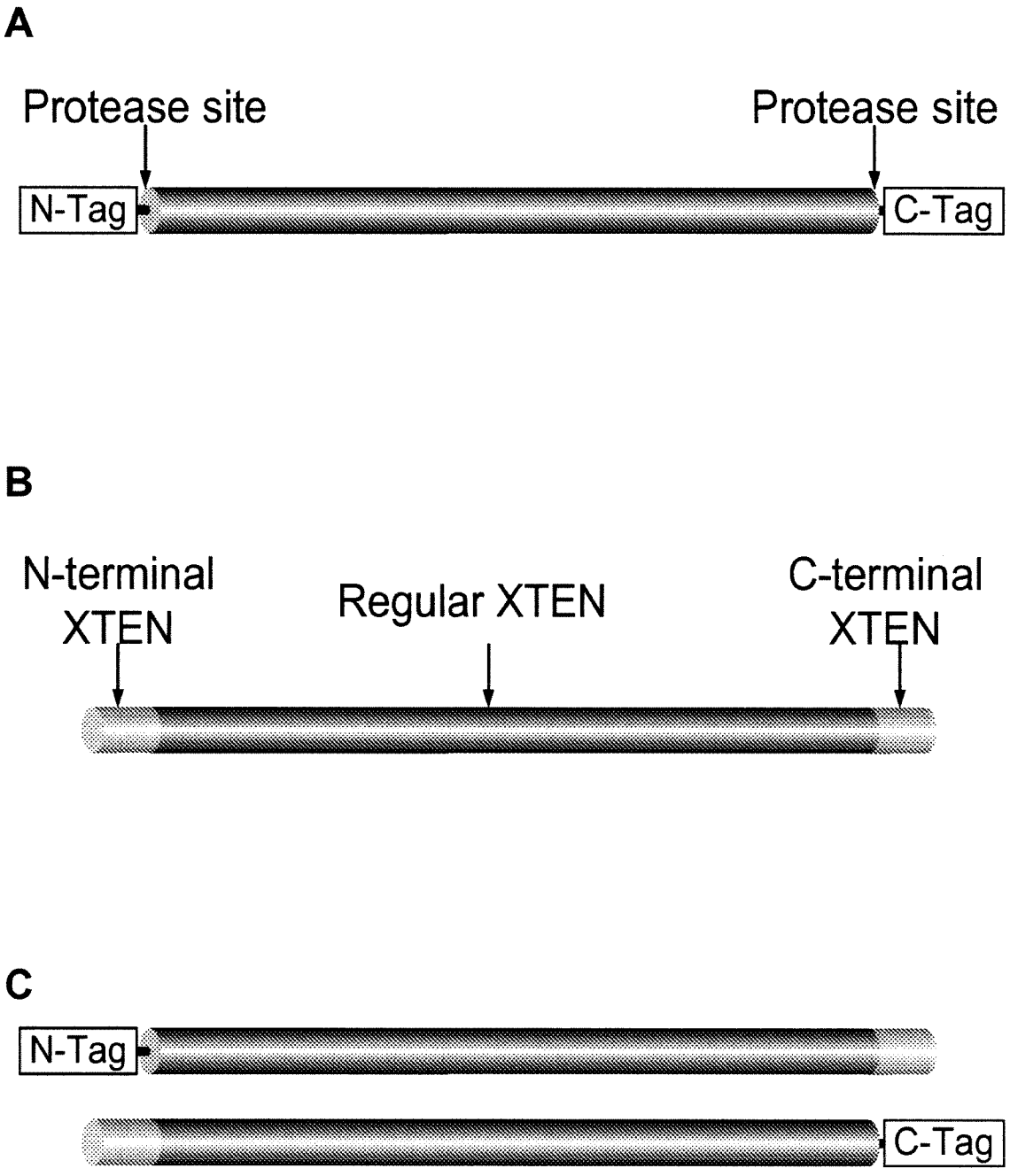

A
Protease site
Protease site
N-Tag
C-Tag
B
N-terminal XTEN
Regular XTEN
C-terminal XTEN
C
N-Tag
C-Tag

FIG. 41

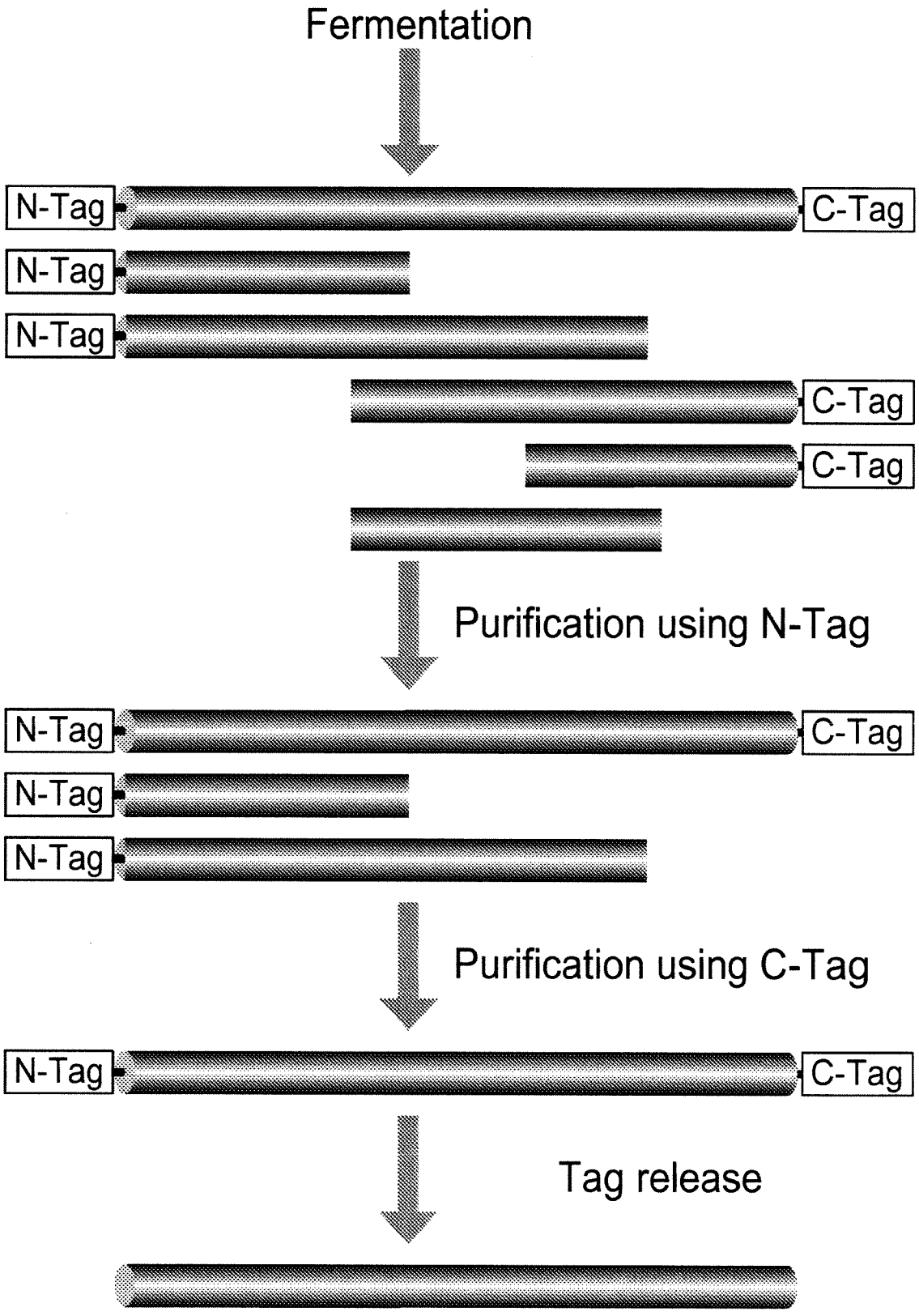

Fermentation
N-Tag
C-Tag
N-Tag
N-Tag
C-Tag
C-Tag
Purification using N-Tag
N-Tag
C-Tag
N-Tag
N-Tag
Purification using C-Tag
N-Tag
C-Tag
Tag release

FIG. 42

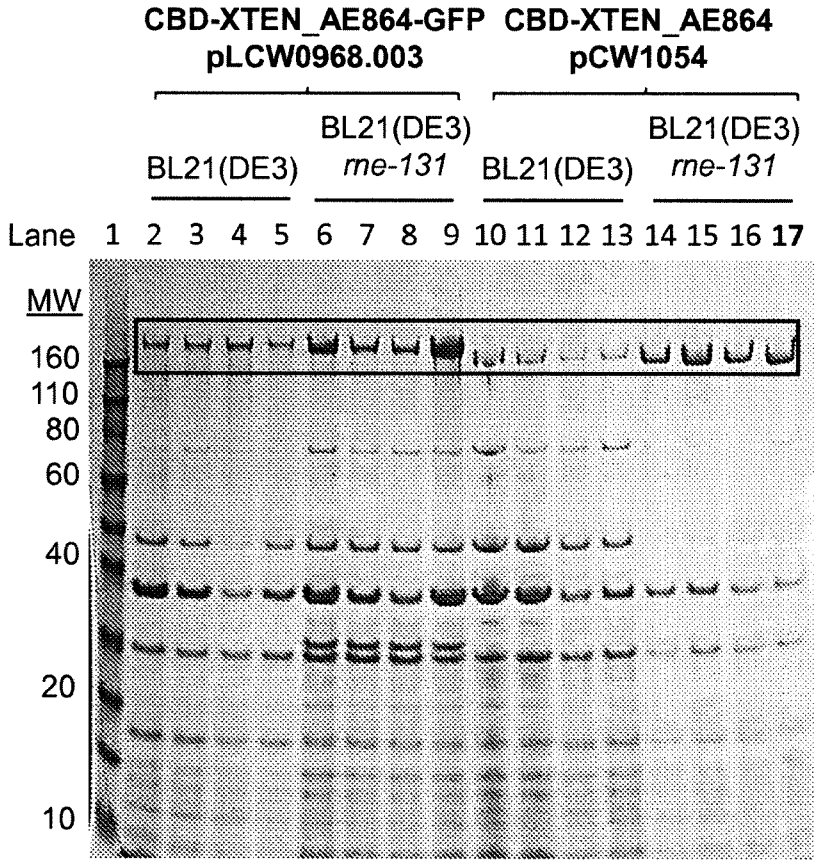

CBD-XTEN_AE864-GFP
pLCW0968.003
CBD-XTEN_AE864
pCW1054
BL21(DE3)
BL21(DE3)
rne-131
BL21(DE3)
BL21(DE3)
rne-131
Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
MW
160
110
80
60
40
20
10

FIG. 43

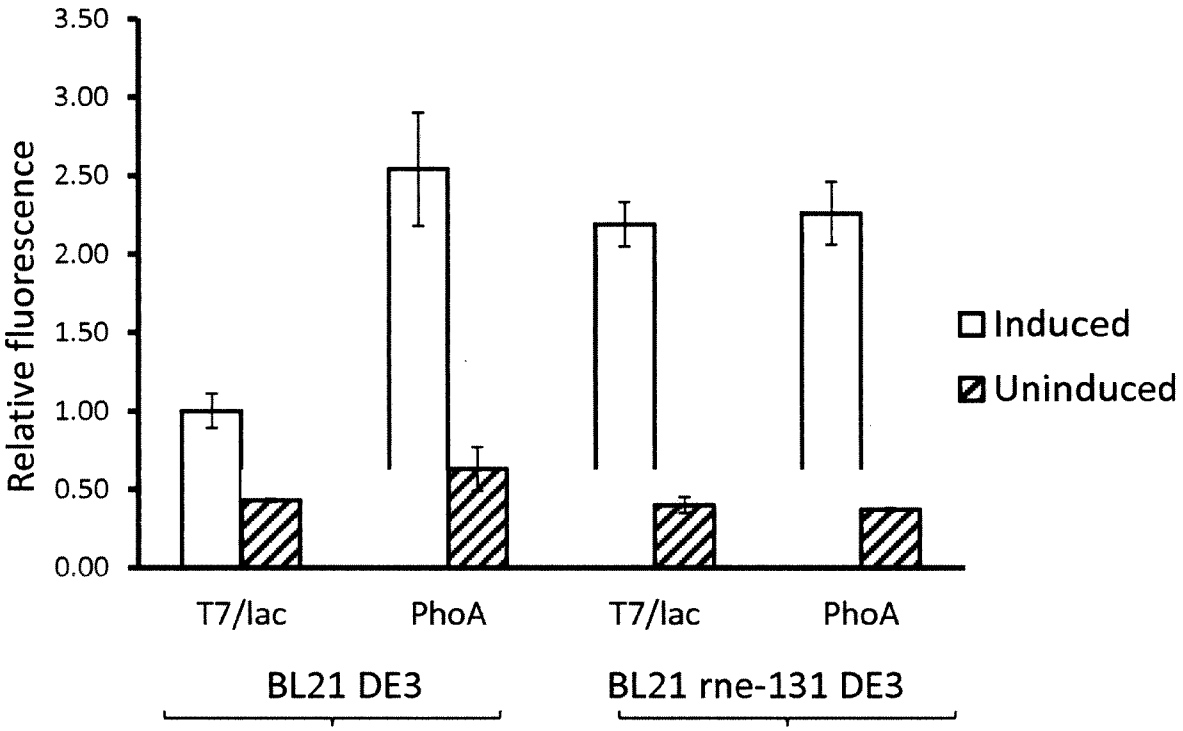

3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Relative fluorescence
Induced
Uninduced
T7/lac
PhoA
T7/lac
PhoA
BL21 DE3
BL21 rne-131 DE3

FIG. 44

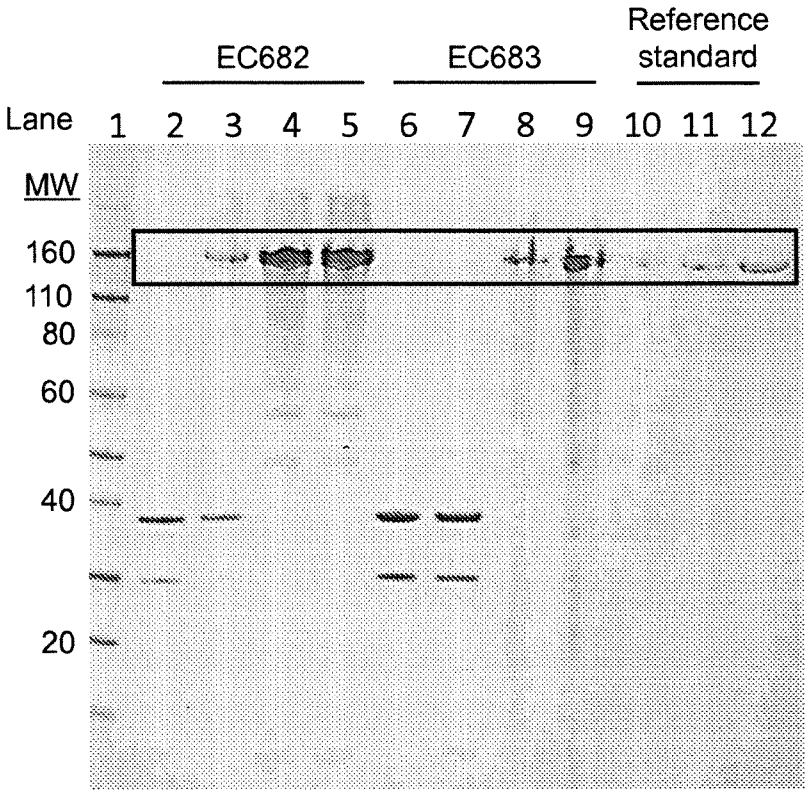

EC682
EC683
Reference standard
Lane
1 2 3 4 5 6 7 8 9 10 11 12
MW
160
110
80
60
40
20

FIG. 45

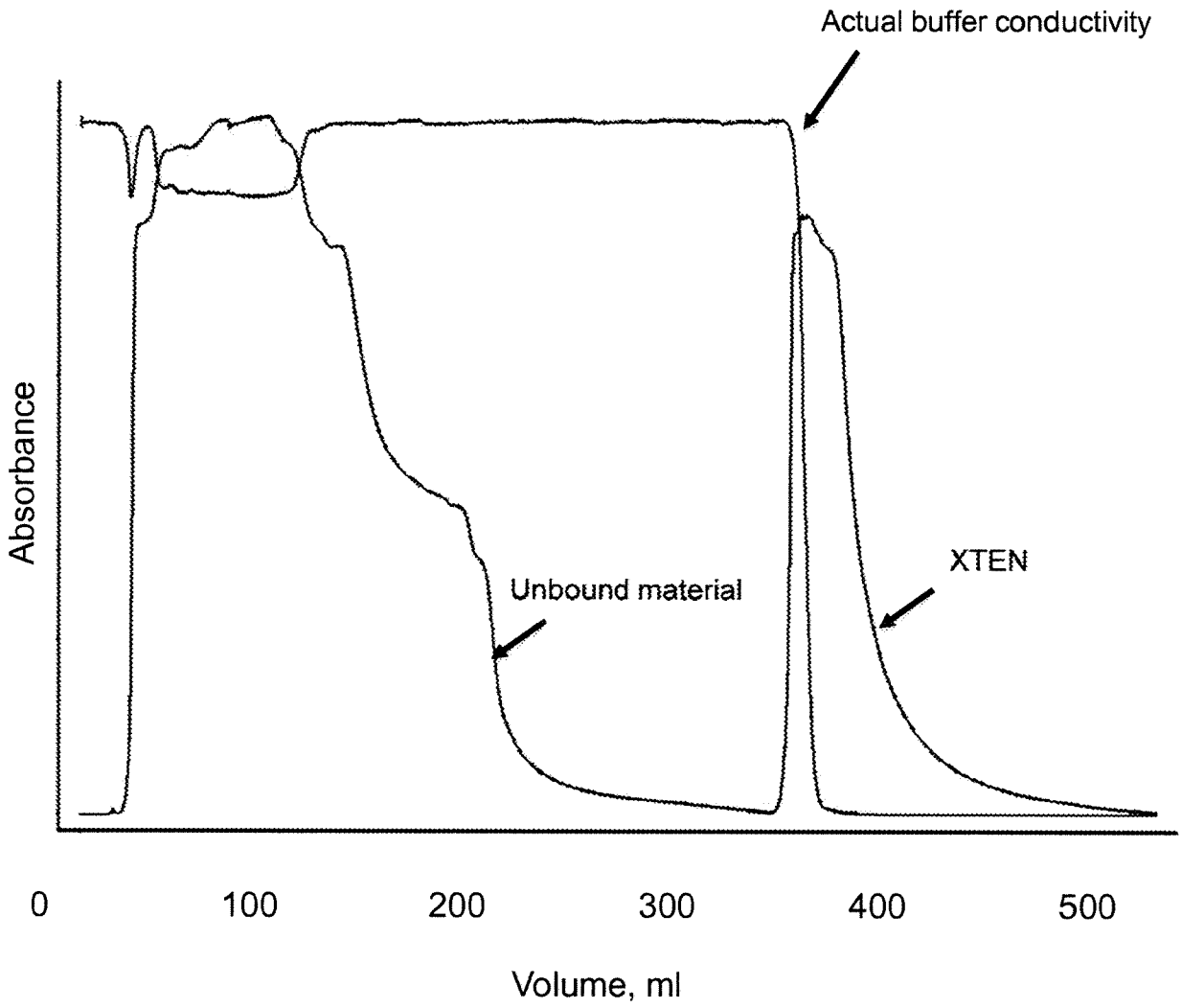

Actual buffer conductivity
Absorbance
Unbound material
XTEN
0
100
200
300
400
500
Volume, ml

FIG. 46

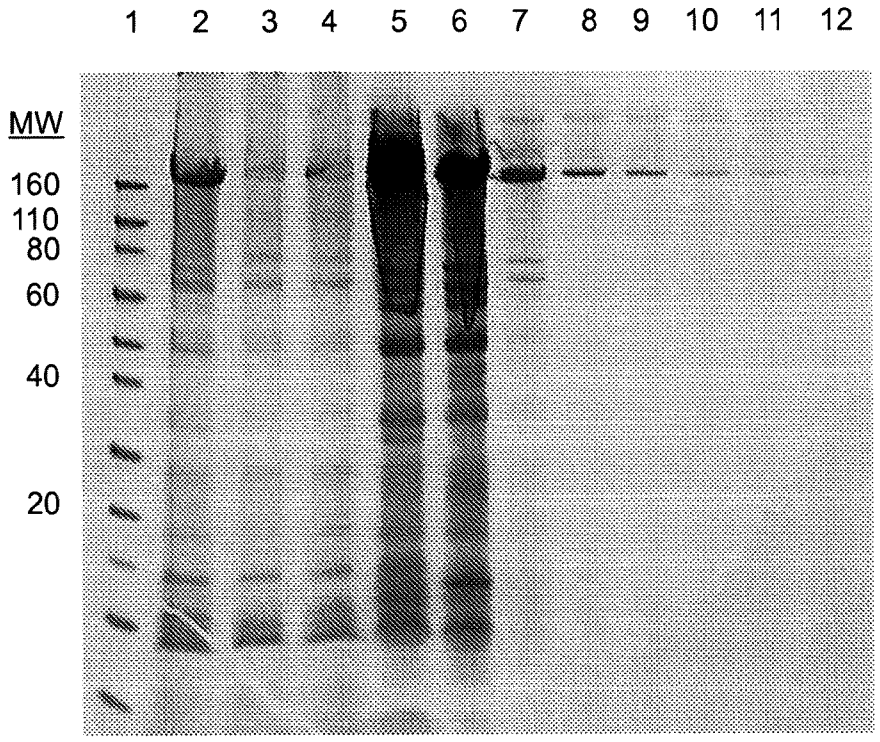


FIG. 47

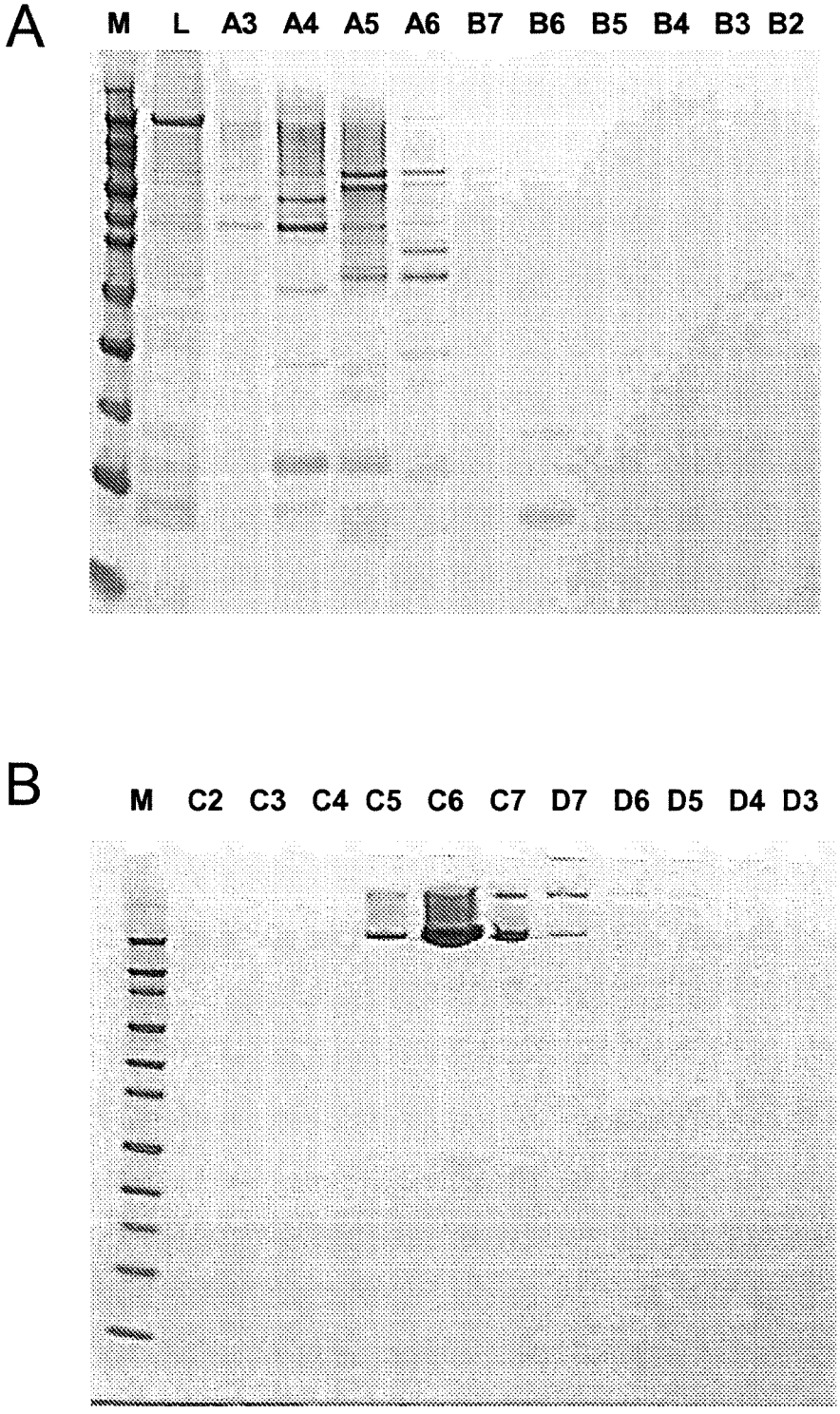


FIG. 48

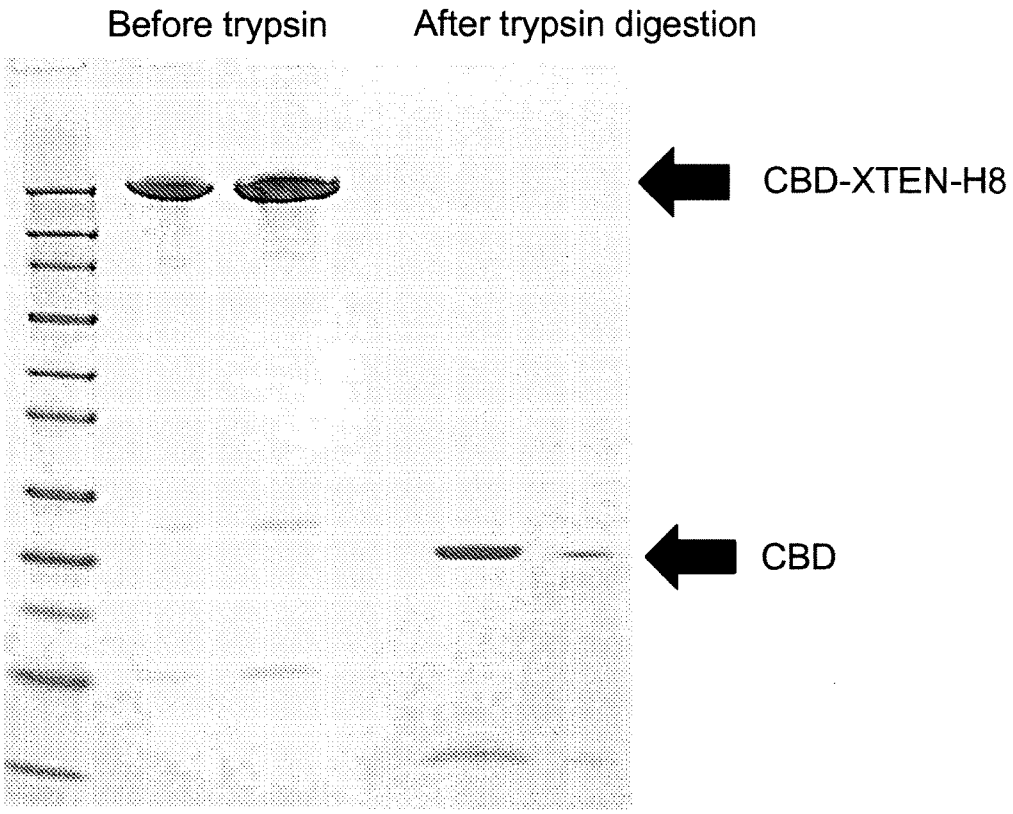


FIG. 49

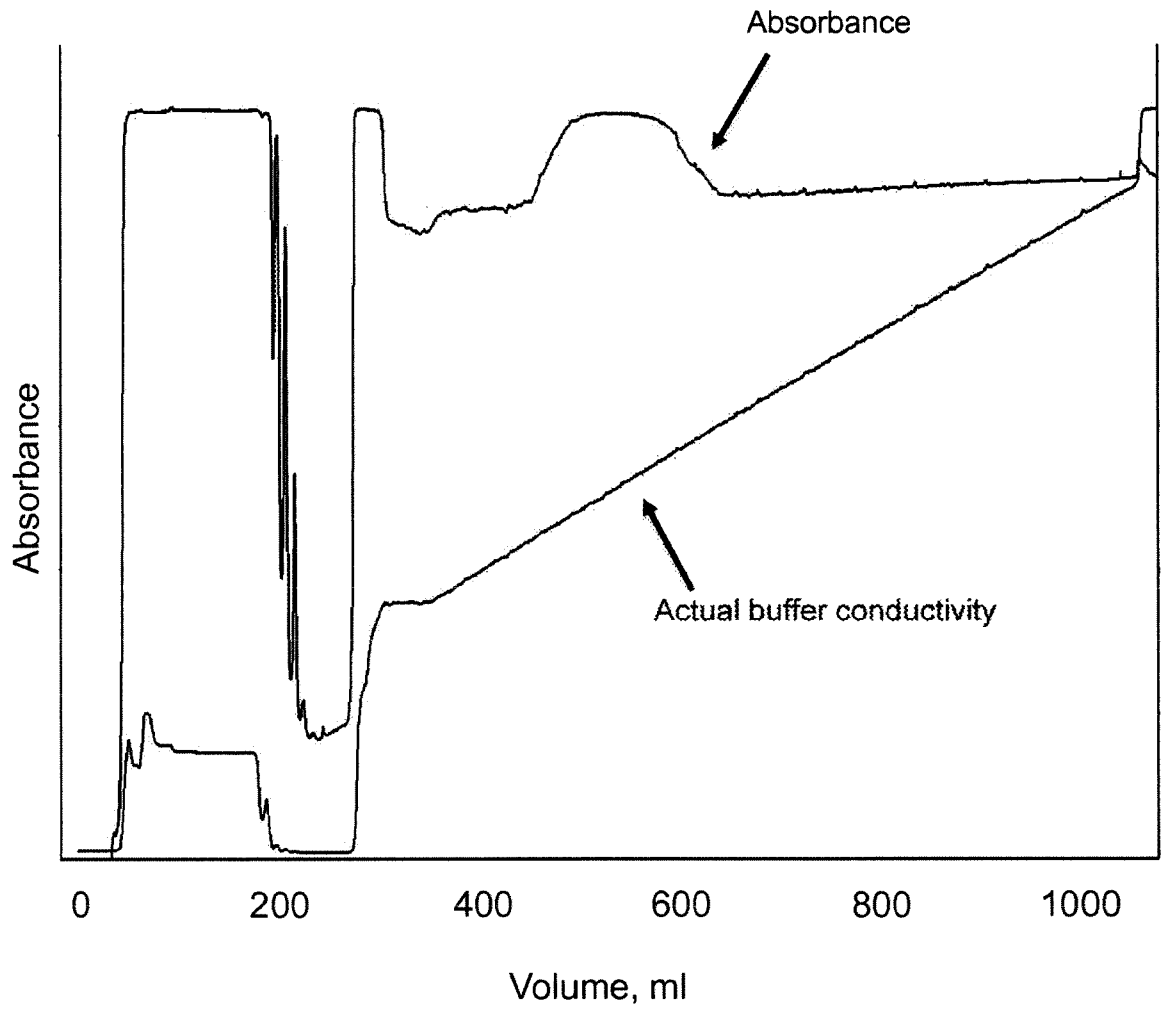
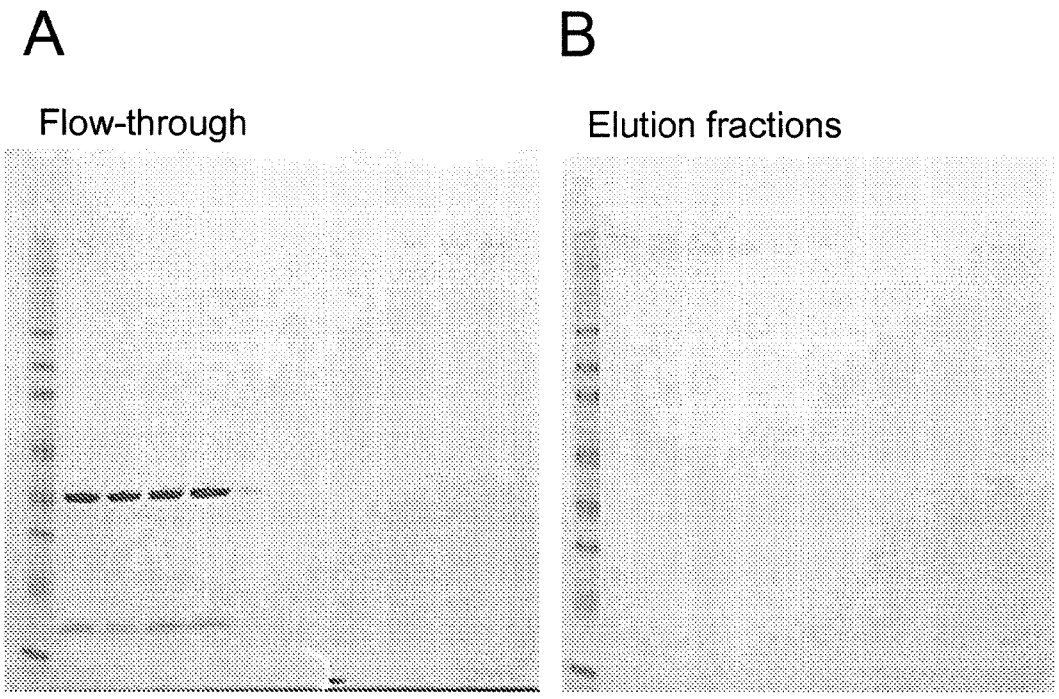

Absorbance
Absorbance
Actual buffer conductivity
0
200
400
600
800
1000
Volume, ml

FIG. 50


A
Flow-through
B
Elution fractions

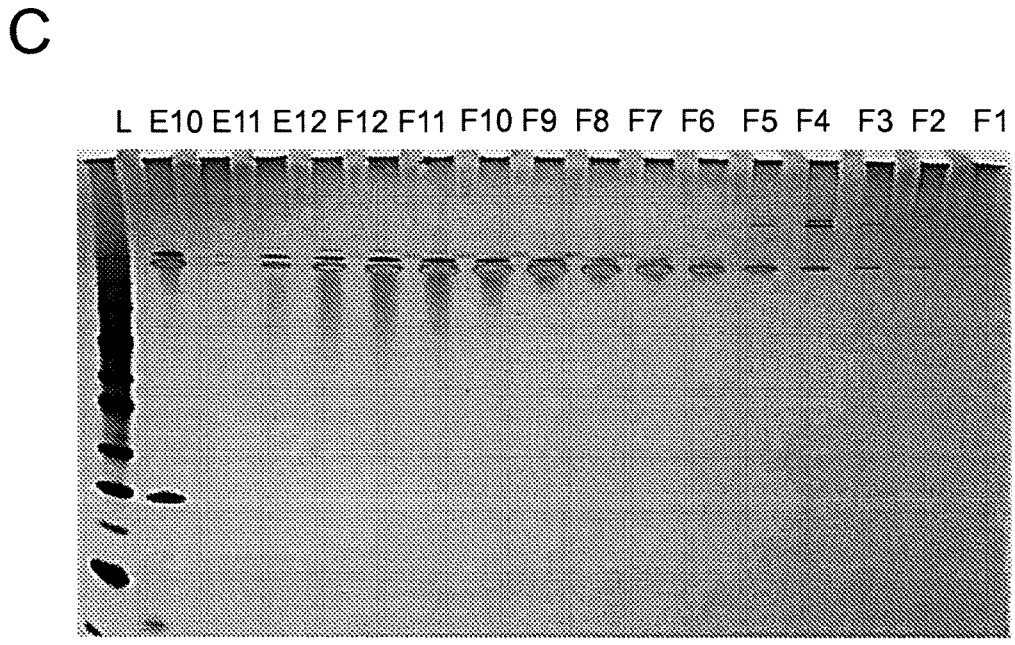

C
L E10 E11 E12 F12 F11 F10 F9 F8 F7 F6 F5 F4 F3 F2 F1

FIG. 51

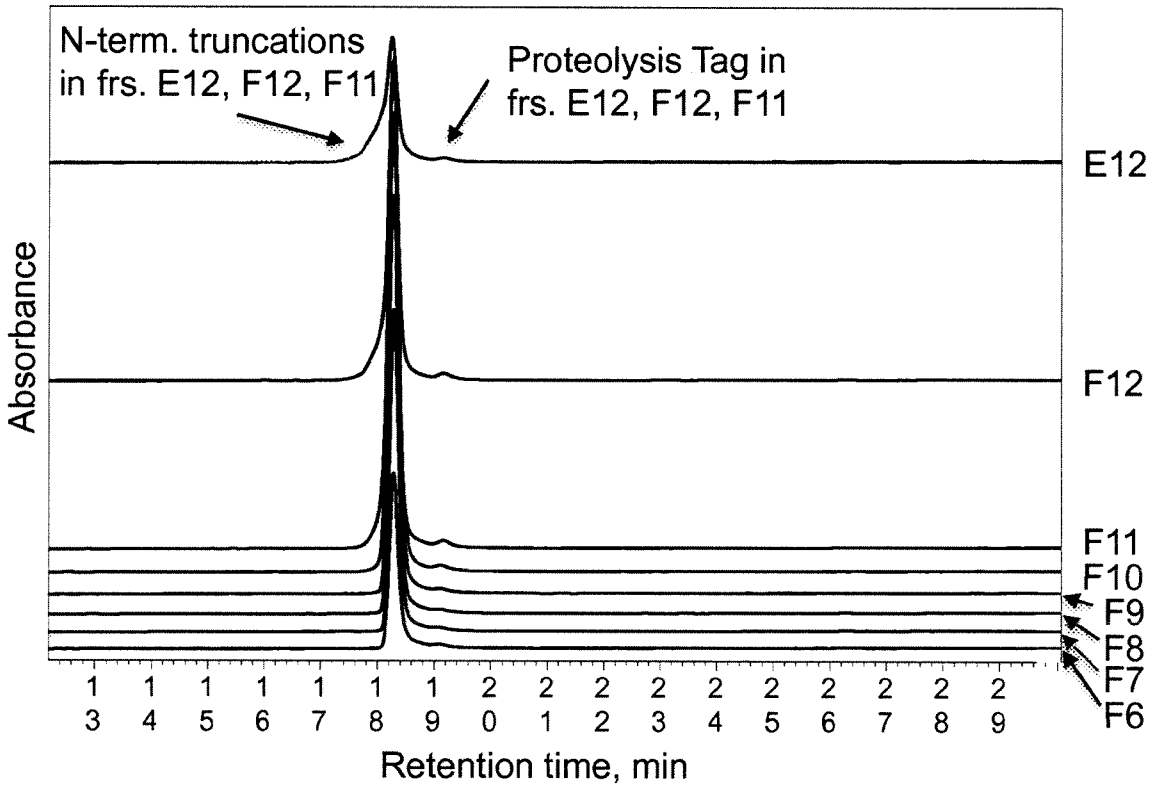

N-term. truncations
in frs. E12, F12, F11
Proteolysis Tag in
frs. E12, F12, F11
E12
F12
F11
F10
F9
F8
F7
F6
Absorbance
13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Retention time, min

FIG. 52

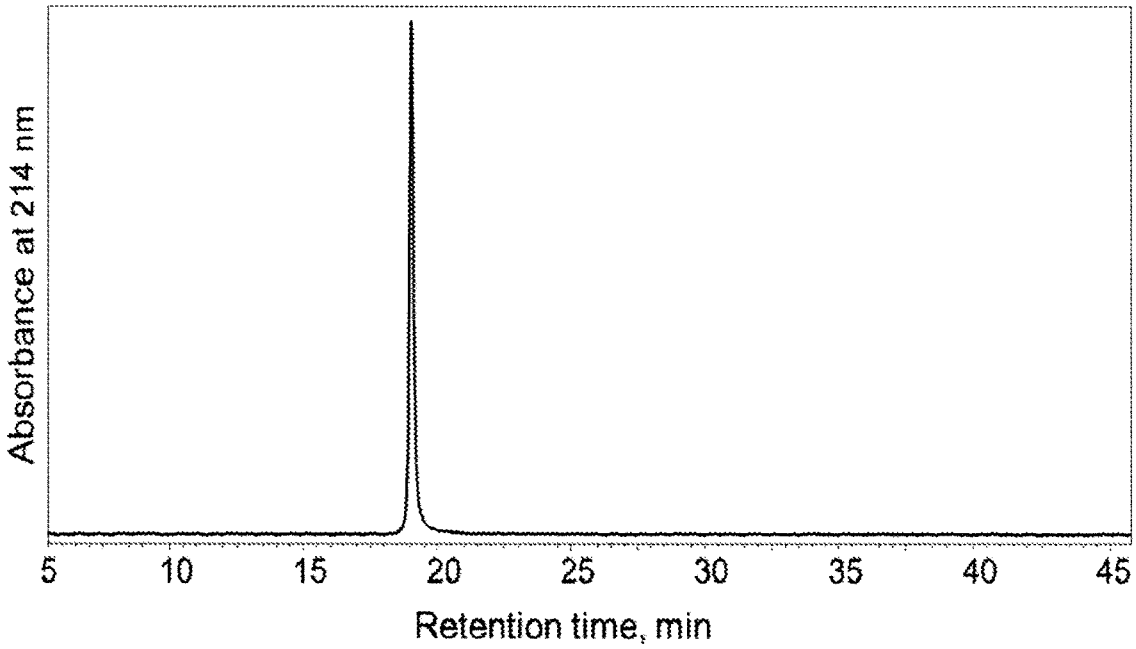

Absorbance at 214 nm
5
10
15
20
25
30
35
40
45
Retention time, min

FIG. 53

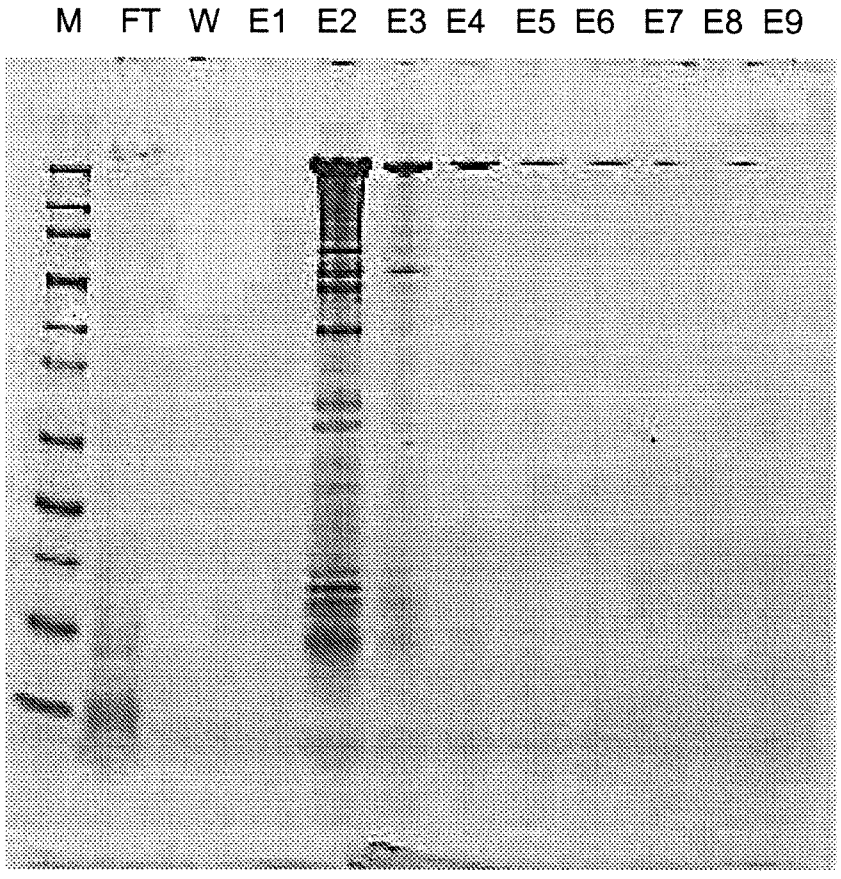


FIG. 54

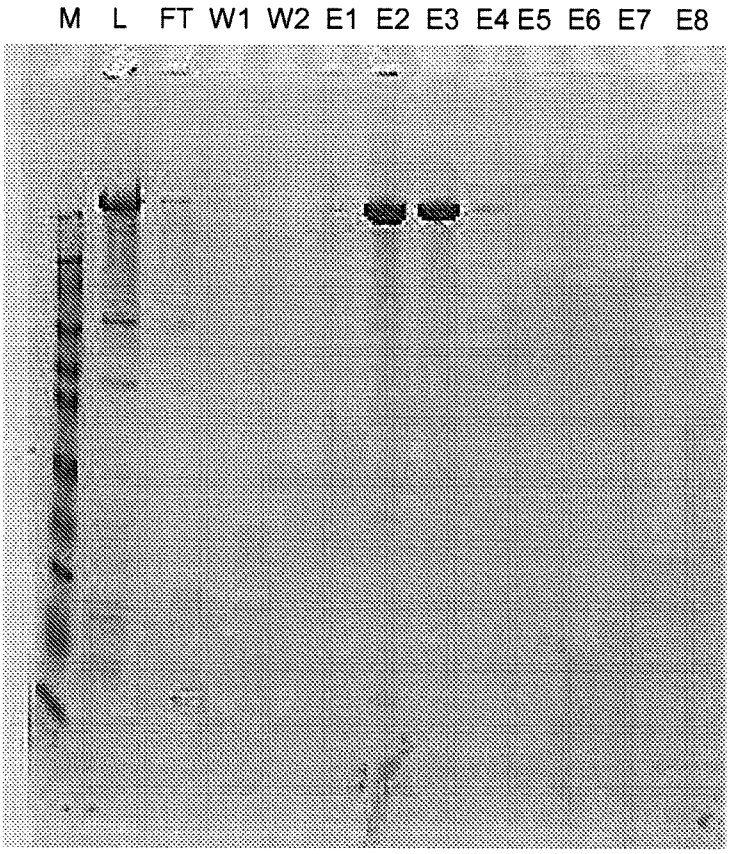


FIG. 55

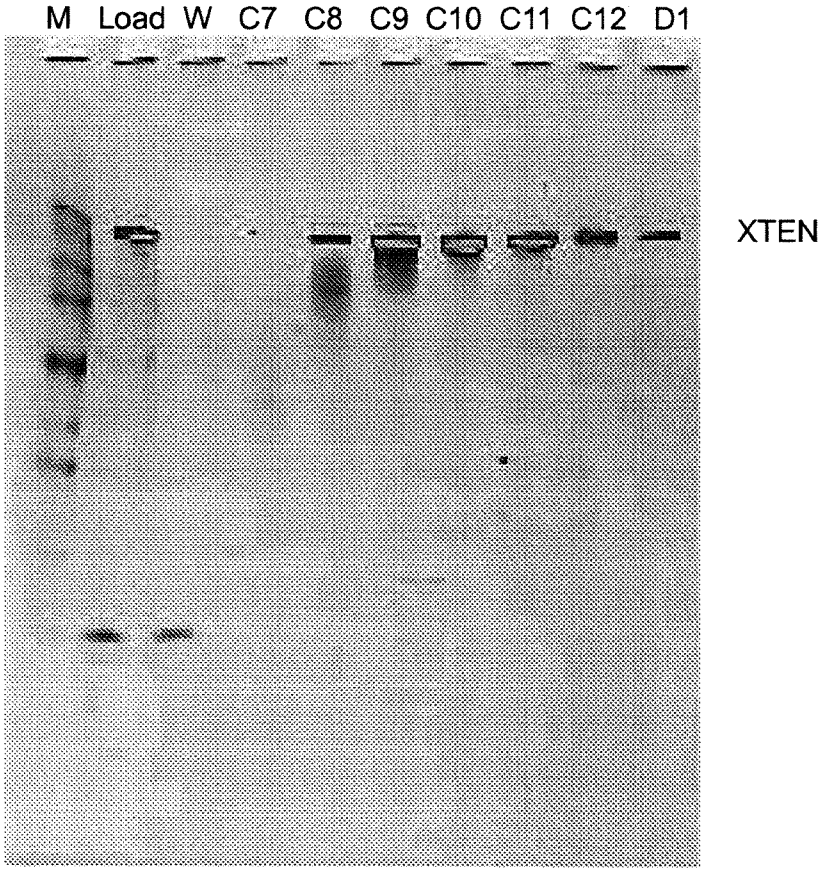


FIG. 56

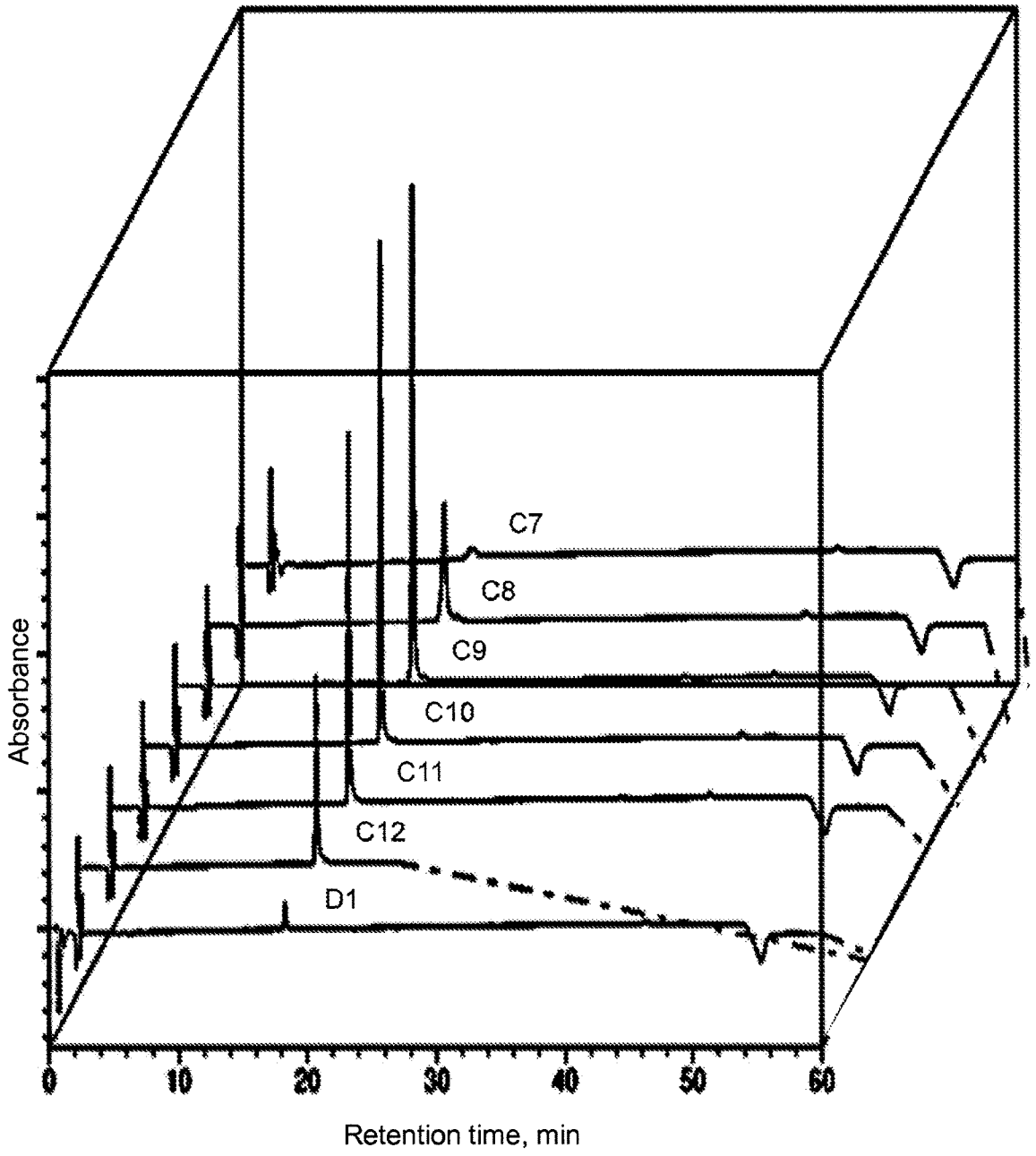

Absorbance
C7
C8
C9
C10
C11
C12
D1
0
10
20
30
40
50
60
Retention time, min

FIG. 57

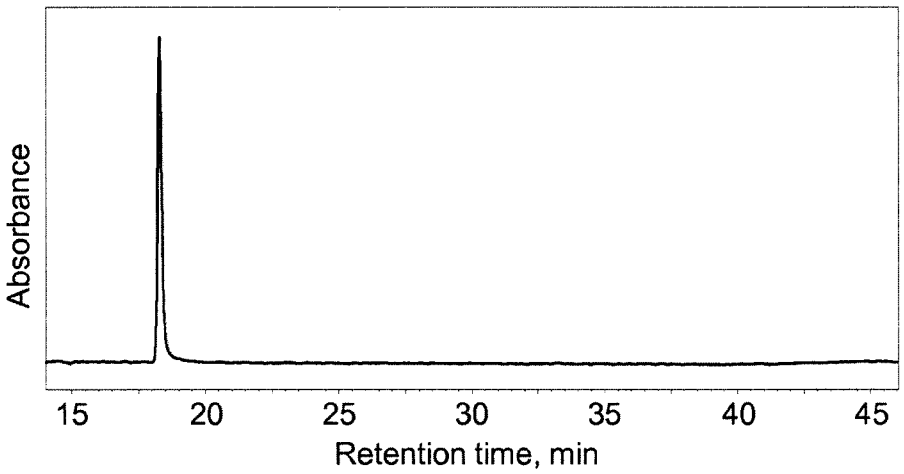


FIG. 58

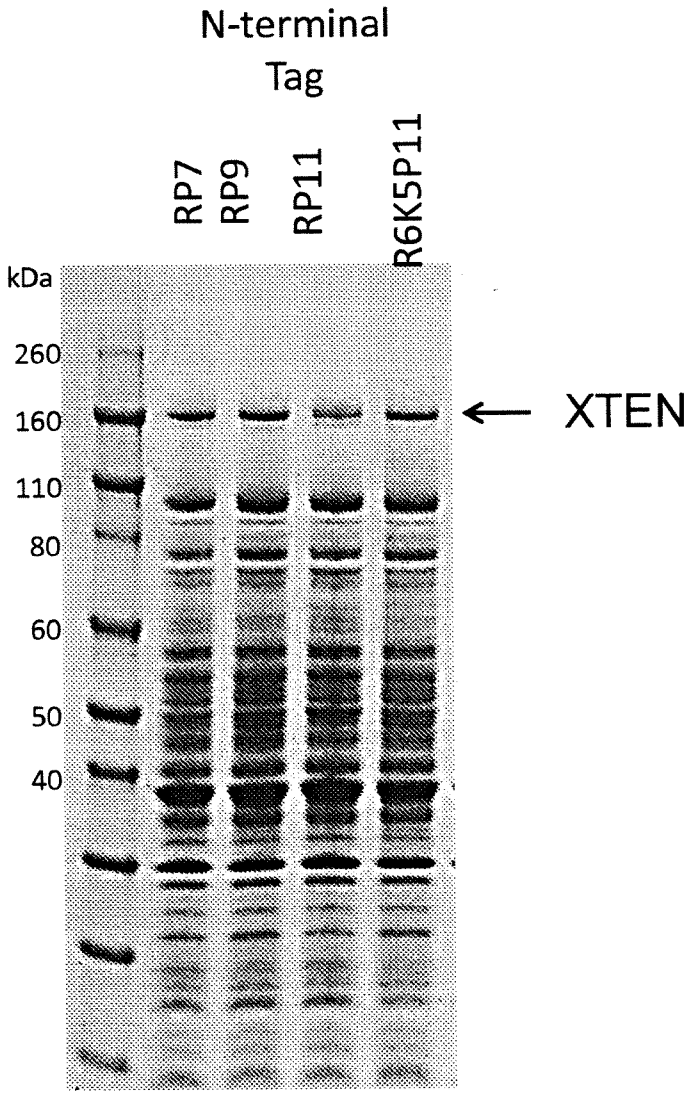

N-terminal
Tag
RP7
RP9
RP11
R6K5P11
kDa
260
160
110
80
60
50
40
XTEN

FIG. 59

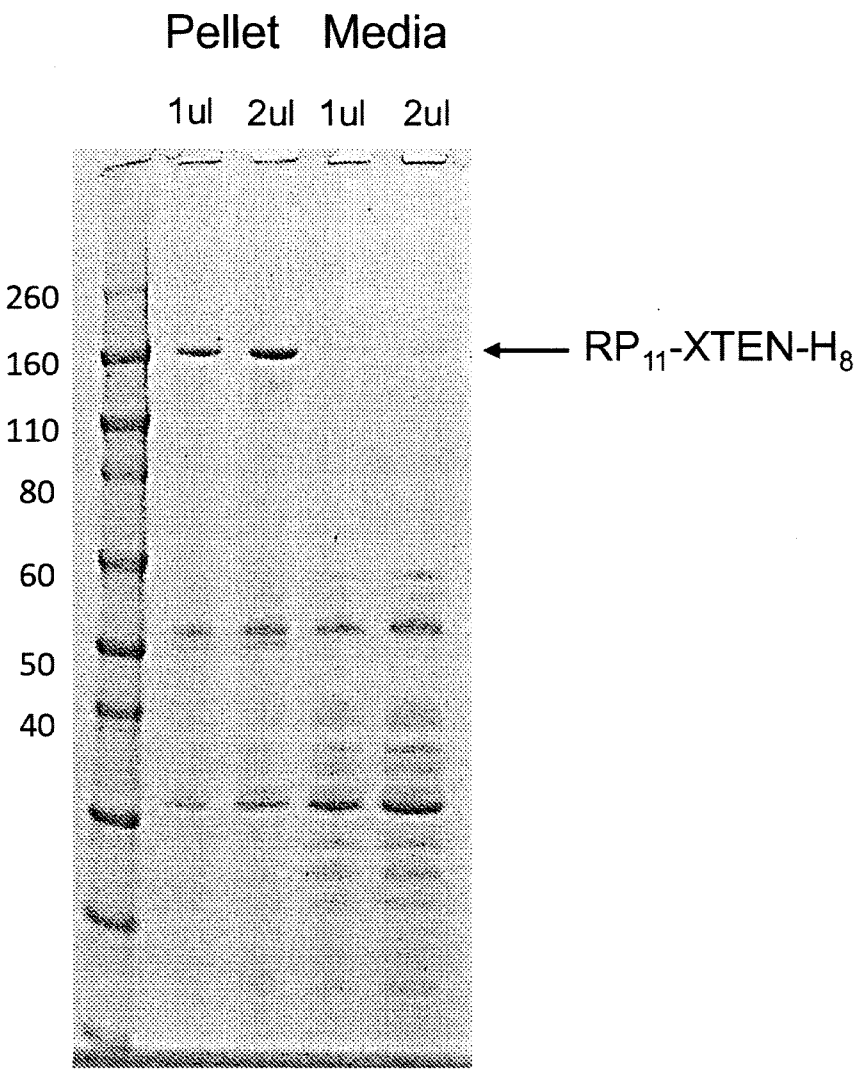

Pellet
Media
1ul
2ul
1ul
2ul
260
160
110
80
60
50
40
RP11-XTEN-H8

FIG. 60

Lane 1	Molecular weight standard
Lane 2	MacroCap SP load
Lane 3	MacroCap SP flow through
Lane 4	MacroCap SP chase
Lane 5	MacroCap SP wash
Lane 6	MacroCap SP elution 1
Lane 7	MacroCap SP elution 2
Lane 8	MacroCap SP elution 3
Lane 9	MacroCap SP elution 4
Lane 10	MacroCap SP elution 5
Lane 11	MacroCap SP elution 6

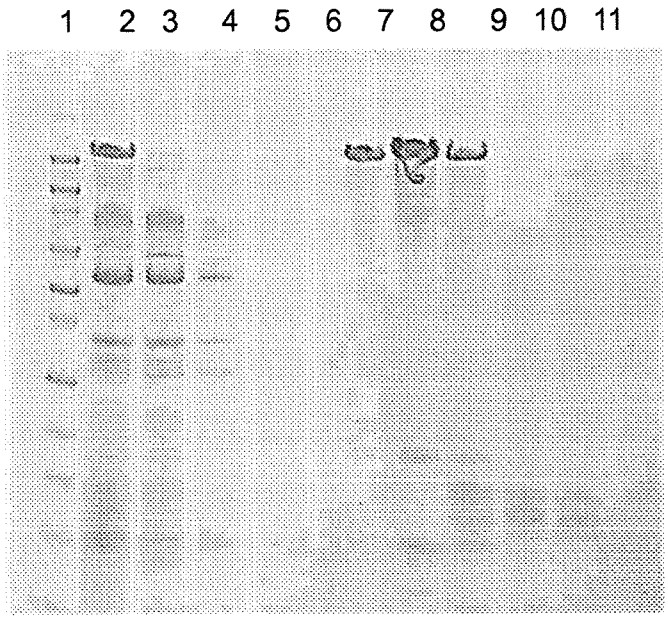


FIG. 61

Lane 1	Molecular weight standard
Lane 2	IMAC load
Lane 3	IMAC flow through
Lane 4	IMAC chase
Lane 5	IMAC wash
Lane 6	IMAC elution 1
Lane 7	IMAC elution 2
Lane 8	IMAC elution 3
Lane 9	IMAC elution 4
Lane 10	IMAC elution 5
Lane 11	IMAC elution 6
Lane 12	IMAC elution 7

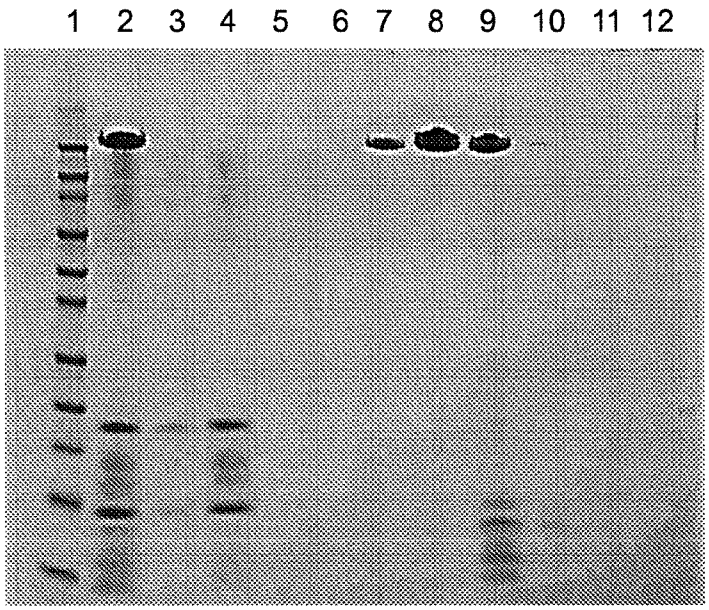
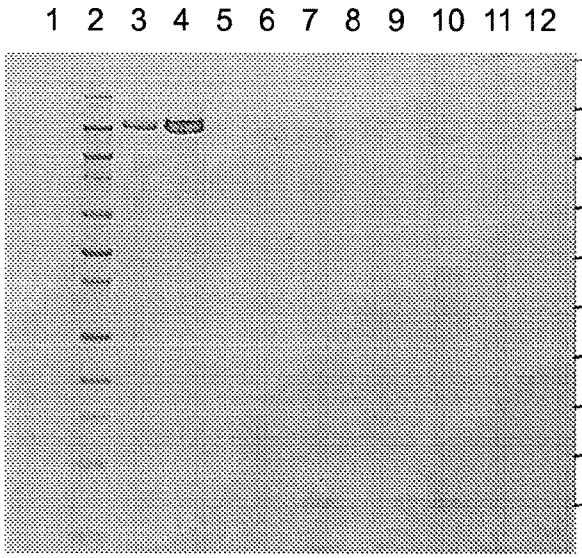


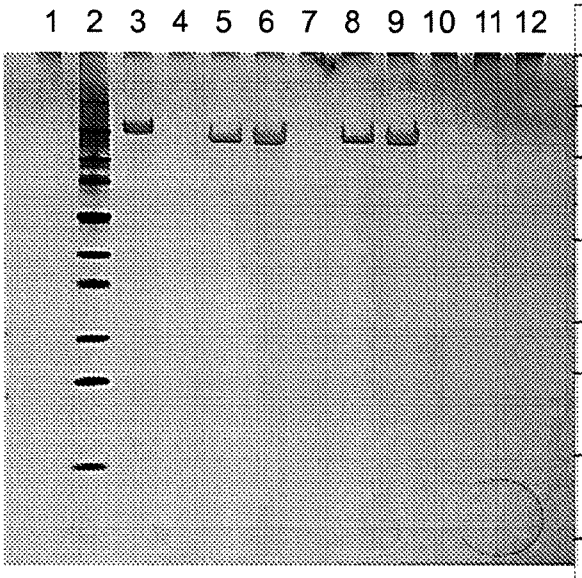
FIG. 62

A



Lane 2	Molecular weight standard
Lane 3	IMAC pool 2 µl
Lane 4	IMAC pool 10 µl
Lane 5	blank
Lane 6	IMAC pool 1:200 trypsin digest 2 µl
Lane 7	IMAC pool 1:200 trypsin digest 10 µl
Lane 8	blank
Lane 9	IMAC pool 1:500 trypsin digest 2 µl
Lane 10	IMAC pool 1:500 trypsin digest 10 µl

B



Lane 2	Molecular weight standard
Lane 3	IMAC pool 1:20 diluted 10 µl
Lane 4	blank
Lane 5	IMAC pool 1:200 trypsin digest 1:5 diluted 5 µl
Lane 6	IMAC pool 1:200 trypsin digest 1:5 diluted 10 µl
Lane 7	blank
Lane 8	IMAC pool 1:500 trypsin digest 1:5 diluted 5 µl
Lane 9	IMAC pool 1:500 trypsin digest 1:5 diluted 10 µl
Lane 10	blank

FIG. 63

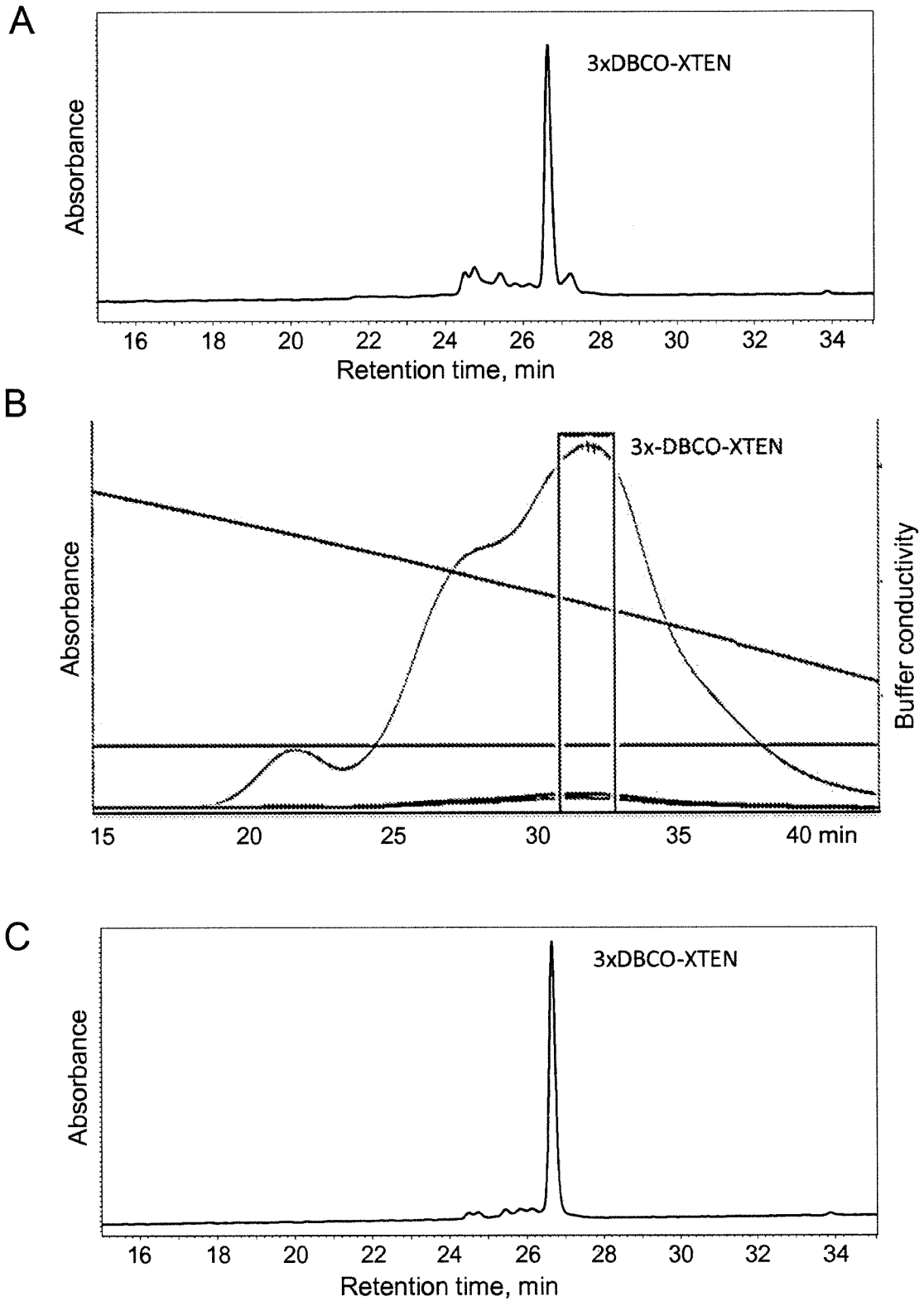

A
3xDBCO-XTEN
Absorbance
16
18
20
22
24
26
28
30
32
34
Retention time, min
B
3x-DBCO-XTEN
Absorbance
Buffer conductivity
15
20
25
30
35
40 min
C
3xDBCO-XTEN
Absorbance
16
18
20
22
24
26
28
30
32
34
Retention time, min

FIG. 64

FIG. 65A

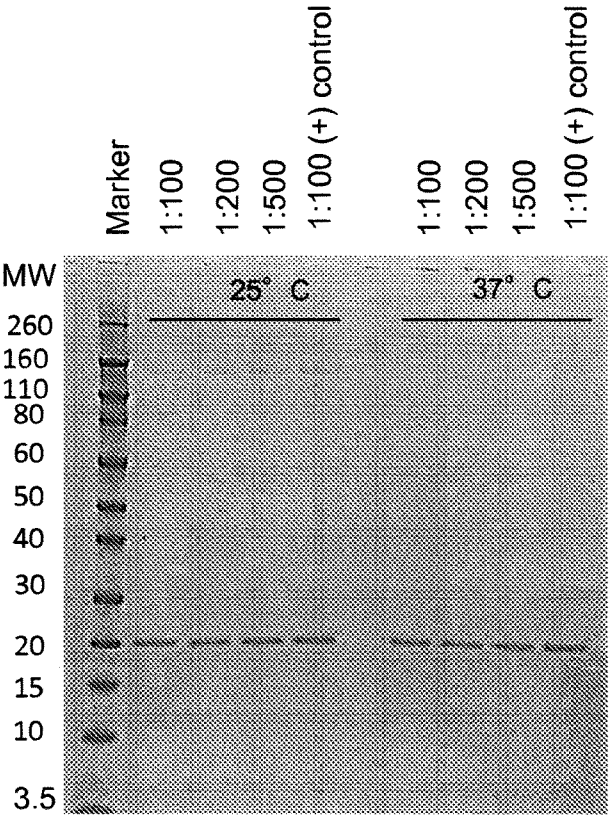


FIG. 65B

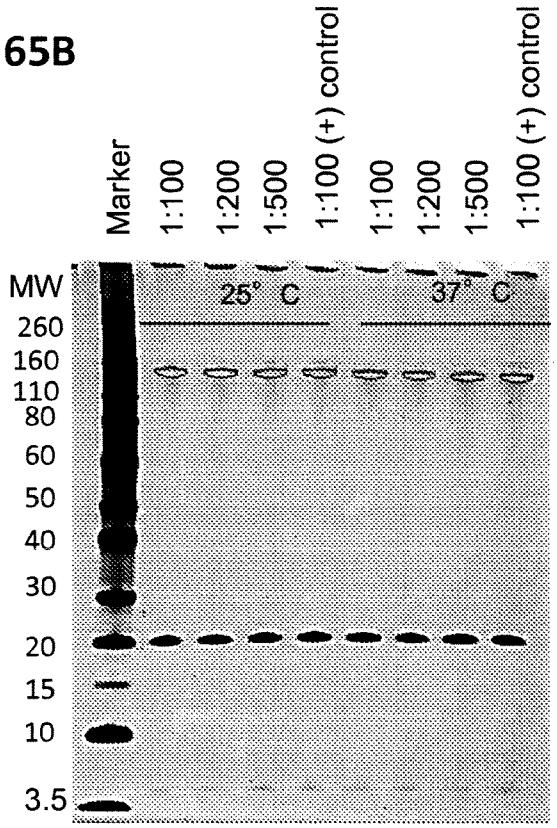


FIG. 65

FIG. 66A

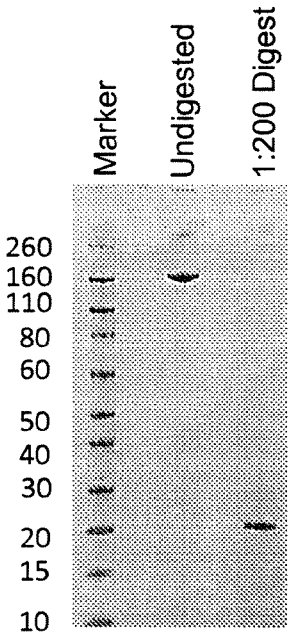


FIG. 66B

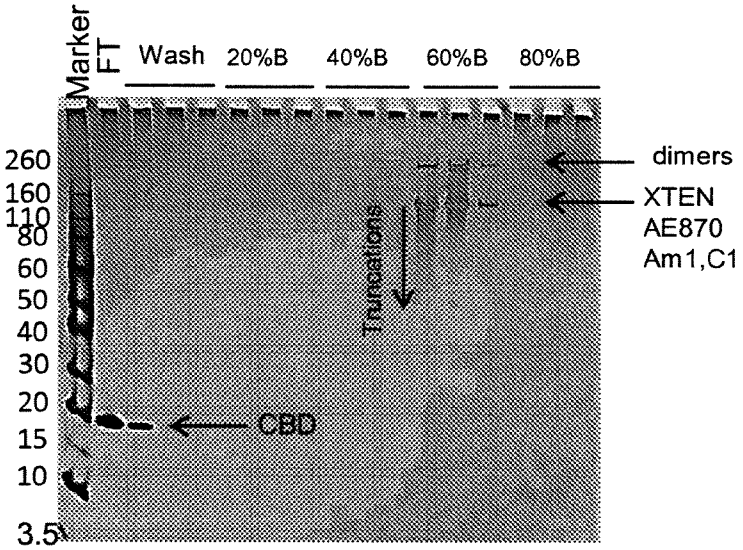


FIG. 66C

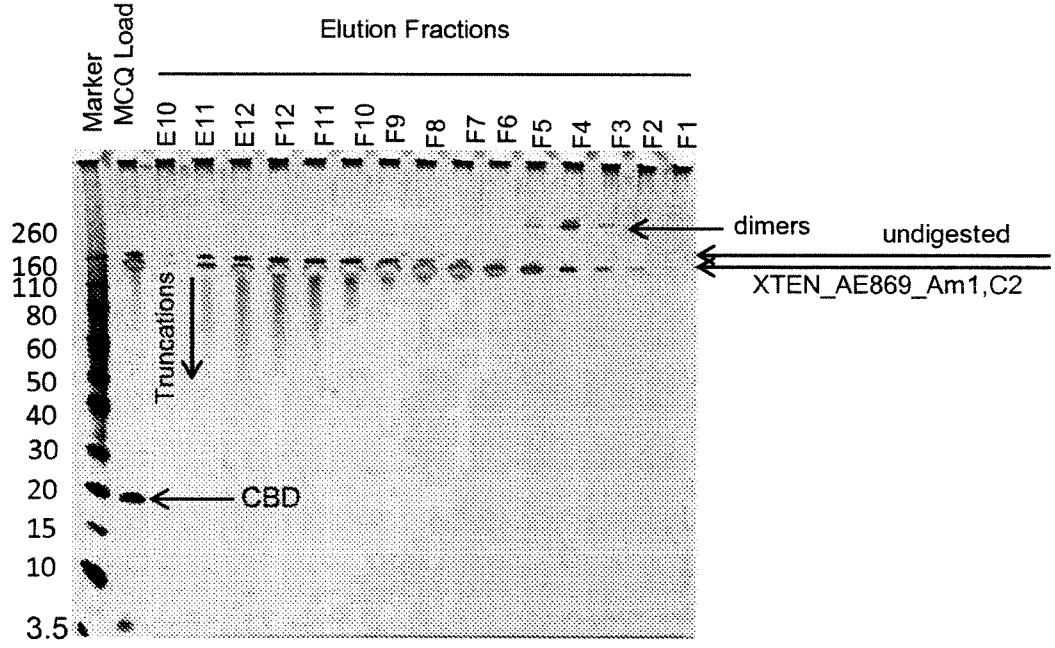


FIG. 66

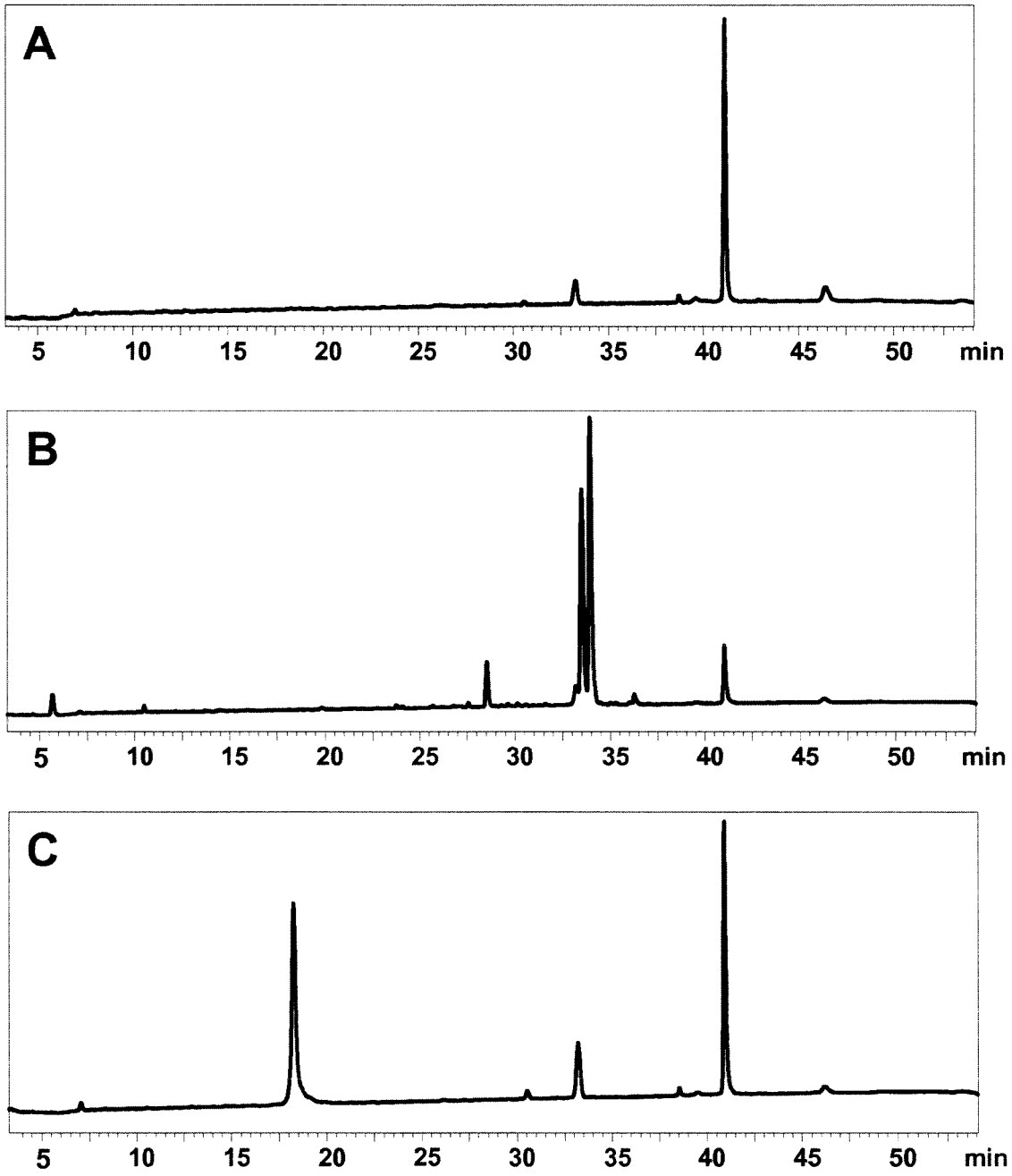

A
5
10
15
20
25
30
35
40
45
50
min
B
5
10
15
20
25
30
35
40
45
50
min
C
5
10
15
20
25
30
35
40
45
50
min

FIG. 67

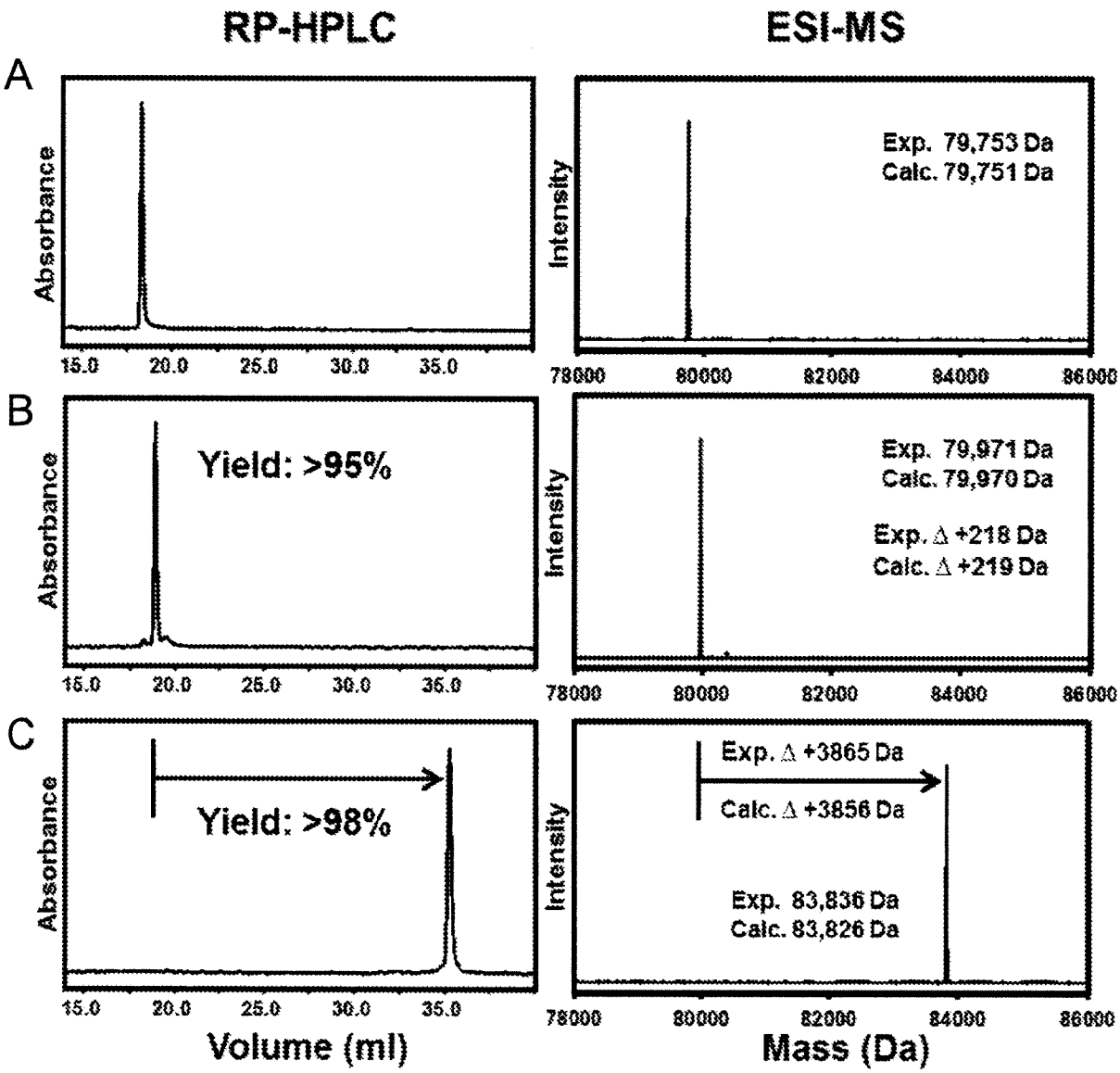

RP-HPLC
ESI-MS
A
Absorbance
Intensity
Exp. 79,753 Da
Calc. 79,751 Da
15.0
20.0
25.0
30.0
35.0
78000
80000
82000
84000
86000
B
Yield: >95%
Exp. 79,971 Da
Calc. 79,970 Da
Exp. Δ +218 Da
Calc. Δ +219 Da
C
Yield: >98%
Exp. Δ +3865 Da
Calc. Δ +3856 Da
Exp. 83,836 Da
Calc. 83,826 Da
Volume (ml)
Mass (Da)

FIG. 68

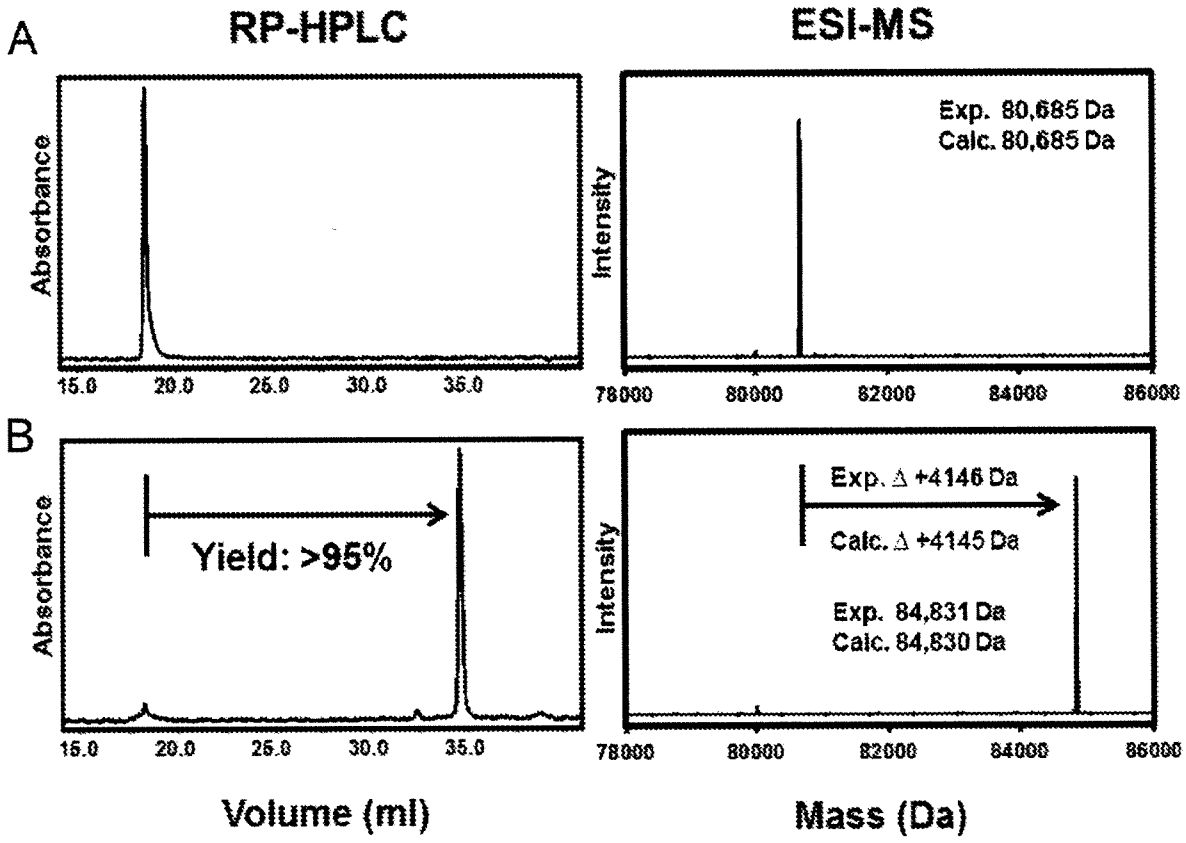

A
RP-HPLC
ESI-MS
Absorbance
Intensity
Exp. 80,685 Da
Calc. 80,685 Da
15.0
20.0
25.0
30.0
35.0
78000
80000
82000
84000
86000
B
Yield: >95%
Exp. Δ +4146 Da
Calc. Δ +4145 Da
Exp. 84,831 Da
Calc. 84,830 Da
Volume (ml)
Mass (Da)

FIG. 69

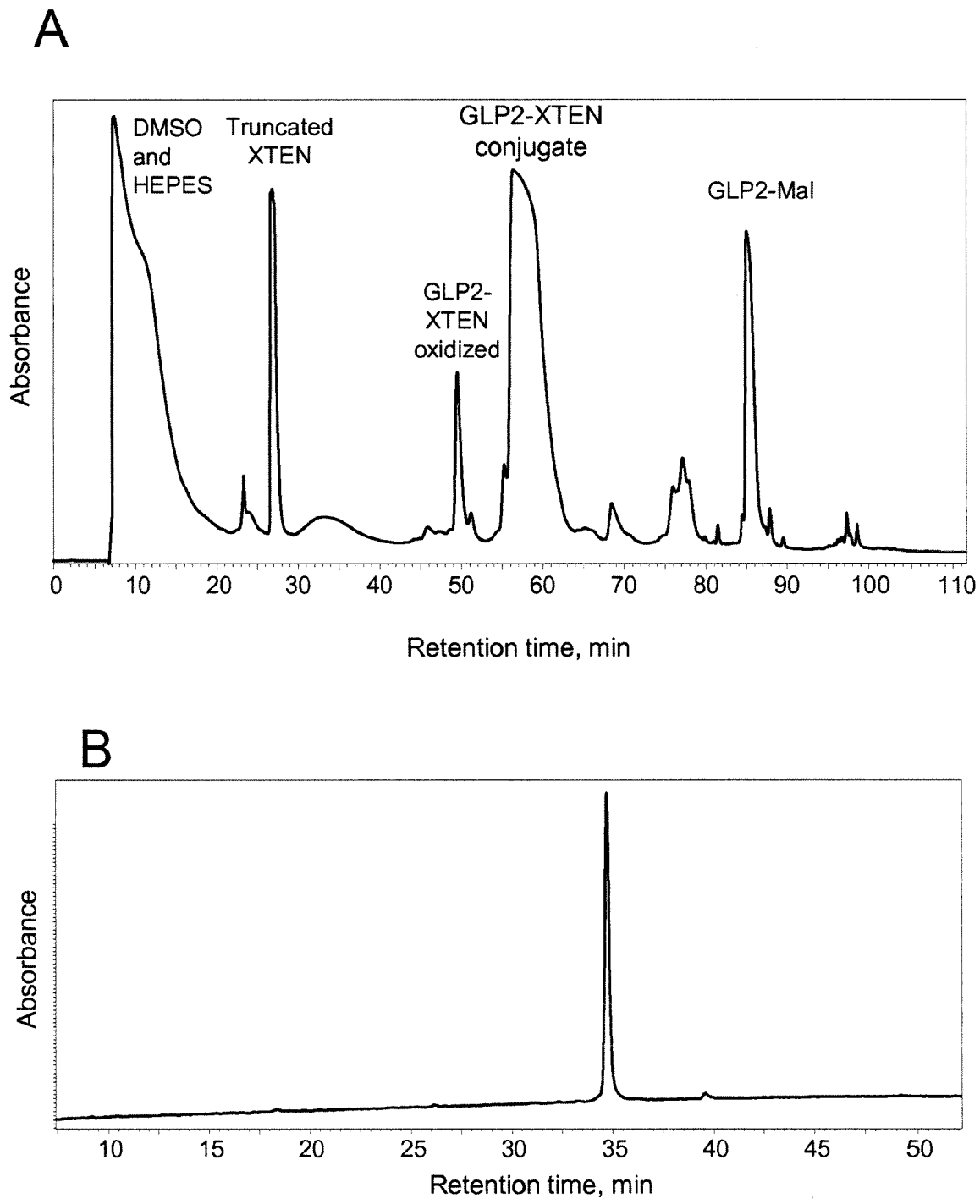

A
DMSO and HEPES
Truncated XTEN
GLP2-XTEN conjugate
GLP2-Mal
GLP2-XTEN oxidized
Absorbance
0
10
20
30
40
50
60
70
80
90
100
110
Retention time, min
B
Absorbance
10
15
20
25
30
35
40
45
50
Retention time, min

FIG. 70

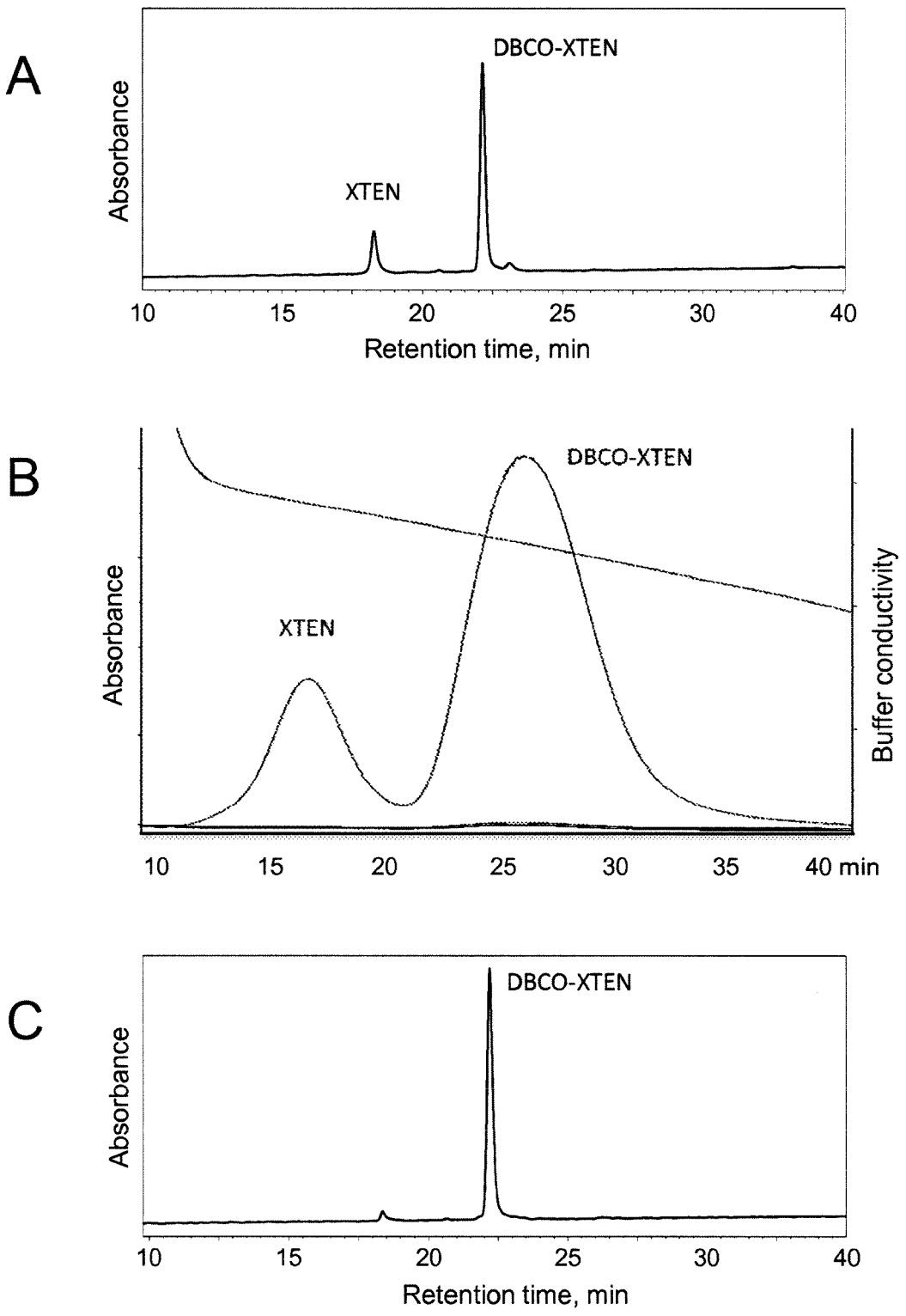
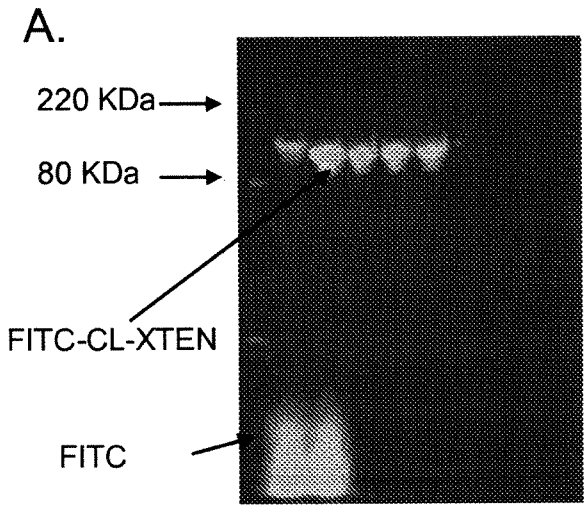

A
Absorbance
DBCO-XTEN
XTEN
10
15
20
25
30
40
Retention time, min
B
Absorbance
DBCO-XTEN
XTEN
Buffer conductivity
10
15
20
25
30
35
40 min
C
Absorbance
DBCO-XTEN
10
15
20
25
30
40
Retention time, min

FIG. 71


A.
220 KDa
80 KDa
FITC-CL-XTEN
FITC

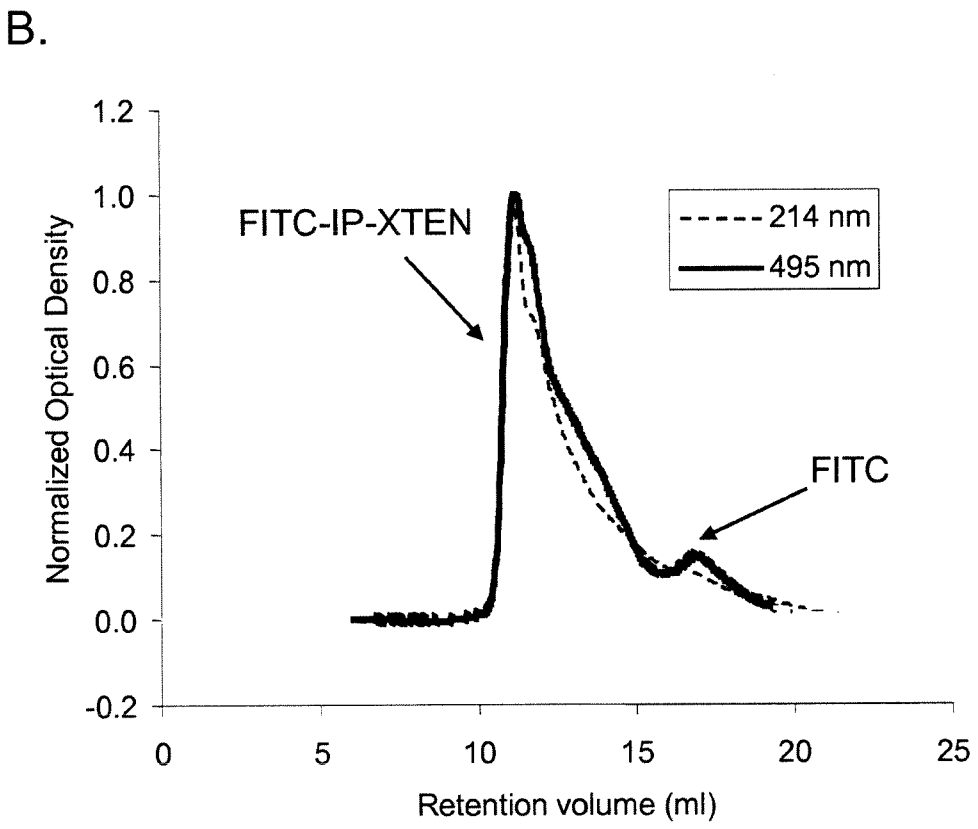

B.
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
Normalized Optical Density
FITC-IP-XTEN
214 nm
495 nm
FITC
0
5
10
15
20
25
Retention volume (ml)

FIG. 72

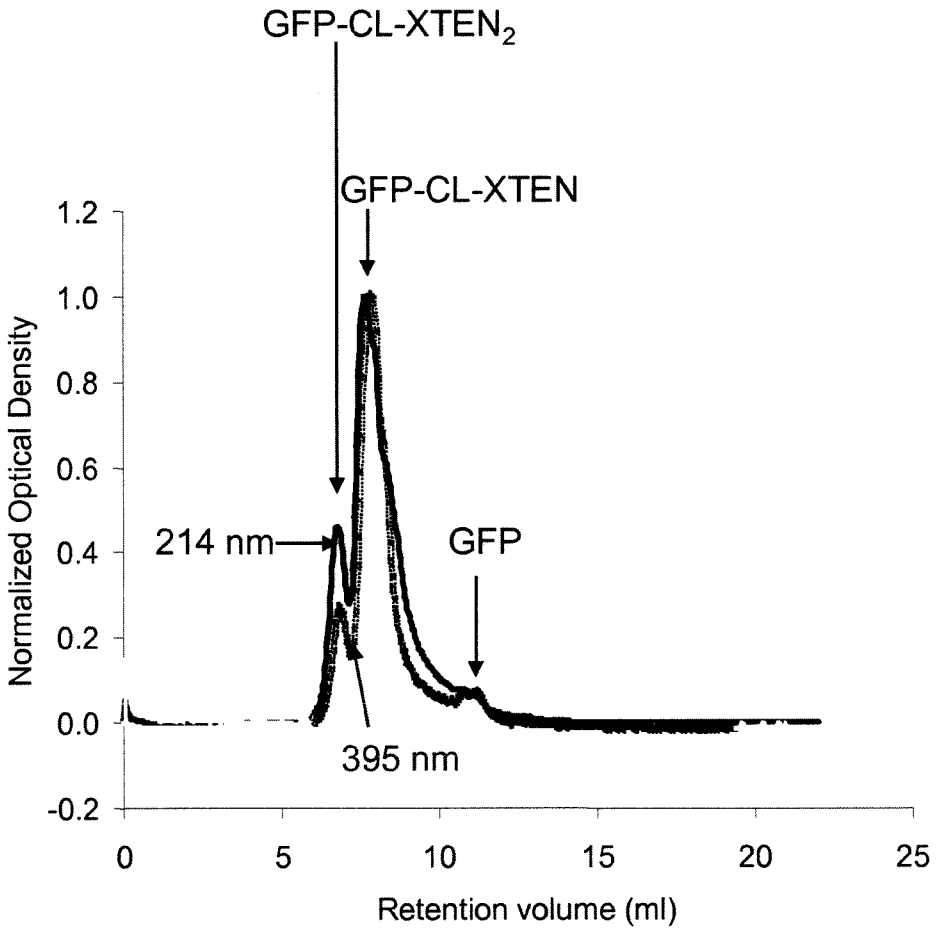

GFP-CL-XTEN2
GFP-CL-XTEN
214 nm
GFP
395 nm
Normalized Optical Density
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
5
10
15
20
25
Retention volume (ml)

FIG. 73

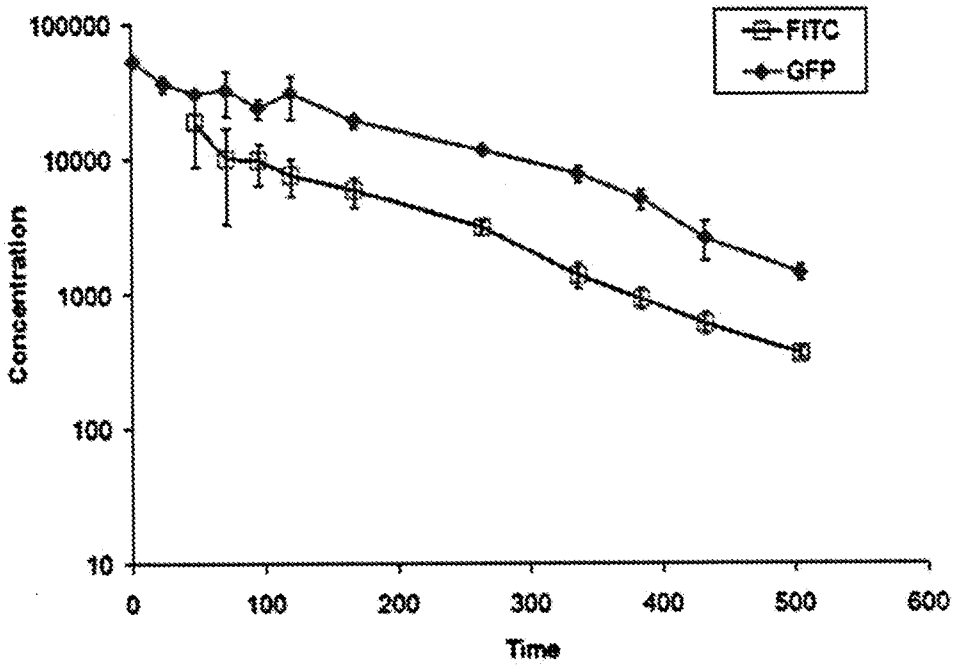

FITC
GFP
100000
10000
1000
100
10
Concentration
0
100
200
300
400
500
600
Time

FIG. 74

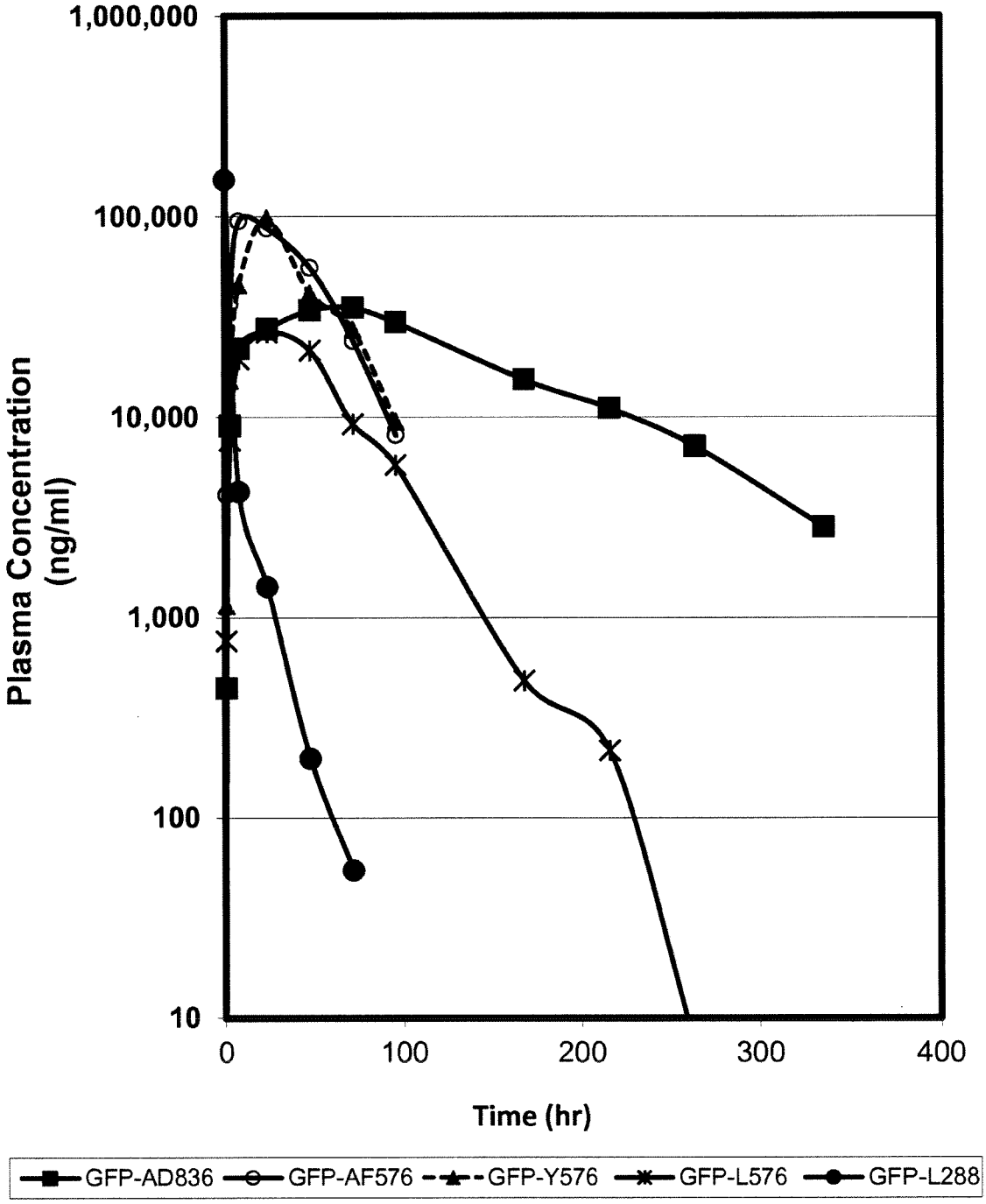

1,000,000
100,000
10,000
1,000
100
10
Plasma Concentration (ng/ml)
0
100
200
300
400
Time (hr)
GFP-AD836
GFP-AF576
GFP-Y576
GFP-L576
GFP-L288

FIG. 75

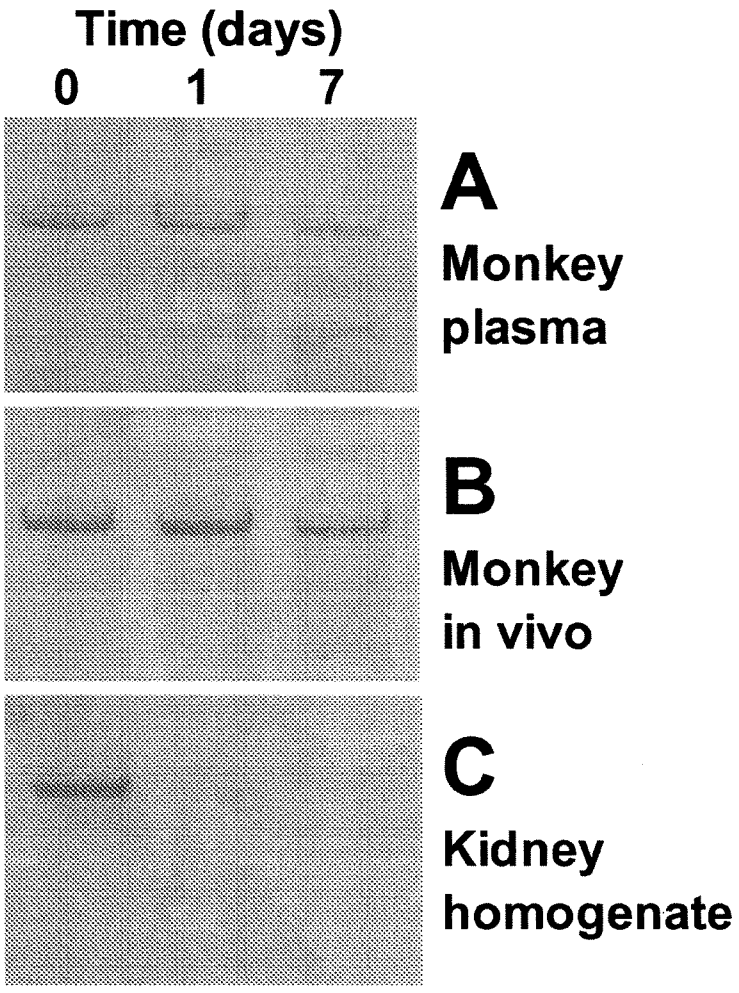

Time (days)
0
1
7
A
Monkey plasma
B
Monkey in vivo
C
Kidney homogenate

FIG. 76

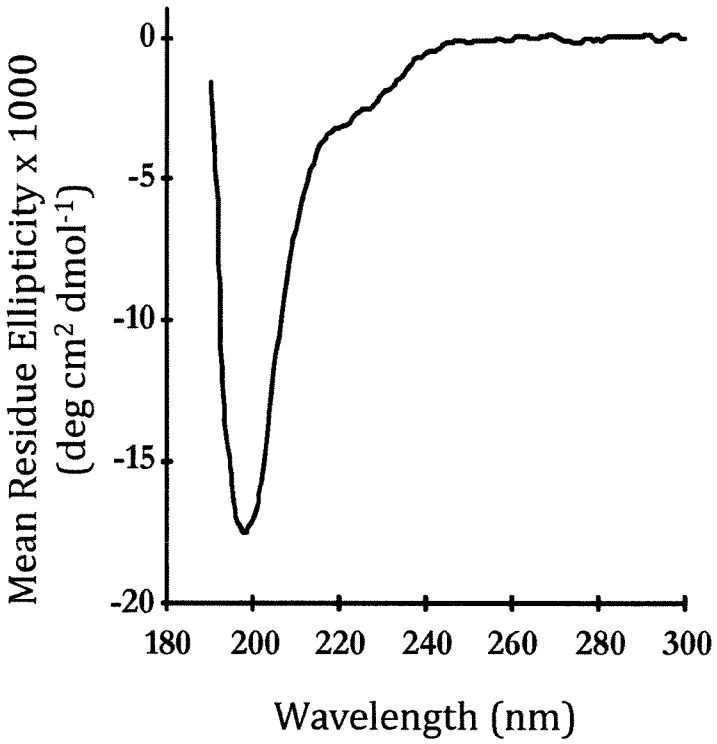
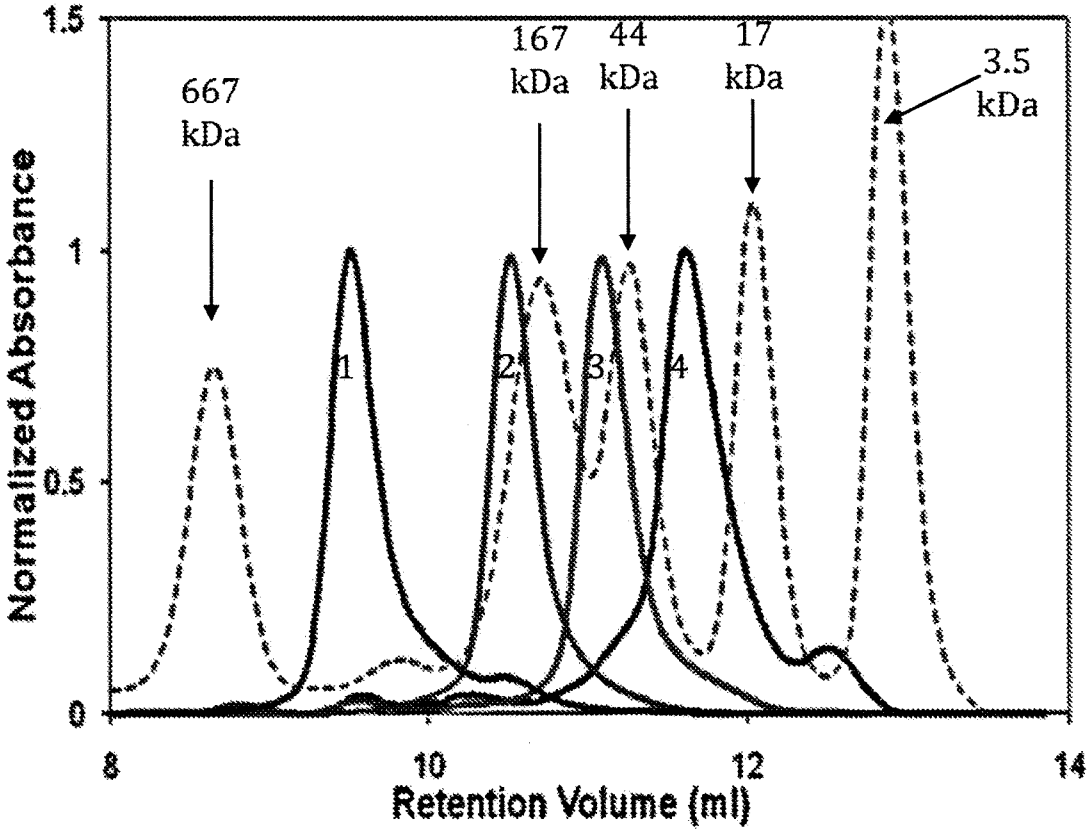

Mean Residue Ellipticity x 1000
(deg cm2 dmol-1)
0
-5
-10
-15
-20
180
200
220
240
260
280
300
Wavelength (nm)

FIG. 77



1. Glucagon-Y288
2. Glucagon-Y144
3. Glucagon-Y72
4. Glucagon-Y36

- - - - = Standards

FIG. 78

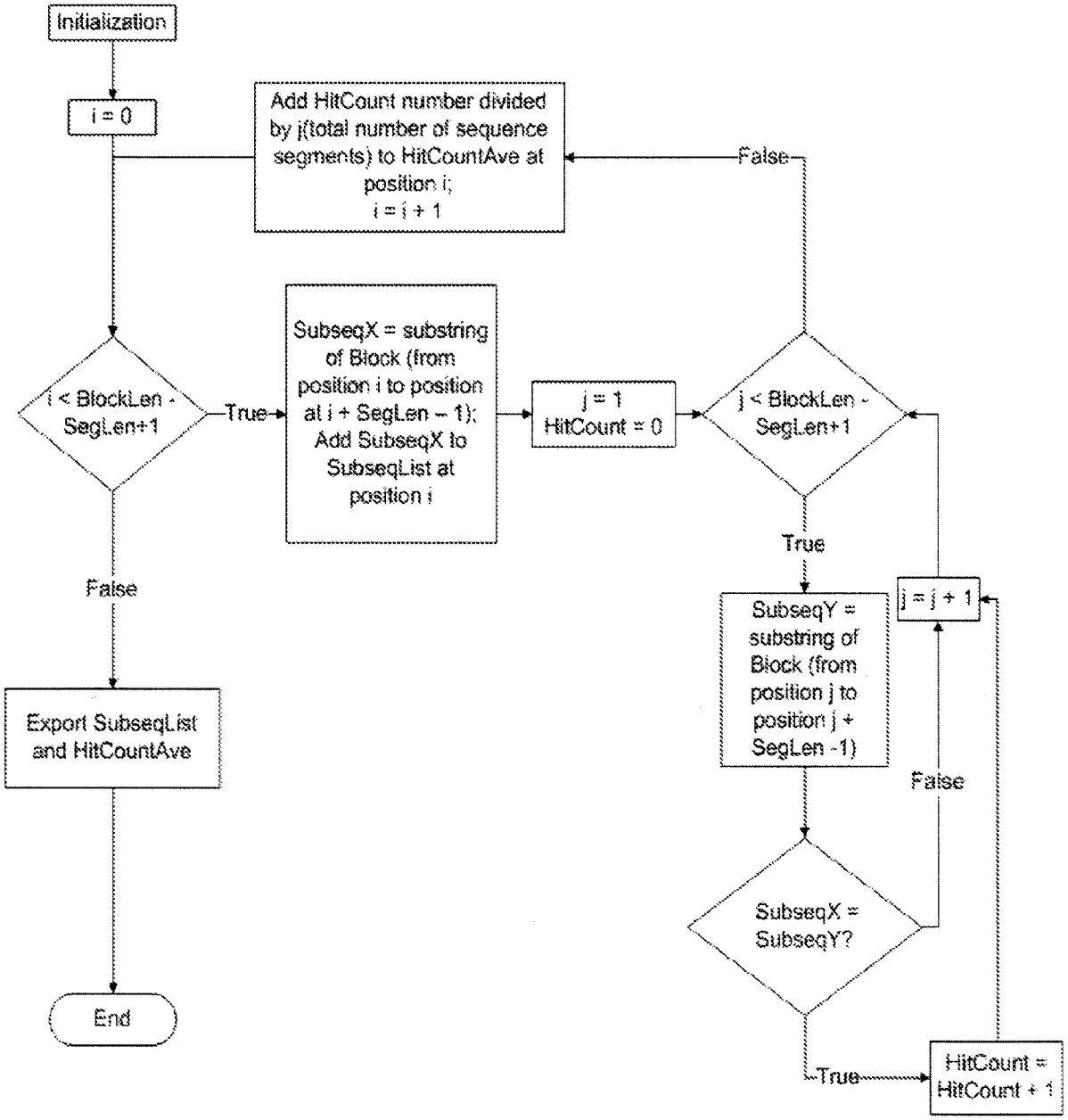

Initialization
i = 0
Add HitCount number divided by j(total number of sequence segments) to HitCountAve at position i;
i = i + 1
False
i < BlockLen - SegLen+1
True
SubseqX = substring of Block (from position i to position at i + SegLen – 1); Add SubseqX to SubseqList at position i
j = 1
HitCount = 0
j < BlockLen - SegLen+1
True
False
SubseqY = substring of Block (from position j to position j + SegLen -1)
j = j + 1
False
SubseqX = SubseqY?
True
HitCount = HitCount + 1
Export SubseqList and HitCountAve
End

FIG. 79

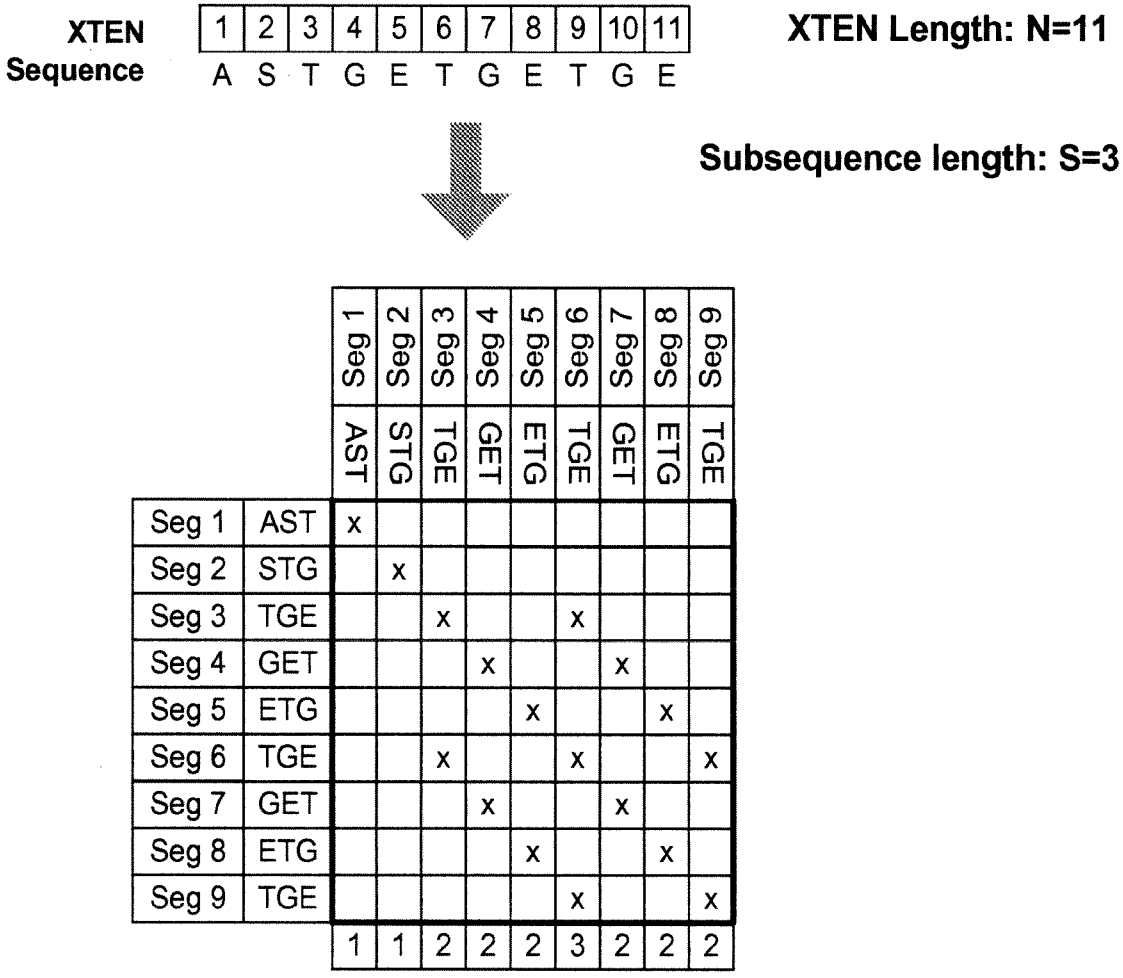

XTEN Sequence
1 2 3 4 5 6 7 8 9 10 11
A S T G E T G E T G E
XTEN Length: N=11
Subsequence length: S=3
Seg 1 AST
Seg 2 STG
Seg 3 TGE
Seg 4 GET
Seg 5 ETG
Seg 6 TGE
Seg 7 GET
Seg 8 ETG
Seg 9 TGE
1 1 2 2 2 3 2 2 2
Subsequence Score = 1.89

FIG. 80

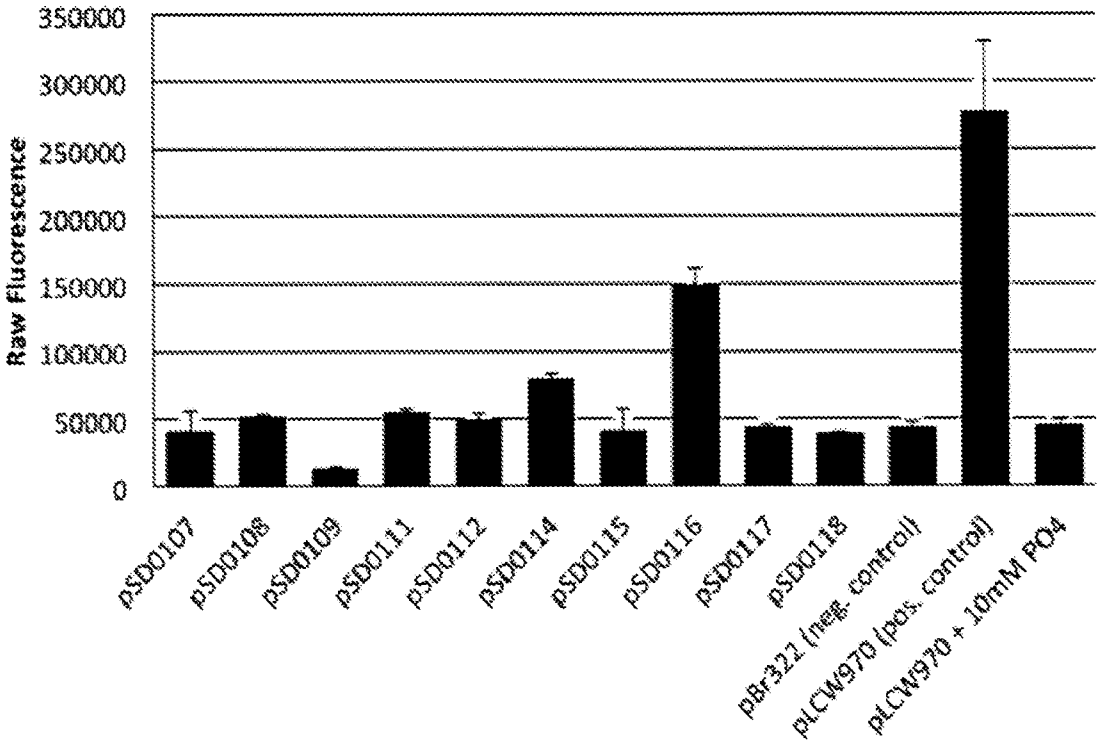

Raw Fluorescence
350000
300000
250000
200000
150000
100000
50000
0
pSD0107
pSD0108
pSD0109
pSD0111
pSD0112
pSD0114
pSD0115
pSD0116
pSD0117
pSD0118
pBr322 (neg. control)
pLCW970 (pos. control)
pLCW970 + 10mM PO4

FIG. 81

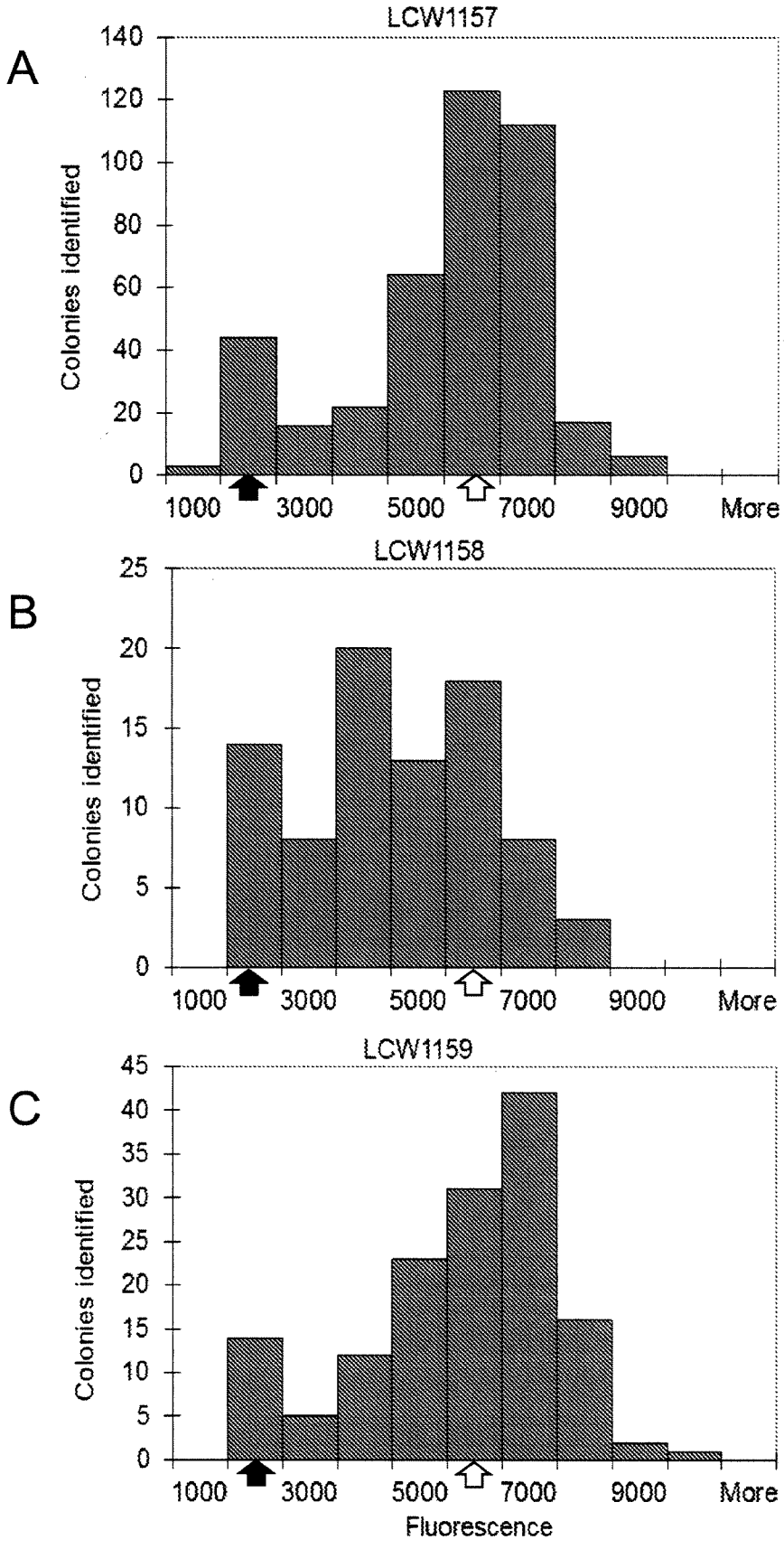

A
LCW1157
Colonies identified
140
120
100
80
60
40
20
0
1000
3000
5000
7000
9000
More
B
LCW1158
Colonies identified
25
20
15
10
5
0
1000
3000
5000
7000
9000
More
C
LCW1159
Colonies identified
45
40
35
30
25
20
15
10
5
0
1000
3000
5000
7000
9000
More
Fluorescence

FIG. 82, A, B, C

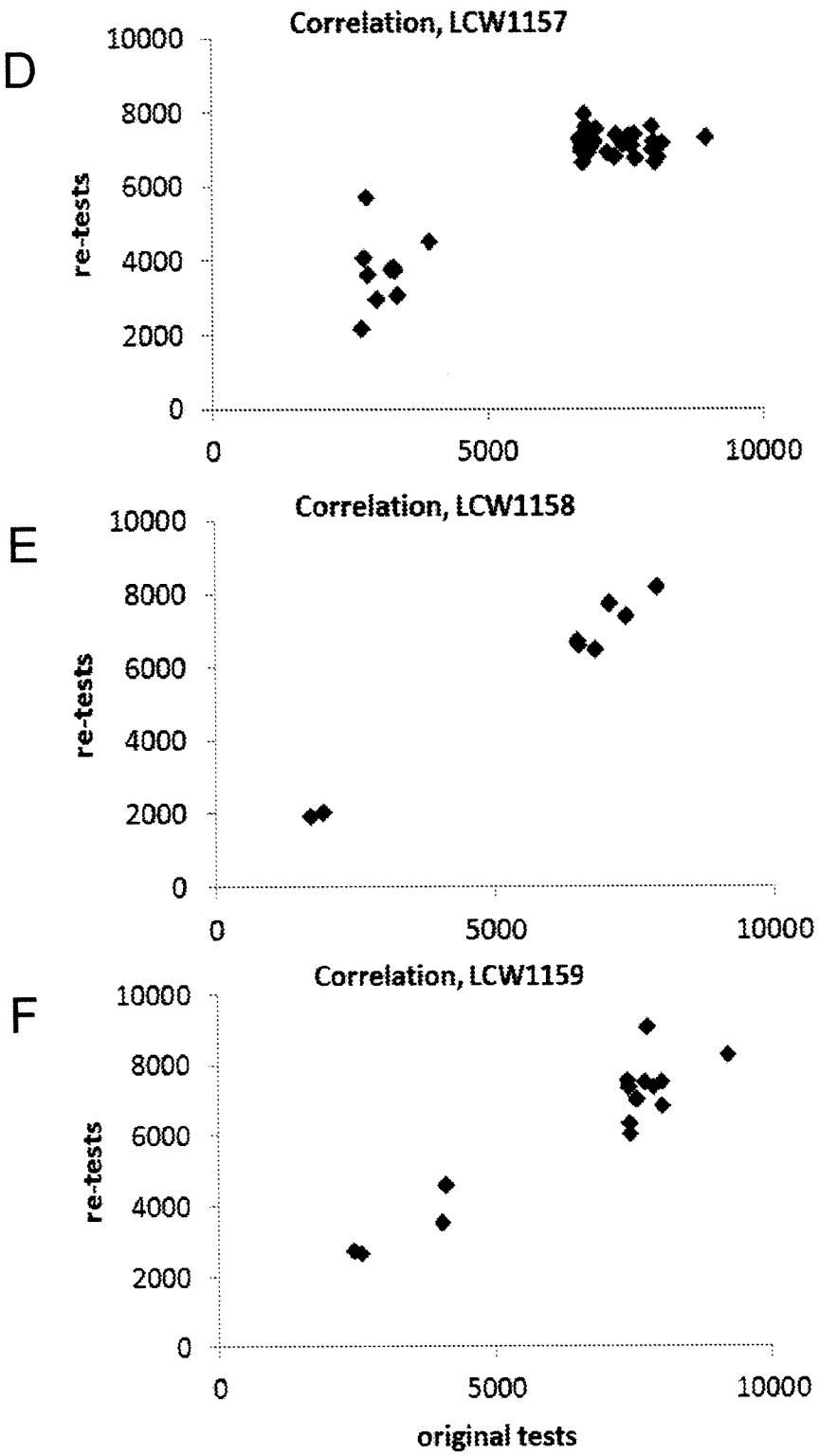

D
Correlation, LCW1157
re-tests
10000
8000
6000
4000
2000
0
0
5000
10000
E
Correlation, LCW1158
re-tests
10000
8000
6000
4000
2000
0
0
5000
10000
F
Correlation, LCW1159
re-tests
10000
8000
6000
4000
2000
0
0
5000
10000
original tests

FIG. 82, D, E, F

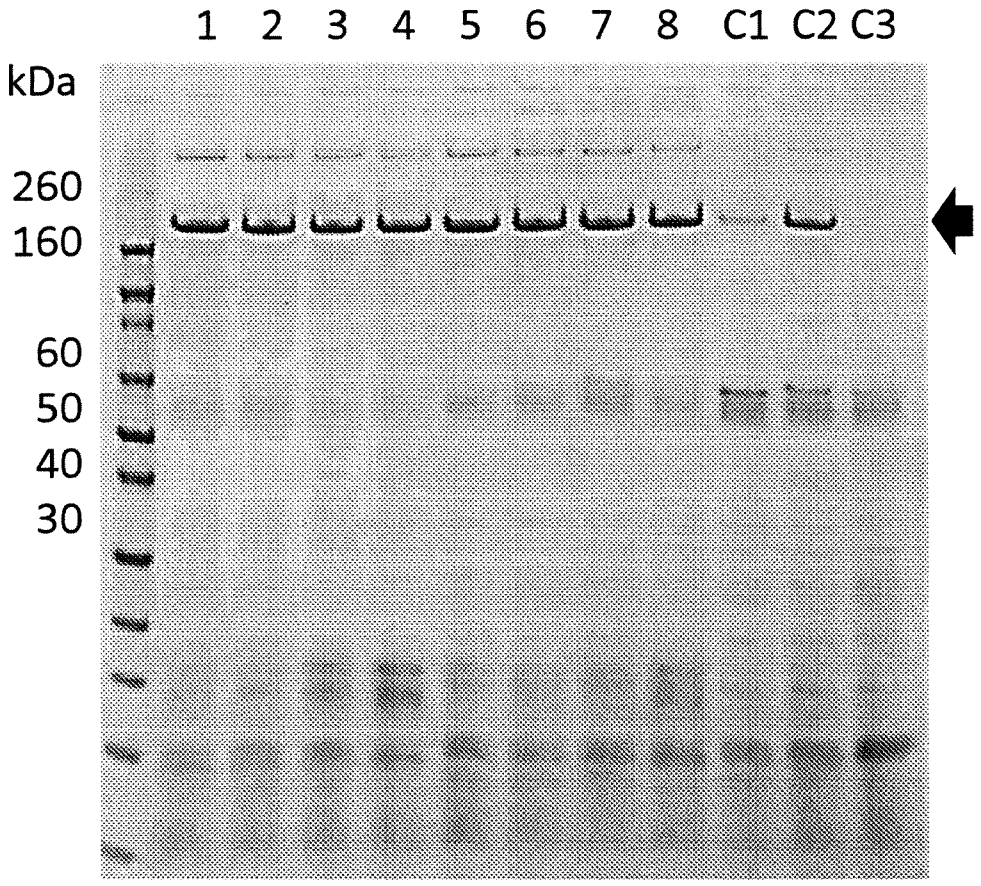


FIG. 83

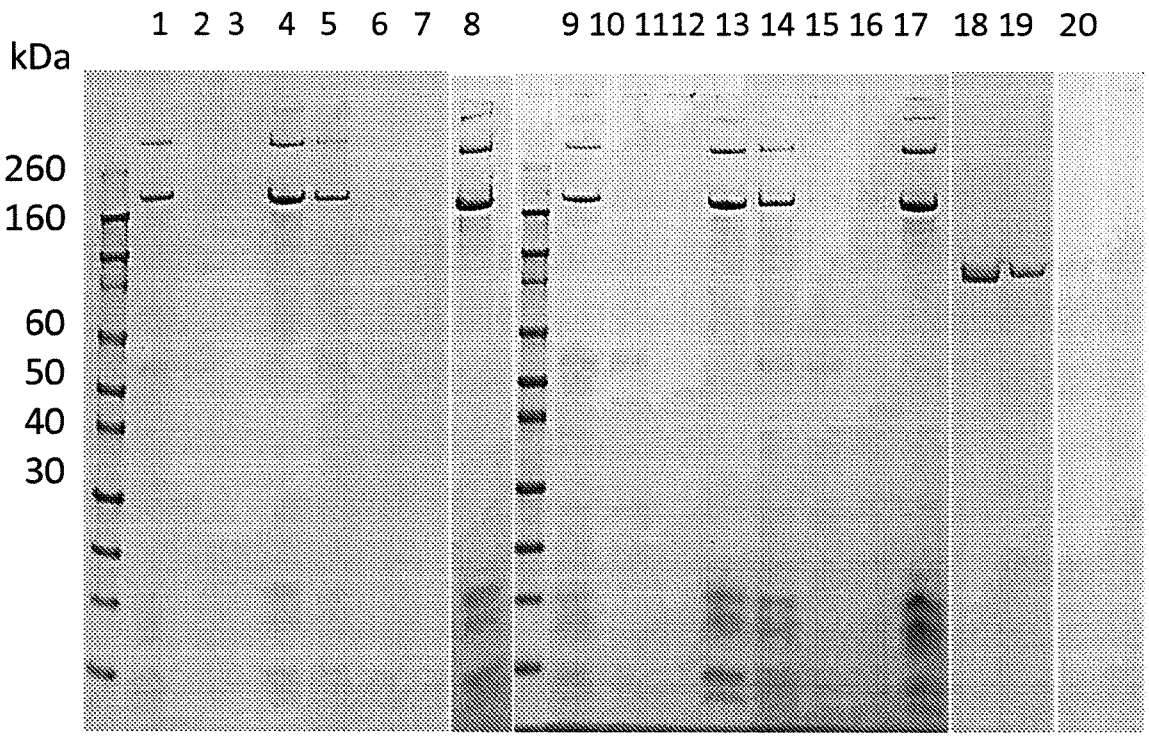


FIG. 84

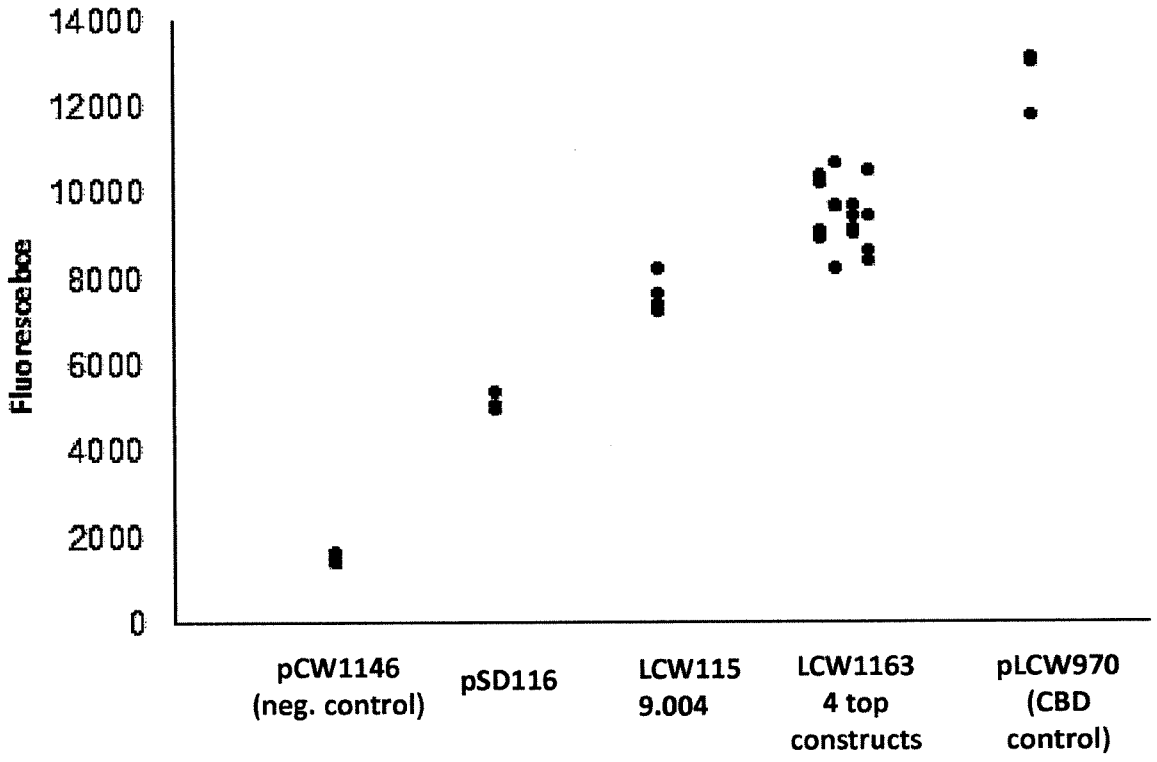

14000
12000
10000
8000
6000
4000
2000
0
Fluorescence
pCW1146 (neg. control)
pSD116
LCW115 9.004
LCW1163 4 top constructs
pLCW970 (CBD control)

FIG. 85

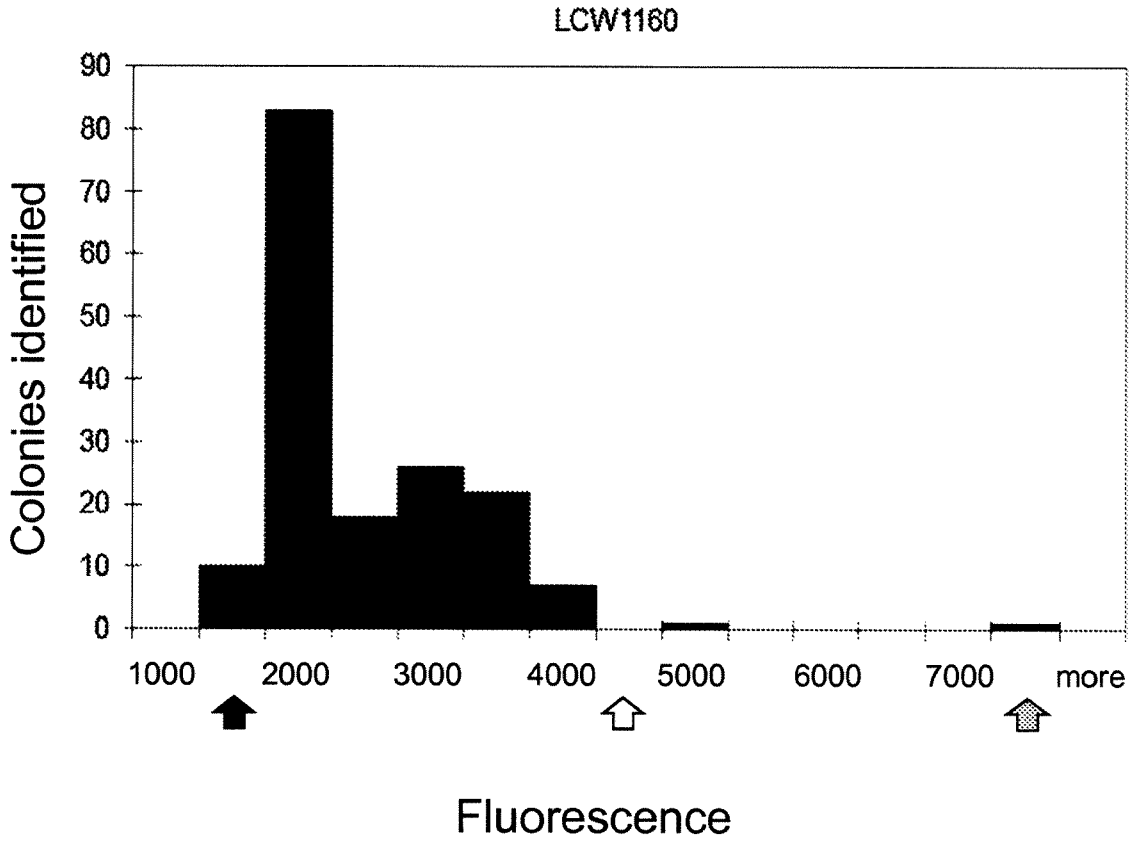

LCW1160
Colonies identified
90
80
70
60
50
40
30
20
10
0
1000
2000
3000
4000
5000
6000
7000
more
Fluorescence

FIG. 86

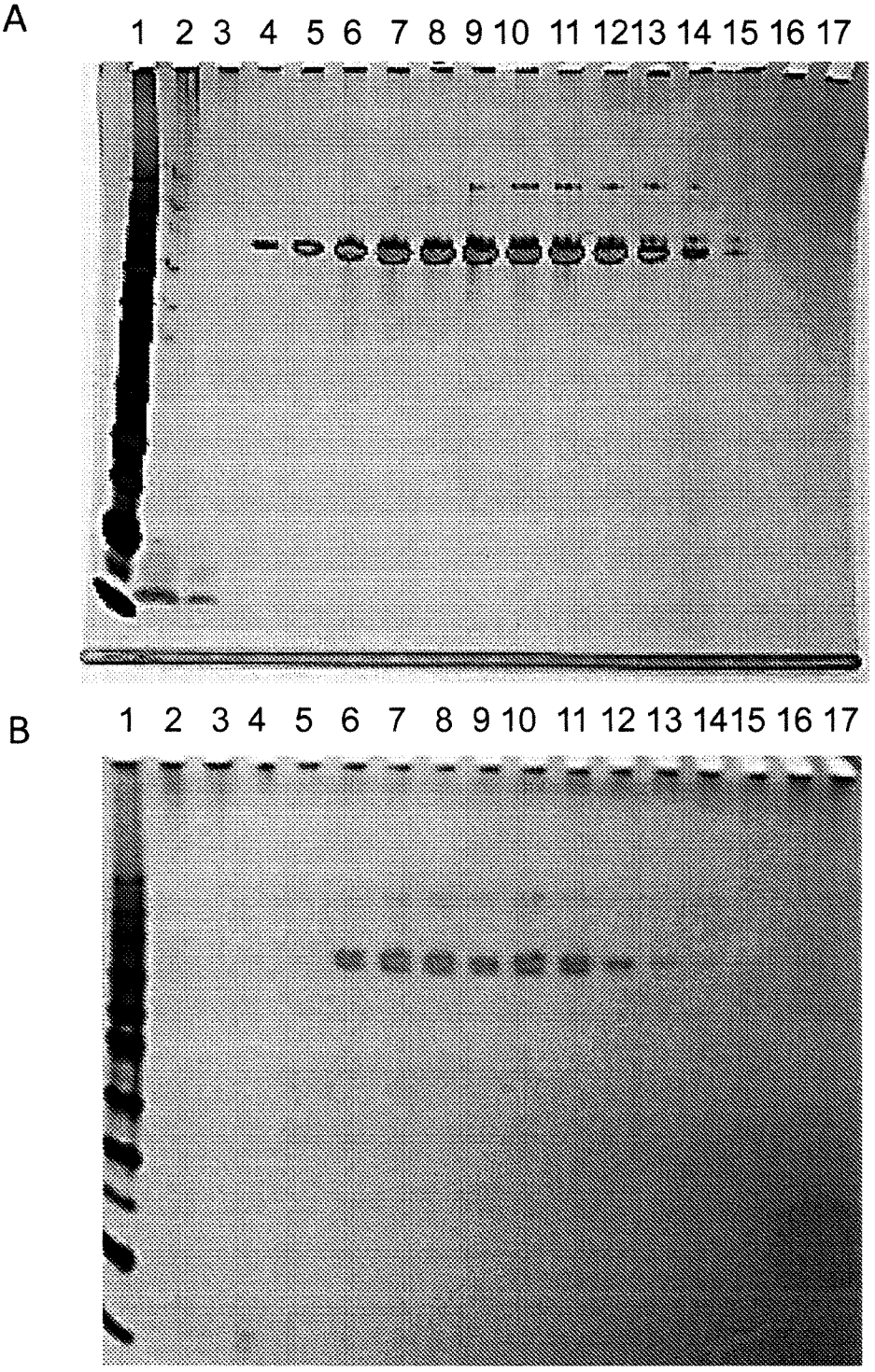

A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
B
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

FIG. 87

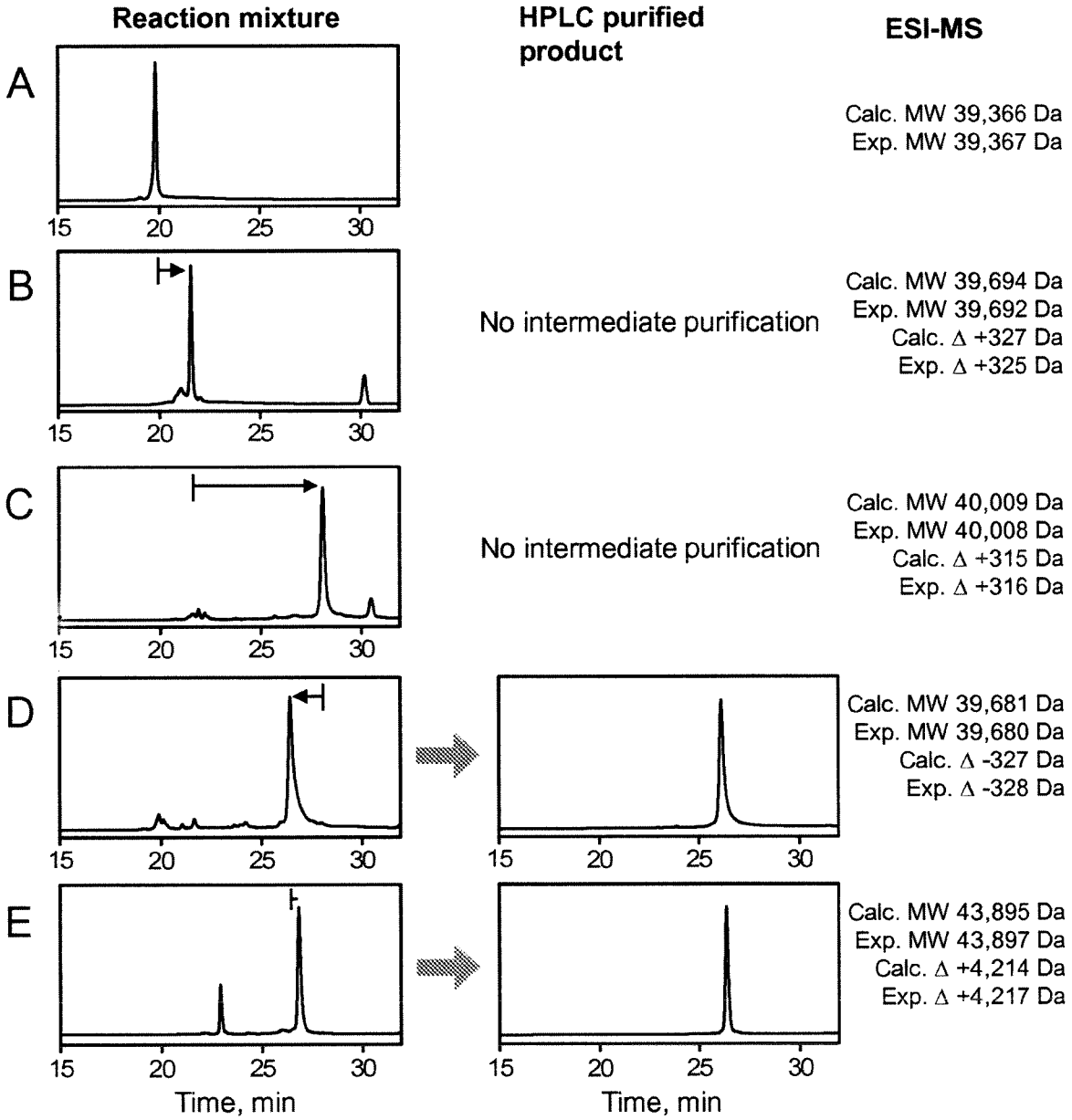

Reaction mixture
HPLC purified product
ESI-MS
A
Calc. MW 39,366 Da
Exp. MW 39,367 Da
B
No intermediate purification
Calc. MW 39,694 Da
Exp. MW 39,692 Da
Calc. Δ +327 Da
Exp. Δ +325 Da
C
No intermediate purification
Calc. MW 40,009 Da
Exp. MW 40,008 Da
Calc. Δ +315 Da
Exp. Δ +316 Da
D
Calc. MW 39,681 Da
Exp. MW 39,680 Da
Calc. Δ -327 Da
Exp. Δ -328 Da
E
Calc. MW 43,895 Da
Exp. MW 43,897 Da
Calc. Δ +4,214 Da
Exp. Δ +4,217 Da
15
20
25
30
Time, min

FIG. 88

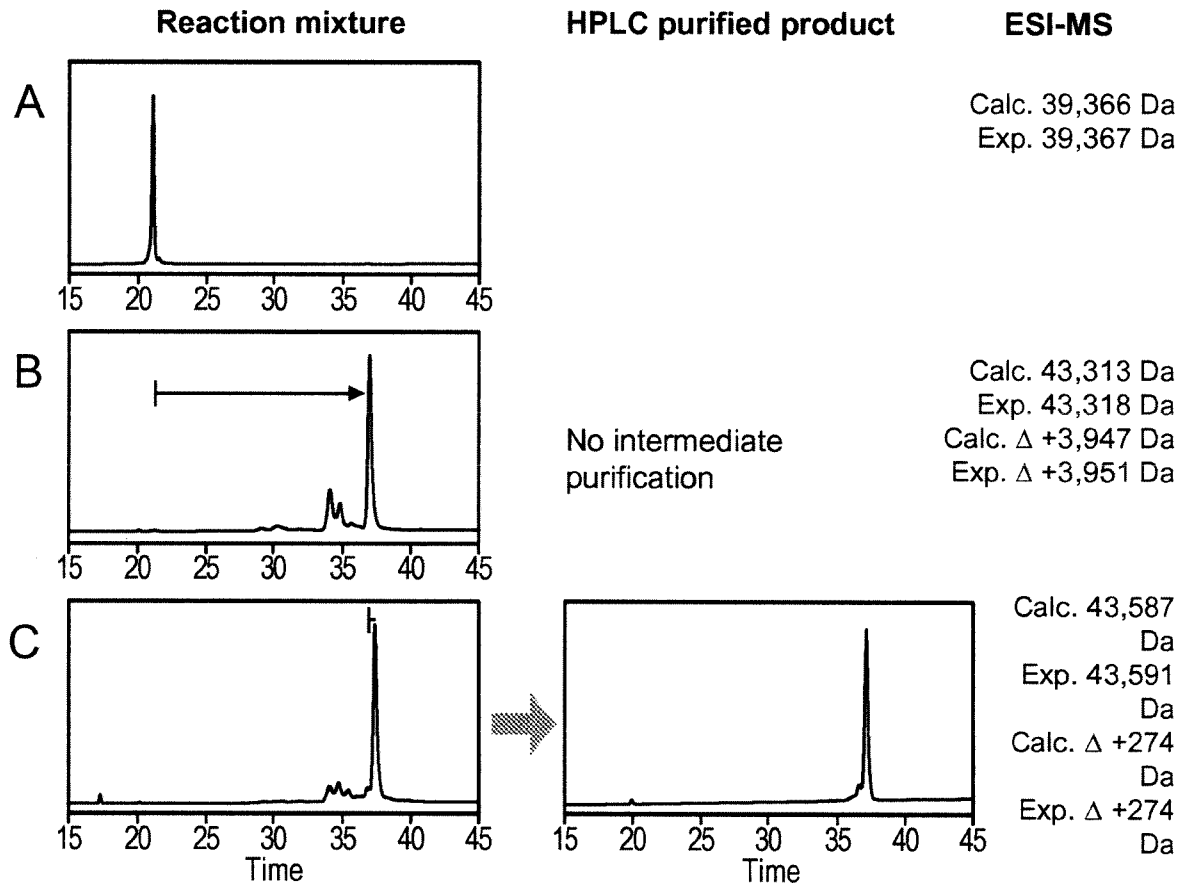

Reaction mixture
HPLC purified product
ESI-MS
A
Calc. 39,366 Da
Exp. 39,367 Da
B
Calc. 43,313 Da
Exp. 43,318 Da
Calc. Δ +3,947 Da
Exp. Δ +3,951 Da
No intermediate purification
C
Calc. 43,587 Da
Exp. 43,591 Da
Calc. Δ +274 Da
Exp. Δ +274 Da
15 20 25 30 35 40 45
Time

FIG. 89

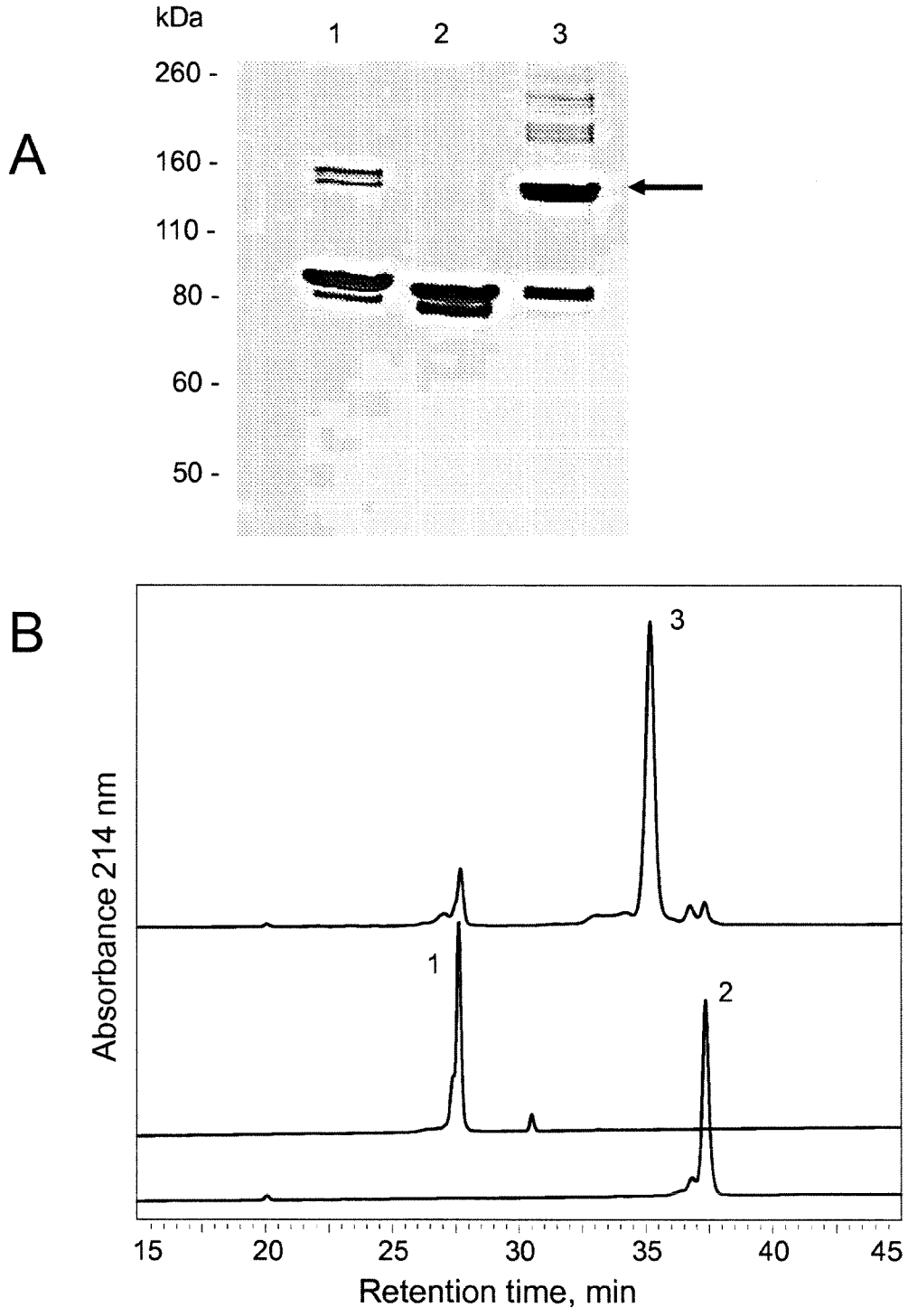

A
kDa
1
2
3
260 -
160 -
110 -
80 -
60 -
50 -
B
Absorbance 214 nm
3
1
2
15
20
25
30
35
40
45
Retention time, min

FIG. 90

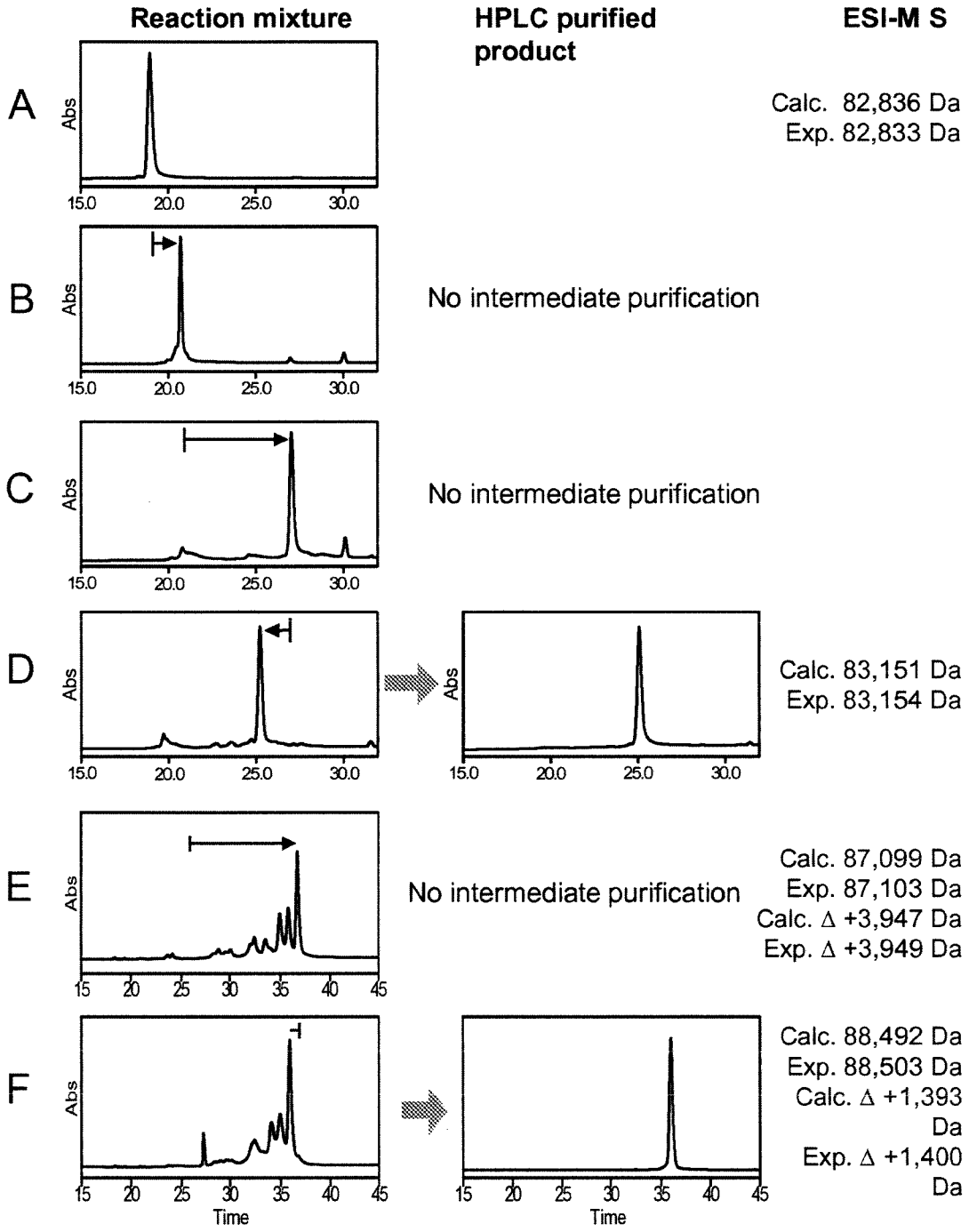

Reaction mixture
HPLC purified product
ESI-M S
A
Abs
15.0
20.0
25.0
30.0
Calc. 82,836 Da
Exp. 82,833 Da
B
No intermediate purification
C
No intermediate purification
D
Calc. 83,151 Da
Exp. 83,154 Da
E
No intermediate purification
Calc. 87,099 Da
Exp. 87,103 Da
Calc. Δ +3,947 Da
Exp. Δ +3,949 Da
F
15
20
25
30
35
40
45
Time
Calc. 88,492 Da
Exp. 88,503 Da
Calc. Δ +1,393 Da
Exp. Δ +1,400 Da

FIG. 91

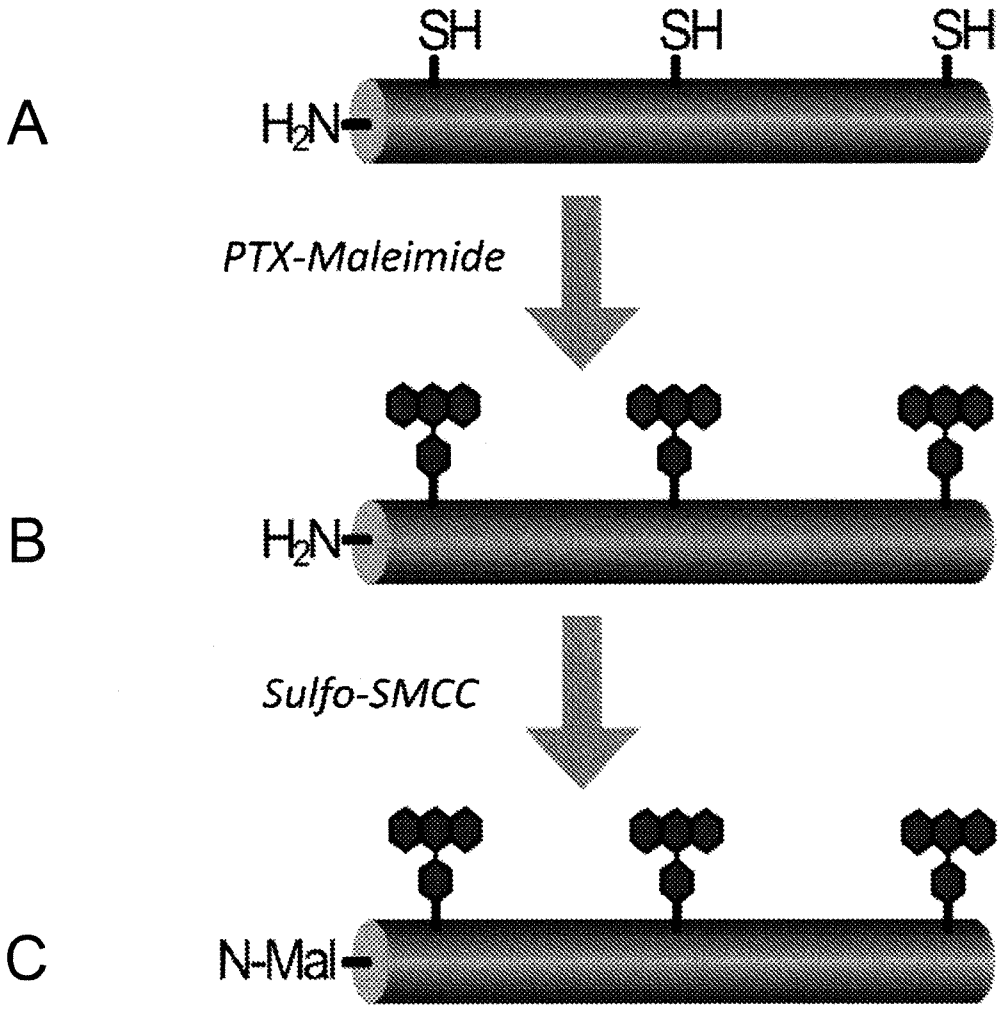


FIG. 92

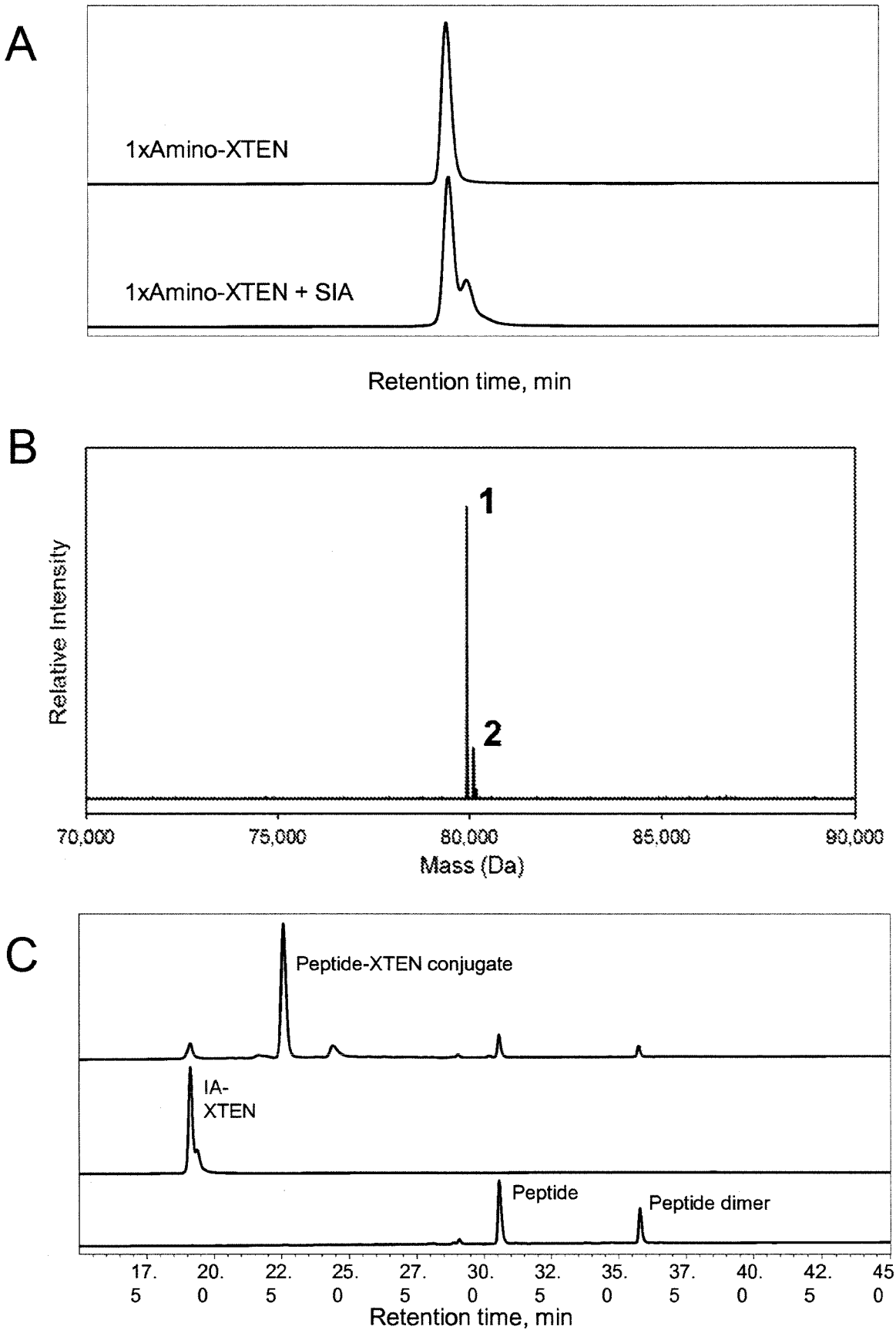

A
1xAmino-XTEN
1xAmino-XTEN + SIA
Retention time, min
B
Relative Intensity
1
2
70,000
75,000
80,000
85,000
90,000
Mass (Da)
C
Peptide-XTEN conjugate
IA-
XTEN
Peptide
Peptide dimer
17.5
20.0
22.5
25.0
27.5
30.0
32.5
35.0
37.5
40.0
42.5
45.0
Retention time, min

FIG. 93

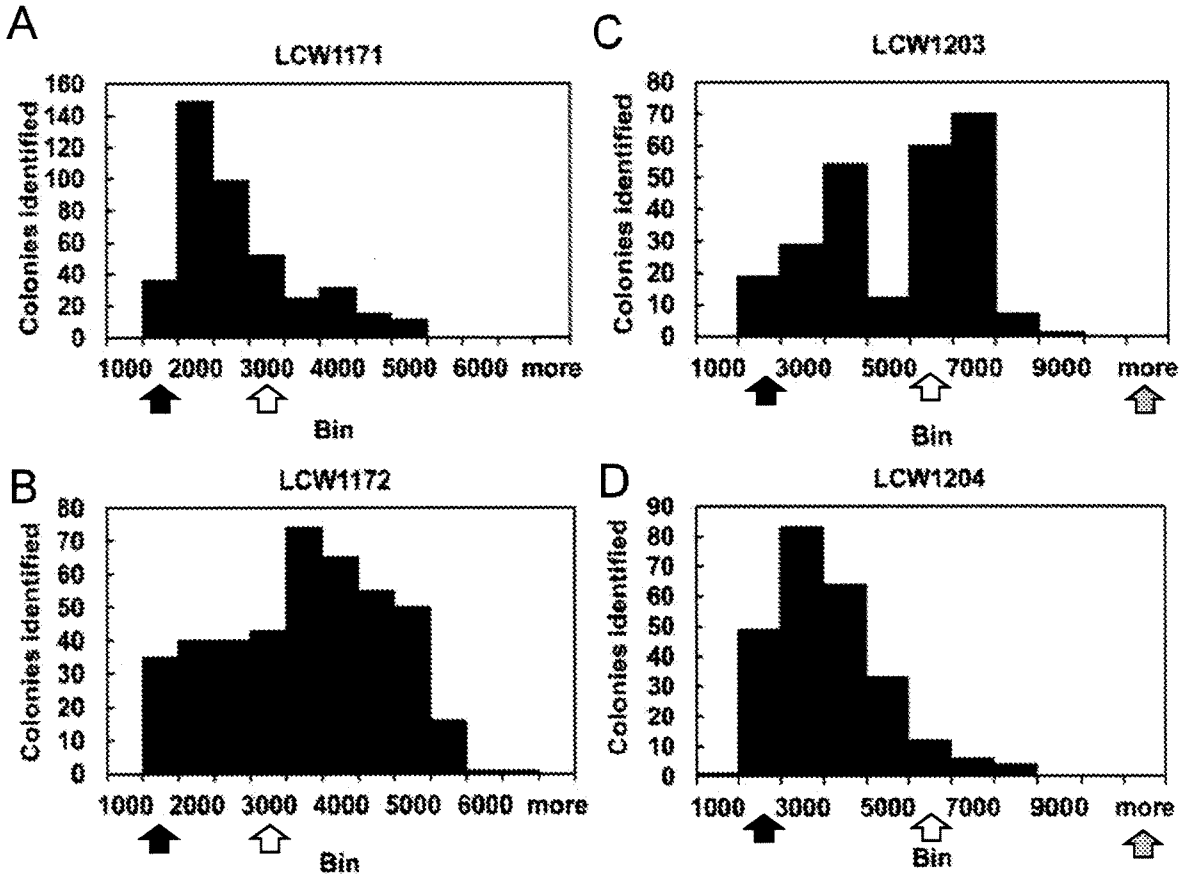

A
LCW1171
Colonies identified
160
140
120
100
80
60
40
20
0
1000 2000 3000 4000 5000 6000 more
Bin
C
LCW1203
Colonies identified
80
70
60
50
40
30
20
10
0
1000 3000 5000 7000 9000 more
Bin
B
LCW1172
Colonies identified
80
70
60
50
40
30
20
10
0
1000 2000 3000 4000 5000 6000 more
Bin
D
LCW1204
Colonies identified
90
80
70
60
50
40
30
20
10
0
1000 3000 5000 7000 9000 more
Bin

FIG. 94

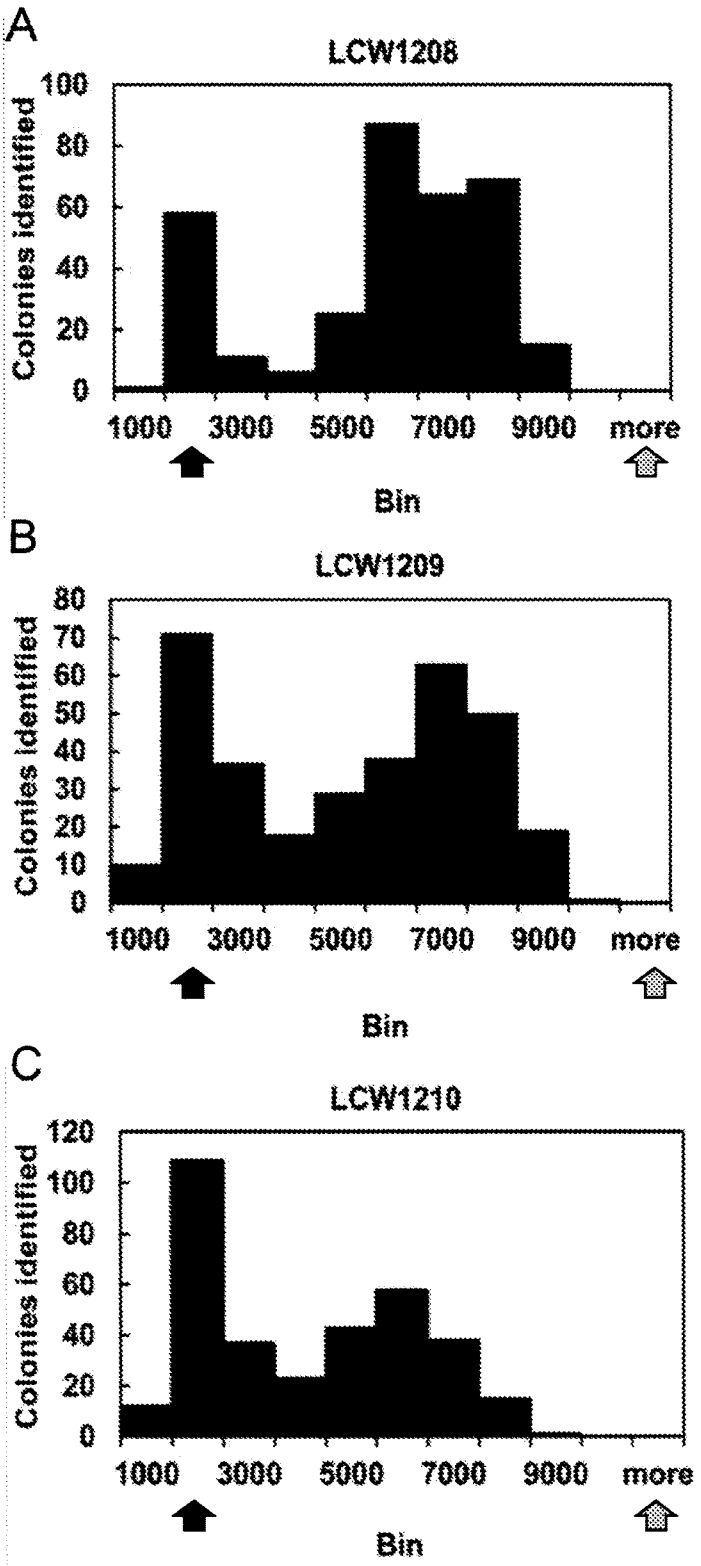

A
LCW1208
Colonies identified
100
80
60
40
20
0
1000
3000
5000
7000
9000
more
Bin
B
LCW1209
Colonies identified
80
70
60
50
40
30
20
10
0
1000
3000
5000
7000
9000
more
Bin
C
LCW1210
Colonies identified
120
100
80
60
40
20
0
1000
3000
5000
7000
9000
more
Bin

FIG. 95

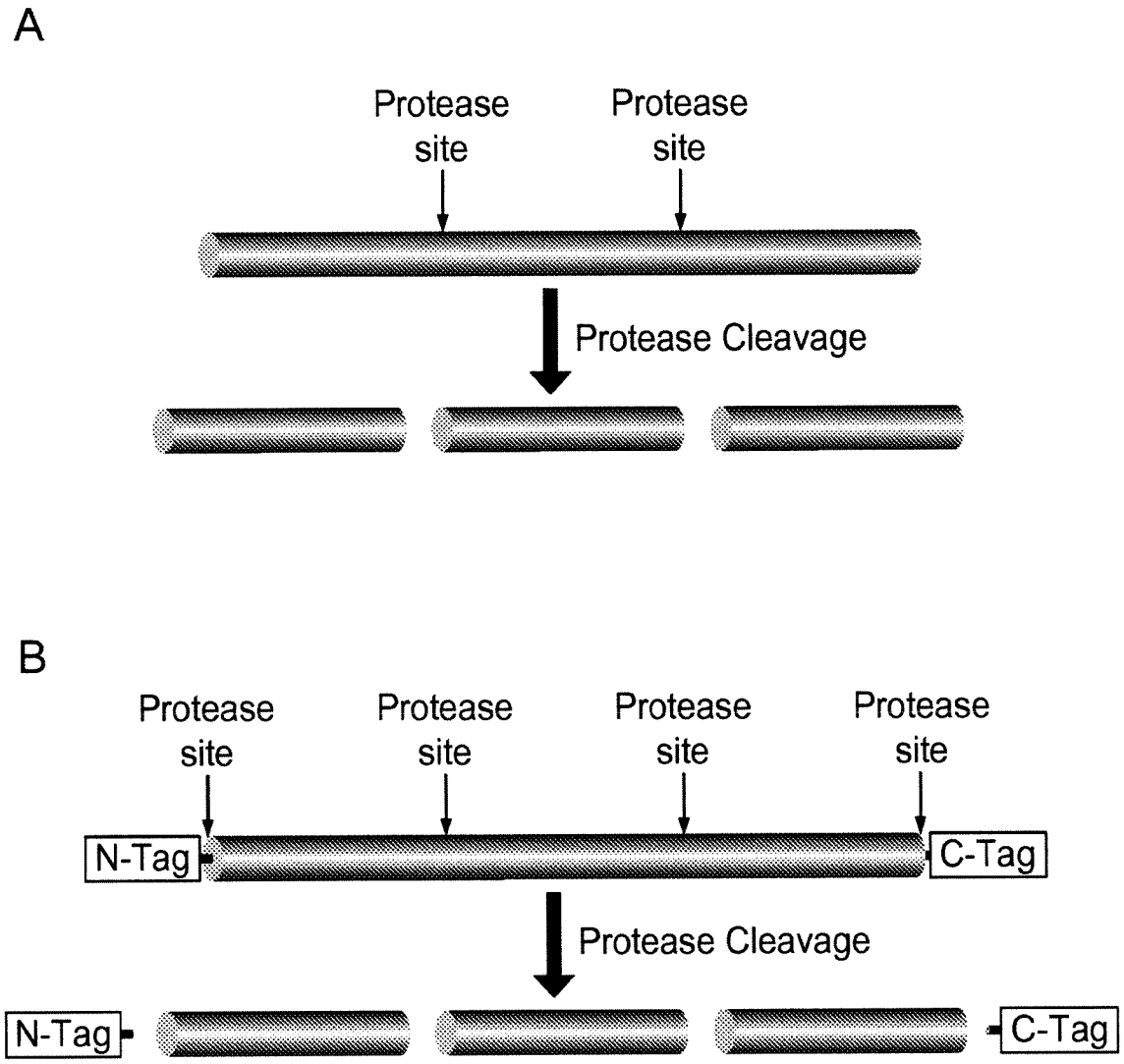

A
Protease site
Protease site
Protease Cleavage
B
Protease site
Protease site
Protease site
Protease site
N-Tag
C-Tag
Protease Cleavage
N-Tag
C-Tag

FIG. 96

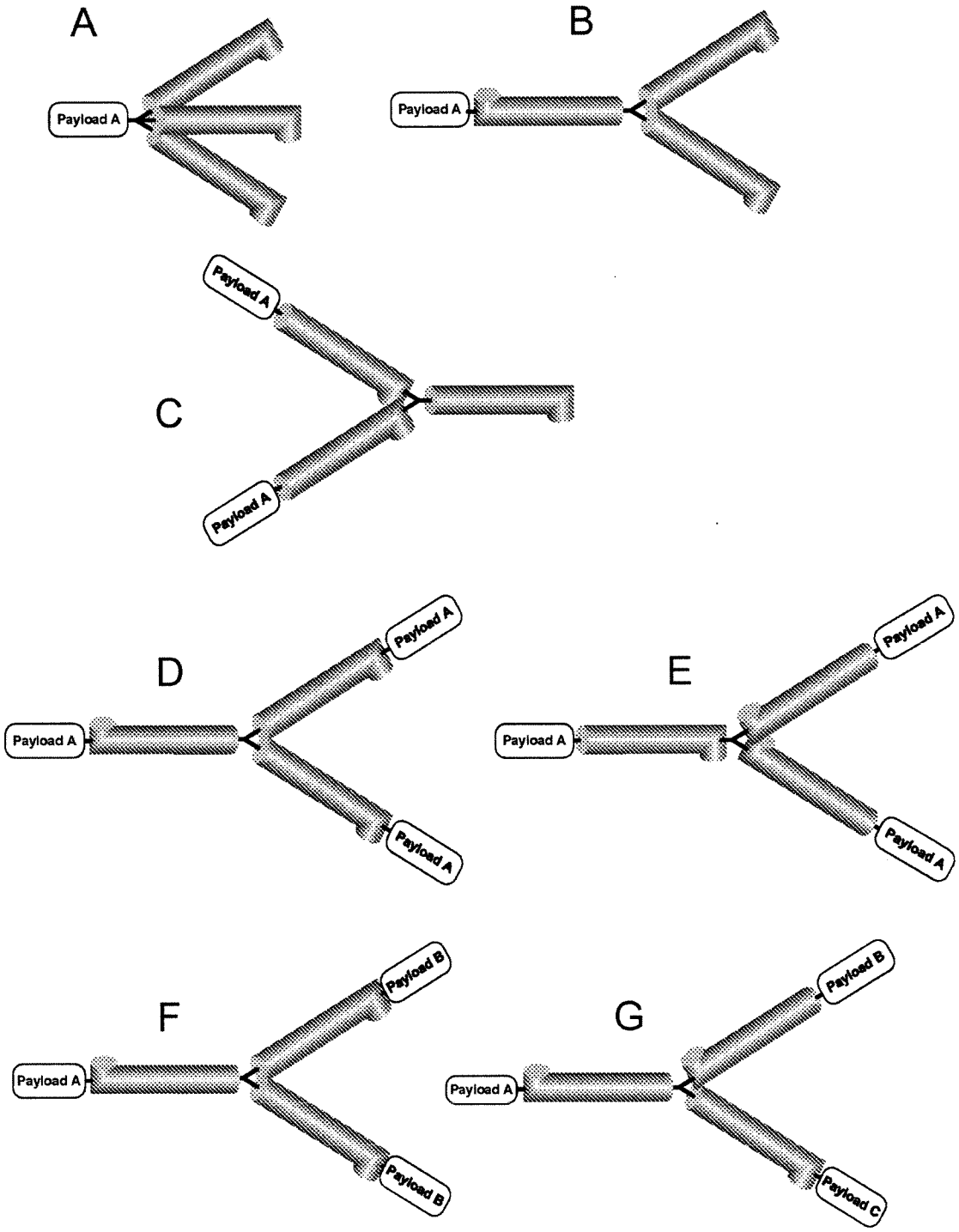

A
Payload A
B
Payload A
C
Payload A
Payload A
D
Payload A
Payload A
Payload A
E
Payload A
Payload A
Payload A
F
Payload A
Payload B
Payload B
G
Payload A
Payload B
Payload C

FIG. 97

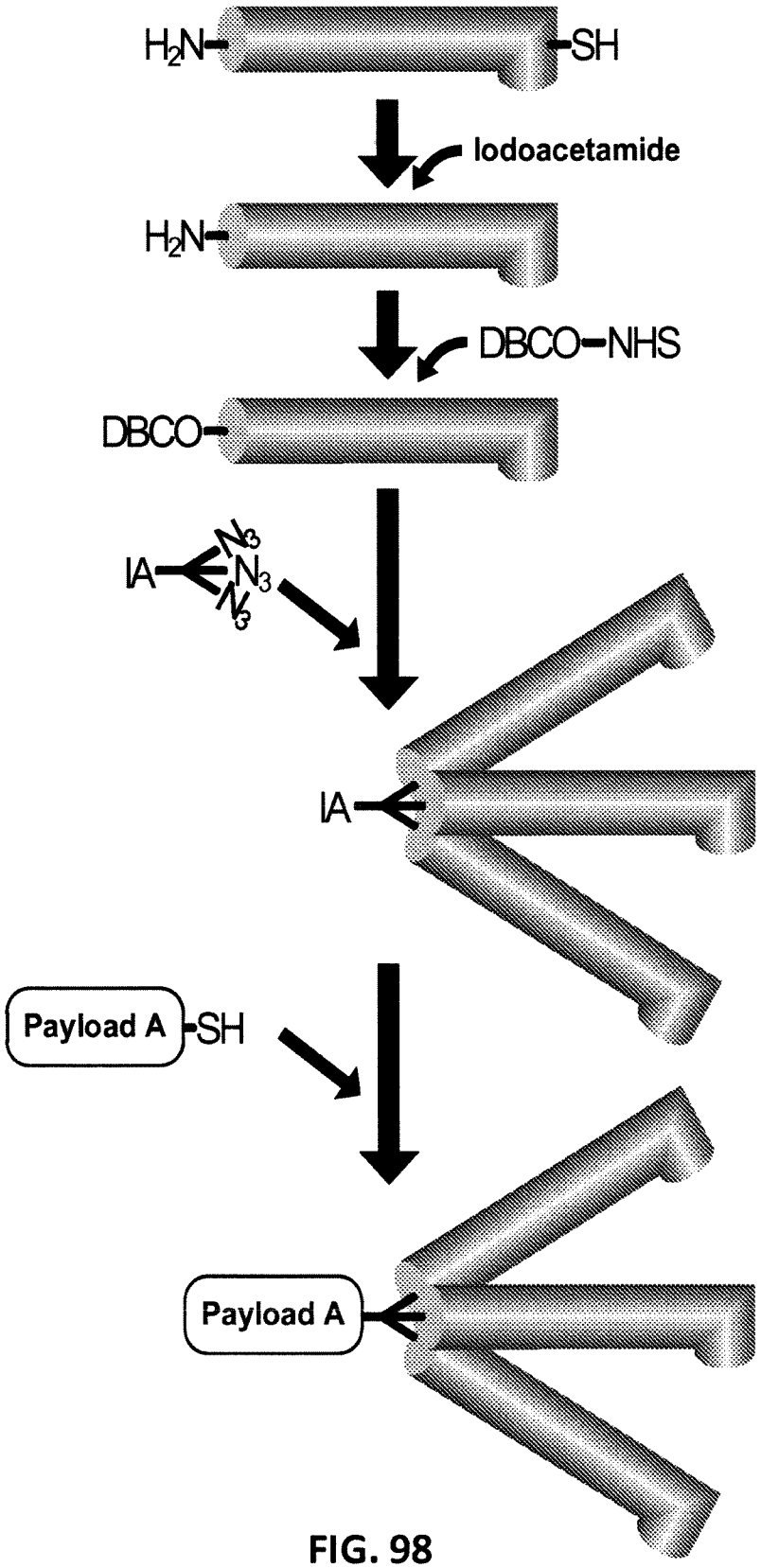

H2N
SH
Iodoacetamide
H2N
DBCO-NHS
DBCO
IA
N3
N3
N3
IA
Payload A
SH
Payload A

FIG. 98

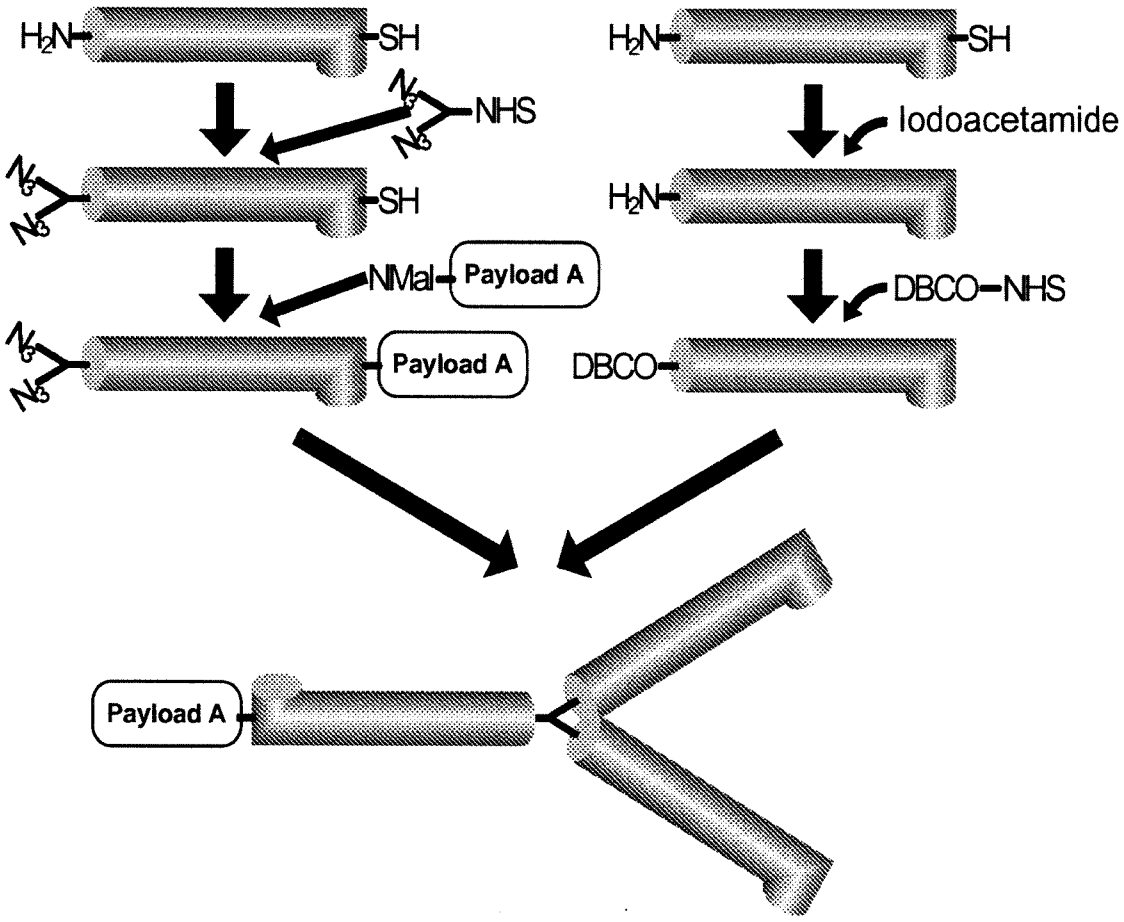

H2N
SH
NHS
SH
NMal
Payload A
Payload A
H2N
SH
Iodoacetamide
H2N
DBCO-NHS
DBCO
Payload A

FIG. 99

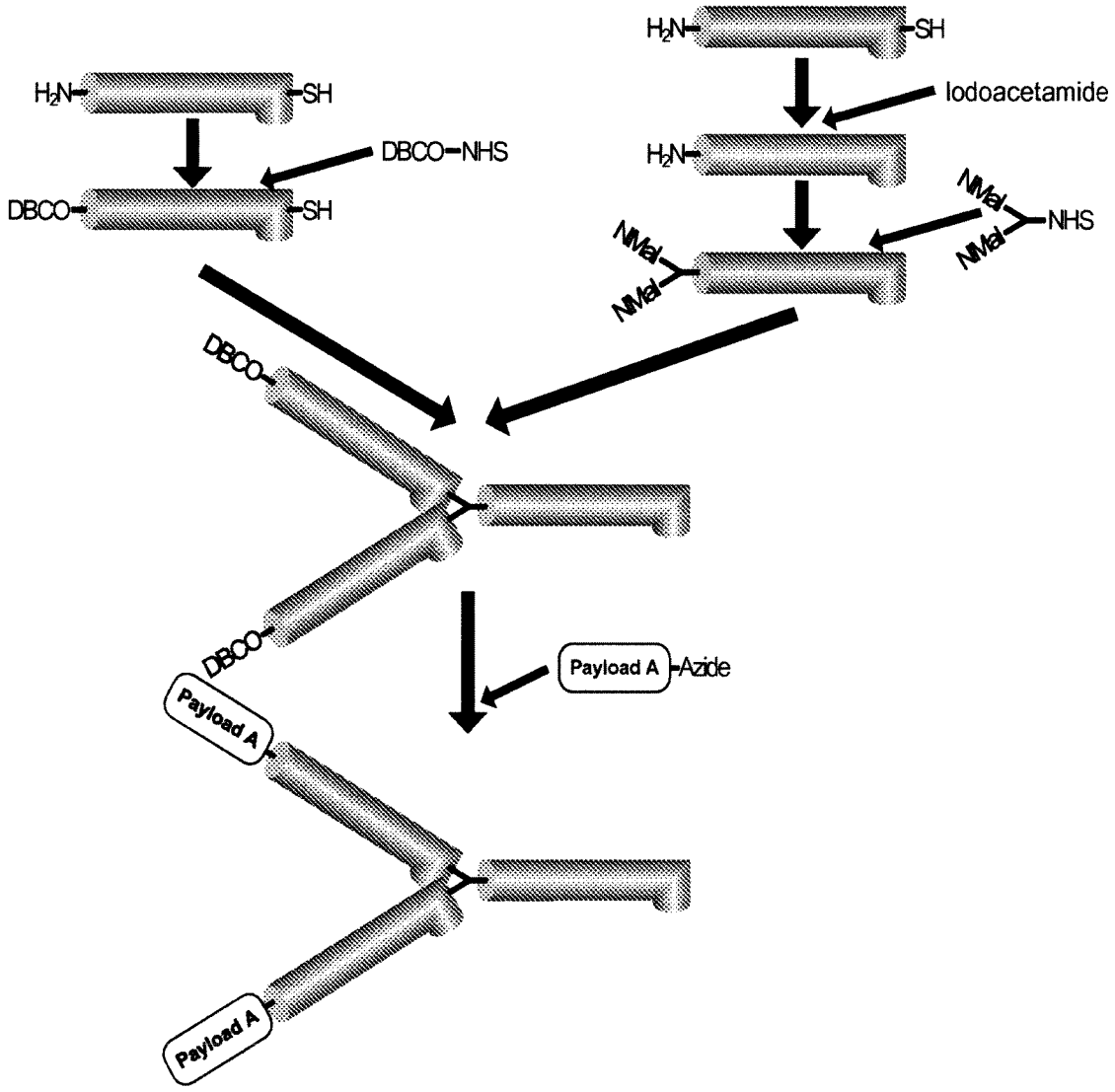

H2N
SH
DBCO-NHS
DBCO
SH
H2N
SH
Iodoacetamide
H2N
NMal
NMal
NMal
NMal
NHS
DBCO
DBCO
Payload A
Azide
Payload A
Payload A

FIG. 100

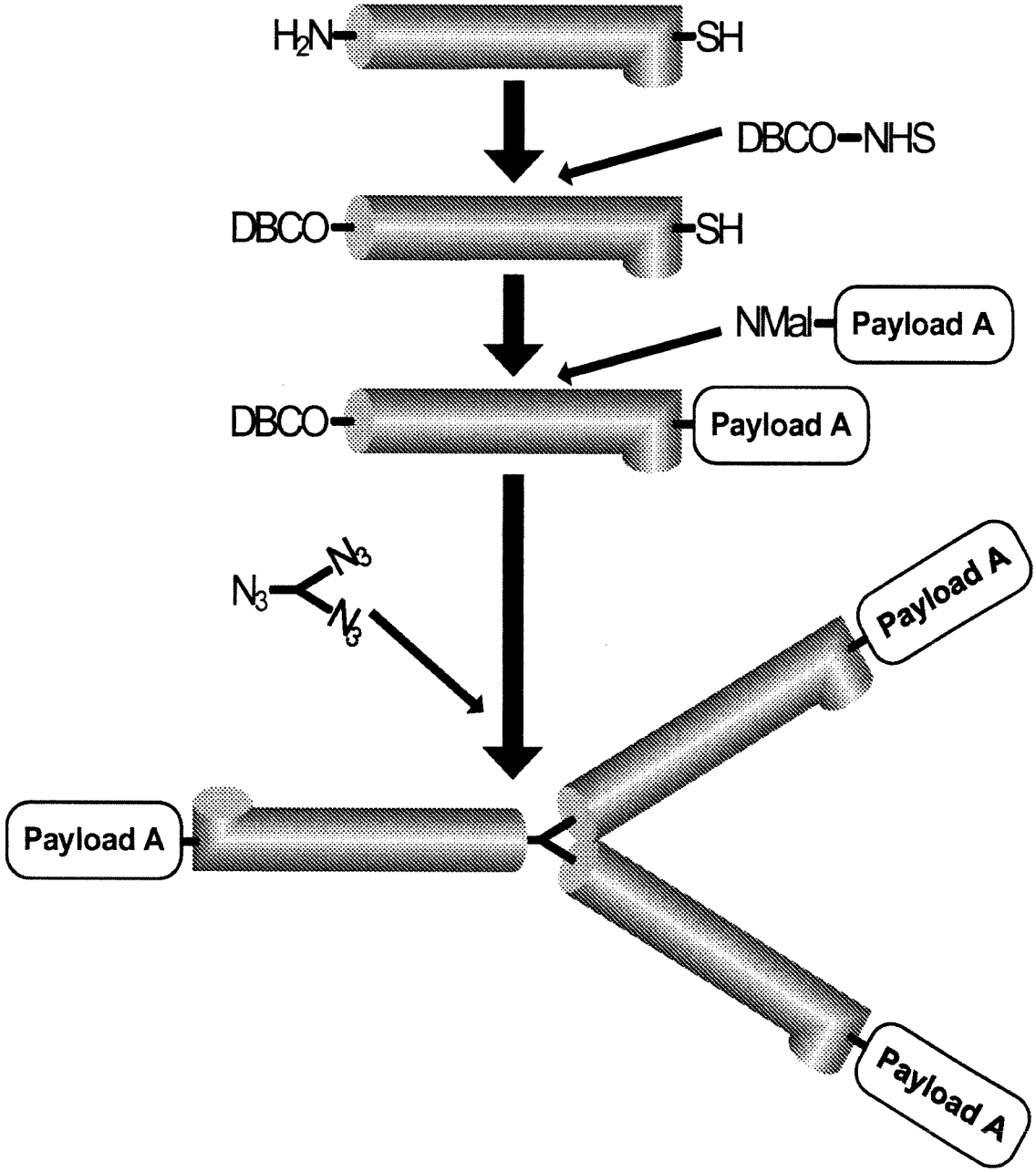

H2N
SH
DBCO-NHS
DBCO
SH
NMal
Payload A
DBCO
Payload A
N3
N3
N3
Payload A
Payload A
Payload A

FIG. 101

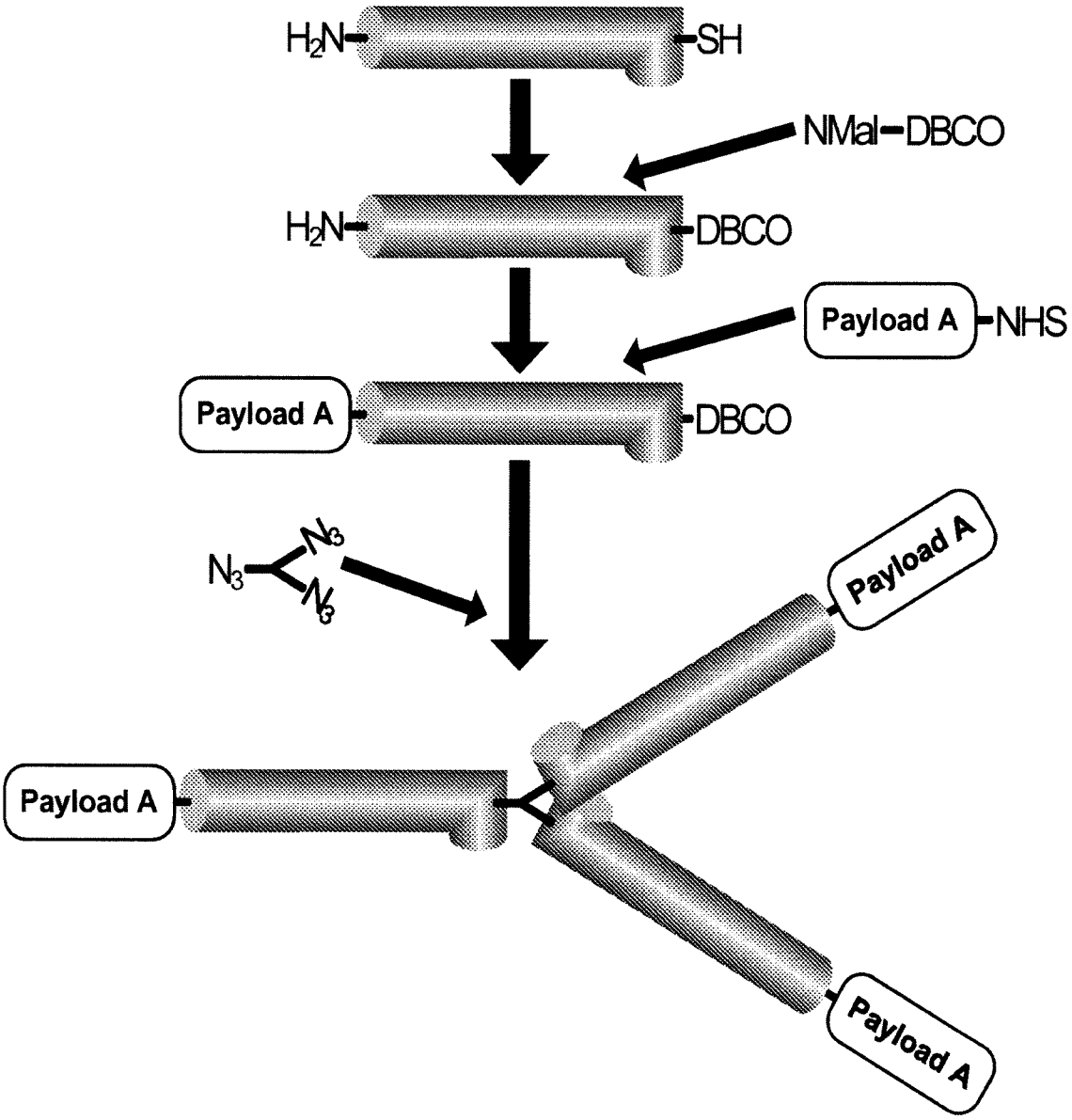

H2N
SH
NMal-DBCO
H2N
DBCO
Payload A
NHS
Payload A
DBCO
N3
N3
N3
Payload A
Payload A
Payload A

FIG. 102

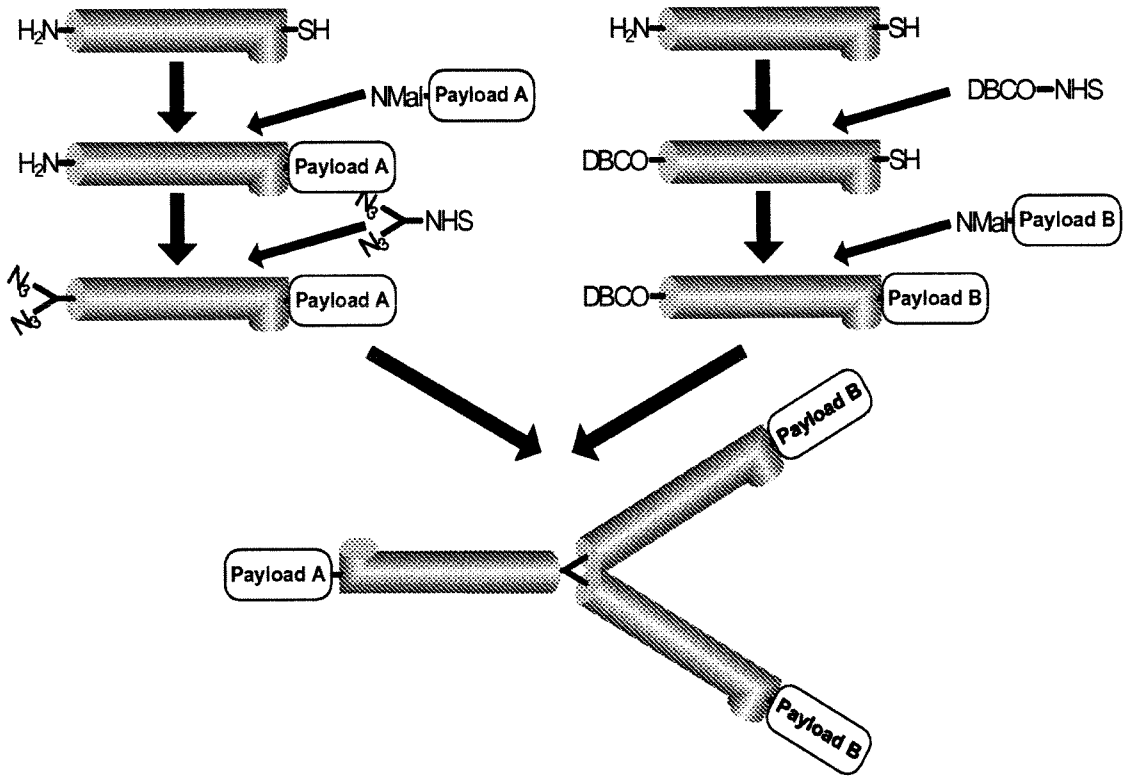

H2N
SH
NMal
Payload A
H2N
Payload A
NHS
Payload A
H2N
SH
DBCO
NHS
DBCO
SH
NMal
Payload B
DBCO
Payload B
Payload B
Payload A
Payload B

FIG. 103

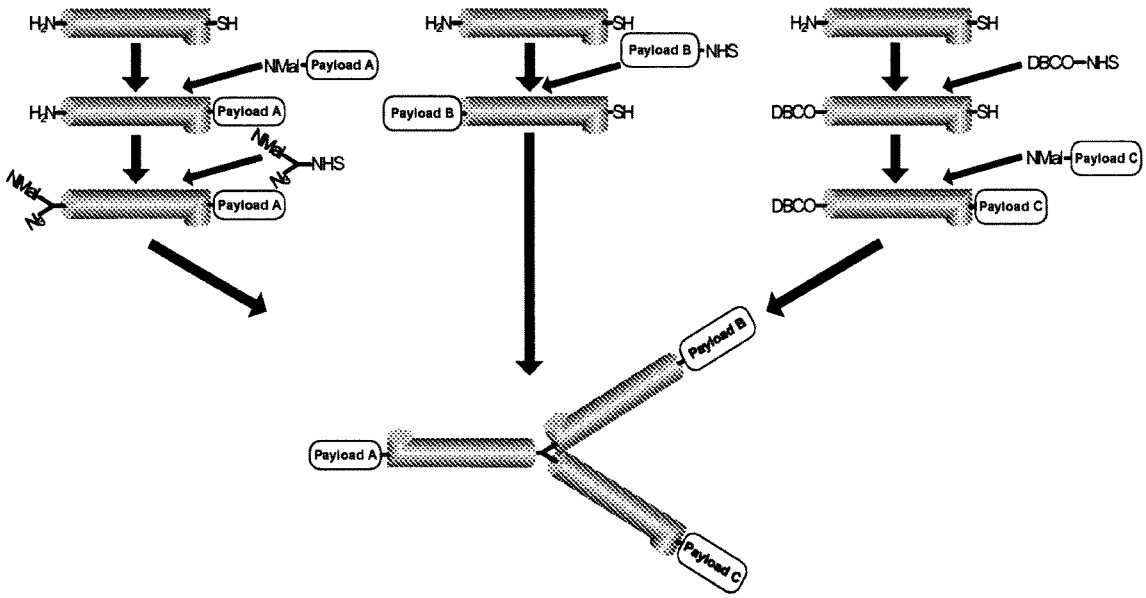


FIG. 104

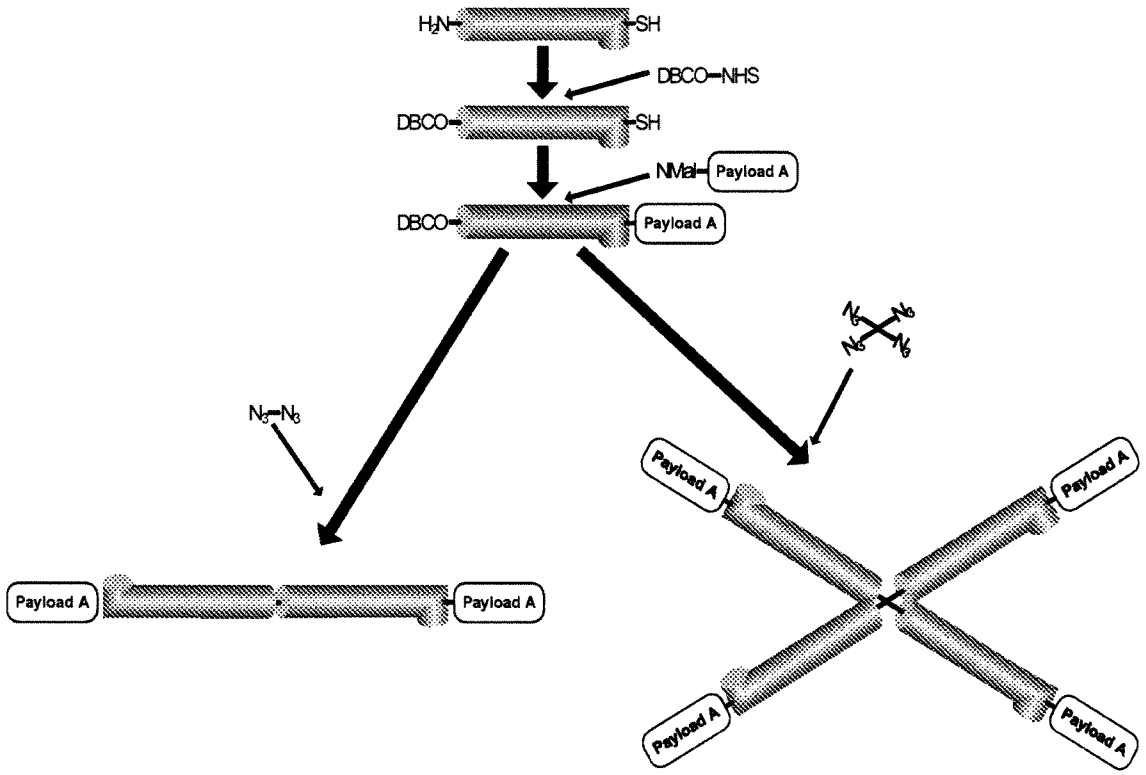

H2N
SH
DBCO
NHS
DBCO
SH
NMal
Payload A
DBCO
Payload A
N3
N3
N3
N3
N3
N3
Payload A
Payload A
Payload A
Payload A
Payload A
Payload A

FIG. 105

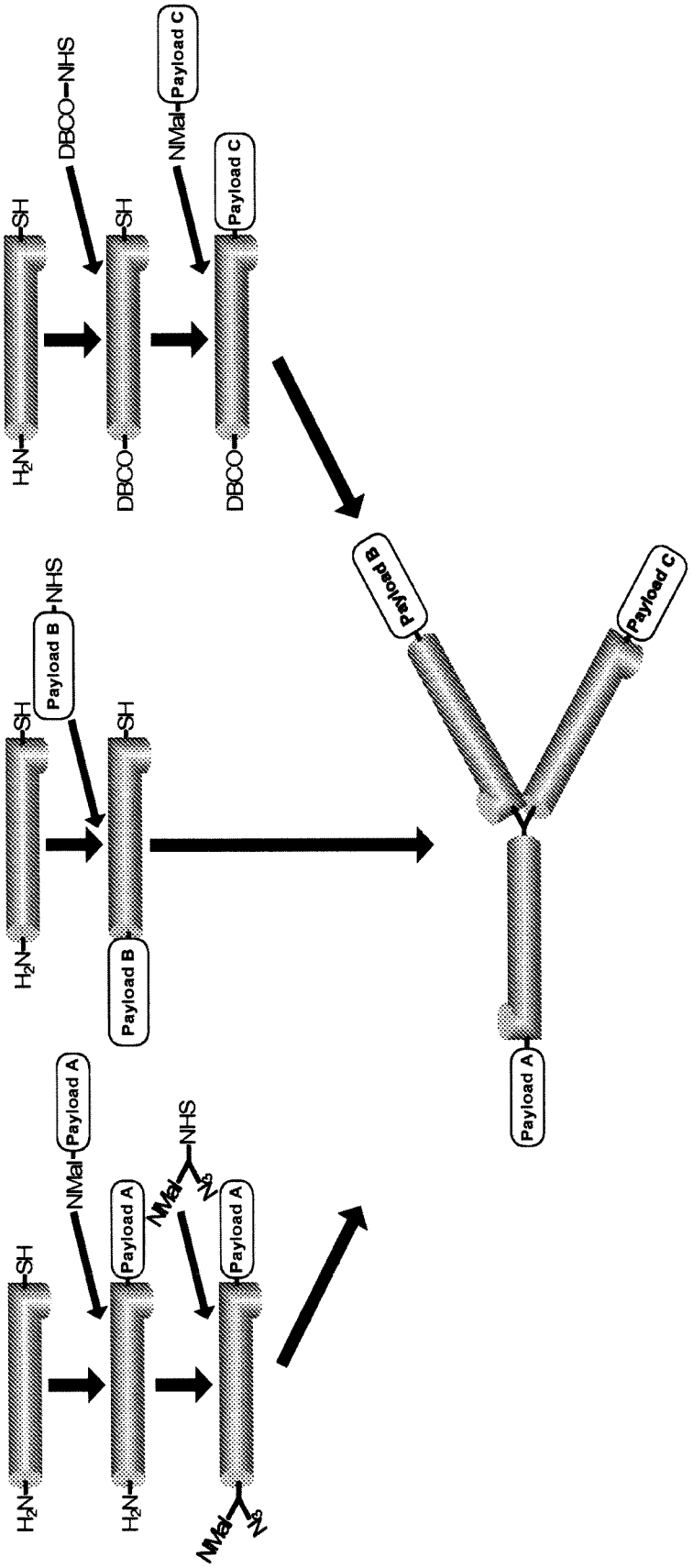

SH
DBCO–NHS
NMal–Payload C
Payload C
H2N
DBCO
DBCO
Payload B
NHS
Payload B
SH
SH
H2N
Payload B
Payload C
Payload A
NMal–Payload A
Mal–NHS
Payload A
Payload A
SH
H2N
H2N
Mal–N
Mal–N

FIG. 106

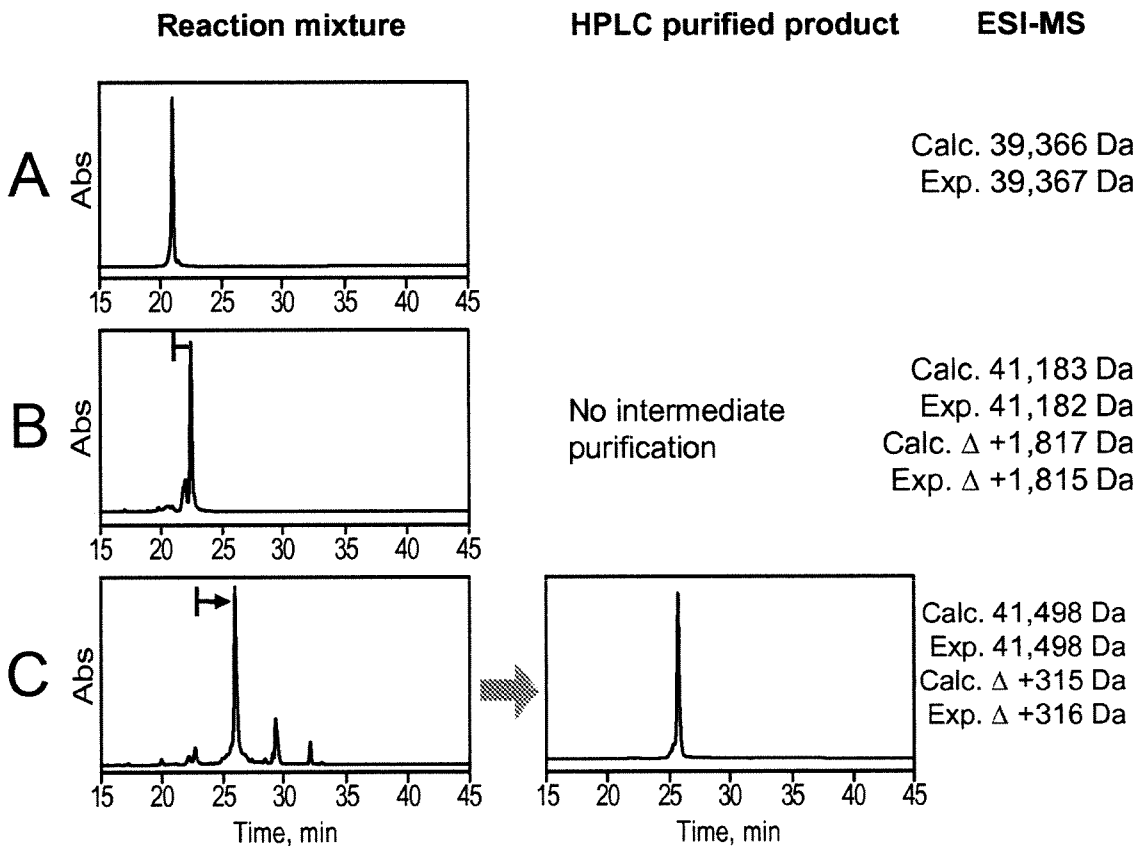

Reaction mixture
HPLC purified product
ESI-MS
A
Abs
15 20 25 30 35 40 45
Calc. 39,366 Da
Exp. 39,367 Da
B
Abs
15 20 25 30 35 40 45
No intermediate purification
Calc. 41,183 Da
Exp. 41,182 Da
Calc. Δ +1,817 Da
Exp. Δ +1,815 Da
C
Abs
15 20 25 30 35 40 45
Time, min
15 20 25 30 35 40 45
Time, min
Calc. 41,498 Da
Exp. 41,498 Da
Calc. Δ +315 Da
Exp. Δ +316 Da

FIG. 107

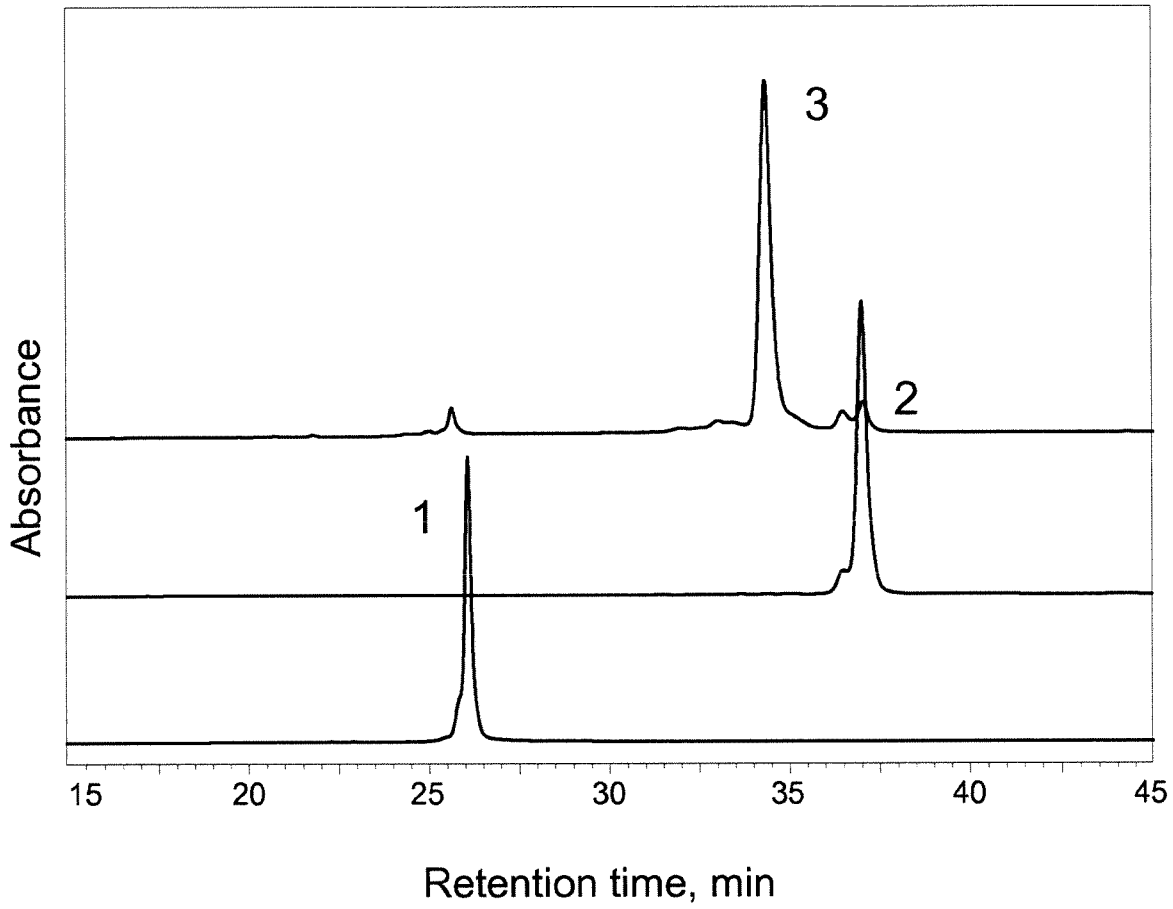


FIG. 108

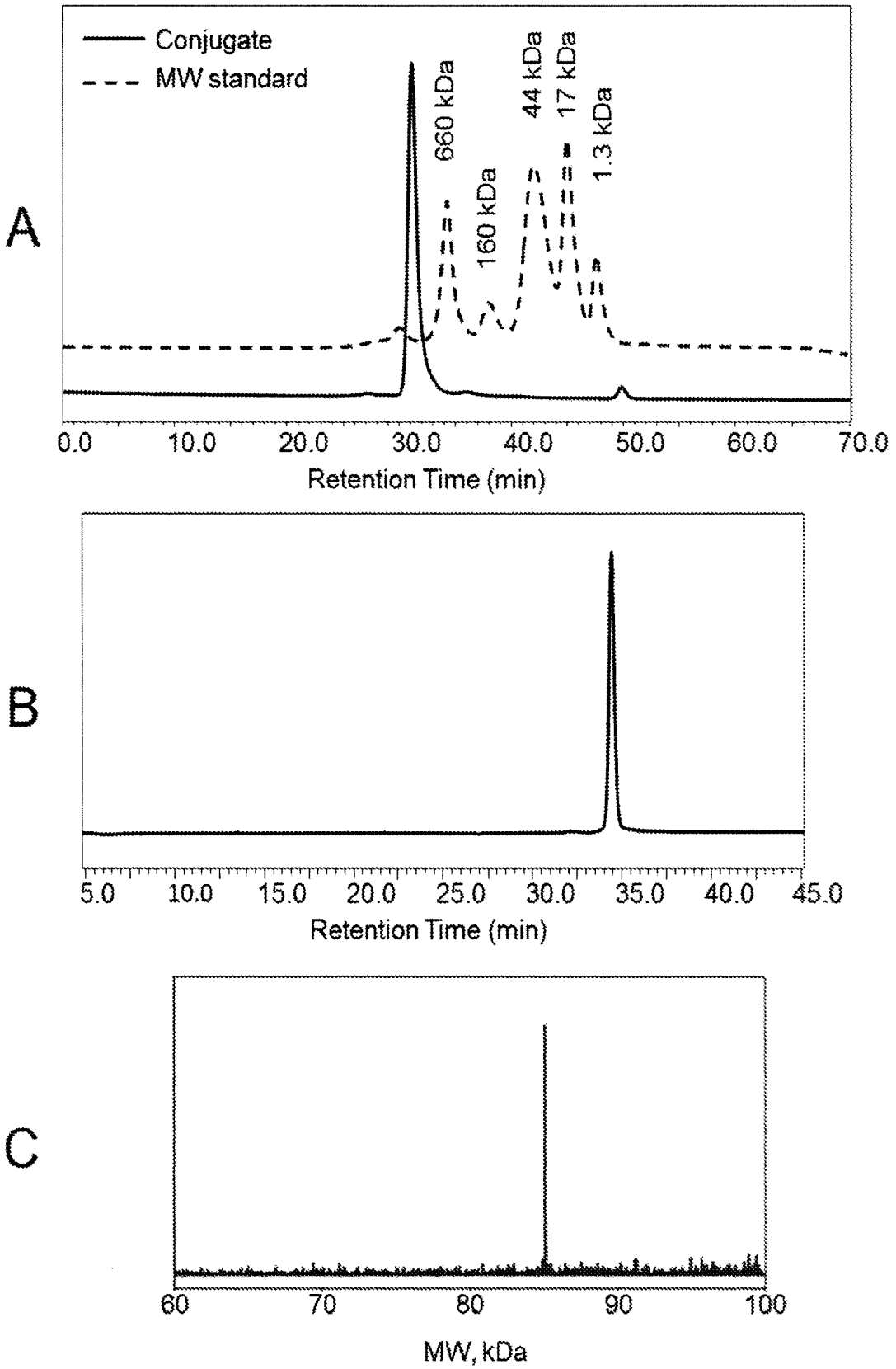

A
Conjugate
MW standard
660 kDa
160 kDa
44 kDa
17 kDa
1.3 kDa
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
Retention Time (min)
B
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
Retention Time (min)
C
60
70
80
90
100
MW, kDa

FIG. 109

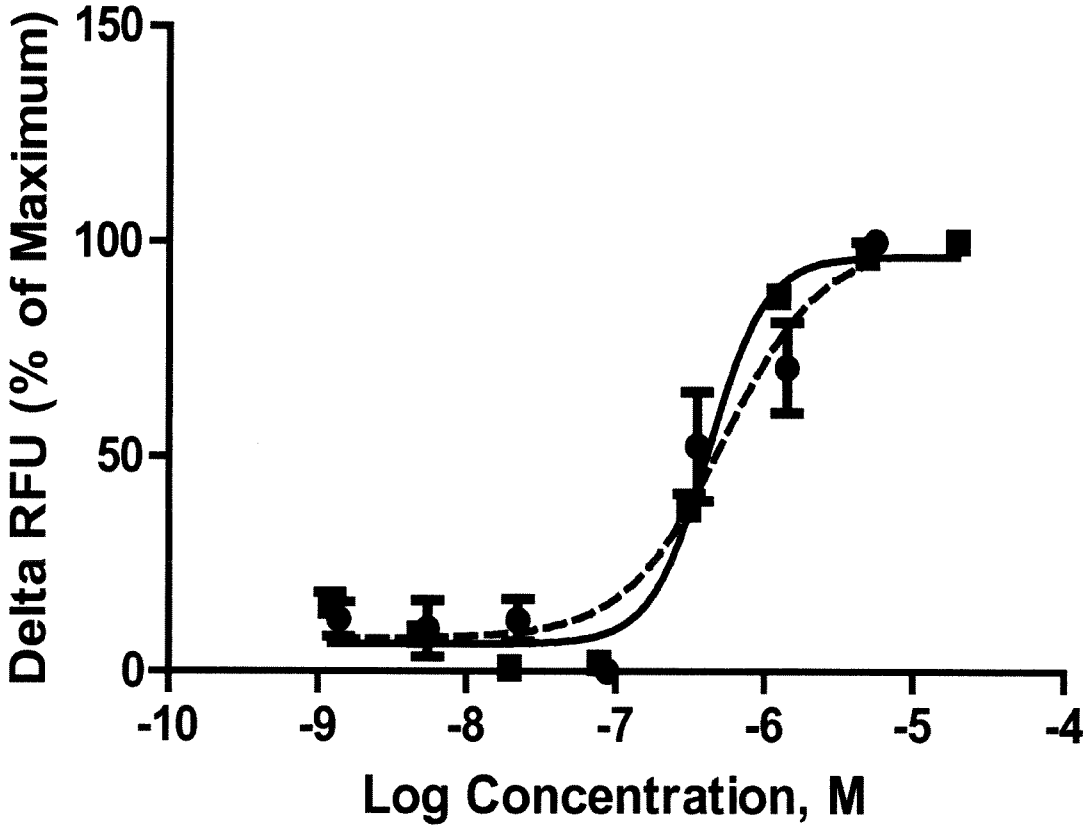

Delta RFU (% of Maximum)
150
100
50
0
-10
-9
-8
-7
-6
-5
-4
Log Concentration, M

FIG. 110

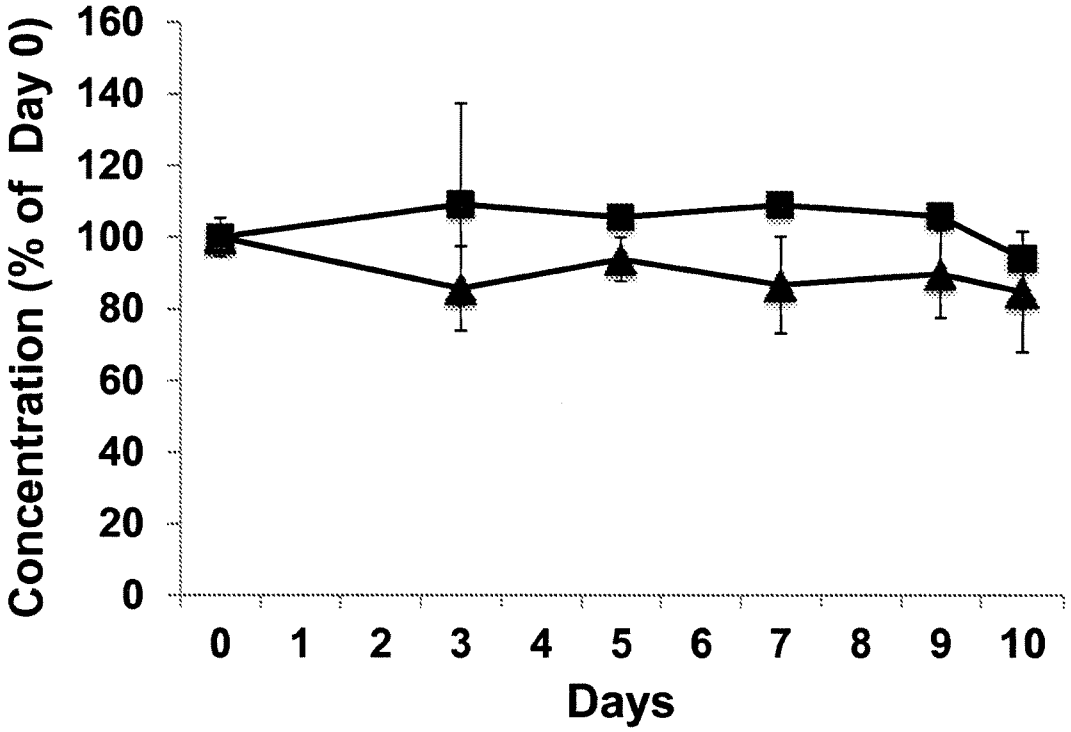

Concentration (% of Day 0)
160
140
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10
Days

FIG. 111

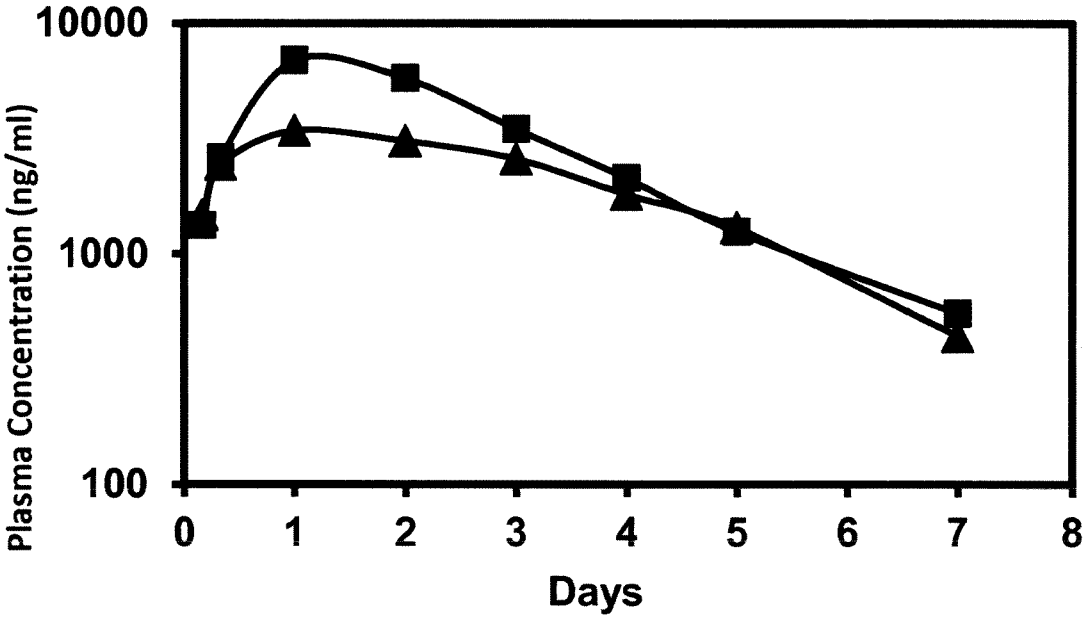


FIG. 112

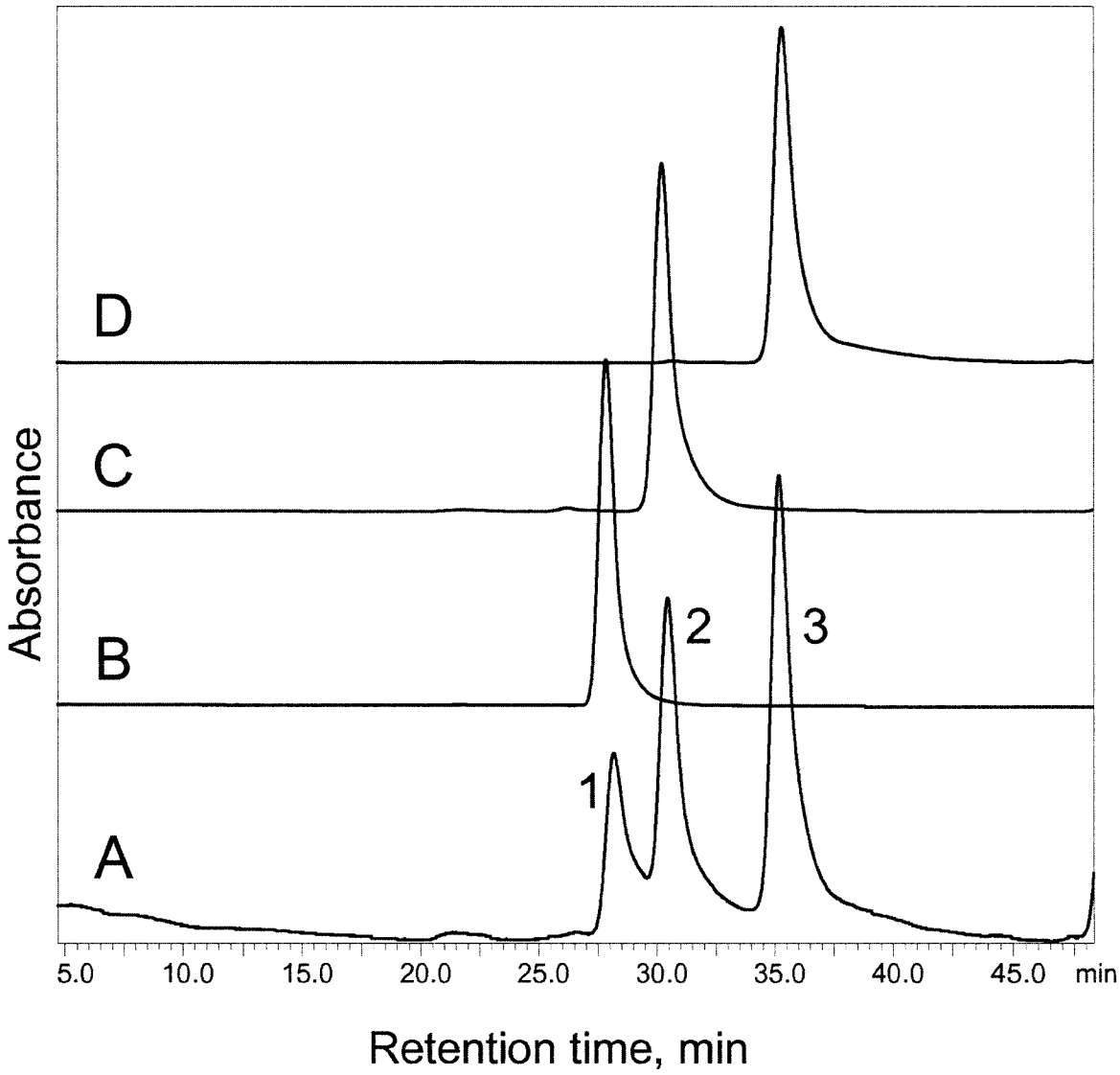
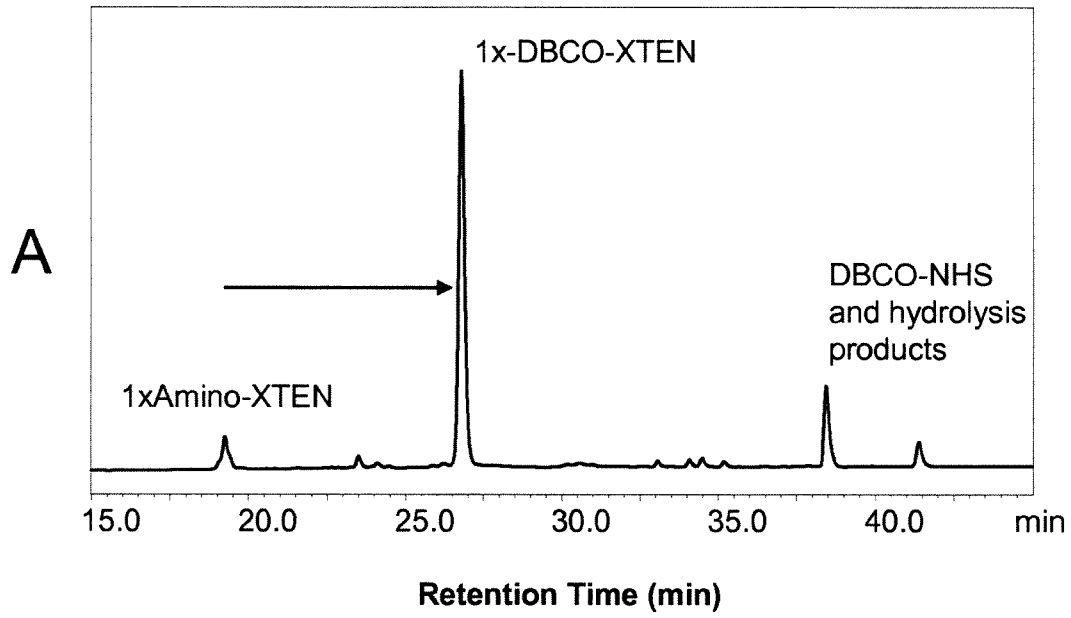

D
C
B
A
1
2
3
Absorbance
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
min
Retention time, min

FIG. 113


A
1x-DBCO-XTEN
DBCO-NHS
and hydrolysis
products
1xAmino-XTEN
15.0
20.0
25.0
30.0
35.0
40.0
min
Retention Time (min)

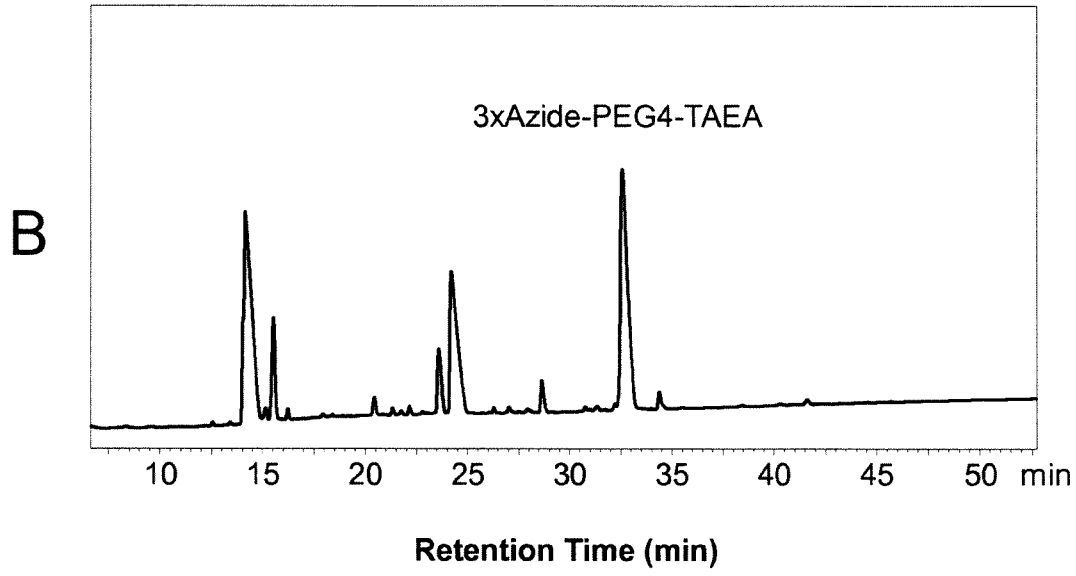

B
3xAzide-PEG4-TAEA
10
15
20
25
30
35
40
45
50
min
Retention Time (min)

FIG. 114

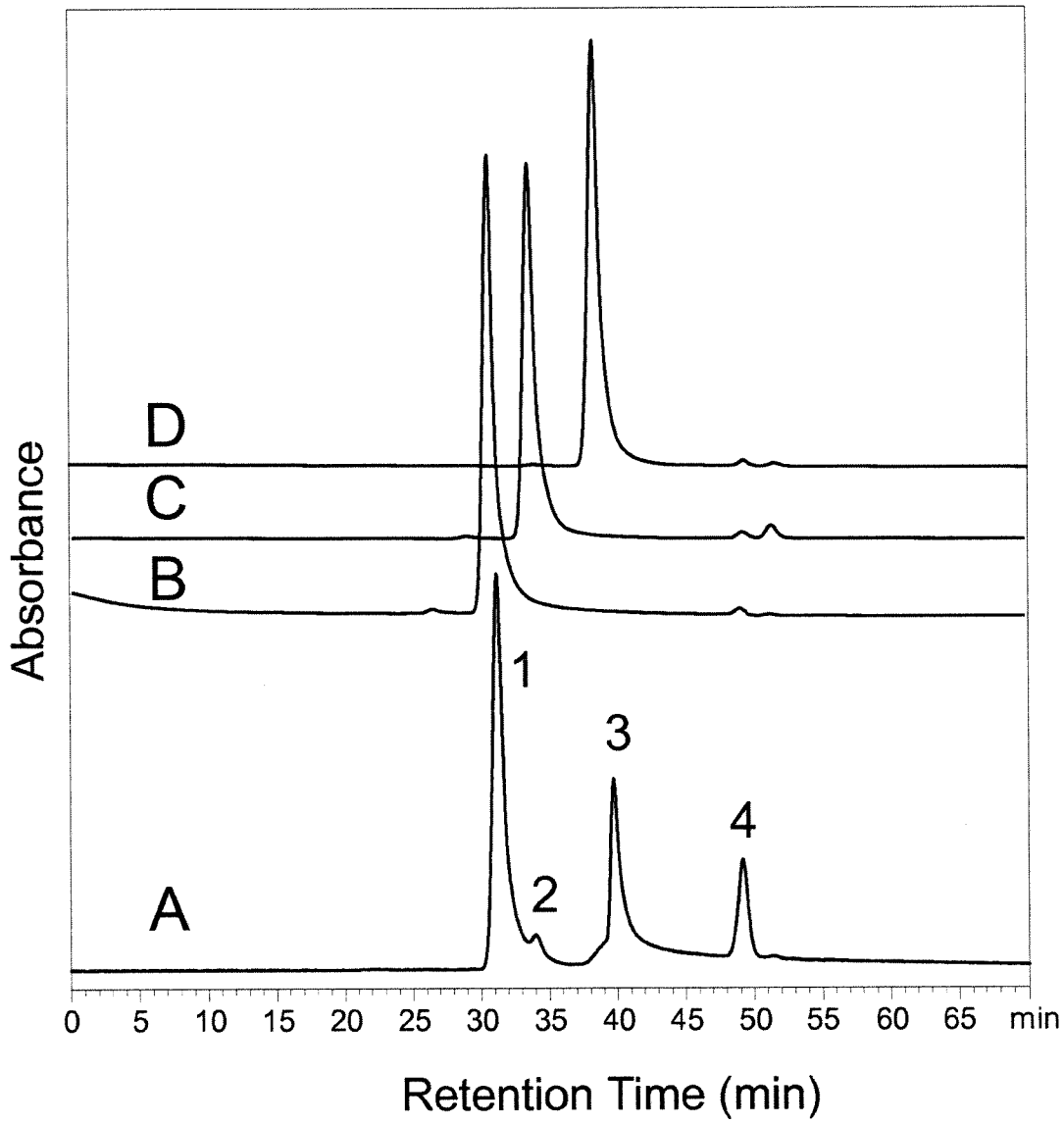

Absorbance
D
C
B
A
1
2
3
4
0
5
10
15
20
25
30
35
40
45
50
55
60
65
min
Retention Time (min)

FIG. 115

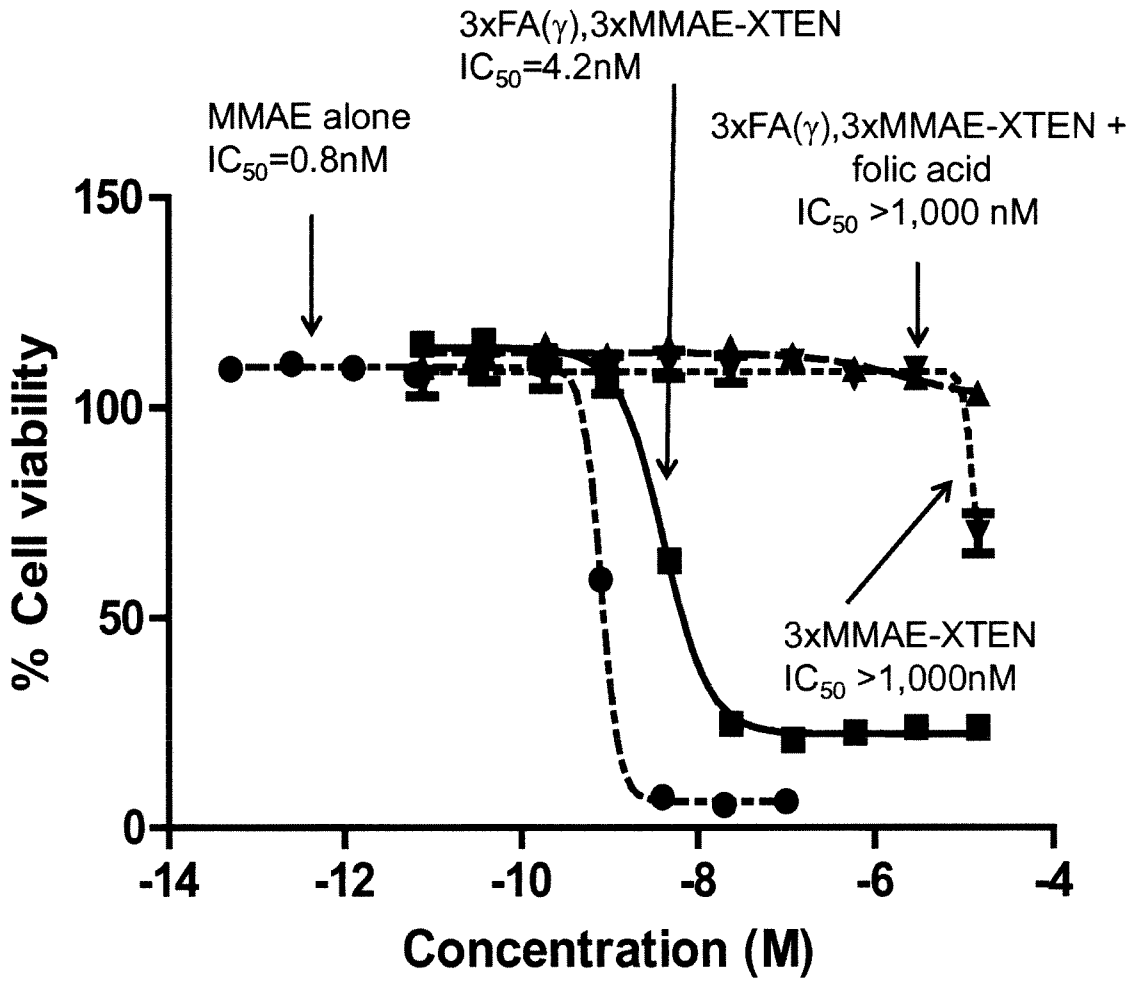
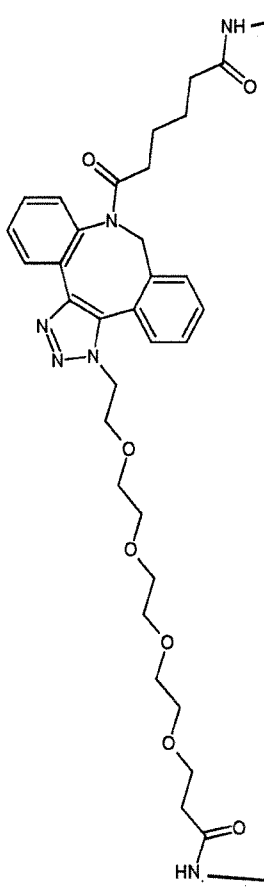

3xFA(γ),3xMMAE-XTEN
IC50=4.2nM
MMAE alone
IC50=0.8nM
3xFA(γ),3xMMAE-XTEN +
folic acid
IC50 >1,000 nM
3xMMAE-XTEN
IC50 >1,000nM
% Cell viability
150
100
50
0
-14
-12
-10
-8
-6
-4
Concentration (M)

FIG. 116

A



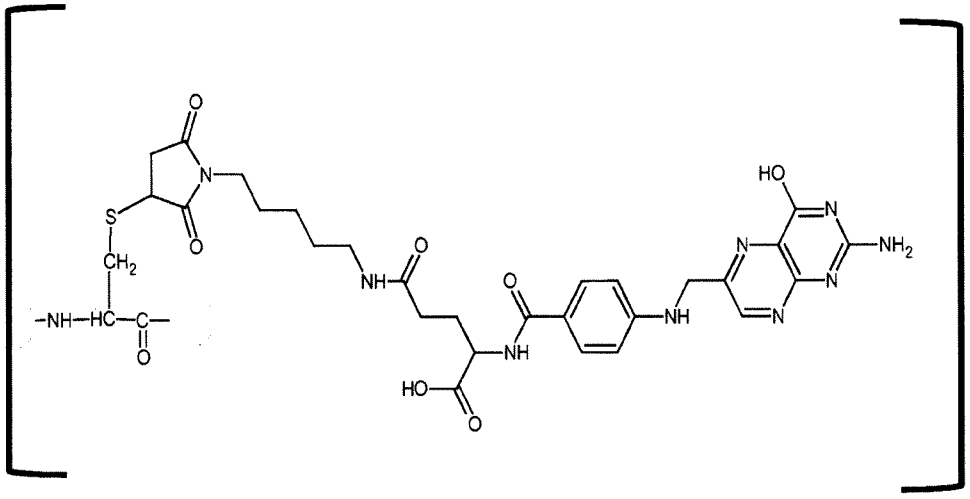
SAGSPTAEAAGXGTAEAAGTSESATPESGPGSEPATS
GSETPGTSESATPESGPGTSTEPSEGSAPGTSESATP
ESGPGSPAGSPTSTEEGSPAGSPTSTEEGSPAGSPTS
TEEGTSESATPESGPGTSTEPSEGSAPGTSESATPES
GPGSEPATSGSETPGTSESATPESGPGSEPATSGSET
PGTSESATPESGPGTSTEPSEGSAPTAEAAGXGTAEA
AGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETP
GTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEG
TSTEPSEGSAPGTSESATPESGPGTSESATPESGPGT
SESATPESGPGSEPATSGSETPGSEPATSGSETPGSP
AGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEP
ATSGSETPTAEAA
GXGTAEAASASR

SAGSPTAEAAGZGTAEAAGTSESATPESGPGSEPATS
GSETPGTSESATPESGPGTSTEPSEGSAPGTSESATP
ESGPGSPAGSPTSTEEGSPAGSPTSTEEGSPAGSPTS
TEEGTSESATPESGPGTSTEPSEGSAPGTSESATPES
GPGSEPATSGSETPGTSESATPESGPGSEPATSGSET
PGTSESATPESGPGTSTEPSEGSAPTAEAAGZGTAEA
AGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETP
GTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEG
TSTEPSEGSAPGTSESATPESGPGTSESATPESGPGT
SESATPESGPGSEPATSGSETPGSEPATSGSETPGSP
AGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEP
ATSGSETPTAEAAGZGTAEAASASR

FIG. 117A

B

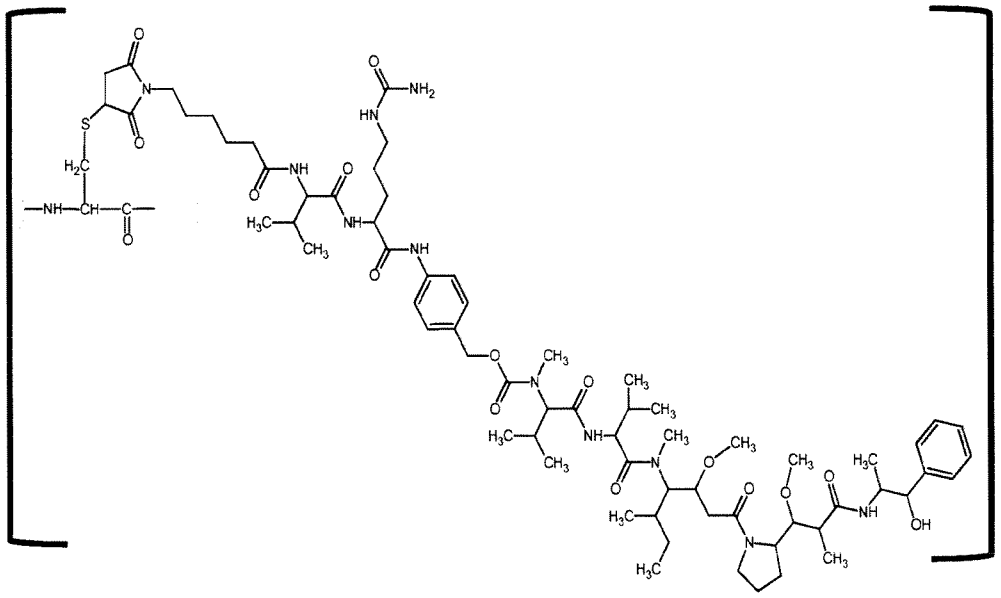
X =



Residue X = residue of Cys modified with Folate-γ-Mal

C

Z =



Residue Z = residue of Cys modified with MC-Val-Cit-PAB-MMAE

FIG. 117B, C