Figure 1

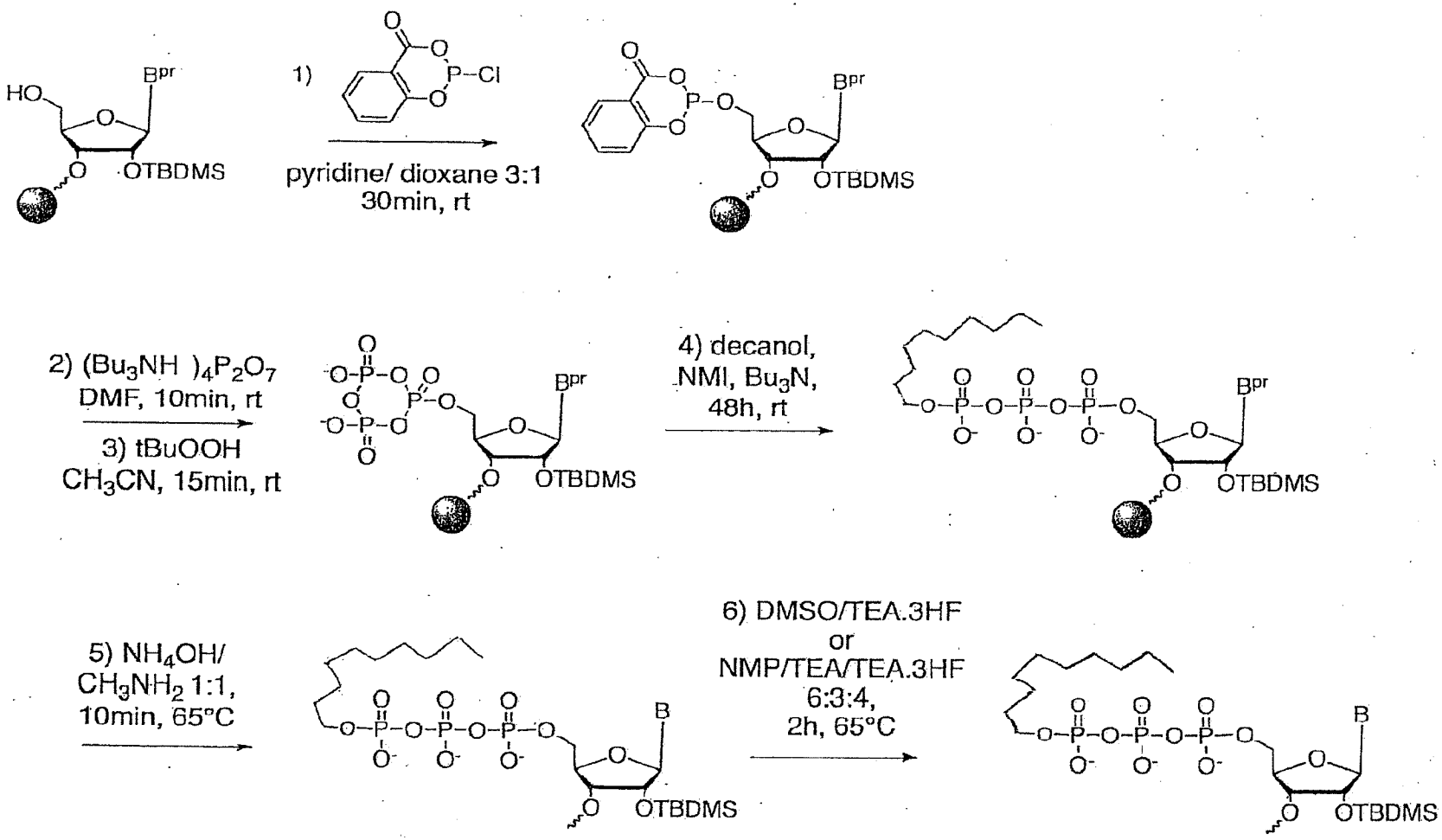
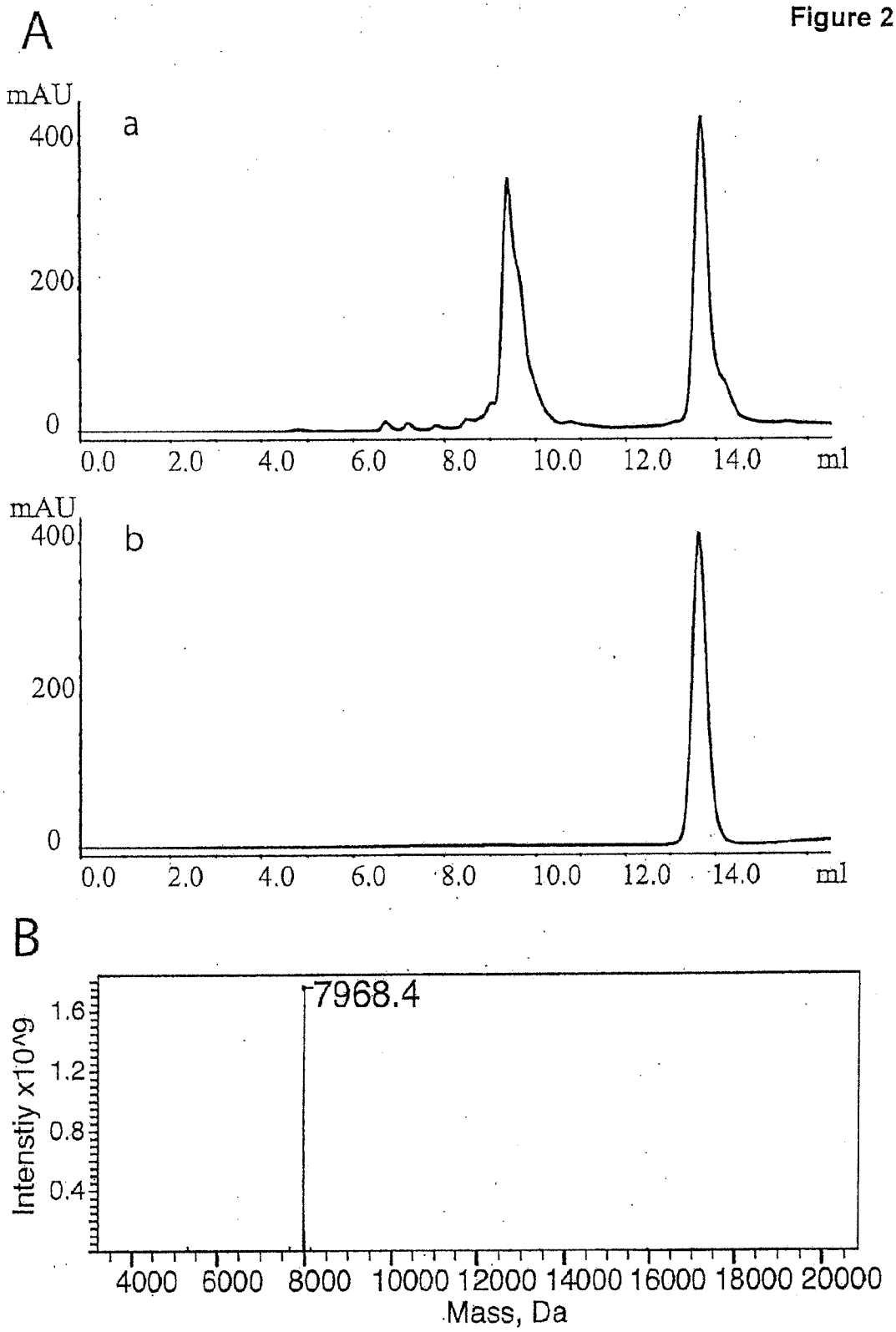

HO
Bpr
OTBDMS
1)
P–Cl
pyridine/ dioxane 3:1
30min, rt
2) (Bu3NH)4P2O7
DMF, 10min, rt
3) tBuOOH
CH3CN, 15min, rt
4) decanol,
NMI, Bu3N,
48h, rt
5) NH4OH/
CH3NH2 1:1,
10min, 65°C
6) DMSO/TEA.3HF
or
NMP/TEA/TEA.3HF
6:3:4,
2h, 65°C
B

Figure 2


A
mAU
400
200
0
a
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
ml
mAU
400
200
0
b
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
ml
B
7968.4
Intenstiy x10^9
1.6
1.2
0.8
0.4
4000
6000
8000
10000
12000
14000
16000
18000
20000
Mass, Da

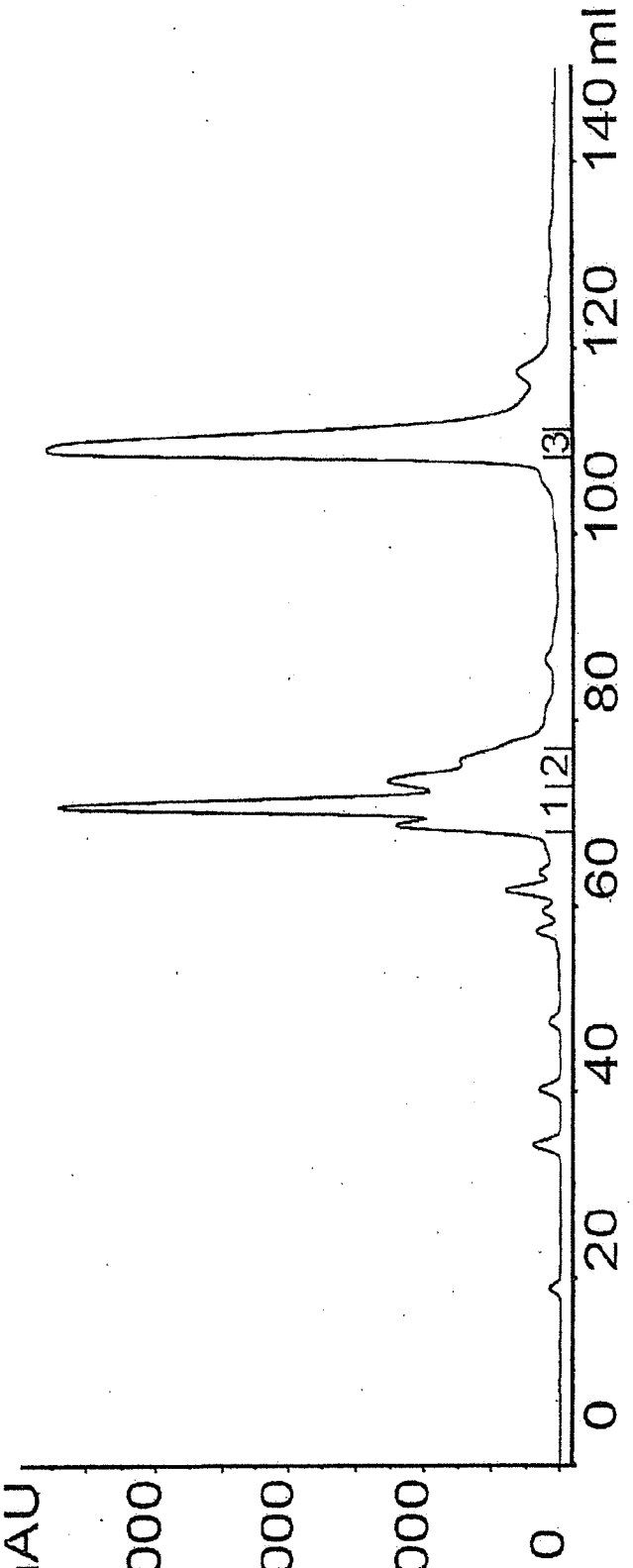


Figure 3

Figure 4

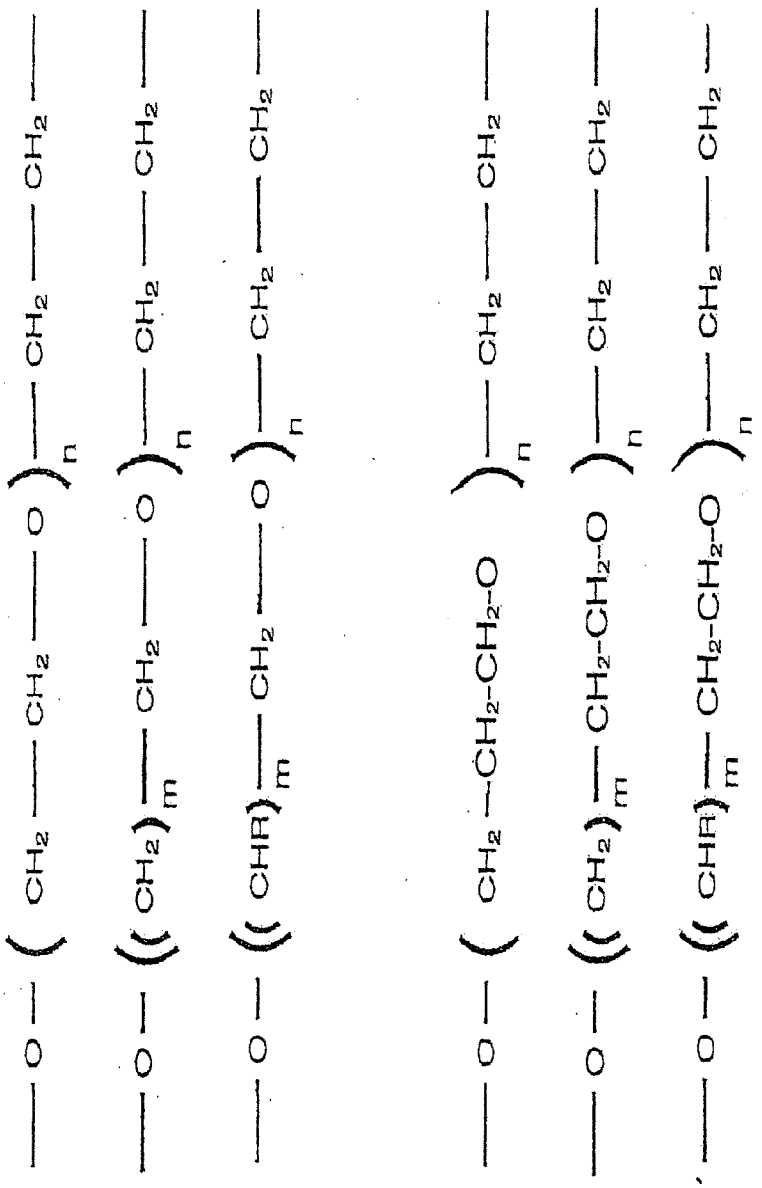
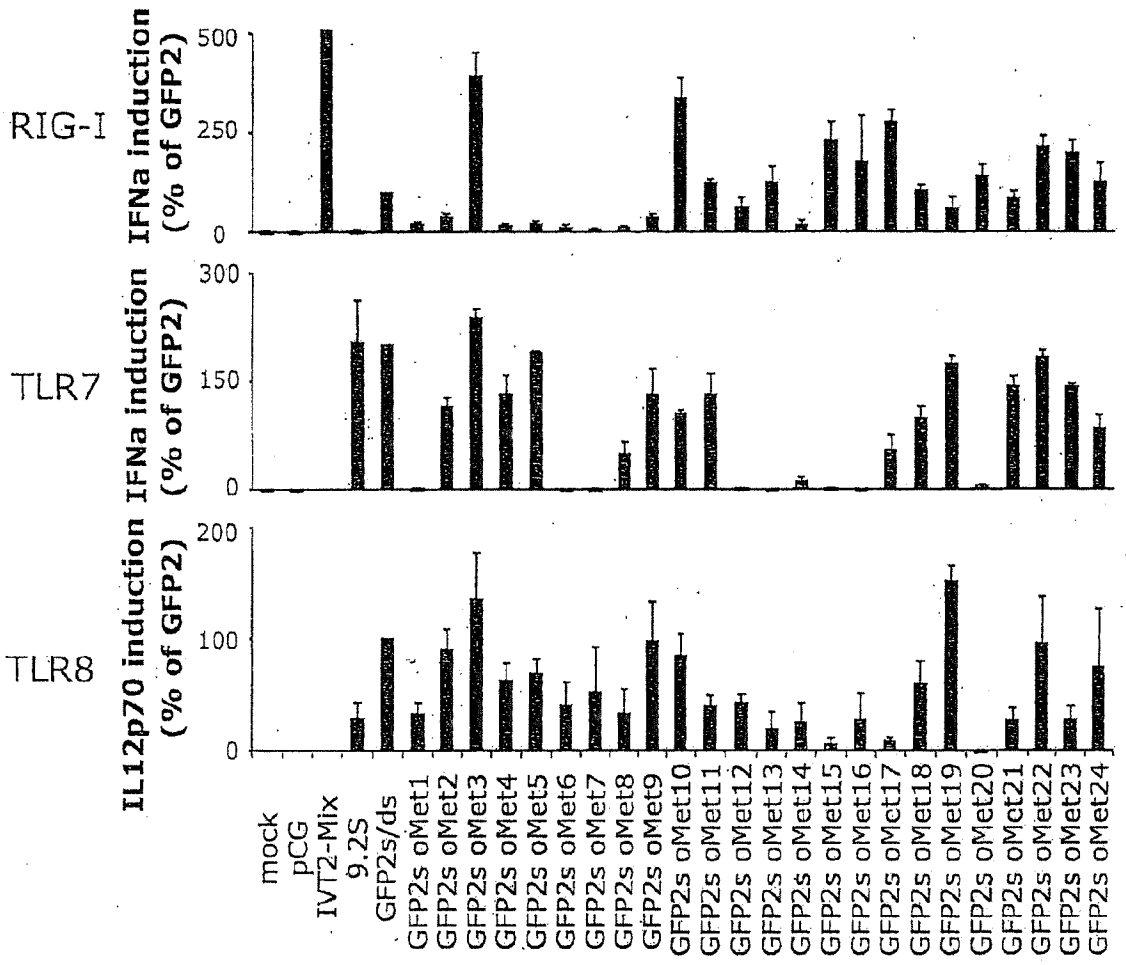

—O—(—CH2—CH2—O—)n—CH2—CH2—
—O—((—CH2—)m—CH2—O—)n—CH2—CH2—
—O—((—CHR—)m—CH2—O—)n—CH2—CH2—
—O—(—CH2—CH2—O—)n—CH2—CH2—
—O—((—CH2—)m—CH2—CH2—O—)n—CH2—CH2—
—O—((—CHR—)m—CH2—CH2—O—)n—CH2—CH2—

Figure 5

A GFP2 sense strand (5'-3')



GFP2 sense strand (5'-3')

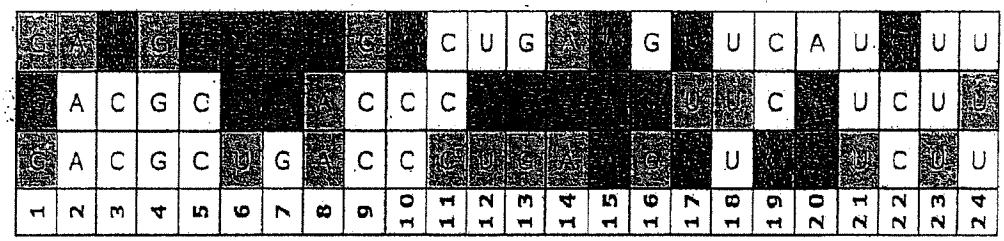
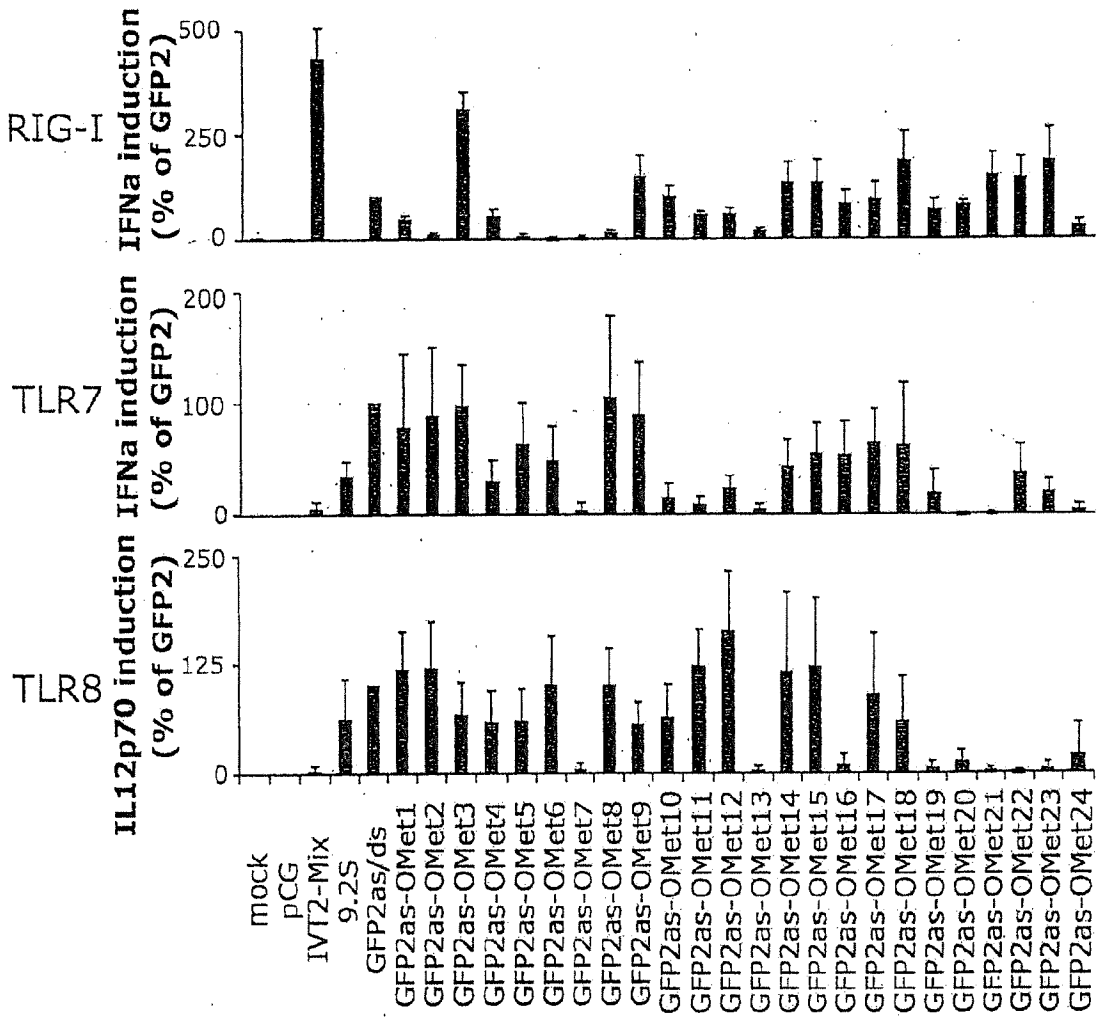


Figure 5 continued

B GFP2 antisense strand (3'-5')



GFP2 antisense strand (3'-5')

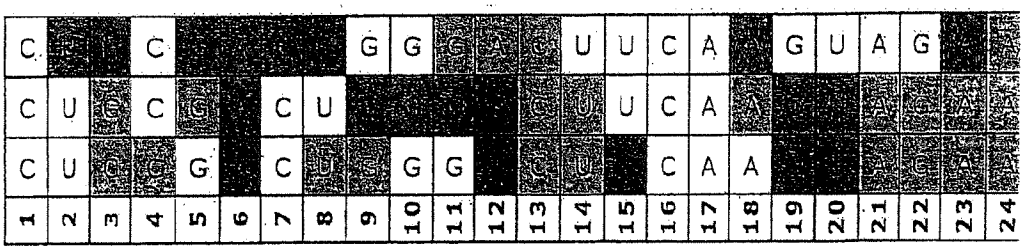


Figure 6

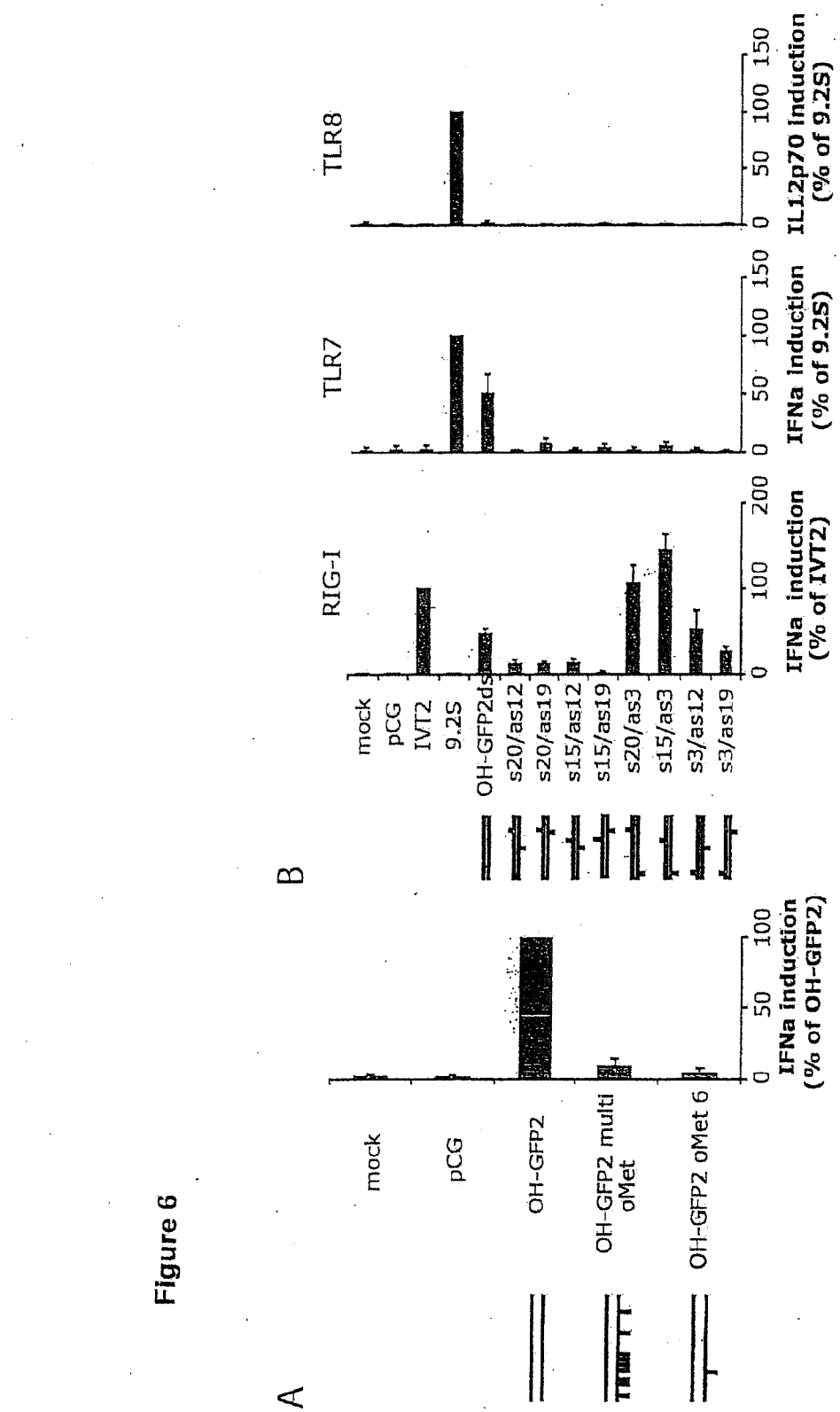

A
mock
pCG
OH-GFP2
OH-GFP2 multi oMet
OH-GFP2 oMet 6
0 50 100
IFNa induction (% of OH-GFP2)
B
RIG-I
TLR7
TLR8
mock
pCG
IVT2
9.2S
OH-GFP2ds
s20/as12
s20/as19
s15/as12
s15/as19
s20/as3
s15/as3
s3/as12
s3/as19
0 100 200
IFNa induction (% of IVT2)
0 50 100 150
IFNa induction (% of 9.2S)
0 50 100 150
IL12p70 induction (% of 9.2S)

Figure 7

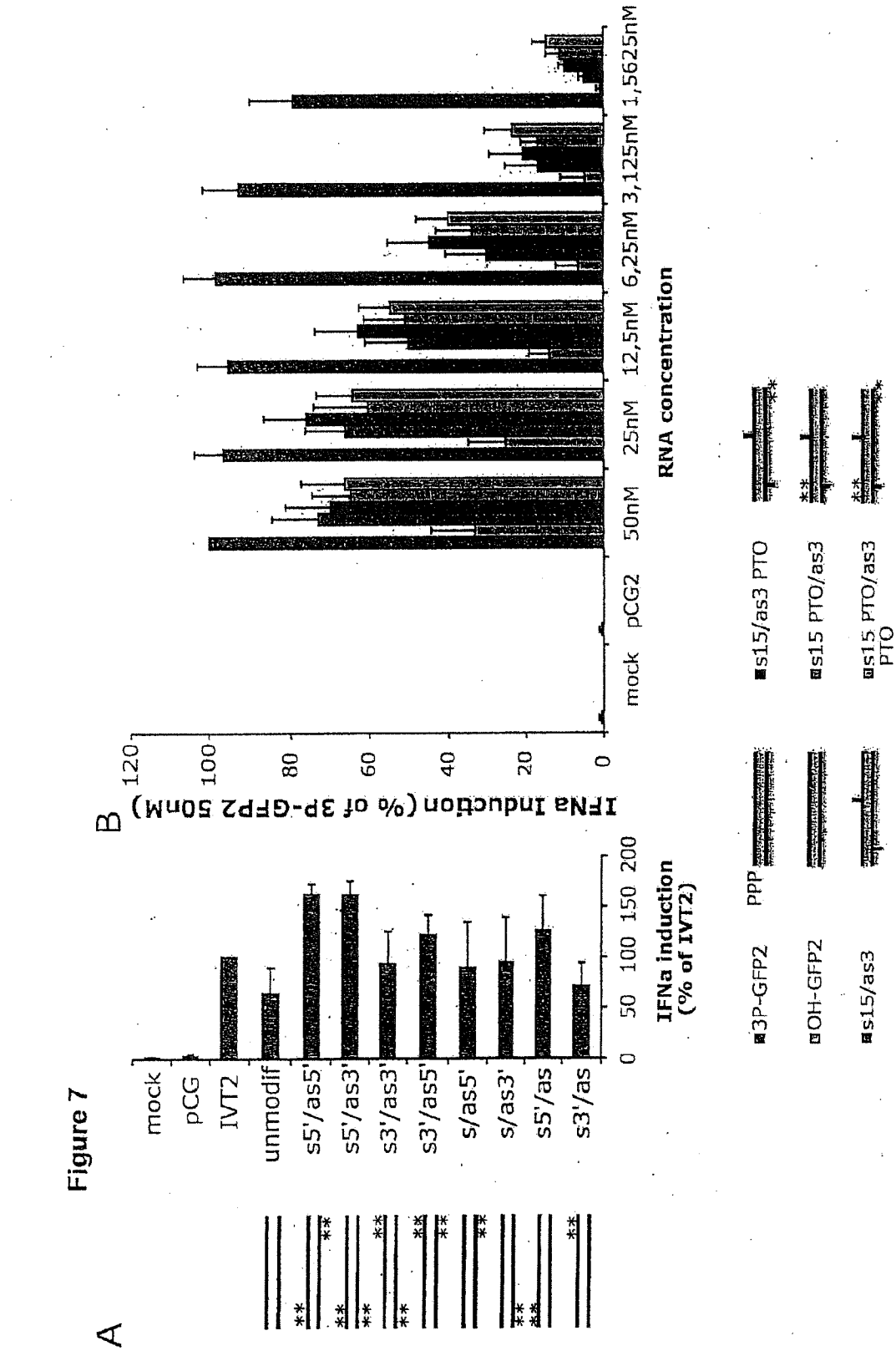

A
mock
pCG
IVT2
unmodif
s5'/as5'
s5'/as3'
s3'/as3'
s3'/as5'
s/as5'
s/as3'
s5'/as
s3'/as
0 50 100 150 200
IFNa induction (% of IVT2)
B
IFNa induction (% of 3P-GFP2 50nM)
0 20 40 60 80 100 120
mock pCG2 50nM 25nM 12,5nM 6,25nM 3,125nM 1,5625nM
RNA concentration
3P-GFP2 PPP
OH-GFP2
s15/as3
s15/as3 PTO
s15 PTO/as3
s15 PTO/as3 PTO
* 2'-oMet
** 2x PTO

Figure 8

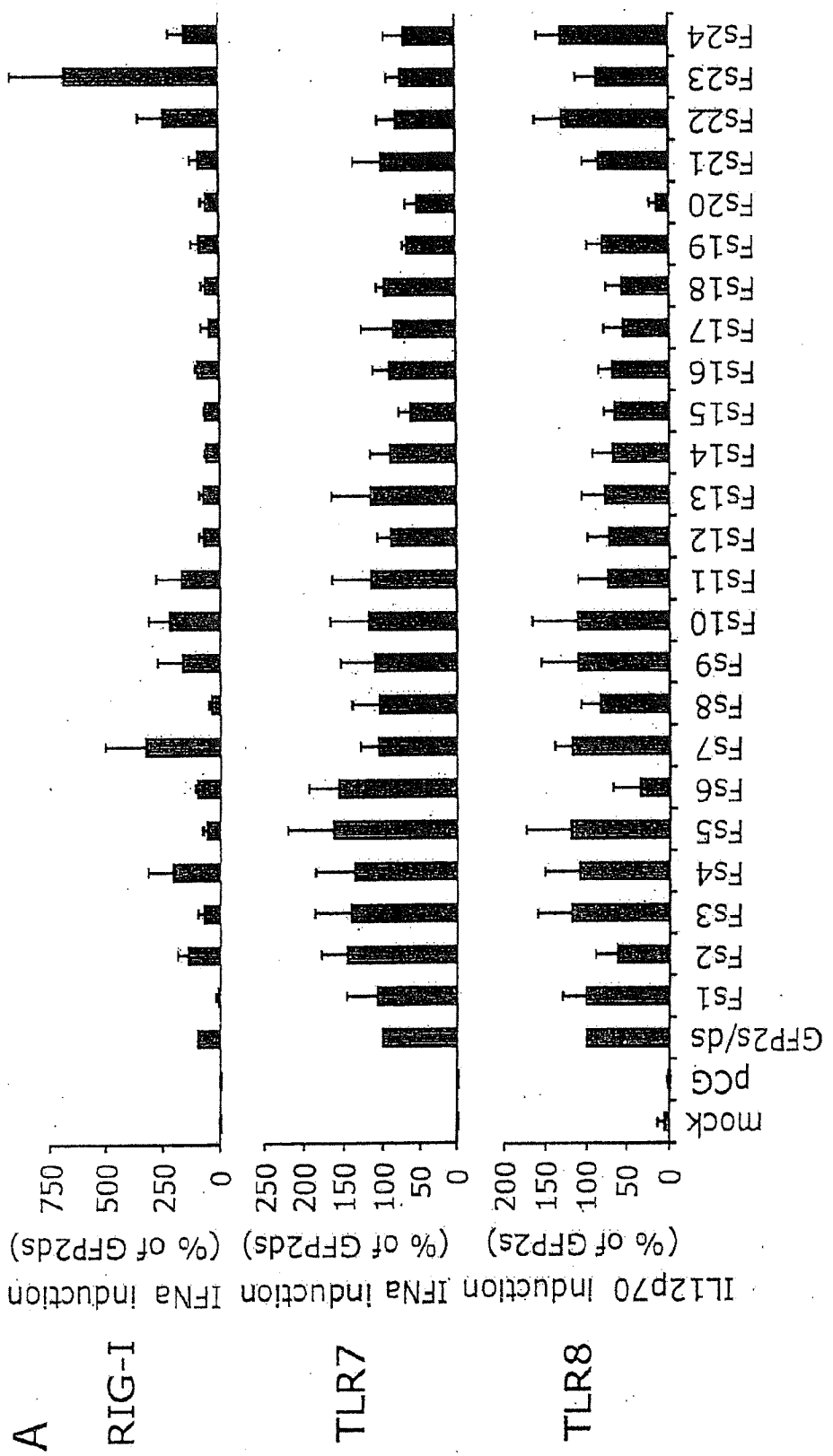
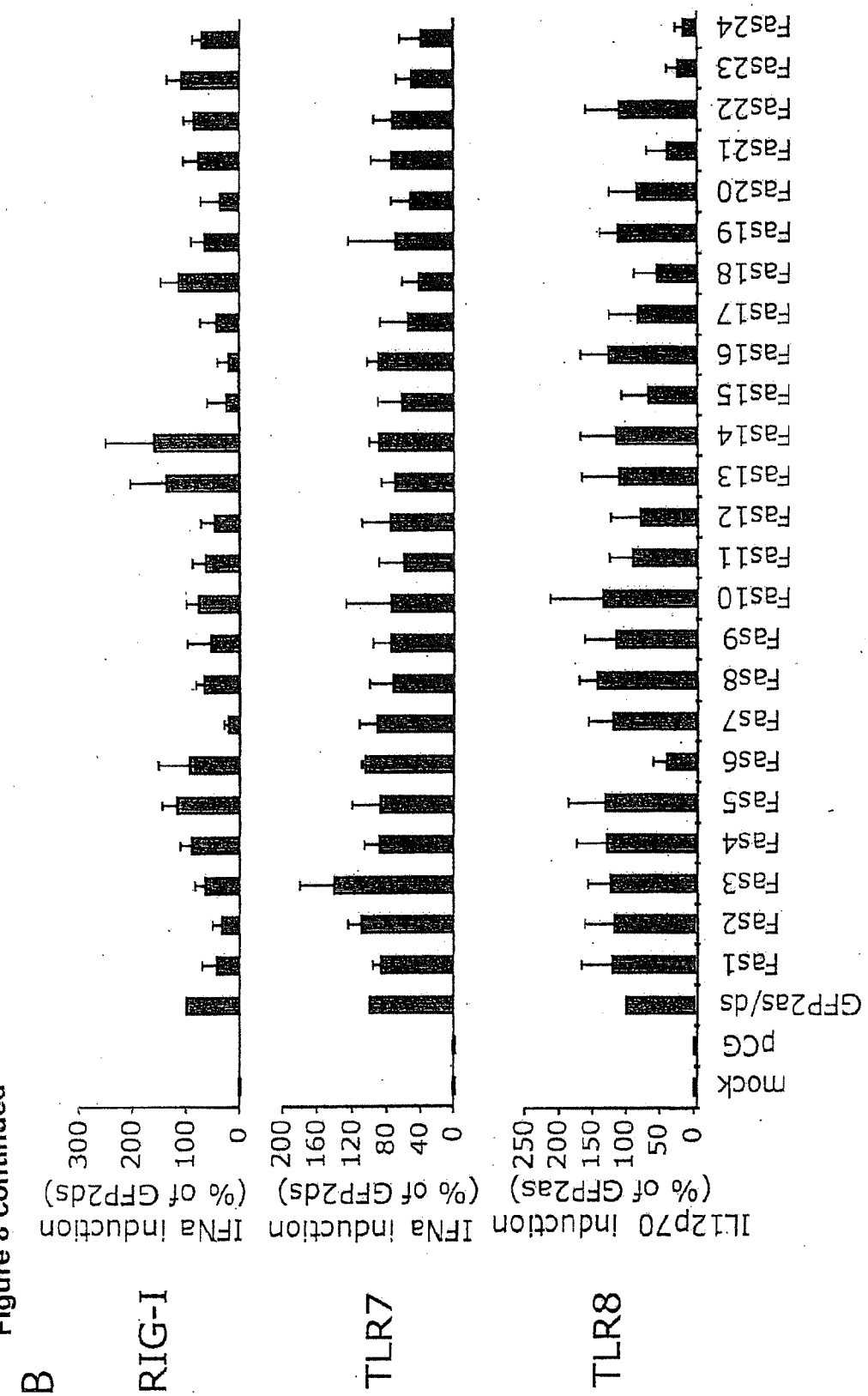

A
RIG-I
TLR7
TLR8
IFNa induction (% of GFP2ds)
IL12p70 induction (% of GFP2s)
750
500
250
0
250
200
150
100
50
0
200
150
100
50
0
mock
pCG
GFP2s/ds
Fs1
Fs2
Fs3
Fs4
Fs5
Fs6
Fs7
Fs8
Fs9
Fs10
Fs11
Fs12
Fs13
Fs14
Fs15
Fs16
Fs17
Fs18
Fs19
Fs20
Fs21
Fs22
Fs23
Fs24

Figure 8 continued



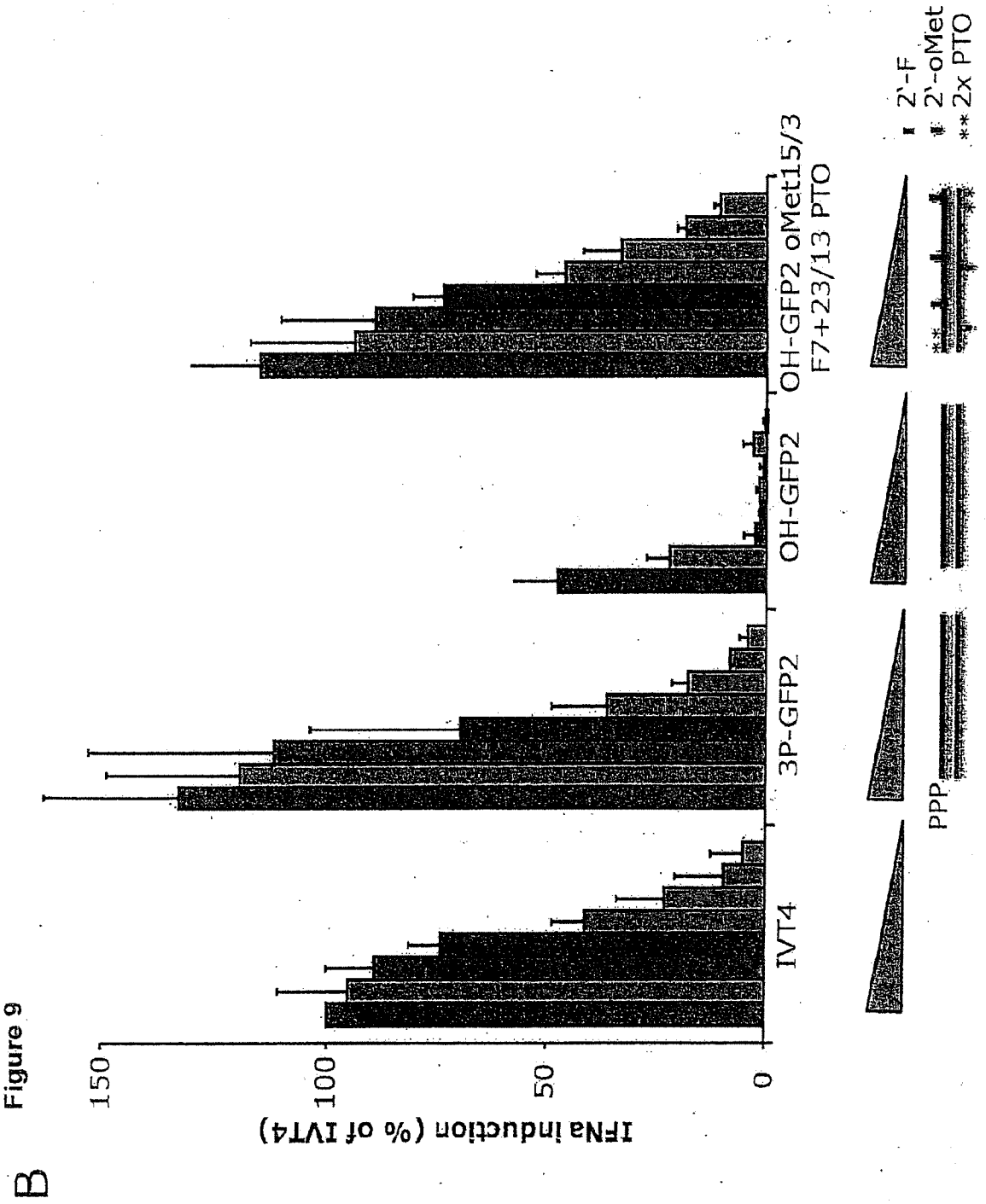

B
IFNa induction (% of IVT4)
150
100
50
0
IVT4
3P-GFP2
OH-GFP2
OH-GFP2 oMet15/3
F7+23/13 PTO
PPP
2'-F
2'-oMet
** 2x PTO

Figure 9

Figure 9 continued

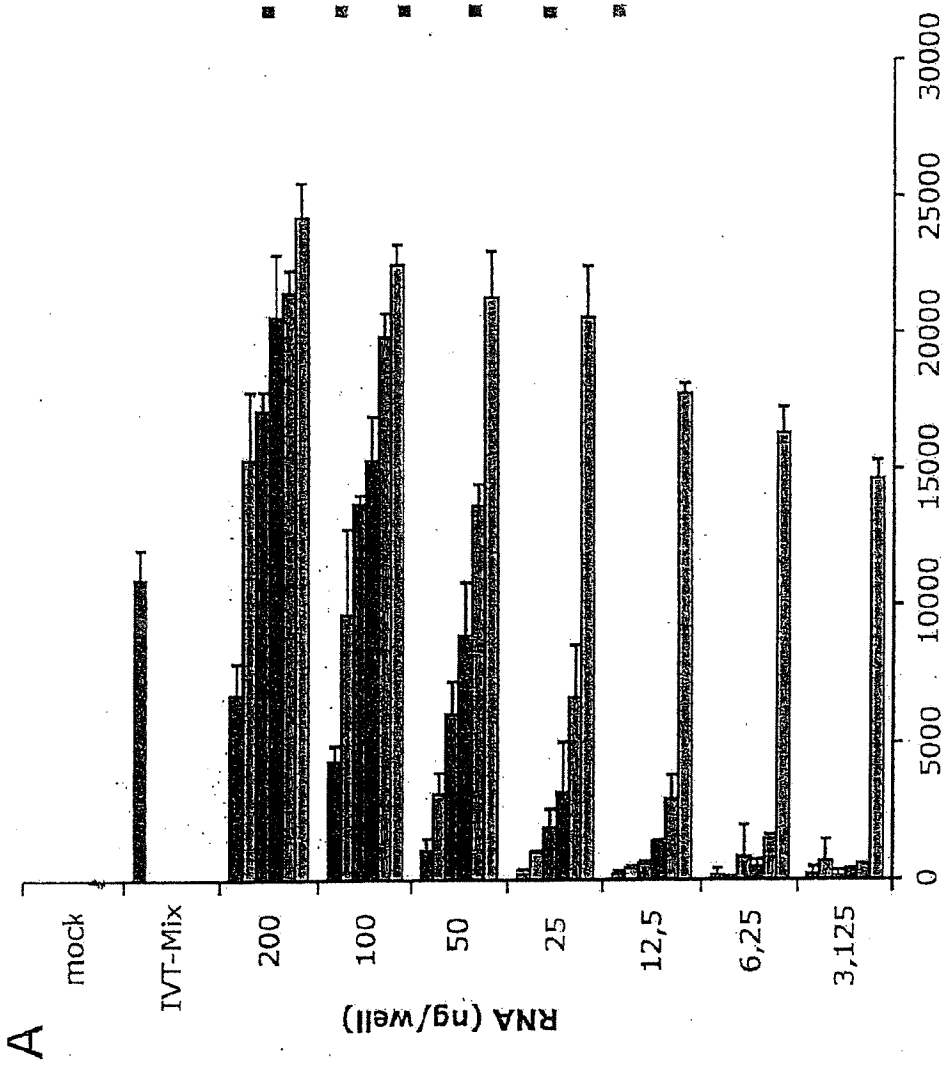


Figure 10

A

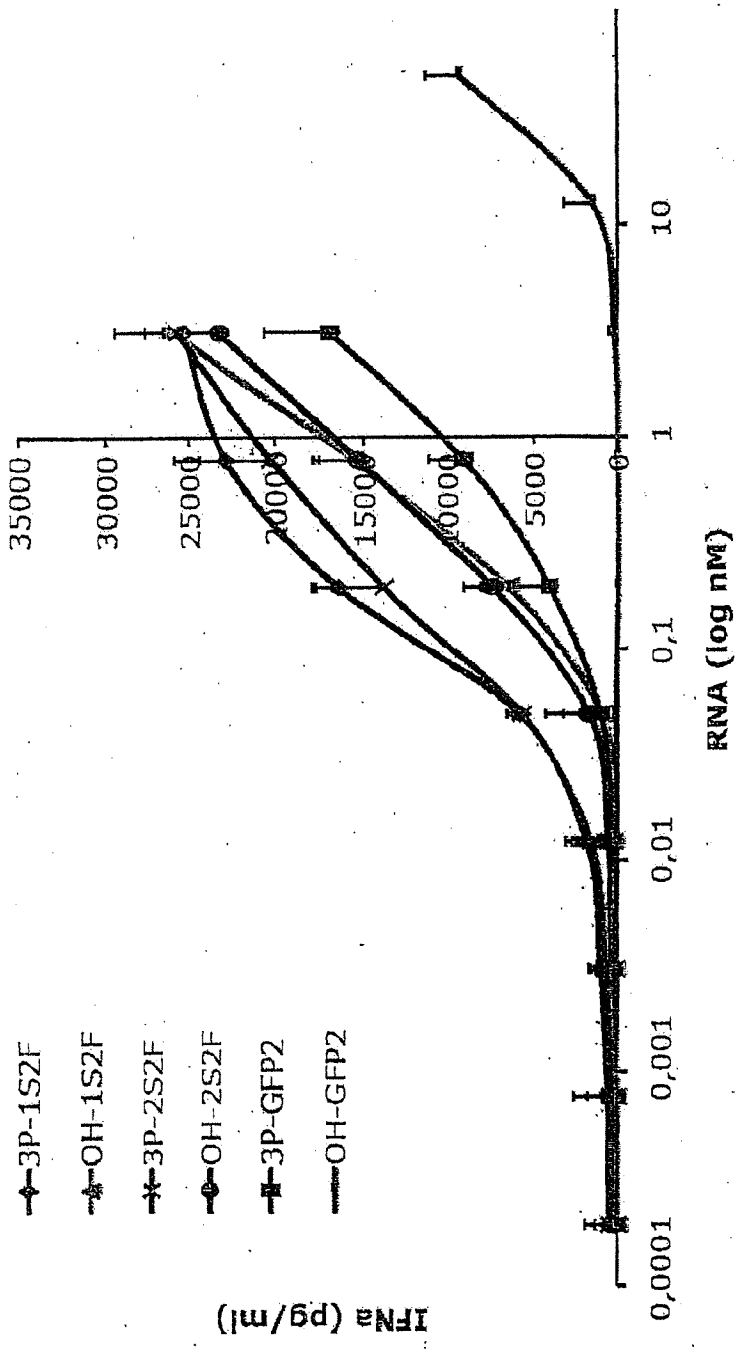
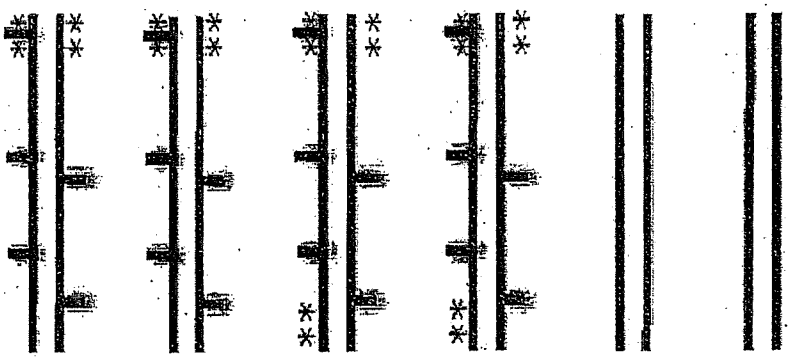

IFNa (pg/ml)
35000
30000
25000
20000
15000
10000
5000
0
0,0001
0,001
0,01
0,1
1
10
RNA (log nM)
3P-1S2F
OH-1S2F
3P-2S2F
OH-2S2F
3P-GFP2
OH-GFP2

Figure 10 continued



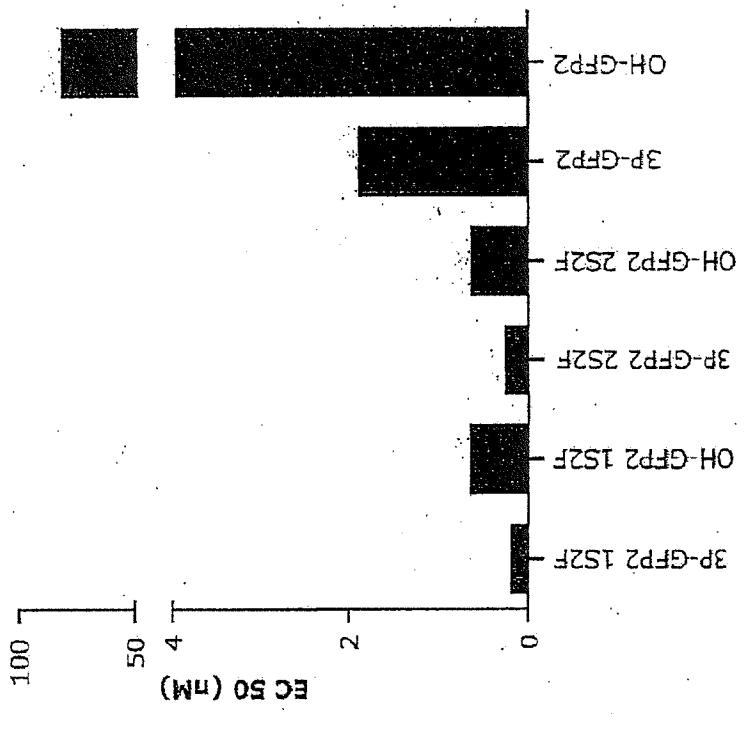
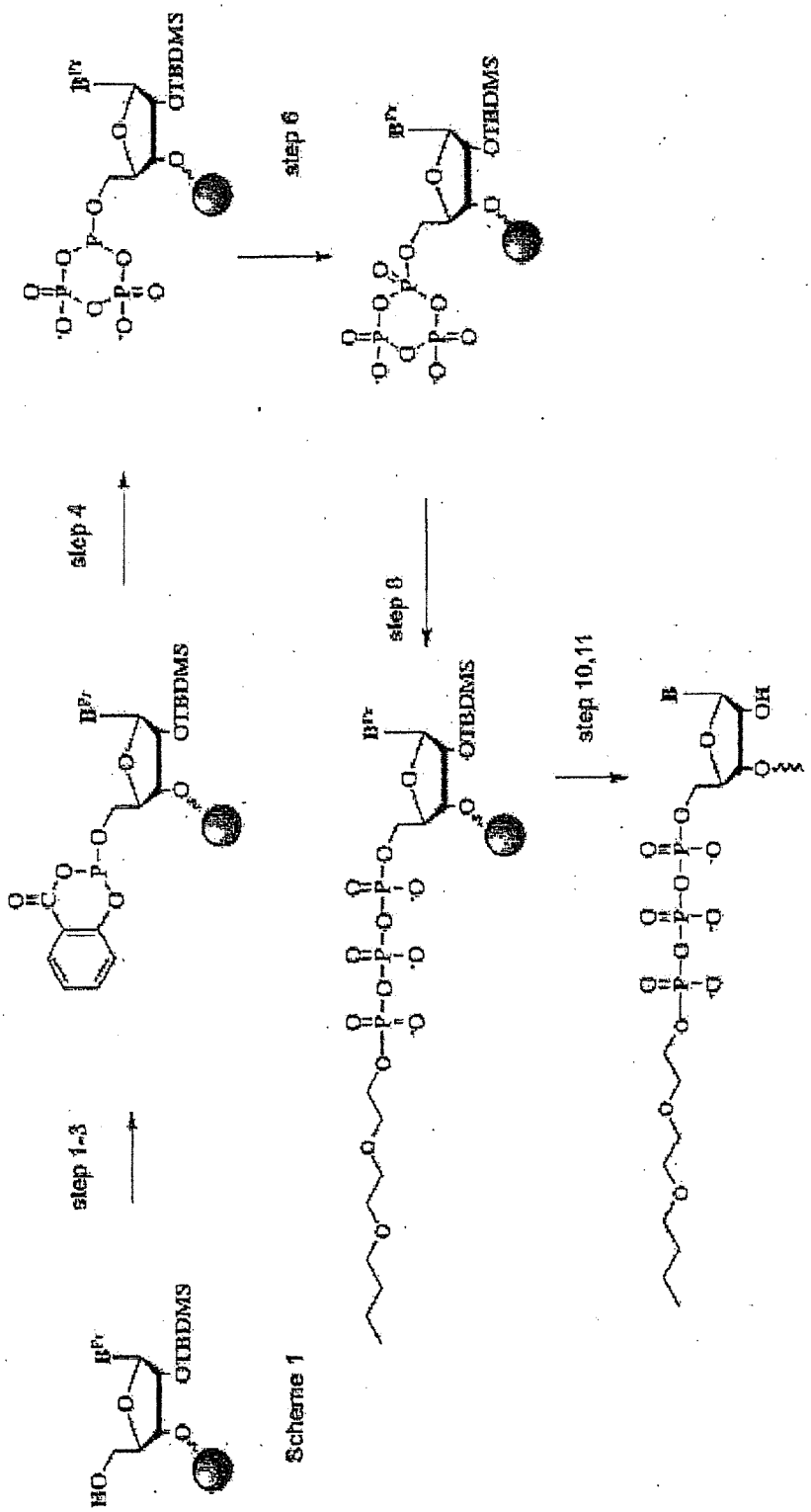


Figure 10 continued

Figure 11



Scheme 1

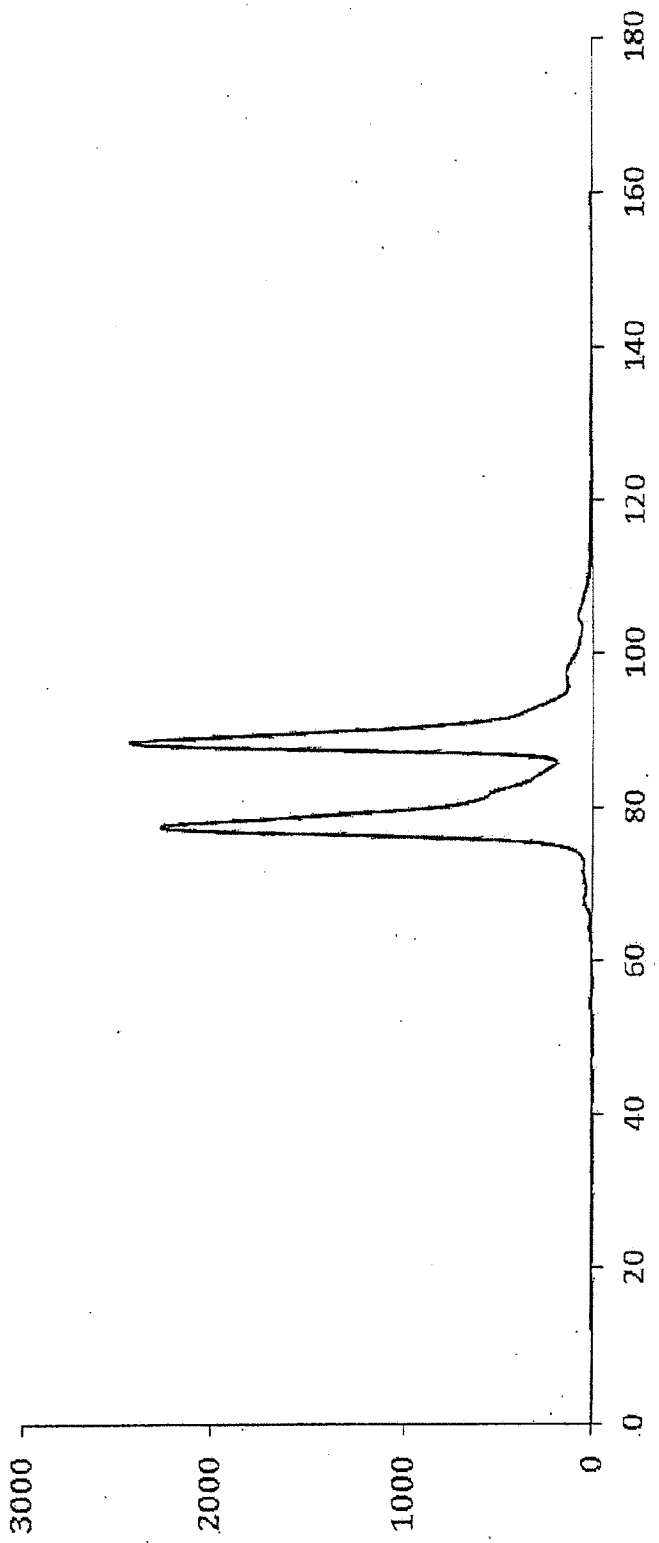


Figure 12

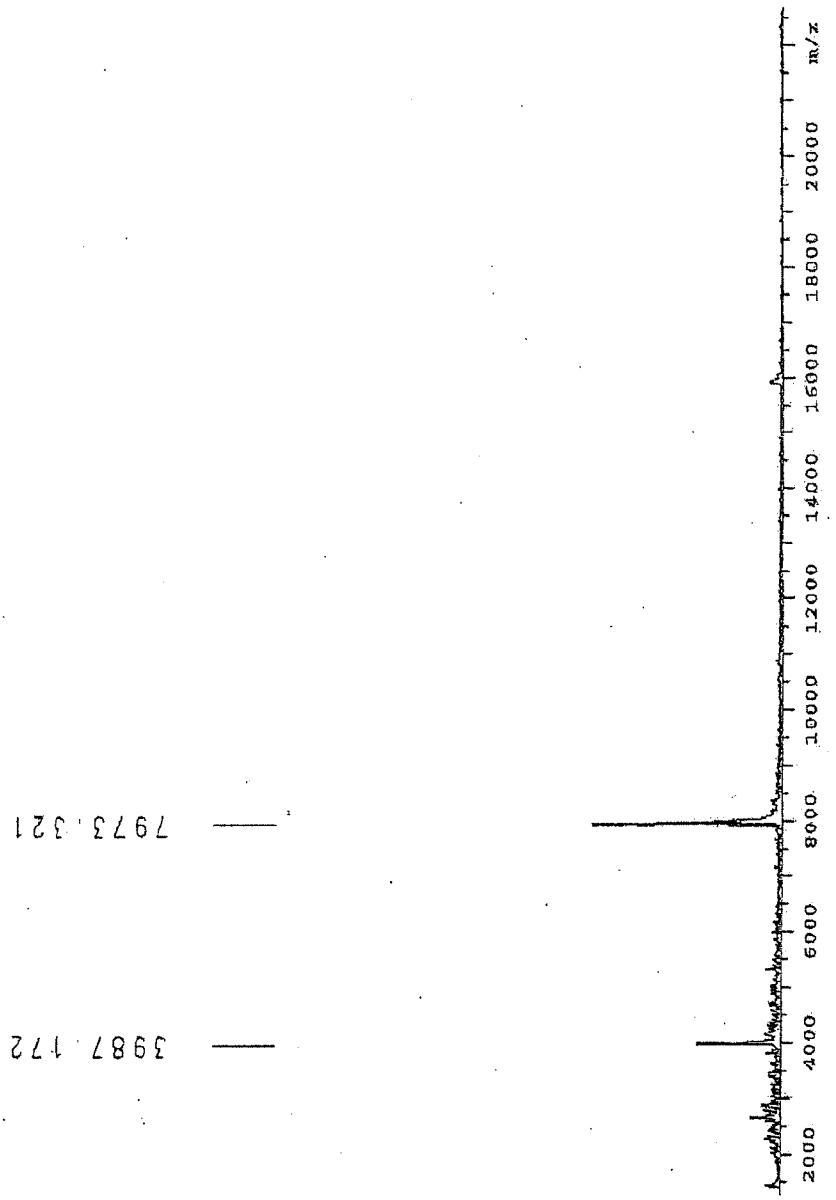
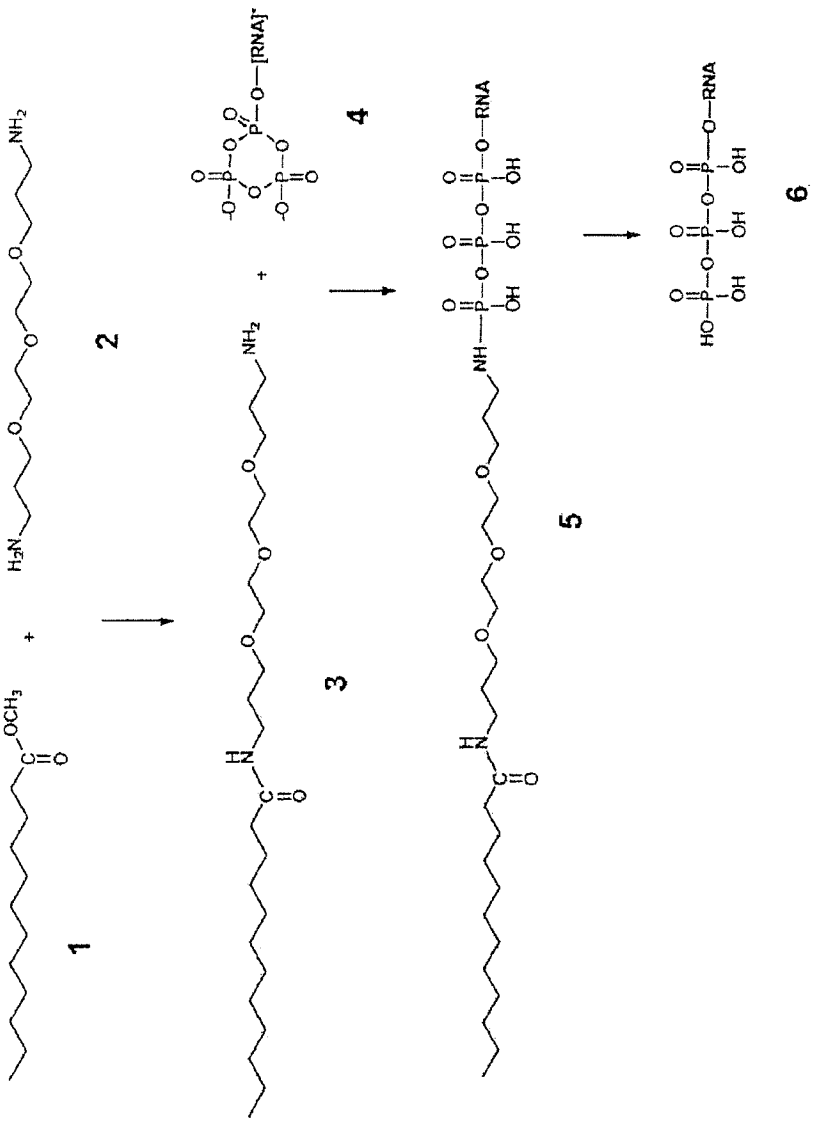

7973.321
3987.172
2000
4000
6000
8000
10000
12000
14000
16000
18000
20000
m/z

Figure 13

Figure 14



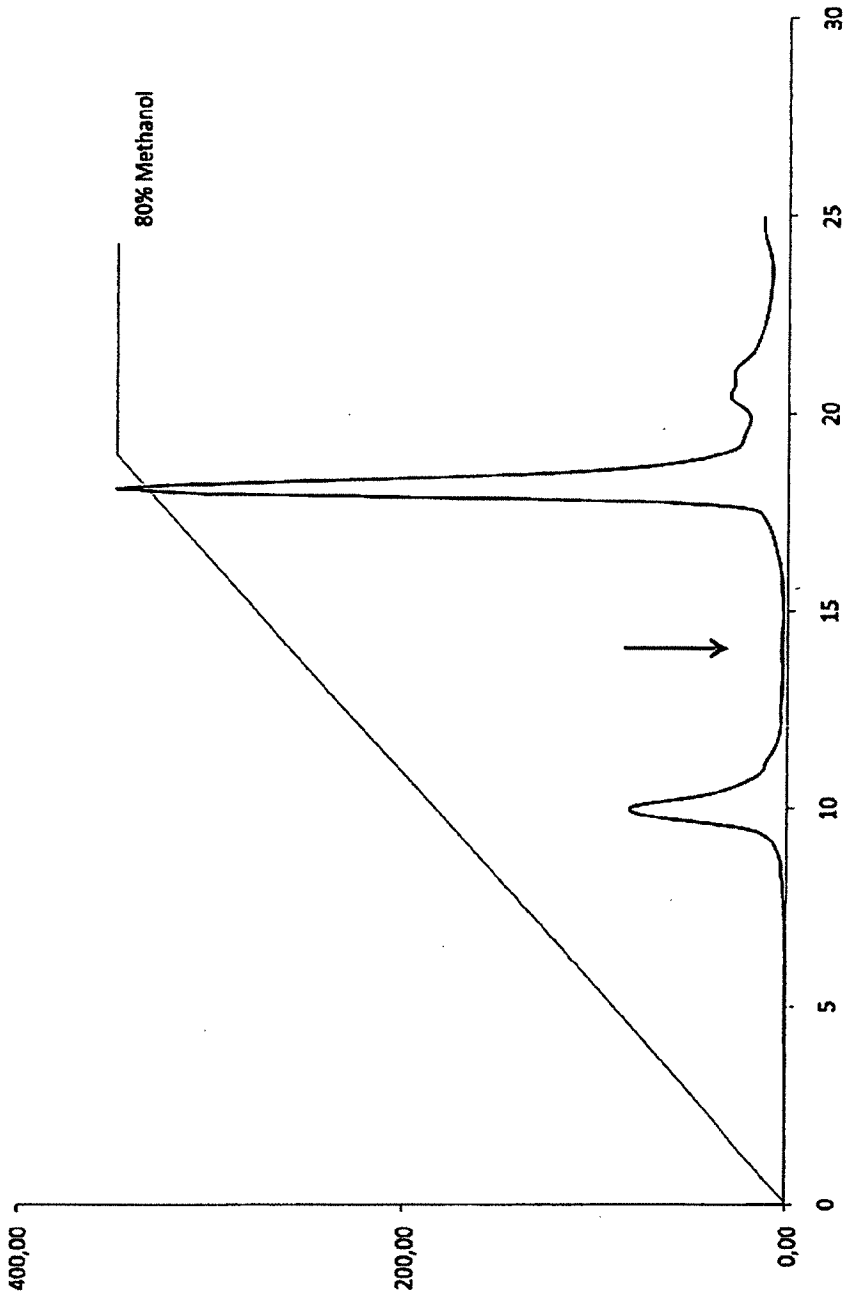


Figure 15

A

Figure 15 continued

B

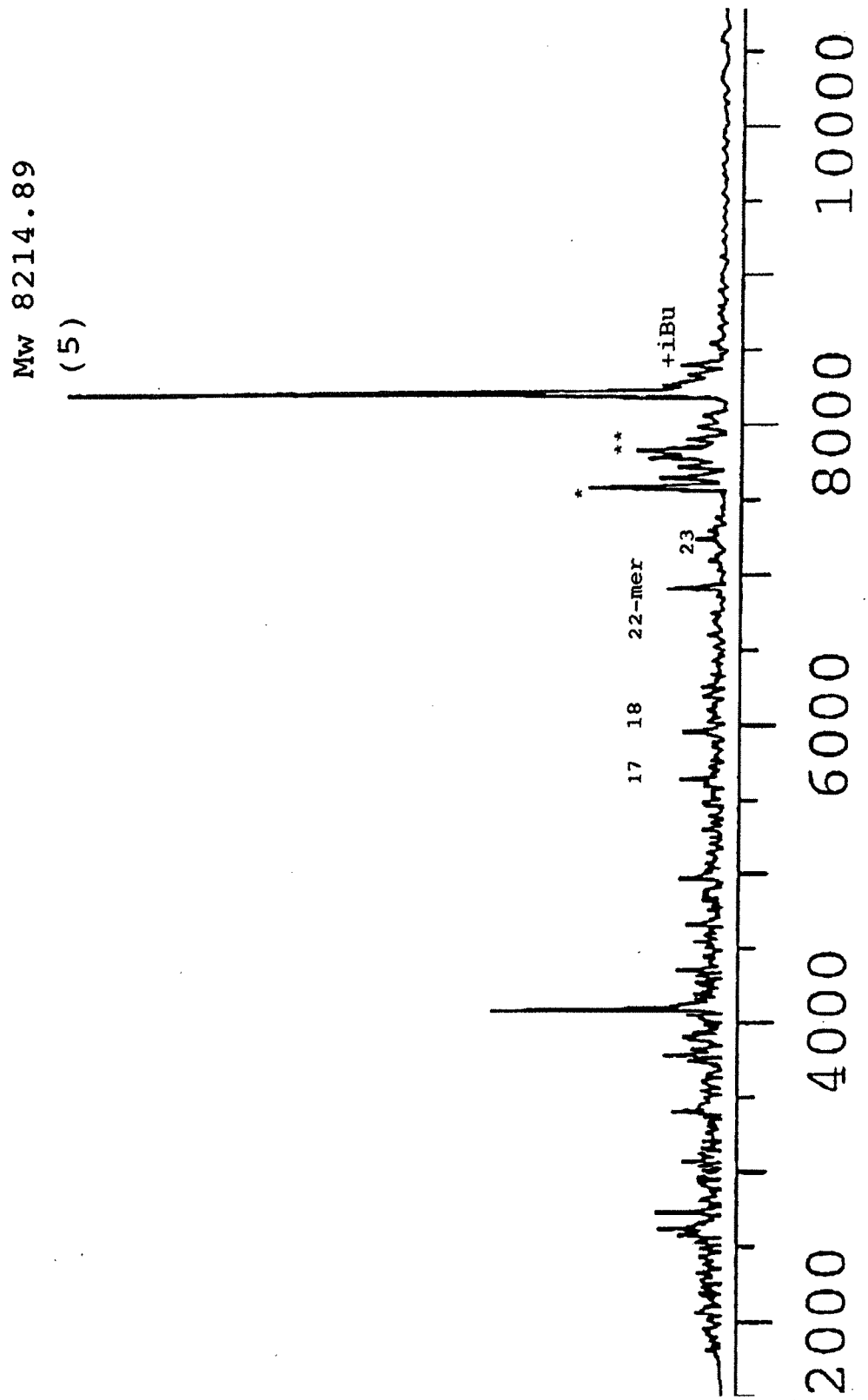
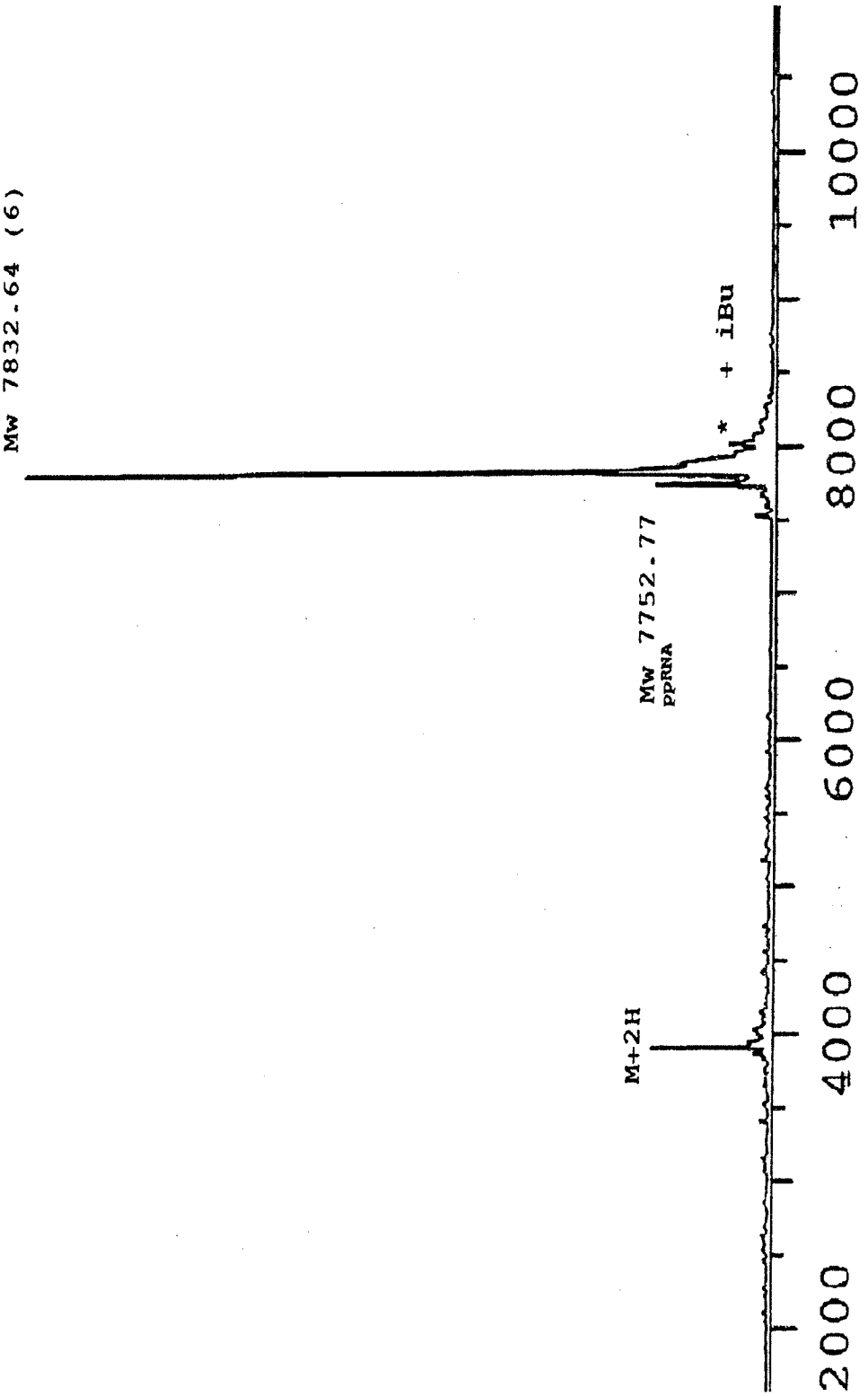


Figure 15 continued

C



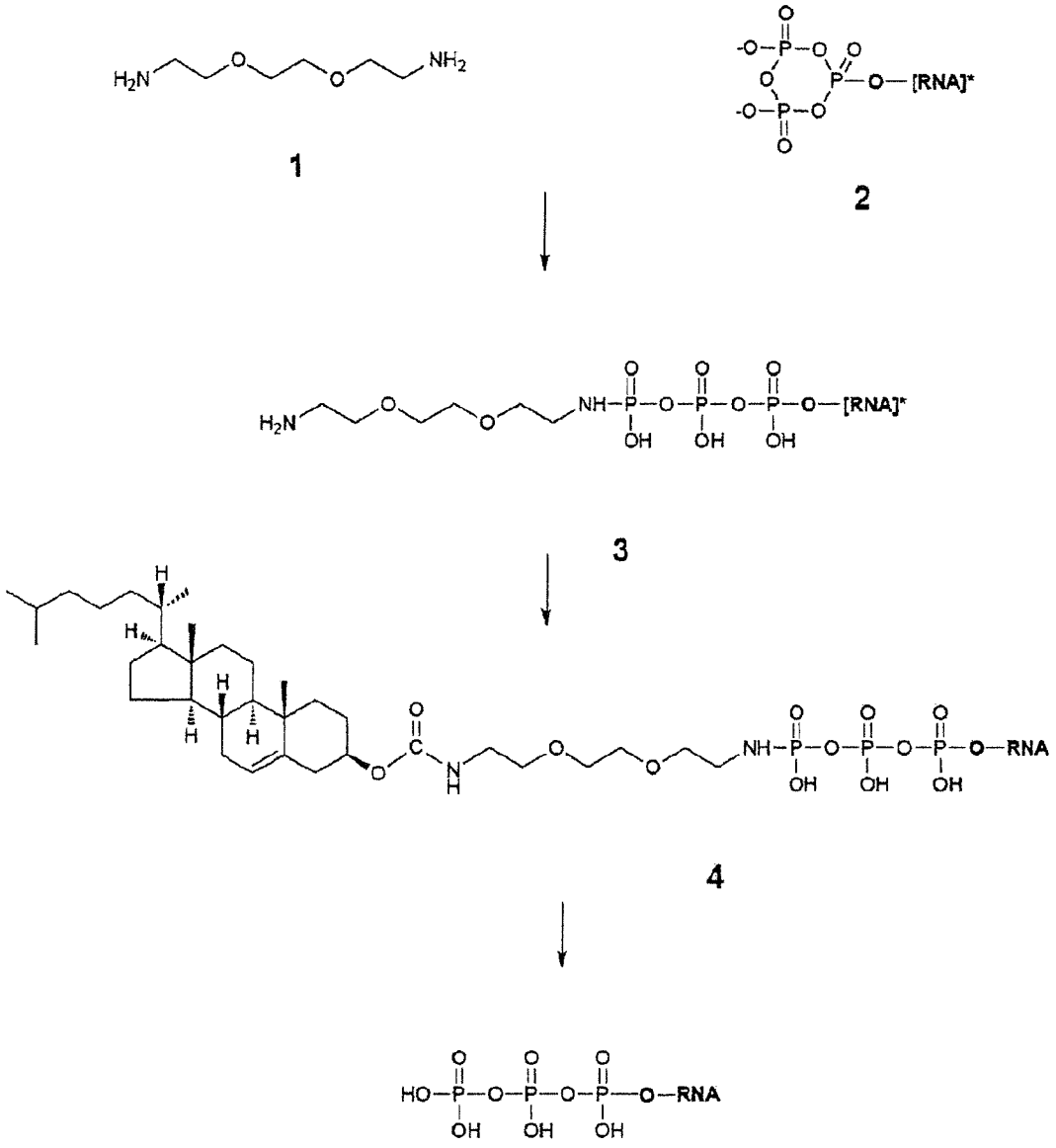
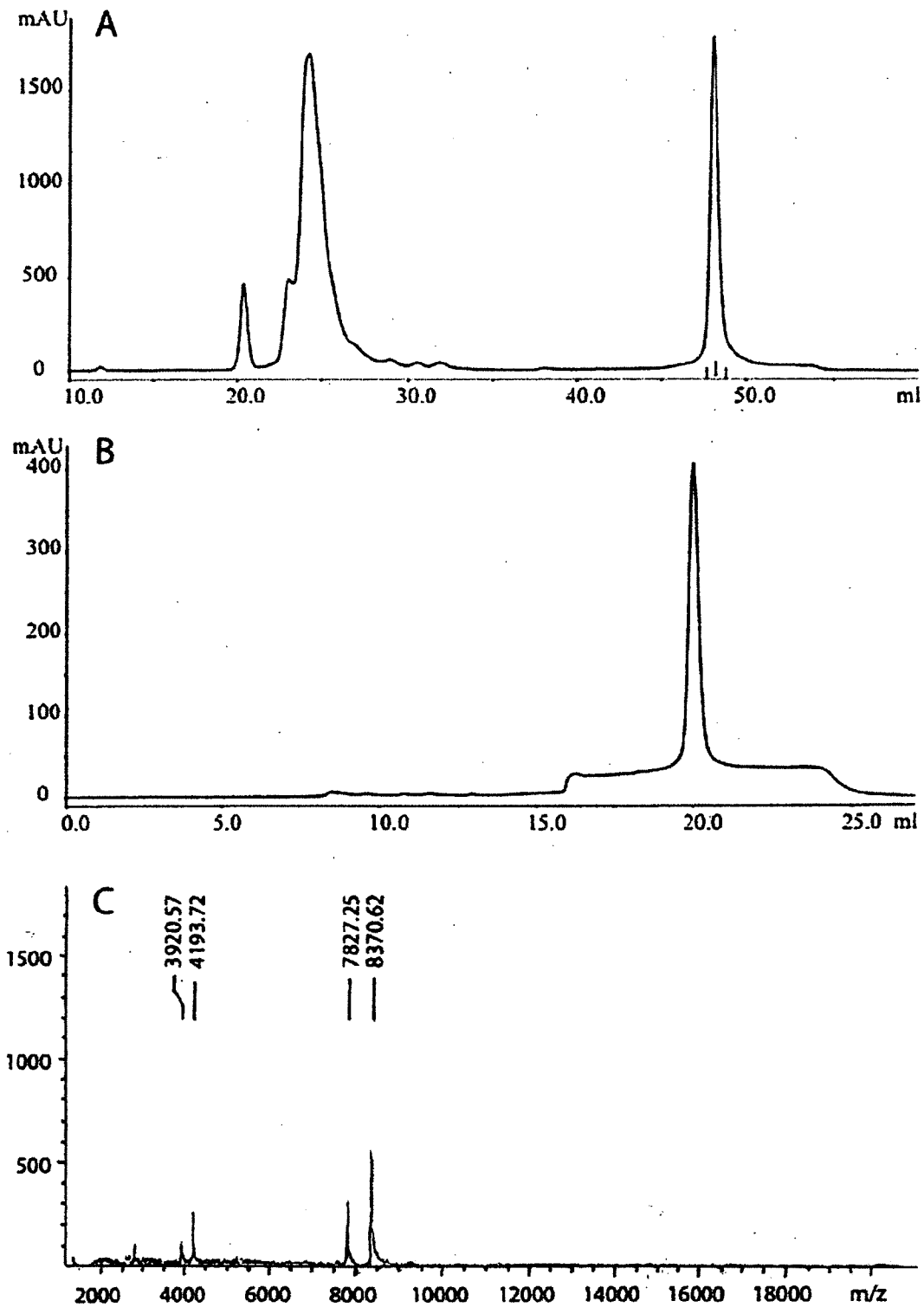


Fig 16

Figure 17


A
mAU
1500
1000
500
0
10.0
20.0
30.0
40.0
50.0
ml
B
mAU
400
300
200
100
0
0.0
5.0
10.0
15.0
20.0
25.0 ml
C
3920.57
4193.72
7827.25
8370.62
1500
1000
500
2000
4000
6000
8000
10000
12000
14000
16000
18000
m/z