Supporting Information

Stereocontrolled Solid-Phase Synthesis of Phosphate/Phosphorothioate (PO/PS) Chimeric Oligodeoxyribonucleotides on an Automated Synthesizer using an Oxazaphospholidine–Phosphoramidite Method

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**General information.** $^{31}$P NMR spectra were obtained at 121.5 MHz with 85% H$_3$PO$_4$ ($\delta$ 0.0) as an external standard.

$^{31}$P NMR analysis of condensation reaction of 1a with 12 in the presence of CMPT. Phosphoramidite 1a (18.6 mg, 25 µmol) and 3'-O-(DMTr)thymidine 12 (13.6 mg, 25 µmol) were placed in an NMR sample tube and dried over P$_2$O$_5$ for 48 h in vacuo. Dry CD$_3$CN (100 µL), dry CH$_3$CN (300 µL), and a CH$_3$CN solution (100 µL) of CMPT (50 µmol), which was dried over MS3A for 48 h, were added into the tube at 22 °C under argon. The reaction was then monitored over 1 h by $^{31}$P NMR at 22 °C.

![Diagram showing the condensation reaction](image)

**Figure S1.** $^{31}$P NMR spectrum obtained by the reaction of 1a with 12 in the presence of CMPT.
Scheme S1. $^{31}$P NMR analysis of stability of phosphorothioate triester in the presence of TBHP.
Figure S2. RP-HPLC profiles of crude 16a,b, 17a,b, and authentic (Rp/Sp)-mixtures obtained by non-stereoselective synthesis (shown as references). RP-HPLC was performed with a linear gradient of 0–20% CH$_3$CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min at a rate of 0.5 mL/min.
**Figure S3.** RP-HPLC profiles of purified 16a,b and 17a,b. RP-HPLC was performed with a linear gradient of 0–20% CH$_3$CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min at a rate of 0.5 mL/min.
purified

\((Rp)-d(CAGTsCAGT) (16a)\)
purified

(§p)-d(CAGT§CAGT) (16b)
purified

(Rp)-d(CG5GCCGCC) (17a)
purified

(Sp)-d(CG₃GCCGCC) (17b)
purified
all-(R$p$)-d(C$_{5}$A$_{5}$G$_{5}$TCAGTCAGT) (18a)
purified all-((Sp)-dC₈AsG₈TCAGTCAGT) (18b)
purified all-\((R_p)-d(CAGTC_{sA5G5}TCAGT)\) (19a)
purified

all-((Sp)-d(CAGTC₈As₄G₅TCAGT)

(19b))
purified all-(Rp)-d(CAGTCAGTCsAsGgT) (20a)
purified

all-(Sp)-d(CAGTCAGTC₈As₈T)

(20b)