

solutions and 300 mL of the Coomassie reagent. The assay samples were allowed to sit for approximately 30 minutes before reading the optical absorbance at 570 nm in a microplate reader (Molecular Probes).

The cells were assayed for CAT protein as described above. The results of the transfection efficiency of a mixture of 3-3 and Lipid P is tabulated in Table 1.

Example B

FITC-Oligonucleotide Uptake Assay

A. Oligomers Used

The oligonucleotides used for the determination of cationic lipid mediated oligonucleotide uptake in all cell lines tested are:

#3498-PS: 5' FITC-ggt-ata-tcc-agt-gat-ctt-ctt-ctc [SEQ. ID NO. 1],

Oligomer 3498-PS has an all-phosphorothioate backbone. This oligonucleotide has 23 negative charges on the backbone and is considered to be 100% negatively charged.

#3498: 5' FITC-ggt-ata-tcc-agt-gat-ctt-ctt-ctc [SEQ. ID NO. 2],

Oligomer 3498 is a chimeric oligonucleoside. The underlined bases were linked by a phosphorothioate backbone, while the other linkages in the oligomer consisted of alternating methylphosphonates and phosphodiester. The oligomer had 11 methylphosphate, 7 diester, and 5 phosphorothioate linkages. The total charge density was 57% of 3498-PS.

#3793-2: 5' FITC-ggu-aau-ucc-agu-gau-cuu-cut [SEQ. ID NO. 3],

Oligomer 3293-2 has an alternating methylphosphonate and diester backbone with all 2'-O-methyl groups on each ribose in the oligonucleotide. The total charge density was 50% of 3498-PS.

Stocks of oligomers 3498-PS and 3498 are prepared at 300 micromolar, while the oligomer 3793-2 stock is prepared at 440 micromolar.

B. Reagents and Cells

The commercially available lipids used in the assays were: Lipofectin® ("LFN") Lot#EF3101 1 mg/mL, Gibco/BRL (Gaithersburg, Md.) LipofectAMINE® ("LFA") Lot#EFN101 2 mg/mL, Gibco/BRL (Gaithersburg, Md.) Transfectam® ("TFM") Lot#437121 1 mg dry, Promega, (Madison, Wis.) and resuspended in 100% ethanol.

The novel lipids of the present invention used in these evaluations, are at 1 mg/mL in 100% ethanol.

The tissue culture cell stocks, SNB-19 (human glioblastoma), C8161 (a human amelanotic melanoma), RD (human rhabdomyosarcoma, ATCC # CCL-136) and COS-7 (African green monkey kidney cells, ATCC # CRL-1651) are maintained in standard cell culture media: DMEM:F12 (1:1) mix from Mediatech, Lot#150901126, 10% fetal bovine serum from Gemini Bioproducts, Lot#A1089K, 100 units/mL penicillin and 100 micrograms/mL streptomycin, from Mediatech, Lot#30001044 and 365 micrograms/mL L-glutamine. The cells are maintained under standard conditions (37° C., 5% CO₂ atmosphere) at all times prior to fixation and microscopic examination.

C. Preparation of Cells and Transfection Mixes

For each FITC labeled oligomer delivery determination, the appropriate cells are plated into 16 well slides (Nunc #178599, glass microscope slide with 16 removable plastic wells attached to the slide surface with a silicone gasket) according to standard tissue culture methods. Each cell line is plated at a starting density (approximately 20,000 cells/well) that allowed them to be healthy and 60–80% confluent

one to two days after plating. The cells are allowed to adhere to the glass and recover from the plating procedure in normal growth medium for 24 to 48 hours before beginning the transfection procedure.

Oligonucleotide transfection mixes are made up in Opti-MEM® without antibiotics as follows: 500 mL aliquots of Opti-MEM® containing a 0.25 micromolar solution of either oligomer 3498-PS, 3498, or 3793-2 (2 micrograms of oligomer per sample) are pipetted into 1.5 mL Eppendorf tubes. Cationic lipid or lipid mixture is then added to the oligomer solution to give a final 9:1 or 6:1 ratio (18 or 12 mg of lipid total) of cationic lipid to oligomer by weight. The tubes are mixed by vortexing immediately after the addition of lipid.

Prior to beginning the transfection reactions the cells are rinsed in 200 μL Opti-MEM®; then, the cells are rinsed with Dulbecco's phosphate buffered saline (PBS) solution, and 200 μL of oligomer transfection mix is added directly to a well to begin each transfection reaction. Transfection reactions are allowed to continue for four to six hours.

At that time, the cells are then rinsed in PBS from Mediatech and fixed for ten minutes in 200 μL of 3.7% formaldehyde (Sigma, St. Louis, Mo.) to terminate the transfection reaction. Then the wells are rinsed again in PBS. The formaldehyde is quenched with 200 μL of 50 mM glycine (Sigma, St. Louis, Mo.) for ten minutes. Finally, the wells are then emptied by shaking out the glycine solution. At that time, the plastic chambers and silicone gasket are removed and the cells are covered with Fluoromount-G mounting medium (from Fisher, Pittsburgh, Pa., with photobleaching inhibitors) and a cover slip.

Intracellular fluorescence is evaluated under 200X magnification with a Nikon Labophot-2 microscope with an episcopic-fluorescence attachment. Using this equipment we can distinguish extracellular from nuclear and endosomal fluorescence.

The cells are scored for uptake of FITC labelled oligomer as follows: No nuclear fluorescence, 0; up to 20% fluorescent nuclei, 1; up to 40% fluorescent nuclei, 2; up to 60% fluorescent nuclei, 3; up to 80% fluorescent nuclei, 4; and up to 100% fluorescent nuclei, 5.

TABLE 1

Demonstration of plasmid delivery and expression in SNB-19 with a lipid 3-3/Lipid P mixture			
Cell line	Lipid	CAT cpm/ug ave.	SDV
SNB-19	Lipofectin	742	72
	3-3/Lipid P	4321	601

We claim:

1. A compound of the formula

