

61

M Triton X-100. The plates were frozen at -80° C. for a minimum of 2 hours, and thawed at room temperature or 37° C. The thawed cell lysates were cleared by centrifugation and the supernatants were assayed for 3-gal activity using the enzymatic substrate ONPG. The concentration of total protein in cell lysates was also determined using a Bradford assay (Bio-Rad Laboratories, Hercules Calif.). β -gal activity in transfected cell extracts was calculated against a standard curve and expressed as ng 3-gal per surface area of tissue culture plate (ng/cm²) to reflect activity per transfection, or as ng β -gal per μ g of total protein (ng/ μ g) to reflect specific activity.

HEK-293 (FIG. 1), COS-7 (FIG. 2), CHO-K1 (FIG. 3), and HeLa (FIG. 4) cells were transfected with 0.4 or 0.8 μ g of pCMV \cdot SPORT- β -gal DNA and 0.2 to 4.0 μ l of transfection reagent. The transfection reagents tested were DHDOS (IV) formulated at 2 mg/ml with the neutral co-lipid, cholesterol (at a ratio of 1:15 (M/M) DHDOS to cholesterol); DHDOS formulated at 2 mg/ml with the neutral co-lipid DOPE (dioleoylphosphatidyl ethanolamine) (at a ratio of 1:1 (M/M) DHDOS to DOPE); LipofectAMINE PLUS (Life Technologies, Rockville Md.); and FuGENETM-6 (Boehringer Mannheim, Germany). DHDOS formulations were tested in the range of 0.2 to 1.5 μ l; LipofectAMINE PLUS and FuGENE-6 were tested in the range of 0.2 to 4.0 μ l.

FuGENE-6 was used according to the manufacturer's recommended protocol. DHDOS and LipofectAMINE PLUS were used according to the above protocol. The data presented in the Figures are expressed as total activity (ng/cm²) to better compare total expression from the transfected DNA. Only data with 0.8 μ g of DNA is shown, since similar results were obtained with 0.4 and 0.8 μ g of DNA.

Example 8

Primary, passaged, normal human fibroblasts (NHF's) were plated in 96-well plates at a density of 1.6×10^4 cells per well and transfected the following day. Cells in each well were transfected with 40 ng pCMV \cdot SPORT- β -gal DNA and 0.1 or 0.2 μ l lipid.

The DNA and lipid were diluted separately into 10 μ l of DMEM. The DNA was either used alone or pre-mixed with PLUS, insulin, transferrin, or an integrin-targeting peptide prior to complexing with the lipid. After 15 minutes of complexing, the DNA-lipid was added to cells. Cells were assayed for p-gal activity as described above.

LIPID	ACTIVITY (ng/ β gal/cm ²)				
	DNA	DNA and PLUS*	DNA and INSULIN	DNA and TRANSFERRIN	DNA and INTEGRIN-TARGETING PEPTIDE**
LipofectAMINE Compound of Formula X	10.36	28.6	ND	17.4	ND
1:1.5 DOPE 1 mg/ml	ND	37.8	ND	ND	40.9
Compound of Formula VII 1:1 DOPE 2 mg/ml	29.4	637.9	195.7	21.7	587.9

ND = no detectable activity

*PLUS Reagent is available from Life Technologies, Inc., Rockville, Maryland.

**Reference: S. L. HART, et al (1998), Human Gene Therapy, 9: 575-585.

62

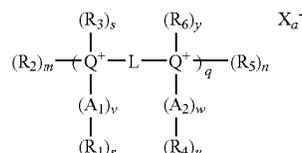
The results show that these cationic lipid formulations can deliver DNA molecules alone, but also that delivery, and ultimately gene expression, may be enhanced when the lipids are used in conjunction with peptides or proteins that bind DNA and/or act as ligands for cell surface receptors, or otherwise enhance cellular and/or nuclear uptake.

Having now fully described the present invention in some detail by way of illustration and examples for purposes of clarity of understanding, it will be obvious to one of ordinary skill in the art that the same can be performed by modifying or changing the invention within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or any specific embodiment thereof, and that such modifications or changes are intended to be encompassed within the scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains, and are herein incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

We claim:

1. A compound or polycation having the formula:



wherein

m, n, q, r, s, u, and y are 1;

v and w are 0;

Q is N;

L is (CH₂)₁, where 1 is an integer from 1 to 4;

R₁ and R₄ are H;

R₂, R₃, R₅ and R₆, independently of one another, are H, or an alkyl or alkyl ether optionally substituted by one or more of an alcohol, an amino alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, an alkylthio, a urea, a thiourea, a guanidyl, and a carbamoyl group;