Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Cationic Modified Nucleic Acids for use in DNA Hairpins and Parallel Triplexes

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Electronic Supplementary Information

pK_a-values of related tertiary amines in H_2O

Amine(s)	Temperature	pK_a^1	pK_a^2
Benzyldimethylamine	25 °C	8.79[a]	-
1,2-Bis(dimetylaminomethyl)benzene	35 °C	10.58[b]	4.97[b]
1,4-Bis(dimetylaminomethyl)benzene	25 °C	9.65[c]	-
N-Benzyl-*N*-methylethanolamine	25 °C	8.41[d]	-
1,4-Dimethylpiperazine	25 °C	7.84[e]	3.45[e]
1-(2-Hydroxyethyl)piperazine	25 °C	8.63[e]	3.60[e]

[a] J. Armstrong and R. B. Barlow, *Br. J. Pharmacol.*, 1976, **57**, 501-516.
[b] J. Hine and W. S. Li, *J. Org. Chem.*, 1975, **40**, 1795-1800.
[c] A. De Roocker and P. De Radzitzky, *Bull. Soc. Chim. Belg.*, 1964, **73**, 181-&.
[d] W. R. Morgan and D. E. Leyden, *J. Am. Chem. Soc.*, 1970, **92**, 4527-4531.
[e] F. Khalili, A. Henni and A. L. L. East, *J. Chem. Eng. Data*, 2009, **54**, 2914-2917.

Gel electrophoresis study with ON2 and increasing concentration of NaCl

			Lane
ON-ref	3′-TGT CAG ACC GGC	100mM NaCl	**A**
D1	3′-TGT CAG ACC GGC 5'-ACA GTC TGG CCG	100mM NaCl	**B**
ON2	3'-GCA CGT-**XX**-ACG TGC	10mM NaCl	**C**
ON2	3'-GCA CGT-**XX**-ACG TGC	100mM NaCl	**D**
ON2	3'-GCA CGT-**XX**-ACG TGC	1000mM NaCl	**E**
D1	3′-TGT CAG ACC GGC 5'-ACA GTC TGG CCG	100mM NaCl	**H**

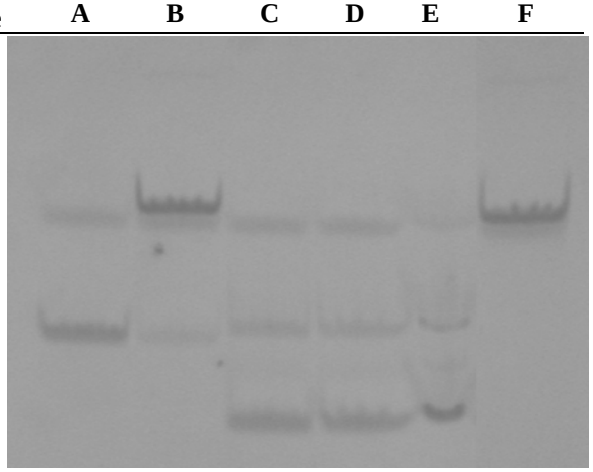


Fig. 1. Nondenaturing 20 % PAGE; 25 µM of **ON-ref**, **D1** and **ON2** in TB-buffer, pH 8.0, 4 °C.

Gel electrophoresis study with ON1 and Mg^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}

			Lane
ON-ref	3′-TGT CAG ACC GGC	10mM $MgCl_2$	**A**
D1	3′-TGT CAG ACC GGC 5'-ACA GTC TGG CCG	10mM $MgCl_2$	**B**
ON1	3'-GCA CGT-**X**-ACG TGC	10mM $MgCl_2$	**C**
ON1	3'-GCA CGT-**X**-ACG TGC	10mM $MgCl_2$	**D**
ON1	3'-GCA CGT-**X**-ACG TGC	250nM $ZnCl_2$	**E**
ON1	3'-GCA CGT-**X**-ACG TGC	250nM $NiCl_2$	**F**
ON1	3'-GCA CGT-**X**-ACG TGC	250nM $CuSO_4$	**G**
ON1	3'-GCA CGT-**X**-ACG TGC	250nM $MnSO_4$	**H**
D1	3′-TGT CAG ACC GGC 5'-ACA GTC TGG CCG	10mM $MgCl_2$	**I**

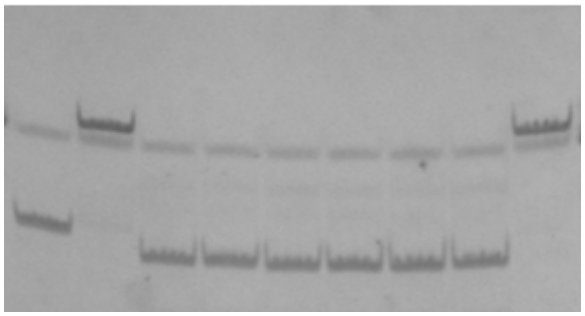


Fig. 2. Nondenaturing 20 % PAGE; 25 µM of **ON-ref**, **D1** and **ON1** in TB-buffer, 100 mM NaCl, pH 8.0, 4 °C.

Gel electrophoresis study with ON2 and Mg^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}

			Lane
ON-ref	3′-TGT CAG ACC GGC	10mM $MgCl_2$	**A**
D1	3′-TGT CAG ACC GGC 5'-ACA GTC TGG CCG	10mM $MgCl_2$	**B**
ON2	3'-GCA CGT-**XX**-ACG TGC	10mM $MgCl_2$	**C**
ON2	3'-GCA CGT-**XX**-ACG TGC	10mM $MgCl_2$	**D**
ON2	3'-GCA CGT-**XX**-ACG TGC	250nM $ZnCl_2$	**E**
ON2	3'-GCA CGT-**XX**-ACG TGC	250nM $NiCl_2$	**F**
ON2	3'-GCA CGT-**XX**-ACG TGC	250nM $CuSO_4$	**G**
ON2	3'-GCA CGT-**XX**-ACG TGC	250nM $MnSO_4$	**H**
D1	3′-TGT CAG ACC GGC 5'-ACA GTC TGG CCG	10mM $MgCl_2$	**I**

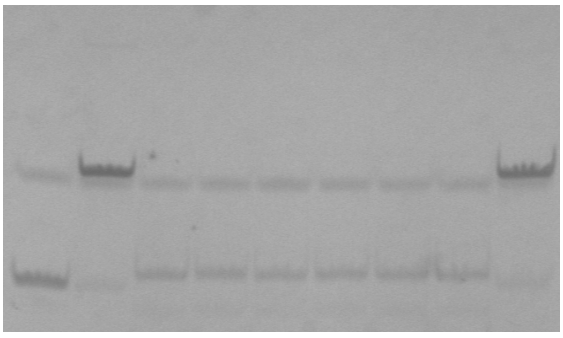


Fig. 3. Nondenaturing 20 % PAGE; 25 µM of **ON-ref**, **D1** and **ON2** in TB-buffer, 100 mM NaCl, pH 8.0, 4 °C.

Gel electrophoresis study with ON3 and Mg^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}

			Lane
ON-ref	3′-TGT CAG ACC GGC	10mM $MgCl_2$	**A**
D1	3′-TGT CAG ACC GGC 5'-ACA GTC TGG CCG	10mM $MgCl_2$	**B**
ON3	3'-GCA CGT-**Y**-ACG TGC	10mM $MgCl_2$	**C**
ON3	3'-GCA CGT-**Y**-ACG TGC	10mM $MgCl_2$	**D**
ON3	3'-GCA CGT-**Y**-ACG TGC	250nM $ZnCl_2$	**E**
ON3	3'-GCA CGT-**Y**-ACG TGC	250nM $NiCl_2$	**F**
ON3	3'-GCA CGT-**Y**-ACG TGC	250nM $CuSO_4$	**G**
ON3	3'-GCA CGT-**Y**-ACG TGC	250nM $MnSO_4$	**H**
D1	3′-TGT CAG ACC GGC 5'-ACA GTC TGG CCG	10mM $MgCl_2$	**I**

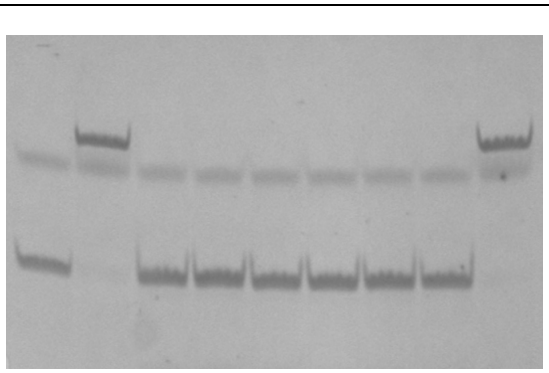


Fig. 4. Nondenaturing 20 % PAGE; 25 µM of **ON-ref**, **D1** and **ON3** in TB-buffer, 100 mM NaCl, pH 8.0, 4 °C.

Thermal denaturation study with Cu^{2+} at pH 5.0 and pH 8.0

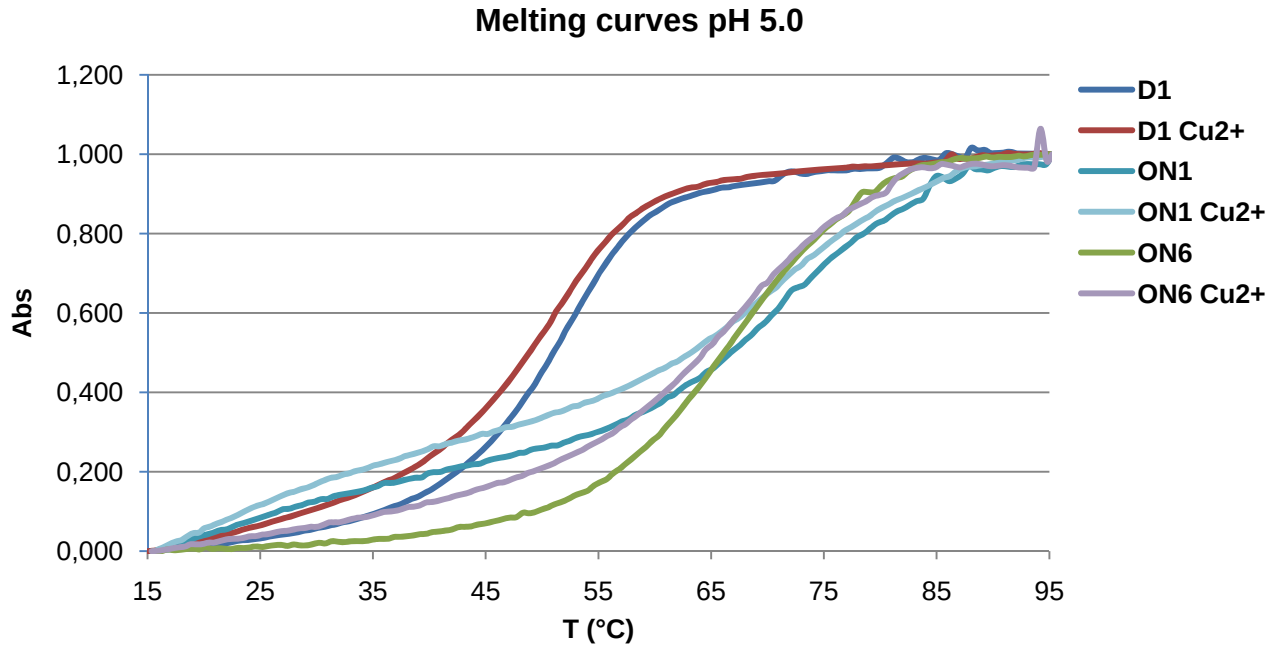


Fig. 5. Melting curves of thermal denaturation experiments of duplex **D1** and hairpin **ON1** and **ON6** recorded with 5µM of each ON in 10mM Na_2HPO_4/NaH_2PO_4 at pH5.0 and 100mM NaCl and with and without 5µM CuSO4 at 260nm versus temperature, with a heating of 1.0 ºC/min.

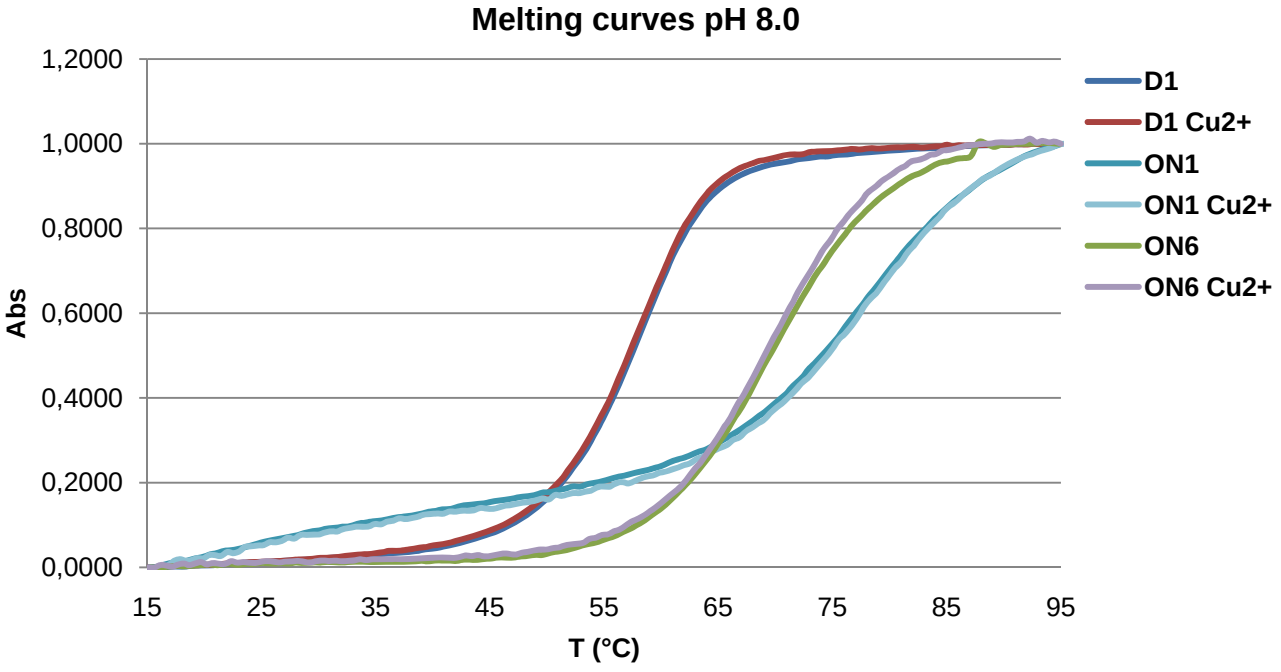


Fig. 6. Melting curves of thermal denaturation experiments of duplex **D1** and hairpin **ON1** and **ON6** recorded with 5µM of each ON in 10mM Na_2HPO_4/NaH_2PO_4 at pH8.0 and 100mM NaCl and with and without 5µM CuSO4 at 260nm versus temperature, with a heating of 1.0 ºC/min.

Thermal denaturation study with increasing ON-concentration

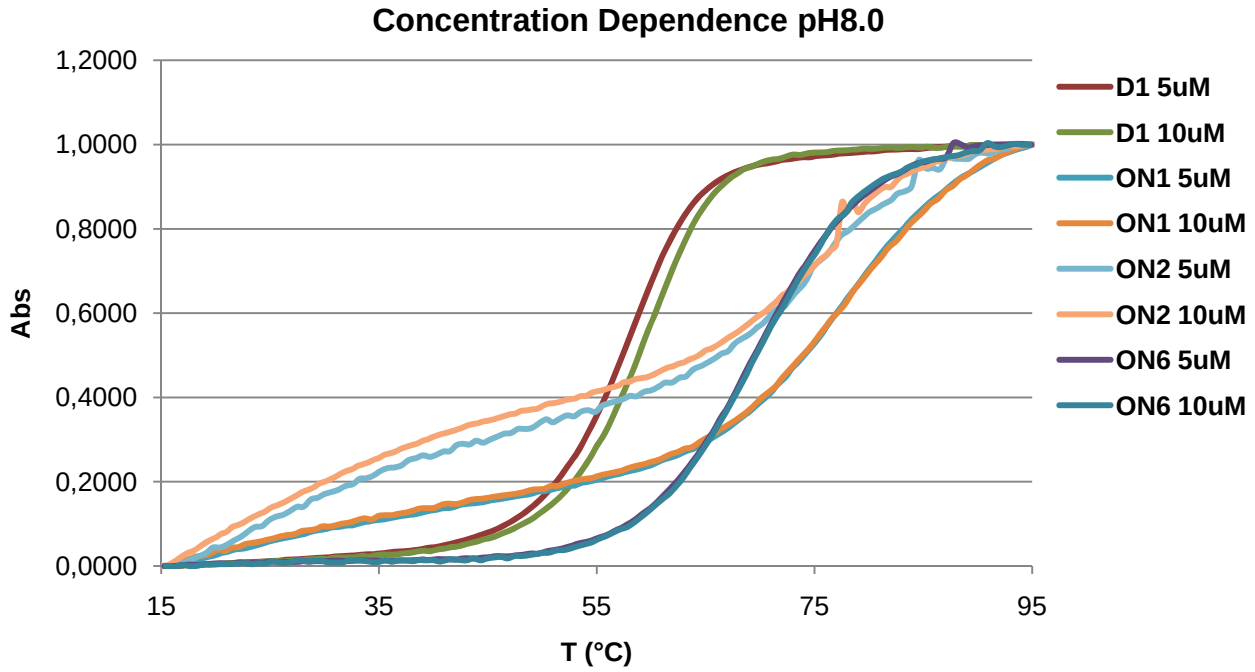


Fig. 7. Melting curves of thermal denaturation experiments of duplex **D1** and hairpin **ON1-2** and **ON6** recorded with 5µM and 10 µM of each ON in 10mM Na_2HPO_4/NaH_2PO_4 at pH8.0 and 100mM NaCl at 260nm versus temperature, with a heating of 1.0 ºC/min.

Thermal denaturation study with X and Y as bulge insertion in parallel triplex

		5′-GAAGCTCTTTTCTCTTTT 3′-CTTCGAGAAAAGAGAAAA	
Entry	Sequence	pH 5.0[a]	ΔT_m
ONS1	3′-TCTTTT-CTCTTTT	56.0*	*ref.*
ONS2	3′-TCTTTT CTCTTTT └**X**┘	36.5	-19.5
ONS3	3′-TCTTTT CTCTTTT └**Y**┘	27.0	-29.0
ONS4	3′-TCTTTT CTCTTTT └C┘	43.0	-13.0

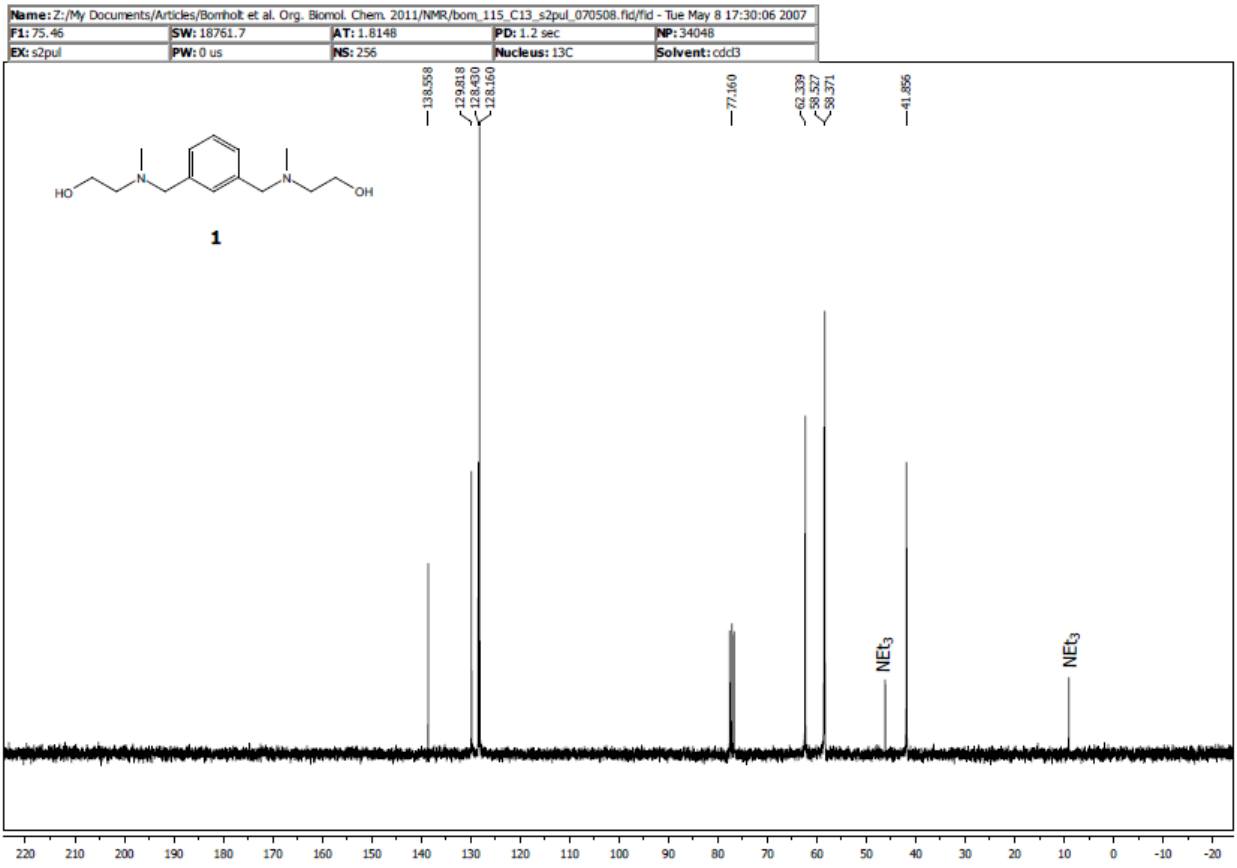
Table 1. a) C = 1.5 µM of **ONS1**-**4** and 1.0 µM of each strand of dsDNA in 20 mM sodium cacodylate, 100 mM NaCl, 10 mM $MgCl_2$, pH 5.0; duplex T_m = 54.5˚C; Target regions are underlined for TFO hybridization. *Triplex-duplex melting overlap.

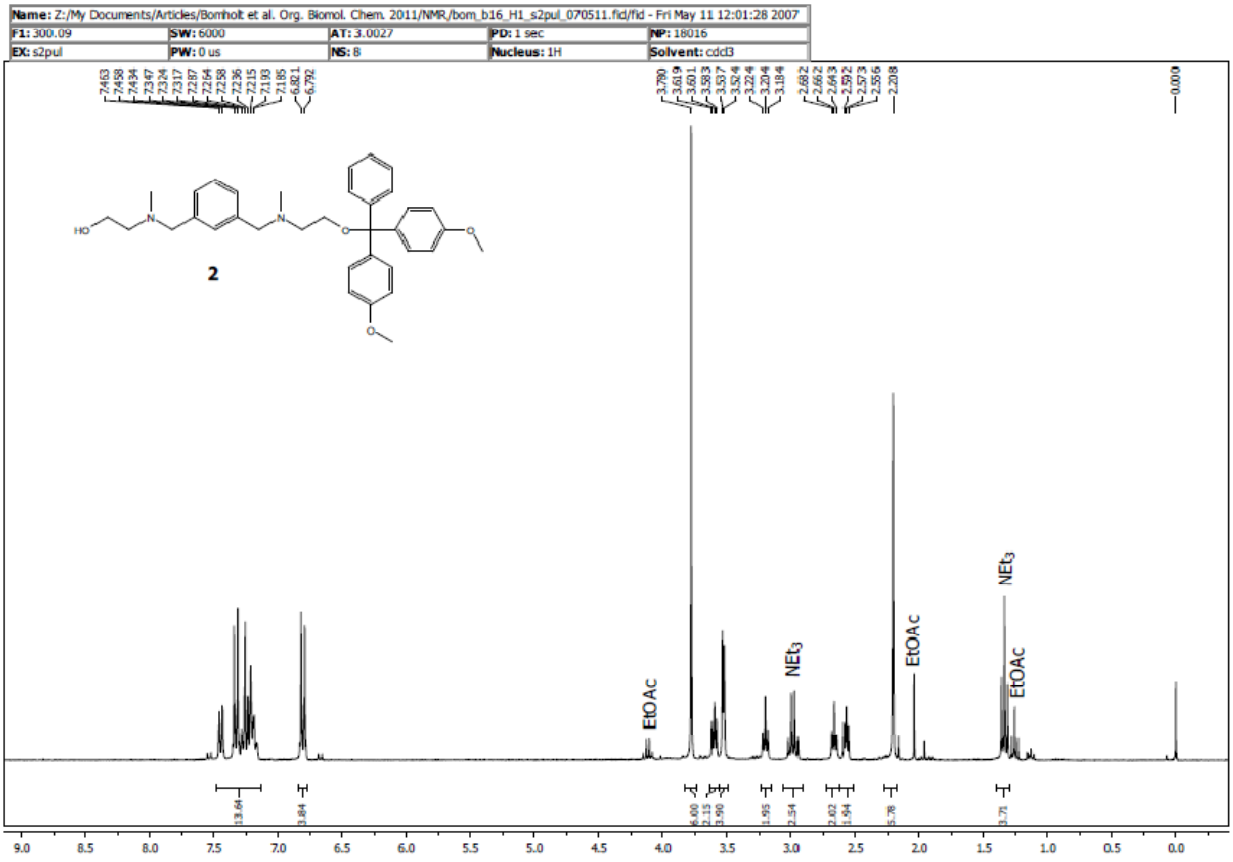
Thermal denaturation study with X and Y as replacement of thymine in parallel triplex

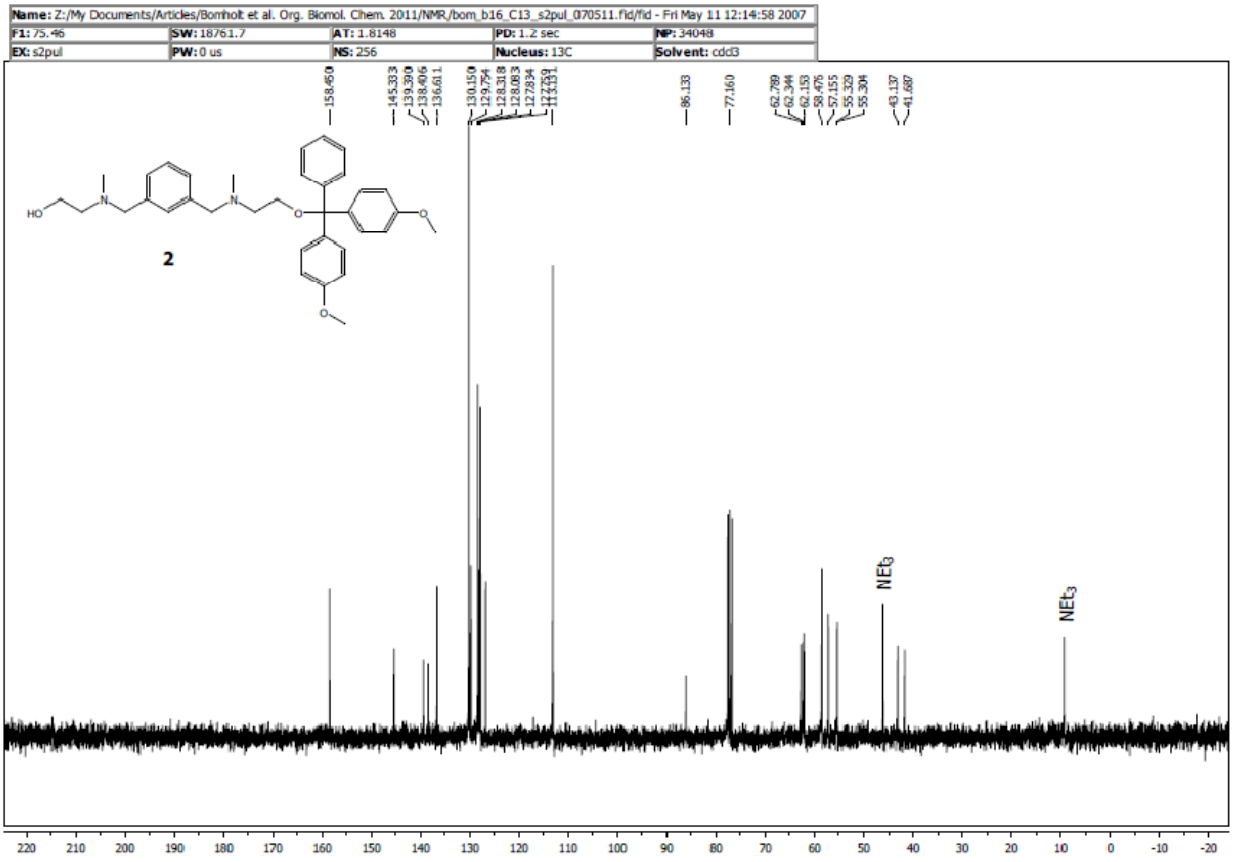
		5′-GAAGCTCTTTTGCTCTTTT 3′-CTTCGAGAAAA***C***GAGAAAA			
Entry	Sequence	pH 5.0[a]	ΔT_m	pH 6.0[a]	ΔT_m
ONS1	3′-TCT TTT **T**CT CTT TT	35.5	*ref.*	20.5	*ref.*
ONS2	3′-TCT TTT **X**CT CTT TT	31.5	-4.0	15.5	-5.0
ONS3	3′-TCT TTT **Y**CT CTT TT	24.5	-11.0	9.0	-11.5

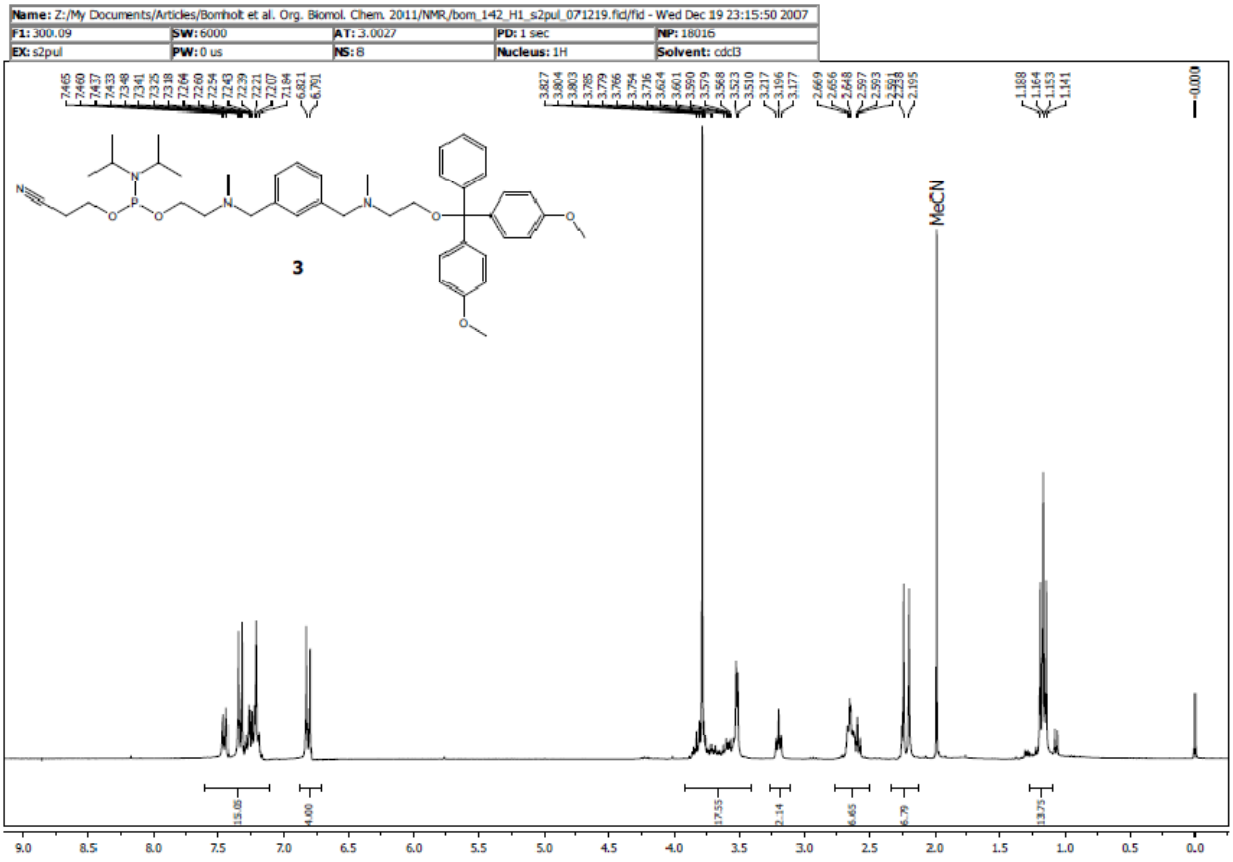
Table 2. a) C = 1.5 µM of **ONS1-3** and 1.0 µM of each strand of dsDNA in 20 mM sodium cacodylate, 100 mM NaCl, 10 mM $MgCl_2$, pH 5.0 and 6.0; duplex T_m = 60.0˚C (pH 5.0), 61.0˚C (pH 6.0); target regions are underlined for TFO hybridization.

NMR spectra of compound 1-6





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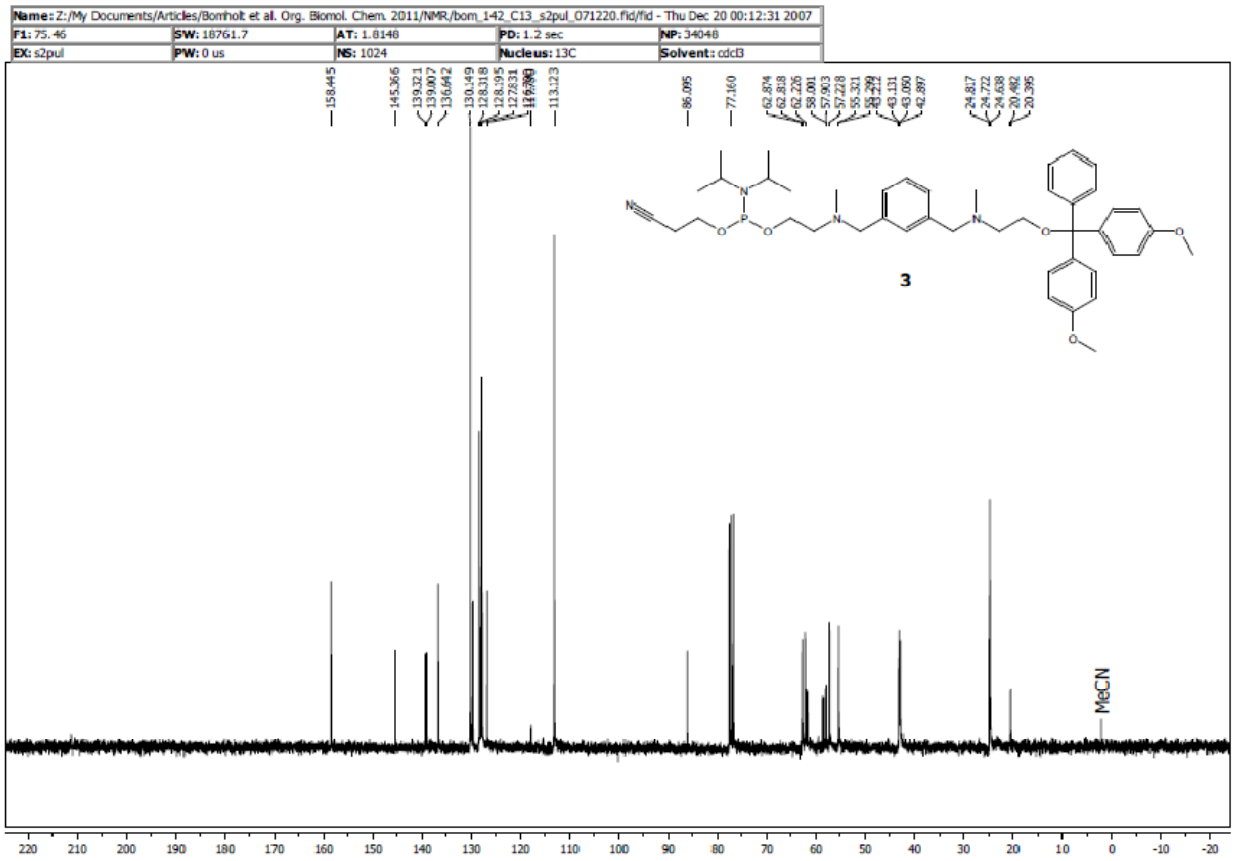
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Solvent: cdcl3
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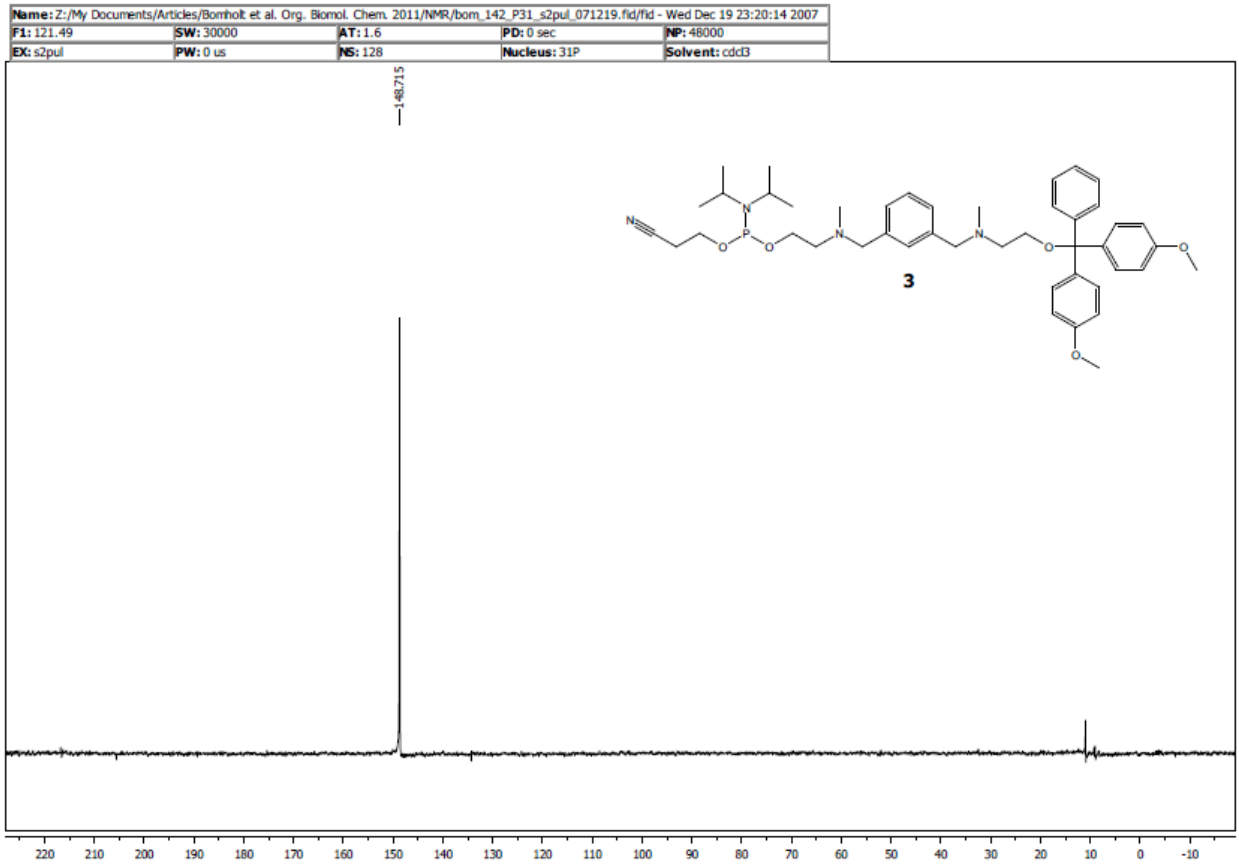
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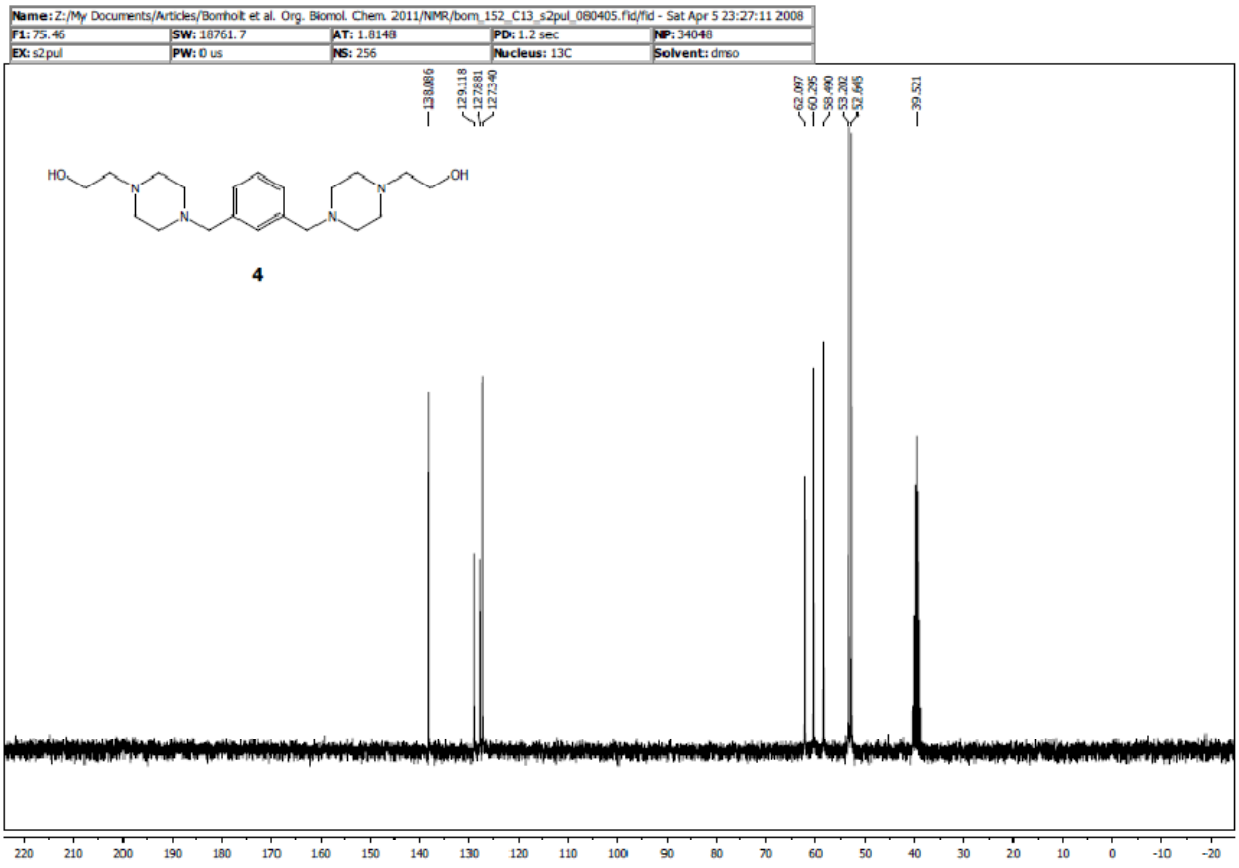
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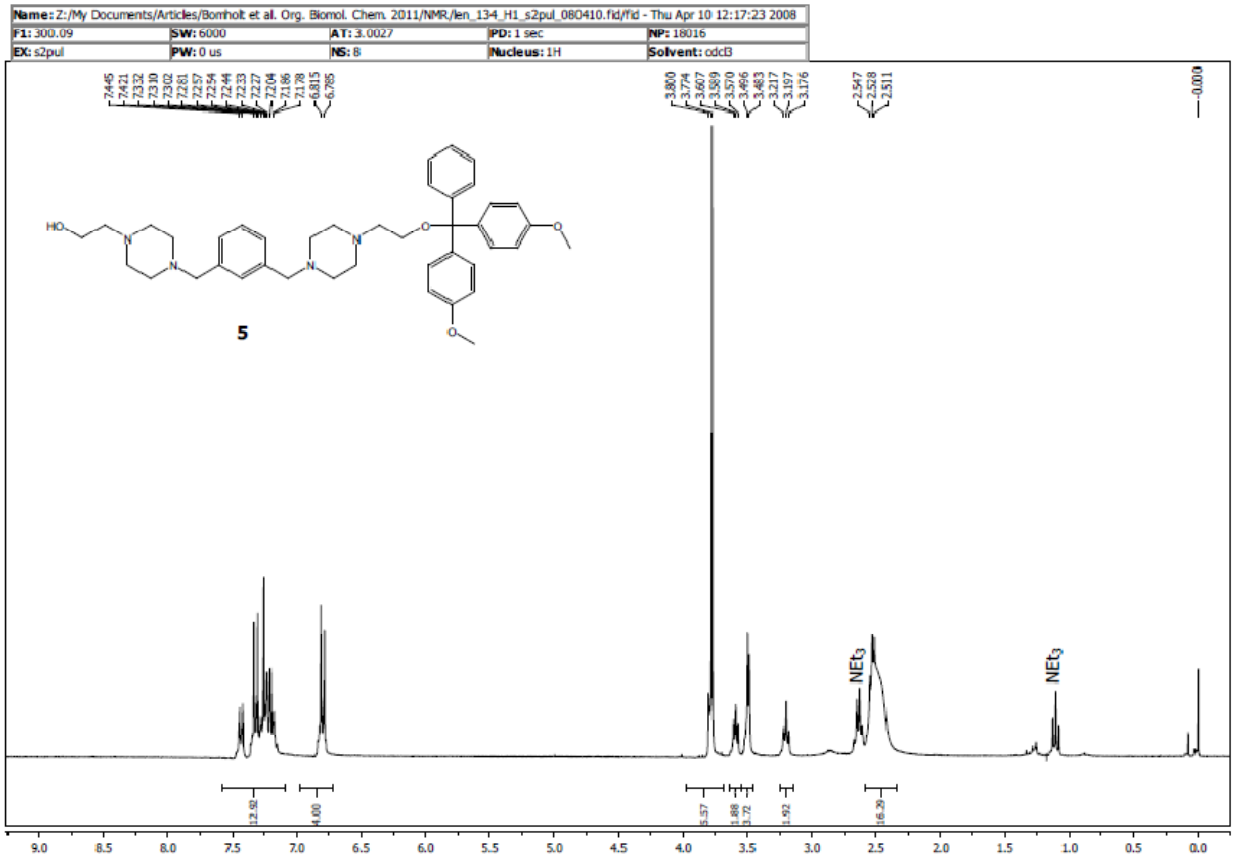
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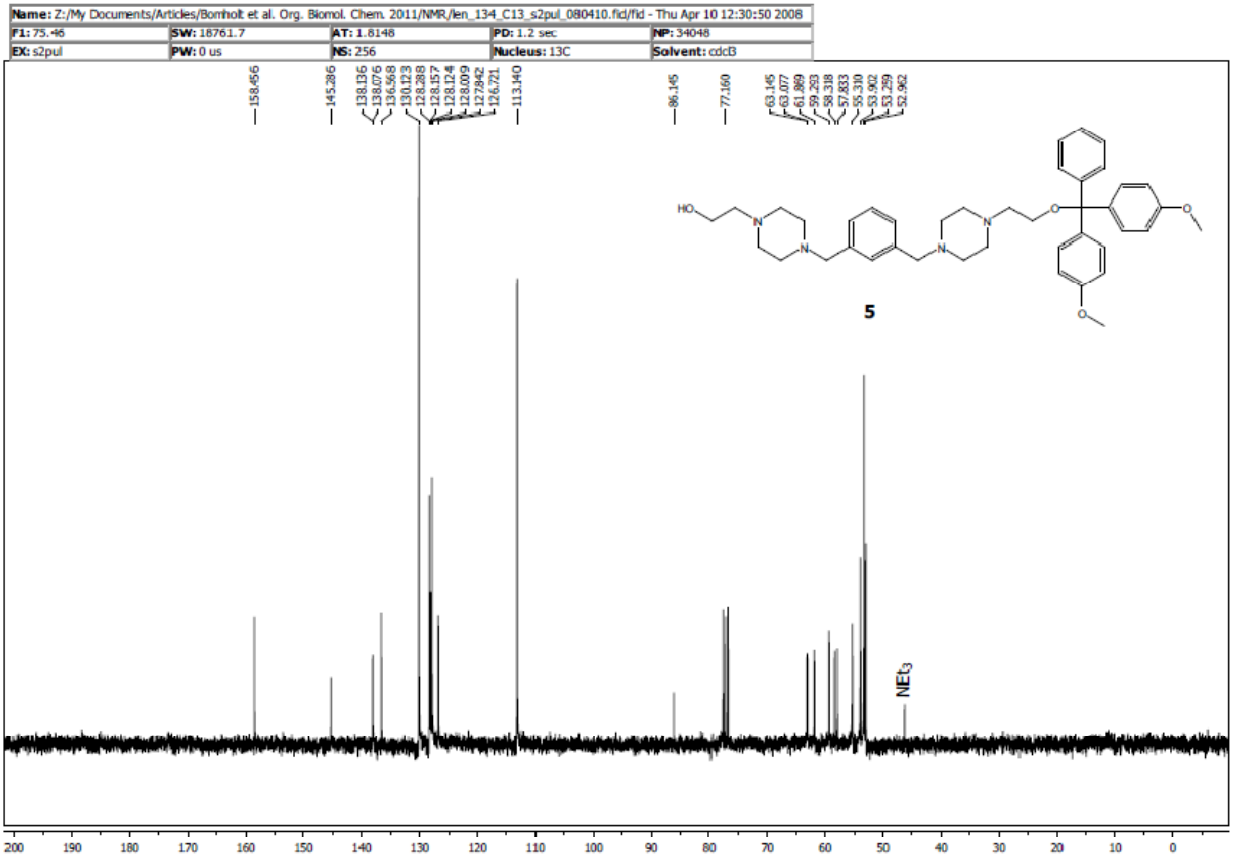
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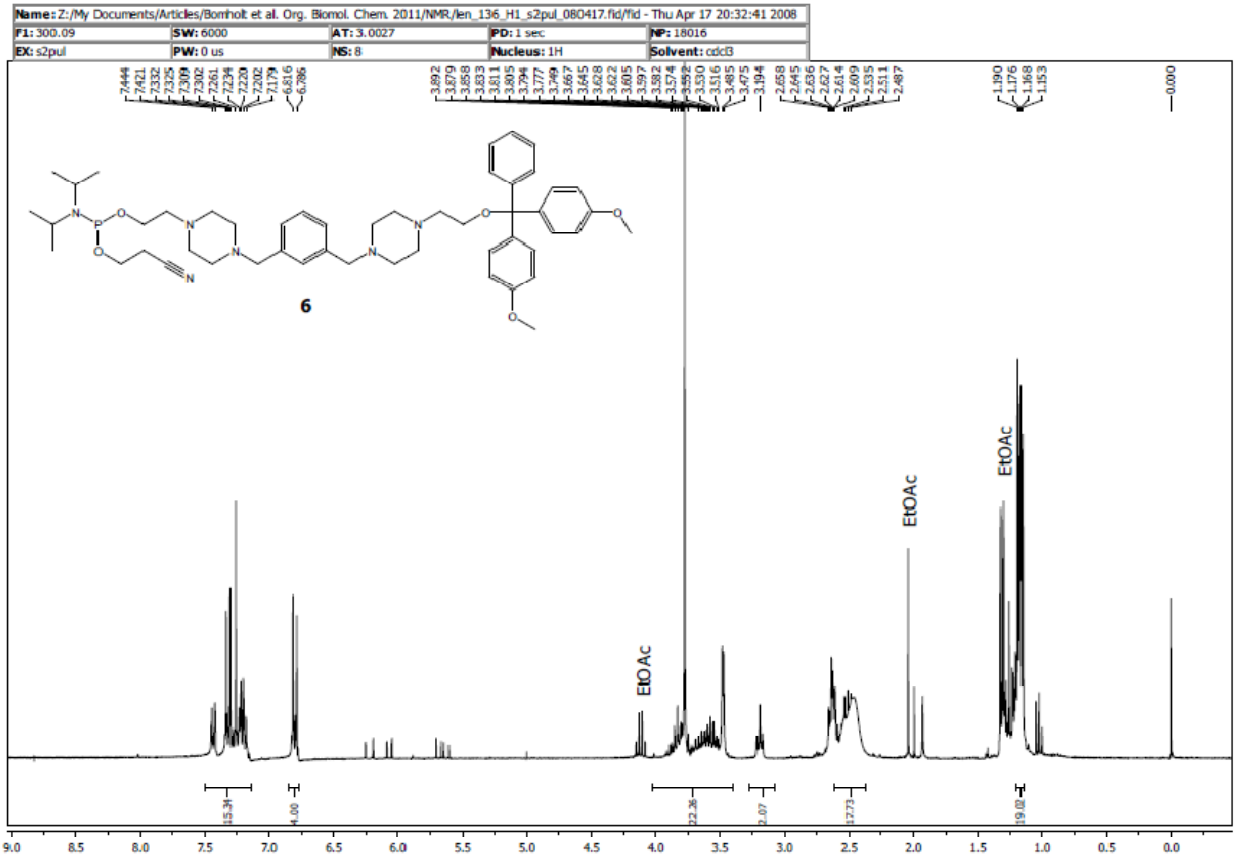
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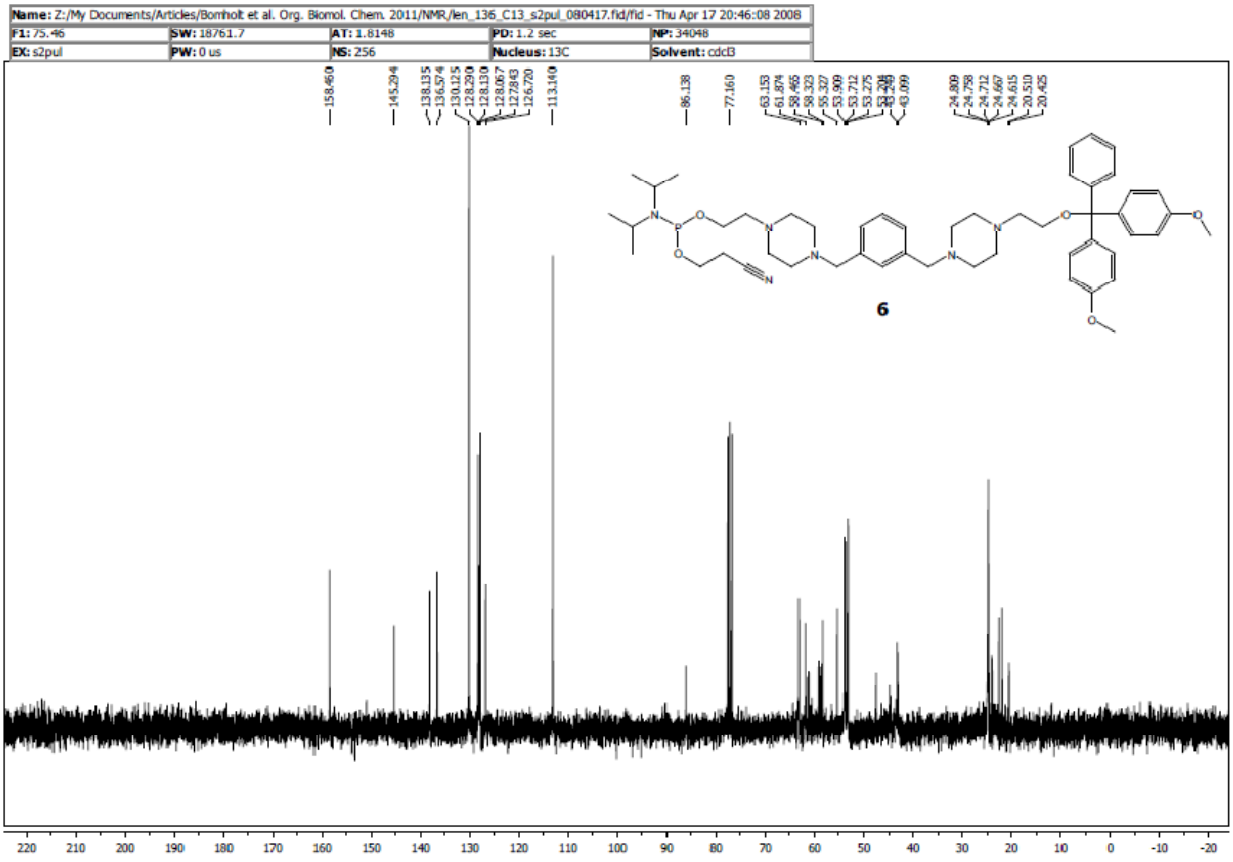

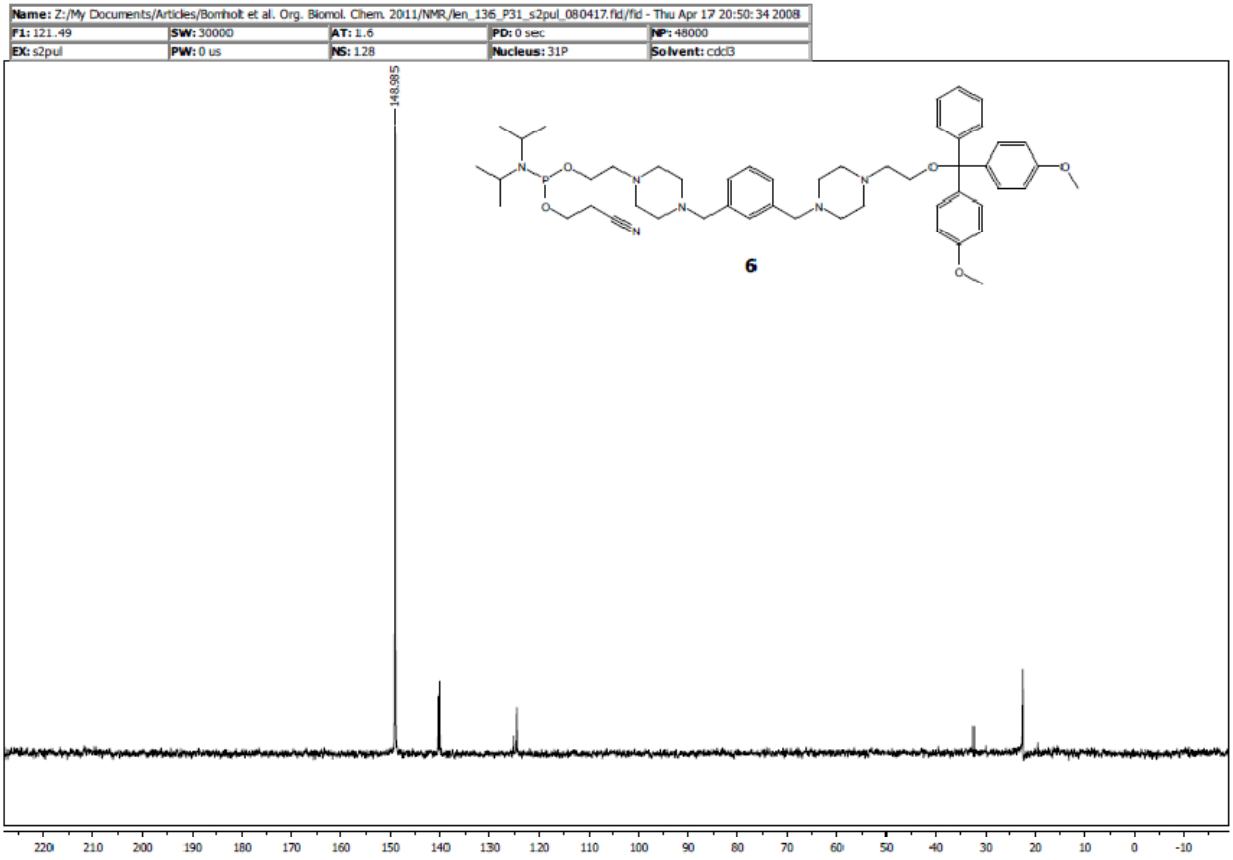
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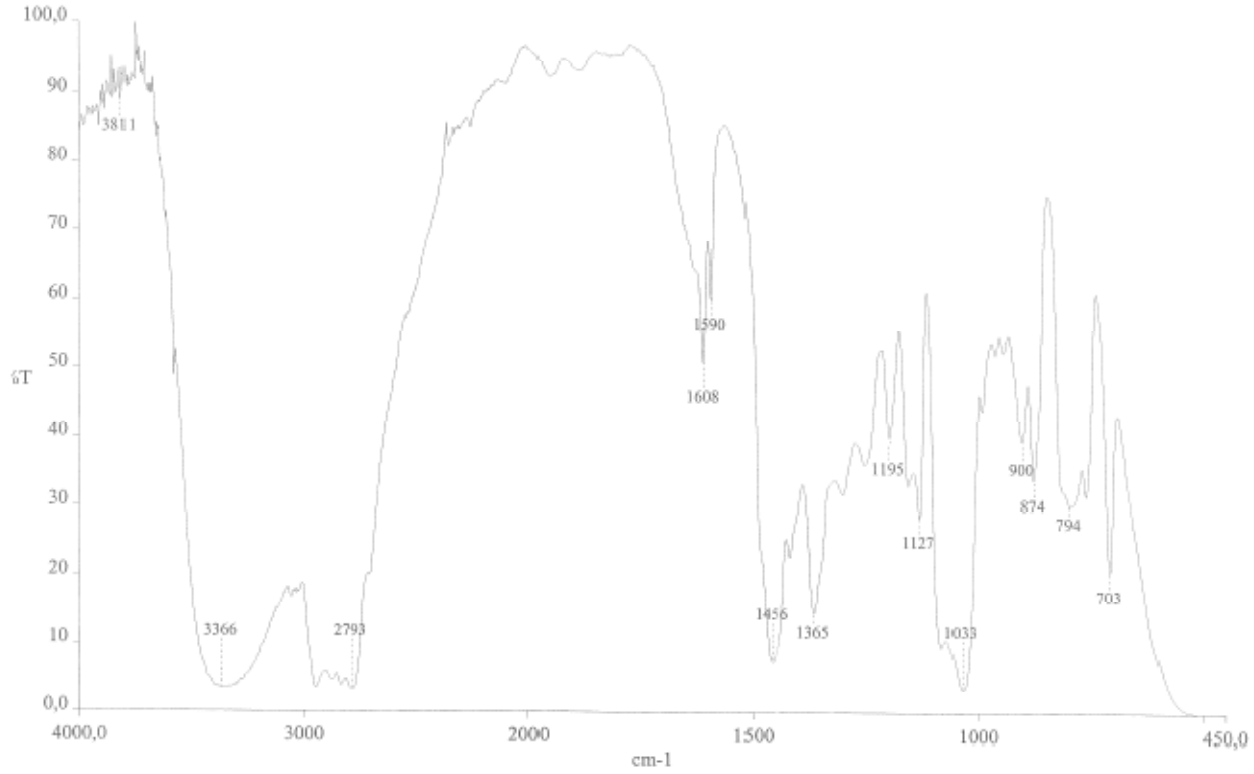
IR spectra of compound 1-6

Compound 1



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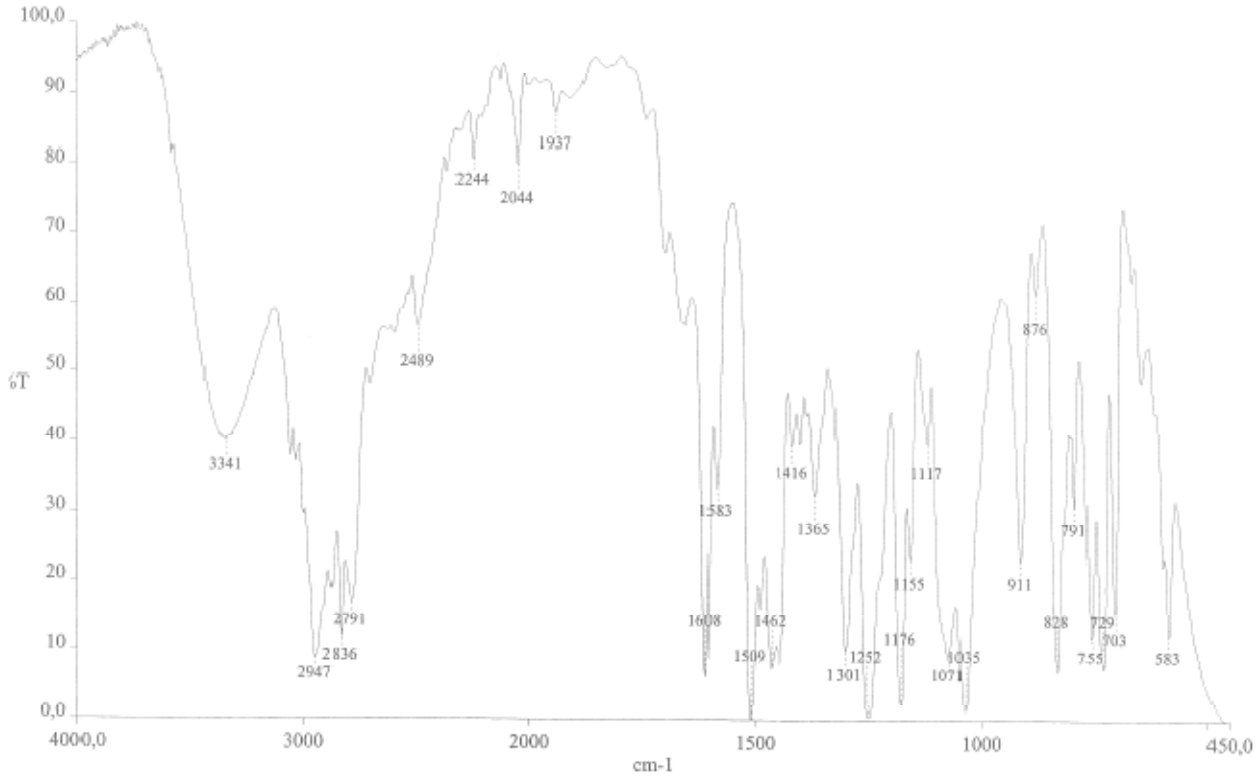

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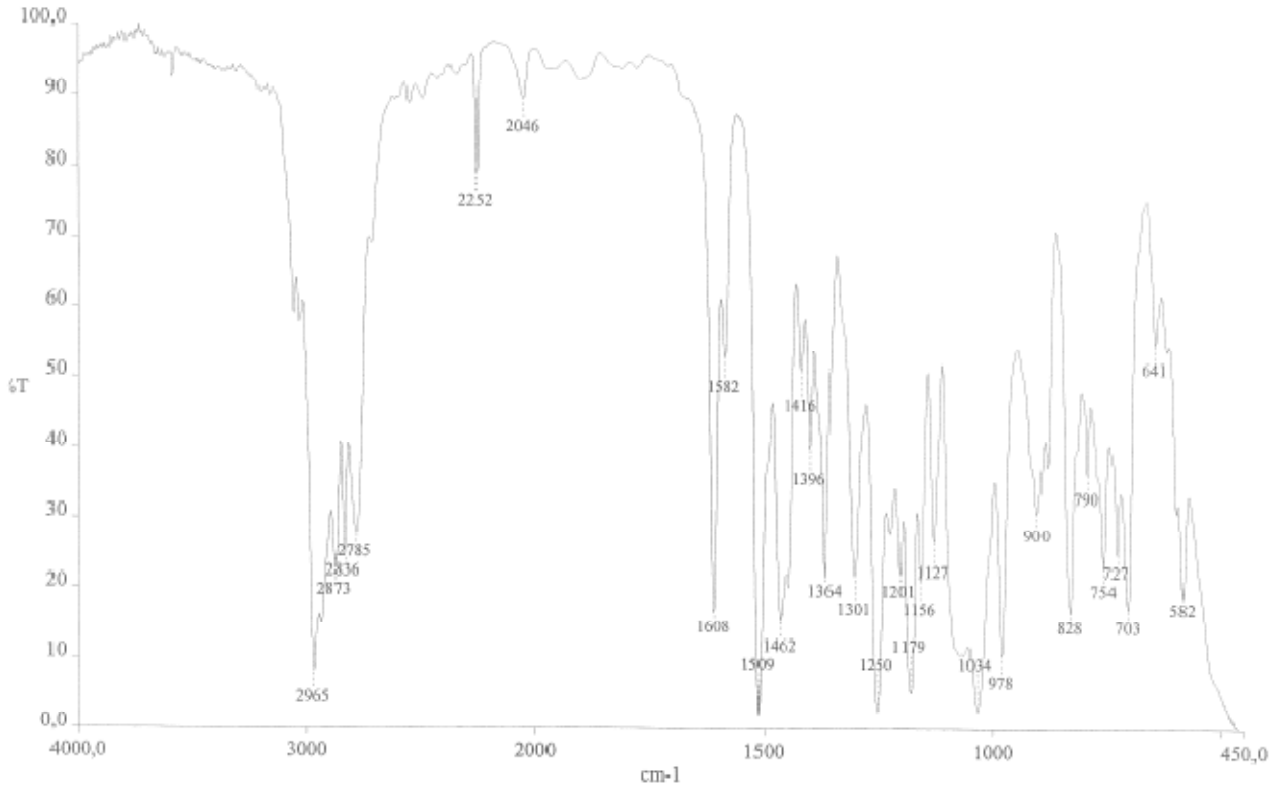

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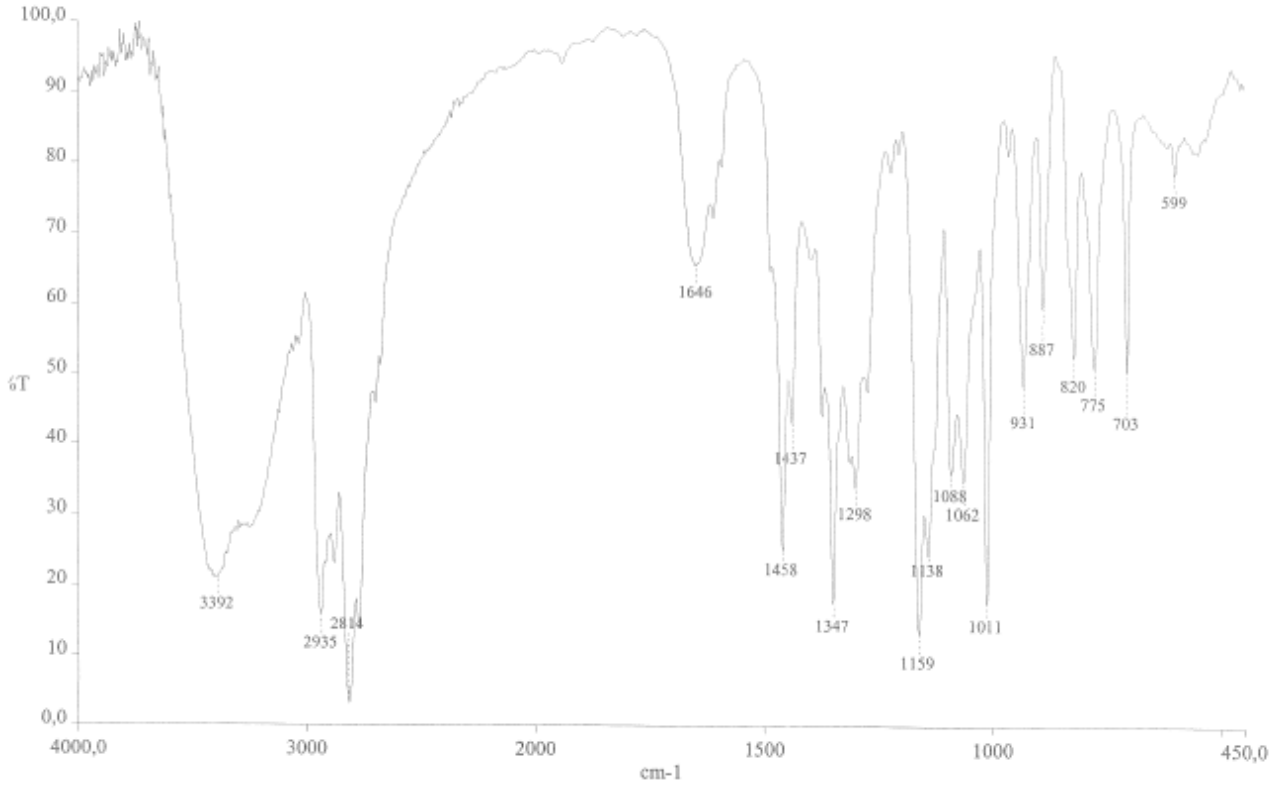

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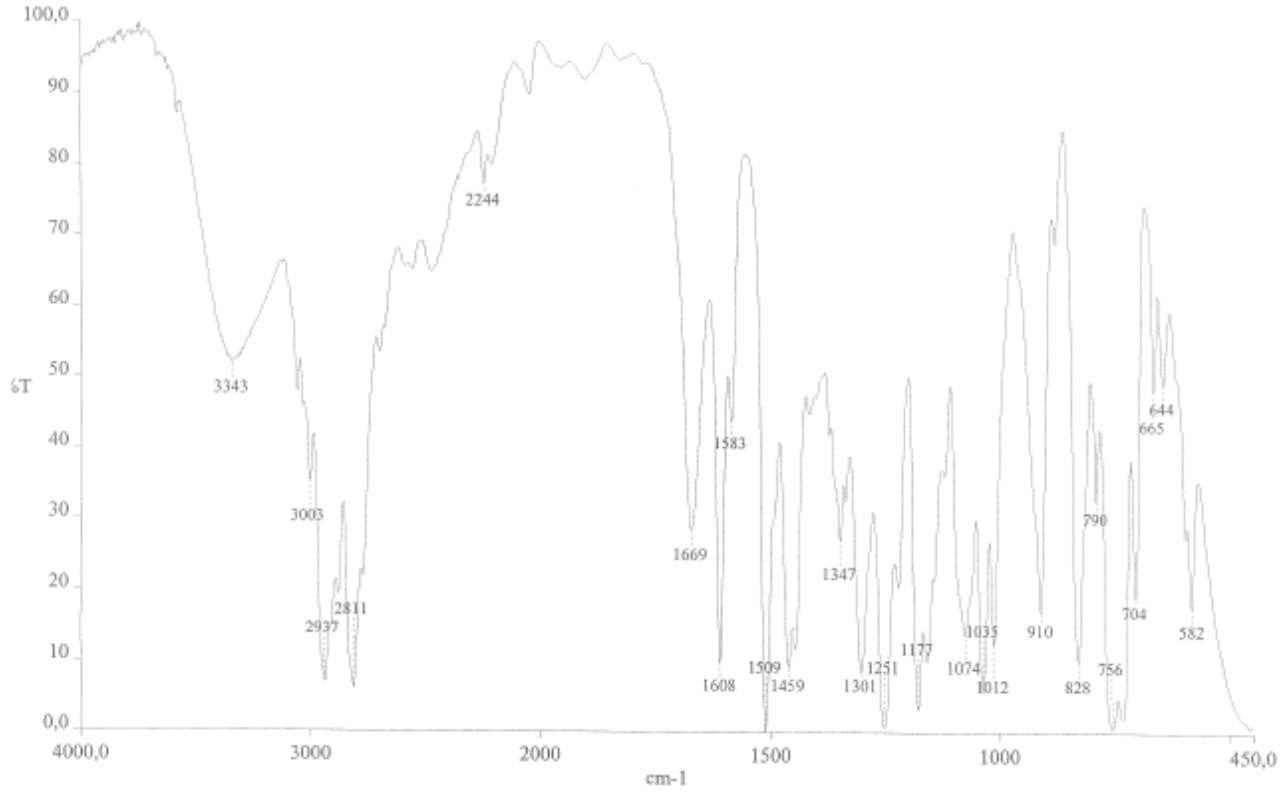

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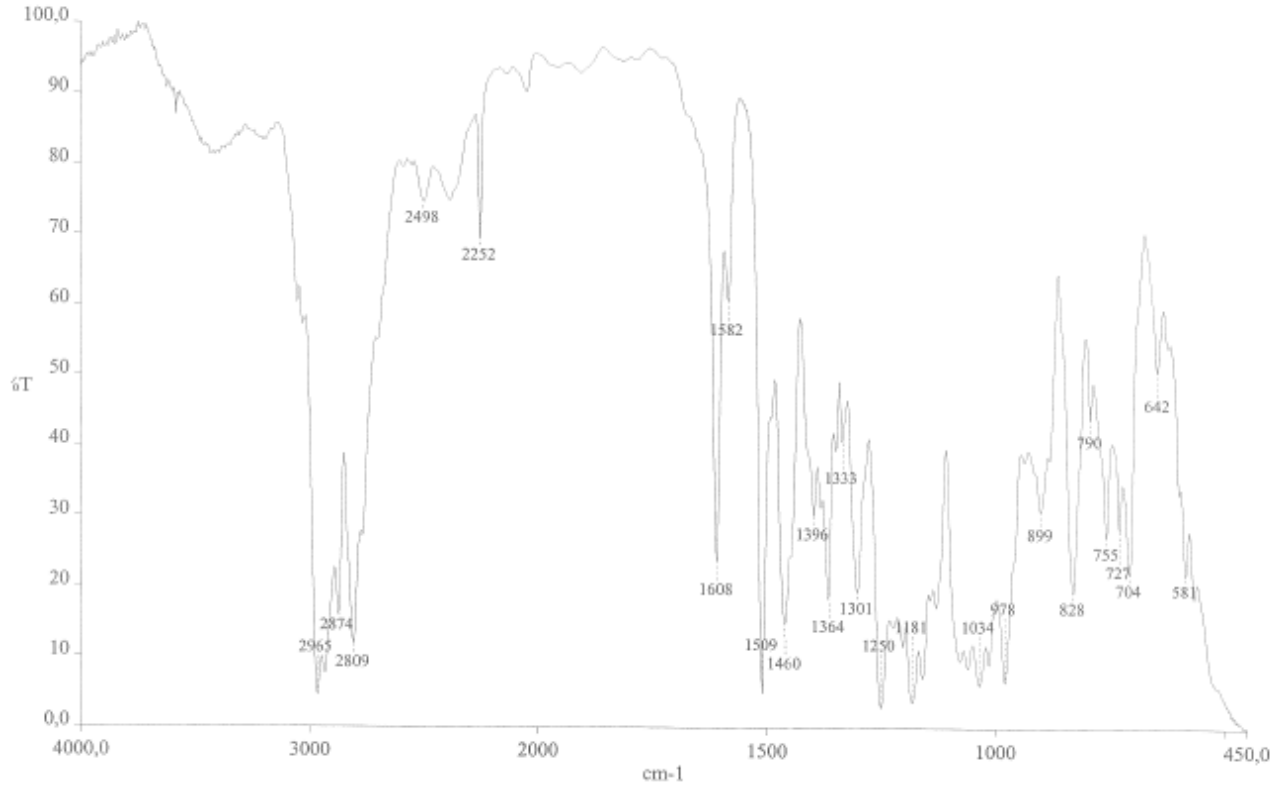
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