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Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

Synthesis of Fluorescent Cationic Lipids for Evaluation of Cellular Pathways and Delivery Mechanisms of Antisense Oligonucleotides

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To cite this Article Bhat, Balkrishen , Heber^t, Normand , Marcusson, Eric G. , Dean, Nicholas M. , Bennett, C. Frank and Manoharan, Muthiah(1999) 'Synthesis of Fluorescent Cationic Lipids for Evaluation of Cellular Pathways and Delivery Mechanisms of Antisense Oligonucleotides', *Nucleosides, Nucleotides and Nucleic Acids*, 18: 6, 1727 — 1728

To link to this Article: DOI: 10.1080/07328319908044835

URL: <http://dx.doi.org/10.1080/07328319908044835>

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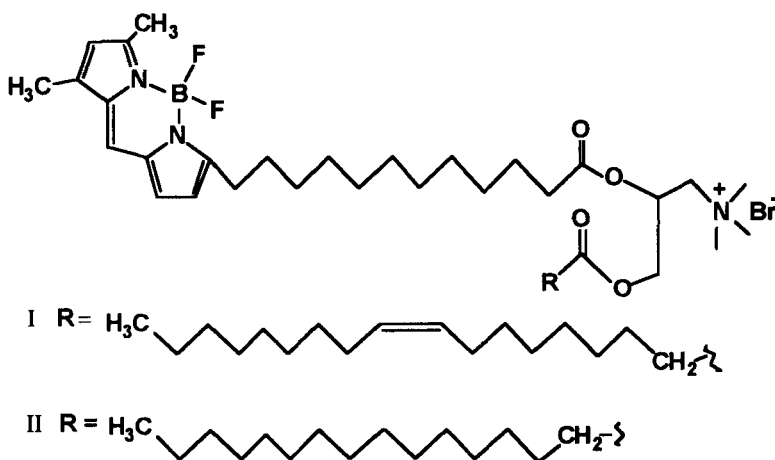
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**Balkrishen Bhat*, Normand Heber^t, Eric G. Marcusson, Nicholas M. Dean
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ABSTRACT: The synthesis of BODIPY conjugated cationic lipids was achieved in three steps from 3-bromopropane-1,2 diol as the starting material. These compounds were evaluated for their ability to enhance cellular uptake of the antisense oligonucleotides.

The use of antisense oligonucleotides to down regulate specific gene products requires oligonucleotides to enter cells and hybridize to target mRNA present in the cytoplasm and/or nucleus of cells ¹⁻⁵. Significant research efforts to increase the absorption of antisense oligonucleotides by cells is ongoing.. In this regard liposomal encapsulation ⁶, conjugation with other ligands such as cholesterol ⁷⁻⁸, cationic lipids ⁹⁻¹⁰ have been successfully used to deliver and increase the cellular uptake of oligonucleotides in cells.



To delineate the cellular trafficking mechanism and pathways several groups have attempted to use fluorescently labeled lipids or oligonucleotides¹¹⁻¹³. In our efforts in understanding the role of cationic lipids as possible delivery agents, the synthesis of two different fluorescent labeled lipids was carried out by the reaction of 3-bromo 1,2 propanediol with the appropriate fatty acid (Fig) followed by reacting the corresponding esters with trimethylamine. The reaction of trimethylammonium esters with BODIPY labeled fatty acid in presence of EDC/DMAP gave labeled cationic lipids. The two lipids showed some differences in their ability to enhance the cellular uptake of rhodamine labeled antisense oligonucleotide in A549 cells .

Acknowledgments:

The authors wish to thank Dr. P. Dan Cook and for his support throughout this project.

REFERENCES

1. Crook, S. T. and Lebleu, B., *Antisense Research and Application*, CRC Press, Boca Raton, FL, **1993**.
2. Crooke, S. T.; Bennett, C. F. *Annu. Rev. Pharmacol. Toxicol.* **1996**, *36*, 107.
3. Gewirtz, A. M. *Anticancer Drug Design.*, **1997**, *12*, 341.
4. Sharma, H. W. and Narayanan, R. *Bioessays*, **1995**, *17*, 1055.
5. Wagner, R. W. *Nature*, **1994**, *372*, 333.
6. Zelphati, O.; Szoka, F. C. *J. Controlled. Release.* **1996**, *41*, 99
7. Alahari, S.K., Dean, N. M., Fisher, M. H., Delong, R., Manoharan, M., Tivel, K. L. and Juliano, R. L. *Mol. Pharmacol.*, **1996**, *50*, 808.
8. Bongartz, J. P., Aubertin, A. M., Milhaud, P. G. and Lebleu, B. *Nucleic. Acid. Research*, **1994**, *22*, 4681
9. Bennett, C. F., Chiang, M.- Y. , Chan, H., Shoemaker, J. E. and Mirabelli, C. K. *Mol. Pharmacol.* **1992**, *41*, 1023.
10. Lewis, J. G. , Lin, K. Y., Kothavale, A., Flanagan, W. M., Matteucci, M. D., DePrince, R. B. , Mook, R. A., Jr, Hendren, R.W. and Wagner, R. W. *Proc. Nat. Acad. Sci.* **1996**, *93*, 3176.
11. Zelphati, O. and Szoka, F. C. *Proc. Natl. Acad. Sci.* **1996**, *93*, 11493
12. Szoka, F. C., Xu, Y., Zelphati, O. J. *Liposome. Research.* **1996**, *6*, 567
13. Marcusson, E. G., Bhat, B., Manoharan, M., Bennett, C.F. and Dean, N. M. *Nucleic. Acid. Research*, **1998**, *26*, 2016.