I. An example of the PS-(DNA/LNA) oligonucleotide synthesis

Figure S1. Upper Panel.

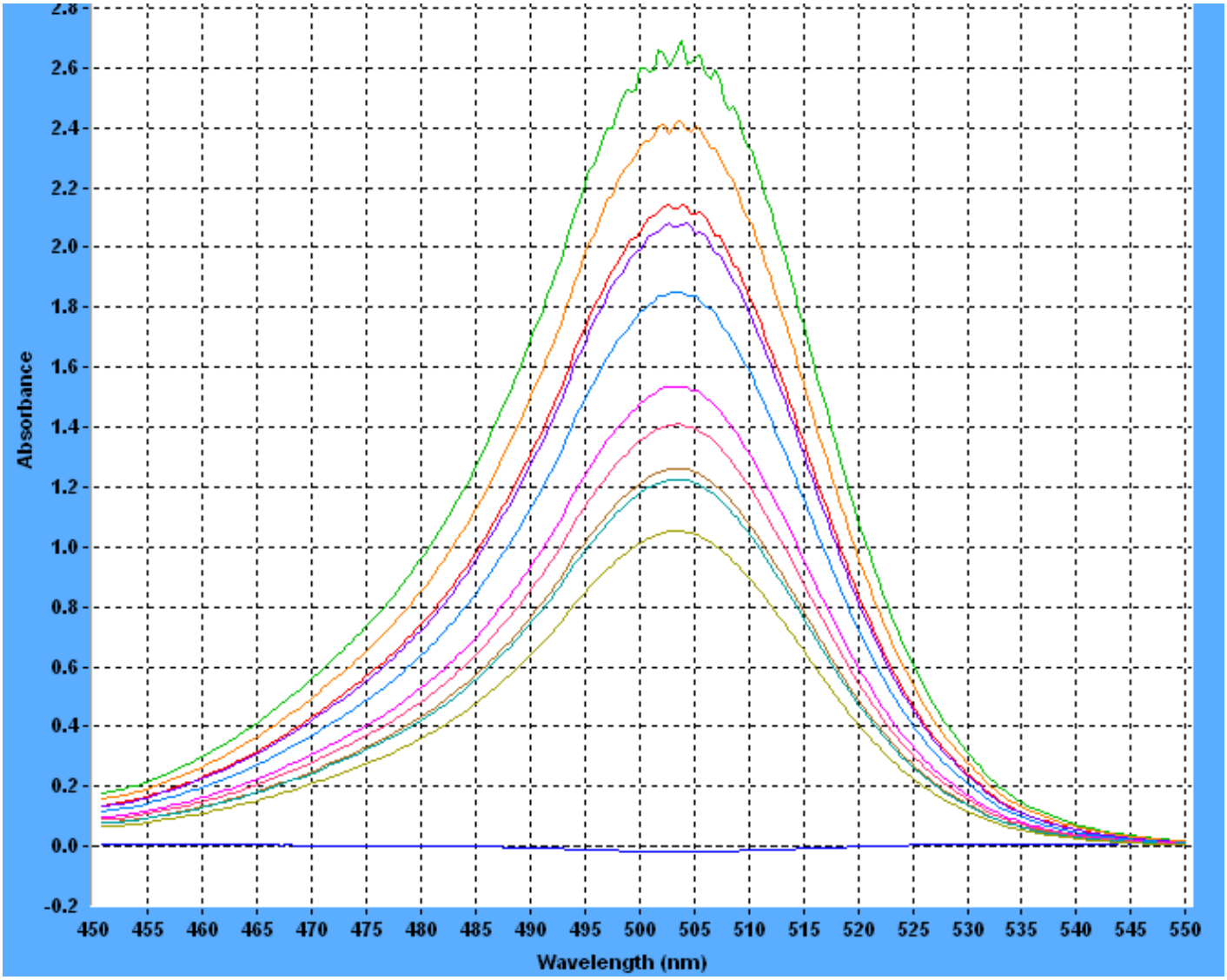

Figure S1. Lower Panel

abs@504nm	normalized abs@504nm	#cycle	calculated abs.	R.I. assumed
2.69	1	0	1.000	0.90
2.42	0.900	1	0.900	
2.14	0.796	2	0.810	
2.08	0.773	3	0.729	
1.82	0.677	4	0.656	
1.54	0.572	5	0.590	
1.41	0.524	6	0.531	
1.26	0.468	7	0.478	
1.23	0.457	8	0.430	
1.05	0.390	9	0.387	

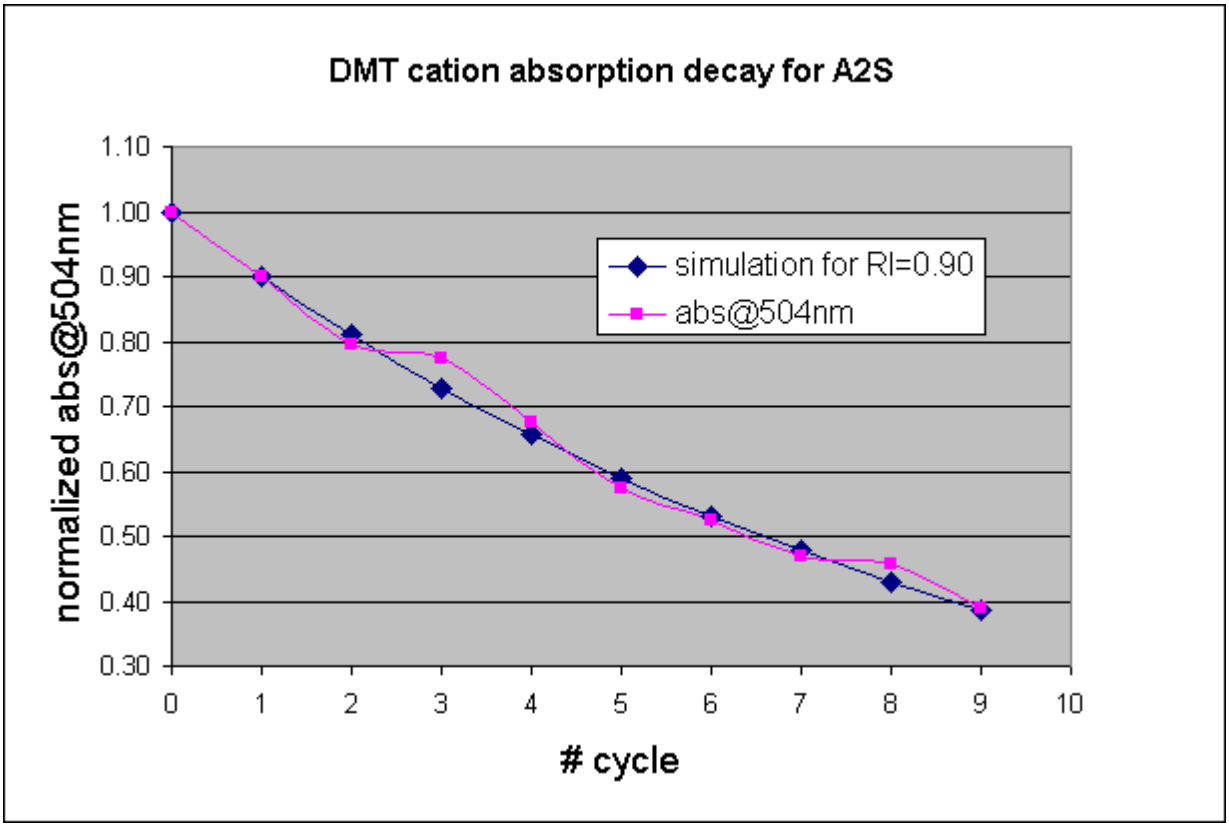

Figure S1. Upper Panel: Overlaid VIS spectra for measurement of the DMT^+ cation absorption (λ_{max}=504 nm) after consecutive detritylation steps during the synthesis of the **A2S** oligomer (5'-[S_P-PS]-d(GACA$_L$TCA$_L$CTAG)-3') at 1 μmole scale. Lower Panel: Calculation of the Average Repetitive Yield (RI) and plot for the normalized absorption measured @ 504 nm (pink rectangles) and values calculated at RI=0.90 (blue diamonds). Cycle #0 – detritylation of the nucleoside attached to the support.

II. Separation of OTP-LNA monomers into P-diastereomers.

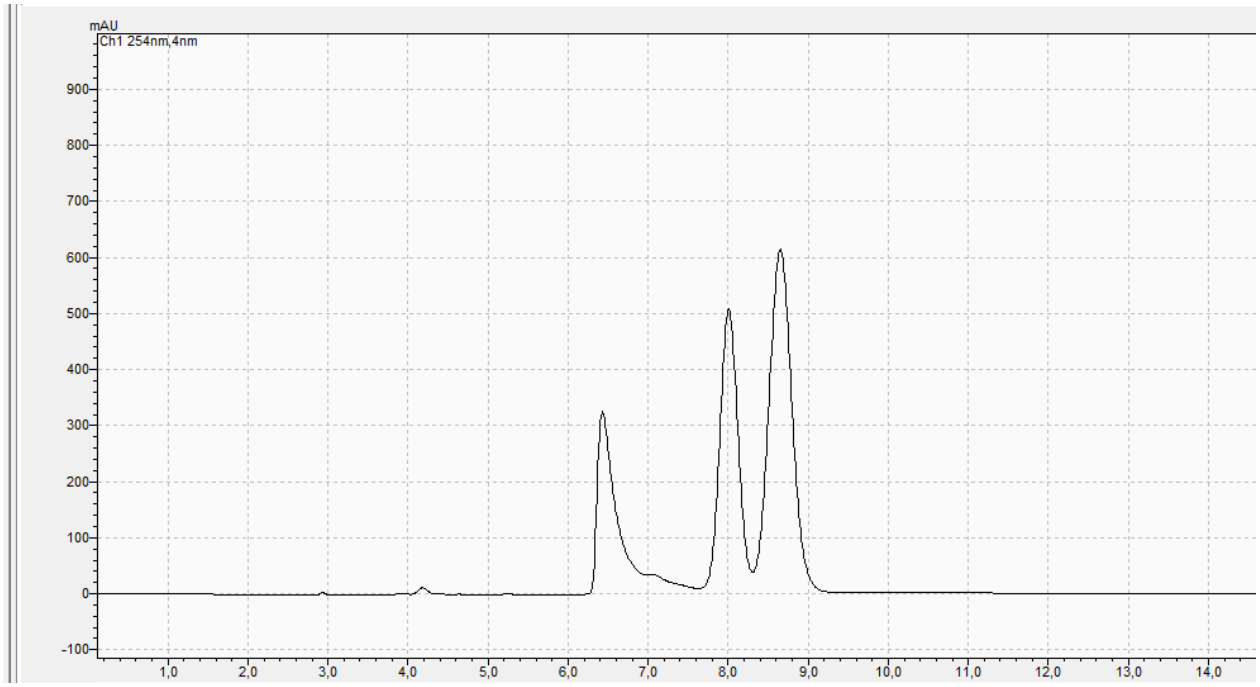

Figure S2. An HPLC profile from search for conditions for semi-preparative separation of P-diastereomers of **2d**. The conditions were identified using a Phenomenex Luna 5u Silica column (100Å; 250×10 mm; flow rate 5 mL/min)

III. Melting experiment

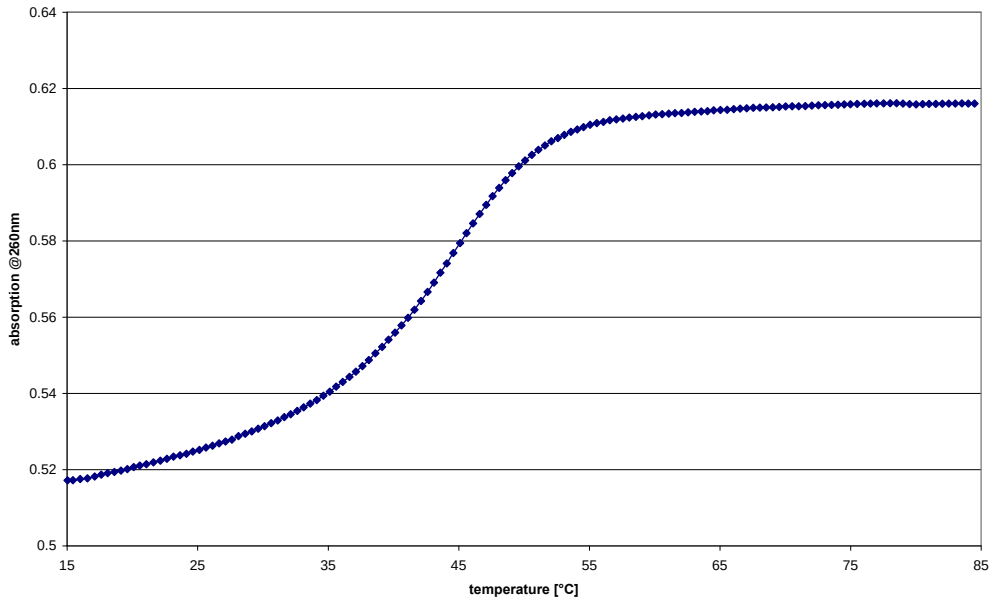

Figure S3. The melting profile for **C2R**/**M1$_D$** in a pH 7.2 buffer containing 10 mM Tris-HCl, 100 mM NaCl, and 10 mM $MgCl_2$. The oligonucleotides were mixed at 2.0 μM concentration each. Temperature gradient 0.5°C/min.

IV. CD measurements

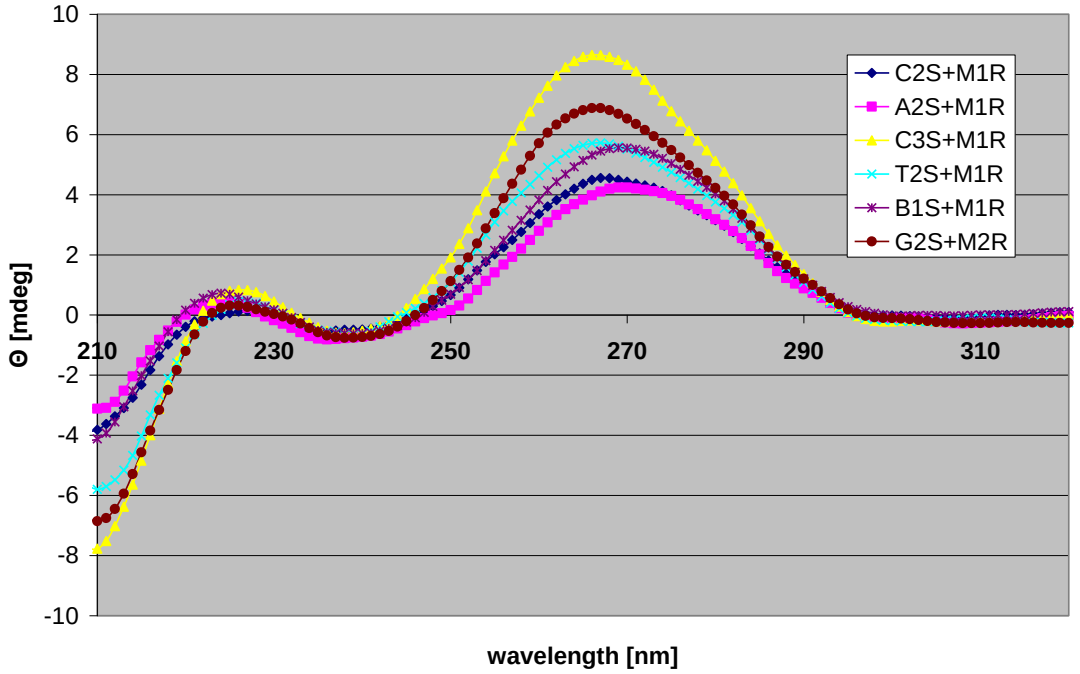

Figure S4. The CD spectra recorded for [S_P-PS]-(DNA/LNA)/RNA duplexes and the reference spectrum.

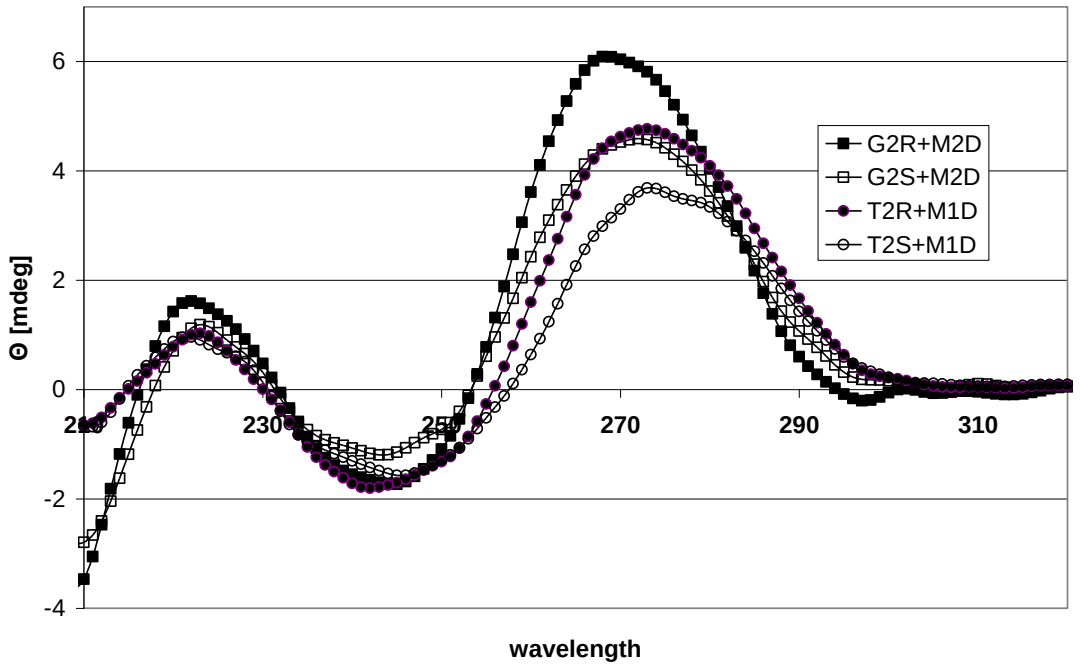

Figure S5. The CD spectra recorded for complexes of **T2R** and **T2S** with $\mathbf{M1_D}$, and **G2R** and **G2S** with $\mathbf{M2_D}$.

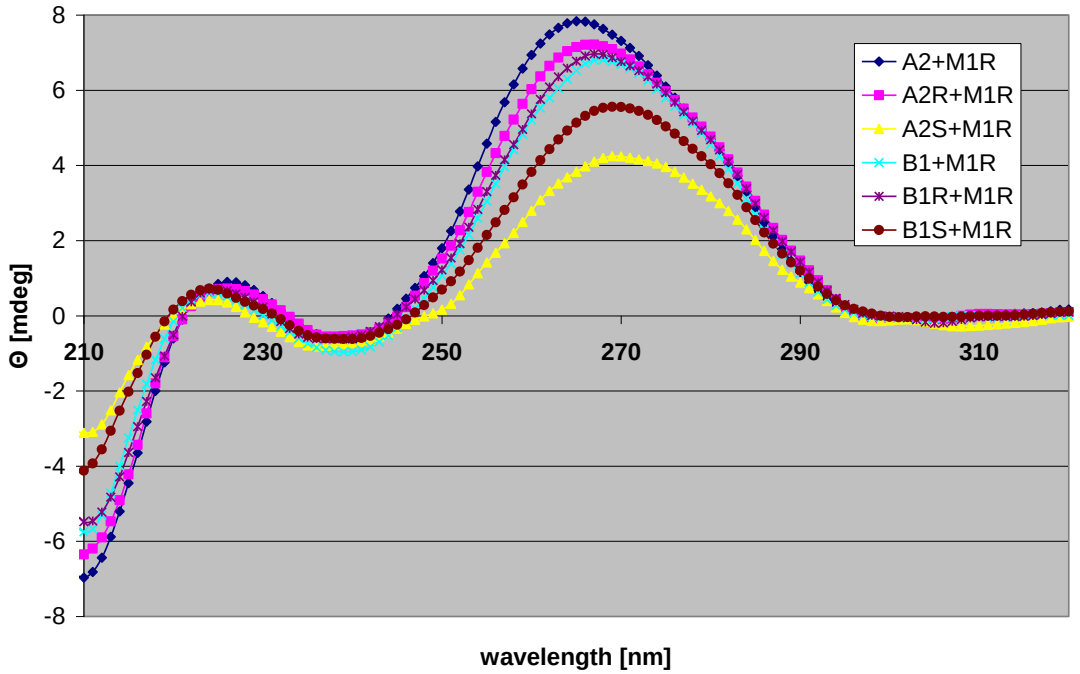

Figure S6. The CD spectra recorded for complexes of **A2**, **A2R** and **A2S** with $\mathbf{M1_R}$ and for the reference duplexes.

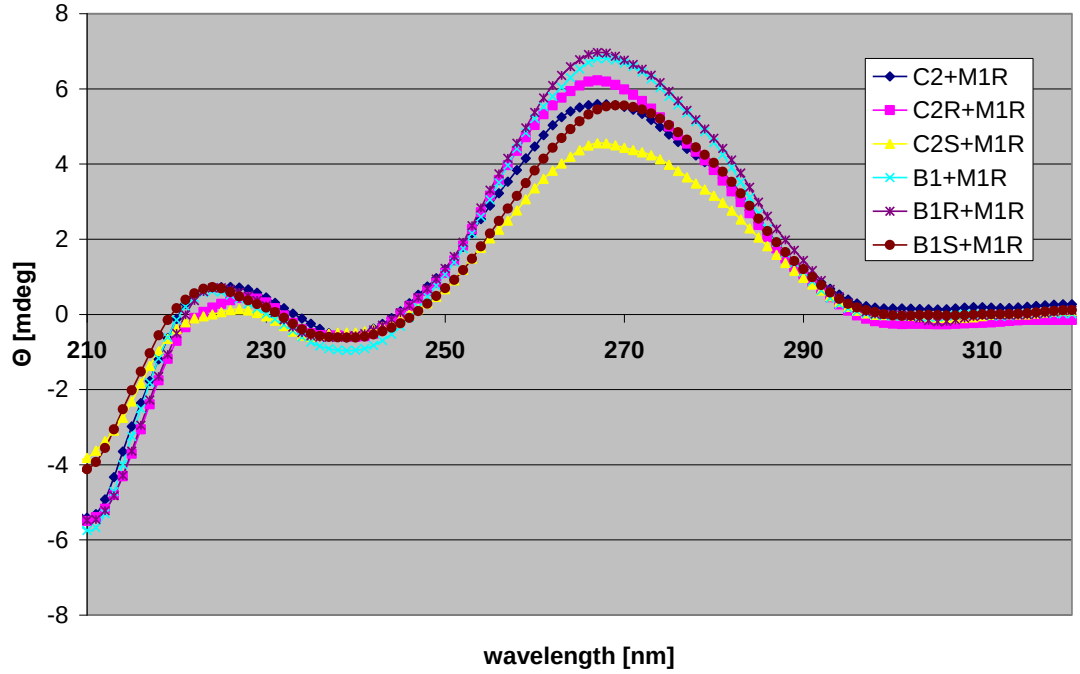

Figure S7. The CD spectra recorded for complexes of **C2**, **C2R** and **C2S** with $\mathbf{M1_R}$ and for the reference duplexes.

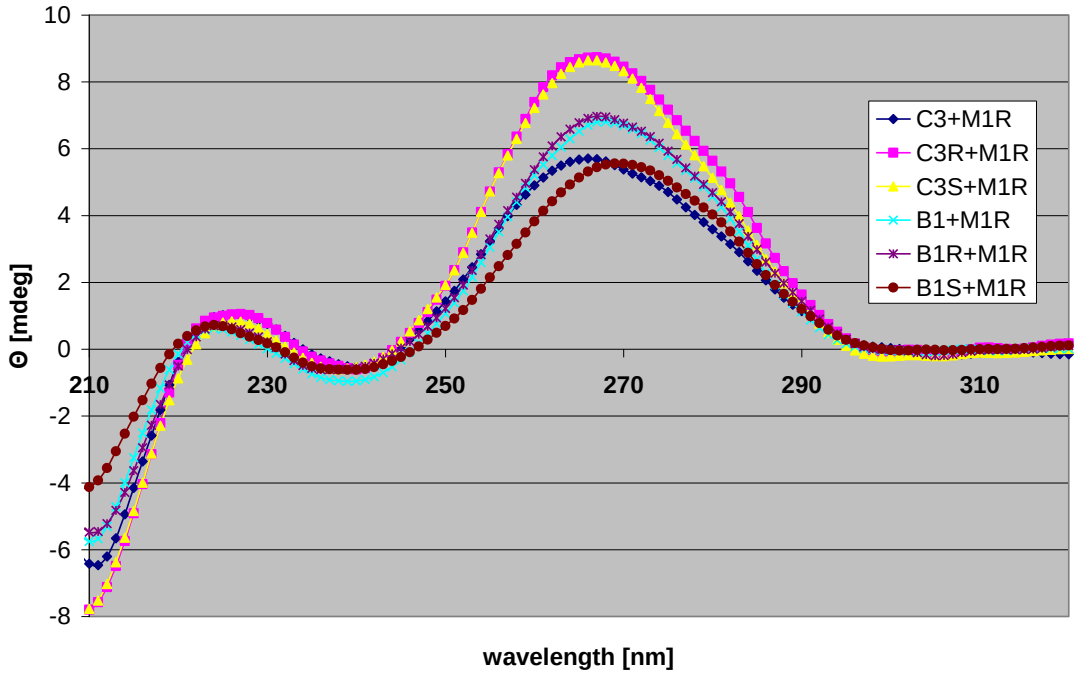

Figure S8. The CD spectra recorded for complexes of **C3**, **C3R** and **C3S** with $\mathbf{M1_R}$ and for the reference duplexes.

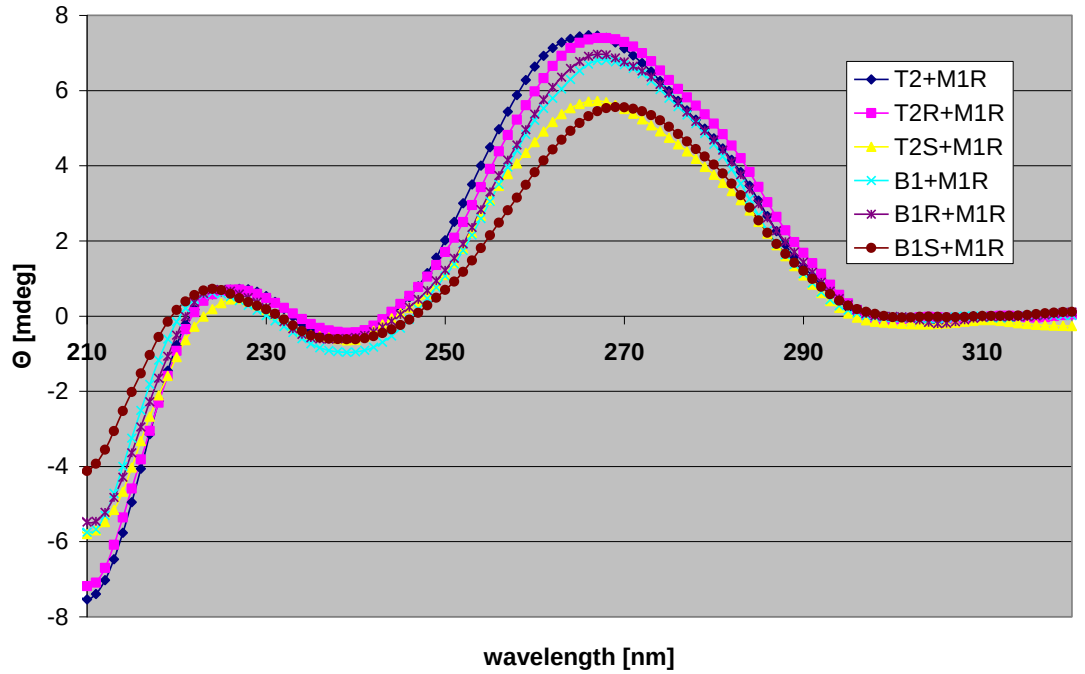

Figure S9. The CD spectra recorded for complexes of **T2**, **T2R** and **T2S** with $\mathbf{M1_R}$ and for the reference duplexes.

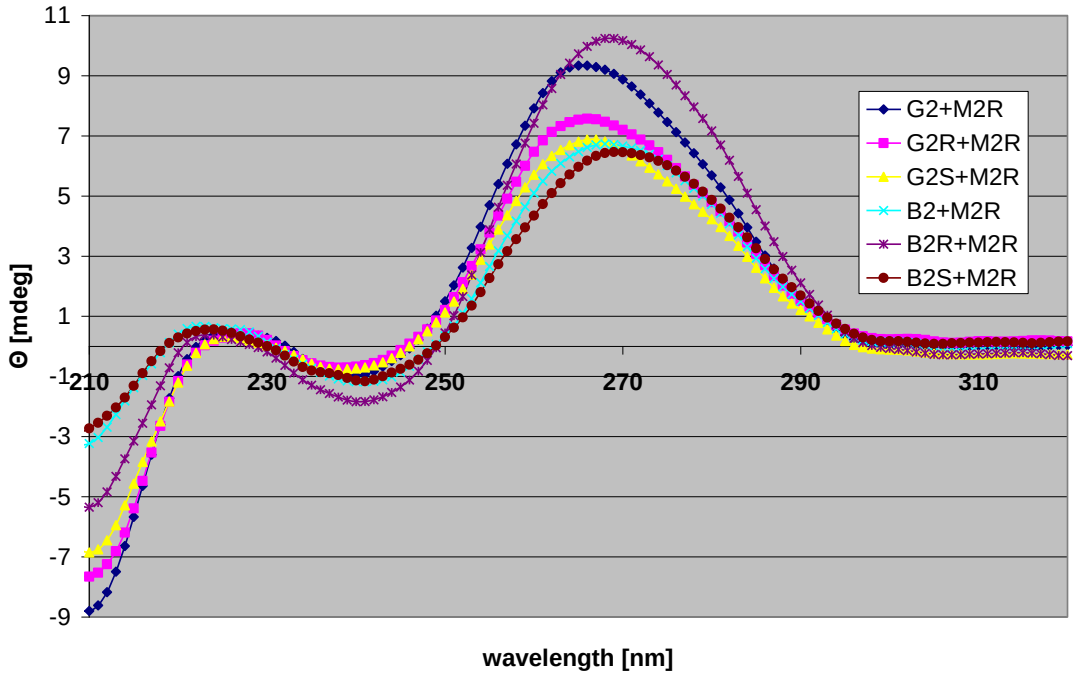

Figure S10. The CD spectra recorded for complexes of **G2**, **G2R** and **G2S** with $\mathbf{M2_R}$ and for the reference duplexes.

V. X-ray analysis of **3**.

Table S1. Crystal data for X-ray analysis of **3**.

Empirical formula	$C_{18}H_{25}N_2O_7PS_2$
Formula weight	476.51
Temperature (K)	100
Wavelength (Å)	1.54184
Crystal system, space group	Orthorombic, $P2_12_12_1$
Unit cell dimensions: a, b, c (Å)	6.3711 (1), 12.1422 (3), 27.2294 (7)
Volume ($Å^3$)	2106.44 (8)
Z	4
Calculated density (Mg m^{-3})	1.502
Absorption coefficient (mm^{-1})	3.41
F(000)	1000
Crystal size (mm^3)	0.10 × 0.05 × 0.02

Table S2. Parameters for data collection and refinement for X-ray analysis of **3**

Data collection	
Diffractometer	SuperNova single-crystal diffractometer (Agilent Technologies) with Cu Kα radiation (λ = 1.5418 Å).
No. of measured reflection	7619
No. of independent reflection	4177
No. of reflections with I>2σ(I)	3851
R_{int}	0.034
$(\sin \theta/\lambda)_{max}$	0.626
Limiting indices	-7 <= h <= 5, -14 <= k <= 13, -34 <= l <= 28
Theta range for data collection	4.0° < θ < 75.0°
Refinement	
Refinement method	Full-matrix least-squares on F^2
Completeness to theta = 75.0°	0.979
Data / restrains / parameters	4177/0/273
Goodness-of-fit on F^2	1.032
Final R indices [I>2sigma(I)]	R1=0.031, wR2=0.073
R indices (all data)	R1=0.036, wR2=0.075
Absolute structure parameter	0.010 (15)
Largest diff. peak and hole (e $Å^{-3}$)	0.268, -0.323

VI. ^{1}H NMR spectra for separated OTP-LNA monomers, recorded with a Bruker AV-200 spectrometer (200 MHz)

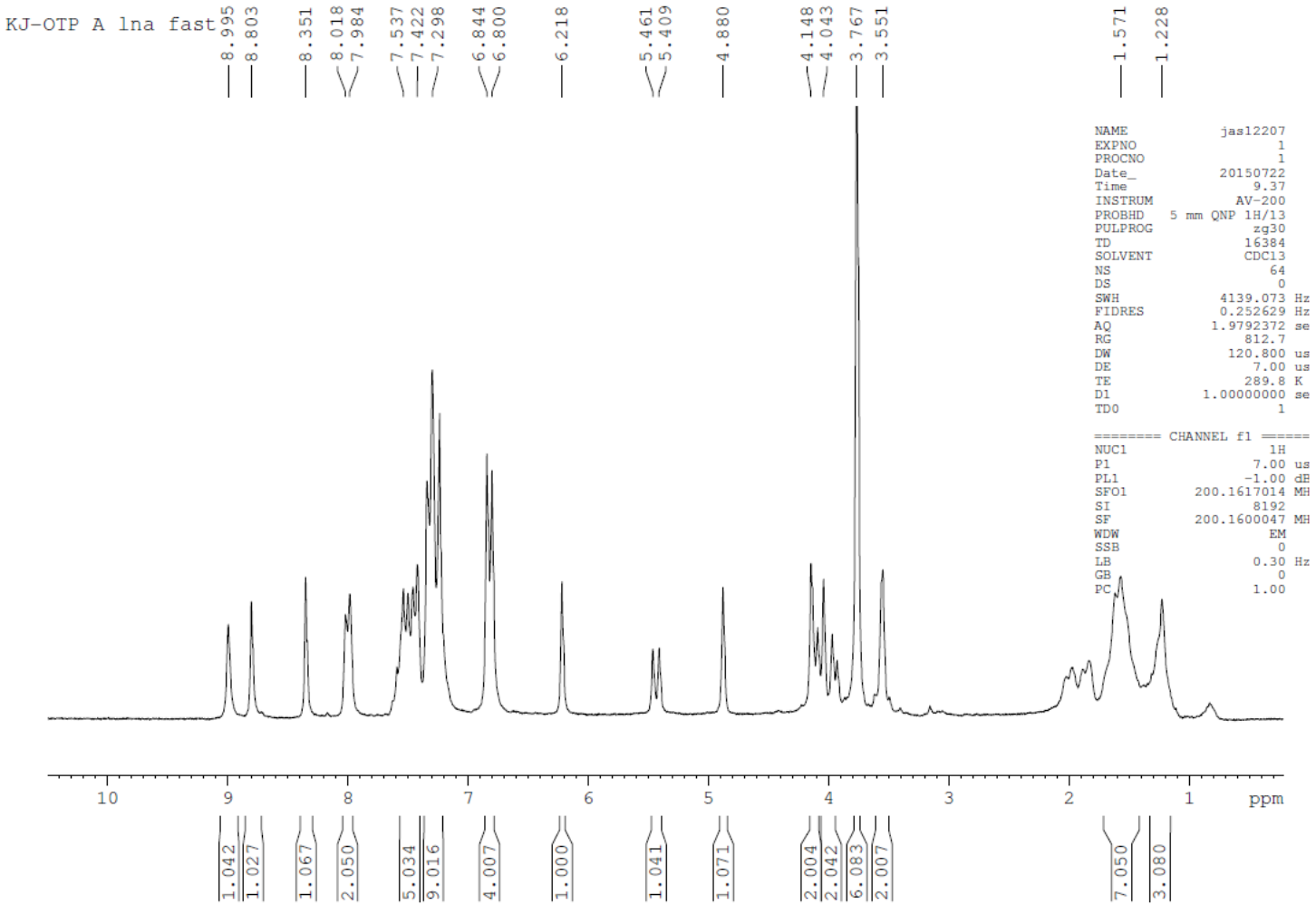

Figure S11. A_L-OTP *fast* (**2b**) *;* δ (ppm, $CDCl_3$) 9.00 (1H, NHCO), 8.80 (1H, C8-H), 8.36 (1H, C2-H), 7.54-6.80 (18H, DMT, Bz), 6.22 (1H, C1'-H), 5.46-5.41 (1H, C3'-H), 4.88 (1H, C2'-H), 4.15-4.04 (2H, P-O-CH_2C-S; 2H, O2'-CH_2-C4'), 3.77 (6H, 2xOCH_3), 3.55 (2H, 5'CH_2), 1.57-1.23 (10H, -$(CH_2)_5$- *„spiro"*)

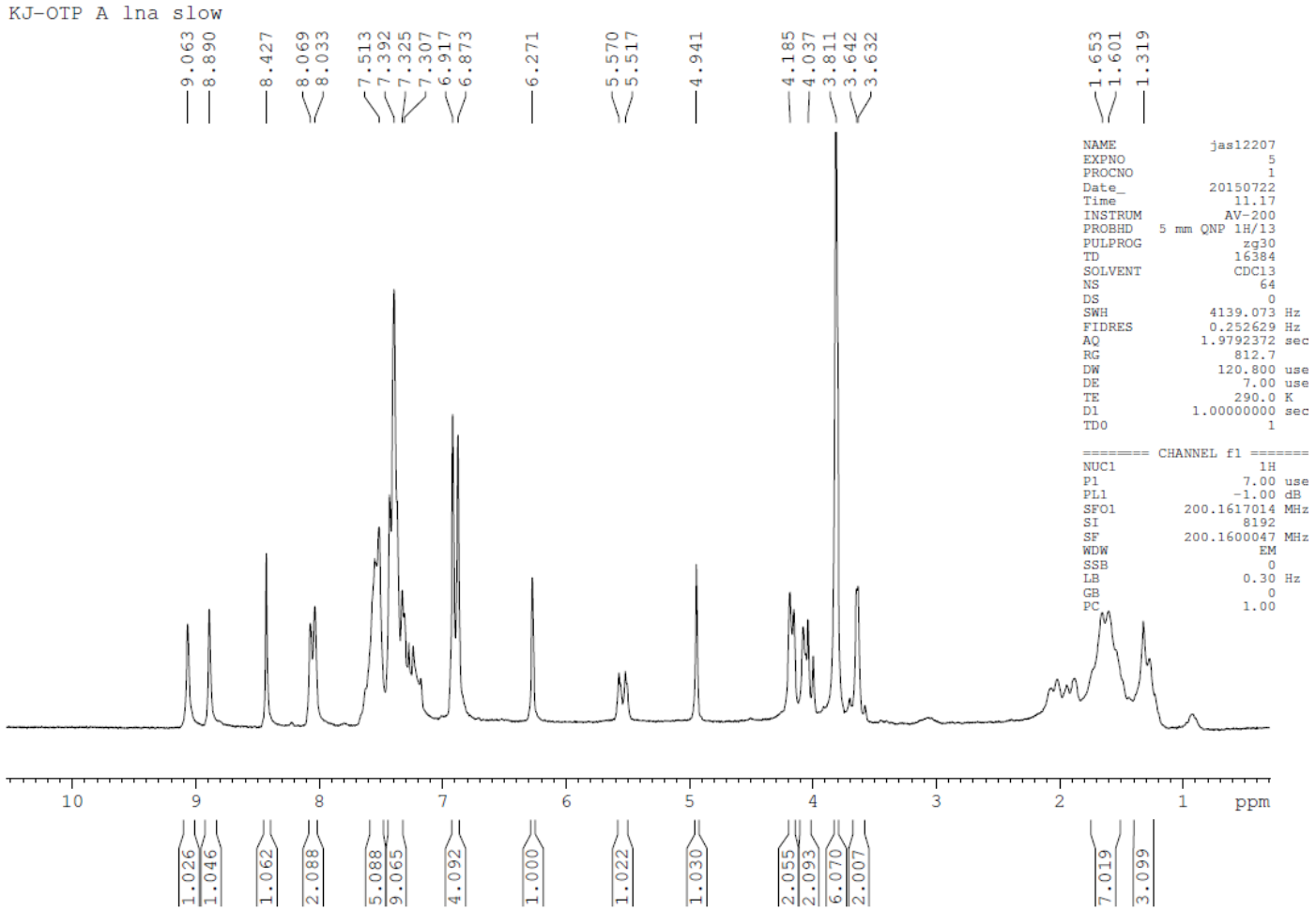

Figure S12. A_L-OTP *slow* (**2b**)*;* δ (ppm, $CDCl_3$) 9.06 (1H, NHCO), 8.89 (1H, C8-H), 8.43 (1H, C2-H), 7.51-6.87 (18H, DMT, Bz), 6.27 (1H, C1'-H), 5.57-5.52 (1H, C3'-H), 4.94 (1H, C2'-H), 4.19-4.00 (2H, P-O-CH_2C-S; 2H, O2'-CH_2-C4'), 3.81 (6H, 2xOCH_3), 3.64 (2H, 5'CH_2), 1.65-1.32 (10H, -$(CH_2)_5$- *„spiro"*)

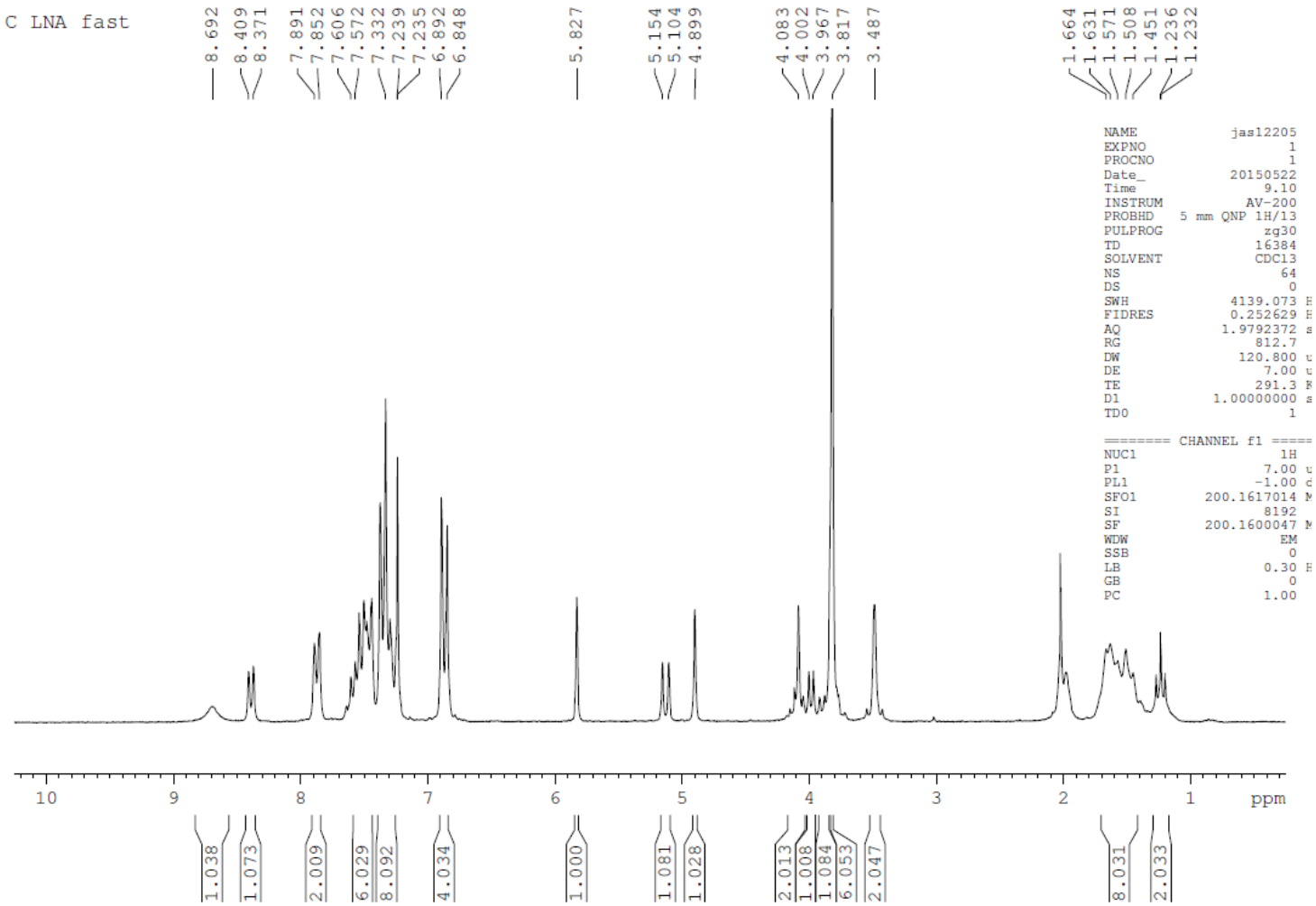

Figure S13. C_L-OTP *fast* (**2c**)*;* δ (ppm, $CDCl_3$) 8.69 (1H, NHCO), 7.89-7.85 (2H, C6-H, C5-H), 7.60-6.85 (18H, DMT, Bz), 5.83 (1H, C1'-H), 5.15-5.10 (1H, C3'-H), 4.90 (1H, C2'-H), 4.08-3.97 (2H, P-O-CH_2C-S; 2H, O2'-CH_2-C4'), 3.82 (6H, 2xOCH_3), 3.49 (2H, 5'CH_2), 1.66-1.23 (10H, -$(CH_2)_5$- *„spiro"*)

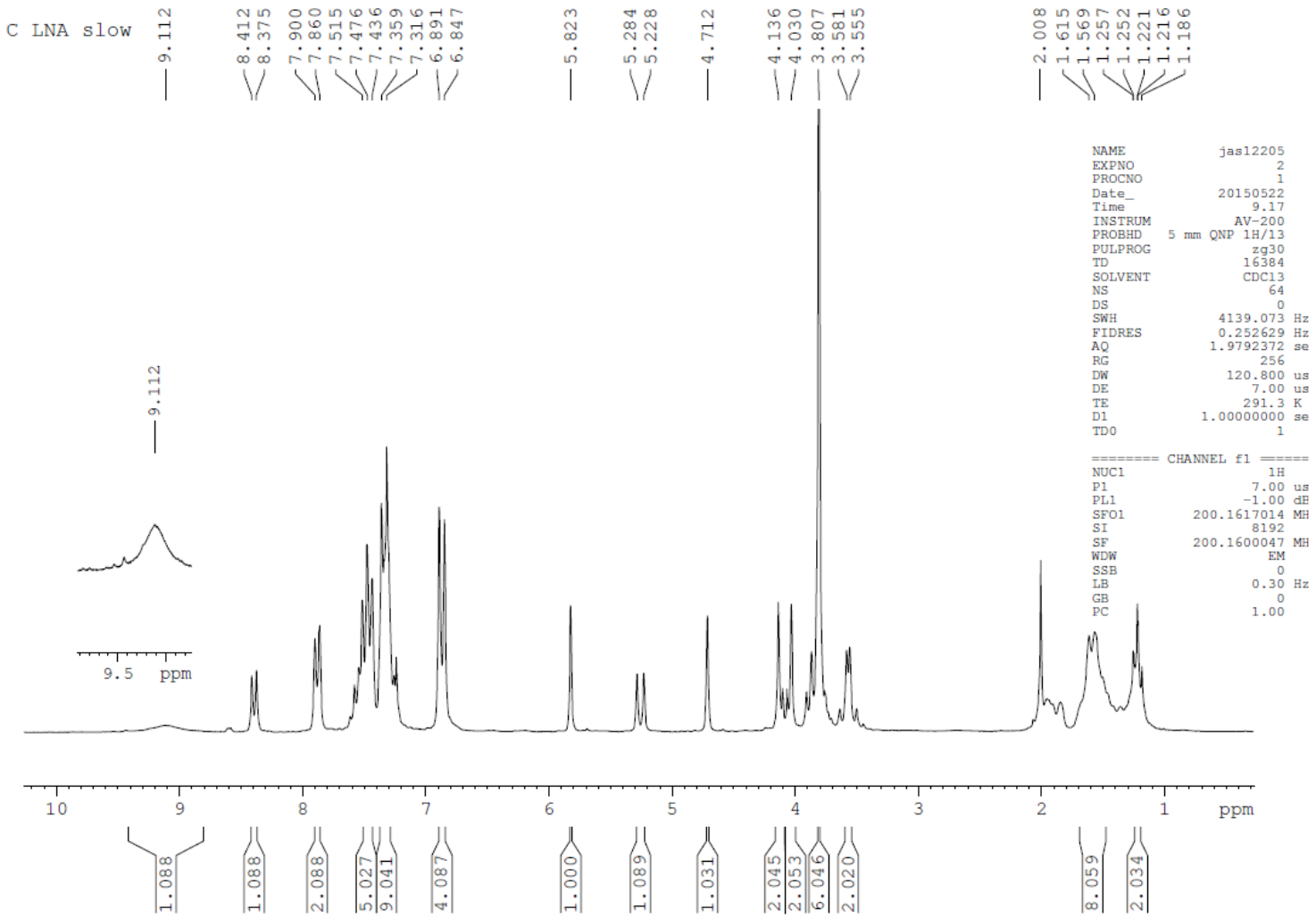

Figure S14. C_L-OTP *slow* (**2c**)*;* δ (ppm, $CDCl_3$) 9.11 (1H, NHCO), 7.90-7.86 (2H, C6-H, C5-H), 7.52-6.85 (18H, DMT, Bz), 5.82 (1H, C1'-H), 5.28-5.23 (1H, C3'-H), 4.71 (1H, C2'-H), 4.14-3.90 (2H, P-O-CH_2C-S; 2H, O2'-CH_2-C4'), 3.81 (6H, 2xOCH_3), 3.58 (2H, 5'CH_2), 1.62-1.19 (10H, -$(CH_2)_5$-*„spiro"*)

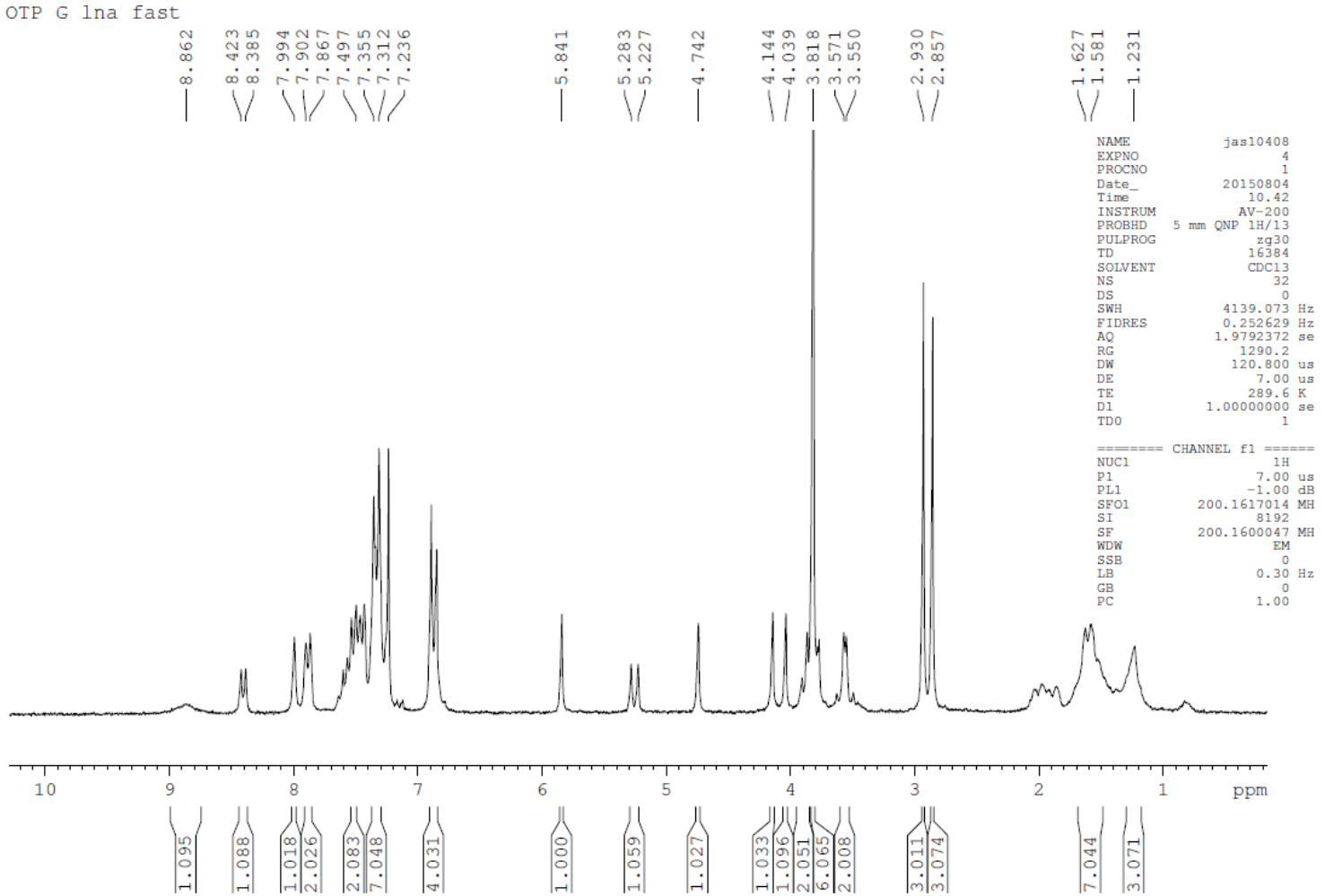

Figure S15. G_L-OTP *fast* (**2d**)*;* δ (ppm, $CDCl_3$) 8.86 (1H, N1-H), 8.40 (1H, N=CH, dmf), 7.90 (1H, C8-H), 7.50-7.24 (13H, DMT), 5.84 (1H, C1'-H), 5.28-5.23 (1H, C3'-H), 4.74 (1H, C2'-H), 4.14-3.80 (2H, P-O-CH_2C-S; 2H, O2'-CH_2-C4'), 3.82 (6H, 2xOCH_3), 3.57-3.55 (2H, C5'CH_2), 2.93-2.86 (6H, 2xNCH_3, dmf), 1.63-1.23 (10H, -$(CH_2)_5$- *„spiro"*)

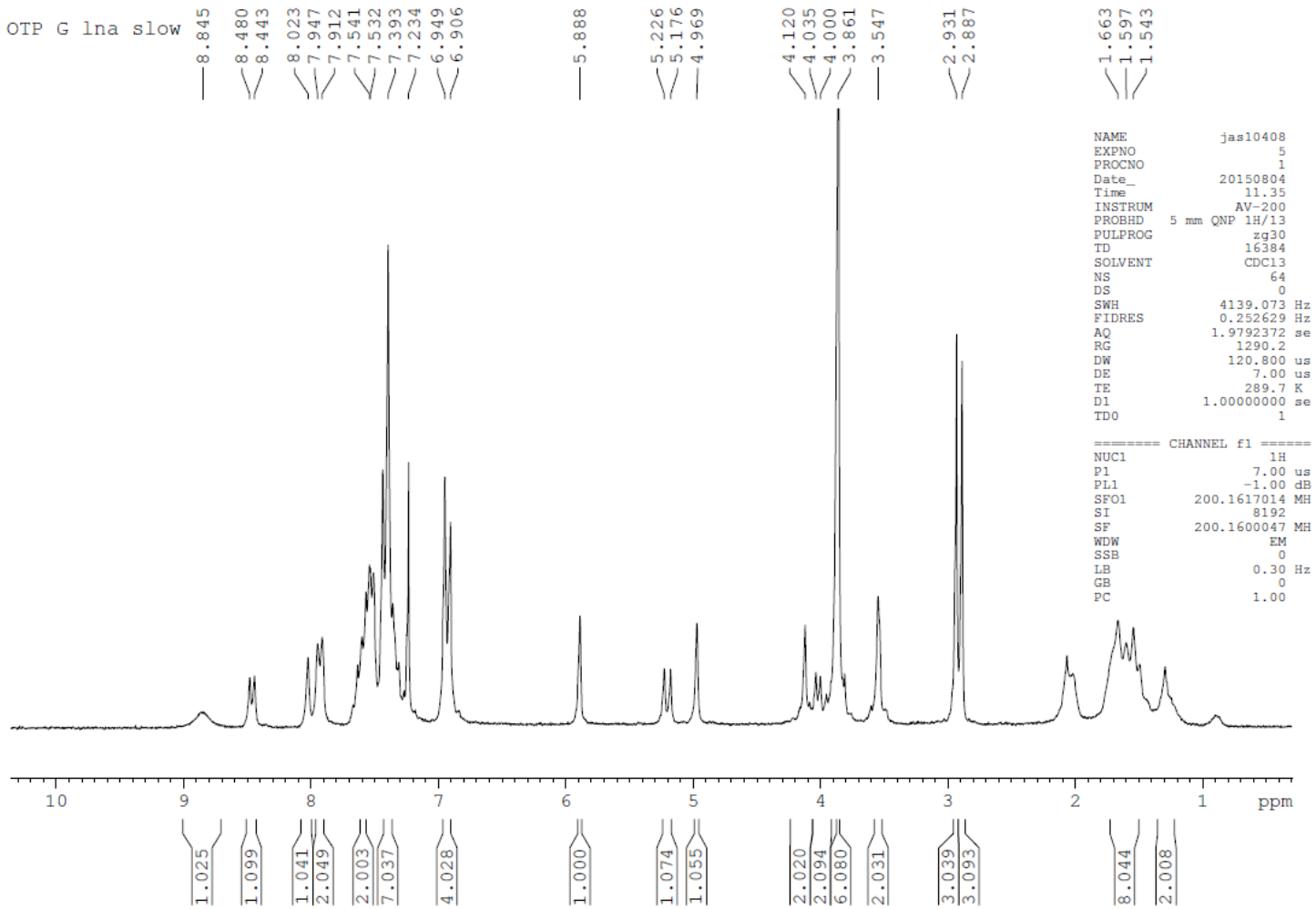

Figure S16. G_L-OTP *slow* (**2d**)*;* δ (ppm, $CDCl_3$) 8.85 (1H, N1-H), 8.45 (1H, N=CH, dmf), 8.02 (1H, C8-H), 7.54-6.91 (13H, DMT), 5.89 (1H, C1'-H), 5.23-5.18 (1H, C3'-H), 4.97 (1H, C2'-H), 4.12-4.00 (2H, P-O-CH_2C-S; 2H, O2'-CH_2-C4'), 3.86 (6H, 2xOCH_3), 3.55 (2H, 5'CH_2), 2.93-2.89 (6H, 2xNCH_3, dmf), 1.66-1.54 (10H, -$(CH_2)_5$- *„spiro"*)

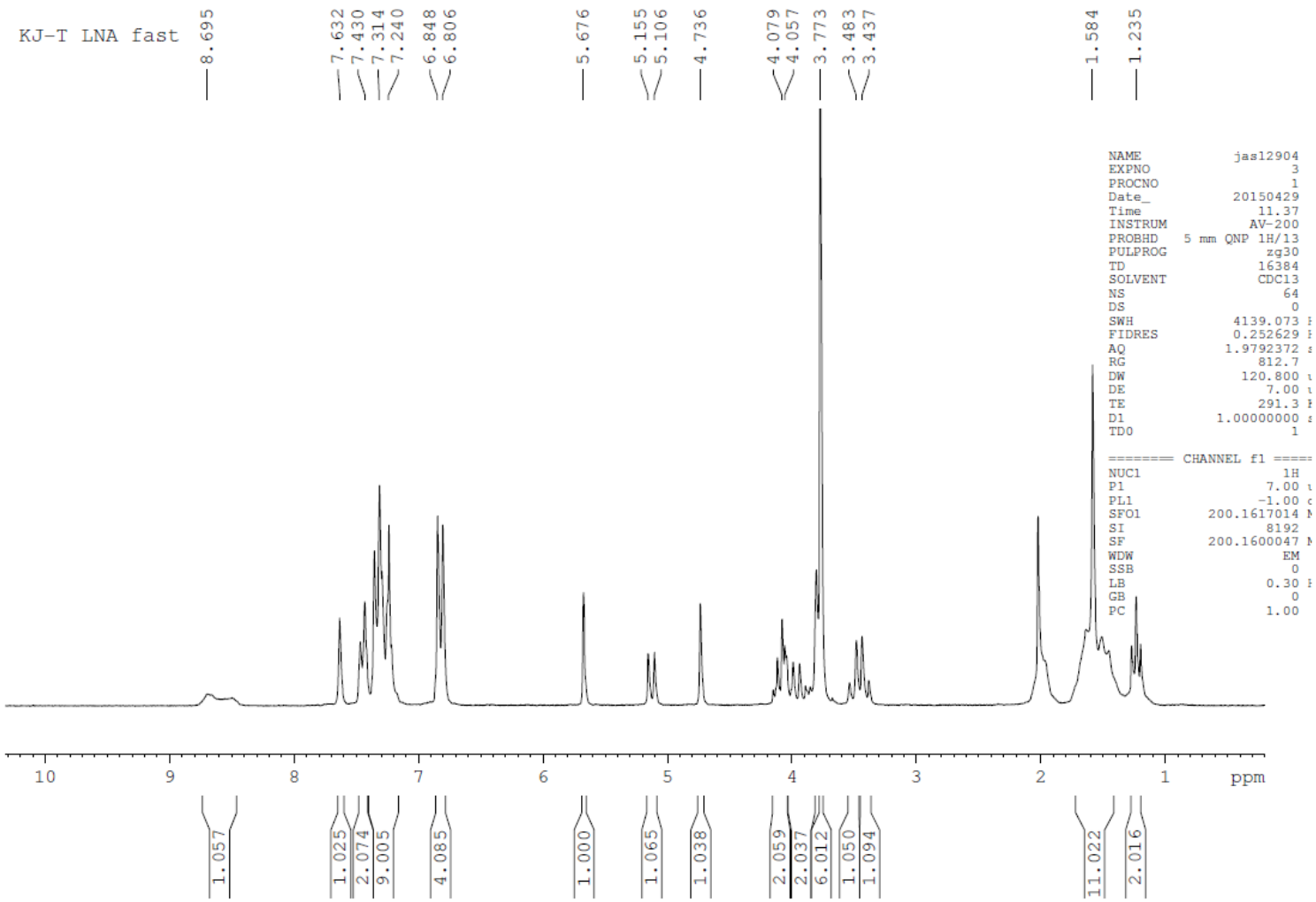

Figure S17. T_L-OTP *fast* (**2a**)*;* δ (ppm, $CDCl_3$) 8.70 (1H, N3-H), 7.63 (1H, C6-H), 7.31-6.81 (13H, DMT), 5.68 (1H, C1'-H), 5.15-5.11 (1H, C3'-H), 4.74 (1H, C2'-H), 4.11-3.90 (2H, P-O-CH_2C-S; 2H, O2'-CH_2-C4'), 3.77 (6H, 2xOCH_3), 3.48-3.44 (2H, 5'CH_2), 1.58-1.23 (3H, C5-CH_3; 10H, -$(CH_2)_5$- *„spiro"*)

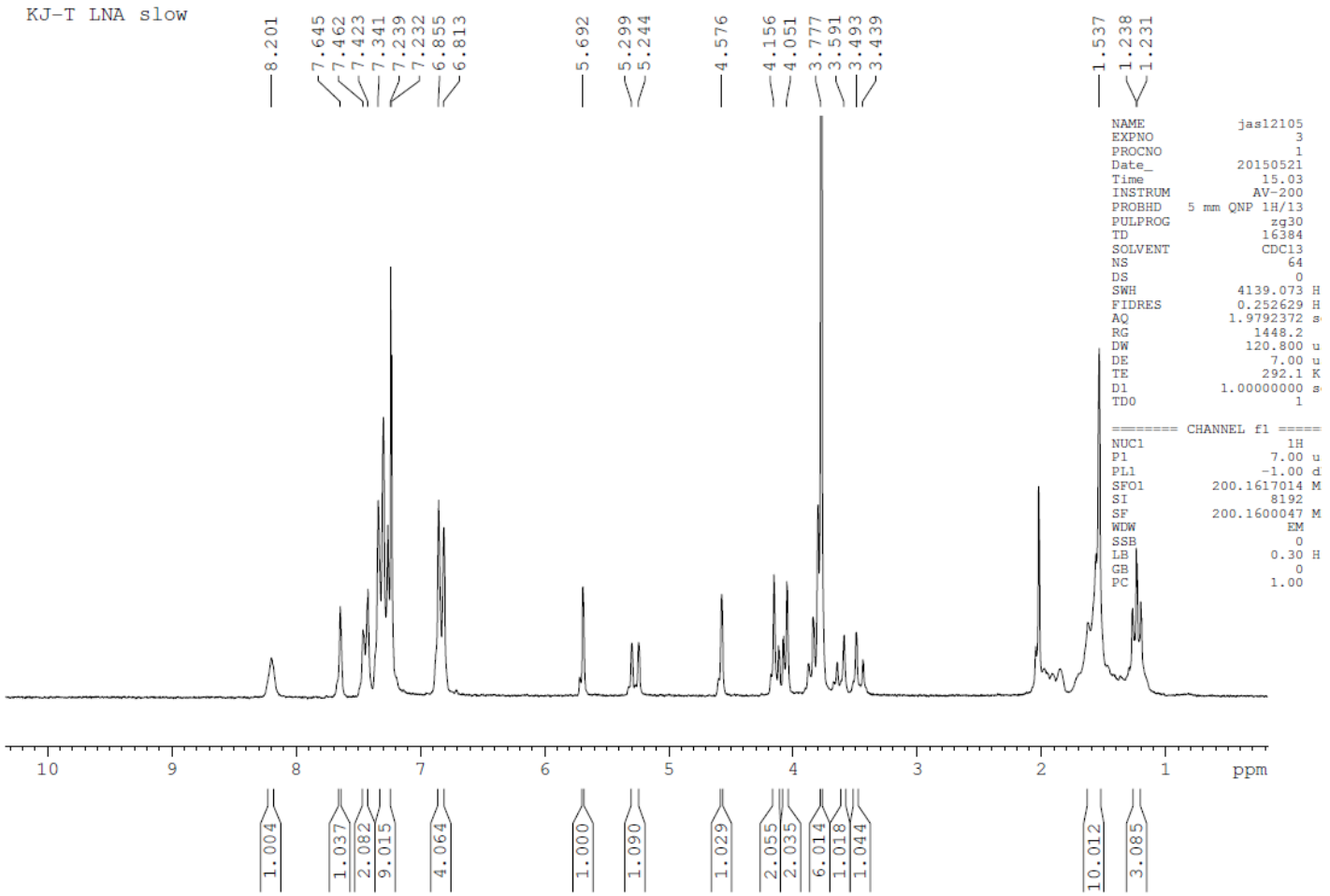

Figure S18. T_L-OTP *slow* (**2a**)*;* δ (ppm, $CDCl_3$) 8.20 (1H, N3-H), 7.65 (1H, C6-H), 7.42-6.81 (13H, DMT), 5.69 (1H, C1'-H), 5.30-5.24 (1H, C3'-H), 4.58 (1H, C2'-H), 4.16-4.05 (2H, P-O-CH_2C-S; 2H, O2'-CH_2-C4'), 3.78 (6H, 2xOCH_3), 3.60-3.44 (2H, 5'CH_2), 1.54-1.23 (3H, C5-CH_3; 10H, -$(CH_2)_5$- *„spiro"*)

VII. ^{31}P NMR spectra for separated OTP-LNA monomers, recorded with a Bruker AV-200 spectrometer (200 MHz for ^{1}H)

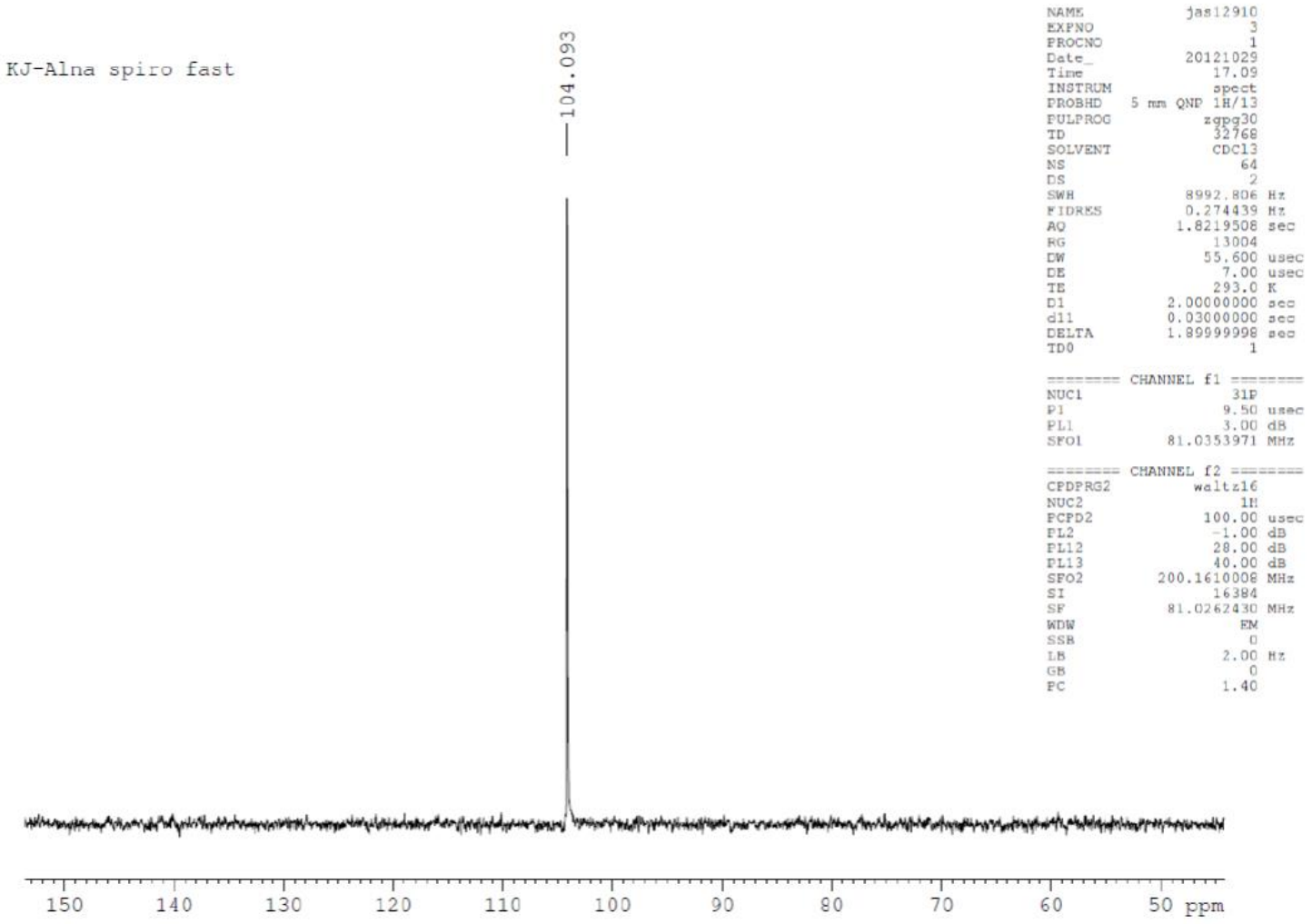

Figure S19. A_L-OTP *fast* (**2b**)*;* δ (ppm, $CDCl_3$)

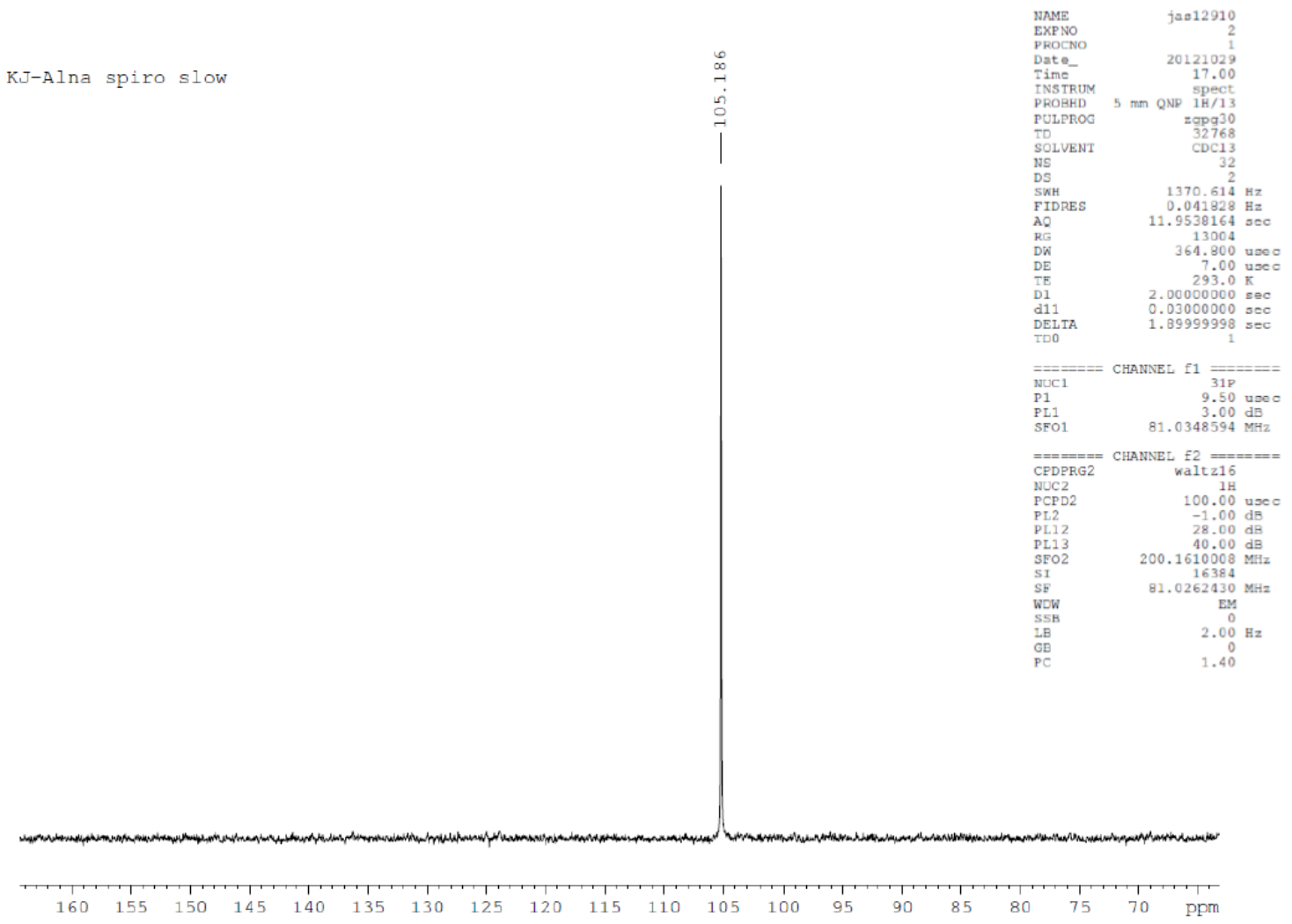

Figure S20. A_L-OTP *slow* (**2b**)*;* δ (ppm, $CDCl_3$)

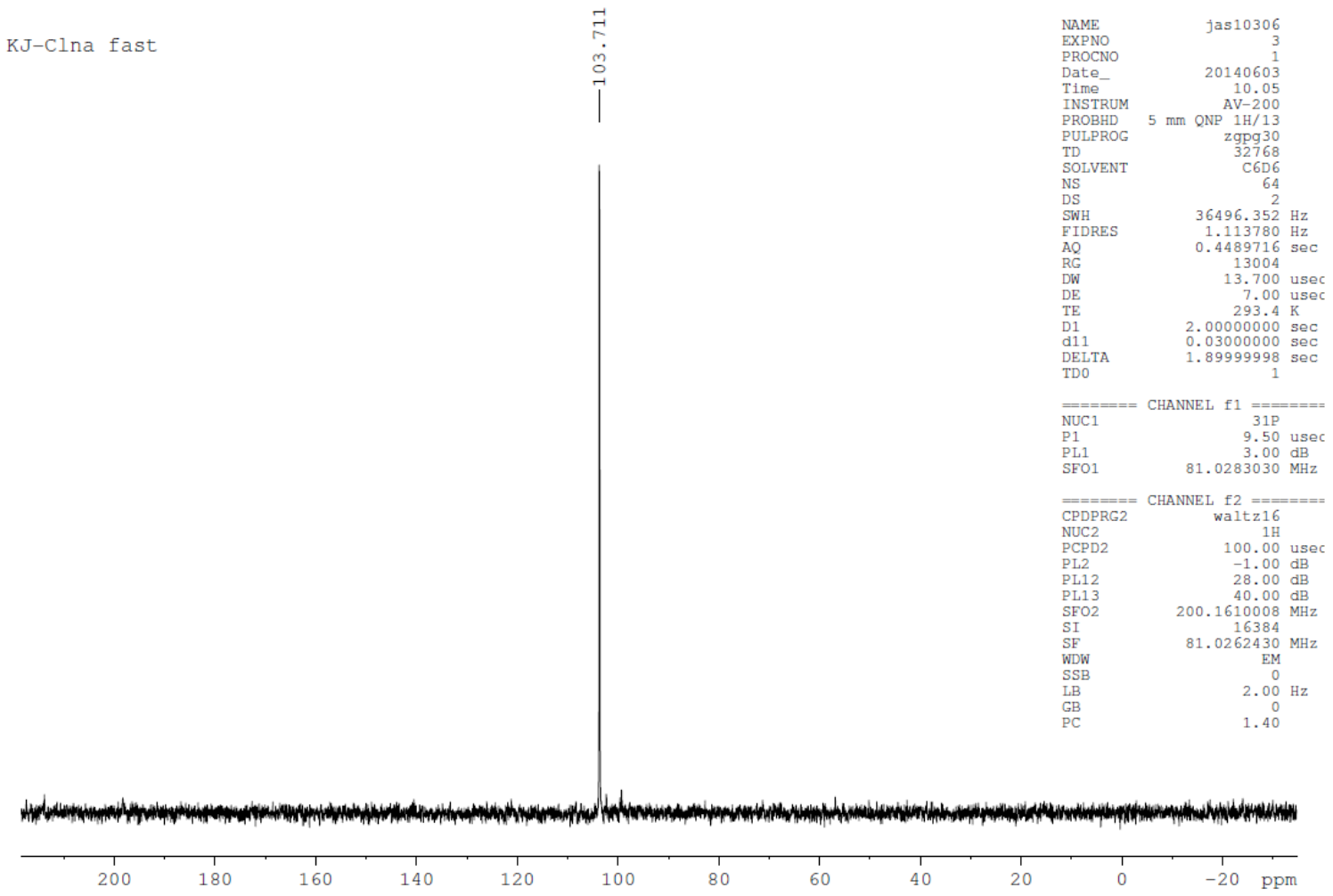

Figure S21. C_L-OTP *fast* (**2c**)*;* δ (ppm, C_6D_6)

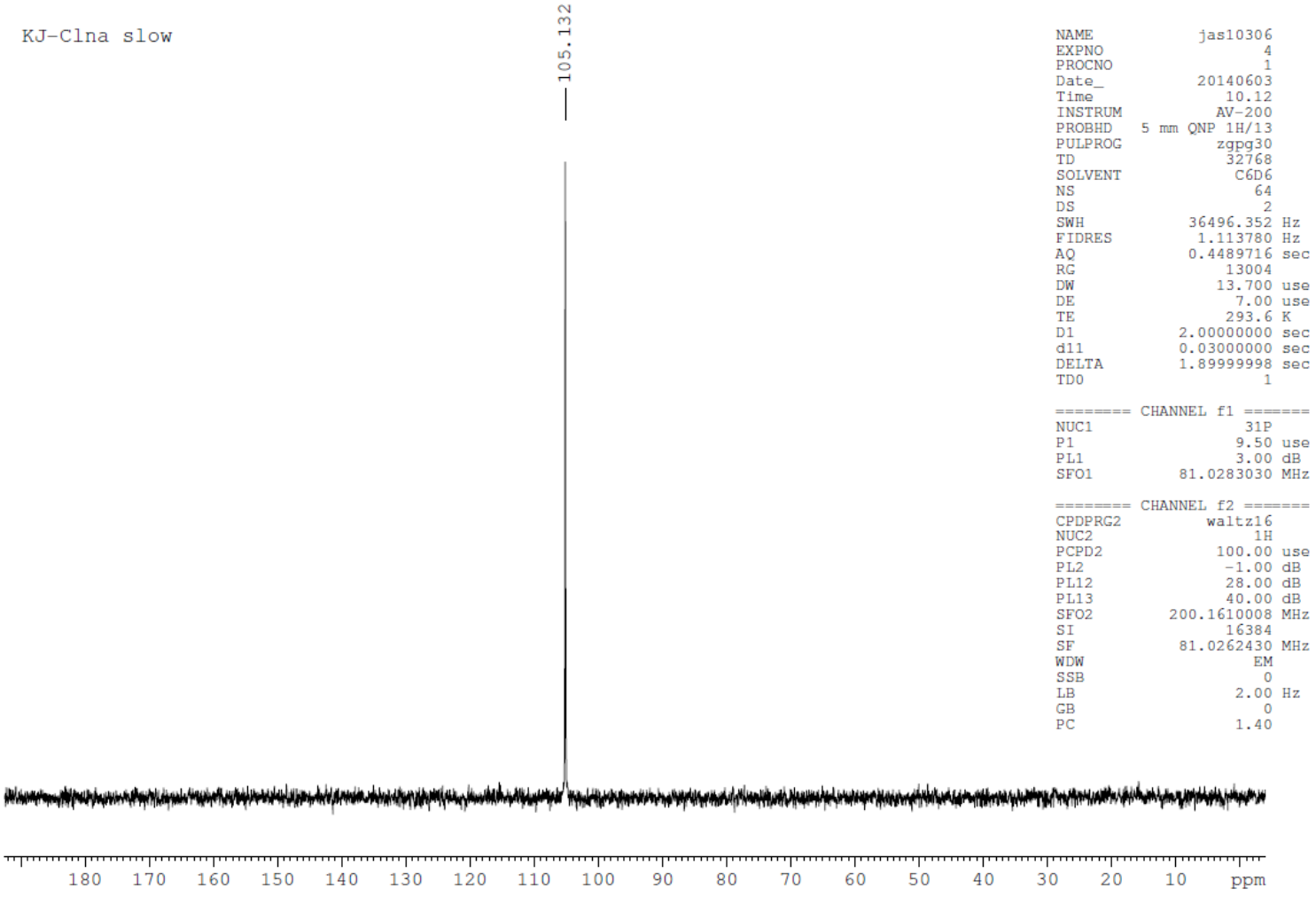

Figure S22. C_L-OTP *slow* (**2c**)*;* δ (ppm, C_6D_6)

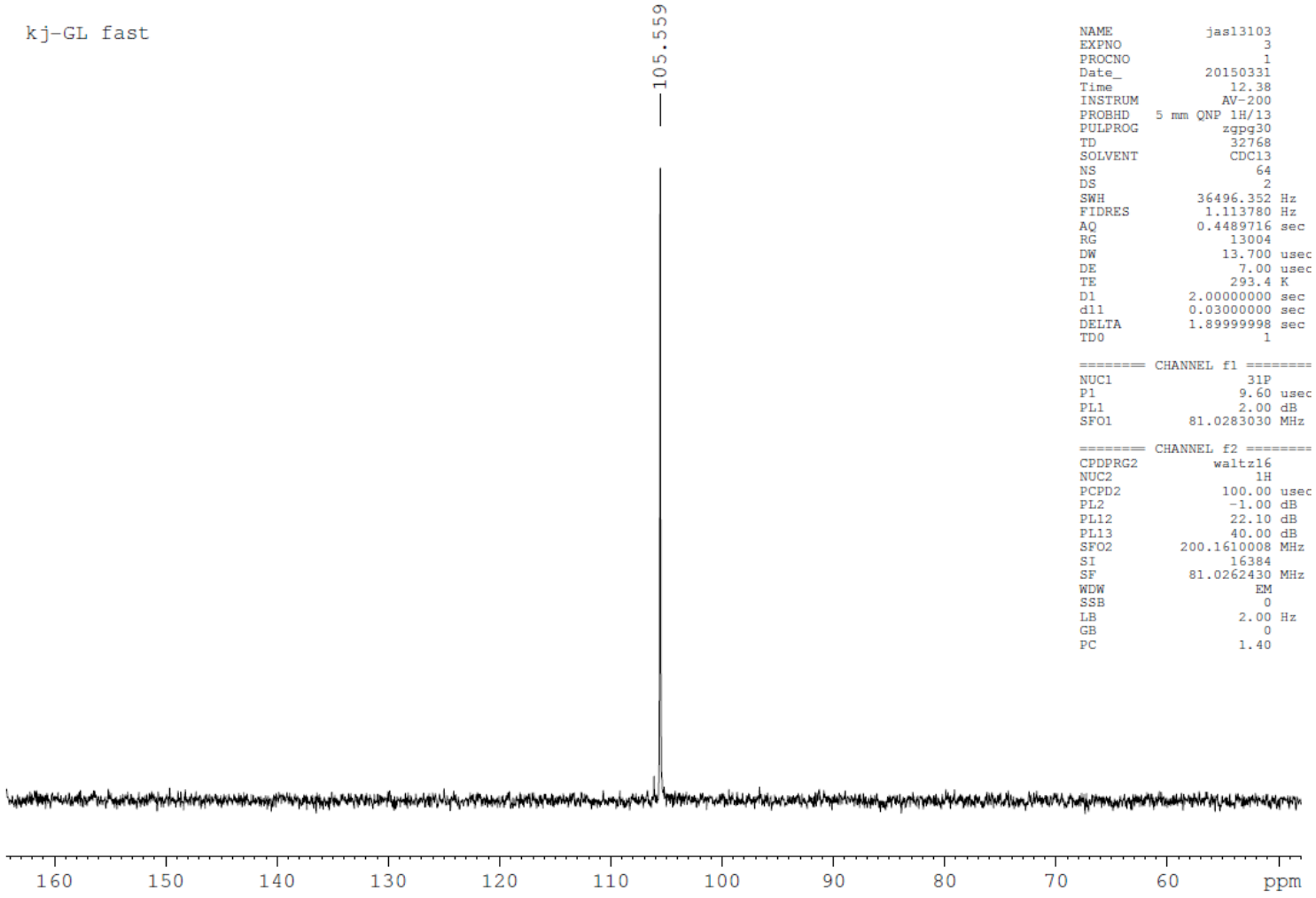

Figure S23. G_L-OTP *fast* (**2d**)*;* δ (ppm, $CDCl_3$)

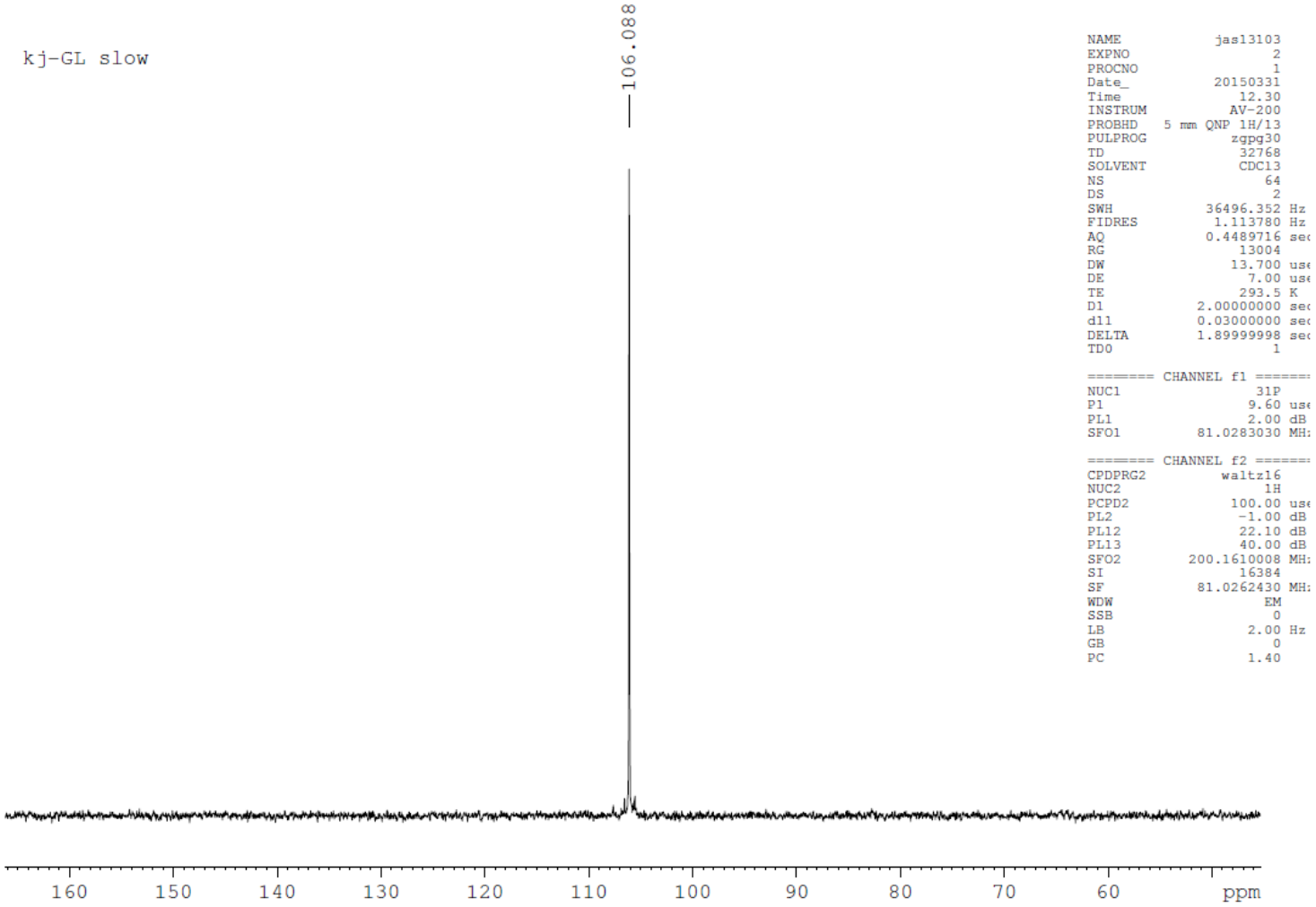

Figure S24. G_L-OTP *slow* (**2d**)*;* δ (ppm, $CDCl_3$)

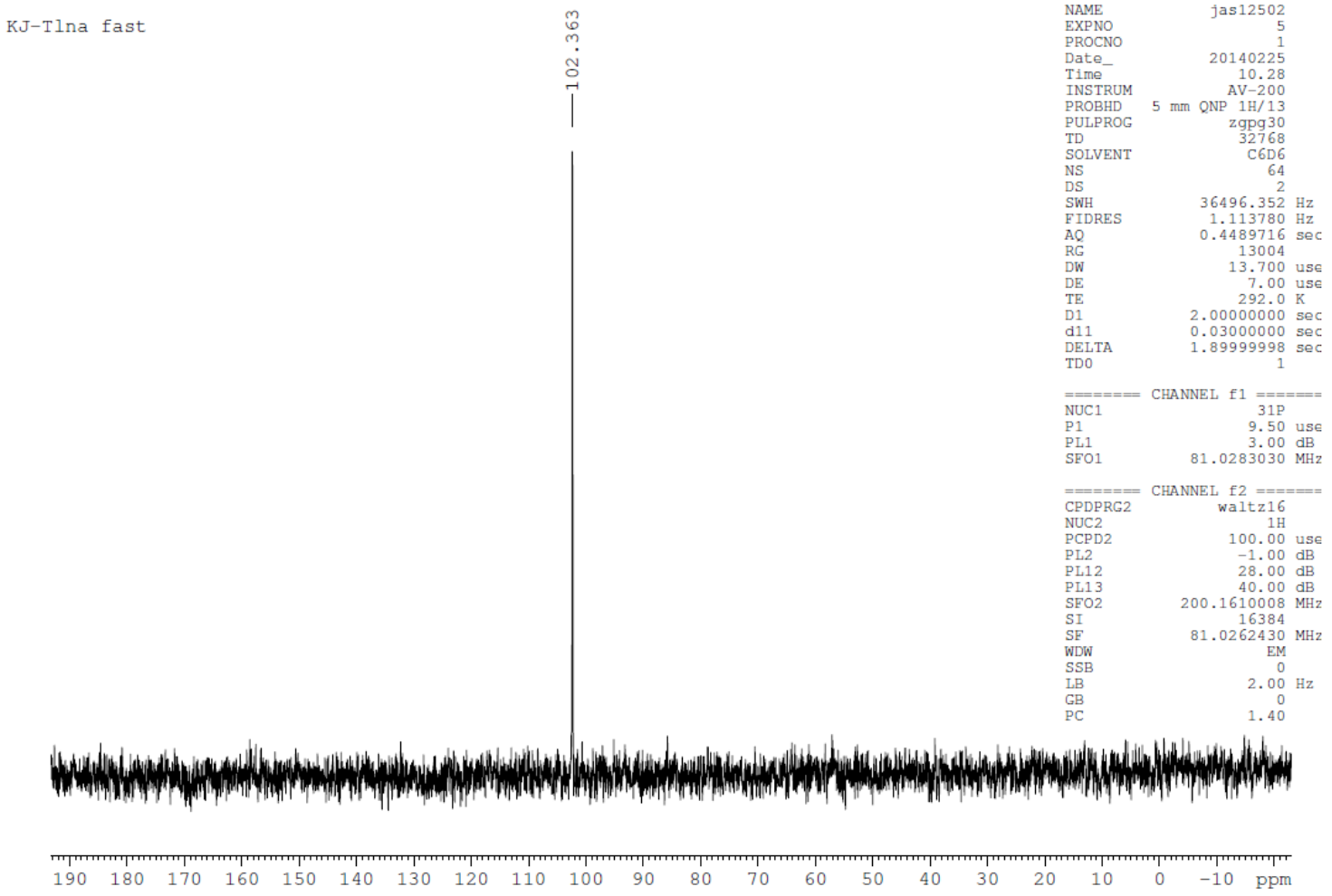

Figure S25. T_L-OTP *fast* (**2a**)*;* δ (ppm, C_6D_6)

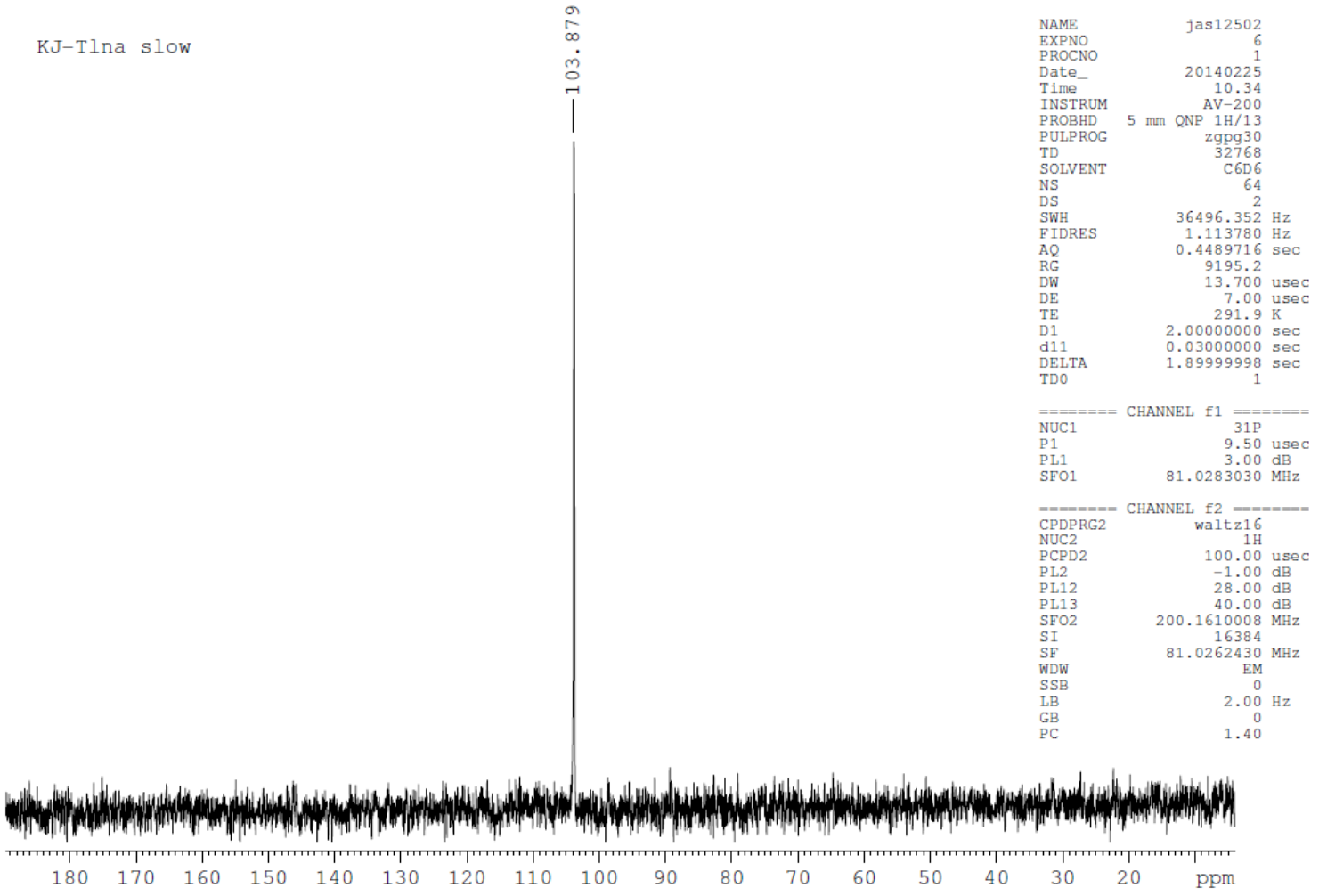

Figure S26. T_L-OTP *slow* (**2a**)*;* δ (ppm, C_6D_6)

VII. ^{13}C NMR spectra for separated OTP-LNA monomers, recorded with a Bruker AV-200 spectrometer (200 MHz for ^{1}H)

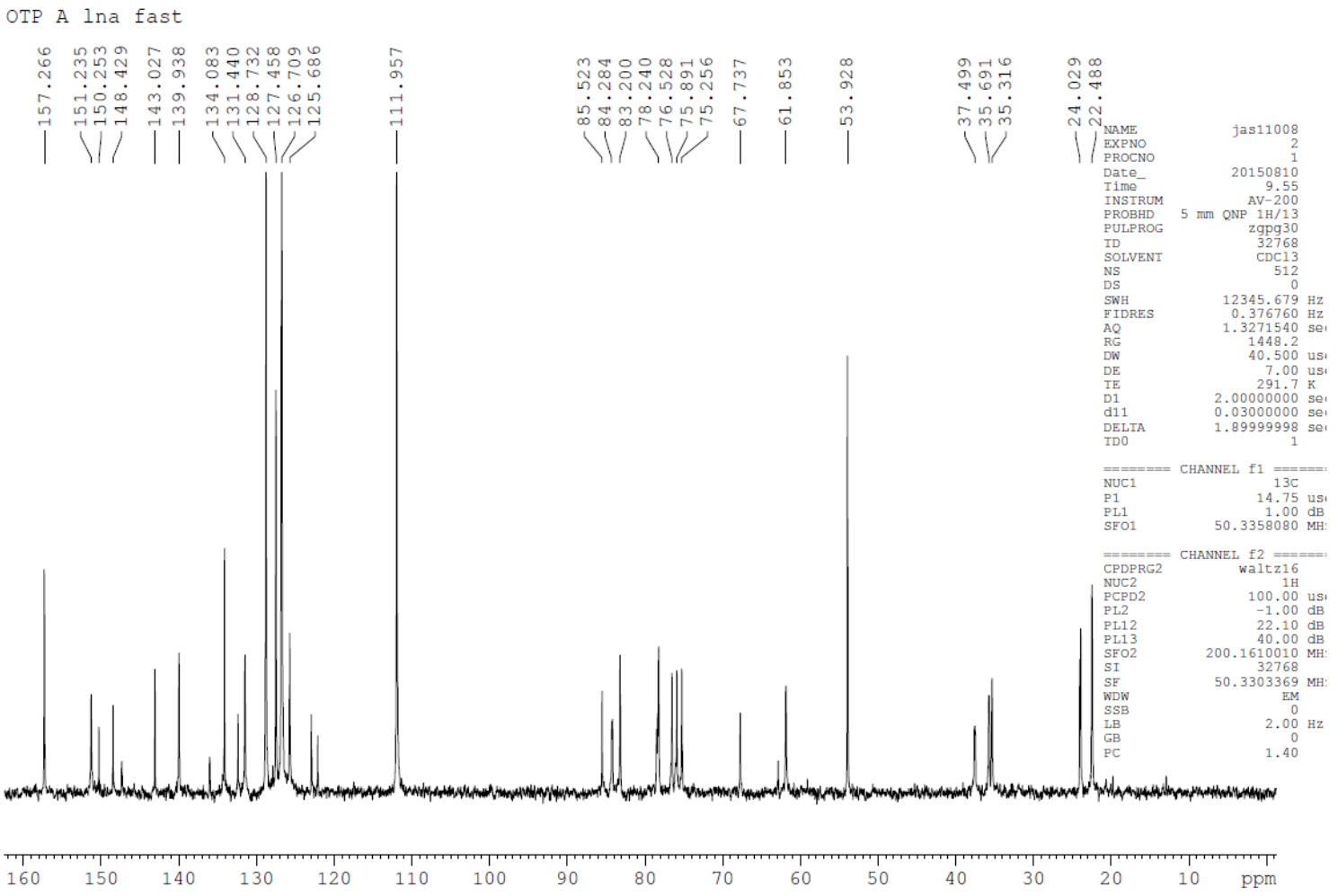

Figure S27. A_L-OTP *fast* (**2b**)*;* δ (ppm, $CDCl_3$) 157.3, 151.2, 150.2, 148.4, 143.0, 139.9, 134.1, 131.4, 128.7, 127.5, 126.7, 125.7, 112.0, 85.5, 84.3, 83.2, 78.2, 76.5, 75.9, 75.3, 67.7, 61.9, 53.9, 37.5, 35.7, 35.3, 24.0, 22.5

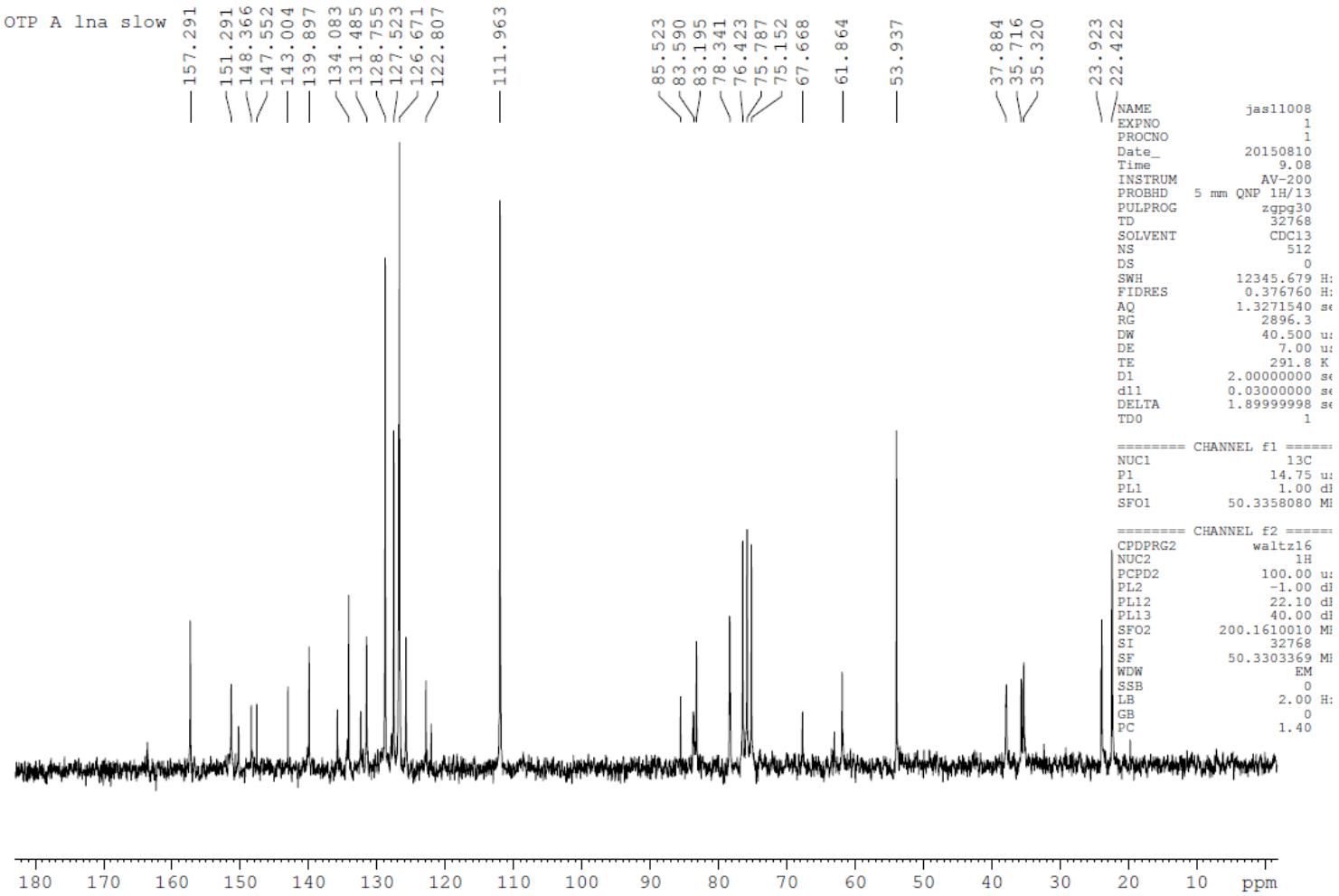

Figure S28. A_L-OTP *slow* (**2b**)*;* δ (ppm, $CDCl_3$) 157.3, 151.3, 148.4, 147.6, 143.0, 139.9, 134.1, 131.5, 128.8, 127.5, 126.7, 122.8, 112.0, 85.5, 83.6, 83.2, 78.3, 76.4, 75.8, 75.2, 67.7, 61.9, 53.9, 37.9, 35.7, 35.3, 23.9, 22.4

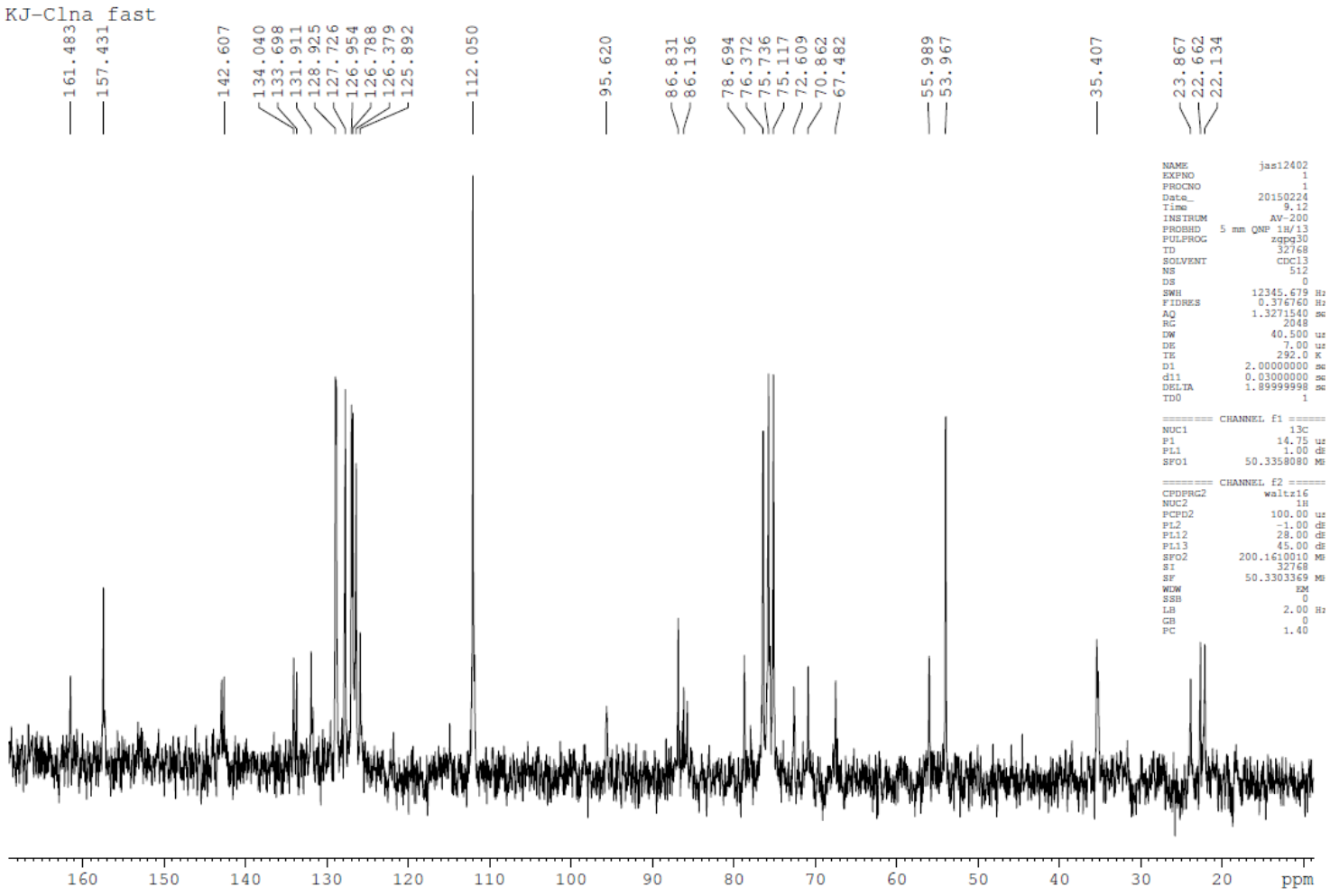

Figure S29. C_L-OTP *fast* (**2c**)*;* δ (ppm, $CDCl_3$) 161.5, 157.4, 142.6, 134.0, 133.7, 131.9, 128.9, 127.7, 127.0, 126.8, 126.4, 125.9, 112.1, 95.6, 86.8, 86.1, 78.7, 76.4, 75.7, 75.1, 72.6, 70.9, 67.5, 56.0, 54.0, 35.4, 23.9, 22.7, 22.1

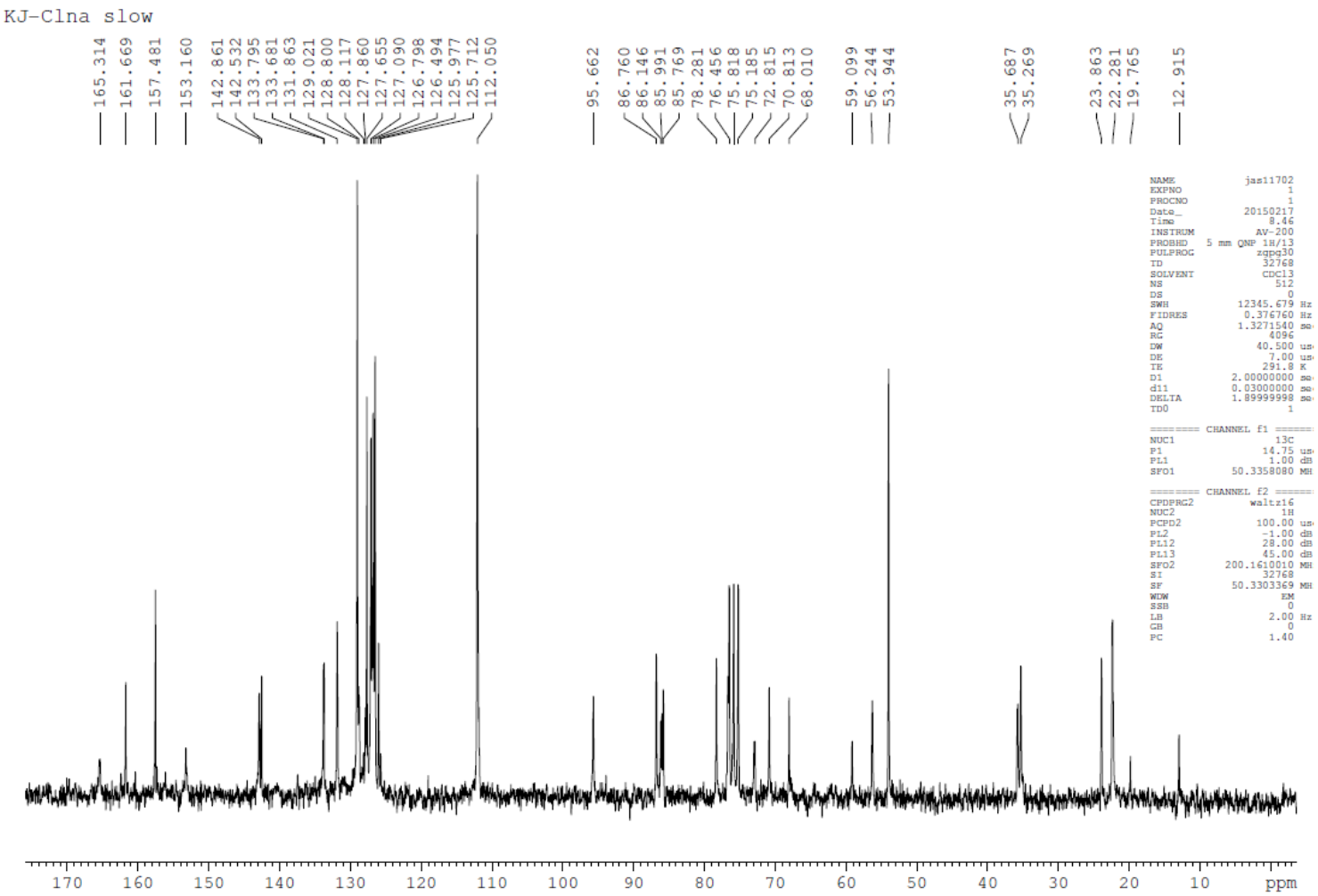

Figure S30. C_L-OTP *slow* (**2c**)*;* δ (ppm, $CDCl_3$) 161.7, 157.5, 142.9, 133.8, 133.7, 131.9, 129.0, 128.8, 127.9, 127.1, 126.8, 126.5, 112.1, 95.7, 86.8, 86.1, 86.0, 85.8, 78.3, 76.5, 75.2, 72.8, 68.0, 56.2, 53.9, 35.7, 35.3, 23.9, 22.3

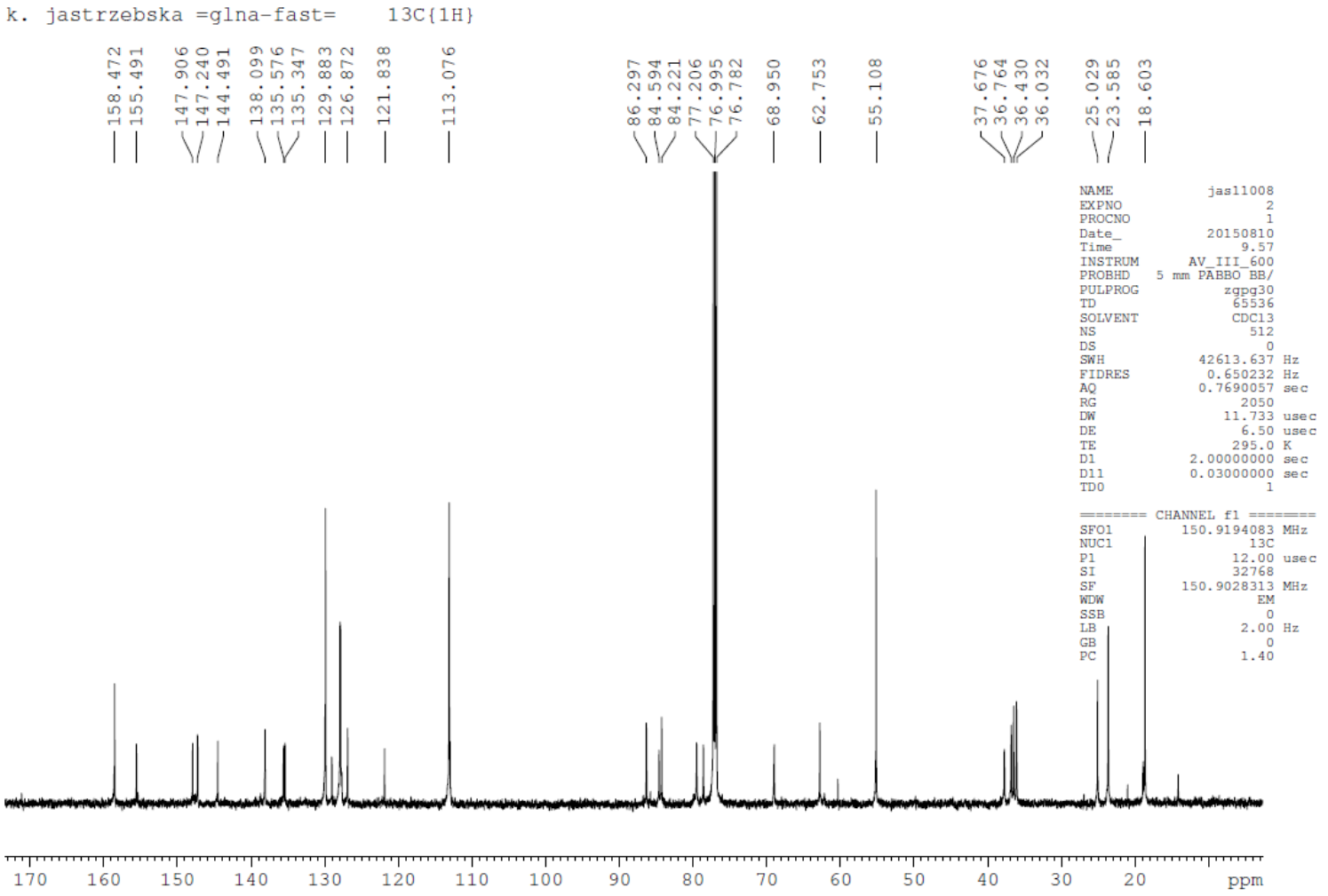

Figure S31. G_L-OTP *fast* (**2d**)*;* SF=600 MHz for ^{1}H; δ (ppm, $CDCl_3$) 158.5, 155.5, 147.9, 147.2, 144.5, 138.1, 135.6, 135.3, 129.9, 126.9, 121.8, 113.1, 86.3, 84.6, 84.2, 77.2, 77.0, 76.8, 69.0, 62.8, 55.1, 37.7, 36.8, 36.4, 36.0, 25.0, 23.6, 18.6

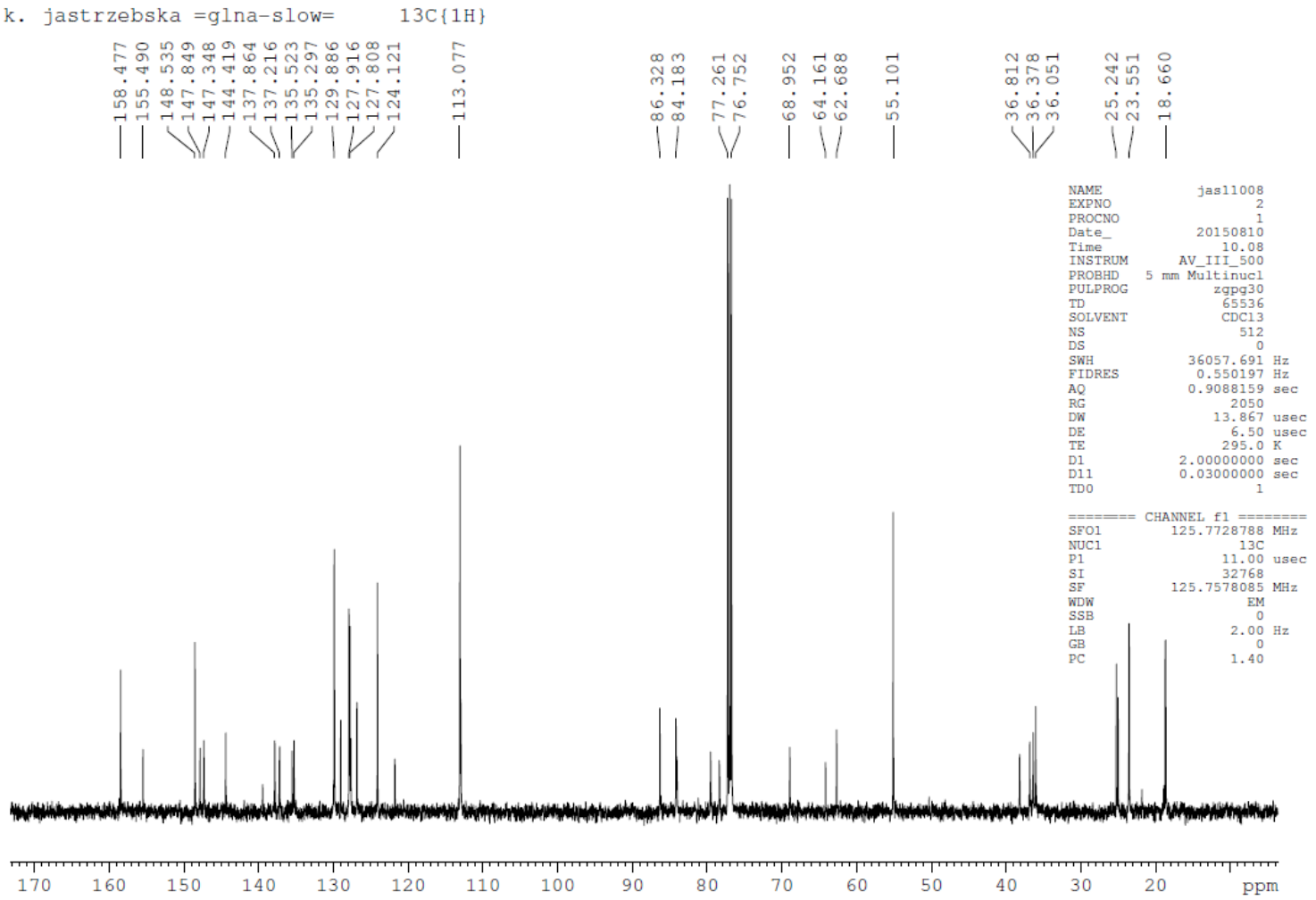

Figure S32. G_L-OTP *slow* (**2d**)*;* SF=500 MHz for ^{1}H; δ (ppm, $CDCl_3$) 158.5, 155.5, 148.5, 147.8, 147.3, 144.4, 137.9, 137.2, 135.5, 135.3, 129.9, 127.8, 124.1, 113.1, 86.3, 84.2, 77.3, 76.8, 69.0, 64.2, 62.7, 55.1, 36.8, 36.4, 36.1, 25.2, 23.6, 18.7

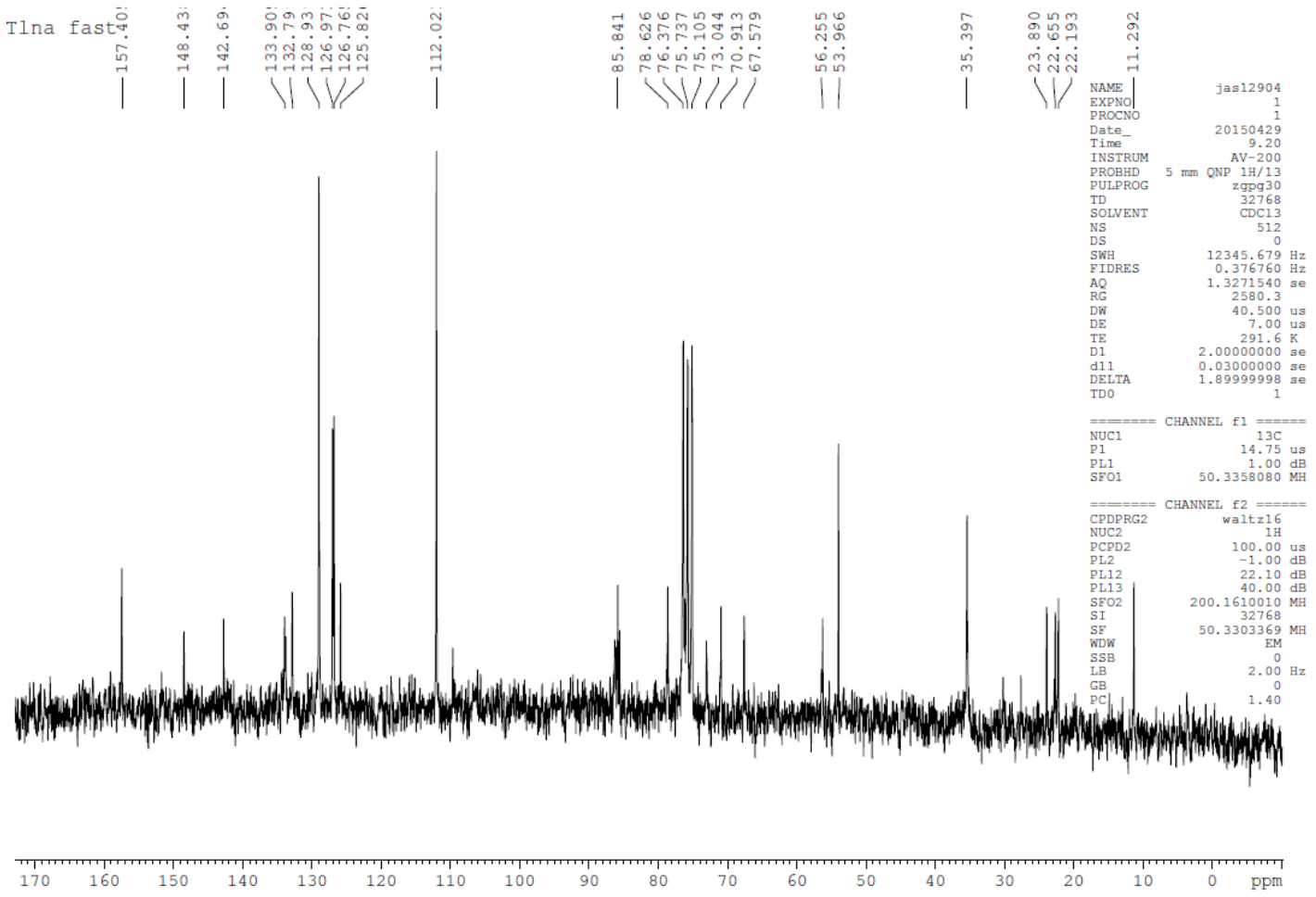

Figure S33. T_L-OTP *fast* (**2a**); δ (ppm, $CDCl_3$) 157.4, 148.4, 142.7, 133.9, 132.8, 128.9, 127.0, 126.8, 125.8, 112.0, 85.8, 78.6, 76.4, 75.7, 75.1, 73.0, 70.9, 67.6, 56.3, 54.0, 35.4, 23.9, 22.7, 22.2, 11.3

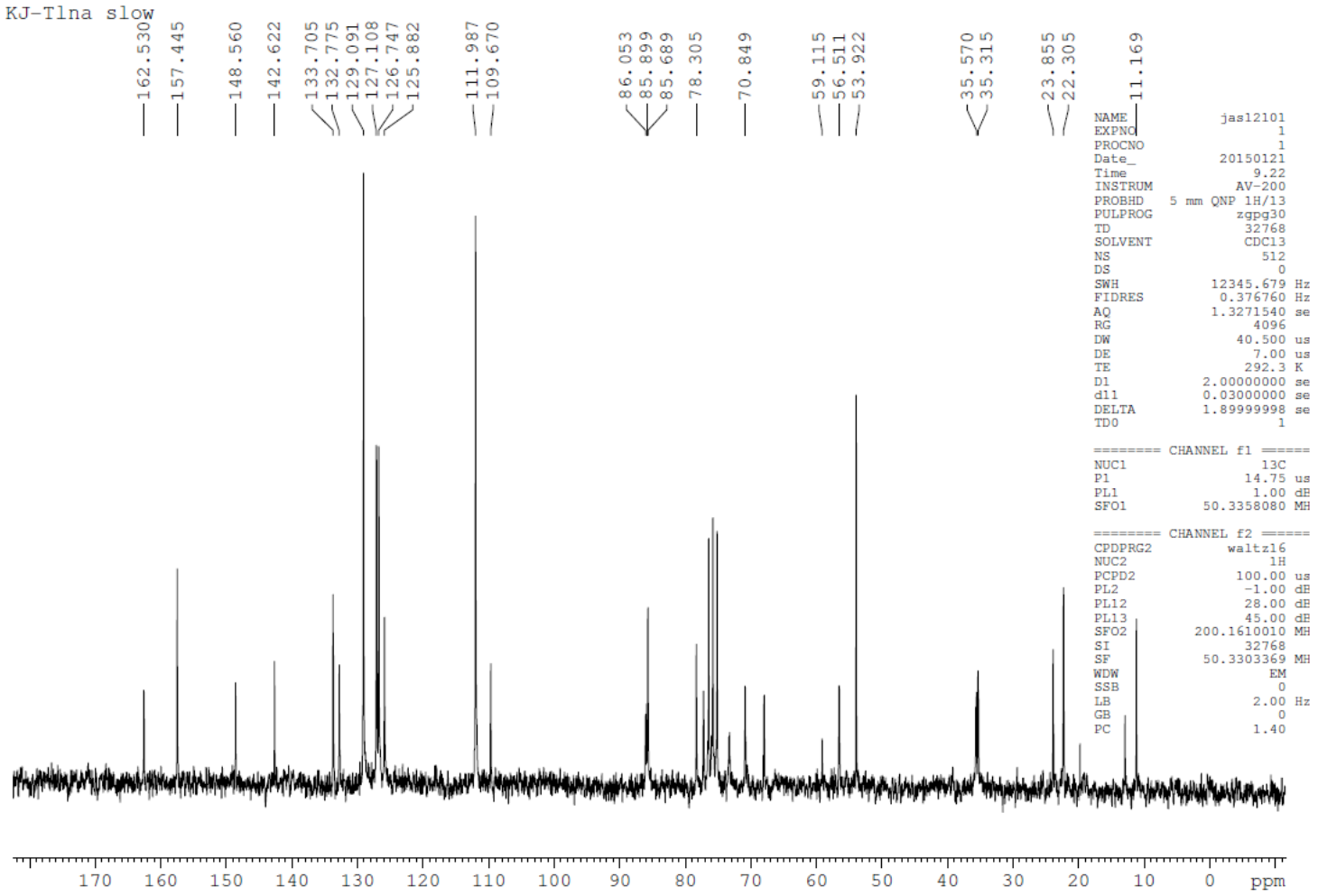

Figure S34. T_L-OTP *slow* (**2a**); δ (ppm, $CDCl_3$) 162.5, 157.4, 148.6, 142.6, 133.7, 132.8, 129.1, 127.1, 126.7, 125.9, 112.0, 109.7, 86.1, 85.9, 85.7, 78.3, 70.8, 56.5, 53.9, 35.6, 35.3, 23.9, 22.3, 11.2

VIII. Profiles from an RP-HPLC analysis of **PS-(DNA/LNA)** oligomers carrying the 5'-*O*-DMT tag.

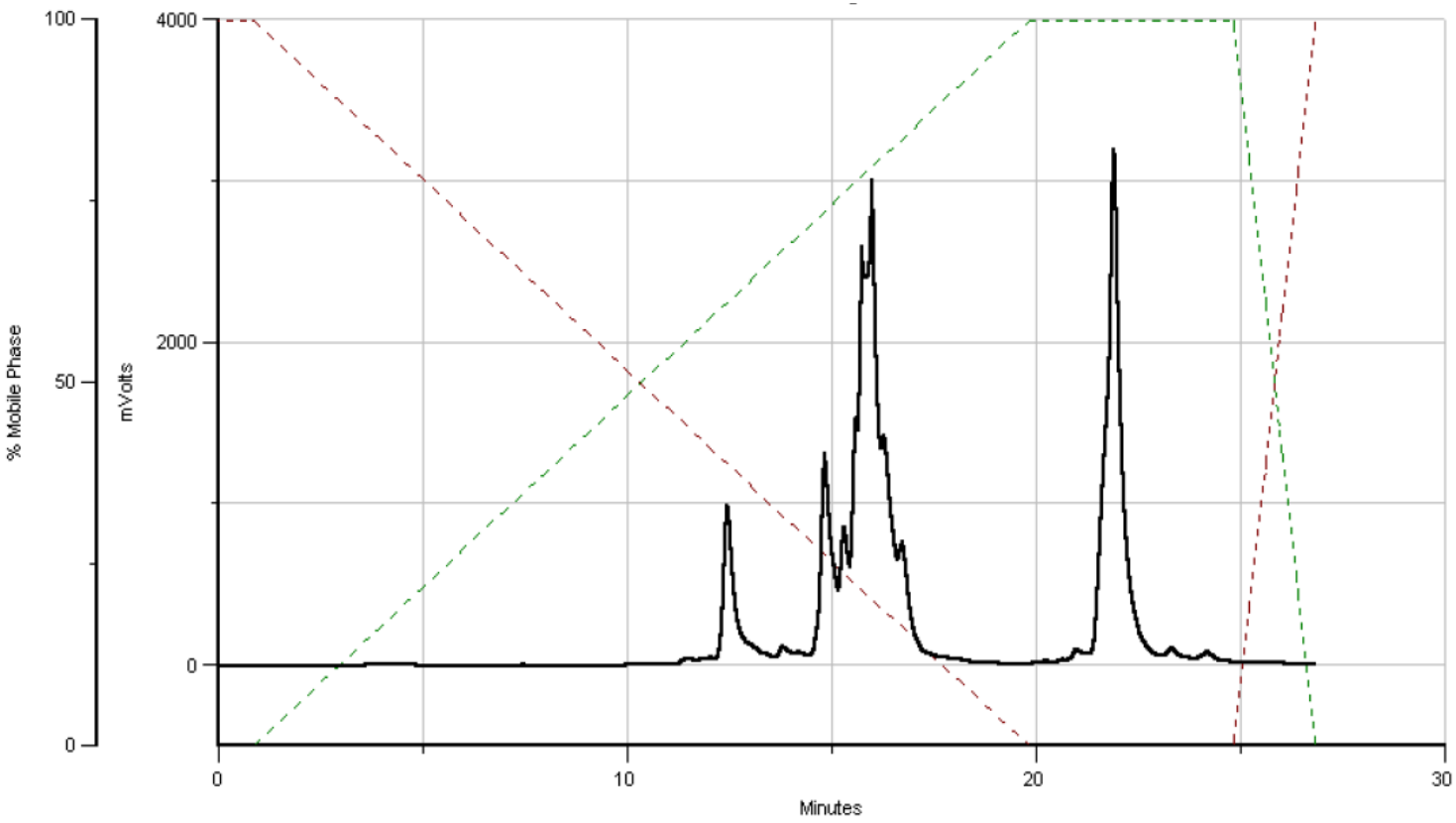

Figure S35. **A2R** [R_P-PS]-d(GACA$_L$TCA$_L$CTAG)

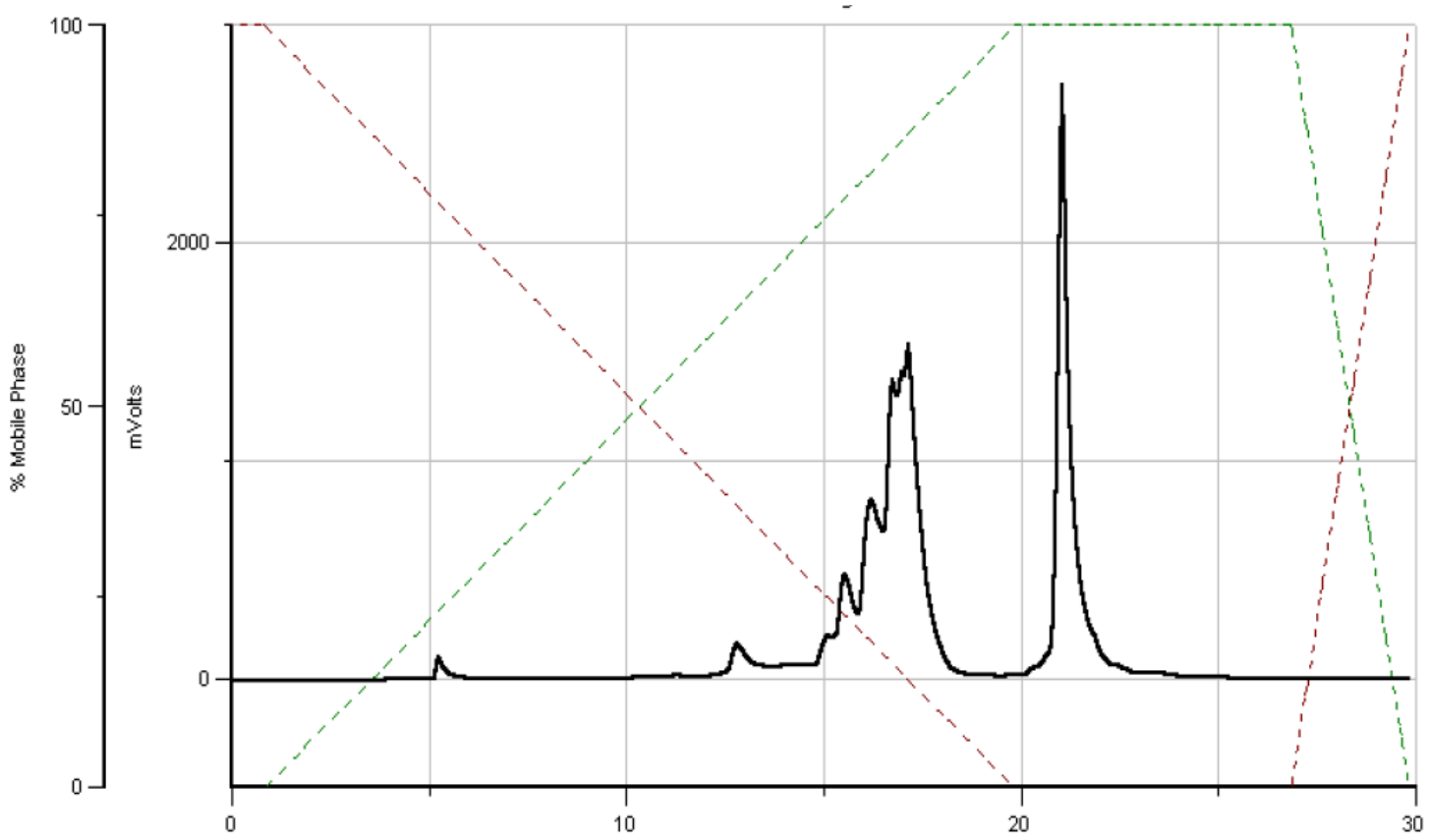

Figure S36. **A2S** [S_P-PS]-d(GACA_LTCA_LCTAG)

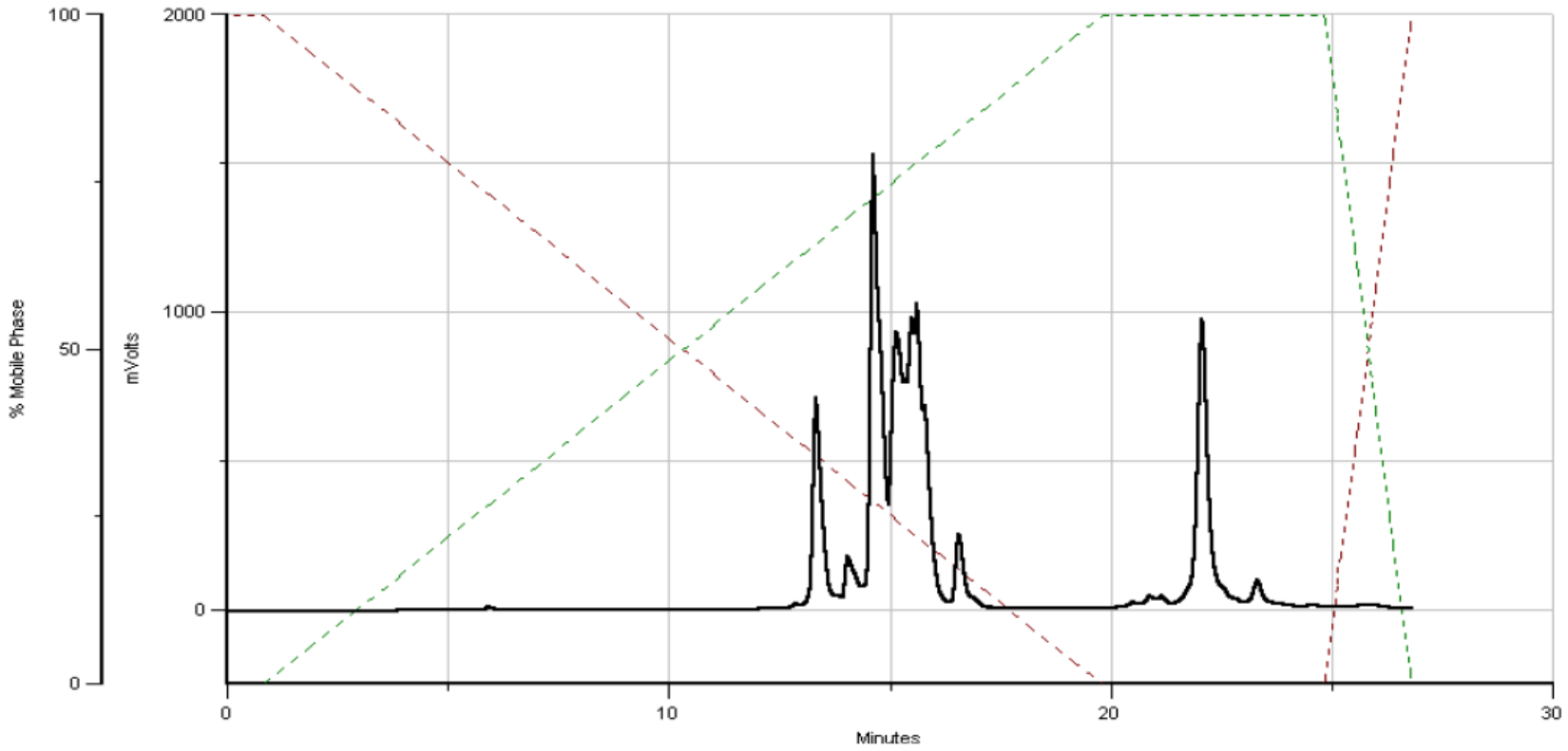

Figure S37. **C2R** [R_P-PS]-d(GAC$_L$ATC$_L$ACTAG)

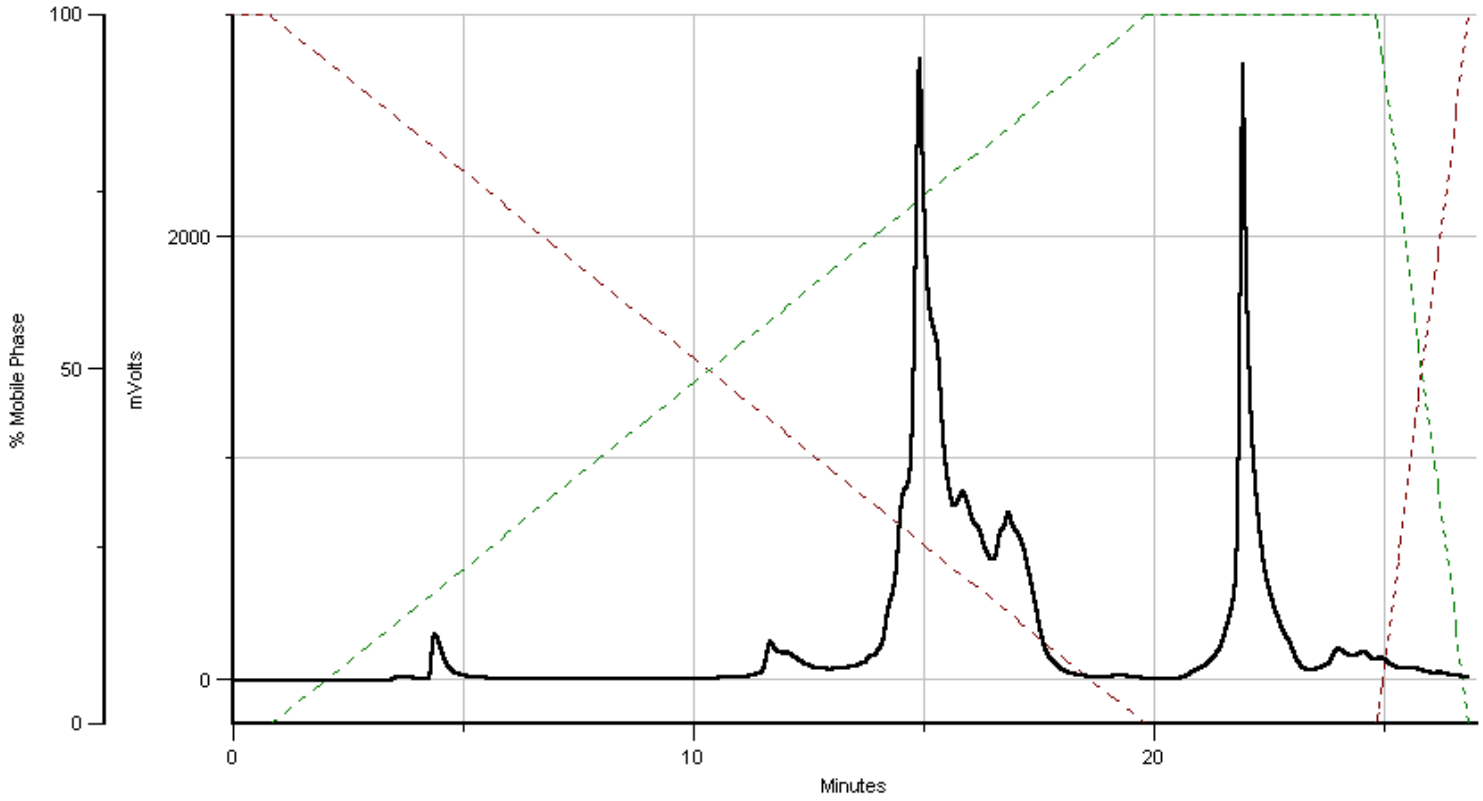

Figure S38. **C2S** [S_P-PS]-d(GAC$_L$ATC$_L$ACTAG)

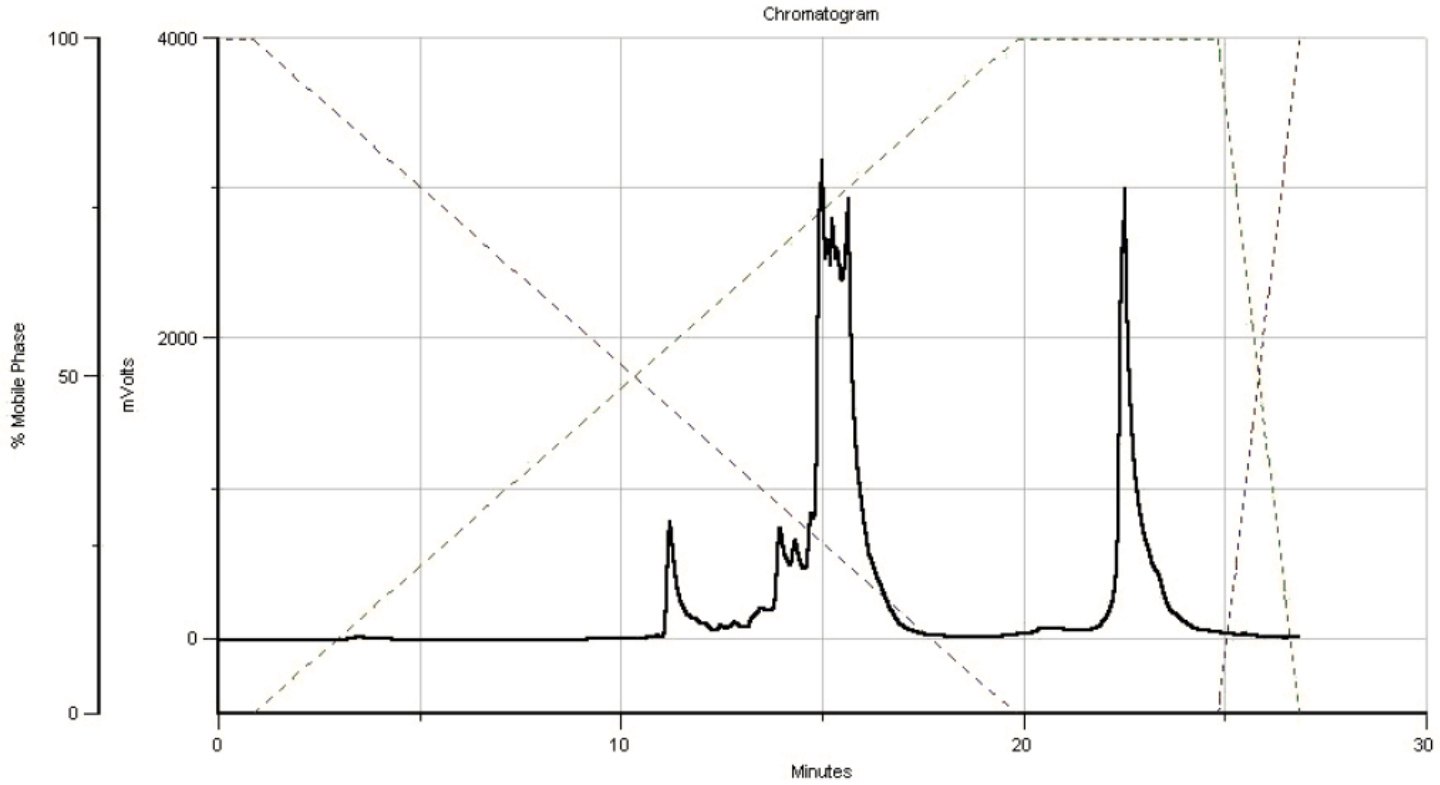

Figure S39. **C3R** [R_P-PS]-d(GAC$_L$ATC$_L$AC$_L$TAG)

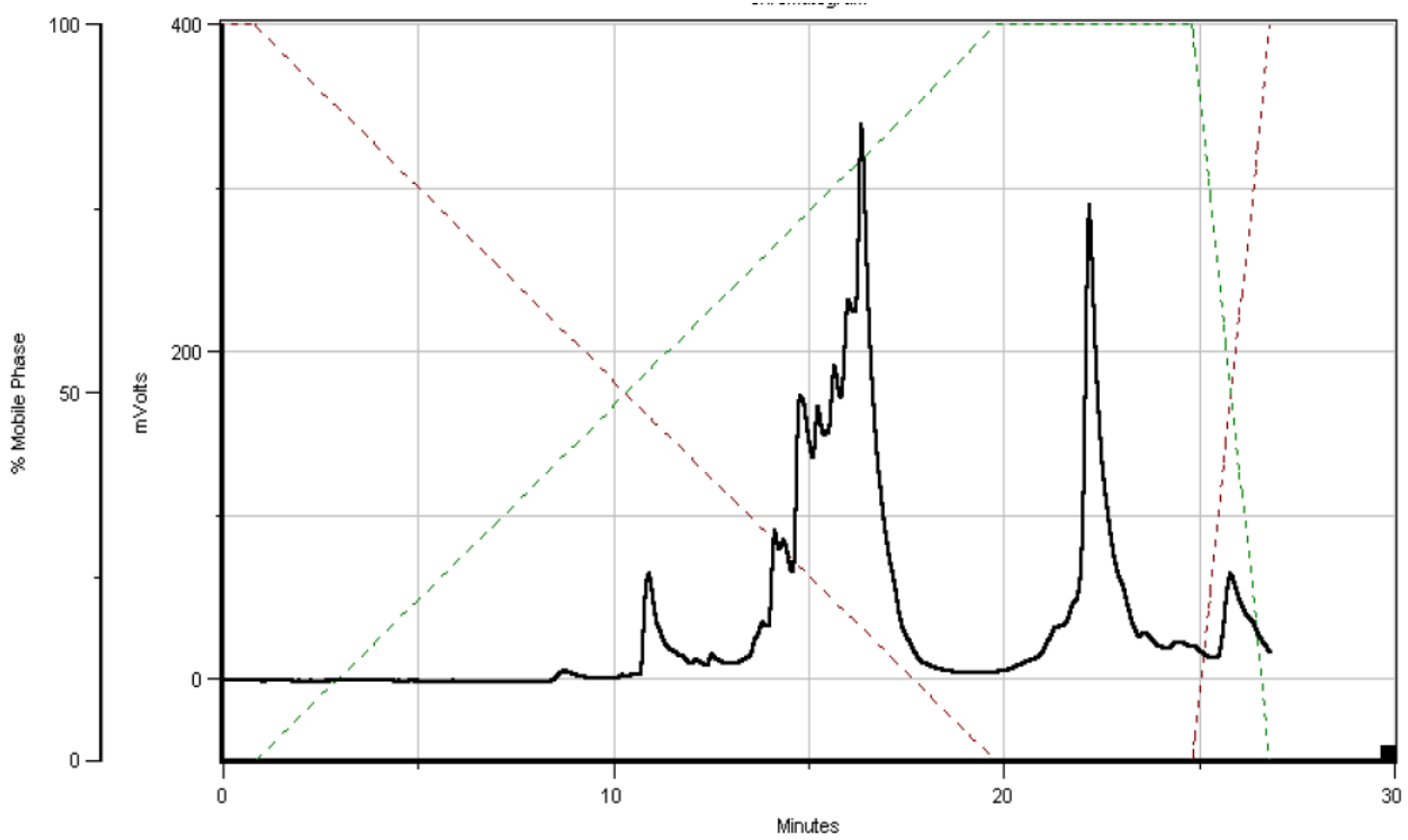

Figure S40. **C3S** [S_P-PS]-d(GAC$_L$ATC$_L$AC$_L$TAG)

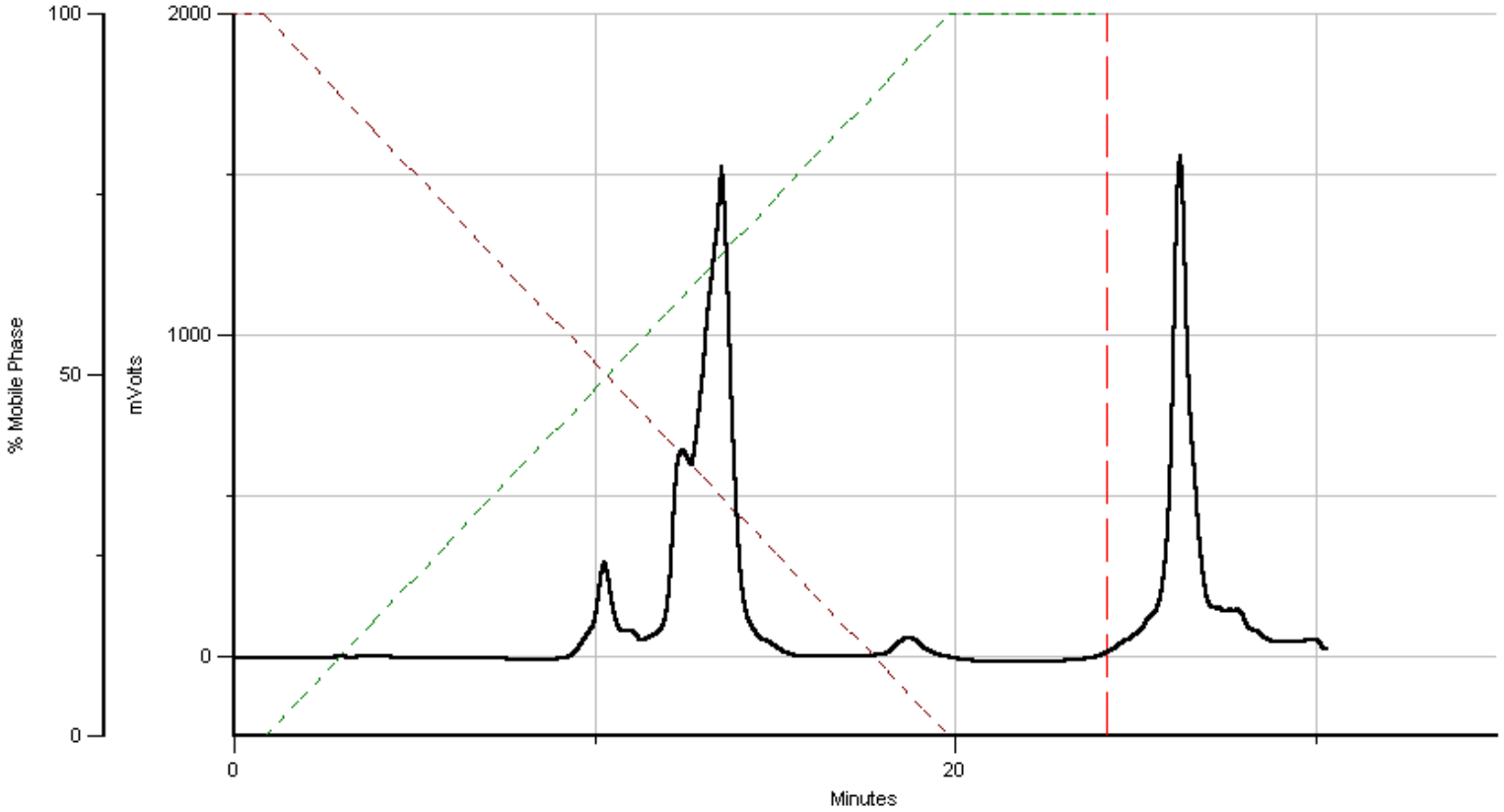

Figure S41. **G2R** [R_P-PS]-d(GAG$_L$ATG$_L$ACTAG)

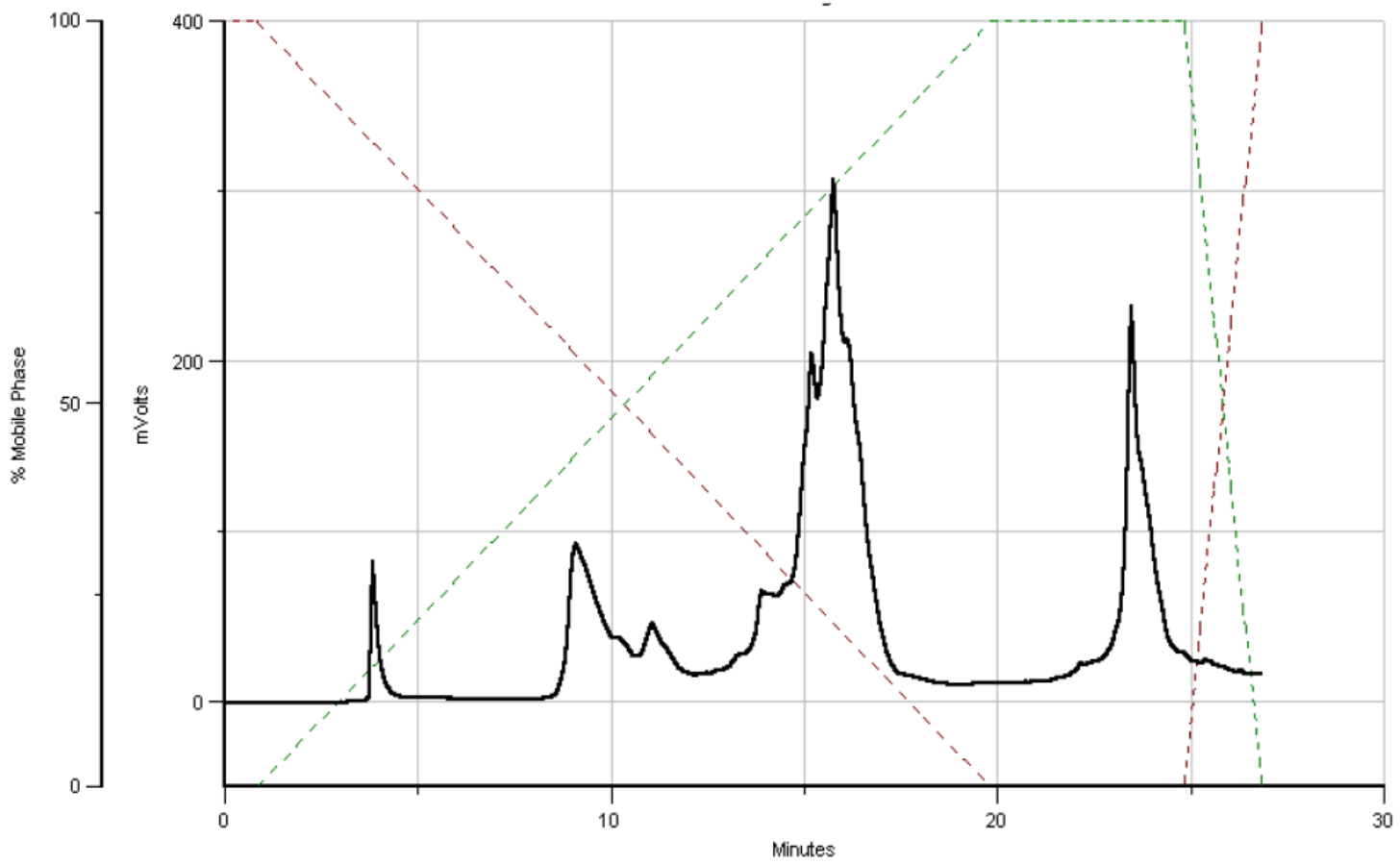

Figure S42. **G2S** [S_P-PS]-d(GAG$_L$ATG$_L$ACTAG)

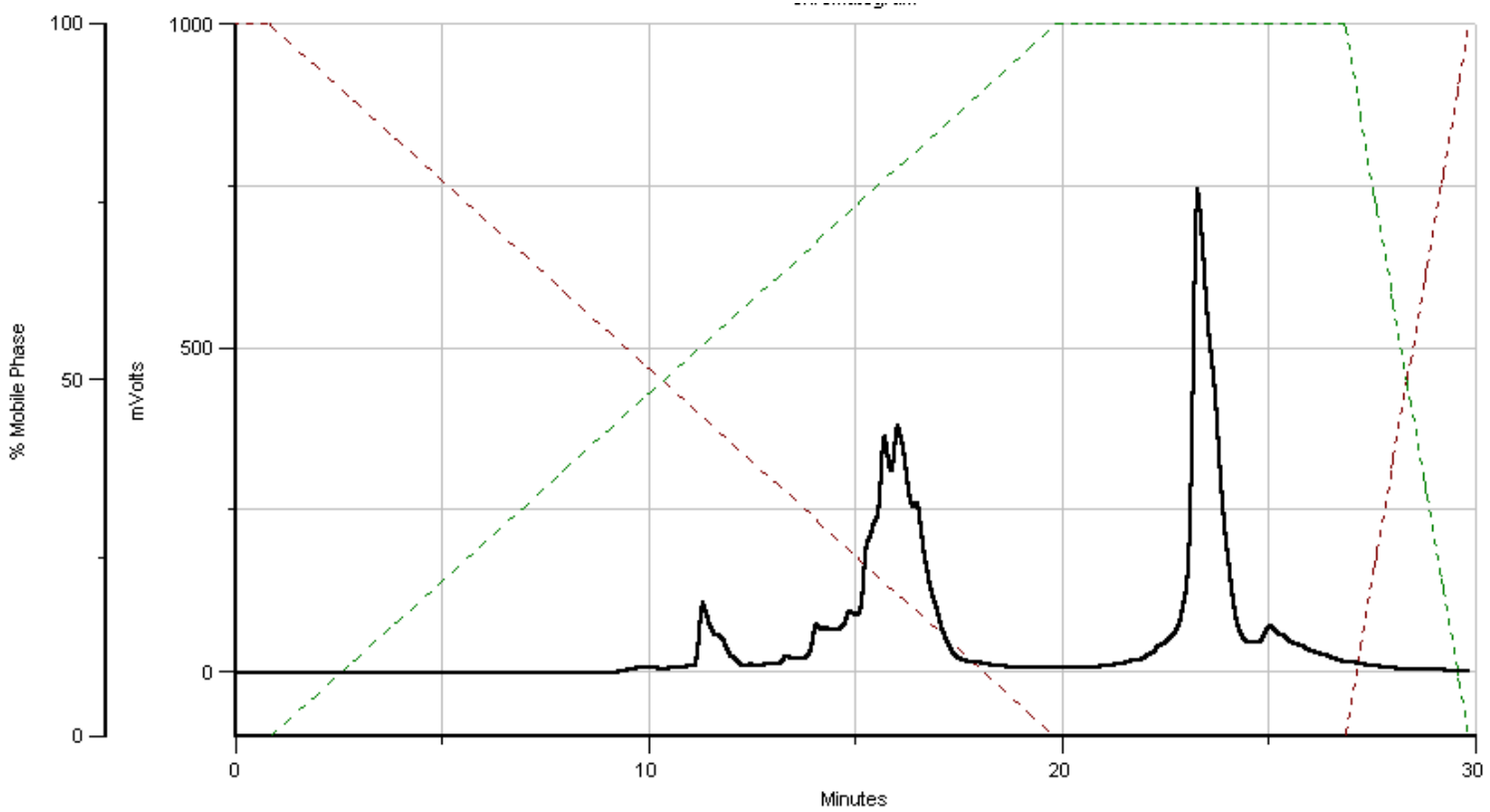

Figure S43. **T2R** [R_P-PS]-d(GACAT$_L$CACT$_L$AG)

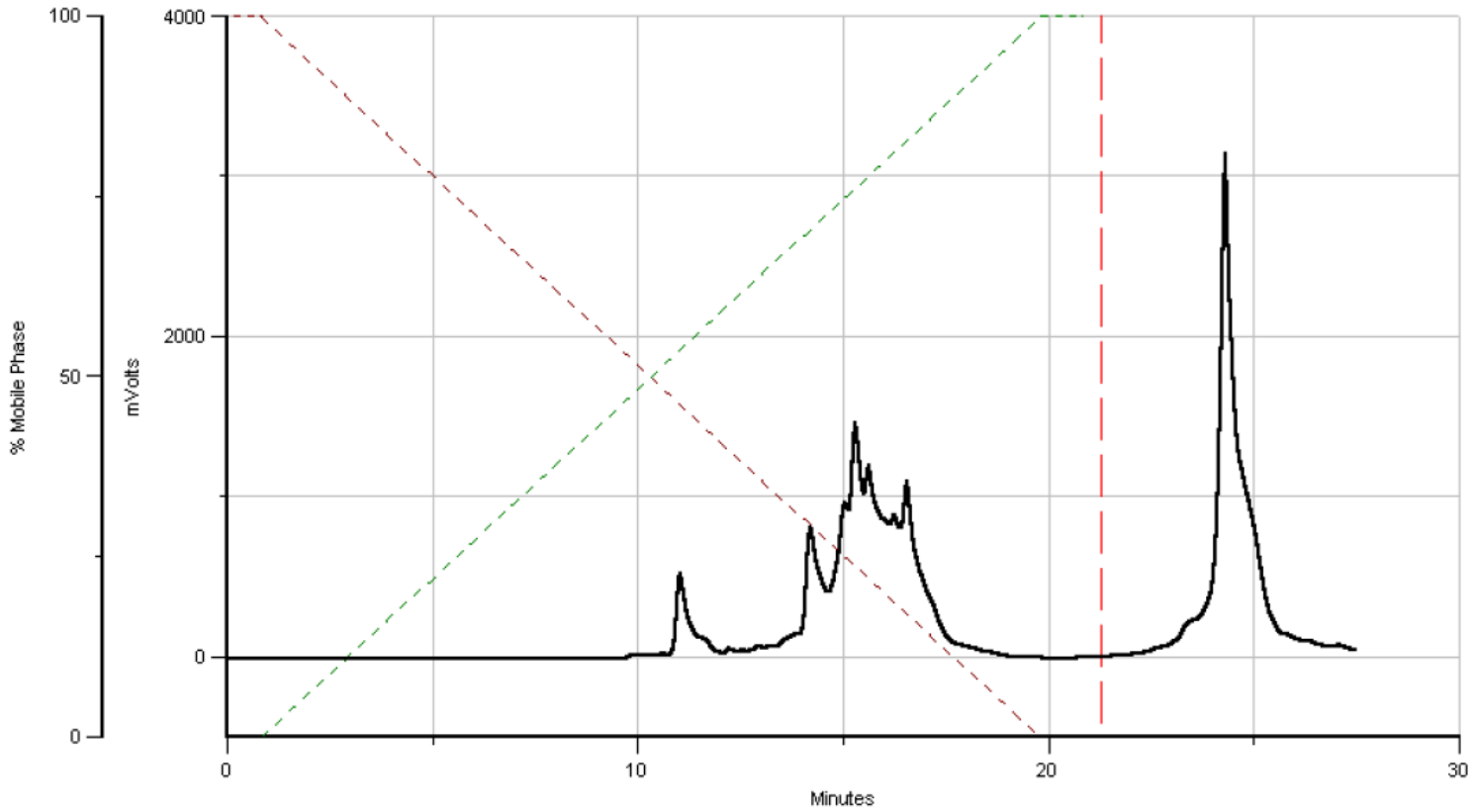

Figure S44. **T2S** [S_P-PS]-d(GACAT$_L$CACT$_L$AG)

IX. Profiles from an RP-HPLC analysis of **PS-(DNA/LNA)** oligomers after removal of the 5'-*O*-DMT tag.

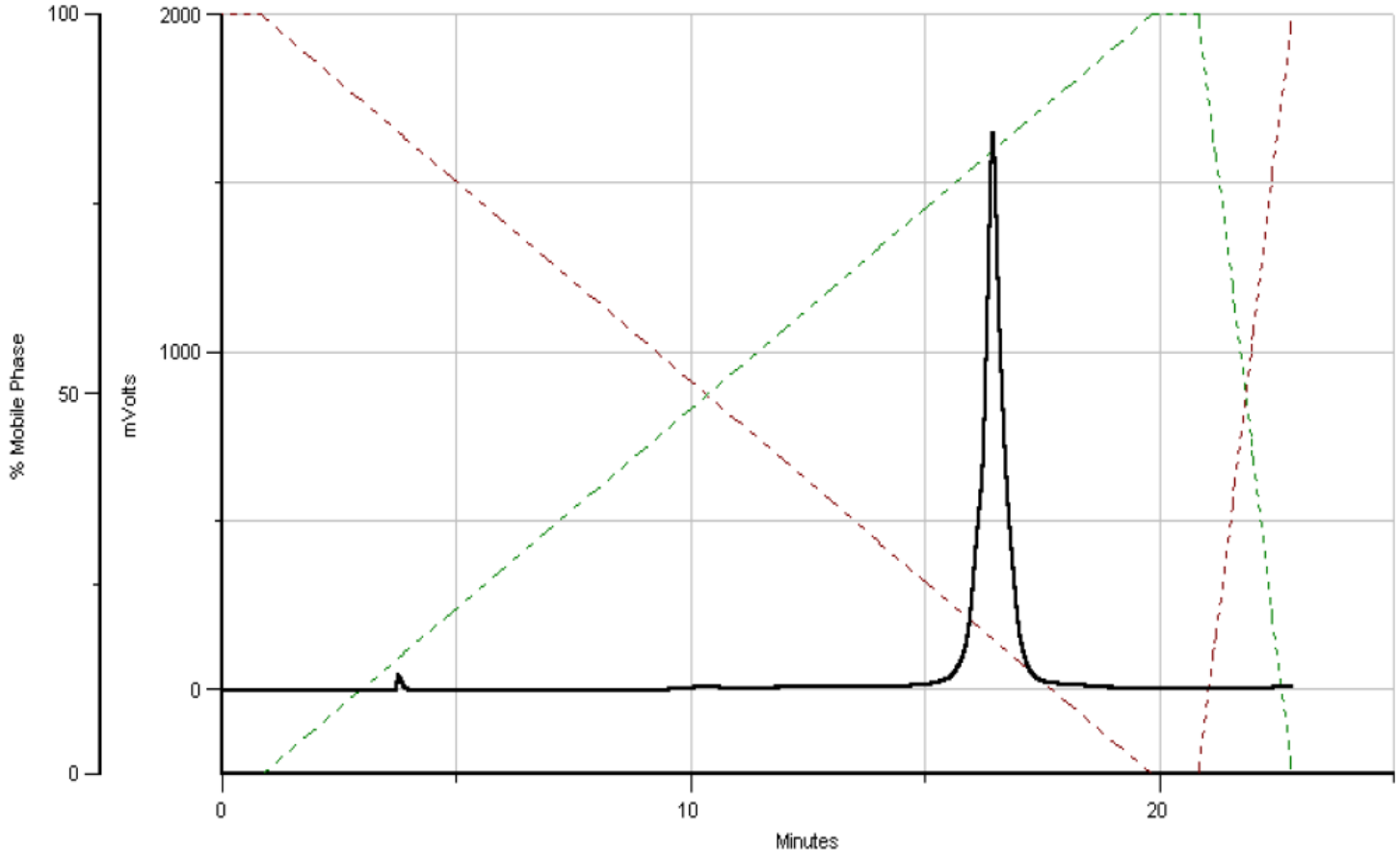

Figure S45. **A2R** [R_P-PS]-d(GACA$_L$TCA$_L$CTAG)

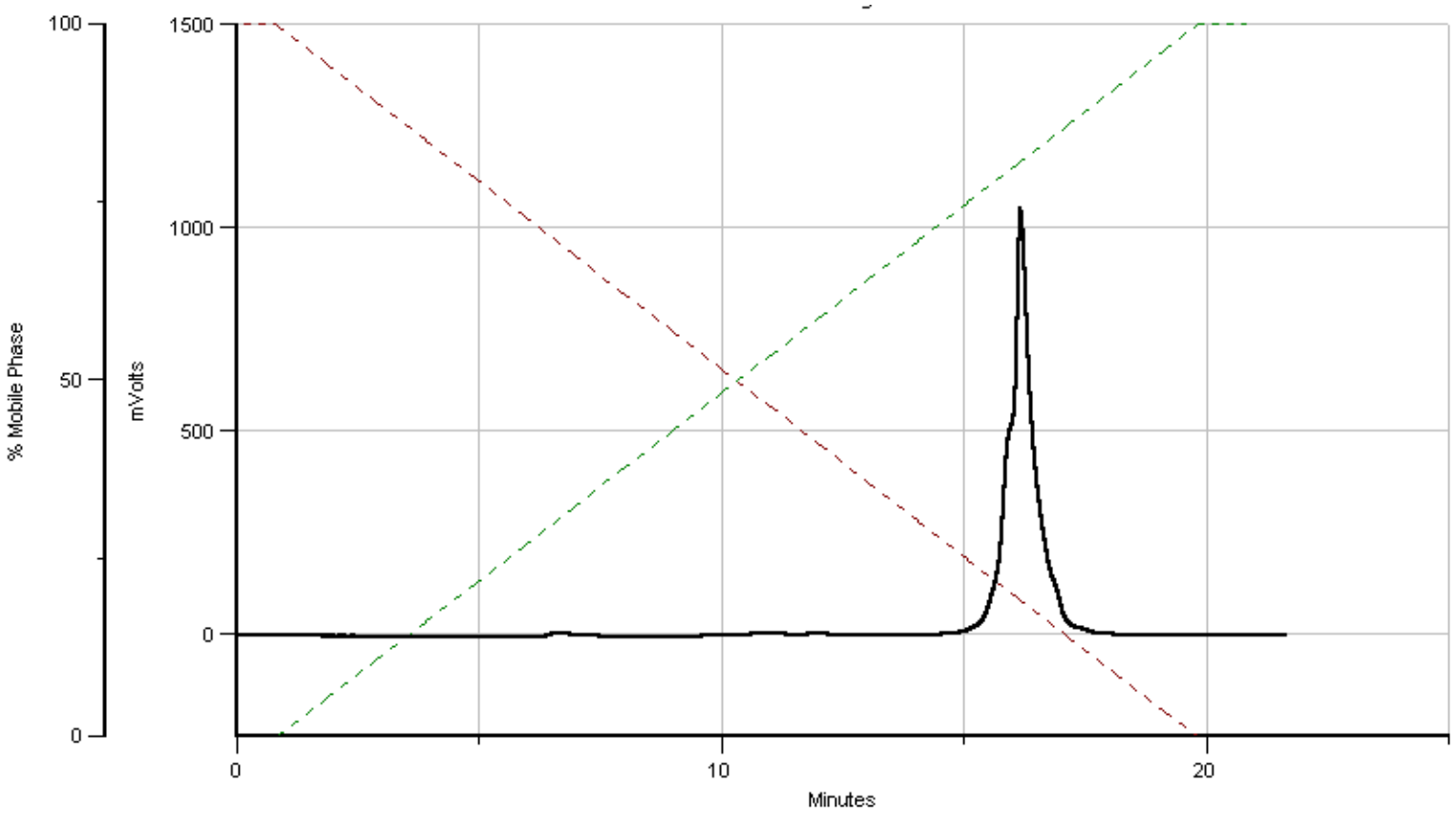

Figure S46. **A2S** [S_P-PS]-d(GACA$_L$TCA$_L$CTAG)

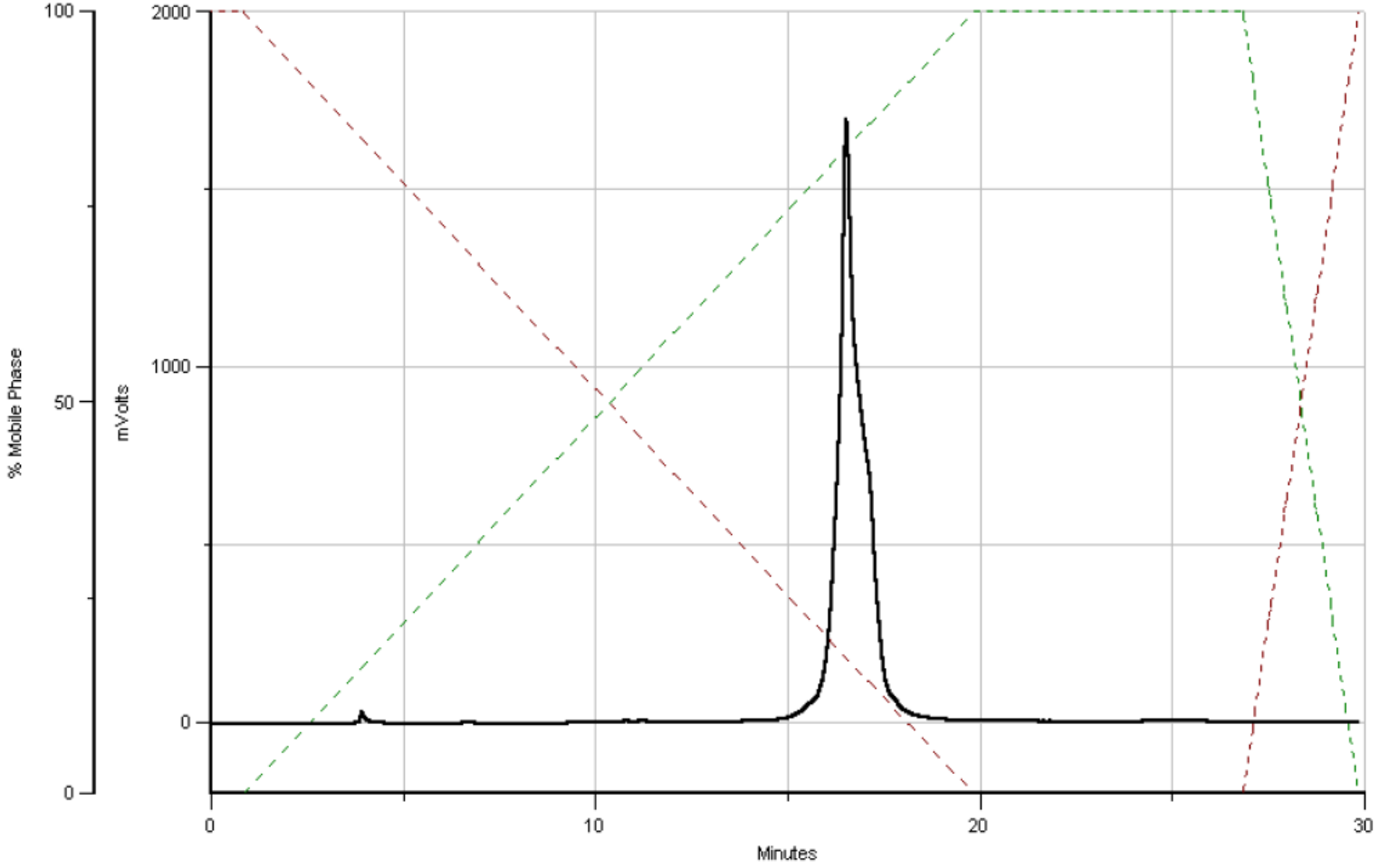

Figure S47. **C2R** [R_P-PS]-d(GAC$_L$ATC$_L$ACTAG)

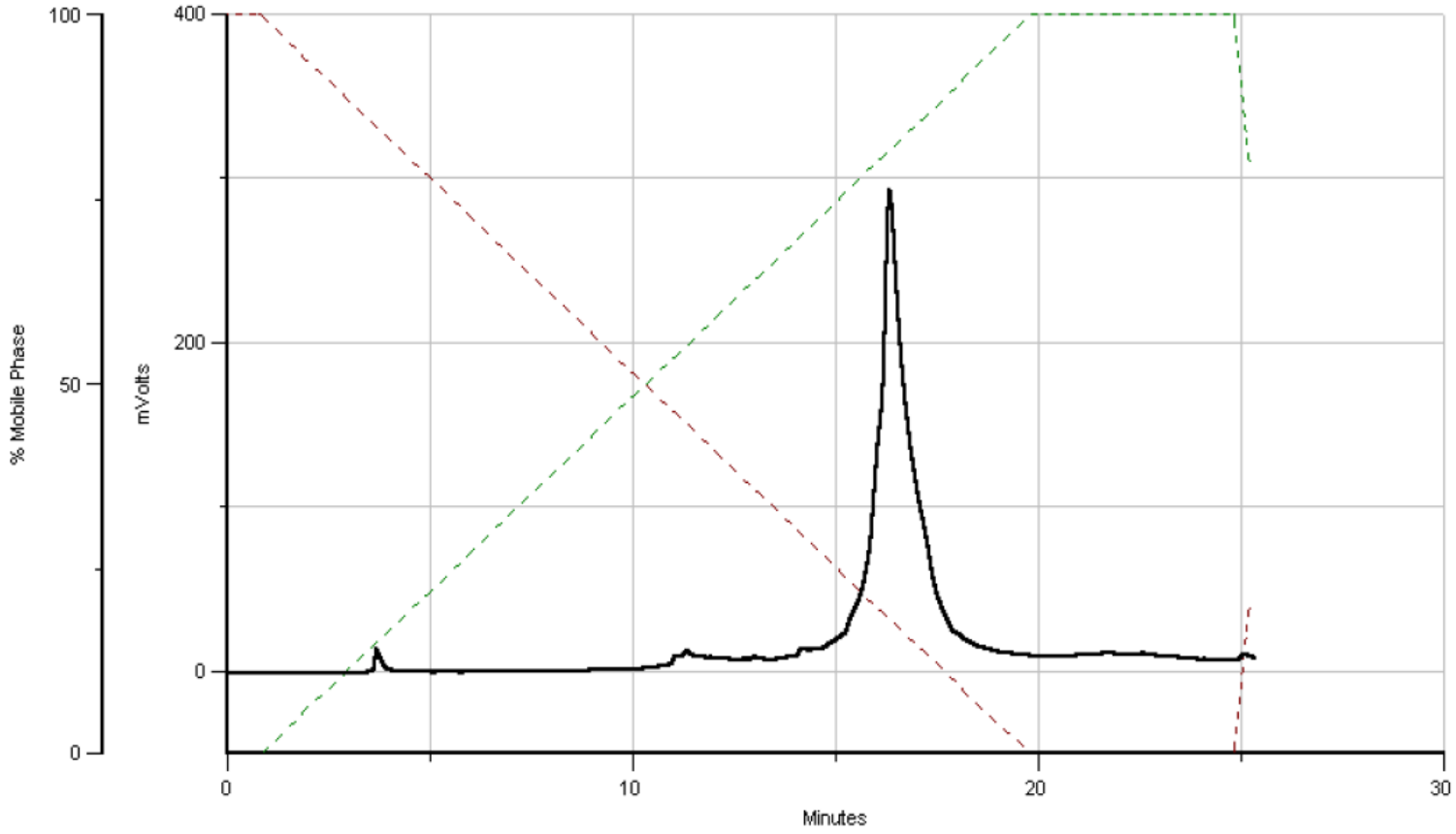

Figure S48. **C2S** [S_P-PS]-d(GAC$_L$ATC$_L$ACTAG)

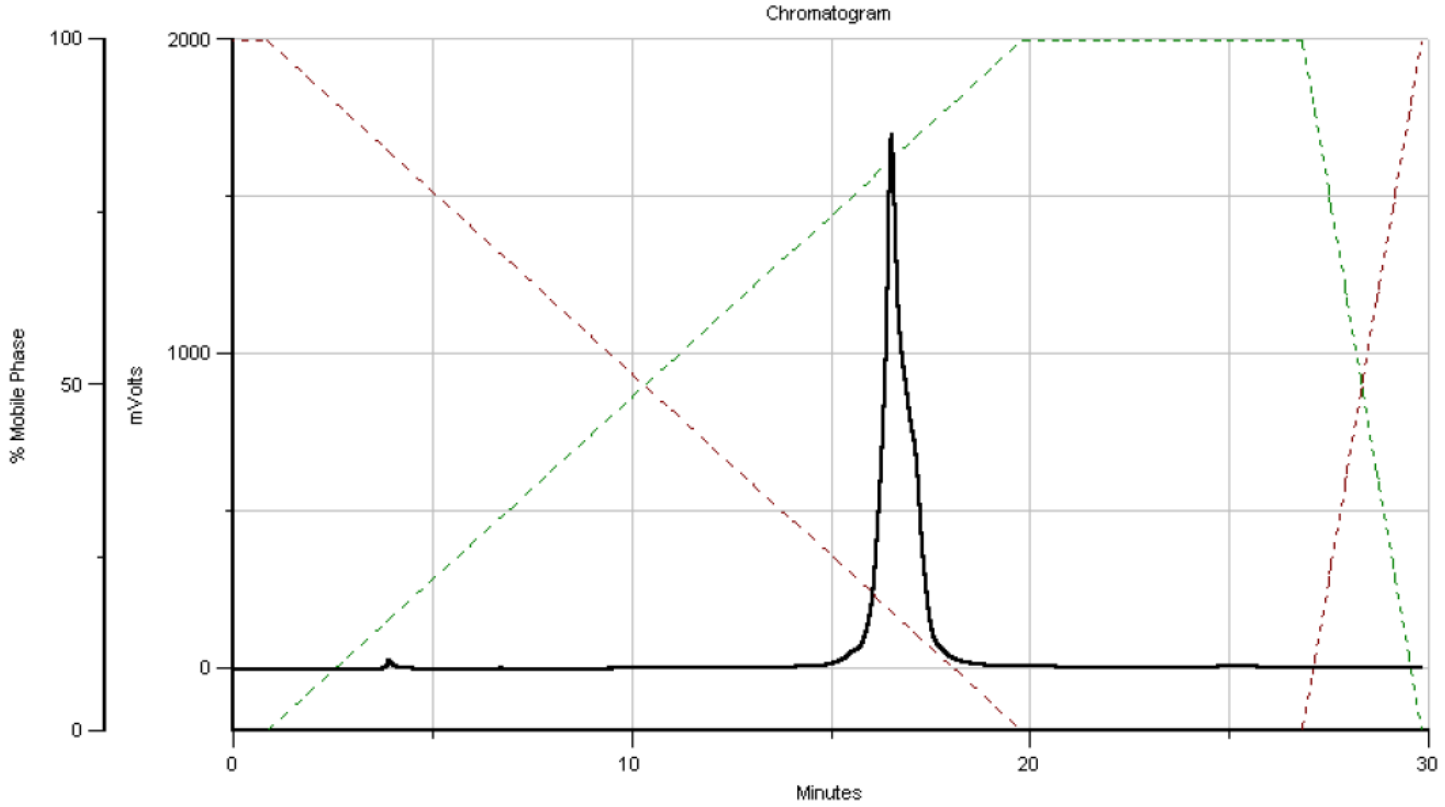

Figure S49. **C3R** [R_P-PS]-d(GAC$_L$ATC$_L$AC$_L$TAG)

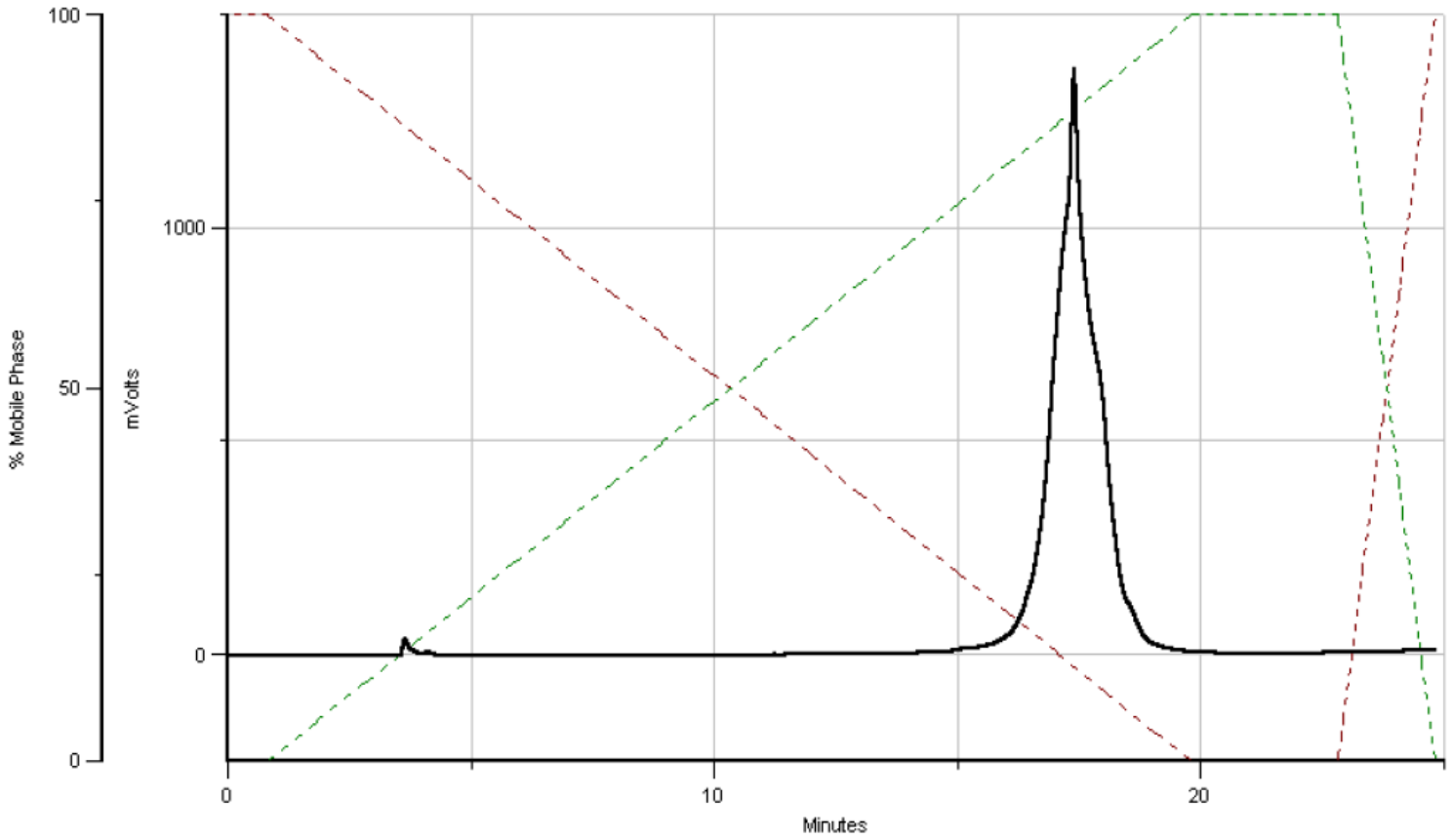

Figure S50. **C3S** [S_P-PS]-d(GAC$_L$ATC$_L$AC$_L$TAG)

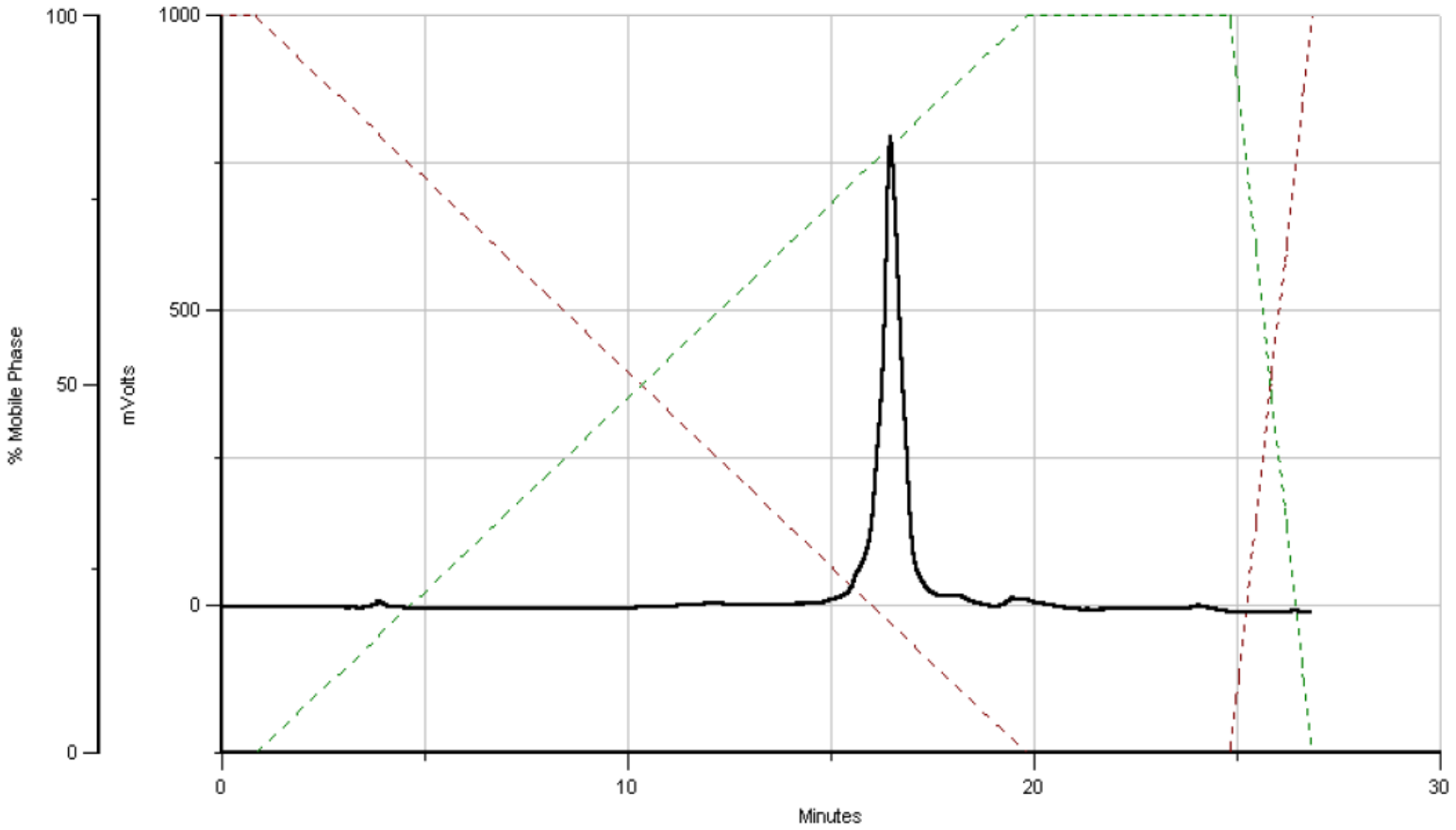

Figure S51. **G2R** [R_P-PS]-d(GAG$_L$ATG$_L$ACTAG)

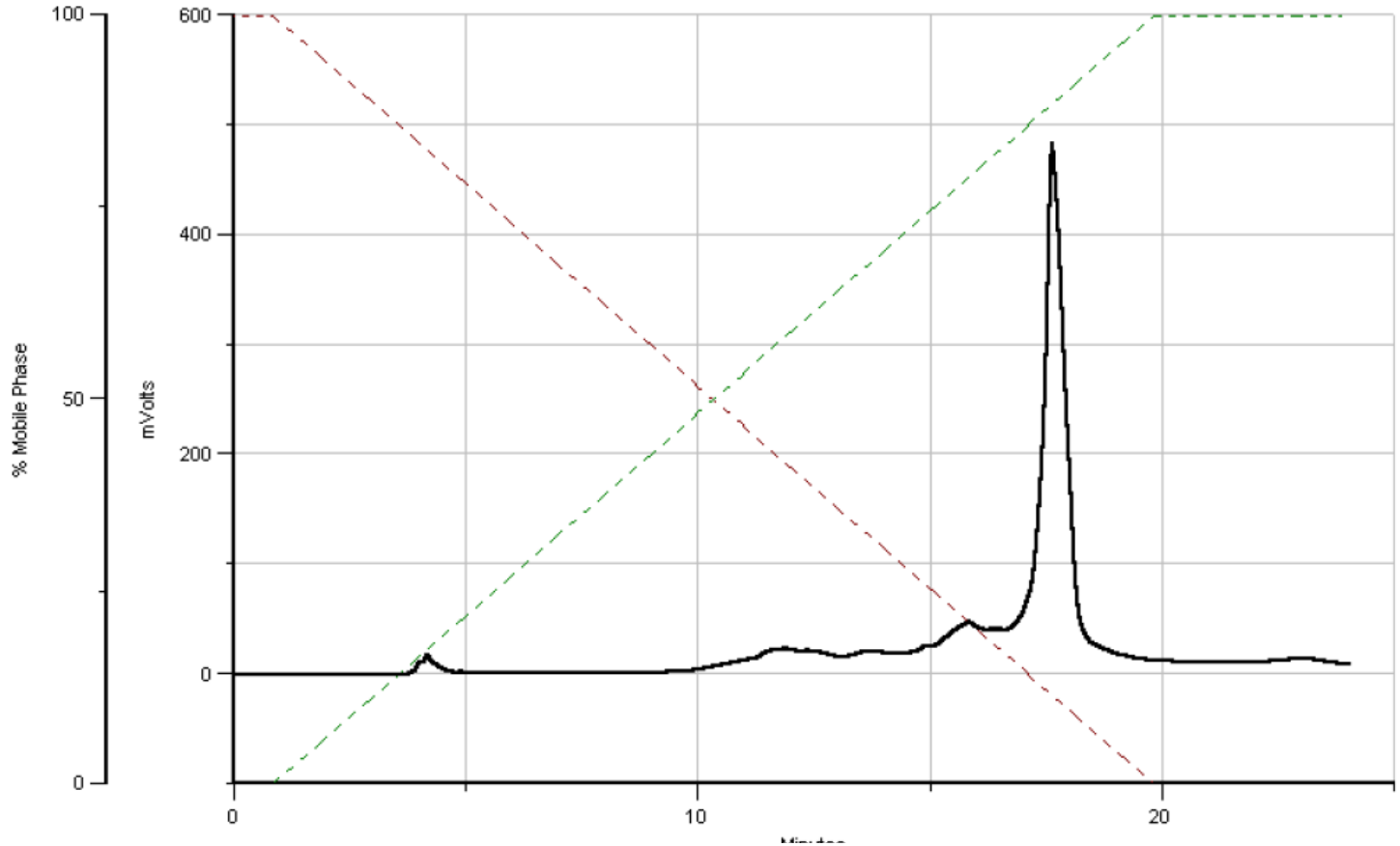

Figure S52. **G2S** [S_P-PS]-d(GAG$_L$ATG$_L$ACTAG)

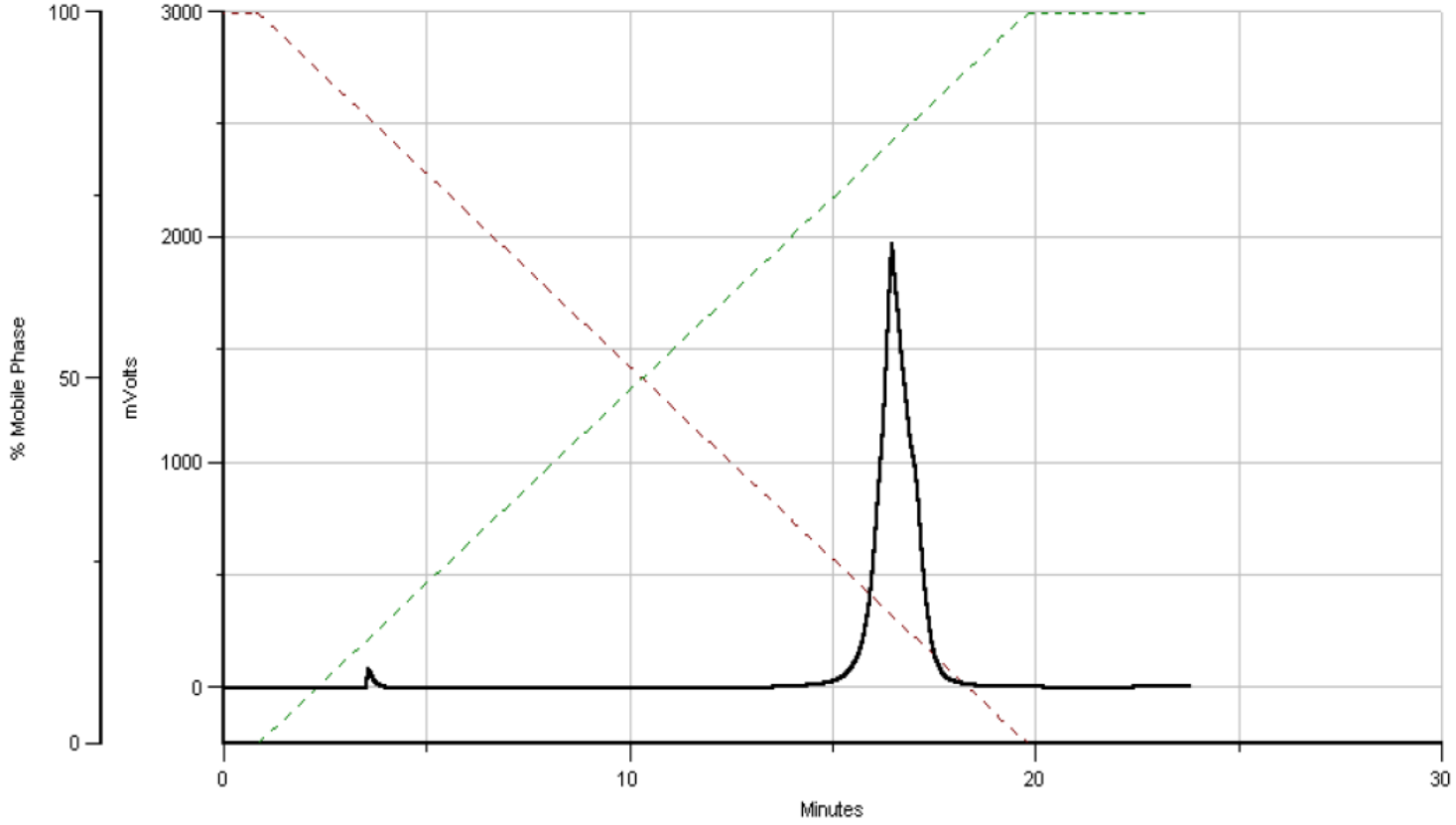

Figure S53. **T2R** [R_P-PS]-d(GACAT$_L$CACT$_L$AG)

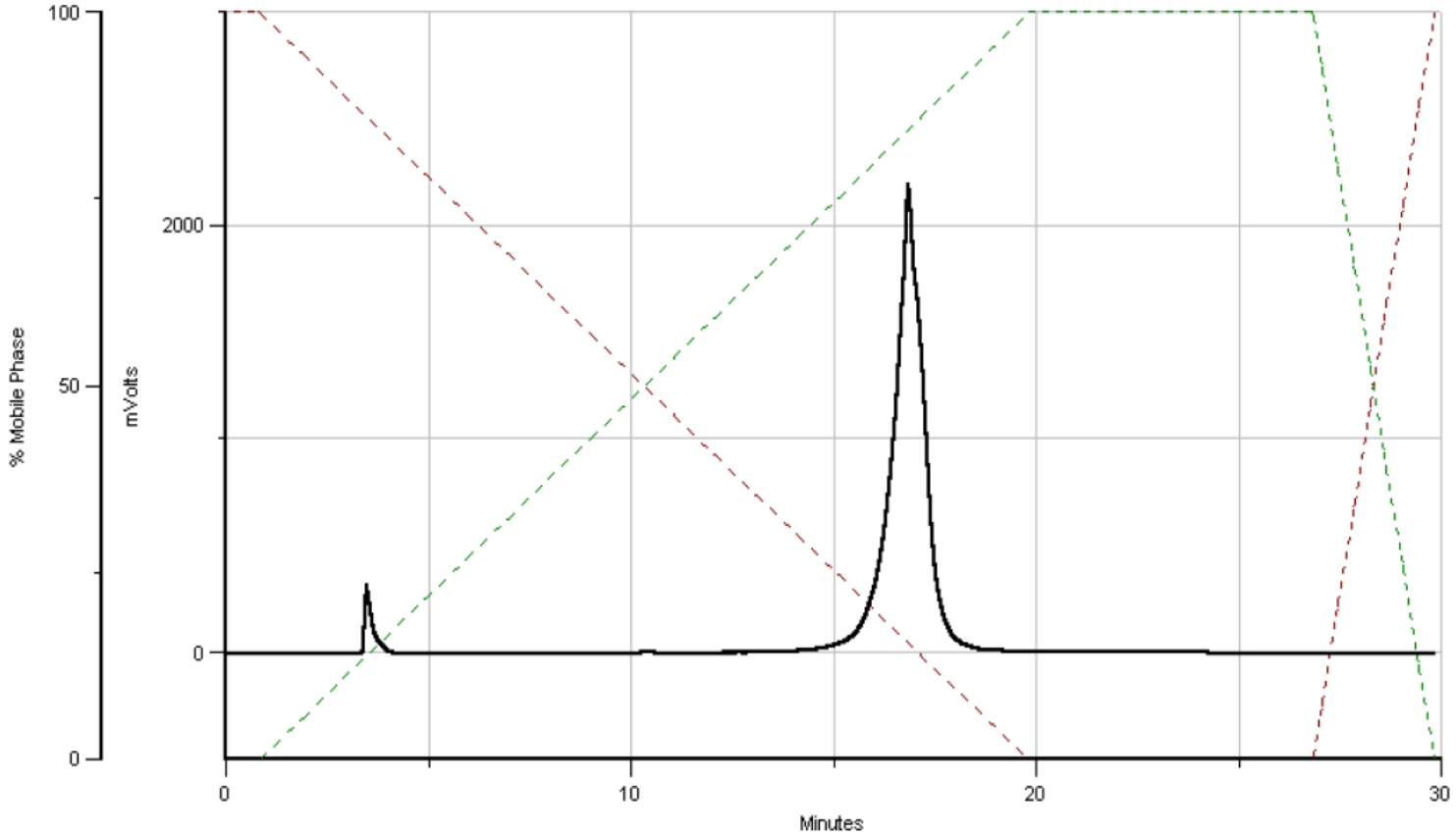

Figure S54. **T2S** [S_P-PS]-d(GACAT$_L$CACT$_L$AG)

X. Enzymatic hydrolysis of $(A_L)_{PS}T$ obtained from **2b** *fast* and **2b** *slow*.

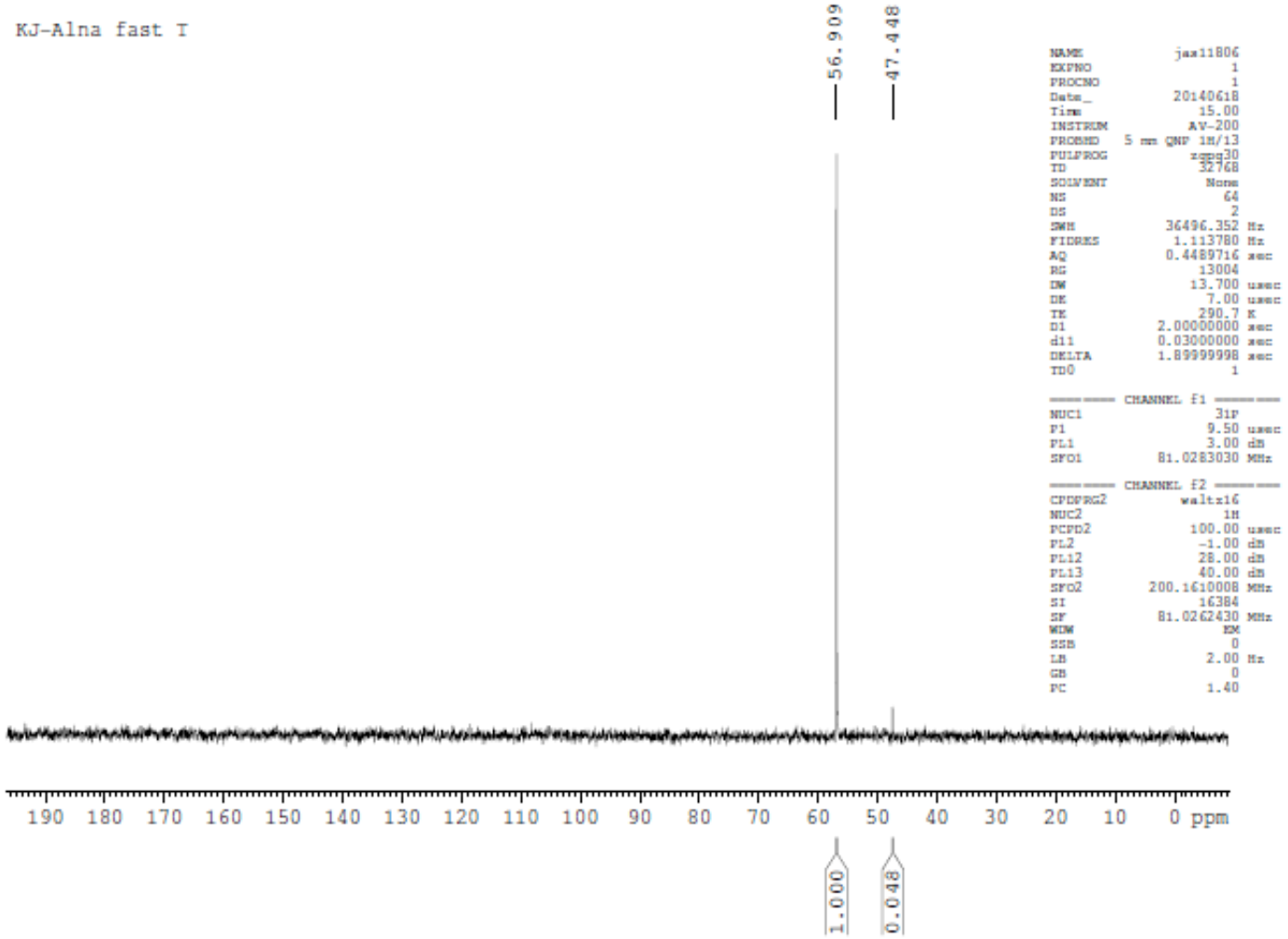

Figure S55. A ^{31}P NMR spectrum for $(A_L)_{PS}T$ obtained from **2b** *fast*. A signal at 47.4 ppm corresponds to the product of hydrolysis and not to the second P-diastereoisomer.

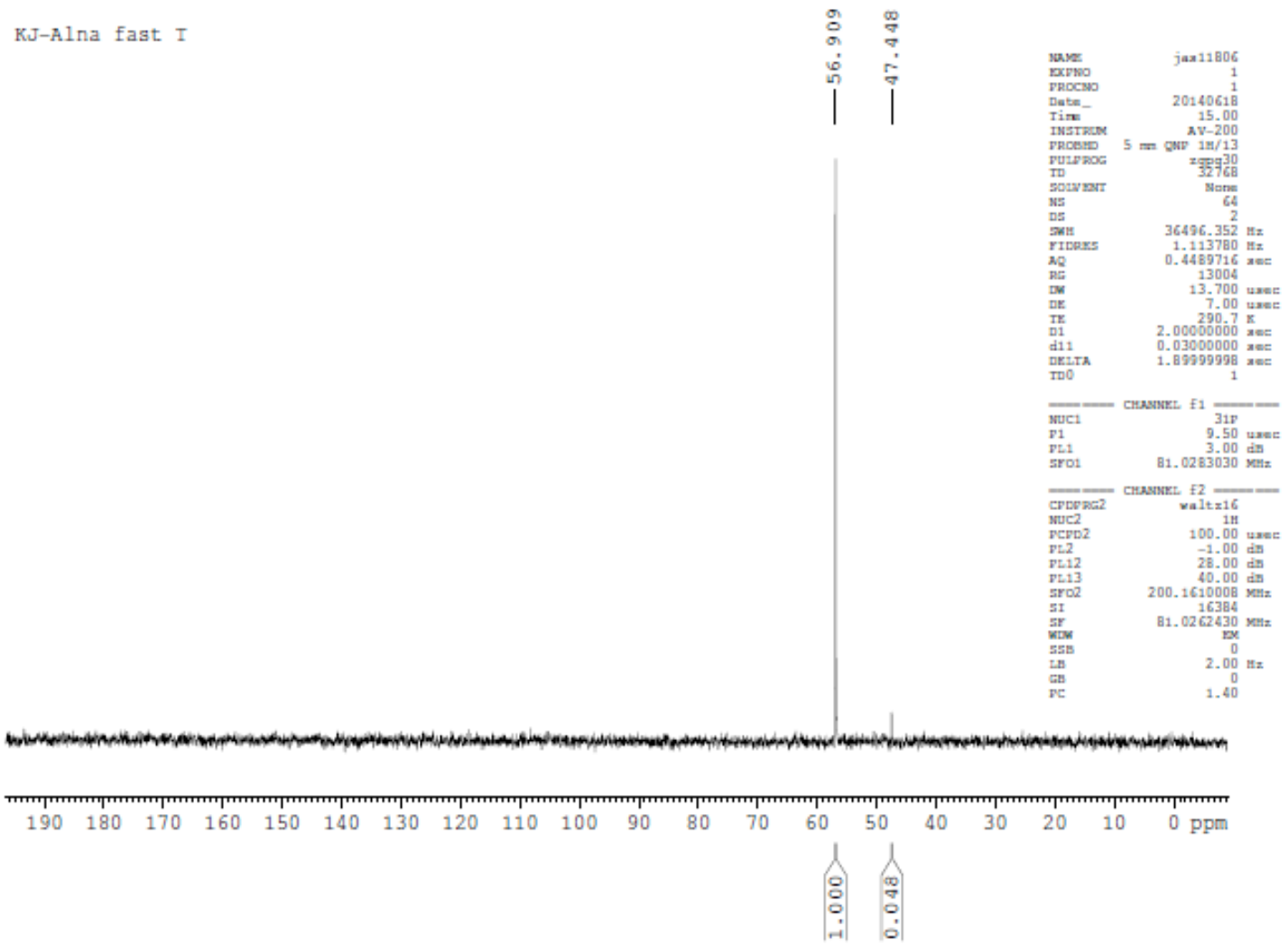

Figure S56. A ^{31}P NMR spectrum for $(A_L)_{PS}T$ obtained from **2b** *slow*. A signal at 47.4 ppm corresponds to the product of hydrolysis and not to the second P-diastereoisomer.

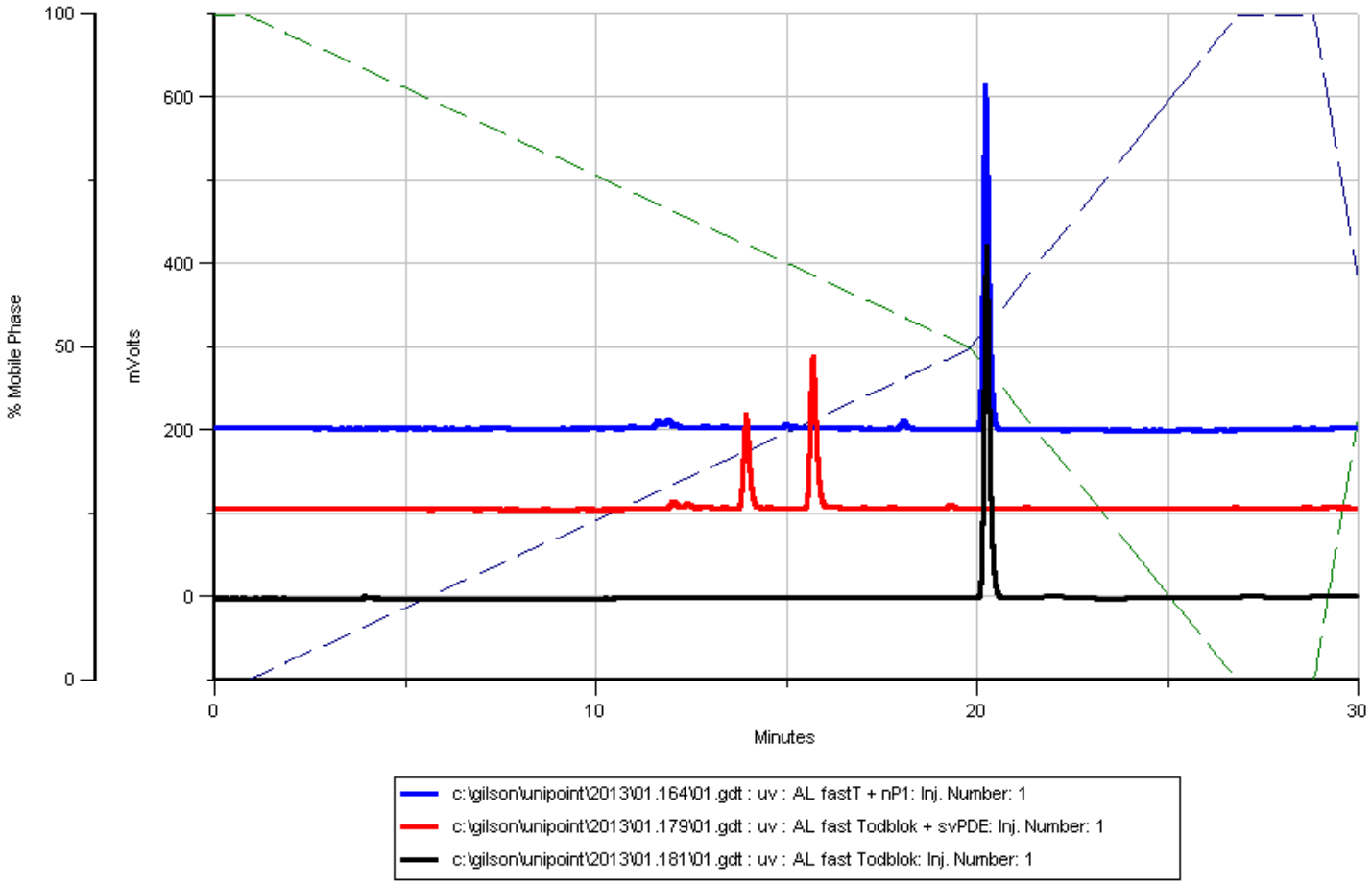

Figure S57. RP HPLC profiles for: $(A_L)_{PS}T$ from **2b** *fast* (black line), $(A_L)_{PS}T$ from **2b** *fast* + *svPDE* (red line), $(A_L)_{PS}T$ from **2b** *fast* + *nP1* (blue line). ACE 5 C 18-AR Column, 250×4.6 mm; flow rate 1mL/min, A buffer: 0.1 M TEAB, B buffer: 40% CH_3CN in 0.1 TEAB, Gradient: 0-50% of B buffer in 20 min, 50-100% of B buffer in 7 min.

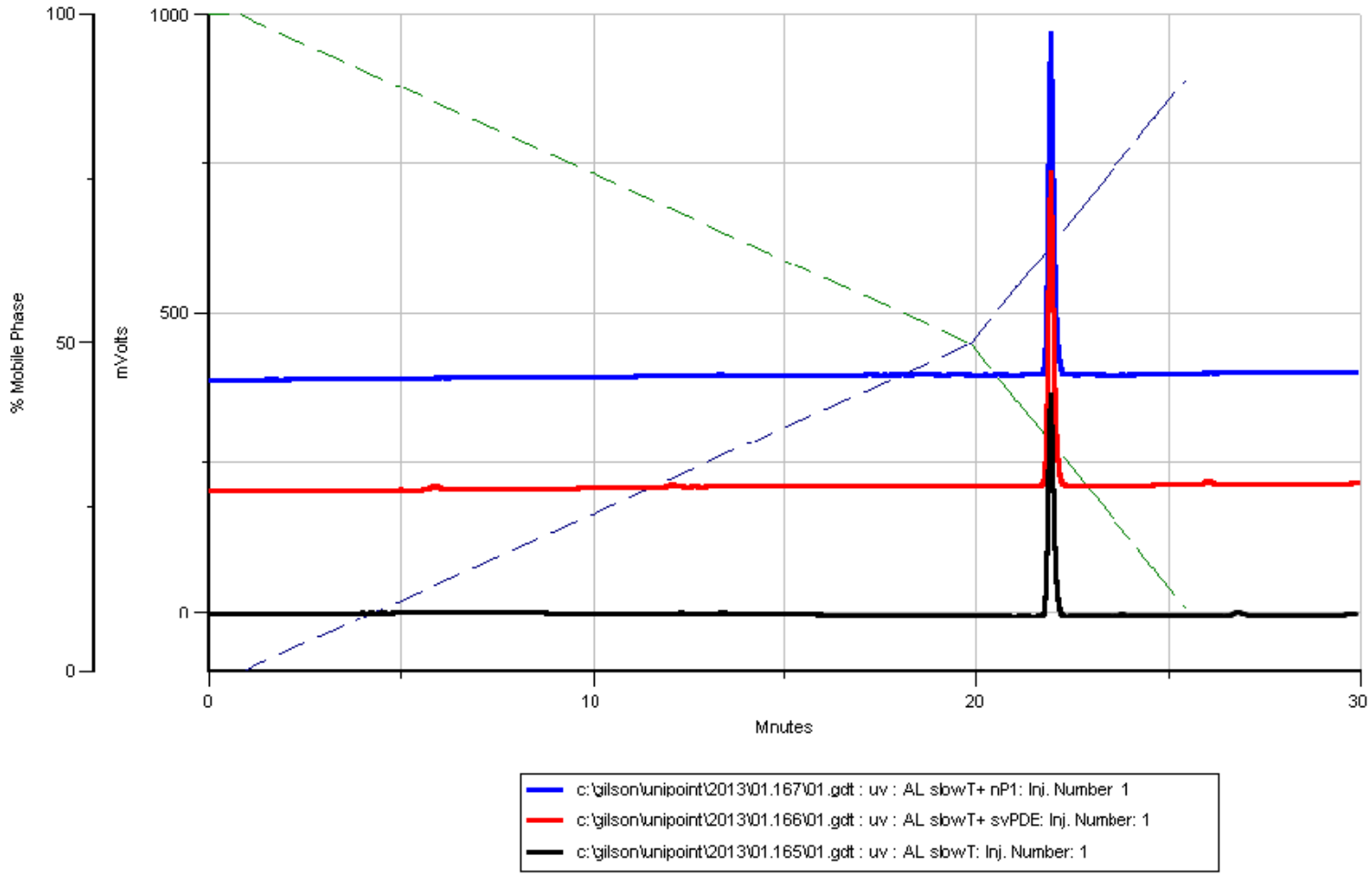

Figure S58. RP HPLC profiles for: $(A_L)_{PS}T$ from **2b** *slow* (black line), $(A_L)_{PS}T$ from **2b** *slow* + *svPDE* (red line), $(A_L)_{PS}T$ from **2b** *slow* + *nP1* (blue line). ACE 5 C 18-AR Column, 250×4.6 mm; flow rate 1mL/min, A buffer: 0.1 M TEAB, B buffer: 40% CH_3CN in 0.1 TEAB, Gradient: 0-50% of B buffer in 20 min, 50-100% of B buffer in 7 min.