Supporting Information

Uridine-Conjugated-Ferrocene DNA Oligonucleoides:

Unexpected Cyclization Reaction of Uridine Base

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Experimental Section

Materials: Diisopropylamine (DIPA), diethylamine, triphenylphosphine, copper(I) iodide, triethylamine(TEA) were purchased from Aldrich and used as received. DMF, pyridine, dichloromethane, silica gel (240-400 mesh), acetonitrile (MeCN, HPLC grade), hexane, methanol were purchased from EM Science and used as received. Acetonitrile (MeCN, DNA synthesis grade) and dichloromethane (DNA synthesis grade) were purchased from Burdick & Jackson. Tetrazole, 5-iodo-deoxyuridine, 2-cyanoethyl N, N, N', N'-tetraisopropylphosphane, 4,4'-dimethoxytrityl chloride (DMT Cl) was purchased from Chemgenes and used as received. All standard phosphromidites and ancillary reagents were purchased from Glen Research and used as received. Phosphodiesterase I, P1 and S1 nuclease were purchased from Phamacia and used as received. Bacterial alkaline phosphatase were purchased from Bibco BRL ans used as received. Diisopropanylammonium tetrazolide was prepared using literature procedure. $Pd(dba)_3$ was prepared following the literature procedure.

Instrumentation: Spectra were recorded on the following spectrometers: GC/MS performed at 70 eV with a 50 m x 0.2 mm capillary column programmed at 140 °C for 1 min and 280 °C at 10 °C min^{-1}, ^{1}H NMR spectra recorded on a 300 MHz machine and ^{31}P NMR spectra recorded on a 400 MHz spectrometer, UV spectra recorded on HP 845X UV-Vis system,. Oligo. R3 HPLC columns were purchased from PerSeptive Biosystems, and C6 and C18 HPLC columns were purchased from Keystone Scientific. Mass spectra for organic compounds were obtained from Mass Consortium at San Diego using either HP 1100 MSD for electrospray or VG ZAB 2-FE for high resolution FAB. MALDI-TOF mass spectra for DNA oligonucleotides were obtained from Caltech Protein/Peptide Micro Analysis Lab, two MALDI-TOF mass spectra of **D3** and **D4** are shown in Fig. **S1**.

All DNA oligonucleotides were synthesized using either an ABI 394 or ABI 392 RNA/DNA synthesizers, for non-modified DNA oligonucleotides, standard protocol was used; for Fc-containing DNA oligonucleotides, standard protocol except a coupling time of 15 min was used. HPLC analyses were performed on Hitachi D7000 systems equipped with a diode array; with a Betasil C_{18} reversed-phase column (25 cm x 4.6 mm I.D.) for compounds **I** and **II**, DNA dimers; with a Betasil C_6 reversed-phase column (25 cm x 4.6 mm I.D.) for modified DNA oligonucleotides; and with an Oligo R3 polystyrene column (10 cm x 4.6 mm I.D.) for non-modified DNA oligonucleotides.

For analyzing **I** and **II**, a gradient of 15 % to 50 % MeCN over 6 min and 50 % to 100 % MeCN over 44 min in 100 mM triethylammonium acetate (TEAA) (pH 6.5) was used; DNA dimers, employing a gradient of 10 % to 35 % MeCN over 32 min and 35 % to 100 % acetonitrile over 10 min in 100 mM TEAA (pH 6.5) was used. For DNAs containing single modification of either **III** or **IV**, the gradient system is 20 % to 45 % MeCN over 32 min and 45

% to 100 % MeCN over10 min in 100 mM TEAA; for DNA containing dual modifications of either **III** or **IV**, the gradient system is 10 % to 35 % MeCN over 32 min and 35 % to 100 % MeCN over10 min in 100 mM TEAA. For standard DNA, the gradient system is 0 to 25 % MeCN over32 min and 25 % to 100 % MeCN over 8 min in 100 mM TEAA.

All Tm values of DNA duplexes were measured under the following conditions: [DNA strand] = 2.0 μM; buffer: 1X SSC, with temperature ramped from 20 °C to 80 °C at a rate of 1 °C per minute. The thermal denaturation curves of four pairs of DNA hybrids; **D1:D2** (perfect match), **D1:D3** (**III** at 12th position), **D1:D4** (**IV** at 12th position), **D1:D5** (AA mismatch at 12th position) are shown in Fig. **S2**. The thermal denaturation curves of another three pairs of DNA hybrids; **D2:D6** (GT mismatch at 12th position), **D3:D6** (either **III** or **IV** pairs to dG at 12th position), **D6:D7** (perfect match) are shown in Fig. **S3**.

One example of digestion procedure: A mixture of 11.9 nmol (20 μL) of D4, 300 U (2 μL) of bacterial alkaline phosphatase, 0.31 U (10μL) of snake venom phosphodiesterase I, 0.75 μL of 1.0 M $MgCl_2$, 3.0 μL of 0.5 M Tris-HCl (pH = 8.0) and 14.25 μL of DI water was incubated overnight at 37 °C. The crude digestion mixture was analyzed by HPLC at 27 °C using a Betasil C_{18} reversed-phase column (25 cm x 4.6 mm I.D.). The analysis gradient is : 1 % MeCN for 6 min, 1 % to 7 % MeCN over19 min, 7 % to 30 % MeCN over 2 min, 30 % to 60 % MeCN over 20 min, 60 % to 100 % MeCN over 3 min in 100 mM TEAA. HPLC profile of the digestion mixture is shown in Fig. **S4**.

Synthesis of Compounds I and II: To a mixture of 6.0 g (9.13 mmol) of 2'-deoxy-5-iodio-5'-*O*-DMT-doxyuridine that was prepared in literature procedure[11], 2.3 g (10.95 mmol) of ferrocenyl acetylene[10], 0.4 g (0.69 mmol) of $Pd(dba)_2$, 0.74 g (2.82 mmol) of PPh_3, and 0.35 g (1.83 mmol) of CuI were added 120 mL of dry DMF and 60 mL of DIPA under argon. The

mixture was stirred at 55 °C for 6h. After removing DMF under reduced pressure, the residue was dissolved in 150 ml of CH_2Cl_2 and the resulting solution was washed with 5 % aqueous $NaHCO_3$ solution (2 x 100 mL) and then dried over Na_2SO_4. Removal of the solvent gave a crude product for purification. The crude product was purified on a 150 g silica gel column, packing with 1% TEA/hexane and eluting with 1% TEA/10-80% ethyl acetate/hexane. The fractions were identified by RP-TLC (CH_3CN : H_2O = 85 : 15). HPLC profiles of compounds **I** and **II** are shown in Fig. **S5**. The fractions containing **I** were pooled and concentrated to give 4.2 g (62.3 %) of the product. Later fractions containing **II** were pooled and concentrated to give 1.8 g (26.5%) of the product. Compound **I**: ^{1}H NMR (300 MH, DMSO-D_6): 2.20-2.38 (m, 2H), 3.70 (s, 6H), 3.92 (s, 1H), 4.15 (s, 5H), 4.31-4.24 (m, 5H), 5.34 (d, J = 2.3 Hz, exchangeable with D_2O), 5.82 (s, 1H), 6.14 (t, J = 6.9 Hz, 1H), 6.85-7.44 (m, 13H), 7.92 (s, 1H), 11.72 (s, 1H, exchangeable with D_2O); ^{13}C NMR (75 MHz, $CDCl_3$): 162.48, 159.08, 150.25, 145.04, 141.70, 136.15, 130.47, 128.62, 127.53, 113.97, 101.89, 93.81, 87.32, 86.53, 72.94, 72.0870.59, 69.30, 65.05, 64.27, 61.03, 55.77, 42.00, 21.61, 14.77. Anal. Calcd for $(C_{42}H_{42}FeN_2O_7 + H)^+$: 739.20, Found: 739. Compound **II**: ^{1}H NMR (300 MHz, DMSO-D_6) δ 2.21-2.30 (m, 1H) 2.42-2.49 (m, 1H), 3.74 (s, 6H), 3.99-4.06 (m, 2H), 4.15 (s, 5H), 4.39-4.42 (m, 2H), 4.47 (b.s, 2H), 4.73-4.74 (m, 2H), 5.44 (d, J = 4.5Hz, 1 H, exchangeable with D_2O), 5.82 (s, 1H), 6.17 (t, J = 5.4 Hz, 1H), 6.90-7.40 (m, 13 H), 8.59 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.06, 159. 25, 157.65, 155.49, 145.01, 136.11, 135.94, 135.61, 130.74, 128.85, 128.62, 127.61, 113.94, 109.14, 96.10, 88.65, 87.59, 86.98, 73.29, 70.52, 70.43, 70.15, 66.93, 66.81, 62.79, 55.80, 42.88, 14.75. Anal. Calcd for $(C_{42}H_{42}FeN_2O_7 + H)^+$: 739.20. Found: 739.

Synthesis of Compound III: To a solution of 1.85 g (2.51 mmol) of **I** in 60 mL of CH_2Cl_2, were added 0.2 g (1.15 mmol) of the catalyst, diisopropylammonium tetrazolide, and 1.06 mL (3.25 mmol) of 2-cyanoethyl bis(N,N-diisopropylamino) phosphoramidite under argon. The mixture was stirred overnight. The reaction mixture was diluted by adding 150 mL of CH_2Cl_2 and washed with 80 mL of 5 % $NaHCO_3$ aqueous solution, and dried over Na_2SO_4. After solvent was removed, the resulting residue was purified on a 65 g silica gel column, packing with 2% TEA/hexane, and eluting with 1 % TEA/10-70 % hexane/CH_2Cl_2. The desired fractions were pooled and concentrated to afford 2.14 g (91.0 %) of the red foam product **III**, which was further precipitated from hexane. ^{31}P NMR (164 MHz, $CDCl_3$) δ 149.54, 149.96. HRMS calcd for $(C_{51}H_{55}FeN_4O_8P + Na)^+$: 961.3005. Found: 961.3011.

Synthesis of Compound IV: To a solution of 1.12 g (1.52 mmol) of **I** in 40 mL of CH_2Cl_2, were added 0.13 g (0.75 mmol) of the catalyst, diisopropylammonium tetrazolide, and 0.63 mL (1.92 mmol) of 2-cyanoethyl bis(N,N-diisopropylamino) phosphoramidite under argon. The mixture was stirred overnight. The reaction mixture was diluted by adding 200 mL of CH_2Cl_2 and washed with 100 mL of 5 % $NaHCO_3$ aqueous solution, and dried over Na_2SO_4. After solvent was removed, the resulting residue was purified on a 55 g silica gel column, packing with 2% TEA/hexane, and eluting with 1 % TEA/40-80 % hexane/CH_2Cl_2. The desired fractions were pooled and concentrated to afford 1.36 g (95.4 %) of the red foam product **IV**, which was further precipitated from hexane. ^{31}P NMR (164 MHz, $CDCl_3$) δ 149.96, 150.66. HRMS calcd for $(C_{51}H_{55}FeN_4O_8P + Cs)^+$: 1071.2161. Found: 1071.2119.

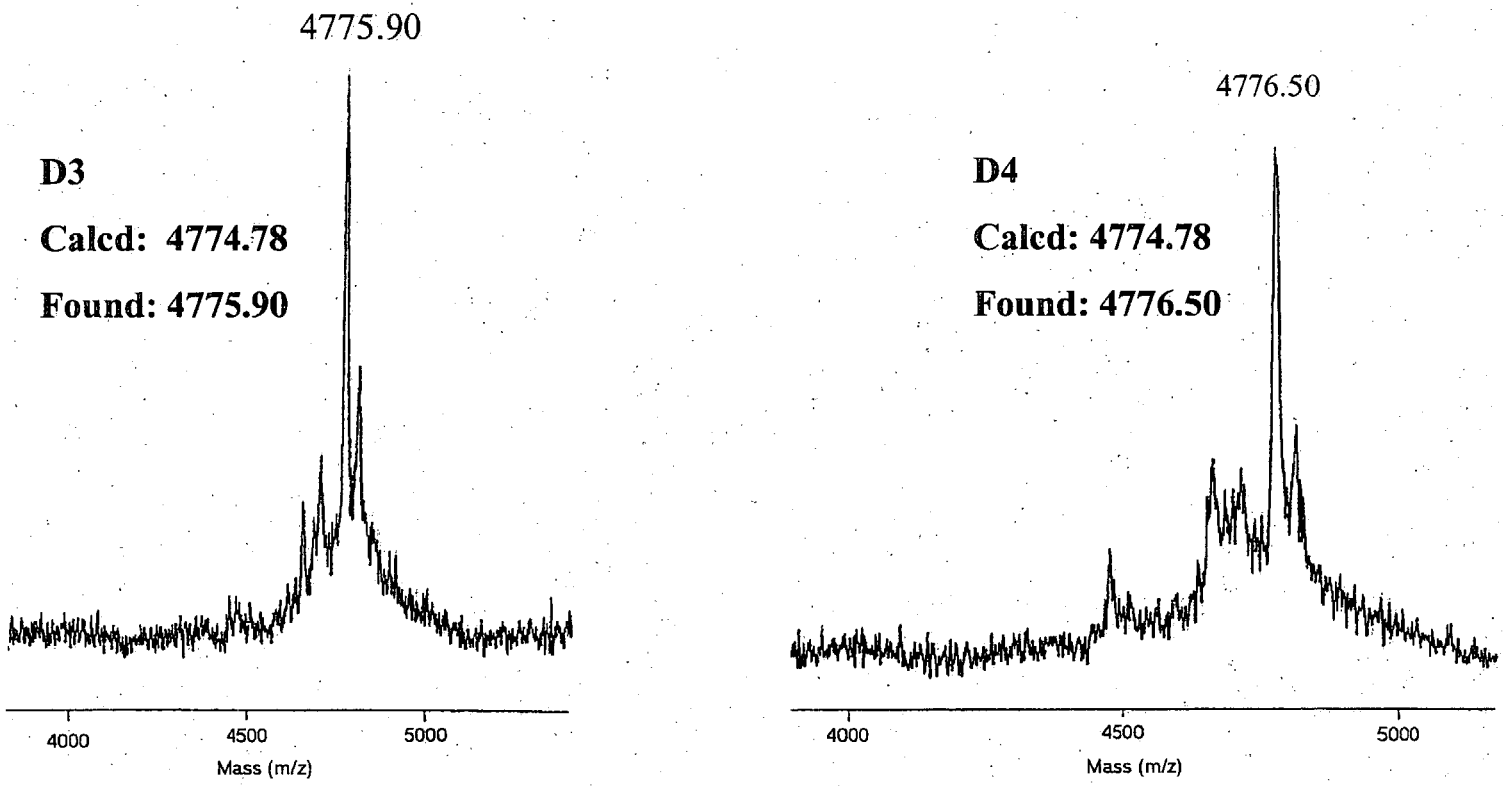

Fig. S1, MALDI-TOF MS Spectra of D3 and D4

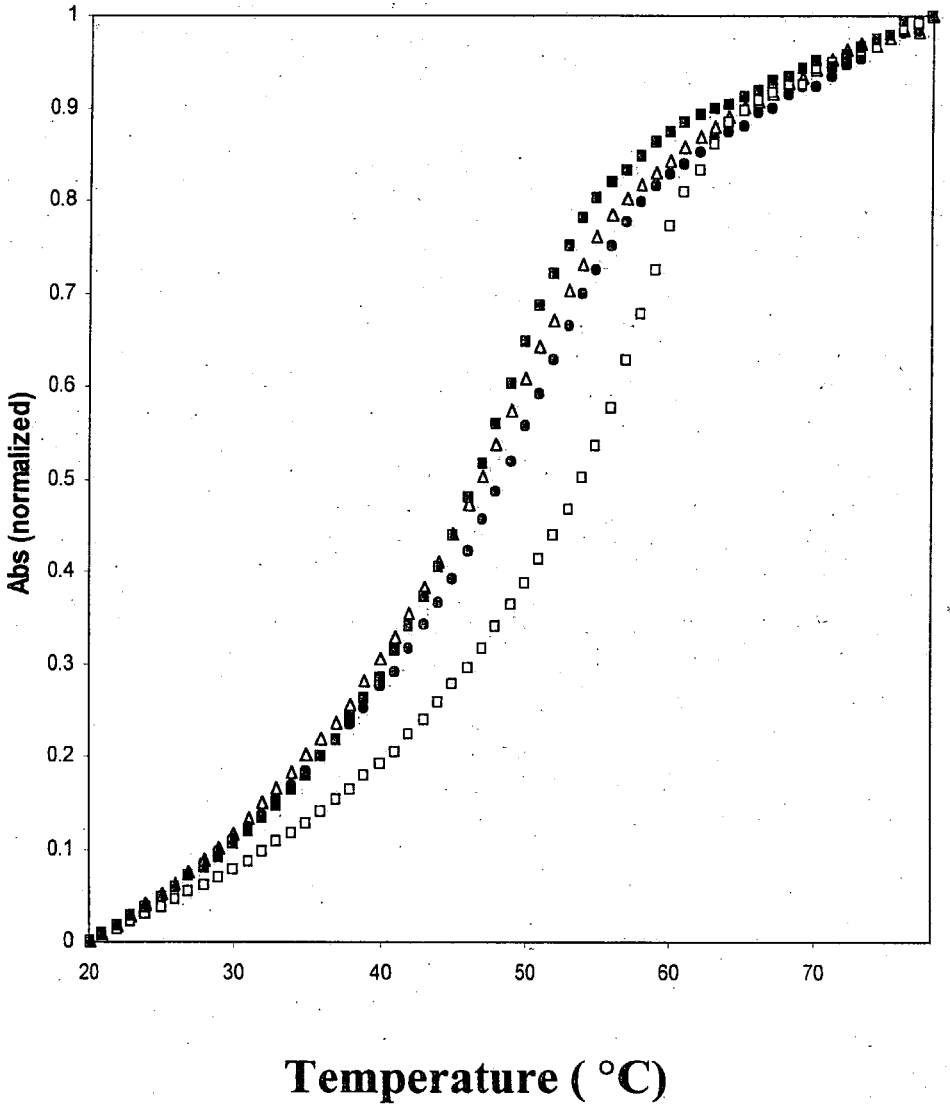

Fig. S2. Thermal Denaturation Curves for **D1:D2** duplex(□, perfect match), **D1:D3** duplex(●, **III** at 12th position), **D1:D4** duplex(Δ, **IV** at 12th position), **D1:D5** duplex(■, **AA** mismatch at 12th postion).

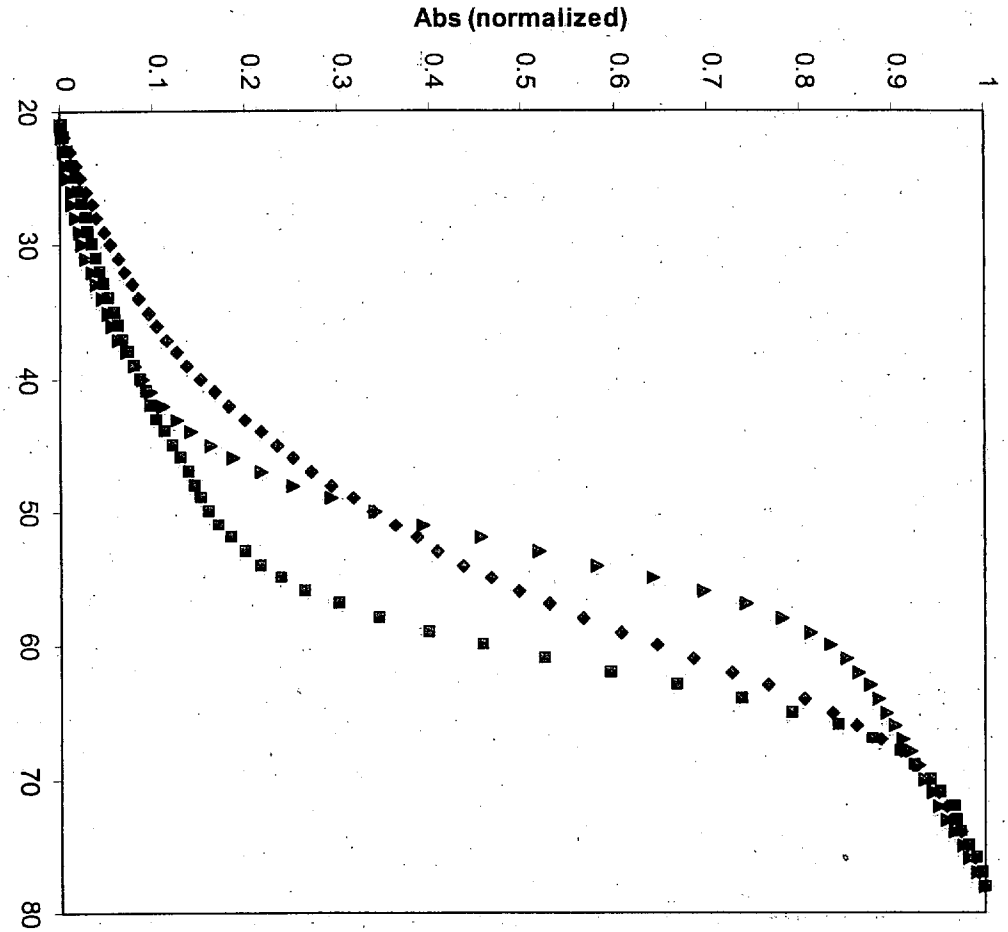

Fig. S3. Thermal Denaturation Curves for **D6**:**D7** (■, Perfect GC match at 12th position), **D3**:**D6** (◆, **III**:dG at 12th position), **D2**:**D6** (▲, GT mismatch at 12th position)

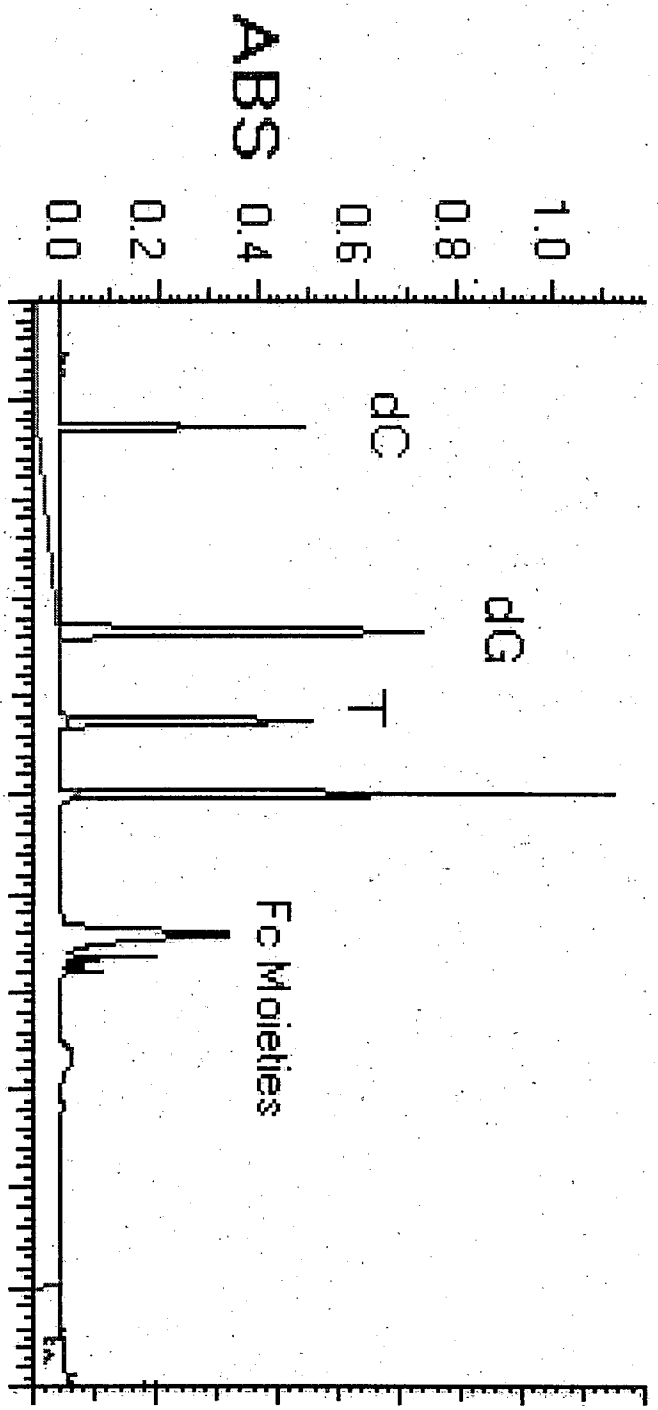

Fig. S4, HPLC Profile of the Crude Digestion Mixture

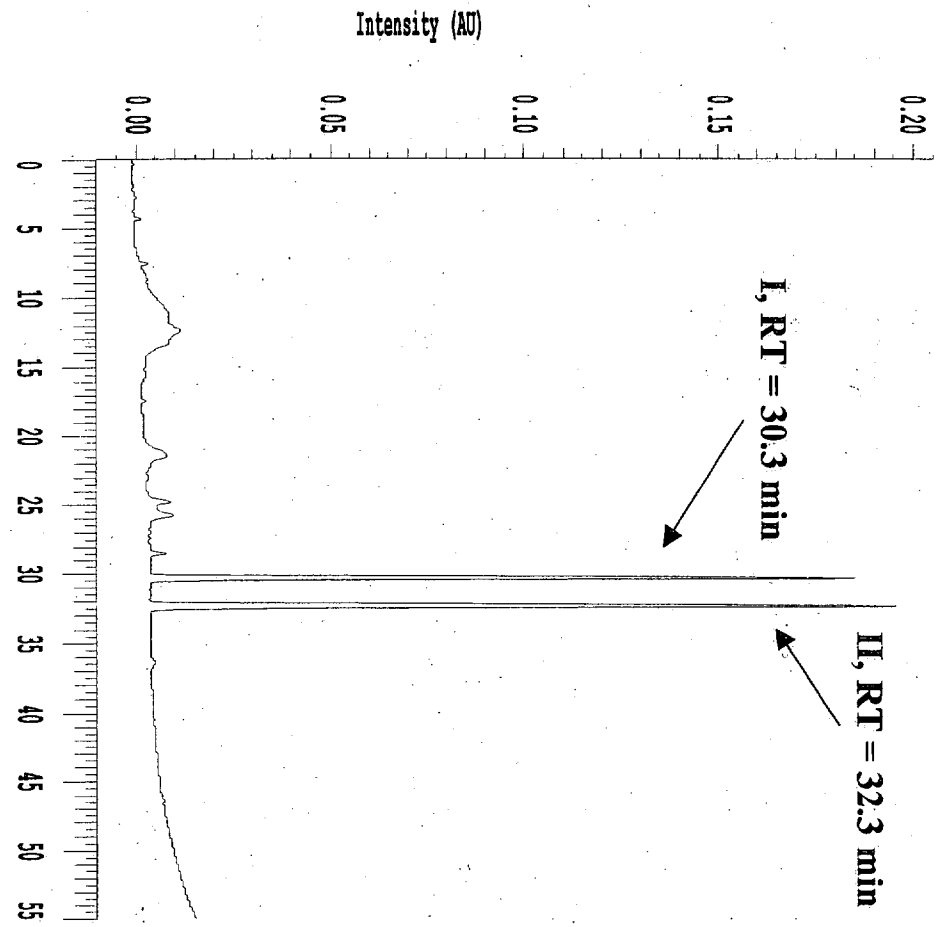

Fig. S5, HPLC Profiles of Compounds I and II

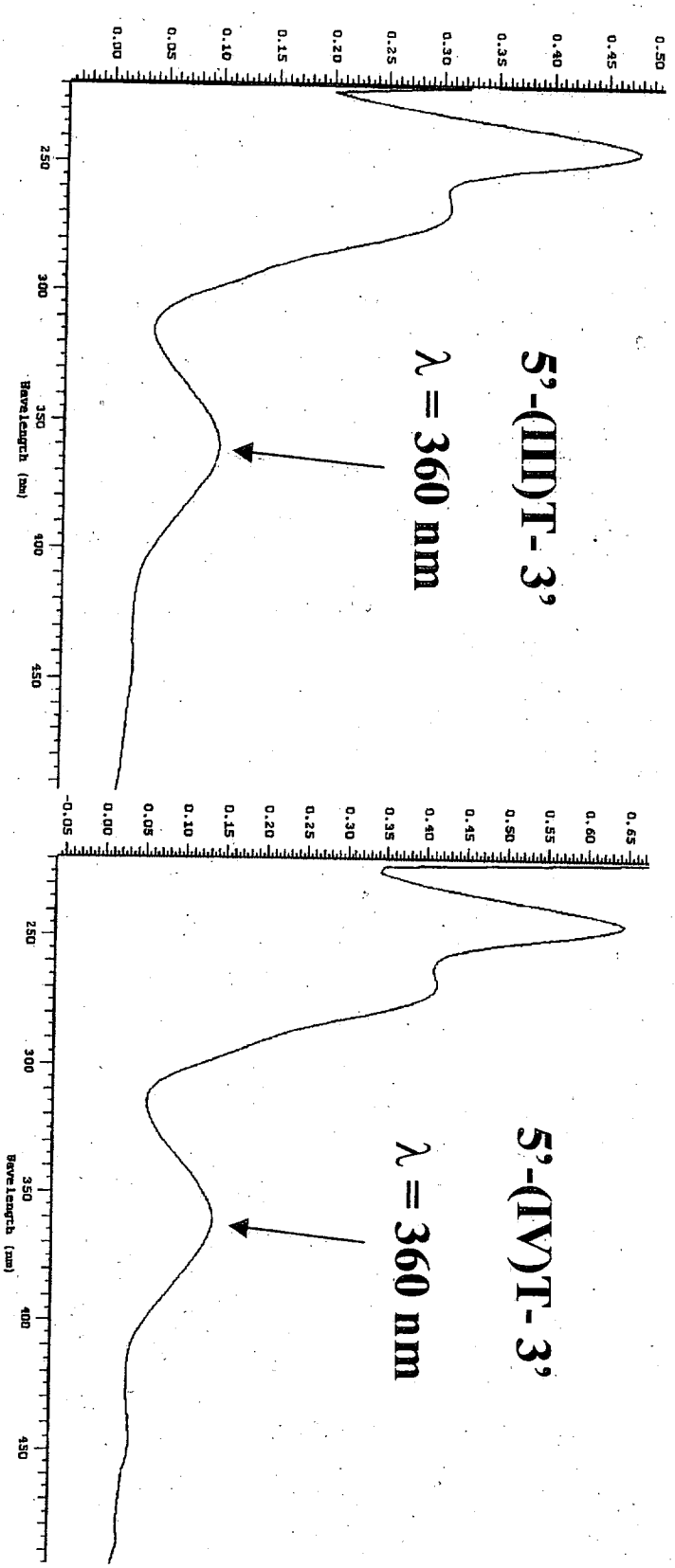

Figure S6. UV-vis spectra of two dimers bearing III and IV