Supporting Information

Templated Synthesis of Nylon Nucleic Acid and Characterization by Nuclease Digestion

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Materials. All buffer solutions were prepared from analytically pure chemicals purchased from Sigma-Aldrich (St. Louis, MO) and doubly distilled water. Phosphodiesterase II, calf spleen phosphodiesterase (CSP, a 5',3' exonuclease) was purchased from Sigma-Aldrich (St. Louis, MO), bacterial alkaline phosphatase (BAP) and 10×BAP buffer were purchased from Invitrogen (Carlsbad, CA) and phosphodiesterase I, snake venom phosphodiesterase (SVP, 3',5' exonuclease) was purchased from USB Corp (Cleveland, OH). The preparation of the modified phosphoramidite uridine monomers and synthesis of nylon nucleic acid precursor strands followed the procedures previously reported,[1] except that strands were cleaved from the resin and deprotected at 50 ^{o}C, instead of at room temperature. Compound dU_8 was synthesized on an automatic DNA synthesizer with deoxyuridine support and phosphoramidite purchased from Glen Research (Sterling, VA). The complementary hairpin DNA 46-mer for templated nylon nucleic acid synthesis, the unmodified DNA **1**, **2**, and dA_8 were obtained from Integrated DNA Technologies (Coralville, IA). All commercial DNA strands and synthesized strands were purified either by denaturing gel electrophoresis (20 % acrylamide; running buffer contained 89 mM Tris•HCl, pH 8.0, 89 mM boric acid, and 2 mM EDTA) or by HPLC (20 mM phosphate buffer, pH 7.0 / methanol). The complementary hairpin DNA and nylon nucleic acid precursor strands were further desalted by HPLC (water / acetonitrile). (**NOTE:** Desalting by HPLC is

essential for templated coupling without side reactions.) Concentrations of oligonucleotides were determined by UV spectroscopy (OD_{260}).

Hairpin **1**:
5'-GCACGTATGCTGTTTTCAGCATACGTGCCGTAT
CAAAAAACAGATG was used as the template for the synthesis of nylon nucleic acids **3b** – **8b**.

Hairpin **2**:
5'-GCACGTATGCTGTTTTCAGCATACGTGCCGTAT
AAAAAAAAAGATG was used as the template for the synthesis of nylon nucleic acid **9b**.

DNA templated nylon nucleic acid synthesis. The nylon nucleic acid precursor strand (15 nmol) and the complementary hairpin DNA strand (16.5 nmol, 1.1 equivalence) were dissolved in 3-(N-morpholino)-propanesulfonic acid (MOPS) buffer (700 µL, 0.1 M MOPS, 1.0 M NaCl, pH 7.0) in an Eppendorf vial. The resulting solution was annealed overnight from 90 ^{o}C to room temperature. An aliquot (2 µL) was subjected to non-denaturing gel electrophoresis to check the formation of duplex. The condensing agent 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM, 13.8 mg, 0.05 mmol) was dissolved in MOPS buffer (200 µL) and the DMTMM solution (150 µL) was added to the nylon nucleic acid precursor strand**:** hairpin DNA duplex solution. The reaction solution was mixed with the pipette and centrifuged before being held at room temperature for 48 h. For nylon nucleic acids **8b** and **9b**, an additional DMTMM aliquot (120 µL) was added after 24 h. After coupling, another aliquot (2 µL) was checked by non-denaturing gel electrophoresis to confirm that the oligonucleotides were still in duplex form. The reaction solution was subjected to ethanol precipitation or filtration through a G25 cartridge in order to remove most of the salt before purification by denaturing gel electrophoresis. Nylon nucleic acids were separated from the template hairpins by denaturing gel electrophoresis (20 % acrylamide; running buffer contains 89 mM Tris.HCl, pH 8.0, 89 mM boric acid, and 2 mM EDTA). Note: formamide should be avoided to minimize the side reaction. The separated nylon nucleic acids were further desalted by HPLC (water / acetonitrile). Strands concentrations were determined by UV spectroscopy (OD_{260}).

MALDI-TOF MS analysis. All MALDI-TOF mass spectra were recorded on a Bruker OmniFLEX MALDI-TOF spectrometer.

Procedure for characterization of nylon nucleic acids and precursor strands: A 3-hydroxypicolinic acid (3-HPA) matrix solution was prepared by dissolving 3-HPA (18 mg) in CH_3CN (150 μL) and H_2O (150 μL). An ammonium citrate co-matrix solution was prepared by dissolving ammonium citrate (35 mg) in H_2O (1 mL). The working matrix solution was obtained by mixing the 3-HPA matrix solution (40 μL) and the ammonium citrate co-matrix solution (10 μL). An oligonucleotide sample (20~100 μM, 2 μL) was mixed with the working matrix solution (2.5 μL) using a vortex mixer, and then centrifuged. The mixture was deposited on a target. ODNs (oligodeoxynucleotides) with known masses were used as calibrants in each measurement.

Procedure for characterization of the complete nuclease digestion products (nylon ribonucleosides): An α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution was prepared by dissolving CHCA (20 mg) in CH_3CN (500 μL) and H_2O (500 μL) containing 0.1 % TFA. The neutral uridine oligoribonucleoside sample (2~10 μM, 2 μL) was mixed with the CHCA solution (2 μL) using a vortex mixer and then centrifuged. The mixture was deposited on a target. Peptides or proteins with known masses were used as calibration standards in each measurement.

Phosphodiesterase II controlled digestion. The controlled digestion procedure followed slight modification of a previously reported protocol.[2] ODN (500 pmol in 5 μL of water) was mixed with phosphodiesterase II (calf spleen phosphodiesterase, CSP) solution (5 milliunits in 5 μL water) and ammonium citrate water solution (1 μL, 50 g/L). After vortex mixing and centrifugation, the mixture was incubated at 37 ^{o}C. Aliquots (1 μL) were removed after 0.5, 2, 4, 9, 15, 20, 30, 40, 60, 240 min and quickly mixed with trihydroxyacetophenone (THAP, as MALDI-TOF matrix) solution (1 μL, 30 mg in 1mL methanol) before being placed in dry ice to quench the reaction. All digestion aliquots were spotted on a target and analyzed by MALDI-TOF MS.

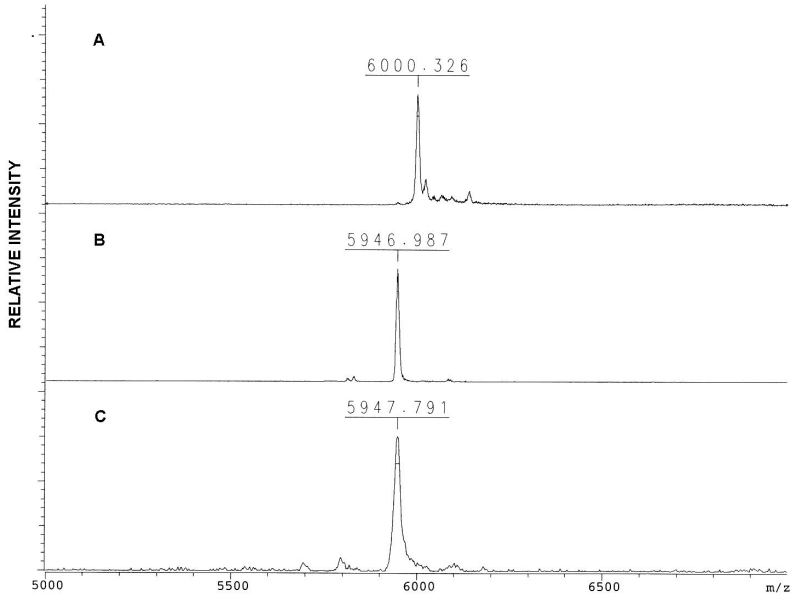
Phosphodiesterase I / bacterial alkaline phosphatase complete digestion. An ODN (200 pmol) was dissolved in 1×BAP buffer (15 μL, diluted from 10×BAP buffer) containing $MgCl_2$ (16 mM), bacterial alkaline phosphatase (BAP, 1 unit) and phosphodiesterase I (snake venom phosphodiesterase, SVP, 0.5 units). The digestion solution was incubated at 37 ^{o}C for 12 h and

then at room temperature for 24 h. Thereafter, additional BAP (0.4 units) and SVP (0.2 units) were added to the digestion solution which was incubated at 37 ^{o}C for another 12 h. Nylon nucleic acids **8b** and **9b** were further incubated for 48 h at room temperature to ensure complete digestion. The final solution was directly subject to LCMS analysis without further purification.

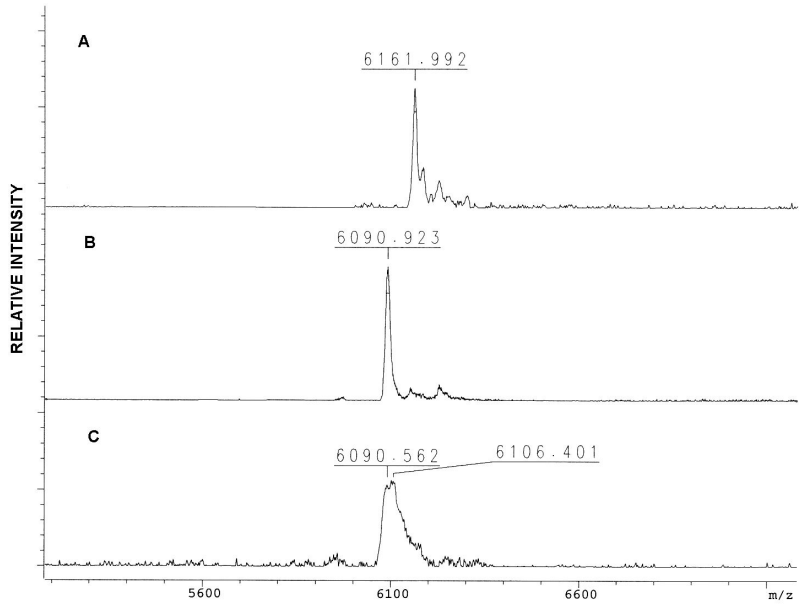
LCMS analysis of complete nuclease digests of ODNs. The analysis of digested products by LCMS was conducted on an Agilent 1100 serial Capillary LCMSD Trap XCT system equipped with an atmospheric pressure electrospray ionization source. Either a Zorbax SB-C18 column (2.1 mm×150 mm, 5 μm, Agilent Technologies) or a Zorbax SB-C8 column (2.1 mm × 50 mm, 5 μm, Agilent Technologies) was used for the separation of the nuclease digestion products. A 6 μL portion of digestion solution was injected onto the analytical column held at 30 ^{o}C. Eluents: A, water containing 0.1 % formic acid; B, methanol containing 0.1 % formic acid. Gradient for nylon nucleic acids and unmodified DNA digests was: 0 min, 95 % A; 2 min, 95 % A; 5 min, 65 % A; 40 min, 15 % A; 45 min, 0 % A. The gradient for precursor strand **6a** digests was: 0 min, 100 % A; 5 min 100 % A; 35 min, 95 % A; 70 min, 15 % A. The flow rate was 200 μL/min. The effluent was monitored by UV absorption detection at 254 nm. Electrospray source conditions were 8 L/min drying gas flow rate, 40.0 psi nebulizer pressure, and 350 ^{o}C drying temperature. Mass spectra were recorded in positive mode and the target mass of the ion trap was set to the mass of uridine oligomer from the corresponding nylon nucleic acid digestion. For analysis of nylon nucleic acids **3b** – **7b** digestions, Ultra Scan mode was applied with 50 – 2200 *m/z* scan range. For analysis of nylon nucleic acids **8b**, **9b** digestions, extended mode was applied with 200 – 4000 *m/z* scan range and concomitant lower resolution. All data were analyzed using the LC/MSD Trap Control 4.0 data analysis software.

HPLC separation of complete nuclease digests of nylon nucleic acids. Complete nuclease digestion products were chromatographed on a Zorbax C8 analytical column (4.6mm×50mm, 5μm Agilent Technologies). Eluents: A, water; B, acetonitrile. Gradient: 0 min, 100 % A; 2 min 100 % A; 3 min, 93 % A; 25 min, 73 % A. Flow rate: 1 mL/min. The effluent was monitored by UV absorption detection at 260 nm. After removal of solvent by vacuum spinner, the fraction containing oligouridine nucleosides was re-dissolved in water and subject to MALDI-TOF MS analysis.

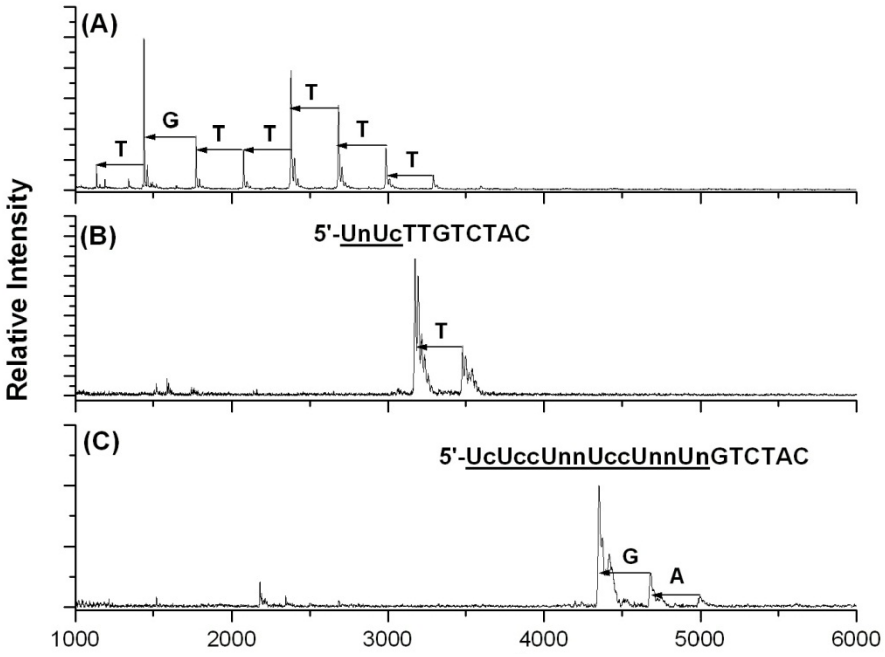
Thermal denaturing studies. Pairs of complementary strands (45 nmol each) were dissolved in a buffer with stated salt concentrations to a final volume of 120 μL. The solution was annealed overnight from 70˚C to room temperature. The samples were transferred to quartz cuvettes with 1 mm path length and 100 μL volume. Buffer was used as a blank. Thermal denaturation was monitored at 260 nm on a Cary 100 Bio spectrometer. At least two consecutive heating-cooling cycles were applied with a linear temperature gradient of 0.1 ˚C/min. Absorbance vs. temperature curves were converted into θ vs. temperature curves (where θ is the fraction of oligomers in the associated state) by subtracting upper and lower base lines. The upper and lower linear base lines were used to define temperature-dependent extinction coefficients for associated and dissociated states. T_m is defined as the temperature at which half of the strands are in the associated form and half in the dissociated form, i.e. $\theta = 0.5$.[3] Only the T_m of the U8 : dA_8 duplex in water, 10 mM phosphate buffer, 100 mM NaCl/10 mM phosphate buffer could be determined. No T_m could be extracted from other melting curves due to the lack of UV transitions or the lack of lower base lines.



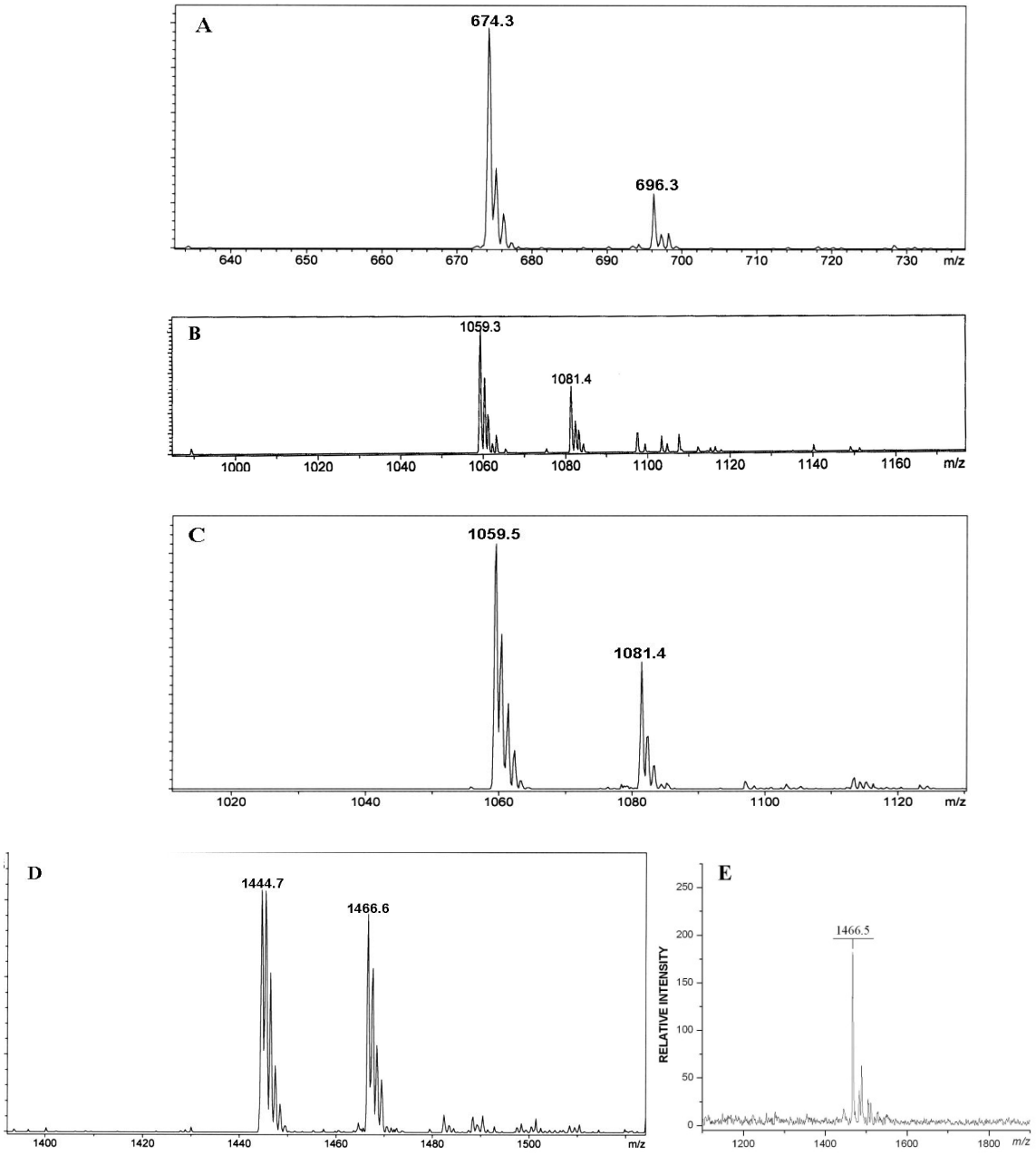
S1. Comparison of MALDI-TOF MS of (A) the precursor strand **6a**, (B) DNA templated synthesis of nylon nucleic acid **6b**, and (C) single-stranded synthesis of nylon nucleic acid **6b**.



S2. Comparison of MALDI-TOF MS of (A) the precursor strand **7a**, (B) DNA templated synthesis of nylon nucleic acid **7b**, and (C) single-stranded synthesis of nylon nucleic acid **7b**.



S3. MALDI-TOF mass spectra of phosphodiesterase II (CSP) digests of DNA **1** at 2 min (A), nylon nucleic acid **3b** at 15 min (B) and **8b** at 15 min (C).



S4. ESI mass spectra (under ultra scan mode) of oligouridine nucleoside fragments from LCMS analysis of nylon nucleic acid complete nuclease digestion. (A) **U2** from digestion of **3b**, (B) **U3a** from digestion of **4b**, (C) **U3b** from digestion of **5b** and (D) **U4** from digestion of **6b**. All mass spectra show $[M+H]^+$ peak and $[M+Na]^+$ peak. (E) MALDI-TOF mass spectrum of **U4** purified by HPLC, only $[M+Na]^+$ and $[M+2Na]^{2+}$ peaks shown.

S5. Relative integrated areas calculated from UV chromatogram of LCMS analysis of DNA and nylon nucleic acids. [a, b, c, d]

ODNs	Deoxycytidine (dC)	Deoxyadenosine (dA)	Deoxyguanosine (dG)	Thymidine (dT)	Oligonucleosides
1	1	3.27 (3.31)	3.84 (3.79)	6.71(6.67)	--
2	1	3.35	1.30	8.20	--
3b	1	3.35	3.81	5.32	1.36 (**U2**)
4b	1	3.16	3.80	4.50	1.68 (**U3a**)
5b	1	3.36	3.65	4.22	1.78 (**U3b**)
6b	1	3.15	3.88	3.69	2.23 (**U4**)
7b	1	3.14	3.61	2.99	2.47 (**U5**)
8b	1	3.31	3.83	2.36	2.56 (**U6**)
9b	1	3.33	1.24	2.35	2.83 (**U8**)

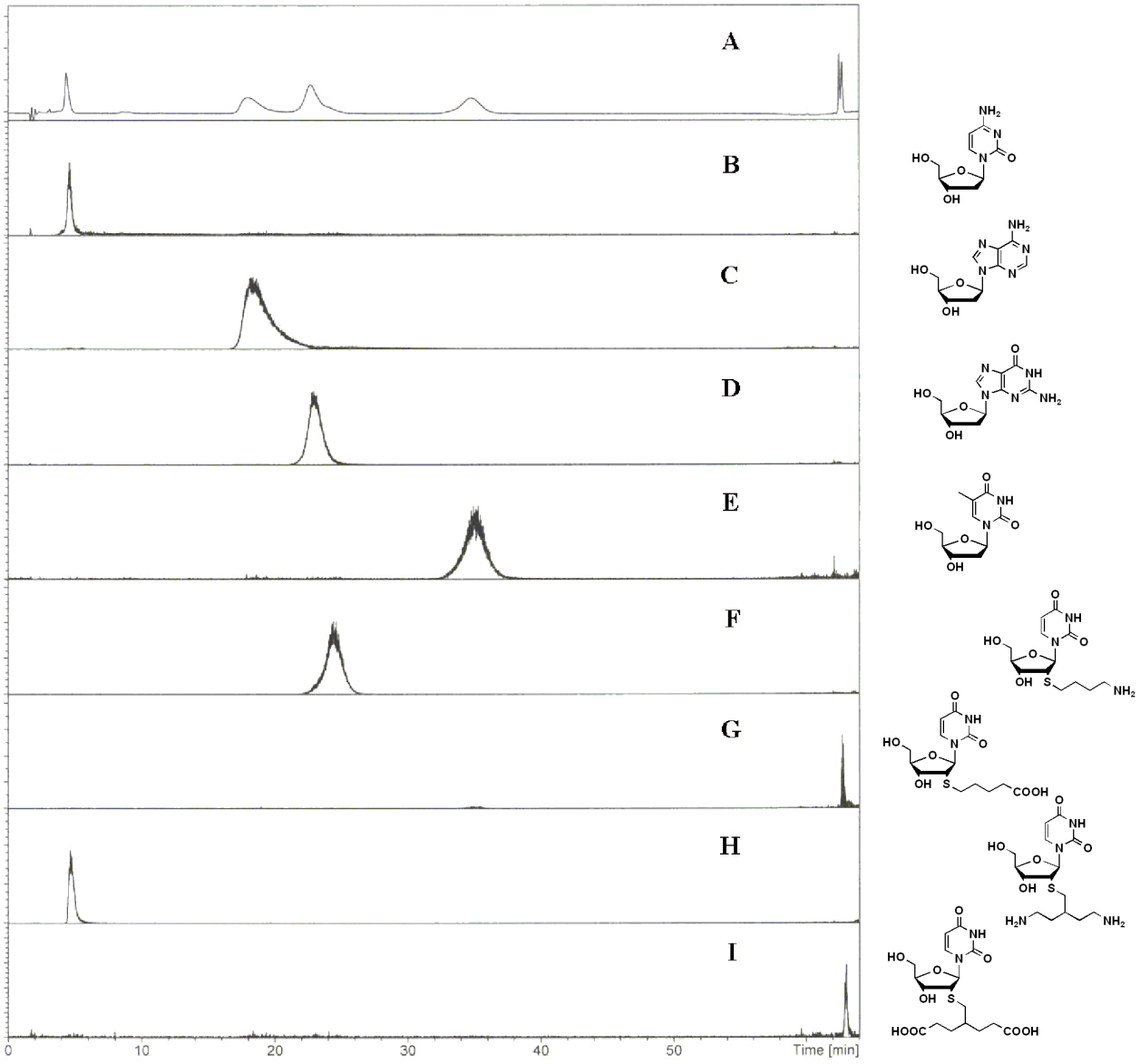
a Relative integration areas for **1**, **3b** – **7b** are obtained from UV chromatogram on C18 column, Relative integration areas for **1** (data in parenthesis), **2**, **8b** and **9b** are obtained from UV chromatogram on C18 column.

b The relative integrated areas refer to the ratio of the integrated areas of individual UV peaks to the integrated area of deoxycytidine (dC) in the same UV chromatogram.

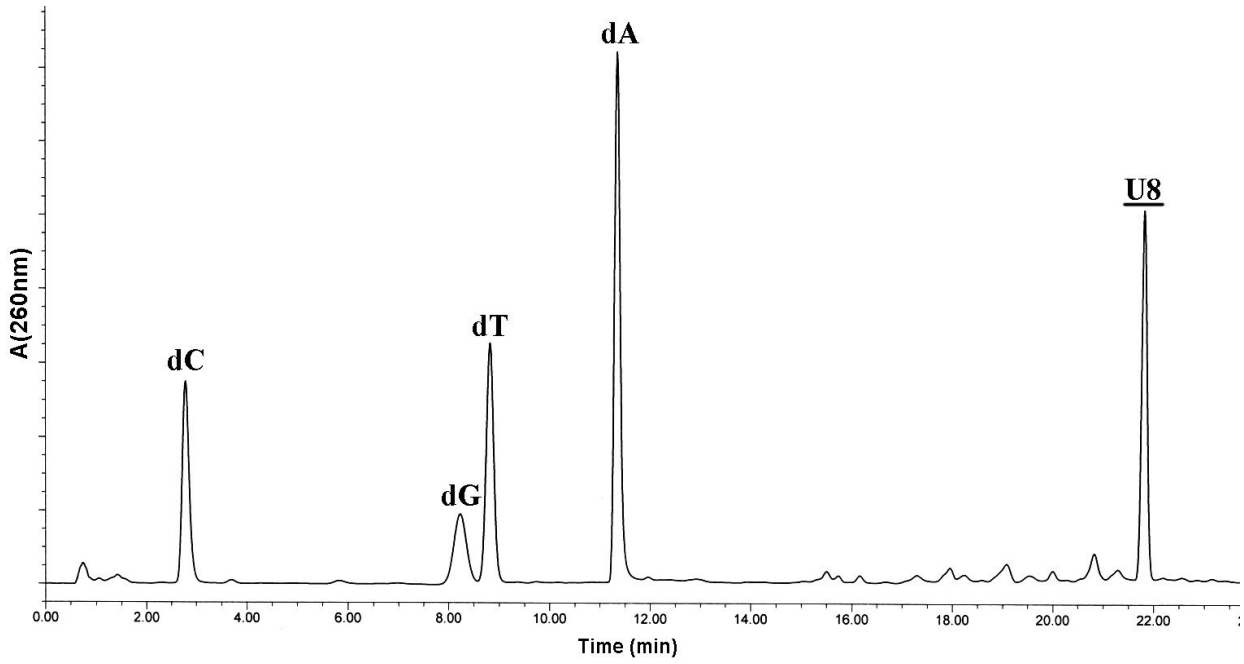
c Measured at pH 4.0, 25 °C, 254nm.

d The released dC, dA and dG residues showed similar ratios in UV chromatograms of **1**, **3b** – **8b**. However, the relative ratios of deoxythymidine (dC of each chromatogram as standard) from **1** to **8b** were: 6.67 (**1**) : 5.32 (**3b**) : 4.50 (**4b**) : 4.22 (**5b**) : 3.69 (**6b**) : 2.99 (**7b**) : 2.36 (**8b**). Taking into account the same number of dT residues in sequences **4b** and **5b**, the decrease of dT ratios was consistent with the number of dT residues in each strand. In contrast, the relative ratios of uridine oligomers were: 1.36 (**U2**) : 1.68 (**U3a**) : 1.78 (**U3b**) : 2.23 (**U4**) : 2.47 (**U5**) : 2.56 (**U6**) : 2.83 (**U8**). Again, except for **U3a** and **U3b** with same number of uridines, there was a

clear trend that longer oligomers have a higher ratio. Comparing UV chromatogram of digests of nylon nucleic acid **9b** and its control **2**, the relative ratio of dT was 2.35(**9b**) : 8.20(**2**) close to 3 : 11 real ratio. Finally, **8b** and **9b** had similar ratios of dC, dA and dT, but dG was in 3.83(**8b**) : 1.24(**9b**), which was compares favorably with the actual 3 : 1 dG ratio.



S6. LCMS analysis of the uncoupled precursor strand **6a** complete nuclease digestion products using Zorbax C18 column (2.1 mm×150 mm, 5 μm, Agilent Technologies). (A) UV chromatogram, (B) extracted ion chromatography with target mass 112.4 (deoxycytosine), (C) extracted ion chromatography with target mass 156.1 (deoxyguanine), (D) extracted ion chromatography with target mass 152.1 (deoxyadenine), (E) extracted ion chromatography with target mass 127.5 (deoxythymine), (F) extracted ion chromatography with target mass 332.1 (**Un** nucleoside), (G) extracted ion chromatography with target mass 375.2 (**Uc** nucleoside), (H) extracted ion chromatography with target mass 383.3 (**Unn** nucleoside) and (I) extracted ion chromatography with target mass 433.1 (**Ucc** nucleoside).



S7. UV chromatogram of HPLC separation of nylon nucleic acid **9b** complete nuclease digestion products using Zorbax C8 column (4.6mm×50mm, 5 μm, Agilent Technologies).

References

1. L. Zhu, P. S. Lukeman, J. W. Canary and N. C. Seeman, *J. Am. Chem. Soc*., 2003, **125**, 10178-10179.
2. C. Chou, Limbach,P.A., *Current Protocols in Nucleic Acid Chemistry, 10.1.1-10.1.25*, 2000.
3. J. Mergny, Lacroix,L., *Oligonucleotides*, 2003, **13**, 515-537.