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SYNTHETIC 6-GLUCOSYL PHOSPHOLIPID AS A DRUG TRANSPORT SYSTEM

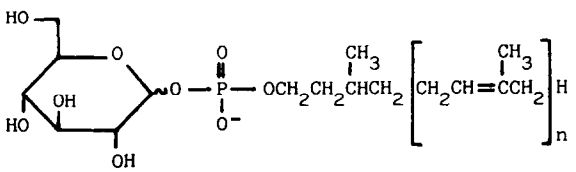
F. IGLESIAS GUERRA[a+], J.M. NEUMANN[b] and T. HUYNH-DINH[a*]

a) Unité de Chimie Organique, UA CNRS 487, Département de Biochimie & Génétique Moléculaire, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France.

b) Service de Biophysique, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette Cedex, France.

Abstract: A glucosyl phospholipid of thymidine is prepared in two steps from 6-glucose phosphate as a model for trans membrane transport.

Among the enormous amount of work devoted to drug carriers accross the blood-brain barrier or the bacterial cell wall, phospholipid-based systems attracted special attention because of their structural relationship to biological membranes[1]. In this paper we report the synthesis of a phosphotriester analogue to the dolichyl phosphate which could be used as a model for drug transport. It was actually demonstrated that 1-glucosyldolichyl monophosphate 1 is a lipd carrier which adds the sugar to preformed peptides during the biosynthesis of glycoproteins of the cell membrane in all living organisms[2-4]



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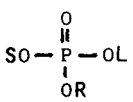

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+Present address: Departamento de Quimica Organica y Farmaceutica, Facultad de Farmacia, Sevilla, Spain

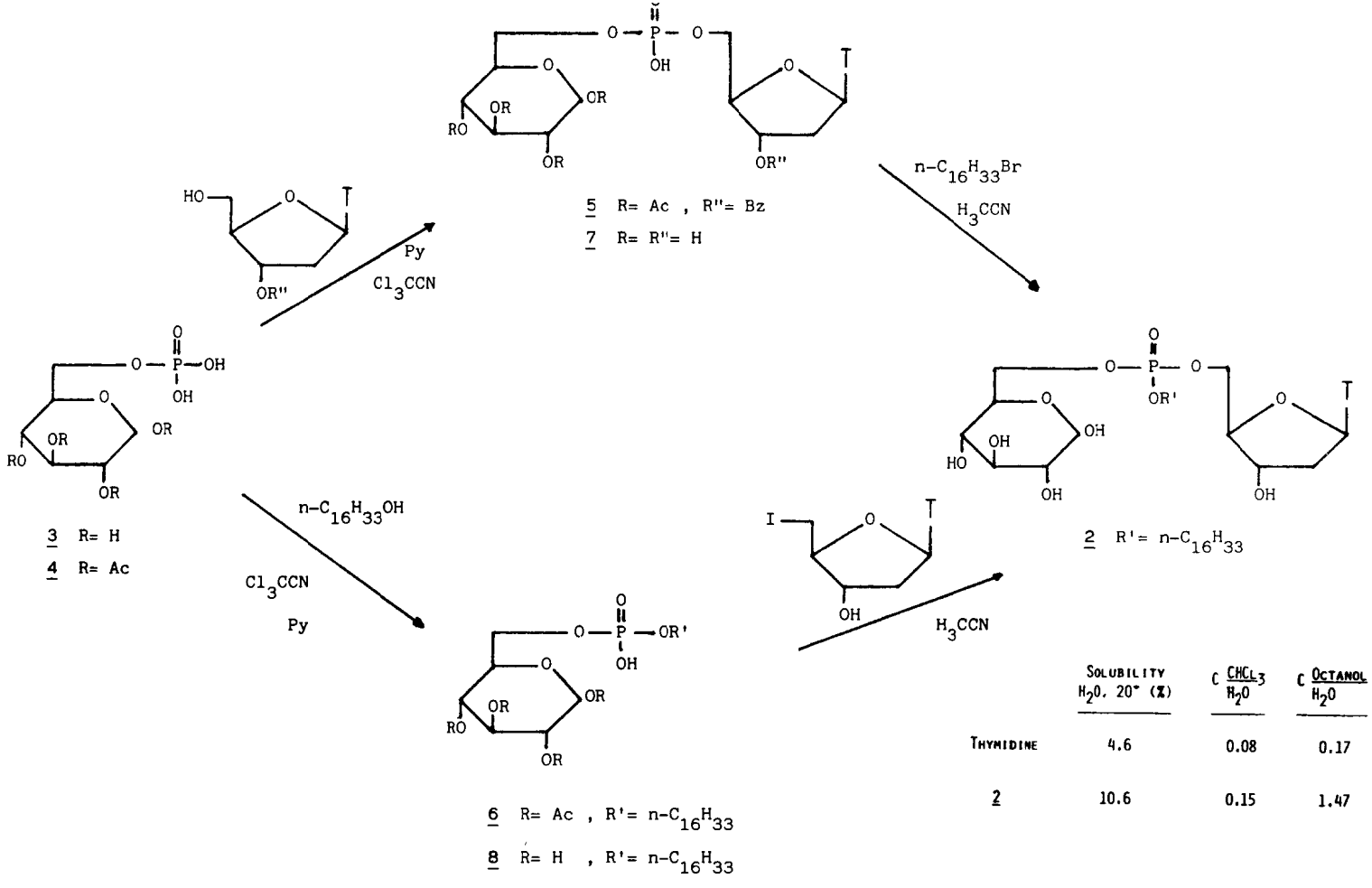
Synthetic procedures are available[5] for alky and aryl phosphotriesters, which are generally described as temporary protective materials for the isolation of the phosphodiester compound. Phosphotriester derivatives of a drug are not well known[6] and we speculate that if a glycosyl phospholipid such as 2 could be synthesized, this molecule would possess the following characteristics: 1) hydrophilic solubility due to the carbohydrate **S** 2) hydrophobicity due to the lipid moiety **L** and to the absence of charge 3) possibility of active transfer by the glucose-phosphate and the dolichol-phosphate transport proteins 4) among the different ways of hydrolysis, one should give the monophosphate of **R** which is often the biologically active entity. This last point is of special interest with antiviral or antitumoral nucleosides which require intracellular kinase into 5'-nucleotides.

In order to assess the validity of this concept, we decided to synthesize a model of phosphotriester 2 with **S** = glucose, **L** = phytol and **R** = thymidine. Our preliminary works in this series started with the energy-rich 1-glucose phosphate and we became rapidly confronted to the high instability of the phosphotriester derivatives[7]. So we switched to the 6-glucose phosphate 3 series, with **R** = hexadecanol. Two different phosphodiesters could be obtained from 1,2,3,4 tetra-O-acetyl 6-glucose phosphate 4 by esterification with 3'-O-benzoyl thymidine into 5 or with hexadecanol into 6.

The main methods previously used for the preparation of carbohydrate containing phosphodiesters is the condensation of a protected sugar monophosphate with an alcohol in the presence of either dicyclohexyl carbodiimide (DCC)[8], triisopropyl-sulfonyl chloride (TPSCl)[9] or activation by trichloroacetonitrile of the alcohol[10] and the phosphate[11-13]. In our hands, the first procedures[9,10] give unreproduced or low yields and we found that the most convenient method was the use of trichloroacetonitrile[7,11] in pyridine at 80°C under inert gas in order to avoid colored degradation compounds: evaporation of the solvent followed by a silica gel chromatography gave the crystalline phosphodiesters 5 (65%) and 6 (82%) which were quantitatively deacylated with sodium methylate into 7 and 8.

The phosphotriester 2 was obtained by direct nucleophilic displacement[13] of the tetrabutylammonium salt of phosphodiesters 7 or 8 with 1-bromohexadecane (45% yield) or 5'-iodothymidine[15] (20%) in acetonitrile at 80°C. The 6-glucosyl hexadecyl 5'-thymidinyl phosphate 2 was isolated as a mixture of 4 stereoisomers (α, β , R, S)[16] which could not be separated by HPLC.

The physico chemical properties of 2 are quite interesting as compared to those of thymidine itself (table): the phosphotriester 2 of thymidine is more soluble in water and more lipophilic as measured by its partition coefficients[17] than the nucleoside. Although these constants are not enough to assure a good transport, this result and the stability of 2 are quite encouraging and we have found with preliminary ^{31}P-NMR experiments that the phosphotriester is transported through the membrane of synthetic large unilamellar



5 R= Ac , R''= Bz

7 R= R''= H

3 R= H

4 R= Ac

2 R'= $n-C_{16}H_{33}$

6 R= Ac , R'= $n-C_{16}H_{33}$

8 R= H , R'= $n-C_{16}H_{33}$

	Solubility H_2O, 20° (%)	C $\frac{CHCl_3}{H_2O}$	C $\frac{Octanol}{H_2O}$
Thymidine	4.6	0.08	0.17
2	10.6	0.15	1.47

vesicles. Beside the nucleoside derivatives, we expect that the concept of glycosyl phospholipid will be a promising one for transmembrane transport or drug-targeting of antibiotics or neurotransmitters by varying the sugar and the lipid moieties.

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16 C,H,N elemental analysis $C_{32}H_{57}N_2O_{13}P$, H_2O Calc C% 52.89; H% 8.12; N% 3.85; P% 4.26. Found: 53.05; 8.35; 3.82; 4.02. Mass spectra (CI) 709 (MH^+). UV (H_2O): 7 400 (254 mn). NMR (500 MHz, ^1H): 4.900 (H_1^{α}); 4.305 (H_1^{β}); 3.975 (CH_2-O-P); 0.834 (CH_3); 6.176, 6.163 ($H_{1'}$); 1.780 (5-CH_3).
17 The partition coefficients were determined by UV spectroscopy at 265 nm.

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