Supporting information

Development of Bioreduction Labile Protecting Groups for the 2′-Hydroxyl Group of RNA

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General information

Chemicals were purchased from FUJIFILM Wako Pure Chemical Corporation (Japan), Sigma–Aldrich (USA), Tokyo Chemical Industry (Japan), and Glen Research (USA) and used without further purification. NMR spectra were recorded on a JEOL (Japan) instrument at 500 MHz and 600 MHz for \(^1\)H NMR, 125 MHz and 151 MHz for \(^{13}\)C NMR, and 202 MHz and 243 MHz for \(^{31}\)P NMR. NMR (\(^1\)H, \(^{13}\)C, \(^{31}\)P) spectra were reported in parts per million (δ) relative to tetramethylsilane (0.00 ppm for \(^1\)H or 0.0 ppm for \(^{13}\)C) or the residual solvent peaks in case of DMSO-d\(_6\) (2.49 ppm for \(^1\)H or 39.5 ppm for \(^{13}\)C) or 85% phosphoric acid (0.0 ppm) for \(^{31}\)P. The coupling constant (\(J\)) was reported in hertz. Abbreviations for multiplicity were: s, singlet; d, doublet; t, triplet; dd, double doublet; td, triplet doublet; m, multiplet; br, broad. Column chromatography was carried out with a silica gel C-60 (Kanto, Japan) or NH silica gel (Fuji Silysia Chemical, Japan). Thin-layer chromatography (TLC) analyses were carried out on Kieselgel 60-F254 plates (Merck). Reverse phase HPLC was carried out with Intersil ODS-3 (4.6 × 250 mm, GL Sciences, Japan). A system composed of SPD-20A, LC-20AT (Shimazu, Japan) and Chromato PRO (Run Time Corporation, Japan) was used for HPLC analysis and purification. The UV–VIS spectrum was recorded on a UV-1650PC Spectrophotometer (Shimadzu, Japan).
Scheme S1. Synthesis of various nitrobenzyl alcohols
Figure S1. HPLC chromatograms of purified ONs used in this study

HPLC condition
Buffer A : 5% CH3CN in 0.1 M TEAA buffer
Buffer B : 50% CH3CN in 0.1 M TEAA buffer
Gradient (B)
5% (0 min) $\rightarrow$ 35% (20 min) $\rightarrow$ 95% (40 min)
<table>
<thead>
<tr>
<th>No.</th>
<th>Sequence</th>
<th>Formula</th>
<th>Calcd. [M-H]^-</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON 1</td>
<td>5'-TTTTU-(NBOM)-3'</td>
<td>C_{27}H_{70}N_{11}O_{37}P_{4}^-</td>
<td>1624.29</td>
<td>1623.20</td>
</tr>
<tr>
<td>ON 2</td>
<td>5'-TTTTU-(MMNBOM)-3'</td>
<td>C_{58}H_{132}N_{22}O_{38}P_{4}^-</td>
<td>1654.30</td>
<td>1654.46</td>
</tr>
<tr>
<td>ON 3</td>
<td>5'-TTTTU-(DMNBOM)-3'</td>
<td>C_{58}H_{132}N_{22}O_{38}P_{4}^-</td>
<td>1684.31</td>
<td>1685.33</td>
</tr>
<tr>
<td>ON 4</td>
<td>5'-TTTTU-(gm-NBOM)-3'</td>
<td>C_{48}H_{124}N_{12}O_{37}P_{4}^-</td>
<td>1652.32</td>
<td>1653.81</td>
</tr>
<tr>
<td>ON 5</td>
<td>5'-CACTGCAU-(DMNBOM)TGGTCAC-3'</td>
<td>C_{159}H_{394}N_{53}O_{98}P_{14}^-</td>
<td>4737.63</td>
<td>4741.66</td>
</tr>
<tr>
<td>ON 6</td>
<td>5'-CACU-(DMNBOM)GCAU-(DMNBOM)U-(DMNBOM)GGU-(DMNBOM)CAC-3'</td>
<td>C_{137}H_{174}N_{27}O_{91}P_{12}^-</td>
<td>5416.96</td>
<td>5413.00</td>
</tr>
<tr>
<td>ON 7</td>
<td>5'-UUUCGAAGUACUCAGCGUAAGU-(DMNBOM)-3'</td>
<td>C_{250}H_{323}N_{54}O_{166}P_{22}^-</td>
<td>7838.36</td>
<td>7835.33</td>
</tr>
<tr>
<td>ON 8</td>
<td>5'-U-(gm-NBOM)TCTGCATTGGTCAC-3'</td>
<td>C_{150}H_{198}N_{47}O_{88}P_{14}^-</td>
<td>4716.81</td>
<td>4716.81</td>
</tr>
</tbody>
</table>

Figure S2. MALDI-TOF mass analysis of synthesized ONs

U-(NBOM) = ![Image](image1.png)
U-(MMNBOM) = ![Image](image2.png)
U-(DMNBOM) = ![Image](image3.png)
U-(gm-NBOM) = ![Image](image4.png)

underline = ![Image](image5.png)
Figure S3. HPLC chromatograms of TiCl$_3$-mediated deprotection of ONs
Figure S4. Stability of ON 4 in the buffer without TiCl₃
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Figure S9. HPLC chromatograms of nitroreductase-mediated deprotection at the 5’-terminal position (ON 8)
### Experimental procedure

**Compound 1**

(Uridine (10.5 g, 43.0 mmol) was co-evaporated with pyridine three times and dissolved in pyridine (200 mL) under Ar. To the mixture was added TIPSCl$_2$ (16.4 mL, 51.3 mmol) at 0 °C and stirred at room temperature for 7 h. After disappearance of the starting material, to the mixture was added $	ext{H}_2	ext{O}$ (20 mL) and evaporated in vacuo. The residue was dissolved in EtOAc and washed with $	ext{H}_2	ext{O}$ and brine. The organic layer was dried (Na$_2$SO$_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with hexane/EtOAc (7:1-3:1, v/v) to give 1 (18.4 g, 37.8 mmol, 88%) as a white foam. $^1$H-NMR (600 MHz, CDCl$_3$) $\delta$ 8.45 (1H, brs), 7.68 (1H, d, $J = 8.3$ Hz), 5.73 (1H, s), 5.69 (1H, dd, $J = 7.9$ Hz), 4.39 (1H, dd, $J = 8.6, 4.8$ Hz), 4.21-4.18 (2H, m), 4.11-4.09 (1H, m), 4.02 (1H, dd, $J = 13.1, 2.8$ Hz), 2.98 (1H, s), 1.10-1.00 (28H, m).

**Compound 2**

(I (4.02 g, 8.26 mmol) was co-evaporated three times each with pyridine and toluene. Compound 1 was dissolved in DMSO (7 mL) under Ar. To the solution were added AcOH (11 mL, 190 mmol) and Ac$_2$O (7 mL, 74.3 mmol). The mixture was stirred at rt for 3 days and then added a saturated solution of Na$_2$CO$_3$ at 0 °C. The mixture was diluted with EtOAc and washed with a saturated solution of NaHCO$_3$ and brine. The organic layer was dried (Na$_2$SO$_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with hexane/EtOAc (3:1, v/v) to give 2 (3.42 g, 6.25 mmol, 76%) as a white foam. $^1$H-NMR (600 MHz, CDCl$_3$) $\delta$ 8.46 (1H, brs), 7.91 (1H, d, $J = 8.3$ Hz), 5.73 (1H, s), 5.69 (1H, d, $J = 8.3$ Hz), 4.98 (2H, m), 4.37 (1H, d, $J = 4.5$ Hz), 4.27-4.22 (2H, m), 4.15 (1H, dd, $J = 9.6, 1.7$ Hz), 3.99 (1H, dd, $J = 13.4, 2.1$ Hz), 2.19 (3H, s), 1.11-0.94 (28H, m).

**Compound 3**

(2 (1.63 g, 2.98 mmol) and 4-nitrobenzyl alcohol (582 mg, 3.80 mmol) were co-evaporated three times each with pyridine and toluene. 2 was dissolved in THF (10 mL) under Ar. To the mixture were added NIS (743 mg, 3.30 mmol), 4Å molecular sieves (520 mg) and TfOH (292 μL, 3.30 mmol) at -40 °C. The mixture was stirred at same temperature for 2 h. TLC indicated the disappearance of starting material and then Et$_3$N (2 mL) was added to the mixture. The mixture was filtered by celite pad and evaporated in vacuo. The residue was dissolved in EtOAc. To the solution was added a saturated sodium thiosulfate. The mixture was washed with washed with a saturated solution of NaHCO$_3$ and brine. The organic layer was dried (Na$_2$SO$_4$), filtered and evaporated in vacuo. The residue was co-evaporated three times each with pyridine and toluene. The residue was dissolved in MeOH (15 mL). To the solution was added NH$_4$F (1.18 g, 31.9 mmol). The mixture was stirred at rt for 13 h and evaporated in vacuo. The 3
residue was purified by column chromatography on silica gel eluted with CHCl₃/MeOH (96:4, v/v) to give 3 (550 mg, 1.34 mmol, 45%) as a white foam. ¹H-NMR (500 MHz, DMSO-d₆) δ 11.3 (1H, brs), 8.18-8.17 (2H, m), 7.92 (1H, d, J = 8.3 Hz), 7.55-7.53 (2H, m), 5.92 (1H, d, J = 5.2 Hz), 5.59 (1H, d, J = 8.3 Hz), 5.30 (1H, d, J = 4.9 Hz), 5.19 (1H, brs), 4.85 (2H, s), 4.73-4.64 (2H, m), 4.24 (1H, 5.2 Hz), 4.14-4.12 (1H, m), 3.89 (1H, q, J = 2.9 Hz), 3.66-3.55 (2H, m).

**Compound 4**

2 (1.11 g, 2.01 mmol) and alcohol 3 (427 mg, 2.23 mmol) were co-evaporated three times each with pyridine and toluene. 2 was dissolved in THF (10 mL) under Ar. To the mixture were added NIS (502 mg, 2.23 mmol), 4Å molecular sieves (512 mg) and TfOH (200 μL, 2.23 mmol) at -40 °C. The mixture was stirred at same temperature for 2 h. TLC indicated the disappearance of starting material and then Et₃N (2 mL) was added to the mixture. The mixture was filtered by celite pad and evaporated in vacuo. The residue was dissolved in EtOAc. To the solution was added a saturated sodium thiosulfate. The mixture was washed with washed with a saturated solution of NaHCO₃ and brine. The organic layer was dried (Na₂SO₄), filtered and evaporated in vacuo. The residue was co-evaporated three times each with pyridine and toluene. The residue was dissolved in MeOH (12 mL). To the solution was added NH₄F (770 mg, 20.3 mmol). The mixture was stirred at rt for 16 h and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with CHCl₃/MeOH (97:3, v/v) to give 4 (394 mg, 0.897 mmol, 45%) as a pale yellow solid. ¹H-NMR (500 MHz, DMSO-d₆) δ 11.24 (1H, brs), 7.86 (1H, d, J = 8.3 Hz), 7.82 (1H, dd, J = 8.3, 2.3 Hz), 7.70 (1H, J = 2.0 Hz), 7.52 (1H, d, J = 8.3 Hz), 5.93 (1H, d, J = 5.7 Hz), 5.52 (1H, d, J = 8.0 Hz), 5.28 (1H, d, J = 5.2 Hz), 5.17 (1H, brs), 4.86 (2H, s), 4.58 (2H, s), 4.24 (1H, t, J = 5.2 Hz), 4.14 (1H, q, J = 4.9 Hz), 3.89-3.88 (4H, m), 3.63-3.55 (2H, m);

¹³C-NMR (126 MHz, DMSO-d₆) δ 162.9, 156.4, 150.6, 147.6, 140.2, 134.2, 127.4, 115.5, 105.0, 101.9, 93.8, 85.9, 85.4, 78.1, 68.7, 63.3, 60.7, 56.1; HRMS (ESI-TOF) m/z: [M+Na⁺] Calcd for C₁₈H₂₁N₃O₁₀⁺: 462.1119, Found: 462.1147.
Compound 5

2 (1.27 g, 2.33 mmol) and alcohol 4 (546 mg, 2.56 mmol) were co-evaporated three times each with pyridine and toluene. 2 was dissolved in THF (10 mL) under Ar. To the mixture were added NIS (630 mg, 2.80 mmol), 4Å molecular sieves (496 mg) and TfOH (250 μL, 2.83 mmol) at -40 °C. The mixture was stirred at same temperature for 2 h. TLC indicated the disappearance of starting material and then Et3N (2 mL) was added to the mixture. The mixture was filtered by celite pad and evaporated in vacuo. The residue was dissolved in EtOAc. To the solution was added a saturated sodium thiosulfate. The mixture was washed with washed with a saturated solution of NaHCO3 and brine. The organic layer was dried (Na2SO4), filtered and evaporated in vacuo. The residue was co-evaporated three times each with pyridine and toluene. The residue was dissolved in MeOH (14 mL). To the solution was added NH4F (884 mg, 23.3 mmol). The mixture was stirred at rt for 16 h and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with CHCl3/MeOH (97:3, v/v) to give 5 (721 mg, 1.54 mmol, 66%) as a pale yellow solid. 1H-NMR (500 MHz, DMSO-d6) δ 11.3 (1H, brs), 7.89 (1H, d, J = 8.0 Hz), 7.46 (1H, s), 7.21 (1H, s), 5.93 (1H, d, J = 5.7 Hz), 5.54 (1H, d, J = 8.0 Hz), 5.29-5.19 (2H, m), 4.88 (2H, m), 4.55 (2H, s), 4.25 (1H, t, J = 5.2 Hz), 4.14 (1H, m), 3.90 (1H, q, J = 2.9 Hz), 3.86 (3H, s), 3.78 (3H, s), 3.65-3.55 (2H, m); 13C-NMR (126 MHz, DMSO-d6) δ 163.0, 150.6, 149.1, 146.4, 140.2, 137.6, 133.6, 113.1, 107.1, 101.8, 93.9, 86.1, 85.4, 78.2, 68.7, 63.3, 60.7, 56.9, 56.2; HRMS (ESI-TOF) m/z : [M+Na+] Calcd for C19H23N3NaO11+: 492.1225, Found : 492.1244.

Compound 6

2 (1.02 g, 1.88 mmol) and alcohol 5 (380 mg, 2.07 mmol) were co-evaporated three times each with pyridine and toluene. 2 was dissolved in THF (10 mL) under Ar. To the mixture were added NIS (466 mg, 2.07 mmol), 4Å molecular sieves (498 mg) and TfOH (183 μL, 2.07 mmol) at -40 °C. The mixture was stirred at same temperature for 2 h. TLC indicated the disappearance of starting material and then Et3N (2 mL) was added to the mixture. The mixture was filtered by celite pad and evaporated in vacuo. The residue was dissolved in EtOAc. To the solution was added a saturated sodium thiosulfate. The mixture was washed with washed with a saturated solution of NaHCO3 and brine. The organic layer was dried (Na2SO4), filtered and evaporated in vacuo. The residue was co-evaporated three times each with pyridine and toluene. The residue was dissolved in MeOH (10 mL). To the solution was added NH4F (740 mg, 18.8 mmol). The mixture was stirred at rt for 16 h and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with CHCl3/MeOH (97:3, v/v) to give 6 (248 mg, 0.567 mmol, 30%) as a pale yellow solid. 1H-NMR (600 MHz, DMSO-d6) δ 11.3 (1H, brs), 8.17 (2H, d, J = 8.9 Hz), 7.95 (1H, d, J = 8.2 Hz), 7.66 (2H, J = 8.9 Hz), 5.94 (1H, d, J = 6.2 Hz), 5.67 (1H, d, J = 8.3 Hz), 5.19-5.16 (2H, m), 4.74 (1H, q, J = 7.6 Hz), 4.29 (1H, t, J = 5.8 Hz), 4.13 (1H, q, J = 5.2 Hz), 3.88 (1H, q, J = 3.1 Hz), 3.64-3.55 (2H, m), 1.50 (3H, s), 1.43 (3H, s); 13C-NMR (151 MHz, DMSO-d6) δ 163.1, 154.7, 150.7, 146.5, 140.7, 126.8, 123.4, 102.2, 89.5, 85.8, 85.7, 78.2, 77.8, 68.7, 61.0, 29.4, 27.4; HRMS (ESI-TOF) m/z : [M+Na+] Calcd for C19H23N3NaO9+: 460.1327, Found : 460.1299.
**Compound 7**


3 (500 mg, 1.22 mmol) was co-evaporated with pyridine (three times) and toluene (twice). 2 was dissolved in pyridine (12 mL) under Ar. To the mixture was added DMTr-Cl (455 mg, 1.47 mmol) and stirred at rt for 3 h. To the mixture was added H2O and the mixture evaporate under reduced pressure. The residue was dissolved in EtOAc and washed with saturated NaHCO3aq and brine. Organic layer was dried (Na2SO4), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with CHCl3/MeOH (98:2, v/v) to give 7 (860 mg, 1.20 mmol, 98%) as a white foam. 1H-NMR (DMSO-d6, 500 MHz) δ 11.3 (1H, brs), 8.18-8.16 (2H, m), 7.72 (1H, d, J = 8.0 Hz), 7.57 (2H, m), 7.38-7.25 (10H, m), 6.88-6.86 (4H, m), 5.88 (1H, d, J = 4.0 Hz), 5.40 (1H, d, J = 6.3 Hz), 5.25 (1H, d, J = 8.0 Hz), 4.91 (2H, s), 4.77-4.68 (2H, m), 4.33-4.31 (1H, m), 4.28 (1H, q, J = 6.0 Hz), 4.01-3.99 (1H, m), 3.72 (6H, s), 3.29-3.22 (2H, m).

**Compound 8**

4 (394 mg, 0.90 mmol) was co-evaporated with pyridine (three times) and toluene (twice). 2 was dissolved in pyridine (9 mL) under Ar. To the mixture was added DMTr-Cl (366 mg, 1.08 mmol) and stirred at rt for 3 h. To the mixture was added H2O and the mixture evaporate in vacuo. The residue was dissolved in EtOAc and washed with saturated NaHCO3aq and brine. Organic layer was dried (Na2SO4), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with CHCl3/MeOH (98:2, v/v) to give 8 (594 mg, 0.80 mmol, 89%) as a pale-yellow foam. 1H-NMR (600 MHz, CDCl3) δ 8.45 (1H, brs), 7.93 (1H, d, J = 8.3 Hz), 7.85 (1H, dd, J = 8.2, 2.1 Hz), 7.70 (1H, d, J = 2.1 Hz), 7.54 (1H, d, J = 8.2 Hz), 7.38 (2H, m), 7.31-7.28 (6H, s), 7.25-7.23 (1H, m), 6.84-6.83 (4H, m), 6.06 (1H, d, J = 3.1 Hz), 5.27 (1H, dd, J = 8.3, 2.1 Hz), 5.13 (1H, d, J = 6.9 Hz), 5.02 (1H, d, J = 6.9 Hz), 4.79 (1H, d, J = 14.1 Hz), 4.72 (1H, d, J = 14.1 Hz), 4.50 (1H, q, J = 6.5 Hz), 4.39-4.37 (1H, m), 4.09-4.08 (1H, m), 3.92 (3H, s), 6.40 (6H, s), 3.57-3.41 (1H, m), 2.64 (1H, d, J = 6.9 Hz); 13C-NMR (151 MHz, CDCl3) δ 163.4, 158.74, 158.71, 156.8, 150.3, 148.4, 144.2, 140.0, 135.2, 135.0, 133.3, 130.2, 130.1, 128.1, 128.04, 128.02, 127.2, 115.9, 113.32, 113.30, 105.0, 102.3, 95.2, 87.5, 87.2, 83.5, 80.3, 69.2, 65.1, 61.8, 55.9, 55.3; HRMS (ESI-TOF) m/z : [M+Na]+ Calcd for C39H39N3NaO12+: 764.2426, Found : 764.2390.
Compound 9

(721 mg, 1.54 mmol) was co-evaporated with pyridine (three times) and toluene (twice). 2 was dissolved in pyridine (16 mL) under Ar. To the mixture was added DMTr-Cl (706 mg, 2.00 mmol) and stirred at rt for 2 h. To the mixture was added H2O and the mixture evaporate in vacuo. The residue was dissolved in EtOAc and washed with saturated NaHCO3aq and brine. Organic layer was dried (Na2SO4), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with CHCl3/MeOH (99:1, v/v) to give 9 (1.08 g, 1.40 mmol, 91%) as a pale-yellow foam.

1H-NMR (600 MHz, DMSO-d6) δ 11.35 (1H, brs), 7.69 (1H, d, J = 7.9 Hz), 7.38-7.36 (2H, m), 7.30-7.28 (2H, m), 6.88-6.87 (4H, m), 5.89 (1H, d, J = 5.2 Hz), 5.34 (1H, d, J = 5.8 Hz), 5.26 (1H, d, J = 5.8 Hz), 4.80 (2H, s), 4.37 (1H, t, J = 5.2 Hz), 4.24 (1H, q, J = 5.2 Hz), 3.97 (1H, q, J = 4.5 Hz), 3.72 (6H, m), 3.28-3.21 (2H, m), 1.53 (3H, s), 1.48 (3H, s); 13C-NMR (151 MHz, DMSO-d6) δ 162.9, 158.1, 154.6, 150.4, 146.4, 144.6, 135.3, 135.0, 130.0, 127.9, 127.7, 126.7, 123.8, 113.3, 101.7, 89.5, 86.6, 86.0, 83.2, 79.2, 77.7, 68.6, 63.0, 55.0, 29.1, 27.5; HRMS (ESI-TOF) m/z: [M+Na+] Calcd for C40H41N3NaO13+: 794.2532, Found: 794.2557.

Compound 10

(230 mg, 0.57 mmol) was co-evaporated with pyridine (three times) and toluene (twice). 2 was dissolved in pyridine (6 mL) under Ar. To the mixture was added DMTr-Cl (233 mg, 0.68 mmol) and stirred at rt for 2 h. To the mixture was added H2O and the mixture evaporate in vacuo. The residue was dissolved in EtOAc and washed with saturated NaHCO3aq and brine. Organic layer was dried (Na2SO4), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with CHCl3/MeOH (98:2, v/v) to give 10 (383 mg, 0.52 mmol, 91%) as a white foam.

1H-NMR (600 MHz, DMSO-d6) δ 11.38 (1H, brs), 8.16-8.15 (2H, m), 7.73-7.67 (3H, m), 7.38-7.22 (9H, m), 5.89 (1H, d, J = 5.2 Hz), 5.34 (1H, d, J = 7.9 Hz), 5.26 (1H, d, J = 5.8 Hz), 4.80 (2H, s), 4.37 (1H, t, J = 5.2 Hz), 4.24 (1H, q, J = 5.2 Hz), 3.97 (1H, q, J = 4.5 Hz), 3.72 (6H, m), 3.28-3.21 (2H, m), 1.53 (3H, s), 1.48 (3H, s); 13C-NMR (151 MHz, DMSO-d6) δ 162.9, 158.1, 154.6, 150.4, 146.4, 144.6, 135.3, 135.0, 130.0, 127.9, 127.7, 126.7, 123.8, 113.3, 101.7, 89.5, 86.6, 86.0, 83.2, 79.2, 77.7, 68.6, 63.0, 55.0, 29.1, 27.5; HRMS (ESI-TOF) m/z: [M+Na+] Calcd for C40H41N3NaO11+: 762.2633, Found: 762.2614.

7 (160 mg, 0.22 mmol) was co-evaporated with pyridine (five times) and toluene (three times). 2 was dissolved in CH$_2$Cl$_2$ (2 mL) under Ar. To the mixture were added i-Pr$_2$NEt (115 µL, 0.66 mmol) and 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (108 µL, 0.48 mmol). The mixture was stirred at rt for 1 h. To the mixture was added H$_2$O and the mixture was evaporated in vacuo. The residue was dissolved in EtOAc and washed with saturated NaHCO$_3$aq and brine. Organic layer was dried (Na$_2$SO$_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on NH-silica gel eluted with hexane/EtOAc (50:50, v/v) to give 11 (135 mg, 0.15 mmol, 67%) as a white foam.

$^{31}$P-NMR (202 MHz, C$_6$D$_6$) δ 151.3, 149.9.

Compound 12

8 (594 mg, 0.80 mmol) was co-evaporated with pyridine (five times) and toluene (three times). 2 was dissolved in CH$_2$Cl$_2$ (8 mL) under Ar. To the mixture were added i-Pr$_2$NEt (419 µL, 2.4 mmol) and 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (393 µL, 1.76 mmol). The mixture was stirred at rt for 3 h. To the mixture was added H$_2$O and the mixture evaporate in vacuo. The residue was dissolved in EtOAc and washed with saturated NaHCO$_3$aq and brine. Organic layer was dried (Na$_2$SO$_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with hexane/EtOAc (40:60, v/v) to give 12 (536 mg, 0.57 mmol, 71%) as a pale-yellow foam.

$^{31}$P-NMR (202 MHz, CDCl$_3$) δ 151.2, 150.5.; HRMS (ESI-TOF) m/z: [M+Na$^+$] Calcd for C$_{48}$H$_{56}$N$_5$NaO$_{13}$P$^+$: 964.3504, Found : 964.3506.

Compound 13

9 (160 mg, 0.22 mmol) was co-evaporated with pyridine (five times) and toluene (three times). 2 was dissolved in CH$_2$Cl$_2$ (2 mL) under Ar. To the mixture were added i-Pr$_2$NEt (115 µL, 0.66 mmol) and 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (108 µL, 0.48 mmol). The mixture was stirred at rt for 3 h. To the mixture was added H$_2$O and the mixture evaporate in vacuo. The residue was dissolved in EtOAc and washed with saturated NaHCO$_3$aq and brine. Organic layer was dried (Na$_2$SO$_4$), filtered and evaporated in vacuo. The residue was purified.
by column chromatography on silica gel eluted with hexane/EtOAc (50:50, v/v) to give 13 (135 mg, 0.14 mmol, 64%) as a white foam. $^{31}$P-NMR (202 MHz, CDCl$_3$) $\delta$ 151.3, 150.3.; HRMS (ESI-TOF) $m/z$: [M+Na$^+$] Calcd for C$_{49}$H$_{58}$N$_5$NaO$_{14}$P$^+$: 994.3610, Found : 994.3651.

**Compound 14**

![Compound 14](image)

Compound 10 (375 mg, 0.51 mmol) was co-evaporated with pyridine (five times) and toluene (three times). 2 was dissolved in CH$_2$Cl$_2$ (5 mL) under Ar. To the mixture were added i-Pr$_2$NEt (267 $\mu$L, 1.53 mmol) and 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (252 $\mu$L, 1.12 mmol). stirred at rt for 1.5 h. To the mixture was added H$_2$O and the mixture evaporate in vacuo. The residue was purified by column chromatography on silica gel eluted with hexane/EtOAc (50:50, v/v) to give 14 (290 mg, 0.31 mmol, 61%) as a white foam. $^{31}$P-NMR (243 MHz, CDCl$_3$) $\delta$ 151.1, 150.9.; HRMS (ESI-TOF) $m/z$: [M+Na$^+$] Calcd for C$_{49}$H$_{58}$N$_5$NaO$_{12}$P$^+$: 962.3712, Found : 962.3678.


2-Methoxy-4-nitrobenzaldehyde 15 (2 g, 11.04 mmol) was dissolved in MeOH (60 mL). The mixture was treated with NaBH$_4$ (520 mg, 13.25 mmol) at 0°C and stirred at the same temperature for 30 min. To the mixture was added acetone (30 mL). The mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc. The mixture was washed with a saturated solution of NaHCO$_3$ and brine. Organic layer was dried (Na$_2$SO$_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with hexane/EtOAc (80:20, v/v) to give 16 (1.79 g, 9.75 mmol, 88%) as a pale-yellow powder. $^1$H-NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.91 (1H, m), 7.70 1H, d, J = 2.3 Hz), 7.64 (1H, d, J = 8.3 Hz), 5.41 (1H, t, J = 5.5 Hz), 4.56 (2H, d, J = 4.9 Hz), 3.90 (s, 3H).


3-Hydroxy-4-nitrobenzoic acid 17 (5 g, 27.3 mmol) was dissolved in 2M NaOH (100 mL). To the solution was added an aqueous solution (150 mL) containing K$_2$S$_2$O$_8$ (7.4 g). The mixture was stirred at rt for 1 h. To the mixture was added conc.H$_2$SO$_4$ until pH 1-2. The resulted mixture was filtered. Using an oil bath, obtained solution was refluxed for 1 h and cooled to rt. The resulted solid was filtered to give 18 (1.52 g, 7.64 mmol, 28%) as a brown solid. $^1$H-NMR (500 MHz, DMSO-$d_6$) $\delta$ 10.55 (1H, brs), 7.48 (1H, s), 7.37 (1H, s).
Compound 19

Compound 18 (1.079 g, 5.42 mmol) was dissolved in DMF (20 mL) under Ar. To the solution were added CH$_3$I (934 μL, 81.3 mmol) and K$_2$CO$_3$ (2.07 g, 108 mmol). The mixture was stirred at rt for 3 days. The mixture was diluted with EtOAc and washed twice with H$_2$O. The organic layer was dried (Na$_2$SO$_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with hexane/EtOAc (9:1, v/v) to give 19 (778 mg, 3.23 mmol, 60%) as a yellow powder.

$^1$H-NMR (500 MHz, DMSO-d$_6$) δ 7.67 (1H, s), 7.54 (1H, s), 3.88 (3H, s), 3.83 (3H, s), 3.82 (3H, s); $^{13}$C-NMR (126 MHz, DMSO-d$_6$) δ 165.0, 150.8, 144.8, 141.2, 125.1, 115.9, 109.1, 57.1, 56.9, 52.6; HRMS (ESI-TOF) m/z: [M+Na$^+$] Calcd for C$_{10}$H$_{11}$NNaO$_6$ $^+$: 264.0479, Found : 264.0454.

Compound 20

Compound 19 (502 mg, 2.08 mmol) was dissolved in THF (20 mL) under Ar. To the solution were added .0 M DIBAL-H in toluene (6.24 mL, 6.24 mmol) at 0°C. The mixture was stirred at 0°C for 2 h. To the mixture were added acetone (4 mL) and a solution of saturated NH$_4$Cl. The organic layer was filtered thorough celite pad and evaporated in vacuo. The residue was dissolved in EtOAc and washed with a solution of saturated NaHCO$_3$ and brine. Organic layer was dried (Na$_2$SO$_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with hexane/EtOAc (60:40, v/v) to give 20 (307 mg, 1.44 mmol, 69%) as a yellow powder.

$^1$H-NMR (500 MHz, DMSO-d$_6$) δ 7.45 (1H, s), 7.35 (1H, s), 5.43 (3H, t, J = 5.5 Hz), 4.53 (2H, dd, J = 5.4, 0.86 Hz), 3.87 (3H, s), 3.79 (3H, s); $^{13}$C-NMR (126 MHz, DMSO-d$_6$) δ 148.7, 146.7, 138.4, 136.9, 112.4, 106.7, 57.6, 56.8, 56.0; HRMS (ESI-TOF) m/z: [M+Na$^+$] Calcd for C$_9$H$_{11}$NNaO$_5$ $^+$: 236.0529, Found : 236.0530.


4-Nitroacetophenone 21 (2 g, 12.1 mmol) was co-evaporated three times each with pyridine and toluene. The compound was dissolved in CH$_2$Cl$_2$ (9 mL). To the solution was added 1.0 M Al(CH$_3$)$_3$ in toluene (6.6 mL, 13.3 mmol) at 0°C. The mixture was stirred at 0°C for 1 h and then added acetone (10 mL) and sat. NH$_4$Claq. The mixture was filtered on the celite pad. The mixture was washed with sat. NaHCO$_3$aq, dried (Na$_2$SO$_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel eluted with hexane/EtOAc (92:8, v/v) to give 22 (1.31 g, 7.24 mmol, 60%) as a light brown oil. $^1$H-NMR (600 MHz, DMSO-d$_6$) δ 8.18-8.17 (2H, m), 7.75-7.74 (2H, m), 5.37 (1H, s), 1.46 (6H, s).

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Oligonucleotide synthesis

Oligonucleotide synthesis was carried out on the NTS-M2 DNA/RNA synthesizer according to the manufacturer’s recommendations. Phosphoramidites are used as 0.1 M solution in dry acetonitrile. Cleavage from universal support and deprotection were performed by NH₄OH at 55 °C in a heat block for 14 h. Cleavage from conventional support and deprotection were performed by NH₄OH at 55 °C in a heat block for 5 h. The CPG solid support was filtered off, and the filtrate was concentrated in vacuo. Crude ODNs were purified by C₁₈ cartridge. Each sample was further purified by using reversed-phase HPLC with Intersil ODS-3 (4.6 × 250 mm, GL Sciences, Japan). The structures of each ODN were confirmed by measurement of MALDI-TOF/MASS spectrometry on the AXIMA-CFR plus (SHIMADZU, JAPAN) by using Refection negative mode. (Matrix for ionizing samples was used as a mixture (10 : 1 : 1; saturated 3-hydroxy-2-picolic acid, 2-picolic acid/H₂O (50 mg/mL) , ammonium citrate/H₂O (50 mg/mL)).

Chemical reduction

ON (10 μM), TiCl₃ (2 mM) and dT (60 μM) in 200 μL of 200 mM sodium citrate (pH 6.0) were incubated at 37 °C. Aliquot of a sample solution was analyzed by reversed-phase HPLC and MALDI-TOF mass spectroscopy.

In case of ON 6, ON (12 μM), TiCl₃ (2 mM) and dT (12 μM) in 200 μL of 200 mM sodium citrate (pH 6.0) were incubated at 37 °C. Aliquot of a sample solution was analyzed by reversed-phase HPLC and MALDI-TOF mass spectroscopy.

HPLC condition; Buffer A : 5% CH₃CN in 0.1 M TEAA buffer, Buffer B : 50% CH₃CN in 0.1 M TEAA buffer, Gradient (B) 5% (0 min)→35% (20 min)→95% (40 min); Flow rate : 1.0 mL/min.

HPLC condition for ON 6; Buffer A : 5% CH₃CN in 0.1 M TEAA buffer, Buffer B : 50% CH₃CN in 0.1 M TEAA buffer, Gradient (B) 5% (0 min)→15% (15 min)→60% (30 min)→95% (40 min); Flow rate : 1.0 mL/min.

Enzymatic reduction

ON (12 μM), NADH (10 mM), nitroreductase from Escherichia coli (20 μg) and N⁶-Bz-dA (12 μM or 24 μM for Figure S7) in 200 μL of 50 mM sodium phosphate (pH 6.5) were incubated at 37 °C. Aliquot of a sample solution was analyzed by reversed-phase HPLC and MALDI-TOF mass spectroscopy.

HPLC condition; Buffer A : 5% CH₃CN in 0.1 M TEAA buffer, Buffer B : 50% CH₃CN in 0.1 M TEAA buffer, Gradient (B) 0% (0 min)→50% (50 min); Flow rate : 1.0 mL/min.

HPLC condition for Figure S7; Buffer A : 5% CH₃CN in 0.1 M TEAA buffer, Buffer B : 50% CH₃CN in 0.1 M TEAA buffer, Gradient (B) 0% (0 min)→60% (60 min); Flow rate : 1.0 mL/min.

HPLC condition for Figure S8; Buffer A : 5% CH₃CN in 0.1 M TEAA buffer, Buffer B : 50% CH₃CN in 0.1 M TEAA buffer, Gradient (B) 0% (0 min)→20% (30 min)→80% (60 min); Flow rate : 1.0 mL/min.
NMR spectra of synthesized compounds

(600 MHz, CDCl$_3$)
(500 MHz, DMSO-$d_6$)
(500 MHz, DMSO-d6)

(126 MHz, DMSO-d6)
(500 MHz, CDCl₃)

(243 MHz, C₆D₆)
HO-\text{NO}_2\text{OCH}_3

16

(500 MHz, DMSO-\text{d}_6)
(500 MHz, DMSO-d6)
19
(500 MHz, DMSO-$d_6$)

19
(126 MHz, DMSO-$d_6$)
(500 MHz, DMSO-d6)

(126 MHz, DMSO-d6)
(600 MHz, DMSO-<i>d</i>6)
References


