Figure 1

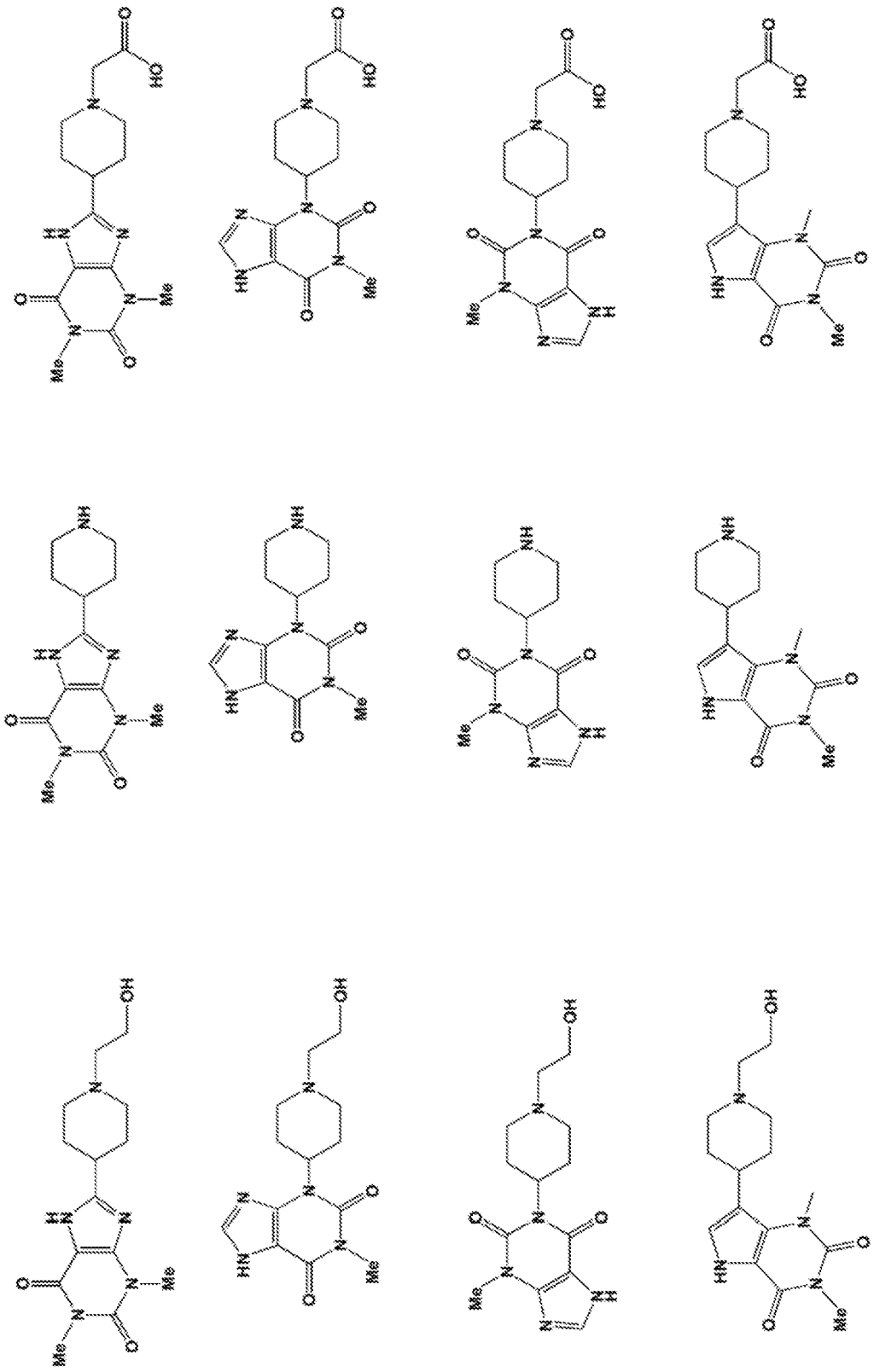


Figure 2

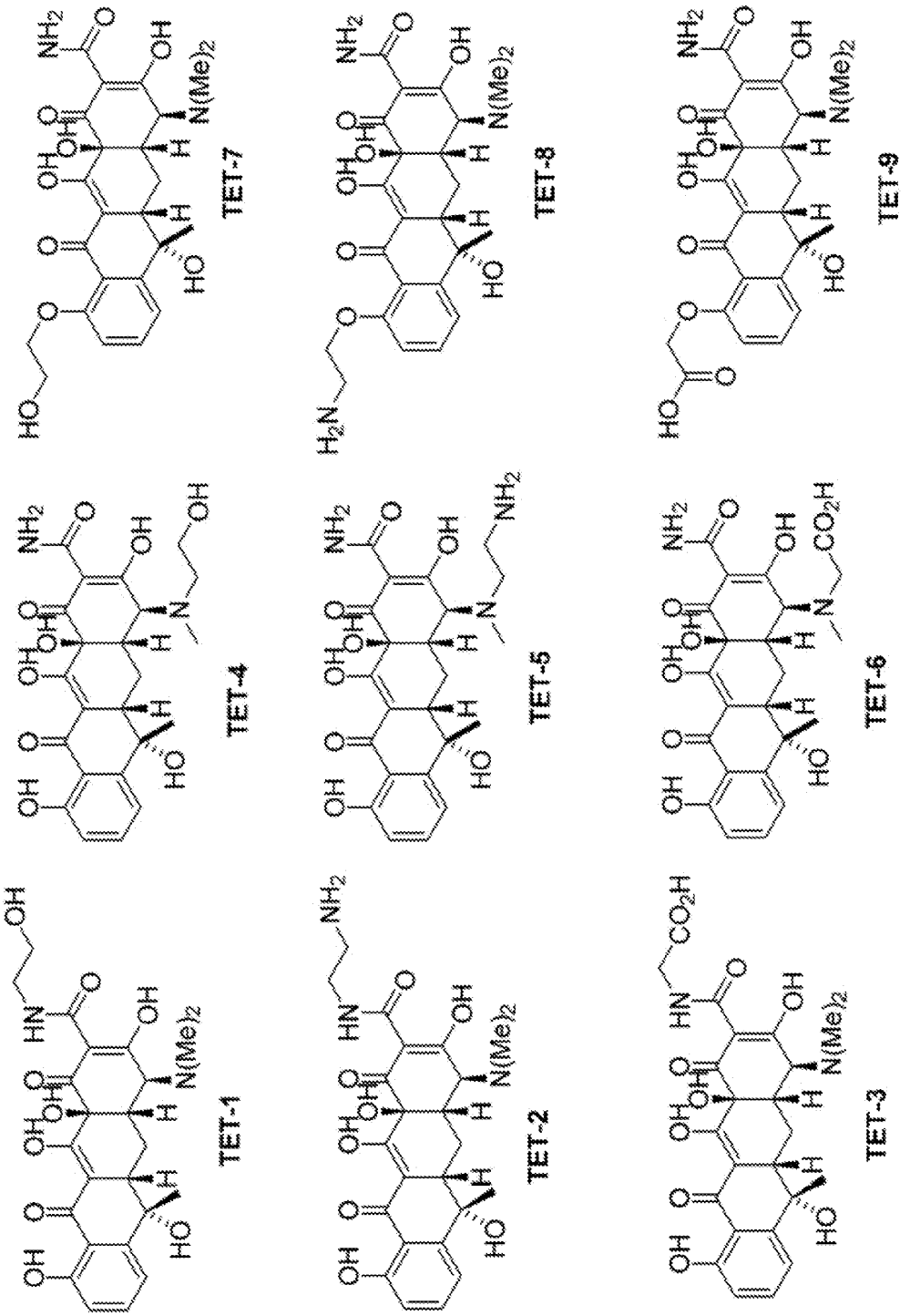


Figure 3

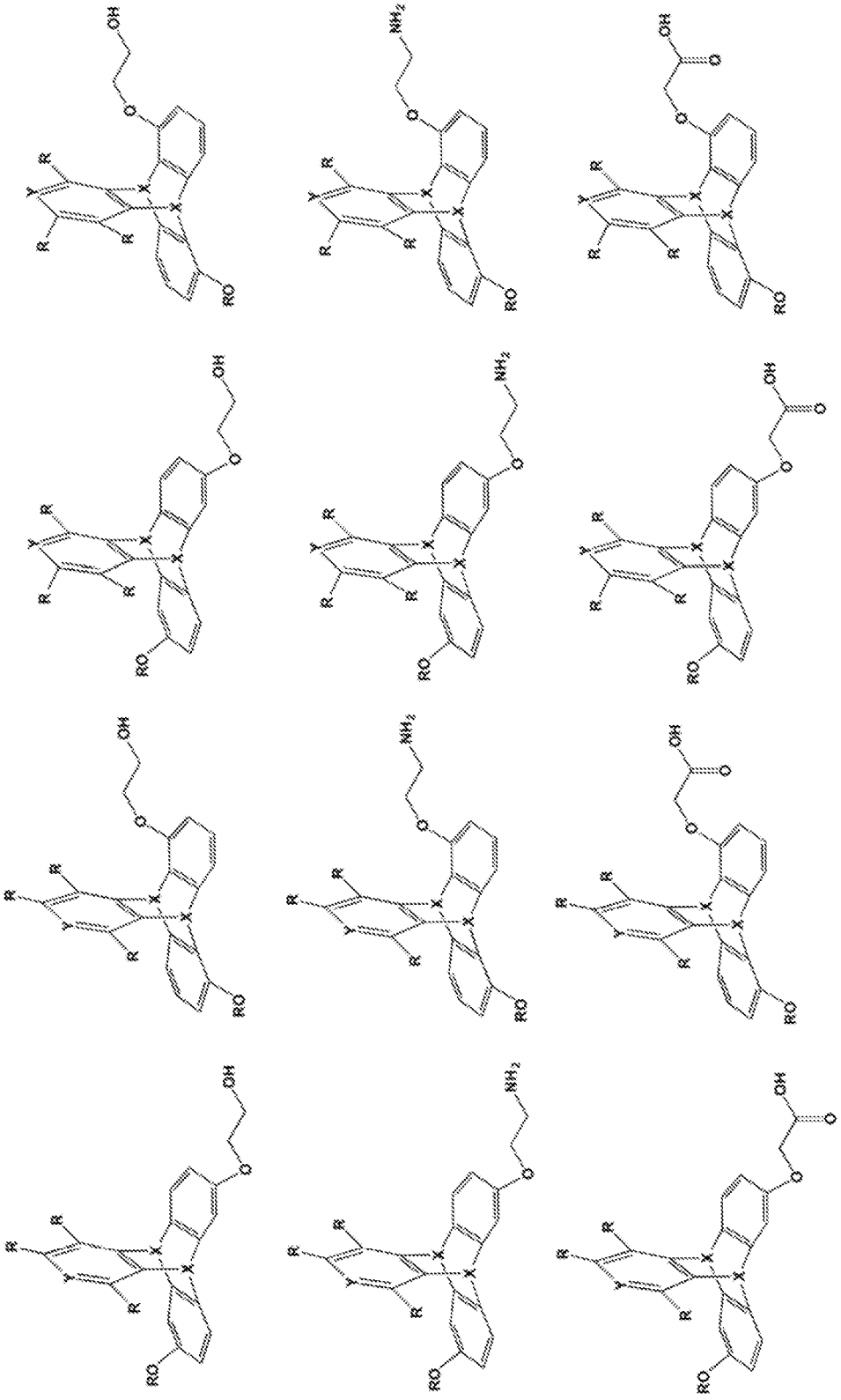
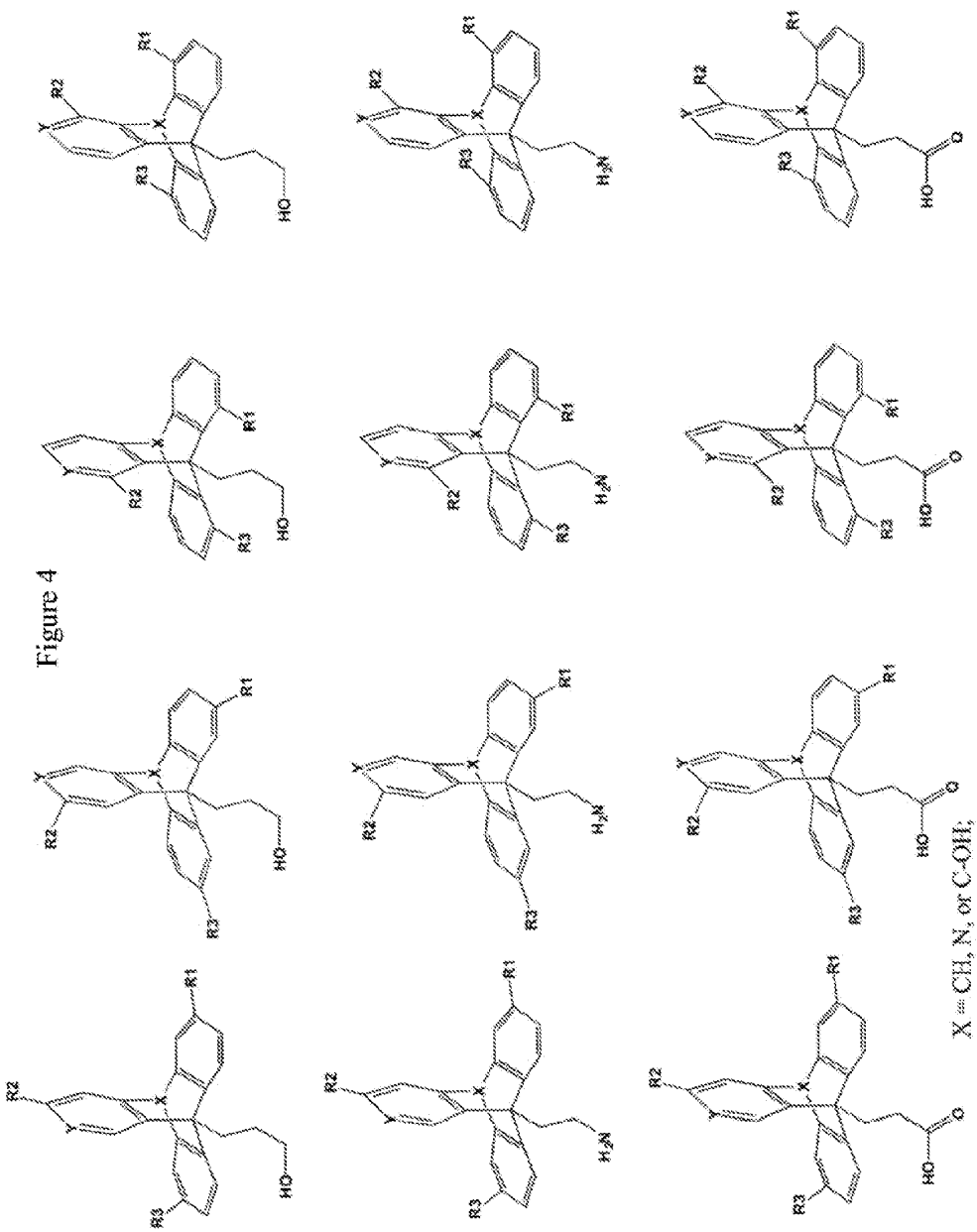
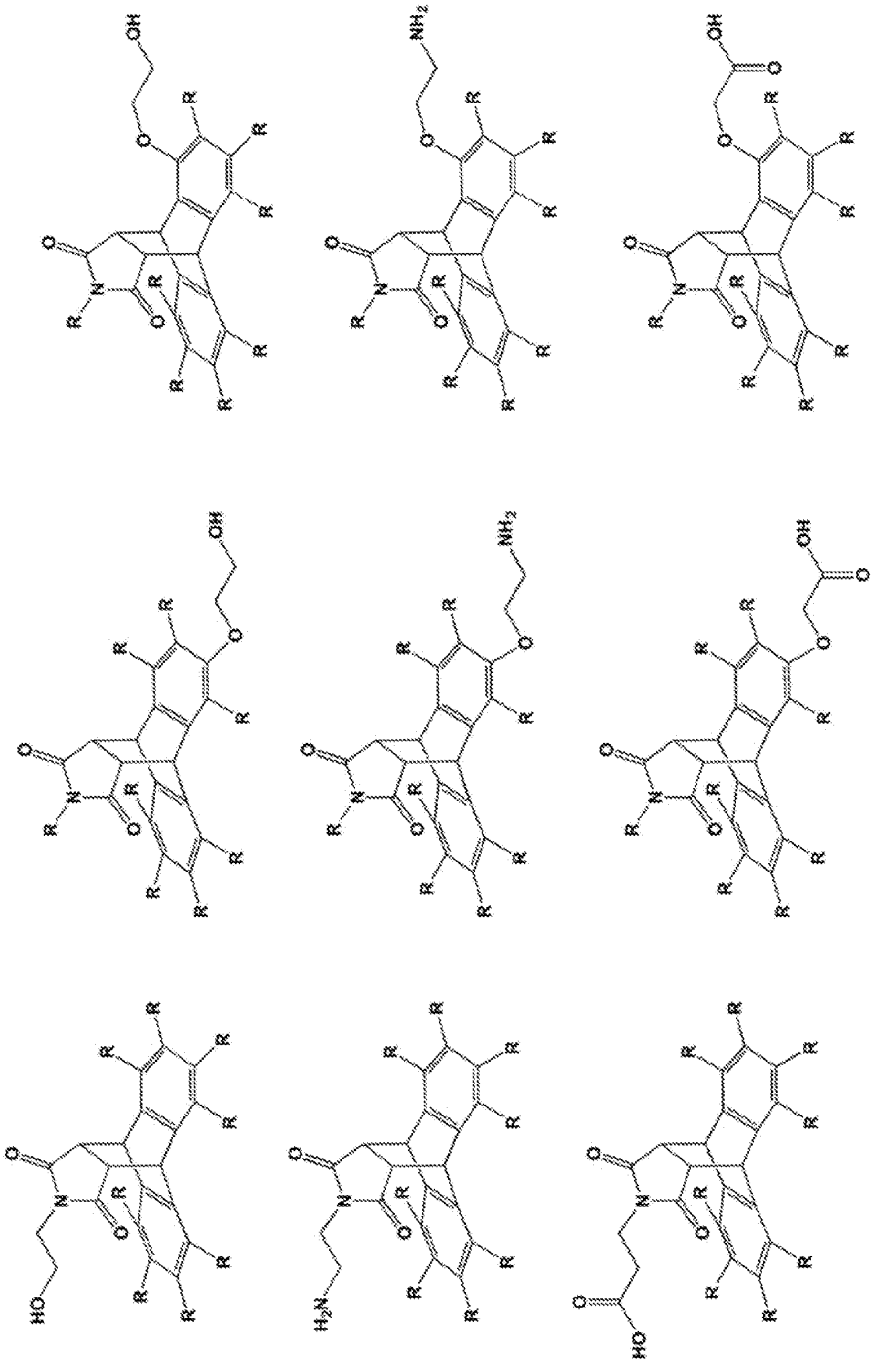


Figure 4



X = CH, N, or C-OH;
Y = CH or N;
R1, R2, R3 = each independently selected from halo, -OH, -OMe, $-NH_2$, -NH-(optionally substituted C_{1-10} aliphatic), optionally substituted C_{1-10} aliphatic, or other described tethering groups. The modifier moiety may be attached at any position on R1, R2, or R3, or at the other functional groups on the above structures.

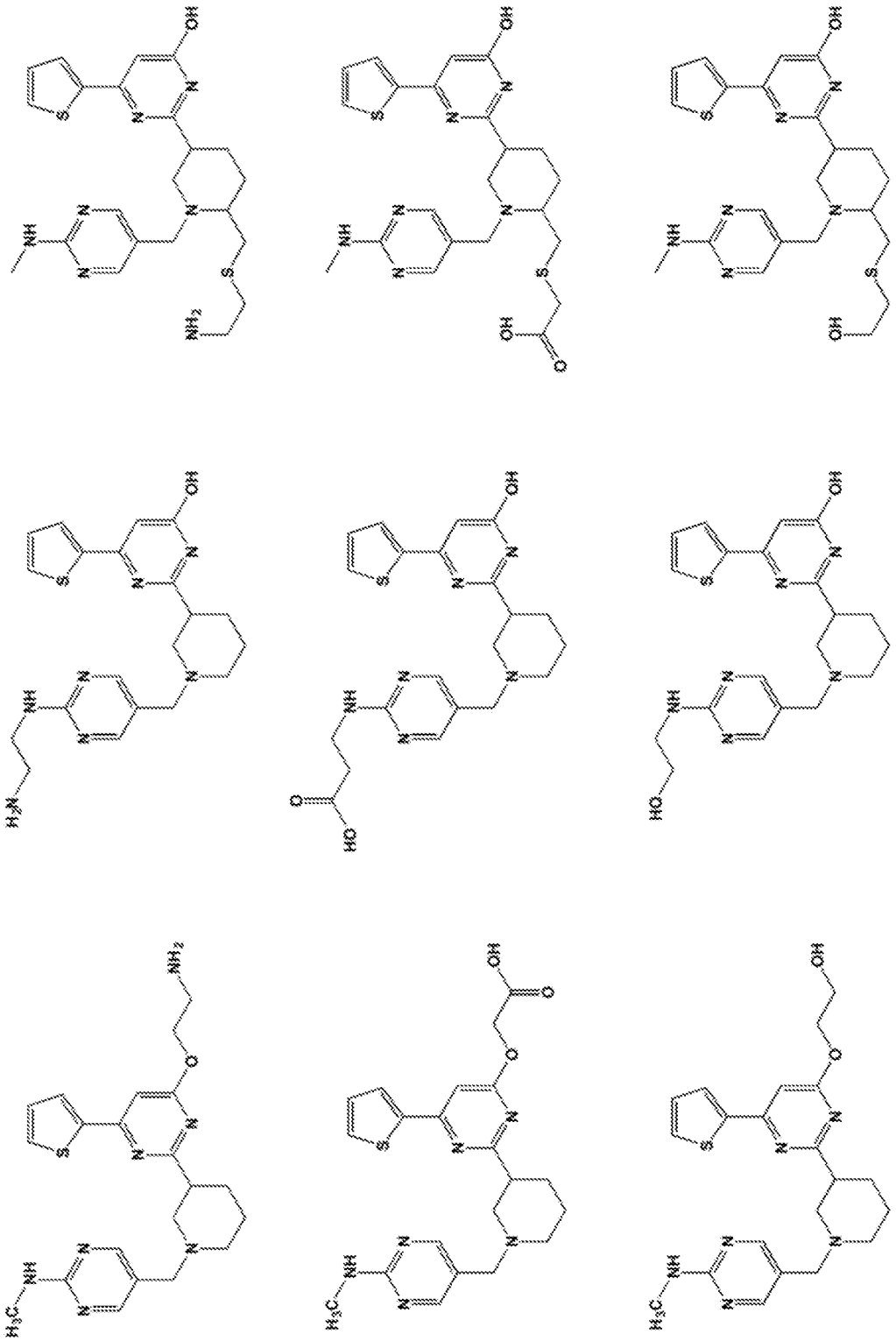
Figure 5



***Note**: The corresponding structures having the succinimido group in the opposite stereochemical orientation may also be prepared.*

Each R is independently selected from halo, -OH, -OMe, $-NH_2$, -NH-(optionally substituted C_{1-10} aliphatic), optionally substituted C_{1-10} aliphatic, or other described tethering groups. The modifier moiety may be attached at any position on R, or at the other functional groups on the above structures.

Figure 6



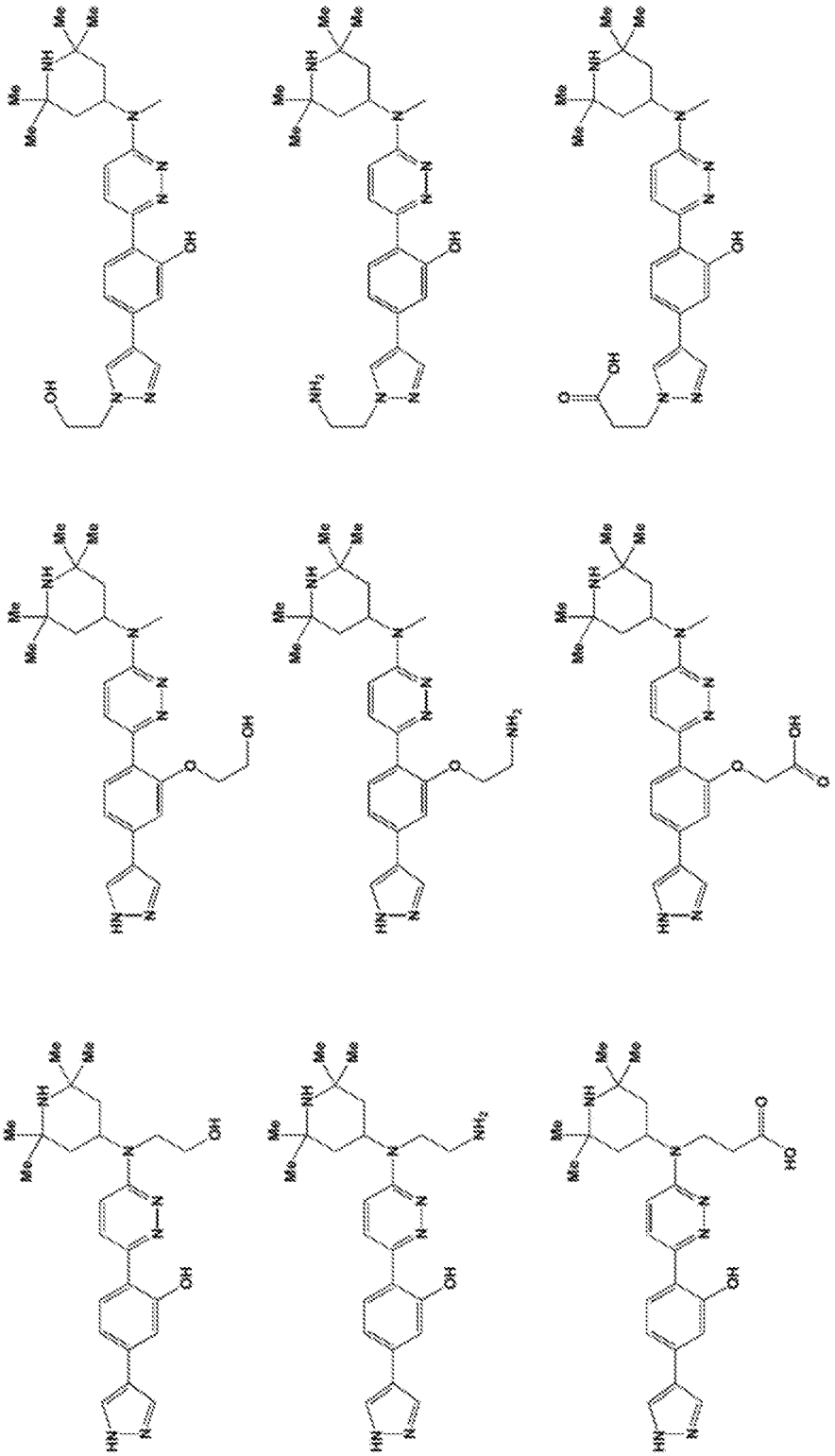


Figure 7

Figure 8

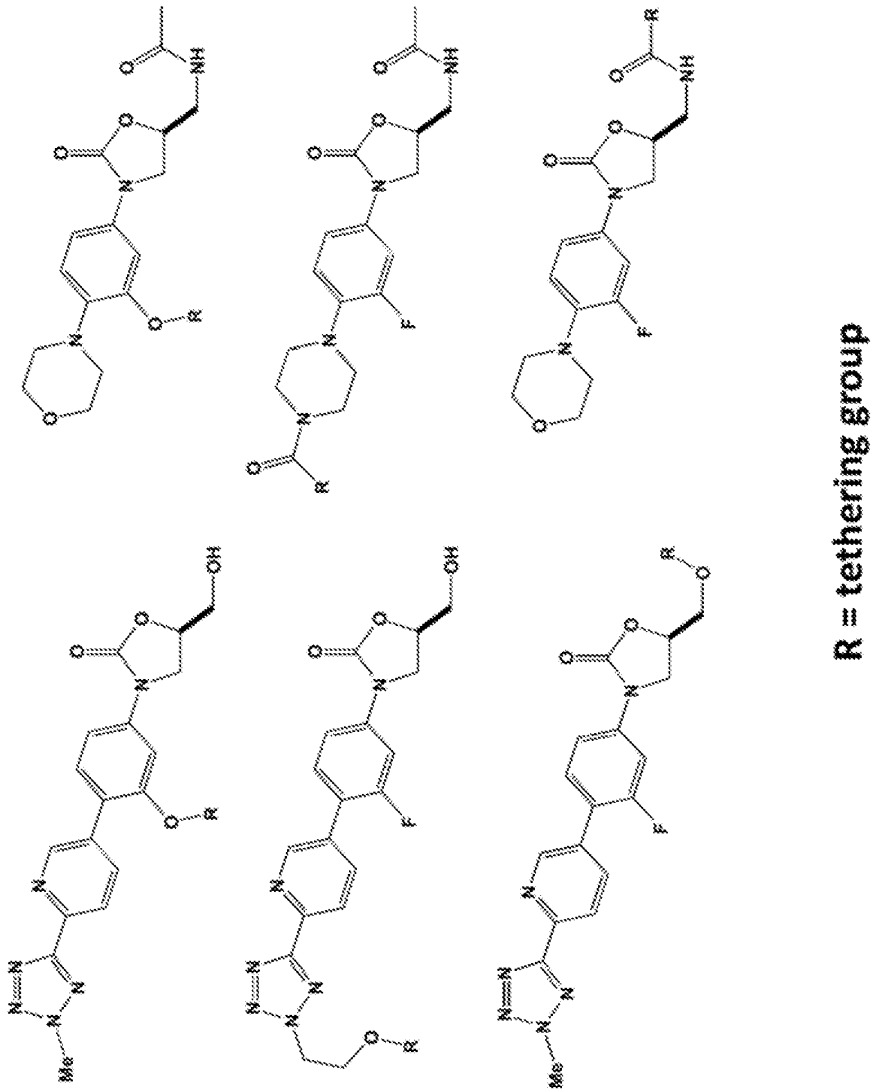


Figure 9

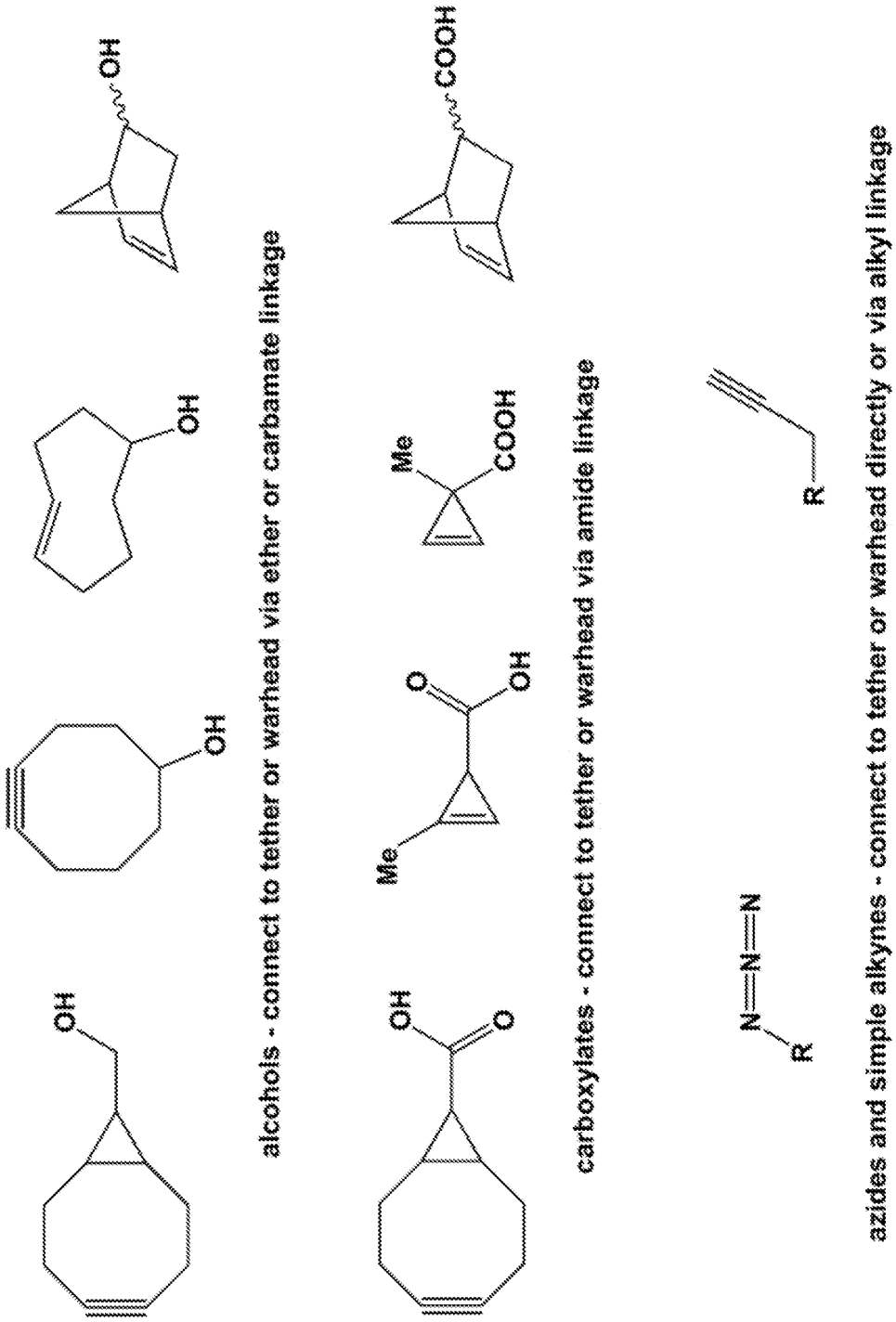
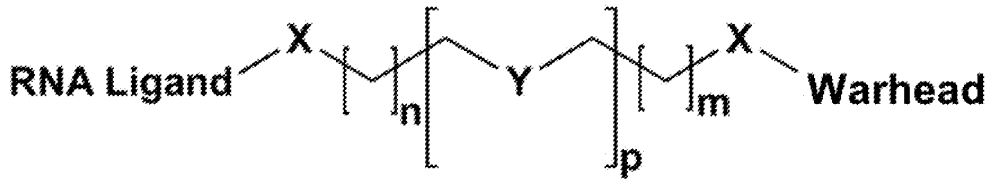

OH
OH
OH
alcohols - connect to tether or warhead via ether or carbamate linkage
COOH
Me
COOH
O
OH
Me
OH
O
carboxylates - connect to tether or warhead via amide linkage
N
N
N
R
R
azides and simple alkynes - connect to tether or warhead directly or via alkyl linkage

Figure 10



RNA Ligand	= described elsewhere
X	= -CO-, -SO_2-, -NH-, -N(alkyl)-, -S-, -O-, -triazole-, -arylene-, -heteroarylene-, etc.
n	= 1, 2, 3, 4, 5; n can = 0 when X = CO or SO_2 or aryl
Y	= a bond, -O-, -S-, -SO-, -SO_2-, -NH-, -N(alkyl)-, -CH_2-, -arylene-, -heteroarylene-, etc.
p	= 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12
m	= 1, 2, 3, 4, 5; m can = 0 when X = -CO- or -SO_2- or -arylene- or -heteroarylene-
Warhead	= described elsewhere

Figure 11

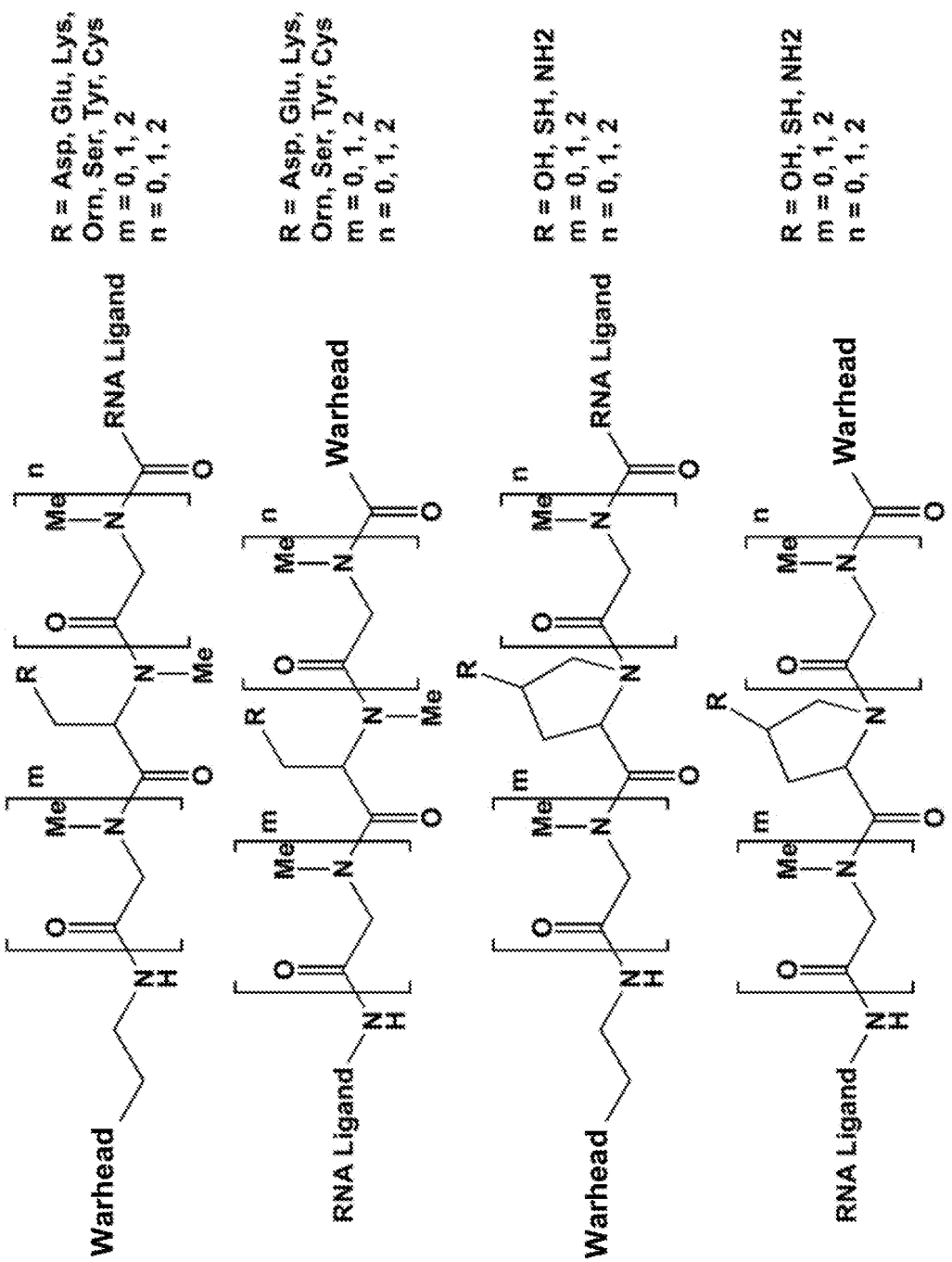

R = Asp, Glu, Lys, Orn, Ser, Tyr, Cys
m = 0, 1, 2
n = 0, 1, 2
RNA Ligand
Warhead
R = Asp, Glu, Lys, Orn, Ser, Tyr, Cys
m = 0, 1, 2
n = 0, 1, 2
Warhead
RNA Ligand
R = OH, SH, NH2
m = 0, 1, 2
n = 0, 1, 2
RNA Ligand
Warhead
R = OH, SH, NH2
m = 0, 1, 2
n = 0, 1, 2
Warhead
RNA Ligand

Figure 12

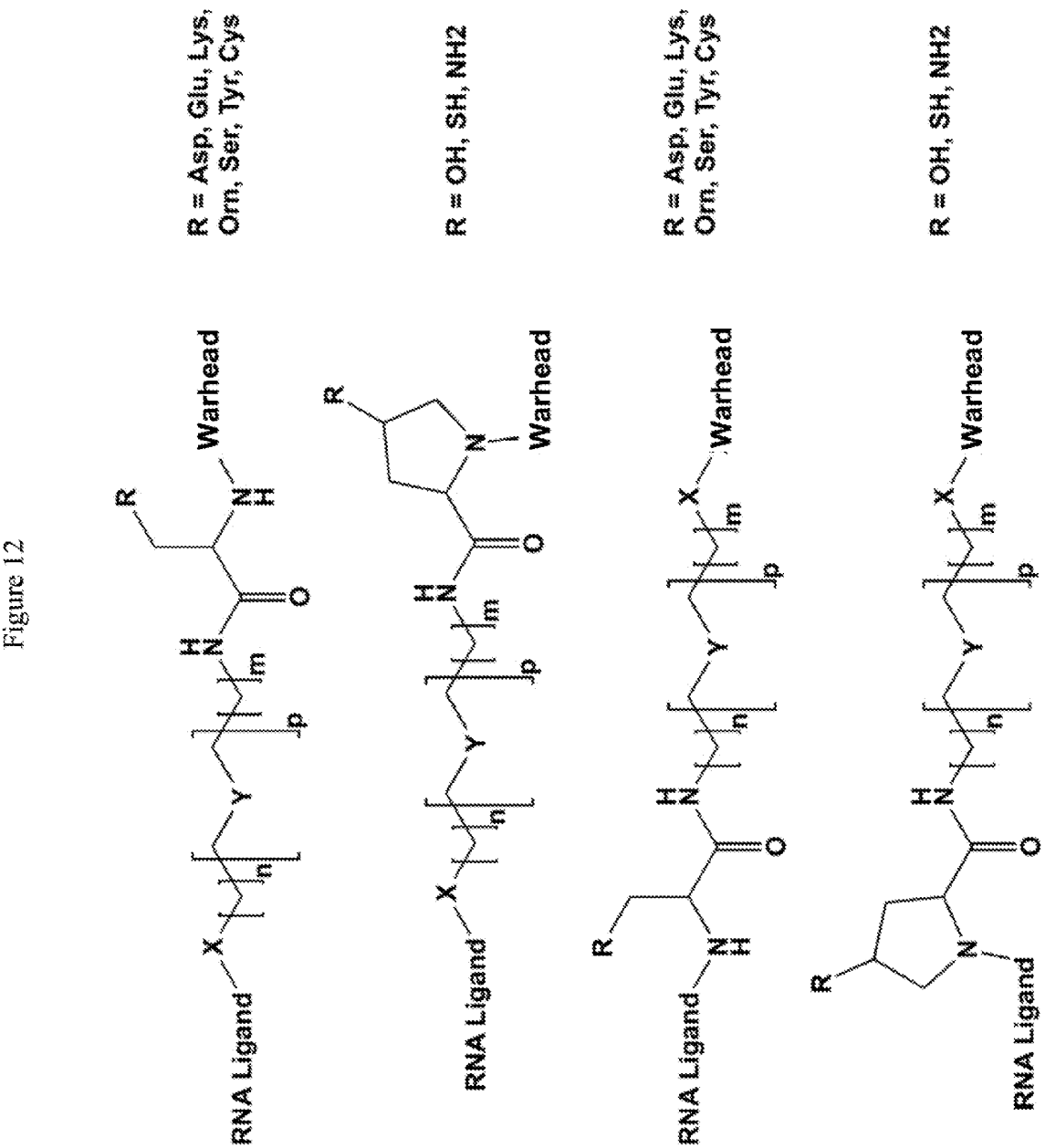

R = Asp, Glu, Lys, Orn, Ser, Tyr, Cys
R = OH, SH, NH2
R = Asp, Glu, Lys, Orn, Ser, Tyr, Cys
R = OH, SH, NH2
Warhead
RNA Ligand
X
Y
n
m
p
H
N
O

Figure 13

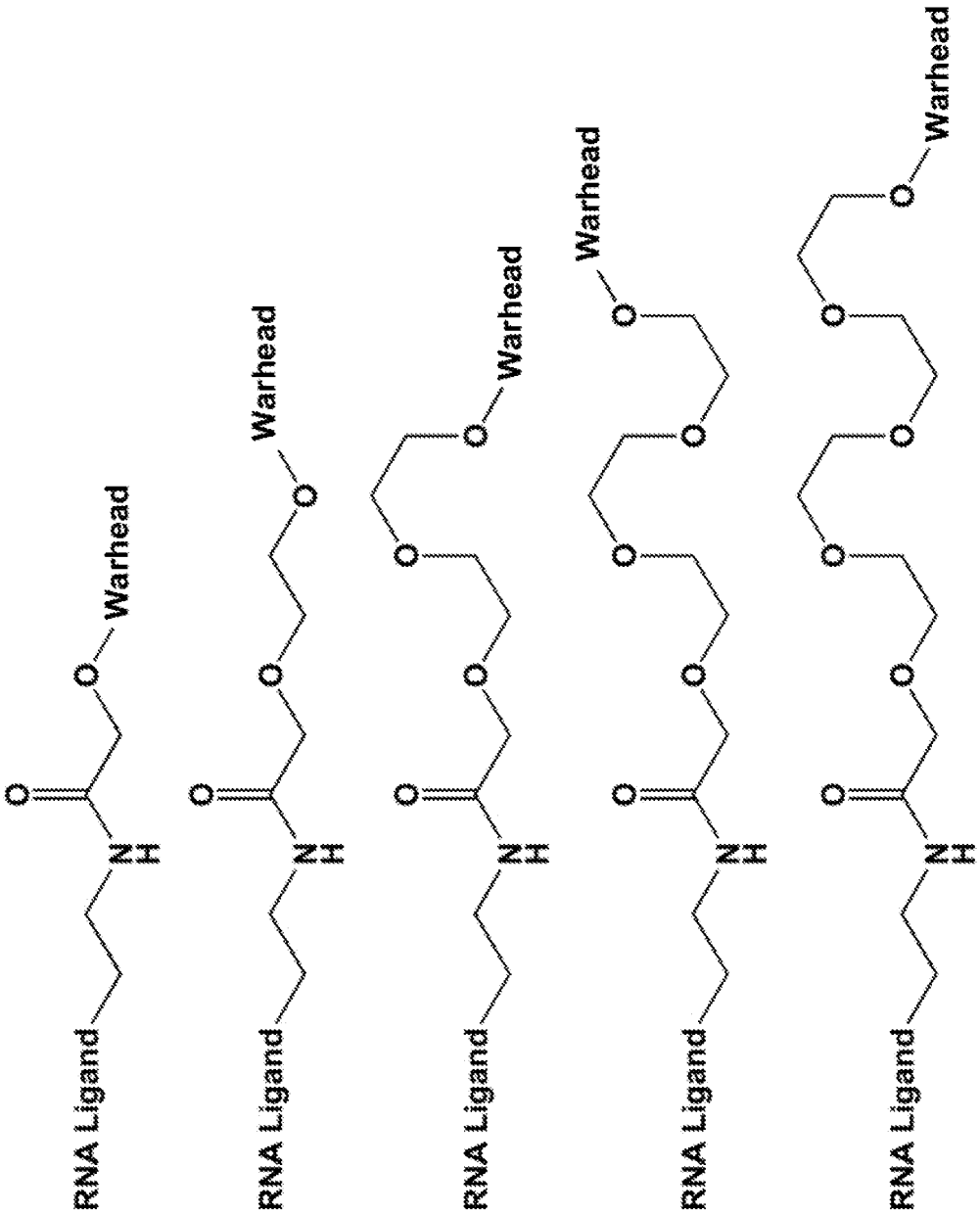

RNA Ligand
Warhead
RNA Ligand
Warhead
RNA Ligand
Warhead
RNA Ligand
Warhead
RNA Ligand
Warhead

Figure 14

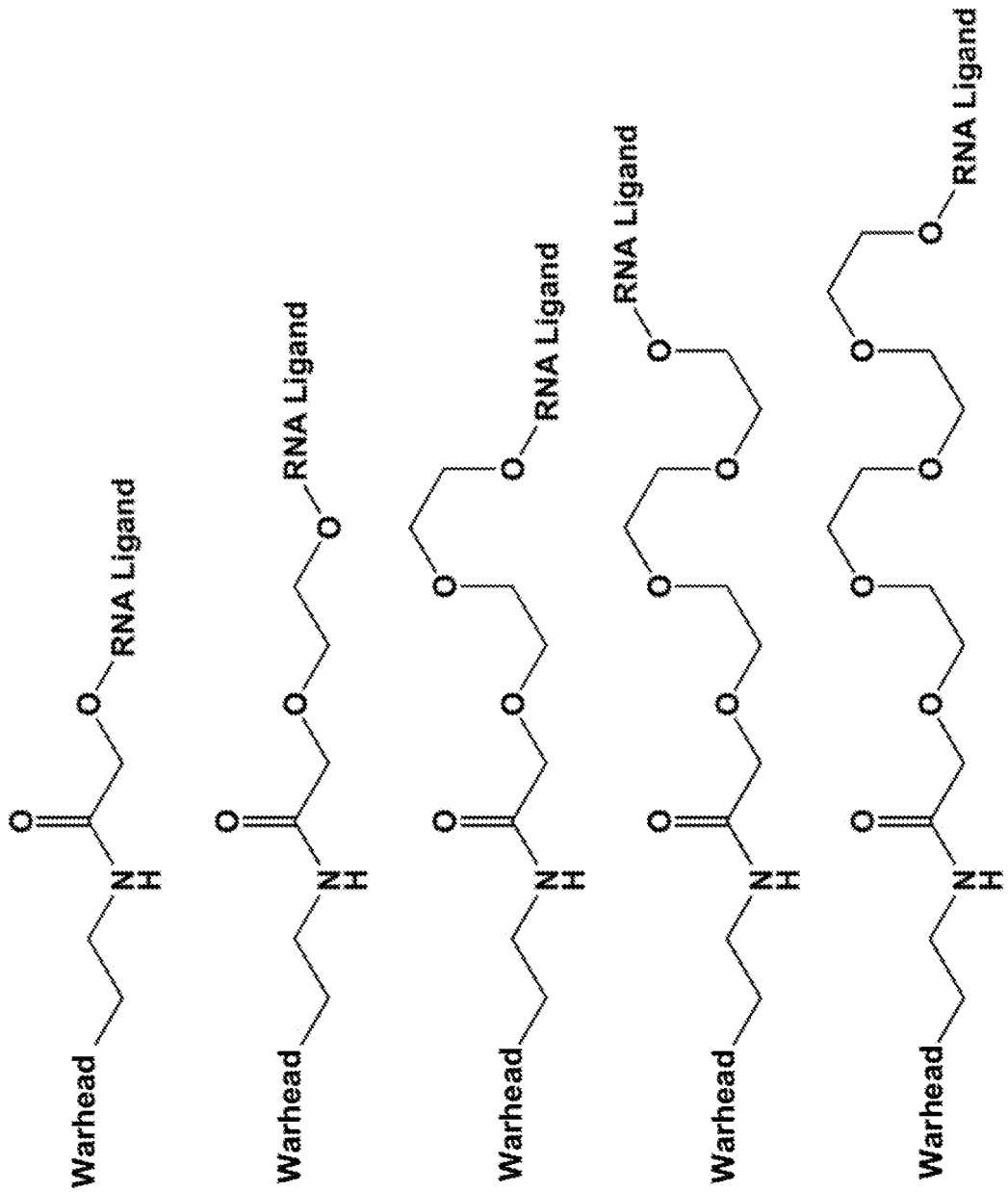

Warhead
RNA Ligand
Warhead
RNA Ligand
Warhead
RNA Ligand
Warhead
RNA Ligand
Warhead
RNA Ligand

Figure 15

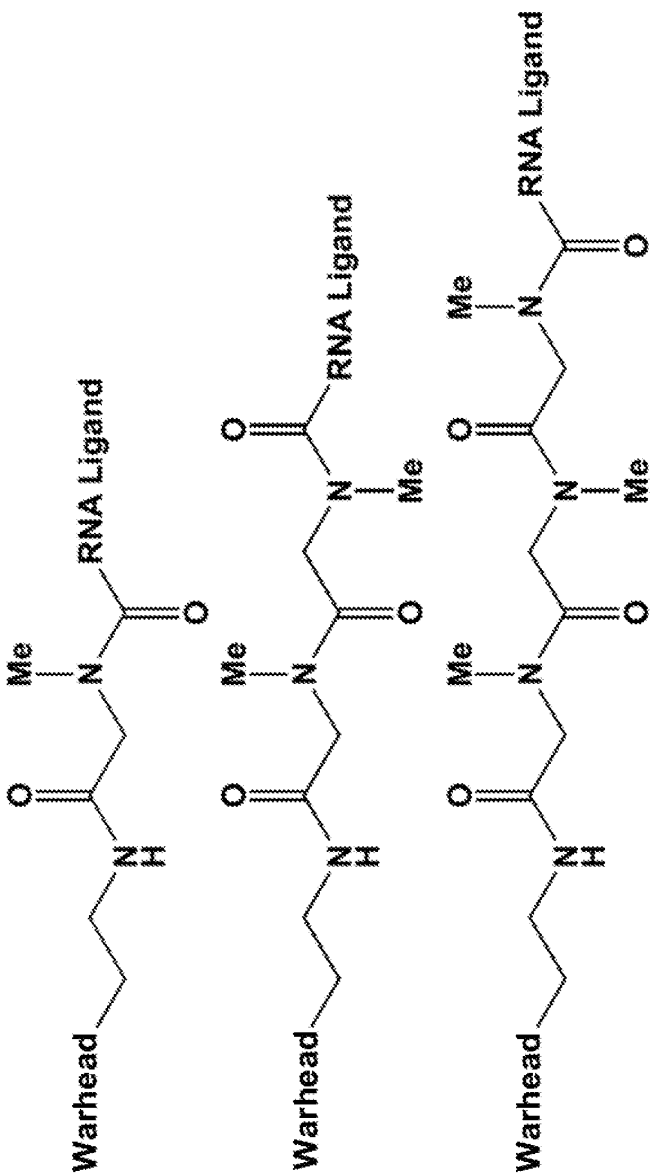

Warhead
N
H
O
O
Me
N
RNA Ligand
O
Warhead
N
H
O
O
Me
N
Me
N
RNA Ligand
O
Warhead
N
H
O
O
Me
N
Me
N
RNA Ligand
O

Figure 16

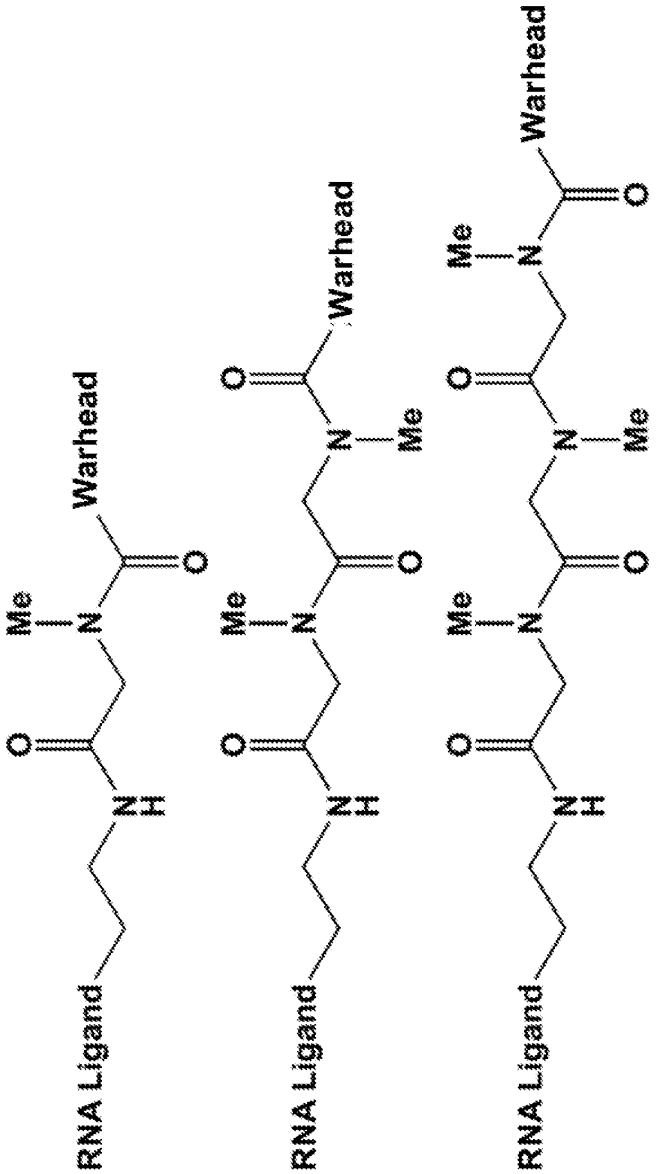

RNA Ligand
Warhead
Me
N
O
H
RNA Ligand
Warhead
Me
N
O
H
RNA Ligand
Warhead
Me
N
O
H

Figure 17

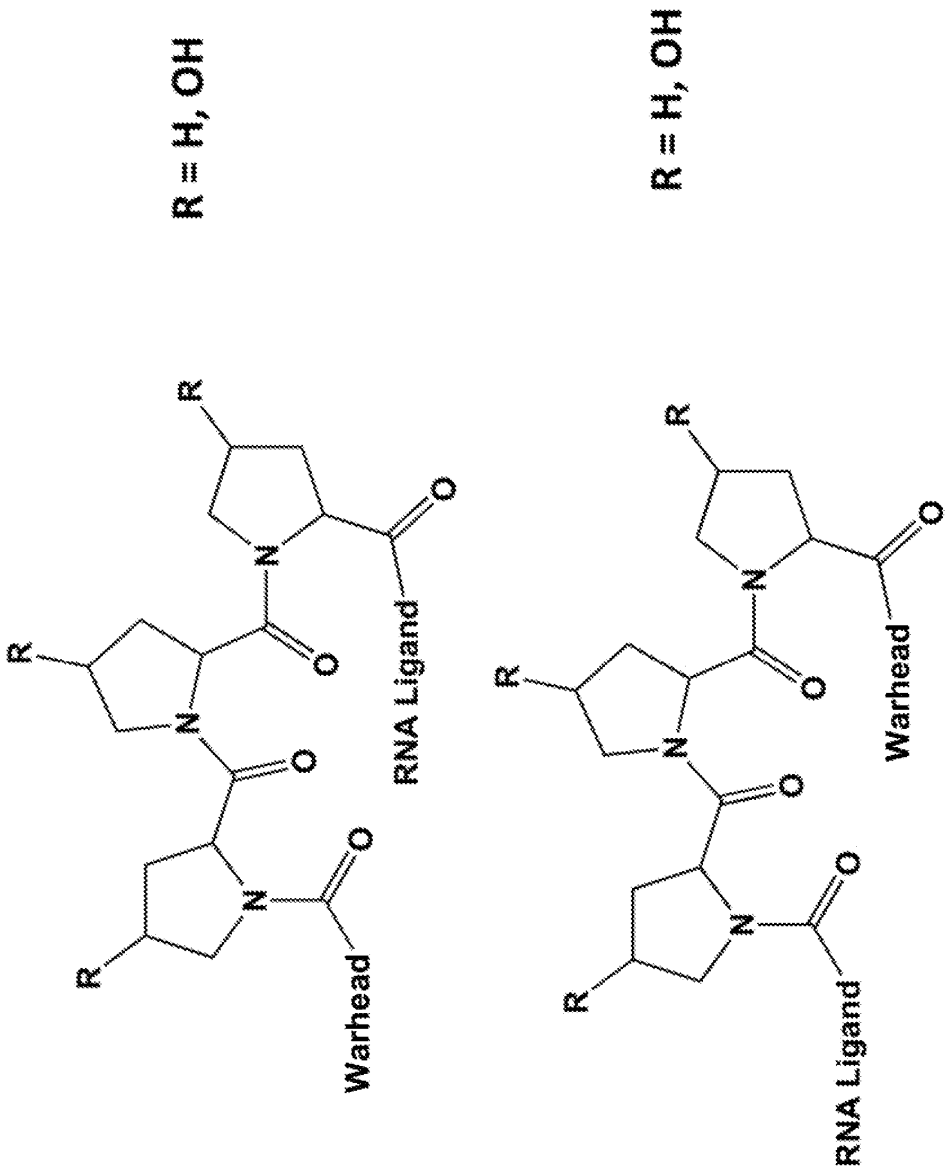


Figure 18

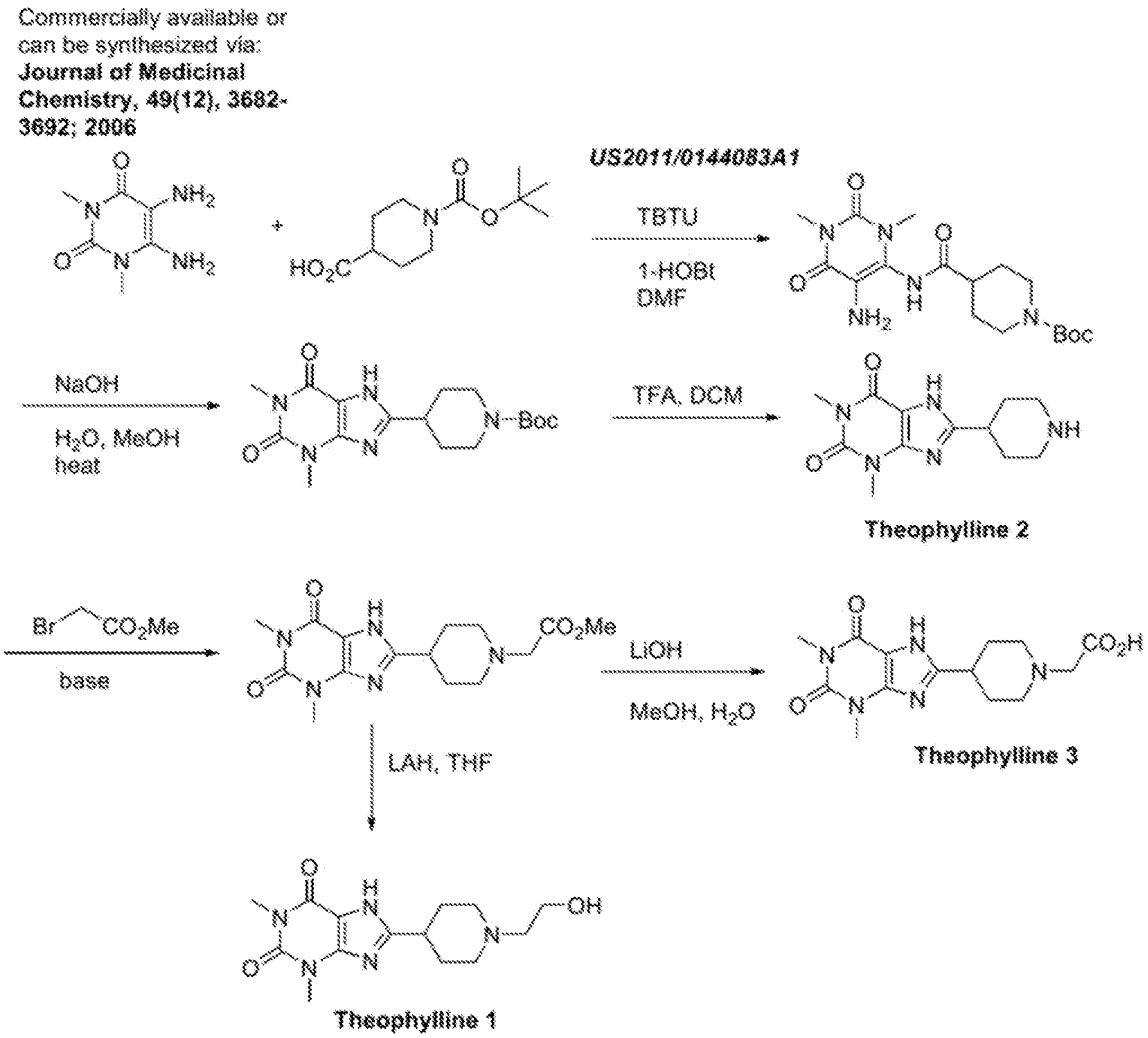

Commercially available or can be synthesized via: Journal of Medicinal Chemistry, 49(12), 3682-3692; 2006
US2011/0144083A1
TBTU
1-HOBt
DMF
NaOH
H2O, MeOH
heat
TFA, DCM
Theophylline 2
Br
CO2Me
base
LiOH
MeOH, H2O
LAH, THF
Theophylline 3
Theophylline 1

Figure 19

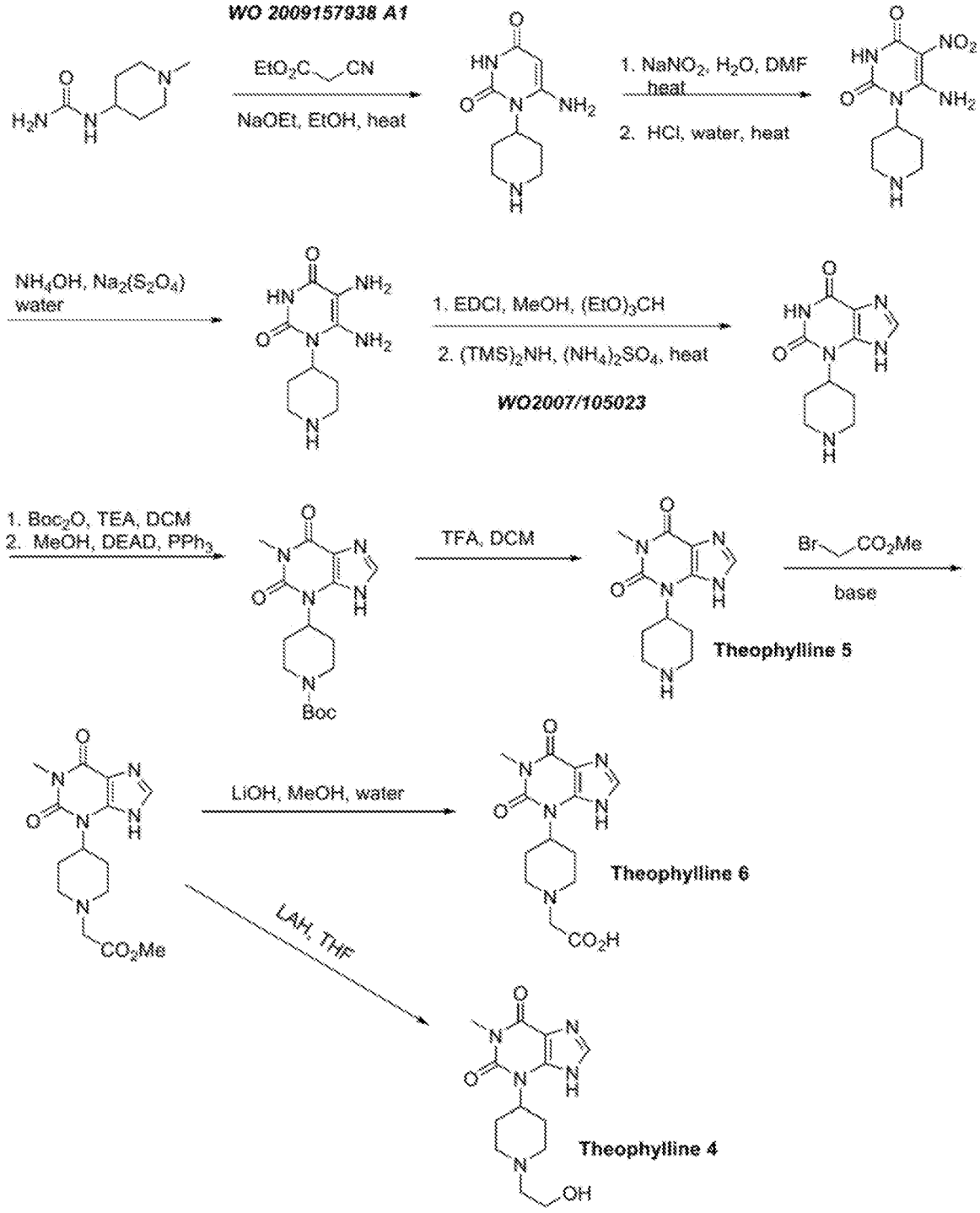

WO 2009157938 A1
EtO2C CN
NaOEt, EtOH, heat
1. NaNO2, H2O, DMF
heat
2. HCl, water, heat
NH4OH, Na2(S2O4)
water
1. EDCI, MeOH, (EtO)3CH
2. (TMS)2NH, (NH4)2SO4, heat
WO2007/105023
1. Boc2O, TEA, DCM
2. MeOH, DEAD, PPh3
TFA, DCM
Br CO2Me
base
Theophylline 5
LiOH, MeOH, water
Theophylline 6
LAH, THF
Theophylline 4

Figure 20

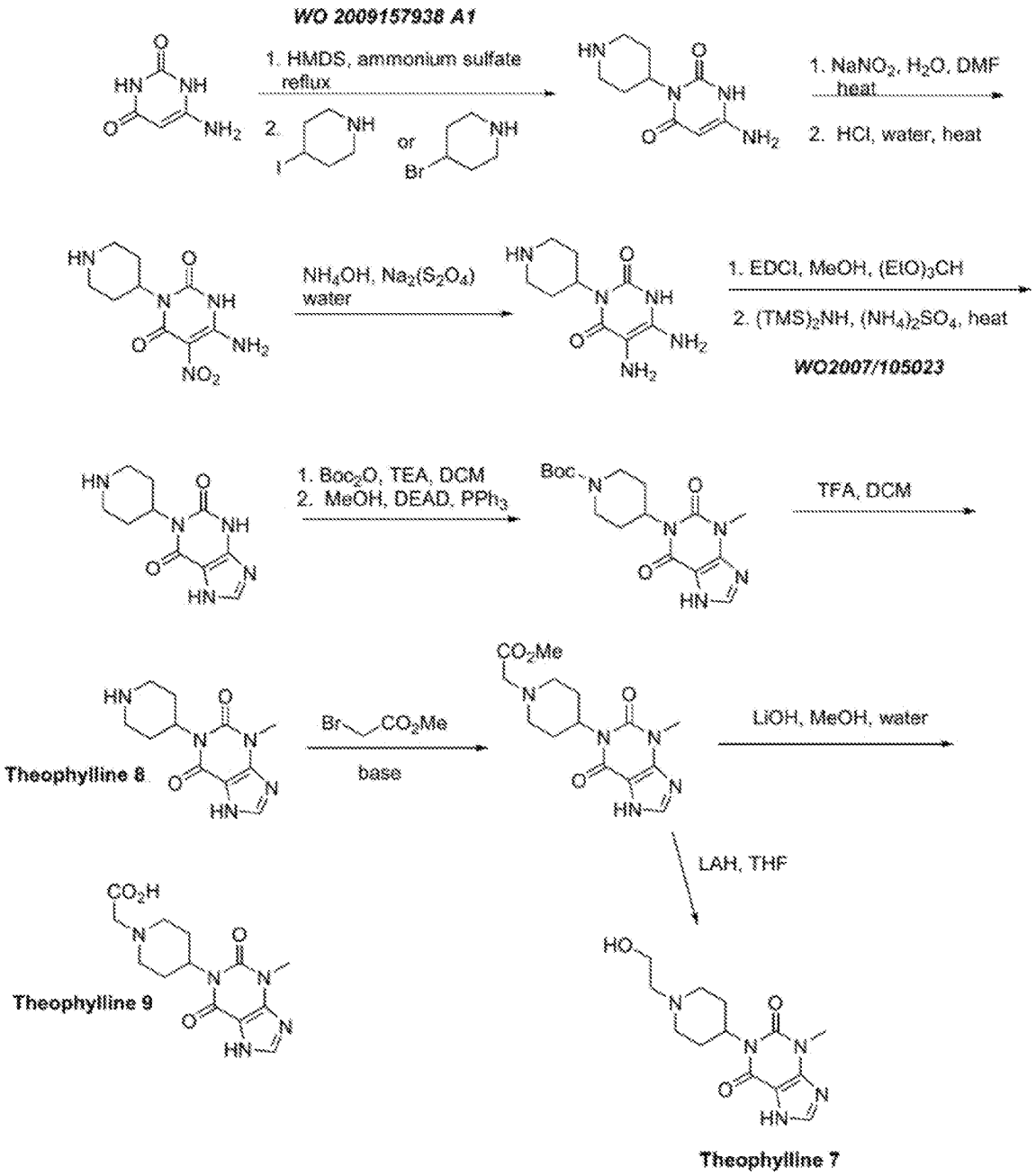

WO 2009157938 A1
1. HMDS, ammonium sulfate
reflux
2.
or
1. NaNO2, H2O, DMF
heat
2. HCl, water, heat
NH4OH, Na2(S2O4)
water
1. EDCI, MeOH, (EtO)3CH
2. (TMS)2NH, (NH4)2SO4, heat
WO2007/105023
1. Boc2O, TEA, DCM
2. MeOH, DEAD, PPh3
TFA, DCM
Theophylline 8
base
LiOH, MeOH, water
LAH, THF
Theophylline 9
Theophylline 7

Figure 21

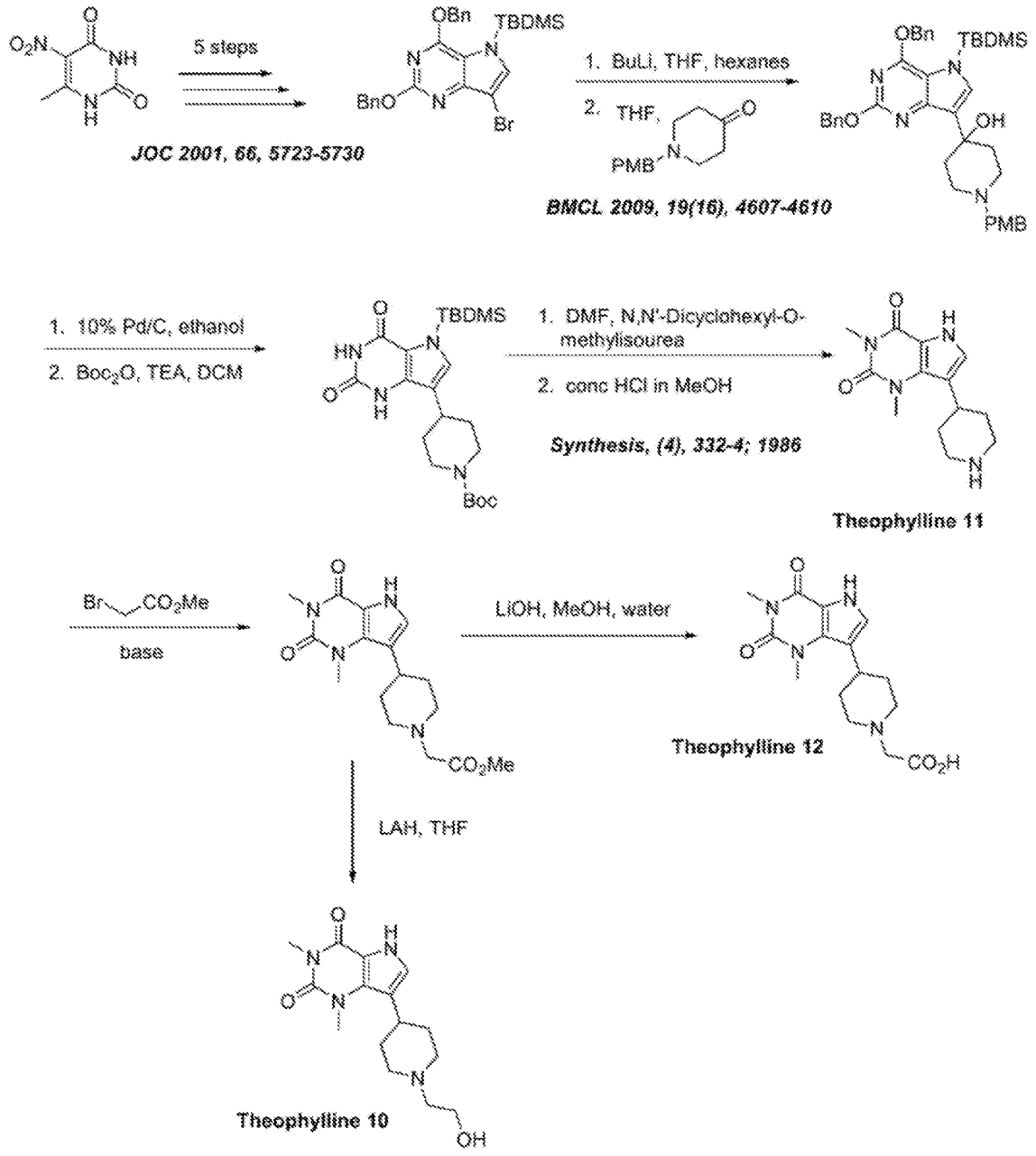
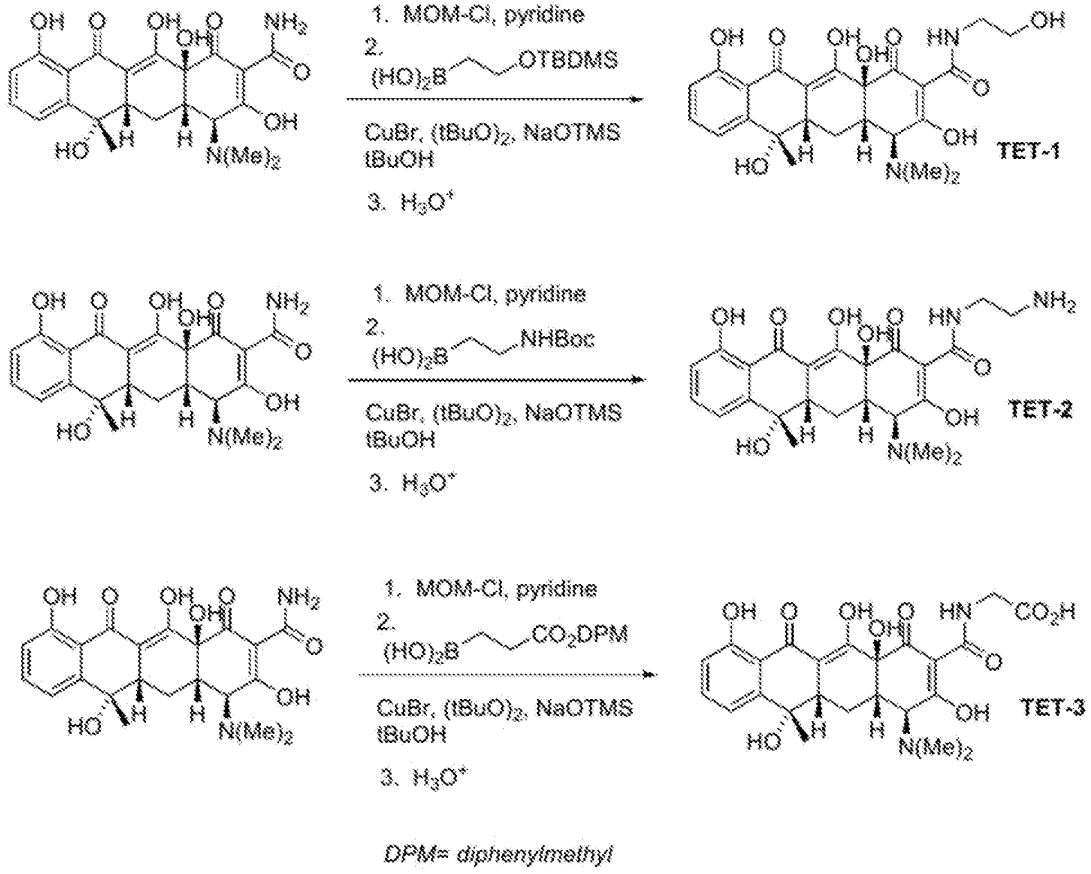

5 steps
JOC 2001, 66, 5723-5730
1. BuLi, THF, hexanes
2. THF,
BMCL 2009, 19(16), 4607-4610
1. 10% Pd/C, ethanol
2. Boc2O, TEA, DCM
1. DMF, N,N'-Dicyclohexyl-O-methylisourea
2. conc HCl in MeOH
Synthesis, (4), 332-4; 1986
Theophylline 11
LiOH, MeOH, water
base
Theophylline 12
LAH, THF
Theophylline 10

Figure 22

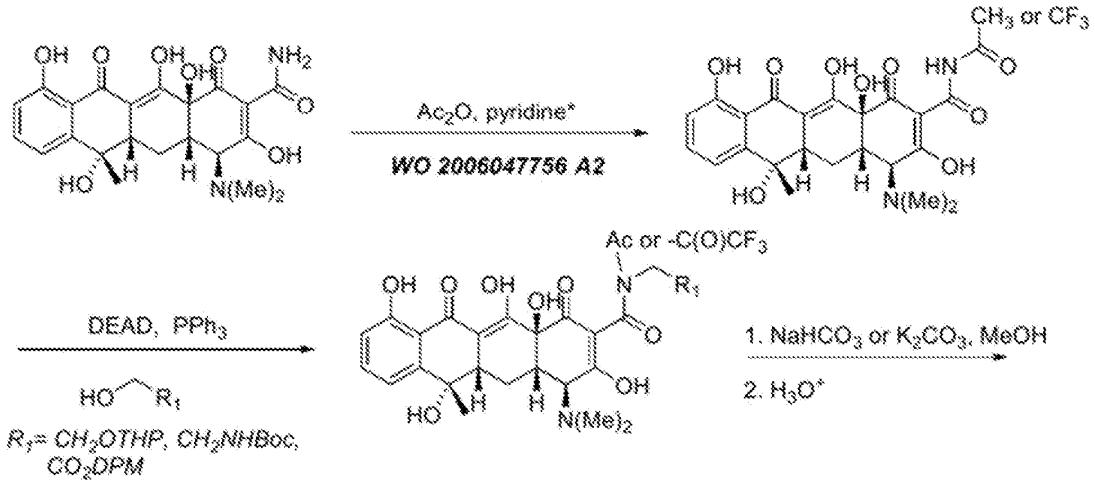
Org Lett 2013, 15, 2314-2317

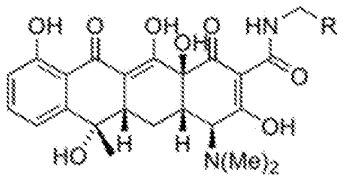


DPM= diphenylmethyl

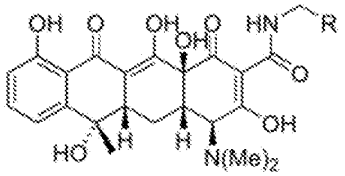
Figure 23

Alternative Scheme for TET1, TET-2 and TET-3





TET-1; R=CH_2OH
TET-2; R=CH_2NH_2
TET-3; R=CO_2H



** It may be necessary to persilylate the phenols via TMS-Cl or another acid labile protecting group such as a MOM-Cl with pyridine before the acylation to ensure a selective Mitsunobu, but both routes can be tried*

Figure 24

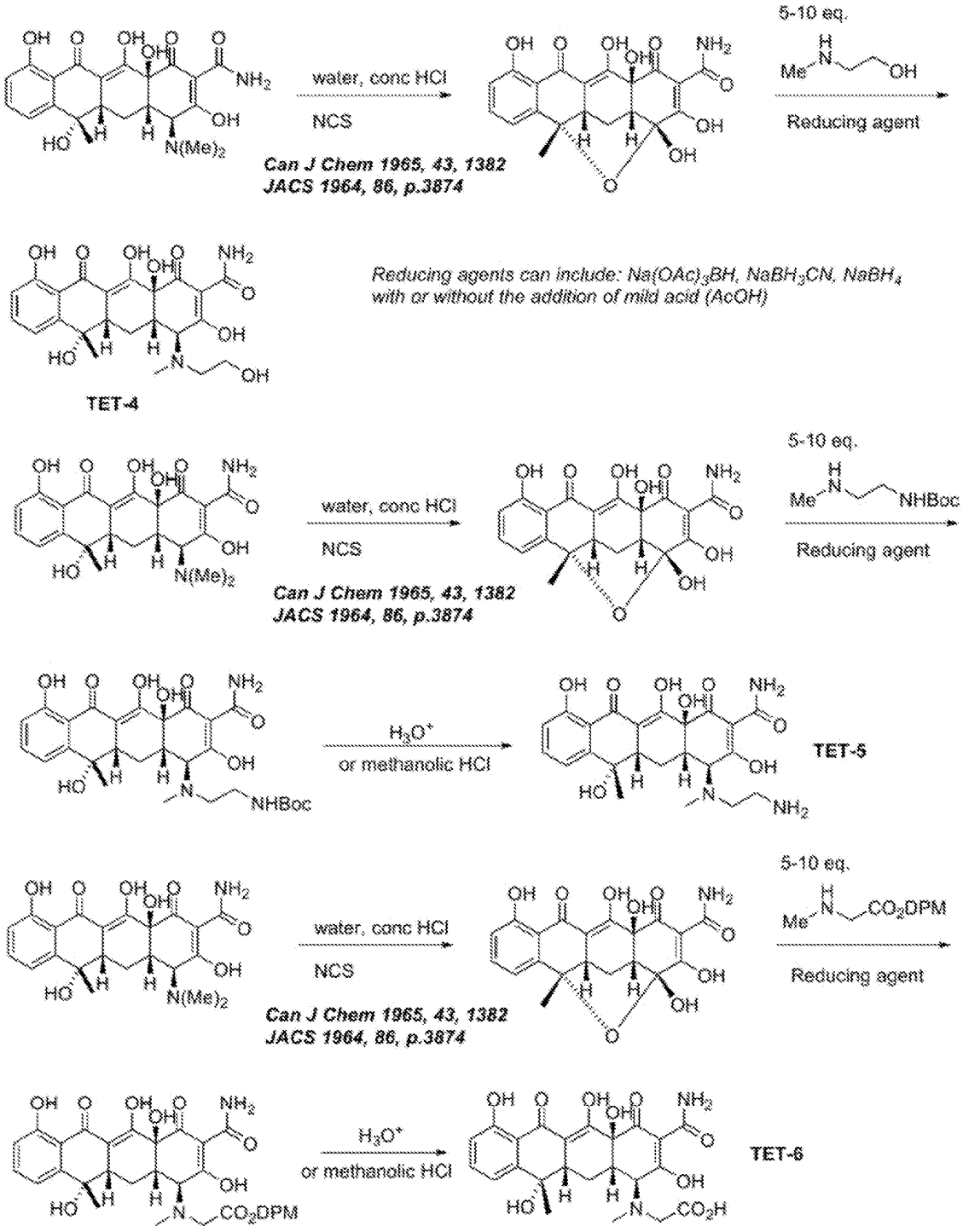

water, conc HCl
NCS
Can J Chem 1965, 43, 1382
JACS 1964, 86, p.3874
5-10 eq.
Reducing agent
Reducing agents can include: Na(OAc)3BH, NaBH3CN, NaBH4 with or without the addition of mild acid (AcOH)
TET-4
H3O+
or methanolic HCl
TET-5
TET-6

Figure 25

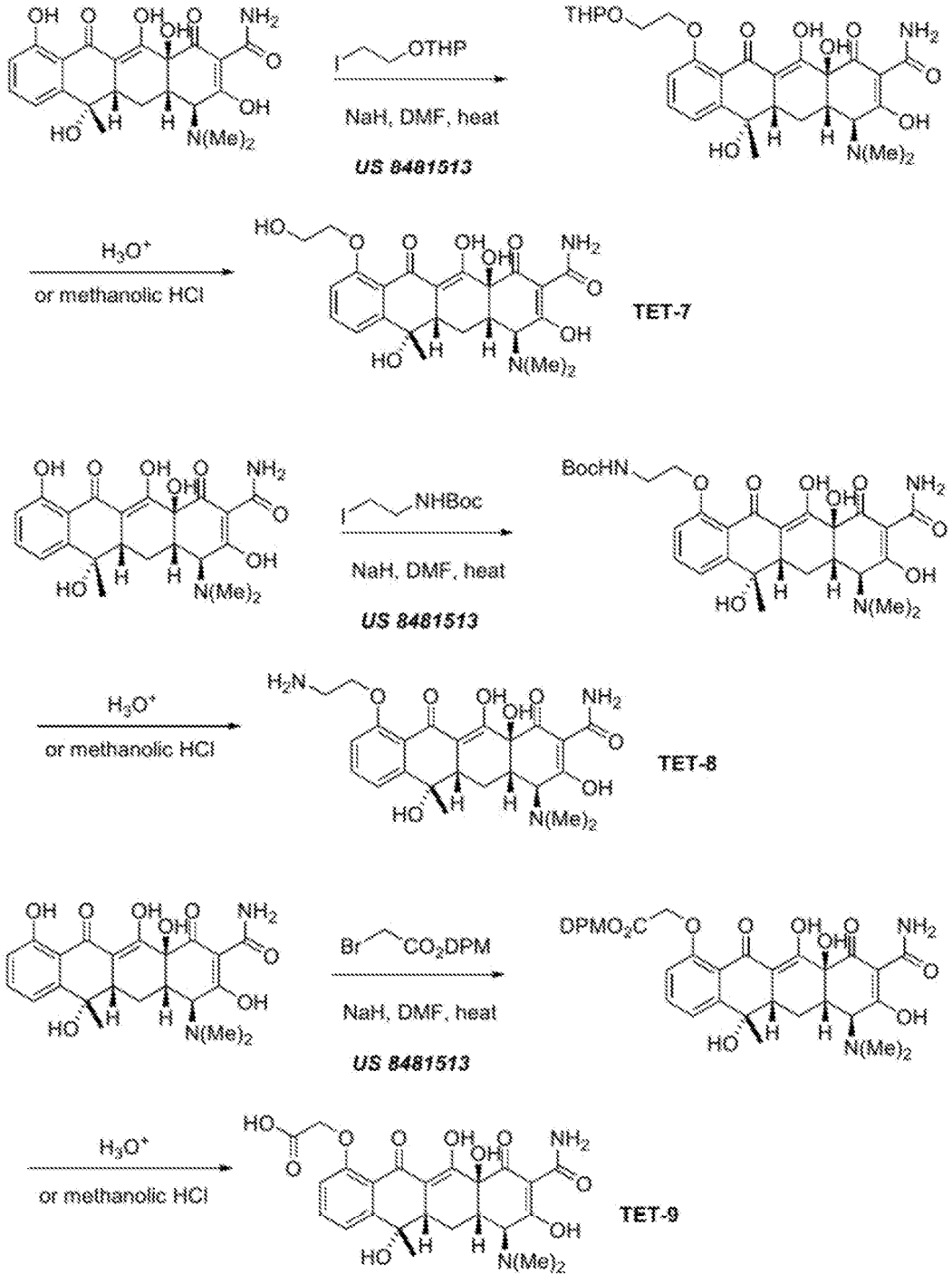

OTHP
NaH, DMF, heat
US 8481513
THPO
H3O+
or methanolic HCl
TET-7
NHBoc
BocHN
TET-8
Br
CO2DPM
DPMO2C
TET-9
N(Me)2
NH2
OH
HO

Figure 26

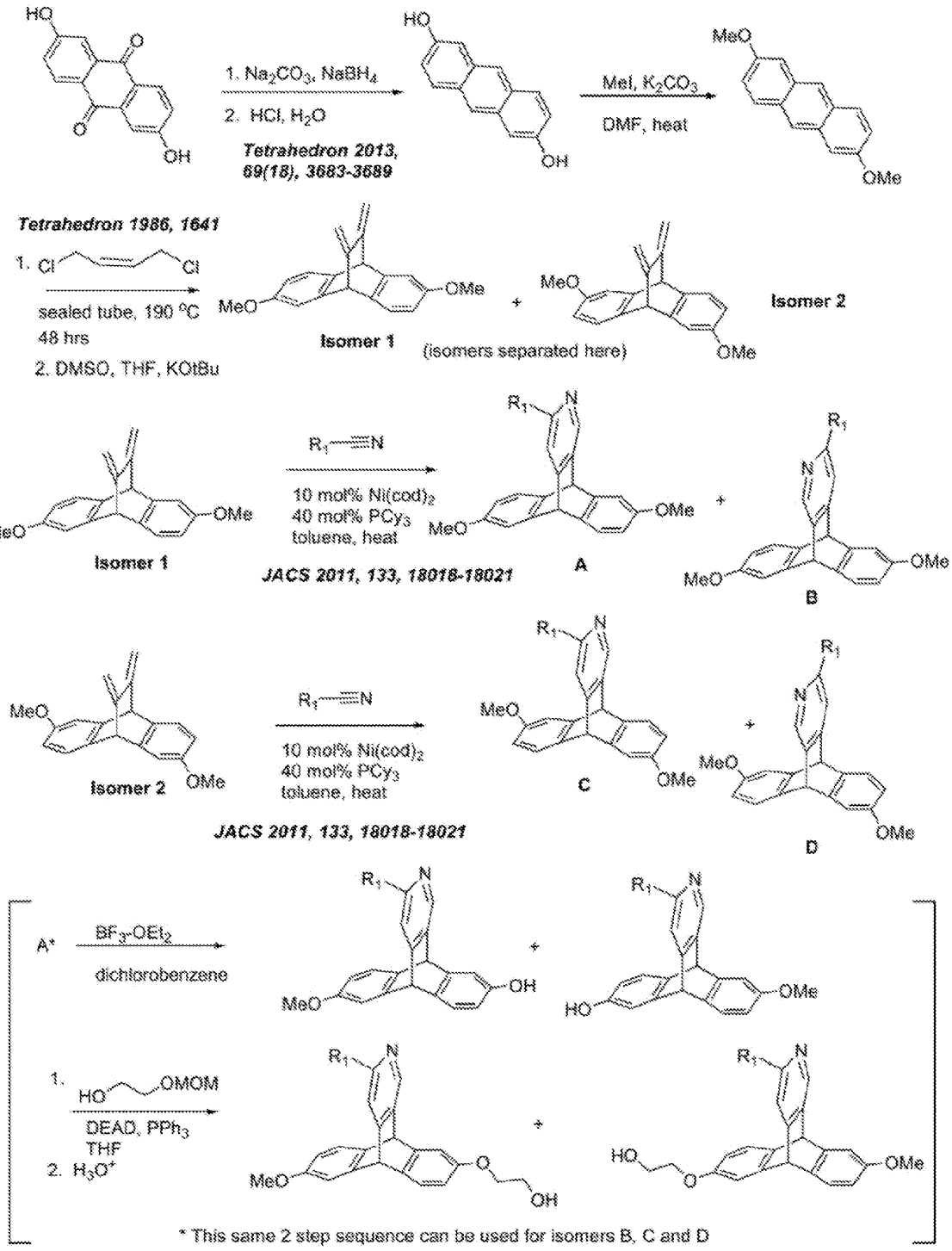
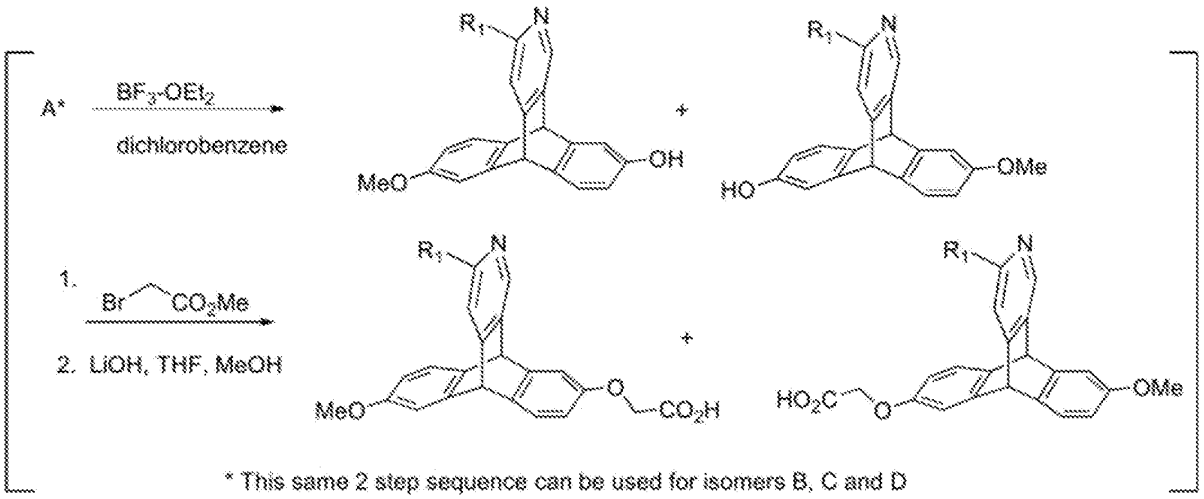

1. Na_2CO_3, $NaBH_4$
2. HCl, H_2O
Tetrahedron 2013, 69(18), 3683-3689
MeI, K_2CO_3
DMF, heat
Tetrahedron 1986, 1641
1. Cl Cl
sealed tube, 190 °C
48 hrs
2. DMSO, THF, KOtBu
Isomer 1
Isomer 2
(isomers separated here)
R_1—≡N
10 mol% $Ni(cod)_2$
40 mol% PCy_3
toluene, heat
JACS 2011, 133, 18018-18021
A
B
C
D
A*
BF_3-OEt_2
dichlorobenzene
1. HO OMOM
DEAD, PPh_3
THF
2. H_3O^+
* This same 2 step sequence can be used for isomers B, C and D

Figure 27


A*
BF3-OEt2
dichlorobenzene
MeO
OH
HO
OMe
1.
Br
CO2Me
2. LiOH, THF, MeOH
CO2H
HO2C
* This same 2 step sequence can be used for isomers B, C and D

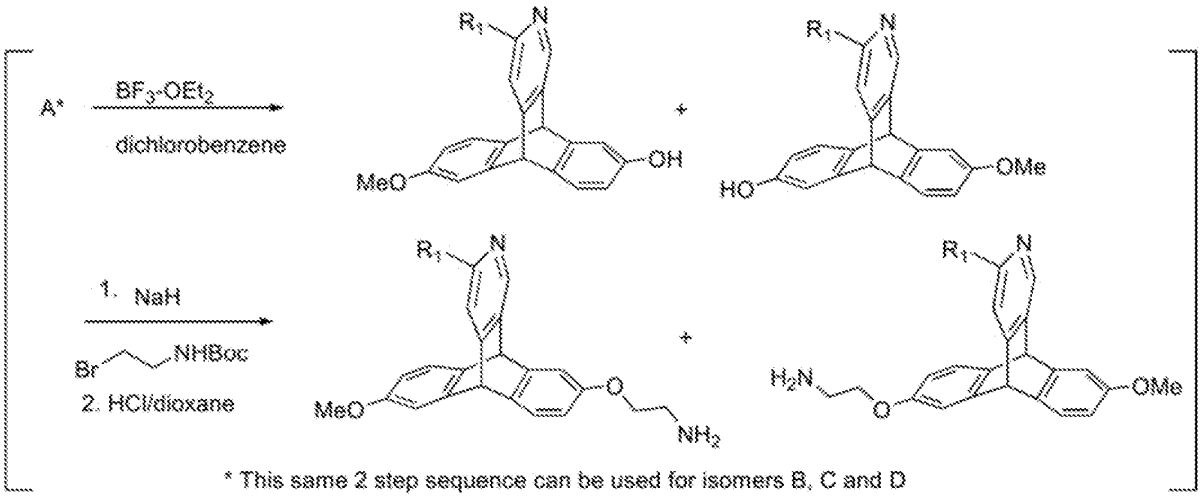

A*
BF3-OEt2
dichlorobenzene
MeO
OH
HO
OMe
1. NaH
Br
NHBoc
2. HCl/dioxane
NH2
H2N
* This same 2 step sequence can be used for isomers B, C and D

Figure 28

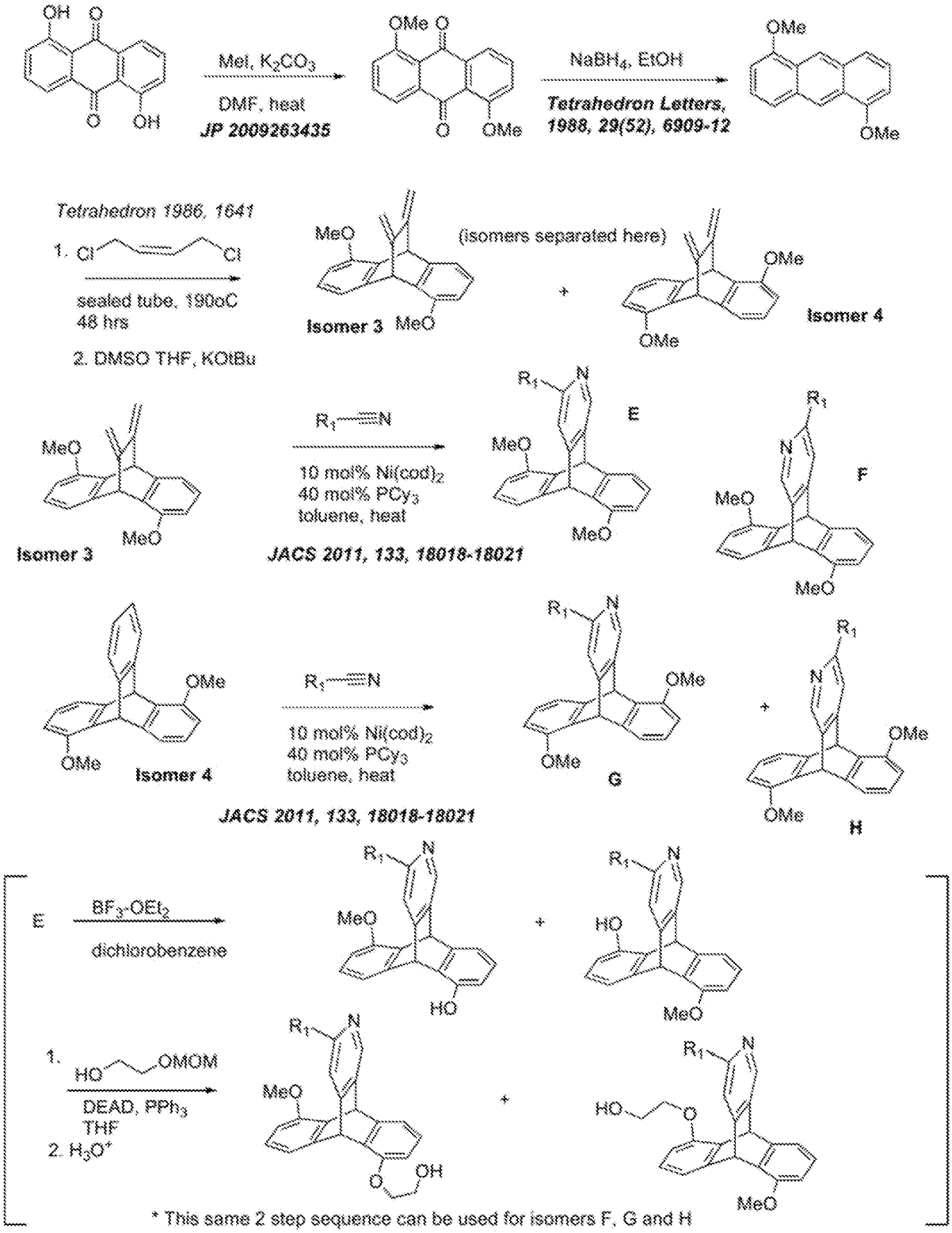
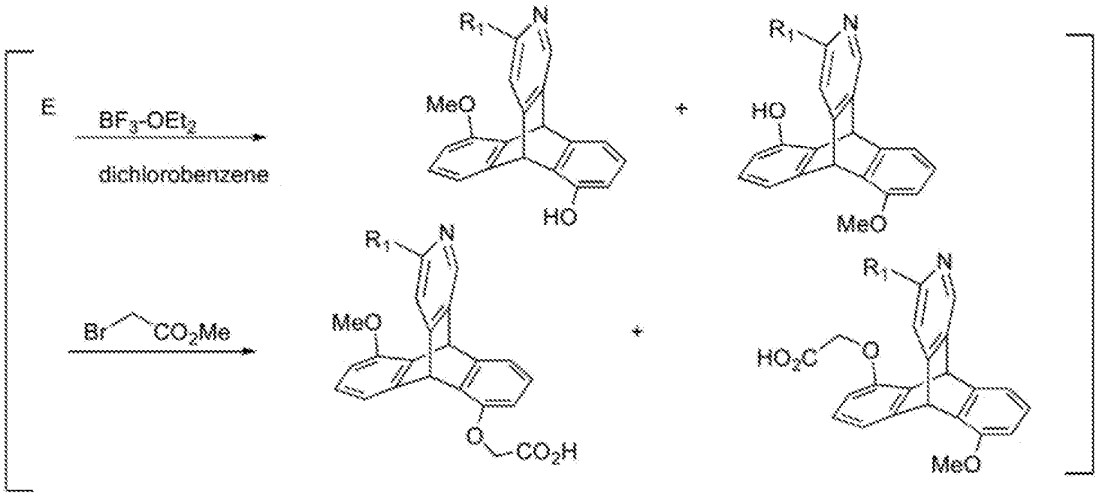
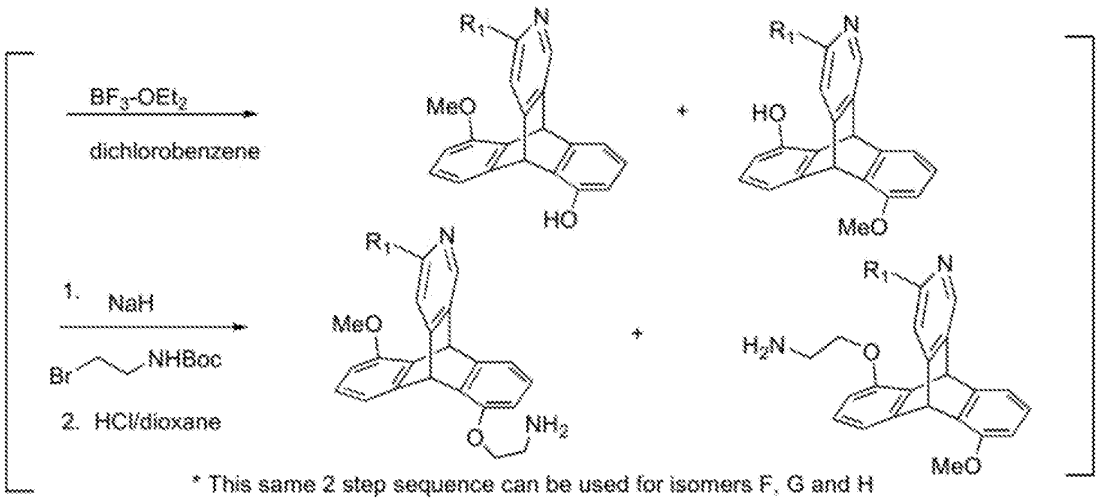

MeI, K2CO3
DMF, heat
JP 2009263435
NaBH4, EtOH
Tetrahedron Letters, 1988, 29(52), 6909-12
Tetrahedron 1986, 1641
1. Cl Cl
sealed tube, 190oC
48 hrs
2. DMSO THF, KOtBu
(isomers separated here)
Isomer 3
Isomer 4
R1—≡N
10 mol% Ni(cod)2
40 mol% PCy3
toluene, heat
JACS 2011, 133, 18018-18021
E
F
G
H
BF3-OEt2
dichlorobenzene
1. HO OMOM
DEAD, PPh3
THF
2. H3O+
* This same 2 step sequence can be used for isomers F, G and H

Figure 29

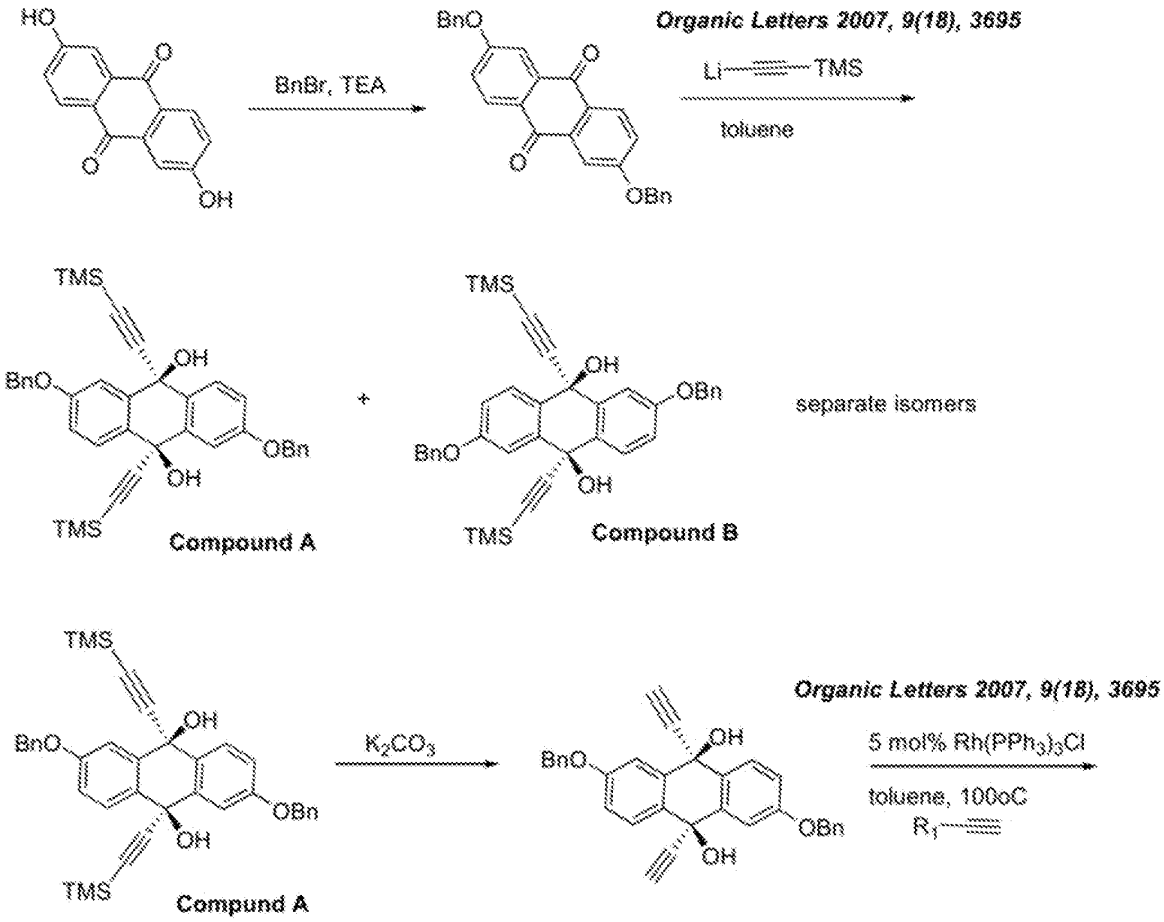


* This same 2 step sequence can be used for isomers B, C and D



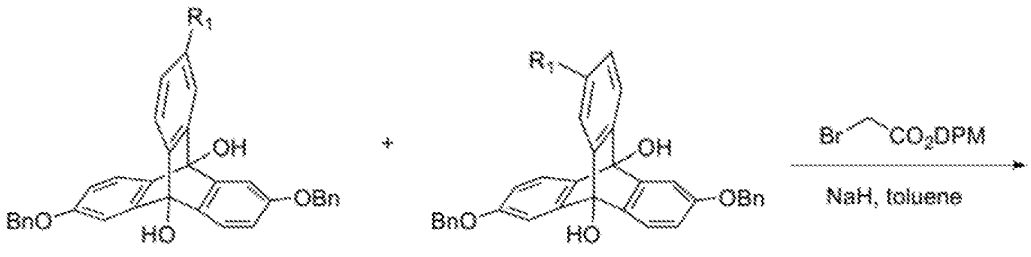
* This same 2 step sequence can be used for isomers F, G and H

Figure 30



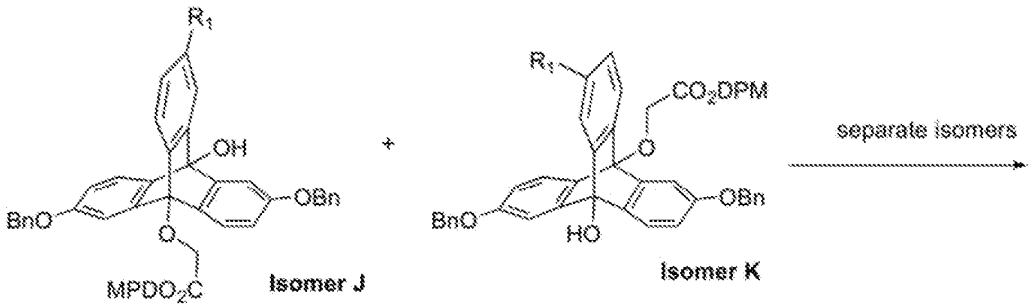
The following Sequence A can also be run on Compound B

Sequence A



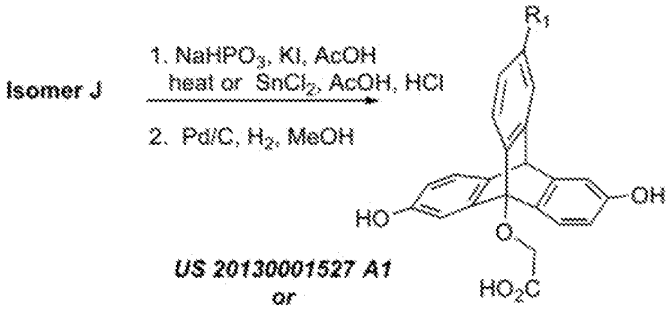
DPM= diphenylmethyl

Figure 31



The following Sequence B also can be run on all generated isomers

Sequence B



US 20130001527 A1
or
Journal of Organic Chemistry, 72(19), 7229-7236; 2007

The following Sequence C can be run on Compound B

Sequence C

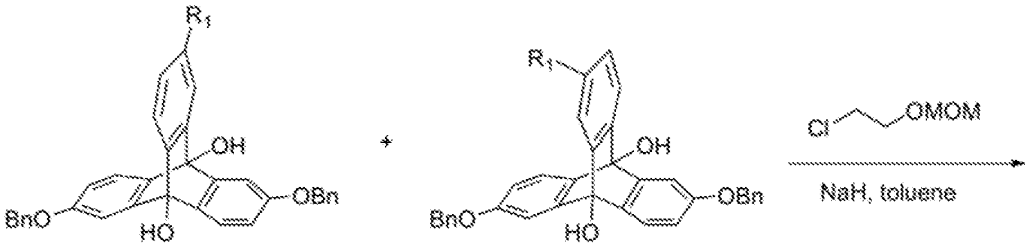
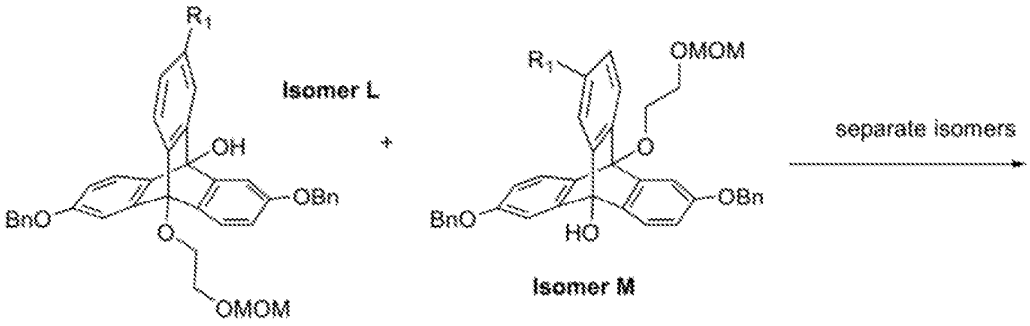
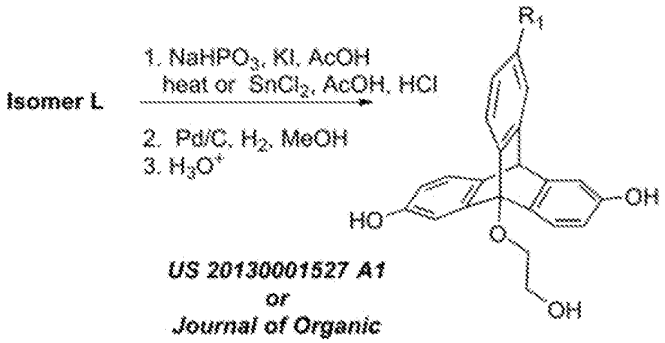


Figure 32



The following Sequence D can also be run on all generated isomers

Sequence D



US 20130001527 A1
or
Journal of Organic Chemistry, 72(19), 7229-7236; 2007

The following Sequence E can also be run on Compound B

Sequence E

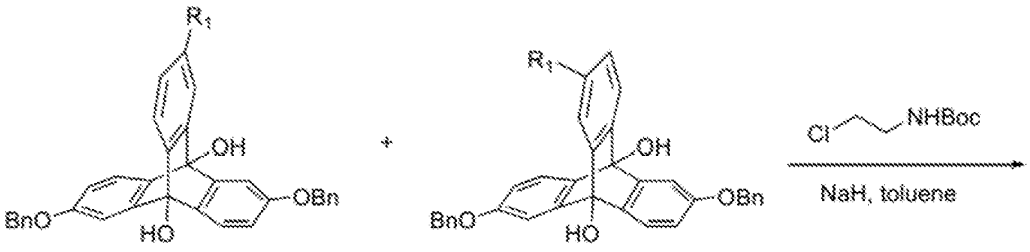
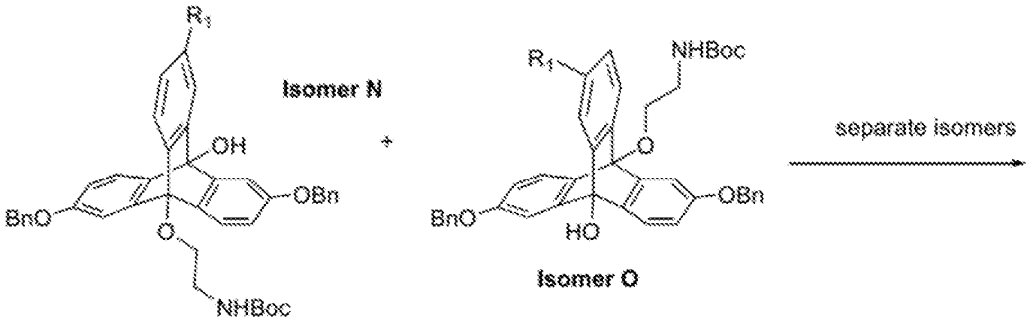
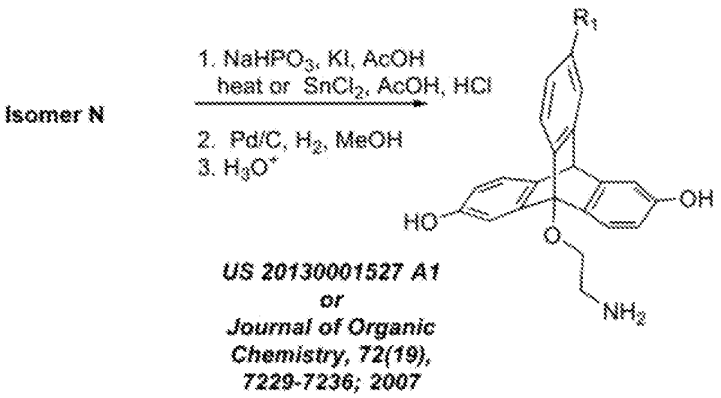


Figure 33



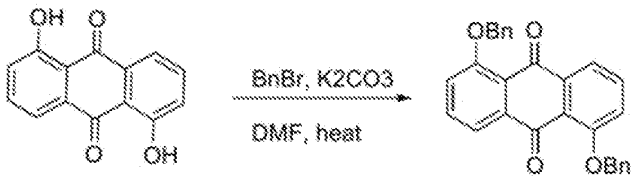
The following Sequence E can also be run on all generated isomers

Sequence F



US 20130001527 A1
or
Journal of Organic Chemistry, 72(19), 7229-7236; 2007

All of these reaction in this Scheme can also be performed starting from this alternative starting material:



alternative starting material

Figure 34

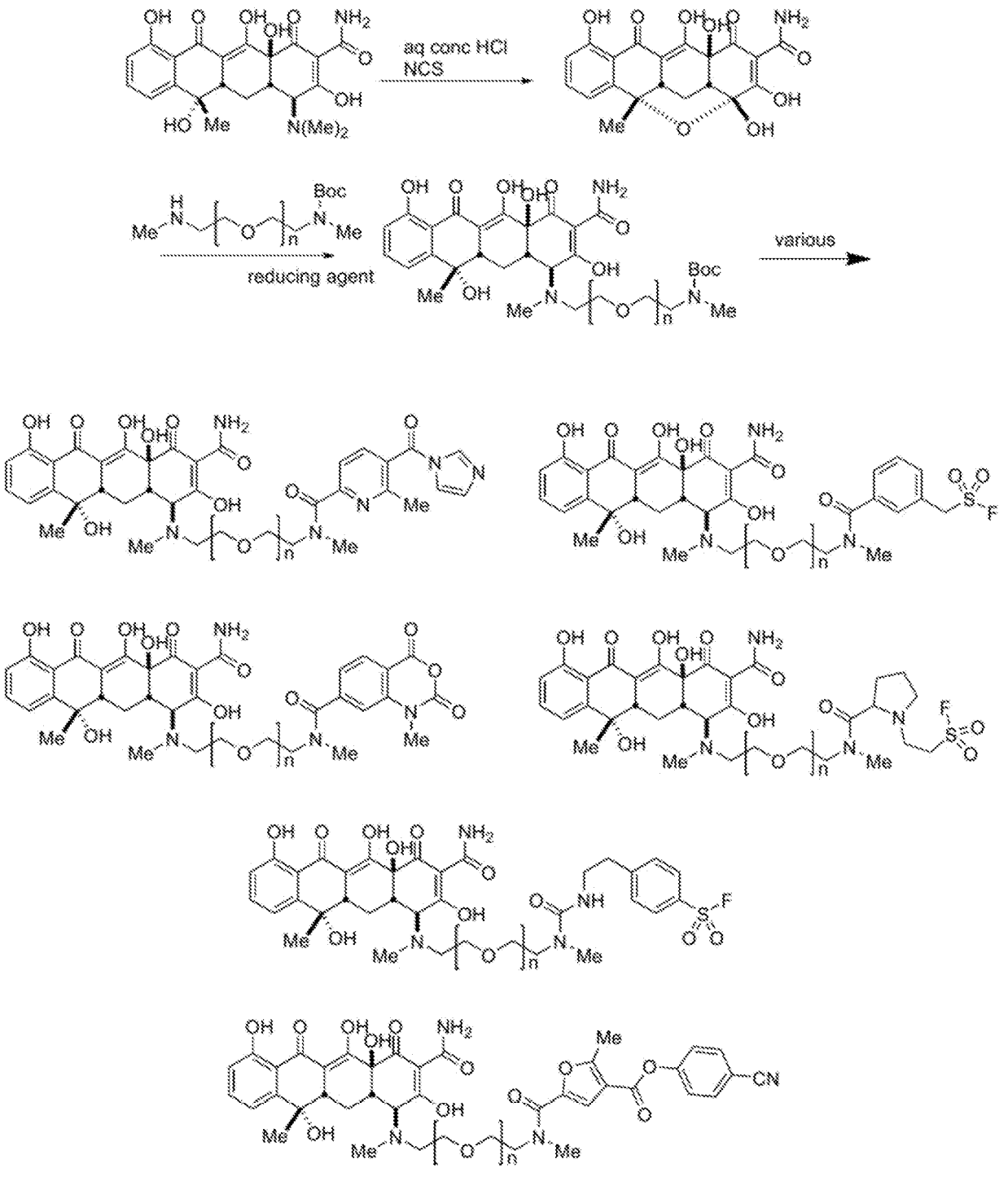


Figure 35

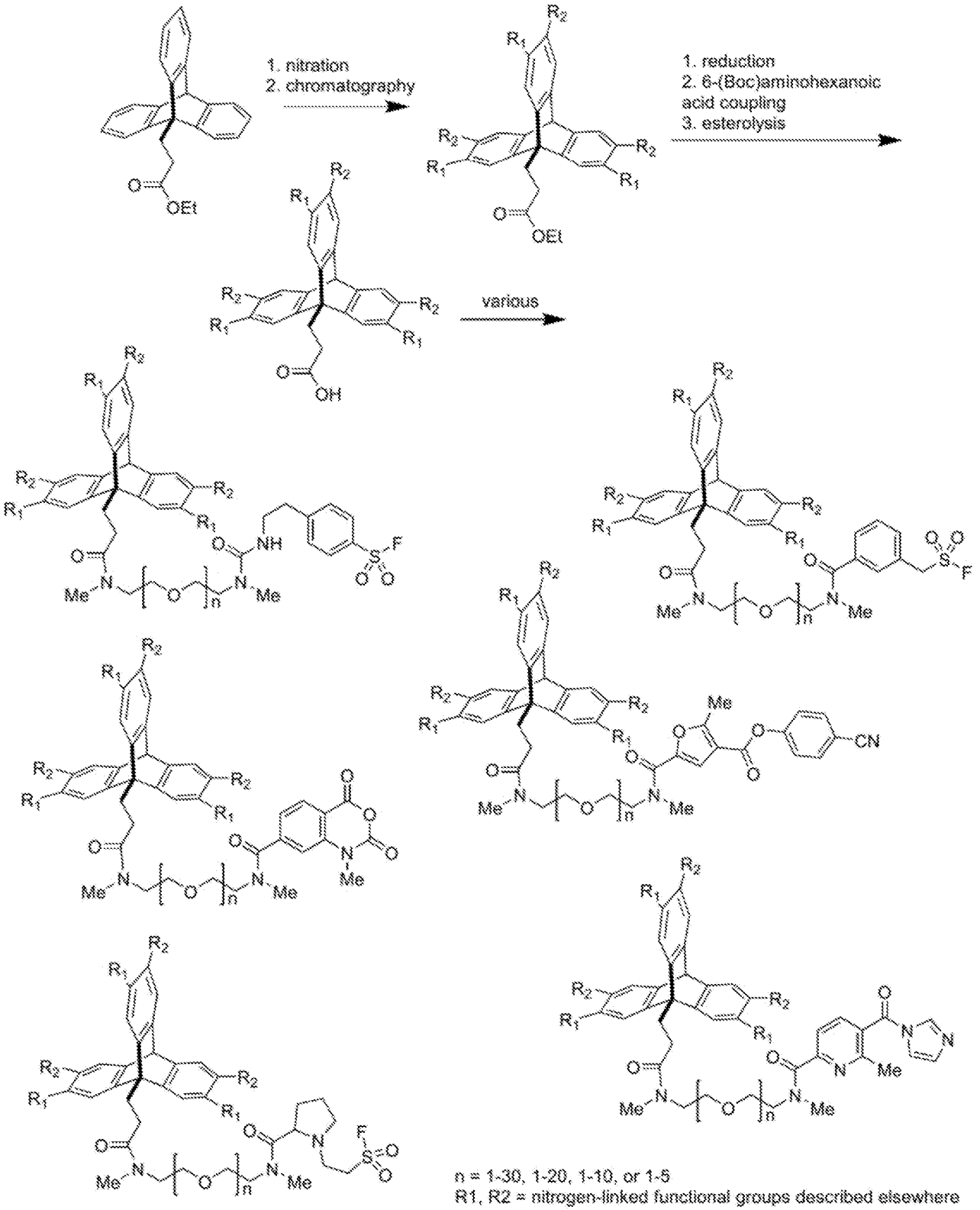

1. nitration
2. chromatography
1. reduction
2. 6-(Boc)aminohexanoic acid coupling
3. esterolysis
various
n = 1-30, 1-20, 1-10, or 1-5
R1, R2 = nitrogen-linked functional groups described elsewhere

Figure 36

ARK-132: Synthetic Route

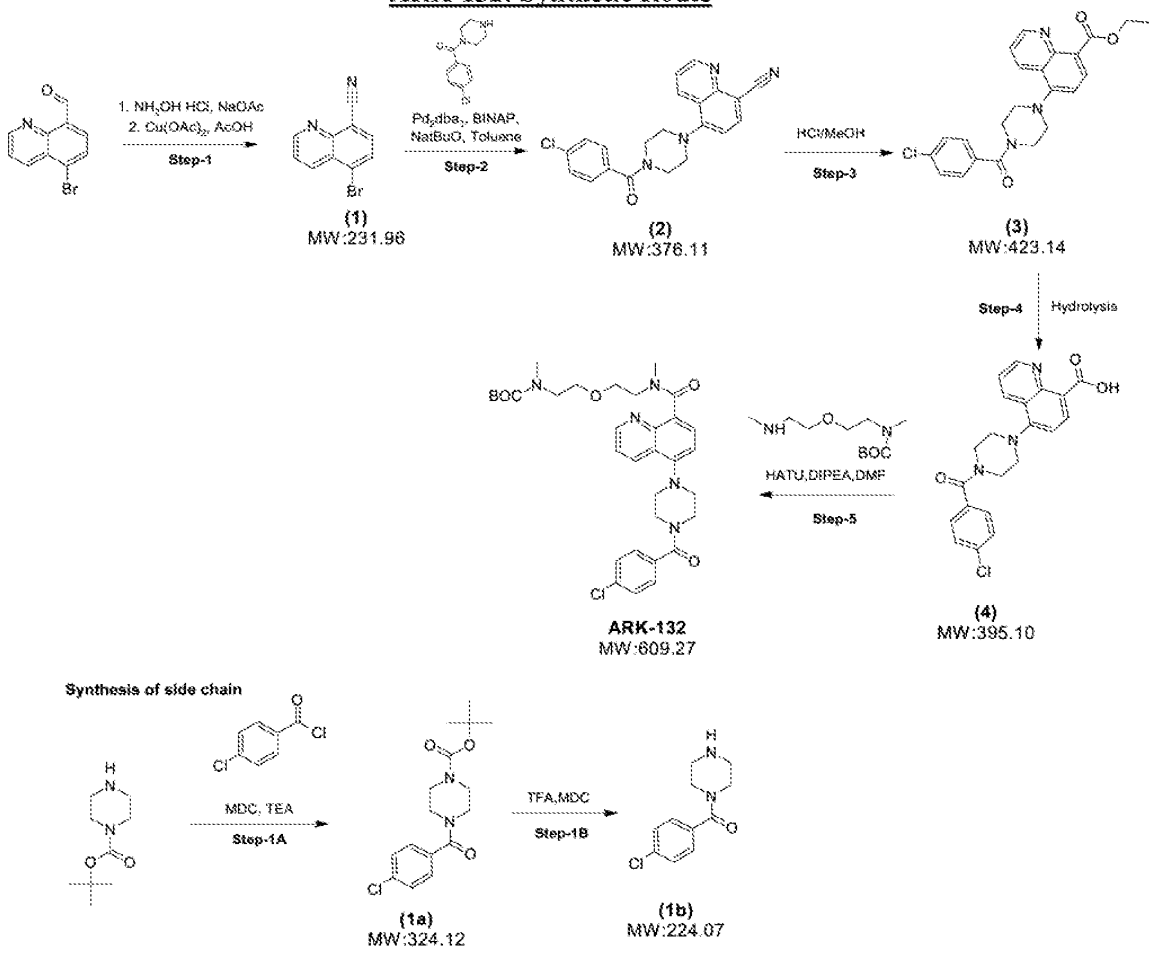


Figure 37

ARK-134 Synthetic Route

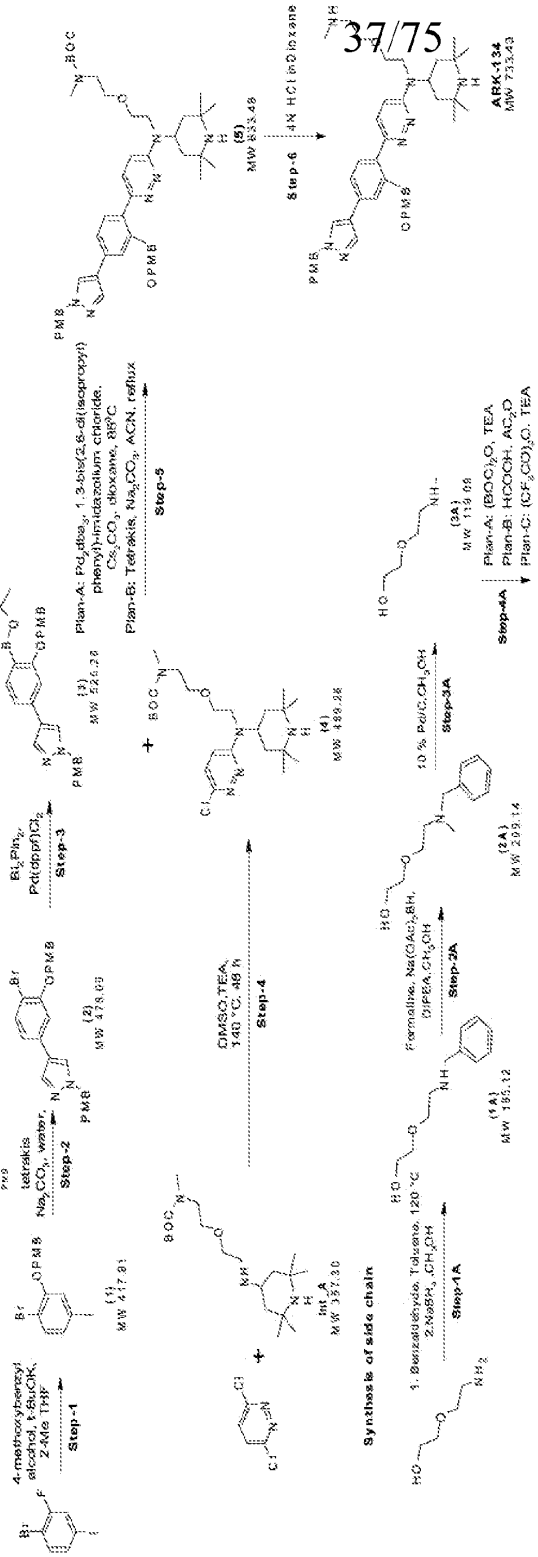


Figure 38

ARK-135 and 136 Synthetic Route

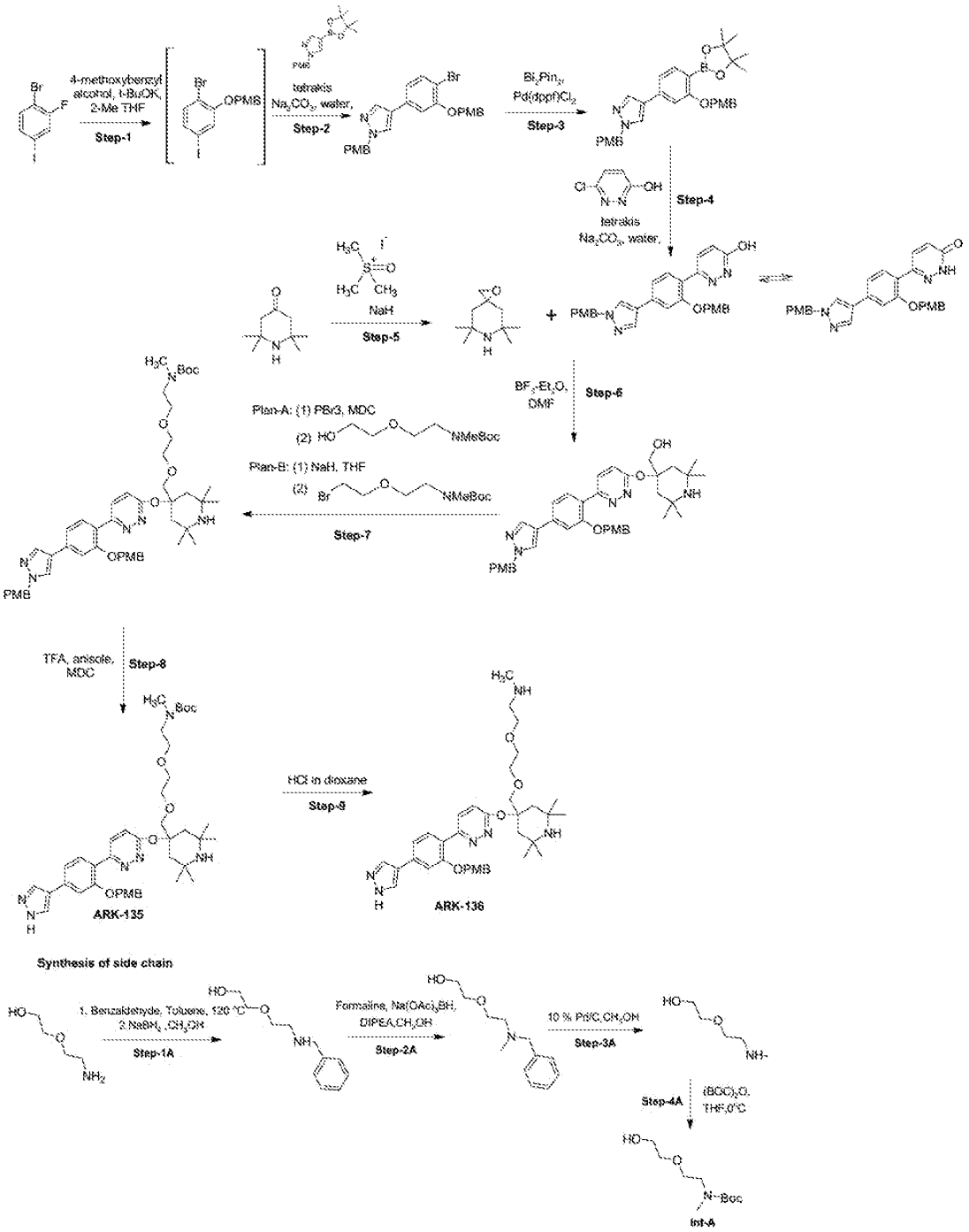


Figure 39

ARK-188 Synthetic Route

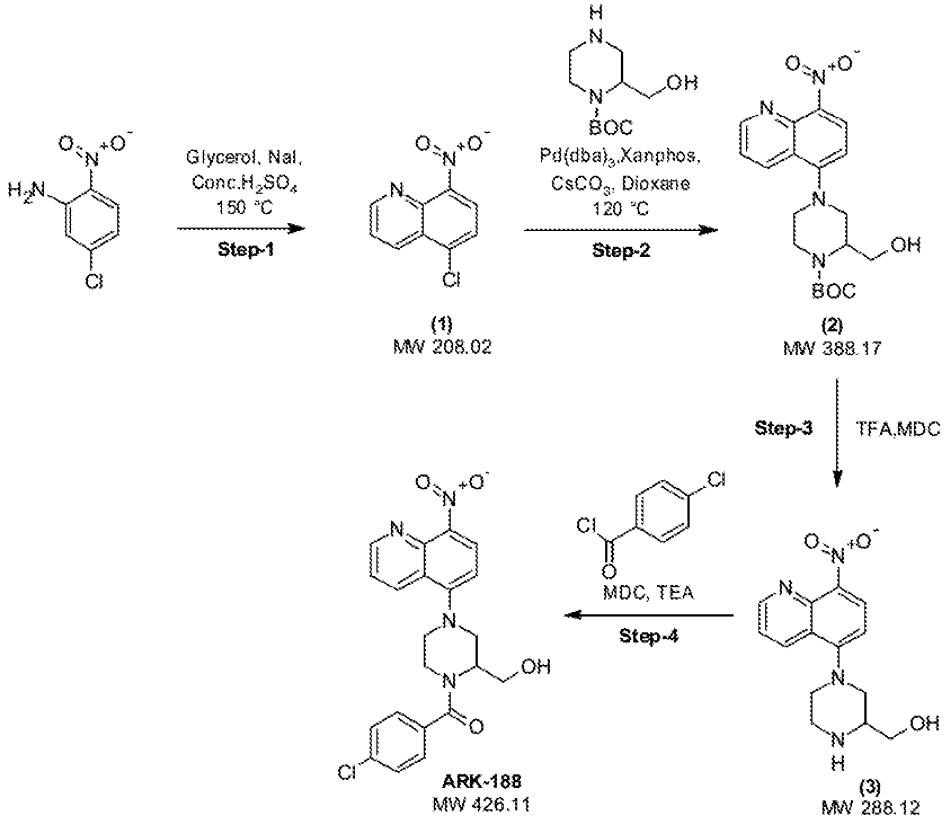


Figure 40

ARK-190 Synthetic Route

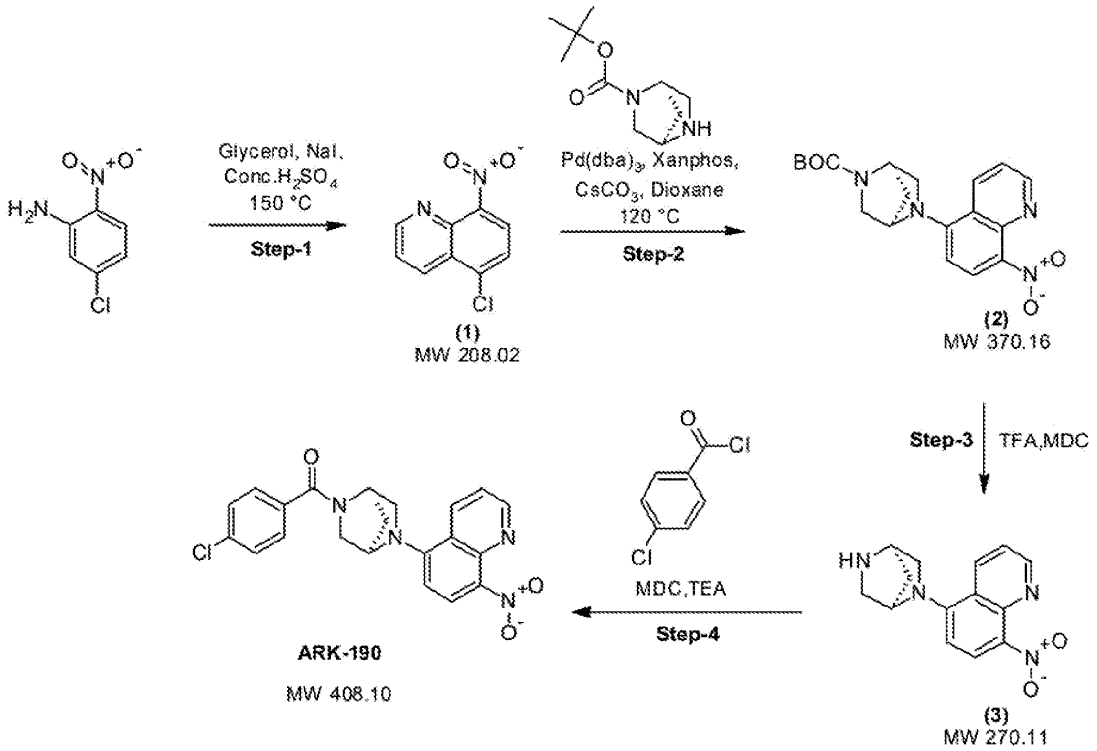


Figure 41

ARK-191 Synthetic Route

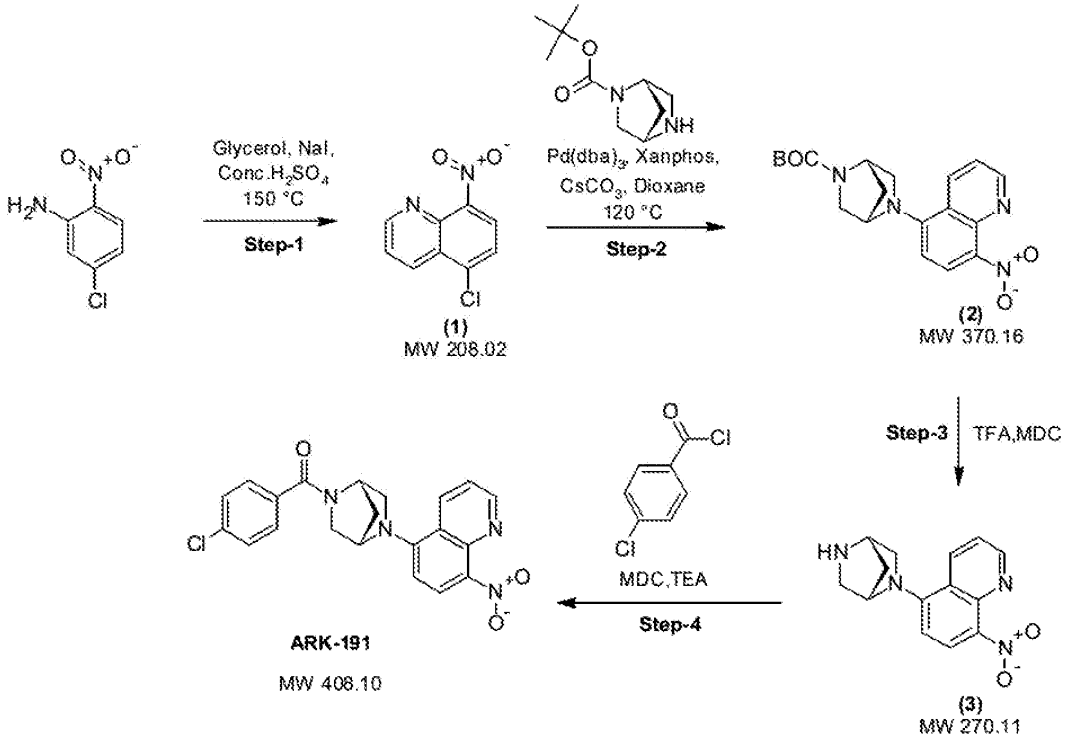


Figure 42

ARK-195 Synthetic Route

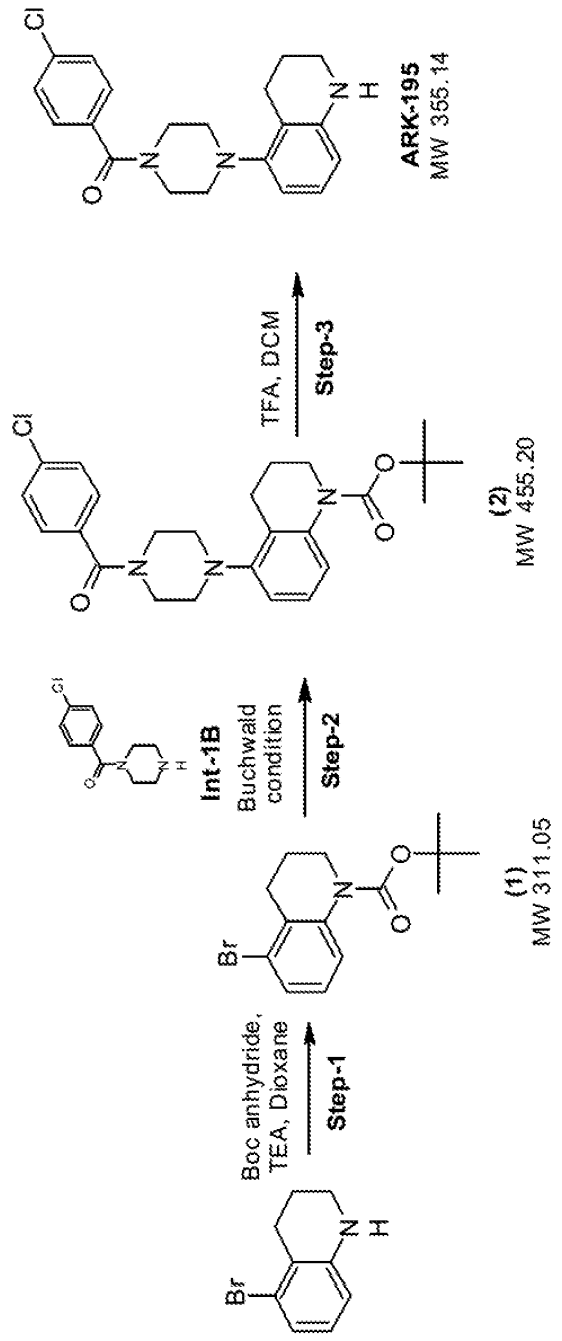


Figure 43

ARK-197 Synthetic Route

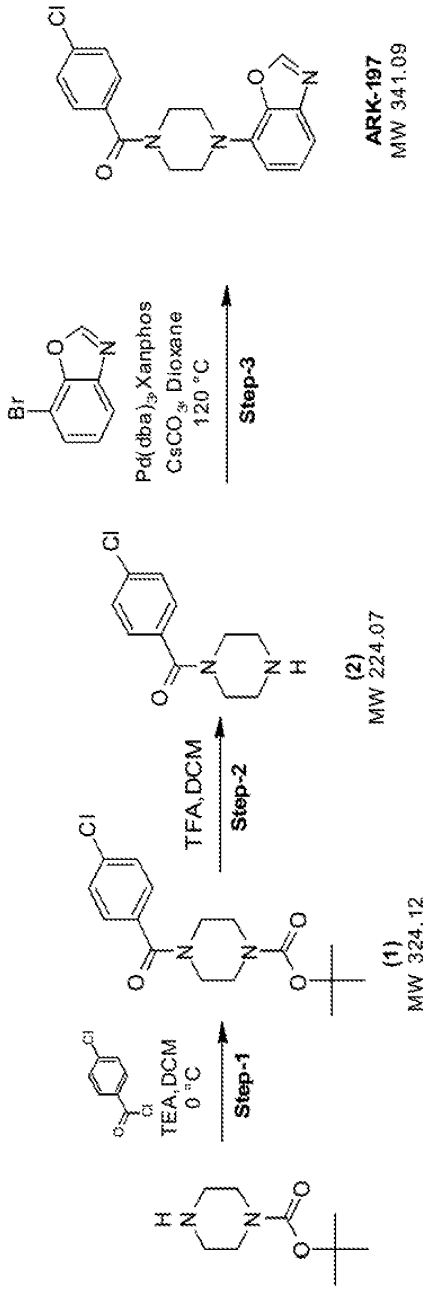


Figure 44

Synthetic Route for Ribocil-Based Ligands

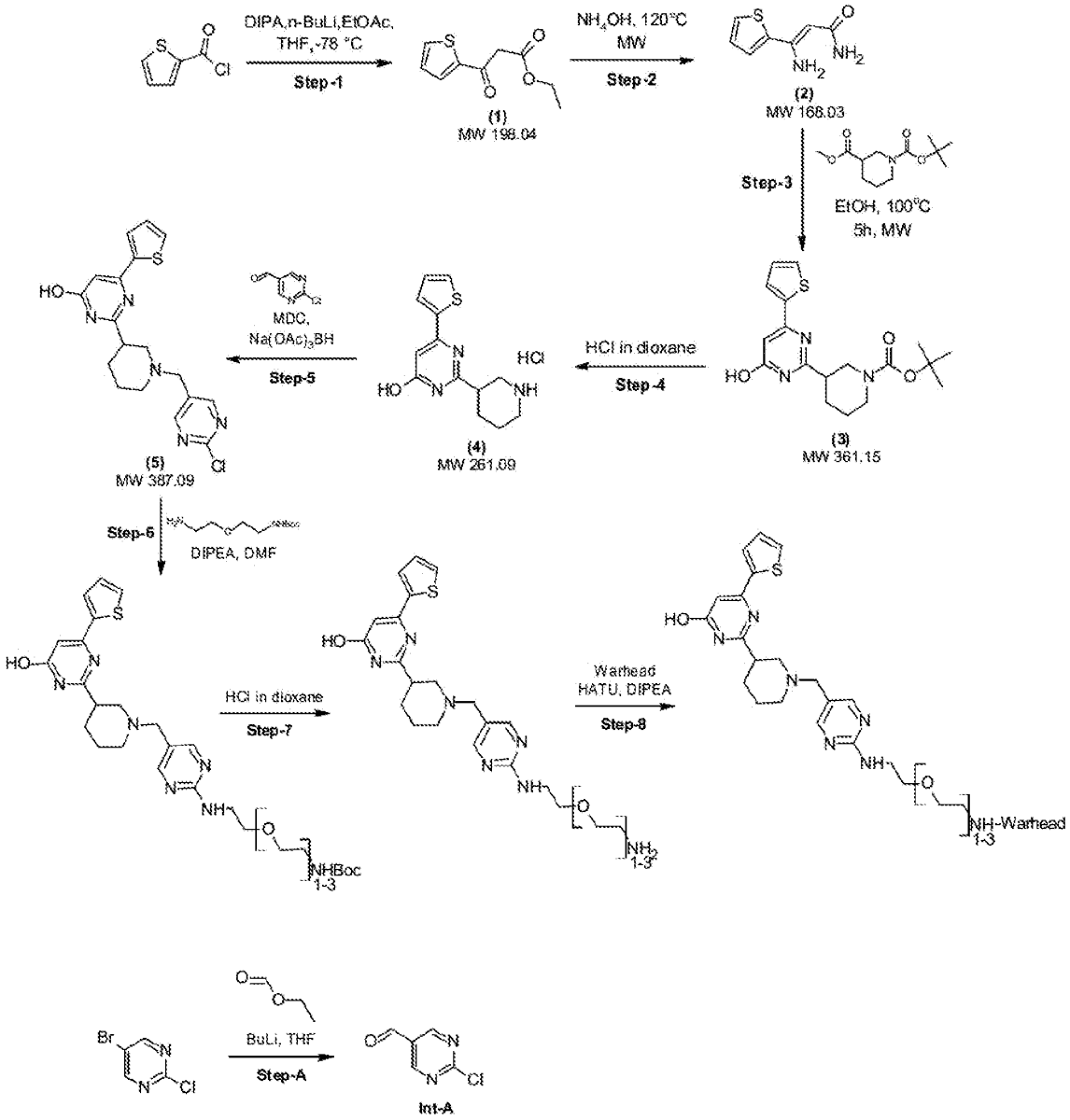


Figure 45

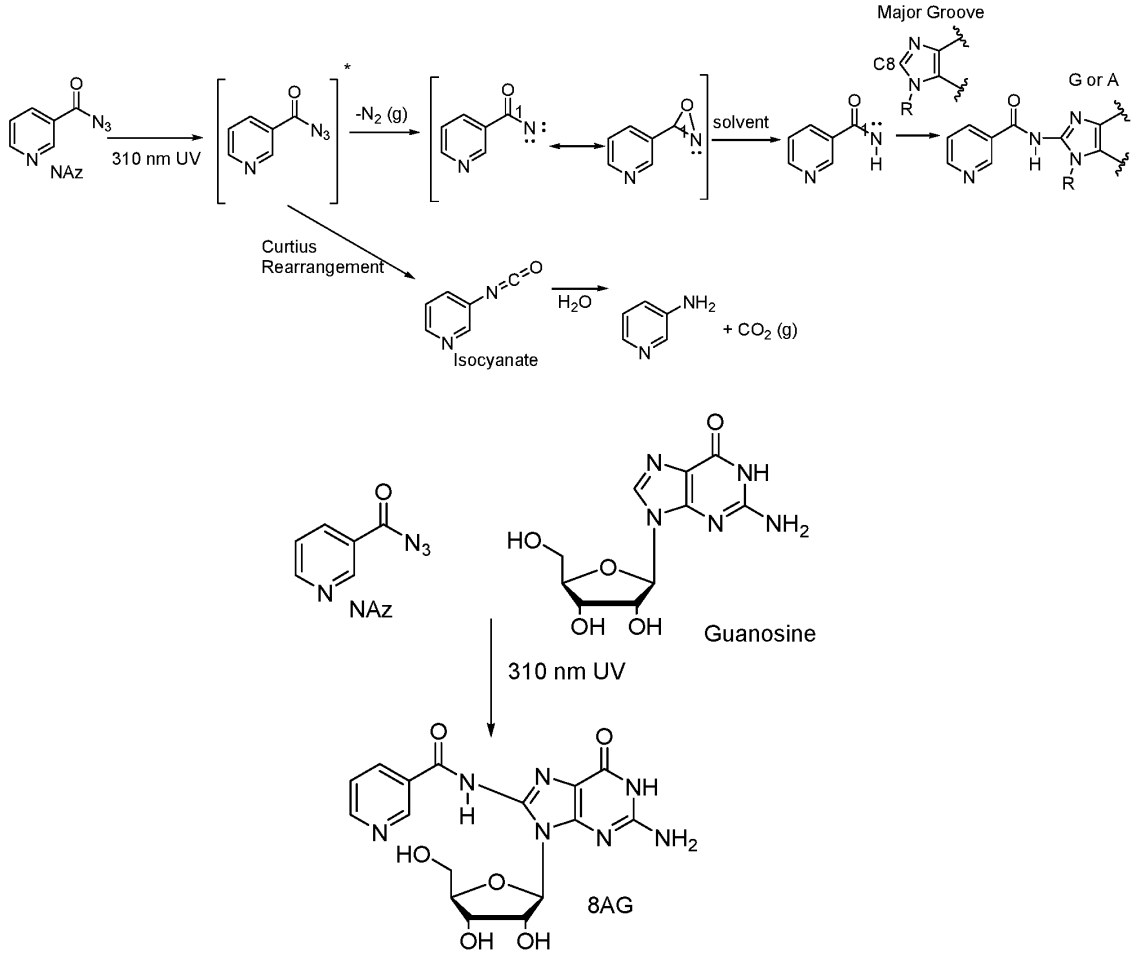
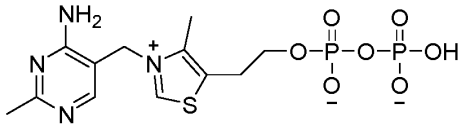

Major Groove
C8
G or A
NAz
310 nm UV
-N2 (g)
solvent
Curtius
Rearrangement
Isocyanate
H2O
+ CO2 (g)
NAz
Guanosine
310 nm UV
8AG

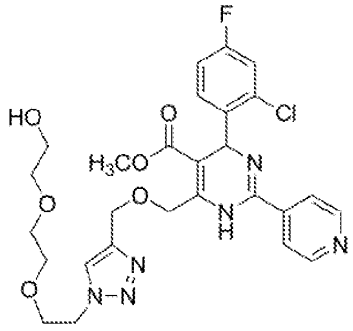
Figure 46



PreQ1 ligand **TPP ligand**



Aptamer 21 and Aptamer 21-E



Compound 1b
(Aptamer 21 ligand)

Figure 47

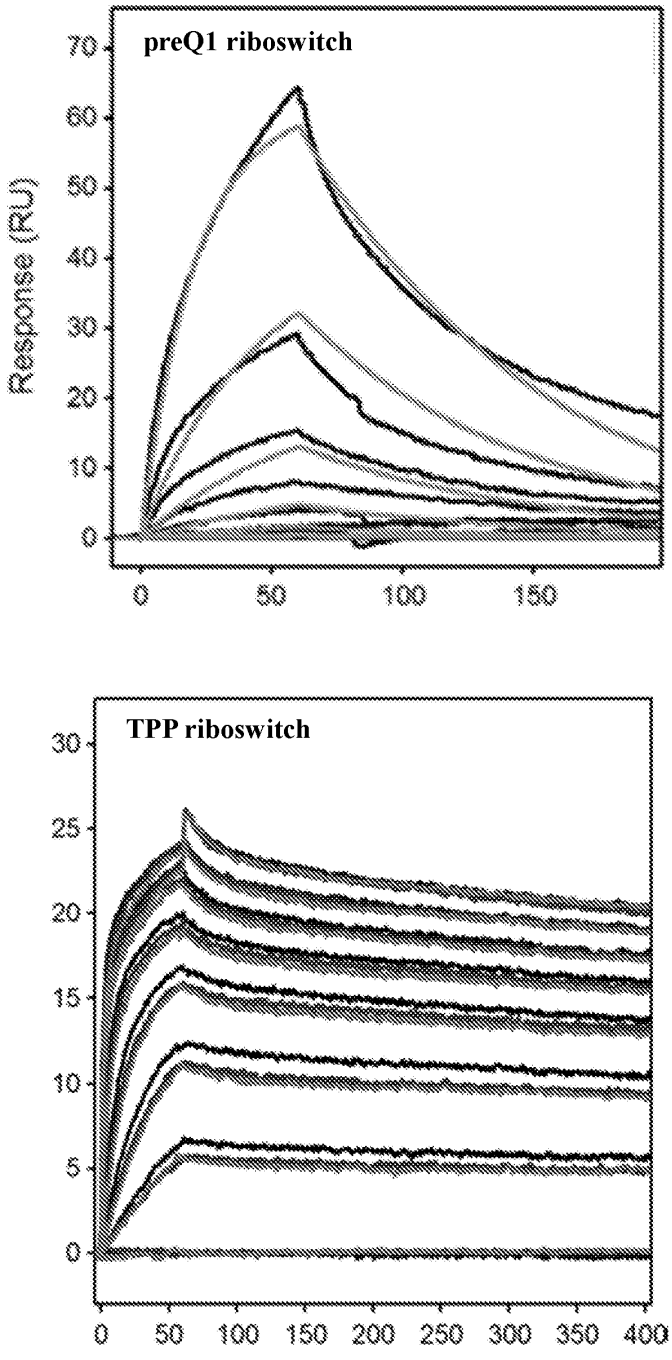

preQ1 riboswitch
Response (RU)
70
60
50
40
30
20
10
0
0
50
100
150
TPP riboswitch
30
25
20
15
10
5
0
0
50
100
150
200
250
300
350
400

Figure 48

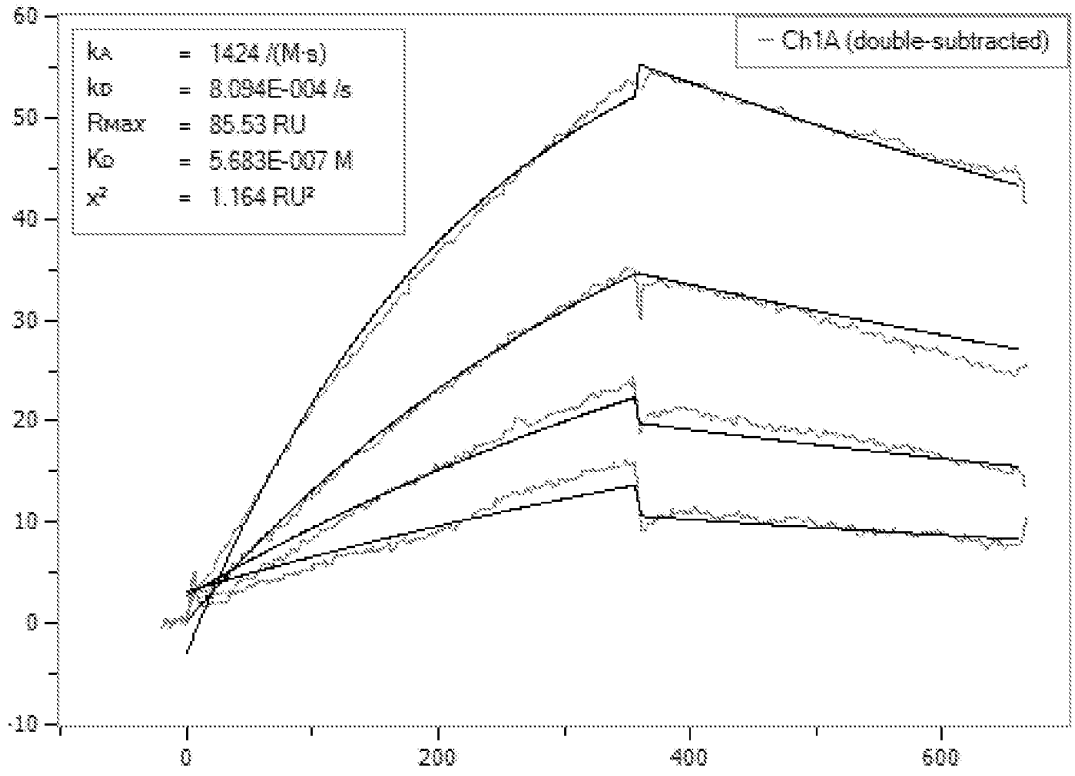

kA = 14.24 /(M·s)
kD = 8.094E-004 /s
Rmax = 85.53 RU
KD = 5.683E-007 M
x² = 1.164 RU²
Ch1A (double-subtracted)
60
50
40
30
20
10
0
-10
0
200
400
600

Figure 49

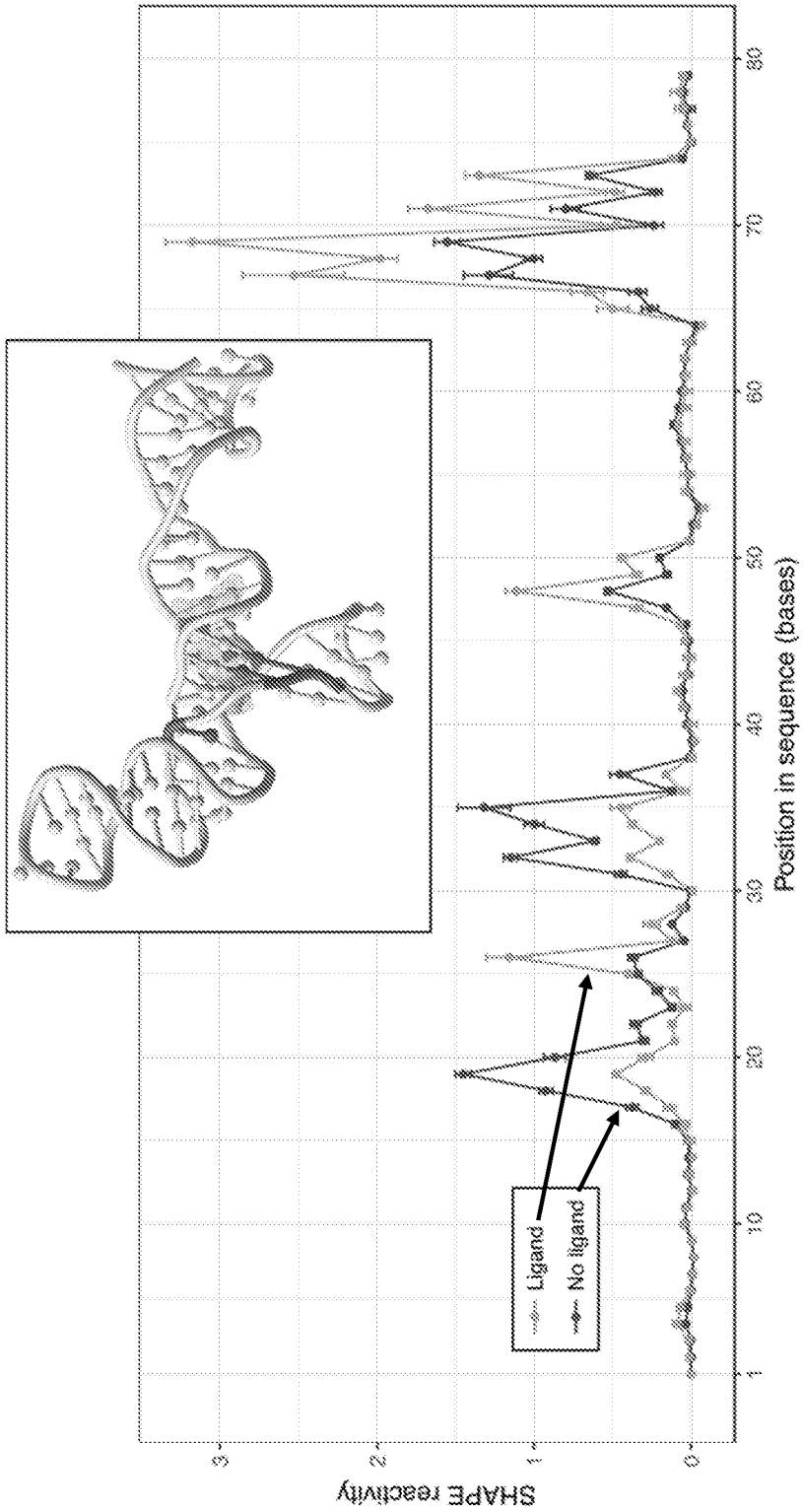
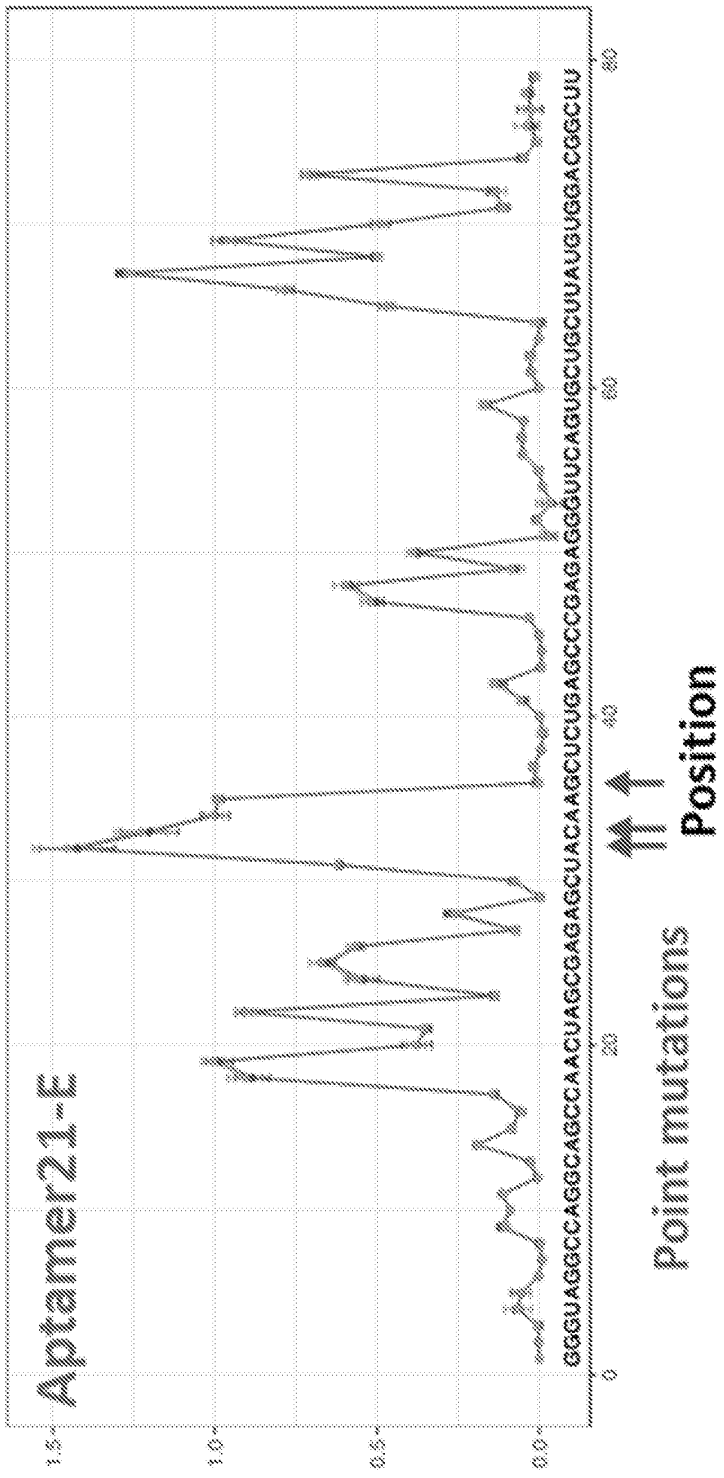

SHAPE reactivity
0
1
2
3
1
10
20
30
40
50
60
70
80
Position in sequence (bases)
Ligand
No ligand

Figure 50


Aptamer21-E
GGGUAGGCCAGGCAGCCAACUAGCGAGAGCUACAAGCUCUGAGCCCGAGAGGGUUCAGUGCUGCUUUAUGUGGACGGCUU
0
20
40
60
80
0.0
0.5
1.0
1.5
Point mutations
Position

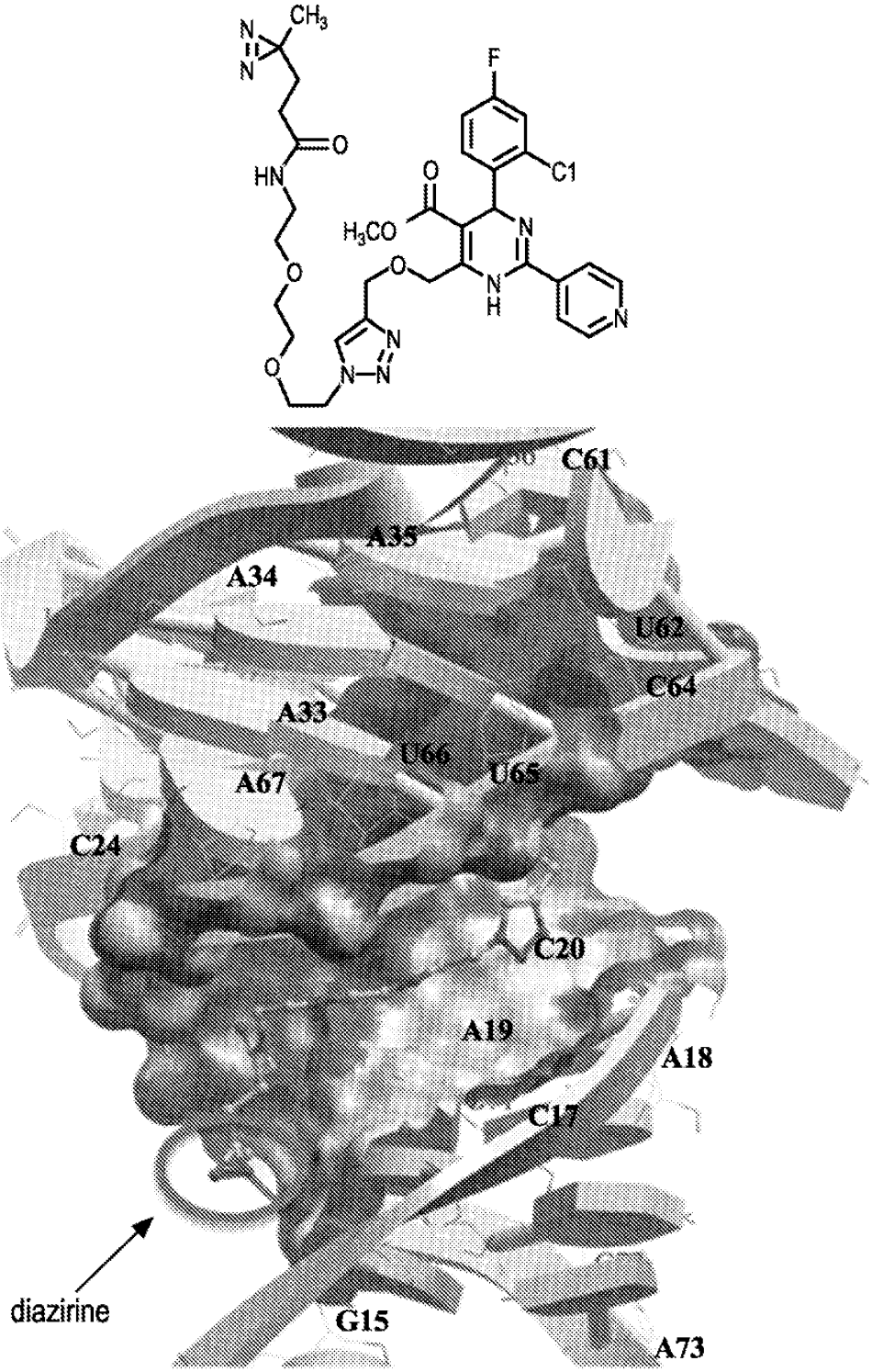

CH3
N
N
O
HN
O
O
O
N
N
N
F
Cl
O
H3CO
O
N
H
N
C61
A35
A34
U62
C64
A33
U66
U65
A67
C24
C20
A19
A18
C17
G15
A73
diazirine

Fig. 51

Figure 52

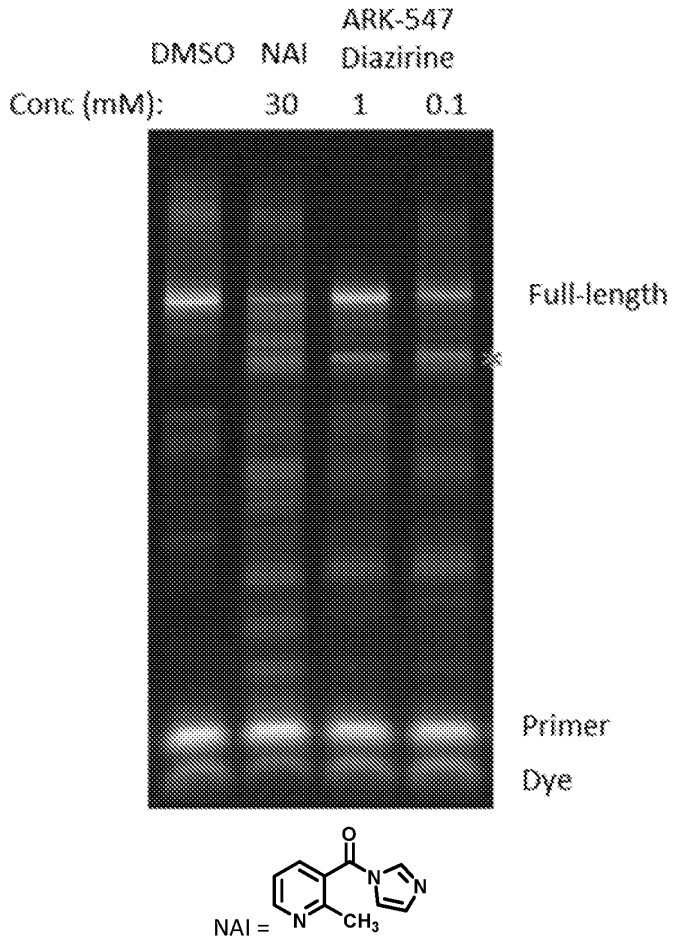
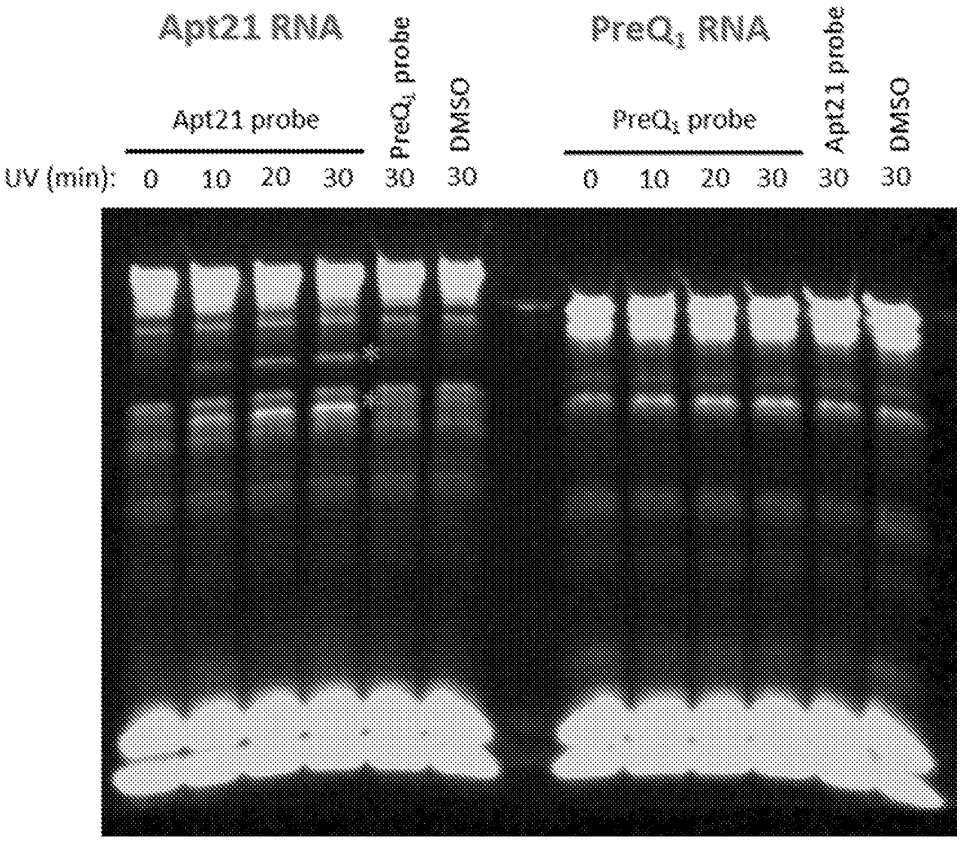
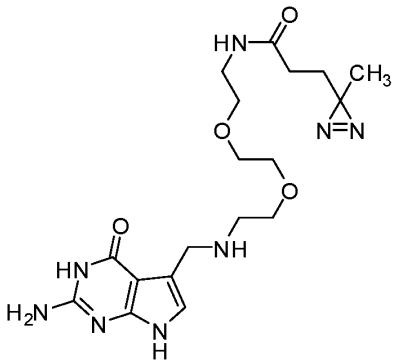

DMSO
NAI
ARK-547
Diazirine
Conc (mM):
30
1
0.1
Full-length
Primer
Dye
NAI =
O
N
N
N
CH3
Functionalized ligand
Cross-link
Reverse
Transcribe
RNA Receptor

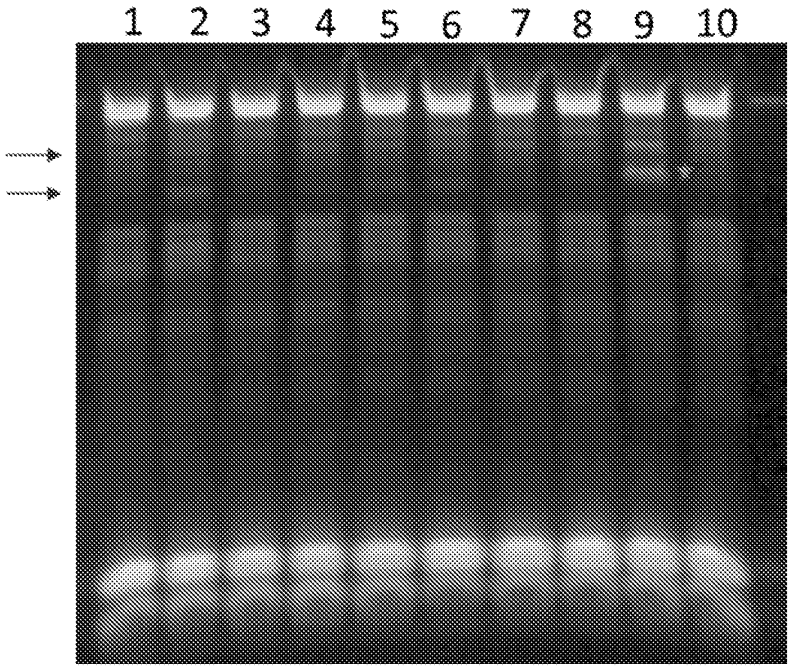
Figure 53





Structure of $PreQ_1$ RNA photoprobe (**I-23**)

Figure 54



Lane	Compound	Warhead
1	ARK-139 (Cpd 1b)	None
2	ARK-547	Diazirine
3	**ARK-547 + 100 μM ARK-139**	
4	ARK-546	Bio-diazirine
5	ARK-579	Bio-diazirine
6	ARK-580	Bio-diazirine
7	ARK-548	CF_3-diazirine
8	ARK-544	Benzophenone
9	ARK-581	Phenyl-N_3
10	ATN-5-36 ($PreQ_1$; compound **I-23**)	Diazirine

Figure 55

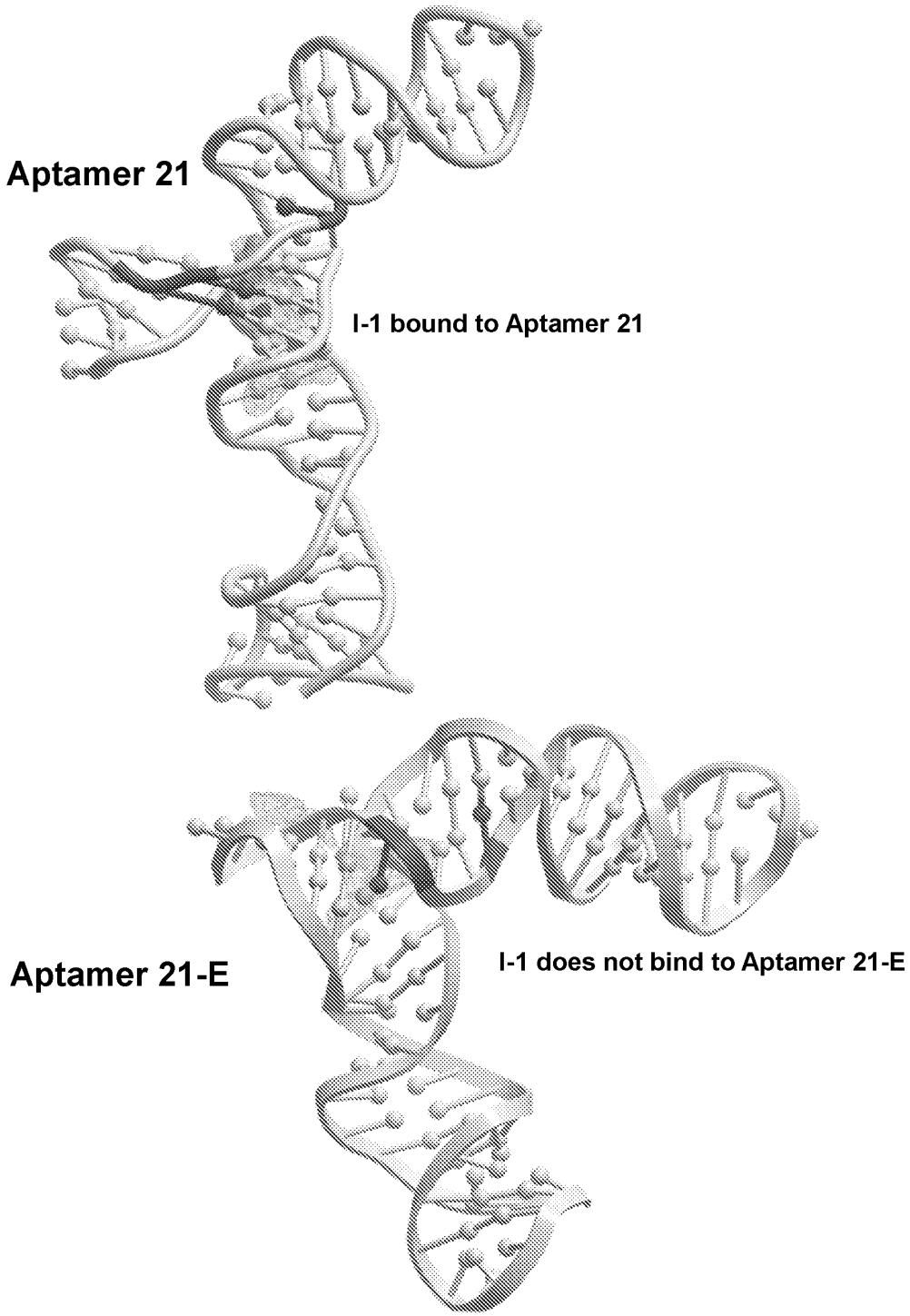

Aptamer 21
I-1 bound to Aptamer 21
Aptamer 21-E
I-1 does not bind to Aptamer 21-E

Figure 56

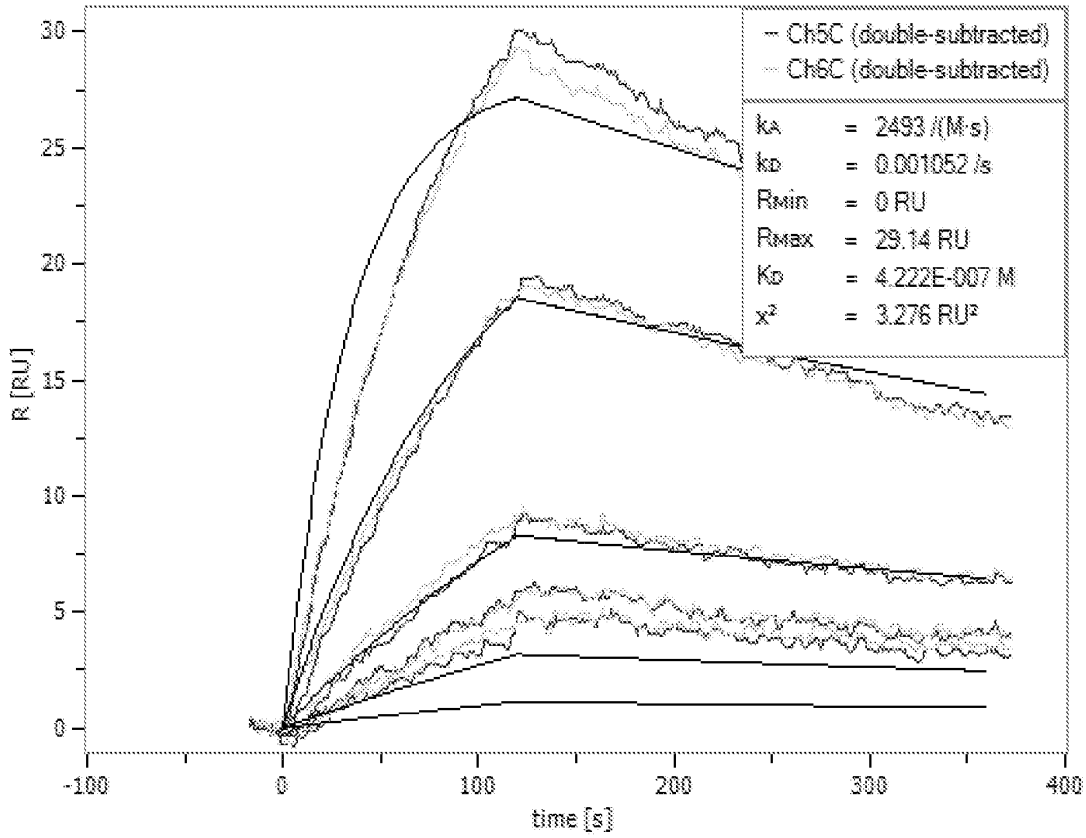

Ch5C (double-subtracted)
Ch6C (double-subtracted)
kA = 2493 /(M·s)
kD = 0.001052 /s
Rmin = 0 RU
Rmax = 29.14 RU
KD = 4.222E-007 M
x² = 3.276 RU²
R [RU]
time [s]
30
25
20
15
10
5
0
-100
0
100
200
300
400

Figure 57

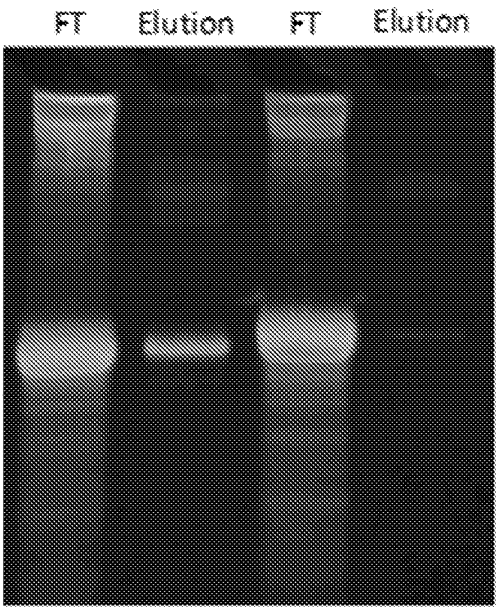
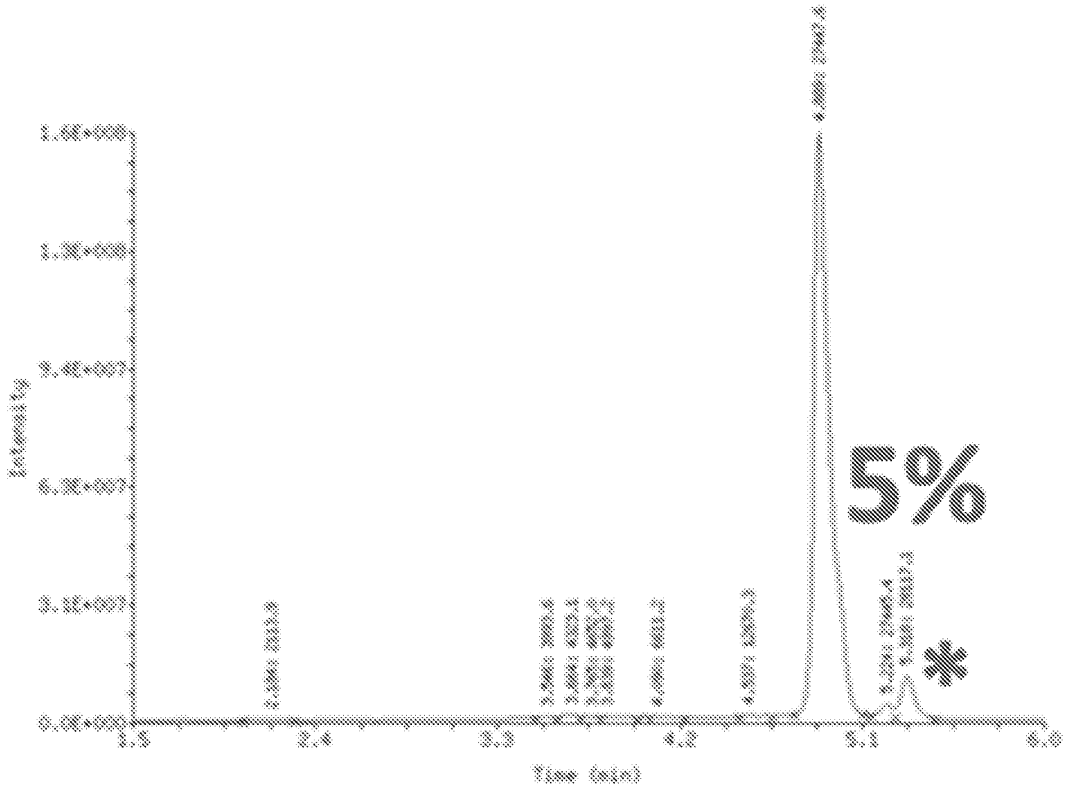
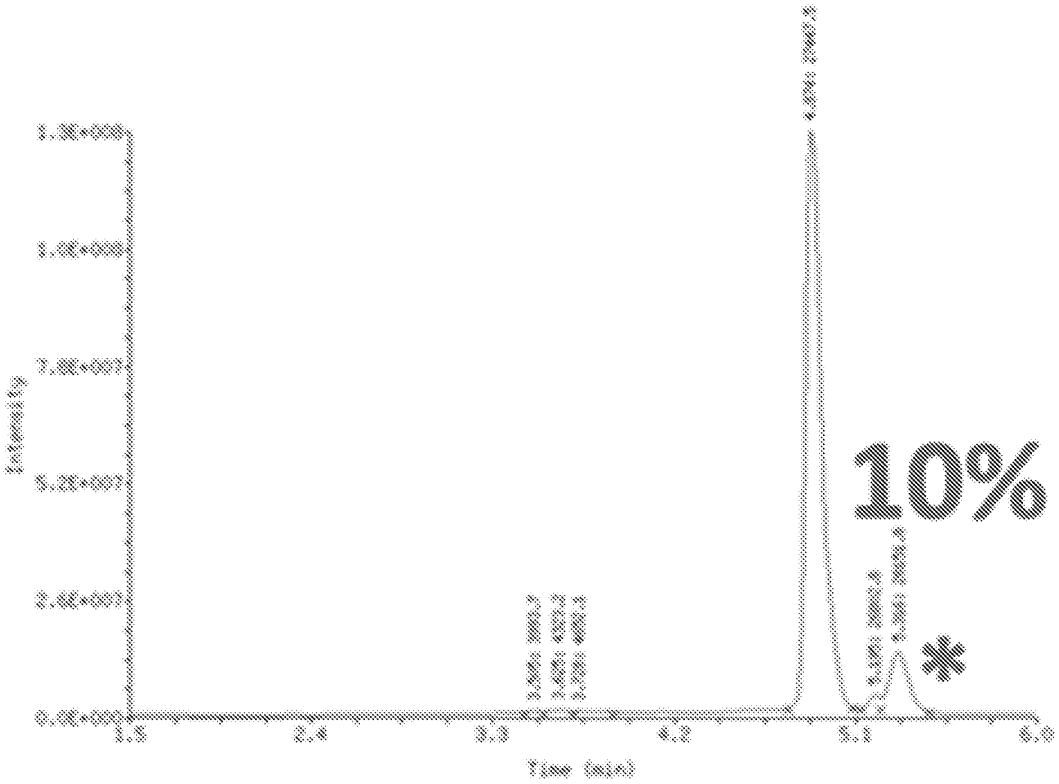


Figure 58A



ARK-547 (diazirine)

Figure 58B
LC-MS Results with Aptamer 21



ARK-581 (phenyl azide)

Figure 59

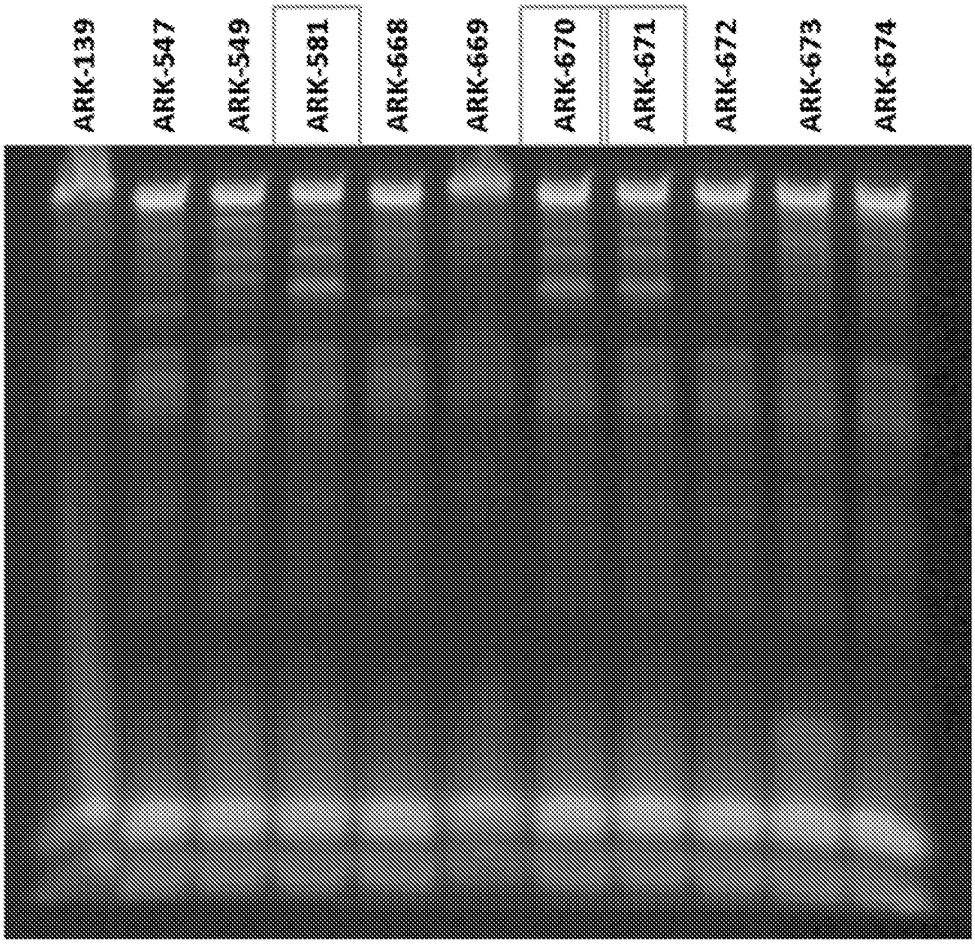
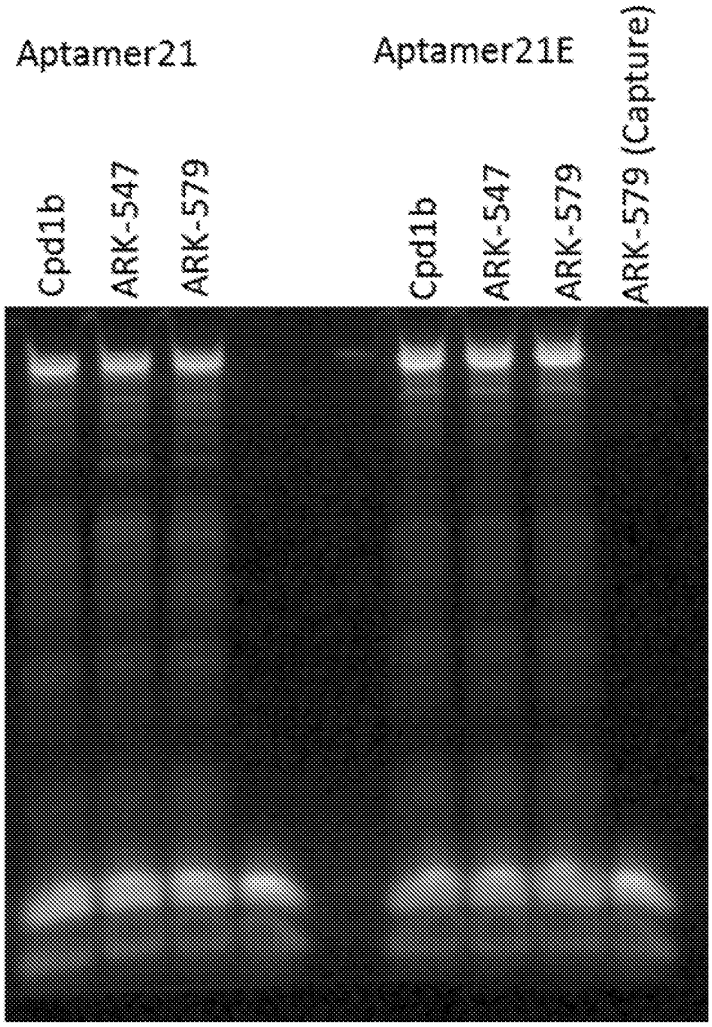

ARK-139
ARK-547
ARK-549
ARK-581
ARK-668
ARK-669
ARK-670
ARK-671
ARK-672
ARK-673
ARK-674

Figure 60


Aptamer21
Aptamer21E
Cpd1b
ARK-547
ARK-579
Cpd1b
ARK-547
ARK-579
ARK-579 (Capture)

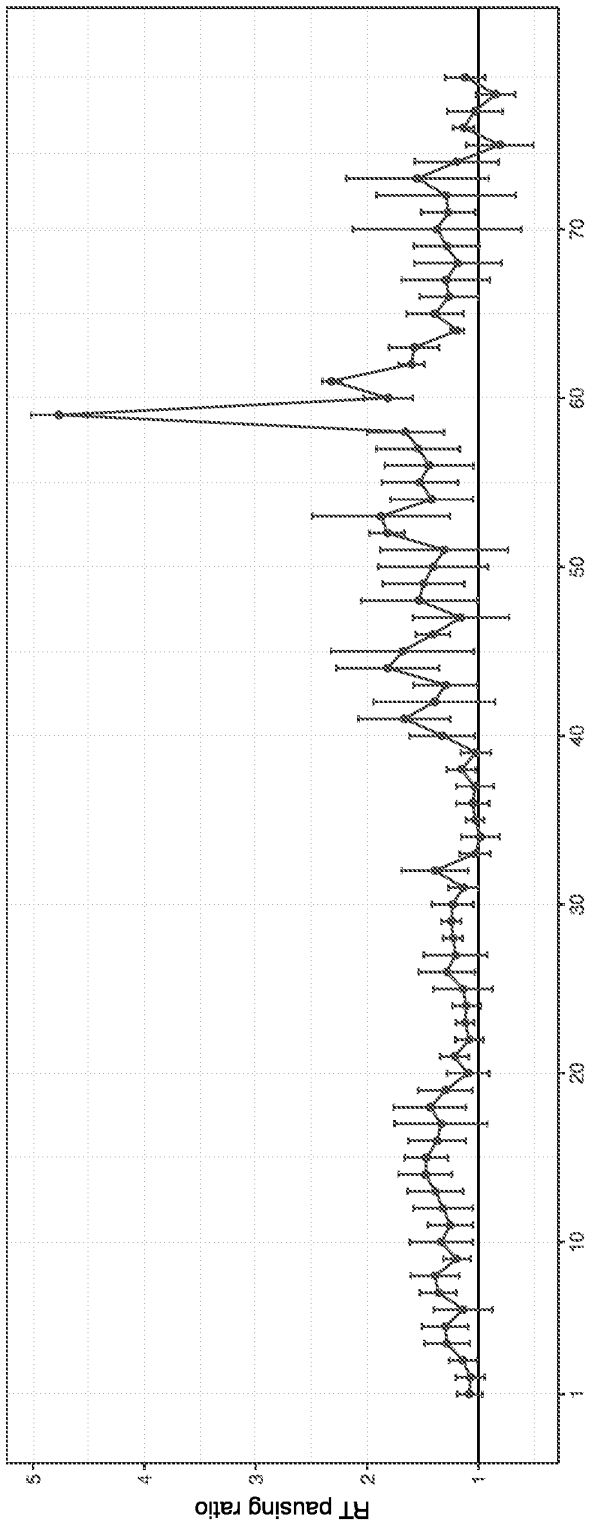


Figure 61A

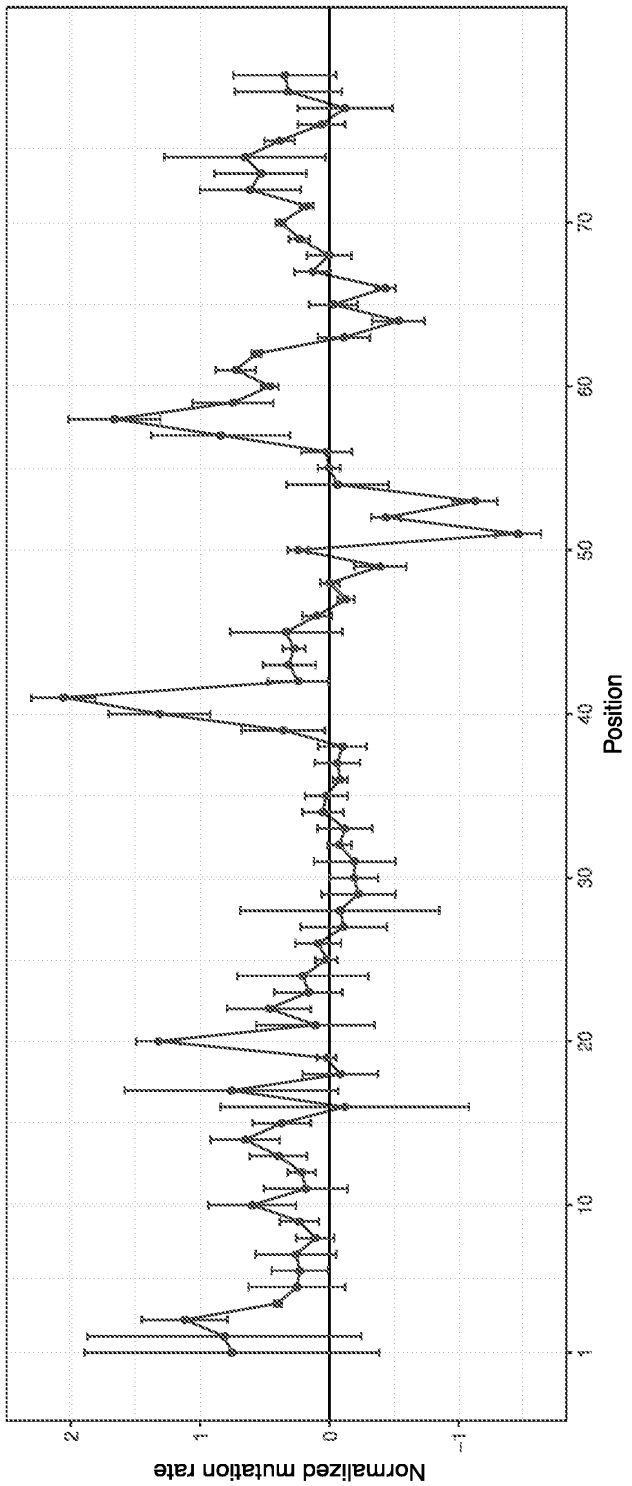


Figure 61B

Figure 62

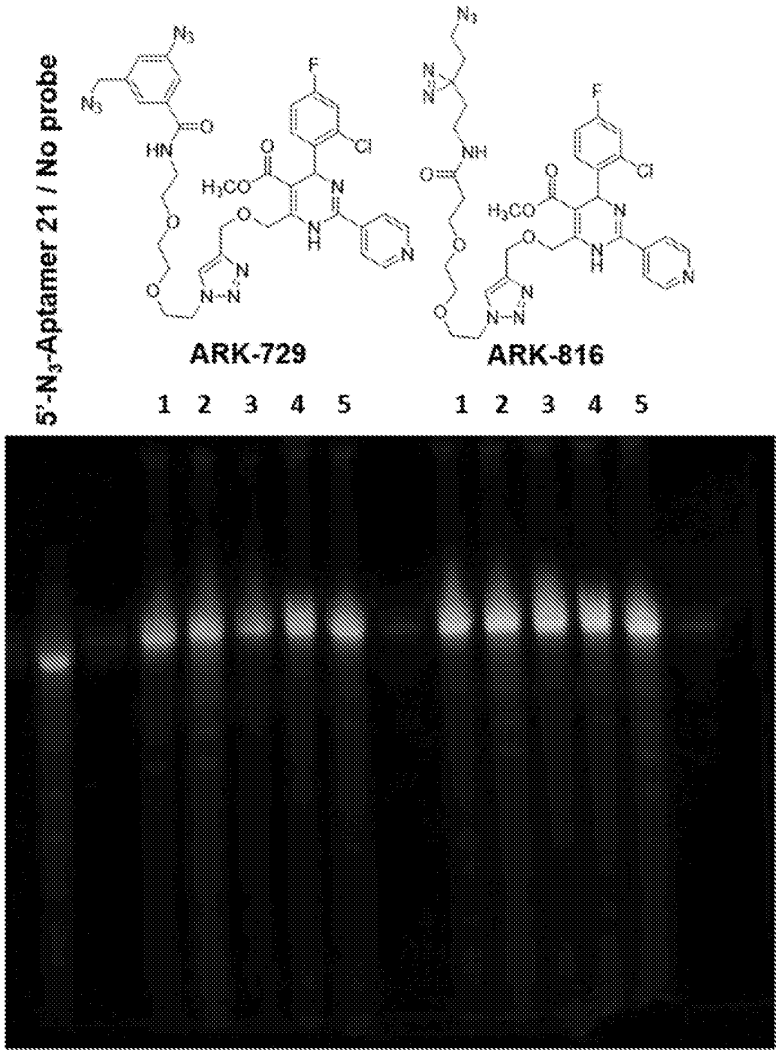
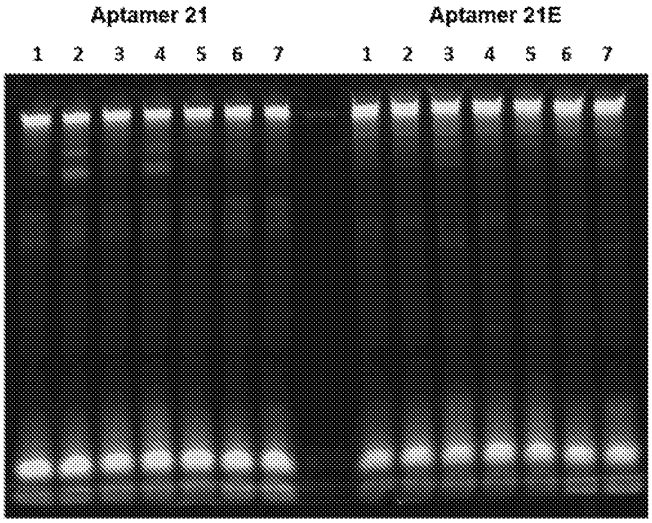
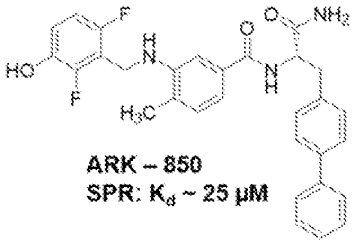


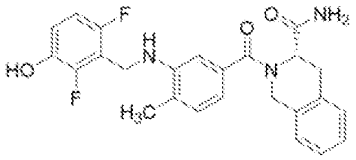
Figure 63



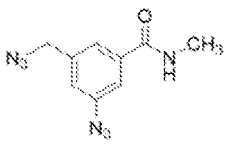
#	Probe(s)
1	ARK – 139
2	ARK – 581
3	ARK – 581 + 10x ARK – 139
4	ARK – 581 + 10x ARK – 850
5	ARK – 581 + 10x ARK – 852
6	ARK – 2058
7	ARK – 2059



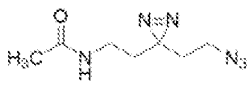
ARK – 850
SPR: K_d ~ 25 µM



ARK – 852
SPR: K_d ~ 600 nM

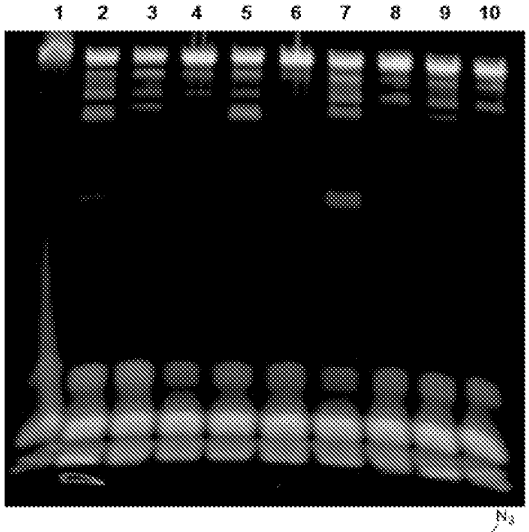


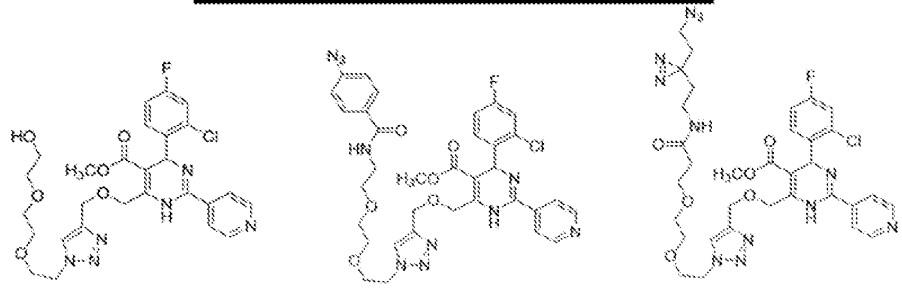
ARK – 2058



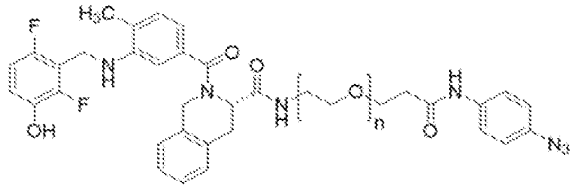
ARK – 2059

Figure 64
RT Pausing

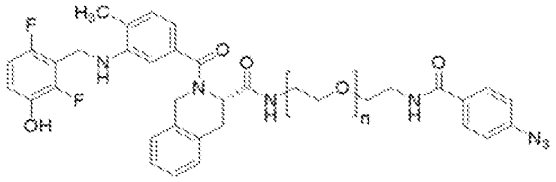




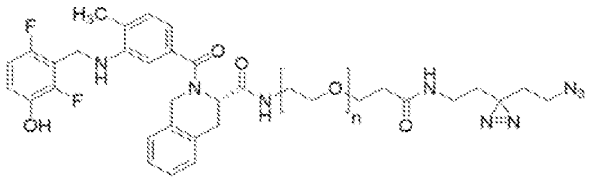
ARK-139 **(1)** ARK-581 **(2)** ARK-816 **(7)**



n = 1 **(3)**
n = 2 **(4)**
n = 4 **(6)**

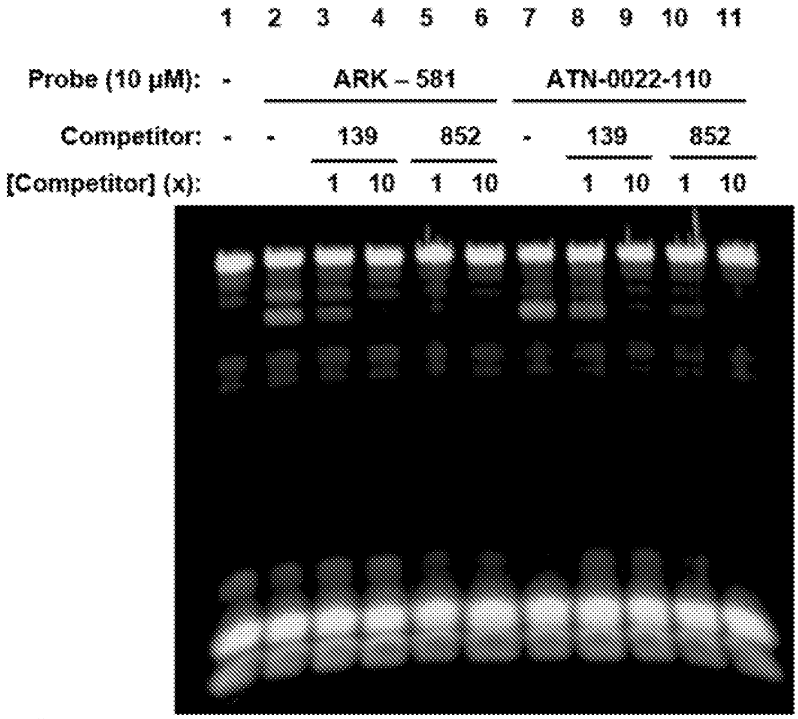


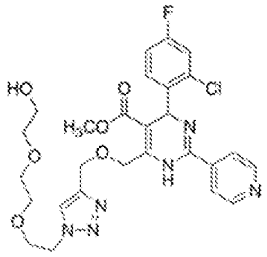
n = 2 **(5)**

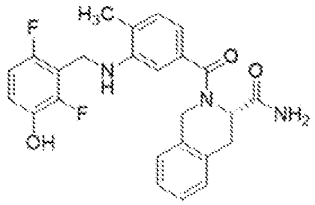


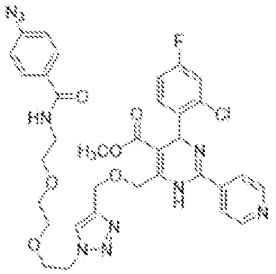
n = 1 **(8)**
n = 2 **(9)**
n = 4 **(10)**

Figure 65
RT Pausing – Competition Assay




ARK-139


ARK-852


ARK-581

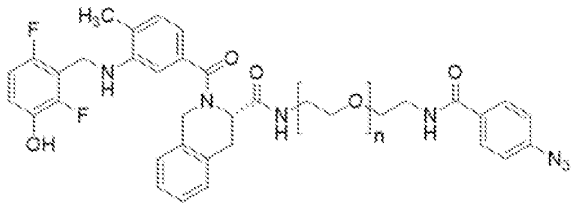
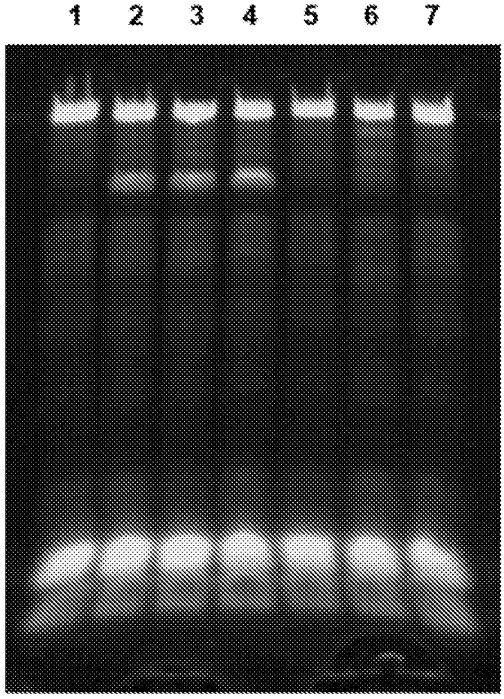
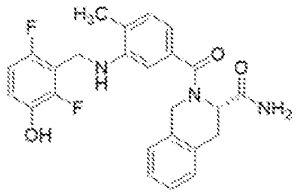
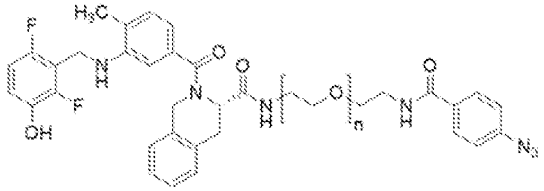

ATN-0022-110 (n = 2)

Figure 66

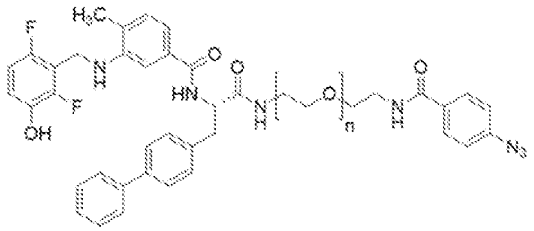




ARK-852 (**1**)



n = 1 (**2**)
n = 2 (**3**)
n = 4 (**4**)



n = 1 (**5**)
n = 2 (**6**)
n = 4 (**7**)

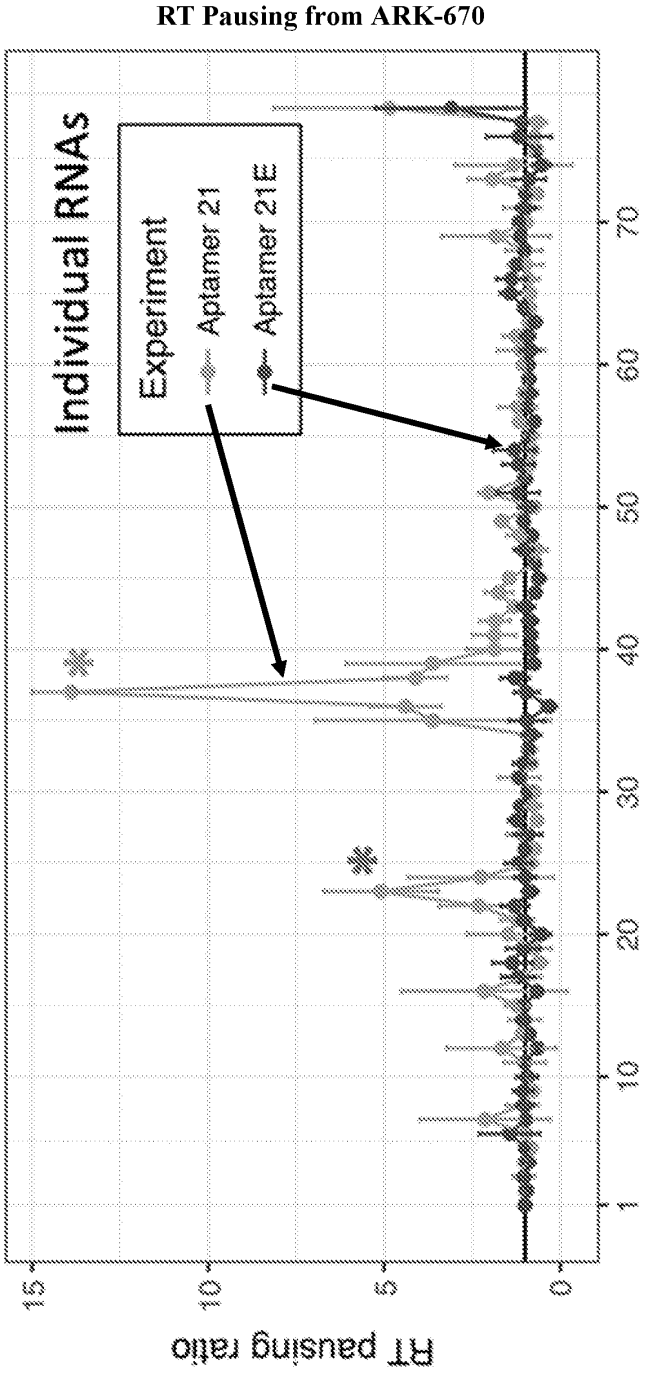

RT Pausing from ARK-670
Individual RNAs
Experiment
Aptamer 21
Aptamer 21E
RT pausing ratio
0
5
10
15
1
10
20
30
40
50
60
70

Figure 67A

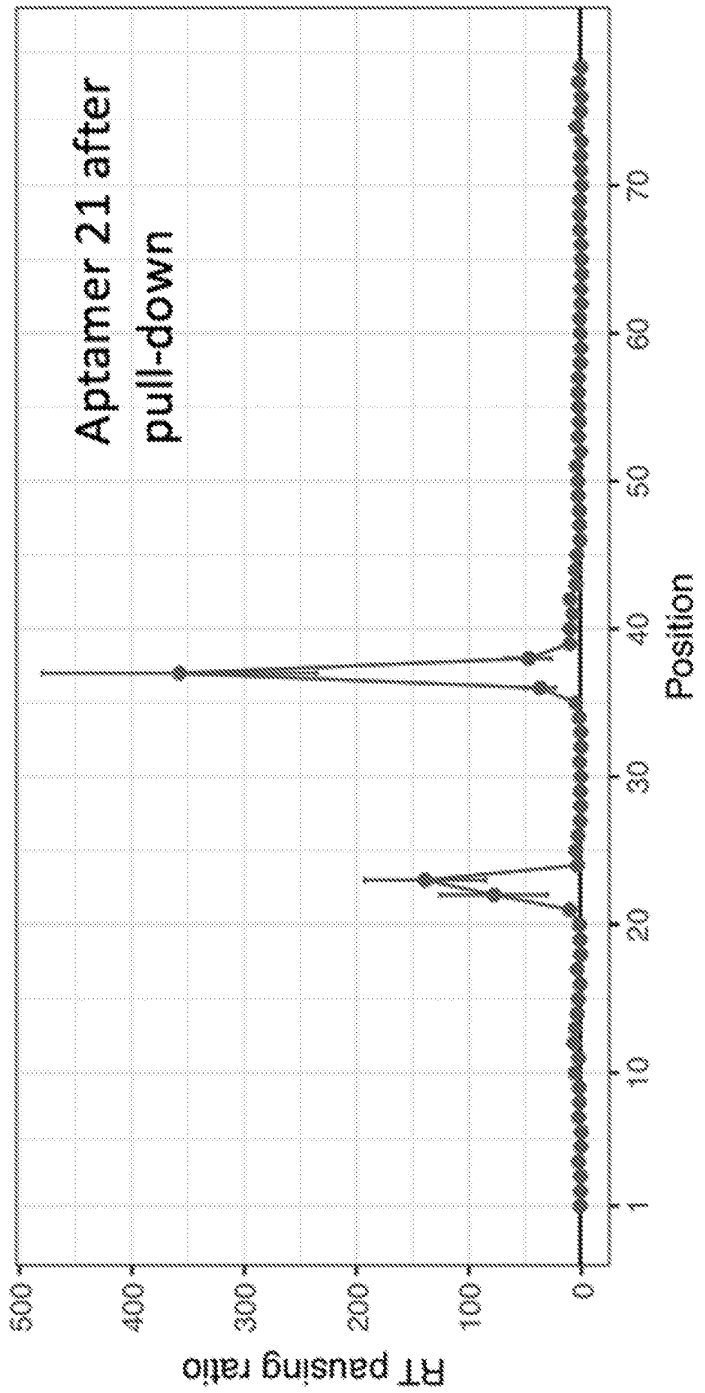

Aptamer 21 after
pull-down
RT pausing ratio
Position
0
100
200
300
400
500
1
10
20
30
40
50
60
70

Figure 67B

Figure 68

Enrichment of Aptamer 21 from a Mixture of RNAs

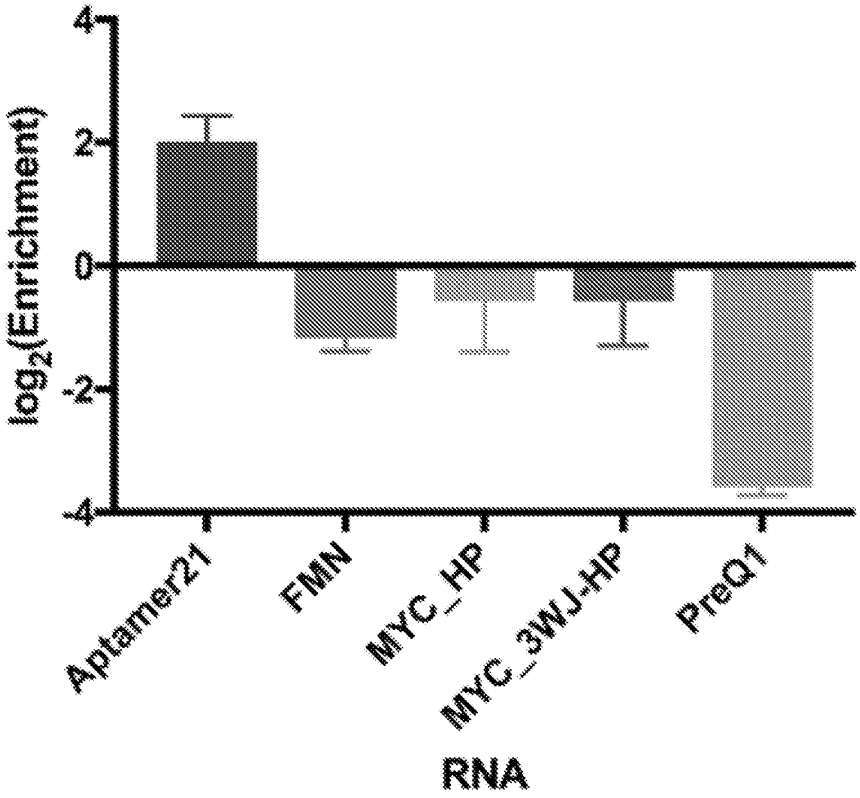


Figure 69

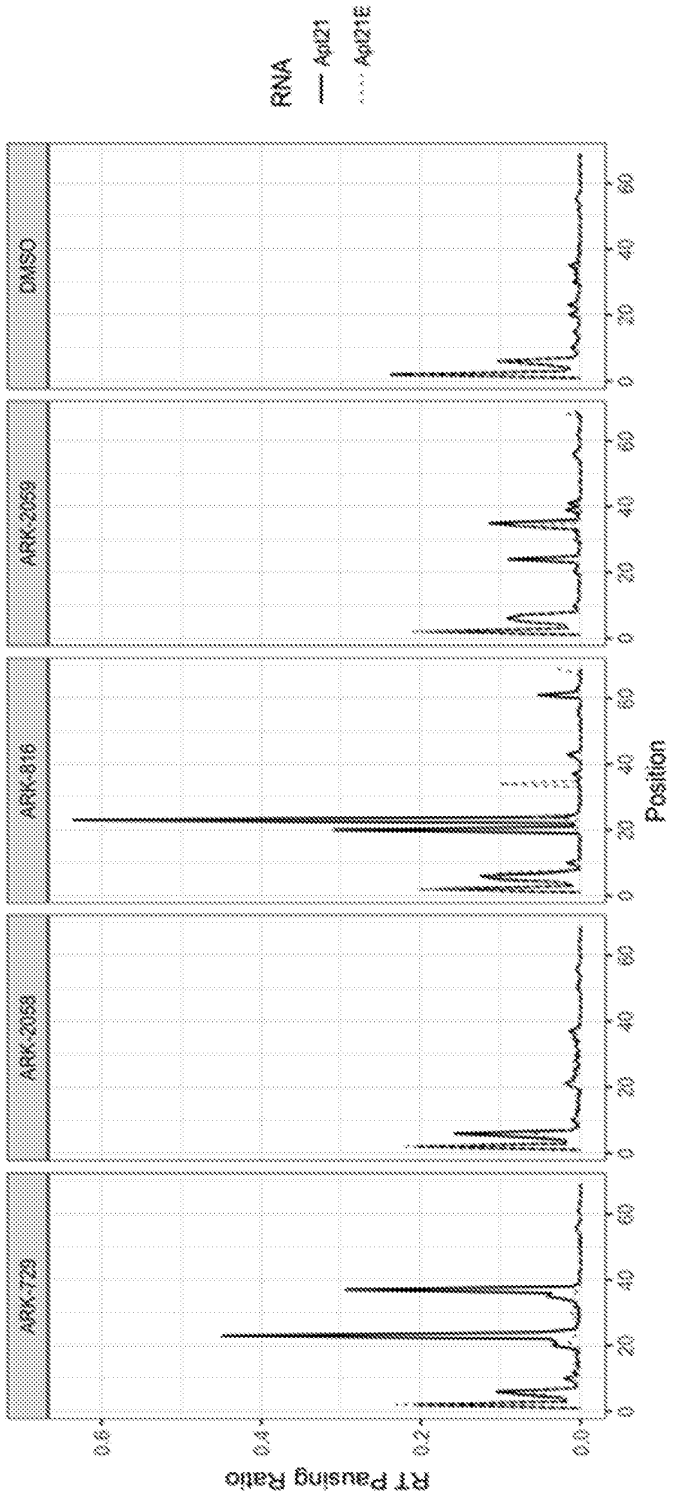

RNA
Aqil21
Aqil21E
DMSO
ARK-2059
ARK-816
ARK-2059
ARK-729
RT Pausing Ratio
0.0
0.2
0.4
0.6
0
20
40
60
Position

Figure 70

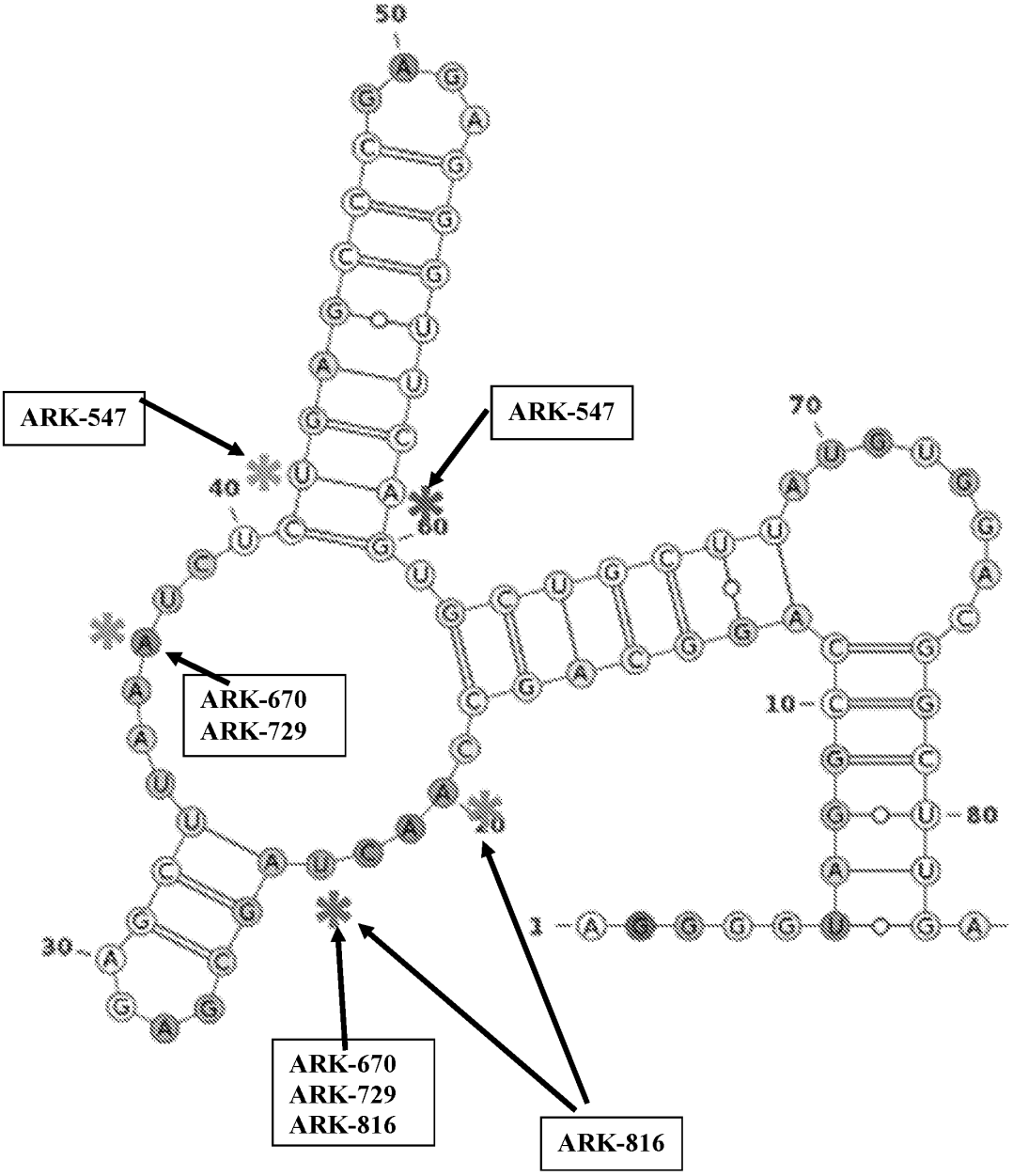

50
ARK-547
ARK-547
40
60
70
ARK-670
ARK-729
10
80
30
1
ARK-670
ARK-729
ARK-816
ARK-816

Figure 71

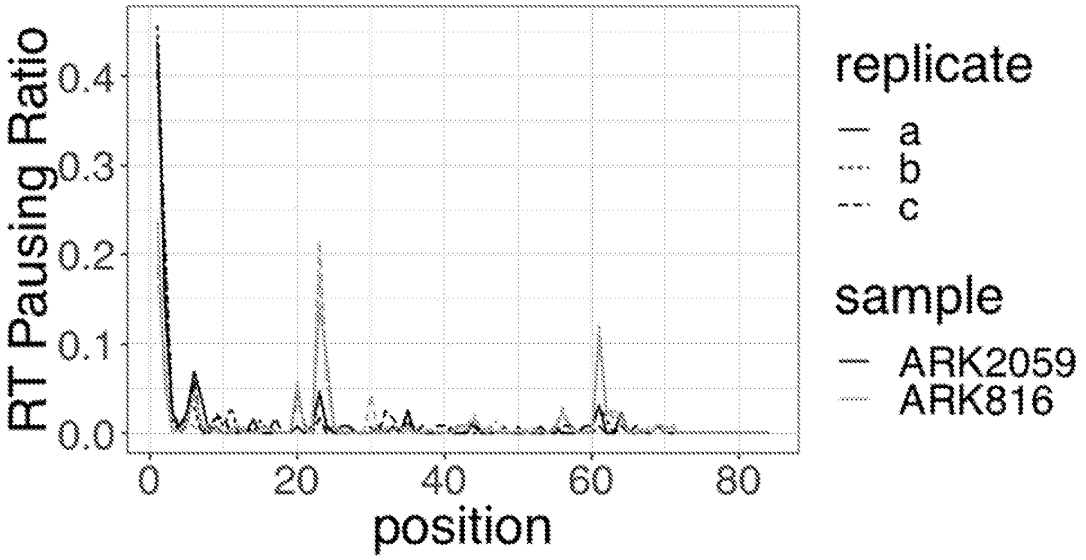
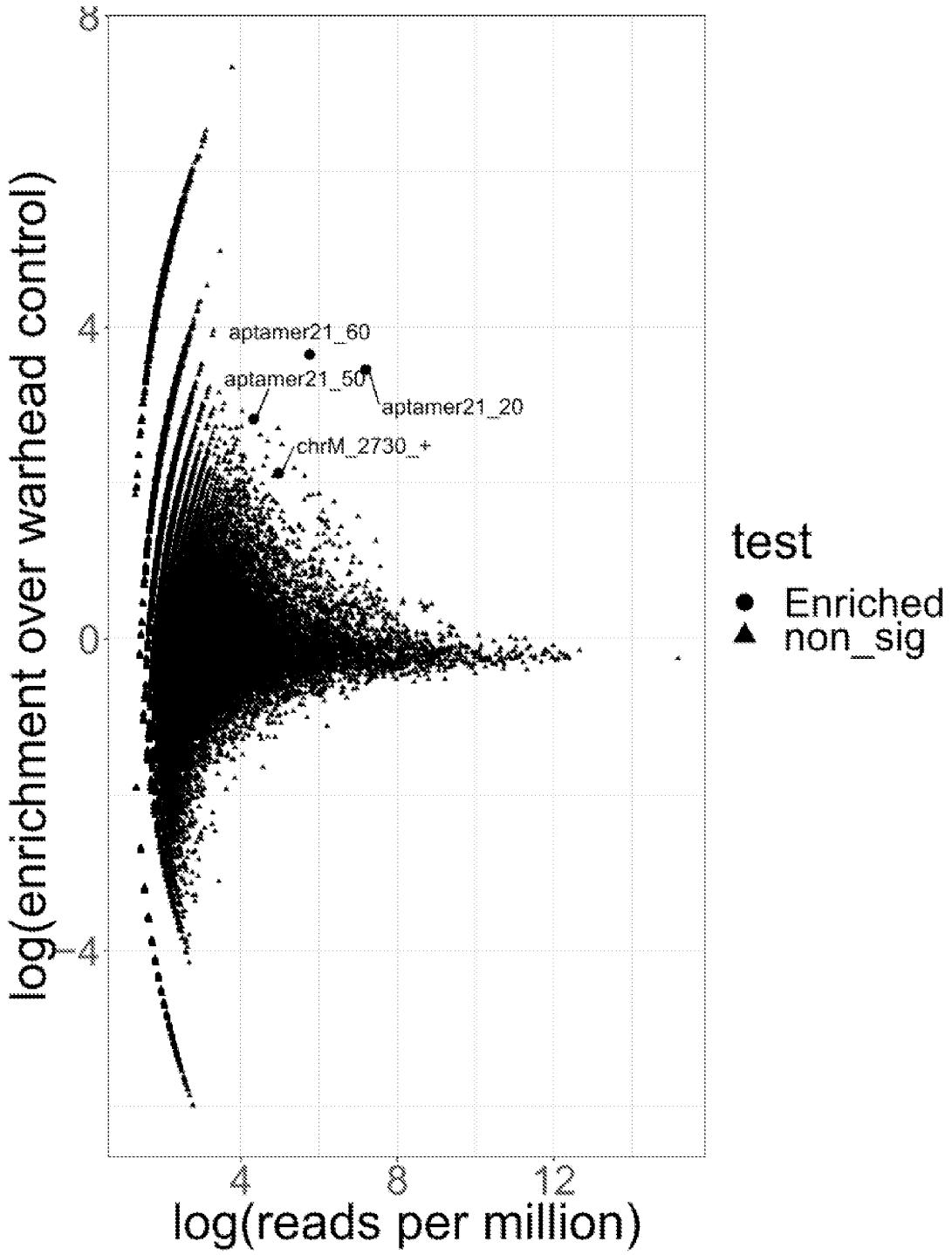

RT Pausing Ratio
0.0
0.1
0.2
0.3
0.4
0
20
40
60
80
position
replicate
a
b
c
sample
ARK2059
ARK816

Figure 72


log(enrichment over warhead control)
log(reads per million)
aptamer21_60
aptamer21_50
aptamer21_20
chrM_2730_+
test
Enriched
non_sig