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Syntheses and fluorescence of RNA conjugates having pyrene-modified adenosine and nitrobenzene-modified uridine base pairs

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ABSTRACT

We prepared RNA duplexes possessing 2′-*O*-(1-pyrenylmethyl)adenosine and 5-(4-nitrophenyl)uridine base pairs. In the duplexes, pyrene serves as a photo-excitable electron donor and 5-(4-nitrophenyl)uridine acts as an electron acceptor. The donor–acceptor-modified RNA duplexes showed very weak fluorescence originating from the pyrene monomer and excimer emissions, which occur due to electron transfer from the excited pyrene to the nitrobenzene acceptor.

Arranging π-aromatic molecules in defined spaces and distances has attracted much research interest. Because the spatially arranged π-aromatic molecules exhibit unique photophysical and electroconductive properties, it should be possible to use them in many applications, such as in molecular electronic and photonic devices.[1] Bicontinuous electron donor and acceptor arrangements are essential for photovoltaics.[2]

DNA and RNA are attractive building blocks for self-assembled nanostructures.[3] In addition, DNA and RNA can be supramolecular scaffolds for arranging multiple aromatics by using a wide variety of strategies to incorporate aromatic compounds.[4] We have shown that RNA molecules having multiple pyrenylmethyl substituents on the 2′-*O*-sugar residues can form duplexes with complementary RNA sequences without the loss of thermal stability. In the RNA duplexes, covalently incorporated pyrenes (Pyrs) can be assembled in a helical manner along the duplex. These helically assembled pyrene arrays exhibit significantly strong excimer emissions.[5] Moreover, we have demonstrated that the charge transfer occurs with double exponential distance dependence in RNA duplexes having continuous rA_n-U_n sequences as bridges, Pyr as a photo-excitable electron donor, and 4-nitrobenzene (NB) as an electron acceptor.[6] Systems with Pyr and NB site specifically introduced at the 2′-*O*-position of U-strands through a one-carbon linker have been shown to undergo photo-induced charge transfer mediated by π-stacked bases along the axis of the helix.[7]

In this Letter, we describe another type of donor–acceptor-modified rA_n-U_n duplexes using Pyr and NB. Pyr groups were incorporated at the 2′-*O*-positions of an rA strand, and NBs were appended at the 5-positions of a U strand in the duplex. As a result, the Pyrs are directed into the minor groove of the duplex, whereas the NBs are positioned at the major groove. Figure 1 shows a schematic illustration of the configuration of Pyr and NB of the A_{Pyr}-U_{NB} base pair in an A-form duplex. In this configuration, photo-induced charge transfer from an excited Pyr to an NB across the base pair of the duplex is expected to occur.[8] In addition, consecutive incorporation of Pyrs and NBs into rA_n and U_n sequences, respectively, in the same manner causes a Pyr-NB bicontinuous array along the duplex. Sequences of the RNA duplexes (rA_n-U_n) having A_{Pyr}-U_{NB} pairs are illustrated in Chart 1.

All the RNAs employed in this work were synthesized by using a conventional phosphoramidite method. 2′-*O*-Pyrenylmethyl-modified adenine phosphoramidite was prepared according to a previously reported method.[4b] 5-(4-nitrophenyl)-2′-deoxyuridine **1** was prepared in 62% yield via Suzuki–Miyaura coupling using Pd(II) acetate in the presence of triphenylphosphine-3,3′,3″-trisulfonic acid trisodium salt (TPPTS) (Scheme 1).[9,10] Nucleoside **1** was reacted with 4,4′-dimethoxytrityl chloride (DMT-Cl) to give **2** in

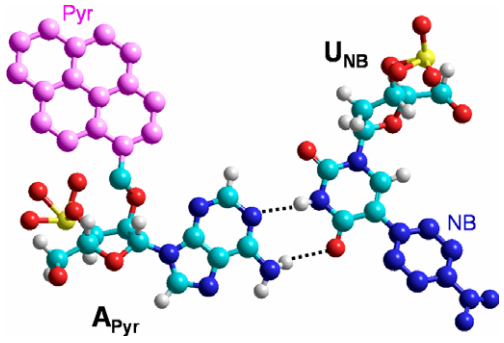

Figure 1. Schematic illustration of the configuration of pyrene (Pyr) and nitrobenzene (NB) of the A–U base pair in an A-form duplex.

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P0: 5'-rAAA AAA AAA AAA AAA AAA AA-3'
P1: 5'-rAAA AAA A_{Pyr}AA AAA AAA AAA AA-3'
P2: 5'-rAAA AAA $A_{Pyr}A_{Pyr}$A AAA AAA AAA AA-3'
N0: 5'-rUUU UUU UUU UUU UUU UUU UU-3'
N1: 5'-rUUU UUU UUU UUU U_{NB}U UUU UU-3'
N2: 5'-rUUU UUU UUU UUU $U_{NB}U_{NB}$U UUU UU-3'

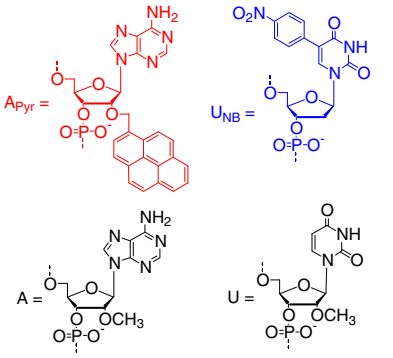


Chart 1. Sequences of Pyr donor- and NB acceptor-modified RNAs.

54% yield after purification on a silica gel column.[11] Protected nucleoside **2** was then converted to **3** by reacting it with 2-cyanoethyl-*N,N,N′N′*-tetraisopropylphosphorodiamidite in dichloromethane containing tetrazole. Purification by using silica gel column chromatography gave **3**, which was used in DNA/RNA-automated synthesis,[12] in 81% yield.

Melting profiles of the RNA duplexes displayed a single melting transition (Fig. 2). The melting temperatures (T_m) of the duplexes were in the range of 42–47 °C. The introduction of A_{Pyr} and U_{NB} into the RNA duplexes (rA_{20}-U_{20}) slightly destabilized the resulting Pyr-NB-modified duplexes.

Figure 3 shows absorption spectra of the Pyr-NB-modified RNA duplexes under identical conditions. For the Pyr-modified duplexes (**P1N0** and **P2N0**), pyrene ^1L_a absorption bands were observed in the region of 300–370 nm. A blue shift and hypochromism in the **P2N0** absorption band in comparison with that of **P1N0** were observed, suggesting that the two Pyrs of **P2N0** interact with each other. For the NB-modified duplexes (**P0N1** and **P0N2**), a broad absorption band was observed in the region of 300–370 nm. No such absorption band was observed in the RNA possessing NB at the 2′-*O*-position of U.[6] In addition, a broad absorption band in that region was seen in the spectra of single-stranded **N1** and single-stranded **N2**, whereas in the spectrum of single-stranded **N0**, no such absorption band was observed (see Supplementary data). Therefore, the broad absorption band is possibly due to the electronic coupling between U and NB in U_{NB}. The broad absorption bands observed for **N1** and **N2** shifted to longer wavelengths and decreased upon hybridization with **P0**, suggesting that the electronically coupled U_{NB}s form base-pairs with complement bases and stack with adjacent bases in the duplexes. In the cases of the Pyr-NB-modified duplexes (**P1N1**, **P2N1**, and **P2N2**), their absorption spectra showed hypochromism in comparison with the sum of the absorption intensities of **P*n*N0** (n = 1 and 2) and **P0N*n*** (n = 1 and 2). The absorption intensities of **P1N1** and **P2N1** were nearly equal to those of **P1N0** and **P2N0**, respectively. The absorption intensity of **P2N2** was slightly lower than the sum of the intensities of the **P2N0** and **P0N2** absorptions bands.

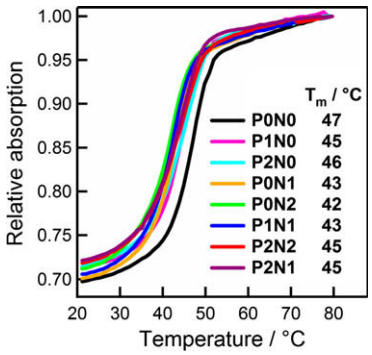


Figure 2. Melting profiles of Pyr-NB-modified RNA duplexes in a buffer of pH 7 containing 0.1 M NaCl and 0.01 M NaH_2PO_4.

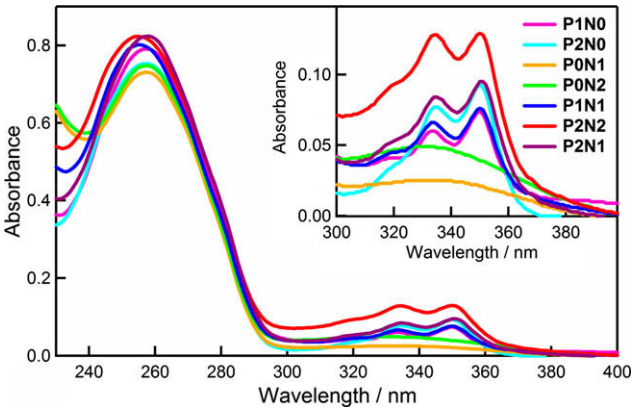
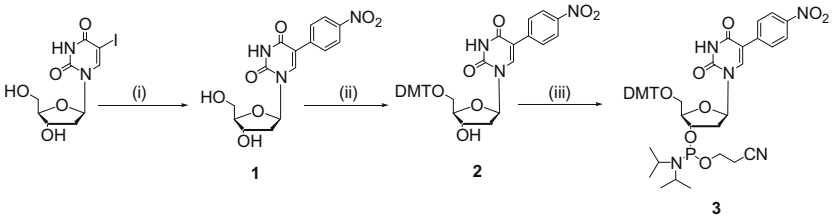


Figure 3. UV–vis spectra of Pyr-NB-modified RNA duplexes measured at 22 °C in a pH 7 buffer containing 0.1 M NaCl and 0.01 M NaH_2PO_4. Inset shows expanded spectra between 300 nm and 400 nm.

In addition to the UV–vis spectral studies, circular dichroism (CD) spectroscopy was performed in order to obtain further structural information about the Pyr and NB chromophores. Figure 4 shows CD spectra of the Pyr-NB-modified RNA duplexes. In the CD spectra of all the modified duplexes, a typical CD pattern (<300 nm) of a right-handed A-form duplex was observed. Duplex **P1N0** exhibited no induced CD in the region between 300 and 370 nm, which is consistent with a Pyr ring directed into the minor groove of the duplex. **P2N0** exhibited negative Cotton effects in the



Scheme 1. Synthesis of 5-(4-nitrophenyl)-2′-deoxyuridine derivatives (**1–3**). Reagents and conditions: (i) 4-nitrophenyl-1-boronic acid pinacol ester, Na_2CO_3, $Pd(OAc)_2$, triphenylphosphine-3,3′,3″-trisulfonic acid trisodium salt in CH_3CN–H_2O, reflux for 20 h; (ii) 4,4′-dimethoxytrityl chloride in pyridine, 6 h, rt; (iii) 2-cyanoethyl-*N,N,N′N′*-tetraisopropylphosphorodiamidite and tetrazole in dichloromethane, 2 h, rt.

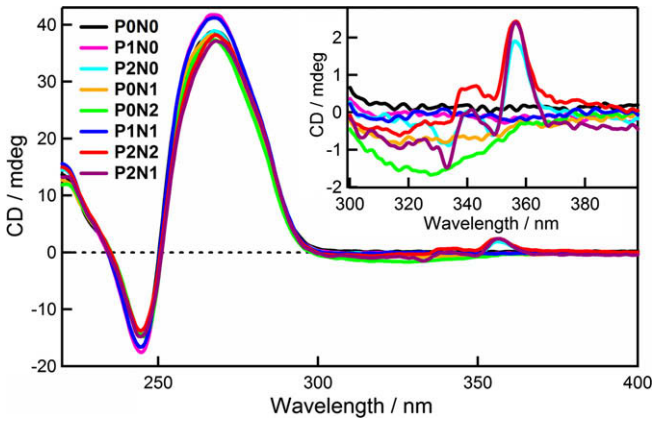


Figure 4. CD spectra of Pyr-NB-modified RNA duplexes measured at 22 °C in a buffer of pH 7 containing 0.1 M NaCl and 0.01 M NaH_2PO_4. Inset shows expanded spectra between 300 nm and 400 nm.

region of 300–350 nm and a positive Cotton effect in the region of 350–370 nm due to exciton coupling between the two Pyr chromophores.[5a,5b] On the other hand, **P0N1** and **P0N2** showed only negative induced CD signals owing to electronically coupled U_{NB}s in the region of 300–370 nm, and the CD amplitude for **P0N2** was about two times that for **P0N1** in this region. In the CD spectra of **P1N1**, **P2N1**, and **P2N2**, negative induced CD signals like those observed in the spectra of **P0N1** and **P0N2** were not observed. In the spectra of **P1N1**, no induced CD signals appeared in the region of 300–370 nm. In contrast, in the spectra of **P2N1** and **P2N2**, positive and negative Cotton effects due to exciton coupling between Pyrs were observed. On the basis of the UV–vis and CD spectra, there are interactions between Pyrs of the $A_{Pyr}A_{Pyr}$ domain and those between U_{NB}s of the $U_{NB}U_{NB}$ domain in the RNA duplexes. However, whether or not there are interactions between Pyr and U_{NB} in the Pyr-NB-modified duplexes is still unclear. The hypochromism in their UV–vis spectra suggests some electronic interaction between Pyrs and U_{NB}s, whereas little information about the interaction could be obtained from the CD spectra.

Fluorescence spectra of the Pyr-NB-modified RNA duplexes are shown in Figure 5. In the spectrum of **P1N1**, Pyr monomer fluorescence (376 and 398 nm) was observed, and its fluorescence intensity was much lower than that for **P1N0**, indicating that the Pyr emission was strongly quenched due to electron transfer (ET) from the excited Pyr (Pyr^*) to the U_{NB} acceptor.[13] The fluorescence

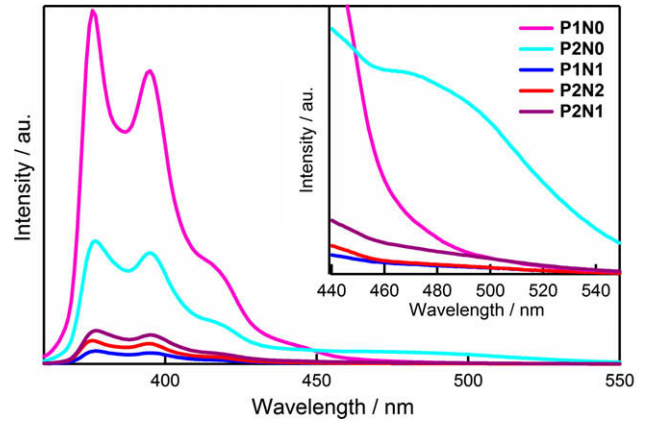


Figure 5. Fluorescence spectra of Pyr-NB-modified RNA duplexes measured at 22 °C in a buffer of pH 7 containing 0.1 M NaCl and 0.01 M NaH_2PO_4. Inset shows expanded spectra between 440 and 550 nm. The excitation wavelength was 350 nm.

intensity of **P1N1** at 376 nm was smaller (35%) than that of **P1N0**, where the distance between Pyr and NB is ca. 13 Å. In our previous work,[6] the fluorescence of the RNA duplex modified with Pyr and NB at the 2′-*O*-positions, where Pyr and NB were separated by 8.7 Å, was quenched by 48%. These results indicate that the fluorescence quenching in the present RNA system is stronger than that in the previous system. **P2N0** exhibited a Pyr excimer fluorescence (480 nm) in addition to Pyr monomer fluorescences (376 and 398 nm). In contrast to **P2N0**, **P2N1** and **P2N2** exhibited virtually no excimer fluorescence and showed only very weak monomer emission. Quenching of the **P2N2** emission was more effective than that of the **P2N1** emission. Scaiano et al. have reported that nitrobenzene can efficiently quench the pyrene intermolecular excimer as well as the monomer fluorescence.[8a] The extreme decrease in the intensity of the excimer fluorescence of **P2N1** and **P2N2** is therefore due to the direct quenching of the excimer by U_{NB} and the decrease in the Pyr^* population is due to the emission quenching. The electronic coupling between U and NB, as well as the base pairing of A_{Pyr}-U_{NB}, may play important roles in the ET.[8d] Further detailed studies to clarify this point are necessary.

In summary, RNA duplexes having A_{Pyr}-U_{NB} base pairs showed significant quenching of Pyr excimer and monomer fluorescences. The present system is the first step toward bicontinuous donor–acceptor arrays to generate charge-separated states by photo-irradiation of RNA duplexes.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.01.081.

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10. To a solution of 5-iodo-2′-deoxyuridine (1.0 g, 2.8 mmol), 4-nitrophenyl-1-boronic acid pinacol ester (0.77 g, 3.1 mmol), and Na_2CO_3 (0.90 g, 8.5 mmol) in CH_3CN–H_2O (20 mL, 10:1), palladium acetate (0.032 g, 0.14 mmol) and TPPTS (0.24 g, 0.42 mmol) were added. The solution was refluxed for 20 h under a N_2 atmosphere, diluted with 30 mL of water, and extracted with ethyl acetate (30 mL × 3). The organic phase was dried over Na_2SO_4, filtered, and evaporated. The crude product was separated on a silica gel column and eluted with 9:1 (v/v) CH_2Cl_2/CH_3OH to afford **1**. Yield, 62% (0.61 g); TLC (9:1, CH_2Cl_2- CH_3OH, v/v), R_f 0.28. ^1H NMR (DMSO-d_6): δ 2.26 (2H, 2′-CH_2), 3.66 (2H, 5′-CH_2), 3.84 (1H, 3′-CH), 4.31 (1H, 4′-CH), 5.25 (1H, 5′-OH), 5.29 (1H, 3′-OH), 6.21 (1H, 1′-CH), 7.88 (2H, Ar), 8.21 (2H, Ar), 8.54 (1H, 6-CH of uracil), 11.69 (1H, 3-NH of uracil).
11. 4,4′-Dimethoxytrityl chloride (0.53 g, 1.6 mmol) was added to a solution of **1** (0.50 g, 0.73 mmol), which was dried by coevaporation with pyridine three times, in pyridine (4.3 mL). After stirring for 6 h at room temperature, the solution was concentrated to near dryness. The residual material was dissolved in dichloromethane (150 mL), and then the solution was extracted with water. The organic phase was dried over Na_2SO_4, filtered, and evaporated to near dryness. The concentrated solution was added to a silica gel column chromatograph and eluted with 9:1 (v/v) CH_2Cl_2/CH_3OH. The appropriate fractions were pooled and then evaporated to near dryness. The residual solution was poured into cooled hexane, and pure **2** was obtained by filtering. Yield 54% (0.50 g). TLC (9:1, CH_2Cl_2/CH_3OH, v/v), R_f 0.42. ^1H NMR (DMSO-d_6): δ 2.25 (2H, 2′-CH_2), 3.66 (2H, 5′-CH_2), 3.67 (6H, OCH_3), 3.95 (1H, 3′-CH), 4.29 (1H, 4′-CH), 5.36 (1H, 3′-OH), 6.22 (1H, 1′-CH), 6.73–7.31 (total 13H, Ar of DMT), 7.58 (2H, Ar of nitrophenyl), 7.89 (2H, Ar of nitrophenyl), 7.91 (1H, 6-CH of uracil), 11.78 (1H, 3-NH of uracil).
12. 2-Cyanoethyl-*N,N,N′N′*-tetraisopropylphosphorodiamidite (0.294 mL, 0.93 mmol) was added to a solution of **2** (0.50 g, 0.78 mmol) and tetrazole (0.055 g, 0.78 mmol) in dry dichloromethane (1 mL). The solution was stirred for 2 h at room temperature, and then dichloromethane (5 mL) was added to the solution. The solution was washed with 10% $NaHCO_3$ aqueous solution, dried over Na_2SO_4, filtered, and then evaporated to near dryness. The product **3** was purified by silica gel column chromatography with 45/45/10 (v/v)CH_2Cl_2/AcOEt/Et_3N as the eluent. Yield, 81% (0.54 g); TLC (45/45/10, CH_2Cl_2–AcOEt–Et_3N, v/v), R_f 0.64.
13. The Pyr fluorescence of **P1** in single stranded state was quenched upon hybridization with **N0** (see Supplementary data).