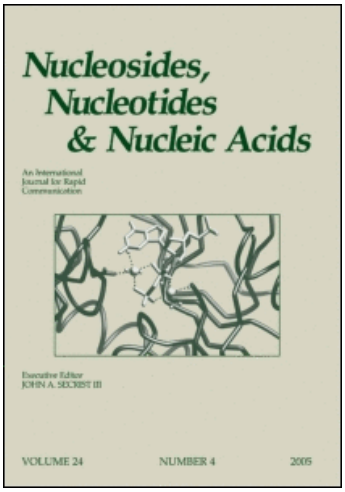

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Studies on the Formation of the Internucleotidic Bond in RNA Synthesis Using Dialkylamino Phosphoroamidites

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STUDIES ON THE FORMATION OF THE INTERNUCLEOTIDIC BOND IN RNA SYNTHESIS USING DIALKYLAMINO PHOSPHOROAMIDITES.

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SUMMARY : Several N,N-dialkylamino-cyanoethyl-phosphoroamidites were tested for use in solid-phase synthesis of homo-uridine RNA fragments. N-ethyl-N-methylamino protection gave the best results. The corresponding monomer is stable for several months and an average coupling yield of 97 % was obtained after 4 minutes of condensation using this agent.

INTRODUCTION

One of the most important achievements in oligodeoxynucleotide synthesis was the introduction of phosphoroamidite chemistry for the formation of the internucleotidic bond [1]. When they are stored under an inert atmosphere, these molecules are stable for several months and then, activated by tetrazole, they become very reactive reagents. Using them, the formation of the internucleotidic bond takes place in a few minutes, reducing the time necessary for the production of a 20 base oligonucleotide to less than 4 hours. For reasons of stability, methoxydimethylamino-phosphoroamidites, originally proposed by Caruthers [2], have been successively replaced by methoxymorpholino- and methoxydiisopropylamino-phosphoroamidites. Köster and coworkers [3,4] proposed the use of the cyanoethyl group in place of the methyl group for the protection of the internucleotidic phosphates, which reduces and considerably simplifies the final deprotection step, avoiding the use of noxious thiophenol.

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Oligoribonucleotide synthesis has not been improved as much. Mainly, this is due to the presence of the hydroxyl function on the 2' position of ribose moiety. This function must be protected and, whatever the protecting group [5-8], it inevitably causes steric hindrance which inhibits the formation of the internucleotidic bond. In RNA synthesis, the average coupling yields currently observed are in the range of 95 %, obtained in 5 - 15 minute coupling times. These insufficient yields eliminate the possibility of the routine preparation of long RNA fragments with acceptable yields in reasonable times. We show here the improvement of condensation conditions in RNA synthesis *via* phosphoroamite methodology.

RESULTS AND DISCUSSION

FIGURE 1 shows the reaction mechanism proposed [9,10] for the formation of the internucleotidic bond in DNA synthesis. The limiting step is the nucleophilic attack of the P-protonated phosphoroamidite (**2**) by tetrazole, to give a tetrazolide (**3**). This intermediate is then displaced by the 5'-OH function of the growing chain. The parameters effecting this coupling have been extensively studied. Schematically, the following observations can be made :

- The use of the cyanoethyl group in place of the methyl group for the protection of the internucleotidic phosphate leads to slower reactions [9]. This phenomenon, which is difficult to show in DNA is well known in RNA synthesis [5-9,11,12], in which steric hindrance due to the 2' protecting group occurs.
- The choice of the amino group used for the temporary protection of the phosphoroamidite is crucial. Dimethylaminophosphoroamidites are not stable enough to safely ensure stability during storage [2,4,9,13-15]. They were successively replaced by morpholino- and diisopropylamino-phosphoroamidites [2,3], the latter being the most popular reagents for solid phase synthesis of DNA and RNA fragments. Dahl and coworkers [9] clearly showed for various phosphoroamidites that the reaction rate decreases through the series NEt_2 > $NiPr_2$ > morpholino. The choice of dialkylamine for the formation of the phosphoroamidite is a compromise between the stability of the monomer and its effciency during the coupling step.
- The nature of the activation agent was also studied. Amine hydrochlorides [1], which are possible activation agents, are not useful because they are hygroscopic. Among the various weak acids potentially useful as condensation activators, tetrazole is the most widely used. Substituted tetrazoles were tried

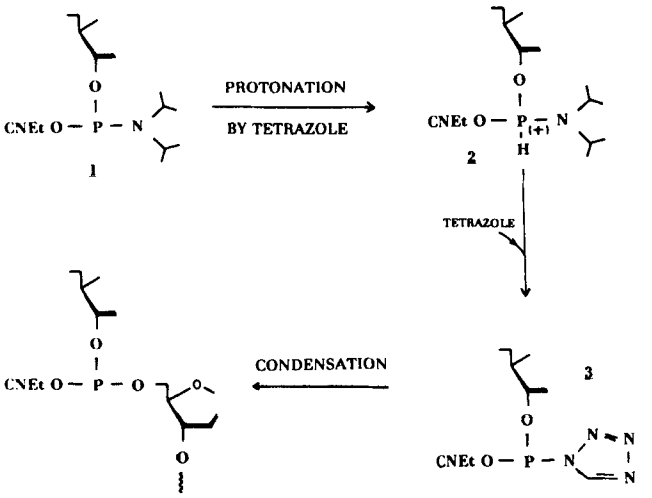


FIGURE 1 : Reaction scheme proposed for the formation of the internucleotidic bond [9]. (CNEt = 2-cyanoeth-1-yl)

in DNA synthesis and demonstrated to be more efficient catalysts [16,17]. For this reason they are more widely employed in RNA synthesis [6-8,11].

- Excessive prolongation of the coupling time should be avoided [9] because accumulation of tetrazolide ion deactivates intermediate **3**. This is corroborated by the lower efficiency of the *in situ* activation methodology [18] over the use of purified amidites and tetrazole.

The improvement of condensation yield and shortening of condensation time in RNA synthesis can be approached using any of these ways. Nevertheless, the second one - the nature of the amino protection of the phosphoroamidite - seemed to be the most promising since :

- The substitution of the cyanoethyl group for the methyl group looks unjudicious because it has been shown [5] that the lower coupling efficiency due to the cyanoethyl group is compensated by its better final deprotection yield.
- Substituted tetrazoles have a low solubility in acetonitrile and their use in combination with dimethylaminopyridine reduces their solubility [11]. There is a risk that they will precipitate if they are used with automated nucleic acid synthesizers. If they are excessively diluted (0.1 M), they give results comparable with those obtained with 0.5 M tetrazole.

Our approach was to prepare a series of cyanoethyl-dialkylamino-chlorophosphines, to condense them with 5'-O-dimethoxytrityl-2'-O-tertiobutyl-dimethylsilyl-

uridine and to test the efficiency of the corresponding uridine phosphoroamidites for the synthesis of $U_{14}T$ on a solid support.

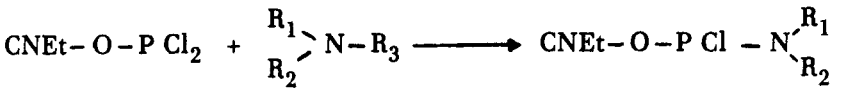
Five dialkylamine derivatives (N,N-dimethylamine, N-ethyl-N-methylamine, N,N-diethylamine, N,N-diisopropylamine and trimethylsilyl-morpholine) were condensed with cyanoethyl dichlorophosphine using published procedures [1,4] to prepare the corresponding cyanoethyl dialkylamino chlorophosphines.(**4 a-e**) depicted in FIGURE 2. These phosphitylating agents were distilled under reduced pressure to give colorless liquids. Yields were from 50 to 85 %, reached for the preparation of compound **4b**. In our hands, distillation of compound **4e** was unsuccessfull . These compounds were analysed by proton and phosphorus NMR. They are stable under strictly inert storage conditions, FIGURE 3 shows NMR spectra of compound **4b** before and after 7 months at -20°C under inert atmosphere.

5'-O-dimethoxytrityl-2'-O-tertiobutyldimethylsilyl-uridine was condensed with phosphitylating agents **4 a-e** to produce uridine phosphoroamidites **5 a-e** (FIGURE 4), in the presence of N-ethyl-N,N-diisopropylamine (4 eq.) and N,N-dimethylaminopyridine (0.2 eq.) in anhydrous tetrahydrofuran, according to a published procedure [5]. Compounds **5 a-e** were purified on silica gel using dichloromethane - triethylamine as the eluent. Compounds **5 b-e** were stable under these purification conditions. Dimethylamino-phosphoroamidites **5a** decomposed on the column and could not be obtained in satisfactory purity. For this reason, compound **5a** was not used in further experiments. Monomers were then precipitated in hexane (-78° C) and stored in sealed bottles, under argon at - 18° C.

The purity of amidites **5 b-e** was checked by ^{1}H, ^{31}P nuclear magnetic resonance and Fast Atom Bombardment mass spectrometry. Physical data concerning these products are listed in TABLE 1.

Although these products have been observed as uncontaminated pairs of diastereoisomers by NMR, the absence of 3'-O-tbdms-2'-phosphoroamidites as contaminents was carefully checked using high performance liquid chromatography. 3'-O-tbdms-2'-O-ethyl-methylphosphoroamidite was synthesized and used to check for its presence in normal phosphoroamidite **5b**. FIGURE 5 shows the analysis of purified nucleotide **5b** onto a Superspher RP 18-e reversed phase column. Within the detection limits of this method, no migration of the tbdms group during the phosphitylation reaction could be detected.

Preparation of phosphitylating agents



4a - e

FIGURE 2 : Reaction scheme for the preparation of cyanoethyl dialkylamine chlorophosphine. **a** : $R_1 = R_2 = CH_3$ - ; **b** : $R_1 = CH_3$ - $R_2 = C_2H_5$ - ; **c** : $R_1 = R_2 = C_2H_5$ - ; **d** : $R_1 = R_2 = isoC_3H_7$ - ; **e** : R_1,R_2 = N-morpholinyl- ; R_3 = H or $SiMe_3$.

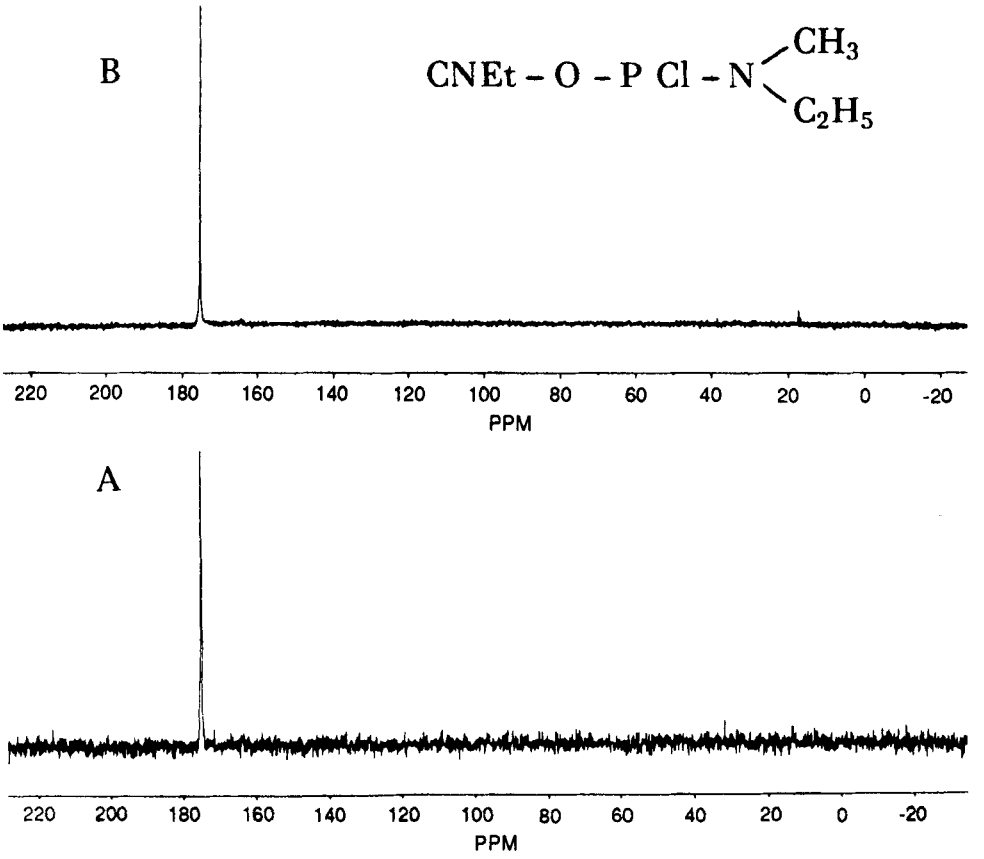
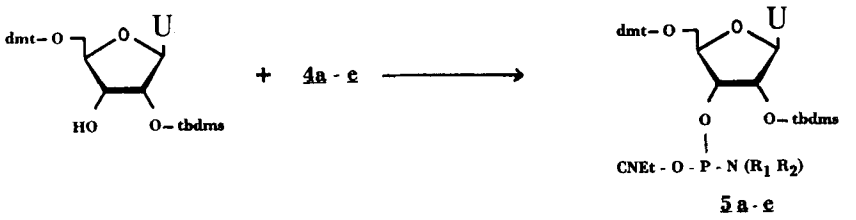


FIGURE 3 : ^{31}P NMR spectra of compound **4b** in $CDCl_3$. A: Freshly distilled ; B : After 7 months under argon atmosphere at -20°C.

Preparation of uridine-phosphoroamidites for solid phase synthesis.



5 a - e

FIGURE 4 : Reaction scheme used for the phosphitylation of fully protected uridine dmt = dimethoxy trityl- ; tbdms = *tert*butyldimethylsilyl-.

The time necessary to obtain a quantitative 3'-O-phosphitylation of protected uridine gave a good idea about the relative reactivity of the studied derivatives. Compounds **5a-c** were obtained in 30 minutes and phosphoroamidites **5d** and **5e** necessited 2 hours for condensation. The evolution of the reaction mixtures was difficult to monitor by T.L.C. so reversed phase H.P.L.C. was used. The starting product was well separated from the diastereoisomers of the phosphoroamidite and the phosphonates generated as minor by-products.

These monomers are quite stable. Compound **5b** was stored for 3 months at -20°C, diluted in acetonitrile and kept in solution for 4 days at room temperature. A ^{31}P-NMR spectrum registred after this treatment showed no modification (data not shown).

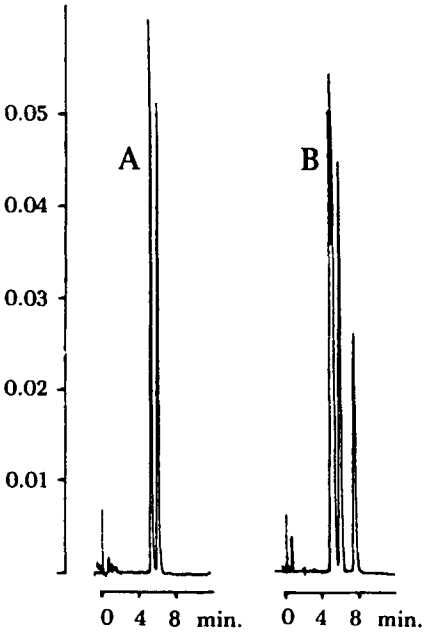


FIGURE 5 : **A** ; Reversed phase column analysis of compound **5b** (2'-O-tbdms-3'-phosphoroamidite), after silica gel purification and precipitation in hexane. **B** ; Control injection of the 3'-O-tbdms-2'-phosphoroamidite in mixture with the desired product. Column : Merck Superspher 100 RP-18e 4 µm, 125 X 4 mm eluted with CH_3CN / H_2O 75 / 25 v/v at 1 ml/min.

Solid phase condensation tests

To evaluate the relative efficiency of uridine nucleotides in oligoribonucleotide synthesis, the preparation of a standard homopolymer was undertaken in analogous conditions. Commercially available cartridges from a single batch, loaded with Control-

Compound	Yield %	^{31}P NMR	FAB MS
5 a	60	149.4 - 148.7	803.2
5 b	75	149.8 - 149.0	817.5
5 c	79	151.6 - 150.5	832
5 d	72	151.6 - 150.8	860
5 e	52	146.5 - 144.9	845.5

TABLE 1 : Physical constants of phosphoroamidites **5 a-e**. Chemical shifts are from 85 % phosphoric acid as external standard in deuterated chloroform. FAB mass measures are in negative mode in a N.B.A. matrix.

REAGENTS	TIME
0.5 M Tetrazole	5
0.1 M **5b-e** / 0.5 M Tetrazole (1/1)	3
0.5 M Tetrazole	3
0.1 M **5b-e** / 0.5 M Tetrazole (1/1)	3
0.5 M Tetrazole	3
WAIT	30 - 960
0.1 M **5b-e** / 0.5 M Tetrazole (1/1)	3
0.5 M Tetrazole	3
0.1 M **5b-e** / 0.5 M Tetrazole (1/1)	3
0.5 M Tetrazole	3
WAIT	30 - 960

TABLE 2 : Condensation steps used for the coupling of RNA monomers. Various condensation times were obtained by modification of the WAIT steps.

led Pore Glass linked with thymidine were used as initiators of all synthesis. 8-14 condensation cycles with the synthetic ribo-monomers were then executed using commercial DNA synthesis reagents (Trichloracetic acid, tetrazole, oxidation and capping solutions). Chain lengths of 9-15 nucleosides were chosen to avoid calculations bases on the first few condensations which are frequently of variable yield. Coupling yields on larger chains are generally quite reproducible [19] allowing precised calculation of the yields.

The coupling cycles used in these experiments were those defined by the manufacturer of the machine (Applied Biosystems- 381A) for DNA synthesis, except in

the coupling step which was modified as indicated in TABLE 2. This pumping sequence corresponds to an average use of 45 equivalents of monomer per cycle.

Coupling yields were calculated by spectrophotometric determination at 500 nm of the amount of dimethoxytrityl cation released during each detritylation step. Average values were plotted versus condensation time, the corresponding curves are given in FIGURE 6.

These curves show that a significant improvement of the coupling yield and time can be obtained by the choice of an appropriate alkylamine for the protection of the phosphoroamidite. They confirm the observation made by Dahl *et al.*[9] that an excessive prolongation of the condensation time does not necessarily lead to quantitative couplings. Low yields obtained with morpholino-phosphoramidite are certainly due to the presence of impurities in the monomer, due to the use of undistilled material for the phosphitylating step. Dimethylamino-phosphoroamidite could not be obtained with acceptable yields, and thus were rejected from our tests. N-ethyl-N-methylamino-.and N,N-diethylamino-phosphoroamidite appear to be promising monomers in oligoribonucleotide synthesis since they allow 96 - 97 % coupling yields in 4 minutes condensation times. Tests for extension of these systems to the four common RNA nucleosides are in progress.

EXPERIMENTAL SECTION :

Material and reagents :

3-hydroxypropionitrile (Fluka - puriss), phosphorus trichloride (Fluka - Pract.), N,N-diisopropylamine (Fluka - puriss), morpholine (Janssen Chimica) and diisopropylethylamine (Fluka - puriss) were distilled before use. N,N-dimethylamine (Prolabo Rectapur), N-ethyl-N-methylamine (Aldrich) and N,N-diethylamine (Aldrich) were used without further purification.

5'-O-dimethoxytrityl-2'-O-tertiobutyldimethylsilyl-uridine was prepared according to reported procedure [5]. The nucleoside was carefully purified and its purity (absence of 3'-O-tbdms isomer) was checked by reversed phase H.P.L.C.

T.L.C were developped on silica-gel Merck 60 F 254 using CH_2Cl_2 / AcOEt / TEA, 50 / 50 / 5 (v/v/v) as the eluent.

^{31}P NMR spectra were recorded on a Bruker WM 250 working at 101.25 MHz. Chemical shifts are given in ppm from 85 % H_3PO_4 as an external standard. Proton NMR spectra were recorded on a Bruker AC 200 spectrometer. Chemical shifts are given in ppm from TMS as an internal standard.

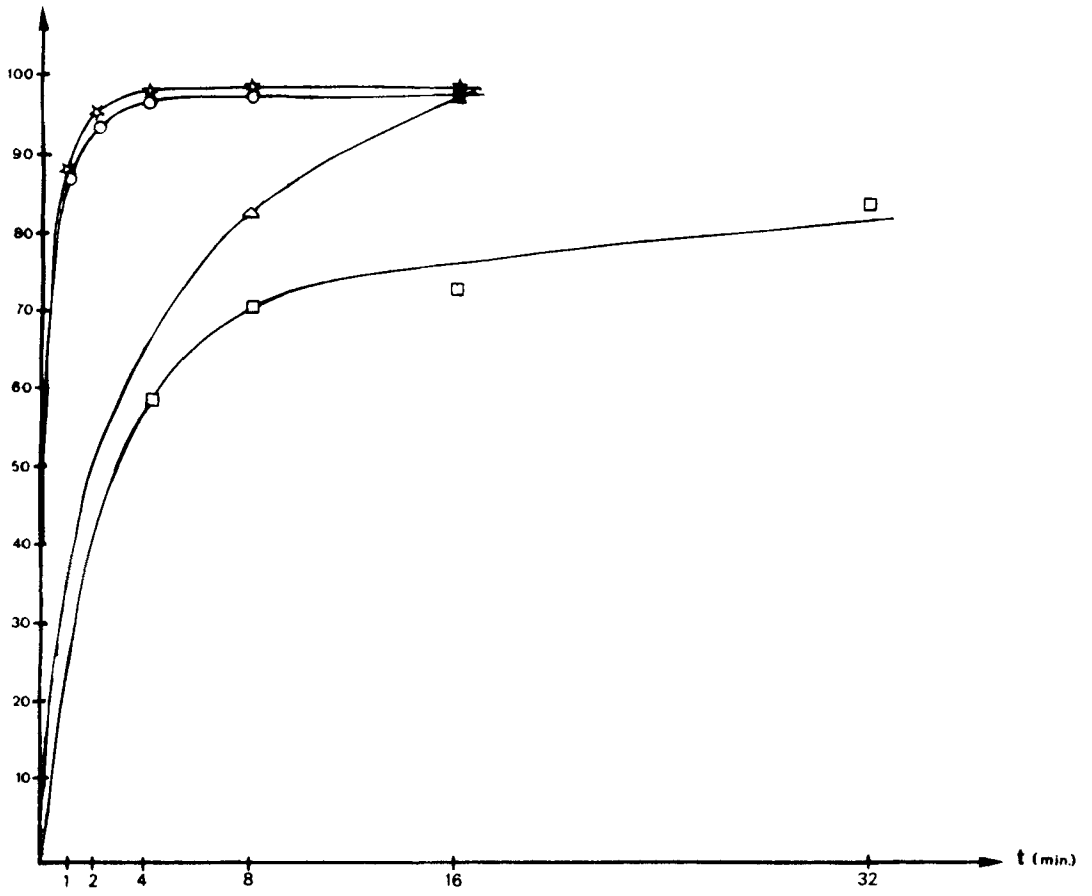


FIGURE 6 : Average coupling yields plotted versus condensation times for various dialkylaminophosphoroamidites tested in this paper. —☆— 5b, —○— 5c, —△— 5d, —□— 5e.

Analytical H.P.L.C were performed with a Waters 600 pump equipped with a Waters 484 tunable absorbance detector and a Rheodyne 7125 injection valve. Columns (Merck) were LiChrospher RP 18 e, 10 µm particle diameter (250 X 4 mm) or Supersher RP 18 e 4, µm particle diameter (125 X 4 mm).

Preparative H.P.L.C. were performed on a Jobin-Yvon axial compression 40 mm diameter column system, equiped with a SM 25 variable wavelength UV detector (Delsi Instruments) and a LKB 2152 H.P.L.C. gradient controller. Silica gel was Kieselgel PF_{254} From Merck.

Solid phase synthesis were performed on an Applied Biosystems 381 A DNA synthesizer. Standard cycle and reagents were used (Applied Biosystems) except for the condensation time which was modified according to TABLE 2.

Preparation of phosphitylating agents (4a-e)

General procedure : In a round bottom flask fitted addition funnel, magnetic stirrer and argon delivery system was placed β-cyanoethyldichlorophosphine (34.4 g, 200 mmol.) in dry ether (200 ml). N,N-dialkylamine (2 eq. 400 mmol.) or N-trimethylsilyl-morpholine (1 eq., 200 mmol.) in solution in ether (100 ml) was added dropwise at -20° C over 1.5 hour. Amine hydrochloride precipited and the suspension was stirred for 20 hours at room temperature. Amine hydrochloride was filtered off and the filtrate and washings were concentrated under reduced pressure at room temperature. Distillation gave the desired compounds.

(2-Cyanoethoxy)-N,N-dimethylamino-chlorophosphine 4a : Bp 90-93° C / 10^{-2} mbar. ^{31}P NMR ($CDCl_3$) 175.8 ppm. ^1H NMR $CDCl_3$ 4.00 ppm (dt, 2H, $POCH_2$), 2.66 ppm (t, 2H, CH_2CN), 2.60 ppm (d, 6H, $N-CH_3$).
(2-Cyanoethoxy)-N-ethyl-N-methylamino-chlorophosphine 4b : Bp 83-84° C / 10^{-2} mbar. ^{31}P NMR $CDCl_3$ 174.9 ppm. ^1H NMR $CDCl_3$ 3.95 ppm (dt, 2H, $POCH_2$), 3.00 (m, 2H, $N-CH_2$) 2.62 ppm (t, 2H, CH_2CN), 2.57 ppm (d, 3H, $N-CH_3$), 1.03 ppm (t, 3H, CH_3 from ethyl)
(2-Cyanoethoxy)-N,N-diethylamino-chlorophosphine 4c : Bp 94-96 ° C / 10^{-2} mbar. ^{31}P NMR $CDCl_3$ 176.5 ppm. ^1H NMR $CDCl_3$ 4.03 ppm (dt, 2H, $POCH_2$), 3.14 (m, 4H, $N-CH_2$) 2.70 ppm (t, 2H, CH_2CN), 1.12 ppm (t, 6H, CH_3 from ethyl)
(2-Cyanoethoxy)-N,N-diisopropylamino-chlorophosphine 4d : Bp 86-90 ° C / 6 10^{-2} mbar. ^{31}P NMR $CDCl_3$ 174.9 ppm. ^1H NMR $CDCl_3$ 4.06 ppm (dt, 2H, $POCH_2$), 3.61 (m, 2H, N-CH) 2.75 ppm (t, 2H, CH_2CN), 1.28 ppm (d, 12H, CH_3 from isopropyl)
(2-Cyanoethoxy)-N-morpholino-chlorophosphine 4e : Not distilled. ^{31}P NMR $CDCl_3$ 168.2 ppm. ^1H NMR $CDCl_3$ 4.06 ppm (dt, 2H, $POCH_2$), 3.66 (t, 4H, $O-CH_2$) 3.18 ppm (m, 4H, $N-CH_2$), 2.72 ppm.(t, 2H, CH_2CN).

Preparation of monomers 5a-e

General procedure : 5'-O-dimethoxytrityl-2'-O-*tert*butyldimethylsilyl-uridine (1.98 g - 3 mmol.) was dried by 2 evaporations of anhydrous pyridine (5 ml) and dry THF (5 ml). The residue was dissolved in dry THF (15 ml) under argon atmosphere. Dimethylaminopyridine (0.2 eq. - 73 mg), N,N,N-ethyldiisopropylamine (4 eq. - 2.05 ml) and dialkylaminochlorophosphine (1.4 eq.) were added through a rubber septum. The reaction mixture was stirred at room temperature and monitored by R.P.L.C. (30 - 120 min. condensation time were determined). The solution was then poured in a separation funnel loaded with ethyl acetate (150 ml) and saturated sodium bicarbonate. The organic phase was washed with water (2 X 150 ml), the combined aqueous phase was extracted with ethyl acetate (150 ml) and the organic extracts were combined, dried over sodium sulfate and evaporated to dryness. Purification was made by silica gel H.P.L.C using dichloromethane/ triethylamine 98 / 2 (v/v) as the eluent. After evaporation of the solvent, mononucleotides were dissolved in toluene (10 ml) and precipited in cold (-78° C) hexane. They were collected as white powders and stored under argon atmosphere at - 20° C.

5'-O-dimethoxytrityl-2'-O-tertiobutyldimethylsilyl-uridine (3'-O-(cyanoethoxy-(N,N-dimethylamino)-phosphoroamidite)) 5a : Rf 0.64. ^{31}P NMR in CD_3COCD_3 148.7 & 149.4 ppm. Proton NMR in CD_3COCD_3 8.04 & 8.03 ppm (d, 1H, H_6), 6.05 & 6.00 ppm (d, 1H, $H_{1'}$), 5.38 & 5.36 ppm (d, 1H, H_5), 4.64 ppm (m, 1H, $H_{2'}$), 4.54 ppm (m, 1H, $H_{3'}$), 4.32 ppm (m, 1H, $H_{4'}$), 4.01 ppm (m, 2H, CH_2-OP), 3.92 ppm (d, 6H, CH_3 trityl), 3.61 ppm (m, 2H, $H_{5'5''}$), 2.87 & 2.76 ppm (t, 2H, CH_2CN), 2.74 & 2.64 ppm (d, 6H, N-CH_3), 1.05 ppm (s, 9H, *tert*-butyl), 0.29 ppm (s, 6H, $SiCH_3$).

5'-O-dimethoxytrityl-2'-O-tertiobutyldimethylsilyl-uridine (3'-O-(cyanoethoxy-(N-methyl-N-ethylamino)-phosphoroamidite)) 5b : Rf 0.60. ^{31}P NMR in CD_3COCD_3 148.9 & 149.8 ppm. Proton NMR in CD_3COCD_3 8.06 & 8.04 ppm (d, 1H, H_6), 6.05 & 6.00 ppm (d, 1H, $H_{1'}$), 5.38 & 5.36 ppm (d, 1H, H_5), 4.64 ppm (m, 1H, $H_{2'}$), 4.60 ppm (m, 1H, $H_{3'}$), 4.39 ppm (m, 1H, $H_{4'}$), 3.98 ppm (m, 2H, CH_2-OP), 3.92 ppm (d, 6H, CH_3 trityl), 3.63 ppm (m, 2H, $H_{5'5''}$), 3.11 ppm (m, 2H, CH_2 N-ethyl), 2.87 & 2.73 ppm (t, 2H, CH_2CN), 2.72 & 2.61 ppm (d, 3H, N-CH_3), 1.20 & 1.12 ppm (t, 3H, CH_3 N-ethyl), 1.04 ppm (s, 9H, *tert*-butyl), 0.30 & 0.29 ppm (s, 6H, $SiCH_3$).

5'-O-dimethoxytrityl-2'-O-tertiobutyldimethylsilyl-uridine (3'-O-(cyanoethoxy-(N,N-diethylamino)-phosphoroamidite)) 5c : Rf 0.72. ^{31}P NMR in CD_3COCD_3 150.4 & 151.5 ppm. Proton NMR in CD_3COCD_3 8.06 & 8.05 ppm (d, 1H, H_6), 6.05 & 6.00 ppm (d, 1H, $H_{1'}$), 5.38 ppm (d, 1H, H_5), 4.66 ppm (m, 1H, $H_{2'}$), 4.62 ppm (m, 1H, $H_{3'}$), 4.38 ppm (m, 1H, $H_{4'}$), 3.99 ppm (m, 2H, CH_2-OP), 3.92 ppm (d, 6H, CH_3 trityl), 3.64 ppm (m, 2H, $H_{5'5''}$), 3.15 ppm (m, 4H, CH_2 N-ethyl), 2.88 & 2.74 ppm (t, 2H, CH_2CN), 1.22 & 1.11 ppm (t, 3H, CH_3 N-ethyl), 1.04 ppm (s, 9H, *tert*-butyl), 0.29 & 0.28 ppm (s, 6H, $SiCH_3$).

5'-O-dimethoxytrityl-2'-O-tertiobutyldimethylsilyl-uridine (3'-O-(cyanoethoxy-(N,N-diisopropylamino)-phosphoroamidite)) 5d : Rf 0.82. ^{31}P NMR in CD_3COCD_3 150.8 & 151.7 ppm. Proton NMR in CD_3COCD_3 8.04 & 7.99 ppm (d, 1H, H_6), 6.11 & 6.06 ppm (d, 1H, $H_{1'}$), 5.42 & 5.40 ppm (d, 1H, H_5), 4.61 ppm (m, 1H, $H_{2'}$), 4.55 ppm (m, 1H, $H_{3'}$), 4.44 ppm (m, 1H, $H_{4'}$), 4.02 ppm (m, 2H, CH_2-OP), 3.92 ppm (d, 6H, CH_3 trityl), 2.92 & 2.73 ppm (t, 2H, CH_2CN), 1.26 ppm (d, 12H, CH_3 N-isopropyl), 1.05 ppm (s, 9H, *tert*-butyl), 0.29 & 0.28 ppm (s, 6H, $SiCH_3$).

5'-O-dimethoxytrityl-2'-O-tertiobutyldimethylsilyl-uridine (3'-O-(cyanoethoxy-N-morpholino-phosphoroamidite) 5e : Rf 0.85. ^{31}P NMR in CD_3COCD_3 144.9 & 146.47 ppm. Proton NMR in CD_3COCD_3 8.08 & 8.07 ppm (d, 1H, H_6), 6.06 & 6.00 ppm (d, 1H, $H_{1'}$), 5.41 & 5.40 ppm (d, 1H, H_5), 4.67 ppm (m, 1H, $H_{2'}$), 4.64 ppm (m, 1H, $H_{3'}$), 4.38 ppm (m, 1H, $H_{4'}$), 4.04 ppm (m, 2H, CH_2-OP), 3.92 ppm (d, 6H, CH_3 trityl) 3.67 & 3.57 ppm (m, 4H, O-CH_2 morpholine), 3.20 & 3.13 ppm (m, 4H, N-CH_2 morpholine), 2.90 & 2.77 ppm (t, 2H, CH_2CN), 1.05 ppm (s, 9H, *tert*-butyl), 0.29 ppm (s, 6H, $SiCH_3$).

Solid phase syntheses :

Commercially available cartridges (Applied Biosystems) loaded with 0.2 µmole 5'-O-dimethoxytrityl-thymidine grafted on C.P.G. 500, were used as initiator of the chain syntheses. 8 to 14 synthesis cycles were performed. Detritylation solution were collected and diluted to 25 ml with 70 % perchloric acid / ethanol 1/1 v/v. Yields were determined by the ratio of A_{500} consecutive detritylation crops.

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