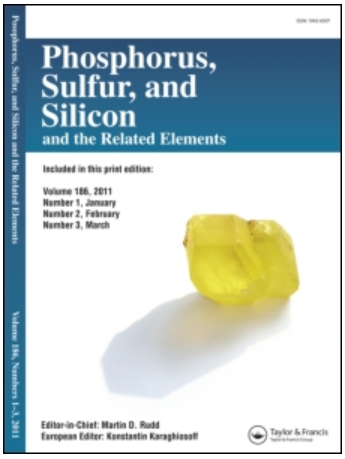

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Cationic Lipids Based on Phosphonate and Phosphoramidate Chemistry: Synthesis and Application to Gene Therapy

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To cite this Article Mével, Mathieu , Yaouanc, Jean-Jacques , Laurent, Pascale , Clément, Jean-Claude , Cartier, Dominique , Jaffrès, Paul-Alain , Montier, Tristan , Delépine, Pascal , Le Gall, Tony , Lehn, Pierre , Pichon, Chantal , Midoux, Patrick and Férec, Claude(2008) 'Cationic Lipids Based on Phosphonate and Phosphoramidate Chemistry: Synthesis and Application to Gene Therapy', Phosphorus, Sulfur, and Silicon and the Related Elements, 183: 2, 460 — 468
To link to this Article: DOI: 10.1080/10426500701761300
URL: http://dx.doi.org/10.1080/10426500701761300

Phosphorus, Sulfur, and Silicon, 183:460–468, 2008

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426500701761300

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Cationic Lipids Based on Phosphonate and Phosphoramidate Chemistry: Synthesis and Application to Gene Therapy

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Cationic lipids having a phosphorus group (phosphonate or phosphoramidate) to link the lipidic part to a cationic head form an efficient family of vectors for DNA delivery. The synthesis of these types of vectors is summarized, including the recent contributions to this field.

Keywords Cationic lipids; DNA carriers; phosphonate; phosphoramidate; synthetic vectors

INTRODUCTION

The delivery of plasmid DNA into cells represents a great potential of development for the treatment of several types of diseases such as monogenic diseases (e.g; cystic fibrosis), infectious pathologies or cancers.[1] The major challenge, in relation with the use of nucleic acid constructs as drugs, consists in its transportation which must be efficient but also achieved without, or at least with a minimum, side effects. As the vectorisation of naked DNA is very limited, the use of carriers has

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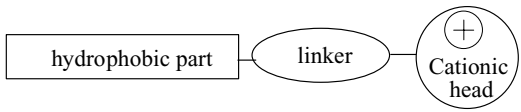
been widely investigated. Recombinant virus and synthetic transfection reagents have been recognized to be efficient carriers of DNA, but each type of vectors has their own limitations. The viral based vectors are characterized by their high in vivo efficiency for the delivery of plasmid but their high immunogenicity and the oncogenic risks limits their administration especially for a long-term treatment. For these reasons the non-immunogenic synthetic vectors, also called non-viral vectors, have been studied over the last 20 years (1987–2007). The synthetic vectors can be classified into two categories formed respectively by the cationic polymers and the cationic lipids. Of note, the cationic lipids are generally formed by three main parts (hydrophobic part, linker, cationic polar headgroup) as depicted in Scheme 1.



SCHEME 1 General structure of cationic lipid.

Whatever the type of vector used (cationic polymer or lipid), a compromise balance have to be found between the transfection efficiency of the vector and the toxicity induced by the transfection reagent by itself. For this reason the design of vectors having some mimesis with natural products can be an interesting strategy to produce carriers having the lowest toxicity as possible. In view of the structural features of the natural phospholipids present into the cell membranes, the design of cationic lipids incorporating phosphorus groups has been investigated, notably by our group. In this account, we report the design of cationic lipids having a phosphorus functional group and their use as DNA carriers. As discussed below, the phosphorus group into the cationic lipid can formed either the linker part or the cationic head. When the phosphorus group acts as a linker, it can be a phosphate, a phosphonate or a phosphoramidate. Our contribution to this field of research, which mainly concerns the carriers having a phosphonate or a phosphoramidate group, is included in the present account.

CATIONIC LIPIDS DESIGNED ON THE BASIS OF PHOSPHORUS CHEMISTRY

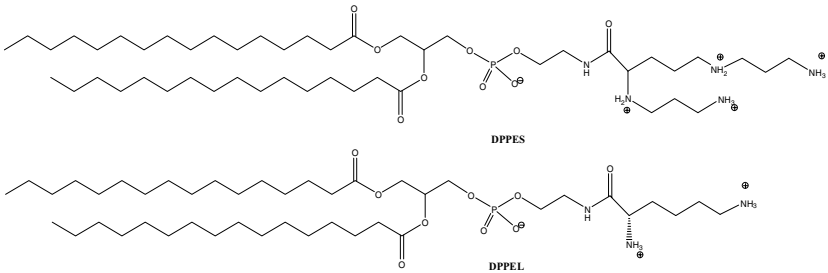
Cationic lipids are known to form, via electrostatic interactions with the negatively charged DNA, a lipoplexe which has some ability to cross the cellular membrane. The structural features of such supramolecular assemblies, which depend of the molecular structure of the cationic lipids,

can be lamellar (L_α^C)[2], inverted hexagonal (H_{II}^C),[3] or hexagonal (H_I^C).[4] The design of cationic lipids for gene delivery,[5] and the mechanisms involved have been previously reviewed.[6] Among all the cationic lipids reported, only few of them have a molecular structure incorporating phosphorus. To produce a low cytotoxic carrier, the cationic phospholipids reported below have some common points with the natural phospholipid present into the cell membranes. The next three parts report the molecular structure of DNA carriers (cationic lipids) having either a phosphate, a phosphonate or phosphoramidate group. Some aspects of their synthesis and their use as DNA carriers are also reported.

Vectors with a Phosphate Group

The first chemical vector for DNA delivery into cells having a phosphate group was calcium phosphate itself which has been used first in the seventies. Even if this method suffers from a low efficiency of transfection and also some variability depending on the size of the particles resulting from the precipitation of the Ca/DNA complexes, recent improvement have been published.[7,8] Nevertheless this vector is still limited for in vitro transfection experiments.

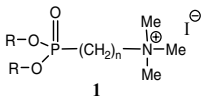
Cationic lipids having a phosphate group have been also prepared. The DPPES[9] (DiPalmitoyl PhosphatidylEthanolamidoSpermine) and DPPEL[10] (DiPalmitoyl Phosphatidyl Ethanolamido L-Lysine) have evident structural similarity with the natural phospholipids present in cell membranes with the presence of two lipidic chains attached to a glycerol unit. These cationic lipids are synthesized by an amide coupling reaction engaging DiPalmitoyl PhosphatidylEthanolamine (DPPE) and BOC-protected spermine or lysine. DPPES was used as carrier of DNA for in vitro transfection on Melanotrophs while DPPEL was evaluated as a vector of DNA for an employed as vectors for antiviral assays.



SCHEME 2 Cationic lipids having a phosphate functional group.

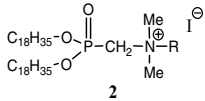
Vectors with a Phosphonate Group

In our group, we have investigated the use of phosphonate group as a linker between the lipid chains and the spacer ended by the cationic head. The use of this function allows designing vectors without the glycerol unit. A first generation of vectors (compounds **1** and **2**) are characterized by the presence of a phosphonate group and an ammonium to form the cationic head.[11]The method of synthesis of the vectors **1** depends on the number of methylene group between the phosphorus atom and the ammonium head. For the preparation of the compound having one methylene (n = 1, compound **1a** or **1d**), the reaction of Field, involving the dioleyl or dimyristyl phosphite, formaldehyde and a secondary amine, was employed. The neutral compound thus obtained was quaternarised by iodomethane to produce the cationic lipids **1** or with another electrophile[12] to produce the cationic lipids **2**. Phophonolipid having a polar head formed by two ammonium groups (**3b**) is isolated after a double quaternarisation step using methyltriflate as alkylating reagent in order to avoid the loss of one lipidic chain, observed when methyliodide is employed.[13,14]



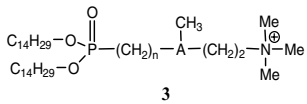
1

1a R = $C_{14}H_{29}$; n = 1
1b R = $C_{14}H_{29}$; n = 2
1c R = $C_{14}H_{29}$; n = 3
1d R = $C_{18}H_{35}$; n = 1
1e R = $C_{18}H_{35}$; n = 2
1f R = $C_{18}H_{35}$; n = 3



2

2a R = CH_2-CO_2-CH_3
2b R = CH_2-CH=CH_2
2c R = CH_2-CN



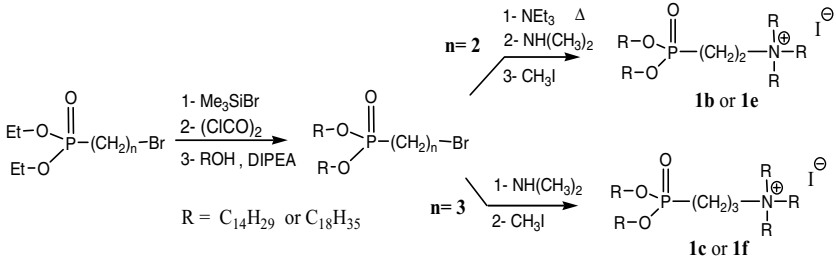
3

3a A = N ; n=1
3b A = N-CH_3 ; n=2

SCHEME 3 Cationic lipids having a phosphonate group and a short linker.

For the production of cationic lipids **1b** and **1e** (n = 2), the first step consists in synthesizing the dioleyl or di myristyl ω-bromoalkylphosphonate from the corresponding diethylphosphonate. The procedure used, to achieve this transesterification like reaction, consists in synthesizing the alkylphosphonyl dichloride, following the Bhongle method, [15] which is then transformed to a phosphonate by reaction with the desired alcohol. For an ethylene spacer (n=2), an elimination reaction occurs in presence of triethylamine at reflux to produce the corresponding vinylphosphonate. This intermediate, which is a good Michael acceptor, reacts with dimethylamine to produce after a quaternarization step the cationic-lipids **1b** or **1e**. Finally the compounds **1c**

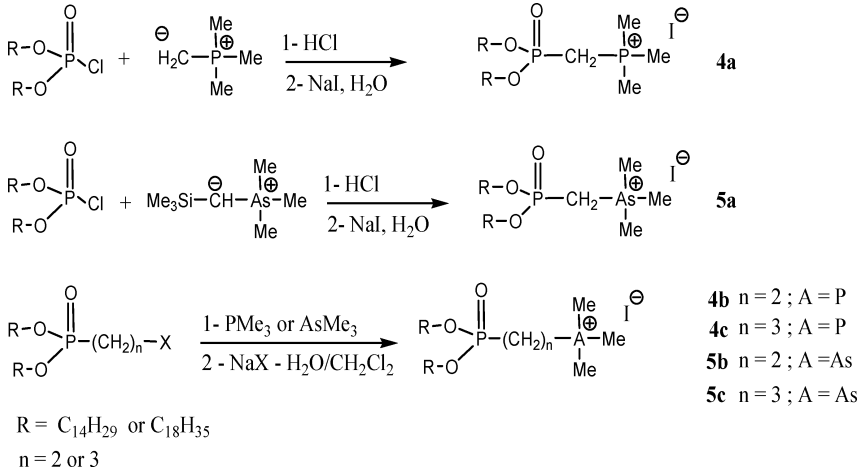
and **1f** (n = 3) were obtained by an amination step followed by a quaternarisation with iodomethane.



SCHEME 4 Cationic lipids with longer linkers.

In order to decrease further the toxicity of the cationic lipids **1** or **2**, we have investigated the replacement of the ammonium head by a phosphonium or an arsonium group. Of note, a similar strategy has been reported by Stekar to decrease the toxicity of some antitumoral agents (Edelfosine, Miltefosine).[16] For the synthesis of the vectors **4a** (phosphonium head) and **5a** (arsonium),[14,17] a strategy different to the one used for the synthesis of compound **1a** and **1d** and based on the chemistry of the phosphonium and arsonium ylides was employed. For the synthesis of the phosphonium (**4b–c**) or arsonium (**5b–c**) having a longer spacer (n = 2 and n = 3) the reaction of the trimethylphosphine or trimethyl arsine on a ω-halogeneoalkylphosphonate can be used. Of note the contribution of the phosphorus chemistry in vectors **4** takes place to design both the cationic head (phosphonium) and the linker (phosphonate group) between the lipidic part and the polar head. It is also worth noting that vectors **4** and **5** constitute the first cationic lipids having a cationic head formed by a phosphonium or an arsonium.

Cationic lipids **1** and **2** have been tested as DNA carriers for the transfection of different cell lines including lung epithelial cells (CFT1) ,[12,18] Human hematopoietic non adherent cell line (K562),[11,19,20] Human epithelial carcinoma cell line (HeLa), epithelial carcinoma cell line (HTB-9),[21] Human hematopoietic non adherent cell line (TF1),[22] and peripheral blood progenitor cells (CD34+).[22] Of note the most efficient vectors with an ammonium head, possess the trimethylammonium group and the toxicity of these vectors appears to be low. These vectors have been also used for in vivo applications. For instance, compound **1d**, was employed for a biodistribution study revealing that the lipoplex was directly located in the lung after administration.[23] One other study demonstrates the efficiency of vectors of type **1** as non viral gene transfer agents in the lungs of mice.[24]



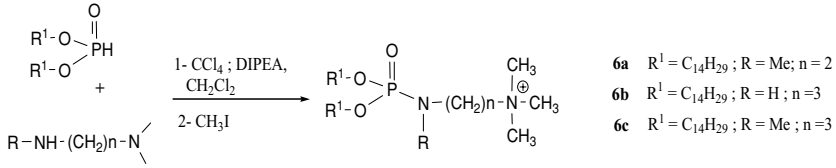
SCHEME 5 Cationic lipids with a phosphonium or arsonium head group.

For the vectors having a phosphonium (**4**) or an arsonium (**5**) cationic head, the number of methylene group separating the phosphonate group from either the phosphonium or the arsonium group must be lengthened to get more efficient vectors. For instance vectors **4b** and **5b** are among the most efficient vectors of the phosphonoloipids. [25] Of note their transfection efficiency is increased compared to the more efficient vector having an ammonium polar head (**1a**). Interestingly, the toxicity of the vectors having a phosphonium or arsenium head is decreased compared to the vectors having ammonium head.[17]Noteworthy, compound **4c** (R = oleyl) has been tested to delivery plasmids to human myoblasts for the ex-vivo gene therapy of Duchene muscular dystrophy.[26]

Vectors with a Phosphoramidate Group

DNA carriers having a phosphoramidate functional group present a P-N bond which is easily cut compared to a P—C bond present into the phosphonolipids (**1–5**). The biodegradability of the vector (chemically or enzymatically) is a crucial point in order to produce vector with a low cytotoxicity. It must be indeed pointed out that the vector, after having accomplished its mission which consists to deliver DNA into cells, must be metabolized into non toxic species in order to avoid the death of the tranfected cell. The access of the phosphoramidate cationic lipids is based on the Todd-Atherton coupling involving a diaakylphosphite (the alkyl group is for instance oleyl, myristyl. . .) and an amine substrate

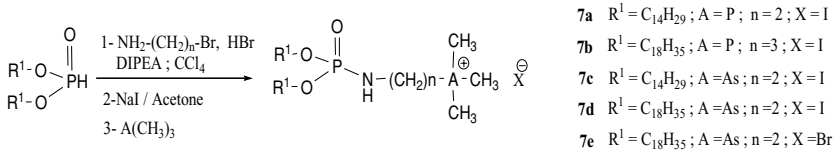
as depicted on Scheme 6. After a quaternarisation with iodomethane, the cationic lipids **6** are isolated.[27]



SCHEME 6 Phosphoramidate cationic lipids with an ammonium cationic head.

Another approach, for the synthesis of phosphoramidate, consists to open a pyrophosphate by an amine in dry pyridine. This approach has been used for the grafting a phosphoramidate moiety on a polyamine compound[28] [spermine, spermidine, or PEI (1800)].

The synthesis of the analogues of compound **6**, having a phosphonium or an arsonium polar head, has been also described (compounds **7**). The synthesis was achieved into a 3 steps process starting with a Todd-Atherton coupling as depicted into the following scheme.[29]



SCHEME 7 Phosphoramidate cationic lipids with a phosphonium or arsonium head group.

In vitro and in vivo transfection assays employing the phosphoramidate as carrier have been reported. The phosphoramidates **6** have been used with success for the transfection of haematopoietic cell lines (CD34+). The best result was obtained with the compound **6c**. Of note this vector is more efficient for the transfection of the same cell line than the phosphonolipid **4b** (with $R = C_{14}H_{29}$) and better than a mixture DOTAP/DOPE which is a commercial carrier agent. Further, the toxicity of this compound is less important than the reference experiment employing DOTAP/DOP mixture.[27]

The presence of a phosphonium or an arsonium polar head in phosphonolipid **7** greatly enhances the transfection efficiency compared to the similar vectors **6** having an ammonium group. Furthermore, the cytotoxicity is also decreased. Of note, compound **7d**, which is one of the best DNA carrier in this series is also efficient for the vectorisation of plasmid in vivo (mice lung). This vector is more efficient when it is use

alone compared to a formulation in presence of DOPE.[29] It is also worth noting that compound **7e**, synthesized by the reaction of trimethylarsine on the bromide substrate, has been used as carrier of plasmide coding for the CFTR protein in the course of ex vivo assays.[30]

CONCLUSION

The design of cationic non viral vectors lipids for DNA delivery involving phosphorus chemistry is rich. Indeed, these vectors are characterized by the presence of a phosphate, a phosphonate or phosphoramidate group acting as a linker between the hydrophobic part and the cationic head. Further, vectors having a cationic head formed by a phosphonium head (or arsonium) have been proved to be a suitable strategy to decrease further the toxicity of the carriers. Nevertheless, further efforts must be devoted to design more efficient and even less toxic vectors to reach the targeted application which is gene therapy.

REFERENCES

[1] J. Gomez-Navarro, D. T. Curiel, and J. T. Douglas *Eur. J. Cancer*, **35,** 2039 (1999).
[2] J. O. Rädler, I. Koltover, T. Salditt, and C. R. Safinya, *Science*, **275,** 810 (1997).
[3] I. Koltover, T. Salditt, J. O. Rädler, and C. R. Safinya, *Science*, **281,** 78 (1998).
[4] K. K. Ewert, H. M. Evans, A. Zidovska, N. F. Bouxsein, A. Ahmad, and C. R. Safinya, *J. Am. Chem. Soc*., **128,** 3998 (2006).
[5] B. Martin, M. Sainlos, A. Aissaoui, N. Oudrhiri, M. Hauchecorne, J. P. Vigneron, J. M. Lehn, and P. Lehn, *Curr. Pharm. Des.*, **11,** 375 (2005).
[6] (a) A. D. Miller, *Angew. Chem. Int. Ed.*, **37,** 1768 (1998); (b) B. Martin, A. Aissaoui, M. Sainlos, N. Oudrhiri, M. Hauchecorne, J.P. Vigneron, J.M. Lehn, P. Lehn, *Gene Ther. Mol. Biol*., **7,** 273 (2003); (c) X. Guo and F. C. Szoka, *Acc. Chem. Res.*, **36,** 335 (2003).
[7] A. Maitra, *Expert. Rev. Mol. Diagn*., **5,** 893 (2005).
[8] S. Bisht, G. Bhakta, S. Mitra, and A. Maitra, *Int. J. Pharm.*, **288,** 157 (2005).
[9] J. P. Behr, B. Demeneix, J. P. Loeffler, and J. Perez-Mutul, *Proc. Natl. Acad. Sci USA*, **86,** 6982 (1989).
[10] C. Puyal, P. Milhaud, A. Bienvenue, and J. R. Philippot, *Eur. J. Biochem*., **228,** 697 (1995).
[11] V. Floch, S. Loisel, E. Guenin, A. C. Hervé, J. C. Clément, J. J. Yaouanc, H. des Abbaues, and C. Férec, *J. Med. Chem*., **43,** 4617 (2000).
[12] M. P. Audrézet, G. Le Bolch, V. Floch, J. J. Yaouanc, J. C. Clément, H. des Abbayes, B. Mercier, A. Paul, and C. Férec, *J. Liposome Res*., **7,** 273 (1997).
[13] V. Floch, G. Le Bolc'h, C. Gable-Guillaume, N. Le Bris, J. J. Yaouanc, H. des Abbayes, C. Férec, and J. C. Clément, *Eur. J. Med. Chem.*, **33,** 923 (1998).
[14] V. Floch, S. Loisel, E. Guenin, A. C. Hervé, J. C. Clément, J. J. Yaouanc, H. des Abbayes, and C. Férec, *J. Med. Chem.*, **43,** 4617 (2000).
[15] N. N. Bhongle, R. H. Notter, and J. G. Turcotte, *Synth. Commun*., **17,** 1071 (1987).
[16] J. Stekar, G. Nössner, B. Kutscher, J. Engel, and P. Hilgard, *Angew. Chem. Int. Ed. Engl*., **34,** 238 (1995).

[17] E. Guénin, A. C. Hervé, V. Floch, S. Loisel, J. J. Yaouanc, J. C. Clément, C. Férec, and H. des Abbayes, *Angew. Chem. Int. Ed. Engl*., **39,** 629 (2000).
[18] P. Delépine, C. Guillaume, V. Floch, S. Loisel, J. J. Yaouanc, J. C. Clément, H. des Abbayes, and C. Férec, *J. Pharm. Sci.*, **89,** 629 (2000).
[19] C. Guillaume, P. Delépine, B. Mercier, E. Gobin, J.P. Leroy, V. Morin, and C. Férec, *J. Pharm. Sci.*, **89,** 639 (2000).
[20] V. Floch, M. P. Audrézet, C. Guillaume, E. Gobin, G. Le Bolch, J. C. Clément, J. J. Yaouanc, H. des Abbayes, B. Mercier, J. P. Leroy, J. F. Abgrall, and C. Férec, *Biochim. Biophys. Acta*, **1371,** 53 (1998).
[21] D. Koumbi, J. C. Clément, Z. Sideratou, J. J. Yaouanc, D. Loukopoulos, and P. Kollia, *Biochim. Biophys. Acta*, **1760,** 1151 (2006).
[22] V. Floch, G. Le Bolc'h, M. P. Audrézet, J. J. Yaouanc, J. C. Clément, H. des Abbayes, B. Mercier, J. F. Abgrall, and C. Férec, *Blood Cells, Mol. Diseases*, **23,** 69 (1997).
[23] P. Delépine, C. Guillaume, T. Montier, J.C. Clément, J. J. Yaouanc, H. des Abbayes, F. Berthou, A. Le Pape, and C. Férec, *J. Gene Med*., **5,** 600 (2003).
[24] C. Guillaume-Gable, V. Floch, B. Mercier, M. P. Audrézet, E. Gobin, G. Le Bolc'h, J. J. Yaouanc, J. C. Clément, H. des Abbayes, J. P. Leroy, V. Morin, and C. Férec, *Human Gene Therapy*, **9,** 2309 (1998).
[25] T. Montier, A. Cavalier, P. Delépine, C. Guillaume, J. J. Clément, J. J. Yaouanc, G. Morel, D. Thomas, and C. Férec, *Blood. Cells, Mol. Diseases*., **30,** 112 (2003).
[26] P. Campeau, P. Chapdelaine, S. Seigneurin-Venin, B. Massie, and J. P. Tremblay, *Gene Ther*., **8,** 1387 (2001).
[27] T. Montier, P. Delépine, K. Le Ny, Y. Fichou, M. Le Bris, E. Hardy, E. Picquet, J. C. Clément, J. J. Yaouanc, and C. Férec, *Biochim. Biophys. Acta*, **1665,** 118 (2004).
[28] T. Dewa, Y. Ieda, K. Morita, L. Wang, R. C. MacDonald, K. Iida, K. Yamashita, N. Oku, and M. Nango, *Bioconjugate Chem*., **15,** 824 (2004).
[29] E. Picquet, K. Le Ny, P. Delépine, T. Montier, J. J. Yaouanc, D. Cartier, H. des Abbayes, C. Férec, and J. C. Clément, *Bioconjugate Chem.*, **16,** 1051 (2005).
[30] T. Montier, P. Delépine, R. Marianowski, K. Le Ny, M. Le Bris, D. Gillet, G. Potard, P. Mondine, I. Frachon, J. J. Yaouanc, J. C. Clément, H. des Abbayes, and C. Férec, *Mol. Biotech*., **26,** 193 (2004).