Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 4326–4330, April 1996
Biochemistry

Design and synthesis of ribonucleic guanidine: A polycationic analog of RNA

(antisense/antigene/triple helix)

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Contributed by Thomas C. Bruice, January 2, 1996

ABSTRACT **Replacement of the phosphodiester linkages of the polyanion RNA with guanidinium linkers (represented by g) provides the polycation ribonucleic guanidine (RNG). An anticipated structure for the triple-helical hybrid [r(Up)$_9$U·r(Ag)$_9$A·r(Up)$_9$U] is presented. A basic strategy for the synthesis of RNG oligomers is described. Synthetic procedures are provided for tetrameric adenosyl RNG [r(Ag)$_3$A].**

We recently reported the synthesis and DNA/RNA binding properties of the putative antisense/antigene agent deoxyribonucleic guanidine (DNG) (1–4). This positively charged DNA analog incorporates a 5′–3′ astereogenic guanidinium linkage, replacing the negatively charged phosphodiester backbone found in naturally occurring nucleic acids. In our model system, pentameric thymidyl DNG associates with complementary strands of poly(dA) and poly(rA) with binding affinities unprecedented for modified oligonucleotide analogs previously reported.

Thymidyl DNG recognizes only complementary adenyl tracts while rejecting noncomplementary polynucleotide strands. Consistent with the fact that electrostatic repulsion exists between adjacent strands of negatively charged DNA and that electrostatic attraction exists between DNG–DNA complexes, the effect of ionic strength has an opposite relationship for DNG complexes with DNA compared to DNA–DNA complexes. At physiological ionic strength, the denaturation temperature of pentameric thymidyl DNG bound as a duplex with poly(dA) is greater than the boiling point of water.

The interesting differences and similarities between DNA and RNA, in addition to possible differential antisense properties, prompted us to explore the properties of DNG versus its RNA analog, ribonucleic guanidine (RNG). In this report we describe the synthetic methodology used to prepare an adenosyl RNG oligomer and provide an anticipated structure of RNG complexed to RNA.

MATERIALS AND METHODS

Synthesis. All TLC was run with Merck silica gel 60 (F_{254}) plates. ^{1}H NMR were obtained on a 200- or 500-MHz instrument in hexadeuterated dimethyl sulfoxide (DMSO-d_6) at 25°C unless otherwise specified. Chemical shifts (ppm) were referenced to DMSO (2.49 ppm). The following nomenclature is used: the guanidyl linkage of DNG oligonucleotides is specified by the letter g, whereas thiourea-linked oligonucleotides are specified by the letter t.

1,2-*O*-Isopropylidene-5-trifluoroacetamido-5-deoxy-α-D-xylofuranose (6). To a stirred solution of 5-amino-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose (5) (20 g, 105.8 mmol) in 250 ml of dry CH_2Cl_2, cooled to 0°C, was added triethylamine (15 ml) followed by dropwise addition of trifluoroacetic anhydride (15 ml, 106.2 mmol). The reaction solution was stirred for 20 min with continued cooling. The CH_2Cl_2 was evaporated off and the residue was suspended in 500 ml of water. The aqueous layer was extracted with hexane (2 × 150 ml). The hexane extracts were discarded and the aqueous suspension was extracted with diethyl ether (3 × 500 ml). The pooled ether extracts were dried over sodium sulfate and evaporated to afford a white solid: 24.3 g (81%) yield; mp = 110°C dec (slow darkening); TLC (ether/hexane, 2:1) R_f = 0.37; IR (KBr) 3451, 3266, 3116, 1712, 1577, 1390, 1217, 1191, 1151, 1070, 1025 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.52 (1 H, br t, amide proton), 5.83 (1 H, d, J = 3.7 Hz, 1-H), 5.39 (1 H, d, J = 4.9 Hz, 3-OH), 4.42 (1 H, d, J = 3.7 Hz, 2-H), 4.17 (1 H, m, 4-H), 3.97 (1 H, m, 3-H), 3.40 (2 H, m, 5-Hs), 1.37 and 1.23 (6 H, 2 × s, isopropylidene). High-resolution mass spectroscopy (HRMS) [fast-atom bombardment (FAB)] m/e 286.0905 (M + H)$^+$, calcd for $C_{10}H_{15}NO_5F_3$, 286.0902.

1,2-*O*-Isopropylidene-5-trifluoroacetamido-5-deoxy-3-*O*-trifluoromethanesulfonyl-α-D-xylofuranose (7). To a cooled solution (0°C) of **6** (see Scheme **II**; 24.3 g, 85.2 mmol) in 250 ml of dry CH_2Cl_2 containing 4-dimethylaminopyridine (25.2 g, 206.2 mmol) was added trifluoromethanesulfonic anhydride (31.5 ml, 187.2 mmol) by dropwise addition over a period of ≈20 min. The reaction solution was stirred for an additional 20 min with continued chilling. Diethyl ether (125 ml) was added to the completed reaction and the resulting mixture was filtered. The filtrate was evaporated and the residue was crystallized from ethanol–water: 28.7 g (81%) yield; mp = 83°C; TLC (50% hexane in ether) R_f = 0.50; IR (KBr) 3344, 1704, 1550, 1419, 1213, 1178, 1095, 993, 931 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.69 (1 H, br t, amide proton), 6.08 (1 H, d, J = 3.8 Hz, 1-H), 5.56 (1 H, d, J = 2.1 Hz, 3-H), 4.95 (1 H, d, J = 3.9 Hz, 2-H), 4.57 (1 H, m, 4-H), 3.46 (2 H, m, 5-Hs), 1.45 and 1.30 (6 H, 2 × s, isopropylidene). HRMS (FAB) m/e 418.0399 (M + H)$^+$, calcd for $C_{11}H_{16}NO_7F_6S$, 418.0395.

3-Azido-1,2-di-*O*-acetyl-5-trifluoroacetamido-3,5-dideoxy-β-D-ribofuranose (10). To a solution of **7** (26.3 g, 63.1 mmol) dissolved in 150 ml of dry DMSO was added sodium azide (24.4 g, 375 mmol) followed by ammonium chloride (24.4 g, 456 mmol). The reaction mixture was stirred at 70°C for 45 min and then diluted with 400 ml of water and extracted with ethyl acetate (3 × 300 ml). The pooled extracts were dried over sodium sulfate and evaporated to afford a 1:1 mixture of **8** and **9**. The mixture was dissolved in 400 ml of 88% formic acid and stirred at 50°C for 1 h. The formic acid was evaporated and the residue was coevaporated with 1-butanol (2 × 250 ml) followed by toluene (2 × 250 ml). The remaining residue was dissolved in 350 ml of dry pyridine, and 260 ml of acetic anhydride was added. The solution was stirred at 25°C for 2.0 h and then poured over 500 ml of ice water. The mixture was extracted with ether (3 × 400 ml), and the pooled extracts were evaporated to dryness. The residue was dissolved in 300 ml of

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Abbreviations: DNG, deoxyribonucleic guanidine; RNG, ribonucleic guanidine; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; FAB, fast-atom bombardment; HRMS, high-resolution mass spectroscopy.

ethyl acetate, and 30 g of silica gel was added. The ethyl acetate was evaporated and the dry silica gel was placed in a fritted filter funnel and rinsed with 500 ml of 30% hexane in ether. Residual amounts of pyridine were removed by washing the filtrate with 3% HCl solution (3 × 150 ml). The organic solution was treated with activated charcoal, filtered through celite, and evaporated to afford crude **10** (5.1 g, 24% yield for the three-step conversion), which was used without further purification for the preparation of **11.** An analytical sample was obtained by preparative TLC by using 33% hexane in diethyl ether as the mobile phase. TLC (33% hexane in ether) R_f = 0.40; IR (KBr) 3338, 2117, 1752, 1726, 1556, 1373, 1217, 1182 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.84 (2 H, br t, amide), 6.13 (1 H, s, 1-H), 5.33 (1 H, d, J = 4.5 Hz, 2-H), 4.21 (1 H, m, 4-H), 3.92 (1 H, m, 3-H), 3.86–3.58 (2 H, m, 5-Hs), 2.18 and 2.09 (6 H, 2 × s, acetates). HRMS (electron impact) m/e 354.0740 (M+), calcd for $C_{11}H_{13}N_4O_6F_3$, 354.0787.

3′-Azido-6-benzamido-2′-*O*-*t*-butyldimethylsilyl-5′-trifluoroacetamido-3′,5′-dideoxyadenosine (12). A suspension of N^6-benzoyladenine (3.0 g, 12.5 mmol) was refluxed in 45 ml of hexamethyldisilizane containing 4.5 ml of trimethylsilyl chloride for 20 h excluding moisture. Excess solvents were distilled away from the homogenous solution *in vacuo*, the residue was dissolved in 40 ml of dry dichloroethane containing **10** (2.90 g, 8.19 mmol), and trimethylsilyl trifluoromethanesulfonate (2.6 ml, 14.4 mmol) was added. The reaction solution was stirred at reflux for 20 h and then diluted with 10 ml of methanol followed by 100 ml of ethyl acetate. The organic solution was washed with 100 ml of water, and the aqueous layer was extracted with ethyl acetate (2 × 100 ml). The pooled organic extracts were dried over sodium sulfate and evaporated to dryness affording a mixture of **11** (TLC, 10% methanol in CH_2Cl_2; R_f = 0.55) and unreacted N^6-benzoyladenine.

The mixture was dissolved in 30 ml of methanol, and 15 ml of 6% aqueous K_2CO_3 solution was added. The resulting solution was stirred at 50°C for 15 min. The reaction was monitored by TLC (10% methanol in CH_2Cl_2). During some runs, the reaction time was extended to ensure reaction completion as judged by TLC analysis. The completed reaction was diluted with 100 ml of water and extracted with ethyl acetate (3 × 100 ml). The pooled organic extracts were dried over sodium sulfate and evaporated to afford a mixture of deacetylated **11** (TLC, 10% methanol in CH_2Cl_2; R_f = 0.48) and unreacted N^6-benzoyladenine.

The mixture was dissolved in 30 ml of dry *N,N*-dimethylformamide (DMF) containing imidazole (1.85 g, 27.2 mmol), and *tert*-butyldimethylsilyl chloride (4.0 g, 26.5 mmol) was added. The reaction solution was stirred at 60°C for 90 min. The DMF was evaporated off and the residue was chromatographed through a silica gel column by eluting with ethyl acetate. The pooled product fractions were evaporated to afford **12** as a glass: 1.31 g (26% yield for the three-step process); mp = 75°C (dec); TLC [ethyl acetate/hexane, 3:2 (vol/vol)], R_f = 0.38; IR (KBr) 3259, 2954, 2933, 2112, 1720, 1612, 1581, 1459, 1255, 1218, 1180, 1155, 840 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.70 (1 H, br t, trifluororacetamide) 8.76 and 8.72 (2 H, 2 × s, adenine protons), 8.05 and 7.59 (5 H, dd and m, J = 1.6 and 6.9 Hz for the dd, phenyl protons), 6.09 (1 H, d, J = 5.2 Hz, 1′-H), 5.31 (1 H, t, J = 5.4 Hz, 2′-H), 4.45 (1 H, t, J = 5.1 Hz, 3′-H), 4.16 (1 H, m, 4′-H), 3.69 (2 H, m, 5′-Hs), 0.79 (9 H, s, *t*-butyl), −0.013 and −0.22 (6 H, 2 × s, methyl protons). HRMS (FAB) m/e 606.2236 $(M + H)^+$, calcd for $C_{25}H_{31}N_9O_4F_3Si$, 606.2220.

5′-Acetamido-3′-amino-2′-*O*-*t*-butyldimethyl-silyl-3′,5′-dideoxyadenosine (2). A solution of **12** (1.20 g, 1.98 mmol), dissolved in 20 ml of 50% ammonium hydroxide in ethanol, was stirred in a sealed tube at 25°C for 12 h. The solvents were removed *in vacuo* to afford crude **13**, which was dissolved in 20 ml of dry DMF containing 0.28 ml of triethylamine. The solution was cooled in an ice bath and acetyl chloride (0.14 ml, 1.98 mmol) was added dropwise. The resulting solution was stirred with continued chilling for 10 min. DMF was removed *in vacuo*, and the residue was chromatographed through a silica gel column by eluting with 10% methanol in ethyl acetate. The pooled product fractions were evaporated to afford **14** as a glass that was used directly in the following step.

A solution of **14** was dissolved in 20 ml of 50% aqueous pyridine, and the solution was saturated with H_2S gas at 25°C. The reaction vial was sealed and the solution was allowed to stir at 25°C for 12 h. Methanol (10 ml) was added to the completed reaction and the resulting solution was filtered. The filtrate was evaporated to give **2** as a solid: 432 mg; TLC [1-butanol/water/acetic acid, 5:3:2 (vol/vol)], R_f = 0.56; IR (KBr) 2954, 2929, 2859, 1649, 1606, 1575, 1473, 1376, 1255, 1135, 838 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.34 and 8.18 (2 H, 2 × s, aromatic-Hs), 8.27 (1 H, t, J = 5.74 Hz, amide N-H), 7.37 (2 H, br s, adenine NH_2), 5.91 (1 H, d, J = 4.1 Hz, 1′-H), 4.70 (1 H, t, J = 5.3 Hz, 2′-H), 3.86 (1 H, m, 4′-H), 3.43 (3 H, m, 3′- and 5′-Hs), 1.85 (3 H, s, acetyl Hs), 0.80 (9 H, s, *t*-butyl Hs), −0.04 and −0.13 (6 H, 2 × s, silyl methyl Hs). HRMS (FAB) m/e 422.2320 $(M + H)^+$, calcd for $C_{18}H_{32}N_7O_3Si$, 422.2336.

3′-Azido-2′-*O*-*t*-butyldimethylsilyl-5′-isothiocyano-3′,5′-dideoxyadenosine (3). A solution of **12** (400 mg, 0.69 mmol), dissolved in 10 ml of 50% ethanol in ammonium hydroxide, was stirred in a sealed tube at 25°C for 12 h. The solvents were removed *in vacuo* to give crude **13**, which was dissolved in 10 ml of dry CH_2Cl_2 containing 0.14 ml of triethylamine and 1,3-dicyclohexylcarbodiimide (143 mg, 0.693 mmol). Carbon disulfide (0.50 ml) was added, and the reaction solution was stirred at 25°C for 10 min. The solution was evaporated, and the residue was chromatographed through a silica gel column by eluting with ethyl acetate. The pooled product fractions were evaporated to afford **3** as glass: 203 mg (66% yield for the two-step conversion); TLC (ethyl acetate) R_f = 0.51; IR (film) 3332, 3182, 2952, 2856, 2206, 2107, 1658, 1602, 1577, 1255, 1153, 1085, 838 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.37 and 8.18 (2 H, 2 × s, aromatic Hs), 7.38 (2 H, br s, adenine NH_2), 5.97 (1 H, d, J = 6.0 Hz, 1′-H), 5.34 (1 H, t, J = 5.8, 2′-H), 4.53 (1 H, m, 3′-H), 4.30–4.01 (3 H, m, 4′- and 5′-Hs), 0.75 (9 H, s, *t*-butyl Hs), −0.03 and −0.29 (6 H, 2 × s, silyl methyl Hs). HRMS (FAB) m/e 448.1699 $(M + H)^+$, calcd for $C_{17}H_{26}N_9O_2SSi$, 448.1699.

2′-Protected Acetamido-r(At)A-azido (4). A solution of **2** (300 mg, 0.713 mmol) and **3** (319 mg, 0.713 mmol) dissolved in 3.0 ml dry DMF was stirred at 80°C for 12 h. The solution was evaporated and the residue was chromatographed through a silica gel column by eluting with 15% methanol in ethyl acetate (containing 1% triethylamine). The product fractions were evaporated, affording **4** as a solid: 502 mg (81% yield); mp = 161°C (dec) slow darkening; TLC (30% methanol in ethyl acetate), R_f = 0.54; IR (KBr) 3315, 3215, 2952, 2929, 2107, 1649, 1601, 1576, 1473, 1255, 1147, 1079, 839, 782 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.41, 8.37, 8.18, and 8.17 (4 H, 4 × s, aromatic Hs), 8.23 (1 H, br t, amide N-H), 7.96 and 7.71 (2 H, 2 × br s, thiourea N-Hs), 7.38 (4 H, br s, adenine NH_2), 5.94 and 5.88 (2 H, 2 × d, J = 6.5 and 3.2 Hz, 1′-Hs), 5.45–3.32 (10 H, 7 × m, 2′, 3′, 4′, and 5′-Hs), 1.83 (3 H, s, acetyl Hs), 0.74 and 0.71 (18 H, 2 × s, *t*-butyl Hs), −0.04, −0.10, −0.15, −0.33 (12 H, 4 × s, silyl methyl Hs). HRMS (FAB) m/e 869.3968 $(M + H)^+$; calcd for $C_{35}H_{57}N_{16}O_5SSi_2$, 869.3957.

2′-Protected Acetamido-r(At)$_3$A-azido (5). A solution of **4** (500 mg, 0.576 mmol) dissolved in 8.0 ml of 50% aqueous pyridine was saturated with H_2S (gas) at 25°C. The resulting solution was stirred in a sealed tube for 12 h. TLC analysis (1-butanol/water/acetic acid, 5:3:2; R_f = 0.68) indicated that the reaction was complete. The solution was degassed with argon and filtered. The filtrate was evaporated affording the 3′-amino dimer, which was dissolved in 3.0 ml of dry DMF containing **3** (280 mg, 0.627 mmol) and subjected to another coupling reaction as described for the preparation of **4.** The

trimer-azido was precipitated from 2-propanol/diethyl ether after evaporation of the DMF (376-mg yield). The supernatant was evaporated to dryness, and an additional crop of product was obtained by precipitation of the residue from diethyl ether/hexane (186-mg yield). Total yield: 562 mg (76%); TLC (1-butanol/water/acetic acid, 5:3:2), R_f = 0.83.

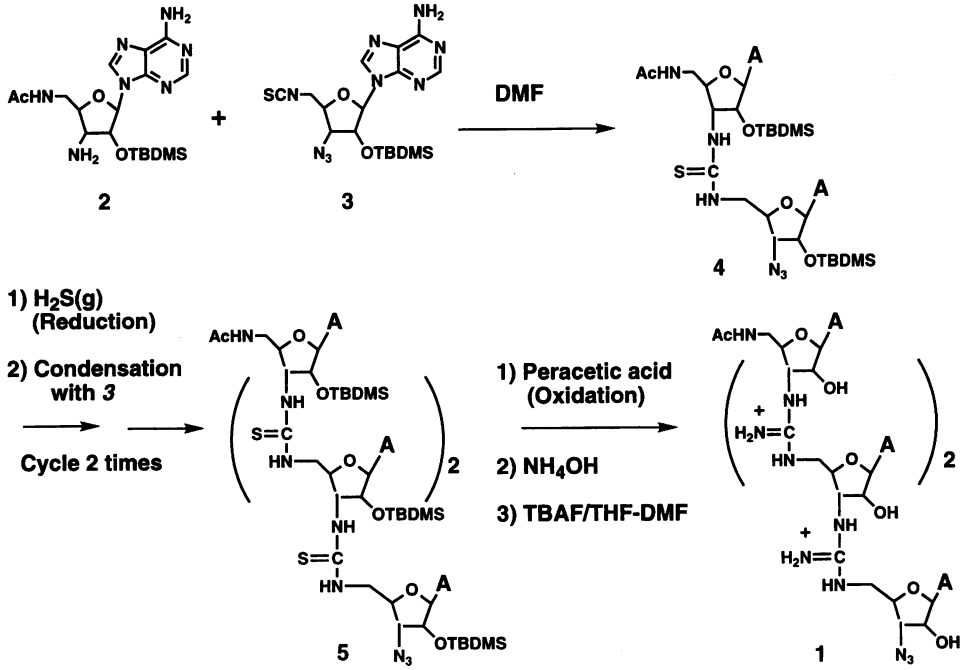
The trimer-azido (550 mg, 0.427 mmol) was reduced with H_2S (gas), as described above, to the amino derivative which was treated with a slight excess of **3**. The tetramer-azido **5** was precipitated from 2-propanol/diethyl ether after removal of DMF: 482 mg (92%) yield; mp > 160°C (slow dec), TLC (1-butanol/water/acetic acid, 5:3:2), R_f = 0.83; IR (KBr) 3195, 2954, 2931, 2107, 1641, 1599, 1577, 1544, 1473, 1255, 1126, 1089, 838 cm^{-1}; ^1H NMR (DMSO-d_6, 60°C) δ 8.35, 8.34, 8.33, and 8.18 (8 H, 5 × s, aromatic Hs), 7.94 and 7.52 (7 H, 2 × m, amide N-H and thiourea N-Hs), 7.15 (8 H, br s, adenine NH_2), 5.94 and 5.87 (4 H, m and d, J = 3.3 Hz for the d, 1′-Hs), 5.44–3.06 (20 H, t and 5 × m, 2′, 3′, and 5′-Hs), 1.84 (3 H, s, acetomido CH_3), 0.77, 0.75, 0.72, and 0.70 (36 H, 4 × s, *t*-butyl Hs), −0.02, −0.05, −0.09, −0.12, −0.14, −0.16, and −0.26 (24 H, 7 × s, silyl methyl Hs). MS (FAB) m/e 1713.4 $(M + H)^+$, calcd for $C_{69}H_{111}N_{30}O_9S_3Si_4$, 1713.2.

Acetamido-r(Ag)$_3$A-azido (1). Compound **5** (50 mg, 0.029 mmol) was added to a solution of 0.5 ml of DMF and 0.14 ml of peracetic acid solution [32% (wt/wt) in dilute acetic acid, (Aldrich)] previously cooled to 0°C. The resulting solution was stirred for 15 min at 0°C. The DMF was evaporated off under a stream of N_2 (gas), and the residue was dissolved in 4.0 ml of dry DMF and saturated with anhydrous ammonia at 25°C. The reaction vial was sealed and the solution was stirred at 80°C for 4.0 h. The DMF was evaporated off and the residue was dissolved in 5.0 ml of 30% DMF in tetrahydrofuran (THF-DMF). Tetrabutylammonium fluoride (TBAF; 0.88 ml) was added and the reaction solution was stirred for 1.0 h. The solution was evaporated to dryness and the solid residue was washed with diethyl ether (2 × 10 ml). The ether extracts were discarded and the remaining solid was triturated with water and collected, affording **1** as a crude solid: 22 mg (63% crude yield for the three-step process). An analytical sample was prepared by preparative HPLC using an Alltech Associates WCX cation-exchange column eluted with 1.0 M ammonium acetate buffer (pH 5.0). A suspension of crude **1** in water was rendered water soluble by addition of dilute acetic acid before HPLC injection. The peak of longest retention time (6.5 min, flow rate = 1.5 ml/min), which was also the major component, was isolated, and the ammonium acetate was removed from the RNG sample by repetitive evaporations from water: IR (KBr) 3413, 2967, 2114, 1648, 1564, 1413, 1093, 652 cm^{-1}; ^1H NMR (D_2O, 50°C) δ 8.21–7.89 (8 H, 7 × s, aromatic Hs), 6.08, 6.01, 5.93 (4 H, d and 2 × s, J = 4.5 Hz for the d, 1′-Hs), 5.19 and 4.83 (4 H, t and m, J = 5.5 Hz for the t, 2′-Hs), 4.55, 4.53, 4.52, and 4.39 (4 H, 4 × m, 3′-Hs); 4.43, 4.35, 4.30, and 4.15 (4 H, 4 × m, 4′-Hs); 3.92, 3.79, 3.76, 3.51, 3.50, 3.48, and 3.38 (8 H, 7 × m, 5′-Hs), 1.91 (3 H, s, acetomido CH_3). MS (FAB) m/e 1204.48 $(M + H)^+$, calcd for $C_{45}H_{58}N_{33}O_9$, 1204.51.

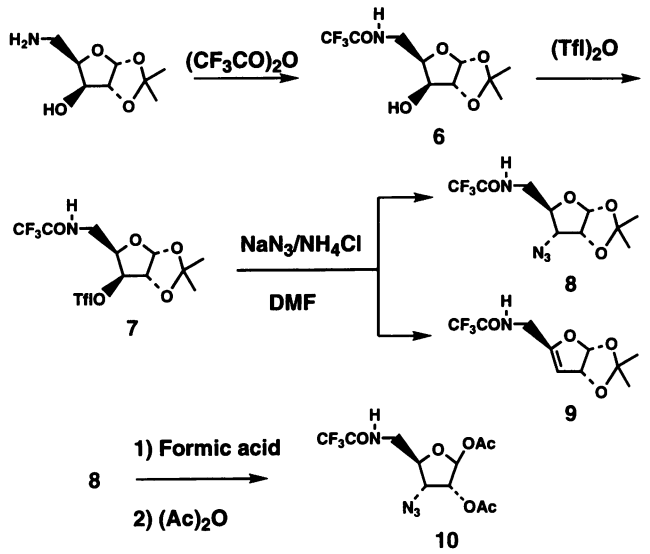
Molecular Modeling. Model building was performed as previously described (1, 4). Before minimizations, sodium ions (charge, +1.0) were placed adjacent to the phosphate moieties, and chloride ions (charge, −1.0) were placed near the guanidinium groups. The CHARMM residue topology file was modified for the adenosyl RNG and termini to construct and parameterize the proposed structural system for the energy minimization calculations. Helical parameters for the minimized structure were analyzed using the NEWHEL93 program (6). Calculation of the major and minor groove widths were based on the shortest distances between the phosphate phosphorus and the guanidinium carbon atoms across the major and minor grooves.

RESULTS AND DISCUSSION

Synthesis. The general synthetic strategy for the formation of adenosyl RNG oligomers is similar to that described for the preparation of DNG oligomers. A thiourea-linked RNG dimer was formed by the reaction of a 5′-terminal residue, the adenosine 3′-amino derivative (**2**) (Scheme I; OTBDMS refers to the *tert*-butyldimethylsilyl ether protecting group) and the 5′-isothiocyanate derivative (**3**). Chain extension proceeded via a two-step process involving hydrogen sulfide-mediated reduction of the 3′-azido moiety to the amine, followed by an



Scheme I



Scheme II

additional coupling reaction with a slight excess of **3**. After construction of an oligomer of desired length, the thiourea linkages were converted to guanidinium linkages by a two-step process involving oxidation of the thiourea moiety to the aminoiminosulfonic acid derivative with peracetic acid and amination with anhydrous ammonia in dry DMF. The 2′-silyl ether groups, present on the RNG oligomer, were then removed using tetrabutylammonium fluoride. Final purification was performed by preparative HPLC using a cation-exchange column with 1.0 M ammonium acetate buffer (pH 5.0) as the mobile phase.

The coupling intermediates **2** and **3** were prepared from a common synthetic pathway starting with 5-amino-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose (Scheme **II**). The amine was protected as the trifluoroacetamide group before conversion of the 3-position to the azide. Thus, treatment of **6** with trifluoromethanesulfonic anhydride [$(Tfl)_2O$] produced the 3-*O*-triflate derivative (**7**) which was then allowed to undergo an S_N2 displacement reaction with ammonium azide in DMF, forming the 3-azido derivative (**8**). The elimination product (**9**) was also formed in this reaction. The ratio of the substitution vs. elimination products was determined to be ≈1:1 by ^1H NMR analysis. A similar product ratio was previously observed for the preparation of 3-azido-1,2-*O*-isopropylidene-5-*O*-(4-methylbenzoyl)-3-deoxy-α-D-ribofuranose (7). Isolation of the desired product was not performed at this point as the elimination product was conveniently removed after two subsequent steps.

Purination at the C-1 position utilizing Vorbrüggen methodology (8) necessitates the presence of a 1,2-diacyl moiety. Thus, acid-catalyzed removal of the 1,2-isopropylidene group with 88% formic acid solution was followed by acylation of the resulting diol with acetic anhydride in pyridine, yielding the β-1,2-diacetyl derivative (**10**) as the predominant product. Isolation of **10** was achieved by filtering the residue through silica gel with 30% hexane in ether. The elimination product decomposed during the formic acid treatment to a highly polar species which was retained on the silica gel.

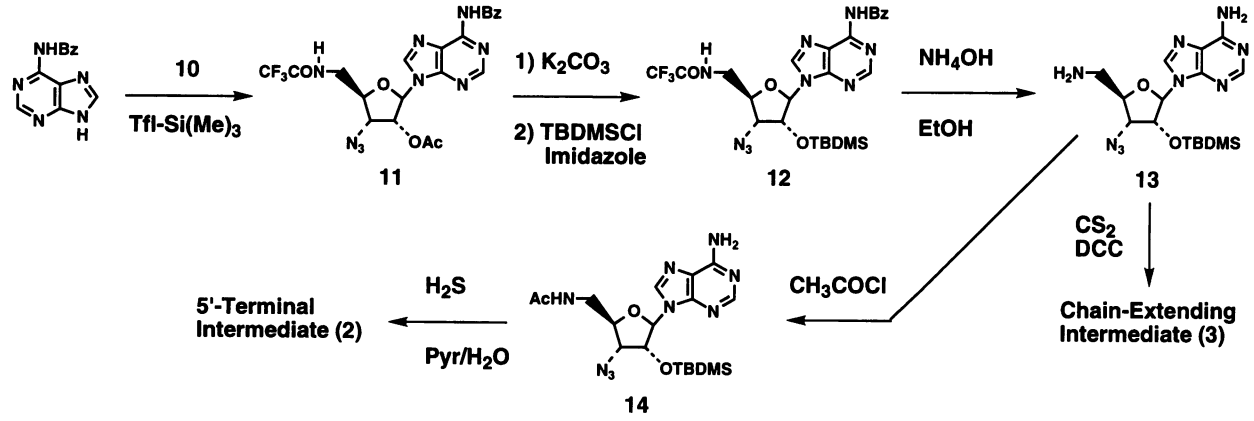
Incorporation of a purine base at the C-1 position using standard Vorbrüggen reaction conditions (8) resulted from the reaction of **10** with presilylated N^6-benzoyladenine in the presence of trimethylsilyl triflate. Formation of an intermediate ribofuranosyl carbocation is assisted by the neighboring effect of the 2′-acetyl group which attacks the α-position of C-1. Subsequent attack of the purine base can only occur at the β-position.

Regioselective ribosylation at the N-9 position of the purine ring is predicted to be the thermodynamic product. After the course of a 22-h reaction time, only one nucleoside product **11** was detected. UV spectral analysis of the subsequent product **2** presented a UV maxima at 260 nm (Scheme **III**).

A concern of ours pertained to a possible intramolecular reaction of the free 2′-hydroxyl at the central carbon of the 3′–5′ backbone after oxidation of the thiourea group. Thus, the 2′-acetyl group was selectively removed under mild basic conditions with 6% aqueous potassium carbonate solution. The free hydroxyl group was subsequently protected as the *tert*-butyldimethylsilyl ether (**12**). Deprotection of the amide protecting groups was accomplished with concentrated ammonium hydroxide in ethanol, affording the amino derivative (**13**).

At this point two separate synthetic pathways emerge forming the 5′-terminal intermediate and the 3′-chain-extending intermediate. The 5′ unit (**2**) was prepared by a two-step process involving capping of the 5′-amino group of **13** as the acetamide (later synthetic strategies will involve connection of this amino group to a polymer for solid-support synthesis), followed by reduction of the 3′-azido moiety to the amine with hydrogen sulfide gas in aqueous pyridine. The 3′-chain-extending intermediate (**3**) was prepared by treating **13** with carbon disulfide in the presence of 1,3-dicyclohexylcarbodiimide. The resulting 5′-isothiocyanate derivative **3** was conveniently purified by flash chromatography.

The cationic nature of **1** was confirmed by its retention on a cation-exchange HPLC column. The ^1H NMR spectrum of **1** identifies four adenosine moieties present in equal ratios.



Scheme III

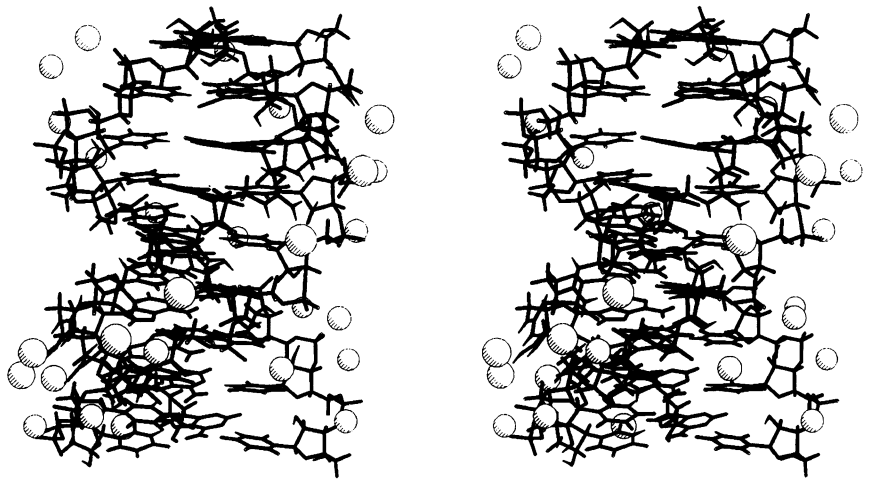


FIG. 1. Stereoview of the triplex structure of $(Up)_9U{\cdot}r(Ag)_9A{\cdot}(Up)_9U$ with counterions. The minor groove can be viewed from the upper left of the figure, where the Watson–Crick-paired RNG strand and the RNA strand situate at the right and the left, respectively. The major groove is at the lower right, and the Hoogsteen-strand RNA runs parallel with the RNG strand in the major groove.

The 5′-terminal acetamido methyl protons are presented at 1.91 ppm. The presence of the 3′-azido terminal moiety was confirmed by an IR absorbance at 2114 cm^{-1}. Mass spectral analysis of **1** provided the correct mass of 1204.48 $(M + H)^+$.

Model of RNG·$(RNA)_2$ Triple Helix. Fig. 1 shows the triple helix structure $(Up)_9U{\cdot}r(Ag)_9A{\cdot}(Up)_9U$, generated using QUANTA and the molecular mechanics program CHARMM. The sugar of the RNG strand is in the O4′-endo and C1′-exo conformation with the exception of the 3′-azido terminal residue, which is in the C1′-endo conformation. The sugar of the RNA strands is C1′-exo with the exception of the 3′-terminal ribose, which is O4′-endo. This is suggestive of a B-type nucleotide oligomer structure as is the 3.25-Å axial rise and 30.7° turn angle, as well as the 11.7 residue helical turn. The hydrogen bonds between the Watson–Crick and Hoogsteen base pairs are all maintained at 1.8 and 1.9 Å from donor to acceptor atoms. Counterions remained proximal to their respective charged groups. The major groove width contracts by 0.8 Å and the minor groove contracts by 0.2 Å, compared with a RNA triplex structure (9). The contractions are caused by electrostatic attractions between the positively charged RNG backbone and the negatively charged RNA backbone.

Conclusion. In this report we present the synthesis of tetrameric adenosyl RNG. The RNG building blocks were prepared by stereoselective ribosylation of presilylated N^6-benzoyl adenine with the 1,2-diacyl intermediate **10** under standard Vorbrüggen reaction conditions (treatment of **10** with the Lewis acid trimethylsilyl triflate affords a resonance stabilized ribofuranosyl carbocation which alkylates at the β-position of C-1). This methodology may be extended to the synthesis of RNG building blocks incorporating any of the other natural nucleobases (U, G, and C) and is currently being used in the preparation of RNG oligomers with mixed nucleotide sequences.

We would like to thank William P. Schulz (Department of Chemistry, Arizona State University) for obtaining NMR spectral data for compound **1**. This study was supported by grants from the National Science Foundation and the Office of Naval Research.

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