Synthesis of Oligonucleotides Containing 2′-Deoxyisoguanosine and 2′-Deoxy-5-methylisocytidine Using Phosphoramidite Chemistry

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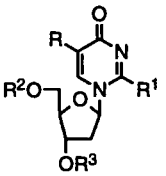
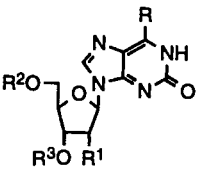
The synthesis of oligonucleotides containing 2′-deoxy-5-methylisocytidine and 2′-deoxyisoguanosine using phosphoramidite chemistry in solid-phase oligonucleotide synthesis is described. Supporting previous observations, the *N*,*N*-diisobutylformamidine moiety was found to be a far superior protecting group than *N*-benzoyl for 2′-deoxy-5-methylisocytidine. 2′-Deoxy-N^2-[(diisobutylamino)methylidene]-5′-(4,4′-dimethoxytrityl)-5-methylisocytidine 3′-(2-cyanoethyl diisopropylphosphoramidite) (**1c**) incorporated multiple consecutive residues during a standard automated synthesis protocol with a coupling efficiency > 99% according to dimethoxytrityl release. Extending coupling times of the standard protocol to ≥ 600 s using 2′-deoxy-N^6-[(diisobutylamino)methylidene]-5′-*O*-(dimethoxytrityl)-O^2-(diphenylcarbamoyl)isoguanosine, 3′-(2-cyanoethyl diisopropylphosphoramidite) (**7e**) led to successful incorporation of multiple consecutive 2′-deoxyisoguanosine bases with a coupling efficiency > 97% according to dimethoxytrityl release.

Introduction. – The *Watson-Crick* base pairing between two complementary oligonucleotide strands remains one of the most remarkable examples of molecular recognition. It follows two rules of complementarity: *i*) a large purine from one strand pairs with a small pyrimidine from the other; *ii*) H-bond donors (NH groups) of one base pair with H-bond acceptors (lone pairs of electrons on an O- or N-atom) of the other. Nature only partially exploits the potential of the *Watson-Crick* formalism, however. Structures for six base pairs can readily be written to conform to *Watson-Crick* geometry [1][2]. Therefore, it is possible, in principle, to have twelve independently replicating 'letters' in the nucleoside 'alphabet'.

One feasible non-standard base pair is formed between 2′-deoxyisocytidine ($isoC_d$, **1a**) and 2′-deoxyisoguanosine ($isoG_d$, **2a**), held together by three H-bonds like a G · C base pair. It was suggested three decades ago by *Rich* [3] that the isoG · isoC base pair might have been a component of primitive nucleic acids early in the development of life. In the late 1980's, the first experimental work was done to explore the oligonucleotide chemistry of this non-standard base pair, including its ability to be incorporated by enzymatic template-directed polymerization. Since then, several other laboratories have made major contributions to developing the chemistry and enzymology of the isoC · isoG base pair, including those of *Tor* and *Dervan* [4], *Horn et al.* [5], *Seela* and *Wei* [6] and *Switzer et al.* [7]. This work has shown, *inter alia*, that these bases can pair in both an antiparallel duplex as well as a parallel duplex, with similar stability to a guanine and cytosine base pair [5][8–10]. Further, a variety of RNA and DNA polymerases have been found that catalyze template-directed incorporation of this base pair into DNA [7].



1a R = R^2 = R^3 = H, R^1 = NH_2
b R = H, R^1 = NHC(O)Ph, R^2 = $(MeO)_2Tr$, R^3 = $P(OCH_2CH_2CN)(N(i\text{-}Pr)_2)$
c R = Me, R^1 = NHC(O)Ph, R^2 = $(MeO)_2Tr$, R^3 = $P(OCH_2CH_2CN)(N(i\text{-}Pr)_2)$
d R = Me, R^2 = R^3 = H, R^1 = NH_2
e R = Me, R^1 = NHC(O)Ph, R^2 = R^3 = H
f R = Me, R^1 = NHC(O)Ph, R^2 = $(MeO)_2Tr$, R^3 = H
g R = Me, R^1 = N = $CHNMe_2$, R^2 = R^3 = H
h R = Me, R^1 = N = $CHN(i\text{-}Bu)_2$, R^2 = R^3 = H
i R = Me, R^1 = N = $CHN(i\text{-}Bu)_2$, R^2 = $(MeO)_2Tr$, R^3 = H
j R = Me, R^1 = N = $CHN(i\text{-}Bu)_2$, R^2 = $(MeO)_2Tr$, R^3 = $P(OCH_2CH_2CH)(N(i\text{-}Pr)_2)$
l R = Me, R^1 = NHC(O)Ph, R^2 = $(MeO)_2Tr$, R^3 = $P(O)H(OCH_2CH_2CN)$
m R = Me, R^1 = NH_2, R^2 = $(MeO)_2Tr$, R^3 = H



2a R = NH_2, R^1 = R^2 = R^3 = H
b R = N = $CHN(i\text{-}Bu)_2$, R^1 = R^2 = R^3 = H
c R = N = $CHN(i\text{-}Bu)_2$, R^1 = H, R^2 = R^3 = Me_3Si
d R = N = $CHN(i\text{-}Bu)_2$, R^1 = OH, R^2 = R^3 = H
e R = N = $CHN(i\text{-}Bu)_2$, R^1 = OH, R^2 = $Si(i\text{-}Pr)_2OSi(i\text{-}Pr)_2OH$, R^3 = H
f R = N = $CHN(i\text{-}Bu)_2$, R^1 = OH, R^2 = R^3 = $(t\text{-}Bu)Me_2Si$
g R = NH_2, R^1 = OH, R^2 = R^3 = H
h R = N = $CHN(i\text{-}Bu)_2$, R^1 = Me_3SiO, R^2 = R^3 = Me_2Si

Despite the structural similarities that isoG and isoC share with the standard bases, it has not been trivial to convert procedures and protecting groups used for the automated solid-phase synthesis of standard oligonucleotides to be suitable for preparing oligonucleotides containing isoC and isoG. For example, *Switzer* and coworkers [9] reported that formamidine-protected 2′-deoxyisocytidine underwent rapid depyrimidination under conditions used routinely for the deprotection of oligonucleotides containing standard nucleobases. In contrast, *Horn et al.* [5] mentioned no such problem in their work. We found that $isoC_d$ as its benzoyl-protected derivative underwent deamination under basic oligonucleotide-deprotection conditions [7]. Likewise, *Strobel et al.* [11] reported that 5-methylisocytidine derivatives deaminated when subjected to these conditions. *Horn et al.* [5], however, reported no deamination upon basic deprotection of synthetic oligonucleotides using formamidine-protected 2′-deoxy-5-methylisocytidine phosphoramidites, although they did report depyrimidination 'precluding incorporation of more than a single disoMeC residue' into an oligonucleotide when the disoMeC (= me^5isoC_d) nucleobase was protected as a benzoate. In contrast, *Tor* and *Dervan* [4] reported neither depyrimidination nor deamination in oligonucleotide syntheses with the same benzoyl-protected me^5isoC_d.

Similar incongruitites can be found in the literature reporting the synthesis of oligonucleotides containing isoG. *Roberts et al.* [8][9] and *Sugiyama et al.* [10] mentioned no difficulties using standard solid-phase procedures to prepare oligonucleotides containing the phosphoramidite of 2′-deoxyisoguanosine protected as the *N*,*N*-formamidine and O^2-diphenylcarbamylate (dpc) ester. *Horn et al.* [5] reported, however, that dpc-protected isoG phosphoramidite 'may be too acid-labile for practical use'. *Seela* and *Wei* [6] noted that solid-phase synthesis with 2′-deoxyisoguanosine using phosphoramidites and

phosphonates gave low yields with consecutive incorporations, and suggested that a modified phosphonate synthesis be used for oligomers containing multiple consecutive isoG residues.

In view of the importance of the non-standard base pair in a variety of areas, most interestingly in DNA-based diagnostics [5][12], it is timely to report a detailed study containing full experimental recipes describing the chemistry for the synthesis of oligonucleotides containing $isoC_d$ and $isoG_d$. In this work, we develop syntheses and evaluate the utility of various protecting groups for phosphoramidites of $isoC_d$ and $isoG_d$ in automated synthesis, exploring and resolving some of the incongruities noted above.

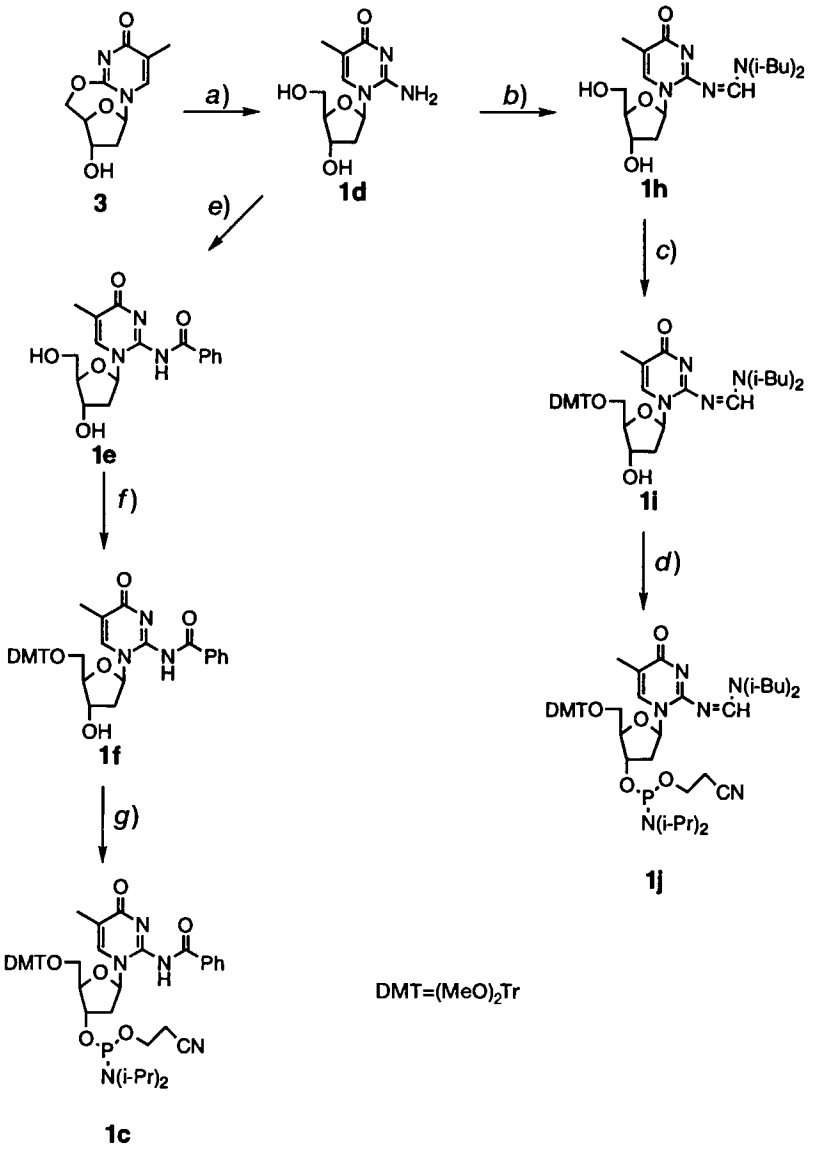
Results and Discussion. – *Isocytidine.* Because of reports that deamination was suppressed by a 5-methyl substituent in isoC [4] (*Scheme 1*), this work focused on protected variants of this derivative. Thus, N^2-benzoyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-methylisocytidine 3′-(2-cyanoethyl diisopropylphosphoramidite) (**1c**) was prepared following the general procedure of *Tor* and *Dervan*, with two improvements that significantly increased the yields. First, use of a *Parr* pressure reactor for the treatment of 2,5′-anhydrothymidine (**3**) with ammonia resulted in an improved, nearly quantitative yield of 2′-deoxy-5-methylisocytidine (**1d**). The synthetic results were also improved by chromatographic purification (silica gel) of the tritylated derivative **1f** immediately following its formation from **1e**, without storage. After modification, the overall yield for the three steps producing **1c** from 2′-deoxy-5-methylisocytidine (**1d**) was 36%.

When used in a standard oligonucleotide synthesis, N^2-benzoyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-methylisocytidine 3′-(2-cyanoethyl diisopropylphosphoramidite) (**1b**) did not perform satisfactorily in our hands. The trityl release following coupling of the non-standard nucleotide derivative dropped precipitously, and very little full-length product was detected upon completion of the synthesis. An analogous observation was made by *Horn et al.* [5]. These authors proposed that the low coupling yield arose due to depyrimidination, and they suggested the use of formamidine protection to avoid this problem.

Depyrimidination occurs during the acidic detritylation steps employed in automated synthesis, so the efficacies of several detritylation reagents on the model compound 5′-*O*-(4,4′-dimethoxytrityl)thymidine ($[(MeO)_2Tr]T_d$) were investigated. Three detritylation reagents proposed for oligonucleotide synthesis were examined: CCl_3COOH, $ZnBr_2$ [13], and ceric ammonium nitrate [14]. CCl_3COOH (1%) in 1,2-dichloroethane and $ZnBr_2$ in $MeNO_2/PhNO_2$ 4:1 both detritylated $[(MeO)_2Tr]T_d$ almost instantaneously at room temperature. Interestingly, a substantial amount of $(MeO)_2Tr$-protected thymidine remained after 15 min when ceric ammonium nitrate in wet MeCN or wet MeCN/DMF was used.

N^2-Benzoyl-2′-deoxy-5-methylisocytidine (**1e**) and the formamidine-derived 2′-deoxy-N^2-[(dimethylamino)methylidene]-5-methylisocytidine (**1g**) were treated with the three detritylating reagents at room temperature (see *Table*). The resulting mixtures were quenched and then analyzed on reversed-phase HPLC, monitoring at 260 nm. The N^2-benzoyl derivative **1e** degraded completely in less than 15 min in the CCl_3COOH and $ZnBr_2$ solutions. In solutions containing ceric ammonium nitrate, **1e** decomposed more slowly, but the detritylation of the corresponding trityl derivative **1f** was also slower, with depyrimidination faster than detritylation. A larger sample of the only decomposition

Scheme 1



a) NH_3, MeOH; 98%. *b*) $(i\text{-Bu})_2NCH(OMe)_2$, DMF; 92%. *c*) $(MeO)_2TrCl$, pyridine, 4-(dimethylamino)pyridine, DMAP; 52%. *d*) 2-Cyanoethyl diisopropylphosphoroamidochloridite, $(i\text{-Pr})_2NEt$, CH_2Cl_2; 79%. *e*) PhCOCl, pyridine, DMAP; 66%. *f*) $(MeO)_2TrCl$, pyridine, DMAP; 73%. *g*) 2-Cyanoethyl diisopropylphosphoramidochloridite, $(i\text{-Pr})_2NEt$, CH_2Cl_2; 74%.

product absorbing at 260 nm observed after exposure of **1e** to the detritylation conditions was generated with CCl_3COOH and confirmed as 5-methylisocytosine by ^1H-NMR.

Table. *Approximate Time Needed for Complete Depyrimidination of 2'-Deoxy-5-methylisocytidine Derivates* (detection level *ca.* 1%)

	1% CCl_3COOH/ 1,2-dichloroethane (r.t.)	$ZnBr_2$ in nitrobenzene/ nitromethane 1:4 (r.t.)	$Ce^{IV}(NH_4)_2(NO_3)_6$ (1.2 mM) in MeCN/DMF/H_2O 6:2:0.05 (reflux)
1e	< 15 min	< 15 min	150 min
1g	36 h	7 days	–

The corresponding formamidine-protected N^2-[(dimethylamino)methylidene] derivative **1g** was considerably more stable. After 1 h in the CCl_3COOH solution, only *ca.* 1% of the compound was degraded (UV). In the presence of $ZnBr_2$, no depyrimidination of **1g** was detected after 1 h at room temperature. Even after a further 16 h at 40°, only *ca.* 2% degradation was detected.

To evaluate the stability of **1g** with respect to deamination, the compound was exposed to standard cleavage/deprotection conditions used in automated synthesis. A small amount of **1g** was placed in 25% aqueous ammonium hydroxide and heated at 60° for 16 h. The solvent was removed under vacuum, the residue dissolved in water, and the mixture resolved by HPLC. Prior to treatment, UV analysis of an HPLC chromatogram showed **1g** to account for 99% of the material absorbing at 260 nm. Following treatment, 2'-deoxy-5-methylisocytidine (**1d**) accounted for 96% of the material. Thymidine (perhaps 2%, verified by addition of authentic thymidine) was the only detectable impurity, corresponding to the deamination product of **1d**.

These results show that N^2-benzoyl-2'-deoxy-5-methylisocytidine (**1e**) is too acid-labile for practical use; it was even labile under non-protic detritylation conditions. The successful use of this compound in oligonucleotide synthesis by *Tor* and *Dervan* [4] might be ascribed to the fact that the oligonucleotide prepared contained a single isoC residue close to the 5'-end of the oligomer, and therefore did not need to survive many cycles of treatment under detritylation conditions.

In contrast, the formamidine-protected 5-methylisocytidine **1g** appears suitable for phosphoramidite automated synthesis, displaying only small amounts of depyrimidination and (perhaps) deamination. The dimethylformamidine derivative **1g** used in the stability study, however, has been reported as labile under DNA-synthesis conditions [15]. Therefore, 2'-deoxy-N^2-[(diisobutylamino)methylidene]-5'-*O*-(4,4'-dimethoxytrityl)-5-methylisocytidine 3'-(2-cyanoethyl diisopropylphosphoramidite) (**1j**), bearing the more stable diisobutylformamidine protecting moiety, was synthesized in three steps from 2'-deoxy-5-methylisocytidine (**1d**). Reaction with *N*,*N*-diisobutylformamidine dimethyl acetal gave the easily-purified N^2-protected isocytidine **1h** after 1 h (*Scheme 4*). The fully protected target phosphoramidite **1j** was readily isolated in an overall yield of 38% for the three steps *via* **1h** and **1i** starting with **1d**.

Additionally, a method adapted from a procedure for recycling unused phosphoramidites from an automated synthesizer [16], proceeding through intermediates **1l** and **1m**, was developed to convert existing benzoyl-protected phosphoramidite into formamidine-protected phosphoramidite (**1c** → **1l** → **1f** → **1m**; see *Exper. Part*).

Phosphoramidite **1j** was examined for utility for synthesizing oligonucleotides containing isoC. Although $ZnBr_2$ was (from the studies above with **1g**) a superior detritylat-

ing reagent without depyrimidination, it was decided to first use standard detritylating conditions (CCl_3COOH), which also performed well in the stability study. The three oligonucleotides **4**–**6** were synthesized to test the efficacy of phosphoramidite **1j**.

A PAGE autoradiogram and an anion-exchange HPLC (*Fig. 1*) of the crude synthetic oligonucleotide **4** showed small amounts of failure products (albeit greater than seen with standard bases). Nevertheless, the synthesis yielded a satisfactory amount of full-length oligonucleotide **4**. The synthesis of crude oligonucleotide **5** gave similar results. After deprotection and desalting, the crude mixtures containing **4** or **5** were purified by ion-exchange HPLC, followed by reversed-phase HPLC. To verify the identity of the major product, the purified oligonucleotides **4** and **5** were digested by snake-venom phosphodiesterase and alkaline phosphatase and analyzed by reversed-phase HPLC. The component nucleosides found were consistent with the expected results for digestion of **4** and **5**, respectively (*Fig. 2*). Notably, no significant peak arising from T_d was present in the chromatograms, indicating that deamination did not occur in the oligonucleotides to the same extent as observed in syntheses with nucleoside **1b**, or even to the extent observed above with the formamidine-protected monomer nucleoside **1g**.

5'-d(A-iC-iC-iC-iC-iC-iC-iC-iC-iC-C) (**4**)

5'-d(A-A-A-A-A-iC-iC-iC-iC-iC-A-A-A-A-A) (**5**)

5'-d(T-T-T-T-T-iC-iC-iC-iC-iC-T-T-T-T-T) (**6**)

5'-d(T-T-T-T-T-iG-iG-iG-iG-iG-T-T-T-T-T) (**10**)

5'- d(C-A-C-A-A-iG-A-iG-A-iG-A-A-C-A) (**11**)

Oligonucleotide syntheses are frequently followed by quantifying the amount of trityl cation released. The $isoC_d$-containing oligonucleotide **6** was synthesized to evaluate coupling during synthesis by analyzing the integrated absorbance of released $(MeO)_2Tr$ protecting group at each added base. The efficiency of synthesis of the first part of oligonucleotide **6** was determined from couplings 2–4, the second part (containing $isoC_d$) from couplings 5–9, and the final part from couplings 10–14. The coupling efficiency of the $isoC_d$-derived phosphoramidites was 99.5%, comparable to the 99.9% efficiency for coupling in the other two sections containing only T_d.

Isoguanosine. Synthesis of oligonucleotides containing 2'-deoxyisoguanosine has been previously reported by *Switzer et al.* [7] using the 2'-deoxy-N^6-[(dibutylamino)-methylidene]-5'-*O*-(4,4'-dimethoxytrityl)-O^2-([2-4-nitrophenyl)ethyl]isoguanosine 3'-(2-cyanoethyl diisopropylphosphoramidite) monomer. To avoid the use of 1,8-diazabicyclo[5.4.0]undec-7-ena (DBU) in the deprotection procedure, the diphenylcarbamoyl moiety has been suggested for protecting the 2-*O*-position [5][9]. This protecting group has been used successfully for incorporation of 2'-deoxyisoguanosine using phosphonate chemistry [6], and evidently helps stabilize the *N*-glycoside bond from acid-catalyzed hydrolysis [6].

Thus, 2'-deoxy-N^6-[(diisobutylamino)methylidene]-5'-*O*-(4,4'-dimethoxytrityl)-O^2-(diphenylcarbamoyl)isoguanosine 3'-(2-cyanoethyl diisopropylphosphoramidite) (**7e**) was first synthesized in 7 steps (*Scheme 2*) from 2'-deoxy-2-aminoadenosine (**7a**). Although this synthesis is satisfactory for small amounts of the target phosphoramidite **7e**,

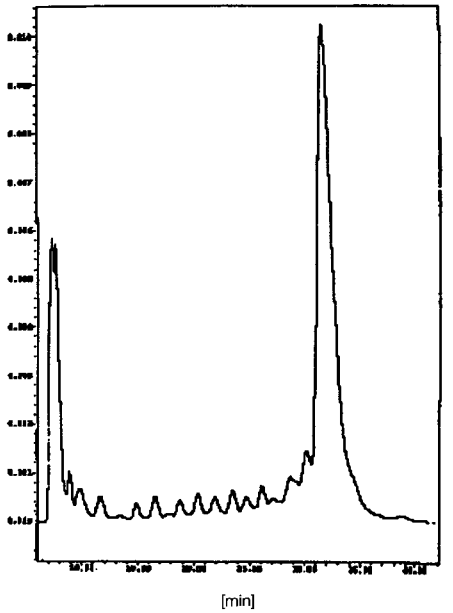


Fig. 1. *Anion-exchange HPLC of the crude synthetic oligonucleotide* **4**

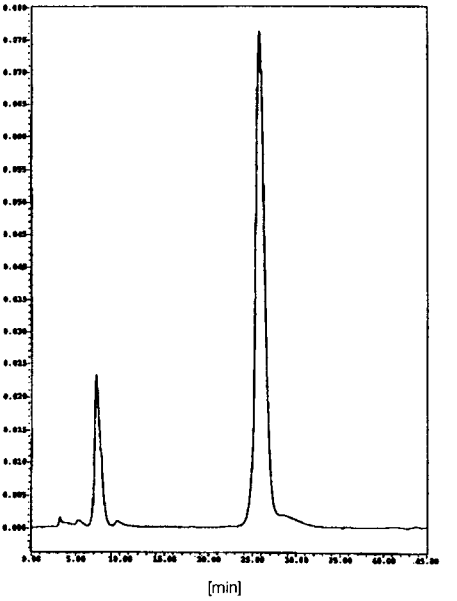
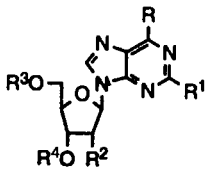
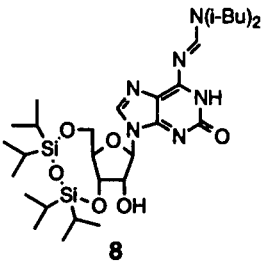


Fig. 2. *Reversed-phase HPLC of the enzymatic digestion products obtained from* **5**. The product of deamination of $isoC_d$, T_d, is present in less than 1%. $isoC_d$ (8.0 min), A_d (26 min).





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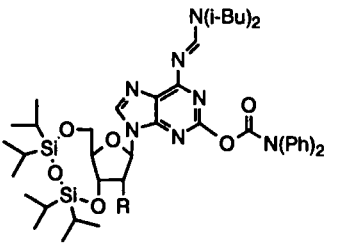
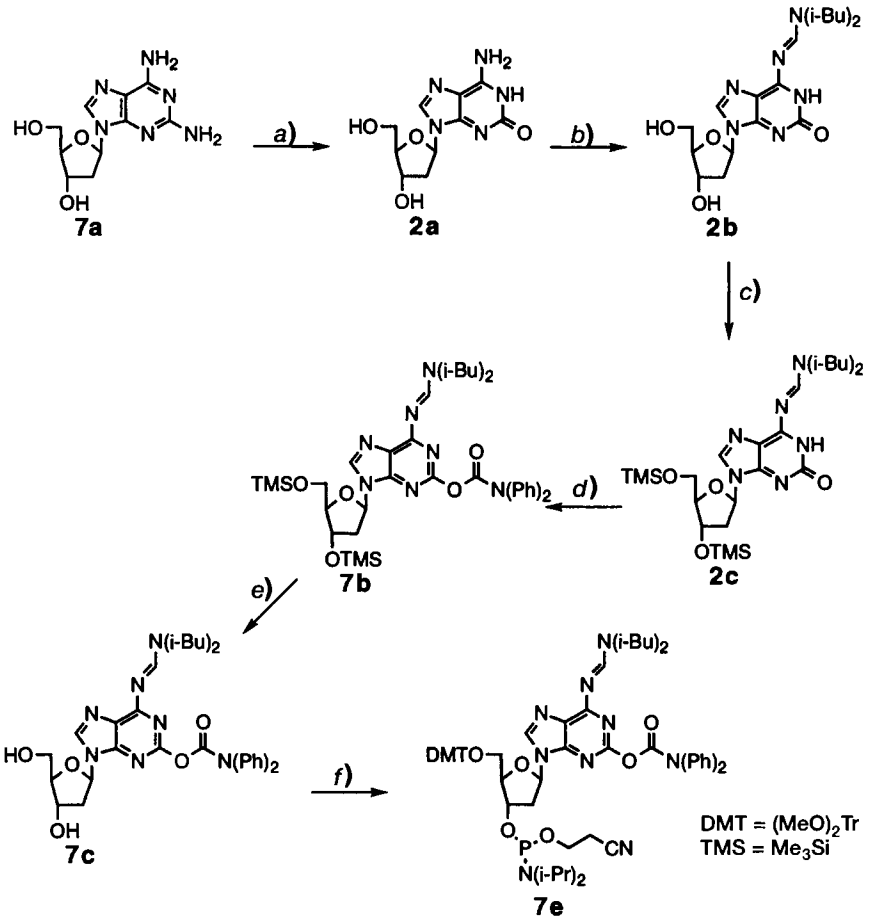


7a R = R^1 = NH_2, R^2 = R^3 = R^4 = H
b R = N = CHN(i-Bu)$_2$, R^1 = OC(O)NPh_2, R^2 = H, R^3 = R^4 = Me_3Si
c R = N = CHN(i-Bu)$_2$, R^1 = OC(O)NPh_2, R^2 = R^3 = R^4 = H
d R = N = CHN(i-Bu)$_2$, R^1 = OC(O)NPh_2, R^2 = H, R^3 = $(MeO)_2$Tr, R^4 = H
e R = N = CHN(i-Bu)$_2$, R^1 = OC(O)NPh_2, R^2 = H, R^3 = $(MeO)_2$Tr, R^4 = $P(OCH_2CH_2CN)(N(i\text{-}Pr)_2)$
f R = R^1 = NH_2, R^2 = OH, R^3 = R^4 = H
g R = N = CHN(i-Bu)$_2$, R^1 = OC(O)NPh_2, R^2 = Me_3SiO, R^3 = R^4 = Me_3Si
h R = N = CHN(i-Bu)$_2$, R^1 = OC(O)NPh_2, R^2 = OH, R^3 = R^4 = H

9a R= OH
b R= OC(S)OPh
c R=H

we required a procedure amenable to large-scale work. Unfortunately, the starting material **7a** in this route is quite expensive. Consequently, an alternative route starting with the relatively inexpensive ribose compound, 2-aminoadenosine (**7f**), was designed.

Scheme 2

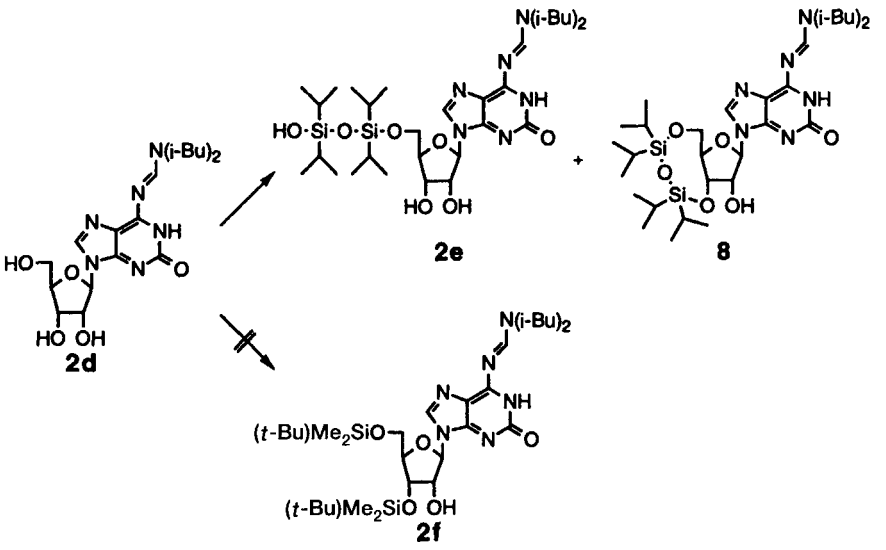


a) AcOH, $NaNO_2$, H_2O; 81%. *b*) $(i\text{-Bu})_2NCH(OMe)_2$, DMF; 96%. *c*) Me_3SiCl, Et_3N, THF; 77%. *d*) Ph_2NCOCl, pyridine, $(i\text{-Pr})_2NEt$; 75%. *e*) THF; 89%. *f*) 1. $(MeO)_2TrCl$, pyridine, DMAP; 76%; 2. 2-cyanoethyl diisopropylphosphoramidochloridite, $(i\text{-Pr})_2NEt$, CH_2Cl_2; 74%.

Originally the intermediate **8** was planned in the synthesis of **7e** from **7f**. Reaction of **2d** with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane gave a *ca.* 1:1 mixture (^{1}H-NMR) of **8** and **2e** (*Scheme 3*), however. Problems forming the eight-membered cycle bridging the O-atoms at C(3′) and C(5′) have been observed in other nucleosides. Silylations at the 3′-*O*- and 5′-*O*-positions to give desired product, as well as at an unprotected carbonyl group of the base and the 5′-*O*-position to give side product, have been observed for unprotected $isoG_d$ [17] and for *N*-protected isoC [18]. Compounds **8** and **2e** were separable, but the resulting poor yield was impracticable. Next, selective 3′,5′-di-*O*-silylation of

2d to **2f** was tried (*Scheme 3*), using a literature procedure for similar reactions [19]; however, NMR analysis of the product revealed a complex mixture of several inseparable silylated derivatives.

Scheme 3

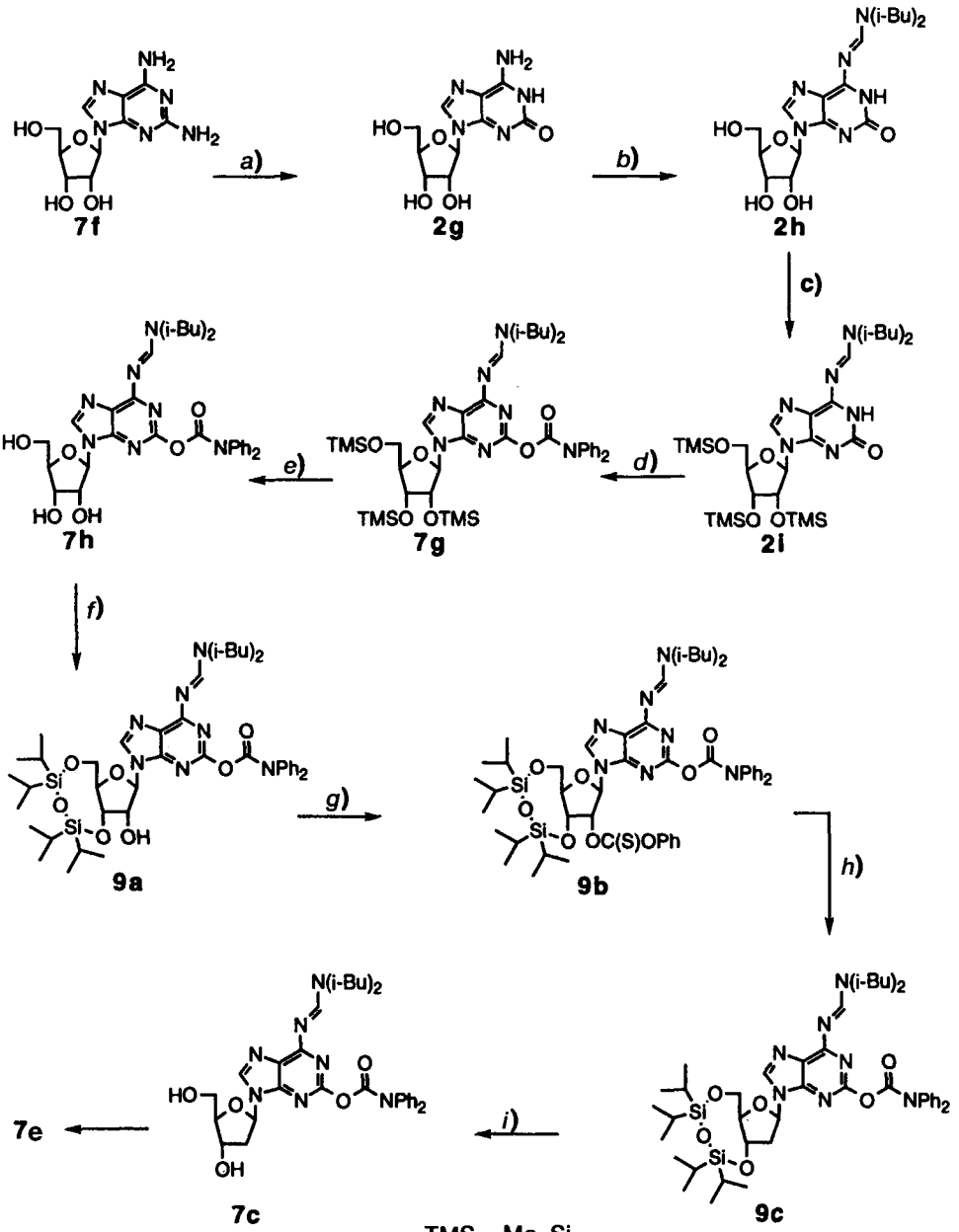


A slightly longer route was then developed to synthesize **7e** from the ribose derivative **7f** (*Scheme 4*). The intermediate **2g** was purified with much greater ease than the corresponding 2′-deoxy compound **2a** (see *Scheme 3*), and not all synthetic intermediates required isolation. An overall yield of 13% for 11 steps, producing **7e** from the inexpensive starting material **7f** *via* the silylated derivatives **9a–c** without side reactions or laborious purifications, make this route suitable for large-scale preparation.

Coupling efficiency with phosphoramidite **7e** was evaluated in the same manner as described for the $isoC_d$-derived phosphoramidite **1j** above. Incorporation of **7e** into oligonucleotide **10** yielded little or no target oligomer using standard coupling times. The situation was greatly improved with extended coupling times (*Fig. 3*). It proved necessary to extend the coupling times to at least 600 s for each cycle incorporating an $isoG_d$ residue to obtain reasonable coupling efficiency. The increase in full-length target oligomer with extended coupling times was verified by ion-exchange HPLC of the crude products. Despite high coupling yields by trityl release, the HPLC indicate significantly more failure products than seen with standard bases, although the full-length oligonucleotide **10** was clearly the major product of the synthesis. The longer coupling time (600 s) needed for the incorporation of the $isoG_d$ residue compared to conventional DNA synthesis (*ca.* 90 s) lacks an explanation. In any case, the coupling time required for oligonucleotide synthesis containing 2′-deoxyisoguanosine is comparable to the coupling time employed in oligoribonucleotide synthesis [20].

Oligonucleotide **11** was synthesized with extended coupling times (900 s) using phosphoramidite **7e**. An anion-exchange HPLC of the crude product indicated that although the trityl-release-monitored coupling efficiency was > 95%, quite significant amounts of

Scheme 4



a) AcOH, $NaNO_2$, H_2O; 91%. *b*) $(i\text{-Bu})_2NCH(OMe)_2$, DMF; 90%. *c*) Me_3SiCl, Et_3N, THF; 96%. *d*) Ph_2NCOCl, pyridine, $(i\text{-Pr})_2NEt$; 77%. *e*) Bu_4NF, THF; 91%. *f*) 1,3-Dichloro-1,1,1,3,3,3-tetraisopropyldisiloxane, pyridine; 92%. *g*) PhOC(S)Cl, pyridine, DMAP, CH_2Cl_2; 94%. *h*) Bu_3SnH, AIBN, toluene; 73%. *i*) Bu_4NF, THF.

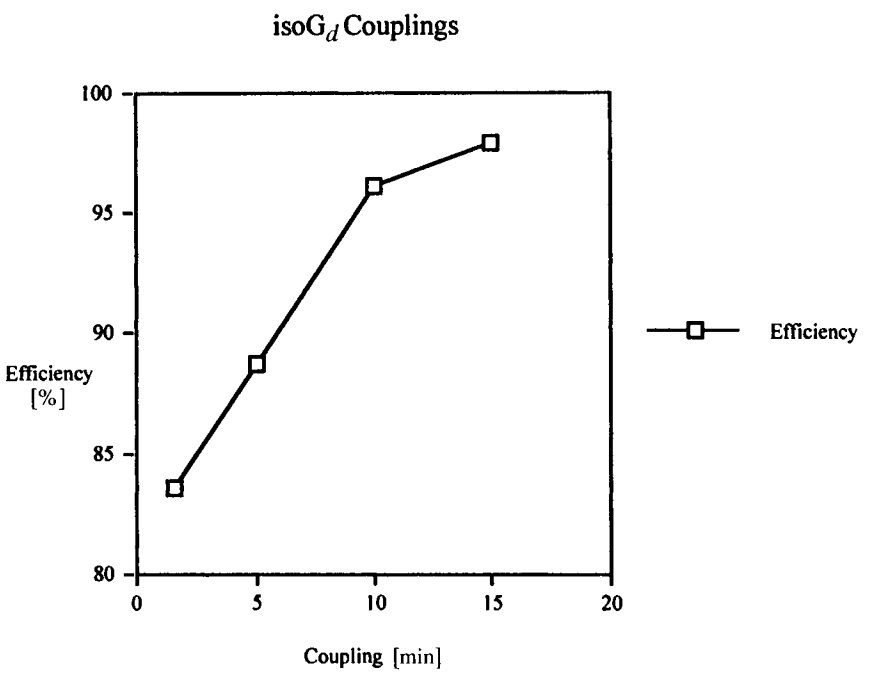


Fig. 3. *The coupling efficiency* [%] *by trityl-release monitoring of isoG$_d$ phosphoramidite* **7e** vs. *coupling time in syntheses of* **10**

failure products were generated. The crude product was purified by anion-exchange HPLC, followed by reversed-phase HPLC, and the purified oligonucleotide **11** was enzymatically digested as described above. The reversed-phase HPLC of the digestion products are consistent with the expected component nucleosides of **11** (*Fig. 4*).

This work was supported by the *Danish Natural Science Research Council*, *National Institutes of Health*, and *Office of Naval Research*. We thank the University of Florida ICBR DNA Synthesis Core for their special effort in the synthesis of all oligonucleotides containing non-standard bases.

Experimental Part

General. The 2-aminoadenosine (**7f**) and 2-amino-2'-deoxyadenosine (**7a**) were purchased from *RI Chemicals, Inc.* (Orange, CA), 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane from *Farchan Laboratories, Inc.* (Gainesville, FL), 1*H*-imidazole from *Kodak*, and all other chemicals from *Aldrich Chemical* or *Fisher Scientific*; they were used without further purification. All materials to make buffer solutions, including K_2HPO_4, KCl, Et_3N, *Tris* (= tris(hydroxymethyl)aminomethane (= 2-amino-2-(hydroxymethyl)propane-1,3-diol)), HCl, and AcOH were from *Fisher*, except for KH_2PO_4, which was from *Mallinckrodt*. TsCl was freshly recrystallized from petroleum ether. Solvents were dried over 4-Å molecular sieves. CH_2Cl_2 used in the synthesis and workup of phosphoramidites was freshly distilled from K_2CO_3. All reactions were carried out under dry Ar in an oven-dry glass system. 'Evaporation' refers to removal of volatile solvents with a membrane pump. Column chromatography (CC): silica gel (230–425 mesh). TLC: silica gel TLC plates from *Whatman*, visualization by staining with a Ce/Mo reagent (2.5% phosphormolybdic acid, 1% $Ce^{IV}(SO_4)_2 \cdot 4H_2O$, 6% H_2SO_4 in H_2O) and heating. HPLC: solvents from *Fisher Scientific* (HPLC grade) and *Milli*-Q-purified water (*Millipore Corp.*); solvents filtered through a 0.45 μm *Whatman* nylon filter just before use; *Waters Alliance* system with a *486* tunable absorbance detector or *Waters* system consisting of a *600S* controller, *616* pump, and a *996* photodiode array detector; for anion exchange,

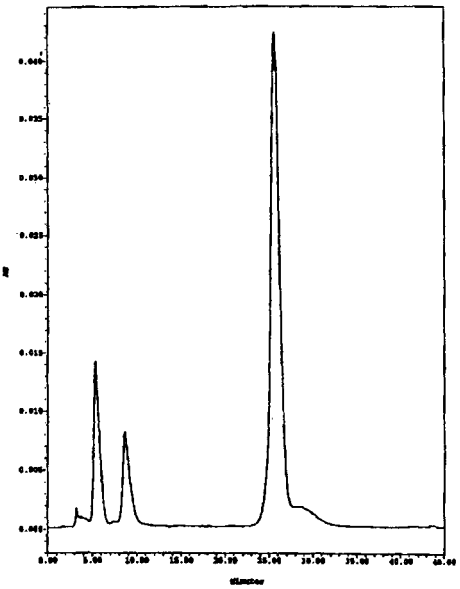


Fig. 4. *Reversed-phase HPLC of the enzymatic digestion products of* **11**. C_d (5.5 min), $isoG_d$ (9.0 min), A_d (26 min).

Alltech Associates column (*Macrosphere 300A WAX*, 7 μm, 4.6 × 250 mm); for reversed-phase chromatography, two *Waters* columns (*Nova-Pak HR C_{18} 60A*, 6 μm, 7.8 × 300 mm, or *Nova-Pak C_{18} 60A*, 4 μm, 3.9 × 150 mm). NMR Spectra: *Varian-XL-300* spectrometer at 300 MHz referenced to $SiMe_4$ (^1H), at 75.4 MHz and referenced to $CDCl_3$ (^{13}C) and at 121.4 MHz with H_3PO_4 as standard (^{31}P).

2,5'-Anhydrothymidine (**3**). The reported procedure [21] with a reaction time of 24 h resulted in formation of decomposition products; the reaction was nearly complete after 3.5 h (TLC (CH_2Cl_2/MeOH 3:1): R_f 0.42). Further, much solid product was discarded in the filtration of the hot reaction mixture following the literature workup. The procedure was improved as follows: According to [21], 5'-*O*-(*p*-tolylsulfonyl)thymidine (75.7 mmol, 30.0 g) was prepared and then suspended in dry MeCN (450 ml). DBU (1.1 equiv., 83.32 mmol, 12.66 g, 12.44 ml) was added and the mixture refluxed for 3.5 h. Then more DBU (0.1 equiv, 1.2 ml) was added, the mixture refluxed for 1 more h, then cooled to r.t., and filtered, and the filtrate collected. The solid was refluxed with MeOH (150 ml) for 5 min and the mixture filtered while hot. The solid was again refluxed with MeOH (50 ml) for 5 min and filtered. The combined filtrates were evaporated and triturated with MeCN. The solid product (9.86 g) was dried *in vacuo* overnight. The MeCN filtrate obtained above was evaporated, acetone (150 ml) added, and the mixture kept for several hours. The product (1.60 g) was isolated as long needles and dried *in vacuo*. The products were combined (11.5 g, 68%). ^1H-NMR (D_2O/CD_3OD): 1.74 (*s*, Me–C(5)); 2.21–2.52 (*m*, 2H–C(2')); 4.00 (*d*, 1H–C(5')); 4.28 (*m*, H–C(4')); 4.38 (*d*, 1H–C(5')); 4.50 (*m*, H–C(3')); 5.84 (*d*, H–C(1')); 7.75 (*s*, H–C(6)). ^{13}C-NMR (D_2O/CD_3OD): 13.4; 43.2; 73.0; 76.5; 87.5; 95.7; 119.7; 142.1; 159.1; 175.5.

2'-Deoxy-5-methylisocytidine (**1d**). The yield of the reported procedure [4] was greatly improved by the use of a 600-ml pressure reactor (heater, mechanical stirrer; *Parr* instrument) which also allowed the reaction to be easily scaled up: Compound **3** (43.0 mmol, 9.63 g) was added to MeOH saturated at 0° with NH_3 (250 ml), and the mixture was placed in the pressure reactor and heated to 103° for 4 h. The reactor was cooled to 0° and opened, the solvent evaporated and the solid product taken up in MeCN and filtered. The white powder (10.3 g, 99%) was dried *in vacuo* overnight. ^1H-NMR (D_2O/CD_3OD): 1.61 (*s*, Me); 2.20 (*m*, 2H–C(2')); 3.58 (*m*, 2H–C(5')); 3.80 (*m*, H–C(4')); 4.21 (*m*, 1H–C(3')); 5.67 (*t*, H–C(1')); 7.39 (*s*, H–C(6)). ^{13}C-NMR (D_2O/CD_3OD): 13.7; 39.2; 61.7; 71.0; 87.9; 89.7; 115.9; 137.3; 155.9; 174.9.

N-*Benzoyl-2'-deoxy-5-methylisocytidine* (**1e**). A modified literature procedure [4] was inimicable to large-scale synthesis because of the large excess of PhCOCl required and the associated cumbersome purification; the excess of benzoyl chloride required could be reduced by addition of 4-(dimethylamino)pyridine (DMAP): Compound **1d** (1.77 mmol, 4.27 mg) was dissolved in anh. pyridine (15 ml) and DMAP (0.5 equiv., 0.886 mmol, 108 mg).

PhCOCl (5 equiv., 8.86 mmol, 1.03 ml) and Et_3N (5 equiv., 8.86 mmol, 1.23 ml) were added at 0°. The mixture was stirred at r.t. for 45 min (TLC ($CHCl_3$/MeOH 9:1): R_f 0.87). Aq. $NaHCO_3$ soln. was added at 0°, adjusting the pH to 7. The soln. was stirred at r.t. for 30 min and then extracted with $CHCl_3$ (3 × 50 ml). The combined org. layers were washed ($NaHCO_3$ soln. and H_2O), dried (Na_2SO_4), and evaporated. The residue was dissolved in pyridine/MeOH 3:1 (9 ml), and a soln. of 9 equiv. NaOH (16.0 mmol, 638 mg) in H_2O/MeOH 1:1 (20 ml) was added at 0°. After 10 min stirring at r.t., phosphate buffer soln. (5 ml) was added and the mixture evaporated. The residue was purified by CC (silica gel, $CHCl_3$/MeOH 9:1): 403 mg (66%) of **1e**. White solid. ^{1}H-NMR (D_2O/CD_3OD): 1.75 (*s*, Me); 2.08, 2.40 (2*m*, 2H–C(2′)); 3.63 (*m*, 2H–C(5′)); 3.92 (*m*, H–C(4′)); 4.28 (*m*, H–C(3′)); 6.58 (*m*, H–C(1′)); 7.20–7.40 (*m*, 3 H, Ph); 7.82 (*s*, H–C(6)); 7.92 (*d*, 2 H, Ph).

N²-Benzoyl-2′-deoxy-5′-(4,4′-dimethoxytrityl)-5-methylisocytidine (**1f**). The reported procedure [4] (12 h at r.t.) gave mainly unreacted starting material **1e**, even after a 20 h reaction time; better results were achieved at higher temp., with increased excess of $(MeO)_2$TrCl, and by extraction with AcOEt instead of Et_2O. Thus, to a soln. of **1e** (1.54 mmol, 530 mg) in dry pyridine (20 ml) and DMAP (0.25 equiv., 0.384 mmol, 46.8 mg), Et_3N (2 equiv., 3.1 mmol, 310 mg, 0.43 ml) and $(MeO)_2$TrCl (1.8 equiv., 2.77 mmol, 936 mg) were added. The mixture was stirred at 40° for 3.5 h and at r.t. for 1 h. MeOH (3 ml) was added to quench the reaction, followed by aq. $NaHCO_3$ soln. (50 ml). The mixture was extracted with AcOEt (1 × 50 ml, 3 × 30 ml), the extract dried (Na_2SO_4) and evaporated, and the residue purified by CC (CH_2Cl_2/MeOH 98.5:1.5; R_f 0.27): yellowish foam (726 mg, 73%). ^{1}H-NMR ($CDCl_3$): 1.49 (*s*, Me–C(5)); 2.42, 2.70 (2*m*, 2H–C(2′)); 3.50 (*dd*, 2H–C(5′)); 3.74 (*s*, 2 MeO); 4.22 (*m*, H–C(4′)); 4.68 (*m*, H–C(3′)); 6.81 (*dd*, 4 H, $(MeO)_2$*Tr*); 6.91 (*t*, H–C(1′)); 7.20–7.46 (*m*, 12 H, Ph, $(MeO)_2$*Tr*); 7.88 (*s*, H–C(6)); 8.18 (*d*, 2 H, Ph). ^{13}C-NMR ($CDCl_3$): 12.3; 41.7; 55.1; 55.2; 63.0; 71.3; 71.4; 86.2; 86.3; 86.9; 113.2; 114.7; 127.1; 127.91; 127.97; 128.0; 129.4; 130.0; 132.1; 135.1; 135.2; 135.5; 144.2; 152.5; 158.6; 161.0; 177.4.

N²-Benzoyl-2′-deoxy-5′-O-(4,4′-dimethoxytrityl)-5-methylisocytidine 3′-O-(2-Cyanoethyl Diisopropylphosphoramidite) (**1c**). The reaction time of the reported procedure [4] was shortened to avoid formation of decomposition products, and the product was purified by reprecipitation to remove traces of phosphoramidite by-products: A soln. of **1f** (co-evaporated from MeCN; 6.16 mmol, 3.98 g) in CH_2Cl_2 was cooled to 0°, and (i-Pr)$_2$EtN (5.5 equiv., 33.9 mmol, 4.37 g, 5.89 ml) and 2-cyanoethyl diisopropylphosphoramidochloridite (1.4 equiv., 8.62 mmol, 2.04 g, 2.01 ml) were added *via* syringe. The mixture was stirred for 10 min at 0° and then 30 min at r.t. Aq. $NaHCO_3$ soln. (100 ml) was added and the mixture extracted with CH_2Cl_2/Et_3N 98:2 (3 × 70 ml). The org. layer was dried (Na_2SO_4) and evaporated and the residue purified by CC (CH_2Cl_2/AcOEt/Et_3N 7:3:1; R_f 0.84 and 0.86, 2 diastereoisomers). The product was dissolved in CH_2Cl_2 (10 ml), this soln. added slowly dropwise to strongly stirred hexane (700 ml) at r.t., the mixture decanted, and the solid product dried *in vacuo*: pale-yellow foam (3.86 g, 74%). ^{1}H-NMR ($CDCl_3$): 1.08–1.24 (*m*, 2 *Me*$_2$CH); 1.47, 1.51 (2*s*, Me–C(5)); 2.34–2.81 (*m*, 2H–C(2′), CH_2CN); 3.37 (*dd*), 3.54–3.89 (*m*, 2H–C(5′), 2 Me$_2$C*H*, OC*H*$_2$CH$_2$CN); 3.78 (*d*, 2 MeO); 4.27 (*m*, H–C(4′)); 4.69 (*m*, H–C(3′)); 6.84 (*dd*, 4 H, $(MeO)_2$*Tr*); 6.91 (*m*, H–C(1′)); 7.22–7.54 (*m*, 12 H, $(MeO)_2$*Tr*, Ph); 7.91, 7.95 (2*s*, H–C(6)); 8.20 (*m*, 2 H, Ph). ^{13}C-NMR ($CDCl_3$): 12.3; 20.2; 24.5; 40.6; 43.1; 43.4; 55.1; 58.0; 62.7; 73.3; 85.7; 86.0; 86.9; 113.2; 114.9; 117.3; 117.4; 127.1; 127.9; 128.0; 129.3; 130.0; 132.0; 135.1; 135.2; 137.0; 137.1; 144.1; 152.7; 152.8; 158.7; 160.7; 177.2. ^{31}P-NMR ($CDCl_3$): 149.24; 150.28.

N,N-*Diisobutylformamide Dimethyl Acetal.* According to a slightly modified literature procedure [22][23], a mixture of *N,N*-dimethylformamide dimethyl acetal (754 mmol, 100 ml) and diisobutylamine (754 mmol, 131 ml) was refluxed in an oil bath at 105–115° for 5 days. After evaporation of the volatile compounds, the product was distilled under reduced pressure using a 35-cm column: 53.3 g (35%). B.p. 92°/17 Torr. ^{1}H-NMR ($CDCl_3$): 0.84–0.92 (*m*, 2 *Me*$_2$CHCH$_2$), 1.69 (*m*, 2 Me$_2$C*H*CH$_2$); 2.35 (*m*, 2 Me$_2$CHC*H*$_2$); 3.34 (*s*, 2 MeO); 4.47 (*s*, CH). ^{13}C-NMR ($CDCl_3$): 20.7, 27.0, 54.0, 56.8, 112.7, 112.8.

2′-Deoxy-N²-[(diisobutylamino)methylidene]-5-methylisocytidine (**1h**). According to [18][23], **1d** (30.9 mmol, 7.45 g) was coevaporated with DMF and then suspended in dry DMF (150 ml). *N,N*-Diisobutylformamide dimethyl acetal (1.5 equiv., 46.4 mmol, 9.41 g) was added, and the suspension became a clear soln. within 10 min. It was stirred at r.t. for 1 h. Then MeOH (5 ml) was added and the mixture evaporated. The residue was purified by CC (silica gel, $CHCl_3$/MeOH 82.5:17.5; R_f 0.54): white solid (10.8 g, 92%). ^{1}H-NMR ($CDCl_3$): 0.90 (*m*, 2 *Me*$_2$CHCH$_2$); 1.95 (*s*, Me–C(5)); 1.98–2.39 (*m*, 1H–C(2′), 2 Me$_2$C*H*CH$_2$); 3.11 (*m*, 1H–C(2′)); 3.26–3.50 (*m*, 2 Me$_2$CHC*H*$_2$), 3.73–3.88 (*m*, 2H–C(5′)); 3.62 (*m*, H–C(4′)); 4.40 (*m*, H–C(3′)); 6.69 (*dd*, H–C(1′)); 8.04 (*s*, H–C(6)); 8.72 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): 14.0; 20.0; 20.6; 27.7; 28.2; 42.6; 54.8; 61.3; 62.6; 71.8; 88.2; 88.3; 89.1; 117.5; 137.0; 137.1; 159.2; 160.6; 175.1.

2′-Deoxy-N²-[(diisobutylamino)methylidene]-5′-(4,4′-dimethoxytrityl)-5-methylisocytidine (**1i**). Compound **1h** (28.18 mmol, 10.71 g) was co-evaporated with pyridine and dissolved in dry pyridine (150 ml). DMAP (0.25 equiv., 7.05 mmol, 860 mg), $(MeO)_2$TrCl (1.15 equiv., 32.4 mmol, 11.0 g; 11.6 g, 95%), and Et_3N (2 equiv., 56.4 mmol, 7.84 ml) were added, and the mixture was stirred at r.t. for 2.5 h. MeOH (5 ml) was added and the

mixture evaporated. The residue was partitioned in aq. $NaHCO_3$ soln./AcOEt and extracted with AcOEt (3 × 200 ml). The combined org. layer was dried (Na_2SO_4) and evaporated and the residue purified by CC (silica gel, AcOEt/MeOH/Et_3N 80:2:1; R_f 0.21): yellow foam (9.97 g, 52%). ^{1}H-NMR ($CDCl_3$): 0.85 (*m*, 2 Me_2CHCH_2); 1.62 (*s*, Me–C(5)); 1.83–2.47 (*m*, 2H–C(2'), 2 Me_2CHCH_2); 2.98–3.55 (*m*, 2H–C(5'), 2 Me_2CHCH_2); 3.78 (2*s*, 2 MeO); 4.09 (*m*, H–C(4')); 4.58 (*m*, H–C(3')); 6.74 (*dd*, H–C(1')); 6.80 (*m*, 4 H, $(MeO)_2$*Tr*); 7.14–7.34 (*m*, 9 H, $(MeO)_2$*Tr*); 7.68 (*s*, H–C(6)); 8.54 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): 13.8; 19.7; 20.3; 26.4; 27.3; 41.9; 53.6; 55.1; 60.2; 63.5; 71.6; 85.9; 86.4; 86.7; 113.1; 117.7; 127.0; 127.9; 128.1; 129.1; 129.0; 133.4; 135.5; 136.0; 144.4; 157.3; 158.6; 159.0; 172.6.

*2'-Deoxy-*N^2*-[(diisobutylamino)methylidene]-5'-(4,4'-dimethoxytrityl)-5-methylisocytidine 3'-(2-Cyanoethyl Diisopropylphosphoramidite)* (**1j**). To a soln. of **1i** (13.4 mmol, 9.11 g) in CH_2Cl_2, *N,N*-diisopropylethylamine (5.5 equiv., 73.5 mmol, 12.8 ml) and 2-cyanoethyl diisopropylphosphoramidochloridite (1.1 equiv., 14.7 mmol, 3.28 ml) were added *via* syringe at 0°. The mixture was stirred at 0° for 5 min and at r.t. for 15 min. Aq. $NaHCO_3$ soln. (150 ml) was added and the mixture extracted with CH_2Cl_2/Et_3N 98:2. The combined org. layer was dried (Na_2SO_4) and evaporated and the residue purified by CC (silica gel, CH_2Cl_2/AcOEt/Et_3N 7:3:1; R_f 0.60). The obtained product was further purified by reprecipitation. A 50% soln. of **1j** in CH_2Cl_2/Et_3N 2:1 was added dropwise into vigorously stirred hexane causing the product to precipitate. Drying under vacuum gave 6.63 g (56%) of white foam. The hexane soln. was evaporated, the residue purified by CC and further purified by reprecipitation to give another 2.64 g (22.4%). Total yield: 9.27 g (79%). ^{1}H-NMR ($CDCl_3$): 0.82–0.96 (*m*), 1.04 (*d*), 1.13–1.20 (*m*), 1.22–1.29 (*m*, 24 H, Me_2CHCH_2, Me_2CH); 1.62 (*s*, Me–C(5)); 1.90–2.61 (*m*, 6 H, 2H–C(2'), CH_2CH_2CN, Me_2CHCH_2); 3.11–3.84 (*m*, 16 H, MeO (3.77), Me_2CH, Me_2CHCH_2, CH_2CH_2CN, 2H–C(5')); 4.20 (*m*, H–C(4')); 4.61 (*m*, H–C(3')); 6.74 (*m*, H–C(1')); 6.82 (*m*, 4 H, $(MeO)_2$*Tr*); 7.16–7.36, 7.40–7.46 (*m*, 9 H, $(MeO)_2$*Tr*); 7.69, 7.77 (2*s*, H–C(6)); 8.88 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): 13.4; 19.5; 19.9; 20.1; 24.2; 26.1; 27.0; 40.6; 40.8; 42.8; 43.0; 53.3; 54.9; 57.8; 58.0; 59.9; 62.7; 63.0; 72.7; 73.6; 85.0; 85.4; 86.1; 86.5; 112.9; 117.1; 117.7; 126.8; 127.7; 127.9; 127.9; 129.8; 133.0; 135.1; 135.2; 144.1; 157.1; 157.1; 158.4; 158.9; 172.3. ^{31}P-NMR ($CDCl_3$): 151.829; 152.591.

N^2*-Benzoyl-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-5-methylisocytidine 3'-*O*-(2-Cyanoethyl Phosphonate)* (**1l**). As described for similar reactions [15], **1c** (1 mmol, 847 mg) was dissolved in MeCN (5 ml), and 1*H*-tetrazole (0.5 mmol, 35 mg) in H_2O (0.1 ml) was added. The mixture was stirred at r.t. for 1.5 h and then evaporated. The residue was extracted ($NaHCO_3$ soln./AcOEt), the org. layer dried (Na_2SO_4) and evaporated, and the residue purified by reprecipitation from CH_2Cl_2 into hexanes (659 mg, 0.863, 86%). TLC (AcOEt/MeOH/Et_3N 80:2:1): R_f 0.55. ^{1}H- and ^{13}C-NMR: no i-Pr group. ^{31}P-NMR: no signals at 149 and 150 (*cf.* **1c**); instead, signals at 11.13 and 11.27 ppm.

N^2*-Benzoyl-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-5-methylisocytidine* (**1f**). Similarly to [16], **1l** (0.685 mmol, 523 mg) was treated with 10 ml of 2M KF in MeOH and stirred at r.t. for 5 h. The reaction was quenched with sulfur, the solvent evaporated, and the residue worked up by extraction with $NaHCO_3$ soln./AcOEt. The org. layer was dried (Na_2SO_4) and evaporated, and the residue purified by CC ($CHCl_3$/MeOH 98.5:1.5, R_f 0.30): light-yellow foam (249 mg, 56%). NMR: identical with that of **1f** obtained above.

2'-Deoxy-5'-(4,4'-dimethoxytrityl)-5-methylisocytidine (**1m**). Compound **1f** (0.167 mmol, 108 mg) was treated with hydrazine monohydrate (1 ml) in pyridine (1 ml). The two-layer mixture was vigorously stirred for 5¾ h, then H_2O was added and the mixture extracted with $CHCl_3$. The org. layer was dried (Na_2SO_4) and evaporated and the residue purified by CC ($CHCl_3$/MeOH, 82.5:17.5, R_f 0.34): white foam (49 mg, 54%). ^{1}H-NMR ((D_6)DMSO): 1.42 (*s*, Me–C(5)); 2.28 (*m*, 2H–C(2')); 3.20 (*m*, 2H–C(5')); 3.77 (*s*, 2 MeO); 3.88 (*m*, H–C(4')); 4.37 (*m*, H–C(3')); 5.40 (br., OH), 5.98 (*dd*, H–C(1')); 6.83 (*m*, 4 H, $(MeO)_2$*Tr*); 7.21–7.40 (*m*, 9 H, $(MeO)_2$*Tr*. ^{13}C-NMR ((D_6)DMSO): 13.2 (C(2') superimposed by DMSO); 55.0; 55.1; 63.3; 69.9; 85.5; 85.8; 113.2; 114.7; 126.8; 127.7; 127.9; 129.7; 132.4; 135.3; 135.4; 144.7; 154.2; 158.1; 168.6.

Nucleoside **1m** can be employed for formamidine protection and further for phosphoramidation.

2'-Deoxyisoguanosine (**2a**) was prepared from 2-amino-2'-deoxyadenosine (**7a**) in 81% yield using a slightly modified literature method [24].

*2'-Deoxy-*N^6*-[(diisobutylamino)methylidene]isoguanosine* (**2b**). Compound **2a** (0.807 mmol, 215.4 mg) was co-evaporated with DMF and dissolved in anh. DMF (5 ml). *N,N*-Diisobutylformamide dimethyl acetal (1.4 equiv., 1.58 mmol, 321 mg) was added and the mixture stirred at r.t. for 6.5 h. After evaporation, the residue was purified by CC (silica gel, $CHCl_3$/MeOH 82.5:17.5, R_f 0.35): off-white foam (315 mg, 96%). ^{1}H-NMR ($CDCl_3$): 0.67, 0.77 (2*s*, 2 Me_2CHCH_2); 1.89–2.21 (*m*, 2 Me_2CHCH_2); 2.40, 2.85 (2*m*, 2H–C(2')); 3.27, 3.45 (2*m*, 2 Me_2CHCH_2); 3.67–3.95 (*m*, 2H–C(5')); 4.17 (*m*, H–C(4')); 4.76 (*m*, H–C(3')); 6.30 (*t*, J = 6.9, H–C(1')); 7.90 (*s*, H–C(8)); 9.42 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): 19.5; 19.9; 26.8; 26.9; 39.8; 52.9; 60.3; 62.6; 71.7; 85.9; 88.6; 115.4; 140.9; 154.2; 156.1; 157.4; 162.4.

2'-Deoxy-N^6-[(diisobutylamino)methylidene]-3',5'-bis-O-(trimethylsilyl)isoguanosine (**2c**). Compound **2b** (0.775 mmol, 315 mg) was co-evaporated with pyridine and dissolved in anh. THF (15 ml). Et_3N (3 equiv., 2.32 mmol, 0.323 ml) and Me_3SiCl (3 equiv., 2.32 mmol, 0.295 ml) were added, and the mixture was stirred at r.t. overnight (15 h). MeOH (2 ml) was added and the mixture evaporated. The residue was extracted (dil. $NaHCO_3$ soln./AcOEt) and the org. layer dried (Na_2SO_4) and evaporated. The product was pure by TLC ($CHCl_3$/MeOH 9:1, R_f 0.52) and the white foam (328 mg, 77%) used without further purification. ^{1}H-NMR ($CDCl_3$): 0.06 (*s*, 2 Me_3Si); 0.87 (*m*, 2 Me_2CHCH_2); 1.84–2.13 (*m*, 2 Me_2CHCH_2, 1H–C(2')); 2.96 (*m*, 1H–C(2')); 3.3 (*m*, Me_2CHCH_2); 3.40 (*m*, Me_2CHCH_2); 3.63 (*m*, 1H–C(5')); 3.84 (*m*, 1H–C(5')); 3.99 (*m*, H–C(4')); 4.56 (*m*, H–C(3')); 6.10 (*dd*, H–C(1')); 7.55 (*s*, H–C(8)); 9.21 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): –0.2; 19.4; 19.8; 26.2; 26.8; 39.9; 52.6; 60.0; 62.8; 73.3; 86.8; 89.9; 115.9; 140.2; 154.3; 156.2; 157.1; 161.6.

2'-Deoxy-N^6-[(diisobutylamino)methylidene]-O^2-(diphenylcarbamoyl)-3',5'-bis-O-(trimethylsilyl)isoguanosine (**7b**). Compound **2c** (0.596 mmol, 328 mg) was co-evaporated with pyridine and dissolved in anh. pyridine (10 ml). Diphenylcarbamoyl chloride (2 equiv., 1.19 mmol, 276 mg) and (i-Pr)$_2$EtN (1.7 equiv., 1.01 mmol, 0.176 ml) were added, and the mixture was stirred at r.t. for 4 h. MeOH (1 ml) was added, the mixture evaporated, the residue extracted ($NaHCO_3$/$CHCl_3$), the org. layer dried (Na_2SO_4) and evaporated, and the residue purified by CC (CH_2Cl_2/(MeOH 98.5:1.5; R_f 0.48): off-white foam (332 mg, 75%). ^{1}H-NMR ($CDCl_3$): 0.02 (*s*, 2 Me_3Si); 0.77 (*m*, 2 Me_2CHCH_2); 1.74–2.15 (*m*, 2 Me_2CHCH_2, 1H–C(2')); 2.75 (*m*, 1H–C(2')); 3.00 (*m*, Me_2CHCH_2); 3.41 (*m*, Me_2CHCH_2); 3.64–3.88 (*m*, 2H–C(5')); 3.96 (*m*, H–C(4')); 4.58 (*m*, H–C(3')); 6.22 (*dd*, H–C(1')); 7.14–7.50 (*m*, 10 H, Ph); 8.89 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): –0.6; 19.1; 19.5; 25.6; 26.5; 40.4; 52.0; 59.3; 62.0; 72.3; 75.8; 88.8; 125.0; 125.8; 126.3; 128.3; 140.8; 141.7; 151.2; 151.5; 155.0; 159.2; 161.0.

2'-Deoxy-N^6-[(diisobutylamino)methylidene]-O^2-(diphenylcarbamoyl)isoguanosine (**7c**). a) *From* **7b**. To a soln. of **7b** (0.445 mmol, 332 mg) in THF (10 ml), 1M Bu_4NF in THF (2.2 equiv., 0.98 mmol, 0.98 ml) was added. The mixture was stirred at r.t. for 1.5 h and then evaporated. The residue was extracted (H_2O/$CHCl_3$), the org. layer dried (Na_2SO_4) and evaporated, and the product purified by CC ($CHCl_3$/MeOH 9:1; R_f 0.38): off-white foam (238 mg, 89%).

b) *From* **9c**. To a soln. of **9c** (2.34 mmol, 1.97 g) in anh. THF (100 ml), 1M Bu_4NF in THF (2.2 equiv., 5.15 mmol, 5.15 ml) was added. The mixture was stirred at r.t. for 2 h and then evaporated. The residue was diluted with H_2O (50 ml) and extracted with $CHCl_3$. The org. layer was dried (Na_2SO_4) and evaporated, and the residue purified by CC (silica gel, $CHCl_3$/MeOH 9:1): light-yellow foam (1.25 g, 89%). ^{1}H-NMR ($CDCl_3$): 0.81–1.02 (*m*, 2 Me_2CHCH_2); 1.86–2.18 (*m*, 2 Me_2CHCH_2); 2.20 (*m*, 1H–C(2')); 2.62 (*m*, 1H–C(2')); 3.12 (*d*, Me_2CHCH_2); 3.43 (*m*, Me_2CHCH_2); 3.75–3.98 (*m*, 2H–C(5')); 4.09 (*m*, H–C(4')); 4.59 (*m*, H–C(3')); 5.92 (*m*, H–C(1')); 7.08–7.50 (*m*, 2 Ph); 7.74 (*s*, H–C(8)); 8.92 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): 19.6; 20.1; 26.0; 27.0; 40.7; 52.4; 59.8; 63.1; 72.6; 72.7; 87.3; 88.5; 125.4; 126.6; 126.6; 128.8; 141.8; 141.9; 150.9; 152.3; 154.8; 159.6; 161.3.

2'-Deoxy-N^6-[(diisobutylamino)methylidene]-5'-(4,4'-dimethoxytrityl)-O^2-(diphenylcarbamoyl)isoguanosine (**7d**). Compound **7c** (2.08 mmol, 1.25 g) was co-evaporated with pyridine, dissolved in anh. pyridine (50 ml) and DMAP (0.25 equiv., 0.521 mmol, 63.5 mg), $(MeO)_2TrCl$ (1.8 equiv., 3.75 mmol; 1.34 g, 95%) and Et_3N (3 equiv., 6.25 mmol, 0.869 ml) were added. The mixture was stirred at r.t. for 4.5 h, then MeOH (5 ml) was added and the mixture evaporated. The residue was diluted with AcOEt and extracted (aq. $NaHCO_3$ soln./AcOEt), the combined org. layer dried (Na_2SO_4) and evaporated, and the residue purified by CC (silica gel, $CHCl_3$/MeOH 98.5:1.5, then 90:10; R_f 0.79): light-yellow foam (1.42 g, 76%). ^{1}H-NMR ($CDCl_3$): 0.90 (*dd*, 2 Me_2CHCH_2); 1.91–2.18 (*m*, 2 Me_2CHCH_2); 2.51 (*m*, 2H–C(2')); 3.18 (*d*, Me_2CHCH_2); 3.30 (*m*, Me_2CHCH_2); 3.54 (*m*, 2H–C(5')); 3.72 (*s*, 2 MeO); 4.09 (*m*, H–C(4')); 4.54 (*m*, H–C(3')); 6.47 (*dd*, H–C(1')); 6.71–6.84 (*m*, 4 H, $(MeO)_2Tr$); 7.11–7.40 (*m*, 19 H, Ph, $(MeO)_2Tr$); 7.97 (*s*, H–C(8)); 8.97 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): 19.7; 20.0; 26.1; 27.0; 40.9; 52.6; 55.0; 55.0; 59.9; 63.8; 71.7; 71.8; 83.5; 83.6; 85.9; 86.3; 113.0; 124.4; 126.3; 126.7; 126.8; 127.6; 127.7; 127.7; 128.0; 128.8; 129.0; 129.8; 129.9; 135.5; 135.6; 139.6; 142.1; 144.5; 152.2; 152.3; 156.0; 158.3; 158.4; 159.5; 161.2.

2'-Deoxy-N^6-[(diisobutylamino)methylidene]-5'-(4,4'-dimethoxytrityl)-O^2-(diphenylcarbamoyl)isoguanosine 3'-(2-Cyanoethyl Diisopropylphosphoramidite) (**7e**). To a soln. of **7d** (0.199 mmol, 180 mg) in CH_2Cl_2 (3 ml) at 0°, (i-Pr)$_2$EtN (5.5 equiv., 1.1 mmol, 0.19 ml) and 2-cyanoethyl diisopropylphosphoramidochloridite (1.1 equiv., 0.22 mmol, 0.049 ml) were added. The mixture was stirred at r.t. for 30 min. Then aq. $NaHCO_3$ soln. was added, the mixture extracted with CH_2Cl_2/Et_3N 98:2, the combined org. layer dried (Na_2SO_4) and evaporated, and the residue purified by CC (silica gel, hexanes/$CHCl_3$/AcOEt/Et_3N 60:20:20:10 (R_f 0.53, long spot), then 40:25:25:10 (R_f 0.36, 0.28 for the two diastereoisomers)). This product was obtained as a light-yellow foam. The obtained product was dissolved in CH_2Cl_2/10% Et_3N and added dropwise into stirred hexanes. The resulting precipitate was dried *in vacuo*: white foam (162 mg, 73.9%). ^{1}H-NMR ($CDCl_3$): 0.87–1.22 (*m*, 24 H, Me_2CHCH_2, Me_2CH); 1.90–2.23 (*m*, 2 Me_2CHCH_2); 2.37–2.71 (3*m*, 4 H, 2H–C(2'), CH_2CH_2CN), 3.18–3.79

(*m*, Me_2CHCH_2, Me_2CH, 2H–C(5'), CH_2CH_2CN, MeO (*s*, 3.78)); 4.23 (*m*, H–C(4')); 4.73 (*m*, H–C(3')); 6.46 (*dd*, H–C(1')); 6.75–6.84 (*m*, 4 H, $(MeO)_2Tr$); 7.14–7.46 (*m*, 19 H, Ph, $(MeO)_2Tr$); 8.02, 8.09 (2*s*, H–C(8)); 8.99 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): 19.7; 20.0; 24.4; 26.1; 27.1; 40.2; 43.0; 43.2; 52.5; 55.0; 55.1; 58.1; 58.3; 59.8; 63.1; 63.3; 73.4; 83.5; 85.5; 86.3; 113.0; 117.3; 117.5; 124.5; 124.6; 126.2; 126.7; 127.7; 127.9; 128.0; 128.8; 129.9; 130.0; 135.5; 135.5; 139.7; 142.2; 144.4; 152.1; 152.3; 152.4; 156.1; 156.1; 158.3; 159.5; 161.3. ^{31}P-NMR ($CDCl_3$): 151.497; 151.527.

Isoguanosine (**2g**). To a suspension of 2-aminoadenosine (**7f**; 18 mmol, 5.0 g) in H_2O (160 ml) at 50°, $NaNO_2$ (3.86 equiv., 68.4 mmol, 4.72 g) in H_2O (30 ml) was added. Then, AcOH (6.93 equiv., 123 mmol, 7.02 ml) was added dropwise at 50°. The resulting clear soln. was stirred for 5 min and then diluted with H_2O (150 ml). Conc. aq. ammonia was added to pH 8, the mixture evaporated, and the remaining solid washed with H_2O: light-yellow powder (4.59 g, 91.4%). ^{1}H-NMR ((D_6)DMSO): 3.50–3.74 (*m*, 2H–C(5')); 3.98 (*m*, H–C(4')); 4.12 (*m*, H–C(3')); 4.54 (*m*, H–C(2')); 5.69 (*m*, H–C(1')); 7.97 (*s*, H–C(8)). ^{13}C-NMR ((D_6)DMSO): 61.9; 71.0; 73.2; 86.3; 87.9; 109.8; 138.38; 152.78; 153.58; 156.8.

N^6-*[(Diisobutylamino)methylidene]isoguanosine* (**2h**). Compound **2g** (16.2 mmol, 4.59 g) was co-evaporated with pyridine followed by DMF and suspended in dry DMF (100 ml). *N,N*-Diisobutylformamide dimethyl acetal (1.4 equiv., 22.7 mmol, 4.61 g) was added, the mixture stirred at r.t. for 23 h, then MeOH (3 ml) added, and the mixture evaporated. The residue was purified by CC (silica gel, $CHCl_3$/MeOH 82.5:17.5; R_f 0.38): light-yellow foam (6.17 g, 90%). ^{1}H-NMR ((D_6)DMSO): 0.81–0.98 (*m*, 2 Me_2CHCH_2); 1.97–2.19 (*m*, 2 Me_2CHCH_2); 3.28–3.50 (*m*, 2 Me_2CHCH_2); 3.60–3.78 (*m*, 2H–C(5')); 4.07 (*m*, H–C(4')); 4.25 (*m*, H–C(3')); 4.59 (*m*, 1H–C(2')); 5.79 (*m*, H–C(1')); 8.18 (*s*, H–C(8)); 9.21 (*s*, N=CH). ^{13}C-NMR ((D_6)DMSO): 20.2; 20.6; 26.8; 27.5; 53.2; 60.6; 62.3; 71.3; 74.1; 86.6; 88.5; 114.7; 141.9; 155.6; 157.4; 157.7; 163.1.

N^6-*[(Diisobutylamino)methylidene]-3',5'*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)isoguanosine* (**8**). Compound **2d** (0.761 mmol, 321 mg) was co-evaporated with pyridine and dissolved in dry pyridine (5 ml), 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.1 equiv., 0.837 mmol, 0.268 ml) added, and the mixture stirred at r.t. for 6 h. Aq. $NaHCO_3$ soln. (10 ml) was added and the mixture extracted ($CHCl_3$). The combined org. layer was dried (Na_2SO_4) and evaporated and the residue used for NMR analysis: mixture of desired **8** and the corresponding 5'-*O*-(3-hydroxy-1,1,3,3-tetraisopropyldisiloxan-1-yl) compound **2e**. Separation was possible but the yield was too poor for large-scale use.

Alternatively, according to [25], **2h** (0.3507 mmol, 148 mg) was co-evaporated with pyridine and dissolved in dry DMF (5 ml), 1*H*-imidazole (4 equiv., 1.40 mmol, 95.4 mg) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.1 equiv., 0.386 mmol, 0.123 ml) were added, and the mixture was stirred at r.t. until the reaction was complete (30 min). Aq. $NaHCO_3$ soln. (10 ml) was added and the mixture extracted ($CHCl_3$). The combined org. layer was dried (Na_2SO_4), and evaporated and the residue used for NMR analysis: **8**/**2e**.

N^6-*[(Diisobutylamino)methylidene]-2',3',5'-tris*-O-*(trimethylsilyl)isoguanosine* (**2i**). Compound **2h** (8.23 mmol, 3.48 g) was co-evaporated with pyridine and dissolved in anh. THF (100 ml). Et_3N (4 equiv., 32.9 mmol, 4.58 ml) and Me_3SiCl (4 equiv., 32.9 mmol, 4.18 ml) were added, and a white precipitate was formed immediately. The mixture was stirred at r.t. overnight (13 h). TLC ($CHCl_3$/MeOH 9:1): R_f 0.48 (**2i**); 0.27 (starting material). MeOH (2 ml) was added, the mixture evaporated, dil. aq. $NaHCO_3$ soln. added, and the mixture extracted with AcOEt. The org. layer was dried (Na_2SO_4), the solvent evaporated, and the obtained light-yellow foam (5.04 g, 96%) used in the next step without further purification. ^{1}H-NMR ($CDCl_3$): 0.10, 0.14, 0.18 (3*s*, 3 Me_3Si); 0.91–1.10 (*m*, 2 Me_2CHCH_2); 2.03–2.32 (*m*, 2 Me_2CHCH_2); 3.32–3.61 (*m*, 2 Me_2CHCH_2); 3.76–4.04 (*m*, 2H–C(5')); 4.16 (*m*, H–C(4')); 4.34 (*m*, H–C(3')); 4.58 (*m*, H–C(2')); 6.07 (*m*, H–C(1')); 8.04 (*s*, H–C(8)); 9.41 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): –0.8; 0.1; 19.7; 20.0; 26.4; 27.1; 52.8; 60.7; 61.0; 70.9; 75.5; 83.9; 87.6; 114.6; 139.5; 153.7; 157.7; 158.0; 161.9.

N^6-*[(Diisobutylamino)methylidene]*-O^2-*(diphenylcarbamoyl)-2',3',5'-tris*-O-*(trimethylsilyl)isoguanosine* (**7g**). Compound **2i** (7.91 mmol, 5.04 g) was co-evaporated with pyridine and dissolved in anh. pyridine (100 ml). $(i\text{-}Pr)_2EtN$ (1.7 equiv., 13.4 mmol, 2.34 ml) and diphenylcarbamoyl chloride (2 equiv., 15.8 mmol, 3.66 g) were added. The dark-orange soln. was stirred at r.t. for 2.25 h, then dil. aq. $NaHCO_3$ soln. added with ice-bath cooling, and the mixture extracted with $CHCl_3$. The org. layer was dried (Na_2SO_4) and evaporated and the residue purified by CC (CH_2Cl_2/MeOH 98.5:1.5): yellow foam (5.11 g, 78%). ^{1}H-NMR ($CDCl_3$): 0.10, 0.14, 0.18 (3*s*, 3 Me_3Si); 0.93 (*dd*, 2 Me_2CHCH_2); 1.93–2.20 (*m*, 2 Me_2CHCH_2); 3.18 (*d*, Me_2CHCH_2); 3.47–3.64 (*m*, Me_2CHCH_2); 3.70–4.05 (2*m*, 2H–C(5')); 4.12 (*m*, H–C(4')); 4.31 (*m*, H–C(3')); 4.51 (*m*, H–C(2')); 6.00 (*m*, H–C(1')); 7.22–7.40 (*m*, 2 Ph); 8.27 (*s*, H–C(8)); 8.94 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): –0.7; 0.1; 19.8; 20.1; 26.1; 27.2; 52.7; 59.9; 60.8; 70.4; 75.8; 83.8; 88.9; 123.7; 126.2; 126.9; 128.8; 140.5; 140.5; 142.3; 152.0; 152.4; 156.0; 159.4; 161.2.

N^6-*[(Diisobutylamino)methylidene]*-O^2-*(diphenylcarbamoyl)isoguanosine* (**7h**). Compound **7g** (6.13 mmol, 5.11 g) was dissolved in anh. THF (100 ml) and 1M Bu_4NF in THF (3.3 equiv., 20.2 mmol, 20.2 ml) added *via*

syringe. The mixture was stirred at r.t. for 1.5 h and then evaporated. H_2O was added and the mixture extracted with $CHCl_3$. The org. layer was dried (Na_2SO_4) and evaporated and the residue purified by CC (silica gel, $CHCl_3$/MeOH 9:1; R_f 0.27): light-yellow foam (3.44 g, 91%). ^{1}H-NMR ($CDCl_3$): 0.88 (*m*, 2 Me_2CHCH_2); 1.83–2.17 (*m*, 2 Me_2CHCH_2); 3.18 (*m*, Me_2CHCH_2); 3.37–3.58 (*m*, Me_2CHCH_2); 3.63–3.80 (2*m*, 2H–C(5′)); 4.16 (*m*, H–C(4′)); 4.29 (*m*, H–C(3′)); 4.82 (*m*, H–C(2′)); 5.37 (*m*, H–C(1′)); 7.18–7.48 (*m*, 2 Ph); 7.63 (*s*, H–C(8)); 8.99 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): 19.6; 20.1; 26.4; 27.0; 52.6; 60.0; 63.1; 72.4; 73.6; 87.1; 90.2; 125.4; 126.7; 127.1; 128.9; 141.8; 142.3; 151.0; 152.8; 154.8; 160.2; 161.4.

N^6-*[(Diisobutylamino)methylidene]*-O^2-*(diphenylcarbamoyl)-3′,5′*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)isoguanosine* (**9a**). Compound **7h** (5.29 mmol, 3.27 g) was co-evaporated with pyridine and dissolved in anh. pyridine (70 ml), 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.1 equiv., 5.82 mmol, 1.86 ml) added *via* syringe, and the mixture stirred for 16.25 h at r.t. The mixture was evaporated, dil. $NaHCO_3$ soln. added. The mixture extracted with $CHCl_3$, the org. layer dried (Na_2SO_4) and evaporated, and the residue purified by CC (silica gel, $CHCl_3$/MeOH 9:1; R_f 0.77): light-yellow foam (4.18 g, 92%). ^{1}H-NMR ($CDCl_3$): 0.90–1.19 (*m*, 40 H, Me_2CH, Me_2CHCH_2); 1.93–2.20 (*m*, 2 Me_2CHCH_2); 3.19 (*d*, Me_2CHCH_2); 3.57 (*m*, Me_2CHCH_2); 4.03–4.12 (*m*, 2H–C(5′), H–C(4′)); 4.49 (*m*, H–C(3′)); 4.78 (*m*, H–C(2′)); 6.00 (*m*, H–C(1′)); 7.18–7.40 (*m*, 2 Ph); 7.99 (*s*, H–C(8)); 8.98 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): 12.5; 17.3; 19.8; 20.1; 26.2; 27.1; 52.7; 60.0; 61.9; 71.0; 75.1; 82.0; 88.9; 89.0; 123.7; 125.0; 126.2; 128.9; 140.0; 140.1; 142.3; 149.8; 152.0; 156.2; 159.6; 161.4.

N^6-*[(Diisobutylamino)methylidene]*-O^2-*(diphenylcarbamoyl)-2′*-O-*(phenoxythiocarbonyl)-3′,5′*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)isoguanosine* (**9b**). Compound **9a** (3.42 mmol, 2.94 g) was co-evaporated with pyridine and dissolved in CH_2Cl_2 (120 ml). Pyridine (4 equiv., 13.7 mmol, 1.11 ml), DMAP (0.25 equiv., 0.855 mmol, 104 mg), and phenyl carbonochloridathioate (1.5 equiv., 5.1 mmol, 0.71 ml) were added. The mixture turned brown-red, was stirred at r.t. for 19.5 h, and then extracted ($CHCl_3$/dil. $NaHCO_3$ soln.). The org. layer was dried (Na_2SO_4) and evaporated and the residue purified by CC (one-step gradient of CH_2Cl_2/MeOH 82.5:1.5; R_f 0.44, then $CHCl_3$/MeOH 9:1): light-yellow foam (3.21 g, 94%). ^{1}H-NMR ($CDCl_3$): 0.87–1.12 (*m*, 40 H, Me_2CH, Me_2CHCH_2); 1.92–2.20 (*m*, 2 Me_2CHCH_2); 3.18 (*d*, Me_2CHCH_2); 3.54 (*m*, Me_2CHCH_2); 4.00–4.28 (*m*, 2H–C(5′), H–C(4′)); 4.95 (*m*, H–C(3′)); 6.26 (*m*, H–C(1′), H–C(2′)); 7.06–7.45 (*m*, 3 Ph); 8.06 (*s*, H–C(8)); 8.96 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): 12.7; 12.8; 12.9; 13.2; 17.0; 17.2; 19.8; 20.0; 26.2; 27.1; 52.7; 60.0; 60.7; 69.7; 82.1; 83.9; 86.4; 115.5; 121.4; 121.7; 124.8; 126.5; 126.9; 128.9; 129.4; 139.5; 142.2; 152.0; 153.4; 156.4; 159.5; 161.4; 193.6.

2′-Deoxy-N^6-*[(diisobutylamino)methylidene]*-O^2-*(diphenylcarbamoyl)-3′,5′*-O-*(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)isoguanosine* (**9c**). Compound **9b** (3.22 mmol, 3.21 g) was co-evaporated with toluene, dissolved in anhy. toluene (75 ml), and degassed with Ar for 30 min. In a second flask, 2,2′-azobis[isobutyronitrile] (AIBN; 0.5 equiv., 1.61 mmol, 264 mg) and Bu_3SnH (3.0 equiv., 9.65 mmol, 2.60 ml) in toluene (15 ml) were degassed with Ar for 30 min. The first flask was then heated to 80°, the AIBN/Bu_3SnH soln. added dropwise *via* syringe, and the mixture stirred at 100° for 3.5 h; gas evolution ceased after 20 min. After cooling to r.t., the mixture was evaporated, the residue extracted with dil. $NaHCO_3$ soln./$CHCl_3$, the org. layer dried (Na_2SO_4) and evaporated, and the residue purified by CC (silica gel, $CHCl_3$/MeOH 9:1; R_f 0.80): yellow oil (1.97 g, 73%). ^{1}H-NMR ($CDCl_3$): 0.78–1.11 (*m*, 40 H, Me_2CH, Me_2CHCH_2); 1.82–2.21, 2.52–2.66 (*m*, 2H–C(2′), 2 Me_2CHCH_2); 3.02–3.58 (*d*, 2 Me_2CHCH_2); 3.71–4.08 (*m*, H–C(4′), 2H–C(5′)); 4.72 (*m*,H–C(3′)); 6.29 (*m*, H–C(1′)); 7.04–7.56 (*m*, 2 Ph); 8.16 (*s*, H–C(8)); 8.90 (*s*, N=CH). ^{13}C-NMR ($CDCl_3$): 12.4; 12.7; 12.9; 13.2; 16.9; 17.0; 17.2; 17.4; 19.6; 19.7; 27.0; 40.1; 52.7; 59.8; 61.5; 69.5; 82.9; 85.0; 119.4; 125.8; 126.5; 127.1; 128.8; 139.7; 142.0; 151.4; 152.2; 156.1; 159.3; 161.1.

Ion-Exchange HPLC. Oligonucleotides were chromatographed by anion-exchange HPLC with an *Alltech Associates* column (*Macrosphere 300A WAx*, 7 μm, 4.6 × 250 mm), using either of the two gradients with A = 25 mM *Tris* · HCl (pH 7.5/MeCN 9:1 and B = 25 mM *Tris* · HCl (pH 7.5) 2M NaCl/MeCN 9:1 or C = 20 mM K_3PO_4 (pH 6.0) and D = 20 mM K_3PO_4 (pH 6.0), 2M KCl; flow rate 0.5 ml/min. For anal. purposes, 0.1 A_{260} units were injected. Semi-prep. separations were done with the 4.6-mm diameter column as well, by injecting up to 6 A_{260} units.

Anal. Reversed-Phase HPLC. Oligonucleotides were analyzed by reversed-phase HPLC with a *Waters Corporation* column (*Nova-Pak C_{18} 60A*, 4 μm, 3.9 × 150 mm), using a gradient with A = 100 mM (Et_3NH)OAc (pH 7.0) and B = MeCN; flow rate 0.5 ml/min; 0.1 A_{260} units were injected.

Semiprep. Reversed-Phase HPLC. After semiprep. ion-exchange HPLC with oligonucleotides, the solvent of collected fractions was lyophilized. The solns. were then further purified with a *Waters Corporation* column (*Nova-Pak HRC_{18} 60A*, 6 μm, 7.8 × 300 mm), using a gradient with A = 25 mM (Et_3NH)OAc (pH 7.0) and B = MeCN; flow rate 2.2 ml/min.

Reversed-Phase HPLC for Analysis of Nucleosides. Following digestion of oligonucleotides, the component nucleosides were separated by reversed-phase HPLC with a *Waters Corporation* column (*Nova-Pak C_{18} 60A*, 4 μm, 3.9 × 150 mm), using a gradient with *A* = 50 mM K_3PO_4 (pH 7.0) and *B* = 50 mM K_3PO_4 (pH 7.0)/MeOH 2:1; flow rate 0.5 ml/min. Order of eluting of 2′-deoxynucleosides: C_d, $isoC_d$, $isoG_d$, G_d, T_d, A_d.

Oligonucleotide Synthesis. Bottles to contain non-standard base phosphoramidites for the DNA synthesizer were washed, rinsed with acetone, and heated overnight at 120°. The bottles were then allowed to cool overnight in a dessicator over P_2O_5 and under vacuum of < 1 Torr. $IsoC_d$ phosphoramidite was introduced and the bottles containing phosphoramidite were again placed in the dessicator under vacuum overnight.

Oligonucleotide synthesis was performed by the DNA Synthesis Core of the University of Florida on a *PerSeptive Biosystems Expedite 8900* synthesizer, at the 0.2-μmol scale. The synthesis was done using the manufacturer's recommended procedure, except the concentrations of standard phosphoramidites were half the manufacturer's recommended concentration. All chemicals were from *PerSeptive Biosystems*. DNA Membrane 0.2 μm columns, also from *PerSeptive*, containing the 3′-terminal base of the desired oligonucleotide, were used (part numbers for each base: C = GEN050014, G = GEN050024, T = GEN050026, A = GEN050004). Anh. MeCN from *Glen Research* (part number 40-4050-45) was used to dilute the phosphoramidites (0.5 g in 10 ml for standard bases, 0.15 g in 1.5 ml for non-standard bases).

The synthesis protocols used are below. $isoC_d$ Phosphoramidite was coupled using the standard protocol, and $isoG_d$ phosphoramidite was coupled with an extended (600 s) coupling time.

At the completion of the synthesis, oligonucleotides were deprotected by incubating in 30% aq. ammonium hydroxide (1.5 ml) at 55° for 12 h. The sample was then centrifuged and the supernatant removed and dried to a pellet. After drying, 0.7 mM NaOAc (0.3 ml) was added, the soln. vortexed, and EtOH added (1 ml) at −20°. This soln. was stored at −20° for 15 min before being centrifuged (15000 *g*, 20 min). The supernatant was discarded and the pellet suspended in 80% $EtOH/H_2O$ (1 ml) and centrifuged again (15000 *g*, 20 min). The desalted oligonucleotide was then redissolved in 10 m*M Tris* · HCl, 0.4 mM EDTA, pH 7.7 (0.5 ml).

Coupling Efficiency by $(MeO)_2Tr$ Monitoring. A short oligomer having the sequence 5′-d(TTTTT-XXXXXTTTTT)-3′ (X = non-standard base) was synthesized at 0.2-μmol scale using the phosphoramidite to be evaluated. $(MeO)_2Tr$ Release at each coupling was monitored using the trityl viewer on an *Expedite 8909* synthesizer. The coupling efficiency of each of the three sections (T_5, X_5, and T_5) was then determined by using $100 \cdot 2^m$, where *m* is the slope of the linear regression line of natural logarithm of the absorbance value *vs.* the coupling number.

Enzymatic Digestion of Oligonucleotides. The enzymes used were alkaline phosphatase from bovine calf intestine (18.2 prot mg/ml in 50% glycerol, 5 mM $MgCl_2$, and 0.1 mM $ZnCl_2$, pH 7.5) and phosphordiesterase I from *Crotalus durissus terrificus* venom (0.16 mg/ml in 50% glycerol, 5 mM *Tris* · HCl, pH 7.5). A stock soln. of alkaline phosphatase was prepared by adding enzyme soln. (4 μl) to a soln. of 50 mM *Tris* · HCl, 10 mM $MgCl_2$, pH 8.3 (198 μl).

The oligonucleotide (0.1 *OD*) was dissolved in 0.1M *Tris* · HCl, 20 mM $MgCl_2$, pH 8.3 (2 μl). H_2O (2 μl), phosphodiesterase I (0.5 μl), and phosphatase stock soln. (0.5 μl) were added. The resulting soln. was incubated at 37° for 3 h, diluted with 1M $(Et_3NH)OAc$, (pH 7; 80 μl), filtered, and analyzed using reversed-phase HPLC. Composition of the oligonucleotide was verified by using the integrated absorbance of the component nucleosides with the following extinction coefficients ($M^{-1}\,cm^{-1}$): 15400 (A_d), 11700 (G_d), 7300 (C_d), 8800 (T_d), 10100 (U_d), 6300 ($isoC_d$), and 4600 ($isoG_d$).

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