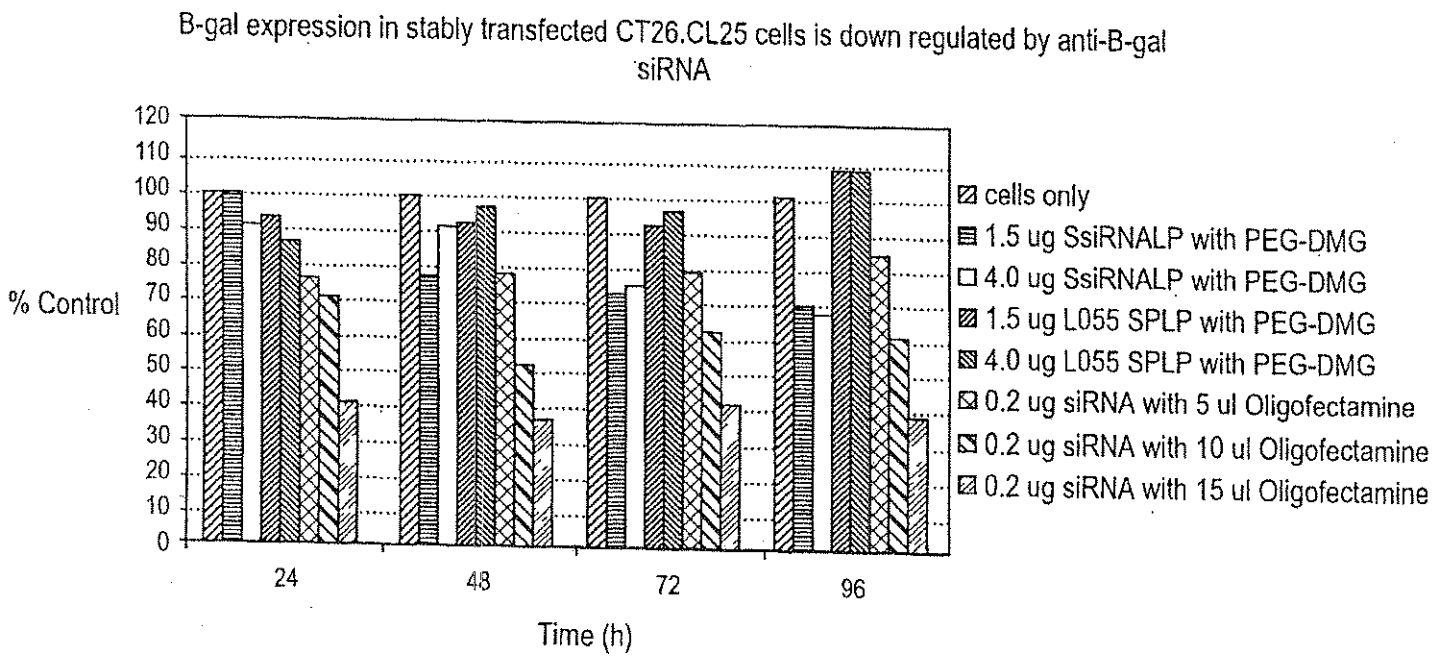
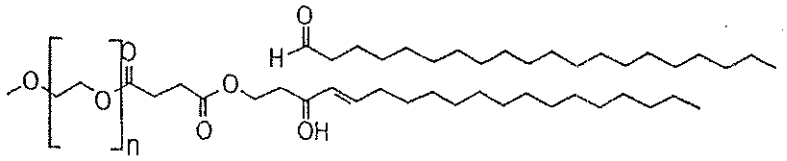
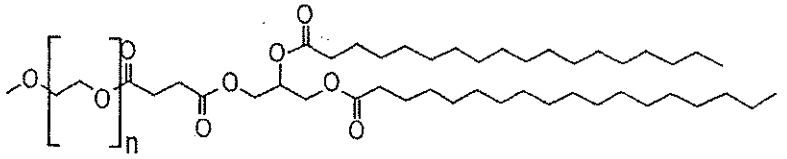

B-gal expression in stably transfected CT26.CL25 cells is down regulated by anti-B-gal siRNA
% Control
120
110
100
90
80
70
60
50
40
30
20
10
0
24
48
72
96
Time (h)
cells only
1.5 ug SsiRNALP with PEG-DMG
4.0 ug SsiRNALP with PEG-DMG
1.5 ug L055 SPLP with PEG-DMG
4.0 ug L055 SPLP with PEG-DMG
0.2 ug siRNA with 5 ul Oligofectamine
0.2 ug siRNA with 10 ul Oligofectamine
0.2 ug siRNA with 15 ul Oligofectamine

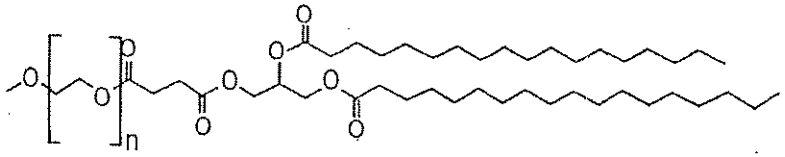
FIG. 1



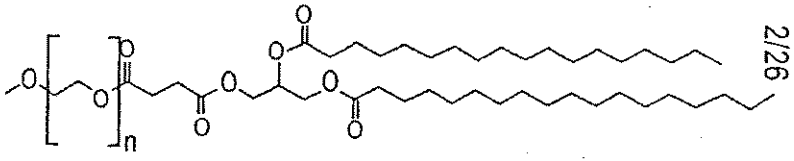
PEG-CeramideC20



PEG-Distearylglycerol (C18)



PEG-Dipalmitoylglycerol (C16)



PEG-Dimyristylglycerol (C14)

FIG. 2

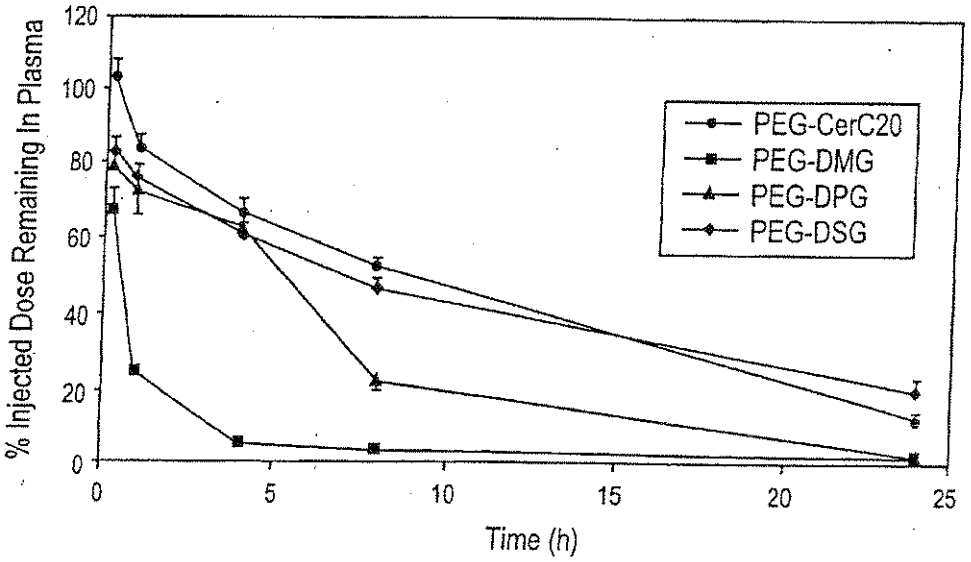

% Injected Dose Remaining In Plasma
120
100
80
60
40
20
0
0
5
10
15
20
25
Time (h)
PEG-CerC20
PEG-DMG
PEG-DPG
PEG-DSG

FIG. 3

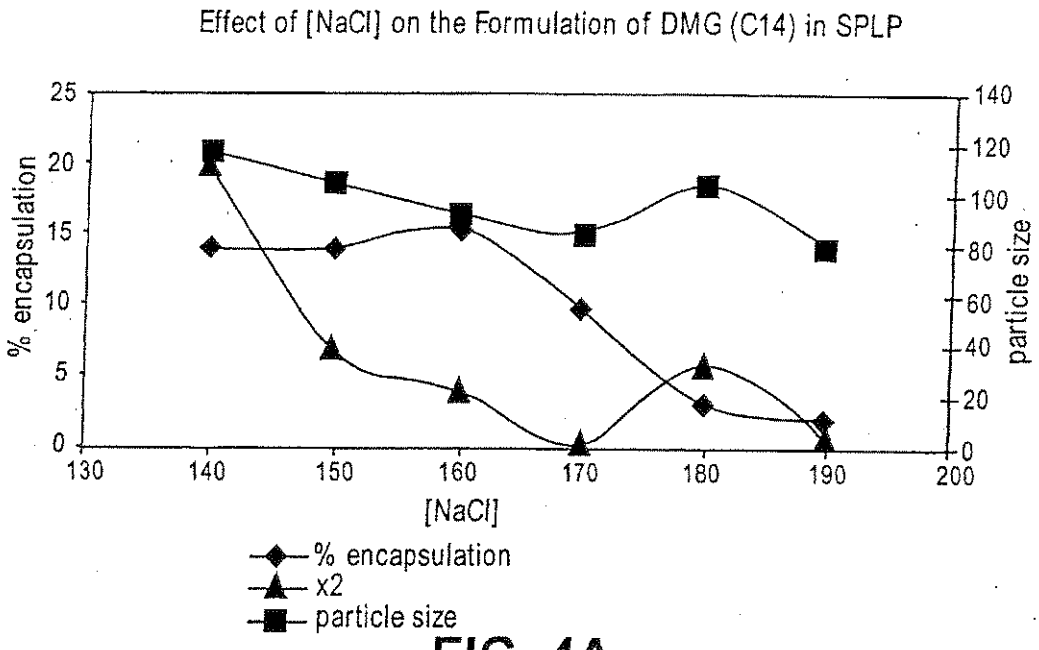

Effect of [NaCl] on the Formulation of DMG (C14) in SPLP
% encapsulation
particle size
[NaCl]
% encapsulation
x2
particle size

FIG. 4A

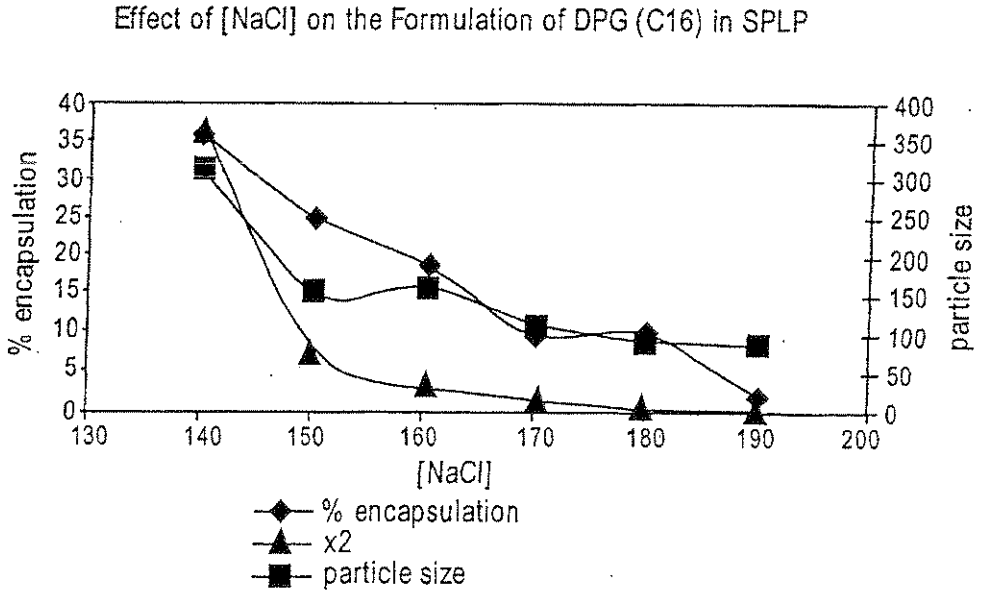

Effect of [NaCl] on the Formulation of DPG (C16) in SPLP
% encapsulation
particle size
[NaCl]
% encapsulation
x2
particle size

FIG. 4B

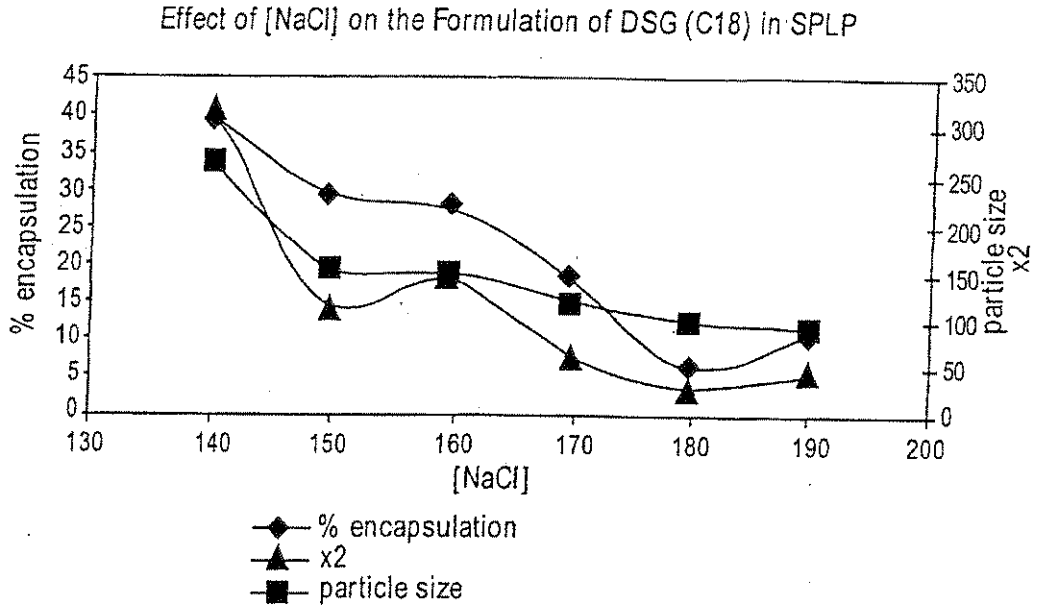

Effect of [NaCl] on the Formulation of DSG (C18) in SPLP
% encapsulation
particle size x2
[NaCl]
% encapsulation
x2
particle size

FIG. 4C

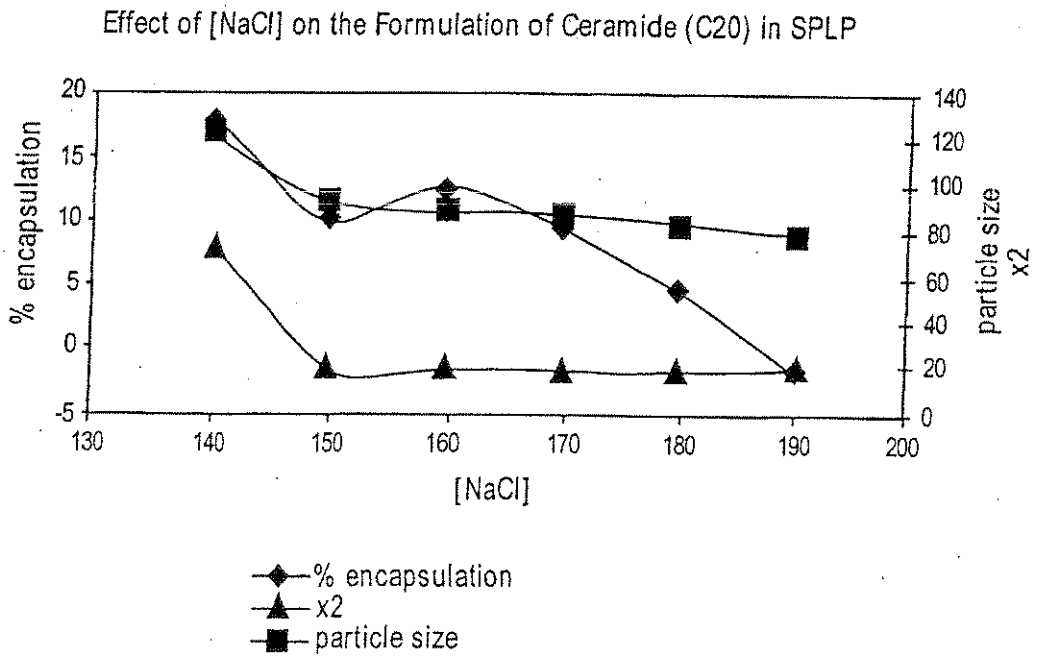

Effect of [NaCl] on the Formulation of Ceramide (C20) in SPLP
% encapsulation
particle size x2
[NaCl]
% encapsulation
x2
particle size

FIG. 4D

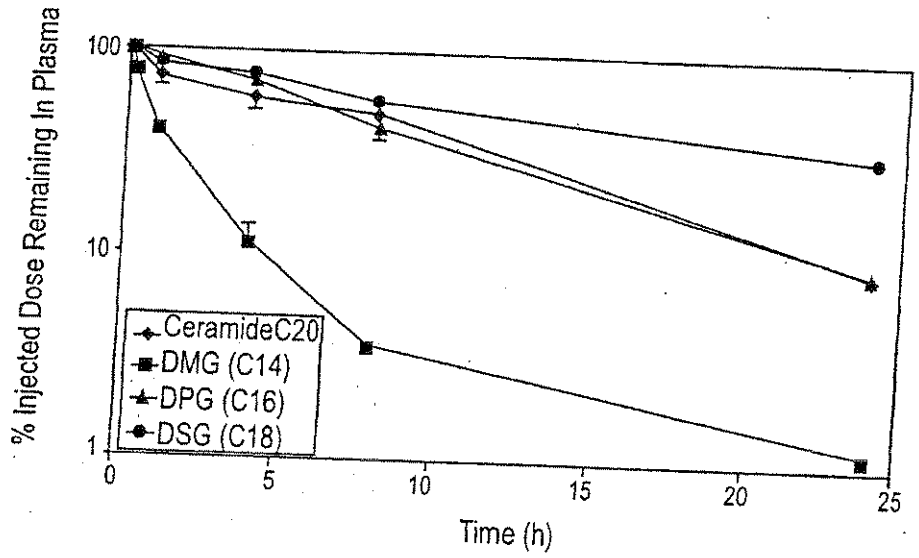

% Injected Dose Remaining In Plasma
100
10
1
0
5
10
15
20
25
Time (h)
CeramideC20
DMG (C14)
DPG (C16)
DSG (C18)

FIG. 5

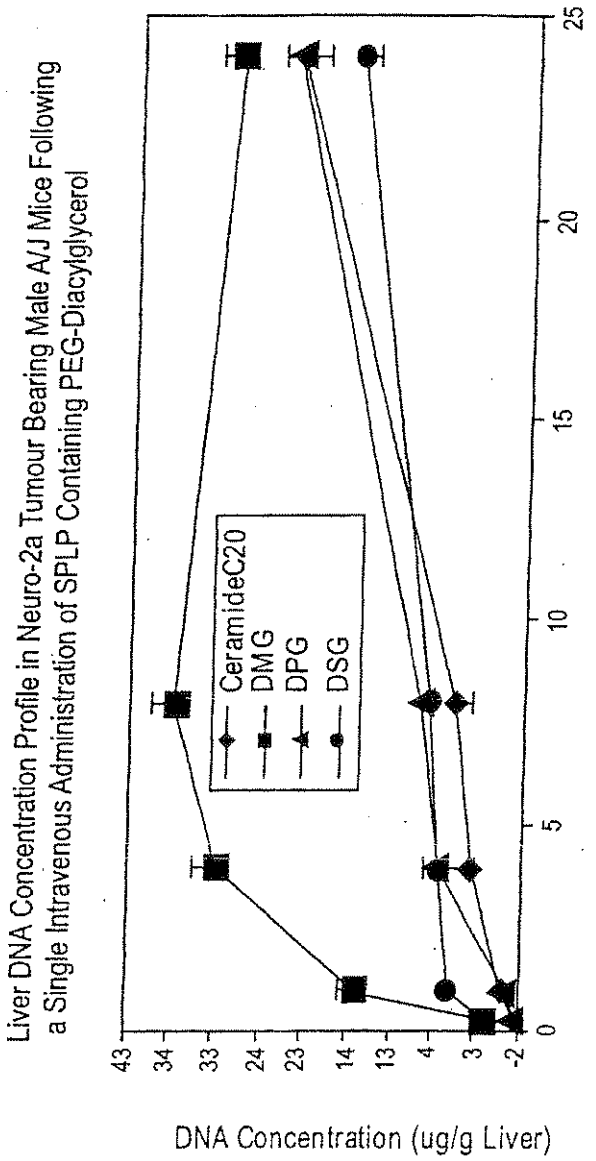

Liver DNA Concentration Profile in Neuro-2a Tumour Bearing Male A/J Mice Following a Single Intravenous Administration of SPLP Containing PEG-Diacylglycerol
DNA Concentration (ug/g Liver)
Time (h)
CeramideC20
DMG
DPG
DSG
43
34
33
24
23
14
13
4
3
-2
0
5
10
15
20
25

FIG. 6A

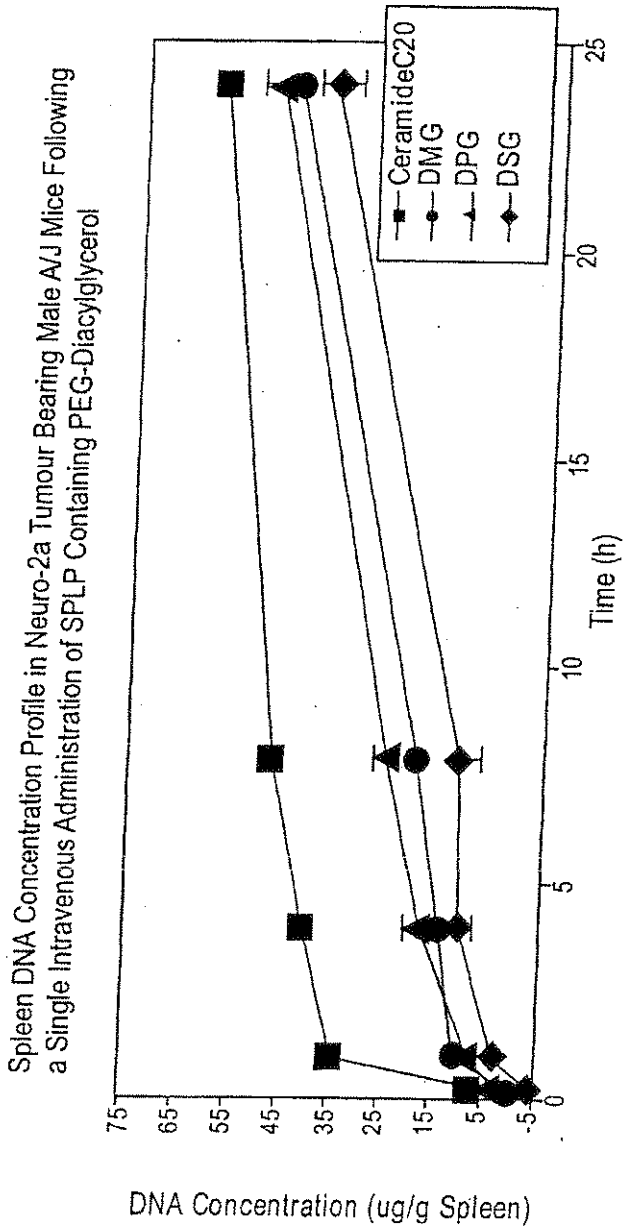

Spleen DNA Concentration Profile in Neuro-2a Tumour Bearing Male A/J Mice Following a Single Intravenous Administration of SPLP Containing PEG-Diacylglycerol
DNA Concentration (ug/g Spleen)
75
65
55
45
35
25
15
5
-5
0
5
10
15
20
25
Time (h)
CeramideC20
DMG
DPG
DSG

FIG. 6B

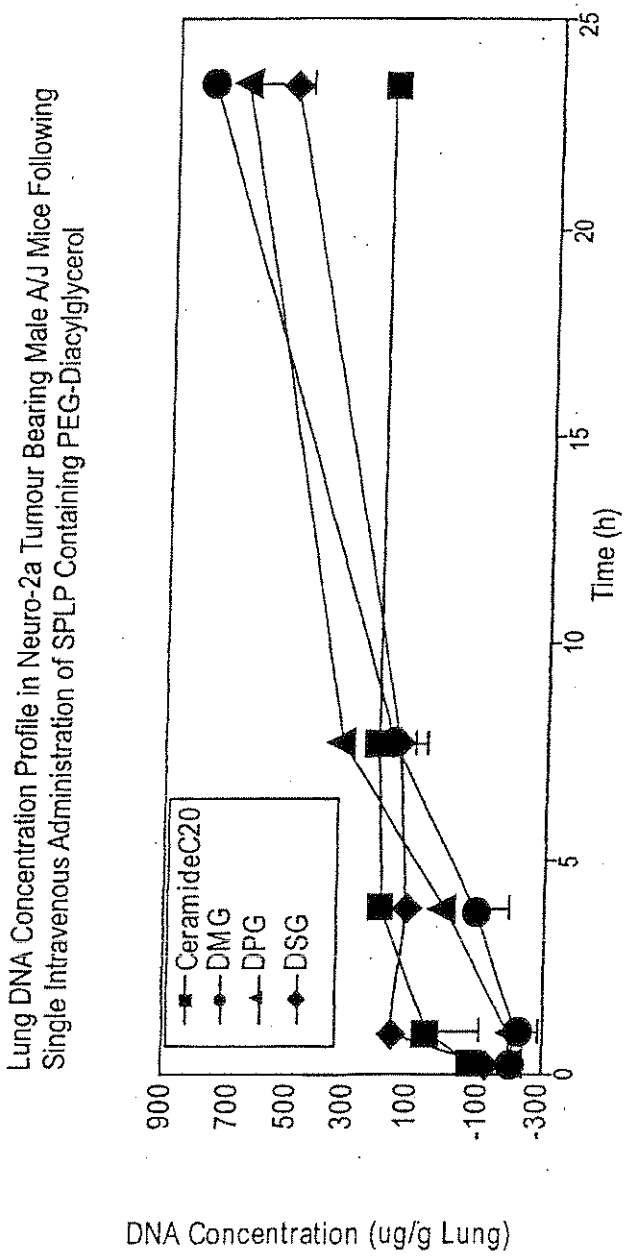

Lung DNA Concentration Profile in Neuro-2a Tumour Bearing Male A/J Mice Following
Single Intravenous Administration of SPLP Containing PEG-Diacylglycerol
CeramideC20
DMG
DPG
DSG
900
700
500
300
100
-100
-300
0
5
10
15
20
25
Time (h)
DNA Concentration (ug/g Lung)

FIG. 6C

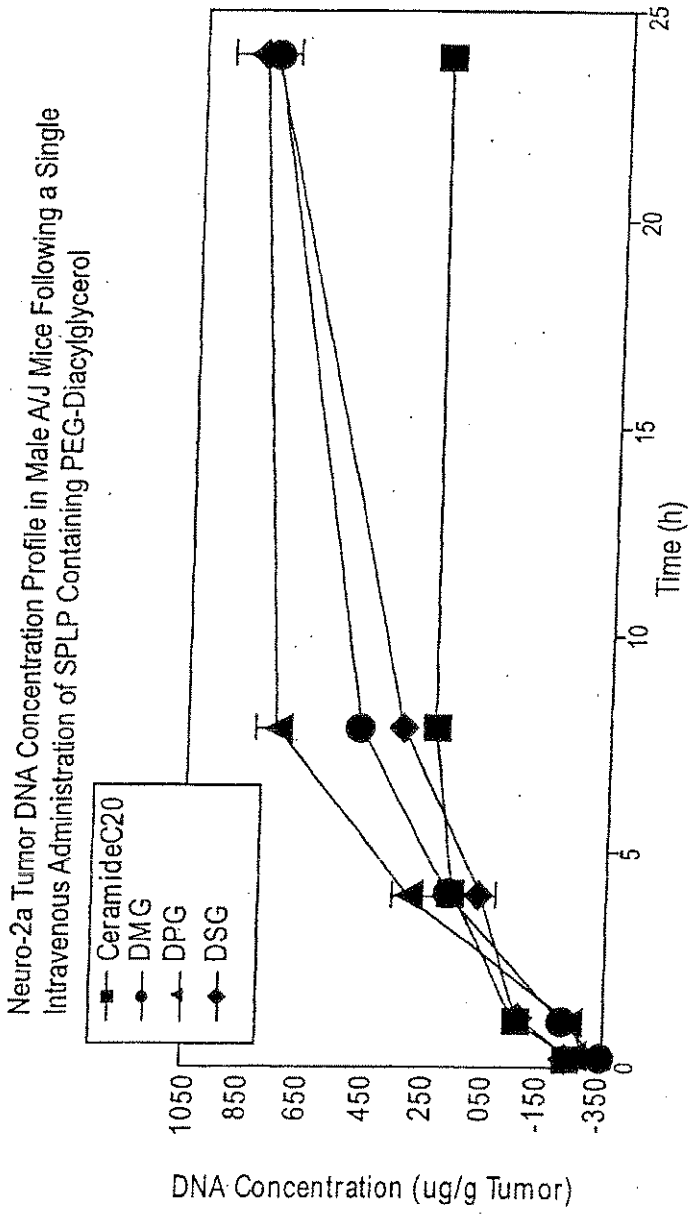

Neuro-2a Tumor DNA Concentration Profile in Male A/J Mice Following a Single Intravenous Administration of SPLP Containing PEG-Diacylglycerol
CeramideC20
DMG
DPG
DSG
DNA Concentration (ug/g Tumor)
1050
850
650
450
250
050
-150
-350
0
5
10
15
20
25
Time (h)

FIG. 6D

Luciferase Gene Expression 24hrs Post IV Administration of SPLP Containing PEG-CeramideC20 verses PEG-diacyllycerols in Neuro-2a Tumour Bearing Male A/J Mice

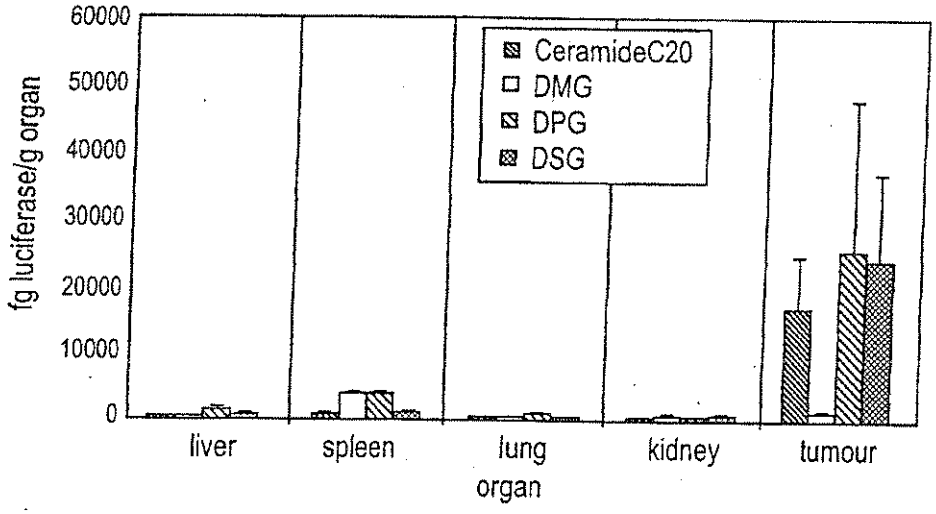


FIG. 7

Luciferase Gene Expression 48hrs Post IV Administration of SPLP Containing PEG-CeramideC20 verses PEG-diacyllycerols in Neuro-2a Tumour Bearing Male A/J Mice

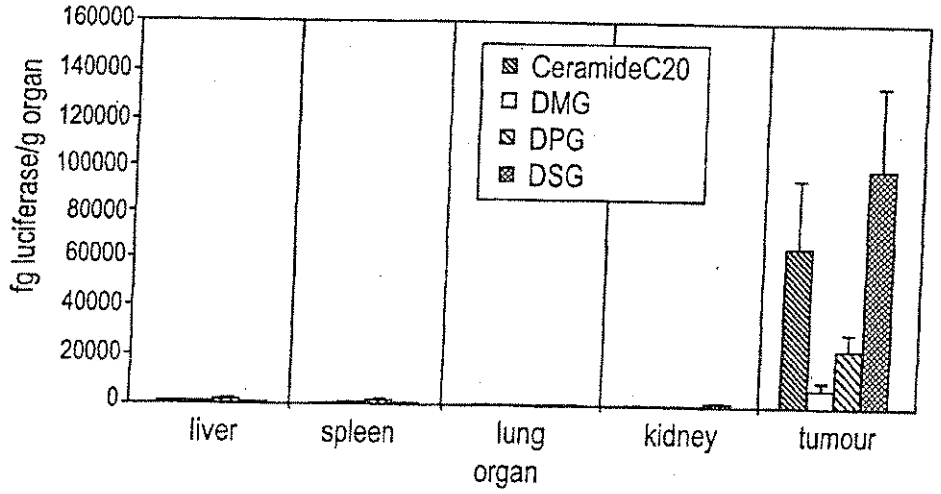


FIG. 8

Luciferase Gene Expression 72hrs Post IV Administration of SPLP Containing PEG-CeramideC20 verses PEG-diacyllycerols in Neuro-2a Tumour Bearing Male A/J Mice

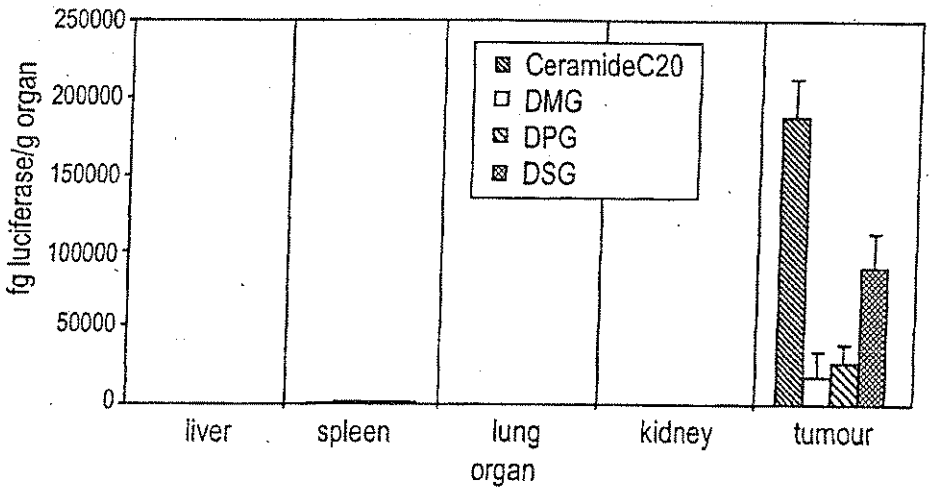
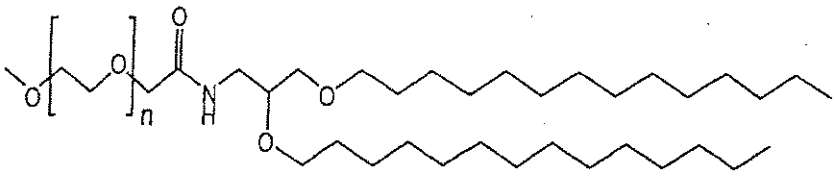
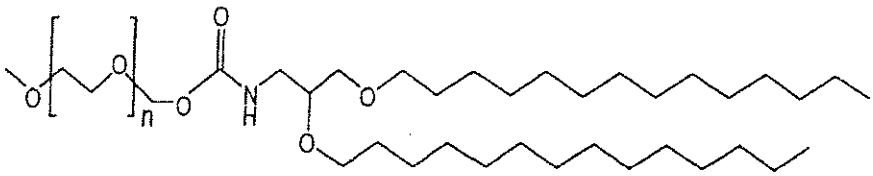


FIG. 9

N-(2,3-dimyristyloxypropyl) amide PEG_{2000} methyl ether (PEG-A-DMA)



N-(2,3-dimyristyloxypropyl) carbamate PEG_{2000} methyl ether (PEG-C-DMA)



N-(2,3-dimyristyloxypropyl) succinamide PEG_{2000} methyl ether (PEG-S-DMA)

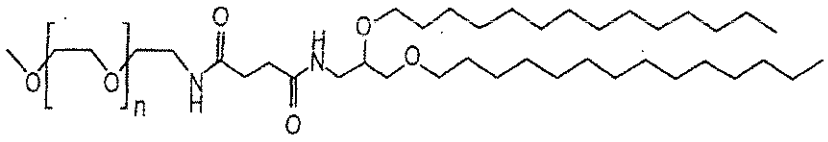


FIG. 10

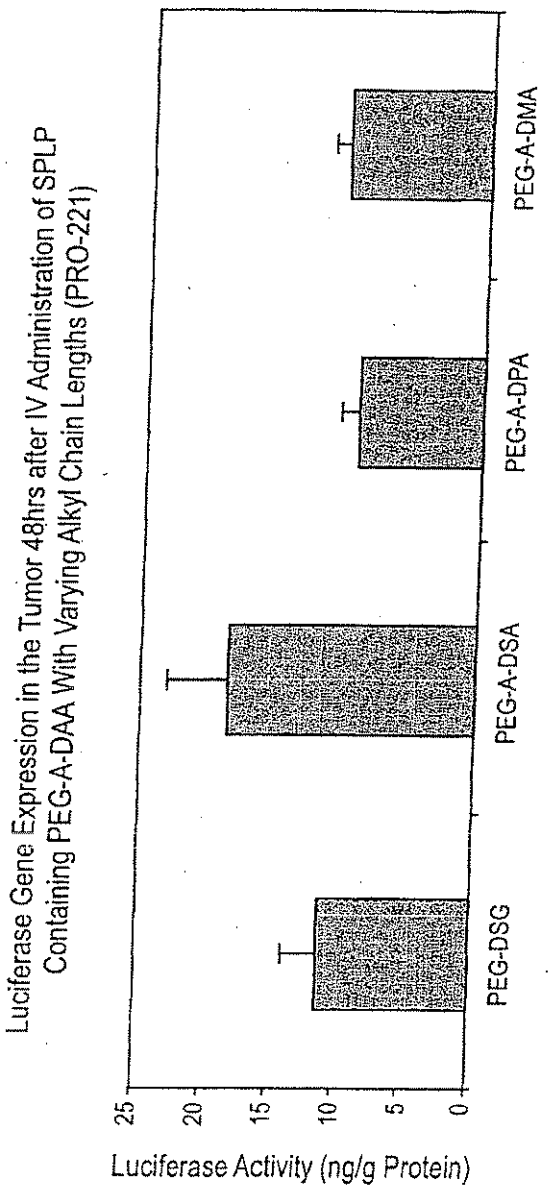

Luciferase Gene Expression in the Tumor 48hrs after IV Administration of SPLP
Containing PEG-A-DAA With Varying Alkyl Chain Lengths (PRO-221)
Luciferase Activity (ng/g Protein)
25
20
15
10
5
0
PEG-DSG
PEG-A-DSA
PEG-A-DPA
PEG-A-DMA

FIG. 11

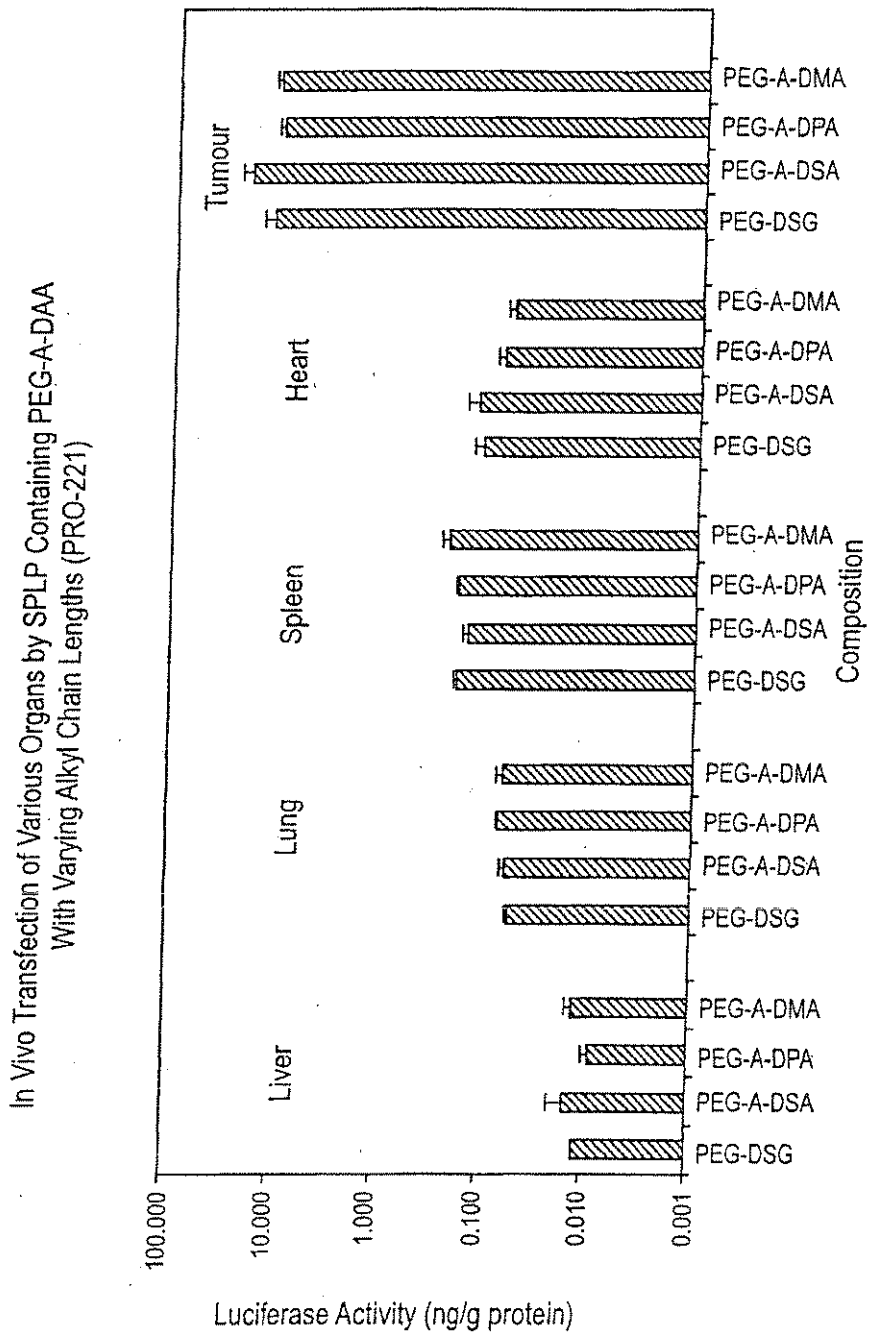

In Vivo Transfection of Various Organs by SPLP Containing PEG-A-DAA
With Varying Alkyl Chain Lengths (PRO-221)
Tumour
Heart
Spleen
Lung
Liver
PEG-A-DMA
PEG-A-DPA
PEG-A-DSA
PEG-DSG
Composition
100.000
10.000
1.000
0.100
0.010
0.001
Luciferase Activity (ng/g protein)

FIG. 12

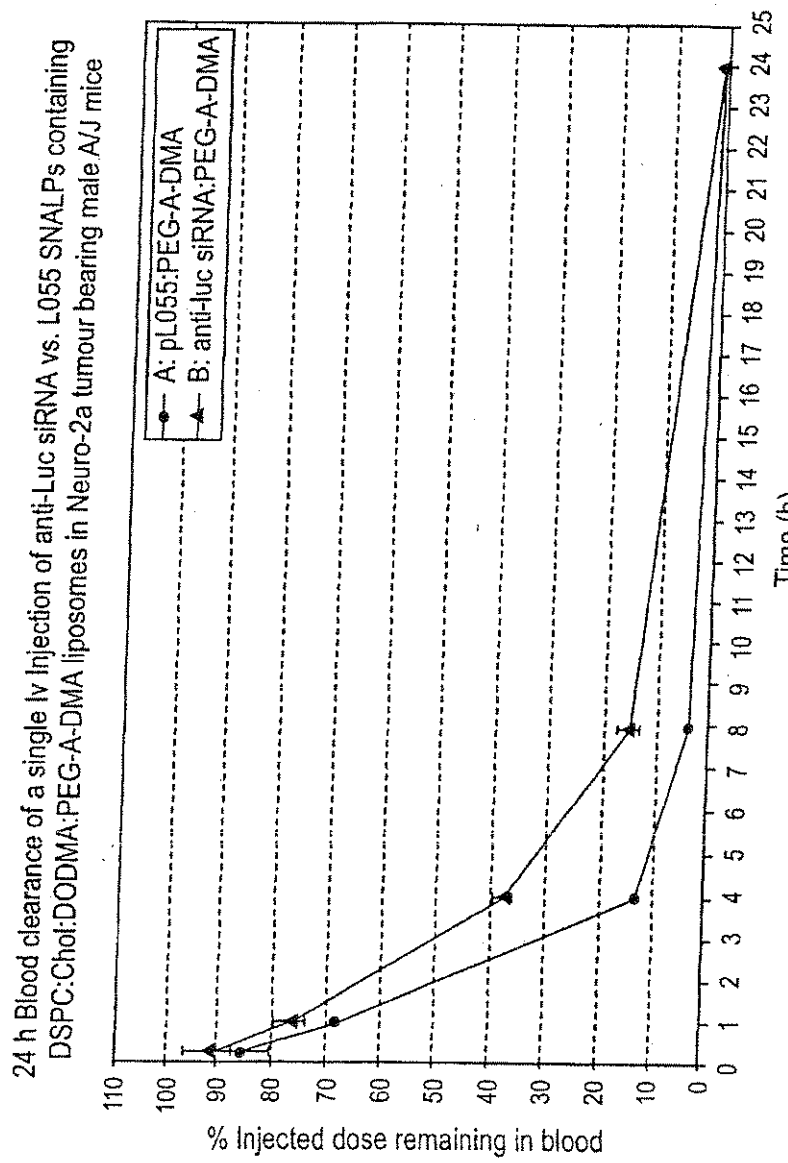

24 h Blood clearance of a single Iv Injection of anti-Luc siRNA vs. L055 SNALPs containing DSPC:Chol:DODMA:PEG-A-DMA liposomes in Neuro-2a tumour bearing male A/J mice
A: pL055:PEG-A-DMA
B: anti-luc siRNA:PEG-A-DMA
% Injected dose remaining in blood
Time (h)
110
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

FIG. 13

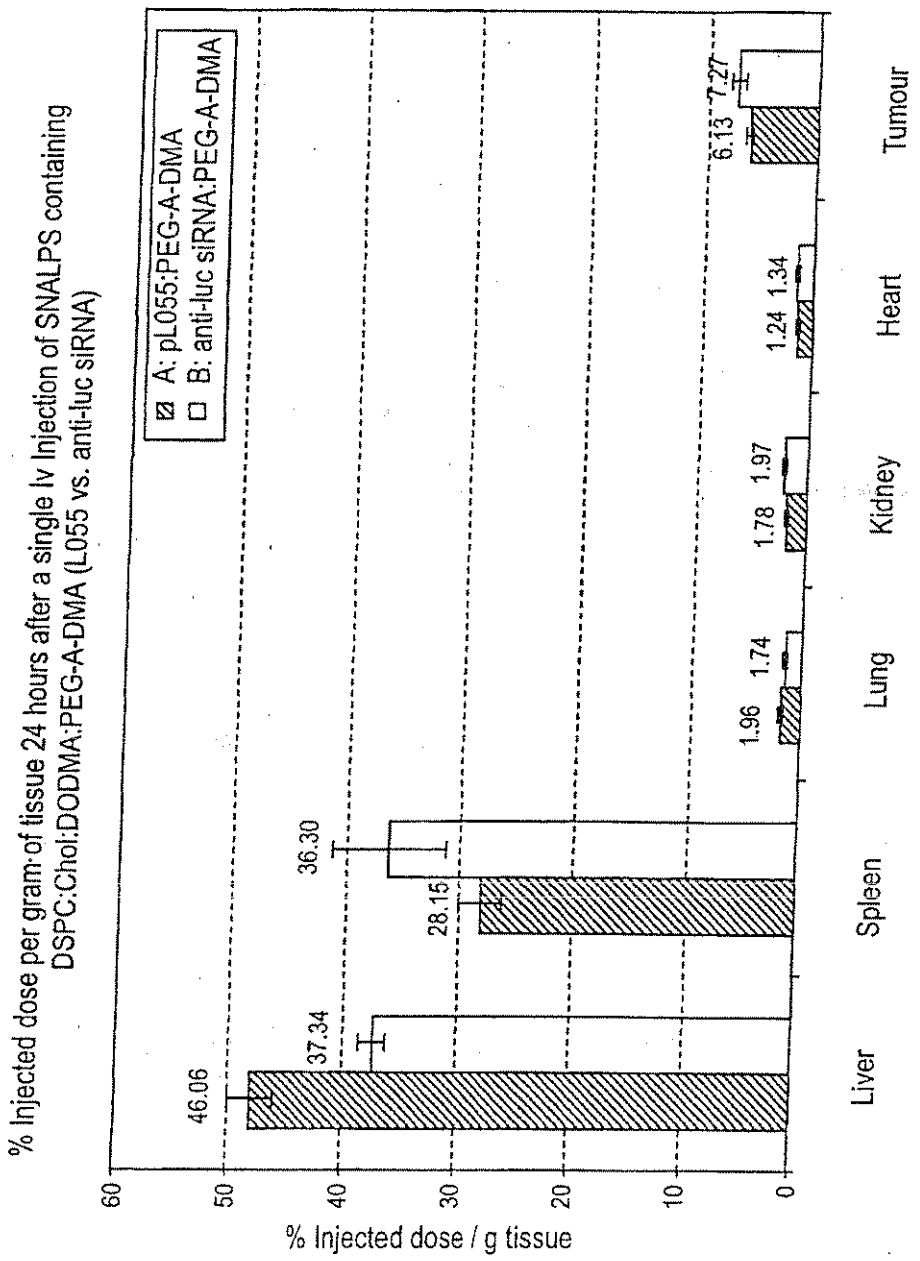

% Injected dose per gram of tissue 24 hours after a single Iv Injection of SNALPS containing DSPC:Chol:DODMA:PEG-A-DMA (L055 vs. anti-luc siRNA)
A: pL055:PEG-A-DMA
B: anti-luc siRNA:PEG-A-DMA
% Injected dose / g tissue
0
10
20
30
40
50
60
46.06
37.34
36.30
28.15
1.96
1.74
1.78
1.97
1.24
1.34
6.13
7.27
Liver
Spleen
Lung
Kidney
Heart
Tumour

FIG. 14

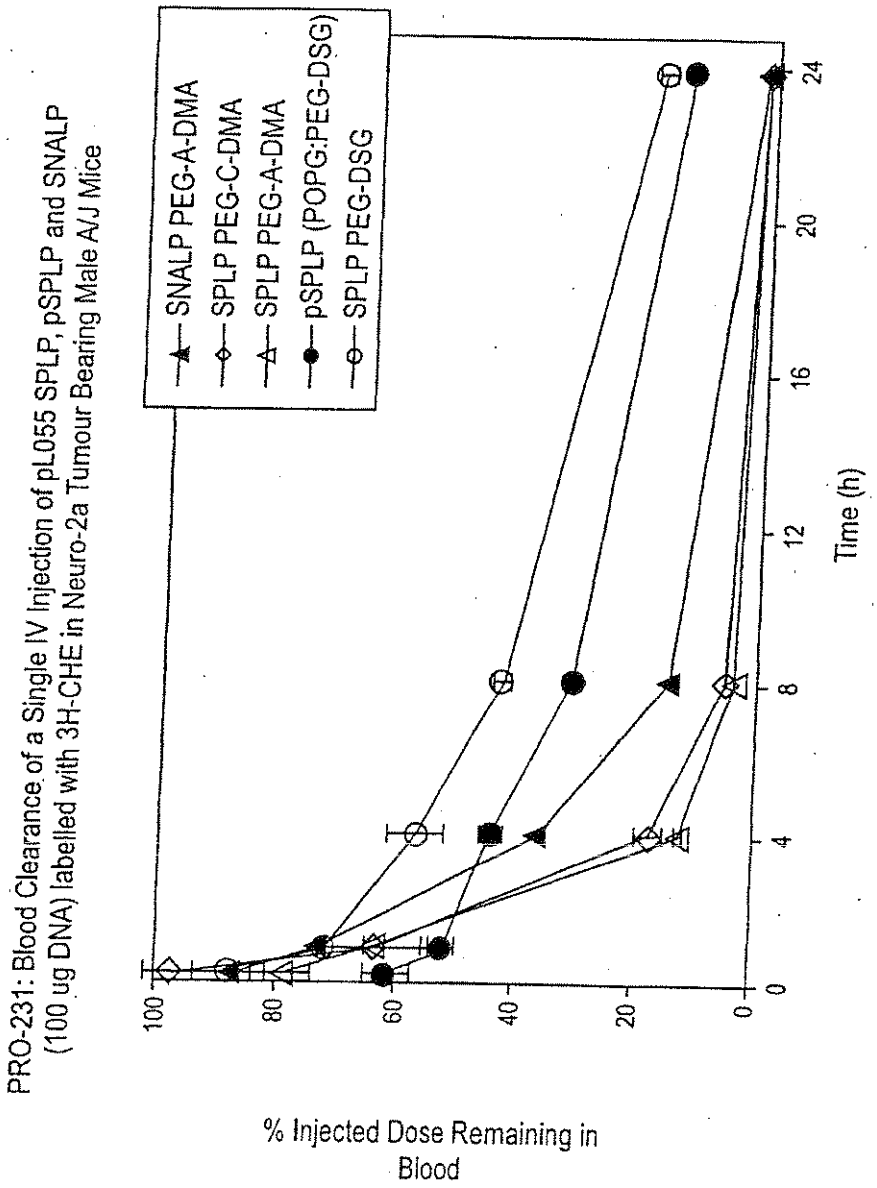

PRO-231: Blood Clearance of a Single IV Injection of pL055 SPLP, pSPLP and SNALP (100 ug DNA) labelled with 3H-CHE in Neuro-2a Tumour Bearing Male A/J Mice
SNALP PEG-A-DMA
SPLP PEG-C-DMA
SPLP PEG-A-DMA
pSPLP (POPG:PEG-DSG)
SPLP PEG-DSG
% Injected Dose Remaining in Blood
Time (h)
0
20
40
60
80
100
0
4
8
12
16
20
24

FIG. 15

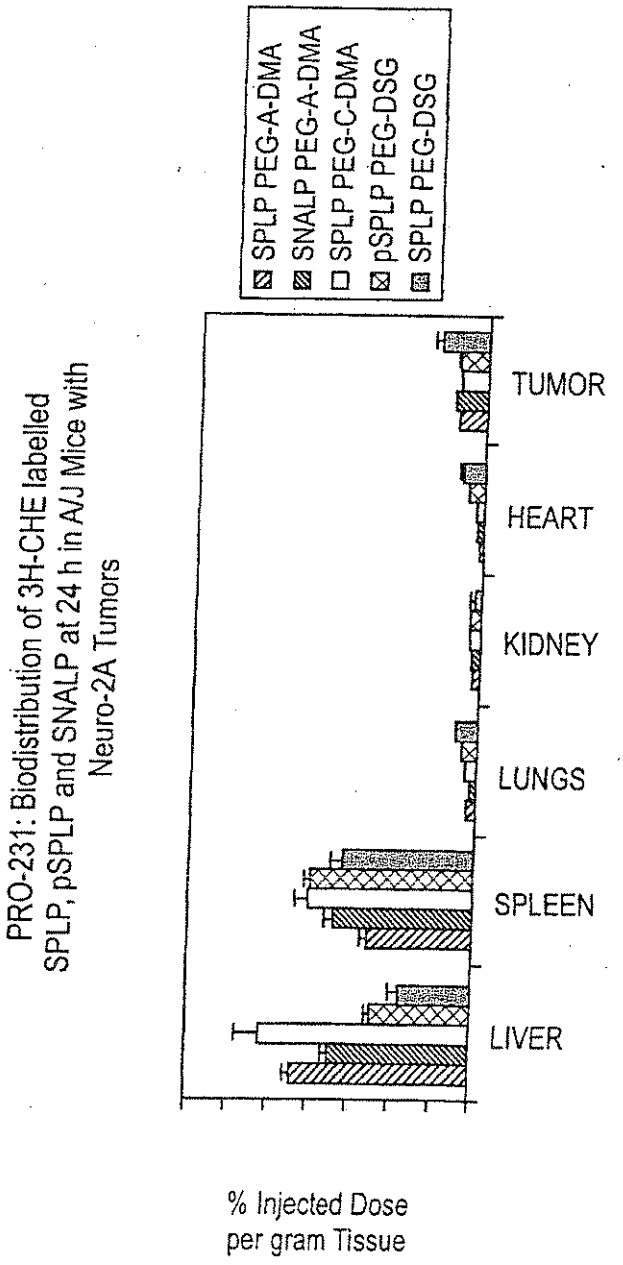

PRO-231: Biodistribution of 3H-CHE labelled
SPLP, pSPLP and SNALP at 24 h in A/J Mice with
Neuro-2A Tumors
SPLP PEG-A-DMA
SNALP PEG-A-DMA
SPLP PEG-C-DMA
pSPLP PEG-DSG
SPLP PEG-DSG
TUMOR
HEART
KIDNEY
LUNGS
SPLEEN
LIVER
% Injected Dose
per gram Tissue

FIG. 16

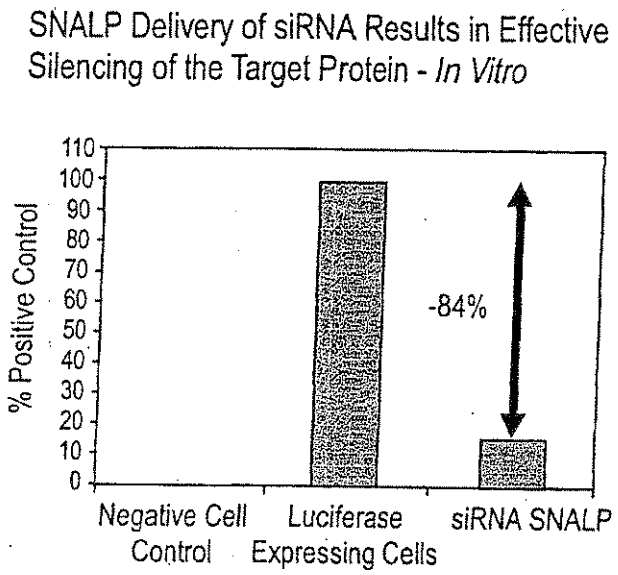

SNALP Delivery of siRNA Results in Effective Silencing of the Target Protein - *In Vitro*
% Positive Control
110
100
90
80
70
60
50
40
30
20
10
0
-84%
Negative Cell Control
Luciferase Expressing Cells
siRNA SNALP

FIG. 17

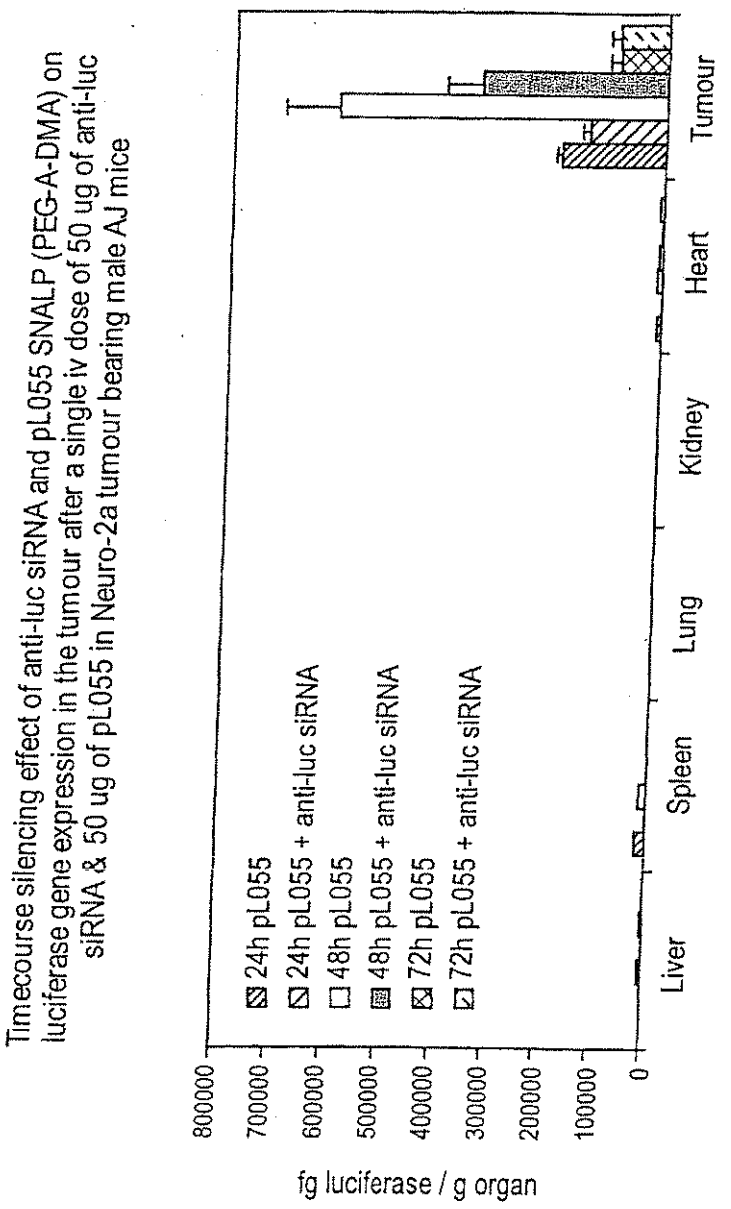

Timecourse silencing effect of anti-luc siRNA and pL055 SNALP (PEG-A-DMA) on luciferase gene expression in the tumour after a single iv dose of 50 ug of anti-luc siRNA & 50 ug of pL055 in Neuro-2a tumour bearing male AJ mice
24h pL055
24h pL055 + anti-luc siRNA
48h pL055
48h pL055 + anti-luc siRNA
72h pL055
72h pL055 + anti-luc siRNA
800000
700000
600000
500000
400000
300000
200000
100000
0
fg luciferase / g organ
Liver
Spleen
Lung
Kidney
Heart
Tumour

FIG. 18

Timecourse silencing effect of anti-luc siRNA and pL055 SNALP (PBG-A-DMA) on luciferase gene expression in the tumour after a single iv dose of 50 ug of anti-luc siRNA & 50 ug of pL055 in Neuro-2a tumour bearing male AJ mice

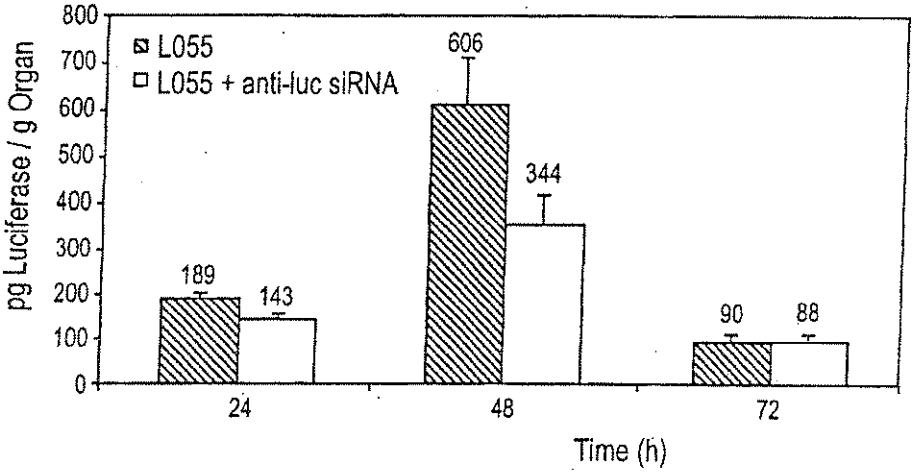


FIG. 19

Timecourse silencing effect of anti-luc siRNA and pL055 SNALP (PBG-A-DMA) on luciferase gene expression in the tumour after a single iv dose of 50 ug of anti-luc siRNA & 50 ug of pL055 in Neuro-2a tumour bearing male AJ mice

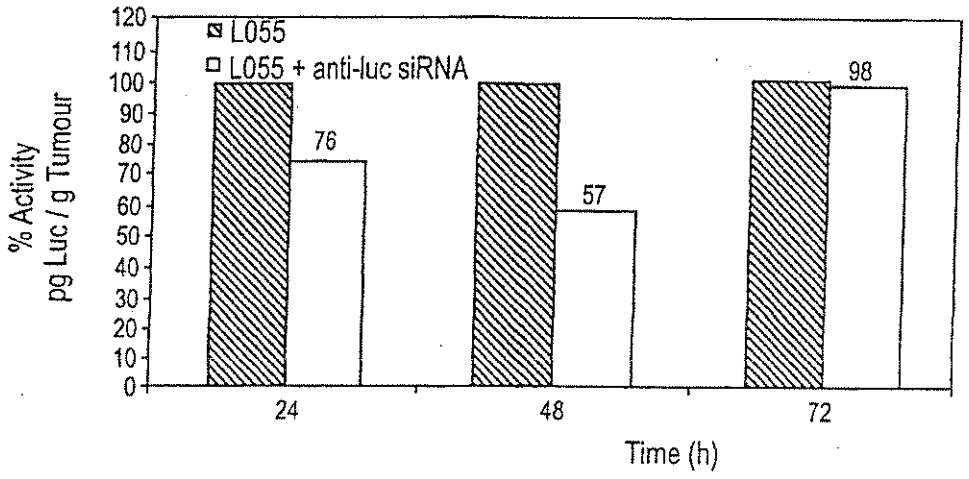


FIG. 20

Timecourse silencing effect of anti-luc siRNA and pL055 SNALP (PBG-A-DMA) on luciferase gene expression in the tumour after a single iv dose of 50 ug of anti-luc siRNA & 50 ug of pL055 in Neuro-2a tumour bearing male AJ mice

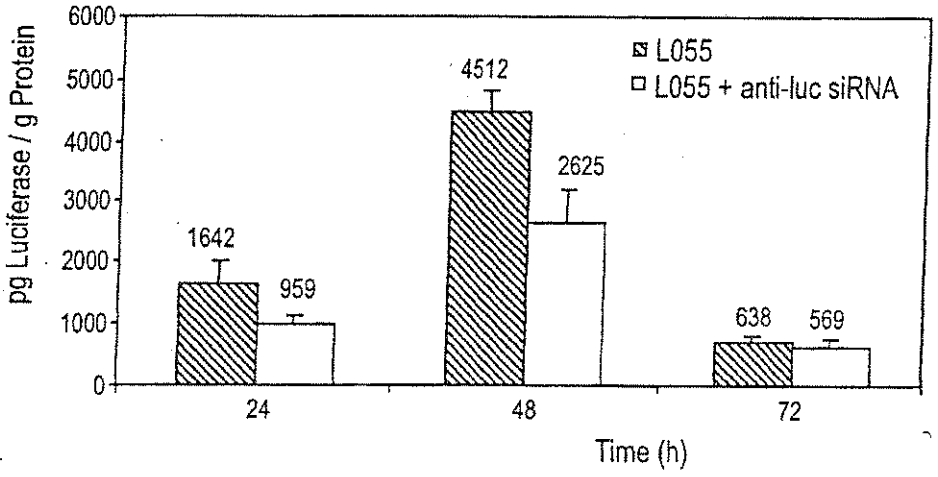


FIG. 21

SNALP Delivery of siRNA Results in Effective Silencing of the Target Protein - *In Vitro*

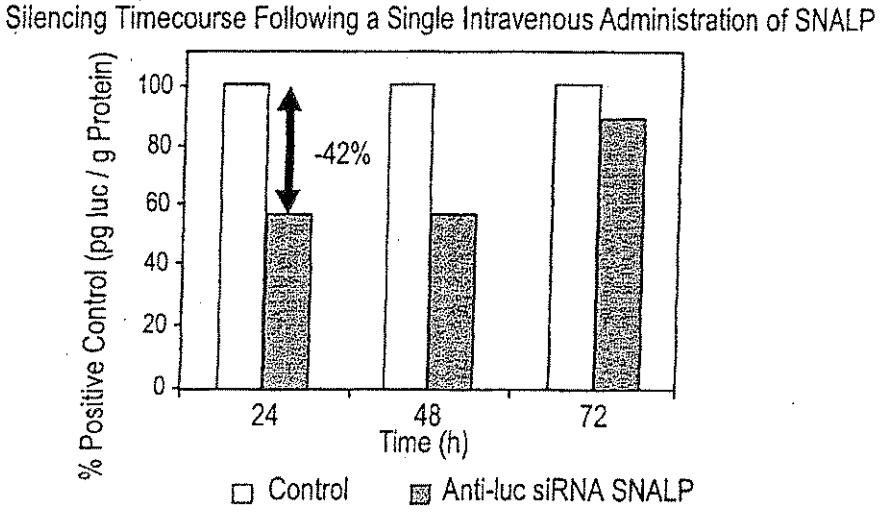


FIG. 22