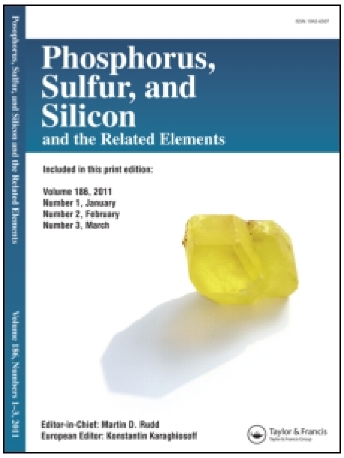

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LDL-LINKED LARIAT-BEARING CYCLOPHOSPHAZENES: AN ATTEMPT AT THE PRODUCTION OF PRECURSORS FOR NEW WATER-SOLUBLE MAGNETIC RESONANCE IMAGING AGENTS

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LDL-LINKED LARIAT-BEARING CYCLOPHOSPHAZENES: AN ATTEMPT AT THE PRODUCTION OF PRECURSORS FOR NEW WATER-SOLUBLE MAGNETIC RESONANCE IMAGING AGENTS

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The linkage of cyclophosphazenic sepulchers to LDL vesicles leads to supermolecules which complex Gd^{3+} cations through water-soluble adducts of interest for further Magnetic Resonance Imaging applications.

Key words: Cyclophosphazene; dry media; regiospecific aminolysis; MRI agents; Gadolinium cryptates.

INTRODUCTION

We recently reported on the design of new lariat-bearing cyclophosphazenes upon aminolysis of hexachlorocyclotriphosphazene by amino-esters hydrochlorides followed by a common alkaline hydrolysis.[1] These cryptands are actually molecular sepulchers capable of complexing gadolinium cations with stability constants larger than 10^{28} but the gadolinium cryptates are too poorly soluble in physiological serum to be used for clinical purposes within the field of magnetic resonance imaging agents.

A possible way to escape from this uncomfortable situation would be to link these non-soluble gadolinium cryptates to some suitable vesicles for generating water-soluble supersystems. Some attempts in this way were recently performed for indium cryptates of interest as imaging agents in clinical scintigraphy[2–4] and for targeting genuine antitumor drugs such as celiptium[5] or methotrexate.[6] LDL (Low Density Lipoproteins) were used as the vesicle and/or the vehicle on this opportunity, the cryptating moieties and/or the drugs being linked to LDL through some highly lipophilic "tails."

Thus, the same approach was envisaged in the case of lariat-bearing cyclophosphazenes (LBC) (Figure 1) and the present contribution reports on the strategy for designing LBC with lipophilic tails (Figure 2) which would be convenient for LDL-linkage.

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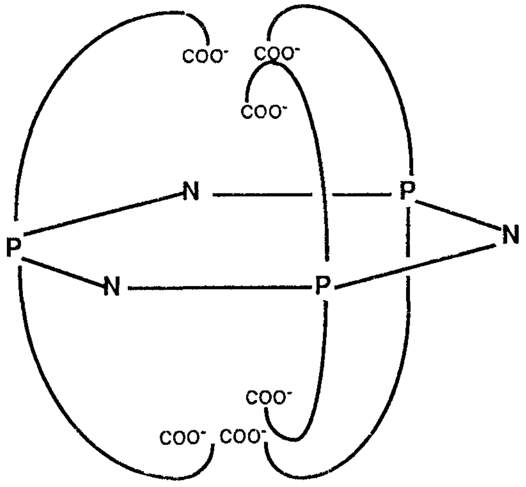

FIGURE 1 General pattern of lariat-bearing cyclophosphazenes (LBC).

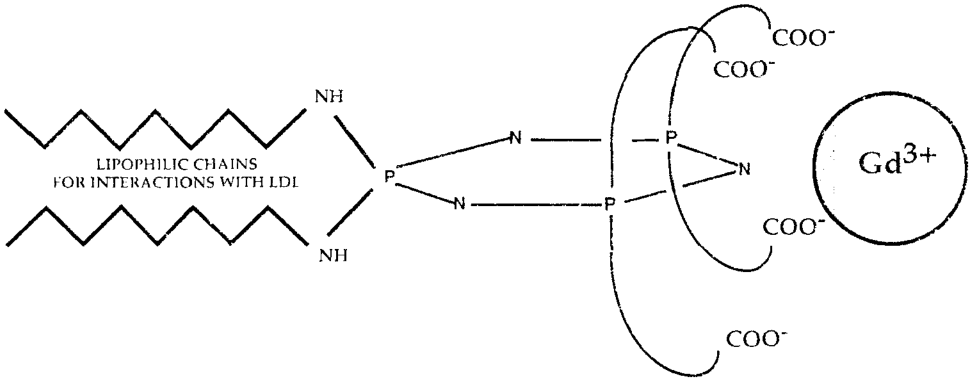

FIGURE 2 General pattern of LBC with lipophilic tails suitable for LDL-linkage.

SYNTHESIS OF LBC WITH LIPOPHILIC TAILS

These peculiar LBC were synthesized according to the following pathway:

$N_3P_3Cl_6 + 2H_2N(CH_2)_nCH_3 \Rightarrow$ gem-$N_3P_3Cl_4[HN(CH_2)_nCH_3]_2 + 2HCl$ ①
($n = 7$, gem-dioctylamino derivative 1; $n = 12$, gem-didodecylamino derivative 2)

1 or 2 + $4[H_2N(X)CO_2R]\cdot HCl \Rightarrow$ gem-$N_3P_3[HN(CH_2)_nCH_3]_2[HN(X)CO_2R]_4$ ②
($n = 7$, $X = (CH_2)_3$ derivative 3; $n = 12$, $X = (CH_2)_3$ derivative 4)

3 or 4 + $6NaOH \Rightarrow$ gem-$N_3P_3[HN(CH_2)_nCH_3]_2[HN(X)CO_2^-Na^+]_4$ ③
($n = 7$, $X = (CH_2)_3$ derivative 5; $n = 12$, $X = (CH_2)_3$ derivative 6)

LDL + 5 or 6 $\Rightarrow$ supersystems 7 or 8 ④

supersystems 7 or 8 + $GdCl_3\cdot 6H_2O \Rightarrow$ water-soluble Gd^{3+} cryptates 9 or 10 ⑤

Step ①: Standard aminolysis of hexachlorocyclotriphosphazene, $N_3P_3Cl_6$, by primary amines such as octylamine or dodecylamine in (1:2) stoichiometric conditions leads regioselectively (in toluene with Et_3N for picking up hydrogen chloride) to geminal disubstituted derivatives (30%) contaminated with their digem-tetrasubstituted cousins (30%) and other substituted products, mainly nongem disubstituted isomers (30%). Such reactions took commonly two days at room temperature and were stopped when the ^{31}P NMR singlet of $N_3P_3Cl_6$ at 20.09 ppm (in $CDCl_3$ with 85% H_3PO_4 as the standard) remained unchanged with time. In other words, these aminolyses are not complete, probably because of the bulky character of octylamine and dodecylamine. Anyhow, the obtention of the expected geminal disubstituted derivatives needs several successive time-and-money consuming SiO_2 column chromatographies which penalize this process for any further industrial application.

We demonstrated recently that such aminolyses could be achieved regiospecifically and instantaneously when working on a peculiar alumina-supported potassium hydroxide [coded as ALPOT (50:11)] as the "dry media."[7] Reactions are so fast and so aggressive that traces of the gem-gem-tetrasubstituted cousins, $N_3P_3Cl_2[HN(CH_2)_nCH_3]_4$, are observed when working in the (1:2) stoichiometry. Then, an actual stoichiometry of (1:1.6) instead of (1:2) has to be preferred. In these conditions indeed, the unreacted $N_3P_3Cl_6$ *remains fixed on the dry support* and the traces of the tetrasubstituted isologs are no more observed. Pure gem moieties are then obtained neat in few minutes. Their ^{31}P NMR spectra show the $\underline{P}Cl_2$ doublet centered at 20.7 ppm and the $\underline{P}(amino)_2$ triplet at 10.2 ppm (Bruker AC 200, $CDCl_3$, 85% H_3PO_4), $^2J_{PP} = 43.9$ Hz. DCI mass spectra reveal the MH^+ molecular ions at m/z 532 and 644, respectively, with satellite distributions indicating the presence of four chlorine atoms in both structures (maximal components of molecular peaks at (MH^+ + 2), i.e. at m/z 534 and 646, respectively).

Step ②: The four remaining chlorine atoms of $\underline{1}$ and $\underline{2}$ were persubstituted upon reaction of the GABA (Gamma Amino Butyric Acid) isopropyl ester hydrochloride, $H_2N(CH_2)_3COO\text{-}iPr.\ HCl$, coded as GABA-iPr. HCl, which was prepared according to Patel and Brice[8]

$$H_2N(CH_2)_3COOH + iPrOH + SOCl_2 \Rightarrow H_2N(CH_2)_3COOiPr \cdot HCl + HCl + SO_2$$

in the (1:60:14) stoichiometry. Reaction took about 24 h and its completion was checked by ^1H 200 MHz. Yield was never less than 60%.

Then, Step ② was achieved in triethylamine as the solvent. Completion of reactions from $\underline{1}$ and $\underline{2}$ were checked by ^{31}P NMR as previously. It may be noticed that a previous dehydrochloration of GABA-iPr · HCl by Et_3N (for 30 min) followed by the reaction of the free GABA-iPr ester on $\underline{1}$ or $\underline{2}$ leads to the expected persubstituted $\underline{3}$ and $\underline{4}$ derivatives with yields larger than 80% and with much higher rates than when GABA-iPr · HCl reacts with $\underline{1}$ or $\underline{2}$. ^{31}P NMR patterns of $\underline{3}$ and $\underline{4}$ at 81.015 MHz (Bruker AC 200) reveal a false singlet at 17.8 ppm for $\underline{3}$ and at 16.3 ppm for $\underline{4}$, respectively. This deceptively simple A_2B system could not be split at 121.496 MHz (Bruker WM 300) and will have to be investigated at 202.458 MHz (Bruker WM 500) or even at 242.950 MHz (Bruker WM 600) for evidencing the expected doublet-triplet systems. Incidentally, such deceptively simple A_2B or ABC ^{31}P NMR spectra in cyclophosphazenes were commonly observed in the past and skillfully circumvented in this way.[9–14]

Step ③ was achieved in methanol as the solvent. Reactions took a few minutes. Methanol was then removed under reduced pressure, leading to light brown powders containing both the expected natrium salt and the unreactive amino-carboxylate. Extraction with 2-propanol yields pure 5 and 6 salts as white powders, the purity of which was controlled by ^1H and ^{31}P NMR together with IR spectroscopy.

Now, the linkage of cyclophosphazenic sepulchers for gadolinium 5 and 6 to LDL vesicles (Step ④) is in progress and preliminary results show that the supersystems so obtained complex Gd^{3+} cations (Step ⑤) as firmly as their parent hexasubstituted molecules, $N_3P_3[HN(X)CO_2^-Na^+]_6$ did,[1] but through water-soluble adducts of interest for further MRI applications.

EXPERIMENTAL

The NMR spectra were recorded on a Bruker AC 200 spectrometer with H_3PO_4 85% as external reference.

Molecular modelings were achieved by using functionalities of the MAD (Molecular Advanced Design) software developed by Lahana (Oxford Molecular SA, X-Pole, Ecole Polytechnique, 91128 Palaiseau Cedex, France).

Hexachlorocyclotriphosphazene was generously provided to us (degree of purity ≥ 99.8%) by SHIN NISSO KAKO Co., subsidiary company of NIPPON SODA Company. FLUKA supplied us with the primary amines (degree of purity ≥ 98%).

Alumina-supported potassium hydroxide: Potassium hydroxide (Prolabo Rectapur, 11 g) in H_2O (250 ml) was mixed with neutral chromatographic alumina (Fluka, type 207 C, 90–110 μ, 50 g). After stirring for 5 min, the water was removed under reduced pressure. The resulting powder was further dried at 70°C for 24 h in oven. This reagent (coded as ALPOT) may be kept in a desiccator without loss of activity during several months.

Step ① General method for the preparation of gem disubstituted moieties such as 1 on ALPOT: A mixture of hexachlorocyclotriphosphazene (2 g; 5.6 mmol) and of the alkylamine (2 eq.; 11.2 mmol) in toluene (80 ml) was added to ALPOT (40 g corresponding to 32 eq. of base). After 1 min stirring, the solvent was removed under reduced pressure at room temperature and the resulting white powder was eluted with 2 × 100 ml of toluene or acetone. After removal of the solvent, 2.32 g (76% yield) were obtained which contained mainly 1 with about 10% of its gem, gem-tetrasubstituted parent. 1 could be purified by a single SiO_2 column chromatography using a *n*-heptane/ethyl acetate (7:3) as the eluant. The NMR spectrum of pure 1 revealed a triplet centered on 10.1 ppm and a doublet centered on 20.3 ppm. Incidentally, the presence of the gem, gem-tetrasubstituted parent as the impurity suggested to decrease slightly the amount of alkylamine to be used. Actually, a (1:1.6) stoichiometry for the reaction of alkylamines on hexachlorocyclotriphosphazene yields indeed pure 2 straightway (90% yield m.p. = 70°C for 1 and 55°C for 2, respectively).

Step ② General method for the preparation of the gem hexasubstituted moiety 3: 0.81 g of 1 in 20 ml Et_3N are added in one shot to a solution of 1.65 g of GABA isopropylester hydrochloride in 100 ml Et_3N in presence of 20 g of ALPOT. After 40 min stirring, the excess of Et_3N was removed under reduced pressure at room temperature and the resulting white powder was eluted with firstly 100 ml of acetone and 100 ml of chloroform in sequence. After removal of the solvents, 1.35 g (0.87 from acetone and 0.45 from chloroform) (89.6% yield) were obtained which contained pure 3 (white waxy oil). ^{31}P NMR patterns of 3 at 81.015 MHz (Bruker AC 200) revealed a false singlet at 17.8 ppm for 3. This deceptively simple A_2B system could not be split at 121.496 MHz (Bruker WM 300) but was actually clarified at 202.458 MHz (Bruker WM 500, CEA, Saclay, France) thanks to Dr. Bruno Perly.

Incidentally, the same persubstitution mechanism of 1 by other esters of amino-acids such as ACA (Amino Caproic Acid) isopropylester could be achieved in the same experimental condition. Conversely, IDA (Imino Diacetic Acid) isopropylester yielded to the gem,gem-tetrasubstituted derivative, $N_3P_3[HN(CH_2)_7CH_3]_2[IDA(CO_2R)]_2Cl_2$, only, as demonstrated by ^{31}P NMR [ABC system with three doublets of doublets centered on 21.1 ($\underline{P}Cl_2$), 18.3 ($\underline{P}NHNH$) and 17.1 ($\underline{P}NN$) ppm, respectively]. This specific behaviour is presumably due both to the bulky character of IDA and to the fact that this amino-acid has to link the cyclophosphazenic ring through a secundary amino group and not through a primary one as in GABA and ACA.

Step ③ General method for the preparation of natrium salts, 5 and 6, of 3 and 4: 24 ml of a solution of NaOH 5 M in methanol (250 ml) are added dropwise to a suspension of 4 mmol of 3 or 4 in 20 ml water. The medium is refluxing 6 h at 80°C till homogeneicity and apparition of an orange color. Then, the water is removed in vacuo and the final crude products containing both the expected natrium salts and the unreactive amino-carboxylates are collected as light brown solids which are perfectly soluble in water (when the starting organic material 3 or 4 was not). Extraction with 2-propanol yields pure 5 and 6 salts [m.p. higher than 240°C (dec.)] as white powders the purity of which was controlled by ^1H and ^{31}P NMR together with IR spectroscopy. Indeed, a comparison of IR spectra before and after hydrolysis shows that the $\nu(C{=}O)$ vibrator is shifted from 1720 (ester form) to 1600 cm^{-1} (carboxylate form) with a broadening of the whole vibrations around 1400 cm^{-1}. Yields were larger than 75% in both cases. Negative ions DCI/NH$_3$ mass spectrometry (RIEBERMAG R-1010 H Quadrupole Mass Spectrometer) revealed the molecular ions M^{4-} of 5 and 6 at m/4 199 (796/4) and 227 (908/4), respectively. Both M^{4-} peaks are parent peaks (intensity 100%).

Micro-analytical data: Compound 1: $N_5P_3Cl_4C_{16}H_{36}$, theor. %: N 13.1, C 36.0, H 6.8; averaged exp. %: N 12.9, C 36.4, H 6.8. Compound 2: $N_5P_3Cl_4C_{24}H_{52}$, theor. %: N 10.8, C 44.7, H 8.1; averaged exp. %: N 10.9, C 44.4, H 8.1. Compound 3: $N_9P_3C_{44}O_8H_{92}$, theor. %: N 13.0, C 54.6, H 9.6; averaged exp. %: N 12.9, C 54.4, H 9.8. Compound 4: $N_9P_3C_{52}O_8H_{108}$, theor. %: N 11.7, C 57.8, H 10.1; averaged exp. %: N 11.9, C 57.4, H 9.8. Compound 5: $N_9P_3C_{32}O_8H_{64}Na_4$, theor. %: N 14.2, C 43.3, H 7.3; averaged exp: %: N 14.9, C 42.8, H 7.8. Compound 6: $N_9P_3C_{40}O_8H_{90}Na_4$, theor. %: N 12.6, C 48.0, H 8.1; averaged exp. %: N 12.9, C 48.8, H 7.8.

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