Supporting Information

Development of Minimal Diguanosinyl Motif toward RNA G-Quadruplex-Like Structures in Solution

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1. General experimental details:

All reactions were performed in oven or flame-dried glassware with magnetic stirring under nitrogen atmosphere using dry, freshly distilled solvents, unless otherwise noted. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm silica gel plates with UV light, I_2 , 7% ethanolic phosphomolybdic acid-heat and 2.5% ethanolic anisaldehyde (with 1% AcOH and 3.3% conc. H_2SO_4)-heat as developing agents. Silica gel finer than 200 mesh was used for flash column chromatography. Yields refer to chromatographically and spectroscopically homogeneous materials unless otherwise stated.

RP-HPLC analysis and purifications were carried out using Shimadzu LC-20AD HPLC instrument and SPD-M20A UV/vis detector using 5 μ m C18 columns, dimension 4.6 $\times$ 250 mm with a flow rate of 1 mL/min for analytical purposes and dimension 20 $\times$ 250 mm with a flow rate of 10 mL/min for preparative separation; with eluants A (0.01% TFA in H_2O) and B (0.01% TFA in CH_3CN) and photodiode array detector setting of λ = 210-254 nm.

Infrared (FT-IR) spectra were recorded on a Perkin Elmer Spectrum BX spectrophotometer, reported as ν_{max} in cm^{-1} and optical rotations were measured on JASCO P-2000 polarimeter. NMR spectra were recorded on Bruker Ultrashield spectrometer at 400 MHz (for 1H -NMR) and 100 MHz (for ^{13}C -NMR). Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as internal standard ($CDCl_3$: δ 7.26, $DMSO-d_6$: δ 2.50 for 1H -NMR and $CDCl_3$: δ 77.16, $DMSO-d_6$: δ 39.52 for ^{13}C -NMR). For 1H -NMR, data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz) and integration. High resolution mass spectrometry was performed on Micromass Q-TOF Micro instrument. The UV spectra were recorded with a SHIMADZU UV-3600 spectrophotometer and CD spectra were recorded with a JASCO J-810 CD spectropolarimeter with a Peltier temperature controller at a scanning speed of 50 nm/min with a quartz cell of 10 mm path-length.

2. Experimental procedures and spectroscopic data of the compounds:

Synthesis of compound 10:

TFA:CH₂Cl₂ (1:1, 11.2 mL) was added to the compound **9** (800 mg, 1.112 mmol) which was prepared according to the literature procedure²⁹ at 0 °C and the reaction mixture was stirred at room temperature for 45 minutes. After completion of the reaction (TLC), the reaction mixture was concentrated in vacuo to remove excess TFA. The reaction mixture was diluted with ethyl acetate and quenched with saturated NaHCO₃ and extracted with ethyl acetate (2×50 mL). The combined organic extracts were washed with H₂O (2×20 mL), brine (20 mL), dried (Na₂SO₄) and concentrated in vacuo. Purification by column chromatography (silica gel, 100% EtOAc) afforded compound **10** (490 mg, 84%) as colourless solid.

R_f: 0.5 (Silica gel, 100 % EtOAc); **IR (neat)**: $\nu_{\max}$ 3468, 3302, 3081, 2919, 2850, 2721, 2564, 2366, 2109, 1739, 1682, 1464, 1219, 1031 cm⁻¹; **¹H NMR (CDCl₃, 400 MHz)**: δ 12.09 (brs, 1H), 10.07 (brs, 1H), 7.82 (brs, 1H), 7.38-7.29 (m, 6H), 7.16 (d, J = 7.4 Hz, 1H), 5.83 (d, J = 3.6 Hz, 1H), 5.71 (brs, 1H), 5.16 (d, J = 4.0 Hz, 2H), 4.49 (t, J = 4.8 Hz, 1H), 4.07 (d, J = 3.5 Hz, 1H), 2.83-2.72 (m, 1H), 2.55-2.44 (m, 1H), 2.26 (s, 3H), 2.14 (s, 3H); **¹³C NMR (CDCl₃, 100 MHz)**: δ 175.1, 172.5, 170.0, 155.6, 147.8, 141.8, 135.3, 129.8, 128.8, 128.7, 128.1, 126.5, 88.0, 81.4, 75.5, 67.3, 62.5, 28.3, 24.2, 22.8, 20.5; **HRMS (ESI)**: calcd for C₂₃H₂₄N₈O₇Na [M+Na]⁺ 547.1666, found 547.1663.

Synthesis of compound 3:

NH₃ saturated methanol (2.0 mL) was added to a solution of compound **10** (35 mg, 0.067 mmol) at 0 °C and the reaction mixture was stirred for 2 h at room temperature. After completion of the reaction (TLC), the reaction mixture was concentrated in vacuo and the crude reaction mixture was purified by reverse phase HPLC to furnish compound **3** (20.7 mg, 85%).

RP-HPLC, t_R = 20.56 mins (initial 5 mins, 5% CH₃CN and then linear gradient 5 - 80% CH₃CN in 30 mins); **IR (neat)**: $\nu_{\max}$ 3116, 2924, 2364, 2329, 2107, 1735, 1689, 1533, 1260, 1176 cm⁻¹; **¹H NMR (DMSO-d₆, 400 MHz)**: δ 10.67 (s, 1H), 7.91 (s, 1H), 6.50 (brs, 2H), 6.24 (d, J = 4.4 Hz, 1H), 5.66 (d, J = 5.1 Hz, 1H), 4.82 (d, J = 4.3 Hz, 1H), 4.20 (t, J = 5.4 Hz, 1H), 3.89 (q, J = 6.1 Hz, 1H), 3.56 (s, 3H), 2.41 (t, J = 7.5 Hz, 2H), 2.0-1.90 (m, 2H); **¹³C NMR (DMSO-d₆, 100 MHz)**: δ 173.3, 157.0, 153.9, 116.9, 87.3, 80.0, 74.0, 63.9, 51.6, 29.5, 28.6; **HRMS (ESI)**: calcd for C₁₃H₁₆N₈O₅Na [M+Na]⁺ 387.1141, found 387.1143.

Synthesis of compound 11:

Ph₃P (416 mg, 1.588 mmol) was added to a solution of compound **10** (416 mg, 0.794 mmol) in dry methanol (3 mL) at room temperature under inert atmosphere. After stirring the reaction mixture for 30 minutes, (Boc)₂O (0.28 mL, 1.191 mmol) was added in the reaction mixture and stirred for another 4h at room temperature. After completion of the reaction as mentioned by TLC, the reaction mixture was concentrated in vacuo and the crude mixture was purified by column chromatography (silica gel, 100% EtOAc) afforded compound **11** (250 mg, 60%) as colourless solid.

Rf: 0.4 (Silica gel, 100 % EtOAc); **IR (neat)**: $\nu_{\max}$ 2926, 2854, 1725, 1679, 1611, 1562, 1366, 1253, 1164 cm⁻¹; **¹H NMR (CDCl₃, 400 MHz)**: δ 12.08 (s, 1H), 10.60 (s, 1H), 7.58 (s, 1H), 7.37-7.27 (m, 5H), 6.67 (brs, 1H), 5.69 (s, 2H), 5.15 (s, 1H), 5.10 (s, 2H), 4.18 (brs, 2H), 2.71-2.58 (m, 1H), 2.57-2.44 (m, 1H), 2.42-2.32 (m, 1H), 2.29 (s, 3H), 2.15-2.06 (m, 1H), 1.41 (s, 9H); **¹³C NMR (CDCl₃, 100 MHz)**: δ 174.7, 173.7, 156.6, 155.5, 147.8, 147.6, 139.4, 135.8, 128.7, 128.4, 128.1, 121.2, 92.2, 83.0, 80.3, 72.3, 66.7, 55.8, 50.6, 30.6, 28.9, 28.4, 24.2; **HRMS (ESI)**: calcd for C₂₆H₃₂N₆O₈Na [M+Na]⁺ 579.2179, found 579.2178.

Synthesis of compound 12:

$\text{Pd}(\text{OH})_2/\text{C}$ (52 mg, 20% by weight) was added to a solution of the compound **11** (262 mg, 0.471 mmol) in dry methanol at 0 °C followed by Et_3SiH (1.50 mL, 9.42 mmol) under inert atmosphere. The reaction mixture was stirred for 3 h at room temperature which point of time complete consumption of starting material was achieved and the reaction mixture was filtered through a short pad of celite and concentrated in vacuo. Purification by column chromatography (silica gel, 10% methanol in chloroform) afforded compound **12** (176 mg, 80%) as colourless solid.

R_r: 0.3 (Silica gel, 20% methanol in CHCl_3); **IR (neat)**: ν_{max} 2961, 2924, 2853, 2363, 2329, 1711, 1462, 1366, 1224 cm^{-1} ; **^1H NMR (DMSO- d_6 , 400 MHz)**: δ 12.08 (brs, 2H), 11.7 (brs, 1H), 8.18 (s, 1H), 6.67 (d, J = 8.2 Hz, 1H), 5.90 (brs, 1H), 5.70 (d, J = 3.5 Hz, 1H), 4.48 (brs, 1H), 4.00-3.90 (m, 1H), 3.90-3.80 (m, 1H), 2.40-2.22 (m, 2H), 2.18 (s, 3H), 1.97-1.88 (m, 1H), 1.88-1.78 (m, 1H), 1.40 (s, 9H); **^{13}C NMR (DMSO- d_6 , 100 MHz)**: δ 174.0, 173.5, 155.3, 154.8, 148.5, 148.0, 137.6, 120.3, 88.5, 80.5, 79.2, 78.3, 72.6, 55.5, 30.0, 28.2, 23.8; **HRMS (ESI)**: calcd for $\text{C}_{19}\text{H}_{26}\text{N}_6\text{O}_8\text{Na}$ $[\text{M}+\text{Na}]^+$ 489.1710, found 489.1711.

Synthesis of amide linked guanosine dimer **4**:

Ph_3P (49 mg, 0.1866 mmol) was added to a solution of compound **10** (49 mg, 0.0933 mmol) in dry methanol (3 mL) at room temperature under inert atmosphere. After stirring the reaction mixture for 30 minutes, the reaction mixture was concentrated under vacuum to furnish the amine compound **13** which was used for the next step without purification.

BOP reagent (82.5 mg, 0.1866 mmol) was added to a solution of compound **12** (29 mg, 0.0622 mmol) in dry CH_2Cl_2 (2 mL) and dry DMF (2 mL) at 0 °C under inert atmosphere. After 15 min., compound **13**, prepared above, dissolved in CH_2Cl_2 (5 mL) was added to the reaction mixture followed by DIPEA (0.06 mL, 0.311 mmol). After stirring for 12 h at room temperature, the reaction mixture was quenched with saturated NH_4Cl solution, extracted with

ethyl acetate, washed with 1(N) HCl, NaHCO₃, water, brine, dried (Na₂SO₄), filtered and concentrated in *vacuo* to furnish compound **14** which was used for the next step without further purification.

NH₃ saturated in methanol (1.0 mL) was added to the above prepared compound **14** at 0 °C and stirred at room temperature for 12h. After completion of the reaction (TLC), the reaction mixture was concentrated in *vacuo* and the crude reaction mixture was purified by reverse phase HPLC to furnish compound **4** (22.7 mg, 49% over 2 steps).

RP-HPLC, *t_R* = 22.27 mins (initial 5 mins, 5% CH₃CN and then linear gradient 5 - 70% CH₃CN in 30 mins); **IR (neat)**: *v*_{max} 3584, 2919, 2852, 2364, 2330, 1652, 1542, 1315, 1192, 1058 cm⁻¹; **¹H NMR (DMSO-*d*₆, 400 MHz)**: δ 10.73 (brs, 2H), 7.87 (brs, 1H), 7.84 (d, *J* = 6.8 Hz, 2H), 6.61 (d, *J* = 8.2 Hz, 1H), 6.51 (brs, 2H), 6.49 (brs, 2H), 5.90-5.80 (m, 2H), 5.64 (q, *J* = 2.9 Hz, 2H), 4.41 (brs, 2H), 4.25 (q, *J* = 7.6 Hz, 1H), 3.95 (q, *J* = 7.8 Hz, 1H), 3.85-3.76 (m, 2H), 3.54 (s, 3H), 2.41-2.30 (m, 3H), 2.03-1.90 (m, 2H), 1.90-1.76 (m, 3H), 1.40 (s, 9H); **¹³C NMR (DMSO-*d*₆, 100 MHz)**: δ 172.9, 172.0, 156.7, 153.7, 151.0, 150.9, 135.2, 116.7, 88.3, 80.2, 78.1, 72.7, 69.7, 53.9, 51.2, 31.6, 31.2, 29.6, 29.0, 28.9, 28.2, 22.0; **HRMS (ESI)**: calcd for C₃₀H₄₀N₁₂O₁₁Na [M+Na]⁺ 767.2837, found 767.2839.

Synthesis of compound **14**:

Ph₃P (38 mg, 0.145 mmol) was added to a solution of compound **10** (38 mg, 0.072 mmol) in dry methanol (3 mL) at room temperature under inert atmosphere. After stirring the reaction mixture for 30 minutes, the reaction mixture was concentrated under vacuum to furnish the amine compound **13** which was used for the next step without purification.

Boc-6-Aminocaproic acid (16.8 mg, 0.0725 mmol) was dissolved in CH₂Cl₂ (2 mL) and cooled to 0 °C. Then it was sequentially treated with HOBt (10.8 mg, 0.0797 mmol) and HBTU (33 mg, 0.087 mmol). After 15 min., compound **13**, prepared above, dissolved in CH₂Cl₂ (1 mL) was added to the reaction mixture followed by DIPEA (0.06 mL, 0.36 mmol). After stirring for

12 h at room temperature, the reaction mixture was quenched with saturated NH₄Cl solution, extracted with ethyl acetate, washed with 1(N) HCl, NaHCO₃, water, brine, dried (Na₂SO₄), filtered and concentrated in *vacuo*. purification by column chromatography (silica gel, 6% methanol in chloroform) afforded compound **14** (32.5 mg, 70%) as white foam.

Rf: 0.5 (Silica gel, 10% methanol in CHCl₃); IR (neat): ν_{max} 3368, 2925, 2854, 1717, 1682, 1611, 1562, 1366, 1254, 1168, 1096 cm⁻¹; **¹H NMR (DMSO-*d*₆, 400 MHz)**: δ 12.07 (brs, 1H), 11.67 (brs, 1H), 8.19 (s, 1H), 7.79 (d, *J* = 8.1 Hz, 1H), 7.38-7.27 (m, 5H), 6.74 (t, *J* = 5.3 Hz, 1H), 5.96 (d, *J* = 5.0 Hz, 1H), 5.75 (d, *J* = 3.3 Hz, 1H), 5.04 (d, *J* = 1.9 Hz, 2H), 4.54-4.48 (m, 1H), 4.30-4.22 (m, 1H), 3.90-3.83 (m, 1H), 2.88 (q, *J* = 6.6 Hz, 2H), 2.47-2.38 (m, 1H), 2.18 (s, 3H), 2.16-2.12 (m, 1H), 2.04-1.85 (m, 2H), 1.54-1.44 (m, 2H), 1.36 (s, 9H), 1.35 (brs, 2H), 1.28-1.17 (m, 4H); **¹³C NMR (DMSO-*d*₆, 100 MHz)**: δ 173.5, 172.6, 172.4, 155.6, 148.7, 137.4, 136.1, 128.4, 127.9, 127.8, 120.3, 88.8, 80.6, 79.2, 77.3, 72.8, 65.4, 56.0, 53.9, 48.6, 35.3, 29.9, 29.3, 28.3, 28.1, 26.0, 25.0, 24.0; **HRMS (ESI)**: calcd for C₃₂H₄₃N₇O₉Na [M+Na]⁺ 692.3020, found 692.3021.

Synthesis of compound 5:

TFA (0.4 mL) was added to a solution of compound **14** (12 mg, 0.018 mmol) in dry CH₂Cl₂ at 0 °C under inert atmosphere. The reaction mixture was stirred at room temperature for 30 min and concentrated in *vacuo* to give TFA salt **15** which were used for the next reaction without further purification.

Compound **12** (8.4 mg, 0.018 mmol) was dissolved in CH₂Cl₂ (2 mL) and dry DMF (2 mL) and cooled to 0 °C. Then it was sequentially treated with HOBt (2.7 mg, 0.0198 mmol) and HBTU (8.2 mg, 0.0216 mmol). After 15 min., compound **15**, prepared above, dissolved in CH₂Cl₂ (1 mL) and dry DMF (1 mL) and was added to the reaction mixture followed by DIPEA (0.02 mL, 0.09 mmol). After stirring for 12 h at room temperature, the reaction mixture was

quenched with saturated NH_4Cl solution, extracted with ethyl acetate, washed with 1(N) HCl , NaHCO_3 , water, brine, dried (Na_2SO_4), filtered and concentrated in *vacuo*. purification by column chromatography (silica gel, 10% methanol in chloroform) afforded protected dimer compound as white foam which was used for the next reaction without further purification.

NH_3 saturated in methanol (1.0 mL) was added to the above prepared protected dimer compound at 0 °C and stirred at room temperature for 12h. After completion of the reaction (TLC), the reaction mixture was concentrated in *vacuo* and the crude reaction mixture was purified by reverse phase HPLC to furnish compound **5** (6.2 mg, 40% over 2 steps).

RP-HPLC, t_R = 23.02 mins (initial 5 mins, 5% CH_3CN and then linear gradient 5 - 70% CH_3CN in 30 mins); **IR (neat)**: ν_{max} 3585, 2923, 2853, 2700, 2554, 2363, 2329, 1696, 1539, 1367, 1169, 1018 cm^{-1} ; **^1H NMR ($\text{DMSO}-d_6$, 500 MHz)**: δ 10.68 (brs, 2H), 7.84 (s, 2H), 7.79-7.75 (m, 2H), 6.60 (d, J = 8.3 Hz, 1H), 6.46 (d, J = 7.3 Hz, 4H), 5.91 (d, J = 4.4 Hz, 1H), 5.82 (d, J = 4.2 Hz, 1H), 5.65 (d, J = 3.2 Hz, 1H), 5.62 (d, J = 3.6 Hz, 1H), 4.42 (brs, 2H), 4.31-4.24 (m, 1H), 4.15-4.08 (m, 1H), 3.96-3.89 (m, 1H), 3.84-3.75 (m, 2H), 3.54 (s, 3H), 3.01-2.96 (m, 2H), 2.37-2.33 (m, 2H), 1.96-1.87 (m, 3H), 1.87-1.82 (m, 1H), 1.82-1.69 (m, 2H), 1.53-1.45 (m, 4H), 1.40 (s, 9H), 1.38-1.33 (m, 3H); **^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz)**: δ 172.9, 172.5, 171.4, 153.7, 150.9, 135.3, 118.6, 116.7, 88.4, 80.3, 79.1, 78.1, 75.0, 72.7, 66.9, 65.4, 64.4, 53.8, 51.2, 35.2, 31.2, 29.6, 29.2, 28.9, 28.2, 26.1, 24.9, 22.0; **HRMS (ESI)**: calcd for $\text{C}_{36}\text{H}_{51}\text{N}_{13}\text{O}_{12}\text{Na}$ $[\text{M}+\text{Na}]^+$ 880.3678, found 880.3676.

Synthesis of compound 6:

Compound **12** (26 mg, 0.056 mmol) was dissolved in CH_2Cl_2 (2 mL) and dry DMF (2 mL) and cooled to 0 °C. Then it was sequentially treated with HOBt (8.3 mg, 0.0616 mmol) and HBTU (25.5 mg, 0.0672 mmol). After 15 min., hexamethylenediamine (3.2 mg, 0.028 mmol) dissolved in CH_2Cl_2 (1 mL) and dry DMF (1 mL) and was added to the reaction mixture

followed by DIPEA (0.05 mL, 0.28 mmol). After stirring for 12 h at room temperature, the reaction mixture was quenched with saturated NH_4Cl solution, extracted with ethyl acetate, washed with 1(N) HCl , NaHCO_3 , water, brine, dried (Na_2SO_4), filtered and concentrated in *vacuo*. purification by column chromatography (silica gel, 10% methanol in chloroform) afforded crude compound which was used for the next reaction without further purification.

NH_3 saturated methanol (1.0 mL) was added to the above prepared compound at 0 °C and stirred at room temperature for 12h. After completion of the reaction (TLC), the reaction mixture was concentrated in *vacuo* and the crude reaction mixture was purified by reverse phase HPLC to furnish compound **6** (26.0 mg, 50% over 2 steps).

RP-HPLC, t_R = 32.58 mins (Initial 5 mins, 5% CH_3CN and then linear gradient 5 - 60% CH_3CN in 40 mins); **IR (neat)**: ν_{max} 2924, 2853, 2364, 2329, 1694, 1638, 1365, 1248, 1165, 1016 cm^{-1} ; **^1H NMR (DMSO- d_6 , 400 MHz)**: δ 10.68 (brs, 2H), 7.83 (s, 2H), 7.73 (t, J = 5.5 Hz, 2H), 6.58 (d, J = 8.2 Hz, 2H), 6.47 (brs, 4H), 5.80 (brs, 2H), 5.61 (d, J = 3.6 Hz, 2H), 4.42 (brs, 2H), 3.92 (q, J = 7.4 Hz, 2H), 3.81-3.74 (m, 2H), 3.03-2.92 (m, 5H), 2.29-2.15 (m, 3H), 2.14-2.00 (m, 3H), 1.98-1.87 (m, 2H), 1.83-1.66 (m, 3H), 1.40 (s, 18H), 1.35-1.28 (m, 4H); **^{13}C NMR (DMSO- d_6 , 100 MHz)**: δ 171.4, 156.8, 155.3, 153.7, 151.0, 135.3, 116.8, 88.2, 80.7, 79.1, 78.1, 72.5, 55.5, 38.4, 31.7, 29.2, 29.0, 28.2, 26.1; **HRMS (ESI)**: calcd for $\text{C}_{40}\text{H}_{60}\text{N}_{14}\text{O}_{12}\text{Na}$ $[\text{M}+\text{Na}]^+$ 951.4413, found 951.4415.

Synthesis of compound 16:

Ph_3P (38 mg, 0.145 mmol) was added to a solution of compound **10** (38 mg, 0.072 mmol) in dry methanol (3 mL) at room temperature under inert atmosphere. After stirring the reaction mixture for 30 minutes, the reaction mixture was concentrated under vacuum to furnish the amine compound **13** which was used for the next step without purification.

Boc-11-Aminoundecanoic acid (21.7 mg, 0.0725 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C. Then it was sequentially treated with HOBt (10.7 mg, 0.0792 mmol) and HBTU

(32.8 mg, 0.086 mmol). After 15 min., compound **13**, prepared above, dissolved in CH₂Cl₂ (1 mL) was added to the reaction mixture followed by DIPEA (0.06 mL, 0.36 mmol). After stirring for 12 h at room temperature, the reaction mixture was quenched with saturated NH₄Cl solution, extracted with ethyl acetate, washed with 1(N) HCl, NaHCO₃, water, brine, dried (Na₂SO₄), filtered and concentrated in *vacuo*. purification by column chromatography (silica gel, 6% methanol in chloroform) afforded compound **16** (40 mg, 75%) as white foam.

Rf: 0.5 (Silica gel, 10 % methanol in CHCl₃); **IR (neat)**: $\nu_{\max}$ 3297, 2926, 2854, 2723, 2562, 1681, 1610, 1561, 1365, 1253, 1165 cm⁻¹; **¹H NMR (CDCl₃, 400 MHz)**: δ 12.18 (brs, 1H), 10.78 (brs, 1H), 7.62 (s, 1H), 7.36-7.27 (m, 5H), 6.68 (d, J = 4.5 Hz, 1H), 6.65-6.51 (m, 1H), 5.75 (d, J = 3.9 Hz, 1H), 5.09 (d, J = 3.8 Hz, 2H), 5.04-4.98 (m, 1H), 4.63-4.51 (m, 2H), 4.20-4.11 (m, 1H), 3.13-3.02 (m, 2H), 2.69-2.59 (m, 1H), 2.56-2.46 (m, 2H), 2.28 (s, 3H), 2.24 (t, J = 7.6 Hz, 2H), 2.20-2.10 (m, 1H), 1.68-1.57 (m, 2H), 1.43 (s, 11H), 1.25 (s, 12H); **¹³C NMR (CDCl₃, 100 MHz)**: δ 174.8, 174.7, 173.5, 156.2, 155.7, 147.9, 147.7, 139.4, 135.8, 128.7, 128.4, 128.1, 121.4, 92.2, 83.0, 79.2, 72.6, 66.7, 54.8, 40.8, 36.7, 32.1, 30.5, 30.2, 29.6, 29.4, 29.3, 28.9, 28.6, 26.9, 25.7, 24.2, 22.8; **HRMS (ESI)**: calcd for C₃₇H₅₃N₇O₉Na [M+Na]⁺ 762.3802, found 762.3805.

Synthesis of compound 7:

TFA (0.4 mL) was added to a solution of compound **16** (11 mg, 0.015 mmol) in dry CH₂Cl₂ at 0 °C under inert atmosphere. The reaction mixture was stirred at room temperature for 30 min and concentrated in *vacuo* to give TFA salt **17** which were used for the next reaction without further purification.

Compound **12** (6.9 mg, 0.015 mmol) was dissolved in CH₂Cl₂ (2 mL) and dry DMF (2 mL) and cooled to 0 °C. Then it was sequentially treated with HOBt (2.2 mg, 0.0165 mmol) and HBTU (6.8 mg, 0.018 mmol). After 15 min., compound **17**, prepared above, dissolved in

CH₂Cl₂ (1 mL) and dry DMF (1 mL) and was added to the reaction mixture followed by DIPEA (0.02 mL, 0.075 mmol). After stirring for 12 h at room temperature, the reaction mixture was quenched with saturated NH₄Cl solution, extracted with ethyl acetate, washed with 1(N) HCl, NaHCO₃, water, brine, dried (Na₂SO₄), filtered and concentrated in *vacuo*. purification by column chromatography (silica gel, 10% methanol in chloroform) afforded protected dimer compound as white foam which was used for the next reaction without further purification.

NH₃ saturated methanol (1.0 mL) was added to the above prepared protected dimer compound at 0 °C and stirred at room temperature for 12h. After completion of the reaction (TLC), the reaction mixture was concentrated in *vacuo* and the crude reaction mixture was purified by reverse phase HPLC to furnish compound **7** (7.0 mg, 50% over 2 steps).

RP-HPLC, *t_R* = 35.95 mins (Initial 5 mins, 5% CH₃CN and then linear gradient 5 - 60% CH₃CN in 40 mins); **IR (neat)**: *v*_{max} 2961, 2923, 2853, 2364, 2329, 1744, 1695, 1607, 1460, 1367, 1172, 1018 cm⁻¹; **¹H NMR (DMSO-*d*₆, 400 MHz)**: δ 10.73 (brs, 2H), 7.84 (d, *J* = 3.0 Hz, 2H), 7.81-7.73 (m, 2H), 6.61 (d, *J* = 8.4 Hz, 1H), 6.55-6.43 (m, 4H), 5.91 (brs, 1H), 5.82 (brs, 1H), 5.66 (d, *J* = 3.2 Hz, 1H), 5.61 (d, *J* = 3.6 Hz, 1H), 4.45-4.37 (m, 2H), 4.31-4.23 (m, 1H), 3.95-3.88 (m, 1H), 3.84-3.74 (m, 2H), 3.54 (s, 3H), 3.02-2.94 (m, 2H), 2.45-2.29 (m, 3H), 2.25-2.02 (m, 5H), 1.98-1.68 (m, 5H), 1.54-1.44 (m, 3H), 1.40 (s, 9H), 1.38-1.29 (m, 4H), 1.23 (s, 6H); **¹³C NMR (DMSO-*d*₆, 100 MHz)**: δ 172.9, 172.6, 171.4, 156.8, 153.8, 151.0, 150.9, 135.3, 116.8, 88.4, 88.2, 80.7, 80.3, 78.1, 72.7, 72.6, 55.5, 53.8, 51.2, 38.4, 35.3, 31.8, 29.6, 29.1, 29.0, 28.9, 28.8, 28.7, 28.6, 28.2, 26.4, 25.2; **HRMS (ESI)**: calcd for C₄₁H₆₁N₁₃O₁₂Na [M+Na]⁺ 950.4460, found 950.4462.

3. Spectra (^1H and ^{13}C NMR) of Compounds:

^1H NMR spectrum (CDCl_3 , 400 MHz) of compound **10**

^{13}C NMR spectrum (CDCl_3 , 100 MHz) of compound **10**

¹H NMR spectrum (DMSO-*d*₆, 400 MHz) of compound **3**

¹³C NMR spectrum (DMSO-*d*₆, 100 MHz) of compound **3**

Chemical structure of compound 10 is shown above the ¹H NMR spectrum. The structure is a bicyclic molecule with a benzodioxane core, a Boc-protected amine, a hydroxyl group, and a pyrimidine ring substituted with an acetamido group and a benzoyl group.

¹H NMR spectrum (CDCl₃) of compound 10. The spectrum shows peaks corresponding to the structure, with chemical shifts (ppm) labeled above the peaks:

- 12.084
- 10.596
- 7.584
- 7.319
- 7.300
- 6.674
- 5.693
- 5.151
- 5.101
- 4.186
- 2.688
- 2.661
- 2.636
- 2.623
- 2.546
- 2.533
- 2.520
- 2.507
- 2.483
- 2.470
- 2.344
- 2.290
- 2.162
- 2.155
- 2.081

Integration values are shown below the baseline:

- 0.960
- 0.871
- 0.988
- 4.856
- 0.745
- 1.983
- 0.907
- 2.100
- 1.909
- 1.326
- 1.179
- 0.879
- 3.328
- 1.133
- 9.000

CC(=O)Nc1nc2nc(NC(=O)O[C@H]3[C@@H](CO[C@H]3C(=O)OCC(=O)OCC4=CC=CC=C4)nc2n1

174.661
 173.696
 156.658
 155.496
 147.842
 147.645
 139.442
 135.777
 128.707
 128.433
 128.085
 121.216
 92.189
 82.994
 80.311
 72.337
 66.683
 55.858
 50.632
 30.585
 28.934
 28.456
 24.186

¹H-NMR spectrum (DMSO-*d*₆, 400 MHz) of compound **12**

¹³C-NMR spectrum (DMSO-*d*₆, 100 MHz) of compound **12**

¹H NMR spectrum (DMSO-*d*₆, 400 MHz) of compound **4**

¹³C NMR spectrum (DMSO-*d*₆, 100 MHz) of compound **4**

¹H-NMR spectrum (DMSO-*d*₆, 400 MHz) of compound **14**

¹³C-NMR spectrum (DMSO-*d*₆, 100 MHz) of compound **14**

¹H-NMR spectrum (DMSO-*d*₆, 400 MHz) of compound **5**

¹³C-NMR spectrum (DMSO-*d*₆, 100 MHz) of compound **5**

¹H-NMR spectrum (DMSO-*d*₆, 400 MHz) of compound **6**

¹³C-NMR spectrum (DMSO-*d*₆, 100 MHz) of compound **6**

¹H NMR spectrum (CDCl₃, 400 MHz) of compound **16**

¹³C NMR spectrum (CDCl₃, 100 MHz) of compound **16**

¹H NMR spectrum (DMSO-*d*₆, 400 MHz) of compound **7**

¹³C NMR spectrum (DMSO-*d*₆, 100 MHz) of compound **7**

4. RP-HPLC Chromatograms:

HPLC trace of compound **3**

HPLC trace of compound **4**

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	22.270	8738047	98.813
2	22.491	104970	1.187
Total		8843017	100.000

HPLC trace of compound 5

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	22.563	194231	0.595
2	23.017	31221034	95.578
3	23.404	1250088	3.827
Total		32665353	100.000

HPLC trace of compound 6

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	32.581	46083336	98.486
2	33.133	165524	0.354
3	40.378	542982	1.160
Total		46791842	100.000

HPLC trace of compound 7

PDA Ch1 254nm

Peak#	Ret. Time	Area	Area%
1	35.269	499526	1.850
2	35.952	26370098	97.653
3	36.457	134285	0.497
Total		27003909	100.000

5. CD study of modified guanosine monomer 3 and dimers (4-7).

Boltzmann fit of the fraction folded vs. temperature curve to determine the melting temperature (T_m) from variable temperature CD study of compound 4.

Model		Boltzmann	
Equation		$y = A2 + (A1 - A2) / (1 + \exp((x - x0)/dx))$	
Adj.R-square		0.96842	
		Value	Standard Error
Theta	A1	1.11081	0.21517
Theta	A2	-0.10749	0.09845
Theta	x0	39.4741	3.53773
Theta	dx	12.24173	3.85745

CD spectrum of 200 μM of compound **3** in presence of 500 mM KCl and 10 mM tris-HCl buffer

CD spectrum of 200 μM of compound **5** in presence of 500 mM KCl and 10 mM tris-HCl buffer

CD spectrum of 200 μM of compound **6** in presence of 500 mM KCl and 10 mM tris-HCl buffer

Effect of salt on the CD spectrum of 500 μM compound **4** in the presence of 10 mM tris-HCl buffer

Concentration-dependent CD study of compound **4** with compound **3** as control in the presence of 500 mM KCl and 10 mM tris-HCl buffer

CD spectrum of 400 μ M of compound **3** in presence of 500 mM NaCl and 10 mM tris-HCl buffer

CD spectrum of 500 μ M of compound **4** in presence of 500 mM NaCl and 10 mM tris-HCl buffer

CD spectrum of 400 μ M of compound **5** in presence of 500 mM NaCl and 10 mM tris-HCl buffer

CD spectrum of 200 μ M of compound **6** in presence of 500 mM NaCl and 10 mM tris-HCl buffer

CD spectrum of 200 μ M of compound **7** in presence of 500 mM NaCl and 10 mM tris-HCl buffer