Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Electronic Supplementary Information

Synthesis and Characterization of Oligonucleotides Containing an O^6-2'-Deoxyguanosine-Alkyl-O^6-2'-Deoxyguanosine Interstrand Cross-Link in a 5'-GNC Motif and Repair by Human O^6-Alkylguanine-DNA Alkyltransferase

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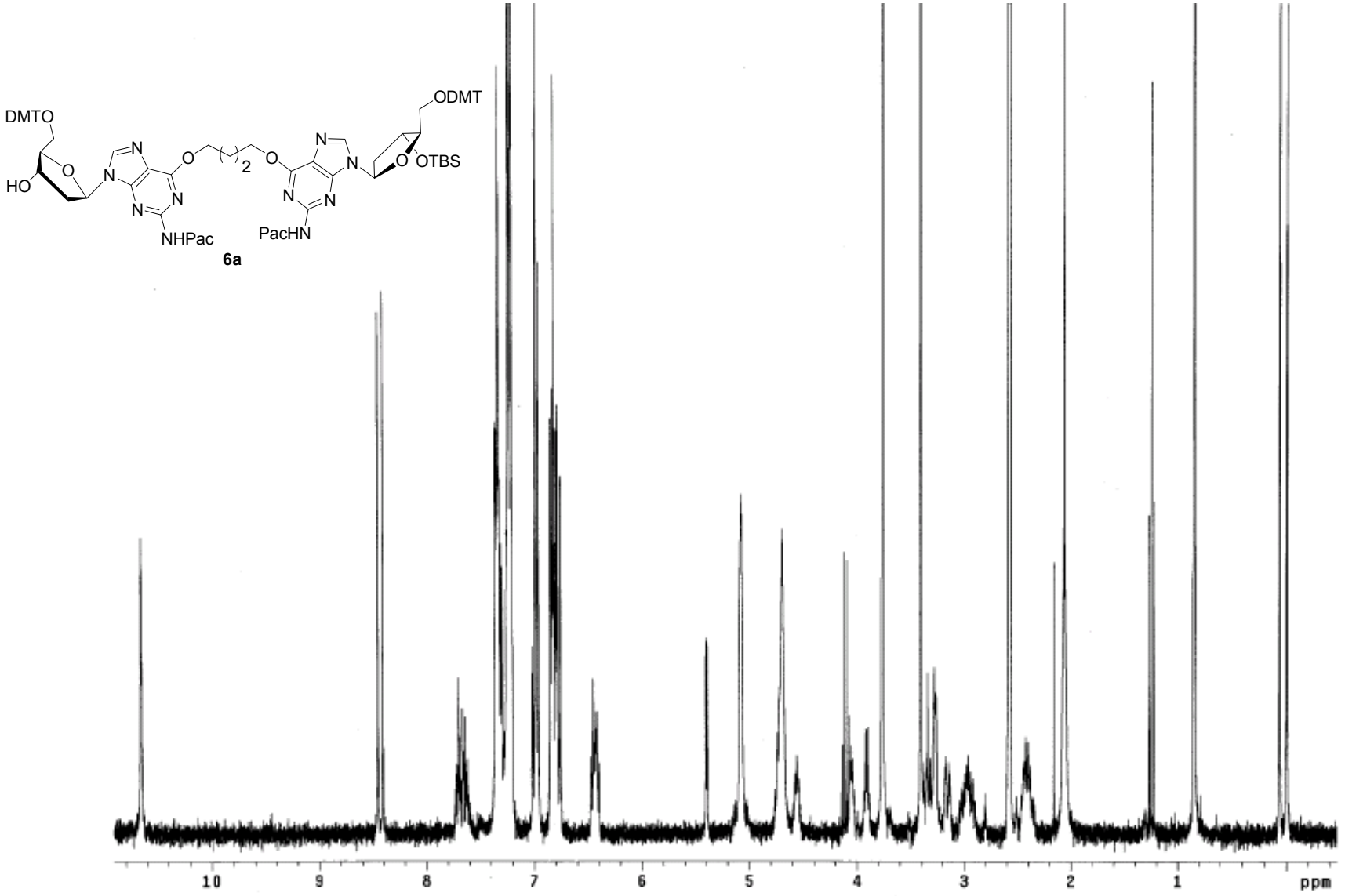
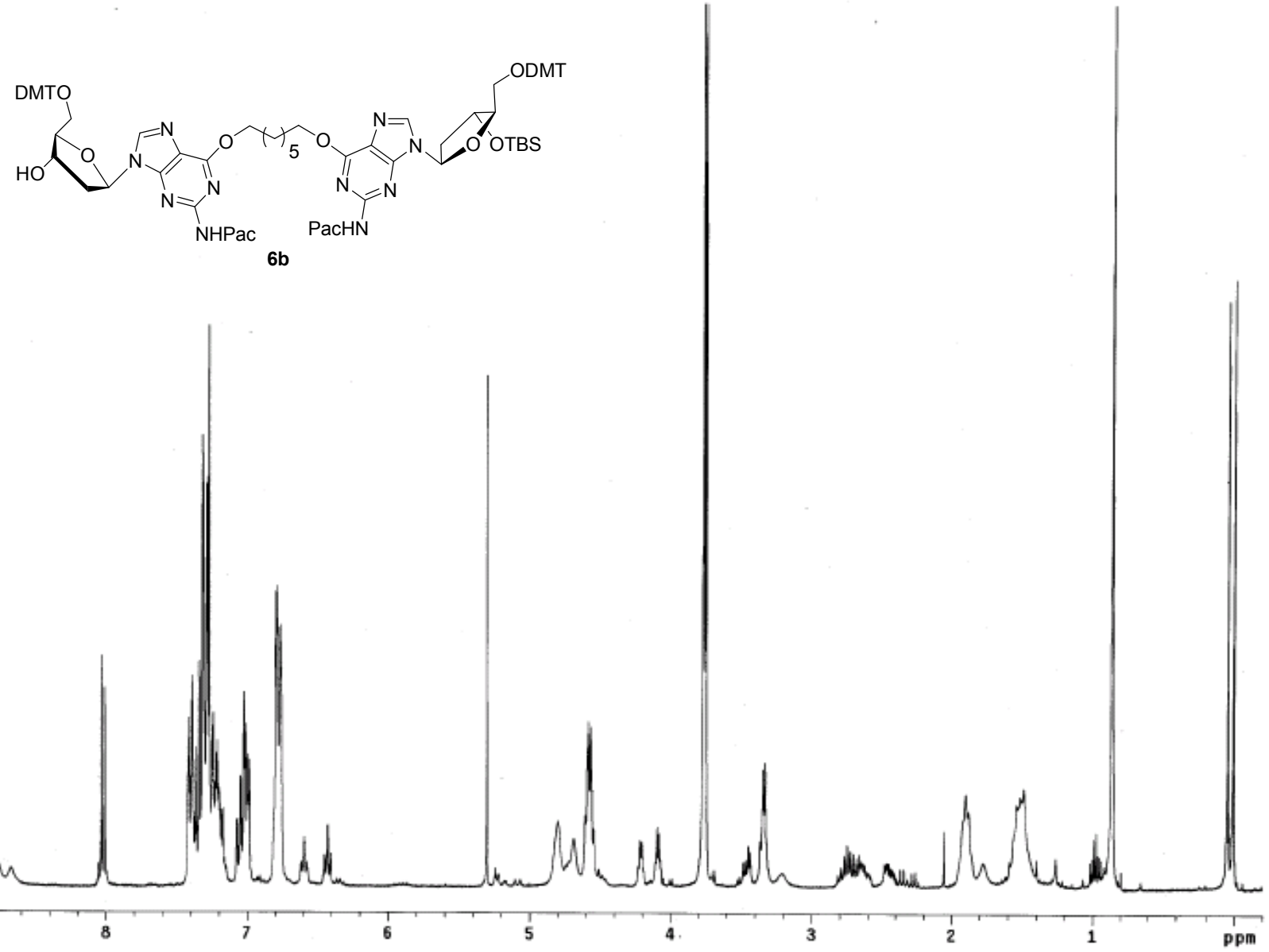
Contents **Page**

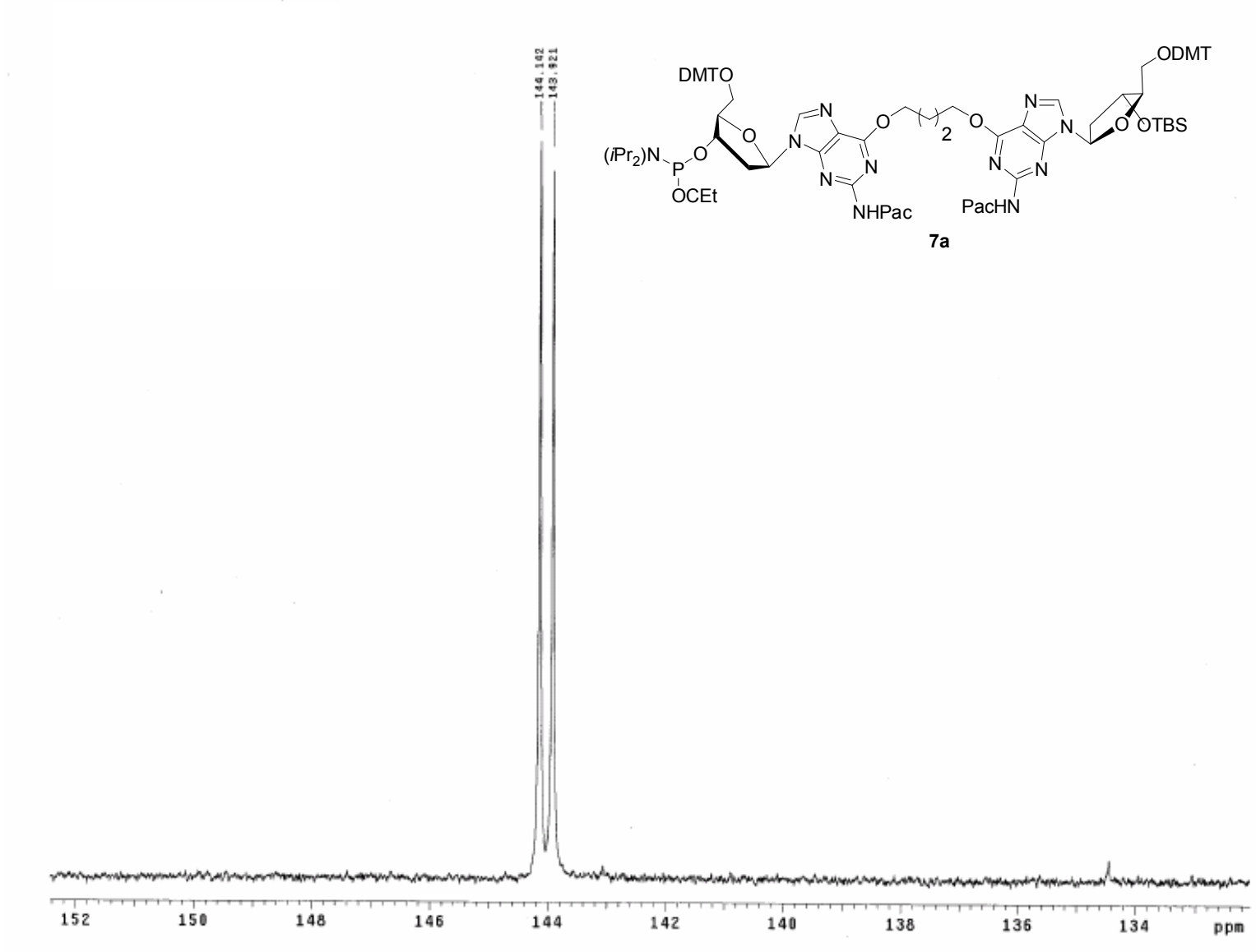
Supplementary Figure 1 - 300 MHz ^{1}H NMR spectrum of compound (**6a**) (in d_6-DMSO)



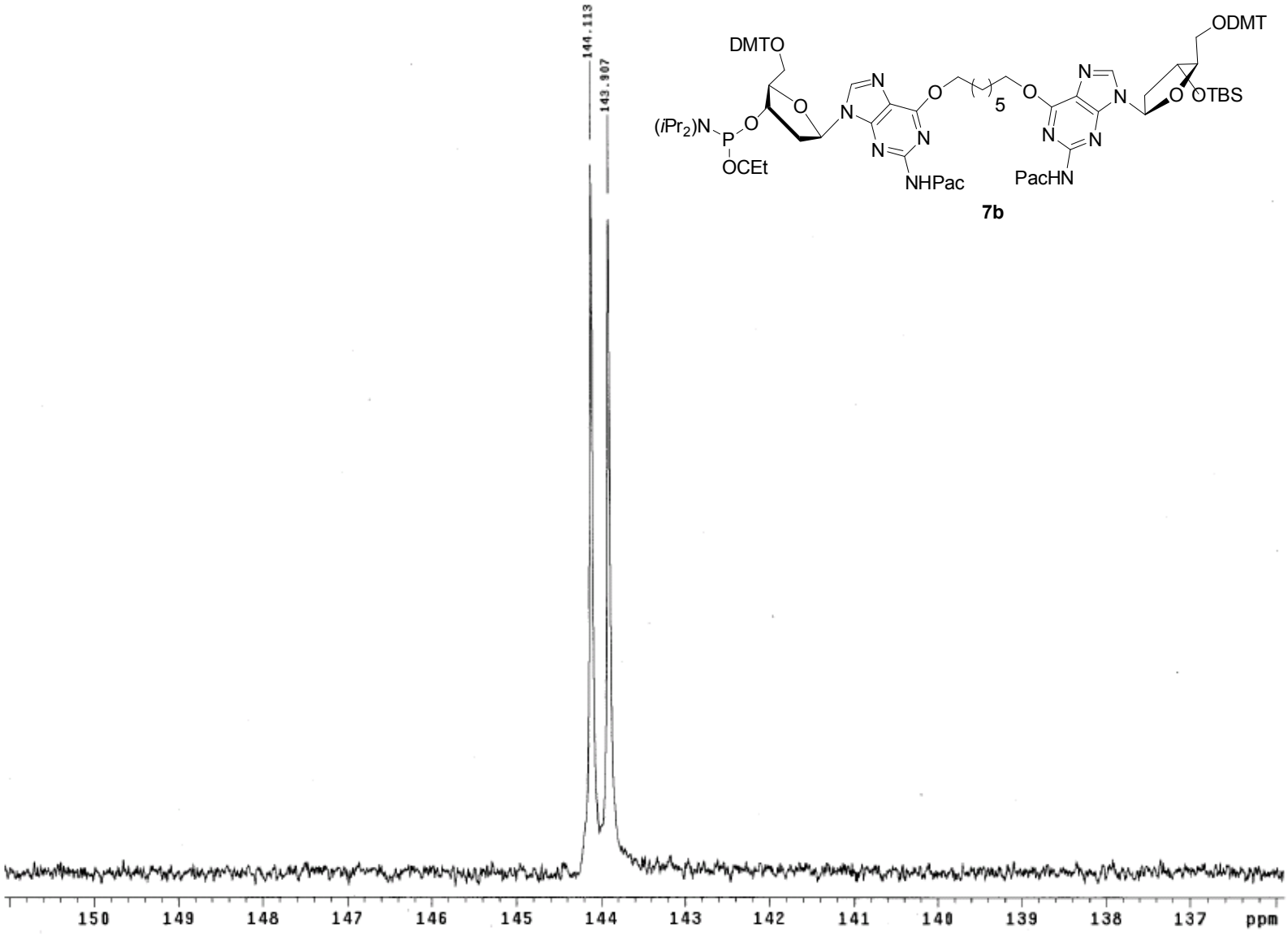
Supplementary Figure 2 - 300 MHz ^{1}H NMR spectrum of compound (**6b**) (in $CDCl_3$)



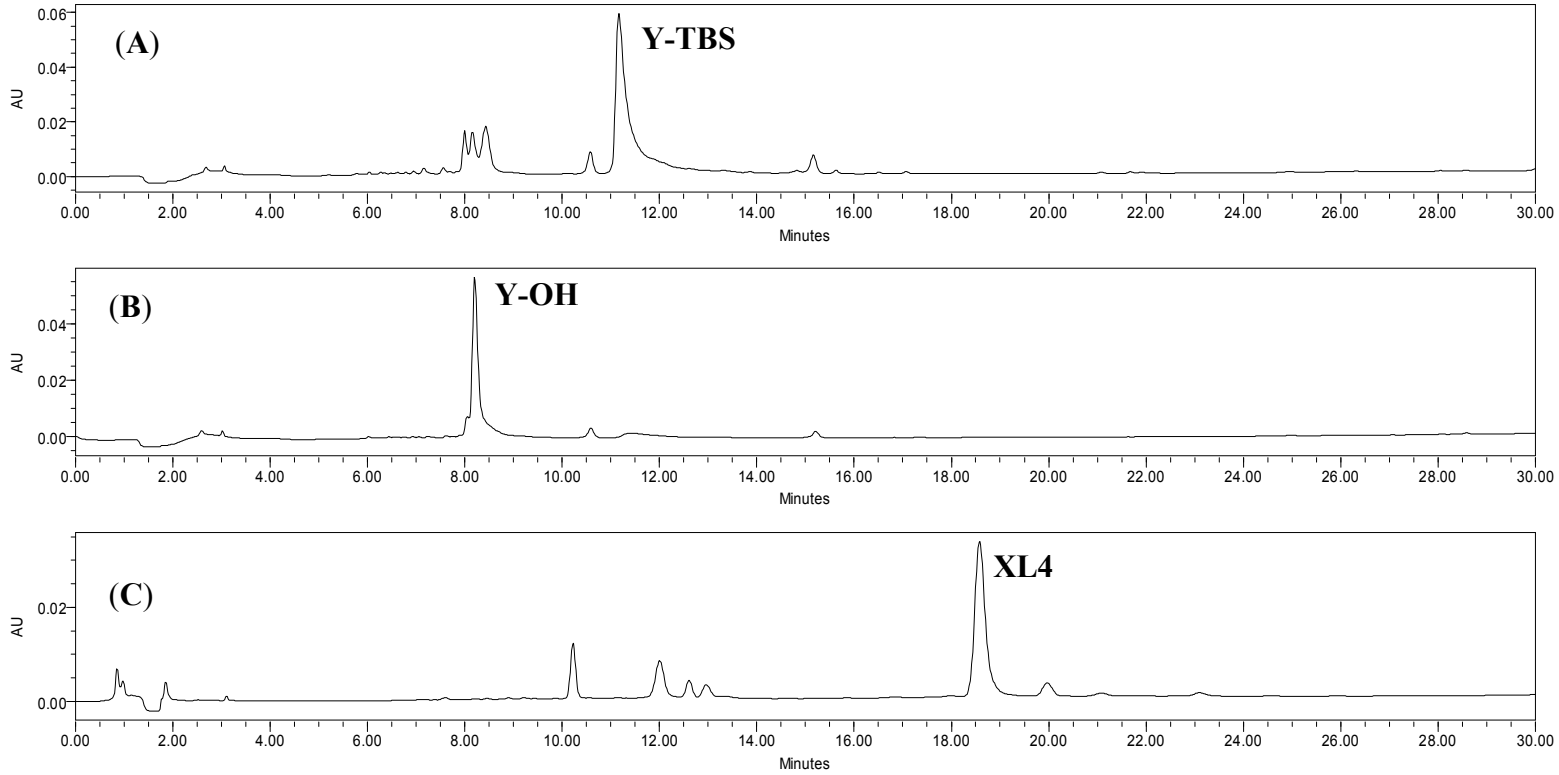
Supplementary Figure 3 - 121.5 MHz ^{31}P NMR spectrum of compound (**7a**) (in d_6-acetone)



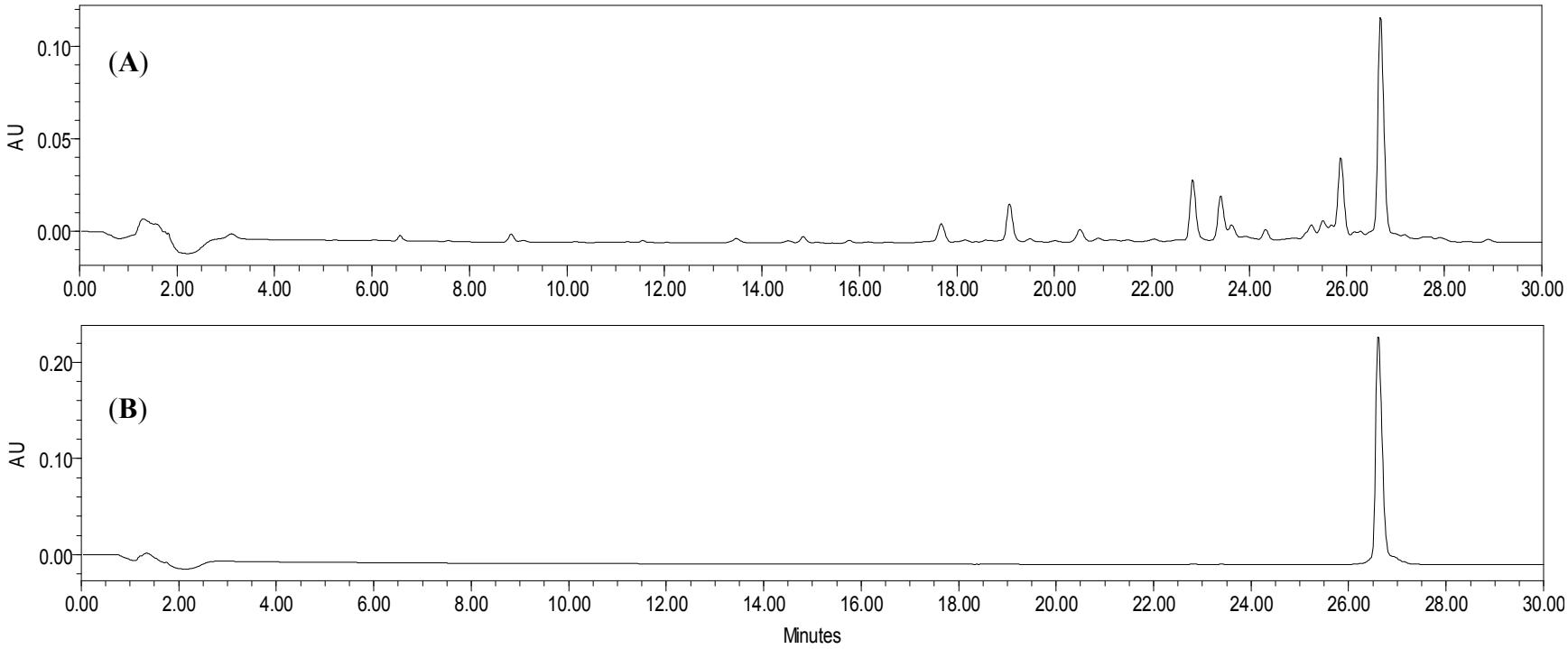
Supplementary Figure 4 - 121.5 MHz ^{31}P NMR spectrum of compound (**7b**) (in d_6-acetone)



Supplementary Figure 5 - C-18 HPLC profiles of (**A**) crude **Y-TBS** (**XL4** precursor), (**B**) **Y-OH** after removal of the TBS group, and (**C**) final extension product **XL4**. Gradient of 0-60% buffer B over 30 min (buffer A: 50 mM sodium phosphate, pH 5.8 and buffer B: 50 mM sodium phosphate, pH 5.8, 50% acetonitrile) at a flow rate of 1.0 mL/min and monitored at 260 nm.

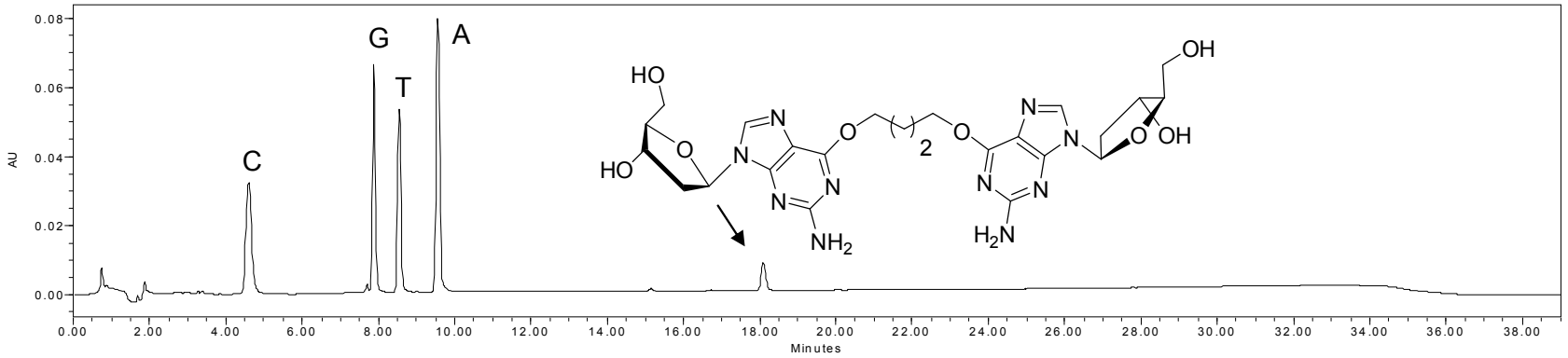


Supplementary Figure 6 - SAX HPLC profiles of crude **XL7** (**A**) and purified **XL7** (**B**). For analytical runs the column was eluted with a linear gradient of 0-60% buffer B over 30 min (buffer A: 100 mM Tris HCl, pH 7.5, 10% acetonitrile and buffer B: 100 mM Tris HCl, pH 7.5, 10% acetonitrile, 1 M NaCl) at a flow rate of 1.0 mL/min over 30 min, monitored at 260 nm.

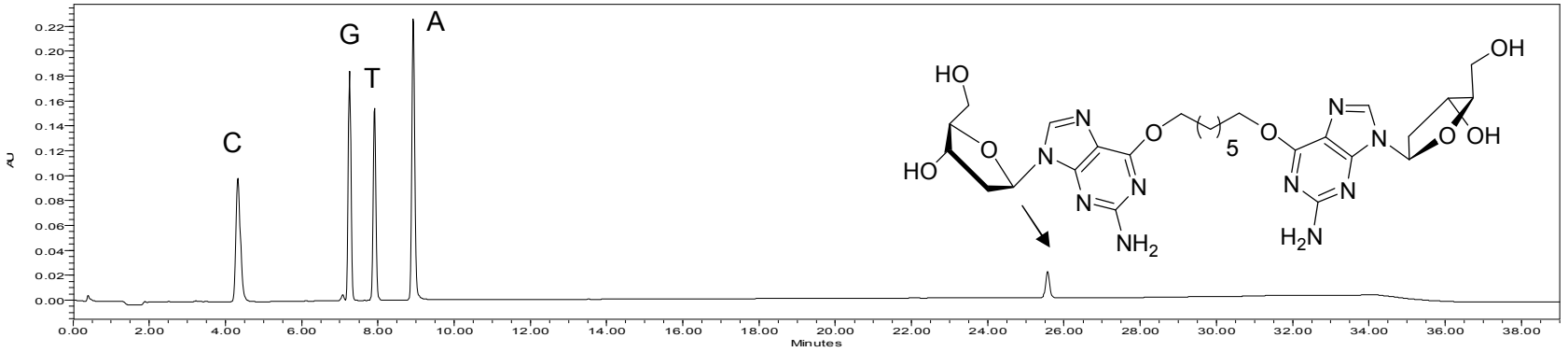


Supplementary Figure 7 - C-18 HPLC profile of digested cross-linked duplex **XL4** (**A**) and **XL7** (**B**). The column was eluted with a linear gradient of 0-60% buffer B over 30 min (buffer A: 50 mM sodium phosphate, pH 5.8 and buffer B: 50 mM sodium phosphate, pH 5.8, 50% acetonitrile) at a flow rate of 1.0 mL/min over 30 min, monitored at 260 nm.

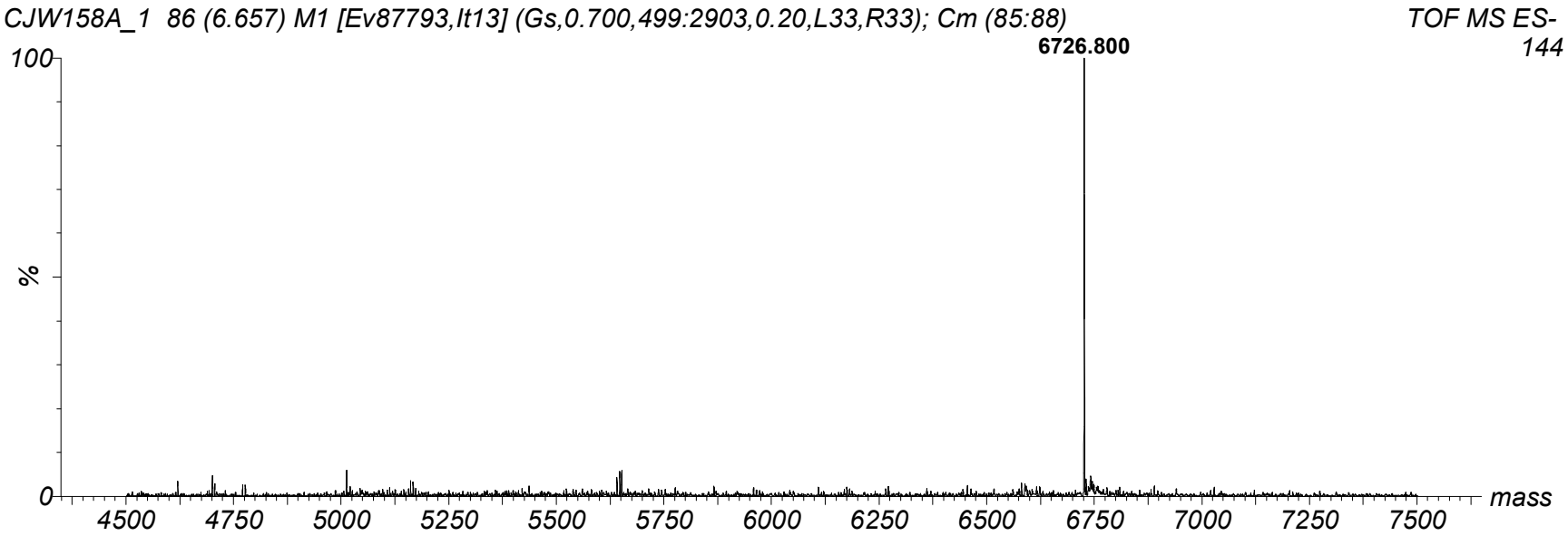
(**A**)



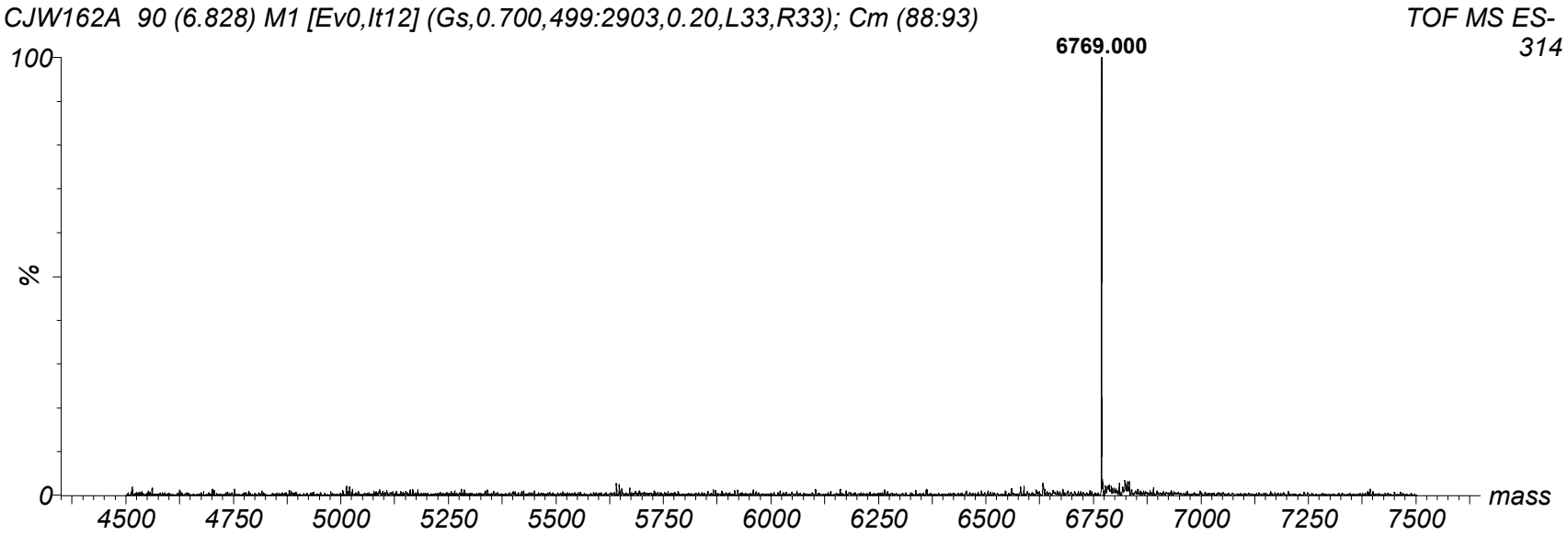
(**B**)



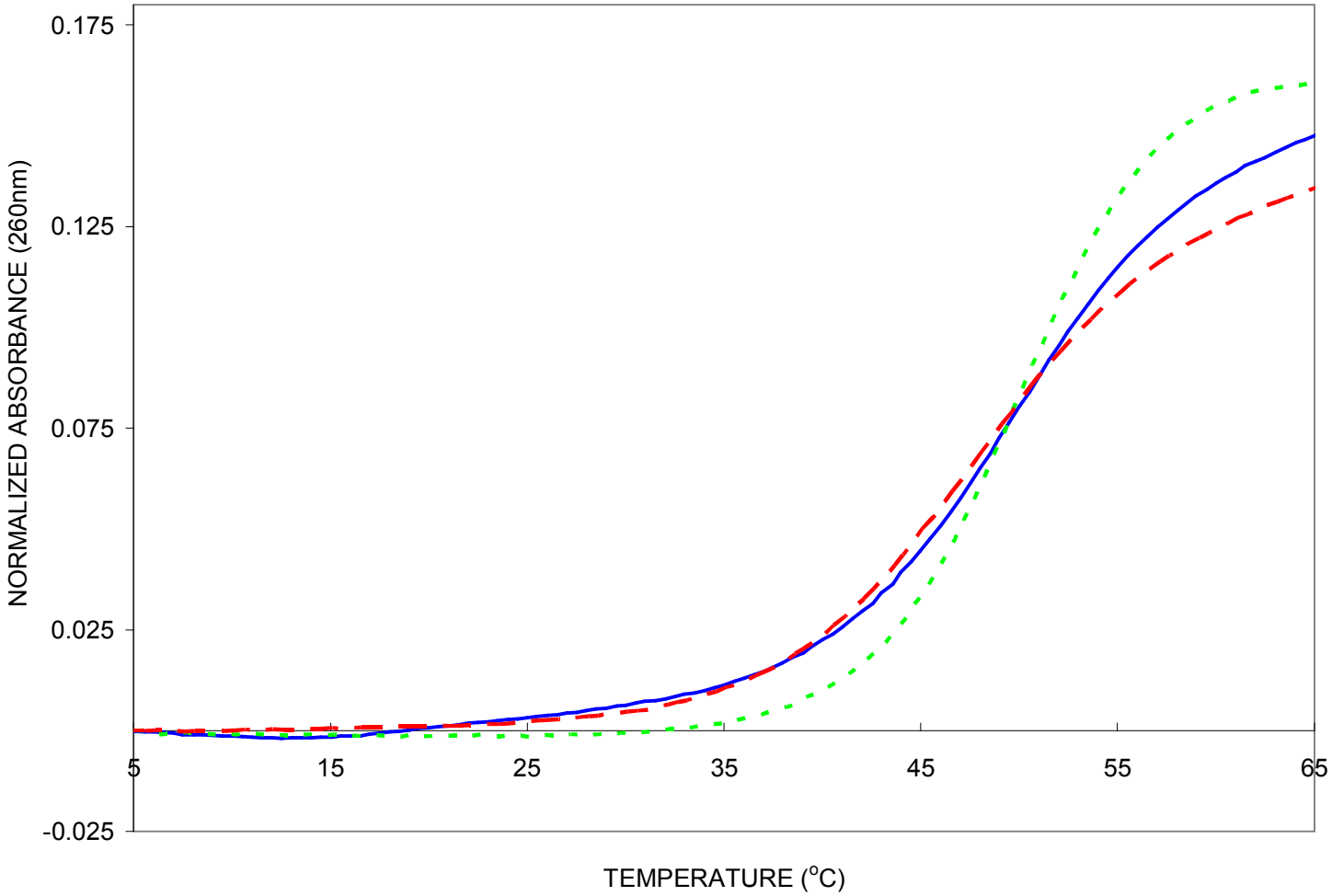
Supplementary Figure 8 - ESI MS spectrum of oligonucleotide **XL4**



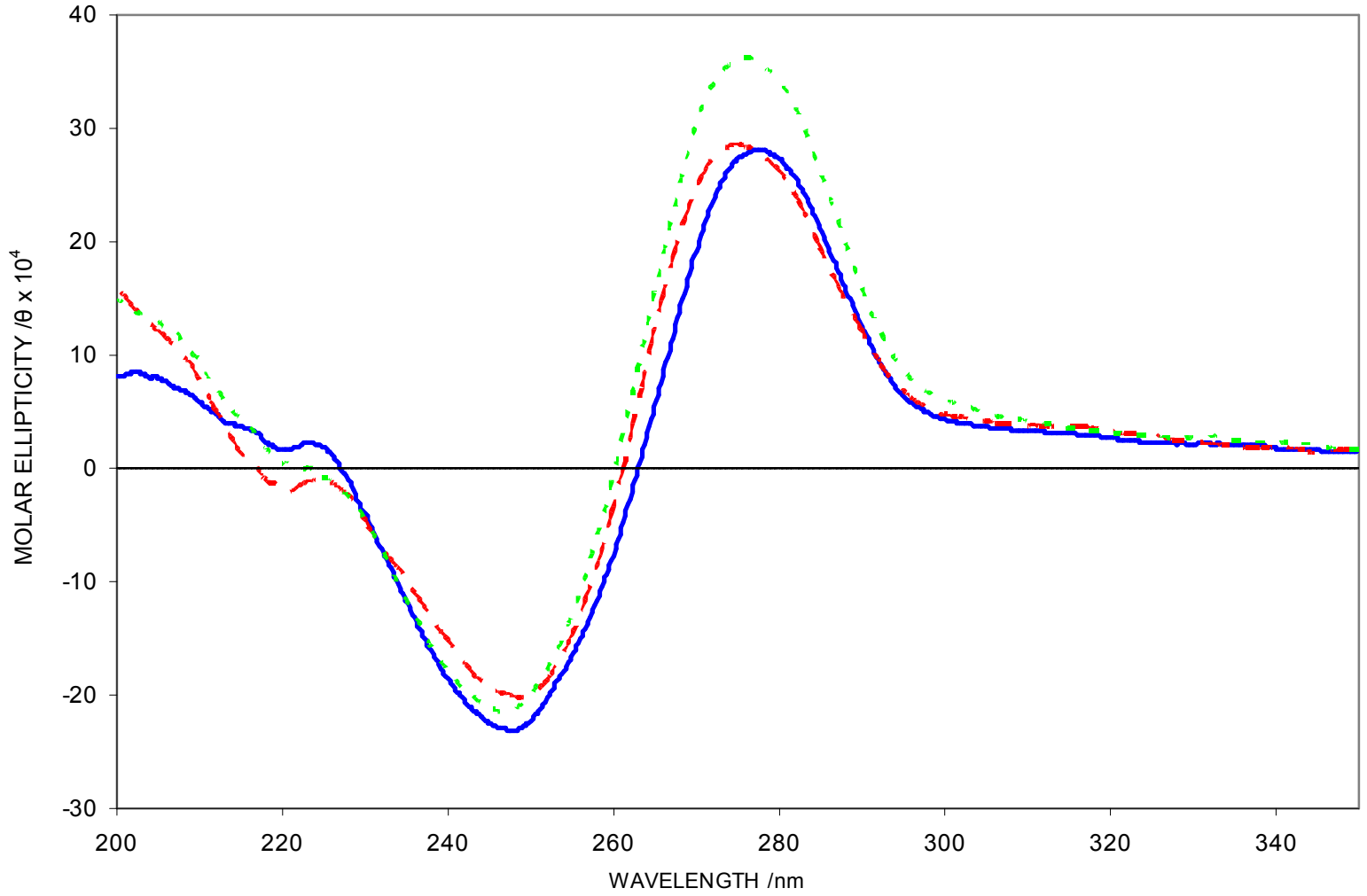
Supplementary Figure 9 - ESI MS spectrum of oligonucleotide **XL7**



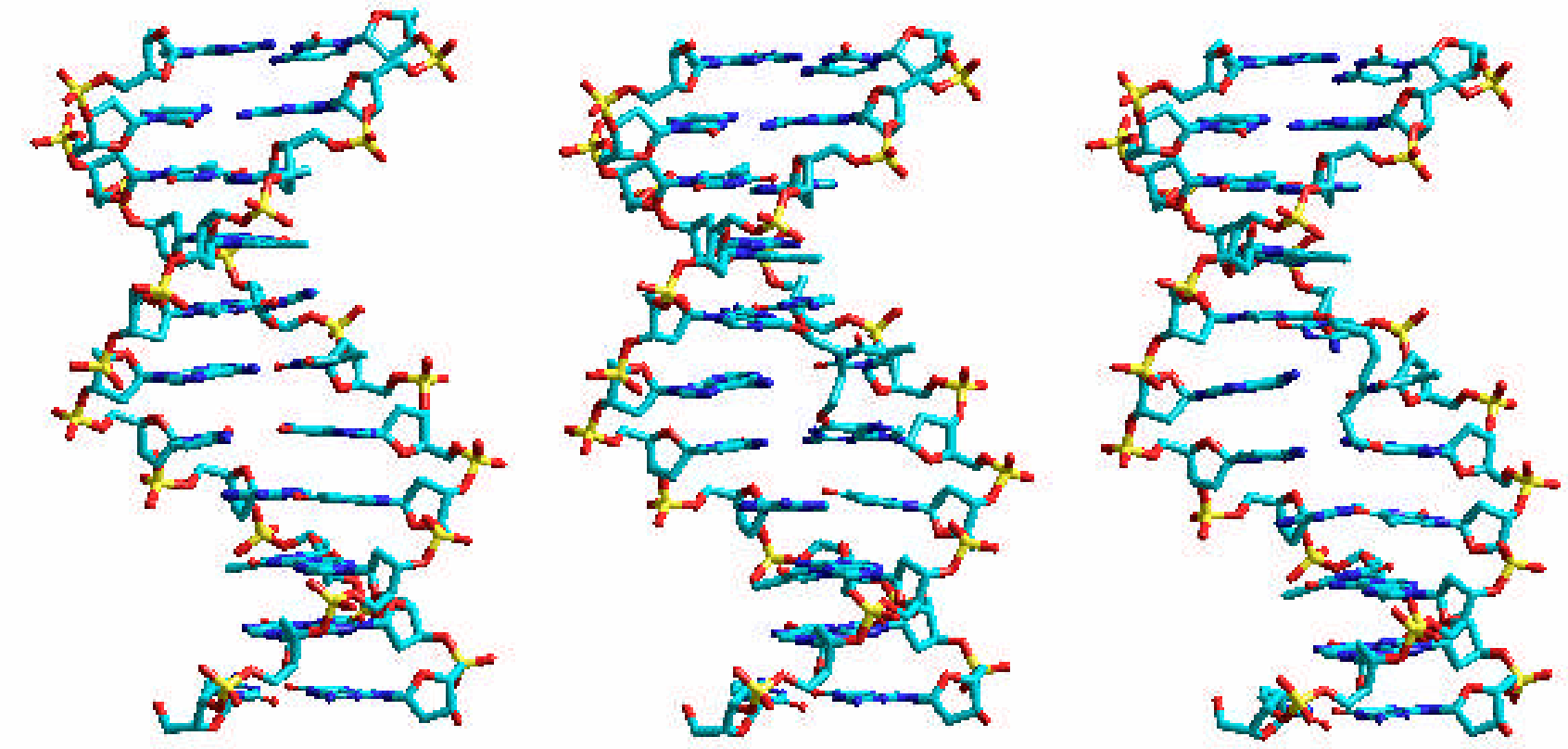
Supplementary Figure 10 - Absorbance (A_{260}) versus temperature profiles of non cross-linked duplex (_____) 5'-dCGATGTCATCG-3'/5'-dCGATGACATCG-3', cross-linked duplex **XL4** (_ _ _ _), and cross-linked duplex **XL7** (……..). Solutions containing a total strand concentration of 2.8 µM for the cross-linked (**XL4** and **XL7**) and non-cross-linked control duplexes in 90 mM sodium chloride, pH = 7.0, 10 mM sodium phosphate, and 1 mM EDTA buffer, were heated at 0.5°C/min.



Supplementary Figure 11 - Circular dichroism spectra of non cross-linked duplex (_____) 5'- dCGATGTCATCG-3'/5'-dCGATGACATCG3', cross-linked duplex **XL 4** (_ _ _ _), and cross-linked duplex **XL 7** (……..). Solutions containing a total strand concentration of 2.8 μM for the cross-linked duplex **XL 4,7** and 2.8 μM of the non-crosslinked control duplexes in 10 mM sodium phosphate, pH 7.0, 90 mM sodium chloride, and 1 mM EDTA. Spectra are the average of 5 scans and were recorded at 10 ºC.

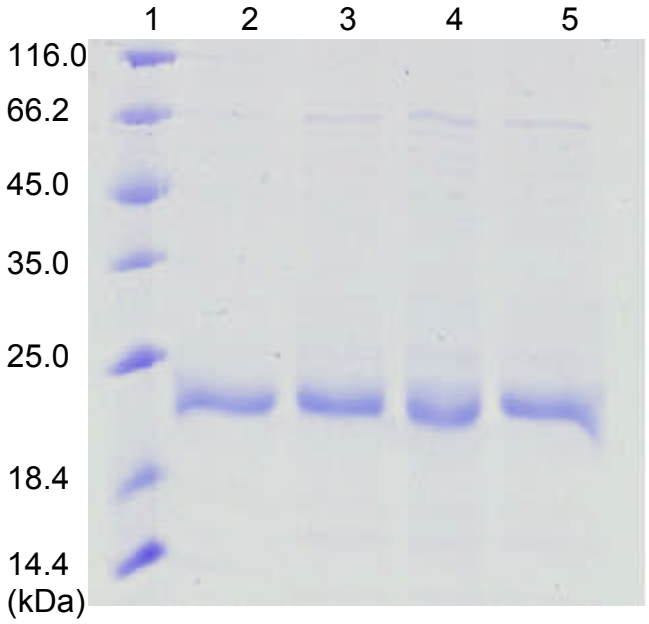


Supplementary Figure 12 – Molecular models of non cross-linked control duplex and cross-linked duplexes **XL4** and **XL7** that were geometry optimized using the AMBER forcefield.

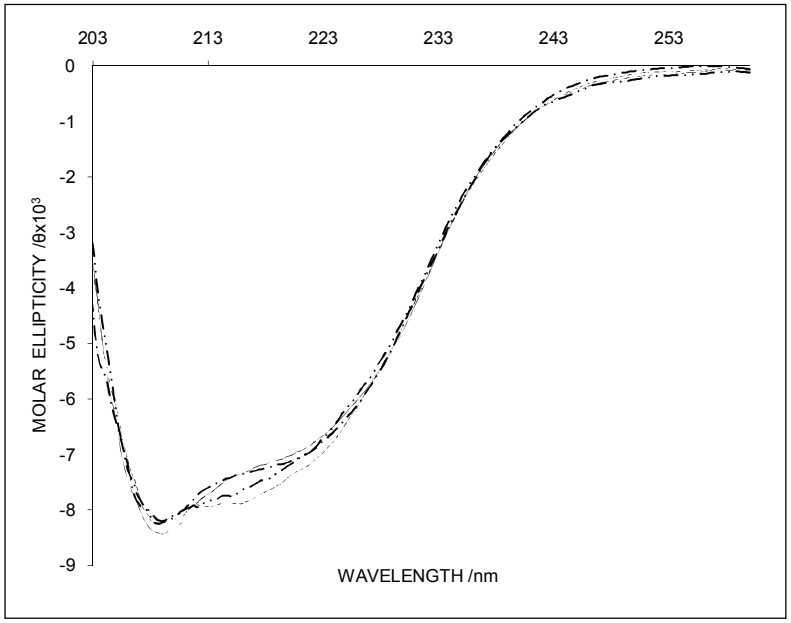


non cross-linked control **XL4** **XL7**

Supplementary Figure 13 - 12% SDS-PAGE Gel of purified hAGT proteins. Loaded: lane 1, 10 µL of Unstained Protein Molecular Weight Marker (Fermentas); lane 2, 7 µg wild-type hAGT protein; lane 3, 7 µg C145S hAGT protein; lane 4, 7 µg P140A hAGT protein; lane 5, 7 µg V148L hAGT protein.

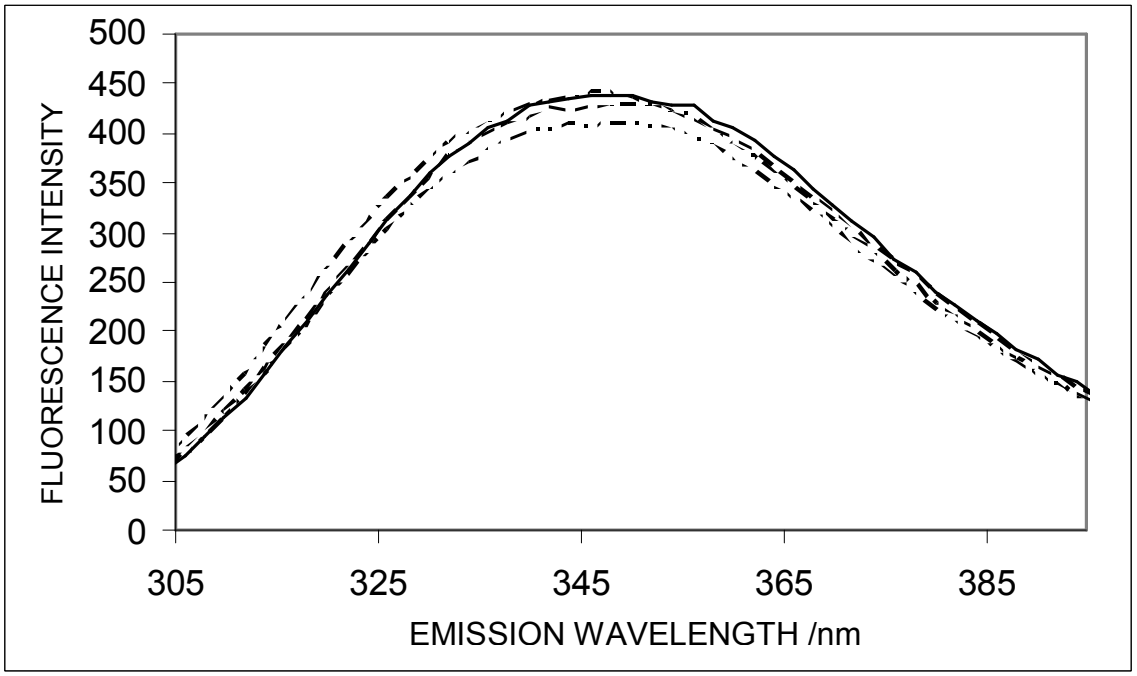


Supplementary Figure 14 - Effects of mutations on secondary structure of hAGT by circular dichroism. Scans were taken with 5 μM wild-type hAGT (_____), C145S (__ _ _ __), P140A (__ _ __) and V148L (__ __) between 260 and 200 nm in CD buffer (260-203 nm shown due to high voltage below 203nm).

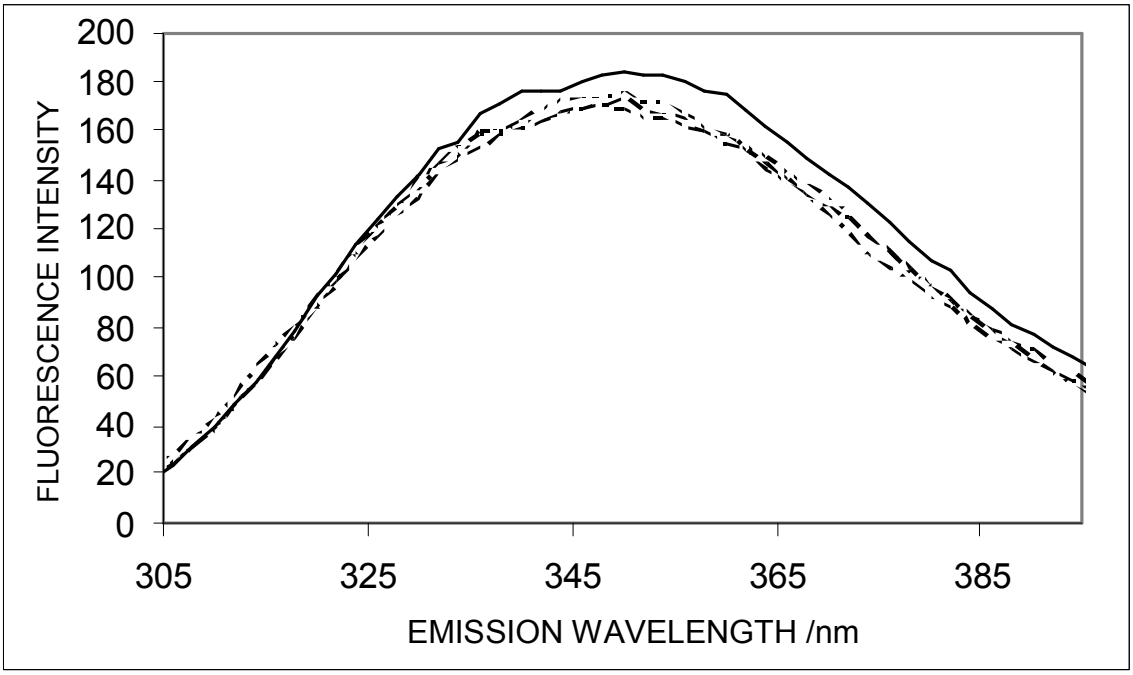


Supplementary Figure 15 - Effects of mutations on tertiary structure of hAGT by studying the intrinsic fluorescence signals of 1μM of wild-type hAGT (_____), C145S (__ _ _ __), P140A (__ _ __) and V148L (__ __). (**A**) Monitoring of intrinsic tryptophan and tyrosine fluorescence. (**B**) Monitoring of intrinsic tryptophan fluorescence.

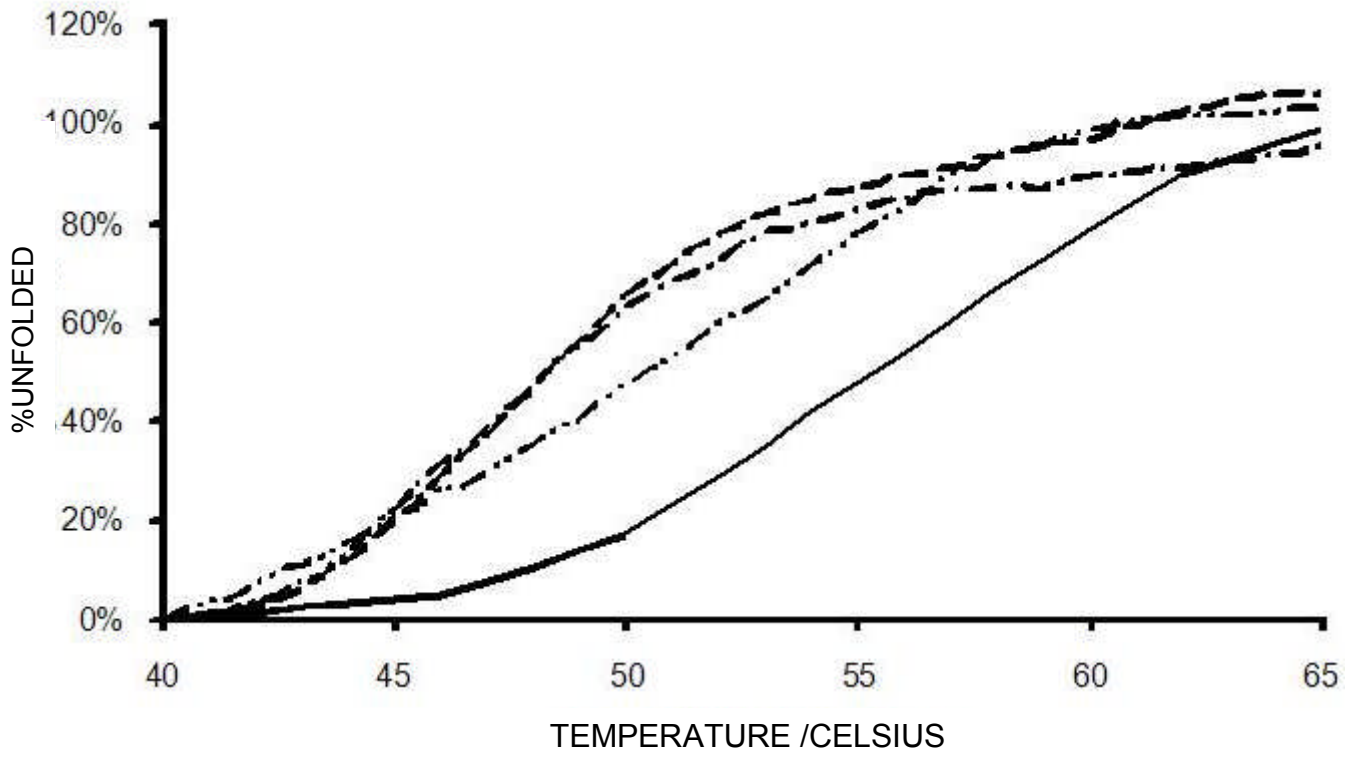
(**A**)



(**B**)

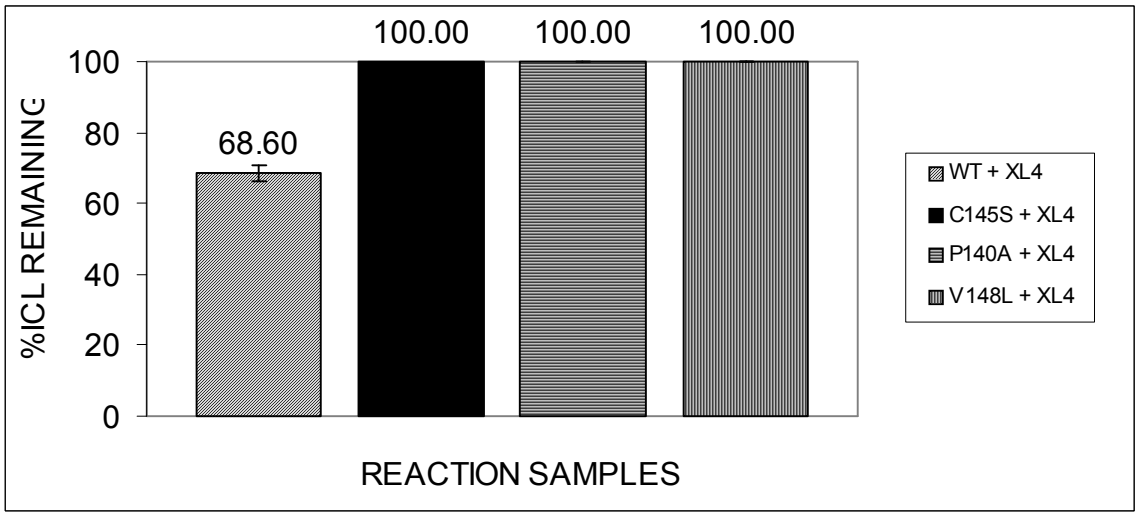


Supplementary Figure 16 - Effect of mutations on protein stability by thermal denaturation of 5 µM wild-type hAGT (_____), C145S (__ _ _ __), P140A (__ _ __) and V148L (__ __) by monitoring the change in molar ellipticity at 222nm.

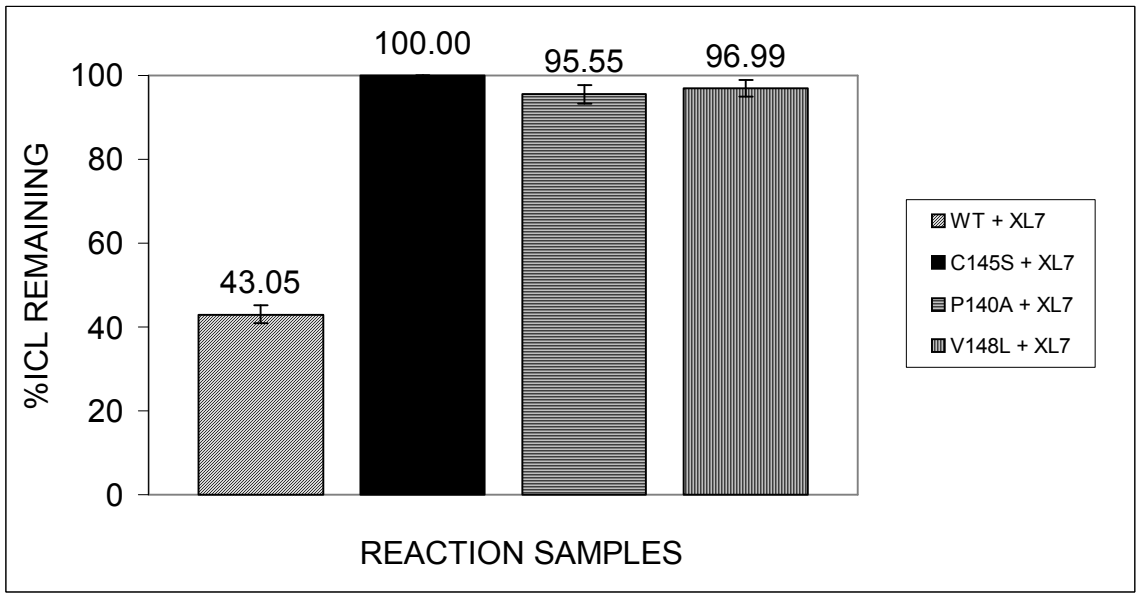


Supplementary Figure 17 – Graphic representation of the % ICL of (A) **XL4** and (B) **XL7** remaining in the reaction tube obtained using ImageQuant™.

(A)

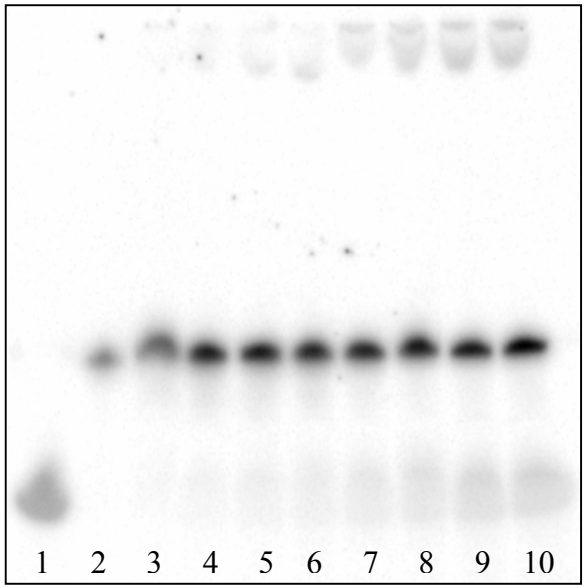


(B)

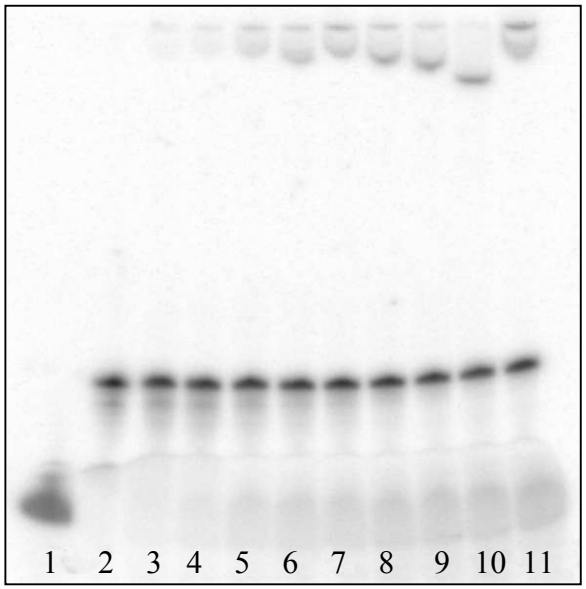


Supplementary Figure 18 – Time course repair of **XL4** and **XL7** by hAGT. (**A**) Denaturing gel of the repair of 2 pmol of **XL4** by 60 pmol hAGT as a function of time: lane 1, 2 pmol Control; lanes 2-10, 2 pmol **XL4** + 60 pmol hAGT incubated for 0, 5, 15, 30, 60, 120, 240, 510 and 540 min, respectively (**B**) Denaturing gel of the repair of 2 pmol of **XL7** by 60 pmol hAGT as a function of time: lane 1, 2 pmol Control; lanes 2-10, 2 pmol **XL7** + 60 pmol hAGT incubated for 0, 1, 2, 5, 10, 15, 30, 60, 120 and 180 min, respectively.

(**A**)

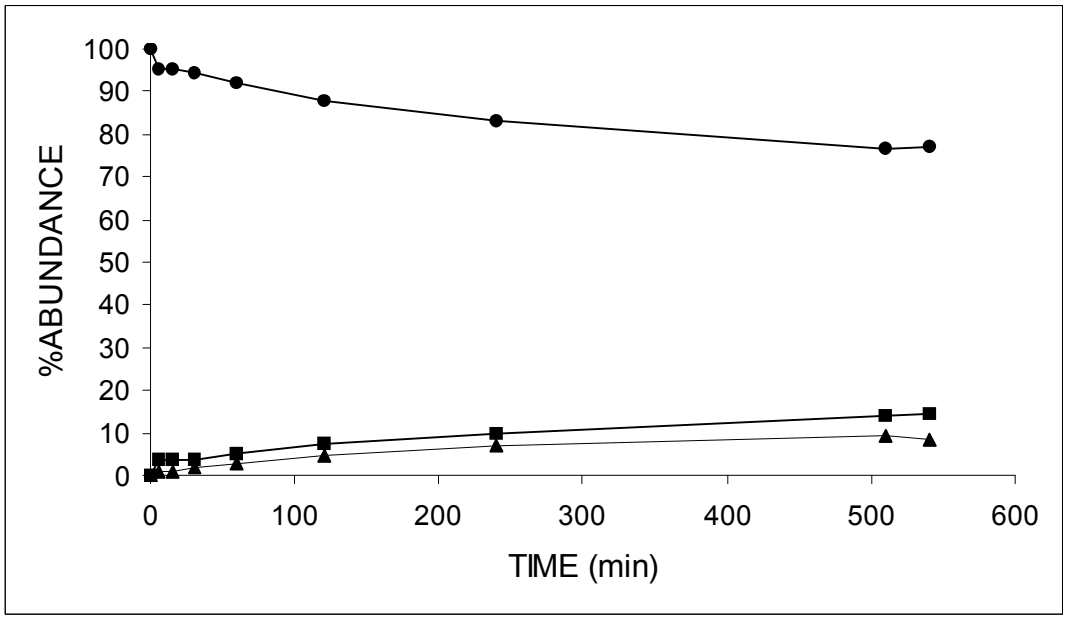


(**B**)

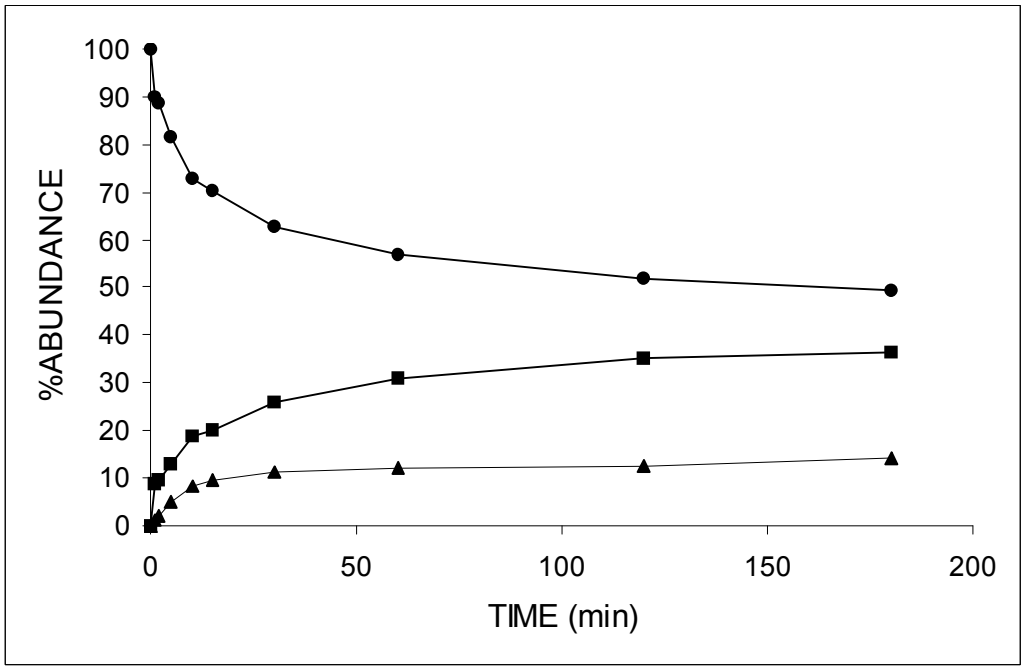


Supplementary Figure 19 – Graphic representation of the % abundance of each species in the time course repair by hAGT of (A) **XL4** and (B) **XL7** (see Supporting Information for denaturing gels). hAGT-DNA complex/partially repaired product (▲); free DNA/ fully repaired product (■); ICL/ unrepaired substrate (●).

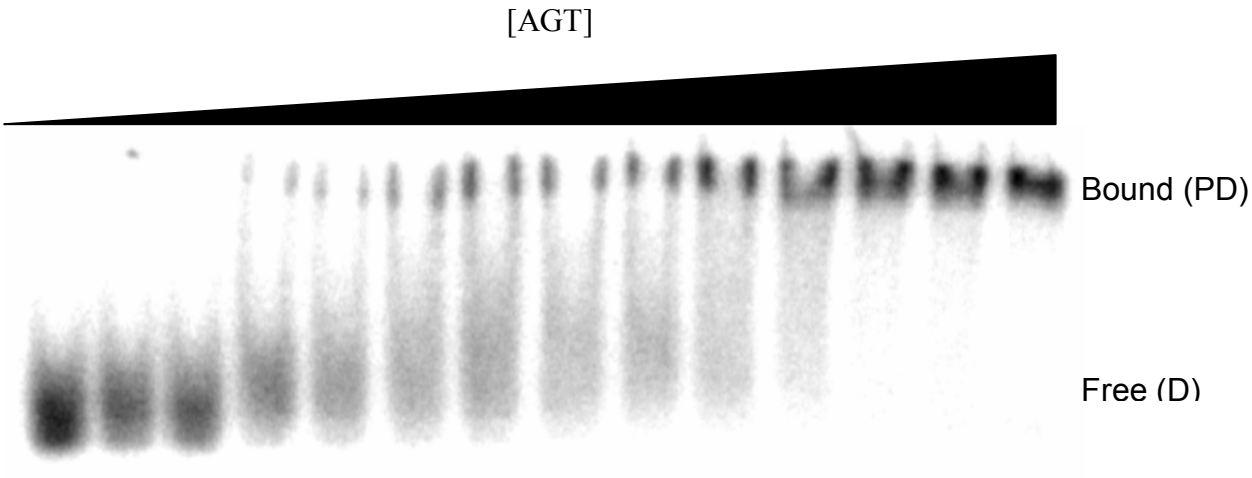
(A)



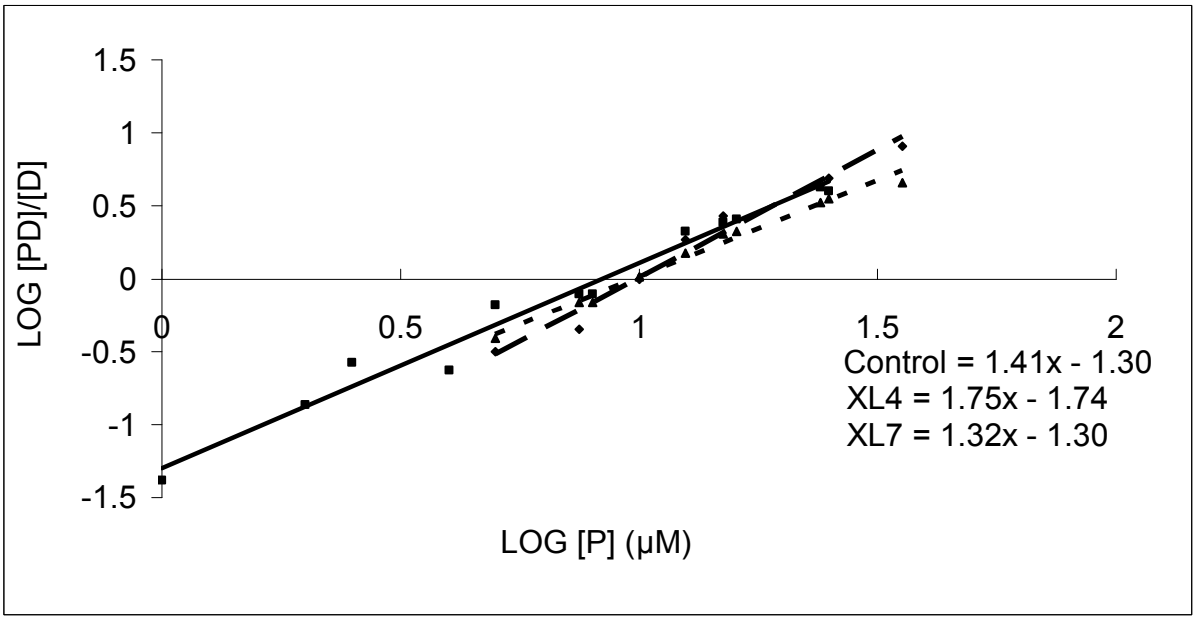
(B)



Supplementary Figure 20 - Electromobility shift assay of C145S hAGT binding to the control DNA duplex. EMSA Gel of C145S hAGT and the control 11-mer DNA duplex. 5 nM control DNA and 0 - 35.69 μM C145S hAGT.



Supplementary Figure 21 - Hill plot representation of log[PD]/[D] versus log[P] for the control duplex (■), **XL4 (♦)** and **XL7** (▲).



Supplementary Table 1 - ESI-MS results of wild-type hAGT and variants

Protein	Molecular Weight (Da)	
	Calculated	Observed
Wild-Type hAGT	21876.2	21875.0
C145S	21860.1	21860.0
P140A	21850.1	21850.5
V148L	21890.2	21889.5

Supplementary Table 2 – Effect of mutations on fluorescence emission signals of intrinsic hAGT on Tryptophan and Tyrosine (λex 280nm) and Trypotophan only (λex 295nm).

	λex 280nm		λex 295nm	
Protein	λem (nm)	Fluorescence Intensity	λem (nm)	Fluorescence Intensity
Wild-Type hAGT	346	438	350	184
C145S	350	409	350	176
P140A	346	440	348	169
V148L	350	428	350	173

Supplementary Table 3 - Difference in T_m between mutants and wild-type hAGT.

Protein	Melting Temperature (^oC)		
	Observed	Wild-Type	Difference
Wild-Type hAGT	56.5	56.5	0.0
C145S	50.7	56.5	-5.8
P140A	47.0	56.5	-9.5
V148L	48.0	56.5	-8.5