 

Selected Paper

# Formation of Pyrene Dimer Radical Cation at the Minor Groove of DNA

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To arrange pyrene at the minor groove of DNA, we synthesized a nucleoside derivative possessing a pyrene group on guanine N2. Doubly pyrene-modified duplex DNA was synthesized and the formation rates of the pyrene dimer radical cation were measured upon one-electron oxidation during pulse radiolysis. For all of the DNA studied here, the formation of a pyrene dimer radical cation was accomplished quickly, within 20 micro seconds after a 8-ns electron pulse during pulse radiolysis. The results show that the minor groove of DNA offers a good space for the formation of pyrene dimer radical cations.

DNA is a dynamic structure with several transient conformations appearing in a short period of time, and the transiently existing conformations are thought to play a variety of important biological roles.[1–5] However, the low population and short lifetime of the transient conformations make their characterization very difficult. One way to identify less frequent motions of biomolecules is to chemically trap such transient conformations. We recently reported the use of a pyrene (Py) dimer radical cation ($Py_2^{\bullet+}$), which is stabilized by the charge resonance (CR) between the two aromatics.[6,7] Two Pys were introduced at the end or at the internal site of a duplex DNA, and then the formation rates of $Py_2^{\bullet+}$ were measured upon the one-electron oxidation by sulfate radical anions ($SO_4^{\bullet-}$) generated from dissociative electron attachment of $S_2O_8^{2-}$ during the pulse radiolysis of an aqueous solution of Py-modified DNA in the presence of $K_2S_2O_8$. The formation rate of $Py_2^{\bullet+}$ reflects the frequency of the occurrence of DNA conformations in which $Py^{\bullet+}$ and Py can associate, and was discussed with relation to the DNA dynamics that allow the interaction between $Py^{\bullet+}$ and Py. As for the structure of the transiently trapped DNA, it was suggested that DNA unwinding and bending allowed the formation of $Py_2^{\bullet+}$ at the minor groove of DNA. The minor groove of DNA has been demonstrated to serve as a good candidate for arranging heterocyclic compounds, and allows $\pi$–$\pi$ dimer formation.[8–11] Herein, to test the plausibility of the formation of $Py_2^{\bullet+}$ at the minor groove of DNA, we synthesized a nucleoside derivative possessing a Py group on guanine N2 to arrange Py at the minor groove of DNA. Doubly Py-modified oligodeoxynucleotides (ODNs) were synthesized and the formation rates of $Py_2^{\bullet+}$ were measured upon one-electron oxidation during pulse radiolysis.

## Experimental

**Synthesis of 2.** Pyrenepropylamine (180 mg, 0.72 mmol)[12] and **1** (390 mg, 0.60 mmol, synthesized as previously reported[13,14]) were dissolved in pyridine and the solution was stirred at room temperature for 3 days. After evaporation of the solvent, the residue was diluted with $CH_2Cl_2$ and the organic phase was washed with water and then with brine. The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated to dryness. The residue was subjected to silica gel flash chromatography ($CH_2Cl_2$/MeOH = 50/1 (v/v)) to yield **2** (84 mg, 17% yield). $^1$H NMR (270 MHz) ($CDCl_3$) $\delta$ 0.00–0.02 (m, 12H), 0.78–0.88 (m, 18H), 2.11–2.22 (m, 3H), 2.24–2.50 (m, 1H), 2.82–2.99 (m, 2H), 3.36–3.52 (m, 4H), 3.67–3.74 (m, 2H), 3.86–3.89 (m, 1H), 4.28–4.40 (m, 2H), 4.46–4.50 (m, 1H), 6.18–6.23 (dd, 1H, $J$ = 7.0, 6.2 Hz), 7.14–7.19 (m, 2H), 7.76–8.20 (m, 12H). FAB MS (positive ion): $m/z$ 887.9 (M + H).

**Synthesis of 3.** To a solution of **2** (80 mg, 0.09 mmol) in THF (5 mL) was added a THF solution (5 mL) of triethylamine·3HF (0.15 mL, 0.45 mmol), and the solution was stirred at room temperature for 24 h. After evaporation of the solvent, the residue was diluted with AcOEt and the organic phase was washed with saturated $NaHCO_3$ solution and then with brine. The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated to dryness. The residue was subjected to silica gel flash chromatography ($CH_2Cl_2$/MeOH = 50/2 (v/v)) to yield **3** (42.8 mg, 72% yield). $^1$H NMR (270 MHz) ($CDCl_3$) $\delta$ 2.14–2.23 (m, 3H), 2.66–2.80 (m, 2H), 2.96–3.07 (m, 1H), 3.42–3.54 (m, 4H), 3.75–3.98 (m, 2H), 4.13–4.21 (m, 3H), 4.66–4.72 (m, 1H), 6.14–6.20 (dd, 1H, $J$ = 9.6, 5.5 Hz), 7.01–7.06 (m, 2H), 7.54 (s, 1H), 7.85–8.27 (m, 11H). FAB MS (positive ion): $m/z$ 659.6 (M + H).

**Synthesis of 4.** **3** (40 mg, 0.061 mmol) was dried by coevaporation with dry pyridine three times. The residue was dissolved in 4 mL of dry pyridine, and then 4,4′-dimethoxytrityl chloride (DMTrCl) (31 mg, 0.091 mmol) was added. The solution was stirred at room temperature for 48 h. The reaction was quenched with MeOH (1 mL) and evaporated to dryness. The residue was diluted with $CH_2Cl_2$ and the organic phase was washed with saturated $NaHCO_3$ solution (two times). The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated to dryness. The residue was subjected to silica gel flash chromatography ($CH_2Cl_2$/MeOH = 50/1 (v/v)) to yield **4** (18 mg, 31% yield). $^1$H NMR (270 MHz) ($CDCl_3$) $\delta$ 2.15–2.21 (m, 2H), 2.28–2.37 (m, 1H), 2.69–2.79 (m, 1H), 2.91–3.01 (m, 2H), 3.31–3.50 (m, 6H), 3.73 (s, 6H), 3.99–4.05 (m, 1H), 4.34–4.44 (m, 2H), 4.54–4.60 (m, 1H), 6.19–6.23 (dd, 1H, $J$ = 6.8, 6.5 Hz), 6.76–8.24 (m, 27H). FAB MS (positive ion): $m/z$ 961.8 (M + H).

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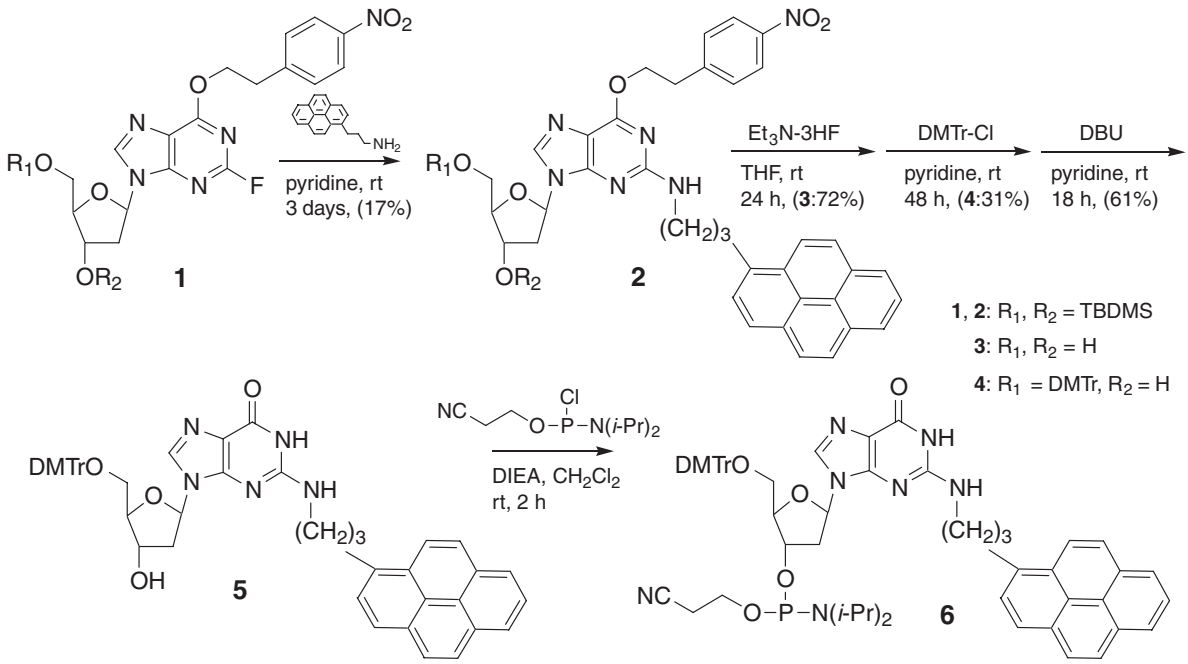



Scheme 1. Synthetic route of the $^{Py}$**G**-phosphoramidite **6**. TBDMS denotes *tert*-butyldimethylsilyl.

**Synthesis of 5.** **4** (204 mg, 0.21 mmol) was dissolved in 15 mL of dry pyridine, and then DBU (263 mg, 1.73 mmol) was added. The solution was stirred at room temperature for 18 h. After evaporation of the solvent, the residue was diluted with $CH_2Cl_2$ (50 mL), and the organic phase was washed with a 0.5 M citric acid solution (30 mL, three times), and then with a 5% $NaHCO_3$ solution (30 mL, two times). The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated to dryness. The residues were subjected to flash chromatography ($CH_2Cl_2$/MeOH/$Et_3N$ = 95/5/1 (v/v)) to yield **5** (105 mg, 61% yield). $^1$H NMR (270 MHz) ($CDCl_3$) $\delta$ 1.94–2.22 (m, 3H), 2.32–2.48 (m, 1H), 3.14–3.52 (m, 6H), 3.70 (s, 6H), 3.82–3.93 (m, 1H), 4.27–4.39 (m, 1H), 5.91–6.00 (m, 1H), 6.74–8.20 (m, 23H). ESI MS (positive ion): $m/z$ 812.2 (M + H).

**Synthesis of 6.** **5** (106 mg, 0.13 mmol) was dried by coevaporation with pyridine (three times) and dissolved in 5.1 mL of $CH_2Cl_2$. To this solution 38 µL (52 mg, 0.4 mmol) of diisopropylamine and 84 µL (99 mg, 0.42 mmol) of 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite were added. The mixture was stirred for 2 h at room temperature under Ar. The reaction was quenched with MeOH (1 mL) and filtered. The reaction mixture was diluted with $CH_2Cl_2$ (30 mL), the organic phase was washed with 5% $NaHCO_3$ solution (20 mL, three times), and then was dried over anhydrous $Na_2SO_4$ and evaporated to dryness to yield crude **6** (90 mg). Crude **6** was dried by coevaporation with $CH_3CN$ (three times), dissolved in $CH_3CN/CH_2Cl_2$ = 1/1 (2 mL) and used in an automated DNA synthesizer without further purification. ESI MS (positive ion): $m/z$ 1034 (M + Na).

**DNA Synthesis.** All of the reagents for DNA synthesis were purchased from Glen Research. The DNA used in this study was synthesized with an Expedite 8909 DNA synthesizer (Applied Biosystems) according to the standard synthetic protocol. After the automated synthesis, the DNA was detached and deprotected by treating it with 28% $NH_3$aq for 24 h at rt. Crude DNA was purified by reverse phase HPLC and lyophilized. All of the Py-modified DNA studied here was characterized by MALDI-TOF mass spectra, and by complete digestion with s. v. PDE, nuclease P1, and AP to 2′-deoxyribonucleosides.

**Melting Temperatures.** Thermal denaturation profiles were recorded on a JASCO V-530 spectrometer equipped with a Peltier temperature controller (ETC-505T). The absorbance of the sample was monitored at 260 nm from 10 to 70 °C with a heating rate of 1 °C min$^{-1}$. The $T_m$ value was determined as the maximum in a plot of $\Delta A_{260}/\Delta T$ versus temperature.

**Fluorescence Spectra.** The fluorescence spectra of Py-modified ODNs were measured in an Ar-saturated aqueous solution in the presence of a 20 mM Na phosphate buffer (pH 7.0) at a total strand concentration of 8 µM at 20 °C on a Hitachi 850 spectrofluorometer.

**Pulse Radiolysis.** $Py^{\bullet+}$ was formed from the reaction with $SO_4^{\bullet-}$, which was generated during the pulse radiolysis (28 MeV, 8 ns) of Ar-saturated $D_2O$ solution containing 10 mM $K_2S_2O_8$, 100 mM *t*-BuOH, 20 mM Na phosphate buffer (pH 7.0), and 0.1 mM (strand conc.) ODN.[6,7,15,16] A xenon flash lamp (Osram, XBO-450), which was synchronized with the electron pulse, was focused through the sample as a probe light for the transient absorption measurement. The monitor light for the measurement of time profiles of the transient absorption in the NIR region was passed through an interference filter (CVI, transmittance of 40%, band width of 10 nm) and its intensity was monitored with a fast InGaAs PIN photodiode equipped with an amplifier (Thorlabs, PDA255) and a digital oscilloscope (Tektronix, TDS 580D).

## Results and Discussion

In order to arrange Py at the minor groove of DNA, a nucleoside derivative that possesses a Py group on guanine N2 ($^{Py}$**G**) was synthesized as shown in Scheme 1. $^{Py}$**G** was converted to the phosphoramidite derivative **6** and $^{Py}$**G**-modified ODNs were synthesized by a DNA synthesizer according to the standard procedure. To check the duplex stability of the $^{Py}$**G**-modified ODNs, melting temperatures were measured (Table 1).

Table 1. Calculated and Measured Mass Values, Melting Temperatures, and Formation Rate Constants ($k_{dimer}$) of Pyrene Dimer Radical Cation of ODNs

| ODNs | | Mass | | $T_m$ [a] | $n$ [b] | $k_{dimer}$ [c] |
|---|---|---|---|---|---|---|
| | | Calcd | Found | /°C | | /$10^5$ $s^{-1}$ |
| **py1** | 5′GCA$^{Py}$**G**ACACG | 2975.1 | 2975.4 | 42 | 1 | 4.4 |
| | 3′CGTCT$^{Py}$**G**TGC | 2948.1 | 2948.5 | | | |
| **py2** | 5′GCA$^{Py}$**G**AACACG | 3288.3 | 3289.3 | 45 | 2 | 4.5 |
| | 3′CGTCTT$^{Py}$**G**TGC | 3252.3 | 3251.8 | | | |
| **py3** | 5′GCA$^{Py}$**G**AAACACG | 3601.6 | 3602.3 | 46 | 3 | 4.6 |
| | 3′CGTCTTT$^{Py}$**G**TGC | 3556.5 | 3556.9 | | | |
| **py4** | 5′GCA$^{Py}$**G**AAAACACG | 3914.8 | 3914.9 | 48 | 4 | 4.7 |
| | 3′CGTCTTTT$^{Py}$**G**TGC | 3860.6 | 3860.7 | | | |
| **pyr2** | 5′GCA$^{Py}$**G**CGCACG | 3280.3 | 3279.1 | 56 | 2 | 3.6 |
| | 3′CGTCGC$^{Py}$**G**TGC | 3262.3 | 3261.5 | | | |
| **m2** | 5′GCAGAACACG | nd | nd | 41 | — | — |
| | 3′CGTCTT$^{Py}$**G**TGC | 3252.3 | 3251.8 | | | |
| **n2** | 5′GCAGAACACG | nd | nd | 41 | — | — |
| | 3′CGTCTTGTGC | nd | nd | | | |

a) UV melting measurements were carried out in a pH 7.0 Na phosphate buffer 20 mM at a total strand concentration of 8 μM. b) Number of intervening A–T base pairs between two $^{Py}$**G**s. c) Determined from the time profiles of transient absorption in Fig. 2.

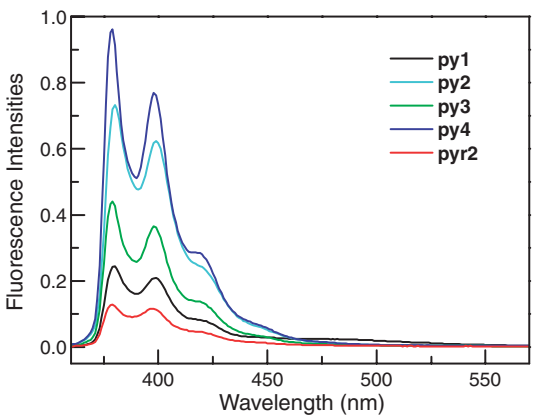
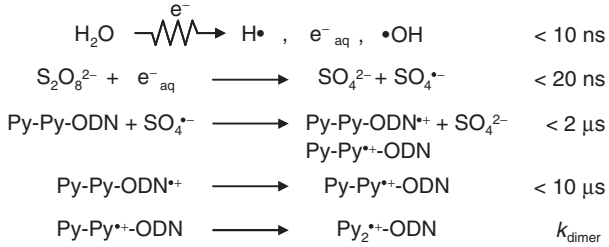



Fig. 1. Fluorescence spectra ($\lambda_{ex} = 345$ nm) for doubly Py-modified ODNs in aqueous solution in the presence of 20 mM Na phosphate buffer (pH 7.0) at a total strand concentration of 8 μM.

Singly $^{Py}$**G**-modified ODN (**m2**) showed a similar $T_m$ value with that of the corresponding unmodified ODN (**n2**). Incorporation of two $^{Py}$**G** (**py2**) showed a slightly higher thermostability compared to that of **n2**. Thus, $^{Py}$**G** was incorporated into DNA without a large disturbance of the duplex stability.

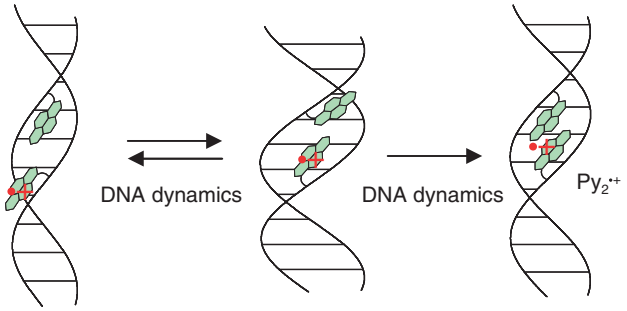
To identify the population of the DNA conformation in which two Pys associate close to each other at the non-oxidized state, the fluorescence spectra of the doubly Py-modified ODNs were measured (Fig. 1). Since formation of the Py excimer requires close cofacial contact between the excited fluorescent probe ($^1$Py*) and its neutral counterpart (Py) within the short lifetime of $^1$Py* in the DNA conformations,[17–20] Py excimer emission can provide structural information about the two Pys. For all of the doubly Py-modified ODNs studied here, excimer emission was not apparent, demonstrating that




Scheme 2. Mechanistic scheme for pulse radiolysis of doubly $^{Py}$**G**-modified ODN.

the population of the sandwich conformation between two Pys is low in the non-oxidized state.

To measure the intramolecular rates of association between $Py^{\bullet+}$ and Py in DNA, Py-modified ODNs were oxidized by $SO_4^{\bullet-}$, which was generated during the pulse radiolysis of a $D_2O$ solution in the presence of $K_2S_2O_8$. As previously reported, $Py^{\bullet+}$ was formed by two processes as shown in Scheme 2: The direct oxidation of Py by $SO_4^{\bullet-}$ and the hole transfer from $G^{\bullet+}$ generated in DNA to Py.[15,16,21] Here, if there is a DNA motion that brings $Py^{\bullet+}$ and Py into contact, $Py_2^{\bullet+}$ will be formed (Scheme 3). The pulse radiolysis of the doubly Py-modified DNA led to the formation of an absorption around 1600 nm that is assigned to $Py_2^{\bullet+}$ (CR band[22–24]), as shown in the inset of Fig. 2. In order to investigate the plausibility of the formation of $Py_2^{\bullet+}$ at the minor groove of DNA, we studied the $Py_2^{\bullet+}$ formation process for the five ODNs shown in Table 1. Interestingly, for all of the DNA studied here, the formation of $Py_2^{\bullet+}$ was accomplished quickly, within 20 micro seconds after a 8-ns electron pulse during pulse radiolysis (Fig. 2), showing that the minor groove of DNA offers a good space for the formation of $Py_2^{\bullet+}$. This was in strong contrast to the results of the previous report, in which Pys were modified




Scheme 3. DNA dynamics and the formation of $Py_2^{\bullet+}$.

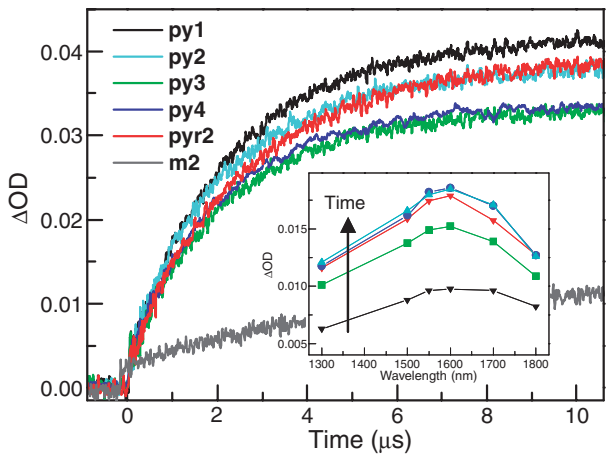



Fig. 2. Time profiles of the transient absorption of $Py_2^{\bullet+}$ monitored at 1550 nm during pulse radiolysis of doubly Py-modified ODNs in Ar-saturated $D_2O$ solution in the presence of 10 mM $K_2S_2O_8$, 100 mM *t*-BuOH, 20 mM Na phosphate buffer (pH 7.0), at a total strand concentration of 200 μM (100 μM in the case of **m2**). The inset shows the transient absorption spectra for **py2** (total strand conc. of 100 μM) observed at 2, 5, 10, 20, and 50 μs after the electron pulse during the pulse radiolysis.

at the 2′-sugar position of the nucleoside (**PyU**), where the formation rate of $Py_2^{\bullet+}$ strongly depended on the number of intervening base-pairs between the two **PyUs** and no formation of $Py_2^{\bullet+}$ was observed for ODNs with more than three intervening A–T base pairs between the two **PyUs** in the time scale of ≈1 ms. This is probably because larger conformational changes are needed for **PyU**-modified ODNs to bring two Pys in contact in the minor groove of DNA. The change of intervening A–T base pairs to G–C base pairs between two Pys (**py2**/**pyr2**) leads to a slight decrease in the formation rate of $Py_2^{\bullet+}$. Since G–C base pairs are known to have a more rigid structure, as can be seen from the higher $T_m$ value for **pyr2** compared to that for **py2**, the slower formation rate of $Py_2^{\bullet+}$ can be interpreted in terms of the reduced flexibility of DNA. Since the formation of $Py_2^{\bullet+}$ was not obvious in the case of the singly Py-modified **m2**, the observed results are accounted for by intramolecular processes as shown in Scheme 3.

In conclusion, to test the plausibility of the formation of $Py_2^{\bullet+}$ at the minor groove of DNA, we synthesized a nucleoside derivative possessing a Py group on guanine N2 to arrange Py at the minor groove of DNA. The formation of $Py_2^{\bullet+}$ was accomplished quickly, within 20 micro seconds after an electron pulse during pulse radiolysis for all the doubly Py-modified ODNs studied here. The results clearly demonstrate that the minor groove of DNA offers a good space for the formation of $Py_2^{\bullet+}$.

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