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Synthesis of 3,4,3 LI 1,2 HOPO Labelled with ^{14}C.

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Summary

3,4,3 LI 1,2 HOPO **1** or LIHOPO (1,5,10,14-tetra (1-hydroxy-2-pyridone-6-oyl)-1,5,10,14-tetraazatetradecane.) is a decorporation agent used against actinides. It was synthesised labelled with carbon 14 with a view to metabolism studies. [6,9-^{14}C]-LIHOPO **2** was obtained in two radioactive steps through a very convenient synthesis in 50% radioactive yield from commercially available [1,4-^{14}C]-spermine, hydrochloride and 1-benzyloxy-6-oxo-1,6-dihydro-pyridine-2-carbonyl chloride **7**. The synthesis of **7** is also described.

Key Words: carbon 14, LIHOPO, decorporation, actinide.

Introduction

Actinides and some other toxic metals such as cobalt introduced into the body in the case of internal contamination or in the event of a nuclear accident by inhalation, ingestion or through wounds are mainly complexed by blood transferrin, which is one of the proteins of iron transport in the blood, and by low molecular weight complexing agents such as citrates, bicarbonates and phosphates. Chelation within the blood is then followed by distribution and retention in target organs such as kidney or liver, which can induce cancers (1). Furthermore, certain substances such as uranium can be chemically toxic in cases of heavy contamination. The toxic metals Pu, Am, Th, Np and Co must be eliminated from the body by administering non-toxic chelating agents (2). These must be highly selective and have a high stability constant so that they can

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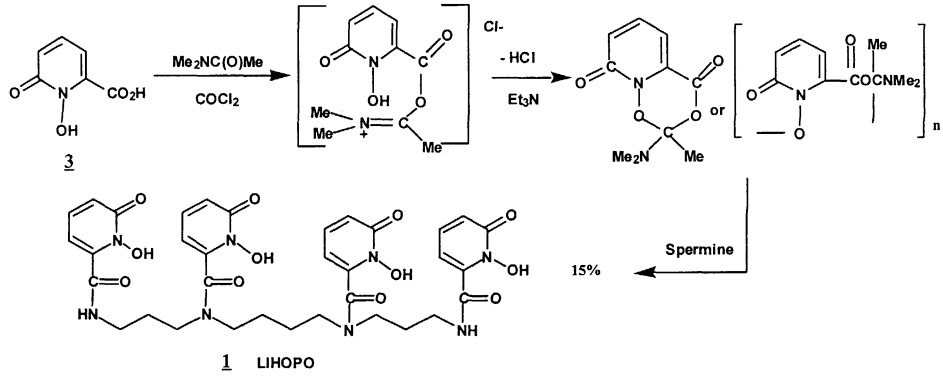
displace the natural complexes rapidly formed with components of blood. The ligand-actinide complex must be soluble in biological fluids and stable in a pH range of 1 to 8 in order to be subsequently eliminated from the body by crossing the renal or hepatic barriers. Treatment of internal contamination is usually referred to as decorporation. The decorporation agent which has been used for the last thirty years is diethylene triamine pentaacetic acid (DTPA), which has the drawback of being ineffective in cases of intoxication with cobalt, uranium and neptunium. More active and more polyvalent ligands are therefore being actively sought.

One of the most promising ligands discovered during the last ten years for the decorporation of the above elements (3-9) consists of four 1-hydroxy-2-pyridinone rings carboxylated at position 6 and fixed by amidic links to a spermine molecule which constitutes the substrate.

Results and discussion

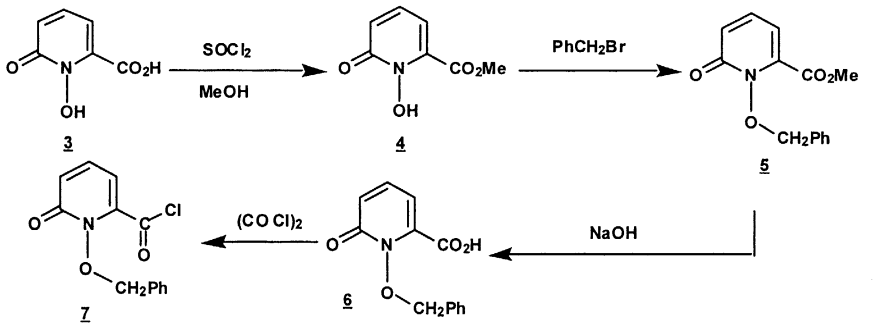
The compound, designated LIHOPO **1** which is the acronym of linear hydroxypyridone, has been synthesised (3) by activating the carboxyl function of pyridone **3** with the phosgene-dimethylacetamide complex and then reacting the resulting mixture with spermine.



Scheme 1

As can be seen in Scheme 1, the intermediate reagent is not clearly established. It mainly consists of polymerised substances, which means that at the end of the reaction a number of purification stages are required, notably HPLC, and the final yield is therefore only 15%, which calls into question the future of this ligand. In a preliminary communication (10), we described a new simple synthetic method for LIHOPO with a yield of around 85%. This method is based, firstly, on a protection reaction concerning the carboxyl group and the nitroxide group of the pyridone **3**, followed by deprotection and activation of the carboxyl group as an acid chloride and, secondly, on coupling of this acid chloride to spermine by a reaction carried out under phase transfer

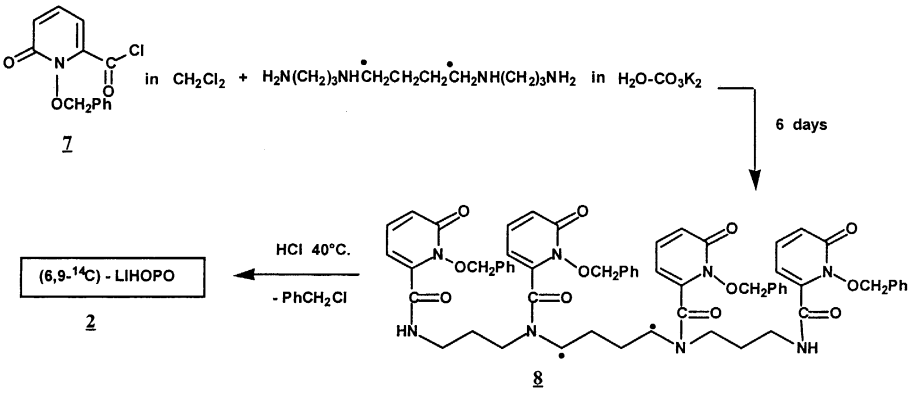
conditions. Here we present the application of this method to the synthesis of LIHOPO labelled at positions 6 and 9 with ^{14}C, the metabolism of which can be studied through *in vivo* experiments, thereby enhancing our understanding of the mechanisms of transport such as fixation and retention in target organs, and clearance.



Scheme 2

Scheme 2 shows the four stages by which pyridone 3 is protected at the carboxylic and the nitroxide groups, deprotected at the carboxylic group and then activated. The protected acid chloride 7 is obtained with an average yield of 95% from the pyridone 3. The advantage of this intermediate is that, in addition to LIHOPO, it enables the synthesis of a wide range of new tetra-, hexa- and octodentate ligands, by coupling with different aliphatic and aromatic polyamine substrates.

As shown in Scheme 3, the coupling reaction carried out between the acid chloride 7 and the labelled spermine, under phase transfer conditions, produces a LIHOPO whose nitroxide groups are benzylated. Deprotection of the nitroxide group by conventional means such as BBr_3 or catalytic hydrogenation is risky with a structure such as the pyridone used here. We observed that treatment with HCl at 30 to 40 °C produces satisfactory results.



Scheme 3

Experimental part

Table 1 gives the physical and chemical parameters of the intermediates **3** to **7**, as recorded by NMR with a Bruker ARX 400instrument. The initial pyridinone **3** has been described earlier (11). The spermine (4 HCl) labelled with ^{14}C: N,N'-bis-(3-aminopropyl)-1,4 ^{14}C) tetramethylene-1,4-diamine (MW 352) was supplied by Amersham Life Science as an aqueous solution at a concentration of 1.85 MBq/mL (50 μCi/mL) and a specific activity of 4.33 GBq/mmol (117 mCi/mmol). The unlabelled spermine base and oxalyl chloride were from Merck. The benzyl bromide and thionyl chloride were from Fluka

The solvents were dried and purified using conventional methods.

Methyl ester 4, MW 245.23: 15 g of pyridinone **3** was dissolved in 900 mL of methanol with magnetic stirring. 7.8 mL of thionyl chloride was added and stirring was maintained for 3 days at 20°C. The solvent was removed under reduced pressure, and the resulting white solid (19 g) was washed with ether and vacuum dried.

Benzyl methyl ester 5, MW 259.26: 1.25 g of dry K_2CO_3 followed by 1.36 g (7.95 mmol) of benzylbromide were added to 1.25 g (6.56 mmol) of ester **4** dissolved in 36 mL of acetone. The mixture was then refluxed for 6 h with vigorous stirring. After cooling, the mixture was filtered, and washed on the filter with 3 times 10 mL of acetone. The acetonic filtrate was first evaporated at 15 mm Hg and then 0.01 mm at 40 to 60°C. for 2 h ; 1.8 g of a light yellow oil was obtained, 94.7% yield.

Free acid 6, MW 245.23: 1.7 G (6.56 mmol) of benzyl ester **5** was dissolved in 15 mL of methanol to which 0.27 g (6.75 mmol) of NaOH in 4 mL of water was added while stirring. The mixture was stirred for 3 h at room temperature. 6N HCl was then added dropwise until pH 1 was attained. The white precipitate obtained was rapidly filtered and washed with chilled water on a filter and then vacuum dried at 100°C, producing 1.3 g of acid. The filtrate was partially evaporated, thus producing another 0.3 g of crystals, total yield 1.6 g (quantitative). The product can be crystallised in methanol.

Acid chloride 7, MW 263.68: A two-neck, round-bottomed flask was fitted with a septum cap and a reflux condenser with an argon line at the top. The material was dried with a burner and allowed to cool to room temperature in a current of argon. 10 ML of methylene chloride and 0.532 g (2.197 mmol) of acid **6** were introduced into the flask, and the mixture was magnetically stirred. 0.8 ML of oxalyl chloride (large excess) was then slowly injected with a microsyringe through the septum cap. When foam was no longer formed, the mixture was refluxed for 4 h, and

then stirred for 20 h at 25°C. Throughout this operation, the circulation of a slow current of argon at the top of the condenser carried away the gases formed and maintained an anhydrous atmosphere. At the end of the reaction, the reaction mixture was evaporated under a vacuum, then re-dissolved and evaporated a further 3 times with dry methylene chloride. The final residue (a yellow oil which can be crystallised) was dissolved in 20 mL of dry methylene chloride (solution A).

NB: The reaction proceeds faster if the chlorination is catalysed with pyridine, DMF or other substances, but the impurities introduced can be difficult to remove and we therefore decided against this procedure.

(6,9-^{14}C)-benzylated LIHOPO 8 MW 1111.22: An aqueous solution of spermine was prepared in a 100 mL flask by dissolving 101 mg (0.499 mmol) of spermine base in 21 mL of an aqueous solution of labelled spermine hydrochloride (32.4 MBq-0.875 mCi; specific activity after isotopic dilution 65 MBq/mmol-1.75 mCi/mmol), and finally 2.64 g of K_2CO_3 was then added to this aqueous solution. Once magnetic stirring had led to dissolution of the solids, the 20 mL of solution A prepared at the previous stage and containing the acid chloride 7 was quickly added and contact between the two phases was maintained by vigorous stirring for 6 days. After this period of time, the organic phase was separated off and washed with 5 mL of water, then twice with 2 mL of water, then with 5 mL of water containing 10% HCl then twice with 2 mL of water. The organic phase was then dried over anhydrous Na_2SO_4 and evaporated under vacuum. The residue consisted of 587 mg of benzyl-protected LIHOPO in the form of sticky crystalline material (measured activity 31.7 MBq-0.857 mCi, radioactive yield: 98%). The product was purified on a silica column (height 20 cm, diameter 1.5 cm, flow rate 25 mL/h, system: ethyl acetate-methanol 3-1 with column previously impregnated with pure methanol. Elution was followed by TLC coupled with UV and radioactivity detection. The fraction containing the pure product was evaporated under vacuum to give 279 mg of pure benzyl LIHOPO in the form of a white solid with a total activity of 16.1 MBq-0.44 mCi (radioactive yield after purification: 50%).
NB: In the case of preparation of larger quantities of unlabelled benzyl LIHOPO, the silica column purification stage, which resulted in significant loss, has been found to be unnecessary.

(6,9-^{14}C)-LIHOPO 2, MW 750.72: 275.9 Mg (0.248 mmol, 16.1 MBq-0.435 mCi) of (^{14}C)-benzyl LIHOPO was treated with 2 mL of 12 N HCl for 2 h at 45°C with magnetic stirring. After 18 h at room temperature, the aqueous mixture was washed with 4 mL of methylene chloride then twice with the same solvent in a 10 mL separating funnel. The aqueous phase was evaporated under reduced pressure at 20-30°C, the residue was then twice dissolved with water and evaporated to eliminate as much of the HCl retained as possible, and finally the residue was maintained for 20 h in a vacuum of 10^{-3} mm of Hg, giving 197 mg of LIHOPO in the form of a

white powder. A quantitative debenzylation yield should have given 186 mg (16.1 MBq-0.43 mCi) of product. The observed difference resulted from the residual HCl and water which we did not attempt to eliminate.

Analyses of (6,9-^{14}C)-LIHOPO 2

The chemical and radiochemical purity of (6,9-^{14}C)-LIHOPO was checked by high-performance liquid chromatography (HPLC). Column: Zorbax SB C18 250*4.6; flow rate: 0.4 mL/min; detection: UV on LDC 3200 apparatus at 310 nm; radioactivity detection: Berthold LB 503 apparatus. The product to be injected was dissolved in a 0.5% aqueous solution of trifluoroacetic acid. Column temperature: 40°C; elution: water with 0.5% of trifluoroacetic acid (A)-acetonitrile with 0.5% of trifluoroacetic acid (B); gradient: plateau (A) 5/(B) 95: 10 min; rise (A) 30/(B) 70: 15 min; plateau (A) 30/(B) 70: 15 min; rise (A) 70/(B) 30: 15 min; plateau (A) 70/(B) 30: 10 min; rise (A) 100/(B) 0: 15 min; plateau (A) 100/(B) 0: 10 min then return to initial conditions (A) 5/(B) 95.

Results: Radiochemical purity 94.5%, retention time 36.06 min,

Chemical purity (UV detection: 310 nm) 95.04%, retention time 35.78 min,

Injected mixture of radioactive and unlabelled LIHOPO, retention time 35.73 min.

N°	δ ^{13}C	δ ^{1}H	Mp°C
3	105; 114.5; 133.6; 147.4 Pyr. 162.3; 170.4 (C:O) (D_2O-NaOH)	6.2 broad d 7.2 Hz (1H); 6.4 broad d 8.5 Hz (1H); 7.15 dd 7.2 and 8.5 Hz.(1H).	216
4	53.29 (OMe) 108.5; 121.2; 136; 136.3 Pyr 158.5; 160.39 (C:O) ($CDCl_3$)	3.87 s OMe; 6.4 broad d 7.1 Hz (1H); 6.6 broad d 9 Hz (1H); 7.12 dd 7.1 and 9 Hz (1H).	90-92
5	53; 78.5 (OMe ,OCH_2).. 107; 125.8; 137.3; 138.7 Pyr. 128.6; 129.1; 130; 133.7 (Ph)158.6; 160.3 (C:O) ($CDCl_3$)	3.8 s OMe; 5.4 s CH_2Ph; 6.5 broad d 6.8 Hz (1H); 6.83 broad d 9.2 Hz (1H); 7.31 dd 9.2 and 6.8 Hz (1H); 7.29-7.33 m; 7.54-7.56 m (5H).	oil
6	78.6 (OCH_2) 108.3; 124; 139; 140 Pyr. 128.3; 129; 130; 133.6 (Ph) 159.8; 161.5 (C:O) (CD_3OD)	5.ll s CH_2Ph; 6.31d 7 Hz (1H); 6.57 d 9 Hz (1H); 7.32-7.34 m and 7.43-7.47 m (1+5 H) (D_2O-NaOH)	200-202
7	78.8 (OCH2) 112.5; 128.7; 128.8; 129.6; 130.3; 130.5; 133; 136.6; 140.4 Pyr + Ph 158.4; 159 (C:O) ($CDCl_3$)	5.37 s CH_2Ph; 6.91 d 7 Hz (1H); 6.99 d 9.2 Hz (1H); 7.38-7.43 m and 7.52-7.55 m (1+5 H)	oil

Percentage Composition

6 $C_{13}H_{11}O_4N$ MW. 245.23 Calc. % C 63.67; H 4.52; N 5.71; O 26.1.
Tr. % C 63.86; H 4.35; N 5.65.

Table 1

Mass spectrometry (Mariner ESI/TOF apparatus, solvent: methanol 90-water 10, quadripole temperature 133°C).

(6,9-^{14}C)-LIHOPO was analysed by mass spectrometry and the spectrum obtained was compared with that of an unlabelled sample. In view of the low specific activity (65 MBq/mmol-1.75 mCi/mmol), the peak corresponding to the contribution of carbon-14 did not appear. Main peak m/z 751 (M+1), 768 (M+18 (H_2O)), 773 (M+23 (Na)), 786 (M+36 (2 H20)).

The spectrum of the radioactive product has the same profile as the control spectrum at the main peak.

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