

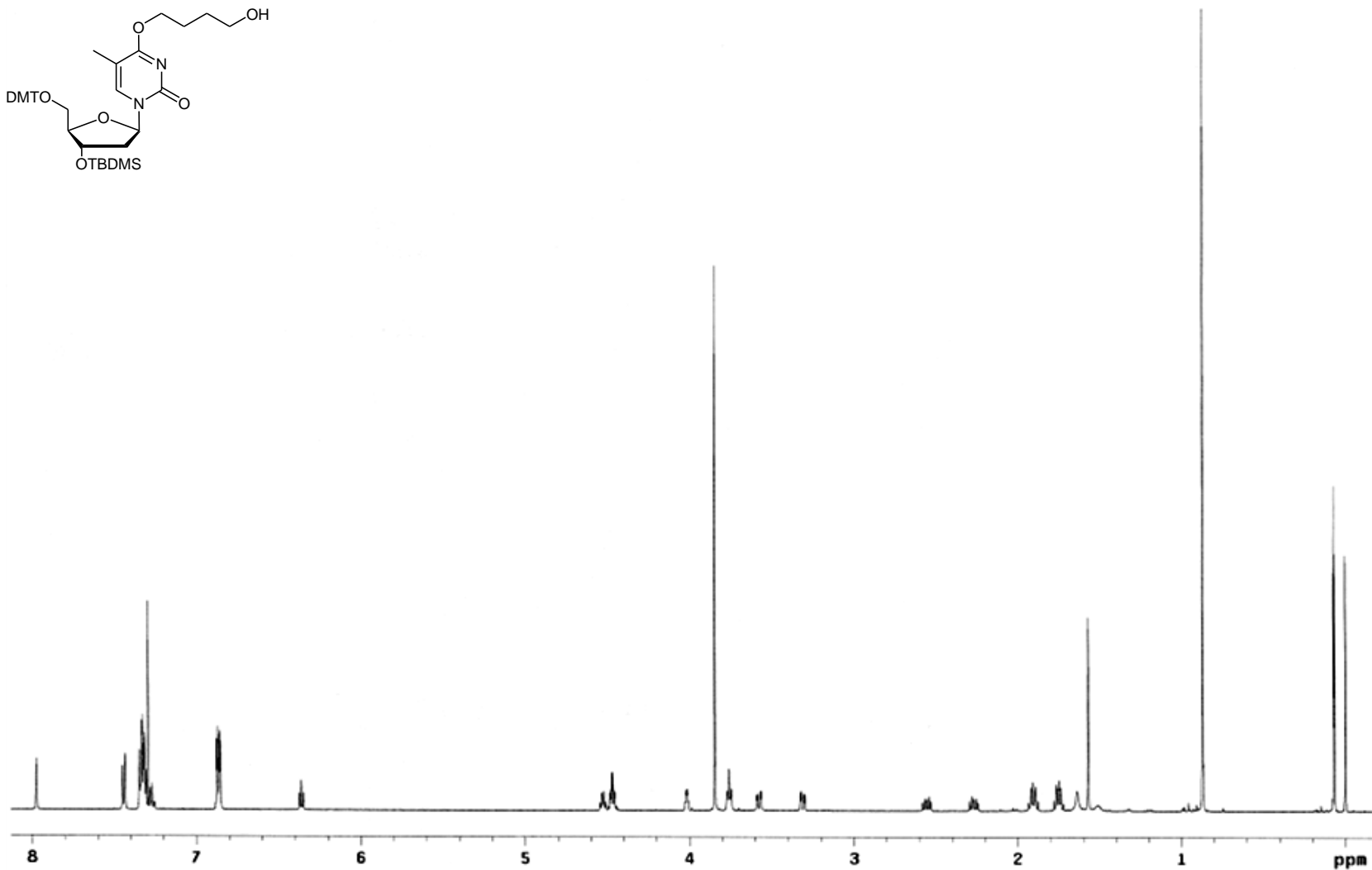
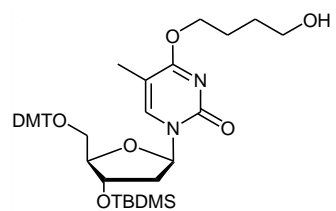
Supplementary Information

***O*4-Alkyl-2'-deoxythymidine Cross-linked DNA to Probe Recognition and Repair by *O*6-Alkylguanine-DNA Alkyltransferases**

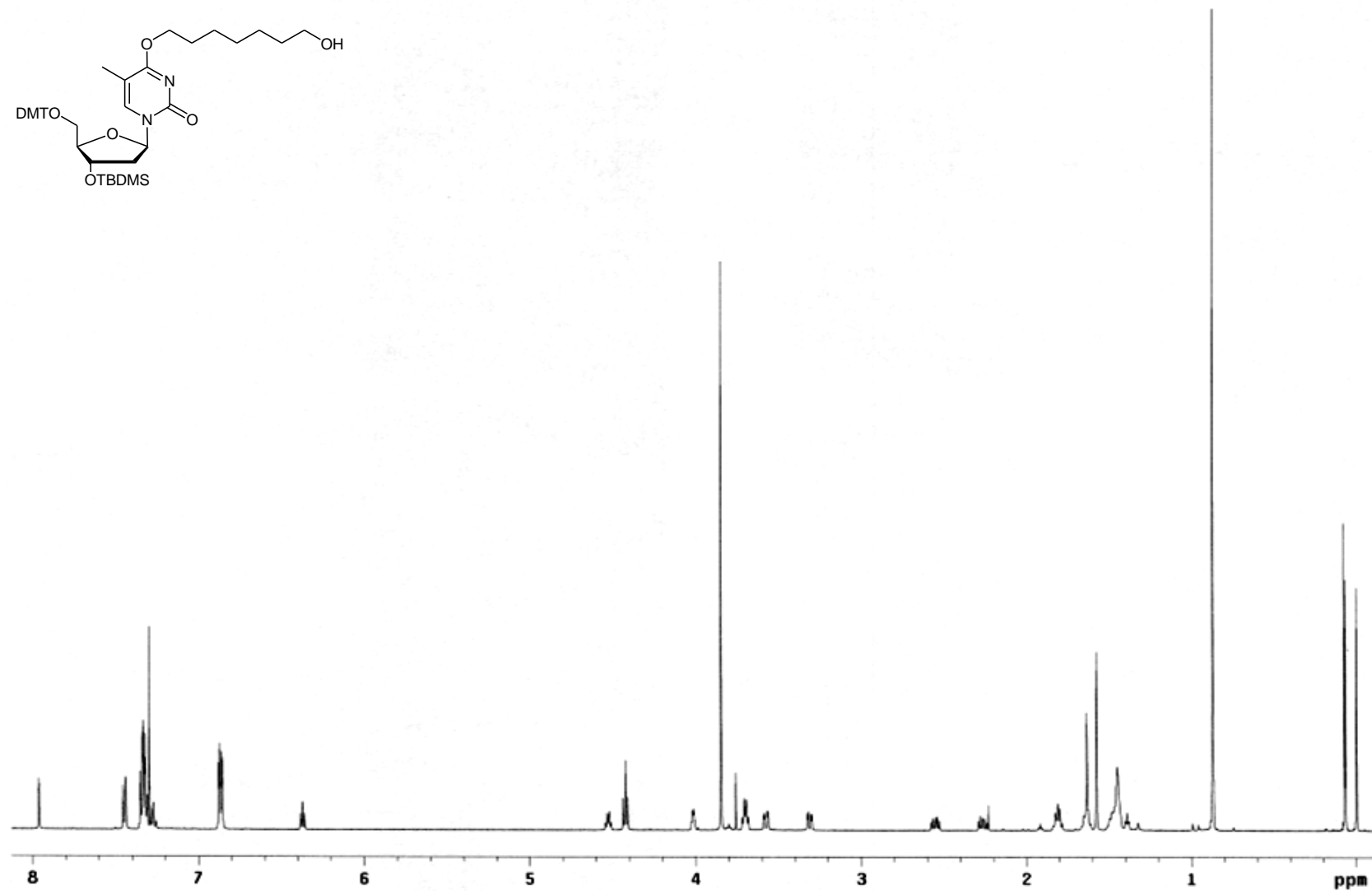
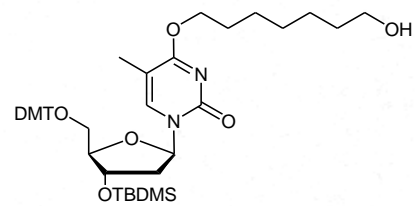
Francis P. McManus, Derek K. O'Flaherty, Anne M. Noronha and Christopher J. Wilds*

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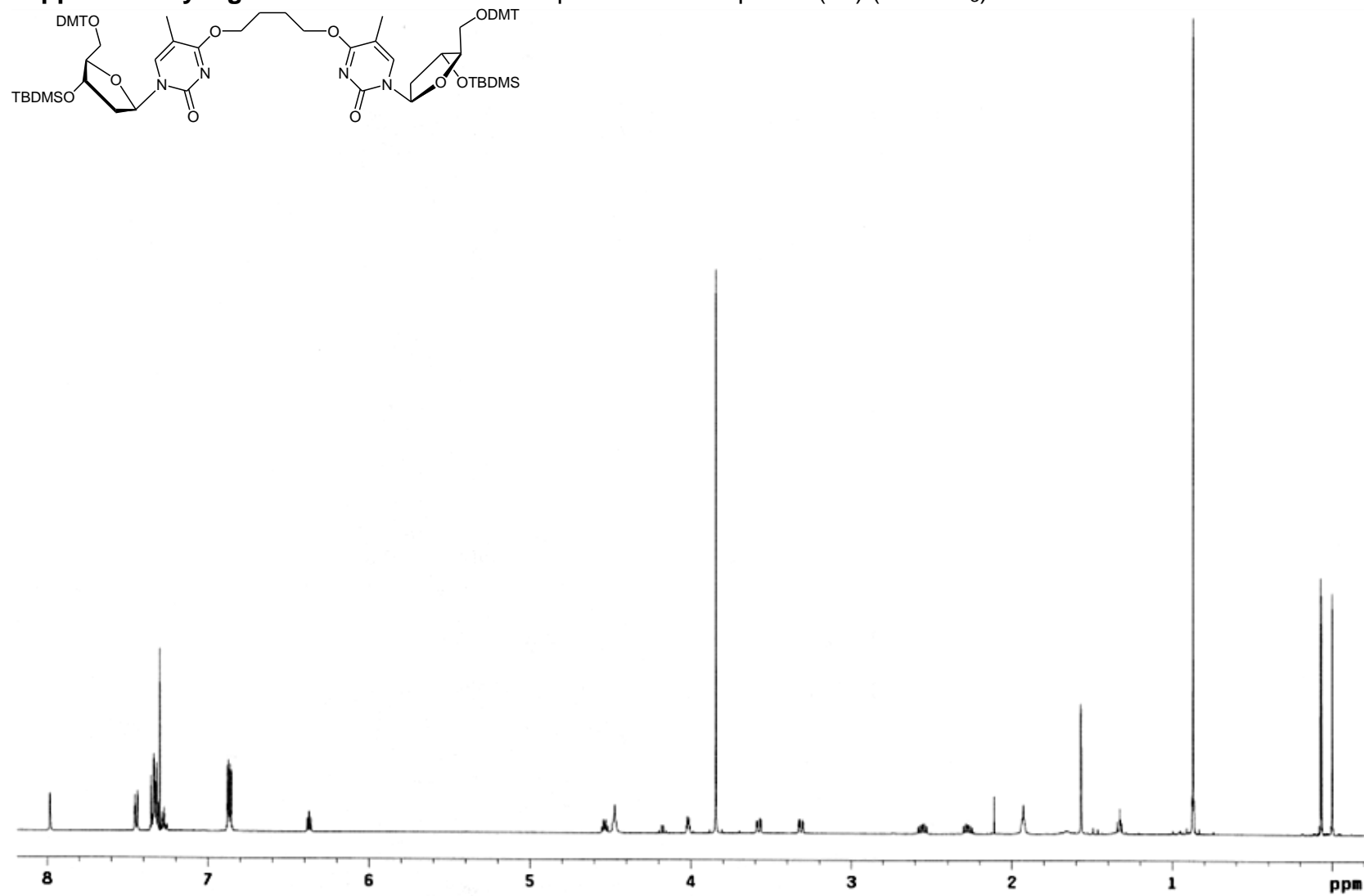
Supplementary Figure 1 - 500 MHz ^1H NMR spectrum of compound (**1a**) (in CDCl_3)



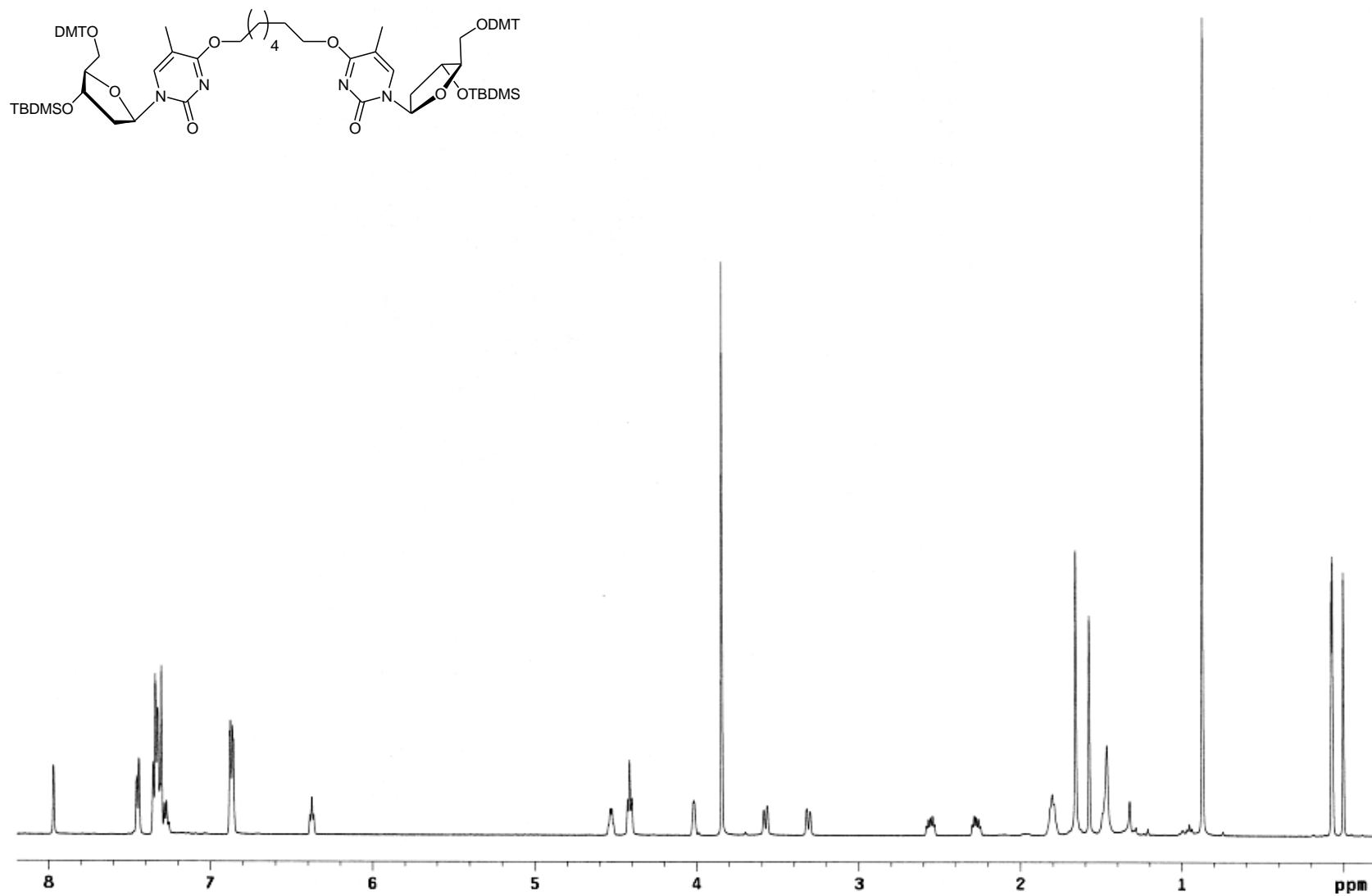
Supplementary Figure 2 - 500 MHz ^1H NMR spectrum of compound (**1b**) (in CDCl_3)



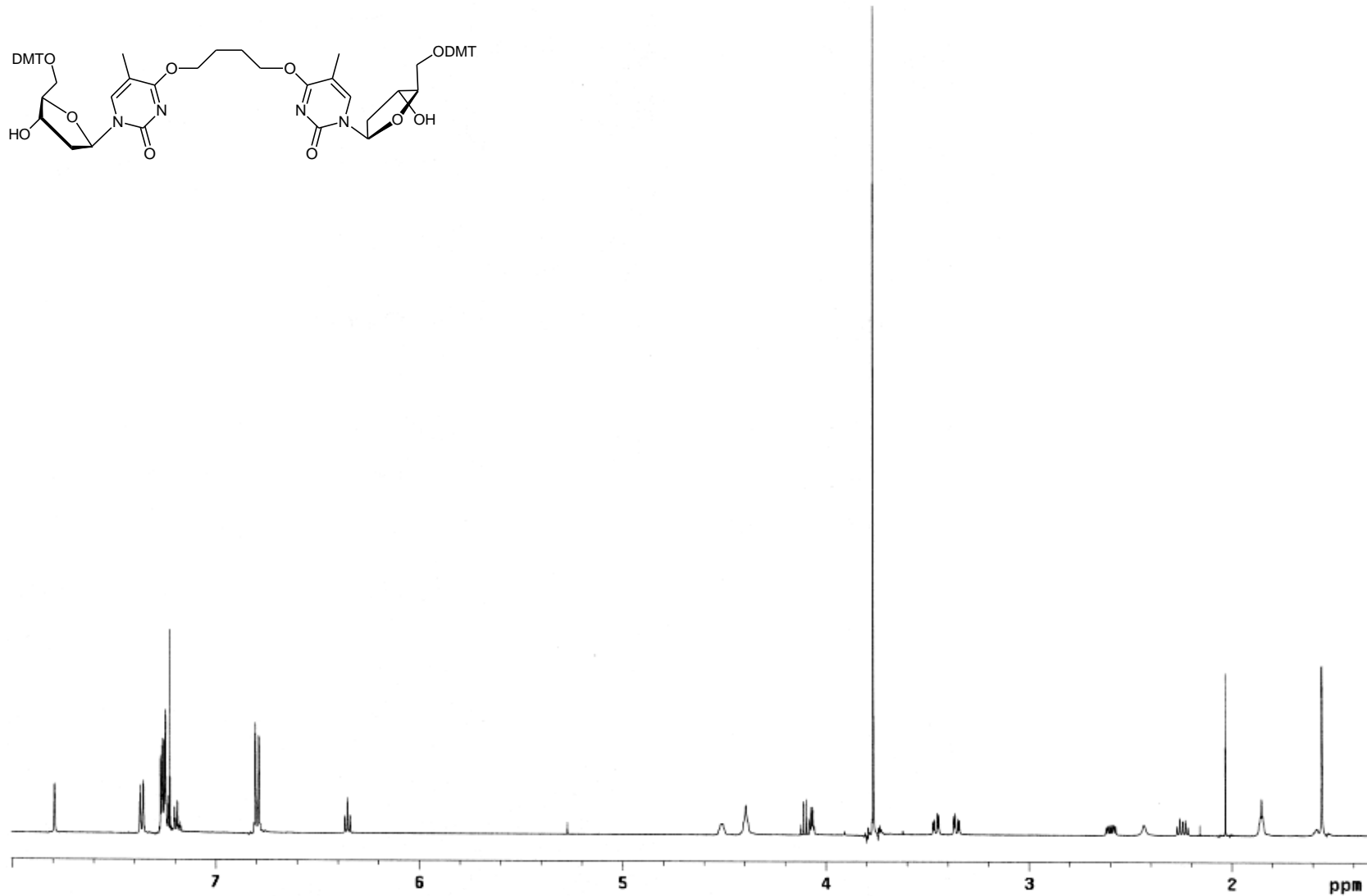
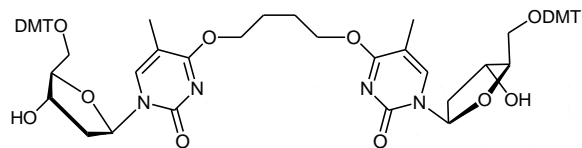
Supplementary Figure 3 - 500 MHz ^1H NMR spectrum of compound (**2a**) (in CDCl_3)



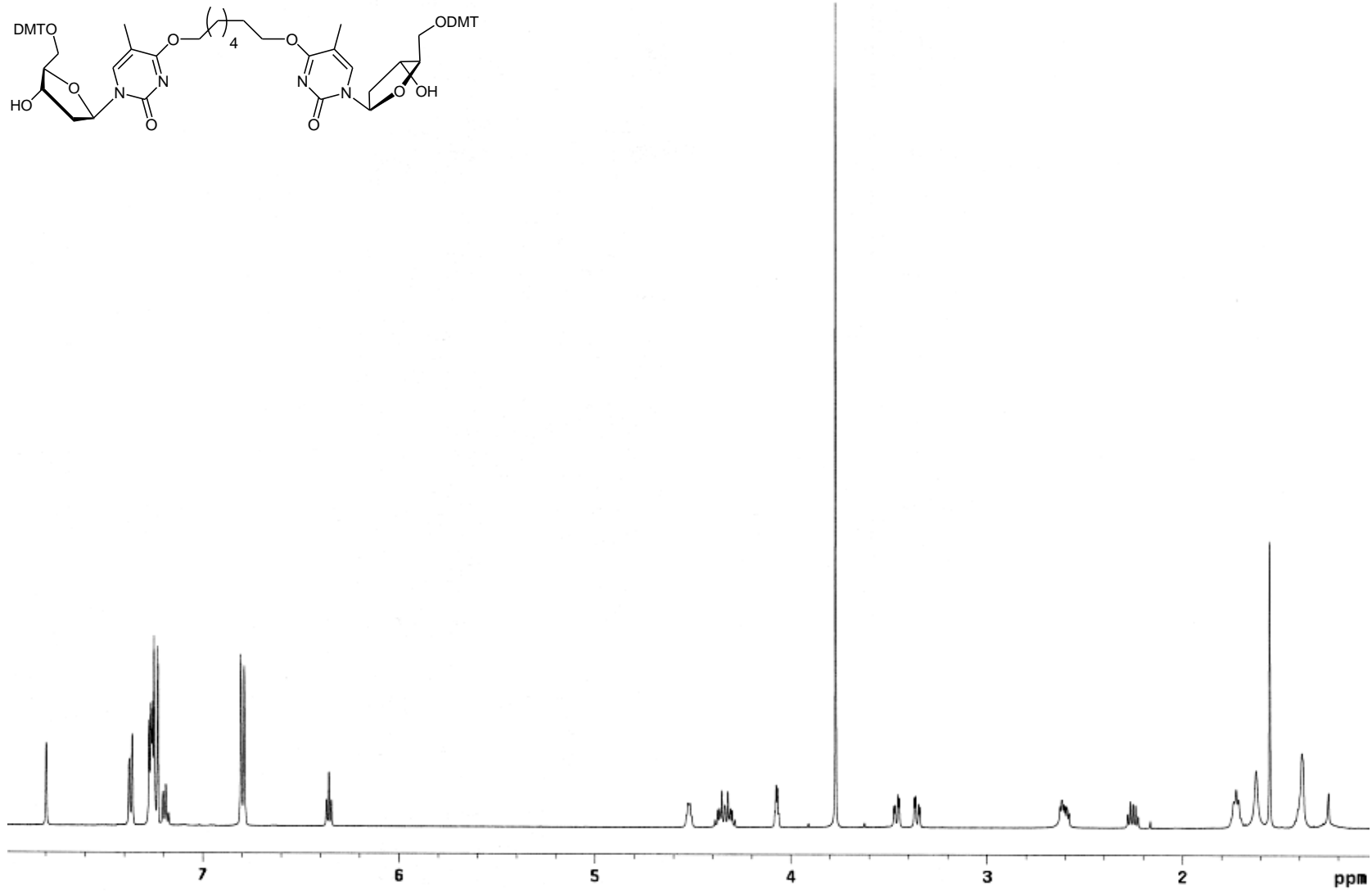
Supplementary Figure 4 - 500 MHz ^1H NMR spectrum of compound (**2b**) (in CDCl_3)



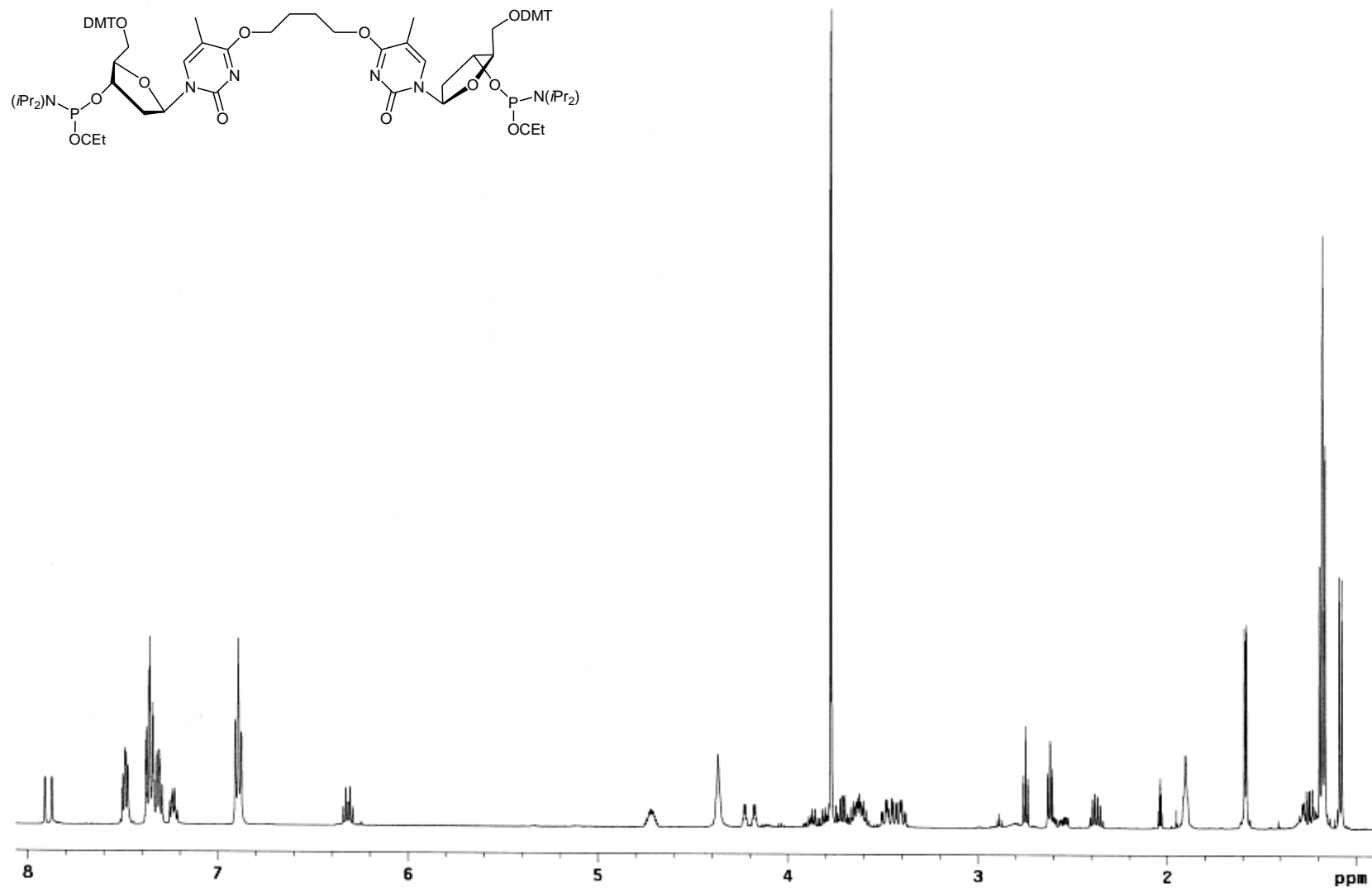
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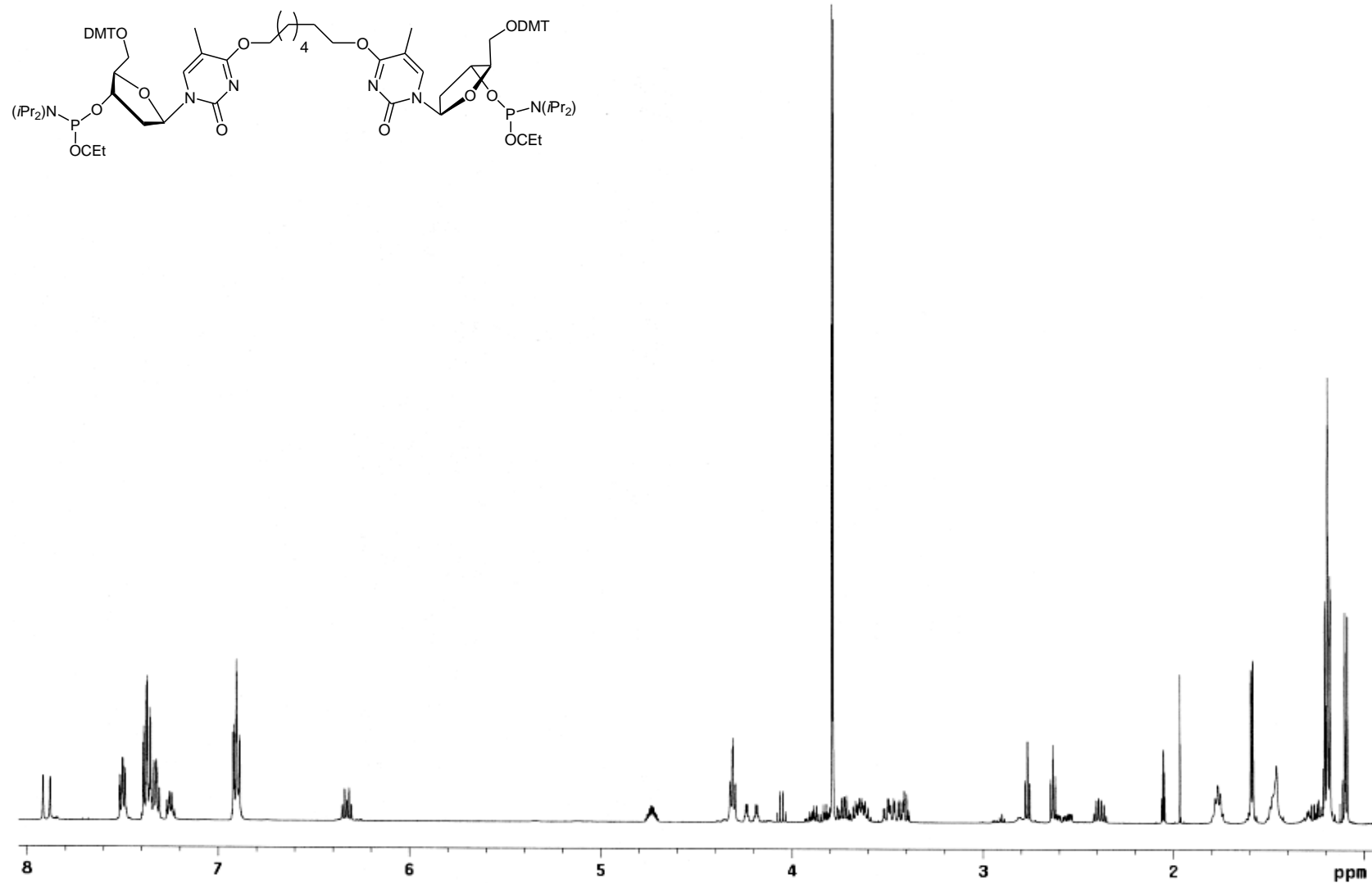
Supplementary Figure 6 - 500 MHz ^1H NMR spectrum of compound (**3b**) (in CDCl_3)



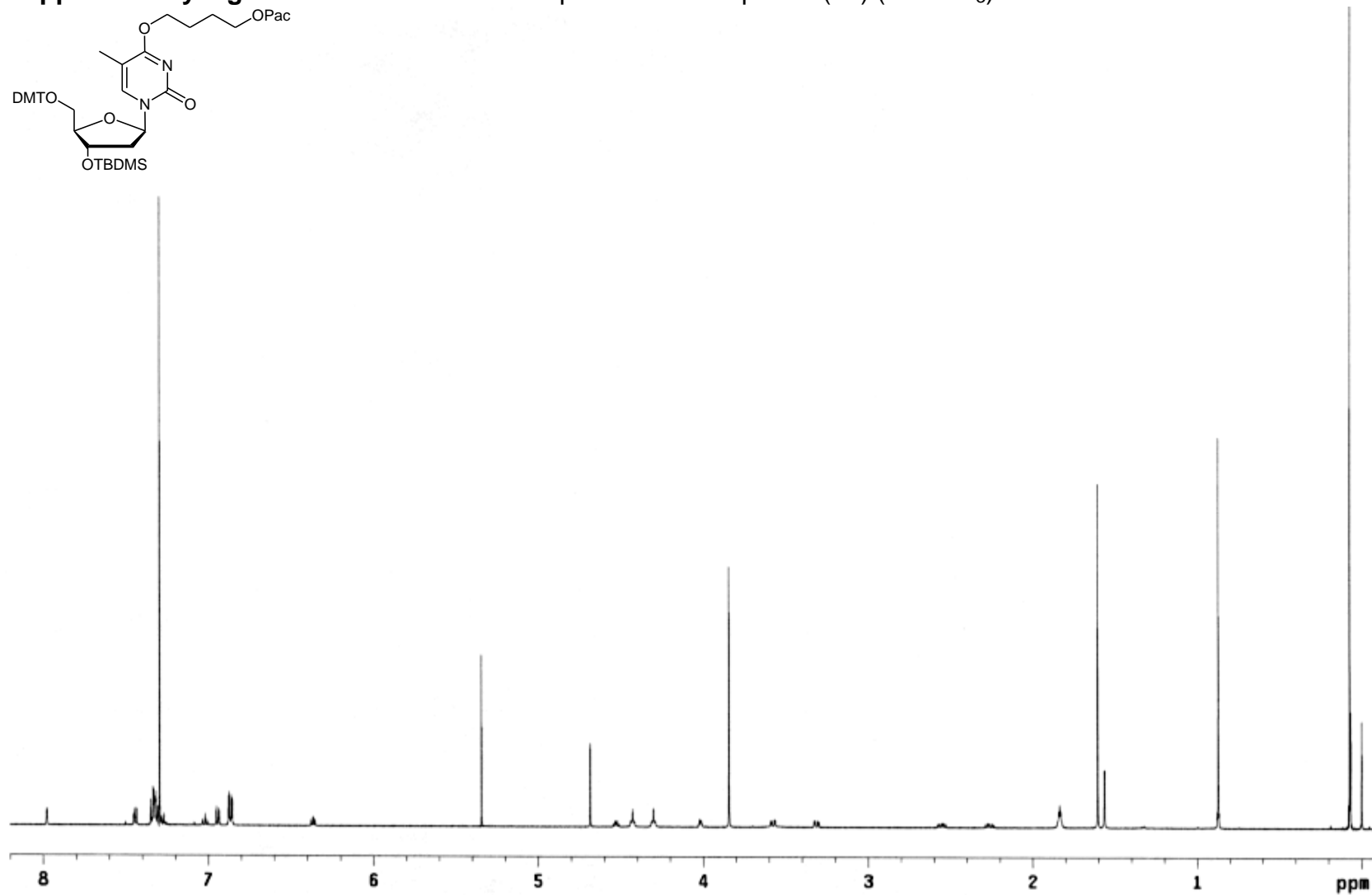
Supplementary Figure 7 - 500 MHz ^1H NMR spectrum of compound (**4a**) (in d_6 -acetone)



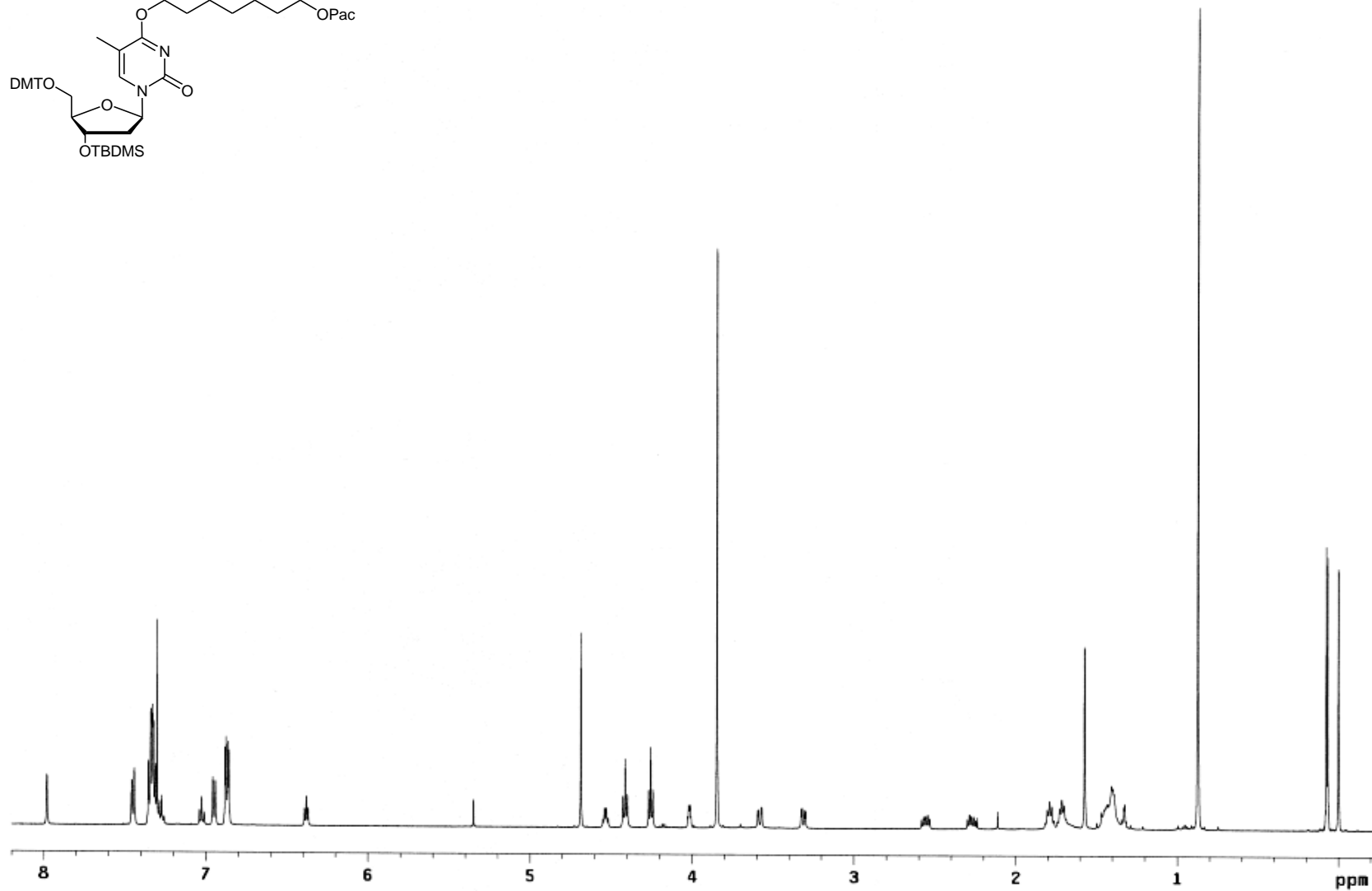
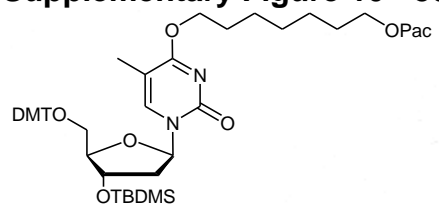
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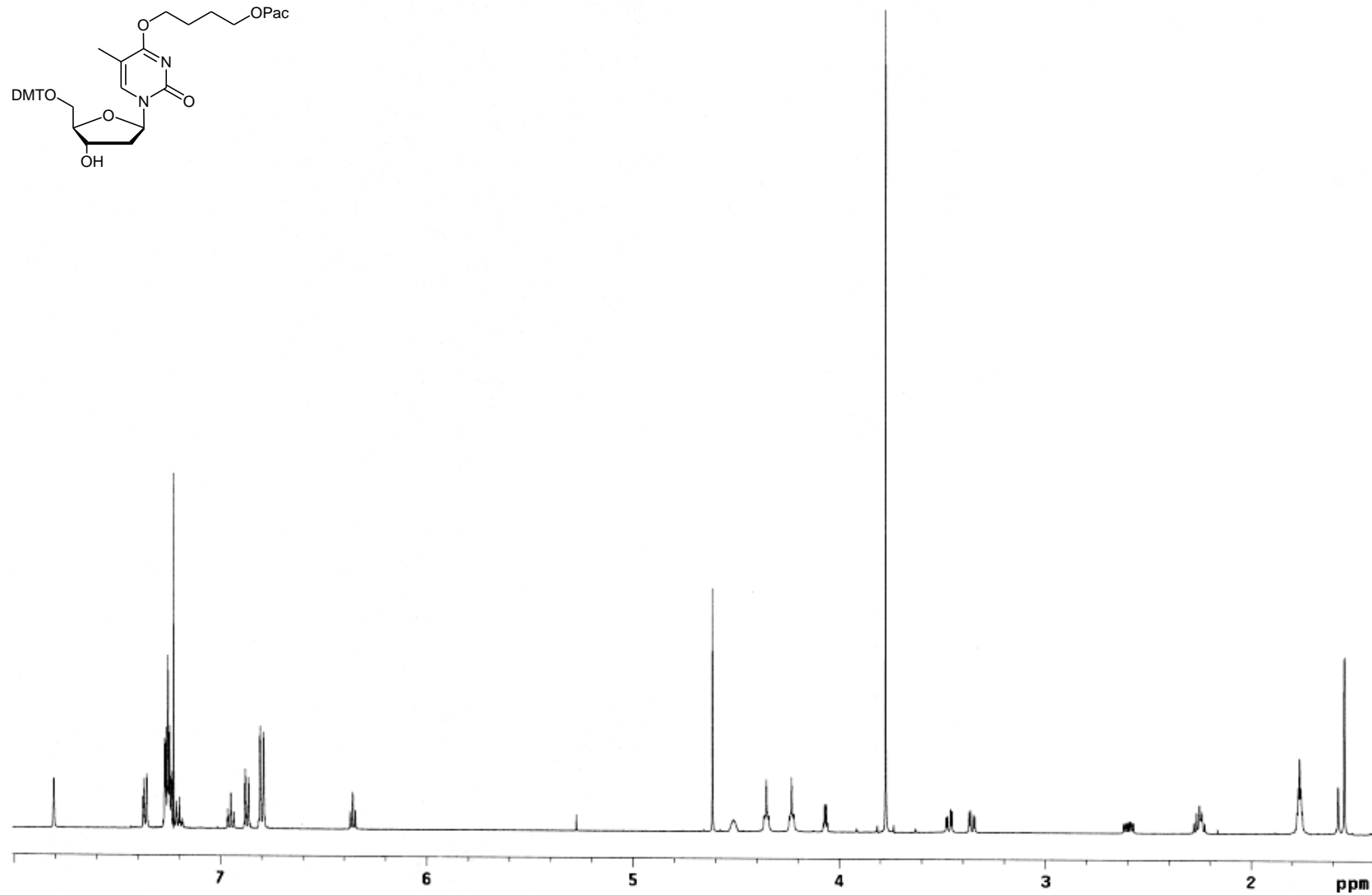
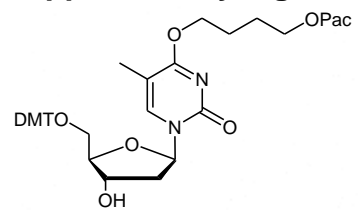
Supplementary Figure 9 - 500 MHz ^1H NMR spectrum of compound (**5a**) (in CDCl_3)



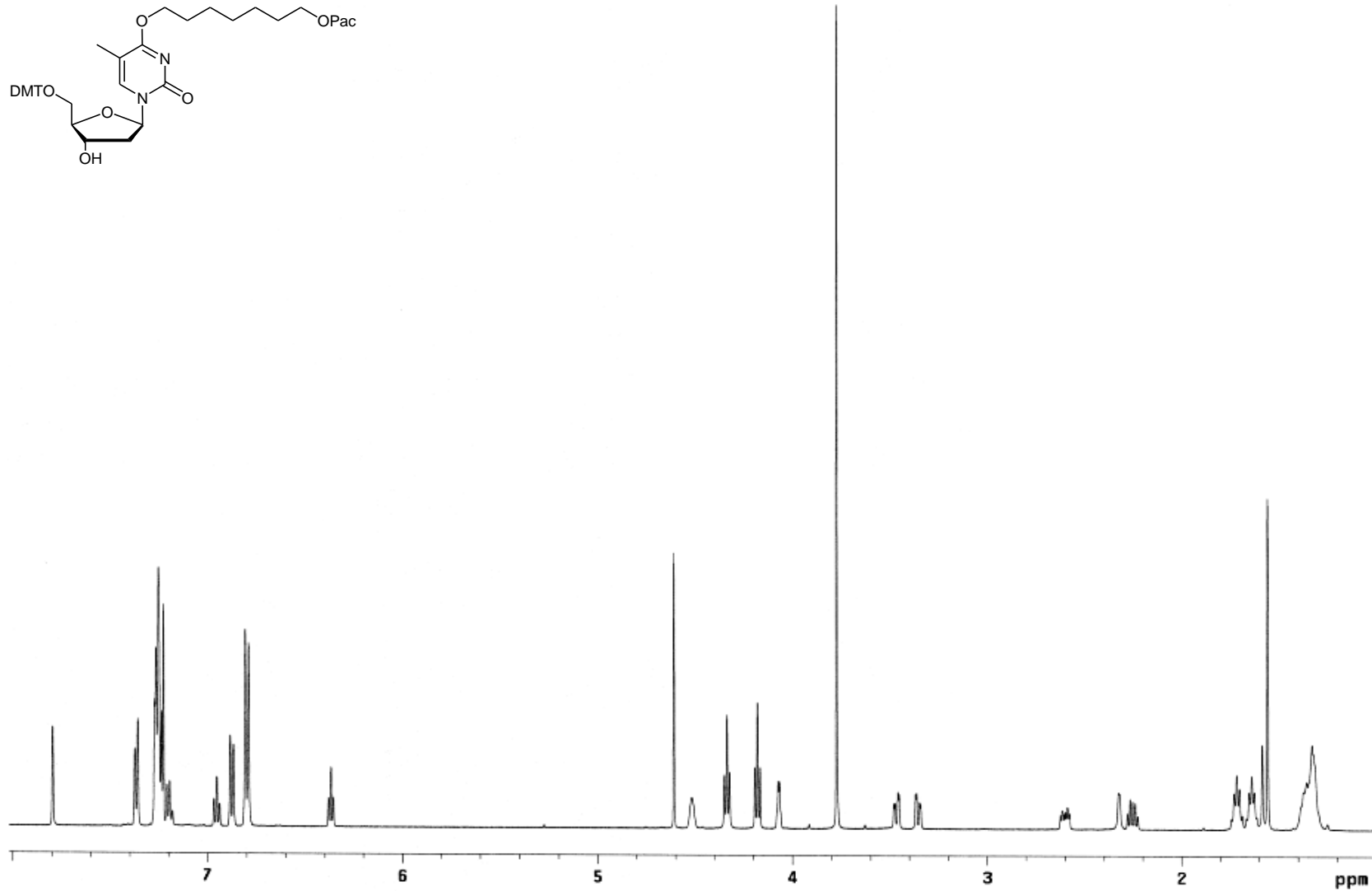
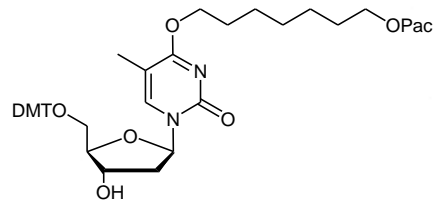
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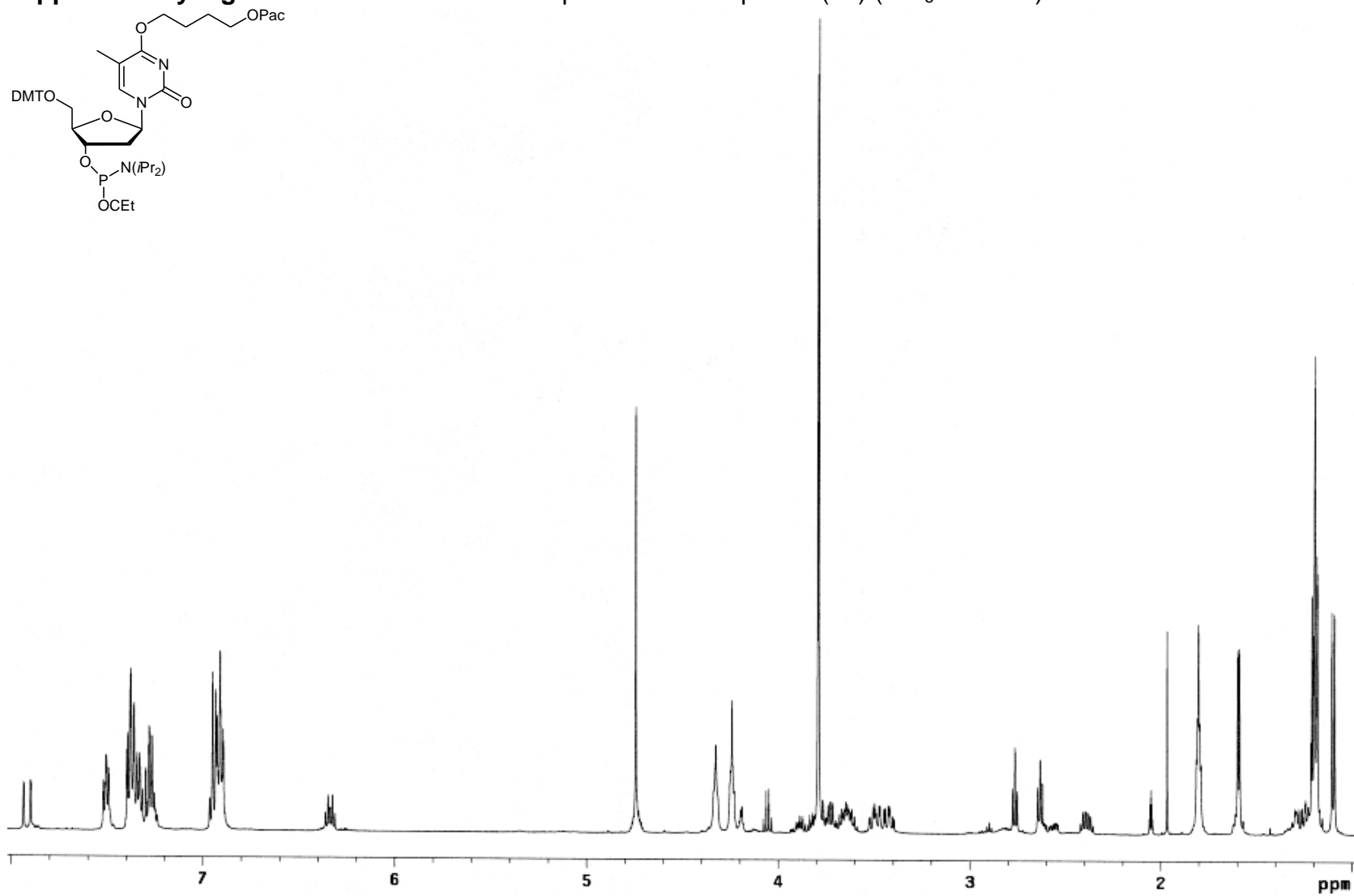
Supplementary Figure 11 - 500 MHz ^1H NMR spectrum of compound (**6a**) (in CDCl_3)



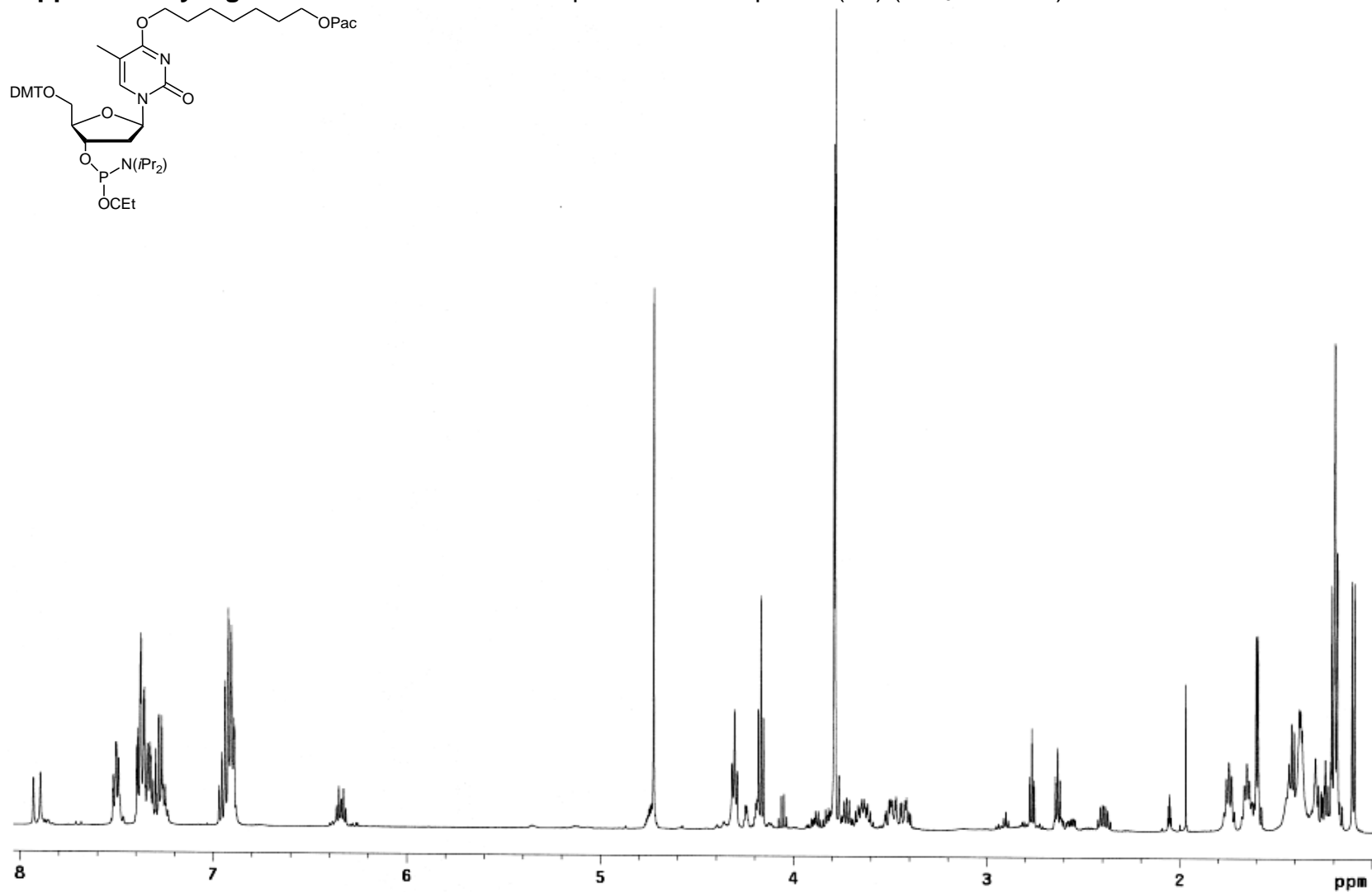
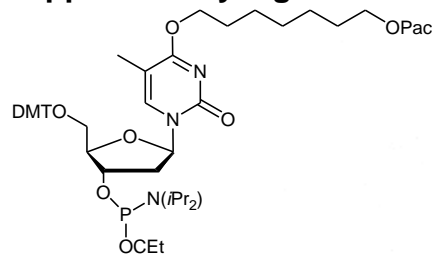
Supplementary Figure 12 - 500 MHz ^1H NMR spectrum of compound (**6b**) (in CDCl_3)



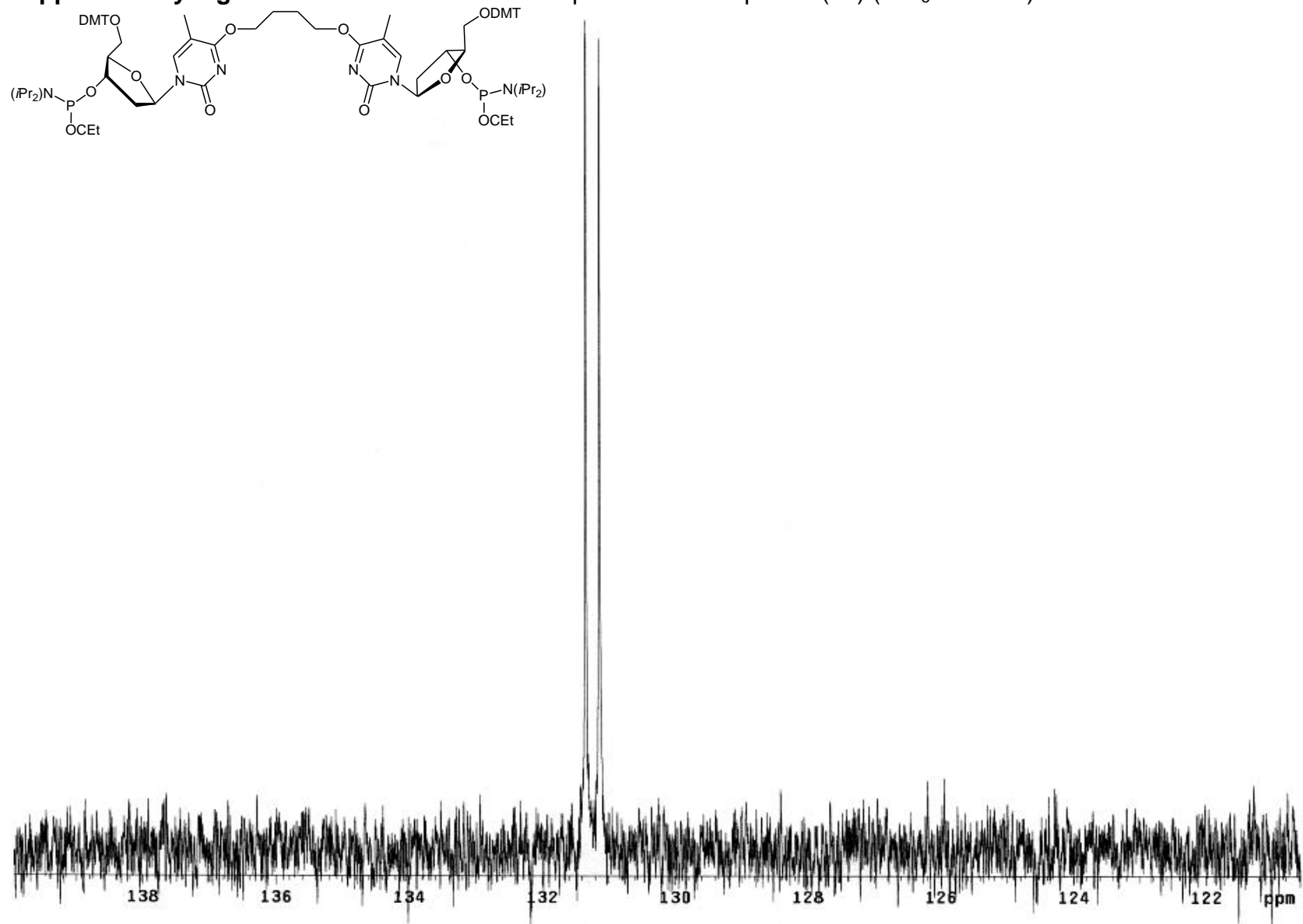
Supplementary Figure 13 - 500 MHz ^1H NMR spectrum of compound (**7a**) (in d_6 -acetone)



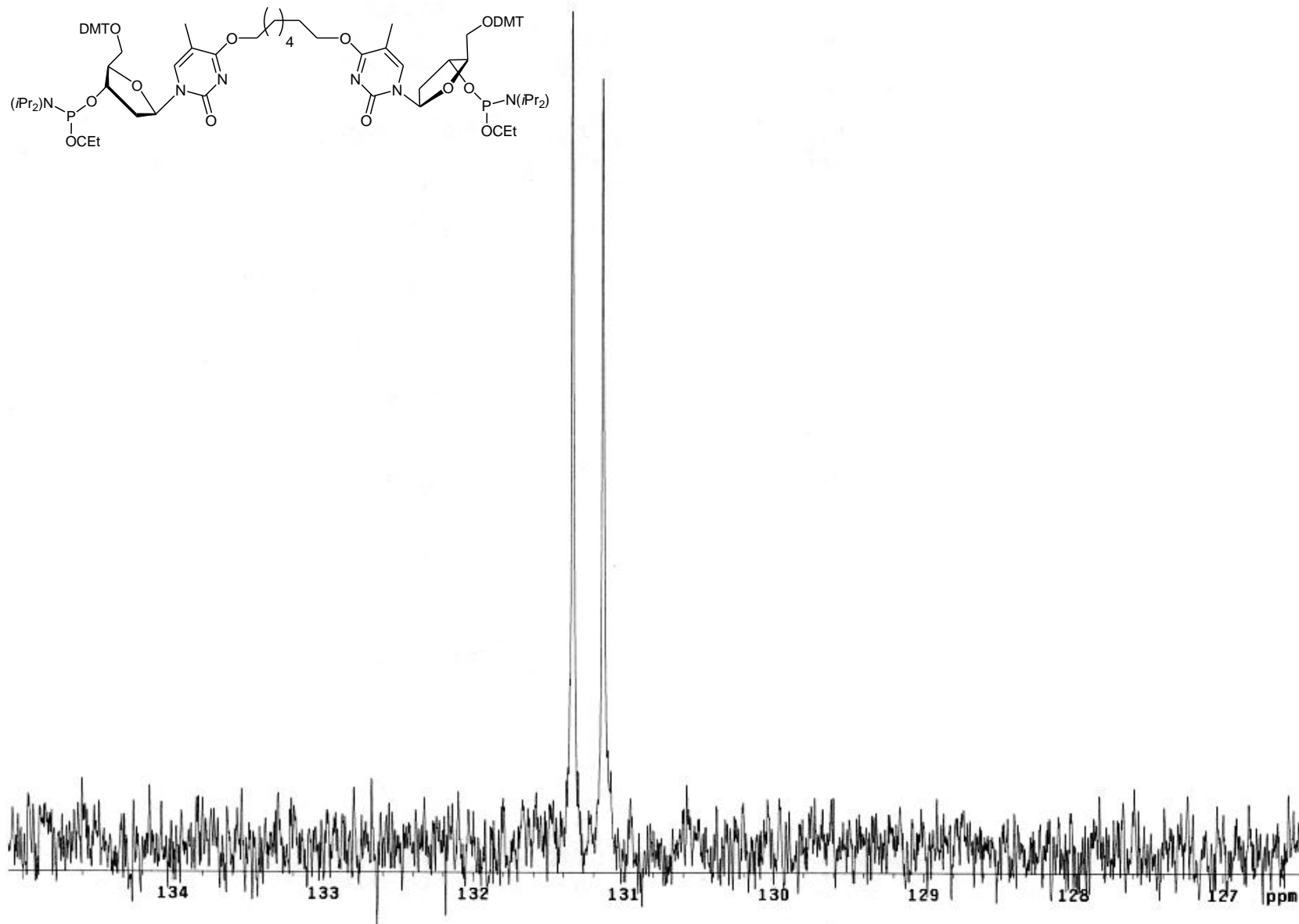
Supplementary Figure 14 - 500 MHz ^1H NMR spectrum of compound (**7b**) (in d_6 -acetone)



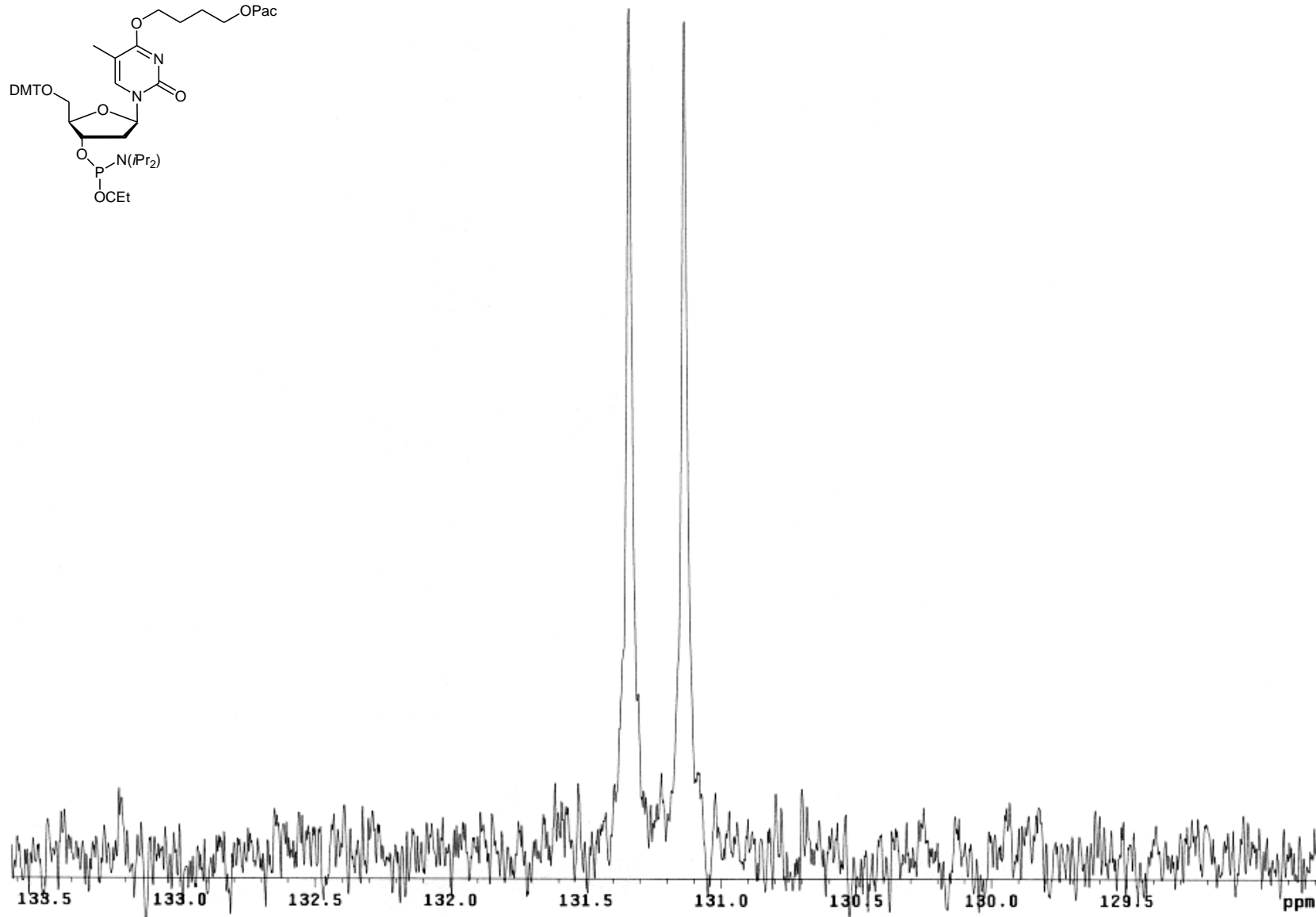
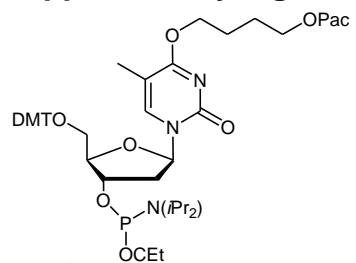
Supplementary Figure 15 - 202.3 MHz ^{31}P NMR spectrum of compound (**4a**) (in d_6 -acetone)



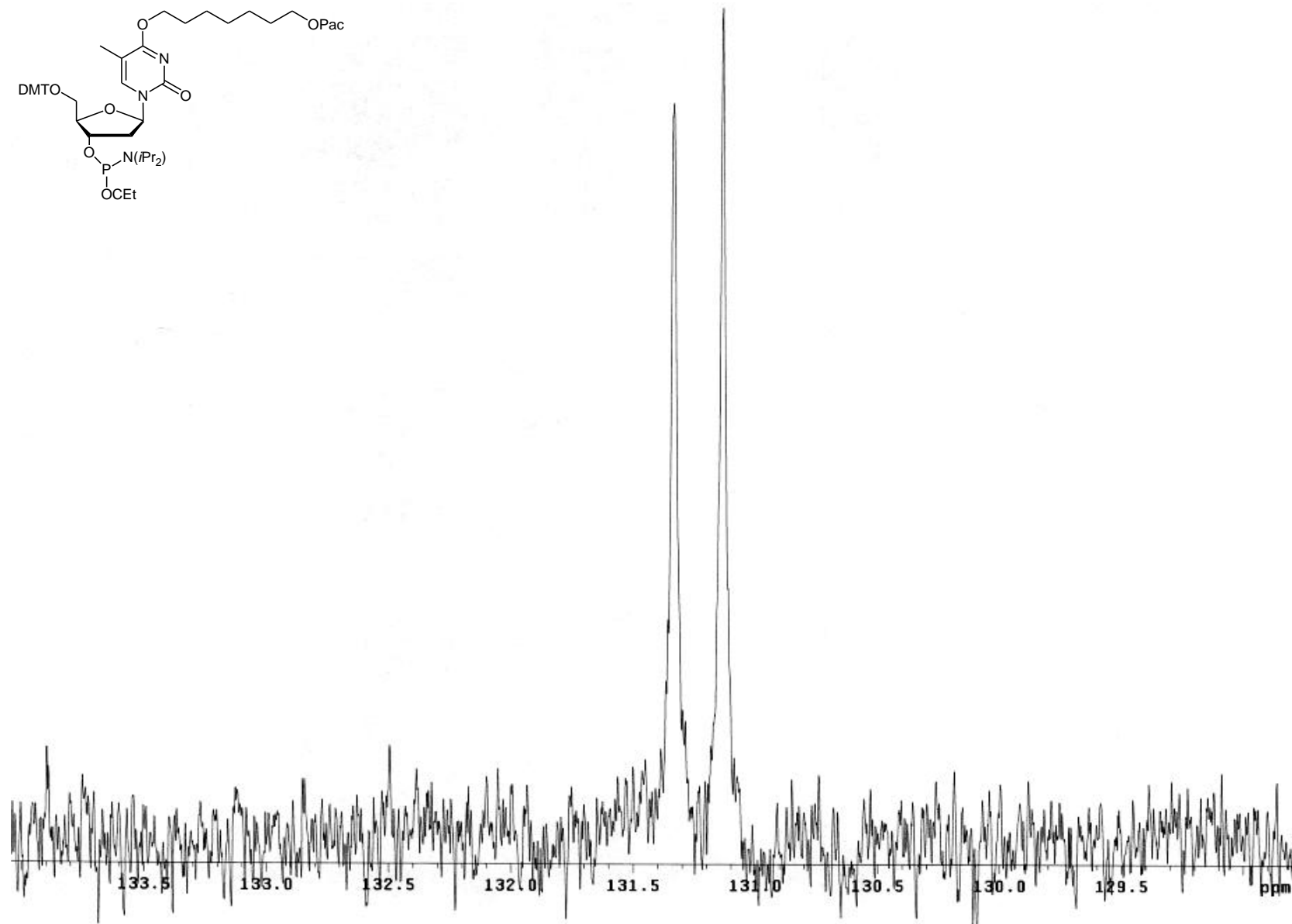
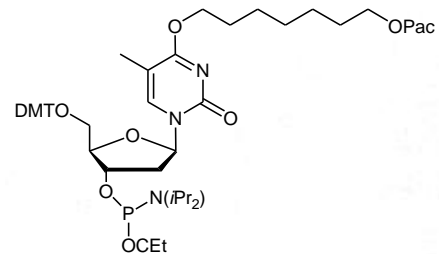
Supplementary Figure 16 - 202.3 MHz ^{31}P NMR spectrum of compound (**4b**) (in d_6 -acetone)



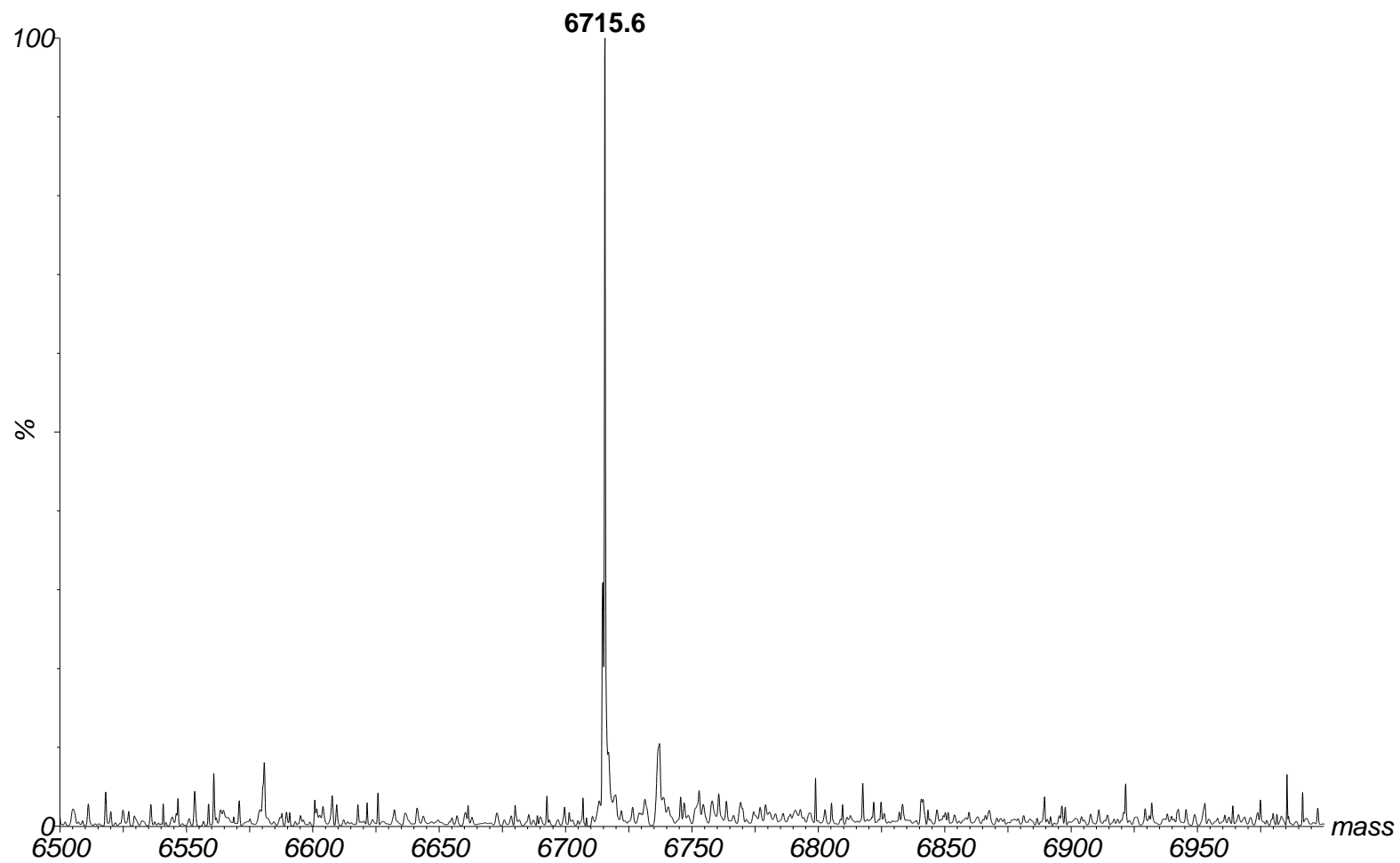
Supplementary Figure 17 - 202.3 MHz ^{31}P NMR spectrum of compound (**7a**) (in d_6 -acetone)



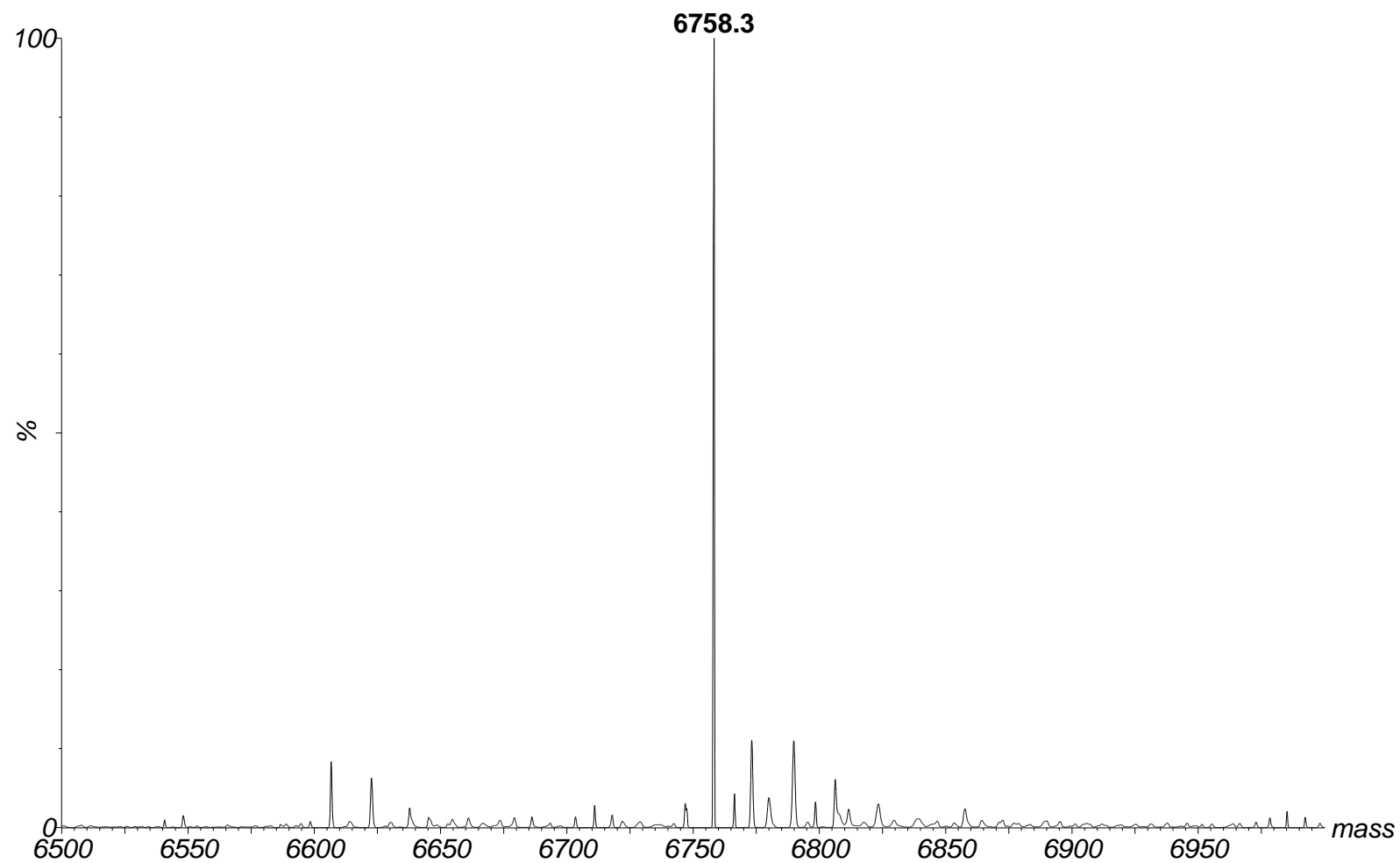
Supplementary Figure 18 - 202.3 MHz ^{31}P NMR spectrum of compound (**7b**) (in d_6 -acetone)



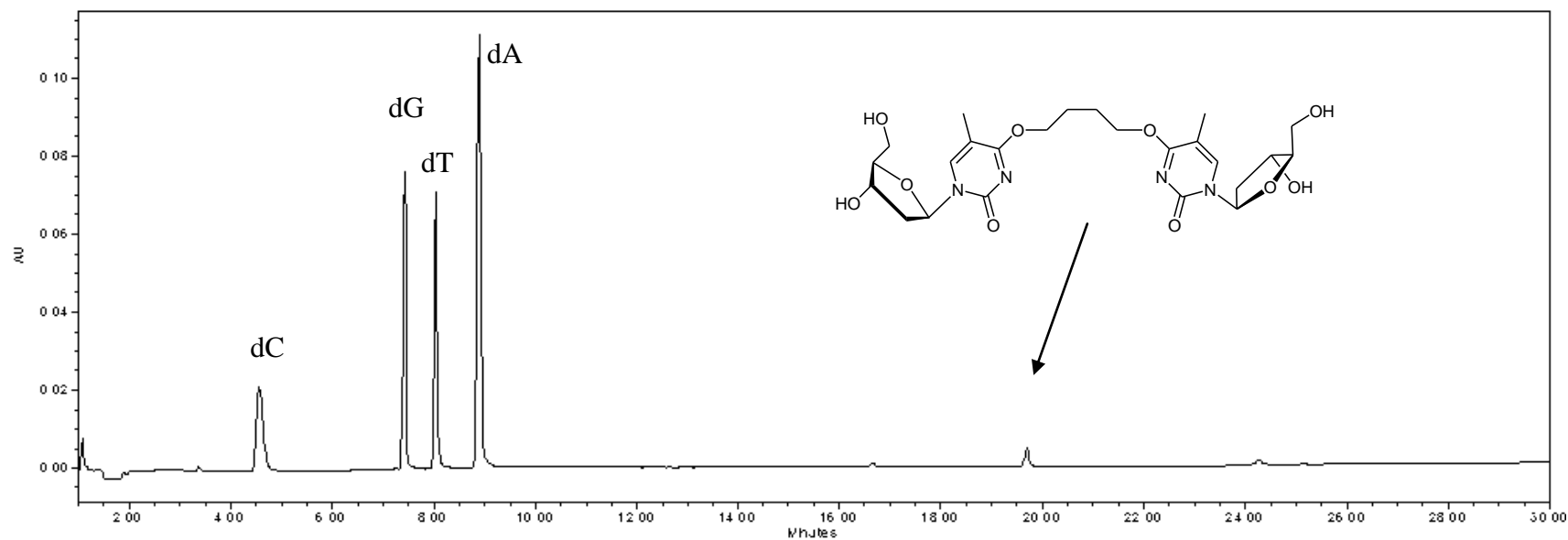
Supplementary Figure 19 - ESI MS of oligonucleotide XLTT4



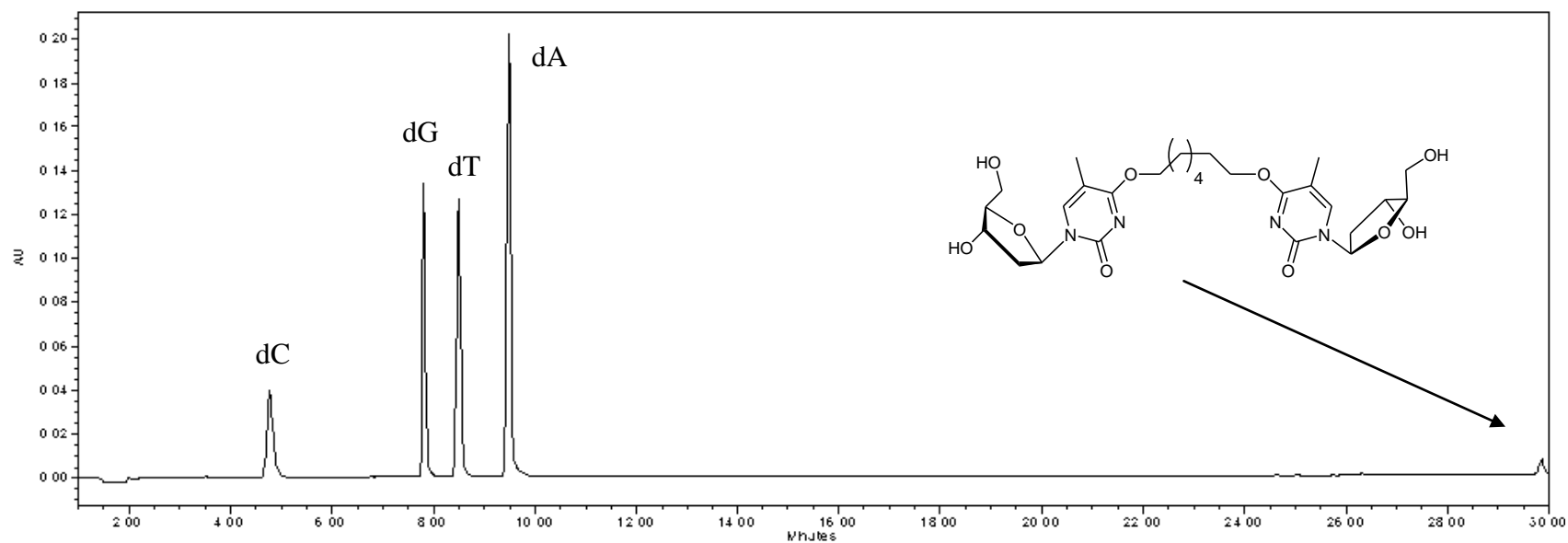
Supplementary Figure 20 - ESI MS of oligonucleotide XLTT7



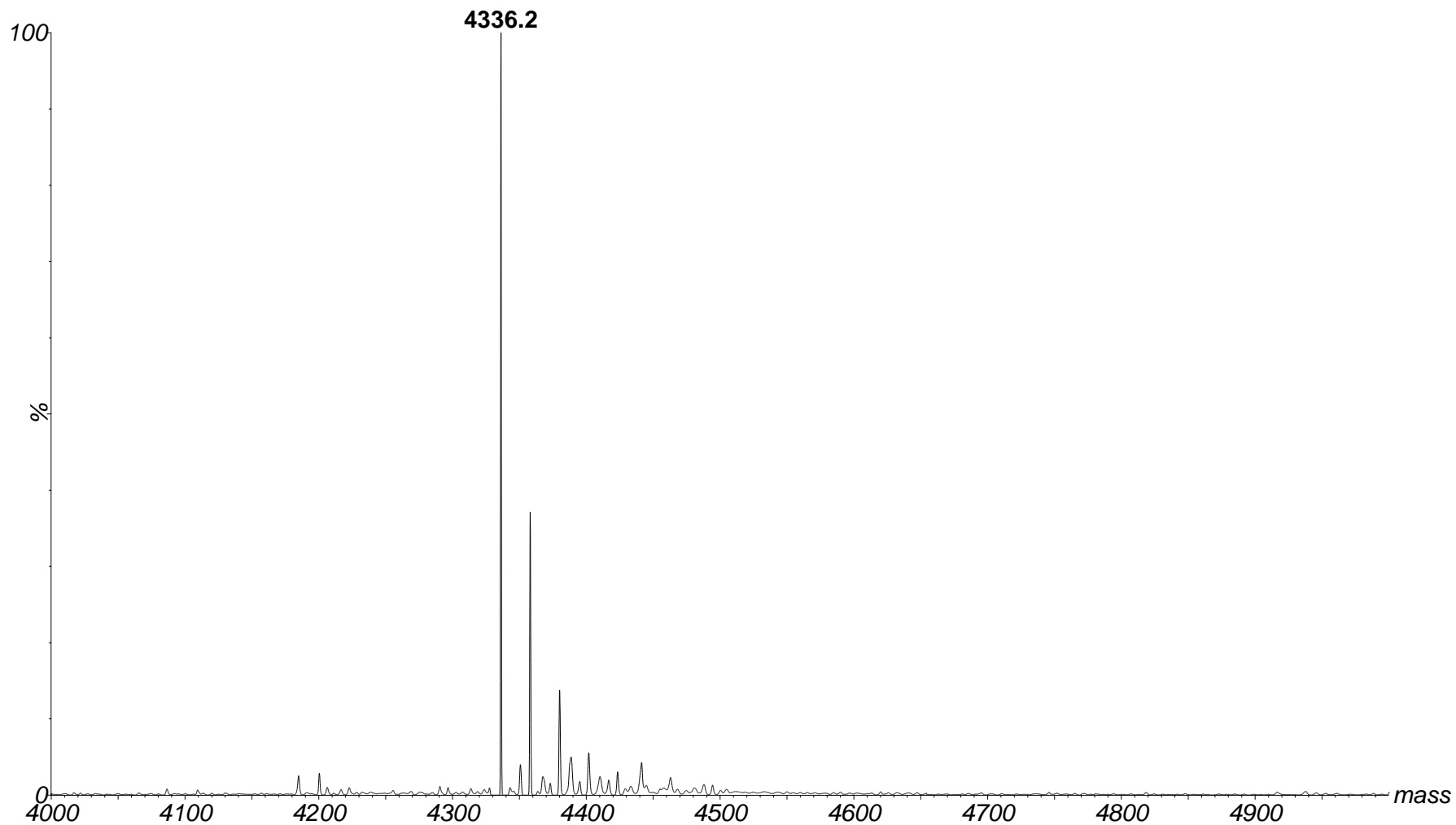
Supplementary Figure 21 - C-18 HPLC profile of digested cross-linked duplex **XLTT4**. The column was eluted with a linear gradient of 0-60% buffer B over 30 min (buffer A: 2% ACN, 50 mM sodium phosphate, pH 5.8 and buffer B: 50 mM sodium phosphate, pH 5.8, 50% acetonitrile).



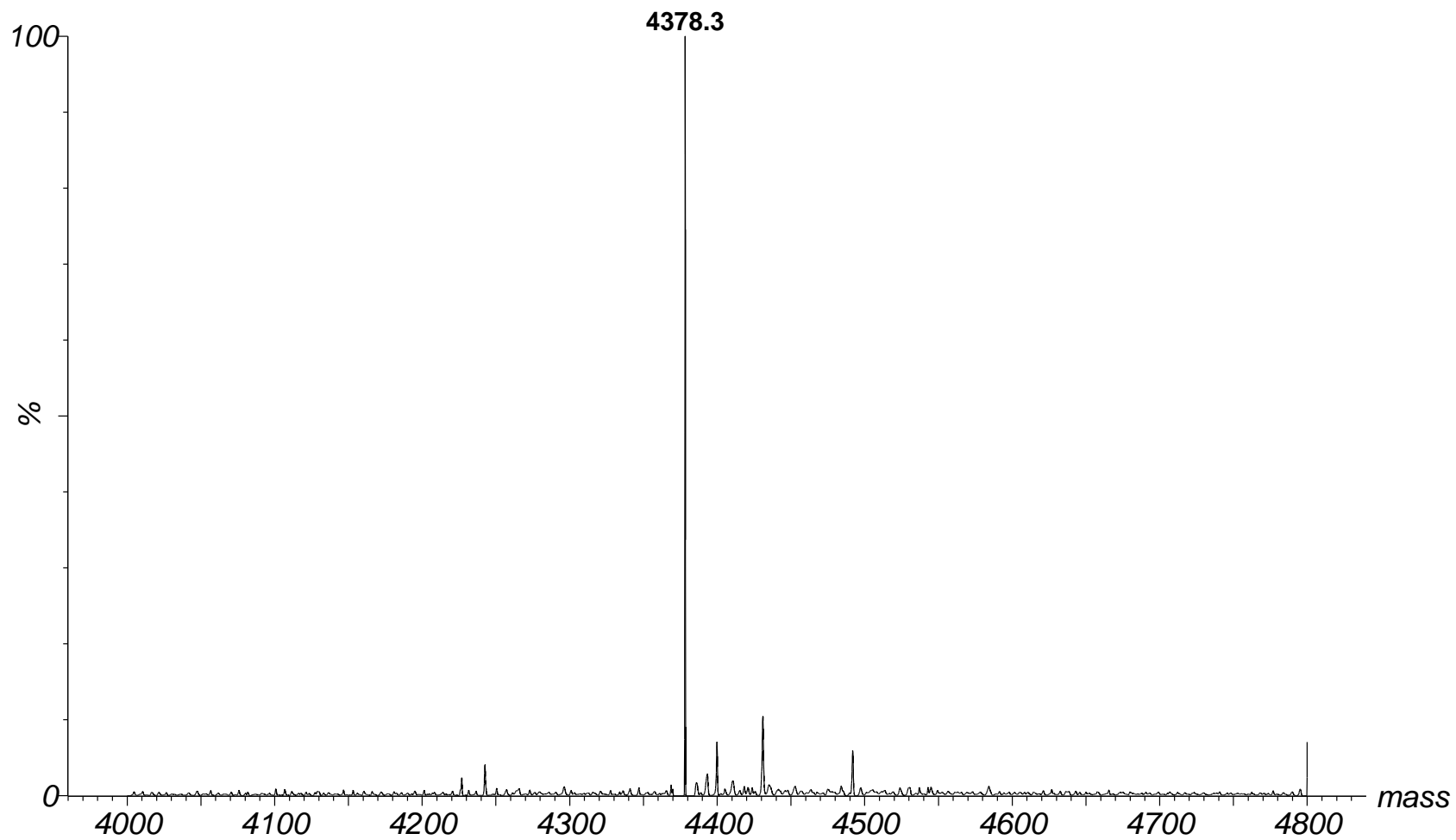
Supplementary Figure 22 - C-18 HPLC profile of digested cross-linked duplex **XLTT7**. The column was eluted with a linear gradient of 0-60% buffer B over 30 min (buffer A: 2% ACN, 50 mM sodium phosphate, pH 5.8 and buffer B: 50 mM sodium phosphate, pH 5.8, 50% acetonitrile).



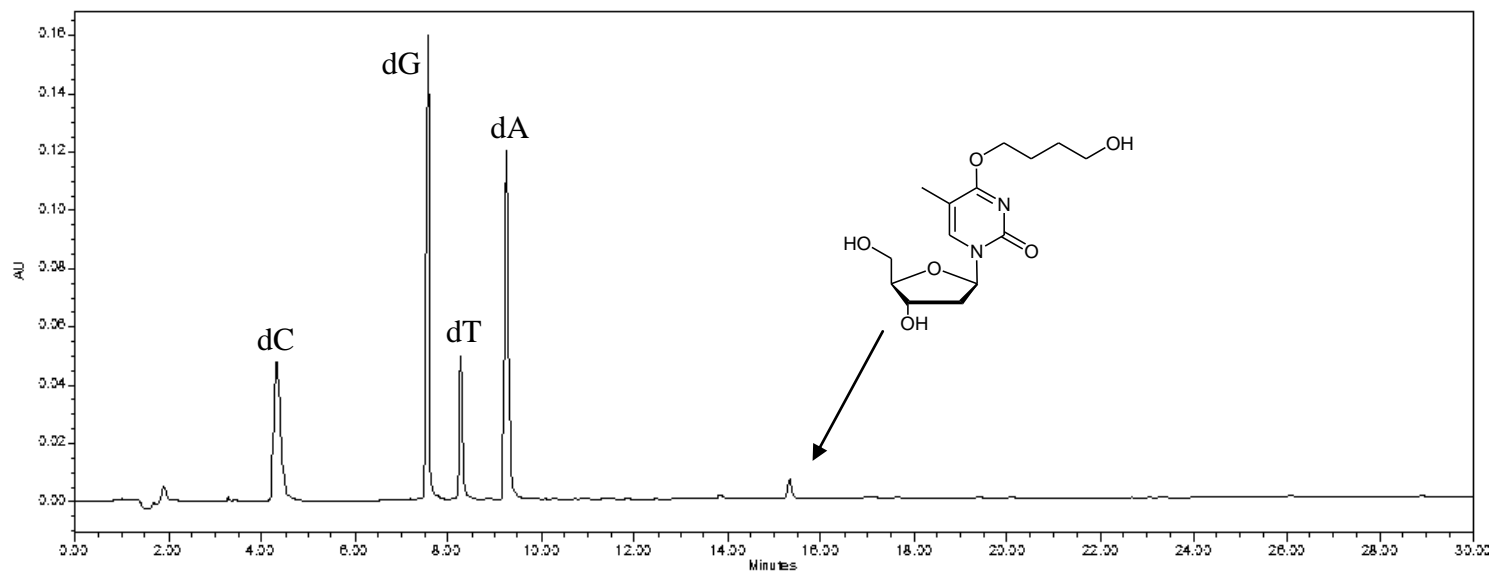
Supplementary Figure 23 - ESI MS of oligonucleotide **S4** containing mono-adduct O^4 -butyl-4-ol-dT.



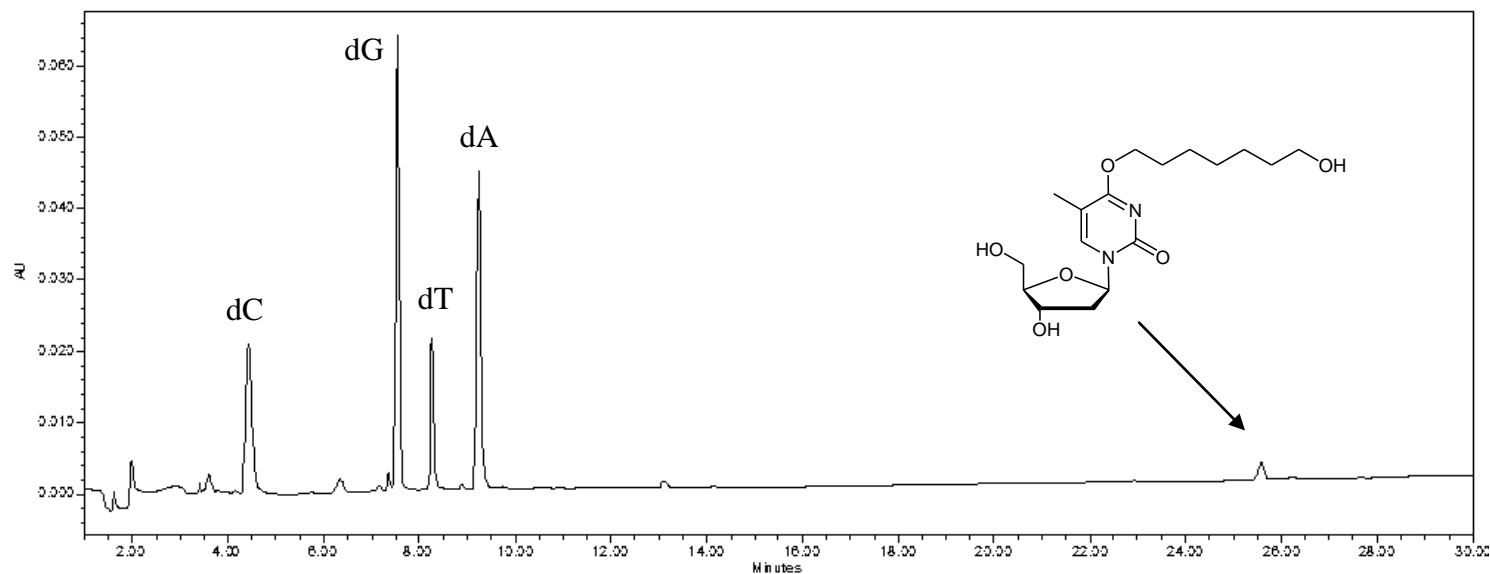
Supplementary Figure 24 - ESI MS of oligonucleotide **S7** containing mono-adduct O^4 -heptyl-7-ol-dT.



Supplementary Figure 25 - C-18 RP HPLC profile of digested oligonucleotide **S4** containing mono-adduct O^4 -butyl-4-ol-dT. The column was eluted with a linear gradient of 0-60% buffer B over 30 min (buffer A: 2% ACN, 50 mM sodium phosphate, pH 5.8 and buffer B: 50 mM sodium phosphate, pH 5.8, 50% acetonitrile).

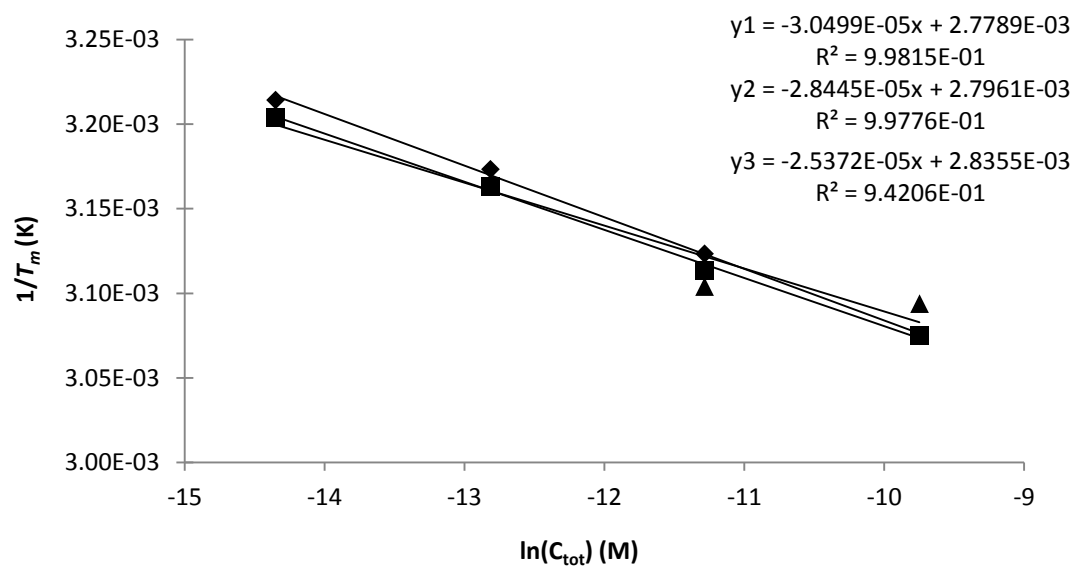


Supplementary Figure 26 - C-18 RP HPLC profile of digested oligonucleotide **S7** containing mono-adduct O^4 -heptyl-7-ol-dT. The column was eluted with a linear gradient of 0-60% buffer B over 30 min (buffer A: 2% ACN, 50 mM sodium phosphate, pH 5.8 and buffer B: 50 mM sodium phosphate, pH 5.8, 50% acetonitrile).

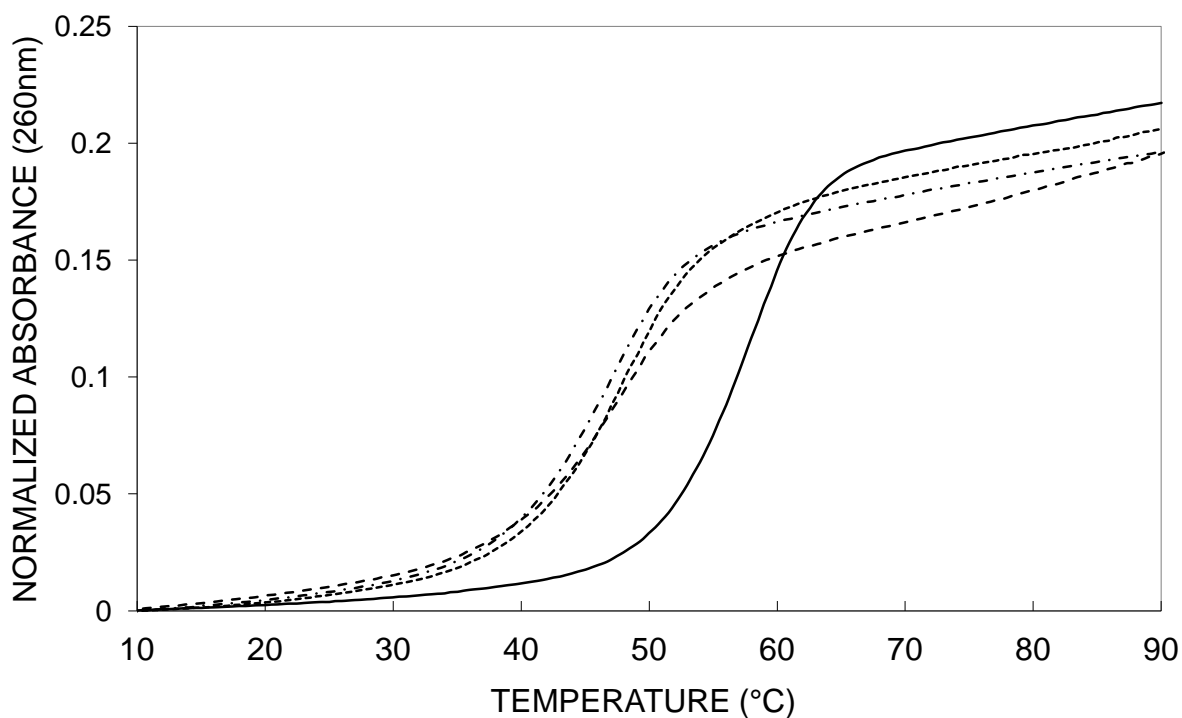


Supplementary Figure 27 - Thermodynamic data for the non-crosslinked control, **XLTT4** and **XLTT7** DNA

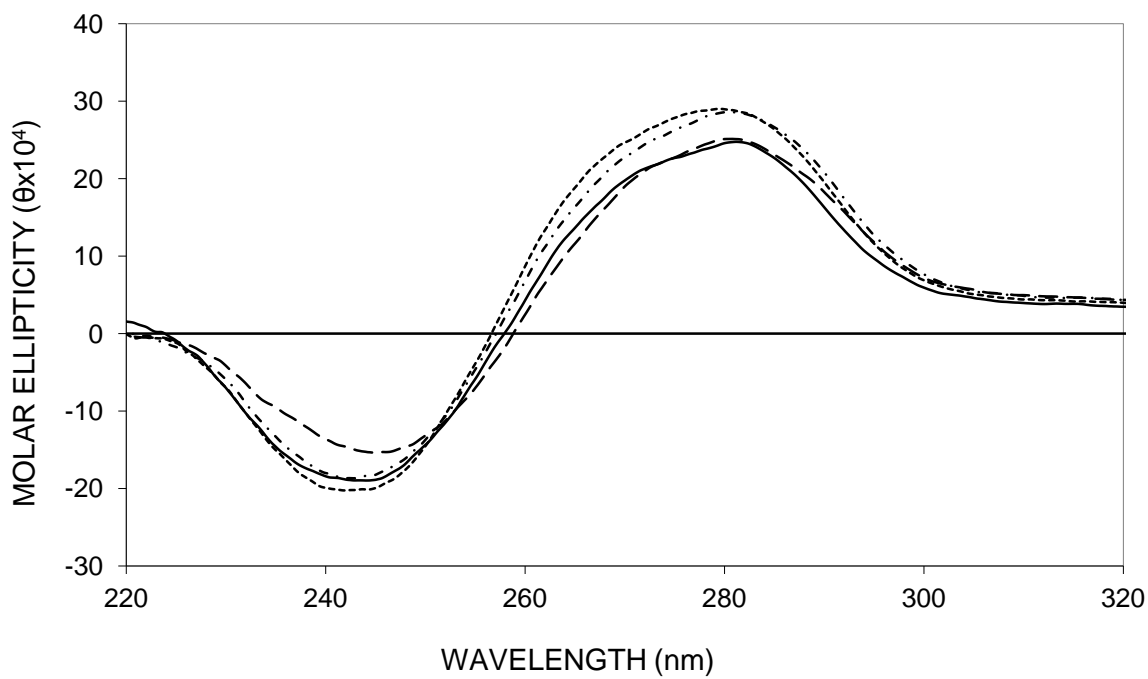
Trial	Control (T-A)				XLTT4		XLTT7	
	0.585 uM	2.719 uM	12.57 uM	58.46 uM	0.585 uM	2.95 uM	0.585 uM	2.95 uM
1	38.0°C	42.0°C	47.0°C	52.0°C	66.5°C	66.0°C	52.0°C	52.0°C
2	38.0°C	43.0°C	48.0°C	52.0°C	65.0°C	67.0°C	53.5°C	51.0°C
3	39.0°C	43.0°C	49.0°C	50.0°C	66.0°C	66.0°C	51.0°C	53.0°C
Avg	38.3°C	42.7°C	48.0°C	51.3°C	65.8°C	66.3°C	52.2°C	52.0°C
StdDev	0.5°C	0.5°C	0.8°C	0.9°C	0.6°C	0.5°C	1.0°C	0.8°C



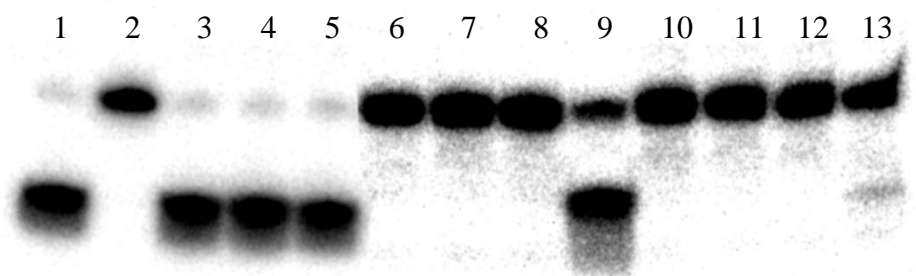
Supplementary Figure 28 - T_m curves of various O^4 -alkyl-dT mono-adducts in DNA duplexes 5' GGCTXGATCACCCAG 3' / 5' CTGGTGATCCAGCC 3' where X is dT (—), O^4 -MedT (.....), O^4 -butyl-4-ol-dT for **S4** (-•-) and O^4 -heptyl-7-ol-dT for **S7** (- - -). Solutions containing a total strand concentration of 2.8 μ M for the 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.



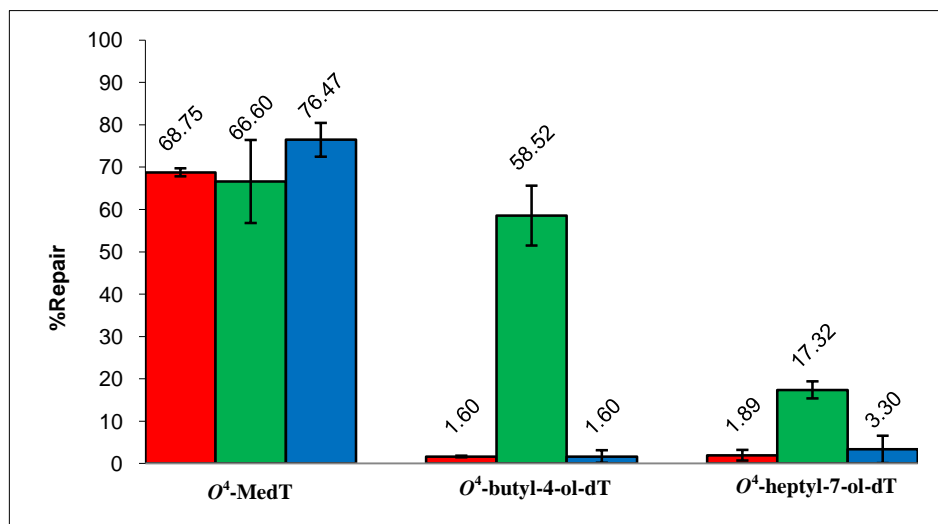
Supplementary Figure 29 - CD profiles of various O^4 -alkyl-dT mono-adducts in DNA duplexes 5' GGCTXGATCACCCAG 3' / 5' CTGGTGATCCAGCC 3' where X is dT (—), O^4 -MedT (.....), O^4 -butyl-4-ol-dT for **S4** (-•-) and O^4 -heptyl-7-ol-dT for **S7** (- - -). Solutions containing a total strand concentration of 2.8 μ M for the duplexes in 90 mM sodium chloride, pH = 7.0, 10 mM sodium phosphate, and 1 mM EDTA buffer.



Supplementary Figure 30 - Repair gel of O^4 MeT, **S4** and **S7** DNA by hAGT, Ada-C and OGT. Denaturing gel of the repair of 2 pmol of DNA by 10 pmol AGT. Lane 1, Control; lane 2, O^4 MeT DNA; lane 3, O^4 MeT DNA + hAGT; lane 4, O^4 MeT DNA + Ada-C, lane 5, O^4 MeT DNA + OGT; lane 6, **S4** DNA; lane 7, **S4** DNA + hAGT; lane 8, **S4** DNA + Ada-C, lane 9, **S4** DNA + OGT; lane 10, **S7** DNA; lane 11, **S7** DNA + hAGT; lane 12, **S7** DNA + Ada-C, lane 13, **S7** DNA + OGT.



Supplementary Figure 31 - Repair of O^4 -MedT, **S4** and **S7** by hAGT (red), OGT (green) and Ada-C (blue).



Supplementary Table 1 - Nucleoside Ratio of the Various Modified Oligonucleotides as Observed by Snake Venom Digestion using RP-HPLC

Oligomer	Nucleoside	Nucleoside ratios	
		Expected	Observed
XLTT4	dC	4.00	4.00
	dG	4.00	4.24
	dT	6.00	5.86
	dA	6.00	6.22
	dT-butyl-dT	1.00	1.55
XLTT7	dC	4.00	4.00
	dG	4.00	4.18
	dT	6.00	5.84
	dA	6.00	6.34
	dT-heptyl-dT	1.00	1.51
S4	dC	4.00	3.88
	dG	4.00	4.00
	dT	2.00	1.98
	dA	3.00	2.99
	O ⁴ -butyl-4-ol-dT	1.00	1.06
S7	dC	4.00	3.85
	dG	4.00	4.05
	dT	2.00	2.00
	dA	3.00	2.90
	O ⁴ -butyl-7-ol-dT	1.00	1.18

Supplementary Table 2 - K_d and stoichiometry of hAGT binding with **XLTT4**, **XLTT7** and control duplex

DNA Duplex	K_d (μ M)	Stoichiometry
Control	30.57 \pm 3.00	1.94 \pm 0.03
XLTT4	2.45 \pm 0.05	1.90 \pm 0.11
XLTT7	2.58 \pm 0.67	2.09 \pm 0.02

Supplementary Table 3 - K_d of hAGT binding to single stranded 5' CGAAAXTTTCG

X	K_d approximate (μ M)
T	>30
G	1-10
C	30
A	30

Supplementary Table 4 - K_d of hAGT binding to duplexes containing various O^4 -Alkyl-dT mono adducts and O^6 MeG

DNA Duplex	K_d (μ M)
Control	6.90 ± 0.04
O^4 MeT	2.87 ± 0.19
S4 (O^4 -Butyl-4-ol-dT)	3.41 ± 0.28
S7 (O^4 -Heptyl-7-ol-dT)	3.68 ± 0.15
O^6 MeG	2.86 ± 0.16