Electronic Supplementary Information

Synthesis and duplex-forming ability of oligonucleotides containing 4’-carboxythymidine analogs

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(2'R)-2'-Deoxy-2'-'N,3'-O-2-(Trichloromethyl)oxazolino-5'-O-trityl-5-methyluridine (S2): Under a nitrogen atmosphere, Et3N (58 µL, 0.41 mmol) was added to a solution of compound S1¹ (1.00 g, 2.07 mmol) in CCl₃CN (4.0 mL) at room temperature. The reaction mixture was refluxed for 12 h. The resulting mixture was concentrated in vacuo and the residue (1.60 g) was purified by column chromatography (silica gel, 30 g, n-hexane:EtOAc = 3:2) to give compound S2 (556 mg, 42%) as a white foam. Mp: 104–108 °C. [α]D° +12.2 (c 1.00, CHCl₃). IR: νmax (KBr): 3168, 3055, 2927, 1695, 1478, 1448, 1368, 1306, 1263 cm⁻¹. 1H NMR (400 MHz, CDCl₃) δ: 1.86 (d, J = 1.0 Hz, 3H), 3.46 (dd, J = 4.0, 10.5 Hz, 1H), 3.59 (dd, J = 7.0, 10.5 Hz, 1H), 4.31 (ddd, J = 4.0, 4.5, 7.0 Hz, 1H), 5.14 (dd, J = 2.5, 8.5 Hz, 1H), 5.38 (dd, J = 4.5, 8.5 Hz, 1H), 5.68 (d, J = 2.5 Hz, 1H), 6.75–7.46 (m, 16H), 9.58 (brs, 1H); 13C NMR (75 MHz, CDCl₃) δ: 29.06, 36.12, 43.84, 52.90, 106.39, 117.95, 123.12, 143.27, 149.98, 163.13, 163.93. MS (EI): m/z = 625 (M⁺, 0.3), 384 (3.1), 382 (3.2), 368 (4.8), 331 (3.1), 366 (5.1), 243 (100). HRMS (EI): Calcd for C₃₁H₂₆Cl₃N₃O₅ [M⁺], 625.0938, found, 625.0963.

(2'R)-2'-Trifluoroacetamidothymidine (S3): A solution of compound S2 (507 mg, 0.809 mmol) in 80% AcOH (10 mL) was stirred for 24 h at room temperature. The resulting mixture was concentrated in vacuo. The obtained residue (515 mg) was dissolved in anhydrous MeOH (10 mL), then Et3N (56 µL, 0.40 mmol) and CF₃CO₂Et (70 µL, 0.89 mmol) were added to this solution at 0 °C. The reaction mixture was stirred for 1.5 h at room temperature. The resulting mixture was concentrated in vacuo and the residue (540 mg) was purified by column chromatography (silica gel, 30 g, CHCl₃:MeOH = 15:1 to 5:1) to give compound S3 (185 mg, 65%, 2 steps) as a white powder. Mp: 172–177 °C. [α]D° +1.1 (c 1.00, MeOH). IR: νmax (KBr): 3494, 3250, 2989, 2699, 2506, 1682, 1474, 1428, 1270, 1205 cm⁻¹. 1H NMR (300 MHz, CD3OD) δ: 1.82 (d, J = 1.0 Hz, 3H), 3.79 (dd, J = 4.0, 12.0 Hz, 1H), 3.85 (dd, J = 3.0, 12.0 Hz, 1H), 4.11–4.12 (m, 1H), 4.37 (dd, J = 2.5, 6.0 Hz, 1H), 4.64 (dd, J = 6.0, 8.0 Hz, 1H), 6.13 (d, J = 8.0 Hz, 1H), 7.88 (d, J = 1.0 Hz, 1H); 13C NMR (75 MHz, CDCl₃) δ: 12.56, 57.34, 62.93, 87.70, 88.82, 112.00, 117.67 (q, J = 290 Hz), 137.85, 152.65, 162.10 (q, J = 39 Hz), 166.06. MS (EI): m/z = 353 (M⁺, 2.9), 264 (2.1), 240 (10.1), 228 (30.6), 203
(10.1), 180 (86.4), 168 (31.9), 138 (15.3), 126 (55.5), 110 (24.8), 95 (19.8), 84 (38.1), 69 (100). HRMS (EI): Calcd for C_{12}H_{14}F_{3}N_{3}O_{6} [M^+] = 353.0835, found, 353.0824.

(2'R)-5'-O-(4,4'-Dimethoxytrityl)-2'-trifluoroacetamidothymidine (S4): Under a nitrogen atmosphere, DMTrCl (253 mg, 0.747 mmol) was added to a solution of compound S3 (132 mg, 0.374 mmol) in anhydrous pyridine (2.0 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2.5 h. The reaction was quenched with sat. NaHCO₃ at 0 °C, and the product was extracted with EtOAc. The combined organic layer was washed with water and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue (422 mg) was purified by column chromatography (silica gel, 15 g, CHCl₃:MeOH = 30:1) to give compound S4 (202 mg, 83%) as a white powder. Mp: 242–243 °C. [α]D²² +7.8 (c 1.00, MeOH). IR: νmax (KBr): 3444, 3263, 3062, 2932, 2838, 1728, 1702, 1607, 1508, 1469, 1382, 1302, 1252 cm⁻¹. 1H NMR (300 MHz, CD₃OD) δ: 1.32 (s, 3H), 3.41–3.44 (m, 2H), 3.80 (s, 6H), 4.21 (m, 1H), 4.46 (dd, J = 5.5, 6.0 Hz, 1H), 4.97 (dd, J = 5.5, 8.5 Hz, 1H), 6.29 (d, J = 8.5 Hz, 1H), 6.87–7.57 (m, 13H), 8.31 (s, 1H). 13C NMR (75 MHz, CDCl₃, CD₃OD) δ: 10.64, 54.45, 55.46, 63.22, 70.56, 84.50, 85.39, 86.83, 111.28, 112.71, 115.27 (q, J = 286 Hz), 120.84, 126.66, 127.48, 127.70, 129.64, 134.48, 134.72, 135.27, 136.60, 134.44, 151.05, 158.28, 157.82 (q, J = 37 Hz), 164.25. MS (EI): m/z = 655 (M⁺, 7.8), 336 (2.1), 303 (100), 288 (8.8), 228 (16.5), 180 (27.3). HRMS (EI): Calcd for C_{33}H_{32}F_{3}N_{3}O_{8} [M⁺] = 655.2141, found, 655.2133.

(2'R)-3'-O-[2-Cyanoethoxy(diisopropylamino)phosphino]-5'-O-(4,4'-dimethoxytrityl)-2'-trifluoroacetamidothymidine (S5): Under a nitrogen atmosphere, DIPEA (0.28 mL, 1.6 mmol) and i-Pr₂NP(Cl)OCH₂CH₂CN (81 μL, 0.37 mmol) were added to a solution of compound S4 (202 mg, 0.310 mmol) in anhydrous CH₂Cl₂ (2.0 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The reaction was quenched with sat. NaHCO₃ at 0 °C, and the product was extracted with CH₂Cl₂. The combined organic layer was washed with sat. NaHCO₃, water and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue (280 mg) was chromatographed (silica gel, 15 g, CHCl₃:MeOH = 30:1) to give compound S5 with a small amount of impurity (201 mg), which was reprecipitated to give compound S5 (188 mg, 71%) as a white powder. Mp: 118–123 °C. 1H NMR (400 MHz, CDCl₃) δ: 1.05–1.19 (m, 12H), 1.32 (s, 1.5H), 1.34 (s, 1.5H), 2.31–2.62 (m, 2H), 3.34–3.97 (m, 12H), 4.27 (m, 0.5H), 4.45–4.50 (m, 1H), 4.66 (dd, J = 5.5, 9.5 Hz, 0.5H), 4.94–5.11 (m, 1H), 6.39 (d, J = 9.5 Hz, 0.5H), 6.42 (d, J = 9.5 Hz, 0.5H), 6.85–7.44 (m, 13H), 7.62 (s, 0.5H), 7.68 (s, 0.5H), 7.84 (d, J = 8.5 Hz, 0.5H), 7.95 (d, J = 8.0 Hz, 0.5H), 9.67 (brs, 1H). 31P NMR (161 MHz, CDCl₃) δ: 150.32, 151.67. MS (FAB): m/z = 856 [M+H]⁺. HRMS (FAB): Calcd for C_{42}H_{50}F_{3}N_{5}O_{9}P [M+H]^+ = 856.3298, found, 856.3301.

Synthesis of oligonucleotide S6: Phosphoramidite S5 was used and the 0.2 μmol scale synthesis of oligonucleotides was performed on an automated DNA synthesizer (Applied Biosystems Expedite™ 8909) using a standard phosphoramidite protocol (DMTr-ON mode). After treatment with 28% NH₃ aq., rt, 1.5 h then 55 °C, 12 h, removal of ammonia was carried out in vacuo. The
crude S6 was purified with Sep-Pak® Plus C18 cartridges (Waters) followed by reversed-phase HPLC (Waters XBridge® MS C18 2.5 μm, 10 x 50 mm). The composition of S6 was confirmed by MALDI-TOF mass analysis. Yield and MALDI-TOF-MS data ([M-H]⁻) for S6; 26% yield, found 3646.68 (calcd 3647.38).

References

ON1a

HPLC

Column: Waters XBridge® MS C$_{18}$ 2.5 μm, 4.6 × 50 mm.
Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.
ON1b

HPLC

Column: Waters XBridge® MS C18 2.5 µm, 4.6 x 50 mm.
Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
ON1c

HPLC

Column: Waters XBridge® MS C18 2.5 μm, 4.6 × 50 mm.
Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.
ON1d

HPLC

Column: Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.
Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
ON2a

HPLC

Column: Waters XBridge® MS C18 2.5 μm, 4.6 × 50 mm.
Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.
**ON2b**

**HPLC**

Column: Waters XBridge® MS C\textsubscript{18} 2.5 μm, 4.6 × 50 mm.

Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate: 1.0 mL/min.

Column temp.: 50ºC.

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**MALDI-TOF-Mass**
ON2c

HPLC

Column: Waters XBridge® MS C\textsubscript{18} 2.5 μm, 4.6 × 50 mm.
Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
ON2d

HPLC

Column: Waters XBridge® MS C$_{18}$ 2.5 μm, 4.6 × 50 mm.
Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50ºC.

MALDI-TOF-Mass
**ON3a**

**HPLC**
- **Column**: Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
- **Gradient**: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
- **Flow rate**: 1.0 mL/min.
- **Column temp.**: 50°C.

![HPLC Chromatogram](image)

**MALDI-TOF-Mass**

![MALDI-TOF-Mass Spectrum](image)

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*S13*
ON3b

HPLC

Column: Waters XBridge® MS C_{18} 2.5 \mu m, 4.6 \times 50 mm.
Gradient: 5-11\% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50ºC.

MALDI-TOF-Mass
**ON3c**

**HPLC**

Column: Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.

Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate: 1.0 mL/min.

Column temp.: 50°C.

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**MALDI-TOF-Mass**
ON3d

HPLC

Column: Waters XBridge® MS C18 2.5 μm, 4.6 × 50 mm.
Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
ON4a

HPLC

Column: Waters XBridge® MS C\textsubscript{18} 2.5 µm, 4.6 × 50 mm.
Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
ON4b

HPLC

Column: Waters XBridge® MS C\textsubscript{18} 2.5 \(\mu\)m, 4.6 \(\times\) 50 mm.

Gradient: 7-13\% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate: 1.0 mL/min.

Column temp.: 50°C.

MALDI-TOF-Mass
ON4c

HPLC

Column: Waters XBridge® MS C18 2.5 µm, 4.6 × 50 mm.
Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
ON4d

HPLC

Column: Waters XBridge® MS C18 2.5 μm, 4.6 × 50 mm.
Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
ON5a

HPLC

Column: Waters XBridge\textsuperscript{®} MS C\textsubscript{18} 2.5 \(\mu\)m, 4.6 \(\times\) 50 mm.
Gradient: 5-11\% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
ON6a

HPLC

Column: Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.
Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.
S6

HPLC

Column: Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
Gradient: 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.
Flow rate: 1.0 mL/min.
Column temp.: 50°C.

MALDI-TOF-Mass
Compound 6

$^1{H}$ NMR

$^{13}C$ NMR
Compound 7

$^1$H NMR

$^{13}$C NMR
Compound 8
Compound 9

\[ ^1H \text{NMR} \]

\[ ^13C \text{NMR} \]
Compound 10
Compound 1 (diastereoisomers on the basis of chirality at the phosphorus)
Compound 12

\(^1\text{H NMR}\)

\(^{13}\text{C NMR}\)
Compound 13

$^1$H NMR

$^{13}$C NMR

single pulse decoupled gated NOE
Compound 14

$^1$H NMR

$^{13}$C NMR
Compound 15

\[ \text{\textsuperscript{1}H NMR} \]

\[ \text{\textsuperscript{13}C NMR} \]
Compound 16

$^1$H NMR

$^{13}$C NMR
Compound 17

\[ \text{Single pulse} \]

\[ \text{Single pulse decoupled gated NOE} \]

\[ {^1}H \text{ NMR} \]

\[ {^{13}}C \text{ NMR} \]
Compound 2 (diastereoisomers on the basis of chirality at the phosphorus)
Compound 19
Compound 20

$^1$H NMR

$^{13}$C NMR
Compound 21

\[ ^1H \text{ NMR} \]

\[ ^{13}C \text{ NMR} \]
Compound 22

$^{1}H$ NMR

$^{13}C$ NMR
Compound 23

$^{1}H$ NMR

$^{13}C$ NMR
Compound 24

$^1$H NMR

$^{13}$C NMR
Compound 25

$^1$H NMR

$^{13}$C NMR
Compound 26

$^1$H NMR

$^{13}$C NMR
Compound 3 (diastereoisomers on the basis of chirality at the phosphorus)

\[ ^{1}H \text{ NMR} \]

\[ ^{31}P \text{ NMR} \]
Compound 4 (diastereoisomers on the basis of chirality at the phosphorus)
Compound S2

$^1$H NMR

$^{13}$C NMR
Compound S3

$^1$H NMR

$^{13}$C NMR
Compound S4

1H NMR

13C NMR

S49
Compound S5 (diastereoisomers on the basis of chirality at the phosphorus)