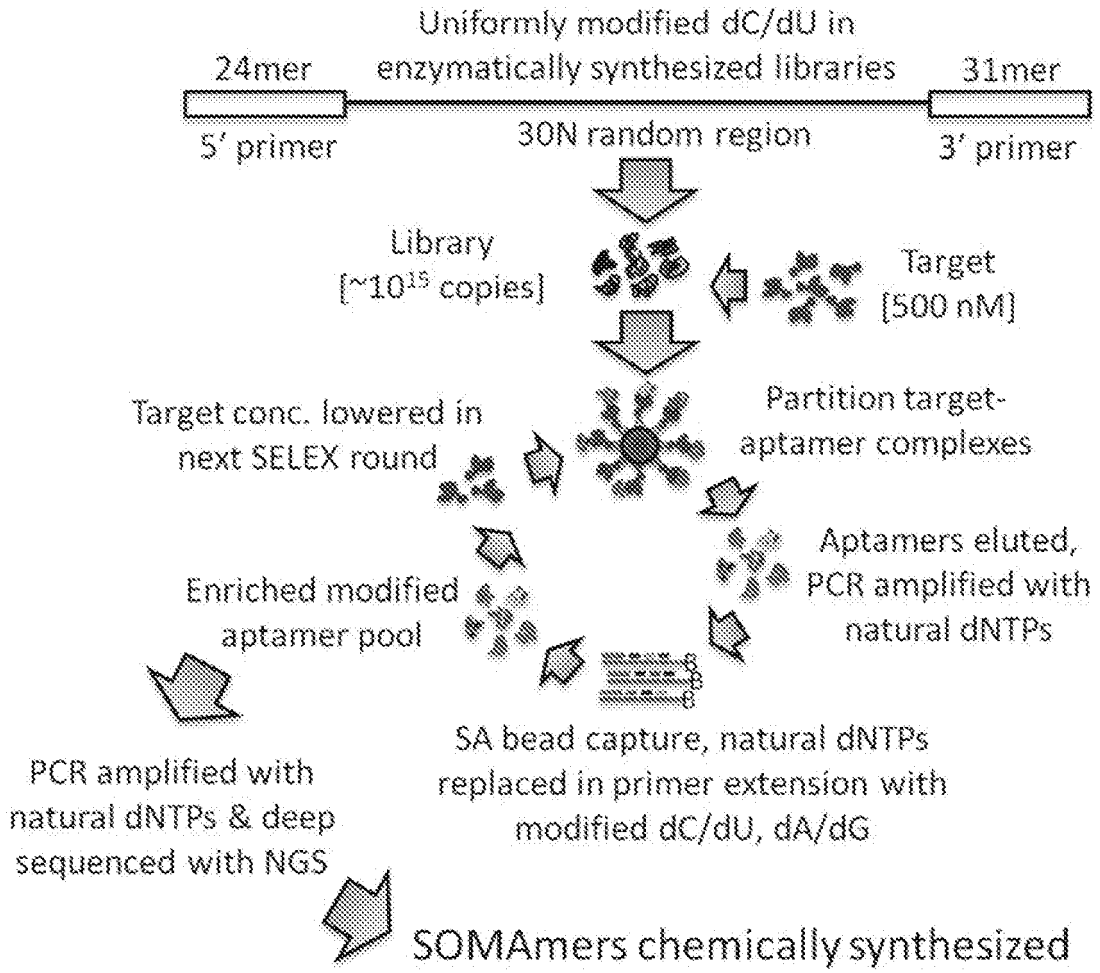

Uniformly modified dC/dU in
enzymatically synthesized libraries
24mer
31mer
5′ primer
30N random region
3′ primer
Library
[~10^15 copies]
Target
[500 nM]
Target conc. lowered in
next SELEX round
Partition target-
aptamer complexes
Aptamers eluted,
PCR amplified with
natural dNTPs
Enriched modified
aptamer pool
SA bead capture, natural dNTPs
replaced in primer extension with
modified dC/dU, dA/dG
PCR amplified with
natural dNTPs & deep
sequenced with NGS
SOMAmers chemically synthesized

FIG. 1

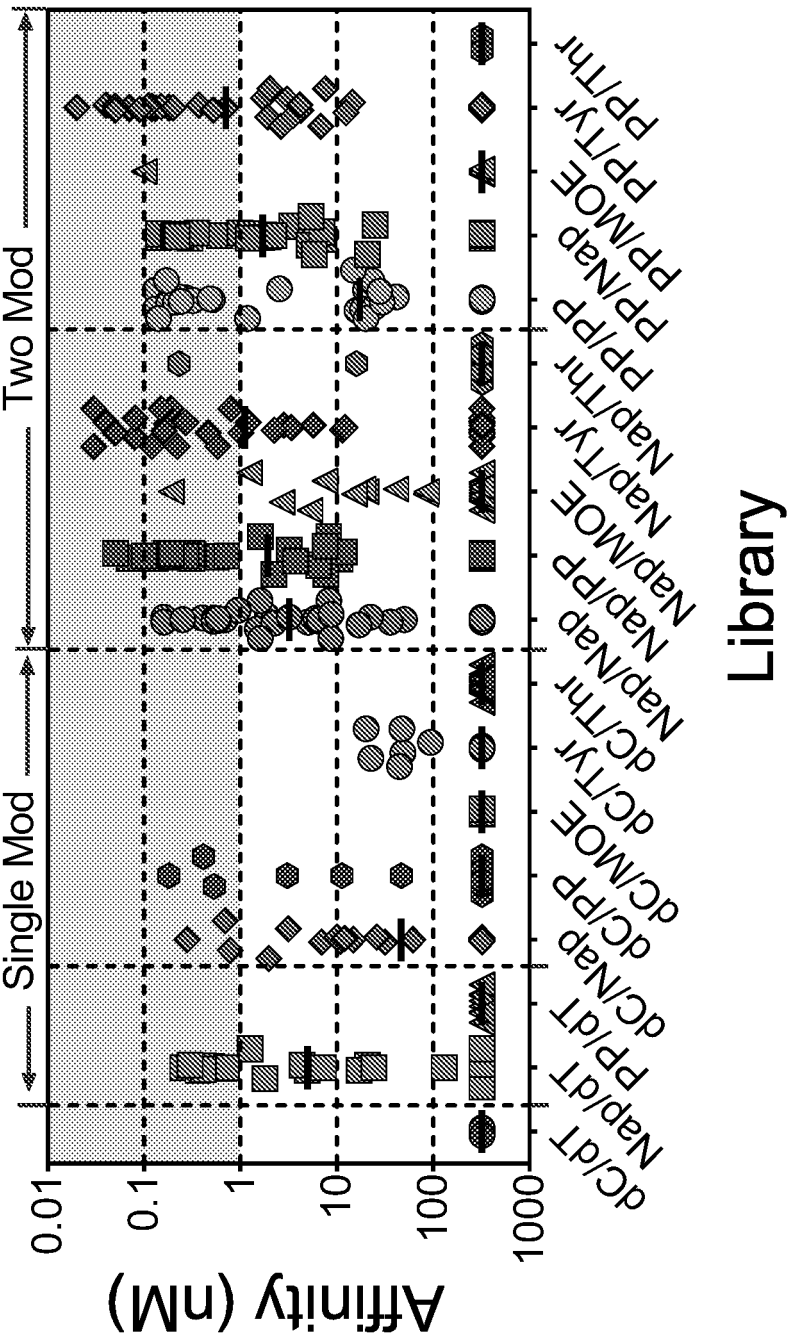

Affinity (nM)
0.01
0.1
1
10
100
1000
Single Mod
Two Mod
dC/dT
Nap/dT
PP/dT
dC/Nap
dC/PP
dC/MOE
dC/Tyr
dC/Thr
Nap/Nap
Nap/PP
Nap/MOE
Nap/Tyr
Nap/Thr
PP/PP
PP/Nap
PP/MOE
PP/Tyr
PP/Thr
Library

FIG. 2

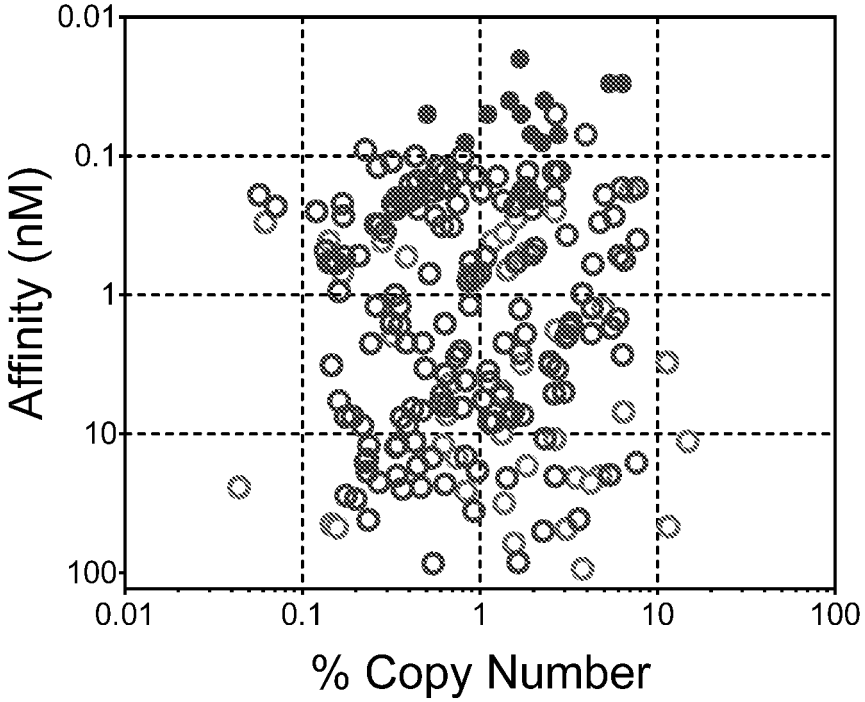

0.01
0.1
1
10
100
Affinity (nM)
0.01
0.1
1
10
100
% Copy Number

FIG. 3

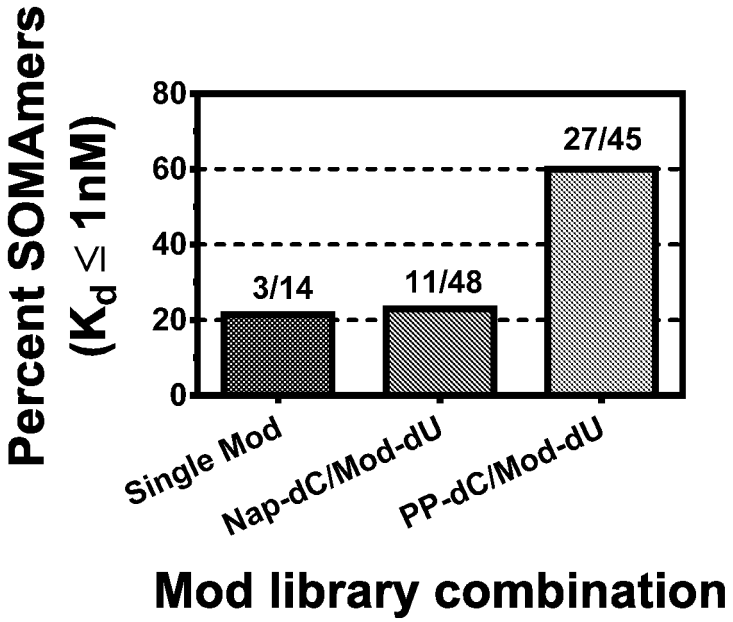

Percent SOMAmers
(Kd VI 1nM)
80
60
40
20
0
3/14
11/48
27/45
Single Mod
Nap-dC/Mod-dU
PP-dC/Mod-dU
Mod library combination

FIG. 4A

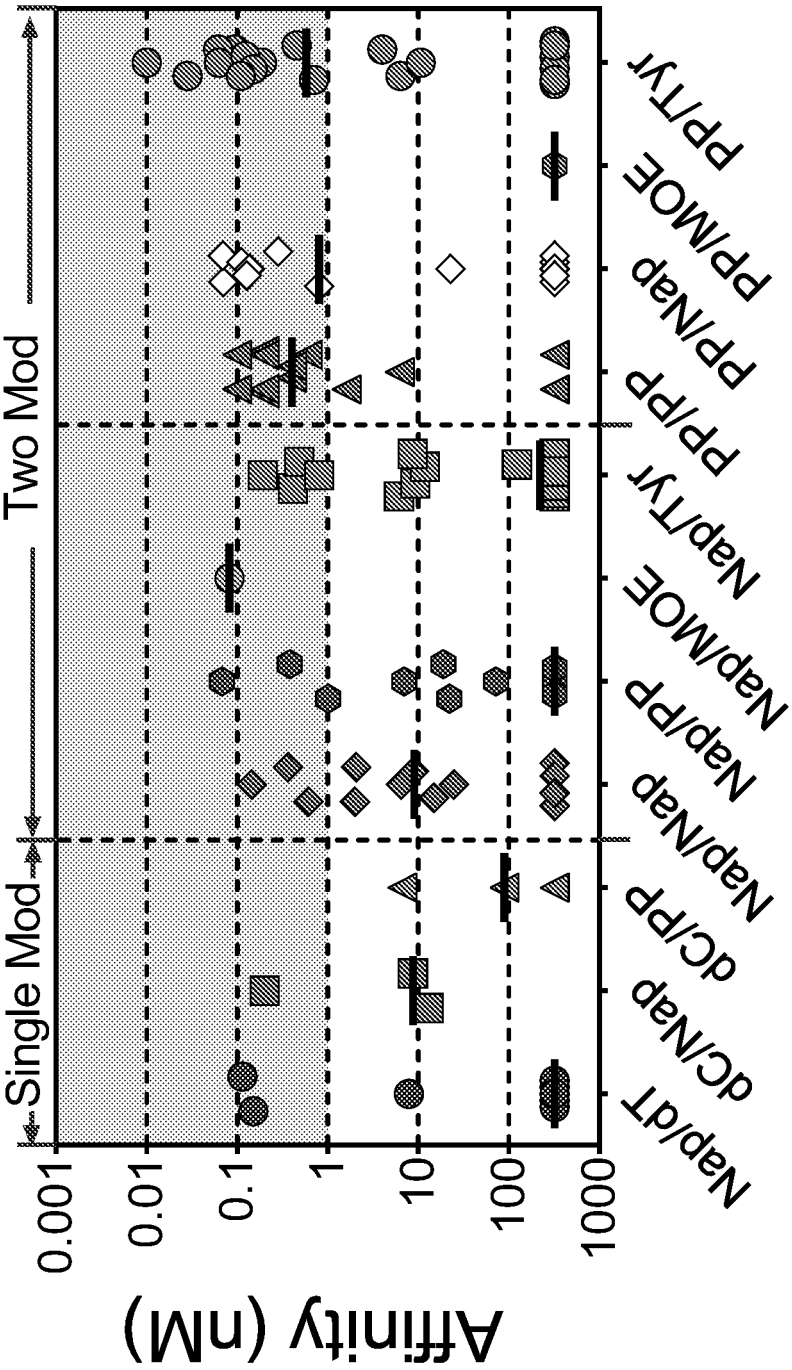

Single Mod
Two Mod
0.001
0.01
0.1
1
10
100
1000
Affinity (nM)
Nap/dT
dC/Nap
dC/PP
Nap/Nap
Nap/PP
Nap/MOE
Nap/Tyr
PP/PP
PP/Nap
PP/MOE
PP/Tyr
Library

FIG. 4B

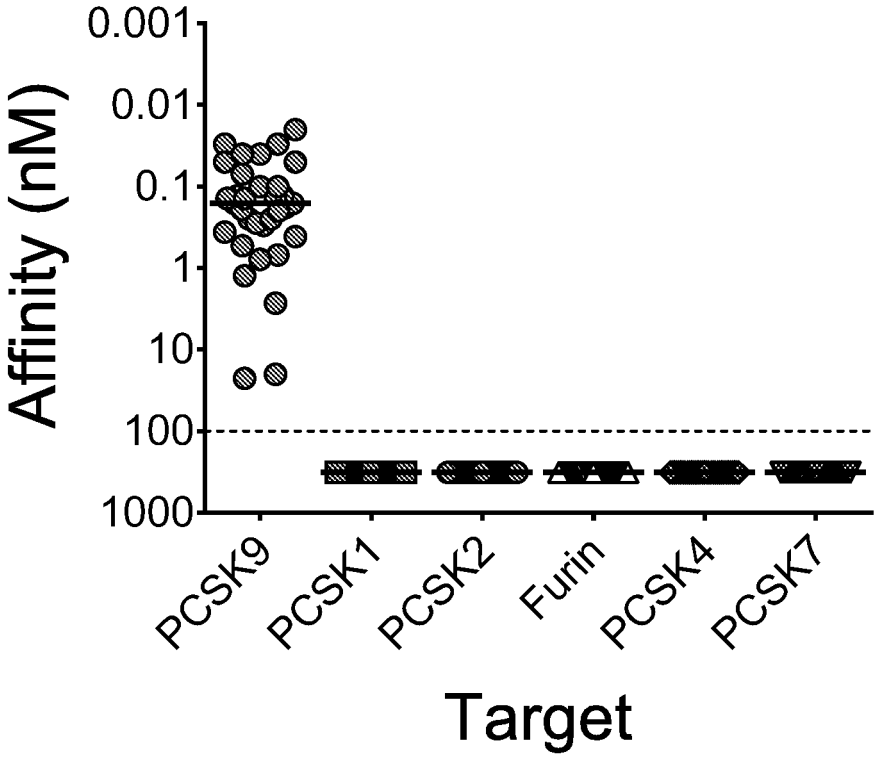

Affinity (nM)
0.001
0.01
0.1
1
10
100
1000
PCSK9
PCSK1
PCSK2
Furin
PCSK4
PCSK7
Target

FIG. 5

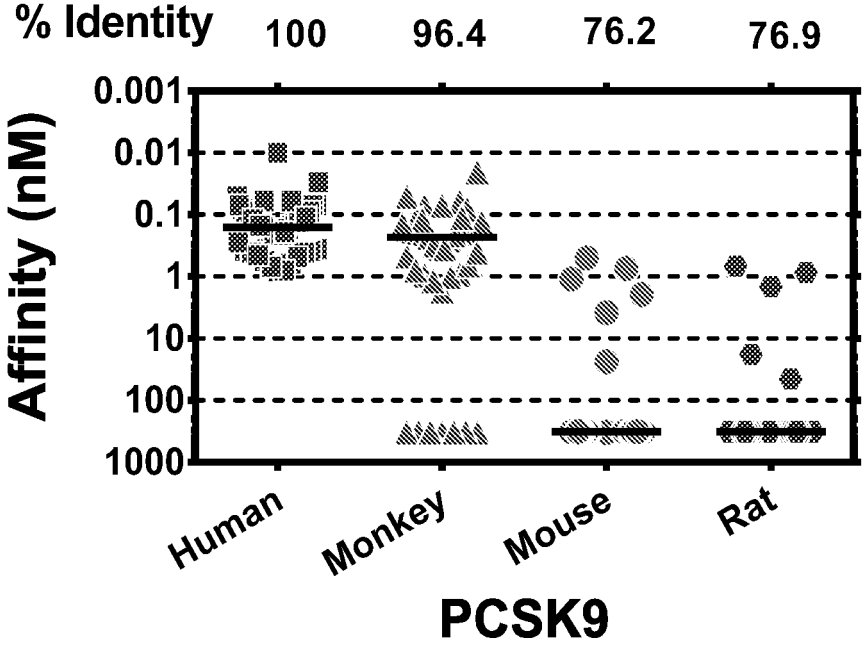

% Identity
100
96.4
76.2
76.9
Affinity (nM)
0.001
0.01
0.1
1
10
100
1000
Human
Monkey
Mouse
Rat
PCSK9

FIG. 6

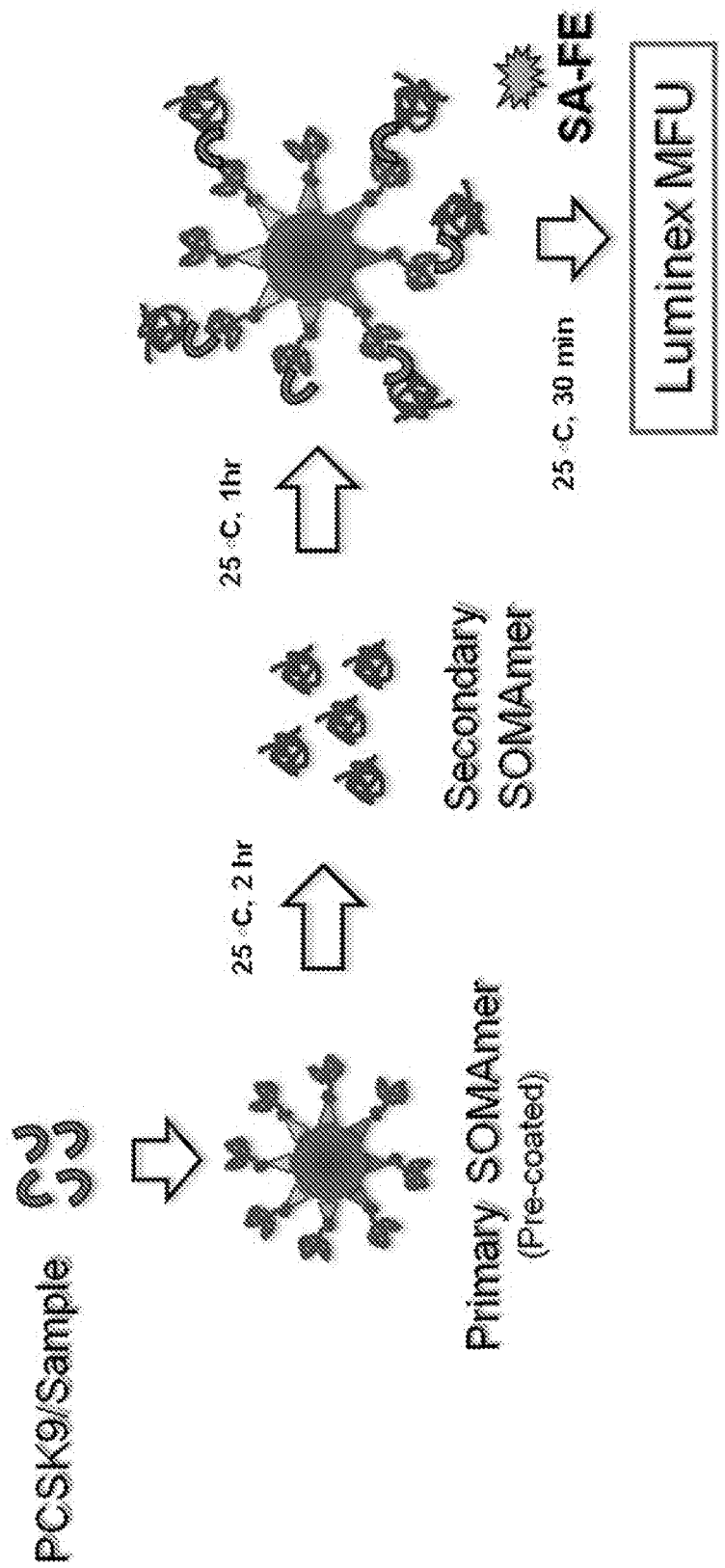

PCSK9/Sample
Primary SOMAmer
(Pre-coated)
25 °C, 2 hr
Secondary
SOMAmer
25 °C, 1hr
SA-FE
25 °C, 30 min
Luminex MFU

FIG. 7A

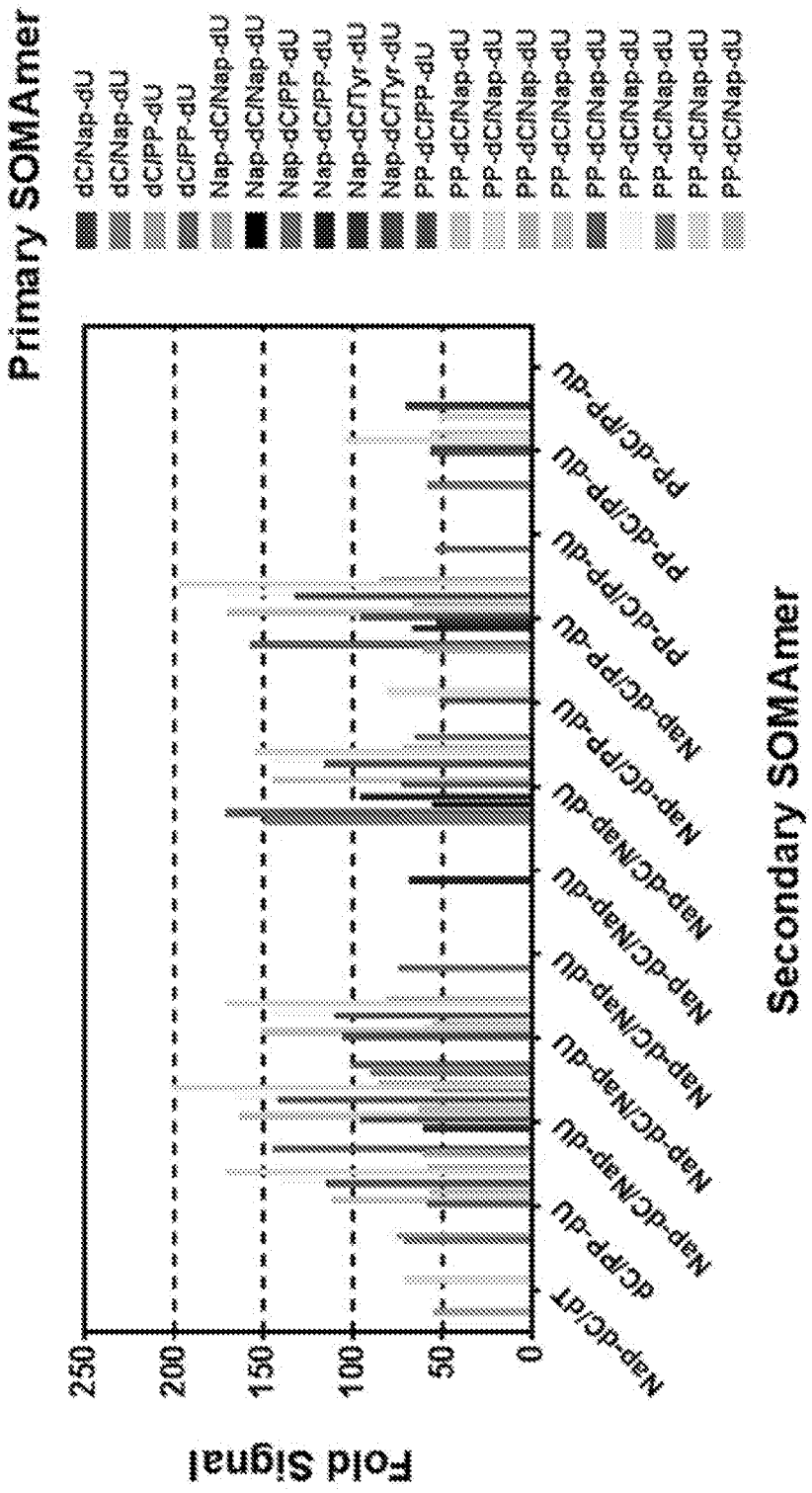

Primary SOMAmer
dCNap-dU
dCNap-dU
dCPP-dU
dCPP-dU
Nap-dCNap-dU
Nap-dCNap-dU
Nap-dCPP-dU
Nap-dCPP-dU
Nap-dCTyr-dU
Nap-dCTyr-dU
PP-dCPP-dU
PP-dCNap-dU
PP-dCNap-dU
PP-dCNap-dU
PP-dCNap-dU
PP-dCNap-dU
PP-dCNap-dU
PP-dCNap-dU
PP-dCNap-dU
Fold Signal
250
200
150
100
50
0
Nap-dC/dT
dC/PP-dU
Nap-dC/Nap-dU
Nap-dC/Nap-dU
Nap-dC/Nap-dU
Nap-dC/Nap-dU
Nap-dC/PP-dU
Nap-dC/PP-dU
PP-dC/PP-dU
PP-dC/PP-dU
PP-dC/PP-dU
Secondary SOMAmer

FIG. 7B

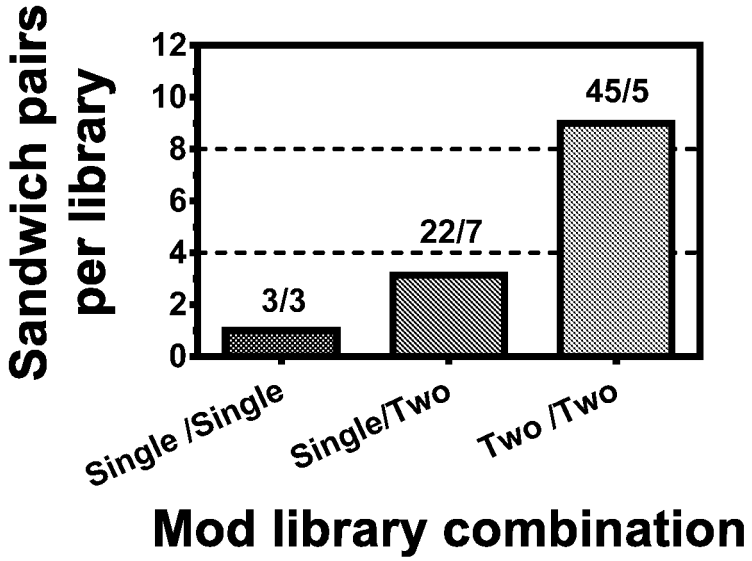

Sandwich pairs per library
12
10
8
6
4
2
0
3/3
22/7
45/5
Single /Single
Single/Two
Two /Two
Mod library combination

FIG. 7C

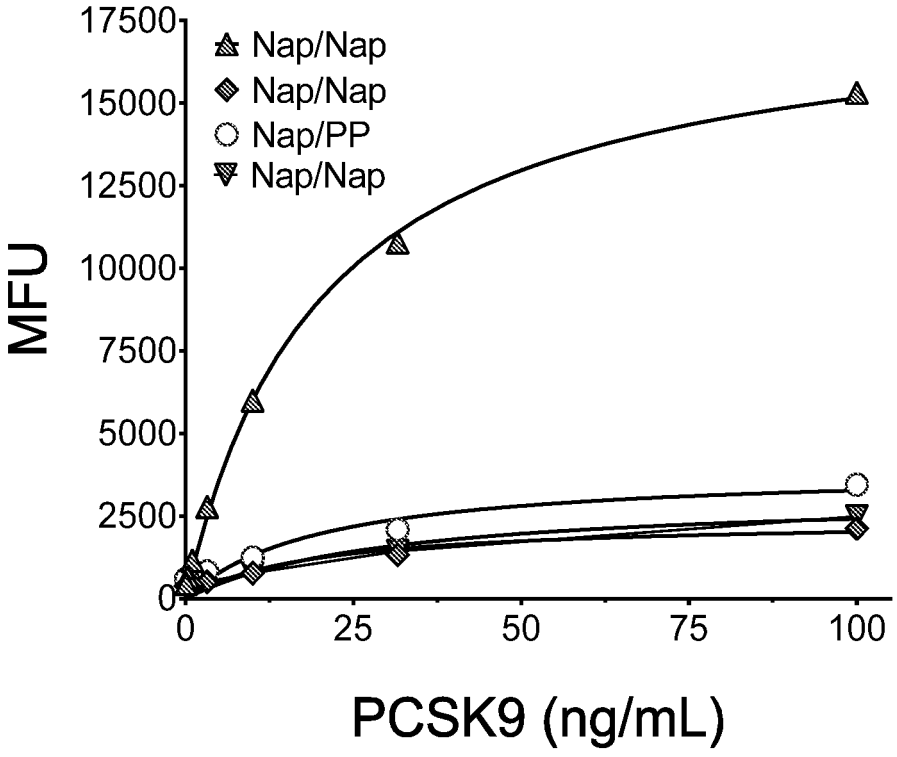

MFU
17500
15000
12500
10000
7500
5000
2500
0
Nap/Nap
Nap/Nap
Nap/PP
Nap/Nap
0
25
50
75
100
PCSK9 (ng/mL)

FIG. 8A

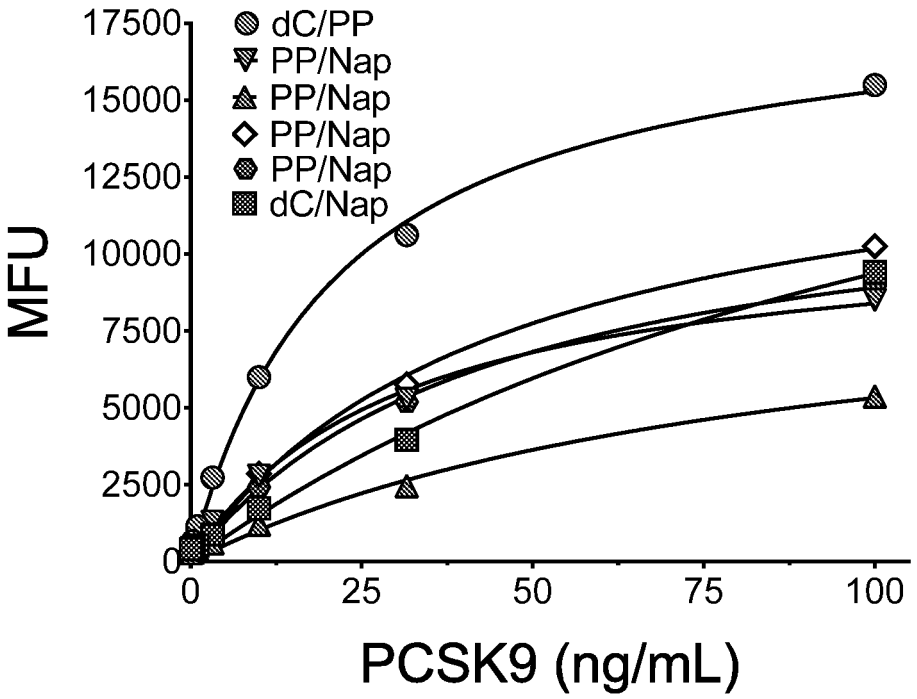

17500
15000
12500
10000
7500
5000
2500
0
MFU
dC/PP
PP/Nap
PP/Nap
PP/Nap
PP/Nap
dC/Nap
0
25
50
75
100
PCSK9 (ng/mL)

FIG. 8B

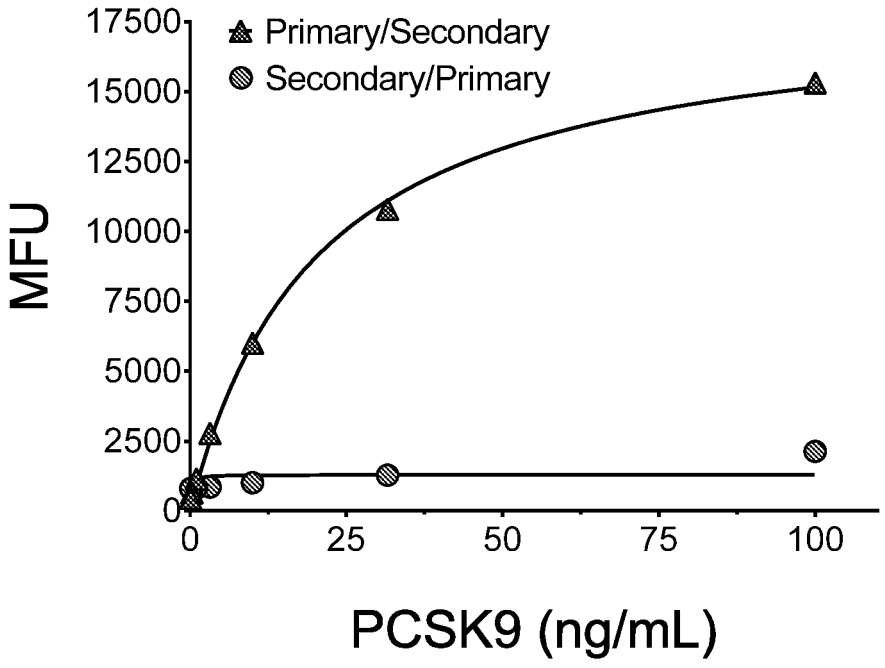

17500
15000
12500
10000
7500
5000
2500
0
MFU
Primary/Secondary
Secondary/Primary
0
25
50
75
100
PCSK9 (ng/mL)

FIG. 8C

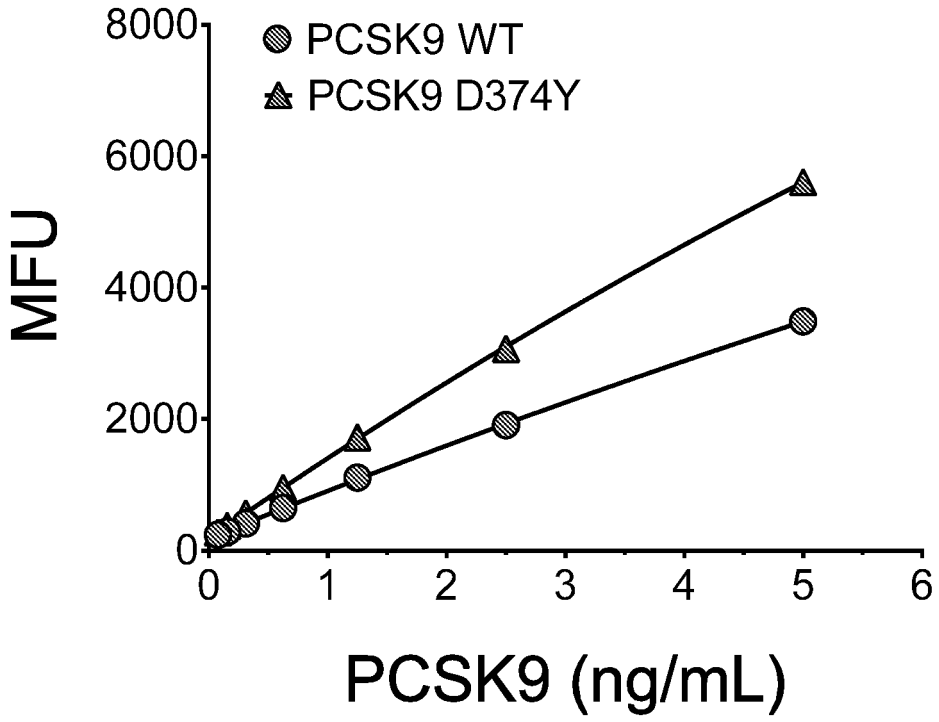

8000
6000
4000
2000
0
MFU
PCSK9 WT
PCSK9 D374Y
0
1
2
3
4
5
6
PCSK9 (ng/mL)

FIG. 8D

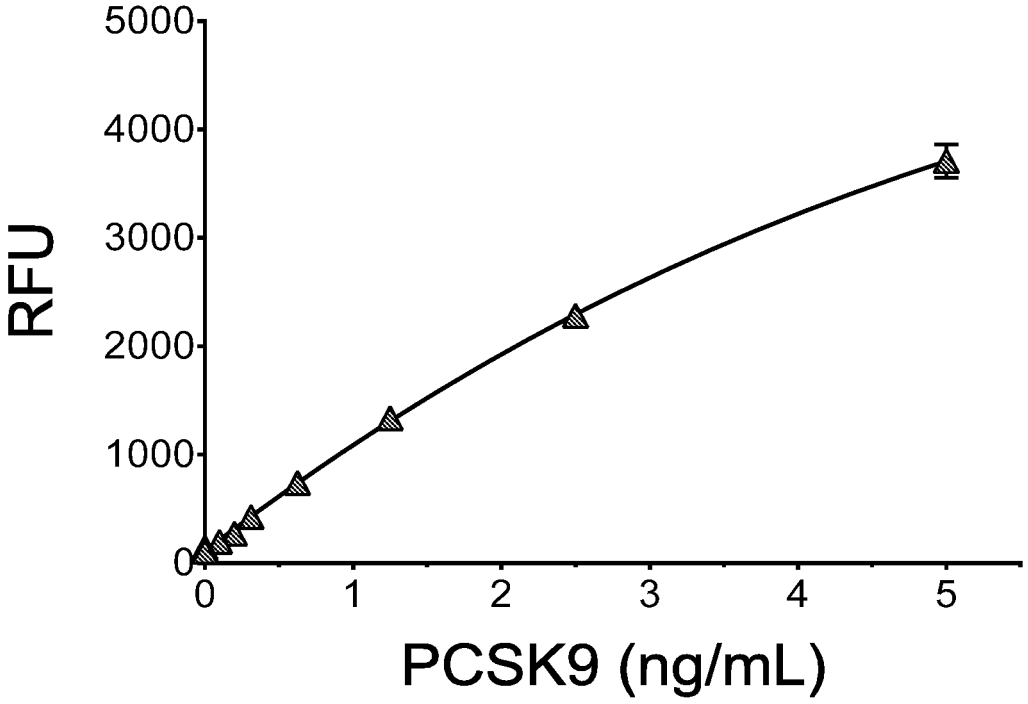

5000
4000
3000
2000
1000
0
RFU
0
1
2
3
4
5
PCSK9 (ng/mL)

FIG. 9A

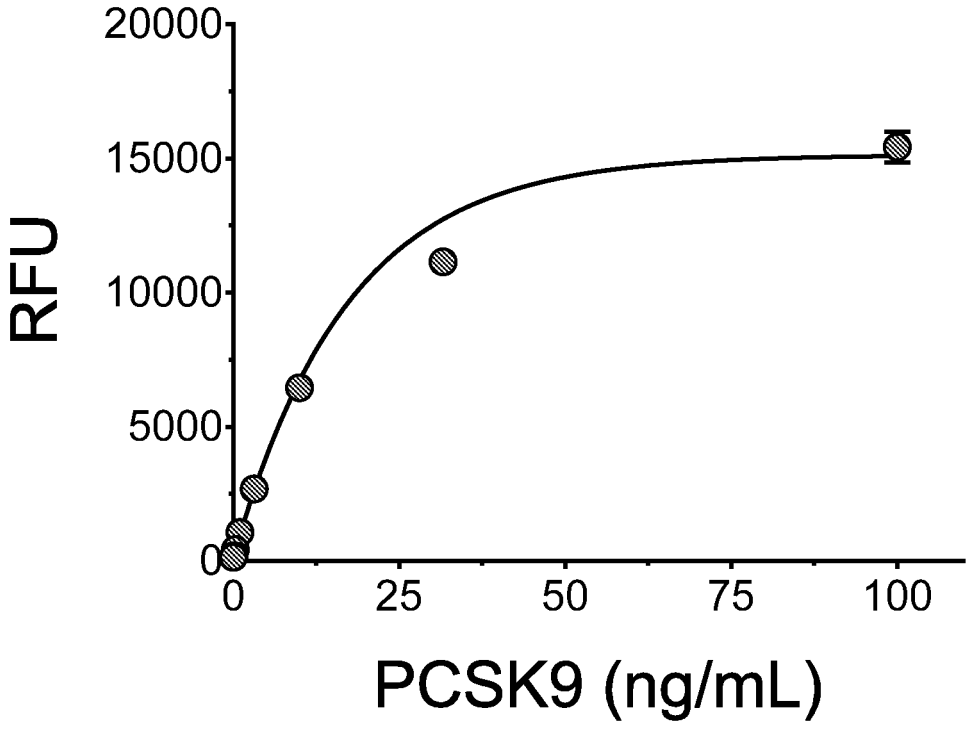

20000
15000
10000
5000
0
RFU
0
25
50
75
100
PCSK9 (ng/mL)

FIG. 9B

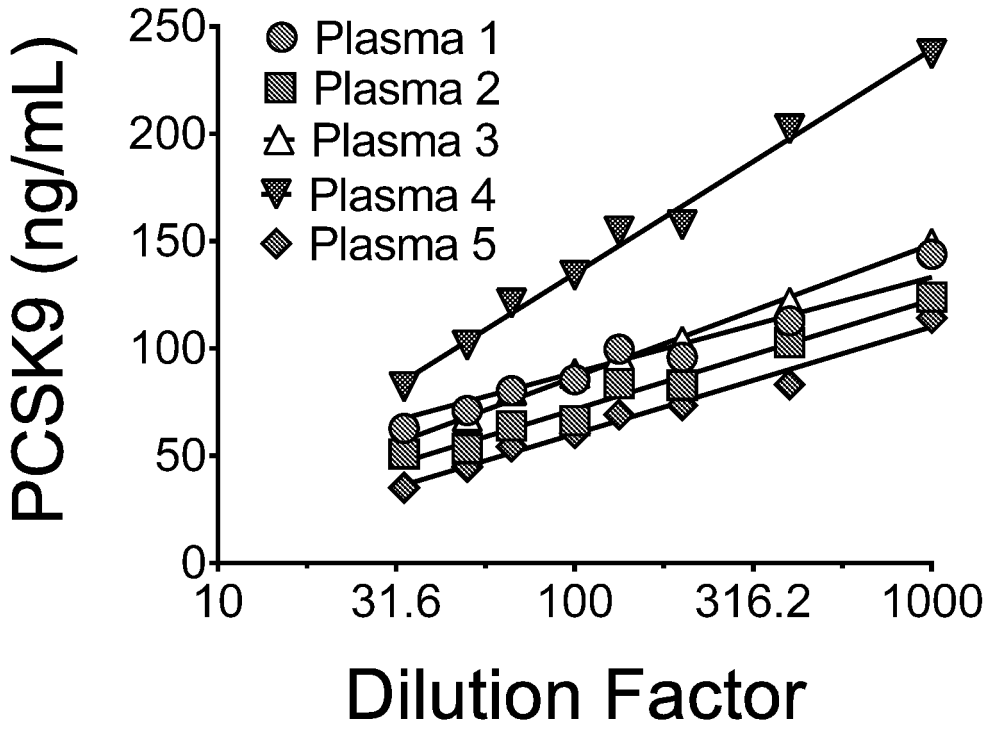

PCSK9 (ng/mL)
250
200
150
100
50
0
Plasma 1
Plasma 2
Plasma 3
Plasma 4
Plasma 5
10
31.6
100
316.2
1000
Dilution Factor

FIG. 10

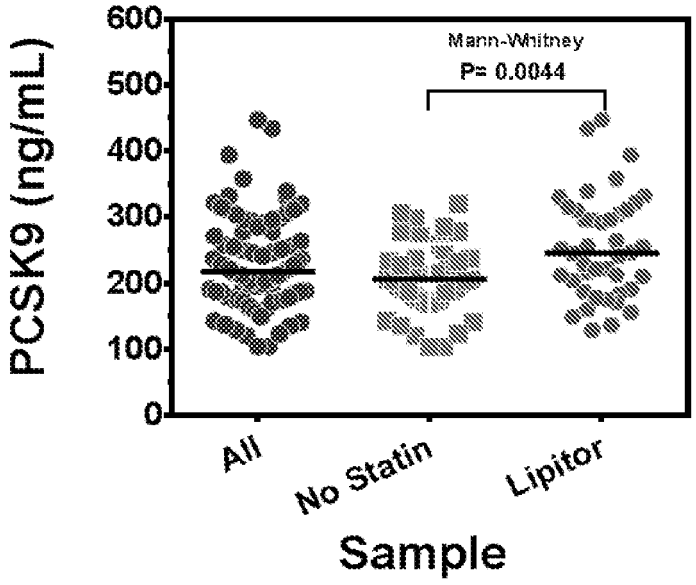

PCSK9 (ng/mL)
600
500
400
300
200
100
0
Mann-Whitney
P= 0.0044
All
No Statin
Lipitor
Sample

FIG. 11

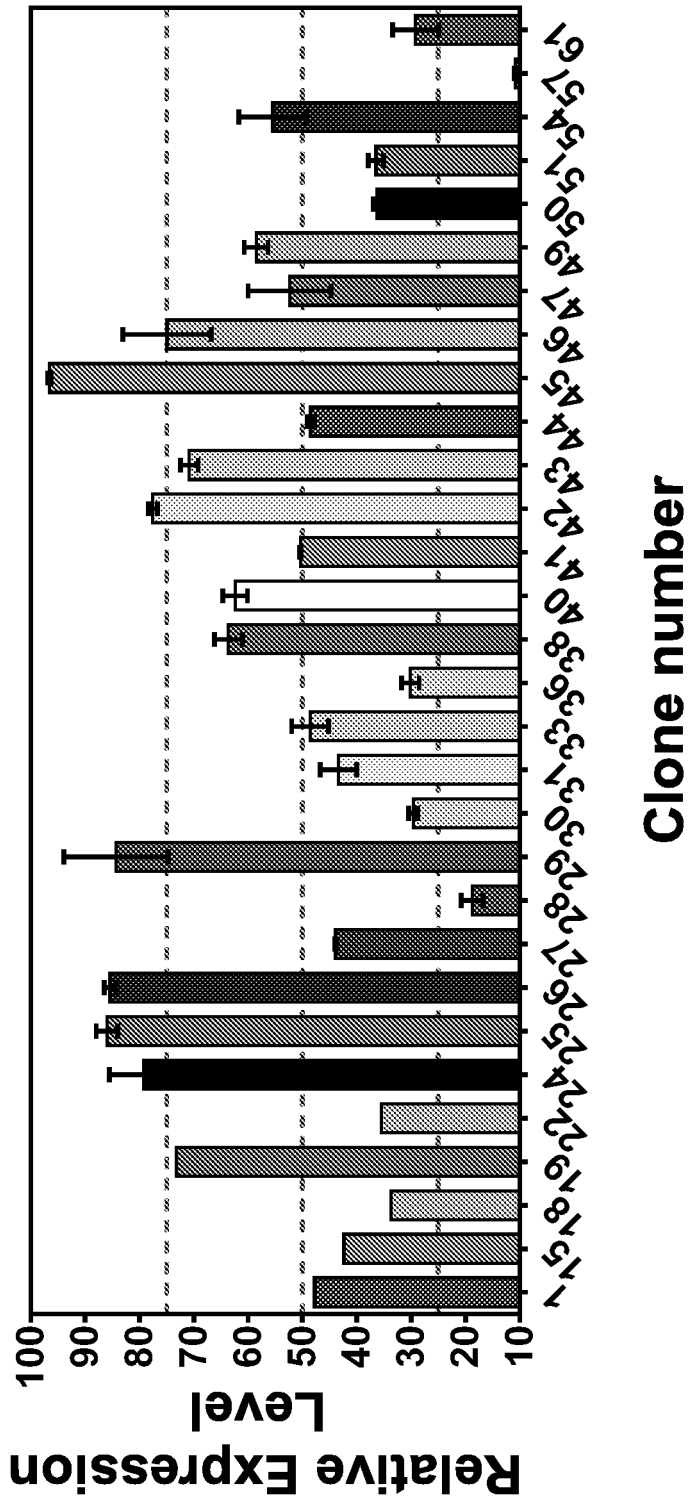

Relative Expression Level
100
90
80
70
60
50
40
30
20
10
Clone number
1 5 1 8 1 9 2 2 2 4 2 5 2 6 2 1 2 8 2 9 3 0 3 1 3 3 3 6 3 8 4 0 4 1 4 2 4 3 4 4 4 5 4 6 4 7 4 9 5 0 5 1 5 4 5 7 6 1

FIG. 12

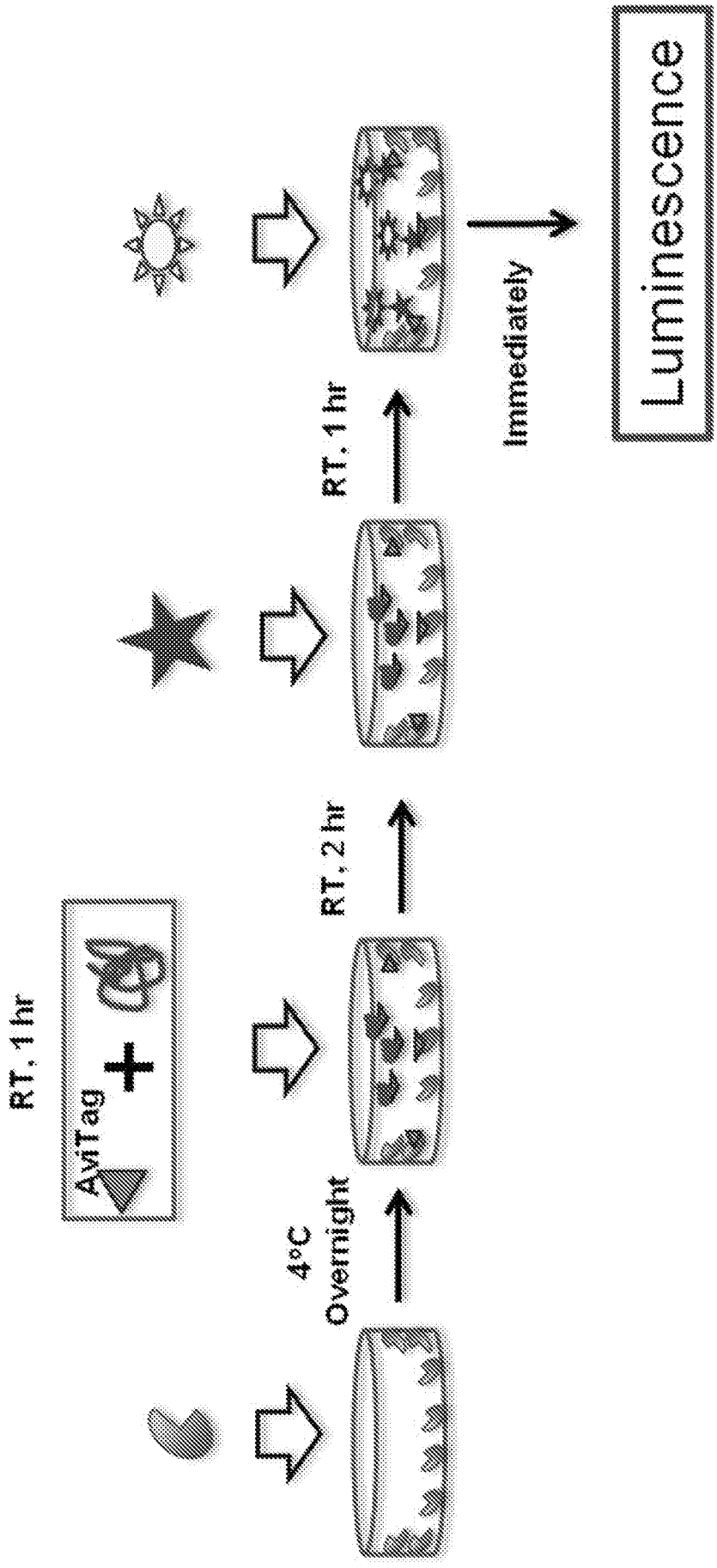

RT, 1 hr
AviTag
4°C
Overnight
RT, 2 hr
RT, 1 hr
Immediately
Luminescence

FIG. 13

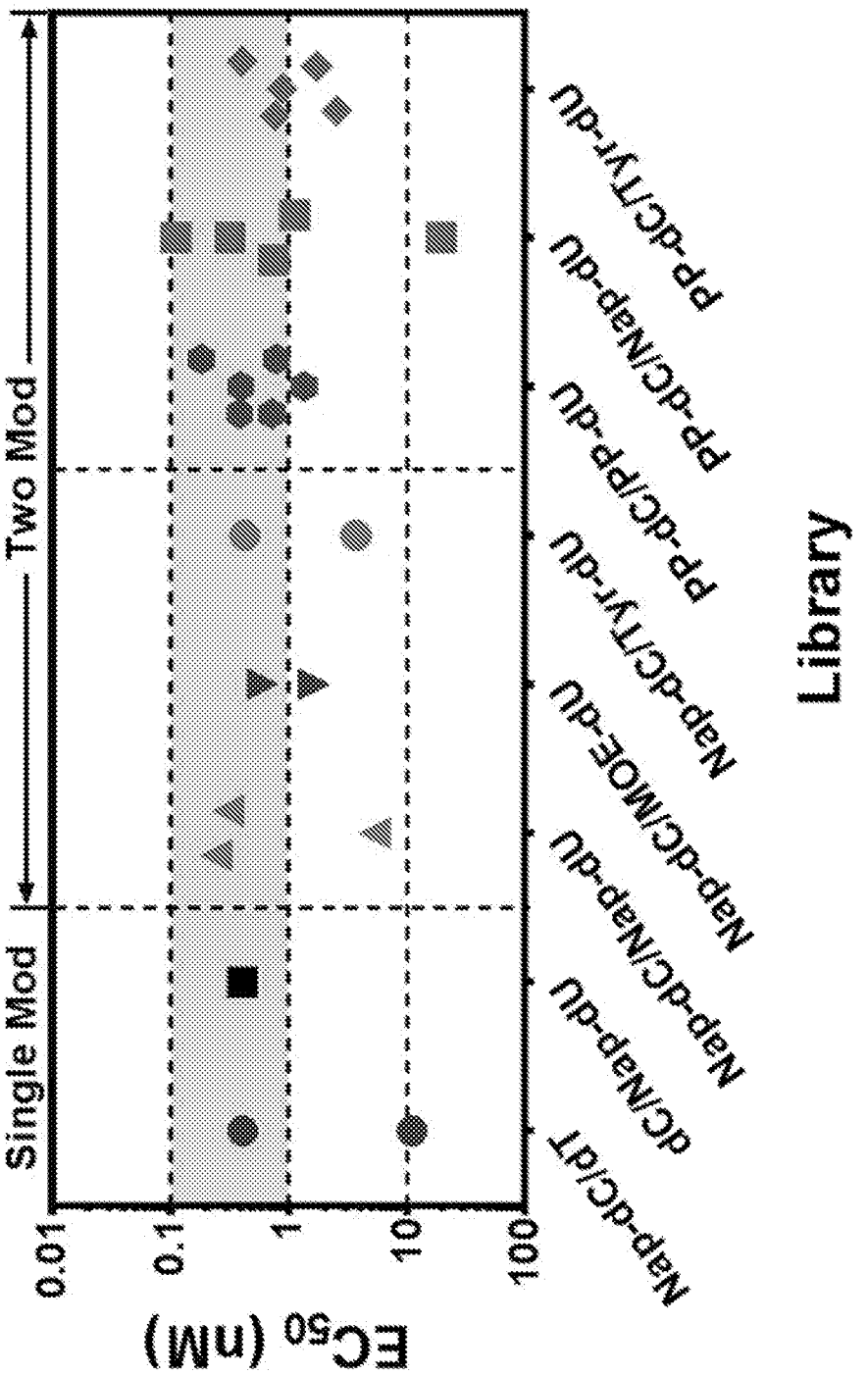

Single Mod
Two Mod
0.01
0.1
1
10
100
EC50 (nM)
Nap-dC/dT
dC/Nap-dU
Nap-dC/Nap-dU
Nap-dC/MOE-dU
Nap-dC/TYr-dU
PP-dC/PP-dU
PP-dC/Nap-dU
PP-dC/TYr-dU
Library

FIG. 14

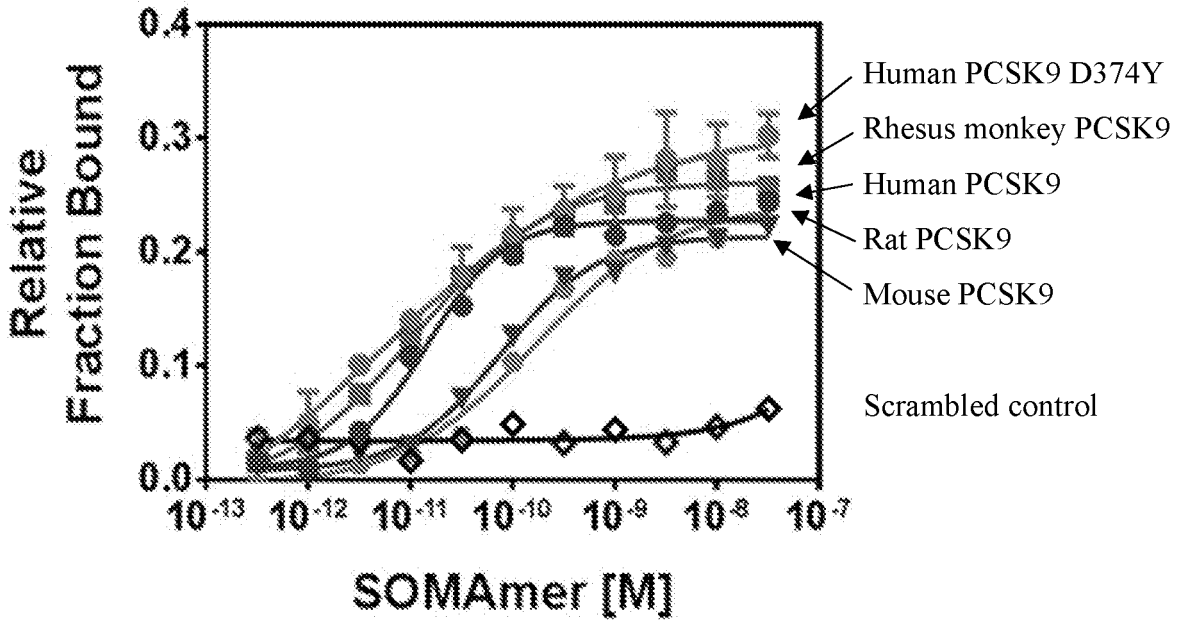


FIG. 15A

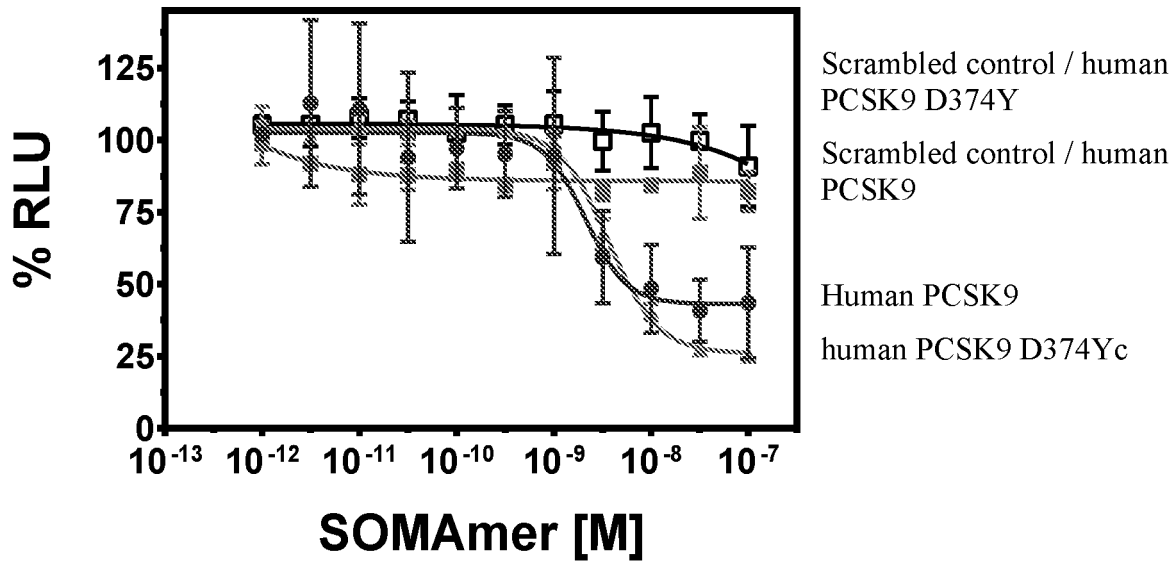


FIG. 15B

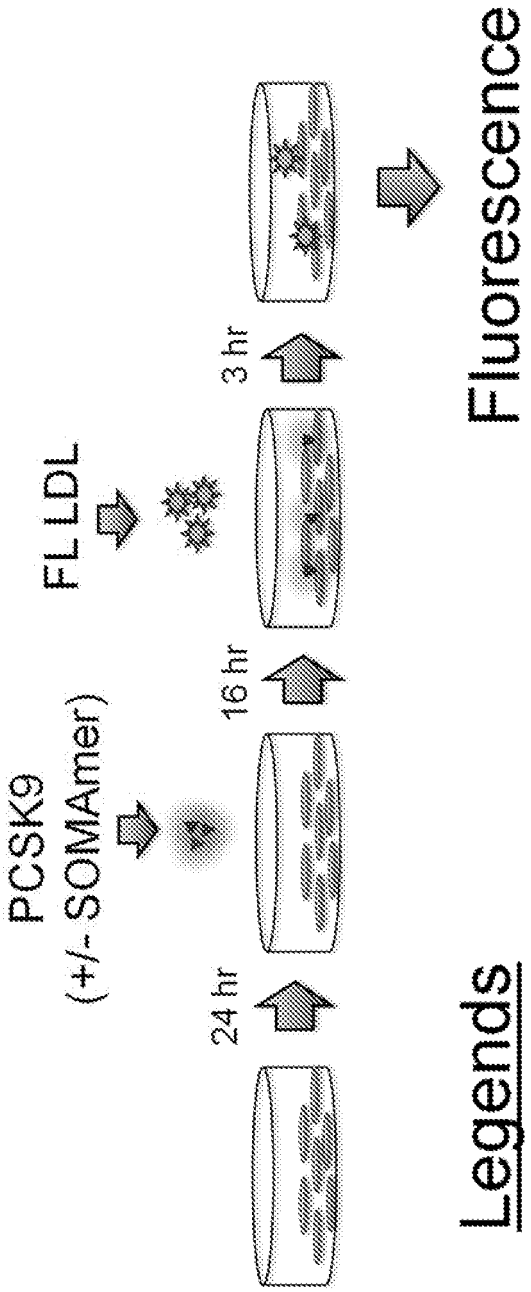

PCSK9
(+/- SOMAmer)
FL LDL
24 hr
16 hr
3 hr
Fluorescence
Legends
HepG2 cells
PCSK9
FL LDL

FIG. 16

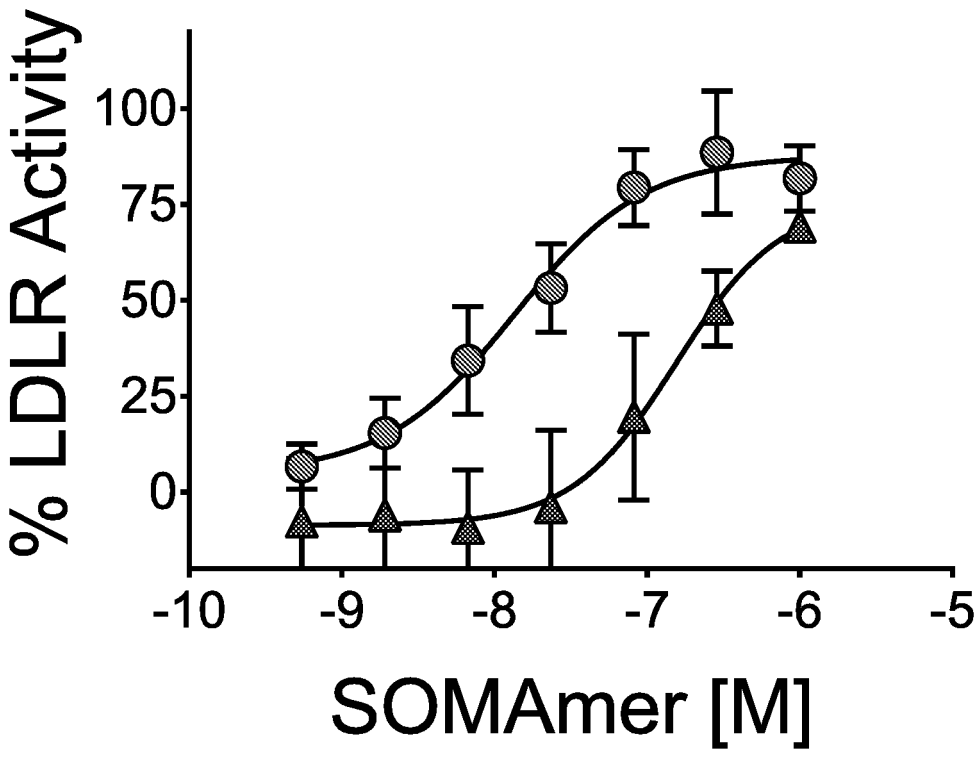


FIG. 17

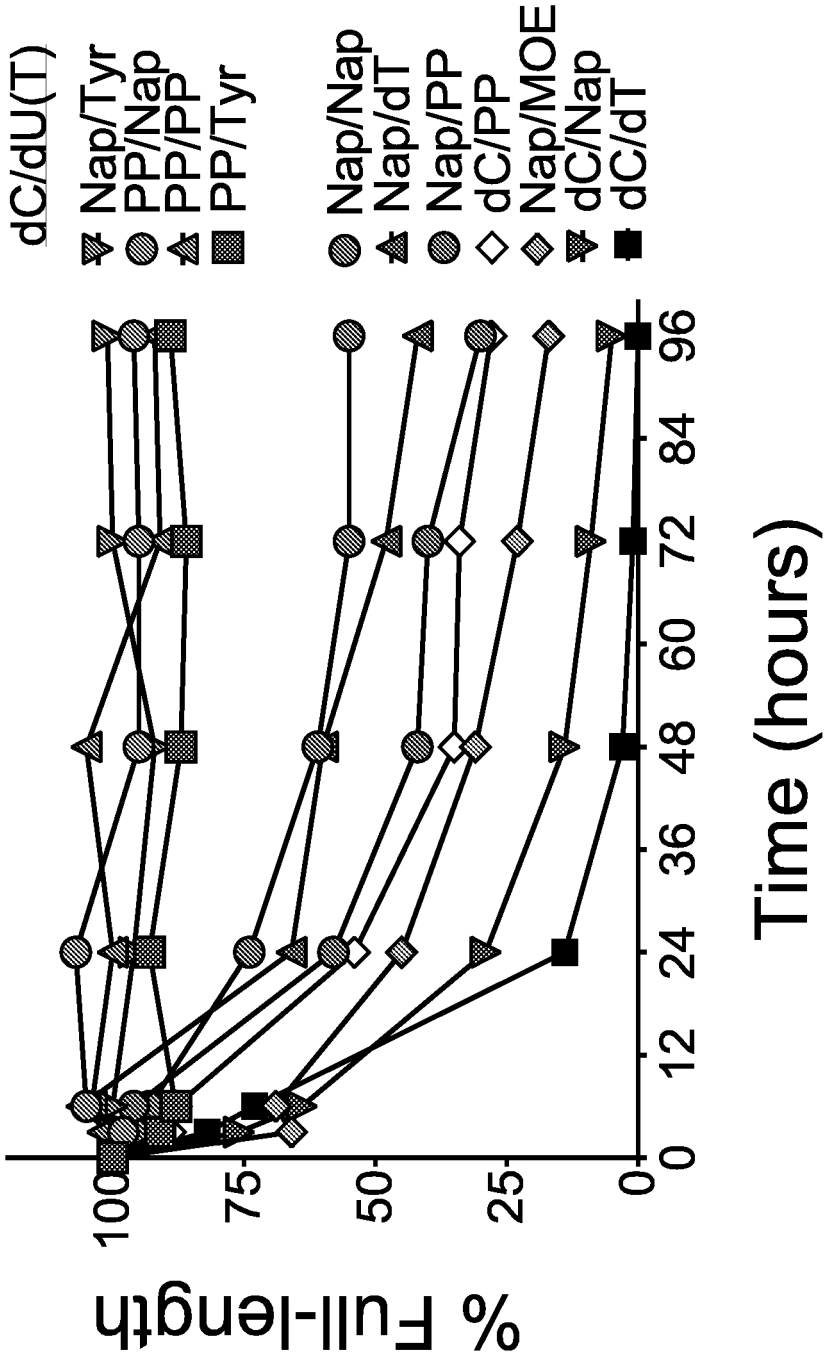

% Full-length
100
75
50
25
0
0
12
24
36
48
60
72
84
96
Time (hours)
dC/dU(T)
Nap/Tyr
PP/Nap
PP/PP
PP/Tyr
Nap/Nap
Nap/dT
Nap/PP
dC/PP
Nap/MOE
dC/Nap
dC/dT

FIG. 18

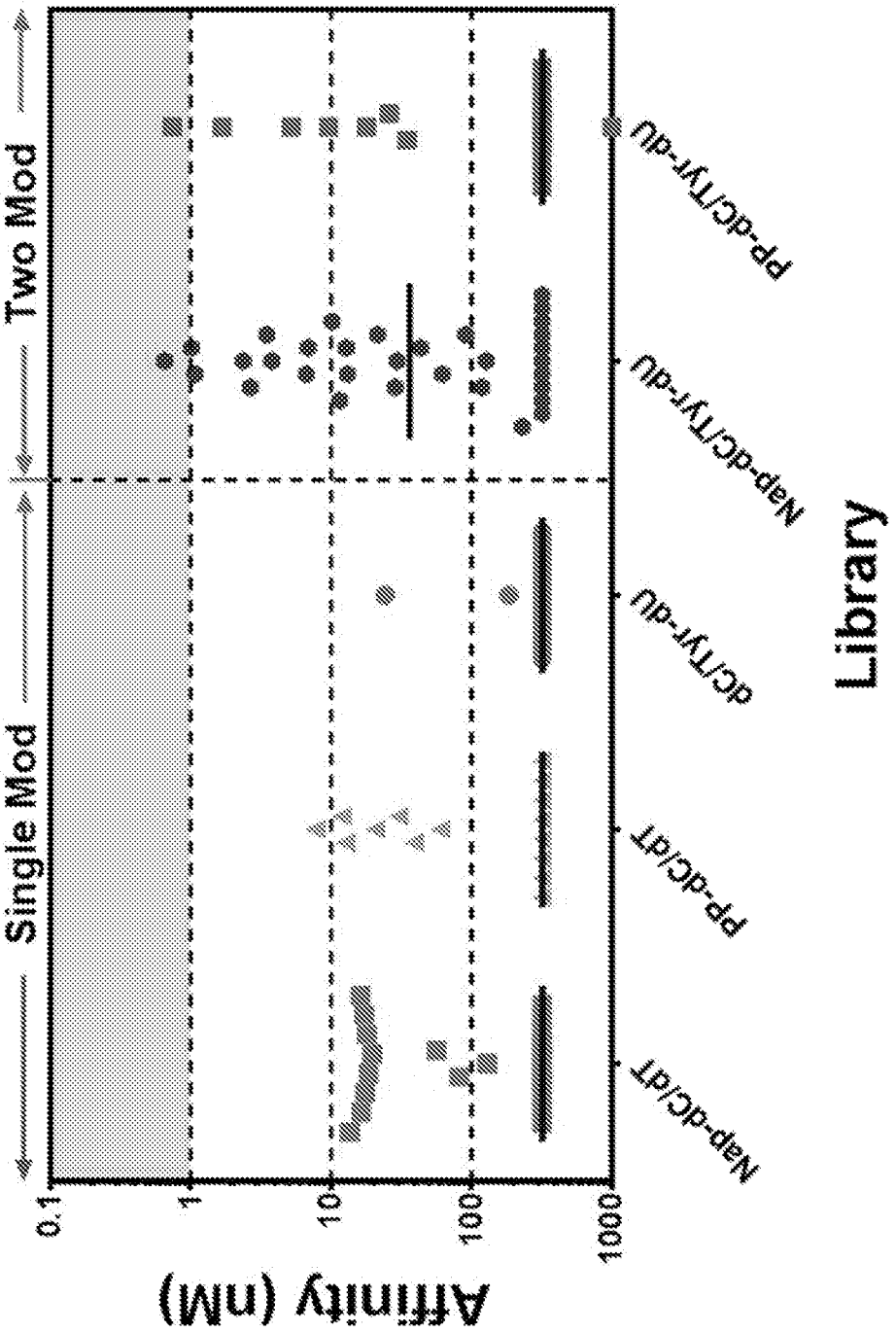

Single Mod
Two Mod
Affinity (nM)
0.1
1
10
100
1000
Nap-dC/dT
PP-dC/dT
dC/Tyr-dU
Nap-dC/Tyr-dU
PP-dC/Tyr-dU
Library

FIG. 19A

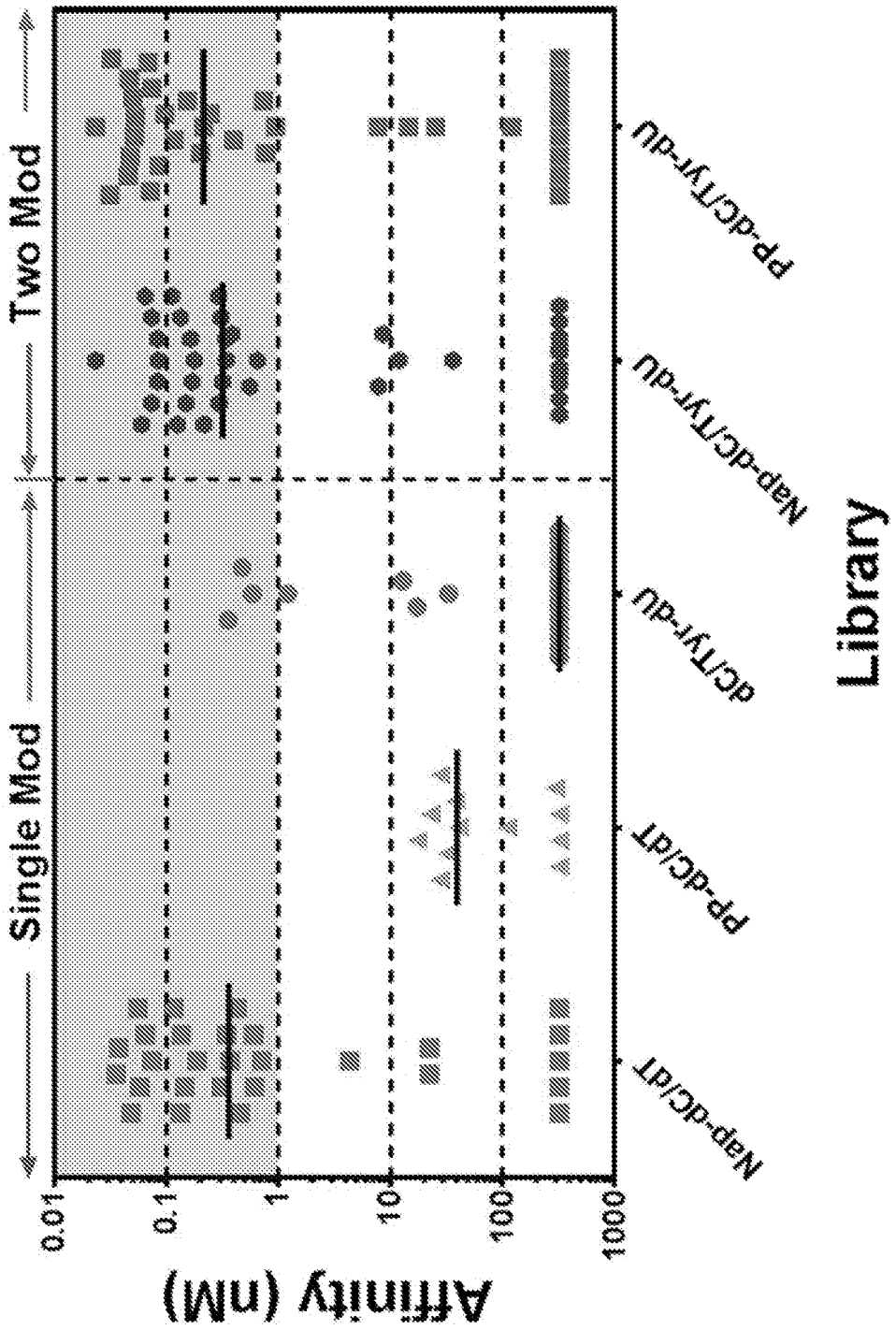

Single Mod
Two Mod
Affinity (nM)
0.01
0.1
1
10
100
1000
Nap-dC/dT
PP-dC/dT
dCTyr-dU
Nap-dCTyr-dU
PP-dCTyr-dU
Library

FIG. 19B

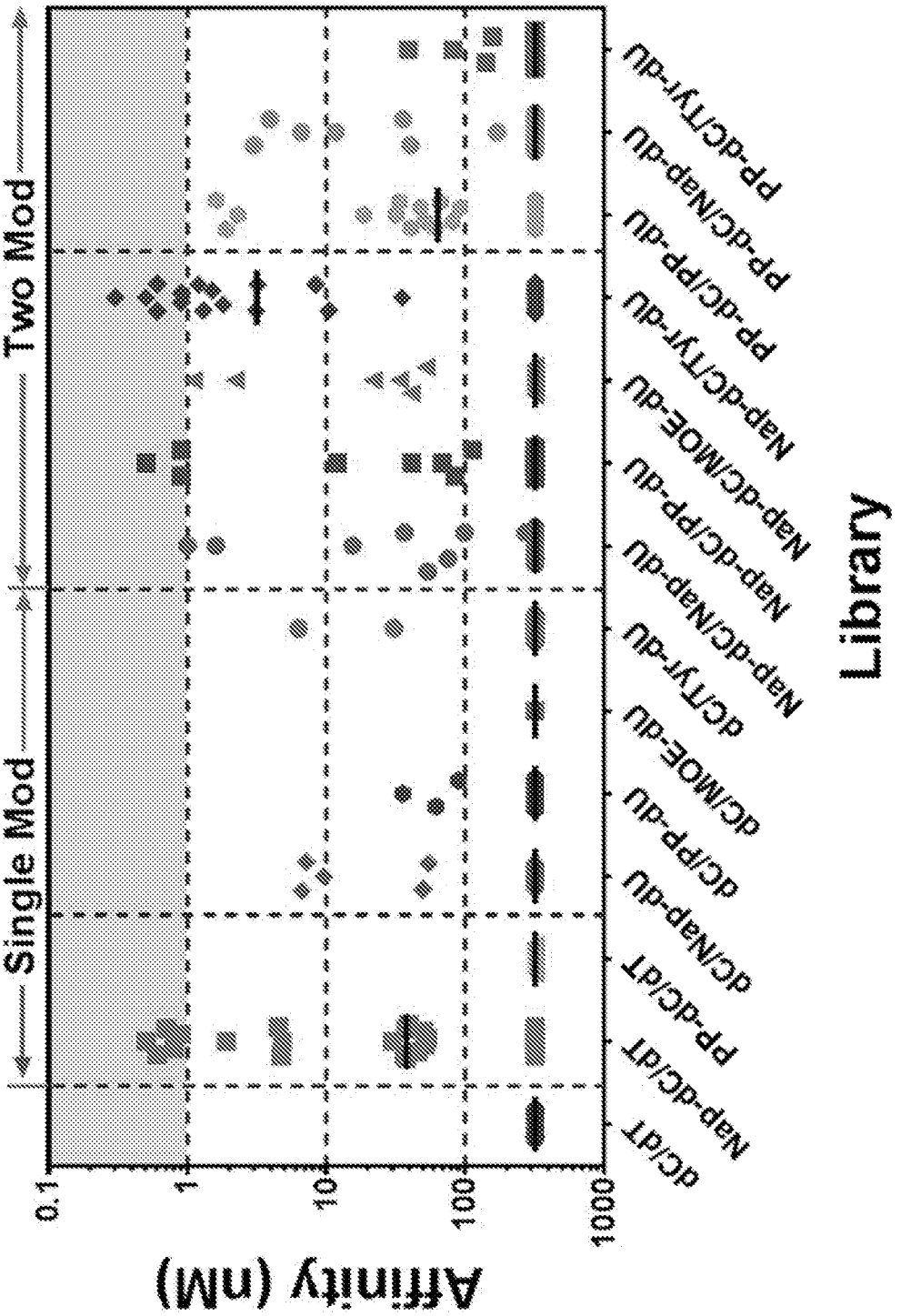
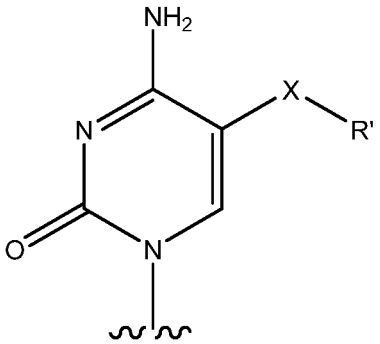
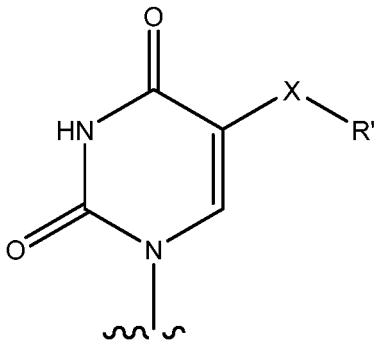

Single Mod
Two Mod
Affinity (nM)
0.1
1
10
100
1000
dC/dT
Nap-dC/dT
PP-dC/dT
dC/Nap-dU
dC/PP-dU
dC/MOE-dU
dC/Tyr-dU
Nap-dC/Nap-dU
Nap-dC/PP-dU
Nap-dC/MOE-dU
Nap-dC/Tyr-dU
PP-dC/PP-dU
PP-dC/Nap-dU
PP-dC/Tyr-dU
Library

FIG. 19C

CYTOSINE



URACIL



 - denotes attachment to backbone of nucleic acid molecules (e.g., phosphate deoxyribose backbone)

X - denotes a linker attached to the 5-position of the pyrimidine molecule (uracil or cytosine)

R' - denotes a moiety attached to the 5-position of the pyrimdine molecule via the linker (X)

FIG. 20

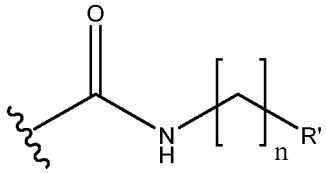
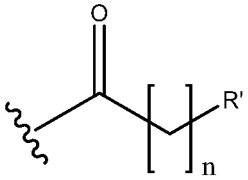
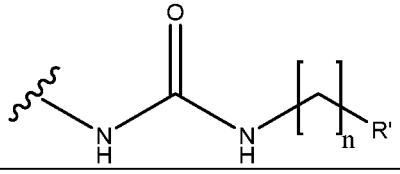
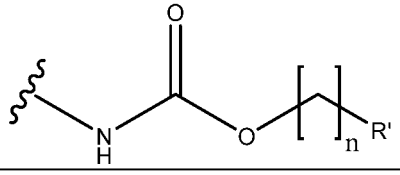
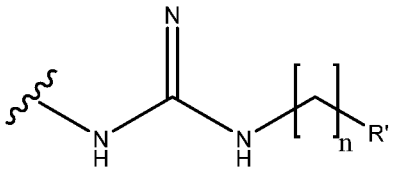
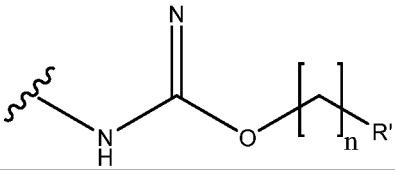
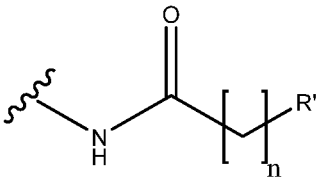
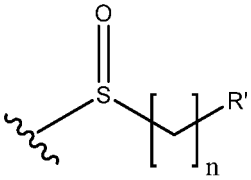
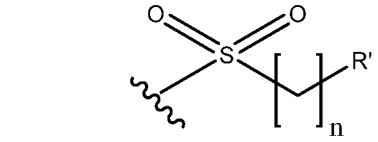
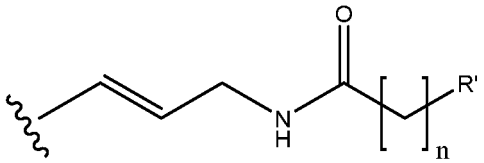
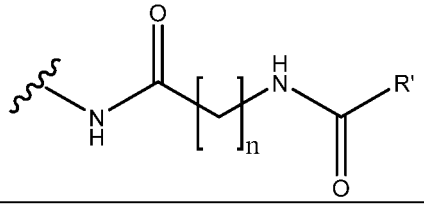
Exemplary Linkers (denoted as X in Figure 20, above) n = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10; R' denotes a moiety ~~~ - denotes attachment to the 5-position of the pyrimidine molecule (uracil or cytosine)









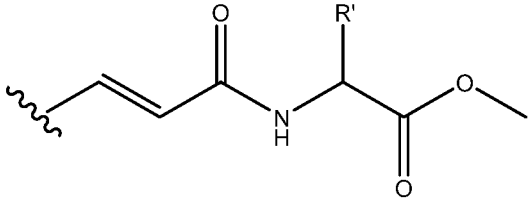
FIG. 20 (cont.)

Exemplary Linkers (denoted as X in Figure 20, above)
n = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10; R' denotes a moiety
~~~ - denotes attachment to the 5-position of the pyrimidine molecule (uracil or cytosine)









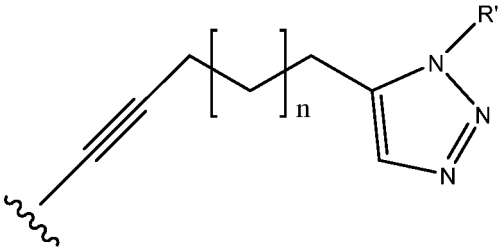


FIG. 20 (cont.)
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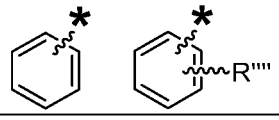
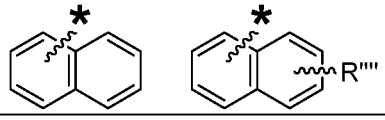
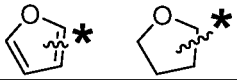
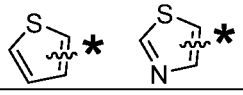
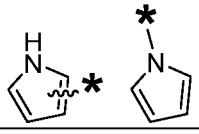
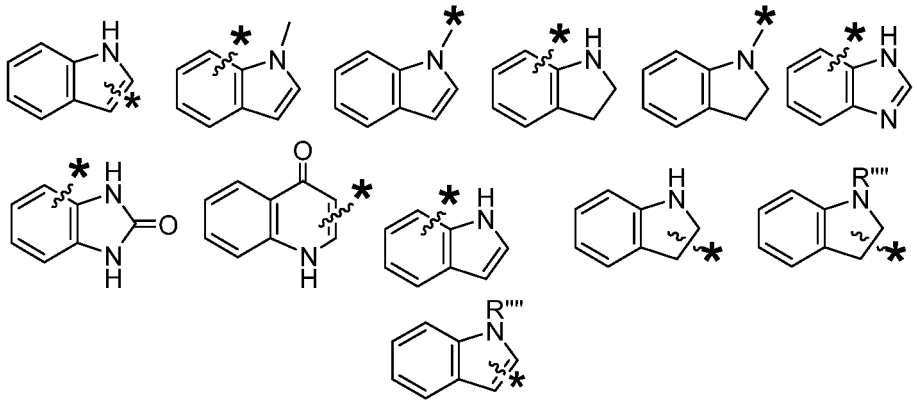
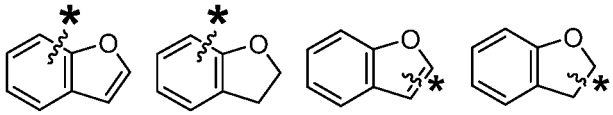
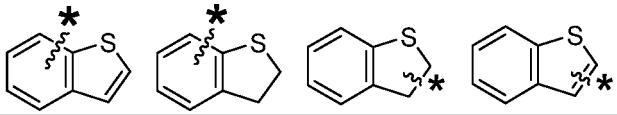
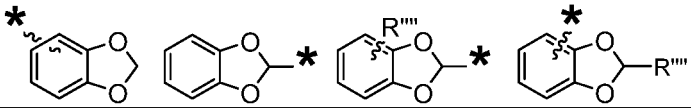
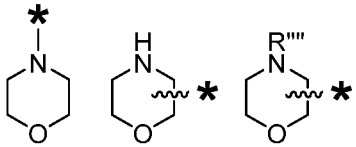
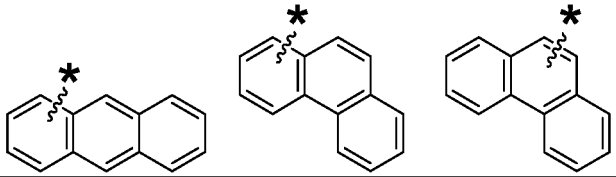
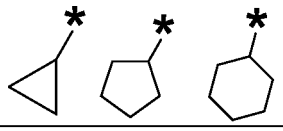
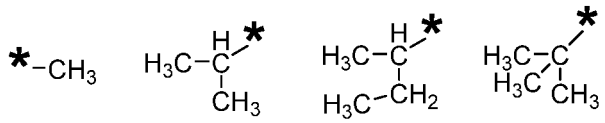
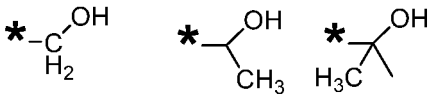
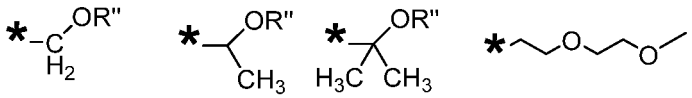
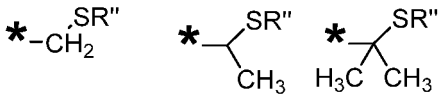
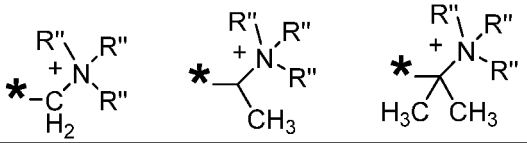
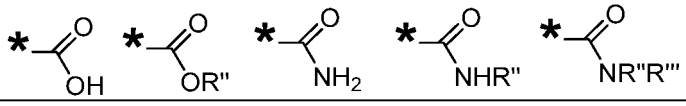
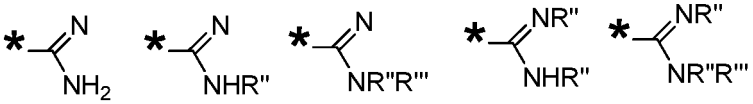
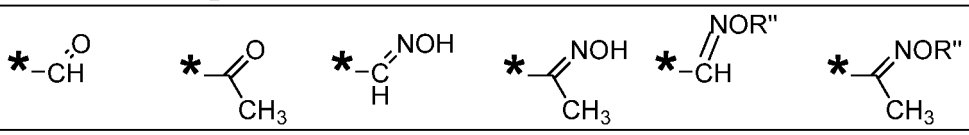
Group	R' Structures (* denotes the point of attachment of the R' group to X (linker))
I	
II	
III	
IV	
V	
VI	
VII	
VIII	
IX	
X	

FIG. 20 (cont.)

Group	R' Structures (* denotes the point of attachment of the R' group to X (linker))
XI	
XII	
XIII	
XIV	
XV	
XVI	
XVII	
XVIII	
XIX	
XX	

R" and R"" are, independently, selected from a halogen (-F, -Cl, -Br, -I); nitrile (-CN); -NH_2; -OH; branched lower alkyl (C1-C20); linear lower alkyl (C1-C20); primary amide; secondary amide; tertiary amide; and alkoxy group.

FIG. 20 (cont.)

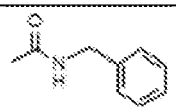
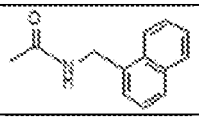
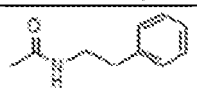
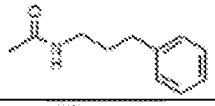
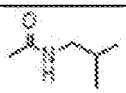
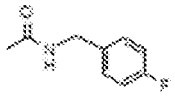
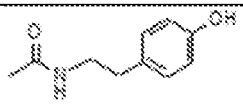
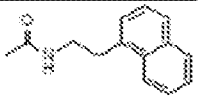
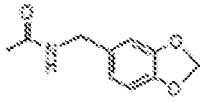
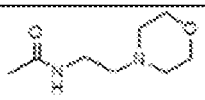
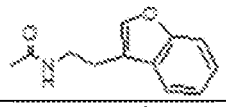
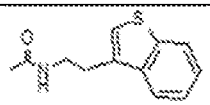
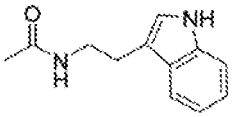
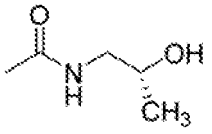
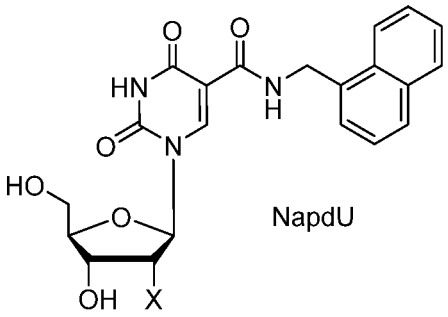
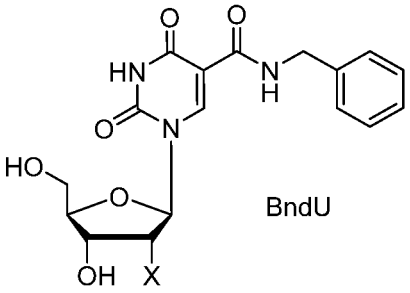
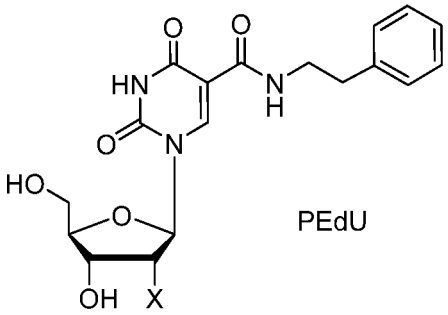
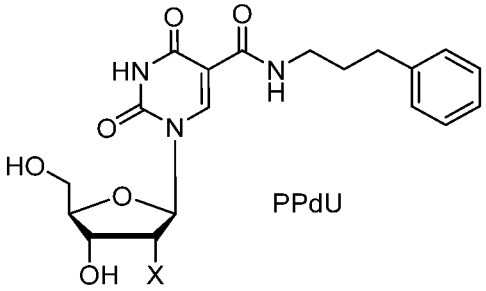
Abbreviation	5-dU Modification Attached via Amide Linkage	Chemical Structure
Bn	benzylmethyl	
Nap	*1*-naphthylmethyl	
PE	*2*-phenylethyl	
PP	*3*-phenylpropyl	
iBu	*iso*-butyl	
FBn	*4*-fluorobenzylmethyl	
2Nap	*2*-naphthylmethyl	
Tyr	tyrosyl	
NE	*1*-naphthylethyl	
MBn	3,4-methylenedioxy benzyl	
MOE	morpholinoethyl	
BF	*3*-benzofuranylethyl	
BT	*3*-benzothiophenylethyl	
Trp	*3*-indole-2-ethyl	
Thr	(R)-2-hydroxypropyl	

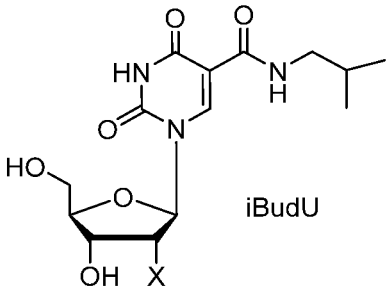
FIG. 21

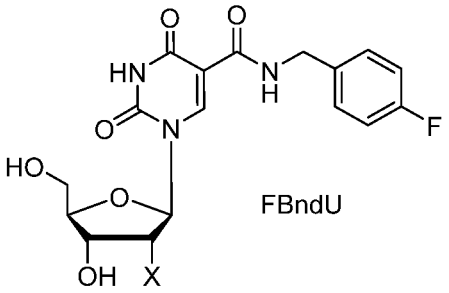


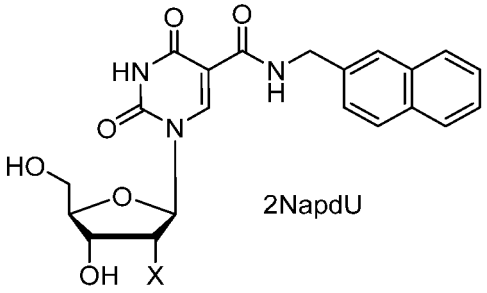












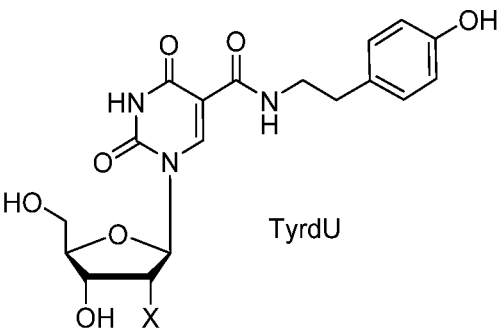
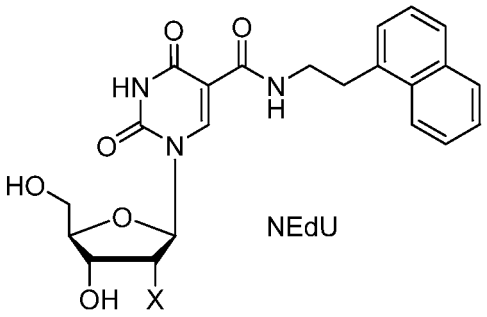
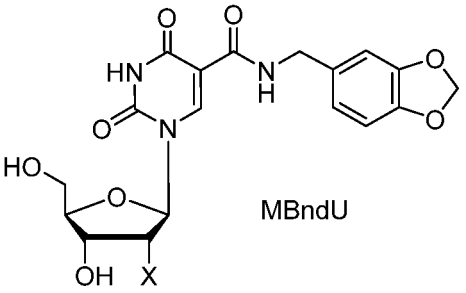
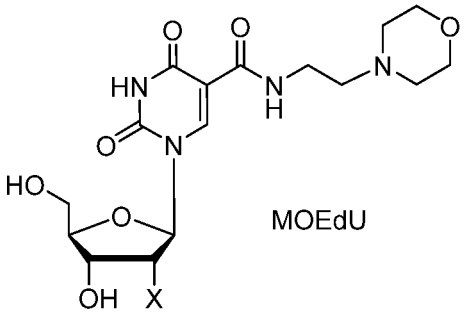
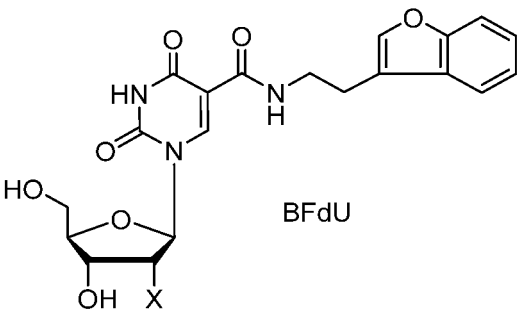


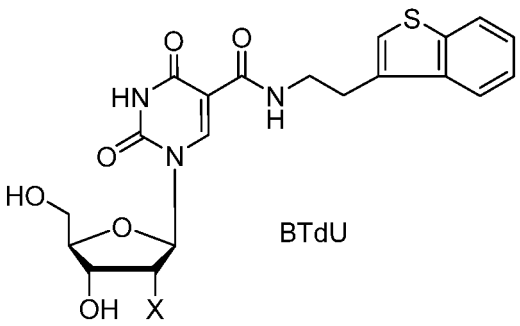
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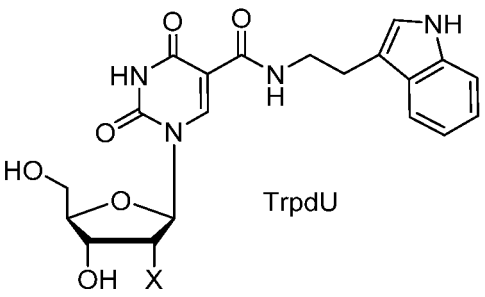












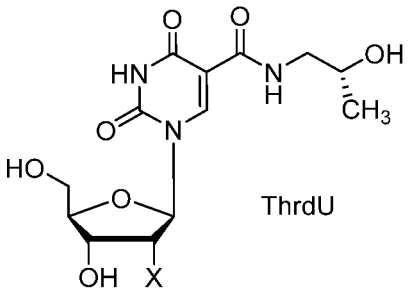


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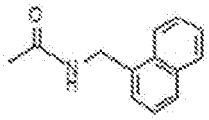
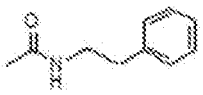
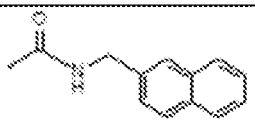
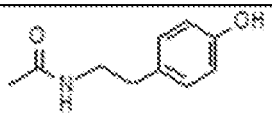
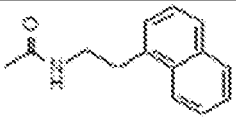
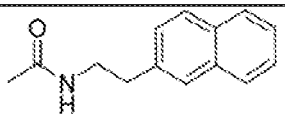
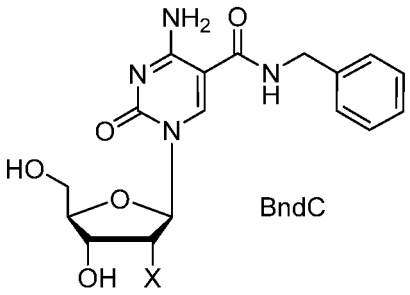
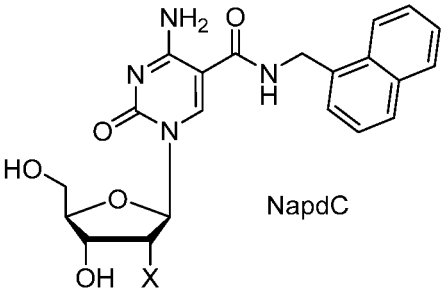
Abbreviation	5-dC Modification Attached via Amide Linkage	Chemical Structure
Bn	benzylmethyl	
Nap	*1*-naphthylmethyl	
PE	*2*-phenylethyl	
PP	*3*-phenylpropyl	
2Nap	*2*-naphthylmethyl	
Tyr	tyrosyl	
NE	*1*-naphthylethyl	
2NE	*2*-naphthyl-2-ethyl	

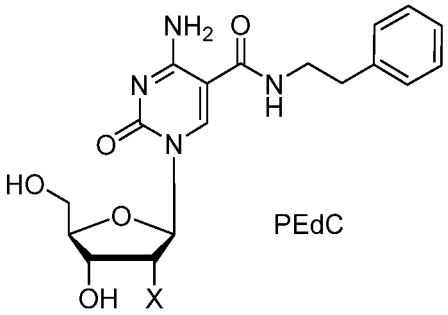
FIG. 22



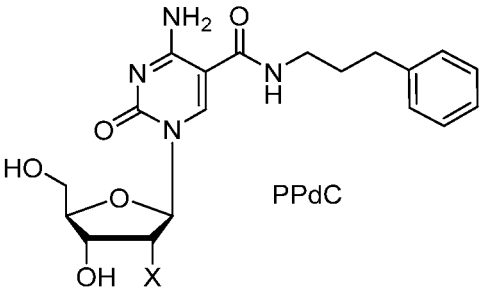
BndC



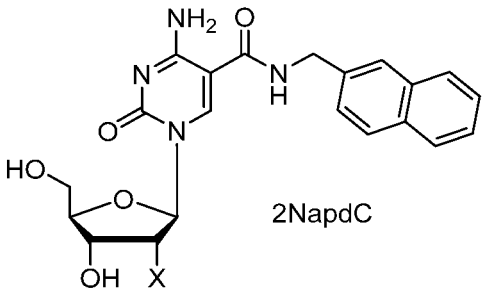
NapdC



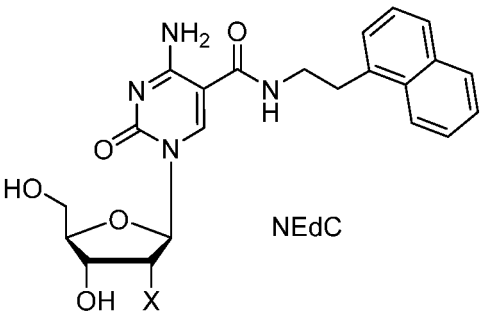
PEdC



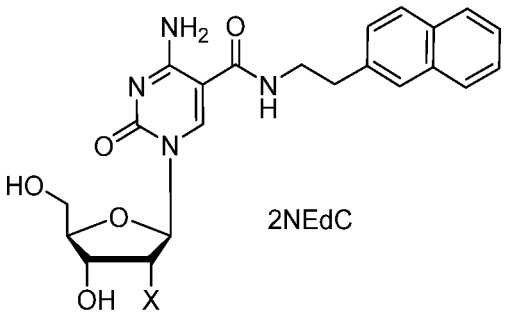
PPdC



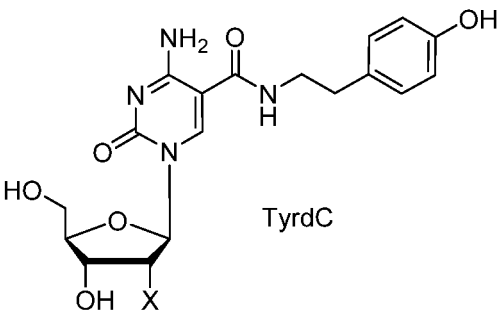
2NapdC



NEdC



2NEdC



TyrdC

FIG. 22 (cont.)