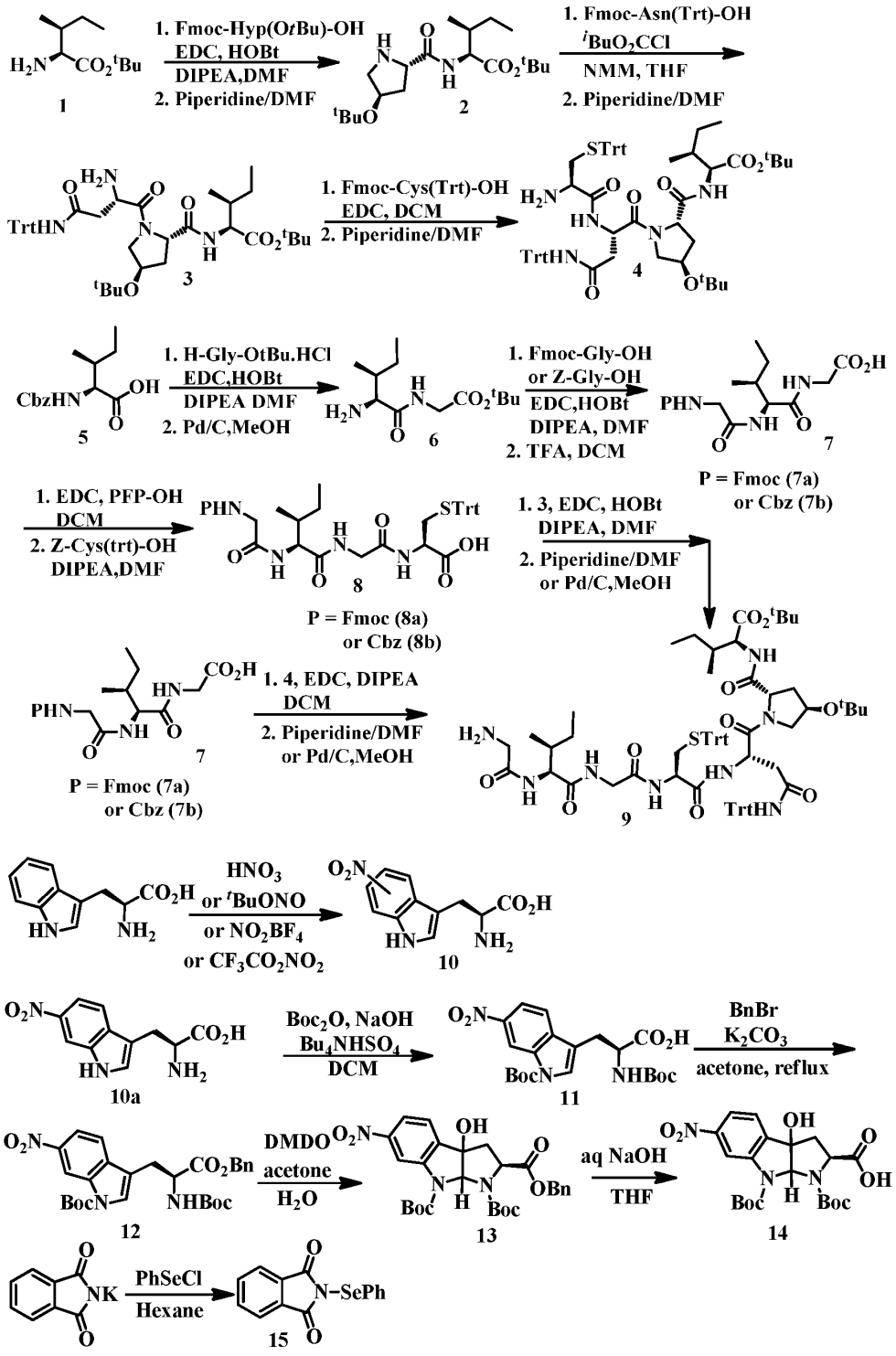

1. Fmoc-Hyp(OtBu)-OH
EDC, HOBt
DIPEA,DMF
2. Piperidine/DMF
1. Fmoc-Asn(Trt)-OH
iBuO2CCl
NMM, THF
2. Piperidine/DMF
1. Fmoc-Cys(Trt)-OH
EDC, DCM
2. Piperidine/DMF
1. H-Gly-OtBu.HCl
EDC,HOBt
DIPEA DMF
2. Pd/C,MeOH
1. Fmoc-Gly-OH
or Z-Gly-OH
EDC,HOBt
DIPEA, DMF
2. TFA, DCM
P = Fmoc (7a)
or Cbz (7b)
1. EDC, PFP-OH
DCM
2. Z-Cys(trt)-OH
DIPEA,DMF
P = Fmoc (8a)
or Cbz (8b)
1. 3, EDC, HOBt
DIPEA, DMF
2. Piperidine/DMF
or Pd/C,MeOH
1. 4, EDC, DIPEA
DCM
2. Piperidine/DMF
or Pd/C,MeOH
HNO3
or tBuONO
or NO2BF4
or CF3CO2NO2
Boc2O, NaOH
Bu4NHSO4
DCM
BnBr
K2CO3
acetone, reflux
DMDO
acetone
H2O
aq NaOH
THF
PhSeCl
Hexane

Fig. 1

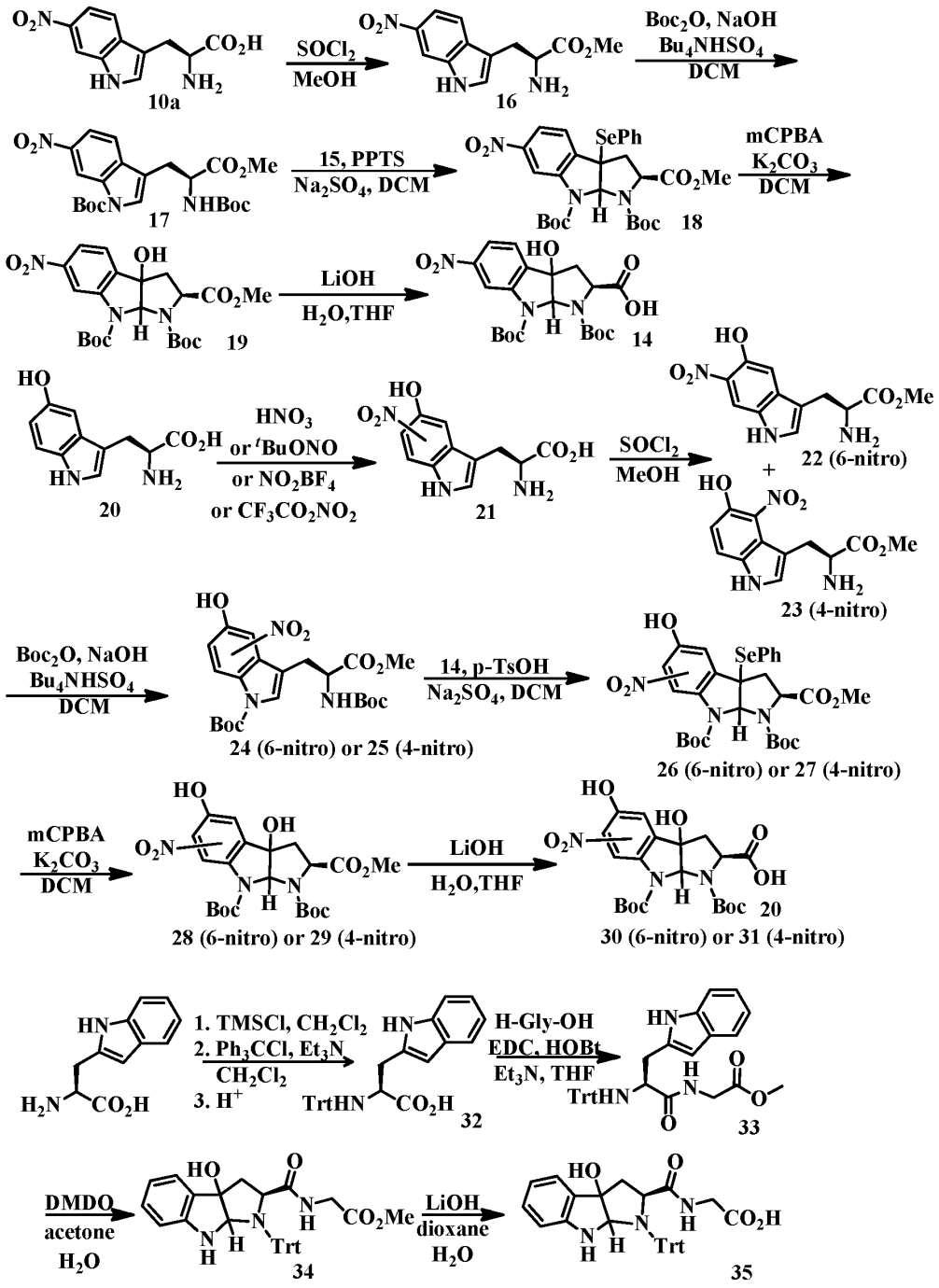

10a
SOCl2
MeOH
16
Boc2O, NaOH
Bu4NHSO4
DCM
17
15, PPTS
Na2SO4, DCM
18
mCPBA
K2CO3
DCM
19
LiOH
H2O,THF
14
20
HNO3
or tBuONO
or NO2BF4
or CF3CO2NO2
21
22 (6-nitro)
23 (4-nitro)
24 (6-nitro) or 25 (4-nitro)
14, p-TsOH
Na2SO4, DCM
26 (6-nitro) or 27 (4-nitro)
28 (6-nitro) or 29 (4-nitro)
30 (6-nitro) or 31 (4-nitro)
1. TMSCl, CH2Cl2
2. Ph3CCl, Et3N
CH2Cl2
3. H+
32
H-Gly-OH
EDC, HOBt
Et3N, THF
33
DMDO
acetone
H2O
34
LiOH
dioxane
H2O
35

Fig. 2

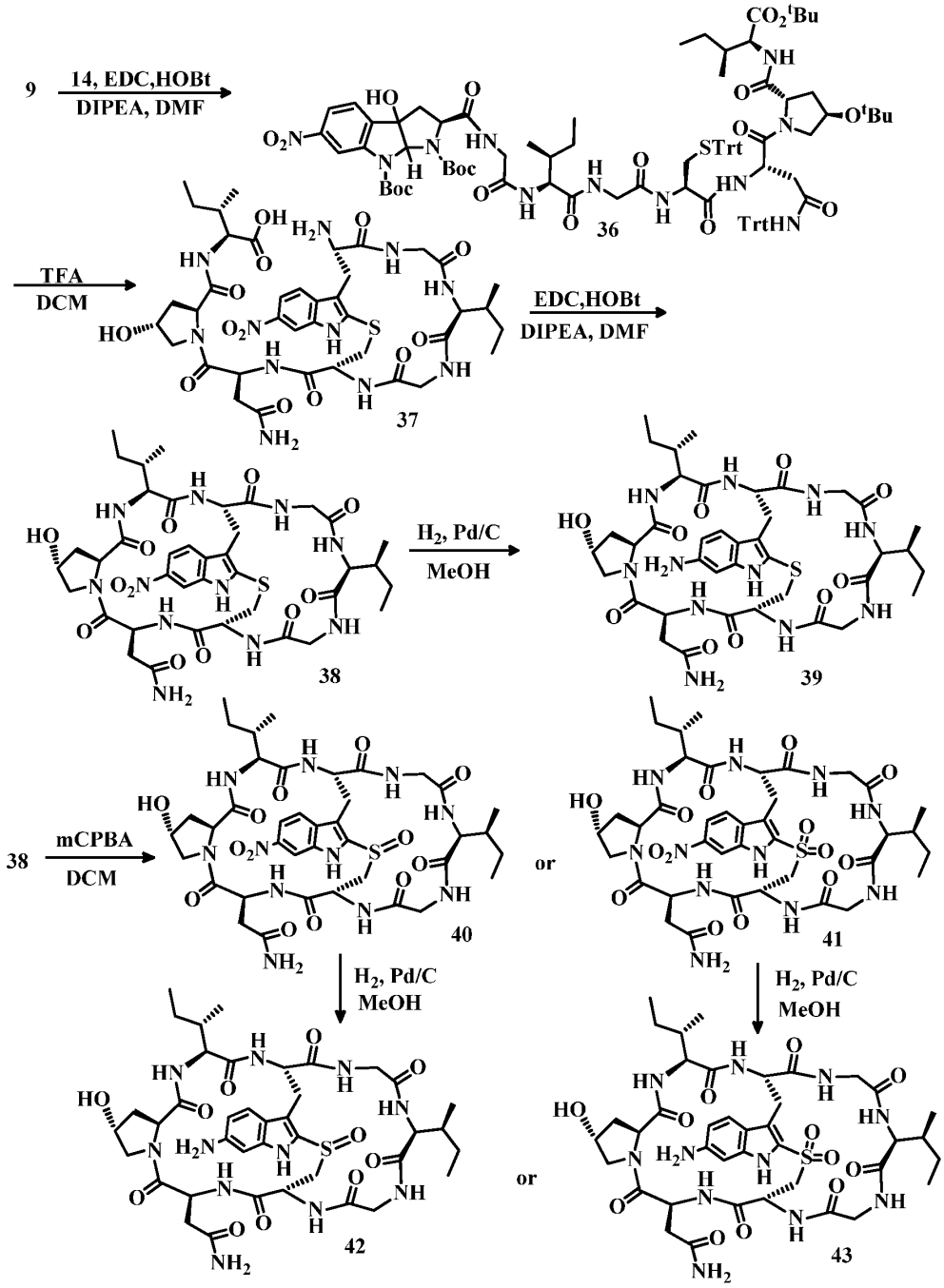

9
14, EDC,HOBt
DIPEA, DMF
36
TFA
DCM
37
EDC,HOBt
DIPEA, DMF
38
H2, Pd/C
MeOH
39
38
mCPBA
DCM
40
or
41
H2, Pd/C
MeOH
H2, Pd/C
MeOH
42
or
43

Fig. 3

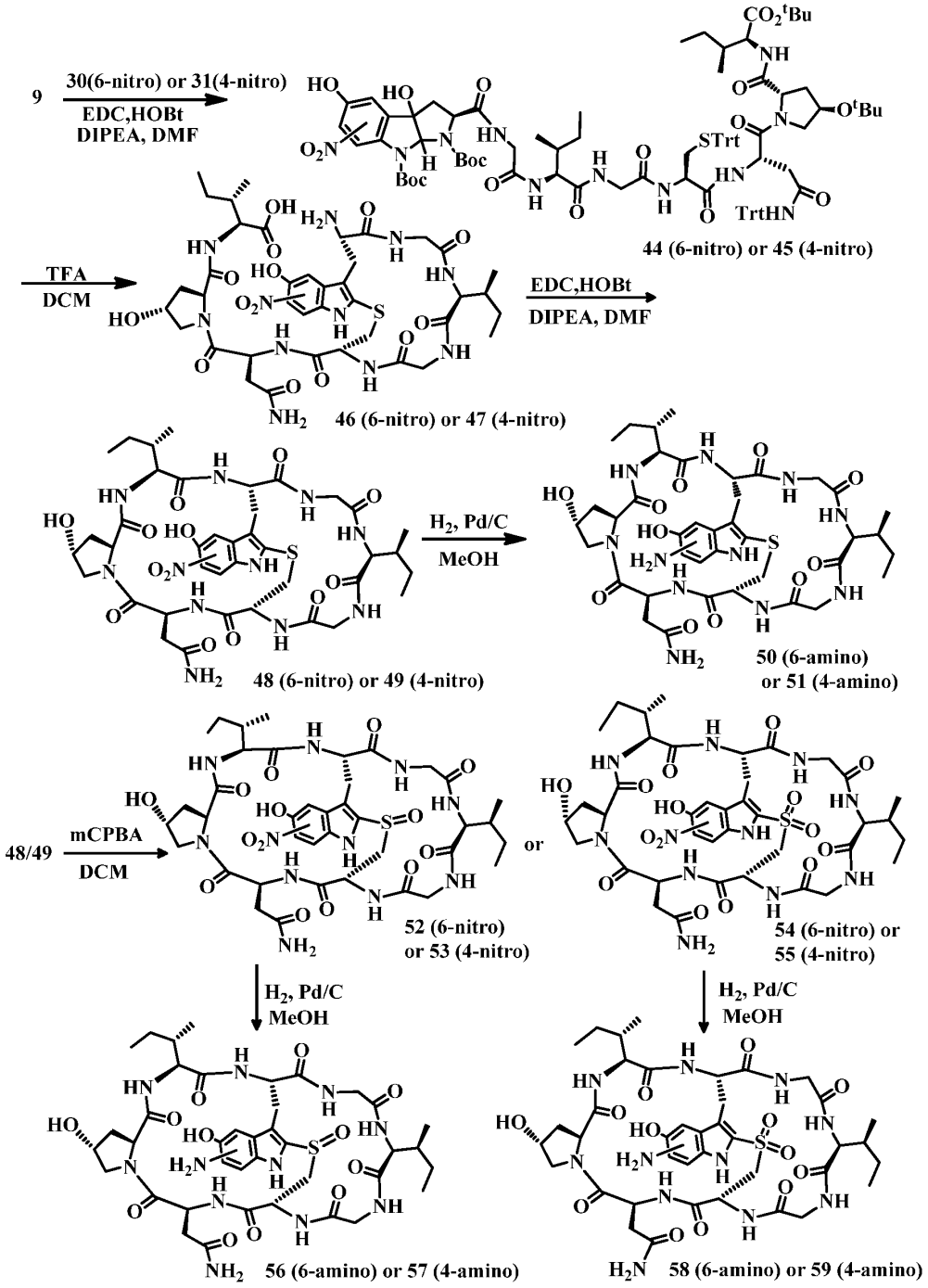

9
30(6-nitro) or 31(4-nitro)
EDC,HOBt
DIPEA, DMF
44 (6-nitro) or 45 (4-nitro)
TFA
DCM
EDC,HOBt
DIPEA, DMF
46 (6-nitro) or 47 (4-nitro)
H2, Pd/C
MeOH
48 (6-nitro) or 49 (4-nitro)
50 (6-amino)
or 51 (4-amino)
48/49
mCPBA
DCM
or
52 (6-nitro)
or 53 (4-nitro)
54 (6-nitro) or
55 (4-nitro)
H2, Pd/C
MeOH
H2, Pd/C
MeOH
56 (6-amino) or 57 (4-amino)
58 (6-amino) or 59 (4-amino)

Fig. 4

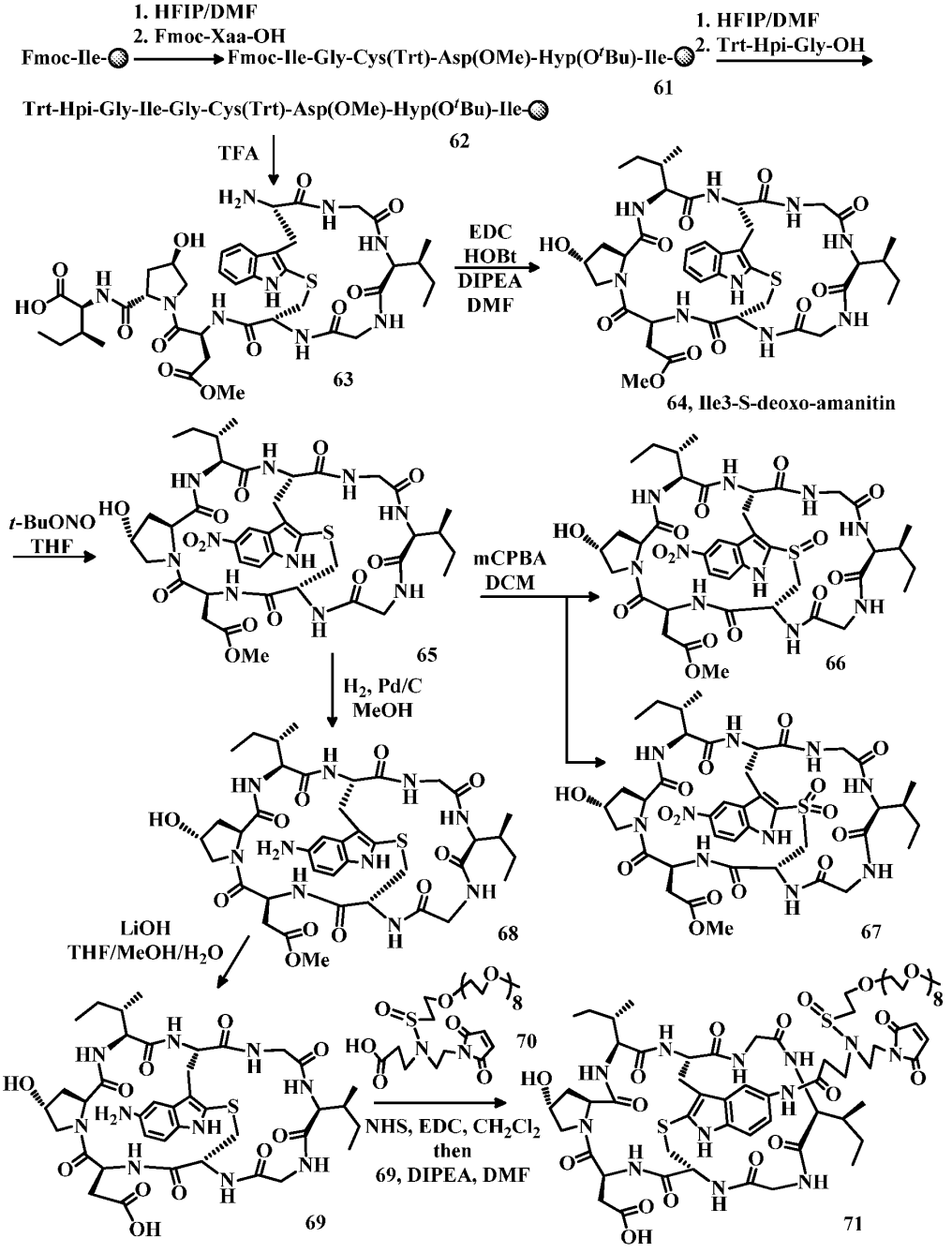

1. HFIP/DMF
2. Fmoc-Xaa-OH
Fmoc-Ile-
Fmoc-Ile-Gly-Cys(Trt)-Asp(OMe)-Hyp(O^{t}Bu)-Ile-
1. HFIP/DMF
2. Trt-Hpi-Gly-OH
61
Trt-Hpi-Gly-Ile-Gly-Cys(Trt)-Asp(OMe)-Hyp(O^{t}Bu)-Ile-
62
TFA
63
EDC
HOBt
DIPEA
DMF
64, Ile3-S-deoxo-amanitin
t-BuONO
THF
65
mCPBA
DCM
66
67
H2, Pd/C
MeOH
68
LiOH
THF/MeOH/H2O
69
70
NHS, EDC, CH2Cl2
then
69, DIPEA, DMF
71

Fig. 5

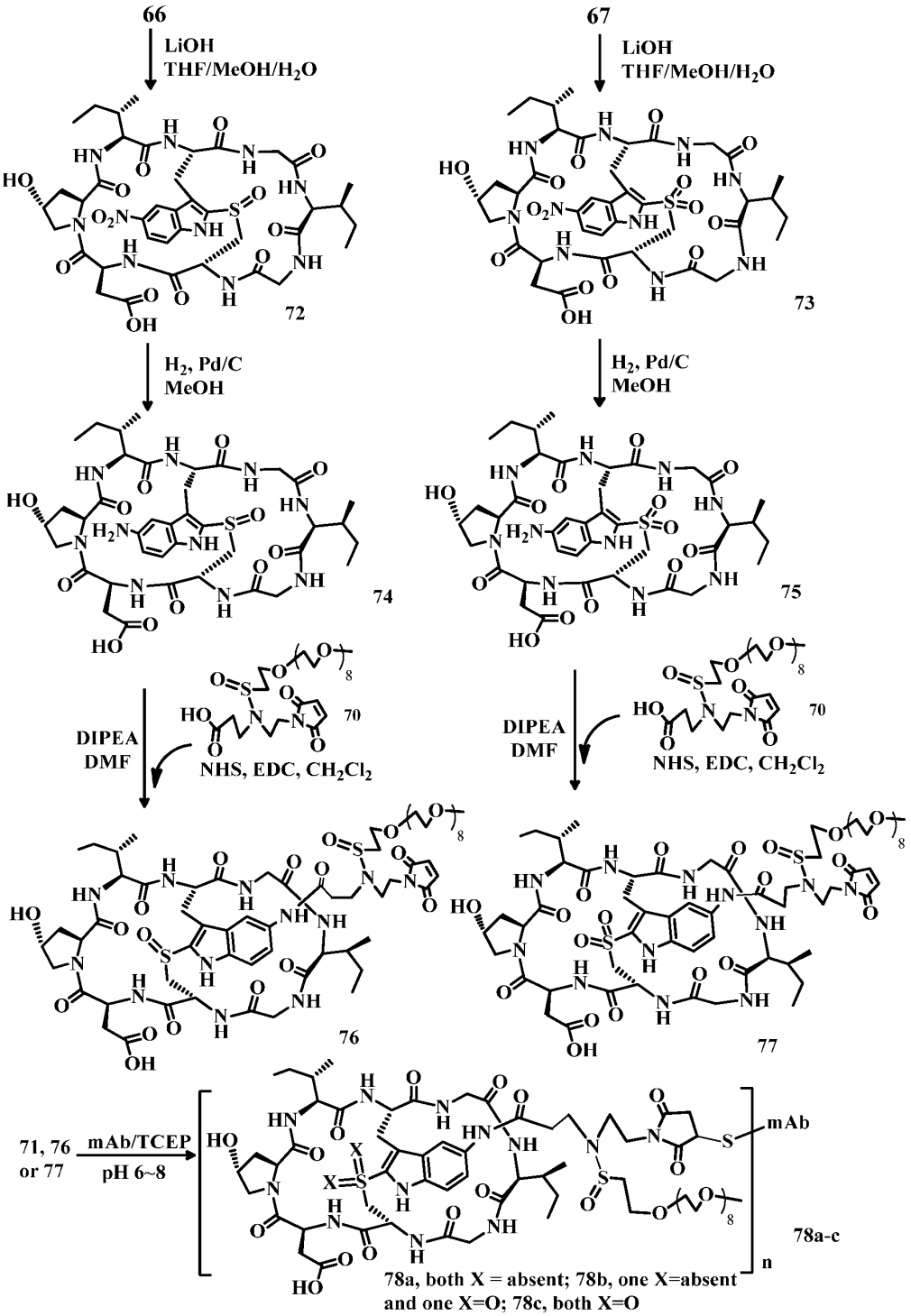

66
LiOH
THF/MeOH/H2O
72
67
LiOH
THF/MeOH/H2O
73
H2, Pd/C
MeOH
74
H2, Pd/C
MeOH
75
DIPEA
DMF
NHS, EDC, CH2Cl2
70
DIPEA
DMF
NHS, EDC, CH2Cl2
70
76
77
71, 76
or 77
mAb/TCEP
pH 6~8
mAb
78a-c
n
78a, both X = absent; 78b, one X=absent
and one X=O; 78c, both X=O

Fig. 6

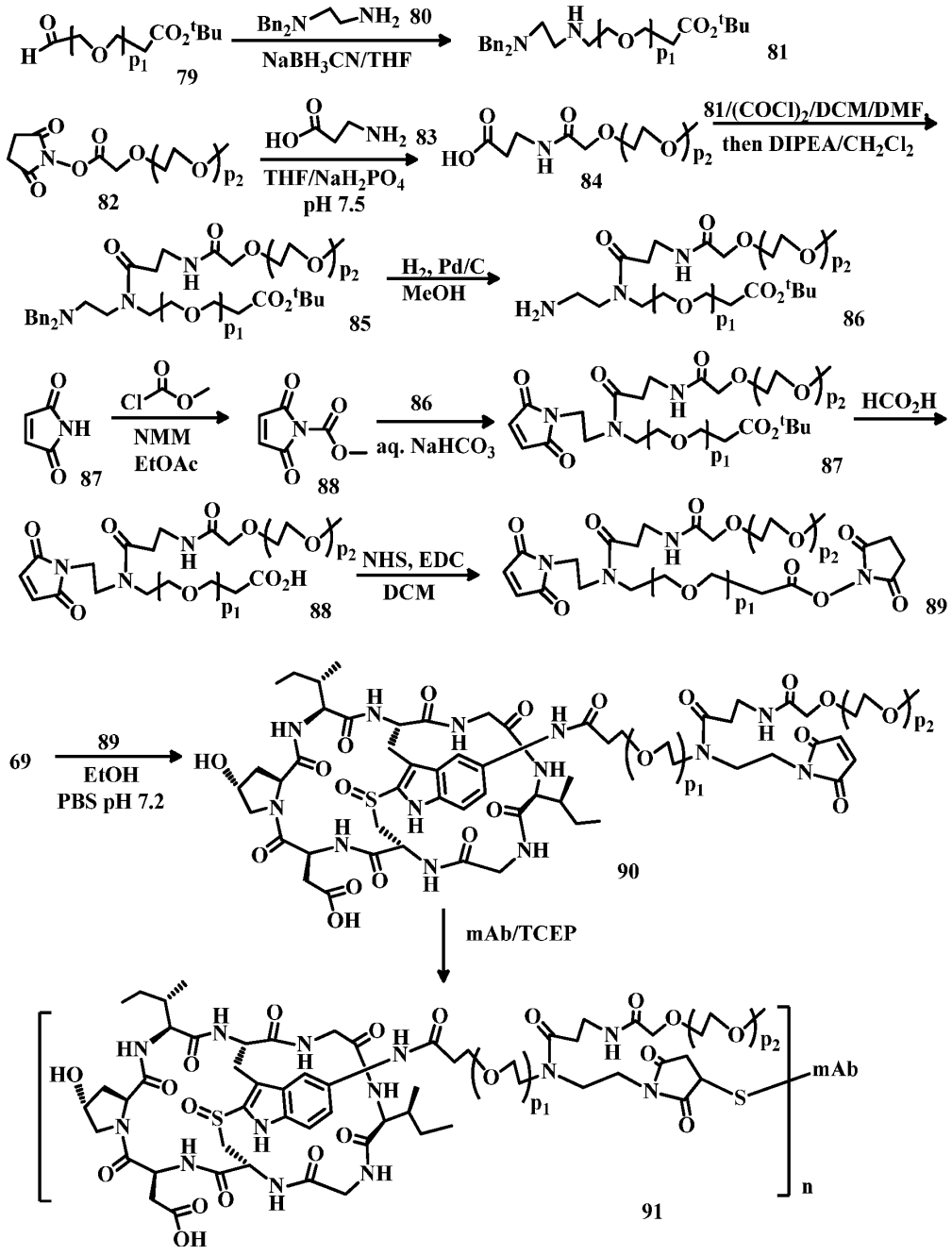

Bn2N NH2 80
NaBH3CN/THF
79
81
HO NH2 83
THF/NaH2PO4
pH 7.5
82
84
81/(COCl)2/DCM/DMF
then DIPEA/CH2Cl2
H2, Pd/C
MeOH
85
86
Cl O
NMM
EtOAc
87
88
aq. NaHCO3
HCO2H
NHS, EDC
DCM
89
69
EtOH
PBS pH 7.2
90
mAb/TCEP
91
mAb
n

Fig. 7

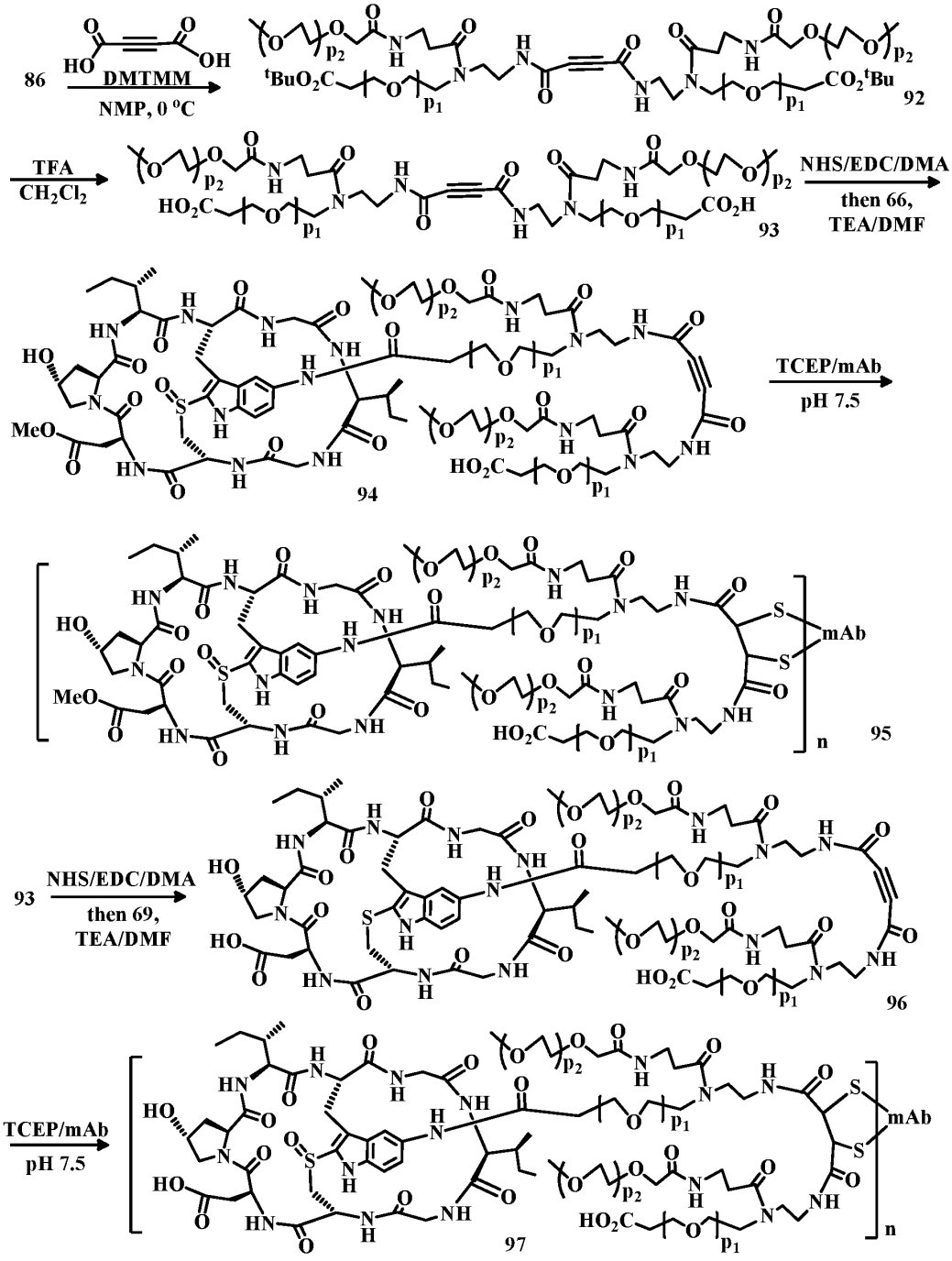

86
DMTMM
NMP, 0 °C
92
TFA
CH2Cl2
93
NHS/EDC/DMA
then 66,
TEA/DMF
94
TCEP/mAb
pH 7.5
95
mAb
n
NHS/EDC/DMA
then 69,
TEA/DMF
96
TCEP/mAb
pH 7.5
97

Fig. 8

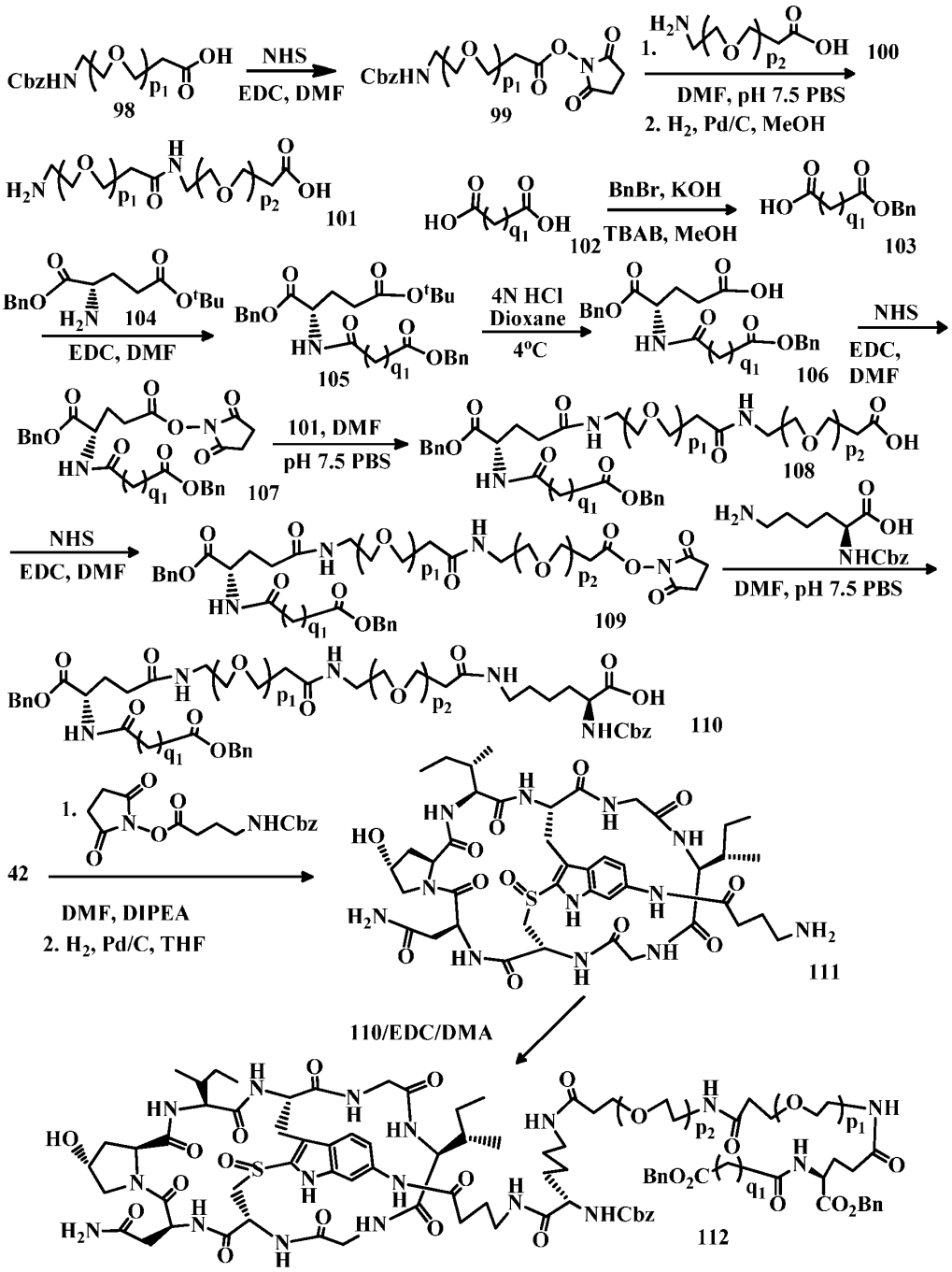

NHS
EDC, DMF
98
99
1.
DMF, pH 7.5 PBS
2. H2, Pd/C, MeOH
100
101
BnBr, KOH
TBAB, MeOH
102
103
EDC, DMF
104
105
4N HCl
Dioxane
4°C
106
NHS
EDC, DMF
107
101, DMF
pH 7.5 PBS
108
NHS
EDC, DMF
109
NHCbz
DMF, pH 7.5 PBS
110
42
DMF, DIPEA
2. H2, Pd/C, THF
111
110/EDC/DMA
112

Fig. 9

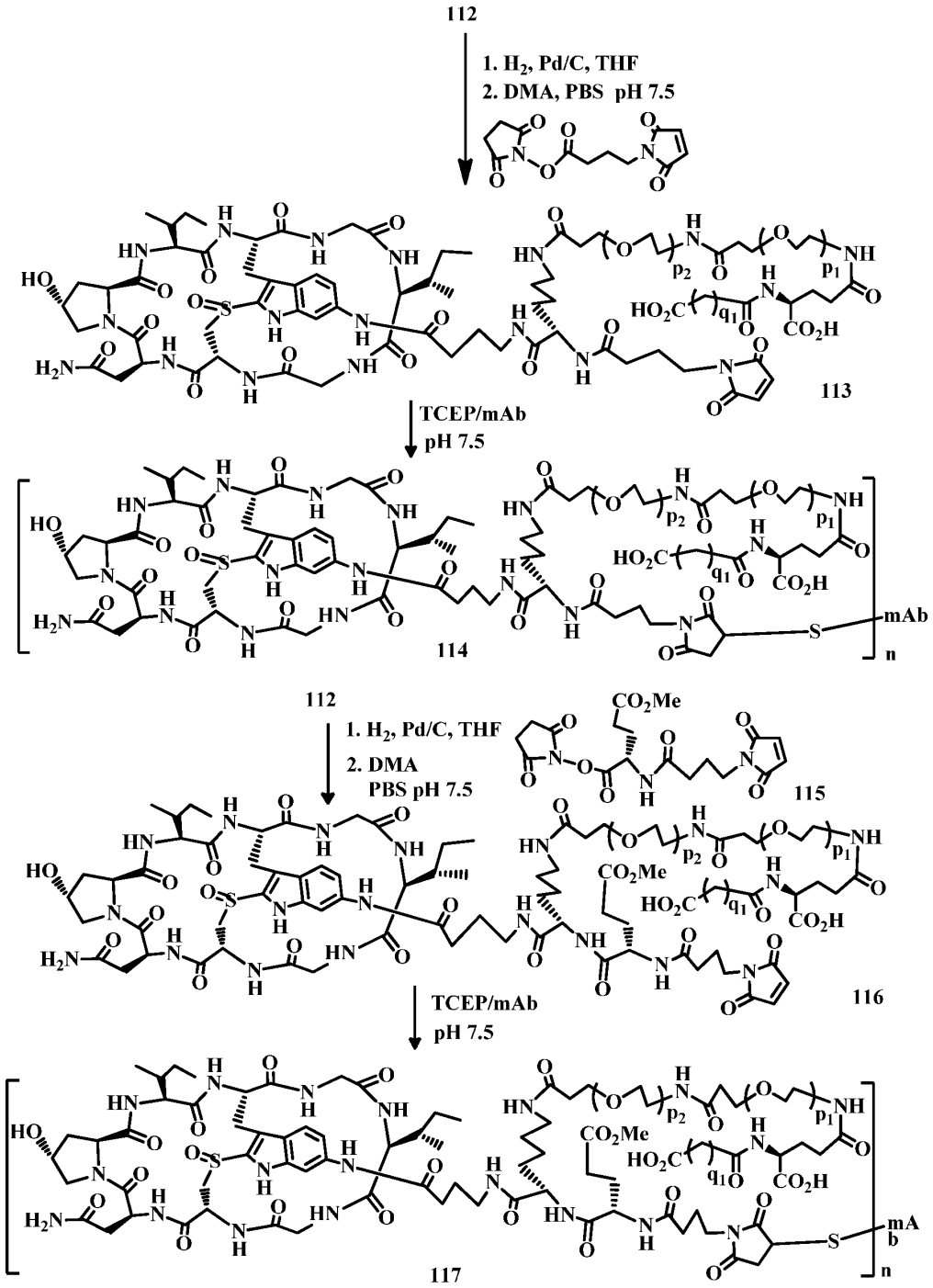

112
1. H2, Pd/C, THF
2. DMA, PBS pH 7.5
113
TCEP/mAb
pH 7.5
114
mAb
n
112
1. H2, Pd/C, THF
2. DMA
PBS pH 7.5
115
116
TCEP/mAb
pH 7.5
117
mAb
n

Fig. 10

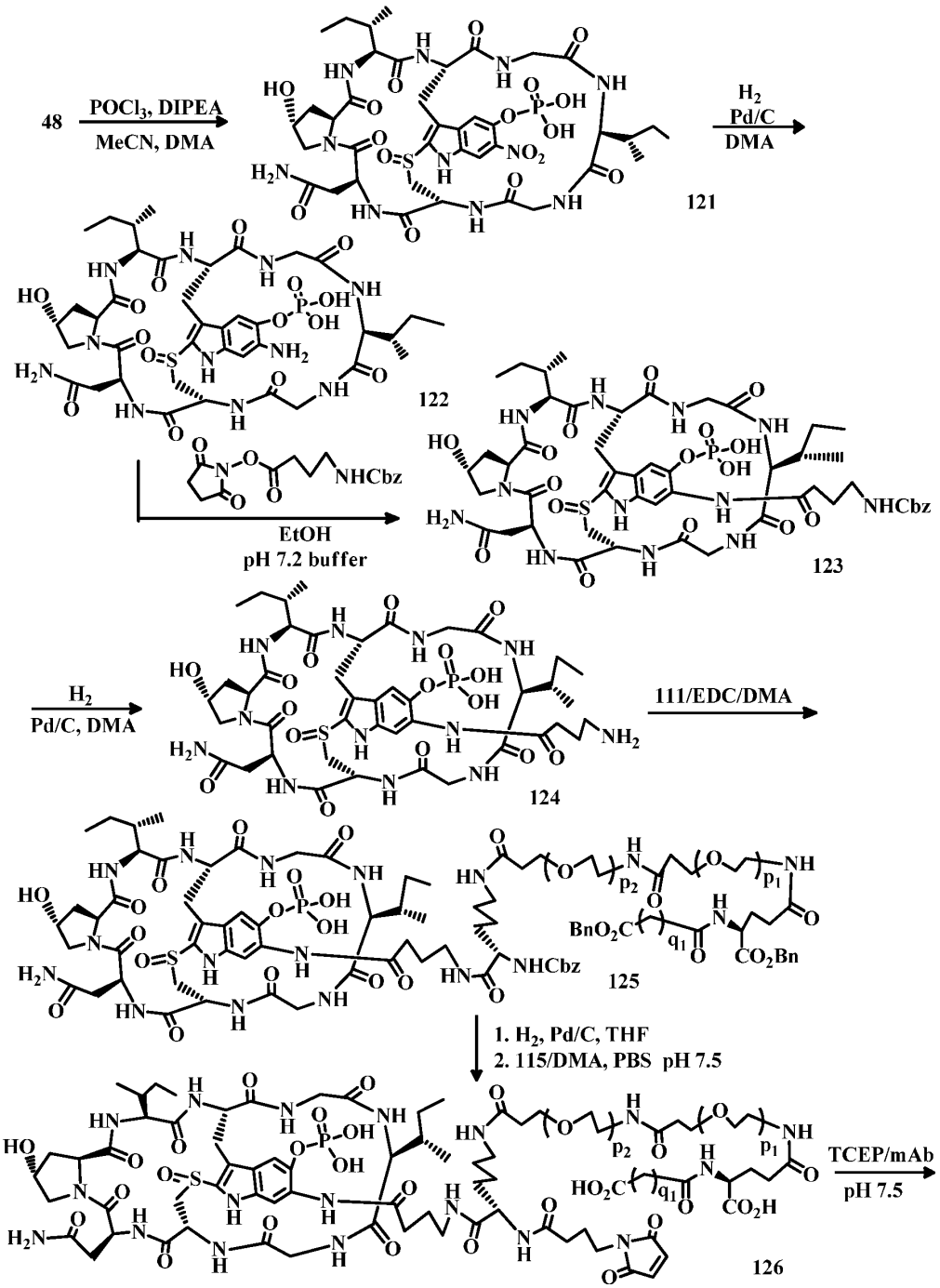

48
POCl3, DIPEA
MeCN, DMA
121
H2
Pd/C
DMA
122
NHCbz
EtOH
pH 7.2 buffer
123
H2
Pd/C, DMA
124
111/EDC/DMA
125
1. H2, Pd/C, THF
2. 115/DMA, PBS pH 7.5
126
TCEP/mAb
pH 7.5

Fig. 11

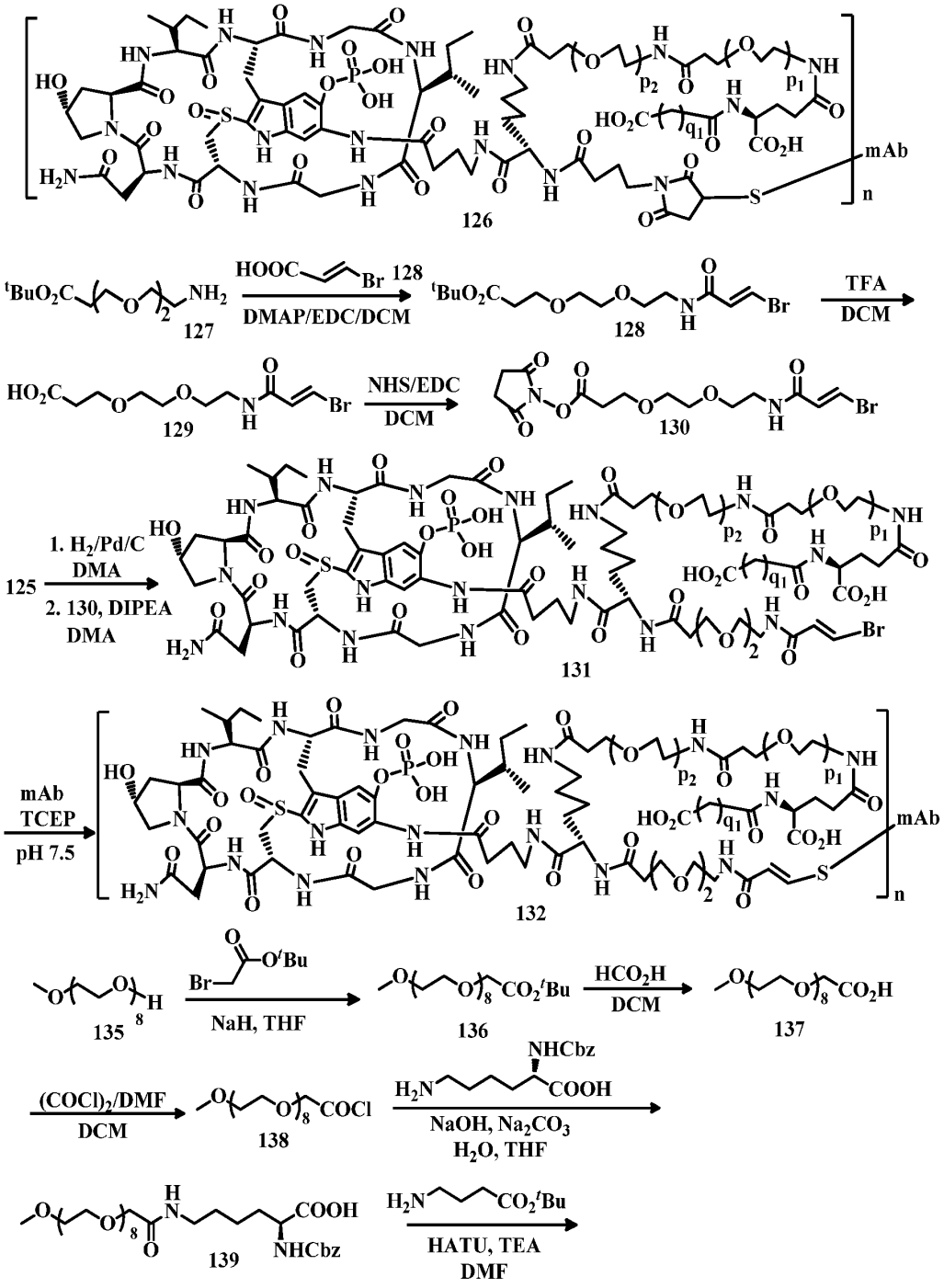

126
HOOC
Br
128
DMAP/EDC/DCM
127
TFA
DCM
NHS/EDC
DCM
129
130
1. H2/Pd/C
DMA
125
2. 130, DIPEA
DMA
131
mAb
TCEP
pH 7.5
132
NaH, THF
135
136
HCO2H
DCM
137
(COCl)2/DMF
DCM
138
NaOH, Na2CO3
H2O, THF
139
HATU, TEA
DMF

Fig. 12

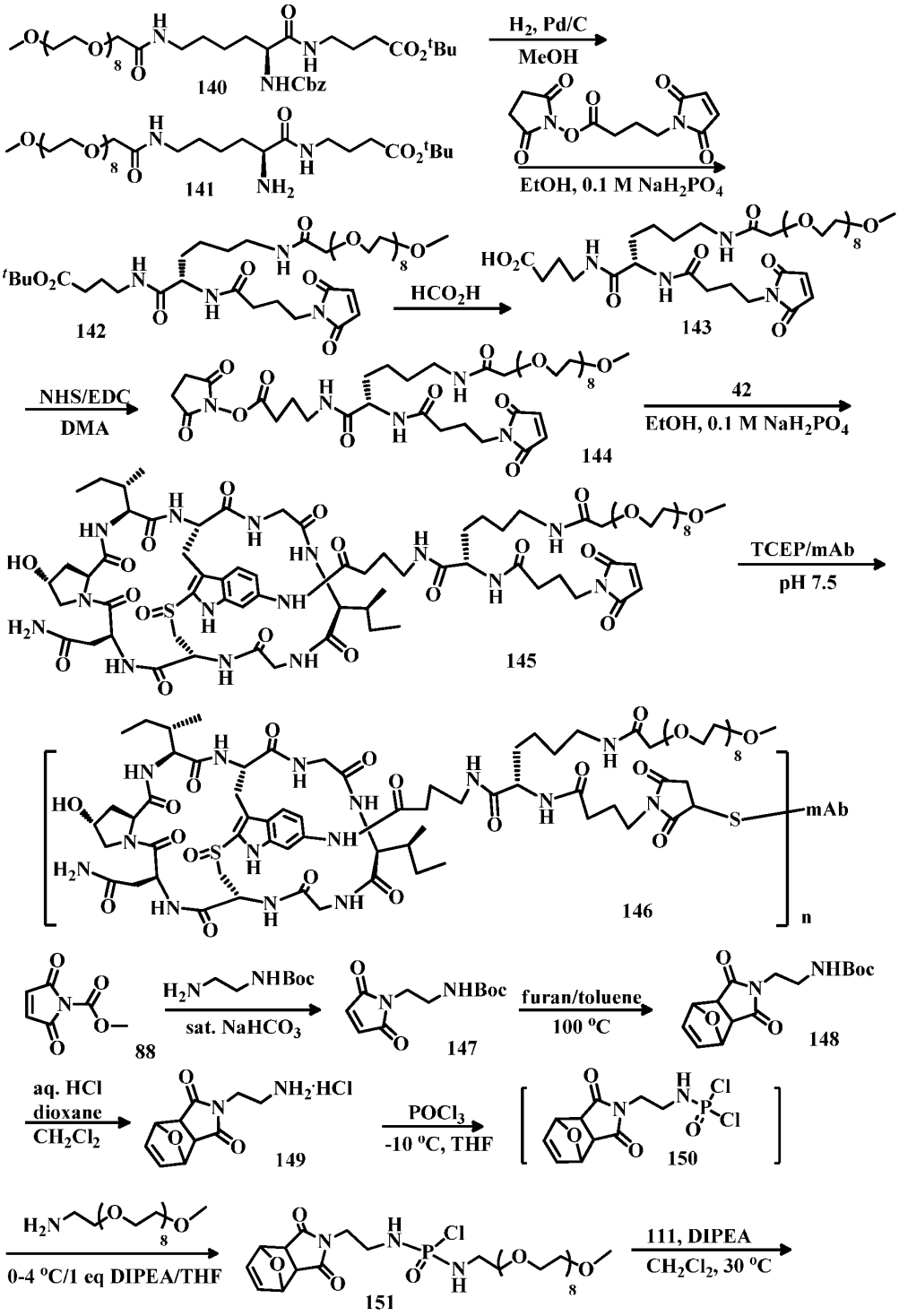

140
141
142
143
144
145
146
147
148
149
150
151
88
H_2, Pd/C
MeOH
EtOH, 0.1 M NaH_2PO_4
HCO_2H
NHS/EDC
DMA
42
EtOH, 0.1 M NaH_2PO_4
TCEP/mAb
pH 7.5
mAb
furan/toluene
100 °C
sat. $NaHCO_3$
aq. HCl
dioxane
CH_2Cl_2
$POCl_3$
-10 °C, THF
0-4 °C/1 eq DIPEA/THF
111, DIPEA
CH_2Cl_2, 30 °C

Fig. 13

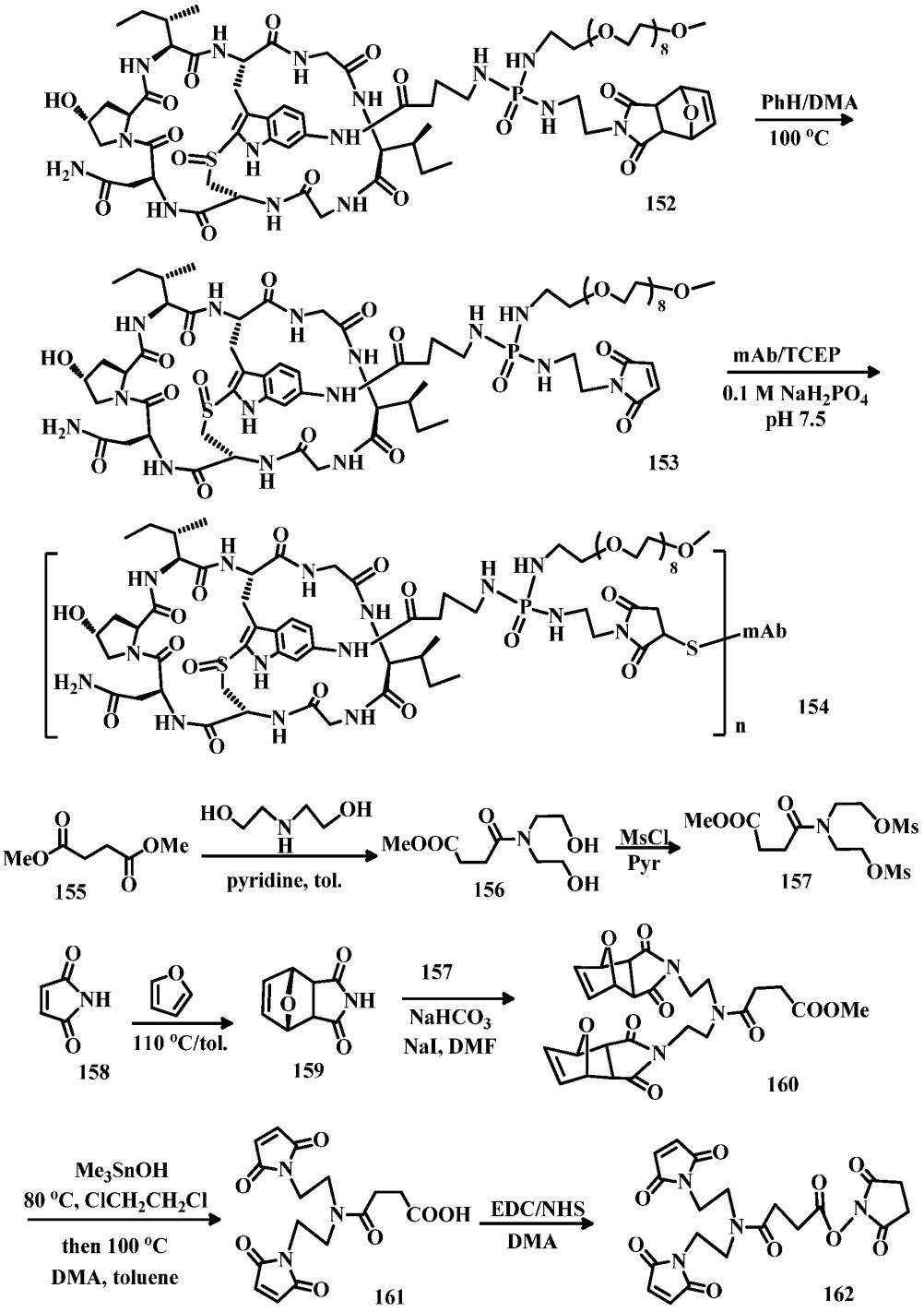

PhH/DMA
100 °C
152
mAb/TCEP
0.1 M NaH2PO4
pH 7.5
153
mAb
n
154
MeO
OMe
155
pyridine, tol.
MeOOC
OH
156
MsCl
Pyr
OMs
157
158
110 °C/tol.
159
NaHCO3
NaI, DMF
COOMe
160
Me3SnOH
80 °C, ClCH2CH2Cl
then 100 °C
DMA, toluene
COOH
161
EDC/NHS
DMA
162

Fig. 14

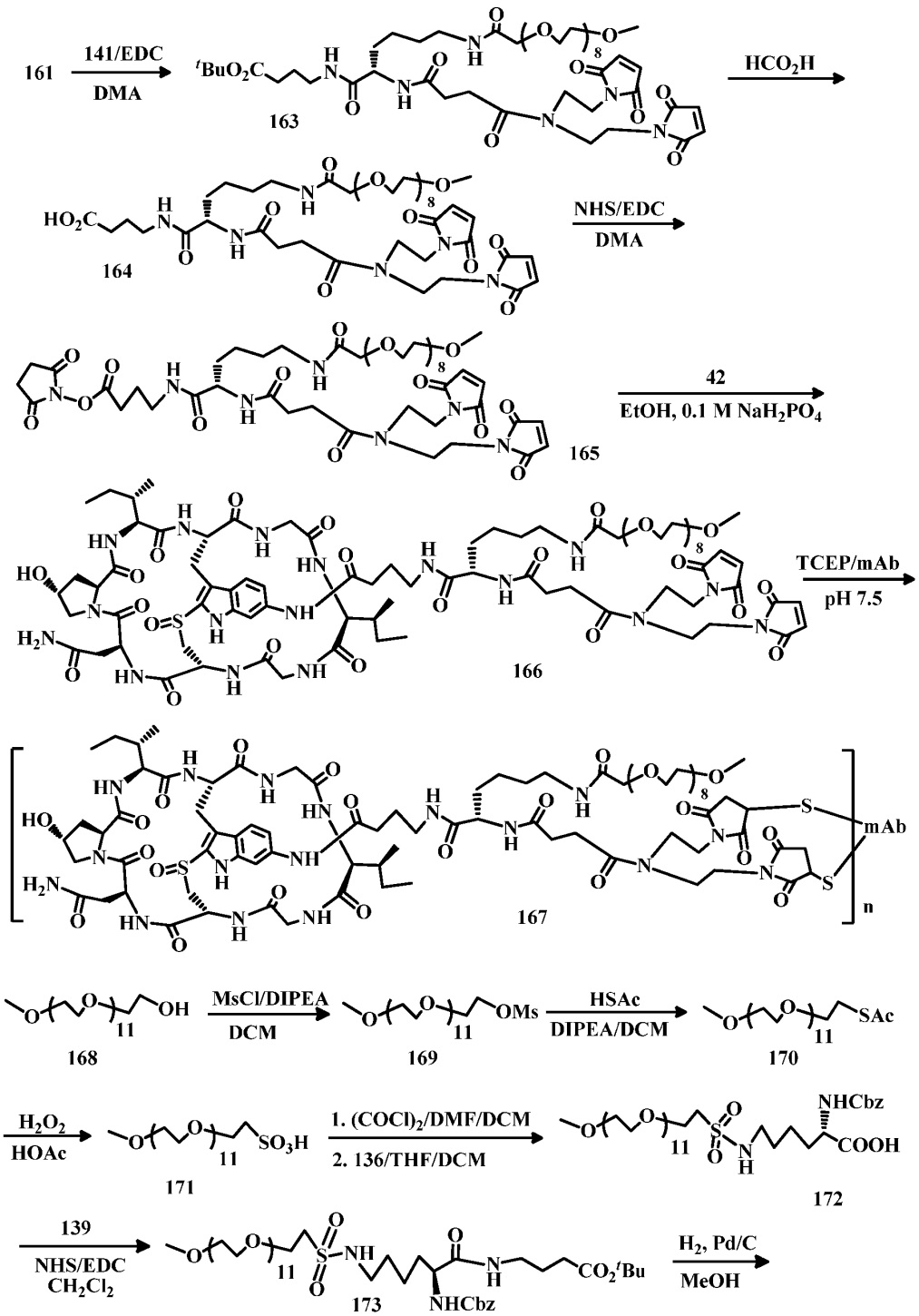

161
141/EDC
DMA
163
HCO2H
164
NHS/EDC
DMA
165
42
EtOH, 0.1 M NaH2PO4
166
TCEP/mAb
pH 7.5
167
mAb
168
MsCl/DIPEA
DCM
169
HSAc
DIPEA/DCM
170
H2O2
HOAc
171
1. (COCl)2/DMF/DCM
2. 136/THF/DCM
172
139
NHS/EDC
CH2Cl2
173
H2, Pd/C
MeOH

Fig. 15

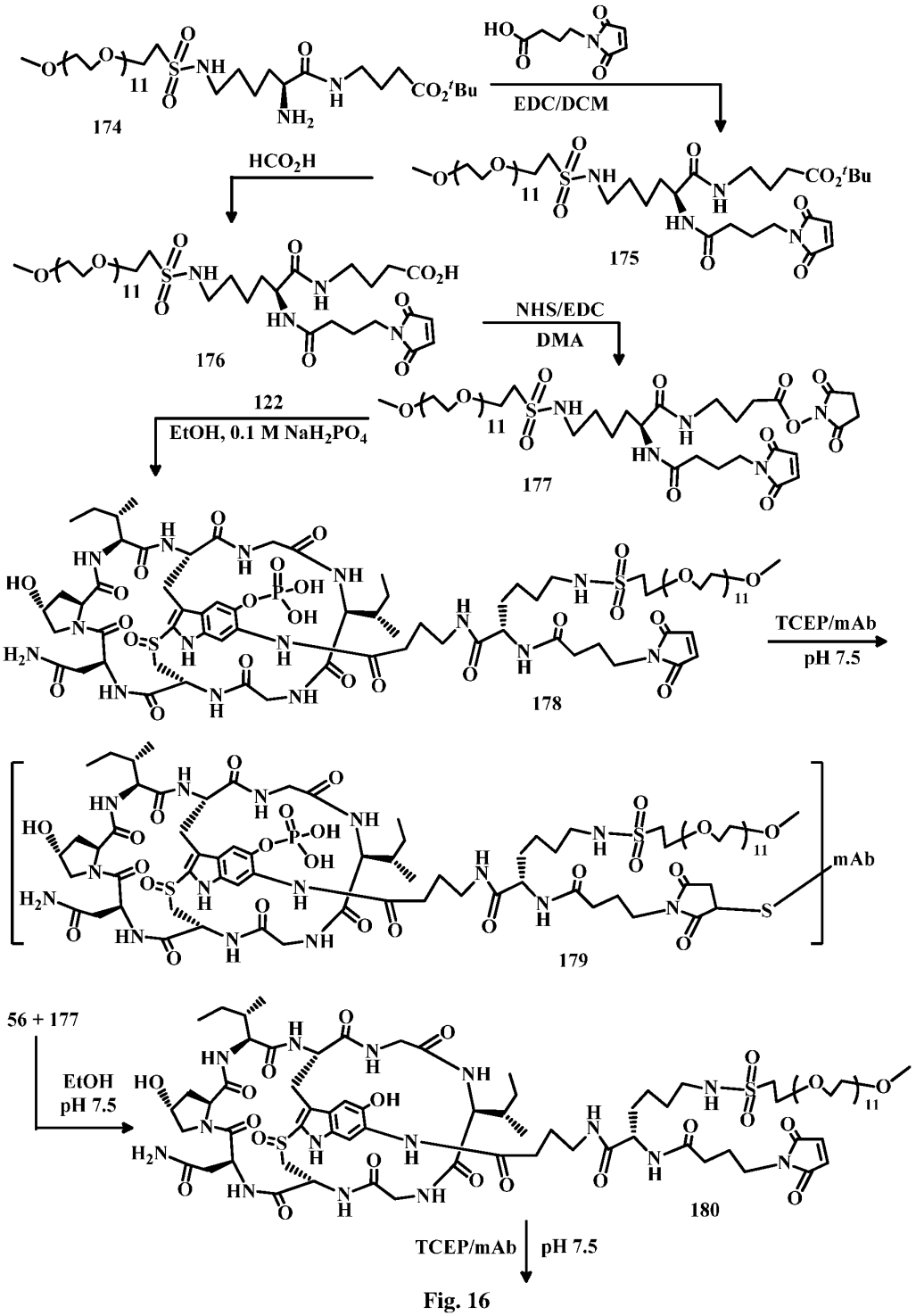

174
EDC/DCM
175
HCO2H
176
NHS/EDC
DMA
177
122
EtOH, 0.1 M NaH2PO4
178
TCEP/mAb
pH 7.5
179
mAb
56 + 177
EtOH
pH 7.5
180
TCEP/mAb
pH 7.5

Fig. 16

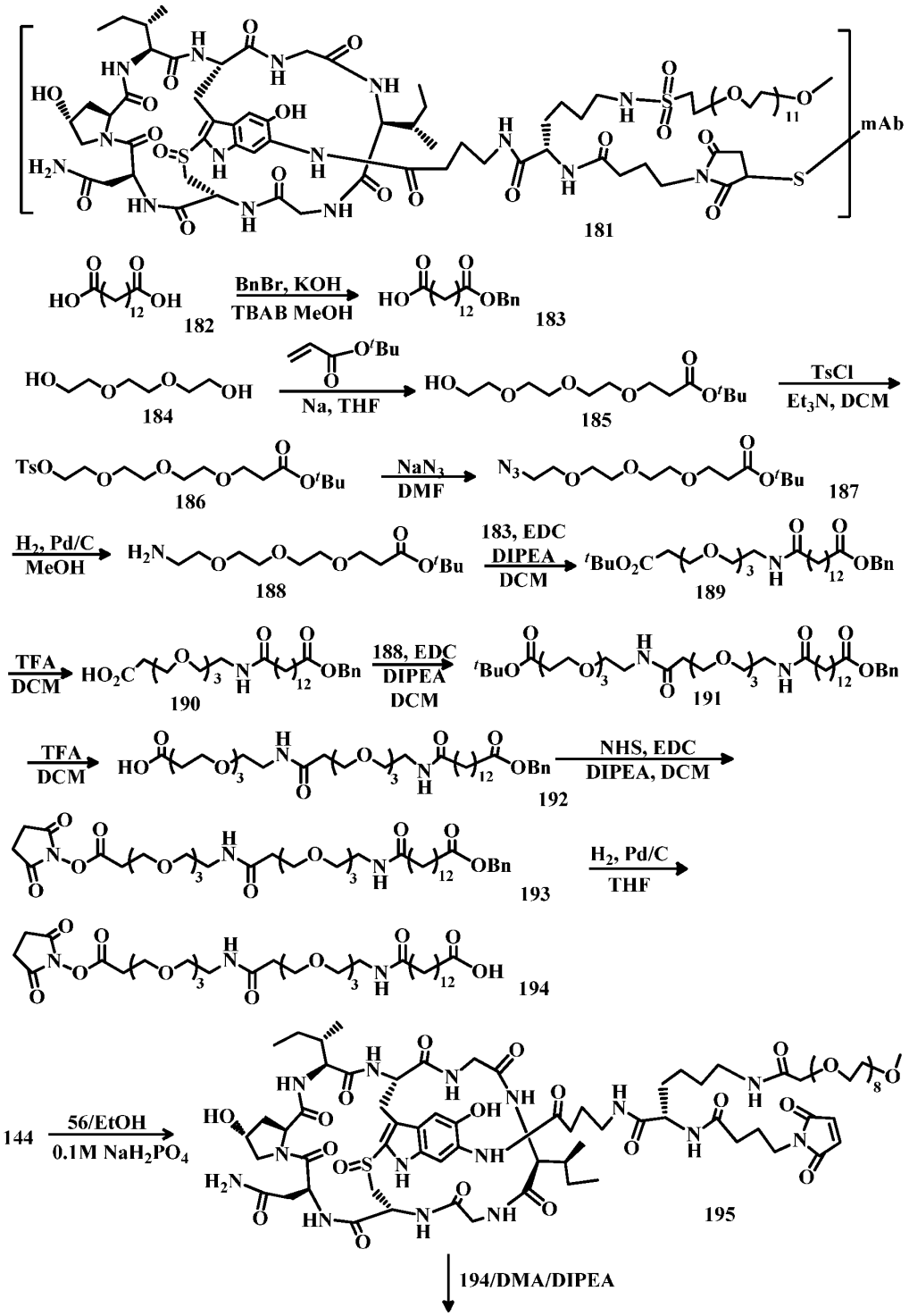

181
182
BnBr, KOH
TBAB MeOH
183
184
Na, THF
185
TsCl
Et3N, DCM
186
NaN3
DMF
187
H2, Pd/C
MeOH
188
183, EDC
DIPEA
DCM
189
TFA
DCM
190
188, EDC
DIPEA
DCM
191
TFA
DCM
192
NHS, EDC
DIPEA, DCM
193
H2, Pd/C
THF
194
144
56/EtOH
0.1M NaH2PO4
195
194/DMA/DIPEA

Fig. 17

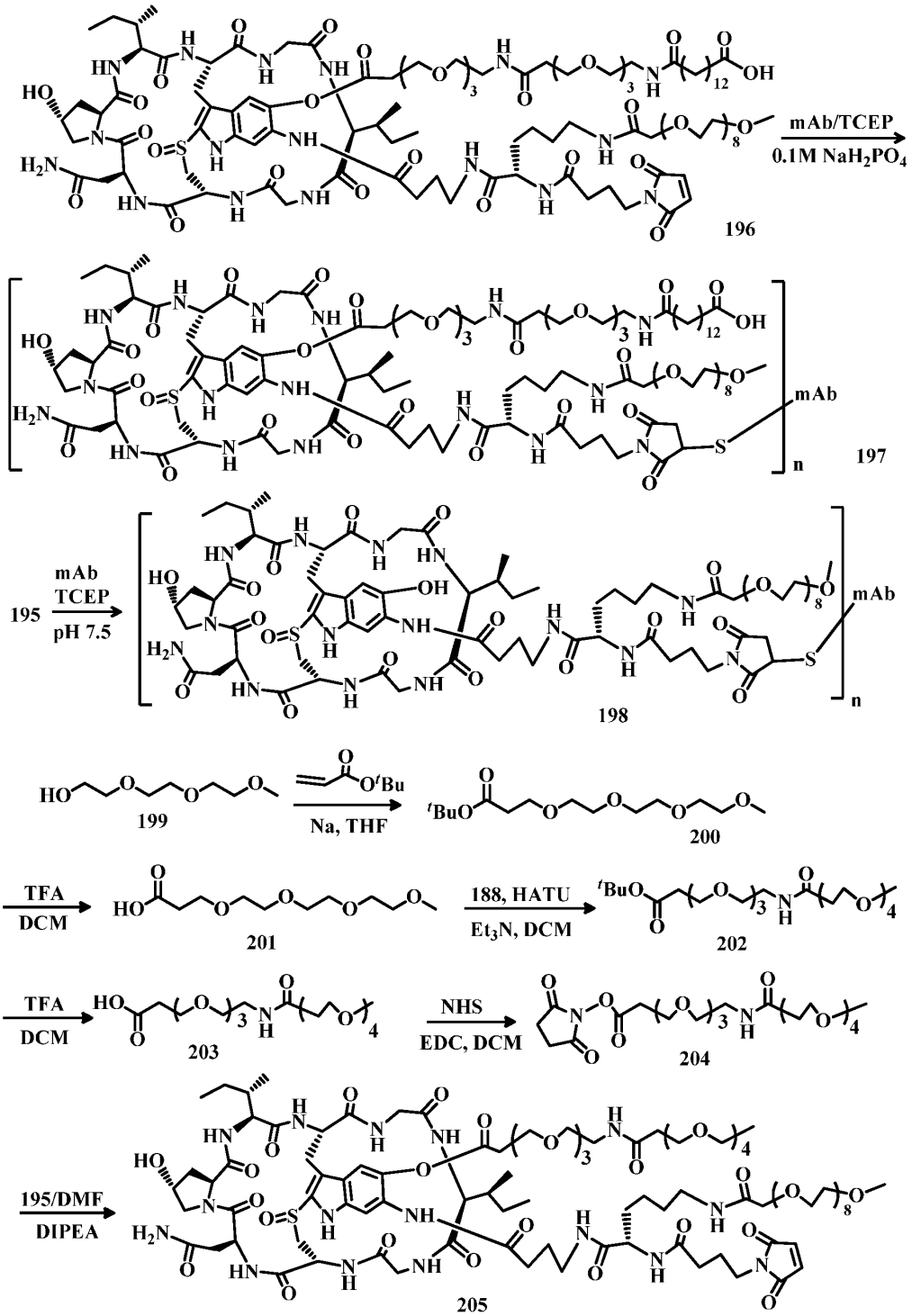

mAb/TCEP
0.1M NaH2PO4
196
197
mAb
TCEP
pH 7.5
195
198
199
Na, THF
200
TFA
DCM
201
188, HATU
Et3N, DCM
202
203
NHS
EDC, DCM
204
195/DMF
DIPEA
205

Fig. 18

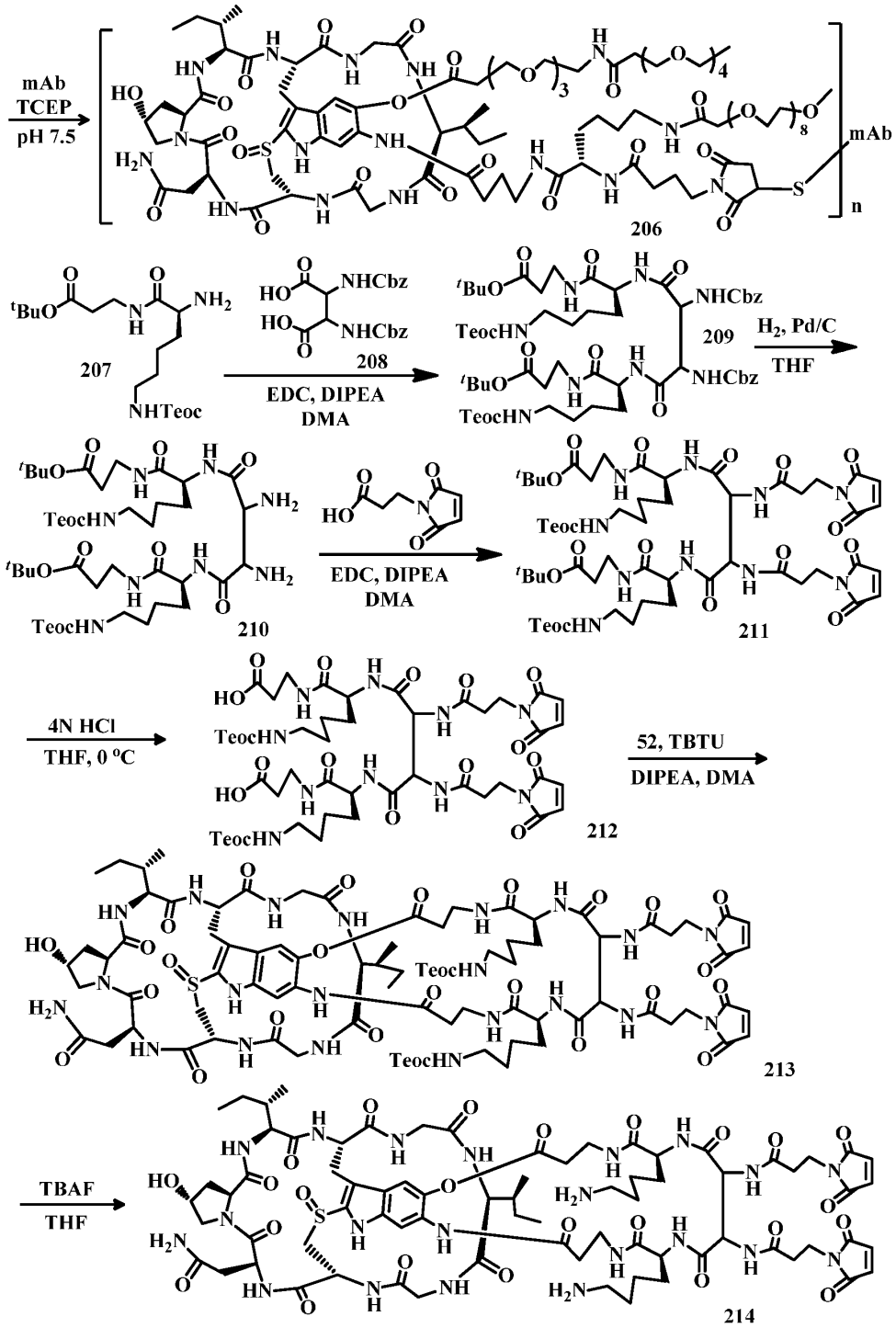

mAb
TCEP
pH 7.5
206
207
208
EDC, DIPEA
DMA
209
H2, Pd/C
THF
210
211
4N HCl
THF, 0 °C
212
52, TBTU
DIPEA, DMA
213
TBAF
THF
214

Fig. 19

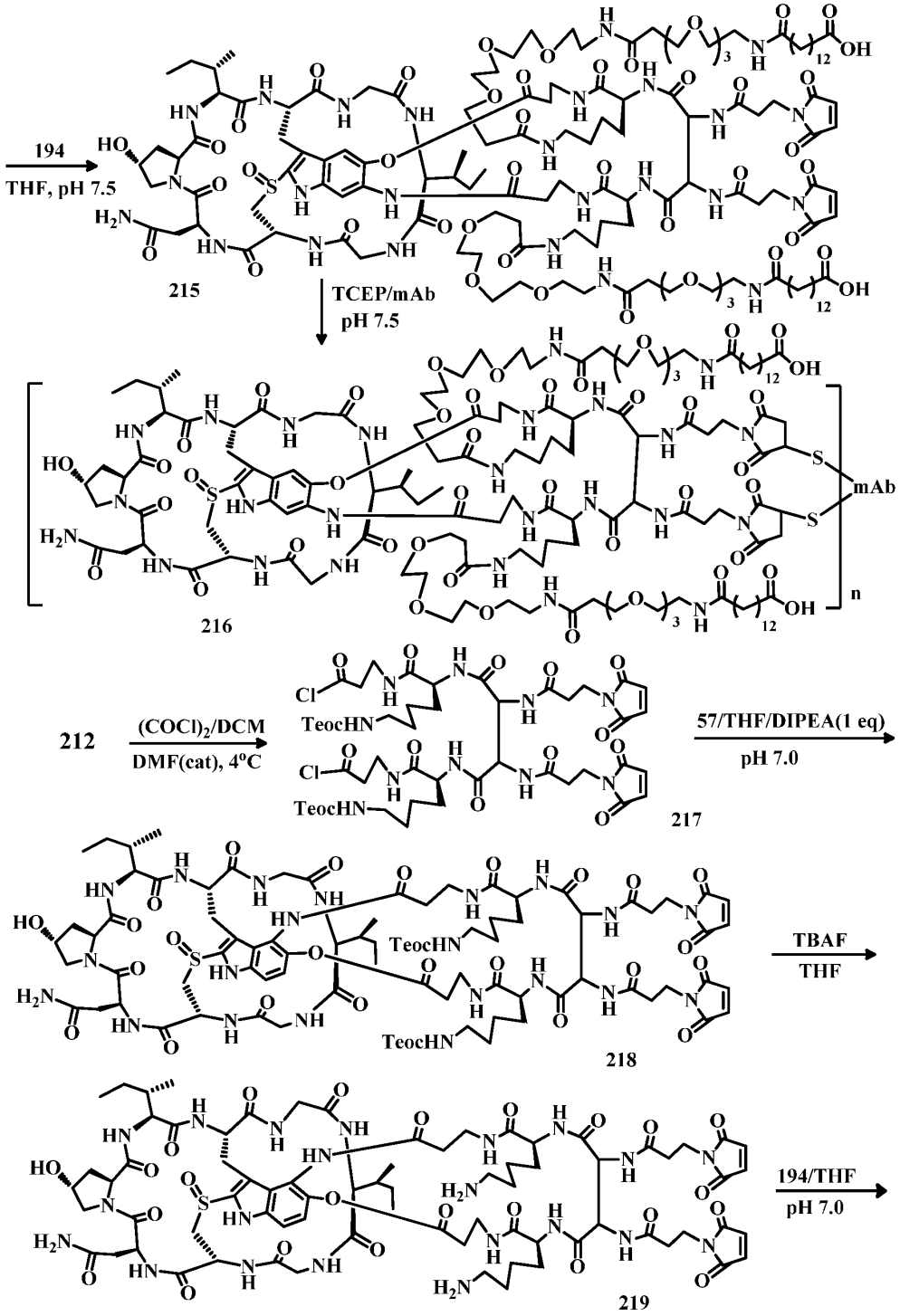

194
THF, pH 7.5
215
TCEP/mAb
pH 7.5
216
mAb
n
212
(COCl)2/DCM
DMF(cat), 4°C
TeocHN
Cl
217
57/THF/DIPEA(1 eq)
pH 7.0
218
TBAF
THF
219
194/THF
pH 7.0

Fig. 20

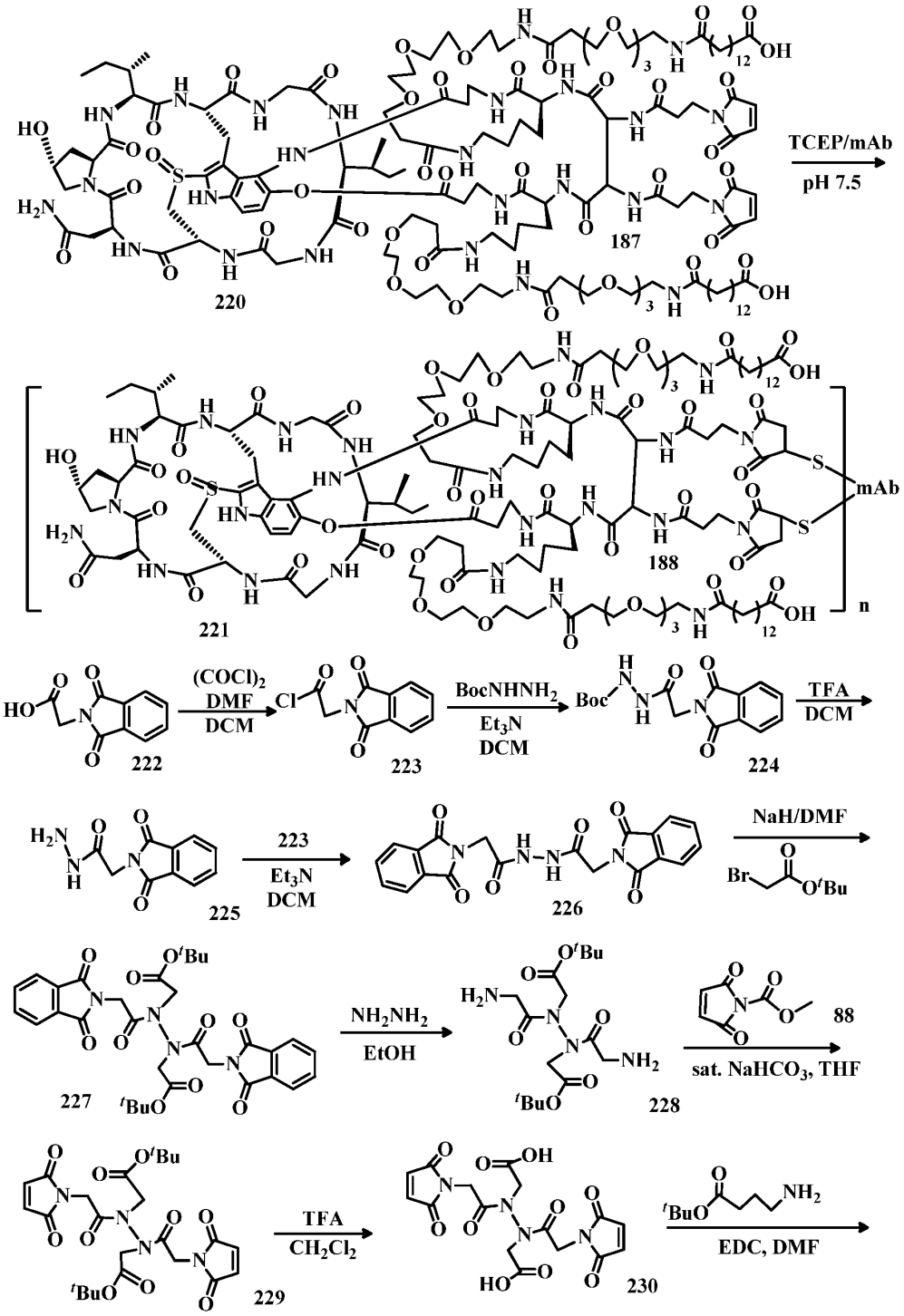

TCEP/mAb
pH 7.5
220
187
221
188
mAb
n
(COCl)2
DMF
DCM
BocNHNH2
Et3N
DCM
TFA
DCM
222
223
224
223
Et3N
DCM
225
226
NaH/DMF
NH2NH2
EtOH
227
228
88
sat. NaHCO3, THF
TFA
CH2Cl2
229
230
EDC, DMF

Fig. 21

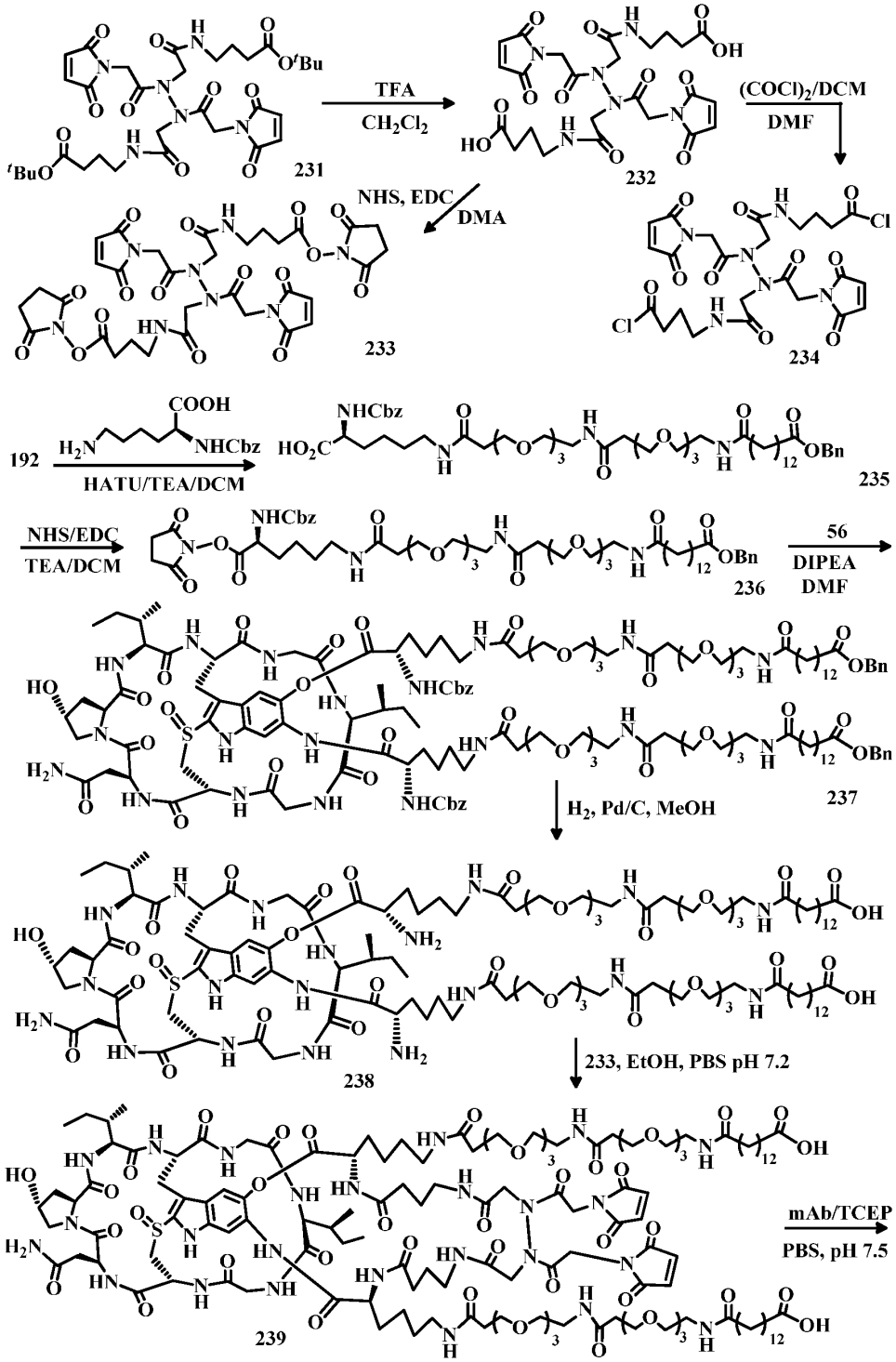

TFA
CH2Cl2
(COCl)2/DCM
DMF
NHS, EDC
DMA
231
232
233
234
192
HATU/TEA/DCM
235
NHS/EDC
TEA/DCM
236
56
DIPEA
DMF
237
H2, Pd/C, MeOH
238
233, EtOH, PBS pH 7.2
239
mAb/TCEP
PBS, pH 7.5

Fig. 22

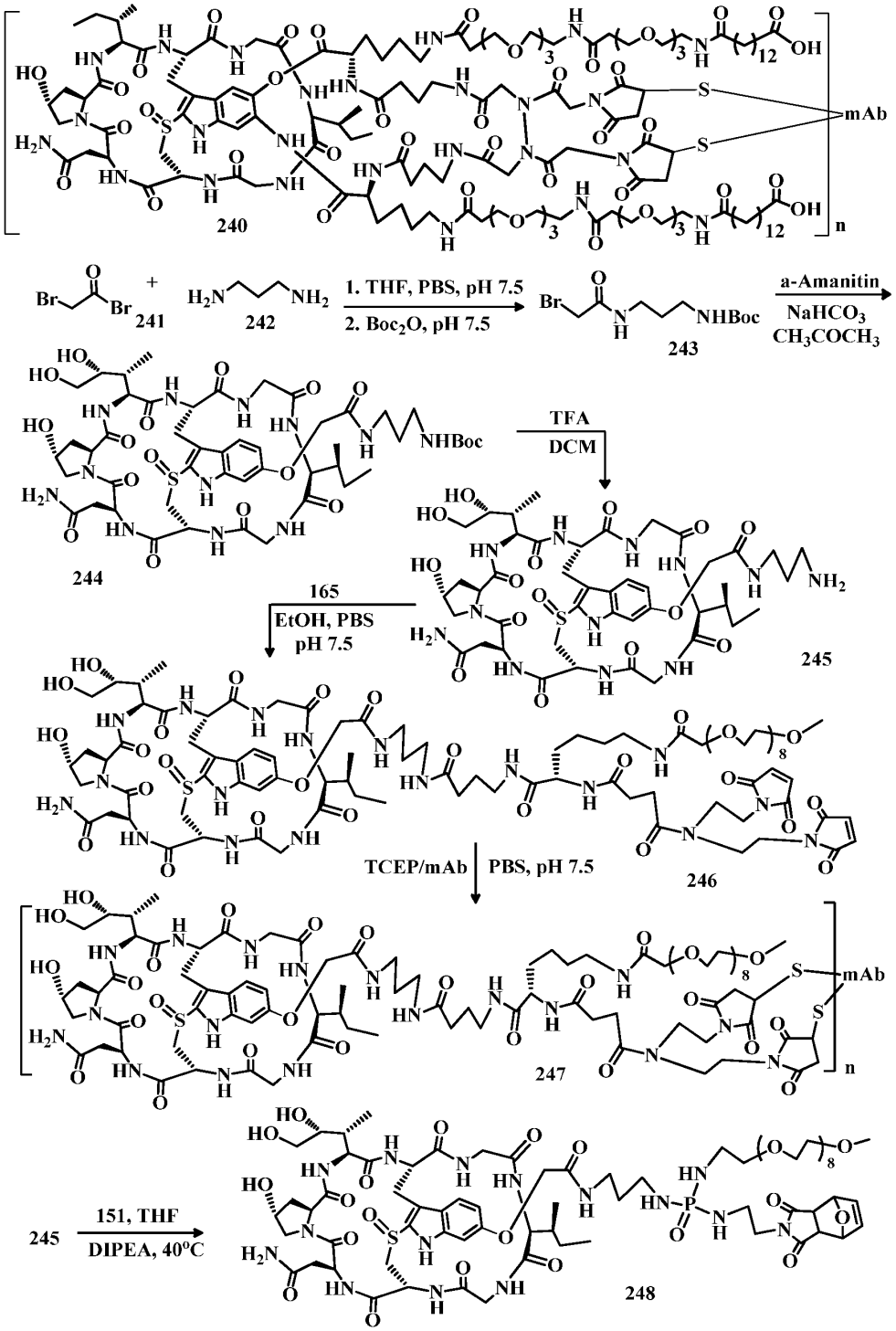

240
241
242
1. THF, PBS, pH 7.5
2. Boc2O, pH 7.5
243
a-Amanitin
NaHCO3
CH3COCH3
244
TFA
DCM
245
165
EtOH, PBS
pH 7.5
246
TCEP/mAb
PBS, pH 7.5
247
mAb
151, THF
DIPEA, 40°C
248

Fig. 23

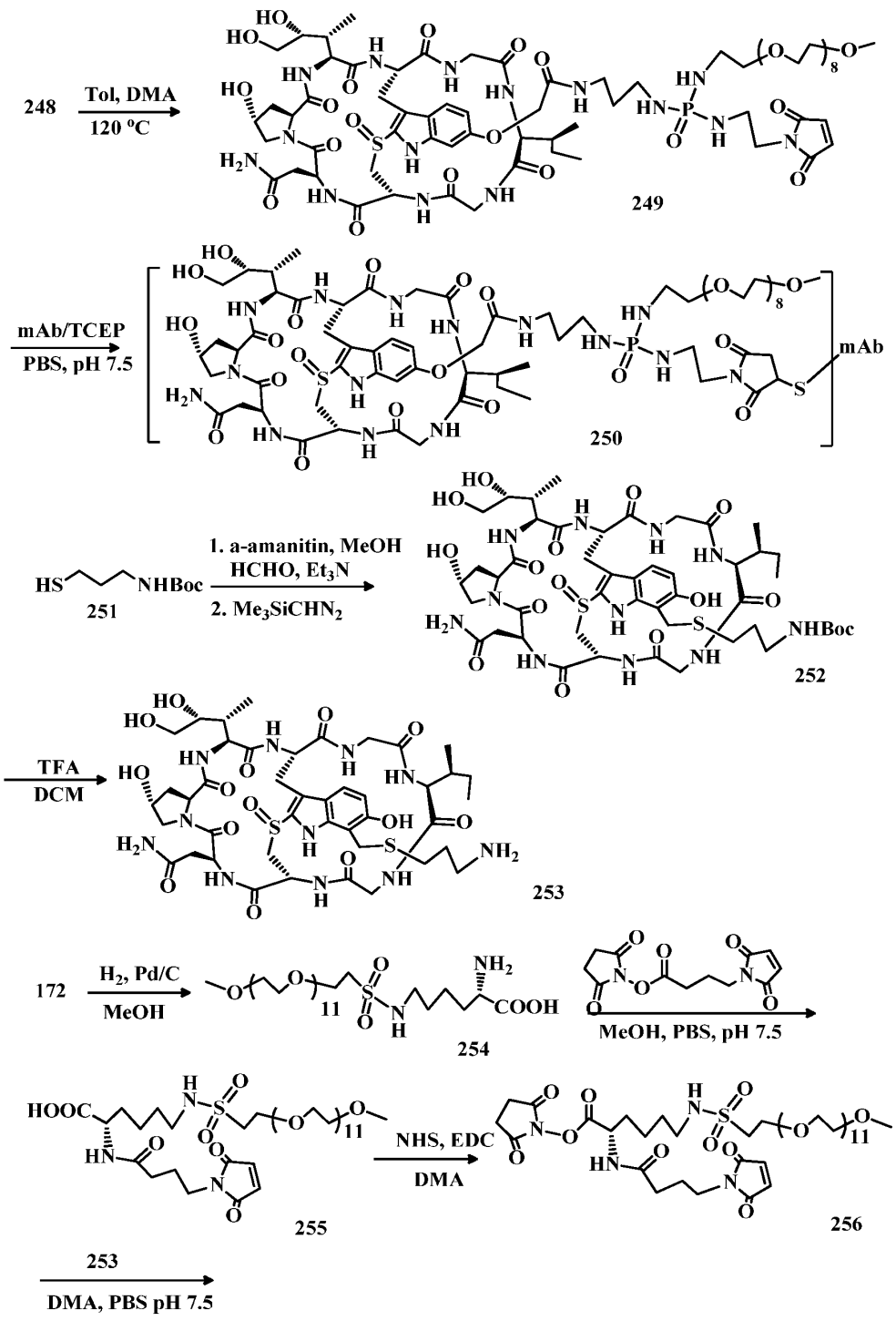

248
Tol, DMA
120 °C
249
mAb/TCEP
PBS, pH 7.5
mAb
250
HS
NHBoc
251
1. a-amanitin, MeOH
HCHO, Et3N
2. Me3SiCHN2
NHBoc
252
TFA
DCM
253
172
H2, Pd/C
MeOH
11
COOH
254
MeOH, PBS, pH 7.5
HOOC
11
255
NHS, EDC
DMA
256
253
DMA, PBS pH 7.5

Fig. 24

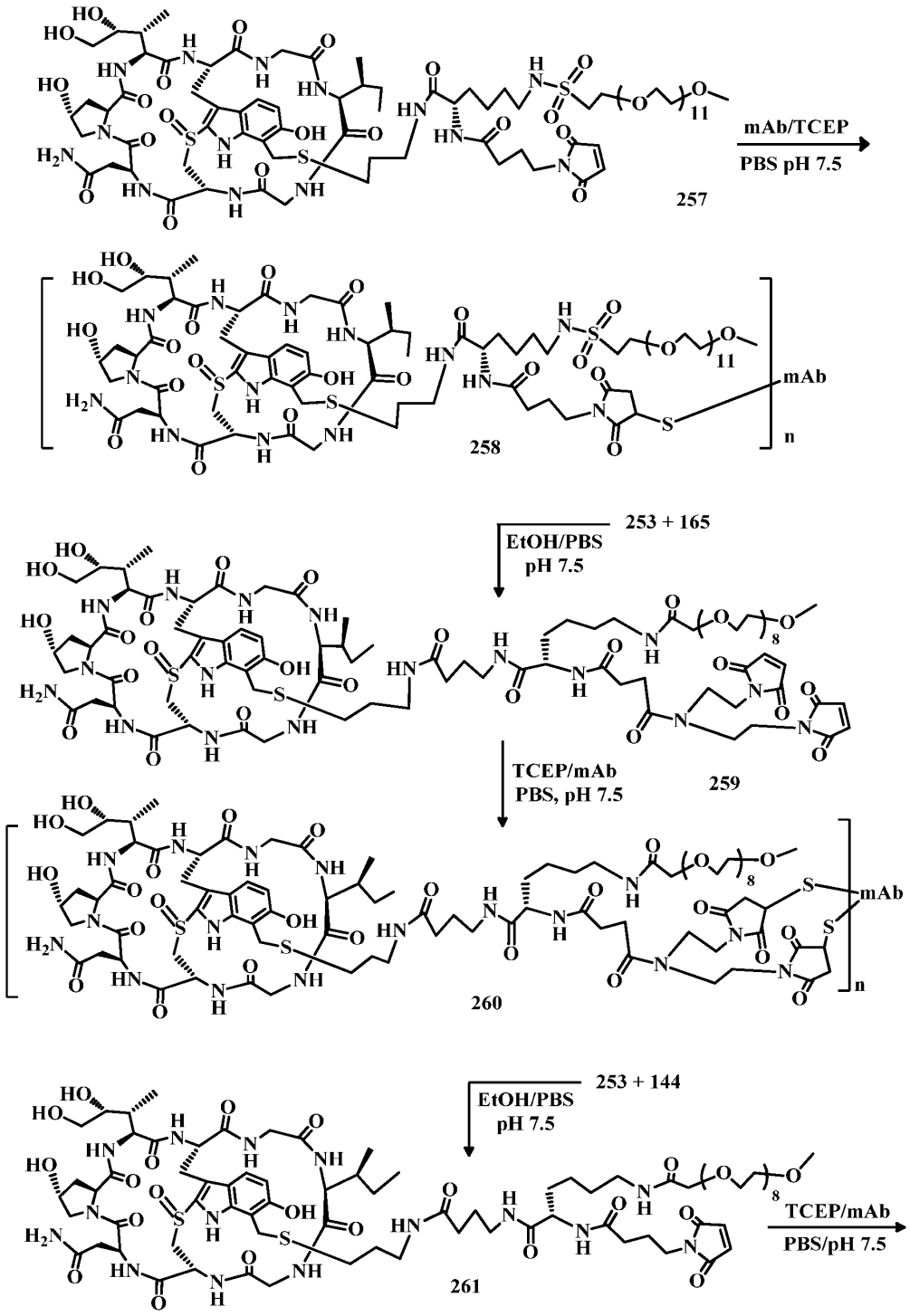
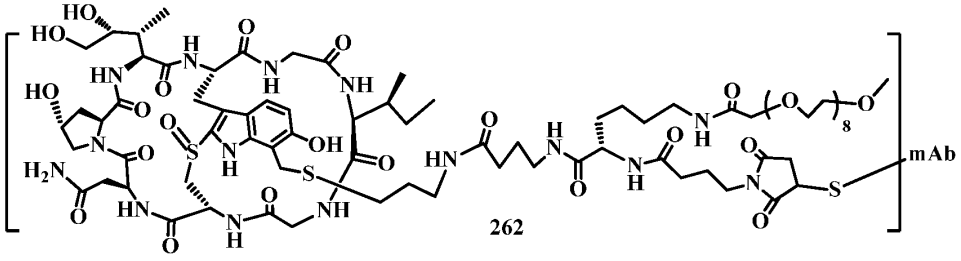

mAb/TCEP
PBS pH 7.5
257
258
mAb
n
253 + 165
EtOH/PBS
pH 7.5
259
TCEP/mAb
PBS, pH 7.5
260
253 + 144
EtOH/PBS
pH 7.5
TCEP/mAb
PBS/pH 7.5
261

Fig.25



262

Fig. 26

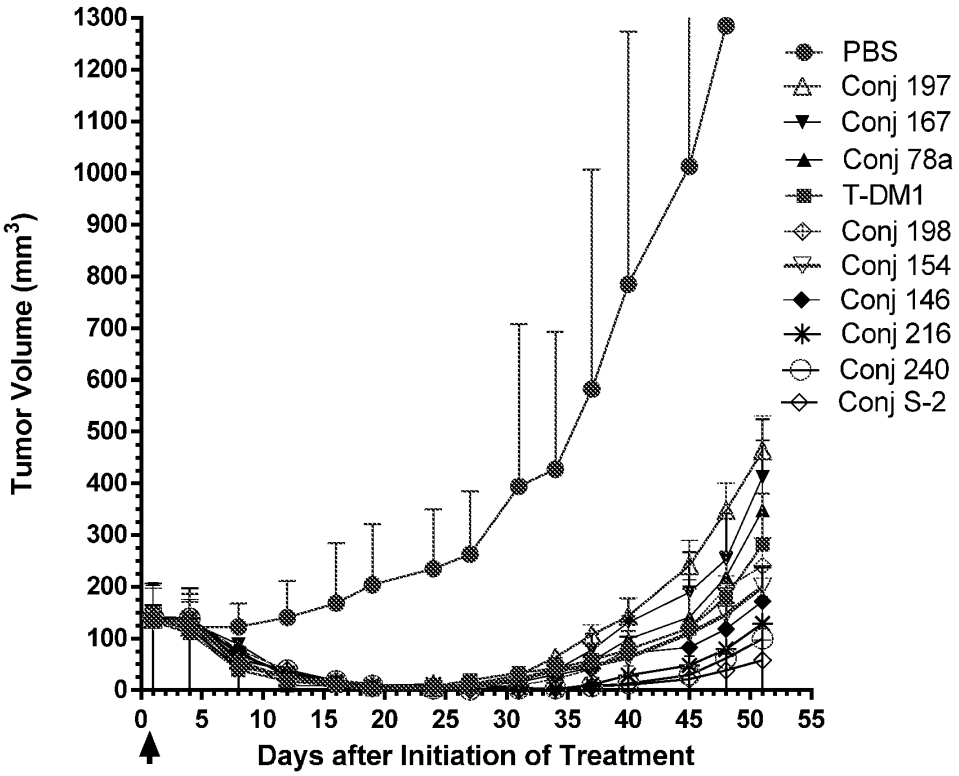


Fig. 27

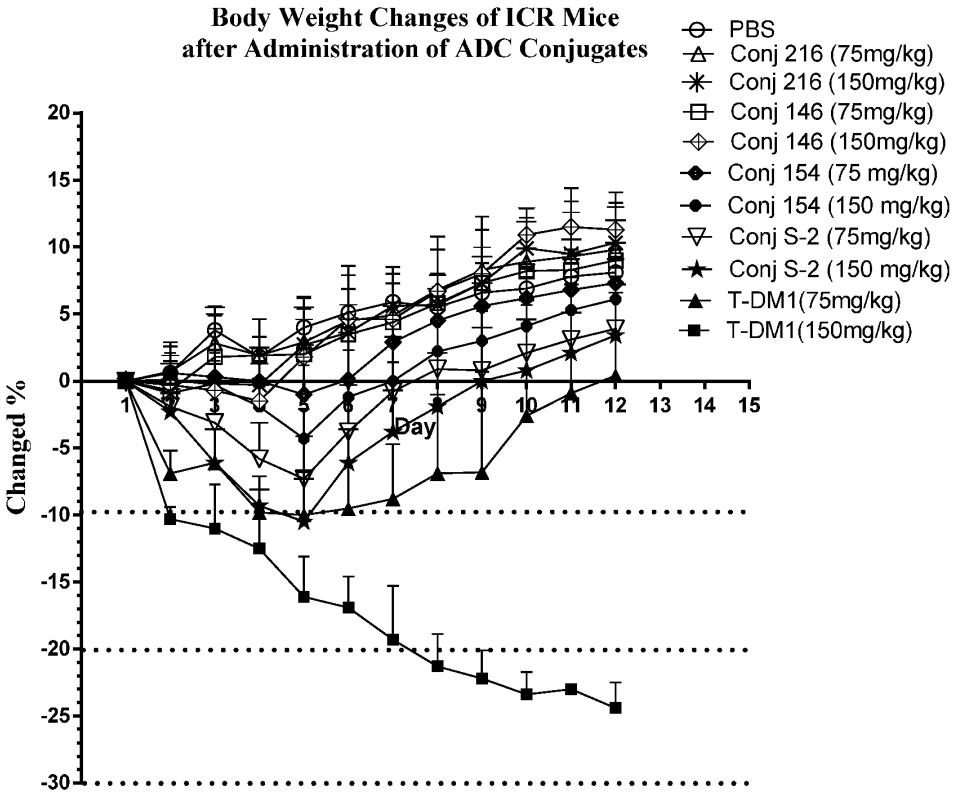

Body Weight Changes of ICR Mice
after Administration of ADC Conjugates
PBS
Conj 216 (75mg/kg)
Conj 216 (150mg/kg)
Conj 146 (75mg/kg)
Conj 146 (150mg/kg)
Conj 154 (75 mg/kg)
Conj 154 (150 mg/kg)
Conj S-2 (75mg/kg)
Conj S-2 (150 mg/kg)
T-DM1(75mg/kg)
T-DM1(150mg/kg)
Changed %
Day
20
15
10
5
0
-5
-10
-15
-20
-25
-30
1
3
5
6
7
8
9
10
11
12
13
14
15

Fig. 28.