Enzymatic Cleavage of 3'-Esterified Nucleotides Enables a Long, Continuous DNA Synthesis

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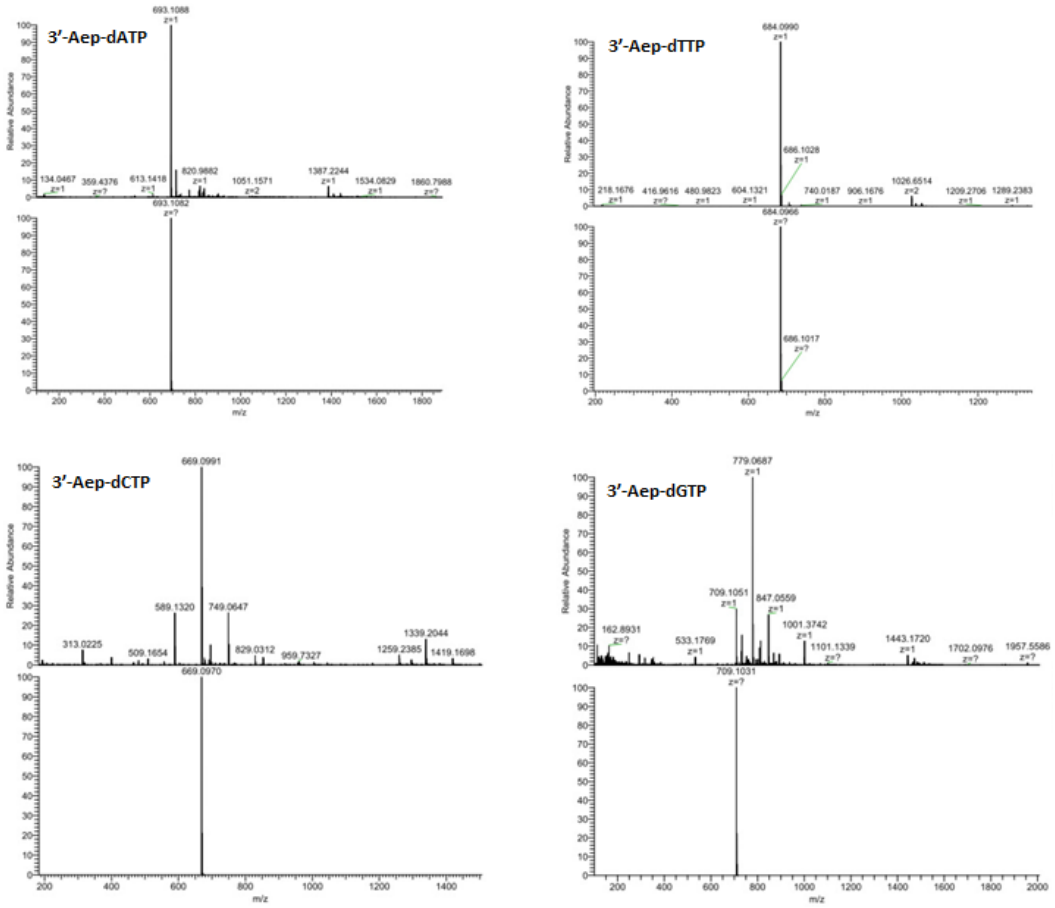
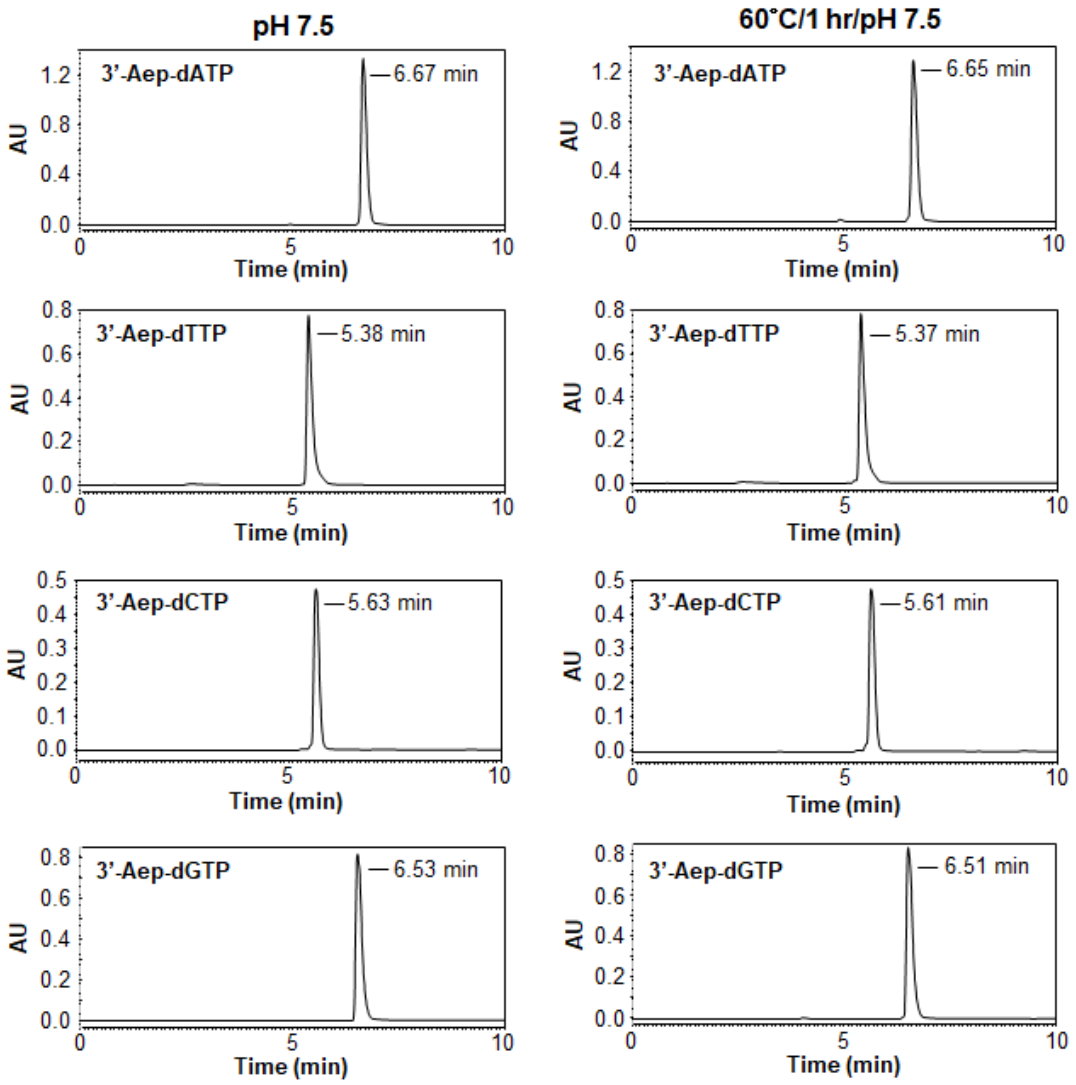


Figure S1. Analysis of 3'-Aep-dNTP using ESI-MS analysis.

The nucleotide analysis was performed as described previously with the Bruker AutoFlex III smartbeam TOF/TOF 200 system (Bruker Daltonics, MA)[30,45].



Conditions	Purity			
	3'-Aep-dATP	3'-Aep-dTTP	3'-Aep-dCTP	3'-Aep-dGTP
pH 7.5	99.32%	99.92%	99.50%	99.75%
pH 7.5/1 hr/60°C	98.75%	99.57%	99.50%	99.33%

Figure S2. Purity and stability of 3'-Aep-dNTP verified by HPLC analysis.

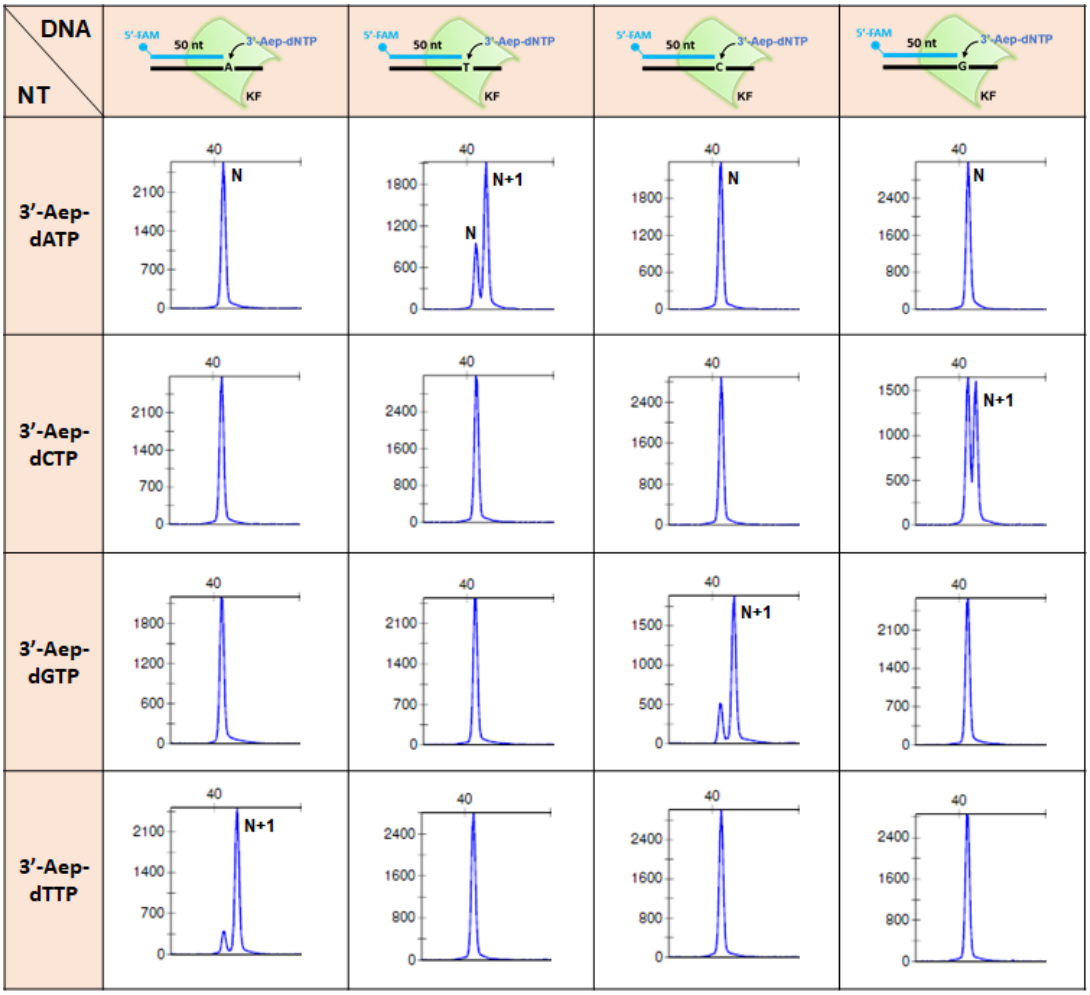


Figure S3. Selectivity of matched and mismatched 3'-Aep-dNTP by KF. Each reaction contains 3 nM of DNA primer, 1 μM of 3'-Aep-dNTP and 0.1U of KF as described in the Methods. Reactions were performed at 60°C for 5 minutes.

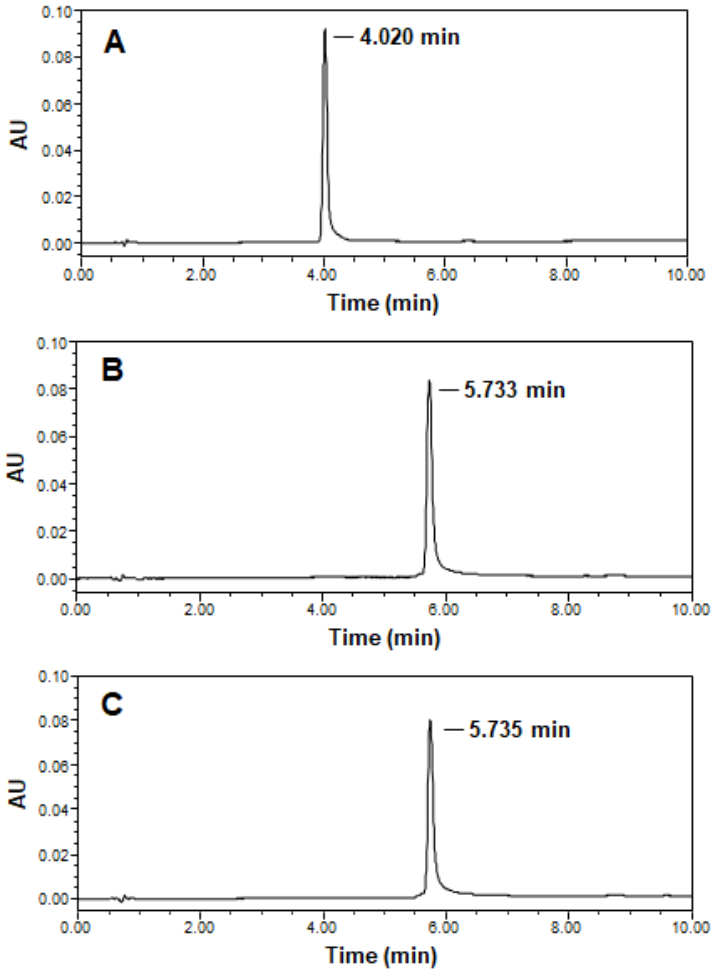


Figure S4. Analysis of 3'-Aep-dATP stability in the presence of BF in the DNA polymerase reaction condition by HPLC. (A) 80 μM of dATP only, (B) 80 μM 3'-Aep-dATP only, and (C) 80 μM of 3'-Aep-dATP and BF. The reactions were performed at 45°C for 10 minutes.

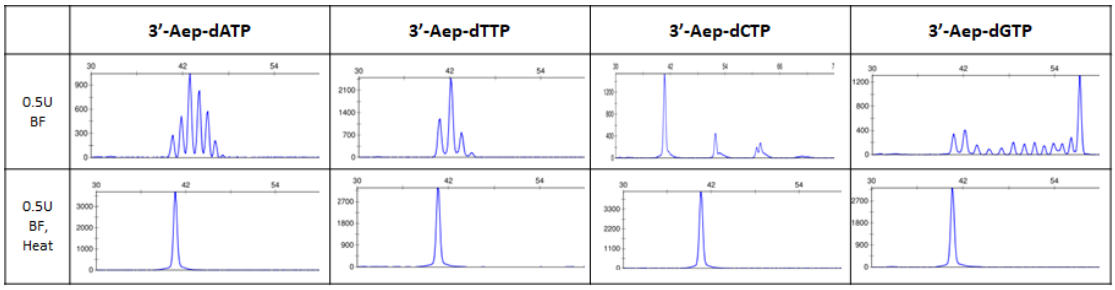


Figure S5. Multiple incorporations of 3'-Aep-dNMP by BF (Top) or heat-inactivated BF (Bottom) on the homopolymeric DNA template. The heat-inactivated BF was pre-treated at 90°C for 15 minutes before added into the reaction mixture.

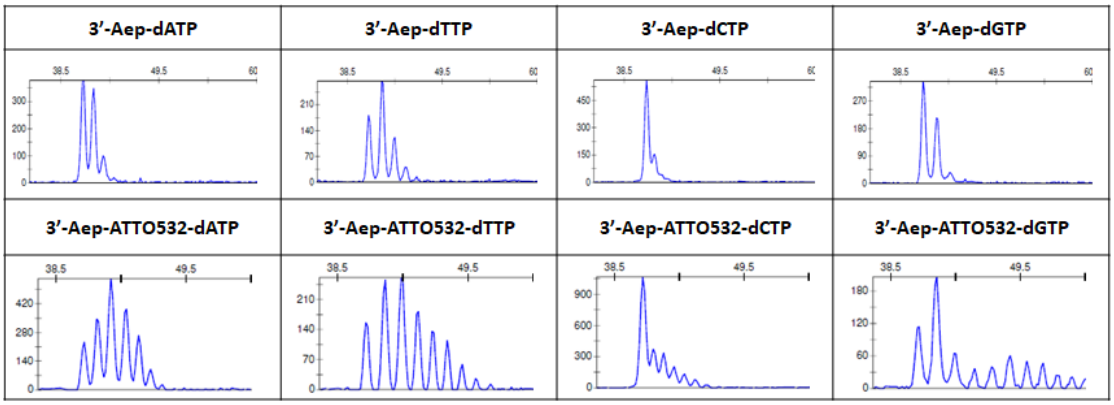


Figure S6. Multiple incorporations of 3'-Aep-dNMP and 3'-Aep-ATTO532-dNMP by BF on the homopolymeric DNA templates. All reactions were performed at 45°C for 30 seconds in the presence of 10 μM nucleotide.

Table S1. List of oligonucleotides used in this study[a].

No.	Sequence (5' to 3')	Experiment
Oligo_P#1	FAM-AGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGC	PE & Kinetics
Oligo_T#1	TTGCTCGTTTGCTGGG**A**GCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACT	PE & Kinetics
Oligo_T#2	TTGCTCGTTTGCTAAA**G**GCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACT	PE & Kinetics
Oligo_T#3	TTGCTCGTTTGCTGGG**T**GCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACT	PE & Kinetics
Oligo_T#4	TTGCTCGTTTGCTGGG**C**GCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACT	PE & Kinetics
Oligo_P#2	CCCTCGCAGCCGTCCAACCAACTCA	MS
Oligo_T#5	TTTGTTCTCC**A**TGAGTTGGTTGGACGGCTGCGAGGG	MS
Oligo_T#6	TTTGTTCTCC**T**TGAGTTGGTTGGACGGCTGCGAGGG	MS
Oligo_T#7	TTTGTTCTGG**C**TGAGTTGGTTGGACGGCTGCGAGGG	MS
Oligo_T#8	TTTGTTCTCC**G**TGAGTTGGTTGGACGGCTGCGAGGG	MS
Oligo_T#9	TTACTCG**AAAAAAAAAAAA**GCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACT	PE of homopolymer
Oligo_T#10	TTACTCG**TTTTTTTTTTTT**GCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACT	PE of homopolymer
Oligo_T#11	TTACTTT**CCCCCCCCCCCC**GCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACT	PE of homopolymer
Oligo_T#12	TTACTTT**GGGGGGGGGGGG**GCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCACT	PE of homopolymer
Oligo_P#3	FAM-CGAGCACGTATAACGTGCTTTCCTCGTTGGAATCAGAGCGGGAGCTAAAC	DNA elongation
Oligo_T#13	ATGGACAGACTCTTTTACTCGGTGGCCTCACTGATTATAAAAACACTTCTCAAGATTCTGGCGTACCGTTCCTGTCTAAAATCCCTTTAATCGGCCTCCTGTTTAGTCCCGCTCTGATTCCAACGAGGAAAGCACGTTATACGTGCTCG	DNA elongation
Oligo_P#4	CTTTTAAGAACCGGACGAACCGAGCACGT**T**TAACGTGCTTTCCTCG	Amplification for sequencing "**T**": watermark
Oligo_T#14	ATGGACAGACTCTTTTACTCGGTGGCCTCACTGATTATAAAAACACTTCTCAAGATTCTGGCGTACCGTTCCTGTCTAAAATCCCTTTAATCGGCCTCCTCGAGGAAAGCACGTTA**T**ACGTGCTCG	Amplification for sequencing "**T**": watermark
Oligo_P#5	CTTTTAAGAACCGGACGAACCGAG	Amplification for sequencing
Oligo_P#6	CTGGATATTACCAGCAAGGCCGAT	Amplification for sequencing

[a]The underlined sequences are complimentary regions, and the bolded bases are the templating bases for specific nucleotide incorporation.