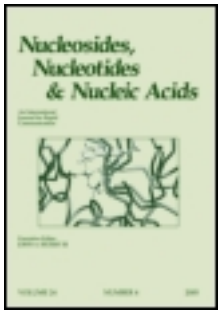

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A Facile Synthesis of the Phosphoramidites of 2-N-Methyl-2′-deoxy (or 2′-O-allyl)-ψ-isocytidine, 1, 3-Dimethyl-2′-deoxy-ψ-uridine and N1-Methyl-2′-O-allyl-ψ-uridine as Synthons Suitable for Oligonucleotide Synthesis

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A FACILE SYNTHESIS OF THE PHOSPHORAMIDITES OF 2-*N*-METHYL-2'-DEOXY (or 2'-O-ALLYL)-ψ-ISOCYTIDINE, 1,3-DIMETHYL-2'-DEOXY-ψ-URIDINE AND *N*1-METHYL-2'-O-ALLYL-ψ-URIDINE AS SYNTHONS SUITABLE FOR OLIGONUCLEOTIDE SYNTHESIS

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ABSTRACT: An efficient and facile syntheses of 5'-O-(4,4'-dimethoxytrityl)-3'-[2-cyanoethyl bis(1-methylethyl)]phosphoramidites of 2-*N*-methyl-2'-deoxy-ψ-isocytidine (**6**), 2-*N*-methyl-2'-deoxy-α-ψ-isocytidine (**13**), 2-*N*-methyl-2'-O-allyl-ψ-isocytidine (**11**), 1,3-dimethyl-2'-deoxy-ψ-uridine (**4**) and *N*1-methyl-2'-O-allyl-ψ-uridine (**19**) have been accomplished in good overall yields. The pyrimidine-pyrimidine transformation reaction was found to be useful for the preparation of 2-*N*-methyl-2'-O-allyl-ψ-isocytidine (**10**). The utility of these novel phosphoramidites is demonstrated by their incorporation into oligonucleotides *via* solid-support, oligonucleotide methodology.

Introduction: Soon after the discovery of the *double helical* structure of DNA,[1] the existence of *triple helical* DNA was recognized.[2,3] Subsequently, it was shown[4-10] that under suitable conditions, short oligonucleotides will bind in a sequence-specific manner to a duplex target and form a local three stranded structure, or triplex. Since triplex forming oligonucleotides (TFOs) bind to duplex DNA in the major groove, they have the potential to interfere with the binding of various proteins. Formation of a triplex at such a site would block access of the protein to the DNA, thus preventing binding.[11-13] Gene expression is known to be regulated by the actions of a variety of proteins, many of which act by binding to DNA sequences. It has been well documented that expression of certain genes is critical for the progression of many diseases, especially viral and malignant diseases. The ability to design an oligonucleotide that would bind

to a specific sequence and shut off (or turn on) a particular gene could have enormous benefit for the treatment of such diseases.

Triplex formation occurs when TFOs wrap into a groove of the duplex, forming specific hydrogen bonded base triplets. Two major triplet motifs are known. In one, T (thymidine) in the third strand binds to A (2'-deoxyadenosine) in the duplex, and protonated 2'-deoxycytidine (C^+) in the third strand binds to G (2'-deoxyguanosine) in the duplex.[14-17] A major drawback of this scheme is that protonation of third strand C, which is required for binding to G in the duplex, optimally requires a pH 5-6 which is well below physiological range. The second scheme involves T in the third strand binding to A in the duplex, and G in the third strand binding to G in the duplex (known as G/T motif). It has been shown that under appropriate conditions, TFOs utilizing a G/T motif can bind with high affinity (apparent $K_d \leq 1$ nM), with high sequence selectivity of G and T residues, and are often biased in favor of G.

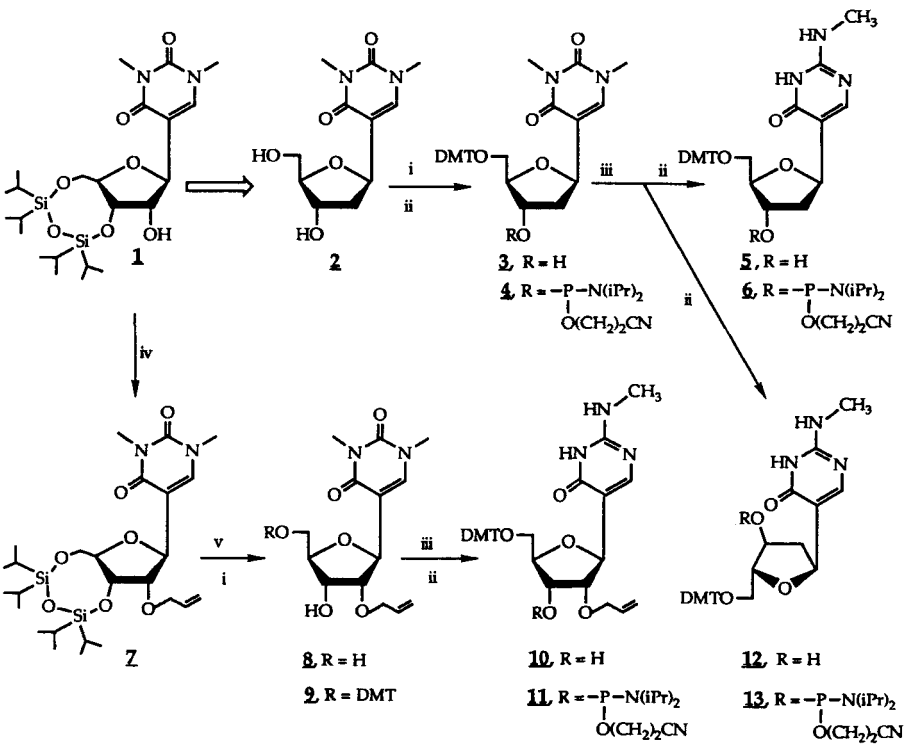
Since protonation of cytosine (C) bases is essential in order to provide the second hydrogen bonding between *N*3 of cytosine to *N*7 of guanine to form a Hoogstein-like base pair in the triad, this C^+-G-C triad is stable in acidic conditions but is not stable in neutral or physiological conditions.[18-20] This requirement prevents the formation of triplex in living cells. To overcome these limitations, Ono et al.[21,22] recently incorporated 2'-O-methyl-ψ-isocytidine in substitution for 2'-deoxycytidine into oligonucleotides and found that these oligonucleotides formed stable triple helices at physiological pH. Furthermore, it has been found recently that poly 5-methyl-2'-deoxycytidine and poly 2'-deoxyguanosine does not form triple helices at neutral condition which suggests that modified nucleosides having extra *N*3 or *N*1 hydrogen should be explored in order to allow for triplex formation at physiological pH. Based on these facts, the incorporation of suitably protected modified nucleosides such as ψ-uridine and ψ-isocytidine into TFOs is of particular interest, since these congeners have an extra hydrogen at the *N*3 (or *N*1) position available for hydrogen bonding with *N*7 of G at physiological pH.

The 2'-O-methyloligonucleotides have emerged recently as a novel nucleic acids probe[23-25] and have shown that these modified oligonucleotides have important applications in studying RNA processing.[26-29] Among the various 2'-O-alkyl substituents, 2'-O-allyl derivatives have been found to be superior to 2'-O-methyl or 2'-O-dimethylallyl analogs.[29] Consequently, modified 2'-O-allyl analogs might be an interesting monomeric building block for oligonucleotide

synthesis. As a part of our ongoing program to incorporate modified nucleosides into TFOs, here we report the efficient synthesis of suitably protected phosphoramidites of 2-*N*-methyl-2'-deoxy-ψ-isocytidine (**6**), 2-*N*-methyl-2'-O-allyl-ψ-isocytidine (**11**), *N*1-methyl-2'-O-allyl-ψ-uridine (**19**) and their incorporation into oligonucleotides *via* solid-support, phosphoramidite method.

Chemistry: Literature survey reveals that the incorporation of ψ-uridine or ψ-isocytidine into oligonucleotides was done *via* *H*-phosphonate method.[30] Recently, Ono et al.[21,22] reported the incorporation of 2'-O-methyl-ψ-isocytidine into oligonucleotides *via* phosphoramidite method. The automated synthesis of oligonucleotides by phosphoramidite method[31,32] is generally superior to *H*-phosphonate method. The phosphoramidites of suitably protected 2-*N*-methyl-2'-deoxy-ψ-isocytidine (**6**) and 2-*N*-methyl-2'-O-allyl-ψ-isocytidine (**11**) were synthesised by the route as shown in *Scheme 1*. The ψ-uridine was protected at *N*1 and *N*3 positions by methyl groups to yield *N*1,*N*3-dimethyl-ψ-uridine by treatment with DMF-dimethyl acetal.[33,34] The *N*1,*N*3-dimethyl-ψ-uridine was further protected at 5'- and 3'- positions with 1,1,3,3-tetraisopropyldisiloxane to give (**1**) which was converted into 1,3-dimethyl-2'-deoxy-ψ-uridine (**2**) in a multistep synthesis *via* a conventional procedure.[34]

The phosphoramidite of (**2**) was obtained by tritylation to yield the 5'-O-DMT derivative (**3**), followed by phosphitylation with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite. The synthesis of 2-*N*-methyl-2'-deoxy-ψ-isocytidine was accomplished *via* a pyrimidine-pyrimidine transformation reaction.[35] We developed an improved method by first introducing an acid labile DMT protecting group at 5'-OH of **2** to give (**3**), which upon heating under reflux in EtOH with methyl guanidine (generated *in situ*) gave a mixture of β/α isomers. Purification of the anomeric mixture by silica gel column chromatography yielded β-isomer (**5**) in 65% yield and the α-isomer (**12**) in 23% yield. However, our attempted pyrimidine-pyrimidine transformation[35] of free nucleoside **2** using excess of methyl guanidine (generated *in situ* from methyl guanidine sulfate and NaOEt) resulted in low yield of the desired β-isomer and also its separation from β/α mixture proved to be rather difficult. It seems to us that the substitution of a DMT group at 5'-position of **2**, improved the yield of the desired β-isomer (**5**) and also facilitated the separation of the β/α anomeric mixture of (**5**) and (**12**). The anomeric configuration of β-isomer (**5**) and α-isomer **12** were assigned by ^{1}H NMR studies. The anomeric proton of **5** resonates at δ

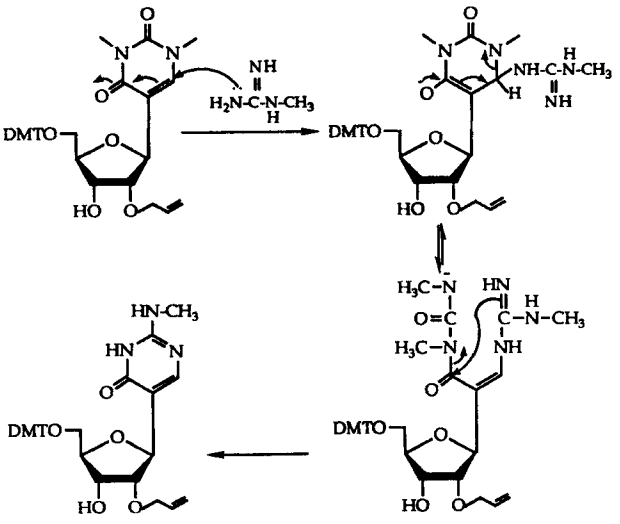


(i) DMT-Cl/Py, (ii) 2-Cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite/DEA/CH_2Cl_2, (iii) Me-guanidine HCl, (iv) TDBAD/BDPB/Allyl ethyl carbonate/THF, (v) *n*-Bu_4NF/THF

Scheme 1

4.81 ppm as a pseudo triplet (ψt= pseudo triplet , refers to a doublet of doublet that has the appearence of a triplet), while the anomeric proton of the α-isomer **12** resonates at δ 4.94 ppm as a quartet (resolved after D_2O exchange). This pyrimidine-pyrimidine transformation reaction of 5'-ODMT derivative **3** seems to be an ideal condition for the synthesis of 2-*N*-methyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-ψ-isocytidine (**5**). The phosphitylation of **5** and **12** with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite afforded the corresponding phosphoramidites **6** and **13** , respectively.

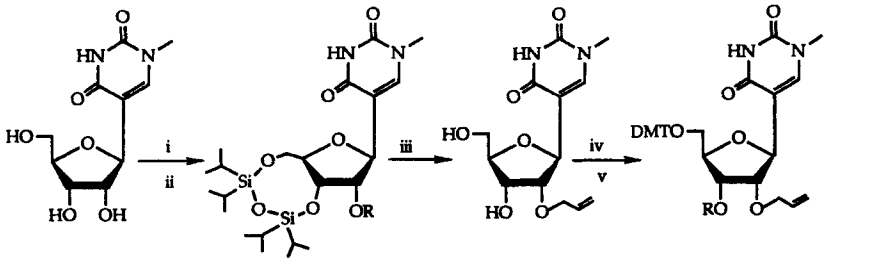
The suitably protected 2'-O-allyl monomeric building block (**11**) was prepared starting from (**1**). Allylation[26] of **1** with allyl ethyl carbonate in the presence of catalysts tris(dibenzylideneacetone)dipalladium(O) (TDBAD) and 2,4-bis(diphenylphosphino)butane (BDPB) in dry THF gave the corresponding 2'-



Scheme 2

O-allyl derivative (7). Our attempted allylation of **1** with allyl bromide in the presence of dibutyltinoxide(DMTO)-tetrabutylammonium bromide (TBAB)[36] resulted in an intractable reaction mixture from which the isolation of the desired 7 was rather difficult.

The 2'-O-allyl phosphoramidite monomer (**11**) was synthesized from 7 by first desilylation with *n*-Bu4NF to obtain the 1,3-dimethyl-2'-O-allyl-ψ-uridine (**8**). Tritylation of **8** with 4,4'-dimethoxytrityl chloride in pyridine gave the 5'-O-trityl derivative (**9**). Treatment of **9** with a large excess of free methyl guanidine afforded 2-*N*-methyl-2'-O-allyl-5'-O-(4,4'-dimethoxytrityl)-ψ-isocytidine (**10**) in a 72% yield, along with a minor amount of a second product presumed to be the α-isomer, which was not isolated. This reaction condition for allylation seems to be ideal and well suited for the synthesis of (**10**) and proceeds *via* pyrimidine-pyrimidine transformation reaction. A plausible reaction mechanism has been shown in *Scheme 2*. This mechanism is similar to that proposed by Oostveen et al.[37] for the conversion of 1-methylpyridinium iodide into 2-substituted pyrimidine with an amidine nucleophile. The 1,3-ambient nucleophile attacks at C6-position of 2'-O-allyl derivative **9** due to anion formation in basic media and opens the ring, which then ring-annulates to form 2-*N*-methyl-2'-O-allyl-5'-O-(4,4'-dimethoxytrityl)-ψ-isocytidine (**10**). This type of reaction mechanism has



(i) TIPS-Cl/Py, (ii) TDBAD/BDPB/Allyl ethyl carbonate/THF, (iii) *n*-Bu_4NF/THF, (iv) DMT-Cl/Py, (v) 2-Cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite/DEA/CH_2Cl_2

Scheme 3

also been proposed earlier by Fox et al.[35] in the case of the transformation of 1,3-dimethyl-ψ-uridine to ψ-isocytidine. The phosphoramidite **11** was obtained by phosphitylation of **10** with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite under the standard condition.

The phosphoramidite (**19**) was obtained from ψ-uridine by a multistep synthesis (*Scheme 3*). The *N*1-methyl-ψ-uridine (**14**) was prepared as reported.[38,39] ψ-Uridine was acetylated using acetic anhydride in dry DMF in the presence of 4-dimethylaminopyridine (DMAP) at -25 °C to give 2,3,5-tri-O-acetyl-ψ-uridine, which on selective methylation at *N*1 position with BSA and iodomethane, followed by deacetylation with NH3/MeOH gave *N*1-methyl-ψ-uridine[39] (**14**). Nucleoside **14** was first protected at 5'- and 3'- positions with 1,1,3,3-tetraisopropyldisiloxane to give (**15**), which was then allylated with allyl ethyl carbonate in the presence of TDBAD and BDPB to give *N*1-methyl-2'-O-allyl-3',5'-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-ψ-uridine (**16**).

Desilylation of **16** with *n*-Bu4NF afforded *N*1-methyl-2'-O-allyl-ψ-uridine (**17**). The tritylation of **17** with 1.3 molar equivalent of DMT-Cl in pyridine in the presence of Et3N gave the 5'-O-DMT derivative (**18**) in a 65% yield. It was observerd that the tritylation of **17** in the absence of Et3N gave a very poor yield (~5%) of **18**. The conventional phosphitylation of **18** gave *N*1-methyl-2'-O-allyl-5'-O-(4,4'-dimethoxytrityl)-ψ-uridine-3'-[2-cyanoethyl bis(1-methylethyl)]phosphoramidite (**19**).

TABLE 1.

Oligonucleotide sequence	Stepwise trityl yield (%)	Overall yield (%)
1. 5'-**TTXTTXTT**-3'	90.1	45.3
2. 5'-**XXXXXXXX**-3'	95.2	60.1
3. 5'-**TTYTTYTT**-3'	90.7	48.2
4. 5'-**YYYYYYYY**-3'	95.4	63.9
5. 5'-**TTZTTZTT**-3'	90.2	48.5

T = Thymidine
X= 2-*N*-methyl-2'-deoxy-ψ-isocytidine
Y = 2-*N*-methyl-2'-deoxy-α-ψ-isocytidine
Z = 2-*N*-methyl-2'-O-allyl-ψ-isocytidine

To demonstrate the utility of these modified phosphoramidites we successfully incorporated them into oligonucleotides *via* solid-support, phosphoramidite method. The oligonucleotide synthesis was done on 1 μM scale. The concentration of phosphoramidite used was 0.1 μM and coupling time was increased to 900 seconds. The coupling efficiency of modified bases during the synthesis of oligonucleotides was measured by UV spectrometric quantitation of released dimethoxytrityl cation at 498 nm on each synthesis cycle. Purification of the crude oligonucleotides was done by HPLC using ion exchange Q-Sepharose (Pharmacia) column.[40] and the purified product was desalted by passage through a C_{18} Sep-Pak (Waters) cartridge. The HPLC purification gave about 85% pure modified oligos which was then purified by polyacrylamide gel and the modified oligonucleotides were found to be >95% pure. These modified oligonucleotides were analyzed on a 20% denaturing polyacrylamide gel after labeling with ^{32}P-ATP using polynucleotide kinase.[41] Unmodified oligonucleotide was used as the standard for comparison of mobility and purity. The stepwise coupling yields and overall yields of some of the oligonucleotides (1-8 mer) synthesised during this study are listed in *TABLE* 1.

In summary, we have synthesized a series of suitably protected phosphoramidites of ψ-isocytidine and incorporated into oligonucleotides using the solid-support, phosphoramidite chemistry.

EXPERIMENTAL

Melting points (uncorrected) were determined in a Thomas-Hoover capillary melting-point apparatus. Elemental analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. The presence of water as indicated by elemental analysis was verified by ^{1}H NMR spectroscopy. Thin layer chromatography (TLC) was performed on aluminum plates coated (0.2 mm) with silica gel 60F_{254} (EM Science). Silica gel (EM Science, 230-400 mesh) was used for flash column chromatography. All solvents used were reagent grade and the solvent mixtures are in volumes. Detection of nucleoside components on TLC was by uv light, and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were conducted under diminished pressure with the bath temperature below 30 °C. Infrared (IR) spectra were recorded in KBr with a Perkin-Elmer 1420 IR spectrophotometer and ultraviolet spectra (UV) were recorded on a Hewlett-Packard 8452 diode array spectrophotometer. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 400 MHz with an Brüker AM400 wide bore NMR spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as the internal standard. Polyphosphoric acid was used as an external standard for ^{31}P NMR spectra (key: s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet, ψ-t = pseudo triplet, refers to a doublet of doublet that has the appearance of a triplet, br = broad). Pseudo (ψ)-uridine was purchased from Kyowa Hakko USA, Inc., New York. The oligonucleotides were synthesized on ABI DNA Synthesizer (Model 380B or 394) using phosphoramide method.

1,3-Dimethyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-ψ-uridine (3). 1,3-Dimethyl-2'-deoxy-ψ-uridine[34] (**2**, 5.75 g , 22.43 mmol) was co-evaporated with pyridine (2 x 30 mL) and then dissolved in pyridine (250 mL). To this solution 4,4'-dimethoxytrityl chloride (9.09 g, 26.83 mmol) was added and the reaction mixture was stirred at room temperature for 2 h with the exclusion of moisture. The mixture was evaporated and the residue was co-evaporated with toluene (3 x 25 mL) to remove last traces of pyridine. The residue was dissolved in CH_2Cl_2 (150 mL), washed with a 15% aqueous solution of $NaHCO_3$, dried (Na_2SO_4) and evaporated to dryness. The residue was purified by flash silica gel column chromatography using CH_2Cl_2:MeOH (98:2) as the eluent to give 9.45 g (75%) of **3**; mp 105 °C; IR ν_{max} 1660 and 1700 (C=O) and 3350 (OH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.98 (m, 1 H, $C_2'H$), 2.15 (m, 1 H, $C_2''H$), 3.12 (m, 2 H, $C_5'H_2$), 3.17 (s, 3 H, CH_3), 3.18 (s, 3 H, CH_3) , 3.73 (s, 6 H, 2 OCH_3), 4.02 (m, 1 H, $C_4'H$), 4.17 (q,

1 H, $C_3'H$), 4.92 (dd, 1 H, $J_{1',2'}$ = 6.2 Hz, $J_{1',2''}$ = 6.16 Hz, $C_1'H$), 5.04 (d, 1 H, J = 4.2 Hz, $C_3'OH$), 6.87-7.41 (m, 13 H, DMT) and 7.47 (s, 1 H, C_6H). *Anal.* Calcd. for $C_{32}H_{34}N_2O_7$: C, 68.80; H, 6.13; N, 5.01. Found: C, 68.70; H, 6.16; N, 4.85.

1,3-Dimethyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-ψ-uridine-3'-[2-cyanoethyl bis(1-methylethyl)]phosphoramidite (4). Compound 3 (1.0 g, 1.79 mmol) was co-evaporated with dry toluene (2 x 20 mL) and dissolved in a mixture of dry CH_2Cl_2 (5 mL) and dry THF (5 mL). To this mixture, *N,N*-diisopropylethylamine (1.04 g, 8.05 mmol) and 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (1.06 g, 4.47 mmol) were added and stirred at ambient temperature for 45 min under nitrogen . The mixture was diluted with CH_2Cl_2 (25 mL) and washed with a 15% solution of $NaHCO_3$ (60 mL), dried (Na_2SO_4) and evaporated to dryness. The residue was dissolved in a mixture of 2% triethylamine in CH_2Cl_2 (2 mL) and loaded on the top of a pre-packed silica gel column in CH_2Cl_2:hexane:Et_3N (10:88:2) and the column was eluted with CH_2Cl_2:EtOAc:Et_3N (90:8:2). The appropriate homogeneous fractions were collected, evaporated and co-evaporated with CH_2Cl_2:hexane (2 x 10 mL) to give 1.12 g (82.5 %) of 4 as a white foam. ^1H NMR (CD_3CN) δ 1.20 {m, 12 H, $N[CH(CH_3)_2]_2$}, 1.89 (m, 2 H, $NCCH_2$), 1.91 (m, 1 H, $C_2'H$), 2.52 (m, 1 H, $C_2''H$), 3.16 (s, 3 H, CH_3), 3.21 (s, 3 H, CH_3), 3.27 (m, 2 H, OCH_2), 3.58 (m, 2 H, $C_5'H_2$), 3.62 (m, 2 H, 2 $CH(CH_3)_2$), 3.75 (s, 6 H, 2 OCH_3), 4.02 (q, 1 H, $C_4'H$), 4.49 (m, 1 H, $C_3'H$), 4.98 (t, 1 H, $C_1'H$), 6.83-7.46 (m, 14 H, DMT and C_6H); ^{31}P NMR (CD_3CN) δ 148.77 and 148 89. *Anal.* Calcd. for $C_{41}H_{51}N_4O_8P$: C, 64.88; H, 6.77; N, 7.38; P, 4.08. Found: C, 64.49; H, 7.09; N, 7.37; P, 4.19.

2-*N*-Methyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-ψ-isocytidine (5) and 2-*N*-Methyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-α-ψ-isocytidine (12). A solution of methyl guanidine hydrochloride (100.0 g, 912 mmol) in freshly prepared NaOEt in EtOH (12.33 g of Na in 450 mL of EtOH) was stirred at ambient temperature for 20 min and the precipitated NaCl was removed by filteration. The filtrate was concentrated and the residual syrup was dissolved in absolute EtOH (150 mL). Compound 3 (3.1 g, 5.54 mmol) was added and the mixture was heated under reflux for 16 h. The reaction mixture was evaporated to dryness and the residue was dissolved in water (500 mL). The pH of the aqueous solution was maintained at 8.5 by the addition of AcOH and the solution was extracted with EtOAc (450 mL). The organic layer was dried (Na_2SO_4) and evaporated to dryness. The crude product was chromatographed on a flash silica gel column using successively CH_2Cl_2:EtOAc (8:2, 7:3, 6:4) as the eluent. The homogeneous

fractions having a R_f of 0.54 in CH_2Cl_2:MeOH (9:1) were collected and evaporated to give 1.95 g (65 %) of **5**, mp 144-146 °C ; IR ν_{max} 1670 (C=O), 2200 ($NHCH_3$) and 2950-3300 (OH, NH) cm^{-1}; UV λ_{max} nm ($\varepsilon \times 10^{-3}$): (pH 1) 234 (20.5), 264 (8.2); (pH 7) 234 (20.0), 292 (7.2); (pH 11) 258 (5.1); ^1H NMR (DMSO-d_6) δ 1.99 (m, 1 H, $C_2'H$), 2.38 (m, 1 H, $C_2''H$), 2.79 (s, 3 H, CH_3), 3.05 (m, 2 H, $C_5'H_2$), 3.75 (s, 6 H, 2 OCH_3), 3.96 (q, 1 H, $C_4'H$), 4.10 (q, 1 H, $C_3'H$), 4.81 (t, 1 H, $J_{1',2'}$ = 7.32 Hz, $J_{1',2''}$ = 7.36 Hz, $C_1'H$), 5.23 (d, 1 H, $C_3'OH$), 6.33 (br s, 1 H, $NHCH_3$), 6.87-7.49 (m, 13 H, DMT), 7.69 (s, 1 H, C_6H) and 10.88 (br s, 1 H, NH). *Anal.* Calcd. for $C_{31}H_{33}N_3O_6$: C, 68.49; H, 6.11; N, 7.72. Found: C, 68.35; H, 6.28; N, 7.41.

The subsequent fractions with a R_f of 0.49 in CH_2Cl_2:MeOH (9:1) were pooled and the solvent was evaporated to give 0.71 g (23%) of **12**; mp 125-128 °C; IR ν_{max} 1665 (C=O), 2150 ($NHCH_3$) and 2900-3300 (OH, NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.82 (m, 1 H, $C_2'H$), 2.13 (m, 1 H, $C_2''H$), 2.78 (s, 3 H, CH_3), 3.09 (m, 2 H, $C_5'H_2$); 3.74 (s, 6 H, 2 OCH_3), 3.84 (m, 1 H, $C_4'H$), 4.09 (d, 1 H, $C_3'H$), 4.94 (m, 2 H, $C_1'H$ and $C_2'OH$, after D_2O exchange gave a quartet for $C_1'H$, $J_{1',2'}$ = 5.8 Hz and $J_{1',2''}$ = 5.42 Hz), 6.47 (br s, 1 H, $NHCH_3$), 6.87-7.42 (m, 13 H, DMT), 7.66 (s, 1 H, C_6H) and 10.81 (br s, 1 H, NH). *Anal.* Calcd. for $C_{31}H_{33}N_3O_6 \cdot 0.5\ H_2O$: C, 67.37; H, 6.19; N, 7.60. Found: C, 67.50; H, 6.38; N, 7.46.

2-*N*-Methyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-ψ-isocytidine-3'-[2-cyanoethyl bis(1-methylethyl)]phosphoramidite (6). Compound **5** (0.55 g, 1.04 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and the solution was stirred at ambient temperature under nitrogen for 10 min. To this solution, *N,N*-diisopropylethylamine (0.59 g, 1.04 mmol) and 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.613 g, 4.74 mmol) were added and the stirring was continued for an additional 45 min. The reaction mixture was diluted with CH_2Cl_2 (35 mL). The organic layer was washed with a 15% solution of $NaHCO_3$ (15 ml), dried (Na_2SO_4) and evaporated to dryness. The residue was dissolved in a mixture of 2% triethylamine in CH_2Cl_2 (2 mL) and the solution was loaded on the top of a silica gel column pre-packed in CH_2Cl_2:hexane:Et_3N (65:35:2). The column was eluted with CH_2Cl_2:EtOAc:Et_3N (90:8:2). The appropriate homogeneous fractions were collected and evaporated to dryness. The residual syrup was dissolved in a mixture of CH_2Cl_2:hexane (2:8, 15 mL) and evaporated to dryness to give 0.27 g (86%) of **6** as a white foam. ^1H NMR (CD_3CN) δ 1.06–1.26 {m, 12 H, $N[CH(CH_3)_2]_2$}, 2.08 (m, 2 H, $C_2'H$), 2.45 (m, 1 H, $C_2''H$), 2.62 (m, 2 H, $C_5'H_2$), 2.87 {m, 4 H, CH_2CN and $[N(CHCH_3)_2]$}, 3.17 (s, 3 H, CH_3), 3.25 (m, 2 H, OCH_2),

3.75 (s, 6 H, 2 OCH_3), 4.28 (m, 1 H, $C_4'H$), 4.45 (m, 1 H, $C_3'H$), 5.08 (t, 1 H, $C_1'H$), 6.89-7.51 (m, 13 H, DMT) and 7.71 (s, 1 H, C_6H); ^{31}P NMR (CD_3CN) δ 148.89 and 149.06. *Anal.* Calcd. for $C_{40}H_{50}N_5O_7P \cdot 1.5\ H_2O$: C, 62.31; H, 6.92; N, 9.09; P, 4.02. Found: C, 62.58; H, 7.21; N, 9.40; P, 4.18.

2-*N*-Methyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxy-α-ψ-isocytidine-3'-[2-cyanoethyl bis(1-methylethyl]phosphoramidite (13). In a similar manner as described for **4**, phosphitylation of **12** (0.75 g, 1.38 mmol) with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.72 g, 5.52 mmol) in the presence of *N,N*-diisopropylethylamine (0.71 g, 5.52 mmol) was carried out in dry CH_2Cl_2 (10 mL). Purification of the product by silica gel column chromatography afforded 0.71 g (82%) of **13** as a white foam. ^1H NMR (CD_3CN) δ 1.11-1.26 {m, 12 H, $N[CH(CH_3)_2]_2$}, 2.02 (m, 1 H, $C_2'H$), 2.41 (m, 1 H, $C_2''H$), 2.64 (m, 2 H, $C_5'H_2$), 2.82 {m, 4 H, CH_2CN and $[N(CHCH_3)_2]$}, 3.18 (s, 3 H, CH_3), 3.28 (m, 2 H, OCH_2), 3.80 (s, 6 H, 2 OCH_3), 4.11 (m, 1 H, $C_4'H$), 4.48 (m, 1 H, $C_3'H$), 5.06 (q, 1 H, $C_1'H$), 6.92-7.62 (m, 13 H, DMT) and 7.71 (s, 1 H, C_6H). ^{31}P NMR (CD_3CN) δ 148.0 and 148.91. *Anal.* Calcd. for $C_{40}H_{50}N_5O_7P \cdot 1.5\ H_2O$: C, 62.31; H, 6.92; N, 9.09; P, 4.02: Found: C, 62.43; H, 7.27; N, 9.38; P, 4.35.

1,3-Dimethyl-2'-O-allyl-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-ψ-uridine (7). To a mixture of tris(dibenzylideneacetone)dipalladium(O) (0.10 g, 0.11 mmol), 1,4-bis(diphenylphosphino)butane (0.18 g, 0.42 mmol) and dry THF (20 mL) was added a solution of **1** (2.0 g, 3.88 mmol) and allyl ethyl carbonate (1.01 g, 7.76 mmol) in dry THF (40 mL) portionwise over a period of 15 min. The mixture was heated under reflux for 4 h. After cooling the reaction mixture to room temperature it was filtered and the filtrate was concentrated. The residue was dissolved in CH_2Cl_2 (60 mL), washed with water (100 mL), dried (Na_2SO_4) and evaporated to dryness. The residual syrup was dissolved in CH_2Cl_2 (2 mL) and loaded on the top of a pre-packed (in CH_2Cl_2) silica gel column. The column was eluted with CH_2Cl_2:EtOAc (10:1) and the appropriate homogeneous fractions were pooled and evaporated to afford 1.78 g (83%) of **7**. Analytical sample was obtained by crystallization of the material from CH_3OH/hexane mixture; mp 105-108 °C, IR ν_{max} 1660 and 1720 (C=O), 2860 ($CH=CH_2$) and 2940 (NCH_3), cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.05 (m, 28 H, 4 i-Pr), 3.17 (s, 3 H, CH_3), 3.28 (s, 3 H, CH_3), 3.81 (d, 1 H, J = 4.44 Hz, $C_5'H$), 3.90 (d, 1 H, J = 15 Hz, $C_5''H$), 4.18 (d, 1 H, J = 12.24 Hz, $C_4'H$), 4.22 (m, 2 H, $C_2'H$ and $C_3'H$), 4.35 (dd, 2 H, J = 4.92 Hz and 8.76 Hz, OCH_2), 4.66 (s, 1 H, $C_1'H$), 5.13 [dd, 1 H, J

= 1.5 Hz and J = 11.1 Hz) and 5.35 (dd, 1 H, J = 1.7 Hz and J = 12.5 Hz), =CH_2], 5.94 (m, 1 H, =C*H*) and 7.45 (s, 1 H, C_6H); ^{13}C NMR (DMSO-d_6) δ 26.79 (NCH_3), 36.09 (NCH_3), 60.08 ($C_{5'}$), 70.02 (OCH_2), 78.65 ($C_{3'}$), 79.31 ($C_{2'}$), 80.81 ($C_{4'}$), 110.42 ($C_{1'}$), 115.17 (=CH_2), 135.10 (=CH) and 140.35 (C_6). *Anal.* Calcd. for $C_{26}H_{46}N_2O_7Si_2$: C, 56.28; H, 8.36; N, 5.05. Found: C, 56.51; H, 8.47; N, 4.88.

1,3-Dimethyl-2'-O-allyl-ψ-uridine (8). To a solution of 7 (1.6 g, 2.88 m mol) in dry THF (25 mL) was added 1.0 *M* solution of *n*-Bu_4NF in THF (15 mL) at 0 °C (ice bath) over a period of 10 min and the temperature of the reaction mixture was allowed to raise to room temperature. After stirring for 1.5 h, the mixture was evaporated and the residual syrup was dissolved in a mixture of pyridine:MeOH:H_2O (3:1:1). To this mixture , Dowex 50xW resin (pyridinium form, 2.5 g) was added, stirred for 30 min and filtered. The filtrate was evaporated, and the residue was co-evaporated with toluene (3 x 20 mL) followed by EtOH (2 x 25 mL). The residue was purified on a silica gel column using CH_2Cl_2:EtOAc (6:4) as the eluent to give 0.79 g (87.2%) of 8; mp 96-98 °C ; IR ν_{max} 1660 and 1700 (C=O), 2860 (NCH_3), 2910 (CH=CH_2) and 3300-3500 (OH) cm^{-1}; UV λ_{max} nm (ε x 10^{-3}): (pH 1) 212 (7.7), 270 (8.2); (pH 7) 210 (9.8), 270 (9.0); (pH 11) 210 (14.2), 272 (7.2); ^{1}H NMR (DMSO-d_6) δ 3.16 (s, 3 H, CH_3), 3.30 (s, 3 H, CH_3), 3.77 (m, 3 H, $C_{4'}H$ and $C_{5'}H_2$), 4.01 (m, 2 H, OCH_2), 4.17 (m, 2 H, $C_{2'}H$ and $C_{3'}H$), 4.66 (d, 2 H, $C_{1'}H$ and $C_{3'}OH$, collapsed to a singlet after D_2O exchange), 4.78 (t, 1 H, $C_{5'}OH$), 5.12 [(dd, 1 H, J = 1.24 Hz and 12.1 Hz) and 5.31 (dd, 1 H, J = 3.7 Hz and 17.4 Hz), =CH_2], 5.93 (m, 1 H, =C*H*) and 7.81 (s, 1 H, C_6H). *Anal.* Calcd. for $C_{14}H_{20}N_2O_6$: C, 53.83; H, 6.45; N, 8.97. Found: C, 53.86; H, 6.64; N, 8.73.

1,3-Dimethyl-2'-O-allyl-5'-O-(4,4'-dimethoxytrityl)-ψ-uridine (9): Compound 8 (0.50 g, 1.60 mmol) was co-evaporated with dry pyridine (3 x 10 mL) and dissolved in anhydrous pyridine (15 mL). To this solution, 4,4'-dimethoxytrityl chloride (0.70 g, 2.14 mmol) was added and the mixture was stirred at ambient temperature for 2.5 h. The mixture was evaporated to dryness and co-evaporated with toluene (2 x 20 mL) to remove last traces of pyridine. The residue was dissolved in CH_2Cl_2 (60 mL), washed with saturated solution of $NaHCO_3$ (150 mL), dried (Na_2SO_4) and evaporated to dryness. The residue on purification by flash silica gel column chromatography using CH_2Cl_2:EtOAc (7:3) gave 0.88 g (89.7%) of 9; mp 120 °C; IR ν_{max} 1660 and 1700 (C=O), 2920 (CH=CH_2) and 3000-3200 (OH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.99 (s, 3 H, CH_3),

3.17 (s, 3 H, CH_3), 3.73 (s, 6 H, 2 OCH_3), 3.80 (m, 2 H, $C_{5'}H_2$), 3.93 (m, 1 H, $C_{4'}H$), 4.10 (m, 2 H, OCH_2), 4.24 (m, 2 H, $C_{2'}H$ and $C_{3'}H$), 4.70 (d, 1 H, $C_{3'}OH$), 4.74 (d, 1 H, J = 1.2 Hz, $C_{1'}H$), 5.13 [(dd, 1 H, J = 1.6 Hz and 10.4 Hz) and 5.33 (dd, 1 H, J = 1.8 Hz and 17.3 Hz), $=CH_2$], 5.95 (m, 1 H, =CH), 6.90-7.42 (m, 13 H, DMT) and 7.57 (s, 1 H, C_6H). *Anal.* Calcd. for $C_{35}H_{38}N_2O_8$: C, 68.38; H, 6.23; N, 4.55. Found: C, 68.71; H, 6.49; N, 4.31.

2-*N*-Methyl-2'-O-allyl-5'-O-(4,4'-dimethoxytrityl)-ψ-isocytidine (10). In a similar manner as described for **5**, to a freshly prepared solution of NaOEt (1.86 g of Na, 81.3 mmol in 100 mL of absolute EtOH) was added methyl guanidine hydrochloride (8.9 g, 81.3 mmol) and the mixture was stirred at room teperature for 20 min. The mixture was filtered, and the filtrate was evaporated to dryness. The residual syrup was dissolved in absolute EtOH (15 mL) and to the solution was added **9** (0.50 g, 0.81 mmol). The reaction mixture was gently heated under reflux for 20 h. After cooling to room temperature, water (150 mL) was added and the aqueous solution was neutralized with acetic acid at ice bath temperature. The mixture was evaporated to dryness and the residue was partitioned between water (100 mL) and EtOAc (80 mL). The organic layer was separated, dried (Na_2SO_4) and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (2 mL) and loaded on top of a silica gel column pre-packed in CH_2Cl_2. The column was eluted with CH_2Cl_2:MeOH (98:2) and two UV absorbing products were isolated. The homogeneous fractions with a R_f of 0.48 (CH_2Cl_2:MeOH; 15:1) were evaporated to give 0.35 g (71.8%) of **10** as a major product; mp 115-116 °C; IR ν_{max} 1665 (C=O), 2910 ($CH=CH_2$) and 3200-3300 (OH , NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.77 (s, 3 H, NCH_3), 3.20 (m, 2 H, $C_{5'}H_2$), 3.79 (m, 1 H, $C_{4'}H$), 3.93 (m, 2 H, OCH_2), 4.14 (m, 2 H, $C_{2'}H$ and $C_{3'}H$), 4.57 (d, 1 H, $C_{3'}OH$), 4.73 (d, 1 H, J = 3.4 Hz, $C_{1'}H$), 5.13 [(dd, 1 H, J = 1.2 Hz and 10.2 Hz) and 5.29 (dd, 1 H, J = 1.8 Hz and 15.6 Hz), $=CH_2$], 5.92 (m, 1 H, =CH), 6.37 (br s, 1 H, $NHCH_3$), 6.85-7.43 (m, 13 H, DMT), 7.68 (s, 1 H, C_6H) and 10.85 (br s, 1 H, NH). *Anal.* Calcd. for $C_{34}H_{37}N_3O_7$: C, 68.09; H, 6.22; N, 7.01. Found: C, 67.78; H, 6.33; N, 6.81.

The subsequent fractions with a R_f of 0.41 (CH_2Cl_2:MeOH, 15:1) were collected and evaporated. The product was presumed to be the α-isomer of **10** and obtained in about 10% yield. This product was found to be about 80% pure as judged by TLC and proved to be difficult to obtain an analytically pure sample.

2-*N*-Methyl-2'-O-allyl-5'-O-(4,4'-dimethoxytrityl)-ψ-isocytidine-3'-[2-cyanoethyl bis(1-methylethyl)]phosphoramidite (11). Compound **10** (0.25 g, 0.43 mmol) was dissolved in anhydrous CH_2Cl_2 (8 mL) and the solution was stirred under argon for 10 min. To this solution, *N,N*-diisopropylethylamine (0.22 g, 0.3 mmol) was added, followed by 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.26 g, 1.11 mmol), and the reaction mixture was stirred at ambient temperature for 1 h. The mixture was diluted with CH_2Cl_2 (50 mL) and was partitioned between 15% aqueous solution of $NaHCO_3$ (100 mL). The organic layer was separated, dried (Na_2SO_4) and evaporated to dryness . The residual syrup was dissolved in a mixture of CH_2Cl_2:Et_3N (95:5, 2 mL) and loaded on the top of a silica gel column pre-packed in a mixture of hexane:CH_2Cl_2:Et_3N (90:5:5). The column was eluted with CH_2Cl_2:EtOAc:Et_3N (75:20:5) and the appropriate homogeneous fractions were pooled and evaporated. The residue was dissolved in CH_2Cl_2 (1 mL) and added with stirring to hexane (-40 °C). The cloudy solution was evaporated to dryness to give 0.28 g (82.3%) of **11** as a white foam. ^{1}H NMR (CD_3CN) δ 1.25 {m, 12 H, N[CH(CH_3)$_2$]$_2$}, 1.94 {m, 2 H, N[C*H*(CH_3)$_2$]$_2$}, 2.98 (s, 3 H, N*HCH*$_3$), 3.25 (m, 2 H, O*CH*$_2$), 3.35 (m, 2 H, C$_{5'}$*H*$_2$), 3.55 (m, 2 H, *CH*$_2$CN), 3.75 (s, 6 H, 2 O*CH*$_3$), 4.07 (m, 2 H, C$_{3'}$*H* and C$_{4'}$*H*), 4.15 (m, 2 H, O*CH*$_2$ of *allyl*), 4.81 (m, 1 H, C$_{2'}$*H*), 5.12 (t, 1 H, C$_{1'}$*H*), 5.31 (dd, 2 H, =*CH*$_2$), 5.92 (m, 1 H, =*CH*), 6.82-7.47 (m, 13 H, DMT) and 7.72 (s, 1 H, C$_6$*H*). ^{31}P NMR (CD_3CN) δ 148.64 and 148.76. *Anal.* Calcd. for $C_{43}H_{54}N_5O_8P\cdot H_2O$: C, 63.13; H, 6.89; N, 8.56; P, 3.78. Found: C, 63.19; H, 7.19; N, 8.40; P, 3.51.

***N*1-Methyl-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-ψ-uridine (15):** *N*1-Methyl-ψ-uridine[39] (**14**, 2.0 g, 7.75 mmol) was co-evaporated with dry pyridine (3 x 25 mL) and dissolved in anhydrous pyridine (50 mL). To the cold (0-5 °C) solution was added 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (2.56 g, 8.13 mmol) and the mixture was stirred for 1 h at 0 °C and then for 4 h at ambient temperature under argon. The mixture was evaporated to dryness and the residue was co-evaporated with toluene (3 x 20 mL). The residue was dissolved in CH_2Cl_2 (85 mL) and washed with water (300 mL), followed by 5% solution of $NaHCO_3$. After drying (Na_2SO_4), the organic layer was evaporated to dryness. The residue was purified on a silica gel column using CH_2Cl_2:EtOAc (6:4) as the eluent to yield 3.65 g (94%) of **15**. IR ν_{max} 1660 and 1720 (C=O), 2835 (NCH_3) and 3250-3450 (OH and NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.05 (m, 28 H, 4 i-Pr), 3.19 (s, 3 H, N*CH*$_3$) 3.9 (m, 2 H, C$_{5'}$*H*$_2$), 4.02 (m, 2 H, C$_{3'}$*H* and C$_{4'}$*H*), 4.2 (m,

1 H, $C_{2'}H$), 4.5 (br s, 1 H, $C_{2'}OH$), 4.72 (d, 1 H, $J_{1',2'}$ = 4.32 Hz, $C_{1'}H$), 7.43 (s, 1 H, C_6H) and 11.14 (br s, 1 H, N*H*). *Anal.* Calcd. for $C_{22}H_{40}N_2O_7Si_2$: C, 52.77; H, 8.05; N, 5.60. Found: C, 52.82; H, 8.11; N, 5.50.

***N*1-Methyl-2'-O-allyl-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-ψ-uridine (16).** In a similar manner as described for 7, allylation of **15** (1.15 g, 2.3 mmol) with allyl ethyl carbonate (0.448 g, 3.44 mmol) in the presence of TDBA (0.10g, 0.11 mmol) and BDPB (0.14 g, 0.33 mmol) in THF (40 mL) gave 0.85 g (68.5%) of **16**. IR ν_{max} 1650 and 1710 (C=O), 2950 (CH=CH_2) and 3200-3500 (OH, NH) cm^{-1} ; ^1H NMR (DMSO-d_6) δ 1.05 (m, 28 H, 4 i-Pr), 3.28 (s, 3 H, NCH_3), 3.86 (m, 2 H, $C_{5'}H_2$), 3.97 (t, 1 H, $C_{4'}H$), 4.06 (dd, J = 2.32 Hz and J = 2.2 Hz, 1 H, $C_{3'}H$), 4.13 (m, 1 H, $C_{2'}H$), 4.45 (d, J = 5.2 Hz, 2 H, OCH_2), 4.55 (s, 1 H, $C_{1'}H$), 5.05 [(dd, 1 H, J = 1.32 Hz and J = 11.12 Hz) and 5.12 (dd, 1 H, J = 1.32 Hz and J = 14.26 Hz), = CH_2], 5.84 (m, 1 H, =C*H*), 7.48 (s, 1 H, C_6H) and 11.45 (br s, 1 H, N*H*). *Anal.* Calcd. for $C_{25}H_{44}N_2O_7Si_2$: C, 55.52; H, 8.20; N, 5.18. Found: C, 55.32; H, 8.46; N, 5.11.

***N*1-Methyl-2'-O-allyl-ψ-uridine (17).** Compound **16** (0.75 g, 1.37 mmol) was treated with 1.0 *M* solution of *n*-Bu_4NF in THF (15 mL) and the mixture was stirred at ambient temperature for 30 min. The precipitated product, after being collected by filtration, was dissolved in a mixture of pyridine:MeOH:H_2O (3:1:1) and stirred with amberlite IRC-50 (pyridinium form) resin for 30 min. The resin was removed by filtration. The filtrate was concentrated, co-evaporated with toluene (3 x 15 mL) and the residue was chromatographed on a flash silica gel column. The column was first eluted with hexane:EtOAc (15:1) and then with CH_2Cl_2:MeOH (9:1) to give 0.36 g (86.2%) of **16** , mp 98-99 °C ; IR ν_{max} 1640 and 1710 (C=O), 2920 (CH=CH_2) and 3300-3400 (OH, NH) cm^{-1}; UV λ_{max} nm (ε x 10^{-3}): (pH 1) 212 (8.4), 272 (9.5); (pH 7) 210 (11.4), 270 (10.6); (pH 11) 208 (21.6), 272 (9.5); ^1H NMR (DMSO-d_6) δ 3.17 (s, 3 H, NCH_3), 3.49 (m, 1 H, $C_{5'}H$), 3.61 (m, 1 H, $C_{5''}H$), 3.72 (m, 1 H, $C_{4'}H$), 3.93 (m, 2 H, $C_{2'}H$ and $C_{3'}H$), 4.40 (d, 2 H, J = 5.32 Hz, OCH_2), 4.52 (d, 1 H, J = 4.01 Hz , $C_{1'}H$), 4.78 (m, 1 H, 3'O*H*), 4.89 (d, 1 H, $C_{5'}OH$), 5.08 [(dd, 1 H, J = 1.08 Hz and J = 10.16 Hz) and 5.11 (dd, 1 H, J = 1.52 Hz and J = 11.32 Hz), =CH_2], 5.84 (m, 1 H, =C*H*), 7.83 (s, 1 H, C_6H) and 11.21 (br s, 1 H, N*H*). *Anal.* Calcd. for $C_{13}H_{18}N_2O_6 \cdot 0.75\ H_2O$: C, 50.03; H, 6.37; N, 8.98. Found: C, 50.23; H, 6.49; N, 8.81

***N*1-Methyl-2'-O-allyl-5'-O-(4,4'-dimethoxytrityl)-ψ-uridine (18).** In a similar manner as described for **3**, tritylation of a solution of **17** (0.55 g, 1.84

mmol) in pyridine (25 mL) with 4,4'-dimethoxytrityl chloride (0.81 g, 2.39 mmol) in the presence of triethylamine (0.33 g, 3.26 mmol) afforded 0.72 g (65%) of the title compound, mp 116 °C; IR ν_{max} 1660 and 1705 (C=O), 2920 (CH=CH_2) and 3300-3500 (OH, NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.04 (s, 3 H, NCH_3), 3.23 (m, 2 H, $C_{5'}H_2$), 3.73 (s, 6 H, 2 OCH_3), 3.87 (m, 1 H, $C_{4'}H$), 3.93 (m, 2 H, $C_{3'}H$ and $C_{2'}H$), 4.41 (d, 2 H, J = 5.32 Hz, OCH_2), 4.60 (d, 1 H, J = 2.08 Hz, $C_{1'}H$), 4.98 (s, 1 H, $C_{3'}$-OH), 5.07 [(dd, 1 H, J = 1.28 Hz and J = 10.5 Hz) and 5.10 (dd, 1 H, J = 1.04 Hz and J = 9.24 Hz), =CH_2], 5.81 (m, 1 H, =CH), 6.87-7.42 (m, 13 H, DMT), 7.53 (s, 1 H, C_6H) and 11.35 (br s, 1 H, NH). *Anal.* Calcd. for $C_{34}H_{36}N_2O_8$: C, 67.98; H, 6.04; N, 4.66. Found: C, 67.78; H, 6.40; N, 4.32.

***N*1-Methyl-2'-O-allyl-5'-O-(4,4'-dimethoxytrityl)-ψ-uridine-3'-[2-cyanoethyl bis(1-methylethyl)]phosphoramidite (19).** Compound **18** (0.08 g, 0.13 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and was treated with *N,N*-diisopropylethylamine and 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (0.063 g, 0.266 mmol). The mixture was stirred at ambient temperature for 40 min and was diluted with CH_2Cl_2 (30 mL). The solution was washed with a 15% solution of $NaHCO_3$ (40 mL), dried (Na_2SO_4) and evaporated to dryness. The residual syrup was dissolved in CH_2Cl_2 (2 mL) and loaded on the top of a pre-packed silica gel column (hexane:CH_2Cl_2:Et_3N; 90:9:1). The colum was eluted with a mixture of CH_2Cl_2:EtOAc:Et_3N (90:6:2). The appropriate homogeneous fractions were pooled and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (1 mL) and added with stirring to cold (-40 °C) hexane. The cloudy solution was evaporated to dryness to give 0.04 g (48%) of **19** as a white foam. ^{31}P NMR (CD_3CN) δ 148.78 and 148.90; ^1H NMR (CD_3CN) δ 1.31 {m, 12 H, N[CH(CH_3)$_2$]$_2$}, 1.93 (m, 2 H, N[CH(CH_3)$_2$], 3.22 (s, 3 H, NCH_3), 3.24 (m, 2 H, OCH_2), 3.57(m, 2 H, $C_{5'}H_2$), 3.65 (m, 2 H, CH_2CN), 3.77 (s, 6 H, 2 OCH_3), 4.05 (m, 4 H, OCH_2 of *allyl* , $C_{3'}H$, and $C_{4'}H$), 4.45 (m, 1 H, $C_{2'}H$), 4.97 (dd, 1 H, J = 6.28 Hz and 5.28 Hz, $C_{1'}H$), 5.29 (dd, 1 H, =CH_2), 5.49 (dd, 1 H, =CH_2) and 6.83-7.46 (m, 14 H, DMT and C_6 H). *Anal.* Calcd. for $C_{43}H_{53}N_4O_9P$: C, 64.48; H, 6.67; N, 6.99; P, 3.87. Found: C, 64.39; H, 7.06; N, 7.31; P, 4.18.

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