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6- AND 1- SUBSTITUTED MANNOSYL PHOSPHOTRIESTERS AS LIPOPHILIC MACROPHAGE-TARGETED CARRIERS OF ANTIVIRAL NUCLEOSIDES

C. Gouyette[(a)], J.M. Neumann[(b)], R. Fauve[(c)], and T. Huynh-Dinh[(a)]*

(a) Unité de Chimie Organique, URA CNRS 487, Institut Pasteur, 75724 Paris Cedex 15, France

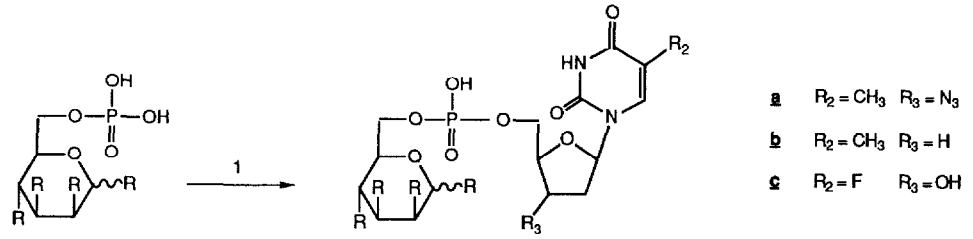
(b) Service de Biophysique, CEN de Saclay, 91191 Gif-sur-Yvette Cedex, France

(c) Unité d'Immunophysiologie Cellulaire, URA CNRS 1113, Institut Pasteur

Abstract : Lipophilic mannose-6 phosphate and (1-α mannosyl)ethyl phosphate derivatives of the antiviral nucleosides 3'-azido thymidine (AZT), 2',3'-dideoxy thymidine (ddT) and 2'-deoxy 5-fluoro uridine (dU^F) were prepared from D-mannose as membrane soluble prodrugs directed towards cells carrying mannosyl receptors.

Starting from the model dolichyl glucose-1 phosphate which serves as a lipid carrier in glycoprotein, we have recently described the synthesis of a hexadecyl 6-D-glucopyranosyl 5'-thymidinyl phosphate[1] and its NMR studies of trans-membrane transport across unilamellar vesicles[2]. A series of antiviral nucleosides derived from thymidine have been prepared and their promising biological activities have increased our interest to another carbohydrate series, that of D-mannose. We wondered wether the mannosyl residue, in addition to its higher water solubility, could also be used as a site-directing moiety towards mannosyl-binding proteins. This ligand-cell recognition is important in the case of macrophages, which are known to be the target of numerous pathogens which replicate in these cells and to present mannose-specific receptors responsible for the pathogens adhesion[3]. Moreover, certain HIV strains, proposed as the common factor of AIDS diseases, can replicate in monocytes/macrophages for a considerable time without inducing a substantial cytopathic effect[4] and it was suggested that these cells may be the reservoirs of the virus *in vivo* [5]. It is therefore hoped that compounds which have a deleterious effect on hematopoieisis will not act on bone marrow cells since most of mannosylated drugs will be endocytosed by liver and spleen macrophages; in these conditions a higher therapeutic index could be obtained, together with lower side effects and toxicity.

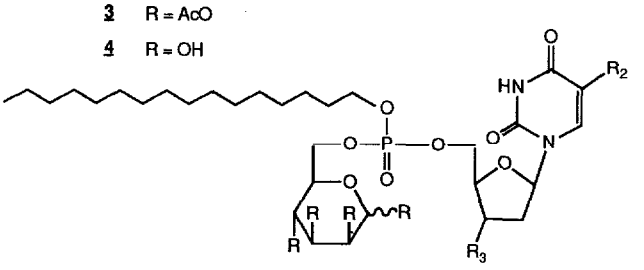
We wish to report here the synthesis of phosphotriesters of nucleosides, of a lipidic chain and of mannose at C-6 or at C-1 through a C_2 linker, as lipophilic macrophage-targeted carriers of antiviral nucleosides (AZT,



1 R = OH
2 R = AcO

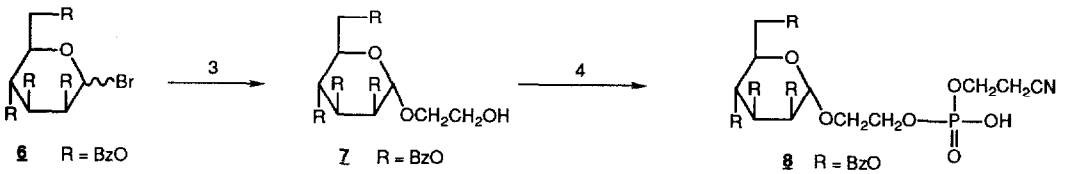
3 R = AcO
4 R = OH

a $R_2 = CH_3$ $R_3 = N_3$
b $R_2 = CH_3$ $R_3 = H$
c $R_2 = F$ $R_3 = OH$



5 R = OH

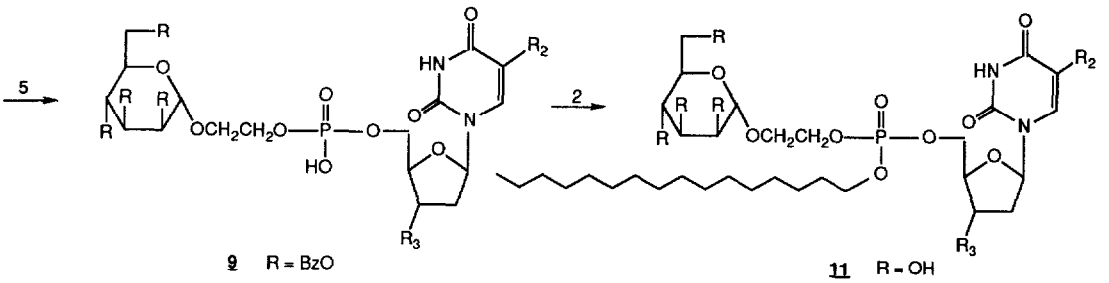
1 CCl_3CN/ nucleoside, 80°C; CH_3ONa
2 $C_{16}H_{33}I/CH_3CN$, 80°C



6 R = BzO

7 R = BzO

8 R = BzO



9 R = BzO
10 R = OH

11 R = OH

3 $C_6H_5CH_2OCH_2CH_2OH$/ $Hg(CN)_2$ /CH_3NO_2; Pd-C/ H_2
4 $OP(OH)_2OCH_2CH_2CN$/ CCl_3CN , 80°C
5 pyridine/ TPSNT/ nucleoside; CH_3ONa

a $R_2 = CH_3$ $R_3 = N_3$
b $R_2 = CH_3$ $R_3 = H$
c $R_2 = F$ $R_3 = OH$

ddT, dU^F). A similar concept of phosphotriester derivatives of nucleosides analogues was recently presented, associating an alkoxy chain with an aminoacid [6].

As in the glucose series [1, 2], the 6-mannosyl phosphotriesters of pyrimidine nucleosides were prepared from D-mannose 6-phosphate 1 : the phosphodiesters 3 were obtained by condensation of the protected phosphate 2 and the different nucleosides 3'-azido thymidine (AZT), 2',3' dideoxy-thymidine (ddT) and 2'-deoxy-5-fluoro uridine (dU^F) with activation of the phosphate function by trichloroacetonitrile. The resulting compounds were deprotected with sodium methylate into 4 a, b, c, exchanged to their tetrabutyl ammonium salts and alkylated by hexadecyl iodide into the triesters 5 a, b, c which were isolated after a short-columm silica gel chromatography[7].

We also decided to explore the 1-mannosyl substituted series, which are more closely to the lipidic carrier dolichyl glucose-1 phosphate. As the pyranosyl sugars are converted into 6-phosphates by group-translocation processes during their active transport inside cells, we wanted to obtain glycosyl phosphates where the 6-OH was free in order to be taken up by the phosphotransferase system. To prevent the instability of the phosphate esters at the anomeric position observed in preliminary work, we added a short linker arm to the sugar : 2-benzyloxyethanol reacted with 1-bromo-2,3,4,6-tetrabenzoyl D-mannose[8] 6 to give, after catalytic hydrogenation, 64 % of hydroxyethyl α-D-mannopyranoside 7. The mannosyl alcohol 7 reacted with cyanoethyl phosphate in a mixture of CCl_3CN-pyridine at 80° C overnight to give the phosphate 8 (67 %) which was condensed with the nucleosides into the protected phosphodiesters 9 a, b, c (56-69 %) using triisopropylbenzenesulfonyl-triazole (TPSNT) as an activating agent[9]. Alkaline deprotection gave the phosphodiesters 10 a, b, c. These mannosyl derivatives are closely related to uridine 5'-diphosphate analogs recently prepared by van Boom et al.[10] as inhibitors of glycolipid biosynthesis. The alkylation of the tetrabutylammonium salts of phosphodiesters 10 gave, as previously, the mannosyl phosphotriesters 11 a, b, c (67-70 %).

The purity of all the phosphotriesters 5 and 11 are checked by analytical reverse-phase HPLC and their structures identified by NMR and mass spectra[7]. Preliminary screening have shown antiviral activities for the described compounds; detailed antiviral and NMR trans-membrane transport studies of these 6- and 1-substituted mannosyl phosphotriesters will be reported elsewhere.

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[7] 5a FAB MS : 734 (MH^+). NMR (DMSO d_6) : ^{1}H chain CH_3 0.87, $(CH_2)_n$ 1.25, $\underline{CH_2}$-CH_2OP 1.63, CH_2-$\underline{CH_2}$-OP 4.01; mannose H-1 α 4.90, H-2 3.58, H-3 3.57, H-4 3.45, H-5 3.72, H-6 4.23, H-6' 4.09 ; nucleotide H-6 7.51 (41 %) and 7.49 (59 %). CH_3 1.82, H-1, 6.14 ; ^{31} P (TMP) : -3.65 (50 %) and -3.84 (50 %). TLC : 0.67 (isopropanol/NH_4OH/H_2O 7/1/2).

5b FAB MS : 716 (MNa^+). NMR (DMSO d_6): ^{1}H chain CH_3 0.87, $(CH_2)_n$ 1.25, $\underline{CH_2}$-CH_2-OP 1.59, CH_2-$\underline{CH_2}$-OP 4.00; mannose H-1 α 4.89, H-2 3.57, H-3 3.53, H-4 3.40, H-5 3.71, H-6 4.07, H-6' 4.20; nucleotide H-6 7.53 (45%) and 7.50 (55%) CH_3 1.81, H-1' 6.02. TLC : 0.63.

5c FAB MS : 735 (MNa^+). NMR (DMSO d_6) : ^{1}H chain CH_3 0.85, $(CH_2)_n$ 1.24, $\underline{CH_2}$-CH_2-OP 1.58, CH_2-$\underline{CH_2}$-OP 3.99 ; mannose H-1 4.90, H-2 3.57, H-3 3.53, H-4 3.41, H-5 3.71, H-6 4.06, H-6' 4.14 ; nucleotide H-6 7.88 (55 %) and 7.85 (55 %), H-1, 6.15 ; ^{31}P (TMP) : -3.45 (55 %) and -3.82 (45 %). TLC : 0.63.

11 a FAB MS : 776 (M^-). NMR (DMSO d_6) : ^{1}H chain CH_3 0.85, $(CH_2)_n$ 1.25, $\underline{CH_2}$-CH_2-OP 1.59, CH_2-$\underline{CH_2}$-OP 4.00 ; mannose H-1 4.69, H-2 3.63, H-3 3.48, H-4 3.35, H-5 3.42, H-6 and H-6' 3.50, $\underline{CH_2}$-CH_2-OP 3.59 and 3.79, CH_2-$\underline{CH_2}$-OP 4.18 ; nucleotide H-6 7.46, CH_3 1.80, H-1, 6.13 ; ^{31}P (TMP) : -3.65 (52 %) and -3.82 (48 %); TLC 0.69.

11b FAB MS : 759 (MNa^+) ; NMR (DMSO d_6): ^{1}H chain CH_3 0.87, $(CH_2)_n$ 1.25, $\underline{CH_2}$-CH_2-OP 1.59, CH_2-$\underline{CH_2}$-OP 4.00 ; mannose H-1 α 4.67, H-2 3.61, H-3 to H-6, H-6' 3.4 ± 0.1; $\underline{CH_2}$-CH_2-OP 3.60 and 3.75; CH_2-$\underline{CH_2}$-OP 4.13 ; nucleotide H-6 7.53 (52%) and 7.51 (48%), CH_3 1.81, H-1' 6.02. TLC 0.69.

11c FAB MS : 780 (MNa^+). NMR (DMSO d_6) : ^{1}H chain CH_3 0.89, $(CH_2)_n$ 1.25, $\underline{CH_2}$-CH_2-OP 1.60, CH_2-$\underline{CH_2}$-OP 3.95 ; mannose H-1 α 4.67, H-2 3.64, H-3 3.46, H-4 3.32, H-5-H-6, H-6' 3.45 ± 0.05 $\underline{CH_2}$-CH_2-OP 3.61 and 3.77 ; CH_2-$\underline{CH_2}$-OP 4.11 ; nucleotide H-6 7.90 (45 %) and 7.88 (55 %), H-1' 6.17 ; ^{31}P (TMP) : -3.57 (48 %) and -3.90 (52 %). TLC : 0.67.

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