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# Short Synthesis of Polyoxygenated Macrocyclic Rings Using Acetal Linkages. Application to the Preparation of a New Lipidic Polyamine

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**Abstract:** A short preparation of polyoxygenated macrocycles can be carried out by combining the formation of an acetal linkage, to introduce long alkyl chains, with a ring closure metathesis. As an example, this methodology was used to synthesize a new polyamino lipid.

Gene therapy is a rapidly evolving field of medicine, in which DNA is the therapeutic agent.[1,2] DNA delivery systems mainly consist of either viral or synthetic vectors, viral vectors being the most widely used. However, the use of viruses is restricted by several drawbacks, such as immunological responses, biological safety, and limitation in the gene size. Synthetic vectors[3,4] offer an alternative to viral transfection. Compared to modified viruses, the major advantages of synthetic delivery systems are their simplicity, ease of production, and relatively low toxicity. However, because they are less efficient than viral systems, are sensitive to blood components, and are prone to toxicity, there is still a need for the development of new models.

Archeobacteria lipids[5] are an attractive class of compounds for drug and nucleic acid delivery. They consist of regularly branched, and usually fully saturated, long phytanyl chains attached via ether bonds to a glycerol backbone(s). Archaeobacteria liposomes[6] (archaeosomes) demonstrate relatively higher stabilities to oxidative stress, high temperature, alkaline pH, the action of phospholipases, bile salts, and serum proteins. Furthermore, archaeosomes are safe and do not invoke any noticeable toxicity.[7] Consequently, several macrocyclic models,[8−12] with or without a phytanyl structure, have been synthesized to overcome the difficulties encountered in isolating gram quantities of naturally occurring archaebacterial lipids and to study structure/activity relationships.

In an extension to our research in synthetic vectors for DNA delivery,[13] we became interested in these unique features. We anticipated that liposomes made from simpler macrocycles could provide, despite the absence of phytanyl groups, some of the properties of archaeosomes that could be of great interest for gene and drug delivery. Therefore, we designed a macrocycle that possesses the functionalities to interact with DNA by means of cationic or polythiourea heads[14] at one end and a targeting moiety at the other (Figure 1).

While searching for a methodology suitable for the connection of a long alkyl chain to an alcohol, we came across a report on the preparation of alkyl chloromethoxy ethers.[15] We have used these reactive species for the synthesis of carbohydrate based cationic lipids.[16] We thus reasoned that the above strategy, combined with ring closure metathesis (RCM), would provide a short and

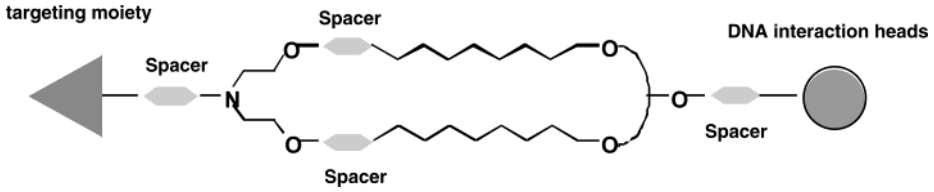


**FIGURE 1.** DNA macrocyclic vectors.

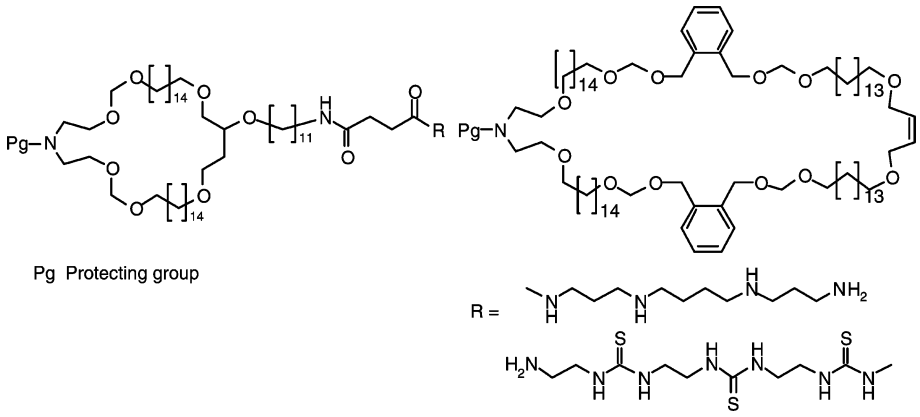


**FIGURE 2.** Structure of polyoxygenated macrocycles.

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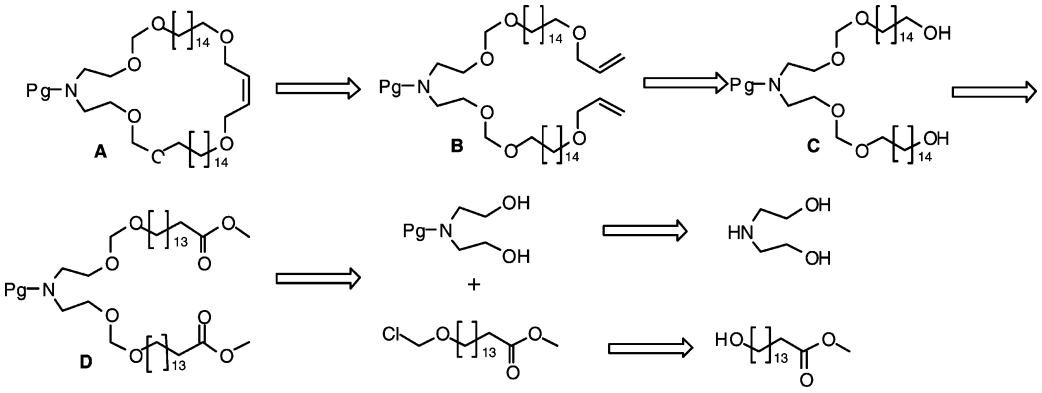
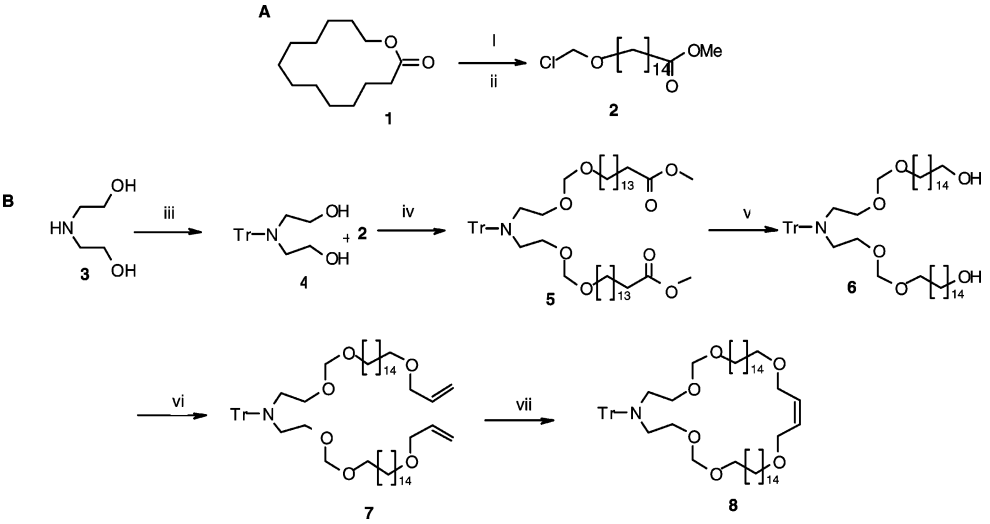
**SCHEME 1. Retrosynthetic Analysis of the 47-Member Macrocycle**



**SCHEME 2. Preparation of the 47-Member Macrocycle**[a]



[a] (i) a MeONa, MeOH, (ii) TMSCl, paraformaldehyde 100%, (iii) TrCl, 85%, (iv) **2**, DIPEA, $tBu_4NI$, THF 60 °C 82%, (v) $LiAlH_4$, reflux THF 91%, (vi) NaH, $tBu_4NI$, allyl iodide, 90%, (vii) $RuCl_2(=CHPh)(PCy_3)_2$, $CH_2Cl_2$ reflux 100%.

simple route to macrocycles. Furthermore, the calculation of the octanol/water partition coefficients (logP) indicated a gain of eight logs between the saturated (16.12) and the oxygenated ring (8.36). These data showed that the polyoxygenated ring will decrease the highly hydrophobic nature of polythiourea lipids and will therefore lead to an easier lipid/DNA complex formulation.[14]

In this note, we describe the preparation of two models (Figure 2), a 47-member macrocycle and a tricyclic 101-atom ring containing two symmetrical benzene groups, in order to avoid folding that has been described for bolaform amphiphile.[17−20] Second, the transformation of the 47-member macrocycle to a polyamino lipid will be depicted.

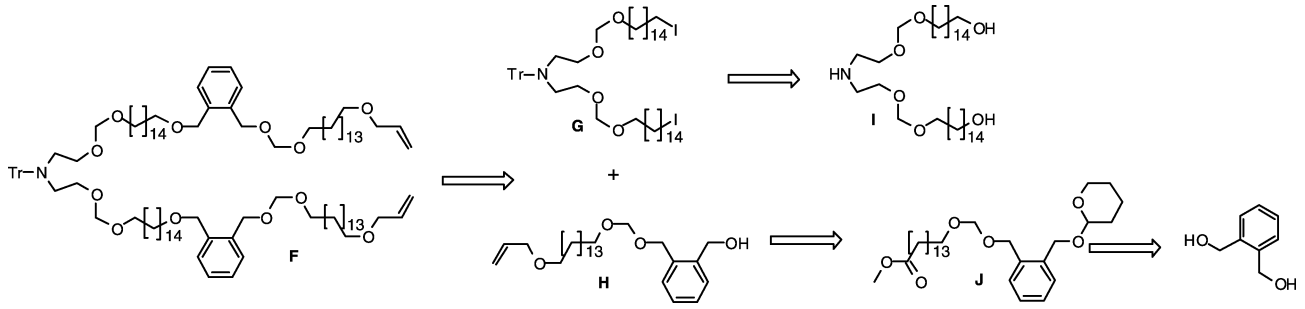
## Synthesis of the 47-Member Macrocycle

Our retrosynthetic analysis for the macrocycle A is shown in Scheme 1. Macrocyclization would occur upon a ring-closing metathesis[21] (RCM) of precursor B, which may be prepared by allylation of diol C. The construction of C would be easily achieved by the reduction of methyl ester D. Accordingly, disconnection of D gives the known compounds diethanolamine and methyl 15-hydroxy-pentadecanoate.

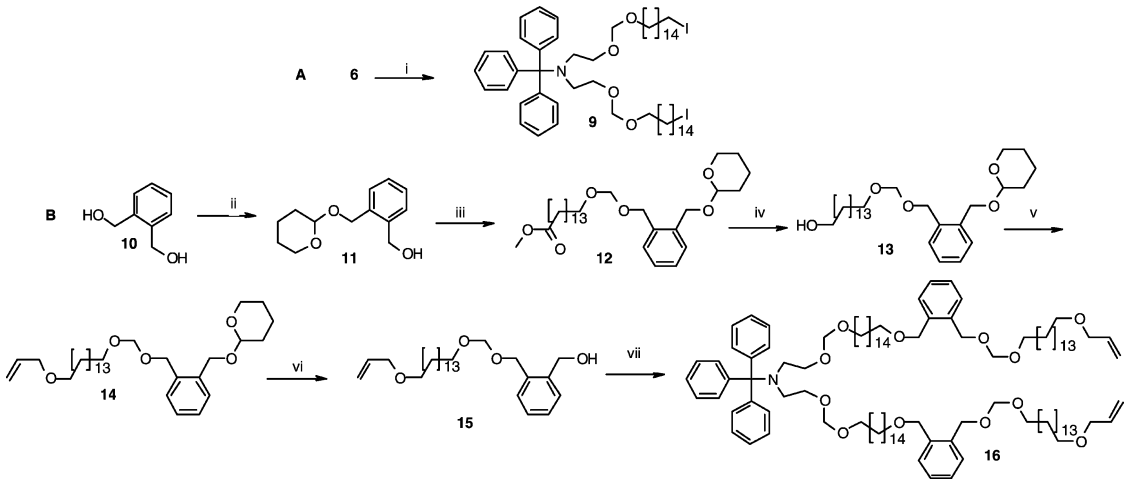
Therefore, we began our synthesis with **2**, which is available in two steps from *ω*-pentadecalactone (Scheme 2). Protection of diethanolamine produced the trityl derivative **4** in 85% yield. Condensation of **4** with the

(1) Rubanyi, G. M. *Mol. Aspects Med.* **2001**, *22*, 113.
(2) Nabel, G. *J. Proc. Nat. Acad. Sci.* **1999**, *96*, 324.
(3) Verma, I. M.; Somia, N. *Nature* **1997**, *389*, 239.
(4) Miller, A. D. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 7, 1768.
(5) (a) De Rosa, M. *Thin Solid Films* **1996**, *284−285*, 13−17. (b) Gliozzi, A.; Relini, A.; Chong, P. L.-G. *J. Membrane Sci.* **2002**, *206*, 131.
(6) Patel, G. B. Sprott, G. D. *Crit. Rev. Biotechnol.* **1999**, *19*, 317.
(7) Patel, G. B; Omri, A.; Deschatelets, L.; Sprott, G. D. *J. Liposome Res.* **2002**, *12*, 353.
(8) Patwardhan, A. P.; Thompson, D. H. *Org. Lett.* **1999**, *1*, 241.
(9) Menger, F. M.; Chen, X. Y. *Tetrahedron Lett.* **1996**, *37*, 323.
(10) (a) Eguchi, T.; Terachi, T.; Kakinuma, K. *J. Chem. Soc., Chem. Commun.* **1996**, 365. (b) Eguchi, T.; Ibaragi, K.; Kakinuma, K. *J. Org. Chem.* **1998**, *63*, 2689. (c) Arakawa, K.; Kano, H.; Eguchi, T.; Nishiyama, Y.; Kakinuma, K. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 1575.
(11) Arakawa, K.; Eguchi, T.; Kakinuma, K. *J. Org. Chem.* **1998**, *63*, 4741.
(12) Eguchi, T.; Arakawa, K.; Terachi, T.; Kakinuma, K. *J. Org. Chem.* **1997**, *62*, 1924.
(13) Carriere, M.; Tranchant, I.; Niore, P.-A.; Byk, G.; Mignet, N.; Escriou, V.; Scherman, D.; Herscovici, J. *J. Liposome Res.* **2002**, *12*, 95−106.
(14) Herscovici, J.; Scherman, D.; Tranchant, I.; Mignet, N.; Girard, C. Patent WO02092558; WO2002FR01626, FR20010006330, US20010297482P, 14/05/2001. Girard, C.; Tranchant, I.; Gorteau, V.; Potey, L.; Herscovici, J. *J. Comb. Chem.* **2004**, *6*, 275.

**SCHEME 3. Retrosynthetic Analysis of the 101-Member Macrocycle Precursor**



**SCHEME 4. Preparation of the 101 Atoms Macrocycle Precursor 16**[a]



[a] (i) $I_2$ $PPh_3$ imidazole 95%, (ii) APTS dihydropyran 85%, (iii) **2**, diisopropylethylamine, tetrabutylammonium iodide reflux, (iv) $LiAlH_4$ THF (80% two steps), (v) NaH, allyl iodide 66%, (vi) Dowex $H^+$ 95%, (vii) NaH, **9** 0°−5 °C then 60 °C, one night 32%.

chloromethoxy ether **2** at 60 °C afforded the diester **5** (82%), which was reduced with $LiAlH_4$ to give the diol **6** (91%). Allylation of **6** with allyl iodide afforded the bis-(2-(15-allyloxy-pentadecyloxymethoxy)-ethyl)-tritylamine (**7**) in 90% yield. Heating **7** for 2 days in the presence of Grubb's catalyst produced the 47-member macrocycle **8** quantitatively.[22,23] NMR data was consistent with the 1,3,9,11,27,32-hexaoxa-6-aza-cycloheptapentacont-29-ene structure for **8**. Examination of the DEPT spectrum showed only CH olefinic carbons ($\delta$ 130.2 ppm), which were correlated to the proton signal at $\delta$ 5.8 ppm.

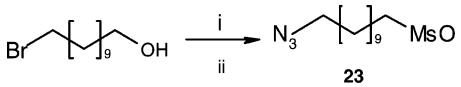
### Preparation of the 101-Member Macrocycle

Having completed the synthesis of **8** we investigated the preparation of the 101-member macrocycle. The retrosynthetic analysis showed that the precursor of macrocycle **F** could be prepared by the condensation between **G** and **H**. Therefore, the diodide **9** was prepared by the reaction of the diol **6** with $I_2/PPh_3$ in the presence of imidazole (Scheme 4). Tetrahydropyranylation of **10** led to the monoprotected derivative **11.** Coupling **11** with chloromethoxy ether **2** followed by $LiAlH_4$ reduction generated **13** (80% yield, two steps). Allylation of alcohol **13** provided **14**. Removal of the THP group using a Dowex resin gave the benzylic alcohol **15**. Condensation of the diiodide **9** with **15** afforded the bis(allyl) ether **16** in 32% yield.

To improve this modest yield, another approach was investigated (Scheme 5). Acetalation of **15** with the chloromethoxy ether **2** and reduction of the resulting ester **17** afforded the bis-acetal **18** in 80% yield (two steps). Reaction of **18** with methanesulfonyl chloride gave the mesyl **19.** Condensation of the diol **4** with **19** in the presence of HMPA produced the macrocycle's precursor **20** in 56% yield. To complete the synthesis, **20** was refluxed in the presence of Grubb's catalyst to quantitatively yield the macrocycle **21** as a white solid.

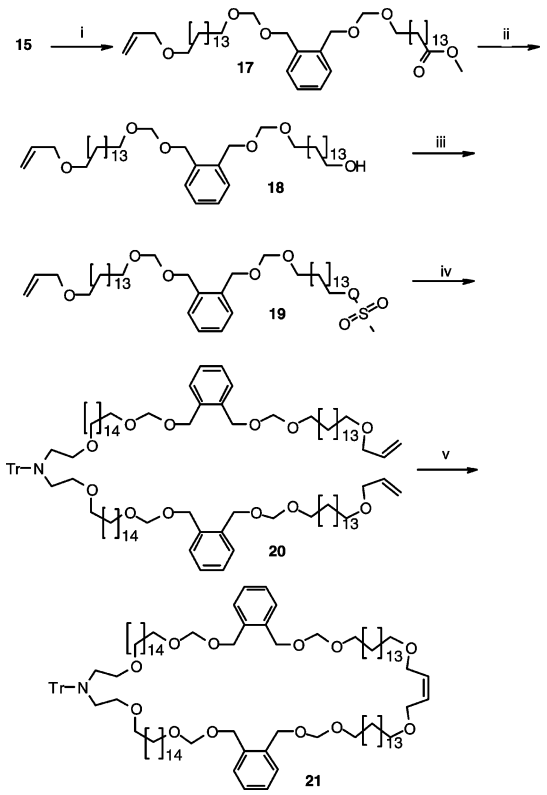
(15) Shipov, A. G.; Savost'yanova, I. A.; Baukov, Y. *I Zh. Obshch. Khim.* **1984**, *54*, 2649.
(16) Herscovici, J.; Egron, M. J.; Quenot, A.; Leclercq, F.; Leforestier, N.; Mignet, N.; Wetzer, B.; Scherman, D. *Org. Lett.* **2001**, 1893.
(17) Yamauchi, K.; Moriya, A.; Kinoshita, M. *Biochim. Biophys. Acta* **1989**, *1003*, 151.
(18) Raguse, B.; Culshaw, P. N.; Prashar, J. K.; Raval, K. *Tetrahedron Lett.* **2000**, *41*, 2971.
(19) Moss, R. A.; Li, J.-M. *J. Am. Chem. Soc.* **1992**, *114*, 9227.
(20) Cornell, B. A.; Braach-Maksvytis, V. L. B.; King, L. G.; Osman, P. D. J.; Raguse, B.; Wieczorek, L.; Pace, R. J. *Nature* **1997**, *387*, 580.
(21) For the preparation of macrocycle using RCM see: (a) Patwardhan, A. P.; Thompson, D. H. *Org. Lett.* **1999**, *1*, 241−244. (b) Lee, C. W.; Grubbs, R. H. *J. Org. Chem.* **2001**, *66*, 7155−7158 and ref 10b.
(22) Maynard, H. D.; Grubbs, R. H. *Macromolecules* **1999**, *32*, 6917.
(23) Schwab, P.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100.

(24) The azido sulfonate **22** was prepared from 11-bromo undecanol in a two step sequence entailing (i) substitution of bromine with sodium azide. (ii) mesylation of the hydroxyl group with methanesulfonyl chloride.



**23**

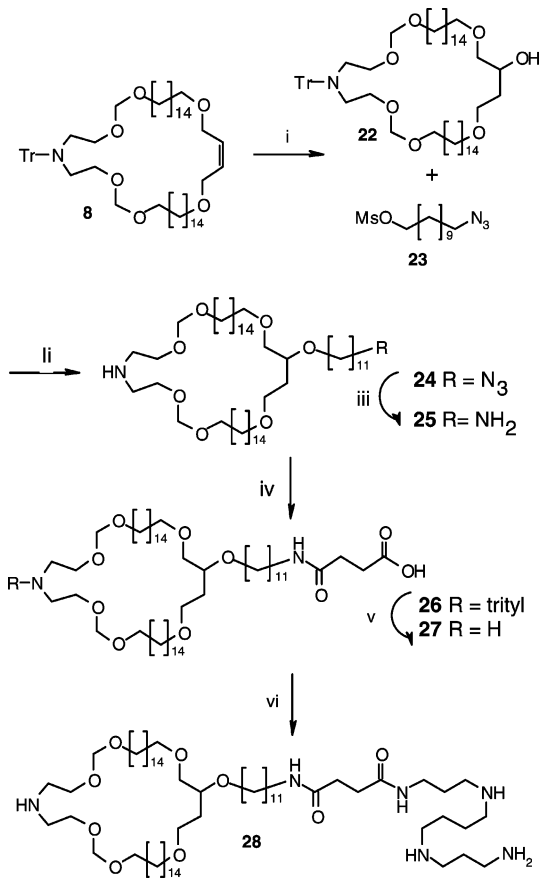
**SCHEME 5. Preparation of the 101-Member Macrocycle**[a]



[a] (i) **2**, diisopropylethylamine tetrabutylammonium iodide, (ii) $LiAlH_4$ 80% (two steps), (iii) methanesulfonyl chloride DMAP RT one night 90%, (iv) **4**, NaH THF HMPA 0−5 °C 2 h then RT, overnight, 56%. (v) benzylidene-bis(tricyclohexylphosphine)dichlororuthenium, $CH_2Cl_2$, 48 h, reflux 100%.

To demonstrate the interest of macrocyclic polycationic vectors, the preparation of polyamine **27** was undertaken (Scheme 6). Hydroboration of **8** with borane-tetrahydrofuran complex gave the 6-trityl-1,3,9,11,27,32-hexaoxa-6-aza-cycloheptapenta-contan-27-ol (**22**) in 70% yield as a yellow oil. Alkylation of **22** with the sulfonate **23**[24] afforded the azide **24**. Treatment of **24** with a polystyrene grafted triphenylphosphine yields the amine **25** quantitatively. Reaction of **25** with succinic anhydride proceeded smoothly to yield the succinate **26** (72%). Hydrogenolysis of **26** afforded the secondary amine **27** quantitatively. Finally, spermine condensation with **27** was performed using BOP. Purification of the resulting product on Sephadex LH-20 afforded the lipopolyamine **28** (yield 46%).

**SCHEME 6. Preparation of the Macrocyclic Polyamino Lipid**[a]



[a] (i)$BH_3$-THF, 0 °C, 70%, (ii) NaH, THF reflux 40% (iii PS−$PPh_3$ $H_2O$, THF 100%, (iv) succinic anhydride, DMAP, $CH_2Cl_2$, pyridine 72%, (v) Pd(C) MeOH 100%, (vi) DIPEA, BOP, $CH_2Cl_2$, 46%.

In conclusion, the work presented here describes methodologies for the preparation of polyoxygenated macrocycles and their transformation into a macrocyclic cationic lipid. Efforts toward the introduction of a targeting moiety[25] and evaluation of their transfecting properties are underway.

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**Supporting Information Available:** Experimental procedures and NMR data for compounds **2** to **28**. This material is available free of charge via the Internet http://pubs.acs.org.

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(25) In an attempt to prepare a lipid possessing an interacting head and a targeting moiety, lipids **29** and **30** were synthesized. However, condensation with spermine has thus far been unsuccessful.