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SUPPORTING INFORMATION

Deoxynucleoside triphosphates bearing histamine, carboxylic acid, and hydroxyl residues – Synthesis and biochemical characterization

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1. General procedures:

All reactions were performed under Ar in flame-dried glassware. Anhydrous solvents for reactions were obtained by filtration through activated aluminum oxide, or by storage over 4Å activated molecular sieves. Flash chromatography was performed using silica gel (230–400 mesh) from Silicycle. Thin layer chromatography was carried out on precoated glass-backed plates of silica gel (0.25mm, UV_{254}) from Macherey-Nagel. All chemicals and solvents used were purchased from Sigma-Aldrich, unless stated otherwise. 4-pentynoic acid methyl ester was synthesized following a literature procedure.[1] 5-iodo-2'-deoxyuridine was purchased from AK Scientific and 5-iodo-2'-deoxycytidine was purchased from Carbosynth. NMR spectra were recorded on a Bruker DRX-400 or a Bruker AC-300 spectrometer (400 or 300 MHz for ^{1}H, 101 or 75.5 MHz for ^{13}C, 121.4 MHz for ^{31}P, and 376.5 MHz for ^{19}F) and all spectra were referenced to the signals of the corresponding solvent. Chemical shifts are given in ppm (δ scale) and coupling constants (J) in Hz. Assignation of the NMR signals was performed by using a combination of $^{1}H/^{1}H$-COSY, ^{13}C-DEPT-135, and $^{13}C/^{1}H$-HMBC experiments. High resolution electrospray ionization (ESI) mass spectra (MS, m/z) were recorded on a Thermo Scientific LTQ Orbitrap XL instrument. MALDI-TOF spectra were recorded on an Applied Biosystems Sciex QSTAR Pulsar instrument. HPLC purification was performed using an Äkta™ basic 10/100 system (Amersham Pharmacia Biotech) equipped with a semi-preparative Phenomenex Jupiter semi-preparative RP-HPLC column (5µ C18 300Å). PCR was carried out on a Gene Q Thermal Cycler from Bioconcept.

Vent (*exo*-) DNA polymerase, DNA polymerase I, Large (Klenow) Fragment, 9°N_m DNA polymerase, and lambda exonuclease were purchased from New England Biolabs. The *Pwo* DNA polymerase was purchased from Peqlab. T4 Polynucleotide kinase and the Terminal deoxynucleotidyl Transferase (TdT) were purchased from Promega. Sequenase Version 2.0 was purchased from Affymetryx. γ-^{32}P-ATP was purchased from Hartmann Analytic and the natural dNTPs from Promega. The dideoxynucleoside triphosphates (ddNTPs) were purchased from TriLink. Streptavidin coated magnetic beads were obtained from New England Biolabs. Oligonucleotides were purchased from Microsynth and gel purified (PAGE 20%). Acrylamide/bisacrylamide (19:1, 40%) was obtained from Serva. Radioactivity was detected using a Storm 820 phosphorimager with the ImageQuant software (both from GE Healthcare).

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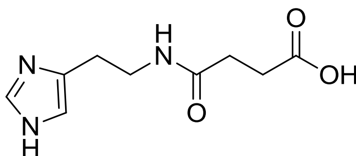
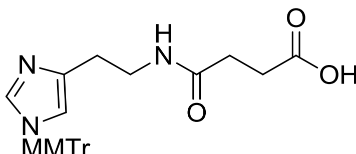
2. Synthesis of compounds S1-S3:



Succinylhistamine S1[2]

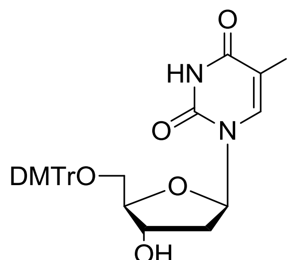
Histamine (1 g, 9 mmol) was dissolved in dry DMF (6 mL). A solution of succinic anhydride (0.99 g, 9.9 mmol) in dry DMF (6 mL) was then added dropwise over 15 min. The reaction mixture was then stirred at rt for 1 h and a white precipitate formed. The solvent was then reduced under vacuum and EtOH (15 mL) was added. After storing the precipitate at 4°C for 3 h, the solid was filtered off and washed with EtOH (2 x 15 mL). Succinylhistamine **S1** was obtained as a white solid (1.73 g, 91%). ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ 2.27-2.32 (m, 2H), 2.39-2.44 (m, 2H), 2.62 (t, 2H, J = 7.4 Hz), 3.24 (q, 2H, J = 6.8 Hz), 6.80 (s, 1H), 7.58 (s, 1H), 7.94 (t, 1H, J = 5.6 Hz), 11.79 (br s, 1H). ^{13}C-NMR (75.5 MHz, DMSO-d$_6$): δ 26.8, 29.4, 30.2, 38.8, 117.0, 134.1, 134.6, 171.0, 174.0. HR MS: *m/z*: calcd for $C_9H_{12}O_3N_3$ ([M-H]$^-$): 210.0884, found: 210.0885.



***N*-(4-methoxytrityl)-succinylhistamine S2**

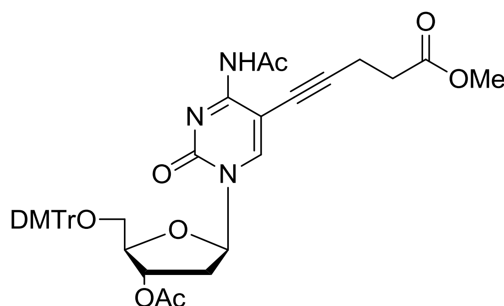
Succinylhistamine **S1** (1 g, 4.7 mmol) was suspended in dry pyridine (30 mL). MMTrCl (1.9 g, 6.2 mmol) was then added portion wise. The now clear solution was allowed to stir at rt for 12 h. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (50 mL) and washed with a diluted solution of citric acid (5% in H_2O, 100 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 50 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and evaporated. The residue was dissolved in CH_2Cl_2 (5 mL), cooled down to 4°C, and triturated with Et_2O (100 mL). R$_f$: 0.28 (CH_2Cl_2/MeOH 9:1). ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ 2.23-2.28 (m, 2H), 2.36-2.40 (m, 2H), 2.57 (t, 2H, J = 7.1 Hz), 3.24 (q, 2H, J = 6.5 Hz), 3.76 (s, 3H), 6.69 (s, 1H), 6.90-6.99 (m, 4H), 7.01-7.08 (m, 4H), 7.32-7.40

(m, 7H), 7.84 (t, 1H, J = 5.6 Hz). ^{13}C-NMR (75.5 MHz, DMSO-d_6): δ 27.8, 29.2, 30.0, 38.4, 55.1, 74.3, 113.4, 118.3, 127.9, 128.2, 129.1, 130.7, 134.1, 137.5, 137.8, 142.5, 158.6, 170.7, 173.8. HR MS: *m*/*z*: calcd for $C_{29}H_{30}O_4N_3$ ([M+H]$^+$): 484.2231, found: 484.2226.



5-iodo-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyuridine S3

5-iodo-2'-deoxyuridine (1.5 g, 4.2 mmol) was dissolved in dry pyridine (50 mL). DMAP (0.05 g, 0.4 mmol) and DMTrCl (1.72 g, 5.1 mmol) were then added in turn. The reaction mixture was allowed to stir at rt for 12 h. The solvent was removed under reduced pressure and co-evaporated with toluene (2 x 30 mL). The residue was then purified by flash column chromatography (CH_2Cl_2/MeOH/NEt_3 97:3:1), yielding **S3** as a white foam (2.57 g, 93%). R_f: 0.30 (CH_2Cl_2/MeOH 95:5 + 1% NEt_3). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.23-2.32 (m, 1H), 2.41-2.50 (m, 1H), 3.32-3.43 (m, 2H), 3.78 (s, 6H), 4.05 (d, 1H, *J* = 2.7 Hz), 4.50-4.54 (m, 1H), 6.28 (t, 1H, *J* = 6.8 Hz), 6.83 (d, 4H, *J* = 9.0 Hz), 7.22-7.33 (m, 7H), 7.37-7.41 (m, 2H), 8.11 (s, 1H). ^{13}C-NMR (75.5 MHz, DMSO-d_6): δ 29.9, 41.7, 45.9, 55.5, 63.7, 69.0, 72.3, 85.9, 86.8, 87.2, 113.6, 124.0, 127.2, 130.2, 130.3, 135.7, 136.4, 144.4, 144.6, 149.7, 158.9, 160.6. HR MS: *m*/*z*: calcd for $C_{30}H_{29}O_7N_2I$ ([M+H]$^+$): 656.1014, found: 656.1010.



Characterization of 5-[5-(pent-1-inylic acid methyl ester)]-5'-O-(4,4'-dimethoxytrityl)-3'-O-acetyl-*N*-acetyl-2'-deoxycytidine S4

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^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.00 (s, 3H), 2.22-2.30 (m, 3H), 2.39-2.44 (m, 2H), 2.63 (s, 3H), 2.76 (dd, 1H, J = 5.6, 6.4 Hz), 3.31 (apparent d, 2H, J = 3.2 Hz), 3.63 (s, 3H), 3.78 (s, 6H), 4.18 (apparent d, 1H, J = 2.0 Hz), 5.33 (d, 1H, J = 6.4 Hz), 6.22 (dd, 1H, J = 5.6, 7.6 Hz), 6.76 (dd, 4H, J = 2.0, 8.8 Hz), 7.12-7.29 (m, 8H), 7.33 (d, 2H, J = 7.2 Hz), 8.26 (s, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 15.5, 21.2, 32.7, 39.9, 52.2, 55.5, 63.7, 70.8, 75.2, 85.2, 87.7, 92.8, 97.0, 113.4, 113.5, 113.6, 127.2, 128.0, 128.1, 128.3, 129.4, 130.2, 135.5, 135.7, 144.7, 145.3, 158.9, 160.5, 170.7, 172.3. HR MS: m/z: calcd for $C_{40}H_{42}O_{10}N_3$ ($[M+H]^+$): 724.2865, found: 724.2846.

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3. Oligonucleotides (shown 5' to 3'):

CAAGGACAAAATACCTGTATTCCTT **P1**

GACATCATGAGAGACATCGCCTCTGGGCTAATAGGACTACTTCTAATCTGTAAGA
GCAGATCCCTGGACAGGCAAGGAATACAGGTATTTTGTCCTTG **T1**

GACATCATGAGAGACATCGC **P2**

GAATTCGATATCAAG **P3**

Phosphate-CACTCACGTCAGTGACATGCATGCCGATGACTAGTCGTCACTAGTGCA-
CGTAACGTGCTAGTCAGAAATTTCGCACCAC **T2**

GTGGTGCGAAATTTCTGAC **P4**

CACTCA-CGTCAGTGACATGC **P5**

Biotin-GTGGTGCGAAATTTCTGAC **P4B**

Biotin-CACTCACGTCAGTGACATGC **P5B**.

4. Additional primer extension reactions:

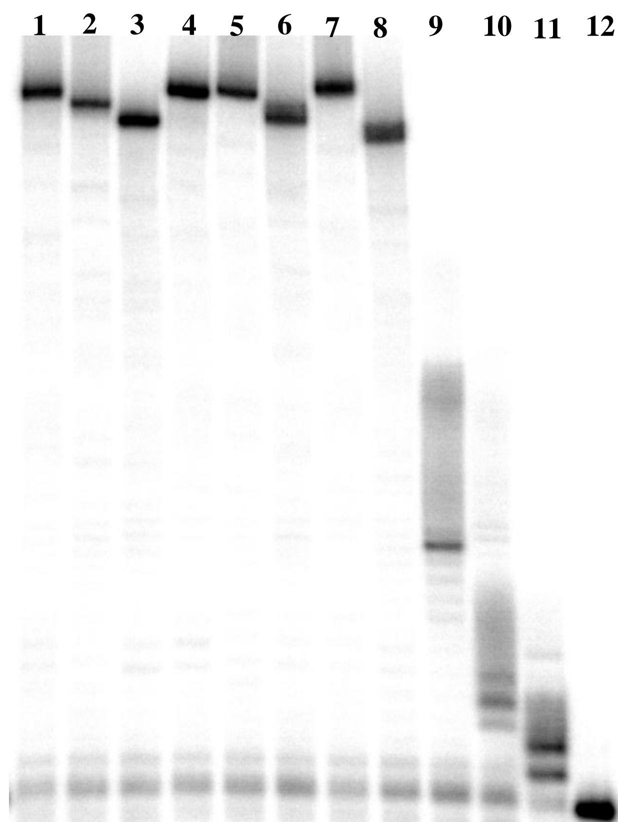

Fig. S1 Gel image (PAGE 15%) of primer extension reactions with primer **P1** and template **T1** using the 9°N_m DNA polymerase. Lane 1: $dA^{Hs}TP$ **1**; Lane 2: $dU^{POH}TP$ **2**; Lane 3: $dC^{Val}TP$ **3**; Lane 4: $dA^{Hs}TP$ **1** and $dC^{Val}TP$ **3**; Lane 5: $dA^{Hs}TP$ **1** and $dU^{POH}TP$ **2**; Lane 6: $dU^{POH}TP$ **2** and $dC^{Val}TP$ **3**; Lane 7: $dA^{Hs}TP$ **1**, $dU^{POH}TP$ **2**, and $dC^{Val}TP$ **3**; Lane 8: Natural dNTPs; Lane 9: Natural dNTPs without dATP; Lane 10: Natural dNTPs without dTTP; Lane 11: Natural dNTPs without dCTP; Lane 12: Primer **P1**.

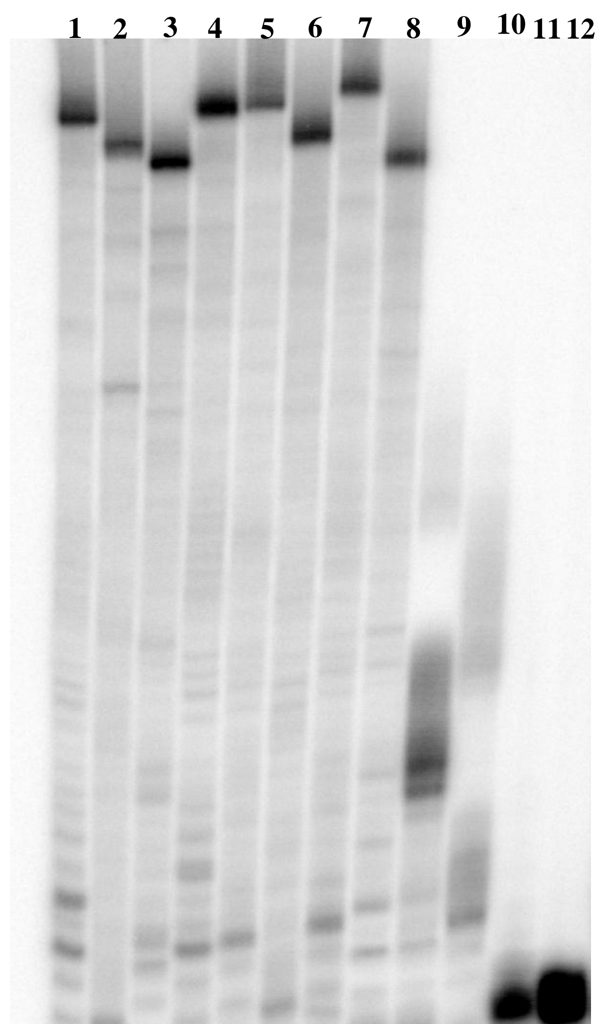

Fig. S2 Gel image (PAGE 15%) of primer extension reactions with primer **P1** and template **T1** using the Klenow fragment of *E. coli* DNA polymerase I. Lane 1: $dA^{Hs}TP$ **1**; Lane 2: $dU^{POH}TP$ **2**; Lane 3: $dC^{Val}TP$ **3**; Lane 4: $dA^{Hs}TP$ **1** and $dC^{Val}TP$ **3**; Lane 5: $dA^{Hs}TP$ **1** and $dU^{POH}TP$ **2**; Lane 6: $dU^{POH}TP$ **2** and $dC^{Val}TP$ **3**; Lane 7: $dA^{Hs}TP$ **1**, $dU^{POH}TP$ **2**, and $dC^{Val}TP$ **3**; Lane 8: Natural dNTPs; Lane 9: Natural dNTPs without dATP; Lane 10: Natural dNTPs without dTTP; Lane 11: Natural dNTPs without dCTP; Lane 12: Primer **P1**.

Fig. S3 Gel image (PAGE 15%) of primer extension reactions with primer **P1** and template **T1** using the *Pwo* DNA polymerase. Lane 1: $dA^{Hs}TP$ **1**; Lane 2: $dU^{POH}TP$ **2**; Lane 3: $dC^{Val}TP$ **3**; Lane 4: $dA^{Hs}TP$ **1** and $dC^{Val}TP$ **3**; Lane 5: $dA^{Hs}TP$ **1** and $dU^{POH}TP$ **2**; Lane 6: $dU^{POH}TP$ **2** and $dC^{Val}TP$ **3**; Lane 7: $dA^{Hs}TP$ **1**, $dU^{POH}TP$ **2**, and $dC^{Val}TP$ **3**; Lane 8: Natural dNTPs; Lane 9: Natural dNTPs without dATP; Lane 10: Natural dNTPs without dTTP; Lane 11: Natural dNTPs without dCTP; Lane 12: Primer **P1**.

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5. Conversion of modified DNA to unmodified DNA:

1) Primer extension reaction:

The 5'-[^{32}P]-labeled primer **P4** (~1 pmol) was annealed to template **T2** (3 pmol) in Thermopol buffer 10x by heating to 95°C and then gradually cooling down to room temperature. Vent (*exo*$^-$) (2 U), and water were then added in turn to the annealed oligonucleotides at 4°C. The various dNTPs were then added (100 μM final concentration) for a total reaction volume of 20 μL. Following incubation at 60°C for 15 min, the reactions were quenched by adding of 20 μL of stop solution. The reaction mixtures were subjected to gel electrophoresis in 15% denaturing polyacrylamide gel containing TBE 1x buffer (pH 8) and 7 M urea. Visualization was performed by phosphorimaging.

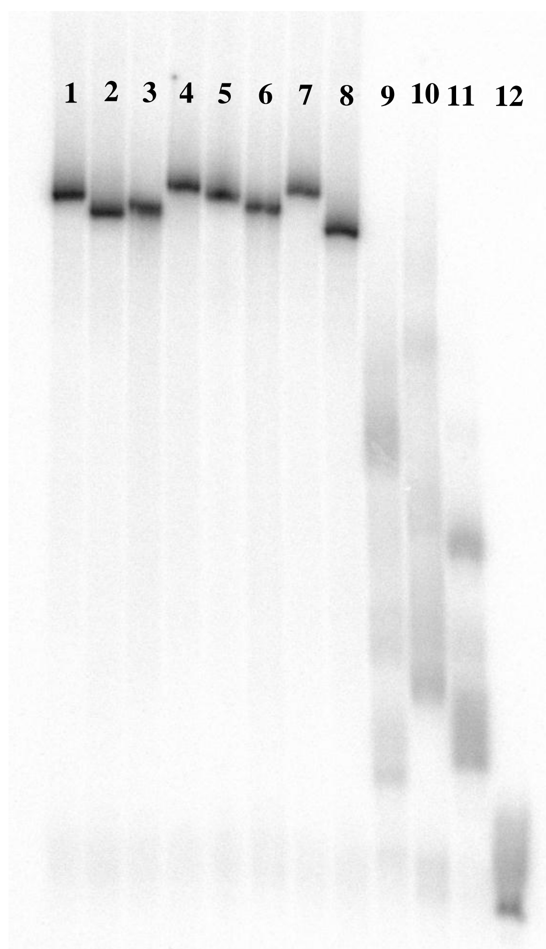

Fig. S4 Gel image (PAGE 15%) of the primer extension reactions with Vent (*exo*$^-$) using primer **P3** and template **T2**: Lane 1: dA^{Hs}TP **1**; Lane 2: dU^{POH}TP **2**; Lane 3: dC^{Val}TP **3**; Lane 4: dA^{Hs}TP **1** and dC^{Val}TP **3**; Lane 5: dA^{Hs}TP **1** and dU^{POH}TP **2**; Lane 6: dU^{POH}TP **2** and dC^{Val}TP **3**; Lane 7: dA^{Hs}TP **1**, dU^{POH}TP **2**, and dC^{Val}TP **3**; Lane 8: Natural dNTPs; Lane 9: Natural dNTPs without dATP; Lane 10: Natural dNTPs without dTTP; Lane 11: Natural dNTPs without dCTP; Lane 12: Primer **P4**.

2) PCR experiment:

Primer **P4** (24 pmol) was annealed to template **T2** (24 pmol) in Thermopol buffer 10x by heating to 95°C and then gradually cooling down to room temperature. Vent (*exo*$^-$) (2 U) and water were added to the annealed oligonucleotides at 4°C. Finally, dNTPs (3 modifications or all natural, final concentration of 200 μM) were added for a total reaction volume of 20 μL. The reaction mixtures were then incubated at 60°C for 30 min. The resulting products were precipitated (phenol–chloroform extraction followed by an EtOH precipitation) and the phosphorylated strand was digested using λ-exonuclease (room temperature, 12h, 1 U). The single stranded DNAs were then precipitated (phenol–chloroform extraction followed by an EtOH precipitation). To the resulting ssDNAs were added 40 pmol of both primers **P4** and **P5**, Vent (*exo*$^-$) (2 U), and natural dNTPs (200 μM final concentration) for a final volume of 20 μL. 30 PCR cycles were then performed (94°C for 1 min, 1 min at 55°C, and 1.5 min at 72°C) with a final extension step of 5 min at 72°C. All PCR products were analyzed by agarose 2% gels in 1x TBE buffer and containing ethidium bromide. The gels were visualized by phosphorimager analysis.

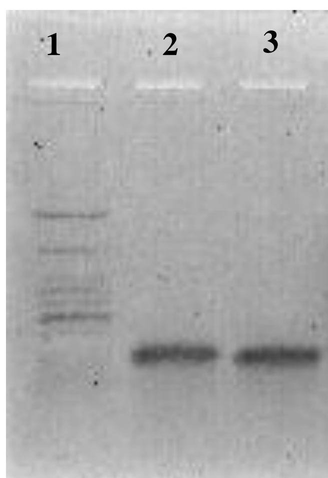

Fig. S5 Agarose (2%) gel electrophoresis of PCR products. Lane 1: Ladder; Lane 2: template containing dA^{Hs}, dU^{POH}, dC^{Val}, and dG; Lane 3: template containing natural nucleotides.

6. **Sequencing reactions:**

1) Primer extension reaction:

Primer **P4** (24 pmol) was annealed to template **T2** (24 pmol) in Thermopol buffer 10x by heating to 95°C and then gradually cooling down to room temperature. Vent (*exo*$^-$) (2 U) and water were added to the annealed oligonucleotides at 4°C. Finally, dNTPs (3 modifications or all natural, final concentration of 200 μM) were added for a total reaction volume of 20 μL. The reaction mixtures were then incubated at 60°C for 30 min. The resulting products were precipitated (phenol–chloroform extraction followed by an EtOH precipitation) and the phosphorylated strand was digested using λ-exonuclease (room temperature, 12h, 1 U). The single stranded DNAs were then precipitated (phenol–chloroform extraction followed by an EtOH precipitation), G25 desalted, and dissolved in 30 μL H_2O.

2) PCR with biotinylated primers:

The oligonucleotides obtained from the primer extension reactions were used in 2 different PCR experiments that included one biotinylated primer:

a) To half of the obtained ssDNAs were added 40 pmol of both primers **P4B** and **P5**, Vent (*exo*$^-$) (2 U), and natural dNTPs (200 μM final concentration) for a final volume of 40 μL. 30 PCR cycles were then performed (94°C for 1 min, 1 min at 55°C, and 1.5 min at 72°C) with a final extension step of 5 min at 72°C.
b) To the other half of the obtained ssDNAs were added 40 pmol of both primers **P4** and **P5B**, Vent (*exo*$^-$) (2 U), and natural dNTPs (200 μM final concentration) for a final volume of 20 μL. 30 PCR cycles were then performed (94°C for 1 min, 1 min at 55°C, and 1.5 min at 72°C) with a final extension step of 5 min at 72°C.

The resulting PCR products were then precipitated (phenol–chloroform extraction followed by an EtOH precipitation), dissolved in 100 μL TEN buffer 1x, and allowed to bind to prewashed streptavidin coated magnetic particles (50 μL, 30 min, 37°C). Following magnetization, the beads were washed with 2 x 100 μL TEN buffer 1x, and the unbiotinylated strands were received by basic elution (3 x 100 μL NaOH 0.1M). After neutralization with 30 μL HCl 1M, the decants were evaporated (speed-vac). The single stranded DNAs thus obtained were dissolved in 20 μL H_2O and G25 desalted.

3) Sequencing reactions:

The 5'-[^{32}P]-labeled primer **P4** (~3 pmol) was annealed in sequenase buffer 5x to template oligonucleotide stemming from the PCR using primer **P4B**, while the 5'-[^{32}P]-labeled primer **P5** (~3 pmol) was annealed to the strand made with primer **P5B**. A mixture of natural dNTPs

(175 μM final concentration) and DTT (4.5 mM final concentration) were then added to the annealed oligonucleotides. The resulting solutions were then split into 6 eppendorfs for the different sequencing reactions. 4 of these vials contained each of the dideoxynucleoside triphosphates (ddNTPs, 20 μM final concentration), while one eppendorf only contained water (negative control), and the last was used for the positive control. A 1:5:1 mixture of thermostable inorganic pyrophosphatase (1.2 U/reaction), Sequenase diluent, and Sequenase (5.2 U/reaction) was added to the vials as a cocktail at 4°C, for a final volume of 10 μL. The reaction mixtures were then incubated at 37°C for 15 min and quenched by adding of 10 μL of stop solution. The reaction mixtures were subjected to gel electrophoresis in 7% denaturing polyacrylamide gel containing TBE 1x buffer (pH 8) and 7 M urea. Visualization was performed by phosphorimaging.

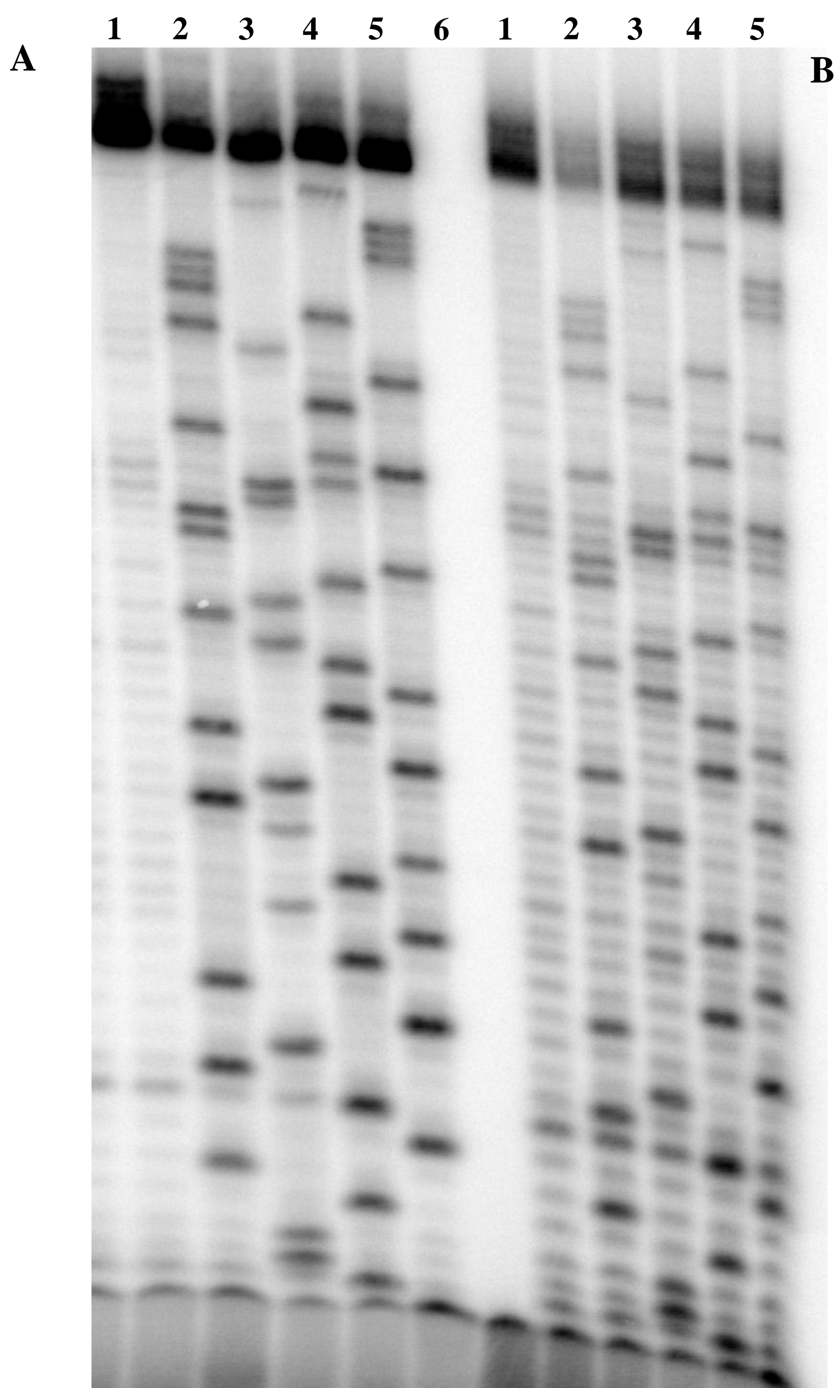

Fig S6 Gel image (PAGE 7%) of the sequencing reactions of the products stemming from the PCR with primer **P5B**. A) Template formed with natural dNTPs; B) template formed with modified dNTPs. Lane 1: only dNTPs, no ddNTPs; Lane 2: with ddATP; Lane 3: with ddCTP; Lane 4: with ddGTP; Lane 5: with ddTTP; Lane 6: primer **P5**.

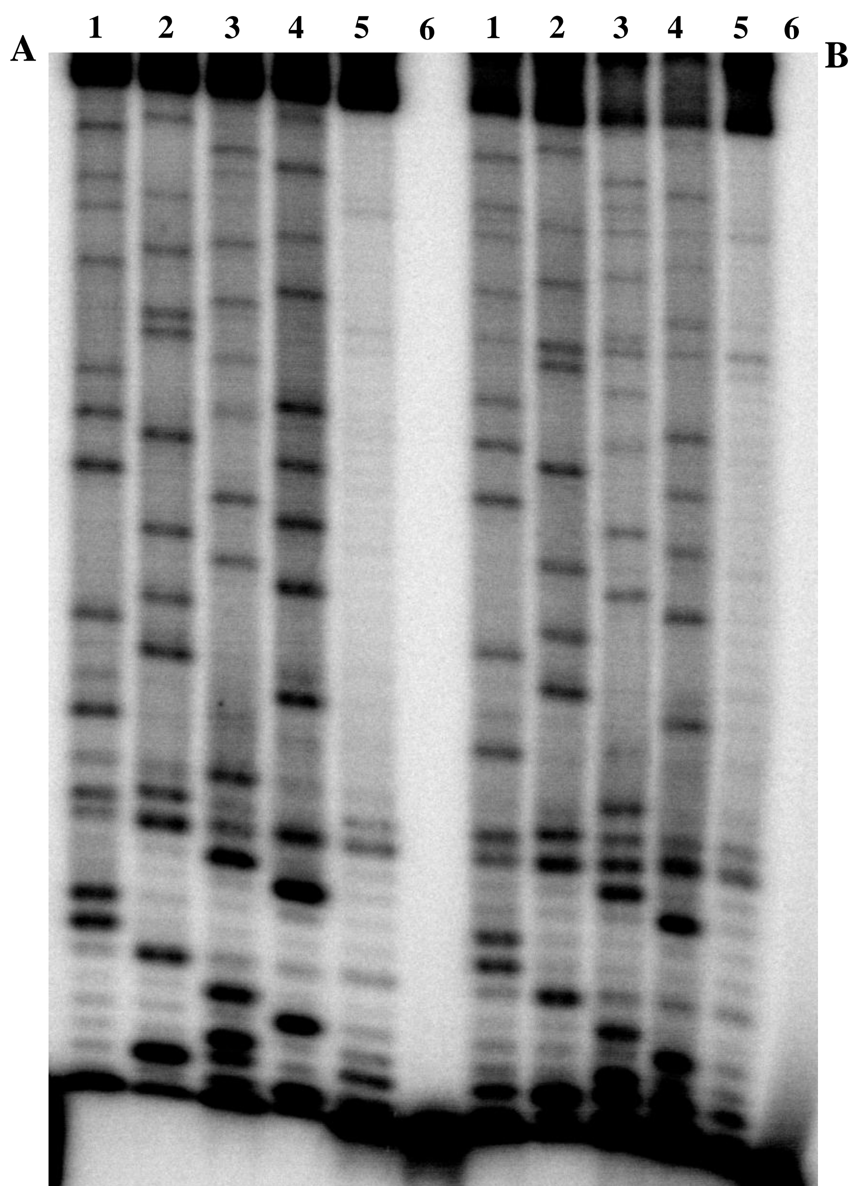

Fig S7 Gel image (PAGE 15%) of the sequencing reactions of the products stemming from the PCR with primer **P4B**. A) Template formed with modified dNTPs; B) template formed with natural dNTPs. Lane 1: with ddTTP; Lane 2: with ddGTP; Lane 3: with ddCTP; Lane 4: with ddATP; Lane 5: only dNTPs, no ddNTPs; Lane 6: primer **P4**.

7. References for the Supporting Information:

1. H. Shimotahira, S. Fusazaki, I. Ikeda and Y. Ozoe, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 1598-1600.
2. L. Morandeau, E. Benoist, A. Loussouarn, A. Ouadi, P. Lesaec, M. Mougin, A. Faivre-Chauvet, J. Le Boterff, J. F. Chatal, J. Barbet and J. F. Gestin, *Bioconjugate Chem.*, 2005, **16**, 184-193.