Cationic Phosphonolipids Containing Quaternary Phosphonium and Arsonium Groups for DNA Transfection with Good Efficiency and Low Cellular Toxicity**

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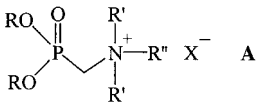
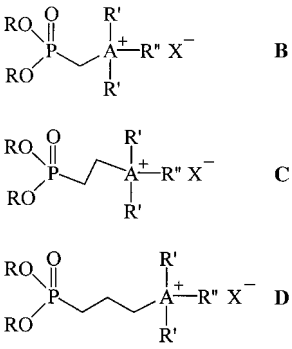
The search for synthetic vectors able to complex DNA, carry the resulting "lipoplex" through cell membranes, and then deliver the DNA in (or close to) the nucleus in order to replace a deficient gene is of current interest. Unlike viral vectors, synthetic vectors do not impose limitations on the size of the encapsulated genetic material and illicit no immunogenic response. Since the pioneering work of Felgner et al.[1] the most studied vectors are cationic lipids, perhaps because of the basic nature of biological amines. One must note that the cationic or polycationic charge is always carried by nitrogen atoms in these lipids. Thus, quaternary ammonium compounds (DOTMA, DDAB, DOTAP, DORIE, GLB), polyamines (DOGS, DPPES, DOSPA, GL67), and guanidines (BGTC) were synthesized and studied.[2]



Scheme 1. Previous phosphonolipids with R = $C_{14}H_{29}$, $C_{18}H_{37}$, $C_{18}H_{35}$; R′, R″ = alkyl and other functional groups; X = halogen.

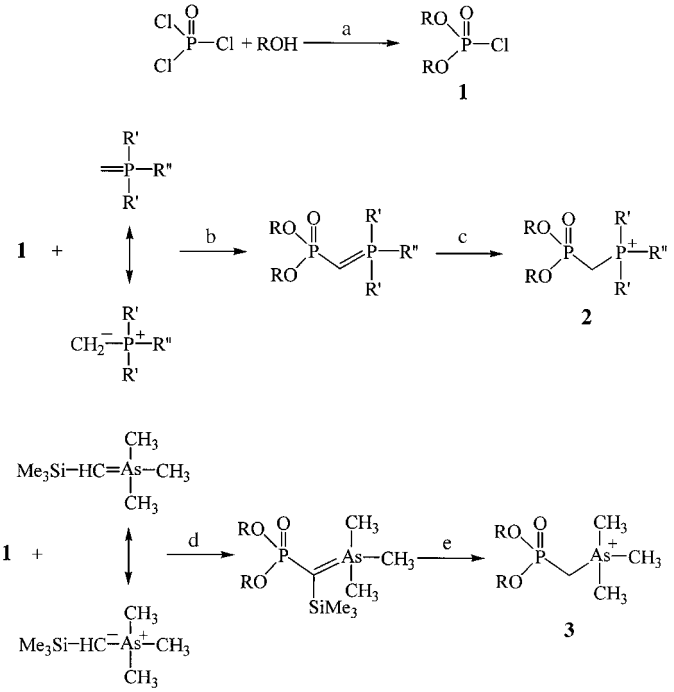
In continuing our work on phosphonolipids **A**[3] (Scheme 1), we paid special attention to the results of Stekar's group: the replacement of the quaternary ammonium polar head in edelfosine and miltefosine[4] (two antineoplastically zwitterionic phospholipids) by a phosphonium or arsonium group resulted in maintained cytostatic activity together with decreased cellular toxicity. It should be noted that unlike arsenic(III) compounds arsonium compounds such as arsenobetaines, which occur abundantly in seafoods, are not cytotoxic for humans.[5] Since changing a nitrogen atom for a phosphorus or arsenic atom in cationic lipids increases the volume of the cationic head, one can expect a modification of the interactions of the vector with the solvent and DNA. Therefore we explored the effects of such a replacement in lipidic ammonium phosphonates. We examined also the influence of the length of the chain between the cation and the phosphoryl group (Scheme 2).

The ammonium phosphonates **A** were easily obtained in two steps from lipidic phosphites by a Mannich reaction followed by quaternization of the resulting aminophosphonates.[3a] But the analogues **B**–**D** in Scheme 2, in which the polar head is replaced and the chain length between the cation and the phosphonate is increased, required other synthetic pathways.



Scheme 2. General formula of the new phosphonolipids (R = $C_{14}H_{29}$, $C_{18}H_{35}$, $C_{18}H_{33}$; R′, R″ = alkyl; A = N, P, As; X = halogen).

Methylenephosphonium and -arsonium analogues were synthesized according to Scheme 3. Fatty dialkylchlorophosphate **1** (obtained from $POCl_3$, 2 equiv fatty alcohol, and 4 equiv diisopropylethylamine) was treated with phosphorus and arsenic ylides,[6] which led after acidification to the crude phosphonium and arsonium methylenephosphonates **2** and **3**, respectively, as powders or waxes depending on the R groups. Purification by successive washing with or precipitation from diethyl ether at low temperature afforded pure **2** and **3** (see Tables 1 and 2 for selected NMR data). Trimethylsilyl-stabilized ylides were used for arsonium compounds.[6] The silyl groups were finally removed with MeOH, Me_3SiOH, or H_2O.



Scheme 3. Synthesis of **2** and **3** (R = $C_{14}H_{29}$, $C_{18}H_{35}$; R′, R″ = alkyl). a) Et_2O, NE*t*Pr_2 (4 equiv), 72 h, 20 °C; b) THF, 1 h, 0 °C; c) THF, HX, 1 h, 20 °C; H_2O/NaX, 24 h, 20 °C; d) Et_2O, 1 h, 0 °C; e) Et_2O, HX, 1 h, 20 °C, then Et_2O, Me_3SiOH, MeOH or H_2O/NaX, 24 h, 20 °C (X = halide).

Ammonium and phosphonium ethylenephosphonates **7** and **8** were obtained in five steps starting from 2-bromoethyldiethylphosphonate (Schemes 4 and 5), which was quantitatively converted into the bis-trimethylsilylated derivative

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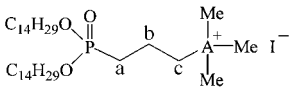
Table 1. Selected ^{31}P NMR data for compounds **1**–**12** with $R = C_{14}H_{29}$, $R' = R'' = Me$; $X = I$ (121.49 MHz, $CDCl_3$, 298 K).

	1[a]	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
δ(P=O)	4.65	16.70	20.38	26.08	17.40	30.84	23.66	27.19	29.11	29.35	27.59	30.24
$\delta(P^+)$	–	25.71	–	–	–	–	–	29.12	–	–	29.64	–
$J_{P\text{-}P}$ [Hz]	–	12.7	–	–	–	–	–	34.0	–	–	5.4	–

[a] In C_6D_6.

Table 2. Selected ^{13}C and ^1H NMR data (100 and 400 MHz, $CDCl_3$, 298 K, δ, J [Hz]) for compounds **2**, **3**, and **7**–**12**.

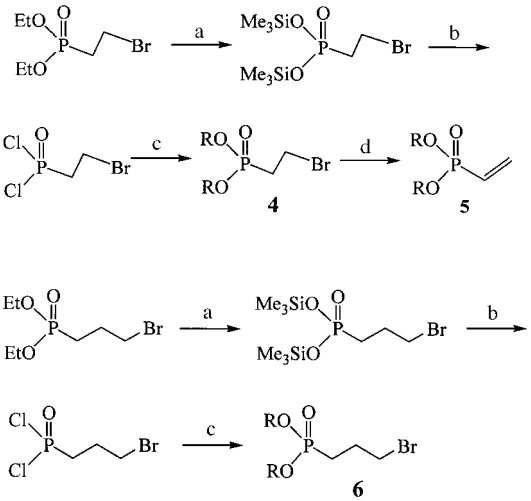


Cmpd	A	H_a	H_b	H_c	C_a	C_b	C_c
2	P	3.59 dd $J^2_{H\text{-}P} = 16.6$ $J^2_{H\text{-}P^+} = 19.2$	–	–	22.08 dd $J_{C\text{-}P} = 133.1$ $J_{C\text{-}P^+} = 47.5$	–	–
3	As	3.56 d $J^2_{H\text{-}P} = 16.4$	–	–	21.47 d $J_{C\text{-}P} = 136.7$	–	–
7	N	2.38 m	3.77 m	–	21.30 d $J_{C\text{-}P} = 138.6$	61.89 s	–
8	P	2.17 m	2.84 m	–	18.40 dd $J_{C\text{-}P} = 143.4$ $J^2_{C\text{-}P^+} = 4.0$	18.50 dd $J_{C\text{-}P^+} = 43.2$ $J^2_{C\text{-}P} = 4.4$	–
9	As	2.35 dt $J^3_{H\text{-}H} = 6.8$ $J^2_{H\text{-}P} = 17.1$	2.95 dt $J^3_{H\text{-}H} = 6.9$ $J^3_{H\text{-}P} = 26.8$	–	19.80 d $J_{C\text{-}P} = 143.4$	20.46 d $J^2_{C\text{-}P} = 4.0$	–
10	N	2.10 m	1.87 m	3.86 m	21.51 d $J_{C\text{-}P} = 143.2$	17.13 s	65.81 m
11	P	1.96 m	1.68 m	2.76 m	25.31 dd $J_{C\text{-}P} = 141.7$ $J^3_{C\text{-}P^+} = 17.1$	15.31 s	24.00 dd $J_{C\text{-}P^+} = 52.1$ $J^3_{C\text{-}P} = 15.1$
12	As	1.99 m	1.89 m	2.88 m	25.84 d $J_{C\text{-}P} = 141.0$	16.73 d $J^2_{C\text{-}P} = 4.4$	25.22 d $J^3_{C\text{-}P} = 16.3$

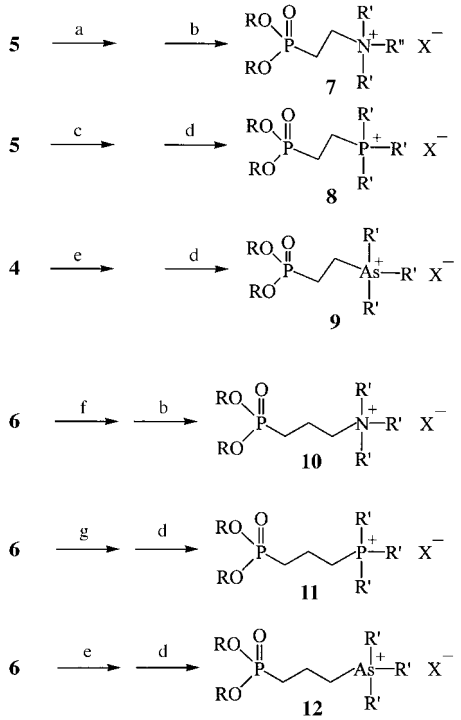
on treatment with bromotrimethylsilane,[7] then into the corresponding dichloride according to the method of Bhongle et al.[8] The fatty bromoethylphosphonate **4** was then easily prepared by reaction of the dichloride with the appropriate alcohol. Elimination of HBr was achieved by treatment with NEt_3 in refluxing THF. The addition of a secondary amine to vinylic **5**, followed by quaternization with R″X led to ammonium phosphonates **7**. Phosphonium phosphonates **8** were obtained by addition of acidic quaternary phosphonium salts. Direct quaternization of AsR_3 by bromophosphonate **4** in a sealed tube led to the corresponding arsonium bromides. Metathesis with NaI converted the bromides into the iodides.

The synthons **6** for propylenephosphonate compounds were synthesized by the same synthetic pathway as that employed for **4** (Scheme 4), then converted into the ammonium phosphonates **10** and to the phosphonium (**11**) and arsonium derivatives (**12**) by direct quaternization with phosphanes or arsanes (sealed tube) (Scheme 5). These syntheses are efficient, with nearly quantitative yields for phosphonates **7** to **12**.

All these onium compounds could be purified by recrystallization from diethyl ether or ethyl acetate at $-20\,^\circ C$ and were obtained in overall yields higher than 60%. They were fully characterized by ^1H, ^{13}C, and ^{31}P NMR spectroscopy (see Tables 1 and 2 for selected values). Their thermotropic properties were studied by differential



Scheme 4. Synthesis of **4**–**6** ($R = C_{14}H_{29}$, $C_{18}H_{35}$, $C_{18}H_{33}$; R′, R″ = alkyl). a) CH_2Cl_2, $BrSiMe_3$, 24 h, 20 °C; b) CH_2Cl_2, oxalyl chloride, dimethylformamide (DMF), 2 h, 20 °C; c) Et_2O, ROH, $NEtiPr_2$, 24 h, 20 °C; d) THF, NEt_3, 48 h, reflux.



Scheme 5. Synthesis of **7**–**12** ($R = C_{14}H_{29}$, $C_{18}H_{35}$, $C_{18}H_{33}$; R′, R″ = alkyl; X = halogen, trifluoromethanesulfonate). a) EtOH, HNR'_2, 96 h, 20 °C; b) Et_2O, R″X, 24 h, 20 °C; c) DMF, $[HR''R'_2P]^+X^-$), 48 h, reflux; d) CH_2Cl_2, NaX/H_2O, 48 h, 20 °C; e) sealed tube, AsR_3, 72 h, 70 °C; f) DMF/H_2O, HNR'_2, 2 h, reflux; g) THF, PR'_3, 24 h, 20 °C.

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scanning calorimetry. They are air and water stable up to 60 °C. At higher temperatures the phosphonium and arsonium compounds are more stable than the corresponding ammonium compounds. It is worth noting that stable aqueous microemulsions (lasting at least three weeks) with concentrations of up to 10 mg mL^{-1} were obtained with phosphonium and arsonium compounds. Under the same conditions ammonium compounds underwent hydration to yield unstable suspensions and should thus be prepared immediately before use.

Ammonium, phosphonium, and arsonium fatty phosphonates were simultaneously assessed for transfection of a reporter gene β-galactosidase on two adherent cell lines (CFT1, Hela) and one hematopoietic nonadherent cell line (K562), with lipofectine as reference, consistent with previously described protocols.[3b] Figure 1 compares the data for

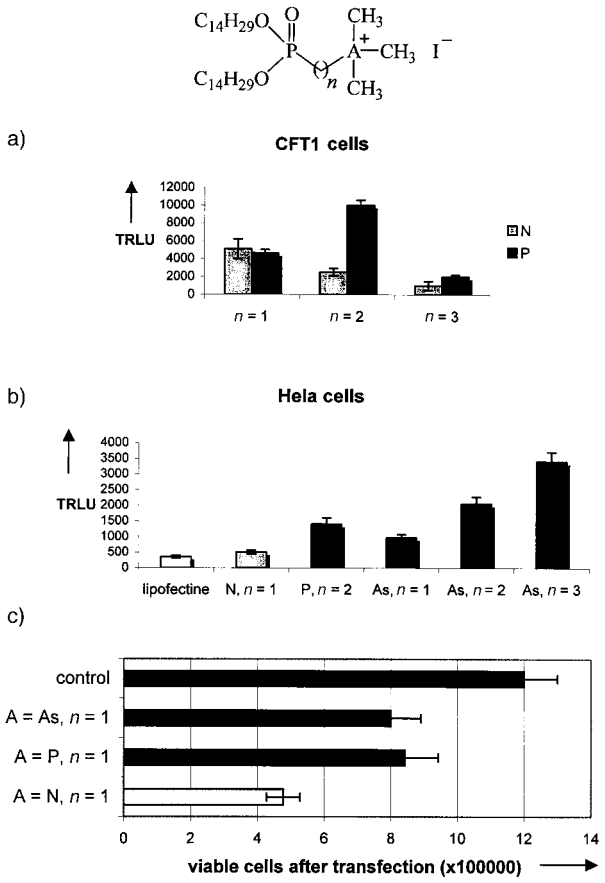
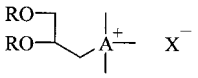


Figure 1. Results of transfection activity on CFT1 (a) and Hela cells (b), and cellular toxicity on K562 cells (c). TRLU = total relative light unit.

oniums (A = N, P, As) in which R = $C_{14}H_{29}$ and R′ = R″ = CH_3. It is clear from the two first histograms that phosphonium and arsonium salts are more efficient than ammonium compounds, especially for transfection into Hela cells. Cellular toxicity was evaluated as the number of cells surviving the transfection experiment using a chemiluminescent assay (Packard);[9] nontransfected cells served as a control. The third histogram indicates clearly that phosphonium and arsonium compounds are less cytotoxic than the ammonium derivatives, which is consistent with the observations of Stekar et al.[4]

A more detailed biological study with a complete biological evaluation of efficiency and toxicity will be published soon elsewhere. Moreover, we have continued with this concept of replacing ammonium in the polar head of cationic lipids and have synthesized the phosphonium and arsonium analogues of DOTMA[1] and DOTAP,[10] (Scheme 6), two well-studied nonviral vectors. These new compounds are currently under biological evaluation.



Scheme 6. Phosphonium and arsonium analogues of DOTAP and DOTMA (A = P, As; X = halogen; R = $C_{18}H_{35}$, $C_{17}H_{33}C(O)$, $C_{15}H_{31}C(O)$).

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Phosphane Sulfide/Octacarbonyldicobalt-Catalyzed Pauson–Khand Reaction Under an Atmospheric Pressure of Carbon Monoxide

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Yoji Yamamoto, Junji Usuki, and Kazuhiko Saigo*

Dedicated to Professor Jean-Marie Lehn on the occasion of his 60th birthday

The Pauson–Khand reaction, a method for constructing the cyclopentenone skeleton from an alkene, an alkyne, and carbon monoxide, has found extensive use in synthetic organic

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