Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information
Phosphorothioate Analogs of Glycol Nucleic Acids

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

Agnieszka Tomaszewska-Antczak, Anna Maciaszek, Katarzyna Jastrzębska, Barbara Mikołajczyk, Piotr Guga

Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Department of Bioorganic Chemistry, Sienkiewicza 112. 90-363 Łódź, Poland

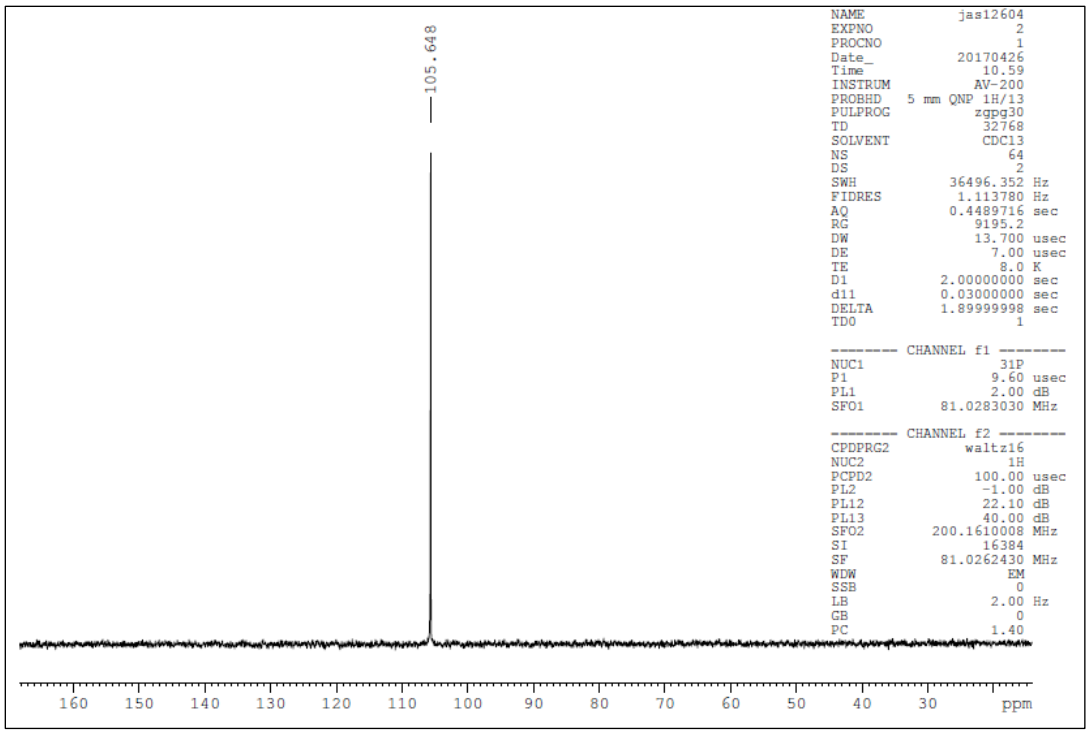
Table 1S. HRMS data for **5a**-**d** (unseparated P-diastereomers) and physico-chemical characteristics of *fast* and *slow*-eluting pairs of enantiomers of **5a**-**d**. A mixture of EtOAc and hexane at given ratio (v/v) was used for TLC and HPLC analyses.

	5a OTP-^{G}T		**5b** OTP-^{G}A^{Bz}		**5c** OTP-^{G}C^{Bz}		**5d** OTP-^{G}G^{iBu}	
HRMS[c] (Da) calc.	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/ slow	*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
δ ^{31}P NMR[b] (ppm)	105.6	106.1	105.8	106.6	105.1	105.7	106.1	106.5

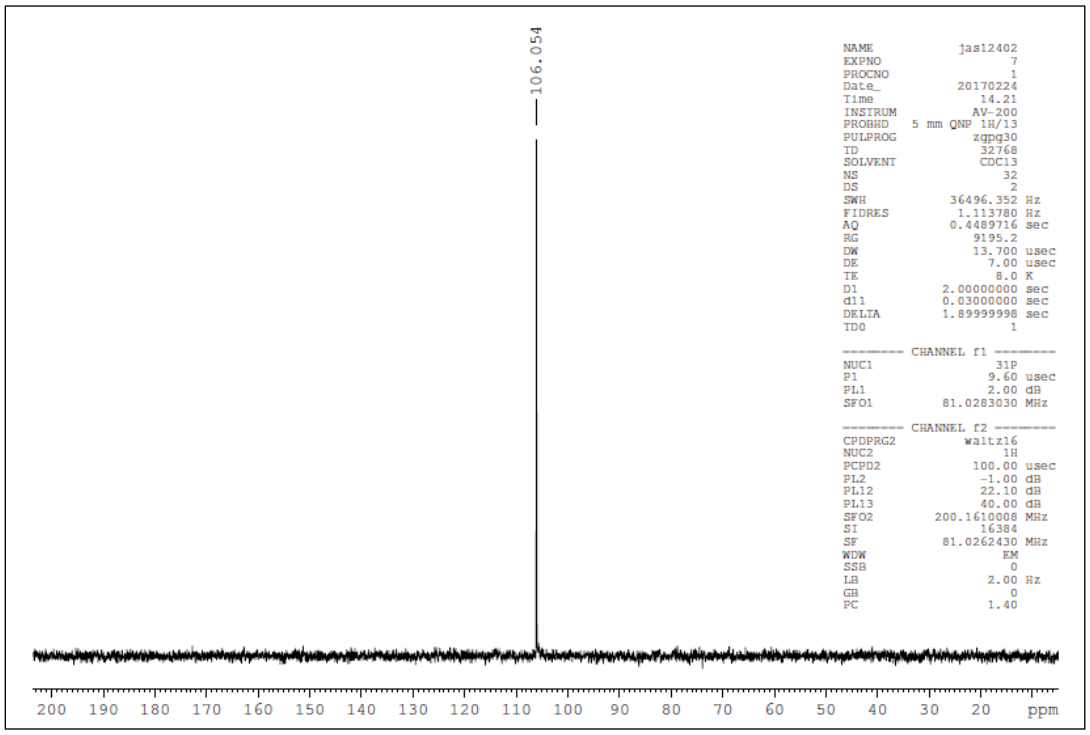
[a] A Pursuit XRs column (10μ silica, 100 Å; 250 × 21.2 mm; flow rate 25 mL min^{-1});

[b] In $CDCl_3$;

In principle, the phosphitylation of eight $^{DMT\text{-}G}$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_PR_C/S_PS_C and S_PR_C/R_PS_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_PS_C and S_PS_C components.

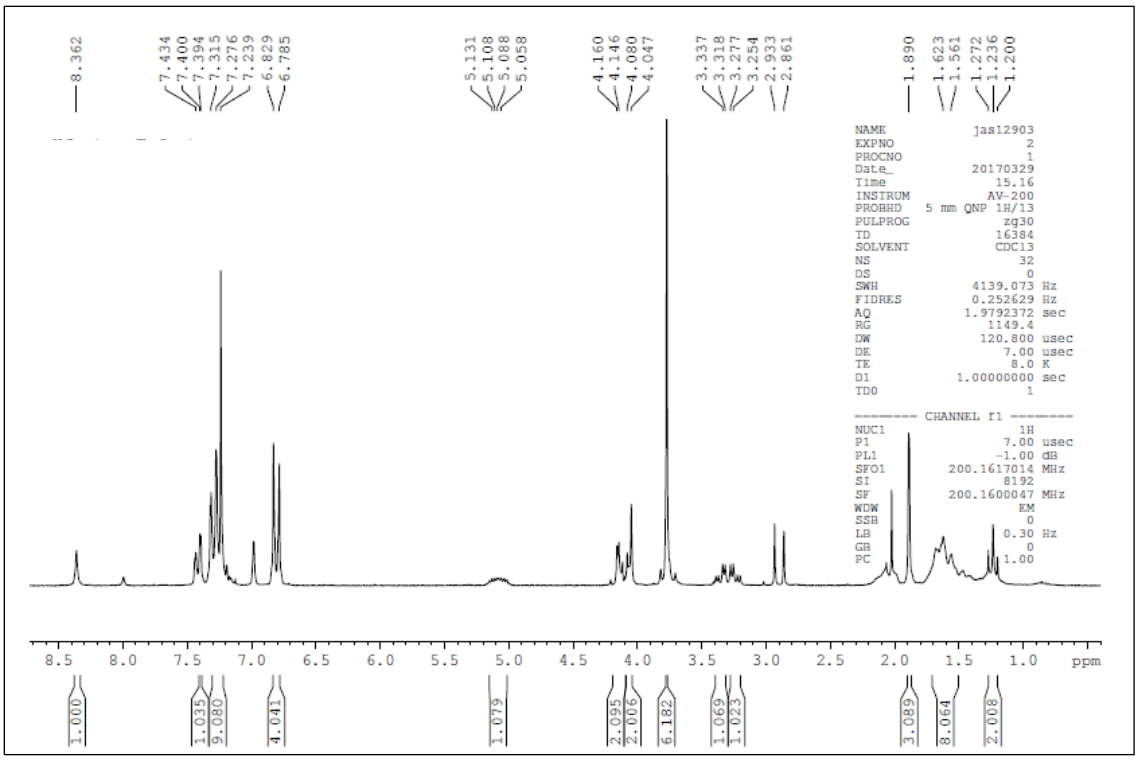


Panel A – isomer *fast*

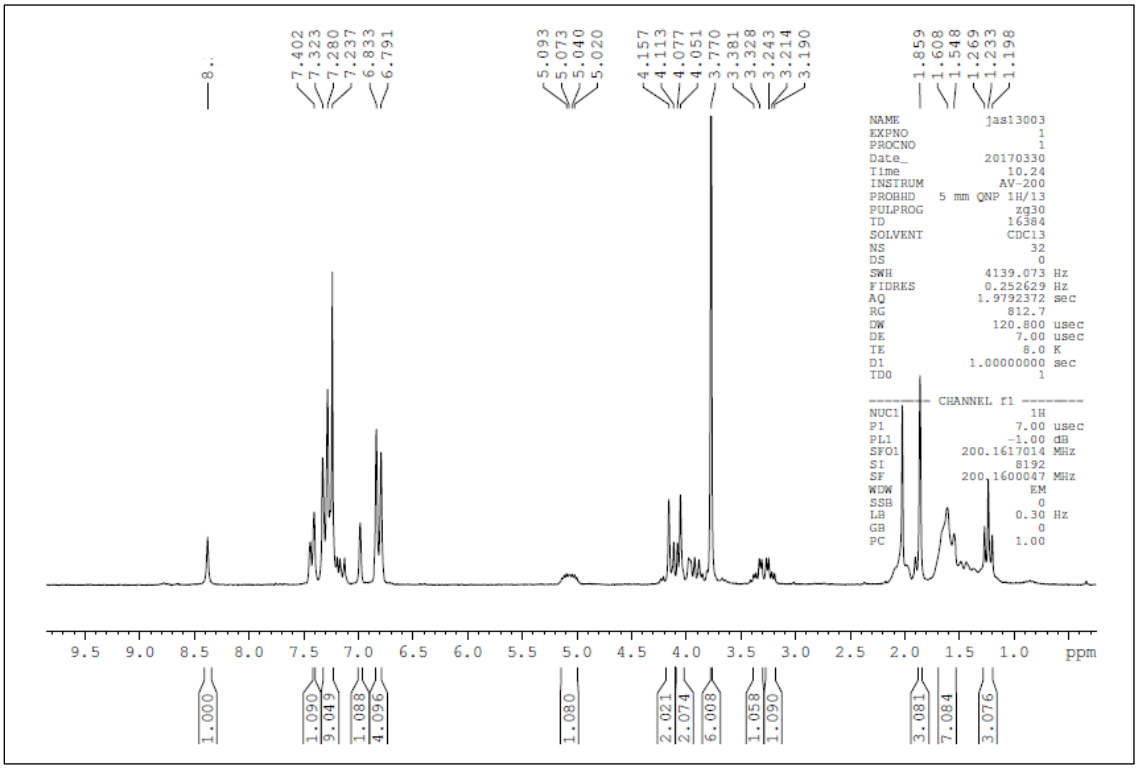


Panel B – isomer *slow*

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).

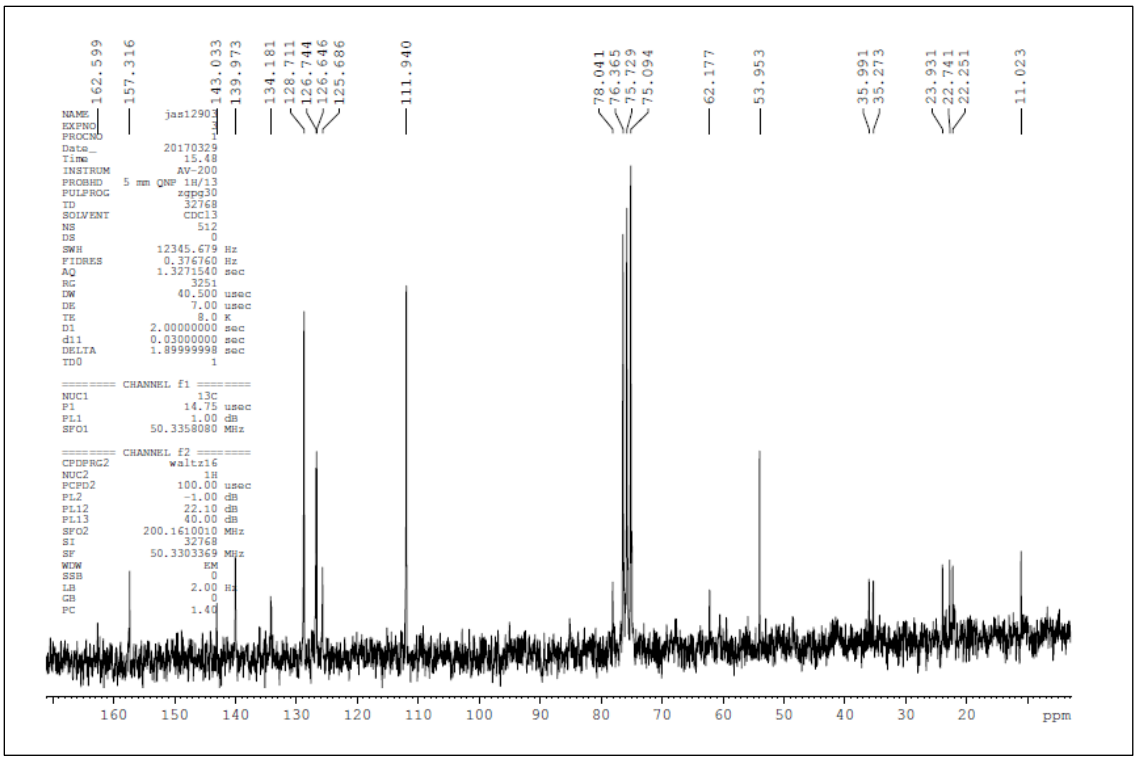


Panel A

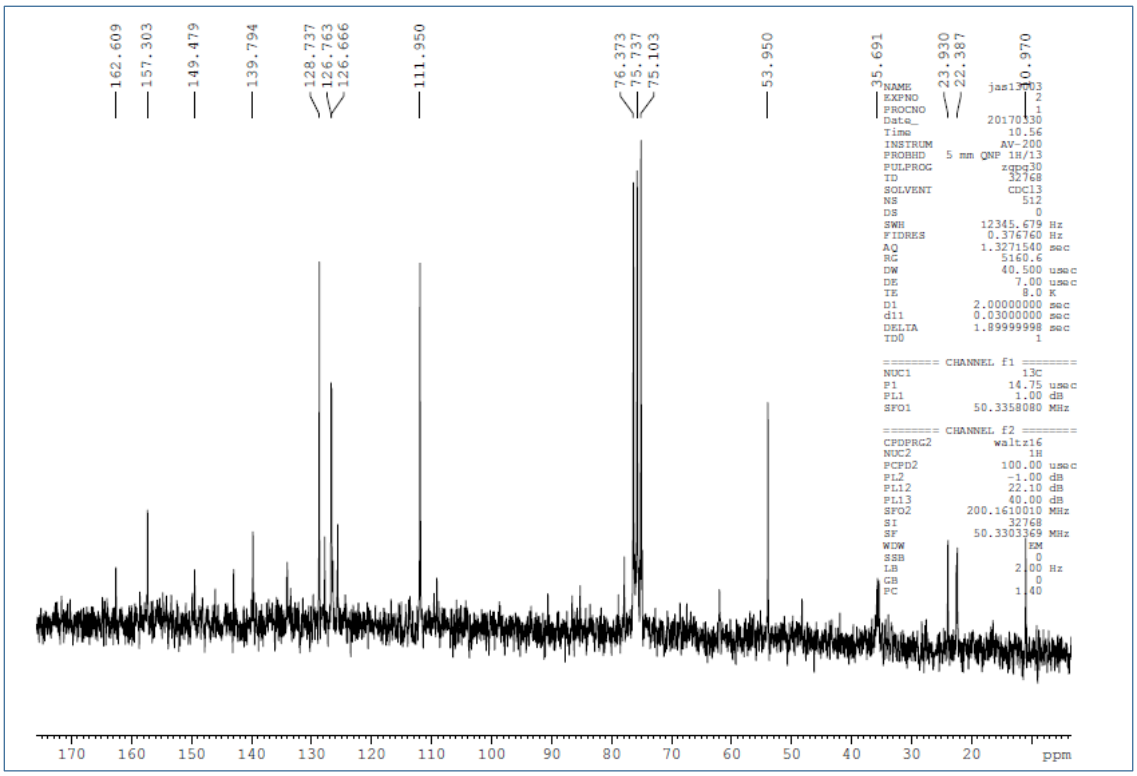


Panel B

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).



Panel A



Panel B

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).

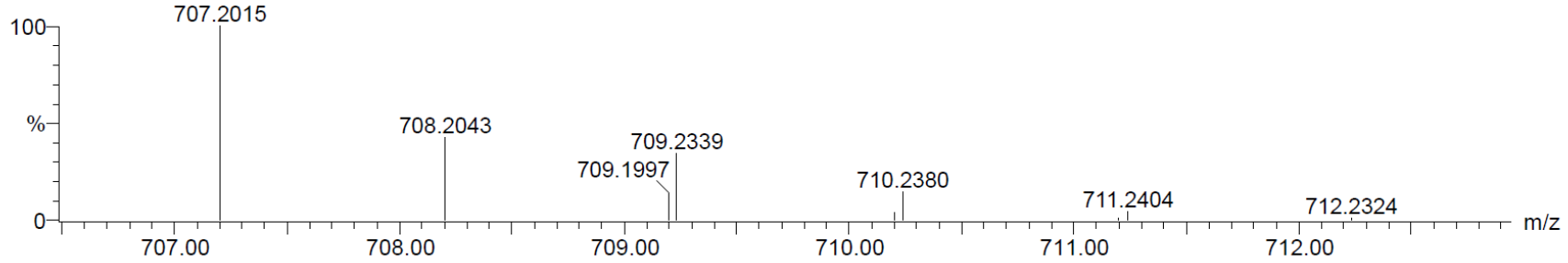
Elemental Composition Report

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



Minimum:	80.00				-1.5				
Maximum:	100.00		5.0	10.0	50.0				
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

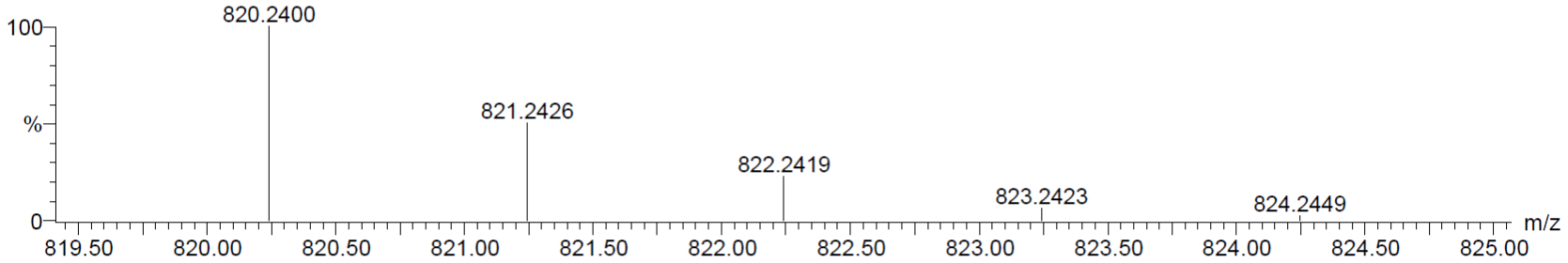
Elemental Composition Report

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
180508_ATA_5_neg 10 (0.265) AM2 (Ar,40000.0,0.00,0.00); Cm (6:18-50:63)

1: TOF MS ES-
4.76e+005



Minimum:	80.00				-1.5				
Maximum:	100.00		5.0	10.0	50.0				
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	3.0	25.5	27.3	11.159	0.00	C44 H44 N3 O7 P2 S

Panel B

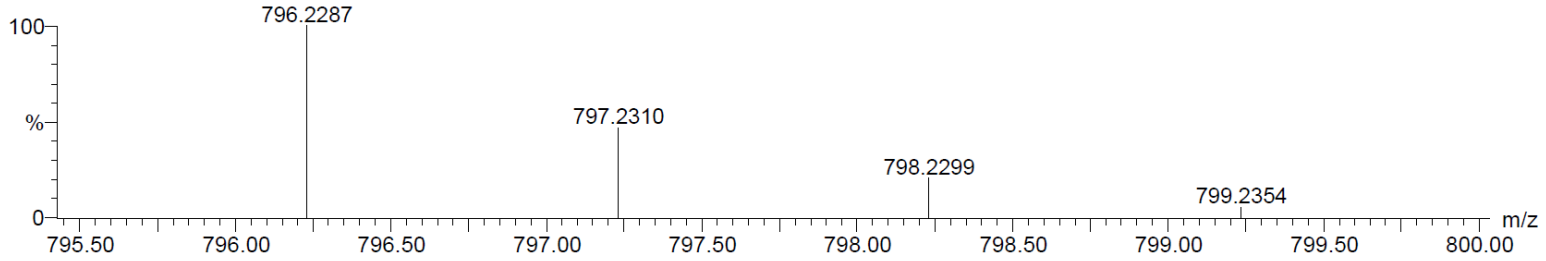
Elemental Composition Report

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
496 formula(e) evaluated with 6 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 S: 0-2 P: 0-2

A. Antczak
180508_ATA_1_neg_2 10 (0.265) AM2 (Ar,40000.0,0.00,0.00); Cm (6:16-19:62)

1: TOF MS ES-
8.52e+004



Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
Minimum:	80.00				-1.5				
Maximum:	100.00		5.0	10.0	50.0				
796.2287	100.00	796.2280	0.7	0.9	23.5	17.3	1.033	35.59	C42 H43 N3 O7 S2 P
		796.2276	1.1	1.4	28.5	22.0	5.716	0.33	C44 H40 N5 O4 S P2
		796.2310	-2.3	-2.9	23.5	16.8	0.485	61.58	C41 H44 N5 O4 S2 P2
		796.2264	2.3	2.9	28.5	20.3	4.008	1.82	C44 H38 N5 O6 S2
		796.2263	2.4	3.0	23.5	21.6	5.277	0.51	C43 H44 N O8 S P2
		796.2246	4.1	5.1	28.5	22.6	6.313	0.18	C45 H39 N3 O7 S P

Panel C

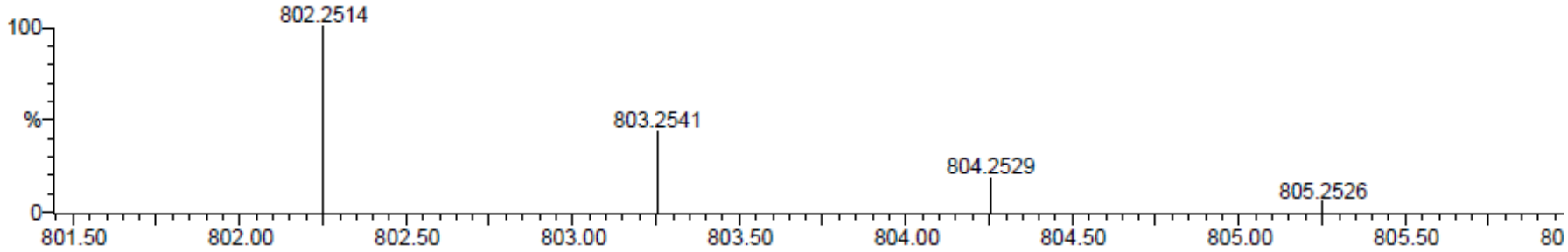
Elemental Composition Report

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

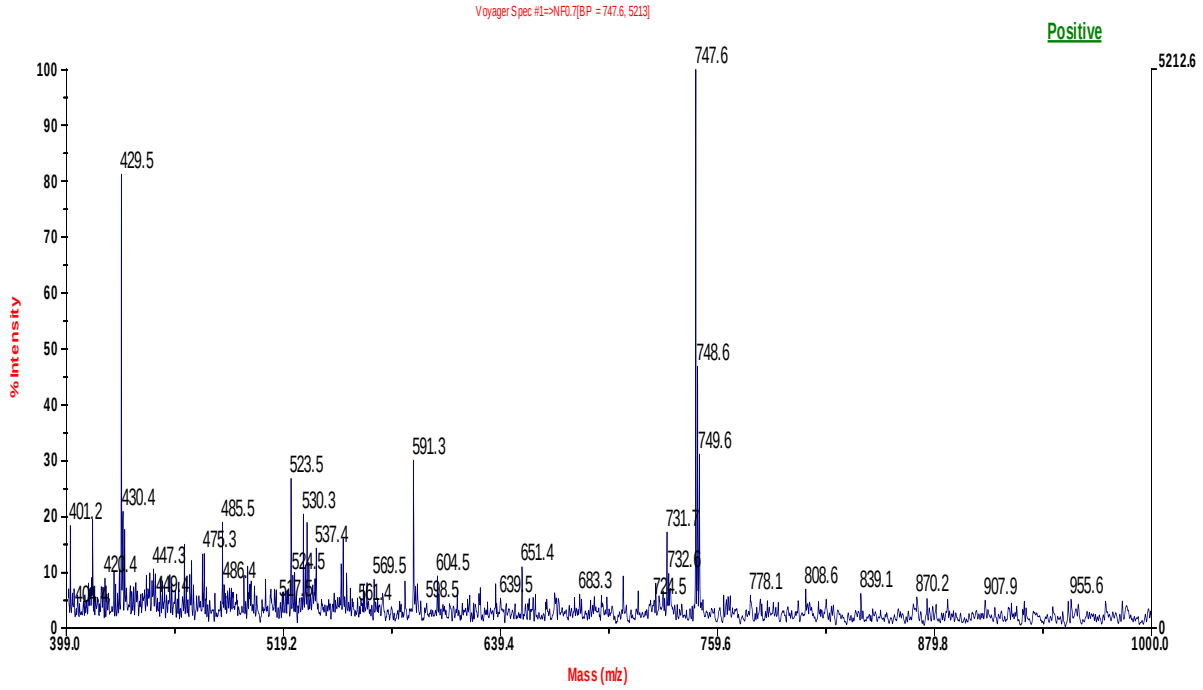
A.Tomaszewska
180518_guaG_OTP 7 (0.194) Cm (5:10-17:30)



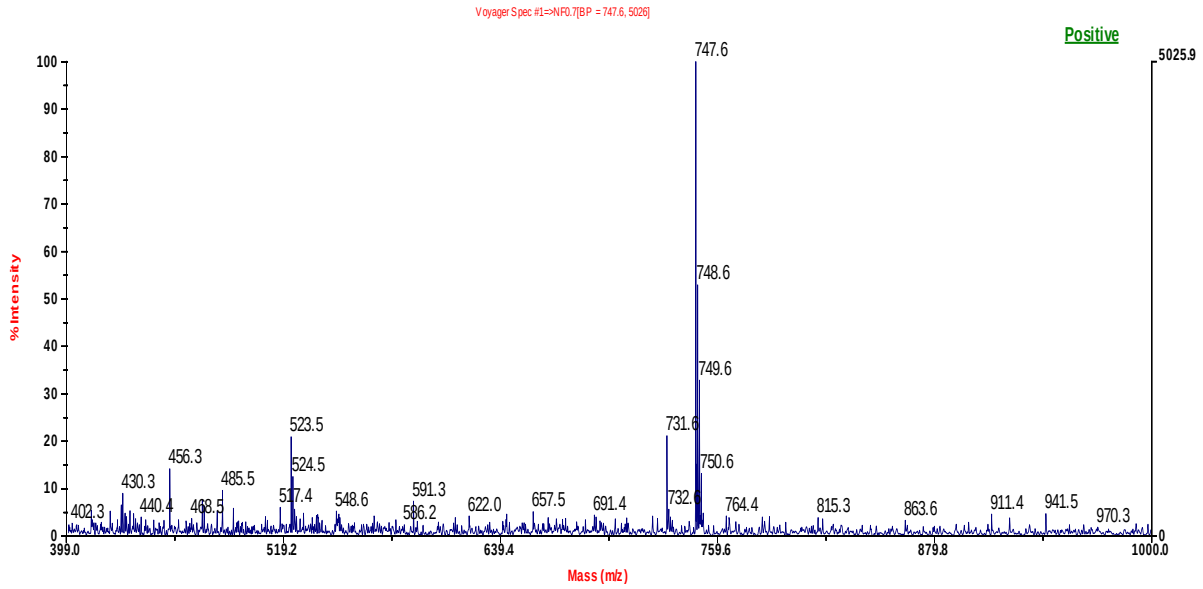
Minimum:				-1.5				
Maximum:		15.0	3.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
802.2514	802.2498	1.6	2.0	21.5	26.9	0.041	96.02	C40 H45 N5 O7 P S2
	802.2532	-1.8	-2.2	16.5	30.1	3.225	3.98	C37 H49 N5 O7 P S3

Panel D

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



Panel A.



Panel B.

Figure 5S. MALDI-TOF MS spectrum for *fast*- and *slow*-eluting **5a** (panel A and B, respectively). 3-hydroxypicolinic acid (50 mg/mL in 50% ACN/H_2O) and ammonium citrate dibasic (50 mg/mL in H_2O) 8:1 (v/v)] used as a matrix.

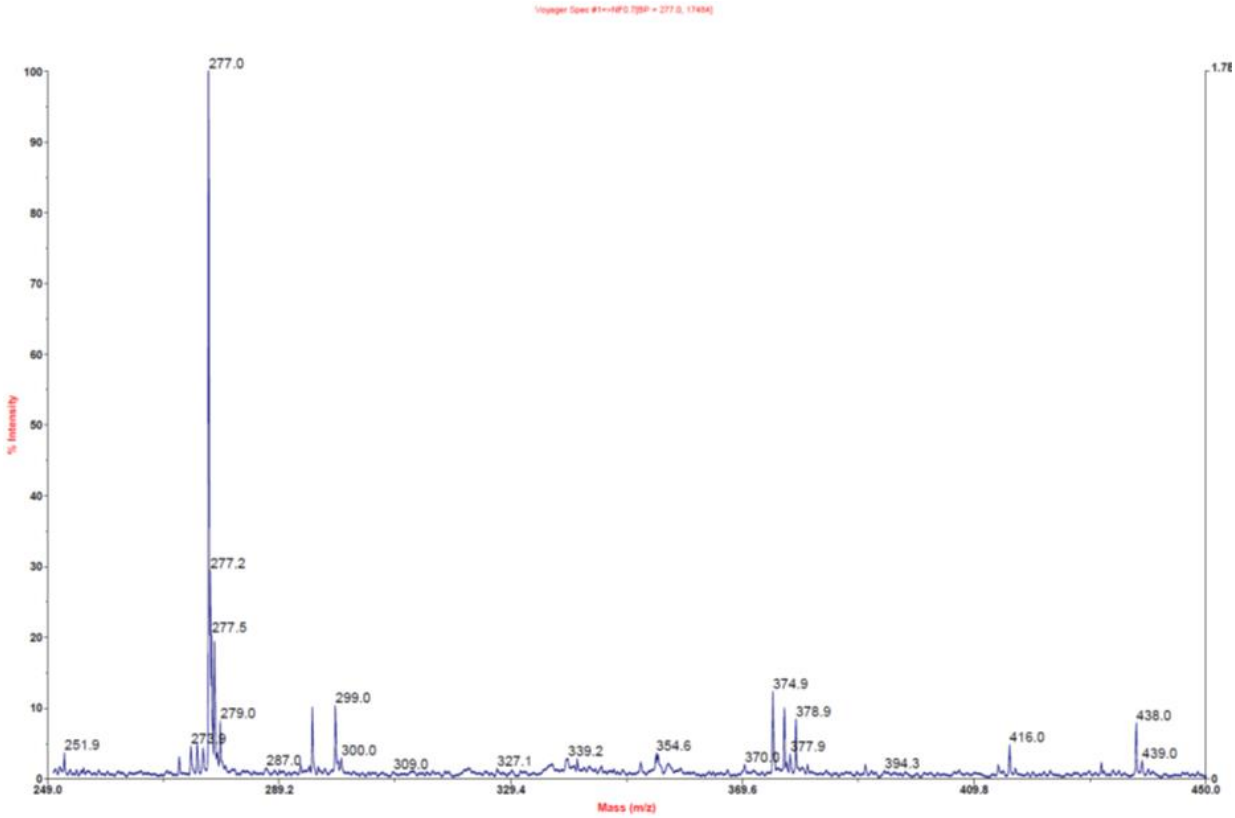
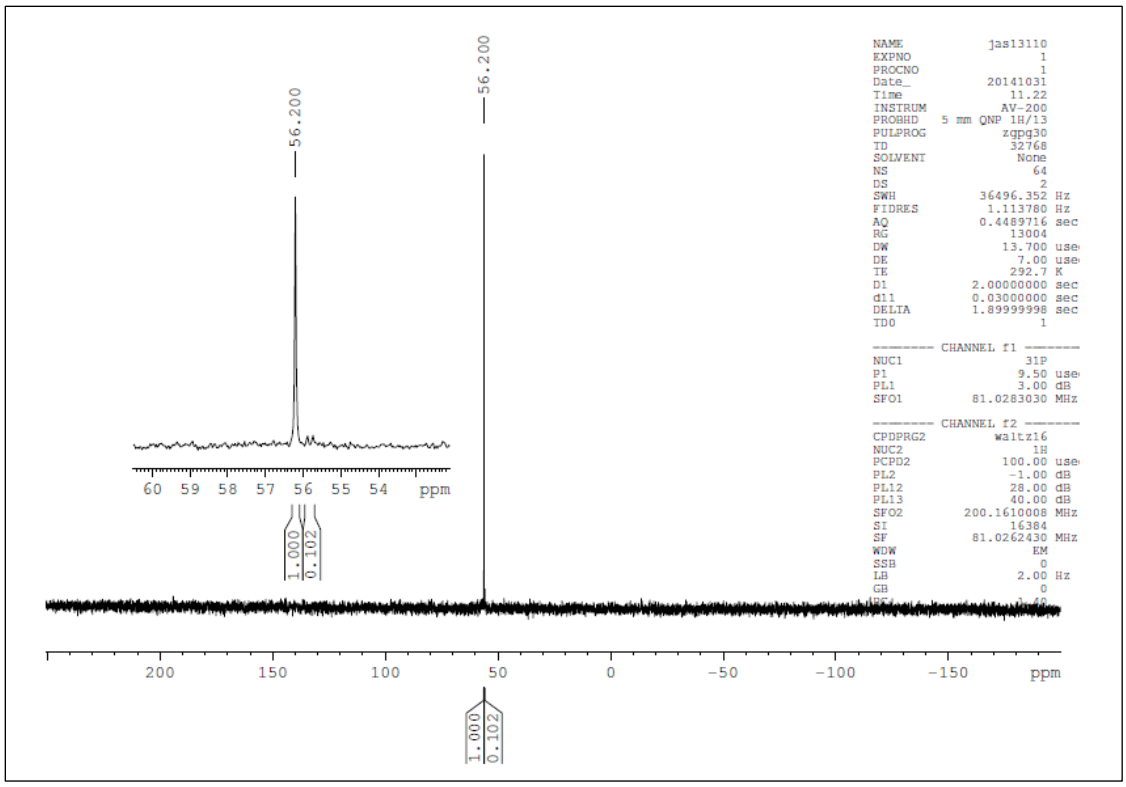
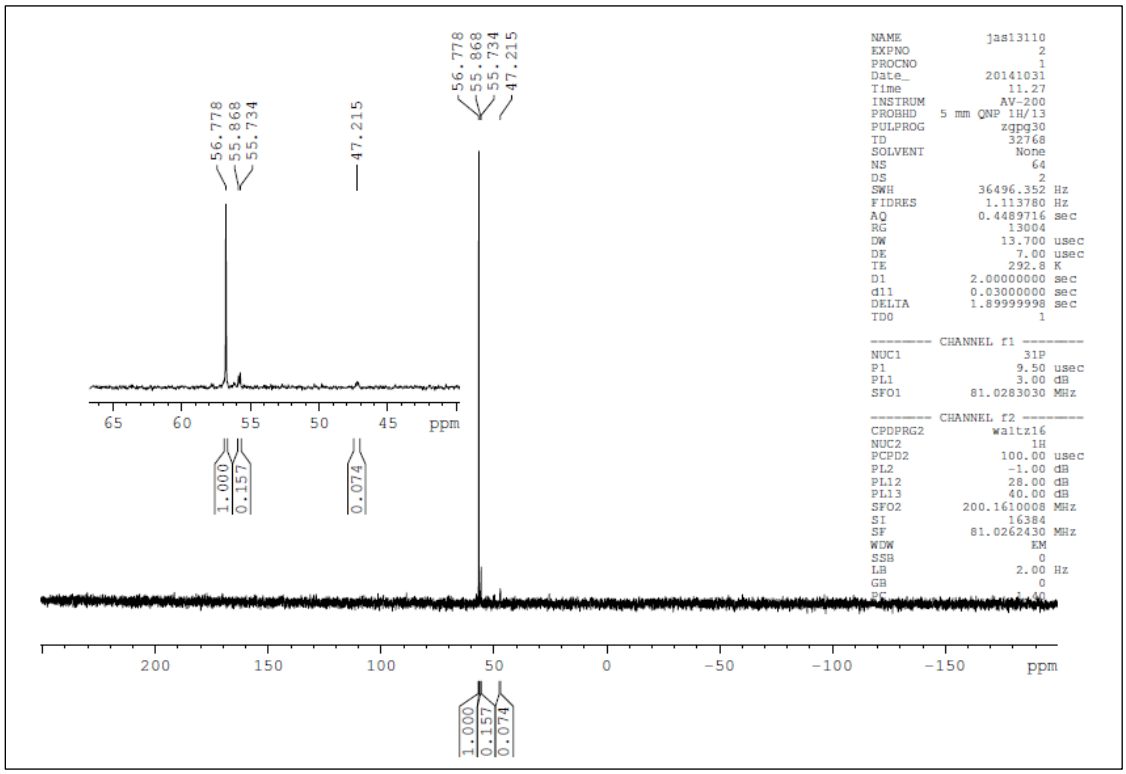


Figure 6S. MS data for c^{G}TMPS isolated from the mixture after detritylation of **5a**.

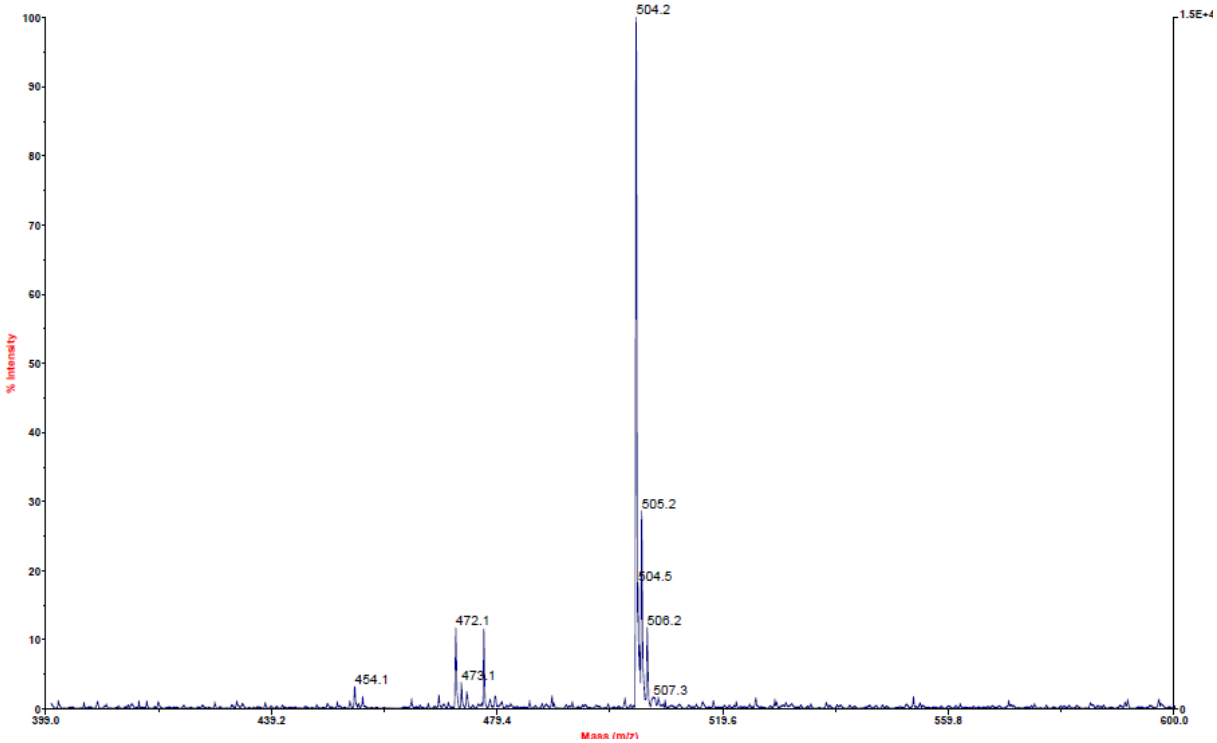


Panel A

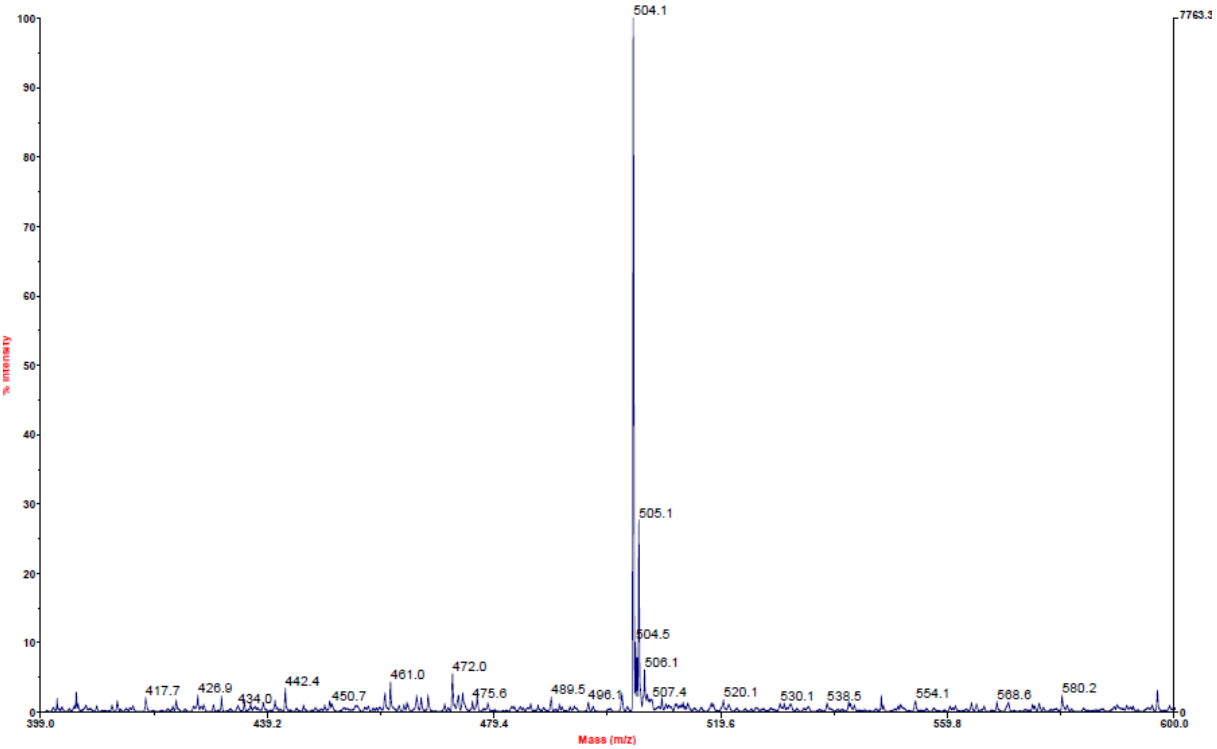


Panel B

Figure 7S. ^{31}P NMR spectra (no deuterated solvent) for $^{DMT\text{-}g}C^{Bz}{}_{PS}T_{Ac}$ **10c** obtained from *fast*-**5c** and *slow*-**5c,** panel A and B, respectively. The monomer **5c** was obtained from *R*-(+)-glycidol.



Panel A



Panel B

Figure 8S. MALDI TOF MS spectra for RP HPLC isolated $^{g}C_{PS}T$ **11c** obtained from *fast*-**5c** (panel A) and *slow*-**5c** (panel B). The monomer **5c** was obtained from *R*-(+)-glycidol. Molecular mass (calc. for $C_{17}H_{23}N_5O_9PS$) 504.44, m/z found 504.2 and 504.1.

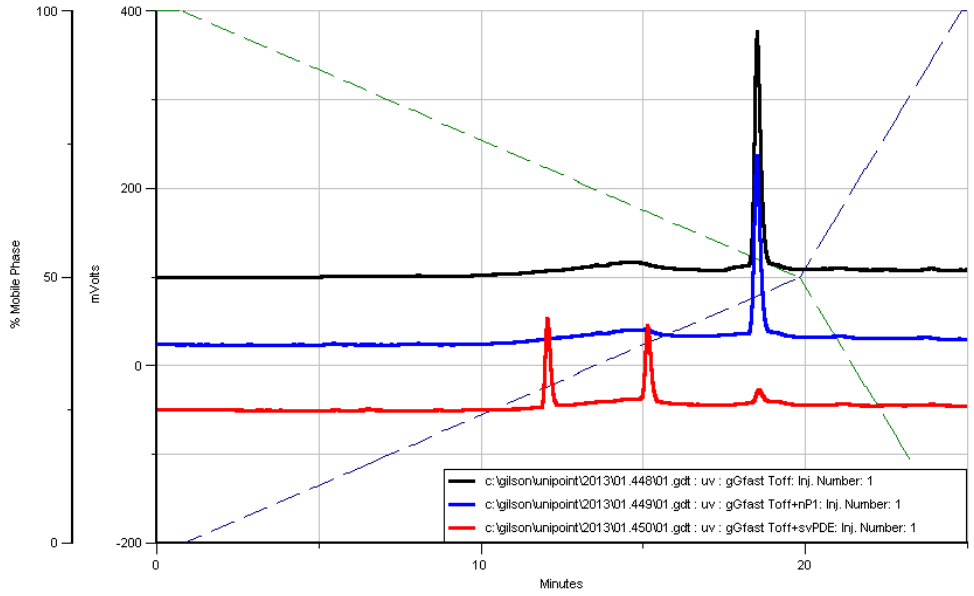


Figure 9S. RP HPLC profiles for three samples: 1) $^{G}G_{PS}T$ **11d** obtained from *fast*-**5d** (a black line), hydrolysis of $^{G}G_{PS}T$ with svPDE (a red line) and hydrolysis $^{G}G_{PS}T$ with nP1 (a blue line). An ACE 5 C 18-AR Column, 250×4.6 mm; flow rate 1mL/min, A buffer: 0.1 M TEAB, B buffer: 40% CH_3CN in 0.1 TEAB, Gradient: 0-50% of B buffer in 20 min, 50-100% of B buffer in 7 min.

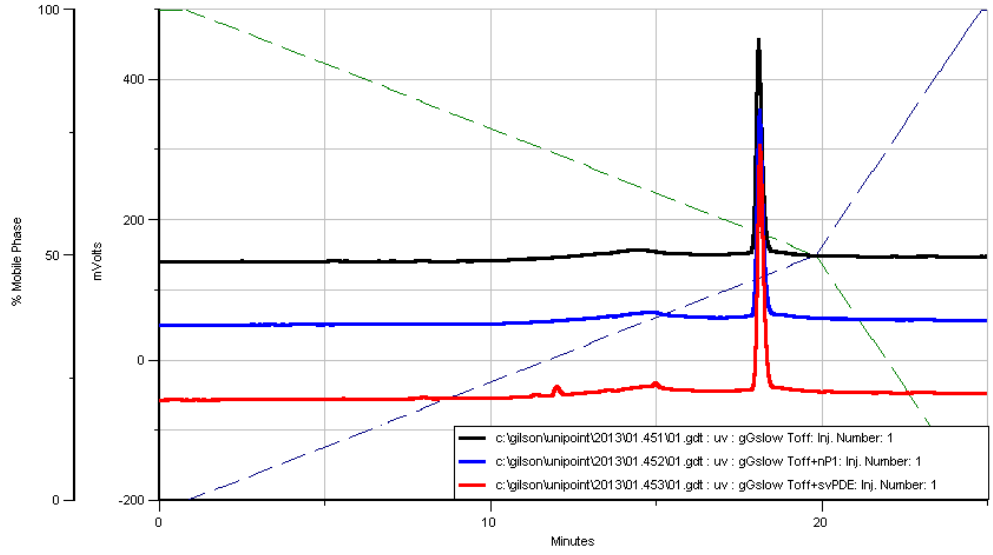


Figure 10S. RP HPLC profiles for three samples: 1) $^{G}G_{PS}T$ **11d** obtained from *slow*-**5d** (a black line), hydrolysis of **11d** with svPDE (a red line) and hydrolysis of **11d** with nP1 (a blue line). An ACE 5 C 18-AR column, 250×4.6 mm; flow rate 1mL/min, A buffer: 0.1 M TEAB, B buffer: 40% CH_3CN in 0.1 TEAB, Gradient: 0-50% of B buffer in 20 min.

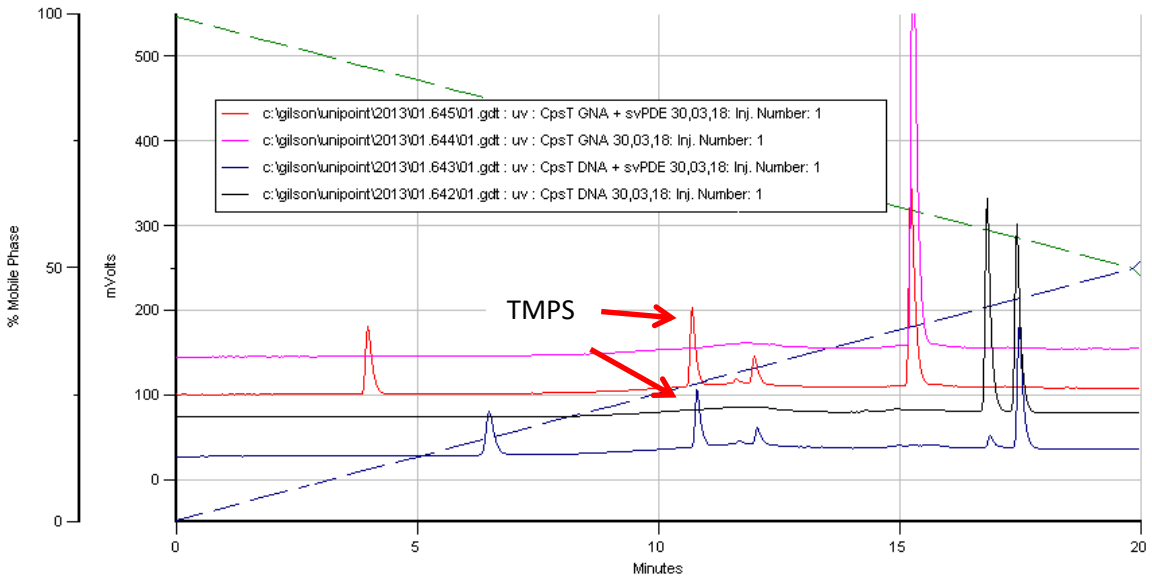


Figure 11S**.** HPLC profiles recorded for four samples: 1) d(C_{PS}T), a mixture of both P-diastereomers – a black line; 2) d(C_{PS}T), a mixture of both P-diastereomers, treated with svPDE – a blue line; 3) $^{G}C_{PS}$T **10c** (derived from *fast*-**5c**) treated with svPDE – a red line; 4) $^{G}C_{PS}$T **10c** (derived from *fast*-**5c**) – a pink line. A Kinetex 5µ C18 column, 250×4.6 mm; flow rate 1mL/min, A buffer: 0.1 M TEAB, B buffer: 40% CH_3CN in 0.1 TEAB, Gradient: 0-50% of B buffer in 20 min.

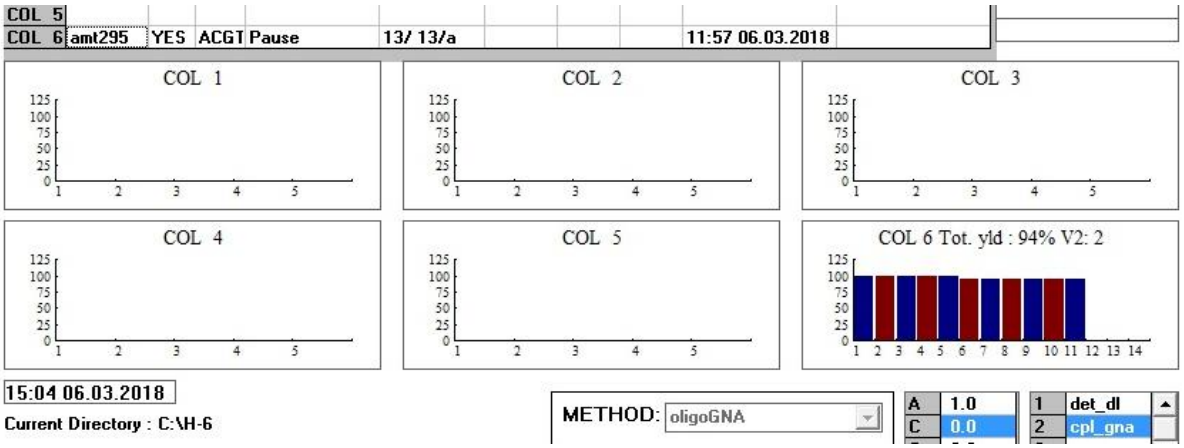


Figure 12S. Decay of the trityl cation absorption (after the 10th coupling) during the synthesis of ($^{G}U_{PS}$)$_{11}$dA as measured photometrically by the internal monitor in the automated H-6 DNA/RNA synthesizer. From the total yield 94% a repetitive yield 99.2% was calculated (0.992^10).

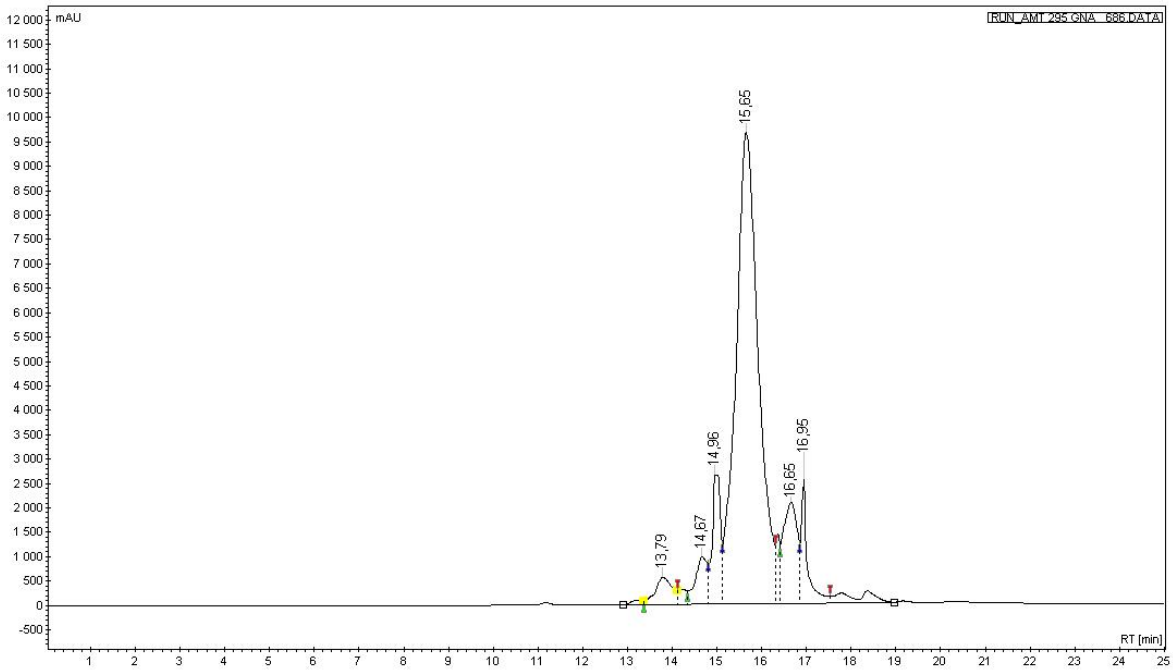


Figure 13S. RP HPLC analysis of $(^{G}U_{PS})_{11}dA$ after treatment with DBU. A Phenomenex Polymer X column, 10um RP-1 100Å, 250x10.0mm; Buffers: A = 0.1 M TEAB, B= 40% CH_3CN in 0.1 MTEAB; flow rate 2.5 ml/min;
Gradient program:

t (min)	%B
0	0
10	50
12	70
14	100
20	100

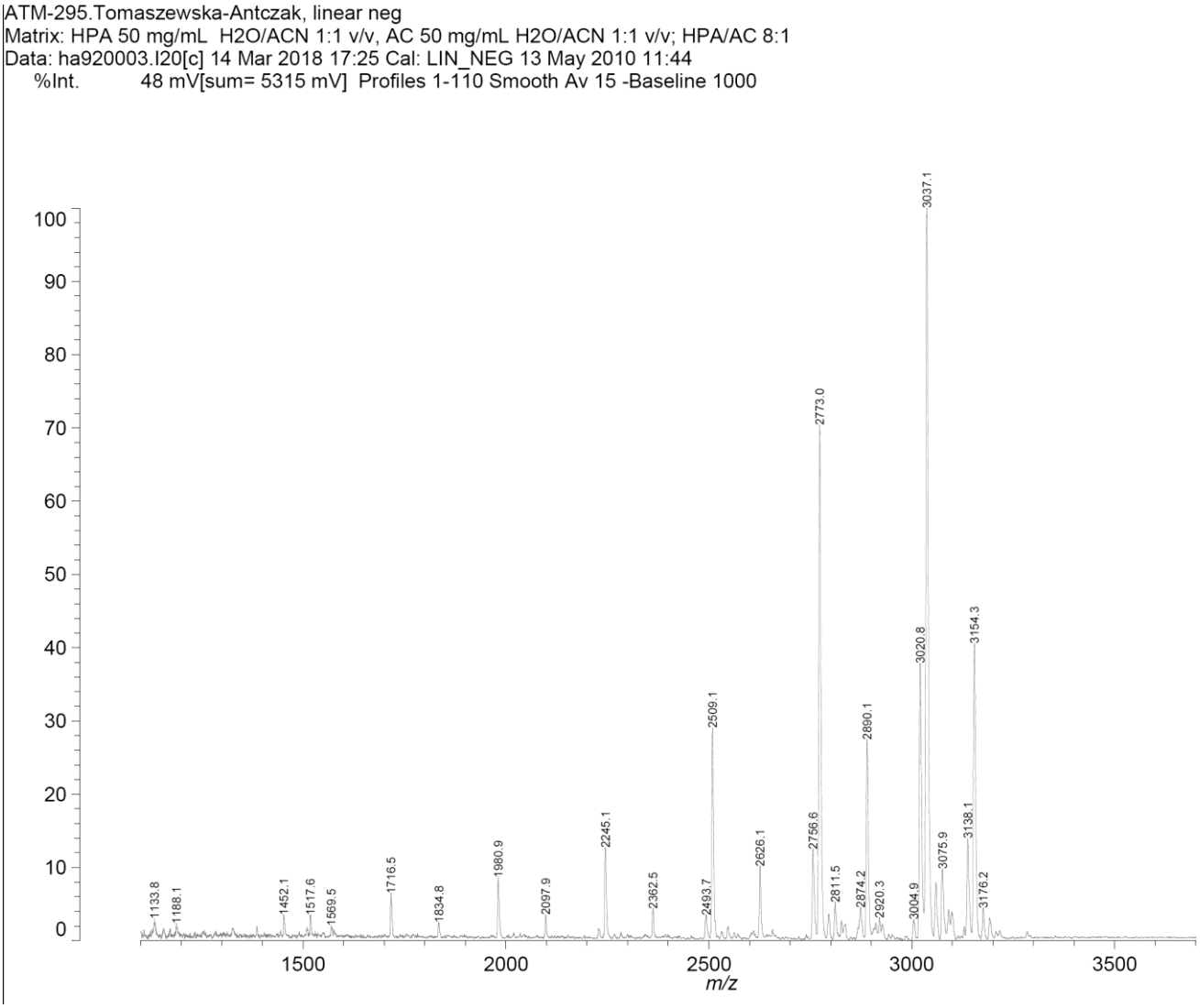


Figure 14S. MALDI-TOF MS analysis of the fraction collected during RP HPLC analysis of $(^{G}U_{PS})_{11}dA$ after treatment with DBU (a broad peak eluting at 15.65 min, see Figure 13S).

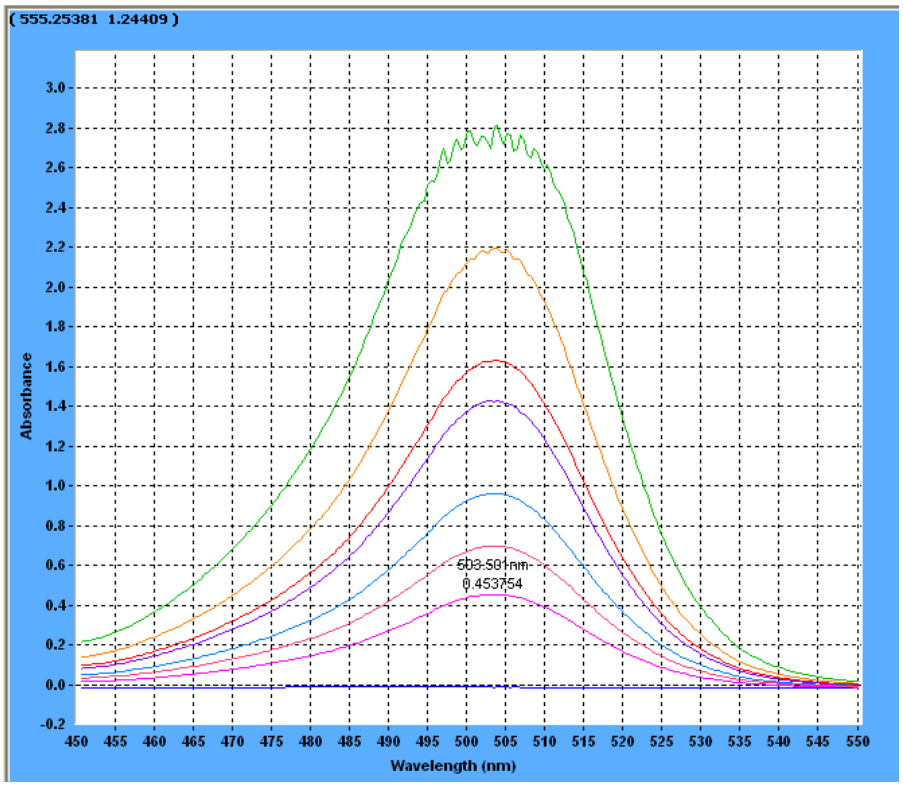


Figure 15S. Decay of the trityl cation absorption during the manual synthesis of S_P-**17** (A^{G}TG^{G}CG^{G}CAT) measured photometrically.

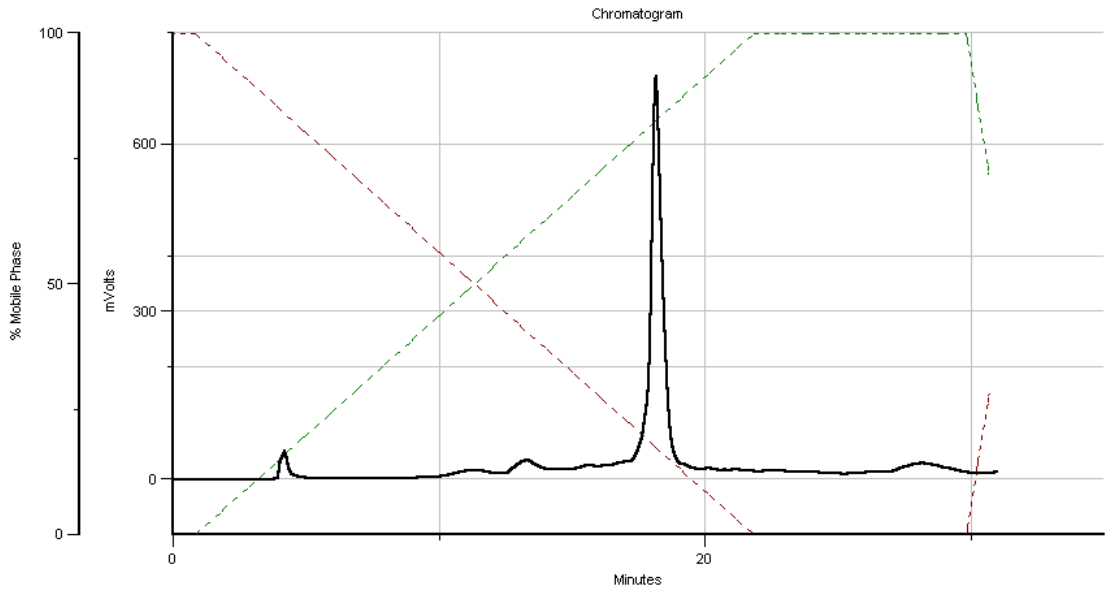


Figure 16S. RP HPLC profile for the detritylated S_P-**17** oligomer. A Kinetex 5µ C18 column, 250×4.6 mm; flow rate 1mL/min, A buffer: 0.1 M TEAB, B buffer: 40% CH_3CN in 0.1 TEAB, Gradient: 0-100% of B buffer in 22 min.

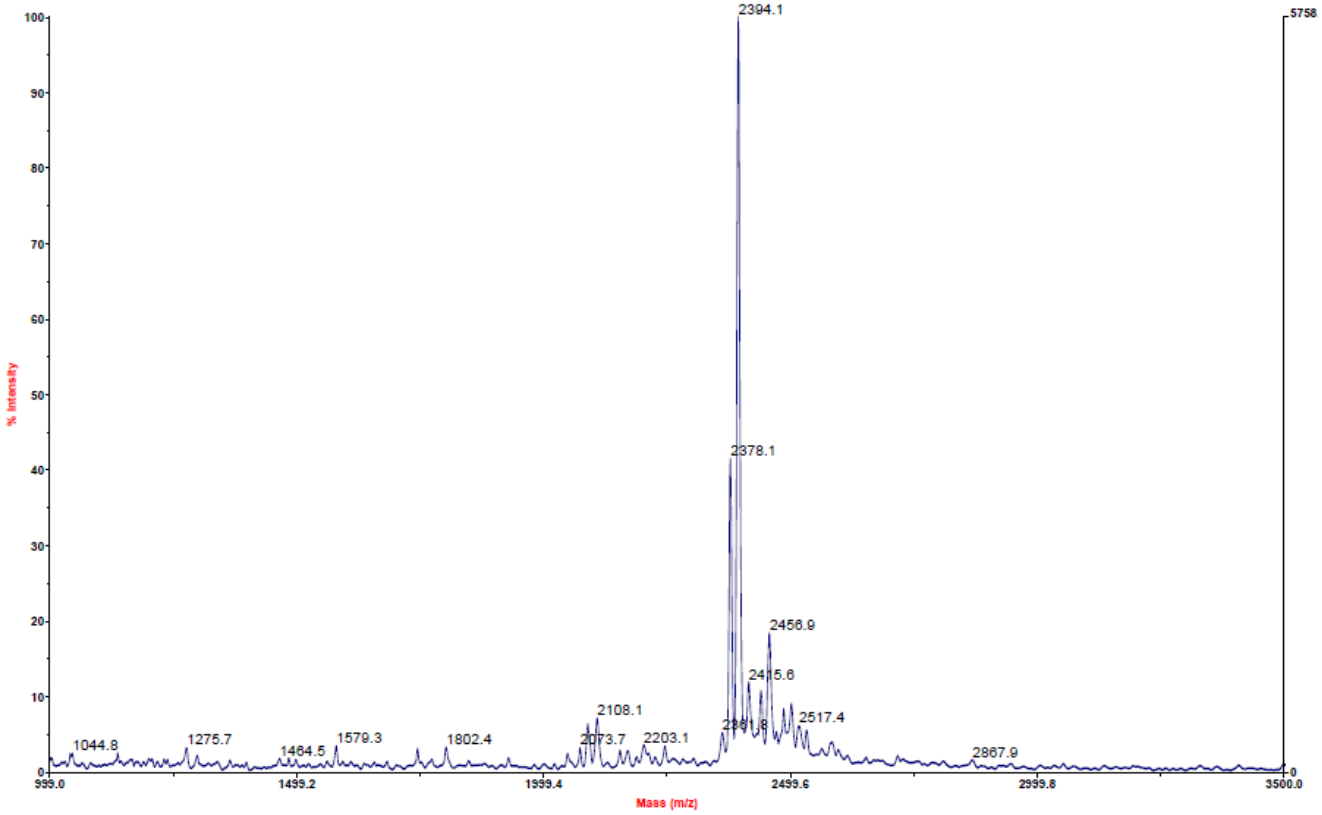


Figure 17S. MALDI-TOF MS spectrum for S_P-**17** oligomer; molecular mass calculated 2395, found 2394.1

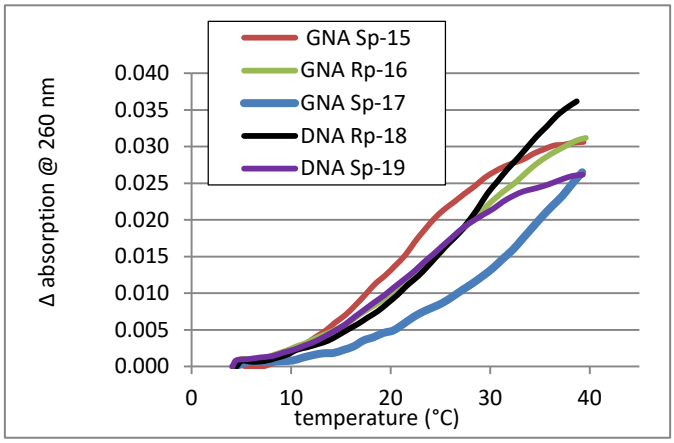


Figure 18S: Increase of UV absorption at 260 nm in melting experiments for selfcomplementary oligomers **15**-**19** (dissolved in pH 7.2 buffer containing 10 mM Tris-HCl, 100 mM NaCl, and 10 mM $MgCl_2$).

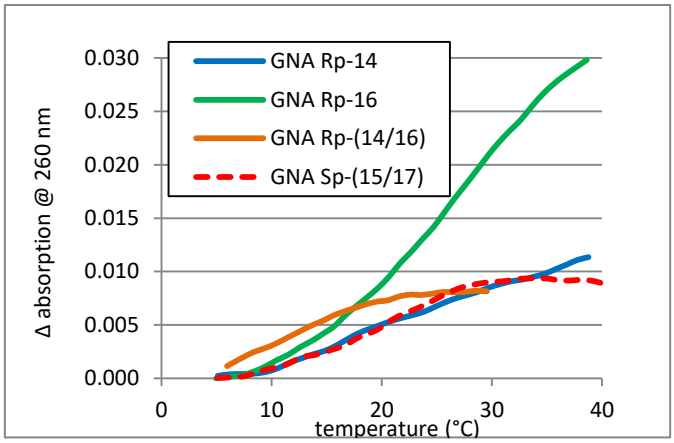


Figure 19S: Increase of UV absorption at 260 nm in melting experiments for selfcomplementary oligomers **14**, **16** and heteroduplexes **14**/**16** and **15**/**17** (dissolved in pH 7.2 buffer containing 10 mM Tris-HCl, 100 mM NaCl, and 10 mM $MgCl_2$).

Table 2S. Melting temperatures for mixtures of R_P-PS-(GNA/DNA) **14** or **16**, and S_P-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.

	T_m (°C)					
template	**DNA**	**(m)RNA**	5'-(^{G}A T^{G}G C^{G}G C^{G}A T)-3'		5'-(A ^{G}T G ^{G}C G ^{G}C A T)-3'	
			R_P-14	**S_P-15**	**R_P-16**	**S_P-17**
DNA d(ATGCGCAT)	43	×	41	42	42	41
(m)RNA (2'-OMe)-AUGCGCAU	×	62	59	60	59	58

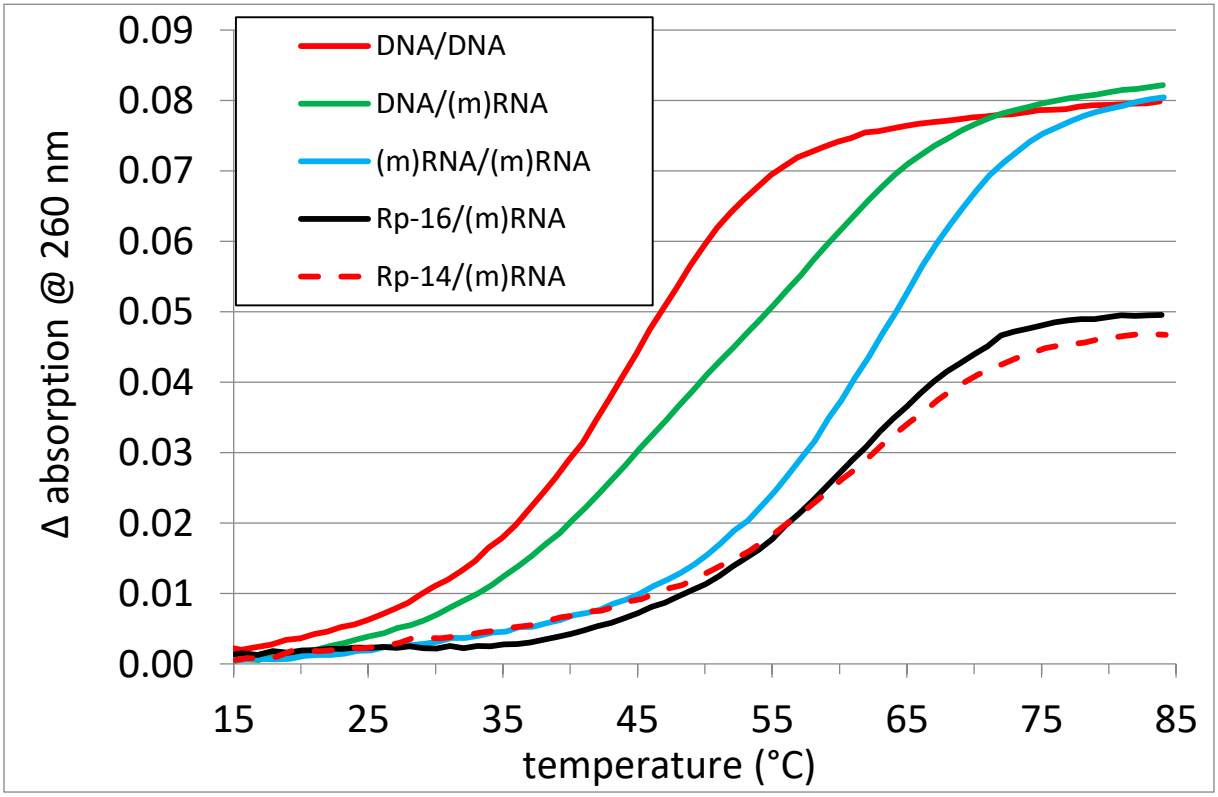


Figure 20S: Increase of UV absorption at 260 nm in melting experiments for selfcomplementary **DNA**, **(m)RNA** and for mixtures **DNA**/**(m)RNA**, **14**/**(m)RNA** and **16**/**(m)RNA** (dissolved in pH 7.2 buffer containing 10 mM Tris-HCl, 100 mM NaCl, and 10 mM $MgCl_2$).

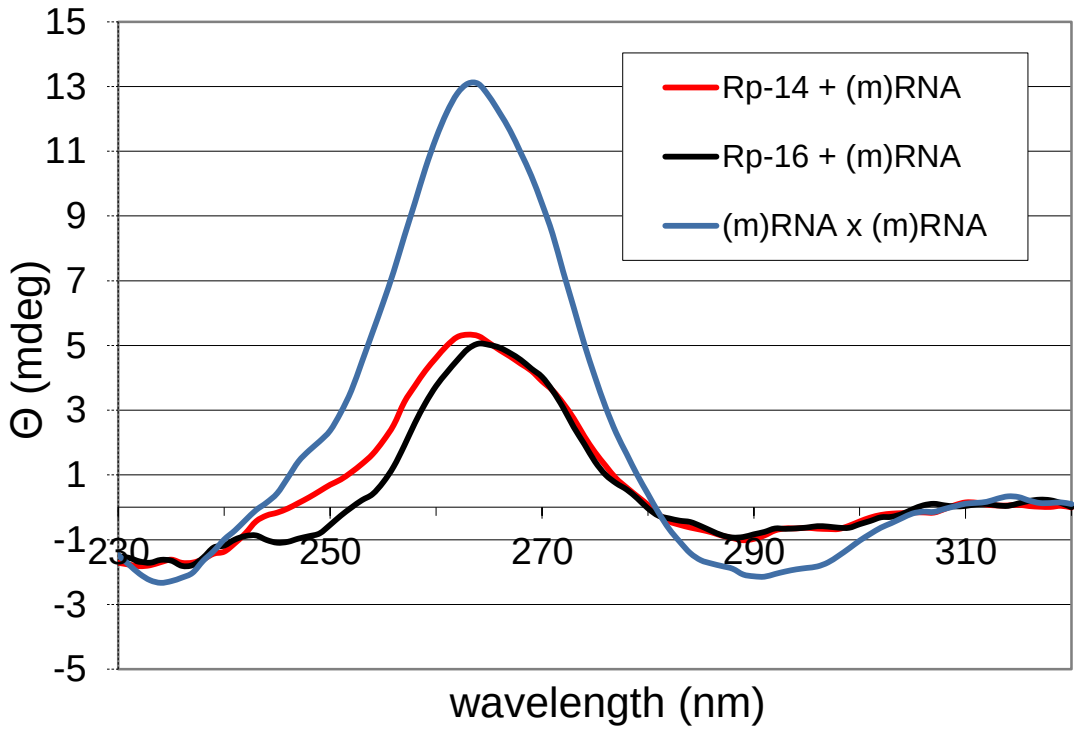


Figure 21S: CD spectra for the selfcomplementary oligomer **(m)RNA** and its mixture with **14** or **16** (dissolved in pH 7.2 buffer containing 10 mM Tris-HCl, 100 mM NaCl, and 10 mM $MgCl_2$) recorded at room temperature.