Lipopolycationic Telomers for Gene Transfer: Synthesis and Evaluation of Their in Vitro Transfection Efficiency

Géraldine Verderone,[†] Nathalie Van Craynest,[†] Otmane Boussif,[‡] Catherine Santaella,[†,§] Rainer Bischoff,[‡,#] Hanno V. J. Kolbe,[‡] and Pierre Vierling*[,†]

Laboratoire de Chimie Bio-Organique, ESA 6001 CNRS, Université de Nice Sophia-Antipolis, Faculté des Sciences, 06108 Nice Cédex 2, France, and Transgène SA, 11 rue de Molsheim, 67082 Strasbourg, France

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We report on the synthesis of a series of lipopolyamine telomers **I-14,*n***, **I-18,*n*,** and **II-18,*n*** and on their in vitro gene-transfer capability. Their structure consists of a polyamine polar moiety, including *n* primary amine functions (from 1 to 70), connected to a hydrophobic moiety, including two hydrocarbon C14 or C18 chains, through a mercaptopropanoyl or mercaptoglyceryl unit and an amide or ether function. They were obtained by telomerization of *N*-{2-[(BOC)-aminoethyl]}acrylamide with *N*,*N*-ditetradecyl- and *N*,*N*-dioctadecylpropanamide-3-thiol and *rac*-1,2-dioctadecyloxypropane-3-thiol, respectively, then BOC deprotection. For *N*/*P* ratios (*N* = number of telomer amine equivalents; *P* = number of DNA phosphates) from 0.8 to 10, these lipopolyamines condensed DNA, with or without the use of DOPE, forming lipopolyplexes or teloplexes of mean sizes less than 200 nm. Some trends, structure−activity and structure−toxicity relationships, were established to achieve both highest in vitro transfection levels and cell viability. Thus, DNA formulations based on telomers **I-14,20** and **I-18,20** and for *N*/*P* ratios lower than 5 led to the most efficient teloplex formulations for plasmid delivery to lung epithelial A549 cells.

Introduction

Gene therapy has been conceived as principally applicable to inherited deficiency diseases (cystic fibrosis, dystrophies, hemophilias, etc.) where permanent cure may be effected by introducing a functional gene. Acquired diseases (cancer, AIDS, multiple sclerosis, etc.) might also be treatable by transiently engineering host cells to produce beneficial proteins. Another application of gene therapy is DNA vaccination.

Successful gene therapy depends on the efficient delivery of genetic material to cells of a living organism and its effective expression within these cells. A functional nucleic acid can be introduced into cells by a variety of techniques resulting in either transient expression of the gene of interest or permanent transformation of the host cells resulting from incorporation of the nucleic acid into the host genome.[1] Viruses have developed diverse and highly sophisticated mechanisms to achieve this goal, including cell targeting, crossing of the cellular membrane by fusion or endocytosis, escape from endosomes and lysosomal degradation, and finally delivery of their genome to the nucleus followed by expression of the viral genome. In consequence, most gene-delivery systems used to date and applied to humans involve recombinant viral vectors, especially adeno- and retroviral vectors.

Although viral vectors are very efficient for gene delivery, their use suffers from a number of disadvantages, including safety and practical issues relating to quality control, high-cost, and problematic large-scale production. Retroviral vectors can only transduce dividing cells; they cannot accommodate large-sized genes: the retroviral genome is integrated into host cell DNA and may thus cause genetic changes in the recipient cell, and infectious viral particles could disseminate within the organism or into the environment. Adenoviral vectors, which do not integrate in the host cell DNA, need, for long-term expression, to be administered repeatedly and thus can induce a strong immune response in treated patients.[1]

Synthetic nonviral gene-transfer vectors, which are free from the risks associated with viral vectors, will be of considerable potential to the gene therapy field. Over the past decade, a large variety of (poly)cationic lipids, liposomes, and (lipo)polymers, eventually associated to molecular conjugates for improving cell targeting, cytoplasmic delivery, and/or nuclear transport, have been used extensively to deliver genes to a large variety of cell lines and tissues (see refs 2−5 and references therein). These (poly)cationic systems are capable of interacting with anionic DNA, condensing or compacting DNA into small-sized complexes (e.g. lipoplexes or polyplexes), neutralizing its negative charges, and thus favoring its entry into the cell. Although the transfection capabilities of such "artificial viruses" are far below those of viral vectors, these systems present potential advantages for the future with respect to low-cost and large-scale production, safety, cell targeting, lower immunogenicity, and capacity to deliver large fragments of DNA.

* Corresponding author. Tel: 33 (0)4 92 07 61 43. Fax: 33 (0)4 92 07 61 51. E-mail: vierling@unice.fr.
[†] Université de Nice Sophia-Antipolis.
[‡] Transgène SA.
[§] Present address: Laboratoire d'Ecologie Microbienne de la Rhizosphère, UMR CNRS-CEA 163, DSV/DEVM, CEN de Cadarache, 13108 Saint Paul lez Durance, France.
[#] Present address: ASTRA-DRACO AB, Dept. of Bioanalytical Chemistry, P.O. Box 34, S-221 00 Lund, Sweden.

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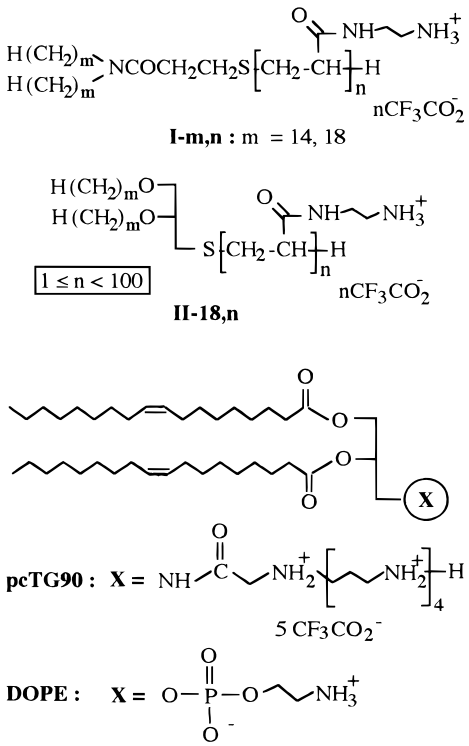
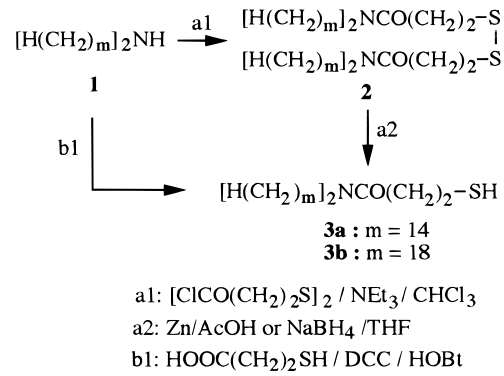


Figure 1. Molecular structure of the lipopolycationic telomers and of the pcTG90/DOPE reference gene-transfer lipids used in this study.

Several studies have shown that the transfer efficiency of the complexed DNA into the cells, especially in the case of in vivo transfer, can vary in function of the interactions between the complexes and the cell membranes (especially with anionic proteoglycans expressed at the cell surface[6,7]), the cell type involved, the cationic components, the lipid composition of the cationic components, the size of the complexes, and, more particularly, the ratio of the positive to negative charges of the different components of the complex. Currently, little is also known concerning the intracellular traffic after endocytosis of the DNA complexes, and the ongoing research remains highly empirical. Consequently, there is a need to develop new compounds, especially cationic compounds, with characteristics and properties different from those already described.

We report herein on the synthesis of new lipopolyamine telomers (Figure 1) and on their capabilities of compacting and transferring DNA into a cell as well as their cellular compatibility. The term "lipopolyamine telomer" designates products obtained from telomerizing (i) one polymerizable monomer (termed taxogen M), for example an acrylamide derivative comprising a (poly)amine part or one of its precursors, in the presence of (ii) a transfer agent (termed telogen), for example hydrophobic long-chain alkane thiols (RS-H), and of (iii) an initiator, which could be a radical generator, for example α,α′-azobis(isobutyronitrile) (AIBN). The lipopolyamine telomers thus obtained are compounds of formula RS-$(M)_n$-H showing low degrees of polymerization ($1 \leq n < 200$) and hence rather low molecular weights, whereas compounds obtainable with classic polymerization processes fit $(M)_n$ structure with high polymerization degrees ($n \gg 200$) and hence high molecular weights. Moreover, the average degree of polymerization (*aDPn*) of the lipopolyamine telomer compounds being particularly linked to the initial taxogen/telogen molar ratio (*Ro*) makes it possible to control and modulate the number of amine functions of the telomers. Theoretically, if the transfer constant is equal to unity, the *aDPn* should be equal to *Ro*.

Scheme 1. Synthesis of Telogens L1-SH **3**



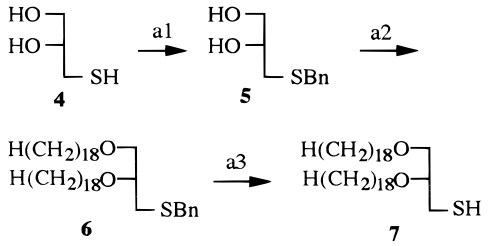
The new cationic lipopolyamine telomer compounds show a modular structure. They consist of a polar moiety, including a variable number of primary amine functions, connected to a hydrophobic moiety, including two hydrocarbon chains of variable length (C18 or C14 chains), through various chemical units (mercaptopropanoyl, mercaptoglyceryl) and functions (amide, ether). Their modular structure is aimed at modulating their physicochemical and biological properties (e.g. chemical stability, biodegradability, toxicity, and transfection efficacy). A hydrophobic moiety composed of two long-aliphatic chains has been introduced as several structure−function studies have shown a correlation of the transfection capability with the hydrophobicity and/or the physical state of the bilayers that theoretically the lipid forms. For instance, cell transfection has been observed with lipopolylysines but not with polylysines[8] and with double-chain lipospermines but not with single-chain lipospermines.[9] A higher cell transfection capability has been observed for some (poly)cationic lipids and co-lipids which form, at the incubation temperature, liquid-crystal rather than crystal bilayers.[10,11] As the use of co-lipids such as DOPE[12,13] and cholesterol[14] (which when used alone do not enhance gene transfer) was found in several cases to enhance transfection activity, we report also on the transfection efficacy of the telomers used in conjunction with DOPE.

Results and Discussion

Telogen Synthesis. Telomerization reactions are more easily performed with transfer agents deriving from primary thiols rather than from sterically hindered secondary or tertiary thiols. Therefore we selected telogens L1-SH **3** and L2-SH **7**, the syntheses of which are outlined in Schemes 1 and 2, respectively.

Two methods were tested for the synthesis of telogens L1-SH. The coupling of the amine directly to unprotected mercaptopropionic acid (step b1 in Scheme 1) afforded in one step L1-SH **3a** in ∼30% yield. The more selective, but time-consuming, two-step procedure which relies on the amidation of 3,3′-dithiodipropanoyl chloride followed by the reduction of the disulfide (steps a1/a2 in Scheme 1) led to L1-SH **3b** in an overall yield of ∼40%.

Scheme 2. Synthesis of Telogens L2-SH **7**



a1: NaOH 2N /EtOH then BnCl, 0°C
a2: $H(CH_2)_{18}OMs$ / Bu_4NHSO_4 , Et_2O, KOH 9N ;
a3: Na, tBuOH

Telogen L2-SH (compound **7** in Scheme 2) was obtained in 40% yield using a three-step procedure which consists, starting from 3-mercaptopropane-1,2-diol, in the selective *S*-benzylation followed by di-*O*-alkylation by phase-transfer catalysis and then *S*-deprotection.

Telomer Synthesis. All telomerization reactions (Scheme 3) were performed under nitrogen by refluxing in acetonitrile a mixture of taxogen **A** and the thiol telogen **3** or **7** in fixed taxogen/telogen concentration and ratio (*Ro*). When the temperature was stabilized, half of the AIBN was added, the other half being added 1 h after. At the end of the reaction, the solvent was evaporated under reduced pressure and the residue purified and fractionated by chromatography on a silica column and gel permeation chromatography on a Sephadex LH20 column. This allows to separate the N-BOC-protected telomers from AIBN and residual disulfide which has formed during telomerization. The BOC-telomers thus obtained were then deprotected in excess TFA at room temperature. The (poly)cationic telomers were isolated as TFA salts by evaporation of excess TFA in the presence of cyclohexane followed by lyophilization. Quantitative deprotection was confirmed by ^{1}H NMR (absence of the signal corresponding to the methyl BOC protons of the starting material). For each telogen as well as *Ro*, the different telomers isolated and their code names are reported in Table 1.

For each fraction collected, the *aDPn* values were determined by ^{1}H NMR on the BOC-protected telomers by comparing the integration of the methylene NCH_2-CH_2NHBOC protons (Hx) with that of the terminal methyl chain protons (Hy): *aDPn* (=*n*) = 3Hx/2Hy. Electrospray ionization mass spectrometry (ESI-MS) was further used to confirm the structure and monodispersity of compounds **I-18,1(BOC)**, **I-18,2(BOC)**, **I-18,1**, **I-18,2**, and **II-18,1** and the polydispersity of telomers **I-18,*n*(BOC)**, **I-14,*n*(BOC)**, **II-18,*n*(BOC)**, **I-18,*n***, **I-14,*n***, and **II-18,*n*** with $n > 2$.

(Lipo)polyplex Formation and Characterization. The capability of the polyamine telomer compounds to condense DNA and to form lipopolyplexes or teloplexes was analyzed with or without an equimolar amount of the helper phospholipid DOPE. These studies were performed with pTG11033 plasmid, which has been used for our in vitro transfection assays (see below). A screening procedure was applied for the teloplex preparation which relies on three formulation methods (dilution from DMSO/EtOH solution in buffer, detergent dialysis, and extrusion) using *N*/*P* ratios of 10, 5, 2.5, 1.25, and 0.8 (*N* = number of amine functions of the telomer; *P* = number of DNA phosphates). With respect to particle size measurements by quasi-elastic light scattering (less than 250 nm), absence of "free" plasmid upon gel electrophoresis, and reproducibility, the dilution method gave the best results (data not shown) and was therefore applied for the formulation of the teloplexes. Table 2 collects the mean particle diameters measured for the different teloplexes.

All the polyamine telomer compounds containing 6−60 primary amines are able, with or without the addition of an equimolar amount of DOPE and for *N*/*P* ratios of 10, 5, and 2.5, to condense DNA into teloplexes of a mean size of less than 200 nm. In most cases, the preparations consist in a single population of particles. Table 2 shows also that smaller (<100 nm) complexes can be obtained frequently for the higher *N*/*P* ratios. For telomers bearing a number of amine groups below 6, one often observes a polydisperse population of large-sized teloplexes that further tend to precipitate. The number of primary amine groups seems indeed to affect the size of the complexes: **I-18,2** with its two amine groups led, without DOPE, to teloplexes of much larger mean size than those obtained with **I-18,*n*** ($n = 10$, 20, 40, 50, or 60) or with **II-18,*n*** ($n = 6$, 11, or 16).

Concerning the impact of the chemical unit connecting the hydrophobic chains to the polar head on teloplex size, one can observe that the mercaptoamidopropanoyl- and mercaptoglyceryl-derived telomers behave very similarly (see Table 2, entries **I-18,10/II-18,*n*** ($n = 6$ or 11) and entries **I-18,20/II-18,16**).

No impact on teloplex size related to the length of the hydrophobic chains could be demonstrated, telomers **I-18,*n*** and **I-14,*n*** leading indeed to complexes of comparable size.

No rationale concerning complex formation and stability (in terms of particle size evolution) can be deduced from the use of DOPE as co-lipid with the telomers. Indeed, and in several cases, the use of DOPE was found either to have no impact on teloplex size or to lead to a teloplex size and polydispersity increase and/or to aggregation. In a few cases only, it led to smaller teloplexes.

In Vitro Transfection. The transfection potency of the telomers, with and without DOPE, was assayed in vitro on lung epithelial A549 cells, from human pulmonary carcinoma. These assays were performed using plasmid pTG11033 (pCMV-intronHMG-luciferase-SV40-pA; 9572 bp) in the presence of 10% fetal calf serum for 24 h. All teloplex formulations that did not precipitate after 15−18 h at 4 °C were tested. The transfection efficiency of the teloplexes (expressed in fg of luciferase/mg of protein) was evaluated for gradual *N*/*P* ratios (0.8, 1.25, 2.5, 5, and 10) and various amounts of DNA (4, 2, 0.5, and 0.1 μg/well) as compared to naked DNA and to the lipoplexes based on pcTG90/DOPE (1/1 molar ratio). The cationic pcTG90 amphiphile (see structure in Figure 1) is among the most efficient lipid for the transfection of A549 cells, the highest level of luciferase expression being observed for the lipoplexes formulated together with DOPE and for a *N*/*P* ratio of 5. [Under the same conditions (plasmid and the A549 cells), but with Transfectam alone or Transfectam/DOPE, luciferase expression levels between 10^5 and 10^6 or 10^6 and 5×10^6 fg of luciferase/mg of protein were measured, respectively (J. Gaucheron, C. Santaella, and P.

Scheme 3. Synthesis of Telomers **I-14,*n***, **I-18,*n***, and **II-18,*n***

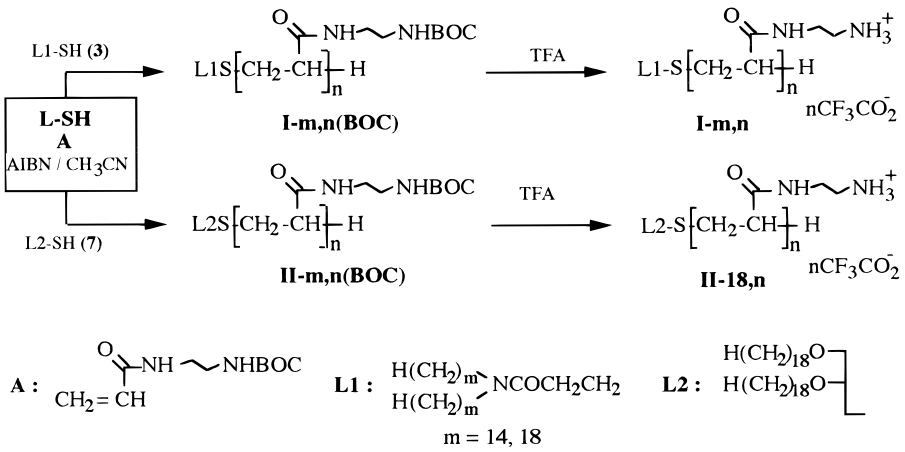


Table 1. Telomerization of Telogens **3** and **7** with Taxogen **A** and Code Names of the Isolated Telomers (after BOC deprotection)[a]

telogen	*Ro*	*aDPn* (*n*)	telomer code name
3b	10	2	**I-18,2**
		10	**I-18,10**
	40	20	**I-18,20**
		40	**I-18,40**
		50	**I-18,50**
		60	**I-18,60**
3a	40	10	**I-14,10**
		20	**I-14,20**
		70	**I-14,70**
7	3	6	**II-18,6**
		11	**II-18,11**
		16	**II-18,16**

[a] *Ro* = [taxogen **A**]/[telogen]; *aDPn* = average degree of polymerization as determined by ^{1}H NMR for the corresponding BOC-protected telomers.

Vierling, unpublished results). The Transfectam lipospermine is a highly efficient synthetic gene-transfer agent widely documented in the literature; see refs 3 and 9.] Cells treated with naked DNA under equivalent conditions showed expression levels of about 10^2 fg of luciferase/mg of protein. The toxicity of the teloplexes was also checked by determining the total protein amount per well of the transfected cells relative to that measured for untreated cells (for which the total protein amount per well is in a 30−60 μg/well range).

The transfection and toxicity results are illustrated in Figures 2−4 for the teloplexes formulated with telomers **I-14,*n*, I-18,*n*,** and **II-18,*n***, respectively. These results indicate that the polyamine telomers do enable transfection of the plasmid into the cells. All the teloplexes tested so far showed indeed a higher efficiency to transfect A549 cells than naked DNA. For the lowest amount of plasmid used (i.e. 0.1 μg/well), the luciferase expression levels stood between 10^2 and 10^4 fg of luciferase/mg of protein. Furthermore, some formulations based on **I-18,2** telomer already peaked out at 10^5−10^6 fg of luciferase/mg of protein for such a low DNA amount (see Figure 3).

The transfection efficiency and the cell tolerance of the teloplexes depend further on the amount of DNA, on the *N*/*P* ratio, on the series of telomers (structure **I** or **II**, polymerization degree), and on the teloplex formulation (DOPE added or not). Some of these parameters are linked and will be discussed together.

Table 2. Mean Sizes, Determined by Light Scattering Spectroscopy, of the Teloplexes Formed by the Dilution Method Applied to Plasmid pTG11033 and the Telomers **I-*m*,*n*** and **II-18,*n***

			teloplex	
			mean diameter in nm (SD)	
telomer	*n*	*N*/*P* ratio[a]	alone	with DOPE
I-14,*n*	10	10	nt[d]	90 (15)
	20	10	55 (20)	37 (100)
	70	10	90 (15)	75 (20)
I-18,*n*	2	10	2640 (250)	85 (25)
	10	10	55 (15)	55 (20)/7150 (700) 75/25%[b]
	20	10	60 (15)	55 (15)
	40	10	65 (20)	70 (15)
	50	10	65 (15)	nt[d]
	60	10	70 (25)	55 (20)
II-18,*n*	6	10	90 (20)	100 (30)
	11	10	50 (15)	100 (40)
	16	10	50 (10)	70 (15)
I-14,*n*	10	5	nt[d]	110 (40)
	20	5	60 (20)	85 (15)
	70	5	90 (25)	65 (15)/700 (90) 75/25%[b]
I-18,*n*	10	5	60 (50)	60 (20)/1100 (230) 85/15%[b]
	20	5	75 (20)	55 (15)
	50	5	60 (25)	nt[d]
	60	5	60 (20)	55 (15)
II-18,*n*	16	5	120 (40)	80 (20)
I-14,*n*	10	2.5	95 (30)	p[c]
	20	2.5	2500 (250)	p[c]
	70	2.5	175 (20)	125 (110)
I-18,*n*	10	2.5	80 (15)	85 (15)
	20	2.5	90 (25)	80 (20)
	40	2.5	p[c]	115 (30)
	50	2.5	150 (110) 80%[b] 1300 (300) 20%[b]	nt[d]
	60	2.5	220 (50)	70 (25)
I-14,*n*	10	1.25	200 (200)	p[c]
	20	1.25	2500 (960)	p[c]
	70	1.25	450 (680)	p[c]
I-14,*n*	10	0.8	p[c]	640 (100)/150 (40) 67/33%[b]
	20	0.8	p[c]	400 (80)/135 (35) 54/46%[b]
	70	0.8	260 (70)	1400 (700)

[a] *N* = amine equivalents; *P* = plasmid phosphate equivalents. [b] Percent (%) of the overall population. [c] p = precipitate. [d] nt = not tested.

The transfection efficiency of the teloplexes usually increased on increasing the DNA amount from 0.1 to 2 μg/well, for which a plateau of luciferase expression seemed to be reached. This plateau was most often attained for a DNA amount of 0.5 μg/well. Concomitantly, their cell toxicity also increased (see Figures 2−4). It remained, however, very low for DNA below 0.5 μg/well. For higher DNA amounts (e.g. 4 μg/well), the transfection, even if higher, was in most cases

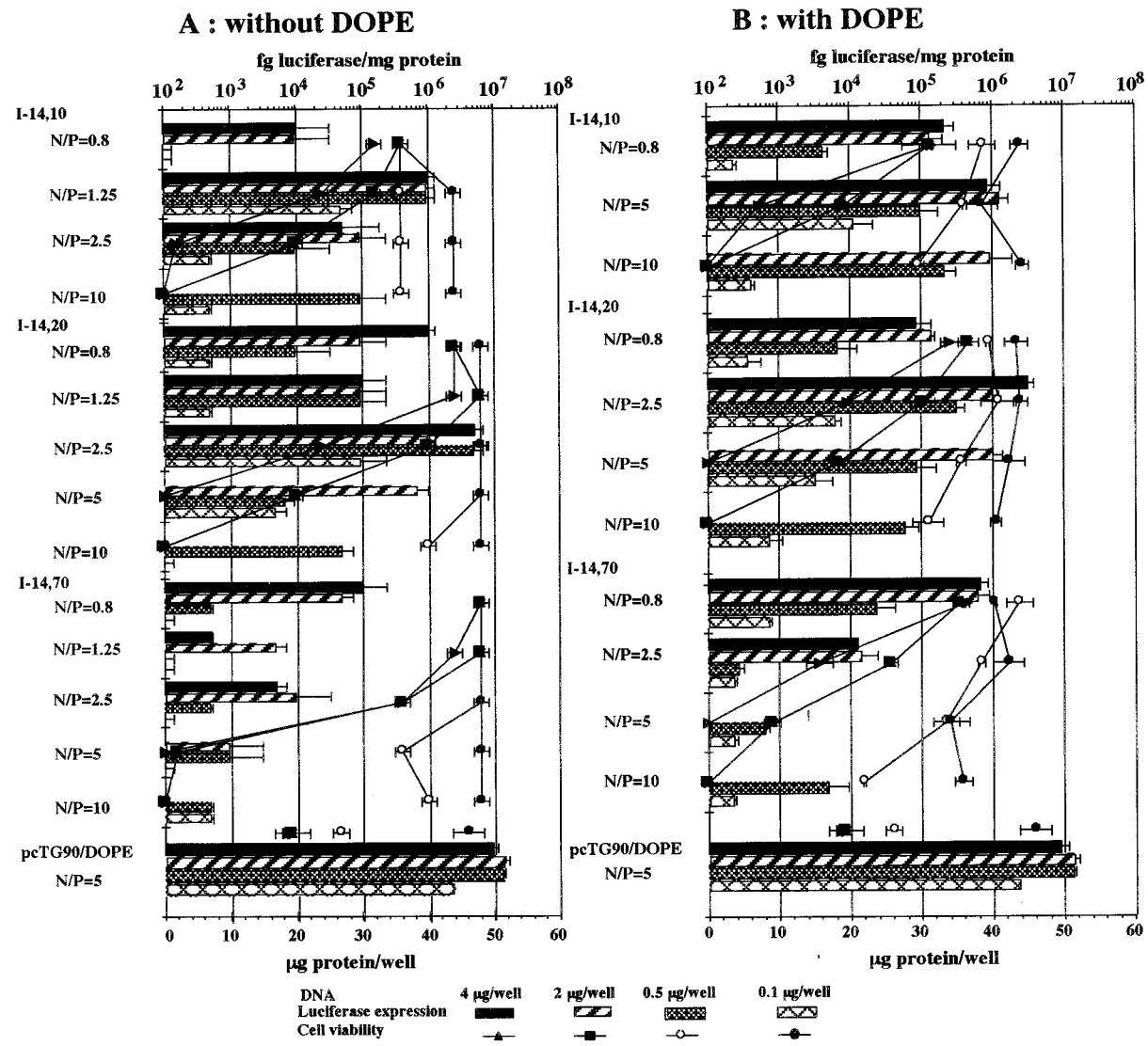


Figure 2. Luciferase expression (bars) and cell viability (points) of the teloplexes made of pTG11033 and **I-14,*n*** telomers (A) or **I-14,*n***/DOPE (1/1 mol) lipids (B), as compared to the reference pcTG90/DOPE (1/1 mol; $N/P = 5$) in A549 cells. Means ± SEM are given.

accompanied by a poor cell survival, except for the lipoplexes based on the telomer **II** series (Figure 2).

Whatever the amount of plasmid used, luciferase expression levels usually grew when the N/P ratio increased from 0.8 to 5 and then slumped. Simultaneously, cell tolerance decreased with the N/P ratio increase, slowly for DNA amounts below 2 μg/well and dramatically when the plasmid amount exceeded 2 μg/well. This transfection efficiency and toxicity increase when raising the cationic charge of the teloplexes is most probably related to an increase of the teloplex cellular uptake which remains at a level compatible with cell growth providing the N/P ratio stayed below 5. For N/P ratios ≥ 5, the teloplexes became too toxic for the cells, likely as a result of a high level of cellular uptake and/or of cell membrane disruption and/or as a result of the presence of a high amount of "free" uncomplexed telomer. As a consequence, reduced cell viability resulted in a drop of luciferase expression. A transfection optimum was reached for plasmid amounts between 0.5 and 2 μg/well for the **I-14,*n*** and **I-18,*n***/DOPE teloplexes, at N/P ratios of 0.8−2.5, and for the **I-14,*n***/DOPE and **I-18,*n*** teloplexes, for N/P ratios of 0.8−5.

Likewise, some trends were found for a moderate toxicity on the A549 cell line. The amount of viable cells increased while DNA amount dropped or when the N/P ratio declined. In most cases, the amount of DNA for which 50% of cell growth inhibition can be observed lies between 2 and 4 μg/well for $N/P \leq 2.5$ and between 0.5 and 2 μg/well for $N/P \geq 5$. An exception are the teloplexes based on the telomer **II** series. These latter formulations displayed indeed higher cell viabilities that were barely affected by the amount of DNA or by the N/P ratio increase. However and likely related, the luciferase expression levels remained low: among the 32 formulations based on telomers **II** tested, only eight of them leveled out at 10^5−10^6 fg of luciferase/mg of protein (see Figure 3 and discussion below).

When compared to the pcTG90/DOPE lipoplex ($N/P = 5$) reference which displays a high transfection efficiency (10^6−10^7 fg of luciferase/mg of protein) already for a DNA amount of 0.1 μg/well, only a few of the formulations investigated here, i.e. **I-18,2** ($N/P = 0.8$), **I-14,10** (1.25), **I-14,20** (2.5), **I-14,20**/DOPE (2.5), and **I-18,20**/DOPE (2.5), were found to be as efficient in mediating DNA transfer and expression (see Figures 2

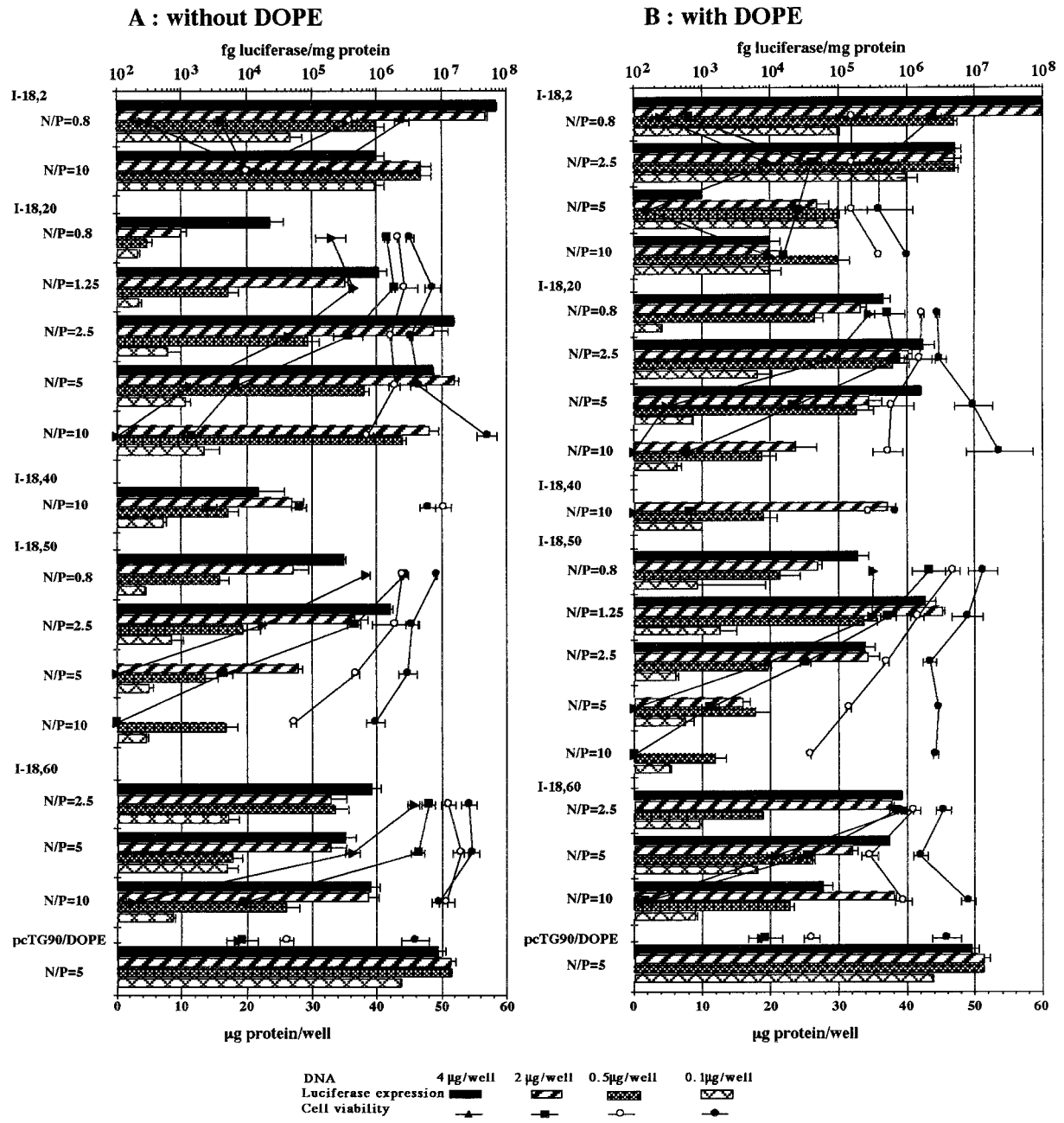


Figure 3. Luciferase expression (bars) and cell viability (points) of the teloplexes made of pTG11033 and **I-18,*n*** telomers (A) or **I-18,*n***/DOPE (1/1 mol) lipids (B), as compared to the reference pcTG90/DOPE (1/1 mol; $N/P = 5$) in A549 cells. Means ± SEM are given.

and 3). However, and with respect to cell toxicity, it is particularly noteworthy that these teloplexes, for DNA amounts ≤ 0.5 μg/well and over a range of N/P ratios lower than 5, allowed luciferase expression levels as high as the reference but with lower cell toxicity. These teloplexes seem to be appealing candidates for in vivo administration.

Concerning the effects on A549 cell survival and transfection (which cannot be analyzed without considering the effects on toxicity) resulting from the formulation of the teloplexes with DOPE, no obvious trend could be evidenced, these effects being rather at random. Indeed, as compared to their respective teloplexes formed without DOPE, the use of DOPE either had no effect on the cell viability for nearly 50% of the cases or increased the toxicity for the other 50% (data not shown). (The increase in cell toxicity resulting from adding DOPE was considered significant if the ratio of protein amount per well measured for the formulation with DOPE vs that measured for the corresponding one without DOPE was lower than 0.8.) The transfection efficiency upon DOPE addition, irrespective of the amount of plasmid used, showed (i) an increase for about 15 of the 100 formulations tested (which also implies that their cell viability has not been affected), (ii) no effect on both transfection and cell viability for 35%, and (iii) a decrease of either transfection and/or cell viability for another 40% range (data not shown). (The effect on transfection of DOPE was considered significant if the ratio of luciferase amount measured for the formulation with DOPE respectively to that measured for the corresponding one without DOPE was out of the 0.2−5 range.) Finally, for less than 10% of the formulations investigated, an increase of transfection could be evi-

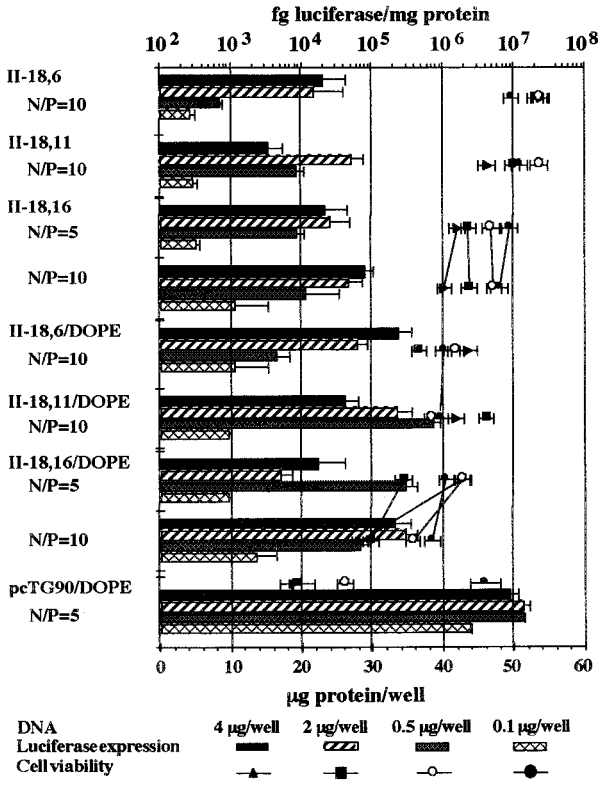


Figure 4. Luciferase expression (bars) and cell viability (points) of the teloplexes made of pTG11033 and **II-18,*n*** telomers or **II-18,*n***/DOPE (1/1 mol) lipids, as compared to the reference pcTG90/DOPE (1/1 mol; $N/P = 5$) in A549 cells. Means ± SEM are given.

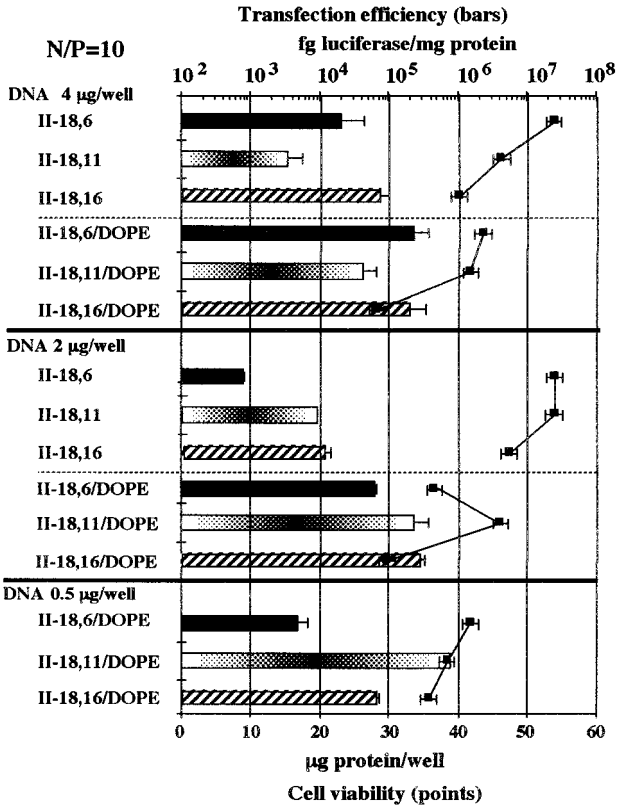


Figure 5. Variation of the **II-18,*n***-based teloplexe's transfection efficiency (bars) and cell viability (points) of the number *n* of amine functions of the **II-18,*n*** telomers.

denced by adding DOPE, together with a consequent toxicity increase. We could not detect any improvement in transfection or in cell viability resulting from the formulation of the teloplexes with DOPE and a given telomer or a series of telomers, with respect to their polymerization degree.

Structure−Activity Relationships. Although a variety of (poly)cationic lipids mediate DNA transfer to cells, few systematic studies have been performed to assess structure−activity relationships of these lipids, partly because the synthesis of the materials is labor-intensive and time-consuming.[15−17] Solid-phase chemistries and combinatorial library approaches have facilitated the rapid generation of a wide array of polyamine lipids[4] and peptoid−phospholipid conjugates.[18] The "telomer" design can access a diverse family of structures that differ in the number of amine functions and in the hydrophobic moiety (chain length and linker), thus enabling the establishment of structure−activity relationships.

An optimum transfection level was measured for a number of amine functions in the 10−20 and 2−20 range for telomers **I-14,*n*** and **I-18,*n***, respectively. For a given N/P ratio and DNA amount, a close analysis of the 34 formulations based on **I-14,10** or **I-14,20** telomers showed indeed that the levels of luciferase expression obtained either with **I-14,10** or with **I-14,20** were in half of the cases comparable. (The effect of the amine function number was considered to significantly increase transfection if the ratio of the luciferase amount measured for the formulations with telomers **I-14,*n*** vs that measured for the corresponding ones with the **I-14,*n′*** homologue is higher than 5 and comparable if this ratio is in the 0.2−5 range.) Almost one-third of the **I-14,20** teloplexes displayed a higher transfection efficiency than their respective **I-14,10** analogues, while one-fifth of the **I-14,10** formulations led to higher transfection levels than the **I-14,20** ones. By contrast, a significant higher level was obtained for 60% or 80% of the **I-14,10-** or **I-14,20**-based teloplexes with respect to their 17 corresponding **I-14,70** formulations, a comparable level of transfection being measured for the complementary 40−20% ones, respectively. This is mainly the case for low N/P ratios. A similar analysis of the **I-18,*n*** formulations showed that up to 85% and 40−50% of the 10 and 19 teloplexes formulated with **I-18,2** or **I-18,20** telomers, respectively, gave a higher level of luciferase expression than their corresponding ones made with the **I-18,40, I-18,50,** or **I-18,60** telomers, a comparable transfection being measured for the complementary 15% and 60−50% ones, respectively. To ensure an optimum transfection level one should preferably use **I-18,2**-based teloplexes rather than **I-18,20** ones. Indeed, 40% of the **I-18,2** formulations were more efficient than their corresponding **I-18,20** ones while the remaining 60% behave very similarly. Finally, no significant difference in transfection was found for a number of amine functions in the 40−60 range.

For the telomer series **II-18,*n***, a significant effect of *n* (transfection increases with raising *n* from 6 to 11 and to 16) could be evidenced only for low amounts of DNA (see Figure 5). Where the effect of the polymerization degree on the teloplex toxicity is concerned, it was also found to be related to its N/P ratio. For low N/P ratios, i.e. <2.5, increasing *n* from 10−20 to 40−70 units did

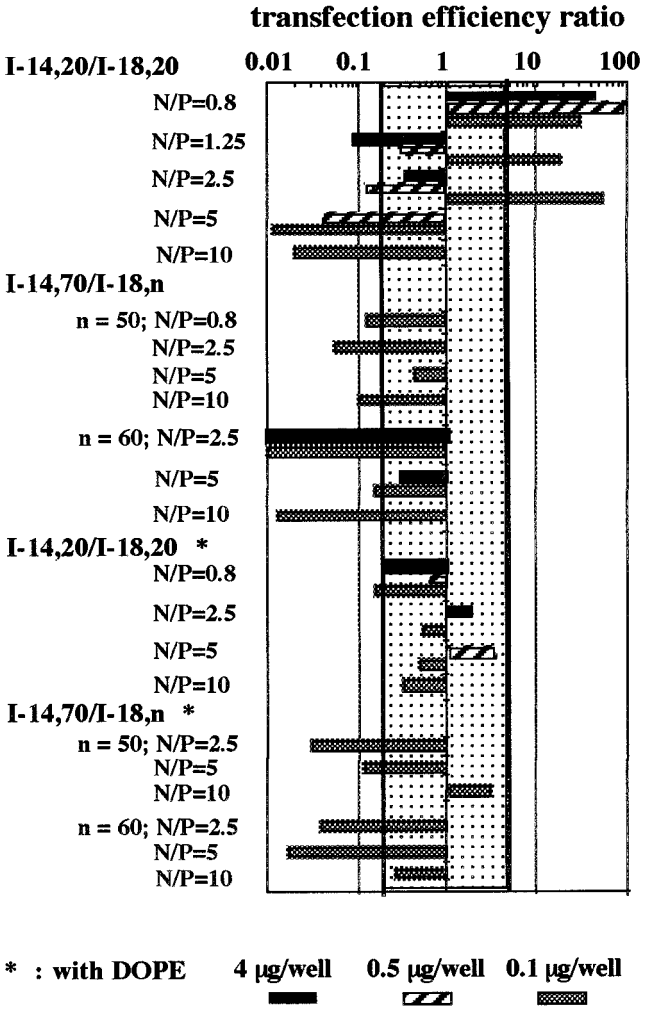


Figure 6. Impact on transfection efficiency of the hydrophobic chain length as estimated by the ratio of luciferase amount measured for the formulations with telomer **I-14,*n*** vs that measured for the corresponding ones with the **I-18,*n*** homologues. The impact is significant if the ratio value is not within the gray zone (see text).

not impair the cell survival and in some cases improved it. Teloplexes formulated with telomers of very small *n*, such as $n = 2$, combined with a low N/P ratio seemed rather more toxic than those made with telomers of higher polymerization degree. Thus, for both an optimum transfection and a low toxicity with the telomers reported here, one should use teloplexes formulated with a telomer having a polymerization degree centered around 20 and having a N/P ratio lower than 5.

Previous work has shown that a single fatty chain in lipopolyamines prevented transfection activity[9] and that the optimal length of the double fatty chain might vary for different products.[4,9,19] Figure 6 displays the ratios of luciferase level measured for the formulations with telomer **I-14,*n*** vs that measured for the corresponding **I-18,*n*** homologues. Assuming that the effect of the chain length (C14 or C18) is significant if this ratio is not in the 0.2−5 range, we found that telomers **I-18,*n*** which possess C18 chains are more efficient in mediating transfection than their **I-14,*n*** counterparts for 42% of 36 formulations investigated. For 47% of them, we found that both series of telomers were as efficient, and only 14% of telomers **I-14,*n*** gave rise to a higher luciferase expression than their **I-18,*n*** analogues. One should mention that no effect on cell viability related to the chain length could be detected. These results indicate that optimum transfection levels are most likely ensured with telomers having C18 chains preferably to C14 ones.

The nature of the linker and the structure (telomers of series **I** as compared to the corresponding molecules of series **II**) was also found to have an impact on toxicity and on transfection as well. As already mentioned above and as illustrated in Figure 7, almost all the ether **II-18,*n***-based teloplexes are well-tolerated by A549 cells, even for a N/P ratio as high as 10 and for a 4 μg/well amount of DNA. By contrast, this is not the case for their most closely related amido **I-18,*n***- or **I-14,*n***-based teloplexes which display an acceptable cell tolerance only for a much lower (0.5 μg/well) amount of DNA. These results make it difficult to compare the transfection efficiency of these two series. Nevertheless, our results strongly suggest a superior transfection efficiency of the amido series **I**-based teloplexes. Indeed, provided some cells are still alive, higher luciferase expression levels (in a range of 10^6-10^7 fg of luciferase/mg of protein) are preferably obtained with the teloplexes of the amido series **I** rather than with the ether **II-18,*n***-based telomers (Figure 7). These gave rise most frequently to moderate levels of luciferase expression (below 10^5 fg of luciferase/mg of protein), even for optimum conditions (high N/P ratio and high amount of DNA, i.e. 4 μg/well). The higher transfection efficiency of the telomers of series **I** is likely confirmed by the high luciferase expression levels ($\sim 10^7$ fg of luciferase/mg of protein) of the **I-18,20** teloplexes which are obtained for a lower N/P ratio (2.5) and for a DNA amount of 2 μg/well, for which a good cell viability is found (see Figure 3).

Conclusion

The present work suggests that our new telomers appear as potential synthetic vectors for gene delivery. The variation of structure parameters allowed the establishment of structure−activity and structure−toxicity relationships and the optimization of the teloplex formulation, where both highest transfection levels and concomitant cell viability were achieved. Thus, a polymerization degree centered around 20 amine units and a N/P ratio lower than 5 leads to the most efficient formulations for plasmid delivery to A549 cells. Some of these telomers are currently being investigated for in vivo studies.

Experimental Section

General Experimental and Analytical Conditions. All the reactions were performed in anhydrous solvents under dry and oxygen-free nitrogen. The purifications by column chromatography were carried out using silica gel 60 (Merck, 70−230 mesh) and chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), methanol (MeOH), diethyl ether (Et_2O), or mixtures thereof as indicated. Advancing of the polymerization reaction was followed by thin-layer chromatography (TLC) on silica plates F_{254} (Merck). The following developing systems were used: UV light; $KMnO_4$; H_2SO_4/MeOH; Dragendorff reagents; DTNB (5,5′-dithiobis(2-nitrobenzoic acid)); ninhydrin reagent (Sigma).

IR spectra were recorded using a Bruker FT-ITS 45 spectrometer. Solid compounds were analyzed using the KBr disk method; liquids were analyzed as thin film between two KBr blocks. ^1H, ^{13}C, and ^{19}F NMR spectra were recorded at 200, 50.3, and 188.3 MHz, respectively, on a Bruker AC-200.

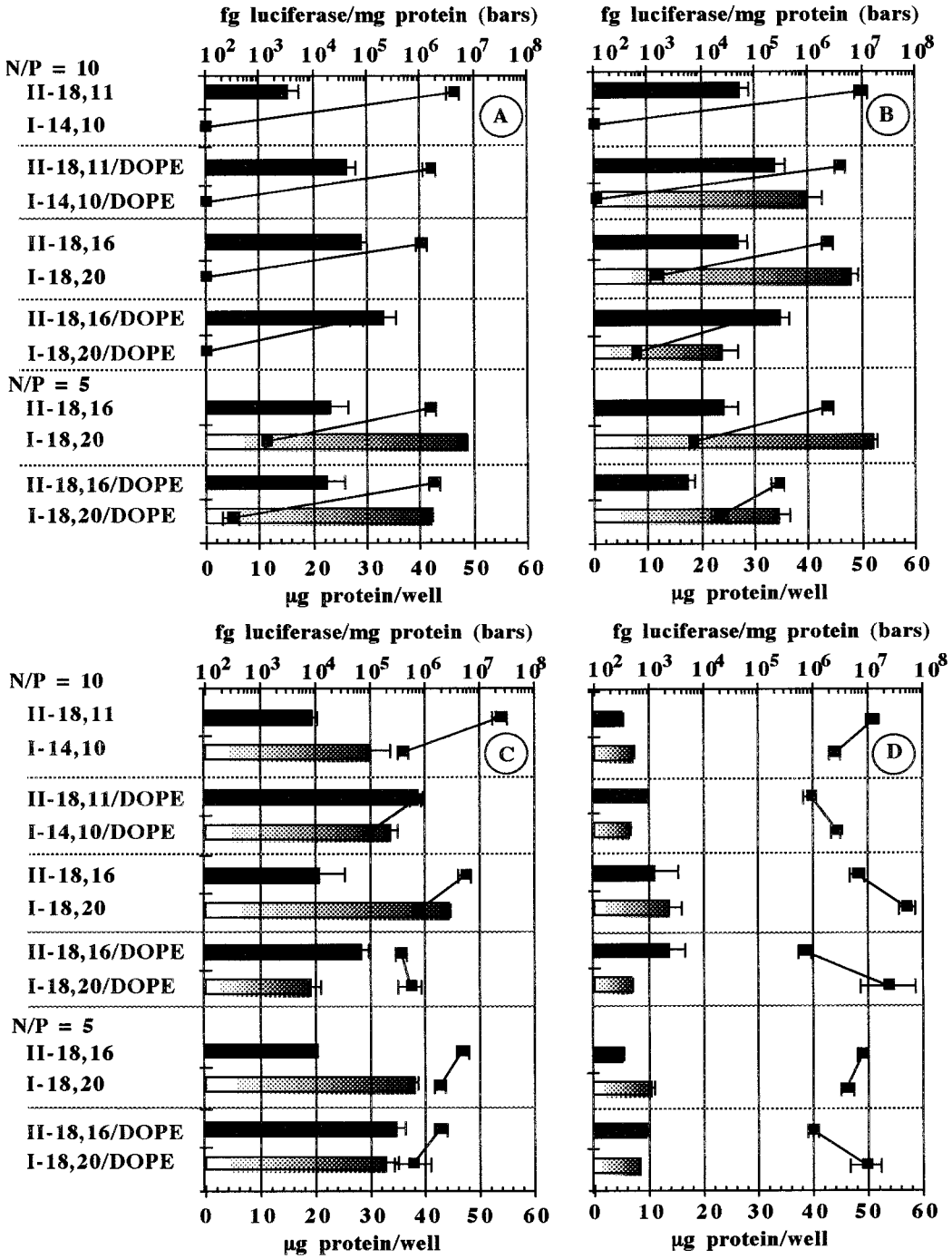


Figure 7. Impact on transfection efficiency (bars) and cell viability (points) of the linker (**II-18,*n***-based teloplexes as compared to their corresponding **I-*m*,*n***-based ones with $m = 14$ or 18). DNA: 4 μg/well (A), 2 μg/well (B), 0.5 μg/well (C), and 0.1 μg/well (D).

Chemical shifts were measured relative to $CHCl_3$ (δ 7.27 ppm) or CH_3OD (δ 3.35 ppm) for ^1H, relative to $CDCl_3$ (δ 76.9 ppm) for ^{13}C and expressed indirectly in relation to TMS, and relative to CCl_3F as internal reference for ^{19}F. The following abbreviations are used to describe the signal multiplicities: s (singlet), d (doublet), t (triplet), q (quadruplet), and m (multiplet). Chemical shifts are expressed in ppm and listed as follows: shift in ppm (multiplicity, integration, coupling, and attribution). Mass spectra (MS) were recorded on a Finningan MAT TSQ 7000 equipped with an atmospheric pressure ionization (API) source. Electrospray ionization mass spectrometry (ESI-MS) was used depending on the polar moiety of the molecules to be studied. This method used in positive mode can give either a $M + H^+$ or a $M + Na^+$ signal. Molecules were also analyzed on a VG-ZAB-HF mass spectrometer in FAB mode or on a Fisons BioQ quadrupol, VG BioTech in positive ionization electrospray mode. Elemental analyses were carried out by the Service Central de Microanalyses du CNRS.

Synthesis of Telogen *N*,*N*-Dioctadecylpropanamide-3-thiol, 3b (Scheme 1). The synthesis of telogen **3b** was carried out in two steps as shown in Scheme 1.

3,3′-Dithiobis(*N*,*N*-dioctadecylpropanamide), 2b. To a solution of 4.0 g (7.2 mmol) dioctadecylammonium hydrochloride (compound **1b**; Fluka) and 8 mL (58 mmol) triethylamine in 50 mL anhydrous $CHCl_3$ was added dropwise 0.88 g (3.6 mmol) 3,3′-dithiodipropanoyl chloride (obtained by reaction of $SOCl_2$ with 3,3′-dithiodipropionic acid (Fluka)) in 3 mL anhydrous $CHCl_3$ at room temperature. After 2 days at room temperature, the reaction mixture was washed with an aqueous solution of 0.1 N HCl. The organic phase was concentrated and the concentrate was chromatographed on a silica column (eluent: CH_2Cl_2). 2.1 g (1.75 mmol, 49%) **2b** was obtained in

the form of a white solid. TLC (hexane/Et_2O 1/1, $KMnO_4$) R_f = 0.4. IR (ν cm^{-1}, KBr): 1639 (CO). ^1H NMR ($CDCl_3$): δ 0.83 (t, 12H, ^3J = 6.0 Hz, CH_3); 0.90−1.40 (m, 120 H, $(CH_2)_{15}$); 1.40−1.65 (m, 8H, C$H_2$$CH_2$NCO); 2.60−2.80 (m, 4H, CH_2CON); 2.80−3.05 (m, 4H, CH_2S); 3.10−3.40 (m, 8H, CH_2N). ^{13}C NMR ($CDCl_3$): δ 14.2 (CH_3); 22.8 (C$H_2$$CH_3$); 27.1, 27.2, 27.7, 29.5, 29.6, 29.7, and 29.9 ($(CH_2)_{14}$); 32.0 (CH_2C$H_2$$CH_3$); 33.5 ($CH_2S$); 34.9 ($CH_2$CO); 46.5 and 48.5 ($CH_2N$); 170.4 (s, CO). MS-ESI ($M + Na^+$) m/z = 1240 (theoretical value for $C_{78}H_{156}N_2O_2S_2 + Na^+$ = 1239).

***N,N*-Dioctadecylpropanamide-3-thiol, 3b.** To a solution of 2.04 g (1.67 mmol) **2b** in 10 mL acetic acid at 60 °C was added 700 mg (10.7 mmol) zinc powder in 4 portions during a period of 3 h. The reaction mixture was stirred at 60 °C for 3 h, then filtered hot. $CHCl_3$ was added and washing with H_2O was carried out until neutrality was reached. The organic phase was dried over Na_2SO_4, then filtered and evaporated. The obtained residue was chromatographed on a silica column (eluent $CHCl_3$). 1.67 g (2.73 mmol, 82%) telogen **3b** was obtained in the form of a white solid. TLC (hexane/Et_2O 2/3, DTNB) R_f = 0.6. ^1H NMR ($CDCl_3$): δ 0.80 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.00−1.35 (m, 60 H, $(CH_2)_{15}$); 1.35−1.60 (m, 4H, CH_2-CH_2N); 1.63 (t, 1H, ^3J = 8.0 Hz, SH); 2.55 (t, 2H, ^3J = 6.5 Hz CH_2CON); 2.65−2.85 (m, 2H, CH_2S); 3.05−3.35 (m, 4H, CH_2N). ^{13}C NMR ($CDCl_3$): δ 14.2 (CH_3); 20.8 (CH_2S); 22.8 (C$H_2$$CH_3$); 27.0, 27.2, 27.9, 29.2, 29.4, 29.5, and 29.8 ($(CH_2)_{14}$); 32.0 (CH_2-C$H_2$$CH_3$); 37.2 (C$H_2$CO); 46.2 and 48.0 ($CH_2N$); 170.2 (CO). MS-ESI m/z = 610 (calcd for $C_{39}H_{79}NOS + H^+$ = 610).

Synthesis of Telogen *N,N*-Ditetradecylpropanamide-3-thiol, 3a. The synthesis of telogen **3a** was carried out in one step as described in Scheme 1. To a solution of 21 μL (0.24 mmol) 3-mercaptopropionic acid (Aldrich) in 2 mL anhydrous $CHCl_3$, kept at 0 °C, were added 51 mg (0.24 mmol) dicyclohexylcarbodiimide (DCC; Aldrich), then 33 mg (0.24 mmol) 1-hydroxy-1*H*-benzotriazole (HOBt, Aldrich), and 100 mg (0.24 mmol) ditetradecylamine **1a** in 8 mL anhydrous $CHCl_3$. The reaction mixture was kept under stirring at room temperature for 12 h, then diluted with $CHCl_3$ and washed with H_2O. The organic phase was dried over Na_2SO_4, filtered and evaporated. The concentrate was chromatographed on a silica column (eluent $CHCl_3$). 37 mg (0.07 mmol, 31%) **3a** was obtained in the form of a white solid. TLC (CH_2Cl_2, DTNB) R_f = 0.5. ^1H NMR ($CDCl_3$): δ 0.81 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.00−1.35 (m, 44 H, $(CH_2)_{11}$); 1.35−1.60 (m, 4H, C$H_2$$CH_2$N); 1.65 (t, 1H, ^3J = 8.2 Hz, SH); 2.55 (t, 2H, ^3J = 6.5 Hz CH_2CO); 2.65−2.85 (m, 2H, CH_2S); 3.05−3.35 (m, 4H, CH_2N). ^{13}C NMR ($CDCl_3$): δ 14.2 (CH_3); 20.5 (CH_2S); 22.8 ($CH_2$$CH_3$); 27.0, 27.2, 27.9, 29.2, 29.4, 29.5, and 29.7 ($(CH_2)_{10}$); 32.0 (CH_2C$H_2$$CH_3$); 37.2 (C$H_2$-CO); 46.2 and 48.0 (C$H_2$NCO); 170.2 (CO).

Synthesis of Telogen *rac*-1,2-Dioctadecyloxypropane-3-thiol, 7 (Scheme 2). Telogen **9** was obtained in three steps from *rac*-3-mercaptopropane-1,2-diol, **4**.

***rac*-3-Benzylthiopropane-1,2-diol, 5.** 3.2 g (29 mmol) *rac*-3-mercaptopropane-1,2-diol (Aldrich) was dissolved in a mixture of 28 mL 2 N NaOH and 35 mL EtOH. To this solution, kept at 0 °C, was added 5.6 g (44 mmol) benzyl chloride dropwise. After complete reaction of the thiol (TLC), the pH was ajusted to 5 with HCl, and the solvent was evaporated. The residue was purified by distillation (bp: 150 °C, 0.4 mmHg) to yield 3.9 g (20 mmol, 68%) **5** in the form of a colorless oil. TLC ($CHCl_3$/MeOH 98/2, DTNB, UV) R_f = 0.3. ^1H NMR ($CDCl_3$): δ 2.59 (d, 2H, ^3J = 6.0 Hz, CH_2SBn [Bn = benzyl]); 3.45−3.78 (m, 2H, CH_2O); 3.78−3.91 (m, 3H, CH and SCH_2Ph); 7.26−7.50 (m, 5H, C_6H_5). ^{13}C NMR ($CDCl_3$): δ 34.5 (CH_2SBn); 36.5 (SCH_2Ph); 65.3 (CH_2O); 70.7 (CH); 127.2 and 128.9 (C ortho and para); 128.6 (C meta); 138.0 (quaternary C).

***rac*-1,2-Dioctadecyloxy-3-benzylthiopropane, 6.** 3.9 g (20 mmol) compound **5** and 15 g (43 mmol) stearylmesylate were solubilized in 30 mL Et_2O (stearylmesylate was obtained by reaction of mesyl chloride with 1-octadecanol in pyridine). TLC (CH_2Cl_2, H_2SO_4, UV) R_f = 0.6. ^1H NMR ($CDCl_3$): δ 0.85 (t, 3H, ^3J = 5.8 Hz, CH_3); 1.15−1.55 (m, 30H, $(CH_2)_{15}$); 1.65−1.85 (m, 2H, C$H_2$$CH_2$OMs); 2.97 (s, 3H, CH_3S); 4.19 (t, 2H, ^3J = 6.6 Hz, CH_2OMs). ^{13}C NMR ($CDCl_3$): δ 14.1 (CH_3); 22.7 (CH_2-CH_3); 25.4 (CH_2CH_2O); 29.0,29.2, 29.3, 29.4, 29.5, 29.6 and 29.7 ($(CH_2)_{13}$); 31.9 (CH_2C$H_2$$CH_3$); 37.4 ($CH_3S$); 70.2 ($CH_2$OMs). To this solution were added 0.94 g (2.8 mmol) tetrabutylammonium hydrogenosulfate (Aldrich) and 25 mL of an aqueous solution of 9 N KOH. The reaction mixture was kept under Et_2O reflux for 96 h. The organic phase was washed with H_2O, then evaporated, and the residue was purified by chromatography on a silica column (eluent: petroleum ether/Et_2O, 95/5). 10.5 g (15 mmol, 76%) compound **6** was obtained in the form of a white solid. TLC (petroleum ether/Et_2O, 95/5, H_2SO_4) R_f = 0.8. ^1H NMR ($CDCl_3$): δ 0.82 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.10−1.36 (m, 60H, $(CH_2)_{15}$); 1.39−1.62 (m, 4H, CH_2-CH_2O); 2.40−2.68 (m, 4H, CH_2SBn); 3.30−3.52 (m, 7H, CH_2O and CHO); 3.68 (s, 2H, SCH_2Ph); 7.11−7.35 (m, 5H, C_6H_5). ^{13}C NMR ($CDCl_3$): δ 14.1 (CH_3); 22.7 ($CH_2$$CH_3$); 26.2, 29.4; 29.5; 29.7; 29.8 and 30.1 ($(CH_2)_{14}$); 32.0 (CH_2C$H_2$$CH_3$); 32.9 (C$H_2$SBz); 37.1 (SC$H_2$Ph);70.4; 71.6 and 71.8 (CH_2O); 78.6 (CHO); 126.9 and 129.0 (C ortho and meta); 128.4 (C para); 138.6 (quarternary C).

***rac*-1,2-Dioctadecyloxypropane-3-thiol, 7.** 2.3 g (3.3 mmol) compound **6** was solubilized in 35 mL anhydrous tBuOH. 0.86 g (38 mmol) Na was added to this solution. The reaction mixture was heated under reflux for 12 h. Then several drops of H_2O were added. After evaporation of the solvent, the residue was taken up in hexane, then washed with H_2O. The organic phase was concentrated and the residue was purified by chromatography on a silica column (eluent: $CHCl_3$) to yield 1.6 g (2.6 mmol, 78%) compound **7** in the form of a white solid. TLC ($CHCl_3$, DTNB) Rf = 0.7. ^1H NMR ($CDCl_3$): δ 0.80 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.00−1.37 (m, 60H, $(CH_2)_{15}$); 1.37−1.70 (m, 4H, C$H_2$$CH_2O$); 2.48−2.76 (m, 2H, CH_2S); 3.27−3.65 (m, 7H, CH_2O and CH). ^{13}C NMR ($CDCl_3$): δ 14.0 (CH_3); 22.6 ($CH_2$$CH_3$); 26.1 ($CH_2S$); 29.3, 29.4, 29.6, 29.7, 30.0 and 31.9 ($(CH_2)_{15}$); 70.3, 71.0 and 71.6 (CH_2O); 79.3 (CH).

Synthesis of Taxogen *N*-{2-[(BOC)aminoethyl]}acrylamide, A. A solution of 1.7 g (18.7 mmol) acryloyl chloride (Aldrich) in 5 mL anhydrous $CHCl_3$ was added dropwise at 0 °C and during 30 min to 2.0 g (12.4 mmol) *N*-(BOC)ethylenediamine (Fluka) and 5.0 g (49,1 mmol) triethylamine in 35 mL anhydrous $CHCl_3$. The reaction mixture was left, under stirring, for 90 min at room temperature, then concentrated to dryness. After purification on a silica column (eluent: $CHCl_3$/MeOH 98/2), one obtained 2.0 g (9,2 mmol, 75%) taxogen **A** as a white solid. TLC ($CHCl_3$/MeOH, 98/2, $KMnO_4$) R_f = 0.2. ^1H NMR ($CDCl_3$): δ 1.36 (m, 9H, CH_3); 3.10−3.34 (m, 2H, CH_2NH); 3.34−3.50 (m, 2H, NCH_2); 5.55 (dd,1H, ^3J = 9,6 Hz, ^2J = 2.2 Hz, CH=CH_2); 6.05 (dd, 1H, ^3J = 9,6 Hz, ^3J = 17.0 Hz, CH=CH_2); 6.20 (dd, 1H, ^3J = 17.0 Hz, ^2J = 2.2 Hz, CH=CH_2). ^{13}C NMR ($CDCl_3$): δ 28.3 (CH_3); 40.1 and 40.7 (CH_2N); 79.6 (C (BOC)); 126.1 (CH_2=CH); 130.9 (CH=CH_2); 157.0 (CO(BOC)); 166.3 (COCH). Anal. Calcd for $C_{10}H_{18}N_2O_3$ (M = 214.2): C 56.05; H 8.46; N 13.07. Found: C 55.79; H 8.47; N 13.16.

Synthesis of Telomers. 1. Telomers I-*m,n*. Telomers I-*m,n*(BOC): General Procedure for the Telomerization Reaction. A solution of 500 mg (0.82 mmol) telogen **3b** and 1.68 g (7.85 mmol) taxogen **A** (i.e. a taxogen/telogen molar ratio of 9.5) in 15 mL anhydrous acetonitrile was heated to 50 °C before adding 40 mg (0.24 mmol) AIBN. The reaction medium was stirred under reflux until the telogen **3b** disappeared (followed by TLC/DTNB) (~24 h). The solution was concentrated and chromatographed on a silica column (eluent: $CHCl_3$ to $CHCl_3$/MeOH 98/2) and fractionated by gel permeation chromatography on a Sephadex LH 20 column (Sigma) with $CHCl_3$ as eluent. In this way, 83 mg telomer **I-18,1(BOC)**, 85 mg telomer **I-18,2(BOC)**, 93 mg telomer **I-18,3(BOC)** of *aDPn* = 3, and 1.64 g telomer **I-18,10(BOC)** of an *aDPn* = 10, as white solids, were isolated. The *aDPn* and structure were determined by ^1H NMR. The *aDPn* is equal to 3Hx/2Hy, Hx being the integration of the signal corresponding to the taxogen ethyldiamido methylenes (from to ppm) and Hy the integration of the signal corresponding to the telogen methyl groups (t at 0.80 ppm). This protocol applied to telogen **3b** and taxogen **A**

at a taxogen/telogen molar ratio of 40 led, after chromatography and fractionation, to telomers **I-18,4(BOC)**, **I-18,5(BOC)**, **I-18,20(BOC)**, **I-18,40(BOC)**, **I-18,50(BOC)**, and **I-18,60-(BOC)** of $aDPn$ = 4, 5, 20, 40, 50, and 60, respectively.

When applied to telogen **3a** and taxogen **A** at a taxogen/telogen molar ratio of 40, this protocol afforded telomers **I-18,-10(BOC)**, **I-18,20(BOC),** and **I-18,70(BOC)** of $aDPn$ = 10, 20, and 70, respectively.

Compound I-18,1(BOC): ^{1}H NMR ($CDCl_3$): δ 0.80 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.00−1.30 (m, 60H, $(CH_2)_{15}$); 1.30−1.75 (m, 13H, CH_2CH$_2$NCO, and CH_3 (BOC)); 2.43 (t, 2H, ^3J = 7.0 Hz, CH_2CO); 2.51 (t, 2H, ^3J = 7.0 Hz, CH_2CO); 2.65−2.85 (m, 4H, CH_2S); 3.05−3.35 (m, 8H, CH_2NCO and CH_2NHCO); 5.20−5.30 (m, 1H, NH (BOC)); 6.55−6.75 (m, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 14.1 (CH_3); 22.7 (CH_2CH_3); 28.5 (CH_3 (BOC)); 27.0, 27.2, 27.3, 27.9, 28.3, 29.2, 29.4, 29.5 and 29.8 ($(CH_2)_{14}$); 32.0 ($CH_2CH_2CH_3$); 33.4 (CH_2S); 36.7 (CH_2CO); 40.5 (CH_2-NHCO); 46.3 and 48.1 (CH_2NCO); 79.5 (C(BOC)); 156.8 (NCO (BOC)); 170.7 and 172.0 (CON). MS-ESI m/z = 847 (calcd for $C_{49}H_{97}N_3O_4S + Na^+$ = 847).

Compound I-18,2(BOC): ^{1}H NMR ($CDCl_3$): δ 0.80 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.00−1.30 (m, 60H, $(CH_2)_{15}$); 1.30−1.75 (m, 22H, CH_2CH$_2$NCO, CH_3 (BOC)); 1.75−1.95 (m, 2H, CHCH_2-CH$_2$CONH); 1.95−2.30 (m, 2H, CHCH$_2$CH_2CONH); 2.30−2.85 (m, 7H, CH_2CON, CHCONH, CH_2S); 3.00−3.60 (m, 12H, CH_2-NCO and CH_2NHCO); 5.35−5.60 (m, 2H, NH (BOC)); 6.75−6.95 (m, 1H, NH); 6.95−7.10 (m, 1H, NH). MS-ESI m/z = 1061 (calcd for $C_{59}H_{115}N_5O_7S + Na^+$ = 1061).

Telomer I-18,3(BOC): ^{1}H NMR ($CDCl_3$): δ 0.80 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.00−1.30 (m, 60H, $(CH_2)_{15}$); 1.30−1.75 (m, (4+9n)H, CH_2CH$_2$NCO, CH_3 (BOC)); 1.75−2.90 (m, (3n+5)H, CHCO, CH_2CON, CH_2S, CH_2CH$_2$CO and CH_2CHCO); 3.00−3.60 (m, (4+4n)H, CH_2NCO and CH_2NHCO); 5.15−5.65 (m, nH, NH (BOC)); 6.45−7.20 (m, nH, NH). ^{13}C NMR ($CDCl_3$): δ 14.1 (CH_3); 22.7 (CH_2CH_3); 28.5 (CH_3 (BOC)); 27.0, 27.2, 27.4, 27.9, 28.1, 28.4, 29.2, 29.4 and 29.8 ($(CH_2)_{14}$ and CHCH_2CH); 32.0 ($CH_2CH_2CH_3$); 33.4 and 34.2 (CH_2S); 35.0 (CH_2CO); 39.9, 40.2, 40.5 and 40.9 (CH_2NHCO); 46.0 (CHCONH); 46.3 and 48.1 (CH_2NCO); 79.5 (C(BOC)); 156.8 (CO (BOC)); 171.0, 173.3 and 174.6 (CO).

Telomer I-18,4(BOC): ^{1}H NMR: identical to that of telomer **I-18,3(BOC)** but with $aDPn$ = 4. ^{13}C NMR ($CDCl_3$): δ 14.1 (CH_3); 22.7 (CH_2CH_3); 28.6 (CH_3 (BOC)); 27.0, 27.2, 27.9, 29.2, 29.4 and 29.8 ($(CH_2)_{14}$ and CHCH_2CH); 32.0 (CH_2CH_2-CH_3); 46.3 and 48.1 (CH_2NCO); 79.6 (C (BOC)); 156.8 (CO (BOC)). The resonances corresponding to the carbons CH_2-NHCO, CH_2CO, CHCO and to the carbons NCO appear, respectively, as large signals between 38 and 43 ppm and between 170 and 177 ppm. The CH_2S carbons are not detectable.

Telomer I-18,5(BOC): ^{1}H NMR: identical to that of telomer **I-18,3(BOC)** but with $aDPn$ = 5. ^{13}C NMR: identical to that of telomer **I-18,4(BOC).**

Telomer I-18,10(BOC): ^{1}H NMR: identical to that of telomer **I-18,3(BOC)** but with $aDPn$ = 10. ^{13}C NMR: identical to that of telomer **I-18,4(BOC).** MS-ESI shows the presence of compounds with n = 3−15: m/z = (610 + 214.2n + 23) with n = 3−15 in agreement with the masses calcd for [$C_{39}H_{79}NOS$ + $n(C_{10}H_{18}N_2O_3)$ + Na^+] with n = 3−15.

Telomer I-18,20(BOC): ^{1}H NMR: identical to that of telomer **I-18,3(BOC)** but with $aDPn$ = 20. ^{13}C NMR: identical to that of telomer **I-18,4(BOC).** MS-ESI shows the presence of compounds with n = 4−33: m/z = (610 + 214.2n + 23) with n = 4−15, in aggreement with the masses calcd for [$C_{39}H_{79}$-NOS + $n(C_{10}H_{18}N_2O_3)$ + Na^+] with n = 4−15; $m/2z$ = (305 + 107.1n + 23) with n = 9−33 in agreement with the masses calcd for [$C_{39}H_{79}NOS$ + $n(C_{10}H_{18}N_2O_3)$ + 2Na^+]/2 with n = 9−33.

Telomers I-18,40(BOC), **I-18,50(BOC), and I-18,60-(BOC):** ^{1}H NMR: identical to that of telomer **I-18,3(BOC)** but with $aDPn$ = 40, 50, and 60, respectively. ^{13}C NMR: identical to that of telomer **I-18,4(BOC).**

Telomer I-14,10(BOC): ^{1}H NMR ($CDCl_3$): δ 0.80 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.00−1.30 (m, 44H, $(CH_2)_{11}$); 1.30−1.75 (m, (9n+4)H, CH_2CH$_2$NCO, and CH_3 (BOC)); 1.75−3.00 (m, (3n+5)H, CHCO, CH_2CON, CH_2S, CH_2CH$_2$CO and CH_2-CHCO); 3.00−3.60 (m, (4+4n)H, CH_2NCO and CH_2NHCO); 5.15−5.65 (m, nH, NH (BOC)). MS-ESI shows the presence of components of n = 2−10: m/z = (498 + 214.2n + 23) with n = 2−10, in agreement with the masses calcd for [$C_{31}H_{63}NOS$ + $n(C_{10}H_{18}N_2O_3)$ + Na^+] with n = 2−10.

Telomer I-14,20(BOC): ^{1}H NMR: identical to that of telomer **I-14,10(BOC)** but with $aDPn$ = 20. MS-ESI shows the presence of components of n = 8−20: $m/2z$ = (249 + 107.1n + 23) in agreement with the masses calcd for [$C_{31}H_{63}$-NOS + $n(C_{10}H_{18}N_2O_3)$ + 2Na^+]/2 with n = 8−20.

Telomer I-14,70(BOC): ^{1}H NMR: identical to that of telomer **I-L1k(BOC)** but with $aDPn$ = 70.

Telomers I-*m*,*n*: General Procedure for the Deprotection of the BOC-Telomers. The deprotection of telomers **I-*m*,*n*(BOC)** was quantitatively achieved by dissolving and keeping these telomers in a large excess of trifluoroacetic acid (TFA; Aldrich) at room temperature for 1 h. The excess of TFA was removed by evaporation in the presence of cyclohexane. The telomers **I-*m*,*n*** were obtained in the form of white solids.

Compound I-18,1: ^{1}H NMR ($CDCl_3$/CD_3OD): δ 0.85 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.15−1.45 (m, 60H, $(CH_2)_{15}$); 1.45−1.60 (m, 4H, CH_2CH$_2$N); 2.51 and 2.57(t, t, 2H, 2H, ^3J = 7.0 Hz, CH_2CO); 2.70−2.90 (m, 4H, CH_2S); 3.04 (t, 2H, ^3J = 5.5 Hz, CH_2NH$_3$); 3.15−3.40 (m, 4H, CH_2N); 3.45 (t, 2H, ^3J = 5.5 Hz, CH_2NH). ^{13}C NMR ($CDCl_3$/CD_3OD): δ 14.2 (CH_3); 22.8 (CH_2-CH_3); 27.1, 27.2, 27.8, 29.5, 29.6, 29.8 ($(CH_2)_{14}$); 32.0 (CH_2CH_2-CH_3); 33.4 (CH_2S); 34.8 (CH_2CO); 37.5 (CH_2NH); 39.9 (CH_2NH_3); 46.3 and 48.1 (CH_2N); 117.0 (q, ^1J = 300 Hz, $CF_3CO_2^-$); 162.8 (q, ^2J = 46 Hz, $CF_3CO_2^-$); 171.0 and 175.2 (CO).

Compound I-18,2: ^{1}H NMR ($CDCl_3$): δ 0.80 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.00−1.30 (m, 60H, $(CH_2)_{15}$); 1.30−1.65 (m, 4H, CH_2CH$_2$NCO); 1.65−1.95 (m, 2H, CHCH_2CH$_2$CONH); 1.95−2.30 (m, 2H, CHCH$_2$CH_2CONH); 2.30−2.85 (m, 7H, CH_2CON, CHCON, CH_2S); 3.00−3.60 (m, 12H, CH_2NH$_3$, CH_2NCO and CH_2NHCO). ^{13}C NMR ($CDCl_3$): δ 14.2 (CH_3); 22.8 (CH_2CH_3); 27.1, 27.2, 27.8, 29.5, 29.6, 29.8 ($(CH_2)_{14}$); 32.0 ($CH_2CH_2CH_3$); 33.4 (CH_2S); 34.8 (CH_2CO); 37.5 (CH_2NH); 39.9 (CH_2NH_3); 46.0 (CHCO); 46.3 and 48.1 (CH_2NCO); 117.0 (q, $^1J_{CF}$ = 300 Hz, $CF_3CO_2^-$); 162.8 (q, $^2J_{CF}$ = 46 Hz, $CF_3CO_2^-$); 171.0, 175.2 and 177.0 (CON). MS-ESI m/z = 839, in agreement with the calcd mass for M[=$C_{39}H_{79}NOS$ + 2($C_5H_{10}N_2O$)] + H^+.

Telomers I-18,*n*, for *n* ≥ 3: ^{1}H NMR (CD_3OD): δ 0.80 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.00−1.35 (m, 60H, $(CH_2)_{15}$); 1.35−2.60 (m, (9+3n)H, CH_2CH$_2$N, CH_2CON, CHCO, CH_2S, CH_2CH$_2$CO and CH_2CHCO); 2.70−3.60 (m, (4n+4)H, CH_2NH$_3^+$, CH_2NCO and CH_2NHCO) with, respectively, an $aDPn$ = 3 (telomer **I-18,3**), 4 (telomer **I-18,4**), 5 (telomer **I-18,5**), 10 (telomer **I-18,-10**), 20 (telomer **I-18,20**), 40 (telomer **I-18,40**), 50 (**telomer I-18,50**), and 60 (telomer **I-18,60**). ^{13}C NMR (CD_3OD): δ 14.3 (CH_3); 23.3 (CH_2CH_3); 29.0, 29.6, 29.9 and 30.3 (CHCH_2CH and $(CH_2)_{14}$); 32.5 ($CH_2CH_2CH_3$); 37.7 (CH_2NCO); 40.0 (CH_2-NH_3^+); 117.0 (q, ^1J = 300 Hz, $CF_3CO_2^-$); 162.8 (q, $^2J_{CF}$ = 46 Hz, $CF_3CO_2^-$); 176.2 (NCO). The resonances corresponding to carbons CHCO and NHCO appear as broad signals between 41 and 45 ppm and between 176.5 and 179 ppm, respectively. The resonances corresponding to carbons CH_2S and CH_2CO are not detectable. MS-ESI of telomer **I-18,10** shows the presence of compounds with n = 3−15: m/z = (610 + 114.1n + 1) with n = 3−10, in agreement with the calcd masses for [$C_{39}H_{79}NOS$ + $n(C_5H_{10}N_2O)$ + H^+] with n = 3−10; $m/2z$ = (305 + 57n + 1) with n = 3−15, in agreement with the calcd masses for [$C_{39}H_{79}NOS$ + $n(C_5H_{10}N_2O)$ + 2H^+]/2 with n = 3−15; $m/3z$ = (203 + 38n + 1) with n = 5−13 in agreement with the calcd masses for [$C_{39}H_{79}NOS$ + $n(C_5H_{10}N_2O)$ + 3H^+]/3, with n = 3−13.

Telomers I-14,*n*, with *n* = 10, 20, 70: ^{1}H NMR (CD_3OD): δ 0.80 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.00−1.35 (m, 44H, $(CH_2)_{11}$); 1.35−2.60 (m, (9+3n)H, CH_2CH$_2$N, CH_2CON, CHCO, CH_2S, CH_2CH$_2$CO and CH_2CHCO); 2.70−3.60 (m, (4n+4)H, CH_2-NH_3^+, CH_2NCO and CH_2NHCO) with respectively an aDP of

10 (telomer **I-14,10**), 20 (telomer **I-14,20**), and 70 (telomer **I-14,70**). ^{13}C NMR: identical to those of telomers **I-18,*n*** with $n \geq 3$.

2. Telomers II-18,*n*. Telomers II-18,*n*(BOC). The telomerization protocol described above for the synthesis of telomers **I-*m*,*n*(BOC)** was applied to telogen **7** and taxogen **A**, at a taxogen/telogen molar ratio of 3 and 5. It led to two series of telomers in the form of white solids after purification by chromatography on a silica column (eluent: $CHCl_3$) and with a yield of 60%. The fractionation on Sephadex LH-20 of the first series led to compound **II-18,1(BOC)** and telomers **II-18,3(BOC)** and **II-18,11(BOC)**; the fractionation of the second series allowed to isolate, in addition to the first three telomers, telomers **II-18,6(BOC)** and **II-18,16(BOC)**. The *aDP* values were determined by ^{1}H NMR, as detailed above for telomers **I-*m*,*n***.

Compound II-18,1(BOC): ^{1}H NMR ($CDCl_3$): δ 0.80 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.02−1.32 (m, 60H, $(CH_2)_{15}$); 1.37 (s, 9H, CH_3 (BOC)); 1.44−1.55 (m, 4H, CH_2CH$_2$O); 2.39 (t, 2H, ^3J = 6.9 Hz, CH_2CO); 2.64 (d, 2H, ^3J = 4.8 Hz, CH_2S); 2.78 (t, 2H, ^3J = 6.2 Hz, SCH_2CH$_2$CO); 3.12−3.60 (m, 11H, CH_2O, CHO and CH_2N); 5.16 and 6.60 (m, 2H, NH). ^{13}C NMR ($CDCl_3$): δ 14.0 (CH_3); 22.7 (*C*H$_2$CH$_3$); 28.4 (CH_3 (BOC)); 26.1, 29.3, 29.5, 29.6, 29.7 and 30.0 ($(CH_2)_{14}$); 31.9 (*C*H$_2$CH$_2$CH$_3$); 33.9 (CH_2S); 36.7 (*C*H$_2$CO); 40.3 and 40.5 (CH_2N); 70.5, 71.5 and 71.7 (CH_2O); 78.5 (CHO); 79.5 (O−C (BOC)); 156.7 (C═O (BOC)); 171.9 (C═O).

Telomer II-18,3(BOC): ^{1}H NMR ($CDCl_3$): δ 0.80 (t, 6H, ^3J = 6.0 Hz, CH_3); 0.98−1.30 (m, 60H, $(CH_2)_{15}$); 1.30−1.60 (m, (9*n*+4)H, CH_3 (BOC) and CH_2CH$_2$O); 1.60−3.00 (m, (3*n*+3)H, CH_2CO, CHCO, CH_2CHCO, CH_2CH$_2$CO and CH_2S); 3.05−3.55 (m, (4*n*+7)H, CH_2O, CHO and CH_2N) with n = 3. ^{13}C NMR ($CDCl_3$): δ 14.1 (CH_3); 22.6 (*C*H$_2$CH$_3$); 28.4 (CH_3 (BOC)); 26.1, 29.3, 29.5, 29.6, 29.7 and 30.1 ($(CH_2)_{13}$ and *C*H$_2$CHCO); 31.9 (*C*H$_2$CH$_2$CH$_3$); 33.8 and 34.5 (CH_2S); 35.6 (*C*H$_2$CO); 35.8 and 36.2 (CHCO); 70.4, 71.6 and 71.7 (CH_2O); 78.5 (CHO); 79.6 (O−C (BOC)); 156.7 (C═O (BOC)); 172.0, 173.1 and 174.4 (CO). The *C*H$_2$N carbons appear as a broad resonance between 39 and 41 ppm.

II-18,*n*(BOC), with *n* = 6, 11, 16: ^{1}H NMR: identical to that of **II-18,3(BOC)** but with n = 6, 11, and 16, respectively. ^{13}C NMR ($CDCl_3$): δ 14.0 (CH_3); 22.6 (*C*H$_2$CH$_3$); 28.4 (CH_3 (BOC)); 26.0, 29.3, 29.4, 29.6 and 29.7 ($(CH_2)_{14}$ and *C*H$_2$CHCO); 31.8 (*C*H$_2$CH$_2$CH$_3$); 33.6 and 34.4 (CH_2S); 70.4, 71.7 and 71.9 (CH_2O); 78.5 (CHO); 79.6 (O−C (BOC)); 156.6 (C═O (BOC)); the *C*H$_2$(C═O), CHC═O, CH_2N, and NHC═O carbons appear as very broad resonance signals between 38 and 44 ppm and between 173 and 176 ppm.

Telomers II-18,*n*. The removal of the BOC protection groups from telomers **II-18,*n*(BOC)** was quantitatively achieved by dissolving and keeping these telomers in a large excess of TFA at room temperature for 1 h. The excess TFA was removed by coevaporation in the presence of cyclohexane. Telomers **II-18,*n*** were obtained in the form of white solids.

Compound II-18,1: IR (ν cm^{-1}, KBr): 1875 (C═O). ^{1}H NMR ($CDCl_3$): δ 0.80 (t, 6H, ^3J = 6.0 Hz, CH_3); 1.00−1.36 (m, 60H, $(CH_2)_{15}$); 1.37−1.70 (m, 4H, CH_2CH$_2$O); 2.43 (t, 2H, ^3J = 6.1 Hz, CH_2CO); 2.62 (d, 2H, ^3J = 5.0 Hz, CH_2S); 2.74 (t, 2H, ^3J = 6.2 Hz, SCH_2CH$_2$CO); 3.00−3.25 (m, 2H, CH_2NH$_3$); 3.30−3.65 (m, 9H, CH_2O, CHO and CH_2N). ^{13}C NMR ($CDCl_3$): δ 14.1 (CH_3); 22.7 (*C*H$_2$CH$_3$); 26.1, 29.3, 29.5, 29.7 and 30.0 ($(CH_2)_{14}$); 31.9 (*C*H$_2$CH$_2$CH$_3$); 33.8 (CH_2S); 36.1 (*C*H$_2$CO); 37.5 (CH_2N); 39.4 (CH_2NH_3); 70.6, 71.6 and 71.8 (CH_2O); 78.3 (CHO); 171.9 (C═O). ^{19}F NMR ($CDCl_3$): δ −76.0 ($CF_3CO_2^-$).

Telomer II-18,3: ^{1}H NMR ($CDCl_3$, CD_3OD): δ 0.84 (t, 6H, ^3J = 5.8 Hz, CH_3); 1.00−1.45 (m, 60H, $(CH_2)_{15}$); 1.45−1.75 (m, 4H, CH_2CH$_2$O); 1.75−3.25 (m, (5*n*+3)H, CH_2CH$_2$CO, CH_2-CHCO, CH_2CO, CHCO, CH_2NH$_3$ and CH_2S); 3.25−3.70 (m, (2*n*+7)H, CH_2O, CHO and CH_2N) with n = 3. ^{13}C NMR ($CDCl_3$. CD_3OD): δ 15.0 (CH_3); 23.7 (*C*H$_2$CH$_3$); 27.2, 29.0, 29.1, 30.4, 30.6, 30.7, 30.8 and 30.9 ($(CH_2)_{14}$ and *C*H$_2$CHCO); 33.0 (*C*H$_2$-CH$_2$CH$_3$); 33.7 (CH_2S); 38.1 (*C*H$_2$CO); 40.7 (CH_2N); 41.0 (CH_2-NH_3); 43.4 (*C*HCO); 70.5, 70.6 and 71.8 (CH_2O); 78.3 (CHO); 163.4 (q, $^2J_{CF}$ = 34 Hz, $CF_3CO_2^-$); 174.8, 175.1 and 177.1 (NC═O). ^{19}F NMR ($CDCl_3$, CD_3OD): δ −76.6 ($CF_3CO_2^-$).

Telomer II-18,6: ^{1}H and ^{19}F NMR ($CDCl_3$, CD_3OD): identical to those of telomer **II-18,3**, but with n = 6. ^{13}C NMR ($CDCl_3$, CD_3OD): δ 14.8 (CH_3); 23.7 (*C*H$_2$CH$_3$); 27.1, 30.4, 30.5, 30.7 and 31.1 ($(CH_2)_{14}$ and *C*H$_2$CHCO); 33.0 (*C*H$_2$CH$_2$CH$_3$); 34.0 (CH_2S); 35.0 (*C*H$_2$CO); 38.1 (CH_2N); 40.7 (CH_2NH_3); 71.5, 72.8 and 72.9 (CH_2O); 79.6 (CHO); 163.0 (q, $^2J_{CF}$ = 36 Hz, $CF_3CO_2^-$); 176.2 and 177.9 (NC═O). The *C*H(C═O) carbons appear as broad resonance signals between 41 and 46 ppm. MS-FAB and MS-ESI show the presence of n = 4−9 oligomers: m/z = 1069.5; 1183.5; 1297.6; 1411.6; 1525.6; 1641.2 in agreement with M[(=$C_{39+5n}H_{80+11n}N_{2n}O_{2+n}S$) + H^+] for n = 4−9, respectively.

Telomer II-18,11: ^{1}H and ^{19}F NMR ($CDCl_3$, CD_3OD): identical to those of telomer **II-18,3**, but with n = 11. ^{13}C NMR ($CDCl_3$, CD_3OD): δ 14.8 (CH_3); 23.7 (*C*H$_2$CH$_3$); 27.1, 30.3, 30.6 and 30.7 ($(CH_2)_{14}$ and *C*H$_2$CHCO); 32.3 (*C*H$_2$CH$_2$CH$_3$); 33.2 (CH_2S); 163.0 (q, $^2J_{CF}$ = 36 Hz, $CF_3CO_2^-$); 175.0 (NC═O). The resonances corresponding to carbons CH_2N, *C*H$_2$CO and *C*HCO appear as broad signals between 38 and 43 ppm; the resonances corresponding to carbons CH_2O and CHO are not detectable. MS-FAB and MS-ESI show the presence of oligomers of n = 4−15: m/z = 1069.9; 1183.9; 1298.0; 1412.1; 1526.1; 1640.2; 1754.3; 1868.4; 1982.4; 2097.5; 2210.5 in agreement with M(=$C_{39+5n}H_{80+11n}N_{2n}O_{2+n}S$) for n = 4−14, respectively; $m/2z$ = 763.8; 821.0; 878.0; 935.0; 992.0; 1049.3; 1106; 1163 in agreement with [M(=$C_{39+5n}H_{80+11n}N_{2n}O_{2+n}S$) + $2H^+$]/2, for n = 8−15, respectively.

Telomer II-18,16: ^{1}H and ^{19}F NMR ($CDCl_3$, CD_3OD): identical to those of telomer **II-18,3**, but with n = 16. ^{13}C NMR ($CDCl_3$, CD_3OD): δ 14.8 (CH_3); 23.7 (*C*H$_2$CH$_3$); 27.1, 30.3, 30.6 and 30.7 ($(CH_2)_{14}$ and *C*H$_2$CHCO); 32.3 (*C*H$_2$CH$_2$CH$_3$); 33.2 (CH_2S); 163.0 (q, $^2J_{CF}$ = 36 Hz, $CF_3CO_2^-$). The resonances corresponding to carbons CH_2N, *C*H$_2$CO, *C*HCO, and NCO, respectively, appear as broad signals between 38 and 43 ppm and between 176 and 179 ppm, respectively; the resonances corresponding to carbons CH_2O and CHO are not detectable. MS-FAB and MS-ESI show the presence of oligomers of n = 6−17: m/z = 1298.0; 1412.1; 1526.2; 1640.3; 1755.3; 1869.4; 1983.5; 2097.5; 2211.6; 2325.4; 2439; 2553; 2667; 2782 in agreement with M(=$C_{39+5n}H_{80+11n}N_{2n}O_{2+n}S$) for n = 6−17, respectively.

Preparation of Complexes Composed of the Polyamine Telomer Compounds and Plasmid pTG11033. The plasmid pTG11033 was produced by Transgene SA. The endotoxin content of the plasmid preparation was checked using a limulus amebocyte lysat kit (Biogenic, France). This value was below 1 endotoxin unit/mg of plasmid, hence below the 5 e.u./mg of DNA recommended for in vivo protocols. The quantities of compound used were calculated according to the desired DNA concentration of 0.1 mg/mL (for in vitro tests), the charge ratio, the molar weight and the number of potential positive charges in the selected compound. The polyamine telomer/DNA complex is formulated by adding a desired volume of telomer preparation at a concentration of 10 mg/mL (in 20 mM HEPES buffer, pH 7.5, containing 10% DMSO and 10% EtOH) to the desired volume of DNA solution to reach the desired DNA concentration (for example 0.1 mg/mL). Thus for the preparation of the polyamine telomer **I-18,40**/DNA complex at charge ratio 5 and 0.1 mg/mL DNA, 40 μL of a polyamine telomer solution (10 mg/mL in $CHCl_3$) was transferred to a borosilicate glass tube (16 × 100 mm). The solvent was evaporated in Rotavap evaporation system (45 °C, 30 pm, 0.2 bar, 40 min). 4 μL of DMSO, 4 μL of EtOH and 32 μL of HEPES 20 mM, pH 7.5, were added to the film obtained. The preparation was heated at 50 °C, while stirring (30 rpm), for 5 min. After cooling to room temperature, 37 μL of this preparation was added to 963 μL DNA solution [100 μL DNA (1 mg/mL) diluted with 863 μL HEPES buffer]. This preparation can be used for further experiments.

When the composition further comprises DOPE, the desired volume of 1 mg/mL chloroform solution (to obtain mole/mole

cationic telomer/DOPE) was added to the volume of polyamine telomer solution (10 mg/mL in $CHCl_3$) and then the mixture was transferred to a borosilicate glass tube. The following steps were as described above.

Measurement of the Size of the Complexes Formed. The average sizes were measured by photon correlation spectroscopy using a Coulter N4Plus particle size analyzer. The analyses were performed at 25 °C, after equilibrating the sample at 25 °C for 20 min. One aliquot of the sample was aspirated and discharged several times prior to pipetting. The sample was diluted with 20 mM HEPES in the measurement tube and homogenized. These analysis were thus carried out on complexes having a 10 μg/mL concentration of DNA. After waiting 180 s, the light diffraction at 90° was measured for 180 s. The range was 3 nm to 10 μm, using 31 bins. The size measurement is valid if the sample gives between 50 000 and 1 000 000 counts/s. The formulations and analyses were reproduced twice.

Agarose Gel Electrophoresis. Each sample (0.2 μg of plasmid) was analyzed by electrophoresis for about 90 min under 75 V/cm, through a 1% agarose gel in TAE 1× (Tris−acetate−EDTA) buffer. The DNA was visualized in TAE buffer containing 12 μL/200 mL ethidium bromide (Sigma).

In Vitro Transfection of A549 Cells. 24 h before transfection, A549 cells (epithelial cells derived from human pulmonary carcinoma) were grown in Dulbeco-modified Eagle culture medium (DMEM), containing 10% fetal calf serum, FCS (Gibco BRL), in 96-well plates (2×10^4 cells/well), in a wet (37 °C) and 5% CO_2/95% air atmosphere. Volume of DNA/polyamine telomer complex (40, 20, 5, and 1 μL, respectively) was diluted to 100 μL in DMEM or DMEM supplemented with 10% FCS in order to obtain various amounts of DNA (4, 2, 0.5, and 0.1 μg, respectively) in the preparation. The culture medium was removed and replaced by 100 μL of DMEM supplemented with 10% FCS and containing the desired amount of DNA. After 2 and 24 h, 50 μL and 100 μL of DMEM supplemented with 10% FCS were added, respectively.

48 h after transfection, the culture medium was discarded and the cells were washed with 100 μL of PBS and then lysed with 50 μL of lysis buffer (Promega). The lysates were frozen at −80 °C awaiting analysis of luciferase activity. This measurement was done for 1 min on 20 μL of the lysis mixture in a Berthold LB96P luminometer in dynamic mode, using the "luciferase" determination system (Promega) in 96-well plates.

The total protein concentration per well was determined using conventional techniques (BCA test, Pierce). For cells grown in the absence of lipoplexes, a well contains around 30−60 μg of proteins. Each formulation was tested twice.

Supporting Information Available: Four figures showing respectively the effect of DOPE on transfection efficiency and cell viability of the teloplexes and the impact of the number of amine functions on transfection efficiency and cell viability for the **I-14,*n*-** and **I-18,*n*-**based teloplexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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