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SYNTHESIS OF PHOSPHODIESTER AND TRIESTER DERIVATIVES OF AZT WITH TETHERED N-METHYL PIPERAZINE AND N,N,N' TRIMETHYLETHYLENEDIAMINE.

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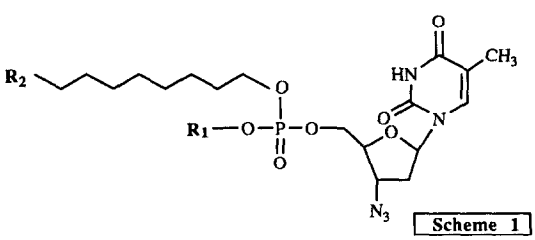
Abstract: N-methyl piperazine and N,N,N' trimethylethylenediamine have been linked to the octyl chain of phosphodiester and triester derivatives of AZT, in order to obtain compounds that may act as combined prodrugs of antiviral nucleoside and putative ribonuclease.

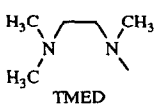
Numerous studies have been developed for prodrugs of antiviral agents that combine plasmatic resistance and good membrane permeability[1]. In our previous work, we have shown that the lipophilic glycosyl phophostriester derivatives of AZT favor the transport of the nucleoside analogue through the membrane bilayer and that it could be hydrolyzed *in situ* releasing the free nucleotide, precursor of the 5' triphosphate active form[2]. In an attempt to improve the pharmacological properties of the prodrug, several groups have inserted different molecules with potential synergistic anti-HIV activity in a phosphoester moiety[3]. With the same purpose, we have undertaken to synthesize phosphate esters derivatives of AZT, combined with tethered compounds able to promote the degradation of RNA viruses (Scheme 1).

Many metal complexes have been used to mimic the artificial restriction enzymes. These nuclease models cleave the phosphate esters through an oxidative[4] or through an hydrolytic[5] mechanism. The models resorting to the second mechanism, which act by a nucleophilic attack on phosphorus substrates, are more appealing as they do not need a redox cofactor ; furthermore, they might release fragments that could become ligated with other nucleotides.

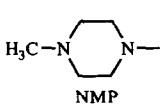
N,N,N' trimethylethylenediamine (TMED) forms complexes with copper salts which have demonstrated an hydrolytic activity toward some activated phosphate derivatives [6]. However, there was so far no example of their ability to catalyze the hydrolysis of a phosphodiester bond such as found in RNA.



Scheme 1



TMED



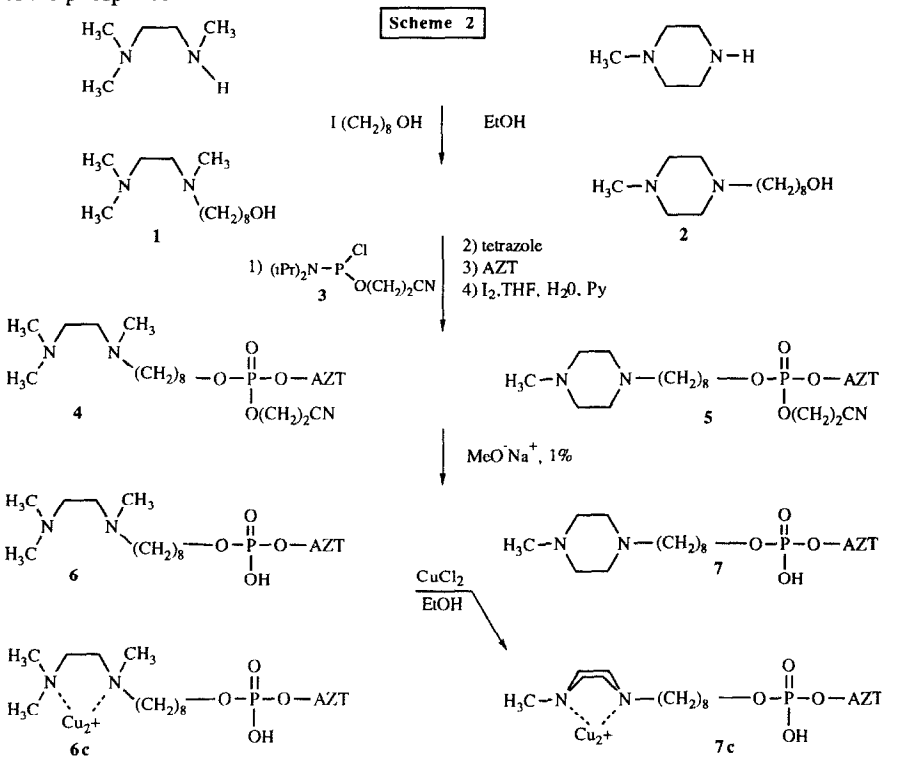
NMP

6: R_1 = H, R_2 = TMED
7: R_1 = H, R_2 = NMP
10: R_1 = 6-Glucopyranosyl, R_2 = NMP
11: R_1 = 6-Glucopyranosyl, R_2 = TMED

Concerning N-methyl piperazine-Cu(II) chelate, no data on the cleavage of a phosphoester bond was published up to now. In this paper, we present the synthesis of phosphoesters **6**, **7**, **10**, **11** (Scheme 1), and of some of their cationic complexes as potential bifunctionnal drugs associating a reverse transcriptase inhibition with a ribonuclease activity.

Both P(III) and P(V) chemistry have been used to synthetize phosphodiesters **6** and **7**. We shall restrict this paper to the description of the synthesis of the phosphodiester derivatives using the phosphoramidite approach[7]which proved successful for all our compounds albeit in low yields. As a first step, N,N,N'trimethyl-N'(8-hydroxyoctyl)-ethylenediamine **1** and N-methyl-N'(8-hydroxyoctyl) piperazine **2** were prepared by nucleophilic substitution of 1,8 iodooctanol as shown in Scheme 2. These alcohols were phosphitylated with β-cyanoethyldiisopropylchlorophosphoramidite reagent **3**. The intermediates were then activated by tetrazole and coupled with AZT. After an hour, the resulting products were oxidized by use of I_2/ THF/ pyridine/ H_2O to obtain the phosphotriesters **4** and **5**. These three steps were carried out as a one-pot reaction owing to the great instability of the phosphites.

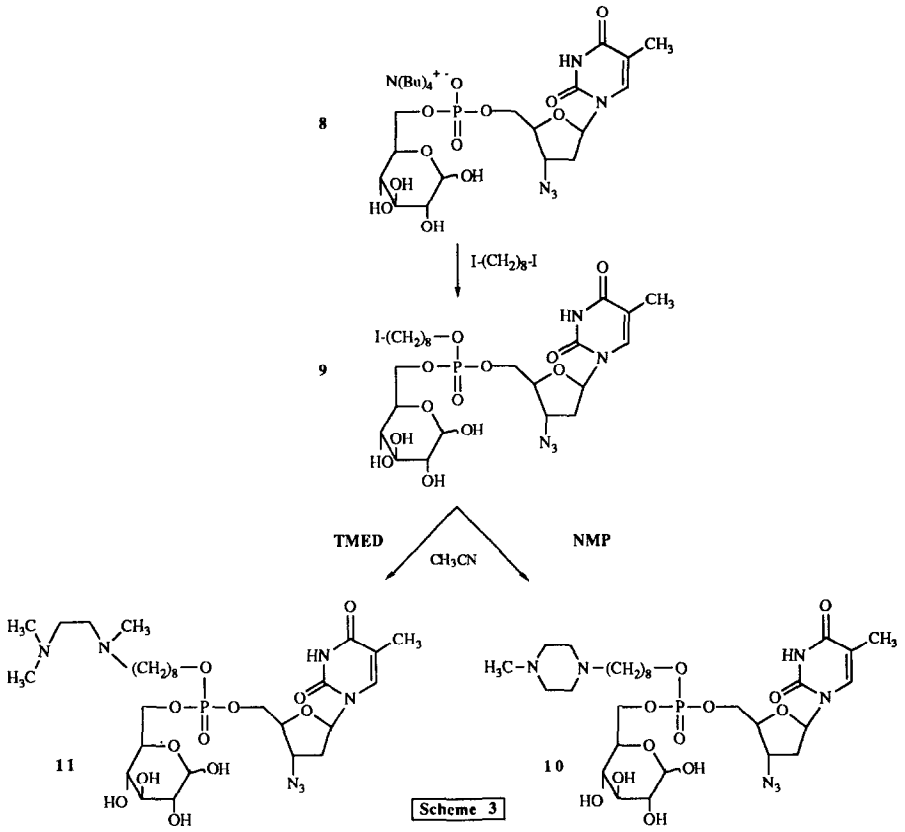
Scheme 2



After a short purification on a silica gel column, the β-cyanoethyl group was removed using a 1% solution of MeONa in MeOH. The diesters were purified by reverse phase chromatography to yield the expected products **6** (20 %) and **7** (22 %). These were characterized by NMR, mass spectroscopy and microanalysis[8].

According to previous papers[9], a convenient preparation of phosphotriester derivatives can be achieved by a nucleophilic substitution between the tetrabutyl ammonium salt of a diester and a halogeno compound.

However, the condensation of phosphodiester **6** and **7** with 6-iodo-glucose[10] was unsuccessful since we failed to obtain the tetrabutyl ammonium form of the diester. We then attempted to synthetize the N,N,N'trimethyl N'(8-iodooctyl) ethylenediamine and N-methyl N'(8-iodooctyl) piperazine in order to condense them with the tetrabutyl-ammonium form of the phosphodiester 6-D glucopyranosyl 5'-(3'-deoxy-3' azido) thymidinyl phosphate (AZT-G6P)[9] **8**. Unfortunatly, the reaction of TMED or NMP with the 1,8 diiodooctane did not give the expected products. Finally, we obtained the nucleophilic displacement of 1,8 diodooctane by the activated form of phosphodiester AZT-G6P into phosphotriester **9** (30% yield).The reaction of the phosphotriester **9** with TMED or NMP at room temperature gave the final products **11** and **10** with respectively 25 % and 41 % yields



The phosphotriesters were purified by reverse phase chromatography on RP-18 and by HPLC.Their structures were confirmed by mass and ^{1}H, ^{31}P and ^{13}C NMR spectroscopies[11]. Up to now, only the phosphodiesters **6** and **7** were precipitated by an ethanolic solution of copper chloride to give the corresponding complexes **6c** and **7c**. Electronic absorption spectroscopy (500-900 nm) was used to characterize the coordination of Cu(II) through TMED and NMP moieties. An aqueous solution of complexed TMED-diester **6c** absorbs at 700 nm which is the absorption of the Cu(II)-TMED itself, whereas $CuCl_2$ or cupric solution of AZT 5'-

monophosphate absorbs at 810 nm. On the other hand, no significative displacement of λ_{max} was observed for the Cu(II) complex of NMP-diester **7c** [12]. HPLC, TLC and UV spectra have been used to monitor the stability of the Cu(II) complexes of phosphodiesters and showed no self-degradation on a 5 day period.

The preliminary capillary electrophoresis assays of hydrolytic cleavage of oligoribonucleotides (25 or 28-mer) with **6c** showed encouraging results. They indicated that the Cu(II) complex of phosphodiester **6c** (10-75 molar excess) was able to promote the hydrolysis (75%) of an RNA fragment at neutral pH after 48 hours of incubation at 37°C. No cleavage occured with the ligand in the absence of copper ion or with a control DNA fragment under the same conditions[13]. Further studies are undertaken with the other complexes of di-and triesters **7c**, **10**, **11**, and attemps are made to identify the hydrolyzed nucleotides.

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8. Spectroscopic data of **6** : ^{1}H-NMR (300MH$_Z$,D$_2$0) :AZT : 1.8 (s,3H) ; 2.5 (m,4H) ; 3.9 (m,2H) ; 4.2 (m,H) ; 4.5 (m, 1H) ; 6.25 (t,1H) ; 7.8 (s,1H). TMED-chain: 1.4 (m,8H) ; 1.6 (m,4H) ; 2.6 (s,9H) ; 2.8 (m,2H) ; 3.0 (m,4H) ; 3.86 (q,2H) ; ^{31} P-NMR (121MH$_Z$,MeOD) ; 2.40. Anal. calcd. for (C$_{23}$ H$_{42}$ O$_7$ N$_7$ P, H$_2$O), CHON, 47.83, 7.70, 22.20, 16.90; found 48.26, 7.35, 22.06, 17.06. MS FAB$^+$ m/e 560.6 (M+1). HPLC: 14.1min (CH3CN / TEAA 0.01 M; pH=7; column: nucleosil C-18).
Spectroscopic data of **7** : ^{1}H-NMR (300 MH$_Z$.D$_2$0): AZT id. NMP-chain : 1.2 (m,8H) ; 1.6 (m,4H) ; 2.5 (s,3H) ; 2.8 (m,2H) ; 3-3.2 (m,8H) ; 3.8 (q,2H) ; ^{31} P-NMR (121MH$_Z$,D$_2$0); 1.00. Anal calcd for (C$_{23}$ H$_{40}$ O$_7$ N$_7$ P, 0.5 H$_2$O), CHN, 48.70, 17.30, 7.29, found 48.40, 17.20, 7.29. MS FAB$^+$ m/e 558.2 (M+1). HPLC: 12.9 min. (idem).
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10. Iodination of 1,2,3,4-tetra-0-acetyl-D-glucopyranose was performed using methyl triphenoxyphosphonium iodide according to Moffat J.G., Verheyden J.P.H., *J Org Chem* , **1970**, *35*, 7, 2319. MS (M+NH$_4^+$) 308; the 6-iodo glucose was then obtained by treatment with 1% sodium methylate solution with 30% overall yield.
11. Spectroscopic data of **11**: ^{1}H-NMR (300MH$_Z$,D$_2$0) : AZT ; 1.8 (s,3H) ; 2.5 (m,2H) ; 4.4 (m,3H) ; 4.5 (m,1H) ; 6.2 (m,1H) ; 7.6 (s,1H).glucose 5.2 (t,1H$_\alpha$) ; 4.8 (d,1H$_\beta$) ; 3.5 (m,2H) ; 3.6 (m,1H$_\beta$) ; 4 (2d,1H$_\alpha$) ; 4.2 (m,2H+1H$_\alpha$+1H$_\beta$). Chain-TMED: 1.3 (m,8H) ; 1.65 (m,4H) ; 2.7 (s,6H) ; 2.8 (s,3H) ; 3 (m,2H) ; 3.2-3.3 (m,4H). MS FAB$^+$ m/e 723 (M+ 1). Spectroscopic data of **10:** ^{1}H-NMR (300MH$_Z$,D$_2$0) ; AZT, glucose: id.NMP : 2.4 (s,3H) ; 2.7 (m,2H) ; 2.8-3.0 (m,8H). MS FAB$^+$ m/e 720.8 (M+1).
12 Microanalysis (Cl) and calibration by capillary electrophoresis (Cl$^-$ and Cu^{2+}) of **6c** and **7c** have confirmed the chelation of one cupric ion on these molecules.
13 The solution contained in a total volume of 100µl, 1.66 µM of synthetic RNA (or DNA), 16µM, 50µM, 83µM or 125µM of ligands (complexed or not) in 250 µM HEPES buffer (pH=7.3). A 50 µl aliquot was removed from the reaction and subjected to capillary electrophoresis to determine the percentage of cleavage of the RNA fragment.