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Oxazaphosphorinane Precursors to the Diastereoselective Synthesis of DNA Phosphorothioates

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Abstract: New chiral oxazaphosphorinanes were synthesized as potential precursors to chiral phosphite triesters. Oxazaphosphorinanes **10** and **14** derived from cholesterol and camphor respectively were obtained as stable compounds. They led to rearrangement products in the acidic conditions required for coupling. Then, oxazaphosphorinane **22** derived from D-xylose was synthesized, and led to the diastereoselective synthesis of a T-T phosphorothioate dimer in a 28.5:1 (*Rp*)/(*Sp*) ratio. © 1997 Elsevier Science Ltd.

The antisense strategy has aroused a lot of interest in the past two decades as a tool to interfere with a disease at the gene level and as a possible approach to rational drug design[1]. Among the many DNA analogues that have been designed and synthesized, DNA phosphorothioates (S-ODN) have stood out[2] and reached clinical studies against various infections. However, one of the major drawbacks of S-ODN is their polydiastereomerism due to the generation of a new stereogenic center at each phosphorothioate internucleotidic linkage. This leads to oligomers that are constituted of a large number of diastereomers (2^n if n is the number of internucleotidic linkages). One may reasonably hypothesize that each diastereomer in the mixture displays discrete physicochemical and consequently pharmacological properties. To address the issue of a diastereoseletive synthesis, several approaches have been designed with various degrees of success[3], the most advanced of which is the oxathiaphospholane method developed by Stec and co-workers[4]. At the present time, a practical method that would allow the synthesis of a phosphorothioate oligomer with a desired sense of stereochemistry in large quantities is still lacking.

The well established phosphoramidite method used in the solid phase synthesis of DNA was initially shown to be inefficient towards a diastereoselective synthesis of S-ODN. Stec and Zon[5] showed that when a diastereomerically pure phosphoramidite building block was reacted with a second nucleoside in the presence of tetrazole as a catalyst, the resulting phosphite triester was obtained as a mixture of diastereomers. The mechanism proposed by Stec and Zon, later supported by Berner *et al.*[6], suggested an acidic as well as a nucleophilic role for tetrazole, involving the intermediacy of a phosphorotetrazolide species.

Our research group focused on the modification of the phosphoramidite approach in order first to synthesize diastereomerically pure and stable precursors to avoid the separation of diastereomers, and second to generate diastereomerically pure phosphite triesters from these precursors. The first goal was reached with the replacement of the phosphoramidite part of the molecule by a cyclic oxazaphosphorinane analogue derived from a γ-aminoalcohol. Xin and Just[7] introduced a nucleoside as the phosphorus exocyclic substituent on this heterocycle. Stable derivatives such as **1** could be obtained as pure diastereomers (Scheme 1), by virtue of the preferred axial orientation dictated by the anomeric effect. However, acid-catalyzed ring opening of the heterocycle was accompanied by a complete epimerization in the presence of tetrazole, and by only a partial epimerization in the presence of 2-bromo-4,5-dicyanoimidazole (DCBI). Then, Jin *et al.*[8] modified precursor **1**

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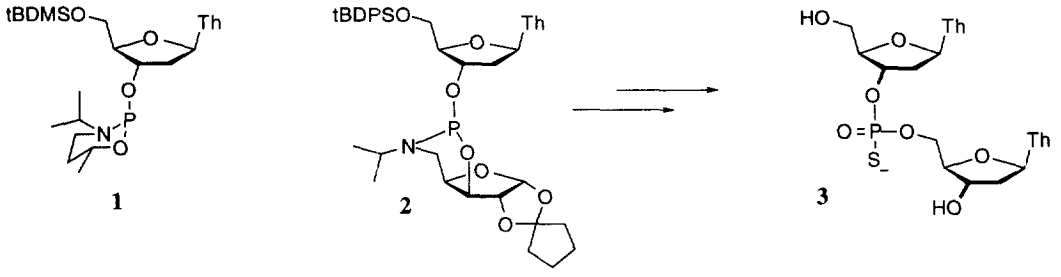
and synthesized oxazaphosphorinane **2**. Chiral oxazaphosphorinane **2** led, in the presence of DCBI and 3'-O-tBDPS-thymidine in chloroform at -15°C, to the intermediate phosphite triester in diastereomeric ratios as high as 70:1 (Scheme 1). After sulfurization, deprotection in 70% TFA led to the desired phosphorothioate dimer **3** in a



Scheme 1

diastereomeric ratio of 70:1. These results clearly demonstrated the validity of this approach for the diastereoselective synthesis of chiral phosphite triesters and the corresponding phosphorothioate triesters and diesters. Besides, it would be reasonable to envisage the diastereoselective synthesis of various P-chiral DNA analogues such as diastereotopically ^{18}O labeled phosphodiesters, phosphoroselenoates, boranophosphates by a simple replacement of the final sulfurization step. However, the deprotection conditions (70% TFA) used to remove the chiral auxiliary at the end of the sequence are very harsh and under these conditions, depurination may be observed when this approach is applied to other nucleotides. We therefore investigated different systems as possible precursors, which could lead to higher diastereoselectivity and be removable under milder conditions (ideally in the presence of ammonia as in the solid phase synthesis of DNA). Our progress is reported in the present article.

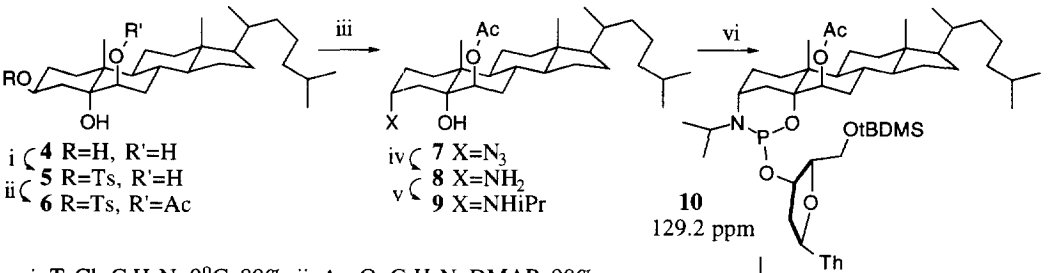
RESULTS AND DISCUSSION

To address this issue, we first considered the use of a chiral γ-aminoalcohol possessing a tertiary alcohol moiety, reasoning that the leaving group ability of the phosphorothioate would make the formation of the corresponding tertiary carbonium ion an easy process. Towards this end, we investigated two types of chiral auxiliaries, possessing a cholestane and a camphane backbone respectively.

Oxazaphosophorinane possessing a cholestane backbone

The first chiral auxiliary of this class was γ-aminoalcohol **9** (Scheme 2). It possessed a tertiary alcohol function on position 5 of the cholestane backbone, as well as an acetate group in an *anti* orientation to the latter, expected to participate[9] and promote the departure of the final phosphorothioate group. The reaction of cholesterol with hydrogen peroxide in formic acid followed by saponification led to triol **4**, as described by Fieser and Rajagopalan[10]. Trihydroxycholestane **4** was then selectively tosylated at position 3 to yield **5** in 80% yield[11]. The alcohol at position 6 was subsequently acetylated under standard conditions to yield **6** in 90% yield[11a]. Lithium azide displacement of the tosylate led to azide **7** in 91% yield[11b], which was then reduced to the corresponding primary amine **8.** The latter directly underwent a reductive alkylation by acetone in the presence of sodium cyanoborohydride, leading to the precursor γ-aminoalcohol **9** in 72% yield from azide **7**.

γ-Aminoalcohol **9** was subsequently reacted with phosphorus trichloride in the presence of triethylamine



i. TsCl, C_5H_5N, 0°C, 80%; ii. Ac_2O, C_5H_5N, DMAP, 90%;
iii. LiN_3, DMF, 100°C, 91%; iv. H_2, Pd/C, EtOH;
v. Me_2CO, $NaBH_3CN$, MeOH, pH 5.5, 72% from **7**
vi. a. PCl_3, 2.2 Et_3N, $CHCl_3$; b. 5'-O-tBDMS-thymidine, 1.1 Et_3N, 65°C, 70% from **9**; vii. 3'-O-tBDPS-thymidine, DCBI, $CHCl_3$

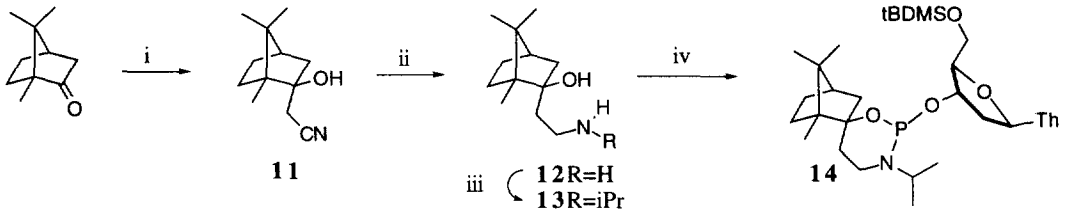
Scheme 2

in chloroform to give immediately the corresponding chloro-substituted oxazaphosphorinane (153.9 ppm by ^{31}P NMR). The chloro substituent was displaced *in situ* by 5'-O-tBDMS-thymidine in the presence of triethylamine, leading to oxazaphosphorinane **10** as a 1:5 mixture of diastereomers (132.3 and 129.2 ppm respectively) when the reaction was performed at ambient temperature. When it was carried out at 65°C, it led to the formation of the major diastereomer only. Presumably, at higher temperature only the thermodynamically favored diastereomer was formed. Flash chromatography allowed the obtention of diastereomerically pure **10** in 70% yield, which turned out to be stable enough to be stored for several months at -20°C. Oxazaphosphorinane **10** turned out to be inert to the presence of tetrazole and 3'-O-tBDPS-thymidine in acetonitrile. When tetrazole was replaced with DCBI, **10** disappeared within 30 min. to give a complex mixture of products having ^{31}P NMR resonance frequencies between 0 and 15 ppm. The same reaction performed in chloroform led, after 15 min., to the formation of about 30% of a product having a chemical shift at δ 141.8 ppm, compatible with the desired phosphite triester. However under these conditions the latter product decomposed to a complex mixture of compounds having resonance frequencies between 0 and 20 ppm. Variation of the temperature and solvent did not improve this result. Disappearance of precursor **10** was also observed in the presence of DCBI alone, leading us to believe that the present structure was too reactive to allow the formation of the desired phosphite triester at C_5.

In order to verify whether the *anti* 6-acetoxy group was responsible for the unstability of precursor **10** in acidic conditions, a structurally similar γ-aminoalcohol lacking this group was synthesized. However, it displayed a reactivity essentially similar to **10** and also led to decomposition in acidic conditions[12].

Oxazaphosphorinane derived from camphor

Following Scheme 3, (*1R*)-(+)-camphor was used as a building block to generate chiral γ- aminoalcohol **13** possessing a hindered, tertiary alcohol function. Initial reaction with cyanomethyllithium at -78°C led to β-hydroxynitrile **11**, which was directly reduced with LAH. The intermediate amine **12** then underwent a reductive alkylation to give the desired γ-aminoalcohol **13** in 63.2% yield starting from camphor. The latter could be purified chromatographically to a waxy gum.



i. $LiCH_2CN$, THF, -78°C; ii. $LiAlH_4$, THF;
iii. $(CH_3)_2CO$, $NaBH_3CN$, MeOH, pH 5.5, 63.2% from camphor
iv. a. PCl_3, 2.2 Et_3N, $CHCl_3$; b. 5'-O-tBDMS-thymidine, 1.1 Et_3N, $CHCl_3$

Scheme 3

Upon reaction of **13** with phosphorus trichloride in the presence of triethylamine in chloroform, a single signal was observed by ^{31}P NMR at 161.5 ppm, corresponding to the desired chloro-oxazaphosphorinane. Subsequent introduction of 5'-O-tBDMS-thymidine and triethylamine into the reaction mixture immediately led to two signals (140.0 and 136.7 ppm), in a 1:1 to 3:1 ratio. However, upon reflux of the mixture, no further change was observed, as opposed to the behavior of oxazaphosphorinanes **1**, **2** and **10** which equilibrated to the thermodynamically favored isomer. The two isomers could be isolated from the reaction mixture but not separated from each other by chromatography.

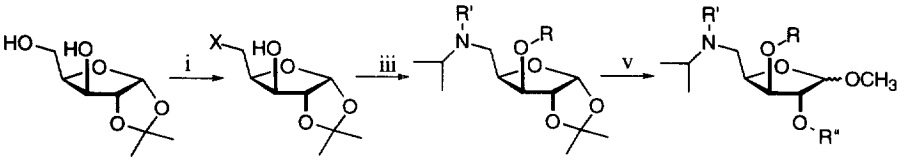
Upon reaction of this pair of isomers in the presence of DCBI with or without 3'-O-tBDPS-thymidine, the same behavior was obtained as with cholestane derivative **10**[13]. A complex mixture of products was obtained (0 to 20 ppm by ^{31}P NMR), which could not be separated by chromatography[14].

We concluded from this study that, even though the desired chiral oxazaphosphorinanes **10** and **14** could be synthesized and purified chromatographically, their ease of decomposition in acidic conditions prevented the coupling with a second nucleoside.

Our efforts were therefore oriented towards a closer direction to that chosen by Jin *et al.*[8], and we considered the use of a xylose precursor such as **2**. However, to modify the deprotection conditions, we planned to introduce a participating group at position 2 of the xylose ring, in an *anti* orientation with respect to the phosphorothioate leaving group, suitable for a neighboring group participation.

Xylose-derived oxazaposphorinane

As a neighboring group, we chose a carbamate as already used by Bernet and Vasella[15] or Knapp *et al.*[16], since it presumably would be able to participate under basic conditions and not interfere under the acidic conditions used for coupling. Scheme 4 summarizes the route followed to synthesize the desired γ-aminoalcohol **21**. Starting from 1,2-O-isopropylidene-D-xylofuranose, a first selective tosylation at position 5 was carried out under standard conditions, giving access to tosylate **15**[17], which was then reacted with isopropylamine at 80°C in a pressure vessel to yield γ-aminoalcohol **16**. The 3-hydroxy group was protected as a benzyl ether to give **17** in 85% yield, and the 5-amino group was protected as a benzyl carbamate, producing **18** in an essentially quantitative yield. Cleavage of the 1,2-O-isopropylidene function was attempted using a variety of conditions[18,19], and was achieved when acetonide **18** was refluxed in a 1% methanolic solution of iodine, as reported by Szarek *et al.*[20]. Two anomers of the resulting alcohol **19** were thus obtained and separated by flash chromatography. The fast-eluting and slow-eluting anomers were obtained in 53% and 44% isolated yields respectively. Assignment of

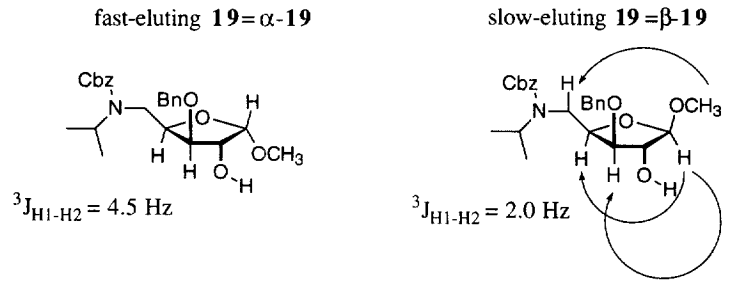


ii (**15** X=OTs, **16** X=NHiPr) iv (**17** R=Bn, R'=H; **18** R=Bn, R'=Cbz) vi (**19** R=Bn, R'=Cbz, R"=H) vii (**20** R=Bn, R'=Cbz, R"=$CONHC_6H_5$; **21** R=R'=H, R"=$CONHC_6H_5$)

i. TsCl, C_5H_5N, 0°C, 92.4%; ii. $iPrNH_2$, 80°C, P, 96%; iii. BnBr, NaH, 0.1 NaI, THF, 85%; iv. BnOCOCl, $KHCO_3$, THF:H_2O, quant.; v. MeOH, I_2, Rfx, 97%; vi. a. $(Cl_3CO)_2CO$, C_5H_5N, 0°C; b. aniline *in situ*, 88.1% from **19**; vii. H_2, Pd/C, EtOH:H_2O:Ac OH, 36h, 77%

Scheme 4

the stereochemistry at C_1 was done unambiguously using a 2D-NOE experiment, in which only slow-eluting **19** showed a cross-peak between the following protons: H_1 and H_3, H_1 and H_4, OCH_3 and H_5. The $^3J_{H1\text{-}H2}$ coupling constants showed the same trend (4.5 Hz for α–**19** and 2.0 Hz for β-**19**), however the difference was not significant enough to assign the configuration unambiguously (Scheme 5).



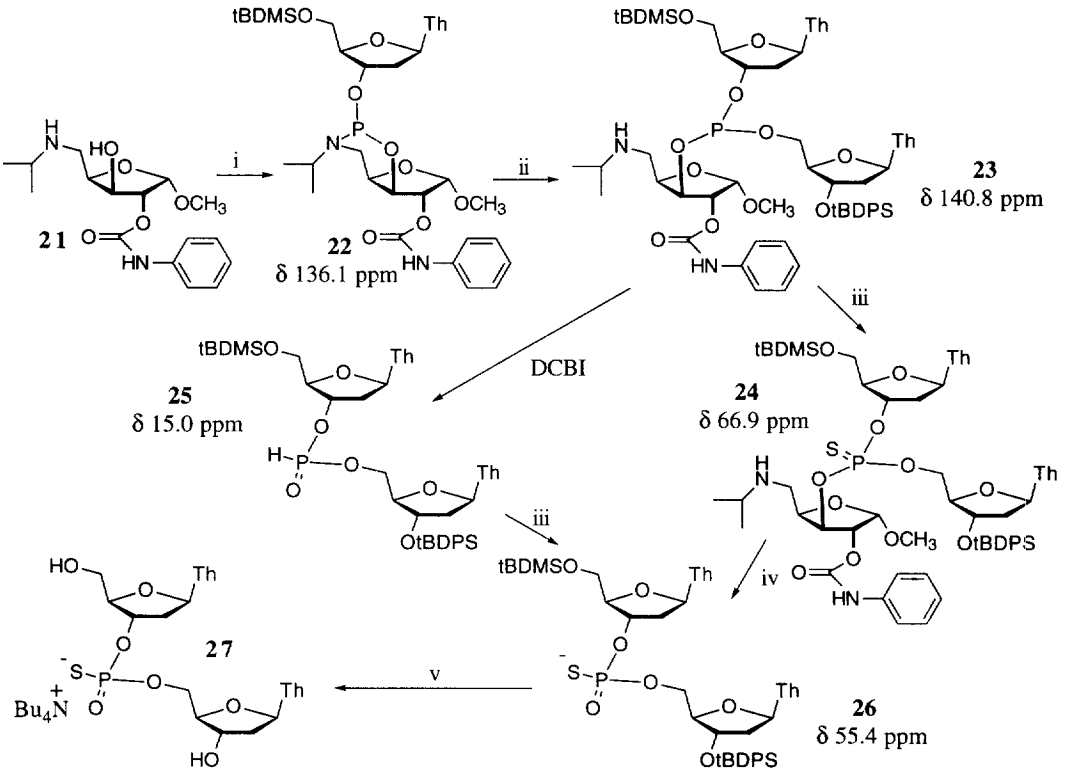
Scheme 5

Each anomer was treated separately with 0.3 eq. of triphosgene in dry pyridine at 0°C followed by aniline, to give the corresponding phenyl carbamates α-**20** and β-**20** in 85-88% yield after flash chromatography. The removal of the 3-Bn and 5-Cbz protections was performed by hydrogenation over 10% Pd/C to yield γ-aminoalcohols α-**21** and β-**21** in 77 to 80% yield.

The next step was to study whether α-**21** and β-**21** would be suitable for the synthesis of an oxazaphosphorinane precursor. Therefore, α-**21** was first reacted with phosphorus trichloride in the presence of triethylamine in chloroform, then with a mixture of 5'-O-tBDMS-thymidine and triethylamine in chloroform, and the solution was subsequently refluxed for 24h, until ^{31}P NMR indicated the presence of a single signal at 136.1 ppm. The desired oxazaphosphorinane **22** was purified by chromatography and obtained as a solid in 67.3% yield. On the other hand, when γ-aminoalcohol β-**21** was reacted with phosphorus trichloride then 5'-O-tBDMS-thymidine in the same conditions as the α anomer, a mixture of at least 4 major compounds was obtained (130-140 ppm by ^{31}P NMR), which did not further evolve. The products could not be separated, and the reason for such a different behavior could thus not be established.

When **22** was reacted with 3'-O-tBDPS-thymidine in the presence of tetrazole in acetonitrile (Scheme 6), it gave very slowly a mixture of products (10-15 ppm by ^{31}P NMR), as well as the desired phosphite triester **23** appearing at 140.8 ppm. When the reaction was carried out in chloroform in the presence of DCBI at -5°C,

precursor **22** quickly gave the desired phosphite triester **23** (δ 140.8 ppm by ^{31}P NMR), as well as several side products (10-15 ppm by ^{31}P NMR). We suspected that H- phosphonates **25** had formed as a side product, and that a faster coupling followed by sulfurization would be suitable. Therefore, the reaction was performed in acetonitrile in the presence of DCBI at ambient temperature. In order to prevent a possible decomposition of the triester in acidic conditions, Beaucage's reagent was introduced into the mixture 1 min. after the coupling. The



i. a. PCl_3, 2.2 Et_3N, $CHCl_3$, Rfx; b. 5'-O-tBDMS-thymidine, 1.1 Et_3N, $CHCl_3$, Rfx, 67.3% from **21**
ii. DCBI, 3'-O-tBDPS-thymidine, CH_3CN, -20°C; iii. Beaucage's reagent; iv. NH_4OH, 91% from **22**
v. 2.2 TBAF/DMF, 89.9% from **26**

Scheme 6

^{31}P NMR spectrum of this mixture showed, on the one hand, two diastereomers of the desired phosphorothioate triester **24** in a 10:1 ratio (67.6 and 66.6 ppm respectively) and on the other hand, two diastereomers of the deprotected diester **26** in a ratio of 6:1 (59.5 and 57.6 ppm respectively). This mixture did not further evolve with time, leading us to believe that the pair of phosphorothioate diesters **26** did not come from the direct decomposition of **24** but rather from the sulfurization of H-phosphonate **25** issued from the decomposition of phosphite triester **23**. When the mixture of **24** and **26** was treated with aqueous ammonia, two peaks were obtained at 55.4 and 55.1 ppm in a ratio of 8:1, corresponding to the desired phosphorothioate diester **26** after elimination of the chiral auxiliary. This indicated that the chiral auxiliary had indeed efficiently been removed under ammonia treatment. The same sequence of reactions was repeated at -20°C and led, after ammonia deprotection, to the desired phosphorothioate dimer **26** in a 28.5:1 diastereomeric ratio. Purification of this

mixture by silica gel chromatography allowed the recovery of the major diastereomer as a pure product in 91% yield. Deprotection of both silyl protecting groups with 2.2 eq. TBAF in DMF allowed the obtention of the desired phosphorothioate dimer **27** as its tetrabutylammonium salt in 89.9% yield after flash chromatography. The (R_p) absolute configuration at the phosphorus atom was assigned based on *Snake venom phosphodiesterase* digestion and HPLC analysis[21].

In conclusion, we have studied a number of structurally diverse oxazaphosphorinanes and evaluated their potential as precursors to chiral phosphite triesters. Cholesterol and camphor derivatives **10** and **14** turned out to be too unstable in the acidic conditions required for coupling, however they could be synthesized and characterized. Xylose-derived oxazaphosphorinane **22** was synthesized and characterized as a stable compound. We further showed that by tuning the coupling conditions (solvent, temperature and catalyst), it was possible to synthesize a T-T phosphorothioate dimer in a good diastereomeric ratio. This chiral auxiliary could be removed by simple ammonia treatment, which represents a significant advantage over the previous precursors of the same type. Even though this new approach does not constitute a practical method, it shows once again that the phosphoramidite technology can be advantageously modified towards a diastereocontrolled synthesis of P-chiral antisense building blocks.

Acknowledgments

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Experimental section

General methods

Melting points were obtained on a Gallenkamp MF-370 electrothermal apparatus and are uncorrected. Optical rotation measurements were recorded on a Jasco DIP-140 digital polarimeter. Mass spectra were recorded on a MS25RFA mass spectrometer, HRMS was performed on a ZAB 2F HS mass spectrometer. ^{1}H NMR, ^{13}C NMR spectra were recorded on a Varian XL200, Unity 500 or a Jeol Eclipse 270 spectrometer and are referenced with respect to the residual signals of the solvent. ^{31}P NMR spectra were recorded on a Varian XL 300 or Unity 500 spectrometer and are referenced to external 85% H_3PO_4 signal. THF was distilled on sodium benzophenone ketyl, acetonitrile and triethylamine from calcium hydride, chloroform and methylene chloride from phosphorus pentoxide, methanol from magnesium and anhydrous DMF was purchased from Aldrich Chemicals Co. in sure-seal bottles and used with no further drying. 3'-O-tBDPS-thymidine, tetrazole and Beaucage's reagent were given by Isis Pharmaceuticals. All other reagents were purchased from Aldrich Chemicals Co.

6β-acetoxy-5-hydroxy-3α-(N-isopropylamino)cholest-5α-ane **9**

A solution of azide **7** (300 mg, 0.6 mmol) and 10% Pd/C (150 ml) in 20 ml ethanol was shaken at RT under 40 psi of hydrogen for 16h. The mixture was filtered on Celite, then evaporated *in vacuo* to a yellow oil. The crude mixture was dissolved in 10 ml dry methanol, the pH of the mixture being adjusted to 5.5 by addition of acetic acid. Acetone (440 µl, 6 mmol) was then added, followed by sodium cyanoborohydride (189 mg, 3

mmol) and the mixture was stirred for 24h at RT. The crude mixture was quenched with sodium bicarbonate, concentrated *in vacuo*, taken up in 30 ml dichloromethane, washed twice with a sodium carbonate solution, dried over magnesium sulfate and evaporated *in vacuo* to a sticky solid. The product was purified by flash chromatography (hexanes:ethyl acetate:triethylamine 80:10:10) and yielded 217 mg (72% from **7**) of pure amine **9**, m.p. 80-82°C.

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.74 (m, 1H); 3.24 (m, 1H); 2.96 (h, 1H, J=6.7 Hz); 2.03 (s, 3H); 2.00-0.70 (multiplets, 52 H); HRMS (EI), M=$C_{32}H_{57}NO_3$ calc: 503.43340, found: 503.43382; $[\alpha]^D_{295}$ -44.9 (c 1.0, ethyl acetate).

Cholesterol-derived oxazaphosphorinane **10**

To a solution of phosphorus trichloride (87 µl, 1 mmol) in 5 ml dry chloroform stirred at 0°C under Ar was added a mixture of **9** (1 mmol) and triethylamine (307 µl, 2.2 mmol) in 5 ml dry chloroform over 1h. At the end of the addition, ^{31}P NMR indicated the presence of a single signal at δ 153.9 ppm, corresponding to the intermediate chlorophosphoramidite. The mixture was warmed up to 60°C and a mixture of 5'-O-tBDMS-thymidine (356 mg, 1 mmol) and triethylamine (153 µl, 1.1 mmol) in 5 ml dry chloroform was added over 5 min. The mixture was allowed to cool down to RT and ^{31}P NMR then indicated a single signal at δ 129.2 ppm (**10**). The crude mixture was concentrated *in vacuo* to a thick oil, then poured into 30 ml of ethyl acetate. The salt was filtered and the filtrate evaporated *in vacuo* to a colorless oil which was purified by flash chromatography (hexanes:ethyl acetate:triethylamine 70:15:15). Oxazaphosphorinane **10** was collected as a white solid in 70% yield, m.p. 91-93°C (dec.).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, 1H, J=1.0 Hz, C=C**H**); 6.35 (dd, 1H, **H$_{1'}$**, J=6.8 Hz; J=6.0 Hz); 4.79 (m, 1H, **H$_6$**); 4.61 (m, 1H, **H$_{3'}$**); 4.05 (m, 1H, **H$_{4'}$**); 3.88 (B of ABX, 1H, **H$_{5'}$**, J=11.5 Hz, J=2.2 Hz); 3.78 (A of ABX, **H$_{5'}$**, J=11.5 Hz, J=2.0 Hz); 3.28 (m, 1H, **H$_3$**); 3.18 (h, 1H, NC**H**$(CH_3)_2$, J=6.8 Hz); 2.43 (ddd, 1H, **H$_{2'}$**, J=13.5 Hz, J=6.0 Hz, J=3.0 Hz); 2.28 (m, 1H, **H$_4$**); 2.16 (ddd, 1H, **H$_{2'}$**, J=13.5 Hz, J=6.8 Hz, J=7.0 Hz); 2.03 (m, 1H, **H$_4$**) ; 2.01 (s, 3H, C**H$_3$**COO); 1.90 (d, 3H, CH=CC**H$_3$**, J=1.0 Hz); 1.82-0.64 (37H); 1.19 (d, 6H, NCH(C**H$_3$**)$_2$, J=6.8 Hz); 0.91 (s, 9H, SiC(C**H$_3$**)$_3$); 0.097, 0.093 (2s, 6H, Si(C**H$_3$**)$_2$); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 169.79; 163.51; 149.92; 135.72; 110.43; 86.75 (d, **C$_{4'}$**, J=5.5 Hz); 85.03 (**C$_{1'}$**); 78.34 (d, **C$_5$**, J=8.2 Hz); 74.87 (**C$_6$**); 72.22 (d, **C$_{3'}$**, J=20.1 Hz); 62.90 (**C$_{5'}$**); 49.10 (d, N**C**H$(CH_3)_2$, J=27.5 Hz); 47.67 (d, **C$_3$**, J=5.5 Hz); 56.03; 55.62; 48.99; 45.96; 44.86; 42.47; 39.68; 39.55; 39.30; 35.98; 35.66; 31.22; 30.16; 29.94; 28.03; 27.81; 25.80; 23.97; 23.92; 23.88; 23.71; 22.62; 22.37; 21.84; 21.19; 20.42; 18.48; 14.53; 12.33; 11.94; 11.12; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 129.2; MS (FAB) m/z 910.47 (8.5%, (M+Na)); 762 (5.3%); 339 (100.0%); 367 (46.1%).

(*1R,2S,4R*)-2-(2-(N-isopropyl)amino)ethyl-2-hydroxy-1,7,7-trimethylbicyclo[2.2.1]heptane **13**

To a solution of dry acetonitrile (210 µl, 4.0 mmol) in 40 ml dry THF stirred at -78°C under Ar was added a 1.6 M solution of n-butyllithium in hexane (2.75 ml, 4.4 mmol) over 30 min. The mixture was stirred for 15 min at -78°C, 30 min. at -40°C, then cooled down to -78°C. At that temperature was added a solution of freshly sublimed (*1R*)-(+)-camphor (533 mg, 3.5 mmol) in 2 ml dry THF over 15 min. The mixture was stirred for 2h at -78°C then 6h at 0°C, until TLC showed complete consumption of camphor. LAH (304 mg, 8 mmol.) was added at 0°C and the mixture was stirred for 14h, until TLC indicated complete disappearance of the intermediate nitrile. To the crude mixture cooled down to 0°C again, was added slowly 310 µl water, then 460 µl

of a 2N sodium hydroxide solution, then 1.2 ml water. The white solid thus formed was filtered, washed with water, and the resulting filtrate evaporated *in vacuo* to a yellow oily residue. This crude product was dissolved in 40 ml dry methanol, the pH was adjusted to 5.5 by addition of acetic acid. Acetone (1.18 ml, 16 mmol) was added to the mixture, followed 1h later by sodium cyanoborohydride. After 4h was added a saturated solution of sodium bicarbonate (2 ml), and the mixture was concentrated *in vacuo*. It was then taken up in 100 ml dichloromethane, washed three times with 10 ml of a 10% solution of sodium carbonate, then dried on magnesium sulfate and evaporated to a yellow sticky gum. The product was purified by flash chromatography (dichloromethane:triethylamine 98:2), to yield 604 mg (63.2% from camphor) of a slightly yellow waxy solid.
^{1}H NMR (500 MHz, $CDCl_3$) δ 2.95 (ddd, 1H, J=12.2 Hz, J=6.8 Hz, J=3.4 Hz); 2.83 (ddd, 1H, J=12.2 Hz, J=9.8 Hz, J=2.9 Hz); 2.73 (h, 1H, J=6.3 Hz); 2.04 (dt, 1H, J=12.7 Hz, J=3.9 Hz); 1.66 (m, 3H); 1.52 (ddd, 1H, J=14.6 Hz, J=3.4 Hz, J=2.9 Hz); 1.34 (m, 3H); 1.04 (d, 6H, J=6.3 Hz); 0.92 (m, 1H); 1.11, 0.87, 0.83 (3s, 9H); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 81.77; 51.92; 49.37; 48.70; 47.21; 44.98; 44.12; 36.95; 30.15; 26.96; 22.78; 22.40; 21.34; 20.84; 11.03; HRMS (FAB) M+H=$C_{15}H_{30}NO$ calc: 240.232740, found: 240.232800; $[\alpha]^D_{295}$ +30.1° (c 1.8, EtOAc).

Camphor-derived oxazaphosphorinane **14**

To a solution of phosphorus trichloride (87 µl, 1.0 mmol) in dry chloroform (3 ml) stirred at 0°C under Ar, was added a solution of **13** (236 mg, 1.0 mmol) and dry triethylamine (307 µl, 2.2 mmol) in dry chloroform (3 ml). After addition, ^{31}P NMR indicated the presence of a single signal (δ 161.5 ppm). At the same temperature, a solution of 5'-O-tBDMS-thymidine (356 mg, 1.0 mmol) and dry triethylamine (154 µl, 1.1 mmol) in 3 ml dry chloroform was added over 1h. The mixture was stirred at RT for 1h, then ^{31}P NMR indicated the presence of two signals (δ 140.0 and 136.7 ppm respectively, ratio 2:1). Refluxing the mixture as long as 48h did not change this ratio. The solvent was evaporated *in vacuo* and the salt precipitated by addition of ethyl acetate. The filtrate was concentrated *in vacuo* and the mixture of diastereomers was isolated from the crude by flash chromatography (hexanes:ethyl acetate:triethylamine 85:15:10) in 77.3% yield (482 mg) as a colorless oil. The two diastereomers could not be separated chromatographically. Analyses were performed on a 2:1 diastereomeric mixture[22].
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, 1H, NC**H**=C(CH_3), J=1.2 Hz); 6.33 (dd, 1H, $\mathbf{H_{1'}}$, J=5.6 Hz, J=8.5 Hz); 4.48-4.51 (m, 1H, $\mathbf{H_{3'}}$); 4.08 (m, 1H, $\mathbf{H_{4'}}$); 3.74 (dd, 1H, $\mathbf{H_{5'}}$, J=11.5 Hz, J=2.1 Hz); 3.74 (dd, 1H, $\mathbf{H_{5'}}$, J=11.5 Hz, J=2.1 Hz); 3.35 (dh, 1H, $\mathbf{H_{13}}$, J=6.6 Hz, J=5.9 Hz); 3.27 (m, 1H, $\mathbf{H_{12}}$); 3.08 (m, 1H, $\mathbf{H_3}$); 2.78 (m, 1H, $\mathbf{H_{12}}$); 2.41 (dt, $\mathbf{H_3}$, J=13.9 Hz, J=3.7 Hz, J=3.2 Hz); 2.32 (ddd, 1H, $\mathbf{H_2}$, J=12.2 Hz; J=5.6 Hz, J=1.5 Hz); 1.99 (ddd, 1H, $\mathbf{H_{2'}}$, J=12.2 Hz, J=8.5 Hz, J=4.4 Hz); 1.88 (b, 3H, CH=C(**C**$\mathbf{H_3}$)); 1.87-0.10 (multiplets); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 163.93; 150.33; 135.43; 110.78; 86.70 (d, J=3.7 Hz); 85.50 (d, J=10.1 Hz); 84.86; 73.16 (d, J=21.1 Hz); 63.40 ; 52.94 (d, J=4.6 Hz); 40.61 (d, J=4.6 Hz); 25.90; 21.76 (d, J=10.1 Hz); 21.44; 18.31; 12.46; -5.43; -5.50; ^{31}P NMR (202.3 MHz, $CDCl_3$) δ 140.1 (major); 136.8 (minor); HRMS (FAB) M+H=$C_{31}H_{55}N_3O_6PSi$ calc: 624.359779, found: 624.359500.
Upon reaction of **14** with DCBI in acetonitrile or chloroform with or without 3'-O-tBDPS-thymidine, a mixture of products was obtained (0-15 ppm by ^{31}P NMR). Chromatographic purification allowed the isolation of three of the components, unseparable from each other. However, HRMS of the mixture gave the same molecular ion as the starting **14** M+H=$C_{31}H_{55}N_3O_6PSi$ calc: 624.359779, found 624.359500.

This result absolutely correlated with the molecular weight of oxazaphosphorinane **14** , leading to the conclusion that it was a rearrangement product from **14.**

5-deoxy-5-N-isopropylamino-1,2-O-isopropylidene-xylofuranose **16**

A solution of 1,2-O-isopropylidene-5-*p*-toluenesulfonyl-xylofuranose **15**[17] (3.0 mmol, 1.032 g) in isopropylamine (20 ml) was heated at 80°C under pressure for 20 h. The solvent was then removed *in vacuo*, and the brown syrup thus obtained was taken up in 100 ml ethyl acetate and washed twice with a 10% sodium carbonate solution. The organic layer was dried over magnesium sulfate and evaporated *in vacuo* to give an amber-colored oil in a 96 % yield (0.665 g). The product was used with no further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.93 (d, 1H, J=3.5 Hz); 4.47 (d, 1H, J=3.5 Hz); 4.28 (d, 1H, J=3.0 Hz); 4.21 (m, 1H); 3.37 (dd, 1H, J=12.5 Hz; J=3.8 Hz); 2.97 (dd, 1H, J=12.5 Hz, J=1.5 Hz); 2.76 (h, 1H, J=6 Hz); 1.47 (s, 3H); 1.31 (s, 3H); 1.07 (2d, 6H, J=6 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 111.37; 105.06; 86.06; 78.19; 76.92; 48.70; 45.80; 26.82; 26.13; 22.58; 22.26; HRMS (EI) M=$C_{11}H_{21}NO_4$ calc: 231.14650, found: 231.14705; $[\alpha]^D_{295}$ +20.6° (c 2.3, EtOAc).

3-O-benzyl-5-deoxy-5-isopropylamino-1,2-O-isopropylidenexylofuranose **17**

To a solution of **16** (3.41 mmol, 788 mg) stirred in dry THF at 0°C under Ar was added sodium hydride (4 mmol, 164 mg of a 60% suspension in mineral oil). After 30 min, benzyl bromide (3.41 mmol, 406 µl) was added to the solution, followed by sodium iodide (45 mg, 0.3 mmol), and the stirring was continued for 6h at 0°C, then at RT for overnight. A 10% solution of ammonium chloride was added dropwise and the mixture was concentrated *in vacuo*, then poured into 100 ml of ethyl acetate, and washed twice with 20 ml of a 10% solution of sodium carbonate. The organic layer was dried over magnesium sulfate and the solvent evaporated to give a yellow oil. The product was purified by flash chromatography (hexanes:ethyl acetate 90:10 to hexanes:ethyl acetate:triethylamine 50:45:5) to give 930 mg (85%) of pure **17** as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28-7.38 (m, 5H); 5.93 (d, 1H, J=4 Hz); 4.70 (B of AB, 1H, J=12 Hz); 4.62 (d, 1H, J=4 Hz); 4.47 (A of AB, 1H, J=12 Hz); 4.28 (ddd, 1H, J=3 Hz, J=5 Hz, J=8 Hz); 3.90 (d, 1H, J=3 Hz); 2.92 (dd, 1H, J=8 Hz, J=12 Hz); 2.85 (dd, 1H, J=5 Hz, J=12 Hz); 2.79 (h, 1H, J=6.5 Hz); 1.47 (s, 3H); 1.31 (s, 3H); 1.01 (t, 6H, J=6 Hz);

^{13}C NMR (125.7 MHz, $CDCl_3$) δ 137.41; 128.51; 127.98; 127.76; 111.50; 104.85; 82.28; 81.74; 79.88; 71.67; 48.82; 45.66; 26.68; 26.24; 23.02; 22.58; HRMS (EI), M-CH_3=$C_{17}H_{24}NO_4$ calc: 306.17029, found: 306.17052; $[\alpha]^D_{295}$ -51.8° (c 1.85, CH_2Cl_2).

3-O-benzyl-5-deoxy-5-(N-isopropyl-N-carboxybenzyloxy)amino-1,2-O-isopropylidene xylofuranose **18**

To a solution of **17** (10.8 mmol, 3.46 g) and potassium bicarbonate (11.0 mmol, 1.10 g) in 100 ml of a THF:water mixture (9:1) stirred at RT was added benzyl chloroformate (10.8 mmol, 1.54 ml). After 1.5h, the mixture was evaporated *in vacuo* and taken up in 350 ml ethyl acetate, washed twice with brine, and the organic layer was dried over magnesium sulfate and evaporated to 4.87 g of a yellow oil in an essentially quantitative yield. The product was used with no further purification.

^{1}H NMR (200 MHz, CD_3SOCD_3, 90°C)[23] δ 7.35 (s, 10H); 5.83 (d, 1H, J=3.9 Hz); 5.08 (s, 2H); 4.68 (d, 1H, J=3.9 Hz); 4.57 (AB, 2H, J=11.8 Hz); 4.22 (m, 1H); 4.00 (h, 1H, J=6.8 Hz); 3.86 (d, 1H, J=3.2 Hz); 3.06 (dd, 1H, J=3.6 Hz, J=15.1 Hz); 3.29 (dd, 1H, J=6.7 Hz, J=15.1 Hz); 1.34 (s, 3H); 1.26 (s, 3H); 1.15 (d, 3H,

J=6.9 Hz); 1.12 (d, 3H, J=6.7 Hz); ^{13}C NMR (67.9 MHz, CD_3SOCD_3, 90°C) δ 168.33; 137.57; 136.81; 128.06; 127.48; 127.43; 127.18; 110.35; 104.03; 81.60; 81.15; 79.33; 70.61; 65.83; 48.30; 42.56; 26.35; 25.92; 20.25; 20.19; HRMS (FAB) M+H=$C_{26}H_{34}NO_6$ calc: 456.238613, found: 456.238690; $[\alpha]^D_{295}$ -28.3° (c 1.5, EtOAc).

1α- and 1β- 3-O-benzyl-5-deoxy-5-(N-carboxybenzyloxy-N-isopropylamino)-1-O-methyl xylofuranose **19**

A 1% solution of iodine in dry methanol (60 ml) containing **18** (8.7 mmol, 3.95 g) was refluxed under Ar for 4h. Two products were observed, a fast and a slow eluting one at R_f = 0.16 and R_f = 0.10 respectively (ethyl acetate:hexanes 30:70). The solution was allowed to cool down to RT and a 1% solution of sodium thiosulfate was added. The mixture was concentrated *in vacuo* and taken up in 400 ml ethyl acetate, washed twice with 50 ml thiosulfate solution and with 50 ml brine. The organic layer was then dried over magnesium sulfate and the solvent was evaporated *in vacuo* to give a yellow oil, which was chromatographed (ethyl acetate:hexanes 15:85 to 20:80), to allow an almost complete separation of the two products. The fast eluting compound was obtained pure in 53.0% yield (1.98 g), the slow eluting one pure in 44% yield (1.64g), both as colorless oils.

- Fast eluting isomer α-**19**

^{1}H NMR (500 MHz, CD_3SOCD_3, 90°C) δ 7.22-7.35 (m, 10H); 5.09 (B of AB, 1H, $CO_2C\mathbf{H_2}$, J=12.5 Hz); 5.06 (A of AB, $CO_2C\mathbf{H_2}$, 1H, J=12.5 Hz); 4.79 (d, 1H, $\mathbf{H_1}$, J=4.5 Hz); 4.66 (B of AB, 1H, $C_3OC\mathbf{H_2}$, J=11.5 Hz); 4.51 (A of AB, 1H, $C_3OC\mathbf{H_2}$, J=11.5 Hz); 4.32 (m, 1H, O**H**, D_2O exchangeable); 4.24 (ddd, 1H, $\mathbf{H_4}$, J=8.0 Hz, J=5.5 Hz, J=3.0Hz); 4.09 (ddd, 1H, $\mathbf{H_2}$, J=4.5 Hz, J=4.5 Hz, J=6.0 Hz); 3.96 (h, 1H, $NC\mathbf{H}(CH_3)_2$, J=7.0 Hz); 3.93 (dd, 1H, $\mathbf{H_3}$, J=6.0 Hz, J=5.5 Hz); 3.59 (dd, 1H, $\mathbf{H_5}$, J=15.0 Hz, J=3.0 Hz); 3.31 (s, 3H, $OC\mathbf{H_3}$); 3.24 (dd, 1H, $\mathbf{H_5}$, J=15.0 Hz, J=8.0 Hz); 1.19 (d, 3H, J=6.5 Hz); 1.17 (d, 3H, J=7.5 Hz); ^{13}C NMR (67.9 MHz, CD_3SOCD_3, 90°C) δ 155.62; 138.88; 137.76; 128.83; 128.72; 128.16; 127.92; 102.50; 84.12; 77.03; 76.76; 71.78; 66.54; 55.24; 49.44; 45.28; 21.00; 20.82; HRMS (EI), M=$C_{24}H_{31}NO_6$, calc: 429.21560, found: 429.21512; $[\alpha]^D_{295}$ +63.2° (c 1.2, methylene chloride)

- Slow eluting isomer β-**19**

^{1}H NMR (500 MHz, CD_3SOCD_3, 90°C) δ 7.26-7.38 (m, 10H); 5.25 (m, exchangeable 1H, O**H**); 5.07 (s, 2H, $CO_2C\mathbf{H_2}$); 4.66 (d, 1H, $\mathbf{H_1}$, J=2.0 Hz); 4.59 (B of AB, 1H, $C_3OC\mathbf{H_2}$, J=12.0 Hz); 4.45 (A of AB, 1H, $C_3OC\mathbf{H_2}$, J=12.0 Hz); 4.28 (ddd, 1H, $\mathbf{H_4}$, J=5.5 Hz, J=8.0 Hz, J=2.5 Hz); 4.04 (ddd, 1H, $\mathbf{H_2}$, J=2.0 Hz, J=4.0 Hz, J=3.0 Hz); 3.94 (h, 1H, $NC\mathbf{H}(CH_3)_2$, J=7.0 Hz); 3.79 (dd, 1H, $\mathbf{H_3}$, J=5.5 Hz, J=3.0 Hz); 3.66 (dd, 1H, $\mathbf{H_5}$, J=15.0 Hz, J=2.5 Hz); 3.28 (s, 3H, $OC\mathbf{H_3}$); 3.20 (dd, 1H, $\mathbf{H_5}$, J=15.0 Hz, J=8.0 Hz); 1.19 (d, 3H, J=7.0 Hz); 1.16 (d, 3H, J=7.0 Hz); ^{13}C NMR (125.7 MHz, CD_3SOCD_3, 90°C) δ 138.83; 137.72; 128.85; 128.70; 128.18; 127.96; 110.40; 84.19; 80.42; 78.43; 71.59; 66.56; 55.51; 49.78; 46.13; 21.07; 20.75; MS (EI) m/z 429 (0.8%, (M^+)); $[\alpha]^D_{295}$ -27.6° (c 2.5, methylene chloride).

α-3-O-benzyl-2-O-carboxyphenylamino-5-deoxy-5-(N-carboxybenzyloxy-N-isopropylamino)-1-O-methyl xylofuranose **20**

To a solution of α-**19** (425 mg, 1.0 mmol) in 10 ml dry pyridine stirred at 0°C under Ar, was added triphosgene (104 mg, 0.35 mmol). After one hour, aniline (103 µl, 1.1 mmol) was added to the solution and the reaction was stirred at 0°C for one hour, then allowed to warm up to room temperature. It was stirred for 12h, then evaporated *in vacuo*. The residual sticky gum was dissolved in 40 ml ethyl acetate, washed twice with 5 ml of a 10% solution of ammonium chloride, then with 5 ml brine. The organic layer was dried over magnesium

sulfate and the solvent evaporated to give a yellow oil that was purified by flash chromatography to yield α-**20** (477 mg, 87.0%) as a clear oil.

^{1}H NMR (500 MHz, CD_3SOCD_3, 90°C) δ 9.52 (b, 1H); 7.47-7.00 (m, 15H); 5.11-5.04 (m, 4H); 4.58 (AB, 2H, J=12.0 Hz); 4.27 (m, 1H); 4.18 (m, 1H); 3.99 (h, 1H, J=6.8 Hz); 3.63 (dd, 1H, J=2.7 Hz, J=15.1 Hz); 3.30 (dd, 1H, J=8.3 Hz, J=15.1 Hz); 3.27 (s, 3H); 1.18, 1.16 (2d, 6H, J=6.8 Hz); ^{13}C NMR (67.9 MHz, CD_3SOCD_3, 90°C) δ 154.65; 152.18; 138.45; 137.33; 136.70; 128.13; 127.82; 127.78; 127.17; 127.09; 127.03; 126.91; 122.18; 118.27; 100.07; 80.49; 77.02; 75.96; 71.06; 65.61; 54.46; 48.41; 43.85; 19.99; 19.83; MS (FAB) m/z 549 (0.2%, $(M+H)^+$); 517 (0.5%); 206 (24.2%); 162 (66.5%); 119 (100.0%); $[\alpha]^D_{295}$ +76.2° (c 0.85, ethyl acetate).

α-2-O-carboxyphenylamino-5-deoxy-5-N-isopropylamino-1-O-methyl-xylofuranose **21**

A solution of α-**20** (548 mg, 1.0 mmol) and Pd/C 10% (660 mg) in a mixture of ethanol (25 ml), water (3 ml) and acetic acid (3 ml) was shaken under a 60 psi pressure of hydrogen for 36h. The mixture was then filtered over celite and evaporated to a yellow oil. Flash chromatography gave the corresponding γ-aminoalcohol α-**21** as a white solid (m.p. 131-133°C).

^{1}H NMR (500 MHz, CD_3OD) δ 7.42-7.00 (m, 5H); 5.07 (m, 1H); 4.81 (m, 1H); 4.44 (m 1H); 4.22 (d, J=5.1 Hz); 3.40 (s, 3H); 2.91 (dd, 1H, J=12.5 Hz, J=4.3 Hz); 2.85-2.77 (m, 2H); 1.10, 1.09 (2d, 6H, J=6.4 Hz); ^{13}C NMR (CD_3OD) δ 152.14; 129.11; 123.82; 123.79; 118.56; 107.70; 83.49; 80.29; 76.58; 55.86; 48.67; 46.57; 22.73; 22.33; HRMS (FAB) M+H=$C_{16}H_{25}N_2O_5$ calc: 325.176347, found: 325.176430; $[\alpha]^D_{295}$ +184.0° (c 0.80, MeOH).

Oxazaphosphorinane **22**

To a solution of freshly distilled phosphorus trichloride (87 µl, 1.0 mmol) in 2 ml dry chloroform stirred at 0°C under Ar, was added a mixture of α-**21** (325 mg, 1.0 mmol) and triethylamine (349 µl, 2.5 mmol) in 3 ml dry chloroform over 1h. After the addition, ^{31}P NMR indicated the presence of several signals between 140 and 170 ppm. The mixture was refluxed for 4h, cooled down to 0°C and a mixture of 5'-O-tBDMS-thymidine (356 mg, 1.0 mmol) and dry triethylamine (168 µl, 1.2 mmol) in 3 ml dry chloroform was added over 5 min. The mixture was refluxed again, and after 24h ^{31}P NMR indicated the presence of a single product (δ 136.1 ppm), as well as about 20% decomposition products (around 10 ppm). To the mixture cooled down to RT was added 20 ml ethyl acetate. The salt was filtered out and the solution concentrated *in vacuo*. The crude mixture was purified by flash chromatography (hexanes:ethyl acetate:triethylamine 60:25:15 to 45:40:15). Pure **22** could thus be obtained in 60% yield (425 mg) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (s, 1H, C=C**H**); 7.38-6.91 (m, 5H, aromatic **H**); 6.34 (dd, 1H, $\mathbf{H_{1'}}$, J=5.6 Hz, J=8.3 Hz); 5.21 (d, 1H, $\mathbf{H_1}$, J=4.4 Hz); 5.14 (m, 1H, $\mathbf{H_2}$); 4.64 (m, 1H, $\mathbf{H_{3'}}$); 4.54 (m, 1H, $\mathbf{H_3}$); 4.27 (m, 1H, $\mathbf{H_4}$); 4.07 (m, 1H, $\mathbf{H_{4'}}$); 3.86 (AB of ABX, 2H, 2X$\mathbf{H_{5'}}$, J=11.5 Hz, J=2.0 Hz, J=2.2 Hz); 3.45 (s, 3H, O$\mathbf{CH_3}$); 3.42-3.35 (m, 2H, $\mathbf{H_5}$, N$\mathbf{CH}(CH_3)_2$); 2.93 (dt, 1H, $\mathbf{H_5}$, J=13.4 Hz, J=5.1 Hz, J=5.1 Hz); 2.38 (ddd, 1H, $\mathbf{H_{2'}}$, J=13.4 Hz, J=5.6 Hz, J=2.0 Hz); 2.10 (ddd, 1H, $\mathbf{H_{2'}}$, J=13.4 Hz, J=8.3 Hz, J=6.1 Hz); 1.91 (s, 3H, CH=CC$\mathbf{H_3}$); 1.15 (2d, 6H, NCH(C$\mathbf{H_3}$)$_2$, J=6.3 Hz); 0.91 (s, 9H, SiC(C$\mathbf{H_3}$)$_3$); 0.11 (s, 6H, Si(C$\mathbf{H_3}$)$_2$); ^{13}C NMR ($CDCl_3$) δ 163.20 (NHC=OC(CH_3)); 152.29 (C_2O**C**=ONHC_6H_5); 150.12 (N**C**=ONH); 135.16 (NCH=**C**(CH_3); 137.72; 129.03; 123.75; 119.00 (NHC=O**C**$_6$H$_5$); 110.83 (NCH=**C**(CH_3)); 102.03 ($\mathbf{C_1}$); 86.59 ($\mathbf{C_{4'}}$, d, J=3.6 Hz); 85.01 ($\mathbf{C_{1'}}$); 78.89 ($\mathbf{C_2}$, d, J=2.1 Hz); 73.40 ($\mathbf{C_{3'}}$, d, J=19.7 Hz); 73.09 ($\mathbf{C_3}$, d, J=4.7 Hz);

72.98 (**C$_4$**, d, ^{3}J=4.1 Hz); 63.12 (**C$_{5'}$**); 56.16 (O**C**H$_3$); 49.79 (N**C**H(CH$_3$)$_2$, d, J=34 Hz); 46.42 (**C$_5$**); 40.38 (**C$_{2'}$**, d, J=5.2 Hz); 25.95 (SiC(**C**H$_3$)$_3$); 22.11 (NCH(**C**H$_3$)$_2$, d, J=9.3 Hz); 21.72 (NCH(**C**H$_3$)$_2$, d, J=5.1 Hz); 18.34 (Si**C**(CH$_3$)$_3$); 12.25 (CH=**C**(CH$_3$)); -5.39, -5.43 (Si(**C**H$_3$)$_2$); ^{31}P NMR (109.38 MHz, CDCl$_3$) δ 136.1; HRMS (FAB), M+Na=$C_{32}H_{49}N_4O_{10}PSiNa$ calc: 731.285331, found: 731.285460.

(5'-O-*ter*butyldimethylsilyl)thymid-3'-yl (3'-O-*ter*butyldiphenylsilylthymid-5'-yl) phosphorothioate **26**

To a solution of oxazaphosphorinane **22** (50 mg, 0.07 mmol) in 0.5 ml dry acetonitrile stirred under Ar at -20°C, was added a solution of 3'-O-tBDPS-thymidine (33 mg, 0.07 mmol) and DCBI (55 mg, 0.28 mmol) in 0.5 ml dry acetonitrile. Exactly 1 min. after the introduction of the second part of the mixture, Beaucage's sulfurizing reagent (14 mg, 0.07 mmol) was introduced as a solid in the reaction mixture. ^{31}P NMR revealed, after 5 min, the presence of two sets of products: one single peak at 66.9 ppm, corresponding to the intermediate phosphorothioate triester, and a second set constituted of two peaks at 55.4 and 55.1 ppm, corresponding to the desired phosphorothioate diester **26**, the two sets of peaks being in a ratio of 5:1 respectively. Dry DMF[24] (0.5 ml) was introduced into the reaction mixture, followed by ammonium hydroxide (0.2 ml of a 28% solution). ^{31}P NMR then indicated the presence of only two peaks at 55.4 and 55.1 ppm, in a ratio of 28.5:1 respectively. The solvents were evaporated *in vacuo* and azeotroped twice with toluene, and the mixture was purified by column chromatography (ethyl acetate to ethyl acetate:methanol 94:6). Only the major diastereomer was obtained pure in 91% yield (59 mg) (yield calculated for the ammonium salt).

^{1}H NMR (500 MHz, CD$_3$OD)[25] δ 7.97 (d, 1H, C**H**=C(CH$_3$), J=1.0 Hz); 7.83 (d, 1H, C**H**=C(CH$_3$), J=1.0 Hz); 7.72-7.36 (m, 10H, **2x**C$_6$**H$_5$**); 6.46 (dd, 1H, **H$_{1'B}$**, J=9.3 Hz, J=5.4 Hz); 6.17 (dd, 1H, **H$_{1'A}$**, J=9.0 Hz, J=5.4 Hz); 4.99 (m, 1H, **H$_{3'A}$**); 4.56 (m, 1H, **H$_{3'B}$**); 4.21 (m, 1H, **H$_{4'A}$**); 4.08 (m, 1H, **H$_{4'B}$**); 3.92 (m, 1H, **H$_{5'B}$**); 3.88 (AB of ABX, 2H, 2x**H$_{5'A}$**, J=11.5 Hz, J=2.5 Hz, J=2.0 Hz); 3.62 (m, 1H, **H$_{5'B}$**); 2.23-2.21 (m, 1H, **H$_{2'A}$**); 2.20-2.15 (m, 1H, **H$_{2'B}$**); 2.09-2.02 (m, 1H, **H$_{2'B}$**); 1.97-1.94 (m, 1H, **H$_{2'A}$**); 1.92 (d, 3H, CH=C(C**H$_3$**), J=1.0 Hz); 1.88 (d, 3H, CH=C(C**H$_3$**); J=1.0 Hz); 1.08 (s, 9H, SiC(C**H$_3$**)$_3$); 0.92 (s, 9H, SiC(C**H$_3$**)$_3$); 0.13, 0.12 (2s, 6H, Si(C**H$_3$**)$_2$).

Tetrabutylammonium thymid-3'-yl thymid-5'-yl phosphorothioate **27**

To a solution of **26** (50 mg, 0.054 mmol) in 1 ml dry DMF was added a 1M solution of TBAF in THF (135 µl, 0.135 mmol). After 3h, TLC indicated the reaction had gone to completion. The solvents were evaporated *in vacuo*, and the desired dimer purified by column chromatography using a short column (acetone to acetone:water 98:3). The product was obtained in 89.9 % yield (39 mg).

^{1}H NMR (500 MHz, CD$_3$OD)[25] δ 7.90 (d, 1H, C**H**=C(CH$_3$); J=1.0 Hz); 7.85 (d, 1H, C**H**=C(CH$_3$), J=1.0 Hz); 6.35 (dd, 1H, **H$_{1'B}$**, J=8.1 Hz, J=6.1 Hz); 6.28 (dd, 1H, **H$_{1'A}$**, J=8.3 Hz, J=5.6 Hz); 5.06 (m, 1H, **H$_{3'A}$**); 4.51 (m, 1H, **H$_{3'B}$**); 4.22 (m, 1H, **H$_{4'A}$**); 4.13 (AB of ABX dedoubled, 1H, **2x**H$_{5'B}$, J=11.3 Hz, J=2.9 Hz, J=2.7 Hz, J=6.6 Hz, J=5.6 Hz); 4.04 (m, 1H, **H$_{4'B}$**); 3.82 (AB of ABX, 1H, **2x**H$_{5'}$, J=12.0 Hz, J=3.2 Hz, J=2.9 Hz); 3.23 (m, 8H, N(C**H$_2$**Pr)$_4$); 2.46 (ddd, 1H, **H$_{2'A}$**, J=13.7 Hz, J=5.6 Hz, J=2.2 Hz); 2.31-2.16 (m, 3H, **2x**H$_{5'B}$, **H$_{5'A}$**); 1.97 (d, 3H, CH=C(C**H$_3$**), J=1.0 Hz); 1.86 (d, 3H, CH=C(C**H$_3$**), J=1.0 Hz); 1.65 (tq, 8H, N(CH$_2$C**H$_2$**Et)$_4$, J=7.6 Hz, J=8.3 Hz); 1.41 (tq, 8H, N(CH$_2$CH$_2$C**H$_2$**CH$_3$)$_4$, J=7.3 Hz, J=7.6 Hz); 1.01 (t, 12H, N(CH$_2$CH$_2$CH$_2$C**H$_3$**)$_4$, J=7.3 Hz); ^{13}C NMR (CD$_3$OD) δ 166.55, 166.42 (2xNH**C**=OC(CH$_3$)); 152.47, 152.29 (2xN**C**=ONH); 138.20, 138.12 (2xCH=**C**(CH$_3$)); 112.09, 111.55 (2xNCH=**C**(CH$_3$)); 87.93 (d, **C$_{4'A}$**, J=5.5 Hz); 87.49 (d, **C$_{4'B}$**, J=8.2 Hz); 86.29 (**C$_{1'A}$**); 86.16 (**C$_{1'B}$**); 77.73 (d, **C$_{3'A}$**, J=5.5 Hz); 72.98 (**C$_{3'B}$**);

66.40 (d, $C_{5'B}$, J=6.4 Hz); 62.94 ($C_{5'A}$); 59.49 (t, $N(CH_2Pr)_4$); 40.97 ($C_{2'B}$); 39.94 (d, $C_{2'A}$, J=4.6 Hz); 24.78 ($N(CH_2CH_2Et)_4$); 20.72 ($N(CH_2CH_2CH_2CH_3)_4$); 13.94 ($N(CH_2CH_2CH_2CH_3)_4$); 12.70, 12.48 ($2xCH=C(CH_3)$); HRMS (FAB) M+Na=$C_{20}H_{27}N_4O_{11}PSNa$ calc: 585.103238, found: 585.103400.

References and Notes

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[12] Data not shown.
[13] As noted by one of the referees, this suggests that the presence of the neighbouring 6-acetoxy group in oxazaphosphorinane **10** is not relevant to any anchimeric effect in the formation and stabilization of the assumed carbocation.
[14] The complexity of this mixture did not allow any relevant NMR analysis. However, MS and HRMS analysis of the mixture gave a molecular ion that had exactly the same molecular wight as the starting oxazaphosphorinane **14**, leading us to believe that an acid-catalyzed rearrangement had indeed taken place.
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[21] Analyses were performed by Ms. Alice Symons, Isis Pharmaceuticals, data not shown.
[22] The ^1H and ^{13}C NMR signals of the major component of the mixture are given. Numbers refer to the camphor part, primed numbers to the nucleoside part.
[23] NMR spectra of compounds **18**, **19**, **20** were recorded at 90°C due to the broadening of their signals at ambient temperature caused by the presence of two rotamers around the N-Cbz bound.
[24] DMF was introduced to help the reaction go to completion, as well as solubilize the final anionic species, poorly soluble in acetonitrile.
[25] The 5'-end nucleoside is referred to as nucleoside A, the 3'-end one as nucleoside B.

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