Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

# Supporting Information



69451 Weinheim, Germany

# Synthesis of 1,8-Naphthyridine *C*-Nucleosides and Their Base Pairing Properties in Oligodeoxynucleotides: Thermally Stable Naphthyridine:Imidazopyridopyrimidine Base Paring Motifs

Sadao Hikishima, Noriaki Minakawa, Kazuyuki Kuramoto, Yuki Fujisawa, Maki Ogawa, and Akira Matsuda

## Synthesis of Naphthyridine *C*-Nucleosides 7 and 9, and Their Conversion into the Corresponding Phosphoramidites 10 and 11.

**2-(*N,N*-Dimethylaminomethylidene)amino-7-hydroxy-6-iodo-1,8-naphthyridine (2).** To a suspension of 2-amino-7-hydroxy-1,8-naphthyridine (**1**; 5.0 g, 31.0 mmol) in DMF (30 mL) was added NIS (7.7 g, 34.1 mmol) at room temperature, and the whole was stirred for 35 h. The resulting precipitate was collected by filtration to give crude 2-amino-7-hydroxy-6-iodo-1,8-naphthyridine. A mixture of the resulting 6-iodo derivative and *N,N*-dimethylformamide dimethylacetal (4.9 mL, 36.7 mmol) in DMF (110 mL) was heated at 80 ˚C for 20 h. The solvent was removed in vacuo, and the residue was purified by a silica gel column, eluted with 0–2% MeOH in $CHCl_3$, to give **2** (2.4 g, 30%, as a yellow solid): EI-LRMS *m/z* 342 ($M^+$); EI-HRMS calcd for $C_{11}H_{11}IN_4O$ ($M^+$) 341.9978, found 341.9982; $^1$H-NMR (DMSO-$d_6$) δ: 12.03 (br s, 1 H), 8.50 (s, 2 H), 7.79 (d, 1 H, *J* = 8.2 Hz), 6.66 (d, 1 H, *J* = 8.2 Hz), 3.11 (s, 3 H), 3.01 (s, 3 H); $^{13}$C-NMR (DMSO-$d_6$) δ: 162.73 (C2), 159.58 (C7), 155.95 (N=C), 149.48 (C1a), 147.03 (C5), 136.31 (C4), 113.27 (C3), 110.49 (C4a), 90.26 (C6), 40.55 (Me), 34.53 (Me).

**2-(*N,N*-Dibutylaminomethylidene)amino-3,6-diiodo-7-hydroxy-1,8-naphthyridine (3).** To a suspension of **1** (413 mg, 2.56 mmol) in DMF (4 mL) was added NIS (1.67 g, 7.42 mmol) at room temperature, and the whole was heated at 80 ˚C for 24 h. The resulting precipitate was collected by filtration to give crude 2-amino-3,6-diiodo-7-hydroxy-1,8-naphthyridine. A mixture of the resulting 3,6-diiodo derivative and *N,N*-dibutylformamide dimethylacetal (0.66 mL, 2.70 mmol) in DMF (5 mL) was stirred at room temperature for 7 h. The solvent was removed in vacuo, and $Et_2O$ was added to the residue. The resulting precipitate was collected by filtration to give **3** (1.1 g, 83%, as a ocher solid): EI-LRMS *m/z* 552 ($M^+$); EI-HRMS calcd for $C_{17}H_{22}I_2N_4O$ ($M^+$) 551.9883, found 551.9889; $^1$H-NMR (DMSO-$d_6$) δ: 12.08 (br s, 1 H), 8.46 (s, 1 H), 8.45 (s, 1 H), 8.34 (s, 1 H), 3.53 (dd, 2 H, *J* = 6.6, 7.9 Hz), 3.37 (dd, 2 H, *J* = 6.6, 7.9 Hz), 1.61 (m, 4 H), 1.31 (m, 4 H), 0.92 (m, 6 H); $^{13}$C-NMR (DMSO-$d_6$) δ: 160.59 (C2), 159.73 (C7), 156.05 (N=C), 149.51 (C1a), 146.13 (C5), 144.89 (C4), 112.49 (C4a), 91.07 (C6), 84.75 (C3), 51.27 (Bu), 45.36 (Bu), 30.55 (Bu), 28.47 (Bu), 19.73 (Bu), 19.20 (Bu), 13.77 (Bu), 13.54 (Bu).

**2-(*N,N*-Dibutylaminomethylidene)amino-7-hydroxy-3-iodo-1,8-naphthyridine (4).** A mixture of $(dba)_3Pd_2\cdot CHCl_3$ (103 mg, 0.1 mmol) and triphenylphosphine (210 mg, 0.8 mmol) in DMF (20 mL) was stirred at room temperature for 30 min to give the yellow suspension. To the resulting suspension were added **3** (1.1 g, 2.0 mmol) and tributyltinhydride (645 μL, 2.4 mmol), and the whole was heated at 60 ˚C for 24 h. The solvent was removed in vacuo, and the residue was purified by a silica gel column, eluted with hexane/AcOEt (3:1-1:1), to give **4** (0.53 g, 63%, as a yellow solid): EI-LRMS *m/z* 426 ($M^+$); EI-HRMS Calcd. for $C_{17}H_{23}IN_4O$ ($M^+$) 426.0917, found 426.0923; $^1$H-NMR (DMSO-$d_6$) δ: 11.67 (br s, 1 H), 8.45 (s, 1 H),

8.36 (s, 1 H), 7.70 (d, 1 H, *J* = 9.6 Hz), 6.26 (d, 1 H, *J* = 9.6 Hz), 3.56 (dd, 2 H, *J* = 7.3, 7.9 Hz), 3.36 (t, 2 H, *J* = 7.3 Hz), 1.61 (m, 4 H), 1.31 (m, 4 H), 0.93 (m, 6 H); $^{13}$C-NMR (DMSO-$d_6$) δ: 162.88 (C7), 160.18 (C2), 155.81 (N=C), 149.27 (C1a), 145.81 (C4), 137.92 (C5), 118.61 (C6), 111.13 (C4a), 84.16 (C3), 51.21 (Bu), 45.29 (Bu), 30.65 (Bu), 28.54 (Bu), 19.83 (Bu), 19.28 (Bu), 13.85 (Bu), 13.64 (Bu).

**2-(*N,N*-Dimethylaminomethylidene)amino-6-(2-deoxy-β-D-ribofuranosyl)-7-hydroxy-1,8-naphthyridine (6).** A mixture of palladium (II) acetate (135 mg, 0.60 mmol) and triphenylarsine (370 mg, 1.20 mmol) in DMF (16 mL) was stirred at room temperature for 30 min. To a suspension of **2** (2.06 g, 6.02 mmol) in DMF (18 mL), **5** (1.53 g, 6.63 mmol), tributylamine (1.58 mL, 6.63 mmol), and the suspension of palladium catalyst were added, and the whole was heated at 60 ˚C for 16 h. The reaction mixture was cooled at 0 ˚C, and AcOH (1.6 mL) and TBAF (1 M in THF; 12 mL, 12 mmol) were added to the mixture. After being stirred for 45 min at the same temperature, the solvent was removed in vacuo. The residue was purified by a short silica gel column chromatography, eluted with 7% MeOH in $CHCl_3$, to give 3′-keto derivative. The resulting blown solid was dissolved in a mixture of acetonitorile (85 mL) and AcOH (85 mL), and sodium triacetoxyborohydride (1.65 g, 7.77 mmol) was added at 0 °C. The suspension was stirred for 1 h at the same temperature, and quenched with acetone. The solvents were removed in vacuo, and the residue was purified by a silica gel column, eluted with 5-20% MeOH in $CHCl_3$, to give **6** (1.56 g, 78%, as a yellow solid): FAB-LRMS *m/z* 333 ($MH^+$); FAB-HRMS Calcd. for $C_{22}H_{33}N_4O_4$ ($MH^+$) 133,1563, found 133.1563; $^1$H-NMR (DMSO-$d_6$) δ: 11.65 (br s, 1 H), 8.47 (s, 1 H), 7.81 (d, 1 H, *J* = 8.3 Hz), 7.80 (s, 1 H), 6.66 (d, 1 H, *J* = 8.3 Hz), 5.02 (dd, 1 H, *J* = 5.6, 10.0 Hz), 4.98 (d, 1 H, *J* = 3.9 Hz), 4.76 (dd, 1 H, *J* = 5.6, 5.8 Hz), 4.14 (m, 1 H),

3.77 (m, 1 H), 3.47 (m, 1 H), 2.22 (ddd, 1 H, *J* = 1.7, 5.6, 12.8 Hz), 1.66 (ddd, 1 H, *J* = 5.7, 10.0, 12.8 Hz); $^{13}$C-NMR (DMSO-$d_6$) δ: 162.06, 161.73, 155.74, 148.47, 137.14, 132.65, 131.09, 113.04, 108.95, 87.15, 74.79, 72.20, 62.34, 41.18, 40.34, 34.39.

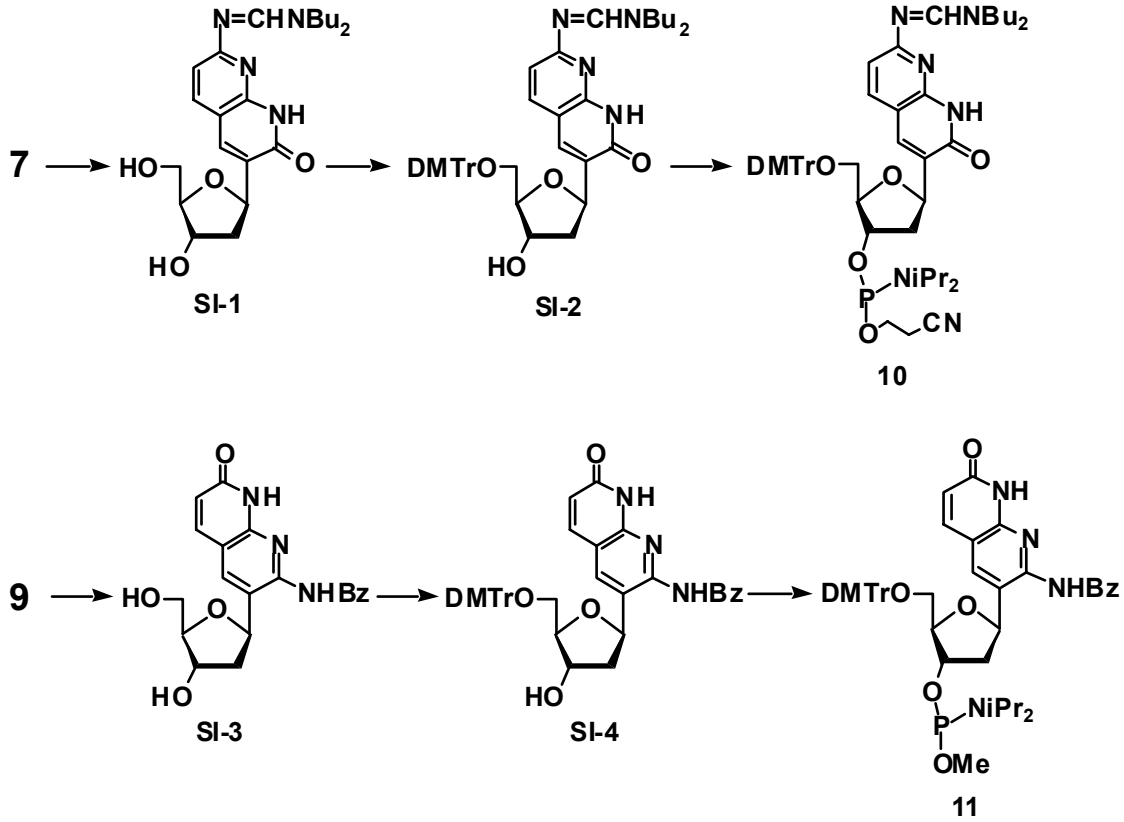
**2-Amino-6-(2-deoxy-β-D-ribofuranosyl)-7-hydroxy-1,8-naphthyridine (7).** A solution of **6** (720 mg, 2,17 mmol) in methanolic ammonia (saturated at 0 ˚C, 50 mL) was heated at 80˚C for 24 h in steel container. The solvent was removed in vacuo, and the residue was purified by a silica gel column, eluted with 10-25% MeOH in $CHCl_3$, to give **7** (512 mg, 85%, crystallized from MeOH): mp 273 °C (colored); UV λmax ($H_2O$) 357 nm (ε = 25,600), 342 nm (ε = 25,000), 210 nm (ε = 33,000); FAB-LRMS *m/z* 278 ($MH^+$); $^1$H-NMR (DMSO-$d_6$) δ: 11.91 (br s, 1 H), 7.72 (s, 1 H), 7.64 (d, 1 H, *J* = 8.4 Hz), 6.92 (br s, 1 H), 6.35 (d, 1 H, *J* = 8.4 Hz), 5.00 (dd, 1 H, *J* = 5.6, 10.1 Hz), 4.98 (d, 1 H, *J* = 3.1 Hz), 4.80 (dd, 1 H, *J* = 5.4, 5.8 Hz), 4.13 (m, 1 H), 3.75 (m, 1 H), 3.45 (m, 2 H), 2.18 (dd, 1 H, *J* = 5.6, 12.4 Hz), 1.67 (ddd, 1 H, *J* = 5.6, 10.1, 12.4 Hz); $^{13}$C-NMR (DMSO-$d_6$) δ: 162.09 (C7), 159.90 (C2), 149.05 (C1a), 136.98 (C4), 133.71 (C5), 127.36 (C6), 105.19 (C3), 104.62 (C4a), 87.07 (C4′), 74.75 (C1′), 72.28 (C3′), 62.39 (C5′), 41.23 (C2′). Anal. Calcd. for $C_{13}H_{15}N_3O_4 \cdot 3/10H_2O$: C, 55.24; H, 5.56; N, 14.86. Found: C, 55.45; H, 5.56; N, 14.70.

**2-(*N,N*-Dibutylaminomethylidene)amino-3-(2-deoxy-β-D-ribofuranosyl)-7-hydroxy-1,8-naphthyridine (8).** In the similar manner as described for **6**, the reaction of 4 (500 mg, 1.17 mmol) with **5** (297 mg, 1.29 mmol), followed by the deprotection and reduction gave **8** (370 mg, 76%, as a yellow solid): EI-LRMS *m/z* 416 ($M^+$); EI-HRMS Calcd. for $C_{22}H_{32}N_4O_4$ ($M^+$) 416.2423, found 416.2424; $^1$H-NMR (DMSO-$d_6$) δ: 11.57 (br s, 1 H),

8.49 (s, 1 H), 7.93 (s, 1 H), 7.73 (d, 1 H, *J* = 9.4 Hz), 6.24 (d, 1 H, *J* = 9.4 Hz), 5.33 (dd, 1 H, *J* = 5.6, 9.5 Hz), 4.94 (d, 1 H, *J* = 3.8 Hz), 4.72 (dd, 1 H, *J* = 4.5, 5.7 Hz), 4.11 (m, 1 H), 3.76 (m, 1 H), 3.49 (m, 4 H), 3.37 (m, 2 H), 2.28 (ddd, 1 H, *J* = 1.9, 5.6, 12.6 Hz), 1.59 (m, 5 H), 1.31 (m, 4 H), 0.92 (m, 6 H); $^{13}$C-NMR (DMSO-$d_6$) δ: 162.67, 158.57, 154.42, 147.98, 138.91, 132.60, 126.54, 117.97, 108.83, 87.01, 74.93, 71.97, 62.32, 51.06, 45.01, 42.43, 30.72, 28.78, 19.80, 19.29, 13.84, 13.66.

**2-Amino-3-(2-deoxy-β-D-ribofuranosyl)-7-hydroxy-1,8-naphthyridine (9).** In the similar mannar as described for **7**, **8** (365 mg, 0.88 mmol) was treated with methanolic ammonia to give **9** (697 mg, 99%, crystallized from MeOH): mp 215–218 °C; UV λmax ($H_2O$) 356 nm (ε = 25,900), 342 nm (ε = 25,000), 210 nm (ε = 32,300); FAB-LRMS *m/z* 278 ($MH^+$); $^1$H-NMR (DMSO-$d_6$) δ: 11.65 (br s, 1 H), 7.68 (s, 1 H), 7.63 (d, 1 H, *J* = 9.3 Hz), 6.81 (br s, 1 H), 6.10 (d, 1 H, *J* = 9.3 Hz), 5.10 (d, 1 H, *J* = 3.5 Hz), 5.06 (dd, 1 H, *J* = 4.7, 5.1 Hz), 5.00 (dd, 1 H, *J* = 5.6, 10.5 Hz), 4.23 (m, 1 H), 3.78 (m, 1 H), 3.54 (m, 2 H), 2.05 (ddd, 1 H, *J* = 6.2, 10.5, 12.5 Hz), 1.95 (ddd, 1 H, *J* = 0.8, 5.6, 12.5 Hz); $^{13}$C-NMR (DMSO-$d_6$) δ: 163.34 (C7), 157.55 (C2), 149.15 (C1a), 139.34 (C5), 134.92 (C4), 115.38 (C3), 115.05 (C6), 104.75 (C4a), 87.65 (C4′), 77.54 (C1′), 71.86 (C3′), 61.38 (C5′), 39.50 (C2′). Anal. Calcd. for $C_{13}H_{15}N_3O_4 \cdot 3/10H_2O$: C, 55.24; H, 5.56; N, 14.86. Found: C, 55.31; H, 5.36; N, 14.95.

**SI-Scheme 1**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O
NH
N
O
HO
SI-1
DMTrO
SI-2
NiPr2
P
CN
10
9
NHBz
SI-3
SI-4
OMe
11

**2-(*N,N*-Dibutylaminomethylidene)amino-6-(2-deoxy-β-D-ribofuranosyl)-7-hydroxy-1,8-naphthyridine (SI-1).** To a solution of **7** (357 mg, 1.29 mmol) in DMF (26 mL), dibutylformamide dimethylacetal (602 μL, 2.57 mmol) was added, and the solution was stirred at room temperature for 3 h. The solvent was removed in vacuo, and the residue was purified by a silica gel column, eluted with 0-5% MeOH in $CHCl_3$, to give **SI-1** (397 mg, 74%, as a pale yellow foam): FAB-LRMS *m/z* 417 ($MH^+$); FAB-HRMS Calcd. for $C_{22}H_{33}N_4O_4$ ($MH^+$) 417.2502, found 417.2511; $^1$H-NMR (DMSO-$d_6$) δ: 8.48 (s, 1 H), 7.80 (s, 1 H), 7.80 (d, 1 H, *J* = 8.3 Hz), 6.65 (d, 1 H, *J* = 8.3 Hz), 5.01 (br s, 1 H), 5.01 (dd, 1 H, *J* = 5.6, 9.9 Hz), 4.80 (br s, 1 H), 4.14 (m, 1 H), 3.77 (m, 1 H), 3.46 (m, 6 H), 2.22 (ddd, 1 H, *J* = 1.2, 5.6, 12.6 Hz), 1.66 (ddd, 1 H, *J* = 5.6, 9.9, 12.6 Hz), 1.52 (m, 4 H), 1.28 (m, 4 H), 0.89 (m, 6 H); $^{13}$C-NMR (DMSO-$d_6$) δ: 162.25, 161.80, 155.50, 148.54, 137.17, 132.79, 131.07, 113.17, 108.98, 87.18, 74.87, 72.28, 62.38, 50.86, 44.42, 41.23, 30.68, 28.72, 19.69, 19.28, 13.81, 13.66.

**2-(*N,N*-Dibutylaminomethylidene)amino-6-[2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-7-hydroxy-1,8-naphthyridine (SI-2).** A mixture of **SI-1** (434 mg, 1.04 mmol) and DMTrCl (424 mg, 1.25 mmol) in pyridine (20 mL) was stirred at room temperature for 4 h. The reaction was quenched by addition of MeOH, and the solvent was removed in vacuo. The residue was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$, and the separated organic layer was washed with brine. The organic layer was dried ($Na_2SO_4$), and concentrated in vacuo. The residue was purified by a silica gel column, eluted with 0-2% MeOH in $CHCl_3$, to give **SI-2** (504 mg, 67%, as a pale yellow foam): FAB-LRMS *m/z* 719 ($MH^+$); FAB-HRMS Calcd. for $C_{43}H_{51}N_4O_6$ ($MH^+$) 719.3808, found 719.3814; $^1$H-NMR ($CDCl_3$)

δ: 9.05 (br s, 1 H), 8.50 (s, 1 H), 7.87 (s, 1 H), 7.48-6.80 (m, 13 H), 7.47 (d, 1 H, *J* = 8.3 Hz), 6.72 (d, 1 H, *J* = 8.3 Hz), 5.34 (d, 1 H, *J* = 6.2, 6.4 Hz), 4.40 (m, 1 H), 4.11 (m, 1 H), 3.78 (s, 6 H), 3.54 (m, 2 H), 3.33 (m, 4 H), 2.59 (dt, 1 H, *J* = 3.2, 6.4 Hz), 2.10 (br s, 1 H), 1.98 (ddd, 1 H, *J* = 6.2, 8.8, 13.2 Hz), 1.62 (m, 4 H), 1.37 (m, 4 H), 0.96 (m, 6 H); $^{13}$C-NMR ($CDCl_3$) δ: 162.82, 162.18, 158.27, 156.03, 147.90, 144.79, 137.11, 135.96, 133.42, 131.40, 129.99, 128.08, 127.71, 126.65, 114.45, 113.01, 109.88, 86.07, 85.43, 75.09, 73.97, 64.25, 55.18, 55.16, 51.71, 45.12, 41.87, 31.11, 29.18, 20.24, 19.82, 13.93, 13.78.

**2-(*N,N*-Dibutylaminomethylidene)amino-6-{2-deoxy-3-*O*-[(*N,N*-diisopropylamino)-2-cyanoethoxyphosphino]-5-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl}-7-hydroxy-1,8-naphthyridine (10).** To a solution of **SI-2** (484 mg, 0.67 mmol) containing *N,N*-diisopropylethylamine (353 μL, 2.02 mmol) in $CH_2Cl_2$ (16 mL) was added 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (300 μL, 1.35 mmol), and the whole was stirred at room temperature for 45 min. The reaction mixture was diluted with AcOEt, and the organic layer was washed with saturated aqueous $NaHCO_3$, followed by brine. The separated organic layer was dried ($Na_2SO_4$), and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (3:1-1:2), to give **10** (437 mg, 71%, as a pale yellow foam): FAB-LRMS *m/z* 919 ($MH^+$); FAB-HRMS Calcd. for $C_{52}H_{68}N_6O_7P$ ($MH^+$) 919.4887, found 919.4841; $^{31}$P-NMR ($CDCl_3$) δ: 149.09, 148.43.

**2-Benzoylamino-3-(2-deoxy-β-D-ribofuranosyl)-7-hydroxy-1,8-naphthyridine (SI-3).** To a suspension of **9** (200 mg, 0.72 mmol) in pyridine (12 mL) was added chlorotrimetylsilane (550 μL, 4.33 mmol) at room temperature. After being stirred for 30 min, BzCl (503 μL, 4.33 mmol)

was added to the reaction mixture. After being stirred for 9 h at the same temperature, the reaction mixture was cooled at 0 ˚C, and then, 28% $NH_4OH$ was added. The reaction mixture was warmed to room temperature and stirred for 2 h. The solvent was removed in vacuo, and the residue was purified by a silica gel column, eluted with 5-12% MeOH in $CHCl_3$, to give **SI-3** (232 mg, 84%, as a pale yellow solid): FAB-LRMS *m/z* 382 ($MH^+$); FAB-HRMS Calcd. for $C_{20}H_{20}N_3O_5$ ($MH^+$) 382.1403, found 382.1392; $^1$H-NMR (DMSO-$d_6$) δ: 12.14 (br s, 1 H), 10.61 (br s, 1 H), 8.31 (s, 1 H), 8.00-7.52 (m, 5 H), 7.94 (d, 1 H, *J* = 9.6 Hz), 6.55 (d, 1 H, *J* = 9.6 Hz), 5.16 (dd, 1 H, *J* = 5.6, 10.2 Hz), 4.99 (d, 1 H, *J* = 4.0 Hz), 4.77 (t, 1 H, *J* = 5.6 Hz), 4.14 (m, 1 H), 3.73 (m, 1 H), 3.50 (m, 1 H), 2,16 (ddd, 1 H, *J* = 1.7, 5.6, 12.8 Hz), 1.76 (ddd, 1 H, *J* = 5.8, 10.2, 12.8 Hz); $^{13}$C-NMR (DMSO-$d_6$) δ: 166.22, 162.91, 148.77, 148.23, 138.71, 135.89, 133.55, 132.04, 129.69, 128.54, 127.78, 122.72, 113.17, 87.48, 74.70, 72.13, 62.21, 42.41.

**2-Benzoylamino-3-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-7-hydroxy-1,8-naphthyridine (SI-4).** In the similar mannar as described for **SI-2**, **SI-3** (450 mg, 1.18 mmol) was treated with DMTrCl (481 mg, 1.42 mmol) in pyridine (23 mL) to give **SI-4** (697 mg, 87%, as a pale yellow foam): FAB-LRMS *m/z* 684 ($MH^+$); FAB-HRMS Calcd. for $C_{41}H_{38}N_3O_7$ ($MH^+$) 684.2709, found 684.2725; $^1$H-NMR ($CDCl_3$) δ: 12.99 (br s, 1 H), 11.39 (br s, 1 H), 8.44 (s, 1 H), 8.12-7.44 (m, 5 H), 7.52-6.78 (m, 13 H), 7.08 (d, 1 H, *J* = 9.5 Hz), 6.48 (d, 1 H, *J* = 9.5 Hz), 5.32 (dd, 1 H, *J* = 5.6, 9.8 Hz), 4.56 (m, 1 H), 4.02 (m, 1 H), 3.39 (dd, 2 H, *J* = 3.7, 10.2 Hz), 3.32 (dd, 1 H, *J* = 3.2, 10.2 Hz), 2.68 (ddd, 1 H, *J* = 0.9, 5.6, 13.4 Hz), 2.38 (ddd, 1 H, *J* = 5.6, 9.8, 13.4 Hz), 1.98 (ddd, 1 H, *J* = 6.2, 8.8, 13.2 Hz); $^{13}$C-NMR ($CDCl_3$) δ: 167.57, 164.12, 158.43, 150.21, 147.69, 144.65, 138.86, 138.10, 135.77, 135.69, 133.07, 132.33,

130.04, 128.42, 128.15, 127.80, 126.87, 121.78, 113.10, 86.46, 86.26, 75.99, 74.28, 64.40, 55.21, 42.80.

**2-Benzoylamino-3-{2-deoxy-*3-O*-[(*N,N*-diisopropylamino)methoxyphosphino]-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl}-7-hydroxy-1,8-naphthyridine (11).** To a solution of **SI-4** (500 mg, 0.73 mmol) containing *N,N*-diisopropylethylamine (380 μL, 2.20 mmol) and 4-(dimethyamino)pyridine (18 mg, 0.15 mmol) in $CH_2Cl_2$ (18 mL) was added methyl *N,N*-diisopropylchlorophosphoramidite (285 μL, 1.46 mmol), and the whole was stirred at room temperature for 45 min. The mixture was diluted with $CHCl_3$, and the organic layer was washed with saturated aqueous $NaHCO_3$, followed by brine. The separated organic layer was dried ($Na_2SO_4$), and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (1:1-1:3), to give **11** (247 mg, 40%, as a pale yellow foam): FAB-LRMS *m/z* 845 ($MH^+$); FAB-HRMS Calcd. for $C_{48}H_{54}N_4O_8P$ ($MH^+$) 845.3676, found 845.3698; $^{31}$P-NMR ($CDCl_3$) δ: 149.32, 147.86.

**Synthesis of ODNs.**

ODNs were synthesized on a DNA synthesizer (Applied Biosystem Model 3400) by the phosphoramidite method. For the incorporation of the naphthyridine *C*-nucleosides into the ODNs, a 0.12 M solution of **10** in $CH_3CN$ and that of **11** in $CH_3CN$:$CH_2Cl_2$ (10:1), and coupling time of 10 min was used. The each ODN linked to the resin (1 μmol) was treated with concentrated $NH_4OH$ at 55 °C for 16 h. In the case of ODNs prepared by **11**, the CPG support was treated with a mixture of thiophenol, triethylamine, and dioxane (1:2:2, 0.5 mL) for 1 h at room temperature and washed with MeOH. The support was then treated with concentrated $NH_4OH$ at 55 °C for 16 h. The released ODN protected by a DMTr group

at 5′-end was chromatographed on a C-18 silica gel column (1 x 12 cm) with a linear gradient of $CH_3CN$ from 0 to 40% in 0.1 M TEAA buffer (pH 7.0). The fractions were concentrated, and the residue was treated with aqueous 80% AcOH at room temperature for 20 min, then the solution was concentrated, and the residue was coevaporated with $H_2O$. The residue was dissolved in $H_2O$ and the solution was washed with AcOEt, then the $H_2O$ layer was concentrated to give a deprotected ODN. The ODN was further purified by reversed-phase HPLC, using a J′sphere ODN M80 column (4.6 x 150 mm, YMC) with a linear gradient of $CH_3CN$ (from 10 to 25% over 30 min) in 0.1 M TEAA buffer (pH 7.0) to give a highly purified ODNs (see SI-Figure 1).

**Hyperchromicities, Extinction Coefficients and Nucleoside Composition of the ODNs.**

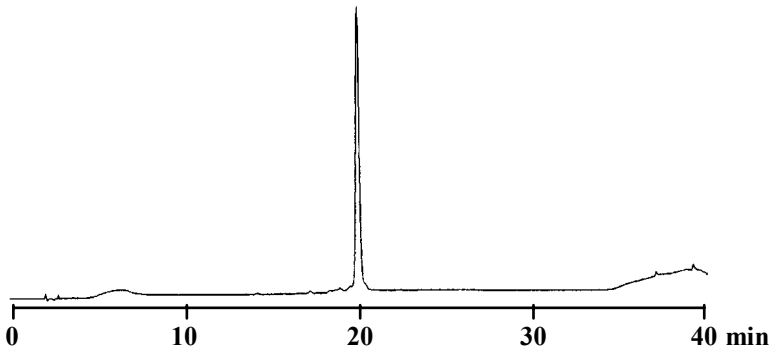
Each ODN (0.25 OD units at 260 nm) was incubated with snake venom phosphodiesterase (12 μg), nuclease P1 (12 μg), and alkaline phosphatase (0.5 units) in a buffer containing 100 mM Tris-HCl (pH 7.7) and 2 mM $MgCl_2$ (total 517 μL) at 37 °C for 12 h. Hyperchromicity of each ODN was determined by comparing UV absorbancies at 260 nm of the solutions before and after hydrolysis. The extinction coefficient (at 260 nm) of each ODN was determined using the following equation: $\varepsilon_{ODN}$ = the sum of $\varepsilon_{nucleoside}$/hyperchromicity. The extinction coefficients (at 260 nm) of the natural nucleosides used for calculations were as follows: dA, 15,400; dC, 7,300; dG, 11,700; T, 8,800. The extinction coefficients for the naphthyridine *C*-nucleosides at 260 nm were determined to be the following: **Na-N$^{o}$**, 2,140; **Na-O$^{N}$**, 1,420. After the reaction mixture was heated in boiling water for 5 min, the enzymes were removed from the reaction mixture by filtration with Micropure®-EZ device (MILLIPORE), and the filtrate was concentrated. Nucleoside composition was determined

by analysis of the residue with reverse-phase HPLC, using a J'sphere ODN M80 column (4.6 x 250 mm, YMC) with linear gradient of $CH_3CN$ (from 2.5 to 25% over 30 min) in 0.1 M TEAA buffer (pH 7.0). The HPLC charts for ODN VI (Y = **Na-O$^N$**) are shown in SI-Figure 1 as an example. Hyperchromicities, extinction coefficients and nucleoside composition of each ODN are listed in SI-Table 1.

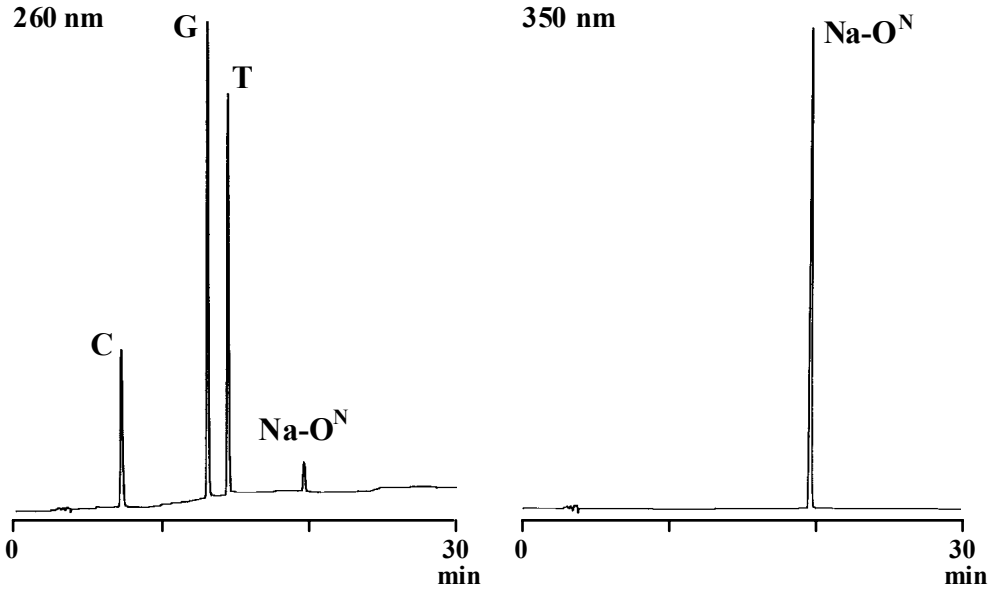
**SI-Figure 1**

**HPLC Analysis of ODN VI (Y = Na-O$^N$)**

**: 5'-CGTGGTTYYYTCGGTGC-3' : Y = Na-O$^N$**




**Determination of Nucleoside Composition by Enzymatic Digestion with Nuclease P1, Snake Venom Phosphodiesterase, and Alkaline Phosphatase**



**SI-Table 1**

| ODN | hyperchromicity | extinction coefficient | nucleoside composition |
|---|---|---|---|
| ODN I : X = Im-O$^N$ | 1.43 | 1.37 x $10^5$ | A:C:G:X = 6.3:6.0:2.9:1.1 |
| Im-N$^O$ | 1.42 | 1.44 x $10^5$ | (7:6:3:1) 6.7:6.6:3.1:1.1 |
| Na-N$^O$ | 1.29 | 1.46 x $10^5$ | 6.9:5.9:3.3:0.9 |
| ODN II : Y = Na-N$^O$ | 1.30 | 1.20 x $10^5$ | T:G:C:Y = 7.0:5.7:3.0:0.8 |
| Na-O$^N$ | 1.19 | 1.30 x $10^5$ | (7:6:3:1) 7.2:5.9:3.2:1.0 |
| Im-N$^O$ | 1.26 | 1.35 x $10^5$ | 7.1:6.2:3.0:0.9 |
| ODN III : X = Im-O$^N$ | 1.58 | 1.17 x $10^5$ | A:C:G:X = 4.8:6.4:3.1:2.8 |
| Im-N$^O$ | 1.65 | 1.27 x $10^5$ | (5:6:3:3) 4.9:6.4:3.4:2.7 |
| Na-N$^O$ | 1.42 | 1.14 x $10^5$ | 5.0:6.4:3.3:2.8 |
| ODN IV : Y = Na-N$^O$ | 1.36 | 1.05 x $10^5$ | T:G:C:Y = 5.1:5.7:3.2:3.2 |
| Na-O$^N$ | 1.36 | 1.03 x $10^5$ | (5:6:3:3) 5.3:5.9:3.2:3.0 |
| Im-N$^O$ | 1.48 | 1.29 x $10^5$ | 5.2:5.7:3.1:2.9 |
| ODN V : X = Im-O$^N$ | 1.45 | 1.27 x $10^5$ | A:C:G:X = 4.7:6.0:3.0:3.3 |
| Im-N$^O$ | 1.58 | 1.33 x $10^5$ | (5:6:3:3) 4.7:6.0:3.0:2.9 |
| Na-N$^O$ | 1.21 | 1.34 x $10^5$ | 5.0:6.2:3.3:2.7 |
| ODN VI : Y = Na-N$^O$ | 1.33 | 1.07 x $10^5$ | T:G:C:Y = 5.3:6.0:3.2:2.5 |
| Na-O$^N$ | 1.19 | 1.18 x $10^5$ | (5:6:3:3) 5.1:5.9:3.2:3.0 |
| Im-N$^O$ | 1.41 | 1.34 x $10^5$ | 5.0:6.1:3.0:3.1 |

**Thermal Denaturation.**

Each sample of the appropriate duplex (3 μM each) in a buffer of 10 mM sodium cacodylate (pH 7.0) containing 1 mM NaCl was heated at 95 °C for 5 min, cooled gradually to an appropriate temperature, and used for the thermal denaturation study. Thermal-induced transitions of the duplex were monitored at 260 nm on a Beckman DU 650 spectrophotometer. The sample temperature was increased by 0.5 °C/min. In the case of the thermal denaturation study between **Na-N$^o$** and natural bases, a buffer of 10 mM sodium cacodylate (pH 7.0) containing 100 mM NaCl was prepared and the measurement was conducted in the same manner as described above.

**Thermodynamic Parameters Determined by Differential Scanning Calorimetry (DSC) Measurements.**

DSC measurements were performed on a VP-DSC Microcalorimeter. The solution of the appropriate duplex (25 μM each) in a buffer of 10 mM sodium cacodylate (pH 7.0) containing 1 mM NaCl was prepared and scanned from 20 to 100 ˚C at a scan rate 0.5 K/min. The apparent molar heat capacity vs. temperature profiles were obtained by subtracting buffer vs. buffer curves from the sample vs. buffer curves. The data were normalized with regard to the concentration and sample volume. The excess heat capacity function, $\Delta C_p$, was obtained after baseline subtraction, assuming that the baseline is given by the linear temperature dependence of the native state heat capacity. The process enthalpies, $\Delta H°$, were obtained by integrating the area under the heat capacity vs. temperature curves. $T_m$ is the temperature corresponding to the maximum of each DSC peak. The process entropies, $\Delta S°$, were determined by integrating the curve obtained and dividing the heat capacity curve by the absolute temperature, i.e., $\Delta S° = \bullet(\Delta C_p/T)\Delta T$. The free energies, $\Delta G°$(25 °C), were determined at $T$ = 298.15 K by $\Delta G°(25\ °C) = \Delta H° - T\Delta S°$. The resulting thermodynamic parameters of each duplex were listed in SI-Table 2.

**SI-Table 2**

| duplex | X | Y | $^{2}H°$ (kcal mol$^{-1}$) | $^{2}S°$ (cal mol$^{-1}$ K$^{-1}$) | $^{2}G°$ (25 °C) (kcal mol$^{-1}$) |
|---|---|---|---|---|---|
| **ODN I:ODN II** | **Im-O$^{N}$** | **Na-N$^{O}$** | –85.16 | –252.70 | –9.82 |
| | **Im-N$^{O}$** | **Na-O$^{N}$** | –86.36 | –257.46 | –9.60 |
| 5'-GCACCGAA**X**AAACCACG-3' | **Im-O$^{N}$** | **Im-N$^{O}$** | –43.16 | –133.53 | –3.35 |
| 3'-CGTGGCTT**Y**TTTGGTGC-5' | **Na-N$^{O}$** | **Na-O$^{N}$** | –48.40 | –144.47 | –5.33 |
| | **G** | **C** | –76.19 | –238.08 | –5.21 |
| | **A** | **T** | –59.07 | –182.30 | –4.72 |
| **ODN III:ODN IV** | **Im-O$^{N}$** | **Na-N$^{O}$** | –128.56 | –333.43 | –29.15 |
| | **Im-N$^{O}$** | **Na-O$^{N}$** | –128.68 | –335.41 | –28.68 |
| 5'-GC**X**CCGAA**X**AAACC**X**CG-3' | **Im-O$^{N}$** | **Im-N$^{O}$** | –82.26 | –251.42 | –7.30 |
| 3'-CG**Y**GGCTT**Y**TTTGG**Y**GC-5' | **Na-N$^{O}$** | **Na-O$^{N}$** | –74.49 | –232.08 | –5.24 |
| | **G** | **C** | –92.29 | –277.70 | –9.49 |
| **ODN V:ODN VI** | **Im-O$^{N}$** | **Na-N$^{O}$** | –118.72 | –306.92 | –27.12 |
| | **Im-N$^{O}$** | **Na-O$^{N}$** | –119.94 | –307.56 | –28.24 |
| 5'-GCACCGA**XXX**AACCACG-3' | **Im-O$^{N}$** | **Im-N$^{O}$** | –96.98 | –273.35 | –15.48 |
| 3'-CGTGGCT**YYY**TTGGTGC-5' | **Na-N$^{O}$** | **Na-O$^{N}$** | –97.28 | –284.87 | –12.35 |
| | **G** | **C** | –83.63 | –244.29 | –10.79 |