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Supporting Information

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Enzymatic Incorporation of an Antibody-Activatable Blue Fluorophore into DNA**

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General Methods. ^{1}H and ^{13}C-NMR spectra were measured on a Bruker AMX-400 or Bruker AMX-500 spectrometer as indicated. Chemical shifts (ppm) were reported relative to internal $CDCl_3$ (^{1}H, 7.26 ppm and ^{13}C, 77.0 ppm), CD_3OD (^{1}H, 3.30 ppm and ^{13}C, 49.2 ppm), and DMSO-d_6 (^{1}H, 2.49 ppm and ^{13}C, 39.0 ppm). HRMS spectra were recorded using electrospray ionization (ESI) or MALDI techniques. Glassware and solvents were dried by standard methods. Flash chromatography was performed on silica gel 60 (230-400 mesh) and thin-layer chromatography on glass plates coated with a 0.02 mm layer of silica gel 60 F-254. All chemical reagents and solvents were from Aldrich Chem. Co., unless otherwise noted, and used without further purification. Pyrene-8-dATP was obtained from PerkinElmer Life Sciences, Inc.

Mono-THP tetraethylene glycol **2**. A mixture of tetraethylene glycol **1** (1.94 g, 10.0 mmol), 3,4-dihydro-2*H*-pyran (0.91 mL, 10.0 mmol) and pyridinium *p*-toluenesulfonate (0.25 g, 1.0 mmol) was stirred in CH_2Cl_2 at room temperature for 2 h. The solvent was evaporated and the residue was purified by chromatography on silica gel to give **2** (1.05 g, 38%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.63 (dd, 1H, J = 4.1, 3.2 Hz), 3.89-3.83 (m, 2H), 3.74-3.58 (m, 15H), 3.52-3.47 (m, 1H), 2.65 (t, 1H, J = 6.4 Hz), 1.87-1.78 (m, 1H), 1.75-1.68 (m, 1H), 1.64-1.48 (m, 4H). ^{13}C ($CDCl_3$, 100 MHz) δ 98.9, 72-5, 70.6, 70.3, 66.6, 62.2, 61.7, 30.5, 25.4, 19.4. FTMS calc. for $C_{13}H_{26}NaO_6$ $[M+Na]^+$: 301.1621, found: 301.1615.

THP tetraethylene glycol methylstilbene **3**. Into a suspension of NaH (60%, 0.16 g, 3.93 mmol) in THF (10 mL), 2 (0.87 g, 3.14 mmol) was added at room temperature under nitrogen. After 30 min a solution of 4-chloromethylstilbene (0.6 g, 2.62 mmol) in THF (10 mL) was added, and the mixture was stirred at 50 ^{o}C for 2 h. The mixture was concentrated and the residue was extracted with CH_2Cl_2, washed with water and dried over Na_2SO_4. After evaporation, the crude product was purified by chromatography on silica gel to give **3** (0.75 g, 61%) as pale yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53-7.48 (m, 4H), 7.38-7.33 (m, 4H), 7.28-7.24 (m, 1H), 7.10 (s, 2H), 4.63 (t, 1H, J = 3.2 Hz), 4.57 (s, 2H), 3.89-3.84 (m, 2H), 3.70-3.58 (m, 15H), 3.52-3.47 (m, 1H), 1.87-1.78 (m, 1H), 1.75-1.68 (m, 1H), 1.64-1.48 (m, 4H). ^{13}C ($CDCl_3$, 100 MHz) δ 137.7, 137.3, 136.7, 128.6, 128.5, 128.3, 128.1, 127.6, 126.4, 98.9, 73.0, 70.6, 70.5, 69.4, 66.6, 62.2, 30.5, 25.4, 19.4. FTMS calc. for $C_{28}H_{38}NaO_6$ $[M+Na]^+$: 493.2560, found: 493.2565.

Tetraethylene glycol methylstilbene **4**. Compound **3** (0.75 g, 1.6 mmol) was dissolved in methanol (15 mL) and treated with *p*-toluenesulfonic acid (6 mg, 0.03 mmol) at 50 ^{o}C for 30 min. The solvent was evaporated and the residue was purified by chromatography on silica gel (EtOAc/ MeOH, 40/1) to give **4** (0.56 g,

86%) as yellowish solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.52-7.48 (m, 4H), 7.37-7.33 (m, 4H), 7.27-7.23 (m,1H), 7.10 (s, 1H), 4.56 (s, 2H), 3.73-3.62 (m, 14H), 3.60-3.58 (m, 2H), 3.03 (t, 1H, J = 6.2 Hz). ^{13}C ($CDCl_3$, 100 MHz) δ 137.5, 137.1, 136.5, 128.5, 128.4, 128.2, 128.0, 127.5, 126.3, 72.8, 72.4, 70.4, 70.1, 69.2, 61.5. FTMS calc. for $C_{23}H_{30}NaO_5$ $[M+Na]^+$: 409.1985, found: 409.1982.

Carboxymethyl tetraethylene glycol methylstilbene **5**. Into a suspension of NaH (60%, 36 mg, 0.9 mmol) in THF (3 mL), bromoacetic acid (63 mg, 0.45 mmol) and **4** (0.115g, 0.3 mmol) in THF (2 mL) were added at 0 ^{o}C under nitrogen. The mixture was stirred overnight and quenched with 1N HCl. After concentration the residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH, 4/1) to give **5** (89 mg, 67%) as a white syrup. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.15 (brs, 1H), 7.51-7.47 (m, 4H), 7.36-7.31 (m, 4H), 7.26-7.23 (m, 1H), 4.55 (s, 2H), 4.04 (brs, 2H), 3.65-3.62 (m, 16H). ^{13}C ($CDCl_3$, 125 MHz) δ 170.0, 137.4, 137.2, 136.7, 128.6, 128.2, 128.1, 127.6, 126.4, 72.7, 70.7, 70.3, 70.2, 69.9, 69.8, 69.7, 69.2, 69.0, 68.4, 68.1. FTMS calc. for $C_{25}H_{32}NaO_7$ $[M+Na]^+$: 467.2040, found: 467.2040.

7-deaza-2'-deoxy-7-aminopropynyl-adenosine **7**. 7-deaza-2'-deoxy-7-[*?*-(trifluoroacet-amido)propynyl]-adenosine 6 (0.13 g, 0.33 mmol) was dissolved in NH_4OH (30%, 40 mL) and methanol (10 mL) and stirred for 3 h. After concentration to dryness, the residue was purified by chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH, 75/20/5) to give **7** (89 mg, 90%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.08 (s, 1H), 7.59 (s, 1H), 6.46 (dd, 1H, J = 7.9, 5.9 Hz), 4.51-4.48 (m, 1H), 4.00-3.98 (m, 1H), 3.80-3.68 (m, 4H), 2.64-2.57 (m, 1H), 2.31 (ddd, 1H, J = 13.2, 5.9, 2.6 Hz). ^{13}C (CD_3OD, 100 MHz) δ 159.2, 153.2, 150.0, 128.3, 104.5, 97.0, 91.1, 89.2, 86.7, 77.0, 73.0, 63.6, 41.6, 32.0, 22.0. FTMS calcd for $C_{14}H_{18}N_5O_3$ $[M+H]^+$: 304.1404, found: 304.1406.

7-deaza-2'-deoxy-7-[*?* -(methylstilbene tetraethylene glycol acetamido)propynyl]-adenosine **8**. Into a solution of **5** (27.5 mg, 0.062 mmol) and N-hydroxysuccinimide (8.8 mg, 0.074 mmol) in CH_2Cl_2 (1.5 mL) and DMF (0.2 mL), EDC-HCl was added, and was stirred for 6 h at room temperature under nitrogen. A solution of **7** (15 mg, 0.05 mmol) in DMF (0.6 mL) was added. The suspension was stirred over night. After concentration, the residue was purified by PTLC (CH_2Cl_2/MeOH, 10/1) to give **8** (13 mg, 36%) as colorless syrup. ^{1}H NMR (CD_3OD, 400 MHz) δ?8.05 (s, 1H), 7.53-7.46 (m, 5H), 7.33-7.27 (m, 4H), 7.23-7.19 (m, 1H), 7.11 (s, 2H), 6.43 (dd, 1H, J = 7.9, 5.8 Hz), 4.49-4.46 (m, 3H), 4.24 (s, 2H), 4.01 (s, 2H), 3.99-3.97 (m, 2H), 3.76 (dd, 1H J = 12.3, 3.2 Hz), 3.71-3.58 (m, 17H), 2.62-2.54 (m, 1H), 2.27 (ddd, 1H, J = 13.2, 5.9, 2.6 Hz). ^{13}C (CD_3OD, 100 MHz) δ ?173.2, 159.2, 155.5, 153.2, 150.0, 139.0, 138.8, 138.2, 130.0, 129.7, 129.3, 129.2, 128.6, 128.0, 127.5, 104.8, 96.9, 89.4, 89.2, 86.7, 76.1, 73.8, 73.0, 72.0, 71.5, 71.4, 71.3, 71.2, 70.6, 63.6, 41.6, 30.2. FTMS calc. for $C_{39}H_{47}N_5NaO_9$ $[M+Na]^+$: 752.3266, found: 752.3291.

7-deaza-2'-deoxy-7-[*?* -(methylstilbene tetraethylene glycol acetamido)propynyl]-adenosine triphosphate **9**. A solution of **8** (13 mg, 0.018 mmol) and proton sponge (5.6mg, 0.027 mmol) in trimethyl phosphate (0.18 mL) was stirred for 10 min at 0 ^{o}C under nitrogen. Phosphorous oxychloride (3.3 μl, 0.036 mmol) was added, and stirred for 2 h at 0 ^{o}C. A mixture of Bu_3N (25 μl, 0.11 mmol) and anhydrous tributylammonium pyrophosphate (40 mg, 0.089 mmol) in DMF (0.18 mL) was added at once. After 1 min, 1.0 M triethylammonium bicarbonate buffer (4 mL) was added, and the clear solution was stirred at room temperature for 30min and lyophilized over night. The crude material was separated by reverse phase HPLC with a DEAE column (0.1 M TEAB/MeCN) to give **9** (9.0 mg, 52%) as a white solid. ^{31}P NMR (50mM Tris, 2mM EDTA, pH 7.5 in D_2O, 140 MHz) δ ? 4.6 (d, *J* = 15.8Hz), -10.1 (d, *J* = 15.8 Hz), -21.0 (t, *J* = 15.8 Hz). ESI calc. for $C_{39}H_{49}N_5O_{18}P_3$ $[M-H]^-$: 968, found: 968.

Polymerase Chain Reaction (PCR) protocol

PCR amplifications were carried out according to standard protocols. A typical reaction (50 µl) mix contained the DNA template (pCGMT-92H2; 1 ng), the primers (1µM each), dNTPs (200 µM each), and the DNA polymerase (2 U for Red*Taq*, *Vent* and *Vent* exo$^-$, and 2.5 U for *Pfu* DNA polymerase) in the buffer supplied by the manufacturer of the DNA polymerase. The reaction were carried out using an Eppendorf Thermocycler Gradient (Eppendorf) at 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 2 min, and 72 °C for 10 min.

Slide preparation and hybridization protocol

The slide preparation was performed as described by Charles D. Bangs and Timothy A. Donlon in "Current protocols in Human Genetics – Unit 4.1". NIH:OVCAR-3 cells were grown in culture and harvested at a density of 10^6/ mL. The cells were spun down and gently resuspended in 6 mL of 75 mM KCl, at room temperature. After an incubation of 15 min at room temperature, 12 drops of fixative (3:1 (v:v) methanol/glacial acetic acid) were added and mixed well. The cells were centrifuged and all but 0.5 mL of the supernatant was removed. The pellet was resuspended in the remaining supernatant, 1 mL fixative was added and the suspension was mixed immediately. The volume was adjusted to 5 mL with fixative and mixed thoroughly. The cells were spun down, the supernatant removed, and the pellet resuspended in 5 mL fixative. After a final centrifugation step, supernatant was removed and the pellet was resuspended in a volume of fixative sufficient to produce a light milky suspension. The suspension was stored overnight at 4 °C. On the next day, the chromosomal DNA was spread onto microscope slides and air dried. The slides were immersed in 1 M sodium thiocyanate (Sigma-Aldrich, St.Louis, USA) at 80 °C for 10 minutes, rinsed with dH_2O and dried. The hybridization solution consisted of 25 µl of solution A (6 mL deionized formamide (Sigma-Aldrich, St.Louis, USA), 2 mL 20x SSC (Sigma-Aldrich, St.Louis, USA), 2 mL dH_20, 2g dextran sulphate), 15 µl deionized formamide, 5 µl stilbene-modified DNA probe (50ng), 0.5 µl salmon sperm DNA

(Invitrogen,Carlsbad, USA), 2 µl dH_2O, and 2.5 µl 20x SSC. After applying the hybridization solution, the slide was incubated at 80 °C for 10 minutes and then transferred to a humid chamber at 37 °C for an overnight incubation. On the next day, the slides were washed in washing solution (60 mL formamide, 30 mL dH_20, 10 mL 20x SSC, 80 µl concentrated HCl and the pH was adjusted to 7) for 10 minutes at 42 °C. The slides were then blocked in blocking solution (100 mL PBS, 2g BSA, 50 µl Tween 20) for 1 h at 37 °C. The antibody solution containing 1 µg/ mL mAb EP2-19G2 in blocking solution was applied and incubated for 1 h at 37 °C. A final washing step with PBS followed, before *SlowFade*® Antifade kit (Molecular Probes, Eugene, USA) was applied.

DNA sequence of the scFv GNC92H2 gene (incl. primer sequences)

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1     TTGTTATTAC TCGCGGCCCA GCCGGCCATG GCAGAGGTGC AGCTGCAGCA
      AACAATAATG AGCGCCGGGT CGGCCGGTAC CGTCTCCACG TCGACGTCGT

51    GTCAGGACCT GAACTGAAGA AGCCTGGAGA GACAGTCAAG ATCTCCTGCA
      CAGTCCTGGA CTTGACTTCT TCGGACCTCT CTGTCAGTTC TAGAGGACGT

101   AGACTTCTGG ATATTCCTTC ACAAACTATG GAATGAACTG GGTGAAGCAG
      TCTGAAGACC TATAAGGAAG TGTTTGATAC CTTACTTGAC CCACTTCGTC

151   GCTCCAGGAA AGGGTTTAAA GTGGATGGGC TGGATAAACA CCTACACTGG
      CGAGGTCCTT TCCCAAATTT CACCTACCCG ACCTATTTGT GGATGTGACC

201   AGAGCCAACA TATGCTGATG ACTTCAGGGG ACGGTTTGCC TTCTCTTTGG
      TCTCGGTTGT ATACGACTAC TGAAGTCCCC TGCCAAACGG AAGAGAAACC

251   CAACCTCTGC CAGCACTGCC TATTTGCAGA TCATCAACCT CAAAAATGAG
      GTTGGAGACG GTCGTGACGG ATAAACGTCT AGTAGTTGGA GTTTTTACTC

301   GACACGGCTA CATATTTCTG TGAAACCTAT GATAGTCCCC TCGGGGACTA
      CTGTGCCGAT GTATAAAGAC ACTTTGGATA CTATCAGGGG AGCCCCTGAT

351   CTGGGGCCAA GGCACCACTC TCACAGTCTC CTCAGGTGGT GGTGGTTCTG
      GACCCCGGTT CCGTGGTGAG AGTGTCAGAG GAGTCCACCA CCACCAAGAC

401   GCGGCGGCGG CTCCGGTGGT GGTGGTTCTG ACATTGTGCT AACTCAGTCT
      CGCCGCCGCC GAGGCCACCA CCACCAAGAC TGTAACACGA TTGAGTCAGA

451   CCTGCTTCCT TAGCTGTATC TCTGGGGCAG AGGGCCACCA TCTCATGCAG
      GGACGAAGGA ATCGACATAG AGACCCCGTC TCCCGGTGGT AGAGTACGTC

501   GGCCAGCAAA AGTGTCAGTA CATCTGGCTA TAATTATATG CACTGGTACC
      CCGGTCGTTT TCACAGTCAT GTAGACCGAT ATTAATATAC GTGACCATGG
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551   AACAGAAACC AGGACAGCCA CCCAAACTCC TCATCTATCT TGCATCCAAC
      TTGTCTTTGG TCCTGTCGGT GGGTTTGAGG AGTAGATAGA ACGTAGGTTG

601   CTAGCATCTG GGGTCCCTGC CAGGTTCAGT GGCAGTGGGT CTGGGACAGA
      GATCGTAGAC CCCAGGGACG GTCCAAGTCA CCGTCACCCA GACCCTGTCT

651   CTTCACCCTC AACATCCATC CTGTGGAGGA GGAGGATGCT GCAACCTATT
      GAAGTGGGAG TTGTAGGTAG GACACCTCCT CCTCCTACGA CGTTGGATAA

701   ACTGTCTGTA TAGTAGGGAG TTTCCTCCGT GGACGTTCGG TGGAGGCACC
      TGACAGACAT ATCATCCCTC AAAGGAGGCA CCTGCAAGCC ACCTCCGTGG

751   AAGCTGGAGC TGAAACGTGG CCTCGGGGGC CGAATTCCAG TCGACGAGGT C
      TTCGACCTCG ACTTTGCACC GGAGCCCCCG GCTTAAGGTC AGCTGCTCCT G
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Primer MVH(sfi): TTG TTA TTA CTC GC*G GCC CAG CCG GCC* ATG GCA
Primer MLJ(sfi): GTC CTC GTC GAC TGG AAT TC*G GCC CCC GAG GCC* AC

M_W: 495.3 kDa; OD_{260} = 1 = 0.098 μM = 48.5 μg (calculated using http://www.basic.nwu.edu/biotools/oligocalc.html)

Agarose Gel Electrophoresis of the **9**-containing PCR products using different DNA polymerases. A typical reaction (50 μl) mix contained the DNA template (pCGMT-92H2; 1 ng), the primers (1μM each), dNTPs (200 μM each), and the DNA polymerase (2 U for Red*Taq*, *Vent* and *Vent* exo$^-$, and 2.5 U for *Pfu* DNA polymerase) in the buffer supplied by the manufacturer of the DNA polymerase. The different lanes show the products obtained from mixtures with varying ratios of **9** (from 2% to 50%) and dATP. The PCR reactions were loaded onto a 1% agarose gel containing 0.0001% ethidium bromide (Invitrogen). 1kp plus DNA ladder (Invitrogen) was used as marker. The experiments were repeated two times for each polymerase, yielding close to identical results, and the gels that are displayed are representative of these experiments.

{Supporting Figure 1}

Gel shift mobility assay of **9**-modified single-chain Fv (scFv) genes
The PCR products were purified with Qiagen's PCR purification kit and eluted in 30μl of EB. The DNA containing 9 was incubated with mAb EP2-19G2 (at varying concentrations) in an Eppendorf tube wrapped in aluminum foil at room

temperature for 30 min and then separated on a 1% agarose gel.

{Supporting Figure 2}

Sandwich ELISA of **9**-modified single-chain Fv (scFv) genes

mAb EP2-19G2 was diluted to a concentration of 10 μg/ mL in PBS. Each well of the ELISA plate was coated with 25 μl of antigen in PBS. The plate was sealed with plastic film and incubated overnight at 4 °C. On the next day the wells were blocked with 5% dry milk in PBS for 1 h at 37 °C. After washing of the plate, 25 μl of the DNA samples (serial dilutions) were added into the wells, followed by an incubation time of 1 h at ambient temperature. After a washing step, 25 μl of recombinantly expressed and affinity-purified single chain antibody (scFv) EP2-19G2 bearing a C-terminal FLAG tag (1 μg/ mL) were added. Following an incubation of 1 h at room temperature, thorough washing of the ELISA plate, 25 μl/well of anti-FLAG-IgG-horseradish peroxidase conjugate (HRP) (1:1000, Sigma-Aldrich) were added, followed by an incubation of 1 h at ambient temperature. Afterwards, the plate was thoroughly washed and HRP substrate solution (TMB, Pierce) for the enzyme was prepared freshly. 50 μl of the substrate solution/well were added and incubated for 10 min at room temperature. The reaction was stopped by addition of 50 μl 2 M H_2SO_4 into each well. The resulting OD_{450} values were read using a Thermomax microplate reader.

{Supporting Figure 3}

Gel shift mobility assay of **9**-labeled X-chromosome specific probes

The PCR products were purified with Qiagen's PCR purification kit and eluted in 30 μl of EB. The DNA containing **9** was incubated with mAb EP2-19G2 (at varying concentrations) in an Eppendorf tube wrapped in aluminum foil for 30 min at room temperature and then separated onto a 1% agarose gel.

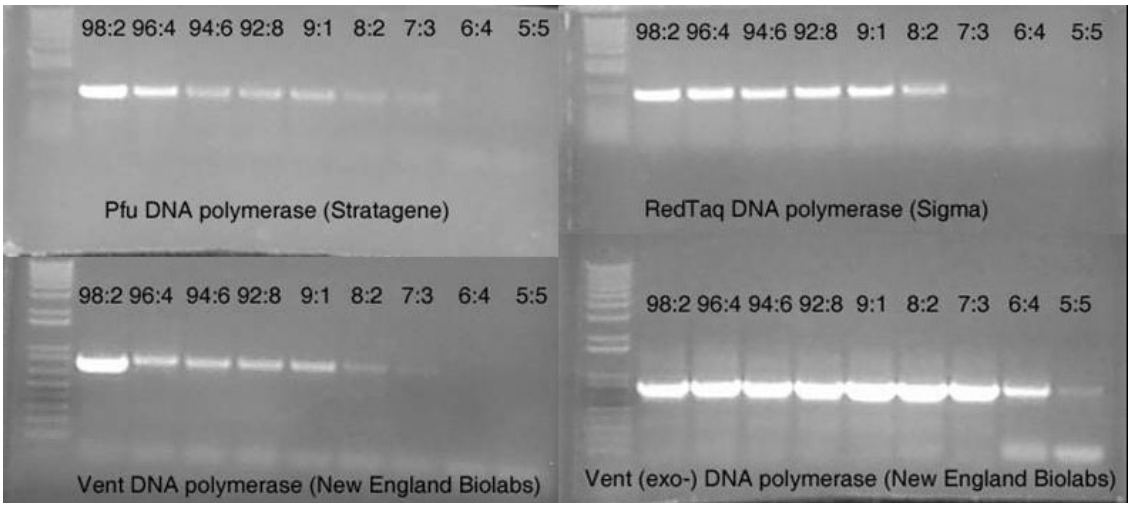
{Supporting Figure 4}

Gel electrophoresis of the PCR products with nucleotides **9** (sATP) and pyrene-8-dATP (pATP) using Vent (exo -) DNA polymerase.

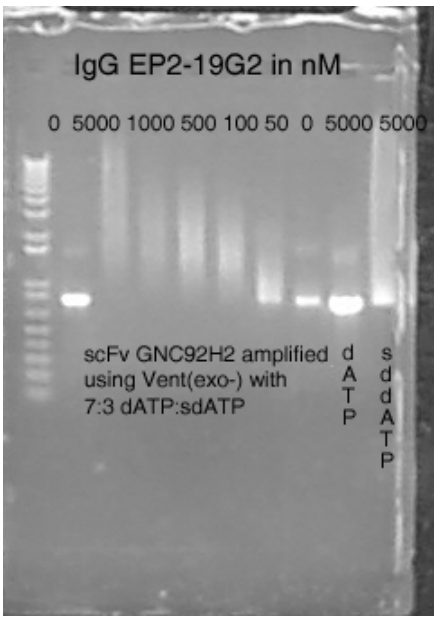
{Supporting Figure 5}

^{1}H- and 13C-NMR spectra of **7** and **8**.

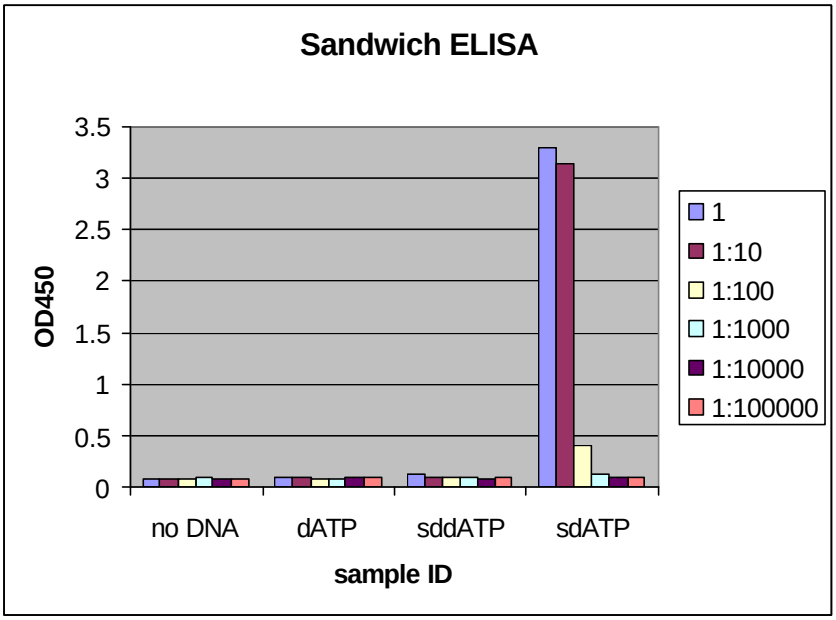
{Supporting Figures 6 A-D}



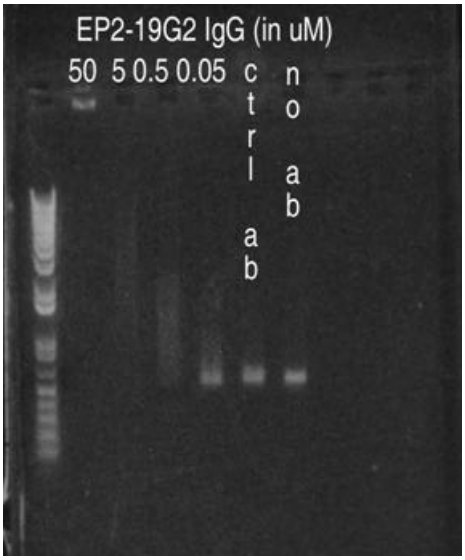
Supporting Figure 1. Agarose Gel Electrophoresis of the **9**-containing PCR products using different DNA polymerases. The PCR reactions were loaded onto a 1% agarose gel containing 0.0001% ethidium bromide (Invitrogen). A 1kp plus DNA ladder (Invitrogen) was used as marker.



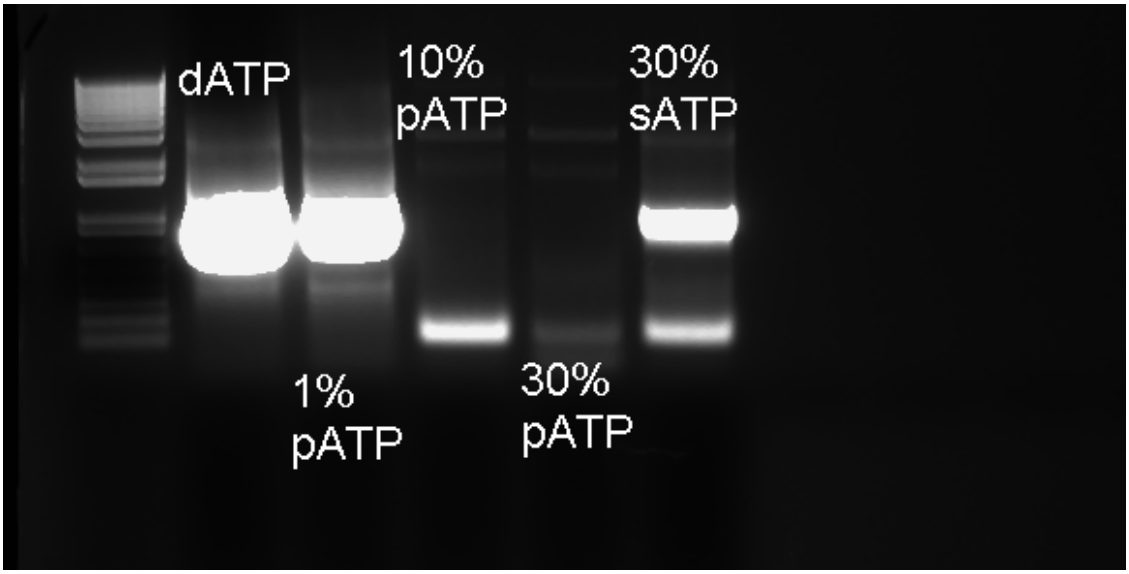
Supporting figure 2. Gel shift mobility assay of **9**-modified (scFv) genes. The PCR reactions were loaded onto a 1% agarose gel containing 0.0001% ethidium bromide (Invitrogen).



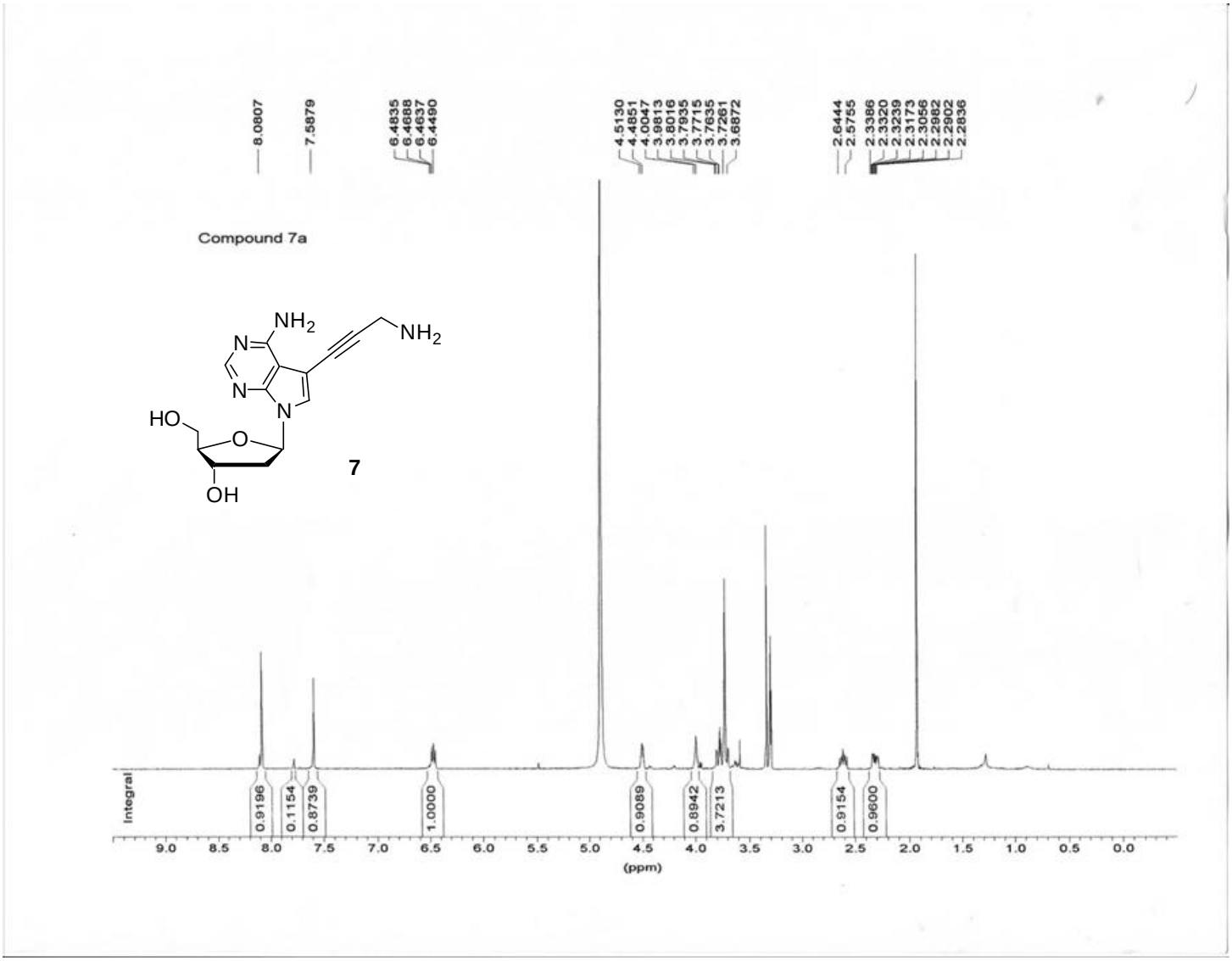
Supporting figure 3. Sandwich ELISA of **9**-modified scFv genes (serial dilutions).



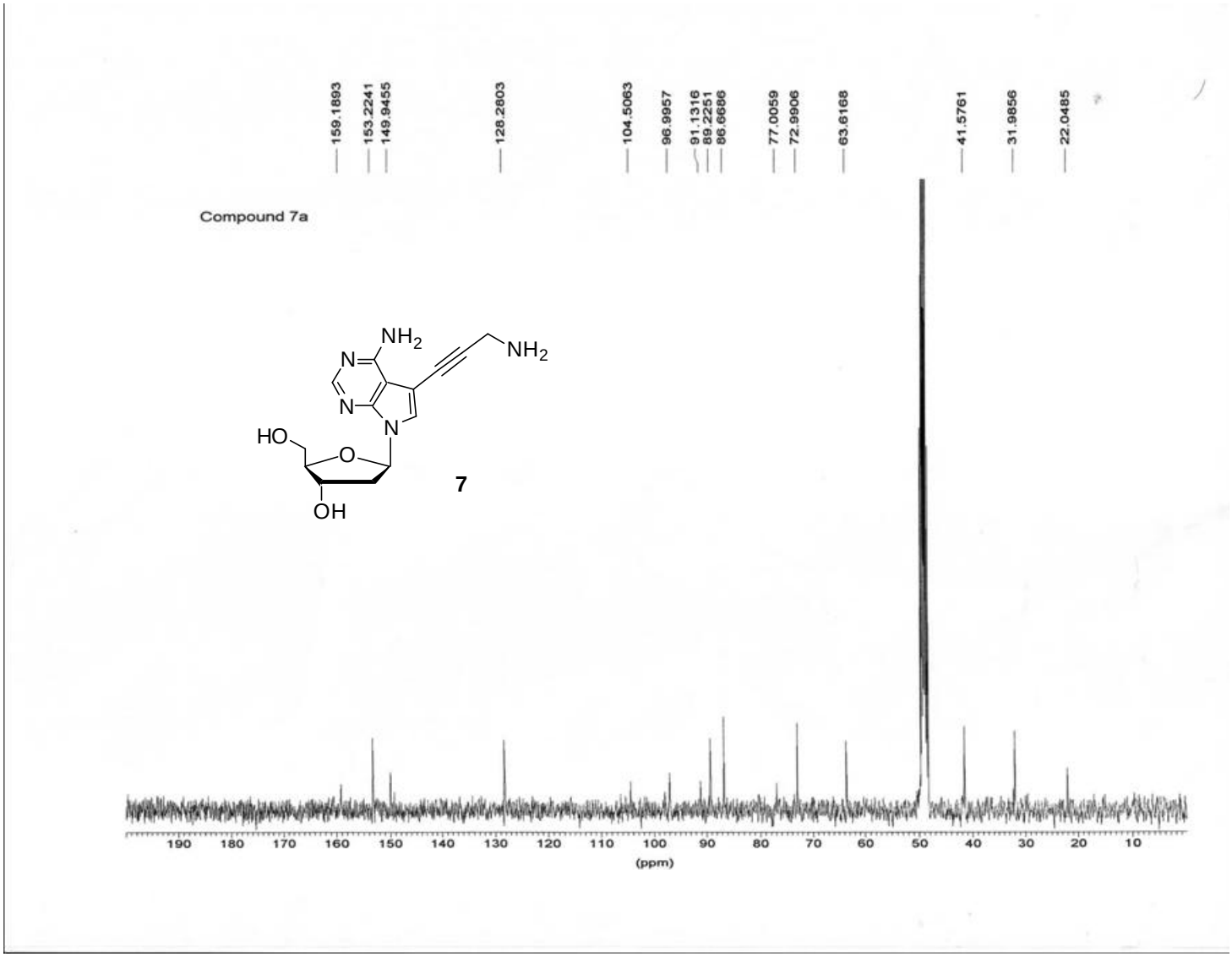
Supporting figure 4. Gel shift mobility assay of sdATP-labeled X-chromosome specific probes. The PCR reactions were loaded onto a 1% agarose gel containing 0.0001% ethidium bromide (Invitrogen).



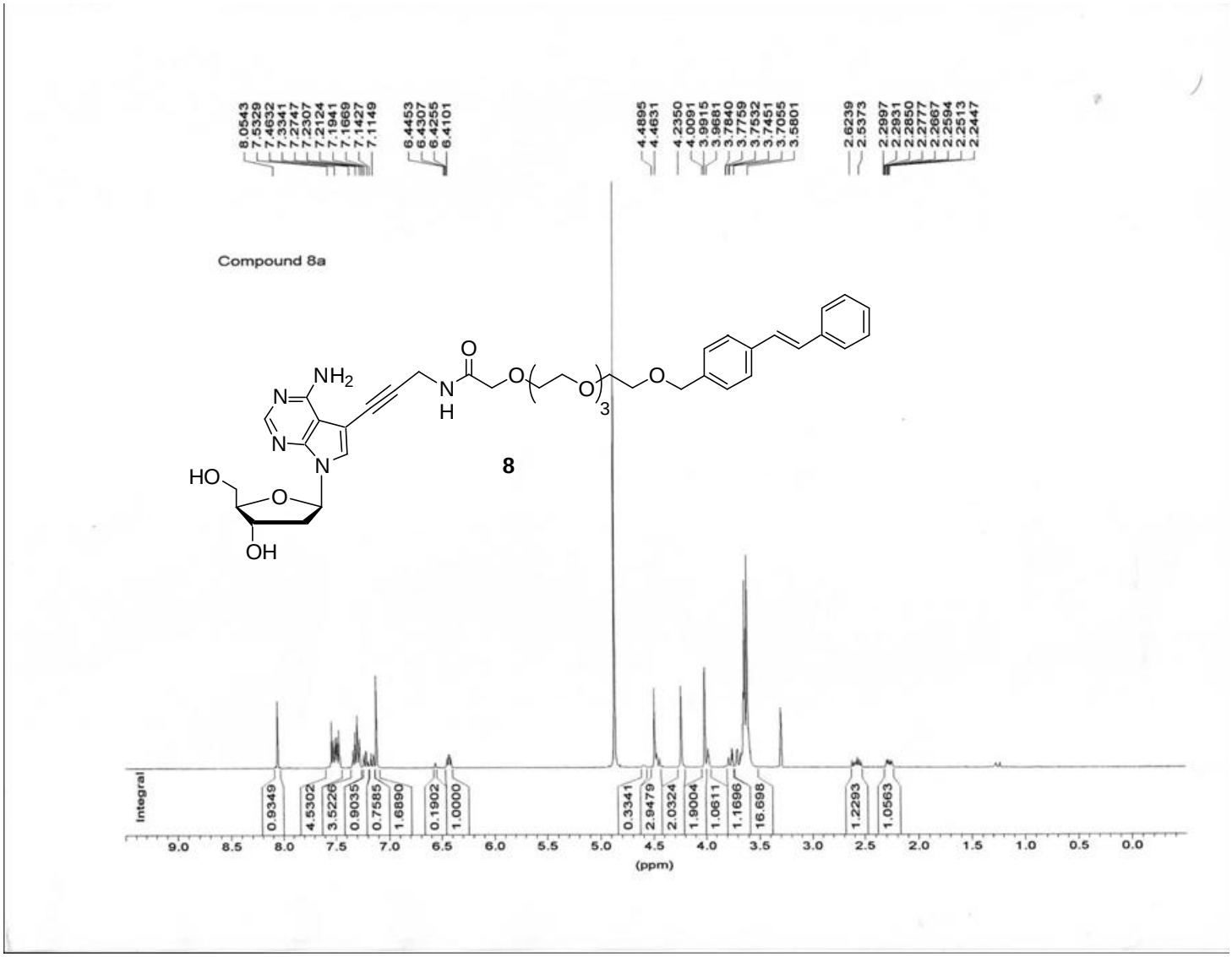
Supporting Figure 5. Gel electrophoresis of the PCR products with nucleotides **9** (sATP) and pyrene-8-dATP (pATP) using Vent (exo-) DNA polymerase.



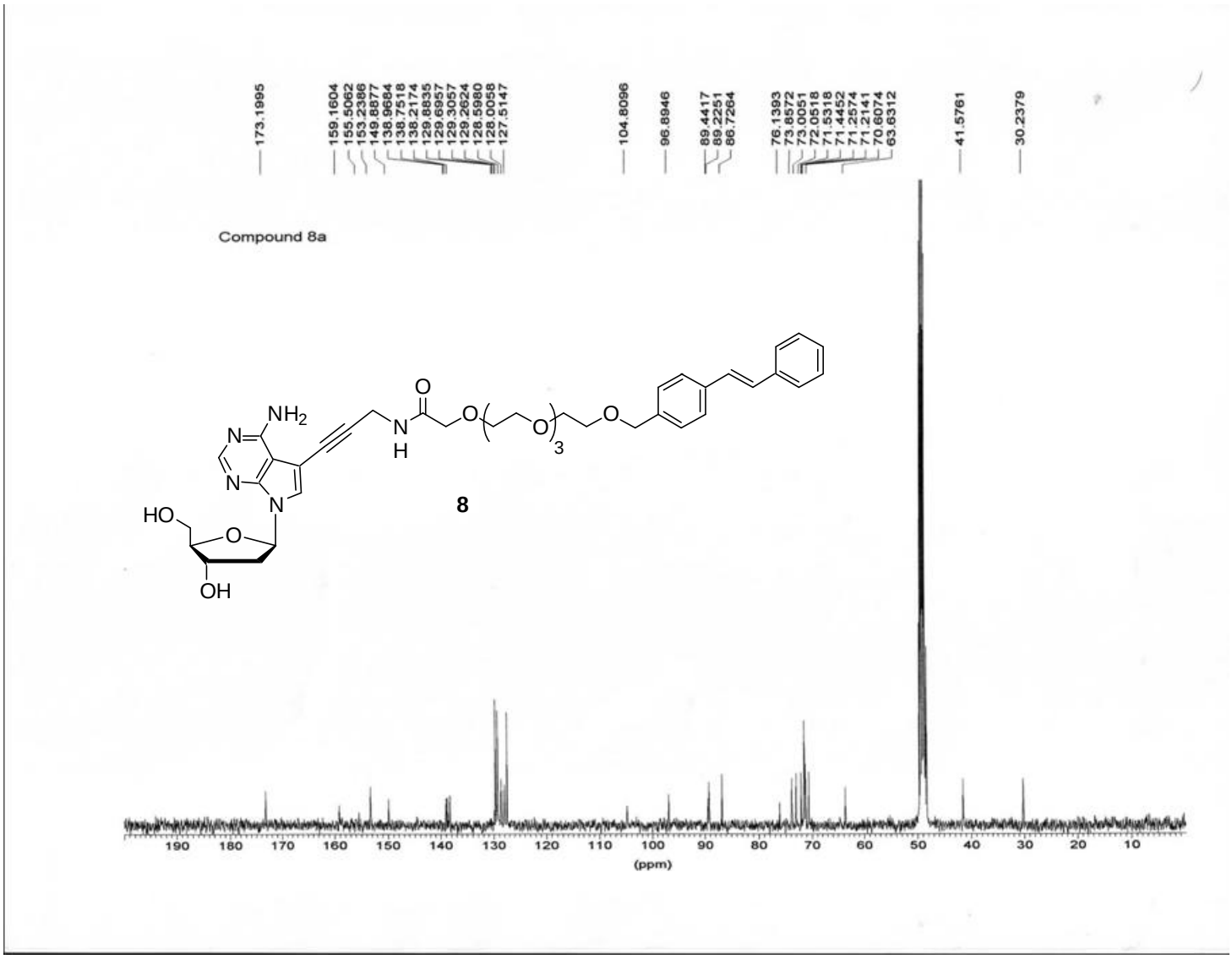
Supporting Figure 6A: ^{1}H-NMR spectrum of **7**.



Supporting Figure 6B: ^{13}C-NMR spectrum of **7**.



Supporting Figure 6C: ^{1}H-NMR spectrum of **8**.



Supporting Figure 6D: ^{13}C-NMR spectrum of **8**.