Supplementary Material (ESI) for Chemical Communications

Synthesis and incorporation of a furan-modified adenosine building block for DNA interstrand crosslinking

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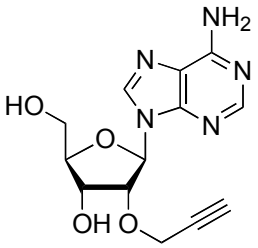
Supporting Information

General Methods

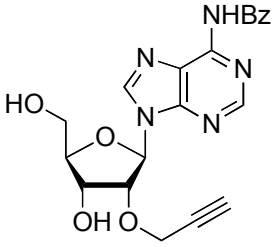
Chemicals were purchased from Sigma-Aldrich and used without further purification. Solvents CH_2Cl_2, acetonitrile, THF, Et_2O and toluene were obtained dry from a MBRAUN SPS-800 solvent purification system, CH_3OH was distilled from magnesium and iodine. All other solvents were purchased from standard chemical suppliers and were distilled under N_2-atmosphere from CaH_2. Analytical thin layer chromatography (TLC) was performed on silica gel-coated plates (Merck 60 F254) with the indicated solvent mixture, visualization was done using ultraviolet (UV) irradiation (λ = 260 nm) and/or staining with $KMnO_4$. Purification by chromatography was carried out using Silicycle silica gel (0.040 – 0.063 mm, and ca. 6 nm pore diameter). ^{1}H NMR spectra were recorded on a Varian Inova 400 (400 MHz), ^{13}C NMR spectra were recorded on a Bruker DMX300 (75 MHz) spectrometer, ^{31}P NMR spectra was recorded on a Bruker DMX300 (121 MHz) spectrometer using 85% H_3PO_4 as an external standard.
RP-HPLC analysis and purification was performed on an Agilent 1200 system equipped with a Phenomenex Clarity column (4.6 mm, 5mm) using the following gradient: 0% MeOH (2 min), 0-30% MeOH (17 min), 30-100% MeOH (20 min), 100% MeOH (27 min), 100-0% MeOH (28 min) and 0% MeOH (33 min).
ESI-MS analysis was carried out on a quadrupole ion trap LC mass spectrometer (Thermofinnigan,San Jose, Ca, USA) equipped with electrospray ionisation (negative mode).

Synthesis



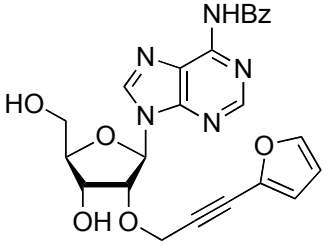
2'-*O*-(1-Propyn-3-yl)adenosine (2)
Adenosine (5 g, 18.72 mmol) was dissolved in hot (50°C) anhydrous DMF (200 mL) under an inert atmosphere. The solution was cooled down to 5 ºC, and NaH (1 g, 25 mmol, 60% dispersion in mineral oil) was added, followed by the addition of TBAI (1.5 g, 4.06 mmol) and propargyl bromide (2.12 mL, 20.92 mmol). The reaction mixture was allowed to stir for 2 days at 55 ºC, and adsorbed on silica gel. Flash-chromatography (CH_2Cl_2/MeOH, 93:7), gave a mixture of 2'-*O*- and 3'-*O*-propargylated products. Selective crystallization from anhydrous ethanol yielded the title compound (2.56 g, 45%) as pale yellow crystals. ^{1}H NMR (400 MHz, DMSO) δ 8.36 (s, 1H), 8.13 (s, 1H), 7.35 (br s, 2H), 6.02 (d, *J* = 6.4 Hz, 1H), 5.49 (m, 1H), 5.34 (d, *J* = 4.8 Hz, 1H), 4.69 (m, 1H), 4.37 (m,

1H), 4.24 (m, 2H), 4.02 (m, 1H), 3.69-3.52 (m, 2H), 3.32 (t, *J* = 2.4 Hz, *J* = 2.4 Hz, 1H). ^{13}C NMR (75 MHz, DMSO) δ 156.2, 152.5, 149.0, 139.7, 119.3, 86.6, 85.9, 79.7, 77.6, 68.9, 61.5, 56.9. HRMS calcd for $C_{13}H_{15}N_5O_4$ ($M+H^+$) 306.1202, found 306.1197.



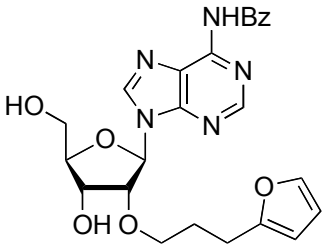
2'-*O*-(1-Propyn-3-yl)-6-*N*-benzoyladenosine (3)

2'-*O*-(1-Propyn-5-yl)adenosine **2** (1.4 g, 4.59 mmol) was coevaporated twice with anhydrous pyridine and dissolved in pyridine (20 mL) followed by the addition of trimethylsilyl chloride (2.4 mL, 18.9 mmol). After 30 min, benzoyl chloride (0.7 mL, 6.0 mmol) was added and stirring was continued for 3 h. The reaction mixture was then cooled down to 0 ºC and diluted with H_2O (10 mL). Subsequently, the reaction mixture was treated with an aqueous concentrated NH_3-solution (15 mL) and stirred for 20 min at room temperature. The mixture was extracted with CH_2Cl_2 (3 x 25 mL), the organic layer was dried over Na_2SO_4 and concentrated to dryness. Flash-chromatography (CH_2Cl_2/MeOH, 95:5), gave the desired compound (1.73 g, 92%) as a colorless foam. ^{1}H NMR (300 MHz, DMSO) δ 11.19 (s, 1H), 8.74 (s, 1H), 8.71 (s, 1H), 8.04-7.53 (m, 5H), 6.17 (d, *J* = 6.0 Hz, 1H), 5.39 (d, *J* = 5.4 Hz, 1H), 5.18 (m, 1H), 4.72 (m, 1H), 4.39 (m, 1H), 4.29 (m, 2H), 4.01 (m, 1H), 3.68-3.58 (m, 2H), 3.30 (m, 1H). ^{13}C NMR (75 MHz, DMSO) δ 165.8, 152.0, 151.7, 150.4, 143.0, 133.2, 132.5, 129.2, 128.5, 128.4, 128.2, 125.6, 86.4, 85.6, 80.0, 79.6, 77.5, 68.8, 61.2, 57.1. HRMS calcd for $C_{20}H_{19}N_5O_5$ ($M+H^+$) 410.1464, found 410.1454.



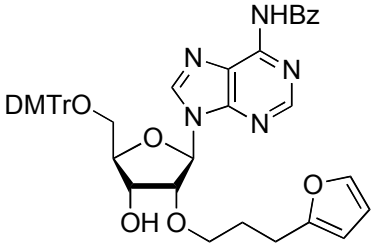
2'-*O*-(3-(Furan-2-yl)-propyn-3-yl)-6-*N*-benzoyladenosine (4)

A mixture of 2'-*O*-(1-propyn-3-yl)-6-*N*-benzoyladenosine **3** (300 mg, 0.73 mmol), 2-iodofuran (240 mg, 1.24 mmol), $PdCl_2(PPh_3)_2$ (24 mg, 0.034 mmol) and CuI (18 mg, 0.095 mmol) in triethylamine and DMF (5 mL, 1:1) was stirred in a microwave at 55 ºC (60 W) for 30 min after which the solvent was evaporated and the crude mixture purified by flash chromatography (CH_2Cl_2/MeOH, 95:5), furnishing the coupled product (329 mg, 94 %) as a yellow oil. ^{1}H NMR (400 MHz, DMSO) δ 11.15 (s, 1H), 8.68 (s, 1H), 8.65 (s, 1H), 8.04-7.50 (m, 6H), 6.66 (m, 1H), 6.47 (m, 1H), 6.17 (d, *J* = 6.0 Hz, 1H), 5.43 (d, *J* = 5.2 Hz, 1H), 5.19 (m, 1H), 4.80 (m, 1H), 4.60 (m, 2H), 4.39 (m, 1H), 4.0 (m, 1H), 3.68-3.55 (m, 2H). ^{13}C NMR (75 MHz, DMSO) δ 165.6, 152.0, 151.6, 150.5, 145.0, 143.1, 135.1, 133.3, 132.5, 128.5, 125.8, 116.3, 111.3, 90.2, 86.4, 85.8, 80.0, 76.5, 68.8, 61.2, 57.7. HRMS calcd for $C_{24}H_{21}N_5O_6$ ($M+H^+$) 476.1570, found 476.1566.



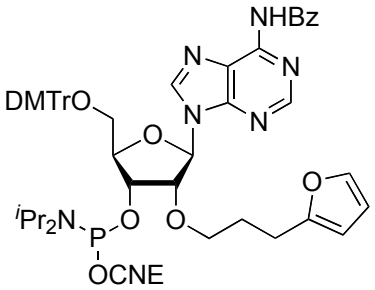
2'-*O*-(3-(Furan-2-yl)-propyl)-6-*N*-benzoyladenosine (5)

A solution of 2'-*O*-(3-(furan-2-yl)-propyn-3-yl)-6-N-benzoyladenosine **4** (250 mg, 0.526 mmol) in MeOH (15 mL) was passed through the H-Cube cartridge (Thalesnano) containing 10% Pd/C, at 20 bar, 30 ºC for 15 min (1 mL/min). The reaction mixture was concentrated to dryness and subjected to flash chromatography (CH_2Cl_2/MeOH, 95:5), giving the desired compound (235 mg, 93%) as a pale yellow foam. ^{1}H NMR (400 MHz, DMSO) δ 11.22 (br s, 1H), 8.76 (s, 1H), 8.75 (s, 1H), 8.06-7.54 (m, 5H), 7.43 (m, 1H), 6.28 (m, 1H), 6.17 (d, *J* = 5.6 Hz, 1H), 5.95 (m, 1H), 5.27 (d, *J* = 5.6 Hz, 1H), 5.17 (m, 1H), 4.53 (m, 1H), 4.36 (m, 1H), 4.02 (m, 1H), 3.71-3.42 (m, 4H), 2.58 (m, 2H), 1.77 (m, 2H). ^{13}C NMR (75 MHz, DMSO) δ 165.6, 155.0, 152.1, 151.7, 150.5, 143.0, 141.2, 133.3, 132.4, 128.5, 125.8, 110.2, 105.0, 86.2, 85.7, 81.1, 68.9, 61.1, 27.7, 23.8. HRMS calcd for $C_{24}H_{25}N_5O_6$ ($M+H^+$) 480.1883, found 480.1869.



5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-2'-*O*-(3-(furan-2-yl)-propyl)-6-*N*-benzoyladenosine (6)

2'-O-(3-(Furan-2-yl)-propyl)-6-N-benzoyladenosine **5** (200 mg, 0.42 mmol) was coevaporated three times with anhydrous pyridine and then dissolved in pyridine (5 mL), followed by the addition of DMTrCl (190 mg, 0.56 mmol). The reaction mixture was stirred for 16 h at room temperature, diluted with CH_2Cl_2 and poured into a 2% aqueous $NaHCO_3$ (15 mL) solution. The aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL), the combined organic layers were dried over Na_2SO_4 and concentrated in *vacuo*. Flash chromatography (acetone /CH_2Cl_2, 15:85) afforded the title compound (250 mg, 77%) as a colorless foam. ^{1}H NMR (400 MHz, DMSO) δ 11.23 (b s, 1H), 8.67 (s, 1H), 8.61 (s, 1H), 8.06 (d, *J* = 7.2 Hz, 1H), 7.65 (m, 1H), 7.57 (m, 2H), 7.44 (s, 1H), 7.38 (m, 2H), 7.27 (m, 7H), 6.85 (m, 4H), 6.28 (m, 1H), 6.19 (d, *J* = 4.8 Hz, 1H), 5.97 (m, 1H), 5.30 (d, *J* = 6.0 Hz, 1H), 4.68 (m, 1H), 4.45 (m, 1H), 4.15 (m, 1H), 3.72 (s, 6H), 3.69 (m, 1H), 3.50 (m, 1H), 3.28 (m, 2H), 2.60 (m, 2H), 1.80 (m, 2H). ^{13}C NMR (75 MHz, DMSO) δ 165.6, 158.0, 155.0, 152.0, 151.6, 150.5, 144.8, 143.3, 141.2, 135.6, 135.4, 133.3, 132.4, 129.7, 128.5, 128.4, 127.8, 127.7, 127.4, 126.6, 125.9, 113.1, 110.2, 105.0, 86.3, 85.5, 83.8, 80.1, 69.1, 69.0, 63.5, 55.0, 27.7, 23.8. HRMS calcd for $C_{45}H_{43}N_5O_8$ ($M+H^+$) 782.3189, found 782.3153.



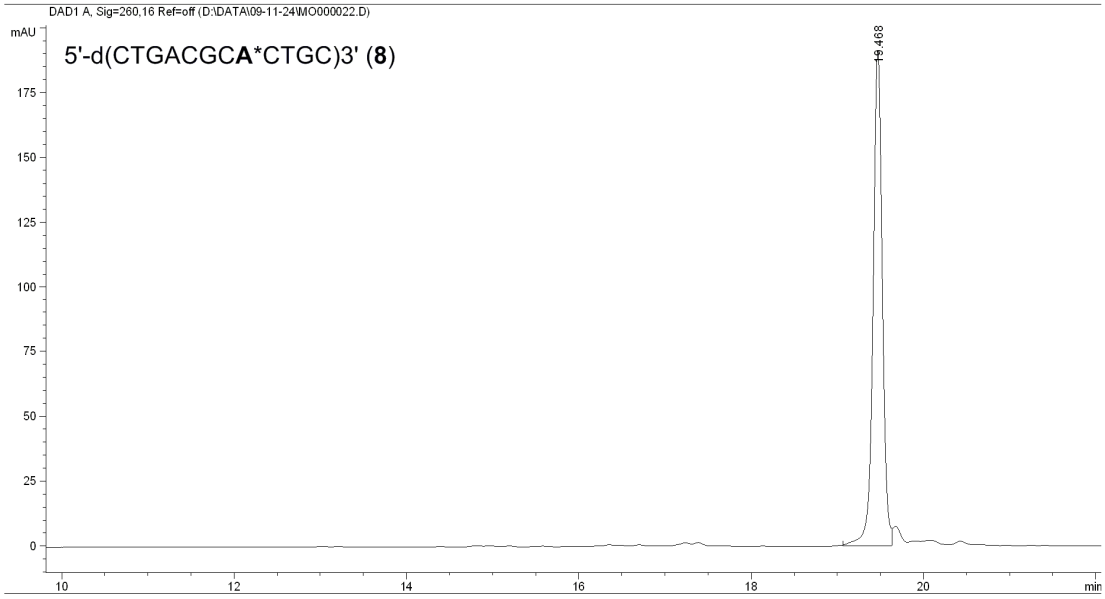
5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-2'-*O*-(3-(furan-2-yl)-propyl)-6-*N*-benzoyladenosine-3'-O-(2-cyanoethyl N, N-diisopropylphosphoramidite) (7)

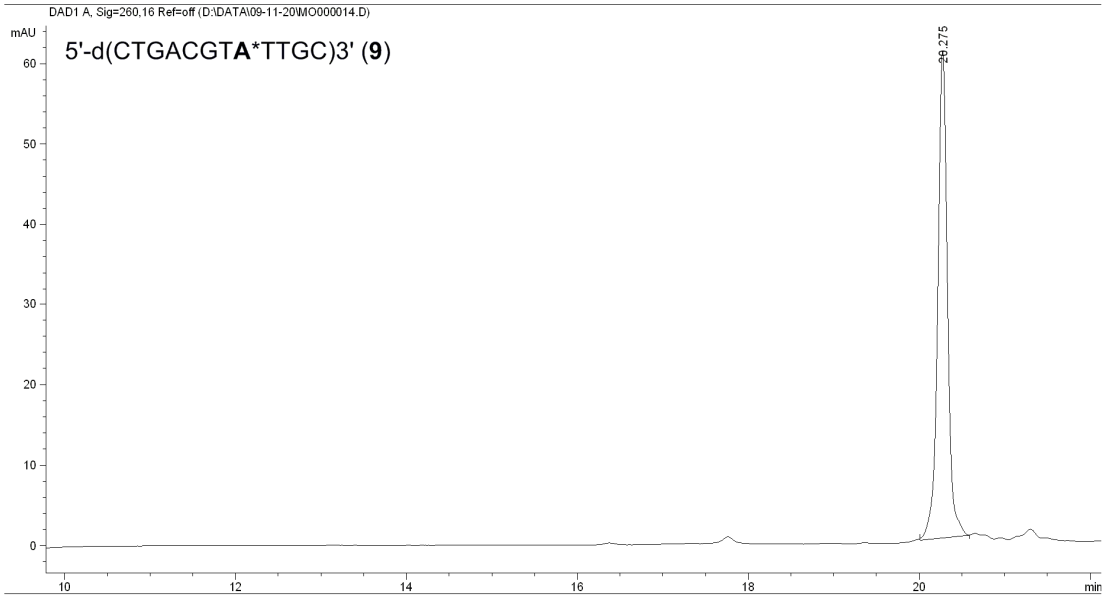
To a solution of **6** (100 mg, 0.128 mmol) in dry CH_2Cl_2 (2 mL) were subsequently added DIPEA (28 µL, 0.16 µmol) and 2-cyanoethyl N, N-diisopropylphosphoramidochloridite (38 µL, 0.17 µmol). The reaction mixture was stirred for 3 h under inert atmosphere, diluted with CH_2Cl_2 (15 mL) and 5% aqueous $NaHCO_3$ solution (5 mL). Extraction was performed with CH_2Cl_2 (2 x 15 mL), the combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (acetone/ CH_2Cl_2, 1:9) to give **7** (115.5 mg, 92%) as a colorless foam. ^{31}P NMR (121 MHz $CDCl_3$) 157.1, 156.4. HRMS calcd for $C_{54}H_{60}N_7O_9P$ ($M+H^+$) 982.4268, found 982.4242.

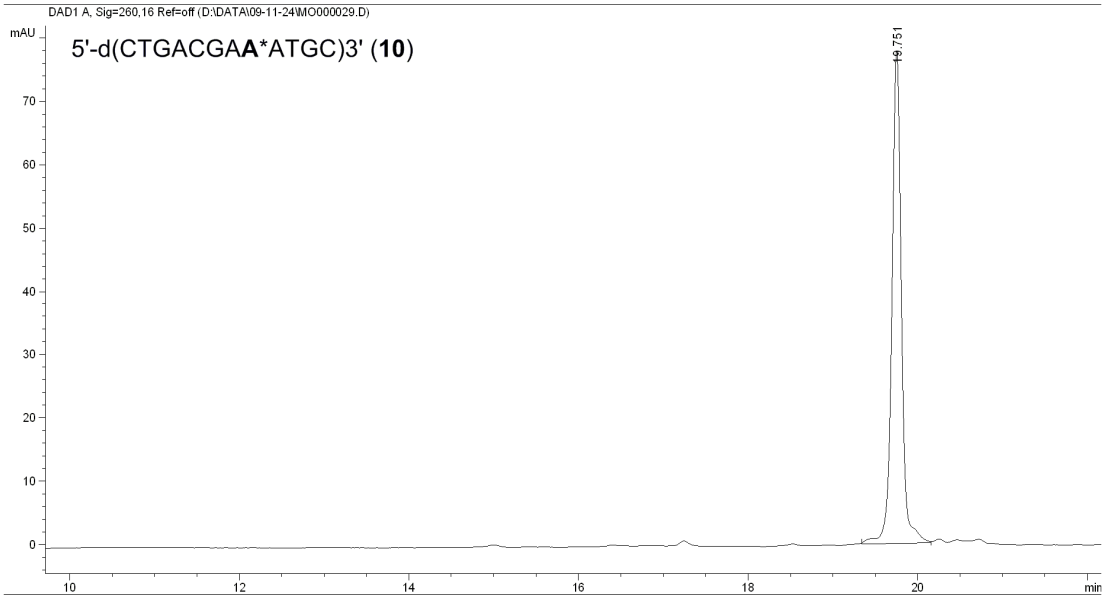
DNA synthesis and purification

All oligonucleotides were synthesized DMT-on on an ABI 394 DNA synthesizer on 1 μmol scale. A standard synthesis protocol was used except for the modified residues. For the manual couplings, a 0.05 M solution of the phosphoramidite in acetonitrile and a 0.1 M solution of dicyanoimidazole (DCI) in acetonitrile were dried on molecular sieves for about 10 min. Then the phosphoramidite solution (0.4 ml) and the DCI solution (0.5 ml) were mixed and pushed over the reaction column. The column was placed back on the DNA-synthesizer and automated synthesis was resumed.

ONs were cleaved from solid support and deprotected by incubation at 55 ºC overnight in concentrated ammonia. DMT groups were removed with 1.5% aqueous TFA during Seppak C18 cartridge purification using standard protocols. Subsequently, synthesized ONs were analyzed via RP-HPLC.



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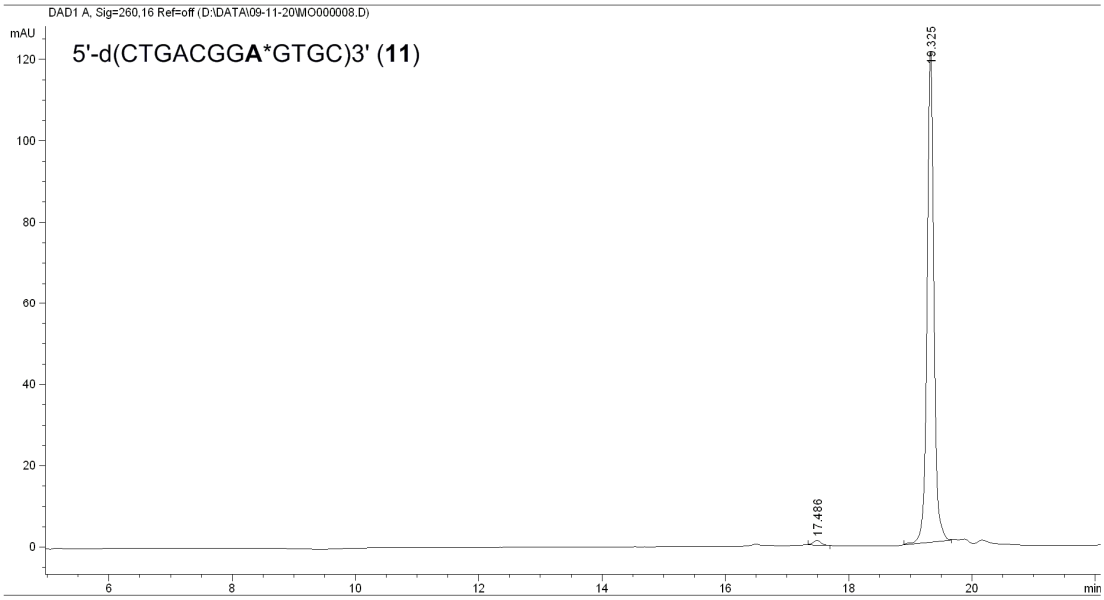
mAU
5'-d(CTGACGA**A***ATGC)3' (**10**)
19.751
0
10
20
30
40
50
60
70
10
12
14
16
18
20
min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5'-d(CTGACGG**A***GTGC)3' (**11**)
19.325
17.486
0
20
40
60
80
100
120
6
8
10
12
14
16
18
20
min

Melting experiments

Complementary ONs were purchased from Eurogentec Liege (see Table **1** for specific sequences). All UV experiments were performed on a Varian Cary 300 Bio equipped with a six-cell thermostatted cell holder. Melting curves were recorded from 20 ºC to 95 ºC at 260 nm with a heating rate of 0.3 ºC/min. Samples were prepared taking 1.8 nmol of each strand, 90 µL each of 0.1 M phosphate buffer and 1 M NaCl in nanopure water (total volume 900 µL). Melting temperatures were calculated from the first derivative of the heating curves using Cary 300 Bio software (Table 1).

C**A***C	A	C	G	T	T**A***T	A	C	G	T
UNMODIFIED	40.5	43.6	50.4	57.2	UNMODIFIED	33.7	35.1	41.6	46.8
MODIFIED	36.3	40.7	43.8	52.3	MODIFIED	28.8	32.2	32.0	40.8
ΔT_m (ºC)	−4.2	−2.9	−6.6	−4.9	ΔT_m (ºC)	−4.9	−2.9	−9.6	−6.0
A**A***A	A	C	G	T	G**A***G	A	C	G	T
UNMODIFIED	35.5	35.5	36.6	49.9	UNMODIFIED	41.0	38.5	45.1	54.8
MODIFIED	33.9	34.3	33.7	46.4	MODIFIED	42.7	43.6	43.4	52.2
ΔT_m (ºC)	−1.6	−0.9	−2.9	−3.5	ΔT_m (ºC)	1.7	5.1	−1.7	−2.6

Table 1. Melting temperatures of oligonucleotides **8**-**11**, paired opposite to A, C, T and G in an unmodified sequence 5'-d(GCAGAXYXTCAG) where X is identical to canonicals complementary to the modified strand and Y is A, C, T or G. **A*** = furan-modified adenosine.

Crosslinking experiments

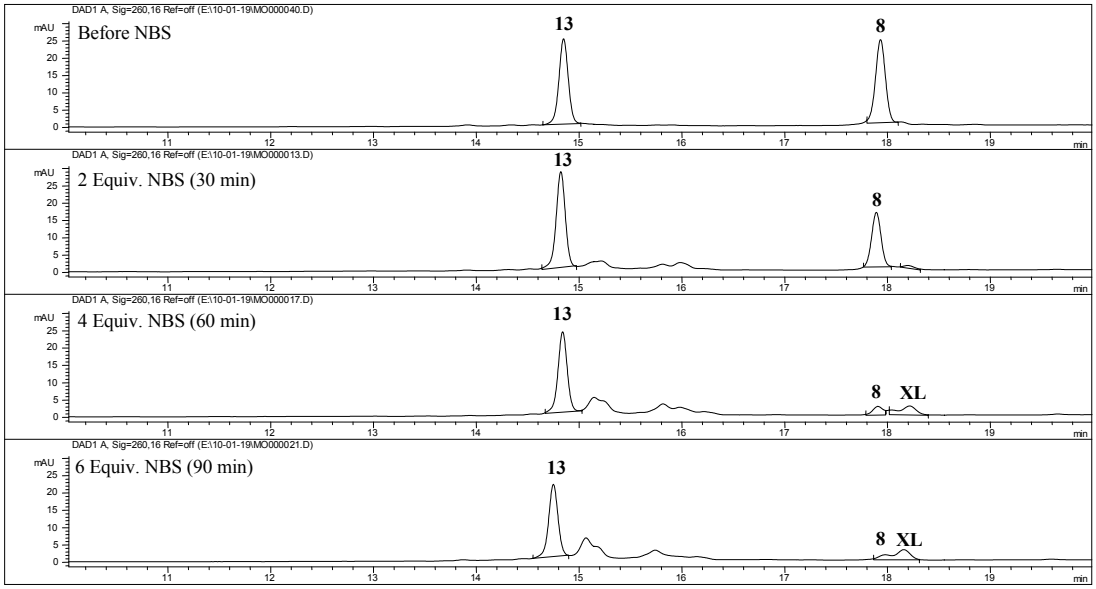
The modified strands were mixed with the respective complements in equimolar amounts in 0.01 M phosphate buffer (pH 7) and 0.1 M NaCl to obtain a final concentration of 20 μM in oligonucleotides. Mixtures were annealed by heating to 90 °C for 5 minutes and cooling down to 25 ºC. Temperature during the crosslink reaction was kept constant in an Eppendorf thermomixer comfort at 25 ºC. A stock solution of NIS (1 equiv./μL) was freshly prepared and 2 μL was added every 30 mins. A total of 3 additions were done. The reaction was monitored by RP-HPLC and by PAGE (polyacrylamide gel electrophoresis) as shown below.

For each HPLC analysis, a 2 μL sample of the crosslink mixture was diluted to 18 μL, of which 15 μL is injected. Crosslinking (XL) yields based on HPLC were calculated by comparing the peak area from the spectra before and after addition of NIS, taking into account the extinction coefficients calculated by the nearest neighbor method. The extinction coefficient of the crosslinked duplex is taken to be 0.9 times the sum of the extinction coefficients of its composing sequences.

DUPLEX	XL Yield (%)	DUPLEX	XL Yield (%)
8 • 12	27	**10 • 18**	15
8 • 13	9	**10 • 19**	9
9 • 16	39	**11 • 20**	17
9 • 17	16	**11 • 21**	8

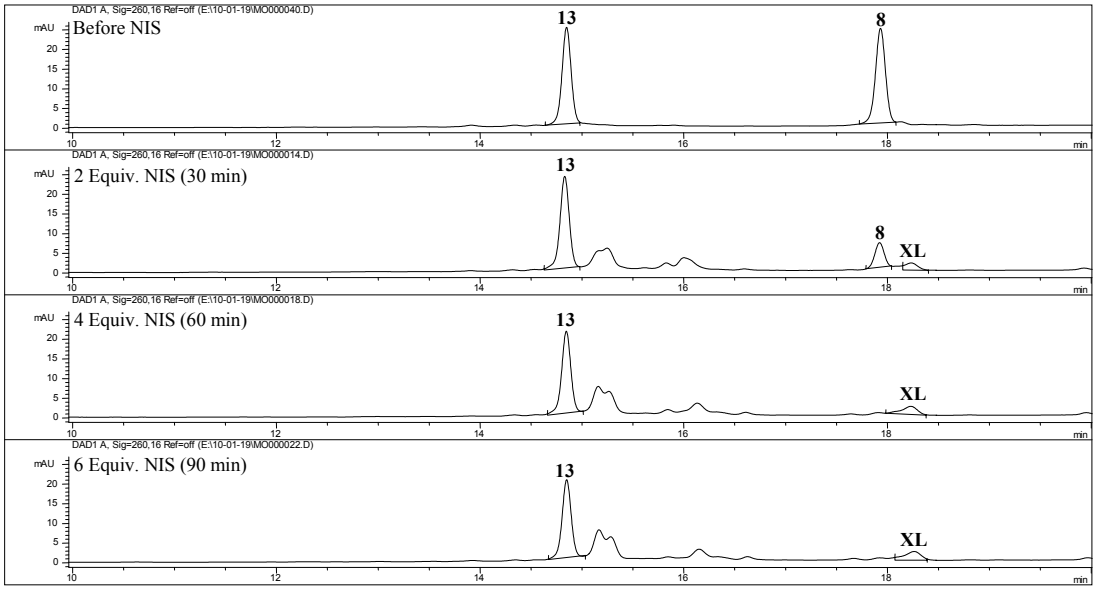
With NBS:

5'-d(CTGACGC**A***CTGC)3' (**8**)
3'-d(GACTGCG**C**GACG)5' (**13**)

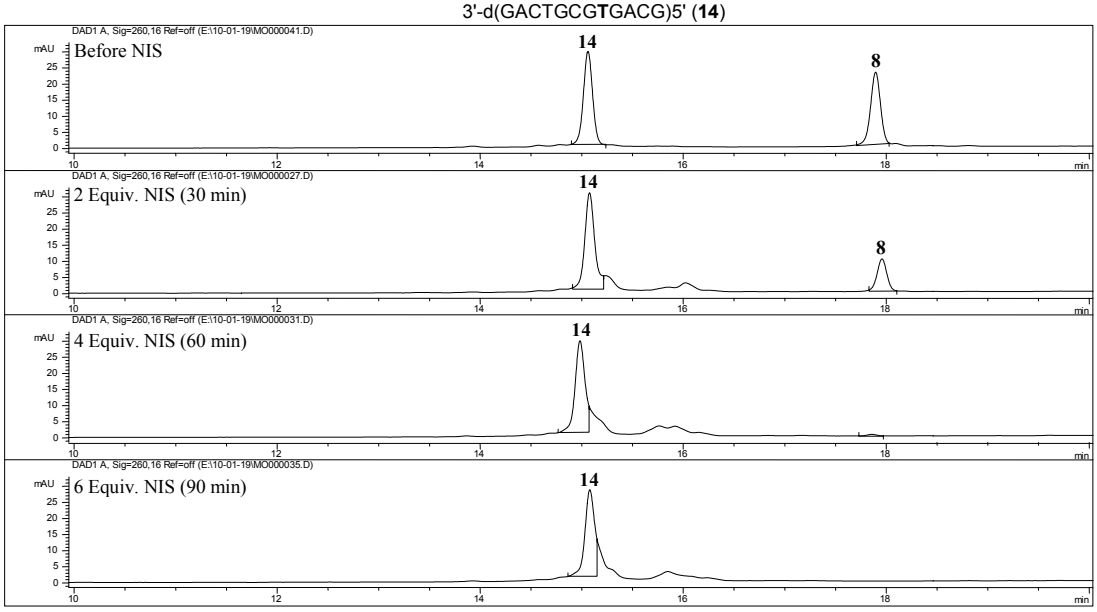


With NIS (remainder of HPLC-traces):

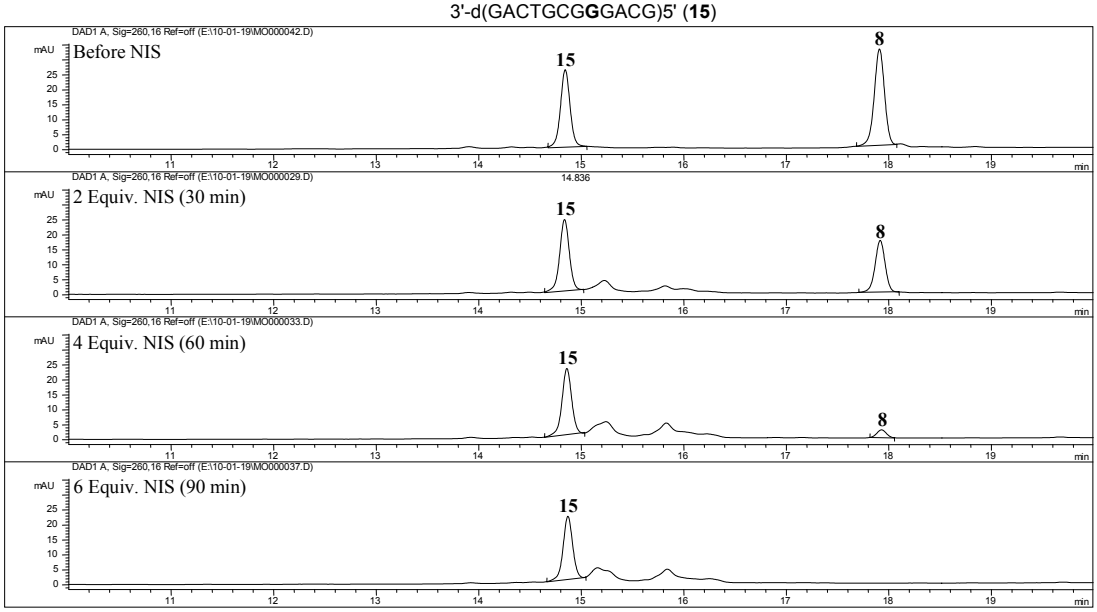
5'-d(CTGACGC**A***CTGC)3' (**8**)
3'-d(GACTGCG**C**GACG)5' (**13**)



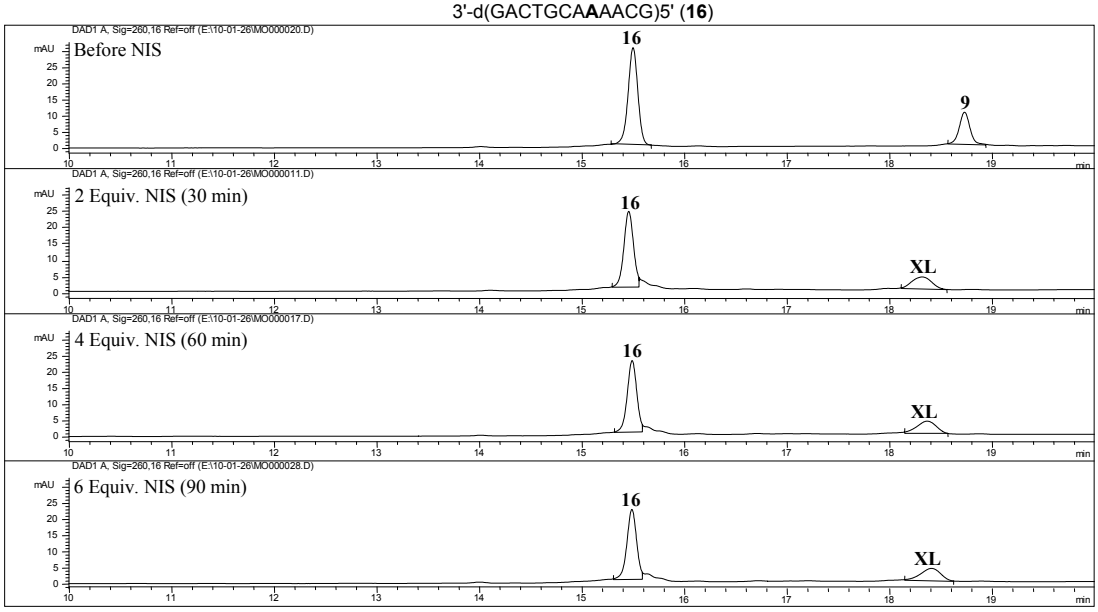
5'-d(CTGACGC**A***CTGC)3' (**8**)
3'-d(GACTGCG**T**GACG)5' (**14**)



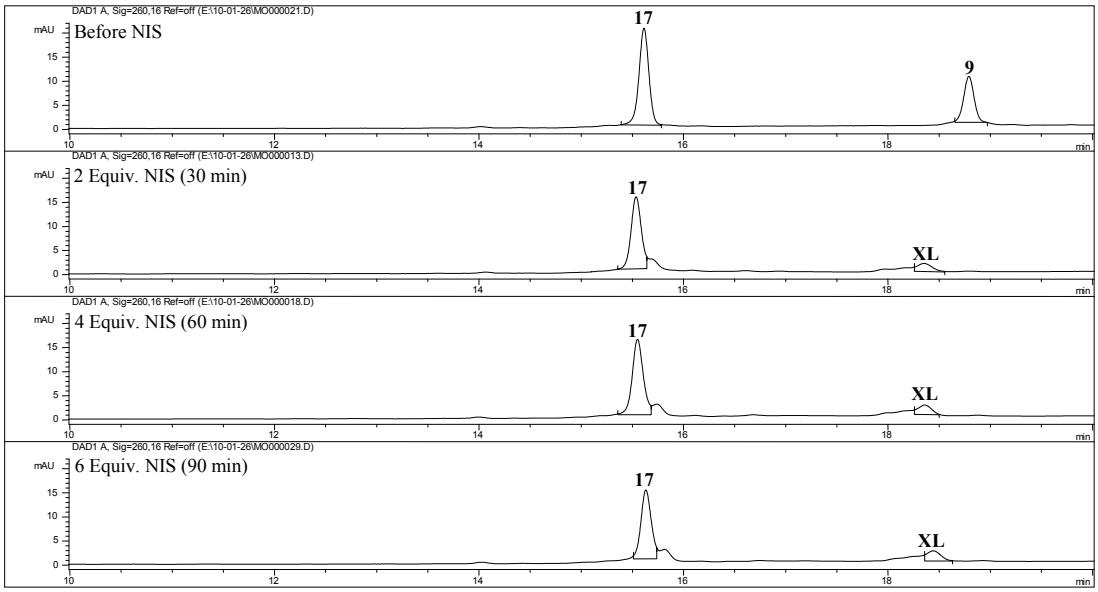
5'-d(CTGACGC**A***CTGC)3' (**8**)
3'-d(GACTGCG**G**GACG)5' (**15**)



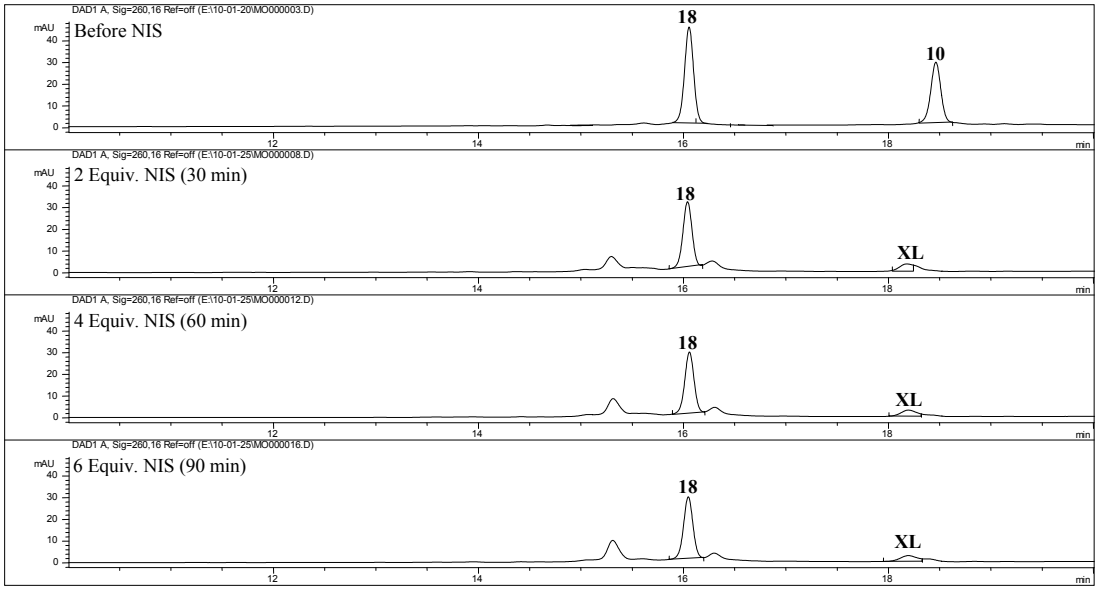
5'-d(CTGACGT**A***TTGC)3' (**9**)
3'-d(GACTGCA**A**AACG)5' (**16**)



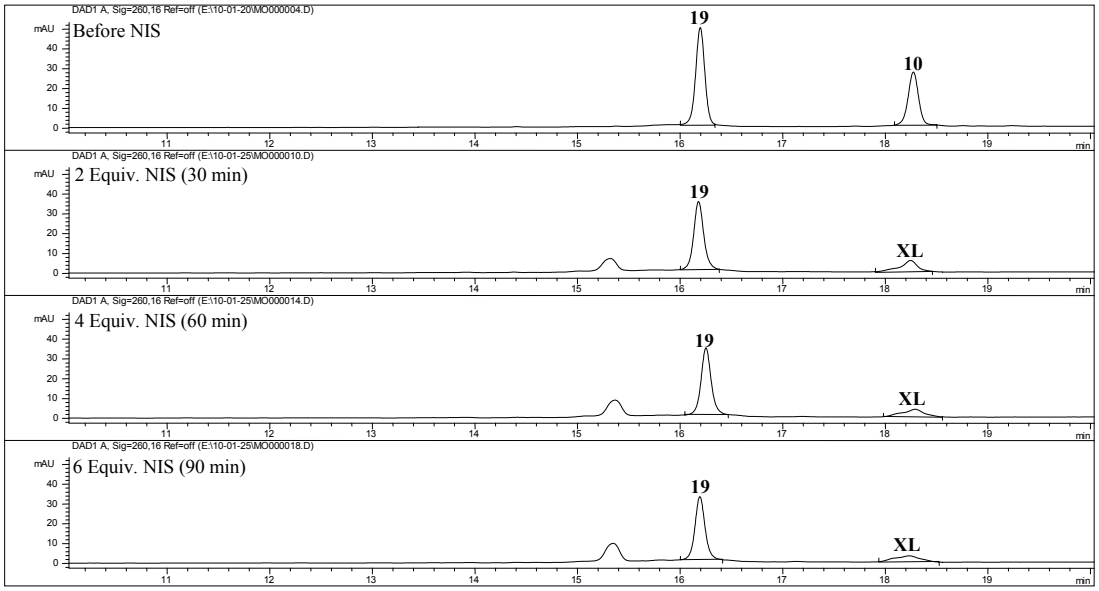
5'-d(CTGACGT**A***TTGC)3' (**9**)
3'-d(GACTGCA**C**AACG)5' (**17**)



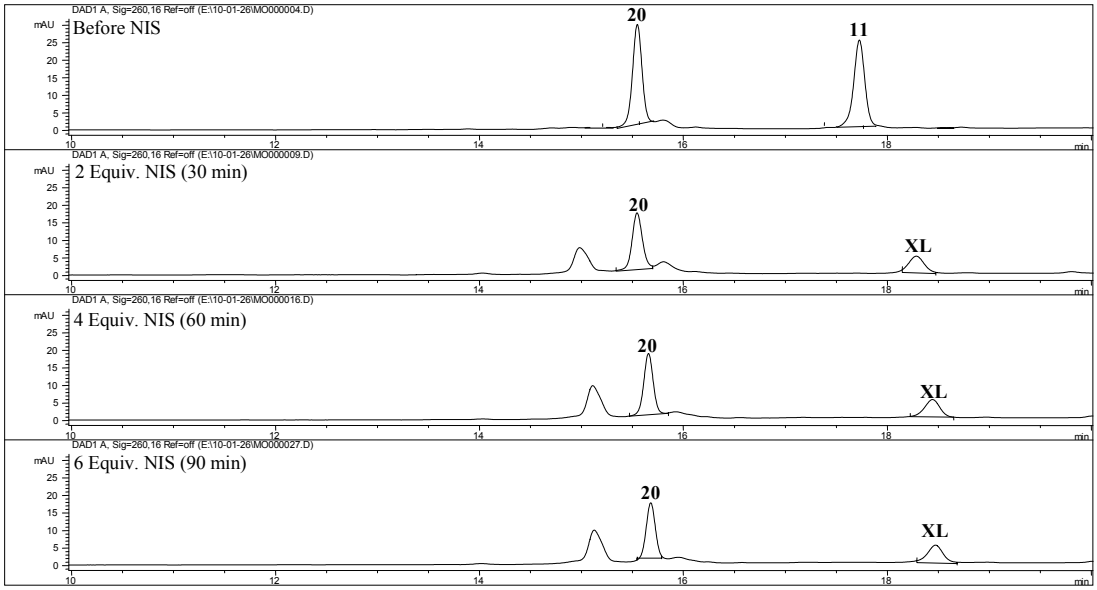
5'-d(CTGACGA**A***ATGC)3' (**10**)
3'-d(GACTGCT**A**TACG)5' (**18**)



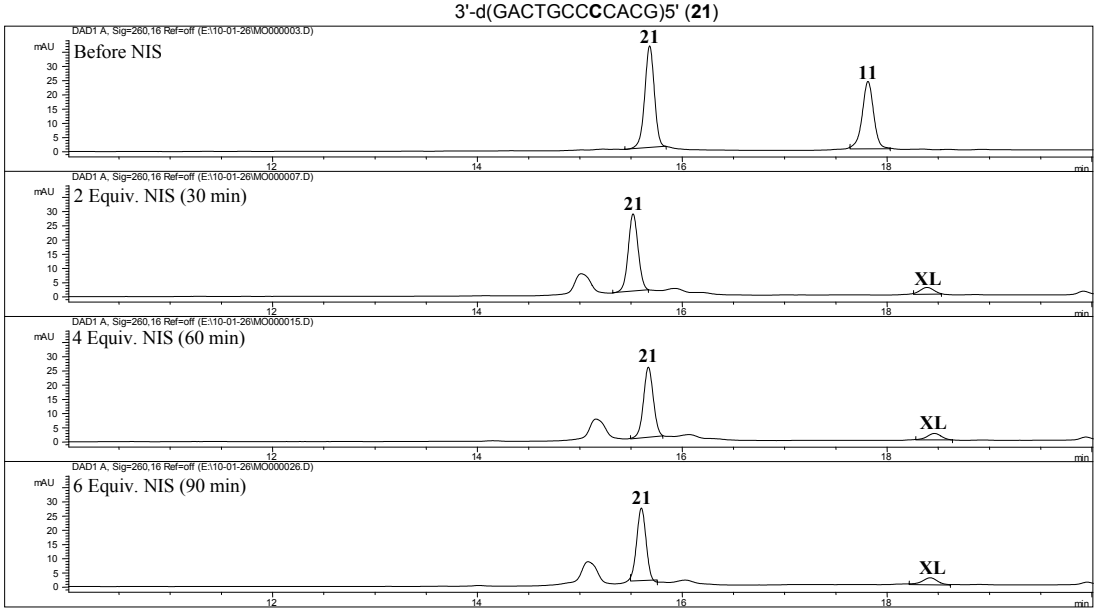
5'-d(CTGACGA**A***ATGC)3' (**10**)
3'-d(GACTGCT**C**TACG)5' (**19**)



5'-d(CTGACGG**A***GTGC)3' (**11**)
3'-d(GACTGCC**A**CACG)5' (**20**)

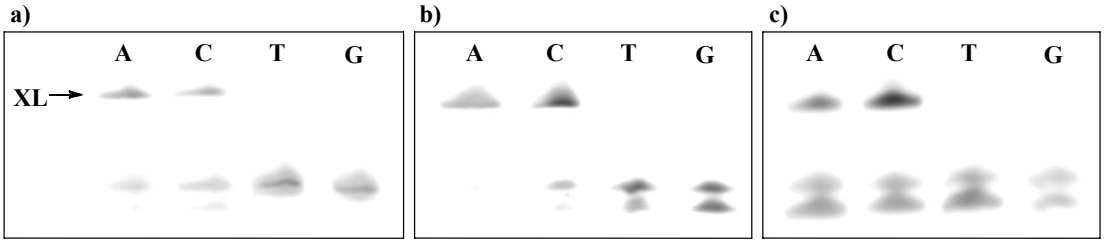


5'-d(CTGACGG**A***GTGC)3' (**11**)
3'-d(GACTGCC**C**CACG)5' (**21**)



Gel-electrophoresis experiments

A 20% polyacrylamide (acrylamide: bisacrylamide 19:1) with 1 x TBE buffer and 7 M urea was used for all analyses. The power supply used for gel-electrophoresis was a consort EV202. Samples were prepared mixing 2 μL probe with 2 μL loading buffer and run at 300 V, 25 ºC, 1.2 h. Gels were stained with GelRed (VWR) and pictures were taken with an Autochemi imaging system (UVP).

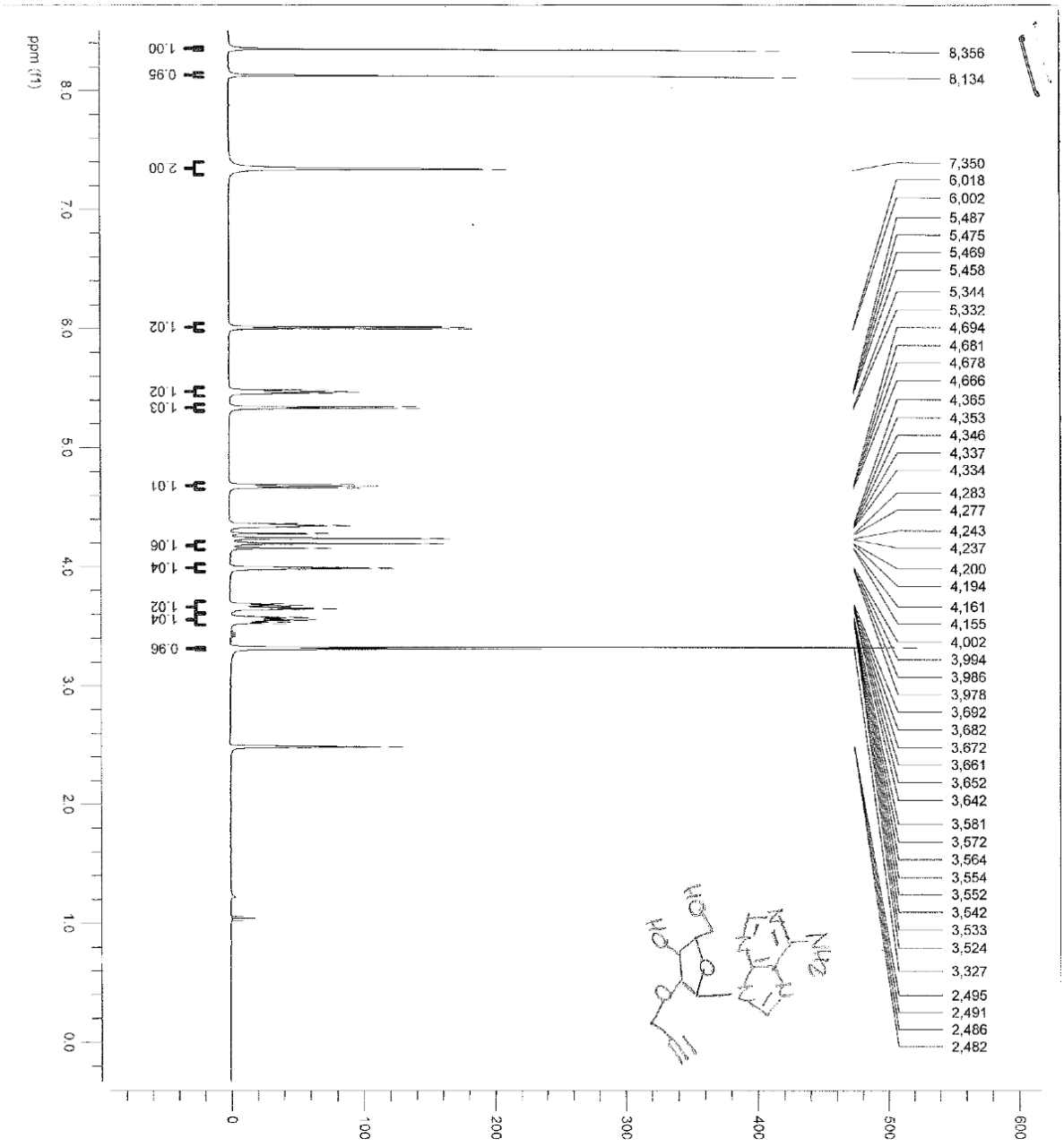


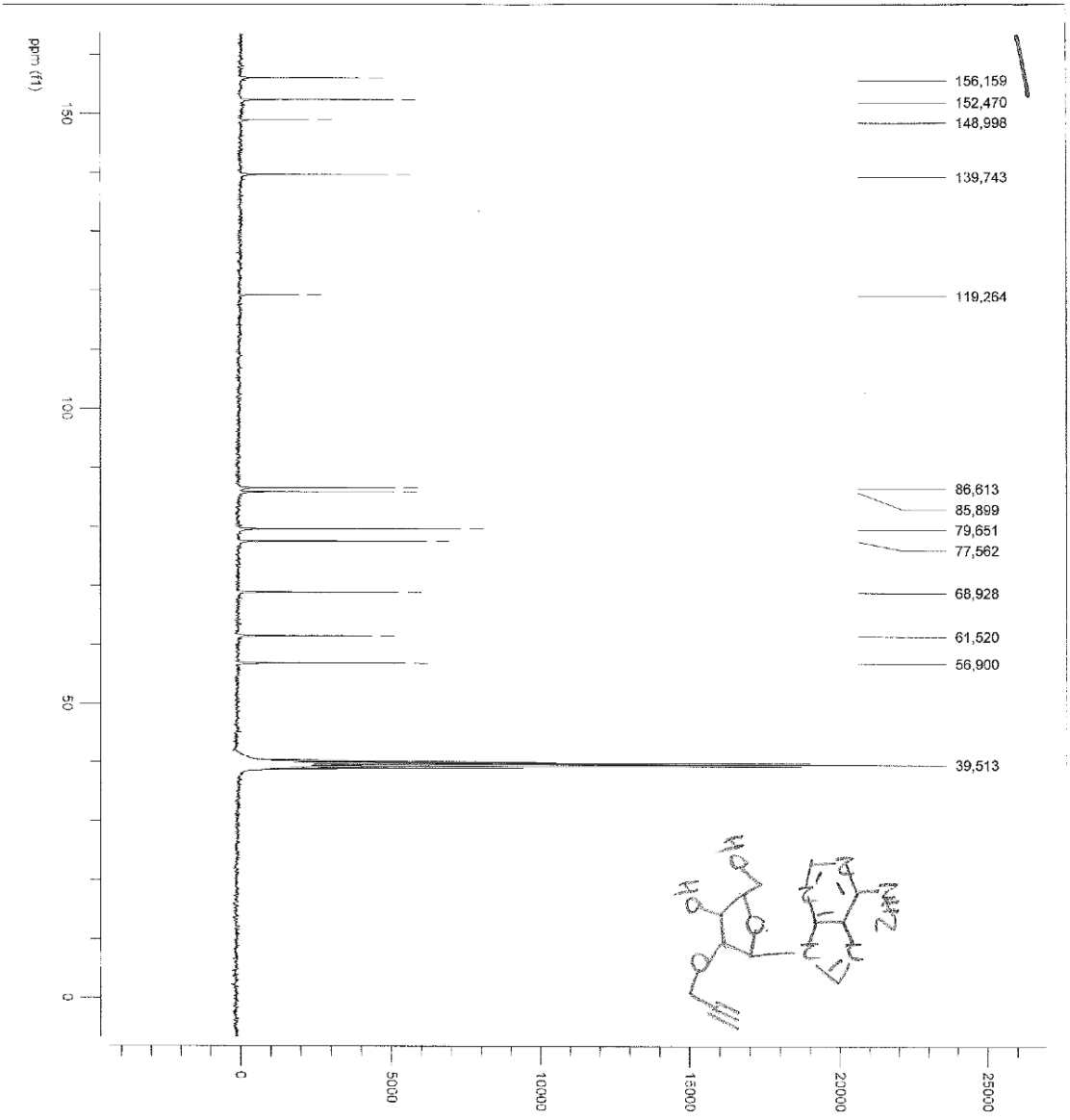
a) 5'-d(CTGACGT**A***TTGC)3' (**9**) • 3'-d(GACTGCA**X**AACG)5', where **X** = A, C, T and G
b) 5'-d(CTGACGAA*ATGC)3' (**10**) • 3'-d(GACTGCT**X**TACG)5', where **X** = A, C, T and G
c) 5'-d(CTGACGGA*GTGC)3' (**11**) • 3'-d(GACTGCC**X**CACG)5', where **X** = A, C, T and G

ESI-MS analysis

For a representative RP-HPLC experiment (**8:13** *vide supra*) fraction collection of all formed species was carried out. ESI-MS analysis confirmed the crosslinked nature of the signal at t_r = 18.158 min. MW of the Na adduct of $C_{238}H_{303}N_{93}O_{143}P_{22}$: Calcd = 7458; Obsd = 7456.

NMR spectra of compounds **2**-**7**.





Date:
12 Nov 2009
Document's Title:
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Spectrum Title:
AJ-B1-69

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Actual Points Count:
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Acquisition Time (sec):
(f1) 1.7302
Spectral Width (ppm):
(f1) 250.933
Pulse Program:
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Temperature:
298
Number of Scans:
2000
Acq. Date:
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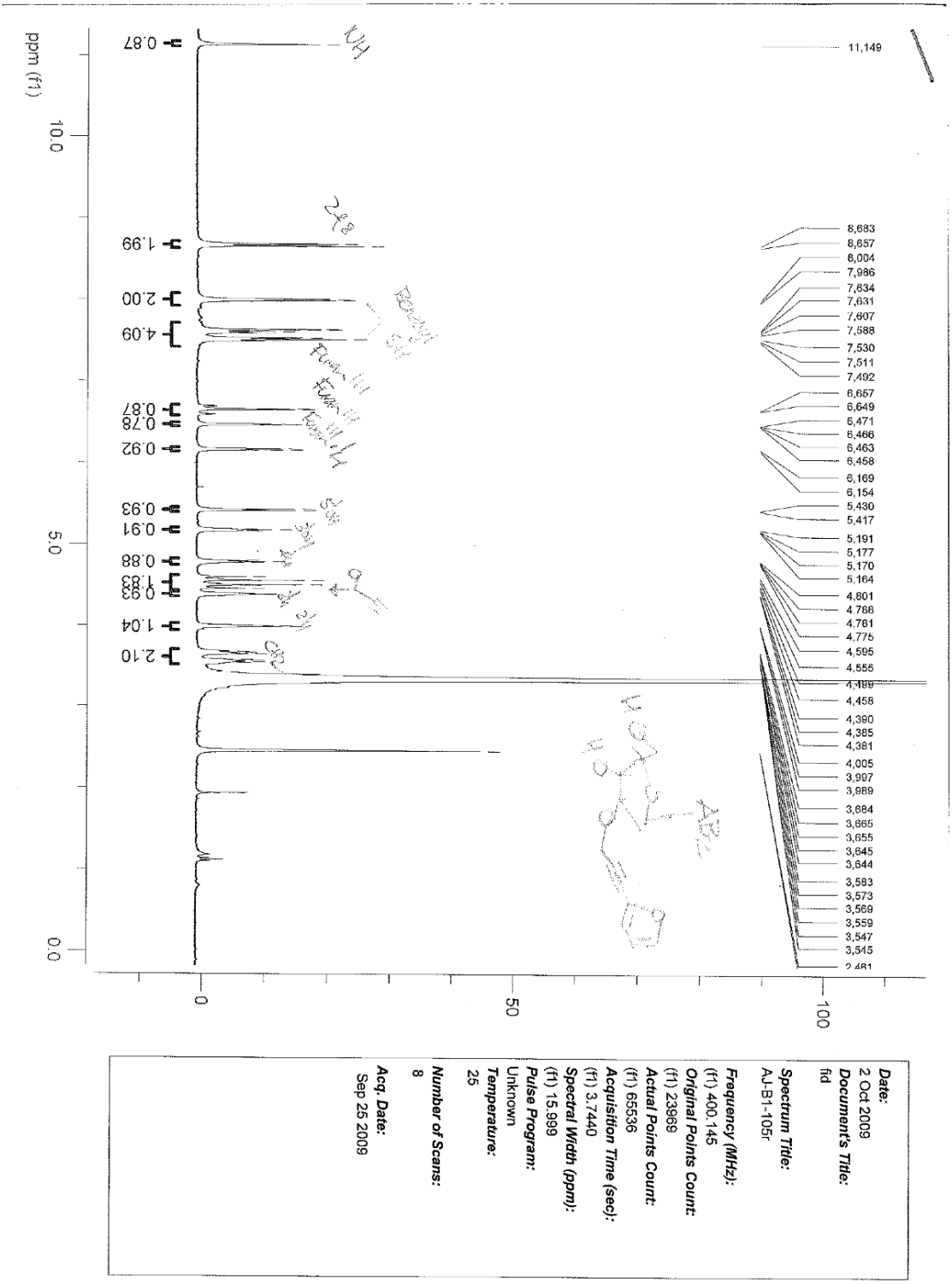

Date:
2 Oct 2009
Document's Title:
fid

Spectrum Title:
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Actual Points Count:
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Spectral Width (ppm):
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Temperature:
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Number of Scans:
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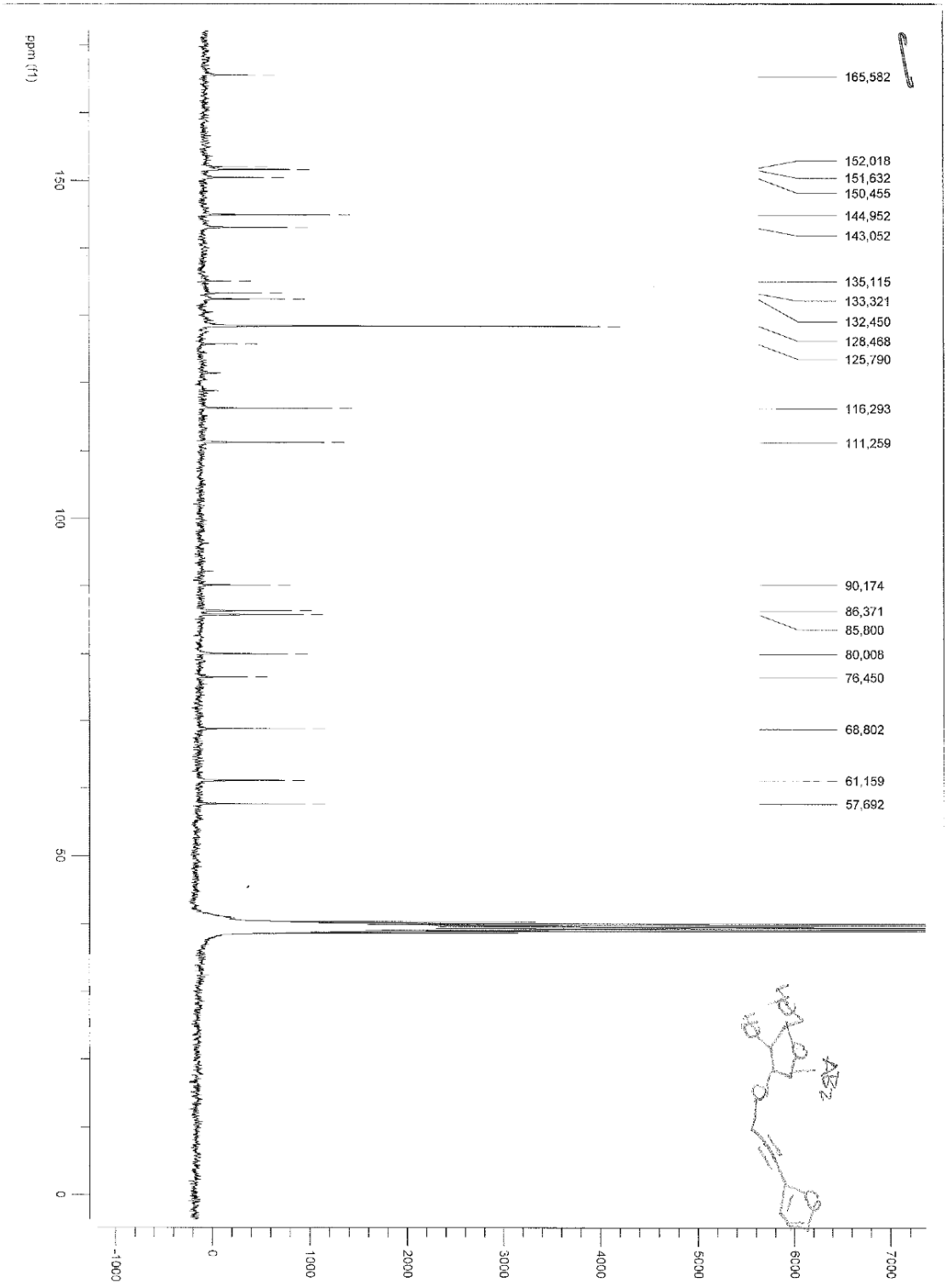
Acq. Date:
Sep 25 2009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132,450
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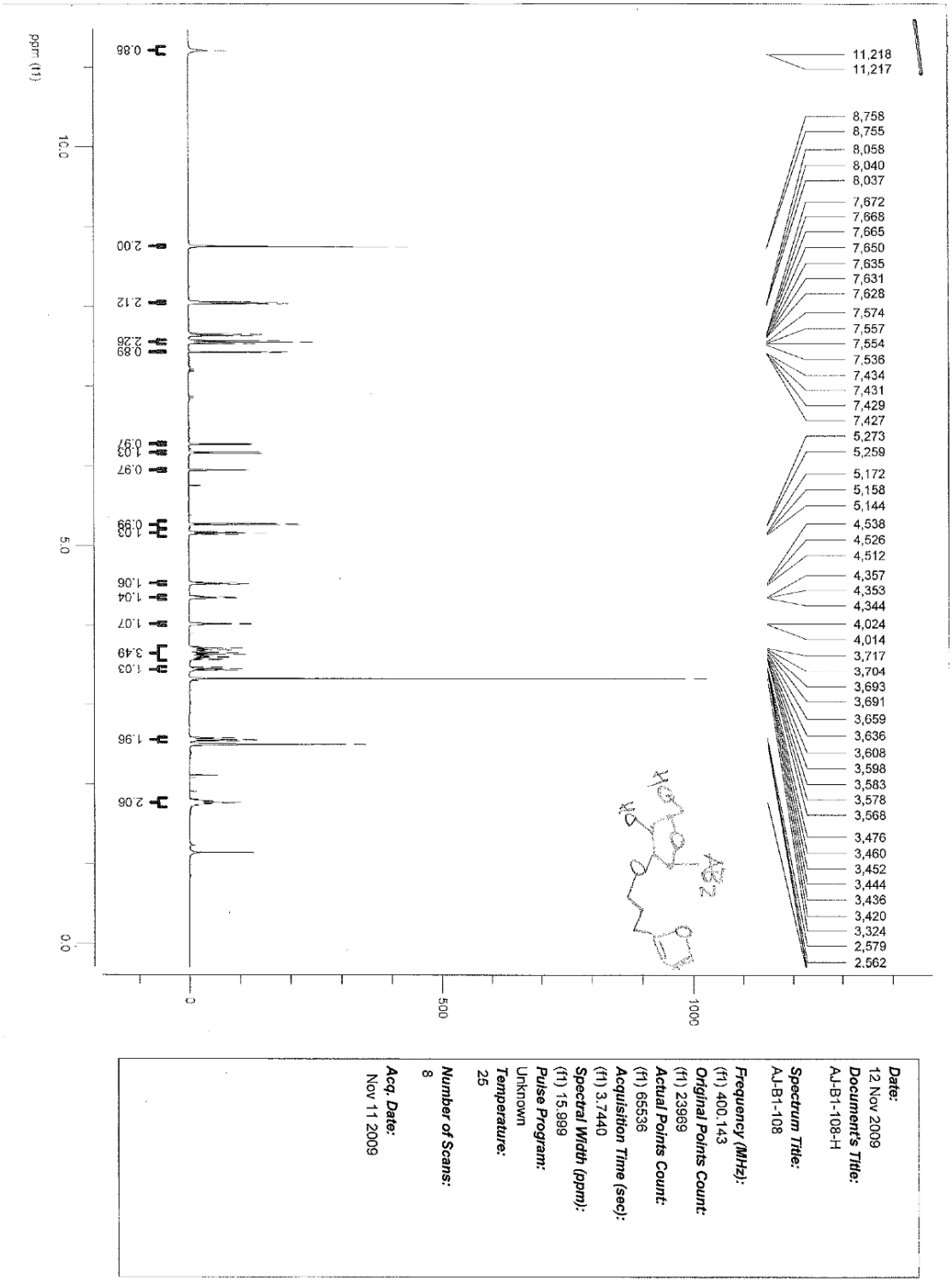
125,790
116,293
111,259
90,174
86,371
85,800
80,008
76,450
68,802
61,159
57,692
AZ2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0.86
2.00
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2.26
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0.97
1.03
0.97
0.99
1.03
1.06
1.04
1.07
3.49
1.03
1.96
2.06
0
500
1000
11,218
11,217
8,758
8,755
8,058
8,040
8,037
7,672
7,668
7,665
7,650
7,635
7,631
7,628
7,574
7,557
7,554
7,536
7,434
7,431
7,429
7,427
5,273
5,259
5,172
5,158
5,144
4,538
4,526
4,512
4,357
4,353
4,344
4,024
4,014
3,717
3,704
3,693
3,691
3,659
3,636
3,608
3,598
3,583
3,578
3,568
3,476
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3,436
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Date:
12 Nov 2009
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Actual Points Count:
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Spectral Width (ppm):
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Temperature:
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Number of Scans:
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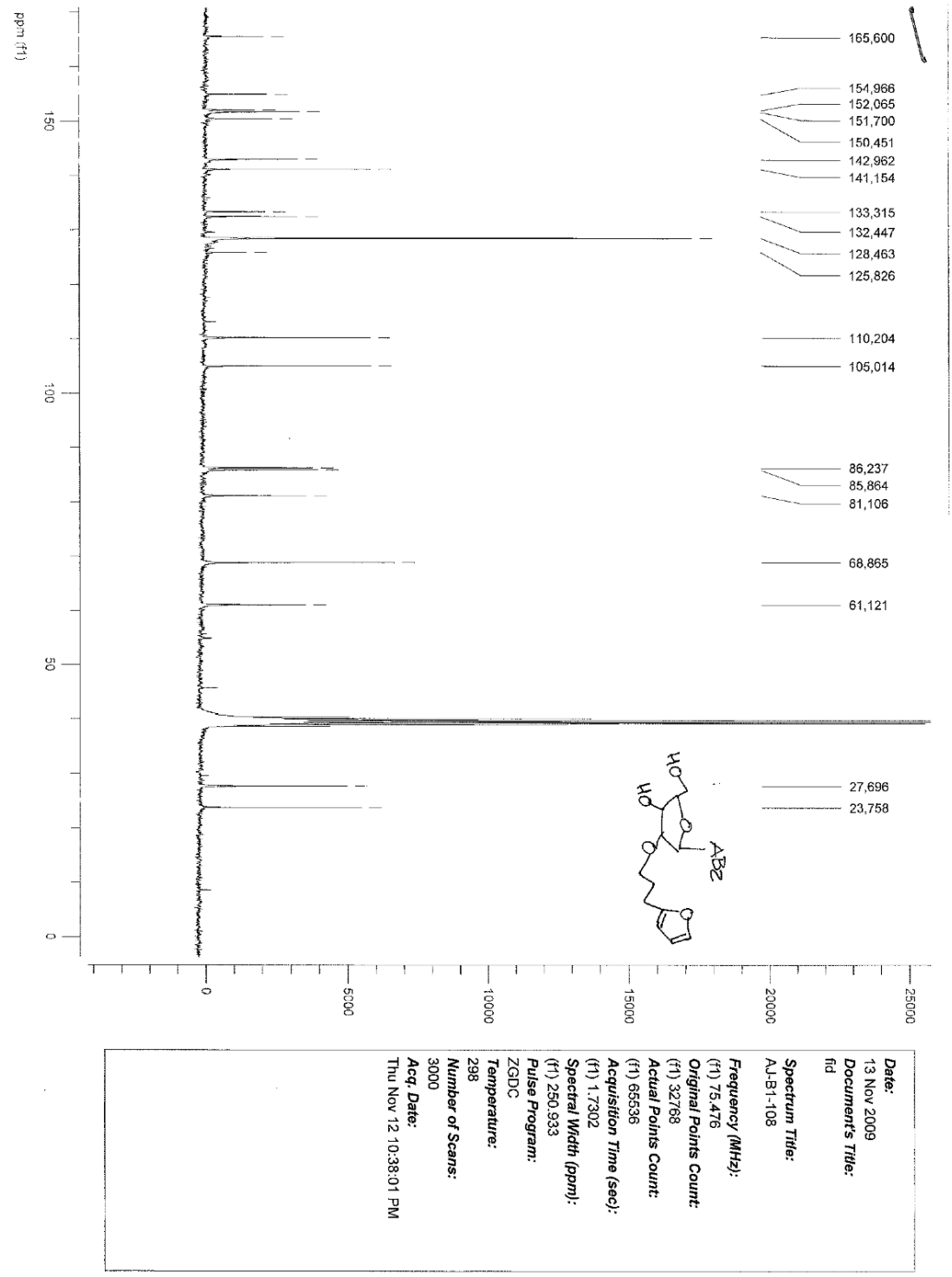
Acq. Date:
Nov 11 2009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50
0
165,600
154,966
152,065
151,700
150,451
142,962
141,154
133,315
132,447
128,463
125,826
110,204
105,014
86,237
85,864
81,106
68,865
61,121
27,696
23,758
HO
HO
ABz
0
5000
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15000
20000
25000
Date:
13 Nov 2009
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Original Points Count:
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Actual Points Count:
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Acquisition Time (sec):
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Spectral Width (ppm):
(f1) 250.933
Pulse Program:
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Temperature:
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Acq. Date:
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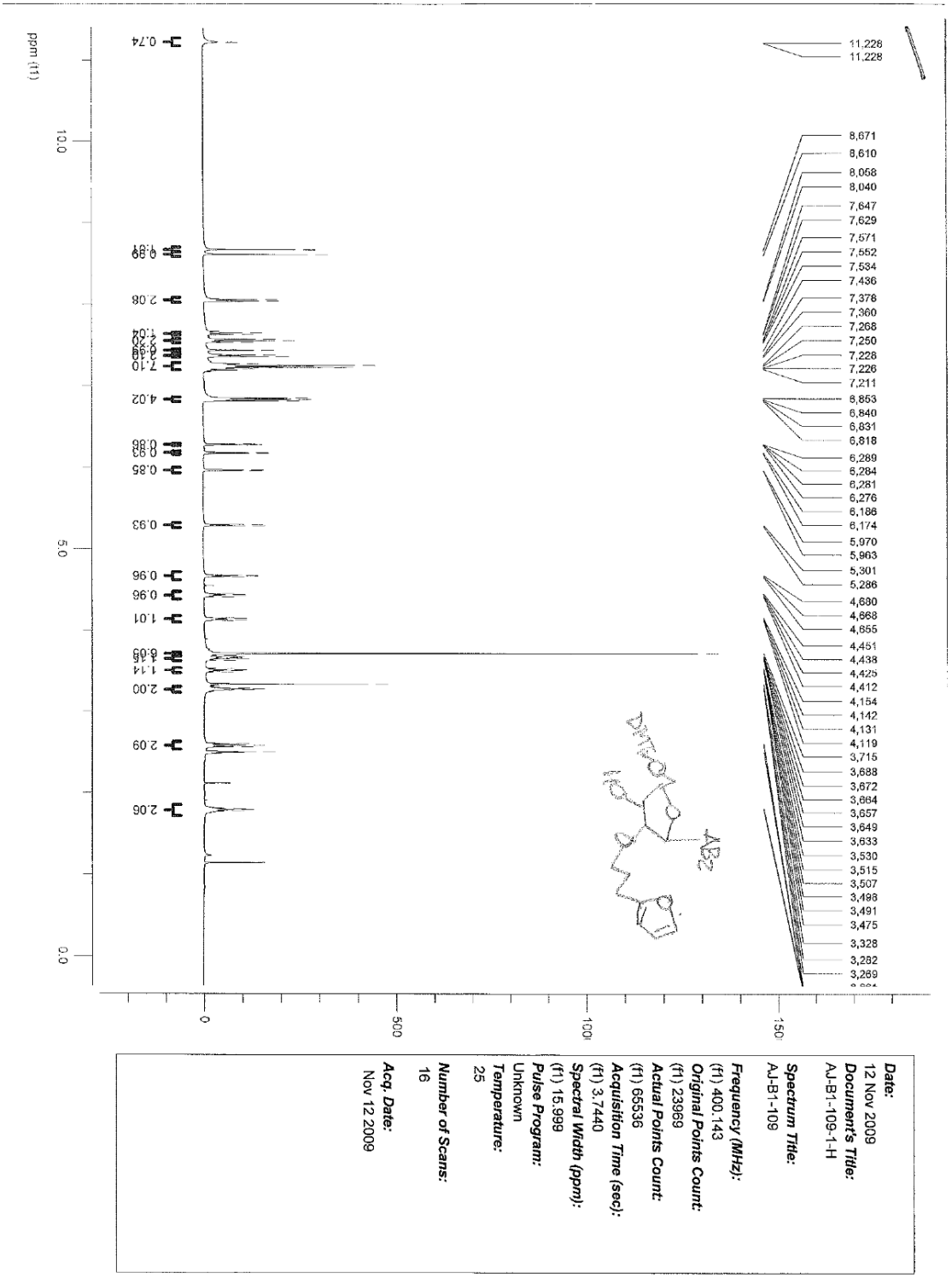

Date:
12 Nov 2009
Document's Title:
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Temperature:
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Number of Scans:
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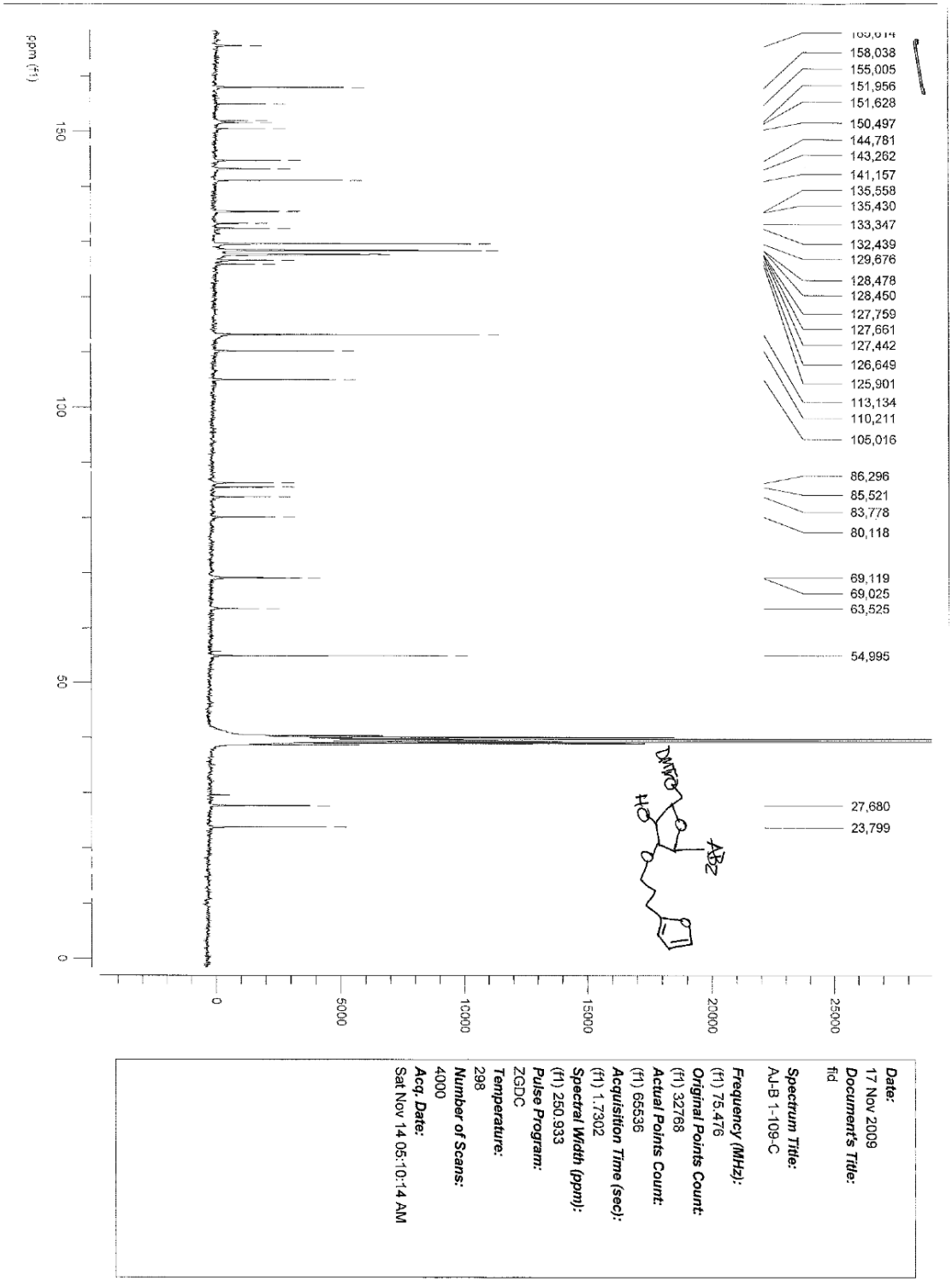
Acq. Date:
Nov 12 2009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50
0
0
5000
10000
15000
20000
25000
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155,005
151,956
151,628
150,497
144,781
143,262
141,157
135,558
135,430
133,347
132,439
129,676
128,478
128,450
127,759
127,661
127,442
126,649
125,901
113,134
110,211
105,016
86,296
85,521
83,778
80,118
69,119
69,025
63,525
54,995
27,680
23,799
HO
ABZ
Date:
17 Nov 2009
Document's Title:
fid
Spectrum Title:
AJ-B 1-109-C
Frequency (MHz):
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Original Points Count:
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Actual Points Count:
(f1) 65536
Acquisition Time (sec):
(f1) 1.7302
Spectral Width (ppm):
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Pulse Program:
ZGDC
Temperature:
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Number of Scans:
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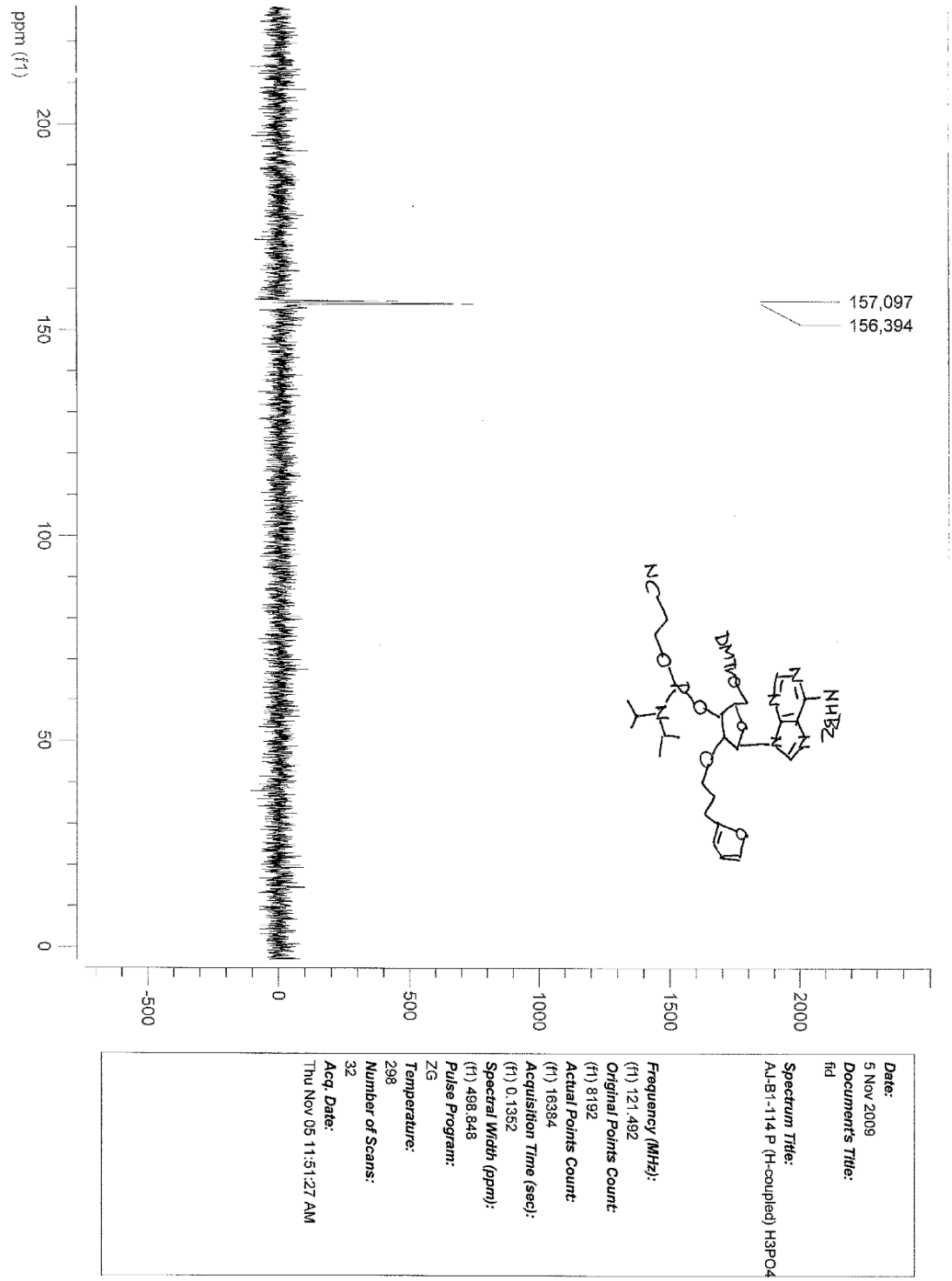
Acq. Date:
Sat Nov 14 05:10:14 AM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100
50
0
-500
0
500
1000
1500
2000
157,097
156,394
NC
DMTrO
NHBz
Date:
5 Nov 2009
Document's Title:
fid
Spectrum Title:
AJ-B1-114 P (H-coupled) H3PO4 as ref
Frequency (MHz):
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Original Points Count:
(f1) 8192
Actual Points Count:
(f1) 16384
Acquisition Time (sec):
(f1) 0.1352
Spectral Width (ppm):
(f1) 498.848
Pulse Program:
ZG
Temperature:
298
Number of Scans:
32
Acq. Date:
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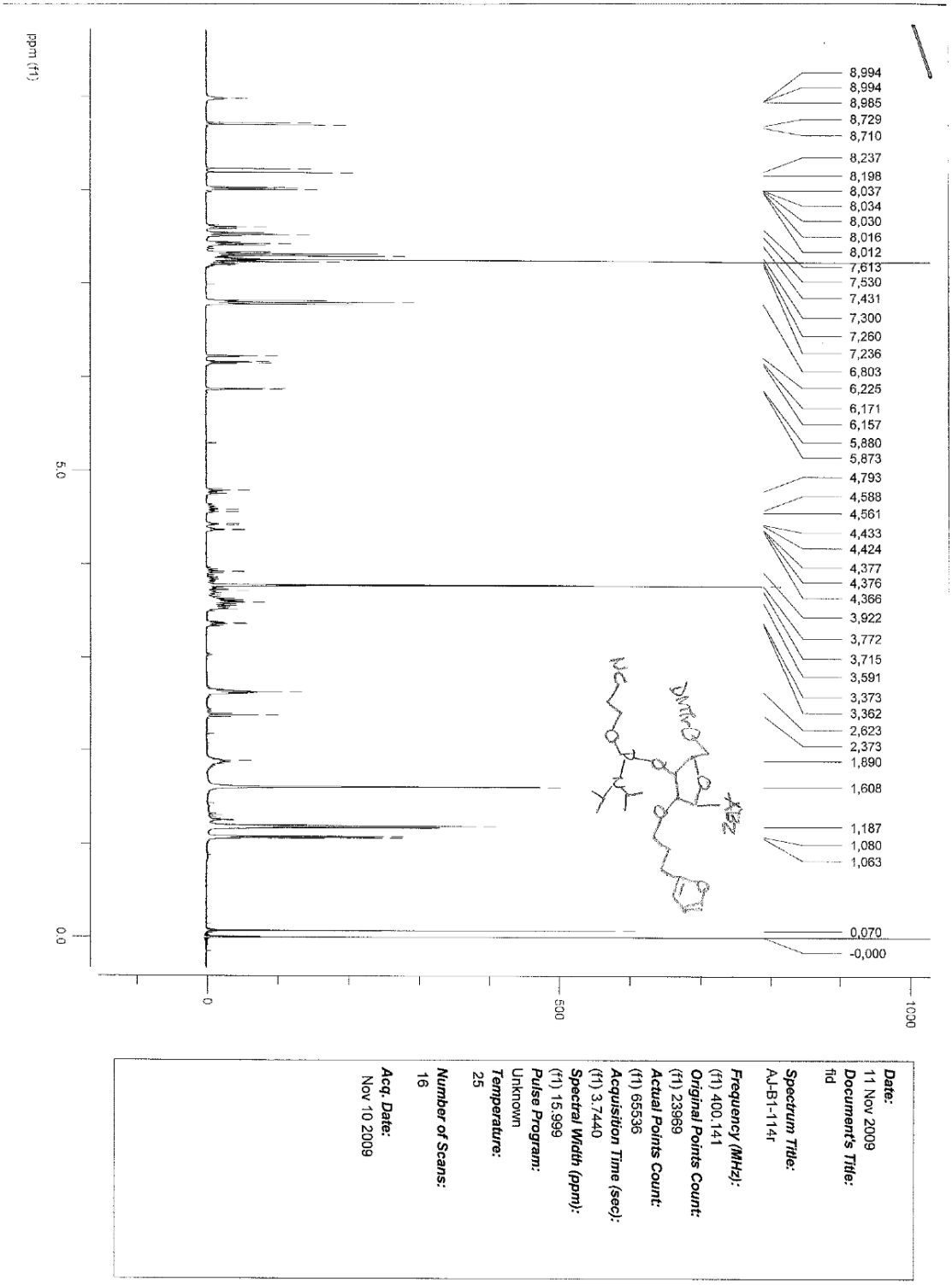
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500
1000
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8,710
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8,198
8,037
8,034
8,030
8,016
8,012
7,613
7,530
7,431
7,300
7,260
7,236
6,803
6,225
6,171
6,157
5,880
5,873
4,793
4,588
4,561
4,433
4,424
4,377
4,376
4,366
3,922
3,772
3,715
3,591
3,373
3,362
2,623
2,373
1,890
1,608
1,187
1,080
1,063
0,070
-0,000
Date:
11 Nov 2009
Document's Title:
fid
Spectrum Title:
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Frequency (MHz):
(f1) 400.141
Original Points Count:
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Actual Points Count:
(f1) 65536
Acquisition Time (sec):
(f1) 3.7440
Spectral Width (ppm):
(f1) 15.999
Pulse Program:
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Temperature:
25
Number of Scans:
16
Acq. Date:
Nov 10 2009