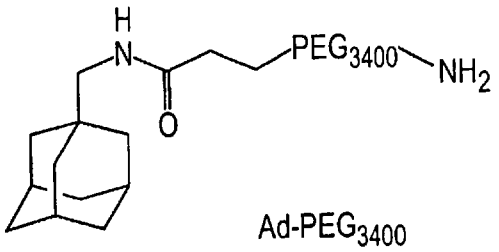
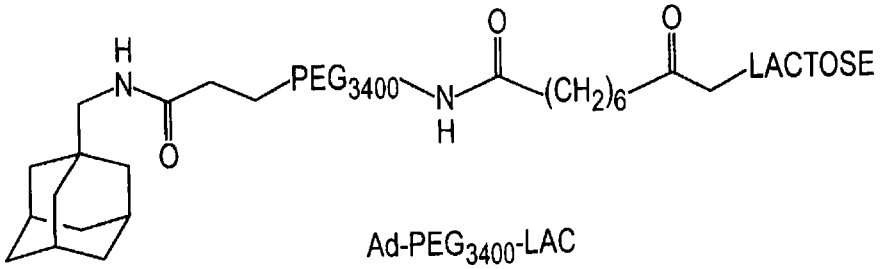
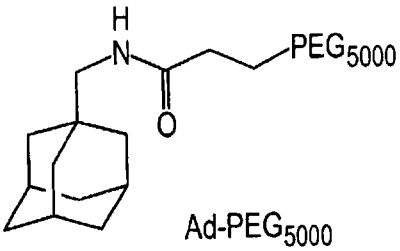
STRUCTURES OF VARIOUS ADAMANTANE-PEG MOLECULES.



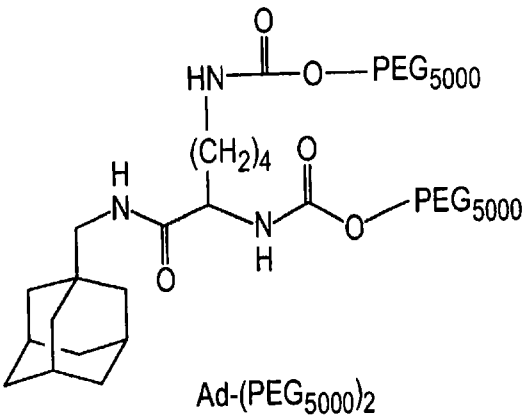
Ad-PEG_{3400}



Ad-PEG_{3400}-LAC



Ad-PEG_{5000}



Ad-$(PEG_{5000})_2$

FIG. 1

163163

G00002

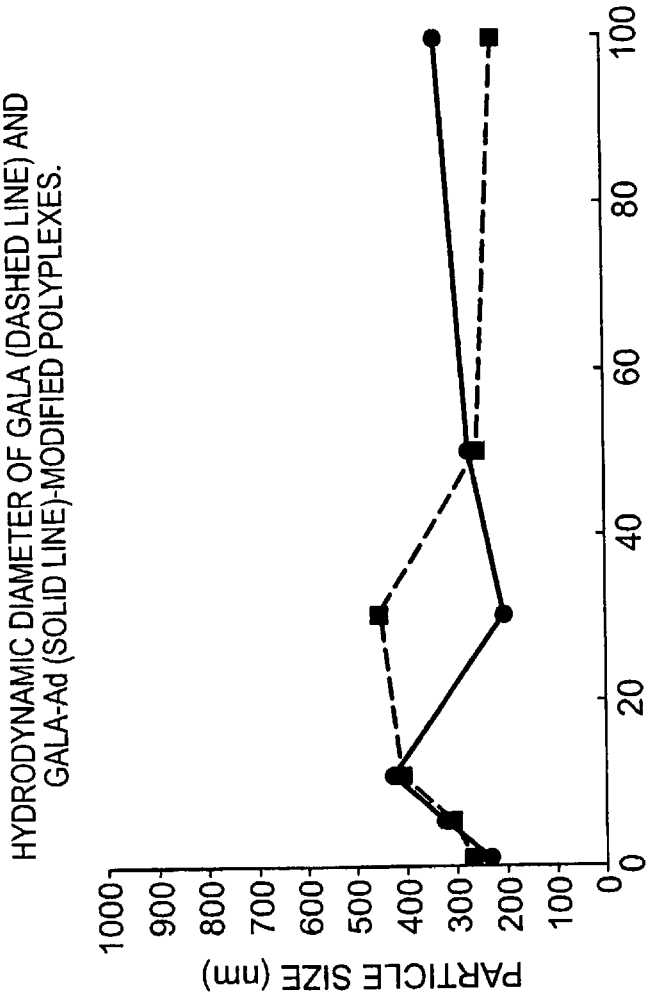

HYDRODYNAMIC DIAMETER OF GALA (DASHED LINE) AND GALA-Ad (SOLID LINE)-MODIFIED POLYPLEXES.
PARTICLE SIZE (nm)
1000
900
800
700
600
500
400
300
200
100
0
0
20
40
60
80
100
% GALA

FIG. 2

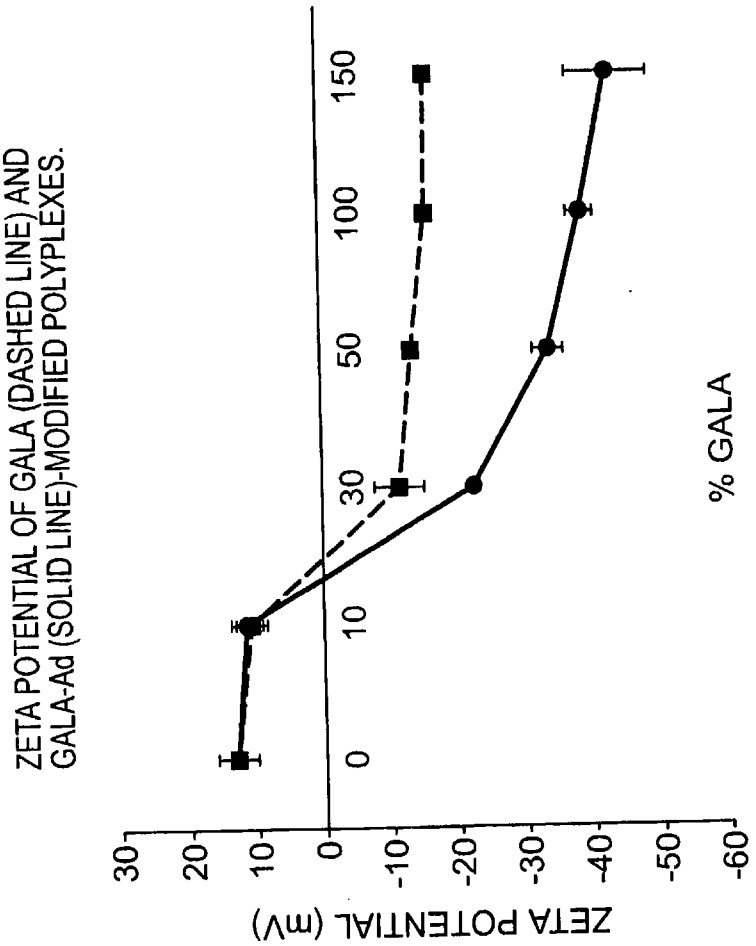
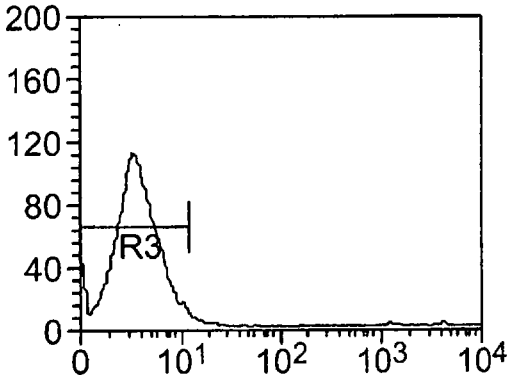

ZETA POTENTIAL OF GALA (DASHED LINE) AND
GALA-Ad (SOLID LINE)-MODIFIED POLYPLEXES.
ZETA POTENTIAL (mV)
30
20
10
0
-10
-20
-30
-40
-50
-60
0
10
30
50
100
150
% GALA

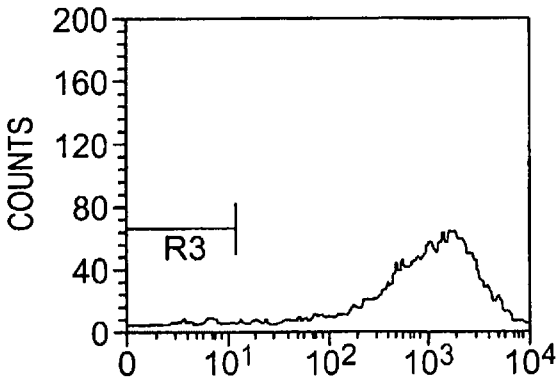
FIG. 3

UPTAKE OF GALA-Ad AND GALA MODIFIED POLYPLEXES BY BHK-21 CELLS.



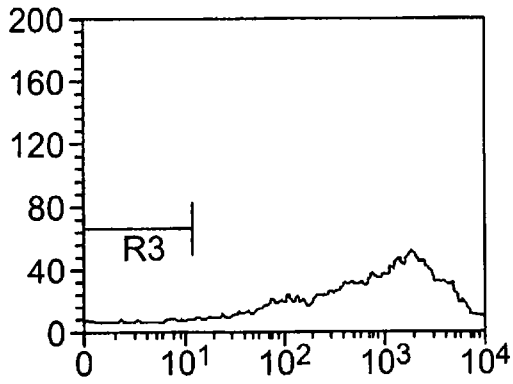
UNTRANSFECTED BHK-21 CELLS

FIG. 4A



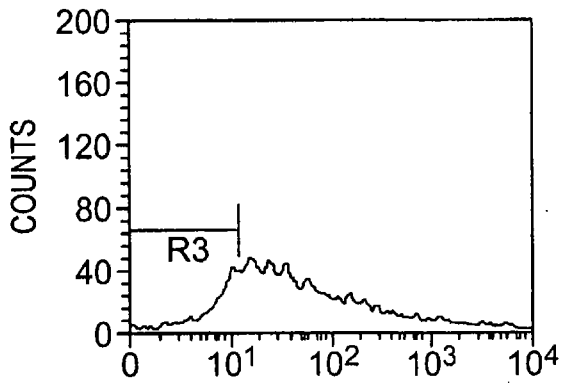
BHK-21 CELLS TRANSFECTED WITH βCDP6/FITC-OLIGO AT 5+/-

FIG. 4B



BHK-21 CELLS TRANSFECTED WITH βCDP6/FITC-OLIGO/50 % GALA

FIG. 4C

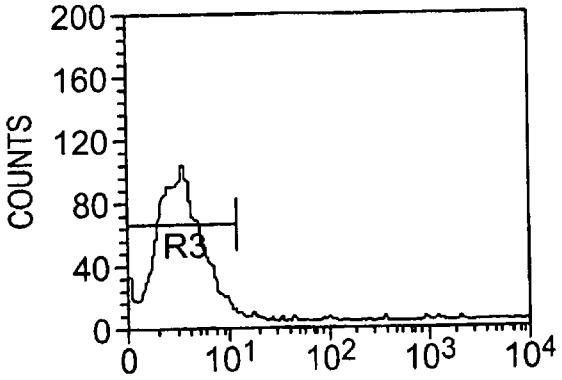


BHK-21 CELLS TRANSFECTED WITH βCDP6/FITC-OLIGO/50 % GALA-AD

FIG. 4D

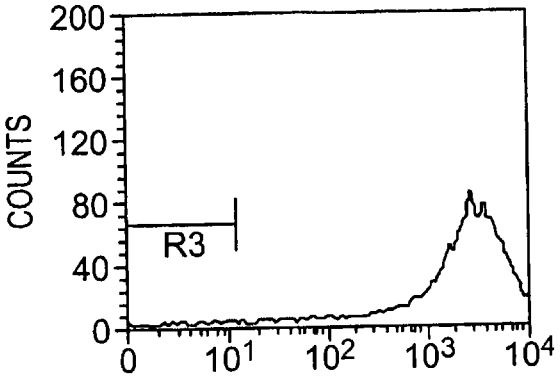
FIG. 4

UPTAKE OF GALA-Ad AND GALA MODIFIED POLYPLEXES BY HUH-7 CELLS



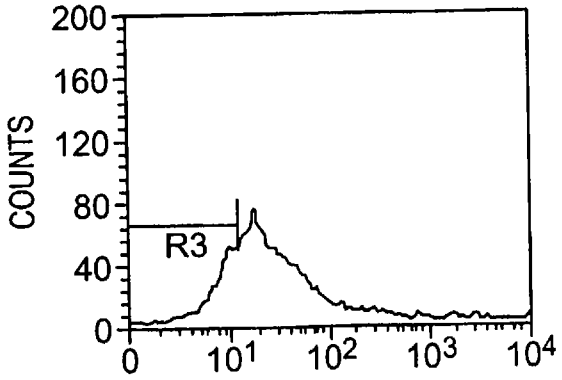
UNTRANSFECTED HUH-7 CELLS

FIG. 5A



HUH-7 TRANSFECTED WITH βCDP6/FITC-OLIGO AT 5+/-

FIG. 5B



HUH-7 TRANSFECTED WITH βCDP6/FITC-OLIGO/ 50 % GALA-Ad

FIG. 5C

FIG. 5

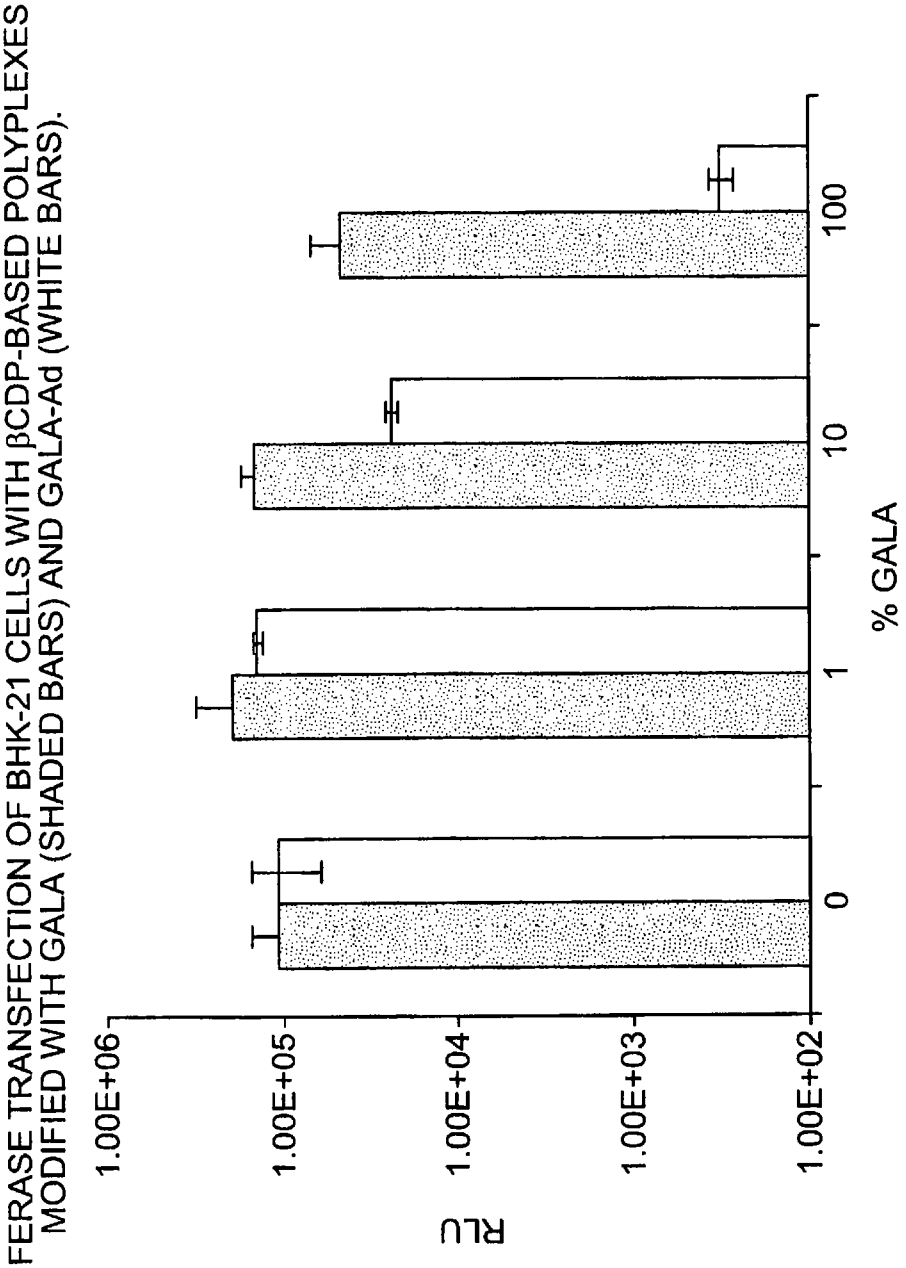

LUCIFERASE TRANSFECTION OF BHK-21 CELLS WITH βCDP-BASED POLYPLEXES MODIFIED WITH GALA (SHADED BARS) AND GALA-Ad (WHITE BARS).
RLU
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
0
1
10
100
% GALA

FIG. 6

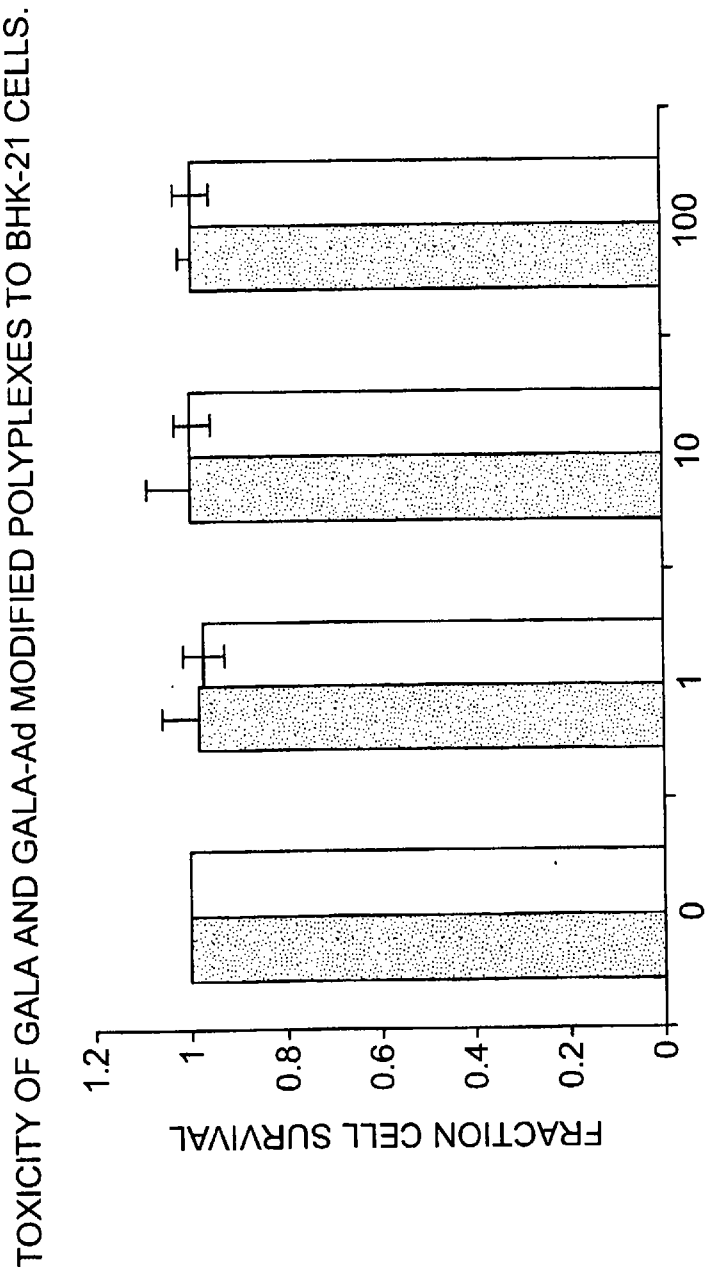

TOXICITY OF GALA AND GALA-Ad MODIFIED POLYPLEXES TO BHK-21 CELLS.
FRACTION CELL SURVIVAL
1.2
1
0.8
0.6
0.4
0.2
0
0
1
10
100
% GALA

FIG. 7

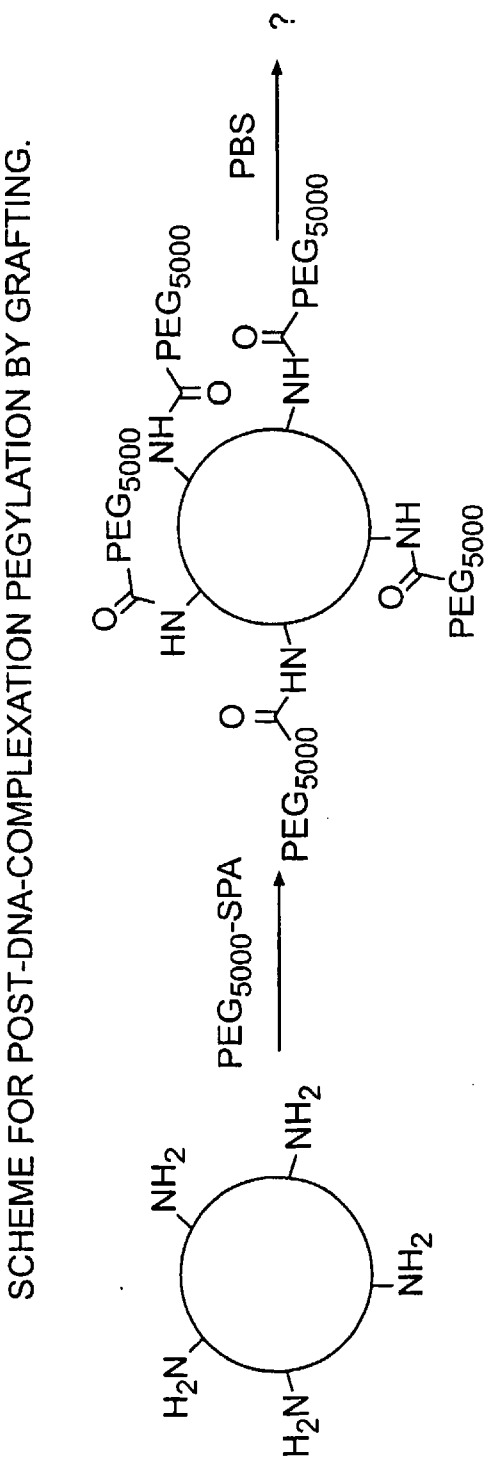

SCHEME FOR POST-DNA-COMPLEXATION PEGYLATION BY GRAFTING.
STAGE 1
PEG5000-SPA
PBS
?
NH2
H2N
PEG5000
NH
HN
O

FIG. 8

PARTICLE SIZES OF PEI AND 12 (βCDP6) POLYPLEXES DURING POST-DNA-COMPLEXATION PEGYLATION OF GRAFTING.

POLYPLEX	PEG	STAGE 1 (nm)	STAGE 2 (nm)	STAGE 3 (nm)
PEI 3+/-	10:1	58	65	115
PEI 6+/-	10:1	55	60	78
βCDP6 5+/-	100%	70	67.4	303
βCDP6 5+/-	150%	70	X*	N/A
βCDP6 5+/-	200%	70	X*	N/A
βCDP6 5+/-	100% PEG**	67	81	700

*POOR CORRELATION FUNCTION; NO SIZE MEASUREMENTS POSSIBLE.
**PEG_{5000} ADDED INSTEAD OF PEG_{5000}-SPA

FIG. 9

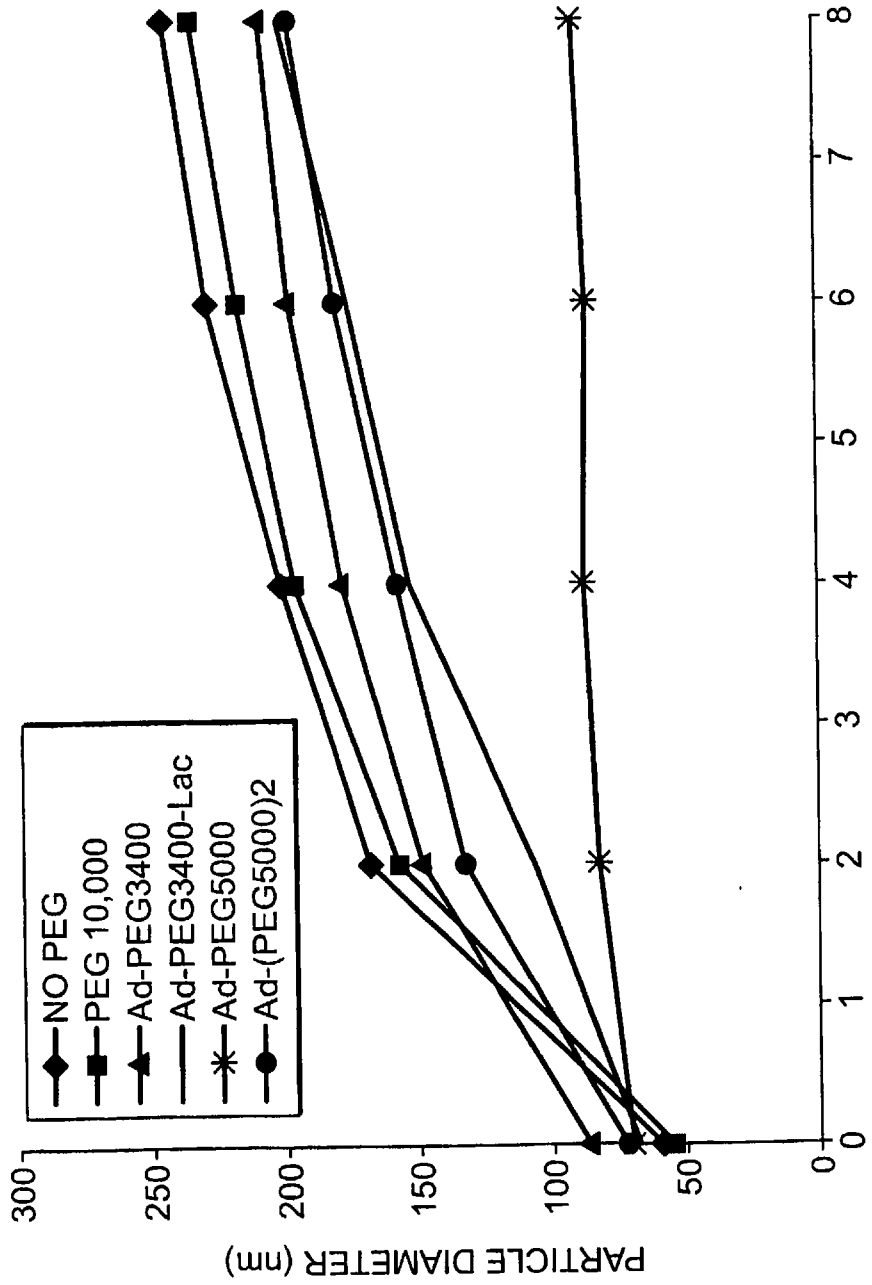

SALT STABILIZATION OF POLYPLEXES BY PEGYLATION.
NO PEG
PEG 10,000
Ad-PEG3400
Ad-PEG3400-Lac
Ad-PEG5000
Ad-(PEG5000)2
PARTICLE DIAMETER (nm)
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
MINUTES AFTER SALT ADDITION

FIG. 10

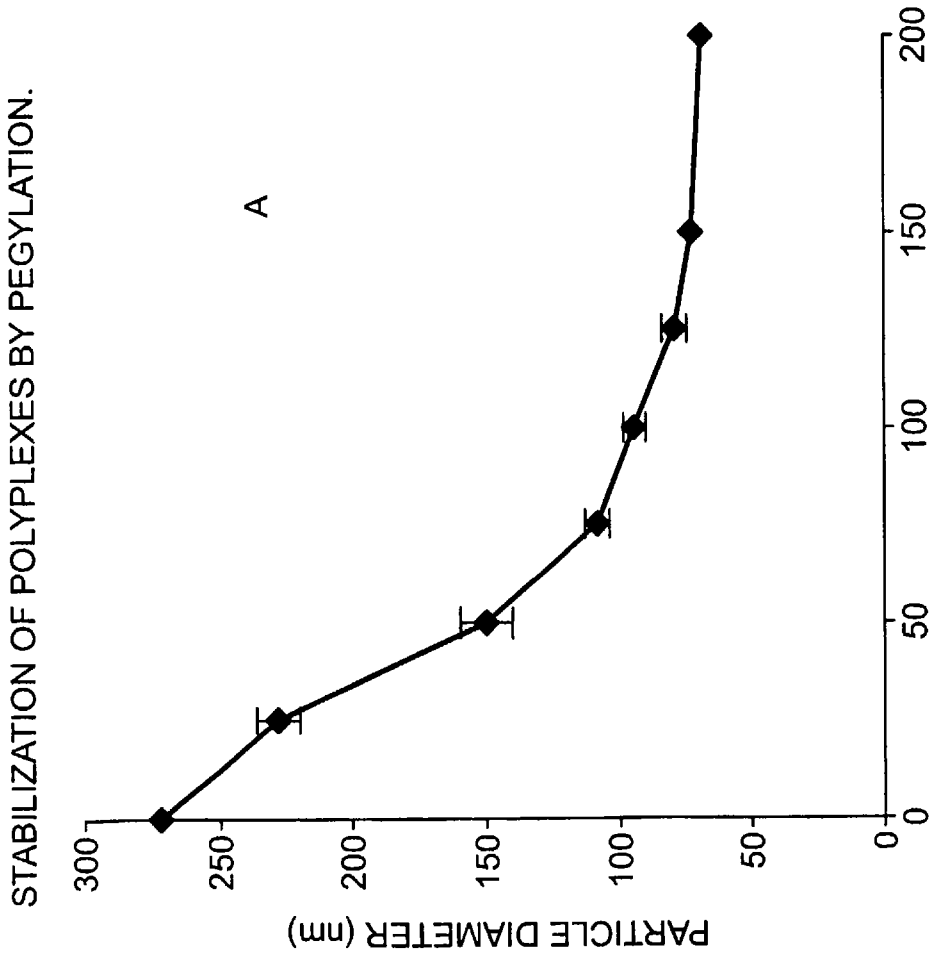
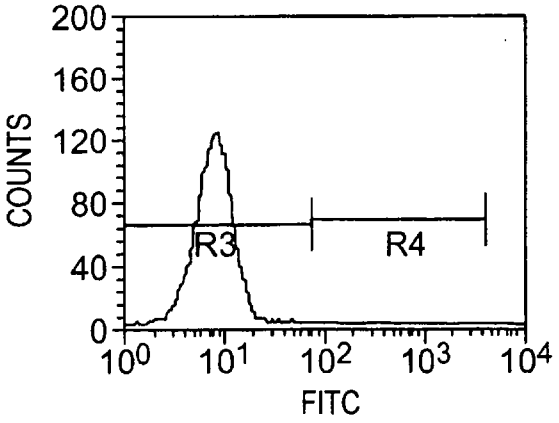

STABILIZATION OF POLYPLEXES BY PEGYLATION.
A
PARTICLE DIAMETER (nm)
300
250
200
150
100
50
0
0
50
100
150
200
% PEG5k-AD

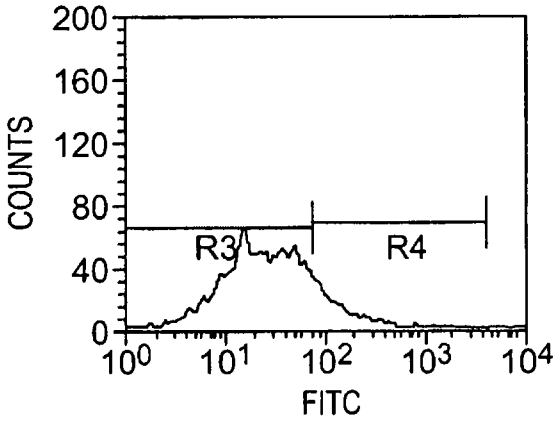
FIG. 10A

CO-DELIVERY OF βCDP6 POLYPLEXES WITH PEG_{3400}-FITC.



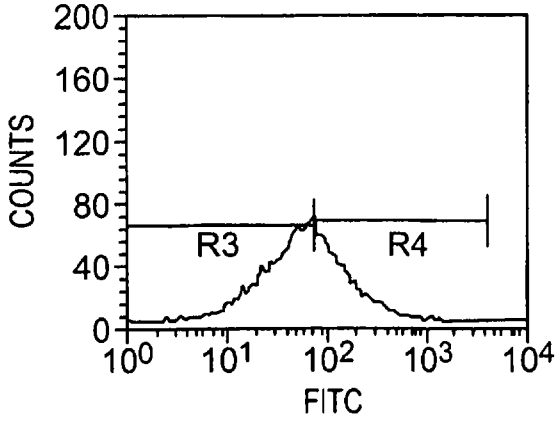
UNTRANSFECTED HUH-7

FIG. 11A



HUH-7 TRANSFECTED WITH βCDP6/OLIGO + FREE PEG_{3400}-FITC

FIG. 11B



HUH-7 TRANSFECTED WITH βCDP6/OLIGO/Ad-PEG_{3400}-FITC

FIG. 11C

FIG. 11

STRUCTURE OF LACTOSE-CDP6

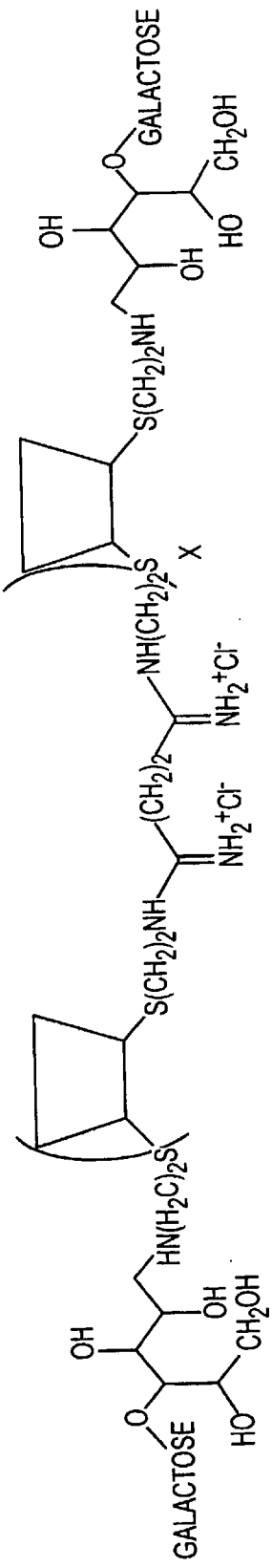


FIG. 12

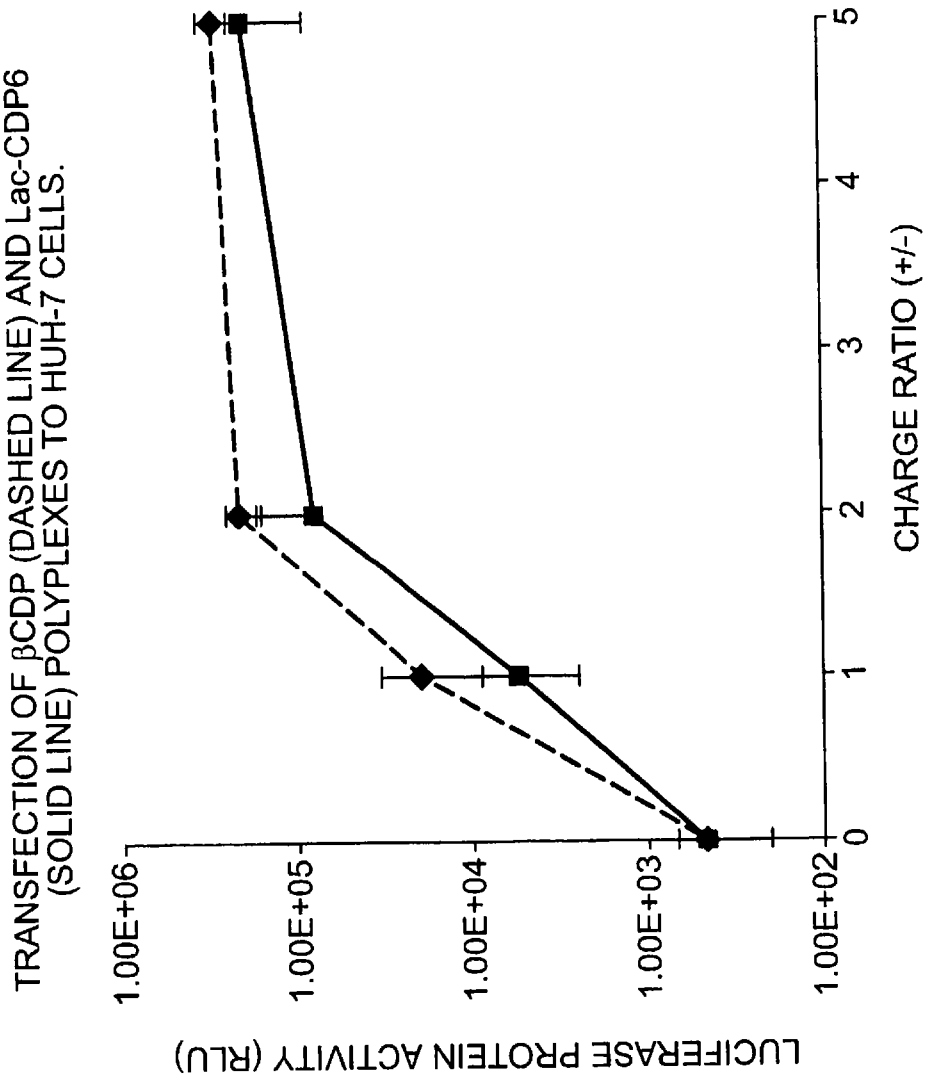

TRANSFECTION OF βCDP (DASHED LINE) AND Lac-CDP6
(SOLID LINE) POLYPLEXES TO HUH-7 CELLS.
LUCIFERASE PROTEIN ACTIVITY (RLU)
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
0
1
2
3
4
5
CHARGE RATIO (+/-)

FIG. 13

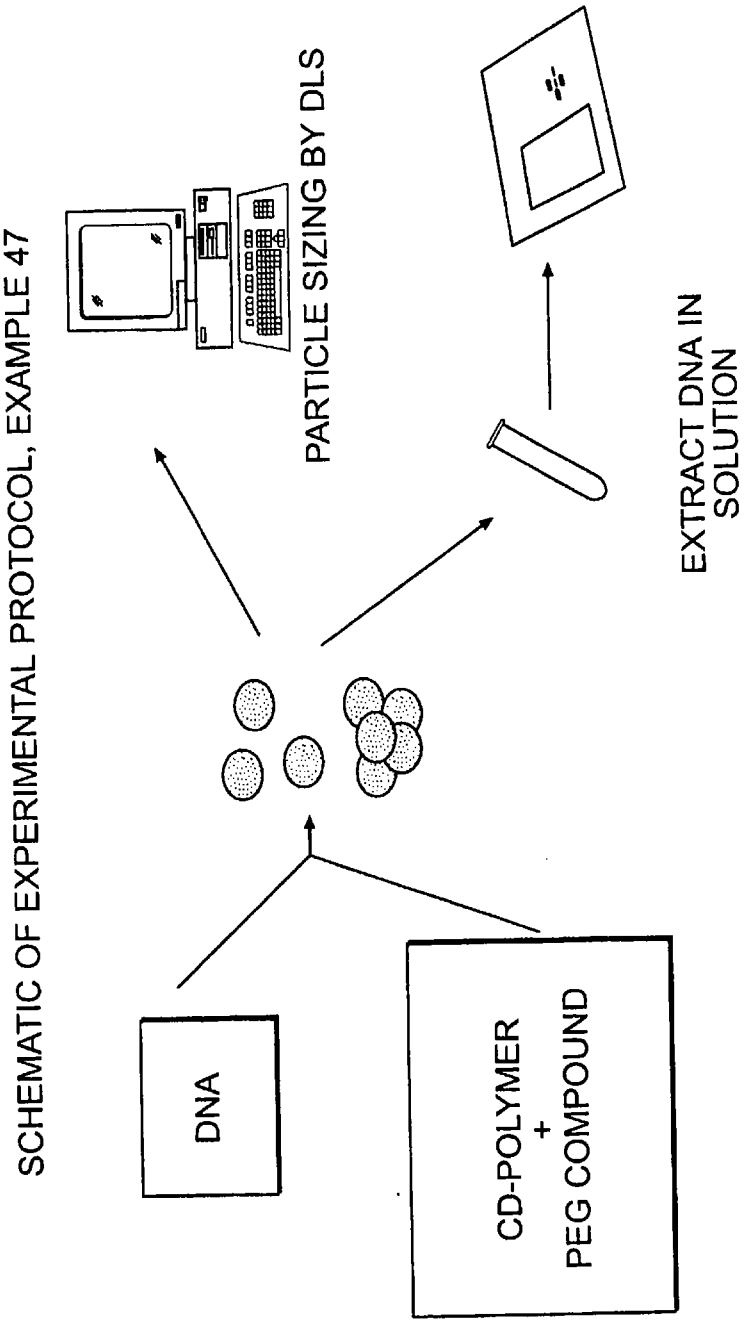

SCHEMATIC OF EXPERIMENTAL PROTOCOL, EXAMPLE 47
DNA
CD-POLYMER
+
PEG COMPOUND
PARTICLE SIZING BY DLS
EXTRACT DNA IN
SOLUTION

FIG. 14

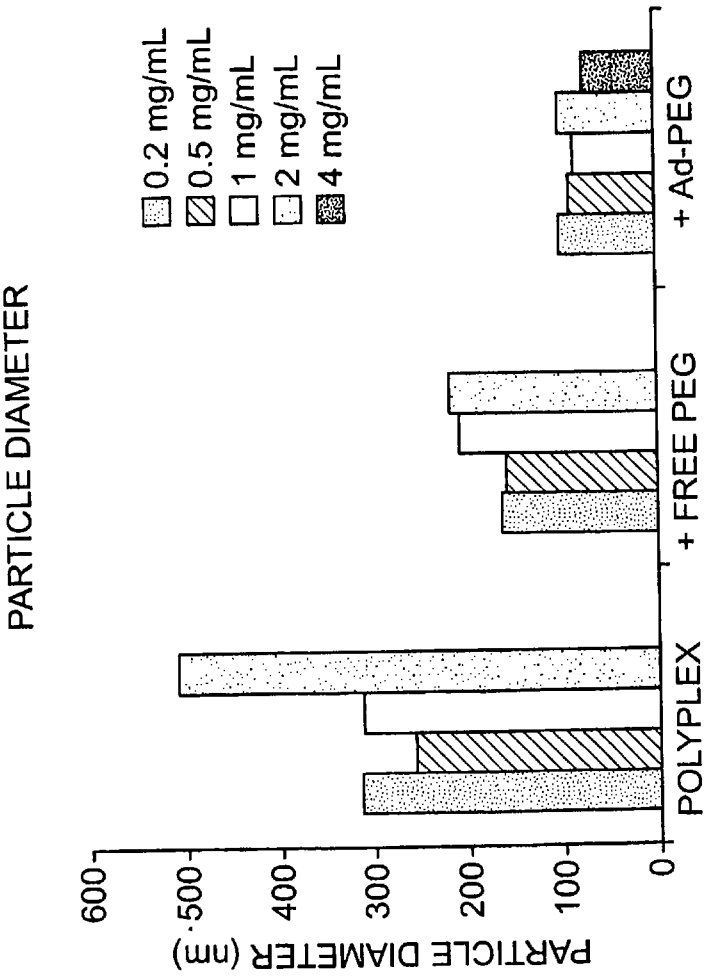

PARTICLE DIAMETER
0.2 mg/mL
0.5 mg/mL
1 mg/mL
2 mg/mL
4 mg/mL
PARTICLE DIAMETER (nm)
600
500
400
300
200
100
0
POLYPLEX
+ FREE PEG
+ Ad-PEG

FIG. 15

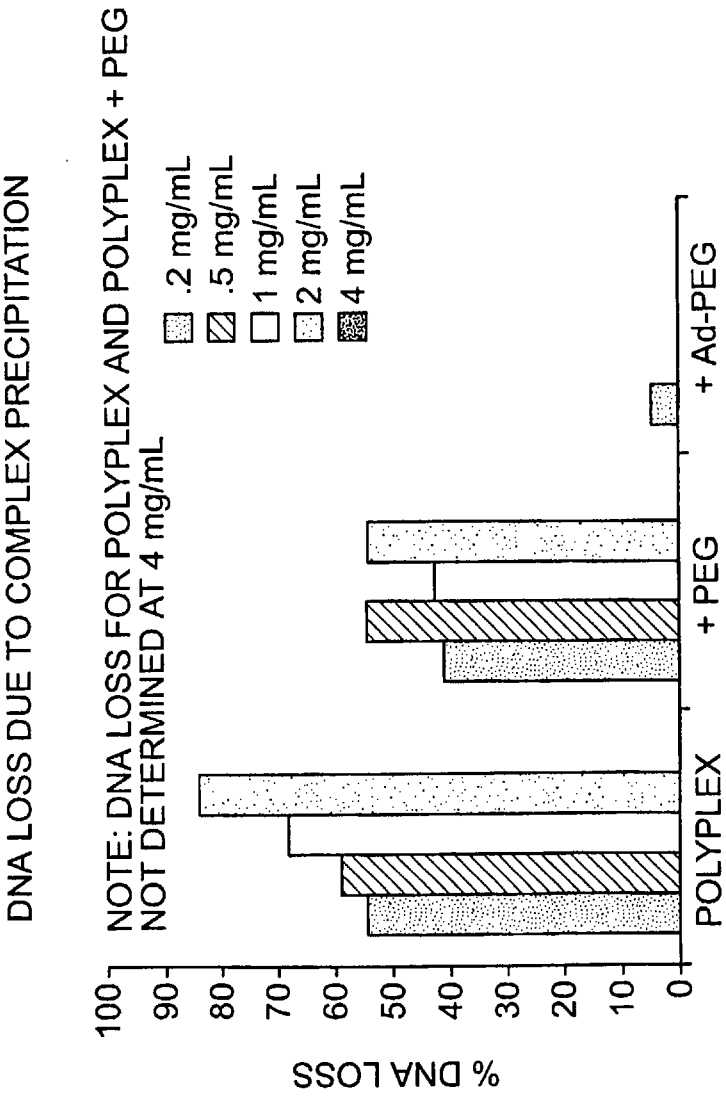

DNA LOSS DUE TO COMPLEX PRECIPITATION
NOTE: DNA LOSS FOR POLYPLEX AND POLYPLEX + PEG
NOT DETERMINED AT 4 mg/mL
.2 mg/mL
.5 mg/mL
1 mg/mL
2 mg/mL
4 mg/mL
% DNA LOSS
100
90
80
70
60
50
40
30
20
10
0
POLYPLEX
+ PEG
+ Ad-PEG

FIG. 16

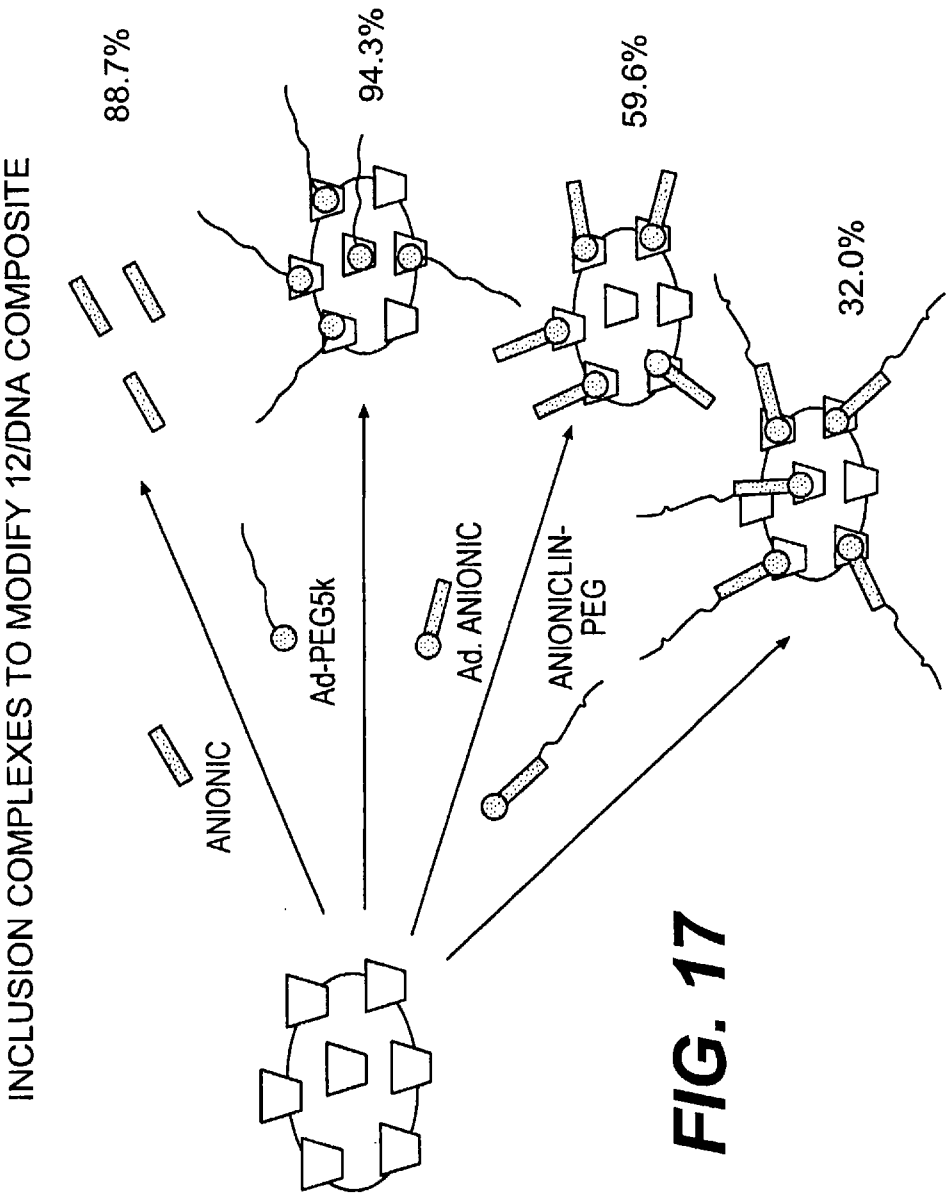

INCLUSION COMPLEXES TO MODIFY 12/DNA COMPOSITE
88.7%
94.3%
59.6%
32.0%
ANIONIC
Ad-PEG5k
Ad. ANIONIC
ANIONICLIN-PEG

FIG. 17

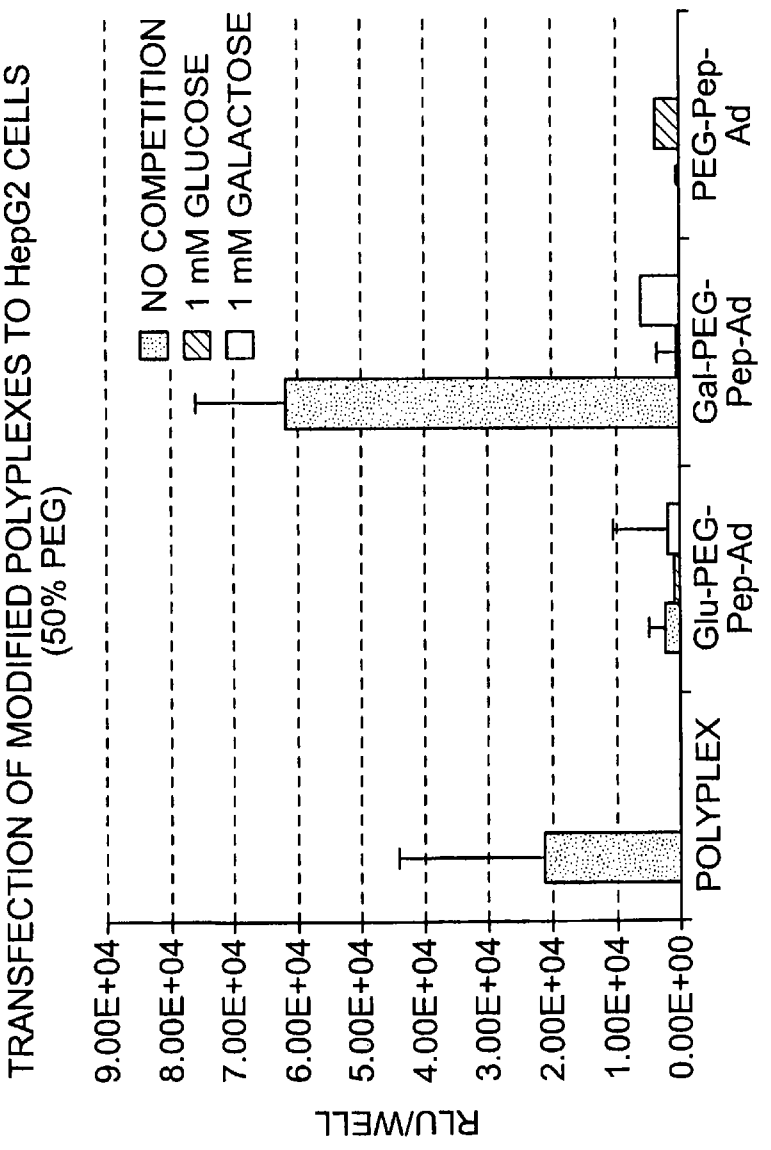

TRANSFECTION OF MODIFIED POLYPLEXES TO HepG2 CELLS
(50% PEG)
NO COMPETITION
1 mM GLUCOSE
1 mM GALACTOSE
RLU/WELL
9.00E+04
8.00E+04
7.00E+04
6.00E+04
5.00E+04
4.00E+04
3.00E+04
2.00E+04
1.00E+04
0.00E+00
POLYPLEX
Glu-PEG-Pep-Ad
Gal-PEG-Pep-Ad
PEG-Pep-Ad

FIG. 18

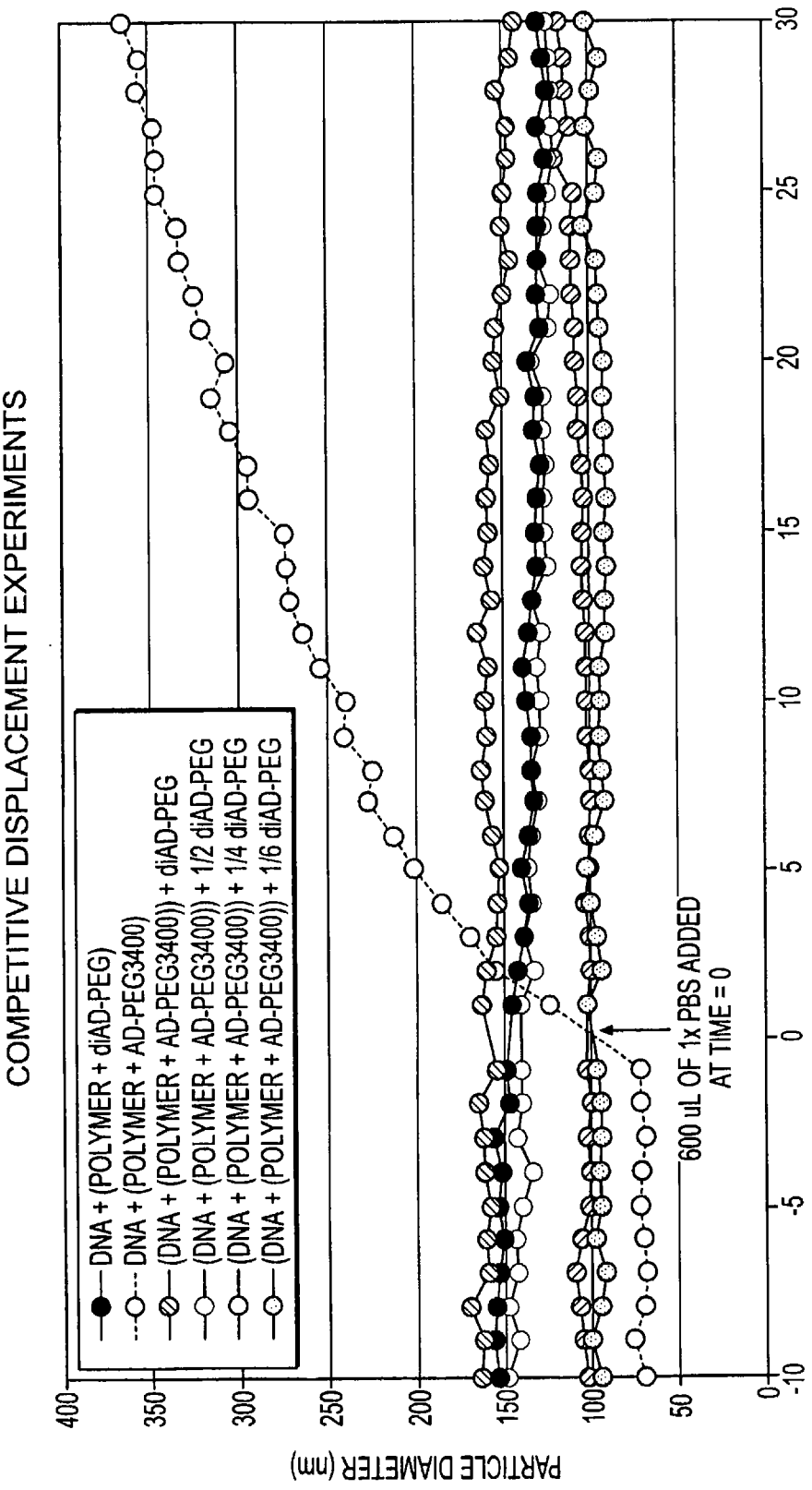

COMPETITIVE DISPLACEMENT EXPERIMENTS
DNA + (POLYMER + diAD-PEG)
DNA + (POLYMER + AD-PEG3400)
(DNA + (POLYMER + AD-PEG3400)) + diAD-PEG
(DNA + (POLYMER + AD-PEG3400)) + 1/2 diAD-PEG
(DNA + (POLYMER + AD-PEG3400)) + 1/4 diAD-PEG
(DNA + (POLYMER + AD-PEG3400)) + 1/6 diAD-PEG
600 uL OF 1x PBS ADDED AT TIME = 0
PARTICLE DIAMETER (nm)
TIME (MINUTES)
400
350
300
250
200
150
100
50
0
-10
-5
0
5
10
15
20
25
30

FIG. 19

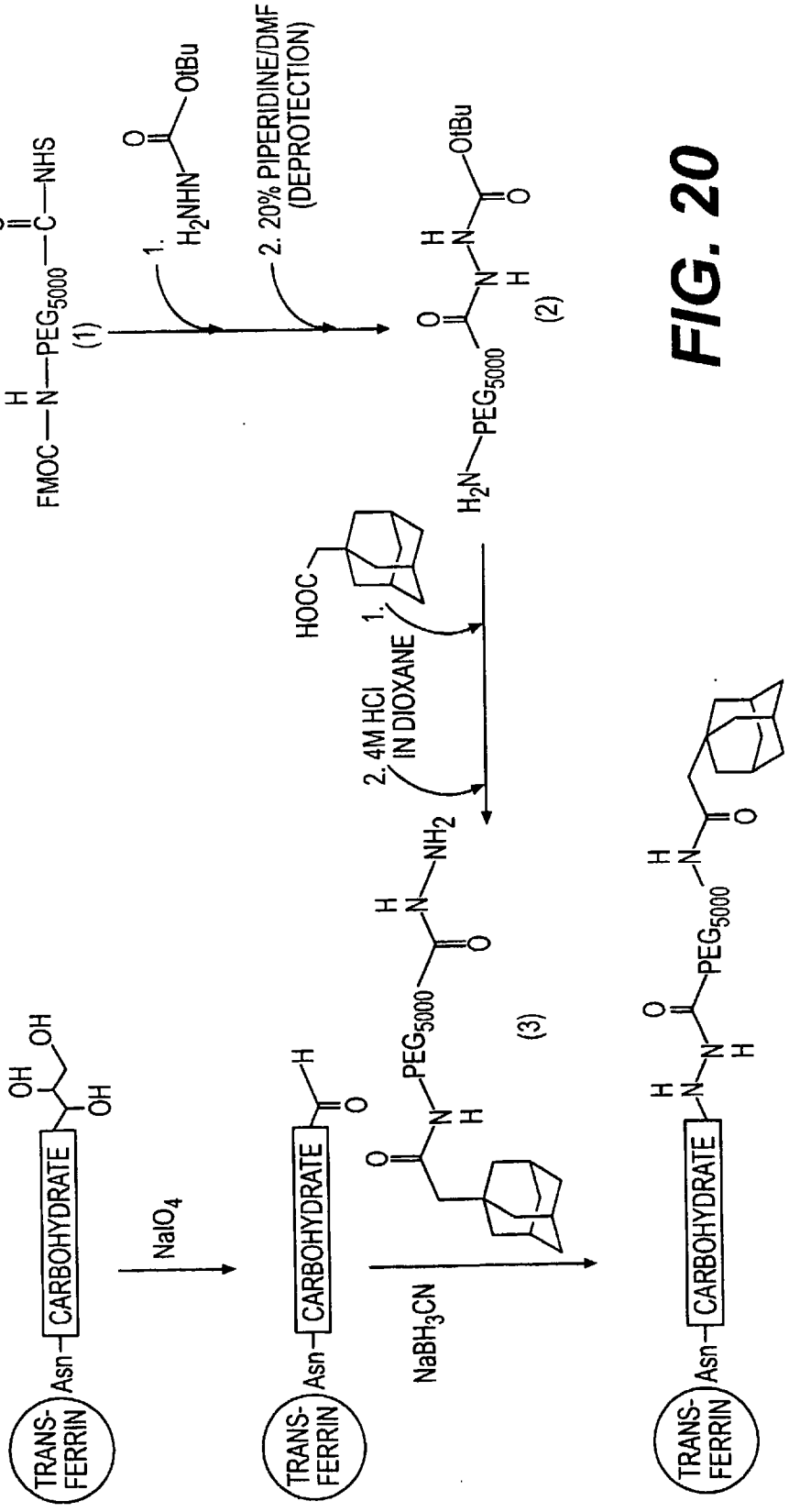

SYNTHESIS OF ADAMANTANE-PEG-TRANSFERRIN (Ad-PEG-Tf)
FMOC—N—PEG5000—C—NHS
(1)
1. H2NHN—OtBu
2. 20% PIPERIDINE/DMF (DEPROTECTION)
(2)
HOOC
1.
2. 4M HCl IN DIOXANE
(3)
NaIO4
NaBH3CN
CARBOHYDRATE
Asn
TRANS-FERRIN

FIG. 20

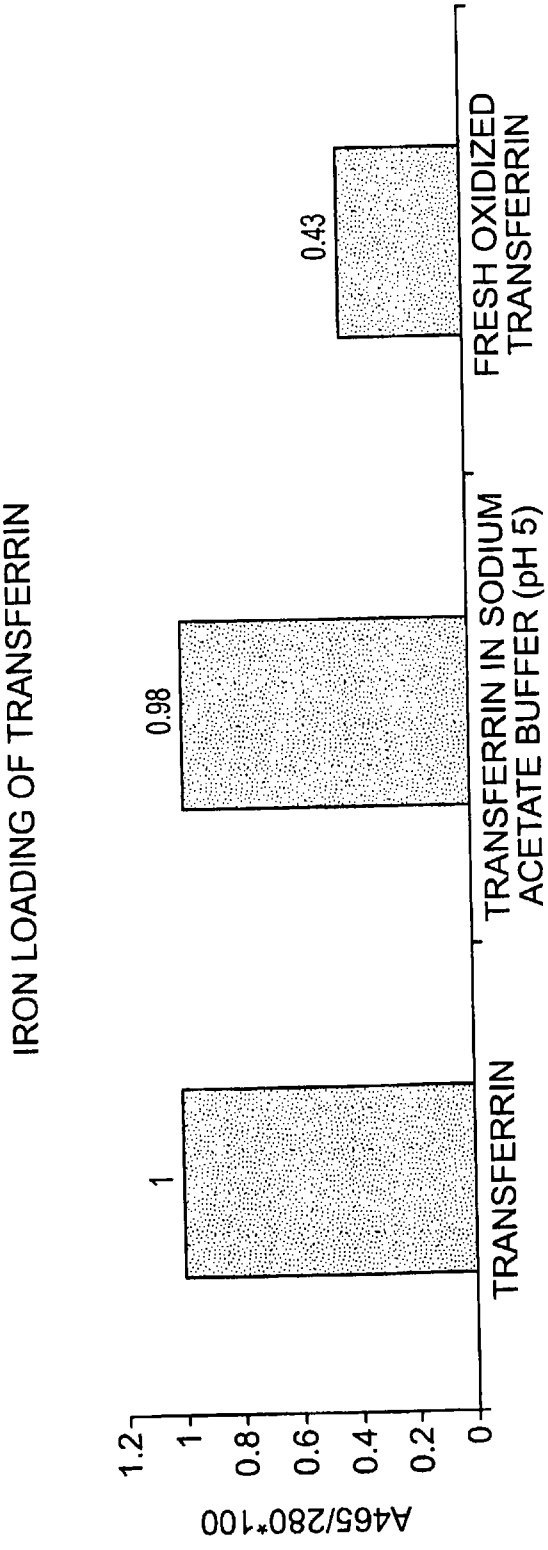

IRON LOADING OF TRANSFERRIN
A465/280*100
1.2
1
0.8
0.6
0.4
0.2
0
1
0.98
0.43
TRANSFERRIN
TRANSFERRIN IN SODIUM ACETATE BUFFER (pH 5)
FRESH OXIDIZED TRANSFERRIN

FIG. 21

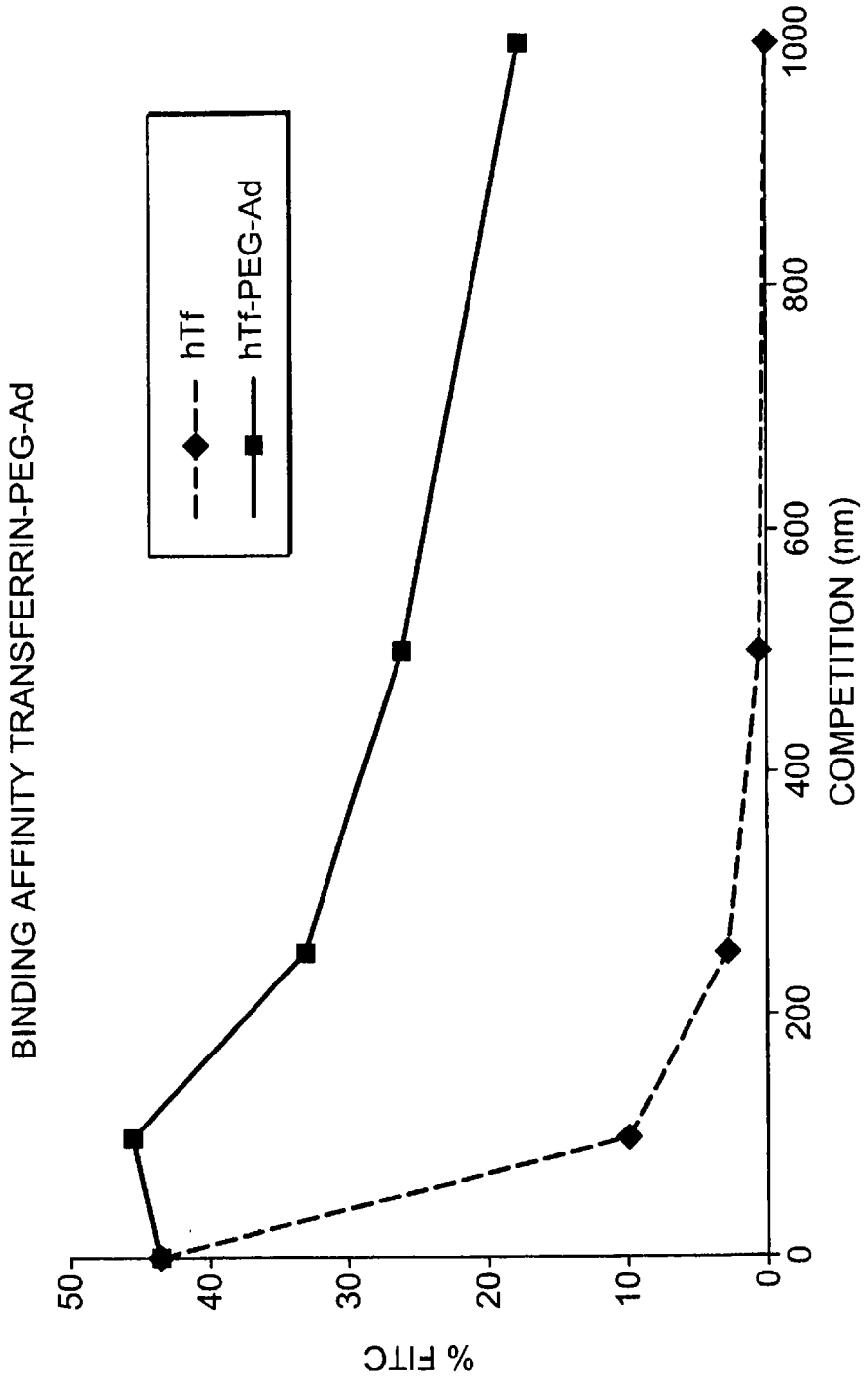

BINDING AFFINITY TRANSFERRIN-PEG-Ad
hTf
hTf-PEG-Ad
% FITC
50
40
30
20
10
0
0
200
400
600
800
1000
COMPETITION (nm)

FIG. 22

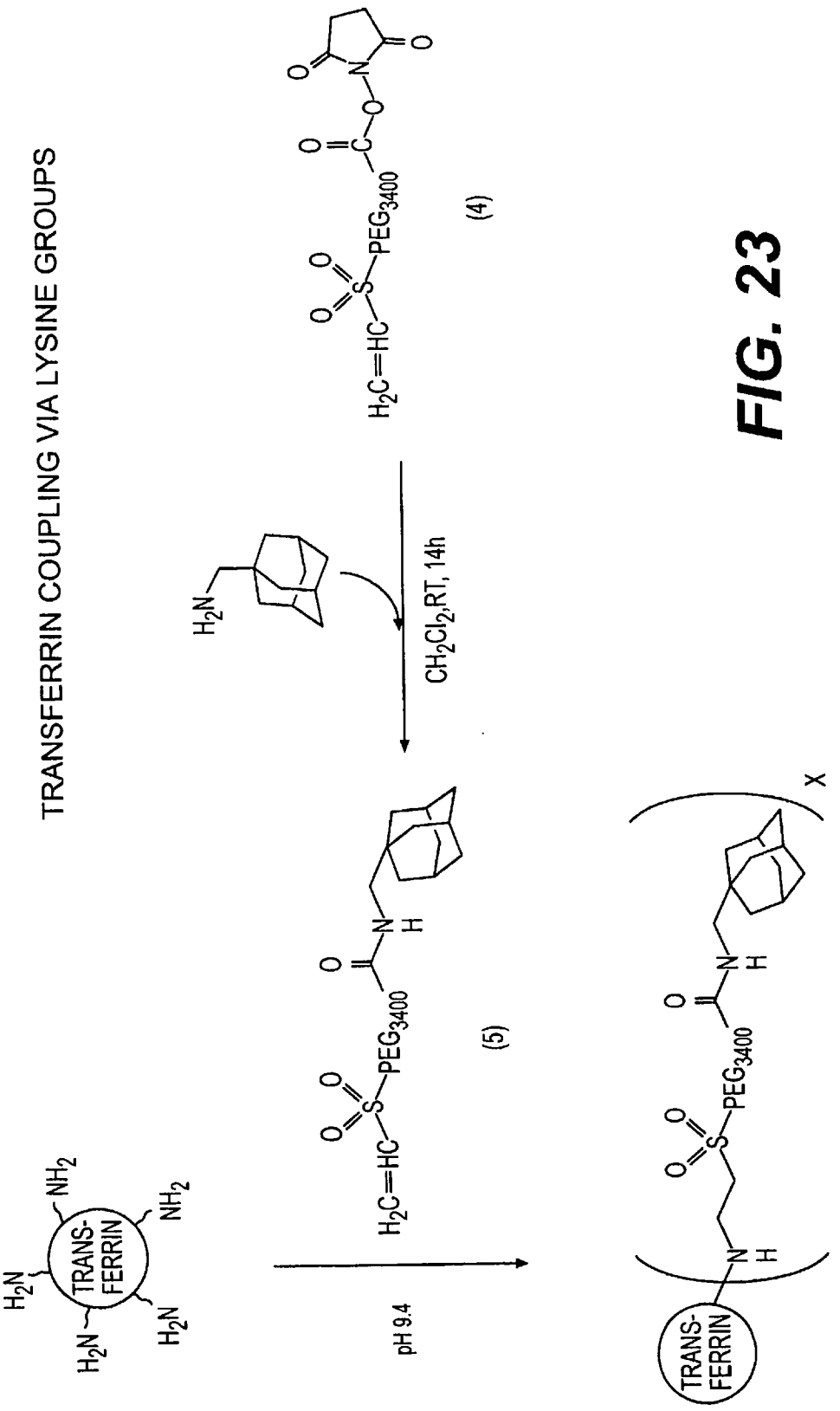

TRANSFERRIN COUPLING VIA LYSINE GROUPS
TRANS-FERRIN
NH2
H2N
(4)
(5)
CH2Cl2,RT, 14h
pH 9.4
PEG3400
H2C=HC
X

FIG. 23

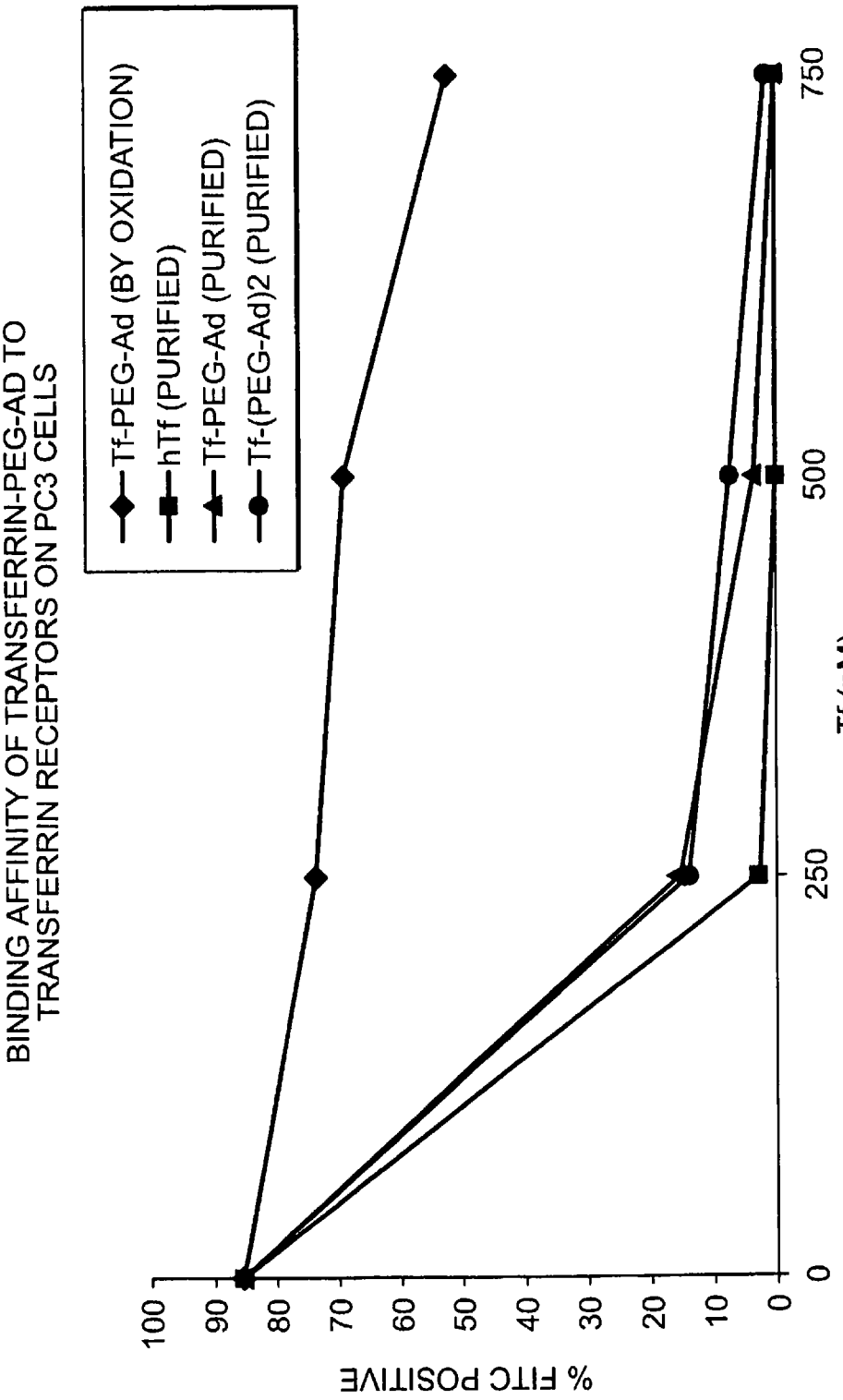

BINDING AFFINITY OF TRANSFERRIN-PEG-AD TO TRANSFERRIN RECEPTORS ON PC3 CELLS
Tf-PEG-Ad (BY OXIDATION)
hTf (PURIFIED)
Tf-PEG-Ad (PURIFIED)
Tf-(PEG-Ad)2 (PURIFIED)
% FITC POSITIVE
100
90
80
70
60
50
40
30
20
10
0
0
250
500
750
Tf (nM)

FIG. 24

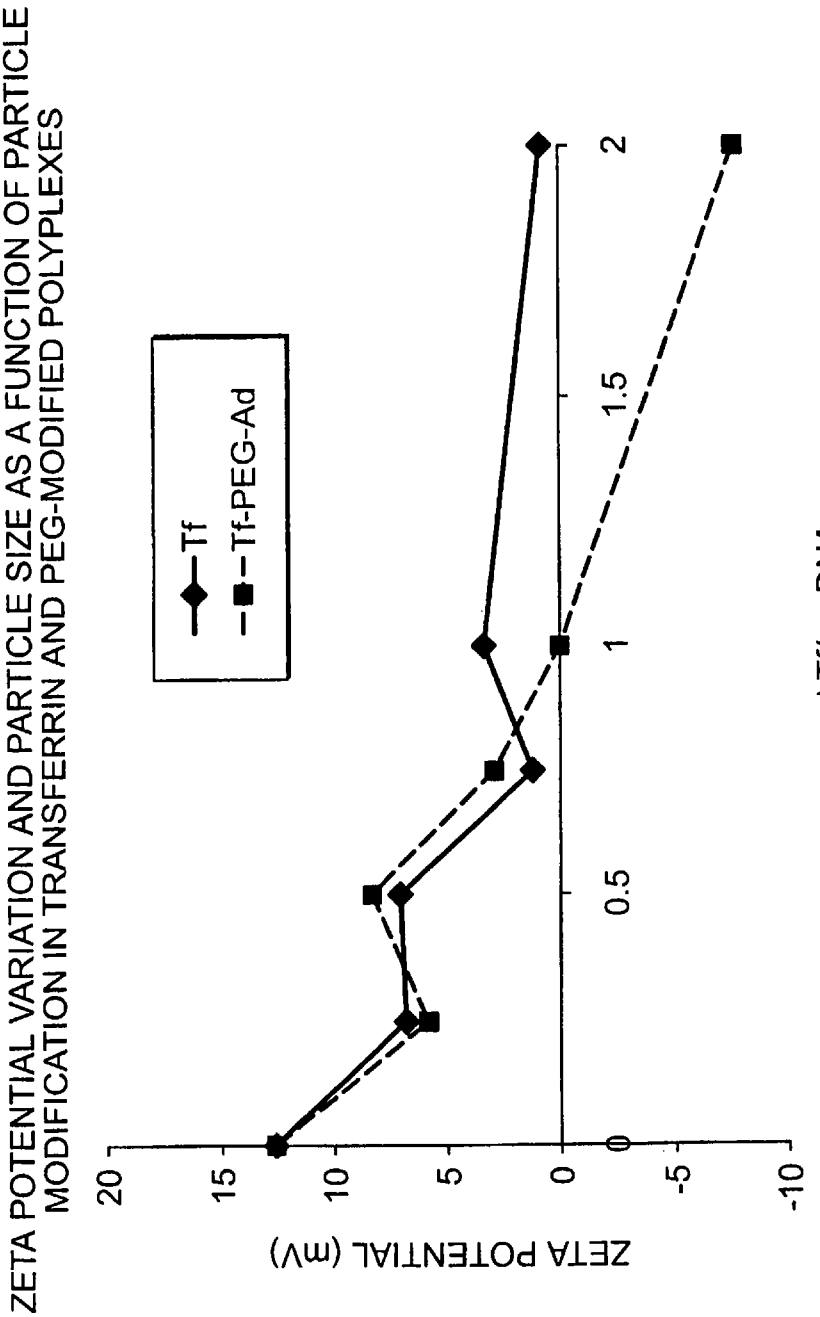

ZETA POTENTIAL VARIATION AND PARTICLE SIZE AS A FUNCTION OF PARTICLE MODIFICATION IN TRANSFERRIN AND PEG-MODIFIED POLYPLEXES
Tf
Tf-PEG-Ad
ZETA POTENTIAL (mV)
20
15
10
5
0
-5
-10
0
0.5
1
1.5
2
nmol Tf/ug DNA

FIG. 25

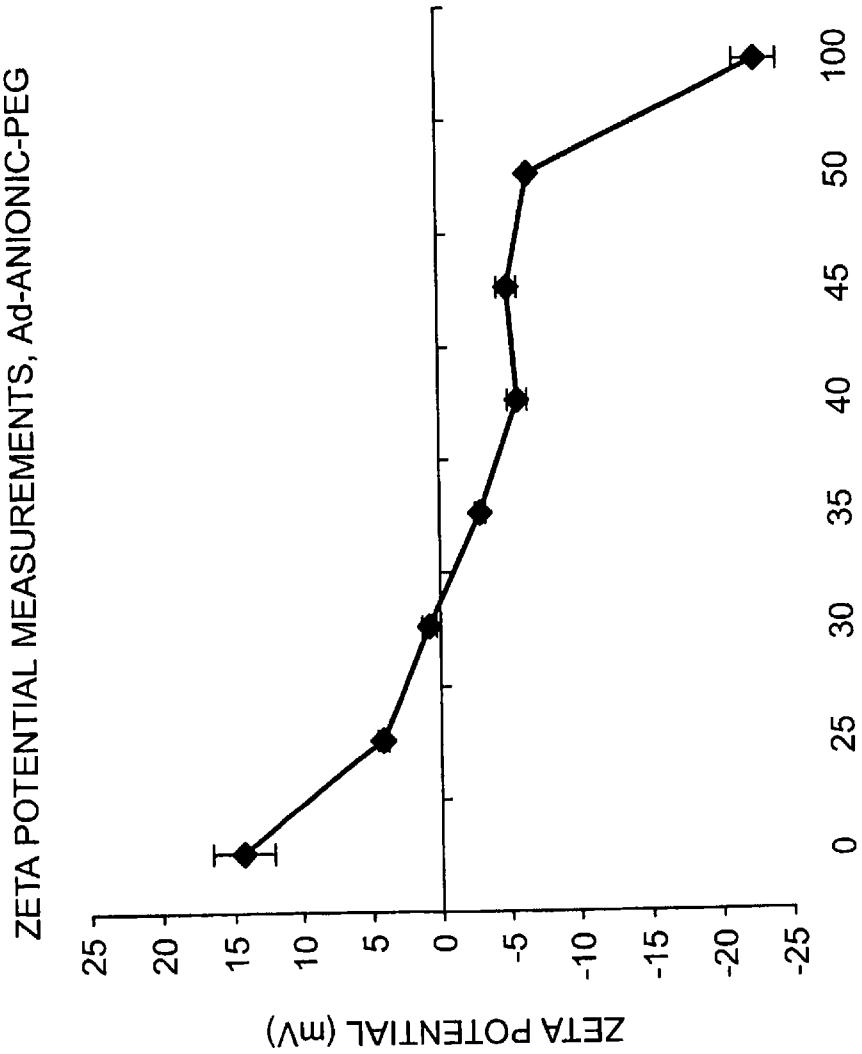

ZETA POTENTIAL MEASUREMENTS, Ad-ANIONIC-PEG
ZETA POTENTIAL (mV)
25
20
15
10
5
0
-5
-10
-15
-20
-25
0
25
30
35
40
45
50
100
% AD-ANIONIC-PEG

FIG. 26

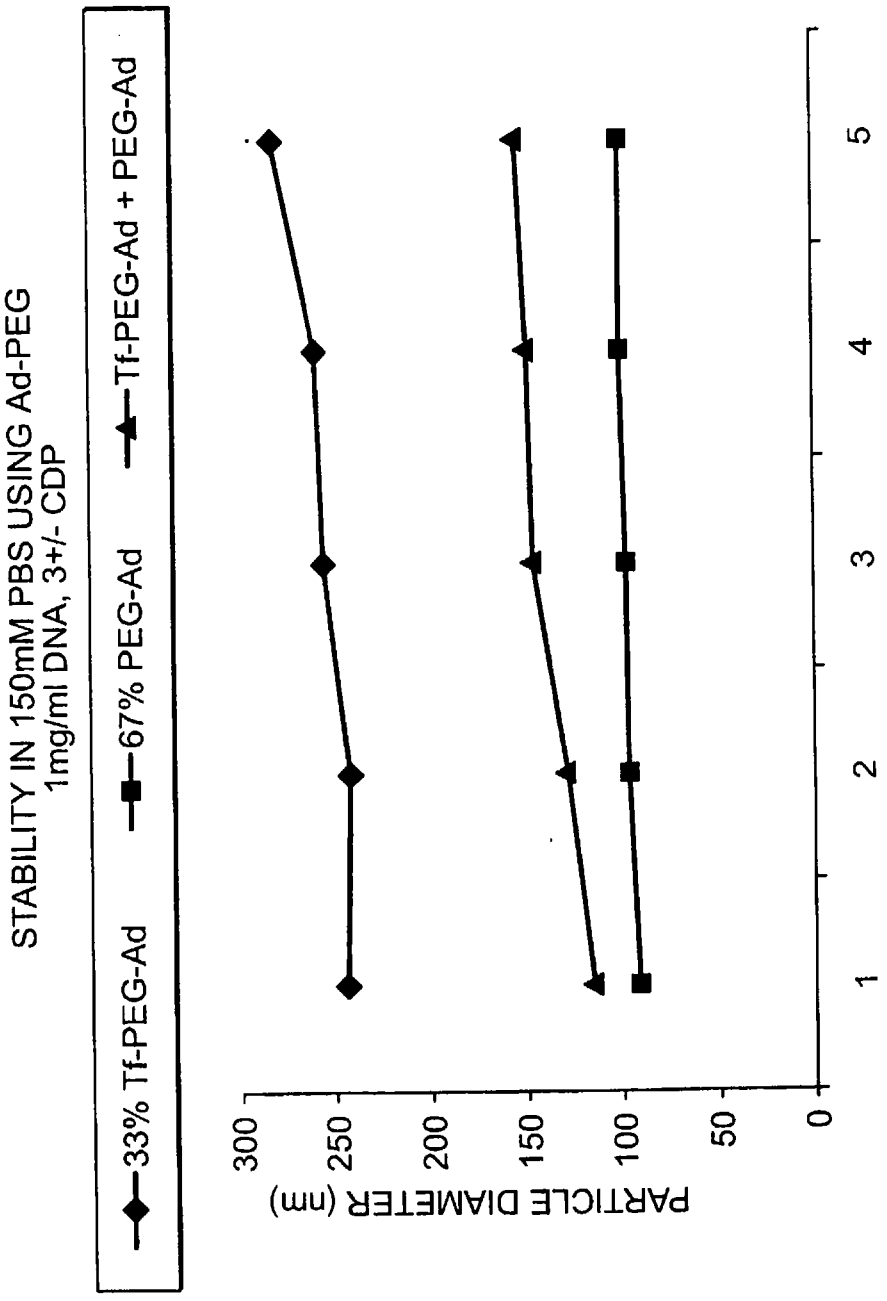

STABILITY IN 150mM PBS USING Ad-PEG
1mg/ml DNA, 3+/- CDP
33% Tf-PEG-Ad
67% PEG-Ad
Tf-PEG-Ad + PEG-Ad
PARTICLE DIAMETER (nm)
300
250
200
150
100
50
0
1
2
3
4
5
TIME (min)

FIG. 27

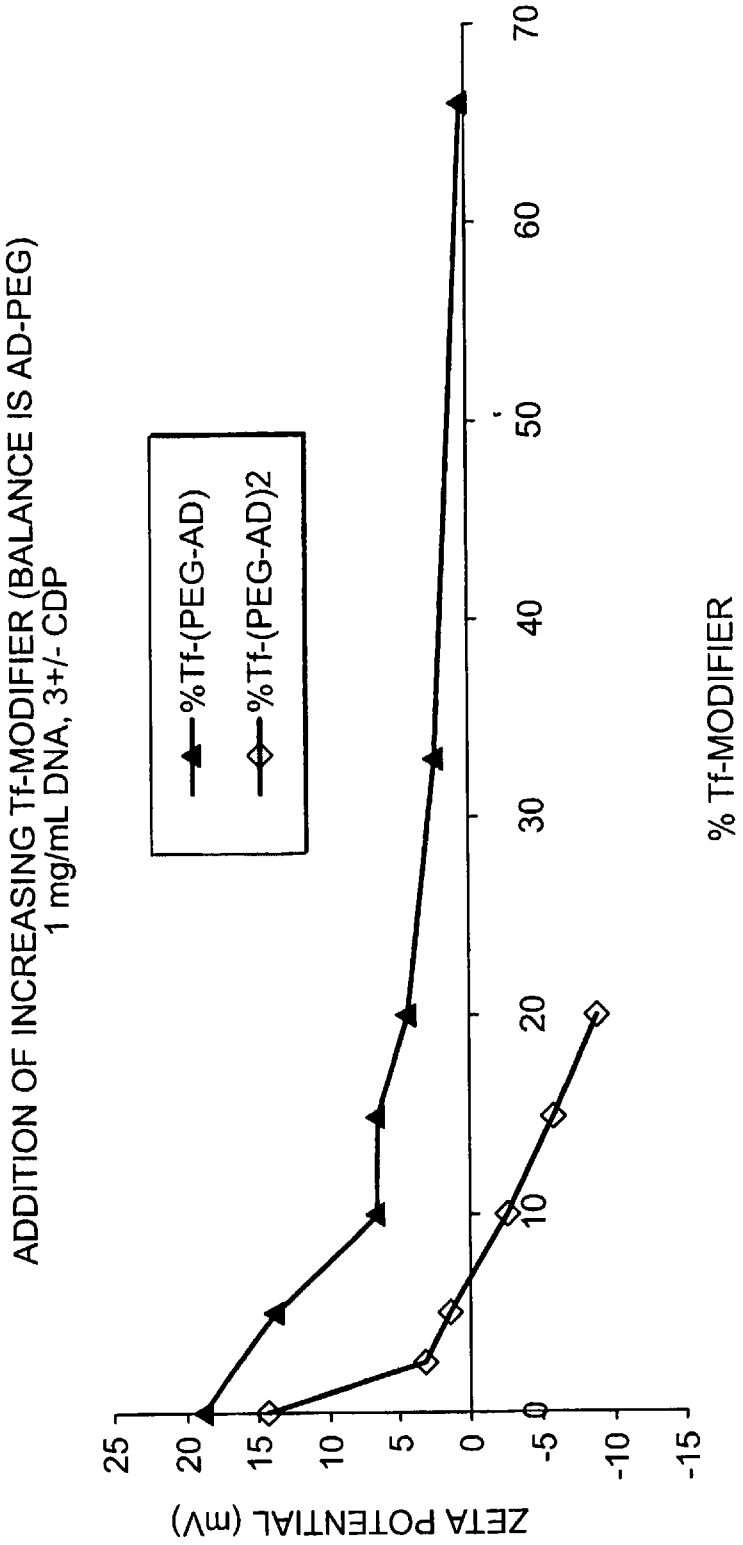

ADDITION OF INCREASING Tf-MODIFIER (BALANCE IS AD-PEG)
1 mg/mL DNA, 3+/- CDP
%Tf-(PEG-AD)
%Tf-(PEG-AD)2
ZETA POTENTIAL (mV)
25
20
15
10
5
0
-5
-10
-15
0
10
20
30
40
50
60
70
% Tf-MODIFIER

FIG. 28

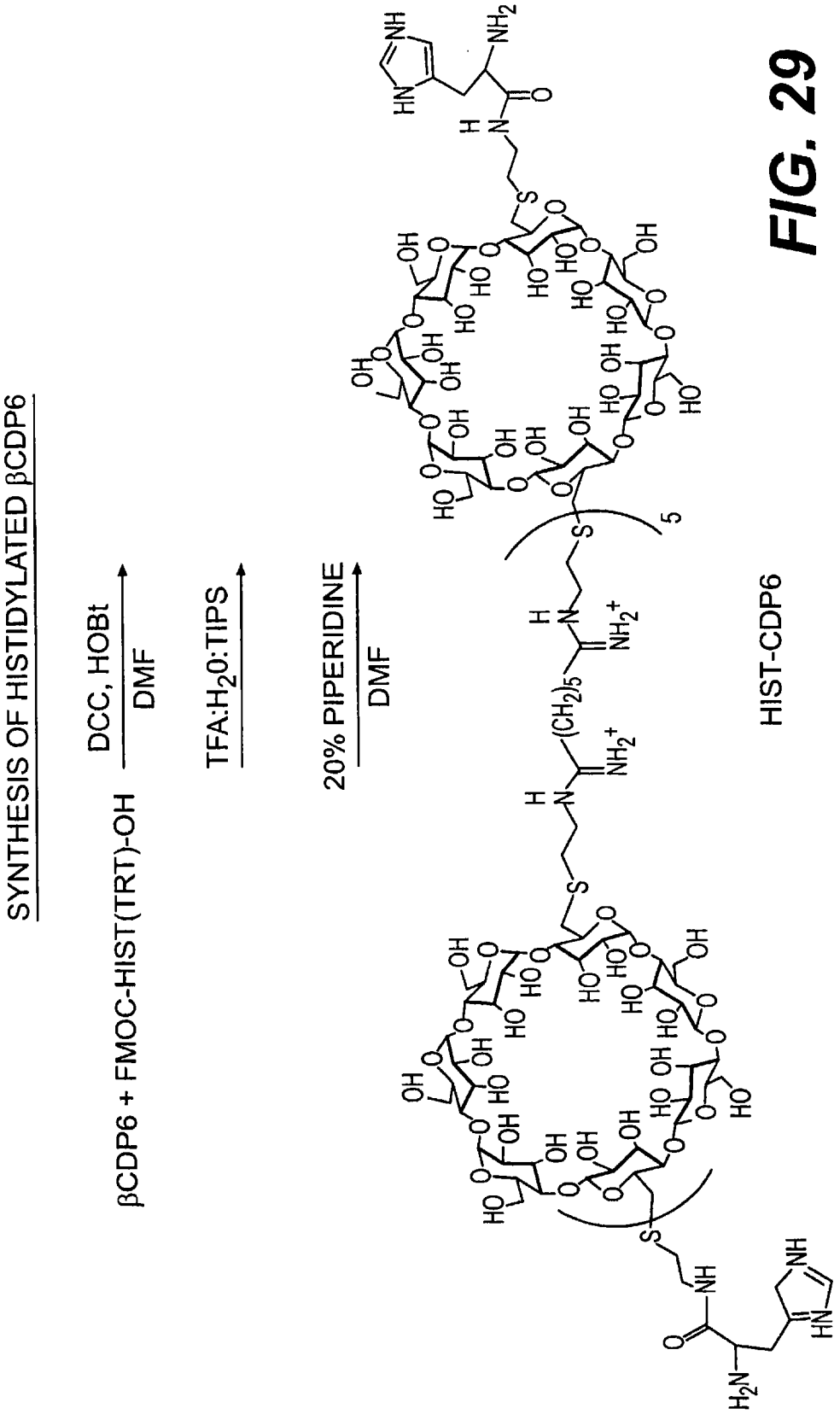

SYNTHESIS OF HISTIDYLATED βCDP6
βCDP6 + FMOC-HIST(TRT)-OH
DCC, HOBt
DMF
TFA:H2O:TIPS
20% PIPERIDINE
DMF
HIST-CDP6

FIG. 29

pH-SENSITIVE POLYMERS FOR ENDOSOMAL ESCAPE

SYNTHESIS OF SECONDARY AMINE CONTAINING POLYMERS

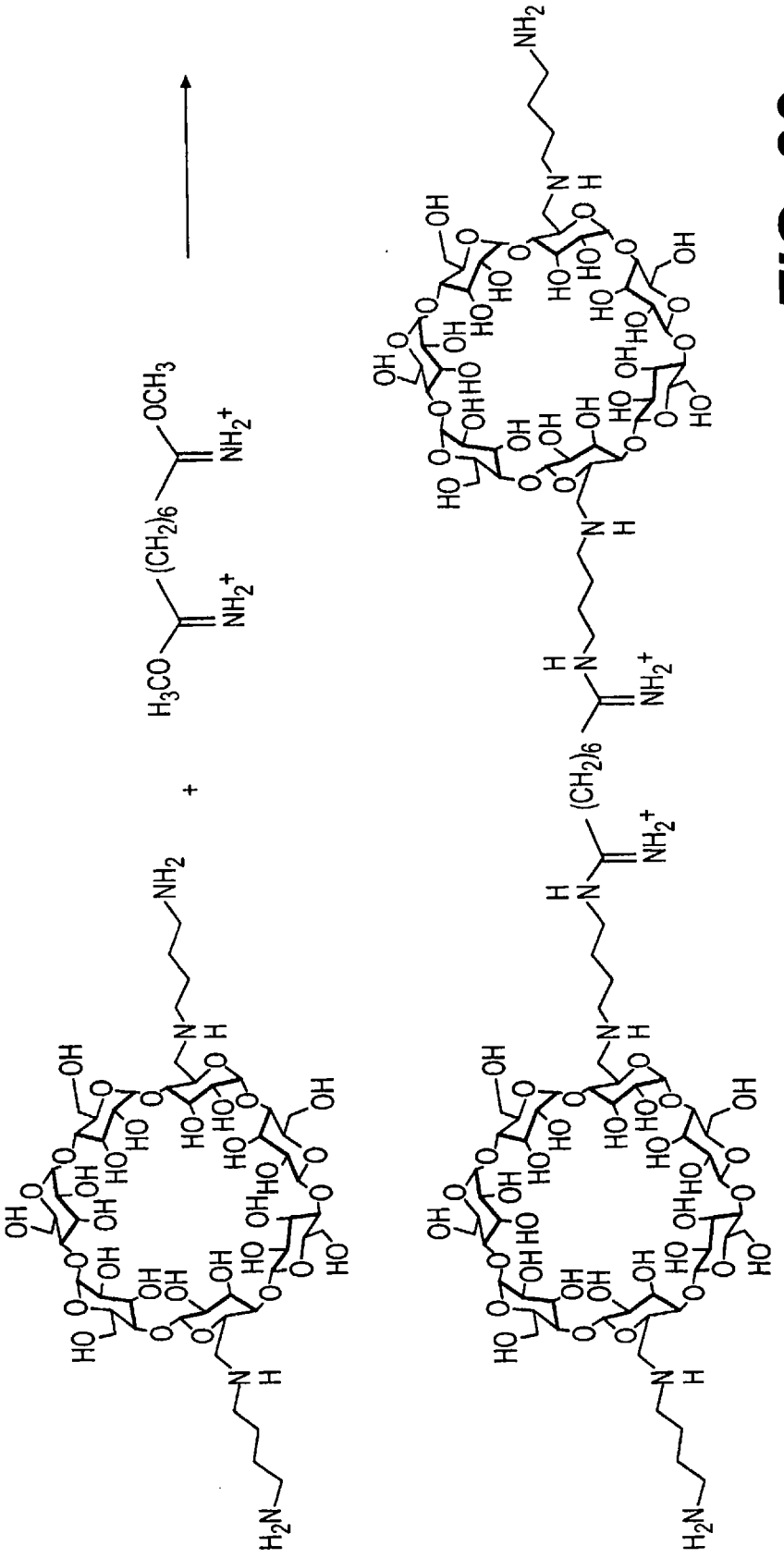


FIG. 30