Supporting Information: Probing the recognition surface of a DNA triplex: Binding studies with intercalator-neomycin conjugates

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Contents:

General Methods. Unless otherwise specified, chemicals were purchased from Aldrich or Fisher Scientific and used without further purification. Neomycin sulfate was purchased from ICN Biomedicals and was used without further purification. Poly(dA)•poly(dT) and poly(dT) were purchased from GE Healthcare Amersham Bioscience. The concentrations of the polynucleotide solutions were determined by UV spectroscopy, using the following molar extinction coefficients: ε_{264} = 8,520 M^{-1} cm^{-1} $base^{-1}$ for poly(dT), ε_{260} = 6,000 M^{-1} cm^{-1} bp^{-1} for poly(dA)•poly(dT). ^{1}H NMR spectra were collected on a JEOL ECA 500 MHz FT-NMR spectrometer. MS (MALDI-TOF) spectra were collected using a Kratos analytical KOMPACT SEQ mass spectrometer. UV spectra were collected on a Varian Cary 1E UV-Vis spectrophotometer. Isothermal titration calorimetric measurements were performed on a MicroCal VP-ITC isothermal titration calorimeter. Circular dichroism spectra were collected on a JASCO J-810 spectropolarimeter equipped with a thermoelectrically controlled cell holder. Differential scanning calorimetric measurements were carried out on a MicroCal VP-DSC differential scanning calorimeter.

Preparation of poly(dA)•2poly(dT). (S5) The poly(dA)•poly(dT) duplex (100 μM/triplet) and poly(dT) (100 μM/triplet) were dissolved in a mixture (1.5 mL) of sodium cacodylate (10 mM, pH 6.8), KCl (150 mM), and EDTA (0.5 mM). The mixture was heated at 90°C for 10 min, slowly cooling down to room temperature, and incubated at 4°C for 12 h to maximize the DNA triplex formation.

Isothermal Titration Calorimetry (ITC). (S5) To a mixture (1.42 mL) of poly(dA)•2poly(dT) DNA solution (100 μM), sodium cacodylate (10 mM, pH 6.8), KCl (150 mM), and EDTA (0.5 mM) in a sample cell at 20°C, was constantly injected an aliquot of mixture (10 μL) of ligand (XX μM), sodium cacodylate (10 mM, pH 6.8), KCl (150 mM), and EDTA (0.5 mM) through a rotary syringe. The interval time between each injection was 300 s and the duration time of each injection was 20 s. The syringe rotated at 260 rpm. Injection of the ligand solution at the same concentration into a mixture of sodium cacodylate (10 mM, pH 6.8), KCl (150 mM), and EDTA (0.5 mM) in a sample cell at 20°C was used as a blank. The calorimetric spectra was recorded after each injection and was processed using Origin (V 5.0). Integration of the area under the each heat burst curve yielded the heat given off upon each injection.

To calculate the actual heat produced from binding of ligand to DNA triplex, the following equation was used.

$$\Delta H_{actual} = \Delta H_{measured} - \Delta H_{blank}$$

ΔH_{actual}: The actual heat produced from binding of ligand to DNA triplex

$\Delta H_{measured}$: The measured heat from the titration curve

ΔH_{blank}: The heat produced from injection of ligand into the buffer solution (blank)

UV denaturation. (S6) The UV denaturation samples (1 mL) were prepared by mixing the poly(dA)•2poly(dT) DNA (15 μM/triplet), sodium cacodylate (10 mM, pH 6.8), KCl (150 mM), and EDTA (0.5 mM) and one of the intercalator-neomycin conjugates (**1-4**) at various concentrations (0, 1, 2, 4, 10, 15, and 25 μM). The UV melting spectra of these samples in 1 cm path length Quartz cuvettes was recorded at 260 nm and 280 nm as a function of temperature (5-95°C, heating rate: 0.2 °C/min). The melting temperature was determined as the one which has the peak value in the first derivative of the melting curve.

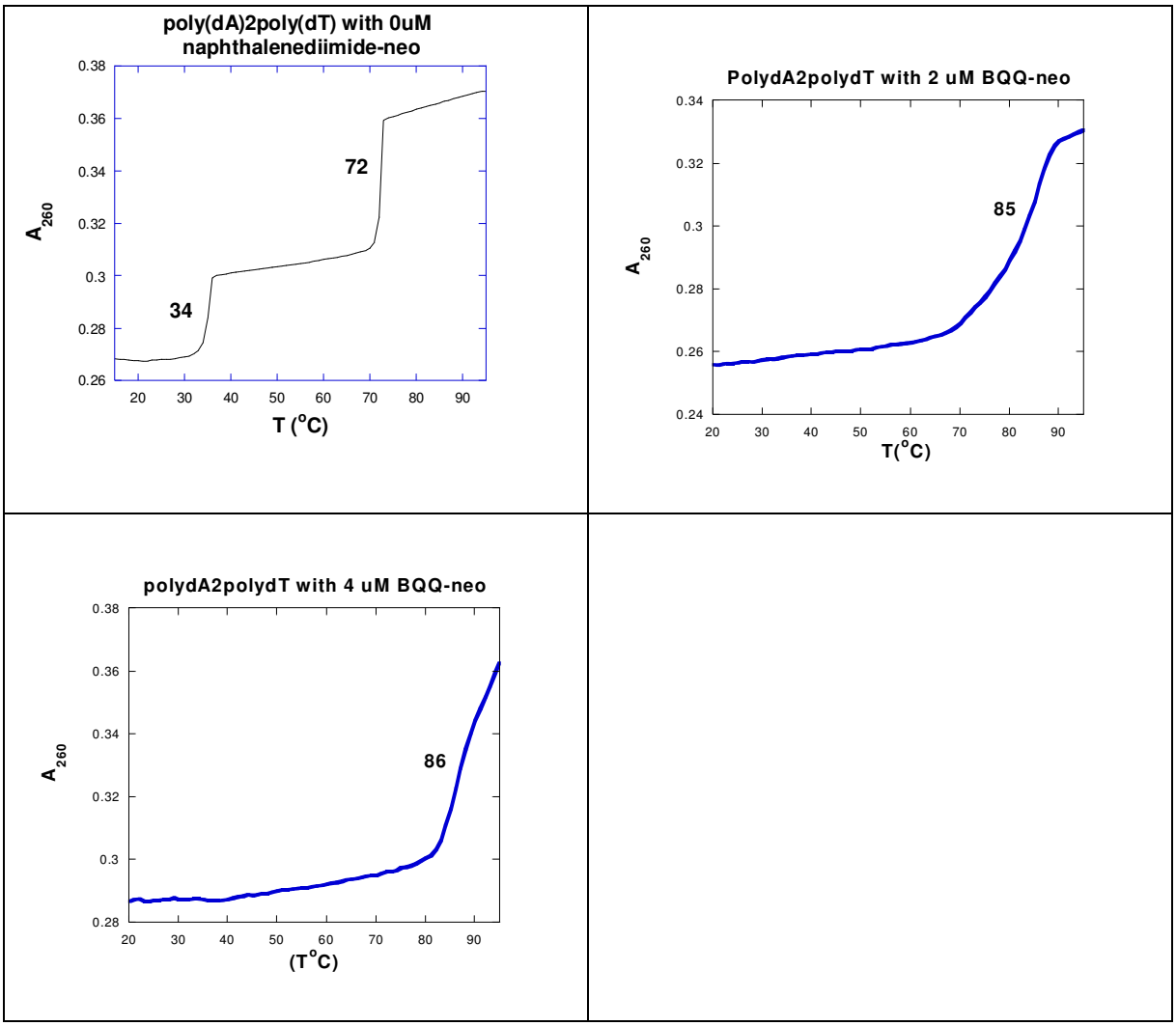
Circular Dichroism (CD) titration measurements. (S7) To a mixture (1.8 mL) of poly(dA)•2poly(dT) DNA solution (15 μM/triplet), sodium cacodylate (10 mM, pH 6.8), KCl (150 mM), and EDTA (0.5 mM) in a 1-cm path length quartz cuvette at 20 °C, were injected aliquots (0.6-40 μL) of intercalator-neomycin stock aqueous solution. The solution was then mixed by gently inversion of the close capped cuvette several times. The interval time between each injection was 10 min for equilibrium. The circular dichroism spectra were recorded as a function of wavelength (200-350 nm). (S1)

DNA Triplex + naphthalenediimide amine	$T_{m3\rightarrow2}$	$T_{m2\rightarrow1}$
0 μM	34	72
1 μM	36	72
2 μM	38	72
4 μM	41	72
8 μM	44	73
10 μM	58	73

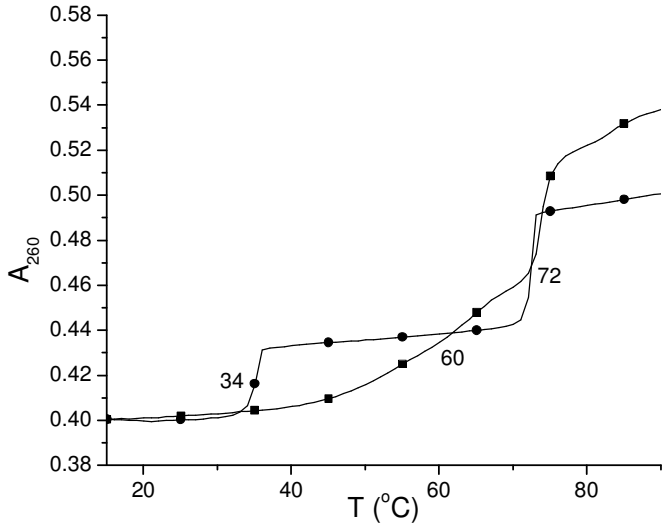
Supporting Information Table 1. UV melting temperatures recorded when poly(dA)•2poly(dT) dissociates in the presence of **7** at various concentrations. (S2)

DNA Triplex + Anthraquinone amine	$T_{m3\rightarrow2}$	$T_{m2\rightarrow1}$
0 μM	34	72
0.5 μM	40	72
1 μM	41	72
2 μM	46	72
4 μM	49	74
10 μM	54	74

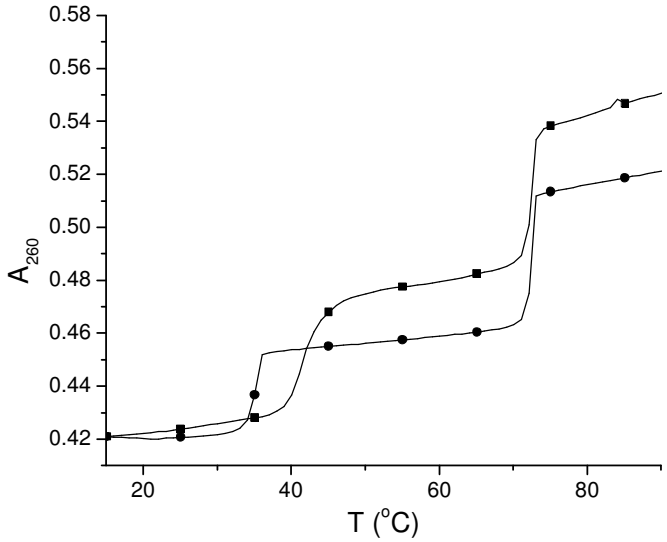
Supporting Information Table 2. UV melting temperatures recorded when poly(dA)•2poly(dT) dissociates in the presence of **8** at various concentrations. (S3)



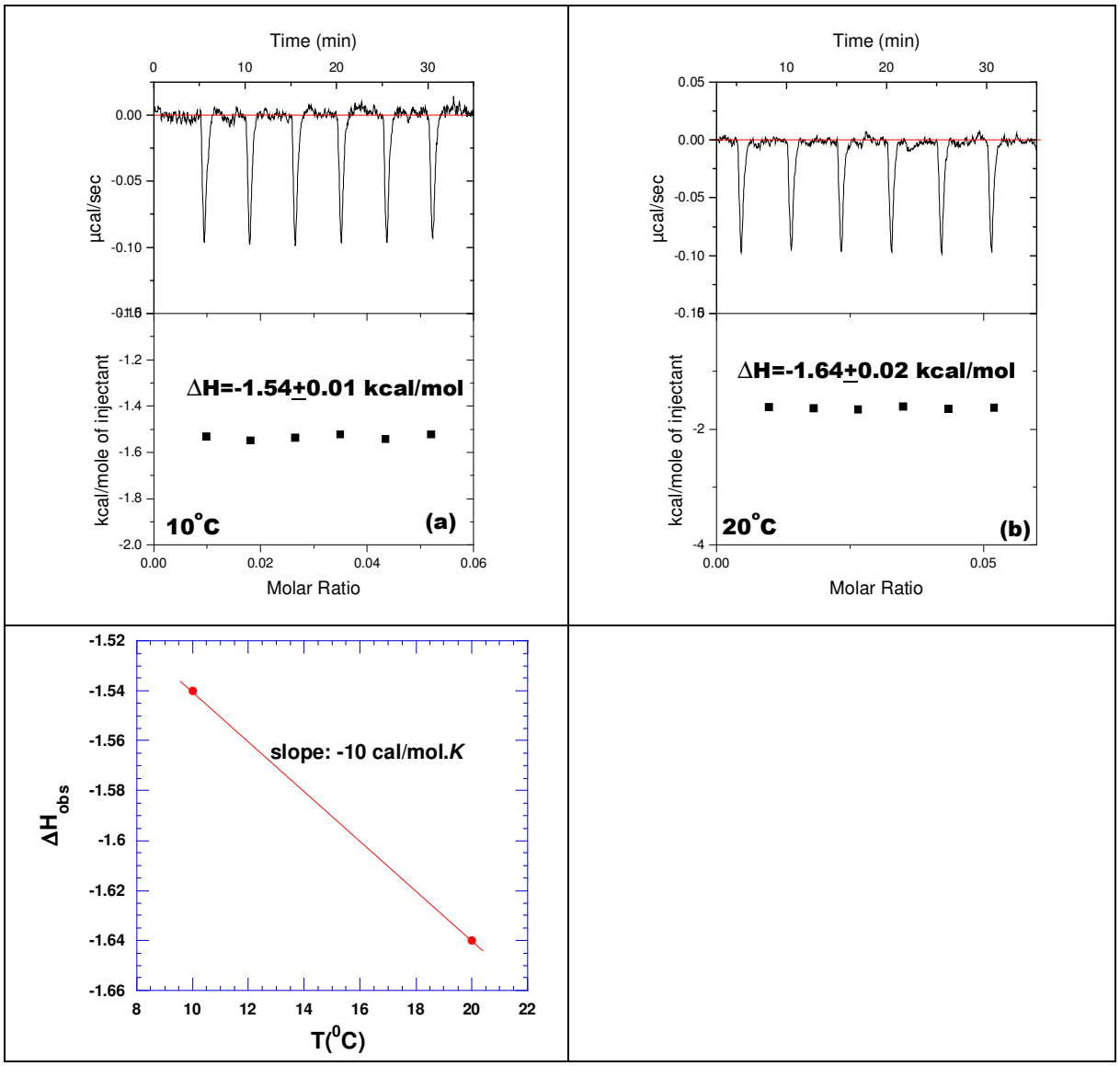
Supporting information Figure 1. UV melting profiles of poly(dA)•2poly(dT) at 260 nm in the absence and presence of **2** (2 μM and 4 μM) . (S4)



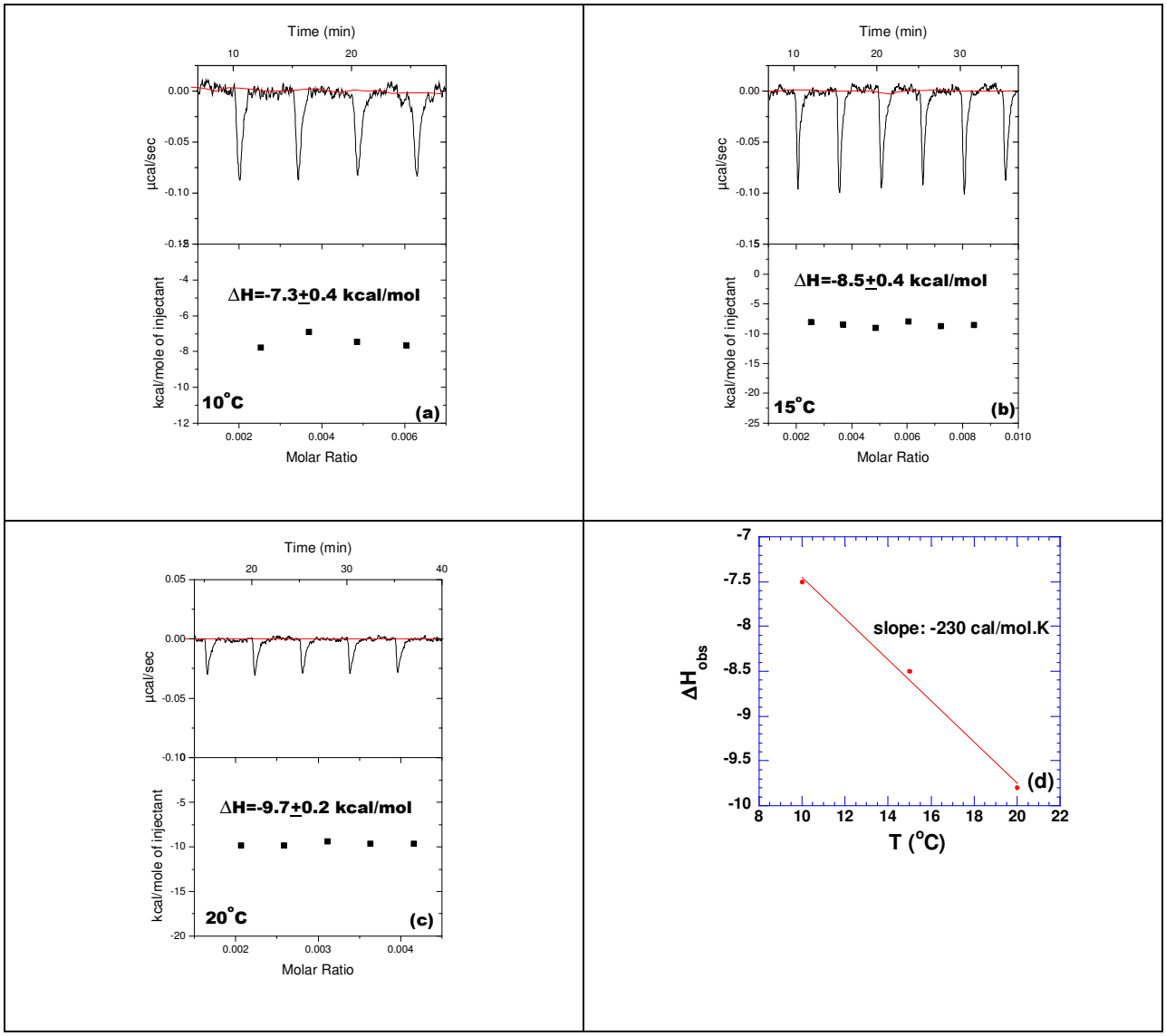
Supporting information Figure 2. UV melting profiles of poly(dA)•2poly(dT) at 260 nm in the absence (●) and presence of **4** (1 μM, ■). (S5)



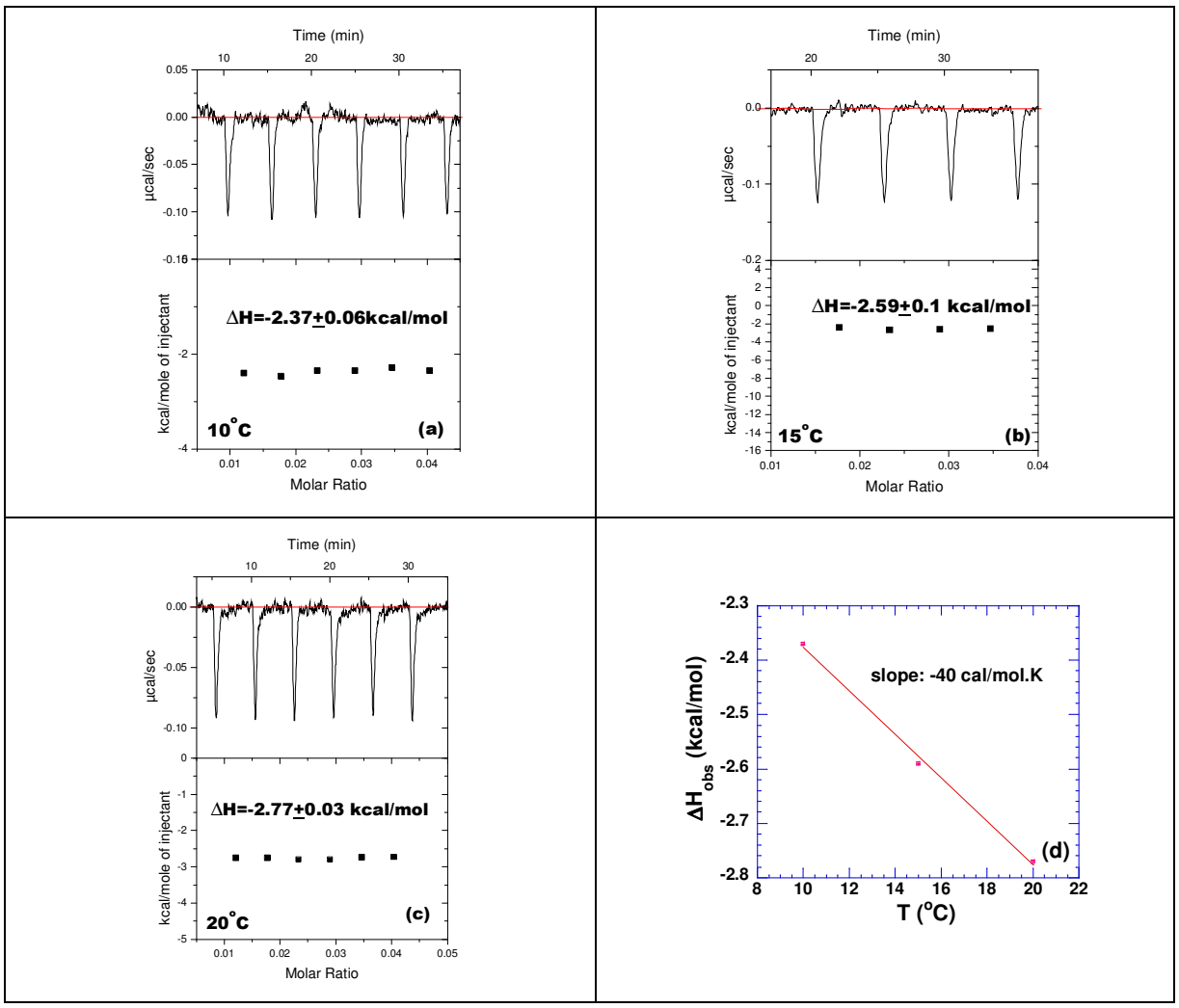
Supporting information Figure 3. UV melting profiles of poly(dA)•2poly(dT) at 260 nm in the absence (●) and presence of **8** (1 μM, ■). (S6)



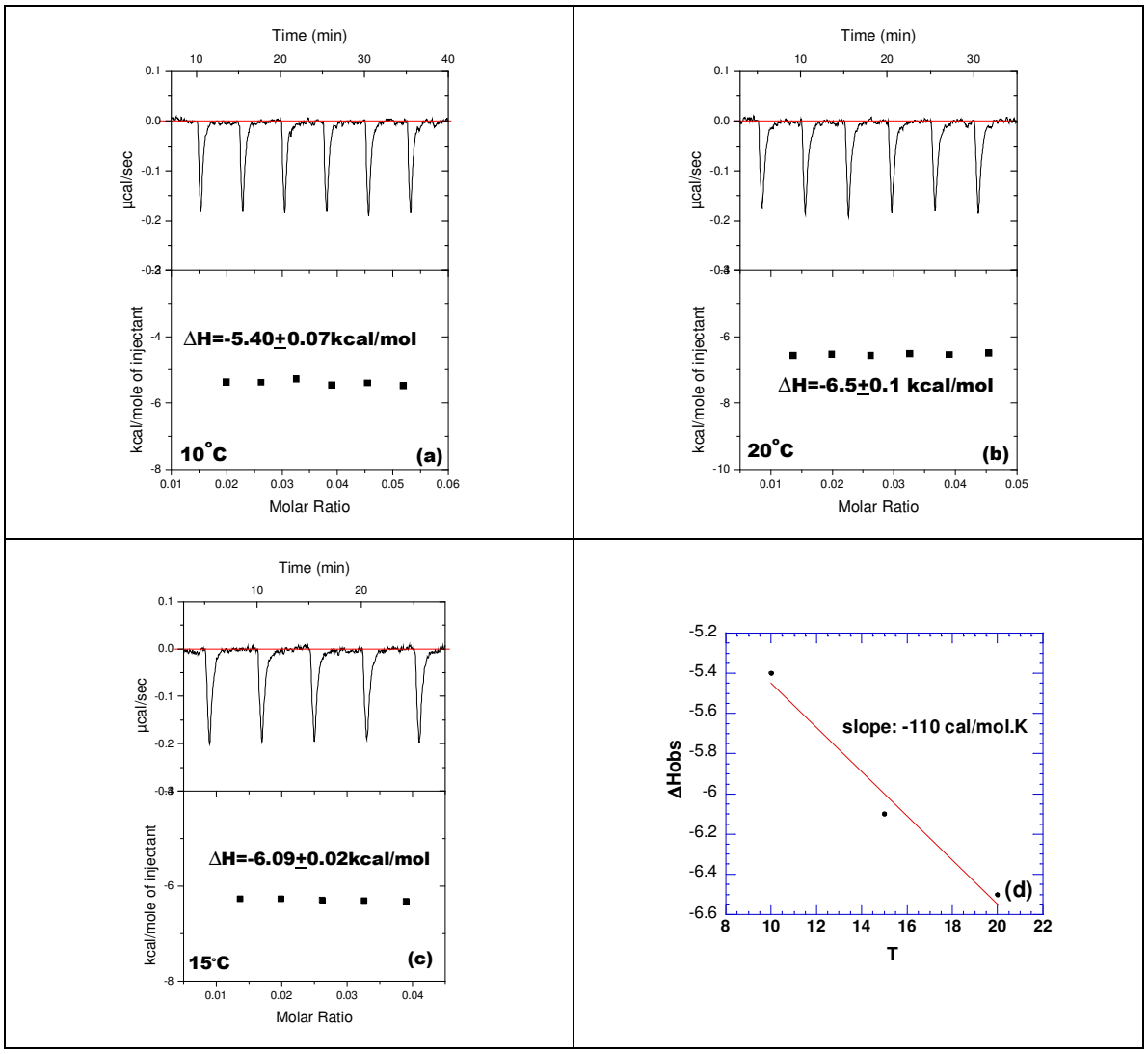
Supporting Information Figure 4. (a) –(b) Heat burst recorded from the ITC titration of **1** (120 µM) into poly(dA)•2poly(dT) (100 µM) at 10 and 20 °C, respectively. (c) A plot of observed binding enthalpy obtained from (a)-(c) as a function of temperature. Slope reveals the heat capacity changes (ΔC_p). Experimental conditions: sodium cacodylate (10 mM, pH 5.5), EDTA (0.5 mM), KCl (150 mM). (S7)



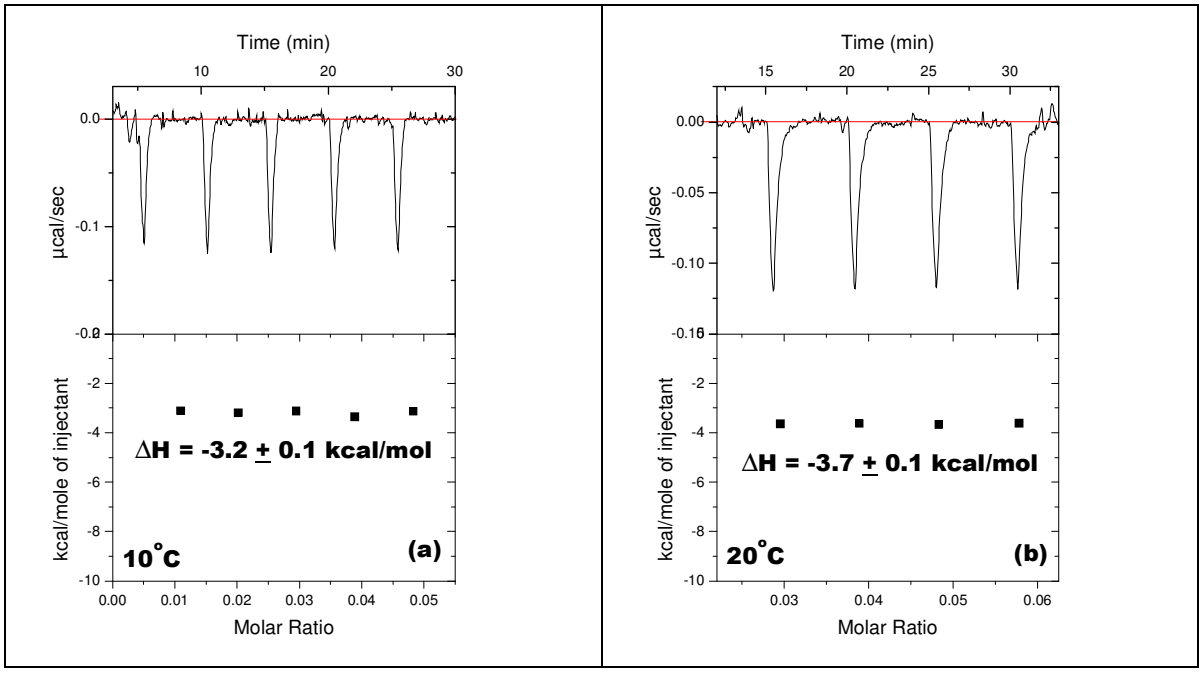
Supporting Information Figure 5. (a) and (b) Heat burst recorded from the ITC titration of **2** (25 µM) into poly(dA)•2poly(dT) (150 µM) at 10 and 15 °C, respectively. (c) Heat burst recorded from the ITC titration of **2** (15 µM) into poly(dA)•2poly(dT) (100 µM) at 20 °C. (d) A plot of observed binding enthalpy as a function of temperature. Slope reveals the heat capacity changes (ΔC_p). Experimental conditions: sodium cacodylate (10 mM, pH 5.5), EDTA (0.5 mM), KCl (150 mM). (S8)



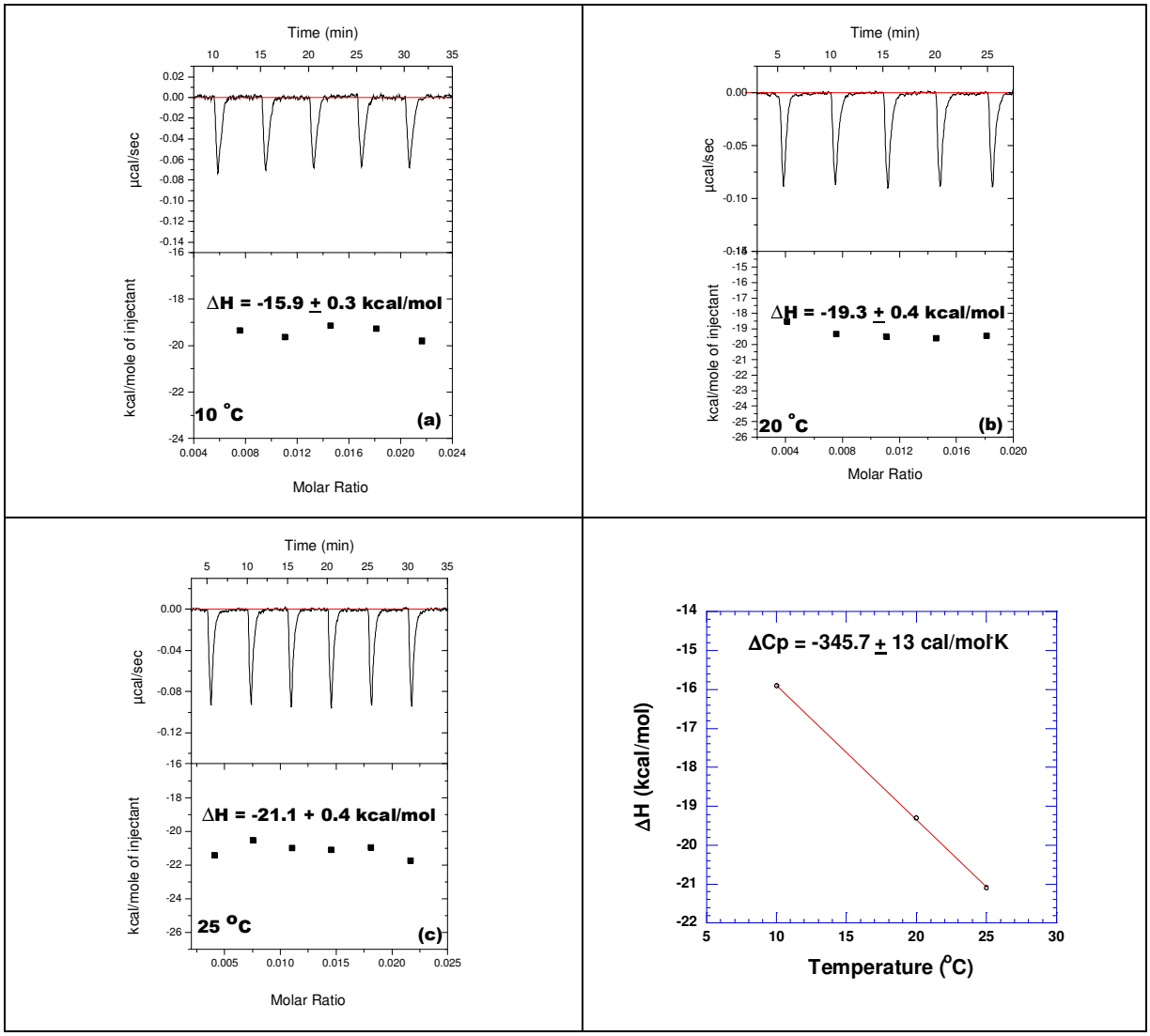
Supporting Information Figure 6. (a) –(c) Heat burst recorded from the ITC titration of **3** (80 µM) into poly(dA)•2poly(dT) (100 µM) at 10, 15, and 20 °C, respectively. (d) A plot of observed binding enthalpy obtained from (a)-(c) as a function of temperature. Slope reveals the heat capacity changes (ΔC_p). Experimental conditions: sodium cacodylate (10 mM, pH 5.5), EDTA (0.5 mM), KCl (150 mM). (S9)



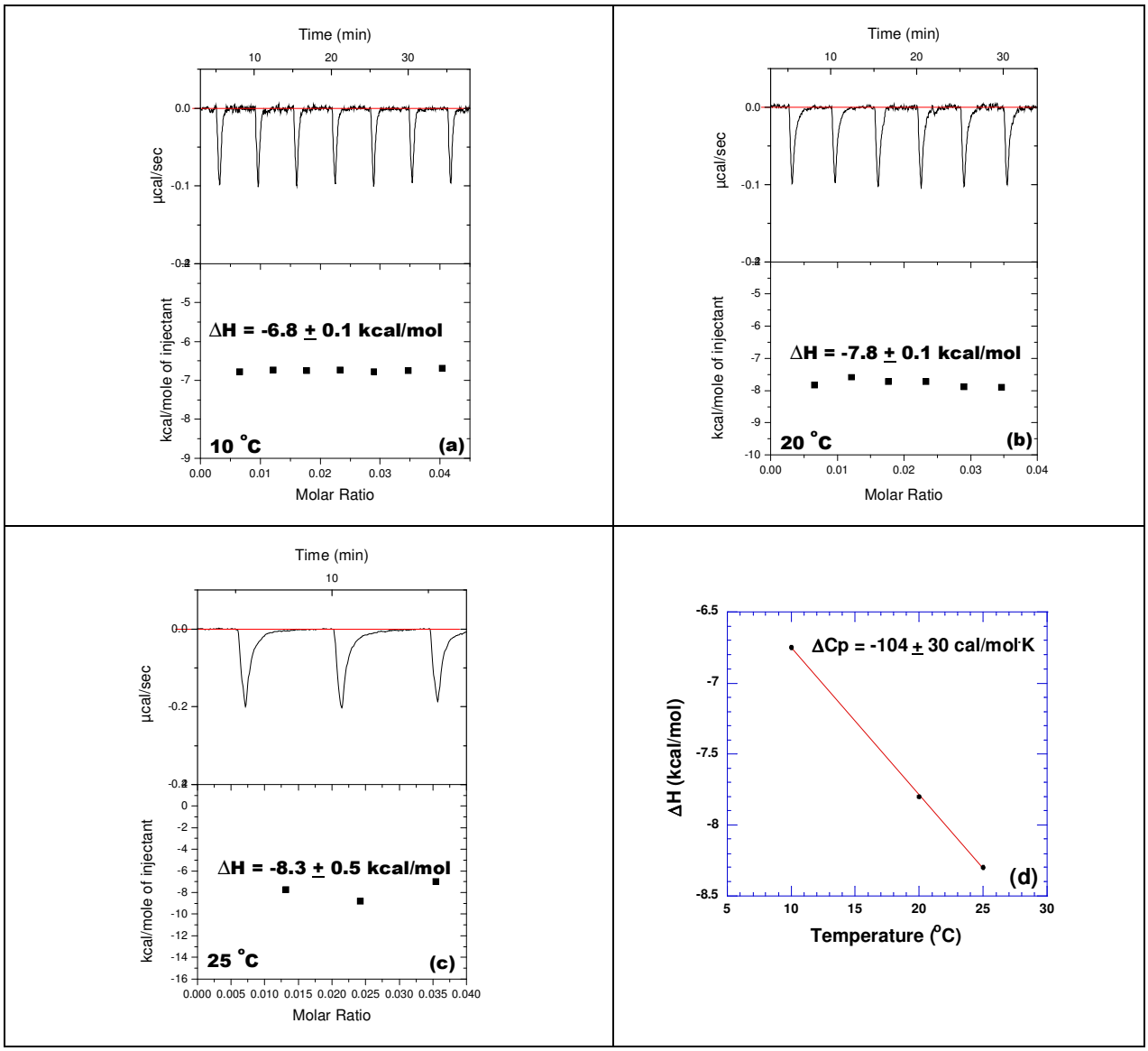
Supporting Information Figure 7. (a) –(c) Heat burst recorded from the ITC titration of **4** (90 µM) into poly(dA)•2poly(dT) (100 µM) at 10, 15, and 20 °C, respectively. (d) A plot of observed binding enthalpy obtained from (a)-(c) as a function of temperature. Slope reveals the heat capacity changes (ΔC_p). Experimental conditions: sodium cacodylate (10 mM, pH 5.5), EDTA (0.5 mM), KCl (150 mM). (S10)



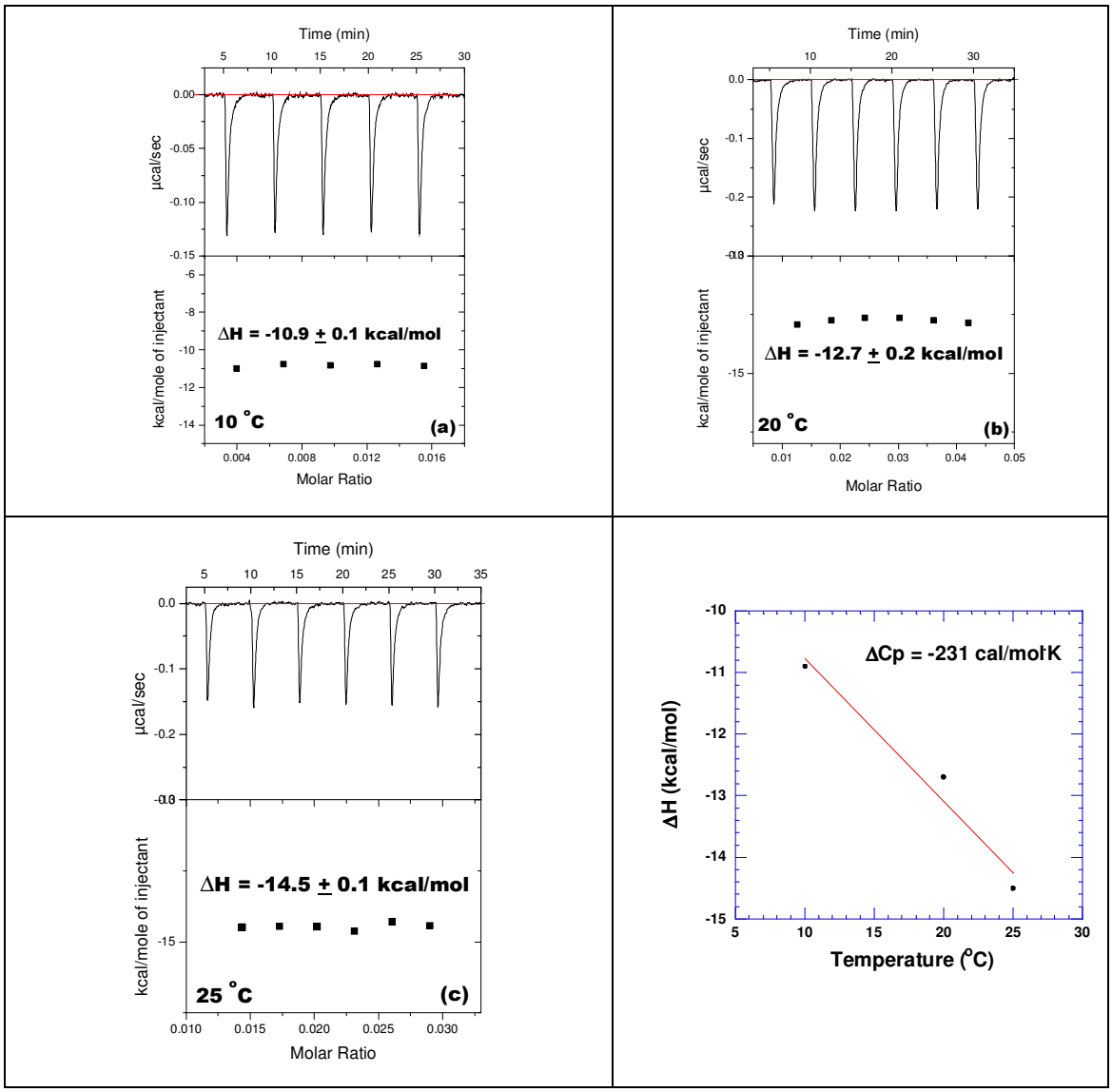
Supporting Information Figure 8. (a) –(b) Heat burst recorded from the ITC titration of **1** (80 µM) into poly(dA)•2poly(dT) (60 µM) at 10 and 20°C, respectively. (d) A plot of observed binding enthalpy obtained from (a)-(b) as a function of temperature. Slope reveals the heat capacity changes (ΔC_p). Experimental conditions: sodium cacodylate (10 mM, pH 6.8), EDTA (0.5 mM), KCl (150 mM). (S11)



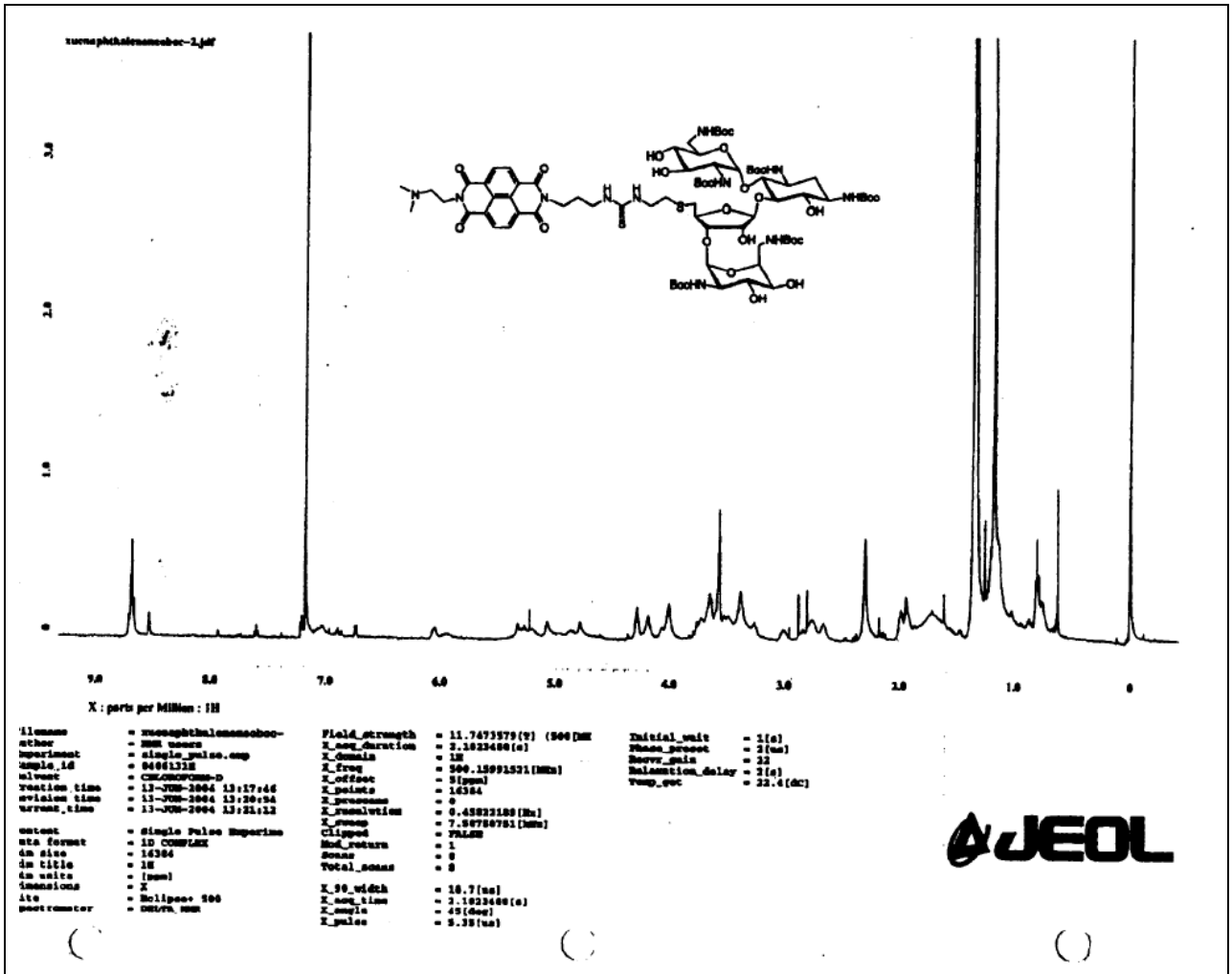
Supporting Information Figure 9. (a) –(c) Heat burst recorded from the ITC titration of **2** (15 µM) into poly(dA)•2poly(dT) (30 µM) at 10, 20, and 25 °C, respectively. (d) A plot of observed binding enthalpy obtained from (a)-(c) as a function of temperature. Slope reveals the heat capacity changes (ΔC_p). Experimental conditions: sodium cacodylate (10 mM, pH 6.8), EDTA (0.5 mM), KCl (150 mM). (S12)



Supporting Information Figure 10. (a) –(c) Heat burst recorded from the ITC titration of **3** (80 µM) into poly(dA)•2poly(dT) (50 µM) at 10, 20, and 25 °C, respectively. (d) A plot of observed binding enthalpy obtained from (a)-(c) as a function of temperature. Slope reveals the heat capacity changes (ΔC_p). Experimental conditions: sodium cacodylate (10 mM, pH 6.8), EDTA (0.5 mM), KCl (150 mM). (S13)



Supporting Information Figure 11. (a) –(c) Heat burst recorded from the ITC titration of **4** (50 µM) into poly(dA)•2poly(dT) (60 µM) at 10, 20, and 25 °C, respectively. (d) A plot of observed binding enthalpy obtained from (a)-(c) as a function of temperature. Slope reveals the heat capacity changes (ΔC_p). Experimental conditions: sodium cacodylate (10 mM, pH 6.8), EDTA (0.5 mM), KCl (150 mM). (S14)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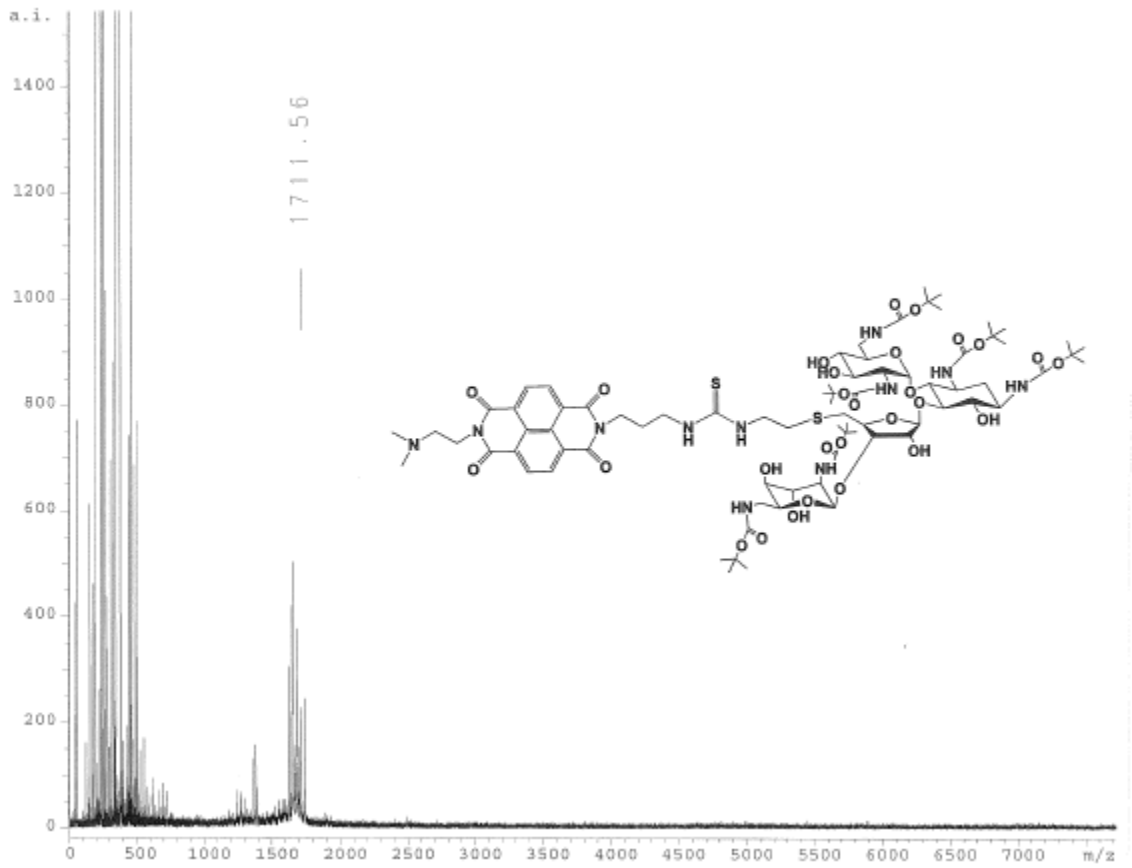
JEOL

^{1}H-NMR of 3a.



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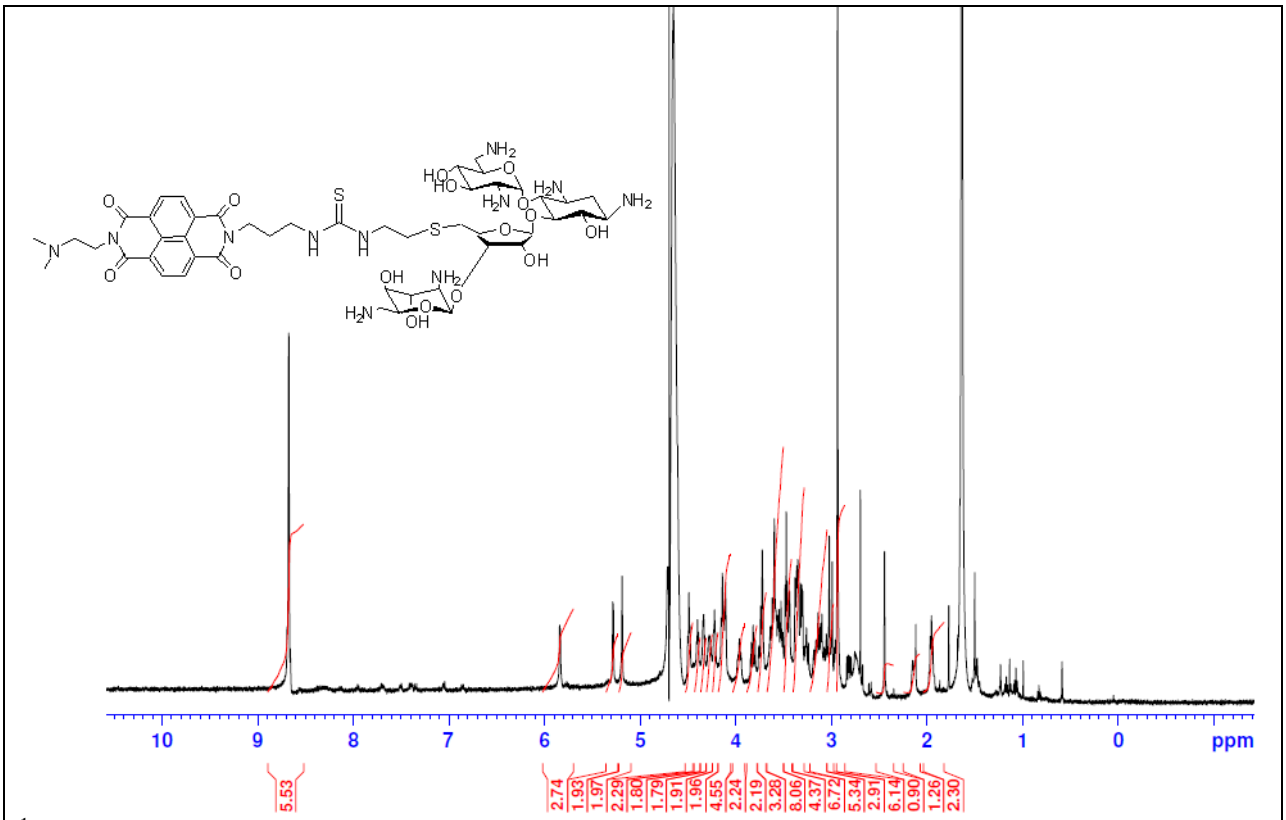
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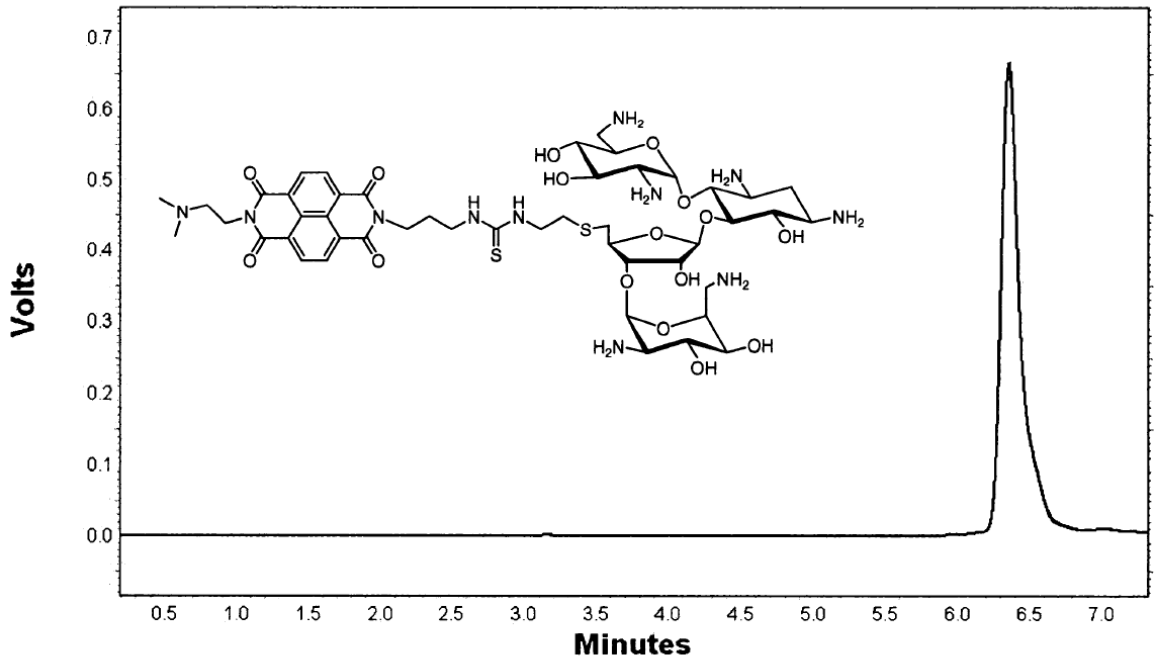
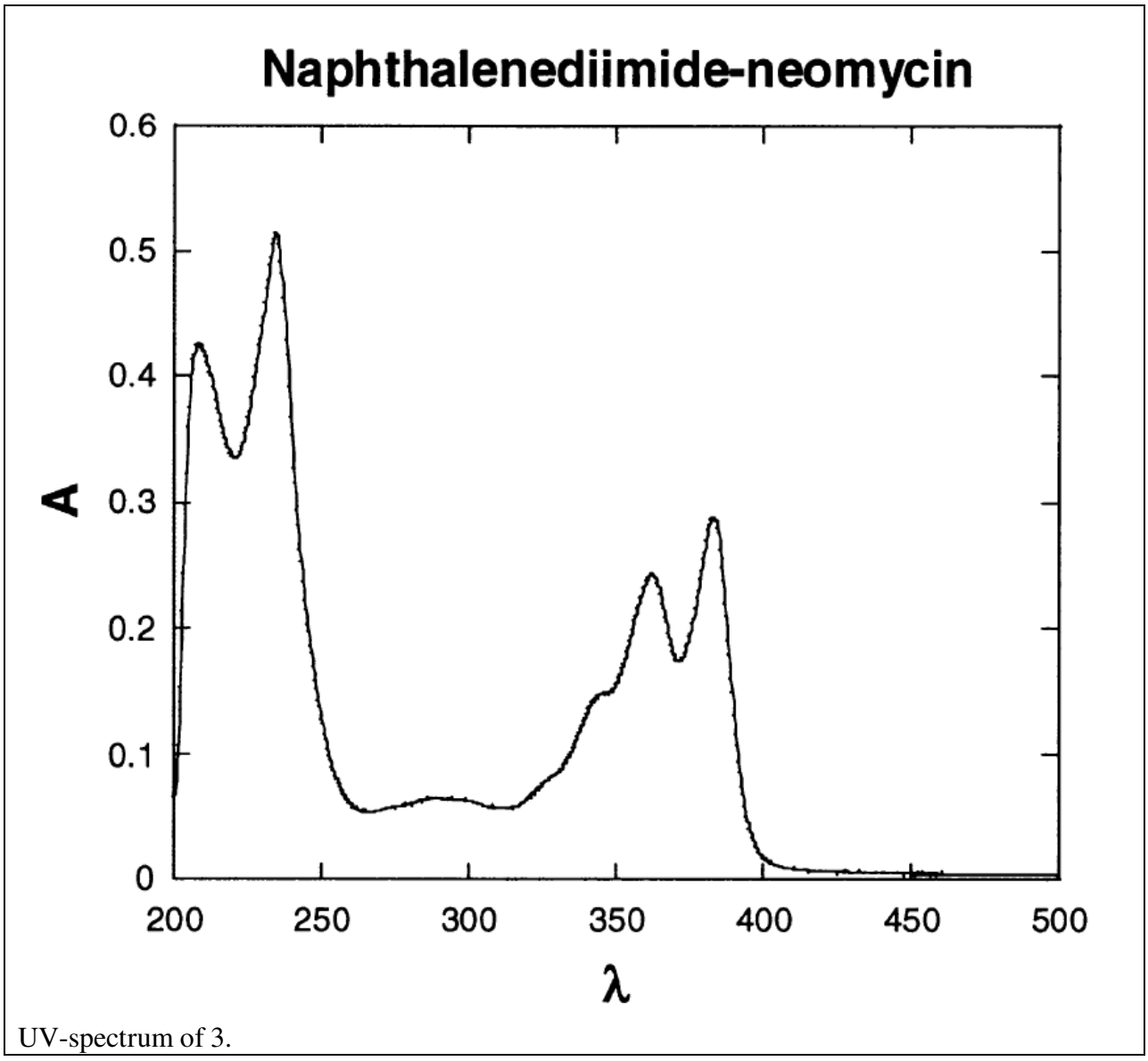
^{1}H-NMR of 3.

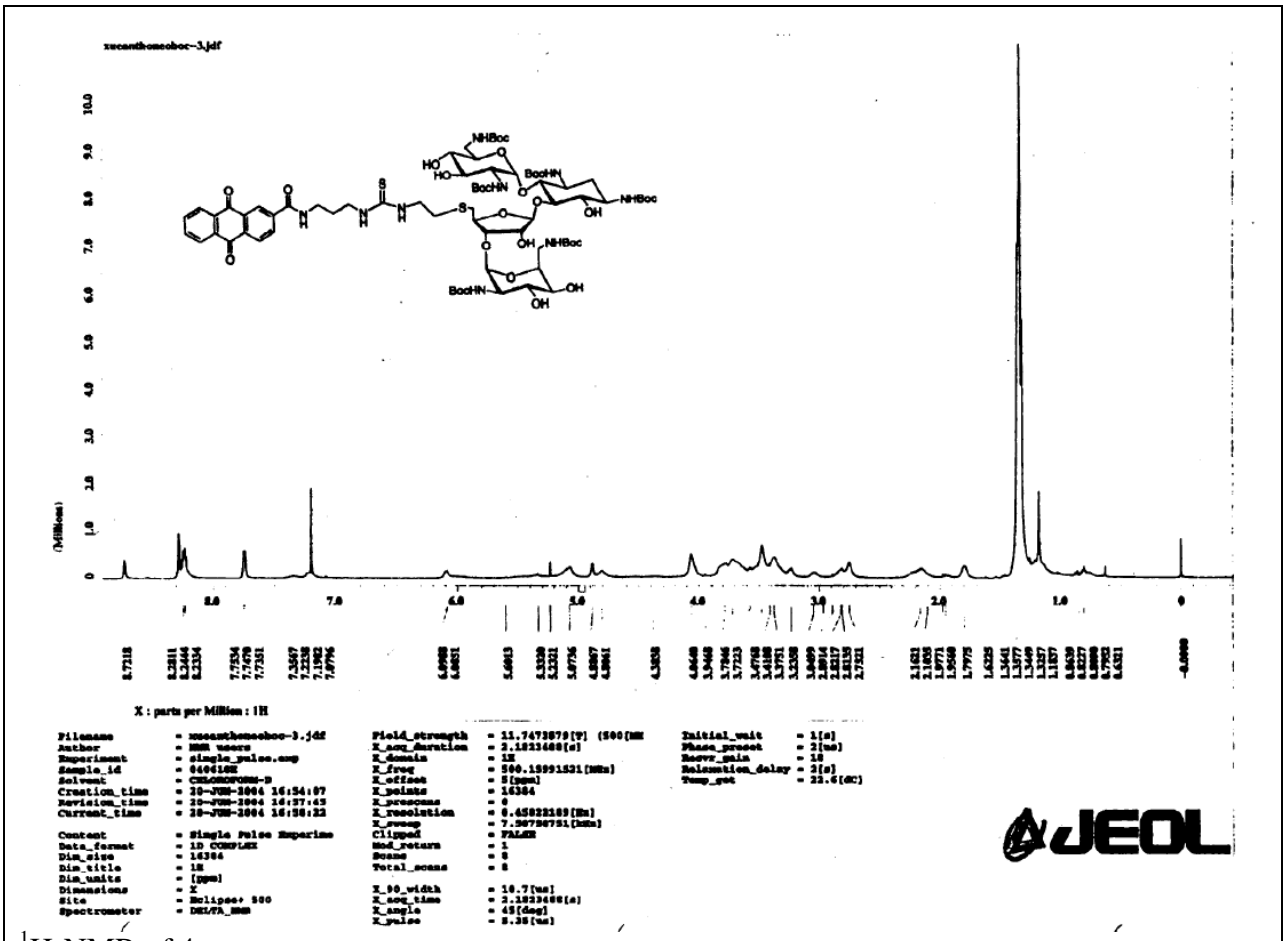


Condition: Buffer A: 0.1% CF_3COOH/H_2O, Buffer B: 0.08% CF_3COOH/CH_3CN; 0-30% of buffer B over buffer A during 2 min, 30%-100% buffer B over buffer A during 23 min.

HPLC of compound 3



UV-spectrum of 3.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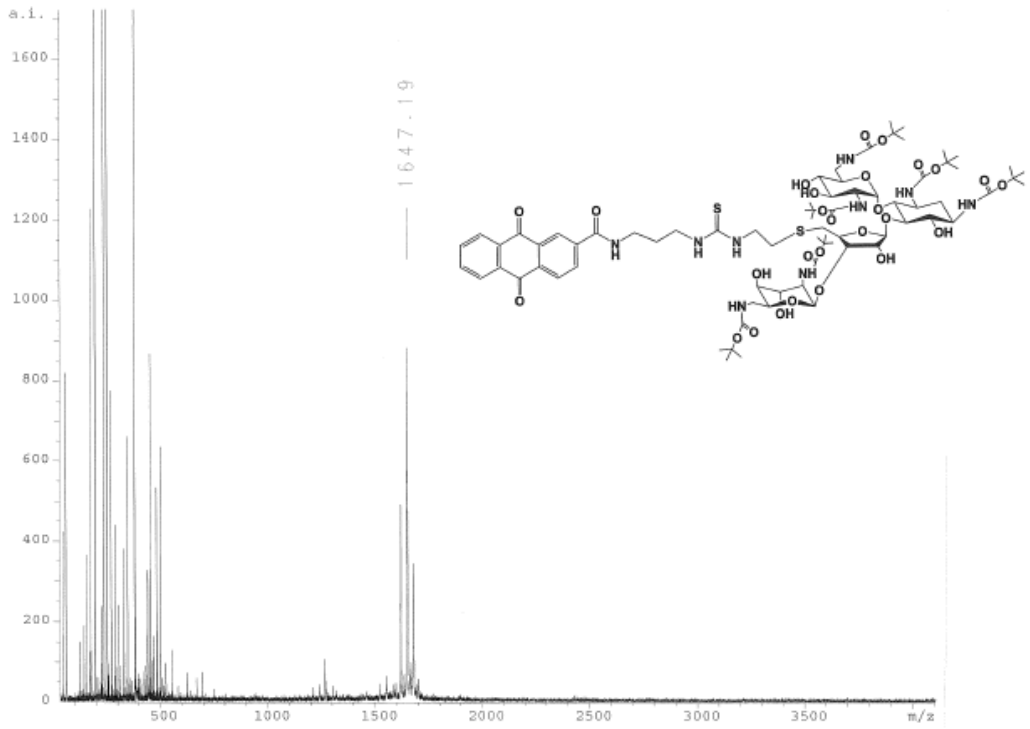
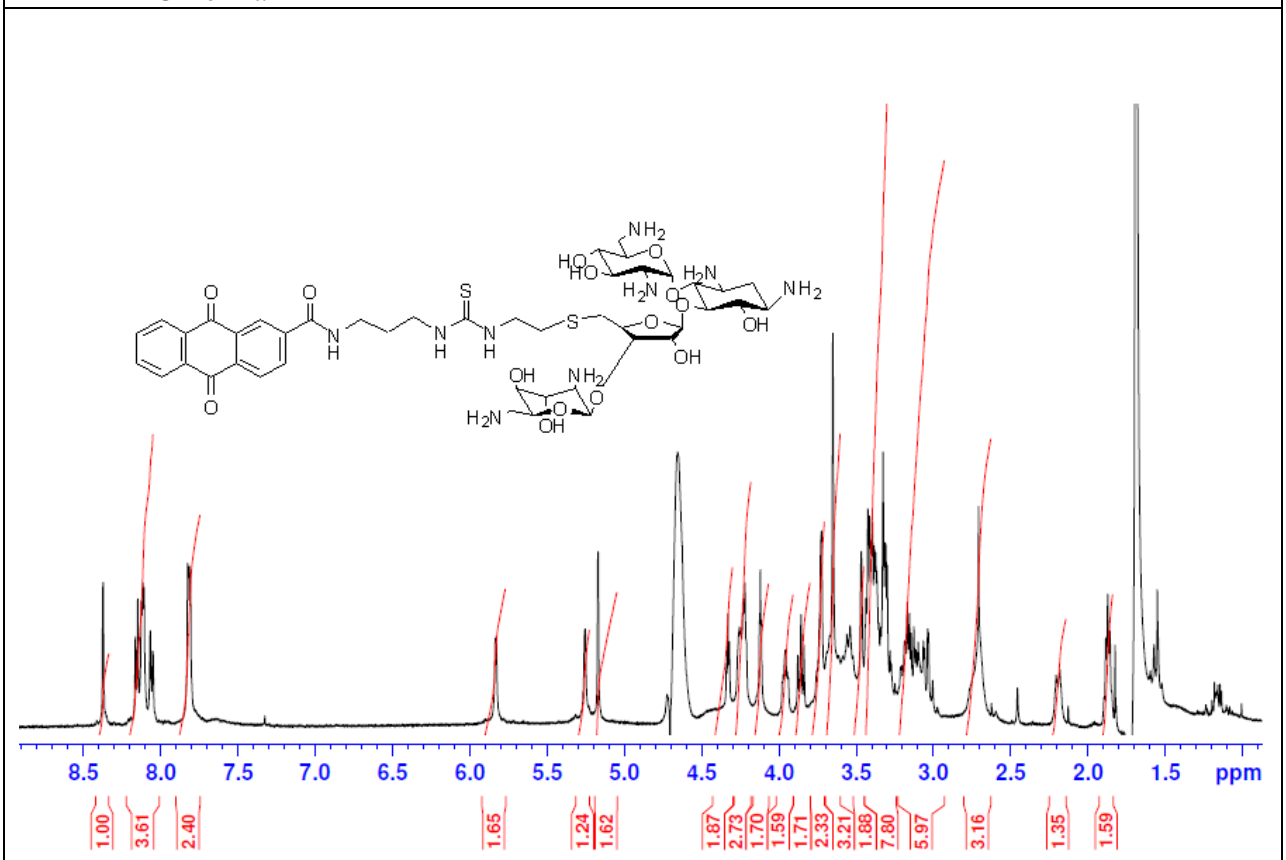
^{1}H-NMR of 4a



MALDI-TOF of 4a



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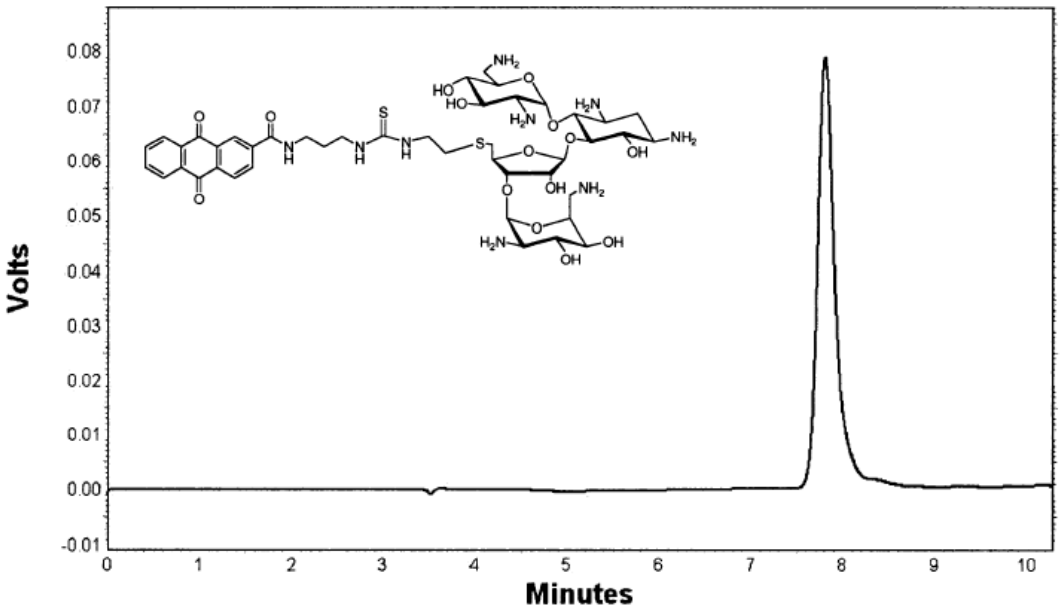
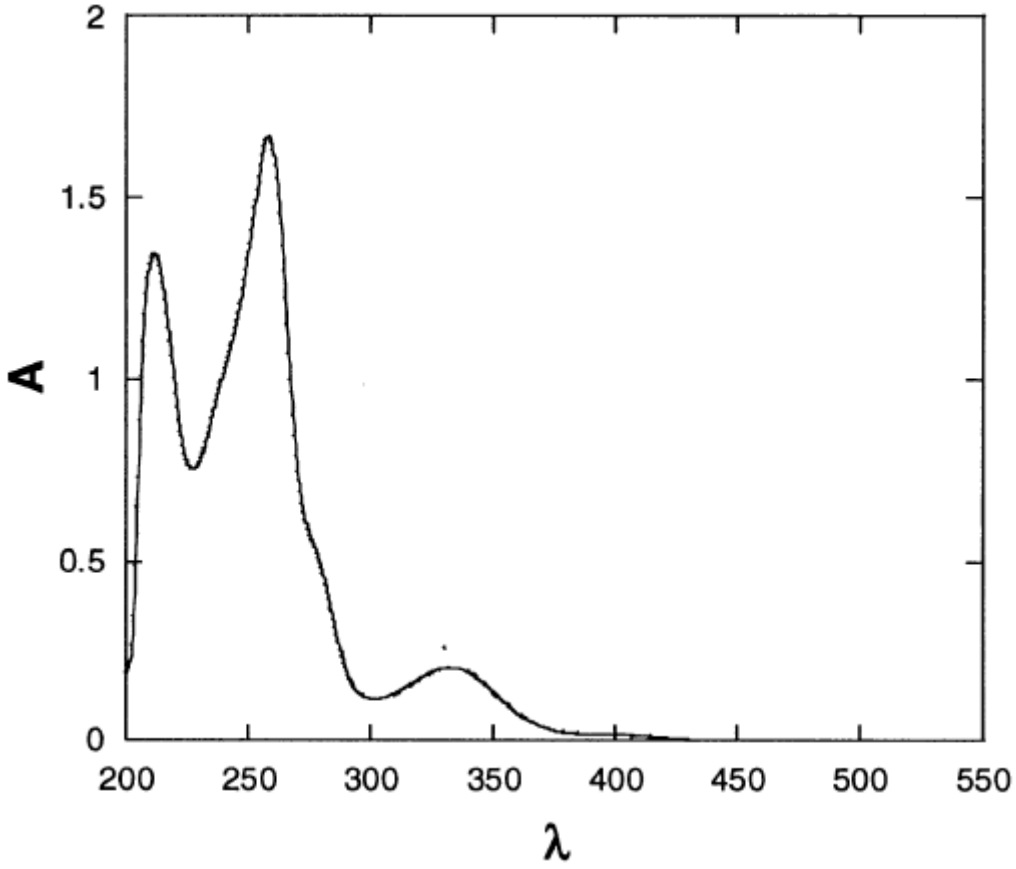


Condition: Buffer A: 0.1% CF_3COOH/H_2O, Buffer B: 0.08% CF_3COOH/CH_3CN; 0-30% of buffer B over buffer A during 2 min, 30%-100% buffer B over buffer A during 23 min.

HPLC of 4



UV-spectrum of 4

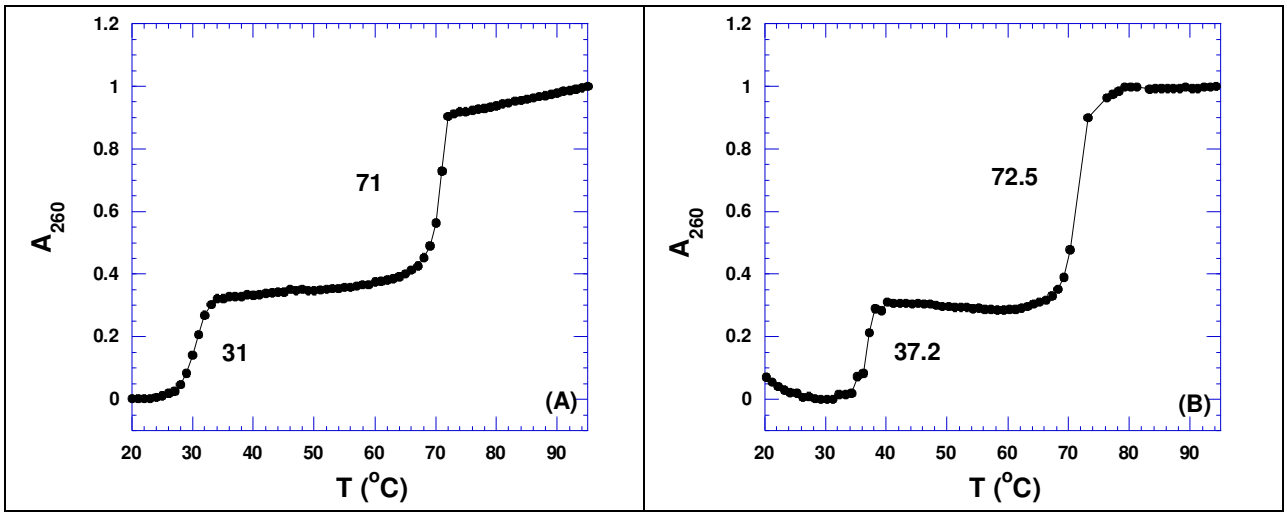


Figure 16. UV melting profiles for poly(dA)•2poly(dT) in the absence (A) and presence (B) of **7**. Experimental conditions: [poly(dA)•2poly(dT)] = 15 μM/bt. [7] = 3.75 μM. Buffer conditions: 150 mM KCl, 10 mM sodium cacodylate, 0.5 mM EDTA, pH 5.5. (S19)

DNA Triplex + naphthalenediimide (7)	$T_{m3\rightarrow2}$	$T_{m2\rightarrow1}$
3.75 μM	37.2	72.5

Table 3. UV melting temperatures recorded when poly(dA)•2poly(dT) dissociates in the presence of **7**. Experimental conditions: [poly(dA)•2poly(dT)] = 15 μM/bt. [7] = 3.75 μM. Buffer conditions: 150 mM KCl, 10 mM sodium cacodylate, 0.5 mM EDTA, pH 5.5. (S20).

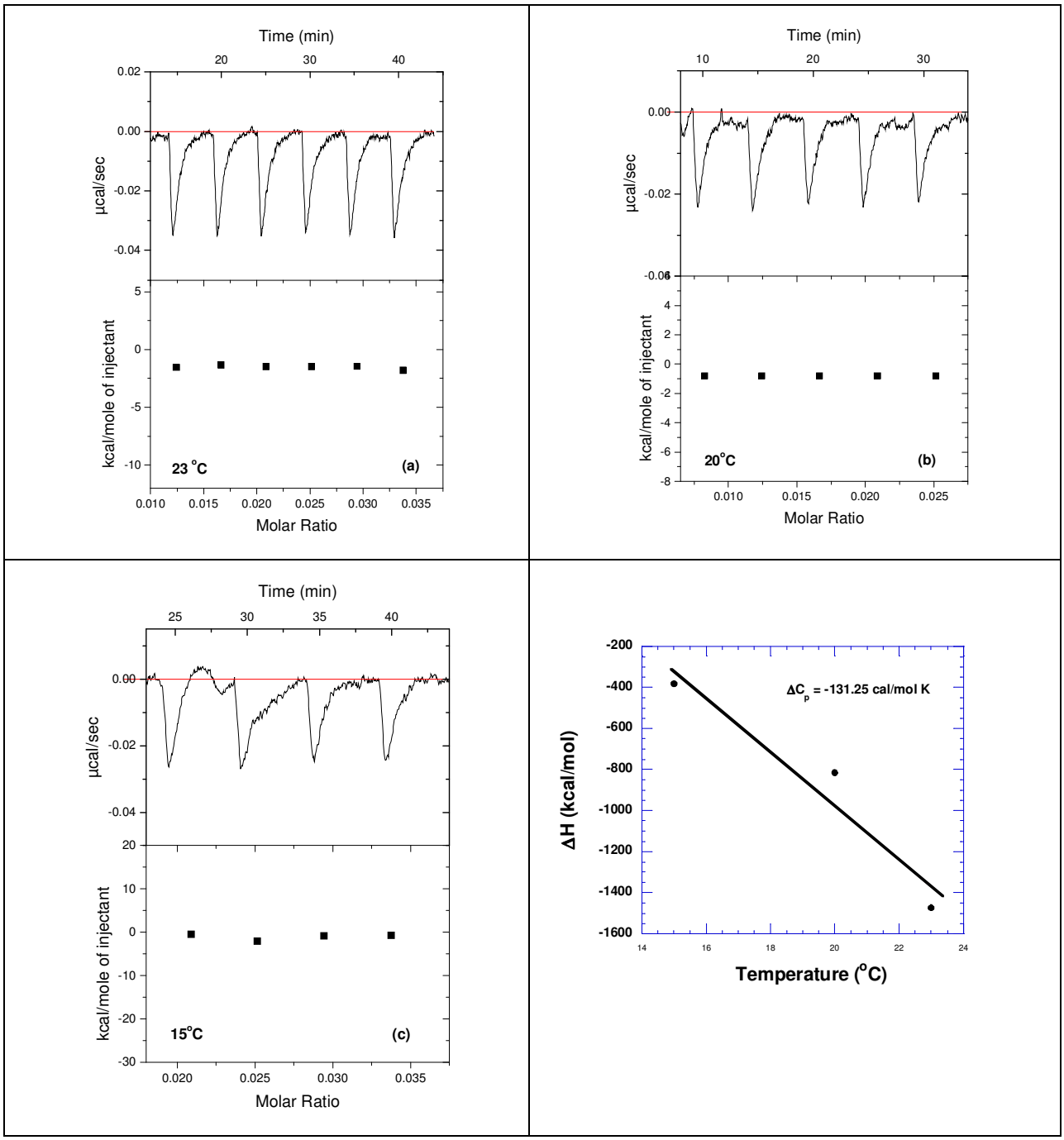


Figure 17. (a-c) Heat burst recorded from the ITC titration of naphthalenediimide (75 µM) into poly(dA)•2poly(dT) (125 µM) at 23, 20, and 15 °C, respectively. (d) A plot of observed binding enthalpy obtained from (a)-(c) as a function of temperature. Slope reveals the heat capacity changes (ΔC_p). Experimental conditions: sodium cacodylate (10 mM, pH 5.5), EDTA (0.5 mM), KCl (150 mM). (S21)