Electronic Supplementary Information
Phosphorothioate Analogs of Glycol Nucleic Acids

Synthesis and Structural Properties of P-Stereodefined Phosphorothioate Analogs of Glycol Nucleic Acids.

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Table 1S. HRMS data for 5a-d (unseparated P-diastereomers) and physico-chemical characteristics of fast and slow-eluting pairs of enantiomers of 5a-d. A mixture of EtOAc and hexane at given ratio (v/v) was used for TLC and HPLC analyses.

|            | 5a | 5b | 5c | 5d |
|------------|----|----|----|----|
|            | OTP-G\(\)T | OTP-G\(\)A\(\)Bz | OTP-G\(\)C\(\)Bz | OTP-G\(\)G\(\)iBu |
| HRMS\(^c\) (Da) | 707.2015 | 820.2392 | 796.2280 | 802.2498 |
|            | found | 707.2015 | 820.2400 | 796.2287 | 802.2514 |
| EtOAc : hexane | 50 : 50 | 45 : 55 | 55 : 45 | 85 : 15 |
| fast/\(s\)low | f | s | f | s | f | s | f | s |
| TLC, \(R_f\) | 0.68 | 0.60 | 0.72 | 0.58 | 0.70 | 0.58 | 0.65 | 0.50 |
| HPLC\(^a\), \(R_t\) (min) | 19.2 | 21.5 | 18.0 | 23.0 | 27.0 | 31.2 | 13.5 | 17.0 |
| \(\delta^{31}\)P NMR\(^b\) (ppm) | 105.6 | 106.1 | 105.8 | 106.6 | 105.1 | 105.7 | 106.1 | 106.5 |

\(^a\) A Pursuit XRs column (10µ silica, 100 Å; 250 × 21.2 mm; flow rate 25 mL min\(^{-1}\));

\(^b\) In CDCl\(_3\);

In principle, the phosphitylation of eight \(^{\text{DMT-G}}\)N’s (obtained from 2 enantiomeric glycidols and 4 nucleobases) should provide 16 OTP-G\(\)N’s, but because 4d was obtained only from \((R)-(+)\)-glycidol we actually have obtained 14 diastereomerically different OTP-G\(\)N’s. However, Table 1S does not contain 14 data sets but only 8 (5a fast/slow to 5d fast/slow) because each of 5a-c consists of two pairs of enantiomers \((R_P\)R\(_C/S_P\)\(S_C\) and \(S_P\)R\(_C/R_P\)\(S_C\)) and within each pair the components cannot be distinguished by the chromatographic and spectroscopic methods applied (no chiral auxiliaries were used). From this perspective 5d consisted of two diastereomeric \(R_P\)\(S_C\) and \(S_P\)\(S_C\) components.
Panel A – isomer fast

Panel B – isomer slow

Figure 1S. $^{31}$P NMR spectra (CDCl$_3$) for fast- and slow-eluting 5a, panel A and B, respectively; recorded with a Bruker AV-200 spectrometer (200 MHz).
Figure 2S. $^1$H NMR spectra (CDCl$_3$) for fast-eluting and slow-eluting 5a, panel A and B, respectively; recorded with a Bruker AV-200 spectrometer (200 MHz).
Figure 3S. $^{13}$C NMR spectra (CDCl$_3$) for fast-eluting and slow-eluting 5a, panel A and B, respectively; recorded with a Bruker AV-200 spectrometer (200 MHz).
### Elemental Composition Report

**Tolerance = 5.0 mDa** / **DBE: min = -1.5, max = 50.0**  
**Element prediction: Off**  
**Number of isotope peaks used for i-FIT = 3**

**Monoisotopic Mass, Even Electron Ions**  
332 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)

Elements Used:
- C: 0-38  
- H: 0-45  
- N: 0-3  
- O: 0-8  
- P: 0-2  
- S: 0-2

**A. Antczak**  
180508_ATA_10_neg_2 9 (0.228) AM2 (Ar,40000.0,0.00,0.00); Cm (7:14-38:63)  
1: TOF MS ES-  
1.09e+005

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**Minimum:** 80.00  
**Maximum:** 100.00

| Mass     | RA  | Calc. Mass | mDa | PPM  | DBE  | i-FIT | Norm  | Conf(%) | Formula       |
|----------|-----|------------|-----|------|------|-------|-------|---------|---------------|
| 707.2015 | 100.00 | 707.2015 | 0.0 | 0.0  | 18.5 | 47.2  | 4.072 | 1.70    | C36 H40 N2 O7 P S2 |
| 707.2031 | -1.6 | -2.3      | 13.5| 47.0 | 3.873| 2.08  | C34 H45 O8 P2 S2 |
| 707.1997 | 1.8  | 2.5       | 18.5| 43.2 | 0.039| 96.22 | C37 H41 O8 P2 S |

Panel A
Elemental Composition Report

Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
444 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)

Elements Used:
C: 0-45  H: 0-47  N: 0-5  O: 0-8  P: 0-2  S: 0-2
A. Antczak
180508ATA_5_neg 10 (0.265) AM2 (Ar,40000,0,0.00,0.00); Cm (6:18-50:63)

Minimum:  80.00  5.0  10.0  -1.5
Maximum:  100.00  0.8  1.0  25.5  16.2

| Mass     | RA   | Calc. Mass | mDa | PPM | DBE | i-FIT | Norm | Conf(%) | Formula |
|----------|------|------------|-----|-----|-----|-------|------|---------|---------|
| 820.2400 | 100.00 | 820.2392   | 0.8 | 1.0 | 25.5| 16.2  | 0.000| 100.00  | C43 H43 N5 O6 P S2 |
| 820.2375 | 2.5   | 820.2352   | 3.0 | 3.0 | 25.5| 27.3  | 11.159| 0.000   | C44 H44 N5 O7 P2 S |

Panel B
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Panel C
Elemental Composition Report

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
614 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0.45  H: 0.50  N: 0.6  O: 0.8  P: 0.1  S: 0.3
A. Tomaszewaka
180518_guaG_OTP 7 (0.194) Cm (5:10-17:30)

Panel D

Figure 4S. HRMS spectra for 5a-d (panel A, B, C, and D, respectively).
Panel A.

Panel B.

Figure SS. MALDI-TOF MS spectrum for fast- and slow-eluting 5a (panel A and B, respectively). 3-hydroxyypicolinic acid (50 mg/mL in 50% ACN/H2O) and ammonium citrate dibasic (50 mg/mL in H2O) 8:1 (v/v) used as a matrix.
Figure 6S. MS data for c\(^{6}\)TMPS isolated from the mixture after detritylation of 5a.
Figure 7S. $^{31}$P NMR spectra (no deuterated solvent) for DMT-$^{15}$C$_{2}$PS$_{10}$c obtained from fast-5c and slow-5c, panel A and B, respectively. The monomer 5c was obtained from R (+)-glycidol.
Figure 8S. MALDI TOF MS spectra for RP HPLC isolated $^4\text{C}_{\text{PS}}\text{T}$ 11c obtained from fast-5c (panel A) and slow-5c (panel B). The monomer 5c was obtained from $R$-($\pm$)-glycidol. Molecular mass (calc. for $\text{C}_{17}\text{H}_{23}\text{N}_5\text{O}_9\text{PS}$) 504.44, m/z found 504.2 and 504.1.
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Figure 9S. RP HPLC profiles for three samples: 1) $^6$G$_{PS}$T 11d obtained from fast-5d (a black line), hydrolysis of $^6$G$_{PS}$T with svPDE (a red line) and hydrolysis $^6$G$_{PS}$T with nP1 (a blue line). An ACE 5 C 18-AR Column, 250×4.6 mm; flow rate 1mL/min, A buffer: 0.1 M TEAB, B buffer: 40% CH$_3$CN in 0.1 TEAB, Gradient: 0-50% of B buffer in 20 min, 50-100% of B buffer in 7 min.

Figure 10S. RP HPLC profiles for three samples: 1) $^6$G$_{PS}$T 11d obtained from slow-5d (a black line), hydrolysis of 11d with svPDE (a red line) and hydrolysis of 11d with nP1 (a blue line). An ACE 5 C 18-AR column, 250×4.6 mm; flow rate 1mL/min, A buffer: 0.1 M TEAB, B buffer: 40% CH$_3$CN in 0.1 TEAB, Gradient: 0-50% of B buffer in 20 min.
Figure 11S. HPLC profiles recorded for four samples: 1) d(CPS-T), a mixture of both P-diastereomers – a black line; 2) d(CPS-T), a mixture of both P-diastereomers, treated with svPDE – a blue line; 3) $^{10c}$CPS-T10c (derived from fast-5c) treated with svPDE – a red line; 4) $^{10c}$CPS-T10c (derived from fast-5c) – a pink line. A Kinetex 5u C18 column, 250x4.6 mm; flow rate 1mL/min, A buffer: 0.1 M TEAB, B buffer: 40% CH$_3$CN in 0.1 TEAB, Gradient: 0-50% of B buffer in 20 min.
Figure 12S. Decay of the trityl cation absorption (after the 10th coupling) during the synthesis of (U$_{25}$)$_{11}$dA as measured photometrically by the internal monitor in the automated H-6 DNA/RNA synthesizer. From the total yield 94% a repetitive yield 99.2% was calculated (0.992$^{10}$).
Figure 13S. RP HPLC analysis of \( (^3\text{G}_{\text{PS}})^{11}\text{dA} \) after treatment with DBU. A Phenomenex Polymer X column, 10um RP-1 100Å, 250x10.0mm; Buffers: A = 0.1 M TEAB, B = 40% CH\(_3\)CN in 0.1 M TEAB; flow rate 2.5 ml/min;
Gradient program:

| t (min) | %B |
|--------|----|
| 0      | 0  |
| 10     | 50 |
| 12     | 70 |
| 14     | 100|
| 20     | 100|
Figure 14S. MALDI-TOF MS analysis of the fraction collected during RP HPLC analysis of (G_U_{PS})_{11}dA after treatment with DBU (a broad peak eluting at 15.65 min, see Figure 13S).
Figure 15S. Decay of the trityl cation absorption during the manual synthesis of \( \text{SP-17} \) (A\(^{\text{T}}\)TG\(^{\text{G}}\)CG\(^{\text{C}}\)CAT) measured photometrically.
Figure 16S. RP HPLC profile for the detritylated S_p-17 oligomer. A Kinetex 5μ C18 column, 250x4.6 mm; flow rate 1mL/min, A buffer: 0.1 M TEAB, B buffer: 40% CH_3CN in 0.1 TEAB, Gradient: 0-100% of B buffer in 22 min.
Figure 17S. MALDI-TOF MS spectrum for Sp-17 oligomer; molecular mass calculated 2395, found 2394.1
Figure 18S: Increase of UV absorption at 260 nm in melting experiments for selfcomplementary oligomers 15-19 (dissolved in pH 7.2 buffer containing 10 mM Tris-HCl, 100 mM NaCl, and 10 mM MgCl₂).

Figure 19S: Increase of UV absorption at 260 nm in melting experiments for selfcomplementary oligomers 14, 16 and heteroduplexes 14/16 and 15/17 (dissolved in pH 7.2 buffer containing 10 mM Tris-HCl, 100 mM NaCl, and 10 mM MgCl₂).
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**Table 2S.** Melting temperatures for mixtures of Rₚ-PS-(GNA/DNA) 14 or 16, and Sₚ-PS-(GNA/DNA) 15 or 17 with DNA (d(ATGCGCAT)) or (m)RNA ((2'-OMe)-AUGCGCAU) templates. Melting temperatures for homoduplexes DNA/DNA and (m)RNA/(m)RNA are given as the reference. Temperature gradients of 1°C/min for annealing and 0.5°C/min for melting were applied.

| template | DNA (m)RNA | 5’-(G A T G G C G) -3’ | 5’-(A G T G G C C G A T) -3’ |
|----------|------------|-------------------------|-----------------------------|
|          |            | Rₚ-14 | Sₚ-15 | Rₚ-16 | Sₚ-17 |
| DNA d(ATGCGCAT) | 43 | × | 41 | 42 | 42 | 41 |
| (m)RNA (2’-OMe)-AUGCGCAU | × | 62 | 59 | 60 | 59 | 58 |
Figure 20S: Increase of UV absorption at 260 nm in melting experiments for selfcomplementary DNA, (m)RNA and for mixtures DNA/(m)RNA, 14/(m)RNA and 16/(m)RNA (dissolved in pH 7.2 buffer containing 10 mM Tris-HCl, 100 mM NaCl, and 10 mM MgCl₂).
Figure 21S: CD spectra for the selfcomplementary oligomer (m)RNA and its mixture with 14 or 16 (dissolved in pH 7.2 buffer containing 10 mM Tris-HCl, 100 mM NaCl, and 10 mM MgCl₂) recorded at room temperature.