A Highly Efficient Approach for the Synthesis of Cationic Lipid DOSPA

Yanhong Li,[a] Robert J. Debs,[b] Timothy D. Heath*[a]

[a] School of Pharmacy, University of Wisconsin, 777 Highland Avenue, Madison, Wisconsin, 53705-2221, USA
Fax +1(608)2625345; E-mail: tdheath@pharmacy.wisc.edu

[b] California Pacific Medical Center Research Institute, 475 Brannan St, Suite 220, San Francisco, CA 94107, USA

Received 23 February 2006

Abstract: A highly efficient strategy has been developed for the synthesis of the cationic lipid, *N*-[2-({2,5-bis[(3-aminopropyl)amino]-1-oxopentyl}amino)ethyl]-*N*,*N*-dimethyl-2,3-bis[(1-oxo-9-octadecenyl)oxy] salt with hydrogen chloride (DOSPA). It involves the linkage of a cationic head group and a hydrophobic moiety in the presence of the standard coupling reagents.

Key words: cationic lipids, DNA, synthesis, liposome, transfection

In the past decade, the near completion of the human genome sequencing project offers an unparalleled opportunity to understand the genetic basis of various disease[1], and makes gene therapy an excellent opportunity to cure cancer or inherited diseases in the future.[2–4] However, gene therapy still faces significant technological hurdles before it becomes an established therapeutic strategy.[5–8] Development is required for systems that can efficiently deliver the therapeutic gene or nucleic acid to the site of disease while minimizing delivery to other sites.[9]

Currently, cationic liposomes, which are formed from either a cytofectin or a combination of a cytofectin and a neutral lipid, show particular promise for the delivery of therapeutic genes.[10] They have many important qualities such as being nonimmunogenic and nontoxic.

Cytofectins are positively charged amphiphiles, which are made up of a cationic head group attached by a linker to a hydrophobic moiety. The cytofectins are classified under several subgroups.[11,12] Among the subgroups, the DOTMA analogues are reported to give high transfection efficiencies, and have achieved the most widespread use. The organic synthesis of most DOTMA analogues has been very well documented,[13] which has certainly aided their utility. The formulation of DNA with multivalent cationic lipid, 2,3-dioleoyloxy-*N*-[2-(spermine-carboxamido)ethyl]-*N*,*N*-dimethyl-1-propanaminum chloride (DOSPA, Figure 1) has proved to be a very promising transfection system. Further investigations of structure–property relationships of DOSPA–DNA complexes and the mechanism of their action are important, so sufficient quantities of multivalent cationic lipid DOSPA are critical for future research. Unfortunately, a synthesis of DOSPA has never been reported in the literature.[14] This prompted us to devise an efficient synthetic procedure for the multivalent cationic lipid DOSPA, which we here report.

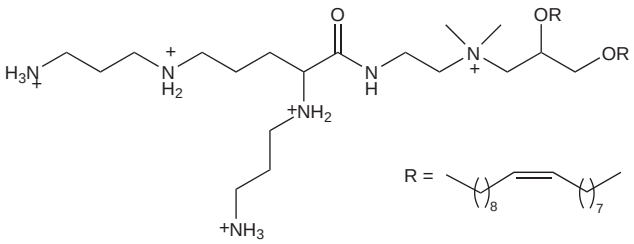
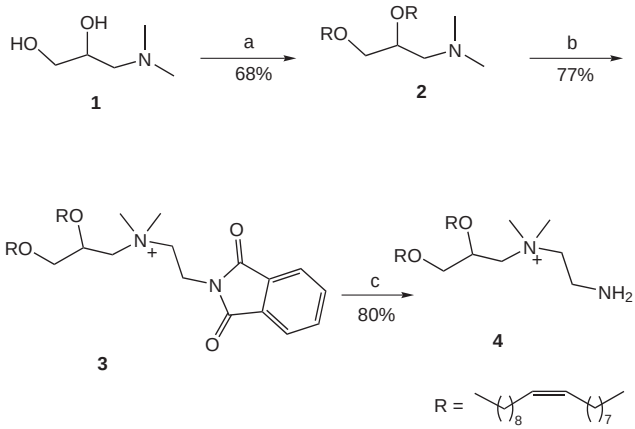


Figure 1 Structure of 2,3-dioleoyloxy-*N*-[2-(spermine-carboxamido)ethyl]-*N*,*N*-dimethyl-1-propanaminum (DOSPA).

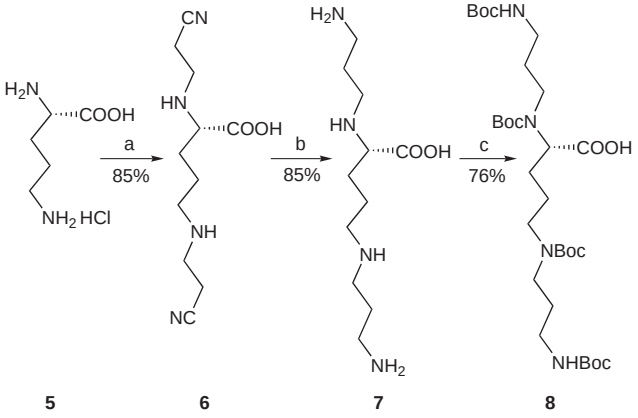
DOSPA is a typical cytofectin and contains a cationic head group attached by a linker to a hydrophobic moiety. The synthesis of the hydrophobic part, **2**, from 3-(dimethylamino)-1,2-propanediol (**1**) is depicted in Scheme 1, and it is identical to the first step of the synthesis of DOTMA.[15] Compound **3** was made by alkylation of **2** with *N*-(2-bromoethyl)phthalimide. The phthalimide (Pht) group is readily removed by refluxing of **3** together with hydrazine and methanol to give **4** with an 80% yield. The free amine group of **4** is the site for linking of the additional portion of the cationic head group.



Scheme 1 *Reagents and conditions*: a) xylene, NaH (2 equiv), oleyl toluenesulfonate (2 equiv), 130 °C, 3 h; b) *N*-(2-bromoethyl)phthalimide (2 equiv), 110 °C, 18 h; c) hydrazine (2 equiv) flux together with MeOH, 18 h.

SYNLETT 2006, No. 14, pp 2284–2286
Advanced online publication: 24.08.2006
DOI: 10.1055/s-2006-949636; Art ID: S02306ST

The additional portion of the cationic head group, tetra(*tert*-butoxycarbonyl)spermine-5-carboxylic acid (Boc_4SperCOOH), was synthesized in three steps as described previously by Remy et al., 1994 (Scheme 2).[16] Instead of using DMF as the solvent in the first step, methanol was used to perform the addition of acrylonitrile to L-ornithine tetramethylammonium salt. The substitution of methanol for DMF increased the yield to 85%. After the hydrogenation and the protection of the amine with a Boc group, the cationic head group precursor **8** has only a free acid group, which can be coupled with the free amine group of the hydrophobic part **4** under the standard coupling condition.



Scheme 2 *Reagents and conditions*: a) MeOH, CH_2CHCN, 24 h; b) EtOH, Raney Ni, H_2, 18 h; c) THF, Boc-ON.

As shown in Scheme 3, by using PyBOP, HOBt and DIPEA as standard coupling reagents, the hydrophobic part **4** and the protected cationic head group **8** can be linked together to give **9** with a yield of 70%. Treatment of **9** with 4 M HCl in dioxane led to the removal of the Boc group of **9** to give the final product, DOSPA (**10**),[17] with a yield of 65%.

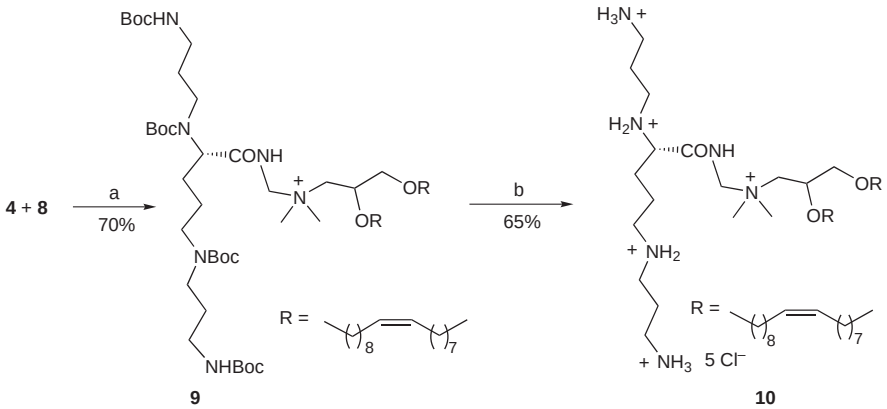
In conclusion, we have developed an efficient and straightforward synthetic strategy to make sufficient multivalent cationic lipid DOSPA for nonviral gene delivery system study. The whole strategy highlights the utility of the temporary amine-protecting group, phthalimide (Pht). This temporary protecting group allows coupling of various multivalent cationic headgroups with the lipid part, which would facilitate the synthesis of new DOSPA-related cytofectins, and would allow detailed investigation of their structure–activity relationships. Ultimately, this may lead to the development of more efficient cytofectins for gene delivery.

Acknowledgment

This work was supported by grant number NIH CA 96666 from the National Institutes of Health, National Cancer Institute

References and Notes

(1) Finkelstein, R.; Baughman, R. W.; Steele, F. R. *Mol. Ther.* **2001**, *3*, 3.
(2) Kayser, O.; Kiderlen, A. F. *Pharm. Biotechnol.* **2004**, 249.
(3) Ozawa, K. *Uirusu* **2004**, *54*, 49.
(4) McGarrity, G. J. *Animal Cell Culture Techniques*; Clynes, M., Ed.; Springer: Berlin, **1998**, 600–612.
(5) Amalfitano, A.; Parks, R. J. *Curr. Gene Ther.* **2002**, *2*, 111.
(6) Hauser, H.; Spitzer, D.; Verhoeyen, E.; Unsinger, J.; Wirth, D. *Cells Tissues Organs* **2000**, *167*, 75.
(7) Suzuki, M.; Matsuse, T.; Isigatsubo, Y. *Curr. Mol. Med.* **2001**, *1*, 67.
(8) Friedmann, T. *Sci. Am.* **1997**, *276*, 96.
(9) Parker, A. L. *J. Drug Targeting* **2005**, *13*, 39.
(10) Miller, A. D. *Medical and Biotechnology Applications*, In *Microspheres, Microcapsules & Liposomes*, Vol. 2; Citus Books: London, **1999**, 545.
(11) Bennett, M. J.; Aberle, A. M.; Balasubramanian, J. G.; Malone, J. G.; Nantz, M. H.; Malone, R. W. *J. Liposome Res.* **1996**, *6*, 545.
(12) Felgner, P. L.; Tsai, Y. J.; Felgner, J. H. *Handbook of Nonmedical Applications of Liposomes*, Vol. 4; CRC Press: Boca Raton, **1996**, 43–56.
(13) Zabner, J. *Adv. Drug Deliv. Rev.* **1997**, *27*, 17.
(14) Miller, A. D. *Angew. Chem. Int. Ed.* **1998**, *37*, 1769.
(15) Felgner, P. L.; Gadek, T. R.; Holm, M.; Roman, R.; Chan, H. W.; Wenz, M.; Northrop, J. P.; Ringold, G. M.; Danielsen, M. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7413.



Scheme 3 *Reagents and conditions*: a) PyBOP, HOBt, DIPEA, CH_2Cl_2 12 h; b) 30% HCl in dioxane, 3 h.

(16) Remy, J. S.; Sirlin, C.; Vierling, P.; Behr, J. P. *Bioconjugate Chem.* **1994**, *5*, 647.

(17) **Selected Data for Compound 4.**
^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.43 (m, 4 H CHCH), 4.11–3.89 (m, 7 H, OCH, OCH_2), 3.59 (s, 6 H, 2 CH_3), 3.51 (m, 6 H, NCH_2CHO, NCH_2), 2.00 (m, 8 H, CH_2CHCH), 1.46–1.20 (m, 48 H, CH_2), 0.93 (t, 6 H, *J* = 5.2 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 130.9, 129.7 (CHCH), 74.6 (CHO), 72.1, 69.5, 68.2 (CH_2O), 65.8, 62.4 (CH_2NCH_2), 53.9, 53.5 (CH_3), 43.1 (CH_2NCO), 32.8-22.8 (CH_2CH_2), 14.3 (CH_3). MALDI-MS: *m/z* = 664.6765 [M + H^+].

Selected Data for Compound 10.
^{1}H NMR (400 MHz, CD_3OD): δ = 5.38 (m, 4 H, CHCH), 3.92–3.02 (m, 30 H, CHCONH, NCH_3, CH_2O, CHO, CH_2N), 2.06–1.20 (m, 70 H, CH_2, CH_3, $CH_2CH_2NH_2$, CH_2CH_2NH, CH_2CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.2 (CO), 130.1, 129.9 (CHCH), 80.6, 79.8, 78.9, 78.5 [$C(CH_3)_3$], 72.9 (CHO), 72.8, 71.2 (CH_2NCH_2), 68.7, 68.0 (OCH_2), 60.9 (NCHCONH), 53.9, 52.8 (NCH_3), 52.1 ($CONHCH_2CH_2$), 47.8–38.2 (CH_2NCO), 32.1–22.1 (CH_2CH_2, CH_2CH_2NHCO, CH_2CHCO), 14.3 (CH_3). MALDI-MS: *m/z* = 1072.1552 [M + H^+].