Chemical Properties of 4,5-Di(ethoxycarbonyl)-1,3-dioxolan-2-yl (DECDO) as a Hydroxyl Protecting Group of the 2'-Hydroxyl Function in Ribonucleosides

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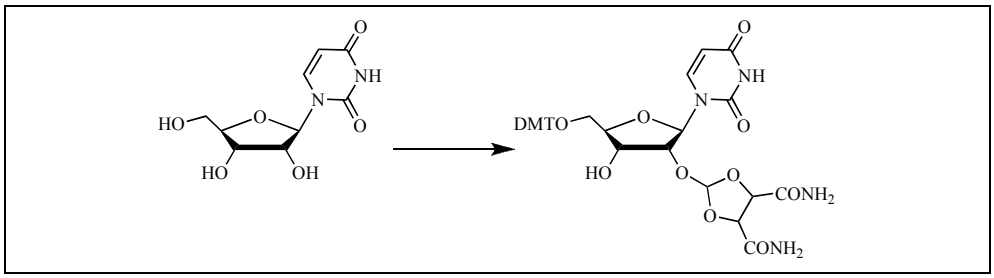

We describe basic chemical properties of 4,5-di(ethoxycarbonyl)-1,3-dioxolan-2-yl (DECDO) in view of its use as a protecting group for the 2'-hydroxyl function of ribonucleosides. The DECDO group is found to be compatible with the DMTr strategy for the currently-used oligonucleotide synthesis. Post-synthetic treatment with ammonia results in the conversion of this protecting group into the 4,5-dicarbamoyl-1,3-dioxolan-2-yl (DCBDO) group which is unexpectedly more stable in aqueous acidic solution.

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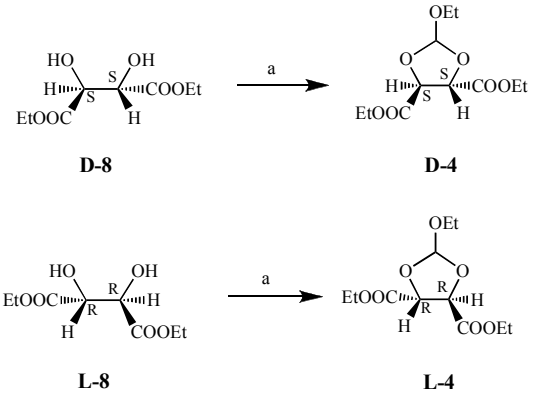
INTRODUCTION

Although a number of hydroxyl protecting groups have been explored, [1-8] only a few have survived in the field of nucleoside and nucleotide chemistry. This is due to the strict conditions which are required for their chemical transformation. For the synthesis of target materials, the protecting group should fulfill the following requirements: 1) The reagent should be easy to prepare; 2) no new chiral centers should be generated; 3) the protecting group should be stable during the desired reaction, 4) removal of the protecting group must be highly selective without any damage to the other functional groups.

The methoxymethylidene group is well-known as the protecting group of ribonucleoside *cis*-2',3'-diol derivatives [9]. Viewed from a different perspective this protection structure can be considered as an OH protecting group in methanol by the 1,3-dioxolan-2-yl group. This idea inspired us to investigate a new method for the protection of the 2'-hydroxyl group of ribonucleosides by exploiting the orthoester structure. In the literature, there are only a few examples of the orthoester-type protecting groups for RNA synthesis, [10-14]. In addition they have several drawbacks such as low yields of the precursors and the necessity to use an expensive co-reagent. [14].

In this paper, we report the chemical properties of 4,5-di(ethoxycarbonyl)-1,3-dioxolan-2-yl (DECDO) as a possible protecting group for the 2'-hydroxyl function of ribonucleoside derivatives.



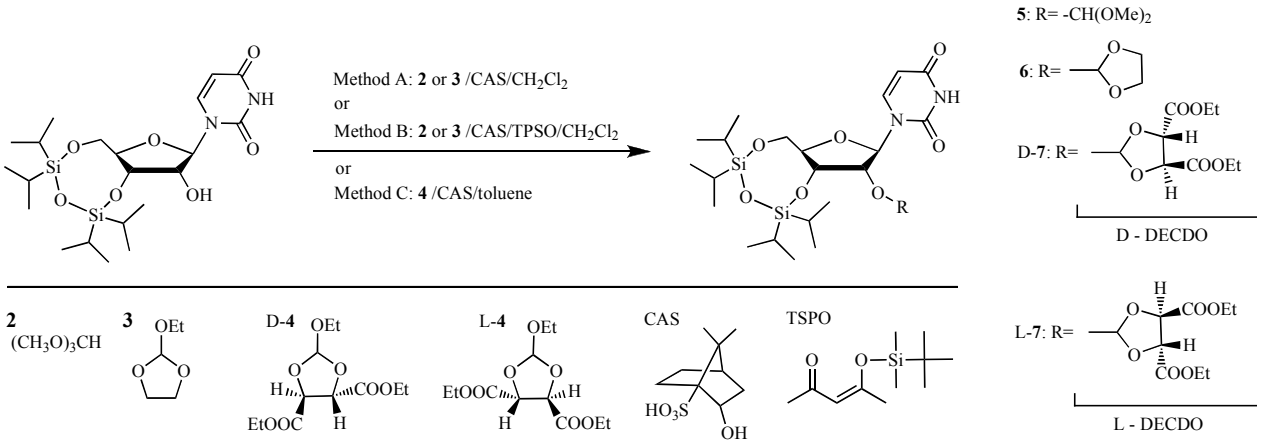
D-8 **D-4**

L-8 **L-4**

Scheme 1 Synthesis of 2-Ethoxy-1,3-dioxolane-4,5-dicarboxylic acid diethyl ester and 2-Ethoxy-1,3-dioxolane-4,5-dicarboxylic acid diamide. a) Toluene, CAS, 95 °C, 95%.

RESULTS AND DISCUSSION

Recently, orthoester type protecting groups, removable by a two-step procedure, such as 3-methoxy-1,5-dicarbomethoxypentan-3-yl [12] or di(2-acetoxyethoxy)-methyl [13-14] have been developed and applied to oligoribonucleotide synthesis. The stability of the ester function can be regulated by changing their electron donating/withdrawing properties. It is expected that during the synthetic cycle of oligoribonucleotides, the ester functions of the protecting groups will be converted into the amide, which are more labile. This idea has prompted us to study new cyclic-type orthoester protecting groups. To verify

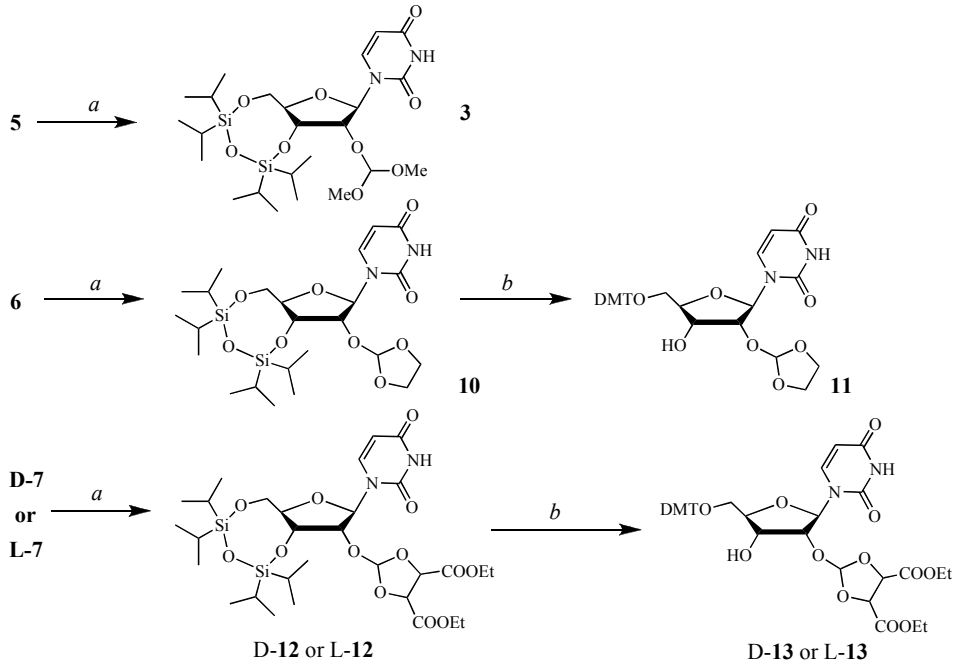


Method A: rt, overnight, 30% (**5**); Method B: rt, overnight, 86% (**5**), 90% (**6**); Method C: reflux, 2 h, 80% (D-**7**), 80% (L-**7**)

Scheme 2 Synthesis of 2'-*O*-dialkoxymethyluridine derivatives.

this hypothesis we have first studied the orthoester exchange reaction of 3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-uridine (**1**) [15] with simple orthoester compounds since the introduction of a protecting group into the 2'-hydroxyl group of ribonucleosides under mild conditions is of primary importance. Our attention turned to trimethoxymethane (**2**) and 2-methoxy-1,3-dioxolane (**3**) as well as a set of enantiomers, D- and L-diethyl 2-ethoxy-1,3-dioxolane-4,5-dicarboxylate (D-**4** and L-**4**) [16-17] as reagents for the introduction of the orthoester functions into the 2'-hydroxyl group of **1** (see Scheme 1). These compounds are commercially available or easily accessible and do not generate new chiral centres.

The ester exchange reactions of **1** with **2** and **3** were studied in detail. As a result, the 2'-*O*-blocked products **5** and **6** were obtained in only *ca*. 30% yield each by the ester exchange reaction in the presence of camphorsulfonic acid. However, it was found that the addition of 4-(*tert*-butyldimethylsilyloxy)pentene-2-one (TPSO) [14] as a scavenger of methanol, formed as a byproduct during the reaction, increased the yield of their synthesis up to *ca*. 90%, as shown in Scheme 2. D-**4** and L-**4** were synthesized in high yields by the reaction of D- and L-tartaric acids, and D-**8** and L-**8** with triethyl orthoformate (as shown in Scheme 1) to allow the synthesis of the 2'-*O*-orthoester modified product **7**.



Scheme 3 a) THF, TBAF, RT, 30 min, 95% (**10**), 95% (D-**12**), 98% (L-**12**); b) DMT-Cl, pyridine, rt, 4h, 64% (**11**), 2 h, 94% (D-**13**), 2h, 96% (L-**13**) .

New protecting groups, D- and L-4,5-(diethoxycarbonyl)-2-ethoxy-1,3-dioxolan-2-yl (D- and L-DECDO), which can be introduced to nucleosides using D-**4** and L-**4**, have been designed as follows (Scheme 2).

We expected that the stability of the DECDO group could be changed after treatment with ammonia (Scheme 4, step *c*). The new created protecting group "4,5-dicarbamoyl-1,3-dioxolan-2-yl" (DCBDO) should be more acid-labile. This lability originates from the superior electron-donating ability of the carbamoyl group over the ethoxycarbonyl group. The differences in the electron-donating ability between the ethoxycarbonyl and carbamoyl groups were estimated from the Hamett constants σ_m in a series of substituted benzoic acids. The values of the ethoxycarbonyl and carbamoyl groups were reported to be 0.37 and 0.28, respectively [18]. Therefore, we expected that the conversion of the DECDO group into the DCBDO would allow a more facile acid deprotection of the DECDO group at the final deprotection step of the RNA synthesis. In addition, the D- or L-DECDO group gave a single product when introducted into the 2'-hydroxyl group of **1** because of the inherent C_2 symmetric property.

All attempts to obtain the uridine derivatives D-**7** by reaction of **1** with D-**4**, under conditions similar to those described in the synthesis of **5** and **6,** failed. The strategy in which we used TPSO, as an ethanol scavenger, did not give successful results. It is likely that the carbonium cation derived from D-**7** by an acid-catalyzed reaction was destabilized because of the presence of two electron-withdrawing ethoxycarbonyl groups at the 4 and 5 positions. However, it was found that the use of an enhanced temperature (refluxing in toluene) together with azeotropic removal of the once-generated ethanol resulted in a satisfactory yield (80%) of the desired product D-**7**. L-**7** was obtained in similar yields by the use of L-**4**.

Synthesis of related compounds. In order to check the stability of the orthoester-type functional groups, several uridine derivatives were functionalized as shown in Scheme 3.

Selective removal of the TIPS group from **5** and **6** was carried out by treatment with Bu_4NF in THF. However, it was difficult to obtain **9** from **5**. Contrary to this result, the 3',5'-free product **10** was isolated with a yield of 95% with respect to **6**. The isolation of **10** by chromatograpy required the addition of 1% pyridine to the eluent, otherwise it decomposed. Moreover, when the reaction of **10** with DMTrCl in pyridine was carried out, the simultaneous loss of the 1,3-dioxolan-2-yl group from the product **11** was observed to a degree of *ca*. 50% after 10 h. This is due to a rather weak acidity of pyridinium hydrochloride formed during the reaction.

Alternatively, desilylation of D-**7** and L-**7** gave high yields of the 3',5'-*O*-free uridine derivatives *i.e*. D-**12** and L-**12**. Similarly, 5'-dimethoxytritylation of these products generated the 5'-*O*-protected compounds D-**13** and L-**13** without any loss of the DECDO group. These results show that the DECDO group proved to be stable during these transformations.

Stability of orthoester-type protecting groups. Since the standard protocol for oligoribonucleotide synthesis requires an acidic promoter for the activation of the phosphoramidite building blocks, we checked the stability of **11** and D-**13** and L-**13** under weak acidic conditions of 1*H*-tetrazole in $CDCl_3$-DMSO (5:1 v/v). As a result, compound **11** was found to gradually decompose after 120 min, while the DECDO group of D-**13** or L-**13** proved to be sufficiently stable.

To test the stability of 5'-*O*-DMTr-2'-*O*-[4,5-di(ethoxycarbonyl)-1,3-dioxolan-2-yl]uridine D-**13** and L-**13,** we chose various acids at various concentrations. It was found that our protecting group was stable during the removal of the DMTr group by 1% DCA in CH_2Cl_2, as evidenced by the ^1H NMR data.

It proved possible to selectively remove the DMTr group from the 5'-*O* position by treatment with 1% of DCA in CH_2Cl_2, therefore we examined the properties of the DECDO group at the dimer level to see if this group could be used as the 2'-protecting group. Therefore, the phosphoramidite derivative **14** with the isomeric ratio of 65:35, was synthesized at a yield of 60% by the reaction of L-**13** with the phosphitylating reagent ($Cl(iPr_2N)P(OCH_2CH_2CN)$) (Scheme 4). We also checked the use of compound **14** for the creation of the internucleotidic phosphate bond by the liquid-phase synthesis of the dinucleotide UpU.

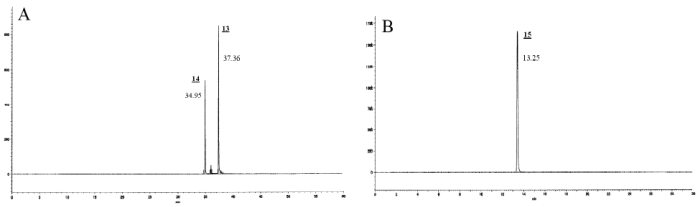


Figure 1 Chart A: RP HPLC trace of the mixture **15** and **16** synthesized using the monomers **14** (solvent system I), Chart B: RP HPLC profile of the mixture obtained by treatment of 16 with 25% CD_3COOD in D_2O (Solvent system II).

During the formation of the internucleotide bond, we did not find any unexpected signals in the ^{31}P NMR spectra. The crude product was analyzed by RP HPLC (ammonium acetate buffer). We found that the product **15** had low water solubility but was easily dissolved in CH_3CN-water (10:90 v/v). The situation dramatically changed when compound **15** was converted by ammonia (28% in water) solution in methanol (1:1 v/v) to compound **16**, due to β-cyanoethyl removal and due to

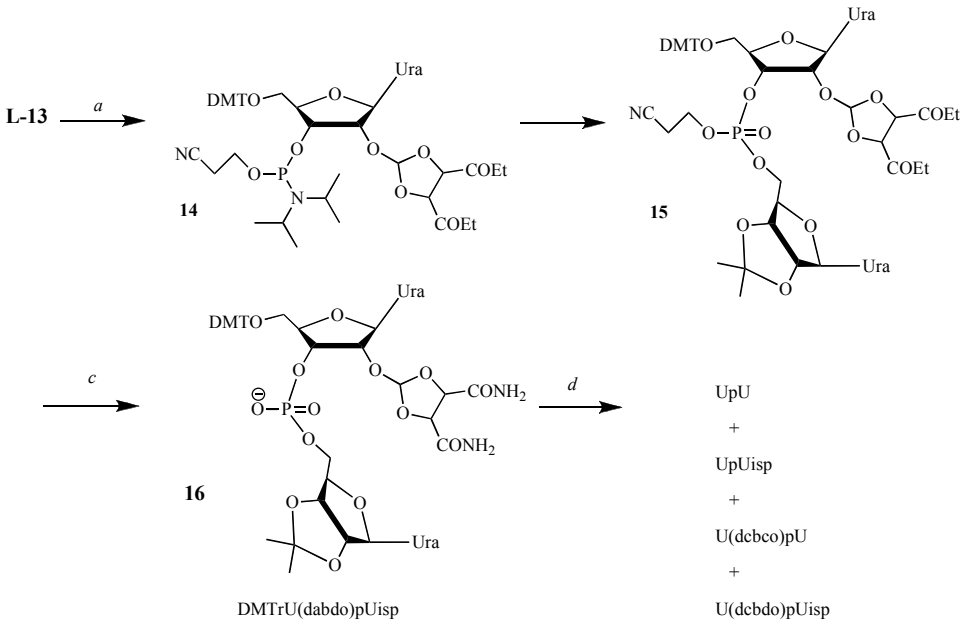
conversion of diester function to diamide one. This compound had good solubility in water so that the retention time (R_t = 35min) of **16** in RP-HPLC became shorter than that of **15** (R_t = 37min) (Fig.1).

This result is promising for future studies; the change of this hydrophilic property enables us to purify them easily. Surprisingly, when compound **16** was treated with 25% CD_3COOD in D_2O at room temperature overnight, the DCBDO group was found to be resistant to this acidic medium. The EPS mass spectrometric analysis of the reaction mixture suggested that the DMTr group was completely removed and UpU, UpUisp, U(dcbco)pU, and U(dcbdo)pUisp were observed in the ratio of 16.5:5.0:3.0:1.8. It was found that the DCBDO group was removed somewhat faster than the isopropylidene group. The latter cannot be used for the current RNA synthesis because strong acidic conditions are required for its removal. These results differ from our initial expectations that the DCBDO group, derived from the DECDO group by ammonia treatment, would be more acid-labile. To clarify these unexpected results, we studied the stability of simpler substrates L-**4** and L-**17** under acidic conditions. Compound L-**17** was prepared according to Scheme 5.

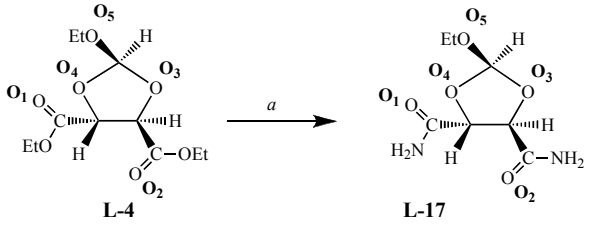
Table 1

Partial charges calculated by the DFT method, B88-PW91/6-31G** basis set.

Atom	Partial charges	Atom	Partial charges
Ester form	L-**4**	Amide form	L-**17**
O_1	-0.437	O_1	-0.499
O_2	-0.481	O_2	-0.497
O_3	-0.399	O_3	-0.365
O_4	-0.406	O_4	-0.367
O_5	-0.389	O_5	-0.384



Scheme 4 Synthesis of UpU by the 1*H*-tetrazole-mediated reaction of 2',3'-*O*-isopropylideneuridine with 1,3-dioxolane-4,5-dicarboxylic acid diethyl ester. a) CH_2Cl_2, rt, $(iPr)_2NEt$, chloro(2-cyanoethoxy)diisopropylaminophosphine, overnight, 60%; b) i) $CHCl_3$, 2',3'-*O*-isopropylideneuridine, 1*H*-tetrazole; ii) I_2-H_2O-Py; **c**) NH_3aq; d) 25% of CD_3COOD in D_2O.



Scheme 5 Synthesis of L-2-ethoxy-1,3-dioxolane-4,5-carboxamide L-**17**. a) CH_3OH, NH_3aq, rt, overnight, 100%.

The stablity of L-**4** and L-**17** was tested in 0.1% camphorsulfonic acid (CSA) in methanol. As a result, the $t_{1/2}$ and t_{com} of the decomposition of L-**4** were 3 min and 60 min, respectively. L-**17** was found to be very stable for 24 h under the same conditions. When the concentration of CSA was increased to 0.2%, the decomposition of L-**17** was observed with $t_{1/2}$ and t_{com} being 6 min and 2 h, respectively.

Quantum Mechanics Calculation. To investigate the partial charges on the oxygen and carbon atoms of L-**4** and L-**17** the density function theory (DFT) with B88-PW91[19-20] was used (the 6-31G** basis set was applied). The data presented in Table 1 show that the partial charges on the three oxygens O_3, O_4, and O_5 of the amide derivative are less negative than the ester group. These results suggest that at the initial step of

deproctection the ester derivative is more susceptible to protonation. It is also important to consider the neighboring electron-rich atoms as possible protonation sites. Since the oxygen of the carbamoyl group is more electron-rich than the carbonyl oxygen of the ester group, the protonation of the O_3, O_4, and O_5 atoms would be suppressed by the presence of more basic carbamoyl oxygens. As a result, it can be expected that when the oxygen of the carbamoyl group is protonated, this group becomes more electron-withdrawing so that the electron density of the two oxygens of the ring and O_5 decreases.

CONCLUSION

In conclusion, the 1,3-dioxolan-2-yl and methoxymethyl groups are weak as secondary alcohol protecting groups, but can be used when mild acidic conditions are necessary. Surprisingly, it was found that the DCBDO group was more stable than the DECDO group under acidic conditions. This result was unexpected, however the skeleton of the DCBDO group would be useful for the introduction of a wide variety of functional groups on the amido nitrogen via an *N*-alkyl spacer. This is promising because the DCBDO group becomes more acid-resistant and thereby such functional groups can remain intact during the standard protocol of RNA or DNA synthesis. Being a typical protecting group for the hydroxyl function, the DECDO and DCBDO groups can also be used as new protecting groups of the orthoester-type in nucleic acid chemistry as well as in carbohydrate chemistry. The stability of the DECDO group changes during the synthesis and increases when the ester groups are transformed into the amide groups. The stability of the resulting DCBDO group rises but is still able to be removed under moderate pH conditions in alcohol/water solutions. Additionally, the purification of the crude post-synthetic oligonucleotides should be fast and easy. Future studies will be continue to adopt this strategy for the solid-phase synthesis of RNA or DNA containing specific functional groups.

EXPERIMENTAL

General methods. ^{1}H, ^{13}C and ^{31}P NMR spectra were obtained on a Varian Unit 500 apparatus at 500, 126, 201 MHz, respectively. The chemical shifts were measured from tetramethylsilane (0 ppm) or DMSO-d_6 (2.49 ppm) for ^{1}H NMR, $CDCl_3$ (77.0 ppm) or DMSO-d_6 (39.7 ppm) for ^{13}C NMR and 85% phosphoric acid (0 ppm) for ^{31}P NMR. Column chromatography was performed by use of Wako silica gel C-200. Reverse-phase HPLC was performed using μBondapak C-18 columns (Waters Co., Ltd., 7.8 x 300 mm) at a flow rate of 1.0 mL/min at 50 °C. Elution was performed with the following solvent systems I and II for 50 min and 20 min, respectively at a flow rate of 1.0 mL/min. Solvent system I: 0.1 *M* Ammonium acetate (pH 7.0)-acetonitrile (100-0 to 70:70 v/v); solvent system II: 0.1 *M* Ammonium acetate (pH 7.0)-acetonitrile (100:0 to 80:20 v/v).

ESI mass spectra were measured on Mariner™. MALDI-TOF mass spectra were measured on Voyager RP. UV spectra were measured by a U-2000 spectrophotometer. TLC was performed with Merck silica gel 60 (F_{254}) plates. Molecular calculation was made by CAChe WorkSystem Pro Version 6.1 2000-2003 Fujitsu Ltd. B88-PW91 (Becke '88; Perdew & Wang '91) with the 6-31G** basic set.

Synthesis of 2'-O-[di(methoxy)methyl]-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (5). In a 30 ml round-bottom flask, 3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (1) (1.0 g, 2.05 mmol) was dissolved in 10 ml of CH_2Cl_2 to give a clear solution. Trimethyl orthoformate (4.0 g, 0.038 mmol) and camphorsulfonic acid (63 mg, 0.27 mmol) were then added to the solution. The reaction mixture was stirred for 20 min and then 4-(*tert*-butyldimethylsilyloxy)-3-pentene-2-one (890 mg, 4.1 mmol) was added. The solution was stirred overnight at room temperature. The reaction was monitored by TLC ($CHCl_3$-CH_3OH, 9:1 v/v). The solvent was evaporated on completion of the reaction. The crude product was purified by flash chromatography on a column of silica gel with $CHCl_3$-MeOH (100:0-99:1 v/v) to give compound 5 as a white foam (0.98 g, 86%); R_f ($CHCl_3$-CH_3OH, 9:1 v/v) 0.38; δ_H (500 MHz; $CDCl_3$; Me_4Si) 0.94-1.10 (m, 24H), 3.39 (s, 3H), 3.43 (s, 3H), 3.97-3.9 (d, 1H, *J* 13.4 Hz), 4.2-4.28 (m, 4H), 5.56 (s, 1H), 5.68-5.7 (d, 1H, *J* 8. 3 Hz), 5.82 (s, 1H), 7.89-7.91 (d, 2H, *J* 8.3 Hz), 9.92 (s, 1H); δ_C□(126 MHz; DMSO-d_6) 12.65, 12.96, 13.01, 13.37, 17.43, 17.48, 17.53, 17.75, 17.82, 17.82, 51.05, 51. 68, 60.31, 68.74, 76.233, 81.68, 89.95, 101.72, 113.53, 124.57, 140.35, 150.29, 150.73, 163.96; ESI-MS (ES^+) m/z 583.331 (M+Na. $C_{24}H_{44}N_2O_9Si_2Na$ requires 583.775).

Stability of 2'-O-[di(methoxy)methyl]-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (5). Compound **5** (10 mg, 0.018 mmol) and 1*H*-tetrazole (25 mg, 0.36 mmol) were dissolved in $CDCl_3$-DMSO-d_6 (5:1 v/v, 0.6 ml) in an NMR tube. The progress of the reaction was monitored by ^{1}H NMR. During the reaction, the disappearance of the signal of the anomeric proton at 5.40 ppm (DMSO) from the 1,3-dioxolane system was observed.

Synthesis of 2'-O-(1,3-dioxolan-2-yl)-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (6). In a 30 ml round-bottom flask, 3',5'-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-uridine (**1**) (4.0 g, 8.0 mmol) was dissolved in 20 ml of CH_2Cl_2 to give a clear solution. 2-Methoxy-1,3-dioxolane (5.2 g, 50 mmol) and camphorsulfonic acid (0.17 g, 0.73 mmol) were added to the solution. The reaction mixture was stirred for 20 min and then 4-(*tert*-butyldimethylsilyloxy)-3-pentene-2-one (2.2 g, 9.3 mmol) was added. The solution was stirred at room temperature overnight. The reaction progress was monitored by TLC ($CHCl_3$-CH_3OH). The solvent was evaporated on completion of the reaction. The crude product was purified by flash chromatography on a column of silica gel in the $CHCl_3$ and 0-1% CH_3OH in $CHCl_3$ to give **6** as a white foam (1.03 g, 90%); R_f ($CHCl_3$-CH_3OH, 9:1, v/v) 0.42; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.0-1.1 (m, 28H), 3.95-4.01 (m, 3H), 4.17-4.26 (m, 6H), 5.66-5.69 (d, 1H, *J* 8.3), 5.75 (s, 1H), 7.87-6.89 (d, 1H, *J* 8.3), 8.79 (s, 1H); δ_C (126 MHz; $CDCl_3$) 12.76, 13.09, 13,23, 13.71, 17.08, 17.19, 17.22, 17.27, 17.47, 17.55, 17.63, 17.72, 31.84, 59.49, 63.84, 63.87, 63.87, 68.18, 76.66, 82.07, 89.69, 101.79, 113.9, 139.48, 150.09, 163.39; ESI-MS (ES^+) m/z 581.110 (M+Na. $C_{12}H_{16}N_2O_8Na$ requires 581.233).

Synthesis of 2'-O-(1,3-dioxolan-2-yl)uridine (10). A solution of 2'-*O*-[1,3-dioxalone]-3',5'-*O*-(1,1,3,3-tetraisopropyl-

disiloxane-1,3-diyl)uridine (**6**) (2.0 g, 3.8 mmol) in dry THF 20 ml was treated with tertbutylamonium fluoride (0.70 g, 2.7 mmol). The mixture was stirred at room temperature for 30 min and the solvent removed under reduced pressure. The residue was purified by column chromatography on a column of silica gel with $CHCl_3$- CH_3OH (20:1 to 20:2.5 v/v) containing 1% pirydine to give **10** as a white foam compound (1.0 g, 95%); R_f ($CHCl_3$-CH_3OH, 9:1 v/v) 0.18; δ_H (500 MHz; DMSO; Me_4Si) 3.54-3.61 (m, 2H), 3.83-3.9 (m, 4H), 3.97-3.99 (m, 1H), 4.05-4.07 (m, 1H), 4.16-4.18 (t, 1H), 5.14-5.16 (t, 1H), 5.19-5.2 (d, 1H, *J* 5.37 Hz), 5.43-5.66 (d, 1H, *J* 8.06 Hz), 5.87-5.88 (d, 1H, *J* 6.10 Hz), 5.93 (s. 1H), 7.87-7.89 (d, 1H, *J* 8.06 Hz), 11,31 (s, 1H); δ_C□(126 MHz; DMSO-d_6) 61.54, 63.91, 64.35, 69.94, 75.86, 86.1, 86.50, 86.56, 102.58, 114.52, 114.6, 141.25, 151.37, 163.78; ESI-MS (ES^+) m/z 338.128 (M+Na. $C_{12}H_{16}N_2O_8Na$ requires 339.080).

Synthesis of 5'-O-(4,4'-O-dimethoxytrityl)-2'-O-(1,3-dioxolan-2-yl)uridine (11). 4,4'-dimethoxytrityl chloride (0.32 g, 0.92 mmol) was added to a solution of compound **10** (0.29 g, 0.92 mmol) in pyridine (5 ml). The reaction solution was stirred at room temperature for 4 hours. The reaction mixture was evaporated under reduced pressure to a 1/3 volume and the residue was partitioned between CH_2Cl_2 and H_2O (1:1 v/v, 15 ml). The organic layer was dried over $MgSO_4$, filtered, evaporated under reduced pressure and the crude product was chromatographed on a column of silica gel with $CHCl_3$ to give the product **11** as a white foam (0.350g, 64%); R_f ($CHCl_3$-CH_3OH, 9:1 v/v) 0.53; δ_H (500 MHz; $CDCl_3$; Me_4Si) 3.19-3.38 (d.m, 2H), 3.74 (s, 6H), 3.88-4.03 (m. 6H), 4.2-4.21 (m, 1H), 4.27-4.29 (m, 1H), 5.35-5.36 (m, 3H), 5.84-5.85 (d, 1H, *J* 4.64), 6.03 (s, 1H), 6.9-6.92 (d, 4H, *J* 8.79), 7.25-7.39 (t, m, 9H); 7.69-7.71 (d, 1H, *J* 8.06), 11.43 (s,1H); δ_C (126 MHz; $CDCl_3$) 55.76, 63.73, 63.89, 64.23, 69.613, 75.64, 79.89, 83.75, 86.66, 87.85, 102.307, 113.98, 114.56, 124.62, 127.53,128.41, 128.64, 130.49, 135.80, 136.06, 136.84, 141.24,145.36, 150.33, 151.15, 158.86, 163.73; ESI-MS (ES^+) m/z 641.366 (M+Na. $C_{33}H_{34}N_2O_{10}Na$ requires 641.211).

Stability of 2'-O-(1,3-dioxolan-2-yl)uridine (10). Compound **10** (16 mg, 0.05 mmol) and 1*H*-tetrazole (148 mg, 2.1 mmol) was dissolved in $CDCl_3$-DMSO-d_6 (5:1 v/v, 1.168 g) in an NMR tube. The progress of the reaction was monitored by ^1H NMR. The disappearance of the signal of the anomeric proton 5.93 ppm was observed during the reaction.

Synthesis of 2-ethoxy-1,3-dioxolane-4,5-dicarboxylic acid diethyl ester (4). To a solution of diethyl D- or L-tartarate (D-**8** or L-**8**) (9.8 g, 48 mmol) in toluene (50 ml) were added triethyl orthoformate (19.4 g, 0.131 mol) and camphorsulfonic acid (20 mg, 0.086 mmol). Triethyl orthoformate (19.4 g, 0.131 mol) and camphorsulfonic acid (20 mg, 0.086 mmol) was added to a solution of diethyl D- or L-tartarate (D-**8** or L-**8**) (9.8 g, 48 mmol) in toluene (50 ml). The mixture was stirred vigorously in a single-neck round-bottom flask equipped with a Dean-Stark receiver and a condenser. The solution was refluxed (95°C) until all ethanol had been azeotropically removed. The reaction progress was monitored by TLC ($CHCl_3$-CH_3OH, 9:1 v/v). The mixture was neutralized by addition of pyridine (1 ml) after cooling and evaporated under reduced pressure. Purification was carried out by flash column chromatography on a column of silica gel with $CHCl_3$ to give the product **4** as an oil (12.2 g, 95%); Compound D-**4**: δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.19-1.29 (t, 3H), 1.3-1.35 (m, 6H), 3.66-3.70 (m, 2H), 4.27-4.32 (m, 4H), 4.726-4.734 (d, 1H, *J* 4.15Hz), 5.05-5.06 (d, 1H, *J* 4.15 Hz), 6.09 (s, 1H). Compound L-**4**: δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.2-1.23 (t, 3H), 1.3-1.35 (m, 6H), 3.67-3.7 (m, 2H), 4.28-4.3 (m, 4H), 4.71-4.72 (d, 1H, *J* 4.40 Hz), 5.04-5.05 (d, 1H, *J* 4.40 Hz), 6.08 (s, 1H).

Synthesis of 2-ethoxy-1,3-dioxolane-4,5-dicarboxylic acid diamide (17). L-(*R,R*)-2-Ethoxy-1,3-dioxolane-4,5-dicarboxylic acid diethyl ester L-**4** (5.0 g, 19 mmol) was dissolved in CH_3OH (10 ml) and a 50% solution of ammonia (28% in water) in CH_3OH (20 ml) was added with stirring at room temperature overnight. The solvent and volatile substances were evaporated under reduced pressure. The product as a white crystal was obtained at a yield of 100%. R_f ($CHCl_3$-CH_3OH, 4:1 v/v) 0.30; δ_H (500 MHz; DMSO; Me_4Si) 1.10-1.13 (t, 3H), 3.59-3.65 (m, 2H), 4.43-4.44 (d, 1H, *J* 4.88 Hz), 4.63(d, 1H, *J* 4.88 Hz), 6.06 (s, 1H), 7.321 (s, 1H), 7.47-7.49 (d, 1H), 7.68 (s, 1H); δ_C (126 MHz; DMSO-d_6) 15.53, 60.43, 77.01, 77.87, 116.95, 171.49, 171.63; ESI-MS (ES^+) m/z 225.879 (M+Na. $C_7H_{12}N_2O_5Na$ requires 225.049).

Synthesis of 2'-O-[(4,5-di(ethoxycarbonyl)-1,3-dioxolan-onic acid (7). The mixture was stirred in a single-neck round-bottom flask equipped with a Dean-Stark receiver and a condenser. The solution was refluxed (105-115 °C) until all the ethanol had been azeotropically removed. The reaction was monitored by TLC ($CHCl_3$-CH_3OH, 9:1 v/v). The mixture was neutralized by addition of pyridine (1 ml) after cooling and evaporated under reduced pressure. The residue was partitioned between $CHCl_3$ (50 ml) and water (50 ml). The $CHCl_3$ solution was collected and the aqueous layer was further extracted with $CHCl_3$ (50 ml). The organic extracts were combined, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel in $CHCl_3$ to give the product D-**7** or L-**7**.

Compound D-7 (561 mg, 80%); R_f ($CHCl_3$-CH_3OH, 9:1 v/v) 0.71; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.28-1.34 (m, 6H), 3.94-4.06 (m, 2H), 4.21-4.29 (m, 4H), 4.45-4.46 (d, 1H, *J* 4.64Hz), 4.74-4.76 (d, 1H, *J* 5,37 Hz), 5.09-5.1 (d, 1H, *J* 5.13 Hz), 5.67-5.68 (d, 1H, *J* 7.79 Hz), 5.81 (s, 1H), 6.50 (s, 1H), 7.8-7.81 (d, 1H, *J* 8.06 Hz), 9.45 (s, 1H); ESI-MS (ES^+) m/z 725.397 (M+Na. $C_{30}H_{50}N_2O_{13}Si_2Na$ requires $C_{30}H_{50}N_2O_{13}Si_2Na$ requires 725.275).

Compound L-7 (562 mg, 80%); R_f ($CHCl_3$-CH_3OH, 9:1 v/v) 0.71; δ_H (500 MHz; $CDCl_3$; Me_4Si) 0.93-1.1 (m, 28H), 1.29-1.35 (m, 6H), 3.95-3.98 (m, 2H), 4.15-4.33 (m, 4H), 4.39-4.41 (d, 1H, *J* 3.35 Hz), 4.78-4.79 (d, 1H, *J* 5.13 Hz), 5.02-5.21 (d, 1H, *J* 5.13 Hz), 5.64 (s, 1H), 5.65-5.67 (d, 1H, *J* 8.06 Hz), 6.52 (s, 1H), 7.8-7.81 (d, 1H, *J* 8.30 Hz), 8.90 (s, 1H). ESI-MS (ES^+) m/z 725.400 (M+Na. $C_{30}H_{50}N_2O_{13}Si_2Na$ requires 725.275).

Synthesis of 2'-O-[4,5-di(ethoxycarbonyl)-1,3-dioxolan-2-yl]uridine (12). Tetrabutylamonium fluoride (0.5 mmol) was added to a solution of compound D-**7** or L-**7**, (1.0 mmol) in dry THF (10 ml). The mixture was stirred at room temperature for 60 min, and then the solvent was removed under reduced pressure. The residue was purified by column chromatography on a column of silica gel with $CHCl_3$-CH_3OH (20:1 to 20:2.5 v/v) containing 1% pyridine.

Compound D-12 (437 mg, 95%); R_f ($CHCl_3$-CH_3OH, 9:1 v/v) 0.21; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.30-1.35 (m, 6H), 3.0-3.04 (b, 2H), 3.82-3.97 (m, 2H), 4.06-4.08 (m, 1H), 4.26-4.31 (m, 4H), 4.45-4.47 (m, 1H), 4.73-4.74 (d, 1H, *J* 3.91 Hz), 4.75-4.78 (m, 1H), 4.97-4.98 (d, 1H, *J* 3.91 Hz), 5.7-5.72 (d, 1H, *J* 8.06 Hz), 5.85-5.86 (d, 1H, *J* 4.64 Hz), 6.32 (s, 1H), 7.69-7.71 (d, 1H, *J* 8.06 Hz), 8.61-8.63 (m, 1H); δ_C (12662-14.34, 17.02, 17.17-17.7, 59.6, 62.29, 62.36, 67.57, 75.74, 76.57, 76.97,

89.62, 101.79, 116.49, 140.13, 150.07, 164.15, 168.68, 168.93; ESI-MS (ES^+) m/z 483.229 (M+Na. $C_{18}H_{24}N_2O_{12}Na$ requires 483.122).

Compound L-12 (451 mg, 98%); R_f ($CHCl_3$-CH_3OH, 9:1 v/v) 0.21; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.29-1.34 (m, 6H), 3.25 (b, signals 2H), 3.7-3.75 (m, 2H), 4.02-4.02 (m, 1H), 4.24-4.29 (m, 4H), 4.3-4.45 (m, 1H), 4.799-4.808 (d, 1H, *J* 4.15 Hz), 5.041-5.049 (d, 1H, *J* 4.15 Hz), 5.60-5.62 (d, 1H, *J* 8.06 Hz), 6.01-6.02 (d, 1H, *J* 5.62 Hz), 6.27 (s, 1H), 7.98-7.99 (d, 1H, *J* 8.06 Hz); δ_C (16 168.16; ESI-MS (ES^+) m/z 483.235 (M+Na. $C_{18}H_{24}N_2O_{12}Na$ requires 483.122).

Synthesis of 5'-O-(4,4'-dimethoxytrityl)-2'-O-[4,5-di-(ethoxycarbonyl)-1,3-dioxolan-2-yl]uridine (13). 4,4'-dimethoxytrityl chloride (508 mg, 1.5 mmol) was added to a solution of compound D-**12** or L-**12** (1.0 mmol) in pyridine (35 ml). The mixture was stirred at room temperature for 2 h. The reaction was monitored by TLC ($CHCl_3$-CH_3OH, 9:1 v/v). The mixture was evaporated under reduced pressure to a 1/3 volume and the residue was taken up by CH_2Cl_2-H_2O (1:1 v/v, 100 ml). The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with $CHCl_3$ to give the product D-**13** or L-**13**.

Compound D-13 (717 mg, 94%); R_f ($CHCl_3$-CH_3OH, 9:1 v/v) 0.63; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.29-1.33 (m, 6H), 3.0 (b.d, 1H), 3.52-3.53 (m, 2H), 3.793-3.796 (d, 6H, *J* 1.47 Hz), 4.01-4.02 (m, 1H), 4.24-4.3 (m, 4H), 4.53-4.55 (m, 2H), 4.77-4.78 (d, 1H, *J* 4.15 Hz), 5.05-5.06 (d, 1H, *J* 4.40 Hz), 5.31-5.32 (d, 1H, *J* 9.77 Hz), 6.05 (d, 1H, *J* 1.47), 6.44 (s, 1H), 6.83-6.86 (d, 4H, 9.03 Hz), 7.23-7.31 (m, 8H), 3.38-3.74 (m, 2H), 7.92-7.93 (d, 1H, *J* 8.30), 9.08 (b, 1H); δ_C (126, 61.50, 62.43, 62.75, 68.53, 75.86, 76.52, 83.33, 86.99, 88.05, 102.05, 113.27, 113.29, 116.43, 123.75, 127.11, 127.99, 128.15, 130.11, 130.19, 135.13, 135.35, 136.01, 140.17, 144.39, 149.79, 150.05, 158.66, 158.66, 158.69, 163.26, 168.26, 169.38; ESI-MS (ES^+) m/z 785.46463 (M+Na. $C_{39}H_{42}N_2O_{14}Na$ requires 785.253).

Compound L-13 (732 mg, 96%); R_f ($CHCl_3$-CH_3OH, 9:1 v/v) 0.63; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.31-1.36 (m, 6H), 3.51-3.53 (m, 2H), 3.785-3.794 (d, 6 Hz, 4.64 Hz), 4.14-4.15 (t, 1H); 4.27-4.33 (m, 4H); 4.44-4.60 (m, 1H), 4.46-4.51 (m, 1H), 4.83-4.84 (d, 1H, *J* 3.91 Hz), 5.02-5.03 (d, 1H, 3.91 Hz), 5.29-5.31 (d, 1H, 8.30 Hz), 6.02-6.03 (d, 1H, *J* 3.42 Hz), 6.38 (s, 1H), 6.8-6.85 (m, 4H,), 7.19-7.45 (m, 10H), 7.88-7.89 (d, 1H, *J* 8.06 Hz), 8.85 (s, 1H); □C(126 170.33; ESI-MS (ES^+) m/z 785.459 (M+Na. $C_{39}H_{42}N_2O_{14}Na$ requires 785.253).

Synthesis of 5'-O-(4,4'-dimethoxytrityl)-2'-O-[4,5-di-(ethoxycarbonyl)-1,3-dioxolan-2-yl]uridine-3'-O-(2-cyanoethyl-N,N-diisopropyl)phosphoramidite (14). Ethyldiisopropylamine (125 mg, 1.1 mmol) was added to a solution of L-13 (760 mg, 1.0 mmol) in CH_2Cl_2 (10 ml). The mixture was stirred at room temperature for 5 min and then chloro(2-cyanoethoxy)-diisopropylaminophosphine (0.28 g, 1.2 mmol) was added. After being stirred at room temperature overnight, the mixture was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with $CHCl_3$ containing 2% triethylamine to give compound 14 as a white foam (0.57 g, 60%); R_f ($CHCl_3$-CH_3OH, 9:1 v/v) 0.53; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.02-1.35 (d.m, 20H), 2.63-2.72 (m, 2H), 3.52-3.87 (m, 11H), 4.19-4.31 (m, 4H), 4.55-5.60 (m, 1H), 5.56-5.66 (m, 1H), 4.69-4.74 (dd, 1H, *J* 4.63 Hz, *J* 5.13 Hz), 5.00-5.04 (dd, 1H, *J* 4.64 Hz, *J* 5.13 Hz), 5.27-5.34 (dd, 1H, *J* 8.06, *J* 8.06 Hz), 6.02-6.03 (d.d, 1H, *J* 2.44 Hz), 6.27-6.34 (d.s, 1H), 6.83-6.86 (m, 4H), 7.24-7.75 (m, 8H), 7.28-7.42 (m, 2H), 7.77-7.81 (dd, 1H *J* 8.30); δ_C (126 MHz; $CDCl_3$) 13.11, 13.35, 14.30, 14.34, 17.35, 17.37, 20.53, 20.58, 23.09, 23.18, 24.7, 24.75, 24.81, 24.87, 43.38, 43.61, 45.50, 45.55, 55.44, 59.16, 59.29, 62.42, 62.48, 75.46, 75.9, 76.67, 76.97, 83.15, 83.19, 87.32, 87.56, 102.58, 113.48, 115.94, 118.28, 123.98, 127.36, 128.17, 128.17-128.54, 130.43, 130.48, 135.2, 135.41, 135.45, 136.29, 140.38, 144.36, 149.9, 150.61, 158.92, 163.69, 168.46, 168.59, δ_P (201 MHz; $CDCl_3$; 85% H_3PO_4) 152.157, 151.205; ESI-MS (ES^+) m/z 985.589 (M+Na. $C_{48}H_{59}N_4O_{15}PNa$ requires 985.361).

Synthesis of dinucleotide UpU in solution. (232 mg, 0.24 mmol), 2',3'-*O*-isopropylideneuridine (76 mg, 0.28 mmol) and 1*H*-tetrazole (40 mg, 0.57 mmol) were dried together in vacuum at 40 °C for 6 h. This mixture was dissolved in dry CH_2Cl_2 (5 ml). The reaction was monitored by ^{31}P NMR ($CDCl_3$). At the beginning of the reaction, the ^{31}P signals (500 MHz; $CDCl_3$; 85% H_3PO_4) of **14** were observed at 152.16 and 151.21 ppm in the ratio of 65:35. After 10 min these peaks disappeared and two other signals at 140.29 and 140.04 ppm derived from the tervalent phosphorus (III) intermediate appeared in the ratio 49:51. These P^{III} derivatives were oxydized by a 1% solution of I_2 in H_2O-pyridine (1:9 v/v, 2 ml). After 5 min, the mixture was quenched by the addition of sat. $Na_2S_2O_3$ (3 ml). The P^V species were formed as shown by ^{31}P NMR which showed the signals derived from **15** were observed at -1.56 and -1.92 ppm at the ratio of 50:50. The diasteromeric product **15** was isolated from the reaction mixture by extraction from the aqueous suspension by CH_2Cl_2 (25 ml). The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel. Compound **15** thus obtained was dissolved in methanol and 28% ammonia (1:1 v/v, 10 ml) for 2 h. Then the mixture was evaporated under reduced pressure. The ^{31}P NMR spectrum of the residue involving **16** showed a signal at 0.067 ppm. After the solvent was evaporated under reduced pressure, the residue was treated with 25% CD_3COOD in D_2O (2 ml). After the solution was evaporated under reduced pressure, the residue containing a mixture of UpU, UpUisp, U(dcbco)pU, and U(dcbdo)pUisp was analyzed by ^{31}P NMR (D_2O) that showed only a signal at -0.299 ppm and also analyzed by ESI mass spectrography.

Compound (15) δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.24-1.38 (m. 9H), 1.53-1.68 (s, 3H), 3.43.3.49 (m, 2H), 3.79 (s, 6H), 4.14-4.43 (m, 11H), 4.7-4.72 (t, 1H, *J* 4.64 Hz), 4.83-4.96 (m, 2H), 4.91-4.94 (d, 1H, *J* 4.88 Hz), 5.00-5.13 (m, 1H), 5.29-5.32 (d.d, 1H), 5.51-5.64 (dm, 1H), 5.60-5.62 (d, 1H, *J* 8.06), 5.69-5.70 (d, 1H, *J* 8.06), 6.08-6.12 (dd, 1H, *J* 6.10, 6.84), 6.26-6.32 (ds, 1H), 6.84-6.86 (d, 4H, *J* 8.79 Hz), 7.8-7.10 (d, 1H, 8.30 Hz), 7.20-7.37 (m, 9H), 7.54-7.62 (dd, 1H, *J* 8.30, 8.06), 9.03 (b, 1H), 9.27-9.37 (db. 1H); δ_C (126 MHz; $CDCl_3$) 14.27, 14.29, 19.65, 19.71, 25.42, 27.27, 55.48, 62.6, 62.57, 62.74, 62.88, 63.13, 63.18, 75.81, 75, 86, 76.69, 77.58, 80.97, 84.55, 85.85, 85.99, 86.44, 87.64, 94.8, 95.54, 102.88, 102.94, 103.00, 103.17, 113.56, 114.7, 114.73, 116.19, 116.35, 116.81, 117.21, 127.46, 128.27, 128.51, 130.42, 130.45, 130.49, 135.07, 135.193, 135.25, 140.293, 142.74, 144.24, 150.44, 150.48, 150.93, 151.17, 158.97, 163.53, 163.66, 163.8, 163.95, 168.2, 168.39, 168.491, 168.55; δ_P (201 MHz; $CDCl_3$; 85% H_3PO_4) -1.557, -1.924; ESI-MS (ES^+) m/z 1182.800 (M+Na. $C_{54}H_{60}N_5O_{22}PNa$ requires 1184.337).

Compound **(16)** δ_H (500 MHz; CD_3OD; Me_4Si) 1.28 (s, 3H), 1.52 (s, 3H), 3.51-3.55 (m, 2H), 3.78 (s, 6H), 3.86-4.06 (dm, 2H), 4.22-4.28 (m, 1H), 4.29-4.30 (m, 1H), 4.64-4.67 (m, 3H),

4.79-4.98 (m, 6H), 5.14-5.16 (d, 1H, *J* 8.06 Hz), 5.66-5.67 (d, 1H, *J* 8.06 Hz), 5.84-5.85 (d, 1H, *J* 2.93Hz), 5.98-5.99 (d, 1H, *J* 3.42 Hz), 6.52 (s, 1H), 6.87-6.89 (d, 4H, *J* 8.03), 7.23-7.33 (m, 8H), 7.41-7.42 (d, 2H), 7.66-7.71 (d, 1H, *J* 8.06 Hz); δ_C (126 MHz; CD_3OD) 24.37, 24.42, 26.38, 35.77, 47.33, 47.33, 54.61, 61.96, 65.51, 71.9, 76.76, 77.13, 78.52, 81.09, 81.23, 82.46, 82.52, 84.37, 84.49, 85.42, 85.35, 85.41, 87.33, 87.99, 92.23, 92.53, 101.53, 101.87, 101.97, 113.15, 113.91, 114.06, 116.74, 117.04, 127.04, 127.86, 128.51, 130.41, 130.47, 135.269, 135.41, 140.76, 142.28, 142.39, 144.55, 150.89, 150.97, 159.15, 159.18, 164.76,164.95, 172.46; 173.13; δ_P (201 MHz; CD_3OD; 85% H_3PO_4) 0.067; ESI-MS (ES^+) m/z 1051.600 (M+H. $C_{47}H_{52}N_6O_{20}P$ requires 1051.297).

UpU: ESI-MS (ES^-) m/z 549.087 (M-H). $C_{18}H_{22}N_4O_{14}P$ requires 549.088.

UpUisp: ESI-MS (ES^-) m/z 589.118 (M-H). $C_{21}H_{26}N_4O_{14}P$ requires 589.119.

U(dcbco)pU: ESI-MS (ES^-) m/z 707.472 (M-H). $C_{23}H_{28}N_6O_{18}P$ requires 707.120.

U(dcbdo)pUisp: ESI-MS (ES^-) m/z 747.536 (M-H). $C_{26}H_{32}N_6O_{18}P$ requires 747.152.

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