Supporting Information for:

A Nucleic Acid Base Analogue FRET-pair Facilitating Detailed Measurements in Nucleic Acid Containing Systems

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RP-HPLC analysis and purification of oligonucleotides

The oligonucleotides were analyzed and purified on a Gilson HPLC system using a Brownlee Aquapore RP-HPLC column (8 mm x 25 cm, Perkin-Elmer). The following protocol was used: run time, 24 min; integration time 21 min; flow rate 4 mL per min; binary system. Gradient: time in mins (% buffer B): 0 (0), 3 (0), 4 (10), 17 (40), 19 (100), 20 (100), 21 (0), 24 (0). Elution buffers: (A) 0.1 M aqueous NH_4OAc pH 7 (B) 0.1 M aqueous NH_4OAc with 50% acetonitrile pH 7. Elution of oligonucleotides was monitored by ultraviolet absorption at 296 nm.

Oligo Code	Oligonucleotide sequences (5'- to 3')	Calculated Mass	Found Mass
A4184	CGATCACACA**X**AAGGACGAGGATAAGGAGGAGG	10394	10394 [M]
A4185	CGATCACA**X**ACAAGGACGAGGATAAGGAGGAGG	10394	10391 [E]
A4186	CGATCA**X**ACACAAGGACGAGGATAAGGAGGAGG	10394	10392 [M]
A4187	CCTCCTCCTTATCCTCGTC**Y**TTGTGTGTGATCG	10115	10124 [E]
A4188	CCTCCTCCTTATCCTCGT**Y**CTTGTGTGTGATCG	10115	10118 [E]
A4189	CCTCCTCCTTATC**Y**TCGTCCTTGTGTGTGATCG	10115	10120 [E]
A4190	CCTCCTCCTTAT**Y**CTCGTCCTTGTGTGTGATCG	10115	10120 [E]
A4191	CGTC**Y**TTTGC	3121	3122 [E]
A4192	CGTT**Y**CTTGC	3121	3123 [E]

Table S2. M = MALDI-TOF, E = Electrospray. X = tC^O, Y = tC_{nitro}

Mass Spectra of oligonucleotides were recorded by negative mode electrospray on a Fisons VG platform mass spectrometer in acetonitrile/water (HPLC grade) or by MALDI-TOF using a ThermoBioAnalysis Dynamo MALDI-TOF mass spectrometer in positive ion mode with oligo-dT standards.[1]

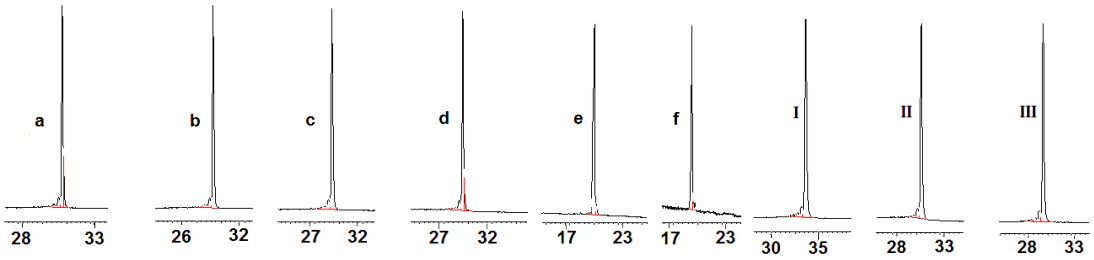

Figure S1. *Capillary Electrophoresis analysis of oligonucleotides. Oligonucleotides a to d are* tC_{nitro} *-containing sequences and I to III contain* tC^{O}*. a: A4187, b: A4188, c: A4189, d: A4190, e: A4191, f: A4192, I: A4184, II: A4185, III: A4186. 0.4 OD/100 µL of each sample in water was injected. ssDNA 100-R Gel, Tris-Borate-7 M Urea were used (Kit No 477480) on a Beckman Coulter P/ACE*^{TM} *MDQ Capillary Electrophoresis System using 32 Karat software. UV-254 nm, injection-voltage 10.0 kV and separation-voltage 9.0 kV (45.0 min. duration). X-axis is time (min), Y-axis is UV absorbance at 254 nm.*

References

(1) G. J. Langley, J. M. H., N. L. Davies, T. Brown, *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1717.