Synthesis and Stability of Oligodeoxynucleotides Containing C8-Labeled 2-Deoxyadenosine: Novel Redox Nucleobase Probes for DNA Mediated Charge-Transfer Studies

Mark T. Tierney and Mark W. Grinstaff*

Department of Chemistry
Paul M. Gross Chemical Laboratory
Duke University, Durham, NC 27708
http://www.chem.duke.edu/~mwg/

Supporting Information

All solvents were dried and freshly distilled prior to use. Absorption spectra were measured on a Hewlett-Packard 8452 diode array spectrometer, and melting curves measured on an AVIV UV-Vis spectrometer with a thermoelectrically controlled cell holder. CD spectra were recorded on a JASCO J-710 Spectropolarimeter. RP HPLC was performed on a Rainin HPLC with a C18 column monitoring at 254 nm. NMR Spectra were recorded on a Varian INOVA spectrometer operating at 400 MHz or a GE QE-300 spectrometer operating at 300 MHz. Chemical ionization mass spectra were obtained on a Hewlett Packard HP 5988A spectrometer using NH_3. Fast atom bombardment mass spectra (FABMS) were obtained on a JOEL JMS-SX102A spectrometer using a 3-nitrobenzyl alcohol matrix. MALDI-TOF mass spectra of oligodeoxynucleotides were obtained using a PerSeptive Biosystems Voyager-DE Biospectrometry Workstation using a hydroxypicolinic acid matrix. DIEA = Diisopropylethylamine, DCC = Dicyclohexylcarbodiimide, HOBt = Hydroxybenzotriazole.

β-(10-phenthiazinyl)propionitrile 2. In a modification of a previous report (Godefroi, E. F.; Wittle, E. L. *J. Org. Chem.* **1956**, *21*, 1163-1168), phenothiazine (2.0 g; 1.0 mmol) was suspended in acrylonitrile (15 mL) and cooled to 0 ^{o}C. Tetrabutylammonium hydroxide (50% in water; 0.1 mL) was added and the mixture allowed to slowly warm to room temperature. A vigorously exothermic reaction occurred. Once the reaction subsided, dioxane, (25 mL) was added and the mixture was heated to reflux for one hour. The mixture was poured into water with vigorous stirring and the resulting tan solid filtered. Recrystallization from acetone gave **2** as a white solid (87%). This material was used directly in the next step.

β-(10-phenothizinyl)-propionic acid 3. This compound was prepared from **2** by the hydrolysis method described in Godefroi, E. F.; Wittle, E. L. *J. Org. Chem.* **1956**, *21*, 1163-1168. (56%).

N'-(2-propynyl)-β-(10-phenthiazinyl)propionamide 4. To a suspension of β-(10-phenthiazinyl)propionic acid (1.024 g; 3.77 mmol) in CH_2Cl_2 (50 mL) was added propargyl amine hydrochloride (0.380 g; 4.15 mmol), HOBt (0.510 g; 3.77 mmol), and DIEA (0.81 mL; 4.53 mmol). DCC (0.934 g; 4.53 mmol) was then added, and the mixture was stirred overnight. The reaction was quenched with 10 mL of 5% aqueous HOAc and stirred vigorously. The mixture was filtered and extracted with several equal portions of 5% aqueous citric acid, water, satd. $NaHCO_3$, water, and brine. Column chromatography (silica gel, $CHCl_3$) gave product as a white solid (75%). ^{1}H NMR (CD_2Cl_2) 7.2 (m, 4H), 6.9 (m, 4H), 6.1 (br, 1H), 4.2 (t, 2H), 3.9 (dd, 2H), 2.6 (t, 2H), 2.2 (t, 1H). FAB-HRMS calc'd for $C_{18}H_{16}N_2OS$ $(M)^+$ 308.0983; found 308.0984.

N-(2-propynyl)-anthraquinone carboxamide 6. To a suspension of anthraquinone 2-carboxylic acid, **5**, (0.846 g; 3.35 mmol) in CH_2Cl_2 (50 mL) was added $(COCl)_2$ (0.12 mL; 13 mmol). Once the reaction became homogeneous, the solvent was removed and the resulting yellow solid was dissolved in CH_2Cl_2 (50 mL). Propargyl amine hydrochloride (0.368 g; 4.02 mmol) was added, and the mixture was cooled to –5 ^{o}C. DIEA (3.04 mL; 16.8 mmol) was then added dropwise. The mixture was allowed to warm to ambient temperature. The yellow precipitate that formed was filtered and dried to give **6** (92%). The solid was recrystallized from CH_3CN/C_6H_6 as pale yellow needles. ^{1}H NMR (DMSO-d_6:$CDCl_3$ 1:1) 9.4 (t, 1H), 8.6 (s, 1H), 8.2 (m, 4H), 7.9 (m, 2H), 4.0 (dd, 2H), 3.2 (t, 1H). FAB-HRMS Calc'd for $C_{18}H_{11}O_3N$ $(M)^+$ 289.0739; found 289.0740.

5'-O-(4,4'-dimethoxytrityl)-8-bromo-2'-deoxyadenosine, 8. 8-Bromo-2'-deoxyadenosine (see Ikehara, M.; Uesugi, S.; Kaneno, M. In *Nucleic Acid Chemistry*; Townsend, L. B., Tipson, R. S., Eds.; John Wiley and Sons: New York, 1978; Vol. II, p 837-841.), **7**, (4.37 g; 13.2 mmol) was dried by the addition of pyridine and then evaporated under high vacuum (10 mtorr) at elevated temperature (ca. 55 ^{o}C). This pre-drying procedure was repeated twice. Pyridine (25 mL) and DMAP (0.20 g) were then added to the solid. 4,4'-Dimethoxytrityl chloride (4.92 g; 14.5 mmol) was dissolved in pyridine (25 mL) and added dropwise over 3 h. to the gently stirring suspension of nucleoside during which time the reaction became homogeneous. Methanol (20 mL) was added and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with equal portions of water and brine. The solution was dried, and the solvent was removed. Column chromatography (silica gel pretreated with 1 % TEA in CH_2Cl_2; 0-5 % MeOH in CH_2Cl_2) followed by evaporation of the solvent gave **8** as a pale yellow foam (81%). ^{1}H NMR (CD_2Cl_2) 8.03 (s, 1H), 7.35 (d, 2H), 7.25-7.14 (m, 6H), 6.73 (m, 4H), 6.36 (t, 1H), 5.64 (s, 2H), 4.92 (m, 1H), 4.07 (q, 1H), 3.74 (d, 6H), 3.52 (m, 1H), 3.37 (m, 2H), 2.50 (br, 1H), 2.32 (m, 1H). FABMS m/z calcd for $C_{31}H_{31}{}^{79}BrN_5O_5$ $(M+H)^+$ 632.1; found 632.1.

N-benzoyl-5'-O-(4,4'-dimethoxytrityl)-8-bromo-2'-deoxyadenosine, 9. 5'-O-(4,4'-dimethoxytrityl)-8-bromo-2'-deoxyadenosine, **8** (1.86 g; 3.49 mmol) was dried by the addition of pyridine and was evaporated under high vacuum (10 mtorr) at elevated temperature (ca. 55 ^{o}C). This pre-drying procedure was repeated twice. Pyridine (50 mL) was added to the solid, and the resulting solution was cooled to –5 ^{o}C. TMS-Cl (2.21 mL; 17.45 mmol) was added slowly, and the mixture was stirred for an additional 1 h during which time the reaction was allowed to warm to room temperature. Next, benzoyl chloride (2.03 mL; 17.5 mmol) was added, and the mixture was stirred overnight. The reaction was quenched with MeOH (20 mL) and the solvent was removed. Methanolic ammonia (200 mL of 2 M soln.) was added to the yellow residue, and the mixture was stirred for 1 h. The solvent was removed under reduced pressure, and the resulting solid dissolved in a mixture of CH_2Cl_2 (200 mL) and water (20 mL). The layers were separated, and the organic layer isolated, washed with equal portions of water and brine, and subsequently dried (Na_2SO_4). Removal of the solvent followed by column chromatography (silica gel pretreated with 1% TEA in CH_2Cl_2; 0-3% MeOH in CH_2Cl_2) and evaporation of the solvent gave **8** as a pale yellow foam (81%). ^{1}H NMR (CD_2Cl_2) 8.45 (s,1H), 8.13 (m, 1H), 7.65-7.35 (m, 6H), 7.30-7.14 (m, 7H), 6.75 (m, 4H), 6.55 (t, 1H), 4.92 (m, 1H), 4.20 (q, 1H), 3.76 (s, 3H), 3.71 (s, 3H), 3.67 (m, 1H), 3.32 (m, 2H), 2.88 (br, 1H), 2.48 (m, 1H), 2.04 (m, 1H). FAB-HRMS calc'd *m/z* for $C_{48}H_{35}O_6N_5{}^{79}Br$ $(M+H)^+$ 736.1774; found 736.1772.

Compound 10. TEA (0.50 mL) was added to a solution of **9** (0.050 g; 0.07 mmol) and **4** (0.042; 0.14 mmol) in DMF (3 mL). Next $Pd(P(C_6H_5)_3)_4$ (0.008 g; 0.007 mmol) and CuI (0.003 g; 0.014 mmol) were added and the flask was immediately immersed in an oil bath (45-50 ^{o}C). The pale yellow solution was stirred for 2.5 h. until TLC showed complete consumption of the nucleoside. The reaction was allowed to cool, and the volatiles were removed under vacuum and the resulting residue dissolved in CH_2Cl_2. Flash chromatography (silica gel pretreated with 1% TEA in CH_2Cl_2; eluent 0-2% MeOH in CH_2Cl_2) followed by precipitation in pentane afforded **10** as a pale yellow solid (0.055 g; 85%). ^{1}H NMR (CD_2Cl_2) (9.13 br, 1H), 8.50 (s, 1H), 7.98 (d, 2H), 7.69-7.20 (m, 15H), 6.85 (m, 4H), 6.74 (dd, 5 H), 6.53 (t, 1H), 4.81 (br, 1H), 4.27-4.12 (m, 5H), 3.74 (s, 3H), 3.73 (s, 3H) 3.40-3.28 (m, 3H), 2.95 (br, 1H), 2.68 (m, 2H), 2.28 (m, 1H). FAB-HRMS calcd *m/z* for $C_{56}H_{45}O_7N_7S$ $(M)^+$ 963.3414; found 963.3428.

Compound 14. DIEA (0.19 mL; 1.0 mmol) was added to a solution of **9** (0.158 g; 0.215 mmol) and **6** (0.120; 0.415 mmol) in DMF (20 mL). Next $Pd(P(C_6H_5)_3)_4$ (0.024 g; 0.021 mmol) and CuI (0.009 g; 0.0474 mmol) were added, and the flask was immediately immersed in an oil bath (45-50 ^{o}C). The pale

yellow solution was stirred for 2.5 h. until TLC showed complete consumption of the nucleoside. The reaction was allowed to cool and the volatiles were removed under vacuum. The resulting residue was dissolved in CH_2Cl_2. Flash chromatography (silica gel pretreated with 1% pyridine in CH_2Cl_2; eluent 0-2% MeOH in CH_2Cl_2) followed by precipitation in pentane afforded **14** as a yellow solid (85%). ^{1}H NMR (DMSO-d_6) 11.25 (s,1H), 9.68 (t, 1H), 8.68 (d, 1H), 8.53 (s, 1H), 8.36 (dd, 1H), 8.26 (d, 1H), 8.23 (m, 2H), 8.21 (m, 2H), 7.95 (m, 2H), 7.69-7.51 (m, 3H), 7.28 (m, 2H), 7.15 (m, 6H), 6.75 (dd, 4H), 6.75 (t, 1H), 5.40 (d, 1H), 4.68 (m, 1H), 4.50 (d, 2H), 4.02 (m, 1H), 3.69 (s, 3H), 3.68 (s, 3H), 3.32 (m, 1H), 3.18 (m, 2H), 2.33 (m, 1H). FAB-HRMS calcd *m/z* for $C_{56}H_{45}O_9N_6$ $(M+H)^+$ 945.3249; found 945.3237.

Compound 12. DIEA (0.032 mL; 0.18 mmol) was added to a solution of **8** (0.075 g; 0.119 mmol) and **4** (0.055 g; 0.178 mmol) in DMF (10 mL). Next $Pd(P(C_6H_5)_3)_4$ (0.014 g; 0.012 mmol) and CuI (0.005 g; 0.024 mmol) were added and the flask was immediately immersed in an oil bath (45-50 ^{o}C). The pale yellow solution was stirred for 2.5 h. until TLC showed complete consumption of the nucleoside. The reaction was allowed to cool and the volatiles were removed under vacuum. The resulting residue was dissolved in CH_2Cl_2. Flash chromatography (silica gel pretreated with 1% pyridine in CH_2Cl_2; eluent 0-5% MeOH in CH_2Cl_2) followed by precipitation in pentane afforded **12** as a white solid (75%). ^{1}H NMR (CD_2Cl_2) 8.07 (s, 1H), 7.37 (m, 2H), 8.62 (m, 4H), 7.19-7.09 (m, 6H), 6.88 (m, 4H), 6.74 (m, 4H), 6.66 (t, 1H), 6.47, (t, 1H), 6.34 (br, 2H), 4.80 (m, 1H), 4.23-4.11 (m, 5H), 3.73 (s, 3H), 3.72 (s, 3H), 3.36 (m, 4H), 2.66 (t, 2H), 2.25 (m, 1H). FAB-MS *m/z* calcd for $C_{49}H_{45}N_7O_6S$ $(M)^+$ 859.3; found 859.3.

Compound 13. To a solution of **12** (0.070 g; 0.083 mmol) in CH_2Cl_2 (2 mL) was added a 2% solution of Cl_3CCOOH in CH_2Cl_2 (10 mL). The mixture was stirred for 5 min and ethanol (2 ml) added. Na_2CO_3 (2 g) was added and the mixture stirred overnight. The mixture was filtered and the solvent removed to give a pale yellow oil. Column chromatography (silica gel, 0-10 % MeOH in CH_2Cl_2) gave **13** as a colorless solid (75%). ^{1}H NMR (DMSO-d_6) 8.16 (t, 1H), 8.10 (s, 1H), 7.11-7.0 (m, 4H), 6.92-6.81 (m, 4H), 6.73 (br, 1H), 6.45 (dd, 1H), 4.86 (m, 1H), 4.50 (m, 1H), 4.19 (d, 2H), 4.10 (t, 2H), 4.00 (d, 1H), 3.73 (dd, 1H), 3.60 (m, 1H), 2.84 (m, 1H), 2.63 (t, 2H), 2.14 (dd, 1H). FAB-HRMS calcd for $C_{28}H_{27}O_4N_7S$ $(M)^+$ 557.1845; found 557.1847.

Compound 16. DIEA (0.032 ml; 0.18 mmol) was added to a solution of **8** (0.075 g; 0.119 mmol) and **6** (0.052 g; 0.178 mmol) in DMF (10 mL). Next $Pd(P(C_6H_5)_3)_4$ (0.014 g; 0.012 mmol) and CuI (0.005 g; 0.024 mmol) were added and the flask was immediately immersed in an oil bath (45-50 ^{o}C). The pale

yellow solution was stirred for 2.5 h. until TLC showed complete consumption of the nucleoside. The reaction was allowed to cool and the volatiles were removed under vacuum. The resulting residue was dissolved in CH_2Cl_2. Flash chromatography (silica gel pretreated with 1% pyridine in CH_2Cl_2; eluent 0-5% MeOH in CH_2Cl_2) followed by precipitation in pentane afforded **16** as a yellow solid (75%). ^{1}H NMR (CD_2Cl_2) 8.52 (s, 1H), 8.21 (m, 2H), 8.06-7.94 (m, 4H), 8.36, (m, 2H), 7.34 (d, 2H), 7.23 (d, 4H), 7.20-7.05 (m, 4H), 6.70 (dd, 4H), 6.59 (t, 1H), 4.83 (br, 1H), 4.53 (m, 2H), 4.26 (m, 1H), 3.73 (d, 1H), 3.66 (s, 3H), 3.65 (s, 3H), 3.48 (m, 3H), 2.41 (br, 1H). FAB-MS *m/z* calcd for $C_{49}H_{41}N_6O_8$ $(M+H)^+$ 841.3; found 841.3.

Compound 17. A 2% solution of Cl_3CCO_2H in CH_2Cl_2 (2 mL) was added to a solution of **16** (0.070 g; 0.083 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 5 min and then methanol (3 drops) was added. The yellow precipitate that formed was collected, washed with CH_2Cl_2 and ether, and dried under vacuum (65%). NMR analysis in $CDCl_3$ or d_6-DMSO was hampered by the low solubility of **17**. FAB-HRMS calc'd for $C_{28}H_{22}O_6N_6$ $(M+H)^+$ 539.1680; found 539.1675.

General Method for phosphoramidite synthesis (11 and 15). DIEA (1.5 eq.) followed by 2-cyanoethylchloro-N,N'-diisopropylphosphoramidite (1.1 eq) was added to a cooled CH_2Cl_2 solution (-5 ^oC) of either **10** or **14** (1 eq.) and protected from light. The mixture was allowed to slowly warm to room temperature over 3-4 hours until TLC showed complete consumption of the starting material. Most of the solvent was removed and the addition of 10% Et_2O/pentane caused the separation of the product as an oil. This material was extensively dried under high vacuum, checked by ^{31}P NMR for P(III) signals at ca. 148.5 ppm, and dissolved to an approximate concentration of 0.1 M with CH_3CN. The resulting solutions were transferred to a reagent bottle under inert atmosphere and loaded on an automated DNA synthesizer.

Oligodeoxynucleotide Syntheses. Oligodeoxynucleotide syntheses were performed on a commercial ABI 395 DNA synthesizer from the 3' to 5' end using standard automated DNA synthesis protocols at the 1.0 μmol scale. A 0.1 M solution of **11**, or **15** in dry acetonitrile was prepared and installed on the DNA synthesizer in a standard reagent bottle. Normal solid-phase oligodeoxynucleotide synthesis was performed. During coupling of the modified dA phosphoramidites, an extended reaction time (5 min.) was employed to ensure reaction completion. Redox labeled oligodeoxynucleotide were deprotected in 30% ammonium hydroxide at 55 ^oC for 16 hours and purified by HPLC. The AQ and PTZ labeled oligodeoxynucleotides exhibited one peak in an HPLC trace, with retention times greater than the

corresponding unlabeled oligodeoxynucleotide. MALDI-TOF or ESI mass spectra confirmed formation of the labeled oligodeoxynucleotide.

18 Calc'd 5187, found 5187.

19 Calc'd 5187, found 5187.

20 Calc'd 5203, found 5203.

21 Calc'd 5167, found 5167.

22 Calc'd 5167, found 5167.

23 Calc'd 5184, found 5184.

HPLC Purification of Oligodeoxynucleotides. HPLC purification of the labeled oligodeoxynucleotides was accomplished on a Rainin HPLC instrument. Reverse phase chromatography was performed on a C18 column (25 cm x 4.6 mm) with acetonitrile (ACN) and 0.1 M triethylamine acetate (TEAA) as eluting solvents. A flow rate of 5 mL/min was used and the concentration of ACN was increased from 5% to 30% over 35 minutes. The retention times of the labeled oligodeoxynucleotides were well separated from the unlabeled oligodeoxynucleotide products (> 2 minutes).

Thermal Denaturation Curves. The concentrations of stock solutions of oligodeoxynucleotide single strands were determined from the UV-vis absorbance. Enzyme digestion with Nuclease G_1 *penicillium citrinum* to give the individual nucleotides was performed by the addition of 1 μL of a 1 mg/mL solution of enzyme to 5.0 μL of oligodeoxynucleotide single strand solution and 44.0 μL of 20 mM sodium acetate buffer at pH 5.5. The solutions were incubated at 55 ^{o}C for 30 min. A sample of 20.0 μL of digest solution was diluted to 2.000 mL with water, and the absorption spectrum measured. The total molar absorptivity was determined from the sum of all the individual contributions of each nucleoside. Back calculation gave the concentrations of the stock solutions. Equimolar amounts of each single strand were measured out of the stock solutions, combined, and diluted with phosphate buffer (5 mM NaH_2PO_4, 50 mM NaCl, pH = 7) to the appropriate concentration for UV-vis measurements. The solution was heated to 90 ^{o}C for 3 min and allowed to slowly cool to room temperature.

After cooling, a thermal denaturation experiment was performed using the following parameters on a AVIV UV-Vis spectrometer: a) monitoring wavelength, 260 nm, b) temperature range, 20 – 90 ^{o}C, c) temperature step, 0.5 ^{o}C, d) overshoot temperature, 0.2 ^{o}C, e) overshoot time, 1 s, f) equilibration time, 30 s, g) averaging time, 45 s. Cooling traces were obtained with similar parameters except for the

temperature range, 90-20 ^{o}C, and the temperature step, – 0.5 ^{o}C. The T_m value was determined from the first derivative of the absorbance as a function of the temperature.

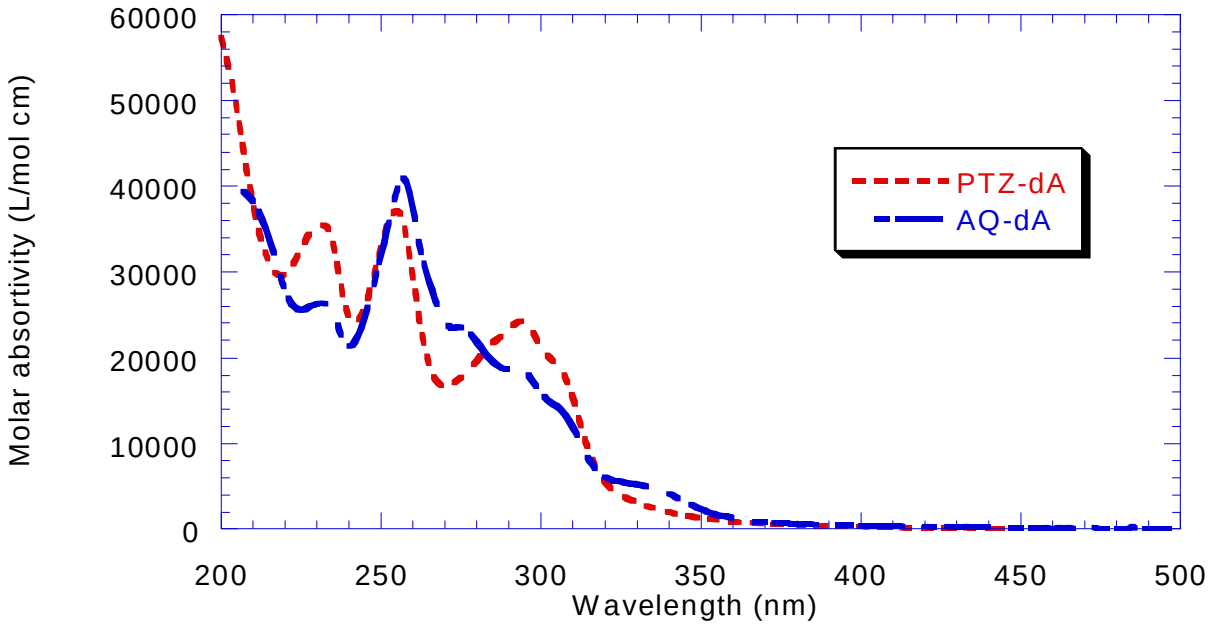

Figure S1. UV-Vis absorbtion spectra for **13 (PTZ-dA)** and **17 (AQ-dA)**.

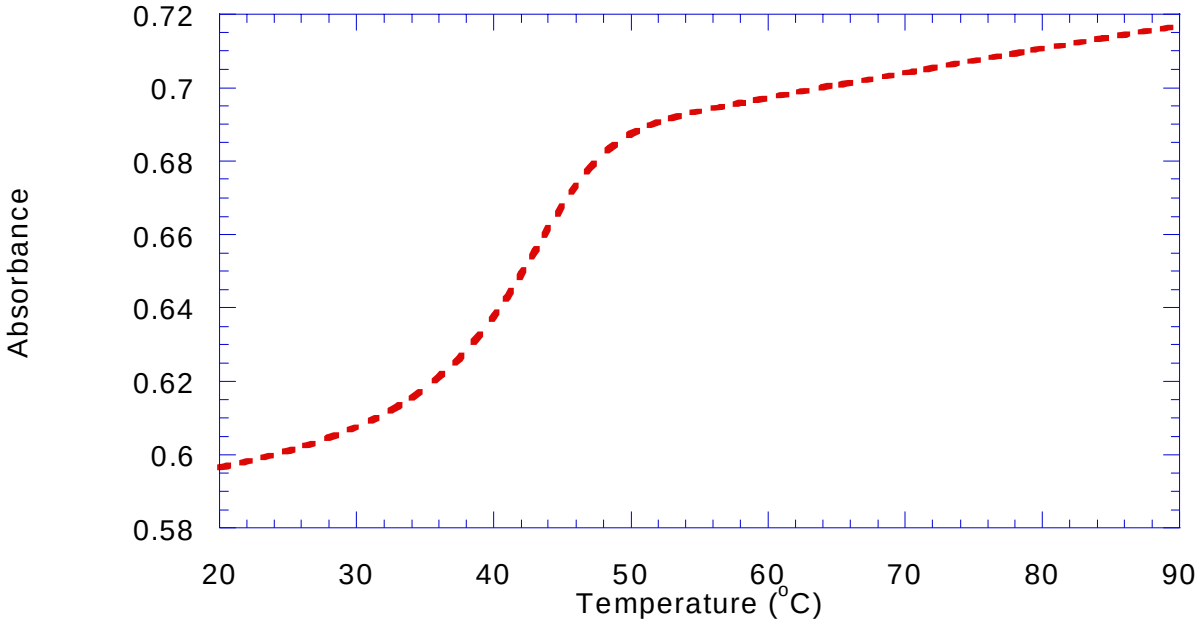

Figure S2. Heating profile for duplex **18-23**.

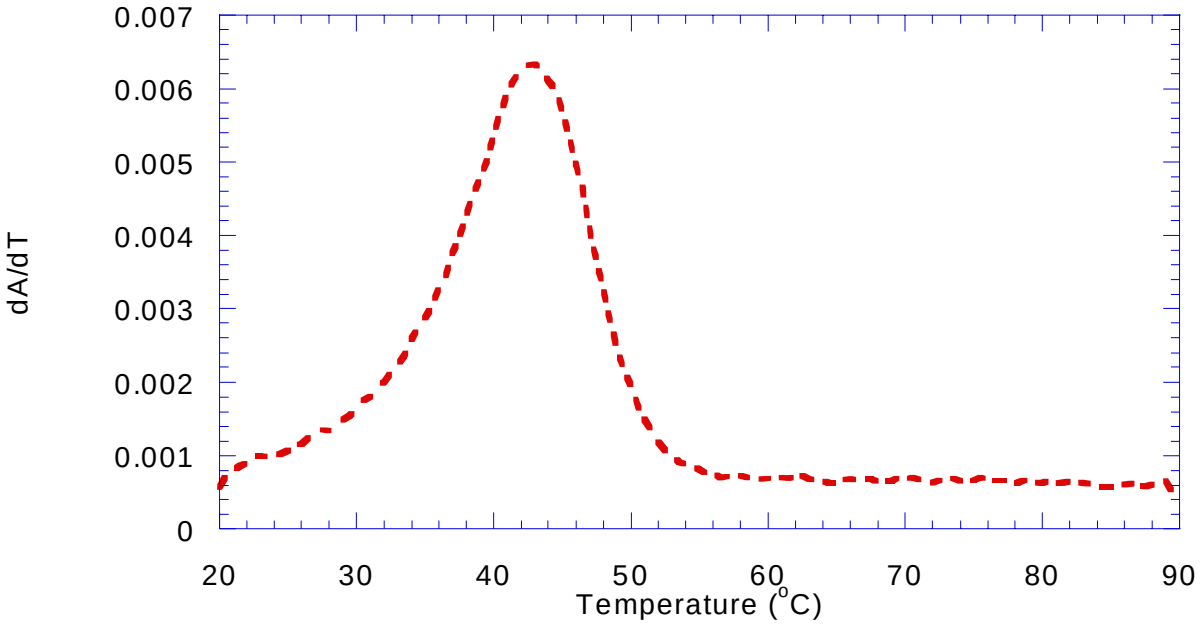

Figure S3. First derivative of heating profile for duplex **18-23**.

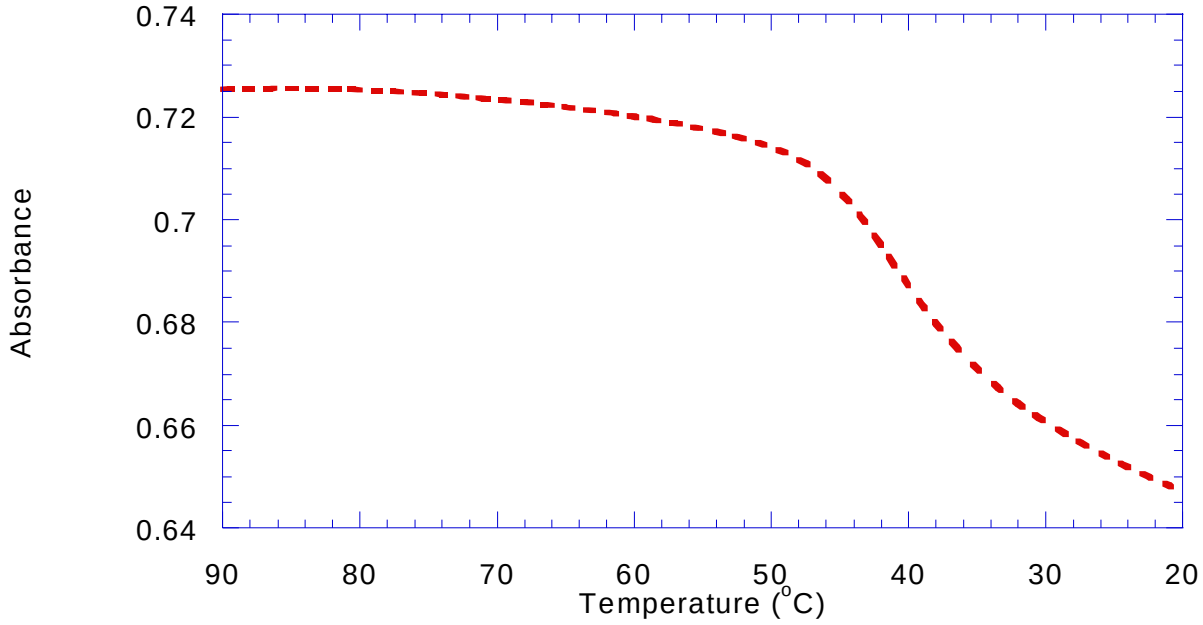

Figure S4. Cooling profile for duplex **18-23**.

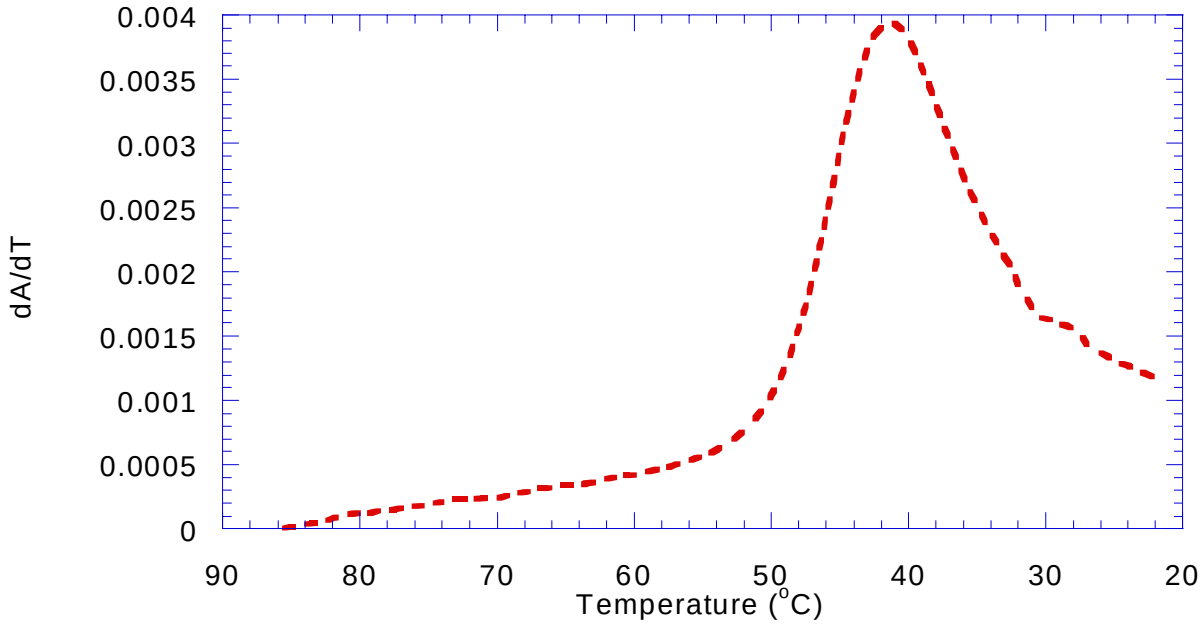

Figure S5. First derivative of cooling profile for duplex **18-23.**

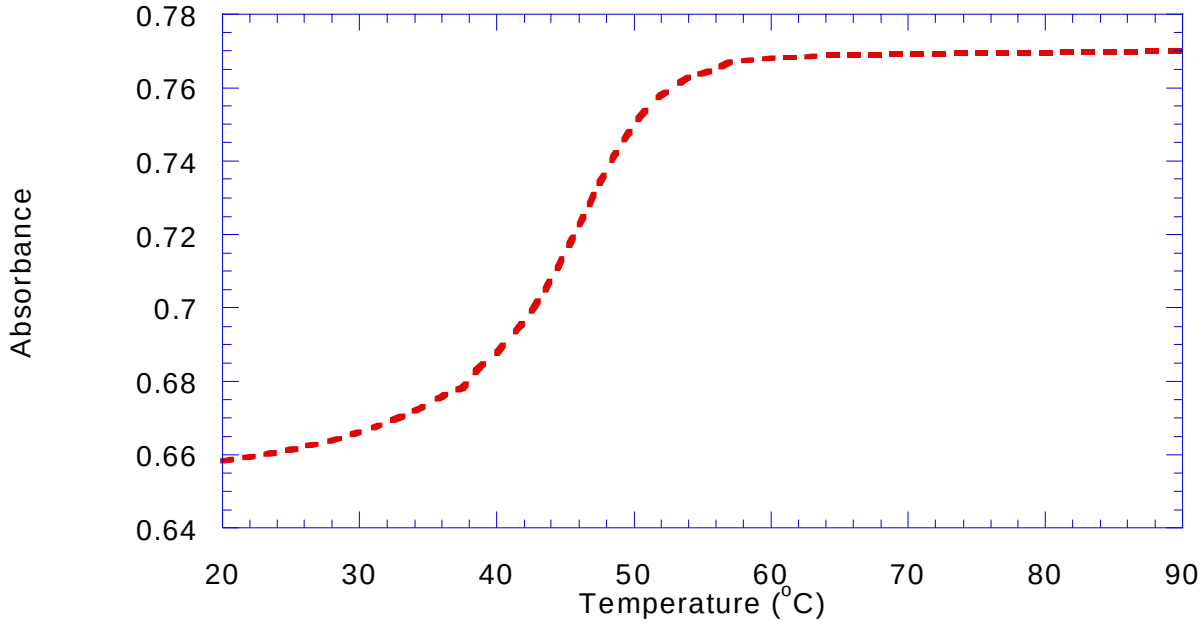

Figure S6. Heating profile for duplex **19-21.**

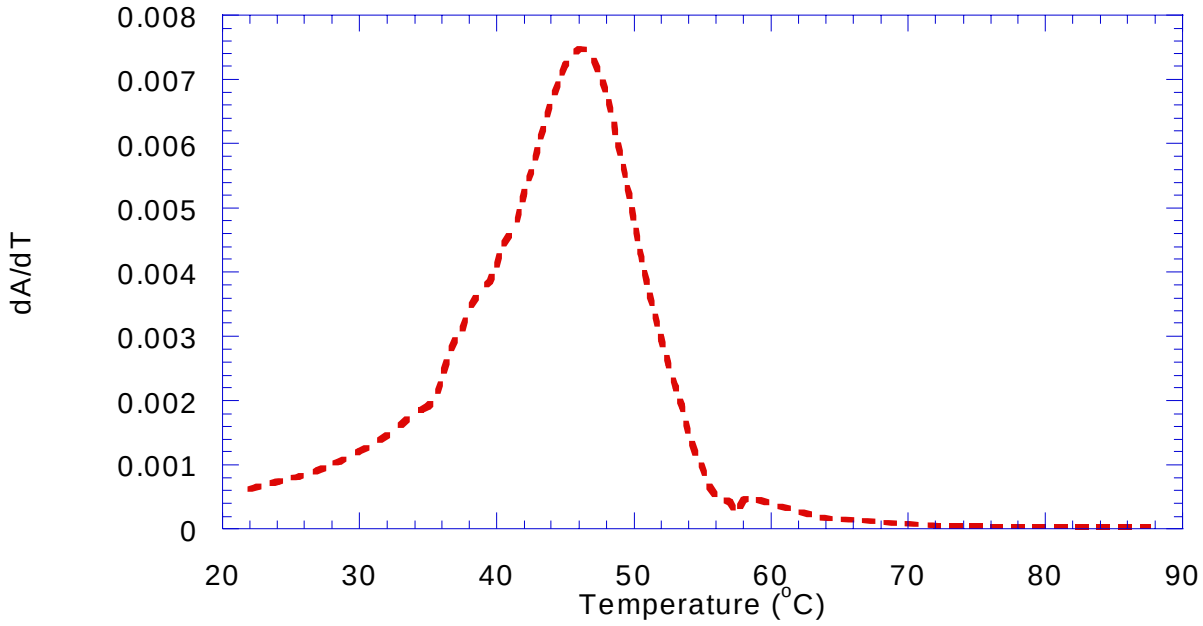

Figure S7. First derivative of heating profile for duplex **19-21.**

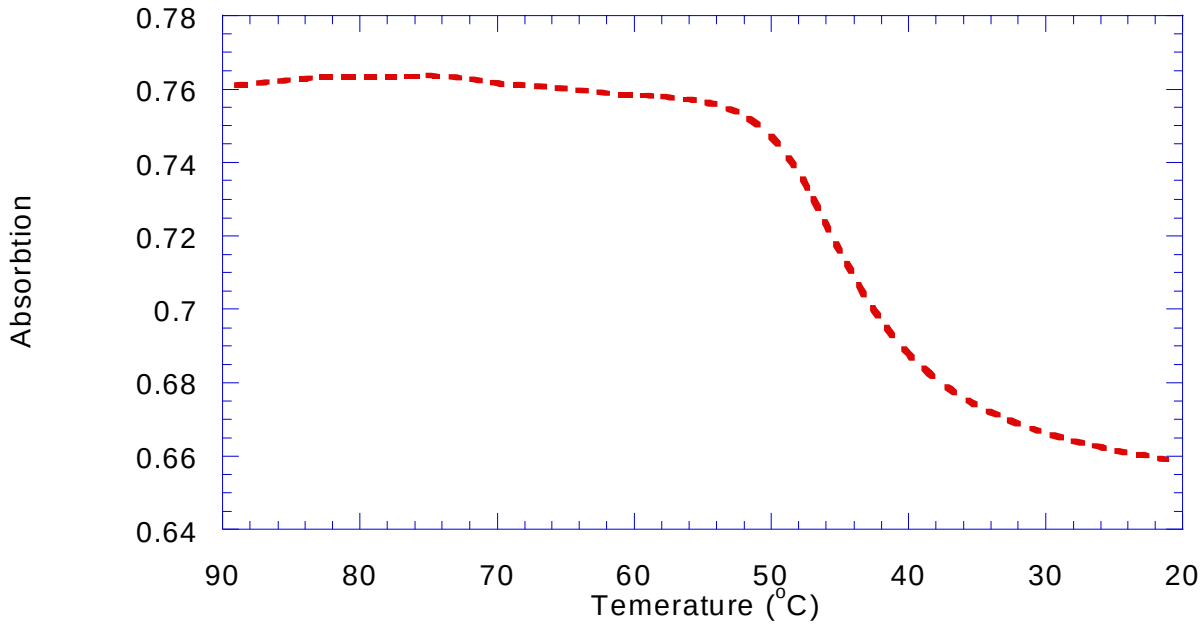

Figure S8. Cooling profile for duplex **19-23.**

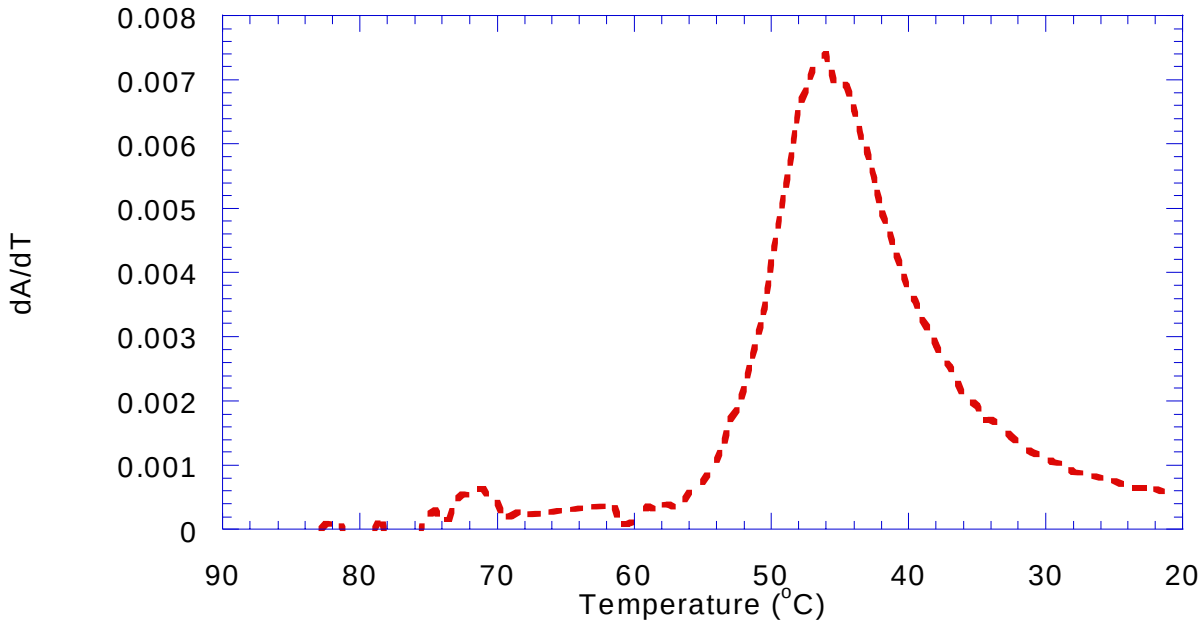

Figure S9. First derivative of cooling profile for duplex **19-23.**

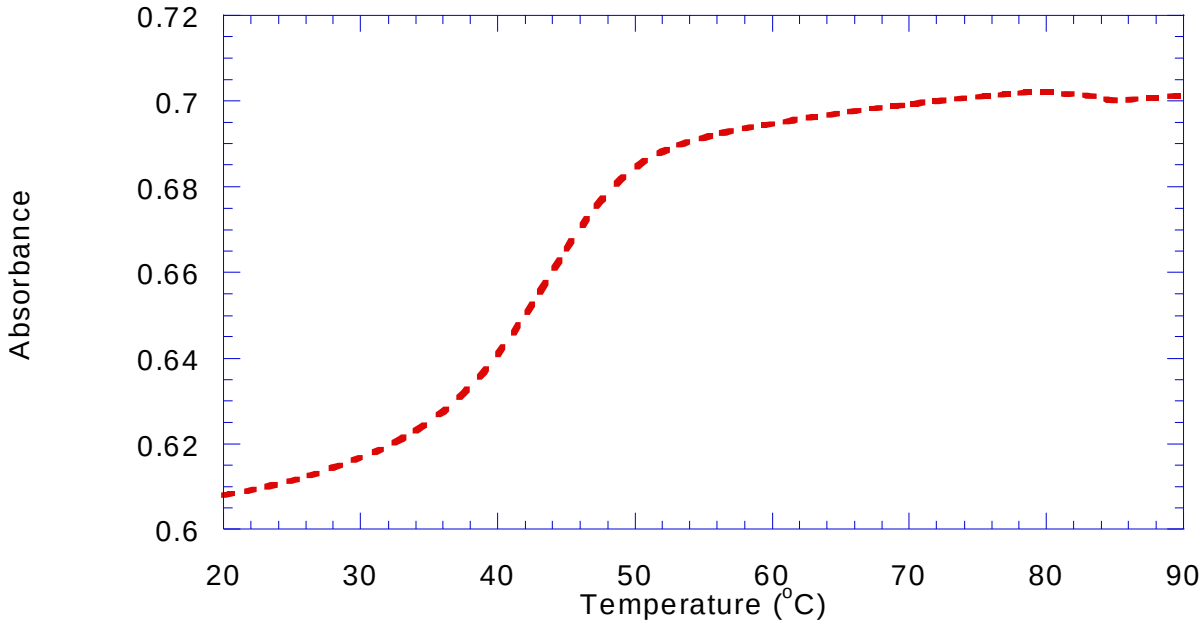

Figure S10. Heating profile for duplex **20-23.**

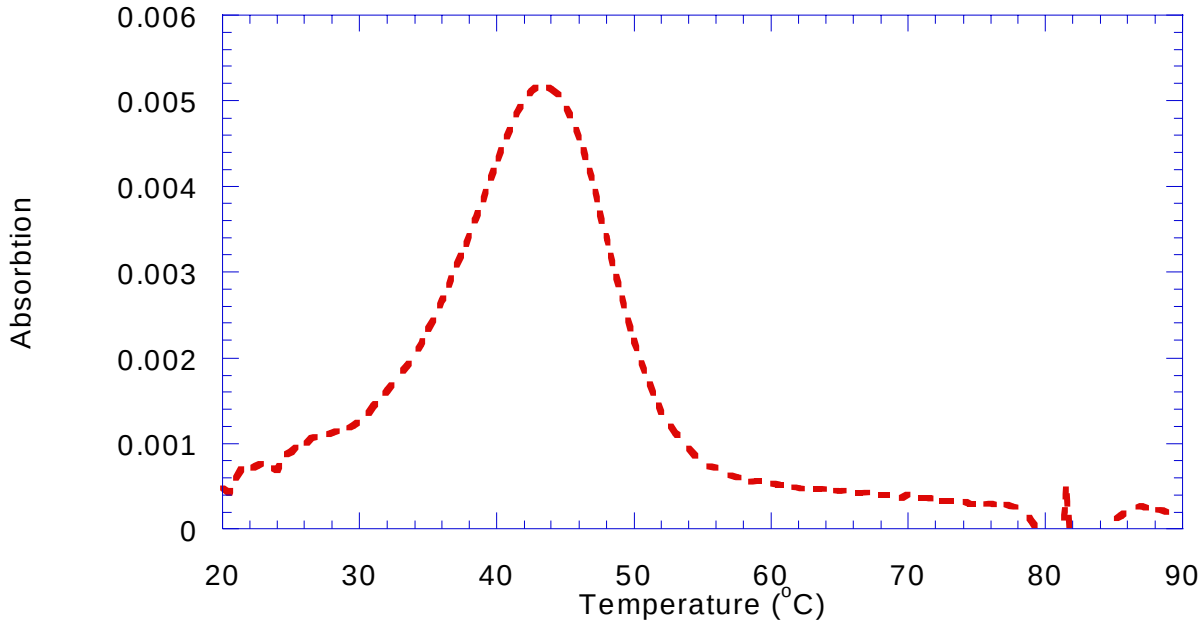

Figure S11. First derivative of heating profile for duplex **20-23.**

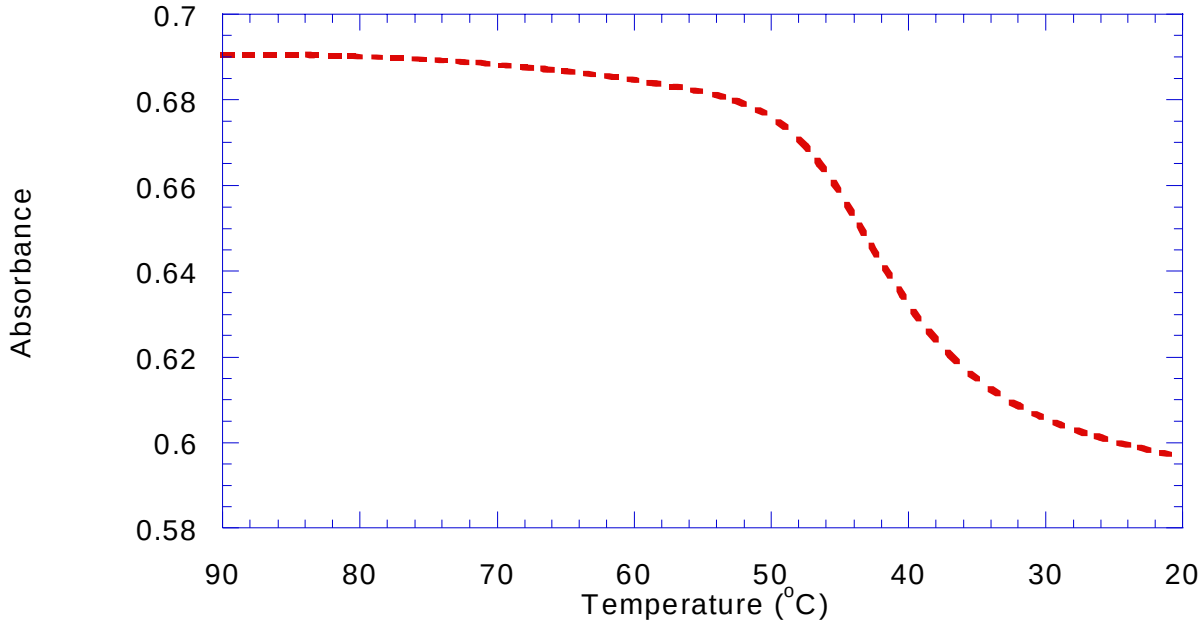

Figure S12. Cooling profile for duplex **20-23.**

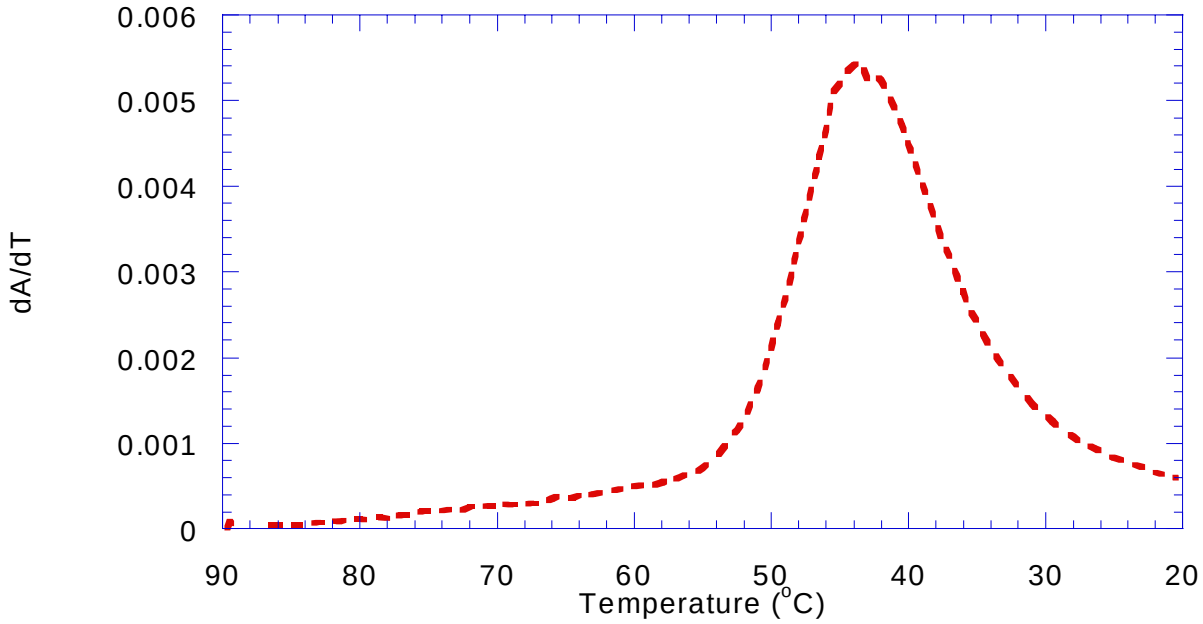

Figure S13. First derivative of cooling profile for duplex **20-23.**

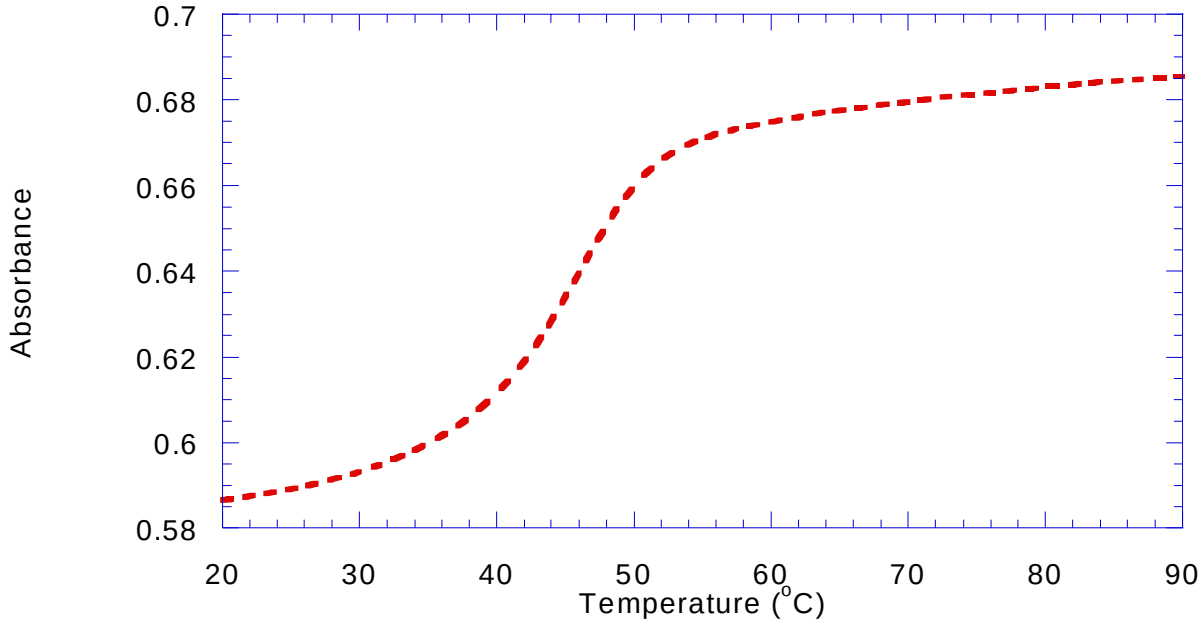

Figure S14. Heating profile for duplex **21-23.**

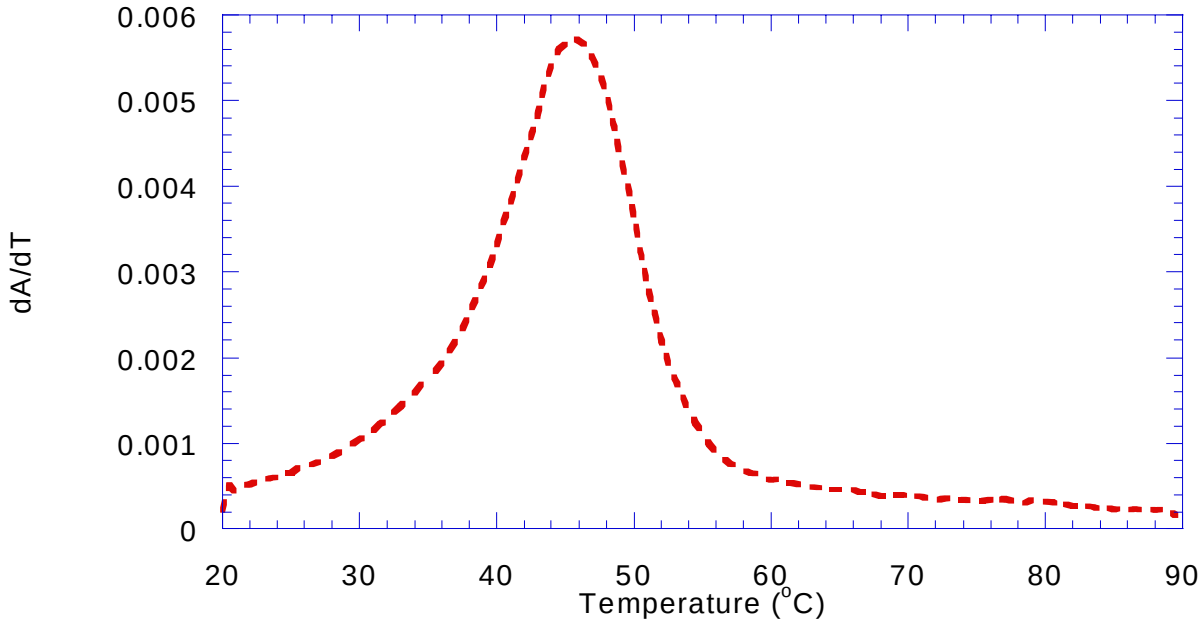

Figure S15. First derivative of heating profile for duplex **21-23.**

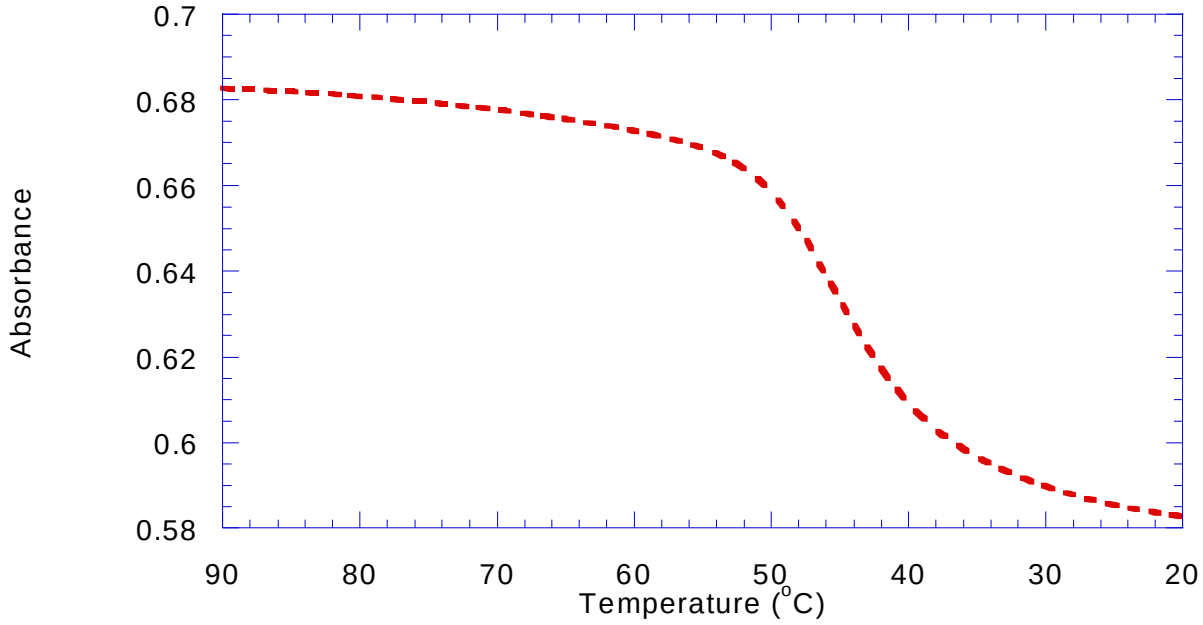

Figure S16. Cooling profile for duplex **21-23.**

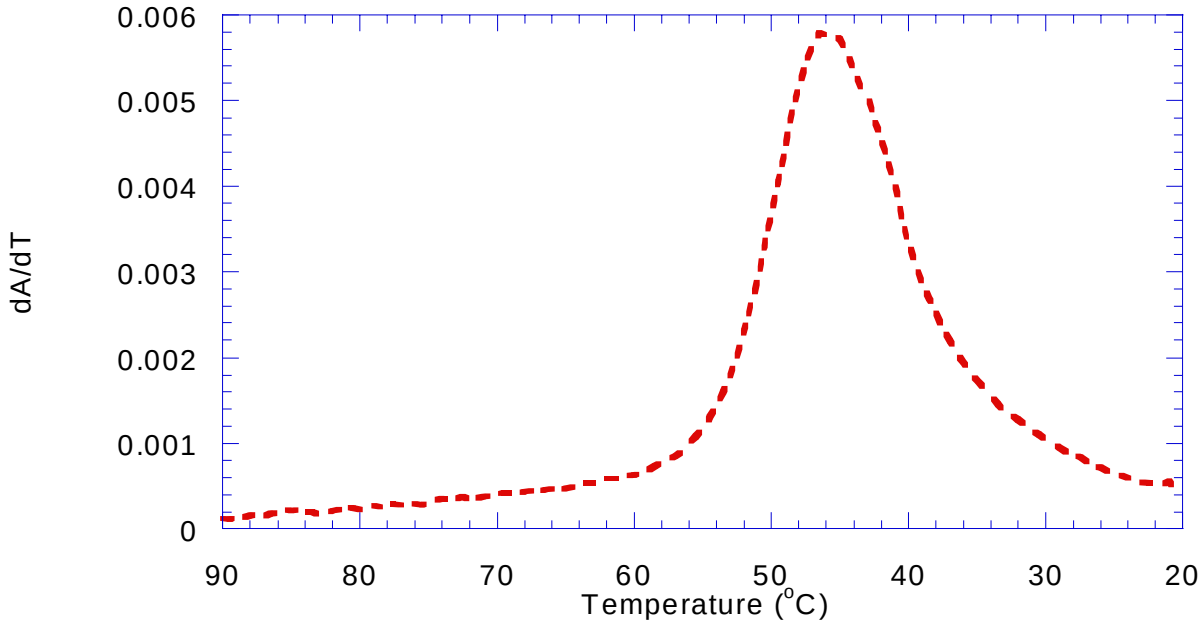

Figure S17. First derivative of cooling profile for duplex **21-23.**

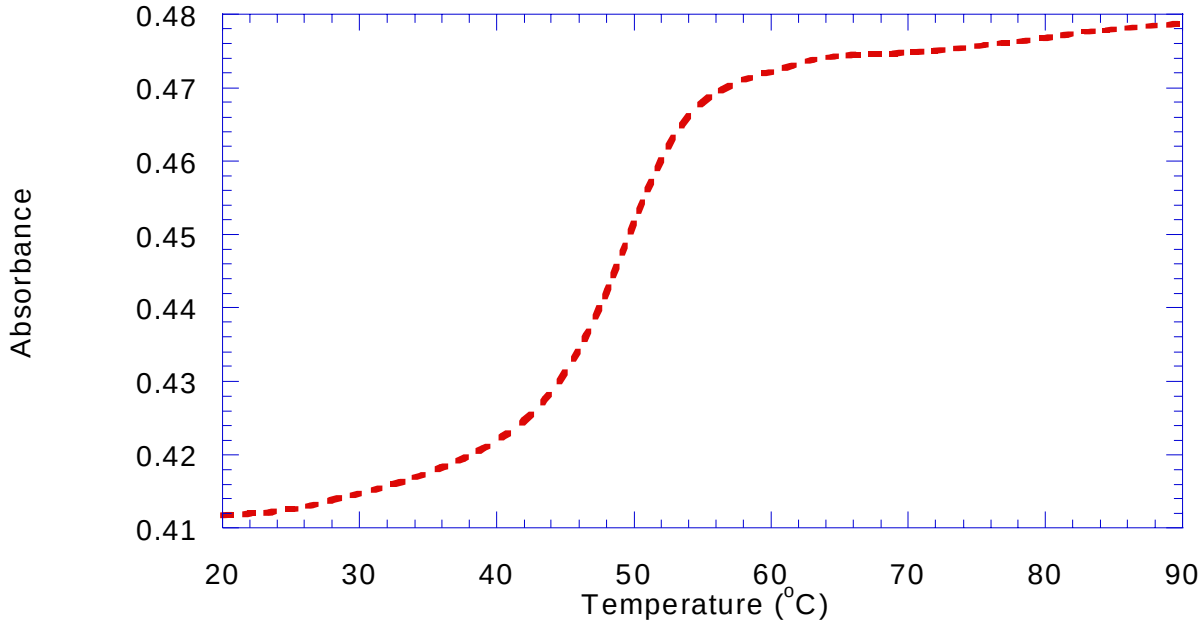

Figure S18. Heating profile for duplex **22-23.**

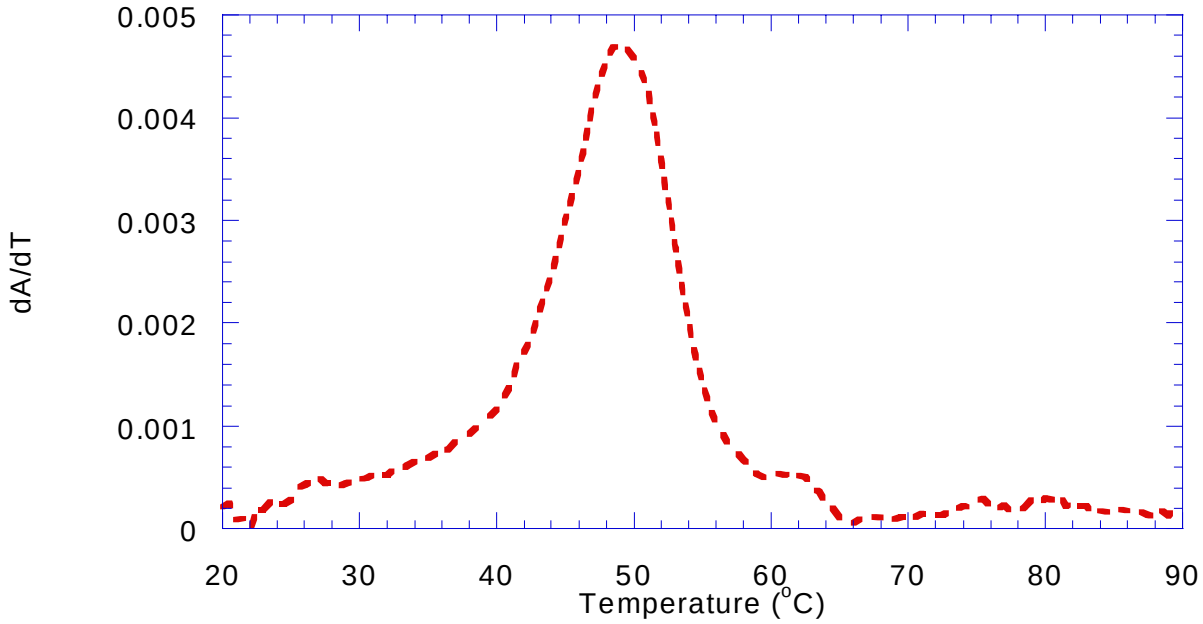

Figure S19. First derivative of heating profile for duplex **22-23.**

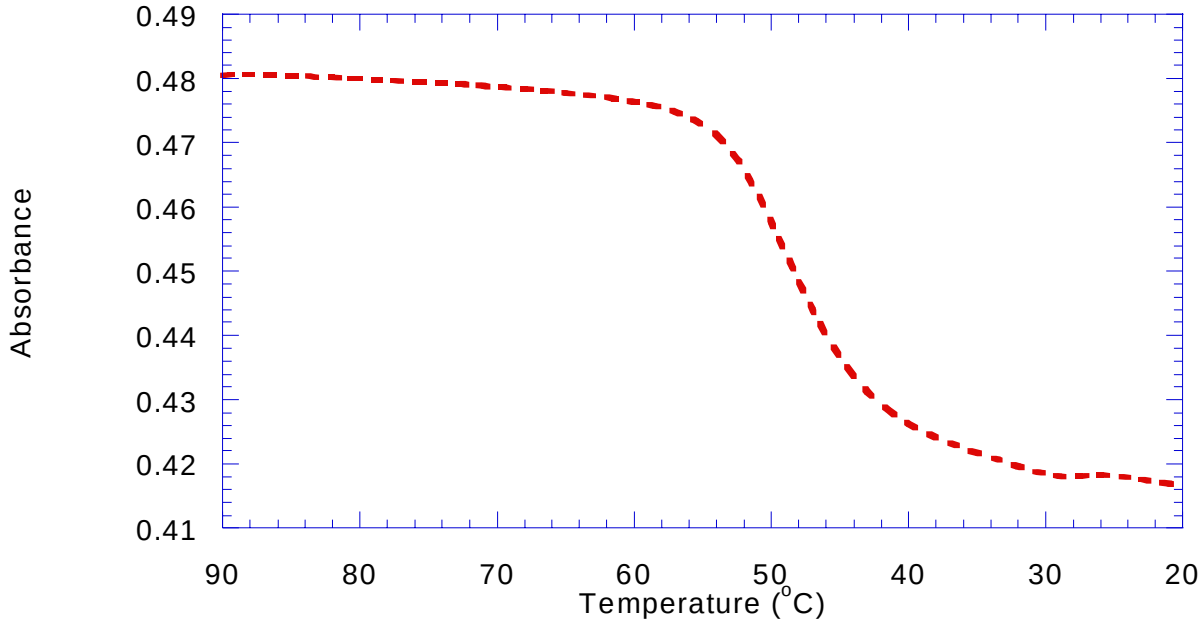

Figure S20. Cooling profile for duplex **22-23.**

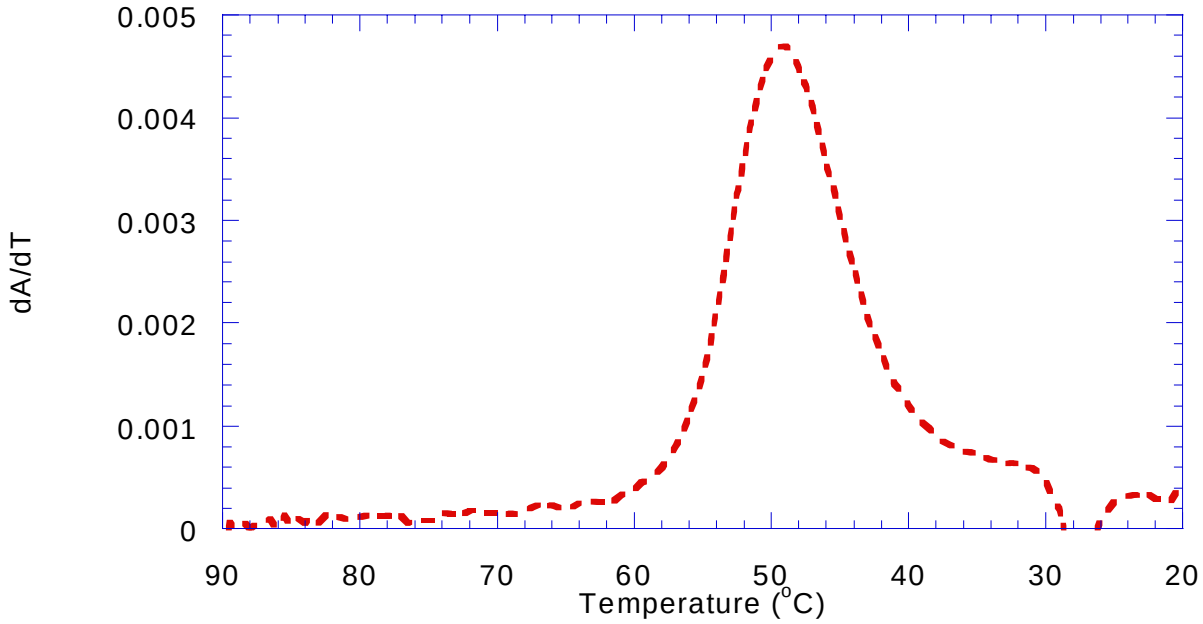

Figure S21. First derivative of cooling profile for duplex **22-23.**