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Alkylphospho-L-serine Analogues: Synthesis of Cytostatically Active Alkylphosphono Derivatives

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Abstract: Hexadecylphosphono-L-serine (**2**) was synthesised by phospholipase-D-catalysed transesterification of hexadecylphosphonocholine in the presence of L-serine. The synthesis of ß-O-hexadecylphosphono-L-alanine (**10**) starting from N-benzyloxycarbonyl-L-serine benzyl ester is reported. The compounds **2** and **10** were found to exhibit cytostatic activity in vitro. © 1997 Elsevier Science Ltd.

Synthetic phospholipid analogues represent a new class of antineoplastic agents[1]. Their antitumor activity differs from that of conventionally used anticancer agents interfering with DNA at the level of replication and transcription. The mechanism of action of phospholipid analogues is not clearly understood so far, but the main target of action appears to be the cell membrane and the intracellular signal transduction pathways at several sites. Most of the cytostatically active phospholipids synthesised have been structural analogues of lysophosphatidylcholine. In recent years a series of glycerophospholipid ethers with modified head groups were also found to exhibit cytostatic properties[2]. Synthetic serine phospholipids appeared to be of special interest in view of the known physiological activities of the naturally occurring phosphatidyl serines (PS), such as activation of several membrane-associated enzymes[3] including the phospholipid-dependent protein kinase C (PKC)[4]. Structurally modified O-alkylglycerophospho-L-serines have been synthesised in our laboratory which exhibited strong cytostatic activity *in vitro* probably due to their interference with PKC[5,9]. Recently it was shown that the presence of the alkylglycerol moiety is not required for the antiproliferative activity of PS analogues. Alkylphospho-L-serines with a long-chain alkyl group instead of the alkylglycerol moiety were also found to be also highly active antineoplastic compounds *in vitro*[6]. Our recent findings indicated that these agents influenced key steps of the proliferative signal transduction[7] such as thrombin-induced inositol-1,4,5-trisphosphate formation, the increase in cytosolic Ca^{++} concentration and inhibition of phosphatidylinositol-specific phospholipase C and D, which may play a role in the tumoricidal action.

In order to obtain more detailed experimental data on the structure-activity relationship, some other alkylphospho-L-serine analogues including the hexadecylphosphono-L-serine **2** and the ß-O-hexadecylphosphono-L-alanine **10** have been prepared. Possessing a P-C bond, these compound represent analogues with increased biostability against hydrolytic cleavage by phospholipase C and phospholipase D, respectively.

The synthesis of compound **2** is based on the phospholipase D-mediated conversion of phosphatidylcholines to phosphatidylserines described previously[8]. This procedure has also been successfully applied for the preparation of novel types of O-alkylglycerophospho-L-serine analogues[9]. In this context it was of interest to find out whether alkylphosphonic esters, such as alkylphosphonocholine[10], lacking a natural

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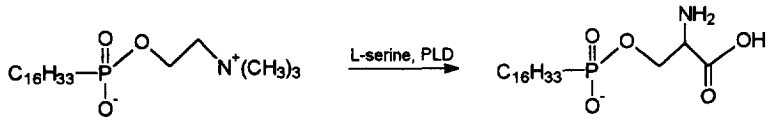
glycerophospholipid component and, in addition, possessing a P-C bond between the hydrophobic lipid group and the P-containing head group might also be suitable substrates for the phospholipase-D-catalysed transfer reaction using L-serine as an acceptor alcohol. This could be confirmed in the case of phosphono derivative **1**.



1 **2**

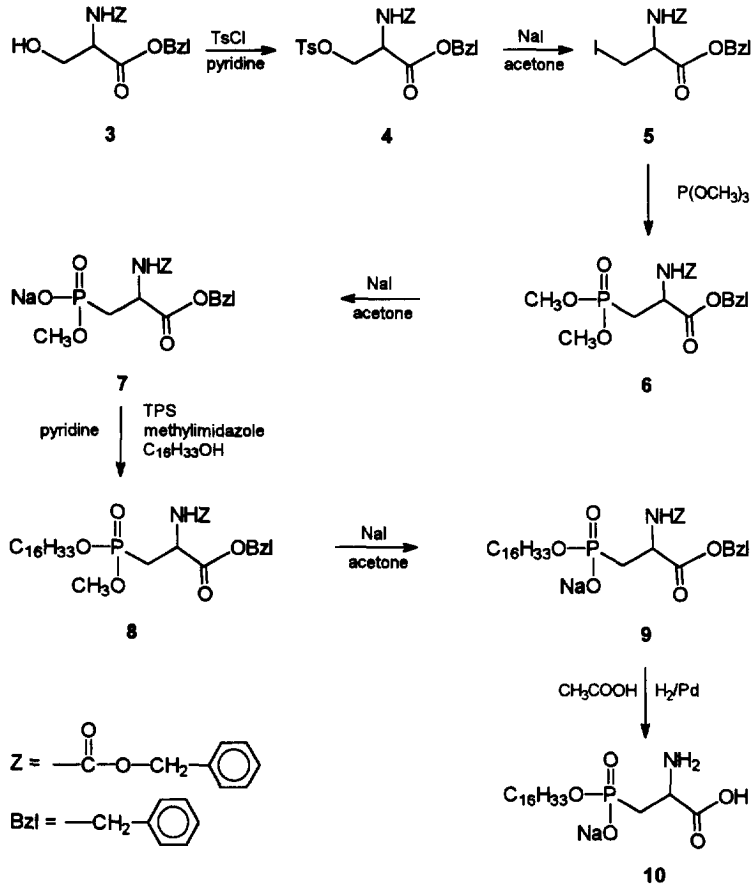
PLD = phospholipase D

Scheme 1

The transesterification reaction (Scheme 1) was carried out by stirring a mixture of hexadecylphosphonocholine[10] (**1**) and excessive L-serine (molar ratio 1:100) in diethyl ether-chloroform (ethanol free) in the presence of cabbage phospholipase D (acetone powder)[11] and acetate-acetic acid buffer (pH 5.6) containing 0.09 M $CaCl_2$ for 2.2 h at 45°C. After work-up[9] the crude substance was chromatographed on a carboxymethyl cellulose column (CM-52-cellulose, Serva). The elution was carried out in succession with chloroform, then mixtures of chloroform-methanol (9:1, 8:2, 7:3, 1:1). The hexadecylphosphono-L-serine was obtained as a white solid (yield 28 %) which was homogeneous by TLC (Merck silica gel 60 plate); R_f 0.06 (solvent A[12]), R_f 0.2 (solvent B[12]); elemental analysis: calcd. for $C_{19}H_{46}N_3O_5P$, di-NH_4-salt (427.57) C 53.37 H 10.84 N 9.83; found: C 53.17 H 10.99 N 9.75; negative FAB-MS calcd. for $C_{19}H_{40}NO_5P$: m/z 392 $(M - H)^-$, m/z 305 $(M - CH_2\text{-}CH(NH_2)COOH)^-$; positive FAB-MS: m/z 416 $(M+Na)^+$. The compound was identical with that obtained by condensation of hexadecylphosphonate with N-t-BOC-serine benzhydryl ester followed by removal of the protective groups from the condensation product by gaseous hydrogen chloride according to the procedure previously described[9, 13].

The phosphono derivative **10** was synthesised as shown in Scheme 2 starting from the commercially available N-benzyloxycarbonyl-L-serine benzyl ester **3**, which was tosylated with tosylchloride in the presence of pyridine to give the p-toluenesulfonate **4**[14]; mp 76-77° C; R_f 0.22 (solvent D[12]); elemental analysis calcd. for $C_{25}H_{25}NO_7S$: C 62.10 H 5.21 N 2.87; found C 62.17 H 5.24 N 2.71; positive ESI-MS: m/z 506 $(M + Na)^+$. Reaction of compound **4** with sodium iodide in acetone (72 h, 20° C) gave the N-benzoxycarbonyl-ß-iodo-L-alanine benzylester **5** (yield 74.6 %, mp 76-77° C (MeOH), Lit.[14] 76-77° C. DC: R_f 0.61 (solvent D[12]). Compound **5** was converted by the Michaelis-Arbusov reaction to the dimethylphosphonate **6** (treatment with trimethylphosphite for 8 h at 60° C) under removal of CH_3I. The crude product was purified by silica-gel chromatography with chloroform-ethyl acetate 1:1, followed by chloroform-ethyl acetate-MeOH 9:9:2. Yield 72 %, TLC: R_f 0.36 (solvent E[12]); positive ESI-MS: m/z 444 $(M+Na)^+$. Subsequent demethylation of the dimethyl ester by stirring with sodium iodide (molar ratio 1:2) in absolute acetone (120 h) led to the monomethyl ester-sodium salt **7**, which was purified on a silica-gel column with chloroform-methanol (9:1 to 3:2) as eluent. Yield: 32 %. TLC: R_f 0.5 (solvent A[12]). Coupling of compound **7** with hexadecanol using 2,4,6-triisopropylbenzenesulfochloride and N-methylimidazole in pyridine as condensing reagents[15] (24 h, r. t.) gave the alanyl benzyl ester **8** as a nearly pure product which was demethylated by sodium iodide/acetone (80 h, r. t.). The resulting compound **9** (51 % yield, TLC: R_f 0.76 (solvent C[12]) was subjected to catalytic hydrogenolysis at atmospheric pressure (acetic acid, 5 % Pd/C) for deprotection of the amino and carboxyl

group. The crude product was purified by silicagel chromatography with chloroform-methanol-water-conc. NH_4OH (65:35:3:0.1) to give the pure target compound **10** in 18 % yield. TLC: R_f 0.33 (solvent A[12]); elemental analysis: calcd. for $C_{19}H_{40}NO_6PNa_2$, di-Na-salt, monohydrate (455.49) C 50.19 H 8.85 N 3.07; found: C 50.31 H 9.19 N 2.61; negative ESI-MS: m/z 392 $(M-H)^-$; IR (KBr): 721 cm^{-1} ($-CH_2-$); 1021 cm^{-1} (P-O-alkyl); 1188 cm^{-1} (P=O); 1618 cm^{-1} (COOH, NH_2); 2850, 2918, 2956 cm^{-1} ($-CH_2-$); ^1H NMR (300 MHz, MeOD, referenced to TMS) δ: 0.88 (t, J = 7 Hz, 3 H, $-CH_3$), 1.20 - 1.50 (m, 30 H, $-(CH_2)_{15}-$), 1.60 - 1.70 (m, 2H, $-CH_2$-P-), 3.80 - 4.40 (m, 5 H, H_2N-CH-COOH, $-CH_2$-O-P, $-NH_2$).



Scheme 2

The alkylphosphono serine analogues **2** and **10** were found to have a strong inhibitory effect on the proliferation of various human tumor cells *in vitro*. Half maximum inhibition was observed at concentrations between 3 and 28 μM. Further studies have revealed that the intensity of the antiproliferative activity as well as the influence on different steps of the mitogenic signal transduction strongly depends on the position of the phosphono group. These results will be published elsewhere in more detail.

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