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EDTA and DTPA Analogues of Dipalmitoylphosphatidylethanolamine as Lipophilic Chelating Agents for Metal Labeling of LDL.

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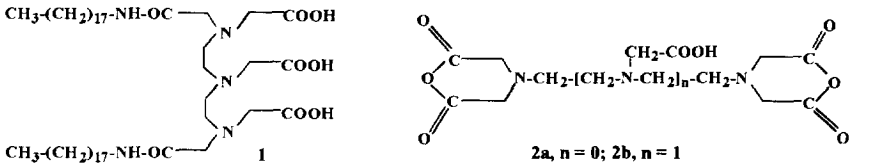
Abstract: *Two lipophilic chelating agents (L) prepared by reaction of dipalmitoylphosphatidyl-ethanolamine with the bis(anhydride) form of ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DTPA) were characterized. L, indium-L or gadolinium-L complexes are soluble in buffered solutions and may be used for metal labeling of LDL.*

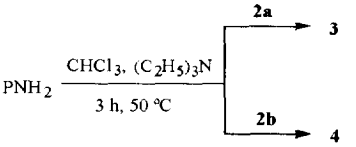
In order to target drugs to cancer sites, increasing attention has been given to drug transport vehicles such as low density lipoproteins (LDL)[1] which are quickly taken up by fast growing tumor cells in the course of their accelerated metabolism.[2-6] To take advantage of the tumor cell's affinity for LDL and improve the delivery of imaging agents to tumor sites, we recently proposed a new method of labeling LDL with indium-111 using a lipid chelating anchor, L, for stabilizing the radionuclide on the LDL particles.[7] The N,N''-bis(stearylamide) of diethylenetriaminepentaacetic acid, **1**, was first anchored into LDL, giving the L-LDL conjugates, before adding indium-111 to obtain the final tracer ^{111}In-L-LDL. *In vitro* and *in vivo* studies[7, 8] demonstrated that these In-L-LDL particles possess suitable properties to be evaluated as potential radiopharmaceuticals for tumor localization.

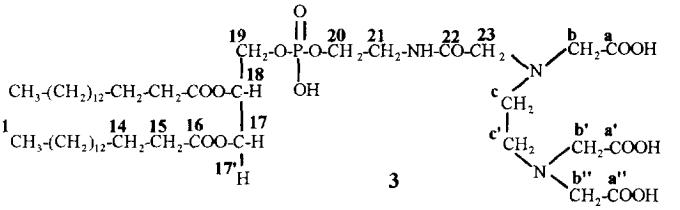
The labeling protocol used to prepare the In-L-LDL conjugates was imposed by the insolubility of the In-L complex in aqueous buffered solution (pH = 7.4). For this reason, **1** was first incorporated into LDL and then labeled with indium-111 to give the ^{111}In-L-LDL particles. To extend this labeling method of LDL to various metal ions, M, and evaluate the possibilities of other diagnostic or therapeutic applications, it is necessary to dispose of lipid chelating agents giving metal complexes, M-L, soluble in buffered solutions. Therefore, EDTA and DTPA analogues of dipalmitoylphosphatidylethanolamine (PNH_2) were prepared.

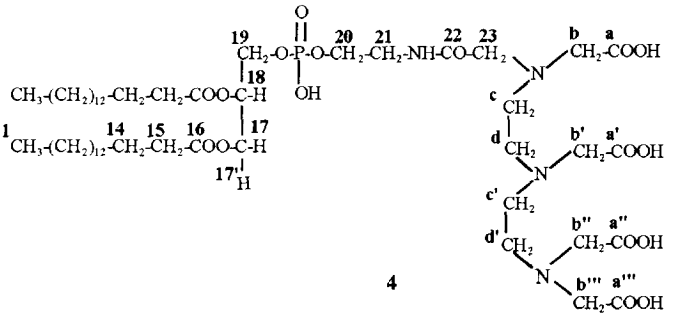
We report here the preparation, the structural characterization and the solubilities of these ligands, together with the solubilities of the M-L complexes (M = In^{3+}, Gd^{3+}) in aqueous buffered solutions and in presence of LDL (pH = 7.4).

Conversion of PNH_2 into EDTA or DTPA analogues was achieved in one reaction[9] using the bis(anhydride) form of EDTA **2a** prepared as previously reported,[10] or the commercially available forms of bis(anhydride) of DTPA **2b**, and PNH_2 in the presence of triethylamine (10 eq). After successive purification steps carried out by precipitation of **3** or **4** from chloroformic solutions with acetone and washing, traces of triethylamine were still visible on NMR spectra. Unfortunately, these traces could not be removed by column chromatography. Finally, triethylamine was eliminated by extraction with aqueous acid solutions. The extraction process was repeated until NMR spectra revealed no more traces of triethylamine giving pure forms of **3** and **4** in 30 % yield.









Preparations of EDTA or DTPA analogues of PNH_2 were previously proposed[11, 12] but spectral data were not given. The preparation reported here allowed a complete spectral characterization of **3** and **4**.[13, 14] ^1H NMR assigments, confirmed by ^1H-^1H COSY spectra and NOE experiments, showed a coupling between

geminal protons H-17 and H-17' for **3** and **4**. The solubility of **3** and **4** in buffer NH_4OH/NH_4Cl (0.1 M; pH = 9.5) is comparable to that of **1** (3.0 mM). In buffered solutions Tris/NaCl (pH = 7.4) (tris(hydroxymethyl)-aminomethane (0.1 M)/NaCl (1.5 M)), **1**, **3** and **4** are insoluble. When **3** and **4** are first solubilized in NH_4OH/NH_4Cl buffer and then added to Tris/NaCl buffer, they remain soluble, in contrast to **1** which is insoluble and precipitates. Then it becomes possible to prepare M-L complexes (L = **3** or **4**; M = In^{3+} or Gd^{3+}) soluble in aqueous buffer Tris/NaCl (pH = 7.4) and to add them to native LDL. Experiments carried out with indium-111 showed that the ^{111}In-L complexes were inserted into LDL in 40% yield. The full labeling procedure and *in vivo* applications will be reported elsewhere.

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9. Preparation of **3** and **4**: To a solution of PNH_2 (0.5 mmol) in $CHCl_3$ (50 mL) **2a** or **2b** (1 eq) was added. After the mixture had been stirred for 30 mn at 50 °C, triethylamine (10 eq) was added to the suspension. The reaction mixture was stirred for 3 h at 50 °C. The final mixture was concentrated to 5 mL by evaporation of $CHCl_3$. Acetone (30 mL) was added to the residue and the mixture was cooled at 4 °C for 48 h. The white precipitate was filtered and washed with acetone (50 mL x 3) and dried (yield 60 %). Traces of triethylamine were removed by successive extractions of chloroformic solutions of **3** or **4** with chlorhydric acid solutions (10^{-5} M) ($CHCl_3$/HCl, 15/5, v/v), adding supplementary $CHCl_3$ (60 mL) at the end to break the emulsion.
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13. **3:** m.p. 155 °C. FAB Mass calcd. for $C_{47}H_{88}N_3O_{15}P$: 965.6; found : 996. IR (KBr) γ_{cm}-1: 3400 (N-H, amide); 2920, 2850, 2740 (C-H, alkyl); 2660 (P-O, phosphoric); 1730 (C=O, ester); 1650 (COOH); 1550 (N-H, amide); 1250 (C-N, amide); 1070 (C-N, amide); 730 (C-H, alkyl). ^{1}H NMR (250 MHz, $CDCl_3$): 0.86 (t, J = 6, 6H, 2 x CH_3); 1.23 (m, 52H, 2 x $(CH_2)_{12}$, H-c, H-c'); 1.56 (m, 4H, 2 x H-14); 2.27 (m, 4H, 2 x H-15); 3.05 (m, 6H, H-b, H-b', H-b"); 3.5 (broad, 5H, H-21, H-23, P-OH); 3.95 (m, 4H, H-19, H-20); 4.15 (dd, J = 12, 1H, H-17); 4.35 (dd, J = 12, 1H, H-17'); 5.19 (m, 1H, H-18); 8.4 (s, 1H, N-H). ^{13}C NMR (62.9 MHz, $CDCl_3$): 13.98 (q, J = 124, CH_3); 22.57 ([b], [b'], [b"], [23]); 24.77 (t, J = 123, 2 x [14]); 24.82, 29.07, 29.10, 29.27, 29.49, 29.63 (t, J = 125, 2 x $(CH_2)_{12}$); 31.82 ([21]); 33.96 (2 x [15]); 34.11 ([c], [c']); 62.22 ([17]); 64.31 ([19], [20]); 69.66 ([18]); 172.97 (2 x [16]); 173.33 ([a], [a'], [a"], [22]).

14. **4:** m.p. 170 °C. FAB Mass calcd. for $C_{51}H_{95}N_4O_{17}P$: 1066.64; found : 1067. IR (KBr) γ_{cm}-1: 3300 (N-H, amide); 2900, 2840 (C-H, alkyl); 2650 (P-O, phosphoric); 1720 (C=O, ester); 1660 (COOH); 1540 (N-H, amide); 1230 (C-N, amide); 1030 (C-N, amide); 730 (C-H, alkyl). ^{1}H NMR (250 MHz, $CDCl_3$): 0.8 (t, 6H, J = 7, 2 x CH_3); 1.24 (m, 56H, 2 x $(CH_2)_{12}$, H-c, H-c', H-d, H-d'); 1.57 (m, 4H, 2 x H-14); 2.27 (td, 4H, 2 x H-15); 3.08 (m, 12H, H-b, H-b', H-b", H-b"', H-23), 3.62 (broad, 3H, H-21, P-OH]); 3.93 (m, 4H, H-19, H-20); 4.12 (dd, J = 11, 1H, H-17); 4.35 (dd, J = 11, 1H, H-17'); 5.2 (m, 1H, H-18); 8.12 (s, 1H, N-H). ^{13}C NMR (62.9 MHz, $CDCl_3$): 14.07 (q, J = 124, CH_3); 22.66 ([b], [b'], [b"], [b"'], [23]); 24.87 (t, J = 126, 2 x [14]); 24.92, 29.19, 29.35, 29.58, 29.72 (t, J = 125, 2 x $(CH_2)_{12}$); 31.90 ([21]); 34.05 (2 x [15]); 34.22 ([c], [c'], [d], [d']); 62.42 ([17], [19], [20]); 69.91 ([18]); 172.98 (2 x [16]); 173.34 ([a], [a'], [a"], [a"'], [22]).

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