Synthesis of 2′-*O*-[2-[(*N*,*N*-Dimethylamino)oxy]ethyl] Modified Nucleosides and Oligonucleotides

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A versatile synthetic route has been developed for the synthesis of 2′-*O*-[2-[(*N*,*N*-dimethylamino)-oxy]ethyl] (abbreviated as 2′-*O*-DMAOE) modified purine and pyrimidine nucleosides and their corresponding nucleoside phosphoramidites and solid supports. To synthesize 2′-*O*-DMAOE purine nucleosides, the key intermediate **B** (Scheme 1) was obtained from the 2′-*O*-allyl purine nucleosides (**13a** and **15**) via oxidative cleavage of the carbon−carbon bond to the corresponding aldehydes followed by reduction. To synthesize pyrimidine nucleosides, opening the 2,2′-anhydro-5-methyl-uridine **5** with the borate ester of ethylene glycol gave the key intermediate **B**. The 2′-*O*-(2-hydroxyethyl) nucleosides were converted, in excellent yield, by a regioselective Mitsunobu reaction, to the corresponding 2′-*O*-[2-[(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)oxy]ethyl] nucleosides (**18**, **19**, and **20**). These compounds were subsequently deprotected and converted into the 2′-*O*-[2-[(methyleneamino)oxy]ethyl] derivatives (**22**, **23**, and **24**). Reduction and a second reductive amination with formaldehyde yielded the corresponding 2′-*O*-[2-[(*N*,*N*-dimethylamino)oxy]ethyl] nucleosides (**25**, **26**, and **27**). These nucleosides were converted to their 3′-*O*-phosphoramidites and controlled-pore glass solid supports in excellent overall yield. Using these monomers, modified oligonucleotides containing pyrimidine and purine bases were synthesized with phosphodiester, phosphorothioate, and both linkages (phosphorothioate and phosphodiester) present in the same oligonucleotide as a chimera in high yields. The oligonucleotides were characterized by HPLC, capillary gel electrophoresis, and ESMS. The effect of this modification on the affinity of the oligonucleotides for complementary RNA and on nuclease stability was evaluated. The 2′-*O*-DMAOE modification enhanced the binding affinity of the oligonucleotides for the complementary RNA (and not for DNA). The modified oligonucleotides that possessed the phosphodiester backbone demonstrated excellent resistance to nuclease with $t_{1/2} > 24$ h.

Introduction

In antisense therapeutic technology, synthetic oligonucleotides that base-pair with messenger RNA encoding a disease-related protein specifically inhibit the synthesis of that protein.[1] Antisense oligonucleotides have demonstrated effective inhibition of many viral and cellular gene products, both in vitro and in vivo.[2] The first successful drug to emerge from this technology was ISIS-2922, "Vitravene," a 2′-deoxyoligonucleotide phosphorothioate.[3]

An ideal antisense oligonucleotide should have high binding affinity for the target RNA, should be resistant to nuclease digestion, should bind selectively to transport proteins, and should be cell permeable in vivo.[4] To achieve these goals, structural analogues of nucleic acids with modified heterocycle, sugar, and phosphodiester backbone moieties have been synthesized.[5] Some of the most successful analogues resulted from modification of the sugar at the 2′-position.[5] The 2′-*O*-modified oligonucleotides used with the "gapmer" technology[6] have emerged as the leading second generation candidates for clinical applications. Gapmer oligonucleotides have one or two 2′-*O*-modified regions and a 2′-deoxyoligonucleotide phosphorothioate region that allows RNase H digestion of mRNA. Among the various 2′-*O*-modified oligonucleotides reported in the literature, the 2′-*O*-(2-methoxyethyl)[7] modified oligonucleotides (2′-*O*-MOE) offer a 2 °C increase in melting temperature (T_m) per modification as a diester (2′-*O*-MOE/P═O) compared to

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(1) (a) Crooke, S. T. In *Basic Principles of Antisense Therapeutics*; Crooke, S. T., Ed.; Antisense Research and Application, Vol. 131, Springer: Berlin, 1998, pp 1−50. (b) Phillips, M. I. Ed.; *Methods Enzymol.* **2000**, *313*, 580; *314*, 646: *Antisense Technology*, Parts A and B. (c) Crooke, S. T.; Lebleu, B. *Antisense Research and Applications*; CRC Press Inc.: Boca Raton, FL, 1993. (d) Mesmaeker, A. D.; Häener, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* **1995**, *28*, 36−374.

(2) *Applied Antisense Oligonucleotide Technology*; Stein, C. A.; Krieg, A. M., Eds.; Wiley-Liss, Inc.: New York, 1998.

(3) Manoharan M. *Biochim. Biophys. Acta* **1999**, *1489*, 117−130.

(4) (a) Cook P. D. *Antisense Medicinal Chemistry*; Crooke, S. T., Ed.; Springer-Verlag: New York, 1998; Antisense Research and Application, Vol. 131, pp 51−101. (b) Cook, P. D. *Nucleosides Nucleotides* **1999**, *18*, 1141−1162. (c) Prakash, T. P.; Manoharan, M.; Fraser, A. S.; Kawasaki, A. M.; Lesnik, E. A.; Owens, S. R. *Tetrahedron Lett.* **2000**, *41*, 4855−4859, and references therein.

(5) Cook, P. D. *Annu. Rep. Med. Chem.* **1998**, *33*, 313−325.

(6) Monia, B. P.; Lesnik, E. A.; Gonzalez, C.; Lima, W. F.; McGee, D.; Guinosso, C. J.; Kawasaki, A. M.; Cook, P. D.; Freier, S. M. *J. Biol. Chem.* **1993**, *268*, 14514−14522.

(7) Martin, P. *Helv. Chim. Acta* **1995**, *78*, 486−504.

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Chart 1. Novel 2′-Carbohydrate Modifications

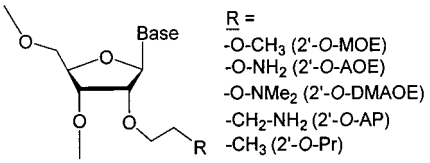
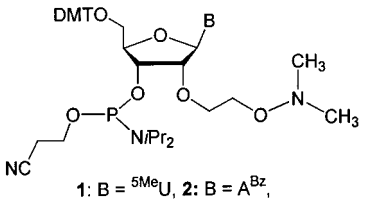


Chart 2. General Structure of 2′-*O*-DMAOE Phosphoramidites



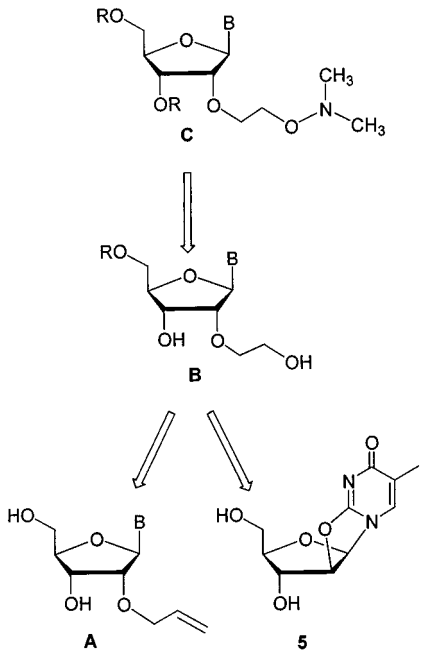
1: B = ^{5Me}U, **2:** B = A^{Bz},
3: B = G^{iBu}, **4**: B = $^{5Me}C^{Bz}$,
Bz = benzoyl, iBu = isobutyryl

the 2′-deoxyphosphorothioate (2′-H/P═S) compounds. This modification with a phosphodiester linkage exhibits resistance to snake venom phosphodiesterase (measured as the half-life of disappearance of the full-length oligonucleotide, $t_{1/2}$) at approximately the same level as a 2′-deoxyoligonucleotide phosphorothioate. Our recent[8] synthesis of the 2′-*O*-[2-[(*N*,*N*-dimethylamino)oxy]ethyl]-5-methyluridine modified oligonucleotides was an attempt to improve upon the 2′-*O*-MOE modification.

This new 2′-*O*-modification (abbreviated as 2′-*O*-DMAOE) was designed to improve the antisense properties of the 2′-*O*-MOE in three ways. First, we envisaged that this modification would maintain the C_3-endo sugar pucker shown by the 2′-*O*-MOE modification[10] thus retaining the "gauche effect."[9] This gauche effect results in preorganization of the 2′-*O*-MOE antisense single strand required for the enhanced hybridization to the RNA target compared to the 2′-deoxy compound. Second, these dialkyl modifications would be more lipophilic than the 2′-*O*-(2-aminoethyl) (abbreviated as 2′-*O*-AOE)[11] and 2′-*O*-MOE modifications, a characteristic affecting the protein binding and cellular permeation properties of oligonucleotides. Finally, the increased steric size would improve the nuclease resistance even more than that observed for the 2′-*O*-MOE and 2′-*O*-AOE modifications (Chart 1).

In this report, we describe the synthesis of all four basic building blocks, namely the 2′-*O*-DMAOE modified nucleosides containing the nucleobases A, ^{5Me}U, G, and ^{5Me}C as well as their 3′-phosphoramidites and their solid supports. We describe the syntheses of fully modified 2′-*O*-DMAOE oligonucleotides with these amidites. In addition, we studied the hybridization of the fully modified 2′-*O*-DMAOE oligonucleotides with complementary RNA and DNA. The stability of 2′-*O*-DMAOE oligonucleotides to snake venom phosphodiesterase (SVPD) was also investigated.

Scheme 1



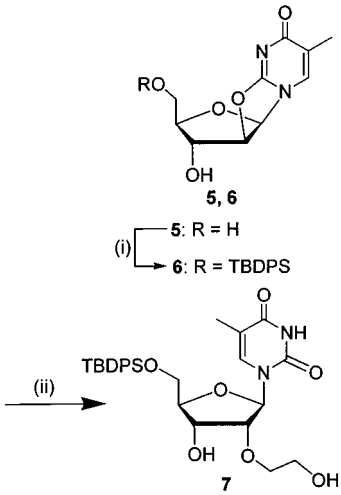
Results and Discussion

Synthesis of 2′-*O*-Modified Nucleoside Phosphoramidites and Solid Supports. The 2′-*O*-DMAOE nucleosides and their phosphoramidites synthesized in this study are shown in Chart 2. A general synthetic strategy planned for the syntheses of these amidites (Chart 2) is described in Scheme 1. The 2′-*O*-(2-hydroxyethyl) derivative (**B**, Scheme 1) was a convenient intermediate for the following reasons. First, this intermediate could be converted to an aminooxy derivative using the Mitsunobu[12] reaction. Second, the resulting, highly reactive aminooxy derivative could be converted into a methyleneaminooxy derivative, reduced to the monomethyl compound, and reductively alkylated with formaldehyde to give the dimethylaminooxy compound.[13] To synthesize pyrimidine nucleosides, **B** was obtained from the 2,2′-anhydro-5-methyluridine (**5**) via ring opening of the anhydro nucleoside by ethylene glycol following a method developed by Ross et al.[14] For the purine nucleosides, preferential 2′-*O*-alkylation gave the 2′-*O*-allyl derivatives (**A**, Scheme 1), which were converted to the 2′-*O*-(2-hydroxyethyl) derivatives. These 2′-modified nucleosides were then converted into the target 2′-*O*-DMAOE nucleosides and their corresponding phosphoramidites (Schemes 2−8).

The synthesis of 2′-*O*-(2-hydroxyethyl)-5-methyluridine (**7**) is described in Scheme 2. The 5′-hydroxy group of 2,2′-anhydro-5-methyluridine (**5**) was silylated with *tert*-butylchlorodiphenylsilane (TBDPSCl) in pyridine to produce **6** in good yield. The *tert*-butyldiphenylsilyl (TBDPS) group was used in preference to other silyl protecting groups[15] because of its high stability under acidic conditions. The product was next subjected to the ring opening

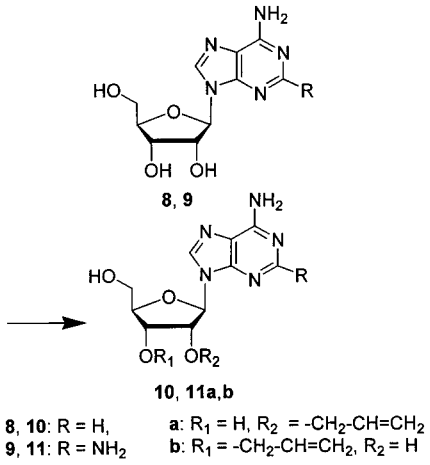
(8) Prakash, T. P.; Manoharan, M.; Kawasaki, A. M.; Lesnik, E. A.; Owens, S. R.; Vasquez, G. *Org. Lett.* **2000**, *2*, 3995−3998.
(9) Wolfe, S. *Acc. Chem. Res.* **1972**, *5*, 102−111.
(10) Teplova, M.; Minasov, G.; Tereshko, V.; Inamati, G. B.; Cook, P. D.; Manoharan, M.; Egli, M. *Nature Struct. Biol.* **1999**, *6*, 535−539.
(11) Kawasaki, A. M.; Casper, M. D.; Prakash, T. P.; Manalili, S.; Sasmor, H.; Manoharan, M.; Cook, P. D. *Tetrahedron Lett.* **1999**, *40*, 661−664.
(12) Mitsunobu, O.; Kimura, J.; Fujisawa, Y. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 245−247. (b) Montero, J. -L.; Criton, M.; Dewynter, G.-F.; Imbach, J.-L. *Tetrahedron Lett.* **1991**, *32*, 5357−5358.
(13) (a) Perbost, M.; Hoshiko, T.; Morvan, F.; Swayze, E.; Griffey, R. H.; Sanghvi, Y. S. *J. Org. Chem.* **1995**, *60*, 5150−5156. (b) Swayze, E. E.; Sanghvi, Y. S. *Synlett* **1997**, 839−841.
(14) Ross, B. S.; Springer, R. H.; Tortorici, Z.; Dimock, S. *Nucleosides Nucleotides* **1997**, *16*, 1641−1643.
(15) Nelson, T. D.; Crouch, R. D. *Synthesis* **1996**, *9*, 1031−1069.

Scheme 2[a]



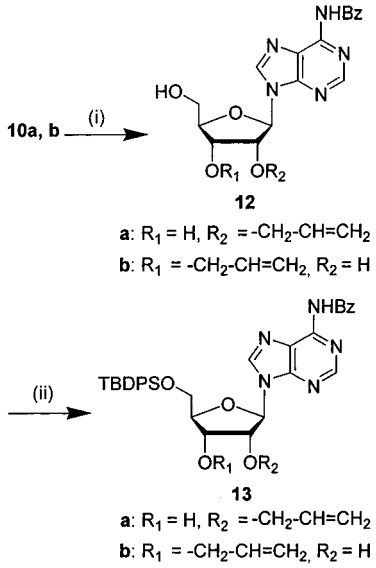
[a] (i) TBDPSCl, pyridine, DMAP, rt; (ii) BH_3.THF, ethylene glycol, 150 °C.

reaction[14] with the borate ester generated from ethylene glycol and borane in THF to obtain the 2′-*O*-(2-hydroxyethyl) nucleoside **7** in 50% yield.



Sproat[16f] and co-workers have carried out 2′-*O*-alkylation with allyl bromide using 3′,5′-bis-protected nucleosides. We attempted the direct alkylation of the unprotected nucleoside at the 2′-*O*-position (pK_a of 2′-OH of adenosine = 12.14)[16g] since this material is quite inexpensive.[16d,16e] Alkylation of adenosine with allyl bromide in the presence of NaH gave a mixture of the 2′ and 3′-*O*-allyl isomers (**10a** and **10b**, in a ratio of 3:1) in 66% isolated yield. However, separation of the two isomers (the 2′-*O*- and 3′-*O*-alkylation products) at this step using silica gel column chromatography proved difficult. Hence, we decided to use the mixture of 2′-*O*- and 3′-*O*- alkylated adenosines (**10a**,**b**) for the benzoylation of the exocyclic amino group under transient

Scheme 3[a]



[a] (i) TMSCl, pyridine, BzCl, NH_4OH, H_2O; (ii) TBDPSCl, CH_2Cl_2, DMAP, triethylamine, rt.

protection conditions[17] to obtain **12a** and **12b** (Scheme 3, 62%) and for the subsequent regioselective silylation at the 5′-position with TBDPSCl in pyridine. It is noteworthy that after silylation of **12a** and **12b**, the 2′-*O*-allyl and 3′-*O*-allyl adenosine derivatives **13a** and **13b**, respectively, could be separated by flash silica gel column chromatography to afford the required 2′-*O*-allyl isomer **13a**. The two isomers were identified by 2D $^1H-^1H$ COSY NMR experiments.

The same approach could not be used for the synthesis of the 2′-*O*-allyl guanosine **14** because of the well-known susceptibility of guanosines to electrophilic attack at the O-6 and N-2 positions of the purine ring. Indeed, direct alkylation of the guanosine with alkyl halides in the presence of NaH in DMF gave inseparable mixtures of products.[16g] In contrast, 2-aminoadenosine, which might be considered as a convenient precursor of guanosine,[18] smoothly underwent alkylation with allyl bromide to give the 2′-*O*- and 3′-*O*-isomers **11a** and **11b** in a ratio of 3:1, respectively, in a total yield of 70%. In the next step depicted in Scheme 4, the mixture of **11a** and **11b** was treated with adenosine deaminase in aqueous buffer at pH 7.5 as reported by Robins et al.[18] and McGee et al.[18] Under these conditions, **11a** was selectively converted to **14**, which precipitated from the reaction mixture to give the product in more than 90% isomeric purity, while the unreacted **11b** remained in solution. Compound **14** was subsequently reacted with TBDPS-Cl in DMF in the presence of imidazole to furnish compound **15** in 72% yield.

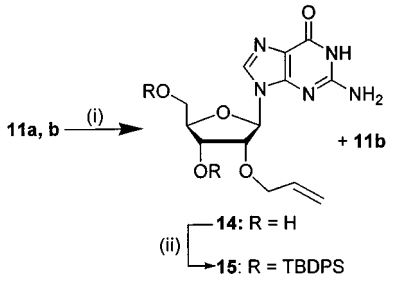
The 2′-*O*-allyl derivatives **13a** and **15** were converted to the 2′-*O*-(2-hydroxyethyl) derivatives **16** and **17**, respectively (Scheme 5). Dihydroxylation of **13a** and **15** with OsO_4 and 4-methylmorpholine *N*-oxide afforded the corresponding *vicinal*-diols.[19] The dihydroxylation reac-

(16) (a) Christensen, L. F.; Broom, A. D. *J. Org. Chem.* **1972**, *37*, 3398−33401. (b) Takaku, H.; Kamaike, K. *Chem. Lett.* **1982**, *2*, 189−192. (c) Yano, J.; Kan, L. S.; Ts'o, P. O. *Biochim. Biophys. Acta* **1980**, *629*, 178−183. (d) Guinosso, C. J.; Woke, G. D.; Frier, S.; Martin, J. F.; Ecker, D. J.; Mirabelli, C. K.; Crooke, S. T.; Cook, P. D. *Nucleosides Nucleotides* **1991**, *10*, 259−262. (e) Manoharan, M.; Guinosso, C. J.; Cook, P. D. *Tetrahedron Lett.* **1991**, *32*, 7171−7174. (f) Sproat, B. S.; Iribarren, A. M.; Garcia, R. G.; Beijer, B. *Nucleic Acids Res.* **1991**, *19*, 733−738. (g) Velikyan, I.; Acharya, S.; Trifonova, A.; Földesi, A.; Chattopadhyaya, J. *J. Am. Chem. Soc.* **2001**, *123*, 2893−2894.

(17) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316−1319.

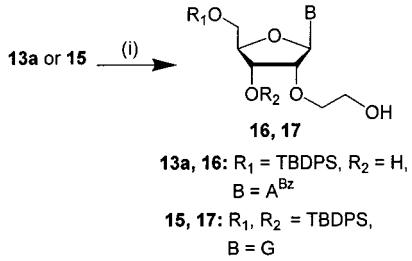
(18) (a) Robins, M. J.; Hanske, F.; Beriner, S. E. *Can. J. Chem.* **1981**, *59*, 3360−3364. (b)McGee, D. P. C.; Cook, P. D.; Guinosso, C. J. US PCT 92−918362, 1994. (c) Robins, M. J.; Zou, R.; Hansske, F.; Wnuk, S. F. *Can. J. Chem.* **1997**, *75*, 762−767. (d)Yokozeki, K.; Tsuji, T. *J. Mol. Catal. B: Enzymol.* **2000**, *10*, 207−213.

Scheme 4[a]



[a] (i) Adenosine deaminase, 100 mM sodium phosphate, pH 7.5, rt; (ii) DMF, TBDPSCl, imidazole, rt.

Scheme 5[a]



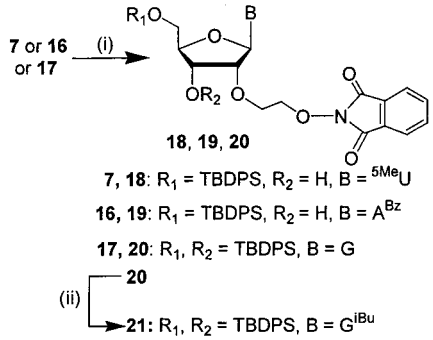
[a] (i) (a) 4-Methylmorpholine *N*-oxide, OsO_4, dioxane for **13a** and $CH_2Cl_2/(CH_3)_2CO$ for **15**; (b) $NaIO_4$ on SiO_2, CH_2Cl_2; (c) 1 M pyridinium *p*-toluenesulfonate (PPTS) in MeOH, $NaBH_3CN$, rt.

tion of 2′-*O*-allyl adenosine **13a** was carried out in dioxane. However, the 2′-*O*-allyl guanosine derivative **15** was not soluble in dioxane. Hence an alternative solvent system containing $CH_2Cl_2/(CH_3)_2CO$ was used for the osmylation of **15** to afford the corresponding *vicinal*-diol which was cleaved in the presence of $NaIO_4$ adsorbed on silica in CH_2Cl_2 to obtain the corresponding aldehyde.[20] Attempts to reduce the aldehyde with $NaBH_3CN$ in acetic acid failed to give the expected products. However, reaction with $NaBH_3CN$ and 1 M pyridinium *p*-toluenesulfonate (PPTS) in MeOH gave the alcohols in high yield. The reduction of the aldehyde with $NaBH_3CN$ in 1 M PPTS in MeOH afforded the 2′-*O*-(2-hydroxyethyl) derivatives **16** and **17** (72−78%). It appears that 1 M PPTS in MeOH produces ideal conditions[21] for the reduction of the above-mentioned aldehydes.

Treatment of **7**, **16** and **17** under Mitsunobu[12] conditions with *N*-hydroxyphthalimide, Ph_3P, and DEAD in THF gave the phthalimido derivatives **18**, **19** and **20** in 69−92% yields. The exocyclic amino group of the guanosine derivative **20** was protected with isobutyryl chloride in anhydrous pyridine (Scheme 6) to afford the N^2-isobutyryl derivative **21** in 65% isolated yield**.**

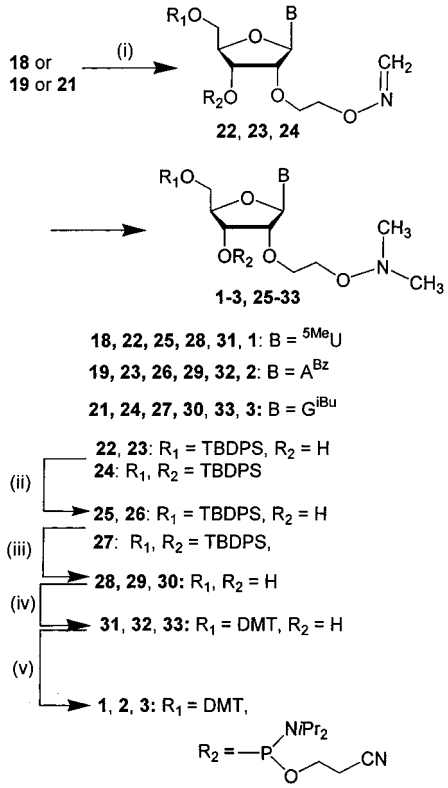
The phthalimido group in compounds **18**, **19**, and **21** (Scheme 7) was deprotected with *N*-methylhydrazine[13a] (NMH) at 0 °C to give the aminooxy derivatives. The use of NMH for deprotection of the phthalimido group[13a] resulted in a thick precipitate of 1,2-dihydro-4-hydroxy-2-methyl-1-oxophthalazine that was readily separated by filtration. Excess NMH was easily removed from the reaction mixture by evaporation. Without further purification, the aminooxy derivatives were converted into the methyleneaminooxy derivatives **22**, **23**, and **24** using formaldehyde in MeOH. Reduction of the compounds **22**, **23**, and **24** with $NaBH_3CN$ in 1 M PPTS in MeOH gave the monomethylaminooxy derivative, which was then treated with formaldehyde under reductive conditions to give the dimethylaminooxy derivatives **25**, **26**, and **27** in 80−96% yield. Reduction of the methyleneaminooxy derivatives **22**, **23**, and **24** proceeded smoothly[13b] and was complete in 2 h at ambient temperature. Desilylation of **25**, **26**, and **27** with triethylamine trihydrofluoride and triethylamine in THF gave compounds **28**, **29**, and **30** in good yield. These nucleosides were then selectively protected at the 5′-position by reaction with 4,4′-

Scheme 6[a]



[a] (i) Ph_3P, *N*-hydroxyphthalimide, THF, DED, rt; (ii) pyridine, isobutyryl chloride.

Scheme 7[a]



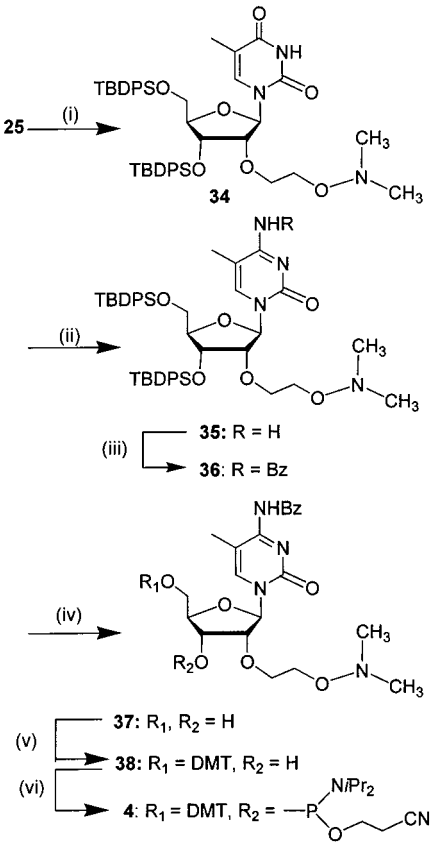
[a] (i) (a) CH_2Cl_2, *N*-methylhydrazine (b) MeOH, HCHO, rt; (ii) (a) 1 M PPTS in MeOH, $NaBH_3CN$, rt; (b) 1 M PPTS in MeOH, HCHO, $NaBH_3CN$, rt; (iii) triethylamine trihydrofluoride, THF, $(C_2H_5)_3N$, rt; (iv) 4,4′-dimethoxytrityl chloride, pyridine, DMAP, rt; (v) 2-cyanoethyl tetraisopropylphosphorodiamidite, *N*,*N*-diisopropylammonium tetrazolide, CH_3CN, rt.

(19) Van Rheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, *23*, 1973−1976.

(20) Zhong, Y.-Li.; Shing, T. K. M. *J. Org. Chem.* **1997**, *62*, 2622−2624.

(21) Borch, F. R.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897−2904.

Scheme 8[a]



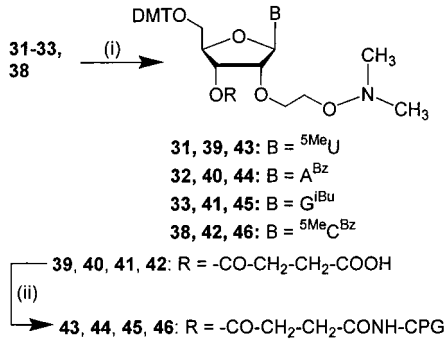
[a] Bz = benzoyl; (i) TBDPSCl, DMF, imidazole, 60 °C; (ii) 1,2,4-triazole, $POCl_3$, $(C_2H_5)_3N$, CH_3CN; (iii) DMF, benzoic anhydride, rt; (iv) triethylamine trihydrofluoride, $(C_2H_5)_3N$, THF, rt; (v) DMTr-Cl, pyridine, DMAP, rt; (vi) diisopropylammonim tetrazolide, 2-cyanoethyl tetraisopropylphosphorodiamidite, CH_3CN, rt.

dimethoxytrityl chloride (DMTCl) and a catalytic amount of DMAP in pyridine to yield the products **31** (78% yield), **32** (75% yield), and **33** (91% yield). Phosphitylation of compounds **31**−**33** at the 3′-position with 2-cyanoethyl *N*,*N*,*N*′*N*′-tetraisopropylphosphorodiamidite in the presence of *N*,*N*-diisopropylamine tetrazolide in CH_3CN afforded the phosphoramidite building blocks **1**−**3** in 52−86% yield.

The synthesis of the 5-methylcytidine phosphoramidite **4** is illustrated in Scheme 8. Compound **25** was silylated at the 3′-*O*- position with TBDPSCl and imidazole in DMF at 60 °C to yield **34** in 85% isolated yield. Compound **34** was converted into the triazole derivative with 1,2,4-triazole, $POCl_3$, and Et_3N.[22] Subsequent treatment with aqueous NH_3:dioxane (1:1) at ambient temperature for 14 h gave the 5-methylcytidine analogue **35** in 94% yield. The exocyclic amino group was protected with a benzoyl group[23] to afford **36** (87% yield), followed by removal of the silyl group to give **37** (97% yield). The dimethoxytritylation of **37** at the 5′-*O*-position yielded **38** (72% yield). Finally, phosphitylation at the 3′-*O*-position gave the phosphoramidite **4** (85% yield) in good overall yield.

Compounds **31**, **32**, **33**, and **38** were also converted into the 3′-*O*-succinyl derivatives **39**-**42** (Scheme 9) and loaded

Scheme 9[a]



[a] (i) Succinic anhydride, 1,2-dichloroethane, DMAP, $(C_2H_5)_3N$, 60 °C; (ii) 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium-tetrafluoroborate (TBTU), 4-methylmorpholine, DMF, aminoalkyl controlled pore glass (CPG), rt.

on to the aminoalkyl controlled pore glass (CPG, Scheme 9) according to the standard synthetic procedure[24] to yield the functionalized solid supports **43**−**46** (55−60 μmol/g).

Synthesis of 2′-*O*-DMAOE Modified Oligonucleotides. The oligonucleotides **48**, **50**, **52**, **54**, **56**, **58**, and **59** (Table 1) were synthesized on a solid-phase DNA synthesizer using the phosphoramidites **1**, **2**, **3**, and **4**. A 0.1 M solution of the phosphoramidites in anhydrous acetonitrile was used for the synthesis. Oxidation of the internucleosidic phosphite to the phosphate was carried out using 1-*S*-(+)-(10-camphorsulfonyl)oxaziridine (CSO).[25] 3-*H*-1,2-Benzodithiol-3-one 1,1-dioxide (the Beaucage reagent)[26] was used as the sulfur-transfer agent for the synthesis of oligonucleotide phosphorothioates. The overall coupling efficiency of all modified phosphoramidites was more than 97%. Oligonucleotides were deprotected with concentrated aqueous ammonium hydroxide. After deprotection, all the 5′-DMTr on oligonucleotides were isolated by reverse phase HPLC. When the 5′-terminal DMTr group was removed, the oligonucleotides **48**, **50**, **52**, **54**, **56**, **58**, and **59** were desalted and characterized by electrospray MS. Their purity was confirmed by HPLC and capillary gel electrophoresis.

Evaluation of Hybridization of the 2′-*O*-DMAOE Modified Oligonucleotides to Complementary RNA and DNA by Thermal Denaturation Studies. A series of oligonucleotides with the 2′-*O*-DMAOE modification were used to investigate the effect of this modification on hybridization affinity for complementary RNA and DNA. Melting temperatures (T_m) of the modified oligonucleotides with target RNA were compared to those of the heteroduplexes formed with unmodified DNA oligonucleotides (Table 1). Free energies of duplex formation $\Delta G°$ were obtained from UV absorbance vs temperature curves assuming a two-state model with a linear sloping baseline.[28] These results are summarized in Table 1.

(22) Divakar, K. J.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, *5*, 1171−1176.
(23) Bhat, V.; Ugarkar, B. G.; Sayeed, V. A.; Grimm, K.; Kosora, N.; Domenico, P. A.; Stocker, E. *Nucleosides Nucleotides* **1989**, *8*, 179−183.
(24) (a) Kumar, P.; Sharma, A. K.; Sharma, P.; Garg, B. S.; Gupta, K. C. *Nucleosides Nucleotides* **1996**, *15*, 879−888. (b) TBTU-mediated synthesis of functionalized CPG synthesis: Bayer, E.; Bleicher, K.; Maier, M. A.; *Z. Naturforsch*. **1995**, *50b*, 1096−1100.
(25) Manoharan, M.; Lu, Y.; Casper,M. D.; Just, G. *Org. Lett.* **2000**, *2*, 243−246.
(26) Iyer, R. P.; Phillips, L. R.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Org. Chem*. **1990**, *55*, 4693−4699.
(27) Freier, S. M.; Altmann, K.-H. *Nucleic Acids Res*. **1997**, *25*, 4429−4443.
(28) Petersheim, M.; Turner, D. H. *Biochemistry* **1983**, *22*, 256−263.

Table 1. Effect of the 2′-*O*-DMOE Modification on Duplex Stability against Complementary RNA Targets

oligo no.	sequence	T_m °C	ΔT_m/modification °C	$\Delta G°_{37}$ kcal mol^{-1}
47	5′ ToCoCoAoGoGoToGoToCoCoGoCoAoToC 3′	62.3		−14.87
48	5′ T*oCoCoAoGoGoT*oGoT*oCoCoGoCoAoT*oC 3′	66.78	1.12	−17.82
49	5′ GoCoGoToToToToToToToToToGoCoG 3′	48.30		−13.00
50	5′GoCoGoT*oT*oT*oT*oT*oT*oT*oT*oT*oT*oGoCoG 3′	62.90	1.46	−17.77
51	5′ToToC§oToC§sGsC§sTsGsGsTsGsAsGsToToToC§oA 3′	58.20		−14.80
52	5′ T*oT*oC*oT*oC*sGsC§sTsGsGsTsGsAsGsT*oT*oT*oC*oA$^{\nabla}$ 3′	69.80	1.16	−23.70
53	5′ TsTsC§sTsC§sGsC§sTsGsGsTsGsAsGsTsTsTsC§sA 3′	53.70		−13.20
54	5′ T*sT*sC*sT*sC*sGsC§sTsGsGsTsGsAsGsT*s T*s T*sC*sA$^{\nabla}$ 3′	65.80	1.21	−20.1
55	5′ ToC§oToGoAoGoToAoGoC§oAoGoAoGoGoAoGo C§oToC§ 3′	59.70		−14.7
56	5′ T*oC*oT*oG*oA*oG*oT*oA*oG*oC*oA*oG*oA*oG*oG*oA*oG*oC*oT*oC* 3′	86.50	1.34	−24.3
57	5′ TsC§sTsGsAsGsTsAsGsC§sAsGsAsGsGsAsGsC§s TsC§ 3′	51.20		−11.7
58	5′ T*sC*sT*sG*sA*sG*sT*sA*sG*sC*sA*sG*s A*s G*sG*sA*sG*sC*sT*sC* 3′	78.00	1.34	−19.20

[a] T* = 2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}-5-methyluridine, A* =2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}adenosine, G* =2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}guanosine, C* = 2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}-5-methylcytidine, A$^{\nabla}$ = 2′-*O*-[2-(methoxy)-ethyl]adenosine,C§ = 5-methylcytidine s = phosphorothioate backbone, o = phosphodiester backbone.

Table 2. T_m Data for the Oligonucleotide Containing 2′-*O*-DMAOE Modification against Complementary DNA Target

oligo no.	no. of modifications	T_m (°C)	ΔT_m (°C)	ΔT_m (°C)/modification
DNA parent		54.2		
50	10	45.4	−8.8	−0.88

Substitution with 2′-*O*-DMAOE nucleosides led to an increase in the melting temperatures relative to unmodified oligonucleotides. A T_m enhancement of 1.12 °C per substitution was observed when modified bases were not contiguous as in the oligonucleotide **48** (Table 1). In the oligonucleotide **50** with 10 adjacent substitutions, the T_m enhancement was 1.46 °C/substitution. The dependence of the stability of the RNA heteroduplexes with 2′-*O*-DMAOE modified oligonucleotides on the base composition and the placement of the modification were verified by studying the hybridization affinity of the gapmer[3,6] oligonucleotides **52** and **54** and the uniformly modified oligonucleotides **56** and **58** to the complementary RNA. The gapmer with a mixed backbone (oligonucleotide **52**) showed a T_m enhancement of 1.16 °C/substitution whereas the gapmer **54** with a full phosphorothioate backbone showed a T_m enhancement of 1.21 °C/substitution. The uniformly modified oligonucleotides **56** and **58** showed an average T_m of 1.34 °C/substitution. These results suggest that the T_m enhancement due to 2′-*O*-DMAOE is sequence independent. In contrast to the increase in the T_m observed with RNA, the hybridization of the oligonucleotide **50** with complementary DNA led to duplexes less stable than those formed with unmodified DNA oligonucleotides (−0.9 °C per modification, Table 2). The favorable stabilization per 2′-*O*-DMAOE modification corresponds to a net stabilization of more than 2 °C per modification compared to the 2′-deoxyoligonucleotide phosphorothioate, taking into account the fact that the 2′-deoxyoligonucleotide phosphorothioate forms a duplex with complementary RNA destabilized by approximately −0.8 °C/modification.[27]

Stability of 2′-*O*-DMAOE Oligonucleotides Against Snake Venom Phosphodiesterase. To evaluate the resistance of the 2′-*O*-DMAOE oligonucleotides toward nucleases, the oligonucleotide **59** was treated with snake venom phosphodiesterase (SVPD).[29] The modifications in the oligonucleotide **59** were placed at the 3′-end and all internucleosidic linkages were phosphodiesters. Figure 1 shows the relative nuclease stability of the modified oligonucleotides compared to the unmodified DNA oligonucleotide, 2′-*O*-MOE, 2′-*O*-propyl, and 2′-*O*-aminopropyl modified oligonucleotides of the same sequence. The half-life of disappearance of the full length 2′-*O*-DMAOE modified oligonucleotide was more than 24 h. The nuclease stability of the 2′-*O*-DMAOE capped oligonucleotide was higher than that of the 2′-*O*-MOE, 2′-*O*- propyl and the 2′-deoxy modified oligonucleotides. Only the 2′-*O*-aminopropyl modified oligonucleotide was more stable than the 2′-*O*-DMAOE oligonucleotide. The amino group in the 2′-*O*-aminopropyl modification is protonated under physiological conditions (pK_a = 9) and leads to enhanced nuclease resistance. The observed order of nuclease stability is consistent with the reported data.[30] The relative nuclease stability of the modified oligonucleotides is in the following order: 2′-*O*-AP > 2′-*O*-DMAOE > 2′-O-MOE > 2′-*O*-Pr > 2′-deoxy.

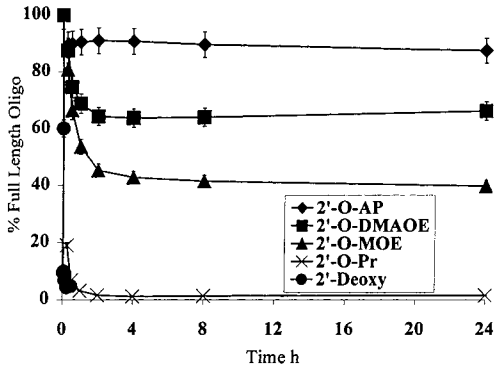


Figure 1. Relative nuclease resistance of the 2′-*O*-DMAOE oligonucleotide versus the 2′-*O*-propylamino, 2′-*O*-MOE, 2′-*O*-propyl and 2′-deoxy oligonucleotides. T* represents 2′-modified ^{5Me}U. The modifications were placed at the 3′ end of the sequence **59** TTT TTT TTT TTT TTT T*T*T* T* and digested with snake venom phosphodiesterase.

In conclusion, efficient synthetic routes have been developed for all four 2′-*O*-DMAOE modified nucleosides,

(29) Cummins, L. L.; Owens, S. R.; Risen, L. M.; Lesnik, E. A.; Freier, S. M.; McGee, D.; Guinosso, C. J.; Cook, P. D. *Nucleic Acids Res.* **1995**, *23*, 2019−2024.

(30) (a) Griffey, R. H.; Monia, B. P.; Cummins, L. L.; Freier, S.; Greig, M. J.; Guinosso, C. J.; Lesnik, E.; Manalili, S. M.; Mohan, V.; Owens, S.; Ross, B. R.; Sasmor, H.; Wancewicz, E.; Weiler, K.; Wheeler, P. D.; Cook, P. D. *J. Med. Chem*. **1996**, *39*, 5100−5109. (b) Manoharan, M.; Prakash, T. P.; Barber-Peoc'h, I.; Bhat, B.; Vasquez, G.; Ross, B. S.; Cook, P. D. *J. Org. Chem*. **1999**, *64*, 6468−6472. (c) Teplova, M.; Wallace, S. T.; Tereshko, V.; Minasov, G.; Symons, A. M.; Cook, P. D.; Manoharan, M.; Egli, M. *Proc. Natl. Acad. Sci. U.S.A*. **1999**, *96*, 14240−14245.

their phosphoramidite building blocks, and solid supports with traditional protecting groups at the exocyclic amino groups. The methods described herein provide general procedures for functionalization of all four nucleosides at the 2′-position. The use of the 2′-*O*-DMAOE building blocks in oligonucleotide synthesis resulted in high coupling efficiency. We have shown the use of these phosphoramidites and solid supports for the syntheses of oligonucleotides with phosphorothioate, phosphodiester, and oligonucleotide-containing phosphodiester and phosphorothioate backbones. These modified oligonucleotides showed RNA-selective, high binding affinity, and excellent nuclease stability and make them ideal candidates for further evaluation for antisense drug development. While this report was in preparation, we also synthesized oligonucleotide gapmers with 2′-*O*-DMAOE modifications at the wings and fully modified oligonucleotides containing 2′-*O*-DMAOE modifications and evaluated their ability to suppress gene expression in several biological targets. In the gapmer format, oligonucleotides with 2′-*O*-DMAOE wings are able to activate RNase H and have been shown to suppress gene expression. The oligonucleotides having the 2′-*O*-DMAOE modification throughout the sequence are able to inhibit translation by the non RNase H mediated mode of action. These results will be reported elsewhere.

Experimental Section

General Procedures. 2,2′-Anhydrothymidine was purchased from Ajinomoto (Tokyo, Japan). Anhydrous MeCN (water content < 0.001%), standard phosphoramidites and ancillary reagents for oligonucleotide synthesis were purchased from PE Biosystems (Foster City, CA). All other reagents and anhydrous solvents were purchased from Aldrich and used without further purification. Adenosine deaminase was purchased from Reliable Biopharmaceuticals, St. Louis, MO. Flash chromatography was performed using silica gel 60 (35−75 μm, EM Science). Thin-layer chromatography was performed on precoated plates (silica gel 60 F254 from EM Science) and visualized with UV light and spraying with a solution of *p*-anisaldehyde (6 mL), H_2SO_4 (8.3 mL), and CH_3COOH (2.5 mL) in C_2H_5OH (227 mL) followed by charring. ^1H NMR spectra were referenced using internal standard $(CH_3)_4Si$ and ^{31}P NMR spectra using external standard 85% H_3PO_4. Microanalyses were performed by Quantitative Technologies Inc., NJ. Mass spectra were recorded by Mass Consortium, San Diego, CA and the College of Chemistry, University of California, Berkeley, CA.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}-5-methyluridine-3′-[(2-cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidite (1). Compound **31** (3.9 g, 6.0 mmol) was coevaporated with toluene (20 mL). To the residue, *N*,*N*-diisopropylammonium tetrazolide (0.97 g, 5.7 mmol) was added and the mixture was dried over P_2O_5 in a vacuum overnight at 40 °C. The reaction mixture was dissolved in anhydrous CH_3CN (28.4 mL) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (7.3 mL, 23.00 mmol) was added. The reaction mixture was stirred at ambient temperature for 4 h under an argon atmosphere. The progress of the reaction was monitored by TLC (hexane:ethyl acetate 1:1). The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (150 mL) and washed with 5% aqueous $NaHCO_3$ (75 mL) and brine (75 mL). The ethyl acetate layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was chromatographed (ethyl acetate as eluent) to afford **1** as a white foam (4.39 g, 86%): ^{31}P NMR (81 MHz, $CDCl_3$) δ 150.73; HRMS (FAB) Calcd for $C_{44}H_{58}N_5O_{10}PNa^+$ 870.3819, found 870.3796.

***N*⁶-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}adenosine-3′-[(2-cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidite (2).** Compound **32** (1.20 g, 1.6 mmol) was coevaporated with toluene (5 mL). The residue obtained was mixed with *N*,*N*-diisopropylamine tetrazolide (0.27 g, 1.6 mmol) and dried over P_2O_5 in a vacuum overnight at 40 °C. The mixture was dissolved in anhydrous CH_3CN (8.2 mL) and 2-cyanoethyl *N*,*N*,*N*′*N*′-tetraisopropylphosphorodiamidite (2.0 mL, 6.3 mmol) was added dropwise. The reaction mixture was stirred at room temperature under an argon atmosphere for 4 h. The reaction was monitored by TLC (ethyl acetate:hexane, 8:2 containing 0.5% pyridine). The solvent was removed under reduced pressure, the resulting residue was dissolved in ethyl acetate (75 mL) and washed with 5% aqueous $NaHCO_3$ (2 × 40 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (ethyl acetate:hexane, 8:2 containing 0.5% pyridine) to afford **2** (1.23 g, 81%): ^{31}P NMR (162 MHz, $CDCl_3$) δ 151.14, 150.72; LRMS (FAB) *m*/*z* 1093 (M + Cs)$^+$, HRMS (FAB) Calcd for $C_{51}H_{62}N_8O_9P^+$ 961.4377; found 961.4360.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}-*N*²-isobutyrylguanosine-3′-[(2-cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidite (3). Compound **33** (1.32 g, 1.8 mmol) was dissolved in CH_2Cl_2 (18 mL), and diisopropylamine tetrazolide (0.15 g, 0.9 mmol) and 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.63 mL, 2.0 mmol) were added. After 2 h at ambient temperature, an additional amount of 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.21 mL, 0.7 mmol) was added and the solution was stirred for 9 h. The solvent was evaporated under reduced pressure to a volume of 5 mL. The resulting solution was purified by flash column chromatography using silica gel [pretreated with 1% triethylamine] eluting with CH_2Cl_2−acetone−triethylamine, 60:40:1, followed by CH_2Cl_2−acetone−$(C_2H_5)_3N$, 30:70:1, to provide the title compound **3** as a white foam (0.87 g, 52%): ^{31}P NMR (81 MHz, $CDCl_3$) δ 150.7, 150.2. HRMS (FAB) Calcd for $C_{48}H_{63}N_8O_{10}PNa^+$ 965.4302, found 965.4341.

***N*⁴-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}-5-methylcytidine-3′-[(2-cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidite (4).** Compound **38** (2.74 g, 3.7 mmol) was coevaporated with toluene (20 mL). It was then mixed with diisopropylamine tetrazolide (0.63 g, 3.7 mmol) and dried over P_2O_5 in a vacuum at 40 °C overnight. The resulting mixture was dissolved in anhydrous CH_3CN (18.3 mL) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (4.64 mL, 14.6 mmol) was added. The reaction mixture was stirred at room temperature for 4 h under an argon atmosphere, followed by removal of the solvent in a vacuum. Ethyl acetate (100 mL) was added to the residue, and it was washed with 5% aqueous $NaHCO_3$ (50 mL) and brine (50 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash silica gel column chromatography and eluted with ethyl acetate:hexane:pyridine, 60:39:1, to yield **4** (2.96 g, 85% yield) as a white foam: ^{31}P NMR (81 MHz, $CDCl_3$) δ 150.83; MS (FAB) *m*/*z* 949 [M − H]$^-$; HRMS (FAB) Calcd for $C_{51}H_{63}N_6O_{10}PCs^+$ 1083.3398, found 1083.3440.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2,2′-anhydro-5-methyluridine (6). 2,2′-Anhydro-5-methyluridine **5** (100.0 g, 416.5 mmol) and DMAP (0.66 g, 5.4 mmol) were dissolved in anhydrous pyridine (500 mL) at room temperature under an argon atmosphere with mechanical stirring. *tert*-Butyldiphenylchlorosilane (119 mL, 458.0 mmol) was added. The reaction was stirred for 16 h at ambient temperature. TLC (R_f 0.22, ethyl acetate) indicated a complete reaction. The solution was concentrated under reduced pressure to a thick oil. This was partitioned between CH_2Cl_2 (1 L), saturated $NaHCO_3$ (2 × 1L), and brine (1 L). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to a thick oil. The oil was dissolved in a mixture of ethyl acetate and diethyl ether (1:1, 600 mL), and the solution was cooled to −10 °C. A crystalline product obtained was collected by filtration, washed with diethyl ether (3 × 200 mL), and dried (40 °C, 1 mmHg, 24 h) to afford **6** (149 g, 74.8%) as a white solid: mp 87−88 °C; R_f 0.14 (5% MeOH in CH_2Cl_2); ^1H NMR

(200 MHz, DMSO-d_6) δ 0.90 (s, 9H), 1.76 (s, 3H), 3.40 (dd, J = 6.9 and 4.44 Hz, 1H), 3.57 (dd, J = 4.78 and 6.52 Hz, 1H), 4.17 (m, 1H), 4.41 (t, J = 3.2 Hz, 1H), 5.23 (d, J = 5.82 Hz, 1H), 5.98 (d, J = 4.66 Hz, 1H), 6.31 (d, J = 5.7 Hz, 1H), 7.35−7.6 (m, 10H), 7.79 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.87, 19.19, 26.66, 63.02, 75.13, 87.89, 89.37, 90.07, 118.85, 127.83, 129.93, 131.30, 132.99, 135.46, 159.49, 173.04; MS (MALDI) m/z 501.2 $[M + Na]^+$.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-*O*-(2-hydroxyethyl)-5-methyluridine (7). To a 2 L stainless steel pressure reactor containing borane in tetrahydrofuran (1.0 M, 622 mL), ethylene glycol (350 mL) was added cautiously with stirring until the evolution of hydrogen gas subsided. Compound **6** (149 g, 311.6 mmol) and $NaHCO_3$ (0.074 g, 0.88 mmol) were added with stirring. The reactor was sealed and heated in an oil bath at internal temperature of 160 °C for 16 h (pressure < 100 psi). The reaction vessel was cooled to ambient temperature and opened. TLC (R_f 0.67 for the desired product and R_f 0.82 for the thymine arabinofuranoside side product, ethyl acetate) indicated about 70% conversion to the product. To avoid additional side product formation, the reaction was stopped and the solution concentrated under reduced pressure (10 to 1 mmHg) in a warm water bath (40−100 °C) with more extreme conditions used to remove the ethylene glycol. The residue was purified by column chromatography (2 kg silica gel, ethyl acetate:hexanes gradient 1:1 to 4:1). The appropriate fractions were combined and concentrated in a vacuum to yield **7** as white crisp foam (84 g, 50%), contaminated starting material (17.4 g), and pure reusable starting material 20 g. Based on consumed starting material, the yield, was 58%: R_f 0.22 (5% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.04 (s, 9H), 1.45 (s, 3 H), 3.55 (m, 4H), 3.78−4.07 (m, 4H), 4.26 (m, 1 H,), 4.76 (t, J = 5.26 Hz, 1H), 5.15 (d, J = 5.7 Hz, 2H), 5.92 (d, J = 5.38 Hz, 1H), 7.39−7.68 (m, 11H), 11.42 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.85, 19.39, 27.07, 61.41, 63.39, 69.02, 72.21, 82.68, 84.73, 86.70, 111.52, 127.94, 130.01, 132.27, 133.02, 135.01, 135.18, 135.48, 151.07, 164.12; MS (API-ES) 539.1 $[M - H]^-$; HRMS (MALDI) Calcd for $C_{28}H_{36}N_2O_7SiNa^+$ 563.2184, found 563.2173. Anal. Calcd for $C_{28}H_{36}N_2O_7Si$: C, 62.20; H, 6.71; N, 5.18. Found: C, 61.91; H, 6.66; N, 5.15.

2′/3′-*O*-Allyl Adenosine (10a,b). Adenosine **8** (20.00 g, 74.8 mmol) was dried over P_2O_5 in a vacuum at 40 °C overnight and then suspended in anhydrous DMF (380 mL). To this was added NaH (3.00 g, 74.8 mmol, 60% dispersion in mineral oil), and the reaction mixture was stirred at room temperature for 10 min. Then allyl bromide (7.2 mL, 83.2 mmol) was added dropwise and the stirring continued at room temperature for additional 18 h. DMF was removed in a vacuum, and the residue was triturated with ethyl acetate (100 mL). The ethyl acetate layer was decanted, and the solid obtained was dissolved in MeOH, adsorbed on silica gel, and purified by flash silica gel column chromatography (10% MeOH in CH_2Cl_2) to yield a mixture of **10a** and **10b** in 66% yield (15.19 g): ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.45−3.91 (m, 2H), 3.95−4.2 (m, 3H), 4.29 (q, J = 4.76 Hz, and 3.04 Hz, 1H), 4.48 (t, J = 5.36 Hz, 1H), 4.97−5.4 (m, 3H), 5.41−5.52 (m, 1H), 5.69−5.9 (m, 1H), 6.01 (d, J = 6.14 Hz, 1H), 7.35 (s, 2H), 8.13 (s, 1H), 8.34 (s, overlapping with peak at 8.37), 8.37 (s, 1H); HRMS (FAB) Calcd for $C_{13}H_{18}N_5O_4^+$ 308.1359, found 308.1360.

2′/3′-*O*-Allyl-2,6-diaminopurine Riboside (11a,b). From 2,6-diaminopurine riboside **9** (30.00 g, 106.4 mmol), DMF (540 mL), sodium hydride (4.26 g, 106.4 mmol, 60% dispersion in mineral oil), and allyl bromide (10.2 mL, 117.9 mmol), a mixture of **11a** and **11b** (26.38 g, 77%) was obtained according to the procedure described for the synthesis of compounds **10a**,**b**: HRMS (MALDI) Calcd for $C_{13}H_{19}N_6O_4^+$ 323.1619, found 323.1468.

2′/3′-*O*-Allyl-*N*^{6}-benzoyladenosine (12a,b). A mixture of compounds **10a,b** (15.19 g, 49.4 mmol) was dried over P_2O_5 in a vacuum overnight at 40 °C. It was then dissolved in anhydrous pyridine (494.4 mL) and protected from moisture. The reaction mixture was cooled to 0 °C in an ice bath. Trimethylchlorosilane (32 mL, 252.3 mmol) was added at 0 °C, and the reaction mixture was stirred for 1 h under an argon atmosphere. Benzoyl chloride (29.3 mL, 252.3 mmol) was added dropwise. The reaction mixture was brought to room temperature and stirred for 4 h and then cooled to 0 °C in an ice bath. Water (100.0 mL) was added, and the reaction mixture was stirred for 30 min at 0 °C. Aqueous NH_3 (30 wt %, 100.0 mL) was added, and stirring was continued for an additional 1 h at 0 °C. The solvent was evaporated and the residue partitioned between water and ether. The product precipitated out as an oil, which was further purified by silica gel column chromatography (5% MeOH in CH_2Cl_2), to yield **12a**,**b** as a white foam (12.61 g, 62%): ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.45−3.77 (m, 2H), 3.95−4.22 (m, 3H), 4.38 (br s, 1H), 4.57 (t, J = 5.38 Hz, 1H), 5.00−5.42 (m, 3H), 5.6−6.00 (m, 1H), 6.08 (d, J = 5.86 Hz, 1H), 6.21 (d, J = 5.76 Hz, 1H), 7.4−7.71 (m, 3H), 8.07 (d, J = 8.1 Hz, 2H), 8.74 (s, 1H), 8.76 (s, 1H), 8.79 (s, 1H); HRMS (MALDI) Calcd for $C_{20}H_{21}N_5O_5Na^+$ 434.1435, found 434.1450.

2′/3′-*O*-Allyl-*N*^{6}-benzoyl-5′-*O*-(*tert*-butyldiphenylsilyl)-adenosine (13a,b). Compound **12a**,**b** (11.17 g, 27.2 mmol) was dried over P_2O_5 in a vacuum at 40 °C and then dissolved in anhydrous CH_2Cl_2 (56 mL). DMAP (0.34 g, 2.8 mmol), triethylamine (23.8 mL, 167.0 mmol), and *tert*-butyldiphenylsilyl chloride (14.5 mL, 55.7 mmol) were added. The reaction was stirred vigorously for 12 h and found to be complete by TLC (ethyl acetate: hexane 1:1). It was diluted with CH_2Cl_2 (50 mL) and washed with water (3 × 60 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated to dryness. The 2′-*O*-allyl derivative **13a** and 3′-*O*-allyl derivative **13b** were separated by flash silica gel column chromatography (ethyl acetate: hexane 1:1). The pure 2′-*O*-allyl isomer **13a** was isolated in 50% yield (8.85 g) as a white foam: R_f 0.35 (ethyl acetate: hexane, 1:1); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.97 (s, 9H), 3.78−4.23 (m, 5H), 4.52 (q, J = 5.26 and 4.82 Hz, 1H), 4.65 (t, 5.1 Hz, 1H), 5.04−5.22 (m, 2H), 5.41 (d, J = 5.92 Hz, 1H), 5.74−5.91 (m, 1H), 6.2 (d, J = 5 Hz, 1H), 7.31−7.69 (m, 13H), 8.04 (d, J = 6.9 Hz, 2H), 8.6 (s, 1H), 8.66 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.49, 27.22, 63.53, 69.64, 72.18, 81.32, 85.45, 87.28, 119.1, 123.5, 128.09, 129.00, 130.17, 132, 88, 133.42, 134.08, 135.83, 141.63, 149.8, 151.69, 152.99, 164.88; MS (FAB) m/z 673 $[M + Na]^+$; HRMS (MALDI) Calcd for $C_{36}H_{39}N_5O_5SiNa^+$ 672.2613, found 672.2430.

2′-*O*-Allylguanosine (14). A mixture of 2′- and 3′-*O*-allyl 2-aminoadenosine **11a**,**b** (20.00 g, 62.1 mmol) was suspended in 100 mM sodium phosphate buffer (640 mL, pH 7.5) and adenosine deaminase (1 g, 2−5 U/mg) was added. The resulting solution was stirred very slowly for 60 h, keeping the reaction vessel open to the atmosphere. The reaction mixture was then cooled in an ice bath for 1 h and the precipitate obtained was filtered and dried over P_2O_5 in a vacuum to yield **14** as a white powder (13.92 g, 69.6% yield): R_f 0.19 (10% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.56 (m, 2H), 3.86−4.2 (m, 3H), 4.26 (m, 2H), 5.08 (m, 2H), 5.2 (d, J = 4.34 Hz, 1H), 5.72−5.9 (m, 1H), 5.82 (d, J = 5.48 Hz, 1H), 6.46 (br s, 2H), 7.95 (s, 1H), 10.64 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 61.34, 69.05, 70.34, 80.82, 84.84, 85.87, 116.79, 134.79, 135.41, 151.23, 153.80, 156.82; MS (FAB) m/z 324 $[M + H]^+$; HRMS (MALDI) Calcd for $C_{13}H_{17}N_5O_5Na^+$ 346.1122, found 346.1155.

2′-*O*-Allyl-3′,5′-bis(*tert*-butyldiphenylsilyl)guanosine (15). 2′-*O*-Allylguanosine **14** (6.00 g, 18.6 mmol) was mixed with imidazole (10.18 g, 149.5 mmol) and dried over P_2O_5 under reduced pressure overnight. To this mixture was added anhydrous DMF (50 mL), and the reaction mixture was stirred at room temperature for 10 min. *tert*-Butyldiphenylsilyl chloride (19.5 mL, 74.8 mmol) was added, and the reaction mixture was stirred at room-temperature overnight under an argon atmosphere. DMF was evaporated under reduced pressure to obtain an oil, which was dissolved in ethyl acetate (100 mL) and washed with water (2 × 75 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue obtained was purified by flash silica gel column chromatography and eluted with 5% MeOH in CH_2Cl_2 to yield **15** (10.84 g, 72% yield) as a white foam: R_f = 0.32 (5% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.89 (s, 9H), 1.06 (s, 9H), 3.43 (m, 1H), 3.62 (dd,

J = 3.52 and 7.82 Hz, 1H), 3.74 (d, J = 4.06 Hz, 2H), 4.1 (m, 1H), 4. 22 (m, 1H), 4. 47 (m, 1H), 5.00 (d, J = 12.82 Hz, 2H), 5.63 (m, 1H), 5.97 (d, J = 6.62 Hz, 1H), 6.45 (br s, 2H), 7.31−7.71 (m, 21H), 10.66 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.15, 19.33, 26.90, 26.98, 63.43, 71.24, 71.42, 80.41, 85.53, 117.40, 117.64, 127.59, 127.65, 127.77, 129.55, 129.85, 129.95, 132.59, 132.85, 133.12, 133.37, 133.82, 134.79, 135.41, 135.53, 135.83, 136.04, 151.77, 153.45, 159.13; MS (FAB) m/z 822 $[M + Na]^+$, HRMS (MALDI) Calcd for $C_{45}H_{53}N_5O_5Si_2Na^+$ 822.3477, found 822.3455. Anal. Calcd for $C_{45}H_{53}N_5O_5Si_2 \cdot H_2O$: C, 66.06, H, 6.78; N, 8.56. Found: C, 66.38; H, 6.60; N, 8.65.

N^6-Benzoyl-5′-*O*-(*tert*-butyldiphenylsilyl)-2′-*O*-(2-hydroxyethyl)adenosine (16). Compound **13a** (5.50 g, 8.5 mmol) and 4-methylmorpholine *N*-oxide (1.43 g, 12.2 mmol) were dissolved in dioxane (45.4 mL). OsO_4 (4% aqueous solution, 2.0 mL, 0.31 mmol) was added. The reaction mixture was protected from light and stirred for 3 h. Reaction was monitored by TLC (5% MeOH in CH_2Cl_2). The reaction mixture was diluted with ethyl acetate (100 mL) and washed with water (1 × 50 mL). The ethyl acetate layer was dried over anhydrous Na_2SO_4 and evaporated to afford dihydroxylated product (5.9 g), R_f 0.17 (5% MeOH in CH_2Cl_2). The crude dihydroxylated compound (5.59 g, 8.17 mmol) was dissolved in anhydrous CH_2Cl_2 (40.4 mL). To this solution, $NaIO_4$ adsorbed on silica gel (16.34 g, 2 g/mmol, prepared as reported in ref 20) was added and stirred at ambient temperature for 30 min. The reaction was monitored by TLC (5% MeOH in CH_2Cl_2), and the silica gel was filtered and washed thoroughly with CH_2Cl_2. The combined organic phase was concentrated under reduced pressure to yield the aldehyde (5.60 g), R_f 0.3 (5% MeOH in CH_2Cl_2). The crude aldehyde (5.55 g, 8.5 mmol) was dissolved in a solution of 1 M pyridinium *p*-toluenesulfonate (PPTS) in anhydrous MeOH (85 mL). The reaction mixture was protected from moisture. $NaBH_3CN$ (1.08 g, 17.3 mmol) was added, and the reaction mixture was stirred at ambient temperature for 8 h, diluted with ethyl acetate (150 mL), and washed with 5% aqueous $NaHCO_3$ (75 mL) and brine (75 mL). The ethyl acetate layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue obtained was purified by flash column chromatography (silica gel, 5% MeOH in CH_2Cl_2) to afford **16** (4.31 g, 78%): R_f 0.21 (5% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.98 (s, 9H), 3.55 (m, 4H), 3.87 (m, 2H), 4.09 (m, 1H), 4.55 (m, 1H), 4.73 (m, 2H), 5.30 (d, J = 5.54 Hz, 1H), 6.20 (d, J = 4.9 Hz, 1H), 7.3−8.1 (m, 15H), 8.62 (s, 1H), 8.68 (s, 1H),11.23 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.24, 26.96, 61.52, 63.37, 69.44, 72.47, 82.76, 85.29, 87.19, 123.40, 127.57, 127.88, 128.37, 128.82, 129.97, 132.58, 132.76, 133.01, 133.48, 133.71, 135.47, 135.59; MS (FAB) m/z 654 $(M + H)^+$; HRMS (MALDI) Calcd for $C_{35}H_{39}N_5O_6SiNa^+$ 676.2562, found 676.2586.

2′-*O*-(2-hydroxyethyl)-3′,5′-bis(*tert*-butyldiphenylsilyl)-guanosine (17). Compound **15** (9.00 g, 11.3 mmol) was dissolved in a mixture of CH_2Cl_2 (80 mL) and acetone (50 mL). To the clear solution obtained, 4-methylmorpholine-*N*-oxide (1.89 g, 16.1 mmol) was added. The reaction flask was protected from light. OsO_4 (2.7 mL, 4 wt % aqueous solution, 0.4 mmol) was added, and the reaction mixture was stirred at room temperature for 6 h. The reaction mixture was concentrated to half of its volume, and ethyl acetate (50 mL) was added. It was then washed with water (30 mL) and brine (30 mL). The ethyl acetate layer was dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue obtained was dissolved in CH_2Cl_2, and $NaIO_4$, adsorbed on silica gel (22.60 g, 2 g/mmol), was added and the reaction mixture stirred for 30 min at room temperature. The reaction mixture was filtered, and the silica gel was washed thoroughly with CH_2Cl_2. The combined CH_2Cl_2 layer was evaporated to dryness under reduced pressure. The residue was then dissolved in 1 M PPTS in anhydrous MeOH (99.5 mL). The reaction mixture was protected from moisture by passing a stream of argon gas through the reaction vessel. To the clear solution, $NaBH_3CN$ (1.14 g, 18.1 mmol) was added and stirred at room temperature for 4 h. Aqueous $NaHCO_3$ (5%, 50 mL) was added to the reaction mixture slowly, and the resulting mixture was extracted with ethyl acetate (2 × 50 mL). The ethyl acetate layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The residue obtained was purified by flash silica gel column chromatography and eluted with 10% MeOH in CH_2Cl_2 to afford **17** (6.46 g, 72% yield)**:** R_f = 0.46 (10% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.89 (s, 9H), 1.07 (s, 9H), 3.15−3.46 (m, 5H), 3.62 (dd, J = 3.2 and 8.3 Hz, J = 1H), 4.11 (br s, 1H), 4.28 (m, 1H), 4.5 (br s, 1H), 4.56 (t, J = 5.14 Hz, 1H), 5.97 (d, J = 6.4 Hz, 1H), 6.48 (br s, 2H), 7.33−7.74 (m, 21H), 10.69 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 18.74, 19.06, 26.67, 26.90, 60.02, 63.74, 71.57, 71.84, 80.88, 84.13, 85.12, 116.88, 127.31, 127.94, 130.05, 132.4, 132.6, 133.01, 134.67, 135.09, 135.49, 135.68, 142.73, 147.30, 151.53, 153.96, 156.84; HRMS (MALDI) Calcd for $C_{44}H_{53}N_5O_6Si_2Na^+$ 826.3427, found 826.3411.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-*O*-[2-[(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)oxy]ethyl]-5-methyluridine (18). Compound **7** (20.00 g, 37.0 mmol) was mixed with triphenylphosphine (11.65 g, 44.4 mmol) and *N*-hydroxyphthalimide (7.24 g, 44.4 mol). It was dried over P_2O_5 under vacuum at 40 °C overnight. The reaction mixture was flushed with argon, and anhydrous THF (370 mL) was added to afford a clear solution. DEAD (7.00 mL, 44.36 mmol) was added dropwise to the reaction mixture. The rate of addition was maintained such that the resulting deep red coloration had just discharged before the next drop was added. After completion of the addition, the reaction mixture was stirred at room temperature for 4 h. The solvent was removed under reduced pressure. The residue obtained was purified by flash silica gel column chromatography and eluted with ethyl acetate:hexane (60:40), to afford **18** (21.82 g, 86%) as a white foam: R_f 0.56 (ethyl acetate:hexane, 60:40); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.01 (s, 9H) 1.44 (s, 3H), 3.81−3.94 (m, 5H), 4.09 (t, J = 5.6 Hz, 1H), 4.25 (m, 1H), 4.31 (br s, 2H), 5.18 (d, J = 5.6 Hz, 1H), 5.90 (d, J = 6 Hz, 1H), 7.34−7.46 (m, 6H), 7.6−7.65 (m, 5H), 7.82 (m, 4H), 11.32 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.8, 19.40, 26.99, 62.62, 68.36, 68.56, 77.64, 83.04, 84.14, 87.50, 110.93, 123.59, 127.86, 129.89, 132.45, 134.59, 134.89, 135.17, 135.44, 150.50, 163.63, 163.97; MS (FAB) m/z 684 $[M - H]^-$; HRMS (MALDI) Calcd for $C_{36}H_{39}N_3O_9SiNa^+$ 708.2348, found 708.2325.

N^6-Benzoyl-5′-*O*-(*tert*-butyldiphenylsilyl)-2′-*O*-[2-[(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)oxy]ethyl]adenosine (19). From compound **16** (3.22 g, 4.9 mmol), triphenylphosphine (1.55 g, 5.9 mmol), *N*-hydroxyphthalimide (0.96 g, 5.9 mmol), anhydrous THF (49.2 mL), and DEAD (0.93 mL, 5.9 mmol), compound **19** (3.60 g, 91.5%) was synthesized according to the procedure used for the synthesis of compound **18**: R_f 0.27 (ethyl acetate: hexane, 7: 3); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.96 (s, 9H), 3.7−4.0 (m, 5H), 4.31 (m, 2H), 4.55 (m, 1H), 4.76 (m, 1H), 5.32 (d, J = 5.4 Hz, 1H), 6.14 (d, J = 5.0 Hz, 1H), 7.3−8.1 (m, 19H), 8.58 (s, 1H), 8.60 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.64, 27.38, 63.86, 69.53, 69.91, 77.96, 83.13, 85.56, 87.72, 124.01, 128.8, 129.14, 130.18, 132.13, 132.37, 132.57, 132.91, 133.34, 133.58, 134.37, 134.98, 135.99, 142.20, 149.98, 151.90, 152.98, 163.89, 164.76; MS (FAB) m/z 821 $(M + Na)^+$; HRMS (MALDI) Calcd for $C_{43}H_{42}N_6O_8SiNa^+$ 821.2726, found 821.2742.

3′,5′-*O*-Bis-(*tert*-butyldiphenylsilyl)-2′-*O*-[2-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)oxy]ethyl]guanosine (20). From compound **17** (3.67 g, 4.6 mmol), triphenylphosphine (1.39 g, 5.3 mmol), *N*-hydroxyphthalimide (0.87 g, 5.3 mmol), anhydrous THF (46 mL), and DEAD (0.83 mL, 5.3 mmol), compound **20** was isolated as a foam (2.98 g, 69%) according to the procedure used for the synthesis of compound **18**. Compound **20** was purified using flash silica gel column chromatography eluting with ethyl acetate, 100%, then ethyl acetate:MeOH, 93:7: ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.85 (s, 9H), 0.99 (s, 9H), 3.5−3.8 (m, 3H), 3.95 (m, 2H), 4.11 (m, 2H), 4.24 (m, 1H), 4.47 (m, 1H), 5.94 (d, J = 5.7 Hz, 1H), 6.41 (br s, 2H), 7.3−7.8 (m, 25H), 10.62 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.1, 19.3, 26.9, 63.2, 68.4, 70.9, 77.2, 81.6, 84.7, 86.0, 123.5, 127.7, 127.8, 128.6, 129.8, 129.9, 130.0, 132.6, 132.9, 133.0, 133.1, 134.5, 135.4, 135.5, 135.7, 135.9, 151.5, 153.3, 159.2, 163.4; HRMS [FAB] calcd for $C_{52}H_{56}N_6O_8Si_2Na^+$

971.3582, found: 971.3596. Anal. Calcd for $C_{52}H_{56}N_6O_8Si_2 H_2O$: C, 64.57; H, 6.04; N, 8.69. Found: C, 64.75, H, 5.85, N, 8.50.

3′,5′-*O*-Bis-(*tert*-butyldiphenylsilyl)-2′-*O*-[2-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)oxy]ethyl]-N^2-isobutyrylguanosine (21). Compound **20** (3.66 g, 3.9 mmol) was dissolved in anhydrous pyridine (40 mL), and the solution was cooled to 5 °C in an ice bath. Isobutyryl chloride (0.81 mL, 7.7 mmol) was added dropwise. The reaction mixture was allowed to warm to 25 °C. After 2h, an additional amount of isobutyryl chloride (0.40 mL, 3.8 mmol) was added at 25 °C. After 1 h, the solvent was evaporated under reduced pressure at 30 °C to give a foam which was dissolved in ethyl acetate (150 mL) to give a fine suspension. The suspension was washed with water (2 × 100 mL) and brine (100 mL), and the organic layer was separated and dried over $MgSO_4$. The solvent was evaporated in a vacuum to furnish a foam which was purified by flash silica gel column chromatography eluting with CH_2Cl_2: MeOH, 94:6, to afford the title compound as a white foam (2.57 g, 65%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.02 (s, 9H), 1.05 (s, 9H), 1.26 (d, J = 3.1 Hz, 3H), 1.30 (d, J = 3.2 Hz, 3H), 2.67 (m, 1H), 3.32 (m, 1H), 3.60 (m, 2H), 3.83 (m, 3H), 4.24 (m, 2H), 4.46 (m, 1H), 5.93 (d, J = 3.3 Hz, 1H), 7.8−7.2 (m, 25H), 8.73 (s, 1H), 11.97 (br s, 1H); HRMS [FAB] calcd for $C_{56}H_{63}N_6O_9Si_2^+$ 1019.4195, found: 1019.4213.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-*O*-[2-[(methyleneamino)oxy]ethyl]-5-methyluridine (22). Compound **18** (12.67 g, 18.5 mmol) was dissolved in anhydrous CH_2Cl_2 ((185 mL). To this was added methylhydrazine (1.3 mL, 24.4 mmol) dropwise at −10 °C. The reaction mixture was stirred at −10 to 0 °C for 1 h. The white precipitate formed was filtered and washed with ice cold CH_2Cl_2 (200 mL). The combined CH_2Cl_2 layer was concentrated to afford the 5′-*O*-(*tert*-butyldiphenylsilyl)-2′-*O*-[2-(aminooxy)ethyl]-5-methyluridine, which was then dissolved in MeOH (277 mL). Formaldehyde (3.1 mL, 20 wt % solution in water, 20.3 mmol) was added, and the mixture was stirred at room temperature for 5 h. The solvent was removed in a vacuum and the residue was purified by silica gel column chromatography and eluted with 5% MeOH in CH_2Cl_2 to afford **22** in 74% isolated yield (7.71 g) as a white foam: R_f 0.32 (5% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.03 (s, 9H), 1.45 (s, 3H), 3.6−4.08 (m, 6H), 4.13 (t, J = 4.3 Hz, 2H), 4.23 (q, J = 5.38 and 4.76 Hz, 1H), 5.20 (d, J = 5.92 Hz, 1H), 5.90 (d, J = 5.42 Hz, 1H), 6.54 (d, J = 7.7 Hz, 1H), 6.99 (d, J = 7.64 Hz, 1H), 7.37−7.67 (m, 11H), 11.39 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 11. 84, 19.41, 27.01, 62.96, 68.57, 70.01, 72.61, 82.67, 84.33, 87.14, 111.12, 127.90, 129.98, 132.37, 133.1, 134.93, 135.18, 135.44, 137.96, 150.50, 164.02; MS (ES) m/z 590 [M + Na]$^+$; HRMS (MALDI) Calcd for $C_{29}H_{37}N_3O_7SiNa^+$ 590.2293, found 590.2304. Anal. Calcd for $C_{29}H_{37}N_3O_7Si$: C, 61.351; H, 6.57; N, 7.40. Found C, 60.53; H, 6.52; N, 7.27.

N^6-Benzoyl-5′-*O*-(*tert*-butyldiphenylsilyl)-2′-*O*-{2-[(methyleneamino)oxy]ethyl)adenosine (23). From compound **19** (3.50 g, 4.4 mmol) in CH_2Cl_2 (44 mL), *N*-methylhydrazine (0.28 mL, 5.3 mmol), MeOH (65.7 mL), and formaldehyde (0.73 mL, 20 wt % solution in water, 4.8 mmol), compound **23** was obtained according to the procedure used for the synthesis of compound **22** in 83% yield (2.47 g): R_f 0.37 (5% MeOH in CH_2Cl_2);^{1}H NMR (200 MHz, $CDCl_3$) δ 1.11 (s, 9H), 3.87 (m, 2H), 4.07 (m, 2H), 4.26 (m, 3H), 4.59 (m, 2H), 6.24 (d, J = 4.0 Hz, 1H), 6.46 (d, J = 7.9 Hz, 1H), 7.02 (d, J = 7.9 Hz, 1H), 7.3−8.1 (m, 15H), 8.31 (s, 1H), 8.78 (s, 1H), 9.15 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.29, 27.01, 63.41, 69.36, 70.18, 72.73, 82.52, 85.22, 87.17, 123.68, 127.86, 128.36, 128.73, 129.94, 131.90, 132.01, 132.65, 133.04, 133.84, 135.52, 135.63, 138.02, 141.67, 149.69, 151.59, 152.71, 164.66; MS (FAB) m/z 681 (M + H)$^+$; HRMS (MALDI) Calcd for $C_{36}H_{40}N_6O_6SiNa^+$ 703.2671, found 703.2640.

3′,5′-*O*-Bis-(*tert*-butyldiphenylsilyl)-2′-*O*-{2-[(methyleneamino)oxy]ethyl)-N^2-isobutyrylguanosine (24). Compound **21** (2.5 g, 2.5 mmol) was dissolved in anhydrous CH_2Cl_2 (25 mL), the solution was cooled to 5 °C in an ice bath, and methylhydrazine (0.14 mL, 2.6 mmol) was added dropwise. After the reaction stirred for 1 h at 5 °C, an additional amount of methylhydrazine (0.03 mL, 0.5 mmol) was added. The reaction mixture was stirred another hour at 5 °C, and the solids were filtered and washed three times with cold CH_2Cl_2. The filtrate was diluted with CH_2Cl_2 (200 mL) and washed with water (2 × 100 mL) and brine (100 mL). The organic phase was dried with $MgSO_4$, and the solvent was evaporated in a vacuum to give a foam which was further dried in a vacuum for 1 h. The deprotected crude product was dissolved in anhydrous MeOH (10 mL) and formaldehyde (0.41 mL, 20 wt % solution in H_2O, 2.7 mmol) was added at ambient temperature. After stirring for 12 h, the solvent was evaporated in a vacuum to give the oxime as the crude foam **24**, which was used for the next step without purification.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-*O*-[2-[(*N*,*N*-dimethylamino)oxy]ethyl]-5-methyluridine (25). Compound **22** (7.71 g, 13.6 mmol) was dissolved in a solution of 1 M PPTS in anhydrous MeOH (136 mL). The reaction mixture was cooled to 10 °C in an ice bath, and $NaBH_3CN$ (1.71 g, 27.2 mmol) was added. The reaction mixture was stirred for 10 min at 10 °C and then allowed to come to room temperature. The reaction mixture was stirred at room temperature for an additional 2 h and monitored by TLC (5% MeOH in CH_2Cl_2). The reaction mixture was concentrated to half of its volume, diluted with ethyl acetate (300 mL), and washed with water (150 mL), aqueous $NaHCO_3$ solution (5%, 200 mL), and brine (200 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue obtained was dissolved in a solution of 1 M PPTS in MeOH (136 mL). Formaldehyde (2.3 mL, 20 wt % solution in water, 15.0 mmol) was added, and the reaction mixture was stirred at room temperature for 10 min. The reaction mixture was cooled to 10 °C in an ice bath, $NaBH_3CN$ (1.71 g, 27.2 mmol) was added, and the reaction mixture stirred at 10 °C for 10 min. The reaction mixture was allowed to come to ambient temperature and stirred for 2 h. It was then concentrated to half of its volume and diluted with ethyl acetate (300 mL). The organic phase was washed with water (240 mL), aqueous $NaHCO_3$ (5%, 250 mL), and brine (250 mL). The ethyl acetate layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue obtained was purified by flash silica gel column chromatography and eluted with 5% MeOH in CH_2Cl_2 to yield compound **25** in 80% yield (6.34 g): R_f 0.35 (5% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.03 (s, 9H), 1.44 (s, 3H), 2.43 (s, 6H), 3.6−4.1 (m, 8H), 4.25 (q, J = 4.4 Hz, 1H), 5.22 (d, J = 5.48 Hz, 1H), 5.94 (d, J = 5.92 Hz, 1H), 7.3−7.7 (m, 11H), 11.41 (s, 1H); ^{13}C (50 MHz, $CDCl_3$) δ 11.65, 19.47, 27.12, 47.53, 63.39, 68.99, 70.14, 70.73, 82.95, 84.66, 87.34, 111.14, 127.68, 127.97, 130.1, 134.62, 135.27, 135.54, 150.47, 163.87; MS (FAB) m/z 584 [M + H]$^+$; HRMS (FAB) Calcd for $C_{30}H_{42}N_3O_7Si^+$ 584.2792 found 584.2795.

N^6-Benzoyl-5′-*O*-(*tert*-butyldiphenylsilyl)-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl)adenosine (26). From compound **23** (2.2 g, 3.2 mmol) in a solution of 1 M PPTS in MeOH (32 mL), $NaBH_3CN$ (0.41 g, 6.5 mmol), formaldehyde (0.55 mL, 20 wt % solution in water, 3.6 mmol), and $NaBH_3CN$ (0.41 g, 6.5 mmol), compound **26** was obtained according to the procedure used for the synthesis of compound **25** in 81.8% yield (1.90 g): R_f 0.29 (5% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.11 (s, 9H), 2.62 (s, 6H), 3.72 (m, 1H), 3.86 (m, 2H), 3.93 (m, 1H), 4.07 (m, 2H), 4.21 (m, 1H), 4.30 (m, 1H), 4.55 (m, 2H), 6.23 (d, J = 3.7 Hz, 1H), 7.4−8.1 (m, 15H), 8.31 (s, 1H), 8.78 (s, 1H), 9.09 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.30, 27.01, 47.53, 63.53, 69.53, 70.13, 70.90, 82.66, 85.39, 87.23, 127.83, 128.34, 128.5, 128.57, 128.82, 129.89, 131.84, 132.01, 132.65, 135.63, 141.70, 149.59, 152.74; MS (FAB) m/z 719 (M + Na)$^+$. Anal. Calcd for $C_{37}H_{44}N_6O_6Si$: C, 63.77; H, 6.36; N, 12.06. Found: C, 63.03; H, 6.31; N, 12.01.

3′,5′-*O*-Bis-(*tert*-butyldiphenylsilyl)-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}-N^2-isobutyrylguanosine (27). Compound **24** (crude product) was dissolved in anhydrous MeOH (24 mL). Pyridinium *p*-toluenesulfonate (6.03 g, 24.0 mmol) was added to the solution, which was then cooled to 5 °C in an ice bath, after which $NaBH_3CN$ (0.31 g, 4.9 mmol) was added. The reaction mixture was allowed to warm to ambient temperature, and after 2 h ethyl acetate (200 mL)

was added. The organic layer was washed with H_2O (100 mL) and brine (2 × 100 mL) and dried with $MgSO_4$. The solvent was evaporated under reduced pressure to give the *N*-methylaminooxyethyl derivative as a crude foam. This mixture was then added to 1 M PPTS in MeOH (24 mL), and formaldehyde (0.41 mL, 20 wt % solution in H_2O, 2.7 mmol) was added. The reaction mixture was stirred at room temperature for 10 min and then cooled to 5 °C in an ice bath. To this was added $NaBH_3CN$ (0.31 g, 4.9 mmol), and the reaction mixture was allowed to warm to room temperature. After 2 h it was concentrated to half of its volume and diluted with ethyl acetate (100 mL) and then washed with 5% aqueous $NaHCO_3$ (75 mL) and brine (75 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. The residue obtained was purified by flash column chromatography using silica gel and CH_2Cl_2−MeOH, 92:7.5, to provide the title compound **27** as a solid (2.15 g, 96%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.98 (s, 9H), 1.11 (s, 9H), 1.25 (d, J = 6.8 Hz, 6H), 2.43 (s, 6H), 3.36 (m, 3H), 3.64 (m, 3H), 4.10 (m, 2H), 4.49 (m, 1H), 6.04 (d, J = 6.4 Hz, 1H), 7.2−7.8 (m, 21H), 8.12 (s, 1H), 11.9 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 18.84, 19.08, 19.24, 26.85, 36.32, 47.47, 63.43, 68.96, 69.87, 71.23, 82.06, 85.54, 127.63, 127.76, 129.88, 132.54, 132.78, 133.08, 135.41, 135.49, 136.67, 136.28, 136.79, 147.23, 148.08, 155.41, 178.13; HRMS (FAB) calcd for $C_{50}H_{64}N_6O_7Si_2Cs^+$ 1049.3429; found 1049.3475. Anal. Calcd for $C_{50}H_{64}N_6O_7Si_2$: C, 65.47; H, 7.03; N, 9.16; found: C, 65.29, H, 6.95, N, 9.09.

2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}-5-methyluridine (28). Triethylamine trihydrofluoride (8.7 mL, 53.2 mmol) was dissolved in anhydrous THF (106 mL), and triethylamine (3.70 mL, 26.6 mmol) was added. This mixture was then added to compound **25** (6.20 g, 10.6 mmol), and the resulting mixture was stirred at room temperature for 24 h. The reaction was monitored by TLC (5% MeOH in CH_2Cl_2). The solvent was removed under reduced pressure. The residue obtained was purified by flash silica gel column chromatography and eluted with 10% MeOH in CH_2Cl_2 to afford compound **28** in 93% yield (3.39 g): R_f 0.27 (5% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.76 (s, 3H), 2.43 (s, 6H), 3.54−3.66 (br s, 6H), 3.84 (d, J = 3.3 Hz, 1H), 3.95 (t, J = 5.1 Hz, 1H), 4.09 (q, J = 4.4 and 4.88 Hz, 1H), 5.08 (d, J = 5.74 Hz, 1H), 5.17 (t, J = 4.88 Hz, 1H), 5.86 (d, J = 5.48 Hz, 1H), 7.78 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.61, 47.66, 61.6, 69.24, 70.32, 70.88, 82.25, 85.51, 90.20, 110.85, 138.01, 150.93, 164.63; MS (FAB) m/z 346 (M + H)$^+$; HRMS (FAB) Calcd for $C_{14}H_{24}O_7N_3^+$ 346.1614, found 346.1614.

***N*^{6}-Benzoyl-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl)-adenosine (29).** From compound **26** (1.8 g, 2.6 mmol), triethylamine trihydrofluoride (4.1 mL, 25.0 mmol), anhydrous THF (25.0 mL), and triethylamine (1.74 mL, 12.5 mmol), compound **29** was synthesized according to the procedure used for the synthesis of compound **28** in 89% yield (1.00 g): R_f 0.40 (10% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.34 (s, 6H), 3.66 (m, 6H), 4.02 (m, 1H), 4.35 (m, 1H), 4.62 (m, 1H), 5.17 (m, 1H), 5.27 (d, J = 5.36 Hz, 1H), 6.16 (d, J = 6.0 Hz, 1H,) 7.5−7.7 (m, 5H), 8.75 (s, 1H), 8.76 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 47.40, 63.16, 70.03, 70.79, 82.26, 88.06, 89.59, 124.54, 127.93, 128.74, 132.81, 133.37, 143.31, 150.34, 150.54, 151.95, 164.78; LRMS (FAB) m/z 481 [M + Na]$^+$; HRMS (MALDI) Calcd for $C_{21}H_{27}N_6O_6^+$ 459.1992, found 459.2001.

2′-*O*-{2-[(*N*,*N*-Dimethylamino)oxy]ethyl}-*N*^{2}-isobutyrylguanosine (30). Triethylamine trihydrofluoride (7.56 mL, 46.4 mmol) was added to anhydrous THF (40 mL) followed by addition of triethylamine (3.24 mL, 23.2 mmol). Compound **27** (2.13 g, 2.3 mmol) was added, and after 24 h the solvent was evaporated in a vacuum to give a gelatin which was dissolved in *n*-BuOH (100 mL). Saturated $NaHCO_3$ was added slowly until evolution of the gas ceased and the pH reached 7. The organic phase was separated and washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL), and then the aqueous washes were combined and extracted with *n*-BuOH (3 × 15 mL). The solvent was evaporated in a vacuum at 50 °C to give an oil, which was dissolved in MeOH and adsorbed onto silica gel (10 g) by evaporation of the solvent. The product adsorbed on silica gel was purified by flash silica gel column chromatography and CH_2Cl_2:MeOH, 85:15, to afford the title compound **30** as a foam (1.35 g), which was contaminated with $(C_2H_5)_3N$·3HF (2.7 mol equiv): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.34 (s, 6H), 2.75 (m, 1H), 3.40−3.73 (m, 6H), 3.93 (m, 1H), 4.27 (m, 1H), 4.40 (m, 1H), 5.09 (m, 1H), 5.19 (d, J = 4.4 Hz, 1H), 5.88 (d, J = 6.8 Hz, 1H), 8.28 (s, 1H);. ^{13}C NMR (100 MHz, CD_3OD) δ 19.44, 36.99, 47.87, 62.52, 70.73, 70.82, 71.65, 84.34, 87.19, 87.82, 121.20, 139.86, 150.00, 150.60, 157.53, 181.73; HRMS (FAB) calcd for $C_{18}H_{28}N_6O_7Na^+$ 463.1917; found 463.1901.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[2-[(*N*,*N*-dimethylamino)oxy]ethyl]-5-methyluridine (31). Compound **28** (2.7 g, 7.8 mmol) was mixed with DMAP (0.20, 1.6 mmol) and dried over P_2O_5 in a vacuum overnight at 40 °C. It was then coevaporated with anhydrous pyridine (20 mL). The residue obtained was dissolved in anhydrous pyridine (18 mL), and 4,4′-dimethoxytrityl chloride (3.18 g, 9.4 mmol) was added. The reaction mixture was stirred at room temperature for 6 h under an argon atmosphere. Pyridine was removed under reduced pressure, and the resulting residue was purified by flash silica gel column chromatography and eluted with 10% MeOH in CH_2Cl_2 (containing 0.5% pyridine) to afford **31** in 78% yield (3.94 g): R_f 0.44 (10% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.35 (s, 3H), 2.60 (s, 6H), 3.43 (dd, J = 2.72, 8.2 Hz, 1H), 3.57 (dd, J = 1.86 Hz, 9.06 Hz, 1H), 3.7−3.89 (m, 3H), 3.80 (s, 6H), 4.03−4.19 (m, 4H), 4. 45 (q, J = 5.38 Hz, 7.36 Hz, 1H), 5.99 (d, J = 3.2 Hz, 1H), 6.84 (d, J = 8.84 Hz, 4H), 7.2−7.44 (m, 9H), 7.68(s, 1H), 8.22 (s, 1H); ^{13}C (50 MHz, $CDCl_3$) δ 11.75, 47.54, 55.23, 62.42, 69.23, 70.10, 70.76, 83.05, 83.67, 86.91, 87.87, 110.92, 113.34, 123.69, 127.10, 127.95, 128.28, 130.17, 135.37, 135.50, 144.45, 149.76, 150.45, 156.81, 163.95; MS (FAB) m/z 648 (M + H)$^+$, MS (ES) m/z 646 [M − H], HRMS (MALDI) Calcd for $C_{35}H_{41}N_3O_9Na^+$ 670.2735, found 670.2731. Anal. Calcd for $C_{35}H_{41}N_3O_9$: C, 64.90; H, 6.38; N, 6.49. Found: C, 64.48, H, 6.41; N, 6.41.

***N*^{6}-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[2-[(*N*,*N*-dimethylamino)oxy]ethyl]adenosine (32).** From compound **29** (1.00 g, 2.3 mmol), DMAP (0.05 g, 0.41 mmol), anhydrous pyridine (5 mL), and 4,4′-dimethoxytrityl chloride (0.88 g, 2.6 mmol), compound **32** was obtained in 75% yield (1.24 g) according to the procedure described for compound **31**, except that the reaction mixture was stirred for 8 h at room temperature under an argon atmosphere: R_f 0.20 (5% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.61 (s, 6H), 3.47 (m, 2H), 3.79 (s, 6H), 3.86 (m, 3H), 4.06 (m, 1H), 4.28 (m, 2H), 4.49 (m, 1H), 4.70 (m, 1H), 6.21 (d, J = 4.5 Hz, 1H), 6.8−8.1 (m, 18H), 8.24 (s, 1H), 8.75 (s, 1H), 9.07 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 47.42, 55.09, 63.11, 69.95, 70.81, 82.29, 84.26, 86.46, 87.19, 113.06, 123.46, 126.82, 127.82, 128.07, 128.64, 129.97, 132.58, 133.57, 135.54, 141.81, 144.42, 149.52, 151.46, 152.52, 158.44, 164.71; MS (FAB) m/z 759 (M − H)$^-$; HRMS (FAB) Calcd for $C_{42}H_{44}N_6O_8Cs^+$ 893.2275, found 893.2252. Anal. Calcd for $C_{42}H_{44}N_6O_8$·H_2O: C, 64.77; H, 5.95; N, 10.79. Found: C, 64.71; H, 5.65; N, 10.71.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[2-[(*N*,*N*-dimethylamino)oxy]ethyl]-*N*^{2}-isobutyrylguanosine (33). From compound **30** (0.85 g, 1.9 mmol), anhydrous pyridine (20 mL), DMAP (0.05 g, 0.4 mmol), and 4,4′-dimethoxytrityl chloride (1.08 g, 3.2 mmol), compound **33** was synthesized according to the procedure described for **31** in 81% yield (1.37 g) after flash silica gel column chromatography using silica (pretreated with 1% NEt_3) eluting with CH_2Cl_2:MeOH:NEt_3, 90:10:1, to afford the title compound as a foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (d, 3H), 0.97 (d, 3H), 2.18 (m, 1H), 2.56 (s, 6H), 3.25 (m, 1H), 3.48 (m, 1H), 3.75 (s, 3H), 3.76 (s, 3H), 3.80 (m, 2H), 4.22 (m, 1H), 4.49 (m, 1H), 4.71 (m, 1H), 5.94 (d, J = 5.9 Hz, 1H), 6.7−7.5 (m, 14), 7.87 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.5, 35.7, 47.3, 55.1, 63.5, 69.5, 69.9, 70.5, 81.8, 84.2, 86.2, 86.6, 113.1, 121.8, 126.9, 127.9, 129.9, 135.5, 135.8, 138.3, 144.7, 147.7, 148.3,156.0, 158.5, 178.8; HRMS (FAB) calcd for $C_{39}H_{46}N_6O_9Na^+$ 765.3224; found 765.3215. Anal. Calcd for $C_{39}H_{46}N_6O_9$: C, 63.06; H, 6.24; N, 11.31. Found: C, 62.61, H, 6.26, N, 11.10.

5′,3′-*O*-Bis(*tert*-butyldiphenylsilyl)-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl)-5-methyluridine (34). Compound

25 (7.1 g, 12.2 mmol) was dissolved in anhydrous DMF (74 mL). To this were added imidazole (11.3 g, 165.7 mmol) and *tert*-butyldiphenylsilyl chloride (21.60 mL, 82.9 mmol), and the reaction mixture was stirred for 14 h at 60 °C. The reaction mixture was allowed to come to room temperature before being poured into water (300 mL). The resulting mixture was extracted with ethyl acetate (2 × 100 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography and eluted with ethyl acetate:hexane (1:1) to yield **34** (8.54 g, 85%): R_f 0.42 (ethyl acetate:hexane, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (s, 9H), 1.09 (s, 9H), 1.43 (s, 3H), 2.5 (s, 6H), 3.27 (dd, J = 2 and 9.6 Hz, 1H), 3.43 (m, 1H), 3.56 (m, 1H), 3.68 (m, 2H), 3.78 (m, 2H), 4.00 (d, J = 2.4 Hz, 1H), 4.39 (m, 1H), 6.27 (d, J = 6.8 Hz, 1H), 7.28−7.76 (m, 21H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.71, 18.95, 19.33, 26.54, 26.86, 47.55, 63.62, 68.69, 70.26, 71.54, 81.67, 85.32, 85.86, 111.131, 127.56, 127.88, 129.48, 129.83, 129.93, 132.08, 132.92, 133.36, 134.77, 135.07, 135.36, 135.76, 136.02; HRMS (MALDI) Calcd for $C_{46}H_{60}N_3O_7Si_2^+$ 822.3964, found 822.3938.

3′,5′-*O*-Bis(*tert*-butyldiphenylsilyl)-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}-5-methylcytidine (35). A suspension of 1,2,4-triazole (17.61 g, 255 mmol) in anhydrous CH_3CN (98 mL) was cooled in an ice bath for 5 to 10 min under an argon atmosphere. To this, a cold suspension of $POCl_3$ (5.6 mL, 60 mmol) was added slowly over 10 min and stirring continued for an additional 5 min. Triethylamine (41.8 mL, 300 mmol) was added slowly over 30 min, keeping the bath temperature around 0−2 °C. The reaction mixture was stirred at 0−2 °C for an additional 30 min. Compound **34** (6.16 g, 7.5 mmol) was added in anhydrous CH_3CN (45.8 mL) in one portion and stirred for 10 min. The reaction mixture was removed from the ice bath and stirred at room temperature for 4 h. It was next concentrated to one-third of its volume, diluted with ethyl acetate (300 mL), and washed with water (2 × 200 mL) and brine (200 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was dissolved in a solution of aqueous NH_3 (24 mL, 30 wt % solution) and dioxane (118 mL). The reaction mixture was stirred at room-temperature overnight in a pressure bottle. The solvent was removed in a vacuum, and the resulting residue was purified by flash silica gel column chromatography and eluted with 5% MeOH in CH_2Cl_2 to yield **35** (5.79 g, 94%) as a white foam: R_f 0.27 (5% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.9 (s, 9H), 1.02 (s, 9H), 1.38 (s, 3H), 2.37 (s, 6H), 3.19 (m, 1H), 3.37 (m, 1H), 3.50 (br s, 3H), 3.69 (m, 2H), 3.96 (br s, 1H), 4.34 (br s, 1H), 6.14 (d, J = 5.98 Hz, 1H), 6.83 (br s, 1H), 7.17 (s, 1H), 7.32−7.68 (m, 21H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.31, 19.26, 19.43, 26.87, 27.03, 47.67, 63.39, 68.31, 70.61, 71.24, 82.21, 84.04, 87.06, 101.89, 127.56, 127.7, 127,82, 129.82, 132.48. 133.1, 133.29, 133.42, 135.22, 135.41, 135.79, 136.04, 137.62, 155.83, 165.69; MS (ES) m/z 821.3 $[M + H]^+$, HRMS (MALDI) Calcd for $C_{46}H_{60}N_4O_6Si_2Na^+$ 843.3944, found 843.3967.

***N*^{4}-Benzoyl-3′,5′-*O*-bis(*tert*-butyldiphenylsilyl)-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}-5-methylcytidine (36).** Compound **35** (5.79 g, 7.1 mmol) was dissolved in anhydrous DMF (18.1 mL). Benzoic anhydride (2.45 g, 10.9 mmol) was added, and the reaction mixture was stirred at room-temperature overnight. Water (500 mL) was added, and the reaction mixture was extracted with ethyl acetate (2 × 150 mL). The organic phase was washed with a saturated solution of $NaHCO_3$ in water (150 mL) and brine (150 mL). The ethyl acetate layer was dried over anhydrous Na_2SO_4 and concentrated in vacuum. The residue obtained was purified by flash silica gel column chromatography and eluted with 5% MeOH in CH_2Cl_2 to yield **36** (5.68 g, 87%) as a white foam: R_f 0.64, (5% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.92 (s, 9H), 1.04 (s, 9H), 1.59 (s, 3H), 2.37 (s, 6H), 3.27 (m, 1H), 3.41−3.6 (m, 4H), 3.77 (d, J = 12.22 Hz, 1H), 3.90 (t, J = 5.86 Hz, 1H), 4.08 (br s, 1H), 4.4 (t, J = 4.14 Hz, 1H), 6.08 (d, J = 5.76 Hz, 1H), 7.33−7.75 (m, 24H), 8.12 (d, J = 6.72 Hz, 2H), 12.59 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.82, 19.31, 19.44, 26.94, 27.08, 47.66, 63.69, 68.83, 70.33, 71.65, 82.03, 85.49, 86.47, 112.11, 127.69, 127.97, 129.90, 132.26, 132.38. 133.02, 133.13, 133.47, 135.21, 135.45, 135.83, 136.09, 136.43, 137.22, 147.99, 159.6, 179.59; MS (ES), m/z 924.39 $[M - H]^-$, HRMS (MALDI) Calcd for $C_{53}H_{64}N_4O_7Si_2Na^+$ 947.4206, found 947.4066.

***N*^{4}-Benzoyl-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}-5-methylcytidine (37).** Compound **36** (5 g, 5.4 mmol) was dried over P_2O_5 under high vacuum. In a 100 mL round-bottom flask, triethylamine trihydrofluoride (8.82 mL, 54.0 mmol) was dissolved in anhydrous THF (39 mL). Triethylamine (3.8 mL, 27 mmol) was added to this solution, and the mixture was quickly poured onto compound **36**. The resulting mixture was stirred at room temperature overnight. The solvent was removed in a vacuum and the residue dissolved in ethyl acetate (100 mL). The organic phase was washed with 5% aqueous $NaHCO_3$ (80 mL) and brine (80 mL). The ethyl acetate layer was dried over anhydrous Na_2SO_4 and concentrated in a vacuum under reduced pressure. The resulting residue was dissolved in CH_2Cl_2 (20 mL) and added dropwise into vigorously stirring hexane (600 mL). The precipitate formed was collected by filtration to afford **37** (2.36 g, 97%) as a white solid: R_f 0.25 (5% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.00 (s, 3H), 2.45 (s, 6H), 3.7 (m, 6H), 3.90 (m, 1H), 4.00 (t, J = 4.5 Hz, 1H), 4.14 (q, t = 5.4 Hz, 1H), 5.09 (d, J = 5.86 Hz, 1H), 5.28 (t, J = 4.76 Hz, 1H), 5.86 (d, J = 3.42 Hz, 1H), 7.52 (m, 3H), 8.19 (m, 3H), 12.98 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.53, 47.50, 61.66, 69.08, 70.08, 70.92, 81.80, 85.50, 91.52, 111.81, 128.12, 129.88, 132.53, 136.94, 139.00, 148.12, 159.60, 179.53; HRMS (FAB) Calcd for $C_{21}H_{28}N_4O_7Na^+$ 471.1856, found 471.1861. Anal. Calcd for $C_{21}H_{28}N_4O_7$: C, 56.24; H, 6.29; N, 12.49. Found: C, 56.01; H, 6.27; N, 12.37.

***N*^{4}-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}-5-methylcytidine (38).** Compound **37** (2.30 g, 5.1 mmol) was dried over P_2O_5 in a vacuum overnight. It was then coevaporated with anhydrous pyridine (10 mL), and the residue obtained was dissolved in anhydrous pyridine (14 mL). To this was added DMAP (0.063 g, 0.5 mmol), and the solution was stirred at room temperature under an argon atmosphere for 4 h. Pyridine was removed in a vacuum, and the residue obtained was dissolved in ethyl acetate (125 mL) and washed with 5% aqueous $NaHCO_3$ (75 mL) and brine (75 mL). The ethyl acetate layer was dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue obtained was purified by flash silica gel column chromatography and eluted with ethyl acetate:hexane: pyridine 60:39:1 to yield compound **38** (2.77 g, 72%): R_f 0.38 (ethyl acetate:hexane; 60:40); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.61 (s, 3H), 2.48 (s, 6H), 3.25−3.4 (m, 2H), 3.77 (br s, 10H), 4.09 (m, 2H), 4.3 (m, 1H), 5.23 (d, J = 6.46 Hz, 1H), 5.9 (d, J = 3.42 Hz, 1H), 6.94 d, J = 8.8 Hz, 4H), 7.22−7.66 (m, 12H), 7.83 (s, 1H), 8.17 (d, J = 6.84 Hz, 2H), 12.96 (s, 1H); ^{13} C NMR (50 MHz, $CDCl_3$) δ 12.79, 47.52, 55.21, 61.97, 68.8, 69.94, 71.03, 83.23, 83.65, 86.81, 88.30, 111.85, 113.25, 127.09, 128.03, 128.18, 129.83, 130.14, 132.36, 135.29, 136.58, 137.13, 144.37, 148.03, 158.79, 159.77, 179.59; HRMS (FAB) Calcd for $C_{42}H_{46}N_4O_9Cs^+$ 883.2319, found 883.2351. Anal. Calcd for $C_{42}H_{46}N_4O_9$: C, 67.19; H, 6.18; N, 7.46. Found: C, 66.96; H, 6.12; N, 7.37.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}-5-methyluridine-3′-*O*-succinate (39). The nucleoside **31** (1.00 g, 1.6 mmol) was mixed with succinic anhydride (0.24 g, 2.4 mmol) and DMAP (0.10 g, 0.8 mmol) and dried in a vacuum at 40 °C overnight. The mixture was dissolved in anhydrous $ClCH_2-CH_2Cl$ (3.90 mL), $(C_2H_5)_3N$ (0.43 mL, 3.1 mmol) was added and the solution stirred at room temperature under argon atmosphere for 4 h. It was then diluted with CH_2Cl_2 (50 mL) and washed with ice cold aqueous citric acid (10 wt %, 50 mL) and water (50 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated to dryness. The residue obtained was purified by flash silica gel column chromatography and eluted with 10% MeOH in CH_2Cl_2 to afford compound **39** in 82% isolated yield (0.95 g): R_f 0.48 (10% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.45 (s, 3H), 2.40 (s, 6H), 2.57 (m, 4H), 3.16−3.38 (m, 2H), 3.61 (m, 4H), 3.74 (s, 6H), 4.15 (m, 1H), 4.36 (m, 1H), 5.29 (m, 1H),

5.87 (d, J = 6.24 Hz, 1H), 6.91 (d, J = 8.78 Hz, 4H), 7.23−7.40 (m, 9H), 7.51 (s, 1H), 11.47 (s, 1H), 12.25 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.75, 29.47, 47.36, 55.29, 62.36, 69.74, 70.32, 70.95, 80.60, 81.31, 87.12, 111.373, 113.38, 127.29, 128.10, 130.18, 135.19, 135.52, 144.14, 150.60, 158.84, 164.29, 171.72, 175.35; MS (FAB) m/z 748 $[M + H]^+$; HRMS (FAB) Calcd for $C_{39}H_{46}N_3O_{12}^+$ 748.3082 found 748.3074.

5′-*O*-(4,4′-Dimethoxytrityl)-*N*^{6}-benzoyl-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}adenosine-3′-*O*-succinate (40). From compound **32** (2.5 g, 3.3 mmol), succinic anhydride (0.49 g, 4.9 mmol), DMAP (0.31 g, 2.6 mmol), triethylamine (0.92 mL, 6.6 mmol), and 1,2-dichloroethane (9.13 mL), compound **40** was isolated in 85% yield (2.4 g), according to the procedure used for the synthesis of compound **39**. Compound **40** was purified by flash silica gel column chromatography (10% MeOH in CH_2Cl_2): R_f 0.11 (5% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.48 (s, 6H), 2.71 (m, 4H), 3.39−3.88 (m, 6H), 3.77 (s, 6H), 4.36 (m, 1H), 5.03 (m, 1H), 5.53 (m, 1H), 6.18 (d, J = 6.2 Hz, 1H), 6.81 (m, 4H), 7.17−7.64 (m, 12H), 8.04 (d, J = 6.6 Hz, 2H), 8.21 (s, 1H), 8.67 (s, 1H), 9.31 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.95, 47.01, 54.95, 62.79, 69.65, 69.92, 71.32, 79.67, 82.14, 86.60, 113.00, 123.55, 126.80, 127.69, 127.90, 128.08, 128.40, 129.82, 132.54, 133.08, 135.19, 141.97, 144.10, 149.73, 151.90, 158.38, 165.09, 171.21, 175.35; MS (ES) m/z 899 $[M + K]^+$; HRMS (FAB) Calcd for $C_{46}H_{49}N_6O_{11}^+$ 861.3459 found 861.3458.

5′-*O*-(4,4′-Dimethoxytrityl)-*N*^{2}-isobutyryl-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]ethyl}guanosine-3′-*O*-succinate (41). From compound **33** (0.6 g, 0.8 mmol), succinic anhydride (0.16 g, 1.6 mmol), DMAP (0.1 g, 0.8 mmol), triethylamine (0.23 mL, 1.6 mmol), and CH_2ClCH_2Cl (3 mL), compound **41** was isolated in 97% yield (0.66 g), according to the procedure used for the synthesis of compound **39**. Compound **41** was purified by flash silica gel column chromatography (10% MeOH in CH_2Cl_2): R_f 0.19 (5% MeOH in CH_2Cl_2; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.12 (d, J = 5.46 Hz, 6H), 2.29 (s, 6H), 2.51−2.65 (m, 4H), 2.74 (m, 1H), 3.18 (dd, J = 3.4 and 7.2 Hz, 1H), 3.42 (m, 5H), 3.72 (s, 6H), 4.18 (br s, 1H), 4.83 (m, 1H), 5.30 (m, 1H), 5.92 (d, J = 7.4 Hz, 1H), 6.82 (m, 4), 7.11−7.4 (m, 9H), 8.15 (s, 1H), 11.58 (s, 1H), 12.08 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 18.36, 29.01, 29.20, 35.68, 46.87, 54.95, 63.00, 69.65, 69.88, 71.07, 79.39, 81.91, 86.31, 113.00, 121.34, 126.89, 127.53, 129.75, 135.14, 135.44, 138.46, 144.39, 147.47, 148.2, 155.29, 158.46, 171.38, 174.54, 178.88; MS (API-ES) m/z 841.3 $[M - H]^-$; HRMS (FAB) Calcd for $C_{43}H_{51}N_6O_{12}^+$ 843.3565 found 843.3575.

***N*^{4}-Benzoyl-2′-*O*-{2-[(*N*,*N*-dimethylamino)oxy]-ethyl}-5′-*O*-(4,4′-dimethoxytrityl)-5-methylcytidine-3′-*O*-succinate (42).** From compound **38** (0.50 g, 0.7 mmol), succinic anhydride (0.10 g, 1.0 mmol), DMAP (0.04 g, 0.34 mmol), $(C_2H_5)_3N$ (0.19 mL, 1.34 mmol), and 1,2-dichloroethane (2.5 mL), compound **42** was isolated in 90% yield (0.51 g), according to the procedure used for the synthesis of compound **39**. Compound **42** was purified by flash silica gel column chromatography (10% MeOH in CH_2Cl_2): R_f 0.27 (10% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.57 (s, 3H), 2.66 (s, 6H), 2.5−2.8 (m, 4H), 3.42 (dd, J = 2.22 and 8.8 Hz, 1H), 3.63 (dd, J = 2.24 and 8.78 Hz, 1H), 3. 82 (s, 6 H) 3.69−4.02 (m, 4H), 4.34 (m, 2H), 5.36 (t, J = 5.36 Hz, 1H), 6.11 (d, J = 4.18 Hz, 1H), 6.87 (d, J = 8.3 Hz, 4H), 7.27−7.55(m, 12H), 7.80 (s, 1H), 8.31 (d, J = 6.68 Hz, 2 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.85, 29.53, 29.84, 47.19, 55.28, 61.98, 69.56, 70.00, 70. 58, 80.78, 81.29, 87.21, 87.69, 112.37, 113.36, 127.27, 128.10, 128.17, 129.91, 130.14, 132.47, 135.11, 136.35, 137.06, 144.10, 148.02, 158.80, 159.59, 171.59, 174.06, 179.58; MS (API-ES$^+$) m/z 873 $[M + Na]^+$, MS (API-ES$^-$) 849 $[M-H]^-$; HRMS (FAB) Calcd for $C_{46}H_{51}N_4O_{12}^+$ 851.3503 found 851.3492.

Oligonucleotide Synthesis. A 0.1 M solution of the amidites **1**, **2**, **3**, and **4** in anhydrous acetonitrile was used for the synthesis of modified oligonucleotides. The oligonucleotides were synthesized on functionalized controlled pore glass (CPG) on an automated solid-phase DNA synthesizer. CPG **43**, **44**, **45**, and **46** functionalized with 2′-*O*-DMAOE modified nucleosides were used wherever necessary. For incorporation of **1**, **2**, **3**, and **4**, phosphoramidite solutions were delivered in two portions, each followed by a 5 min coupling wait time. All other steps in the protocol supplied by the manufacturer were used without modification. Oxidation of the internucleotide phosphite to the phosphate was carried out using CSO {[1-*S*-(+)-(10-camphorsulfonyl)oxaziridine], 0.5 M in acetonitrile}. The Beaucage reagent (0.1 M in acetonitrile) was used as a sulfurizing agent. The coupling efficiencies were more than 97%. After completion of the synthesis, the solid support was suspended in aqueous ammonium hydroxide (30 wt %) and kept at room temperature for 2 h. The solid support was filtered, and the filtrate was heated at 55 °C for 6 h to complete the removal of all protecting groups. Crude oligonucleotides were purified by high performance liquid chromatography (HPLC, C-4 column, Waters, 7.8 × 300 mm, A = 100 mM ammonium acetate, B = acetonitrile, 5−60% of B in 55 min, flow 2.5 mL min^{-1}, λ 260 nm). Detritylation with aqueous 80% acetic acid followed by desalting gave 2′-modified oligonucleotides in 30−40% isolated yield. The oligonucleotides were characterized by ESMS, and their purity was assessed by HPLC (Table 3) and capillary gel electrophoresis.

Table 3. HPLC and Mass Spectral Analysis of the 2′-*O*-DMAOE Oligonucleotides Used for T_m Analysis

oligo. no.	mass		HPLC retention time, min[a]
	calcd	found	
48	5246.36	5245.60	23.58
50	5906.36	5905.60	26.21
52	7018.43	7017.69	27.95
54	7148.16	7147.15	29.99
56	8300.50	8301.67	27.93
58	8605.56	8605.50	31.54

[a] Water C-4, 3.9 × 300 mm, A = 50 mM triethylammonium acetate, pH 7, B = acetonitrile, 5 to 60% B in 55 min, flow 1.5 mL min^{-1}, λ = 260 nm.

T_m Analysis. The thermal stability of the duplexes formed by oligonucleotides with the 2′-*O*-DMAOE modification and complementary RNA was studied by measuring the UV absorbance versus temperature curves as described previously.[31] Each sample contained 100 mM Na^+, 10 mm phosphate, 0.1 mM EDTA, 4 μM oligonucleotides, and 4 μM complementary length matched RNA. Each T_m reported was an average of two experiments. ΔT_m per modification was calculated by subtracting the T_m of the unmodified DNA−RNA parent duplex and dividing by the number of modified residues in the sequence.

Nuclease Stability Assay. The nuclease stability of the 2′-modified oligonucleotides was evaluated using snake venom phosphodiesterase as described previously.[29]

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Supporting Information Available: Copies of the ^{1}H NMR, ^{13}C NMR, and mass spectra of compounds **7**, **13**, **15**−**17**, **25**−**33**, **35**, **37**, and **38**. ^{31}P NMR spectra and mass spectra of **1**−**4**. ESMS data and HPLC profiles for oligonucleotides **48**, **50**, **52**, **54**, **56**, and **58**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(31) (a) Monia, B. P.; Johnston, J. J.; Ecker, D. J.; Zounesli, M. A.; Lima, W. F.; Freier, S. M. *J. Biol. Chem.* **1992**, *267*, 19954−19962. (b) Lesnik, E. A.; Guinosso, C. J.; Kawasaki, A. M.; Sasmor, H.; Zounes, M.; Cummins, L. L.; Ecker, D. J.; Cook, P. D.; Freier, S. M. *Biochemistry* **1993**, *32*, 7832−7838. (c) Lesnik, E. A.; Freier, S. M. *Biochemistry* **1998**, *37*, 6991−6997.