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Synthesis of ribonucleic guanidine: replacement of the negative phosphodiester linkages of RNA with positive guanidinium linkages

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Abstract—Replacement of the negatively charged phosphodiester linkages of RNA with positively charged guanidinium linkages provides the polycationic ribonucleic guanidine (RNG). RNG is designed to bind strongly to target DNA/RNA through the specific interactions of nucleobases and the attractive electrostatic interactions of backbones, thus RNG is a putative antisense/antigene agent. Preparation of uridine and adenine building blocks for the RNG synthesis, as well as the stepwise synthesis of oligomeric RNG are reported. Pentameric homo uridyl, adenyl, and a pentameric mixed base sequence have been synthesized. The methods were also applied to a convenient solid-phase synthesis of RNG. © 2002 Elsevier Science Ltd. All rights reserved.

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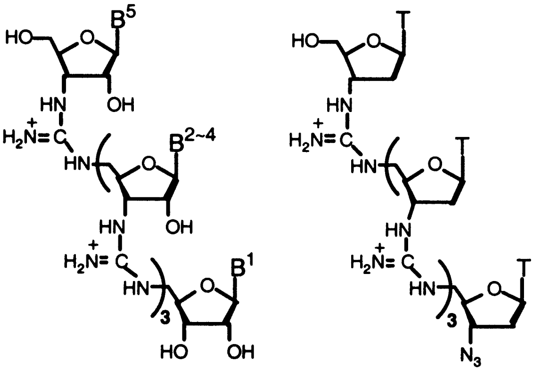
1. Introduction

Putative drugs consisting of oligonucleotide analogs capable of inhibiting cellular processes at the transcriptional or translational level via recognition and binding to complementary DNA or RNA are known as antigene and antisense agents, respectively.[1] Important goals in designing antisense/antigene compounds include increasing the binding affinity while maintaining the sequence specificity, and having resistance to degradation by nucleases. Numerous structural analogs of DNA/RNA designed to be effective antisense/antigene agents have been reported.[2] Replacement of the negatively charged phosphodiester linkages with neutral linkages eliminates the electrostatic repulsion that exists in natural duplex DNA, thus increased binding affinity can be expected. Oligonucleotides linked by methylphosphonate,[3] methylenemethylimino (MMI),[4] and amides[5] are representative of this strategy. An alternative approach involves replacing the sugar–phosphate backbone entirely, such as in the case of PNA,[6] PHONA,[7] or PNAA.[8] These oligonucleotide analogs with modified backbones are resistant to nuclease degradation. On the other hand, incorporation of positive charges into oligonucleotides has been correctly anticipated to increase binding of analogs to negative DNA/RNA strands. Aminoalkyl linkers attached through the bases,[9] the sugar moieties,[10] and the phosphate backbones[11] have been synthesized and used for biological and biochemical studies.

We have recently reported the synthesis and binding properties of deoxyribonucleic guanidine (DNG), wherein the negatively charged phosphodiester linkages of DNA have been entirely replaced by positively charged guanidinium linkages (Fig. 1).[12] DNG is anticipated to, and does, bind strongly to target DNA/RNA because the repulsive electrostatic effects in duplex DNA are replaced by close attractive electrostatic interactions. A pentameric thymidyl oligomer of DNG (**II**), with four positively charged guanidinium linkages, has been synthesized and shown to bind to poly(dA) and poly(dA) with unprecedented high affinity in



RNG. Ia: $B^{1\sim5} = U$
b: $B^{1\sim5} = A$
c: $B^{1,3,5} = A$, $B^{2,4} = U$

DNG. II

Figure 1.

Keywords: antisense; oligonucleotides; RNA; catioinic.

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a 2:1 (T/A) complex.[12c] Since interactions with poly(dG), poly(dC), and poly(dT) were not observed, **II** exhibits a fidelity of base-pair recognition. In addition, our recent studies indicate that the highly positively charged octameric thymidyl DNG oligomer was able to discriminate between complementary and noncomplementary base pairs. A single mismatch at the terminal of complementary DNA lowered the melting points by approximately 4°C, while a mismatch at the center lowered the T_m by 15°C.[13] Although the double helical structure of the duplex formed between DNG and natural DNA/RNA is unknown, computational molecular modeling suggests that the DNG/DNA duplex primarily retains a B-DNA conformation while the DNG/RNA duplex adopted an A-type structure.[12b] In addition to these results, the guanidinium linkage was shown to have nuclease resistance,[14] and the positive charges of the backbone may give rise to cell membrane permeability through electrostatic attraction of the guanidinium moiety to the negatively charged phosphate groups of the cell surface. Because of these potential antisense/antigene aspects of DNG, further studies on the synthesis and properties of guanidinium linked oligonucleotides are warranted.

The interesting differences and similarities between the nature of DNA and RNA prompted us to explore the synthesis and properties of ribonucleic guanidine (RNG **I**). Even though RNA possesses a strong affinity for DNA, its susceptibility to the various nucleases and the labile nature of the phosphodiester backbone limit its applications.[15] The RNG structure should be better suited as an antisense/antigene agent because the guanidinium linkages are stable to both nuclease degradation and intramolecular nucleophilic attack by the ribose C2′–OH. This paper presents the synthesis of uridine (**8**,**10**) and adenine (**20**,**24**) building blocks for the synthesis of RNG. By using these monomers, the stepwise solution-phase synthesis of uridyl, adenyl, and mixed sequence RNG oligomers (**Ia**–**Ic**) are reported. Also, these methods have been adapted to more convenient solid-phase RNG synthesis.[16]

2. Results and discussion

We have recently established an effective method for the synthesis of internucleoside guanidinium linkages, which can be used with both solution phase and solid phase synthesis.[13,17] This method includes the abstraction of the sulfur atom from an fluorenylmethyloxycarbonyl(Fmoc)-protected thiourea by Hg^{2+} to provide an activated carbodiimide (Fig. 2) which can react with a free amino group to give a protected guanido group.[18]

The Fmoc-protected uridine building block (**8**), required for synthesis of the body of the polynucleotide, was obtained from 3′-deoxy-3′-(hydroxyamino) uridine derivative **3** (Scheme 1). Compound **3** was prepared from the di-*tert*-butyldimethylsilyl (TBS) protected uridine **1** according to a literature procedure.[19] This involves a stereoselective reduction of the 3′-oxime moiety. Initially, the 3′-hydroxy group of compound **1** was oxidized to give 3′-keto uridine,

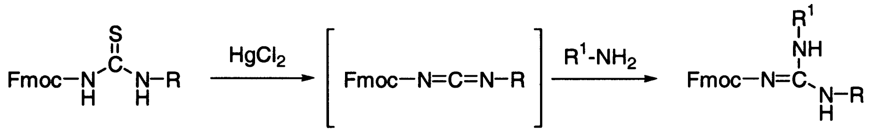
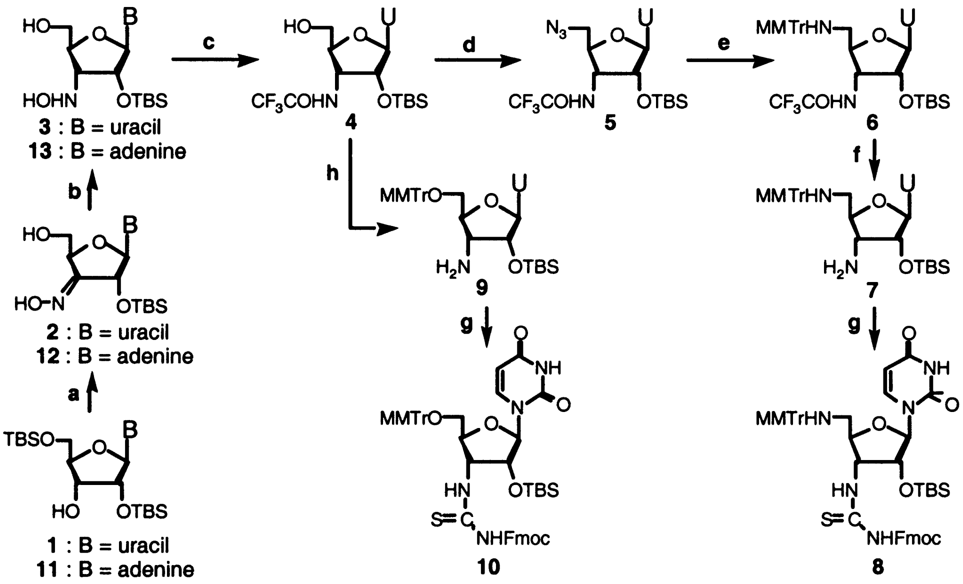


Figure 2.



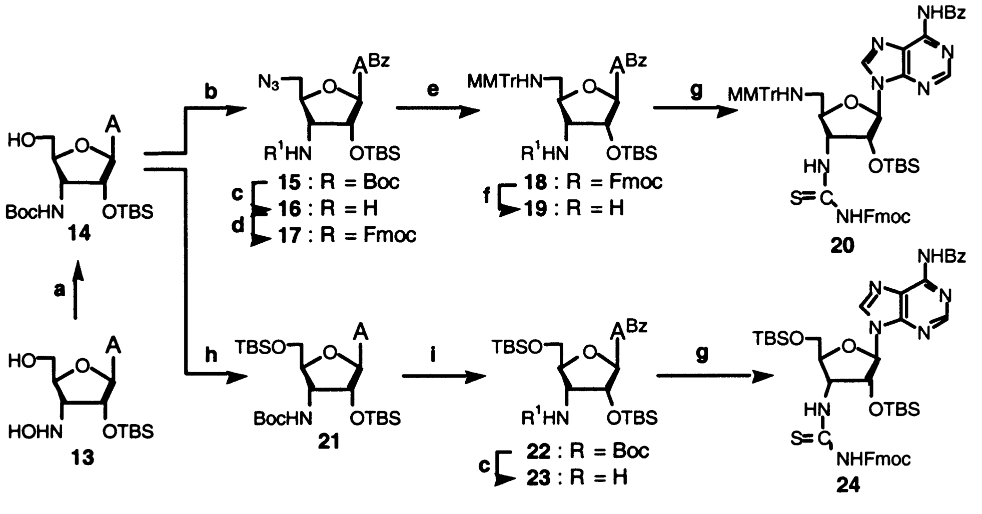
Scheme 1. *Reagents and conditions*: (a) (1) CrO_3, pyridine, Ac_2O, MS 4 Å, CH_2Cl_2, 0°C, (2) $NH_2OH\cdot HCl$, pyridine, rt, (3) 90% aqueous TFA, 0°C; (b) $NaBH_4$, AcOH, 5°C; (c) (1) H_2, 10% Pd/C, 90% aqueous AcOH, rt, (2) CF_3CO_2Et, Et_3N, MeOH, rt; (d) (1) TsCl, pyridine, rt, (2) NaN_3, NH_4Cl, DMF, 80°C; (e) (1) H_2S (gas), 60% aqueous pyridine, rt, (2) MMTrCl, pyridine, rt; (f) NH_3/MeOH, rt; (g) Fmoc-NCS, CH_2Cl_2, rt; (h) (1) MMTrCl, pyridine, rt, (2) NH_3/MeOH, rt.

which was subsequently converted into the 3′-oxime derivative by treatment with hydroxylamine hydrochloride. The 5′-*O*-TBS group was removed to give compound **2** and stereoselective reduction of the 3′-hydroxylimino group to a 3′-α-hydroxylamine, by using $NaBH(OAc)_3$, gave **3**.[20] Further reduction of compound **3** by catalytic hydrogenation, in aqueous acetic acid, gave the 3′-amino derivative, which was then protected with a trifluoroacetyl group to provide **4**. The 5′-hydroxy group was converted into an azide via the 5′-*O*-tosylate. Hydrogen sulfide reduction of the 5′-azido functional group of compound **5** was followed by protection of the subsequent amine with a 4-monomethoxytrityl (MMTr) group, providing **6**. MMTr protection of this amino moiety is ideal because it can be selectively removed, on RNG synthesis, by brief acid treatment. Removal of the trifluoroacetyl protection on the 3′-amine by methanolic ammonia provides a free 3′-amino nucleoside **7**, which was allowed to react with fluorenylmethyloxycarbonyl isothiocyanate (Fmoc-NCS)[21] in dichloromethane to give the desired building block (**8**). To place a hydroxy group at the 5′-end of the growing RNG oligomer, a building block with a 5′-protected-hydroxy group was required. This compound **10** was synthesized from the intermediate **4** by protecting the 5′-hydroxy moiety with a 4-monomethoxytrityl group followed by conversion of the 3′-amino group to a Fmoc-protected 3′-thioureido group.

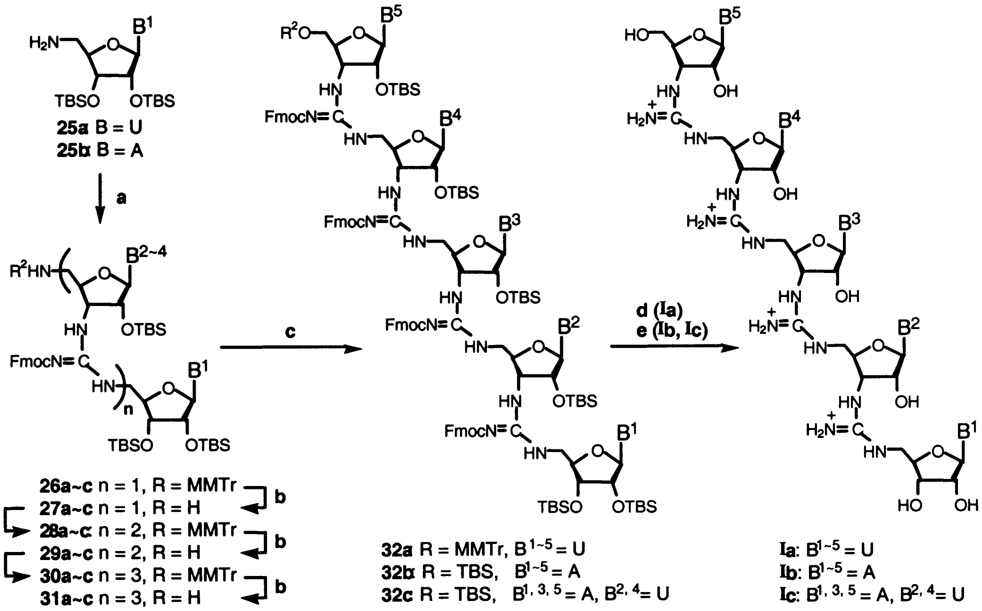
Adenine nucleosides were also modified in a similar fashion (Schemes 1 and 2). The benzoyl group was selected to block the N^6-amino group of adenine because of its stability to acidic conditions and ease of removal by ammonia treatment. The 3′-hydroxy group of 2′,5′-*O*-silylated adenosine (**11**)[22] was oxidized to its 3′-keto derivative and directly converted into the 3′-oxime nucleoside. The TBS group on the 5′-hydroxy position was then selectively removed by treating with 90% aqueous trifluoroacetic acid at 0°C providing **12**. Stereoselective reduction of **12** with $NaBH(OAc)_3$ gave **13** in 76% yield. The resulting 3′-hydroxylamino group was reduced to a 3′-amino group by catalytic hydrogenation and then protected with a *tert*-butyloxycarbonyl (Boc) group providing **14**. Next the 5′-hydroxy group of **14** was converted into an azido group through a tosyl intermediate to give **15**. The Boc group on the 3′-amine of compound **15** was successfully removed by either Lewis acid ($AlCl_3$ in CH_2Cl_2, 0°C, 73% yield) or by a convenient acid treatment (7% TFA in CH_2Cl_2, room temperature, 85% yield). At this stage, introduction of a MMTr group to the di-amino compound, which was obtained by reduction of compound **16**, selectively derivatizes the 5′-amino function. However, the desired mono-tritylated compound **19** was only obtained in low yield (35%, data not shown). In this regard, the 3′-free-amino group of compound **16** was protected with an Fmoc group to give **17**. The reduction of the 5′-azido group with hydrogen sulfide, followed by protection with MMTr gave **18** in 81% yield. The Fmoc group was selectively removed by piperidine–DMF treatment at room temperature to provide **19**, which was then reacted with Fmoc-NCS to give the adenine internal building block (**20**). An adenine building block (**24**) for the 5′-terminus of the RNG oligomer was also synthesized from **14** as shown in Scheme 2. The 5′-end-hydroxy group of **14** was protected with a TBS, instead of a MMTr group, to provide **21**. Next the N^6-amino group of the adenine moiety was benzoylated giving **22**. The removal of the Boc group from the pentose 3′-amine was successfully achieved by treating **22** with $AlCl_3$ in anhydrous dichloromethane at 0°C. The resulting compound **23** was finally reacted with Fmoc-NCS to give the desired 5′-terminal-adenine building block (**24**).

2.1. Solution-phase synthesis of RNG

We have developed a solution-phase method for the synthesis of RNG. Pentameric uridyl-RNG was synthesized by this method and a general schematic solution-phase synthesis is outlined in Scheme 3. The synthesis of uridyl RNG was accomplished via a cyclic process starting with a condensation reaction between the 3′-terminus of the uridine building block (**25a**)[23] and internal monomer (**8**), affording the protected guanido linked dimer **26a**. Typically, a mixed solution of compound **25a** and **8** was treated



Scheme 2. *Reagents and conditions*: (a) (1) H_2, 10% Pd/C, 90% aqueous AcOH, rt, (2) $(Boc)_2O$, Na_2CO_3, THF/H_2O, rt; (b) (1) i) TMSCl, pyridine, rt, ii) BzCl, rt, iii) aqueous NH_4OH, rt, (2) TsCl, pyridine, rt, (3) NaN_3, NH_4Cl, DMF, 80°C; (c) method A (for **16** and **23**): $AlCl_3$, CH_2Cl_2, 0°C, method B (for **16**): TFA, CH_2Cl_2, rt; (d) FmocCl, Na_2CO_3, THF/H_2O, rt; (e) (1) H_2S (gas), 60% aqueous pyridine, rt, (2) MMTrCl, pyridine, rt; (f) piperidine, DMF, rt; (g) Fmoc-NCS, CH_2Cl_2, rt; (h) TBSCl, imidazole, DMF, rt; (i) (1) BzCl, pyridine, rt, (2) aqueous NH_4OH, rt.



Scheme 3. Solution-phase synthesis. *Reagents and conditions*: (a) coupling: **8** or **20**, $HgCl_2$, DIEA, DMF, rt; (b) deblocking: 80% aqueous AcOH, rt; (c) coupling of 5′-terminal monomer: **10** or **24**, $HgCl_2$, DIEA, DMF, rt; (d) deprotection (1) 80% aqueous AcOH, rt, (2) 1.0 M TBAF in THF, rt; (e) deprotection (1) NH_3/MeOH, rt, (2) 1.0 M TBAF in THF, rt.

with $HgCl_2$ in DMF in the presence of *N*,*N*-diisopropylethylamine as base. The carbodiimide intermediate, which was generated in situ by the mercury(II)-mediated abstraction of the sulfur atom from the protected thiourea (**8**), was allowed to react with free 5′-amino group of **25a** to give a dimer with an Fmoc-protected guanido group (**26a**). The resulting guanido moiety was kept protected until the end of RNG synthesis. Chain extension from the dimer (**26a**) followed a cyclic two-step process involving removal of the MMTr protecting group from the 5′-amino terminus with 80% aqueous acetic acid. The 5′-free-amino oligomer (**27a**) was then reacted with another internal monomer (**8**) to give a longer RNG oligomer (**28a**). Excess amount (2–3 equiv.) of incoming monomer **8** was used in each coupling reaction. The isolated reaction yields for these chain extensions were between 77 and 90%. The monomer with 5′-protected-hydroxy group (**10**) was used for the 5′-terminus of the growing chain to give a fully protected uridyl RNG oligomer (**32a**). At the end of each coupling and deprotection cycle, the product was purified by silica gel column chromatography, and the structure was confirmed by mass spectrometry (listed in Section 4).

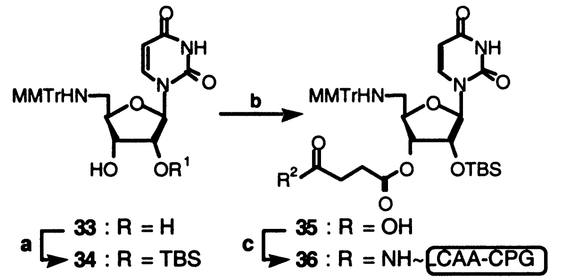
The first step in deprotection of the fully protected oligomer (**32a**) is removal of the MMTr group on the 5′-termini hydroxy by 80% aqueous acetic acid treatment. Then the Fmoc groups on the guanido group and the silyl groups on the hydroxy moieties were simultaneously removed by treating with 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran at room temperature. The resulting crude oligomer was then analyzed and purified by RP-HPLC using the solvent system of 3% acetic acid in CH_3CN to give pentameric uridyl RNG oligomer (**Ia**).

Pentameric adenyl RNG oligomer (**Ib**) was also synthesized by the identical method as mentioned earlier, using a 3′-terminal (**25b**),[23] internal (**20**), and 5′-terminal (**24**) monomers. The coupling yields for each step were similar to those for the coupling of uridine building blocks. Deprotection of the adenyl oligomer was accomplished as follows. The fully protected oligomer (**32b**) was first treated with methanolic ammonia at room temperature to remove the benzoyl protecting group from the adenine N^6-amino group as well as the Fmoc group from the guanido group. The TBS groups were removed from hydroxyls by treating with a 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran. The resulting free oligomer was purified by RP-HPLC to give pentameric adenyl RNG (**Ib**).

The synthesis of an adenine and uracil mixed sequenced RNG (**Ic**) began with the coupling of adenine 3′-terminal building block (**25b**) with the uridine internal building block (**8**). This was followed by coupling with the adenine internal building block (**20**), then the uridine internal building block (**8**), and finally the 5′-terminal adenine building block (**24**). On completion of the synthesis of the protected pentameric RNG oligomer (**32c**), the guanido protecting Fmoc groups as well as the benzoyl groups on the N^6-amino nucleobases were removed by treating with methanolic ammonia. This was followed by treatment with tetrabutylammonium fluoride to remove TBS groups from both the terminal 3′ and 5′-terminal hydroxy groups to provide the fully deprotected mixed sequence oligomer (**Ic**). All synthesized RNG oligomers (**Ia**–**Ic**) were analyzed by mass spectrometry. The observed molecular weights supported their structures (listed in Section 4).

2.2. Solid-phase synthesis of RNG

To synthesize RNG on solid support, it is required that the 3′-terminal building block be attached to a support with a linkage that is cleavable after completion of the synthesis. The most useful method of cleavage from the solid support is the one that simultaneously removes both Fmoc and

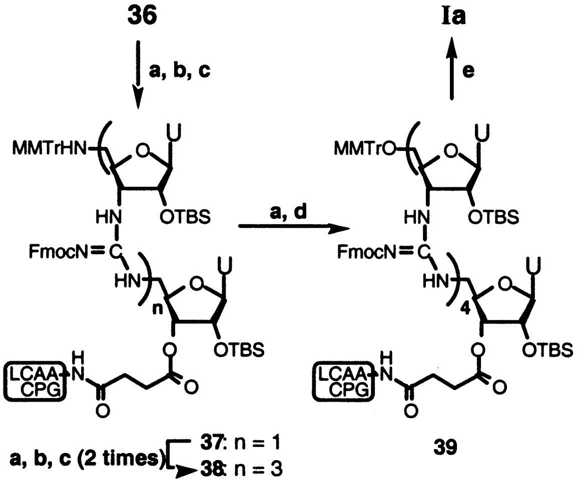


Scheme 4. *Reagents and conditions*: (a) TBSCl, $AgNO_3$, pyridine, THF, rt; (b) succinic anhydride, DMAP, pyridine, rt; (c) LCAA-CPG, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, DMF, rt.

benzoyl protecting groups. The solid-phase condensation cycle was identical to the solution-phase method described earlier, except for the addition of a thiophenol treatment to remove mercurous sulfide precipitate that resulted from the reaction between $HgCl_2$ and the sulfur atom of the thiourea.

Long chain alkylamine controlled pore glass (LCAA-CPG) was chosen for the solid support. The attachment of 3′-terminal nucleoside onto LCAA-CPG followed the standard procedure for DNA/RNA synthesis (Scheme 4).[9] Selective silylation of 5′-monomethoxytritylamino derivative **33** was accomplished using TBSCl, $AgNO_3$, and pyridine in tetrahydrofuran to give 2′-*O*-protected derivative **34** in 70% yield.[22] After the compound **34** was converted into 3′-succinate **35**, it was condensed with LCAA-CPG to provide solid-support attached 3′-terminal monomer **36** (support loading was 23.5 μmol/g).

Pentameric uridyl RNG (**Ia**) was synthesized on a 2.0 μmol scale (Scheme 5). 3′-terminal-LCAA attached nucleoside (**36**) was placed in a solid-phase synthesis vessel and treated with 3% trichloroacetic acid in dichloromethane to remove the 5′-MMTr substituent. The coupling of the internal monomer (**8**) to the uridine loaded solid support was conducted by treating with 15 mol equiv. of **8** in the presence of 2 mol equiv. of $HgCl_2$ and 4 mol equiv. of *N*,*N*-diisopropylethylamine at room temperature for 4 h. Preliminary study indicated that this single coupling reaction gave a yield of 77%, as determined from the released MMTr cation. When the coupling reaction was repeated the average yield rose to 90%. At this point, a black precipitate of mercurous sulfide forms. The latter is removed by treating the CPG resin with a 20% thiophenol solution in DMF. Next, the unreacted 5′-amino group was capped by acylation with trifluoroacetic anhydride. The deblocking/coupling/capping cycles were repeated two more times before coupling the 5′-terminal monomer **10**. The MMTr groups on the 5′-end of the completed RNG oligomer (**39**) were removed prior to the cleavage of oligomer from CPG support, however, the MMTr groups can be left on throughout the purification steps if desired. Treatment of CPG-bound RNG oligomer with an NH_4OH/EtOH (3:1) mixture resulted in cleavage of oligomer from the CPG support as well as removal of Fmoc groups from guanido moieties. TBS groups were then removed using TBAF to provide the fully de-protected RNG oligomer, which was purified by RP-HPLC as mentioned earlier for the solution-phase method. Final yield of HPLC purified oligomer (**Ic**) obtained from the LCAA-CPG bound monomer (**36**) was 15%. Characterization of uridyl RNG (**Ic**) synthesized by the solid-phase method revealed that its spectrum was identical to the oligomer synthesized by solution-phase method, and mass spectrometry confirmed the correct mass.



Scheme 5. Solid-phase synthesis. *Reagents and conditions*: (a) deblocking 3% TCA in CH_2Cl_2, rt; (b) coupling (1) **8**, $HgCl_2$, DIEA, DMF, rt, (2) 20% thiophenol in DMF, rt; (c) capping CF_3CO_2Et, DIEA, DMF, rt; (d) coupling of 5′-terminal monomer (1) **10**, $HgCl_2$, DIEA, DMF, rt, (2) 20% thiophenol in DMF, rt; (e) cleavage and deprotection (1) 3% TCA in CH_2Cl_2, rt, (2) NH_4OH/EtOH, rt, (3) 1.0 M TBAF in THF, rt.

3. Conclusion

We have developed a method for the synthesis of RNG, a polycationic analog of RNA in which the phosphodiester linkages have been replaced with positively charged guanidinium moieties. The synthesis of modified uridine and adenine building blocks along with the liquid-phase and solid-phase synthesis of oligoribonucleotides is reported. Uridine and adenine building blocks for RNG synthesis were prepared from 2′ and 5′-*O*-protected uridine and adenosine, respectively. Homo-oligomeric RNG sequences, consisting of pentameric adenyl and uridyl tracks, as well as a mixed-base pentamer (5′-AUAUA-3′) were synthesized using a solution-phase method. This method was also applied to standard solid-phase techniques, resulting in pentameric uridyl RNG. Future plans include synthesis of the remaining guanosine and cytidine RNG analogs and greater length mixed sequences to determine their antisense potential.

Despite the exposure of Fmoc-protecting groups to 5′-amines during the course of monomer synthesis and oligomer coupling, they remained remarkably stable. This stability was confirmed by the thorough characterization of monomer **18** and a mixed RNG uridyl–adenyl Fmoc-protected dimer **40** (Fig. 3; synthesis not included) resulting from a single solution-phase coupling. High-resolution mass spectroscopy, ^1H and ^{13}C NMR data for **18** and **40** are included in Section 4. High-resolution mass spectroscopy data is also included for the six protected and de-protected RNG dimers, **26a–c** and **27a–c**, indicating that Fmoc groups remain even after the 5′-MMTr protecting group is removed, exposing the free amines. Thus we believe Fmoc

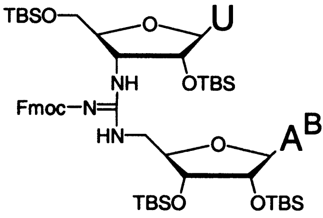


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Figure 3.

to be effective in protecting the guanido group and we consider this to be a more elegant and efficient synthesis compared to past methods. Previous RNG oligomers had to be isolated after each coupling reaction, the result of which was a thiourea-linkage. The guanidinium-linked oligomer was then derived from the thiourea-linked oligomer via oxidation with peracetic acid, followed by amination with ammonia. In combination with the outlined solid-phase procedure, the new Fmoc-protected synthesis requires no isolation after each coupling and exposure of the completed oligomer to ammonia simultaneously cleaves the oligomer from the solid support, de-protects the guanido groups, and will remove N^6-benzoyl protecting groups from any adenyl moieties present. Final exposure to TBAF yields the completely de-protected, guanidinium-linked oligomer.

4. Experimental

4.1. General methods

Physical data were measured as follows: ^1H and ^{13}C NMR spectra were recorded on 200, 400, and 500 MHz instruments using $CDCl_3$ or DMSO-d_6 as the solvent with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by their disappearance on addition of D_2O. TLC was done on Silica Gel 60 F_{254} pre-coated plates (EM Science, Germany). Silica gel used for column chromatography was Scientific Silica Gel (particle size 63–200) (Selecto Scientific, Georgia, USA). RP-HPLC was performed on a Hewlett Packard 1050 system equipped with a quaternary solvent delivery system, a UV detector set at 260 nm, and an Alltech macrosphere RP C8 column.

4.1.1. 1-[2-*O*-(*tert*-Butyldimethylsilyl)-3-deoxy-3-(hydroxyimino)-β-D-*erythro*-pentofuranosyl]uracil (2) from 2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)uridine (1). Pyridine (4.50 mL, 55.2 mmol) was added dropwise to a mixture of CrO_3 (2.76 g, 27.6 mmol) and 4 Å molecular sieves (powder, 7.0 g) in CH_2Cl_2 (60 mL) at 0°C. After stirring for 30 min, Ac_2O (4.50 mL, 55.2 mmol) was added and the mixture was allowed to stir at 0°C for another 15 min. A solution of **1** (4.34 g, 9.18 mmol) in CH_2Cl_2 (20 mL) was added at room temperature and 2 h later the reaction mixture was poured into AcOEt (500 mL). After filtering through Celite the filtrate was concentrated in vacuo then dissolved in pyridine (90 mL) containing hydroxylamine hydrochloride (3.20 g, 46.0 mmol) and allowed to stir at room temperature for 20 h. The solvent was removed in vacuo and residue was dissolved in AcOEt (350 mL), washed with H_2O (150 mL×2) and brine (150 mL), then dried (Na_2SO_4) and concentrated in vacuo. A solution of the residue in aqueous TFA (90%, 30 mL) was stirred at 0°C for 20 min. The solvent was removed in vacuo, co-evaporated with toluene (15 mL×3), and the residue was purified by silica gel column chromatography (4.5×12 cm, 70% AcOEt in hexane) to give **2** (1.96 g, 58%) as a white foam: LRMS (FAB) *m/z* 372 $[(M+H)^+$, 60%]; HRMS (FAB) calcd for $C_{15}H_{26}N_3O_6Si$ $(M+H)^+$ 372.1585, found 372.1577; ^1H NMR (200 MHz, $CDCl_3$) 7.54 (d, 1H, J=8.1 Hz), 5.82 (d, 1H, J=8. 1 Hz), 5.60 (d, 1H, J=6.5 Hz), 5.05 (d, 1H, J=6.5 Hz), 5.03 (m, 1H), 4.16 (dd, 1H, J=1.7, 12.5 Hz), 3.88 (dd, 1H, J=2.0, 12.5 Hz), 0.85 (s, 9H), 0.08 (s, 3H), 0.03 (s, 3H).

4.1.2. 2′-*O*-(*tert*-Butyldimethylsilyl)-3′-(hydroxyamino)-3′-deoxyuridine (3). **2** (1.90 g, 5.11 mmol) was added to a 5°C solution of AcOH (60 mL) containing $NaBH_4$ (390 mg, 21.3 mmol). After the mixture was stirred at the same temperature for 2 h, the solvent was removed in vacuo. The residue was dissolved in AcOEt (180 mL), washed with H_2O (60 mL×2) and brine (60 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (3.5×10 cm, 15% EtOH in $CHCl_3$) to give **3** (1.83 g, 96%) as a white foam: LRMS (FAB) *m/z* 374 $[(M+H)^+$, 100%]; HRMS (FAB) calcd for $C_{15}H_{28}N_3O_6Si$ $(M+H)^+$ 374.1747, found 374.1759; ^1H NMR (200 MHz DMSO-d_6) 11.35 (br s, 1H), 8.01 (d, 1H, J=8.2 Hz), 7.59 (s, 1H), 5.81 (d, 1H, J=4.6 Hz), 5.66 (d, 1H, J=8.2 Hz), 5.42 (br s, 1H), 5.25 (br s, 1H), 4.31 (dd, 1H, J=4.6, 5.7 Hz), 3.98 (m, 1H), 3.70 (dd, 1H, J=2.2, 12.2 Hz), 3.54 (dd, 1H, J=2.5, 12.2 Hz), 3.37 (dd, 1H, J=4.6, 5.7 Hz), 0.83 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H).

4.1.3. 2′-*O*-(*tert*-Butyldimethylsilyl)-3′-(trifluoroacetamido)-3′-deoxyuridine (4). A mixture of **3** (1.95 g, 5.22 mmol) and Pd/C (10%, 390 mg) in aqueous AcOH (90%, 50 mL) was stirred under atmospheric pressure of H_2 at room temperature for 3 h. The catalyst was filtered through Celite and the filtrate was concentrated in vacuo. A mixture of the residue, ethyl trifluoroacetate (3.11 mL, 26.1 mmol), and triethylamine (3.64 mL, 26.1 mmol) in MeOH (50 mL) was stirred at room temperature for 12 h and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (4.5×10 cm, 6% EtOH in $CHCl_3$) to give **4** (2.22 g, 94%) as a white foam: LRMS (FAB) *m/z* 454 $[(M+H)^+$, 10%]; HRMS (FAB) calcd for $C_{17}H_{27}N_3F_3O_6Si$ $(M+H)^+$ 454.1621, found 454.1601; ^1H NMR (400 MHz, $CDCl_3$) 9.46 (br s, 1H), 8.01 (d, 1H, J=8.1 Hz), 6.93 (br d, 1H, J=7.3 Hz), 5.77 (d, 1H, J=8.1 Hz), 5.75 (d, 1H, J=1.5 Hz), 4.48 (ddd, 1H, J=5.2, 7.3, 8.0 Hz), 4.44 (dd, 1H, J=1.5, 5.2 Hz), 4.13 (ddd, 1H, J=8.0, 1.6, 2.2 Hz), 4.08 (dd, 1H, J=1.6, 13.2 Hz), 3.81 (dd, 1H, J=2.2, 13.2 Hz), 2.99 (br s, 1H), 0.94 (s, 9H), 0.26 (s, 3H), 0.17 (s, 3H).

4.1.4. 5′-Azido-2′-*O*-(*tert*-butyldimethylsilyl)-3′-(trifluoroacetamido)-3′,5′-dideoxyuridine (5). A mixture of **4** (1.55 g, 3.42 mmol) and *p*-TsCl (1.32 g, 6.90 mmol) in pyridine (35 mL) was stirred at room temperature for

24 h. H_2O (5 mL) was added to the mixture and the solvent was removed in vacuo. The residue was dissolved in AcOEt (200 mL), which was washed with H_2O (70 mL), aqueous $NaHCO_3$ (saturated, 70 mL) and brine (70 mL), then dried (Na_2SO_4) and concentrated in vacuo. A solution of the residue, NaN_3 (670 mg, 10.3 mmol) and NH_4Cl (730 mg, 13.7 mmol) in DMF (20 mL) was heated at 80°C for 1 h. The mixture was diluted with AcOEt (200 mL) and washed with H_2O (70 mL×3) and brine (70 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (3.5×10 cm, 40% AcOEt in hexane) to give **5** (1.22 g, 75%) as a white solid: LRMS (FAB) *m/z* 479 [$(M+H)^+$, 40%]; HRMS (FAB) calcd for $C_{17}H_{26}N_6F_3O_5Si$ $(M+H)^+$ 479.1686, found 479.1690; ^1H NMR (400 MHz, $CDCl_3$) 9.41 (br s, 1H), 7.65 (d, 1H, *J*=8.2 Hz), 7.00 (br d, 1H, *J*=7.1 Hz), 5.84 (d, 1H, *J*=8.2 Hz), 5.82 (d, 1H, *J*=2.9 Hz), 4.45 (ddd, 1H, *J*=7.1, 6.2, 7.3 Hz), 4.38 (dd, 1H, *J*=2.9, 6.2 Hz), 4.15 (ddd, 1H, *J*=7.3, 2.6, 3.5 Hz), 3.88 (dd, 1H, *J*=2.6, 13.5 Hz), 3.70 (dd, 1H, *J*=3.5, 13.5 Hz), 0.92 (s, 9H), 0.18 (s, 3H), 0.12 (s, 3H).

4.1.5. 2′-*O*-(*tert*-Butyldimethylsilyl)-3′-(trifluoroacetamido)-5′-(4-monomethoxytritylamino)-3′,5′-dideoxyuridine (6). Hydrogen sulfide gas was bubbled into a solution of **5** (1.73 g, 3.62 mmol) in aqueous pyridine (60%, 80 mL) for 15 min. After the mixture was sealed and stirred at room temperature for 5 h, argon gas was bubbled through to remove the hydrogen sulfide gas. The solvent was removed in vacuo, and the residue was dissolved in AcOEt (200 mL) then washed with H_2O (60 mL×2) and brine (60 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. A solution of the residue and monomethoxytrityl chloride (2.25 g, 7.30 mmol) in pyridine (25 mL) was stirred at room temperature for 3 h, after which time EtOH (3 mL) was added to the mixture and the solvent was removed in vacuo. The residue was dissolved in AcOEt (200 mL), washed with H_2O (60 mL), followed by aqueous $NaHCO_3$ (60 mL) and brine (60 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (4.5×17 cm, 40% AcOEt in hexane) to give **6** (2.21 g, 84%) as a pale yellow foam: LRMS (FAB) *m/z* 725 [$(M+H)^+$, 5%]; ^1H NMR (400 MHz, $CDCl_3$) 9.10 (br d, 1H, *J*=1.6 Hz), 8.45 (d, 1H, *J*=8.2 Hz), 7.61–7.18 (m, 12H), 6.84–6.80 (m, 2H), 6.61 (br d, 1H, *J*=8.6 Hz), 5.82 (s, 1H), 5.71 (dd, 1H, *J*=8.2, 1.6 Hz), 4.42 (ddd, 1H, *J*=4.8, 9.9, 8.6 Hz), 4.31 (d, 1H, *J*=4.8 Hz), 4.08 (ddd, 1H, *J*=9.9, 2.7, 3.9 Hz), 3.78 (s, 3H), 2.87 (dd, 1H, *J*=2.7, 14.1 Hz), 2.64 (br s, 1H), 2.22 (dd, 1H, *J*=3.9, 14.1 Hz), 0.92 (s, 9H), 0.29 (s, 3H), 0.15 (s, 3H).

4.1.6. 3′-Amino-2′-*O*-(*tert*-butyldimethylsilyl)-5′-(4-monomethoxytritylamino)-3′,5′-dideoxyuridine (7). Compound **6** (2.05 g, 1.15 mmol) was dissolved in methanolic ammonia (saturated at 0°C, 100 mL) and kept at room temperature for 6 days. The solvent was removed in vacuo and the residue was purified by silica gel column chromatography (4.5×20 cm, 3% EtOH in $CHCl_3$) to give **7** (1.48 g, 83%) as a white foam: LRMS (FAB) *m/z* 629 [$(M+H)^+$, 3%]; HRMS (FAB) calcd for $C_{35}H_{45}N_4O_5Si$ $(M+H)^+$ 629.3159, found 629.3189; ^1H NMR (400 MHz, $CDCl_3$) 8.78 (br s, 1H), 7.49–7.19 (m, 12H), 7.36 (d, 1H, *J*=8.2 Hz), 6.84–6.81 (m, 2H), 5.66 (s, 1H), 5.56 (d, 1H, *J*=8.2 Hz), 4.03 (d, 1H, *J*=4.5 Hz), 3.97 (ddd, 1H, *J*=9.5, 3.0, 6.9 Hz), 3.78 (s, 3H), 2.98 (dd, 1H, *J*=4.5, 9.5 Hz), 2.79 (dd, 1H, *J*=3.0, 12.7 Hz), 2.33 (dd, 1H, *J*=7.0, 12.7 Hz), 1.57 (br s, 2H), 0.94 (s, 9H), 0.23 (s, 3H), 0.13 (s, 3H).

4.1.7. 2′-*O*-(*tert*-Butyldimethylsilyl)-3′-[(*N*-(9-fluorenylmethyloxycarbonyl)thiocarbamoyl)amino]-5′-(4-monomethoxytritylamino)-3′,5′-dideoxyuridine (8). A solution of **7** (905 mg, 1.44 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a solution of fluorenylmethyloxycarbonyl isothiocyanate (445 mg, 1.58 mmol) in CH_2Cl_2 (30 mL) at room temperature. After the mixture was stirred for 15 min, the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (4.5×14 cm, 40% AcOEt in hexane) to give **8**(1.22 g, 93%) as a white foam: LRMS (FAB) *m/z* 910 [$(M+H)^+$, 5%]; HRMS (FAB) calcd for $C_{51}H_{56}N_5O_7SSi$ $(M+H)^+$ 910.3670, found 910.3698 (3.1 ppm); ^1H NMR (400 MHz, DMSO-d_6) 11.67 (s, 1H), 11.45 (d, 1H, *J*=1.9 Hz), 10.12 (d, 1H, *J*=8.0 Hz), 8.02 (d, 1H, *J*=8.0 Hz), 7.89 (m, 2H), 7.80 (m, 2H), 7.45–7.14 (m, 16H), 6.83 (m, 2H), 5.69 (d, 1H, *J*=2.6 Hz), 5.60 (dd, 1H, *J*=8.0, 1.9 Hz), 5.06 (ddd, 1H, *J*=6.1, 8.6, 8.0 Hz), 4.67 (dd, 1H, *J*=2.6, 6.1 Hz), 4.42–4.34 (m, 2H), 4.27 (t, 1H, *J*=7.3 Hz), 4.08 (ddd, 1H, *J*=8.6, 4.2, 4.1 Hz), 3.70 (s, 3H), 3.05 (t, 1H, *J*=8.1 Hz), 2.53 (ddd, 1H, *J*=4.2, 12.8, 8.1 Hz), 2.36 (ddd, 1H, *J*=4.1, 12.8, 8.1 Hz), 0.82 (s, 9H), 0.07 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (500 MHz, DMSO-d_6) 179.9, 163.0, 157.4, 153.5, 150.2, 146.1, 143.2, 143.1, 141.4, 140.7, 140.7, 137.6, 129.6, 128.3, 127.9, 127.7, 127.1, 126.0, 125.4, 125.4, 120.2, 113.0, 101.8, 91.2, 81.0, 73.7, 69.8, 67.4, 56.7, 54.9, 46.0, 44.7, 25.5, 17.6, −5.1, −5.2.

4.1.8. 3′-Amino-2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(monomethoxytrityl)-3′-deoxyuridine (9). A mixture of **4** (907 mg, 2.00 mmol) and monomethoxytrityl chloride (1.54 g, 5.00 mmol) in pyridine (20 mL) was stirred at room temperature for 20 h. After EtOH (3 mL) was added to the mixture, the solvent was removed in vacuo. The residue was dissolved in AcOEt (200 mL), which was washed with H_2O (70 mL), aqueous $NaHCO_3$ (saturated, 70 mL), brine (70 mL), then dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in methanolic ammonia (saturated at 0°C, 40 mL) and kept at room temperature for 5 days. The solvent was removed in vacuo and the residue was purified by silica gel column chromatography (4.5×12 cm, 60% AcOEt in hexane) to give **9** (1.09 g, 87%) as a white foam: LRMS (FAB) *m/z* 630 [$(M+H)^+$, 5%]; ^1H NMR (400 MHz, $CDCl_3$) 8.89 (br s, 1H), 8.20 (d, 1H, *J*=8.2 Hz), 7.44–7.25 (m, 12H), 6.87–6.85 (m, 2H), 5.74 (s, 1H), 5.26 (d, 1H, *J*=8.2 Hz), 4.10 (d, 1H, *J*=4.6 Hz), 3.89 (ddd, 1H, *J*=9.5, 2.0, 2.6 Hz), 3.80 (s, 3H), 3.64 (dd, 1H, *J*=2.0, 11.4 Hz), 3.53 (dd, 1H, *J*=4.6, 9.5 Hz), 3.51 (dd, 1H, *J*=2.6, 11.4 Hz), 1.16 (br s, 2H), 0.92 (s, 9H), 0.25 (s, 3H), 0.16 (s, 3H).

4.1.9. 2′-*O*-(*tert*-Butyldimethylsilyl)-3′-[(*N*-(9-fluorenylmethyloxycarbonyl)thiocarbamoyl)amino]-5′-*O*-(4-monomethoxytrityl)-3′-deoxyuridine (10). A solution of **9** (1.05 g, 1.67 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a solution of fluorenylmethyloxycarbonyl isothiocyanate

(520 mg, 1.84 mmol) in CH_2Cl_2 (30 mL) at room temperature. After the mixture was stirred at the same temperature for 15 min, the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (4.5×13 cm, 40% AcOEt in hexane) to give **10** (1.47 g, 96%) as a pale yellow foam: LRMS (FAB) *m*/*z* 911 $[(M+H)^+$, 15%]; HRMS (FAB) calcd for $C_{51}H_{55}N_4O_8SSi$ $(M+H)^+$ 911.3510, found 911.3535 (2.7 ppm); ^1H NMR (400 MHz, DMSO-d_6) 11.73 (s, 1H), 11.48 (d, 1H, *J*=2.0 Hz), 10.09 (d, 1H, *J*=7.3 Hz), 7.90 (m, 2H), 7.85 (d, 1H, *J*=8.3 Hz), 7.81 (m, 2H), 7.46–7.20 (m, 16H), 6.89 (m, 2H), 5.76 (d, 1H, *J*=2.9 Hz), 5.35 (dd, 1H, *J*=8.3, 2.0 Hz), 5.14 (dt, 1H, *J*=6.0, 7.3 Hz), 4.58 (dd, 1H, *J*=2.9, 6.0 Hz), 4.39 (dd, 1H, *J*=8.0, 10.2 Hz), 4.35 (dd, 1H, *J*=7.4, 10.2 Hz), 4.27 (dd, 1H, *J*=7.4, 8.0 Hz), 4.18 (ddd, 1H, *J*=7.3, 3.6, 2.2 Hz), 3.72 (s, 3H), 3.44 (dd, 1H, *J*=3.6, 11.0 Hz), 3.37 (dd, 1H, *J*=2.2, 11.0 Hz), 0.80 (s, 9H), 0.06 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (500 MHz, DMSO-d_6) 180.2, 162.9, 158.3, 153.6, 150.3, 144.1, 143.7, 143.2, 140.8, 140.7, 140.2, 134.4, 130.2, 128.0, 128.0, 127.9, 127.2, 127.1, 127.0, 125.5, 120.2, 113.3, 101.8, 90.0, 86.5, 81.0, 73.8, 67.4, 62.6, 55.6, 55.0, 46.00, 44.9, 25.4, 17.6, −5.2, −5.3.

4.1.10. 9-[2,5-Bis-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-3-(hydroxyimino)-β-D-*erythro*-pentofuranosyl]adenine and 9-[2-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-3-(hydroxyimino)-β-D-*erythro*-pentofuranosyl]adenine (12) from 2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)adenosine (11). Pyridine (5.82 mL, 72.0 mmol) was added dropwise to a mixture of CrO_3 (3.60 g, 36.0 mmol) and molecular sieves 4 Å (powder, 8.0 g) in CH_2Cl_2 (80 mL) at 0°C. After 30 min Ac_2O (5.82 mL, 72.0 mmol) was added and the mixture was stirred at 0°C for another 15 min. To this mixture was added a solution of **11** (5.95 g, 12.0 mmol) in CH_2Cl_2 (40 mL) at room temperature. After the mixture was stirred at room temperature for a further 2 h, the mixture was poured into AcOEt (500 mL). The mixture was filtered through Celite pad and the filtrate was concentrated in vacuo. The residue was dissolved in pyridine (150 mL) containing hydroxylamine hydrochloride (4.17 g, 60.0 mmol), the mixture was stirred at room temperature for 14 h and the solvent was removed in vacuo. The residue was dissolved in AcOEt (350 mL), which was washed with H_2O (150 mL×2) and brine (150 mL), then dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (4.5×14 cm, 60% AcOEt in hexane) to give 9-[2,5-bis-*O*-(*tert*-butyldimethylsilyl)-3-deoxy-3-(hydroxyimino)-β-D-*erythro*-pentofuranosyl]adenine (5.16 g, 84%) as a white foam. A solution of this compound (5.15 g, 10.1 mmol) in aqueous TFA (90%, 40 mL) was stirred at 0°C for 20 min. The solvent was removed in vacuo, co-evaporated with toluene (15 mL×3), and the residue was purified by silica gel column chromatography (4.5×13 cm, 10% EtOH in $CHCl_3$) to give **12** (3.34 g, 70% from **11**) as a white foam. *Physical data for 9-[2,5-bis-O-(tert-butyldimethylsilyl)-3-deoxy-3-(hydroxyimino)-β-D-erythro-pentofuranosyl]adenine*: LRMS (FAB) *m*/*z* 509 $(M+H)^+$; HRMS (FAB) calcd for $C_{22}H_{41}N_6O_4Si_2$ $(M+H)^+$ 509.2728, found 509.2748; ^1H NMR (200 MHz, $CDCl_3$) 10.64 (br s, 1H), 8.37 (s, 0.5H), 8.36 (s, 0.5H), 8.31 (s, 0.5H), 8.12 (s, 0.5H), 6.13 (d, 0.5H, *J*=2.7 Hz), 6.11 (d, 0.5H, *J*=7.0 Hz), 6.03 (br s, 2H), 5.49 (m, 0.5H), 5.18 (m, 0.5H), 5.13 (dd, 0.5H, *J*=7.0, 1.7 Hz), 4.92 (m, 0.5H), 4.26 (dd, 0.5H, *J*=1.7, 11.0 Hz), 4.08 (dd, 0.5H, *J*=2.4, 11.7 Hz), 3.94 (m, 0.5H), 3.91 (m, 0.5H), 0.94 (s, 4.5H), 0.86 (s, 4.5H), 0.85 (s, 4.5H), 0.72 (s, 4.5H), 0.13 (s, 1.5H), 0.13 (s, 1.5H), 0.10 (s, 1.5H), 0.03 (s, 1.5H), 0.02 (s, 1.5H), 0.02 (s, 1.5H), −0.10 (s, 1.5H), −0.30 (s, 1.5H). *Physical data for 9-[2-O-(tert-butyldimethylsilyl)-3-deoxy-3-(hydroxyimino)-β-D-erythro-pentofuranosyl]adenine (12)*: LRMS (FAB) *m*/*z* 395 $(M+H)^+$; HRMS (FAB) calcd for $C_{16}H_{27}N_6O_4Si$ $(M+H)^+$ 395.1863, found 395.1864; ^1H NMR (200 MHz, DMSO-d_6) 8.53 (s, 1H), 8.25 (s, 1H), 7.93 (br s, 2H), 5.90 (d, 1H, *J*=7.4 Hz), 5.27 (dd, 1H, *J*=7.4, 1.5 Hz), 4.98 (ddd, 1H, *J*=1.5, 2.8, 2.0 Hz), 3.82 (dd, 1H, *J*=2.8, 12.2 Hz), 3.73 (dd, 1H, *J*=2.0, 12.2 Hz), 0.67 (s, 9H), −0.14 (s, 3H), −0.33 (s, 1H).

4.1.11. 2′-*O*-(*tert*-Butyldimethylsilyl)-3′-(hydroxyamino)-3′-deoxyadenosine (13). To a solution of AcOH (110 mL) containing $NaBH_4$ (960 mg, 25.3 mmol) was added **12** (3.33 g, 8.44 mmol) at 5°C. After the mixture was stirred at the same temperature for 2 h the solvent was removed in vacuo. The residue was dissolved in $CHCl_3$ (300 mL), which was washed with H_2O (100 mL×2) and brine (100 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (4.5×14 cm, 20% EtOH in $CHCl_3$) to give **13** (2.76 g, 82%) as a pale yellow foam: LRMS (FAB) *m*/*z* 397 $[(M+H)^+$, 25%]; HRMS (FAB) calcd for $C_{16}H_{29}N_6O_4Si$ $(M+H)^+$ 397.2020, found 397.2011; ^1H NMR (200 MHz, DMSO-d_6) 8.39 (s, 1H), 8.12 (s, 1H), 7.65 (s, 1H), 7.40 (br s, 2H), 5.94 (d, 1H, *J*=5.8 Hz), 5.67 (dd, 1H, *J*=3.7, 7.6 Hz), 4.87 (dd, 1H, *J*=5.8, 5.2 Hz), 4.16 (m, 1H), 3.75 (ddd, 1H, *J*=3.7, 2.5, 12.2 Hz), 3.58–3.51 (m, 2H), 0.74 (s, 9H), −0.14 (s, 3H), −0.27 (s, 3H).

4.1.12. 3′-(*tert*-Butyloxycarbonylamino)-2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxyadenosine (14). A mixture of **13** (2.75 g, 6.94 mmol) and Pd/C (10%, 550 mg) in aqueous AcOH (90%, 100 mL) was stirred under atmospheric pressure of H_2 at room temperature for 20 h. The catalyst was filtered off with Celite and the filtrate was concentrated in vacuo. To a solution of the residue and Na_2CO_3 (1.47 g, 13.9 mmol) in THF/H_2O (4:1, 100 mL) was added $(Boc)_2O$ (3.03 g, 13.9 mmol) at 0°C. After the mixture was stirred at room temperature for 2 h the solvent was removed in vacuo. The residue was dissolved in AcOEt (300 mL), which was washed with H_2O (100 mL×2) and brine (100 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (4.5×14 cm, 6% EtOH in $CHCl_3$) to give **14** (2.22 g, 67%) as a white foam: LRMS (FAB) *m*/*z* 481 $[(M+H)^+$, 35%]; HRMS (FAB) calcd for $C_{21}H_{37}N_6O_5Si$ $(M+H)^+$ 481.2595, found 481.2597; ^1H NMR (200 MHz, DMSO-d_6) 8.41 (s, 1H), 8.13 (s, 1H), 7.34 (br s, 2H), 7.14 (br d, 1H, *J*=8.2 Hz), 5.91 (d, 1H, *J*=4.2 Hz), 5.37 (t, 1H, *J*=5.5 Hz), 4.56 (dd, 1H, *J*=4.2, 5.8 Hz), 4.15 (ddd, 1H, *J*=8.2, 5.8, 1.8 Hz), 4.05 (ddd, 1H, *J*=1.8, 2.2, 3.0 Hz), 3.70 (ddd, 1H, *J*=5.5, 2.2, 12.4 Hz), 3.52 (ddd, 1H, *J*=5.5, 3.0, 12.4 Hz), 1.37 (s, 9H), 0.74 (s, 9H), −0.07 (s, 3H), −0.14 (s, 3H).

4.1.13. 5′-Azido-N^6-benzoyl-3′-(*tert*-butyloxycarbonylamino)-2′-*O*-(*tert*-butyldimethylsilyl)-3′,5′-dideoxyadenosine (15). To a solution of **14** (1.80 g, 3.75 mmol) in pyridine (50 mL) was added TMSCl (1.43 mL, 11.3 mmol) at room temperature. After the mixture was stirred for 30 min, benzoyl chloride (2.18 mL, 18.8 mmol) was added and stirring was continued at room temperature for 2 h. The mixture was then cooled in an ice bath and H_2O (10 mL) was added. After 5 min, 28% aqueous ammonia (20 mL) was added and the mixture was stirred at room temperature for 30 min. Solvent was removed in vacuo and the residue dissolved in AcOEt (300 mL), washed with H_2O (100 mL), aqueous $NaHCO_3$ (100 mL) and brine (100 mL), dried (Na_2SO_4) and concentrated in vacuo to give crude N^6-benzoyl-3′-(*tert*-butyloxycarbonylamino)-2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxyadenosine as a pale yellow foam [LRMS (FAB) *m/z* 585 [$(M+H)^+$, 20%]; HRMS (FAB) calcd for $C_{28}H_{41}N_6O_6Si$ $(M+H)^+$ 585.2857, found 585.2854]. To a solution of this compound in pyridine (40 mL) was added *p*-TsCl (2.15 g, 11.3 mmol) and the mixture was stirred at room temperature for 20 h. After H_2O (3 mL) was added the solvent was removed in vacuo. The residue was dissolved in AcOEt (300 mL), which was washed with H_2O (100 mL), aqueous $NaHCO_3$ (saturated, 100 mL) and brine (100 mL), dried (Na_2SO_4) and concentrated in vacuo. A solution of the residue, NaN_3 (1.22 g, 18.8 mmol), and NH_4Cl (1.20 g, 22.5 mmol) in DMF (35 mL) was heated at 80°C for 1 h. The mixture was diluted with AcOEt (300 mL), which was washed with H_2O (100 mL×3) and brine (100 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (4.5×12 cm, 50% AcOEt in hexane) to give **15** (1.62 g, 71%) as a white foam: LRMS (FAB) *m/z* 610 [$(M+H)^+$, 15%]; HRMS (FAB) calcd for $C_{28}H_{40}N_9O_6Si$ $(M+H)^+$ 610.2922, found 610.2921; ^1H NMR (400 MHz, DMSO-d_6) 11.24 (br s, 1H), 8.79 (s, 1H), 8.72 (s, 1H), 8.05 (dd, 2H, J=1.3, 7.3 Hz), 7.65 (tt, 1H, J=1.3, 7.3 Hz), 7.55 (dt, 2H, J=1.3, 7.3 Hz), 7.15 (d, 1H, J=8.4 Hz), 6.11 (d, 1H, J=3.8 Hz), 4.81 (dd, 1H, J=3.8, 6.1 Hz), 4.32 (ddd, 1H, J=6.1, 7.6, 8.4 Hz), 4.22 (ddd, 1H, J=7.6, 2.8, 6.3 Hz), 3.67 (dd, 1H, J=2.8, 13.7 Hz), 3.61 (dd, 1H, J=6.3, 13.7 Hz), 1.40 (s, 9H), 0.79 (s, 9H), −0.01 (s, 3H), −0.12 (s, 3H).

4.1.14. 3′-Amino-5′-azido-N^6-benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-3′,5′-dideoxyadenosine (16). *Method A* (with $AlCl_3$): to a solution of **15** (1.55 g, 2.54 mmol) in CH_2Cl_2 (35 mL) was added $AlCl_3$ (1.03 g, 7.70 mmol) at 0°C. After the mixture was stirred at the same temperature for 20 min the mixture was poured into aqueous $NaHCO_3$ (saturated, 100 mL) in an ice bath. The mixture was further partitioned by the addition of AcOEt (250 mL) then the organic layer was washed with aqueous $NaHCO_3$ (saturated, 70 mL) and brine (70 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (4.5×12 cm, 5% EtOH in $CHCl_3$) to give **16** (944 mg, 73%) as a white foam. *Method B* (with TFA): to a solution of **15** (1.76 g, 2.89 mmol) in CH_2Cl_2 (44 mL) was added TFA (3.3 mL, 43 mmol) at 0°C. After the mixture was stirred at room temperature for 40 h, aqueous $NaHCO_3$ (saturated, 70 mL) was added. The mixture was diluted with $CHCl_3$ (250 mL) and the organic layer was washed with aqueous $NaHCO_3$ (saturated, 70 mL) and brine (70 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (4.5×12 cm, 5% EtOH in $CHCl_3$) to give **16** (1.25 g, 85%) as a white foam: LRMS (FAB) *m/z* 510 [$(M+H)^+$, 15%]; HRMS (FAB) calcd for $C_{23}H_{32}N_9O_3Si$ $(M+H)^+$ 510.2397, found 510.2447; ^1H NMR (400 MHz, DMSO-d_6) 11.23 (br s, 1H), 8.78 (s, 1H), 8.69 (s, 1H), 8.04 (dd, 2H, J=1.3, 7.2 Hz), 7.65 (t, 1H, J=7.2 Hz), 7.55 (t, 2H, J=7.2 Hz), 6.10 (d, 1H, J=2.6 Hz), 4.68 (dd, 1H, J=2.6, 5.2 Hz), 3.91 (dt, 1H, J=7.8, 4.6 Hz), 3.68 (m, 2H), 3.64 (dd, 1H, J=5.2, 7.8 Hz), 1.72 (br s, 2H), 0.86 (s, 9H), 0.05 (s, 3H), 0.00 (s, 3H).

4.1.15. 5′-Azido-N^6-benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-3′-(9-fluorenylmethyloxycarbonylamino)-3′,5′-dideoxyadenosine (17). To a solution of **16** (1.24 g, 2.43 mmol) and Na_2CO_3 (515 mg, 4.86 mmol) in THF–H_2O (4:1, 50 mL) was added fluorenylmethyloxycarbonyl chloride (670 mg, 2.60 mmol) at 0°C. After the mixture was stirred at room temperature for 10 min, it was diluted with AcOEt (150 mL), which was washed with H_2O (70 mL×2) and brine (70 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (3.0×14 cm, 50% AcOEt in hexane) to give **17** (1.65 g, 93%) as a white foam: LRMS (FAB) *m/z* 732 [$(M+H)^+$, 30%]; ^1H NMR (400 MHz, DMSO-d_6) 11.24 (br s, 1H), 8.78 (s, 1H), 8.75 (s, 1H), 8.04 (d, 2H, J=7.3 Hz), 7.90 (d, 2H, J=7.4 Hz), 7.72 (d, 1H, J=7.4 Hz), 7.69 (d, 1H, J=7.4 Hz), 7.65 (t, 1H, J=7.3 Hz), 7.55 (t, 2H, J=7.3 Hz), 7.43 (t, 2H, J=7.4 Hz), 7.34 (t, 2H, J=7.4 Hz), 6.12 (d, 1H, J=4.3 Hz), 4.86 (dd, 1H, J=4.3, 6.1 Hz), 4.44 (dd, 1H, J=6.4, 10.5 Hz), 4.36 (dt, 1H, J=6.1, 7.4 Hz), 4.31 (dd, 1H, J=6.7, 10.5 Hz), 4.23 (m, 2H), 3.68 (dd, 1H, J=2.9, 13.4 Hz), 3.61 (dd, 1H, J=6.0, 13.4 Hz), 0.72 (s, 9H), −0.10 (s, 3H), −0.20 (s, 3H).

4.1.16. N^6-Benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-3′-(9-fluorenylmethyloxycarbonylamino)-5′-(4-monomethoxytritylamino)-3′,5′-dideoxyadenosine (18). Hydrogen sulfide gas was bubbled through a solution of **17** (1.60 g, 2.19 mmol) in aqueous pyridine (60%, 60 mL) for 15 min. After the mixture was sealed and stirred at room temperature for 20 h, argon gas was bubbled through to remove the hydrogen sulfide gas. The solvent was removed in vacuo and the residue was dissolved in AcOEt (200 mL), which was washed with H_2O (70 mL×2) and brine (70 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. A solution of the residue and monomethoxytrityl chloride (1.40 g, 4.50 mmol) in pyridine (30 mL) was stirred at room temperature for 16 h. After EtOH (5 mL) was added to the mixture, the solvent was removed in vacuo. The residue was dissolved in AcOEt (200 mL), which was washed with H_2O (70 mL), aqueous $NaHCO_3$ (70 mL) and brine (70 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (4.0×15 cm, 50% AcOEt in hexane) to give **18** (1.73 g, 81%) as a pale yellow foam: LRMS (FAB) *m/z* 978 [$(M+H)^+$, 5%]; HRMS (ESI) calcd for $C_{58}H_{59}N_7O_6Si$ $(M+H)^+$ 978.4374, found 978.4333 (4.2 ppm); ^1H NMR (400 MHz, DMSO-d_6) 11.21 (br s, 1H), 8.68 (s, 1H), 8.03 (d, 2H, J=7.3 Hz), 8.00 (s, 1H), 7.90 (d, 2H, J=7.4 Hz), 7.74 (d, 1H, J=7.4 Hz), 7.71 (d, 1H, J=7.4 Hz), 7.65 (t, 1H, J=7.3 Hz), 7.55 (t, 2H, J=7.3 Hz), 7.46–7.16 (m, 2H), 6.82 (m, 2H), 5.97 (d, 1H, J=5.7 Hz), 5.16 (dd, 1H, J=5.7, 6.4 Hz), 4.76 (m, 1H), 4.45 (dd, 1H, J=6.4, 10.5 Hz), 4.38 (dd, 1H, J=6.7, 10.5 Hz), 4.25 (dd, 1H, *J*=6.4, 6.7 Hz), 4.18 (m, 1H), 3.70 (s, 3H), 2.49 (m, 1H), 2.44 (m, 1H), 2.21 (m, 1H), 0.68 (s, 9H), −0.10 (s, 3H), −0.23 (s, 3H); ^{13}C NMR (500 MHz, DMSO-d_6) 165.5, 157.4, 156.1, 151.7, 150.7, 146.3, 146.8, 143.9, 143.8, 140.8, 137.6, 133.3, 132.5, 129.6, 128.8, 128.6, 128.5, 128.3, 127.8, 127.6, 127.1, 126.2, 126.1, 125.1, 125.1, 120.2, 113.1, 89.1, 82.3, 73.0, 69.6, 65.6, 63.5, 59.8, 55.1, 55.0, 53.4, 46.7, 45.2, 40.0, 39.8, 39.7, 39.5, 39.4, 39.2, 39.0, 38.9, 38.7, 30.2, 25.5, 25.4, 25.3, 20.7, 18.6, 17.6, 14.1, 13.5, −5.4, −5.7.

4.1.17. 3′-Amino-*N*^{6}-benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-5′-(4-monomethoxytritylamino)-3′,5′-dideoxyadenosine (19). To a solution of **18** (1.13 g, 1.15 mmol) in DMF (17.5 mL) was added piperidine (0.35 mL, 3.50 mmol) at room temperature. After the mixture was stirred at room temperature for 45 min it was taken up in AcOEt (200 mL), and the solution washed with H_2O (70 mL×4) and brine (70 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was precipitated from AcOEt/hexane and the precipitate was collected to give **19** (805 mg, 92%) as a white powder: LRMS (FAB) *m/z* 756 [(M+H)$^+$, 5%]; ^{1}H NMR (400 MHz, DMSO-d_6) 11.20 (br s, 1H), 8.62 (s, 1H), 8.03 (d, 2H, *J*=7.3 Hz), 7.95 (s, 1H), 7.65 (t, 1H, *J*=7.3 Hz), 7.55 (t, 1H, *J*=7.3 Hz), 7.46–7.17 (m, 12H), 6.84 (m, 2H), 6.02 (d, 1H, *J*=5.1 Hz), 5.05 (t, 1H, *J*=5.1 Hz), 3.94 (ddd, 1H, *J*=5.1, 4.4, 3.1 Hz), 3.84 (t, 1H, *J*=5.1 Hz), 3.71 (s, 3H), 3.46 (dd, 1H, *J*=10.3, 4.9 Hz), 2.46 (ddd, 1H, *J*=4.4, 12.6, 10.3 Hz), 2.28 (ddd, 1H, *J*=3.1, 12.6, 4.9 Hz), 1.80 (br s, 2H), 0.82 (s, 9H), −0.02 (s, 3H), −0.12 (s, 3H).

4.1.18. *N*^{6}-Benzoyl-2′-*O*-(*tert*-butyldimethylsilyl)-3′-[(*N*-(9-fluorenylmethyloxycarbonyl)thiocarbamoyl)amino]-5′-(4-monomethoxytritylamino)-3′,5′-dideoxyadenosine (20). A solution of **19** (1.05 g, 1.39 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a solution of fluorenylmethyloxycarbonyl isothiocyanate (430 mg, 1.53 mmol) in CH_2Cl_2 (50 mL) at room temperature. After the mixture was stirred for 10 min the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (3.0×15 cm, 60% AcOEt in hexane) to give **20** (1.44 g, quantitative) as a white foam: LRMS (FAB) *m/z* 1037 [(M+H)$^+$, 15%]; HRMS (FAB) calcd for $C_{59}H_{61}N_8O_6SSi$ (M+H)$^+$ 1037.4204, found 1037.4226 (2.1 ppm); ^{1}H NMR (400 MHz, DMSO-d_6) 11.79 (s, 1H), 11.23 (s, 1H), 10.54 (d, 1H, *J*=5.4 Hz), 8.75 (s, 1H), 8.03 (d, 2H, *J*=7.4 Hz), 7.92 (d, 2H, *J*=7.7 Hz), 7.84 (d, 1H, *J*=7.7 Hz), 7.81 (d, 1H, *J*=7.7 Hz), 7.65 (t, 1H, *J*=7.4 Hz), 7.60 (s, 1H), 7.55 (t, 2H, *J*=7.4 Hz), 7.50–7.20 (m, 16H), 6.84 (m, 2H), 5.99 (d, 1H, *J*=6.1 Hz), 5.59 (dd, 1H, *J*=6.1, 7.0 Hz), 5.48 (ddd, 1H, *J*=7.0, 3.4, 5.4 Hz), 4.46–4.40 (m, 3H), 4.31 (t, 1H, *J*=7.1 Hz), 3.85 (br d, 1H, *J*=8.3 Hz), 3.71 (s, 3H), 2.87 (dd, 1H, *J*=9.9, 8.3 Hz), 2.21 (d, 1H, *J*=9.9 Hz), 0.73 (s, 9H), −0.01 (s, 3H), −0.18 (s, 3H); ^{13}C NMR (500 MHz, DMSO-d_6) 180.3, 165.6, 153.7, 151.8, 150.6, 143.2, 143.1, 141.7, 140.8, 140.7, 133.3, 132.5, 128.6, 128.4, 127.9, 127.1, 125.6, 125.5, 125.4, 120.2, 88.8, 83.5, 74.4, 67.4, 62.0, 55.9, 46.0, 40.0, 39.9, 39.7, 39.5, 39.4, 39.2, 39.0, 25.8, 25.3, 18.1, 17.5, −5.4, −5.5, −5.5.

4.1.19. 3′-(*tert*-Butyloxycarbonylamino)-2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-3′-deoxyadenosine (21). A mixture of **14** (1.63 g, 3.40 mmol), *tert*-butyldimethylsilyl chloride (770 mg, 5.10 mmol), and imidazole (700 mg, 10.2 mmol) in DMF (20 mL) was stirred at room temperature for 1.5 h. After EtOH (3 mL) was added to the mixture, the mixture was diluted with AcOEt (200 mL), which was washed with H_2O (70 mL×4) and brine (70 mL). The organic layer was dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel column chromatography (4.0×10 cm, 60% AcOEt in hexane) to give **21** (1.73 g, 86%) as a white solid: LRMS (FAB) *m/z* 595 [(M+H)$^+$, 10%]; HRMS (FAB) calcd for $C_{27}H_{51}N_6O_5Si_2$ (M+H)$^+$ 595.3449, found 595.3432; ^{1}H NMR (400 MHz, DMSO-d_6) 8.32 (s, 1H), 8.14 (s, 1H), 7.34 (br s, 2H), 7.14 (d, 1H, *J*=8.0 Hz), 5.96 (d, 1H, *J*=3.0 Hz), 4.43 (dd, 1H, *J*=3.0, 5.3 Hz), 4.19 (ddd, 1H, *J*=8.0, 5.3, 7.7 Hz), 4.11 (ddd, 1H, *J*=7.7, 2.2, 2.9 Hz), 3.96 (dd, 1H, *J*=2.2, 11.8 Hz), 3.76 (dd, 1H, *J*=2.9, 11.8 Hz), 1.37 (s, 9H), 0.89 (s, 9H), 0.80 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H), −0.01 (s, 3H), −0.03 (s, 3H).

4.1.20. *N*^{6}-Benzoyl-3′-(*tert*-butyloxycarbonylamino)-2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-3′-deoxyadenosine (22). A mixture of **21** (1.63 g, 2.74 mmol) and benzoyl chloride (0.96 mL, 8.30 mmol) in pyridine (30 mL) was stirred at room temperature for 2 h. The mixture was then cooled in an ice bath and H_2O (2 mL) was added. After 5 min, 28% aqueous ammonia (10 mL) was added and the mixture was stirred at room temperature for another 30 min. The solvent was removed in vacuo. The residue was dissolved in AcOEt (200 mL), which was washed with H_2O (70 mL), aqueous $NaHCO_3$ (70 mL) and brine (70 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (4.0×10 cm, 30% AcOEt in hexane) to give **22** (1.82 g, 95%) as a white solid: LRMS (FAB) *m/z* 699 [(M+H)$^+$, 10%]; HRMS (FAB) calcd for $C_{34}H_{55}N_6O_6Si_2$ (M+H)$^+$ 699.3722, found 699.3711; ^{1}H NMR (400 MHz, DMSO-d_6) 11.23 (s, 1H), 8.76 (s, 1H), 8.62 (s, 1H), 8.04 (d, 2H, *J*=7.3 Hz), 7.65 (t, 1H, *J*=7.3 Hz), 7.55 (t, 2H, *J*=7.3 Hz), 7.17 (d, 1H, *J*=8.3 Hz), 6.12 (d, 1H, *J*=3.0 Hz), 4.56 (dd, 1H, *J*=3.0, 5.2 Hz), 4.24 (ddd, 1H, *J*=8.3, 5.2, 7.8 Hz), 4.15 (ddd, 1H, *J*=7.8, 2.1, 2.9 Hz), 3.97 (dd, 1H, *J*=2.1, 11.8 Hz), 3.79 (dd, 1H, *J*=2.9, 11.8 Hz), 1.38 (s, 9H), 0.88 (s, 9H), 0.81 (s, 9H), 0.07 (s, 6H), −0.01 (s, 3H), −0.03 (s, 3H).

4.1.21. 3′-Amino-*N*^{6}-benzoyl-2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-3′-deoxyadenosine (23). To a solution of **22** (1.36 g, 1.95 mmol) in CH_2Cl_2 (30 mL) was added $AlCl_3$ (790 mg, 5.90 mmol) at 0°C. After the mixture was stirred for 45 min, the mixture was poured into aqueous $NaHCO_3$ (saturated, 100 mL) in an ice bath. The mixture was diluted with $CHCl_3$ (200 mL) and the organic layer was washed with aqueous $NaHCO_3$ (saturated, 70 mL) and brine (70 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (3×12 cm, 75% AcOEt in hexane) to give **23** (960 mg, 82%) as a white foam: LRMS (FAB) *m/z* 599 [(M+H)$^+$, 80%]; HRMS (FAB) calcd for $C_{29}H_{47}N_6O_4Si_2$ (M+H)$^+$ 599.3197, found 599.3210; ^{1}H NMR (400 MHz, DMSO-d_6) 11.22 (br s, 1H), 8.75 (s, 1H), 8.62 (s, 1H), 8.04 (dd, 2H, *J*=1.3, 7.3 Hz), 7.65 (t, 1H, *J*=7.3 Hz), 7.55 (t, 2H, *J*=7.3 Hz), 6.09 (d, 1H, *J*=2.2 Hz), 4.49 (dd, 1H, *J*=2.2, 4.8 Hz), 3.98 (dd, 1H, *J*=2.5, 11.5 Hz), 3.83 (dd, 1H, *J*=3.5, 11.5 Hz), 3.81 (ddd, 1H, *J*=7.4, 2.5, 3.5 Hz), 3.56 (dd, 1H, *J*=4.8, 7.4 Hz), 1.63 (br s, 2H), 0.88 (s, 9H), 0.87 (s, 9H), 0.06 (s, 6H), 0.05 (s, 3H), 0.04 (s, 3H).

4.1.22. *N*^{6}-Benzoyl-2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-3′-[(*N*-(9-fluorenylmethyloxycarbonyl)thiocarbamoyl)-amino]-3′-deoxyadenosine (24). A solution of **23** (1.05 g, 1.75 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a solution of fluorenylmethyloxycarbonyl isothiocyanate (540 mg, 1.93 mmol) in CH_2Cl_2 (50 mL) at room temperature. After the mixture was stirred for 10 min, the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (3.0×14 cm, 40% AcOEt in hexane) to give **24** (1.47 g, 95%) as a pale orange foam: LRMS (FAB) *m*/*z* 880 [$(M+H)^+$, 10%]; HRMS (FAB) calcd for $C_{45}H_{58}N_7O_6SSi_2$ $(M+H)^+$ 880.3708, found 880.3744 (4.1 ppm); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) 11.73 (s, 1H), 11.27 (s, 1H), 10.30 (d, 1H, *J*=7.0 Hz), 8.77 (s, 1H), 8.64 (s, 1H), 8.05 (dd, 2H, *J*=1.3, 7.4 Hz), 7.91 (d, 2H, *J*=7.5 Hz), 7.83 (d, 1H, *J*=7.5 Hz), 7.80 (d, 1H, *J*=7.5 Hz), 7.65 (t, 1H, *J*=7.4 Hz), 7.56 (t, 2H, *J*=7.4 Hz), 7.44 (t, 2H, *J*=7.5 Hz), 7.35 (t, 2H, *J*=7.5 Hz), 6.16 (d, 1H, *J*=3.9 Hz), 5.13 (dt, 1H, *J*=6.0, 7.0 Hz), 4.95 (dd, 1H, *J*=3.9, 6.0 Hz), 4.41 (m, 2H), 4.33 (ddd, 1H, *J*=7.0, 2.2, 3.5 Hz), 4.29 (t, 1H, *J*=7.3 Hz), 4.04 (dd, 1H, *J*=2.2, 11.5 Hz), 3.94 (dd, 1H, *J*=3.5, 11.5 Hz), 0.88 (s, 9H), 0.75 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H), −0.07 (s, 3H), −0.09 (s, 3H); ^{13}C NMR (500 MHz, DMSO-*d*$_6$) 180.4, 165.5, 157.5, 153.7, 151. 4, 151.0, 150.9, 146.4, 143.2, 140.8, 140.7, 137.6, 133.3, 132.5, 129.8, 128.5, 127.9, 127.8, 127.2, 126.3, 126.1, 125.5, 120.2, 113.1, 89.3, 84.0, 71.8, 69.8, 67.4, 63.5, 59.8, 57.1, 55.0, 46.1, 45.3, 40.0, 39.8, 39.7, 39.5, 39.4, 39.2, 39.0, 30.2, 25.3, 20.7, 17.6, 14.0, 13.5, −5.6.

4.2. Solution-phase synthesis—general procedure

4.2.1. Coupling reaction. To a mixture of the uridine 3′-terminal building block **25a** (104 mg, 0.22 mmol) and the uridine internal building block **8** (182 mg, 0.20 mmol) in DMF (6.0 mL) was added *N*,*N*-diisopropylethylamine (87 μL, 0.50 mmol) and $HgCl_2$ (68 mg, 0.25 mmol), and the mixture was stirred at room temperature for 3 h. The mixture was filtered through Celite pad. The filtrate was diluted with AcOEt (100 mL), which was washed with H_2O (30 mL×4), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (2.7×12 cm, 40% AcOEt in hexane) to give 5′-*N*-MMTr-dimer **26a** (220 mg, 82%) as a yellow foam.

4.2.2. Deblocking reaction. The 5′-*N*-MMTr-dimer **26a** (200 mg, 0.15 mmol) was dissolved in 80% aqueous acetic acid (3.0 mL) and the mixture was kept at room temperature for 10 min. The solvent was removed in vacuo, and the residue was co-evaporated with EtOH and toluene. The residue was purified by silica gel column chromatography (2.7×10 cm, 25% EtOH in $CHCl_3$) to give 5′-NH_2-dimer **27a** (153 mg, 96%) as a white solid.

4.2.3. Synthesis of longer sequence. Each pentameric RNG oligomer (**Ia**–**Ic**) was synthesized in the similar two-step cycle as described for the dimer synthesis except that 2–3 equiv. of the incoming monomer (**8**,**10**,**20**, or **24**) was used for each coupling reactions.

4.2.4. Deprotection. Fully protected uridyl oligomer **32a** (3 mg, approximately 1 μmol) was dissolved in aqueous acetic acid (80%, 5.0 mL) and kept at room temperature for 24 h. The solvent was removed in vacuo and the residue was taken up in EtOH (3 mL×3) and then evaporated. The residue was dissolved in 1.0 M TBAF in THF (0.5 mL, 0.5 mmol) at room temperature and the mixture was stirred at the same temperature for 16 h. Acetic acid (0.3 mL) was added and the mixture was diluted with H_2O (10 mL), which was subsequently washed with Et_2O (5 mL×2). The aqueous phase was concentrated in vacuo. The residue was purified by RP-HPLC (Alltech Macrosphere RP C8 column, 0–10% acetonitrile in 3% aqueous acetic acid) to give pentauridyl RNG (**Ia**). For the adenyl (**Ib**) and mixed-based (**Ic**) oligomer, each fully protected oligomer **32b**,**32c** (approximately 1 μmol) was dissolved in methanolic ammonia (saturated at 0°C, 5 mL) and kept at room temperature for 60 h. After the solvent was removed in vacuo, the residue was dissolved in 1.0 M TBAF in THF (0.5 mL, 0.5 mmol) at room temperature and the mixture was stirred at the same temperature for 16 h. Acetic acid (0.3 mL) was added and the mixture was diluted with H_2O (10 mL), which was washed with Et_2O (5 mL×2). The aqueous phase was concentrated in vacuo. Each residue was purified by RP-HPLC (Alltech Macrosphere RP C8 column, 0–40% acetonitrile in 3% aqueous acetic acid) to give penta-adenyl RNG (**Ib**) and mixed-based RNG (**Ic**).

4.2.5. Characterization. At the end of each coupling and deblocking step, the compounds were confirmed by electrospray ionization mass spectrometry (ESI-MS), and accurate mass (HRMS) is provided for **26a**–**c** and **27a**–**c**. The purified RNG oligomers (**Ia**–**Ic**) were also analyzed by ESI-MS.

4.2.6. Synthesis of Ia. **26a** $C_{72}H_{94}N_8O_{12}Si_3$: calcd for $(M+H)^+$ 1347.6371, found 1347.6360 (0.8 ppm); **27a** $C_{52}H_{78}N_8O_{11}Si_3$: calcd for $(M+H)^+$ 1075.5171, found 1075.5128 (3.9 ppm); **28a** $C_{103}H_{131}N_{13}O_{18}Si_4$: calcd for $(M+H)^+$ 1950.89, found 1950.85; **29a** $C_{83}H_{115}N_{13}O_{17}Si_4$: calcd for $(M+H)^+$ 1678.77, found 1678.74, calcd for $(M+2H)^{2+}$ 839.89, found 839.88; **30a** $C_{134}H_{168}N_{18}O_{24}Si_5$: calcd for $(M+H)^+$ 2554.14, found 2554.12, calcd for $(M+2H)^{2+}$ 1277.58, found 1277.56; **31a** $C_{114}H_{152}N_{18}O_{23}Si_5$: calcd for $(M+H)^+$ 2282.02, found 2281.96, calcd for $(M+2H)^{2+}$ 1141.51, found 1141.50, calcd for $(M+3H)^{3+}$ 761.35, found 761.34; **32a** $C_{165}H_{204}N_{22}O_{31}Si_6$: calcd for $(M+H)^+$ 3158.37, found 3158.30, calcd for $(M+2H)^{2+}$ 1579.69, found 1579.68; **Ia** $C_{49}H_{64}N_{22}O_{22}$: calcd for $(M+H)^+$ 1313.46, found 1313.53, calcd for $(M+2H)^{2+}$ 657.24, found 657.27, calcd for $(M+3H)^{3+}$ 438.49, found 438.51.

4.2.7. Synthesis of Ib. **26b** $C_{88}H_{104}N_{14}O_{10}Si_3$: calcd for $(M+H)^+$ 1601.7439, found 1601.7445 (0.4 ppm); **27b** $C_{68}H_{88}N_{14}O_9Si_3$: calcd for $(M+H)^+$ 1329.6239, found 1329.6217 (1.7 ppm); **28b** $C_{127}H_{146}N_{22}O_{15}Si_4$: calcd for $(M+H)^+$ 2332.05, found 2331.97, calcd for $(M+2H)^{2+}$ 1166.53, found 1166.65; **29b** $C_{107}H_{130}N_{22}O_{14}Si_4$: calcd for $(M+H)^+$ 2059.92, found 2059.61, calcd for $(M+2H)^{2+}$ 1030.46, found 1030.49; **30b** $C_{166}H_{188}N_{30}O_{20}Si_5$: calcd for

$(M+H)^+$ 3062.35, found 3062.27, calcd for $(M+2H)^{2+}$ 1531.68, found 1531.68, calcd for $(M+3H)^{3+}$ 1021.45, found 1021.44; **31b** $C_{146}H_{172}N_{30}O_{19}Si_5$: calcd for $(M+H)^+$ 2790.23, found 2790.16, calcd for $(M+2H)^{2+}$ 1395.62, found 1395.61, calcd for $(M+3H)^{3+}$ 930.75, found 931.08; **32b** $C_{191}H_{227}N_{37}O_{25}Si_7$: calcd for $(M+H)^+$ 3635.60, found 3635.58, calcd for $(M+2H)^{2+}$ 1818.30, found 1818.31, calcd for $(M+3H)^{3+}$ 1212.54, found 1212.22, calcd for $(M+4H)^{4+}$ 909.66, found 909.43; **Ib** $C_{54}H_{69}N_{37}O_{12}$: calcd for $(M+2H)^{2+}$ 714.80, found 714.84, calcd for $(M+3H)^{3+}$ 476.87, found 476.55.

4.2.8. Synthesis of Ic. **26c** $C_{80}H_{99}N_{11}O_{11}Si_3$: calcd for $(M+H)^+$ 1474.6905, found 1474.6923 (1.2 ppm); **27c** $C_{60}H_{83}N_{11}O_{10}Si_3$: calcd for $(M+H)^+$ 1202.5705, found 1202.5687 (1.5 ppm); **28c** $C_{119}H_{141}N_{19}O_{16}Si_4$: calcd for $(M+H)^+$ 2204.99, found 2205.18; **29c** $C_{99}H_{125}N_{19}O_{15}Si_4$: calcd for $(M+H)^+$ 1932.88, found 1932.85, calcd for $(M+2H)^{2+}$ 966.95, found 967.43; **30c** $C_{150}H_{178}N_{24}O_{22}Si_5$: calcd for $(M+H)^+$ 2808.24, found 2808.24, calcd for $(M+2H)^{2+}$ 1404.62, found 1404.62; **31c** $C_{130}H_{162}N_{24}O_{21}Si_5$: calcd for $(M+H)^+$ 2536.12, found 2536.09, calcd for $(M+2H)^{2+}$ 1268.56, found 1269.54, calcd for $(M+3H)^{3+}$ 846.38, found 846.72; **32c** $C_{175}H_{217}N_{31}O_{27}Si_7$: calcd for $(M+H)^+$ calcd for $(M+3H)^{3+}$ 962.76, found 962.76, found 3381.44, calcd for $(M+2H)^{2+}$ 1691.25, found 1691.25, calcd for $(M+3H)^{3+}$ 1127.84, found 1127.51, calcd for $(M+4H)^{4+}$ 846.13, found 846.39; **Ic** $C_{52}H_{67}N_{31}O_{16}$: calcd for $(M+H)^+$ 1382.54, found 1382.57, calcd for $(M+2H)^{2+}$ 691.78, found 691.79, calcd for $(M+3H)^{3+}$ 461.52, found 461.53.

4.2.9. Characterization of r(AgU) dimer (40). HRMS calcd for $C_{66}H_{96}N_{10}O_{11}Si_4$ $(M+H)^+$ 1317.6415, found 1317.6380 (2.7 ppm); (500 MHz, DMSO-d_6); ^{13}C NMR 159.6, 152.2, 144.2, 142.6, 140.7, 139.4, 137.4, 132.4, 129.0, 128.9, 128.8, 128.5, 128.4, 128.3, 127.4, 127.3, 127.3, 127.2, 127.2, 126.9, 125.9, 125.1, 125.0, 121.4, 121.4, 121.3, 120.1, 120.0, 119.9, 109.6, 74.6, 73.4, 73.1, 65.9, 46.7, 40.0, 39.8, 39.7, 39.5, 39.3, 39.2, 39.0, 25.7, 25.6, 25.5, 25.4, 25.0, 24.8, 17.9, 17.9, 17.6, 17.6, 17.4, −4.8, −5.1, −5.6, −5.7, −5.9.

4.2.10. 2′-*O*-(*tert*-Butyldimethylsilyl)-5′-(4-monomethoxytritylamino)-5′-deoxyuridine (34). To a THF solution of 5′-(4-monomethoxytritylamino)-5′-deoxyuridine (**33**, 1.47 g, 2.85 mmol) and $AgNO_3$ (730 mg, 4.30 mmol) was added pyridine (1.04 mL, 12.9 mmol) at room temperature. After the mixture was stirred for 10 min, *tert*-butyldimethylsilyl chloride (650 mg, 4.3 mmol) was added, the mixture stirred for 20 h. When EtOH (2 mL) was added and the mixture filtered through a Celite pad, the filtrate was concentrated in vacuo. The residue was dissolved in AcOEt (180 mL), which was washed with H_2O (60 mL×2) and brine (60 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (4.5×15 cm, 30% AcOEt in hexane) to give **34** (1.26 g, 70%) as a white foam: LRMS (FAB) *m/z* 630 [$(M+H)^+$, 30%]; HRMS (FAB) calcd for $C_{35}H_{44}N_3O_6Si_1$ $(M+H)^+$ 629.2921, found 629.2932; ^1H NMR (400 MHz, $CDCl_3$) 8.61 (br s, 1H), 7.48–7.18 (m, 12H), 7.22 (d, 1H, *J*=8.2 Hz), 6.83–6.81 (m, 2H), 5.74 (d, 1H, *J*=2.8 Hz), 5.63 (d, 1H, *J*=8.2 Hz), 4.19 (dd, 1H, *J*=2.8, 5.2 Hz), 4.09 (dd, 1H, *J*=6.8, 3.1 Hz), 3.90 (ddd, 1H, *J*=8.1, 5.2, 6.8 Hz), 3.79 (s, 3H), 2.71 (dd, 1H, *J*=3.1, 12.8 Hz), 2.45 (d, 1H, *J*=8.1 Hz), 2.35 (dd, 1H, *J*=6.8, 12.8 Hz), 2.07 (br s, 1H), 0.93 (s, 9H), 0.18 (s, 3H), 0.13 (s, 3H).

4.2.11. 2′-*O*-(*tert*-Butyldimethylsilyl)-5′-(4-monomethoxytritylamino)-3′-*O*-succinyl-5′-deoxyuridine (35). A mixture of **34** (440 mg, 0.70 mmol), succinic anhydride (210 mg, 2.10 mmol), and DMAP (86 mg, 0.70 mmol) in pyridine (10 mL) was stirred at room temperature for 2 days, and the solvent was removed in vacuo. The residue was dissolved in AcOEt (60 mL), which was washed with H_2O (20 mL×2) and brine (20 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel column chromatography (2.5×9 cm, 5% EtOH in $CHCl_3$) to give **35** (320 mg, 62%) as a white foam: LRMS (FAB) *m/z* 730 [$(M+H)^+$, 10%]; HRMS (FAB) calcd for $C_{39}H_{47}N_3O_9Si$ $(M+H)^+$ 730.3160, found 730.3149; ^1H NMR (400 MHz, $CDCl_3$) 9.35 (br s, 1H), 7.84 (d, 1H, *J*=8.2 Hz), 7.45–7.18 (m, 12H), 6.84–6.80 (m, 2H), 5.72 (d, 1H, *J*=2.6 Hz), 5.64 (d, 1H, *J*=8.2 Hz), 4.99 (dd, 1H, *J*=4.6, 7.3 Hz), 4.37 (dd, 1H, *J*=2.6, 4.6 Hz), 4.32 (ddd, 1H, *J*=7.3, 3.3, 5.2 Hz), 3.78 (s, 3H), 2.74 (dd, 1H, *J*=3.3, 13.4 Hz), 2.70–2.61 (m, 5H), 2.22 (dd, 1H, *J*=5.2, 13.4 Hz), 0.89 (s, 9H), 0.12 (s, 3H), 0.06 (s, 3H).

4.2.12. 2′-*O*-(*tert*-Butyldimethylsilyl)-5′-(4-monomethoxytritylamino)-5′-deoxyuridine LCAA-CPG unit (36). To a solution of **35** (120 mg, 0.16 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC, 31 mg, 0.16 mmol) in DMF (5.0 mL) was added LCAA-CPG (Sigma, 400 mg) and the mixture was agitated at room temperature for 4 days. The resin was washed with DMF (5 mL×2) and pyridine (5 mL×3). To the resin was added capping solution (0.1 M DMAP in pyridine/acetic anhydride 9:1, 6.0 mL) and the mixture was agitated at room temperature for 24 h. The resin was washed with EtOH (5 mL×3) and acetone (5 mL×3) to give **36** (23.5 μmol/g).

4.2.13. Solid-phase synthesis of Ia. LCAA-CPG attached 3′-terminal monomer **36** (85 mg, 2.0 μmol) was placed in a solid-phase synthesis vessel and 3% trichloroacetic acid in dichloromethane (2.0 mL, 10 s, 3 times) added, the resin drained and washed with dichloromethane (2.0 mL×3) and dried under vacuum at room temperature for 30 min. To the vessel was added **8** (27.3 mg, 0.030 mmol) in DMF (0.5 mL), followed by a solution of *N*,*N*-diisopropylethylamine (120 mM, in DMF, 0.5 mL) and mercury(II) chloride (60 mM in DMF, 0.5 mL). The vessel was agitated at room temperature for 4 h. After the CPG resin was washed with DMF (2.0 mL×3), the same coupling reaction was repeated to give **37**. The CPG resin was washed with DMF (2.0 mL×3), thiophenol (20% in DMF, 10 s, 3 times), and DMF (2.0 mL×3). To the vessel was added a mixed solution of ethyl trifluoroacetate/*N*,*N*-diisopropylethylamine (1:1, 120 mM, in DMF, 2.0 mL), and the mixture was agitated at room temperature for 30 min. The CPG resin was washed with DMF (2.0 mL) and dichloromethane (2.0 mL×3). The deblocking/coupling/capping cycles were repeated until the final coupling of the 5′-terminal monomer **10** yielded **39**. The CPG resin **39** was placed in a glass tube (5 mL), to which was added 28% NH_4OH (1.5 mL) and EtOH

(0.5 mL). The mixture was kept at room temperature for 12 h then filtered through glass filter. The filtrate was concentrated in vacuo, then dissolved in 1.0 M TBAF in THF (1.0 mL, 1.0 mmol) and stirred at room temperature for 16 h. Acetic acid (0.5 mL) was added and the mixture was diluted with H_2O (15 mL), which was washed with Et_2O (8 mL×2). The aqueous phase was concentrated in vacuo.

The residue was purified by RP-HPLC (Alltech Macrosphere RP C8 column, 0–10% acetonitrile in 3% aqueous acetic acid) to give pentauridyl RNG (**Ia**). A representative RP-HPLC of the pentameric uridyl oligomer, before purification, has been included (Fig. 4) and is typical of that for crude all RNG pentamers as purified by this method. Only two significant absorbencies were noted. The de-protected uridyl pentamer has the retention time corresponding to 8.024 min, based on the solvent system and flow rate chosen (0–10% acetonitrile over 50 min).

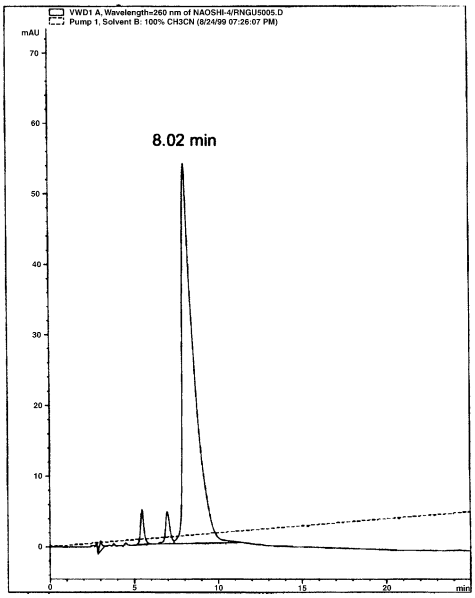

Figure 4. RP-HPLC analysis of crude RNG-U5 (0–10% acetonitrile in 3% aqueous acetic acid): the fully de-protected uridyl pentamer elutes at 8.024 min.

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