Click chemistry as a reliable method for the high-density post-synthetic functionalisation of alkyne-modified DNA*

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Supporting Information

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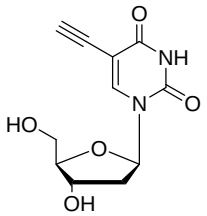
Synthesis and characterisation of building blocks.

The preparation of nucleoside **1** and its phosphoramidite **2** has been reported elsewhere.[1,2]



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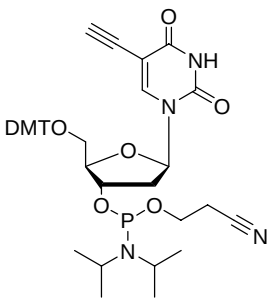

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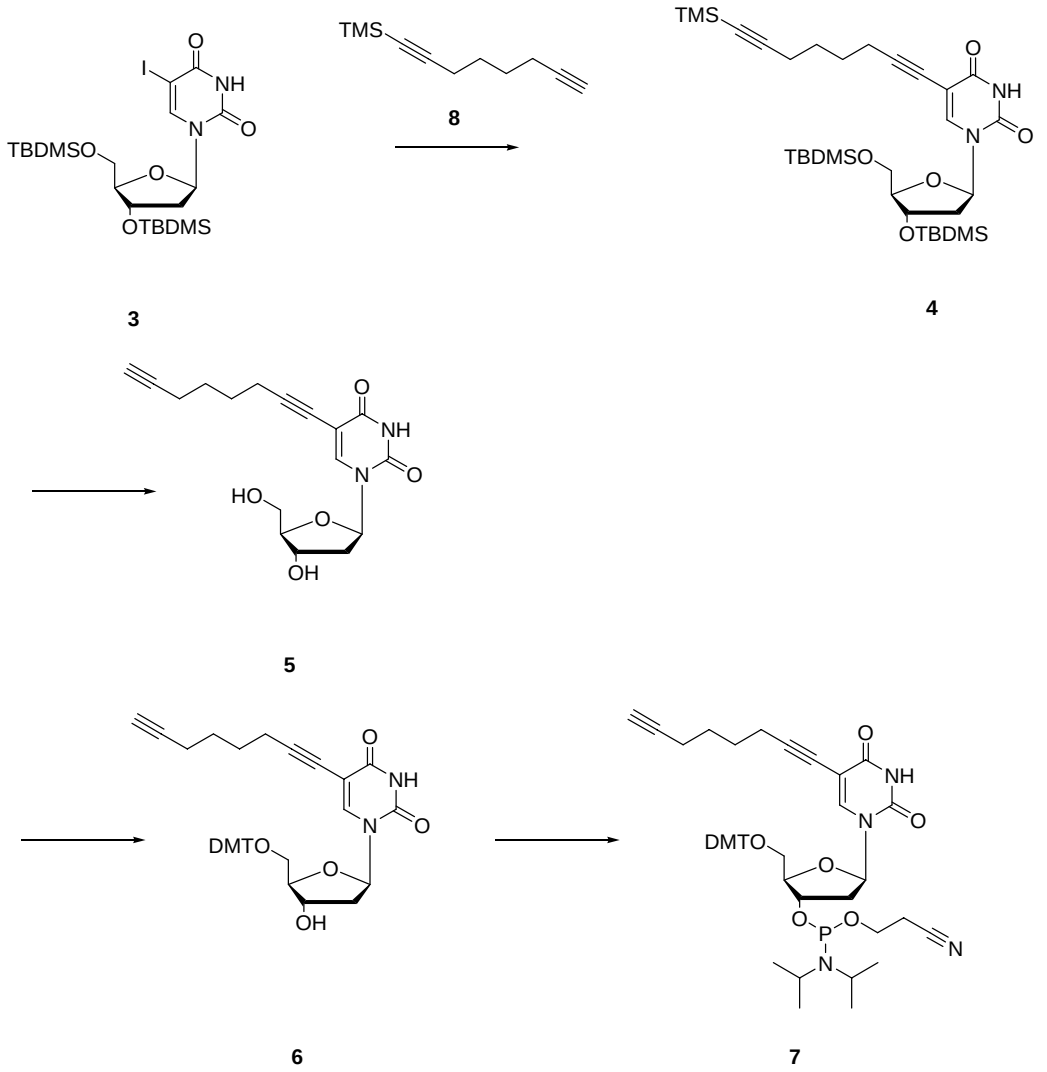
Scheme 1



Preparation of compound **4**

To a thoroughly degassed solution of **3** (2.70 g, 4.26 mmol.), $PdCl_2(PPh_3)_2$ (0.299 g, 0.426 mmol.) and CuI (0.161 g, 0.852 mmol.) in DMF (3 mL) was added degassed N,N-diisopropylethyl amine (3.9 mL, 21.3 mmol.) and the reaction mixture stirred at room temperature for 10 minutes. A degassed solution of trimethylsilyl-1,7-octadiyne **8** (0.908 g, 5.54 mmol.) in DMF (1 mL) was added dropwise to the reaction mixture over 1 hour. After complete addition, the reaction mixture was stirred at room temperature overnight. After concentration *in vacuo*, the crude mixture was diluted with ethyl acetate (200 mL) and the organic layer was washed with brine (3 x 50 mL) followed by water (50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2) eluting with a gradient of ethyl acetate : isohexane (isohexane →1 : 9) provided **4** (2.63 g, 92 %) as a pale yellow foam. ^{1}H NMR ($CDCl_3$, 600 MHz): δ – 0.06 (s, 3 H, OSiCH_3), -0.05 (s, 3 H, OSiCH_3), -0.00 (s, 12 H, OSiCH_3, Si(CH_3)$_3$), 0.01 (s, 3 H, OSiCH_3), 0.76 (s, 9 H, C(CH_3)$_3$), 0.80 (s, 9 H, C(CH_3)$_3$), 1.51 (m, 4 H, -CH_2-), 1.89 (m, 1 H, -CH_2-), 2.11 (t,

2 H, *J* = 6.5 Hz, -C≡C-C*H*$_2$-), 2.16 (m, 1 H, *H*2'/2''), 2.19 (m, 1 H, *H*2'/2''), 2.26 (t, 2 H, *J* = 6.5 Hz, -C≡C-C*H*$_2$-), 3.63 (dd, 1 H, *J* = 9.8, 1.4 Hz, *H*-5'/5''), 3.76 (dd, 1 H, *J* = 9.8, 1.4 Hz, *H*-5'/5''), 3.82 (d, 1 H, *J* = 1.4 Hz, *H*-4'), 6.16 (t, 1 H, *J* = 6.8 Hz, *H*-1'), 7.8 (s, 1H, *H*-6), 9.64 (s, 1 H, N-*H*). ^{13}C NMR ($CDCl_3$, 150.8 MHz): δ -5.7 (Si*C*H$_3$), -5.5 (Si*C*H$_3$), -5.0 (Si*C*H$_3$), -4.8 (Si*C*H$_3$), 0.0 (Si(*C*H$_3$)$_3$), 17.8 (Si*C*(CH$_3$)$_3$), 18.2 (Si*C*(CH$_3$)$_3$), 19.0 (-*C*H$_2$-), 19.2 (-*C*H$_2$-), 25.6 (SiC(*C*H$_3$)$_3$), 25.9 (SiC(*C*H$_3$)$_3$), 27.4 (C≡C-*C*H$_2$-), 27.9 (C≡C-*C*H$_2$-), 41.7 (*C*-2'), 62.9 (C-5'), 71.6 (C≡*C*), 72.0 (C-3'), 84.4 (C≡*C*), 85.4 (C-1'), 88.0 (C-4'), 94.2 (C≡*C*), 100.6 (C-5), 106.8 (C≡*C*), 141.3 (C-6), 149.4 (C-2), 162.1 (C-4). HRMS (ESI, +ve) calcd. for $C_{32}H_{56}N_2NaO_5Si_3$ 655.3389 $[M+Na]^+$ found 655.3400.

Preparation of compound **5**

To a cooled solution (0 °C) of **4** (2.10 g, 3.40 mmol.) in THF (5 mL) was added TBAF (1.0 M in THF, 10.9 mL, 10.9 mmol.) under a nitrogen atmosphere. The reaction mixture was stirred for 3 hours, quenched with glacial acetic acid (1.0 mL) and concentrated *in vacuo*. Column chromatography (flash silica) eluting with 10 % methanol in ethyl acetate afforded **5** (1.18 g, 85 %) as a colourless oil. ^{1}H NMR (d$_6$-DMSO, 400 MHz) δ 1.57 (m, 4 H, -C*H*$_2$CH$_2$-), 2.11 (m, 2 H, *H*-2'/2''), 2.19 (m, 2 H, -C≡C-C*H*$_2$-), 2.38 (m, 2 H, -C≡C-C*H*$_2$-), 2.75 (m, 1 H, -C≡C-*H*), 3.55-3.65 (m, 2 H, H-5'/5''), 3.78 (dd, 1 H, *J* = 6.6, 3.5 Hz, H-4'), 4.23 (m, 1 H, *H*-3'), 5.06 (t, 1 H, J = 4.8 Hz, O-*H*), 5.22 (b4d, 1 H, *J* = 4.0 Hz, O-*H*), 6.10 (t, 1 H, *J* = 6.5 Hz, *H*-1'), 8.10 (s, 1 H, *H*-6), 11.3 (bs, 1 H, N-*H*). ^{13}C NMR (d$_6$-DMSO, 100.6 MHz): δ 17.1 (C≡C-*C*H$_2$CH$_2$), 18.2 (C≡C-*C*H$_2$CH$_2$), 27.0 (C≡C-CH$_2$*C*H$_2$), 27.1 (C≡C-CH$_2$*C*H$_2$), 39.9 (*C*-2'), 60.9 (C-5'), 70.1 (C-3'), 71.2 (C≡*C*-H), 72.9 (C≡*C*), 84.1 (C-1'), 84.5 (C-4'), 87.4 (C≡*C*), 92.8 (C≡*C*), 98.9 (C-5), 142.6 (C-6), 149.4 (C-2), 161.6 (C-4). HRMS (ESI, -ve) calcd. for $C_{17}H_{19}N_2O_5$ 331.1299 $[M- H]^-$ found 331.1295.

161.639, 149.353, 142.573, 98.879, 92.791, 87.452, 84.485, 84.179, 72.903, 71.217, 70.075, 60.900, 39.915, 27.137, 27.007, 18.207, 17.132,

Preparation of compound **6**

To a solution (0 °C) of **5** (1.58 g, 4.97 mmol.) in dry pyridine (5 mL) was added DMAP (cat.), followed by 4,4'-dimethoxytriphenylmethyl chloride (1.85 g, 5.47 mmol.) under a nitrogen atmosphere and the reaction mixture was stirred overnight. After concentration *in vacuo*, the crude mixture was diluted with ethyl acetate (200 mL) and the organic layer was washed with brine (3 x 50 mL) followed by water (50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2) eluting with a gradient of ethyl acetate : isohexane (+ 1 % pyridine) (1:9 → 1 : 1) provided **6** as a pale yellow foam (1.41 g, 46 %). ^{1}H NMR (d$_2$-CH_2Cl_2, 400 MHz): δ 1.42 (m, 4 H, -C*H*$_2$CH$_2$-), 1.96 (t, 1 H, *J* = 2.5 Hz, -C≡C-*H*), 2.07 (td, 2 H, *J* = 6.8, 2.5 Hz, -C*H*$_2$C≡C-H), 2.15 (td, 2 H, *J* = 7.1, 1.6 Hz, -C*H*$_2$C≡C-), 2.28 (m, 1 H, *H*-2'/2''), 2.46 (m, 1 H, *H*-2'/2''), 3.34 (d, 2 H, *J* = 3.3 Hz, *H*-5'/5''), 3.79 (s, 6 H, Ar-O-C*H*$_3$), 4.08 (dd, 1 H, *J* = 6.2, 3.3 Hz, *H*-4'), 4.55 (m, 1 H, *H*-3'), 6.29 (dd, 1 H, *J* = 7.7, 5.8 Hz, *H*-1'), 6.87 (d, 4 H, *J* = 8.5 Hz, Ar-*H*), 7.24 (m, 1 H, Ar-*H*), 7.31 (m, 2 H, Ar-*H*), 7.36 (dd, 4 H, *J* = 8.5, 1.9 Hz, Ar-*H*), 7.45 (dd, 2 H, *J* = 8.6, 1.5 Hz, Ar-*H*), 7.97 (s, 1 H, *H*-6). O-H and N-H not observed. ^{13}C NMR

(d_2-CH_2Cl_2, 150.8 MHz): δ 20.1 (C≡C-*C*H_2CH$_2$), 21.2 (C≡C-*C*H_2CH$_2$), 29.7 (C≡C-*C*H_2-), 30.0 (C≡C-*C*H_2-), 45.7 (*C*-2'), 59.6 (O-*C*H_3), 67.9 (C-5'), 72.6 (C≡*C*-H), 75.6 (C-3'), 76.5, 88.4, 89.6 (C-1'), 90.9 (C-4'), 91.3, 98.6, 105.0, 117.6 (4 C), 128.0, 128.2, 131.3 (2 C), 132.2, 132.4, 134.3, 134.4, 139.9, 140.0, 140.4, 146.0, 146.2, 149.1, 153.8, 153.9, 163.0, 163.3. HRMS (ESI, +ve) calcd. for $C_{38}H_{38}N_2NaO_7$ 657.2571 $[M+Na]^+$ found 657.2570.

Preparation of compound **7**

To a solution (0 ° C) of **6** (0.200 g, 0.32 mmol.) in dry dichloromethane (5 mL) was added triisopropyl tetrazolide (0.068 g, 0.40 mmol.) followed by 2-cyanoethyl tetraisopropyl phosphoramidite (306 μL, 0.97 mmol.) under a nitrogen atmosphere and the reaction mixture was stirred at room temperature for 4 hours. After concentration *in vacuo*, the crude mixture was purified by flash column chromatography (SiO_2) eluting with ethyl acetate : isohexane (1 : 3; + 1 % pyridine) to afford **7** (0.188 g, 70 %) as a white solid. ^{13}P NMR (d_2–CD_2Cl_2, 80.9 MHz): δ 149.9, 149.6. MS (MALDI-TOF, +ve): 857 [*M* + Na]$^+$.

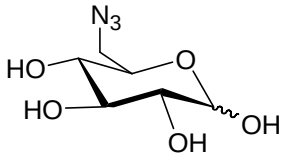
Preparation of compound **8** [3]

To a solution of 1,7-octadiyne (12.86 mL, 96.9 mmol) in dry THF (150 mL) at -78 C was added LiHMDS (1 M, 96.9 mL). The solution was stirred for 30 min, and TMS-Cl (12.3 mL, 96.9 mmol) was added over 30 min. The solution was warmed to r.t. and stirred for 4 h, quenched with water (100 mL), extracted with diethyl ether and washed with 1 M HCl. The organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was distilled (bath temp = 120 °C, 4 mbar) to give 7.25 g (40.7 mmol, 42%) of **8** as a clear liquid. R_f ($CHCl_3$/Methanol 50:1): 0.47. ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 0.12 (s, 9H, $Si(CH_3)_3$), 1.61 (quin, 4H, *J* = 3.4 Hz, C_{sp3}-CH_2-CH_2-C_{sp3}), 1.93 (t, 1H, *J* = 2.7 Hz, CCH), 2.16-2.26 (m, 4H, CC-CH_2).
HRMS (EI-). 177.1106 calcd. for $C_{10}H_{17}Si$ $[M-H]^-$: 177.1099

Solid phase synthesis of alkyne-modified ODNs

ODNs were prepared by the DMT- and β-(cyanoethyl) phosphoramidite method on CPG supports with an Expedite DNA synthesiser (Applied Biosystems). A double coupling protocol and elongated de-blocking times were applied for the coupling of modified bases and the coupling time of each base coupled after the modified base was elongated. As activator, benzylthiotetrazole (BTT) gave the best coupling yields. After automated synthesis, ODNs were cleaved from the solid support by soaking in concentrated aqueous ammonia : ethanol solution (3 : 1) for 3 hours at 40 ºC. The aqueous ammonia was removed by evaporation, and the crude ODN were purified by reverse phase HPLC, lyophilised and redissolved in pure water. UV and MALDI-TOF spectroscopy determined the concentration and purity of each ODN respectively. ODN-**9** was synthesised with the last DMT-group on, cleaved off the solid support as mentioned above and purified by HPLC. Then the DMT-group was cleaved by absorbing the DNA on a SePac-cartridge (Waters) and treating with 2% TFA before eluting. The DNA from the column was re-purified by HPLC.

Azide building blocks



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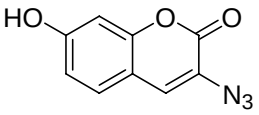

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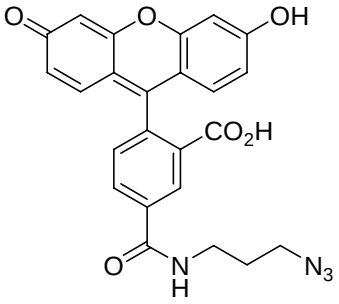

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The azides **9**,[4] **10** [5] and **11** [6,7] were prepared according to literature procedures.

Click procedure for short DNA oligos

Using TCEP:

To 25 µL of a 0.5 mM DNA solution (12.5 nmol) in water 6.25 µL of an azide solution (0.1 N, 625 nmol, 50 eq.) and 10 µL of a solution containing Cu(II)-salt ($CuSO_4$) and Ligand in a 1:1 ratio in 4:3:1 water : DMSO : tBuOH was added (0.05 N, 250 nmol, 20 eq). The mixture was vortexed and as the last component 5 µL of a freshly prepared TCEP solution in water was added (0.1 N, 500 nmol, 40 eq.). The solution was shaken at 15 °C over night. The reaction was diluted with water (200 µL) and used for gel electrophoresis without further purification.

Using CuBr:

To 25 µL of a 0.5 mM DNA solution (12.5 nmol) in water 6.25 µL of an azide solution (0.1 N, 625 nmol, 50 eq.) and 10 µL of a freshly prepared solution containing CuBr and Ligand in a 1:1 ration in 4:3:1 water : DMSO : tBuOH was added (0,05 N, 250 nmol, 20 eq.). The mixture was vortexed and shaken at 15 °C for 1 hour. The reaction was diluted with water (200 µL) and used for the gel without further purification.

MALDI analysis:

For MALDI analysis sample volumes of approx. 2 µL were desalted with dialysis membranes (Millipore) before spotting them onto the target with crystallized matrix (3-hydroxypicolinic acid, citric acid and 18-crown-4).

If the result was not satisfying the whole sample was desalted using SePak-cartridges (Waters).

Gel electrophoreses

The click reactions on the short DNA oligomers was analyzed by 22.5 % TBE-urea-PAGE using the Protean-Mini-Gel system (Bio-Rad). The bands were visualized using a Raytest LAS-3000 Imager with appropriate cut-off filters (515 nm for SYBR

Green and fluorescein, 460 nm for coumarin, 605 nm for ethidium bromide). As the light source 312 nm UV or 460 nm epi-illumination was used)

To wash off the excess fluorescein azide the gels were shaken in 1:1 water : methanol for 1 hour and then in pure water for another hour before taking the image.

PCR conditions

For PCR amplification two different templates were used. Template **A** is the Gateway expression vector *pExp007-pol η* with the polymerase η gene from Rad30 *S. cervisiea.*[8] Template **B** is the Gateway entry vector *pENTR201-FPG* with the FPG repair protein gene from *Lactococcus lactis subsp. cremoris*
The table shows the combination of primers used and the length of the resulting PCR product.

Primer 1 ODN-**9**
Primer 2 5'-TTA ATT GAA TTC GAT TTG GGC CGG ATT TGT TTC-3'
Primer 3 5'-ATT AAG AAT TCT TTT ATG CTA TCT CTG ATA CCC TTG-3'
Primer 4 5'-TGA TGC CCT TGT ACG CAA CTG-3'
Primer 5 5'-GGA AGA TGT AAC TTG TTT CTT CTG-3'
Primer 6 5'-GGG TTA TTG TCT CAT GAG CG-3'
Primer 7 ODN-**10**
Primer 8 5'-GGG GAC AAG TTT GTA CAA AAA AGC AGG CTT CAT GCC AGA GTT ACC AGA AG-3'
Primer 9 5'-GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTA TTT TTG CTG ACA GAA TGG G-3'

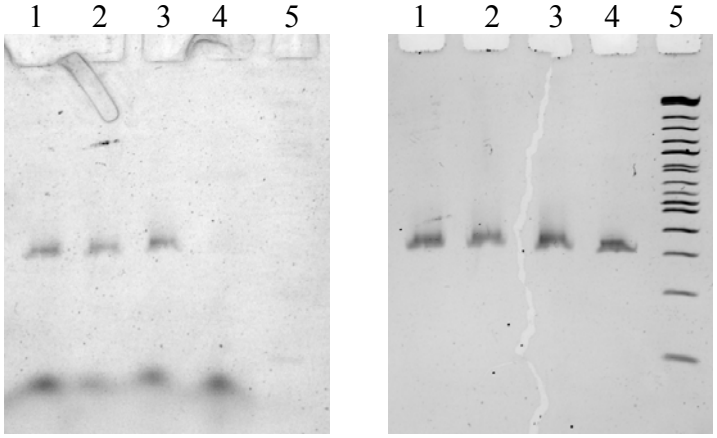
Forward Primer	Reverse Primer	Template	PCR product length (bp)
Primer 1	Primer 3	A	305
Primer 2	Primer 3	A	305
Primer 1	Primer 4	A	934
Primer 2	Primer 4	A	934
Primer 1	Primer 5	A	1398
Primer 2	Primer 5	A	1398
Primer 1	Primer 6	A	2149
Primer 2	Primer 6	A	2149
Primer 7	Primer 9	B	800
Primer 8	Primer 9	B	822

PCR conditions: The PCR was run with 0.18 µM of each dNTP, 0.09 µM of each primer, 300 ng of template and 1.25 U Taq Polymerase (Promega) in the reaction buffer provided by the supplier (50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton® X-100, 1.5mM $MgCl_2$). Total Volume was 22 µL. For production PCRs the volume of the PCR was doubled keeping all concentrations constant.
Temperature program for 300 bp PCR Products : 96°C for 1:30 min, then 29 cycles of 94 °C for 0:10 min 58 °C (-0.3 °C each cycle) for 0:15 min, 72 °C for 1:00 min; then 24 cycles of 95 °C for 0:10 min, 55 °C for 0:15 min, 72 °C for 1:00 min. For the long PCR products the elongation time was increased to 2:00 min.

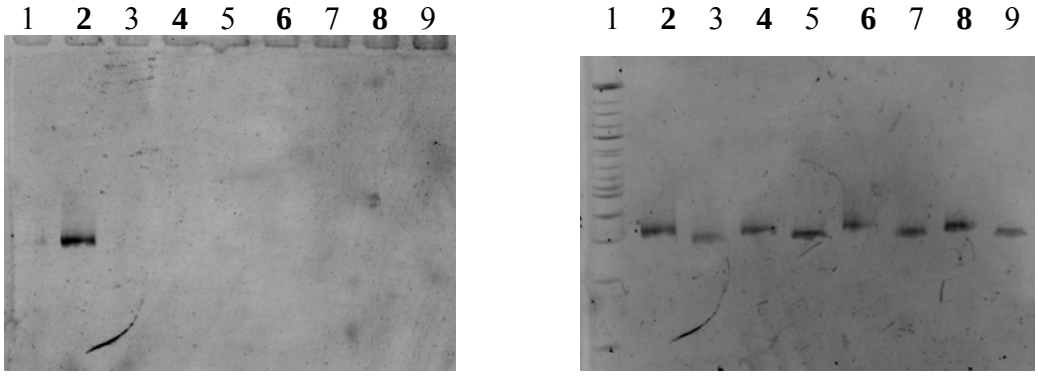
The PCR product was purified using QIAquick PCR Purification Kit (QIAGEN) and the concentration determined by UV absorption.

Click on PCR product

Click on the 300 bp product: To 10 µL DNA solution (1 to 4 pmol DNA, 10 mM Tris), 10 µL fluorescein azide solution (5 mM, diluted with 10 mM Tris with 5% tBuOH from a stock of 0.1 N in DMSO) and 10 µL precomplexed Cu(I) was added (10 mM; 1 mg CuBr (99.99%) dissolved in 700 µL 10 mM Ligand in tert-BuOH : DMSO 1:3) The sample was shaken at 37 °C for 2 hours. Then formamide buffer was added and the samples analyzed with a 5% PAGE gel.
Control experiments showed that the reaction is over in less than 30 minutes.



Lane 1: 30 min, lane 2: 1h, lane 3: 2h, lane 4: 2h but without CuBr, lane 5: DNA 100 bp ladder (New England Biolabs) The lower spot is due to fluorescein azide in the reaction mixture. On the right is the same gel stained with SYBR Green II to visualise all DNA fragments.



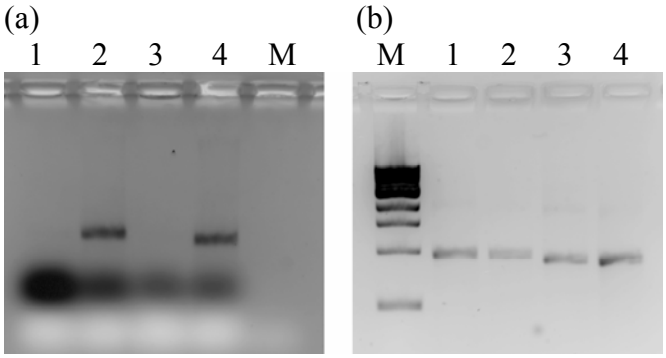
The gel shows different control experiments

lane	**2**	3	**4**	5	**6**	7	**8**	9
Cu/Lig	+	+	-	-	+	+	-	-
Azide	+	+	+	+	-	-	-	-

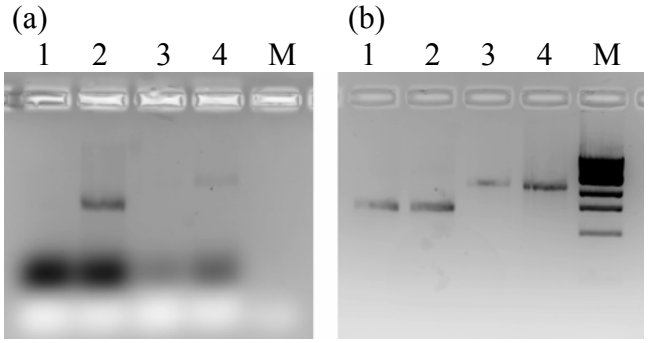
Lane 1 is 2-Log DNA Ladder (New England Biolabs). Lanes 2,4,6,8 contain alkyne-primer DNA (in bold) with one Biotin on the reverse primer. Lanes 3,5,7,9 contain normal DNA without any modification.
The right gel is stained with SYBR Green to visualize all DNA strands.

Click on the long DNA strands:

To 10 µL DNA solution (1 to 4 pmol DNA, 10 mM Tris), 10 µL fluorescein azide solution (5 mM, diluted with 10 mM Tris with 5% tBuOH from a stock of 0.1 N in DMSO) and 10 µL precomplexed Cu(I) was added (10 mM; 1 mg CuBr (99.99%) dissolved in 700 µL 10 mM Ligand in tert-BuOH : DMSO 1:3) The sample was shaken at 20 °C for 2 hours. Then reaction mixture was purified using QIAquick PCR Purification Kit (QIAGEN).

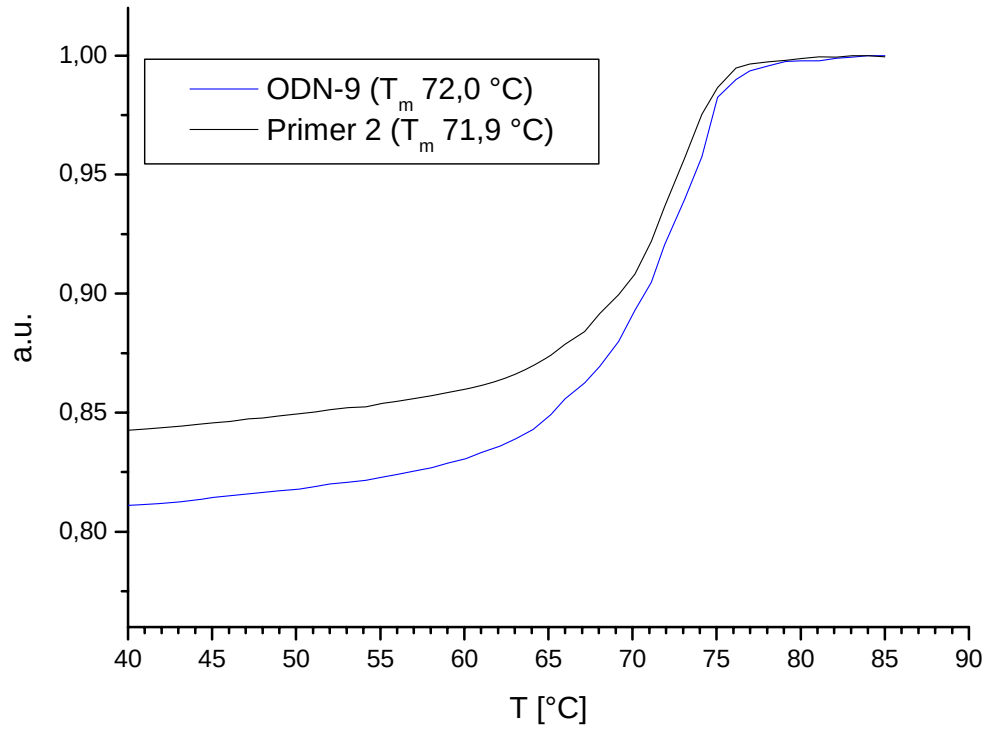


2 % Agarose gel of the click on PCR products using following primer combinations: lane 1: 2+4, lane 2: 1+4 both template **A**, lane 3: 8+9, lane 7+9 both template **B** (a) Gel without staining using 0,6 pmol DNA per lane (ca. 600 ng). The black spots on the front are free fluorescein azide, the white one bromphenolblue. The picture was taken using 460 epi-illumination with 515 nm cutoff filter. (b) Agarose gel of click samples using the same conditions stained with ethidium bromide using less DNA due to overloading. The picture was taken using 312 nm UV through and 605nm cutoff filter. As Marker Quick-Load™ 1 kb DNA Ladder (New England Biolabs) was used.



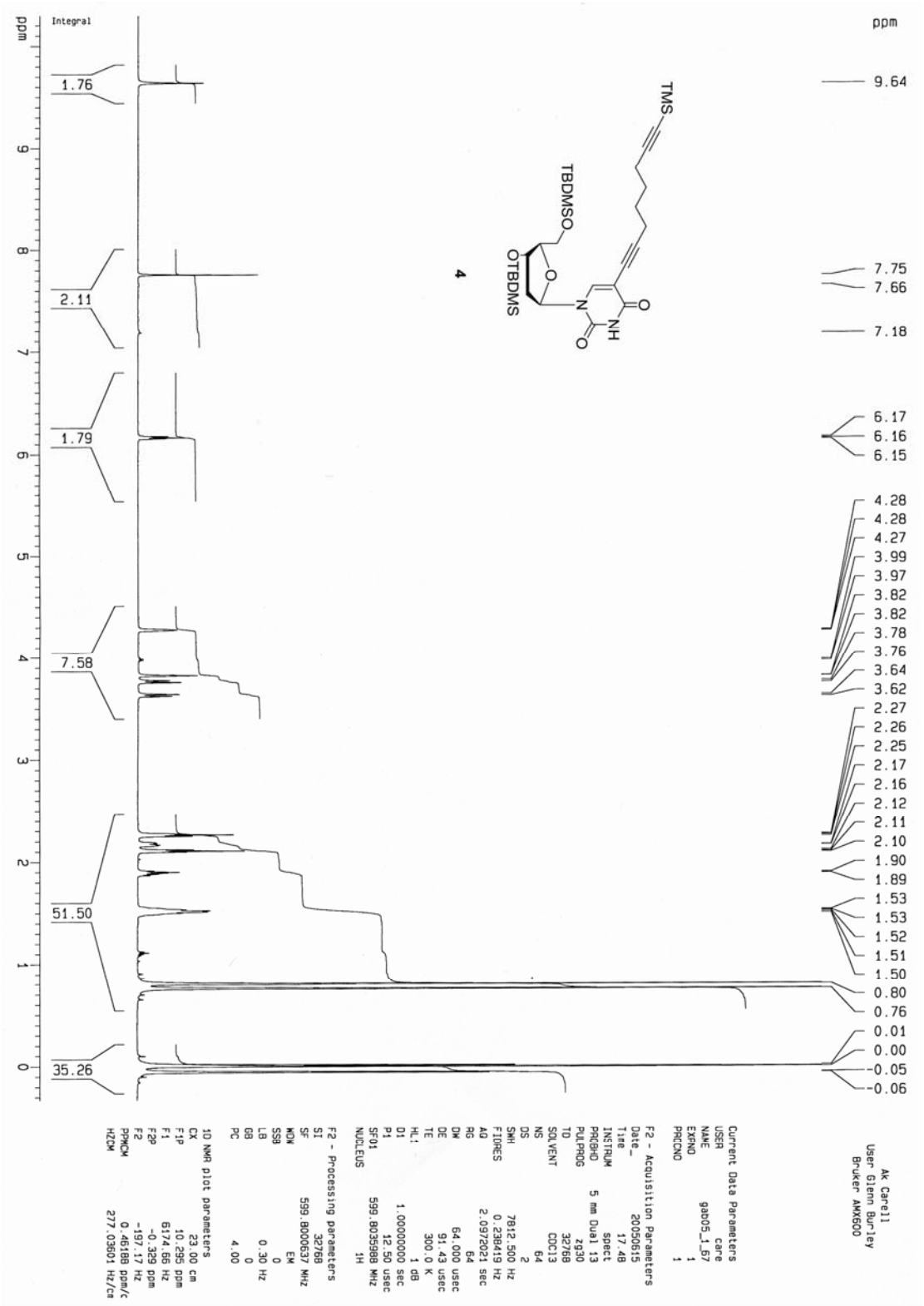
2% Agarose gel of the click on PCR products using the following primer combinations: lane 1: 2+5, lane 2: 1+5, lane 3: 2+6, lane 4: 1+6 all template **A**; All gel conditions are identical to the conditions above.

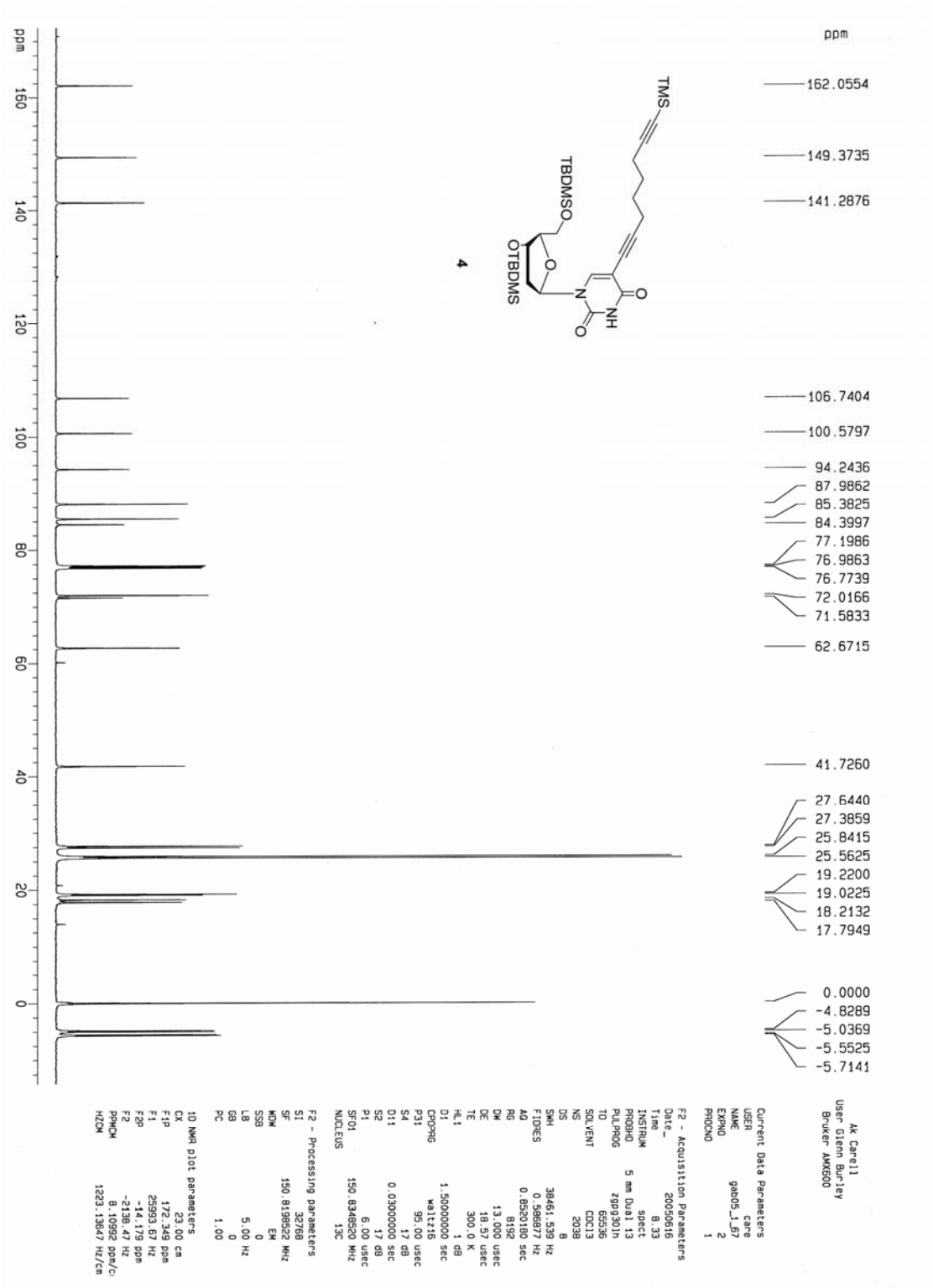
Melting point analysis of ODN-9



The melting points were measured using a Cary 100 Bio spectral photometer. The probes (3 µM DNA, 150 mM NaCl, 10 mM Tris pH 7.4) were cycled five times between 85 °C and 0°C (0.5 °C/min) starting at 85 °C. The melting points were derived from the average of five measurements at 260 nm corrected by the background absorption.

NMR data of compounds 4-7

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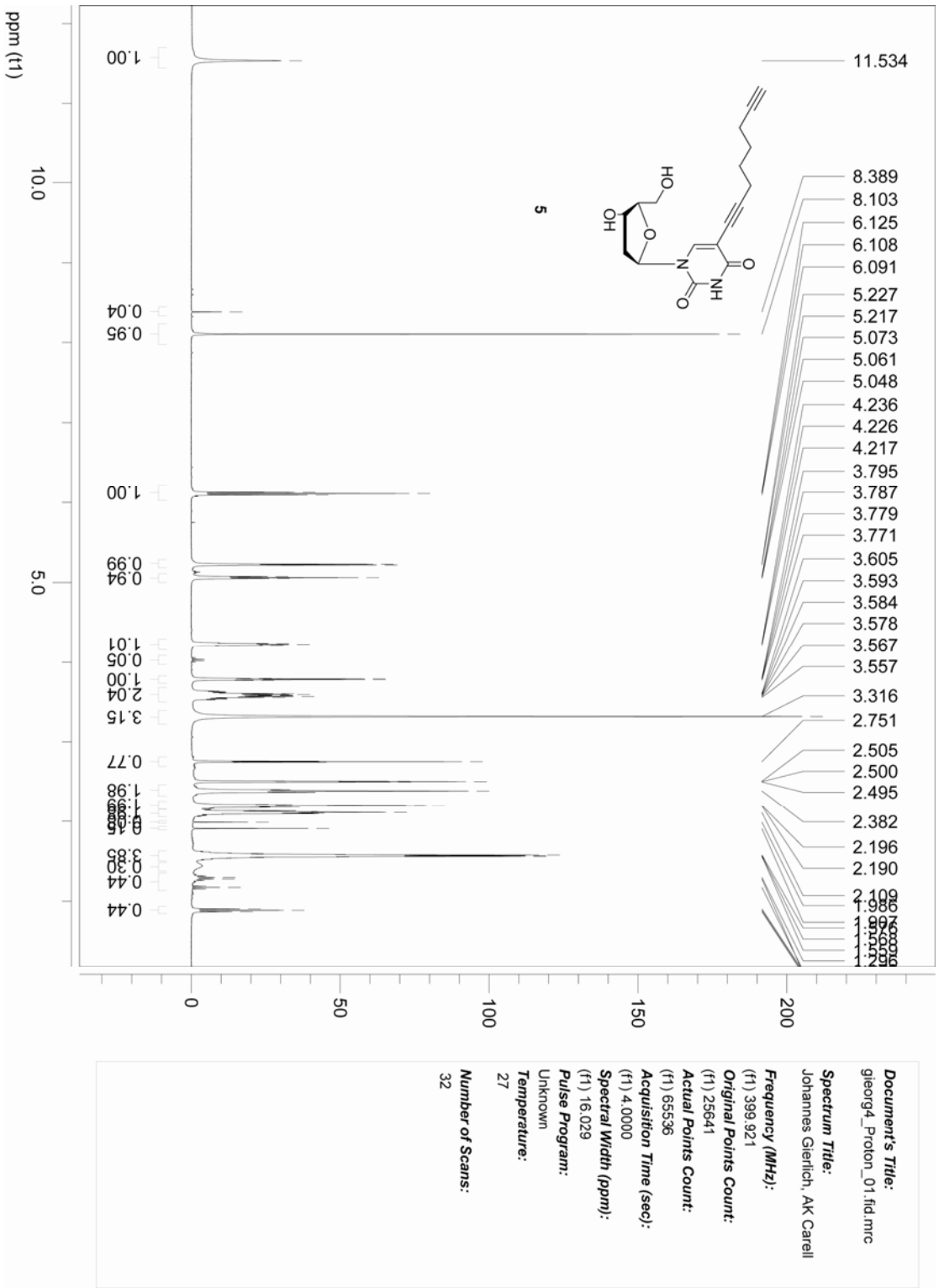

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gleorg4_Proton_01.fid.mrc

Spectrum Title:
Johannes Gierlich, AK Carell

Frequency (MHz):
(f1) 399.921

Original Points Count:
(f1) 25641

Actual Points Count:
(f1) 65536

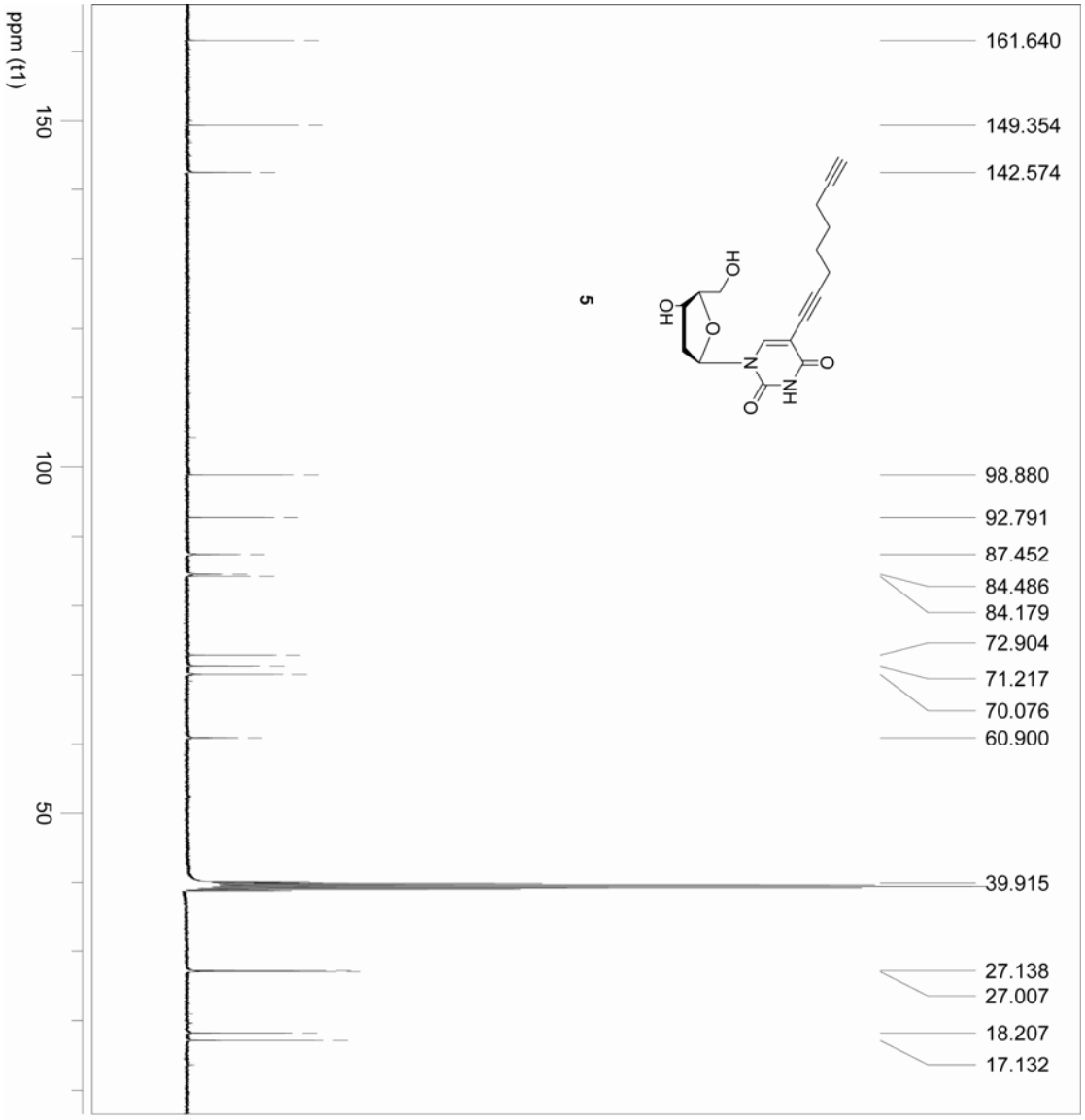
Acquisition Time (sec):
(f1) 4.0000

Spectral Width (ppm):
(f1) 16.029

Pulse Program:
Unknown

Temperature:
27

Number of Scans:
32



Document's Title:
gleorg4_Carbon_01.fid.mrc

Spectrum Title:
Johannes Gierlich, AK Carell

Frequency (MHz):
(f1) 100.570

Original Points Count:
(f1) 39216

Actual Points Count:
(f1) 131072

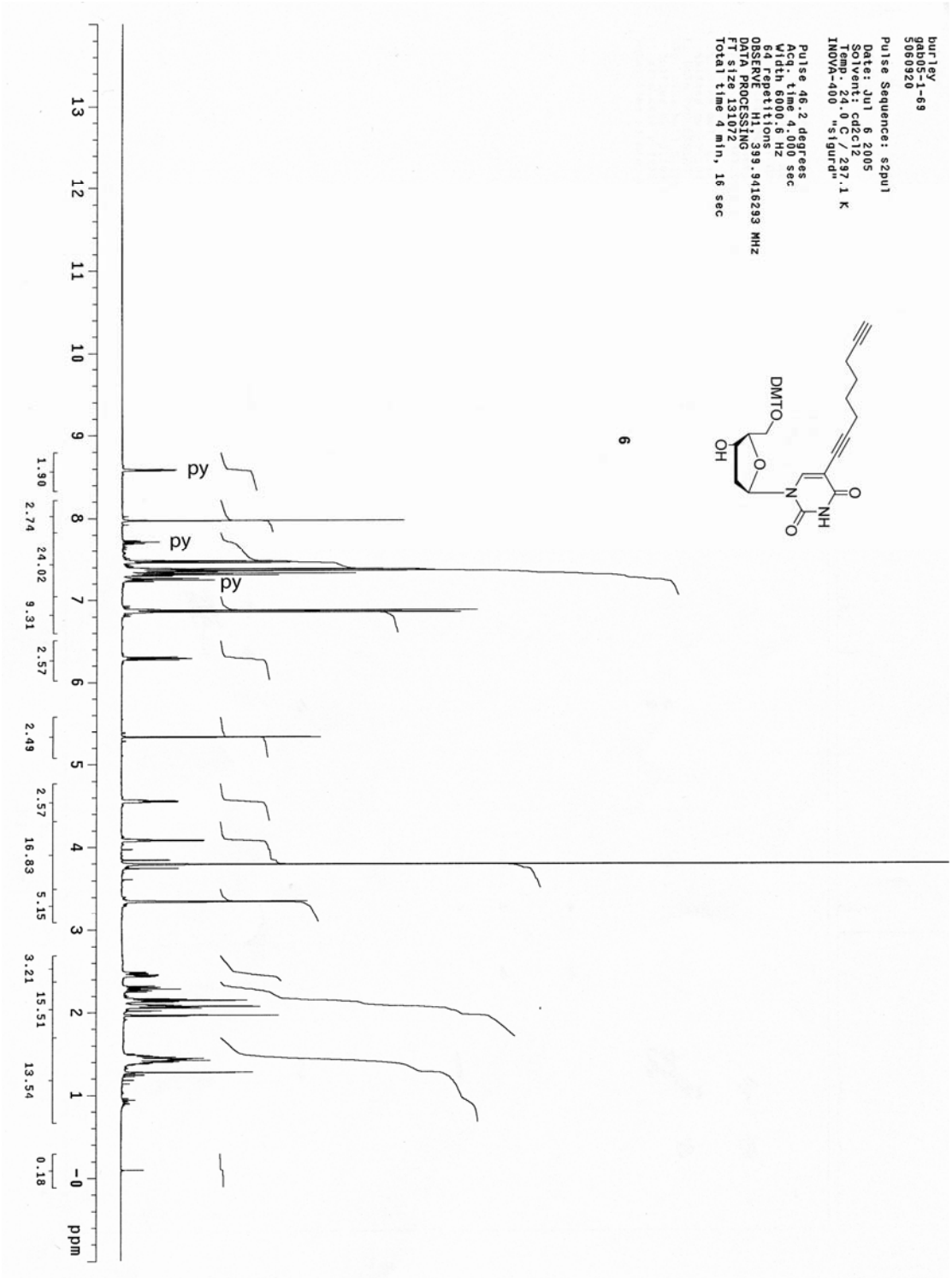
Acquisition Time (sec):
(f1) 1.6000

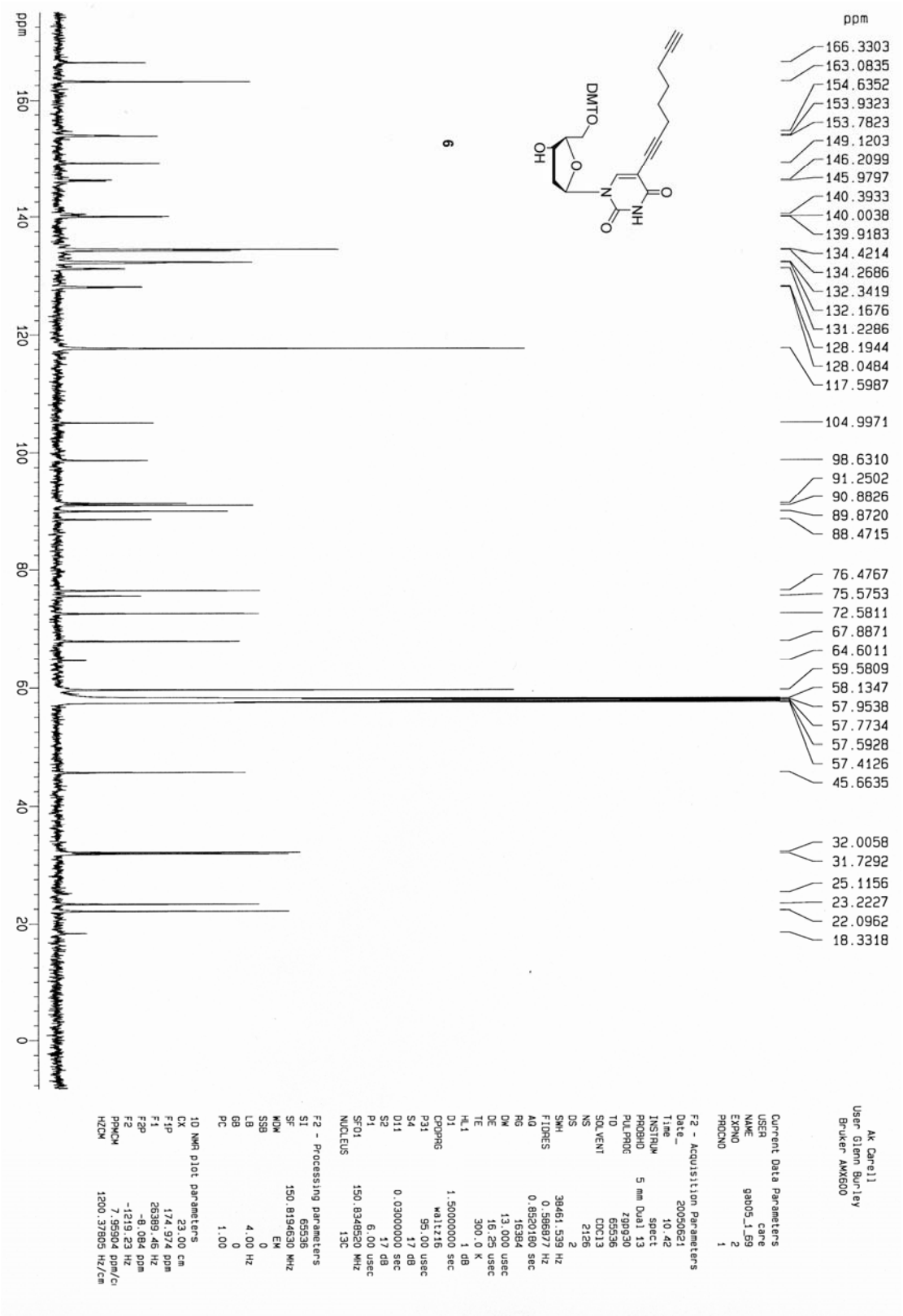
Spectral Width (ppm):
(f1) 243.709

Pulse Program:
Unknown

Temperature:
27

Number of Scans:
4096


burley
gab05-1-69
5060920
Pulse Sequence: s2pul
Date: Jul 6 2005
Solvent: cd2cl2
Temp. 24.0 C / 297.1 K
INOVA-400 "sigurd"
Pulse 45.2 degrees
Acq. time 4.000 sec
Width 6000.6 Hz
64 repetitions
OBSERVE H1, 399.9416293 MHz
DATA PROCESSING
FT size 131072
Total time 4 min, 16 sec
DMTO
OH
O
N
NH
O
O
6
py
py
py
1.90
2.74
24.02
9.31
2.57
2.49
2.57
16.83
5.15
3.21
15.51
13.54
0.18
13
12
11
10
9
8
7
6
5
4
3
2
1
-0
ppm



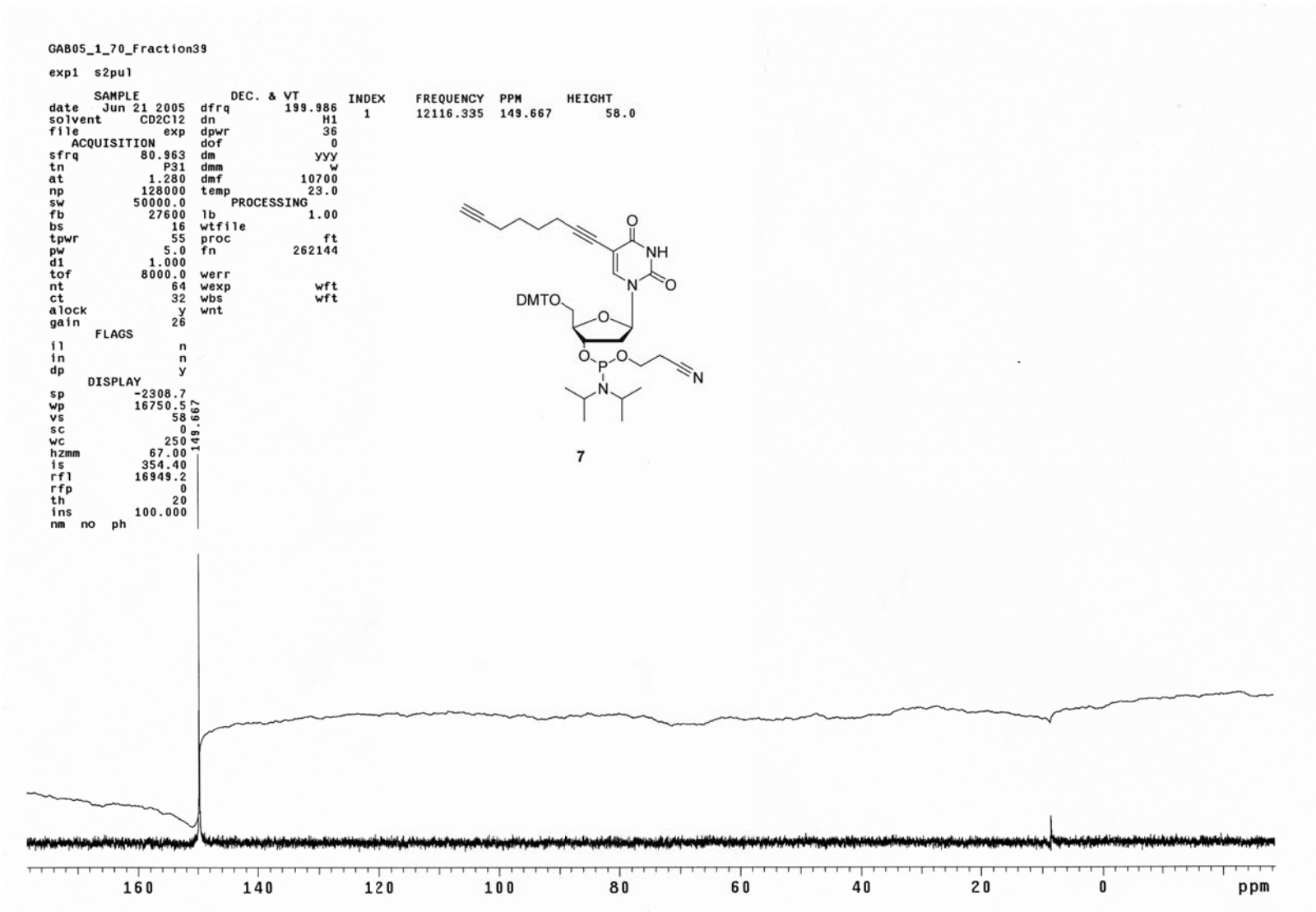
Ak Carell
User Glenn Burley
Bruker AMX600

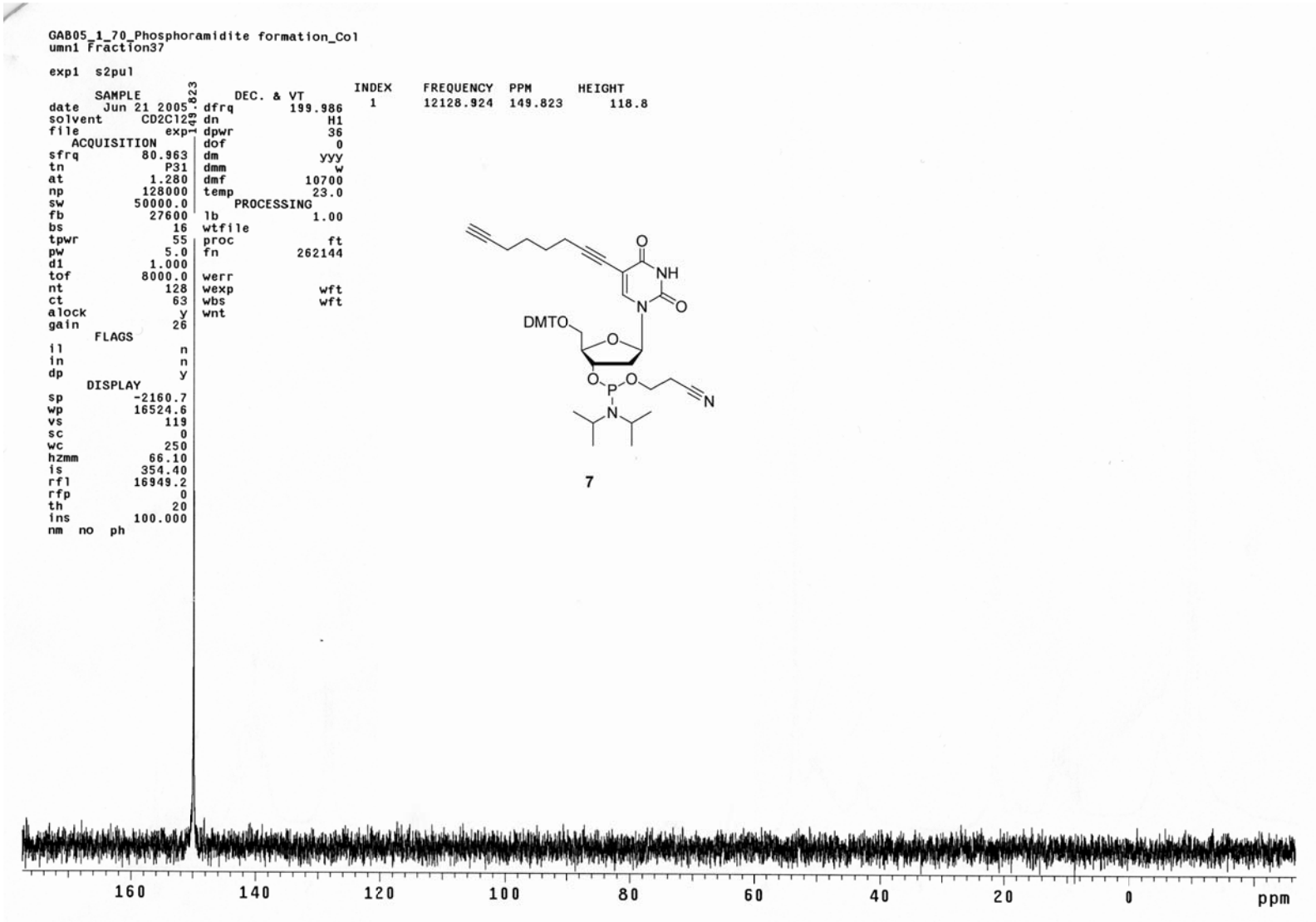
Current Data Parameters
USER care
NAME ga005_1_69
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20050621
Time 10.42
INSTRUM spect
PROBHD 5 mm Dual 13
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 2126
DS 2
SWH 38461.539 Hz
FIDRES 0.586877 Hz
AQ 0.8520180 sec
RG 16384
DW 13.000 usec
DE 16.25 usec
TE 300.0 K
HL1 1 dB
D1 1.50000000 sec
CPDPRG waltz16
P31 95.00 usec
S4 17 dB
D11 0.03000000 sec
S2 17 dB
P1 6.00 usec
SFO1 150.8348520 MHz
NUCLEUS 13C

F2 - Processing parameters
SI 65536
SF 150.8194630 MHz
WDW EM
SSB 0
LB 4.00 Hz
GB 0
PC 1.00

1D NMR plot parameters
CX 23.00 cm
F1P 174.974 ppm
F1 26389.46 Hz
F2P -8.084 ppm
F2 -1219.23 Hz
PPMCM 7.95904 ppm/cm
HZCM 1200.37805 Hz/cm


GAB05_1_70_Fraction39
exp1 s2pul
SAMPLE
date Jun 21 2005
solvent CD2Cl2
file exp
ACQUISITION
sfrq 80.963
tn P31
at 1.280
np 128000
sw 50000.0
fb 27600
bs 16
tpwr 55
pw 5.0
d1 1.000
tof 8000.0
nt 64
ct 32
alock y
gain 26
FLAGS
il n
in n
dp y
DISPLAY
sp -2308.7
wp 16750.5
vs 58
sc 0
wc 250
hzmm 67.00
is 354.40
rfl 16949.2
rfp 0
th 20
ins 100.000
nm no ph
DEC. & VT
dfrq 199.986
dn H1
dpwr 36
dof 0
dm yyy
dmm w
dmf 10700
temp 23.0
PROCESSING
lb 1.00
wtfile
proc ft
fn 262144
werr
wexp wft
wbs wft
wnt
INDEX FREQUENCY PPM HEIGHT
1 12116.335 149.667 58.0
149.667
O
NH
N
O
DMTO
O
O
P
O
N
N
7
160
140
120
100
80
60
40
20
0
ppm

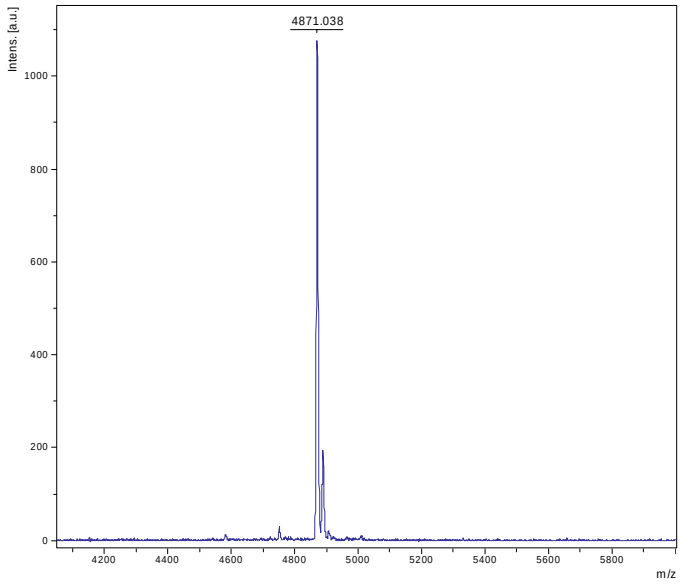

GAB05_1_70_Phosphoramidite formation_Col
umn1 Fraction37
exp1 s2pul
SAMPLE
date Jun 21 2005
solvent CD2Cl2
file exp1
ACQUISITION
sfrq 80.963
tn P31
at 1.280
np 128000
sw 50000.0
fb 27600
bs 16
tpwr 55
pw 5.0
d1 1.000
tof 8000.0
nt 128
ct 63
alock y
gain 26
FLAGS
il n
in n
dp y
DISPLAY
sp -2160.7
wp 16524.6
vs 119
sc 0
wc 250
hzmm 66.10
is 354.40
rfl 16949.2
rfp 0
th 20
ins 100.000
nm no ph
149.823
DEC. & VT
dfrq 199.986
dn H1
dpwr 36
dof 0
dm yyy
dmm w
dmf 10700
temp 23.0
PROCESSING
lb 1.00
wtfile
proc ft
fn 262144
werr
wexp wft
wbs wft
wnt
INDEX FREQUENCY PPM HEIGHT
1 12128.924 149.823 118.8
O
NH
N
O
DMTO
O
O
P
O
N
N
7
160
140
120
100
80
60
40
20
0
ppm

MALDI-TOF data of starting ODNs

ODN-1

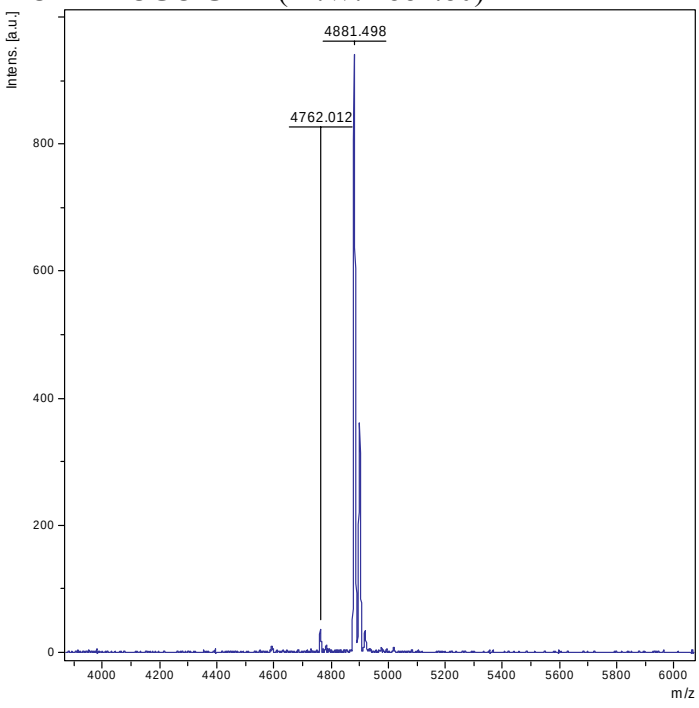
X = 1

5’-GCG CTG T**X**C ATT CGC G (M.W. 4871.81)



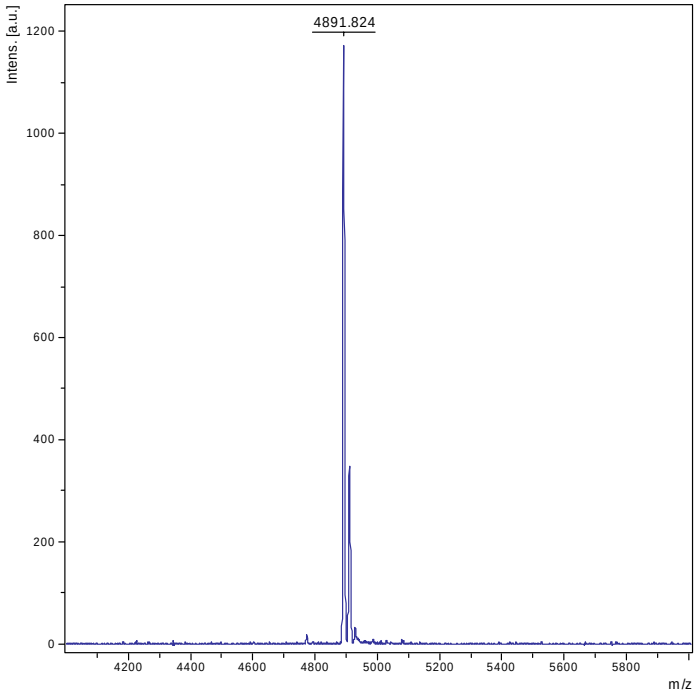
ODN-2

5’-GCG CTG **XX**C ATT CGC G (M.W. 4881.80)



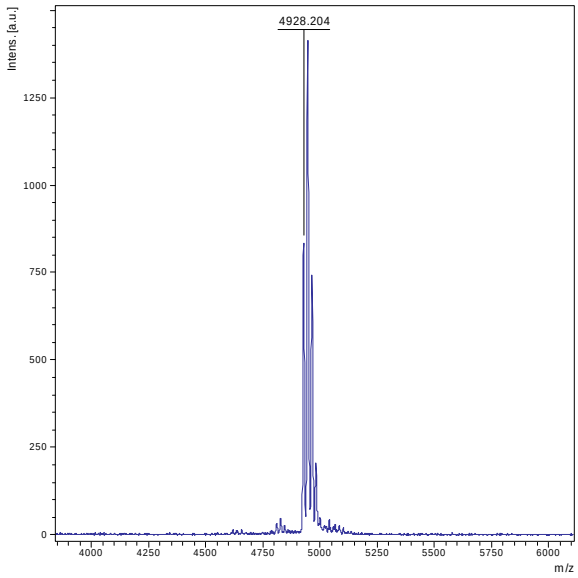
ODN-3

5’-GCG C**X**G T**X**C A**X**T CGC G (M.W. 4866.77; $[M+ Na]^+$: 4889)



ODN-4

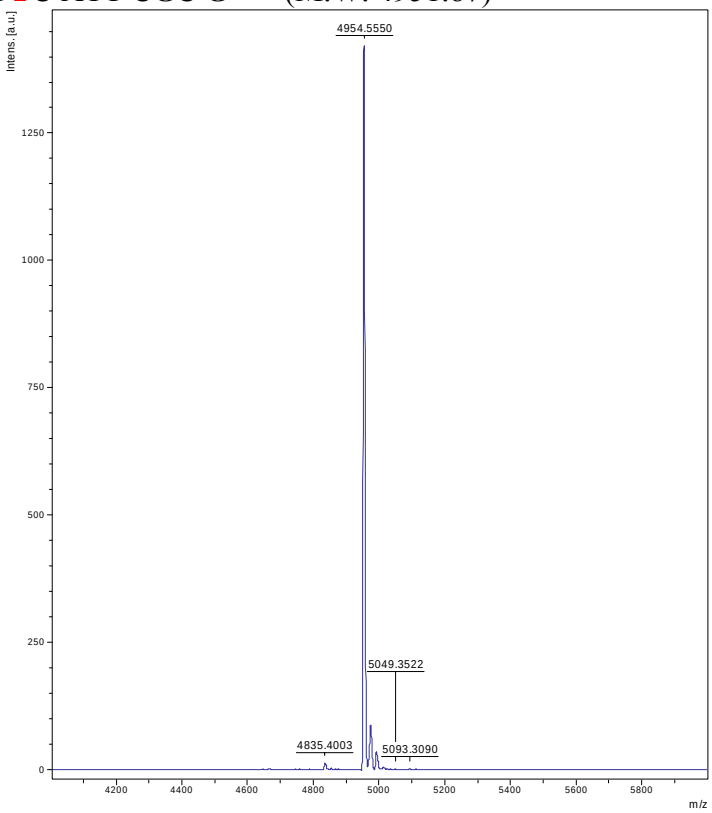
5’-GCG C**XX XXX X**GT CGC G (M.W. 4927.72)



ODN-5

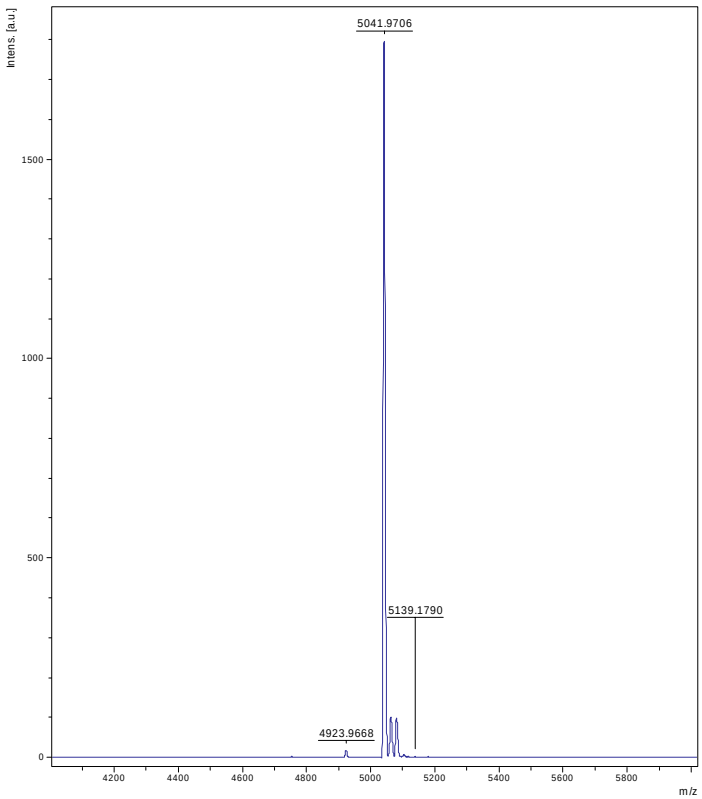
Y = 2

5’-GCG CTG T**Y**C ATT CGC G (M.W. 4951.87)



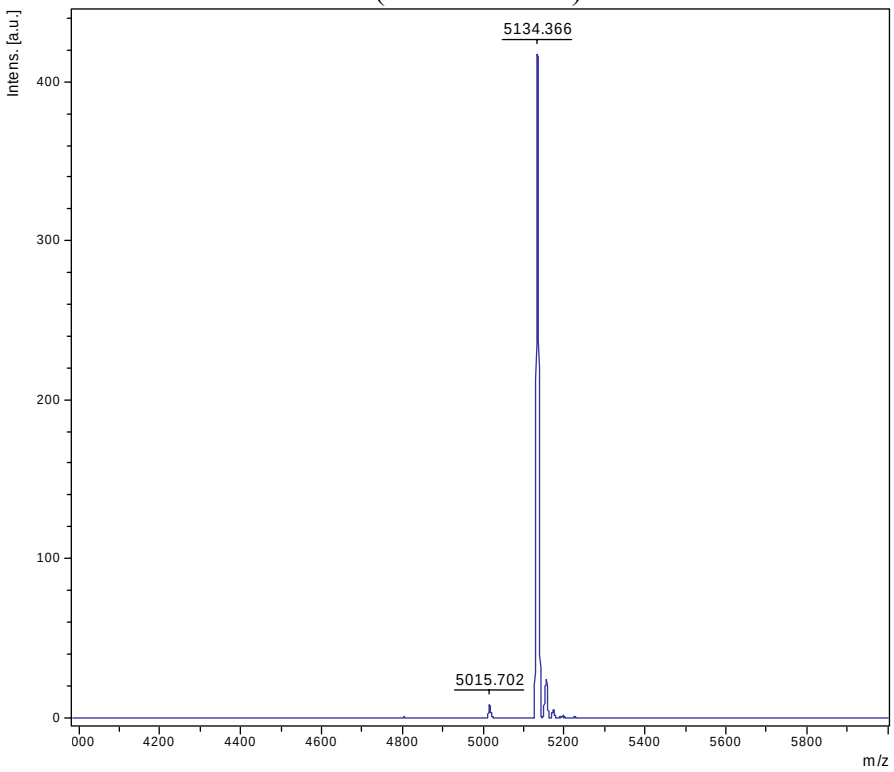
ODN-6

5’-GCG CTG **YY**C ATT CGC G (M.W. 5041.92)



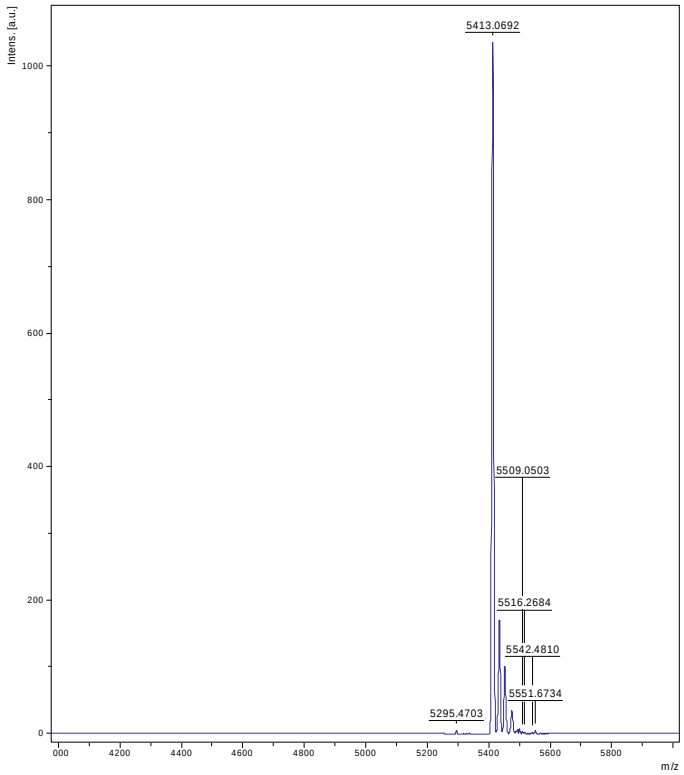
ODN-7

5’-GCG C**Y**G T**Y**C A**Y**T CGC G (M.W. 5131.97)



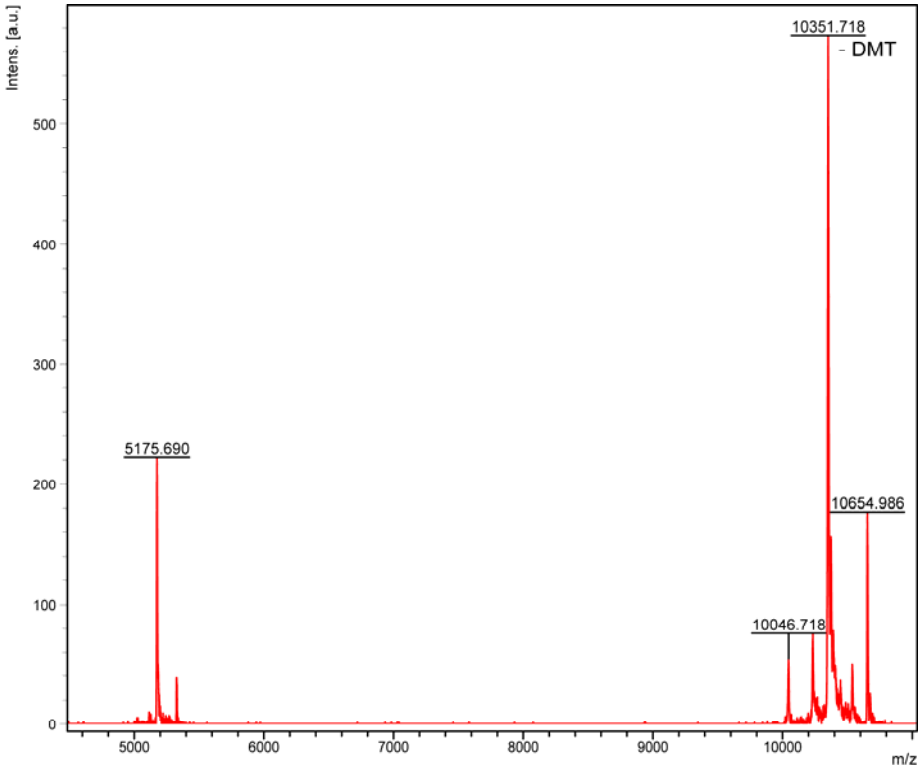
ODN-8

5’-GCG C**YY YYY Y**GT CGC G (M.W. 5408.10)



ODN-9

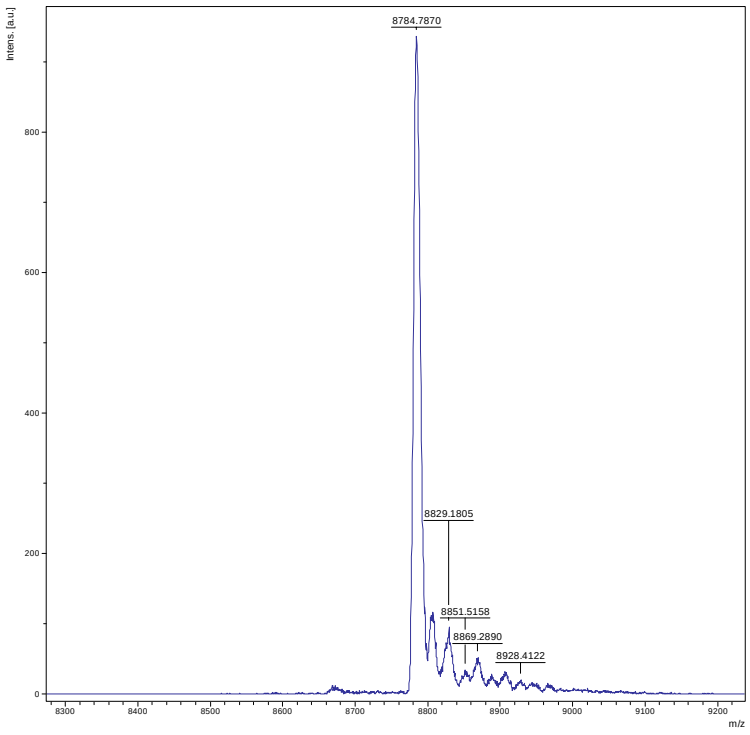
5’-DMT-TTA ATT GAA TTC GAT TYG GGC CGG AYT TGT TTC
(M.W. 10648.92; M.W.(-DMT) 10345.78)



The DMT-group is partly cleaved by the acid in the MALDI matrix.

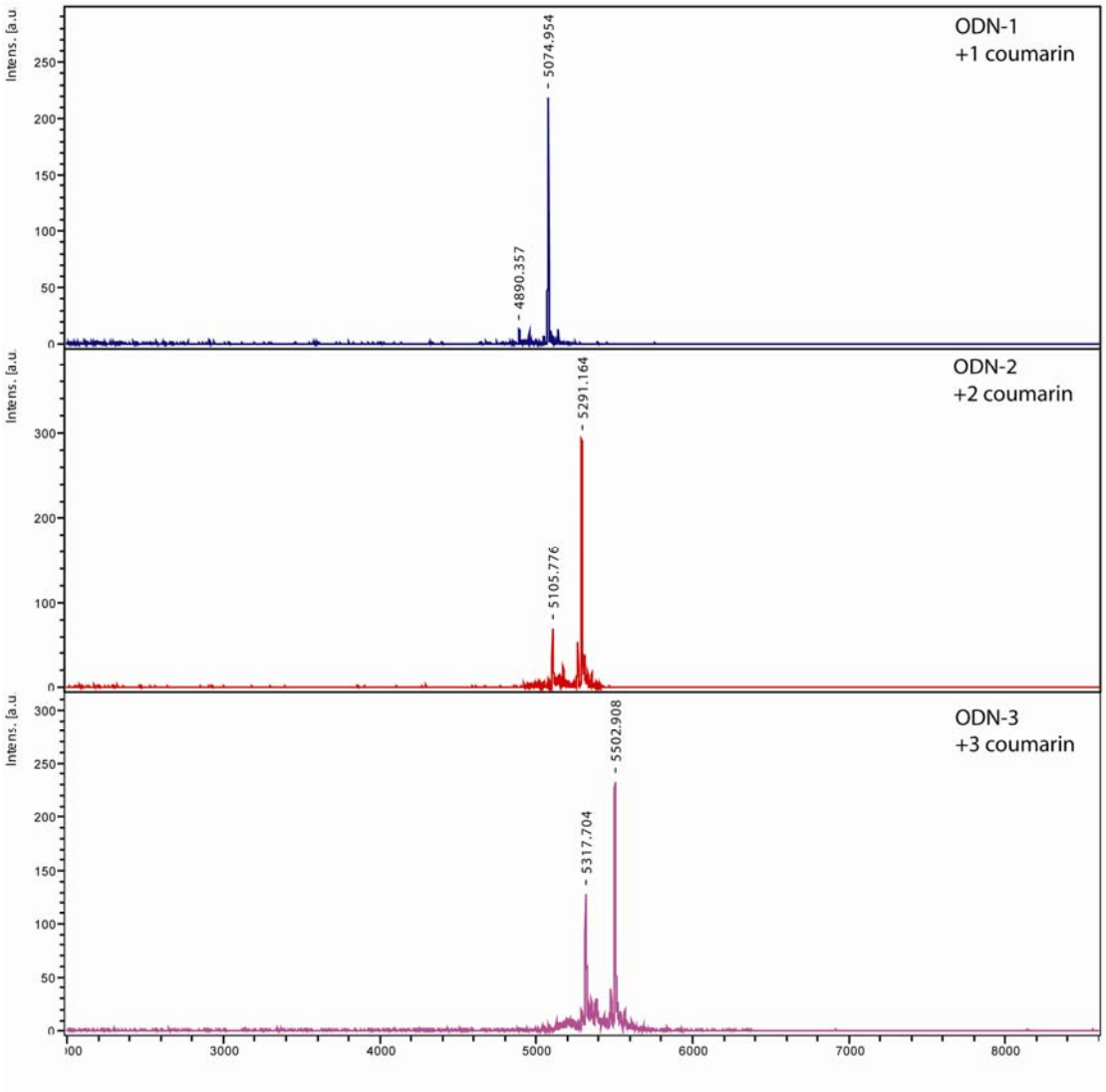
ODN-10

GCA GGC YTCA YGC CAG AAT TAC CAG AAG (M.W. 8782,58)

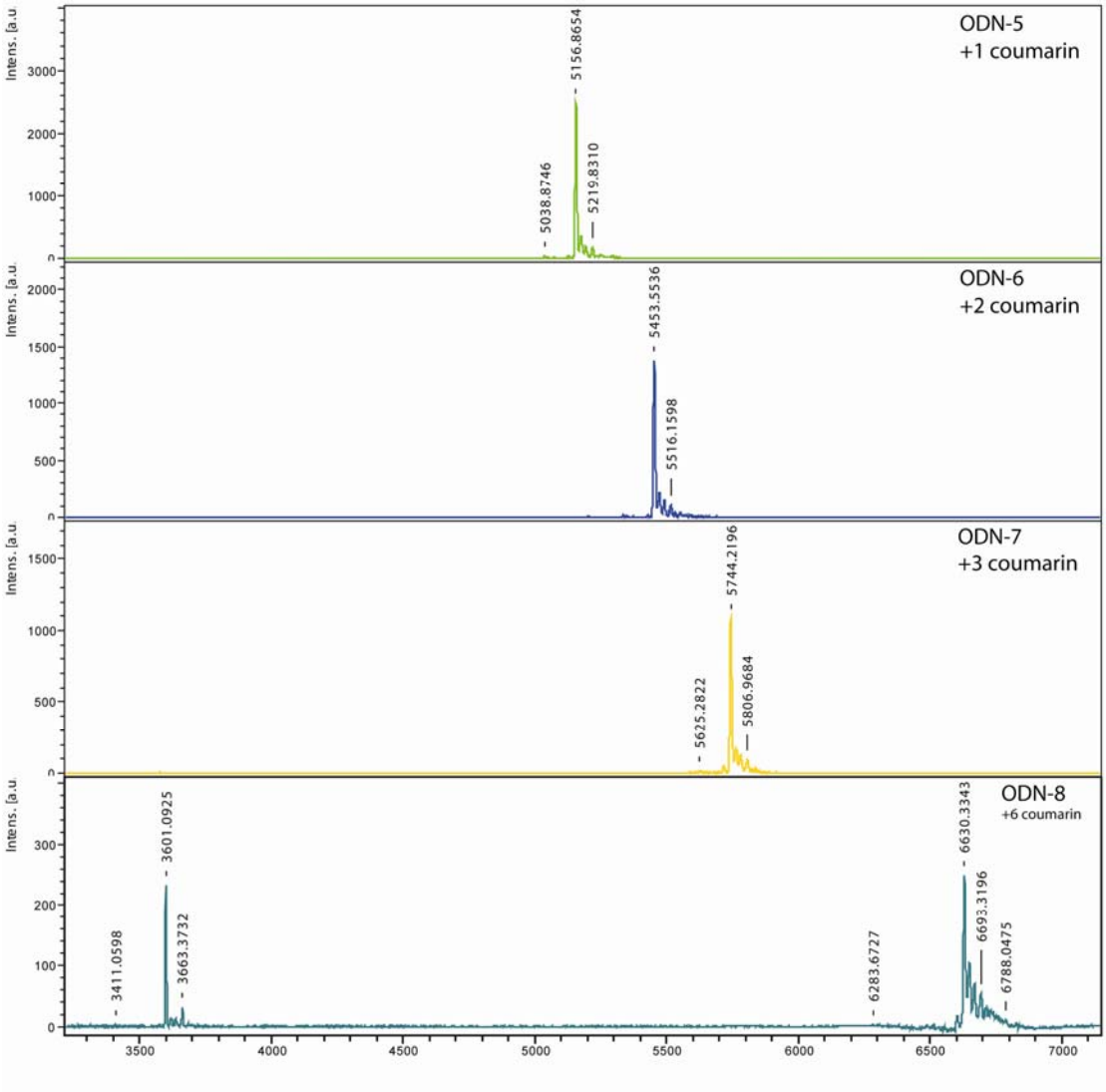


MALDI-TOF data of coumarin click

Series ODN-**1** to ODN-**3**



Series ODN-**5** to ODN-**8**



References

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(5) Sivakumar, K.; Xie, F.; Cash, B. M.; Long, S.; Barnhill, H. N.; Wang, Q. *Org. Lett.* **2004**, *6*, 4603-4606.

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