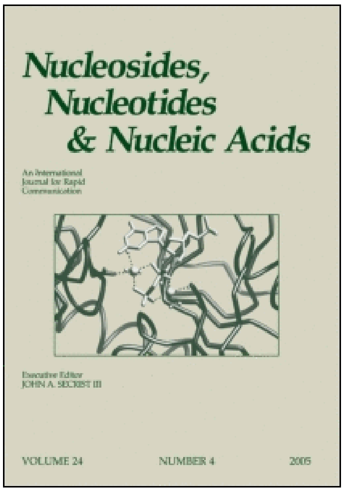

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STUDIES ON THE INHIBITION OF MOLONEY MURINE LEUKEMIA VIRUS REVERSE TRANSCRIPTASE BY N-TRITYLAMINO ACIDS AND N-TRITYLAMINO ACID-NUCLEOTIDE COMPOUNDS

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□ *N-Acylated derivatives of 8-(6-aminohexyl) amino-adenosine-5′-phosphate were prepared and studied with regard to their effect on DNA synthesis by the Moloney leukemia virus reverse transcriptase. N-palmitoyl and N-nicotinyl derivatives and bis-8-(6-aminohexyl) amino-5′-AMP inhibited the enzyme partially using poly (rA).oligo* $d(pT)_{16\text{-}18}$ *as template-primer with* $[^3H]$*dTTP. In order to increase hydrophobicity in the acyl component tethered to the 8-(6-aminohexyl) amino group on the adenine nucleotide, N-trityl-L-phenylalanine and the N-trityl derivatives of the o, m, and p-fluoro-DL-phenylalanine were initially examined for inhibition of the enzyme using the above template-primer system. The compounds all inhibited the reverse transcriptase with* IC_{50} *values of approximately 60–80* μM*. However, when N-trityl-m-fluoro-DL-phenylalanine was coupled to the nucleotide 8-(6-aminohexyl) amino-adenosine-5′-phosphate, the inhibitory activity of this compound increased significantly (*$IC_{50} = 5\ \mu M$*).*

Keywords N-tritylamino acid-nucleotide; reverse transcriptase; inhibitor

INTRODUCTION

Reverse transcriptase is a key enzyme involved in the replication of retroviruses. It is used in the conversion of genomic viral RNA into proviral DNA. Reverse transcriptase enzymes purified from a number of retroviruses including Moloney murine leukemia virus (M-MuLV) and the AIDS virus

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(HIV-1) all possess (i) a DNA polymerase activity using either RNA or DNA templates and (ii) a RNase activity capable of degrading RNA from RNA-DNA hybrids.

Inhibitors of the polymerase (chemicals/drugs) fall into a number of categories–(i) nucleoside analogues and derivatives,[1–3] (ii) naturally occurring compounds,[4,5] and (iii) non-nucleoside inhibitors (NNI).[6–18] Nucleoside inhibitors and nucleoside-like inhibitors act as chain elongation terminators. The non-nucleoside inhibitors (structurally varied group of compounds) on the other hand, all bind to a common hydrophobic allosteric site near the polymerase active centre. The classical non-nucleoside inhibitor is Nevirapine (dipyridodiazepinone), discovered during a random screening of various chemical compounds.

In our studies, we envisaged the preparation of compounds consisting of a nucleotide component covalently linked to a hydrophobic grouping through a spacer of approximitaley 10–15 A which is the distance of the hydrophobic binding pocket area from the active centre of the reverse transcriptase polymerase enzyme. The resulting compound would thus have a ribonucleotide component which could presumably go to the active centre of the enzyme while the hydrophobic section could conceivably interact or attach at the hydrophobic pocket of the enzyme. The compounds mentioned above can thus be thought of as chimeras of a nucleotide and a non-nucleotide inhibitor (NNI).

In the present experiments, compounds consisting of 8-(6-aminohexyl) amino adenosine-5-phosphate coupled to N-trityl-amino acids via a spacer, were therefore investigated for their possible effect on the activity of M-MuLV reverse transcriptase. These compounds in essence consist of a hydrophobic grouping (NN) linked covalently to a nucleotide residue via a spacer. These particular chimera structures are illustrated in Figures 1 and 5 of the manuscript. Chimeras of deoxynucleosides and non-nucleoside reverse transcriptase inhibitors previously prepared by a number of groups and tested for inhibition of the reverse transcriptase are mentioned in the Discussion section. Results of our experiments are reported in the present communication.

MATERIALS AND METHODS

[^{3}H]dTTP (126 Ci/mmol) as the NH_4^+ salt was obtained from Amersham, U.K. Poly (rA), d(pT)$_{16–18}$, tritylchloride, tritanol, 8-(6-aminohexyl) amino adenosine-5′-phosphate (Li^+ salt) and N-hydroxysuccinimide (NHS) were supplied by Sigma (St. Louis, MO, USA). Oligo d(A)$_{10}$ and d(A)$_{20}$ were prepared by Metabion (Germany). These substances and reverse transcriptase (M-MuLV) were supplied by Roche (Germany). All other chemical reagents were of analytical grade.

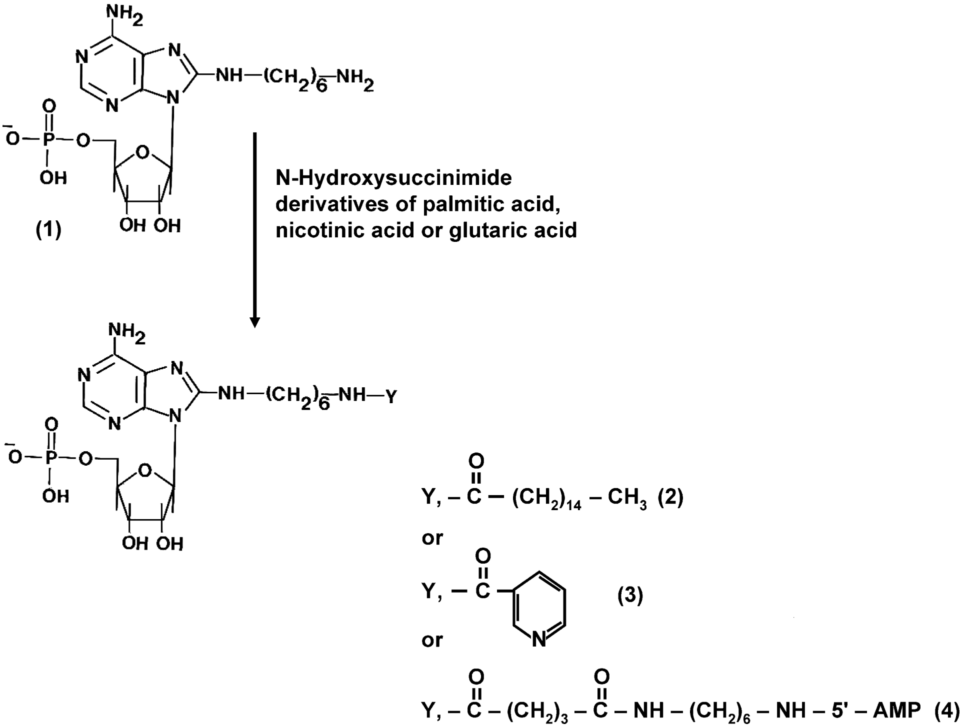


FIGURE 1 Preparation of N-acyl derivatives of 8-(6-aminohexyl) amino-5′-AMP. 8-(6-aminohexyl) amino-5′-AMP (1); palmitoyl derivative (2); nicotinyl derivative (3) and bis-nucleotide with glutaryl spacer (4).

N-Tritylglycine (9) and the N-trityl derivatives of o-(10), m-(11) and p-(12) fluorophenylalanine were prepared by Nel and Ariatti[19] using the method of Zervas and Theodoropoulos.[21] The N-hydroxysuccinimide derivative of N-trityl-m-fluorophenylalanine (6) was prepared from 11 by the procedure of Nel and Ariatti.[19]

Assay for RNA-Dependent DNA Polymerase Activity of M-MuLV Reverse Transcriptase

The incubation system contained 50 mM Tris-HCl (pH 8.3), 20 mM KCl, 10 mM dithiothreitol, 5 mM $MgCl_2$, reverse transcriptase (10 units), poly rA (1.3 μg), $d(pT)_{16}$ primer (0.15 μg), $[^3H]$dTTP (1.5 μCi), and dTTP (0.45 μg) in a final volume of 20 μl. Reactions were carried out at 37°C. Freshly prepared reagents were stored in crushed ice-water. Preincubated poly rA-$d(pT)_{16}$ was added to the enzyme-$[^3H]$dTTP mixture at zero time. At regular intervals thereafter, aliquots (2–6 μl) were removed and mixed into 18–30 μl 0.1 M EDTA (pH 7.6). From these solutions, aliquots (5–10 μl) were streaked on discs of DE-81 paper, dried at 37°C and then immersed in 0.5 M sodium phosphate (pH 6.8) for 5 minutes. This procedure was repeated with fresh 0.5 M phosphate. Discs were dried at 60°C and counted for radioactivity by liquid scintillation.

Test compounds were dissolved either in H_2O, EtOH: H_2O (1:1, v/v) or EtOH. Final concentration of EtOH in test runs or controls was 10% (v/v).

The nucleotide-palmitate compound (2, Figure 1) was also solubilized in an aqueous solution as follows: An EtOH solution of nucleotide-palmitate (400 μg/40 μl) was taken to dryness and the residue suspended in 500 μl H_2O. This suspension was adjusted to pH 7.5 by careful addition of 0.05 M Tris (free base). Water was then added to bring the volume to 850 μl (only partial solubilization). Sodium chloride (1 M, 150 μl) was then rapidly added with stirring to produce a clear solution. This was filtered through a 0.22 μM sterile filter. Spectral analysis (UV) revealed a 60% solubilization of the compound.

8-(6-Aminohexyl) Amino-adenosine-5′-phosphate-palmitate (2)

To palmitoyl-N-hydroxysuccinimide (7.2 mg, 0.02 mmol) in 3.2 ml pyridine:H_2O (9:1, v/v) was added a slightly warm solution of 8-(6-aminohexyl) amino-adenosine-5′-phosphate (9.2 mg, 0.02 mmole of I) in 3.2 ml pyridine: H_2O (9:1,v/v). The clear solution was kept at room temperature for 20 hours in the dark. Thereafter, the reaction mixture was taken to dryness under N_2 at 40°C. The residue was treated with toluene (5 ml) and again taken to dryness under N_2. This was repeated once more. The resulting dry residue was dissolved in ethanol (40°C) and applied to silica gel $60F_{254}$ plates (20 × 20 cm) which were then developed with n-butanol : AcOH : H_2O (12:3:5, v/v/v, solvent A). The title compound (R_f, 0.48) was extracted into EtOH:AcOH (95:5) at 40°C, which was filtered and taken to dryness under a stream of N_2 at 40°C to give a clear white crystalline residue. UV (EtOH): λ_{max} 278 nm and λ_{min} 245 nm, λ_{max} 278 nm (log ε 4.36). Silica gel TLC on $60F_{254}$ plates gave a $R_f = 0.48$ (solvent A). Mass spectrum: m/z 698 $(M\text{-}H)^-$ (sample in 50% acetonitrile).

8-(6-Aminohexyl) Amino-adenosine-5′-phosphate-nicotinamide (3)

To a solution of nicotinic acid-N-hydroxysuccinimide (6 mg 0.02 mmol) in 2.6 ml pyridine: H_2O (9:1, v/v) was added a warm solution (40°C) of 8-(6-aminohexyl) amino-AMP (9.2 mg, 0.02 mmol) in 2.4 ml pyridine : H_2O (9 :1, v/v). After mixing, the solution was kept at room temperature (20°C) in the dark for 24 h. The solution was then taken to dryness under a stream of N_2 at 40°C. The residue was treated with 4 ml of toluene and again taken to dryness. The final residue was dissolved in 2.0 ml of ethanol (96%) at 40°C and purified on silica gel $60F_{254}$ plates developed in solvent A. The required band (R_f 0.18) was extracted from the silica gel into a solution containing ethanol: H_2O:conc. NH_4OH (5:4:2, v/v/v) at 37°C. The extract was filtered and dried in vacuo at 37°C. The residue was dissolved in EtOH:H_2O (1:2, v/v, 3 ml) at 37°C and centrifuged (10 000 × g, 4 min). Portions of the supernatant were used for analysis and assays with reverse transcriptase. The sample was chromatographically pure (R_f 0.18, solvent A), UV: λ_{max} 278 nm, (278/260 ratio, 1.28). Mass spectrum: m/z 567 (molecular mass, 566.5) (sample in 50% acetonitrile containing 0.1% formic acid).

Bis-8-(6-aminohexyl) Amino-5′-AMP-glutarate (4)

This was prepared in the same manner as the palmitoyl (2) and nicotinyl (3) derivatives. A solution of 8-(6-aminohexyl) amino-5′-AMP (9.2 mg, 0.02 mmol) and bis-N-hydroxysuccimimide ester of glutaric acid (3.0 mg, 0.01 mmol) in 7.0 ml pyridine: H_2O (9:1, v/v) was kept at 20°C for 22 hours. Thereafter, the reaction mixture was worked up as described for compound 3. Purification was effected on silica gel $60F_{254}$ plates (solvent A). Compound 4 was extracted into EtOH: H_2O (4:1, v/v) containing 5% acetic acid. The product was freely soluble in H_2O. UV (H_2O, pH 7.5): λ_{max} 278 nm and λ_{min} 242 nm. Mass spectrum: m/z, 1017 $(M\text{-}H)^-$ (sample in 50% acetonitrile). Molecular mass, 1018.

Preparation of Nicotinyl-trismethylolamide (5)

To a solution of the NHS ester of nicotinic acid (8.8 mg, 0.04 mmol) in DMF:pyridine (3:1, v/v, 0.25 ml) was added a solution of tris (hydroxymethyl) aminomethane (4,84 mg, 0.04 mmol) in 0.45 ml of the same solvent. The clear solution was stored in the dark for 20 hours at 20°C. It was then taken to dryness at 35°C under a stream of N_2. Traces of pyridine in the product were removed by repeated co-evaporation with toluene. The final residue was dissolved in EtOH (0.3 ml) and applied to a silica gel $60F_{254}$ TLC plate (20 × 20 cm) which was developed in solvent A. Compound 5 (R_f 0.44) was extracted into EtOH (25 ml) at 30°C. The extract was filtered and concentrated to dryness under N_2 (30°C). The residue was dissolved in H_2O (2 ml) and stored at −20°C. UV (H_2O, pH 5.5): λ_{max} 260 and 219 nm, λ_{max} 260 nm (log ε 3.48). Mass spectrum: m/z 227 (M^+).

R_f values (silica gel $60F_{254}$ plates in chloroform:methanol, 4:1 v/v) for tris (hydroxymethyl) amino-methane, N-hydroxysuccinimide ester of nicotinic acid, nicotinic acid and nicotinyl-tris-methylolamide were 0.06, 0.46, 0.27, and 0.15, respectively.

8-(6-Aminohexyl) Amino-adenosine-5′-monophosphate-N-trityl-m-fluoro-DL-phenylalanine (7)

To a solution of 8-(6-aminohexyl) amino-adenosine-5′-monophosphate (Li^+ salt, 4.6mg, 0.01 mmol) in 0.4 ml pyridine:H_2O (7:3, v/v) was added the N-hydroxysuccinimide ester of N-trityl-m-fluoro-DL-phenylalanine (5.3 mg, 0.01 mmole) in 0.2 ml pyridine:H_2O (7:3, v/v). The reaction mixture was left in the dark at room temperature for 20 hours. It was then taken to dryness under a stream of N_2 at 30°C and traces of pyridine removed by co-evaporation with toluene using N_2. The crude product was taken up in dioxane:EtOH: H_2O (2:2:1, v/v/v, 0.6 ml) and chromatographed on silica gel $60F_{254}$ plates (20 × 20 cm) in $CHCl_3$:CH_3OH (4:1, v/v). The product, which remained at the origin with unreacted 8-(6-aminohexyl) amino-AMP) was selectively extracted into ethanol (5 ml) at 30°C. Chromatography (silica gel $60F_{254}$ in n-propanol: NH_4OH:H_2O, 55:10:35, v/v/v) gave a single UV_{254} absorbing spot (R_f, 0.74) which was also positive for the trityl group (2% aqueous $HClO_4$, 100°C), while 8-(6-aminohexyl) amino-AMP and N-hydroxysuccinimide gave R_f values of 0.55 and 0.66 respectively. UV (EtOH): λ_{max} 278 nm, 278/240 ratio 2.19, λ_{max} 278 nm (log ε 4.25). Mass spectrum: m/z 243.1 (trityl), 299.1, 353.3, 381.4, 409.3, 537.4, 627.3, 869.5 (molecular mass 869.5) (sample in methanol containing 0.1% formic acid).

The compounds 8-(6-aminohexyl) amino-AMP-N-trityl-m-fluoro-DL-phenylalanine and 8-(6-aminohexyl) amino-AMP gave R_f values on silica gel $60F_{254}$ (EtOH:H_2O, 5:1, v/v) of 0.60 and 0.09, respectively.

RESULTS AND DISCUSSION

Our studies commenced originally with the idea of coupling various chemical groups to the terminal primary amino group of the aminohexyl chain at position 8 in 8-(6-aminohexyl) amino-adenosine-5′-phosphate (I in Figure 1). These derivatives could then be examined to explore possible inhibitory or stimulatory activity against the reverse transcriptase enzyme of Moloney mouse leukemia virus. In the first phase of this work we attached (i) palmitic acid, (ii) nicotinic acid and (iii) a further 8-(-6-aminohexyl) amino-adenosine-5′-phosphate to the aminohexyl moiety with glutaric acid spacer (Figure 1). All couplings were by N-hydroxy-succinimide activation of carboxyl groups. Synthesized compounds were purified by TLC chromatography on silica gel $60F_{254}$ plates.

With poly (rA)-oligo d$(pT)_{16}$ as template-primer, incorporation of $[^3H]$dTTP into poly dT was inhibited to varying extents with the compounds mentioned above. Figure 2 shows time curves for incorporation of $[^3H]$dTTP where the palmitoyl derivative (2) gave approximately 35% inhibition at 10^{-4} M and the bis-nucleotide with glutaryl spacer (4), 30% inhibition at 10^{-4} M. It is noteworthy that 8-(6-aminohexyl)-amino-AMP (1) stimulated incorporation at the early phase of the time curve (up to 10 minutes).

In Figure 3, it is seen that 8-(6-aminohexyl)-amino-AMP-nicotinamide (3) at 10^{-4} M inhibited incorporation by some 60%. At 10^{-5} M, incorporation was inhibited by approximately 35%. Nicotinic acid, nicotinamide and

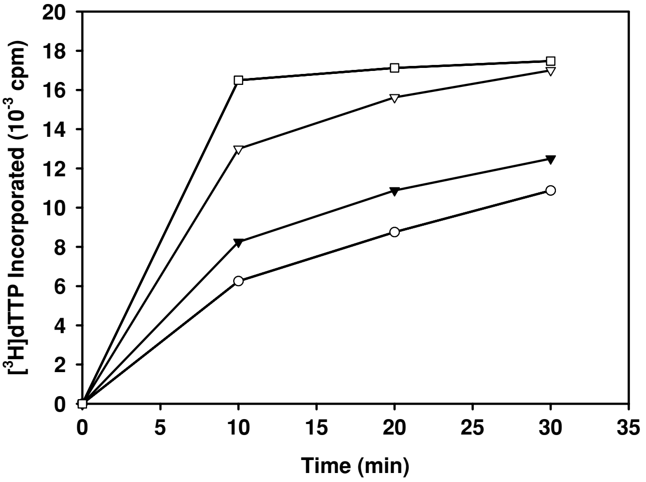


FIGURE 2 Time course of incorporation of [^{3}H]dTTP into DNA using poly(rA).d(pT)$_{16}$ as polymer template and M-MuLV reverse transcriptase in the absence (∇) and presence of different compounds; (□), 8-(6-aminohexyl) amino-5′-AMP (10^{-4}M); (O), palmitoyl derivative (10^{-4}M); (▼), bis-AMP-glutarate (10^{-4}M).

sodium palmitate (all at 10^{-4} M) had no effect on the activity of the reverse transcriptase (results not shown).

In addition to 8-(6-aminohexyl) amino-5′-AMP (1, Figure 1) nicotinyl-trismethylolamide (5, Figure 4) was prepared as a control in this study. This non-nucleotide compound displayed an initial stimulation of

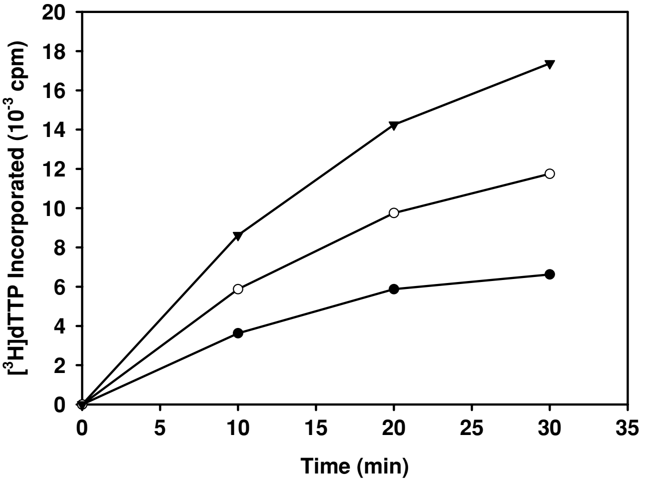
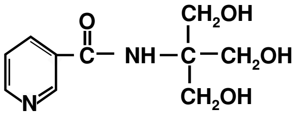


FIGURE 3 Time course of incorporation of [^{3}H]dTTP into DNA using poly(rA).d(pT)$_{16}$ as polymer template and the reverse transriptase in the absence (▼) and presence of the nicotinyl derivative of 8-(6-aminohexyl) amino-5′-AMP at 10^{-5}M (○), and 10^{-4}M (•).



(5)

FIGURE 4 Structure of the non-nucleoside derivative nicotinyl-trismethylolamide used as a control in the incorporation studies with the nucleotidyl compounds illustrated in Figure 1.

incorporation although this effect was less marked at 20 minutes and thereafter. The result paralleled the time curve of incorporation in the presence of 8-(6-aminohexyl) amino-5′-AMP (Figure 2). Results with nicotinyl-trismethylolamide are not shown.

Having shown that attachment of certain groups (palmitoyl, nicotinyl, glutaryl-AMP) to the aminohexyl side chain of nucleotide I, enabled the resulting modified nucleotides to inhibit partially the DNA polymerase activity of MuLV reverse transcriptase (Figures 1 through 3), we were encouraged to attempt further modification of nucleotide I.

In view of the very detailed research carried out over the last few years on non-nucleoside inhibitors of reverse transcriptase, it was considered of interest to attach more hydrophobic groups (molecules) to the aminohexyl side chain of nucleotide I. It was therefore decided to attach N-tritylamino acids to the side chain amino group of the nucleotide (Figure 5). In previous work in our laboratory,[19,20] N-trityl derivatives of different substituted phenylalanines, glycine and histidine were prepared by the method of Zervas and Theodoropoulos[21] and attached to puromycin amino nucleoside (PANS) for studies on protein synthesis. Couplings of N-trityl amino acids to PANS were facilitated by NHS activation of carboxyl groups. This approach has been employed in the present study to prepare 7 (Figure 5).

Using poly (rA)-d(pT)$_{16}$ as template-primer, M-MuLV reverse transcriptase-catalyzed incorporation of [^{3}H]dTTP into DNA was assayed in the absence or presence of various N-trityl amino acids and the 5′-AMP derivative 7. The results are shown in Table 1 and are reported as IC$_{50}$ values. It is seen that at the N-trityl amino acid level, m-fluoro- DL-phenyl-alanine is the most active (IC$_{50}$ = 65 μM). However, if this particular N-trityl amino acid is coupled to the nucleotide 8-(6-aminohexyl) amino-5′-AMP, the resulting compound (7) showed increased inhibitory activity (IC$_{50}$ = 5 μM). The IC$_{50}$ value varied between 2 and 5 for different preparations of compound (7). These results are interesting in that they demonstrate that linkage of a hydrophobic aromatic structure such as N-trityl-m-fluoro-DL-phenylalanine to the C-8 position of the purine nucleotide 5′-AMP via a 1,6-diaminohexyl spacer brings about increased inhibitory activity over the starting N-trityl amino acid. The distance between the purine C-8 position and the methane carbon of the N-trityl group is approximately 10 Å. Detailed x-ray studies by a number of groups on co-crystals of HIV-1

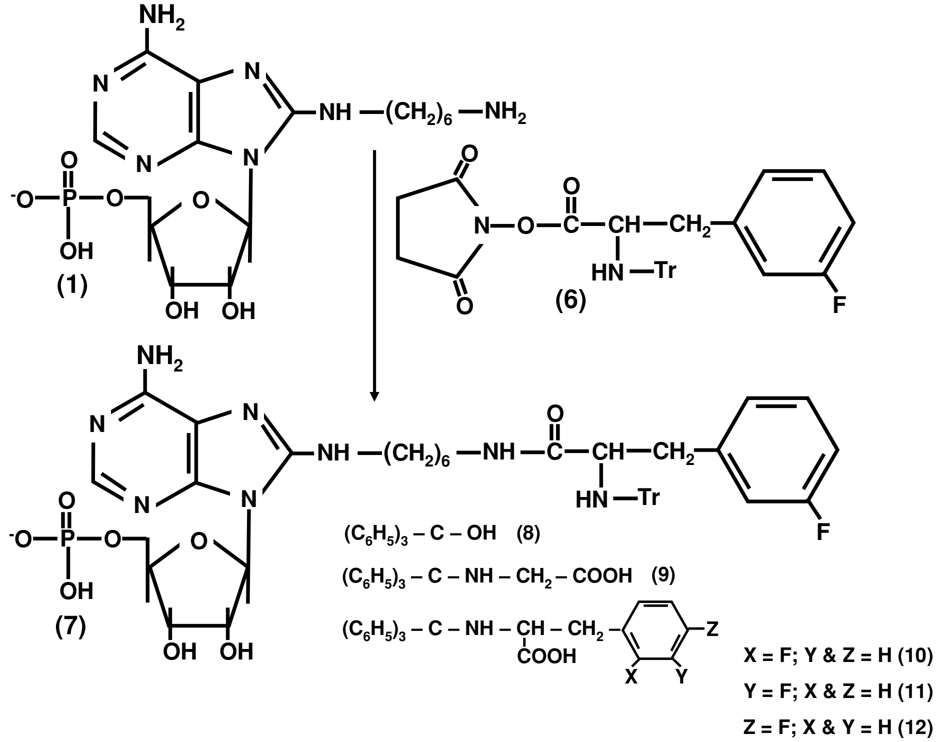


FIGURE 5 N-tritylamino acids coupled to 8-(6-aminohexyl)-amino-5′-AMP (1). N-tritylamino acids were activated as their N-hydroxysuccinimide derivatives. N-hydroxysuccinimide derivative of N-trityl-m-fluoro-DL-phenylalanine (6). N-trityl-m-fluoro-DL-phenylalanine-8-(6-aminohexyl) amino-5′-AMP (7). Tritanol (8). N-tritylglycine (9). N-trityl-(o, m, p)-fluoro-DL-phenylalanines (10, 11, 12). Tr = trityl.

reverse transcriptase and nevirapine (non-nucleoside inhibitor) give an approximate distance of 10 A between the non-nucleoside binding site and the active center.[22]

It is of interest to note that Steitz and co-workers[22,23] suggested the idea of linking a non-nucleoside RT inhibitor (NNRTI) to substrates of the reverse transcriptase such as the primer-template itself or a dNTP. The design features of the nucleotide-N-trityl-m-fluoro-DL-phenylalanine derivative are consistent with the idea of Steitz and co-workers. At present, we do not fully understand how 8-(6-aminohexyl) amino-AMP-N-trityl-m-fluoro-DL-phenylalanine elicits its effect. We believe that

i The N-trityl-phenylalanine portion of the molecule may be binding to the hydrophobic non-nucleoside area of the reverse transcriptase.

ii The 5′-AMP component of the large inhibitor may be somewhere near the active centre of the enzyme. As mentioned above, the spacer between the purine ring and the trityl group of the inhibitor is approximately 10 A. It should also be noted that 5′-AMP is a ribose-containing molecule.

TABLE 1 The effect of different N-trityl amino acids, N-trityl-m-fluoro-DL-phenylalanyl-8-(6-aminohexyl) amino-5′-AMP and tritanol on DNA synthesis by Moloney leukemia virus reverse transcriptase[a]

N-trityl derivatives	IC_{50} (μM)
Tritanol	110
N-trityl-glycine	95
N-trityl-L-phenylalanine	75
N-trityl-o-fluoro-DL-phenylalanine	80
N-trityl-m-fluoro-DL-phenylalanine	65
N-trityl-p-fluoro-DL-phenylalanine	74
N-trityl-m-fluoro-DL-phenylalanyl-8 (6-aminohexyl)-5′-AMP	5

[a] Incubations all contained poly (rA).d(pT)$_{16}$ as template-primer and [^{3}H] dTTP. $IC_{50} = 50\%$ inhibition of activity. Tritanol and all the N-trityl amino acids and derivatives were dissolved in ethanol. Final concentration in incubations was 10% (v/v). Controls contained the same percentage of ethanol. Each compound was assayed at three concentrations.

iii There is a possibility that 8-(6-aminohexyl) amino-5′-AMP-N-trityl-m-fluoro-DL-phenylalanine may be displacing the poly (rA) template at the growth point. However, competition experiments using varying concentrations of poly (rA)-d(pT)$_{16}$ at fixed concentrations of nucleotide-N-trityl-m-fluoro-DL-phenylalanine showed the compound to act as a non-competitive inhibitor (results not shown).

It is important to note that various groups have previously reported on the preparation and biological evaluation of chimeras consisting of dideoxy nucleosides (ddN) and non-nucleoside reverse transcriptase inhibitors (NNRTI). The Spanish-Belgian research groups[24,25] have carried out very detailed studies on this group of compounds. These dimers have the general formula shown below:

[ddN]-Spacer-[NNRTI]

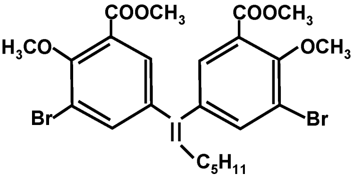
[ddN] = AZT or Thymidine

[NNRTI] = TSAO-T or HEPT (T represents thymidine)

Spacer = $-(CH_2)_n$-where n = 3−9

As shown above, the two classes of inhibitors were linked at the N-3 position of the thymidine base of each component compound by an appropriate spacer [-N-$(CH_2)_n$-N-]. The spacer is an aliphatic bridge of varying length. Because the spacer is linked through the N-3 position of each thymidine ring, these modified bases in the dimers are incapable of base pairing with A residues of the template-primer. The compounds prepared in our work on the other hand used a spacer linked at the 8 position of the A (purine) ring and therefore are still capable of base pairing with a thymidine (T) residue of the template-primer. Further, our compounds are mono-phosphorylated at the 5′-position of the ribose ring. A French research group has also investigated various conjugates containing both a nucleoside analog (AZT or ddC) and a non-nucleoside type inhibitor (HepT analogues) for their ability to block HIV replication.[26] Interesting work has also been carried out with synthetic phospholipid-AZT conjugates and their effects on HIV replication.[27,28]

Of further interest in respect of the hydrophobic trityl group, is the interesting work reported by Cushman and co-workers[29] on the synthesis and biological evaluation of some alkenyl diaryl methanes (ADAMS). These compounds behave as NNRTIs. One of the most active was 3′,3″-dibromo-4′,4″-dimethoxy-5′,5″-bis (methoxycarbonyl)-1,1-diphenyl-1-heptene shown below



This molecule inhibited the reverse transcriptase-catalyzed extension of oligo (dG) primer with poly (rC) as template (IC_{50}, 1 μM) but was without effect in the poly (rA).oligo (dT) template-primer system. It should be noted that compounds presented in this study gave inconsistent results using poly (rU).oligo $(dA)_{10-20}$ as the template-primer system with and without the N-tritylated amino acid nucleotide compounds.

As mentioned by Cushman,[29] the NNRTIs are made up of a large and structurally diverse set of compounds. It is interesting to note, however, that many of these compounds possess two substituted aromatic ring systems, which may also contain hetero atoms in the rings (pyridine, pyrimidine-dione). Examples include, HEPT derivatives (acyclouridine derivatives) and the non-nucleoside reverse transcriptase inhibitor DABO and others.It is noted that the ADAM shown above has two substituted benzene rings attached to a methene carbon. Results obtained in the work presented herein demonstrate that the strongly hydrophobic triphenyl methyl (trityl) group plays a crucial role in potentiating the inhibitory effect on Moloney murine leukemia virus reverse transcriptase by C-8 substituted 5′-AMP derivatives. Moreover, findings are consistent with the notion that the purine nucleotide component of 7 resides near the active centre of the enzyme while the trityl component may be located in the nearby hydrophobic pocket.

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