Methods S3

DNA base analogues for DNA cleavage
We found that the DNA base analogues described below can cause the same DNA cleavage as that induced by EU. Their synthetic schemes are shown following their respective synthetic procedures.

![Chemical structures of T1, C1, C2, A1, and G1]

Preparation of $T_6(T1)T_6$
$T_6(T1)T_6$ was postsynthetically prepared from the DNA oligonucleotide containing 5-ethynyluracil (PU) [17]. AgNO$_3$aq (100 mM, 200 μL) was added to $T_6(\text{PU})T_6$ aqueous solution (1 mM strand concentration, 200 μL) [18]. The solution was mixed and left at 25°C for 16 hours. After addition of NaOHaq (10 M, 20 μL) [19], the solution was mixed and left at 25°C for 2 hours. H$_2$O (600 μL) containing acetic acid (20 μL) was added to the solution. The solution was filtered with 0.45 nm filter. The product was purified by reversed-phase HPLC with a linear gradient over 20 minutes from 8 to 20% CH$_3$CN/50 mM AF. The retention time of the product was 12.5 minutes. After desalination, the product was identified by MALDI TOF mass spectrometry: $T_6(T1)T_6$, calcd for $C_{137}H_{173}N_{26}O_{90}P_{12}$ 3995.6 [M−H]$^{-}$; found 3995.6.
6-N-(3-propynyl)-5-methyl-2'-deoxycytidine (1)

Thymidine (1.21 g, 5.00 mmol) was suspended in CH$_3$CN (20 mL). Triethylamine (5.58 mL, 40.0 mmol) and acetic anhydride (1.89 mL, 20.0 mmol) were added to the suspension. After stirring at 25°C for 21 hours, methanol (10 mL) was added to the solution and stirred for 2 hours. The solvent was removed by evaporation. AcOEt (100 mL) and saturated NaClaq (100 mL) were added to the residue and the product was extracted to the organic layer. The organic layer was washed with saturated NaClaq (100 mL) twice, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation and the residue was dried under reduced pressure. 1,2,4-triazole (3.45 g, 50 mmol), CH$_3$CN (30 mL), and triethylamine (7.67 mL, 55.0 mmol) were added to the residue. POCl$_3$ (932 μL, 10.0 mmol) was slowly added to the solution under constant stirring. After stirring for 1 hour, the solvent was removed by evaporation. AcOEt (100 mmL) and saturated NaClaq (100 mL) were added to the residue and the product was extracted to the organic layer. The organic layer was washed with saturated NaClaq (100 mL) twice, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation and the residue was dried under reduced pressure.
1,4-dioxane (30 mL) was added to the residue and the solution was stirred. Propargylamine (961 μL, 15.0 mmol) was added to the solution. After stirring at 25°C for 2 hours, methanol (30 mL) and 28% NH₃aq (30 mL) were added to the solution. The solution was stirred at 25°C for 24 hours. The solvent was removed by evaporation and the product 1 was purified by silica gel column chromatography (10–15% MeOH/CH₂Cl₂). The product 1 was obtained as a white powder (873 mg, 3.13 mmol, 63%): ¹H NMR (DMSO-d₆) δ 7.65 (d, J = 0.9, 1H), 7.57 (t, J = 5.7, 1H), 5.19 (d, J = 4.6, 1H), 5.01 (t, J = 5.3, 1H), 4.23-4.18 (m, 1H), 4.14-4.01 (m, 2H), 3.76-3.74 (m, 1H), 3.62-3.50 (m, 2H), 3.04 (t, J = 2.5, 1H), 2.12-2.05 (m, 1H), 1.99-1.92 (m, 1H), 1.84 (s, 3H); ¹³C NMR (DMSO-d₆) δ 162.4, 154.9, 138.0, 101.7, 87.2, 84.8, 81.5, 72.6, 70.4, 61.4, 40.3, 29.4, 13.0; HRMS (ESI) calcd for C₁₃H₁₇N₃O₄Na ([M+Na]⁺) 302.1116, found 302.1117.

5'-O-dimethoxytrityl-6-N-(3-propynyl)-5-methyl-2'-deoxycytidine (2)
Pyridine (10 mL) was added to 1 (312 mg, 1.12 mmol) and DMTrCl (454 mg, 1.34 mmol). The solution was stirred at 25°C for 1 hour. After addition of methanol (10 mL), the solvent was removed by evaporation. The residual pyridine was removed by coevaporation with CH₂Cl₂ and hexane. The product 2 was purified by silica gel column chromatography (2–5% MeOH, 2% Et₃N/CH₂Cl₂). The product 2 was obtained as a white powder (631 mg, 0.924 mmol, 83%): ¹H NMR (DMSO-d₆) δ 7.63 (t, J = 5.7, 1H), 7.51 (s, 1H), 7.39-7.35 (m, 2H), 6.90-6.86 (m, 4H), 6.21 (t, J = 6.6, 1H), 5.34 (d, J = 4.1, 1H), 4.31-4.25 (m, 1H), 4.14-4.02 (m, 2H), 3.90-3.86 (m, 1H), 3.72 (s, 6H), 3.22-3.16 (m, 2H), 3.09-3.00 (m, 6H), 2.21-2.06 (m, 2H), 1.50 (s, 3H), 1.23-1.15 (m, 9H); ¹³C NMR (DMSO-d₆) δ 162.3, 158.1, 157.9, 154.8, 144.7, 137.4, 135.5, 135.3, 130.0, 127.9, 127.7, 126.8, 113.2, 113.0, 101.9, 85.8, 85.4, 84.6, 81.4, 72.6, 70.5, 63.6, 55.0, 45.3, 29.4, 8.4; HRMS (ESI): calcd for C₃₄H₃₅N₃O₆Na ([M+Na]⁺) 604.2525, found 604.2424.

Preparation of T₆(C1)T₆
2 (291 mg, 0.426 mmol) was dissolved in CH₃CN (5 mL). Disopropylethylamine (261 μL, 1.50 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (223 μL, 1.00 mmol) was added to the solution. The solution was stirred at 25°C for 30 minutes. AcOEt (25 mL) and saturated NaClaq (25 mL) were added to the suspension. The product was extracted to the organic layer. The organic layer was washed with saturated NaClaq (25 mL) twice, dried over magnesium sulfate,
and filtered. The solvent was removed by evaporation and the residue was dried by coevaporation with acetonitrile and further dried under reduced pressure. After dissolving the residue to CH$_3$CN (10 mL), the phosphoramidite solution was used directly for automated DNA synthesis. T$_6$(C1)T$_6$ was synthesized by standard phosphoramidite method on the DNA synthesizer. The protected oligonucleotide was cleaved by 28% NH$_3$aq from the solid support and deprotected at 25°C for 16 hours in 28% NH$_3$aq. After removal of ammonia by speed-vac, the aqueous solution was filtered through 0.45 μm filter. T$_6$(C1)T$_6$ was purified by reversed-phase HPLC with a linear gradient over 20 minutes from 0 to 50% CH$_3$CN/50 mM AF. The retention time of the product was 10.6 minutes. After the desalination, the product was identified by MALDI TOF mass spectrometry: T$_6$(C1)T$_6$, calcd for C$_{133}$H$_{172}$N$_{27}$O$_{88}$P$_{12}$ 3928.6 [M–H]$^-$; found 3928.8.
4-N-[N,N-di(n-butyl)aminomethylene]-5-phenylethynyl-2′-deoxycytidine (3)

5-phenylethynyl-2′-deoxycytidine (708 mg, 2.16 mmol) [21] was dissolved in methanol (10 mL). N,N-di(n-butyl)formamide dimethyl acetal (2.0 mL, 8.6 mmol) [22] was added to the solution. The solution was stirred at 25°C for 2 hours. The solvent was removed by evaporation. The product 3 was purified by silica gel column chromatography (0–10% MeOH/CH$_2$Cl$_2$). The product 3 was obtained as
a pale brown oil (700 mg, 1.50 mmol, 69%): \(^1\)H NMR (DMSO-d6) \(\delta\) 8.64 (s, 1H), 8.44 (s, 1H), 7.43-7.34 (m, 5H), 6.12 (t, \(J = 6.2\), 1H), 5.23 (d, \(J = 4.1\), 1H), 5.13 (t, \(J = 4.8\), 1H), 4.26-4.21 (m, 1H), 3.84-3.81 (m, 1H), 3.70-3.44 (m, 6H), 2.26-2.20 (m, 1H), 2.08-2.01 (m, 1H), 1.66-1.52 (m, 4H), 1.32-1.22 (m, 4H), 0.90 (t, \(J = 7.3\), 3H), 0.82 (t, \(J = 7.3\), 3H); \(^{13}\)C NMR (DMSO-d6) \(\delta\) 169.7, 157.4, 153.4, 145.3, 130.7, 128.6, 128.1, 123.2, 97.5, 91.6, 87.6, 85.8, 84.5, 69.7, 60.7, 54.9, 51.4, 45.4, 41.0, 30.4, 28.5, 19.6, 19.1, 13.5; HRMS (ESI) calcd for \(C_{26}H_{35}N_4O_4\) ([M+H]+) 467.2658, found 467.2653.

5'-O-dimethoxytrityl-4-N-[N,N-di(n-butyl)aminomethylene]-5-phenylethynyl-2'-deoxycytidine (4)

Pyridine (10 mL) was added to 3 (700 mg, 1.50 mmol) and DMTrCl (763 mg, 2.25 mmol). The solution was stirred at 25°C for 1 hour. After addition of methanol (10 mL), the solvent was removed by evaporation. The residual pyridine was removed by coevaporation with CH\(_2\)Cl\(_2\) and hexane. The product 4 was purified by silica gel column chromatography (0–5% MeOH, 2% Et\(_3\)N/CH\(_2\)Cl\(_2\)). The product 4 was obtained as white powder (924 mg, 1.20 mmol, 80%): \(^1\)H NMR (DMSO-d6) \(\delta\) 8.65 (s, 1H), 8.17 (s, 1H), 7.44-6.81 (m, 18H), 6.14 (t, \(J = 6.6\), 1H), 5.32 (d, \(J = 4.6\), 1H), 4.31-4.26 (m, 1H), 4.02-3.97 (m, 1H), 3.644 (s, 3H), 3.637 (s, 3H), 3.57-3.49 (m, 4H), 3.23-3.16 (m, 2H), 2.36-2.30 (m, 1H), 2.17-2.10 (m, 1H), 1.66-1.53 (m, 4H), 1.32-1.22 (m, 4H), 0.91 (t, \(J = 7.3\), 3H), 0.82 (t, \(J = 7.3\), 3H); \(^{13}\)C NMR(DMSO-d6) \(\delta\) 169.7, 158.03, 158.01, 157.5, 153.4, 144.7, 144.3, 135.6, 135.4, 130.6, 129.7, 129.6, 128.3, 127.93, 127.86, 127.6, 126.6, 123.0, 113.2, 97.9, 91.7, 86.2, 85.9, 83.9, 70.6, 63.5, 54.9, 54.5, 45.4, 41.2, 30.4, 28.5, 19.6, 19.1, 13.5; HRMS (ESI): calcd for \(C_{47}H_{53}N_4O_6\) ([M+H]+) 769.3965, found 769.3985.

**Preparation of \(T_6(C2)T_6\)**

4 (109 mg, 0.142 mmol) was dissolved in CH\(_3\)CN (5 mL). Triethylamine (157 \(\mu\)L, 1.13 mmol) and 2-cyanoethyl \(N,N\)-disopropylchlorophosphoramidite (126 \(\mu\)L, 0.565 mmol) was added to the solution. The solution was stirred at 25°C for 30 minutes. AcOEt (25 mL) and saturated NaClaq (25 mL) were added to the suspension. The product was extracted to the organic layer. The organic layer was washed with saturated NaClaq (25 mL) twice, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation and the residue was dried by coevaporation with acetonitrile and further dried under reduced pressure.
After dissolving the residue to CH$_3$CN (4.7 mL), the phosphoramidite solution was used directly for automated DNA synthesis. T$_6$(C2)T$_6$ was synthesized by the standard phosphoramidite method on the DNA synthesizer. The last DMTr group was not deprotected on the DNA synthesizer. The protected oligonucleotide was cleaved by 28% NH$_3$aq from the solid support and deprotected at 25°C for 16 hours in 28% NH$_3$aq. After removal of ammonia by speed-vac, the aqueous solution was filtered through a 0.45 μm filter. 5'-DMTr-T$_6$(C2)T$_6$ was purified by reversed-phase HPLC with a linear gradient over 20 minutes from 5 to 50% CH$_3$CN/50 mM AF. The retention time of the 5'-DMTr-T$_6$(C2)T$_6$ was 15.2 minutes. After speed-vac and freeze-drying to remove the solvent and AF, the DMTr group was removed at 25°C for 30 minutes in 80% AcOHaq (100 μL). The solution was diluted with H$_2$O to be ≈1 mL and passed through a 0.45 μm filter. T$_6$(C2)T$_6$ was purified by reversed-phase HPLC with a linear gradient over 20 minutes from 8 to 20% CH$_3$CN/50 mM AF. The retention time of the product was 13.6 minutes. After the desalination, the product was identified by MALDI TOF mass spectrometry: T$_6$(C2)T$_6$, calcd for C$_{137}$H$_{172}$N$_{27}$O$_{88}$P$_{12}$ 3976.6 [M−H]$^-$; found 3977.1.
8-(2-formylphenyl)-2'-deoxyadenosine (5)
8-bromo-2'-deoxyadenosine (2.20 g, 6.66 mmol) [23], 2-formylbenzene boronic acid (2.00 g, 13.3 mmol), palladium (II) acetate (150 mg, 0.666 mmol), triphenylphosphine (525 mg, 2.00 mmol), and potassium carbonate (1.84 g, 13.3 mmol) were stirred in acetonitrile (40 mL) and H₂O (20 mL) at 80°C for 4 hours [24,25]. The solvent was removed by evaporation. Methanol (200 mL) was added to the residue. The resulting solution was filtered. The solvent was removed by evaporation. The product 5 was purified by silica gel column chromatography (10% MeOH/CH₂Cl₂). The product 5 was obtained as white powder (1.81 g, 5.09 mmol, 76%): ¹H NMR (DMSO-d₆) δ 9.93 (s, 1H), 8.18 (s,
1H), 8.09-8.07 (m, 1H), 7.90-7.80 (m, 2H), 7.74-7.72 (m, 1H), 7.46 (br, 2H), 5.90 (dd, $J = 8.5$, 6.2, 1H), 5.49 (dd, $J = 8.3$, 4.1, 1H), 5.17 (d, $J = 4.1$, 1H), 4.39-4.35 (m, 1H), 3.82-3.79 (m, 1H), 3.65-3.59 (m, 1H), 3.50-3.44 (m, 1H), 3.19 (ddd, $J = 12.8$, 8.7, 6.0, 1H), 2.06 (ddd, $J = 13.0$, 6.2, 1.8, 1H); $^{13}$C NMR (DMSO-d$_6$) $\delta$ 191.7, 156.2, 152.3, 149.6, 147.4, 135.4, 133.8, 131.5, 131.4, 130.8, 129.5, 119.3, 88.3, 85.7, 71.3, 62.2, 37.4; HRMS (ESI) calcd for C$_{17}$H$_{18}$N$_5$O$_4$ ([M+H]$^+$) 356.1359, found 356.1364.

6-N-[N,N-di(n-butyl)aminomethylene]-8-(2-formylphenyl)-2'-deoxyadenosine (6)

5 (1.55 g, 4.36 mmol) was dissolved in methanol (20 mL). N,N-di(n-butyl)-formamide dimethyl acetal (8.14 mL, 34.8 mmol) was added to the solution [22]. The solution was stirred at 25°C for 2 hours. The solvent was removed by evaporation. The product 6 was purified by silica gel column chromatography (2–5% MeOH CH$_2$Cl$_2$). The product 6 was obtained as a pale brown oil (1.40 g, 2.83 mmol, 65%): $^1$H NMR (DMSO-d$_6$) $\delta$ 9.92 (s, 1H), 8.91 (s, 1H), 8.45 (s, 1H), 8.09 (d, $J = 7.8$, 1H), 7.91-7.82 (m, 2H), 7.74 (d, $J = 7.3$, 1H), 5.94 (dd, $J = 7.8$, 6.8, 1H), 5.28 (dd, $J = 7.6$, 4.4, 1H), 5.18 (d, $J = 4.1$, 1H), 4.40-4.36 (m, 1H), 3.81-3.77 (m, 1H), 3.64-3.41 (m, 6H), 3.25-3.18(m, 1H), 2.09-2.04 (m, 1H), 1.62-1.52 (m, 4H), 1.33-1.22 (m, 4H), 0.94-0.83 (m, 6H); $^{13}$C NMR (DMSO-d$_6$) $\delta$ 191.8, 159.5, 157.9, 151.7, 151.6, 149.3, 135.5, 133.8, 131.5, 131.3, 130.9, 129.7, 125.8, 88.2, 85.5, 71.2, 62.1, 50.9, 44.4, 37.2, 30.4, 28.7, 19.6, 19.1, 13.7, 13.5; HRMS (ESI) calcd for C$_{26}$H$_{35}$N$_6$O$_4$ ([M+H]$^+$) 495.2720, found 495.2718.

5'-O-dimethoxytrityl-6-N-[N,N-di(n-butyl)aminomethylene]-8-(2-formylphenyl)-2'-deoxyadenosine (7)

Pyridine (10 mL) was added to 6 (1.31 g, 2.64 mmol) and DMTrCl (991 mg, 2.92 mmol). The solution was stirred at 25°C for 1 hour. After addition of methanol (10 mL), the solvent was removed by evaporation. The residual pyridine was removed by coevaporation with CH$_2$Cl$_2$ and hexane. The product 7 was purified by silica gel column chromatography (2% MeOH, 2% Et$_3$N/CH$_2$Cl$_2$). The product 7 was obtained as a white powder (1.24 g, 1.56 mmol, 59%): $^1$H NMR (DMSO-d$_6$) $\delta$ 9.89 (s, 1H), 8.88 (s, 1H), 8.25 (s, 1H), 8.06-8.04 (m, 1H), 7.88-7.78 (m, 3H), 7.33-7.31 (m, 2H), 7.21-7.15 (m, 7H), 6.81-6.73 (m, 4H), 5.98 (t, $J = 6.9$, 1H), 5.25 (d, $J = 4.6$, 1H), 4.57-4.53 (m, 1H), 3.94-3.90 (m, 1H), 3.71 (s, 3H), 3.69 (s, 3H), 3.59-3.53 (m, 2H), 3.44-3.32 (m, 3H), 3.20-3.12 (m, 2H),
2.11 (ddd, \( J = 13.0, 7.3, 4.3, 1H \)), 1.62-1.52 (m, 4H), 1.34-1.23 (m, 4H), 0.90-0.87 (m, 6H); 13C NMR(DMSO-d6) \( \delta \) 191.6, 159.2, 157.9, 157.9, 157.7, 151.8, 149.5, 145.0, 135.7, 135.60, 135.55, 133.6, 131.7, 131.4, 130.7, 129.7, 129.5, 129.1, 127.6, 126.5, 125.6, 113.0, 112.9, 85.8, 85.2, 84.8, 71.0, 63.6, 54.94, 54.90, 50.9, 44.4, 36.3, 30.4, 28.7, 19.6, 19.1, 13.7, 13.5; HRMS (ESI): calcd for C\(_{47}\)H\(_{53}\)N\(_6\)O\(_6\) ([M+H]\(^+\)) 797.4027, found 797.4046.

**Preparation of T\(_6\)(A1)T\(_6\)**

7 (159 mg, 0.200 mmol) was dissolved in CH\(_3\)CN (5 mL). Triethylamine (223 \( \mu \)L, 1.60 mmol) and 2-cyanoethyl \( N,N \)-diisopropylchlorophosphoramidite (178 \( \mu \)L, 0.800 mmol) were added to the solution. The solution was stirred at 25°C for 30 minutes. AcOEt (25 mL) and saturated NaCl aq (25 mL) were added to the suspension. The product was extracted to the organic layer. The organic layer was washed with saturated NaCl aq (25 mL) twice, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation and the residue was dried by coevaporation with acetonitrile and further dried under reduced pressure. After dissolving the residue in CH\(_3\)CN (10 mL), the phosphoramidite solution was used directly for automated DNA synthesis. T\(_6\)(A1)T\(_6\) was synthesized by the standard phosphoramidite method on the DNA synthesizer. The last DMTr group was not deprotected on the DNA synthesizer. The protected oligonucleotide was cleaved from the solid support using 28% NH\(_3\) aq and deprotected at 25°C for 16 hours in 28% NH\(_3\) aq. After removal of ammonia by speed-vac, the aqueous solution was filtered through a 0.45 \( \mu \)m filter. 5'-DMTr-T\(_6\)(C2)T\(_6\) was purified by reversed-phase HPLC with a linear gradient over 20 minutes from 0 to 50% CH\(_3\)CN/50 mM AF. The retention time of the 5'-DMTr-T\(_6\)(A1)T\(_6\) was 16.5 minutes. After speed-vac and freeze-drying to remove the solvent and AF, the DMTr group was removed at 25°C for 30 minutes in 80% AcOHaq (100 \( \mu \)L). The solution was diluted with H\(_2\)O to 1 mL and filtered through a 0.45 m filter. T\(_6\)(C2)T\(_6\) was purified by reversed-phase HPLC with a linear gradient over 20 minutes from 5 to 20% CH\(_3\)CN/50 mM AF. The retention time of the product was 14.9 minutes. After the desalination, the product was identified by MALDI TOF mass spectrometry: T\(_6\)(A1)T\(_6\), calcd for C\(_{137}\)H\(_{172}\)N\(_{29}\)O\(_{88}\)P\(_{12}\) 4004.7 [M−H]\(^+\); found 4005.2.
8-(2-formylphenyl)-2'-deoxyguanosine (8)
8-bromo-2'-deoxyguanosine (3.46 g, 10.0 mmol) [26], 2-formylbenzene boronic acid (3.00 g, 20.0 mmol), palladium (II) acetate (224 mg, 1.00 mmol),
triphenylphosphine (787 mg, 3.00 mmol), and potassium carbonate (2.76 g, 20 mmol) were stirred in acetonitrile (80 mL) and H$_2$O (40 mL) at 80°C for 4 hours [24,25]. The solvent was removed by evaporation. Methanol (200 mL) was added to the residue. The resultant solution was filtered. The solvent was removed by evaporation. The product 8 was purified by silica gel column chromatography (10–20% MeOH/CH$_2$Cl$_2$). The product 8 was obtained as a pale yellow powder (2.97 g, 8.00 mmol, 80%): $^1$H NMR (DMSO-d$_6$) $\delta$ 10.80 (br, 1H), 9.85 (s, 1H), 9.78 (dd, $J$ = 7.8, 1.4, 1H), 7.84-7.80 (m, 1H), 7.75-7.67 (m, 2H), 6.47 (br, 2H), 5.92 (dd, $J$ = 8.2, 6.4, 1H), 5.10 (d, $J$ = 4.1 Hz, 1H), 4.89-4.86 (m, 1H), 4.21-4.18 (m, 1H), 3.72-3.69 (m, 1H), 3.49-3.38 (m, 2H), 2.98 (ddd, $J$ = 13.2, 8.2, 5.9, 1H), 4.20 (ddd, $J$ = 13.3, 6.4, 2.3, 1H); $^{13}$C NMR (DMSO-d$_6$) $\delta$ 191.7, 156.5, 153.3, 151.8, 143.4, 135.5, 133.5, 132.5, 131.4, 130.1, 128.4, 117.5, 87.8, 84.6, 71.0, 61.9, 36.8; HRMS (ESI) calcd for C$_{17}$H$_{17}$N$_5$O$_4$Na ([M+Na]$^+$) 394.1127, found 394.1115.

2-N-[N,N-di(n-butyl)aminomethylene]-8-(2-formylphenyl)-2'-deoxyguanosine (9)

8 (2.32 g, 6.25 mmol) was dissolved in methanol (20 mL). N,N-di(n-butyl)-formamide dimethyl acetal (11.7 mL, 50.0 mmol) was added to the solution [22]. The solution was stirred 25°C for 2 hours. The solvent was removed by evaporation. The product 9 was purified by silica gel column chromatography (5–10% MeOH/CH$_2$Cl$_2$). The product 9 was obtained as a pale yellow powder (2.10 g, 4.11 mmol, 66%): $^1$H NMR (DMSO-d$_6$) $\delta$ 11.51 (s, 1H), 9.89 (s, 1H), 8.53 (s, 1H), 8.02-8.00 (m, 1H), 7.87-7.83 (m, 1H), 7.77-7.73 (m, 1H), 7.69-7.67 (m, 1H), 5.92 (dd, $J$ = 7.8, 6.9, 1H), 5.16 (d, $J$ = 4.2, 1H), 4.85-4.82 (m, 1H), 4.33-4.29 (m, 1H), 3.76-3.73 (m, 1H), 3.58-3.35 (m, 6H), 3.14 (ddd, $J$ = 13.7, 7.3, 6.4, 1H), 2.05 (ddd, $J$ = 13.3, 6.4, 2.8, 1H), 1.63-1.54 (m, 4H), 1.35-1.23 (m, 4H), 0.94-0.89 (m, 6H); $^{13}$C NMR (DMSO-d$_6$) $\delta$ 191.7, 157.8, 157.4, 157.3, 150.5, 144.6, 135.5, 133.6, 132.1, 131.3, 130.2, 128.7, 120.5, 87.8, 85.0, 71.0, 62.0, 51.2, 44.9, 37.3, 30.3, 28.5, 19.6, 19.1, 13.7, 13.5; HRMS (ESI) calcd for C$_{26}$H$_{34}$N$_6$O$_5$Na ([M+Na]$^+$) 533.2488, found 533.2481.

5'-O-dimethoxytrityl-2-N-[N,N-di(n-butyl)aminomethylene]-8-(2-formylphenyl)-2'-deoxyguanosine (10)

Pyridine (16 mL) was added to 9 (2.10 g, 4.11 mmol) and DMTriCl (1.53 g, 4.52 mmol). The solution was stirred at 25°C for 1 hour. After addition of methanol (10 mL), the solvent was removed by evaporation. The residual pyridine was
removed by coevaporation with CH$_2$Cl$_2$ and hexane. The product 10 was purified by silica gel column chromatography (80–98% AcOEt, 2% Et$_3$N/n-hexane). The product 10 was obtained as a pale yellow powder (2.20 g, 2.71 mmol, 66%): ¹H NMR (DMSO-d$_6$) δ 11.52 (s, 1H), 9.88 (s, 1H), 8.38 (s, 1H), 7.98 (d, J = 7.8 Hz, 1H), 7.80-7.79 (m, 2H), 7.74-7.70 (m, 1H), 7.31-7.29 (m, 2H), 7.20-7.15 (m, 7H), 6.79-6.72 (m, 4H), 6.00 (dd, J = 8.0 Hz, 4.8 Hz, 1H), 5.19 (d, J = 5.1 Hz, 1H), 4.54-4.48 (m, 1H), 3.84-3.80 (m, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.47-3.43 (m, 2H), 3.31-3.26 (m, 1H), 3.21-3.13 (m, 3H), 3.07-3.04 (m, 1H), 2.17 (ddd, J = 13.7, 8.0, 5.8 Hz, 1H), 1.59-1.51 (m, 2H), 1.45-1.36 (m, 2H), 1.33-1.24 (m, 2H), 1.15-1.09 (m, 2H), 0.93-0.89 (m, 3H), 0.79-0.75 (m, 3H); ¹³C NMR(DMSO-d$_6$) δ 191.6, 158.0, 157.9, 157.5, 157.3, 156.9, 150.3, 144.9, 144.8, 135.7, 135.6, 133.4, 132.5, 131.2, 130.1, 129.6, 129.4, 128.3, 127.6, 126.5, 120.4, 112.9, 85.4, 85.2, 84.0, 70.6, 63.6, 55.0, 54.9, 51.2, 45.0, 37.6, 30.2, 28.5, 19.6, 19.0, 13.7, 13.5; HRMS (ESI): calcd for C$_{47}$H$_{53}$N$_6$O$_7$ ([M+H]$^+$) 813.3976, found 813.3976.

**Preparation of T$_6$(G1)T$_6$**

10 (163 mg, 0.200 mmol) was dissolved in CH$_3$CN (5 mL). Triethylamine (223 μL, 1.60 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (178 μL, 0.800 mmol) were added to the solution. The solution was stirred at 25°C for 30 minutes. AcOEt (25 mL) and saturated NaCl aq (25 mL) were added to the suspension. The product was extracted to the organic layer. The organic layer was washed with saturated NaCl aq (25 mL) twice, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation and the residue was dried by coevaporation with acetonitrile and further dried under reduced pressure. After dissolving the residue in CH$_3$CN (10 mL), the phosphoramidite solution was used directly for automated DNA synthesis. T$_6$(G1)T$_6$ was synthesized by standard phosphoramidite method on the DNA synthesizer. The last DMTr group was not deprotected on the DNA synthesizer. The protected oligonucleotide was cleaved by 28% NH$_3$ aq from the solid support and deprotected at 25°C for 16 hours in 28% NH$_3$ aq. After removal of ammonia by speed-vac, the aqueous solution was filtered through a 0.45 μm filter. 5'-DMTr-T$_6$(G1)T$_6$ was purified by reversed-phase HPLC with a linear gradient over 20 minutes from 5 to 50% CH$_3$CN/50 mM AF. The retention time of the 5'-DMTr-T$_6$(G1)T$_6$ was 15.2 minutes. After speed-vac and freeze-drying to remove the solvent and AF, the DMTr group was removed at 25°C for 30 minutes in 80% AcOH aq (100 μL). The solution was diluted with H$_2$O to 1 mL and filtered through 0.45 μm filter. T$_6$(C2)T$_6$
was purified by reversed-phase HPLC with a linear gradient over 20 minutes from 5 to 20% CH₃CN/50 mM AF. The retention time of the product was 14.6 minutes. After desalination, the product was identified by MALDI TOF mass spectrometry: T₆(G1)T₆, calcd for C₁₃₇H₁₇₂N₂₉O₈₉P₁₂ 4020.7 [M−H]⁻; found 4022.0.
DNA cleavage reaction using designed DNA base analogues

DNA cleavage was carried out using an aqueous solution of DNA oligonucleotides (100 μM) in a screw-cap tube. The same volume of 40% methylamine was added to the sample and the resulting solution was heated at 70°C in a water bath. After removal of methylamine by speed-vac, the sample was directly analyzed by reversed-phase HPLC with a linear gradient over 20 min from 8% to 20% CH3CN in 50 mM AF. The cleavage products, P1 and P2 were purified, desalted by the HPLC and identified by MALDI TOF mass spectrometry: P1 (pT6), calcd for C60H79N12O43P6 1843.2 [M−H]−; found 1841.6 (P1(T6(T1)T6)), 1841.8 (P1(T6(C1)T6)), 1842.0 (P1(T6(C2)T6)), 1842.0 (P1(T6(A1)T6)), and 1842.2 (P1(T6(G1)T6)); P2 (T6pR), calcd for C66H90N13O45P6 1971.3 [M−H]−; found 1971.0 (P2(T6(T1)T6)), 1971.4 (P2(T6(C1)T6)), 1971.1 (P2(T6(C2)T6)), 1971.2 (P2(T6(A1)T6)), and 1971.0 (P2(T6(G1)T6)).