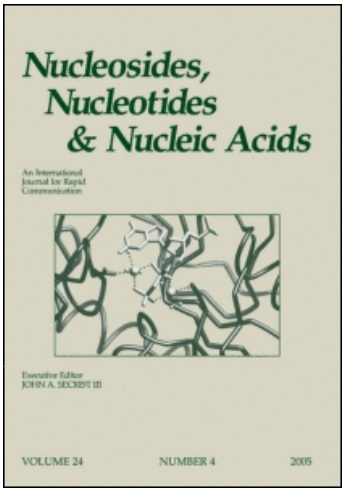

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N,N'-Bis-(2-(cyano)ethoxycarbonyl)-2-methyl-2-thiopseudourea: A Guanylating Reagent for Synthesis of 2'-*O*-[2-(Guanidinium)ethyl]-Modified Oligonucleotides

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N,N′-BIS-(2-(CYANO)ETHOXYCARBONYL)-2-METHYL-2-THIOPSEUDOUREA: A GUANYLATING REAGENT FOR SYNTHESIS OF 2′-*O*-[2-(GUANIDINIUM)ETHYL]-MODIFIED OLIGONUCLEOTIDES

Thazha P. Prakash, Ask Püschl, and Muthiah Manoharan □ *Department of Medicinal Chemistry, Isis Pharmaceuticals Inc., Carlsbad, California, USA*

□ *A guanylating reagent, N,N-bis-(2-(cyano)ethoxycarbonyl)-2-thiopseudourea, was synthesized and used for synthesis of 2′-O-[2-(guanidinium)ethyl] (2′-O-GE) modified oligonucleotides. A convenient deprotection method for the 2′-O-GE oligonucleotides was developed.*

Numerous nucleic acid analogues have been synthesized and their biochemical properties analyzed for therapeutic as well as diagnostic applications.[1,2] In order to improve upon the activity of nucleic acid-based drugs, several chemistries have been developed.[2–4] Among these, the most promising resulted from the chemical modification at the 2′-position of the oligonucleotide.[2,3] The 2′-position is attractive for chemical modification because it offers enhanced binding affinity to a complementary RNA strand and imparts nuclease resistance.[3] For example, the 2′-*O*-aminopropyl (2′-*O*-AP, Figure 1) provides extremely high nuclease resistance and good hybridization properties.[5] The high nuclease resistance of the 2′-*O*-AP oligonucleotides has been attributed to their cationic nature. The pK_a of the primary amino group is around 9 and, thus, the group is protonated under physiological conditions. This is termed the charge effect.

Another interesting highly basic, cationic group is the guanidinium, with a pK_a of 12.5. The guanidinium group plays an important role in fundamental organic and biological processes such as catalysis and protein-nucleic acid interactions. The guanidinium group contains three nitrogen atoms in a plane, remains protonated over a wide pH range, and can form up to five hydrogen bonds when present within the arginine side chain.[6] Recently, efficient postsynthesis methods of guanidination of the phosphate linkage and the 2′-position of nucleic acid were reported.[7] Cationic oligonucleotides containing a guanidinium backbone show increased affinity to

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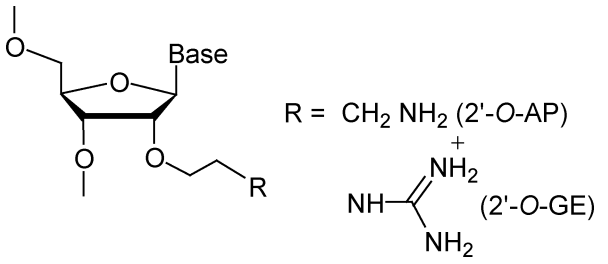
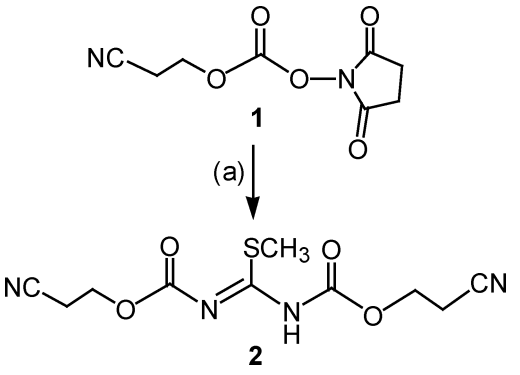


FIGURE 1 2′-*O*-(2-(guanidinium)ethyl) (2′-*O*-GE) and 2′-*O*-(3-aminopropyl) (2′-*O*-AP) RNA.

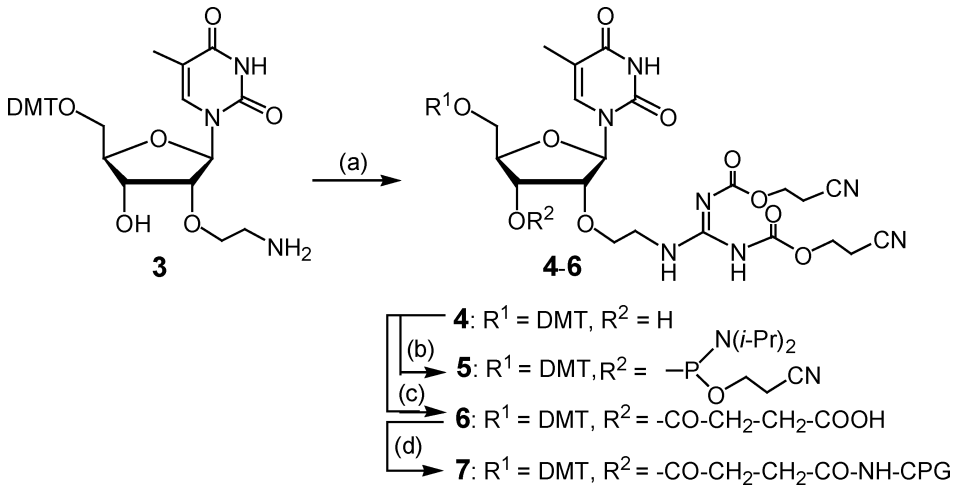
target RNA.[8] Nucleic acids with guanidinium functionality at 5-position of pyrimidine stabilize a triplex structure with a DNA duplex.[9]

A suitable protecting strategy for the guanidinium group that is compatible with solid-phase DNA synthesis protocols is essential for the synthesis of 2′-*O*-GE modified oligonucleotides. Unfortunately, conventional guanidinium protecting groups such as benzyloxycarbonyl (Cbz) or *tert*-butyloxycarbonyl (Boc)[10] are not compatible with solid-phase DNA synthesis. Here we report a guanylating reagent with a base labile protecting group and its use in synthesis of 2′-*O*-(2-guanidinium)ethyl modified oligonucleotides. We recently reported the stabilizing effect of 2′-*O*-(2-guanidinium)ethyl-modified oligonucleotides on duplex and triplex nucleic acid structure.[11] The full account of synthetic strategy used for the synthesis of 2′-*O*-GE-modified oligonucleotides and their biophysical properties are described in this report.

We previously reported the use of an amino protecting group compatible with oligonucleotide synthesis, the *N*-(2-(cyano)ethoxycarbonyl) group (CEOC).[12] Here we report use of the CEOC protecting group in the synthesis of 2′-*O*-GE-modified oligonucleotides. The guanylating reagent was prepared by treatment of CEOE-succinimide **1**[12] with *S*-methylisothiourea hemisulfate to yield *N,N*-bis-CEOC-2-methyl-2-thiopseudourea **2** (Scheme 1).



SCHEME 1 [a](a) *S*-methylisothiourea hemisulfate, CH_2Cl_2, $NaHCO_3$, rt.



SCHEME 2 [a]DMT = 4,4′-dimethoxytrityl; (a) **2**, anhydrous DMF, triethylamine, rt; (b) *N,N*-diisopropylammonium tetrazolide, (2-cyanoethyl)-*N,N,N′,N′*-tetraisopropylphosphorodiami-dite, CH_3CN, rt; (c) succinic anhydride, pyridine, CH_2Cl_2, DMAP, rt; (d) 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumtetrafluoroborate (TBTU), DMF, amino alkyl controlled pore glass (CPG), rt.

Scheme 2 shows the synthesis of the CEOC-protected 2′-*O*-GE-5-methyluridine-3′-phosphoramidite **5** and solid support **7**. The 2′-*O*-[2-(amino)ethyl]-5′-*O*-(4,4′-dimethoxytrityl)-5-methyluridine **3** was synthesized using previously reported procedures.[12] Compound **3** was treated with guanylating reagent **2** and triethylamine in DMF at room temperature to yield **4** (66%) with a protected guanidinium functionality. Compound **4** was converted into 3′-phosphoramidite **5** using standard procdures.[5] Compound **4** was converted into the 3′-*O*-succinyl derivative **6** and loaded onto amino alkyl controlled pore glass (CPG) according to standard synthetic procedures[13] to yield solid support **7** (32.8 μmol g^{-1}). Oligonucleotides shown in the Table 1 were synthesized using phosphoramidite **5** and solid support **7**, and the standard phosphoramidites and solid supports for incorporation of adenine (A), thymine (T), guanine (G), and cytocine (C) residues. Oxidation of the internucleosidic phosphite groups was carried out using 1-*S*-(+)-(10-camphorsulfonyl)oxaziridine[14] or *tert*-butylhydroperoxide/acetonitrile/water (10:87:3). The step-wise coupling efficiency of the modified phosphoramidites was more than 97%.

When the oligonucleotides modified with CEOC-protected 2′-*O*-(2-guanidinium)ethyl were heated with aqueous ammonia at 55°C, one major additional product along with expected oligonucleotide was observed in the electrospray mass spectrometry (ES-MS) analysis. The additional product had a molecular weight of 68 mass units per modification greater than molecular weight of the expected oligonucleotide, suggesting the formation

TABLE 1 2′-*O*-GE modified oligonucleotides

Number	Sequence
8	5′-d(T*CC AGG T*GT* CCG CAT* C)-3′
9	5′-d(CTC GTA CT*T* T*T*C CGG TCC)-3′
10	5′-d(T*T*T* T*T*C TCT CTC TCT)-3′
11	5′-d (T*TT* TT*C TCT CTC TCT)-3′
12	5′-d(TT*T TT*C TCT* CTC T*CT)-3′
13	5′-d(TTT TTT TTT TTT TTT TTT T*)-3′
14	5′-d(TTT TTT TTT TTT TTT TT*T T*)-3′
15	5′-d(TTT TTT TTT TTT TTT T*T*T* T*)-3′

All oligonucleotides were phosphodiesters;
T* indicates 2′-*O*-[2-(guanidinium)ethyl]-5-methyluridine.

of a triazine derivative. One possible mechanism for the formation of a triazine derivative is the nucleophilic attack of the ammonia at one of the carbonyls bearing the 2-cyanoethoxy group, followed by intramolecular cyclization to form **16** (Figure 2).

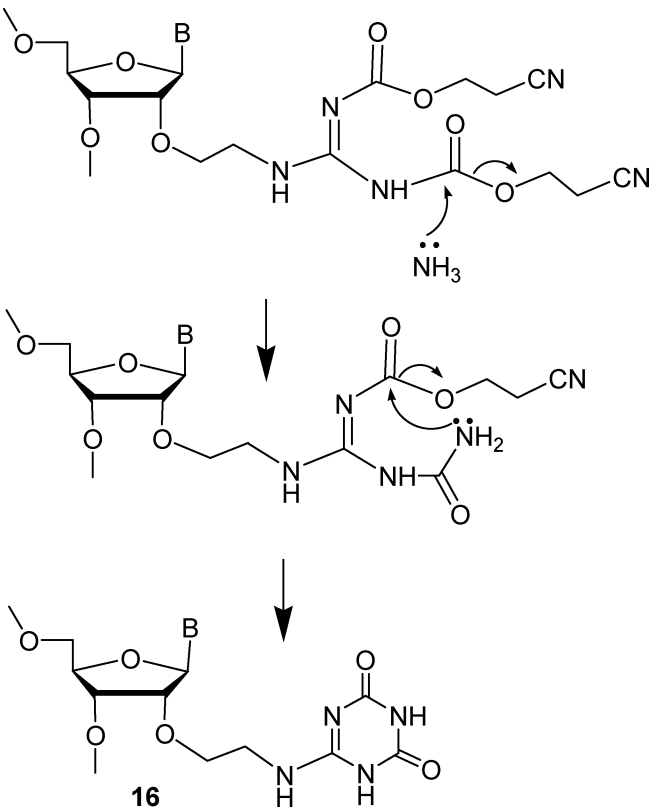


FIGURE 2 Possible mechanism of formation of triazine derivative during deprotection of oligonucleotides with a CEOC-protected guanidinium group at the 2′-position with aqueous ammonia.

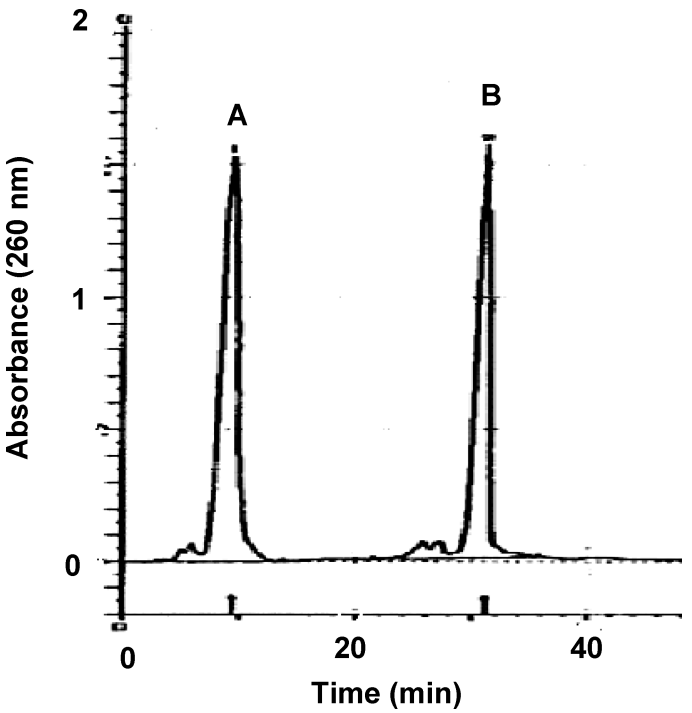


FIGURE 3 HPLC profile of oligonucleotide **17** after deprotection with aqueous ammonia. HPLC conditions: Altech SAX 4.6 × 250 mm column; mobile phase A = 10% acetonitrile, mobile phase B = 10% acetonitrile in 2 M ammonium acetate; gradient 0–100% B in 40 minutes; flow rate 1 mL min-1; detection at 260 nm.

In order to confirm this mechanism, we synthesized a pentamer oligonucleotide **17**, 5′-d(GAT*CT)-3′, where T* is 2′-*O*-GE-5-methyl-uridine, using phosphoramidite **5** to introduce the 2′-*O*-GE residue. The solid support carrying the oligonucleotide was heated with aqueous ammonia at 55°C for 6 hours. The solid support was filtered and the filtrate was concentrated. The residue was dissolved in water and analyzed by HPLC (Figure 3) on a SAX column. The two products formed were separated and analyzed by ES-MS. The fraction from Peak A in Figure 3 gave a mass corresponding to the molecular weight of oligonucleotide **17** (Peak A: Calcd. MS 1579.31, found 1579.95). However, the Peak B fraction gave a mass 69 units greater than expected for oligonucleotide **17** (Peak B: Calcd. MS 1648.4, found 1648.9). This molecular weight corresponds to the triazine-modified oligonucleotide **18** (Figure 4), presumably generated during the ammonium hydroxide-mediated deprotection by the mechanism shown in Figure 2. Deprotection with ammonium hydroxide at room temperature also resulted in the formation of triazine derivative.

Removal of the 2-cyanoethoxycarbonyl group from modified oligonucleotides with 50% piperidine[15] prior to heating with aqueous ammonia

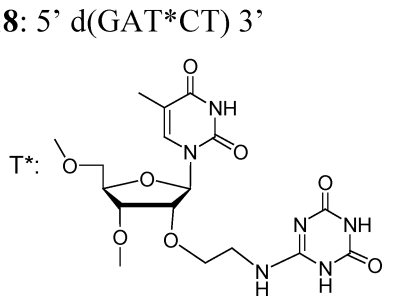


FIGURE 4 2′-Triazine modified oligonucleotide **18** formed when solid support bearing oligonucleotide **17** was heated with agueous ammonia at 55°C.

prevented the formation of the triazine derivative as shown by ES-MS analysis. Piperidine is a base, but is a poor nucleophile. Hence, it may initiate the elimination of acrylonitrile, resulting in the formation of 2′-*O*-GE modified oligonucleotides while suppressing the competing nucleophilic reaction.

A two-step deprotection condition was developed for the synthesis of 2′-*O*-GE oligonucleotides with a CEOC-protected guanidinium group. The oligonucleotides on solid support were treated with 50% piperidine in water and kept at room temperature for 24 hours to remove the CEOC protecting groups from the guanidinium groups, and the cyanoethyl group from the internucleoside phosphates and simultaneously release the oligonucleotides from solid supports. The solid supports were removed by filtration and the filtrates were concentrated to dryness. The oligonucleotides were then heated with aqueous ammonia (28–30 wt%) at 55°C for 6 hours to complete deprotection of the protecting groups from the exocyclic amino groups of the bases. The oligonucleotides (Table 1) were purified by reverse-phase HPLC and characterized by ES-MS (Table 2) and purity was assessed using HPLC (Table 2) and capillary gel electrophoresis.

Our previous work showed that the 2′-*O*-GE modification imparted stabilization of 2°C per modification to a duplex with complementary RNA, relative to an unmodified DNA of the same sequence, when modifications were dispersed as in oligonucleotide **8**.[11] Consecutive modifications, as in sequence **9**, destabilized the duplex with RNA slightly relative to unmodified DNA. This behavior also was observed for oligonucleotides bearing 2′-*O*-aminopropyl or homologous groups.[5] The loss of stability observed for duplexes between RNA and oligonucleotides with consecutive 2′-*O*-GE or other cationic modifications is most likely due to repulsions between positively charged moieties in the minor groove. Hybridization with the complementary DNA led to duplexes less stable than those formed with the complementary RNA.[11]

We also evaluated the affinity of 2′-*O*-GE-modified oligonucleotides for a double-stranded DNA target.[11] An increase in the midpoint of the dissociation of the third strand (T) of 4.1°C per modification was observed

TABLE 2 ES-MS and HPLC analysis of oligonucleotides with 2′-*O*-GE modification

Number	ES-MS		HPLC Retention Time, min[a]
	Calcd	Found	
8	5238.22	5238.21	18.69
9	5797.49	5797.15	18.06
10	4931.41	4931.46	21.93
11	4729.73	4729.47	19.11
12	4830.80	4830.29	16.07
13	5818.47	5818.62	19.46
14	5920.59	5920.21	19.32
15	6122.93	6122.60	18.21

[a]Conditions were Waters C-4 3.9 × 300 mm column; mobile phase A was 50 mM triethylammonium acetate; pH 7; mobile phase B was acetonitrile; gradient was 5% to 60% B in 55 minutes; flow rate 1.5 mL min^{-1}; detection at $\lambda = 260$.

for oligonucleotide **10** with sequential modification as compared to an unmodified DNA third strand. The observed enhancement in T was 0.6°C per modification higher than observed with the 2′-*O*-aminoethyl-modified oligonucleotide.[11,16] When the modifications were dispersed as in oligonucleotides **11** and **12**, the T was increased relative to unmodified DNA, but was considerably less than that of the oligonucleotide with consecutive modifications.[11]

The stability of 2′-*O*-GE oligonucleotides against nucleases also was evaluated.[11] A phosphodiester oligonucleotide with a single 2′-*O*-GE residue at the 3′ end (Table 1, **13**) was almost completely degraded after 4 hours in the presence of snake venom phosphodiesterase. Oligonucleotides with two or four 3′ terminal 2′-*O*-GE residues (Table 1, **14–15**) were stable at >95% full length after 24 hours in the presence of nuclease.

Previous work indicated that 2′-*O*-GE-modified oligonucleotides showed high affinity to complementary RNA and double-stranded DNA and exceptional nuclease resistance.[11] These properties make the 2′-*O*-GE modification of interest for many nucleic acid-based applications, including antisense, RNA interference (RNAi), decoy, and aptamer therapeutic and diagnostic technologies. Initial synthetic routes to 2′-*O*-GE-modified oligonucleotides were not compatible with solid-phase oligonucleotide synthesis protocols. We have developed a guanylating reagent with a CEOC protecting group that is compatible with solid-phase synthesis. A two-step deprotection protocol was required to avoid formation of a triazine side product. This guanylating reagent will make synthesis of 2′-*O*-GE-containing oligonucleotides practical and will hopefully lead to further investigation of this promising modification.

EXPERIMENTAL

General Procedures

Anhydrous CH_3CN, standard phosphoramidites and reagents for oligonucleotide synthesis were purchased from Glen Research, Inc. (Sterling, VA, USA). All other reagents and anhydrous solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. ^{1}H NMR spectra were referenced using internal standard $(CH_3)_4Si$ and ^{31}P NMR spectra were referenced using external standard 85% H_3PO_4. Microanalyses were performed by Quantitative Technologies Inc. (Whitehouse, NJ, USA). Mass spectra were recorded by Mass Consortium (San Diego, CA, USA) and the College of Chemistry, University of California (Berkeley, CA, USA).

***N,N*′-Bis-CEOC-2-methyl-2-thiopseudourea (2).** *S*-methylisothiourea hemisulfate (5.29 g, 38.0 mmol) was suspended in CH_2Cl_2 (250 mL) and sat. $NaHCO_3$ (250 mL). CEOC-*O*-succinimide **1** (20.2 g, 95.2 mmol) was added and the reaction mixture was stirred for 2 hours at room temperature. The organic phase was separated from the aqueous phase. The aqueous phase was extracted with dichloromethane (2 × 200 mL) and the combined organic phase was dried (Na_2SO_4), filtered, and evaporated. The crude product was purified by flash silica gel column chromatography with ethyl acetate/dichloromethane (95:5) as eluent to yield **2** (3.78 g, 35%) as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 11.80 (brs, 1H), 4.39 (p, J = 6.0 Hz, 4H), 2.80 (t, J = 6.0 Hz, 4H), 2.45 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 160.3, 150.7, 117.0, 116.5, 60.9, 60.6, 18.0, 14.7; HRMS (FAB) Calcd for $C_{10}H_{13}N_4O_4S^+$ 285.0656, found: 285.0658.

2′-*O*-[2-(*N,N*′-Bis-CEOC-guanidinium)ethyl]-5′-*O*-(4,4′-dimethoxytrityl)-5-methyl uridine (4). Compound **3** (1.56 g, 2.58 mmol) was dissolved in anhydrous DMF (9 mL). To this, guanylating reagent **2** (0.81 g, 2.85 mmol) and then triethylamine (0.36 ml, 0.2.58 mmol) were added and the reaction mixture was stirred at room temperature for 4 hours. The reaction was quenched by addition of 5% aqueous $NaHCO_3$ (40 mL), extracted with ethyl acetate (2 × 60 mL), and the combined organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated. The crude product was purified by flash silica gel column chromatography with ethyl acetate as eluent to yield **4** (1.44 g, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.67 (s, 1 H), 9.80 (s, 1 H), 8.59 (t, J = 6.0 Hz, 1H), 7.63 (s, 1H), 7.42–7.21 (m, 9H), 6.84 (d, J = 9 Hz, 4H), 6.01 (d, J = 3 Hz, 1H), 4.47 (brs, J = 6 Hz, 2H), 4.36 (t, J = 6 Hz, 2H), 4.27 (t, J = 6 Hz, 2H) 4.15–3.97 (m, 3H), 3.93–3.84 (m, 1H), 3.78 (s, 6H), 3.73 (m, 2H) 3.57–3.42 (m, 2H), 2.73 (m, 4H), 1.34 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5, 162.9, 158.7, 156.1, 153.0, 150.8, 144.4, 135.5, 135.3, 130.2, 128.1, 127.7, 127.2, 117.5, 116.9, 113.3, 111.1, 87.1, 86.8, 83.5, 82.4, 69.2, 68.9, 62.4, 61.1, 59.9, 55.3, 40.9, 18.1, 17.9, 11.9; HRMS (FAB) Calcd for $C_{42}H_{45}N_7O_{12}Na^+$ 862.3024, found: 862.2991.

2′-*O*-[2-(*N′*,*N′*,-bis-CEOC-guanidinium)ethyl]-5′-*O*-(4,4′-dimethoxytrityl)-5-methyl uridine-3′-[(2-cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidite (5). Compound **4** (0.78 g, 0.92 mmol) and diisopropylamine tetrazolide (0.16 g, 0.92 mmol) were dried by coevaporation with anhydrous CH_3CN. The residue obtained was re-dissolved in anhydrous CH_3CN (6 mL). 2-cyanoethyl-*N*,*N*,*N′*,*N′*-tetraisopropylphosphorodiamidite (0.54 mL, 1.74 mmol) was added and the reaction mixture was stirred at room temperature under argon for 5 hours. The solvent was evaporated and the crude product purified by flash silica gel column chromatography with ethyl acetate/hexane as eluent to give **5** (0.62 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.66 (s, 1 H), 8.58 (t, $J = 6.0$ Hz, 1H), 8.31(s, 1H), 7.65–7.61 (m, 1H), 7.43–7.23 (m, 9H), 6.86–6.81 (m, 4H), 6.04–6.01 (m, 1H), 4.55–4.46 (m, 1H), 4.41–4.28 (m, 5H), 4.22–4.08 (m, 2H), 4.06–3.87 (m, 3H), 3.79 (s, 6H), 3.70–3.47 (m, 6H) 3.37–3.29 (m, 1H), 2.76–2.72 (m, 4H),2.65 (t, $J = 6$ Hz, 1H), 2.37 (t, $J = 6$ Hz, 1H), 1.38–1.36 (m, 3H), 1.17–1.14 (m, 9H), 1.01 (d, $J = 6$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5, 164.4, 163.0, 158.9, 156.1, 156.0, 153.0, 150.8, 144.4, 135.4, 135.3, 130.4, 130.3, 128.5, 128.4, 128.1, 127.4, 118.0, 117.6, 117.2, 116.6, 113.4, 111.4, 111.3, 87.5, 87.4, 87.2, 87.1, 83.2, 82.9, 82.1, 70.8, 70.7, 70.6, 69.1, 68.5, 62.4, 62.1, 61.0, 59.9, 58.1, 58.0, 57.8, 55.4, 43.5, 43.4, 43.2, 40.9, 24.9, 24.8, 24.7, 24.6, 20.4, 20.3, 18.2, 18.1, 12.0, 11.9; ^{31}P NMR (80 MHz, $CDCl_3$): δ 150.87 and 150.78; HRMS (FAB) Calcd for $C_{51}H_{63}O_{13}N_9PCs^+$ 1172.3259, found: 1172.3203.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[2-(*N*,*N*′-CEOC-guanidinium)ethyl]-5-methyluridine-3′-*O*-succinate (6). Compound **4** (0.25 g, 0.3 mmol) was co-evaporated with anhydrous CH_3CN. To this, succinic anhydride (0.06 g, 0.6 mmol), DMAP (0.02 g, 0.16 mmol), anhydrous pyridine (0.05 mL, 0.6 mmol) and CH_2Cl_2 (1 mL) were added and stirred at room temperature under argon atmosphere for 4 hours. The reaction mixture was diluted with CH_2Cl_2 (25 mL) washed with cold 10% aqueous citric acid (20 mL) and brine (25 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated to yield **6** (0.25 g, 88%) as a foam. R_f (0.25, 5% MeOH in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.34 (s, 3H), 2.20–2.50 (m, 8H), 3.45 (d, $J = 5.4$ Hz, 1H), 3.64 (d, $J = 11.6$ Hz, 1H), 3.72–4.01 (m, 10H), 4.15 (brs, 1H), 4.26–4.38 (m, 6H), 5.40 (d, $J = 4.4$ Hz, 1H), 5.93 (d, $J = 2.1$ Hz, 1H), 6.83 (d, $J = 7.2$ Hz, 4H), 7.24–7.41 (m, 9H), 7.77 (s, 1H), 8.57 (s, 1H), 10.51 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.4, 171.7, 165.8, 163.1, 159.0, 156.2, 153.0, 150.4, 144.2, 135.9, 135.2, 130.3, 128.2, 127.4, 117.3, 116.6, 113.5, 110.8, 88.0, 87.3, 81.1, 80.8, 70.0, 69.5, 61.6, 60.9, 60.0, 55.5, 41.1, 28.8, 18.3, 18.1, 11.9; HRMS (FAB) Calcd for $C_{46}H_{50}N_7O_{15}^+$ 940.3365, found: 940.3346.

5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[2-(*N*,*N*′-CEOC-guanidinium)ethyl]-5-methyluridine-3′-*O*-succinyl-CPG (7). The 3′-*O*-succinate **6** (0.23 g, 0.24 mmol) was dried over P_2O_5 in vacuo at 40°C overnight. Anhydrous

DMF (0.62 mL) was added, followed by 2-(1H-benzotriazole)-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, 0.08 g, 0.24 mmol) and *N*-methylmorpholine (53 μL, 0.48 mmol). The solution was vortexed until a clear solution was obtained. To this, anhydrous DMF (2.38 mL) and amino alkyl CPG (1.03 g, 115.2 mmol g^{-1}, particle size 120/200, mean pore diameter 520 Å) were added. The mixture was allowed to shake for 18 hours at room temperature. The functionalized solid support was filtered and washed thoroughly with DMF, CH_3CN, and Et_2O and dried under reduced pressure overnight. The functionalized sold support (**7**) was suspended in capping solution (10% acetic anhydride, 10% pyridine, 10% *N*-methylimidazole in THF) and was allowed to shake for 2 hours at room temperature. It was filtered and washed with CH_3CN and Et_2O. After drying under reduced pressure, the loading capacity was determined using standard trityl assay (32.8 μmol g^{-1}).

Synthesis of Oligonucleotides Containing 2′-*O*-[2-(Guanidinium)ethyl] modification. Oligonucleotides with 2′-*O*-GE modifications were synthesized (Table 1) using phosphoramidite building block **5** and functionalized CPG **7**. A 0.1 M solution of phosphoramidite **5** in anhydrous CH_3CN was used for the synthesis of the modified oligonucleotides. For incorporation of **5**, phosphoramidite solutions (12 equivalents per coupling) were delivered in two portions, each followed by a 5-minute coupling wait time. The standard phosphoramidites and commercial solid supports were used for the incorporation of A, C, G, and T residues. Oxidation of the internucleosidic phosphite to the phosphate was carried out using CSO [1-*S*-(+)-(10-camphorsulfonyl)oxaziridine] with 4-minute oxidation wait time or *tert*-butylhydroperoxide/acetonitrile/water (10:87:3) with 10 min oxidation wait time. All other steps in the protocol supplied by Millipore (Billerica, MA, USA) were used without modification. The DMT group at the 5′-end of the modified oligonucleotides was retained after the final step of the synthesis. After completion of the synthesis, solid support bearing the oligonucleotides was suspended in 50% piperidine in water (2 mL for a 2 μmol scale synthesis) and kept at room temperature for 24 hour to deprotect the CEOC group from the guanidinium functionality at 2′-position as well as cyanoethyl group from the phosphate. Under these conditions oligonucleotides also were removed from the solid support. The solid support was filtered and washed with water (2 × 0.5 mL). The combined washes and the filtrate were evaporated to dryness. To the residue, aqueous ammonia solution (2 mL for a 2 μmol scale synthesis, 28–30 wt%) was added and the solution was heated at 55°C for 6 hours to complete the deprotection of exocyclic amino protecting groups of the bases. After evaporation to dryness, the residue was purified by High Performance Liquid Chromatography (HPLC, Waters (Milford, MA, USA) C-4, 7.8 × 300 mm column, A = 50 mM triethylammonium acetate, pH = 6.5–7, B = CH_3CN, 5 to 60% B in 55 minutes, flow rate 2.5 mL min^{-1}, detection

λ = 260 nm). Detritylation with aqueous 80% acetic acid followed by desalting gave the 2′-modified oligonucleotides (Table 1). A second HPLC purification, after detritylation, was carried out using the same conditions described above to increase the purity of the modified oligonucleotide to >90%. Oligonucleotides were characterized by ES-MS analysis. HPLC and CGE analysis assessed the purity.

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