Synthesis and properties of peptide nucleic acids containing psoralen unit

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Supporting Information

Experimental Section

General Techniques. ^{1}H NMR spectra were measured with Varian Mercury (400 MHz) spectrometers and JNM a-400 (400 MHz) spectrometers. Coupling constants (*J* values) are reported in Hz. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform (δ = 7.24 in ^{1}H NMR) and residual dimethylsulfoxide (δ = 2.49 in ^{1}H NMR) as internal standards. The following abbreviations were used to explain the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Melting points were obtained on a Yanaco MP-500D micro melting point apparatus and are uncorrected. Electron impact mass spectra, fast atom bombardment mass spectra and high-resolution mass spectra were recorded on JEOL JMS-DX 300 or JEOL JMS-SX 102A. All reactions were monitored by thin layer chromatography carried out on 0.25-mm E. Merck silica gel plates (60F-254) using UV light, 5% ethanolic phosphomolybdic acid, or *p*-anisaldehyde solution and heat as developing agent. Wako gel (C-200, particle size 75–150 μm, Wako) was used for column chromatography. All reagents were purchased at highest commercial quality and used without further purification unless otherwise stated.

9-Hydroxyfurano[3,2-*g*]2*H*-chromen-2-one (8-Hydroxypsoralen, 2). To a solution of boron tribromide (2.78 mL, 1 M in dichloromethane, 2.78 mmol) in dichloromethane was added 8-methoxypsoralen (**1**) at 0 ˚C, and the mixture was stirred for 2 h. After diluted with 2M *aq*. NaOH the reaction mixture was warmed to ambient temperature and extracted with chloroform. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 50 % ethyl acetate/hexane) to give **2** (133 mg, 71 %) as colorless solid: mp 244–246 ˚C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 9.7 Hz, 1H), 7.70 (d, *J* = 2.2 Hz, 1H), 7.25 (s, 1H), 6.80 (d, *J* = 2.2 Hz, 1H), 6.35 (d, *J* = 9.7 Hz, 1H); MS (EI) *m/e* (%) 202 (M^+, 100), 174 (57); HRMS (EI) calcd for $C_{11}H_6O_4$ (M^+), 202.0266; found, 202.0262.

Ethyl 2-(2-Oxofurano[3,2-*g*]2*H*-chromen-9-yloxy)acetate (3). To a solution of **2** (280 mg, 1.39 mmol) in DMF (6 mL) was added ethyl bromoacetate (694 mg, 4.16 mmol) and potassium carbonate (574 mg, 4.16 mmol) at 0 ˚C, and the mixture was stirred for 4 h at

ambient temperature. After diluted with water, the reaction mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by washing with ethylether to give **3** (323 mg, 81 %) as pale yellow solid: mp 107–110 ˚C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 9.7 Hz, 1H), 7.66 (d, J = 2.2 Hz, 1H), 7.36 (s, 1H), 6.80 (d, J = 2.2 Hz, 1H), 6.35 (d, J = 9.7 Hz, 1H), 5.12 (s, 2H), 4.23 (q, J = 7.1 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H); MS (EI) *m/e* (%) 288 (M^+, 100), 215 (36), 201 (43); HRMS (EI) calcd for $C_{15}H_{12}O_6$ (M^+), 288.0633; found, 288.0645.

2-(2-Oxofurano[3,2-*g*]2*H*-chromen-9-yloxy)acetic Acid (4). To a solution of **3** (320 mg, 1.11 mmol) in EtOH–H_2O (2:1, 9 mL) was added LiOH•H_2O (155 mg, 3.69 mmol) and the mixture was stirred for 10 min at 0 ˚C. After acidification with 2M *aq*. HCl, the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and filtered. Removal of the solvent under reduced pressure gave **4** (285 mg, 99 %) as ivory solid: mp 207–210 ˚C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (d, J = 9.6 Hz, 1H), 8.09 (d, J = 2.4 Hz, 1H), 7.63 (s, 1H), 7.07 (d, J = 2.4 Hz, 2H), 6.43 (d, J = 9.6 Hz, 1H), 5.11 (s, 2H); MS (EI) *m/e* (%) 260 (M^+, 100), 215 (20), 201 (51); HRMS (EI) calcd for $C_{13}H_8O_6$ (M^+), 260.0320; found, 260.0309.

Ester 6. To a solution of **4** (280 mg, 1.08 mmol) in DMF (8 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide monohydrochloride (619 mg, 3.23 mmol) and 1-hydroxybenzotriazole (436 mg, 3.23 mmol) at 0 ˚C, and the mixture was stirred for 1 h. To the reaction mixture added ethyl *N*-[2-(*tert*-butoxycarbonylamino)ethyl]aminoacetate (**5**) (399 mg, 1.62 mmol), and the mixture was stirred for 3 h at ambient temperature. After diluted with water, the reaction mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 9 % methanol/chloroform) to give **6** (461 mg, 87 %) as colorless solid: mp 162–166 ˚C; ^{1}H NMR (400 MHz, $CDCl_3$): due to restricted rotation around secondary amide bond, several sets of the signals were doubled; δ 7.76 and 7.74 (d_2, J = 9.5 Hz, 1H), 7.71–7.66 (m_2, 1H), 7.38 and 7.36 (s_2, 1H), 6.79 (d_2, J = 2.2 Hz, 1H), 6.35 and 6.34 (d_2, J = 9.7 Hz, 1H), 6.05–5.91 (m_2, 1H), 5.08 and 5.22 (s_2, 2H), 4.23 and 4.16 (q_2, J = 7.1 Hz, 2H), 4.41 and 4.07 (s_2, 2H), 3.74–3.67 and 3.57–3.51 (m_2, 2H), 3.46–3.39 and 3.30–3.27 (m_2, 2H), 1.40 and 1.38 (s_2, 9H), 1.27 and 1.24 (t_2, J = 7.1 Hz, 3H); MS (FAB) *m/e* 489 [(M+H)$^+$]; HRMS (FAB) calcd for $C_{24}H_{29}N_2O_9$ [(M+H)$^+$], 489.1871; found, 489.1883.

Acid 7. To a solution of **6** (76 mg, 156 μmol) in EtOH–H_2O (2:1, 2.25 mL) was added LiOH•H_2O (30 mg, 842 μmol) and the mixture was stirred for 4 h at ambient temperature. The reaction mixture was poured into water and organic impurities were removed by extraction with ethyl acetate. After acidification of the alkaline aqueous layer with 2M *aq*. HCl, the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and filtered. Removal of the

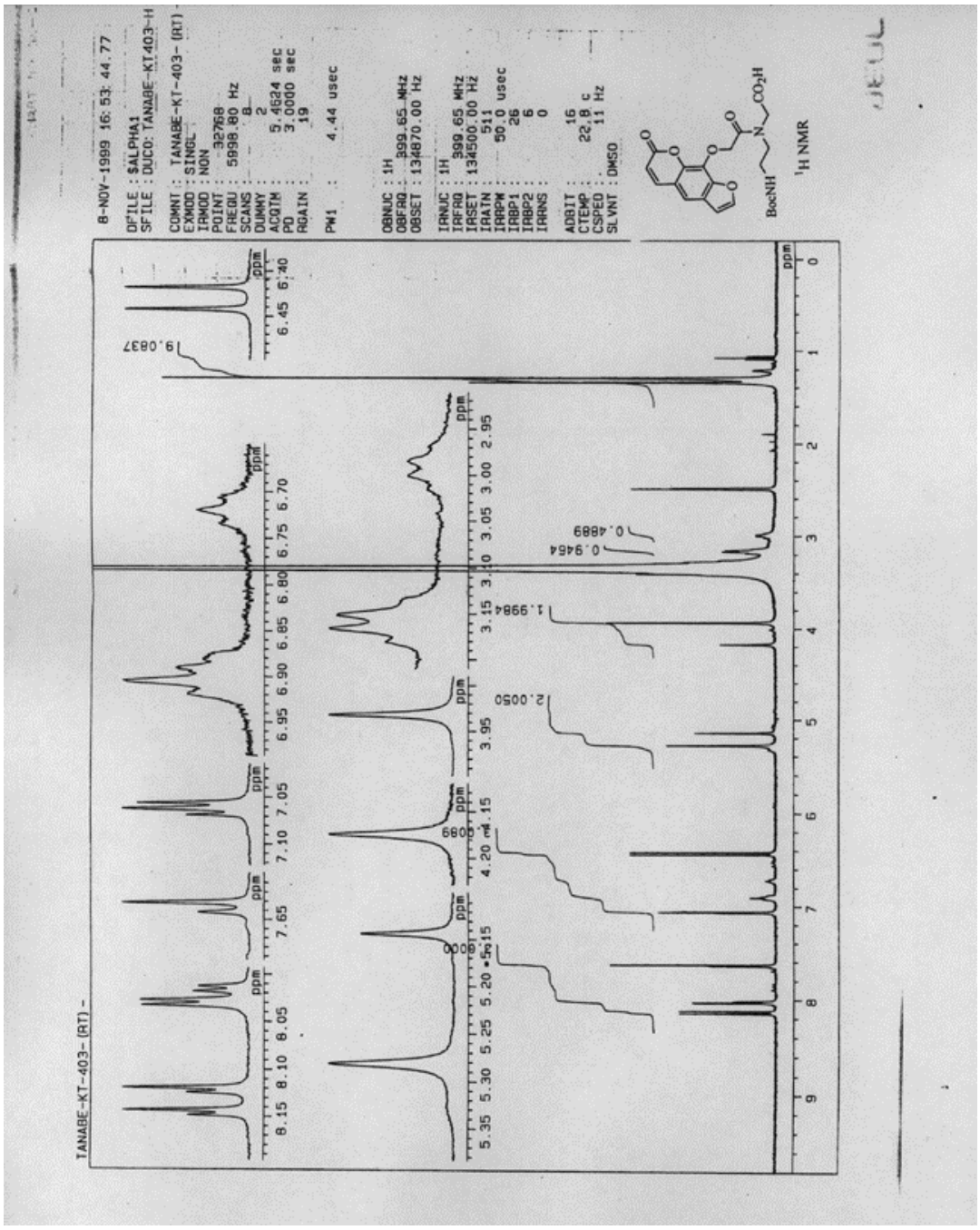
solvent under reduced pressure gave **7** (62 mg, 86 %) as ivory solid: mp 104–105 ˚C; ^{1}H NMR (400 MHz; DMSO-d_6): due to restricted rotation around secondary amide bond, several sets of the signals were doubled; δ 8.14 and 8.13 (d_2, J = 9.7 Hz, 1H), 8.03 and 8.02 (d_2, J = 2.2 Hz, 1H), 7.64 and 7.63 (s_2, 1H), 7.06 (d_2, J = 2.4 Hz, 1H), 6.94–6.86 and 6.76–6.68 (m_2, 1H), 6.43 (d, J = 9.7 Hz, 1H), 5.28 and 5.14 (s_2, 2H), 4.17 and 3.93 (s_2, 2H), 3.47–3.21 (2H), 3.20–3.11 and 3.03–2.94 (m_2, 2H), 1.34 and 1.29 (s_2, 9H); MS (FAB) *m/e* 461 [(M+H)$^+$], 361 [(M–Bu+H)$^+$]; HRMS (FAB) calcd for $C_{22}H_{25}N_2O_9$ [(M+H)$^+$], 461.1558; found, 461.1559; UV (methanol) λ_{max} (nm) 299 (ε = 7.33), 248 (ε = 14.56), 216 (ε = 16.32).

Synthesis of PNA oligomer. PNA oligomers were synthesized by solid phase ^{t}Boc chemistry on a MBHA resin as described by Koch *et al*. (Koch, T.; Hansen, H. F.; Andersen, P.; Larsen, T.; Batz, H. G.; Otteson, K.; Ørum, H. *J. Peptide Res*. **1997**, *49*, 80–88.). After the completion of PNA oligomer synthesis, the resin was treated with a solution of trifluoroacetic acid, trifluoromethanesulfonic acid, thioanisole, and *p*-cresol (6:2:1:1 v/v/v/v) for the cleavage of PNA oligomer from the resin and for the deprotection. The solution was filtered and precipitated in ethyl ether, centrifuged, and decanted. The residue was redissolved in a slight of trifluoroacetic acid, reprecipitated in ethyl ether, centrifuged, and then decanted to give the crude product. The crude oligomer was purified by reversed phase HPLC on a Wakosil II 5-C18-AR (20_150 mm) using a linear gradient of 0–20% acetonitrile in aqueous 0.05% trifluoroacetic acid.

Melting Temperature (T_m) Measurement. All T_ms of the PNA–DNA duplexes (2.5 μM, duplex concentration) were taken in a buffer containing 10 mM sodium cacodylate, pH 7.0. Absorbance *vs* temperature profiles were measured at 260 nm using a JASCO TPU-550 UV/VIS spectrometer connected with a JASCO TPU-436 temperature controller. The absorbance of the samples was monitored at 260 nm from 2 °C to 80 °C with a heating rate of 1 °C/min. From these profiles, first derivatives were calculated to determine T_m values.

Fluorescence Measurement. All fluorescence spectra of single-stranded PNAs (20 μM, strand concentration) and PNA–DNA duplexes (20 μM, duplex concentration) were taken in a buffer containing 10 mM sodium cacodylate, pH 7.0. Fluorescence spectra were obtained at 330 nm excitation using a SHIMADZU RF-5300PC spectrofluorophotometer.

^{1}H NMR of **7**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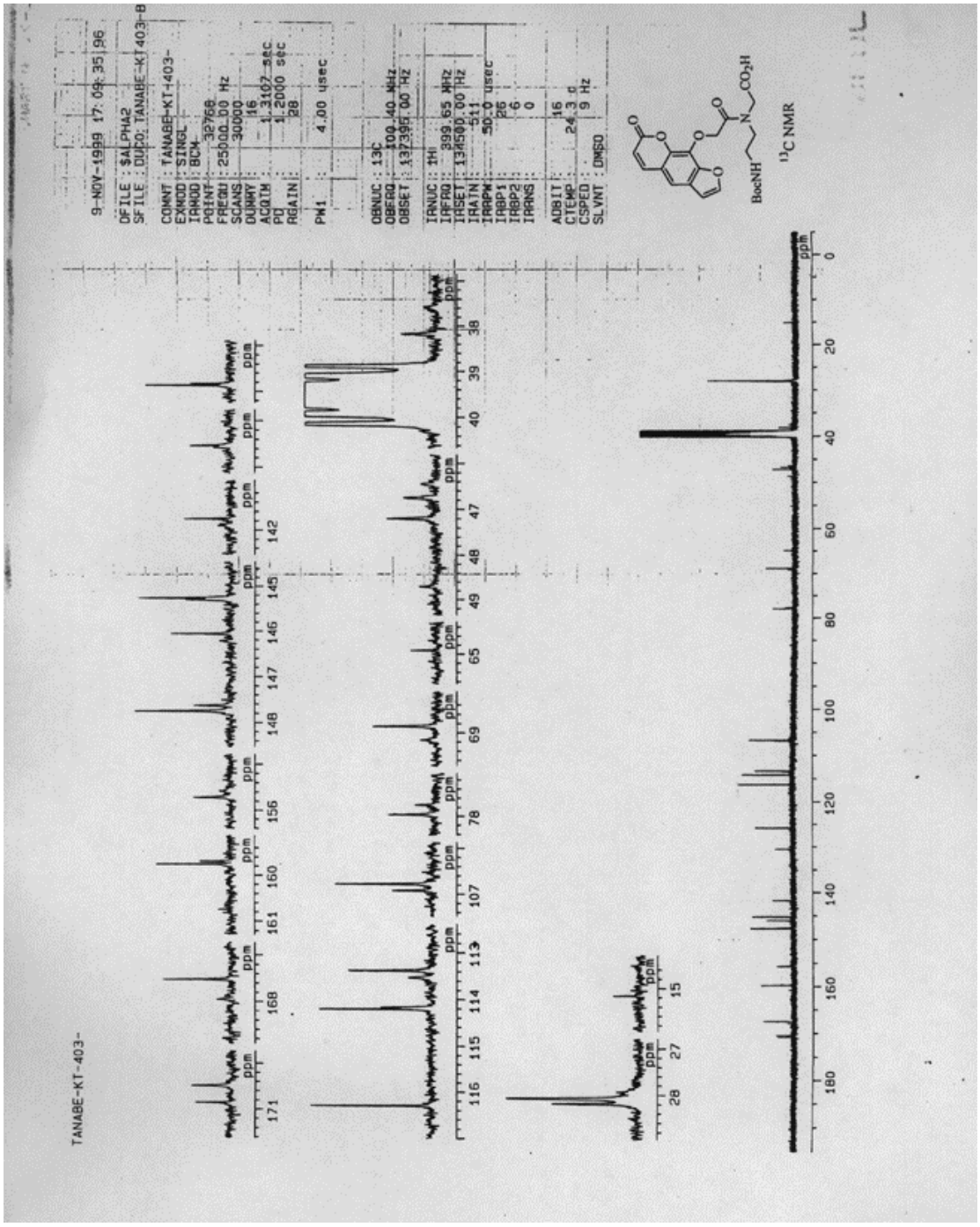
^{13}C NMR of **7**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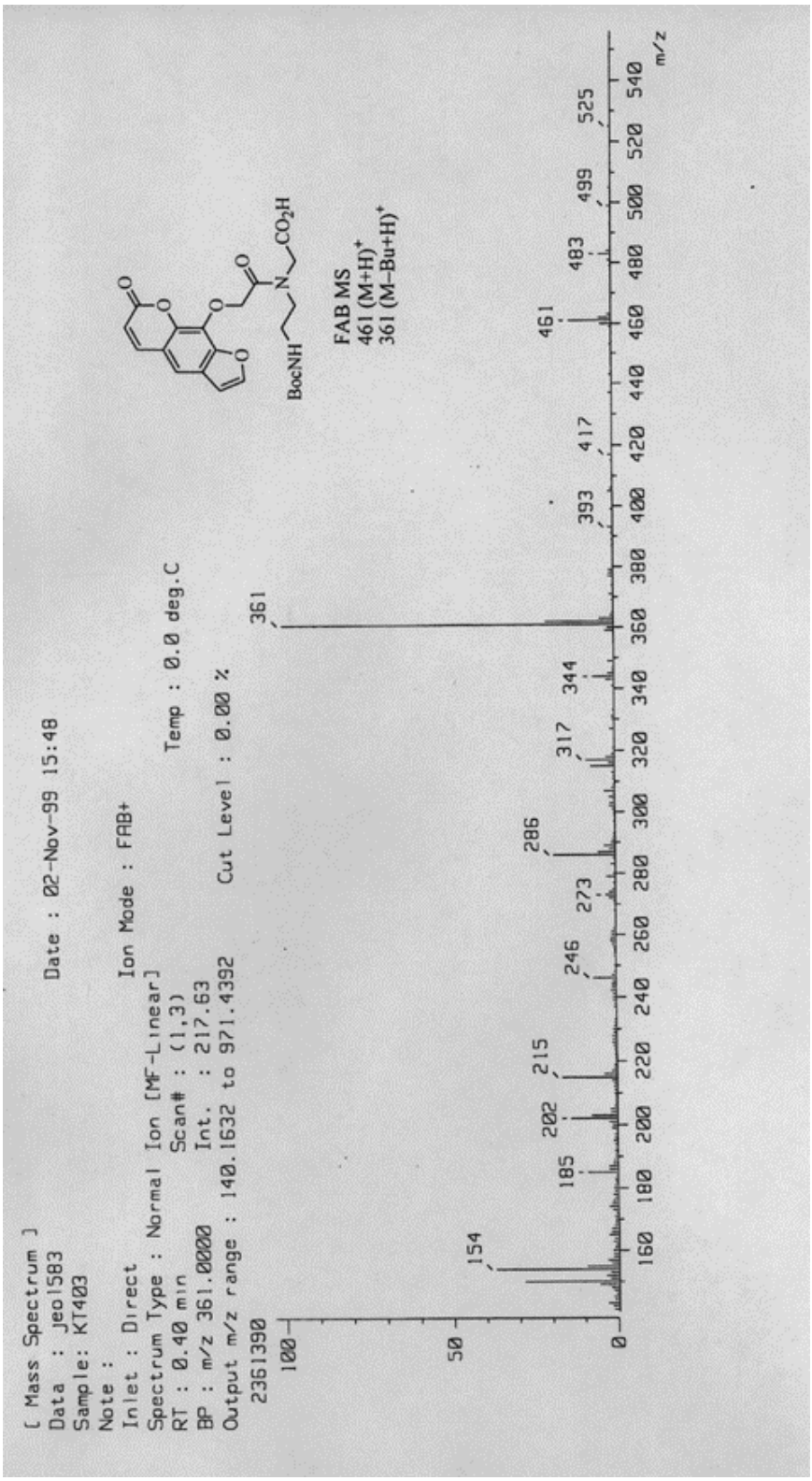
FABMS of **7**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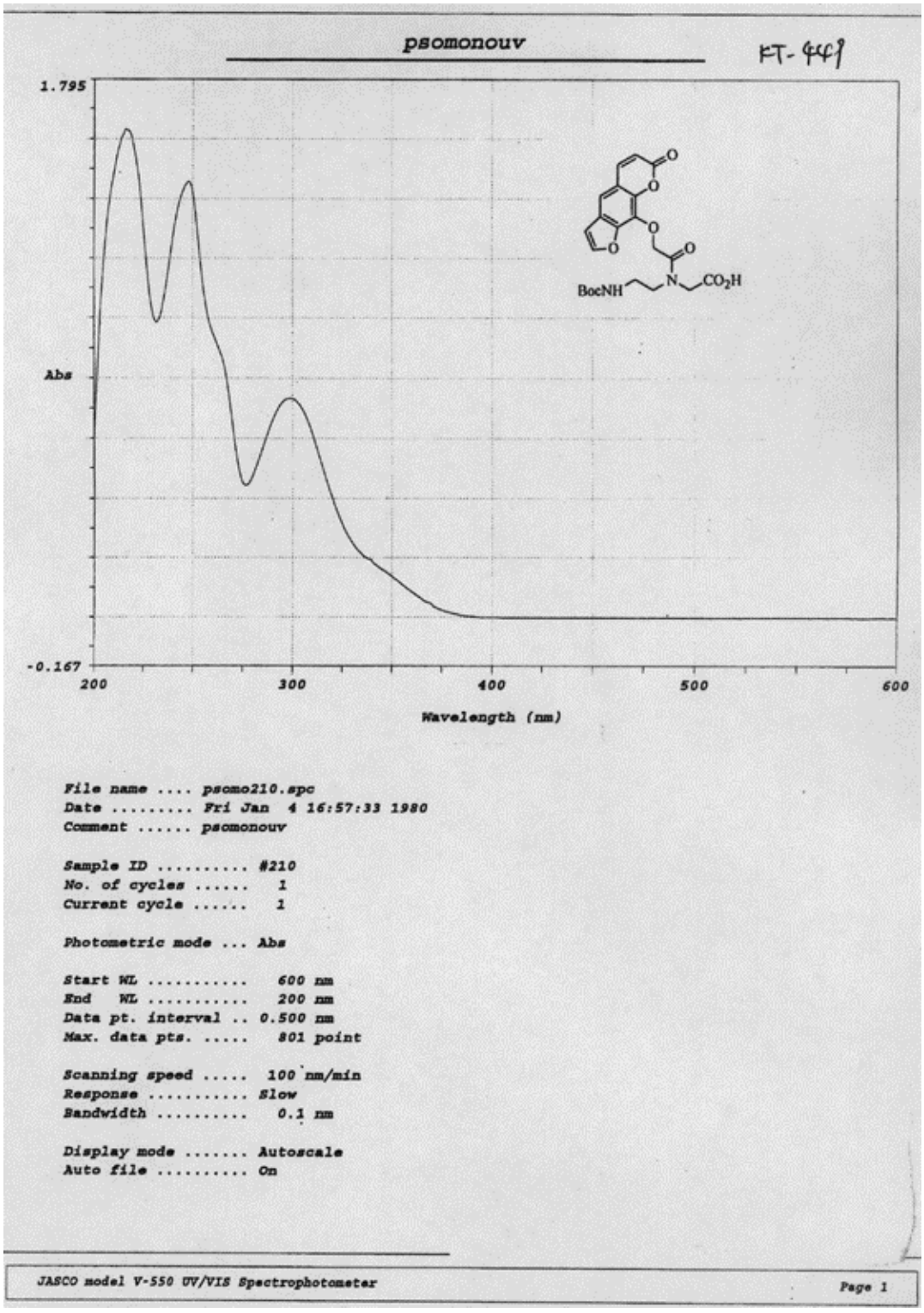
UV spec of **7**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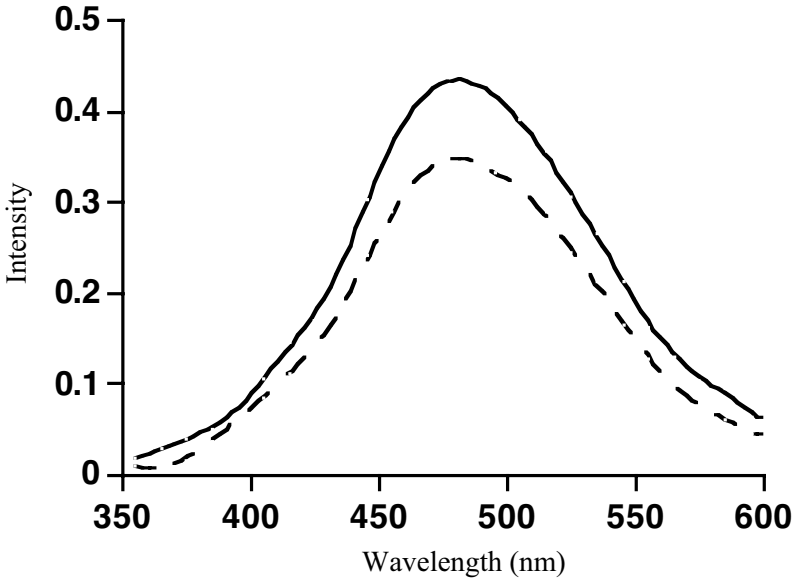

Figure S1. Fluorescence spectral changes caused by **P-PNA 4**–DNA hyblid formation. A solution of 20 μM **P-PNA 4** or **P-PNA 4**–DNA full-matched duplex in 10 mM sodium cacodylate (pH 7.0) was used. The fluorescence spectra were measured at 330 nm excitation at 19 °C. Solid line, single stranded **P-PNA 4**; dashed line, **P-PNA 4**–5'-d(CGCGGAACC)-3' (matched duplex).