Supporting Information

Divergent Synthesis of 5-Substituted Pyrimidine 2’-Deoxynucleosides and Their Incorporation into Oligodeoxynucleotides for the Survey of Uracil DNA Glycosylases

Ai Tran†, Song Zheng†, Dawanna S. White†, Alyson M. Curry†, Yana Cen‡,#,*

†Department of Pharmaceutical Sciences, Albany College of Pharmacy and Health Sciences, Colchester, VT 05446
‡Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23219
#Institute for Structural Biology, Drug Discovery and Development, Virginia Commonwealth University, Richmond, VA 23219

*Correspondence: ceny2@vcu.edu, phone: 804-828-7405
Synthesis of 1

To a solution of 5-ido-2'-deoxyuridine (1 g, 2.82 mmol) in 10 mL of DMF were added TBDMSI (1.275 g, 8.46 mmol) and imidazole (861 mg, 12.66 mmol). The reaction mixture was stirred at r.t. until completion. Solvent was evaporated in vacuo. Residue was redissolved in ethyl acetate, washed with water, sat. NaHCO₃, brine and dried over anhydrous Na₂SO₄. Solvent was removed in vacuo. Crude product was purified by column chromatography (hexanes:ethyl acete 4:1) to afford 1 (1.49 g, 2.56 mmol, 91% yield) as a white foam. ¹H NMR (300 MHz, CDCl₃) δ: 8.21 (s, 1H), 8.07 (s, 1H), 6.25 (t, J = 6.4 Hz, 1H), 4.38 (m, 1H), 3.97 (m, 1H), 3.86 (m, 1H), 3.74 (m, 1H), 2.28 (m, 1H), 1.96 (m, 1H), 0.93 (s, 9H), 0.88 (s, 9H), 0.13 (s, 6H), 0.06 (s, 6H). HRMS (m/z): calculated for C₂₁H₄₁N₂O₅Si₂ (M+H): 583.1515; found: 583.1523.

Synthesis of 2

To a solution of 1 (420 mg, 0.722 mmol) in 3 mL of THF was added NaH (26 mg, 1.083 mmol) at r.t. The solution was stirred for another 30 min before it was cooled down to -78°C and nBuLi (1.35 mL, 2.17 mmol) was added. After 15 min, DMF (316 mg, 4.332 mmol) was then added dropwise. The reaction mixture was kept at -78°C for another hour before it was quenched with the addition of NH₄Cl solution. Organic layer was washed with brine and dried over Na₂SO₄. Solvent was removed in vacuo. Crude product was purified by column chromatography (hexanes:ethyl acete 5:1) to afford 2 (280 mg, 0.579 mmol, 80% yield) as a white foam. ¹H NMR
(300 MHz, CDCl$_3$) $\delta$: 10.18 (s), 9.93 (s), 8.47 (s), 6.16 (t, $J = 6.1$ Hz, 1H), 4.35 (m, 1H), 4.00 (m, 1H), 3.82 (d, $J = 11.4$ Hz, 1H), 3.71 (d, $J = 11.4$ Hz, 1H), 2.39 (m, 1H), 1.98 (m, 1H), 0.82 (s, 18H), 0.017~0.036 (stack, 12H). HRMS ($m$/z): calculated for C$_{22}$H$_{41}$N$_2$O$_6$Si$_2$ (M+H): 485.7475; found: 485.7476.

**Synthesis of 3 (5-formyl-2'-deoxyuridine)**

![Reaction Scheme]

To a solution of 2 (340 mg, 0.70 mmol) in 1 mL of THF was added TBAF (1 M in THF, 1.75 mmol). The reaction was kept at r.t. and monitored by TLC. Upon completion (about 1h), solvent was removed under reduced pressure. Crude product was purified by column chromatography (CH$_2$Cl$_2$ with 8~10% methanol) to afford 3$^2$ (138 mg, 0.54 mmol, 77% yield) as a white solid. $^1$H NMR (300 MHz, DMSO) $\delta$: 9.76 (s, 1H), 8.71 (s, 1H), 6.09 (t, $J = 6.3$ Hz, 1H), 5.27 (br, 1H), 5.11 (br, 1H), 4.23 (dd, $J = 4.2$, 8.9 Hz, 1H), 3.85 (dd, $J = 3.5$, 6.9 Hz, 1H), 3.62 (m, 2H), 2.21 (m, 2H). HRMS ($m$/z): calculated for C$_{10}$H$_{13}$N$_2$O$_6$ (M+H): 257.0768; found: 257.0772.

**Synthesis of 4**

![Reaction Scheme]

Compound 3 (138 mg, 0.54 mmol) was co-evaporated with dry pyridine (3 x 3 mL) and dissolved in dry pyridine (5 mL). 4,4'-Dimethoxytrityl chloride (220 mg, 0.648 mmol) was added
and the reaction mixture was stirred at r.t. for overnight. MeOH (1 mL) was added and the reaction mixture was stirred for an additional 10 min, and then evaporated to dryness. The residue was redissolved in CH₂Cl₂, washed with water, sat. NaHCO₃, and dried over Na₂SO₄. Crude product was purified by column chromatography (CH₂Cl₂ with 2% methanol) to afford 4 (238 mg, 0.43 mmol, 79% yield) as a white foam. ¹H NMR (300 MHz, CDCl₃) δ: 9.65 (s, 1H), 8.42 (s, 1H), 7.24 (m, 2H), 7.22 (m, 7H), 6.80 (dd, J = 0.84, 9 Hz, 4H), 6.15 (t, J = 6.6 Hz, 1H), 4.42 (m, 1H), 4.10 (m, 1H), 3.73 (s, 6H), 3.38 (m, 2H), 2.59 (m, 1H), 2.25 (m, 1H). ¹³C NMR (75 MHz, CDCl₃) δ: 185.3, 161.6, 158.6, 149.3, 145.8, 144.3, 135.4, 135.3, 130.0, 127.9, 127.0, 113.3, 111.1, 87.06, 87.00, 86.8, 72.4, 63.4, 55.2, 41.5. HRMS (m/z): calculated for C₃₁H₃₁N₂O₈ (M+H): 559.2075; found: 559.2078.

Synthesis of 5-fodU-CE phosphoramidite

To a solution of 4 (40 mg, 0.072 mmol) in 1 mL of anhydrous CH₃CN were added diisopropylethylamine (28 mg, 0.215 mmol), tetrazole in CH₃CN (0.24 mL, 0.108 mmol) and 2-cyanoethyl N,N,N',N'-tetraisopropyl phosphorodiamidite (33 mg, 0.108 mmol). The reaction was kept at r.t. and monitored by TLC. Ten minutes later, precipitate appeared. Upon completion, the reaction was quenched with the addition of 0.5 mL of absolute ethanol. Reaction was diluted with ethyl acetate with 1% triethylamine, washed with 5% NaHCO₃, water, brine, and dried over anhydrous Na₂SO₄. Solvent was removed under reduced pressure. Crude product was purified by column chromatography (CH₂Cl₂ with 2% methanol and 1% triethylamine) to afford 5-fodU-CE
phosphoramidite (48 mg, 0.063 mmol, 88% yield) as a white foam. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 9.64 (d, J = 3.34 Hz, 1H), 8.46 (s, 0.53H), 8.42 (s, 0.47H), 7.99 (s, 1H), 7.21 (m, 10H), 6.81 (m, 4H), 6.14 (m, 1H), 5.86 (s, 1H), 4.49 (m, 1H), 4.15 (m, 1H), 3.76 (s, 6H), 3.61 (m, 8H), 2.72 (m, 2H), 2.58 (m, 1H), 2.42 (m, 1H), 1.23 (m, 12H). $^{13}$C NMR (75 MHz, CD$_2$OD) $\delta$: 187.5, 163.7, 160.4, 151.1, 149.0, 146.2, 137.1, 136.9, 131.53, 131.50, 129.4, 129.0, 128.1, 114.4, 112.2, 88.9, 88.8, 88.3, 72.7, 64.8, 55.9, 55.0, 41.7, 26.7, 26.4, 19.4, 19.0. $^{31}$P NMR (120 MHz, CDCl$_3$) $\delta$: 149.2, 148.8. HRMS ($m/z$): calculated for C$_{40}$H$_{48}$N$_4$O$_9$P (M+H): 759.3153; found: 759.3158.

**Synthesis of 5**

To a solution of 1 (0.6 g, 1.03 mmol) in 4 mL of THF was added NaH (37 mg, 1.55 mmol) at r.t. The solution was stirred for another 30 min before it was cooled down to -78°C and nBuLi (1.93 mL, 3.09 mmol) was added. After 15 min, dimethyl carbonate (556 mg, 6.18 mmol) was then added dropwise. The reaction mixture was kept at -78°C for another hour before it was quenched with the addition of NH$_4$Cl solution. Organic layer was washed with brine and dried over Na$_2$SO$_4$. Solvent was removed in vacuo. Crude product was purified by column chromatography (hexanes:ethyl acetate 3:1) to afford 5 (375 mg, 0.73 mmol, 71% yield) as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 9.37 (broad, 1H), 8.48 (s, 1H), 6.15 (t, J = 6.2 Hz, 1H), 4.36 (m, 1H), 4.25 (m, 2H), 3.99 (m, 1H), 3.70 (s, 3H), 2.39 (m, 1H), 2.03 (m, 1H), 0.85 (s, 9H), 0.83 (s, 9H), 0.04 (s, 12H). HRMS ($m/z$): calculated for C$_{23}$H$_{43}$N$_2$O$_7$Si$_2$ (M+H): 515.2603; found: 515.2609.

**Synthesis of 6**
To a solution of 5 (375 mg, 0.73 mmol) in 1 mL of THF was added TBAF (1 M in THF, 1.825 mmol). The reaction was kept at r.t. and monitored by TLC. Upon completion (about 1h), solvent was removed under reduced pressure. Crude product was purified by column chromatography (CH$_2$Cl$_2$ with 8% methanol) to afford 6$^3$ (171 mg, 0.60 mmol, 82% yield) as a white solid. $^1$H NMR (300 MHz, DMSO) $\delta$: 8.81 (s, 1H), 6.08 (t, $J$ = 6.3 Hz, 1H), 5.23 (br, 1H), 5.03 (br, 1H), 4.23 (m, 1H), 3.83 (m, 1H), 3.68 (m, 3H), 3.58 (m, 2H), 2.20 (m, 2H). HRMS ($m/z$): calculated for C$_{11}$H$_{15}$N$_2$O$_7$ (M+H): 287.0874; found: 287.0871.

**Synthesis of 7**

To a solution of 6 (250 mg, 0.874 mmol) in 20 mL of anhydrous pyridine was added 4,4'-dimethoxytrityl chloride (355 mg, 1.049 mmol) under argon atmosphere. The mixture was stirred overnight at room temperature before it was quenched with the addition of 1 mL of methanol. Solvent was evaporated under reduced pressure. The residue was redissolved in CH$_2$Cl$_2$, washed with 5% NaHCO$_3$, water, brine and dried over anhydrous Na$_2$SO$_4$. Solvent was removed \textit{in vacuo}. Crude product was purified by column chromatography (CH$_2$Cl$_2$ with 3% methanol) to afford 7$^4$ (450 mg, 0.765 mmol, 88% yield) as a white foam. $^1$H NMR (300 MHz, CD$_3$COCD$_3$) $\delta$: 8.61 (s,
1H), 7.51 (m, 2H), 7.31 (m, 7H), 6.88 (m, 4H), 6.24 (t, \( J = 6.8 \) Hz, 1H), 4.47 (m, 1H), 4.11 (m, 1H), 3.79 (s, 6H), 3.42 (dd, \( J = 3.3, 10.5 \) Hz, 1H), 3.33 (dd, \( J = 4.3, 10.5 \) Hz, 1H), 3.31 (s, 3H), 2.37 (m, 2H). HRMS \((m/z)\): calculated for C\(_{32}\)H\(_{33}\)N\(_2\)O\(_9\) (M+H): 589.2181; found: 589.2189.

Synthesis of 5-cadU-CE phosphoramidite

To a solution of 7 (80 mg, 0.136 mmol) in 2 mL of anhydrous CH\(_3\)CN were added diisopropylethylamine (53 mg, 0.408 mmol), tetrazole in CH\(_3\)CN (0.45 mL, 0.204 mmol) and 2-cyanoethyl \( N,N,N',N' \)-tetraisopropylphosphordiamidite (61 mg, 0.204 mmol). The reaction was kept at room temperature and monitored by TLC. Ten minutes later, a white precipitate appeared. Upon completion, the reaction was quenched with the addition of 0.5 mL of absolute ethanol. Reaction was diluted with ethyl acetate with 1% triethylamine, washed with 5% NaHCO\(_3\), water, brine, and dried over anhydrous Na\(_2\)SO\(_4\). Solvent was removed under reduced pressure, crude product was purified by column chromatography (CH\(_2\)Cl\(_2\) with 1.5% methanol and 1% triethylamine) to afford 5-cadU-CE phosphoramidite\(^4\) (105 mg, 0.133 mmol, 98% yield) as a white foam. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \): 8.69 (s, 0.52H), 8.65 (s, 0.48H), 7.39 (m, 2H), 7.23 (m, 7H), 6.79 (m, 4H), 6.22 (m, 1H), 4.48 (m, 1H), 4.20 (m, 1H), 3.74 (s, 6H), 3.54 (m, 5H), 3.21 (m, 1H), 3.18 (s, 1.5H), 3.17 (s, 1.5H), 2.72 (m, 2H), 2.57 (m, 1H), 2.22 (m, 1H), 1.23 (m, 12H). \(^{31}\)P NMR (120 MHz, CDCl\(_3\)) \( \delta \): 149.2, 148.6. HRMS \((m/z)\): calculated for C\(_{41}\)H\(_{50}\)N\(_4\)O\(_{10}\)P (M+H): 789.3259; found: 789.3264.
Synthesis of 8

To a solution of 1 (0.4 g, 0.687 mmol) in 3 mL of THF was added NaH (25 mg, 1.03 mmol) at r.t. The solution was stirred for another 30 min before it was cooled down to -78°C and nBuLi (1.29 mL, 2.06 mmol) was added. After 15 min, paraformaldehyde (124 mg, 4.12 mmol) was added. The reaction mixture was then allowed to slowly warm to r.t. and stir overnight before it was quenched with the addition of NH₄Cl solution. Organic layer was washed with brine and dried over Na₂SO₄. Solvent was removed in vacuo. Crude product was purified by column chromatography (hexanes:ethyl acetate 3:1) to afford 8 (287 mg, 0.59 mmol, 86% yield) as a white solid. ¹H NMR (300 MHz, CD₃OD) δ: 7.68 (s, 1H), 6.25 (dd, J = 5.9, 7.9 Hz, 1H), 4.48 (m, 1H), 4.31 (d, J = 0.6 Hz, 2H), 3.92 (m, 1H), 3.80 (m, 2H), 2.20 (m, 1H), 2.15 (m, 1H), 0.93 (s, 9H), 0.92 (s, 9H), 0.12 (s, 12H). ¹³C NMR (75 MHz, CD₃OD) δ: 165.1, 152.3, 138.7, 115.5, 89.4, 86.6, 74.1, 64.4, 58.0, 41.7, 26.7, 26.4, 19.4, 19.0, -4.3, -4.4, -5.05, -5.12. HRMS (m/z): calculated for C₂₂H₂₈N₂O₅Si₂ (M+H): 487.2654; found: 487.2655.

Synthesis of 9

To a solution of 8 (87 mg, 0.18 mmol) in 1 mL of THF was added TBAF (1 M in THF, 0.45 mmol). The reaction was kept at r.t. and monitored by TLC. Upon completion (about 1h), solvent was
removed under reduced pressure. Crude product was purified by column chromatography (CH$_2$Cl$_2$ with 15% methanol) to afford 9\textsuperscript{5} (39 mg, 0.151 mmol, 85% yield) as a white foam. $^1$H NMR (300 MHz, CD$_3$OD) $\delta$: 7.96 (s, 1H), 6.29 (t, $J = 7.0$ Hz, 1H), 4.40 (m, 1H), 4.32 (s, 2H), 3.91 (m, 1H), 3.75 (m, 2H), 2.25 (m, 2H). $^{13}$C NMR (75 MHz, CD$_3$OD) $\delta$: 165.3, 152.4, 139.6, 115.4, 89.0, 86.6, 72.4, 63.0, 58.1, 41.4. HRMS ($m/z$): calculated for C$_{10}$H$_{15}$N$_2$O$_6$ (M+H): 259.0925; found: 259.0928.

**Synthesis of 5-hmdU-CE phosphoramidite**

5-hmdU-CE phosphoramidite was synthesized as described previously.$^5$

**Synthesis of 10**

To a solution of 1 (1.49 g, 2.56 mmol) in 10 mL of THF was added NaH (92 mg, 3.84 mmol) at r.t. The solution was stirred for another 30 min before it was cooled down to -78°C and nBuLi (4.8 mL, 7.68 mmol) was added. After 15 min, methyl iodide (1.86 g, 12.8 mmol) was then added dropwise. The reaction mixture was kept at -78°C for another hour before it was quenched with the addition of NH$_4$Cl solution. Organic layer was washed with brine and dried over Na$_2$SO$_4$. Solvent was removed in vacuo. Crude product was purified by column chromatography (hexanes:ethyl acetate 3:1) to afford 10\textsuperscript{6} (1.12, 2.38 mmol, 93% yield) as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 8.03 (s, 1H), 7.45 (s, 1H), 6.31 (t, $J = 6.3$ Hz, 1H), 4.39 (m, 1H), 3.92 (m, 1H), 3.85 (d, $J = 11.6$ Hz, 1H), 3.74 (d, $J = 11.6$ Hz, 1H), 2.23 (m, 1H), 1.99 (m, 1H), 1.75 (s, 3H), 0.91 (s, 9H), 0.88 (s, 9H), 0.09 (s, 6H), 0.06 (s, 6H). HRMS ($m/z$): calculated for C$_{22}$H$_{43}$N$_2$O$_5$Si$_2$
Synthesis of 11

To a solution of 10 (638 mg, 1.36 mmol) in 2 mL of THF was added TBAF (1 M in THF, 3.4 mmol). The reaction was kept at r.t. and monitored by TLC. Upon completion (about 1h), solvent was removed under reduced pressure. Crude product was purified by column chromatography (CH₂Cl₂ with 8~10% methanol) to afford 11 (303 mg, 1.25 mmol, 92% yield) as a white foam. ¹H NMR (300 MHz, CD₃OD) δ: 7.80 (d, J = 1.1 Hz, 1H), 6.27 (t, J = 6.9 Hz, 1H), 4.38 (m, 1H), 3.89 (m, 1H), 3.75 (m, 2H), 2.21 (m, 2H), 1.87 (d, J = 1.1 Hz, 3H). HRMS (m/z): calculated for C₁₀H₁₅N₂O₅ (M+H): 243.0975; found: 243.0979.

Synthesis of dT-CE phosphoramidite

dT-CE phosphoramidite was synthesized as described previously.⁸

Synthesis of 12

To a solution of 2 (212 mg, 0.44 mmol) in CH₂CN (7 mL) were added TEA (141 mg, 1.39 mmol), DMAP (164 mg, 1.35 mmol) and TPSCl (409 mg, 1.35 mmol). The reaction was stirred at r.t. for 21 h before NH₄OH (25%, 10 mL) was added. The reaction was kept stirring at r.t. for
another 1.5 h. Solvent was removed under vacuum. The residue was partitioned between water and ethyl acetate. The aqueous layer was extracted with ethyl acetate 3 times. Combined organic layer was washed with 5% NaHCO$_3$, brine and dried over Na$_2$SO$_4$. Crude product was purified by column chromatography (CH$_2$Cl$_2$ with 5% methanol) to afford 12$^9$ (191 mg, 0.395 mmol, 90% yield) as a white solid. $^1$H NMR (300 MHz, CD$_3$OD) $\delta$: 9.56 (s, 1H), 8.62 (s, 1H), 6.14 (t, $J$ = 6.5 Hz, 1H), 4.46 (m, 1H), 4.10 (m, 1H), 3.90 (m, 2H), 2.55 (m, 1H), 2.15 (m, 1H), 0.93 (s, 9H), 0.90 (s, 9H), 0.131 (s, 3H), 0.125 (s, 3H), 0.11 (s, 6H). HRMS ($m/z$): calculated for C$_{22}$H$_{42}$N$_3$O$_5$Si$_2$ (M+H): 484.2658; found: 484.2663.

**Synthesis of 13**

![Diagram of synthetic process](image)

To a solution of 12 (60 mg, 0.124 mmol) in 1 mL of DMF was added Ac$_2$O (15.2 mg, 0.15 mmol). The reaction mixture was stirred at r.t. overnight. MeOH (0.5 mL) was added to quench the reaction. After the mixture was stirred for additional 15 min, the solvents were removed under reduced pressure. The residue was purified by column chromatography (CH$_2$Cl$_2$ with 2~4% methanol) to afford 13$^{10}$ (60 mg, 0.114 mmol, 92% yield) as a white foam. $^1$H NMR (300 MHz, CD$_3$OD) $\delta$: 9.58 (s, 1H), 8.83 (s, 1H), 6.15 (t, $J$ = 6.7 Hz, 1H), 4.47 (m, 1H), 4.15 (m, 1H), 3.93 (m, 2H), 2.63 (m, 1H), 2.59 (s, 3H), 2.22 (m, 1H), 0.93 (s, 9H), 0.90 (s, 9H), 0.13 (s, 6H), 0.11 (s, 6H). HRMS ($m/z$): calculated for C$_{24}$H$_{44}$N$_3$O$_6$Si$_2$ (M+H): 526.2763; found: 526.2766.

**Synthesis of 14**

To a solution of 13 (28 mg, 0.053 mmol) in 0.5 mL of THF was added TBAF (1 M in THF,
0.133 mmol). The reaction was kept at r.t. and monitored by TLC. Upon completion (about 1h), solvent was removed under reduced pressure. Crude product was purified by column chromatography (CH₂Cl₂ with 10% methanol) to afford 14 \(^\text{10}\) (13 mg, 0.043 mmol, 81% yield) as a white foam. \(^1\)H NMR (300 MHz, CD₃OD) δ: 9.58 (s, 1H), 9.06 (s, 1H), 6.23 (t, \(J = 5.9\) Hz, 1H), 4.44 (m, 1H), 4.06 (m, 1H), 3.95 (dd, \(J = 3.0, 12.1\) Hz, 1H), 3.82 (dd, \(J = 3.5, 12.1\) Hz, 1H), 2.49 (m, 1H), 2.39 (s, 3H), 2.29 (m, 1H). HRMS (m/z): calculated for C₁₂H₁₆N₃O₆ (M+H): 298.1034; found: 298.1038.

Synthesis of 5-fodC-CE phosphoramidite

5-fodC-CE phosphoramidite was synthesized as described previously.\(^\text{10}\)

Synthesis of 15

To a solution of 5 (258 mg, 0.50 mmol) in CH₃CN (7.7 mL) were added TEA (157 mg, 1.55 mmol), DMAP (183 mg, 1.50 mmol) and TPSCl (454 mg, 1.50 mmol). The reaction was stirred at r.t. for 21 h before NH₄OH (25%, 11 mL) was added. The reaction was kept stirring at r.t. for another 1.5 h. Solvent was removed under vacuum. The residue was partitioned between water and ethyl acetate. The aqueous layer was extracted with ethyl acetate 3 times. Combined organic layer was washed with sat. NaHCO₃, brine and dried over Na₂SO₄. Crude product was purified
by column chromatography (CH$_2$Cl$_2$ with 2~4% methanol) to afford 15$^{11}$ (185 mg, 0.36 mmol, 72% yield) as a white foam. $^1$H NMR (300 MHz, CD$_3$OD) $\delta$: 8.65 (s, 1H), 6.09 (dd, $J = 5.9, 7.4$ Hz, 1H), 4.45 (m, 1H), 4.12 (m, 1H), 3.84 (m, 2H), 3.66 (s, 3H), 2.56 (m, 1H), 2.09 (m, 1H), 0.93 (s, 9H), 0.87 (s, 9H), 0.133 (s, 3H), 0.125 (s, 3H), 0.10 (s, 3H), 0.08 (s, 3H). HRMS ($m/z$): calculated for C$_{23}$H$_{44}$N$_3$O$_6$Si$_2$ (M+H): 514.2763; found: 514.2760.

Synthesis of 16

$$
\text{TBDMSO} \quad \text{Ac}_2\text{O}, \text{DMF} \quad \text{TBDMSO} \\
\text{15} \quad \text{(88%)} \quad \text{16}
$$

To a solution of 15 (185 mg, 0.36 mmol) in 2.3 mL of DMF was added Ac$_2$O (44 mg, 0.432 mmol). The reaction mixture was stirred at r.t. overnight. MeOH (0.5 mL) was added to quench the reaction. After the mixture was stirred for additional 15 min, the solvents were removed under reduced pressure. Crude product was purified by column chromatography (CH$_2$Cl$_2$ with 10% methanol) to afford 16$^{11}$ (176 mg, 0.317 mmol, 88% yield) as a white foam. $^1$H NMR (300 MHz, CD$_3$OD) $\delta$: 8.80 (s, 1H), 6.07 (dd, $J = 6.1, 7.2$ Hz, 1H), 4.46 (m, 1H), 4.17 (m, 1H), 3.85 (m, 2H), 3.78 (s, 3H), 2.65 (m, 1H), 2.53 (s, 3H), 2.17 (m, 1H), 0.94 (s, 9H), 0.87 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H), 0.10 (s, 3H), 0.08 (s, 3H). HRMS ($m/z$): calculated for C$_{25}$H$_{46}$N$_3$O$_7$Si$_2$ (M+H): 556.2869; found: 556.2875.

Synthesis of 17

$$
\text{TBDMSO} \quad \text{TBAF/THF} \quad \text{16} \quad \text{17} \\
\text{AcHN} \quad \text{AcHN} \quad \text{(85%)} \quad \text{OH} \quad \text{OH}
$$


To a solution of 16 (176 mg, 0.317 mmol) in 0.5 mL of THF was added TBAF (1 M in THF, 0.793 mmol). The reaction was kept at r.t. and monitored by TLC. Upon completion (about 1h), solvent was removed under reduced pressure. Crude product was purified by column chromatography (CH₂Cl₂ with 10% methanol) to afford 17¹⁰ (88 mg, 0.269 mmol, 85% yield) as a white foam. ¹H NMR (300 MHz, DMSO-δ₆) δ: 10.63 (s, 1H), 9.22 (s, 1H), 0.04 (t, J = 5.7 Hz, 1H), 4.23 (m, 1H), 3.90 (m, 1H), 3.78 (s, 3H), 3.63 (m, 2H), 2.41 (s, 3H), 2.35 (m, 1H), 2.13 (m, 1H). HRMS (m/z): calculated for C₁₃H₁₈N₃O₇ (M+H): 328.1139; found: 328.1144.

Synthesis of 5-cadC-CE phosphoramidite

5-cadC-CE phosphoramidite was synthesized as described previously.¹⁰

Synthesis of 5-hmdC-CE phosphoramidite

5-hmdC-CE phosphoramidite was synthesized as described previously.¹²

Synthesis of 18

To a solution of 10 (600 mg, 1.276 mmol) in CH₃CN (15 mL) were added TEA (407 mg, 4.02 mmol), DMAP (478 mg, 3.919 mmol) and TPSCI (1.16 g, 3.919 mmol). The reaction was stirred at r.t. for 21 h before NH₄OH (25%, 15 mL) was added. The reaction was kept stirring at r.t. for another 1.5 h. Solvent was removed under vacuum. The residue was partitioned between water and ethyl acetate. The aqueous layer was extracted with ethyl acetate 3 times. Combined organic layer was washed with 5% NaHCO₃, brine and dried over Na₂SO₄. Crude product was purified by column chromatography (CH₂Cl₂ with 5% methanol) to afford 18 (509 mg, 1.09 mmol,
85% yield) as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 8.27 (s, 1H), 6.1 (t, $J = 6.6$ Hz, 1H), 4.35 (m, 1H), 4.01 (m, 1H), 3.88 (dd, $J = 2.5$, 11.6 Hz, 1H), 3.74 (dd, $J = 2.5$, 11.6 Hz, 1H), 2.57 (m, 1H), 1.97 (m, 1H), 1.88 (s, 3H), 0.91 (s, 9H), 0.88 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H). HRMS ($m$/z): calculated for C$_{22}$H$_{44}$N$_3$O$_4$Si$_2$ (M$+$H): 470.2865; found: 470.2867.

Synthesis of 19

![Diagram](image1)

To a solution of 18 (250 mg, 0.533 mmol) in 2 mL of DMF was added Ac$_2$O (66 mg, 0.645 mmol). The reaction mixture was stirred at r.t. overnight. MeOH (1 mL) was added to quench the reaction. After the mixture was stirred for additional 15 min, the solvents were removed under reduced pressure. The residue was purified by column chromatography (CH$_2$Cl$_2$ with 2% methanol) to afford 19 (253 mg, 0.496 mmol, 93% yield) as a white foam. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 8.25 (s, 1H), 6.15 (t, $J = 6.4$ Hz, 1H), 4.34 (m, 1H), 4.04 (m, 1H), 3.90 (m, 1H), 3.74 (m, 1H), 2.63 (m, 1H), 2.30 (s, 3H), 2.06 (m, 1H), 1.89 (s, 3H), 0.87 (s, 9H), 0.86 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H), 0.06 (s, 3H), 0.04 (s, 3H). HRMS ($m$/z): calculated for C$_{24}$H$_{46}$N$_3$O$_5$Si$_2$ (M$+$H): 512.2971; found: 512.2976.

Synthesis of 20

![Diagram](image2)
To a solution of 19 (250 mg, 0.489 mmol) in 1 mL of THF was added TBAF (1 M in THF, 1.22 mmol). The reaction was kept at r.t. and monitored by TLC. Upon completion (about 1h), solvent was removed under reduced pressure. Crude product was purified by column chromatography (CH₂Cl₂ with 10% methanol) to afford 20 (130 mg, 0.46 mmol, 94% yield) as a white foam. ¹H NMR (300 MHz, CD₃OD) δ: 7.95 (d, J = 1.1 Hz, 1H), 6.29 (t, J = 6.2 Hz, 1H), 4.38 (m, 1H), 3.91 (m, 1H), 3.75 (m, 1H), 2.38 (s, 3H), 2.24 (m, 2H), 1.92 (d, J = 1.1 Hz, 3H). HRMS (m/z): calculated for C₁₂H₁₈N₃O₅ (M+H): 284.1241; found: 284.1242.

Synthesis of 5-mdC-CE phosphoramidite

5-mdC-CE phosphoramidite was synthesized as described previously.¹³
**Figure S1.** Substrate selectivity of recombinant eUDG (A), hTDG (B), hSMUG1 (C) and hMBD4 (D).

### A

|       | C:G | mC:G | hmC:G | foC:G | caC:G | U:G | T:G | hmU:G | foU:G | caU:G |
|-------|-----|------|-------|-------|-------|-----|-----|-------|-------|-------|
| **eUDG** |     |      |       |       |       |     |     |       |       |       |

### B

|       | C:A | mC:A | hmC:A | foC:A | caC:A | U:A | T:A | hmU:A | foU:A | caU:A |
|-------|-----|------|-------|-------|-------|-----|-----|-------|-------|-------|
| **hTDG** |     |      |       |       |       |     |     |       |       |       |

### C

|       | C:G | mC:G | hmC:G | foC:G | caC:G | U:G | T:G | hmU:G | foU:G | caU:G |
|-------|-----|------|-------|-------|-------|-----|-----|-------|-------|-------|
| **hSMUG1** |     |      |       |       |       |     |     |       |       |       |

### D

|       | C:A | mC:A | hmC:A | foC:A | caC:A | U:A | T:A | hmU:A | foU:A | caU:A |
|-------|-----|------|-------|-------|-------|-----|-----|-------|-------|-------|
| **hMBD4** |     |      |       |       |       |     |     |       |       |       |
| C:G | mC:G | hmC:G | foC:G | caC:G | U:G | T:G | hmU:G | foU:G | caU:G |
|-----|------|-------|-------|-------|-----|-----|-------|-------|-------|
| hMBD4 | - | + | - | + | - | + | + | - | + |
| hMBD4 | - | + | + | - | - | - | + | + | - | + | + | + |

**Legend:** S = signal, P = background
Figure S2. DNA glycosylase activity in HeLa (A), U87 (B) and hES (C) cell extracts.
Table S1. Sequence, theoretical mass and experimentally determined mass for ODNs used in the current study.

| Name     | Sequence                        | [M+H]^+ (expected) | [M+H]^+ (detected) |
|----------|---------------------------------|--------------------|--------------------|
| 24mer-C  | 5′-GGCTATCGTGCCGGCCACGACGG-3′   | 7396.8150          | 7397.1624          |
| 24mer-mC | 5′-GGCTATCGTGCC<sup>m</sup>CGGCCACGACGG-3′ | 7410.8422          | 7410.8543          |
| 24mer-hmC| 5′-GGCTATCGTGCC<sup>hm</sup>CGGCCACGACGG-3′ | 7426.8409          | 7426.9180          |
| 24mer-foC| 5′-GGCTATCGTGCC<sup>fo</sup>CGGCCACGACGG-3′ | 7424.8252          | 7425.3954          |
| 24mer-caC| 5′-GGCTATCGTGCC<sup>ca</sup>CGGCCACGACGG-3′ | 7440.8236          | 7440.9183          |
| 24mer-U  | 5′-GGCTATCGTGCGUGGCCACGACGG-3′  | 7397.7990          | 7398.7812          |
| 24mer-T  | 5′-GGCTATCGTGGCCTGGCCACGACGG-3′ | 7411.8260          | 7413.2112          |
| 24mer-hmU| 5′-GGCTATCGTGCC<sup>hm</sup>UGGCCACGACGG-3′ | 7427.8249          | 7427.9502          |
| 24mer-foU| 5′-GGCTATCGTGCC<sup>fo</sup>UGGCCACGACGG-3′ | 7425.8089          | 7425.9761          |
| 24mer-caU| 5′-GGCTATCGTGCC<sup>ca</sup>UGGCCACGACGG-3′ | 7441.8078          | 7441.9971          |
| Complementary-G | 5′-CCGTCGTGCGCCGGCCACGATAGCC-3′ | 7316.7933          | 7316.8995          |
| Complementary-A | 5′-CCGTCGTGCGCCGCCACGATAGCC-3′ | 7300.7934          | 7300.9025          |
1. Y. El Safadi, J. C. Paillart, G. Laumond, A. M. Aubertin, A. Burger, R. Marquet and V. Vivet-Boudou, *J Med Chem*, 2010, **53**, 1534-1545.
2. K. Sato, W. Hirose and A. Matsuda, *Curr Protoc Nucleic Acid Chem*, 2008, Chapter 1, Unit 1 21.
3. A. Ono, N. Haginoya, M. Kiyokawa, N. Minakawa and A. Matsuda, *Bioorganic & Medicinal Chemistry Letters*, 1994, **4**, 361-366.
4. N. Haginoya, A. Ono, Y. Nomura, Y. Ueno and A. Matsuda, *Bioconjug Chem*, 1997, **8**, 271-280.
5. L. C. Sowers and G. P. Beardsley, *The Journal of Organic Chemistry*, 1993, **58**, 1664-1665.
6. M. Aso, T. Kaneko, M. Nakamura, N. Koga and H. Suemune, *Chem Commun (Camb)*, 2003, DOI: 10.1039/b301425h, 1094-1095.
7. P. Herdewijn, L. Kerremans, P. Wigerinck, F. Vandendriessche and A. Van Aerschot, *Tetrahedron Letters*, 1991, **32**, 4397-4400.
8. J. Nielsen, M. Taagaard, J. E. Marugg, J. H. van Boom and O. Dahl, *Nucleic Acids Res*, 1986, **14**, 7391-7403.
9. M. Munzel, D. Globisch, C. Trindler and T. Carell, *Org Lett*, 2010, **12**, 5671-5673.
10. Q. Dai and C. He, *Org Lett*, 2011, **13**, 3446-3449.
11. M. Munzel, U. Lischke, D. Stathis, T. Pfaffeneder, F. A. Gnerlich, C. A. Deiml, S. C. Koch, K. Karaghiosoff and T. Carell, *Chemistry*, 2011, **17**, 13782-13788.
12. S. Tardy-Planechaud, J. Fujimoto, S. S. Lin and L. C. Sowers, *Nucleic Acids Res*, 1997, **25**, 553-559.
13. W. L. Sung, *Nucleic Acids Res*, 1981, **9**, 6139-6151.