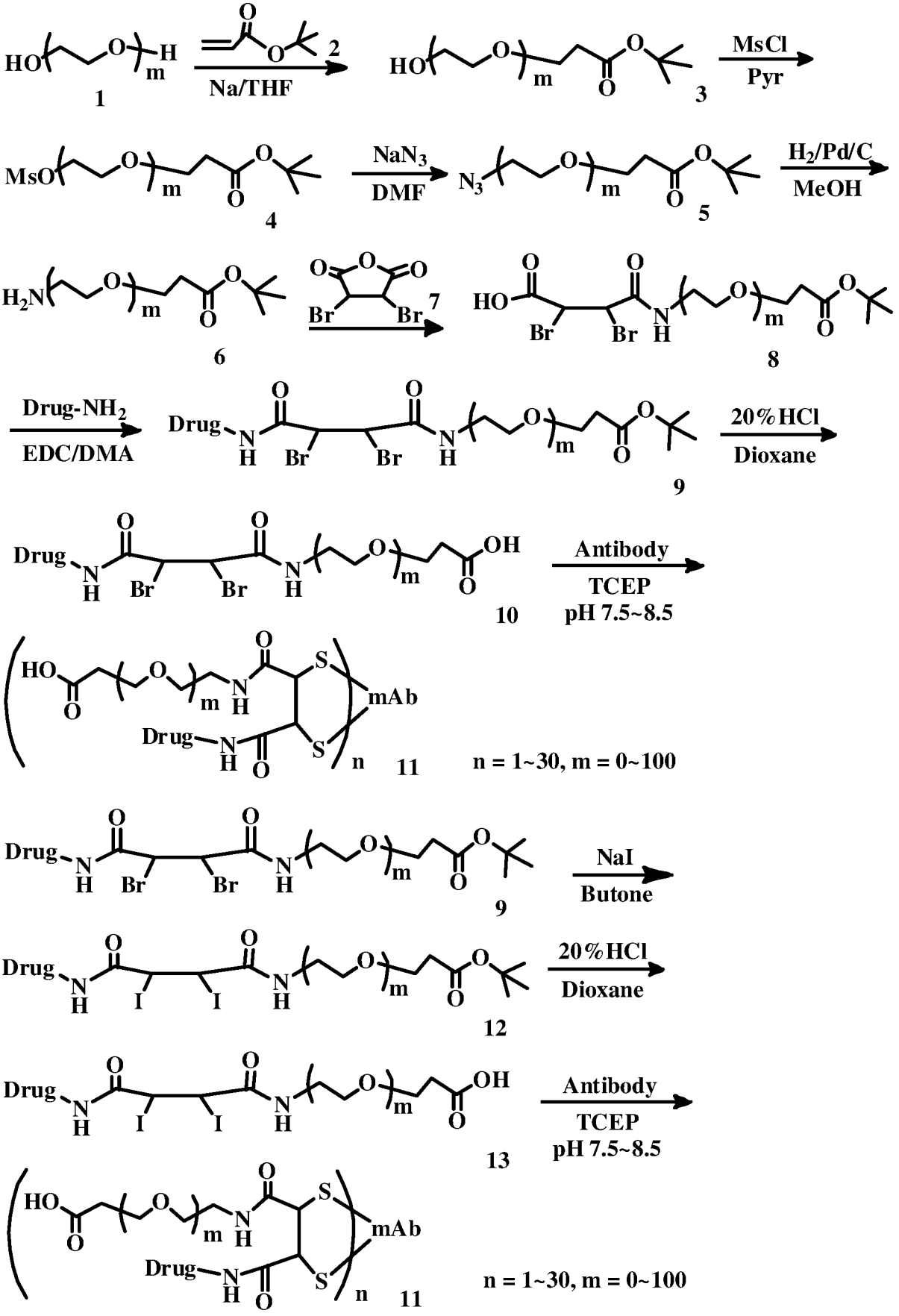

HO
O
H
m
1
O
O
2
Na/THF
HO
O
m
O
3
MsCl
Pyr
MsO
O
m
O
4
NaN3
DMF
N3
O
m
O
5
H2/Pd/C
MeOH
H2N
O
m
O
6
O
O
O
Br
Br
7
HO
O
Br
O
Br
N
H
O
m
O
8
Drug-NH2
EDC/DMA
Drug
N
H
O
Br
Br
O
N
H
O
m
O
9
20%HCl
Dioxane
Drug
N
H
O
Br
Br
O
N
H
O
m
OH
O
10
Antibody
TCEP
pH 7.5~8.5
HO
O
O
m
N
H
S
mAb
Drug
N
H
O
S
n
11
n = 1~30, m = 0~100
Drug
N
H
O
Br
Br
O
N
H
O
m
O
9
NaI
Butone
Drug
N
H
O
I
I
O
N
H
O
m
O
12
20%HCl
Dioxane
Drug
N
H
O
I
I
O
N
H
O
m
OH
O
13
Antibody
TCEP
pH 7.5~8.5
HO
O
O
m
N
H
S
mAb
Drug
N
H
O
S
n
11
n = 1~30, m = 0~100

Figure 1.

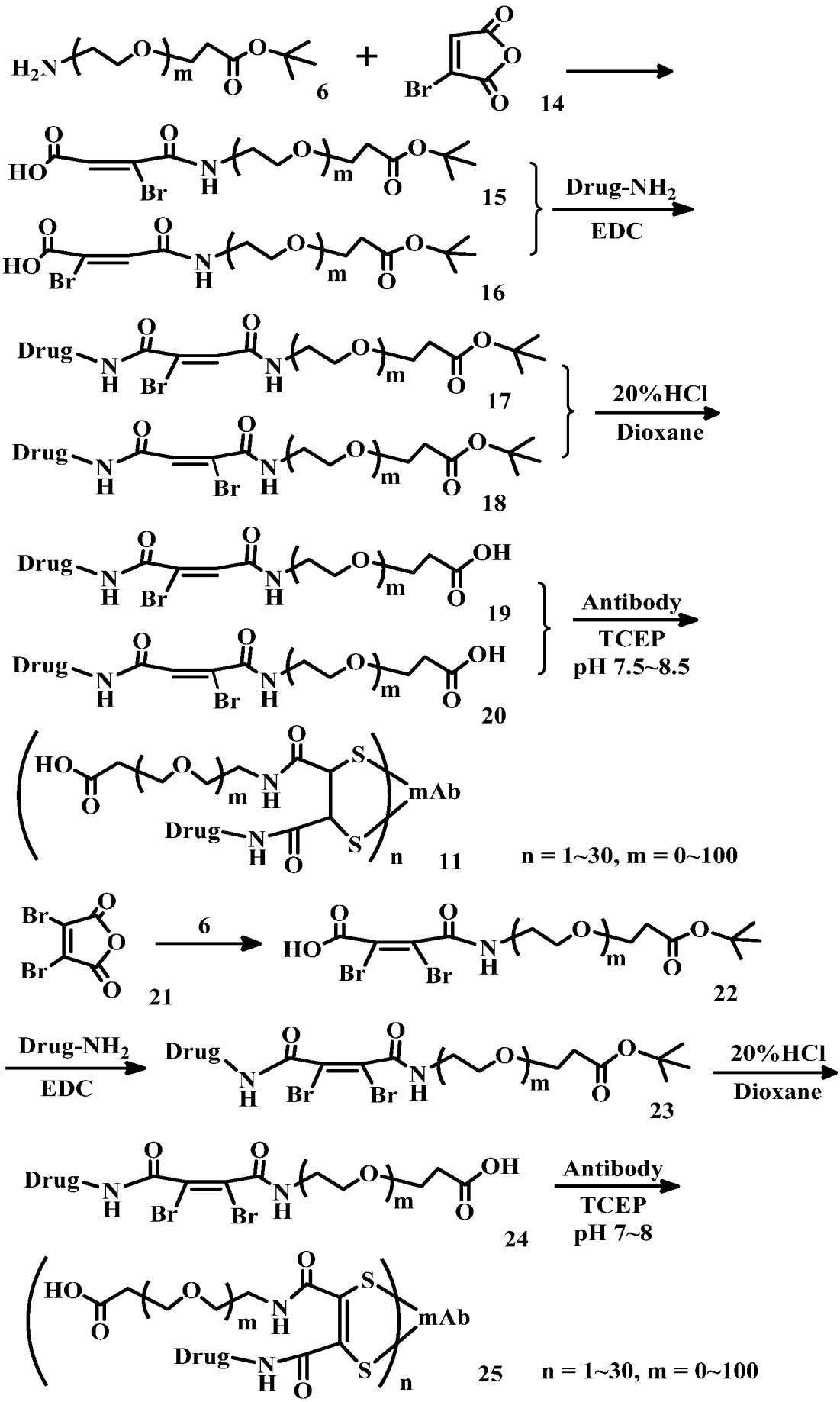

6
14
15
16
Drug-NH2
EDC
17
18
20%HCl
Dioxane
19
20
Antibody
TCEP
pH 7.5~8.5
11
n = 1~30, m = 0~100
21
22
23
24
25
pH 7~8

Figure 2.

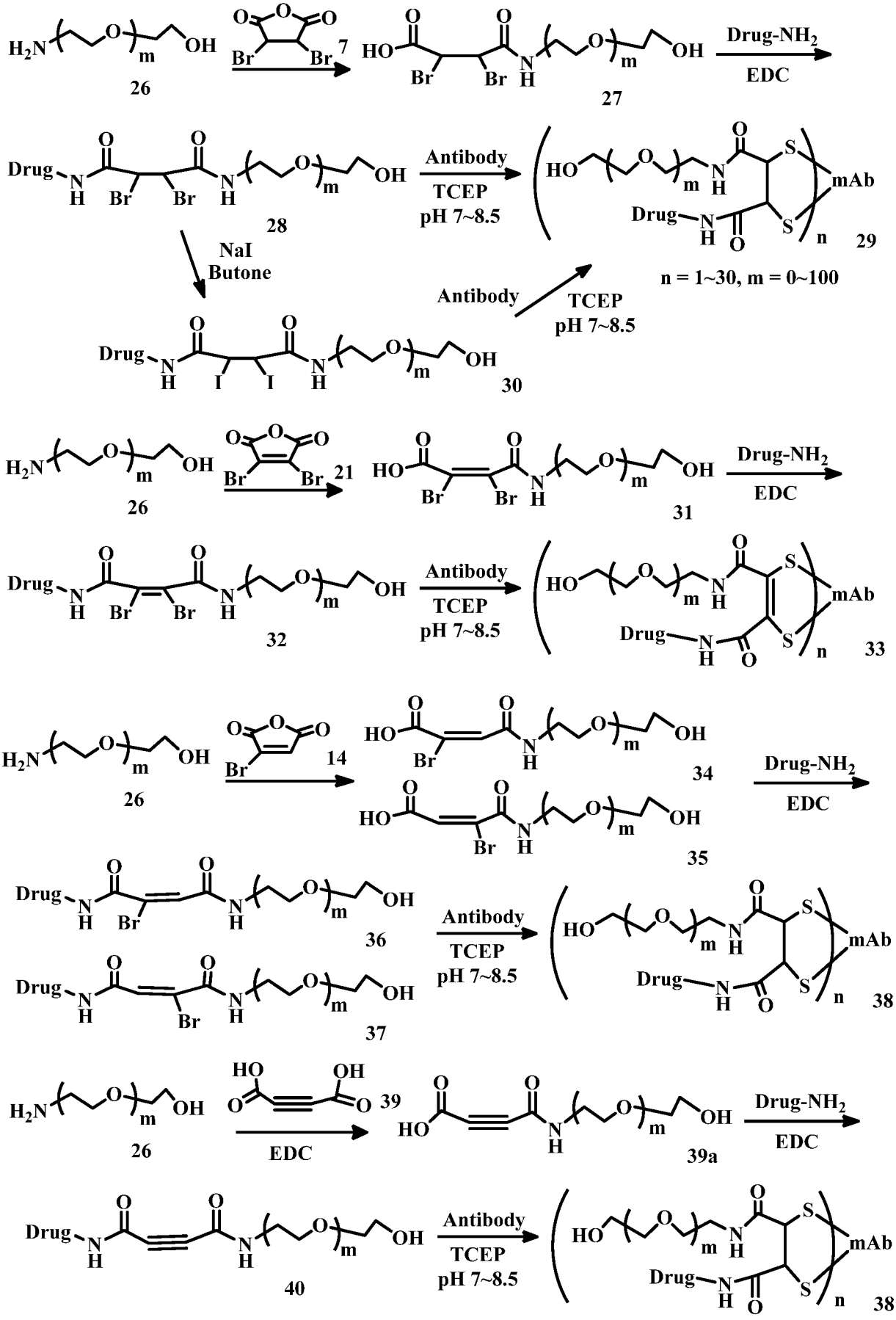

H2N
OH
m
26
Br
Br
7
HO
N
H
27
Drug-NH2
EDC
Drug
28
Antibody
TCEP
pH 7~8.5
mAb
S
n
29
n = 1~30, m = 0~100
NaI
Butone
I
30
21
31
32
33
14
34
35
36
37
38
39
39a
40

Figure 3

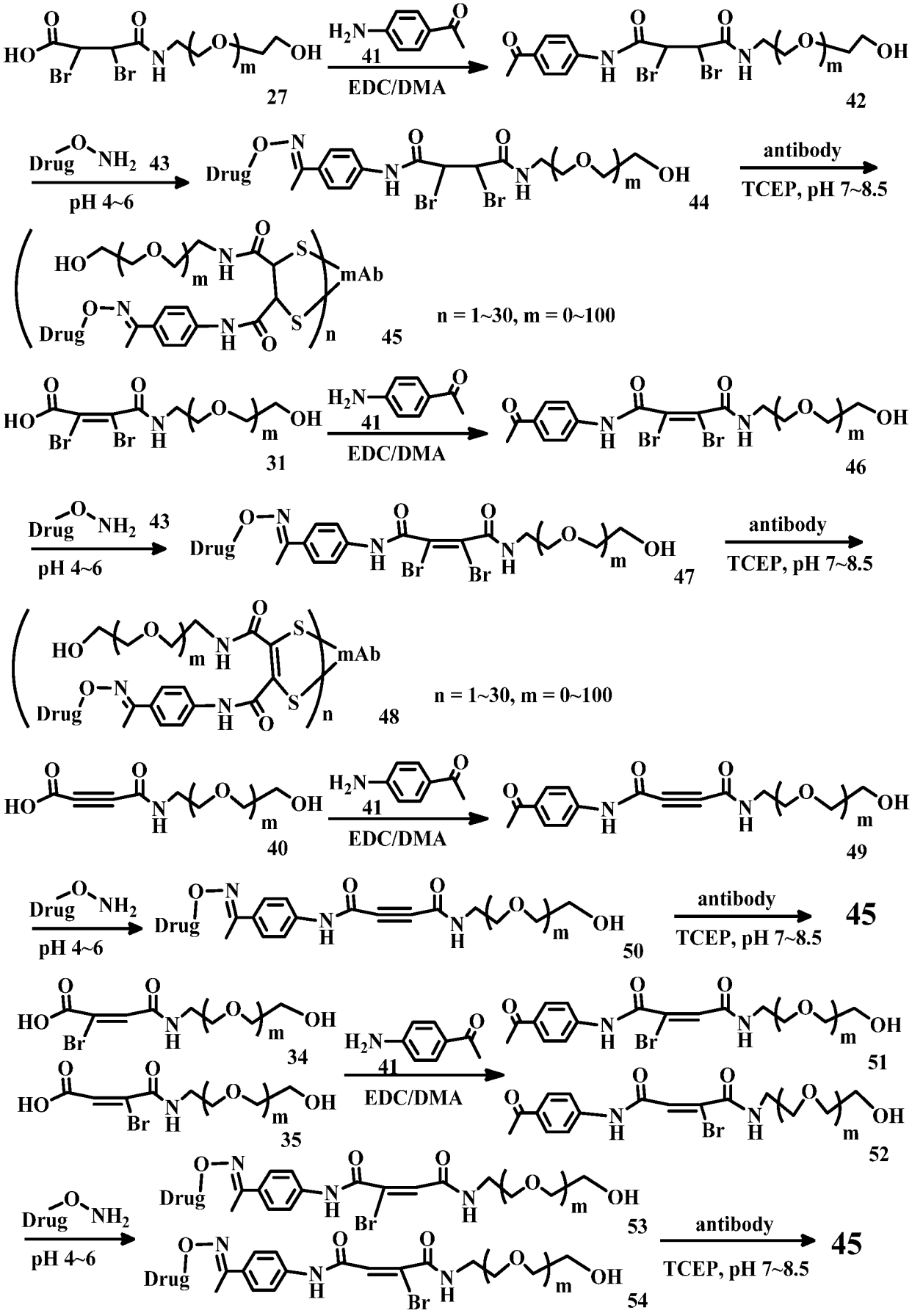

27
41
EDC/DMA
42
Drug NH2 43
pH 4~6
44
antibody
TCEP, pH 7~8.5
mAb
n = 1~30, m = 0~100
45
31
46
47
48
40
49
50
34
35
51
52
53
54

Figure 4

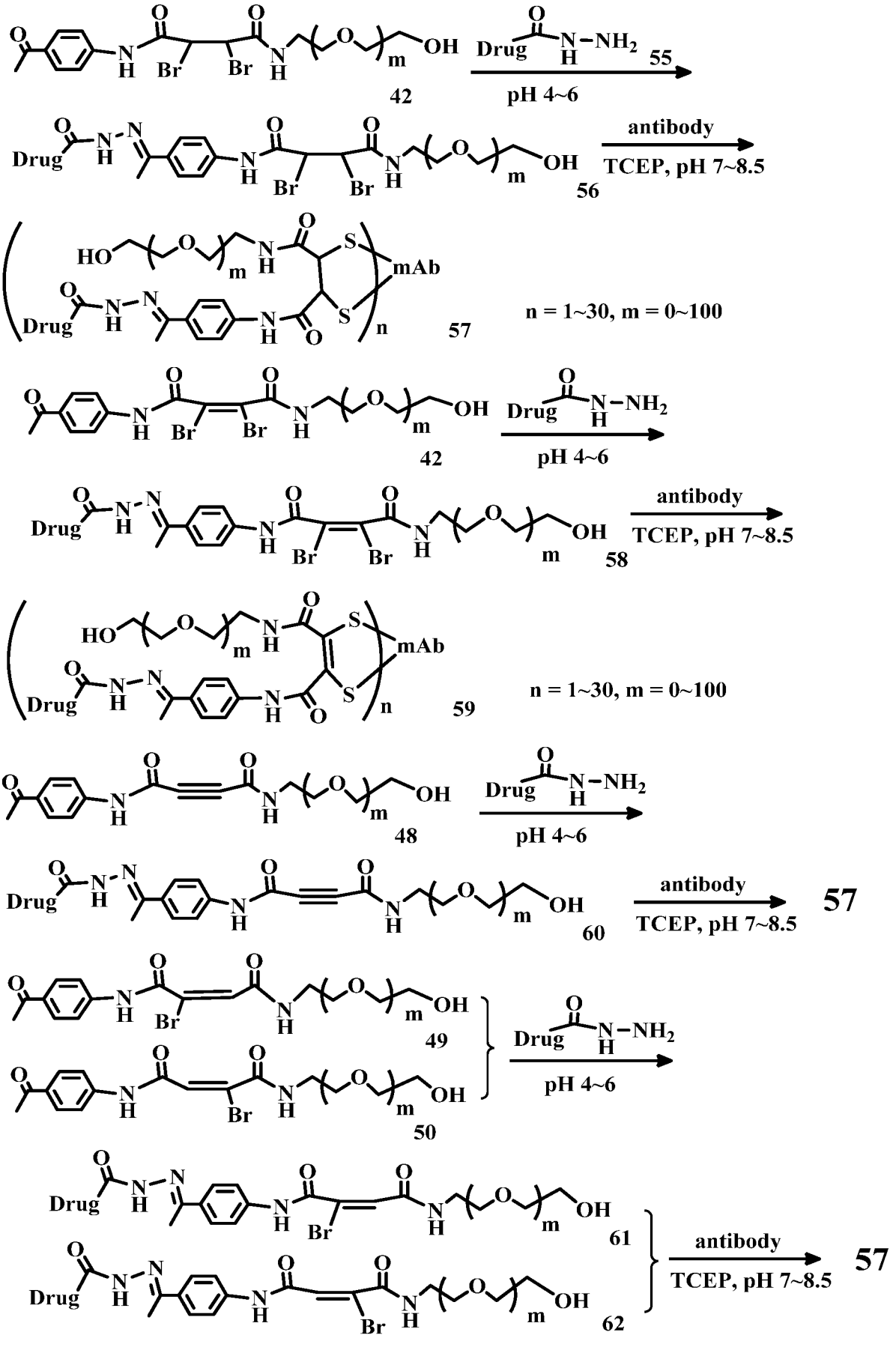

42
55
pH 4~6
antibody
TCEP, pH 7~8.5
56
57
n = 1~30, m = 0~100
42
pH 4~6
antibody
TCEP, pH 7~8.5
58
59
n = 1~30, m = 0~100
48
pH 4~6
antibody
TCEP, pH 7~8.5
60
57
49
50
pH 4~6
61
62
antibody
TCEP, pH 7~8.5
57

Figure 5

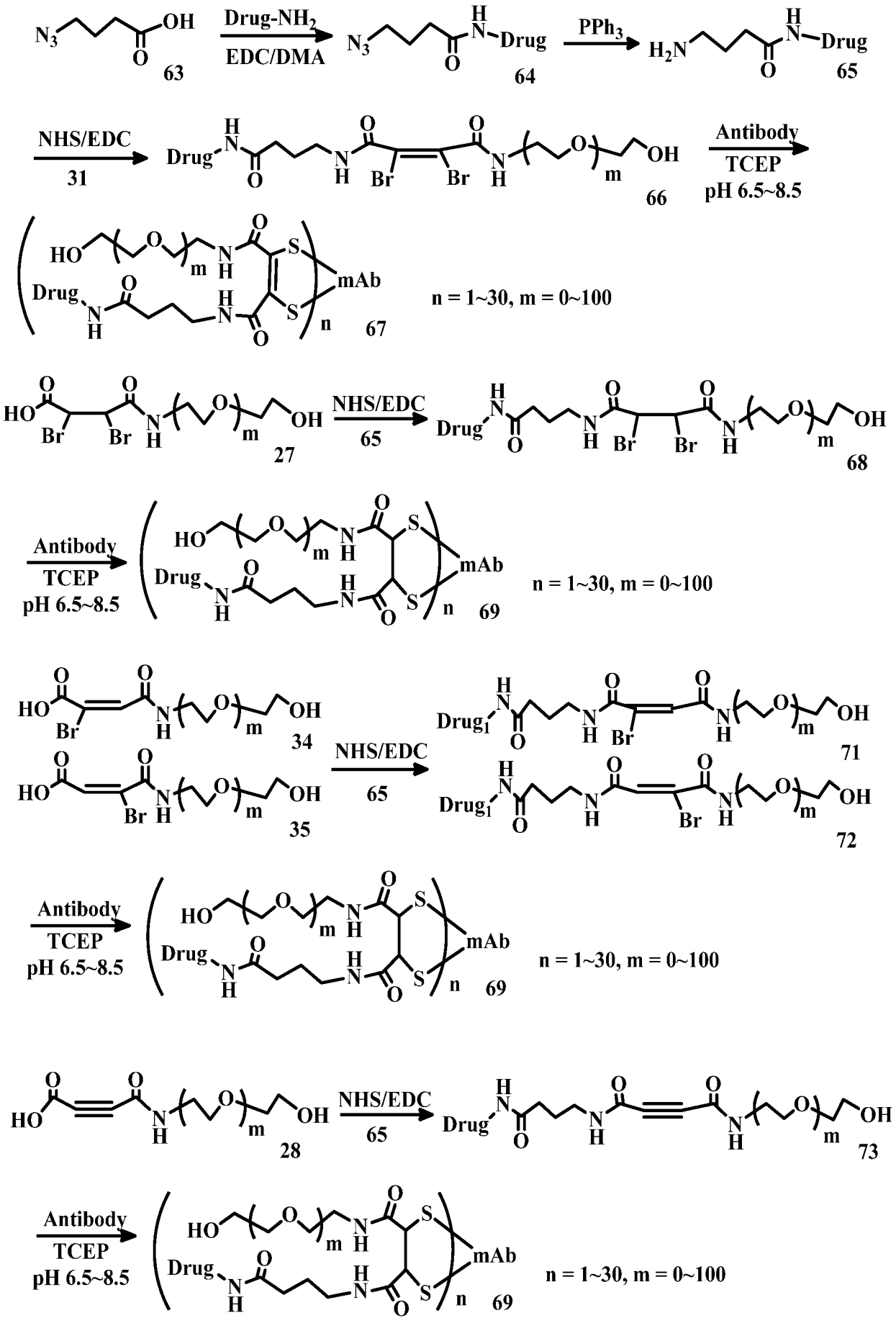

Drug-NH2
EDC/DMA
PPh3
NHS/EDC
31
Antibody
TCEP
pH 6.5~8.5
n = 1~30, m = 0~100
NHS/EDC
65
Antibody
TCEP
pH 6.5~8.5
n = 1~30, m = 0~100
NHS/EDC
65
Antibody
TCEP
pH 6.5~8.5
n = 1~30, m = 0~100
NHS/EDC
65
Antibody
TCEP
pH 6.5~8.5
n = 1~30, m = 0~100

Figure 6

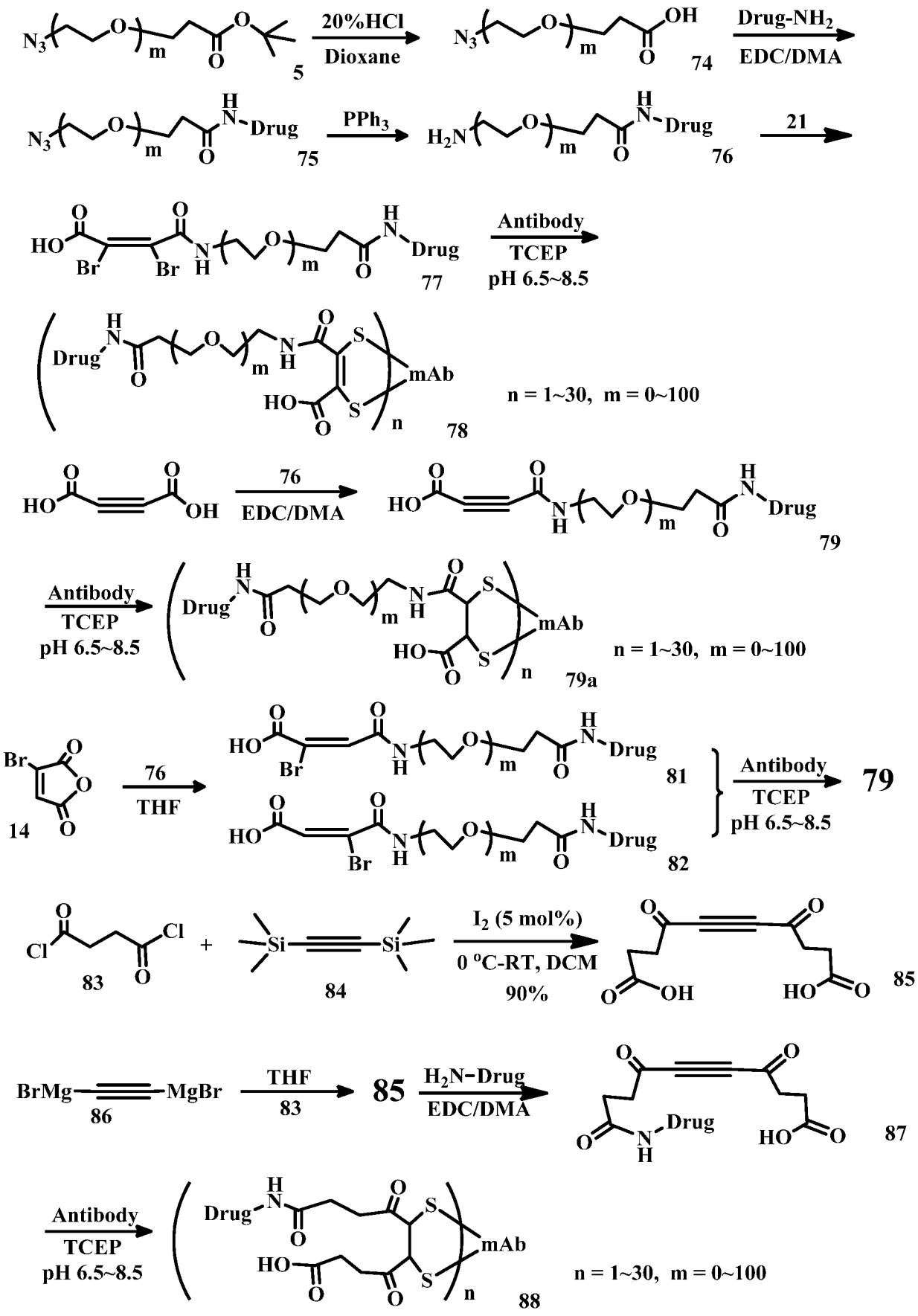

20%HCl
Dioxane
5
74
Drug-NH2
EDC/DMA
PPh3
75
76
21
77
Antibody
TCEP
pH 6.5~8.5
mAb
n = 1~30, m = 0~100
78
76
EDC/DMA
79
Antibody
TCEP
pH 6.5~8.5
79a
n = 1~30, m = 0~100
14
76
THF
81
82
Antibody
TCEP
pH 6.5~8.5
79
83
84
I2 (5 mol%)
0 °C-RT, DCM
90%
85
BrMg
MgBr
86
THF
83
85
H2N-Drug
EDC/DMA
87
Antibody
TCEP
pH 6.5~8.5
88
n = 1~30, m = 0~100

Figure 7

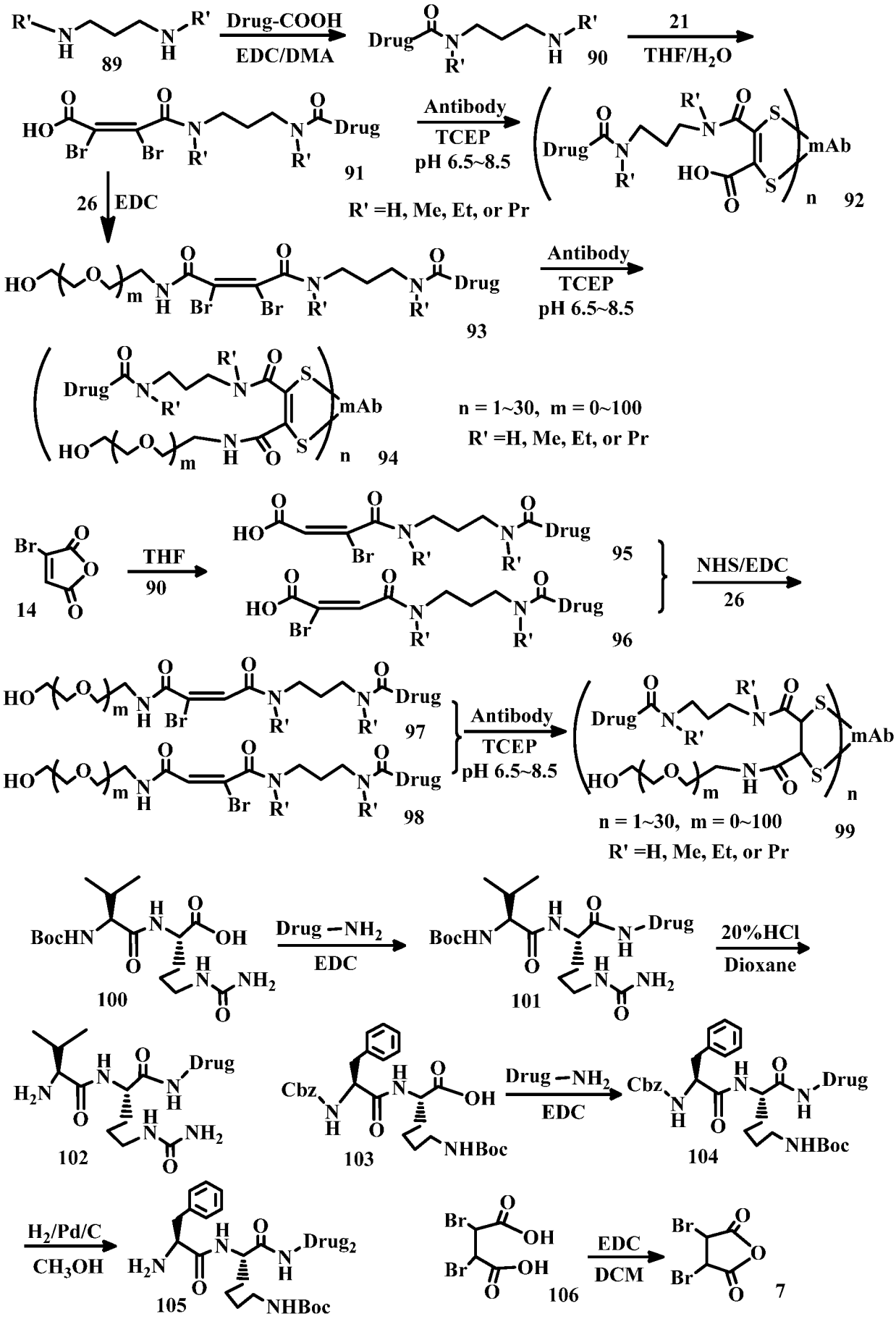

Drug-COOH
EDC/DMA
21
THF/H2O
Antibody
TCEP
pH 6.5~8.5
26
EDC
R' =H, Me, Et, or Pr
n = 1~30, m = 0~100
THF
NHS/EDC
20%HCl
Dioxane
Drug—NH2
H2/Pd/C
CH3OH
EDC
DCM

Figure 8

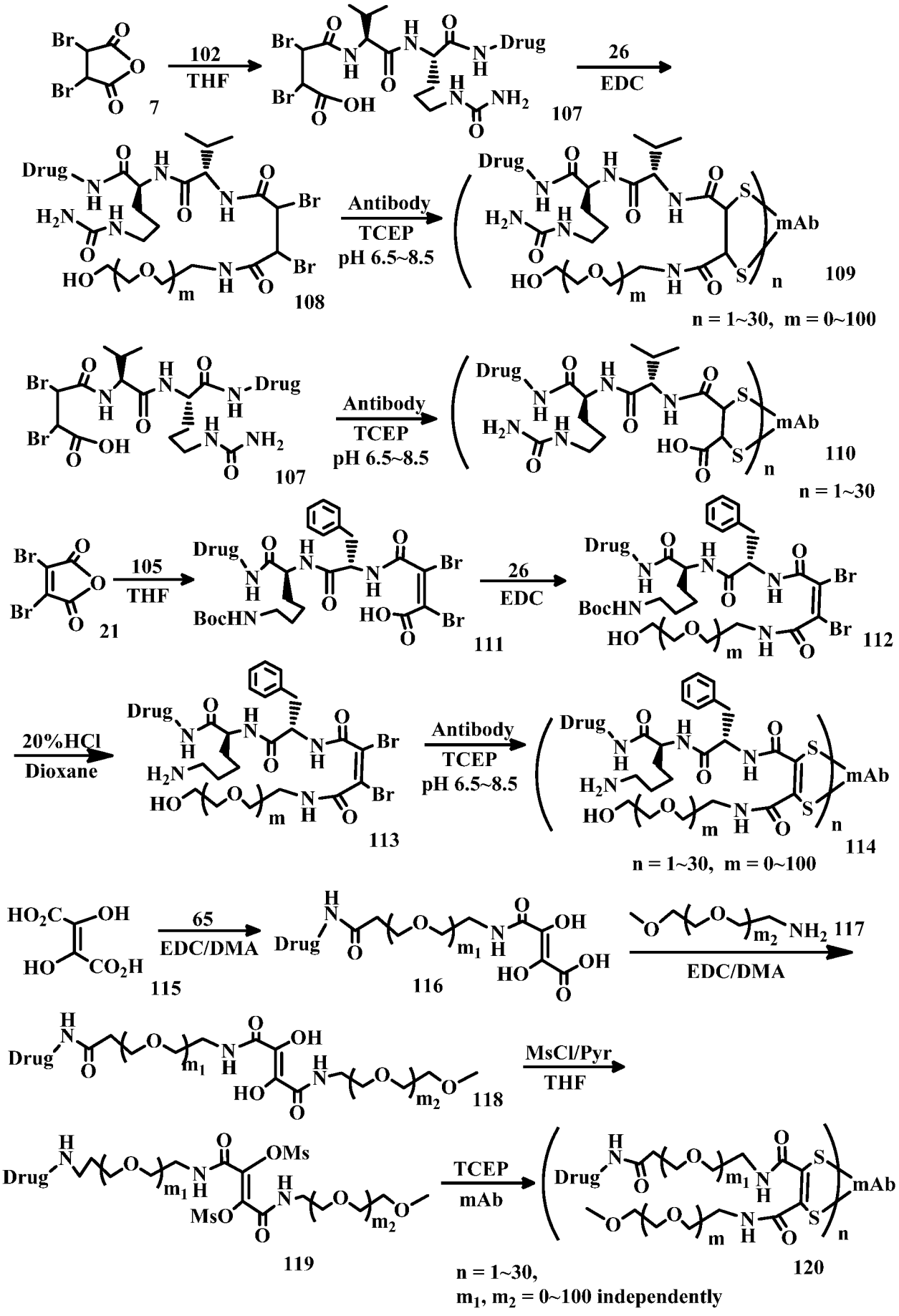

102
THF
26
EDC
Antibody
TCEP
pH 6.5~8.5
n = 1~30, m = 0~100
n = 1~30
105
THF
20%HCl
Dioxane
65
EDC/DMA
MsCl/Pyr
THF
n = 1~30,
m1, m2 = 0~100 independently

Figure 9

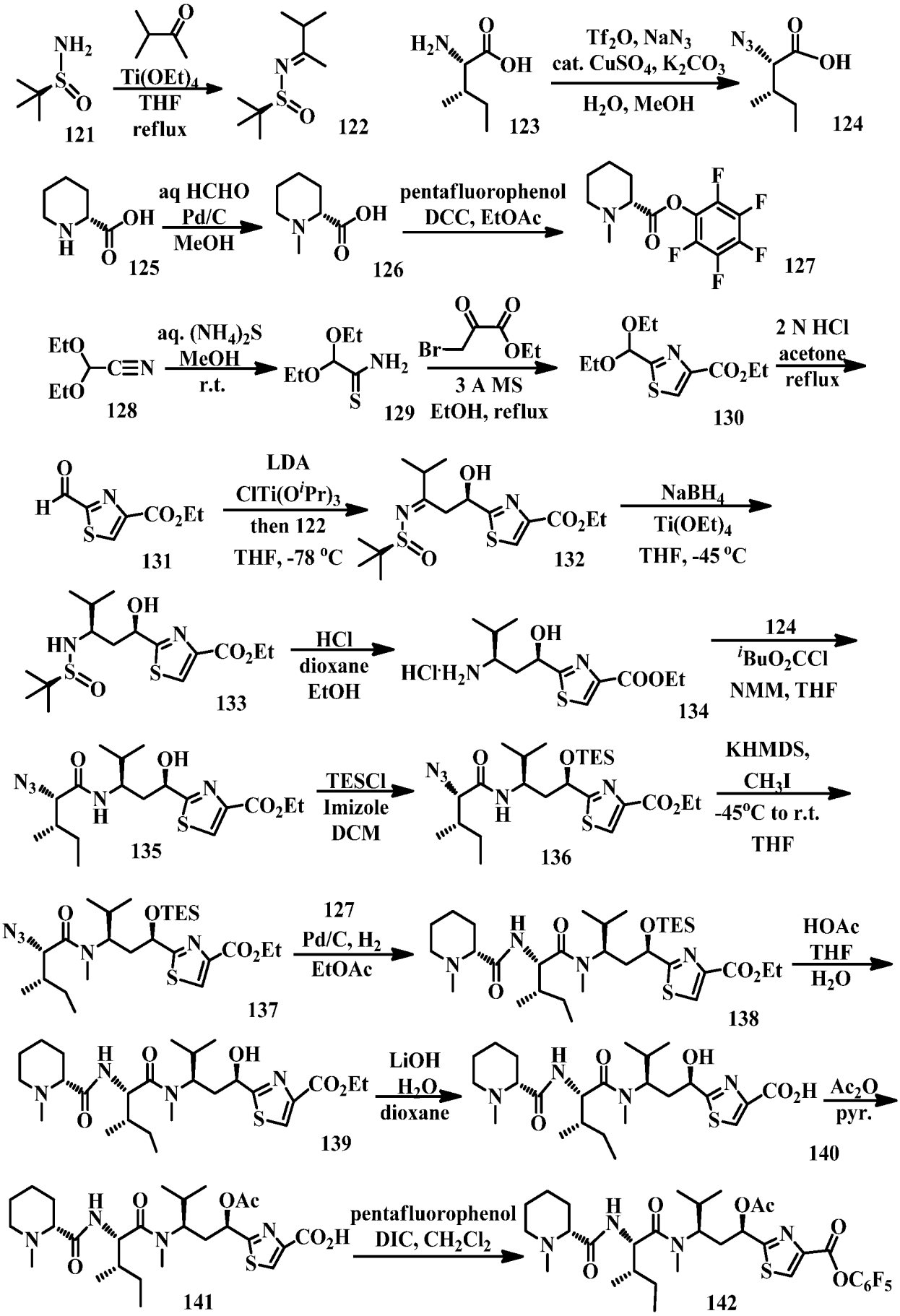

Ti(OEt)4
THF
reflux
121
122
Tf2O, NaN3
cat. CuSO4, K2CO3
H2O, MeOH
123
124
aq HCHO
Pd/C
MeOH
125
126
pentafluorophenol
DCC, EtOAc
127
aq. (NH4)2S
MeOH
r.t.
128
129
3 A MS
EtOH, reflux
130
2 N HCl
acetone
reflux
LDA
ClTi(OiPr)3
then 122
THF, -78 °C
131
132
NaBH4
Ti(OEt)4
THF, -45 °C
HCl
dioxane
EtOH
133
134
124
iBuO2CCl
NMM, THF
TESCl
Imizole
DCM
135
136
KHMDS,
CH3I
-45°C to r.t.
THF
127
Pd/C, H2
EtOAc
137
138
HOAc
THF
H2O
LiOH
H2O
dioxane
139
140
Ac2O
pyr.
pentafluorophenol
DIC, CH2Cl2
141
142

Figure 10

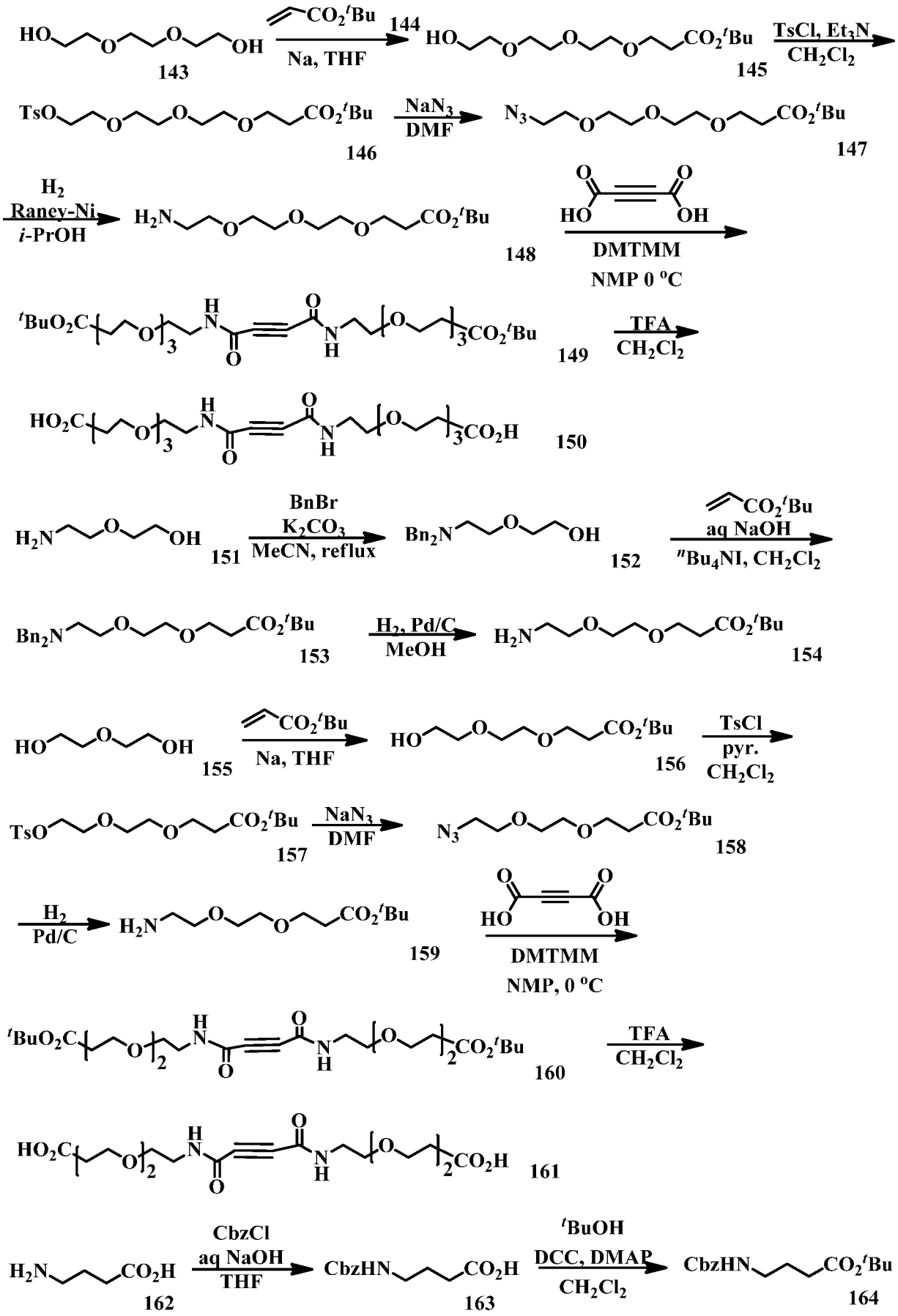

HO
OH
143
$CO_2{}^tBu$
144
Na, THF
HO
$CO_2{}^tBu$
145
TsCl, Et_3N
CH_2Cl_2
TsO
$CO_2{}^tBu$
146
NaN_3
DMF
N_3
$CO_2{}^tBu$
147
H_2
Raney-Ni
i-PrOH
H_2N
$CO_2{}^tBu$
148
HO
OH
DMTMM
NMP 0 °C
$^tBuO_2C$
149
TFA
CH_2Cl_2
HO_2C
CO_2H
150
H_2N
OH
151
BnBr
K_2CO_3
MeCN, reflux
Bn_2N
OH
152
aq NaOH
$^nBu_4NI$, CH_2Cl_2
Bn_2N
$CO_2{}^tBu$
153
H_2, Pd/C
MeOH
H_2N
$CO_2{}^tBu$
154
HO
OH
155
$CO_2{}^tBu$
Na, THF
HO
$CO_2{}^tBu$
156
TsCl
pyr.
CH_2Cl_2
TsO
$CO_2{}^tBu$
157
NaN_3
DMF
N_3
$CO_2{}^tBu$
158
H_2
Pd/C
H_2N
$CO_2{}^tBu$
159
HO
OH
DMTMM
NMP, 0 °C
$^tBuO_2C$
160
TFA
CH_2Cl_2
HO_2C
CO_2H
161
H_2N
CO_2H
162
CbzCl
aq NaOH
THF
CbzHN
CO_2H
163
^tBuOH
DCC, DMAP
CH_2Cl_2
CbzHN
$CO_2{}^tBu$
164

Figure 11

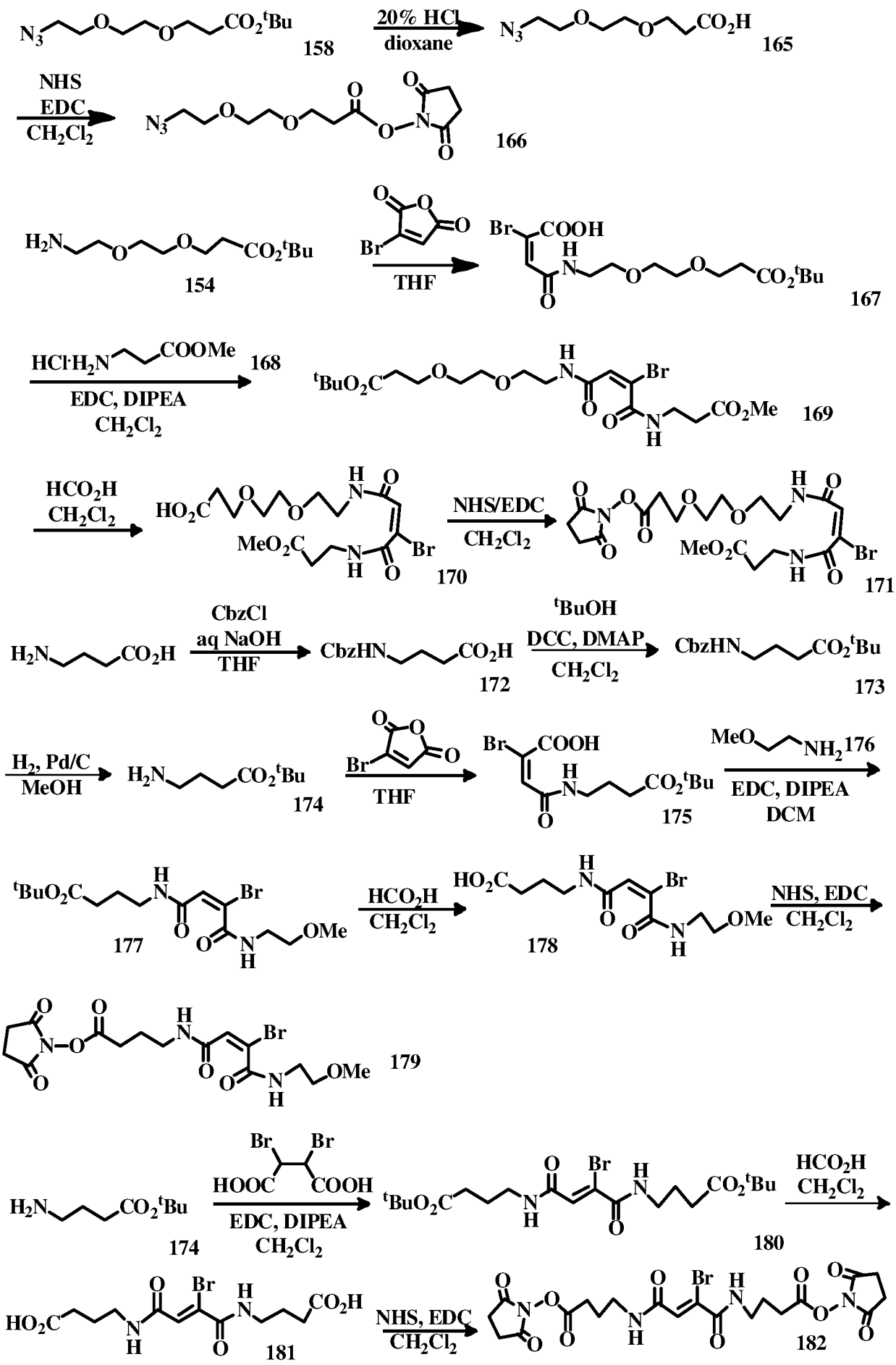


Figure 12

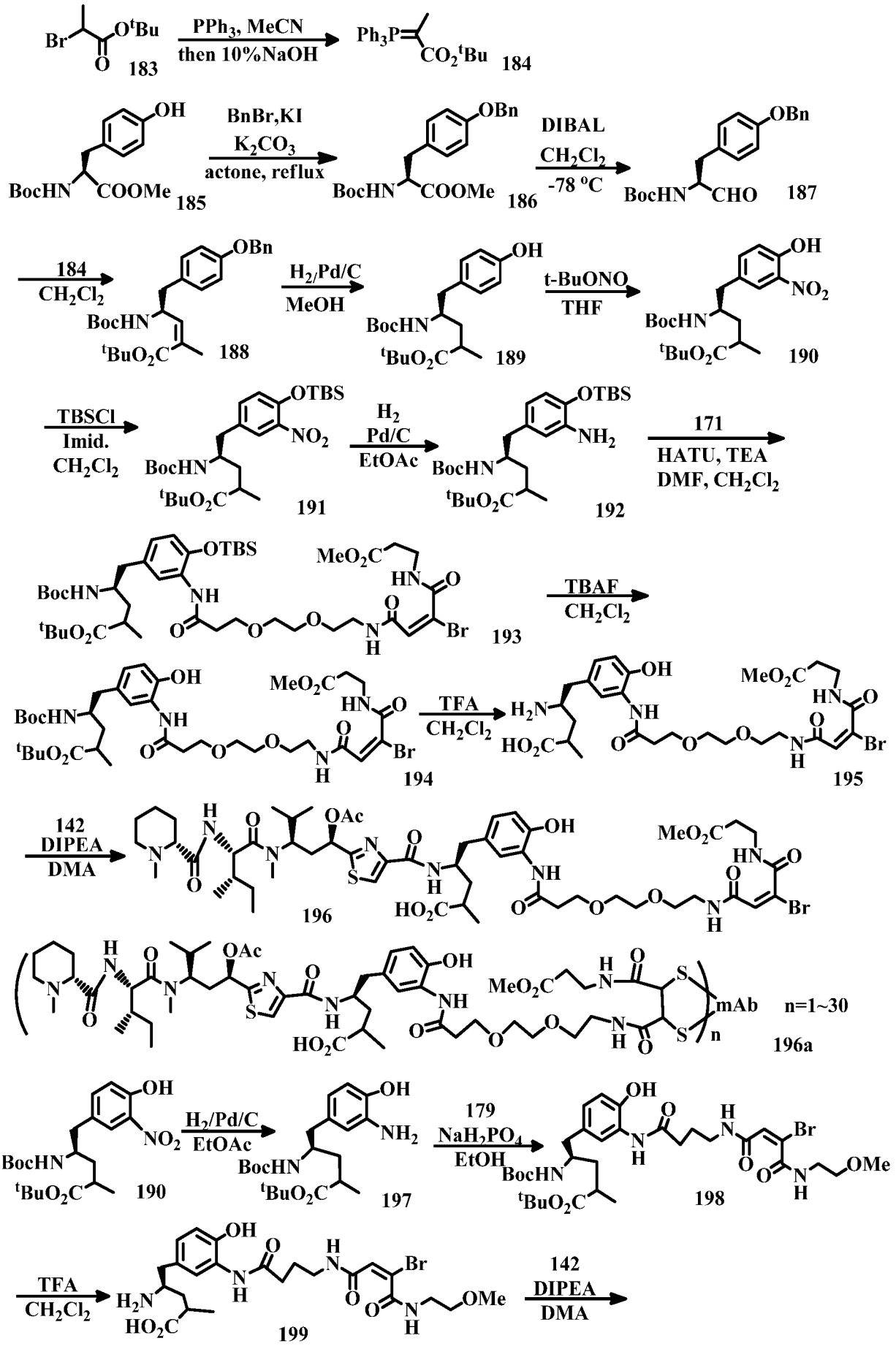

PPh3, MeCN
then 10%NaOH
183
184
BnBr,KI
K2CO3
actone, reflux
185
DIBAL
CH2Cl2
-78 °C
186
187
184
CH2Cl2
188
H2/Pd/C
MeOH
189
t-BuONO
THF
190
TBSCl
Imid.
CH2Cl2
191
H2
Pd/C
EtOAc
192
171
HATU, TEA
DMF, CH2Cl2
193
TBAF
CH2Cl2
194
TFA
CH2Cl2
195
142
DIPEA
DMA
196
mAb
n=1~30
196a
H2/Pd/C
EtOAc
197
179
NaH2PO4
EtOH
198
TFA
CH2Cl2
199
142
DIPEA
DMA

Figure 13

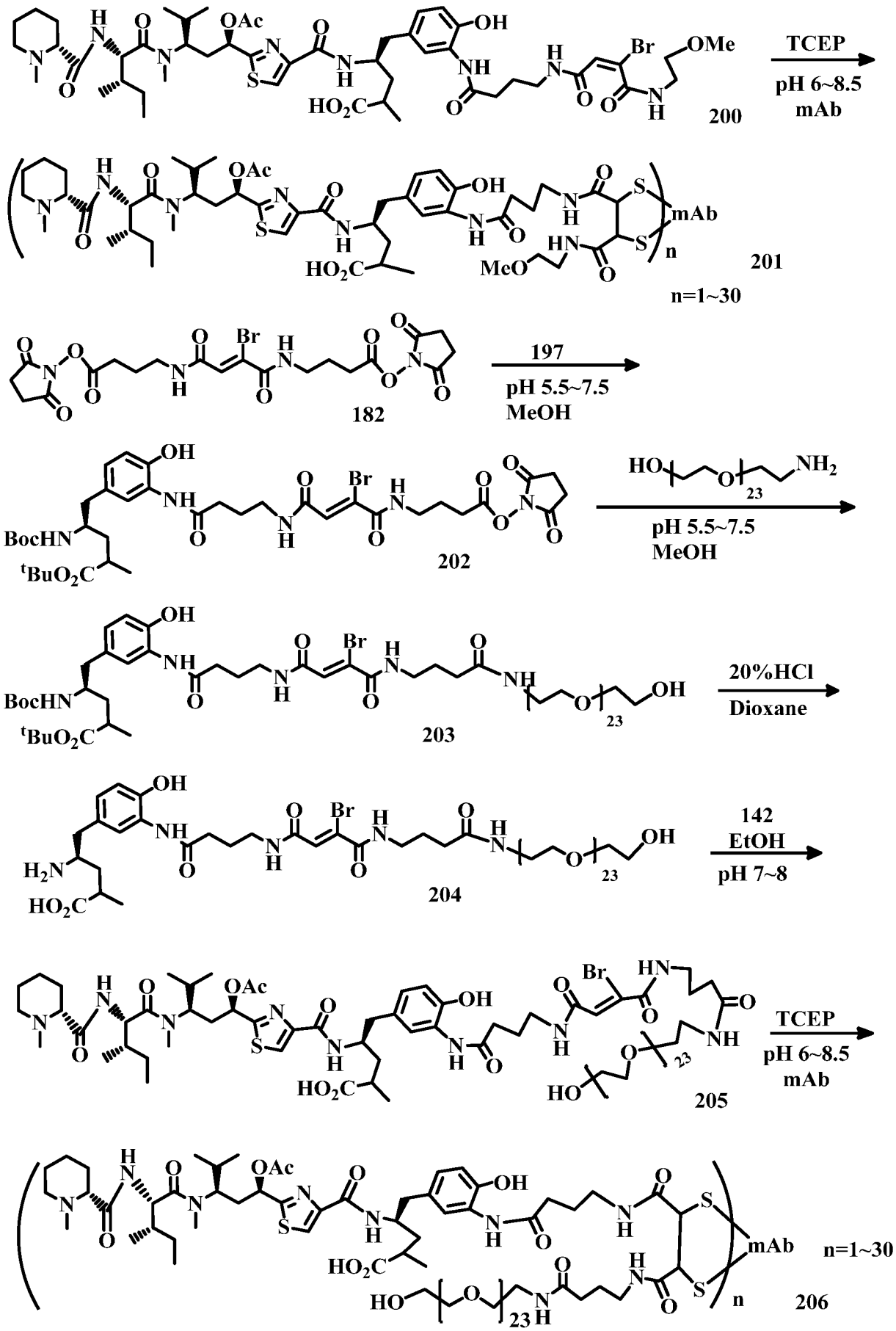

TCEP
pH 6~8.5
mAb
200
201
n=1~30
197
pH 5.5~7.5
MeOH
182
202
pH 5.5~7.5
MeOH
203
20%HCl
Dioxane
204
142
EtOH
pH 7~8
205
TCEP
pH 6~8.5
mAb
206
n=1~30

Figure 14

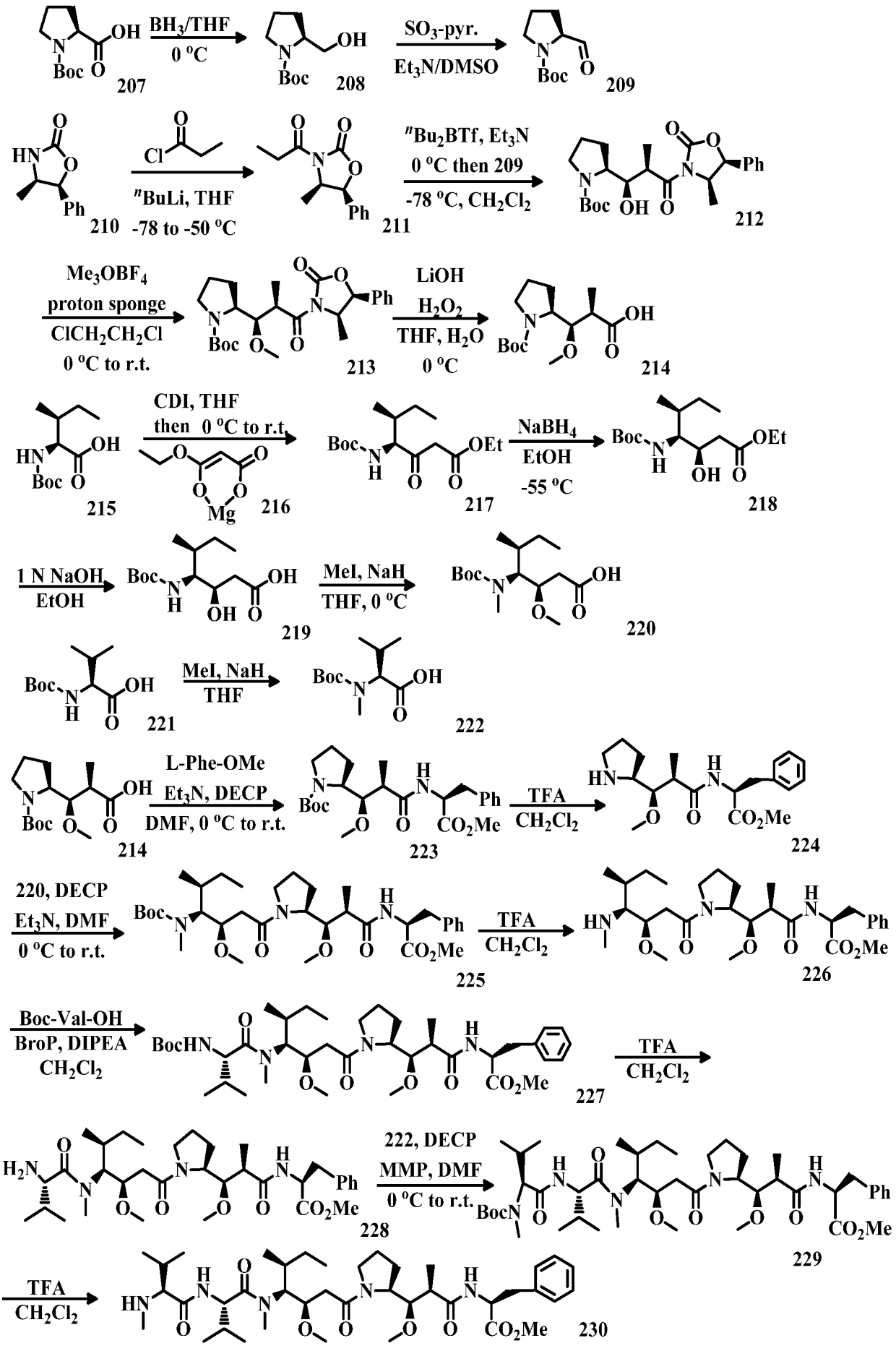

BH3/THF
0 °C
SO3-pyr.
Et3N/DMSO
207
208
209
210
nBuLi, THF
-78 to -50 °C
211
nBu2BTf, Et3N
0 °C then 209
-78 °C, CH2Cl2
212
Me3OBF4
proton sponge
ClCH2CH2Cl
0 °C to r.t.
213
LiOH
H2O2
THF, H2O
0 °C
214
CDI, THF
then 0 °C to r.t.
215
216
NaBH4
EtOH
-55 °C
217
218
1 N NaOH
EtOH
219
MeI, NaH
THF, 0 °C
220
MeI, NaH
THF
221
222
L-Phe-OMe
Et3N, DECP
DMF, 0 °C to r.t.
223
TFA
CH2Cl2
224
220, DECP
Et3N, DMF
0 °C to r.t.
225
226
Boc-Val-OH
BroP, DIPEA
CH2Cl2
227
222, DECP
MMP, DMF
0 °C to r.t.
228
229
230

Figure 15

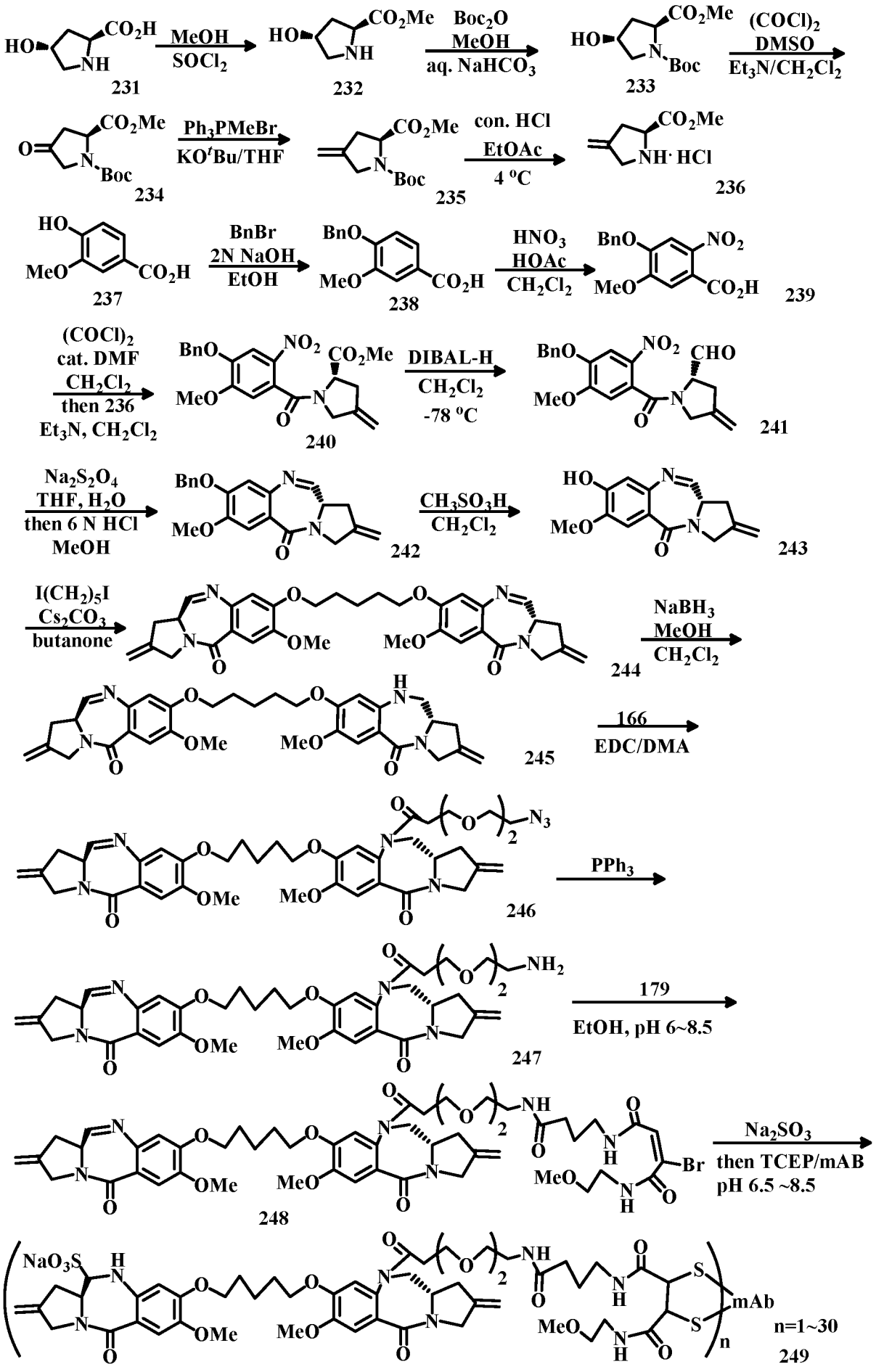

MeOH
SOCl2
Boc2O
MeOH
aq. NaHCO3
(COCl)2
DMSO
Et3N/CH2Cl2
Ph3PMeBr
KOtBu/THF
con. HCl
EtOAc
4 °C
BnBr
2N NaOH
EtOH
HNO3
HOAc
CH2Cl2
(COCl)2
cat. DMF
CH2Cl2
then 236
Et3N, CH2Cl2
DIBAL-H
CH2Cl2
-78 °C
Na2S2O4
THF, H2O
then 6 N HCl
MeOH
CH3SO3H
CH2Cl2
I(CH2)5I
Cs2CO3
butanone
NaBH3
MeOH
CH2Cl2
166
EDC/DMA
PPh3
179
EtOH, pH 6~8.5
Na2SO3
then TCEP/mAB
pH 6.5 ~8.5
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
n=1~30

Figure 16

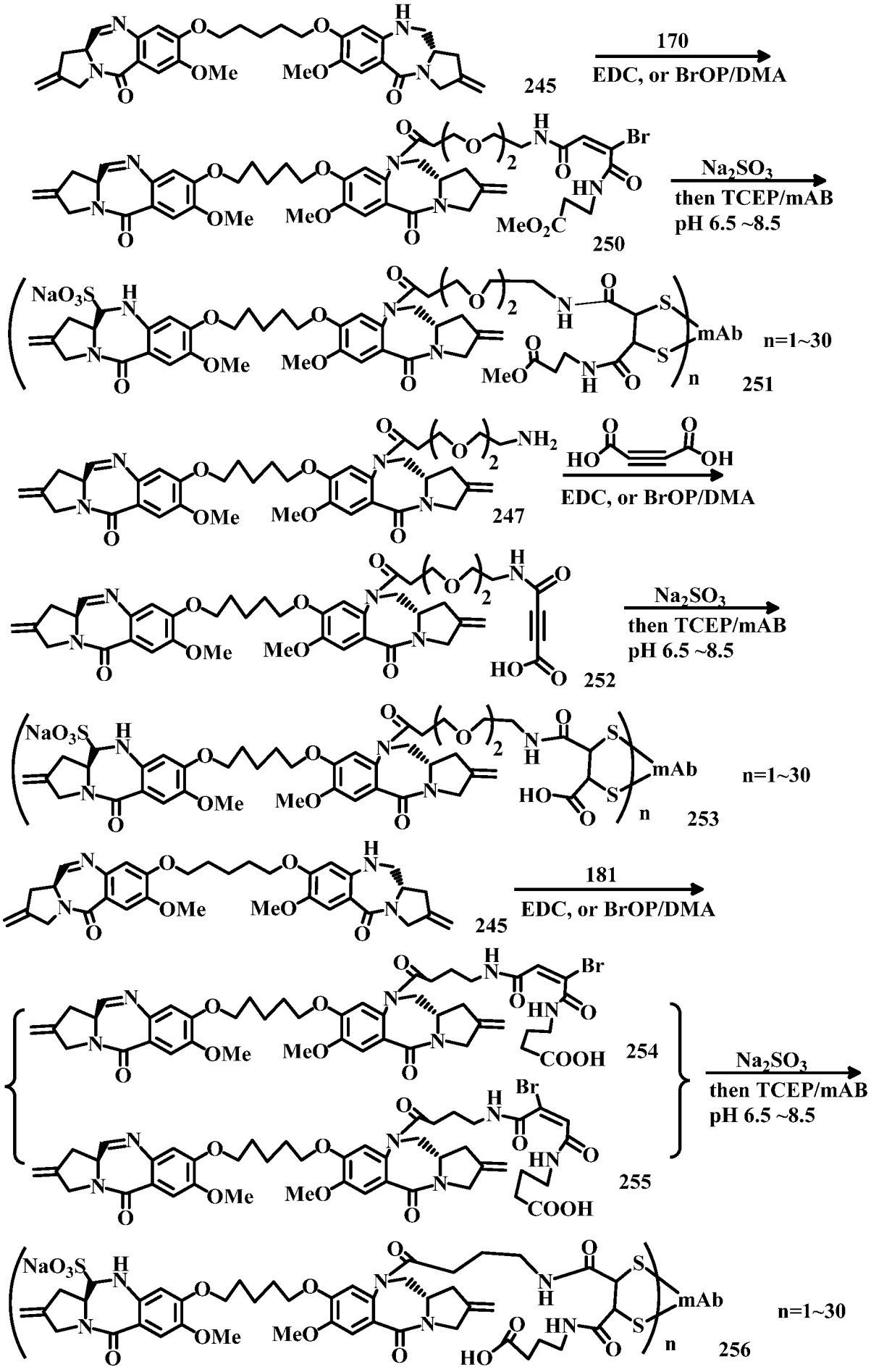

170
EDC, or BrOP/DMA
245
Na2SO3
then TCEP/mAB
pH 6.5 ~8.5
250
mAb
n=1~30
n
251
EDC, or BrOP/DMA
247
Na2SO3
then TCEP/mAB
pH 6.5 ~8.5
252
mAb
n=1~30
n
253
181
EDC, or BrOP/DMA
245
254
255
Na2SO3
then TCEP/mAB
pH 6.5 ~8.5
mAb
n=1~30
n
256

Figure 17

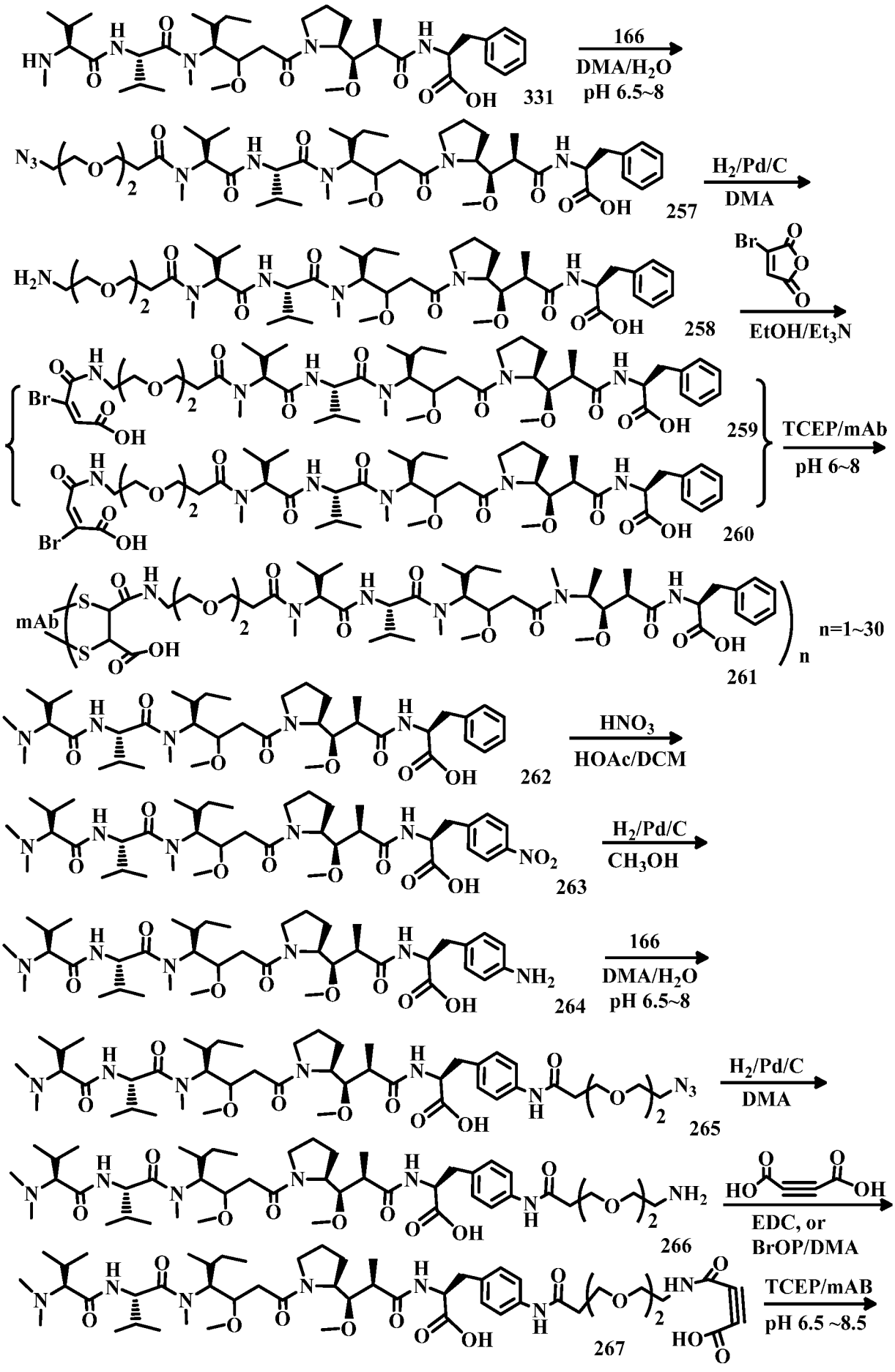

166
DMA/H2O
pH 6.5~8
331
H2/Pd/C
DMA
257
Br
EtOH/Et3N
258
259
260
TCEP/mAb
pH 6~8
mAb
n=1~30
261
HNO3
HOAc/DCM
262
H2/Pd/C
CH3OH
263
166
DMA/H2O
pH 6.5~8
264
H2/Pd/C
DMA
265
EDC, or
BrOP/DMA
266
TCEP/mAB
pH 6.5 ~8.5
267

Figure 18

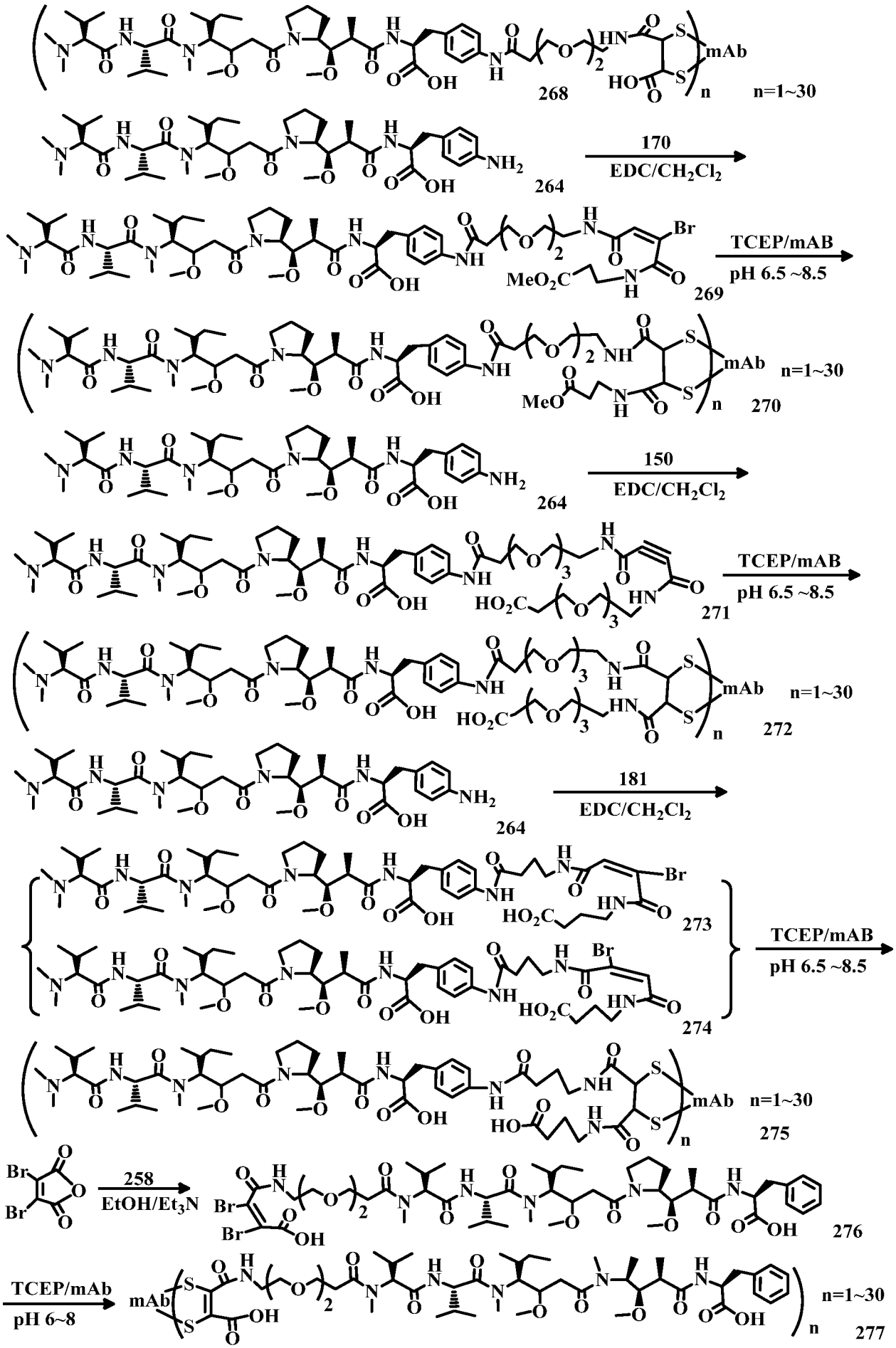

268
n=1~30
170
EDC/CH2Cl2
264
TCEP/mAB
pH 6.5 ~8.5
269
270
n=1~30
150
271
272
181
273
274
275
258
EtOH/Et3N
276
TCEP/mAb
pH 6~8
277

Figure 19

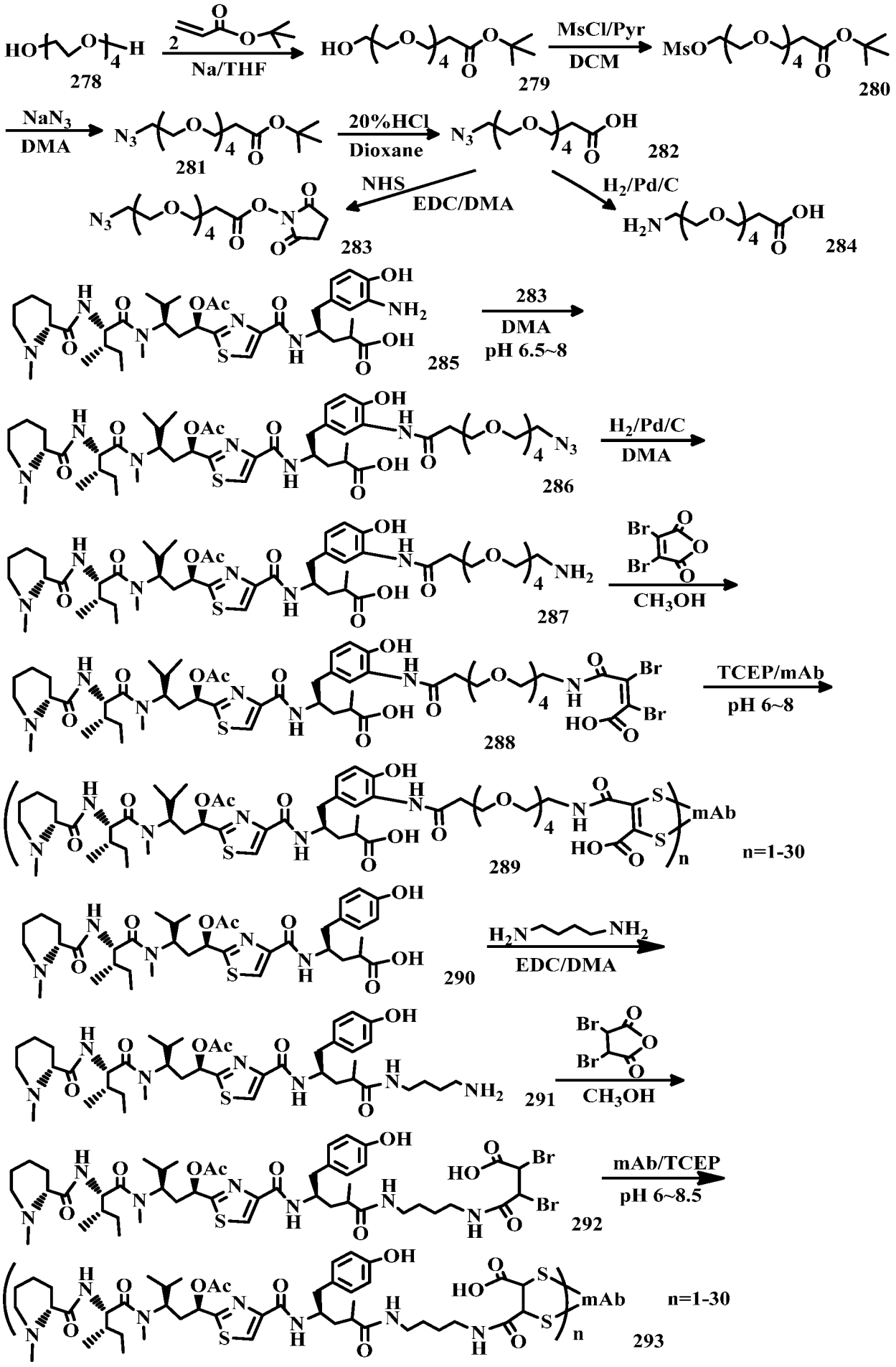

278
Na/THF
279
MsCl/Pyr
DCM
280
NaN3
DMA
281
20%HCl
Dioxane
282
NHS
EDC/DMA
283
H2/Pd/C
284
283
DMA
pH 6.5~8
285
H2/Pd/C
DMA
286
287
CH3OH
TCEP/mAb
pH 6~8
288
289
n=1-30
290
EDC/DMA
291
CH3OH
mAb/TCEP
pH 6~8.5
292
n=1-30
293

Figure 20

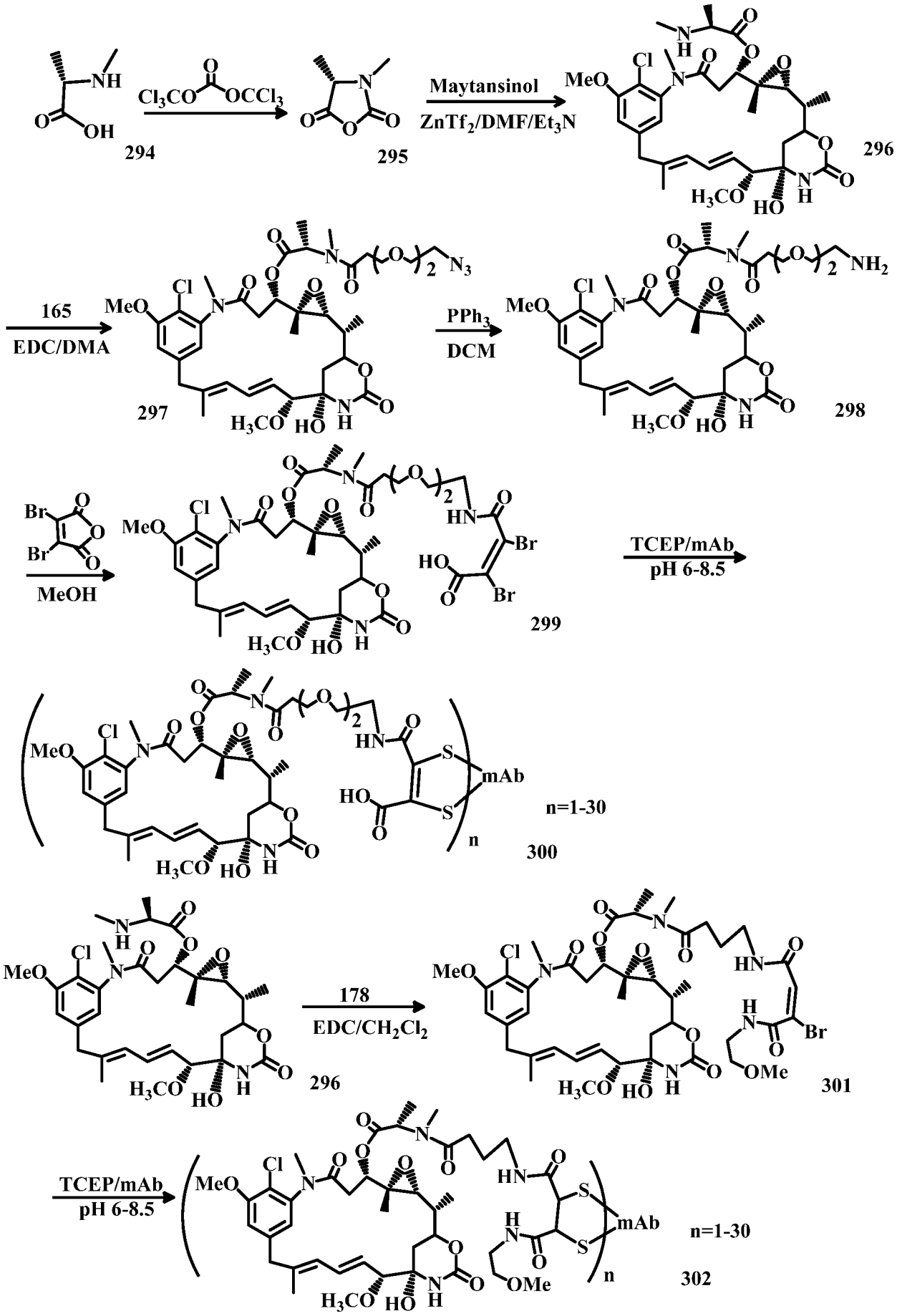

294
295
Maytansinol
ZnTf2/DMF/Et3N
296
165
EDC/DMA
297
PPh3
DCM
298
MeOH
299
TCEP/mAb
pH 6-8.5
n=1-30
300
178
EDC/CH2Cl2
301
302

Figure 21.

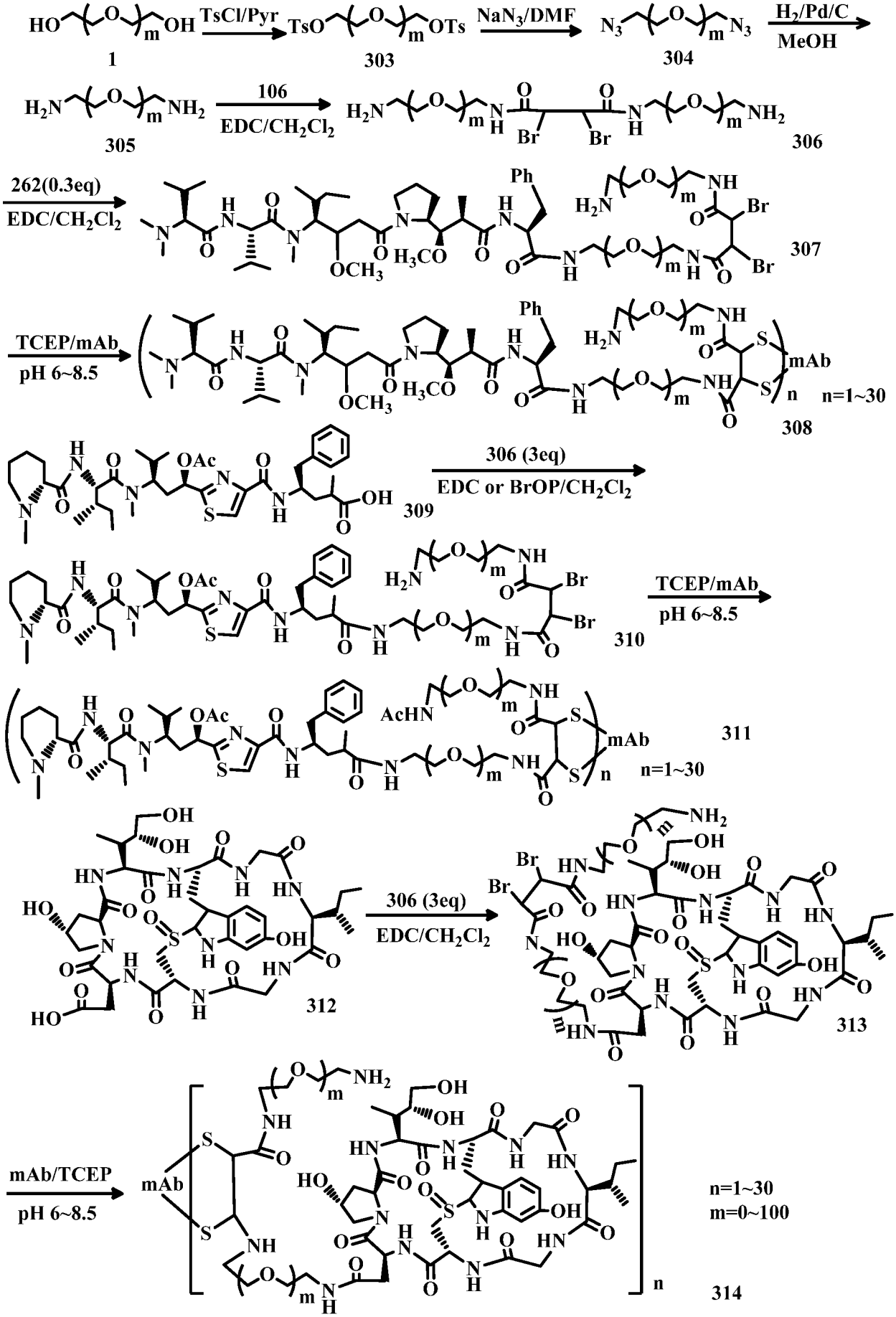

HO OH TsCl/Pyr TsO OTs NaN3/DMF N3 N3 H2/Pd/C MeOH
1
303
304
H2N NH2 106 EDC/CH2Cl2
305
306
262(0.3eq) EDC/CH2Cl2
307
TCEP/mAb pH 6~8.5
mAb
n=1~30
308
309
306 (3eq) EDC or BrOP/CH2Cl2
310
TCEP/mAb pH 6~8.5
311
n=1~30
312
306 (3eq) EDC/CH2Cl2
313
mAb/TCEP pH 6~8.5
n=1~30
m=0~100
314

Figure 22

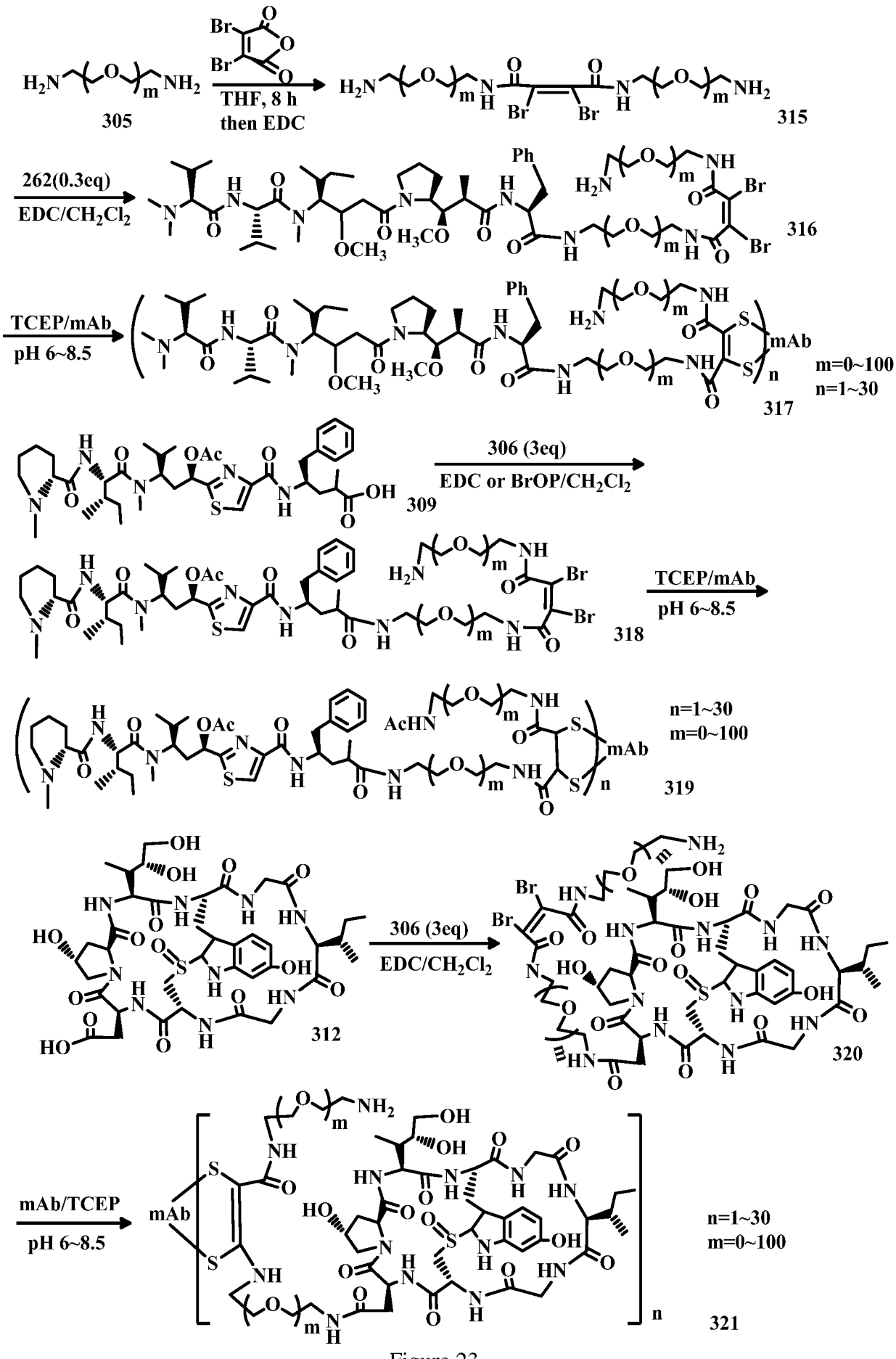

THF, 8 h
then EDC
305
315
262(0.3eq)
EDC/CH2Cl2
316
TCEP/mAb
pH 6~8.5
m=0~100
n=1~30
317
309
306 (3eq)
EDC or BrOP/CH2Cl2
318
TCEP/mAb
pH 6~8.5
n=1~30
m=0~100
319
312
306 (3eq)
EDC/CH2Cl2
320
mAb/TCEP
pH 6~8.5
n=1~30
m=0~100
321

Figure 23.

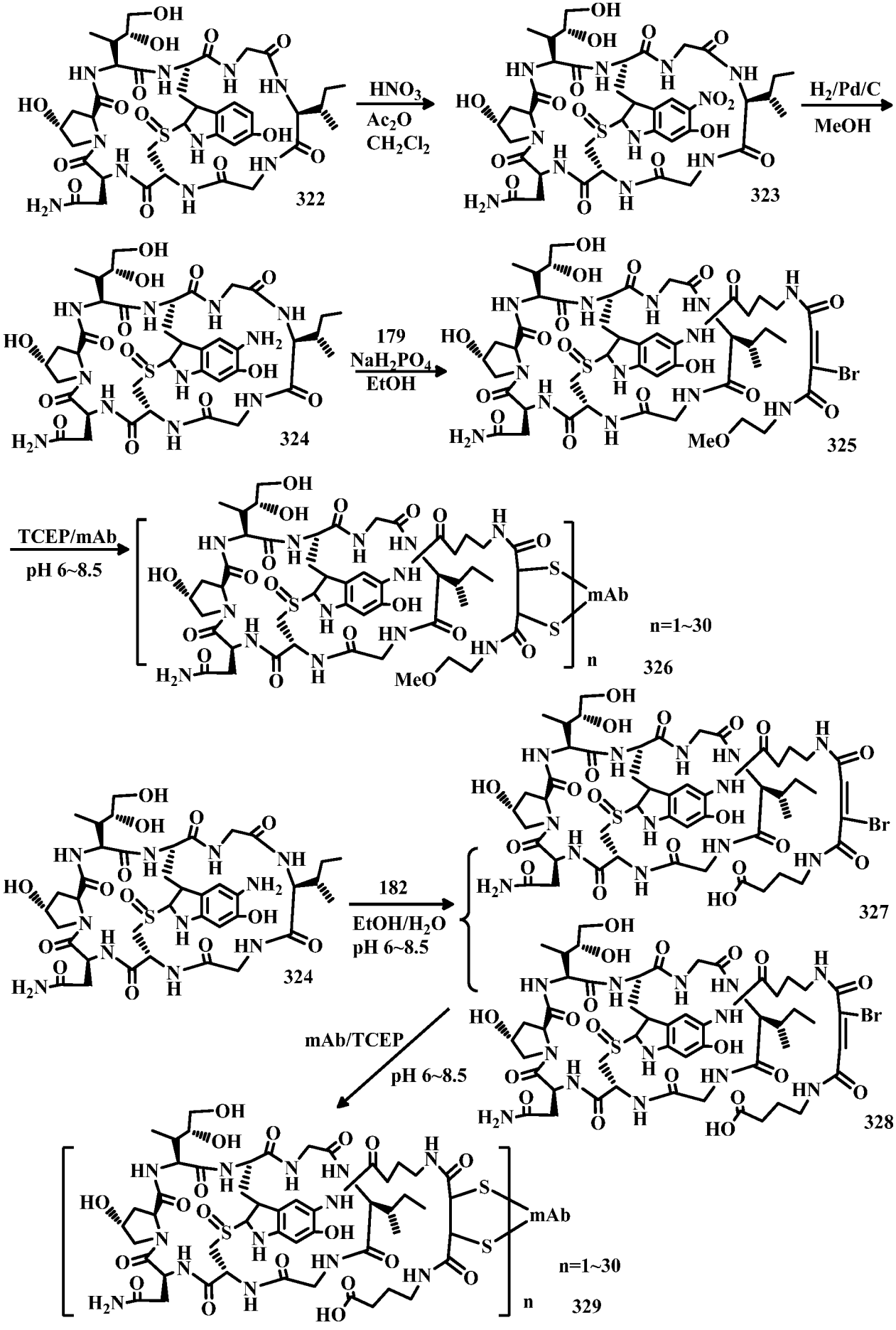

HNO3
Ac2O
CH2Cl2
H2/Pd/C
MeOH
322
323
179
NaH2PO4
EtOH
324
325
TCEP/mAb
pH 6~8.5
mAb
n=1~30
n
326
182
EtOH/H2O
pH 6~8.5
327
328
mAb/TCEP
pH 6~8.5
329

Figure 24.

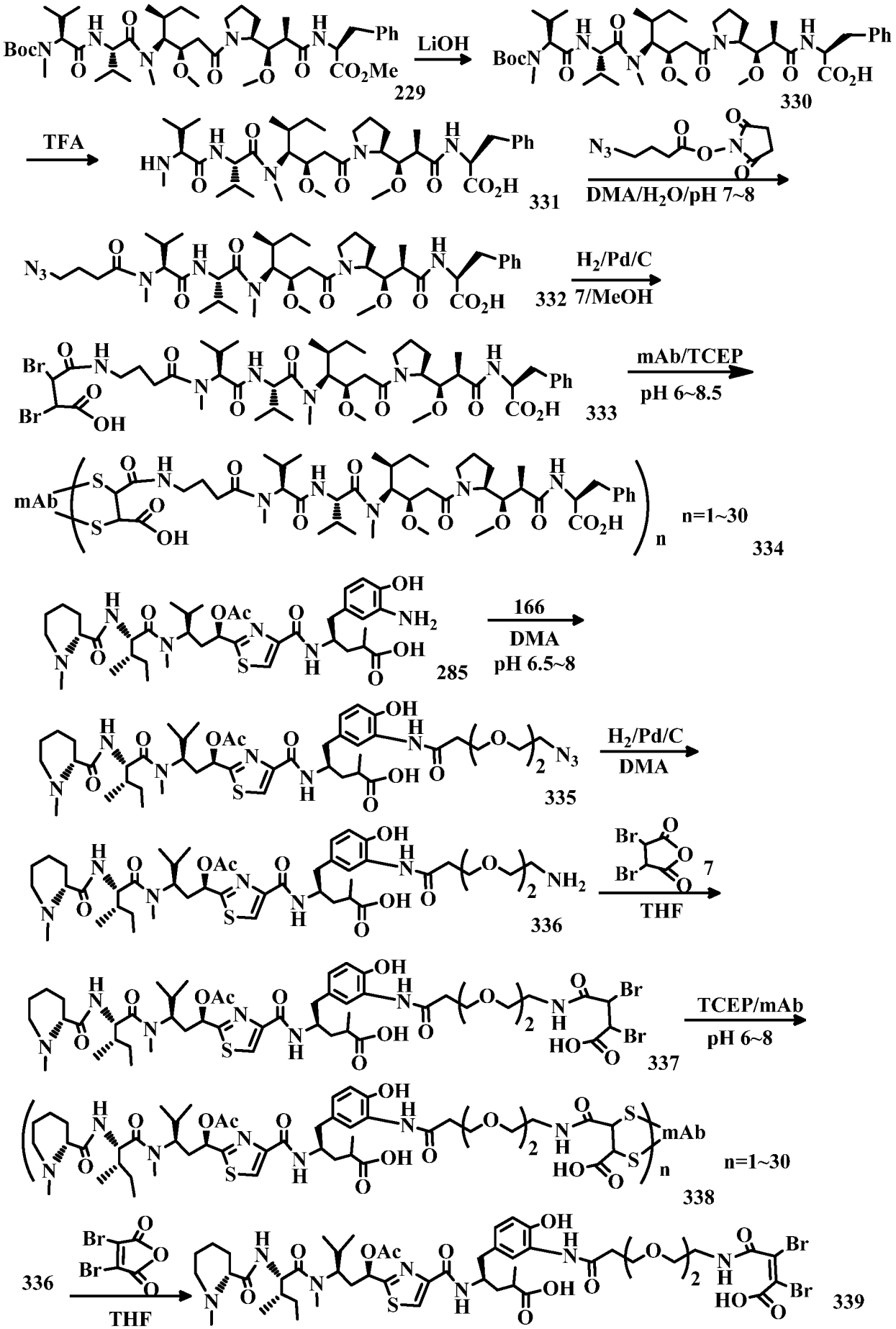

LiOH
TFA
DMA/H2O/pH 7~8
H2/Pd/C
7/MeOH
mAb/TCEP
pH 6~8.5
n=1~30
166
DMA
pH 6.5~8
H2/Pd/C
DMA
THF
TCEP/mAb
pH 6~8
n=1~30
THF

Figure 25

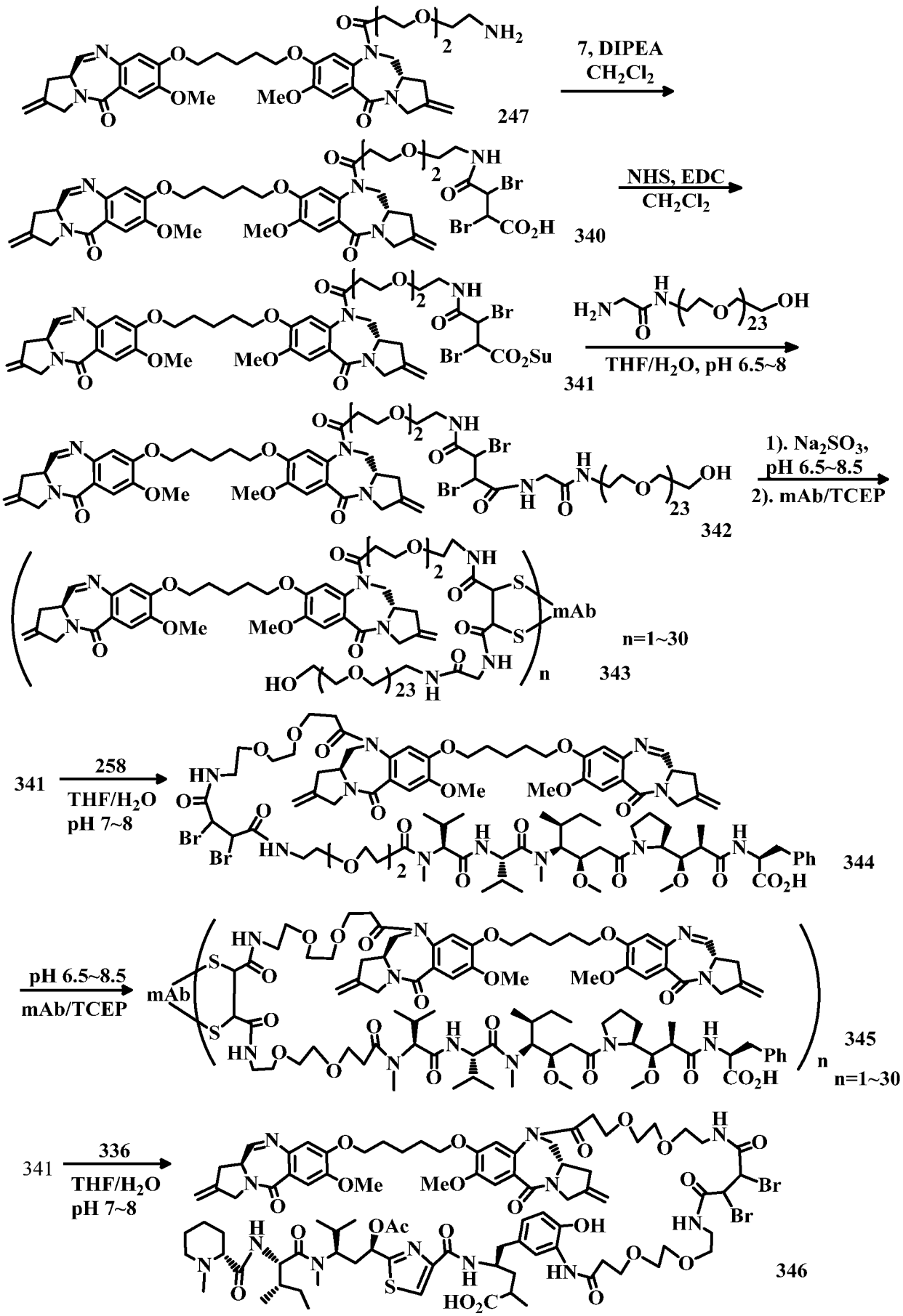

7, DIPEA
CH2Cl2
247
NHS, EDC
CH2Cl2
340
H2N
OH
THF/H2O, pH 6.5~8
341
1). Na2SO3,
pH 6.5~8.5
2). mAb/TCEP
342
mAb
n=1~30
343
341
258
THF/H2O
pH 7~8
344
pH 6.5~8.5
mAb/TCEP
345
n=1~30
341
336
THF/H2O
pH 7~8
346

Figure 26

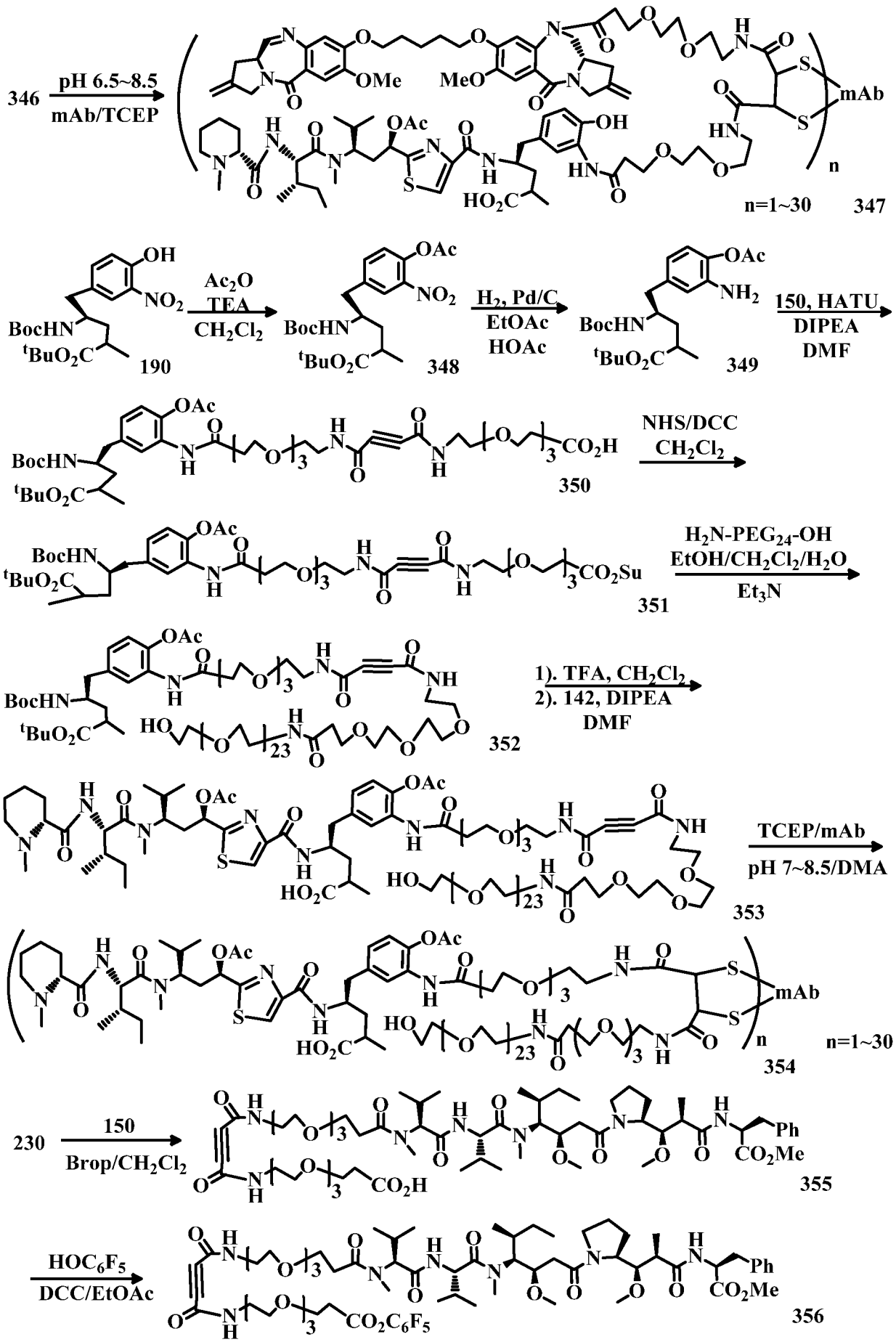

346
pH 6.5~8.5
mAb/TCEP
mAb
n
n=1~30
347
190
Ac2O
TEA
CH2Cl2
348
H2, Pd/C
EtOAc
HOAc
349
150, HATU
DIPEA
DMF
NHS/DCC
CH2Cl2
350
351
H2N-PEG24-OH
EtOH/CH2Cl2/H2O
Et3N
352
1). TFA, CH2Cl2
2). 142, DIPEA
DMF
353
TCEP/mAb
pH 7~8.5/DMA
354
n=1~30
230
150
Brop/CH2Cl2
355
HOC6F5
DCC/EtOAc
356

Figure 27

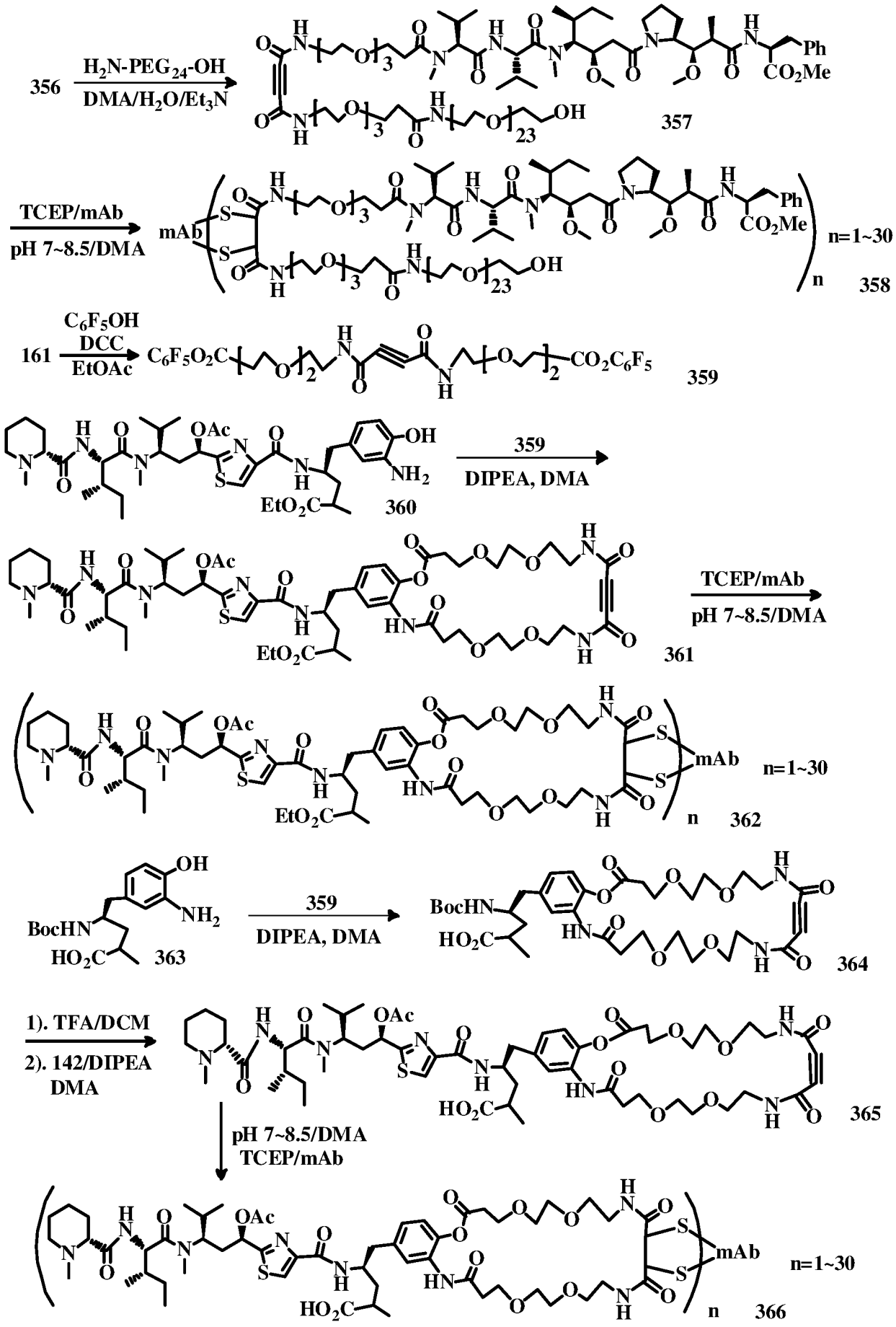

356
H2N-PEG24-OH
DMA/H2O/Et3N
357
TCEP/mAb
pH 7~8.5/DMA
mAb
n=1~30
n
358
161
C6F5OH
DCC
EtOAc
C6F5O2C
CO2C6F5
359
OAc
OH
NH2
EtO2C
360
359
DIPEA, DMA
TCEP/mAb
pH 7~8.5/DMA
361
mAb
n=1~30
362
BocHN
HO2C
363
364
1). TFA/DCM
2). 142/DIPEA
DMA
365
pH 7~8.5/DMA
TCEP/mAb
366

Figure 28

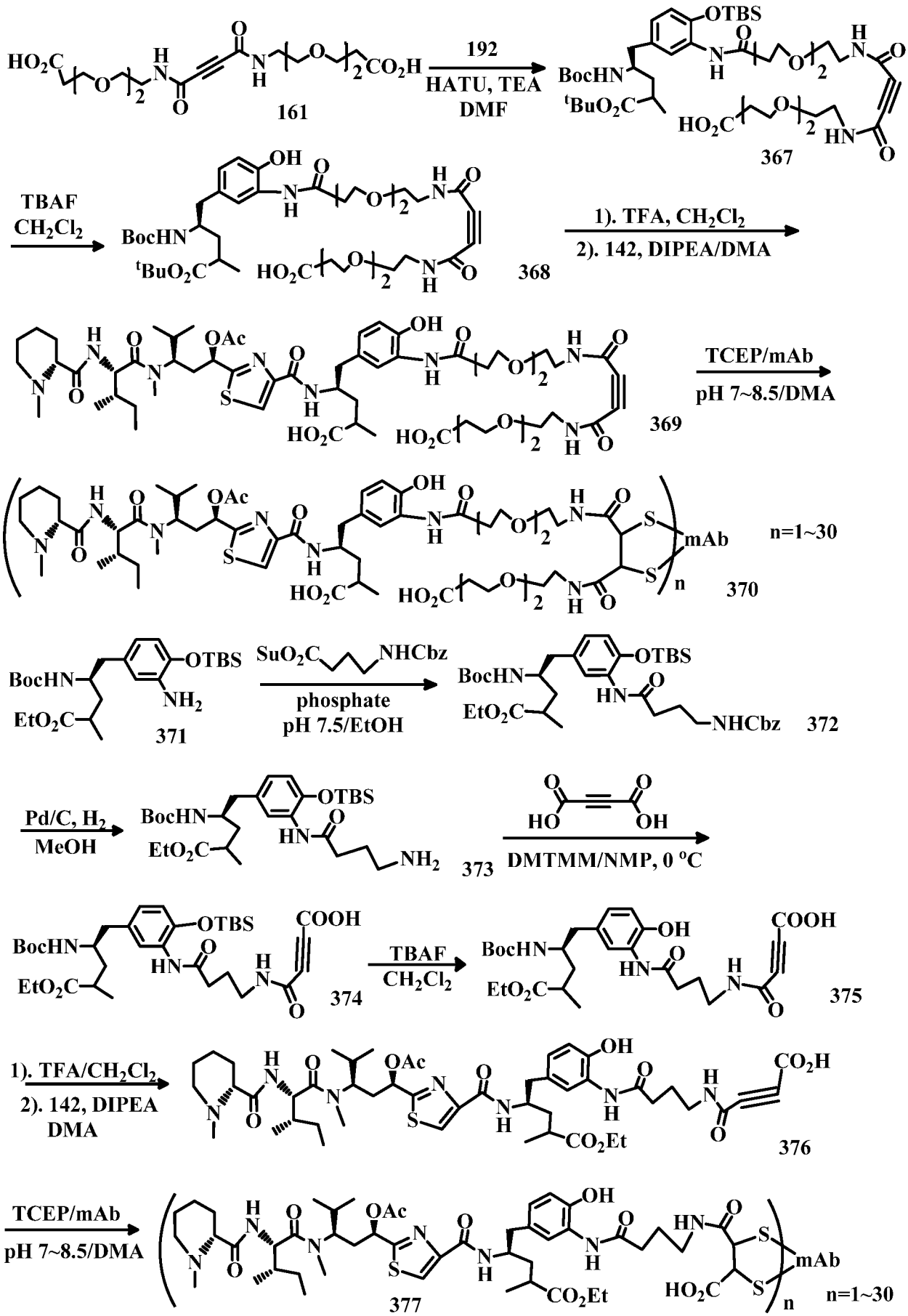

192
HATU, TEA
DMF
161
367
TBAF
CH2Cl2
368
1). TFA, CH2Cl2
2). 142, DIPEA/DMA
369
TCEP/mAb
pH 7~8.5/DMA
n=1~30
370
SuO2C
NHCbz
phosphate
pH 7.5/EtOH
371
372
Pd/C, H2
MeOH
373
DMTMM/NMP, 0 °C
374
375
1). TFA/CH2Cl2
2). 142, DIPEA
DMA
376
377

Figure 29

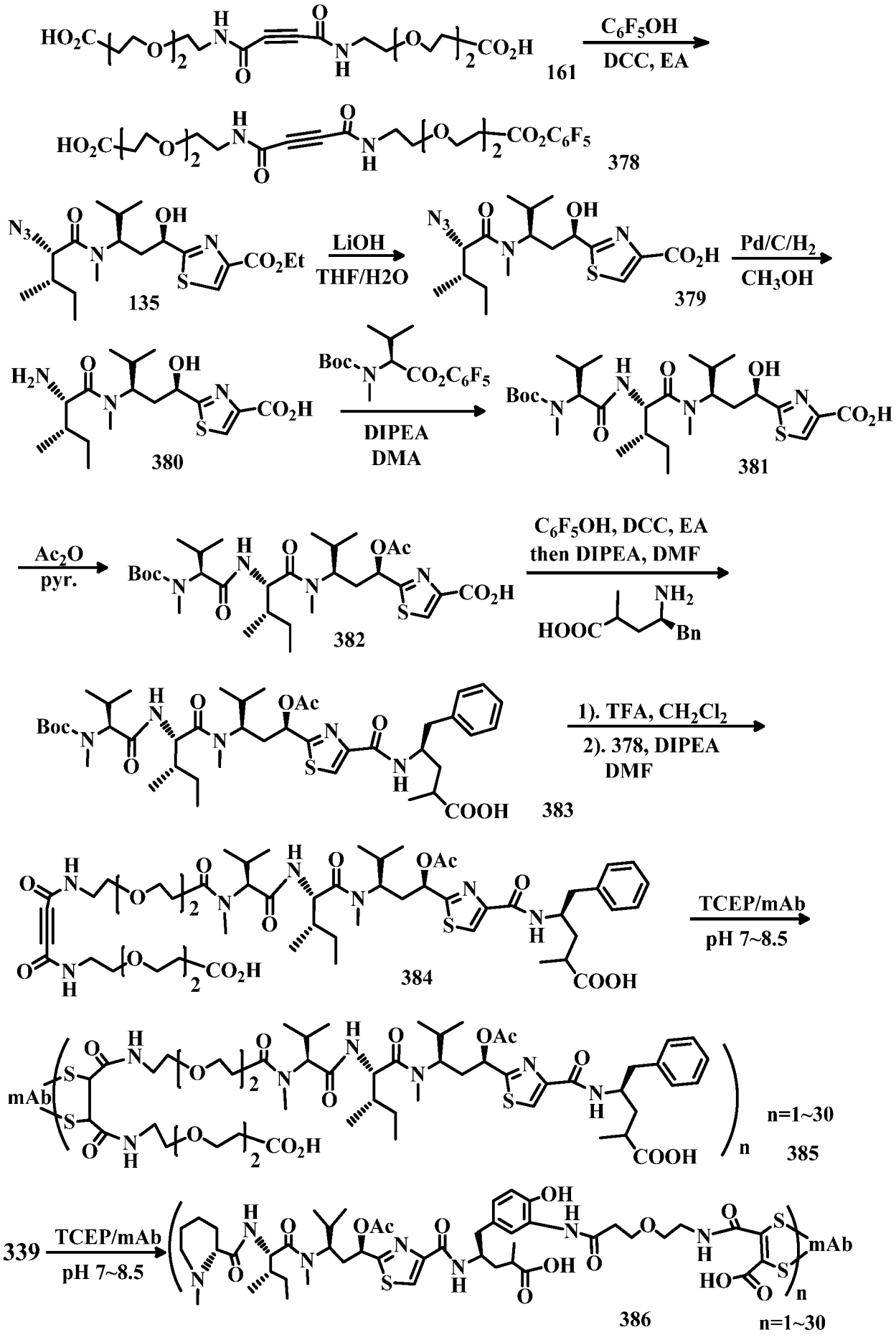

C6F5OH
DCC, EA
161
378
LiOH
THF/H2O
135
379
Pd/C/H2
CH3OH
DIPEA
DMA
380
381
Ac2O
pyr.
382
C6F5OH, DCC, EA
then DIPEA, DMF
1). TFA, CH2Cl2
2). 378, DIPEA
DMF
383
384
TCEP/mAb
pH 7~8.5
n=1~30
385
339
386

Figure 30

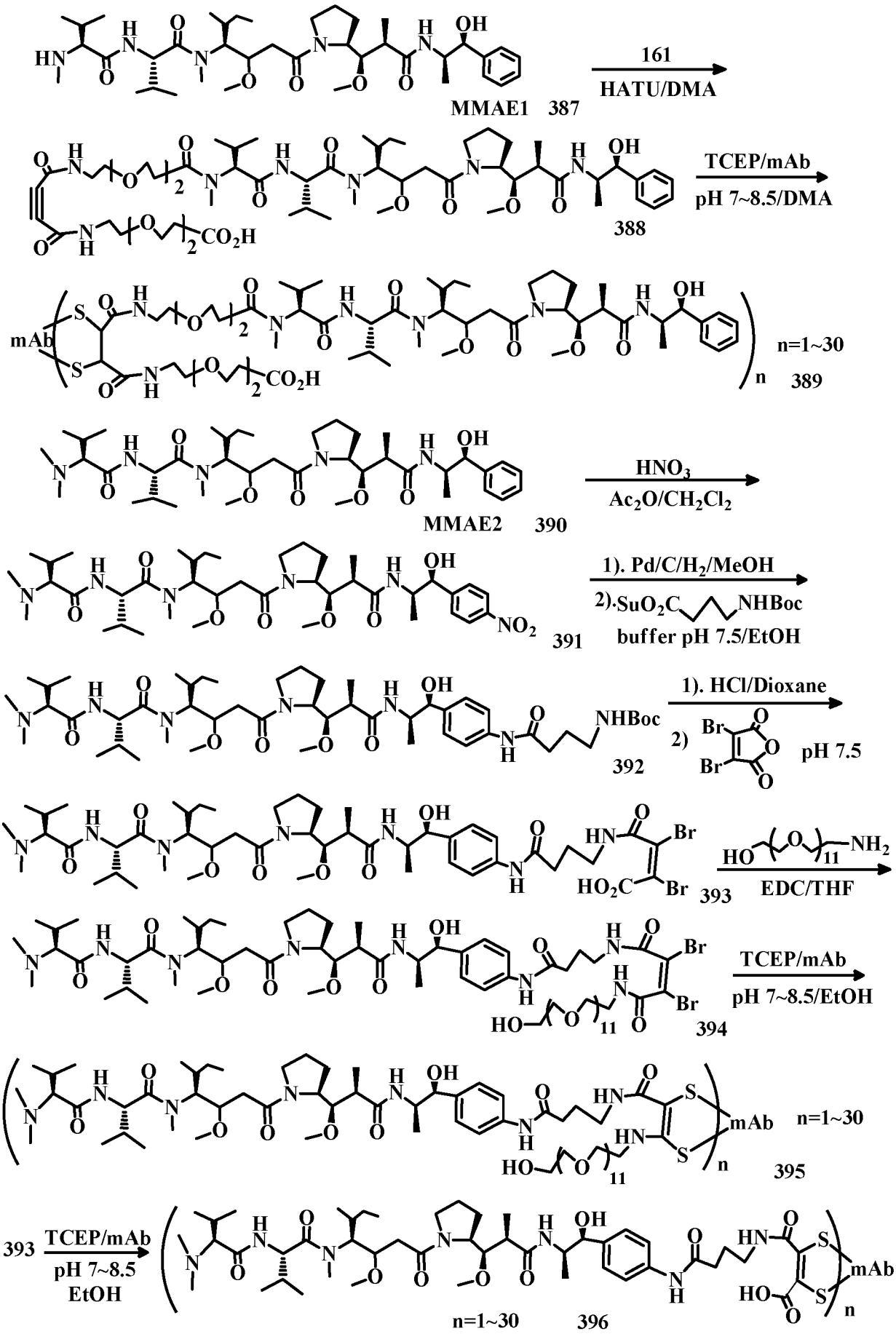

MMAE1 387
161
HATU/DMA
388
TCEP/mAb
pH 7~8.5/DMA
mAb
n=1~30
n
389
MMAE2 390
HNO3
Ac2O/CH2Cl2
391
1). Pd/C/H2/MeOH
2).SuO2C NHBoc
buffer pH 7.5/EtOH
392
1). HCl/Dioxane
2)
pH 7.5
393
HO2C
EDC/THF
394
TCEP/mAb
pH 7~8.5/EtOH
395
n=1~30
393
TCEP/mAb
pH 7~8.5
EtOH
n=1~30
396

Figure 31

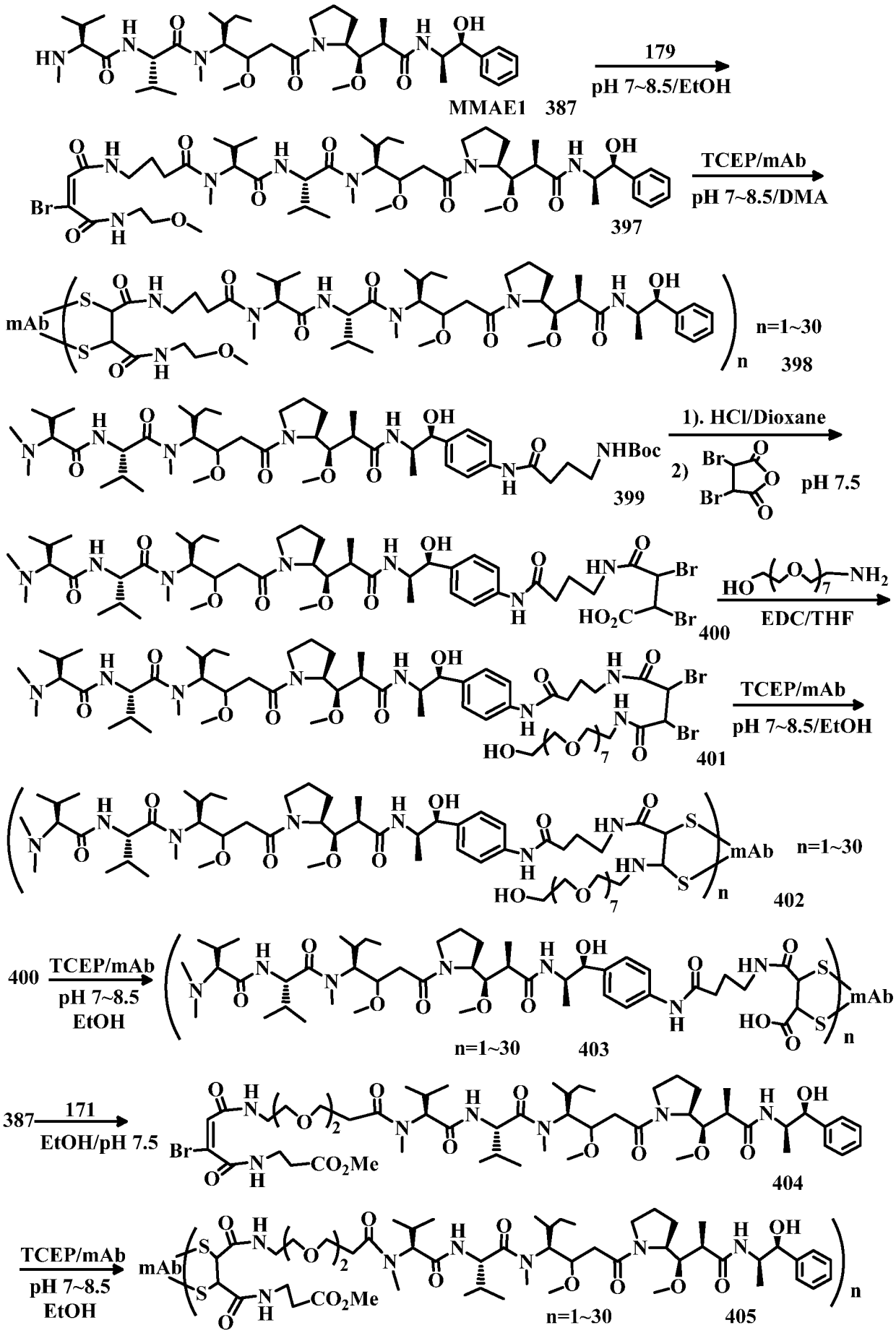

179
pH 7~8.5/EtOH
MMAE1 387
TCEP/mAb
pH 7~8.5/DMA
397
mAb
n=1~30
n
398
1). HCl/Dioxane
2)
pH 7.5
NHBoc
399
HO
NH2
EDC/THF
400
TCEP/mAb
pH 7~8.5/EtOH
401
mAb
n=1~30
402
400
TCEP/mAb
pH 7~8.5
EtOH
n=1~30
403
387
171
EtOH/pH 7.5
CO2Me
404
TCEP/mAb
pH 7~8.5
EtOH
n=1~30
405

Figure 32

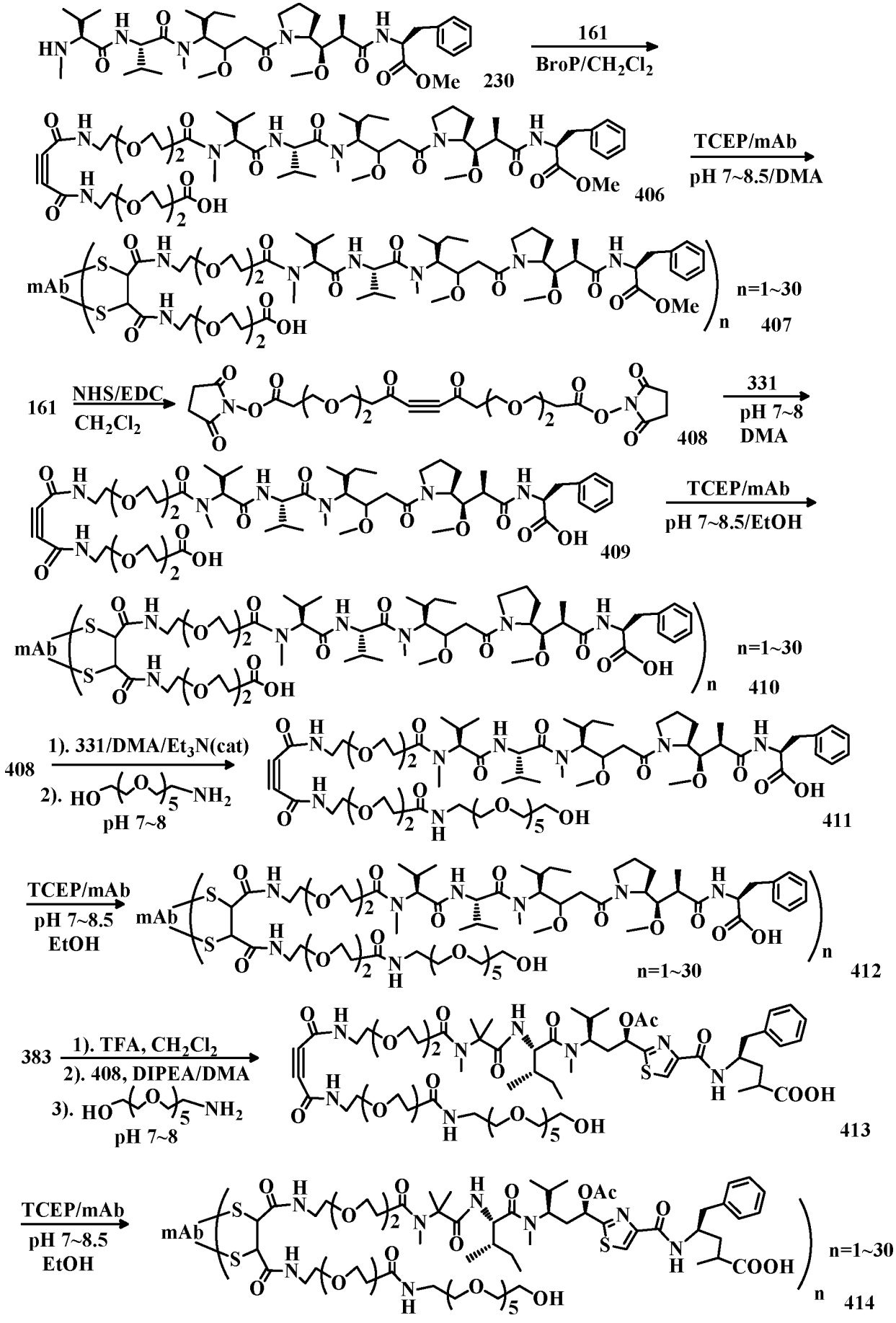

161
BroP/CH2Cl2
230
OMe
TCEP/mAb
pH 7~8.5/DMA
406
407
mAb
n=1~30
161
NHS/EDC
CH2Cl2
408
331
pH 7~8
DMA
409
TCEP/mAb
pH 7~8.5/EtOH
410
1). 331/DMA/Et3N(cat)
2). HO
NH2
pH 7~8
411
412
383
1). TFA, CH2Cl2
2). 408, DIPEA/DMA
3). HO
NH2
pH 7~8
OAc
COOH
413
414

Figure 33

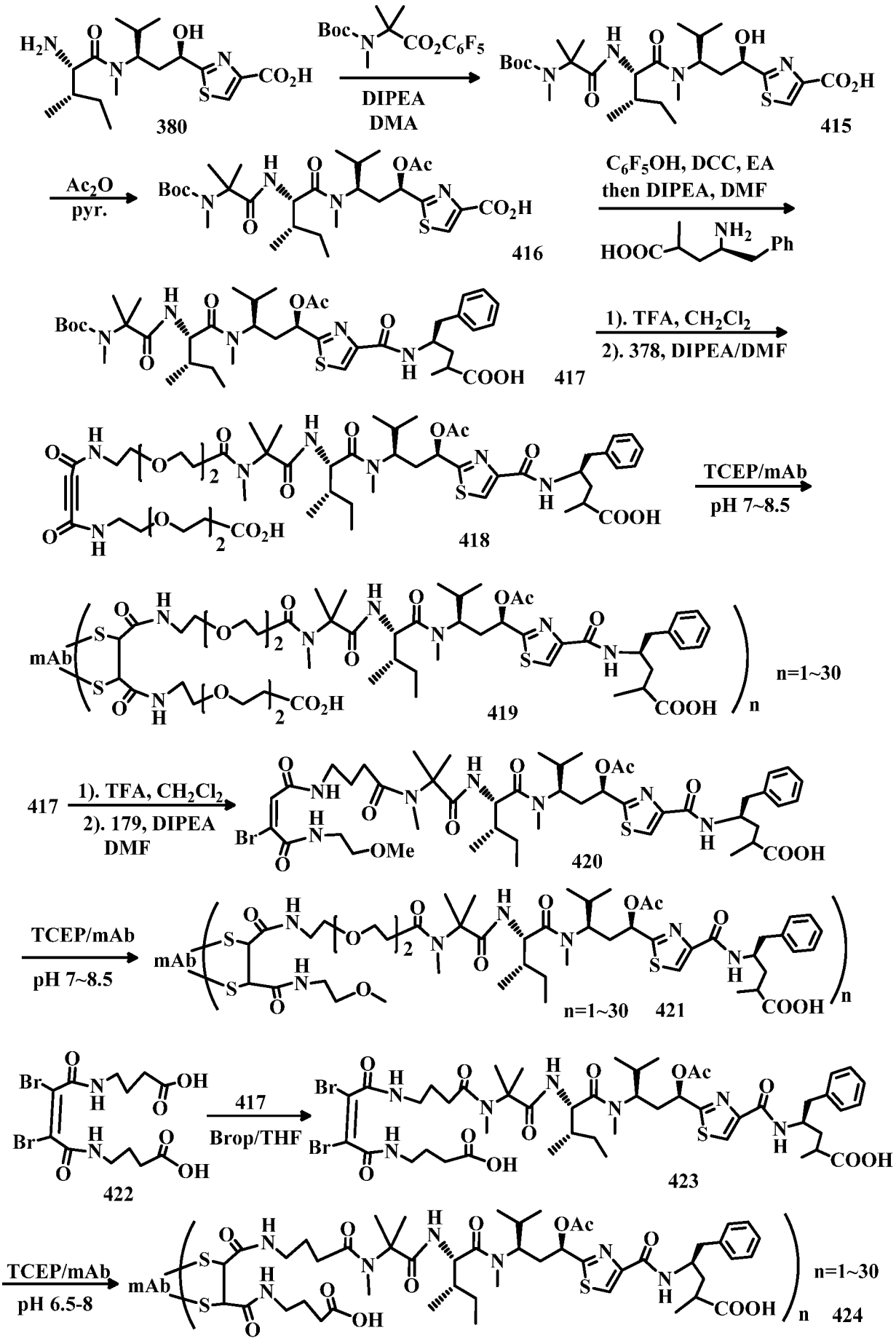

380
DIPEA
DMA
415
Ac2O
pyr.
416
C6F5OH, DCC, EA
then DIPEA, DMF
1). TFA, CH2Cl2
2). 378, DIPEA/DMF
417
418
TCEP/mAb
pH 7~8.5
n=1~30
419
1). TFA, CH2Cl2
2). 179, DIPEA
DMF
420
TCEP/mAb
pH 7~8.5
n=1~30
421
422
417
Brop/THF
423
TCEP/mAb
pH 6.5-8
n=1~30
424

Figure 34

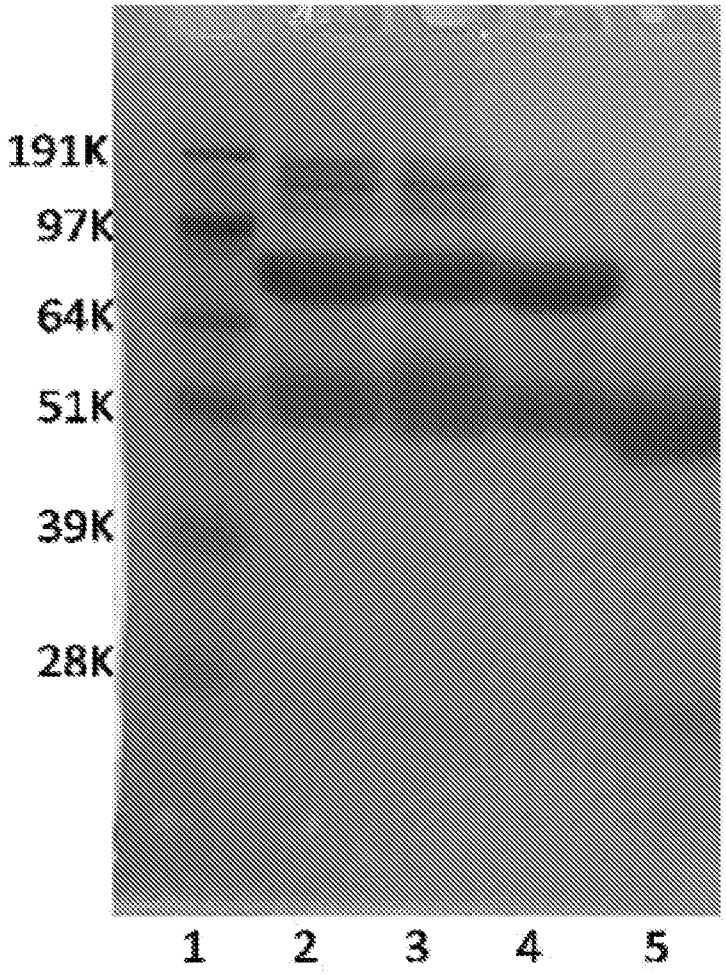
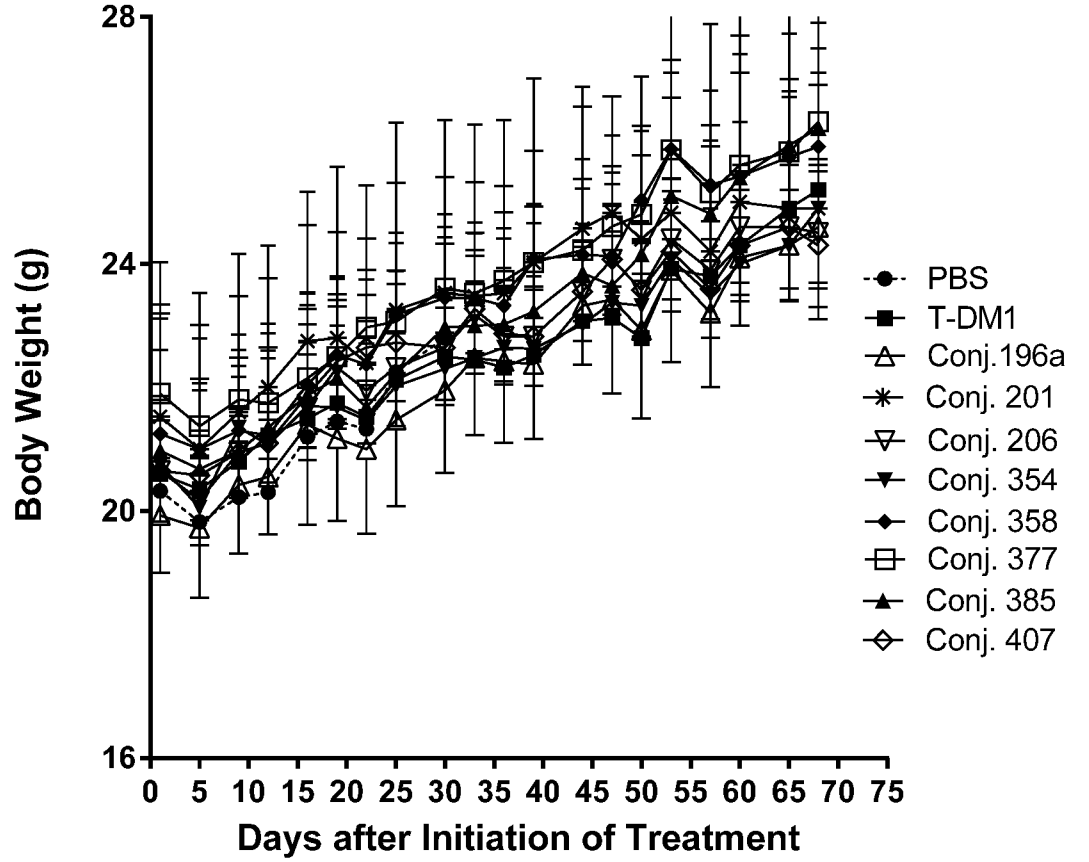


Figure 35


Body Weight (g)
28
24
20
16
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Days after Initiation of Treatment
PBS
T-DM1
Conj.196a
Conj. 201
Conj. 206
Conj. 354
Conj. 358
Conj. 377
Conj. 385
Conj. 407

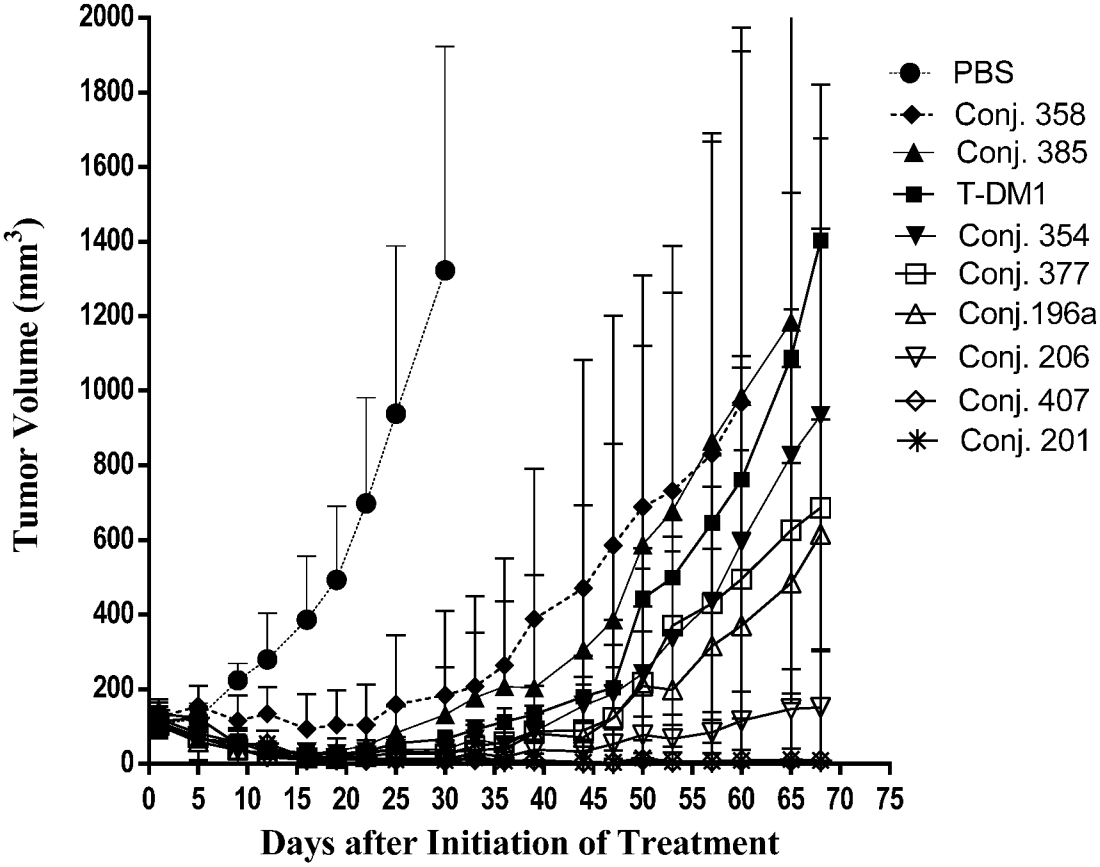

Tumor Volume (mm3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Days after Initiation of Treatment
PBS
Conj. 358
Conj. 385
T-DM1
Conj. 354
Conj. 377
Conj.196a
Conj. 206
Conj. 407
Conj. 201

Figure 36