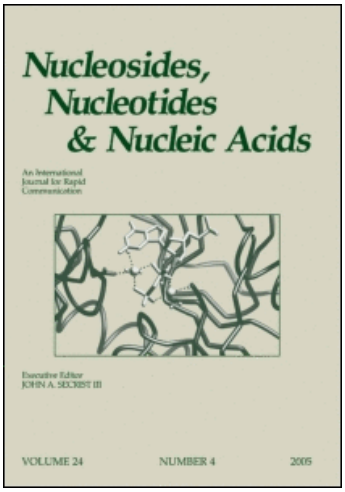

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SYNTHESIS AND ANTI-HIV ACTIVITY OF ALKYL STEROIDAL 3'-AZIDO-3'-DEOXYTHYMIDIN-5'-YL PHOSPHOTRIESTERS AS PRODRUGS OF AZT

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Abstract : Alkyl steroidal AZT 5'-monophosphate triesters are designed as lipophilic prodrugs of AZT to improve its therapeutic efficiency. We have synthesized four phosphotriesters of AZT, in one-pot, using phosphoramidite-phosphite triester methodology. This method afforded the desired prodrugs in high yields under mild conditions. The *in vitro* evaluation of anti-HIV activity of these prodrugs is also reported.

The replication of human immunodeficiency virus (HIV)[1] requires a unique enzyme, human immunodeficiency viral reverse transcriptase (HIVRT). The 3'-azido-3'-deoxythymidine (AZT, **1**),[2] a potent inhibitor of HIV replication, is the first clinically approved agent for treatment of acquired immunodeficiency syndrome (AIDS)[3] patients. However, AZT has serious side effects, particularly bone marrow suppression.[4] Pharmacokinetic studies in humans showed that AZT had short plasma-half life[5] and was excreted in urine as its 5'-O-glucuronidate.[6] The short plasma-half life necessitates frequent high doses of AZT to maintain the adequate therapeutic drug level in plasma. In an effort to circumvent these drawbacks AZT 5'-steroidal conjugates were synthesized as AZT prodrugs. The rationale for the synthesis of steroidal AZT prodrugs were as follows; i) the steroidal conjugates of AZT would increase the lipophilicity of AZT, ii) the steroidal moiety would also protect the 5'-OH of AZT from glucuronidation. These would increase metabolic stability, enhance bioavailability and reduce dose-related toxicity. Based on the above rationale 5'-steroidal carboxylic esters of AZT were synthesized in our laboratories.[7] These prodrugs showed comparable anti-HIV activity with that of AZT and were also found to be viable under the conditions of **in vitro** anti-HIV screening protocol.

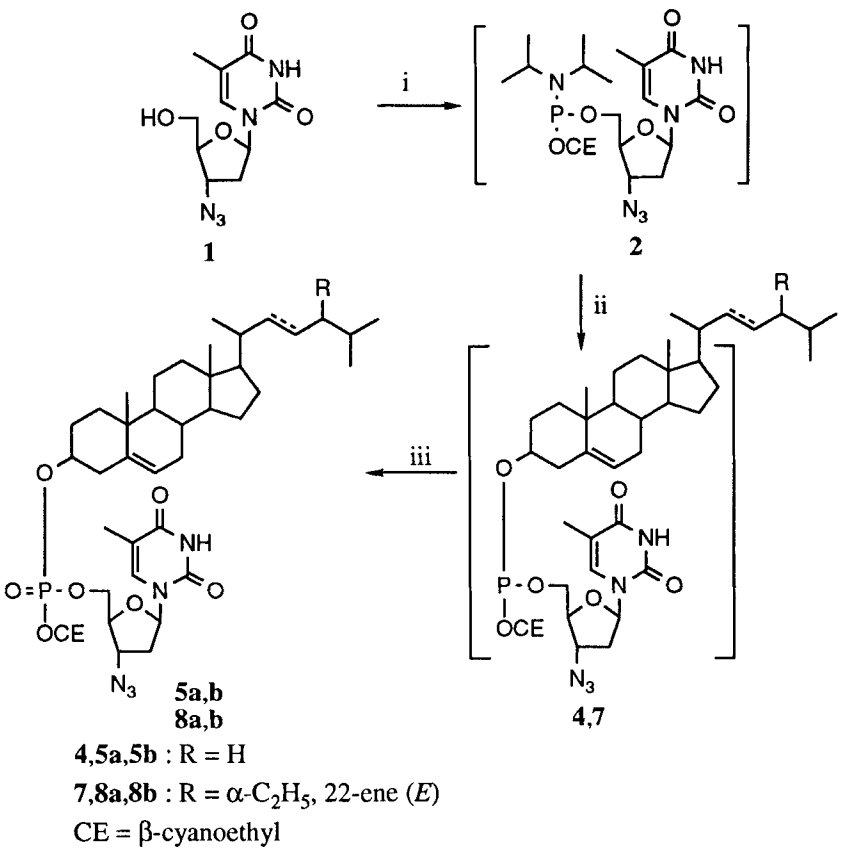
In an effort to further improve the therapeutic profile of the steroidal esters of AZT, we have synthesized steroidal phosphotriester conjugates of AZT. AZT is known to show its antiviral activity **in vivo** after its metabolic conversion to its 5'-triphosphate by cellular

kinases.[8-10] In addition to the low activity of nucleoside kinases, dideoxy nucleoside (DDN) analogs have poor affinity for kinases.[11,12] Hence, the first phosphorylation step in the cell is a limiting step. The dependence on cellular phosphorylation can be overcome by appropriate nucleotide delivery in its masked non-polar form as phosphotriester. These phosphotriesters could easily diffuse through membrane barriers and release the AZT monophosphate (AZTMP) in the target cell after being hydrolyzed by the cellular enzymes. The hydrolysis of these prodrugs by cellular enzymes, such as phosphotriesterase, was found to be similar to alkaline hydrolysis.[13] The title prodrugs, upon cellular hydrolysis, are expected to release AZTMP and biologically inactive by-products. In recent years a similar prodrug approach of nucleotides has been reported.[14-20] While simple dialkyl phosphotriesters[16] of AZT were found to be inactive, haloalkyl[16] and other lipophilic long chain alkyl phosphotriesters[18,19] of AZT showed comparable activity with that of AZT. Interestingly, dialkyl phosphotriesters of anti-HIV agent, 2′,3′-didehydro-3′-deoxythymidine (D4T) bearing a steroid in one of the alkyl groups through ether linkage, showed greater anti-HIV activity than their parent nucleoside.[21] In this report, we describe a rapid one-pot synthesis of various novel 5′-alkyl steroidal phosphotriesters of AZT in high yields using phosphoramidite-phosphite triester methodology.[23,24]

Results and Discussion

The synthesis of the phosphotriesters, 3′-azido-3′-deoxythymidine-5′-[(cholest-5-en-3β-yl) (β-cyanoethyl)] phosphate **5(a,b)** and 3′-azido-3′-deoxythymidine-5′-[(β-cyanoethyl) (24α-ethylcholesta-5,22*E*-dien-3β-yl)] phosphate **8(a,b)** involves three steps (Scheme 1, Method A) : (i) phosphitylation of AZT using (β-cyanoethyl)N,N-diisopropylchlorophosphoramidite to give its phosphoramidite, (ii) conjugation of AZT phosphoramidite with a steroid, by activating the phosphoramidite with a weak acid, 1H-tetrazole, to give a phosphite triester and (iii) oxidation of the phosphite triester to obtain phosphotriester of AZT.

AZT (**1**) was phosphitylated at 5′-OH using (β-cyanoethyl)N,N-diisopropylchorophosphoramidite (1.2 equiv.) to give the phosphoramidite intermediate **2**. To this reaction mixture, cholest-5-ene-3β-ol (**3**, 0.7 equiv.) was added along with 1H-tetrazole (4.8 equiv.). The activation of **2** by 1H-tetrazole allowed further coupling with 3-OH of **3** to give the phosphite triester intermediate **4**. The phosphite triester **4** was directly oxidized using I_2/H_2O to give the phosphotriester **5** in 36% yield. The progress of the reactions was monitored by TLC. Under the same condition, reaction of 24α-ethylcholesta-5,22E-diene (**6**) and **1** gave the corresponding conjugate **8** in 39% yield. Each of the target phosphotriesters, **5** or **8**, was obtained as a 1:1 mixture of two P-chiral diastereomers. We



Scheme 1 : i, $N(iPr)_2(OC_2H_4CN)PCl$, $N(iPr)_2Et$, CH_3CN, O°C, 10 min.; ii, R'OH, 1H-tetrazole, THF, r.t., 10 min.; iii, I_2/H_2O, r.t., 30 min.; R'OH = cholest-5-ene-3β-ol (**3**) or 24α-ethylcholesta-5,22*E*-diene-3β-ol (**6**); **a** = fast moving isomer, **b** = slow moving isomer.

were able to completely separate the (**a**) fast and (**b**) slow moving diastereomers in pure form on silica gel using radial chromatography. All four phosphotriesters, **5(a,b)** and **8(a,b)**, were characterized by IR, ^1H, ^{13}C and ^{31}P NMR analyses as well as FAB-MS.

The ^1H NMR spectra (Experimental) of these phosphotriesters, as a group, presented interesting signal shifts of certain protons in comparison to the spectra of the starting materials,[25-27] AZT and the steroids. The signals of 4'- and 5'-protons of AZT shifted approximately 0.12 and 0.6 ppm down-field respectively, upon phosphorylation. The 3α-proton of the steroids was greatly deshielded, as shown by a signal with a down-field shift of approximately 0.8 ppm. The remaining signals of the phosphotriesters were practically

identical to those of the starting materials indicating the congruency of the structures of phosphotriesters with those of starting materials. ^{13}C NMR spectra of these phosphotriesters (Table 1) were also informative with reference to the those of the starting materials.[28-30] The 5'-carbon of AZT and 3-carbon of the steroids shifted 4.4 and 8.2 ppm down-field respectively, whereas 4'-carbon of AZT was shifted 4.75 ppm up-field. The structures of the products were evident from ^{13}C NMR spectra, by the similarity of the remaining signals of AZT and steroid carbons. The carbons separated by two bonds from Phosphorous (POC) showed splittings due to phosphorous coupling. The carbons separated by three bonds (POCC) of AZT and cyanoethyl units also showed phosphorous coupling, while the same was not noticed for the 2- and 4-carbons of the steroids. Greater phosphorous coupling was noticed for POCC carbons than for POC carbons. The ^{1}H and ^{13}C NMR spectra did not show any significant differences within the pairs of diastereomers. However, ^{31}P NMR showed two distinct signals for each of the fast and slow moving diastereomers. The mass of (MH^+ - CN), obtained by FAB MS, for each diastereomeric mixture was matched with its corresponding theoretical mass.

In order to improve the yields,[31] we reversed the above mentioned synthetic sequence. In this attempt (Scheme 2, Method B), the steroid (**3** or **6**) was phosphitylated with 1.2 equivalents of phosphitylating agent to give the intermediate **9**. This phosphoramidite **9** was further allowed to react with 0.8 equivalents of **1**, in presence of 1H-tetrazole (4.8 equiv.) to give the phosphite triester intermediate **4**. On oxidation, **4** gave a diastereomeric mixture (1:1) of the corresponding phosphotriesters, **5** (**a** and **b**), identical in all respects to that obtained by Scheme 1. By this method **5** (**a** and **b**) were obtained in 86% yield. Similarly, **6** gave a 1:1 diastereomeric mixture of its corresponding phosphotriesters in 75% yield, which was also found to be identical, in all respects, with **8** (**a** and **b**) obtained by Scheme 1. It is interesting to note the report that a similar reversed synthetic sequence, in synthesizing cordycepin long chain alkylphosphotriesters of AZT, was not successful.[19] In contrast to the low yields obtained in a similar synthesis by phosphoramidite method,[22] we found this method to be very promising in giving high yields without any cross products.

All the reaction steps (i and ii , Schemes 1 & 2, Methods A & B) were completed within 10 minutes while the oxidation of the phosphite triester (iii, Schemes 1 & 2, Methods A & B) required 30 min.

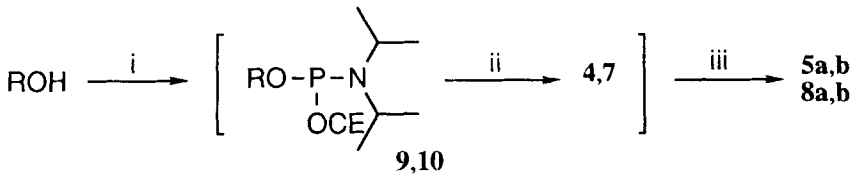
In vitro Anti-HIV Activity

All the compounds **5**(**a**&**b**) and **8**(**a**&**b**) were tested for their *in vitro* anti-HIV activity using T_4 lymphocytes (CEM cell line) at the National Cancer Institute. As shown in Table 2, all compounds are active against HIV. The activity (EC_{50}) of these compounds is

TABLE 1. Proton Decoupled ^{13}C NMR Spectral Data of the Conjugates in ppm.

St.	5a	5b	8a	8b	AZT	5a	5b	8a	8b
1	37.25	37.16	37.16	37.28	2	163.65	163.77	163.74	163.46
2	31.75	31.75	31.75	31.84	4	150.20	150.29	150.26	150.14
3	79.95	79.92	79.85	80.01	5	111.46	111.49	111.37	111.49
	(6.23)	(6.23)	(6.23)	(6.23)	6	135.32	135.35	135.32	135.32
4	42.26	42.23	42.11	42.20	Me	12.38	12.38	12.32	12.44
5	138.68	138.65	138.65	138.68	1'	85.07	85.00	85.00	85.00
6	123.56	123.53	123.44	123.62	2'	36.30	36.30	36.70	36.82
7	31.81	31.75	31.75	31.84	3'	59.92	60.10	59.92	60.17
8	31.81	31.75	31.75	31.84	4'	82.07	82.02	82.01	82.10
9	49.90	49.87	49.87	49.99		(8.30)	(6.22)	(8.30)	(8.30)
10	36.73	36.73	36.27	36.40	5'	66.37	66.53	66.37	66.58
11	21.00	20.97	21.09	21.21		(4.15)	(6.23)	(4.15)	(4.15)
12	39.63	39.60	39.45	39.57					
13	42.26	42.23	42.11	42.20	C_3-unit				
14	56.56	56.53	56.62	56.71					
15	24.21	24.21	24.24	24.33	C-1	62.00	62.03	62.00	62.05
16	28.12	28.12	28.73	28.82		(4.15)	(4.15)	(4.15)	(6.23)
17	56.10	56.10	55.86	55.95	C-2	19.70	19.68	19.63	19.78
18	11.80	11.77	11.92	12.05		(6.23)	(8.31)	(6.22)	(8.30)
19	19.13	19.13	19.10	19.20	CN	116.32	116.35	116.37	116.29
20	36.12	36.12	40.28	40.40					
21	18.68	18.65	20.94	21.03					
22	35.69	35.69	183.07	183.17					
23	23.78	23.78	129.24	129.37					
24	39.45	39.45	51.09	51.21					
25	27.93	27.90	31.75	31.84					
26	22.49	22.46	20.94	21.03					
27	22.71	22.71	18.89	18.98					
28	-----	-----	24.24	25.37					
29	-----	-----	12.11	12.20					

Data in parentheses are coupling constants (J_{POC} or J_{POCC}) in Hz.



9,10

9 : R = cholesteryl

10 : R = stigmasteryl

CE = β-cyanoethyl

Scheme 2 : i, $N(iPr)_2(OC_2H_4CN)PCl$, $N(iPr)_2Et$, THF, 0°C, 10 min.; ii, **1**, 1H-tetrazole, CH_3CN, r.t., 10 min.; iii, I_2/H_2O, r.t., 30 min.; ROH = cholest-5-ene-3β-ol (**3**) or 24α-ethylcholesta-5,22*E*-dinene-3β-ol (**6**); **a** = fast moving isomer, **b** = slow moving isomer.

Table 2. *In vitro* anti-HIV activity.*

Compound #	IC_{50} (Molar)[1]	EC_{50} (Molar)[1]	TI_{50} (IC/EC)[2]
5a	$>2.00 \times 10^{-4}$	5.67×10^{-6}	$>0.46 \times 10^{2}$
5b	$>2.00 \times 10^{-4}$	3.40×10^{-6}	$>0.98 \times 10^{2}$
8a	$>2.00 \times 10^{-4}$	1.65×10^{-6}	1.30×10^{2}
8b	$>2.00 \times 10^{-4}$	1.52×10^{-5}	0.63×10^{2}
AZT	1.00×10^{-6}	1.10×10^{-8}	0.90×10^{2}

* Tested in T_4 lymphocytes (CEM cell line); after incubation, cell viability was determined microscopically and by tetrazolium salt procedure.

1 Average of 4 assays. IC_{50} : inhibitory concentration, concentration of test compound required to inhibit 50% cell growth compared to control. EC_{50} : effective concentration, concentration of test compound required to protect 50% cells from HIV-1 induced cell death compared to control. The IC_{50} and EC_{50} values of AZT were obtained from the National Cancer Institute.

2 TI : therapeutic index.

slightly lesser than that of AZT. The chirality difference at P of the phosphotriester diastereomers **8(a&b)** showed a ten-fold difference in the activity while a similar difference was not noticed for **5(a&b)**.

Experimental Section

Acetonitrile, pyridine of anhydrous grade (Sure/Seal™), (β-cyanoethyl)N,N-diisopropylchlorophosphoramidite and 1H-tetrazole were purchased from Aldrich Chemical Co. Steroids were purchased from Steraloids, Inc. AZT was obtained from Division of AIDS, National Institute of Health (NIH). Tetrahydrofuran (THF) and toluene were freshly distilled from Na/Benzophenone and N,N-diisopropylethyl amine from CaH_2, under N_2 atmosphere. Reaction flasks were dried by flame heating and cooling under Nitrogen. AZT, steroids and 1H-tetrazole were dried just before the reaction by repeated coevaporation with pyridine followed by toluene. Thin layer chromatography (TLC) was performed on a precoated silica gel plastic sheets $60F_{254}$ (0.2 mm) EM Reagents and compounds were detected under short wavelength UV light and also by heating, after spraying 3% sulfuric acid in ethanol (v/v). Silica gel 60 (70-230 mesh ASTM) EM Science, was used for column chromatography. Products were purified on a chromatotron, Harrison Research Ltd., using rotars (2 mm) coated with silica gel $60GF_{254}$ EM Reagents. Duration of the

reactions was monitored by TLC in ethyl acetate- hexane (1:1), unless otherwise mentioned. Organic solvent extracts were dried over anhydrous Na_2SO_4 and concentrated by evaporating under vacuum at 30°C.

Melting points (uncorrected) were recorded using a Mel-Temp apparatus. IR spectra were recorded in chloroform on a Shimadzu-460 infrared spectrophotometer. ^1H NMR spectra were recorded in $CDCl_3$ on a Bruker/IBM-SY200 spectrometer at 270 MHz using tetramethylsilane (TMS) as an internal standard. ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker-270SY at 67.93 MHz using TMS as an internal standard. ^{31}P NMR spectra were recorded in $CDCl_3$ on Bruker/IBM-WP270SY spectrometer at 109 MHz using TMS and 85% phosphoric acid as internal and external standards respectively. The chemical shifts of NMR spectra were expressed in parts per million (ppm). FAB MS spectra were obtained on a Finnigan MAT95Q using a 3:1 mixture of dithiothreitol and dithioerythritol as matrix. Steroid protons were denoted with double prime in the text.

3′-Azido-3′-deoxythymidine-5′-[(cholest-5-en-3β-yl) (β-cyanoethyl)] phosphate (5a,b).

METHOD A :

A mixture of **1** (0.3 g, 1.12 mmol) and N,N-diisopropylethyl amine (0.195 ml, 1.34 mmol) in acetonitrile (2 ml) was cooled to 0°C. A precooled (0°C) solution of (β-cyanoethyl)N,N-diisopropylchlorophosphoramidite (0.3 ml, 1.34 mmol) in acetonitrile (1 ml) was added in portions with vigorous stirring during 2 min. Approximately 2 min. after addition of the phosphitylating agent, turbidity of the reaction mixture disappeared. The reaction mixture was stirred for 10 min. at 0°C. TLC controls showed complete conversion of **1** into **2** (R_f **1**, 0.14; **2**, 0.43). To the solution of **2**, a mixture of **3** (0.304 g, 0.786 mmol) and 1H-tetrazole (0.378 g, 5.39 mmol) in 15 ml of THF was added at 0°C during 3 min. The cooling bath was immediately removed and continued stirring for another 10 min. Again, TLC controls showed complete conversion of **2** into **4** (R_f **4**, 0.51; **3**, 0.71). **4** was oxidized by adding standard 0.1 M iodine reagent until iodine color persisted. After 30 min. excess iodine was destroyed by stirring the reaction mixture with a small amount of $NaHSO_3$ crystals. The reaction mixture was concentrated and extracted with ethyl acetate (100 ml). The extract was washed with water (3 x 100 ml), dried and concentrated to give a solid which showed single spot on TLC, R_f 0.15. The product was initially purified on a short silica gel column by eluting with ethyl acetate to eliminate base line impurities. Further purification on chromatotron gave colorless crystalline solid (0.278 g) of phosphotriester **5**. By repeated elution on chromatotron, **5** was resolved into **5a** and **5b** (R_f, **5a**, 0.69; **5b**, 0.64, ethyl acetate / hexane 3:1). Mp 120-2°C (**5a,b**). **MS FAB**

m/e 743.420 ($C_{39}H_{62}N_5O_7P$ requires 743.439) (MH^+ - CN, 85). **5a : IR** ν 2112 cm^{-1}; **^{1}H NMR** δ 0.65 (s, 3H, 18''-H), 0.84 (d, 3H, *J* = 4.92 Hz, 27''-H), 0.85 (d, 3H, *J* = 4.92 Hz, 26''-H), 0.89 (d, 3H, *J* = 6.34 Hz , 21''-H), 0.98 (s, 3H, 19''-H), 1.03-1.83 (m, steroid protons), 1.93 (s, 3H, 5-CH_3), 2.35-2.44 (m, 4H, 2'- & 7''-H), 2.75 (t, 2H, *J* = 5.86 Hz , CH_2CN), 3.99-4.02 (m, 1H, 4'-H), 4.19-4.37 (m, 6H, OCH_2, 3''-, 3'- & 5'-H), 5.37 (m, 1H, 6''-H), 6.18 (t, 1H, *J* = 6.35 Hz, 1'-H), 7.33 (m, 1H, 6-H) 8.86 (bs, 1H, NH); **^{13}C NMR** : see Table 1; **^{31}P NMR** δ -1.72. **5b: IR** ν 2112 cm^{-1}; **^{1}H NMR** δ 0.64 (s, 3H, 18''-H), 0.83 (d, 3H, *J* = 4.92 Hz, 27''-H), 0.85 (d, 3H, *J* = 4.92 Hz, 26''-H), 0.89 (d, 3H, *J* = 6.35 Hz, 21''-H), 0.98 (s, 3H, 19''-H), 1.00-1.82 (m, steroid protons), 1.92 (s, 3H, 5-CH_3), 2.28-2.47 (m, 4H, 2'- & 7''-H), 2.76 (t, 2H, *J* = 5.86 Hz, CH_2CN), 3.98-4.03 (m, 1H, 4'-H), 4.19-4.36 (m, 6H, OCH_2, 3''-, 3'- & 5'-H), 5.36 (m, 1H, 6''-H), 6.19 (t, 1H, *J* = 6.35 Hz, 1'-H), 7.33 (m, 1H, *J* = 0.98 Hz, 6-H), 8.87 (bs, 1H, NH); **^{13}C NMR** : see Table 1; **^{31}P NMR** δ -1.96.

METHOD B :

A mixture of **3** (0.3 g, 0.776 mmol) and N,N-diisopropylethyl amine (0.162 ml, 0.931 mmol) in THF (15 ml) was cooled to 0°C. To this mixture, acetonitrile solution of (β-cyanoethyl)N,N-diisopropylchlorophosphoramidite (0.207 ml, 0.931 mmol) was added with vigorous stirring during 2 min. at 0°C. The reaction mixture became transparent after approximately 2 min. and was stirred for a further 10 min. at 0°C. **3** was completely converted into **9** (R_f **3**, 0.14; **9**, 0.55, ethyl acetate / hexane 1:9). A mixture of **1** (0.1657 g, 0.621 mmol) and 1H-tetrazole (0.2608 g, 3.723 mmol) in acetonitrile (5 ml) was added at once to the reaction mixture at 0°C followed by removing the cooling bath and the stirring was continued for 10 min. TLC controls showed complete conversion of **9** into **4**. Usual oxidation of **4** gave the phosphotriesters **5(a,b)**. After work-up and purification, as described above, **5** (**a** and **b**) was obtained as colorless crystalline solid (0.407 g). These were similarly separated into **5a** and **5b** by chromatotron.

3'-Azido-3'-deoxythymidine-5'-[(β-cyanoethyl) (24α-ethylcholesta-5,22*E*-dien-3β-yl)] phosphate (8a,b).

METHOD A :

This was prepared by applying Method A used for the preparation of **5(a,b)**. The progress of the reaction steps was followed by TLC (R_f **8**, 0.06; **4**, 0.42; **6**, 0.69). Thus, 0.3 g of **1** gave 0.3479 g of **8** as colorless crystalline solid. **8** was similarly separated into **8a** and **8b** (R_f **8a**, 0.72; **8b**, 0.68, ethyl acetate / hexane 3:1). Mp. 122-4°C **8(a,b)**. **MS FAB** m/e 769.416 ($C_{41}H_{64}N_5O_7P$ requires 769.454) (MH^+ - CN, 68). **8a : IR** ν 2112 cm^{-1}; **^{1}H NMR** δ 0.64 (s, 3H, 18''-H), 0.73 (d, 3H, *J* = 7.25 Hz, 27''-

H), 0.78 (t, 3H, J = 7.25 Hz, 29''-H), 0.81 (d, 3H, J = 7.25 Hz, 26''-H), 0.96 (s, 3H, 21''-H), 0.98 (s, 3H, 19''-H), 1.00-1.86 (m, steroid protons), 1.90 (d, 3H, J = 0.98 Hz, 5-CH_3), 2.02-2.46 (m, 4H, 2'- & 7''-H), 2.72 (t, 2H, J = 5.86 Hz, CH_2CN), 3.96-4.00 (m, 1H, 4'-H), 4.18-4.34 (m, 6H, OCH_2, 3''-, 3'- & 5'-H), 5.01 (dd, 1H, J = 15.13 & 8.3 Hz, 23''-H), 5.10 (dd, 1H, J = 15.13 & 8.3 Hz, 22''-H), 5.34 (m, 1H, 6''-H), 6.15 (t, 1H, J = 6.35 Hz, 1'-H), 7.30 (m, 1H, J = 0.98 Hz, 6-H), 8.68 (bs, 1H, NH); **^{13}C NMR** : see Table 1; **^{31}P NMR** δ -1.72. **8b : IR** ν 2112 cm^{-1}; **^{1}H NMR** δ 0.66 (s, 3H, 18''-H), 0.75 (d, 3H, J = 7.25 Hz, 27''-H), 0.78 (t, 3H, J = 7.25 Hz, 29''-H), 0.83 (d, 3H, J = 7.25 Hz, 26''-H), 0.98 (s, 3H, 21''-H), 1.00 (s, 3H, 19''-H), 1.07-1.81 (m, steroid protons), 1.92 (d, 3H, J = 0.98 Hz, 5-CH_3), 2.31-2.43 (m, 4H, 2'- & 7''-H), 2.76 (t, 2H, J = 5.86 Hz, CH_2CN), 3.99-4.03 (m, 1H, 4'-H), 4.19-4.36 (m, 6H, OCH_2, 3''-, 3'- & 5'-H) 4.98 (dd, 1H, J = 15.13 & 8.3 Hz, 23''-H), 5.13 (dd, 1H, J = 15.13 & 8.3 Hz, 22''-H), 5.36 (m, 1H, 6''-H), 6.18 (t, 1H, J = 6.35 Hz, 1'-H), 7.33 (m, 1H, J = 0.97 Hz, 6-H), 9.05 (bs, 1H, NH); **^{13}C NMR** : see Table 1; **^{31}P NMR** δ -1.96.

METHOD B :

This was prepared by applying METHOD B used for the preparation of **5(a,b)**. The intermediate stages of the reaction was followed by TLC (R_f **6**, 0.41; **10**, 0.77, ethyl acetate / hexane 1:4). Thus, 0.156 g of **1** gave 0.349 g of **8(a,b)**.

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31. All the yields reported were overall yields with respect to **1**.

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