Figure 1

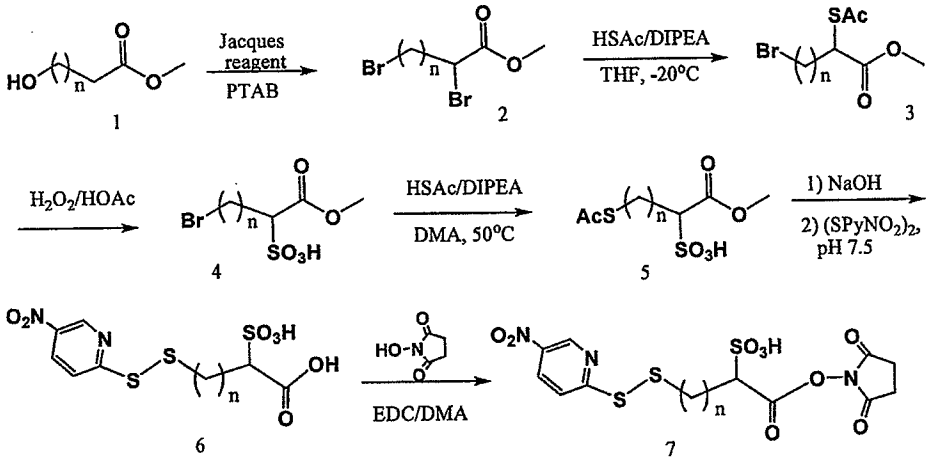


Figure 2

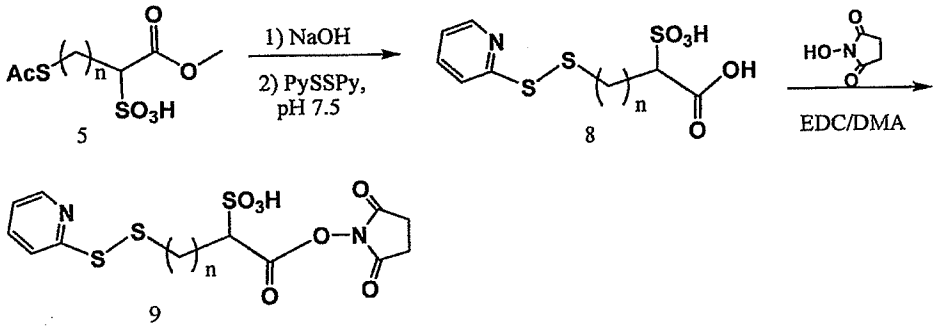


Figure 3

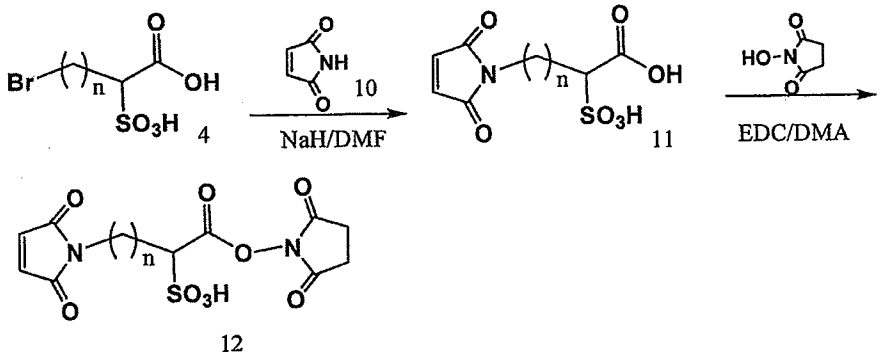


Figure 4

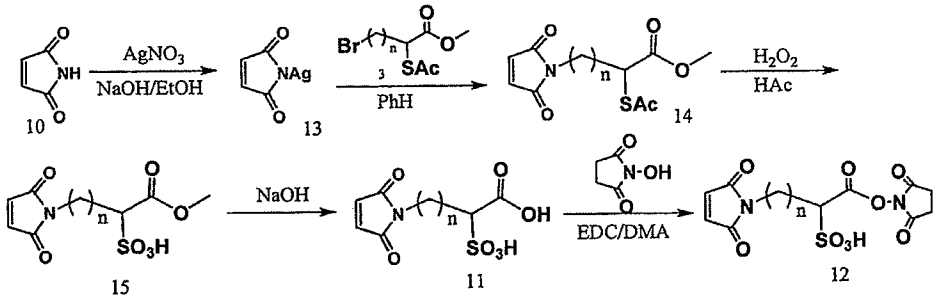


Figure 5

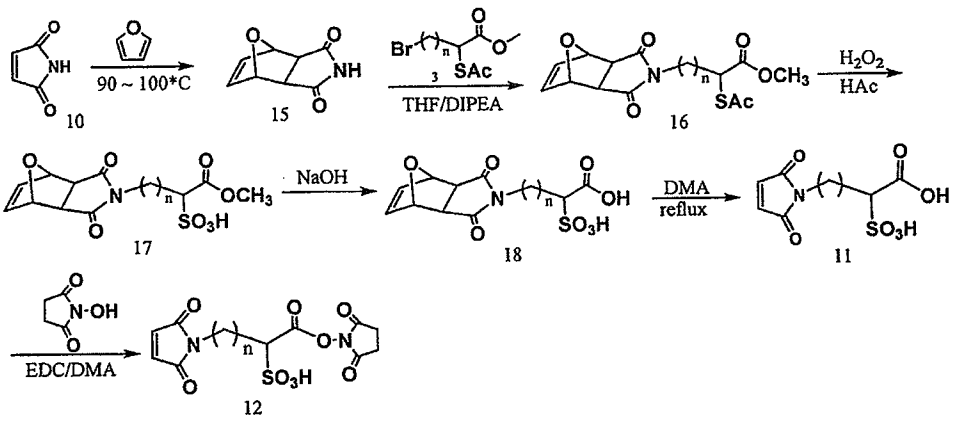


Figure 6

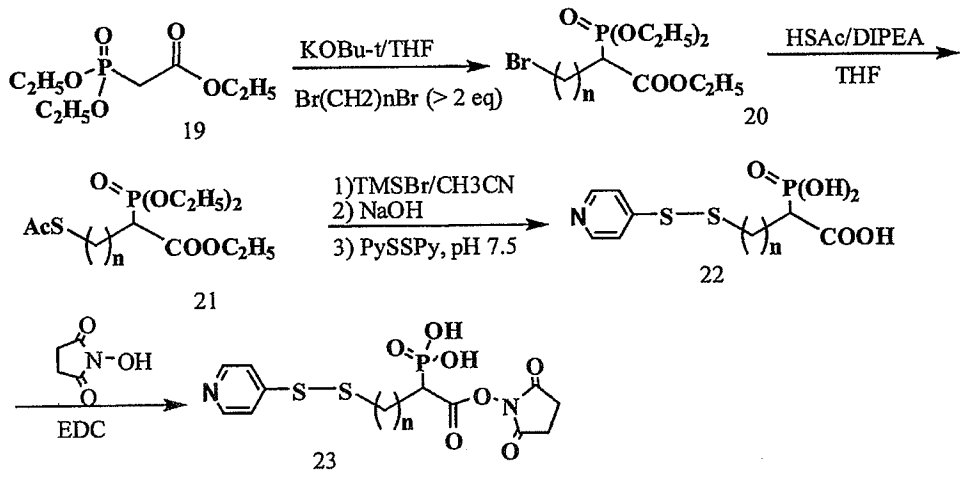


Figure 7.

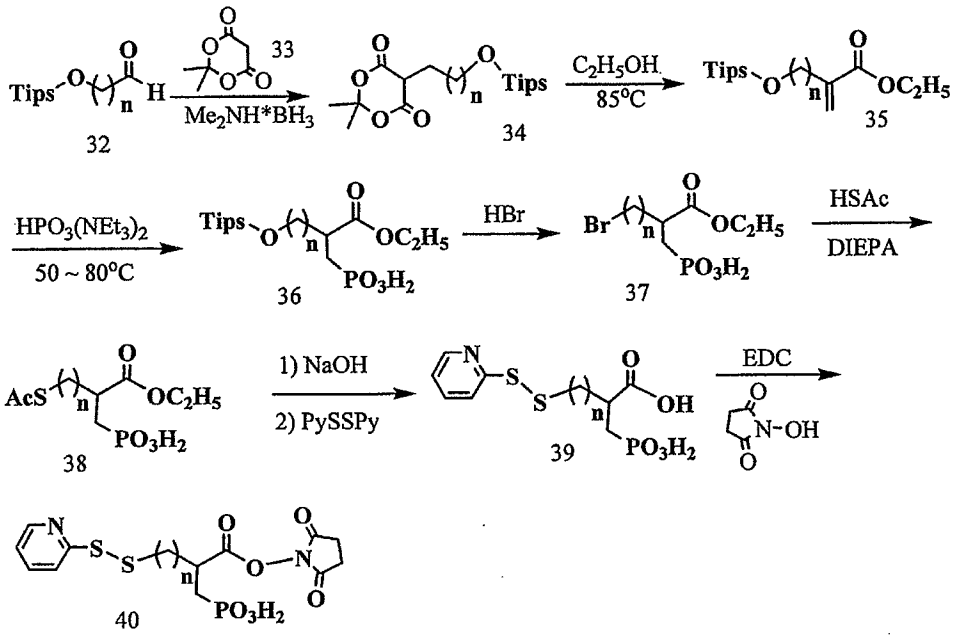


Figure 8.

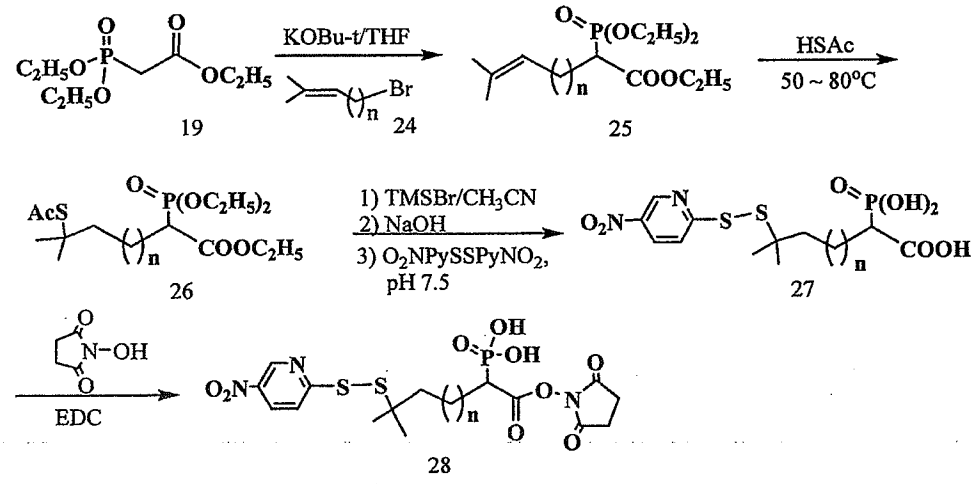


Figure 9

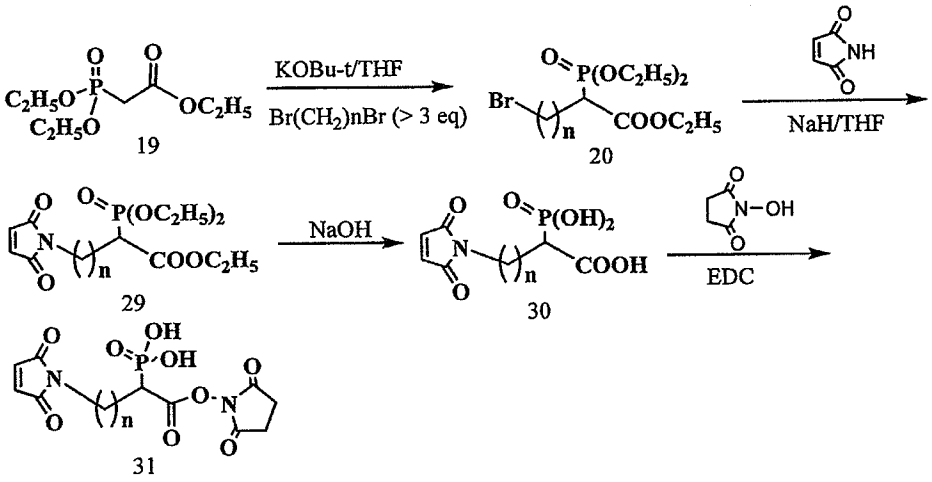


Figure 10

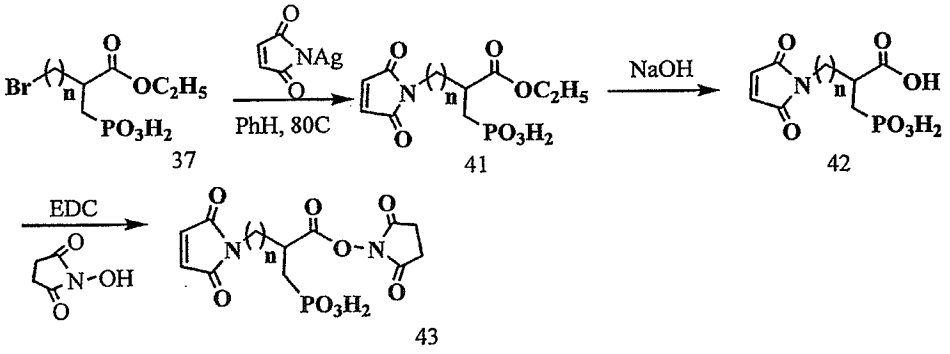


Figure 11

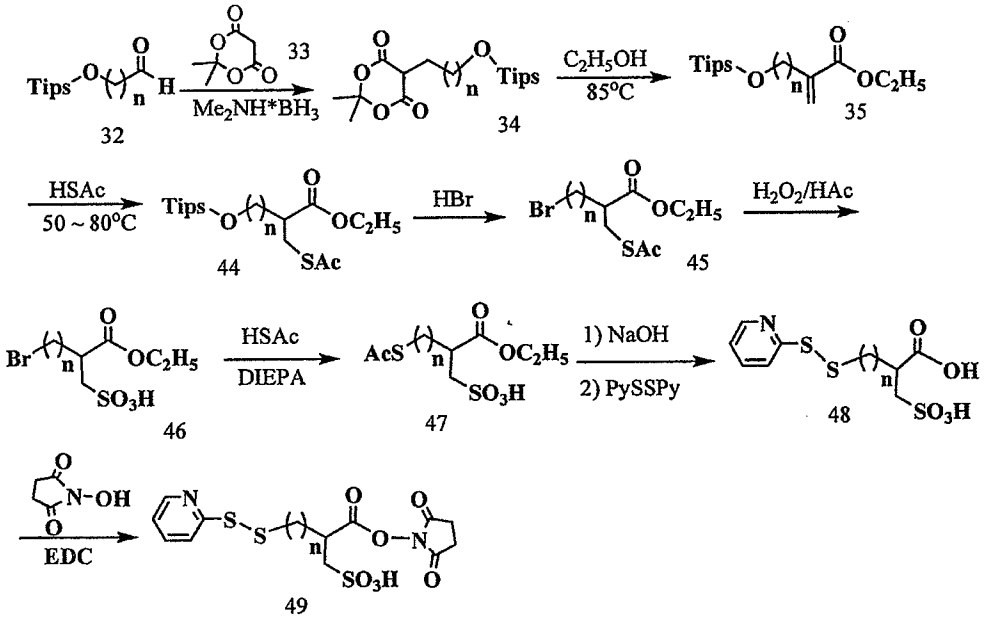


Figure 12

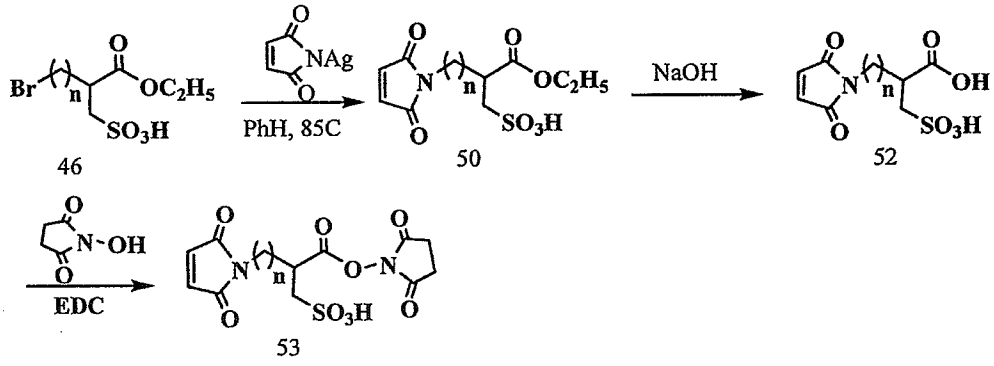


Figure 13

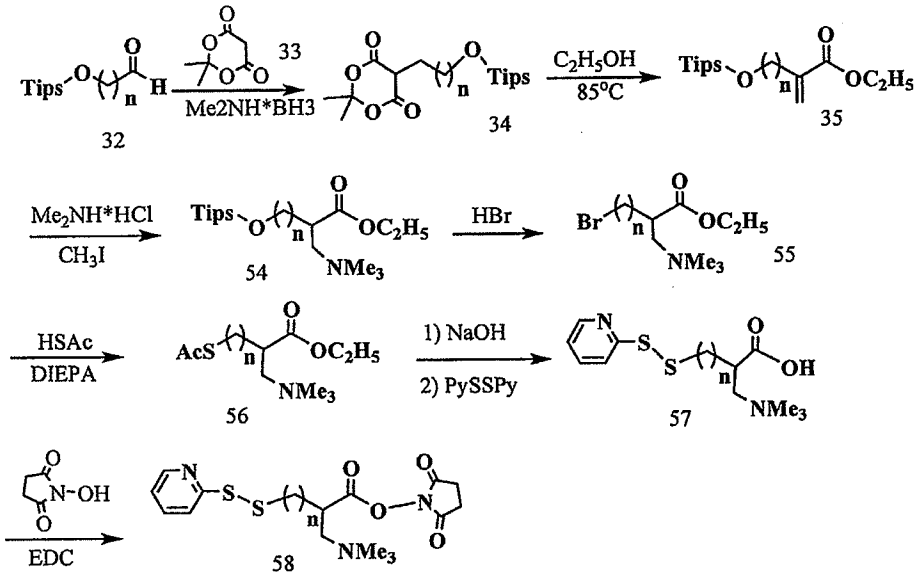


Figure 14

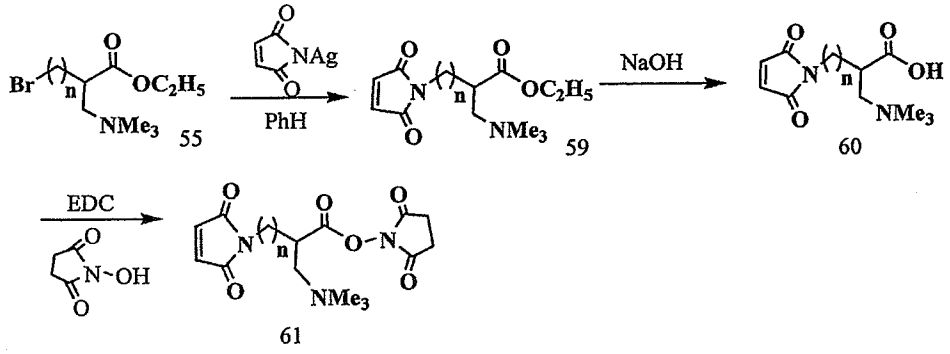


Figure 15

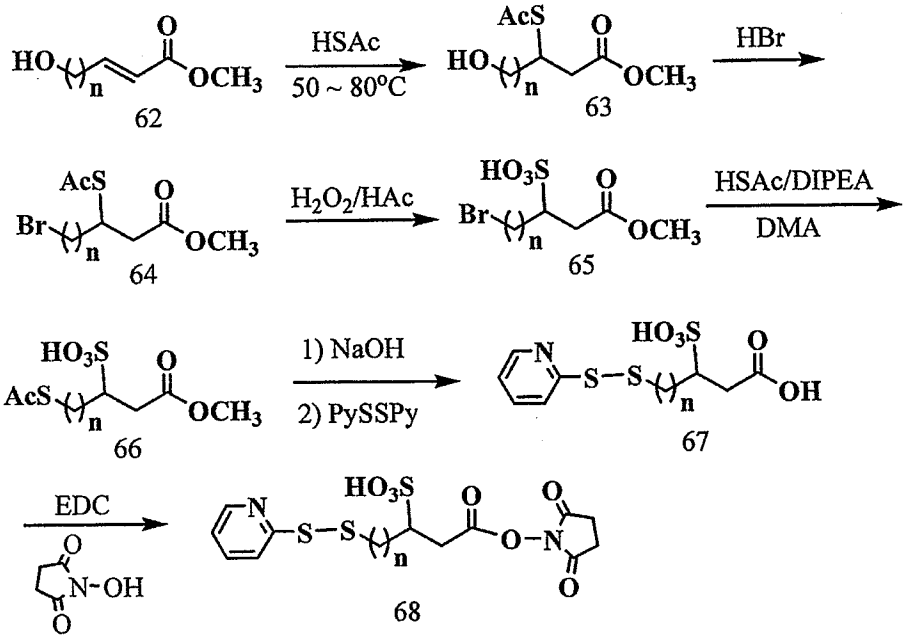


Figure 16

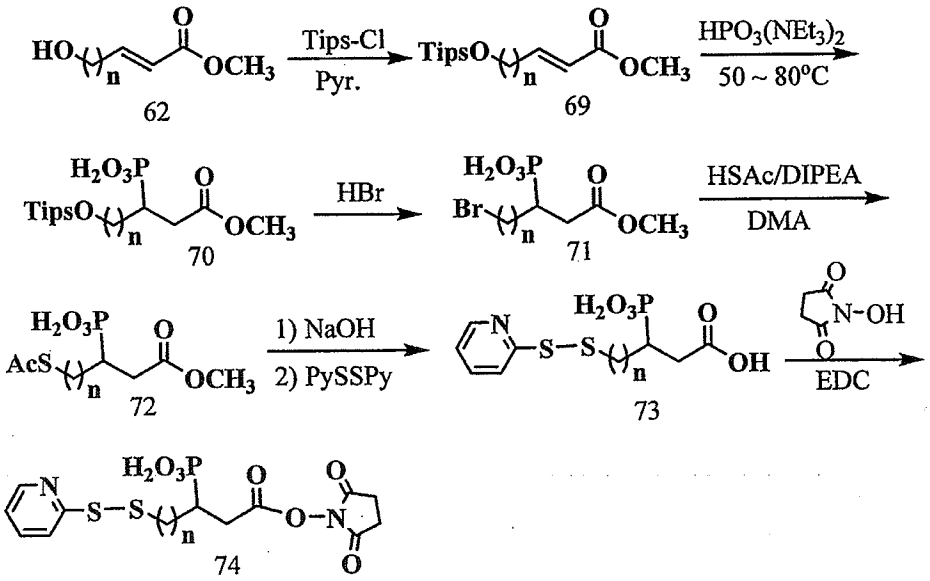


Figure 17

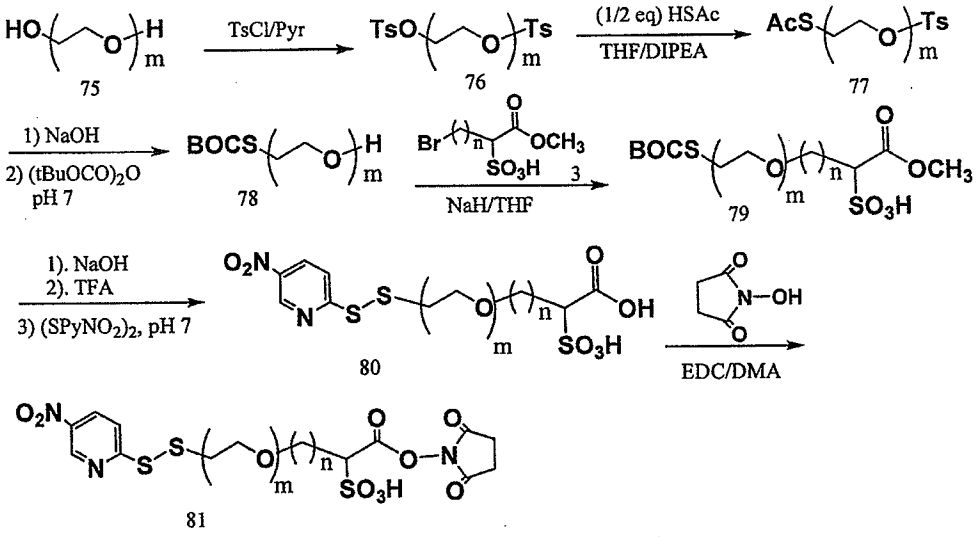


Figure 18

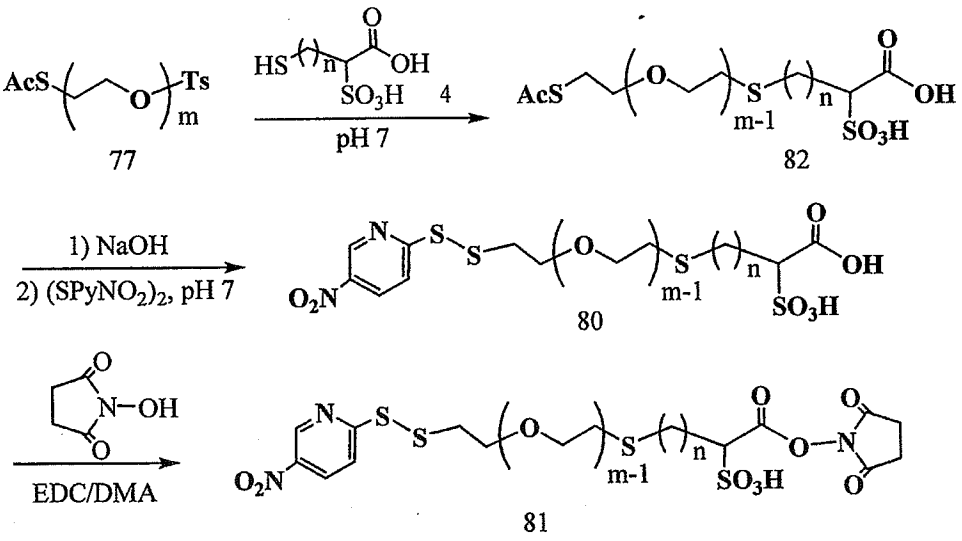


Figure 19

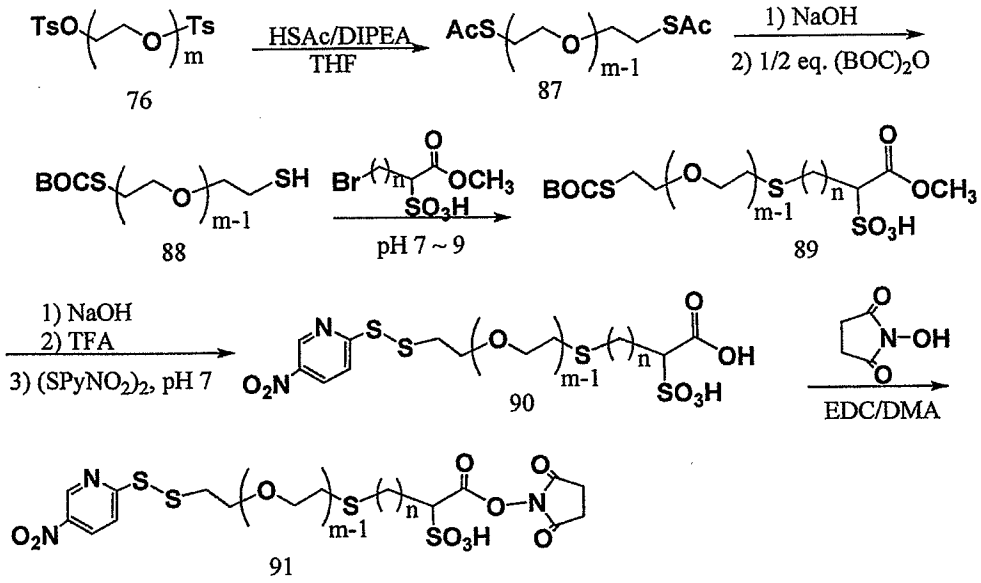


Figure 20

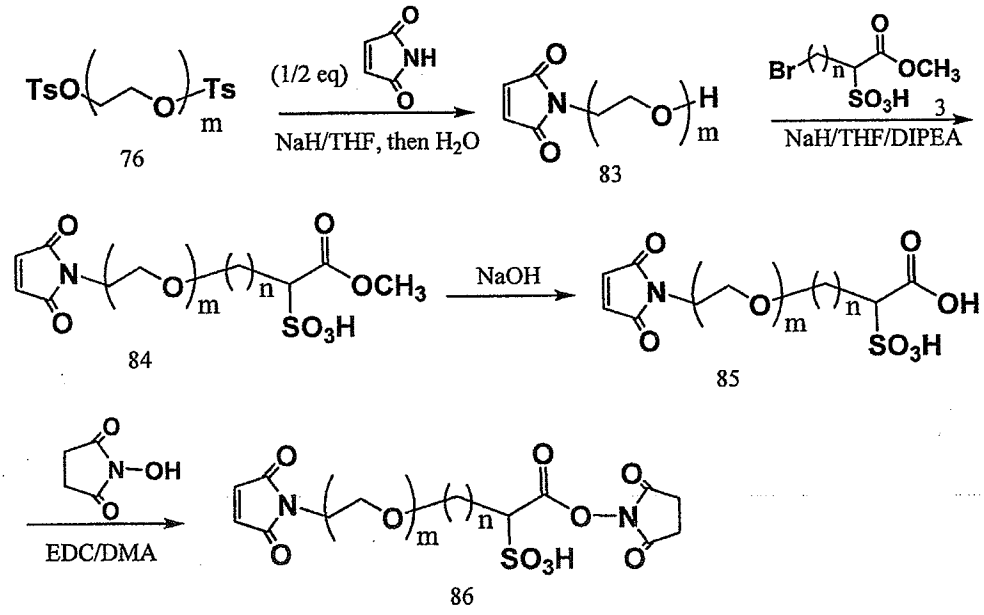


Figure 21

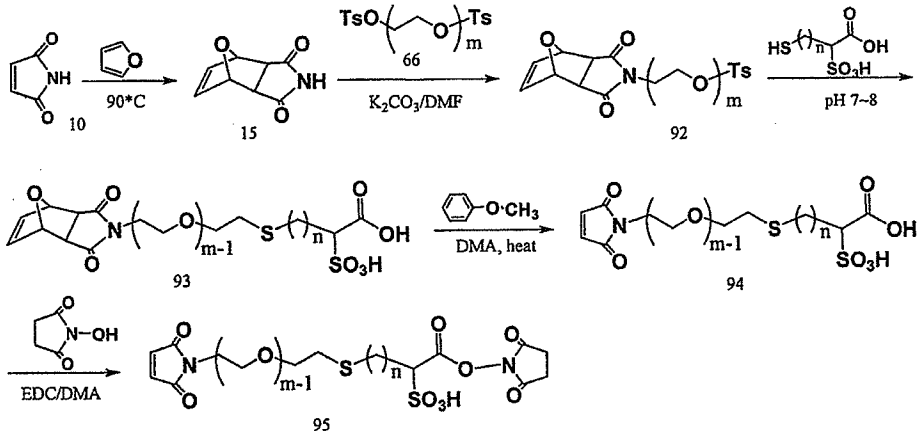


Figure 22

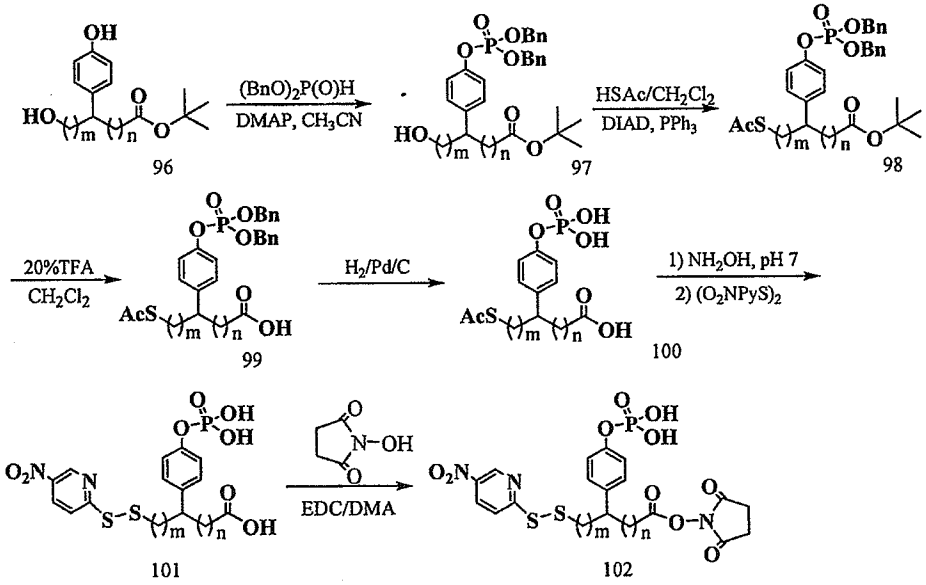


Figure 23

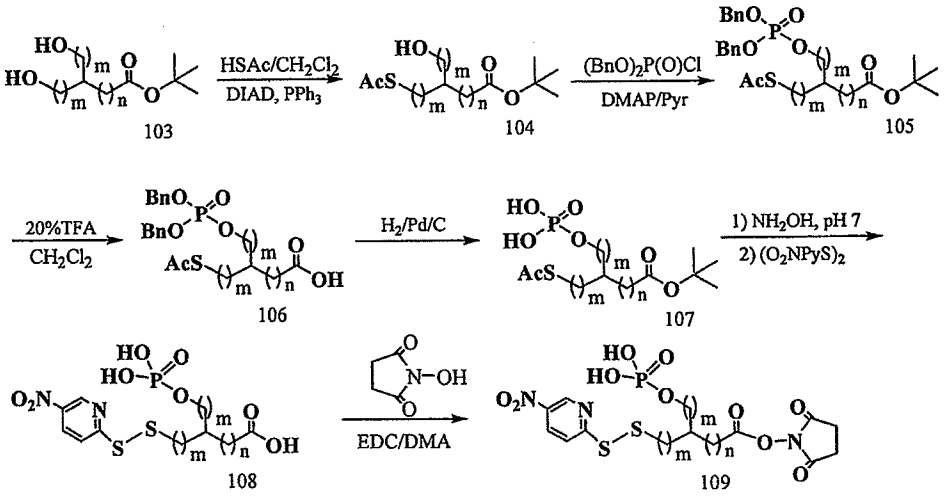


Figure 24

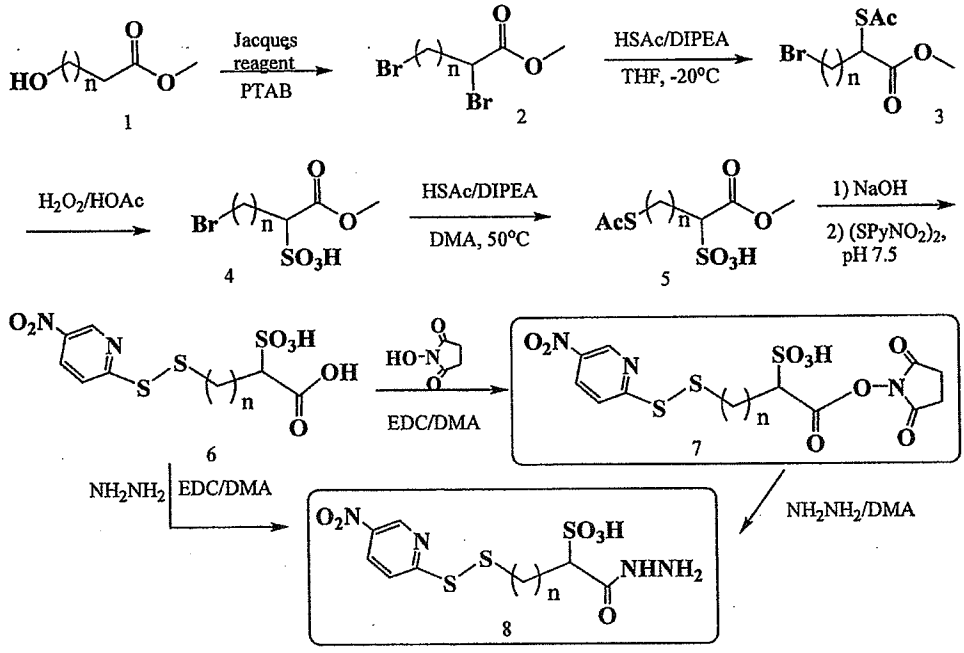


Figure 25

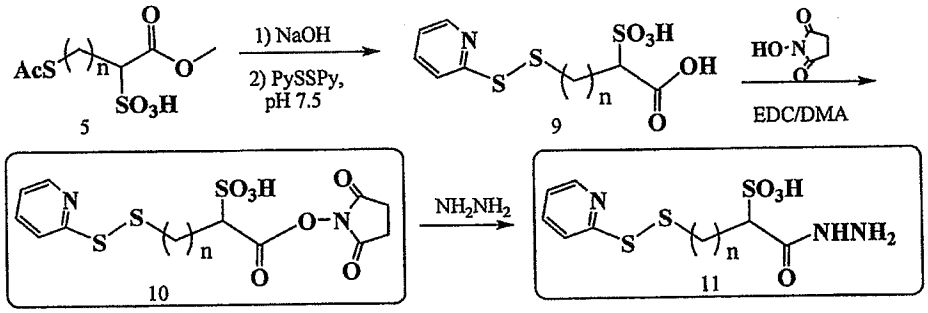


Figure 26

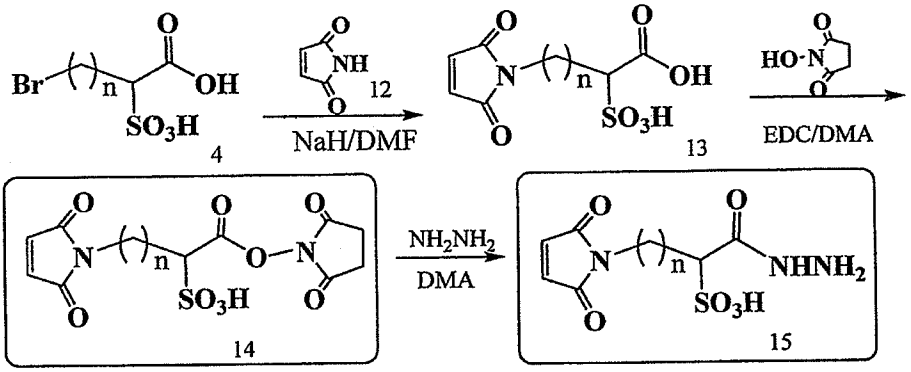


Figure 27

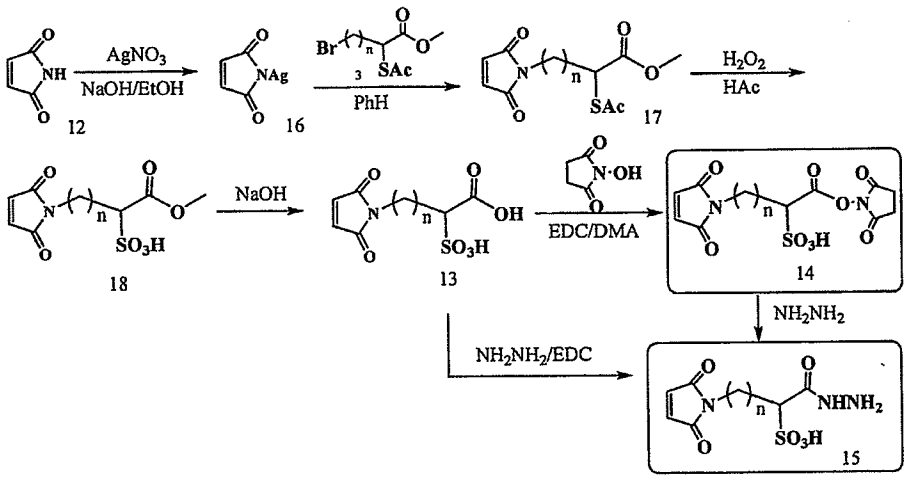


Figure 28

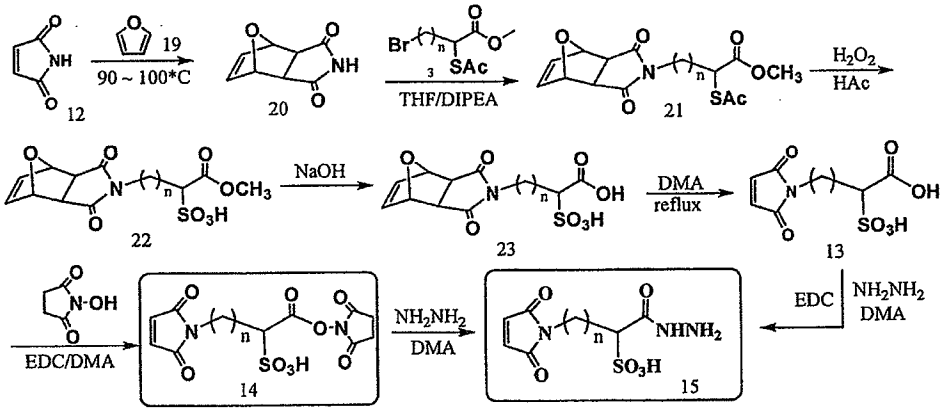


Figure 29

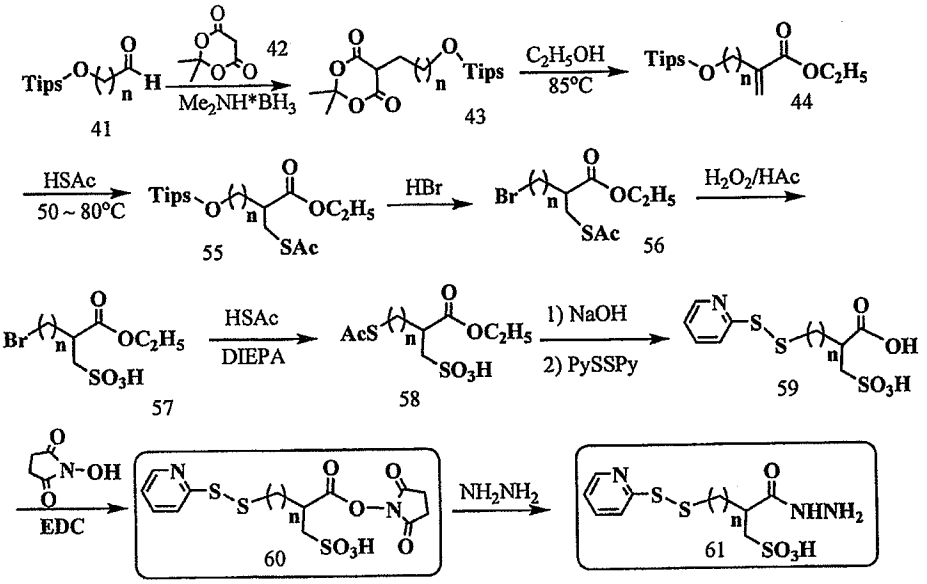


Figure 30

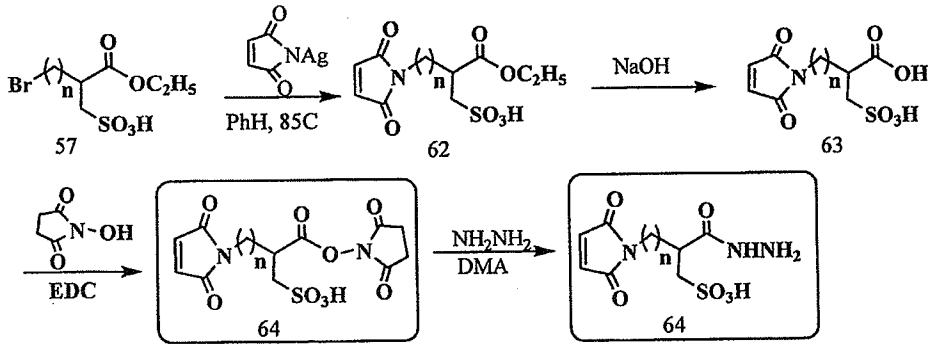


Figure 31

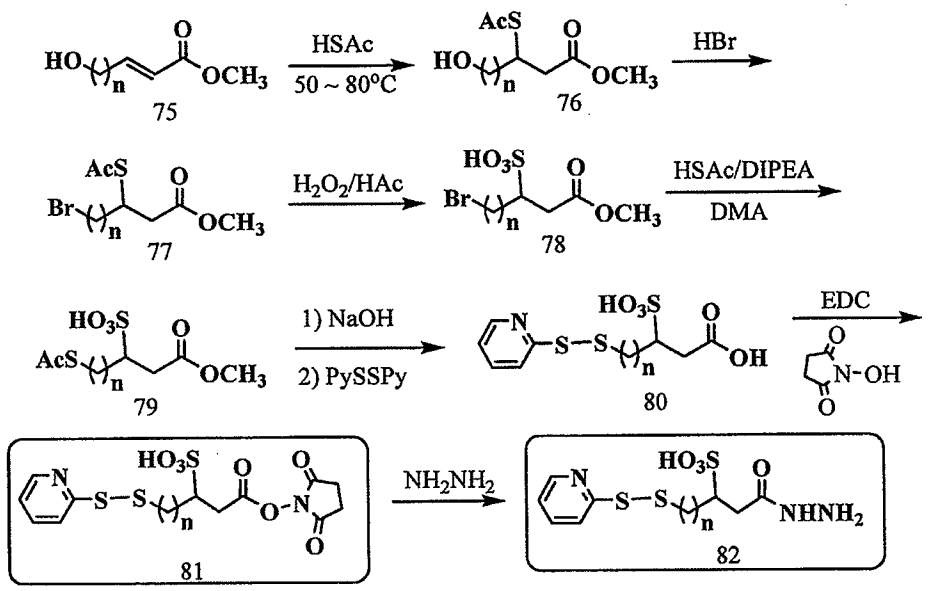


Figure 32

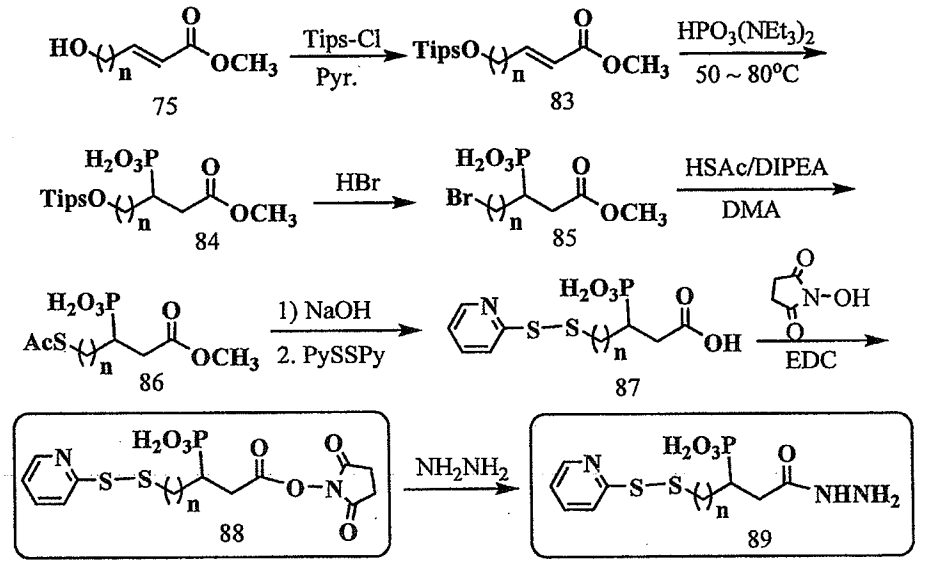


Figure 33

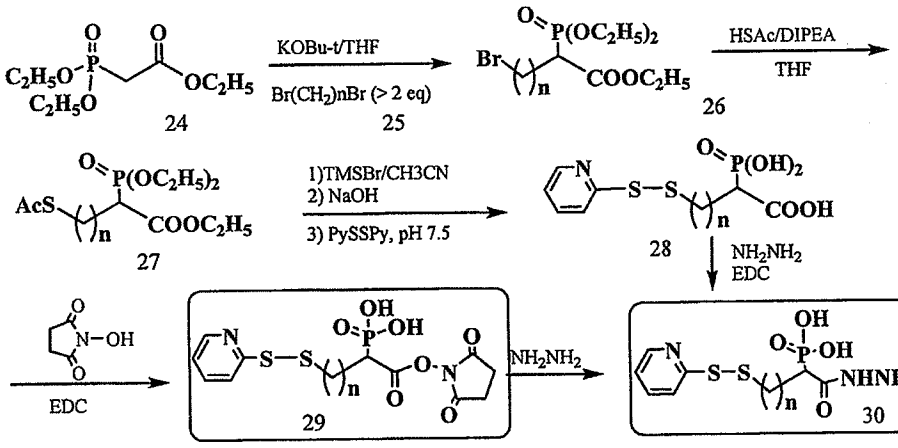


Figure 34

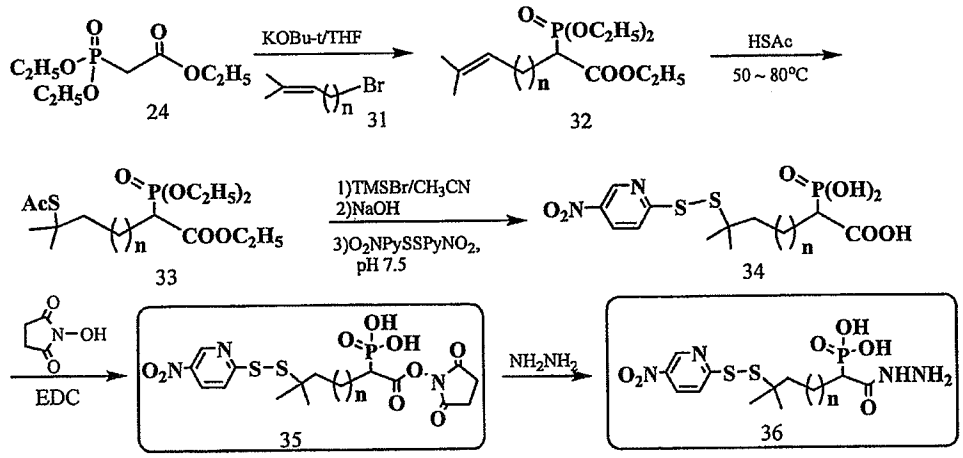


Figure 35

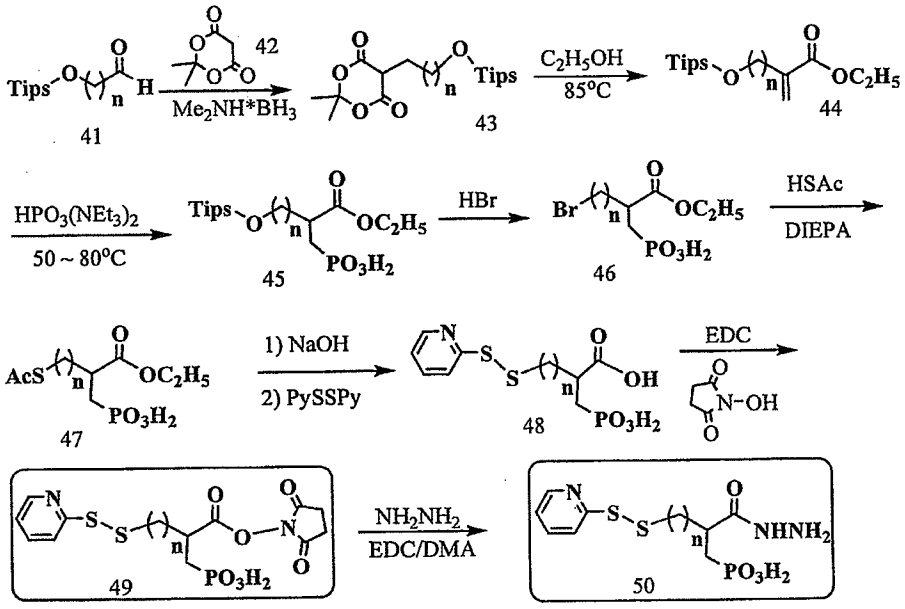


Figure 36

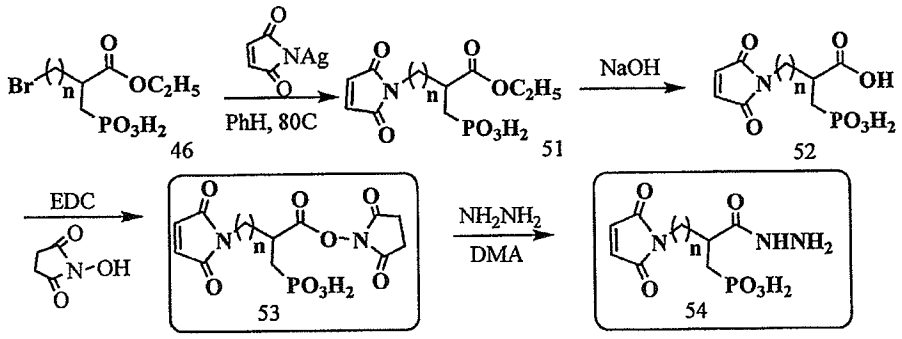


Figure 37

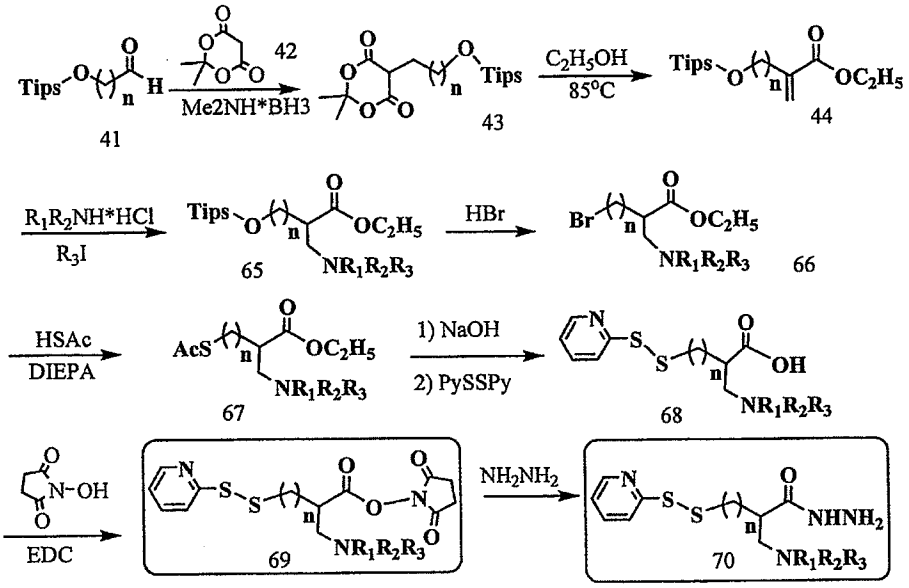


Figure 38

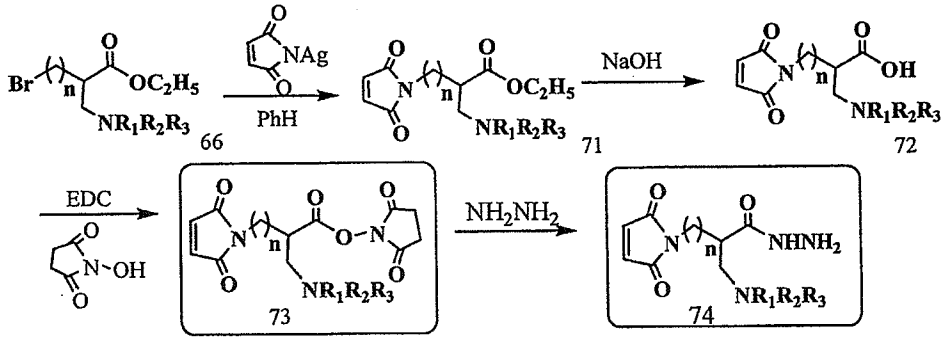


Figure 39

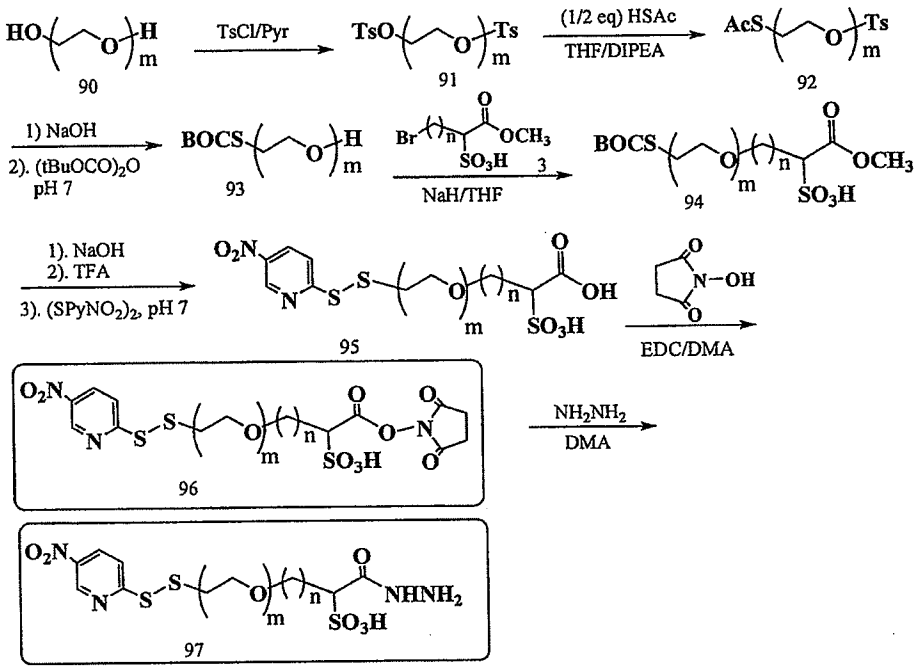

TsCl/Pyr
(1/2 eq) HSAc
THF/DIPEA
1) NaOH
2). (tBuOCO)2O
pH 7
NaH/THF
1). NaOH
2). TFA
3). (SPyNO2)2, pH 7
EDC/DMA
NH2NH2
DMA
90
91
92
93
94
95
96
97

Figure 40

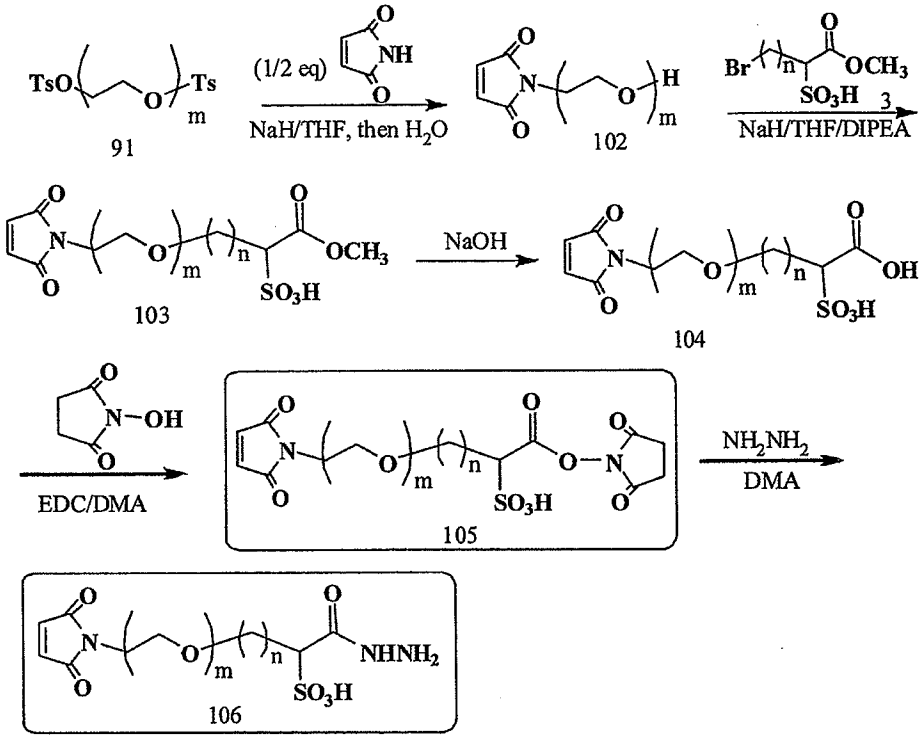

TsO
Ts
m
91
(1/2 eq)
NH
O
NaH/THF, then H2O
102
Br
OCH3
SO3H
3
NaH/THF/DIPEA
OCH3
SO3H
103
NaOH
OH
104
N–OH
EDC/DMA
105
NH2NH2
DMA
NHNH2
106

Figure 41

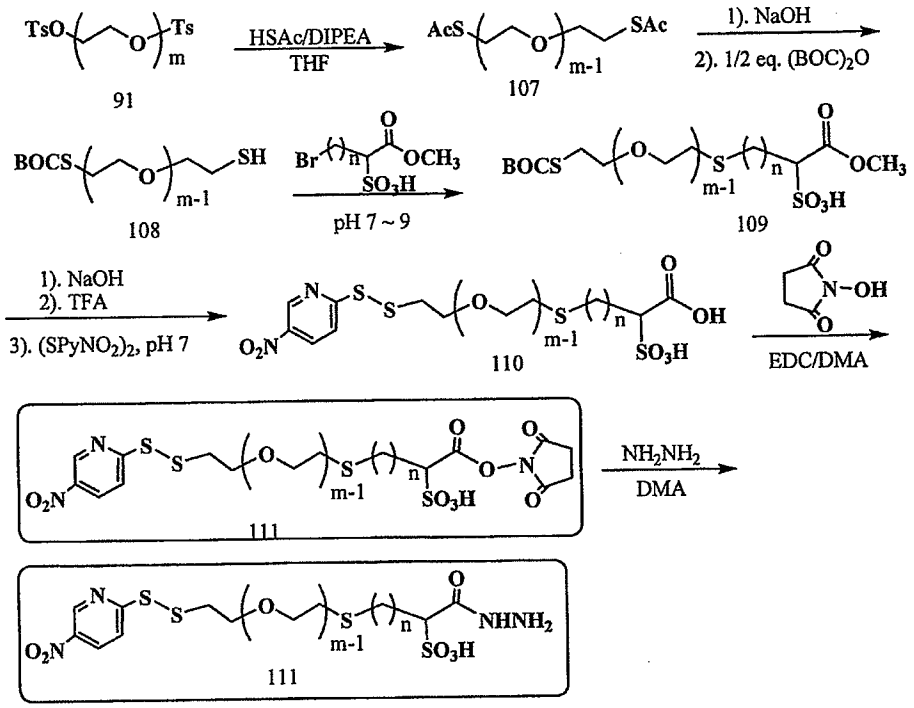

TsO
Ts
m
91
HSAc/DIPEA
THF
AcS
SAc
m-1
107
1). NaOH
2). 1/2 eq. (BOC)2O
BOCS
SH
m-1
108
Br
n
OCH3
SO3H
pH 7 ~ 9
BOCS
S
n
OCH3
m-1
SO3H
109
1). NaOH
2). TFA
3). (SPyNO2)2, pH 7
O2N
N
S
S
O
S
n
OH
m-1
SO3H
110
N-OH
EDC/DMA
111
NH2NH2
DMA
NHNH2
111

Figure 42

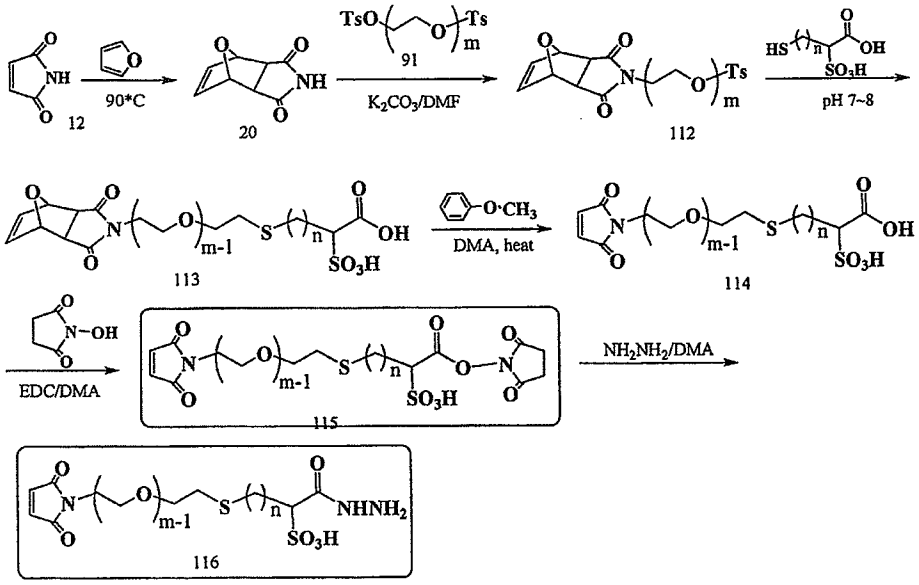

12
90*C
20
TsO
Ts
m
91
K2CO3/DMF
112
HS
OH
SO3H
pH 7~8
113
m-1
n
DMA, heat
114
EDC/DMA
115
NH2NH2/DMA
116
NHNH2

Figure 43

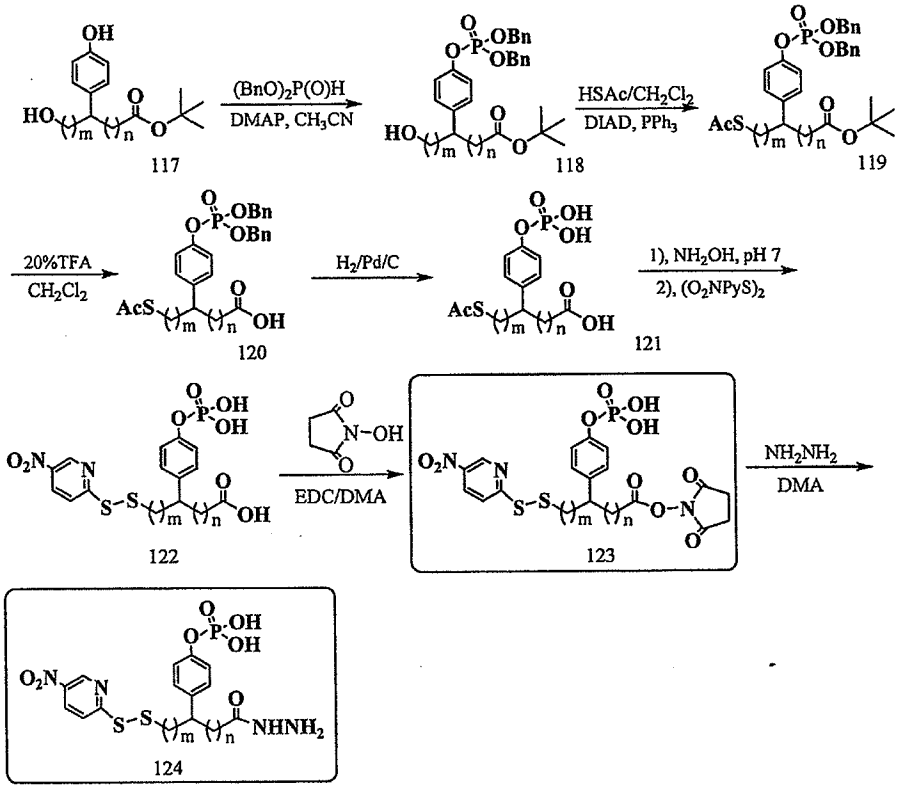

(BnO)2P(O)H
DMAP, CH3CN
HSAc/CH2Cl2
DIAD, PPh3
117
118
119
20%TFA
CH2Cl2
H2/Pd/C
1), NH2OH, pH 7
2), (O2NPyS)2
120
121
EDC/DMA
122
123
NH2NH2
DMA
124

Figure 44

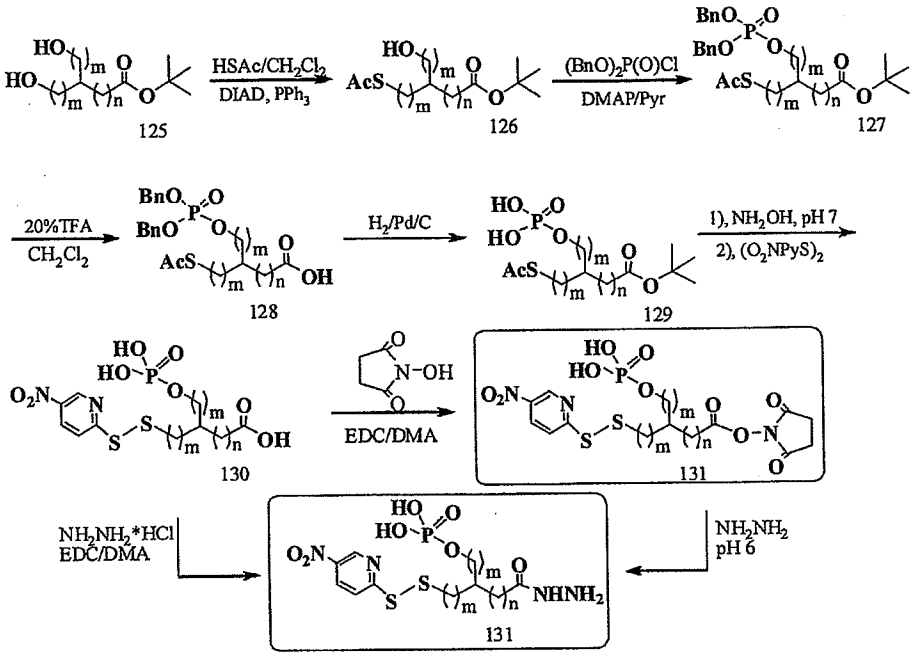

HSAc/CH2Cl2
DIAD, PPh3
(BnO)2P(O)Cl
DMAP/Pyr
125
126
127
20%TFA
CH2Cl2
H2/Pd/C
1), NH2OH, pH 7
2), (O2NPyS)2
128
129
EDC/DMA
130
131
NH2NH2*HCl
EDC/DMA
NH2NH2
pH 6
131

Figure 45

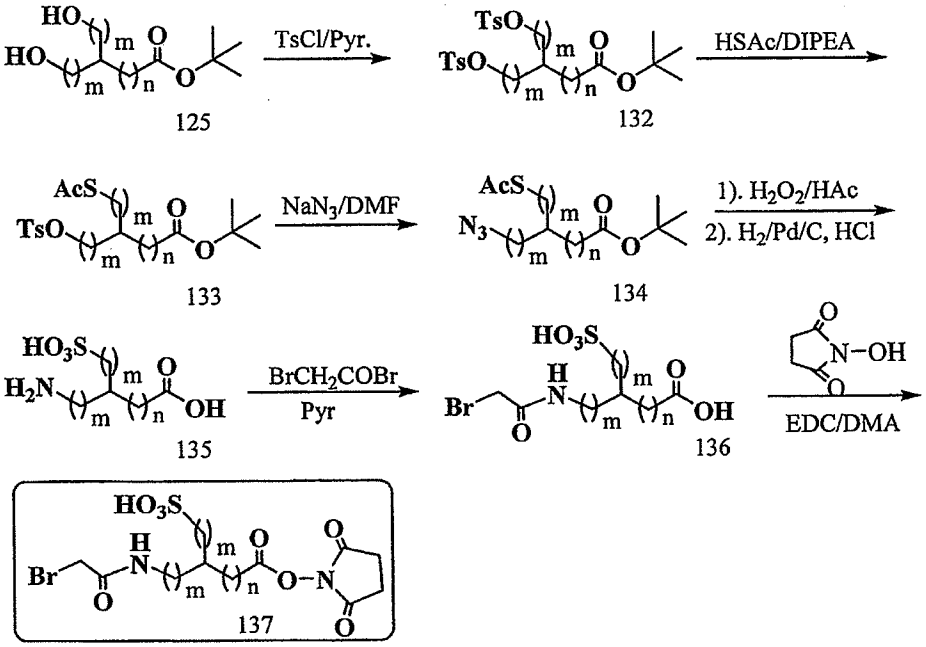


Figure 46

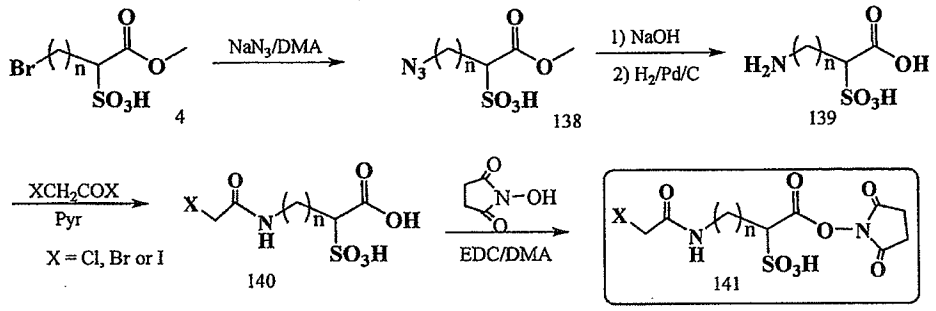


Figure 47

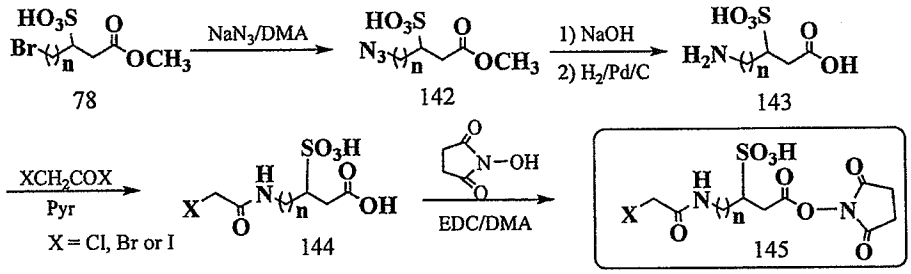


Figure 48

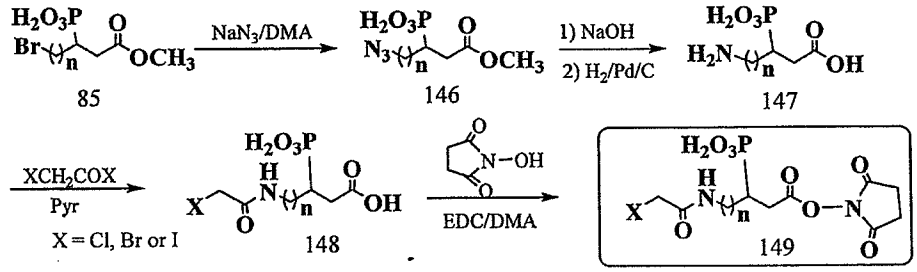
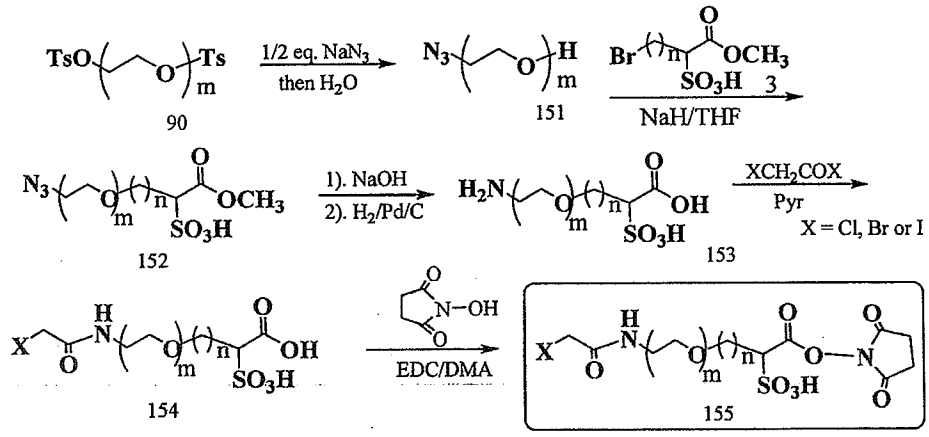


Figure 49



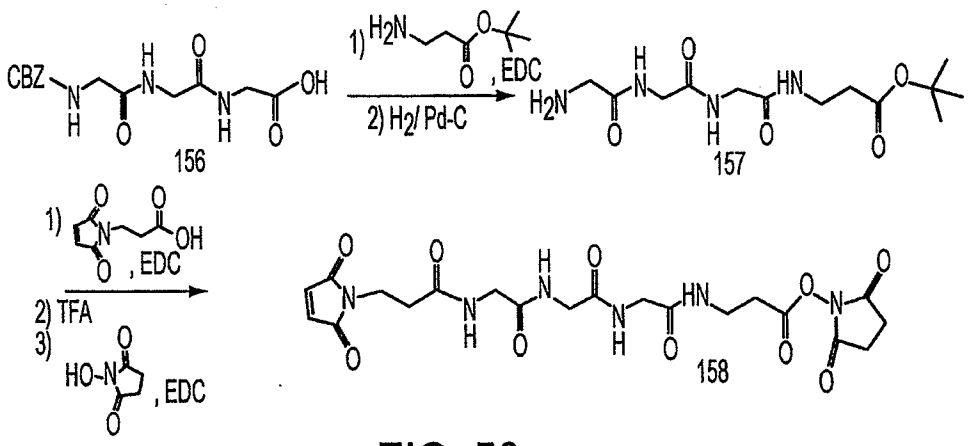

CBZ
OH
156
1) H2N
, EDC
2) H2/ Pd-C
H2N
HN
157
1)
, EDC
2) TFA
3)
HO-N
, EDC
158

FIG. 50

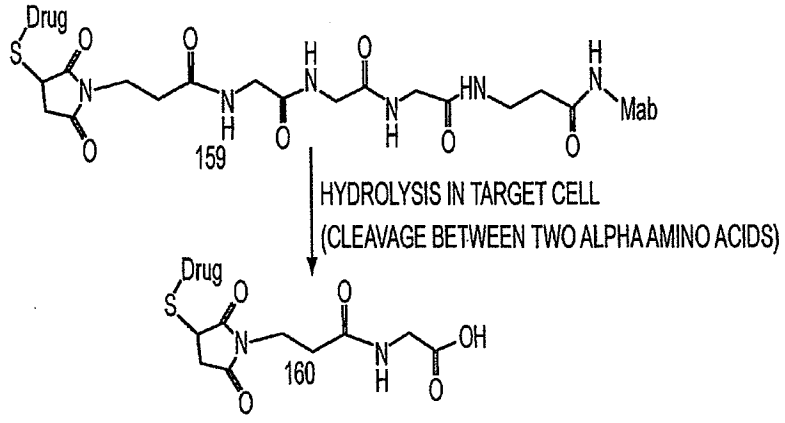

Drug
159
Mab
HYDROLYSIS IN TARGET CELL
(CLEAVAGE BETWEEN TWO ALPHA AMINO ACIDS)
Drug
160
OH

FIG. 51

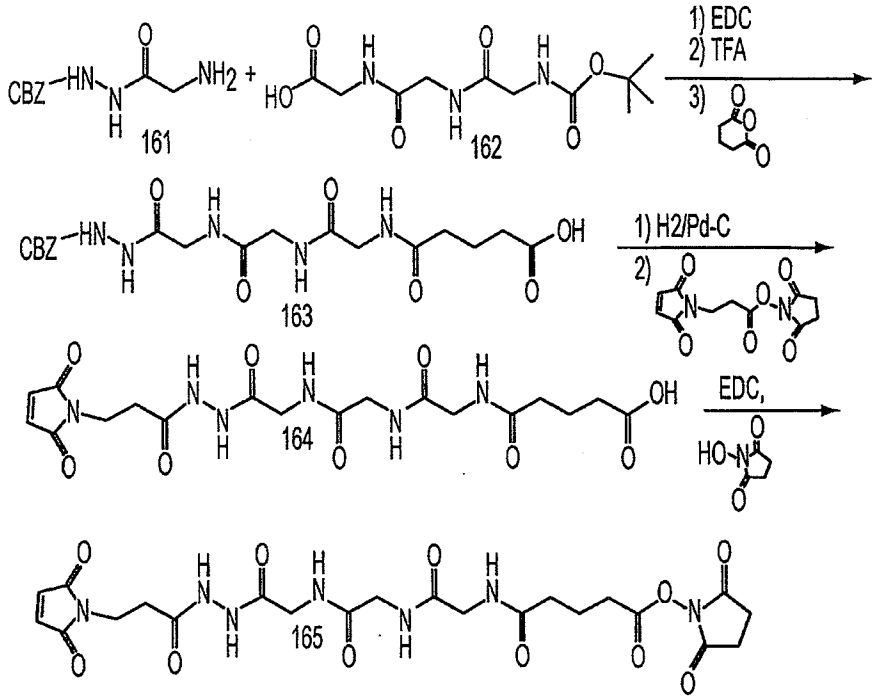

CBZ
HN
NH2
161
HO
162
1) EDC
2) TFA
3)
163
OH
1) H2/Pd-C
2)
164
EDC,
HO
165

FIG. 52

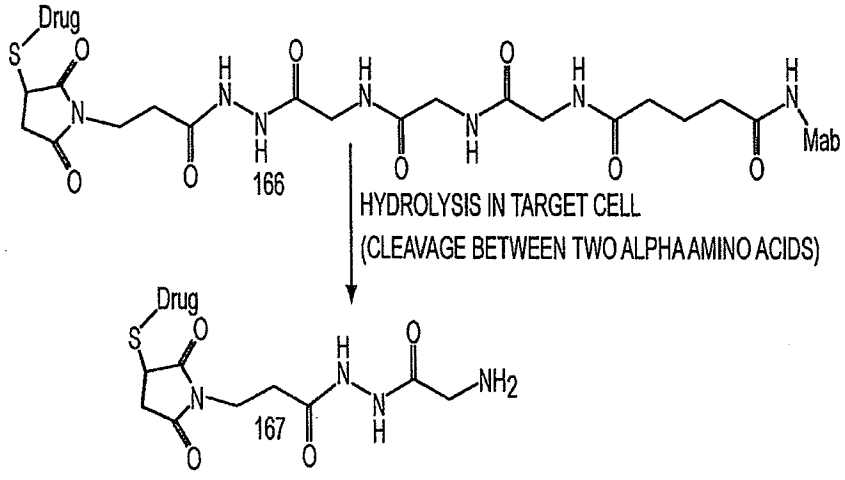

Drug
S
166
Mab
HYDROLYSIS IN TARGET CELL
(CLEAVAGE BETWEEN TWO ALPHA AMINO ACIDS)
Drug
S
167
NH2

FIG. 53

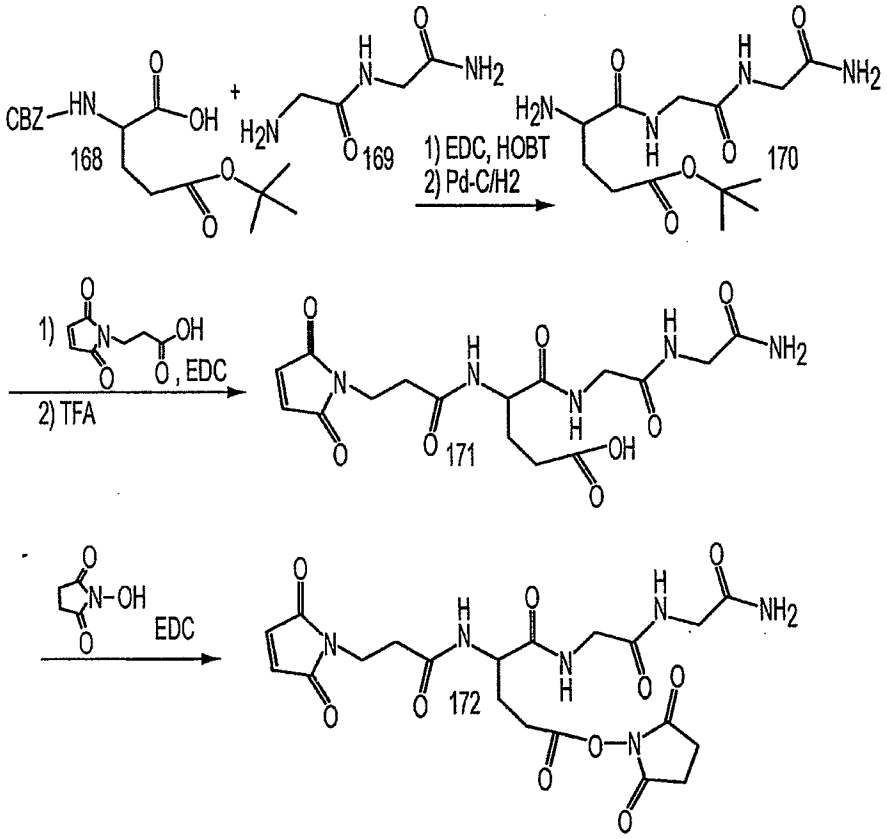

CBZ
HN
OH
168
H2N
169
1) EDC, HOBT
2) Pd-C/H2
170
1)
OH
, EDC
2) TFA
171
N-OH
EDC
172

FIG. 54

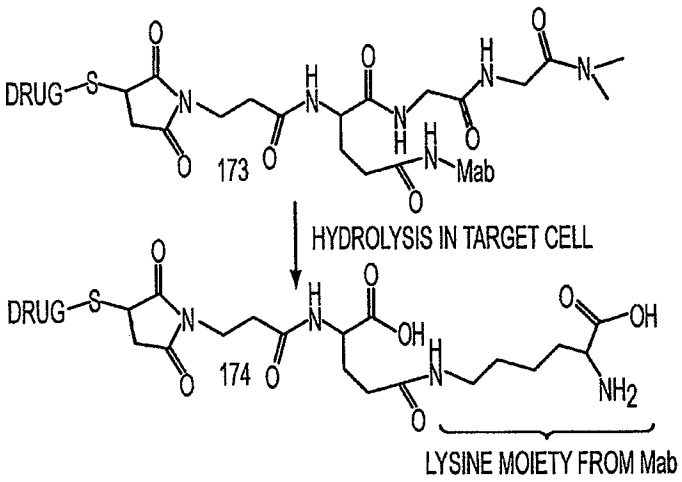

DRUG
S
173
Mab
HYDROLYSIS IN TARGET CELL
DRUG
S
174
OH
OH
NH2
LYSINE MOIETY FROM Mab

FIG. 55

Figure 56

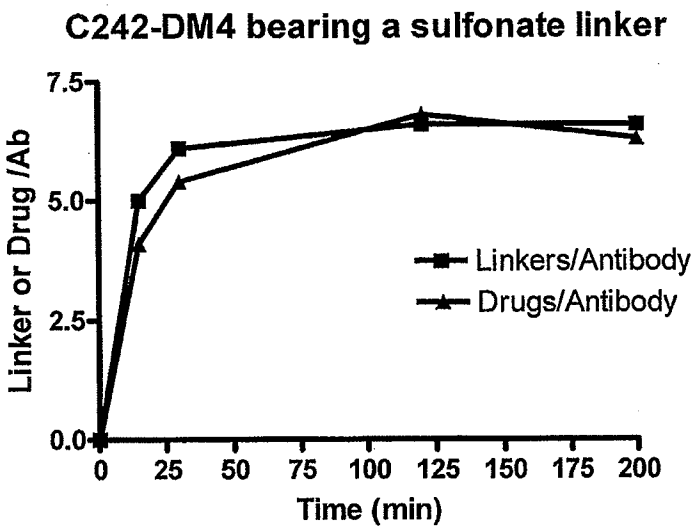

C242-DM4 bearing a sulfonate linker
Linker or Drug /Ab
7.5
5.0
2.5
0.0
0
25
50
75
100
125
150
175
200
Time (min)
Linkers/Antibody
Drugs/Antibody

Figure 57(A) Cytotoxicity of anti-CD56(huN901) antibody-maytansinoid conjugates

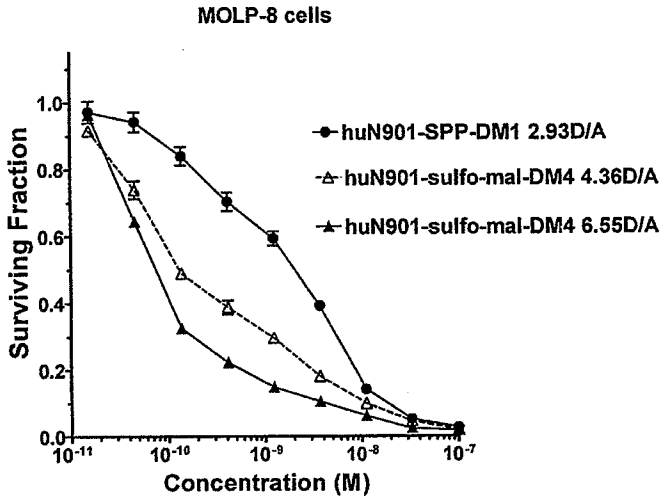


Figure 57(B) Cytotoxicity of anti-CD56(huN901) antibody-maytansinoid conjugates

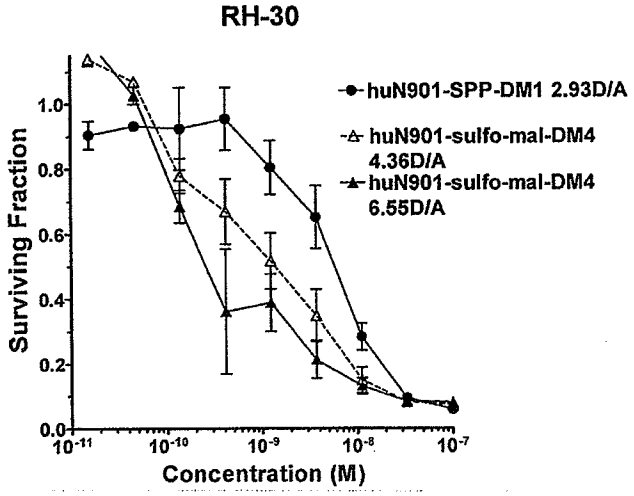
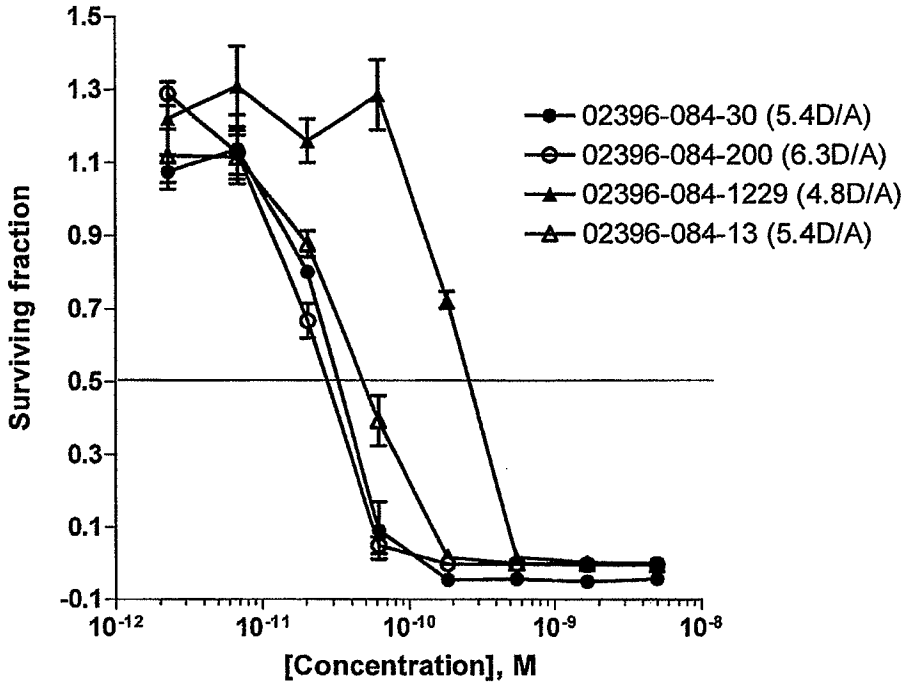
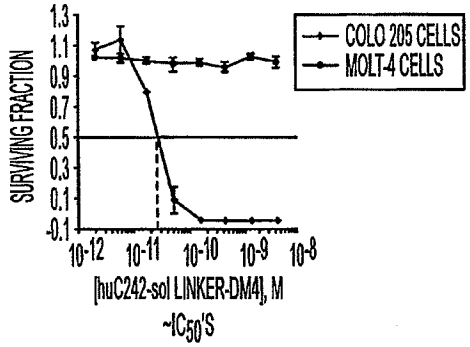


Figure 57(C) Cytotoxicity of anti-CD56(huN901) antibody-maytansinoid conjugates



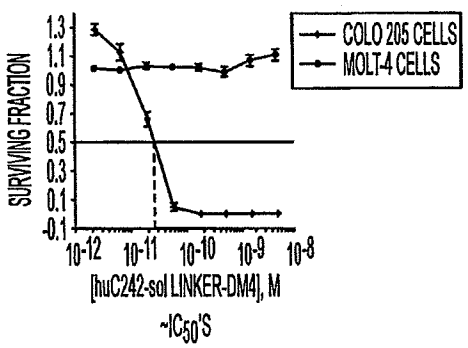
CYTOTOXICITY: huC242-SULFONATE LINKER-DM4, (5.4 D/A)' VS. COLO 205 & MOLT-4 CELLS



~IC_{50}'S

COLO 205 CELLS	3.3e-11 M
MOLT-4 CELLS	> 6.0e-9 M

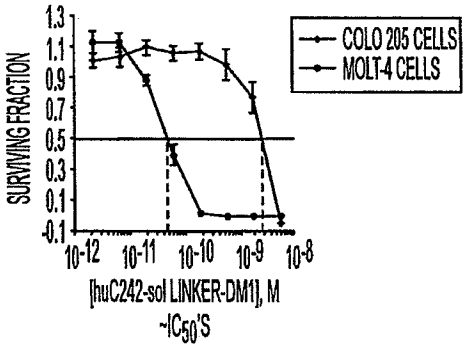
CYTOTOXICITY: huC242-SULFONATE LINKER-DM4, (6.3 D/A)' VS. COLO 205 & MOLT-4 CELLS



~IC_{50}'S

COLO 205 CELLS	2.9e-11 M
MOLT-4 CELLS	> 6.0e-9 M

CYTOTOXICITY: huC242-SULFONATE LINKER-DM1, (5.4 D/A) VS. COLO 205 & MOLT-4 CELLS



~IC_{50}'S

COLO 205 CELLS	4.8e-11 M
MOLT-4 CELLS	2.3e-9 M

FIG. 58

MASS SPECTRAL ANALYSIS OF huC242-SULFONATE LINKER-DM1 CONJUGATE

DEGLYCOSYLATTED C242-SPDB()SO3)-DM1 (DAPENG)
ACL_010507_SEC_C242_SPDB_DM1_3 35 (7.805) Sb (25, 10.00); M1 [Ev-96612,It11]
(Gs, 1.700,2406:4000,2.00,L40,R40): Sb (25,10.00); Cm (31:49) 1: TOF MS ES+3.72e3

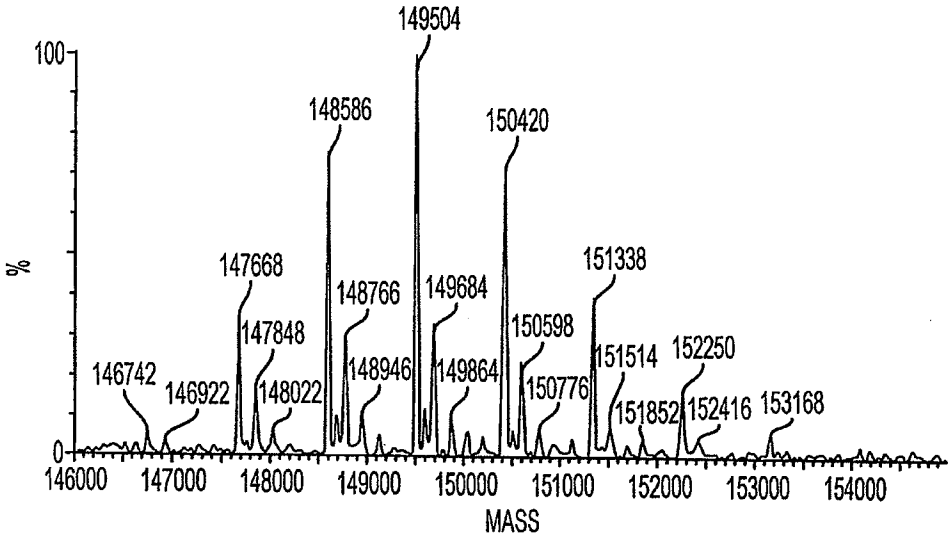


FIG. 59

Figure 60

Cytotoxicity of Anti-CanAg (huC242) -sulfonate linker-maytansinoid conjugates with increasing maytansinoids load (E:A) toward COLO205 cells.

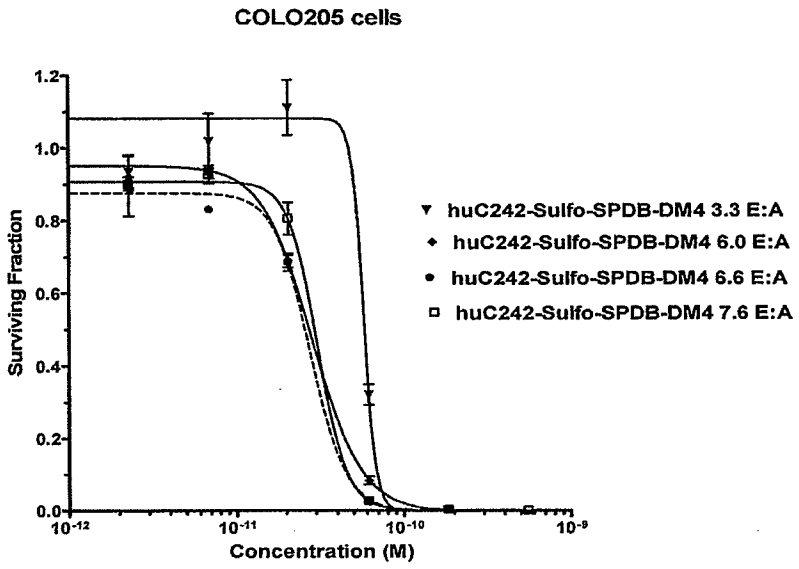


Figure 61

Cytotoxicity of Anti-CanAg (huC242) -sulfonate linker-maytansinoid conjugates with increasing maytansinoids load (E:A) toward multi-drug resistant COLO205-MDR cells.

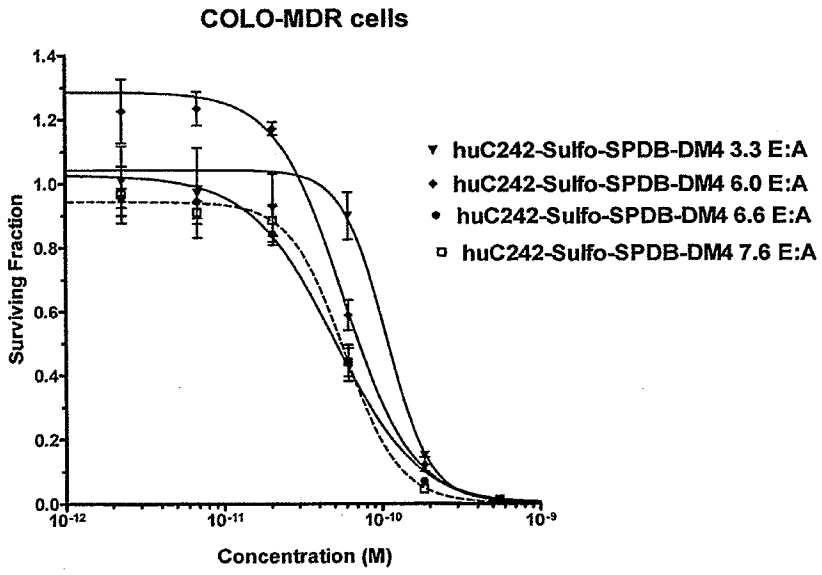


Figure 62

Cytotoxicity of Anti-CanAg (huC242) - maytansinoid conjugates with or without sulfonate linker toward multi-drug resistant COLO205-MDR cells.

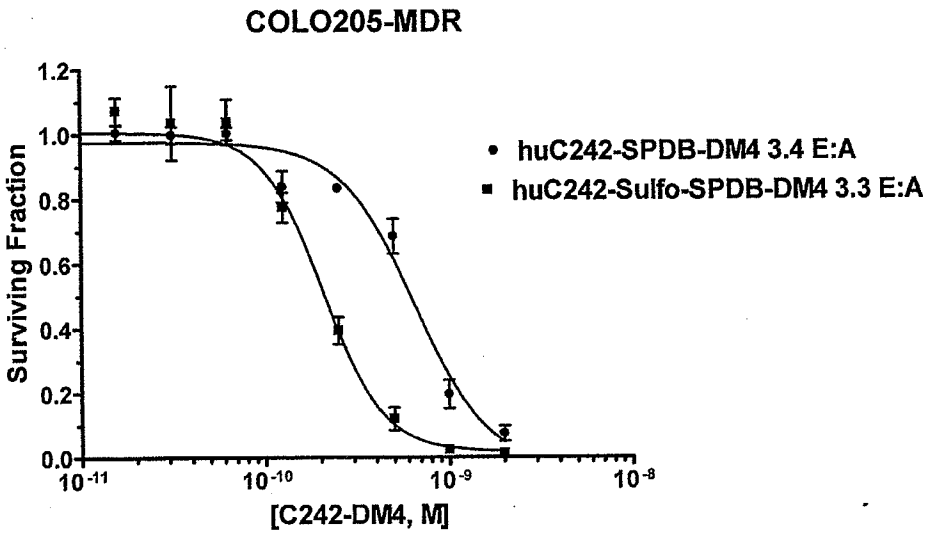


Figure 63

Cytotoxicity of Anti-EpCAM (B38.1) - maytansinoid conjugates with or without sulfonate linker toward multi-drug resistant COLO205-MDR cells.

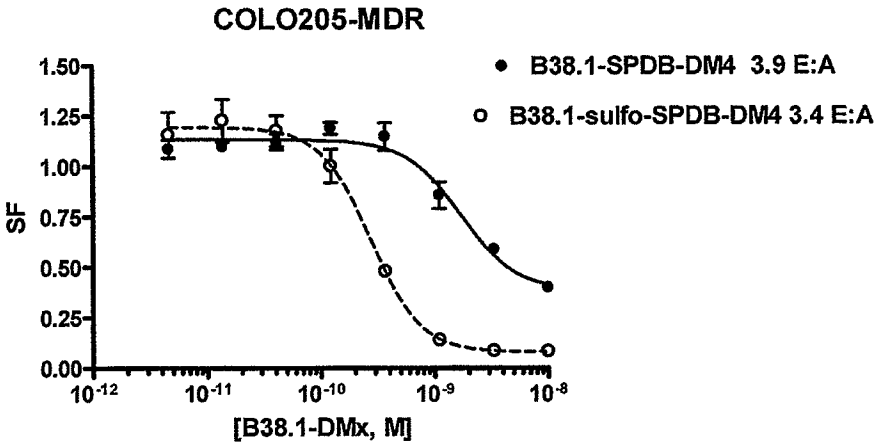


Figure 64

Cytotoxicity of Anti-EpCAM (B38.1)- maytansinoid conjugates with or without sulfonate linker toward multi-drug resistant HCT15 cells.

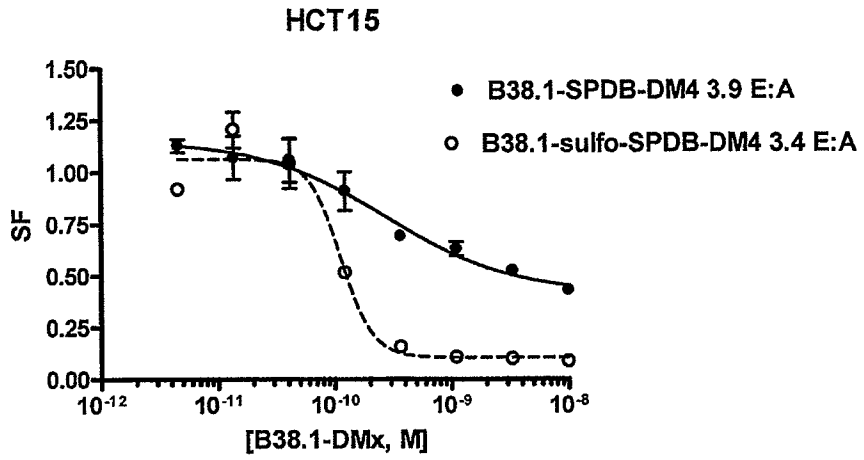


Figure 65

Cytotoxicity of Anti-EpCAM (B38.1) - maytansinoid conjugates with or without sulfonate linker toward multi-drug resistant COLO205-MDR cells.

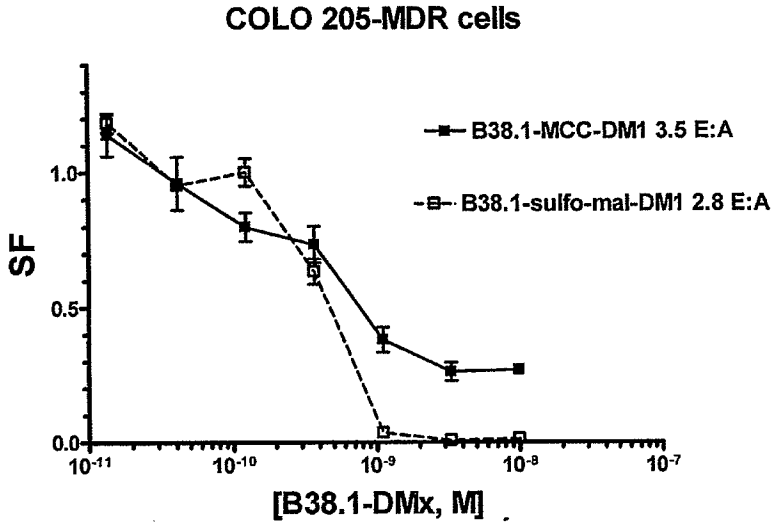
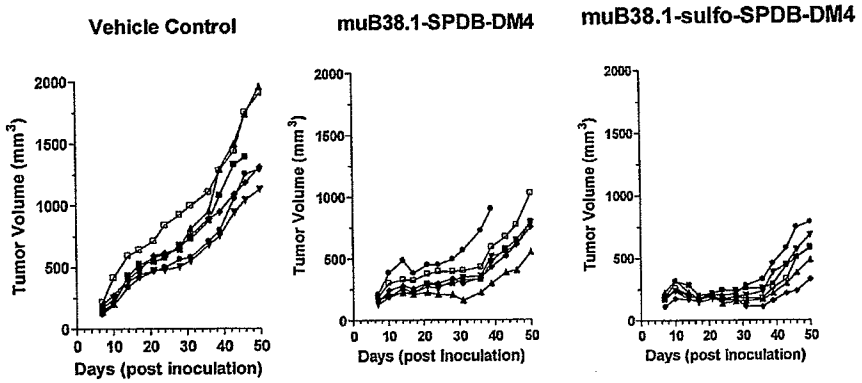


Figure 66

In vivo anti-tumor activity of anti-EpCAM antibody-maytansinoid conjugates on COLO205 *mdr* xenografts (individual tumors).



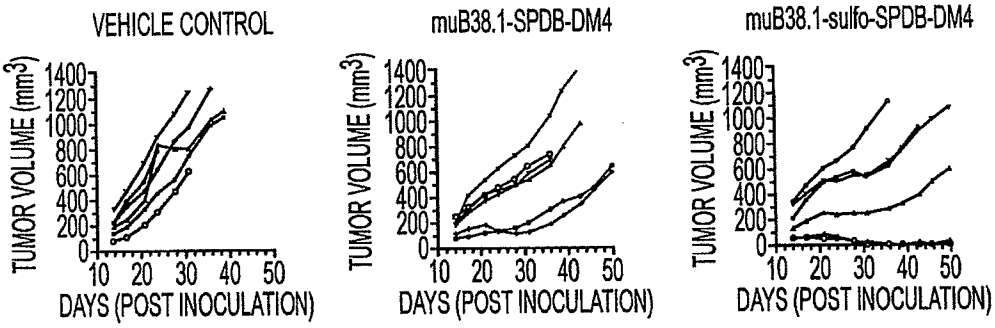

VEHICLE CONTROL
muB38.1-SPDB-DM4
muB38.1-sulfo-SPDB-DM4
TUMOR VOLUME (mm3)
DAYS (POST INOCULATION)

FIG. 67

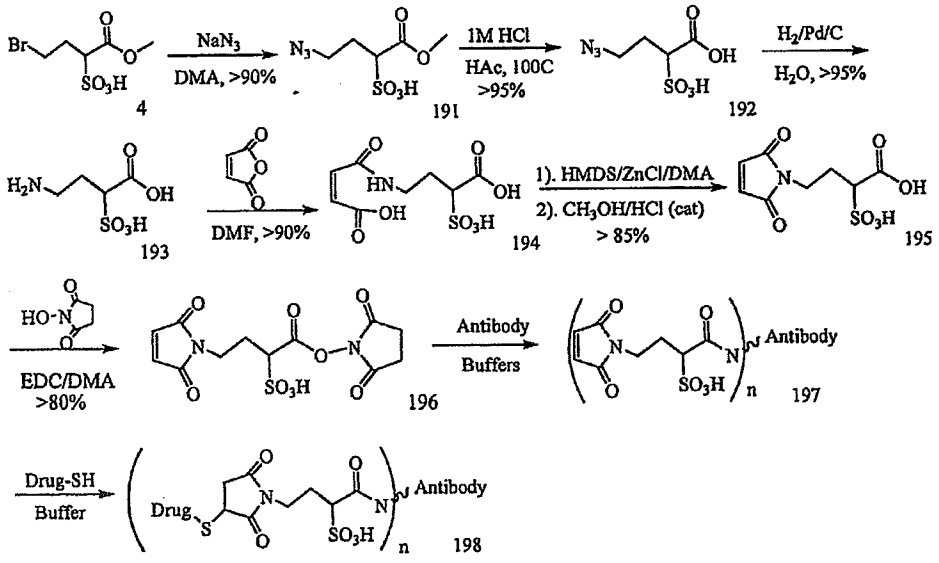

NaN3
DMA, >90%
1M HCl
HAc, 100C
>95%
H2/Pd/C
H2O, >95%
DMF, >90%
1). HMDS/ZnCl/DMA
2). CH3OH/HCl (cat)
> 85%
HO-N
EDC/DMA
>80%
Antibody
Buffers
Drug-SH
Buffer
Drug
4
191
192
193
194
195
196
197
198

FIG. 68

Figure 69

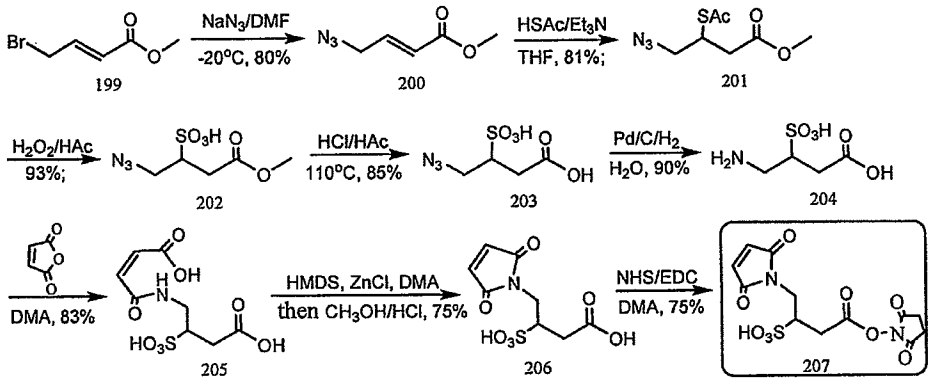


Figure 70

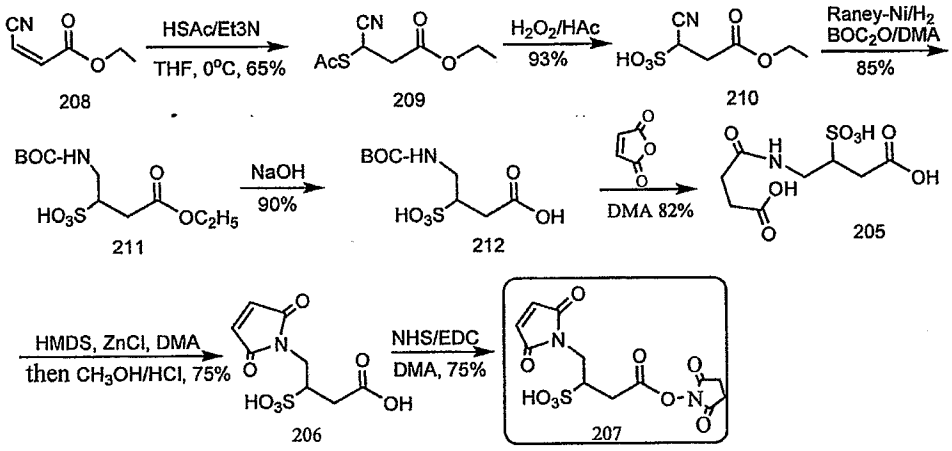
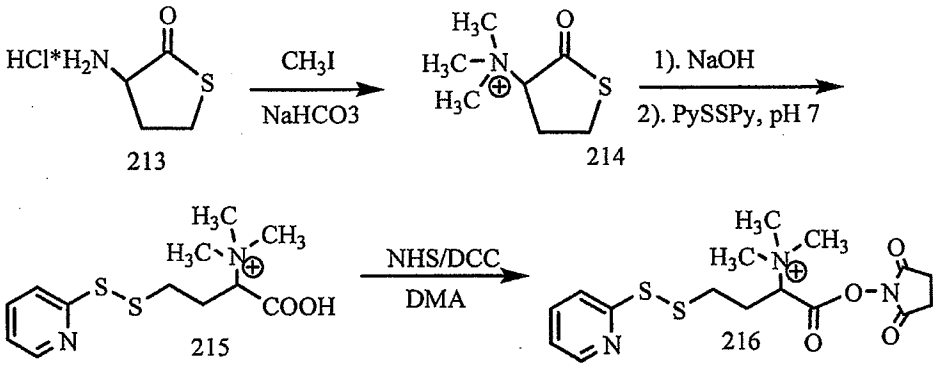
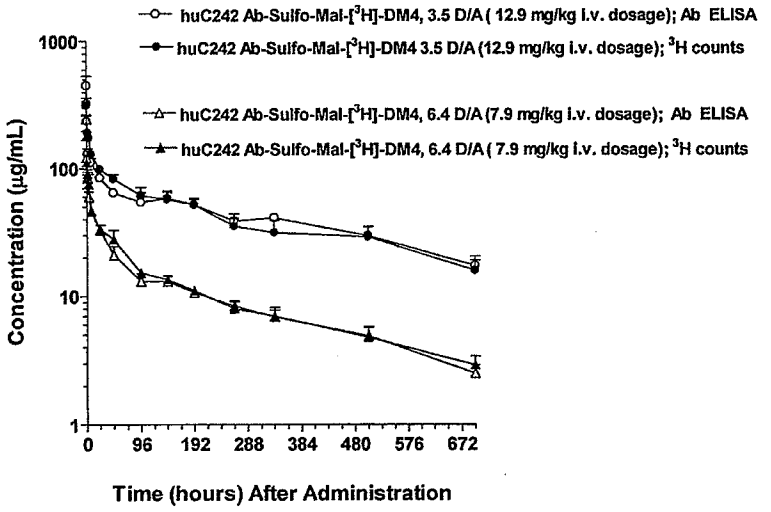


Figure 71

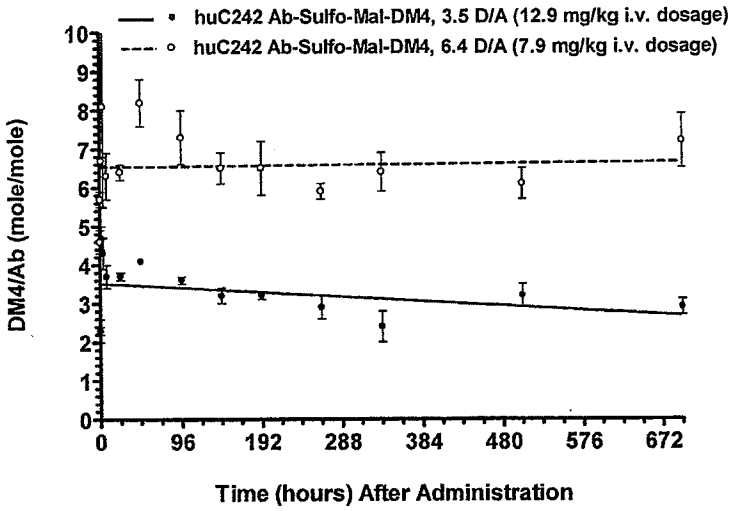

HCl*H2N
O
S
213
CH3I
NaHCO3
H3C
H3C–N
H3C
O
S
214
1). NaOH
2). PySSPy, pH 7
H3C
H3C–N
CH3
S
S
N
COOH
215
NHS/DCC
DMA
H3C
H3C–N
CH3
S
S
N
O
O–N
O
O
216

FIGURES 72 (A) and (B). Plasma pharmacokinetics of huC242 Antibody-Sulfo-Mal-[^{3}H-labeled]-DM4 conjugates with 3.5 DM4/Ab or 6.4 DM4/Ab dosed at 12.9 mg/kg and 7.9 mg/kg (i.v.) respectively in CD-1 mice. A. Ab concentrations (measured by ELISA or by 3H counts) versus time after administration. B. Maytansinoid (DM4)/Antibody (Ab) ratio versus time after administration.

FIGURE 72(A)



. FIGURE 72 (B)


huC242 Ab-Sulfo-Mal-DM4, 3.5 D/A (12.9 mg/kg i.v. dosage)
huC242 Ab-Sulfo-Mal-DM4, 6.4 D/A (7.9 mg/kg i.v. dosage)
DM4/Ab (mole/mole)
0
1
2
3
4
5
6
7
8
9
10
0
96
192
288
384
480
576
672
Time (hours) After Administration