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Lariat-bearing cyclophosphazenes: potential molecular sepulchers for magnetic resonance imaging agents

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Abstract

Lariat-bearing cyclophosphazenes were prepared by aminolysis of hexachlorocyclotriphosphazene with amino ester hydrochlorides followed by standard alkaline hydrolysis. These cryptands are actually capable of complexing gadolinium cations with stability constants larger than 10^{28}; this is much larger than the values for classical magnetic resonance imaging agents. Unfortunately, these cryptates are much too insoluble in physiological serum to be used for clinical purposes in a facile way.

Introduction

Several macrocyclic precursors of contrast agents for NMR imaging have been extensively reported and patented within the last decade. These agents commonly belong to classical coordination chemistry, the metals and/or metal ions basically being attached to the ligand through dative bond systems (Fig. 1(a)). All of these studies attempt to produce complexes as stable as possible, with the aim of providing materials which would have as little toxicity as possible when injected in vivo, the high toxicity of the metals involved currently being masked by complexation.

However, certain metal ions, such as trivalent gadolinium, Gd^{3+}, cannot be inserted in supramolecular cryptates via standard dative bonds because of their "rare gas-like" electron configuration. Such closed-shell guests need special cage-like structures with suitable electrostatic sites capable of trapping the anions. The chemistry of gadolinium shows that carboxylate groups COO^- have the most affinity for this metal amongst cationic groups. Thus, we can envisage designing new gadolinium cryptates consisting of branches with COO^- termini grafted onto a rigid molecular support. Such a supramolecule, coded Dotarem (Fig. 1(b)), is currently being developed by Guerbet SA, the four carboxylate-bearing branches (or lariats [1]) being fixed in the most convenient spatial arrangement for Gd^{3+} complexation because of the exceptional rigidity and stability in vivo of a tetraaza crown ether carrier.

Similar supramolecular structures can be envisaged with cyclotriphosphazenic six-membered rings as the carriers (Fig. 1(c)). It is well known that such N_3P_3 rings are planar, highly rigid and perfectly stable in vivo (no metabolization at all) [2]. Moreover, non-degradable nucleophilic groups with COO^- termini can be easily grafted onto the phosphorus atoms of N_3P_3 rings leading to "double-basket" (Fig. 2) supramolecules for Gd^{3+} cryptation.

This paper reports the synthesis and structure of such lariat-bearing cyclophosphazenes.

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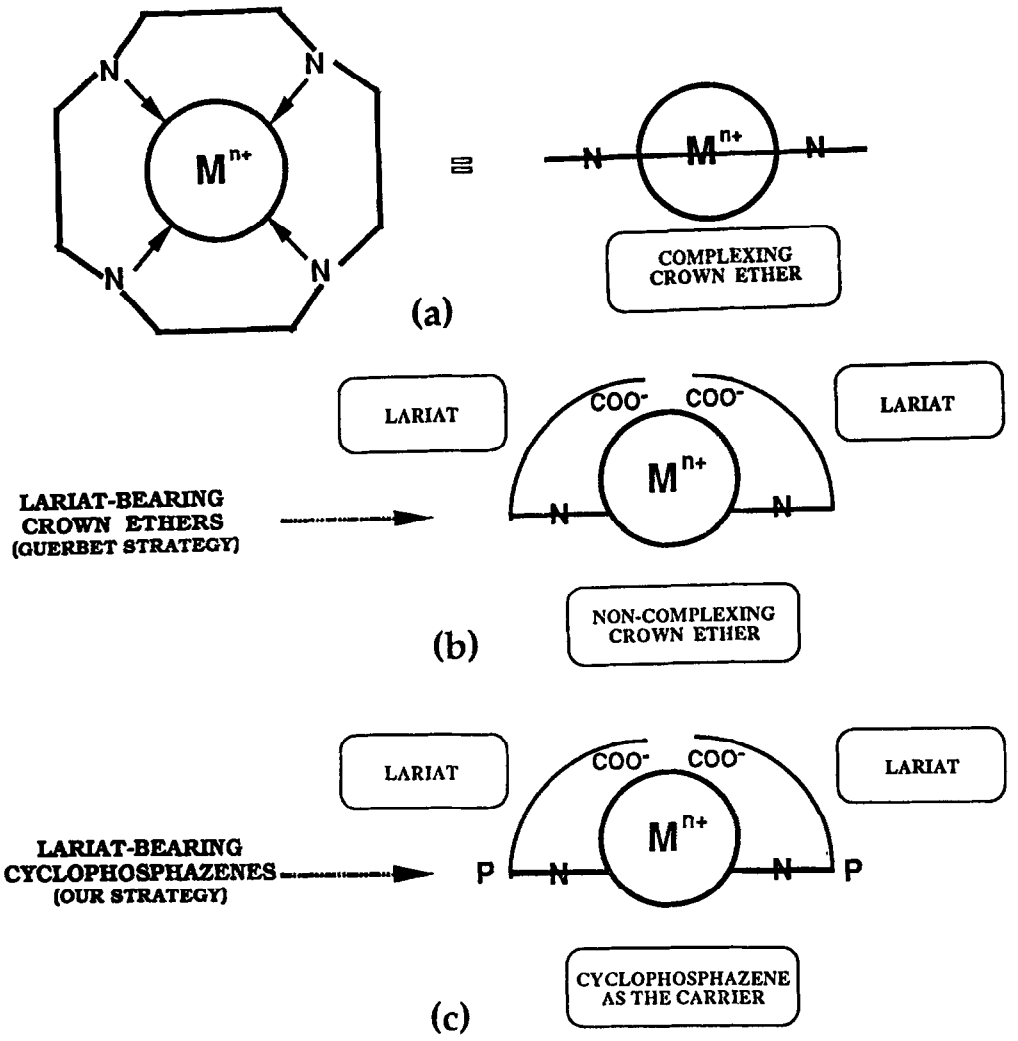


Fig. 1. Various types of molecules for metal cryptation.

Table 1
Reagents and yields of aminoester hydrochloride preparations

Acid	Alcohol	Hydrochloride	Yield (%)
GABA	φCH_2OH	$H_2N(CH_2)_3CO_2CH_2\varphi$, HCl	98
GABA	$CH_3CHOHCH_3$	$H_2N(CH_2)_3CO_2CH(CH_3)_2$, HCl	67–97
GABA	$CH_3(CH_2)_3OH$	$H_2N(CH_2)_3CO_2(CH_2)_3CH_3$, HCl	81
GABA	CH_3OH	$H_2N(CH_2)_3CO_2CH_3$, HCl	91
ACA	$CH_3CHOHCH_3$	$H_2N(CH_2)_5CO_2CH(CH_3)_2$, HCl	75–84
IDA	φCH_2OH	No esterification	–
IDA	$CH_3CHOHCH_3$	$HN[CH_2CO_2CH(CH_3)_2]_2$, HCl	62–98
PipA	$CH_3CHOHCH_3$	$HN{-}CO_2CH(CH_3)_2$, HCl	85

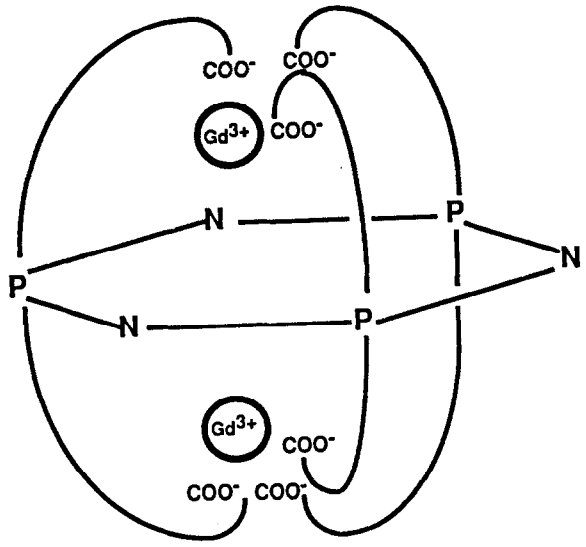


Fig. 2. A basic lariat cyclophosphazene for gadolinium cryptation.

Synthesis of lariat-bearing cyclophosphazenes

Syntheses were achieved according to the following two-step procedure:

$$N_3P_3Cl_6 + 6H_2N(X)CO_2R.HCl + Et_3N \rightarrow N_3P_3[HN(X)CO_2R]_6 + Et_3N.HCl \quad (1)$$

$$N_3P_3[HN(X)CO_2R]_6 + 6NaOH \rightarrow N_3P_3[HN(X)CO_2^-Na^+]_6 + 6ROH \quad (2)$$

Amino ester hydrochlorides, $H_2N(X)CO_2R.HCl$ (Table 1), were prepared from the amino acids of Table 2 according to the procedure of Patel and Brice [3]:

$$H_2N(X)CO_2H + ROH + SOCl_2 \rightarrow H_2N(X)CO_2R.HCl + HCl + SO_2$$

using the stoichiometry (1 amino acid: 60 alcohol: 14 thionylchloride). Reactions took about 24 h and their completion was checked by ^1H 200 MHz NMR. Yields were not less than 60% (Table 1) except for the benzylic ester of IDA where no esterification was observed.

Table 2
Synopsis of abbreviations and nomenclature

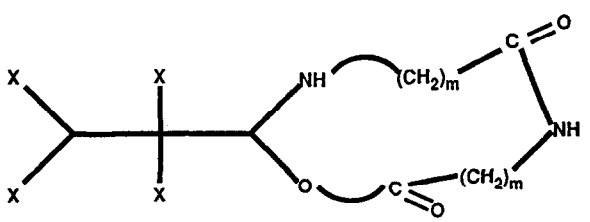
Formula	IUPAC nomenclature	Common name	Abbreviation
$H_2N(CH_2)_5CO_2H$	6-Aminohexanoic acid	Aminocaproic acid	ACA
$H_2N(CH_2)_3CO_2H$	4-Aminobutanoic acid	γ-Aminobutyric acid	GABA
$HN{-}CO_2H$	4-Piperidinecarboxylic acid	Isonipecotic acid	PipA
$HN(CH_2CO_2H)_2$	Iminodiethanoic acid	Iminodiacetic acid	IDA

Table 3
NMR data for some lariat cyclophosphazenes

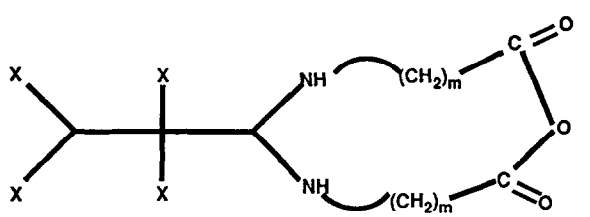
Amino-ester	Stoichiometry (1 $N_3P_3Cl_6$/12 or 18 amino ester)	Duration (days)	$N_3P_3X_6$	$N_3P_3X_4Y_2$	
			Singlet (ppm)	Doublet (ppm)	Triplet (ppm)
$GABACH_2\varphi$	1/18	5	14.5	14.0	3.0
$GABACH(CH_3)_2$	1/12	3	17.9	14.5	2.3
$GABA(CH_2)_3CH_3$	1/18	1	17.7	14.0	3.0
$ACACH(CH_3)_2$	1/12	3	17.3	14.3	3.7
$PipACH(CH_3)_2$	1/12	21	20.8	–	–

Step (1) was achieved using triethylamine as the solvent. The ratio of starting materials and duration of reaction were found to depend on the nature of reactants (Table 3). Completion of reaction was checked by ^{31}P NMR, the expected singlet for $N_3P_3[HN(X)CO_2R]_6$ moieties being detected in the 14.5–20.8 ppm range (Table 3). A by-product (doublet centered on 14 ppm and triplet centered on 3 ppm) was observed in all cases, except for the isopropyl ester of PipA, and could not be fully eliminated by SiO_2 column chromatography. DCI mass spectrometry showed that the MH^+ molecular peak of this impurity was commonly 100 mass units lower than that of the related $N_3P_3[HN(X)CO_2R]_6$ moiety, thus corresponding to either an anhydride-like or to a lactam-like by-product (Fig. 3). This impurity remained unaltered after step (2), that is after alkaline hydrolysis, so one may conclude that a lactam moiety seems to be more likely than an anhydride-like one.

Step (2) was carried out with methanol as the solvent; reactions took a few minutes. Methanol was removed under reduced pressure, leading to a white powder containing both the expected sodium salt and the unreactive amino carboxylate. Extraction with 2-propanol yielded $N_3P_3[HN(X)CO_2^-Na^+]_6$ salts as light-brown powders, the purity of which was determined by ^1H and ^{31}P NMR together with IR spectroscopy. A comparison of IR spectra before and after hydrolysis showed that the ν(C=O) vibra-



LACTAM-LIKE BY-PRODUCT



ANHYDRIDE-LIKE BY-PRODUCT

Fig. 3. Possible structures of the common by-product detected upon reaction of amino ester hydrochlorides with $N_3P_3Cl_6$.

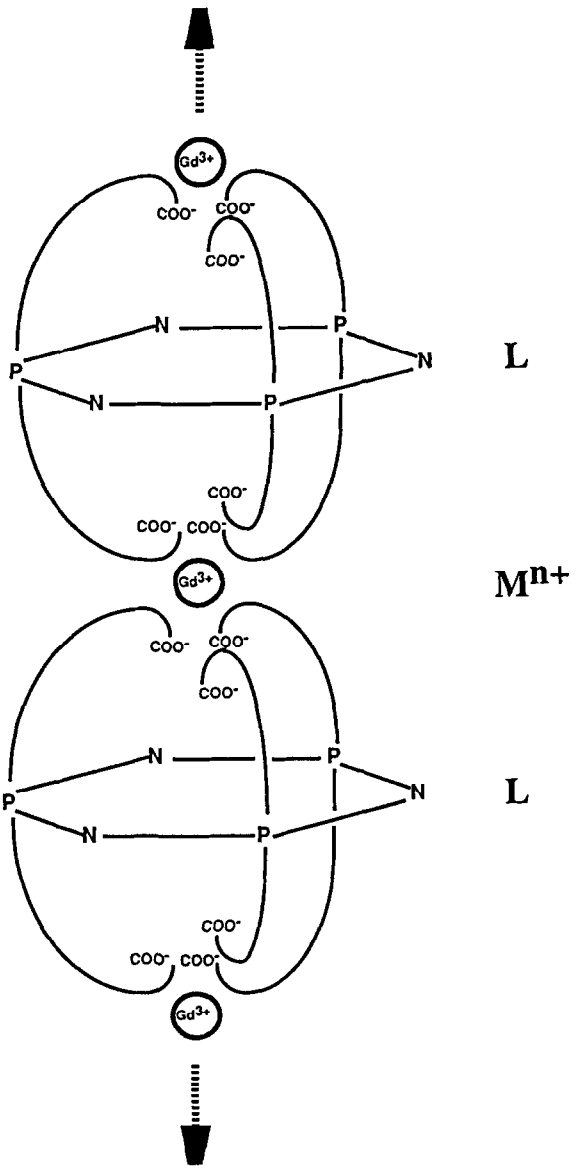


Fig. 4. Probable $Gd^{3+}L_2$ oligomeric structure for the insoluble lariat cyclophosphazenic adduct of the gadolinium cation.

tion is shifted from 1720 (ester form) to 1600 cm^{-1} (carboxylate form) with a broadening of all the vibrations around 1400 cm^{-1} which are caused by (C=O) and (C–N) stretching frequencies. Yields ranged from 57 to 65%.

Gadolinium cryptates

Trapping of the gadolinium cation occurs via the following pathway:

$$N_3P_3[HN(X)CO_2^-Na^+]_6 + 2GdCl_3 \cdot 6H_2O \rightarrow N_3P_3[HN(X)CO_2^-]_6 \cdot 2Gd^{3+} + 6NaCl$$

A solution of $GdCl_3 \cdot 6H_2O$ (3.35 g, 9 mmol) in 10 ml of water was added dropwise to a solution of $N_3P_3[HN(X)CO_2^-Na^+]_6$ (4.5 mmol) in 20 ml of water. The gadolinium adduct, $N_3P_3[HN(X)CO_2^-]_6 \cdot 2Gd^{3+}$, which precipitates instantaneously, is insoluble in water. Moreover, gadolinium cannot be removed from the adduct even upon addition of the Dotarem precursor, indicating that this cryptate has a stability constant larger than that of Dotarem, i.e. larger than 10^{28}, a value which constitutes a real challenge in the design of potential magnetic resonance imaging agents.

Unfortunately, the water insolubility of these gadolinium cations, which is probably due to an oligomeric $Gd^{3+}L_2$ structure (Fig. 4), does not allow their direct use for medical applications. A possible method for solving this problem and making the gadolinium cations water-soluble may involve forming a covalent linkage to vesicles such as low density lipoproteins (LDLs), an approach which is now in progress in our laboratory.

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