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APPLICATION OF THE PHOSPHORAMIDITE-PHOSPHITE TRIESTER APPROACH FOR THE SYNTHESIS OF COMBINATIONS BETWEEN OXYGENATED STEROLS AND NUCLEOSIDE ANALOGUES LINKED BY PHOSPHODIESTER BONDS

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Abstract

The recently developed phosphoramidite-phosphite triester approach applied in solid phase DNA synthesis seems to be the technique of choice for the preparation of labile multifunctional compounds linked by phosphodiester bonds in solution. Thus, to make the lipophilic oxysterols water-soluble for biological studies, we have prepared several combinations between polyoxygenated sterols and nucleoside analogues using (β-cyanoethoxy)bis(diisopropylamine) phosphine **2** as phosphorylating agent. This approach afforded the desired compounds of type **1** under very mild conditions and in reasonably high yields (> 60%).

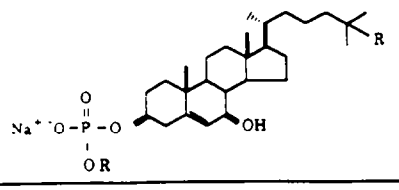
Among the numerous studies devoted to drug carriers for the target cells, the use of macromolecules as drug vectors (1,2) and the preparation of suitable prodrugs from leading compounds are two principal approaches (3,4).

In our research program on oxygenated sterols, we are interested, in particular, in the latter approach for modifying the physicochemical properties of these lipophilic compounds and thereby making them bioavailable. Previously, we have demonstrated that several cholesterol derivatives bearing hydroxyl groups on the nucleus or on the side chain, among them 7β-hydroxycholesterol, 7β,25- dihydroxycholesterol and 7α,22S-dihydroxycholesterol, are selectively cytotoxic towards tumor cells in vitro (5,6). However their high lipophilicity makes them difficult to be studied in vivo. The use of their bishemisuccinate derivatives, which are moderately water-soluble, has given interesting results only in some cases (7). Therefore, recent work has been carried out with derivatives of oxysterols conjugated with nucleoside analogues by a phosphodiester linkage. Such a coupling of two compounds, which are physicochemically quite different, constitutes a very important strategy to synthesize amphiphilic molecules which could improve hydrophilicity of oxysterols and, in the meanwhile, enhance the lipophilicity of nucleosides. By hydrolysis in biological condition, the compound of type **1** (Figure 1) should give a monophosphate which could be a 5'-phosphate nucleoside (8,9). This is of particular interest with some antitumoral nucleosides, as they should be transformed to the 5'-nucleotide as active entity by intracellular kinase (10). So, from a point of view of chemotherapy, such conjugated compounds may overcome the problem of drug resistance due to the lack of kinase for some tumor cells (11,12).

In principle, the phosphorus containing compounds could be synthesized based on P(V) and P(III) chemistry. Since the introduction of phosphotriester methodology by Letsinger (13,14), the synthesis of various phosphodiester compounds in particular oligonucleotides has been quickly

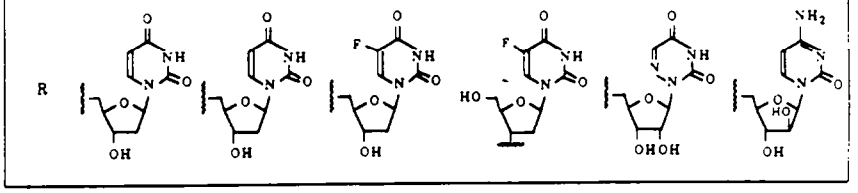
developed in conjunction with the development of new condensation agents (15,16), protecting groups(15) and purification procedures.

Figure 1



1 (a - f)

Compounds	1a	1b	1c	1d	1e	1f
R	H	OH	H	H	H	H

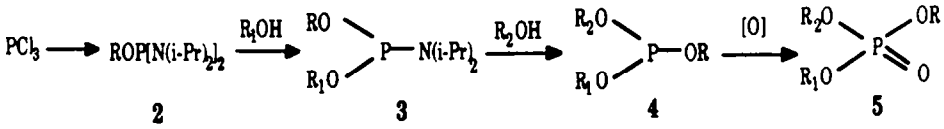


We have initially explored the feasibility of synthesizing the compounds of type **1** by using phosphodi- or triester methodology. The sterols **18** were firstly phosphorylated with 2-chlorophenylphosphorodi (1,2,4-triazolide) (17) and then coupled with 5'-OH of nucleosides directly or using MSNT (1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole) or DMAP (dimethylamino-pyridine) as a condensing agent. By this approach, another side product corresponding a symmetrical phosphotriester : 2-chlorophenyl bis-3-(7β-triethylsiloxycholesteryl) phosphotriester (~8%) was obtained in the first step together with the desired compound : 2-chlorophenyl 3-(7β-triethylsiloxycholesteryl phosphodiester (26%). Although the latter compound could be obtained in a relatively high yield (~70%) by using 2-chlorophenyl phosphorobishydroxybenzotriazolide (18) as a phosphorylating reagent, the second step to couple with nucleoside gave a low yield (<30%). Moreover, after removing the phosphorus-protecting group : 2-chlorophenyl by a treatment with oximate (19), a delicate purification, by chromatography on silical gel, of final phosphodiester **1a** or **1b** as ionic salt made this approach difficult to be carried out (20).

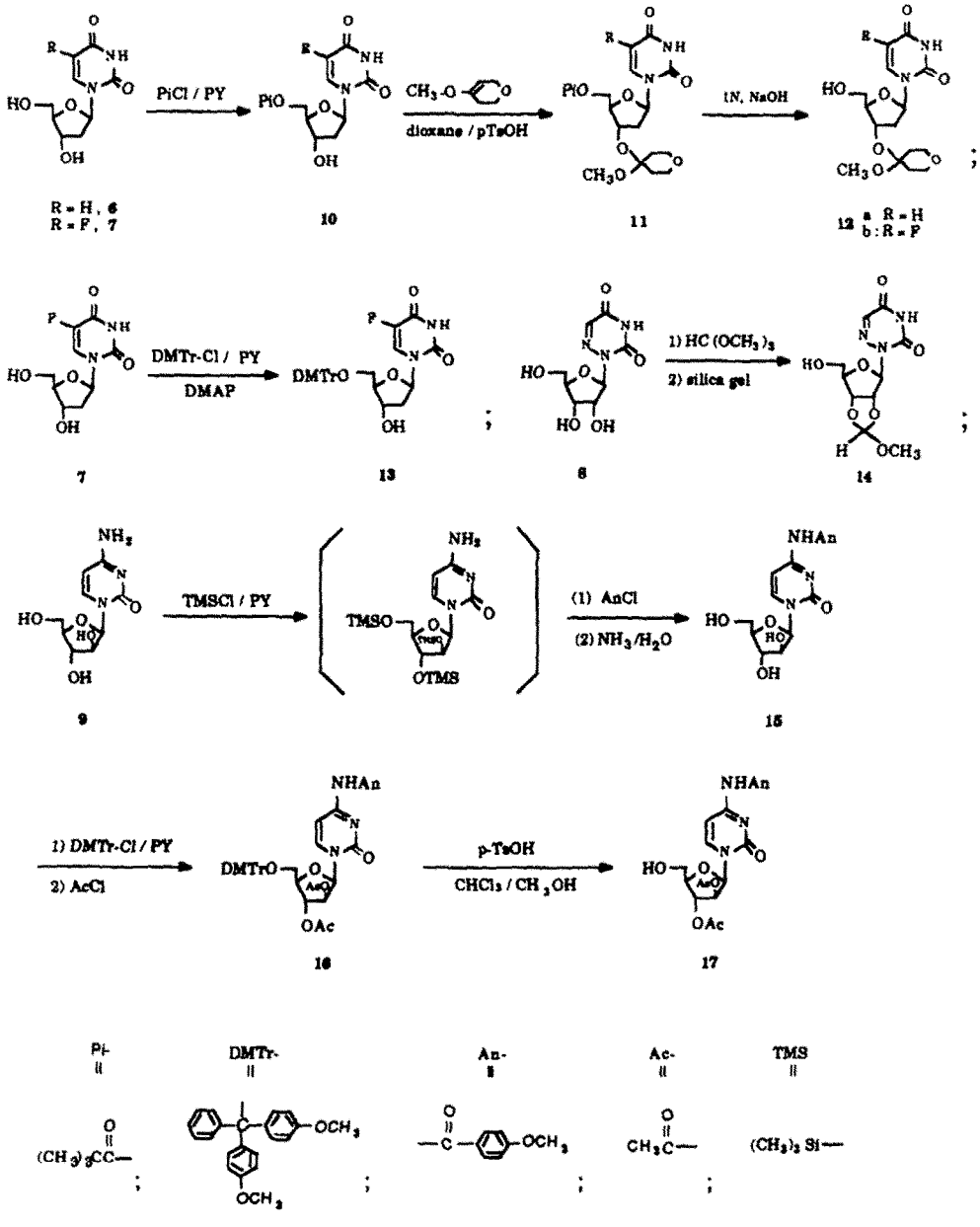
Due to the recent development of the phosphite triester approach by Letsinger et al (21), the strategy for synthesizing biological phosphorus-containing compounds can be modified in such way that P(III)-chemistry can be applied. Furthermore, the introduction of phosphoramidites by Caruthers (22,23) made this technique the method of choice for such compounds.

The principle of phosphoramidite method is outlined in scheme I. The procedure is initiated by the reaction of alkoxy bis(dialkylamino)phosphines **2**, which are easily prepared from PCl_3 (24), with an alcohol (R_1OH) in the presence of diisopropylammonium tetrazolide to give the phosphoramidite **3**. Following activation by a weak acid such as tetrazole, the intermediate **3** is

Scheme I



Scheme II


PiCl / PY
dioxane / pTsOH
1N, NaOH
R = H, 6
R = F, 7
10
11
12 a: R = H
b: R = F
DMTr-Cl / PY
DMAP
7
13
8
1) HC(OCH3)3
2) silica gel
14
TMSCl / PY
9
(1) AnCl
(2) NH3/H2O
15
1) DMTr-Cl / PY
2) AcCl
16
p-TsOH
CHCl3 / CH3OH
17
Pi-
DMTr-
An-
Ac-
TMS

allowed to couple with another alcohol (R_2OH) and affords the phosphite triester **4**, which could be transformed to the corresponding phosphotriester **5** by an additional oxidation. The whole procedure is carried out under very mild conditions and in general, gives an overall yield higher than that obtained via phosphodiester and triester methods.

In this type of chemistry, another challenging problem is the use of appropriate protecting groups, which allow the selective phosphorylation of a desired hydroxyl group in a polyfunctional molecule. In our case, it concerns the selective protection of allylic 7β-hydroxy group of sterol, thus making the 3-hydroxy group accessible to a phosphorylating reagent, and the partial protection of different hydroxy or amino groups of nucleoside.

Owing to the progress made in the oligonucleotide chemistry, a great deal of protecting groups were developed. Most of them can be removed in basic or acidic conditions. However, only a few of them are suitable for our purpose, because of the labile allylic 7β-hydroxy group to be eliminated in acidic condition and of the easy hydrolysis of phosphotriester **5** under basic condition. All this emphasizes that the appreciated protecting groups should be cleaved off under very mild conditions.

The 7β-hydroxy group of sterol **18** was selectively protected as triethylsilyl ether as described (25,20). The deprotection was performed with 0.36%~0.18% HCl in THF without any elimination of the allylic 7β-hydroxyl group.

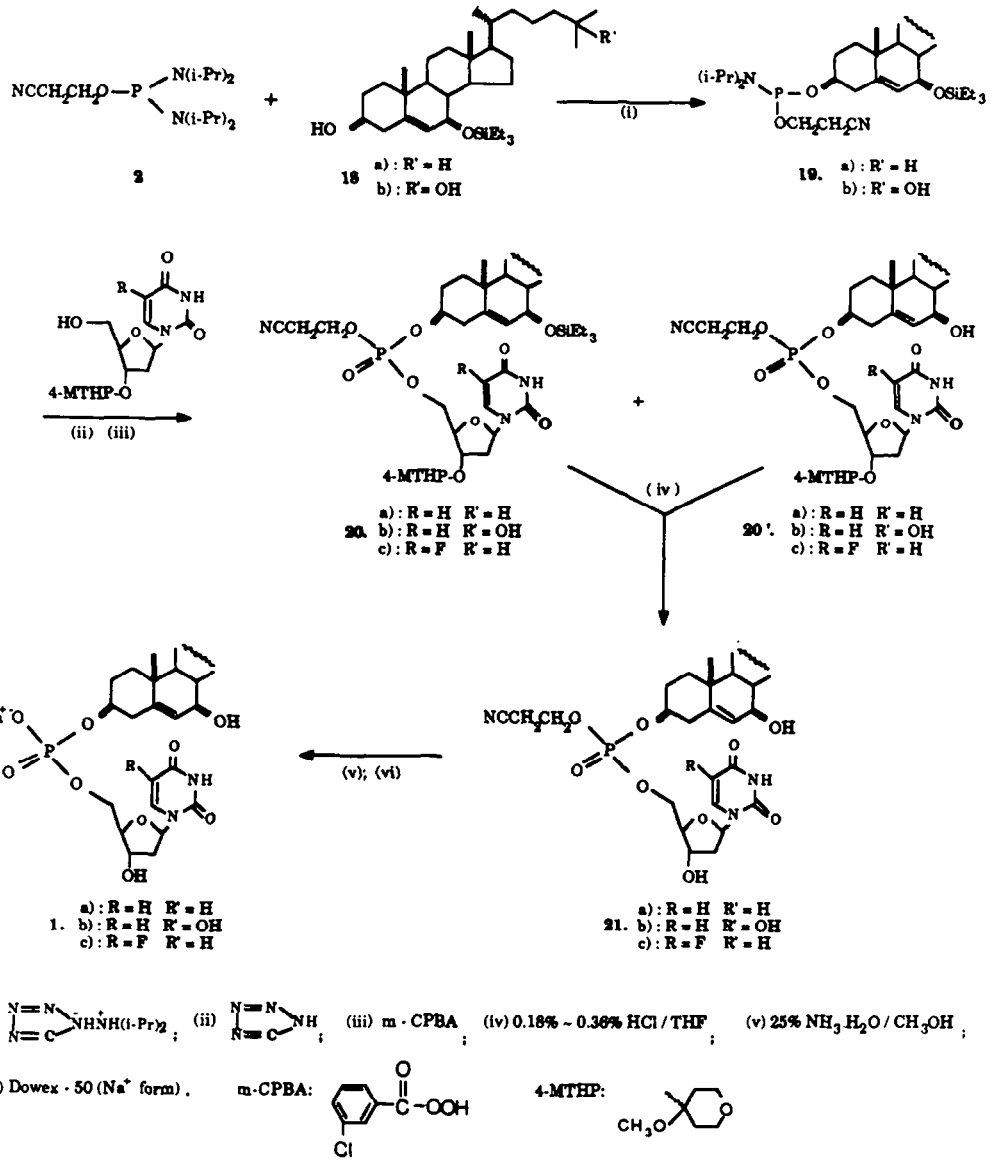
The 3'-hydroxy function of deoxynucleoside (**7** and **8**) was protected by an indirect procedure. Firstly, the 5'-hydroxy group was selectively transformed to pivaloyl ester (26) **10** under the controled conditions : at -30°C and for 3 hours, to avoide the formation of 3',5'-diacylated compound. The intermediate **10** reacted subsequently with an excess of 4-methoxy-5,6-dihydro-2H-pyran (27) in the presence of p-TsOH to give the compound **11**. The specific hydrolysis of pivaloyl ester with 1N NaOH afforded **12** with free 5'-OH and 3'-OH protected as a ketal. We used 4-methoxytetrahydropyranyl but not tetrahydropyranyl as protecting group, because the first one could be removed under the same conditions as the 7β-triethylsilyl ether and no diasteromers are formed. However, the latter one must be cleaved off under more acidic conditions in which the elimination of 7β-OH group of sterol might occur.

The 5'-hydroxy function was protected as 4,4'-dimethoxytrityl (DMTr) ether (28) (Scheme II). This protecting group can be introduced regioselectively into the 5'-position of nucleosides and can be removed under weakly acidic conditions.

The protection of vicinal 2'- and 3'-hydroxy groups of nucleoside **8** was accomplished by the formation of the 2',3'-methoxymethylidene (29) derivative **14** . 6-Aza-uridine underwent acid-catalysed orthoester exchange with trimethyl orthoformate to give a mixture of 2' or 3'-mono and 5'- and 2',3'-bisorthoester. By an excess of acid-catalysed treatment with silical gel, the reaction mixture was converted to an unique derivative, 2',3'-methoxymethylidene-6-aza-uridine **14**. The compound **14** could be transformed to 2'- or 3'-O-formyl nucleoside by acid, under very mild conditions. Such formate esters are then easily hydrolysed and give the parent nucleoside.

For cytosine arabinoside (aracytidine), the introduction of protecting group into the exocyclic amino function is performed via a transient protection in an one-pot procedure (30). The 2',3',5'-hydroxy

Scheme IIIA



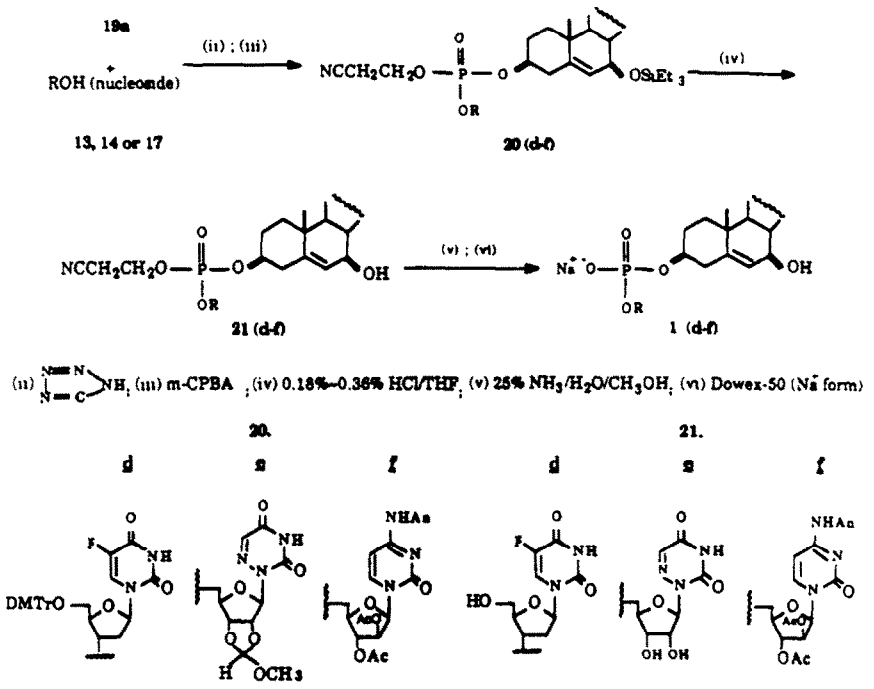
(i) ; (ii) ; (iii) m-CPBA ; (iv) 0.18% ~ 0.36% HCl / THF ; (v) 25% NH_3 H_2O / CH_3OH ;

(vi) Dowex - 50 (Na^+ form).

m-CPBA:

4-MTHP:

Scheme IIIB



functions were converted intermediately to trimethylsilyl ethers. After acylation with 4-methoxybenzoyl chloride and cleavage of the silyl ethers, the N-protected nucleoside **15** was obtained. A regioselective formation of 5'-dimethoxytrityl derivative allowed a further acylation of 2'- and 3'-hydroxy functions as acetyl esters. A subsequent treatment with p-TsOH regenerated the free 5'-hydroxyl group and afforded the partially protected aracytidine **17** to be phosphorylated.

All the introduced protecting groups linked as ester or amide are easy to be cleaved off by a treatment with ammonia.

For the reagent of type **2**, a diversity of phosphorus protecting group R was reported (24). They should be stable during all steps involved in the phosphorylation and should be removed at the end of the synthesis to give an ionic salt of phosphodiester. For our purpose, the reason for the choice of β-cyanoethyl as R is its easy cleavage by a quantitative β-elimination in concentrated ammonia solution. Followed by a simple evaporation, the desired phosphodiester was obtained as its ammonium salt, without further purification. Furthermore, for the linkage of some nucleoside such as aracytidine, all acyl protecting groups can be removed together with β-cyanoethyl group.

The general procedure for the preparation of phosphodiester of type **1** is illustrated in the scheme III (A and B).

At first, (β-cyanoethoxy)bis(diisopropylamino) phosphine **2** was reacted with the 3-hydroxy function of oxysterols **18** in the presence of diisopropylaminonium tetrazolide as activating agent. The phosphoramidite **19** was relatively stable and could be purified by a short column

chromatography on silica-gel. The activation of **19** by a weak acid such as tetrazol allowed a further coupling with a partially protected nucleoside. After an oxidation with m-chloroperbenzoic acid (m-CPBA), the corresponding phosphotriester **20** was obtained as a mixture of diastereoisomers at P level. In this step, we isolated another slightly more polar compound, which corresponded to the partially triethylsilyl-deprotected phosphotriester **20'**. Then, a treatment with 0.36% HCl in THF at 0°C led to the cleavage of all acid labile protecting groups. The removal of base labile protecting groups was achieved by a treatment with concentrated aqueous ammonia in CH_3OH and gave the ammonium salts of phosphodiester of type **1**. A subsequent ion-exchange chromatography converted them to sodium salts. In some cases, a further purification by a gel filtration on sephadex LH-20 was needed. These procedures afforded the desired compounds with an overall yield, in general, higher than 60%

Experimental

General Pyridine and CH_3CN were dried by reflux over CaH_2 for several hours and distilled just before use. Et_2O was dried over Na. CH_2Cl_2 was dried over Al_2O_3. DMF was distilled under reduced pressure and stored over molecular sieves (4Å) in tightly closed bottles. Tetrazole (Fluka) was purified by sublimation. All the commercial reagents were purchased from Aldrich or Fluka. The short-column chromatography separation was carried out by using silica gel (40-63 µm, Merck G60). TLC's were run on pre-coated plates of silica gel (60F254, Merck) and HPTLC-silica plates (Merck). The plates were dipped in a solution of vanillin 1g/l) in EtOH-H_2SO_4 (95-5) and heated on a hot plate to detect the compounds. Phosphorus-containing compounds were also visualized on TLC by Zinzadze solution (31,32). Dowex-50 X 8 resin (20-50 mesh. sodium salt) was used for ion-exchange chromatography. Sephadex LH-20 (Pharmacia) was used for gel permeation chromatography. Evaporation was performed under reduced pressure at 30°C. ^{1}H-NMR spectra were recorded with a Brucker SY (200 or 400 MHz) apparatus with TMS as internal standard. ^{13}C-NMR spectra were recorded with the same apparatus taking $CDCl_3$ (76.9 ppm) or CD_3OD (50.2 ppm) as internal standard. Mass spectra were run on a LKB 9000S apparatus by direct introduction using an ionization potential of 70 eV. FAB-MS were obtained on a VG analytical ZAB-HF double-focusing mass spectrometer using triethanolamine (TEA), 1-thioglycerol, or m-nitrobenzyl alcohol (m-NBA) as matrix. $[\alpha]_D$ were measured on a Perkin-Elmer 141 polarimeter. Melting point (mp) were measured on a Reichert microscope and are uncorrected. Microanalyses were performed by the Service Central de Microanalyse du CNRS (Vernaison) and the Strasbourg Local Section.

Protection of oxysterol

7β-Triethylsilyloxycholesterol (18a) Compound **18a** was prepared as described in Ref. (20).

7β-Triethylsilyloxy-25-hydroxycholesterol (18b) 7β,25-Dihydroxycholesteryl 3β-acetate (460 mg, 1 mmol) was dissolved in dry DMF (5 ml). Diisopropyl- ethylamine (218 µl, 1.25 mmol) and triethylsilyl chloride (185 µl, 1.1 mmol) were added, and the mixture was kept overnight at room temperature under anhydrous conditions. The reaction was quenched by addition of 10% $NaHCO_3$ (3 ml) and extracted with ether (3 x 25 ml). The organic layer was washed with brine and concentrated under vaccum. The crude product was then dissolved in THF (10 ml). 1N NaOH (5 ml) and tetrabutylammonium bromide (100 mg) were added for hydrolysis. This mixture was vigorously stirred for three days at r.t.. After being diluted with ether (100 ml) and washed with brine until pH was stabilized at 7~8, the organic phase was dried over Na_2SO_4 and concentrated to dryness. The oily residue was chromatographed on silica gel using AcOEt / hexane (10/90--->15/85) as eluent. Compound **18b** was obtained in 99% yield (532 mg). **mp** = 63-65°C. $[\alpha]_D$ = +28° (c = 0.63% in $CHCl_3$). **^{1}H-NMR** ($CDCl_3$): 0.68 (s, 3H, CH_3-18); 0.56~0.68 (m, 6H, Si($\underline{CH_2}CH_3)_3$); 0.93~1.01 (m, 12H; 3H: CH_3-21, 9H: Si ($CH_2\underline{CH_3})_3$); 1.05 (s, 3H, CH_3-19); 1.22 (s, 6H, CH_3-26,27); 3.54 (m, 1H, H-3); 3.94 (d, 1H, J = 7.9 Hz, H-7); 5.26 (s, 1H, H-6). **^{13}C-NMR** ($CDCl_3$): see Table 1. **MS** (EI, 70eV), m/e: 533 (MH^+, 25); 532 (M^+, 54); 514 (18); 400 (100); 383 (18); 365 (18). **Anal.** Found. C, 74.0; H, 11.3; Si, 4.8. Calc. for $C_{33}H_{60}O_3Si$ (532.9): C, 74.37; H, 11.35; Si, 5.25.

Protection of (deoxy) nucleoside

5'-O-Pivaloyl-2'-deoxyuridine (10) Pivaloyl chloride (1.24 ml, 11 mmol) was added to a solution of 2'-deoxyuridine (2.28 g, 10 mmol) in anhydrous pyridine (50 ml). The reaction was kept at -20°C for 3 h under Ar and quenched by introducing 1 ml of water. After a further stirring for 15 min, the reaction mixture was evaporated to dryness under reduced pressure. The residue was applied to a short-column chromatography over silica gel (CH_2Cl_2 /CH_3OH:

95/5---> 92/8). 5'-O-Pivaloyl-2'-deoxyuridine (3.06 g) was obtained in 98% yield. **mp** = 154-155°C. $[\alpha]_D$ = +44° (c = 0.1% in CH_3OH). **^{1}H-NMR** (CD_3OD): 1.24 (s, 9H, $(CH_3)_3CO$); 2.15~2.47 (m, 2H, H-2'); 4.10~4.16 (m, 1H, H-4'); 4.29~4.33 (m, 2H, H-5'); 4.35~4.40 (m, 1H, H-3'); 5.73 (d, 1H, J = 8.1Hz, H-5); 6.24 (t, 1H, J = 6.6Hz, H-1'); 7.69 (d, 1H, J = 8.1Hz, H-6). **^{13}C-NMR** (CD_3OD): see Table 1. **MS** (EI, 70eV), m/e: 312 [M^+, 16]; 285 [(MH^+ - CO), 100]; 256 [(MH^+ - $(CH_3)_3C$)], 40]; 228 [(MH^+ - $(CH_3)_3CCO$), 18]; 201 [(M - base)$^+$, 89]. Anal. Found. C, 54.0; H, 6.4; N, 9.0. Calc. for $C_{14}H_{20}N_2O_8$ (312.3): C, 53.84; H, 6.41; N, 8.97.

5'-O-Pivaloyl-3'-O-(4-methoxytetrahydropyranyl)-2'-deoxyuridine (11) 4-Methoxy-5,6-dihydro-2H-pyran (5 g, 43.8 mmol) was added to a stirred solution of 5'-O-pivaloyl-2'-deoxyuridine (3.4 g, 11 mmol) and p-toluene sulphonic acid (209 mg, 1.1 mmol, freshly melted on a flame) in anhydrous dioxane (50 ml). After stirring for 3h at r.t. and under Ar., the reaction mixture was poured into a solution of 20% $NaHCO_3$ (5 ml) and extracted with CH_2Cl_2 (3x50 ml). The combined organic layers were dried over Na_2SO_4 and evaporated. A purification of the residue by chromatography on a silica gel column (CH_2Cl_2/CH_3OH: 96/4) yielded compound 11 (4.21 g) in 90% yield. **mp** = 60~61°C. $[\alpha]_D$ = +63.1° (c = 0.69% in $CHCl_3$). **^{1}H-NMR** ($CDCl_3$): 1.24 (s, 9H, $(CH_3)_3C$); 1.69~1.81 (m, 4H, $2CH_2$-3" of 4-methoxypyranyl); 2.03~2.16, 2.44~2.56 (2m, 2H, H-2'); 3.22 (s, 3H, CH_3O); 3.57~3.82 (m, 4H, $2CH_2$-2" of 4-methoxypyranyl); 4.21~4.27 (m, 1H, H-4'); 4.28~4.31 (m, 2H, H-5'); 4.41~4.48 (m, 1H, H-3'); 5.75 (d, 1H, J = 8.1Hz, H-5); 6.24 (t, 1H, J = 6.6Hz, H-1'); 7.54 (d, 1H, J = 8.1Hz, H-6). **^{13}C-NMR** ($CDCl_3$): see Table 1. **FAB-MS** negative (matrix: TEA): 851.5 [(2M - H)$^-$, 47]; 574.3 [(M - H+TEA)$^-$, 28]; 425.2 [(M - H)$^-$, 100]; 392.3 [(M - H - CH_3OH)$^-$, 8]. **FAB-MS** positive (matrix: 1-thioglycerol): 449.2 [MNa^+, 5]; 427.2 [MH^+, 2]; 295.1 [(M-131), 100]. **Anal.** Found. C, 56.0; H, 7.0; N, 6.6 Calc. for $C_{20}H_{30}N_2O_8$ (426.46): C, 56.28; H, 7.03; N, 6.57.

3'-O-(4-Methoxytetrahydropyranyl)-2'-deoxyuridine (12a) 1N NaOH (20 ml) was added to a solution of compound 11 (3.83 g, 9 mmol) and tetrabutylammonium bromide (300 mg) in THF (60 ml). The reaction mixture was stirred vigorously for 3 h and then concentrated under vaccum. The continuous coevaporation with toluene for several times gave a dried residue which was purified by chromatography on silica gel (CH_2Cl_2/CH_3OH: 96/4--->90/10) and afforded the compound 12 (2.74g, 89%). **mp** = 65°C (dec.). $[\alpha]_D$ = +42.9° (c = 1.34% in $CHCl_3$). **^{1}H-NMR** (CD_3OD): 1.80~1.93 (m, 4H, $2CH_2$-3" of 4-methoxypyranyl); 2.17~2.45 (m, 2H, H-2'); 3.27 (s, 3H, CH_3O); 3.64~3.72 (m, 4H, $2CH_2$-2" of 4-methoxypyranyl); 3.75~3.80(m, 2H, H-5'); 4.05~4.10 (m, 1H, H-4'); 4.58~4.66 (m, 1H, H-3'); 5.73 (d, 1H, J = 8.1Hz, H-5); 6.27 (t, 1H, J = 6.3Hz, H-1'); 7.99 (d, 1H, J = 8.1Hz, H-6). **^{13}C-NMR** (CD_3OD): see Table 1. **FAB-MS** positive (matrix: 1-thioglycerol): 685.4 [(2M+H^+), 12]; 621.3[(2M+H^+-2CH_3OH), 30]; 343.2 [MH^+, 92]; 311.2 [(MH^+-CH_3OH), 88]; 229 [(M-115+2H^+), 100]. **Anal.** Found. C, 52.5; H, 6.4; N, 7.9. Calc. for $C_{15}H_{22}N_2O_7$ (342.35) C, 52.62; H, 6.48; N, 8.19

3'-O-(4-Methoxytetrahydropyranyl)-5-fluoro-2'-deoxyuridine (12b) This compound was prepared from 5-fluoro-2'-deoxyuridine (2.46 g, 10 mmol) by the same procedure as described for **12a**. The final product **12b** was obtained in a overal yield of 92% (2.98 g). **^{1}H-NMR** (CD_3OD): 1.76~1.90 (m, 4H, $2CH_2$-3" of 4-methoxypyranyl); 2.14~2.42 (m, 2H, H-2'); 3.23 (s, 3H, CH_3O); 3.53~3.85 (m, 6H, 4H: $2CH_2$-2" of 4-methoxypyranyl; 2H: H-5'); 4.04 (m, 1H, H-4'); 4.58 (m, 1H, H-3'); 6.22 (t, 1H, J = 6.5Hz, H-1'); 8.21 (d, 1H, J = 6.9Hz, H-6). **^{13}C-NMR** (CD_3OD): see Table 1. **FAB-MS** positive (matrix: m-NBA): 361 [MH^+, 15]; 329 [(MH^+-CH_3OH), 28]; 247.1 [(M-115+2H^+), 29]; 229.1 [(M-131)$^+$, 100]. **Anal.** Found. C, 49.9; H, 5.9; N, 7.4; F, 5.1. Calc. for $C_{15}H_{21}N_2O_7F$ (360.34): C, 49.99; H, 5.88; N, 7.78; F, 5.27.

5'-Dimethoxytrityl-5-fluoro-2'-deoxyuridine (13) 5-Fluoro-2'-deoxyuridine (228 mg, 1 mmol) was dried twice by coevaporation with pyridine and then dissolved in 5 ml of pyridine. Dimethylaminopyridine (18 mg, 0.15 mmol) and dimethoxytrityl chloride (DMTrCl) (407 mg, 1.2 mmol) were added. The reaction was kept for 4 h at r.t. and poured into 20% $NaHCO_3$ (3 ml) and extracted with Et_2O (3x20 ml). The organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by chromatography on silica gel with CH_2Cl_2/CH_3OH (97/3) as eluent. Compound **13** (498 mg) was obtained in 94% yield. **mp** = 101~103°C. $[\alpha]_D$ = +34.1° (c = 1.33% in $CHCl_3$). **^{1}H-NMR** ($CDCl_3$): 2.22~2.32, 2.41~2.53 (2m, 2H, H-2'); 3.43 (d, 2H, J = 3.3Hz, H-5'); 4.05 (m, 1H, H-4'); 4.54 (m, 1H, H-3'); 6.29 (m, 1H, H-1'); 6.84 (d, 4H, J = 8.4Hz, aromatic protons of DMTr); 7.22~7.43 (m, 7H, aromatic protons of DMTr); 7.81 (d, 1H, J = 6.1Hz, H-6). **^{13}C-NMR** ($CDCl_3$): see Table 1. **FAB-MS** negative (matrix: 1-thioglycerol): 547.5 [(M-H)$^-$, 100]; 245.2 [(M-DMTr)$^-$, 17]. **Anal.** Found. C, 65.7; H, 5.4; N, 4.9. Calc. for $C_{30}H_{29}N_2O_7F$ (548.55): C, 65.68; H, 5.33; N, 5.12.

2',3'-O-Methoxymethylidene-6-azauridine (14) 6-Azauridine (1.44 g, 5.9 mmol) and p-TsOH (0.17 g, 0.85 mmol) were added to trimethyl orthoformate (20 ml) under stirring at r.t.. A homogeneous solution occured within 45 min and it was kept overnight. The reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in CH_3OH/CH_2Cl_2 (15/85) and 2 g of silica gel was then put into this solution. After 3 days, the mixture was filtered and the silica gel was washed by the same solvent several times. The combined fractions was evaporated. A chromatography over silica gel (CH_2Cl_2/CH_3OH: 95/5) afforded the compound **14** (1.5 g) as a mixture of

Table 1 : ^{13}C-NMR of nucleoside derivatives

N C°	10	12 a	12b	13	14		15	17
solvent	CD_3OD	CD_3OD / $CDCl_3$	CD_3OD / $CDCl_3$	$CDCl_3$	CD_3OD / $CDCl_3$		CD_3OD / $CDCl_3$	$CDCl_3$
base					diastereoisomers			
2	152.02	152.24	150.86	149.42	150.07		156.11	155.07
4	166.09	166.21	159.25 a	157.65 a	159.09		162.87 *	162.89 *
5	102.80	102.75	141.73 b	140.72 b	138.42		96.21	96.08
6	152.02	142.44	125.91 c	123.85 c			148.09	145.50
sugar								
1'	86.98	86.72	86.64	85.41	93.63	94.79	87.89	85.08
2'	40.99	40.59	40.65	41.09	83.52	83.80	74.87	74.38
3'	72.10	70.97	70.52	71.93	84.88	85.65	76.81	75.86
4'	85.93	87.84	87.62	86.28	89.63	90.10	85.49	83.19
5'	65.21	62.64	62.21	63.29	64.53	64.75	61.18	61.69
others	Pi- d	4-MTHP- d		DMTr- d	CH_3OCH=		An- d	
	$\underline{C}H_3$:	$O\underline{C}H_3$:		$O\underline{C}H_3$:	$\underline{C}H_3$: 52.33; 53.40		$O\underline{C}H_3$:	
	27.58	48.60	48.56	55.25	C: 119.72; 121.06		55.05	55.56
	$\underline{C}O$:	2). 66.01	2). 85.83	$\underline{C}$: 87.17			$\underline{C}O$:166.40	$\underline{C}O$:165.80
	179.66	3). 35.70	3). 35.47	1).135.23			1). 124.77	1). 125.00
	$\underline{C}$. 39.88	4). 100.19	4). 99.96	2).130.01			2). 129.72	2). 129.77
		5). 36.07	5). 35.82	3).113.38			3). 113.67	3). 114.27
		6). 66.01	6). 85.83	4).158.72			4).163.28*	4).163.67 *
				1').144.25				CH_3CO-
				2').127.98				$\underline{C}H_3$: 20.50
				3').128.03				20.74
				4').127.11				$\underline{C}O$: 168.89
								170.21

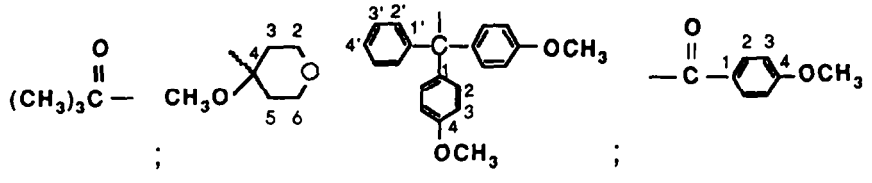
Notes of the table 1 :

a. b. c : doublet ; J_{C-F} coupling constants:

	a	b	c
12b	26.1 Hz	233.5 Hz	34.5 Hz
13	25.9 Hz	238.5 Hz	34.0 Hz

d:

Pi- 4-MTHP- DMTr- An-



Notes of the table 2 :

a, b, c : boublet, J = coupling constants.

	19 a	19b	21 c	1 c	21 d	1 d
	J_{C-P}	J_{C-P}	J_{C-F}	J_{C-F}	J_{C-F}	J_{C-F}
a	18.7 Hz	18.3 Hz	26.2 Hz	26.0 Hz		
b	18.4 Hz	18.7 Hz	236.6 Hz	234.9 Hz	235.5 Hz	235.3 Hz
c	7.1 Hz	7.0 Hz	35.9 Hz	34.5 Hz	34.3 Hz	35.4 Hz

d : see table 1.

e : For compound **21f**, different conformations or diastereoisomers at P level may exist in the ^{13}C-NMR experimental conditions and these could explain the doublet for some carbon atoms.

* : interchangeable assignments.

diastereoisomers in 91% yield. **mp** = 55~58°C. **^{1}H-NMR** (CD_3OD): 3.33, 3.41 (2s, 3H, CH_3O); 3.64, 3.67 (2m, 2H, H-5'); 4.14~4.22, 4.32~4.40 (2m, 1H, H-4'); 4.81, 4.85 (2m, 1H, H-3'); 5.15, 5.21 (2m, 1H, H-2'); 5.96, 6.05 (2s, 1H of methoxyorthoester); 6.25, 6.34 (2d, 1H, H-1'); 7.47 (s, 1H, H-5). **^{13}C-NMR** (CD_3OD): see Table 1. **MS** (EI, 70eV): 256 [(M-CH_3O)$^+$, 32]; 175 (6); 143 (21); 112 (100). **Anal.** Found. C, 41.6; H, 4.4; N, 14.4. Calc. for $C_{10}H_{13}N_3O_7$ (287.22): C, 41.81; H, 4.53; N, 14.63.

4-N-Anisoylaracytidine (15) Aracytidine (1.5 g, 6.2 mmol) was dried by repeated coevaporation with anhydrous pyridine and was suspended in 40 ml of pyridine. Under stirring, trimethylchlorosilane (12 ml, 96 mmol) was added dropwise to this suspension. After several minutes, a homogeneous solution appeared. The stirring was continued for an additional periode of 50 min and the reaction flask was placed into an ice-bath, and then anisoyl chloride (1.4 ml, 10.4 mmol) was introduced dropwise. After this addition, the reaction was removed from ice-bath and kept at r.t. for 4 h. Cold water (12 ml) was added to the chilled reaction (ice-bath) and 20 min later, the concentrated (25%) aqueous ammonia (30 ml) was added to give a soluton approximately 4N in ammonia. Following a further stirring for 30 min, the mixture was concentrated by evaporation under reduced pressure to an oil, which could be dissolved in water and be washed with ethylacetate, and the layers separated immediately. Crystallization occured within minutes. Further recrystallisation of the aqueous phase from a mixture of water and methanol gave compound **15** (2.22 g) in 95% yield. **mp** = 123~125°C. $[\alpha]_D$ = +125° (c = 0.03% in CH_3OH). **^{1}H-NMR** (DMSO-D_6): 3.61~3.64 (brd., 2H, H-5'); 3.84 (s, 3H, OCH_3); 3.85 (brd., 1H, H-4'); 3.94 (brd., 1H, H-3'); 4.08 (brd., 1H, H-2'); 6.08 (d, 1H, J = 3.9Hz, H-1'); 7.18 (d, 2H, J= 8.9Hz, H-3" of anisoyl); 7.31, (d, 1H, J= 7.5Hz, H-5); 8.03 (d, 2H, J = 8.9Hz, H-2" of anisoyl); 8.11 (d, 1H, J = 7.5Hz, H-6). **^{13}C-NMR** (DMSO-D_6): see Table 1. **MS** (EI, 70eV): 378 (MH$^+$, 25); 377 (M$^+$, 100); 135 (An$^+$, 18). **Anal.** Found. C, 49.7; H, 5.7; N, 10.1. Calc. for $C_{17}H_{19}N_3O_7 \cdot 2H_2O$ (413.37): C, 49.39; H, 5.57; N, 10.17.

4-N-Anisoyl-2',3'-diacetyl-5'-dimethoxytritylaracytidine (16) After coevaporation with anhydrous pyridine for 3 times, N-anisoylaracytidine **15** (2.3 g, 6 mmol) was dissolved in pyridine (60 ml) and DMTrCl (3.4 g, 10 mmol) and triethylamine (7 ml, 21.5 mmol) were added. The reaction mixture was kept overnight and followed by addition of acetic anhydride (3 ml, 31.8 mmol) for acetylation. After 3 h, the work-up was carried out by introducing saturated $NaHCO_3$ solution (15 ml) and extracting with CH_2Cl_2 (3x60 ml). The organic layers were dried over $MgSO_4$ and evaporated under vacuum. The crude product was purified by a short-column of silica gel eluted with $CH_2Cl_2/CH_3OH/Et_3N$ (98/1/1), (3.99 g, 87%). **mp** = 109~111°C. $[\alpha]_D$ = +182° (c = 0.02% in $CHCl_3$). **^{1}H-NMR** ($CDCl_3$): 1.83, 2.12 (2s, 6H, $2CH_3CO$); 3.42~3.54 (m, 2H, H-5'); 3.82 (s, 6H, $2CH_3O$ of DMTr); 3.90 (s, 3H, CH_3O of anisoyl); 4.23 (m, 1H, H-4'); 5.26 (m, 1H, H-3'); 5.54 (m, 1H, H-2'); 6.40 (d, 1H, J = 4.2Hz, H-1'); 6.87, 7.29~7.50 (d, m, 10H: 9H of DMTr and 1H: H-5); 7.00 (d, 2H, J = 8.9Hz, H-3" of anisoyl); 7.86 (d, 2H, J = 8.9Hz, H-2" of anisoyl); 7.90 (d, 1H, J = 7.5Hz, H-6). **MS** (EI, 70eV): 476 [(M-base-An)$^+$, 18]; 444 [(M-DMTr)$^+$, 12]; 304 [80]; 303 [DMTr$^+$, 100]; 135 [An$^+$, 53]. **Anal.** Found. C, 65.9; H, 5.5; N, 5.6. Calc. for $C_{42}H_{41}N_3O_{11}$ (763.77): C, 66.04; H, 5.41; N, 5.50.

4-N-Anisoyl-2',3'-diacetylaracytidine (17) The detritylation of compound **16** was performed as follows: the compound **16** (3.8 g, 5 mmol) was taken up in 5% p-TsOH solution (80 ml) in CH_2Cl_2-CH_3OH (9:1). Following a stirring for 10 min, the reaction was quenched by saturated $NaHCO_3$ solution (10 ml) and extracted with CH_2Cl_2 (3x50 ml). After being dried over $MgSO_4$, the organic phase was evaporated and the residue was chromatographed on silica gel and eluted with CH_2Cl_2/CH_3OH (100/0--->94/6). The appropriate fractions were concentrated and recrystalized in CH_2Cl_2 / Et_2O to give pure **17** (2.25 g, 97.6 %). **mp** = 176~177°C. $[\alpha]_D$ = +81° (c = 0.17% in $CHCl_3$). **^{1}H-NMR** ($CDCl_3$): 1.95, 2.12 (2s, 6H, $2CH_3CO$); 3.87 (s, 3H, CH_3O); 3.92~4.00 (m, 2H, H-5'); 4.11 (m, 1H, H-4'); 5.23 (dd, 1H, $J_{2,3}$ = 2.7Hz, $J_{3,4}$ = 4.5Hz, H-3'); 5.63 (dd, 1H, $J_{1,2}$ = 4.4Hz, $J_{2,3}$ = 2.7Hz, H-2'); 6.36 (d, 1H, $J_{1,2}$ = 4.4Hz, H-1'); 6.97 (d, 2H, J = 8.9Hz, H-3" of anisoyl); 7.26 (brd., 1H, H-5); 7.87 (d, 2H, J = 8.8Hz, H-2" of anisoyl); 8.10 (d, 1H, J = 7.6Hz, H-6). **^{13}C-NMR** ($CDCl_3$): see Table 1. **MS** (EI, 70eV): 462 [MH$^+$, 26]; 461 [M$^+$,100]; 342 [(MH$^+$ - 2AcOH), 66]; 312 [(MH$^+$ - NHAn), 30]. **Anal**; Found. C, 53.4; H, 5.0; N, 8.8. Calc. for $C_{21}H_{23}N_3O_9 \cdot 1/2H_2O$ (470.42): C, 53.61; H, 5.14; N, 8.93.

General procedure for the preparation of conjugated phosphodiesters

(2-Cyanoethoxy) bis(diisopropylamino) phosphine (2) The phosphorylating reagent **2** was prepared according to the procedure described in Ref. (24). At -78°C, 3-hydroxypropanenitrile (68 ml, 1 mol) was added dropwise within 90 min to a stirred solution of PCl_3 (85.5 ml, 1 mol) and dry pyridine (81 ml, 1 mol) in Et_2O (200 ml) under argon. After continuous stirring for 1 h, the temperature was allowed to raise to r.t. and the reaction mixture was kept overnight under stirring. The removal of formed precipitate of salts was performed by filtration under Ar., followed by washing twice with anhydrous Et_2O (100 ml). After evaporation of Et_2O, the concentrated oily was further dried under vaccum for 1 h and was used immediately for the next reaction.

Diisopropylamine (700 ml, 5 mol) was introduced dropwise, during 2 h, into a stirred solution of the above-cited compound in Et_2O (1 l) at -40°C. After being at -10°C for 1 h, the reaction mixture was kept overnight at r.t. with stirring. The precipitated salt was removed by a rapid filtration under anhydrous atmosphere and washed with Et_2O (150 ml) for three times. The combined Et_2O solution was evaporated and thus obtained oily residue was distilled rapidly over CaH_2

(1 g) under vacuum. The appropriate fractions with bp: 116~135°C / 0.2~0.5 mmHg gave the compound **18** (146 g, 48.5% based on PCl_3). This distillation procedure should be carried out carefully and very quickly because of the instability of the compound. **Anal.** Found. C, 59.7; H, 10.5; N, 14.0; Calc. for $C_{15}H_{32}N_3OP$ (301.42): C, 59.77; H,10.70; N, 13.94.

3-(7β-Triethylsilyloxycholesteryl) 2-cyanoethyl N,N-diisopropylphosphoramidite (19a) Repeatedly for three times, 7β-triethylsilyloxycholesterol **18a** (2 g, 3.9 mmol) was dissolved in minimum anhydrous dioxane and coevaporated with CH_3CN (freshly distilled over CaH_2) and then was taken up in anhydrous CH_2Cl_2 (60 ml). Bis(diisopropylammonium) tetrazolide (33) (344 mg, 2 mmol) as well as phosphine **2** (1.44 g, 4.8 mmol) were added. After a continuous stirring for 3 h, the reaction mixture was poured into $NaHCO_3$ saturated solution (40 ml) and extracted with CH_2Cl_2 for several times. The combined organic layer was dried over Na_2SO_4 and evaporated under vacuum. A purification of thus obtained residue by a short chromatography on silica gel (hexane/Et_2O: 9/1 + 1% Et_3N) furnished the compound **19a** as a mixture of diastereoisomers in 90% yield (2.6 g). **^{1}H-NMR** ($CDCl_3$): 0.58~0.67 (m, 6H, $Si(\underline{CH_2}CH_3)_3$); 0.67 (s, 3H, CH_3-18); 0.86, 0.87 (2d, 6H, J = 6.6Hz, $2CH_3$-26, 27); 0.90~0.99 (m, 12H, CH_3-21 and $Si(CH_2\underline{CH_3})_3$); 1.04 (s, 3H, CH_3-19); 1.18 (d, 12H, J = 6.8Hz, $N[CH(\underline{CH_3})_2]_2$); 2.15~2.36 (m, 2H, H-4); 2.64, 2.65 (2t, 2H, J = 6.5Hz, $OCH_2\underline{CH_2}CN$); 3.56~3.68 (m, 3H: H-3 and $N[\underline{CH}(CH_3)_2]_2$); 3.72~3.84 (m, 2H, $O\underline{CH_2}CH_2CN$); 3.92 (d, 1H, J = 7.9Hz, H-7); 5.22, 5.26 (2s, 1H, H-6). **^{13}C-NMR** ($CDCl_3$): see Table 2. **FAB-MS** negative (matrix: m-NBA): 782.8 [(M - i-Pr_2N - CH_2CH_2CN - H^+ + (O) + m-NBA)$^-$, 29]; 730.8 [M - H + (O))$^-$, 26]; 714.8 [(M - H)$^-$, 41]; 648.7 [(M - i-Pr_2N + 2(O))$^-$, 100]; 595.7 [(M - i-Pr_2N - CH_2CH_2CN + (O) + OH)$^-$, 91]; 579.7 [(M - i-Pr_2N - CH_2CH_2CN + OH)$^-$, 33]. **Anal.** Found. C, 70.5; H, 10.7; N, 3.9. Calc. for $C_{42}H_{77}N_2O_3SiP$ (717.12): C, 70.28; H, 10.74; N, 3.92.

3-(7β-Triethylsilyloxy-25-hydroxycholesteryl) 2-cyanoethyl N,N-diisopropylphosphoramidite (19b) Compound **19b** was synthesized from **18b** according the procedure for **19a**. A chromatography on silica gel (hexane/Et_2O: 8/2--->6/4) afforded pure **19b** in 98% yield (diastereoisomers). **^{1}H-NMR** ($CDCl_3$): 0.58~0.67 (m, 6H, $Si(\underline{CH_2}CH_3)_3$); 0.67 (s, 3H, CH_3-18); 0.91~0.99 (m, 12H, CH_3-21 and $Si(CH_2\underline{CH_3})_3$); 1.04 (s, 3H, CH_3-19); 1.19 (d, 12H, J = 6.8Hz, $N[CH(\underline{CH_3})_2]_2$); 1.21 (s, 6H, $2CH_3$-26, 27); 2.17~2.36 (m, 2H, H-4); 2.64 (2t, 2H, J = 6.6Hz, $CH_2\underline{CH_2}CN$); 3.52~3.87 (m, 5H: H-3; $N[\underline{CH}(CH_3)_2]_2$; $O\underline{CH_2}CH_2CN$); 3.92 (d, 1H, J = 8.0Hz, H-7); 5.22 (s, 1H, H-6). **^{13}C-NMR** ($CDCl_3$): see Table 2. **FAB-MS** negative (matrix: TEA): 678.5 [(M - CH_2CH_2CN)$^-$, 98]; 595.4 [(M - i-Pr_2N - CH_2CH_2CN + OH)$^-$, 100]; 217.1 [(M - St)$^-$, 26]. **Anal.** Found. C, 69.1; H, 10.9; N, 3.8; Si, 3.8; P, 4.3. Calc. for $C_{42}H_{77}N_2O_4SiP$ (733.12): C, 68.80; H, 10.58; N, 3.82; Si, 3.82; P, 4.23.

3-(7β-Triethylsilyloxycholesteryl) 5'-[3'-(4-methoxytetrahydropyranyl)-2'-deoxyuridylyl] 2-cyanoethyl phosphotriester (20a) Compound **19a** (1.43 g, 2 mmol) was dissolved in minimum anhydrous ether and coevaporated with CH_3CN. This procedure was repeated for three times and then compound **19a** was taken up in 3 ml of ether. Separately, 3'-(4-methoxytetrahydropyranyl)-2'-deoxyuridine **12** was dried three times by coevaporation with CH_3CN and was dissolved in CH_3CN (50 ml), in which the above ether solution of **19a** and tetrazole (140 mg, 2 mmol) were added. The reaction mixture was stirred at r. t. for 4 h, under Ar. When the TLC showed the disappearence of compound **19a**, m-CPBA (55%) (818 mg, 4.8 mmol) was introduced. After an additional stirring for 2 h, the mixture was poured into saturated $NaHCO_3$ solution (15 ml) and extracted several times with CH_2Cl_2. The combined organic layer was washed once with 10% $NaHSO_3$ solution (20 ml) and then dried over Na_2SO_4 and evaporated. The oily residue was applied to a short-column of silica gel and eluted with CH_3OH/CH_2Cl_2 (1/99--->8/92). The appropriate fractions with a Rf = 0.44 (CH_3OH/CH_2Cl_2: 1/9) yielded pure compound **20a** (diastereoisomers, 1.34 g, 69%). **mp** = 90~91°C. **^{1}H-NMR** ($CDCl_3$): 0.60~0.64 (m, 6H, $Si(\underline{CH_2}CH_3)_3$); 0.67 (s, 3H, CH_3-18); 0.86 (2d, 6H, J = 6.6Hz, $2CH_3$-26, 27); 0.90~0.97 (m, 12H, CH_3-21 and $Si(CH_2\underline{CH_3})_3$); 1.05 (s, 3H, CH_3-19); 2.77, 2.78 (2t, 2H, J = 5.7Hz, $OCH_2\underline{CH_2}CN$); 3.22 (s, 3H, CH_3O); 3.59~3.64, 3.73~3.76 (2m, 4H, H-2''' of 4-methoxytetrahydropyranyl); 3.92 (d, 1H, J = 8.1Hz, H-7); 4.17 (m, 1H, H-4''); 4.23~4.31 (m, 5H: H-3; 2H-5''; $O\underline{CH_2}CH_2CN$); 4.54~4.57 (m, 1H, H-3''); 5.30 (s, 1H, H-6); 5.76 (d, 1H, J = 8.1Hz, H-5'); 6.30 (m, 1H, H-1''); 7.56, 7.57 (2d, 1H, J = 8.2Hz, H-6'). **^{13}C-NMR** ($CDCl_3$): see Table 2. **FAB-MS** negative (matrix: TEA): 972.8 [(M - H)$^-$, 15]; 919.8 [(M - CH_2CH_2CN)$^-$, 96]; 648.6 [(M - Nuc.)$^-$, 23]; 297.3 [100]. **Anal.** Found. C, 62.0; H, 8.8; N, 4.4; P, 3.1; Si, 2.7. Calc. for $C_{51}H_{84}N_3O_{11}PSi$ (974.26) C, 62.87; H, 8.69; N, 4.31; P, 3.18; Si, 2.89.

The other fraction with a Rf = 0.37 gave the partially deprotected compound corresponding to the cleavage of 7β-triethylsilyl group: 3-(7β-hydroxycholesteryl) 5'-[3'-(4-methoxytatrahydropyranyl)-2'-deoxyuridylyl] 2-cyanoethyl phosphotriester **20'a** (185 mg, 10%). **^{1}H-NMR** ($CDCl_3$): 0.70 (s, 3H, CH_3-18); 0.87 (d, 6H, J = 6.6Hz, $2CH_3$-26, 27); 0.92 (d, 3H, J = 6.4Hz, CH_3-21); 1.06 (s, 3H, CH_3-19); 2.78 (t, 2H, J = 6.0Hz, $OCH_2\underline{CH_2}CN$); 3.23 (s, 3H, CH_3O); 3.60~3.68, 3.70~3.78 (2m, 4H, $2CH_2$-2''' of 4-methoxytetrahydropyranyl); 3.85 (d, 1H, J = 6.3Hz, H-7); 4.18 (m, 1H, H-4''); 4.25~4.35 (m, 5H: 1H-3; 2H-5''; $O\underline{CH_2}CH_2CN$); 5.34 (s, 1H, H-6); 5.78 (d,1H, J = 8.0Hz, H-5'); 6.29 (t, 1H, J = 6.5Hz, H-1''); 7.57 (d, 1H, J = 8.0Hz, H-6'). **^{13}C-NMR** ($CDCl_3$): see Table 2. **FAB-MS** negative (matrix: TEA): 858.7 [(M - H)$^-$, 5.3]; 805.6 [(M - CH_2CH_2CN)$^-$, 100]; 773.6 [(M - CH_2CH_2CN - CH_3OH)$^-$, 36]; 691.6 [(M - CH_2CH_2CN - $C_6H_{11}O_2$ + H^+)$^-$,

Table 2 : ^{13}C-NMR of oxygenated sterols and their phosphorylated derivatives (the notes of a, b, c, d, e are under the table 1)

CN°	18a	18b	19a	19b	20a	20'a	21a	1a	21 b	1b	21 c	1 c	21 d	1 d	21 f °	1 f
solvent	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	CD_3OD	$CDCl_3$ / CD_3OD	$CDCl_3$ / CD_3OD	$CDCl_3$	$CDCl_3$ / CD_3OD	$CDCl_3$	$CDCl_3$ / CD_3OD	$CDCl_3$	$CDCl_3$ / $NH_3 \cdot H_2O$
St.																
1	38.57	37.15	37 04	37.16	36.64	36.35	36.51	38.57	36.29	38.32	36.53	37.90	36.50	38.07	36.31 ; 36.41	38.01
2	32.57	31.67	30.51	30.64	29.66	29.45	29.58	31.68	29.27	29.41	29.58	30.95	29.64	31.18	29.39	30.92
3	72.34	71.57	73.70 a	73.83 a	79.49	79.17	79.46	77.13	79.37	74.69	79.60	77.12	79.57	76.59	79.15	76.59
4	42.86	41.92	40.75	40.80	39.67	39.40	39.55	41.74	39.04	39.10	40.28	41.12	39.31	40.51 °	39.42	40.95
5	144.10	142.17	142.10	143.26	140.17	141.09	141.31	144.20	140 74	141.93	141.41	143.57	141.33	143.61	141.2 ; 140.9	143.73
6	127.41	126.41	126.38	126.46	129.24	127.30	127.46	128.43	127.43	126.05	127.39	127.58	127.31	127.92	127.6 ; 127.3	127.85
7	76.47	74.85	74.77	74.86	74.60	72.90	73.00	74.24	72.37	72.07	73.08	73.89	73.08	73.73	72.87	73.66
8	41.82	40.52	40.39	40.53	40.46	40.51	40.51	41.70	39.91	39.49	40.56	41.28	40.45	41.11	40.42	41.09
9	49.75	48.21	48.05	48.19	48.04	47.96	48.13	49.79	47.99	47.97	48.15	49.54	48.10	49.95	48.01	49.86
10	37 90	36.50	36.52	36.63	36.29	36.24	36.36	38.06	36.00	35.81	36.42	37.48	36.29	37.37	36.32 ; 36.41	37.39
11	22.54	21.12	20.98	21.10	21.11	20.96	21.11	22.83	20.64	20.41	21.16	22.26	21.11	22.27	20.94	22.22
12	41.01	39.57	39.46	39.57	39.57	39.40	40.17	41.22 °	39.27 °	39.40	39.58	40.72	39.54	40.71	39.42	40.62
13	44.44	43.06	42.89	43.07	43.04	42.80	42.96	44.58	42.64	42.34	43.03	44.03	42.91	43.99	42.78	43.97
14	56.94	56.23	55.35 °	55.39 °	56.47	55.34	55.53	57.37	56.25	55.06	55.57	56.70	55.50	56.96	55.37	56.83
15	28.08	26.65	26.58	26.67	26.65	26.28	26.43	27.82	25.98	25.84	26.46	27.38	26.42	27.35	26.25 ; 26.32	27.31
16	30.00	28.63	28.55	28.67	28.62	28.46	28.60	30.14	28.30	28.01	28.65	29.69	28.60	29.67	28.46	29.62
17	57.86	56.23	56.13 °	56.23 °	56.13	55.76	55.95	58.03	55.78	55.88	55.99	57.27	55.94	57.72	55.76 ; 55.83	57.59
18	13.28	12.00	11.89	12.02	11.99	11.75	11.90	13.18	11.55	11.14	11.95	12.90	11.90	12.42	11.74	12.47
19	20.23	19.03	18.91	19.03	18.93	18.92	19.04	20.24	18.61	18.21	19.05	19.97	19.04	19.48	18.86	19.57
20	37.24	35.72	35.88	35.75	35.79	35.65	35.81	37.56	35.55	35.31	35.84	37.90	35.80	37.14	35.67	37.01
21	20.21	18.88	18.80	18.90	18.93	18.69	18.84	20.13	18.45	18.04	18.88	19.86	18.83	19.48	18.68	19.57
22	37.68	36.53	36.15	36.55	36.26	36.10	36.27	37.19	36.29	38.06	36.30	37.36	36.25	37.48	36.12	37.34
23	25.30	20.79	23.76	20.60	23.88	23.76	23.93	25.48	20.86	20.60	23.99	25.00	23.91	25.02	23.82	24.81
24	40.97	44.47	39.46	44.49	39.57	39 40	39.55	41.41 °	44.03	43.88	39.58	40.93	39.54	40.71	39.42	40.62
25	29.41	71.11	27 94	71.10	28.04	27.93	28.07	29.87	70.75	70 20	28.11	29.16	28.08	29.17	27.94	29.05
26	23.89	29.21	22.78	29.24	22.62	22.49	22.63	23.77	28.45	27.90	22.66	23.59	22.89	23.03	22.50	23.10
27	24.11	29.42	22.53	29.45	22.86	22.76	22.88	24.02	28.60	28.01	22.91	23.85	22.62	23.28	22.77	23.37
Nuc.																
2'					150.10	150 21	150.41	152.77	150.35	150.51	148.95	150.56	149 12	151.16	154.42	158.40
4'					162.62	163.18	163.45	166.54	163.96	164.34	157.02 a	159.31 a	157.21 a	158.51 a	162.78	167.58
5'					102.89	102.78	102.76	103.93	102.37	101 77	140.72 b	141 73 b	140.59 b	142.85 b	96.13	95.72
6'					139.67	139.76	140.17	142.99	140.05	140.71	124.20 c	126.09 c	124.88 c	126.17 c	145.39	144.62
1"					85.36	85.31	85.54	86.77	85.11	84.52	85.66	86.60	85.65	86.76	85.59	87.27
2"					39.57	39.40	39.55	41.22 °	39.27 °	39.51	39.58	40.67	39.54	41.02 °	73.93 ; 74.10	76.39
3"					68.93	68.69	70 67	72.88	69.87	70.07	70.42	71.54	78.40	76.59	76.16 ; 76.69	76 85
4"					83.77	83.51	84.69	86.06	84.38	85.59	84.96	87.23	85.95	88.13	81.71	83.58
5"					66.97	66.74	67.20	66.43	66.94	64 02	67.13	65.88	61.84	62.72	66.40 ; 66.18	64.99
$SiCH_2$-CH_2-CH_3	7.33	6 01	5.88	6.00	5.99											
	8.41	7.19	7.11	7.20	7.21											
OCH_2-CH_2-CN			58.13 b	58.25 b	62.11	61.96	62.30		62 04		62.41		62 35		62.02	
			20.29 c	20 40 c	19.92	19.75	19.87		19.41		19.89		19.81		20.52	
			117.60	117.62	116.32	116.37	116.74		116.52		116.77		16.79		116.47	
others			-N(CH(CH3)2)2 CH 42 88 / 42 99; 43.04 / 43.23; CH3 24 29 / 24 37; 24.36 / 24.52; 24.61 / 24.69; 24 68 / 24 84		4-MTHP- d OCH3 48.34 / 48.23; 2). 64 84 / 2). 64.70; 3). 34.52 / 3). 34 33; 4). 99.37 / 4). 99.18; 5). 34 88 / 5). 34.72; 6). 64.84 / 6). 64.70										An - d CO : 165.77; OCH_3 : 56.52; 1). 124.75; 2). 129.82; 3). 114.20; 4). 163.64; CH_3CO- CO : 168.58; 169.73; 169.82; CH_3 : 20.52; 20.65	

10]; 579.6 [(M - CH_2CH_2CN - $C_6H_{11}O_2$ - base)$^-$, 47]; 534.5 [(M - Nuc.)$^-$, 19]; 481.5 [(M - CH_2CH_2CN - Nuc. + H^+)$^-$, 74]. **Anal.** Found. C, 60.6; H, 8.1; N, 4.9; P,3.4. Calc. for $C_{45}H_{70}N_3O_{11}P{\cdot}2H_2O$ (896.03): C, 60.32; H, 8.32; N, 4.69; P, 3.46.

3-(7β-Hydroxycholesteryl) 5'-(2'-deoxyuridylyl) 2-cyanoethyl phosphotriester (21a) Compound **20a** (974 mg, 1 mmol) was dissolved in THF (10 ml) and 10 ml of 0.36% HCl solution in THF was added. After stirring at r. t. for 2 h, the reaction mixture was poured into saturated $NaHCO_3$ solution (5 ml) and extracted with CH_2Cl_2 (3x30 ml). All organic layers were dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by a short column chromatography on silica gel (CH_3OH/CH_2Cl_2: 5/95-->10/90). The pure compound **21a** (708 mg) was obtained in 95% yield (diastereoisomers). **^{1}H-NMR** ($CDCl_3$): 0.68 (s, 3H, CH_3-18); 0.87 (d, 6H, J = 6.6Hz, $2CH_3$-26, 27); 0.92 (d, 3H, J = 6.3Hz, CH_3-21); 1.05 (s, 3H, CH_3-19); 2.80 (2t, 2H, J = 6.0Hz, $OCH_2\underline{CH_2}CN$); 3.84 (d, 1H, H-7); 4.17 (m, 1H, H-4"); 4.23~4.45 (m, 5H: H-3; 2H-5"; $O\underline{CH_2}CH_2CN$); 4.49 (m, 1H, H-3"); 5.33 (s, 1H , H-6); 5.80, 5.81 (2d, 1H, J = 8.0Hz, H-5'); 6.32 (t, 1H, J = 6.5Hz, H-1"); 7.62, 7.66 (2d, 1H, J = 8.0Hz, H-6'); 9.92 (brd., 1H, NH). **^{13}C-NMR** ($CDCl_3$): see Table 2. **FAB-MS** negative (matrix: TEA): 744.4 [(M - H)$^-$, 12]; 691.4 [(M - CH_2CH_2CN)$^-$, 100]; 534.3 [(M - Nuc.)$^-$, 26]; 481.3 [(M - CH_2CH_2CN - Nuc. + H^+)$^-$, 85]; 360.0 [(M - St.)$^-$, 37]; 307.0 [(M - CH_2CH_2CN - St. + H^+)$^-$, 64]. **Anal.** Found. C, 61.5; H, 8.3; N, 5.4; P, 4.1. Calc. for $C_{39}H_{60}N_3O_9P \cdot H_2O$ (763.88): C, 61.32; H, 8.18; N, 5.05; P, 4.05.

Sodium salt of 3-(7β-hydroxycholesteryl) 5'-(2'-deoxyuridylyl) monophosphate (1a) Concentrated 25% aqueous ammonia (4 ml) was added to a solution of **21a** (373 mg, 0.5mmol) in CH_3OH (16 ml). After 2 h, TLC indicated the complete conversion of **21a** and the mixture was evaporated under reduced pressure to dryness. The residue was then taken up in water (1 ml) and was applied to a ion-exchange chromatography over Dowex-50 resin eluted with bidistilled water. The combined fraction was lyophilized to give the pure compound **1a** (357 mg) as a white powder in 100% yield. **^{1}H-NMR** (CD_3OD): 0.75 (s, 3H, CH_3-18); 0.91 (d, 6H, J = 6.6Hz, $2CH_3$-26, 27); 0.98 (d, 3H, J = 6.5Hz, CH_3-21); 1.09 (s, 3H, CH_3-19); 3.77 (d, 1H, J = 8.4Hz, H-7); 3.98~4.08 (m, 4H: 1H-3; 1H-4"; 2H-5"); 4.55 (m, 1H, H-3"); 5.31 (s, 1H, H-6); 5.78 (d, 1H, J = 8.1Hz, H-5'); 6.34 (t, 1H, J = 6.8Hz, H-1"); 8.01 (d, 1H, J = 8.1Hz, H-6'). **^{13}C-NMR** (CD_3OD): see Table 2. **FAB-MS** positive (matrix: 1-thioglycerol): 737 [MNa^+, 5]; 715 [MH^+, 5]; 383 [19]; 367 [33]. **FAB-MS** negative (matrix: 1-thioglycerol): 691 [(M - Na^+)$^-$, 36]; 481 [(M - Na^+ - Nuc. + H^+)$^-$, 9]; 307 [(M - Na^+ - St. + H^+)$^-$, 35]. **Anal.** Found. C, 57.7; H, 8.1; N, 3.7; P, 3.8; Na, 3.1. Calc. for $C_{36}H_{56}N_2O_9PNa \cdot 2H_2O$ (750.83): C, 57.58; H, 8.06; N, 3.73; P, 4.13; Na, 3.06.

3-(7β-Triethylsilyloxy-25-hydroxycholesteryl) 5'-[3'-(4-methoxytetrahydropyranyl)-2'-deoxyuridylyl] 2-cyanoethyl phosphotriester (20b) **20b** was obtained in 69% yield as described for **20a**.

^{1}H-NMR ($CDCl_3$): 0.68 (s, 3H, CH_3-18); 0.56~0.68 [m, 6H, $Si(\underline{CH_2}CH_3)_3$]; 0.94 (d, 3H, J = 6.5Hz, CH_3-21); 0.97 [t, 9H, J = 8.2Hz, $Si(CH_2\underline{CH_3})_3$]; 1.07 (s, 3H, CH_3-19); 1.23 (s, 6H, $2CH_3$-26, 27); 2.79 (m, 2H, $OCH_2\underline{CH_2}CN$); 3.23 (s, 3H, CH_3O); 3.61~3.82 (m, 4H, $2CH_2$-2"' of 4-methoxytetrahydropyranyl); 3.93 [d (br.), 1H, H-7]; 4.19 (m, 1H, H-4"); 4.22~4.29 (m, 5H: H-3; 2H-5"; $O\underline{CH_2}CH_2CN$); 4.56 (m, 1H, H-3"); 5.31 [s (br.), 1H, H-6]; 5.78 (2d, 1H, J = 8.0Hz, H-5'); 6.31 [t (br.), 1H, J = 6.7Hz, H-1"]; 7.59 (2d, 1H, J = 8.1Hz, H-6'). **FAB-MS** negative (matrix: TEA): 988.8 [(M - H)$^-$, 10]; 935.7 [(M - CH_2CH_2CN)$^-$, 97]; 664.5 [(M - Nuc.)$^-$, 40]; 611.5 [(M - CH_2CH_2CN - Nuc. + H^+)$^-$, 94]; 474.2 [(M - St.)$^-$, 58]; 421.2 [(M - CH_2CH_2CN - St. + H^+)$^-$, 100].

In this step, another partially triethylsilyl-deprotected compound: 3-(7β, 25-dihydroxycholesteryl) 5'-[3'-(4-methoxytetrahydropyranyl)-2'-deoxyuridylyl] 2-cyanoethyl phosphotriester **20'b** was also obtained and identified. **^{1}H-NMR** ($CDCl_3$): 0.70 (s, 3H, CH_3-18); 0.95 (d, 3H, J=6.4Hz, CH_3-21); 1.06 (s, 3H, CH_3-19); 1.23 (s, 6H, $2CH_3$-26, 27); 2.79 (2t, 2H, J=6.4Hz, $OCH_2\underline{CH_2}CN$); 3.23 (s, 3H, CH_3O); 3.61~3.87 (m, 4H, $2CH_2$-2"' of 4-methoxytetrahydropyranyl); 3.85 [d (br.), 1H, J=8.4Hz, H-7]; 4.19 (m, 1H, H-4"); 4.22~4.32 (m, 5H: H-3; 2H-5"; $O\underline{CH_2}CH_2CN$); 4.55 (m, 1H, H-3"); 5.34 [s (br.), 1H, H-6]; 5.75, 5.77 (2d, 1H, J=8.0Hz, H-5'); 6.30 [t (br.), 1H, H-1"]; 7.58 (d, 1H, J=8.1Hz, H-6'); 8.10 (br., 1H, NH).

3-(7β, 25-Dihydroxycholesteryl) 5'-(2'-deoxyuridylyl) 2-cyanoethyl phosphotriester (21b)

21b was obtained from **20b** by an acidic deprotection under the same conditions as for **21a** (yield: 70%). **^{1}H-NMR** ($CDCl_3$): 0.70 (s, 3H, CH_3-18); 0.95 (d, 3H, J=6.3Hz, CH_3-21); 1.07 (s, 3H, CH_3-19); 1.22 (s, 6H, $2CH_3$-26, 27); 2.80 (2t, 2H, $OCH_2\underline{CH_2}CN$); 3.85 (d, 1H, J=8.8Hz, H-7); 4.09 (m, 1H, H-4"); 4.24~4.36 (m, 5H: H-3; 2H-5"; $O\underline{CH_2}CH_2CN$); 4.56 (m, 1H, H-3"); 5.34 [s (br.), 1H, H-6]; 5.76 (d, 1H, J=8.2Hz, H-5'); 6.26, 6.28 [2t, 1H, J=6.4Hz, H-1"]; 7.55 (d, 1H, J=8.1Hz, H-6'). **^{13}C-NMR** ($CDCl_3$): see Table 2. **FAB-MS** negative (matrix: TEA): 760.5 [(M - H)$^-$, 28]; 707.5 [(M - CH_2CH_2CN)$^-$, 100]; 550.4 [(M - Nuc.)$^-$, 31]; 360.1 [(M - St.)$^-$, 48]; 307.1 [(M - CH_2CH_2CN - Nuc. + H^+)$^-$, 43]. **Anal.** Found. C, 59.9; H, 7.7; N, 5.1; P, 3.9. Calc. for $C_{39}H_{60}N_3O_{10}P \cdot H_2O$ (779.87): C, 60.06; H, 8.01; N, 5.39; P, 3.97.

Sodium salt of 3-(7β, 25-dihydroxycholesteryl) 5'-(2'-deoxyuridylyl) monophosphate (1b)

1b was obtained quantitatively by a treatment of **21b** with concentrated aqueous ammonia and then passing through an ion-exchange chromatography. **^{1}H-NMR** ($CDCl_3/CD_3OD$): 0.61 (s, 3H, CH_3-18); 0.85 (d, 3H, J=6.3Hz, CH_3-21);

0.95 (s, 3H, CH_3-19); 1.12 (s, 6H, $2CH_3$-26, 27); 3.71 [d (br.), 1H, J=8.1Hz, H-7); 3.90~3.98 (m, 4H: H-3; H-4"; 2H-5"); 4.40 (m, 1H, H-3"); 5.16 (s, 1H, H-6); 5.67 (d, 1H, J=8.1Hz, H-5'); 6.12 (t, 1H, J=6.7Hz, H-1"); 7.76 (d, 1H, J=8.1Hz, H-6'). **^{13}C-NMR** ($CDCl_3$/CD_3OD): see Table 2. **FAB-MS** positive (matrix: m-NBA): 753.6 [MNa^+, 86]; 735.6 [(MNa^+ - H_2O), 64]; 731.7 [MH^+, 100]; 713.6 [(MH^+ - H_2O), 75]. **Anal.** Found. C, 54.2; H, 7.9; N, 3.6; P, 3.7; Na, 3.1. Calc. for $C_{36}H_{56}N_2O_{10}PNa \cdot 3\ ^1/_2H_2O$ (793.85): C, 54.46; H, 8.00; N, 3.53; P, 3.90; Na, 2.90.

3-(7β-Triethylsilyloxycholesteryl) 5'-[5-fluoro-3'-(4-methoxytetrahydropyranyl)-2'-deoxyuridylyl] 2-cyanoethyl phosphotriester (20c) The compound **20c** was synthesized from **19a** and **12b** according to the procedure used for the preparation of **20a** (yield: 85%). **^{1}H-NMR** ($CDCl_3$): 0.67 (s, 3H, CH_3-18); 0.61~0.64 [m, 6H, $Si(CH_2CH_3)_3$]; 0.86, 0.87 (2d, 6H, J=6.7Hz, $2CH_3$-26, 27); 0.91 (d, 3H, J=6.4Hz, CH_3-21); 0.94~0.98 [2t, 9H, $Si(CH_2CH_3)_3$]; 1.06 (s, 6H, CH_3-19); 2.79 (2t, 2H, OCH_2CH_2CN); 3.22 (s, 3H, CH_3O); 3.59~3.78 (m, 4H, $2CH_2$-2"' of 4-methoxytetrahydropyranyl); 3.92 [d (br.), 1H, H-7]; 4.18 (m, 1H, H-4"); 4.27~4.37 (m, 5H: H-3; 2H-5"; OCH_2CH_2CN); 4.57 (m, 1H, H-3"); 5.30 [s (br.), 1H, H-6]; 6.31 [t (br.), 1H, H-1"]; 7.73, 7.74 (2d, 1H, J= 6.2Hz, H-6'). **FAB-MS** negative (matrix: TEA): 990.6 [(M - H)$^-$, 22]; 937.6 [(M - CH_2CH_2CN)$^-$, 100]; 915.6 [(M - CH_2CH_2CN - CH_3OH)$^-$, 22]; 648.5 [(M - Nuc.)$^-$, 21]; 595.5 [(M - CH_2CH_2CN - Nuc. + H^+)$^-$, 79]; 492.2 [(M - St.)$^-$, 16]; 438.1 [(M - CH_2CH_2CN - St. + H^+)$^-$, 32].

3-(7β-Hydroxycholesteryl) 5'-(5-fluoro-2'-deoxyuridylyl) 2-cyanoethyl phosphotriester (21c) The compound **21c** derived from **20c** by an acidic treatment according to the procedure used for the preparation of **21a** (yield: 81%). **mp** = 124~125°C. **^{1}H-NMR** ($CDCl_3$): 0.68 (s, 3H, CH_3-18); 0.87 (2d, 6H, J=6.6Hz, $2CH_3$-26, 27); 0.95 (d, 3H, J=6.7Hz, CH_3-21); 1.05 (s, 3H, CH_3-19); 2.86, 2.87 (2t, 2H, OCH_2CH_2CN); 3.86 [d (br.), 1H, J=7.8Hz, H-7); 4.13 (m, 1H, H-4"); 4.30 (m, 5H: H-3; 2H-5"; OCH_2CH_2CN); 4.48 (m, 1H, H-3"); 5.33 (s, 1H, H-6); 6.24 [t (br.), 1H, H-1"]; 7.72 (2d, 1H, J=6.1Hz, H-6'). **^{13}C-NMR** ($CDCl_3$): see Table 2. **FAB-MS** negative (matrix: TEA): 762.4 [(M - H)$^-$, 24]; 709.4 [(M - CH_2CH_2CN)$^-$, 48]; 534.4 [(M - Nuc.)$^-$, 23]; 481.4 [(M - CH_2CH_2CN - Nuc. + H^+)$^-$, 100]; 378.1 [(M - St.), 39]; 325.1 [(M - CH_2CH_2CN - St. + H^+)$^-$, 40]. **Anal.** Found. C, 59.6; H, 7.9; N, 5.6. Calc. for $C_{39}H_{59}N_3O_9FP \cdot H_2O$ (783.87): C, 59.75; H, 7.84; N, 5.36.

Sodium salt of 3-(7β-hydroxycholesteryl) 5'-(5-fluoro-2'-deoxyuridylyl) monophosphate (1c) The compound **1c** was obtained quantitatively from **21c** in the same way as described for **1a** . **^{1}H-NMR** (CD_3OD): 0.75 (s, 3H, CH_3-18); 0.91 (d, 6H, J=6.5Hz, $2CH_3$-26,27); 0.98 (d, 3H, J=6.4Hz, CH_3-21); 1.10 (s, 3H, CH_3-19); 3.76 (d, 1H, J=8.1Hz, H-7); 4.08 (m, 4H: H-3; H-4"; 2H-5"); 4.50 (m, 1H, H-3"); 5.29 (s, 1H, H-6); 6.33 (t, 1H, J=6.4Hz, H-1"); 8.07 (d, 1H; J=6.5Hz, H-6'); **^{13}C-NMR** (CD_3OD+$CDCl_3$): see Table 2. **FAB-MS** negative (matrix: 1-thioglycerol): 709 [(M - Na^+)$^-$, 19]; 481 [(M - Na^+ - Nuc. + H^+)$^-$, 6]; 325 [(M - Na^+ - St. + H^+)$^-$, 13]; 129 [base, 100]. **Anal.** Found. C, 56.2; H, 7.7; N, 3.5; F, 2.9; P, 3.9; Na, 2.8. Calc. for $C_{36}H_{55}N_2O_9FPNa \cdot 2H_2O$ (768.82): C, 56.24; H, 7.74; N, 3.65; F, 2.47; P, 4.03; Na, 2.99.

3-(7β-Triethylsilyloxycholesteryl) 3'-[5-fluoro-5'-dimethoxytrityl-2'-deoxyuridylyl] 2-cyanoethyl phosphotriester (20d) The compound **20d** was obtained by coupling **19a** with 5-fluoro-5'-dimethoxytrityl-2'-deoxyuridine **13** according to the general procedure for the preparation of **20a** (66% yield).

^{1}H-NMR ($CDCl_3$): 0.67 (s, 3H, CH_3-18); 0.59~0.67 [m, 6H, $Si(CH_2CH_3)_3$]; 0.84~0.98 (m, 18H: CH_3-21; $2CH_3$-26, 27; $Si(CH_2CH_3)_3$]; 1.03, 1.05 (2s, 3H, CH_3-19); 2.79 (2t, 2H, OCH_2CH_2CN); 3.38~3.53 (m, 2H, 2H-5"); 3.79 (s, 6H, $2CH_3O$); 3.92 [d (br.), 1H, J=8.0Hz, H-7); 4.16~4.28 (m, 3H: H-3; OCH_2CH_2CN); 4.32 (m, 1H, H-4"); 5.13 (m, 1H, H-3"); 5.29 (s, 1H, H-6); 6.33 [2t (br.), 1H, H-1"]; 6.85 (2d, 4H of trityl); 7.29 ~7.36 (m, 9H of trityl); 7.79, 7.80 (2d, 1H, J=5.9Hz, H-6'). **FAB-MS** negative (matrix: TEA): 1178.7 [(M - H)$^-$, 10]; 1125.8 [(M - CH_2CH_2CN)$^-$, 15]; 995.7 [(M - CH_2CH_2CN - DMTr + H^+)$^-$, 15]; 680.3 [(M - St.)$^-$, 6]; 648.5 [(M - Nuc.)$^-$, 61]; 595.5 [(M - CH_2CH_2CN - Nuc. + H^+)$^-$, 100].

3-(7β-Hydroxycholesteryl) 3'-(5-fluoro-2'-deoxyuridylyl) 2-cyanoethyl phosphotriester (21d) **20d** was treated by 0.36% HCl solution in THF for 1 h. A general work-up and a short chromatography on silica gel afforded **21d** in 92% yield. **^{1}H-NMR** ($CDCl_3$): 0.68 (s, 3H, CH_3-18); 0.85, 0.86 (2d, 6H, J=6.5Hz, $2CH_3$-26, 27); 0.91 (d, 3H, J=6.2Hz, CH_3-21); 1.04 (s, 3H, CH_3-19); 2.80, 2.82 (2t, 2H, J=5.7Hz, OCH_2CH_2CN); 3.66~3.88 (br., 3H: 2H-5"; H-7); 4.25~4.29 (br., 4H: H-4"; H-3; OCH_2CH_2CN); 5.13 (br., 1H, H-3"); 5.33 (s, 1H, H-6); 6.27 (br., 1H, H-1"); 8.09 (2d, 1H, J=5.8Hz, H-6'). **^{13}C-NMR** (CD_3OD): see Table 2. **FAB-MS** negative (matrix: TEA): 762.8 [(M - H)$^-$, 10]; 709.8 [(M - CH_2CH_2CN)$^-$, 31]; 534.7 [(M - Nuc.)$^-$, 45]; 481.6 [(M - CH_2CH_2CN - Nuc. + H^+)$^-$, 100]; 378.3 [(M - St.)$^-$, 31]; 325.3 [(M - CH_2CH_2CN - St. + H^+)$^-$, 11].

Sodium salt of 3-(7β-hydroxycholesteryl) 3'-(5-fluoro-2'-deoxyuridylyl) monophosphate (1d) **21d** was quantitatively converted to **1d** in the same manner as described for the preparation of **1a**. **^{1}H-NMR**

($CDCl_3/CD_3OD$): 0.71 (s, 3H, CH_3-18); 0.87 (2d, 6H, J=6.6Hz, $2CH_3$-26, 27); 0.93 (d, 3H, J=6.4Hz, CH_3-21); 1.07 (s, 3H, CH_3-19); 2.20~2.27, 2.31~2.37, 2.48~2.53 (3m, 4H: 2H-4; 2H-2"); 3.71 (d, 1H, J=8.0Hz, H-7); 3.80 (m, 2H, 2H-5"); 3.94~4.00 (br., 1H, H-3); 4.17 (m, 1H, H-4"); 4.82 (br., 1H, H-3"); 5.28 (s, 1H, H-6); 6.29 (t (br.), 1H, J=5.8Hz, H-1"); 8.27 (d, 1H, J=6.8Hz, H-6'). **^{13}C-NMR** (CD_3OD+$CDCl_3$): see Table 2. **FAB-MS** negative (matrix: TEA): 731.7 [(M - H), 89]; 709.8 [(M - Na^+)$^-$, 100]; 481.6 [(M - Na^+ - Nuc. + H^+)$^-$, 65]; 347.3 [(M - St.)$^-$, 15]; 325 [(M - Na^+ - St. + H^+)$^-$, 11]. **Anal.** Found. C, 54.7; H, 7.7; N, 3.6. Calc. for $C_{36}H_{55}N_2O_9FPNa \cdot 3H_2O$ (786.41): C, 54.98; H, 7.82; N, 3.56.

3-(7β-Triethylsilyloxycholesteryl) 5'-(2',3'-methoxymethylidene-6-azauridylyl) 2-cyanoethyl phosphotriester (20e) **20e** (689 mg, 75%) was prepared from **19a** (860 mg, 1,2 mmol) and **14** (288 mg, 1 mmol), according to the general procedure used for the preparation of **20a**. **^{1}H-NMR** ($CDCl_3$): 0.67 (s, 3H, CH_3-18); 0.59~0.65 (m, 6H, $Si(\underline{CH_2}CH_3)_3$); 0.86, 0.87 (2d, 6H, J=6.6Hz, $2CH_3$-26, 27); 0.91 (d, 3H, J=6.4Hz, CH_3-21); 0.95, 0.96 (2t, 9H, $Si(CH_2\underline{CH_3})_3$); 1.03 (s, 3H, CH_3-19); 2.76 (2t, 2H, $OCH_2\underline{CH_2}CN$); 3.33, 3.41 (2s, 3H, CH_3O); 3.92 (d (br.), 1H, H-7); 4.17~4.24 (m, 5H: H-3; 2H-5"; $O\underline{CH_2}CH_2CN$); 4.39, 4.56 (2m, 1H, H-4"); 4.83~5.00 (m, 1H, H-3"); 5.15 (m, 1H, H-2"); 5.30 (s (brd.), 1H, H-6); 5.93, 5.98 (2s, 1H of methoxyformate); 6.30, 6.32 (2d (brd.), 1H, J=9.7Hz, H-1"); 7.42 (2s, 1H, H-5'). **FAB-MS** negative (matrix: TEA): 917.5 [(M - H)$^-$, 20]; 864.5 [(M - CH_2CH_2CN)$^-$, 30]; 648.5 [(M - Nuc.)$^-$, 18]; 595.4 [(M - CH_2CH_2CN - Nuc. + H^+)$^-$, 100]; 419.1 [(M - St.)$^-$, 6]; 366.1 [(M - CH_2CH_2CN - St. + H^+)$^-$, 18].

3-(7β-Hydroxycholesteryl) 5'-(6-azauridylyl) 2-cyanoethyl phosphotriester (21e)
A treatment with 0.36% HCl solution in THF at r. t. for 2 h transformed **20e** (380 mg, 0.41mmol) to **21e** (270 mg, 86%). **^{1}H-NMR** ($CDCl_3$): 0.70 (s, 3H, CH_3-18); 0.87 (d, 6H, J=6.6Hz, $2CH_3$-26, 27); 0.92 (d, 3H, J=6.5Hz, CH_3-21); 1.05 (2s, 3H CH_3-19); 2.80 (brd., 2H, $OCH_2\underline{CH_2}CN$); 3.86 (brd., 1H, H-7); 4.19~4.34 (m, 6H: H-3; H-4"; 2H-5"; $O\underline{CH_2}CH_2CN$); 4.43 (brd., 1H, H-3"); 4.51 (br., 1H, H-2"); 5.31 (s (brd.), 1H, H-6); 6.17 (brd., 1H, H-1"); 7.44 (s (brd.), 1H, H-5'). **FAB-MS** negative (matrix: TEA): 761.4 [(M - H)$^-$, 40]; 708 [(M - CH_2CH_2CN)$^-$, 31]; 534.4 [(M - Nuc.)$^-$, 18]; 481.3 [(M - CH_2CH_2CN - Nuc. + H^+)$^-$, 100]; 377.1 [(M - St.)$^-$, 18]; 324.1 [(M - CH_2CH_2CN - St. + H^+)$^-$, 31].

Sodium salt of 3-(7β-hydroxycholesteryl) 5'-(6-azauridylyl) monophosphate (1e)
1e was obtained quantitatively from **21e** in the same manner as described for the preparation of **1a**. **^{1}H-NMR** (CD_3OD): 0.71 (s, 3H, CH_3-18); 0.87 (d, 6H, J=6.6Hz, $2CH_3$-26, 27); 0.94 (d, 3H, J=6.5Hz, CH_3-21); 1.04 (2s, 3H CH_3-19); 3.70~3.78 (brd., 1H, H-7); 3.90~4.07 (m (brd.), 4H: H-3; H-4"; 2H-5"); 4.32 (t, 1H, H-3"); 4.45 (t, 1H, H-2"); 5.25 (s (brd.), 1H, H-6); 6.08 (d, 1H, J=3.8Hz, H-1"); 7.45 (s, 1H, H-5'). **FAB-MS** negative (matrix: TEA): 730.4 [(M - H)$^-$, 100]; 708.4 [(M - Na^+)$^-$, 68]; 503.3 [(M - Nuc.)$^-$, 5]; 481.0 [(M - Na^+ - Nuc. + H^+)$^-$, 44]; 346.0 [(M - St.)$^-$, 16]; 324.1 [(M - Na^+ - St. + H^+)$^-$, 10]. **Anal.** Found. C, 51.2; H, 7.7; N, 5.0. Calc. for $C_{35}H_{55}N_3O_{10}PNa \cdot 5H_2O$ (821.85): C, 51.15; H, 7.97; N, 5.11.

3-(7β-Triethylsilyloxycholesteryl) 5'-(4N-anisoyl-2',3'-diacetylaracytidylyl) 2-cyanoethyl phosphotriester (20f) The compound **20f** (1.0g, yield: 65%) was prepared in the same manner as described for **20a**, by coupling **19a** (1g, 1.4 mmol) and **17** (0.7g, 1.5 mmol). **mp** = 98~99°C. **^{1}H-NMR** ($CDCl_3$): 0.68 (s, 3H, CH_3-18); 0.56~0.67 (m, 6H, $Si(\underline{CH_2}CH_3)_3$); 0.85~1.00 (m, 18H: CH_3-21; $2CH_3$-26, 27; $Si(CH_2\underline{CH_3})_3$); 1.05, 1.07 (2s, 3H, CH_3-19); 2.02, 2.16 (2s, 6H, $2CH_3CO$); 2.79, 2.81 (2t, 2H, J=6.0Hz, $OCH_2\underline{CH_2}CN$); 3.89 (s, 3H, CH_3O); 3.93 (d (brd.), 1H, H-7); 4.26~4.42 (2m, 6H: H-3; H-4"; 2H-5"; $O\underline{CH_2}CH_2CN$); 5.13 (m, 1H, H-3"); 5.32 (s (brd.), 1H, H-6); 5.54 (m, 1H, H-2"); 6.45 (2d, 1H, H-1"); 7.00 (d, 2H, J=8.9Hz, 2H-3''' of anisoyl); 7.64 (brd., 1H, H-5'); 7.87 (d, 2H, J=8.4Hz, 2H-2''' of anisoyl); 8.11, 8.16 (2d, 1H, J=8.3Hz, H-6'). **FAB-MS** negative (matrix: m-NBA): 1091.9 [(M-H)$^-$, 32]; 1038.8 [(M-CH_2CH_2CN)$^-$, 23]; 648.7 [(M-Nuc.)$^-$, 18]; 595.6 [(M-CH_2CH_2CN-Nuc.+ H^+)$^-$, 72]; 540.3 [(M-St.)$^-$, 18]; 244.2 [base, 100].

3-(7β-Hydroxycholesteryl) 5'-(4N-anisoyl-2',3'-diacetylaracytidylyl) 2-cyanoethyl phosphotriester (21f) The compound **20f** (500 mg, 0.46 mmol) was treated by 0.18% HCl solution in THF for 1.5 h. A general work-up and a short-column chromatography on silica gel (CH_3OH/CH_2Cl_2: 4/96 ---> 8/92) furnished **21f** as a mixture of diastereoisomers (432 mg, 96%). **mp** = 105~106°C. **^{1}H-NMR** ($CDCl_3$): 0.66, 0.68 (2s, 3H, CH_3-18); 0.86, 0.87 (2d, 6H, J=6.6Hz, $2CH_3$-26, 27); 0.89 (d (br.), 3H, CH_3-21); 1.04, 1.05 (2s, 3H, CH_3-19); 2.01, 2.15 (2s, 6H, $2CH_3CO$); 2.50 (m, 2H, H-4); 2.78, 2.80 (2t, 2H, J= 6.0Hz, $OCH_2\underline{CH_2}CN$); 3.83 (br., 1H, H-7); 3.88 (s, 3H, CH_3O); 4.21~4.31 (m, 4H: H-3; H-4"; $O\underline{CH_2}CH_2CN$); 4.39~4.44 (m, 2H, 2H-5"); 5.11~5.14 (m, 1H, H-3"); 5.35, 5.41 (2s (br.), 1H, H-6); 5.52~5.54 (m, 1H, H-2"); 6.42~6.43 (2d, 1H, H-1"); 7.00 (d, 2H, J=8.8Hz, 2H-3''' of anisoyl); 7.62 (br., 1H, H-5'); 7.87 (d, 2H, J=8.5Hz, 2H-2''' of anisoyl); 8.14 (d, 1H, J=7.6Hz, H-6'); 8.82 (br., 1H, NH). **^{13}C-NMR** ($CDCl_3$): see Table 2. **FAB-MS** negative (matrix: TEA): 977.5 [(M - H)$^-$, 14]; 924.5 [(M - CH_2CH_2CN)$^-$, 44]; 882.5 [(M - CH_2CH_2CN - CH_3CO + H^+)$^-$, 12]; 790.4 [(M - CH_2CH_2CN - An + H^+)$^-$, 12]; 593.2 [(M - St.)$^-$, 12]; 540.1 [(M - CH_2CH_2CN - St. + H^+)$^-$, 26]; 481.3 [(M - CH_2CH_2CN - Nuc. + H^+)$^-$, 100]; 406.0 [(M - CH_2CH_2CN - St. - An + $2H^+$)$^+$, 10]; 244 [base, 93]. **Anal.** Found. C, 59.5; H, 7.3; N, 5.7. Calc. for $C_{51}H_{71}N_3O_{13}P \cdot 3H_2O$ (1033.13): C, 59.61; H, 7.51; N, 5.42.

Sodium salt of 3-(7β-hydroxycholesteryl) 5'-(aracytidylyl) monophosphate (1f) The concentrated aqueous ammonia (20 ml) was added to a solution of 21f (392 mg, 0.4 mmol) in CH_3OH (20 ml). After obtaining a homogeneous solution, the mixture was kept for 24 h and then was evaporated to dryness under vaccum. The residue was taken up in 1 ml of CH_3OH/CH_2Cl_2 (4/6) and passed through Sephadex LH-20 chromatography eluted with CH_3OH/CH_2Cl_2 (4/6). The appropriate fractions were collected and evaporated. The obtained residue was converted to 1f as its sodium salt after a ion-exchange chromatography over Dowex-50 (Na^+ form) (119 mg, 41%).

^{1}H-NMR (CD_3OD +$NH_3 \cdot D_2O$): 0.69 (s, 3H, CH_3-18); 0.87 (d, 6H, J=6.6Hz, 2CH_3-26, 27); 0.95 (d, 3H, J=6.4Hz, CH_3-21); 1.04 (s, 3H CH_3-19); 3.73 (d, 1H, J=8.1Hz, H-7); 3.94~3.98 (m, 2H: H-3; H-4"); 4.08~4.10 (m, 2H: 2H-5"); 4.16 (t, 1H, J=4.7Hz, H-3"); 4.21 (brd., 1H, H-2"); 5.28 (s, 1H, H-6); 5.96 (d, 1H, J=7.5Hz, H-5'); 6.21 (d, 1H, J=5.0Hz, H-1"); 7.85 (d, 1H, J=7.5Hz, H-6'). **^{13}C-NMR** (CD_3OD+$NH_3 \cdot D_2O$): see Table 2. **FAB-MS** negative (matrix: TEA): 706.1 [(M - Na^+)$^-$, 100]; 481.0 [(M - Na^+ - Nuc. + H^+)$^-$, 32]; 322.1 [(M - Na^+ - St. + H^+)$^-$, 34]. **Anal.** Found. C, 51.3; H, 8.1; N, 5.0; P, 3.5. Calc. for $C_{36}H_{57}N_3O_9PNa \cdot 6H_2O$ (837.90): C, 51.61; H, 8.30; N, 5.01; P, 3.69.

Abbreviation: m-NBA: m-nitrobenzyl alcohol; Nuc.: nucleoside part; St.: sterol part; TEA: triethanolamine.

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