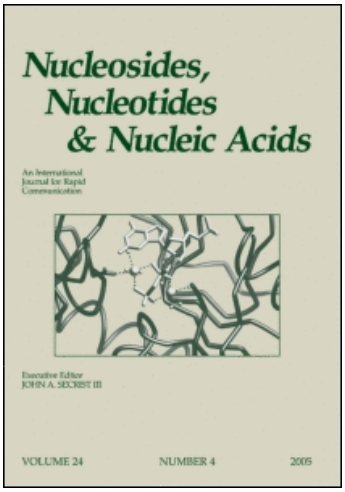

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ISOTACTIC GLYCERO OLIGOTHYMIDYLATE. A CONVENIENT PREPARATION OF (R) AND (S) 1', 2'-*SECO* 2'-*NOR* THYMIDINE

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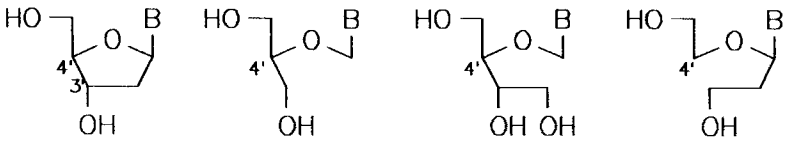
Abstract:(R) and (S) dimethoxytrityl derivatives of 1', 2'-*seco* 2'-*nor* thymidine were synthesized in an efficient way. Isotactic dodecaoligoglycerothymidylate was obtained by a solid support phosphoramidite approach. The lack of hybridization with *poly* rA makes this *acyclo*oligonucleotide useless as antisense or sense agent.

Chirality and biological activity are often intricate properties as encountered in the nucleoside or *pseudo*nucleoside series. For example certain *acyclo* nucleosides such as (S)-DHPA[1,2], (S)-*iso*NDG[3] and some phosphonomethyl derivatives[4,5] exhibit antiviral activities only in a defined configuration.



Scheme 1

Flexible oligonucleotides built from monomeric acyclic nucleosides units may prove to be interesting in two different ways: the first one is associated with the antisense technology[6,7] in which a specific and stable mRNA-antisense hybrid may avoid the translation of the given mRNA. Flexible oligonucleotides need to be synthesized and checked for their potential activity. The second interest for acyclic oligonucleotides is related to the numerous hypothesis on the origin of life[8-10]; such nucleotides have been postulated to be involved in the prebiotic synthetic phase and would be valuable compounds to be examined in this respect.

The design for *acyclo*oligonucleotides can involve various acyclic monomeric precursors as shown in scheme 1 depending on the desired potential chirality at the C-3' and/or C-4' (as referred to ribose numbering) in each unit of the growing oligonucleotide strand.

As an example, the synthesis of oligonucleotides from the glyceronucleoside I implies the use of a chiral phosphoramidite synthon of defined (R) or (S) configuration as it will be related to the tacticity of the newly synthesized oligomer chain. If the C-4' of I is prochiral it becomes chiral in the oligomer but in the latter each monomeric unit adopt randomly a D or L configuration; therefore the resulting oligonucleotide will be atactic and optically heterogeneous. On the other hand, if the compound I is chiral at C-4', one enantiomer will led to an isotactic oligonucleotide.

Two notes on the synthesis of such flexible oligonucleotides from I were recently reported. In the first one[11], this chirality aspect was apparently neglected as the authors used a racemic mixture of the corresponding phosphoramidite synthons. The others[12] obtained the required chiral phosphoramidite derivative but through a lengthy procedure which involved the separation of two pivaloyl isomeric derivatives on silica gel.

We would like to present herewith a general and unequivocal synthetic approach giving rise to the (R) or (S) phosphoramidite synthon **16**. Complementary data on the corresponding oligonucleotides will be also presented.

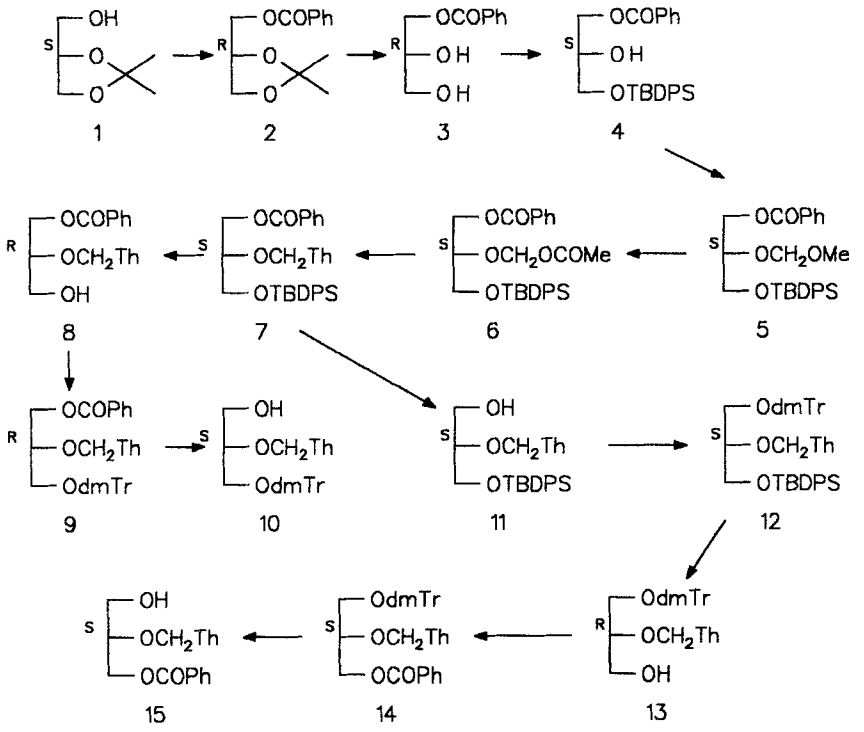
Synthesis of homochiral thymidine *acyclo*nucleosides: In order to obtain the synthons **10** and **13** of defined configuration the synthetic reaction sequence presented on scheme 2 was followed.

The 1, 2-(S)-O-isopropylidene-D-glycerol **1** was quantitatively benzoylated in order to afford **2**. This compound gave 1-benzoyl-D-glycerol **3** in 85% yield by removal of the dioxolane protecting group. Further protection of the primary hydroxyl group of **3** was performed in 98% yield by *tert*-butyldiphenylchlorosilane in pyridine. The resulting compound **4** reacted with formaldehyde dimethylacetal and phosphorus pentoxyde in anhydrous dichloromethane and afforded the methoxymethylether **5** in 80% yield. This derivative was transformed into **6** (93%) by reaction with acetic anhydride in the presence of boron trifluoride diethyl ether at -20°C.

The condensation between **6** and persilylated thymine was performed according to our new N-glycosylation procedure which afforded the N-1 regioisomer **7** in 98% yield by means of a phase transfer catalysis of KI with dibenzo-18-crown-6 ether in a mixture of acetonitrile and toluene (1/1, v/v). The blocked nucleoside **7** was the key-compound used for the synthesis of the desired (R) or (S) phosphoramidite synthon **16**. At this stage of the synthesis, the enantiomeric pair of oligonucleotidic precursors **10** and **13** deserve some comments: if one needs to synthesize an isotactic homo*acyclo*oligomer having free hydroxyl groups at its *pseudo* 3' or 5' *termini*, one of the two enantiomers **10** or **13** only has to be used; but in the event of two differentiated terminal substitutions (for example insertion of this acyclic oligo into a hetero-oligo*deoxyribo* or *ribo* oligomeric chain) or in the case of a substitution at one of the two *termini* of the homo-oligomeric acyclic strand (*e.g.* derivatization with an effector agent[7]) then both enantiomeric phosphoramidite synthons will be required.

The desired compounds **10** and **13** were synthesized according to the scheme 2. The nucleoside **7** (S configuration) was desilylated (90% yield) by TBAF in anhydrous THF and gave **8** which was dimethoxytritylated (90% yield) into **9** which in turn afforded quantitatively the desired compound **10** after a debenzoylation step performed with saturated methanolic ammonia. This compound bears the chiral (S) carbon which is found at the C-4' of 5'-dimethoxytritylated D-*ribo* or 2'-deoxy-D-*ribo* nucleosides. The synthon **10** will be the starting material used for the *acyclo*oligonucleotide synthesis.

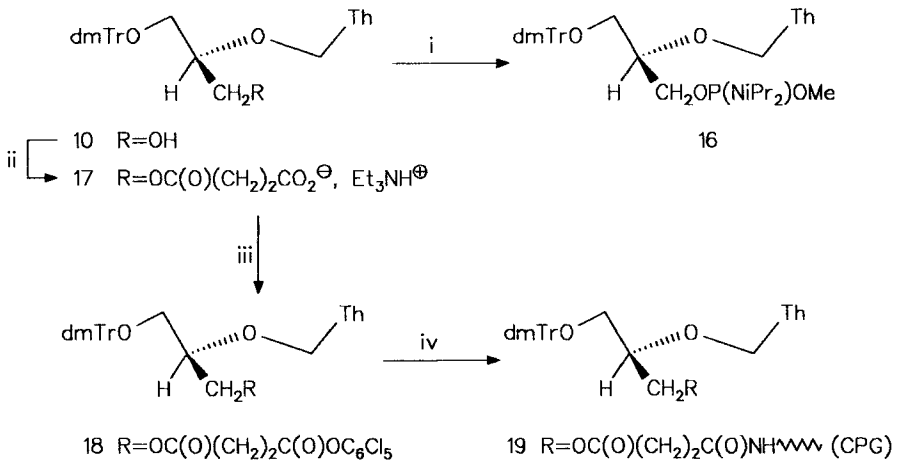
The enantiomeric derivative **13** was obtained from **7** by successive debenzoylation giving **11** (75%yield) which was dimethoxytritylated in **12** (98%) and finally desilylated quantitatively.



Scheme 2

At this stage it turned out that **10** and **13** do not show the exact opposite specific optical rotation. It is known that dimethoxytritylated nucleosides can be contaminated by traces of carbinol even after precipitation by anhydrous petroleum ether; then it appeared necessary to check the optical purity of the enantiomeric pair of untritylated compounds found at an earlier stage of the synthetic sequence. This was done for the benzoylated nucleoside **8** and its enantiomer **15**. This later derivative was obtained by benzoylation of **13** and subsequent demethoxytritylation of **14** according to scheme 2. The opposite values obtained for the specific optical rotations of **8** and **15** suggest that stereochemical integrity has been maintained throughout the whole synthetic sequence.

Synthesis of an isotactic dodeca*acyclo*thymidylate. This synthesis was performed as depicted on scheme 3 starting with the nucleoside **10** (S



Scheme 3- i:ClP(NiPr$_2$)OMe; ii:DMAP, succinic anhydride, NEt$_3$; iii:pentachlorophenol, DCC; iv:LCA-C.P.G.

configuration) which was in part transformed into an activated phosphoramidite synthon **16** by means of chlorodiisopropyl-aminomethoxyphosphine with N-ethyldiisopropylamine in dichloro-methane.

The overall yield of **16** after a flash column chromatography was 68% and its purity was checked by ^{31}P n.m.r spectroscopy. The second part of **10** was *hemi*succinoylated by succinic anhydride and triethylamine and gave **17** which was activated to **18** by pentachlorophenol within a total yield of 64%. Finally, long chain alkylamine controlled pore glass (LCA-C.P.G.) was functionalized by means of **18**. Loading measurement by spectrometric titration of acidic release of dimethoxytrityl cation gave a value of 29 μmol/g. The dodeca*acyclo*thymidylate was synthesized following the automated methoxy phosphoramidite approach on solid support. During deprotection an additional treatment with concentrated ammonia at 55°C for 18 h was necessary to remove all the protecting groups on the phosphate functions. A similar difficulty was observed during the synthesis of a dodeca*acarbo*thymidylate[13]. Assays of hybridization between this *acyclo*oligomer and *poly* rA were performed in 10mM cacodylate sodium pH 7 and 1M sodium chloride as buffer and in the same buffer extended with $MgCl_2$ (10mM). In the both buffers no

transition was observed. These data suggest that this *acyclo*oligonucleotide was too flexible to be able to form a stable duplex structure with complementary strand and corroborated the results of Schneider *et al.*[12] Although such oligonucleotides are expected to be as certain sugar modified oligomers, stable against enzymatic degradation, their lack of hybridization with complementary sequences does not allow to use them as antisense or sense agents.

This work deals with a straightforward synthesis of antipodal glyceronucleoside derivatives. One of them was used for the synthesis of a modified isotactic oligothymidylate.

Experimental.

Mp's were obtained with a Büchi (capillary) apparatus and were uncorrected. Tlc was performed on a silica-gel F-254 (Merck) with detection by u.v. and charring with sulphuric acid. UV spectra were determined on a Cary 1186 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Elemental analyses were performed by the 'Service de Microanalyse du CNRS, Division de Vernaison'. ^{1}H NMR spectra were determined on a Brüker AC250, or a Varian EM 390 spectrometer. *J* Values are given in Hz. Mass spectra were obtained with a Jeol JMS-DX300 by the FAB ionization method. 1, 2(S)-O-Isopropyliden-D-glycerol was purchased from Fluka Chemical Biochemical.

Assays of hybridization were carried out by following the reported procedure described[14]. They were performed between the 12 mer *acyclo*oligomer and *poly* rA at a total base concentration of 240μM in buffers I and II. Buffer I: 10mM cacodylate sodium pH 7 and 1M sodium chloride. Buffer II: 10mM cacodylate sodium pH 7, 1M sodium chloride and 10mM magnesium chloride.

3-O-Benzoyl-1,2(R)-O-isopropylidene glycerol (2).- This compound was obtained from (1) in quantitative yield in following the described procedure[15]. R_f 0.31 (dichloromethane); $[\alpha]_D^{20}$ +14.4° (neat) δ_H(90 MHz; $CDCl_3$) 1.3 (3H, s, Me), 1.4 (3H, s, Me), 3,6-4.25 (5H, m, $2CH_2$, CH), 7.2-7.8 (5H, m, aromatic).

(R)-1-Benzoyloxypropan-2,3-diol (**3**).- A mixture of trifluoroacetic acid-water 2:8 (300 cm^3) and compound (**2**) (13 g, 55 mmol) was stirred at room temperature for 1 h. The solution was neutralized with saturated aqueous $NaHCO_3$ and extracted with dichloromethane. The organic layer was washed with water and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residue was flash chromatographed on a column of silica gel with methanol-dichloromethane (1:99) as the eluting system. Compound (**3**) was obtained as an oil (8 g, 85% yield). R_f 0.1 ($MeOH/CH_2Cl_2$ 1:99), $[\alpha]_D^{20}$ -15.1°(c 10.6, pyridine)(Lit.[15] $[\alpha]$ -15°(c 10, pyridine), δ_H(90 MHz; $CDCl_3$) 3.74 (2H, s, OH), 3.8 (2H, m, CH_2OH), 4.1 (1H, m, CH), 4.4 (2H, m, CH_2OBz), 7.0-8.0 (5H, m, aromatic).

*(S)-1-Benzoyloxy-3-*tert*-butyldiphenylsilyloxypropan-2-ol* (**4**).- To a solution of (**3**) (8 g, 40 mmol) in dry pyridine (60 cm^3) was added dropwise *tert*-butyldiphenylchlorosilane (12.5 cm^3, 48.9 mmol). The solution was stirred at room temp. for 2 h. The solvent was evaporated under reduced pressure and the residue was extracted with dichloromethane (200 cm^3). The organic phase was washed successively with a solution of 1N HCl, a saturated aqueous solution of $NaHCO_3$, water and then dried (Na_2SO_4). The crude product was flash chromatographed on a silica gel column with dichloromethane as the eluent and (**4**) was obtained in 98% yield (17.5 g). R_f 0.47 (methanol-dichloromethane 1:99); δ_H(90 MHz, $CDCl_3$) 1.05 (9H, s, 3Me), 2.67 (1H, s, OH), 3.70 (2H, m, CH_2), 4.1 (1H, m, CH), 4.33 (2H, m, CH_2), 7.1-7.95 (15H, m, aromatic). (Found: C, 71.68; H, 6.92; Si, 6.58. $C_{26}H_{30}O_4Si$ requires C, 71.85; H, 6.96; Si, 6.46%).

*(S)-1-Benzoyloxy-2-methoxymethylenoxy-3-*tert*-butyldiphenylsilyloxy-propane* (**5**).- To a solution of (**4**) (15 g, 34 mmol) in anhydrous chloroform (150 cm^3) and formaldehyde dimethyl acetal (5.5 cm^3, 63 mmol) was added portionwise phosphorous pentaoxide (3.4 g), under vigorous stirring with the temperature maintained at 40-45°C. The mixture was then stirred at room temperature for 24 h. The supernatant was diluted with dichloromethane (300 cm^3), decanted and washed successively with water, saturated aqueous $NaHCO_3$ and with water. The organic layer was

dried ($MgSO_4$) and evaporated under reduced pressure to give (5) as an oil. The crude product was flash chromatographed on a silica gel column using cyclohexane/dichloromethane (4:6) as the eluting system and afforded pure *title compound* (13.3 g, 80% yield); R_f 0.53 (dichloromethane) , $[\alpha]_D^{20}$ -12° (c 2, pyridine), δ_H(90 MHz, $CDCl_3$) 1.05 (9H, s, 3Me), 3.2 (3H, s, OMe), 3.7-4.1 (3H, m, CH_2OSi, CH), 4.4 (2H, m, CH_2OBz), 4.5 (2H, s, OCH_2), 7.15-7.95 (15H, m, aromatic). (Found: C, 70.28; H, 7.26; Si, 5.75. $C_{28}H_{34}O_5Si$ requires C, 70.26; H, 7.15; Si, 5.86%).

*(S)-1-Benzoyloxy-2-acetoxymethylenoxy-3-*tert*-butyldiphenylsilyloxy-propane* (6).- A solution of (5) (11.8 g, 24.6 mmol), acetic anhydride (3.2 cm^3, 34.1 mmol) and boron trifluoride diethyl ether (0.88 cm^3, 7.06 mmol), was stirred at 4°C for 1 h. The solution was poured in ice-water, neutralized with saturated aqueous $NaHCO_3$ and extracted with diethyl ether (500 cm^3). The organic layer was washed with 10% aqueous $NaHCO_3$, water and dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude oil was flash chromatographed on a silica gel column using diethyl ether-cyclohexane (1:9) as the eluent and afforded pure (6) (11.5 g, 93%). R_f 0.44 (diethyl ether-cyclohexane, 3:2), $[\alpha]_D^{20}$ -7.5° (c 2, pyridine), δ_H(90 MHz, $CDCl_3$) 1.05 (9H, s, 3Me), 1.90 (3H, s, MeCO), 3.70-4.1 (3H, m, CH_2OSi, CH), 4.4 (2H, m, CH_2OBz), 5.3 (2H, s, OCH_2), 7.20-8.25 (m, 15H, aromatic). (Found: C, 68.73; H, 6.66; Si, 5.55. $C_{29}H_{34}O_6Si$ requires C, 68.74; H, 6.76; Si, 5.54%).

*1-[(1'-Benzoyloxy-3'-*tert*-butyldiphenylsilyloxy-2'(S)-propoxy)methyl] thymine* (7).- A mixture of KI (1.04 g, 6.3 mmol) and dibenzo-18-crown-6 ether (0.566 g, 1.57 mmol) in anhydrous toluene (100 cm^3) was refluxed for 1 h. Toluene (30 cm^3) was removed by azeotropic distillation and to the remaining solution was added persilylated thymine[16] (1.19 g, 944 mmol) and compound (6) (4 g, 7.86 mmol) in anhydrous acetonitrile (70 cm^3). The reaction was stirred for 4 h at 80°C. The solvents were evaporated under reduced pressure. The residue was chromatographed on a silica gel column and compound (7) was obtained (4.4 g, 80% yield) by elution with methanol-dichloromethane (0.5:99.5). R_f 0.35 (methanol-dichloro-methane, 3:97) $[\alpha]_D^{20}$ -4° (c 1, pyridine) δ_H(250 MHz; $CDCl_3$) 1.06 (9H, s, 3Me), 1.71 (3H, d, *J* 1.2, Me), 3.78 (2H, d, *J* 5.37, CH_2),

4.15 (1H, m, CH), 4.45 (2H, m, CH_2), 5.10 (1H, ,d, *J* 10.7, OCHaN), 5.29 (1H, d, OCHbN), 7.02 (1H, d, *J* 1.26, H-6), 7.33-7.95 (15H, m, aromatic), 8.17 (1H, s, NH). FAB-MS (thioglycerol) m/e 571 $(M-H)^-$, 125 $(BH-H)^-$.

1-[(1'-Benzoyloxy-3'-hydroxy-2'(R)-propoxy)methyl]thymine (**8**).- To a solution of (**7**) (1.4 g, 2.44 mmol) in anhydrous THF (7 cm^3) was added under stirring a solution (0.9 cm^3) of tetrabutylammonium fluoride (1.1M in THF). After 1 h at room temp. the solvent was removed under vacuum and the residue was flash chromatographed on a silica gel column. Elution with methanol-dichloromethane (3:97) afforded pure (**8**) (0.73 g, 90% yield). R_f 0.13 (methanol-dichloromethane, 5:95), $[\alpha]_D^{20}$ -5.8° (c 0.8, methanol); δ_H(250 MHz, $CDCl_3$) 1.70 (3H, d, *J* 1.2, Me), 2.09 (1H, s, OH), 3.78 (2H, m, CH_2), 4.10 (1H, m, CH), 4.43 (2H, m, CH_2), 5.28 (2H, q, *J* 10.6, OCH_2N), 7.11 (1H, d, *J* 1.2, H-6), 7.45-8.00 (5H, m, aromatic), 8.90 (1H, s, NH). (Found: C, 57.38; H, 5.49; N, 8.37. $C_{16}H_{18}N_2O_6$ requires C, 57.48; H, 5.42; N, 8.37%).

1-[(1'-Benzoyloxy-3'-dimethoxytrityloxy-2'(R)-propoxy)methyl]thymine (**9**).- To a solution of (**8**) (0.6 g, 1.8 mmol) in anhydrous pyridine (12 cm^3) was added 4,4'-dimethoxytrityl chloride (2.5 mmol). The reaction was maintained under stirring at room temperature for 2 h. The excess of reagent was quenched by methanol (2 cm^3) and the reaction mixture was poured in an aqueous solution of saturated $NaHCO_3$ and extracted with dichloromethane (200 cm^3). The organic layer was washed with water, dried (Na_2SO_4) and the solvent was removed under reduced pressure. Flash chromatography on a silica gel column using dichloromethane/triethylamine (99:1) as the eluting system afforded (**9**) (1.03 g, 90% yield). R_f 0.54 (methanol-dichloromethane, 5:95); δ_H(250 MHz, $CDCl_3$) 1.72 (3H, d, *J* 1.2, Me), 3.27 (2H, m, CH_2), 3.78 (6H, s, 2OMe), 4.16 (1H, m, CH), 4.42 (2H, d, *J* 5.24, CH_2), 5.17 (1H, d, *J* 10.7, OCHaN), 5.33 (1H, d, *J* 10.7, OCHbN), 6.79 (4H, m, aromatic), 7.06 (1H, d, *J* 1.25, H-6), 7.18-7.44 (12H, m, aromatic), 7.91 (2H, m, aromatic), 8.07 (1H, s, NH). FAB-MS (thioglycerol) m/e 635 $(M-H)^-$, 125 $(BH-H)^-$.

1-[(1'-Dimethoxytrityloxy-2'(S)-propoxy)methyl]thymine (**10**).- A solution of (**9**) (0.88 g, 1.38 mmol) in saturated methanolic ammonia (100 cm^3) was stirred at room temperature for 3 d. The solution was evaporated to dryness under reduced pressure and the crude product was flash chromatographed on a silica gel column buffered with triethylamine 1%. The *title compound* was obtained (0.73 g, 98% yield) with methanol/triethylamine/dichloromethane (1:1:98) as the eluting system. R_f 0.28 (methanol-dichloromethane, 3:97); $[\alpha]_D^{20}$ -7° (c 3, pyridine), λ max (EtOH, 95%)/nm 265. δ_H(250 MHz, $CDCl_3$) 1.87 (3H, d, *J* 1.2, Me), 2.35 (1H, s, OH), 3.23 (2H, m, CH_2), 3.61 (2H, m, CH_2), 3.78 (6H, s, 2OMe), 3.84 (1H, m, CH), 5.26 (2H, q, *J* 10.2 , OCH_2N), 6.83 (4H, s, aromatic), 7.12 (1H, d, *J* 1.2 , H-6), 7.18-7.41 (9H, m, aromatic), 8.62 (1H, s, NH). FAB-MS (thioglycerol) m/e 531 $(M-H)^-$, 125 $(BH-H)^-$.

1-[(1'-Hydroxy-3'-tert-*butyldiphenylsilyloxy-2'(S)-propoxy)methyl] thymine* (**11**).- A solution of (**7**) (2.4 g, 4.19 mmol) in saturated methanolic ammonia (120 cm^3) was stirred at room temperature for 4 d. The solvent was evaporated under reduced pressure and the residue was chromatographed on a column of silica gel. Elution with methanol-dichloromethane (2:98) affords the *title compound* (1.47 g, 75% yield). R_f 0.27 (methanol-dichloromethane 5:95) $[\alpha]_D^{20}$ - 6.6° (c 0.9, pyridine); δ_H(250 MHz, $CDCl_3$) 1.05 (9H, s, 3Me), 1.83 (1H, s, OH), 1.87 (3H, d, *J* 1.2, Me), 3.75 (5H, m, $2CH_2$, CH), 5.23 (2H, q, *J* 7.0, OCH_2N), 7.04 (1H, d, *J* 1.2, H-6), 7.45 (6H, m, aromatic), 7.67 (4H, m , aromatic), 8.43 (1H, s, NH). (Found: C, 65.07; H, 6.88; N, 5.97; Si, 5.99. $C_{25}H_{32}N_2O_5Si$ requires C, 65.09; H, 7.16; N, 5.77; Si, 5.94%).

1-[(1'-Dimethoxytrityloxy-3'-tert-*butyldiphenylsilyloxy-2'(S)-propoxy)-methyl]thymine* (**12**).- To a solution of (**11**) (0.806 g, 1.71 mmol) in anhydrous pyridine (12 cm^3) was added 4,4'-dimethoxytrityl chloride (2.5 mmol). The reaction mixture was maintained under stirring for 2h at room temp. after which it was quenched with methanol (2 cm^3) and then poured in a solution of saturated aqueous $NaHCO_3$ and extracted with dichloromethane (200 cm^3). The organic layer was washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. Chromatography of the crude product on a silica gel column afforded the *title compound*

(1.3 g, 98% yield) with dichloromethane/triethylamine (99:1) as the eluting system. R_f 0.51 (methanol-dichloromethane, 3:97); δ_H(250 MHz, $CDCl_3$) 0.97 (9H, s, 3Me), 1.80 (3H, d, *J* 1.2, Me), 3.19 (2H, m, CH_2), 3.68 (2H, d, *J* 5.4, CH_2), 3.78 (6H, s, 2OMe), 3.91 (1H, m, CH), 5.26 (2H, q, *J* 10.4, OCH_2N), 7.11 (1H, d, *J* 1.2, H-6), 7.22-7.43 (16H, m, aromatic), 7.60 (3H, m, aromatic), 8.30 (1H, s, NH). (Found: C, 71.80; H, 6.52; N, 3.50. $C_{45}H_{50}N_2O_7Si$ requires C, 71.66; H, 6.53; N, 3.63; Si, 3.64%).

1-[(1'-Dimethoxytrityloxy-3'-hydroxy-2'(R)-propoxy)methyl]thymine (**13**).- To a solution of (**12**) (1.2 g, 1.55 mmol) in anhydrous THF (6 cm^3) was added under stirring a solution (1.2 cm^3) of tetrabutylammonium fluoride (1.1M in THF). After 2 h at room temp. the solvent was removed under reduced pressure and the residue was flash chromatographed on a silica gel column. Elution with methanol/triethylamine/dichloromethane (1:1:98) afforded (**13**) (0.82 g, 99% yield). R_f 0.28 (methanol-dichloromethane, 3:97); $[\alpha]_D^{20}$ +7.6° (c 3, pyridine); δ_H(250 MHz, $CDCl_3$) 1.87 (3H, d, *J* 1.2, Me), 2.35 (1H, s, OH), 3.61 (2H, m, CH_2), 3.78 (6H, s, 2OMe), 3.84 (1H, m, CH), 3.23 (2H, m, CH_2), 5.26 (2H, q, *J* 10.2 , OCH_2N), 7.12 (1H, d, *J* 1.2, H-6), 6.82 (4H, m, aromatic), 7.41-7.20 (9H, m, aromatic). FAB-MS (thioglycerol) m/e 531 $(M-H)^-$, 125 $(BH-H)^-$.

1-[(1'-Benzoyloxy-3'-dimethoxytrityloxy-2'(S)-propoxy)methyl]thymine (**14**).- To a solution of (**13**) (0.08 g, 0.15 mmol) in anhydrous pyridine (2 cm^3) was added benzoyl chloride (0.02 cm^3, 0.22 mmol) at 0°C and under stirring. After 4 h at room temperature the solution was neutralized with a cold aqueous saturated $NaHCO_3$ and extracted with dichloromethane. The organic layer was washed with water, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was flash chromatographed on a silica gel column and afforded the pure *title compound* (0.095 g, 95% yield) with dichloromethane/triethylamine (99:1) as the eluting system. R_f 0.54 (methanol-dichloromethane, 5:95), FAB-MS (thioglycerol) m/e 635 $(M-H)^-$, 125 $(BH-H)^-$.

1-[(1'-Benzoyloxy-2'(S)-propoxy)methyl]thymine (**15**).- To a solution of (**14**) (0.08 g, 0.12 mmol) in dichloromethane (5 cm^3) was added under

stirring a solution (10 cm^3) of trichloroacetic acid (3% in dichloromethane, v/v). After 4 h at room temp. the solution was neutralized with cold aqueous saturated $NaHCO_3$ and extracted with dichloromethane (50 cm^3). The organic layer was washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. A flash chromatography of the residue on a silica gel column afforded pure (15) (0.038 g, 95% yield) with methanol-dichloromethane as the eluting system. R_f 0.13 (methanol-dichloromethane, 5:95), $[\alpha]_D^{20}$ +5.8° (c 0.8, methanol), n.m.r. spectrum identical to (8). (Found: C, 57.43; H, 5.53; N, 8.34. $C_{16}H_{18}N_2O_6$ requires C, 57.48; H, 5.42; N, 8.37%).

1-[(1'-Dimethoxytrityloxy-3'-diisopropylaminomethoxyphosphinyloxy-2'(R)-propoxy)methyl]thymine (**16**).- Compound (**10**) (0.341 g, 0.64 mmol) R_f0.31 (triethylamine-ethyl acetate, 10:90) was dissolved in dry dichloromethane (2 cm^3) and N-ethyldiisopropylamine (0.446 cm^3, 2.56 mmol) in a round-bottom flask preflushed with dry argon. Chlorodiisopropylaminomethoxyphosphine (0.162 cm^3, 0.83 mmol) was added dropwise by a syringe to the solution, under argon, at room temp. and stirred for 20 mn. The reaction mixture was diluted with 5% of an aqueous solution of $NaHCO_3$ (50 cm^3) and extracted with ethyl acetate (30 cm^3). The organic layer was washed with brine (3x50 cm^3), dried (Na_2SO_4) and evaporated to dryness under vacuum. The resulting foam was fractionated by flash column chromatography using triethylamine-cyclohexane-dichloromethane (1.5:90:8.5 to 1.5:0:98.5) as the eluting system. The fractions containing the pure *title compound* were pooled and evaporated to dryness. The residue was lyophilized from benzene affording a colourless powder (0.302 g, 0.435 mmol) the purity of which was checked by ^{31}P n.m.r spectroscopy (149.59 and 149.49 ppm). R_f 0.59 (triethylamine-ethyl acetate, 10:90). FAB-MS (PEG 400) m/e 692 $(M-H)^-$.

1-[(1'-Dimethoxytrityloxy-3'-succinoylpentachlorophenyl-2'(R)-propoxy)-methyl]thymine (**18**).- To a solution of (**10**) (0.16 g, 0.30 mmol) and N,N-dimethylaminopyridine (0.055 g, 0.45 mmol) in dry dichloromethane (1.4 cm^3) were added succinic anhydride (0.045 g, 0.45 mmol) and triethylamine (0.063 cm^3, 0.45 mmol). The mixture was stirred for 1.5 h, poured on a 1M TEAB (30 cm^3) and extracted with dichloromethane (3x30 cm^3). The combined organic layers were washed with water (20

Table 1: Elongation cycle used for the synthesis of *acyclo*oligothymidylate. MeIm: N-methyl-imidazole.

N°	Step	Solvent or reagent	Time (s)
1	Wash and flush	CH_3CN, Argon	52
2	Detritylation	3%TCA in CH_2Cl_2	100
3	Wash and flush	CH_3CN, Argon	225
4	Coupling	0.1M amidite (20 eq.) in CH_3CN + 0.5M tetrazole (100 eq.) in CH_3CN	45
5	Flush	Argon	10
6	Capping	6.5% MeIm in THF+ Ac_2O/ lutidine/THF (1/1/8)	143
7	Flush	Argon	20
8	Oxidation	0.1M I_2 in THF/Pyr/H_2O (40/10/1)	55
9	Wash	CH_3CN	100

cm^3), dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel using methanol-triethylamine-dichloromethane (0:1:90 to 10:1:89) as the eluting system. The fractions containing the pure product were evaporated to dryness. R_f .005 (methanol-dichloromethane, 5:95). The 3'-O-*hemi*succinate (17) (0.3 mmol) was dissolved in 1,2-dimethoxyethane (4 cm^3) and pentachlorophenol (0.879 g, 0.33 mmol) and dicyclohexylcarbodiimide (0.681 g, 0.33 mmol) were successively added to the reaction mixture which was stirred overnight at room temperature. The precipitate was filtered off and the filtrate evaporated to dryness. The residue was taken up in dichloromethane and precipitated from *n*-hexane to yield the *title compound* (0.169 g, 64% yield). R_f 0.47 (methanol-dichloromethane, 5:95).

Functionalization of the solid support (19).- Long chain alkylamine controlled pore glass (0.64 g) was activated with triethylamine (0.039 cm^3)

in dry pyridine (2 cm^3). After evaporation of the solvent, the residue was suspended in a solution of the pentachlorophenyl *hemi*succinate (**18**) (0.169 g, 0.19 mmol) in dry pyridine (2 cm^3). The mixture was stirred gently for 3.5 d at room temp. and the solid material was then collected by suction and washed thoroughly with pyridine and dichloromethane and dried. The glass beads were suspended for 15 mn in a capping solution (4 cm^3) made of acetic anhydride (0.25M), 2, 6-lutidine (0.25M) and N,N-dimethylaminopyridine (0.25M) in tetrahydrofuran and then washed with tetrahydrofuran (3x6 cm^3) and dichloromethane (3x6 cm^3) and dried under vacuum. Spectrometric measurement of the amount of dimethoxytrityl cation released by treating an aliquot of the *title compound* with 0.1M *p*-toluene sulfonic acid in acetonitrile indicated a loading of 29 μmol/g.

Solid -phase synthesis of oligo-1', 2'-seco *2'*-nor *dodecathymidilylate.*- The 12 mer was synthesized on an Applied Biosystems 381A DNA synthesizer using the cycle described in table 1. The synthesis was carried out on a 1 μmol scale. The solid support was then treated with thiophenol-triethylamine-dioxan (1/2/2, v/v/v) for 2.3 h at room temp. After washing with methanol (10 cm^3) the support was treated with aqueous concentrated ammonia (3x30 mn) at room temperature. At this step HPLC analysis indicates that the phosphate deprotection was incomplete and therefore the supernatant (2 cm^3) was kept for 18h at 55°C in a sealed tube. The mixture was evaporated to yield the crude oligonucleotide (65 OD^{260nm}) which was purified by HPLC using a reverse phase C18 column (Nucleosil 5μm, 10x250mm, SFCC) and a linear gradient of increasing acetonitrile concentration (9 to 15%) over 20 mn in a 0.05M triethylammonium acetate (pH 7) as buffer (34 OD^{260nm}). λ max(H_2O)/nm 263. Capillary electrophoresis analysis of 1',2'-*seco*, 2'-*nor*-dT_{12} was performed on a 270A-HT capillary electrophoresis system (Applied Biosystems) on a microgel column (ABI), using 75 mM tris-phosphate, 10% methanol, pH 7.6 as buffer and 300 V/cm. R_t 9.66 mn. The spectroscopic purity at 260nm was better than 98% (less than 2% of n-1).

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REFERENCES

1. I. Votruba and A. Holy, *Collect. Czech. Chem. Commun.*, **1980**, *45*, 3039.
2. E. de Clercq and R. T. Walker, *Pharmac. Ther.*, **1984**, *26*, 1.
3. W. T. Ashton, L. F. Canning, G. F. Reynolds, R. L. Tolman, J. D. Karkas, R. Lion, M. E. M. Davies, C. M. De Witt, H. C. Perry and A. K. Field, *J. Med. Chem.*, 1985, *28*, 926.
4. E. de Clercq, *TiPS*, **1990**, *11*, 198.
5. I. Rosenberg and A. Holy, *Collect. Czec. Chem. Commun.*, 1987, *52*, 2792.
6. E. Uhlmann and A. Peyman, *Chem. Rev.*, **1990**, *90*, 544.
7. J. Goodchild, *Bioconjugate Chemistry*, **1990**, *1*, 165.
8. C. A. A. van Boeckel, G. M. Visser, R. A. Hegstrom and J. H. van Boom, *J. Mol. Evol.*, **1987**, *25*, 100.
9. G. F. Joyce, *Nature*, **1989**, *338*, 217.
10. G. F. Joyce, A. W.Schwartz, S. L. Miller and L. E. Orgel, *Proc. Natl. Acad. Sci. USA*, **1987**, *84*, 4398.
11. N. Usman, C. D. Juby and K. K. Ogilvie, *Tetrahedron Lett.*, **1988**, *29*, 4831.
12. K. C. Schneider and S. A. Benner, *J. Am. Chem. Soc.*, **1990**, *112*, 453.
13. M. Perbost, M. Lucas, C. Chavis, A. Pompon, H. Baumgartner, B. Rayner, H. Griengel and J.-L. Imbach, *Biochem. Biophys. Res. Commun.*,**1989**, *165*, 742.
14. J. Paoletti, D. Bazile, F. Morvan, J.-L. Imbach and C. Paoletti, *Nucl. Acids Res.*,**1989**, *17*, 2693.
15. T. Padmakumari Amma and J. K. Stille, *J. Org. Chem.*, **1982**, *47*, 468.
16. M. Azymah, C. Chavis, M. Lucas and J.-L. Imbach, *Tetrahedron Lett.*, **1989**, *30*, 6165.

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