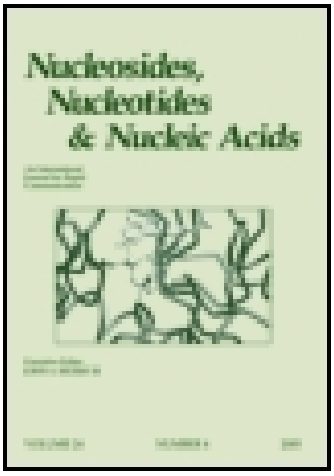

Nucleosides and Nucleotides
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn19

Syntheses of 2′-O-Methylisocytidine Phosphoramidite and Methylphosphonamidite Synthons

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Published online: 21 Aug 2006.

To cite this article: Daguang Wang & Paul O. P. Ts'o (1996) Syntheses of 2′-O-Methylisocytidine Phosphoramidite and Methylphosphonamidite Synthons, Nucleosides and Nucleotides, 15:1-3, 387-397

To link to this article: http://dx.doi.org/10.1080/07328319608002392

NUCLEOSIDES & NUCLEOTIDES, 15(1-3), 387-397 (1996)

SYNTHESES OF 2'-*O*-METHYLISOCYTIDINE PHOSPHORAMIDITE AND METHYLPHOSPHONAMIDITE SYNTHONS

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ABSTRACT. The 2'-*O*-methylisocytidine phosphoramidite synthon **7** and methylphosphonamidite synthon **8** are synthesized from 2'-*O*-methyluridine. The N^2-(*N'*, *N'*-dimethylformamidine) protected 2'-*O*-methylisocytidine is stable to basic deamination and acidic depyrimidination. Synthon **7** and synthon **8** have been incorporated into oligomers via the automated solid state procedure.

INTRODUCTION

The use of antisense oligonucleotides represents a powerful new strategy in the development of biological tools and therapeutic agents.[1,2] Besides the natural nucleosides, the non-natural novel nucleosides are being used for a number of applications including structural studies and the development of new base pairs.[3] It was suggested three decades ago that isocytidine could form a base pair with isoguanosine.[4] Previously, 2'-deoxyisocytidine[5], 2'-deoxy-5-methylisocytidine[6] and isocytidine[7] have been synthesized and 2'-deoxyisocytidine and 2'-deoxy-5-methylisocytidine have been studied in enzymatic systems[8] and 2'-deoxy-5-methylisocytidine hybridization properties with isoguanosine has been reported.[9] In our group, isocytidine is proposed for constructing novel double and triple oligonucleotide complexes. Besides 2'-deoxyisocytidine synthons, 2'-*O*-methylisocytidine synthons are synthesized with the expectation of higher stability towards chemical degradation and of higher affinity towards RNA target when it is incorporated into oligomers. Thus, the 2'-*O*-methylisocytidine synthons have been prepared and incorporated into oligomers having both phosphodiester and nonionic methylphosphonate backbones.[10] The synthetic chemistry is guided by three considerations:

This paper is dedicated to the 75th birthday of Dr. Yoshihisa Mizuno.

1) The integrity of the nucleoside base is the first major consideration. There should be no deamination under deblocking conditions or in the cleaving process of the synthesized oligomers from the solid support. According to prior report, under the alkaline conditions [aqueous ammonia, (25%, 12h, 60°C)] used for base deprotection after automated oligonucleotide synthesis, the deoxyisocytidine, formamidine-protected deoxyisocytidine and *N*-benzoylated deoxyisocytidine were found to undergo large amounts of deamination, yielding up to 12-15% 2'-deoxyuridine.[8,11] Also, no depyrimidination should take place under acidic detritylation conditions during the automated oligomer synthesis. It is reported that the glycosyl bond of isocytidine and related ribonucleoside are more acid labile than that of cytidine.[9]

2) The second consideration is the stability of backbone. The methylphosphonate backbone is more base labile than the phosphodiester linkage. Hence, the deprotection of the oligonucleoside methylphosphonate should be performed under milder conditions than those of phosphodiester oligonucleotides.

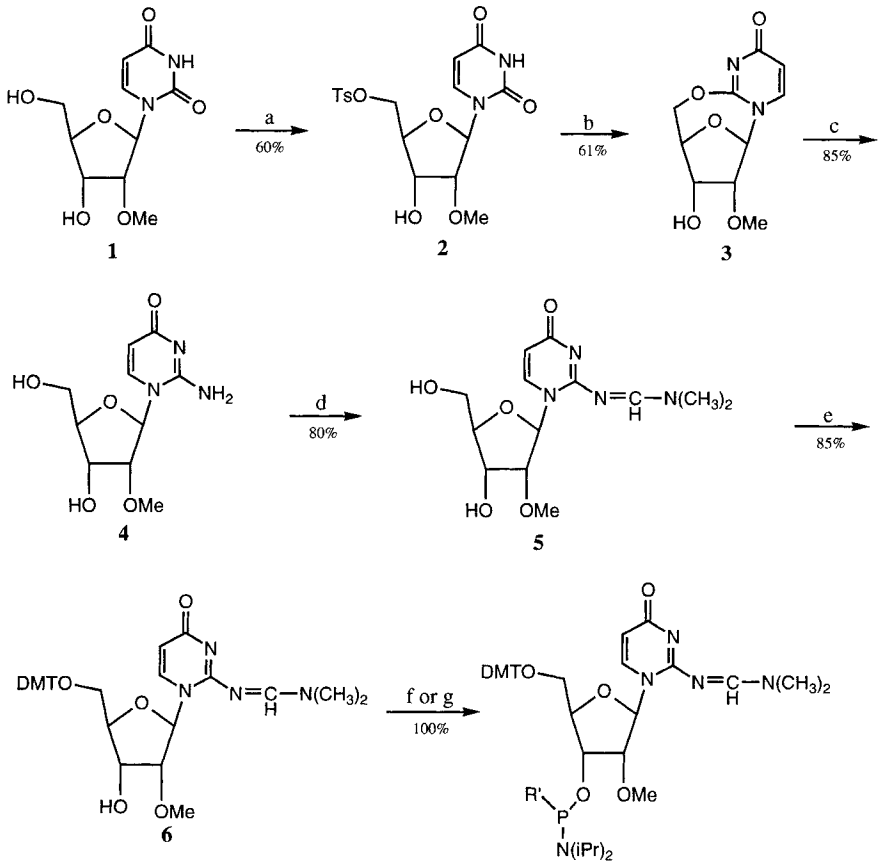
3) The third consideration is the protecting groups themselves. They should be stable during the solution chemistry and solid phase syntheses while being labile under the deblocking and cleaving conditions after automated oligomer synthesis. Mild deprotecting conditions should be used to preserve the methylphosphonate backbone, while simultaneously avoiding deamination of the isocytidine base.

The *N, N*-dimethylformamidine protecting group is chosen due to its protection against deamination, and its rapid removal in the deprotection process. Also, dimethylformamide dimethyl acetal reacts selectively with the exocyclic amine of isocytidine, with no necessity to protect the 2', 3', and 5' hydroxyls, thereby facilitating the synthetic procedure.[12]

This report presents the synthesis of the 2'-*O*-methylisocytidine phosphoramidite (synthon **7**) and methylphosphonamidite (synthon **8**) from 2'-*O*-methyluridine and the proper protecting groups of these synthons.

RESULTS AND DISCUSSION

The 2'-*O*-methylisocytidine phosphoramidite (synthon **7**) and the methylphosphonamidite (synthon **8**) are synthesized by the procedures shown in Scheme 1. 2'-*O*-Methylisocytidine **4** is synthesized from 2'-*O*-methyluridine[13] according to the reported method for 2'-deoxyisocytidine synthesis.[5,14] Tosylation of 2'-*O*-methyluridine gives 2'-*O*-methyl-5'-*O*-*p*-tolylsulfonyluridine **2** in 60 % yield, which is similar to the yield of the tosylation of 2'-deoxyuridine. The key intermediate, 2'-*O*-methyl-2,5'-anhydrouridine **3**, is obtained from 2 days reflux of the 2'-*O*-methyl-5'-*O*-*p*-



7 R' = OCH_2CH_2CN cyanoethyldiisopropyl phosphoramidite
8 R' = CH_3 methylphosphonamidite

a. *p*-toluenesulfonyl chloride, pyridine; b. DBU, acetonitrile, reflux; c. NH_3, methanol;
d. *N,N*-dimethylformamidine dimethyl acetal, DMF; e. 4,4'-dimethoxytrityl chloride;
f. 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, dichloromethane, triethylamine;
g. dichloromethylphosphine, diisopropylamine, dichloromethane, diisopropylethylamine.

SCHEME 1

tolylsulfonyluridine **2** with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in anhydrous acetonitrile. This reaction takes longer than the formation of 2'-deoxy-2,5'-anhydrouridine. Aminolysis of the 2'-*O*-methyl-2,5'-anhydrouridine **3** gives 2'-*O*-methylisocytidine **4**. Further reactions are carried out with crude compound **4** without purification. The exocyclic amine of compound **4** is protected by the dimethylformamidine group[12,15] giving 2'-*O*-methyl-N^2-(*N'*, *N'*-dimethylformamidine)-isocytidine **5** . Dimethylformamide dimethyl acetal reacts selectively with the exocyclic amine of isocytidine without protection of the 3' and 5' hydroxyl groups.

The stabilities of 2'-*O*-methyl-N^2-(*N'*, *N'*-dimethylformamidine)isocytidine **5** under basic and acidic conditions are studied. The first test is the stability of N^2-protecting group under mild deprotection conditions. Our study shows that the N^2-protecting group, dimethylformamidine, can be removed by the Genta one-pot deprotection conditions[16] which preserve the methylphosphonate backbone, a 0.5 hr treatment with EtOH/CH_3CN/NH_4OH (4.5:4.5:1), followed by a 6 hr treatment with ethylenediamine. No deamination is observed after the above deprotection procedure. The second test undertaken is the stability of the isocytidine under acidic detritylation conditions. Studies show that 2'-*O*-methyl-N^2-(*N'*, *N'*-dimethylformamidine)isocytidine is stable for a 24 hour period in dichloromethane containing 2.5 % dichloroacetic acid (DCA), which is the conventional detritylation conditions. No depyrimidination is observed by analytical HPLC. At higher DCA concentration, 5% DCA in dichloromethane, 2'-O-methylisocytidine decomposes slowly. The depyrimidinated base, N^2-(*N'*, *N'*-dimethylformamidine)isocytosine is isolated and characterized by NMR and m.s.

5'-*O*-tritylation of compound **5** gives high yield of compound **6**. The functionalization of 3'-*OH* of **6** yields the cyanoethyldiisopropyl phosphoramidite and methylphosphonamidite synthons. The cyanoethyl diisopropylphophoramidite **7** is synthesized by addition of 2-cyanoethyl *N*, *N*-diisopropylchlorophosphoramidite to a stirred solution of tritylated compound **6** in dry dichloromethane containing triethylamine. ^{31}P-NMR shows the characteristic peaks at 149.34 and 153.54 ppm. The phosphoramidite synthon **7** is incorporated into oligonucleotides by automated solid state synthesis procedure. The coupling yield is very low by using conventional 20 second coupling time. The coupling yield is improved significantly (>97%) when the coupling time is increased to 10 minutes.

The methylphosphonamidite **8** is obtained by the addition of compound **6** in diisopropylethylamine and dichloromethane to a solution of the chloro-*N,N*-diisopropyl-aminomethylphosphine, which is formed by the reaction of diisopropylamine and dichloromethylphosphine in dichloromethane. ^{31}P-NMR gives the characteristic peaks at

122.93 and 132.48 ppm. The methylphosphonamidite **8** is also incorporated into oligomers. The 10 minute coupling gives high yield (>96%).

Another synthesis route (Scheme 2), starting from isocytidine, has also been investigated. The isocytidine **9** is synthesized according to the reported method. The exocyclic amine of compound **9** is protected by the dimethylformamidine group giving N^2-(*N', N'*-dimethylformamidine)isocytidine **10** in high yield. Then the 3', 5'-hydroxyl groups are protected with tetraisopropyldisiloxane 1,3-diyl group.[17] The silylation of 3', 5'-hydroxyl groups by 1,1,3,3-tetraisopropyldisiloxane usually gives high yield (>90%). In our case, the silylation of N^2-(*N', N'*-dimethylformamidine)isocytidine **10** gives only 63% of desired product. The 5'-monosilylated by-product **14**, with 16% yield, is isolated and identified (Scheme 2). The formation of monosilylated compound **14** may be due to the presence of the N^2-(*N', N'*-dimethylformamidine) protecting group, which is rigidly conjugated to the pyrimidine base. This phenomenon may also explain the reason of the slow coupling during the incorporation of the 2'-*O*-methylisocytidine synthons into oligomers. The N^2-(*N', N'*-dimethylformamidine) group may interfere with the coupling reaction during the oligomer synthesis. Longer coupling time is required (10 minutes) to obtain the satisfactory oligomer yield.

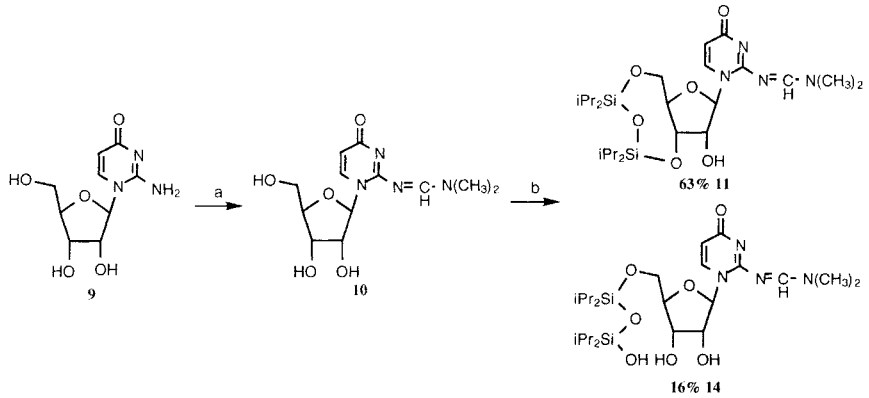
The 2'-*O*-methylation process of N^2-(*N', N'*-dimethylformamidine)-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-isocytidine **11** is not satisfactory, as the N^2- (*N', N'*-dimethylformamidine) group is transformed into an aldehyde group under the methylation conditions, giving compound **13** exclusively (Scheme 3). No desired compound **12** is obtained.

The structure of compound **13** is confirmed by ^{1}H-NMR and mass spectrometry. The possible mechanism of the formation of aldehyde **13** is shown in Scheme 4.

EXPERIMENTAL SECTION

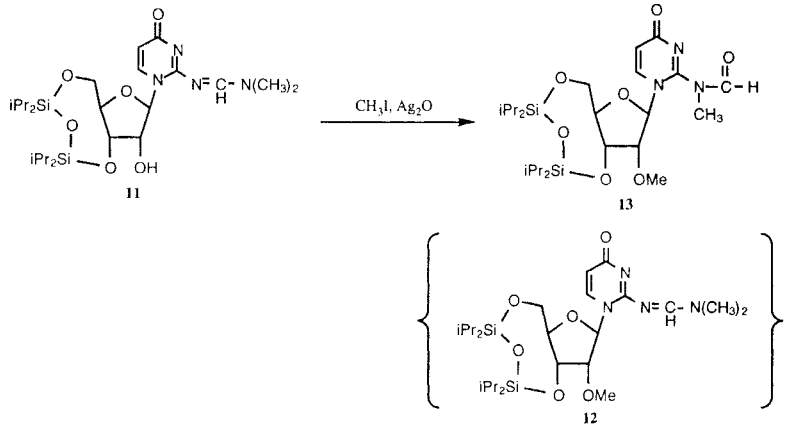
General Method

^{1}H-NMR spectra were recorded on a Bruker WM-300 spectrometer and the assignments are based on homonuclear decoupling and / or COSY experiments. When deuteriochloroform was employed as solvent, internal tetramethylsilane (TMS) was used as the reference. The residual proton signal methanol, assigned a value of 3.30 ppm, was used as reference in this case. The multiplicities are recorded using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. ^{13}C-NMR spectra were all obtained at 75.4 MHz using a Bruker WM-300 spectrometer. The $^{13}CD_3OD$ signal, assigned values of δ49.00, was used as reference in this solvent. ^{31}P-NMR spectra were all obtained at 121.44 MHz using a Bruker WM-300 spectrometer and chemical

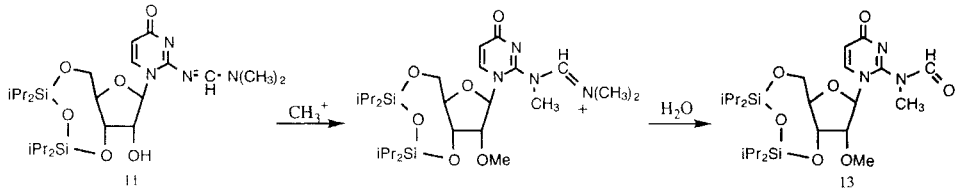


a. *N,N*-dimethylformamide dimethyl acetal, DMF; b. 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine.

SCHEME 2



SCHEME 3



SCHEME 4

shifts were given with respect to 85% phosphoric acid. Mass spectra (CI, FAB) were obtained on KRATOS-COMCETT mass spectrometer and mass spectra (MALDI) were obtained on KRATOS-MALDI-3 mass spectrometer.

Dichloromethane, pyridine, diisopropylamine, and diisopropylethylamine were distilled from calcium hydride. Other solvents were purchased as anhydrous from Aldrich Chemical Company and used without further purification. Thin-layer chromatography (t.l.c.) was performed using Kieselgel 60 F_{254} aluminum-backed plates (0.2 mm thickness) and visualized by UV and / or dipping in a solution of ammonium molybdate (2.5 g) and ceric sulfate (1 g) in 10 % v/v aqueous sulphuric acid (100 mL), followed by heating. Kieselgel 60 (Merck 230-400 mesh) silica gel was employed for column chromatography.

2'-*O*-Methyl-5'-*O*-*p*-tolylsulfonyluridine (2). 2'-*O*-Methyluridine **1** (395 mg, 1.57 mmol) was dissolved in pyridine (4.0 ml) and *p*-toluenesulfonyl chloride (312 mg, 1.68 mmol) was added. The mixture was stirred overnight at room temperature. The solvent was removed *in vacuo*. The residue was dissolved in chloroform (100 ml) and washed successively with saturated aqueous sodium bicarbonate solution (100 ml) and water (100 ml), dried (sodium sulfate), and evaporated to dryness. Traces of pyridine were removed by several times of coevaporation with ethanol, and the residue was crystallized from methanol (378 mg, 60% yield). : ^{1}H-NMR ($CDCl_3$) δ: 2.46 (s, 3H, C**H**$_3$-Ts), 3.61 (s, 3H, 2'-*O*C**H**$_3$), 3.84-4.27 (m, 5H, H2', 3', 4', and 5'), 5.75 (d, 1H, H5), 5.94 (fine d, 1H, H1'), 7.38 (d, 2H, Ts, J = 8.2 Hz), 7.58 (d, 1H, H6, *J* = 7.6 Hz), 7.85 (d, 2H, Ts); MS (FAB) m/e 413 ([MH$^+$]).

2'-*O*-Methyl-2,5'-anhydrouridine (3). Compound **2** (647 mg, 1.5 mmol) was dissolved in dry acetonitrile (30 ml) and then DBU (0.6 ml) was added. The mixture is refluxed with stirring for 2 days, during which time colorless crystals were precipitated. The solution was filtered while hot. The filtrate was evaporated to dryness and the residue was chromatographed over a column of silica gel (10:1, CH_2Cl_2/MeOH, v/v) giving compound **3** in 61% yield (230 mg): ^{1}H-NMR (CD_3CN) δ: 3.56 (s, 3H, 2'-*O*C**H**$_3$), 3.77-4.55 (m, 5H, H2', 3', 4', and 5'), 5.85 (d, 1H, H1'), 6.03 (d, 1H, H5), 7.77 (d, 1H, H6, *J* = 7.7 Hz); MS (FAB) m/e 241 ([MH$^+$]).

2'-*O*-Methylisocytidine (4). Compound **3** (425 mg, 1.77 mmol) was dissolved in dry methanol (15 ml). The solution was saturated with ammonia at 0 °C. The reaction was then left at room temperature for 5 days. The solvent was removed *in vacuo* and the residue was chromatographed over a column of silica gel (100:15, CH_2Cl_2 / MeOH, v/v)

yielding **4** (470 mg, 85%). UV(H_2O) λmax 254 nm (ε = 5700): ^{1}H-NMR (CD_3OD) δ: 3.46 (s, 3H, 2'-*O*C**H**$_3$), 3.89 (d, 2H, H5'), 3.95 (dd, 1H, H2'), 4.13 (dt, 1H, H4'), 4.30 (dd, 1H, H3'), 5.50 (d, 1H, H1'), 5.82 (d, 1H, H5), 7.82 (d, 1H, H6, *J* = 7.6 Hz); ^{13}C NMR (CD_3OD) δ: 58.53 (2'-*O***C**H$_3$), 60.77 (C5'), 73.75 (C3'), 84.39 (C4'), 91.17 (C2'), 94.72 (C1'), 106.75 (C5), 142.20 (C6), 156.41 (C4), 174.40 (C2);
MS (FAB) m/e 258 ([MH$^+$]).

2'-*O*-Methyl-*N*^{2}-(*N', N'*-dimethylformamidine)isocytidine (5). *N, N*-Dimethylformamide dimethyl acetal (1.65 ml) and 2'-*O*-methylisocytidine **4** (279 mg, 1.09 mmol) were dissolved in DMF (5 ml), and the solution was kept at room temperature for 1 h. Ethanol (1 ml) was added to the solution, and the mixture was concentrated *in vacuo*. The residue was chromatographed over a column of silica gel (10:1, CH_2Cl_2 / MeOH, v/v) affording compound **5** (269 mg, 80%): ^{1}H-NMR (CD_3OD) δ: 3.16 and 3.23 (2s, 6H, 2 C**H**$_3$), 3.47 (s, 3H, 2'-*O*C**H**$_3$), 3.76-4.20 (m, 5H, H2', 3', 4', 5'), 5.95 (d, 1H, H5), 6.23 (s, 1H, H1'), 8.06 (d, 1H, H6, *J* = 7.8 Hz), 8.66 (s, 1H, *N*=C**H**); MS (FAB) m/e 313 ([MH$^+$]).

5'-*O*-Dimethoxytrityl-2'-*O*-methyl-*N*^{2}-(*N', N'*-dimethylformamidine)-isocytidine (6). 4,4'-Dimethoxytrityl chloride (236 mg, 0.69 mmol) was added to a stirred solution of compound **5** (180 mg, 0.58 mmol) in dry pyridine (6.0 ml) containing 4-dimethylaminopyridine (13 mg, 0.01 mmol), and then triethylamine (0.4 ml) was added. After stirring for 8 h, the reaction mixture was poured into water (50 ml) and extracted with dichloromethane (3 x 50 ml). The organic phase was dried over Na_2SO_4 and evaporated *in vacuo* to a syrup which was then chromatographed over silica gel (100:5:1, CH_2Cl_2 / MeOH / Et3N, v/v) giving **6** as a white foam (381 mg, 85 %): ^{1}H-NMR (CD_3OD) δ: 3.12 and 3.17 (2s, 6H, 2 C**H**$_3$), 3.42 (2s, 6H, 2'-*O*C**H**$_3$), 3.57-4.36 (m, 5H, H2', 3', 4', and 5'), 5.76 (d, 1H, H5), 6.20 (s, 1H, H1'), 6.78-7.47 (m, 13H, DMTr), 7.77 (d, 1H, H6, *J* = 7.6 Hz), 8.64 (s, 1H, *N*=C**H**); MS (FAB) m/e 636 ([M+Na$^+$]), 615 ([MH$^+$]), 303 ([DMTr$^+$]).

Phosphoramidite synthon (7). 2-Cyanoethyl-*N, N*-diisopropylchloro-phosphoramidite (61 μL, 0.54 mmol) was slowly added to a stirred solution of tritylated compound **6** (166 mg, 0.27 mmol) in dry dichloromethane (3.0 ml) containing triethylamine (150 μl, 1.08 mmol). After 20 h of stirring at ambient temperature under a nitrogen atmosphere, the solution was quenched with methanol (0.5 ml). Then the solvent was removed *in vacuo*. The residue was chromatographed over silica gel (100:5:1, CH_2Cl_2 / MeOH / Et3N, v/v) giving the phosphoramidite **7** as a colorless foam in

quantitative yield (220 mg) which was used as such in the subsequent solid-phase syntheses: ^{1}H-NMR (CD_3OD) δ: 1.30 (dd, 12H, 2 *N*CH(C**H**$_3$)$_2$), 2.69 (m, 2H, CH_2CN), 3.18, 3.21, and 3.23 (3s, 9H, 3 C**H**$_3$), 3.42-3.54 (m, 2H, 2 *N*C**H**(CH$_3$)$_2$), 3.78 (2s, 6H, 2 *O*C**H**$_3$), 3.56-4.40 (m, 5H, H2', 3', 4', and 5'), 5.80 (m, 1H, H5), 6.15 (m, 1H, H1'), 6.82-7.79 (m, 14H, DMT and H6), 8.50 (fine d, 1H, *N*=C**H**); ^{31}P-NMR (CD_3OD) δ 149.34 and 153.54; MS (FAB) m/e 837 ([M+Na$^+$]), 815 ([MH$^+$]), 303 ([DMTr$^+$]).

Methylphosphonamidite synthon (8).

To a stirred solution of dichloromethylphosphine (55 µl, 0.60 mmol) in anhydrous CH_2Cl_2 (0.9 ml) was added diisopropylamine (174 µl, 1.20 mmol). The mixture was allowed to stir at room temperature under N_2 for 1 h to generate a chloro-*N,N*-diisopropylaminomethylphosphine solution. 5'-*O*-Dimethoxytrityl-2'-*O*-methyl-*N*^{2}-(*N'*, *N'*-dimethylformamidine)isocytidine **6** (125 mg, 0.20 mmol), which was dried overnight to a foam on a vacuum pump, was dissolved in anhydrous CH_2Cl_2 (2.0 ml) and diisopropylethylamine (356 µl). This solution was placed under N_2, cooled to O°C in an ice bath, and the chloro-*N,N*-diisopropylaminomethylphosphine solution (prepared as described) was added dropwise over 5 minutes. The ice bath was removed, and the reaction was stirred for 8 h at room temperature. The solution was evaporated to dryness. The residue was partitioned between a saturated Na_2CO_3 solution (100 ml) and CH_2Cl_2 (100 ml). The organic layers were collected, dried over Na_2SO_4, and evaporated to dryness. This crude material was dissolved in dichloromethane and precipitated at -78°C with hexanes. The solvents were decanted off and the residue was washed with hexanes at -78°C and dried *in vacuo* affording methylphosphonamidite synthon **8** in quantitative yield (154 mg), which was used as such in the subsequent solid-phase syntheses: ^{1}H-NMR, δ, (CD_3CN); 0.79-1.28 (m, 15H, *P*C**H**$_3$ and C**H**$_3$, *i*Pr), 3.19, 3.22, and 3.28 (3s, 9H, 3 C**H**$_3$), 3.42-3.49 (m, 2H, 2 C**H**, *i*Pr), 3.59-4.45 (m, 5H, H2', 3', 4', and 5'), 3.75 (fine d, 6H, 2 *O*C**H**$_3$), 5.79 (m, 1H, H5), 6.10-6.22 (m, 1H, H1'), 6.83-7.79 (m, 14H, DMT and H6), 8.51 (s, 1H, *N*=C**H**); ^{31}P-NMR (CD_3OD), 122.93 and 132.48; MS (FAB) m/e 760 ([MH$^+$]), 303 ([DMTr$^+$]).

***N*^{2}-(*N'*, *N'*-Dimethylformamidine)isocytidine (10).** *N, N*-Dimethylformamide dimethyl acetal (4.0 ml) and isocytidine **9** (576 mg, 2.37 mmol) were dissolved in DMF (14 ml), and the solution was kept at room temperature for 1 h. Ethanol was added to the solution, and the mixture was concentrated *in vacuo*. The residue was chromatographed over a column of silica gel (10:1, CH_2Cl_2/MeOH, v/v) giving compound **10** in 89% yield (314 mg): ^{1}H-NMR (CD_3OD) δ: 3.17 and 3.22 (2s, 6H, 2 C**H**$_3$), 3.84 (m, 2H, H5'),

4.03-4.86 (m, 3H, H2', 3', and 4'), 5.96 (d, 1H, H5), 6.37 (fine d, 1H, H1', J = 2.3), 8.22 (d, 1H, H6, J = 7.7), 8.68 (s, 1H, N=C**H**); MS (FAB) m/e 321 ([M+Na$^+$]), 299 ([MH$^+$]).

N^2-(N', N'-Dimethylformamidine)-3',5'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxane-diyl)-isocytidine (11). Compound **10** (150 mg, 0.50 mmol) was dissolved in dry pyridine (2.0 ml) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (159 µl, 0.55 mmol) was added. The reaction was stirred at room temparature for 20 h. TLC (10:1, CH_2Cl_2/MeOH, v/v) showed that the reaction finished and two products formed. The mixture was concentrated *in vacuo*. The residue was chromatographed over a column of silica gel (10:1 CH_2Cl_2/MeOH v/v). The less polar compound is the desired product **11** (171 mg, 63%). R_f = 0.45 in dichloromethane:methanol (8:1): ^{1}H-NMR ($CDCl_3$) δ: 1.00-1.11 (m, 28H, TIPDS), 3.17 (s, 6H, 2 x C**H**$_3$), 3.98-4.32 (m, 5H, H2', 3', 4', and 5'), 5.98 (d, 1H, H5), 6.18 (s, 1H, H1'), 7.82 (d, 1H, H6, J = 7.8), 8.84 (s, 1H, N=C**H**); MS (FAB) m/e 541 ([MH$^+$]).

The more polar compound is N^2-(N',N'-dimethylformamidine)-5'-O-TIPDS-isocytidine**14** (46 mg, 16%). R_f = 0.33 in dichloromethane:methanol (8:1): ^{1}H-NMR (CD_3OD) δ: 1.05-1.11 (m, 28H, TIPDS), 3.19 and 3.23 (s, 6H, 2 C**H**$_3$), 4.02-4.26 (m, 5H, H2', 3', 4', and 5'), 5.92 (d, 1H, H5), 6.35 (s, 1H, H1'), 8.17 (d, 1H, H6, J = 7.6), 8.67 (s, 1H, N=C**H**); MS (FAB) m/e 559 ([MH$^+$]).

By-product Aldehyde (13). Compound **11** (117 mg, 0.22 mmol) was dissolved in methyl iodide (2.0 ml) containing silver oxide (504 mg, 2.17 mmol) and the reaction was stirred at room temperature. TLC (5:1, hexanes/ethyl acetate, v/v) showed the completion of the reaction in 1 h. The reagent was removed *in vacuo* and the residue was chromatographed over silica gel (3:1, hexanes/ethyl acetate, v/v) giving **13** as an oil (82 mg, 70%): : ^{1}H-NMR ($CDCl_3$) δ: 0.86-1.12 (m, 28H, TIPDS), 3.81 (s, 3H, NC**H**$_3$), 4.00 (s, 3H, OC**H**$_3$), 3.83-4.30 (m, 5H, H2', 3', 4', and 5'), 6.05 (d, 1H, H5), 6.06 (s, 1H, H1'), 8.39 (d, 1H, H6, J = 7.6), 9.90 (s, 1H, CO**H**); MS (FAB) m/e 542 ([MH$^+$]), 154 ([base+H$^+$]).

ACKNOWLEDGEMENTS

This work was supported by NCI Grant No. CA42762-07 and a fellowship grant to D. Wang from Genta Inc. We wish to thank Sara Kipp for oligonucleotide synthesis and Dr. Yuanzhong Zhou for reading this paper.

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