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Synthesis of Phosphoric Acid Diesters of 7β-Hydroxycholesterol and of Carbohydrates

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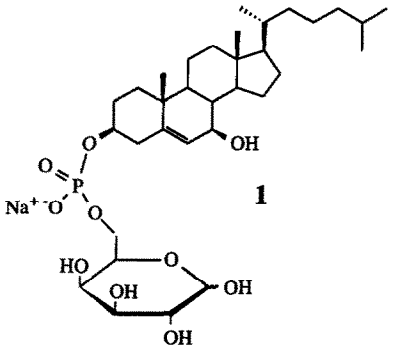
Abstract : In order to enhance the antitumor efficacy of 7β-hydroxycholesterol by targetting its action to defined organs, the phosphoric acid esters of 3(7β-hydroxycholesteryl) and of 6(galactopyranosyl) **1** or 6(mannopyranosyl) **2** were synthesized by the phosphoramidite method (with protected C-1, 2, 3, 4 hydroxyl groups for the carbohydrates). As the protection of the sugars increased the length of the synthesis, we decided to use the hydrogen-phosphonate methodology which leads to a selective phosphorylation at the primary alcohol of carbohydrates and avoids the use of protected carbohydrates. Compound **2** was synthesized in good yield. However compound **1**, probably due to steric hindrance, could not be obtained by this second method.

Introduction

The preparation of suitable prodrugs from lead compounds is considered to be an important approach for carrying drugs to target organs[1-2].

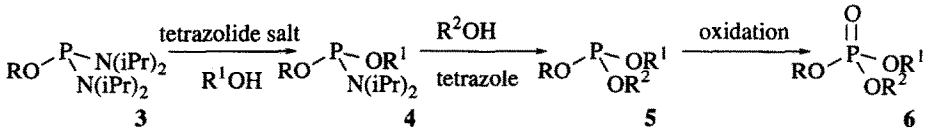
In our research program on oxygenated sterols, we are interested in using this approach to modify the physicochemical properties of these lipophilic compounds and thereby to make them more bioavailable. Previously, we have demonstrated that 7β-hydroxycholesterol is selectively cytotoxic towards tumor cells *in vitro*[3-4]. However its high lipophilicity makes it difficult to be studied *in vivo*. The use of derivatives of 7β–hydroxycholesterol conjugated with nucleoside analogues by a phosphodiester linkage overcomes these difficulties[5-6]. This type of molecules presents several advantages owing to the enhancement of the hydrophilicity of 7β–hydroxycholesterol and a good bioavailability due to the abundance of phosphatases in the living organism. Pharmacokinetic studies[7] have proved that these water-soluble derivatives of oxysterols act as prodrugs, releasing the free oxysterol in the organs and not in blood after i.p. or i.v. injections in rat. In addition, they display anticancer activity when injected i.p. in mice bearing experimental tumors[5,6,8]. We assumed that the phosphoric acid esters of 7β-hydroxycholesterol and of molecules which show a good affinity for a well-defined organ could target the effect of 7β-hydroxycholesterol to this organ. This would reduce the amount of prodrug to administer to have a similar biological effect and consequently decrease the cytotoxicity and the secondary effects of the drug. As receptors for galactose or for mannose are present in higher amount in the liver or in the kidneys respectively than in the other organs, we decided to synthesize the monophosphoric acid esters of 3(7β-hydroxycholesteryl) and of 6(galactopyranosyl) **1** or 6(mannopyranosyl) **2** to target the antitumoral action of oxysterol to liver or to kidney respectively.



These phosphorus-containing products could be synthesized based on P(V) and P(III) chemistry. Three usual methodologies are generally used to produce asymetric phosphodiesters of steroids : the phosphotriester methodology introduced by Letsinger[8-10] seems not to be as efficient as the phosphoramidite one recommended by Caruthers for oligonucleotide synthesis[11-12]. The latter was adapted to the oxysterol phosphorylation by Ji *et al*[13]. A new methodology, the hydrogen-phosphonate approach, seems to be a method of choice since protection of carbohydrate is no longer needed[14].

Results and Discussion

The principle of the phosphoramidite method is outlined in Scheme 1.



Scheme 1

The procedure is initiated by the reaction of alkoxy bis (dialkylaminophosphines) **3**, which are easily prepared from PCl_3[15] and an alcohol R^1OH in neutral conditions, to give the phosphoramidite **4**. Activated by a weak acid such as tetrazole, the intermediate **4** is allowed to couple with another alcohol (R^2OH) and affords the phosphite triester **5**, which can be transformed to the corresponding phosphotriester **6** by an additional oxidation. The whole procedure is carried out under very mild conditions and in general gives a higher overall yield than that obtained via phosphodiester and triester methods.

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

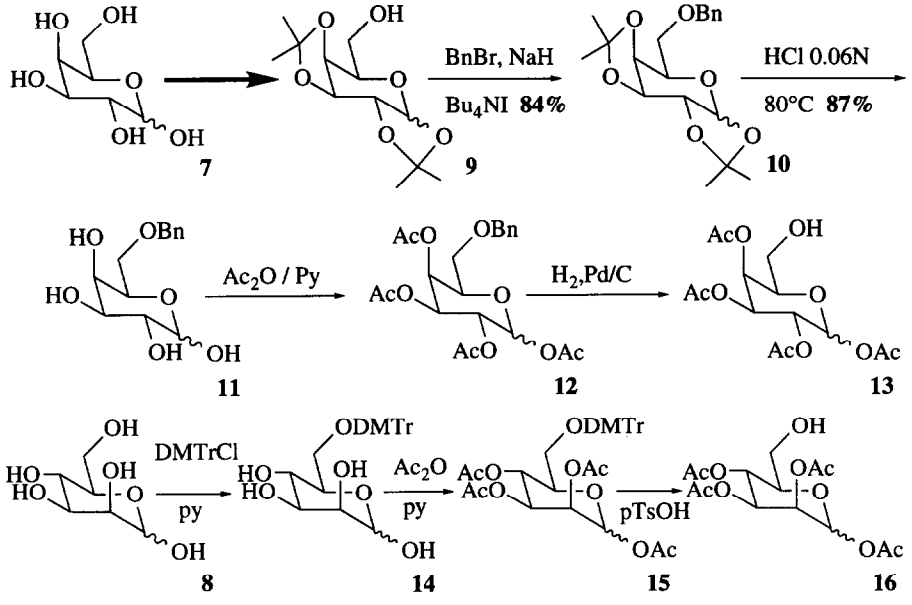
Owing to the progress made in oligosaccharide chemistry, many protective groups have been developed. Most of them are removed only under drastic basic or acidic conditions which are not compatible with our

purpose, because of the lability of the allylic 7β-hydroxyl group in acidic conditions and of the easy hydrolysis of phosphotriester **6** under basic conditions.

The 7β-hydroxyl group of 7β-hydroxycholesterol[16] was selectively protected as its triethylsilyl ether **17**[10]. Deprotection was performed with 0.36% ~ 0.18% HCl in THF without any elimination of the allylic 7β-hydroxyl group.

After several unsuccessful attempts for the protection of C-1, 2, 3 and 4 hydroxyl groups of the carbohydrates with protective groups compatible with the phosphodiester synthesis (triethylsilyl, 4-methoxy benzyl and 4-methoxy tetrahydropyranyl ethers), we turned to acetate as protective group.

The C-1, -2, -3, -4 hydroxyl groups of the carbohydrates (**7** and **8**) were protected by an indirect procedure (Scheme 2).



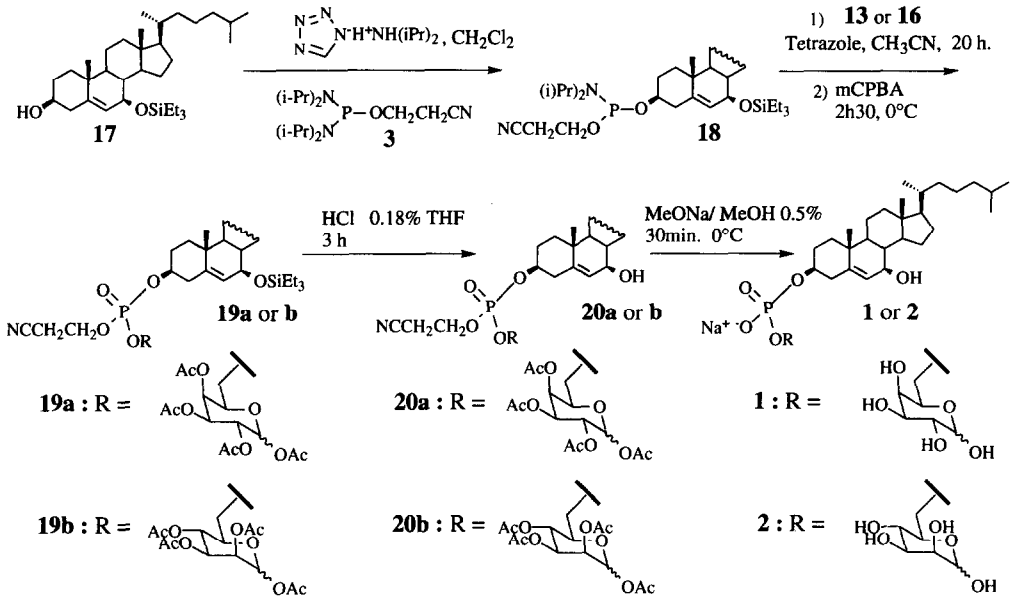
Scheme 2

For galactose, firstly the C-6-hydroxyl group of 1, 2 - 3, 4 diisopropylidene D-galactopyranoside **9** (starting material) was protected as its benzyl (Bn) ether **10** which was hydrolysed to compound **11**. The C-1, 2, 3 and 4 hydroxyl goups were then protected as their acetates to afford intermediate **12**. The latter was then hydrogenolysed in the presence of palladium over charcoal affording the desired compound **13** with free C-6-OH and C-1, 2, 3, and 4-OH protected as acetates. For mannose, the 6-hydroxyl group was selectively transformed to its 4,4' dimethoxytrityl (DMTr) ether **14** under controlled conditions, at - 30°C and for 2 h, to avoid the formation of polytritylated products. The intermediate **14** reacted subsequently with an excess of

acetic anhydride in the presence of pyridine to give the compound **15**. The specific hydrolysis of 4,4' dimethoxytrityl ether with 0,5% p-TsOH in methylene chloride afforded, after 30 minutes, **16** with free C-6-OH and C-1, 2, 3 and 4-OH protected as acetyl esters.

For phosphines of type **3**, a diversity of phosphorus protective groups R has been reported[15]. We have chosen β–cyanoethyl group because of its easy cleavage by a quantitative β-elimination under the same basic conditions as the removal of the protective acetyl groups of the carbohydrates.

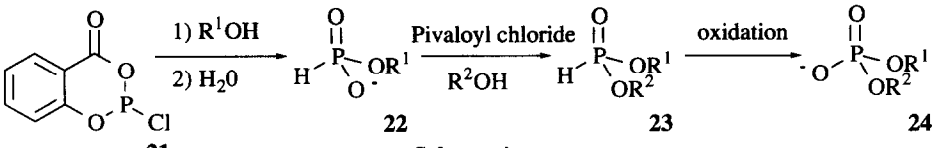
The general procedure for the preparation of phosphodiesters of type **1** or **2** is illustrated in the scheme 3.



Scheme 3

(β-Cyanoethoxy) bis (diisopropylamino) phosphine **3** reacted with the C-3-hydroxyl function of oxysterol **17** in the presence of diisopropylammonium tetrazolide as activating agent. The phosphoramidite **18** was relatively stable and could be purified by chromatography on a short column of silica gel. The activation of **18** by a weak acid such as tetrazole allowed a further coupling with a partially protected carbohydrate. After oxidation with m-chloroperbenzoic acid (m-CPBA), the corresponding phosphotriester **19** was obtained. In this step, we isolated another slightly more polar compound, which corresponds to the partially triethylsilyl-deprotected phosphotriester **20**. Treatment with 0,18% HCl in THF led to the hydrolysis of the triethylsilyl ether group of the sterol. The removal of base labile protective groups was achieved by a treatment with 0.5% CH_3ONa in CH_3OH and gave the sodium salts of phosphodiesters **1** or **2**. These procedures afforded the desired compounds with an overall yield of 47% for **1** and 33% for **2**.

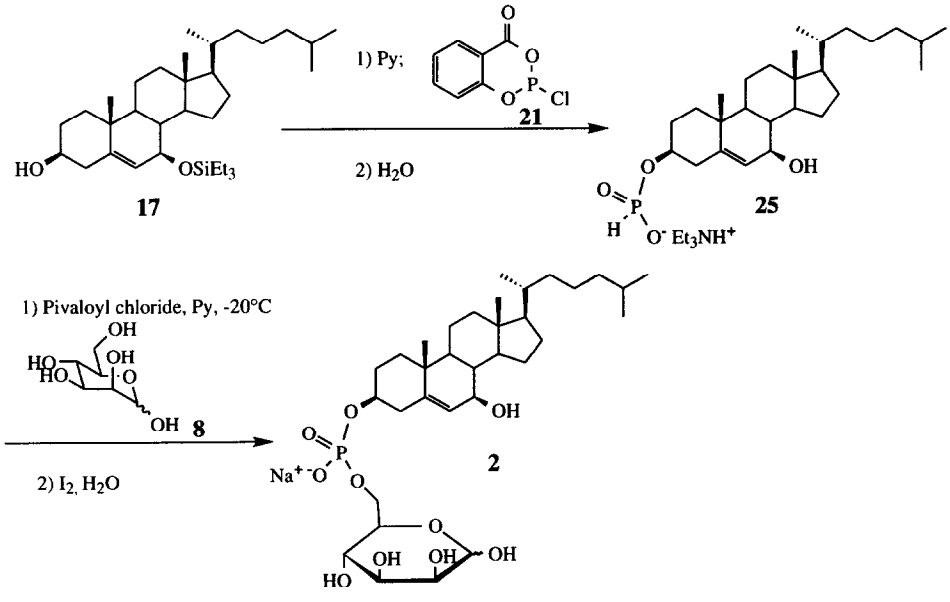
Due to the difficulties of synthesis of phosphodiesters **1** and **2**, we decided to use the hydrogen-phosphonate approach (Scheme 4), which does not require the protection of the carbohydrate[14].



Scheme 4

This procedure is initiated by the reaction of **21** with an alcohol (R^1OH) in pyridine followed by addition of water to give the hydrogen phosphonate **22**[17]. Following formation of the mixed anhydride with pivaloyl chloride[18], the intermediate **22** is allowed to couple with another alcohol (R^2OH) and affords the hydrogen-phosphonate diester **23**, which can be transformed, after oxidation[19], to the corresponding phosphoric acid diester **24**. Because of preferential phosphorylation of primary alcohols over secondary ones, differential protection of hydroxyl groups of the carbohydrate (R^2OH) is no longer necessary.

The synthesis of compounds **1** and **2** was undertaken using the hydrogen-phosphonate methodology. The synthesis of compound **2** is discussed in Scheme 5.



Scheme 5

Phosphite **21** was reacted with the C-3 hydroxyl group of oxysterol **17**, and gave, after hydrolysis and purification, compound **25**. The activation of **25** by pivaloyl chloride allowed at -20°C the coupling at the primary alcohol of mannose **8**. After oxidation with iodine in the presence of water, the corresponding phosphate **2** was obtained in an overall yield of 70%.

Compound **1** could not be obtained : the coupling of galactose **7** to the mixed anhydride formed by **25** and pivaloyl group at -20°C failed, probably due to the steric hindrance at the C-6 hydroxyl group by the axial C-4 hydroxyl group. At higher temperature, many products were obtained and no purification was possible.

Conclusion

The hydrogen-phosphonate method seems to be better adapted than the phosphoramidite one for the synthesis of phosphodiester **2** (better yield : 70% instead of 33%, and faster synthesis), since no protection of mannopyranose is needed. But this method cannot be applied to the synthesis of phosphodiester **1**.

Acknowledgments

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Experimental

Pyridine, dioxane, THF, CH_2Cl_2 and CH_3CN were dried by reflux over CaH_2 and distilled just before use. All the commercial reagents were purchased from Aldrich or Fluka. Tetrazole (Fluka) was purified by sublimation.

TLC were run on pre-coated silica gel plates (Merck, 60F254, 0.25 mm). 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 reveal the compounds. The plates were also dipped in a Dittmer solution[20] in order to visualise phosphorus-containing compounds. Short-column chromatography was carried out by using silica gel (40-63 µm, Merck G60 or Biosil A) columns.

Optical rotations ($[\alpha]_D$) were measured on a Perkin-Elmer 141 polarimeter in DMSO or $CHCl_3$. IR spectra were recorded in $CHCl_3$ on a Perkin-Elmer 881 infrared spectrophotometer. NMR spectra were recorded with Bruker SY (200 MHz) and AM (400 MHz) spectrometers using $CDCl_3$ (7.26 ppm), DMSO-d_6 (2.50 ppm) or CD_3OD (3.34 ppm) as internal standard for ^{1}H-NMR; $CDCl_3$ (77.0 ppm), DMSO-d_6 (39.46 ppm) or CD_3OD (49.0 ppm) as internal standard for ^{13}C-NMR and H_3PO_4 (0 ppm) as internal standard for ^{31}P-NMR. FAB-MS were obtained on a VG analytical ZAB-HF double-focussing mass spectrometer using triethanolamine (TEA), 1-thioglycerol, or m-nitrobenzyl alcohol (m-NBA) as matrix. Microanalyses were performed by the Service Central de Microanalyse du CNRS (Vernaison and Strasbourg local section).

6-O-benzyl-1,2-3,4-di-O-isopropylidene-D-galactopyranoside (10)

NaH 55% (1.97 g, 45 mmol), benzyl bromide (2.56 g, 15 mmol) and tetrabutylammonium iodide (55.2 mg, 0.15 mmol) was added to a solution of compound **9** (3.81 g, 14.63 mmol) in anhydrous THF (20 ml) under Ar. After stirring at r. t. overnight, the reaction mixture was poured into a saturated NaCl solution (50 ml) and extracted with CH_2Cl_2 (3x50 ml). All organic layers were dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was purified by chromatography on silica gel (CH_2Cl_2/CH_3OH: 100/0--->92/8) and afforded compound **10** (4.27g, 84%).

(**10**) **^{1}H-NMR** ($CDCl_3$) : 1.34-1.54 (m, 12H, 2 C($\underline{CH_3}$)$_2$); 3.67 (m, 2H, H-6); 4.01 (m, 1H, H-5); 4.29 (m, 2H, H-2, H-4); 4.61 (m, 3H, H-3, CH_2-benzyl); 5.54 (d, 1H, J = 4.6 Hz, H-1α); 7.32 (m, 5H, H aromatic). **^{13}C-NMR** ($CDCl_3$) : 24.42, 24.92, 25.96, 26.06 (2 C($\underline{C}H_3$)$_2$); 66.86 (C-5); 68.85 (C-6); 70.56 (C-4); 70.61 (C-2); 71.14 (C-3); 73.25 (CH_2-benzyl); 96.32 (C-1); 108.48, 109.16 (2 $\underline{C}$(CH$_3$)$_2$); 127.48-128.26 (CH-benzyl); 138.31 (C-benzyl). **Microanalysis** : found C : 65.37, H : 7.40; calc for $C_{19}H_{26}O_6$ (350.4) C : 65.12, H : 7.48.

6-O-benzyl-D-galactopyranoside (11)

Compound **10** (92.5 mg, 0.26 mmol) was solubilized in 10 ml aqueous HCl solution (0.06N, 0.6 mmol). The reaction mixture was heated at 80°C for 4 h. After addition of $NaHCO_3$ (227.5 mg, 2.7 mmol) at r.t., it was concentrated to dryness under reduced pressure. 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: 4/1) and afforded compound **11** (62 mg, 87%) (diastereoisomers).

(**11**) **^{1}H-NMR** (DMSO-d_6) : 3.24-3.64 (b, 5H, H-2, H-3, H-4, H-5, H-6a); 4.21-4.48 (m, 4H, H-6b, OH-4, OH-2, OH-3); 4.63-4.66 (b, 2H, CH_2-benzyl); 4.93 (b, 1H, H-1α); 6.53 (d, 1H, J = 3.2 Hz, OH-1); 7.32 (m, 5H, H aromatic). **^{13}C-NMR** (DMSO-d_6) : 68.58 (C-5); 69.25 (C-4); 69.81 (C-6); 71.95 (C-2); 72.22 (CH_2-benzyl); 73.01 (C-3); 92.57 (C-1); 127.32-128.17 (CH-benzyl); 138.45 (C-benzyl). **Microanalysis** : found C : 52.63, H : 6.83; calc for C_{13} H_{18} O_6, 1.5 H_2O (297.3) C : 52.52, H : 7.06.

6-O-benzyl-1,2,3,4-tetraacetate-D-galactopyranoside (12)

Acetic anhydride (50ml) was added to a solution of compound **11** (3.48 g, 12.8 mmol) in anhydrous pyridine (50 ml) under Ar. After stirring at r. t. overnight, the reaction mixture was poured into water (100 ml) and extracted with CH_2Cl_2 (3x100 ml). All organic layers were dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was purified by chromatography on silica gel (CH_2Cl_2/CH_3OH: 100/0--->90/10) and afforded compound **12** (4.76g, 85%) (diastereoisomers).

(**12**) **^{1}H-NMR** ($CDCl_3$) : 1.98-2.18 (m, 12H, 4 CH_3CO); 3.52 (m, 2H, H-6); 4.35-4.52 (m, 3H, H-5, CH_2-benzyl); 5.07-5.26 (m, 2H, H-2, H-4); 5.53 (b, 1H, H-3); 6.35 (d, 1H, J = 4.6 Hz, H-1α); 7.28 (m, 5H, H aromatic). **^{13}C-NMR** ($CDCl_3$) : 20.40-20.85 (4 $\underline{C}H_3CO$); 66.73 (C-6); 67.13 (C-5); 68.05 (C-4); 70.97 (C-2); 72.77 (C-3); 73.45 (CH_2-benzyl); 92.24 (C-1); 127.43-128.34 (CH-benzyl); 137.30 (C-benzyl); 168.91-169.94 (4 $CH_3\underline{C}O$). **Microanalysis** : found C : 56.98, H : 6.15; calc for C_{21} H_{26} O_{10} (440.3) C : 57.29, H : 5.95.

1,2,3,4-tetraacetate-D-galactopyranoside (13)

Pd on charcoal 10% (400 mg) was added to a solution of compound **12** (4.76 g, 10.88 mmol) in ethyl acetate (200 ml). Then H_2 was added and stirred at r. t. for 2 days. the reaction mixture was filtered on Celite. All organic layers were concentrated to dryness under reduced pressure. The residue was purified by chromatography on silica gel (Hex/AcOEt: 50/50) and afforded compound **13** (3.19 g, 85%) (diastereoisomers).

(**13**) $[\alpha]_D$ = + 67 (c = 1, $CHCl_3$). **^{1}H-NMR** ($CDCl_3$) : 1.98-2.18 (m, 12H, 4 CH_3CO); 3.87 (m, 1H, H-6a); 4.06-4.26 (m, 2H, H-5, H-6b); 4.98-5.45 (m, 3H, H-2, H-3, H-4); 6.35 (d, 1H, J = 4.6 Hz, H-1α). **^{13}C-NMR** ($CDCl_3$) : 20.55-20.90 (4 $\underline{C}H_3CO$); 62.06 (C-6); 66.67 (C-5); 68.13 (C-4); 69.70 (C-2); 73.07 (C-3); 92.21 (C-1); 168.61-169.83 (4 $CH_3\underline{C}O$). **MS** positive : 348 [M^+ ; 15]; 289 [M - AcO; 90]; 245 [M - AcO - Ac; 60]; 229 [M - 2 AcO; 50]; 169 [M - 3 AcO, 70]. **Microanalysis** : found C : 48.02, H : 6.02; calc for C_{14} H_{20} O_{10} (348.3) C : 48.28, H : 5.79.

6-O-Dimethoxytrityl-D-mannopyranoside (14)

Dimethoxytrityl chloride (10 g, 29.5 mmol) was added to a -20°C solution of D-mannopyranose **8** (4.5 g, 25 mmol) in anhydrous pyridine (50 ml) and triethylamine (17.42 ml, 125 mmol). The reaction was kept at -20°C for 4 h under Ar and quenched by introducing 2 ml of water. The reaction mixture was poured into saturated $NaHCO_3$ solution (100 ml) and extracted with CH_2Cl_2 (3x100 ml). All organic layers were dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was purified by a short-column chromatography on silica gel (CH_2Cl_2 /CH_3OH: 93/7). Compound **14** (10.76 g) was obtained in 90% yield (diastereoisomers).

(**14**) **^{1}H-NMR** (CD_3OD) : 3.35-3.42 (m, 3H, H-5, 2 H-6); 3.75, 3.76 (2s, 6H, 2 OCH_3); 3.80-4.05 (m, 3H, H-2, H-3, H-4); 5.11 (d, 1H, J = 3 Hz, H-1α); 6.79-6.86, 7.15-7.49 (m, 13H, H-aromatic). **^{13}C-NMR** (DMSO-d_6) : 54.97 (O-CH_3); 64.00 (C-5); 67.43 (C-4); 70.88 (C-6); 71.42 (C-2); 73.15 (C-3); 84.92 (C non aromatic of DMT group); 94.06 (C-1); 112.99, 127.38-136.05, 157.92 (C aromatic of DMT group). **Microanalysis** : found C : 64.82, H : 6.18; calc for C_{27} H_{30} O_8, 1 H_2O (500.5) C : 64.79, H : 6.44.

6-O-dimethoxytrityl-1,2,3,4-tetraacetate-D-mannopyranoside (15)

Acetic anhydride (40ml) was added to a solution of compound **14** (1.78 g, 3.7 mmol) in anhydrous pyridine (40 ml) under Ar. After stirring at r. t. overnight, the reaction mixture was poured into water (100 ml) and extracted with CH_2Cl_2 (3x100 ml). All organic layers were dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was purified by chromatography on silica gel (Hex/AcOEt: 8/2--->2/8) and afforded compound **15** (1.88 g, 79%) (diastereoisomers).

(**15**) **^{1}H-NMR** ($CDCl_3$) : 1.97-2.24 (m, 12H, 4 CH_3CO); 3.33 (m, 2H, 2 H-6); 3.55-3.70 (m, 1H, H-5); 3.78, 3.79 (2s, 6H, 2 OCH_3); 5.24-5.49 (m, 3H, H-2, H-3, H-4); 5.86 (d, 1H, J = 6 Hz, H-1β); 6.81-6.84, 7.16-7.45 (m, 13H, H-aromatic). **^{13}C-NMR** ($CDCl_3$) : 20.51-20.84 (4 $\underline{C}H_3CO$); 54.94 (O-CH_3); 61.42 (C-6); 65.84 (C-5); 68.51 (C-4); 69.09 (C-2); 72.06 (C-3);

84.85 (C non aromatic of DMT group); 90.60 (C-1); 112.99, 126.57-135.86, 158.40 (C aromatic of DMT group); 168.19-170.01 ($CH_3\underline{C}O$). **Microanalysis** : found C : 64.53, H : 6.11; calc for C_{35} H_{38} O_{12} (650.6) C : 64.61, H : 5.89.

1,2,3,4-tetraacetate-D-mannopyranoside (16)

Compound **15** (550 mg, 0.84 mmol) was solubilized in 55 ml solution p-TsOH(0.5%, 1.45 mmol) in CH_2Cl_2/CH_3OH : 95/5 at 0°C. The reaction mixture was stirred for 30 min at 0°C, then quenched by adding saturated $NaHCO_3$ solution (35 ml) and extracted with CH_2Cl_2 (3x100 ml). All organic layers were dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The residue was purified by a short-column chromatography on silica gel (CH_2Cl_2 /CH_3OH: 93/7). Compound **16** (278 mg) was obtained in 95% yield (diastereoisomers).

(16) $[\alpha]_D$ = + 30 (c = 0.5, CHCl3). **^{1}H-NMR** ($CDCl_3$) : 1.99-2.18 (m, 12H, 4 CH_3CO); 3.60-3.72 (m, 2H, H-6a, H-5); 3.84 (m, 1H, H-6b); 5.13-5.39 (m, 3H, H-2, H-3, H-4); 6.07 (d, 1H, J = 2 Hz, H-1α). **^{13}C-NMR** ($CDCl_3$) : 20.50-20.82 (4 $\underline{C}H_3CO$); 61.07 (C-6); 65.77 (C-5); 68.36 (C-4); 68.55 (C-2); 72.84 (C-3); 90.64 (C-1); 168.53-169.77 (4 $CH_3\underline{C}O$). **FAB-MS** positive (matrix : NBA) : 331.1 [M -$H_2$0 + H; 8]; 289.1 [M - AcO; 100]; 229 [M - 2 AcO; 50]; 169 [M - 3 AcO, 43]. **Microanalysis** : found C : 48.13, H : 5.86; calc for C_{14} H_{20} O_{10} (348.3) C : 48.28, H : 5.79.

3-(7β-Triethylsilyloxycholesteryl) 2-cyanoethyl N,N-diisopropylphosphoramidite (18).

Compound **18** was prepared as described in Ref. (13).

3-(7β-triethylsilyloxycholesteryl) 6-[1,2,3,4-tetraacetate-D-galactopyranosyl] 2-cyanoethyl phosphotriester (19a).

Compound **18** (3.11 g, 4.33 mmol) was dissolved in minimum anhydrous CH_2Cl_2 and coevaporated with CH_3CN. This procedure was repeated three times and then compound **18** was taken up in 10 ml of CH_2Cl_2. Separately, compound **13** (1.51 g, 4.33 mmol) was dried three times by coevaporation with CH_3CN and was dissolved in CH_3CN (60 ml), in which the above ether solution of **18** and tetrazole (350 mg, 5 mmol) were added. The reaction mixture was stirred at r. t. overnight, under Ar. When the TLC showed the disappearence of compound **2**, m-CPBA (50%) (1.50 g, 4.33 mmol) was introduced. After an additional stirring for 4 h, the mixture was poured into saturated NaCl solution (100 ml) and extracted several times with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The residue was purified by a short column chromatography on silica gel (CH_3OH/CH_2Cl_2: 0/100--->1.5/98.5). Compound **19a** (3.51 g) was obtained in 83% yield.(diastereoisomers).

(19a) $[\alpha]_D$ = + 34 (c = 1, $CHCl_3$). **^{1}H-NMR** ($CDCl_3$) : 0.60 (q, 6H, J = 8 Hz, Si($\underline{C}H_2CH_3)_3$); 0.65 (s, 3H, CH_3-18); 0.84 (d, 3H, J = 6.4 Hz, CH_3-26 and 27); 0.89 (d, 3H, J = 6.4 Hz, CH_3-21); 0.94 (t, 9H, Si($CH_2\underline{C}H_3)_3$); 1.03 (s, 3H, CH_3-19); 1.97-2.18 (m, 12H, 4 CH_3CO); 2.74 (b, 2H, $OCH_2\underline{C}H_2CN$); 3.90 (d, 1H, J = 8.0 Hz, H-7); 4.05-4.28 (m, 6H, H-3, $O\underline{C}H_2CH_2CN$, H-5', 2 H-6'); 5.28-5.69 (m, 4H, H-6, H-2', H-3', H-4'); 6.34 (b, 1H, H-1'α). **^{13}C-NMR** ($CDCl_3$) : 5.91 (($\underline{C}H_3CH_2)_3Si$); 7.11 (($\underline{C}H_3CH_2)_3Si$); 11.91 (C-18); 18.82 (C-21); 18.86 (C-19); 20.47-21.03 (4 $\underline{C}H_3CO$); 22.53 (C-26); 22.78 (C-27); 55.40 (C-14); 56.09 (C-17); 61.99 ($O\underline{C}H_2CH_2CN$); 66.44 (C-6'); 66.47 (C-5'); 67.41 (C-4'); 67.68 (C-2'); 70.73 (C-3'); 74.58 (C-7); 79.23 (C-3); 92.15 (C-1'); 117.69 ($OCH_2\underline{C}H_2CN$); 127.90 (C-6); 142.28 (C-5); 169.80 (4 $CH_3\underline{C}O$). **^{31}P-NMR** ($CDCl_3$) : -2.46; -2.15 (diastereoisomers). **Microanalysis** : Found C : 61.50, H : 8.54; Calc. for C_{50} H_{82} N O_{14} P Si (980.3) C : 61.26, H : 8.43.

3-(7β-triethylsilyloxycholesteryl) 6-[1,2,3,4-tetraacetate-D-mannopyranosyl] 2-cyanoethyl phosphotriester (19b).

Compound **19b** was obtained from **16** and **18** in 78% yield.(diastereoisomers), according to the procedure used for the preparation of **19a**.

(19b) $[\alpha]_D$ = + 30 (c = 1.1, $CHCl_3$). **^{1}H-NMR** ($CDCl_3$) : 0.60 (q, 6H, J = 8 Hz, Si($\underline{C}H_2CH_3)_3$); 0.65 (s, 3H, CH_3-18); 0.84 (d, 3H, J = 6.4 Hz, CH_3-26 and 27); 0.89 (d, 3H, J = 6.4 Hz, CH_3-21); 0.93 (t, 9H, Si($CH_2\underline{C}H_3)_3$); 1.03 (s, 3H, CH_3-19); 1.99-2.18 (m, 12H, 4 CH_3CO); 2.75 (b, 2H, $OCH_2\underline{C}H_2CN$); 3.91 (d, 1H, J = 8.0 Hz, H-7); 4.04-4.26 (m, 6H, H-3, $O\underline{C}H_2CH_2CN$, H-5', 2 H-6'); 5.24-5.38 (m, 4H, H-6, H-2', H-3', H-4'); 6.04 (b, 1H, H-1'α). **^{13}C-NMR** ($CDCl_3$) : 5.90 (($\underline{C}H_3CH_2)_3Si$); 7.10 (($\underline{C}H_3CH_2)_3Si$); 11.91 (C-18); 18.82 (C-21); 18.85 (C-19); 20.58-21.02 (4 $\underline{C}H_3CO$); 22.53 (C-26); 22.78 (C-27); 55.39 (C-14); 56.08 (C-17); 61.82 ($O\underline{C}H_2CH_2CN$); 65.28 (C-5'); 65.83 (C-6'); 68.26 (C-4'); 68.63 (C-2'); 71.03 (C-3'); 74.59 (C-7); 79.03 (C-3); 90.34 (C-1'); 117.62 ($OCH_2\underline{C}H_2CN$); 127.84 (C-6); 140.33 (C-5); 168.10-169.88 (4 $CH_3\underline{C}O$). **^{31}P-NMR** ($CDCl_3$) : -2.68; -2.17 (diastereoisomers). **Microanalysis** : Found C : 61.42, H : 8.56; Calc. for C_{50} H_{82} N O_{14} P Si (980.3) C : 61.26, H : 8.43.

3-(7β-hydroxycholesteryl) 6-[1,2,3,4-tetraacetate-D-galactopyranosyl] 2-cyanoethyl phosphotriester (20a).

Compound **19a** (1.94 g, 1.98 mmol) was dissolved in THF (20 ml) and 20 ml of 0.36% HCl solution in THF was added. After stirring at r. t. for 2 h, the reaction mixture was poured into water (40 ml) and extracted with CH_2Cl_2 (3x50 ml). All organic layers were dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The residue was purified by a short column chromatography on silica gel (CH_3OH/CH_2Cl_2: 0/100--->7/93). Pure compound **20a** (1.46 g) was obtained in 85% yield (diastereoisomers).

(20a) **^{1}H-NMR** ($CDCl_3$) : 0.68 (s, 3H, CH_3-18); 0.86 (d, 3H, J = 6.4 Hz, CH_3-26 and 27); 0.91 (d, 3H, J = 6.4 Hz, CH_3-21); 1.05 (s, 3H, CH_3-19); 1.99-2.18 (m, 12H, 4 CH_3CO); 2.74 (b, 2H, $OCH_2\underline{CH_2}CN$); 3.84 (d, 1H, J = 8.0 Hz, H-7); 4.08-4.37 (m, 6H, H-3, $O\underline{CH_2}CH_2CN$, H-5', 2 H-6'); 5.01-5.54 (m, 4H, H-6, H-2', H-3', H-4'); 6.36 (b, 1H, H-1'α). **^{13}C-NMR** ($CDCl_3$) : 11.83 (C-18); 18.77 (C-21); 19.02 (C-19); 20.63-21.04 (4 $\underline{C}H_3CO$); 22.55 (C-26); 22.81 (C-27); 55.45 (C-14); 55.90 (C-17); 62.02 ($O\underline{C}H_2CH_2CN$); 64.96 (C-6'); 66.37 (C-5'); 67.31 (C-4'); 67.73 (C-2'); 70.75 (C-3'); 73.10 (C-7); 79.19 (C-3); 92.17 (C-1'); 117.65 ($OCH_2CH_2\underline{C}N$); 127.11 (C-6); 139.88 (C-5); 169.86 (4 $CH_3\underline{C}O$). **^{31}P-NMR** ($CDCl_3$) : -2.40; -2.24 (diastereoisomers). **Microanalysis** : Found C : 61.04, H : 8.20; Calc. for $C_{44}H_{68}NO_{14}P$ (866.0) C : 61.03, H : 7.92.

3-(7β-hydroxycholesteryl) 6-[1,2,3,4-tetraacetate-D-mannopyranosyl] 2-cyanoethyl phosphotriester (20b).

Compound **20b** was obtained from **19b** in 80% yield (diastereoisomers), according to the procedure used for the preparation of **20a**.

(20b) **^{1}H-NMR** ($CDCl_3$) : 0.65 (s, 3H, CH_3-18); 0.84 (d, 3H, J = 6.4 Hz, CH_3-26 and 27); 0.88 (d, 3H, J = 6.4 Hz, CH_3-21); 1.03 (s, 3H, CH_3-19); 1.96-2.18 (m, 12H, 4 CH_3CO); 2.75 (b, 2H, $OCH_2\underline{CH_2}CN$); 3.82 (d, 1H, J = 8.0 Hz, H-7); 4.04-4.34 (m, 6H, H-3, $O\underline{CH_2}CH_2CN$, H-5', 2 H-6'); 5.21-5.47 (m, 4H, H-6, H-2', H-3', H-4'); 6.06 (b, 1H, H-1'α). **^{13}C-NMR** ($CDCl_3$) : 11.82 (C-18); 18.83 (C-21); 18.89 (C-19); 20.77-21.02 (4 $\underline{C}H_3CO$); 22.52 (C-26); 22.82 (C-27); 55.39 (C-14); 55.94 (C-17); 61.62 ($O\underline{C}H_2CH_2CN$); 65.21 (C-5'); 66.21 (C-6'); 68.44 (C-4'); 68.64 (C-2'); 71.85 (C-3'); 73.02 (C-7); 79.20 (C-3); 90.36 (C-1'); 117.62 ($OCH_2CH_2\underline{C}N$); 127.65 (C-6); 140.35 (C-5); 168.33-170.13 (4 $CH_3\underline{C}O$). **^{31}P-NMR** ($CDCl_3$) : -2.52; -2.22 (diastereoisomers). **Microanalysis** : Found C : 61.01, H : 7.97; Calc. for $C_{44}H_{68}NO_{14}P$ (866.0) C : 61.03, H : 7.92.

Triethylammonium salt of 3β(7β-hydroxycholesteryl) hydrogen phosphonate (25)

2-Chloro-1,3,2-benzodioxaphosphorin-4-one (**21**) (10 mmol, 10 ml of stock solution of 1M in anhydrous dioxane) was added to a -20°C solution of compound **17** (1.75 g, 3.39 mmol) in anhydrous pyridine (7.5 ml) and dioxane (20 ml). The reaction was kept at -20°C for 20 min under Ar, then hydrolysed with 0.5 ml of water. The reaction mixture was stirred for another 15 min and concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel (Biosil A, desactivated with NEt_3). Elution (CH_2Cl_2/CH_3OH : 95/5 to 85/15) gave the triethylammonium salt of 3β(7β-hydroxycholesteryl) hydrogen phosphonate (**25**), 1.76 g (92% yield).

(25) **^{1}H-NMR** ($CDCl_3$) : 0.60 (s, 3H, CH_3-18); 0.77 (d, 3H, J = 6.4 Hz, CH_3-26*); 0.78 (d, 3H, J = 6.4 Hz, CH_3-27*); 0.83 (d, 3H, J = 6.4 Hz, CH_3-21); 0.95 (s, 3H, CH_3-19); 1.31 (t, $HN^+(CH_2C\underline{H}_3)_3$); 3.02 (q, $HN^+(C\underline{H}_2CH_3)_3$); 3.74 (d, 1H, J = 8.0 Hz, H-7); 3.95 (b, 1H, H-3); 5.19 (s, 1H, H-6); 6.81 (d, 1H, J = 616.0 Hz, P-$\underline{H}$). **^{13}C-NMR** ($CDCl_3$) : 8.5 ($HN^+(CH_2\underline{C}H_3)_3$); 11.7 (C-18); 18.6 (C-21); 18.9 (C-19); 22.4 (C-26); 22.6 (C-27); 45.6 ($HN^+(\underline{C}H_2CH_3)_3$); 55.3 (C-14); 55.8 (C-17); 72.9 (C-7); 73.3 (C-3); 125.8 (C-6); 142.8 (C-5). **^{31}P-NMR** ($CDCl_3$) : 2.46 (d, J = 616.0 Hz). **Microanalysis** : Found C : 69.26, H : 11.22; Calc. for $C_{33}H_{62}NO_4P$ (567.8) C : 69.80, H : 11.00.

Sodium salt of 3(7β-hydroxycholesteryl) 6-D-galactopyranosyl monophosphate (1).

36 ml sodium methanolate 1% (6.33 mmol) in methanol was added to a 0°C solution of **20a** (1.45 g, 1.68 mmol) in CH_3OH (36 ml). After 30 min at 0°C, the reaction was quenched by addition of NH_4Cl (317 mg, 5.94 mmol). The mixture was concentrated under reduced pressure to dryness. The residue was adsorbed on Biosil A (100-200 mesh) and purified by a short column chromatography on Biosil A (200-400 mesh) (CH_3OH/CH_2Cl_2: 3/97--->40/60). Compound **1** (750 mg) was obtained in 67% yield (diastereoisomers).

(1) $[\alpha]_D$ = + 29 (c = 1, DMSO). **^{1}H-NMR** (CD_3OD) : 0.71 (s, 3H, CH_3-18); 0.87 (d, 6H, J = 6.5 Hz, CH_3-26 and 27); 0.94 (d, J = 6.5 Hz, 3H, CH_3-21); 1.07 (s, 3H, CH_3-19); 3.45 (m, 1H, H-4'); 3.64-3.76 (b, 2H, H-7, H-2'); 3.81-4.06 (m, 5H, H-3, H-3', H-5', 2 H-6'); 4.42 and 5.12 (d, 1H, H-1'β 55%, J = 7.4 Hz, H-1'α 45%, J = 3.2 Hz); 5.28 (b, 1H, H-6). **^{13}C-NMR** (CD_3OD) : 13.32 (C-18); 20.30 (C-21); 20.33 (C-19); 23.24 (C-26); 23.27 (C-27); 58.01 (C-14); 58.74 (C-17); 65.71 (C-6'); 66.23 (C-5'); 71.75 (C-4'); 72.84 (C-2'); 72.95 (C-3'); 74.84 (C-7); 77.63 (C-3); 99.73 (C-1'); 128.82 (C-6); 144.67 (C-5). **^{31}P-NMR** (DMSO-d_6) : 0.54 (s). **FAB-MS** negative (matrix: NBA): 643.3 [M - Na; 100]; 523.3 [6]; 481.3 [M - sugar - Na^+ + H; 15]; 259.0 [M - Steroid - Na^+ + H; 8],. **Microanalysis** : found C : 55.19, H : 8.42, Na : 3.60, P : 4.62; calc for $C_{33}H_{56}O_{10}NaP$, 3 H_2O (720.7) C : 55.00, H : 8.67, Na : 3.20, P : 4.30.

Sodium salt of 3(7β-hydroxycholesteryl) 6-D-mannopyranosyl monophosphate (2).

Synthesis of 2 by phosphoramidite methodology.

Compound **2** was obtained from **20b** in 53% yield.(diastereoisomers), according to the procedure used for the preparation of **1**.

Synthesis of 2 by hydrogen-phosphonate methodology.

Pivaloyl chloride (246 µl, 2 mmol) in 3 ml anhydrous pyridine was added dropwise during 20 min to a -20°C solution of compound **25** (400 mg, 0.61 mmol) and mannose **8** (244 mg, 1.36 mmol) in anhydrous pyridine (15 ml). The reaction was kept at -20°C for 20 min under Ar, then quenched by introducing 1 ml of water and was warmed to r.t.. Iodine (155 mg, 0.61 mmol) was added and kept reacting during 2 h. After addition of $NaHSO_3$ (100 mg) and $NaHCO_3$ (100 mg), the reaction mixture was concentrated to dryness under reduced pressure. The residue was adsorbed on Celite and applied to a short-column chromatography over silica gel (Biosil A) (CH_2Cl_2 /CH_3OH: 95/5---> 85/15). Compound **2** (282 mg) was obtained in 70% yield (diastereoisomers).

(**2**) $[\alpha]_D$ = + 4 (c = 1, DMSO). **^{1}H-NMR** (DMSO-d_6) : 0.62 (s, 3H, CH_3-18); 0.83 (d, 6H, J = 6.5 Hz, CH_3-26 and 27); 0.88 (d, J = 6.4 Hz, 3H, CH_3-21); 0.96 (s, 3H, CH_3-19); 3.51-3.90 (m, 7H, H-7, H-3, H-6'a, H-5', H-4', H-3', H-2'); 4.20 (d, 1H, J = 8 Hz, H-6'b); 4.51-4.62 (m, 3H, OH-4', OH-3', OH-2'); 4.84 (b, 1H, H-1'α); 5.16 (b, 1H, H-6); 6.26 (b, 1H, OH-1'). **^{13}C-NMR** (DMSO-d_6) : 11.66 (C-18); 18.59 (C-21 et C-19); 22.32 (C-26); 22.59 (C-27); 55.00 (C-14); 55.89 (C-17); 64.98 (C-6'); 65.93 (C-5'); 66.47 (C-4'); 70.01 (C-2'); 71.26 (C-3'); 74.12 (C-7); 76.88 (C-3); 94.12 (C-1'); 127.47 (C-6); 140.73 (C-5). **^{31}P-NMR** (DMSO-d_6) : 0.97. **FAB-MS** negative (matrix: NBA): 643.3 [M - Na; 100]; 495.2 [60]; 481.3 [M - sugar - Na^+ + H; 30]; 259.0 [M - Steroid - Na^+ + H; 13],. **Microanalysis** : found C : 54.73, H : 8.33, P : 4.44; calc for C_{33} H_{56} O_{10} Na P, 3 H_2O (720.7) C : 55.00, H : 8.67, P : 4.30.

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