Preparation of an Aminoxyl Analog of the Anticancer Agent Miltefosine

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The synthesis and a few properties of hexadecyl 2-[N,N-dimethyl-N-(2,2,6,6-tetramethyl-1-oxyl-piperidin-4-yl)ammonio] ethyl phosphate are described. This compound is a spin labeled analog of the antineoplastic drug hexadecylphosphocholine (Miltefosine).

Introduction

A decade ago in quest of developing new suitable contrast agents for the clinical magnetic resonance imaging tomography (MRI), among a variety of compounds [1–3] also were synthesized two aminoxyls (nitroxyls, “nitroxides”) 1 and 3 containing quaternary ammonium moieties. While compound 1 was used in a study [4] concerning the membrane permeability of nitroxyl moieties through human erythrocyte membranes, compound 3 was somewhat neglected and not published.

Recently, it came to our attention [5] that hexadecyl (cetyl) phosphocholine (Miltefosine, 2), and related analogs were found [5] to have potent in vitro and in vivo antineoplastic activities against several cancer lines. These results prompted us to disclose the synthesis and the available properties of the nitroxyl labeled analog 3. It is believed that this information would be of interest to the scientific community participating in the development of anticancer drugs.

Results and Discussion

The key intermediate N-(2-hydroxyethyl)-N,N-dimethyl-N-(2,2,6,6-tetramethyl-1-oxyl-piperidin-4-yl)-tetraphenyl borate (7) was synthesized via the known compound N-(2-hydroxyethyl)-N,N-dimethyl-N-(2,2,6,6-tetramethyl-1-oxyl-piperidin-4-yl) ammonium iodide (6) [6]. 6 was prepared from 4-oxo-2,2,6,6-tetramethyl-piperidin-1-oxyl (4) and N-methylethanolamine by the reductive amination reaction mediated by sodium cyanoborohydride, followed by the methylation of the intermediate N-(2-hydroxyethyl)-N-methyl-N-(2,2,6,6-tetramethyl-1-oxyl-piperidin-4-yl) (5) with methyl iodide to give 6. The reaction of 6 with sodium tetr phenylborate gave the desired key intermediate 7.

The other key intermediate hexadecylphosphate (cetyl phosphate, 8) was obtained by using a known method [7] from cetyl alcohol and phosphorus oxychloride.

The condensation of 7 with 8 to give hexyl 2-[N,N-dimethyl-N-(2,2,6,6-tetramethyl-1-oxyl-pi-

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peridin-4-yl)ammonio] ethyl phosphate (3) was achieved using a mixture of 2,4,6-trisopropylbenzenesulfonfyl chloride (TPS) and dimethylamino- pyridine (DMAP) in pyridine.

The solid target compound 3 was readily soluble in water, and crystallized with two moles of water, melting at 103–104 °C. The analytical data corresponded to expectations.

As we have shown on several occasions in the past [8], the introduction of a nitroxyl moiety into various anticancer drugs resulted in lowering of toxicity and elevation of activity i.e. causing an increase of the therapeutic indices of those new nitroxyl labeled drugs.

Hence, we hypothesize that the nitroxyl labeled compound of type 3 could provide desirable properties to this class of potential anticancer agents.

**Experimental**

**Materials**

All reagents were of the best quality commercially available. Solvents were dried by standard procedure [9]. Compounds 4 [10], 6 [6] and hexadecyl phosphate (cetyl phosphate, 8) [7] were synthesized by literature methods.

**Analytical procedures**

All melting points were obtained with a Thomas Hoover capillary melting point apparatus model 6406-K using a calibrated thermometer.

The IR spectra were recorded on a Nicolet-10 MX FT-IR spectrophotometer using KBr pellets. Microanalyses were obtained on a Perkin-Elmer elemental analyzer model 240C. Silica gel 60 (Fluka) finer than 230 mesh was used for flash column chromatography [11]. TLC analyses were performed on silica gel F254 precoated sheets (EM Reagents), layer thickness 0.2 mm, with visualization using UV light and/or spraying with the Zin- zadse reagent composed of 1.0 g of molybdic acid dissolved in 25 ml of 10 N sulfuric acid.

The relaxation times were measured at 24 °C using the saturation recovery method for the spin lattice ($T_1$) relaxation time and the spin echo technique for the spin spin ($T_2$) relaxation time using a Praxis pulsed NMR analyzer, model PR-103,
10.7 MHz, 0.251 Tesla, The Praxis Co., San Antonio, Texas, USA. The concentration of each sample was 10 mM. The values for $T_1$ and $T_2$ of deionized water in the absence of the aminoxyl radical were 2150 msec and 182 msec, respectively.

Preparation of N-(2-hydroxyethyl)-N,N-dimethyl-N-(2,2,6,6-tetramethyl-1-oxyl-piperidin-4-yl)tetraphenyl borate (7)

A solution of 6 [6] (3.71 g, 10.0 mmol) in water (50 ml) was rapidly added to a stirred solution of sodium tetraphenylborate (3.60 g, 10.5 mmol) in water (25 ml). The thick, light orange slurry was stirred for 5 min at 20 °C, then filtered. The collected material was washed with water (3x25 ml), then air dried. Crystallization of this product from a mixture of acetone and ethyl ether (v/v, 1:1) gave 4.0 g (71%) of 7, m.p. 215-217 °C.

Analysis for $C_{37}H_{48}N_4C_2B$ (563.66)  
Calcd C 78.83 H 8.60 N 4.97% ,  
Found C 78.63 H 9.13 N 4.71%.

Preparation of hexadecyl 2-[N,N-dimethyl-N-(2,2,6,6-tetramethyl-1-oxyl-piperidin-4-yl)-ammonio] ethyl phosphate (3)

2,4,6-Tri-isopropylbenzene sulfonyl chloride (TPS, 0.910 g, 3.0 mmol) was added in one portion to a stirred mixture of cetyl phosphate (8, 0.32 g, 1.0 mmol, 7 (1.126 g, 2.0 mmol) and 4-dimethylaminoypyridine (DMAP, 0.122 g, 1.0 mmol) in dry pyridine (10 ml). The resultant red homogeneous solution was stirred for 30 min at 70 °C and 4 h at 40 °C. After cooling to 20 °C, water (1 ml) was added and the mixture stirred for 1 h. The mixture was then concentrated on a rotating evaporator at 50 °C/20 torr. The concentration of combined fractions containing 3 on a rotating evaporator at 40 °C/20 torr gave a red oily residue which crystallized on trituration with acetone (2 ml). Collection of the solid by filtration gave 0.165 (30%) of the product 3, m.p. 103-104 °C.

Analysis for $C_{29}H_{60}N_2O_5P$ (583.78)  
Calcd C 59.65 H 10.83 N 4.79% ,  
Found C 59.24 H 10.62 N 4.81%.

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