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SYNTHESIS AND PROPERTIES OF PHOSPHODIESTER AND TRIESTER DERIVATIVES OF AZT WITH TETHERED POTENTIAL RIBONUCLEASES

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Abstract: The synthesis of phosphate ester derivatives of AZT with tethered N,N,N'trimethylethylenediamine, N-methylpiperazine and 2,6-diacetyl pyridine as prodrugs of AZT with potential ribonuclease activity was described. The metal complexes of these compounds should combine a reverse transcriptase inhibition and the ability to hydrolyze the RNA of HIV. Different phosphorus chemistries have been used to synthetize phosphodiesters. The synthesis of phosphotriesters was achieved by a nucleophilic displacement of the halogeno moieties by an activated phosphodiester in one or two chemical steps. The copper complexes of N,N,N'trimethylethylenediamine derivatives were fully characterized and their ribonuclease activity toward 25-mer and 28-mer oligoribonucleotides was demonstrated by capillary electrophoresis. The *in vitro* anti HIV preliminary assays revealed no synergistic effect with copper chelate on the activity of the prodrugs.

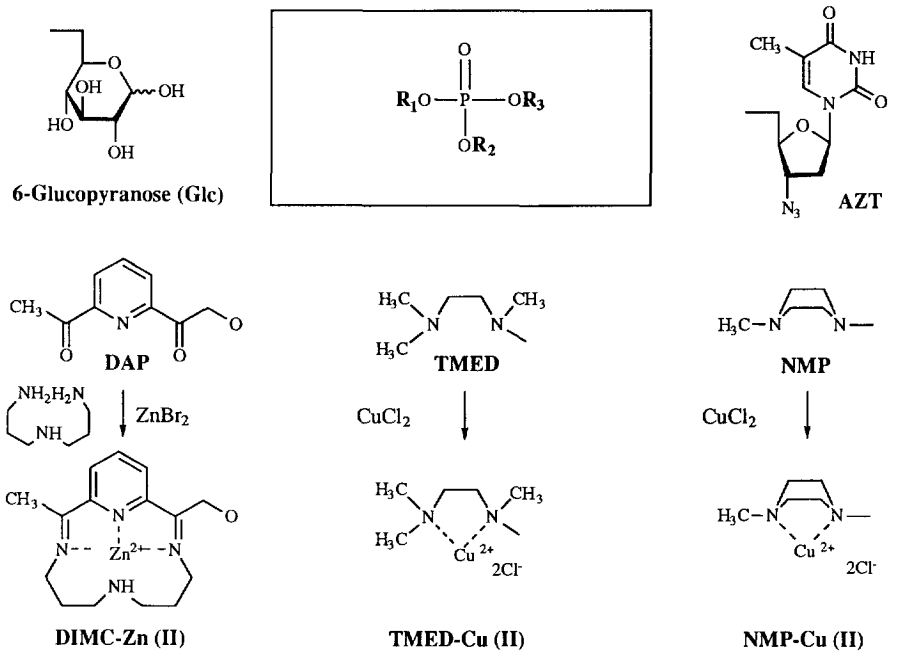
3'-Azido-3'-deoxythymidine (AZT) remains the most widely prescribed therapeutic agent in anti-HIV treatment[1-3], used alone or in combination with 2', 3'-dideoxyinosine (ddI) or 2', 3'-dideoxycytidine (ddC). However, its high toxicity[4,5] may restrict its oral administration to doses which do not permit an effective intracellular or brain concentration[6,7], and numerous studies have been developed to synthetize more lipophilic or brain-targeted prodrugs of this antiviral nucleoside[8-19]. We have previously described the synthesis of a glycosyl phosphotriester of AZT which demonstrated an *in vitro* transmembrane transport and an antiviral activity comparable to AZT[20]. We further demonstrated, with *in vivo* studies, that (i) this prodrug generated AZT-5'-phosphate as its main metabolite; (ii) the brain concentration of AZT derivatives, after an oral administration, was higher than that in mice serum[21].

As a development of this promising pro-drug concept, we anticipated that the introduction of other moieties with potential anti-HIV activity in the phosphoester would increase the antiviral effects. This combination therapy approach has already been described for antiviral nucleosides linked via a phosphate bridge and for a mixed phosphotriester of antiviral and antibiotic nucleosides[22,23]. In order to demonstrate the validity of this approach, we have attempted to synthetize phosphate ester derivatives of AZT with tethered ribonuclease models which could be able to promote the degradation of viral RNAs[33].

These potential ribonucleases are metallic ion complexes of N,N,N'-trimethylethylenediamine (TMED), N-methylpiperazine (NMP) and 2,6-diacetyl pyridine (DAP) (scheme 1). Metal complexes have been developed to mimic restriction enzymes ; most of them cleave the phosphate backbone of nucleic acids through an oxidative mechanism as the well-known copper complexes of *o*-phenanthroline or ferric complexes of EDTA[24,25]. Other chemical nucleases promote the degradation of unactivated phosphate esters through a hydrolytic process[26].

The models resorting to the last mechanism, which act by a nucleophilic attack on phosphorus substrates,[27-29] are appealing as they do not involve the use of a redox cofactor. Furthermore RNA is more susceptible than DNA to such hydrolytic reaction while the oxidative mechanism cleaves both RNA and DNA.

The complexed forms of TMED[30,31] and DAP[32] have demonstrated an hydrolytic activity vis-à-vis some activated phosphate derivatives. However, there was so far no example of their abilities to catalyze the hydrolysis of a phosphodiester bond such as found in RNA. No data were found in the literature concerning Cu(II)-NMP ability to degrade phosphate ester moiety.



Phosphodiester:

1 : R_1= $(CH_2)_8$DAP, R_2= H, R_3= AZT
2 : R_1= $(CH_2)_8$TMED, R_2= H, R_3= AZT
2c: R_1= $(CH_2)_8$TMED-Cu(II), R_2= H, R_3= AZT
3 : R_1= $(CH_2)_8$NMP, R_2= H, R_3= AZT
3c: R_1= $(CH_2)_8$NMP-Cu(II), R_2= H, R_3= AZT
4 : R_1= $(CH_2)_8$TMED, R_2= Glc, R_3=H
4c: R_1= $(CH_2)_8$TMED-Cu(II) , R_2= Glc, R_3= H
5 : R_1= $(CH_2)_8$NMP, R_2= Glc, R_3= H
6 : R_1= H, R_2= Glc, R_3= AZT

Phosphotriester:

7 : R_1= $(CH_2)_8$DAP, R_2= Glc, R_3= AZT
9 : R_1= $(CH_2)_8$NMP, R_2= Glc, R_3= AZT
8 : R_1= $(CH_2)_8$TMED, R_2= Glc, R_3= AZT
8c: R_1= $(CH_2)_8$TMED-Cu(II), R_2= Glc, R_3= AZT

scheme 1

RESULTS AND DISCUSSION

Chemistry

Our strategy was first to synthetize the mixed phosphodiesters chelate-AZT (**1**, **2**, **3**), AZT-Glc (**6**) and chelate-Glc (**4**, **5**) in order to alkylate them in a second step with the halogeno derivative of Glc, chelate or AZT respectively. This approach has proved to be successful during former synthesis in our laboratory[34], and moreover, provided the intermediary moieties that could be helpful for analyzing biological activity.

Phosphodiesters synthesis.

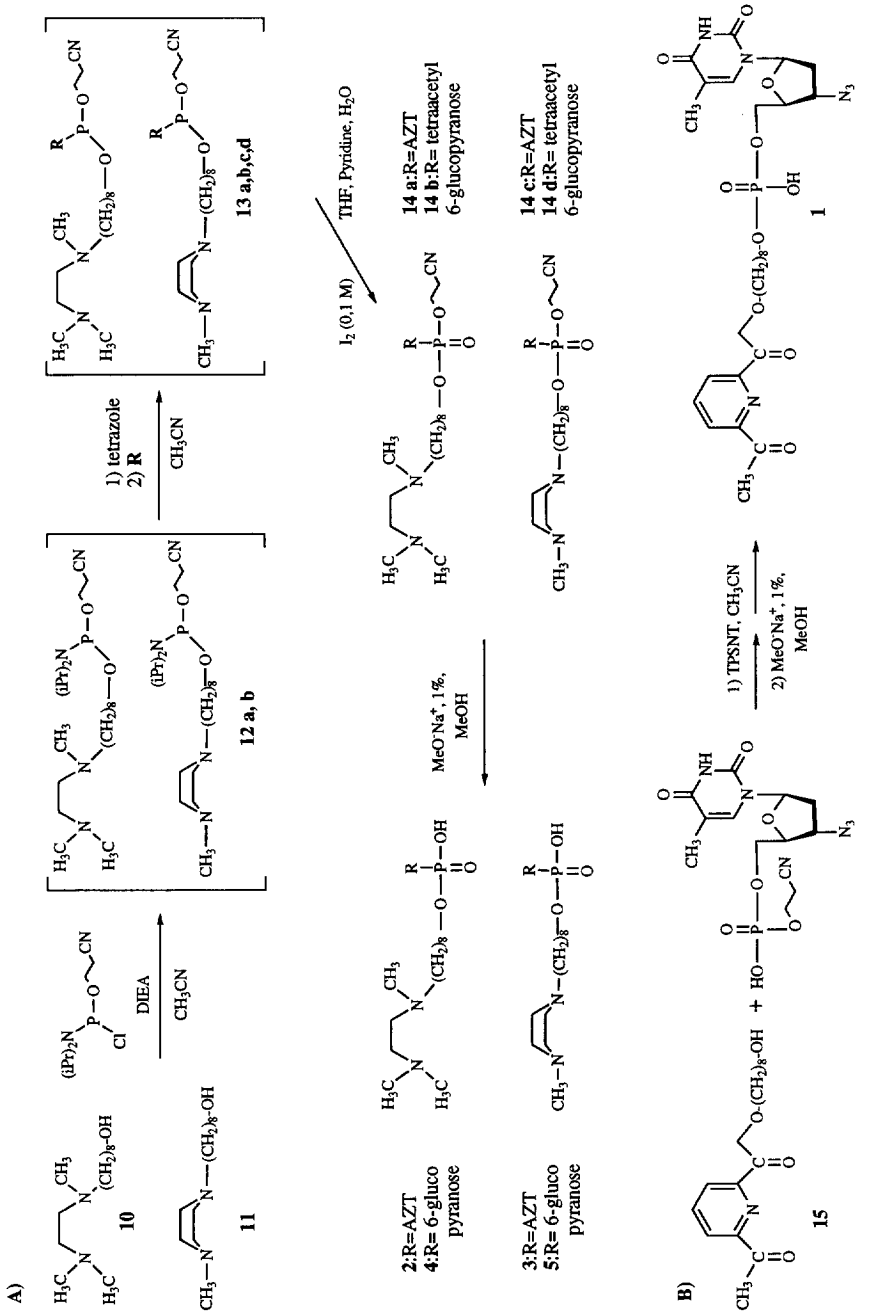
For the synthesis of the phosphodiester, several methods were attempted. The synthesis of diesters TMED-AZT **2**, TMED-Glc **4**, NMP-AZT **3** and NMP-Glc **5** using phosphoramidite chemistry offered the best yields. The alkyl chain derivatives N,N,N'-trimethyl-N'-(8-hydroxyoctyl)-ethylenediamine **10** and N-methyl-N'-(8-hydroxyoctyl)-piperazine **11** were obtained by reaction of TMED and NMP with bromooctanol in ethanol. They were then phosphitylated using β-cyanoethyl-diisopropyl-phosphoramidite[35] (A, scheme 2). This reaction gave **12a,b**, which upon activation with tetrazole, reacted with AZT or with the 1,2,3,4 acetylated 6-glucopyranose to yield phosphites **13a,b,c,d**. Their oxidation under standard conditions produced phosphotriesters **14a,b,c,d**. Because of the great instability of the intermediates **12** and **13**, these steps were carried out as a one-pot reaction. It should be noted that the inverse reaction sequence with addition of **10** and **11** to the phosphitylated nucleoside or protected carbohydrate did not give compounds **12a,b**. The basic treatment of **14a,b,c,d** led to the β elimination of the cyanoethyl residue as well as the complete loss of the acetyl protective groups, and gave, after neutralization, phosphodiesters **2**, **3**, **4**, **5** in overall yields of 23%, 20%, 15% and 15% respectively (from **10** and **11**). Their structures were confirmed by ^{1}H, ^{13}C and ^{31}P NMR spectroscopies.

NMP-AZT **3** was also obtained, with the same overall yield, by coupling N-methyl-N'-(8-hydroxyoctyl)-piperazine **11** with AZT-5'-cyanoethylphosphate in the presence of 2, 4, 6-triisopropylbenzene-sulfonyl-3-nitro-triazolide (TPSNT) as the activating agent according to phosphotriester DNA synthesis[36, 40].This P(V) chemistry approach allowed us to isolate the phosphodiester DAP-AZT **1** (B, scheme 2); the operational octyl chain derivative of diacetyl pyridine DAP-OH **15**, obtained after four steps[37], reacted with AZT-5'-cyanoethylphosphate and TPSNT to give the protected intermediate phosphotriester with a 33% yield. Unfortunately, the removal of the β-cyanoethyl with a triethylamine/ pyridine / water solution led to partial loss of DAP, thus explaining the low yield (23%) observed for the final product **1**. Thus, no attempt was made to obtain the phosphodiester associating the DAP-OH **15** with the carbohydrate.

Phosphodiester AZT-Glc **6** was synthetized by condensation of the protected glucosyl monophosphate with AZT in trichloroacetonitrile as described in a previous paper[34].

Phosphotriesters synthesis.

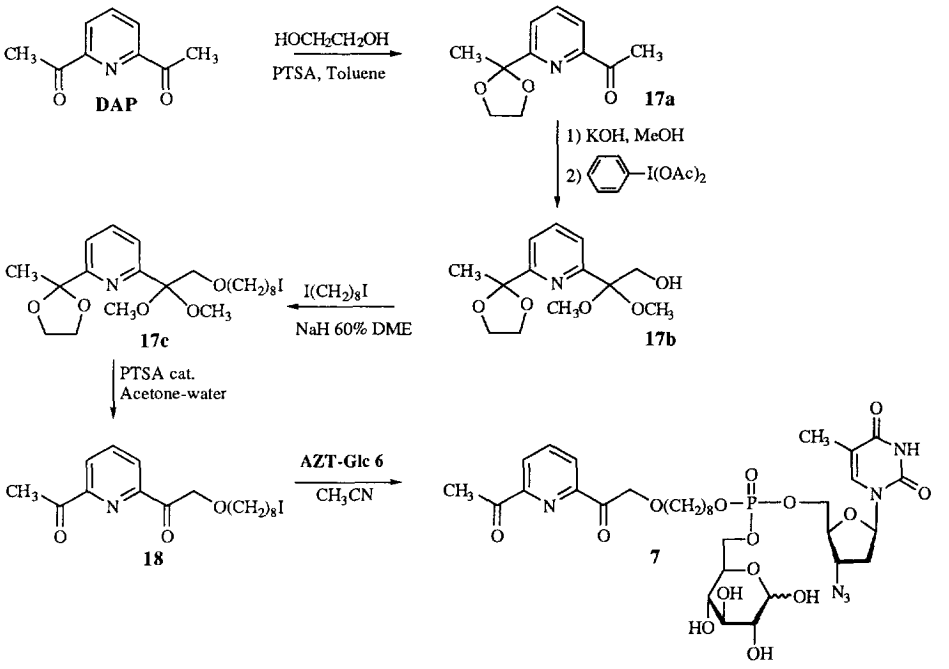
As demonstrated in the preliminary work, the preparation of phosphotriester derivatives can be achieved with a nucleophilic displacement of an halogeno compound by phosphodiester tetrabutylammonium salts. However, we did not to obtain the tetrabutylammonium form of phosphodiesters **2**, **3**, **4** and **5**: it seemed that these products remain adsorbed on the Dowex 50W cation exchange resin. We then attempted to synthetize N,N,N'-trimethyl-N'-(8-iodooctyl)-ethylenediamine and N-methyl-N'-(8-iodooctyl)-piperazine in order to condense them with the tetrabutylammonium salt form of the phosphodiester AZT-GLc **6**.


A)
10
11
DIEA
CH3CN
12 a, b
1) tetrazole
2) R
CH3CN
13 a,b,c,d
I2 (0.1 M)
THF, Pyridine, H2O
14 a:R=AZT
14 b:R= tetraacetyl
6-glucopyranose
14 c:R=AZT
14 d:R= tetraacetyl
6-glucopyranose
2:R=AZT
4:R= 6-gluco
pyranose
3:R=AZT
5:R= 6-gluco
pyranose
MeO-Na+, 1%,
MeOH
B)
15
1) TPSNT, CH3CN
2) MeO-Na+, 1%,
MeOH
1

scheme 2

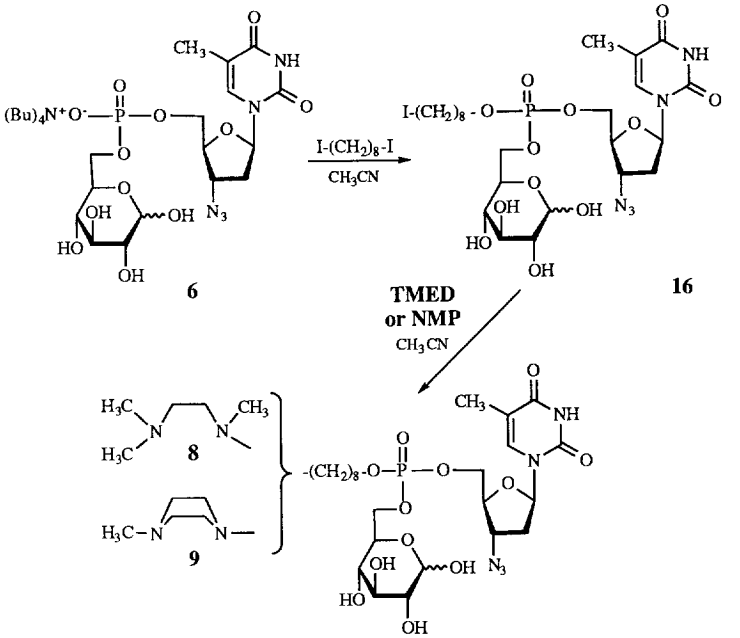
Unfortunately, we did not succeed in isolating the expected products of the reaction of TMED or NMP with 1,8-diiodooctane.

Conversely, the synthesis of DAP **18** iodo derivative could be achieved as shown in scheme 3, with an overall yield of 49%[37]. Reaction between the tetrabutylammonium salts of phosphodiester **6** and a five fold excess of **18** was performed overnight in acetonitrile at 80°C; phosphotriester **7** was then isolated after chromatography on silica gel and Sephadex LH 20 columns (35% yield). The structure of phosphotriester **7** was confirmed by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy.



scheme 3

As our initial strategy of synthesis has proved inappropriate for the phosphotriester derivatives of TMED and NMP, we decided to modify the chemical steps by introducing a supplementary reaction. In fact, we undertook the displacement of 1,8-diiodooctane by the activated form of phosphodiester **6** into phosphotriester **16** in boiling acetonitrile (scheme 4). Then, the reaction of phosphotriester **16** with TMED and NMP at room temperature gave the final phosphotriesters **8** and **9** which were purified by reverse phase chromatography on RP-18 silica and by HPLC. The structures of **8** and **9**, obtained with yields of 19% and 40% respectively, were confirmed by ^{1}H, ^{13}C and ^{31}P NMR spectra.



scheme 4

Formation and characterization of complexes

The Cu(II) complexes of TMED and NMP derivatives were easily obtained by precipitation of **2**, **3**, **4**, **8**, **10**, and **11** in the presence of one equivalent of copper chloride in ethanolic solution. In the case of DAP moiety, the creation of Zn(II) complexes implied the formation of a tetraaza macrocycle by addition, in ethanol, of 3,3'-diamino-di-n-propylamine on the diketone DAP[32] (scheme 1).

The Cu(II) complexes were studied by electronic absorption spectroscopy (500 to 900 nm). These measurements showed that all TMED-Cu(II) ligands in aqueous solution present a characteristic absorption at 690 nm, whereas $CuCl_2$ in same conditions absorbs nearly 800nm.These properties are summed up in table 1 (column A). To verify that the transition metal was not coordinated on the phosphate nor on the nucleoside moiety, we compared these results with the electronic absorption of aqueous solution of AZT-5'-phosphate or phosphodiester AZT-Glc **6** and copper chloride in equimolar amount; the λ_{max} values observed were the same that those given by copper chloride alone (λ_{max} = 820 nm, ε = 12). On the opposite, the addition of an aqueous solution of $CuCl_2$ to a solution of phosphodiester **2** caused an absorption shift at 700nm. Accordingly, it can be concluded that the coordination of the transition metal take place through the TMED moiety. The presence of a unique equivalent of copper chloride salt by TMED ligand was confirmed by elemental analysis for TMED-Cu(II)-AZT **2c** and TMED-Cu(II) **10c** and by capillary electrophoresis quantitative analysis.

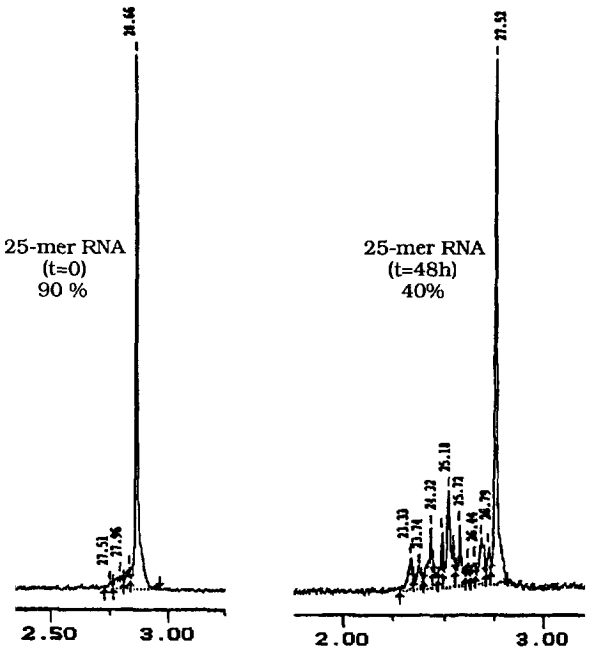
Complexes	A Electronic absorption characteristics		B % of oligonucleotides hydrolyzed by 83μM of complexes		
	λ_{max} (nm)	ε = OD/C.l ($M^{-1}cm^{-1}$)	RNA (1.66μM) 25-mer	28-mer	DNA(1.66μM) 25-mer
$CuCl_2$	810	13	49	75	0
Tetramethylethylenediamine -Cu(II)	680	32	64	85	0
TMED-OH-Cu(II) **10c**	690	30	22	nd	0
TMED-AZT-Cu(II) **2c**	690	37	56	72	0
TMED-AZT-Glc-Cu(II) **8c**	690	55	nd	80	0
TMED-Glc-Cu(II) **4c**	690	41	nd	79	0
TMED-AZT **2** + $CuCl_2$	690	42	51	nd	0
TMED-AZT **2**	-	-	0	0	0

Table 1: *properties of complexes. Column A: spectroscopic characteristics of complexes in water (range of 500-900 nm.), column B: percentage of oligonucleotides hydrolyzed after 48 hours of incubation with 83 μM of complexes at 37°C and neutral pH. The cleavage percentage reported is the mean of three reactions (±5%). Nd: values not determined .*

An interesting point is that no such data were obtained with NMP-copper complexes **11c** and **3c** with absorption values of λ_{max} = 790 nm (ε = 3) and λ_{max} = 810 nm (ε = 7) respectively. Despite correct elemental analysis of complex **3c**, no significant difference of spectroscopic absorption between copper chloride and **3c** was observed. Therefore, the experiment with copper phosphotriester chelate was not carried out.
Concerning the Zn complexes, we encountered the same problem we have already mentioned *ie* the loss of the tetraaza macrocycle occurring during the creation of Zn chelate. Consequently, the amount of Zn complex of purified phosphotriester **7** was insufficient to be fully characterized.
As a conclusion, the TMED derivatives **2c**, **4c**, **8c** and **10c** were the only compounds to give satisfactory results, thus allowing us to continue our study. HPLC, TLC, ^{13}C NMR and UV spectra have been used to monitor the stability of TMED-Cu(II) complexes. They exhibited no self-degradation on a 5 day period of incubation in water at neutral pH and at 37°C (data not shown).

Cleavage of RNA by Cu(II) complexes 2c, 4c, 8c and 10c.

The hydrolytic activity of complexes **2c**, **4c**, **8c** and **10c** was tested on synthetic RNA fragments. The 25-mer or 28-mer RNA at the final concentration of 1.66μM and various concentrations, from 1.66μM to 166μM, of Cu(II) complexes **10c**, **2c**, **4c** and **8c** were incubated at 37°C and neutral pH for 24 hours (data not shown) and 48 hours. The oligomer degradation was monitored by capillary electrophoresis. Typical examples of capillary electrophoresis analysis were shown in scheme 5.



A

Scheme 5:

A -

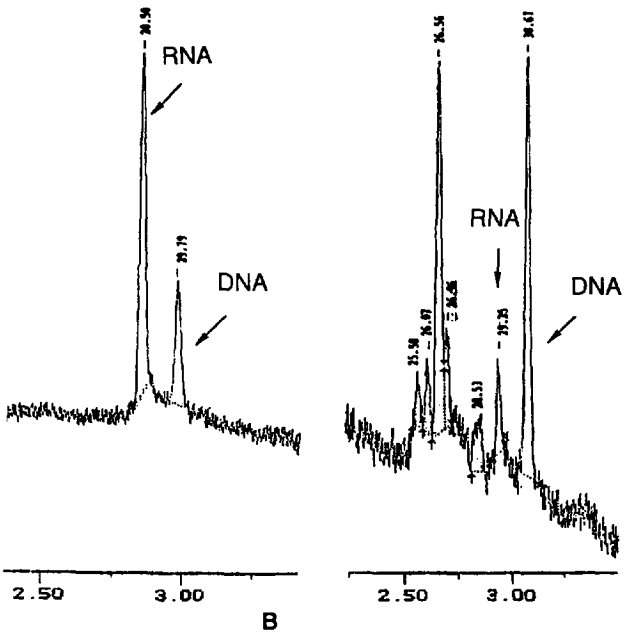
Capillary electrophoresis profiles of the RNA 25-mer (1.66μM) in the presence of 83 μM of TMED-AZT-Cu(II) 2c complex, at initial time (left) and after 48 hours (right) of incubation at 37°C and neutral pH.

B -

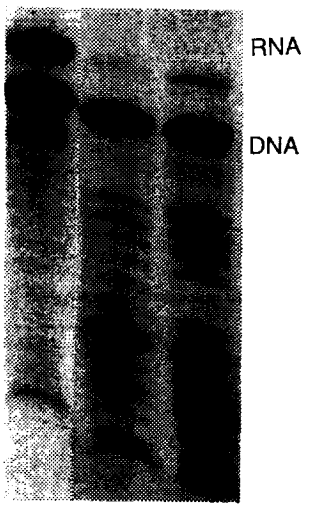
Capillary electrophoresis profiles of RNA 28-mer and DNA 25-mer in the absence (left) and in the presence (right) of 1.66μM of TMED-AZT-Cu(II) 2c complex, after 48 hours of incubation at 37°C and neutral pH.

C-

Scanned autoradiography of the end-labeled aliquots from experiment B. Polyacryl-amide electrophoresis gel of RNA 28-mer (0.05 OD) and DNA 25-mer (0.01), after 48 hours of incubation at 37°C and neutral pH, without copper chelate (a) and with 83μM (b) or 166μM (c) of TMED-AZT-Cu(II) 2c complex.



B



C

This method allowed to demonstrate with a high reproducibility that all metal compounds are able to cleave RNA after 48 hours of incubation at 37°C and neutral pH, with the best rates of degradation as soon as a 83 µM concentration of metal chelate compounds is reached. The percentages measured with each complex at this concentration are contained in table 1, column B. From these values, it appeared that (i) copper chloride alone hydrolyzed RNA fragments as previously described[28, 29], (ii) copper complexes **2c**, **4c** and **8c** cleaved ribonucleotidic fragments as well as $CuCl_2$ alone, but, (iii) their activity could not be attributed to the possible presence of free copper ion since no trace of them had been observed in elemental analysis. Control reaction run in the absence of $CuCl_2$, or in the presence of the phosphodiester **2** only, showed no noticeable RNA degradation in comparison with the RNA alone in solution after 48 H of incubation (near 5% in the two experiments).

It can be noted, on one hand, that different values of degradation were observed between the 25-mer RNA and 28-mer RNA (table 1) , and that, on the other hand, the hydrolysis of 28-mer RNA yielded major fragments (scheme 5). These observations are interesting, knowing that the 25-mer is a single-strand RNA while the 28-mer RNA may fold back on itself and may then present a tertiary conformation of triple helice. The base-paired helice is probably less sensitive to cleavage than the single-strand loop. Consequently, the amount of hydrolysis appeared to be dependent of the oligoribonucleotide conformation .

The values observed for Cu(II) complexes **2c**, **4c** and **8c** were similar to the value obtained with copper chloride alone. Thus the chelation of copper by the TMED ligand does not enhance the cleavage property of the transition metal. Regarding the compounds **10c**, the percentage of degradation was even lower than the percentage obtained with the copper salts alone (22% versus 49% for **2c**). According to Guftavson and Martell[30], this low hydrolytic rates could be explained by possible additional coordination of the copper ion by the hydroxy alkyl group which might decrease the catalytic power of the metal[30].

In a recent publication, Modak et al[38] also reported varying degrees of activity of different Cu(II)-bipyridine complexes attached to a nucleoside towards a homopolymer poly A $_{12\text{-}18}$; some of these complexes presented lower hydrolytic activity than $CuCl_2$ or free Cu(II)-bipyridine complex themselves. The authors suggested that this attenuation of reactivity was due to steric constraints. In our study, tethering the TMED-Cu(II) complexe to phosphodiester or triester seemed to have no significant influence on its hydrolytic capacity but, in contrast with Modak's findings, none of our models showed enhancement of the catalytic activity in comparison with $CuCl_2$. Since Cu(II) ions could not cross a lipophilic membrane, the synthetized phosphotriester complexes could be however a convenient way to introduce Cu(II) inside cells in order to obtain an hydrolytic effect.

It is noteworthy that in identical test conditions, TMED-Cu(II) complexes were unable to degrade a 25-mer DNA substrate. This observation suggests that the reaction of degradation proceeds through an hydrolytic mechanism rather than an oxidative one[39]. In order to confirm this idea, we proceeded to an end-labeling of the cleaved fragments obtained after 48 hours of incubation with **2c** or $CuCl_2$. Referring to known hydrolytic mechanisms[28,29], the existence of free 5'-OH extremity is an argument in favor of this cleavage process. We used the polynucleotide T4 kinase to add ^{32}P radioactive phosphate moiety to free 5'-OH ends of our nucleotidic molecules and the results of the labeling were visualized by autoradiography. The polyacrylamide electrophoresis gel obtained (scheme 5), clearly exhibited the existence of 5'-labeled fragments. Consequently, a hydrolytic mechanism of the cleavage could be assumed.

Antiviral Activity

The antiviral activity of phosphodiesters **2**, **2c**, **3**, **4**, **4c** and phosphotriesters **8**, **8c**, **7** was measured on CEM 4 lymphocytic cell lines infected with HIV-1 (Lai strain). To estimate the antiviral activity of these compounds, we studied (i) inhibition of the HIV-1 induced cytophatic effects (CPE) using the MTT cell viability to determine the CD_{50} and ED_{50} and (ii) the reverse transcriptase (RT) activity in culture supernatants that determine the ED_{50RT}. We report in table 2 the calculated values of the selectivity index SI (ratio CD_{50} / ED_{50}).

On day 6 post-infection, AZT, the reference compound, exhibited a CD_{50} equal to 5μM and a ED_{50} equal to 0.002μM (SI: 2500). In comparison, the complexed trimethylethylenediamine exhibited no antiviral activity with a CD_{50} superior to 500μM and a ED_{50} equal to 250μM. The phosphodiesters **2**, **2c**, **3**, **4**, **4c** and phosphotriesters **8**, **8c**, **7** displayed various patterns of antiviral activity (ED_{50} from 0.07 to 1μM). However, owing to a very low toxicity (50-100 times less than AZT), the non-complexed phosphodiesters **2** and **3** or phosphotriester **8** exhibited an identical or even better selectivity index than AZT (table 2). Concerning the phosphodiester TMED-Glc **4**, a low SI value (250) was calculated, despite a lack of cytotoxicity. These biological datas indicated that the antiviral effect is related to AZT (or its monophosphate) release. This anti-HIV activity could be dependent on the nature of the tethered amine if we compared the SI of phosphotriester **8** (3571) and phosphotriester **7** (312).

The presence of the metal ion appeared to increase the toxicity of the chelated compounds **2c**, **4c**, **8c**, accounting for the lower values of SI observed. Consequently, the synergistic effect of the combination of the chemical ribonuclease and the reverse transcriptase inhibitor could not be showed in these *in vitro* models.

Compounds	MTT Dosage (μM)			RT Dosage(μM)	
	CD50	ED50	SI	ED50	SI
Nevirapine	> 30	0.02	1500	0.03	1000
AZT	> 5	0.002	2500	0.003	1667
Tetramethylethylene diamine-Cu(II)	> 50	250	-	-	-
TMED-AZT **2**	> 250	0.11	2273	0.2	1250
TMED-Glc **4**	> 250	1	250	1	250
TMED-AZT-Glc **8**	> 250	0.07	3571	0.05	5000
TMED-AZT-Cu(II) **2c**	> 50	0.15	333	0.08	625
TMED-Glc-Cu(II) **4c**	90	0.8	112	1	90
TMED-AZT-Glc-Cu(II) **8c**	> 50	0.13	385	0.2	250
NMP-AZT **3**	> 500	0.23	2174	0.4	1250
DAP-AZT-Glc **7**	> 250	0.8	312	2	125

Table 2: *Comparative in vitro anti-HIV selectivity index (SI) of phosphodiesters and triesters compounds. SI: ratio of concentration inducing 50% cell viability (CD50) inhibition measured by the MTT method in CEM 4 after 6 days and an effective antiviral concentration producing 50 % reduction of HIV induced cytopathic effect (ED 50) measured by the MTT method or by RT method in CEM 4 after 6 days.*

CONCLUSION

Both P(III) and P(V) chemistry have been used to synthetize the different phosphodiesters **1**, **2**, **3**, **4**, **5** and **6**. Contrary to our expectations, most of these moieties could not be used for the synthesis of phosphotriesters. We finally started from the same activated phosphodiester AZT-Glc **6** to prepare the final phosphotriesters **7**, **8** and **9** in one or two chemical steps.

Among the different metal complexes isolated, only TMED derivatives exhibited no ambiguity concerning the coordination of the metal ion on the ligand. By capillary electrophoresis, we demonstrated with a high reproducibility that these Cu(II)-TMED complexes (**2c**, **4c**, **8c** and **10c**) were able to promote the cleavage of oligoribonucleotides (25-mer and 28-mer) in physiological conditions, probably through a hydrolytic mechanism.

Preliminary *in vitro* assays showed that this ribonuclease-like activity did not bring an increase of antiviral activity of the mixed phosphoester in standard test conditions. Our works provided however some indications concerning the use of chemical nucleases with therapeutic objectives. Additonal experiments should be undertaken to determine whether Cu(II)-TMED complexes actually reduce viral RNA proliferation *in vivo*.

EXPERIMENTAL SECTION

Dichloromethane was distilled from sodium carbonate; pyridine was twice distilled from calcium hydride and *p*-tolylsulfonyl-chloride. AZT was prepared according to the literature[41]. Glucose 6-phosphate, N,N-diisopropylchlorophosphoramidite, cyanoethyl phosphate, trichloroacetonitrile, 1,8 bromooctanol, 1,8 diiodooctane and 2,6-diacetyl pyridine were purchased from Aldrich. N,N,N'-trimethylethylenediamine and N-methylpiperazine were purchased from Sigma. Merck silica gel plates ($60F_{254}$) were used for analytical thin-layer chromatography and the spots were examined with UV light and anisaldehyde-sulfuric acid or phosphomolybdic acid sprays. Chromatographic separations were carried out on 230-400 mesh Merck silica gel and Pharmacia Sephadex LH20 or G10. UV spectroscopy was carried out using a Perkin-Elmer 550 S spectrophotometer. Mass spectra were obtained using a VG70-250 instrument. Analytical HPLC were performed (i) on a Perkin-Elmer 3B series, equiped with a LC75 detector and a Nucleosil C_{18} column (5µm) with gradients of acetonitrile in 0.01 M triethylammonium acetate (pH=7), (ii) on a Perkin-Elmer 410 series with a SEDEX 45 detector. Capillary electrophoresis was carried out using a Waters Quanta 4000 Millipore. NMR spectra were recorded on a Bruker AC 300 Spectrometer. Capillary electrophoresis data were acquired on NEC Power mate Sx/16 computer and UV detector. Extensive precautions were taken to avoid ribonuclease contamination in the hydrolysis reaction. All buffers were made with distilled-deionized water.

1-(11-(2-Acetyl 6-pyridinyl) 10-one 8-oxa) undecanyl, 3'-azido-3'-deoxy- 5'-thymidinyl phosphate, **DAP-AZT 1**

TPSNT (530mg) was added to a mixture of AZT-5'-cyanoethylphosphate (270mg, 0.65 mmol) and **15** (200mg, 1eq) in 10 mL of anhydrous pyridine. After 3 hours at room temperature, the solution was evaporated to dryness. The residue was dissolved in dichloromethane and washed with $NaHCO_3$ and water successively. The organic layer was dried over $MgSO_4$, filtered and evaporated. The intermediate phosphotriester was chromatographed on silica gel column eluted with dichloromethane-methanol and deprotected by treatment with a ternary solution of triethylamine / pyridine / water (1:3:1) . After one hour, a TLC showed that all the starting

material (Rf: 0.26 ; MeOH/ CH_2Cl_2, 20:80) had disappeared. Lyophilization and chromatographies on G10 and reverse phase C_{18} columns gave 27mg (23% yield) of the final product **1**. Rf: 0.59 (iPrOH/NH_4OH/ H_2O, 6:1:2). HPLC: 17.3 mn (grad 5-50), 100%. MS(FAB$^+$)= 637.3 ($M+H^+$), 659.4 ($M+Na^+$); 675 ($M+K^+$); ^1H NMR (D_2O), δ (ppm): 1.1 (s, 8H); 1.6 (m, 4H); 1.8 (s, 3H); 2.4 (m, 2H); 2.7 (s, 3H); 3.6 (t, 2H); 3.9 (m, 2H); 4 (m, 3H); 4.25 (m, 1H); 5.15 (s, 2H); 6.15 (t, 1H); 7.7 (s, 1H); 8.05 (m, 3H).^{13}C NMR (D_2O),δ (ppm): AZT; 166.8, 152.7, 137.9, 85.4, 83.8, 83.7, 67, 66.9, 61.1, 37.1, 12.4. DAP and chain: 199.6, 152, 152.2, 139.7, 126.7, 125.9, 73.6, 72.4, 65.4, 30.4, 30.3, 29.1 to 25.7 (five carbons), 25.5.

8-(N,N,N'-trimethylethylenediamine) N'-octyl, 3'-azido-3'-deoxy- 5'-thymidinyl phosphate, ***TMED-AZT 2.***
To a solution of N,N,N'-trimethyl-N'(8-hydroxyoctyl)-ethylenediamine **10** (344 mg, 1.5mmol) in 10mL of dry acetonitrile were added diisopropylethylamine (506 µL, 1.7 eq) and β-cyanoethyldiisopropyl-chlorophosphoramidite (598 µL, 1.7 eq) at room temperature. Approximatively 15 mn after addition of the phosphitylating agent, a TLC control (MeOH/ CH_2Cl_2 , 20:80) showed the complete conversion of **10** into **12a**. A mixture of tetrazole (525 mg, 5eq) and AZT (1.5 mmol) was added and the solution stirred at room temperature. A TLC control showed complete conversion of **12a** into **13a** (MeOH/ CH_2Cl_2 , 20:80), which was directly oxidized by addition of 40mL of the standard 0.1 M iodine reagent (I_2, pyridine, THF, H_2O). After 15mn, the mixture was evaporated in vacuo. The residue was then triturated in petroleum ether. The brown precipitate was purified on a silica gel column eluted with dichloromethane-MeOH to give **14a**.Rf: 0.4 (MeOH/ CH_2Cl_2, 20:80).^1H NMR (DMSO d_6), δ (ppm): 1.2 (s, 8H); 1.4 (m, 4H); 1.8 (s, 3H); 2.5 (2s + m, 11H); 2.8 (m, 2H); 2.9 (t, 2H); 3.0 (m, 4H); 4.0-4.3 (m, 7H); 4.4 (m, 1H); 6.1 (t, 1H); 7.75 (s, 1H).
Phosphotriester **14a** was dissolved in a 1% solution of sodium methoxide in methanol and stirred at room temperature for 15mn. After neutralization of the resulting mixture with Dowex 50 W (H^+ form), the resin was filtered, washed with methanol and water and the filtrate lyophilized to a white solid which was first chromatographed on Sephadex G10 eluted with water and then on reverse phase C_{18} using a gradient water/MeOH. 180 mg (23%) of phosphodiester **2** were isolated (hygroscopic). Rf: 0.58 (iPrOH/NH_4OH/ H_2O, 7:1:2). HPLC: 14.1 mn (grad 5-50), 100%. MS(FAB$^+$)= 560.6 ($M+H^+$), 582.8 ($M+Na^+$); 604.5 ($M+2Na^+-H^+$); ^1H NMR (MeOD), δ (ppm): 1.4 (m, 8H); 1.7 (m, 4H); 1.9 (s, 3H); 2.3-2.5 (m, 2H); 2.6 (s, 9H); 2.85 (m, 2H); 3.05 (m, 4H); 3.9 (q, 2H); 4.1 (m, 3H); 4.5 (m, 1H); 6.25 (t, 1H); 7.8 (s, 1H).^{13}C NMR (MeOD),δ (ppm): AZT; 166.4, 152.4, 138, 112, 85.8, 84.8, 84.7, 66.7, 66.6, 62.5, 38, 12.7. TMED and chain: 66.1, 66.05, 58.35, 54.8, 53.2, 44.6, 44.3, 31.6, 31.5, 30.1 to 26.2 (five carbons).^{31}P NMR (MeOD),δ (ppm):+ 2.4. Anal. ($C_{23}N_7H_{42}O_7P$, H_2O) C, H, N: calcd 47.83, 7.70, 16.9; found 48.19, 7.35, 17.06.

8-(N-methyl piperazine) N'-octyl, 3'-azido-3'-deoxy- 5'-thymidinyl phosphate, ***NMP-AZT 3.***
280mg of phosphodiester NMP-AZT **3** was prepared as described above for the phosphodiester **2**.
14c: Rf: 0.33 (MeOH/ CH_2Cl_2, 20:80).^1H NMR ($CDCl_3$), δ (ppm): 1.3 (s, 8H); 1.7 (m, 4H); 1.9 (s, 3H); 2.4 (m, 2H); 2.6 (s, 3H); 2.8 (m, 4H); 3.0-3.2 (broad, 8H); 4.15 (m, 2H); 4.2-4.4 (m, 6H); 6.2 (q, 1H); 7.4 (d, 1H). ^{31}P NMR (DMSO d_6),δ (ppm):+ 1.4, 1.34. **3**: 20% yield. Rf: 0.67 (iPrOH/NH_4OH/ H_2O, 7:1:2). HPLC: 9.2 mn (grad 5-25), 100%. MS(FAB$^+$)= 558.2 ($M+H^+$); ^1H NMR (D_2O), δ (ppm): 1.2 (s, 8H); 1.6 (m, 4H); 1.9 (s, 3H); 2.5 (m, 3H); 2.7-3.2 (m, 10H); 3.8 (q, 2H); 4.1 (m, 3H); 4.5 (q, 1H); 6.25 (t, 1H); 7.75 (s, 1H).^{13}C NMR (D_2O),δ (ppm): AZT; 167.1, 152.3, 138.2, 112.3, 85.5, 83.8, 83.7, 67.1, 67, 61.2, 37.07, 12.5. NMP and chain: 65.6, 65.5, 57.5, 52.4, 51.3, 44.2, 30.46 to 26.2 (five carbons).

^{31}P NMR (D_2O),δ (ppm):+ 1.0. Anal. ($C_{23}N_7H_{40}O_7P$, $0.5H_2O$) C, H, N: calcd 48.7, 17.3, 7.29; found 48.4, 17.2, 7.29.

8-(N,N,N'-trimethylethylenediamine) N'-octyl, 6-D glucopyranosyl phosphate, ***TMED-GLC 4***

The procedure described below for phosphodiester **5,** from **10** and 1,2,3,4-tetraacetyl glucopyranose, was used for the synthesis of **4**. After column chromatography on G10 and reverse phase C_{18}, phosphodiester **4** was isolated with 15% overall yield. Rf: 0.4 (iPrOH/NH_4OH/ H_2O, 7:1:2). MS(FAB+)= 473.5 (M+H+), 495.5 (M+Na+); ^1H NMR (D_2O), δ (ppm): 1.4 (s, 8H); 1.6 (m, 4H); 2.4 (s, 6H); 2.6 (s, 3H); 2.9 (t, 4H); 3.1 (m, 2H); 3.25 (t, Hβ); 3.6-3.42 (m, 3H); 3.7 (t,1H); 3.8-4.15 (m, 5H), 4.6 (d, 1H); 5.1 (d, 1H). ^{13}C NMR (D_2O),δ (ppm): 96.7, 92.9,76.3, 75.5, 75.4, 74.8, 73.3, 72.2, 71.2, 71.1, 70.0, 69.9, 67.2, 67.1, 65.1, 64.9, 57.3, 53.5, 52.5, 44.3, 41, 30.9, 30.4, 28.7, 28.6, 26.5, 25.4, 24.6. ^{31}P NMR (D_2O),δ (ppm):+ 1.6. Anal. ($C_{19}N_2H_{41}O_9P$, $1.5H_2O$) C, H, N: calcd 45.68, 8.87, 5.60; found 45.65, 8.69, 5.13.

8-(N-methyl piperazine) N'-octyl, 6-D glucopyranosyl phosphate, ***NMP-GLC 5****.*

A solution of N-methyl-N'(8-hydroxyoctyl)-piperazine **11** (460mg, 2mmol), diisopropylethylamine (703µL, 4mmol) and β-cyanoethyldiisopropylchloro phosphoramidite (673µL, 3mmol) in 10mL of dry acetonitrile was stirred 15mn at room temperature. A TLC control showed the transformation of the starting product into **12b** (MeOH/ CH_2Cl_2, 20:80). Tetrazole (700mg, 5eq) and 1,2,3,4 tetraacetyl glucopyranose (2mmol) were added to the mixture. After one hour at room temperature, another TLC indicated that **12b** had completely reacted to give **13d** (Rf = 0.56) which was then oxidized by addition of the standard 0.1 M iodine reagent. After 15mn, dichloromethane was added and the mixture was washed twice with a 20% solution of sodium hydrogen sulfite and with water. The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was triturated in petroleum ether. The crude product **14d** was directly hydrolyzed with a 1% solution of sodium methoxide in methanol and stirred at room temperature for 15mn. After neutralization of the resulting mixture with Dowex 50 W (H^+ form), the resin was filtered, washed with methanol and water. The filtrate were lyophilized. The resulting solid was chromatographed on Sephadex G10 eluted with water and reverse phase C_{18} using a gradient water/MeOH. 145 mg (15%) of phosphodiester **5** were isolated (hygroscopic). Rf: 0.3 (iPrOH/NH_4OH/ H_2O, 7:1:2). MS(FAB+)= 470.3 (M+H+), 493.3 (M+Na+); ^1H NMR (D_2O), δ (ppm): 1.4 (s, 8H); 1.7 (m, 4H); 2.5 (s, 3H); 2.9-2.8 (m, 10H); 3.3-4.2 (m); 4.8 (d, 1Hβ); 5.1 (d, 1Hα); ^{13}C NMR (D_2O),δ (ppm): 96.7, 92.9,76.3, 75.5, 75.4, 74.8, 73.3, 72.2, 71.2, 71.1, 70.0, 69.9, 67.2, 67.1, 65.1, 57.5, 52.3, 51.3, 44.3, 30.4, 30.3, 28.7, 28.6, 26.5, 25.4, 24.6.^{31}P NMR (D_2O),δ (ppm):+ 1.6. Anal. ($C_{19}N_2H_{39}O_9P$, $2H_2O$) C, H, N: calcd 47.59, 8.34, 5.82; found 47.55, 8.10, 5.82.

1-(11-(2-acetyl 6-pyridinyl) 10-one 8-oxa) undecanyl, 3'-azido-3'-deoxy- 5'-thymidinyl phosphate, 6-D glucopyranosyl phosphate ***DAP-AZT-Glc 7***

Dowex 50 W cation exchange resin (n-$C_4H_9)_4N^+$ form was added to a solution of **6** (500mg, 0.66 mmol) in water, and the mixture was stirred for one hour at room temperature. After filtration, the filtrate was lyophilized to a white solid. The tetrabutylammonium salt of the phosphodiester **6** was dissolved in anhydrous acetonitrile, **18** was added in excess and the mixture was heated overnight at 80°C. The solvent was evaporated in vacuo and the residue was triturated in petroleum ether in order to eliminate the excess of **18.** It was then fractionned on a column of silica gel eluting with dichloromethane-methanol and chromatographed on a column of Sephadex LH20 with THF as eluent to yield 210mg (35%) of **7**. Rf: 0.43 (MeOH/ CH_2Cl_2, 15:85). HPLC: 12.7 mn (grad 5-95), 100%. MS(FAB+)= 799.6 (M+H+); ^1H NMR (MeOD), δ (ppm): 1.4 (m, 8H); 1.7 (m,

4H); 1.9 (s, 3H); 2.5 (m, 2H); 2.7 (s, 3H); 3.1 (m, 1H); 3.5 (m, 1H); 3.6 (t, 2H); 3.7 (m, 1H); 4.1 (m, 3H); 4.3 (m, 2H); 4.5 (m, 2H); 5.1 (t, 1H); 5.2 (s, 2H); 6.2 (m, 1H); 7.5 (s, 1H); 8.2 (m, 3H).^{13}C NMR (D_2O),δ (ppm): AZT; 166.3, 154, 137.7, 112, 86.5, 83.6, 83.7, 68.8, 68.7, 61.8, 37.8, 12. DAP and chain: 190, 152.6, 152.2, 139.9, 126.3, 125.9, 73.7, 72.9, 68.2, 68.1, 31.4, 31.2, 30.6, 30.4, 30.3, 30.2, 27.3, 26.7 , 25.7.Glucose: 98.3, 94.0, 71.4-71.1, 77.9, 72.9. ^{31}P NMR (MeOD),δ (ppm):+ 0.98; 0.93; 0.91; 0.7. Anal. ($C_{33}N_6H_{47}O_{15}P$-0.5H_2O) C, H, N,O: calcd 49.7, 5.94, 10.79, 30.70; found 49.98, 5.92, 10.13, 30.48.

8-(N,N,N'-trimethylethylenediamine) N'-octyl, 3'-azido-3'-deoxy-5'-thymidinyl, 6-D glucopyranosyl phosphate, ***TMED-AZT-Glc 8***

8-(N-methyl piperazine) N'-octyl, 3'-azido-3'-deoxy- 5'-thymidinyl, 6-D glucopyranosyl phosphate, ***NMP-AZT-Glc 9***

0.227 mmol of **16** and 0.29 mmol of N,N,N'-trimethylethylenediamine or N-methylpiperazine were dissolved in 3 mL of dimethylacetamide (DMA). The mixture was stirred overnight at room temperature. The solvent was removed by lyophilization and the residue was purified by Sephadex G10 (water) and reverse phase C_{18} (gradient: MeOH in water) chromatographies. 31mg of phosphotriester **8** (19% yield) and 27 mg of phosphotriester **9** (40% yield) were isolated as hygroscopic compounds.

TMED-AZT-Glc **8**: Rf: 0.32 (iPrOH/NH_4OH/ H_2O, 7:1:2). HPLC: 9.35 (69%), 9.48 (31%), grad 5-95. MS(FAB$^+$)= 723 (M+H$^+$), 745 (M+Na$^+$); ^{1}H NMR (D_2O), δ (ppm): 1.4 (m, 8H); 1.6 (m, 4H); 1.9 (s, 6H); 2.5 (m, 2H); 2.7 (s, 6H); 2.75 (s, 3H); 3.0 (m, 2H); 3.3-3.2 (m, 4H); 3.55-3.4 (m, 4H); 3.6 (m, 1H); 4.0 (m, 1H); 4.2-4.1 (m, 3H); 4.4 (m, 4H); 4.5 (m, 1H); 4.65 (d, 1H); 5.2 (t, 1H); 6.25 (m, 1H); 7.6 (d, 1H).^{13}C NMR (D_2O),δ (ppm): AZT; 167.1, 152.3, 138.1, 112.1, 85.9, 82.8, 67.7, 67.5, 60.3, 36.7, 12.5. TMED and chain: 67.8, 67.5, 57.5, 52.9, 51.7, 44.3, 40.9, 30.1, 30, 28.9, 28.7, 26.4, 25.3, 24.7; Glucose: 96.7, 92.8, 76.3, 74.7, 73.3, 72, 69.8, 69.7.^{31}P NMR (D_2O),δ (ppm):+ 0.06, 0.05, -1.6, -3. Anal. ($C_{29}N_7H_{52}O_{12}P$- 2H_2O) C, H, N: calcd 45.25, 7.53, 12.73; found 45.36, 7.13, (10.98).

NMP-AZT-Glc **9**: Rf: 0.47 (iPrOH/NH_4OH/ H_2O, 7:1:2). HPLC: 10.6 and, 10.7, grad 5-25. MS(FAB$^+$)= 720.8 (M+H$^+$), 743 (M+Na$^+$); ^{1}H NMR (D_2O), δ (ppm): 1.25 (s, 8H); 1.6 (m, 4H); 1.9 (s, 3H); 2.5 (s, 4H); 2.8-3.1 (s broad, 10H); 3.4-3.6 (m, 3H); 3.7 (t, 1H); 4.0 (m, 1H); 4.2-4.1 (m, 3H); 4.2 (m, 4H); 4.5 (m, 1H); 4.65 (d, 1H); 5.2 (t, 1H); 6.25 (m, 1H); 7.6 (d, 1H).^{13}C NMR (D_2O),δ (ppm): AZT; 167.5, 152.5, 138, 112.1, 85.9, 82.8, 70.5, 70.4, 60.3, 36.7, 12.5. NMP: 67.7, 57.7, 52.8, 51.5, 44.2. Glucose: 96.7, 92.8, 76.3, 74.7, 73.3, 72, 69.8, 69.7. Anal. ($C_{29}N_7H_{50}O_{12}P$- 1.5H_2O) C, H, N: calcd 44.64, 7.15, 13.12; found 46.39, 7.12, (12.08).

N,N,N'-trimethyl-N'-(8-hydroxyoctyl)-ethylenediamine, ***TMED-OH 10:***

N,N,N'-trimethylethylenediamine (5g, 48.9 mmol) and 1,8 bromooctanol (5g, 23.9 mmol) were dissolved in 25 mL of absolute ethanol. After 45 minutes at 80°C, the solution was cooled and a NaOH solution (866mg in 3.5mL of water) was added to the reaction mixture. The resulting solution was then extracted with dichloromethane. The organic layer was dried over $MgSO_4$, filtered and evaporated to a crude product which was further purified on silica gel eluting with dichloromethane-methanol and chromatographed on a column LH20 with THF-methanol (9:1) as eluent to yield 1,5 g of colorless oil (27%); MS(FAB$^+$)= 231.28 (M+H$^+$); ^{1}H NMR ($CDCl_3$), δ (ppm): 1.3 (m, 12H); 1.4-1.6 (m, 4H); 2.1 (s, 9H); 2.3 (m, 2H); 2.4-2.6 (m, 4H); 3.6 (t, 2H). Anal. ($C_{13}N_2H_{30}O$) C, H, N: calcd 67.76, 13.38, 11.96; found 67.79,13.24, 11.85.

N-methyl-N'-(8-hydroxyoctyl)-piperazine, ***NMP-OH 11*****:**

N-methylpiperazine (5g, 50.5 mmol) and 1,8 bromooctanol (7.5g, 35.8 mmol) were dissolved in 40 mL of absolute ethanol. The solution was heated at 80°C. After one hour, the solvent was removed by evaporation. The residue was dissolved in dichloromethane and washed with a saturated solution of sodium bicarbonate. The organic layer was dried over $MgSO_4$, filtered and evaporated. The product was then purified in the same condictions as described for N,N,N' trimethyl-N'(8-hydroxyoctyl)-ethylenediamine.2.5g of white solid were obtained (30%); MS(FAB$^+$)= 229.3 (M+H$^+$). ^{1}H NMR ($CDCl_3$), δ (ppm): 1.3 (s, 8H); 1.4-1.6 (m, 4H); 2.25 (s, 3H); 2.3 (t, 2H); 2.4-2.6 (broad, 8H); 3.6 (t, 2H). Anal. ($C_{13}N_2H_{28}O$) C, H, N: calcd 68.33, 12.28, 12.26; found 68.19, 12.46, 12.10.

11-(2-acetyl 6-pyridinyl) 10-one 8-oxa 1-ol-undecane, ***DAP-OH 15***

Compound**15** was obtained from **17b** and 8-bromo 1-t-butyldimethylsilyloctane following the same procedure as described for compound **18**. 8-bromo 1-t-butyldimethylsilyloctane was obtained by reaction between 1,8-bromooctanol (5g, 23.9 mmoles), with 2.2 eq of imidazole and 1.1 eq of t-butyldimethylsilyl chloride in DMF one night at room temperature. A saturated solution of NaCl was added to the reaction mixture which was extracted with dichloromethane (twice 150mL). The organic layers were dried over $MgSO_4$, filtered and evaporated. Chomatography on a silica gel column with hexane-ethyl acetate as eluent gave 8-bromo 1-*t*-butyl-dimethylsilyl with 75% yield. Rf: 0.55 (hexane/ ethyle acetate, 97: 3).^{1}H NMR ($CDCl_3$), δ (ppm): 0 (s, 6H); 0.9 (s, 9H); 1.5-1.1 (m, 12H); 3.2 (t, 2H); 3.45 (t, 2H).

15: (56%); Rf: 0.55 (ether/ CH_2Cl_2 (20:80)).^{1}H NMR ($CDCl_3$), δ (ppm): 1.4-1.6 (m, 10H); 1.8 (m, 2H); 2.8 (s,3H); 3.65 (t, 4H);.5.1 (s, 2H); 8.1 (t, 1H); 8.3 (d, 2H). Anal. ($C_{17}NH_{25}O_4$) C, H, N: calcd 66.44, 8.14, 4.56; found 66.28, 8.14, 4.60.

3'-azido-3'-deoxy- 5'-thymidinyl, 6-D glucopyranosyl, 8-iodooctyl phosphate ***AZT-Glc-I 16***

A solution of **6** tetrabutylammonium form (590 mg, 0.78 mmol) and 1.5 g of diiodooctane (10 eq) in anhydrous acetonitrile was heated overnight at 80°C. After evaporation of the solvent, the residue was triturated in petroleum ether and purified on a silica gel column eluting with dichloromethane-methanol. 175 mg of diester **16** was isolated (30%). Rf: 0.37 (MeOH/ CH_2Cl_2, 15:85). MS(FAB$^+$)= 747.6 (M+H$^+$); ^{1}H NMR (MeOD), δ (ppm): 1.4 (m, 8H); 1.7 (m, 4H); 1.9 (s, 2H); 2.45 (m, 2H); 3.27 (t, 2H); 3.7 (t, 1H); 3.95 (m, 1H); 4.2 (m, 4H); 4.3 (m, 4H); 4.5 (m, 1H); 4.55 (d, 1H); 5.1 (s, 1H); 6.2 (m, 1H); 7.5 (s, 1H).^{13}C NMR (MeOD),δ (ppm): AZT; 163, 156, 138, 114, 86.5, 83.7, 68.9, 68.8, 61.8, 37.9, 12.7; chain: 68.6, 68.2, 31.4, 31.3, 34.6, 31.2, 30, 29.5, 26 , 7.1; Glucose: 98.3, 94.0, 77.9, 76.2, 74.7, 73.7, 71.3, 71.2, 71, 69.9, 69.8.

6-(α-hydroxydimethylacetal) 2-(2-methyl 1,3 dioxolane 2-yl) pyridine ***17b***

To a solution of 2,6-diacetylpyridine (20g, 0.122 mol) in *p*-toluene (100mL), were added ethylene glycol (8.06g, 0.13 mol) and *p*-toluylsulfonic acid in catalytic amount. The solution was heated at 120°C overnight with a Dean-Stark apparatus. The cooled reaction mixture was washed successively with $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was then chromatographed on silica gel columns eluted with cyclohexane-ethyl acetate and petroleum ether-dichloromethane gradients to yield 6-(2-methyl 1,3 dioxolane 2-yl) 2-diacetylpyridine **17a** (30%).Rf: 0.36 (ethyl acetate/CH_2CL_2, 2.5:4.5).^{1}H NMR ($CDCl_3$), δ (ppm): 1.8 (s, 3H); 2.8 (s, 3H); 4.0-4.2 (m, 4H); 7.8 (d, 1H); 7.9 (t, 1H); 8.0 (d, 1H).

A solution of 2.4g of the compound **17a** (0.012 mol) in anhydrous methanol (6mL) was added dropwise to a solution of KOH (5 eq) in methanol stirred at 0°C. The iodobenzene diacetate (4g, 1eq) was then added slowly to the reaction mixture. After one night at room temperature, the red solution was evaporated in vacuo. The residue was dissolved in a saturated solution of NaCl and extracted three times by dichloromethane. Organic layers were dried over $MgSO_4$, filtered and evaporated. The product was purified by chromatography on silica gel columns with dichloromethane-methanol and cyclohexane-ethylic ether as eluents. 1.6 g of **17b** were obtained (49%). Rf: 0.3 (ether/ cyclohexane, 25:75).^{1}H NMR ($CDCl_3$), δ (ppm): 1.8 (s, 3H); 2.2 (s, 6H); 4.05 (s, 2H); 4.1-3.9 (m, 4H); 7.6 (dd, 2H); 7.8 (t, 1H).

11-(2-acetyl 6-pyridinyl) 10-one 8-oxa 1-iodo-undecane, ***DAP-I 18***

17b (1g, 3.8 mmol) was dissolved in dimethoxyethane (DME) and mixed under nitrogen atmosphere to a solution of NaH 60% (1.2 eq) in DME. The mixture was stirred at room temperature for 20mn. Then, 5g (3.5 eq) of 1,8 diiodooctane diluted in DME (5mL) were added. After one night at room temperature, 5mL of water was added to the reaction and the DME was removed by evaporation. The residue was dissolved in 40mL of water and extracted three times with dichloromethane. Organic layers were dried over $MgSO_4$, filtered and evaporated. The crude product was purified on a silica gel column eluted with petroleum ether-dichloromethane: yield 830mg of **17c** (43%), oil. Rf: 0.44 (MeOH/ CH_2Cl_2, 2.5:97.5).^{1}H NMR ($CDCl_3$), δ (ppm): 1.3 (m, 8H); 1.8-1.6 (m, 4H); 1.8 (s, 3H); 3.2 (s, 6H); 3.15 (t, 2H); 3.45 (t, 2H); 3.9 (s, 2H); 3.8 (m, 2H); 4.1 (m, 2H); 7.5 (m, 1H); 7.65 (m, 2H).

A solution of 800mg of **17c** in a mixture of 8mL of acetone, 1mL of water and *p*-toluylsulfonic acid in catalytic amount was stirred at 50°C for 3 hours. Evaporation of the solvant gave a residue which was dissolved in dichloromethane; the organic layer was washed with a 5% aqueous solution of $NaHCO_3$ and dried over $MgSO_4$, filtered and evaporated. 425 mg of **18** (65%) were obtained after purification on a silica gel column eluted with petroleum ether-dichloromethane and recrystallization in ether. Rf: 0.37 (MeOH/ CH_2Cl_2, 1.5:98.5).^{1}H NMR ($CDCl_3$), δ (ppm): 1.3 (m, 8H); 1.8-1.6 (m, 4H); 2.6 (s,3H); 3.1 (t, 2H); 3.5 (t, 2H); 5.0 (s, 2H); 7.9 (t, 1H); 8.1 (dd, 2H). Anal. ($C_{17}NH_{24}O_3$) C, H, N: calcd 49.81, 6.19, 3.17; found 49.84, 5.89, 3.22.

NMP-AZT-Cu(II) 3c , TMED-AZT-Cu(II) 2c and TMED-AZT-Glc-Cu(II) 8c *complexes*

The phosphodiesters **2** and **3** were precipitated by an ethanolic solution of copper chloride to give the corresponding complexes **2c** and **3c** with 78% and 70% yield, according to the procedure described for **10c** and **11c**. The phosphoester **8** and **4** gave the chelated compounds **8c** and **4c**.

Electronic absorption spectroscopy (500-900nm), in water:

2c = 14,8 mM, λ_{max} = 690nm; ε = 37 M^{-1} cm^{-1}. **3c** = 12,3 mM, λ_{max} = 810 nm; ε = 7 M^{-1} cm^{-1}.

4c = 41,0 mM, λ_{max} = 690nm; ε = 41 M^{-1} cm^{-1}. **8c**= 46,0 mM, λ_{max} = 690nm; ε = 55 M^{-1} cm^{-1}.

2c: Anal. ($C_{23}N_7H_{42}O_7P$, $CuCl_2$, $3H_2O$) C, H, N: calcd 36.93, 6.41, 12.81; found 37.05, 6.34, 13.10.

3c: Anal. ($C_{23}N_7H_{40}O_7P$, $CuCl_2$, H_2O) C, H, N, Cl: calcd 38.18, 5.85, 13.55, 10.78; found 38.08, 5.55, 13.22, 10.3.

Zn(II) complex of phosphotriester 7 (***DIMC-AZT-Glc-Zn(II)*** *)*

100 mg of phosphodiester DAP-AZT-Glc **7** (0.125 mmol) were dissolved in acetonitrile with 0.125 mmol of bis-(aminopropyl-3) amine (16.4 mg) and 0.125 mmol zinc bromide (28.15 mg)[37]. The mixture was stirred one hour at 50°C, and cooled. An orange complex precipitated. In the supernatant were added some drops of diethyl

ether. A second orange complex appeared; the combined precipitates, isolated by filtration, were crystallized in acetonitrile to give 25 mg of complexe contaminated with Zn salts (17%).

IR, cm^{-1} (H_2O, EtOH, 1:1); starting diketone: 1701, DIMC: 1654, 1585.

Anal. ($C_{39}N_9H_{60}O_{13}P$, $ZnBr_2$, $2Zn(OH)_2$, $2H_2O$) C, H, N: calcd 34.6, 5.06, 9.31; found 34.63, 4.79, 9.36.

NMP-OH-Cu(II) 11c and TMED-OH-Cu(II) 10c complexes

N,N,N'-trimethyl-N'(8-hydroxyoctyl)-ethylenediamine **10** or N-methyl-N'-(8-hydroxyoctyl)-piperazine **11** (100mg, 0.43 mmol and 0.44 mmol, respectively) were dissolved in absolute ethanol. A solution of one equivalent of copper chloride in ethanol was prepared, filtered and added dropwise to the solutions of **10** and **11** to form a green precipitate in the case of **10c** and a brown one in the case of **11c**. These solids were suspended in ethanol, washed three times by successive centrifugations and dried under argon. 115 mg of **10c** (73%) and 98 mg of **11c** (61%) were obtained.

Electronic absorption spectroscopy 500-900nm (water):

10c = 0,035 M, λ_{max} = 690 nm; ε = 28,8 M^{-1} cm^{-1}. **11c** = 0,039 M, λ_{max} = 790 nm; ε = 3 M^{-1} cm^{-1}

10c: Anal. ($C_{13}N_2H_{30}O$, $CuCl_2$) C, H, N, Cl: calcd 42.80, 8.23, 7.51, 19.48; found 42.65, 8.33, 7.51, 19.66. **11c:** Anal. ($C_{13}N_2H_{28}O$, $CuCl_2$, H_2O) C, H, N: calcd 30.20, 5.80, 5.40; found 30.08, 5.33, 6.05.

Hydrolysis of RNAs by Cu(II) complexes 2c, 3c, 4c, 8c, 10c and 11c.

Hydrolysis reactions were run in 0.5 mM HEPES buffer (pH = 7.3) at 37°C. In a typical reaction, the assay solution contained in a total volume of 100µL, 1.66 µM RNA 25 or 28-mer, 16.6 or 41.5 or 83 or 124.5 or 166 µM of complexes and 0.5 mM HEPES buffer.The reaction mixture was incubated at 37°C. 30 µL aliquot was removed from the reaction at different times and analyzed by capillary electrophoresis. Control reactions were run under identical conditions but in the absence of copper complexes or in the presence of non-complexed ligands. The DNA cleavage assay was carried according to the same procedure as described above for RNAs.

Identification of free 5'-OH oligoribonucleotides.

The assay solution contained 1.66 µM of RNA 28-mer, 83 µM of complexe **2c** or copper chloride in water (pH = 6.9). 100µL of the reaction mixture were incubated at 37°C for 48h and a 30 µL aliquot was analyzed by capillary electrophoresis analysis. A 50 pmoles aliquot of probe were recovered and lyophilized. The sample was incubated in a total volume of 70µL of water with 3µCi (4.95 pmol) of ATPγ^{32}P, 6.6mmol of ATP and 2.4 µL of polynucleotide T4 kinase (13U/µL) in 7µL of kinase 10X buffer. The mixture was kept for 30min at 37°C and a solution of 0.1 m EDTA/ 5% glycerol/ 0.1% bromophenol was added and subjected to electrophoresis for 1.5 h (denaturing polyacrylamide gel 20%, urea 7M; 2000v; 40W) and then autoradiographed.

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