Bioorganic & Medicinal Chemistry Letters 20 (2010) 3240–3243

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Double stranded DNA discrimination by di-pyrene modified γ-cyclodextrin

Akane Takeda [a], Kazuhiko Akimoto [c], Yoshihiko Kondo [a,b], Fumio Hamada [a,b,*]

[a] Department of Material-Process and Applied Chemistry for Environments, Graduate School of Engineering and Resource Science, Akita University, Akita 010-8502, Japan
[b] Department of Life Science, Faculty of Engineering and Resource Science, Akita University, Akita 010-8502, Japan
[c] Electronic Materials Research Laboratories, Nissan Chemical Industries Ltd., 635 Sasakura Fuchu-Machi, Toyama 939-2792, Japan

ARTICLE INFO

Article history:
Received 21 January 2010
Revised 14 April 2010
Accepted 16 April 2010
Available online 18 April 2010

Keywords:
Cyclodextrin
Pyrene
Fluorescence
Double strand DNA
Discrimination

ABSTRACT

Neutral fluorescent active di-pyrene modified γ-cyclodextrin (**1**) was synthesized in order to discriminate with single and double strand DNA (ssDNA and dsDNA, respectively) with high selectivity. The binding and selectivity of **1** for dsDNA was indicated by increase of the fluorescent intensity in an addition of dsDNA. On the other hand, an increase of fluorescent intensity of **1** was not recognized in an addition of ssDNA.

Recently, the genetic diagnosis has attracted significant attention as key of preventive medicine where the fluorescence reagent has been used for DNA detection.[1] However, there are some problems such as 'the distinction between ssDNA and dsDNA is difficult' and 'the longevity of the fluorescence reagent is short and the handling is not easy' in detection with the fluorescence reagent.[2] To get solution of the problem, our strategy is using of cyclodextrin (CyD) because CyD has many advantages such as protection or increase of water solubility of organic molecules. We synthesized fluorescence active modified CyD in order to study intercalative capability into ssDNA and dsDNA. CyD is tours shaped cyclic oligosaccharides composed of six, seven and eight D-glucopyranose units (α, β, γ-CyD, respectively). A variety of organic compounds can be included in their center cavities in aqueous media. Therefore, fluorescence reagent can be stable by interaction with CyD cavity. In previous Letter, we reported synthesis of pyrene modified β-CyD that was linked between pyrene unit and CyD with polyethylene amine chain. Unfortunately, this compound showed no selective discrimination for ssDNA and dsDNA.[3] Because it might be supposed that secondary amine moiety of the linker can interact with the phosphoric acid part of dsDNA and dsDNA through electrostatic interaction. To get solution this problem, we synthesized the pyrene modified β-CyD with an ether chain (β-**1**) (Fig. 1) because ether chain was hydrophilic and no interactive with the phosphoric acid residue of DNA. Unfortunately, the compound showed no interaction with both of ssDNA and dsDNA.

We planed synthesis of another type of pyrenes modified CyD, where bis pyrene units were introduced into CyD, because particular excimer fluorescence of pyrene can work as unique recognition for DNA. In the synthesis, γ-CyD was used because water

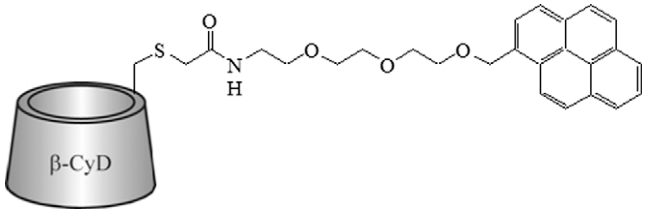


Figure 1. Structure of di-6^{A}-deoxy-6^{A}-[{8-(1-pyrene methoxy)-3,6-(dioxa)octa-1-amino}-(thioacetyl)]-β-CyD (β-**1**).

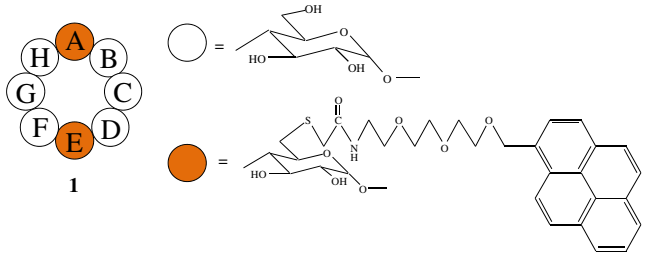


Figure 2. Structure of di-6^{A},6^{E}-deoxy-6^{A},6^{E}-[{8-(1-pyrene methoxy)-3,6-(dioxa)-octa-1-amino}-(thioacetyl)]-γ-CyD (**1**).

* Corresponding author. Tel.: +81 18 889 2440; fax: +81 18 837 0404.
E-mail address: hamada@ipc.akita-u.ac.jp (F. Hamada).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.04.066

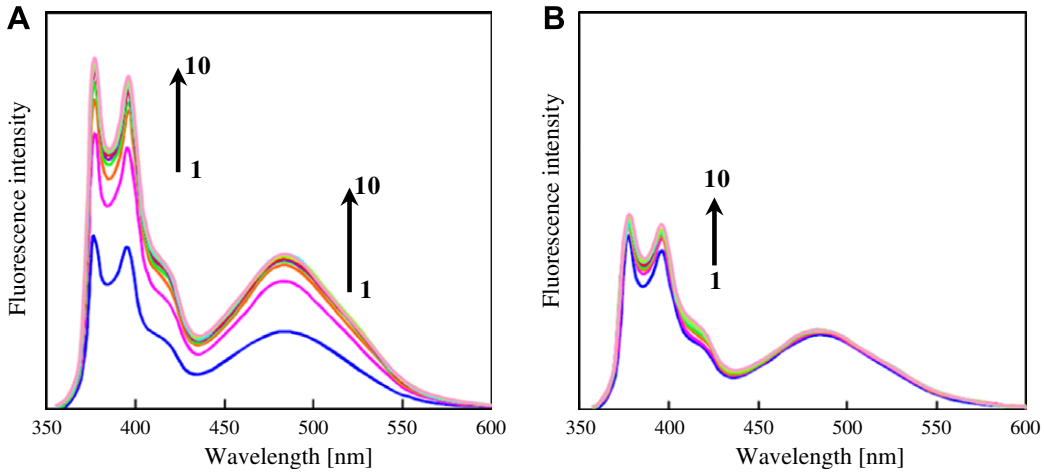
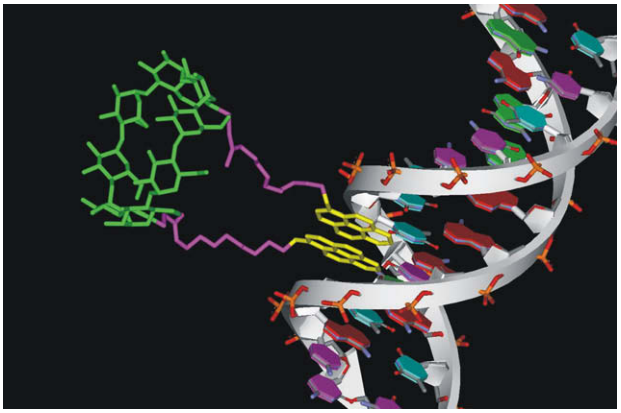


Figure 3. Fluorescence spectra of **1** in a 10 vol % DMSO aqueous solution (3.0×10^{-7} M) at various concentration of dsDNA or ssDNA. (A) dsDNA (1:0, 2:9.38×10^{-11}, 3:1.88×10^{-10}, 4:3.75×10^{-10}, 5:7.50×10^{-10}, 6:1.50×10^{-9}, 7:3.00×10^{-9}, 8:6.00×10^{-9}, 9:1.20×10^{-8}, 10:2.50×10^{-8} M), (B) ssDNA (1:0, 2:4.69×10^{-10}, 3:9.38×10^{-10}, 4:1.88×10^{-9}, 5:3.75×10^{-9}, 6:7.50×10^{-9}, 7:1.50×10^{-8}, 8:3.00×10^{-8}, 9:6.00×10^{-8}, 10:1.20×10^{-7} M).

solubility of di-pyrenes modified γ-CyD can be increased in comparison with those of α- and β-CyD derivatives.[4] The γ-CyD has four isomers when it was modified with double functional groups[5] as shown in Figure 2. In this study, we synthesized the di-pyrene modified γ-CyD (di-6^A, 6^E-deoxy-6^A, 6^E-[{8-(1-pyrenemethoxy)-3,6-(dioxa)octa-1-amino}-(thioacetyl)]-γ-CyD (**1**) by using the AE form of γ-CyD where the substitution sites were away most, and examined intercalative capability into ssDNA and dsDNA by using a spectroscopy technique.



Scheme 1. Energy-minimized complex structure of **1** with dsDNA.

The four isomers of di-(*p*-tosyl)γ-CyD (γ-1, γ-2, γ-3, and γ-4) as precursor for 6^A, 6^E-di(thioacetic acid) γ-CyD (**2**) were prepared as previously reported.[6] Compound **2** was prepared from 6^A, 6^E-di(*p*-tosyl) γ-CyD and mercaptoacetic acid in the presence of sodium carbonate as same procedure as previously reported.[6] Compound **1** was synthesized from **2** and 8-(1-pyrenemethoxy)3,6-(dioxa)octa-1-amine[7–9] in a yield of 6.14%.

Figure 3 shows the fluorescent spectra of **1** alone and with ssDNA or dsDNA.[10] The fluorescence spectra shows monomer (at 377 nm) and excimer (at 479 nm) emissions, where those of intensities of the emission were increased upon an addition of dsDNA. The fluorescence intensity of **1** was increased rapidly when the concentration of dsDNA reached at 7.5×10^{-10} M and then the increase of the intensity was not recognized. On the other hand, an increase in the fluorescence intensity of **1** was not observed in an addition of ssDNA. The addition of dsDNA to the solution of **1**

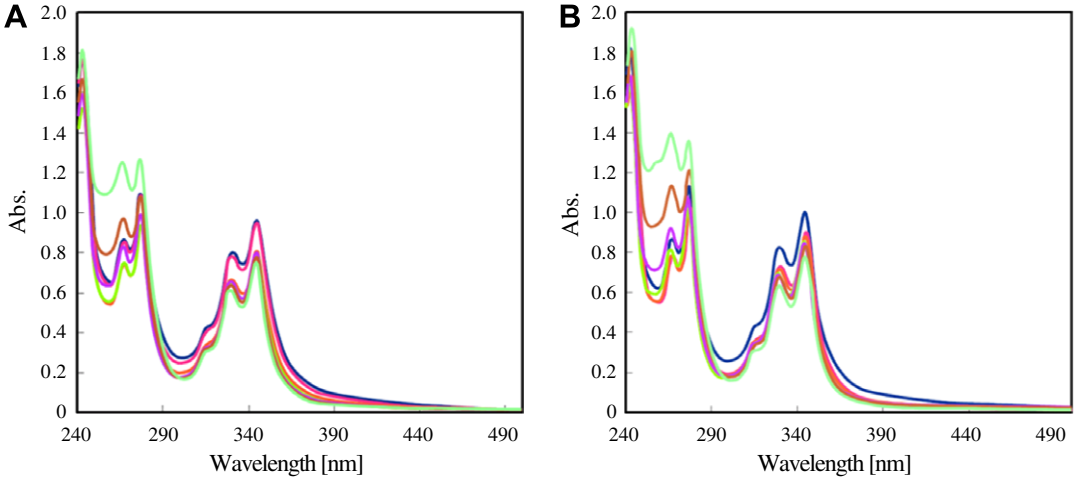


Figure 4. UV–Vis spectra of **1** in a 10 vol % DMSO aqueous solution (3.0×10^{-5} M) at various concentrations of dsDNA or ssDNA. (A) dsDNA (1:0, 2:9.38×10^{-9}, 3:1.88×10^{-8}, 4:3.75×10^{-8}, 5:7.50×10^{-8}, 6:1.50×10^{-7}, 7:3.00×10^{-7} M), B) ssDNA (1:0, 2:4.69×10^{-8}, 3:9.38×10^{-8}, 4:1.88×10^{-7}, 5:3.75×10^{-7}, 6:7.50×10^{-7}, 7:1.50×10^{-6} M).

caused an increase in fluorescence intensity, which indicates the binding of **1** with dsDNA. It is not yet known that **1** is DNA-intercalating agent or groove binder. Based on the distance between two neighboring bases that are perpendicular to the main axis of dsDNA is 3.4 Å, it is highly unlikely that two pyrenes were included into the cavity. On the other hand, a major grove of the dsDNA would be too large for excimer formation. The energy-minimized structure of the complex between **1** and dsDNA obtained using

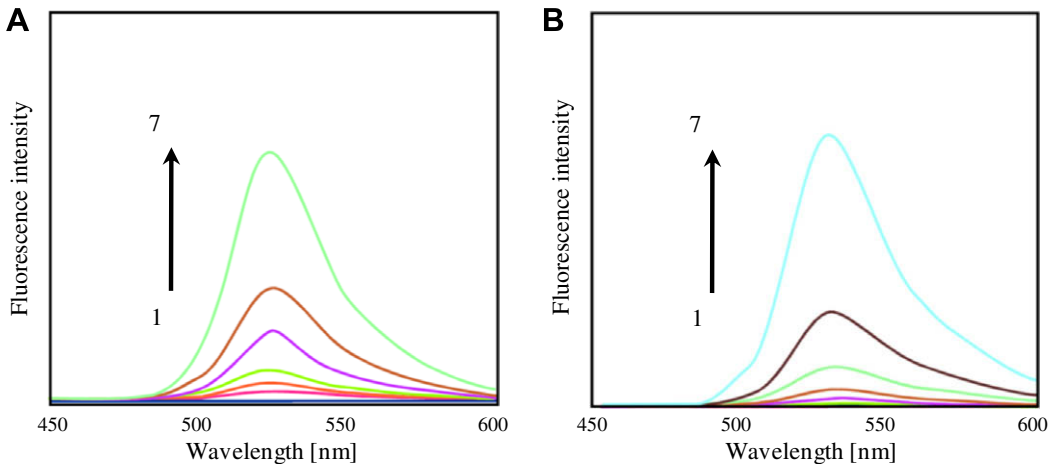


Figure 5. Fluorescence spectra of **3** in a 10 vol % DMSO aqueous solution at various concentrations of dsDNA or ssDNA. (A) dsDNA (1:0, 2:9.38 × 10^{-11}, 3:1.88 × 10^{-10}, 4:3.75 × 10^{-10}, 5:7.50 × 10^{-10}, 6:1.50 × 10^{-9}, 7:3.00 × 10^{-9} M), (B) ssDNA (1:0, 2:4.69 × 10^{-10}, 3:9.38 × 10^{-10}, 4:1.88 × 10^{-9}, 5:3.75 × 10^{-9}, 6:7.50 × 10^{-9}, 7:1.50 × 10^{-8}, 8:3.00 × 10^{-8}, 9:6.00 × 10^{-8} M).

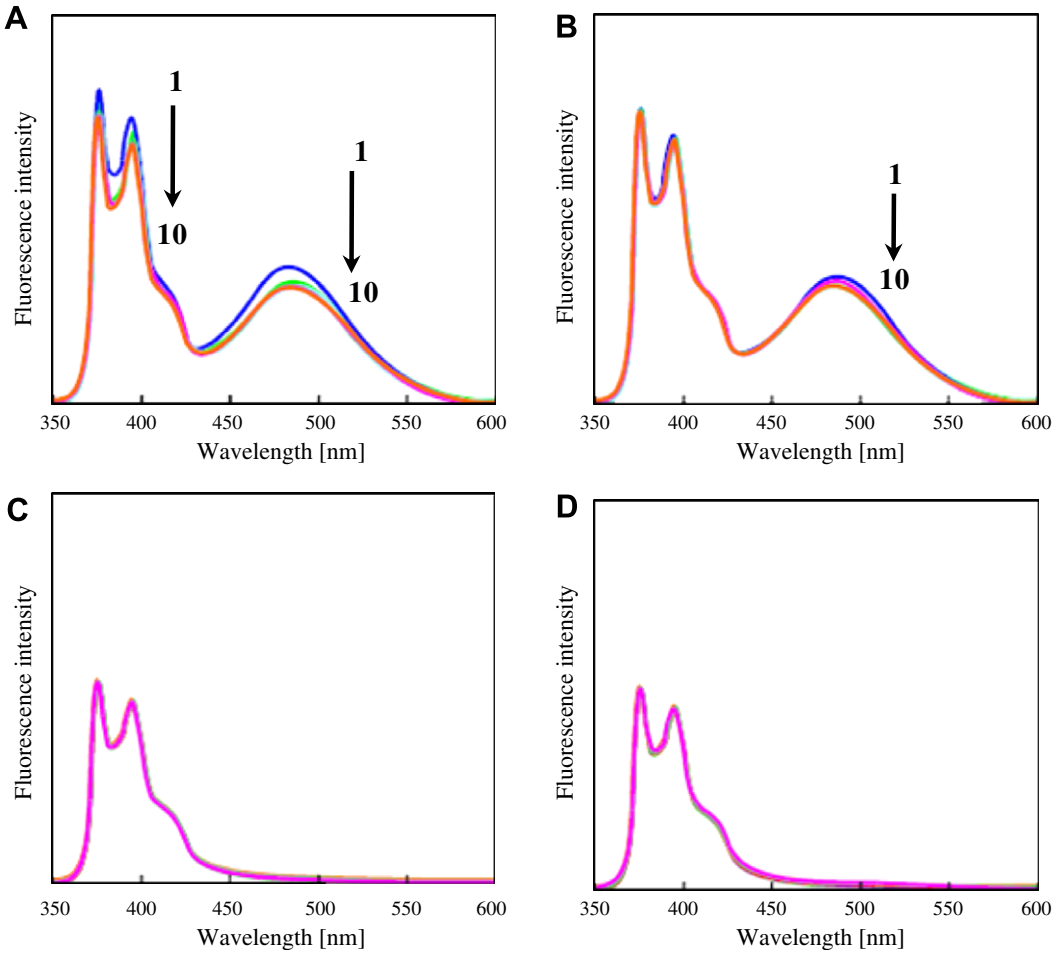


Figure 6. Fluorescence spectra of **4** (A and B) and **5** (C and D) in a 10 vol % DMSO aqueous solution (**4**:6.0 × 10^{-7}, **5**:1.0 × 10^{-7} M) at various concentration of dsDNA or ssDNA. (A) dsDNA (1:0, 2:1.88 × 10^{-10}, 3:3.75 × 10^{-10}, 4:7.50 × 10^{-10}, 5:1.50 × 10^{-9}, 6:3.00 × 10^{-9}, 7:6.00 × 10^{-9}, 8:1.20 × 10^{-8}, 9:2.50 × 10^{-8}, 10:4.80 × 10^{-8} M), (B) ssDNA (1:0, 2:9.38 × 10^{-10}, 3:1.88 × 10^{-9}, 4:3.75 × 10^{-9}, 5:7.50 × 10^{-9}, 6:1.50 × 10^{-8}, 7:3.00 × 10^{-8}, 8:6.00 × 10^{-8}, 9:1.20 × 10^{-7}, 10:2.40 × 10^{-7} M), (C) dsDNA (1:0, 2:3.10 × 10^{-11}, 3:6.30 × 10^{-11}, 4:1.25 × 10^{-10}, 5:2.50 × 10^{-10}, 6:5.00 × 10^{-10}, 7:1.00 × 10^{-9}, 8:2.00 × 10^{-9}, 9:4.00 × 10^{-9}, 10:8.00 × 10^{-9} M), D) ssDNA (1:0 ,2:1.56 × 10^{-10}, 3:3.13 × 10^{-10}, 4:6.25 × 10^{-10}, 5:1.25 × 10^{-9}, 6:2.50 × 10^{-9}, 7:5.00 × 10^{-9}, 8:1.00 × 10^{-8}, 9:2.0 0 × 10^{-8}, 10:4.0 × 10^{-8} M).

molecular mechanics in CS Chem3D, which was illustrated as shown in Scheme 1.

These results suggest that the two pyrene units assembled in a minor grove of dsDNA with locating as face-to-face orientation because the intensity of excimer emission was increased with large extent in addition of dsDNA. On the other hand, Figure 4 shows the UV–Vis spectra of **1** alone and with ssDNA or dsDNA.[11] The absorbance of **1** was decreased by an addition of ssDNA or dsDNA, where a decrease of an absorbance of the presence of dsDNA was slightly larger than that of presence of ssDNA. It was suggested that **1** can interact with dsDNA with larger than that of ssDNA.

Figure 5 shows the fluorescent spectra of SYBER Green I (**3**) alone and with ssDNA or dsDNA.[12] The fluorescent intensity was increased when dsDNA or ssDNA was added to **3**. In addition of ssDNA to **3**, the increase of the fluorescent intensity was not seen when the total DNA concentration reached at 7.5×10^{-10} M and after that the fluorescence intensity was increased as well as dsDNA. Thus, it has been understood that **3** can discriminate between ssDNA and dsDNA, but not completely such as that of **1**.

We also studied binding capability of analogs of **1**, which are di-6^{A}, 6^{C}-deoxy-6^{A}, 6^{C}-[{8-(1-pyrenemethoxy)-3,6-(dioxa)octa-1-amino}-(thioacetyl)]-γ-CyD (**4**)[13] and di-6^{A}, 6^{D}-deoxy-6^{A}, 6^{D}-[{8-(1-pyrenemethoxy)-3,6-(dioxa)octa-1-amino}-(thioacetyl)]-β-CyD (**5**).[14] As shown in Figure 6, the intensities of fluorescent spectra were not almost changed in an addition of both ssDNA and dsDNA, which indicate that compounds **4** and **5** hardly can detect with both of ssDNA and dsDNA.[10] In a case of **4**, the small excimer emission was recognized, which means both pyrene units of **4** were oriented as face-to-face configuration than that of **5**. It seems that large binding formation with dsDNA by those analogs needs face-to-face orientation of the pyrene units of the analogs.

In this study, we studied synthesis of di-pyrene modified γ-CyD (**1**) and binding capability with dsDNA and ssDNA was examined by various spectrum measurements. It is shown that fluorescence intensity of **1** was increased only for dsDNA in a fluorescent spectrum measurements and it was understood that there was an interaction with DNA in the UV–Vis spectrum measurement. In addition, the discrimination ability of dsDNA and ssDNA of **1** is better than **3** when DNA concentration is high. Moreover, γ-CyD works as scaffold of the pyrene and facilitates binding more. From these results, **1** can be effective new type of detection reagent to discriminate with ssDNA and dsDNA completely.

References and notes

1. Park, H.; Song, J.; Park, K.; Kim, M. *Chem. Eng. Sci.* **2006**, *61*, 954.
2. Zipper1, H.; Brunner, H.; Bernhagen, J.; Vitzthum, F. *Nucleic Acids Res.* **2004**, *32*, 103.
3. Matsumura, N.; Kagaya, M.; Kondo, Y.; Akagami, Y.; Hamada, F. *Int. J. Soc. Mater. Eng. Resour.* **2009**, *16*, 16.
4. Bender, M. L.; Komiyama, M. In *Cyclodextrin Chemistry*; Springer: Berlin, Heidelberg, New York, 1978; pp 2–9.
5. Narita, M.; Hamada, F.; Suzuki, I.; Osa, T. *J. Chem. Soc., Perkin Trans. 2* **1998**, 2751.
6. Fujita, K.; Tahara, T.; Koga, T.; Imoto, T. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 3150.
7. Ueno, A.; Moriwaki, F.; Osa, T.; Hamada, F.; Murai, K. *Tetrahedron* **1987**, *43*, 1751.
8. Klausner, Y. S.; Bodansky, M. *Synthesis* **1972**, 453.
9. *Preparation of* **1**: To solution of **2** (0.1479 g, 0.10 mmol) and 1-hydroxybenzotriazole (1-HOBt, 0.0626 g, 0.46 mmol) in a mixture dry-DMF (15 mL), *N,N′*-dicyclohexylcarbodiimide (DCC, 0.0879 g, 0.42 mmol) was added at 0 °C. The reaction mixture was stirred for 2 h and then 8-(1-pyrenemethoxy)-3,6-(dioxa)octa-1-amine (0.1646 g, 0.45 mmol) was added. The reaction mixture was warmed up to a room temperature and then stirred for 3 days at 80 °C. The mixture was concentrated in vacuo which was treated with 200 mL of acetone. The resulting precipitates were filtered and dried up to give crude product was obtained (0.1628 g). The crude product was dissolved in 5 mL of water and 5 mL dry-DMF. The soluble fraction was applied to a reversed—phase column (Lobar column LiChroprep RP-18, Merck Ltd., 310 mm × 10 mm). Stepwise elution with 80–100% aqueous methanol gave pure **1**, which was collected and dried up in vacuo to give pure **1** (0.0134 g, yield; 6.14%). R_f: 0.56 (1-BuOH/EtOH/H_2O = 5:4:3, TLC; silica gel 60F_{254}). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.5–4.7 (6H, m, OH of C^6 of CyD), 4.8–5.0 (8H, d, H of C^1 of CyD), 5.1–5.3 (2H, s, CH_2 of pyrenemethoxy), 5.8–6.2 (16H, s, OH of C^2 and C^3 of CyD), 7.9–8.0 (2H, t, H of amide), 8.0–8.5 (18H, m, aromatic H of pyrene), FAB MS *m*/*z* 2136 [M+H]$^+$.
10. Fluorescence spectra were measured using Perkin–Elmer LS40B Fluorescein the fluorescence spectra was 343 nm and excitation and emission slit were 5 nm. DMSO aqueous solution (10 vol %) was used as solvent for the **1** (or **4**, **5**) and DNA. When DNA solution added to the **1** (or **4**, **5**) solution (**1**: 3.0×10^{-7} M, **4**: 6.0×10^{-7} M, **5**: 1.0×10^{-7} M), the spectra change was recorded. The sequences of ssDNA and dsDNA are TTAAAAATAG GCTATCCCTT ATTAAGTAAA ATAGGGAGTT (40 bp) and CTGAGTTCCA AATGTCCCAG CTGTTTTATG CTTTGTCTCT GTTTCCCAGA GACCCTGAGT GTGGTCTAGA GTTGGGATGA GCATTGGTCT CTAATGGTTC TGAAATAATT GTATATTCCT GCAAAAACAT TAAGTCTATT AGAAACCAGC TAATTTCATT TTGTCATTTT TATAGGTAAC ATATTCTGGT GCAGG (200 bp), respectively.
11. *UV–Vis measurement of* **1** *and DNA*: UV–Vis spectra were measured using SHIMAZU-3600 Spectrophotometer. DMSO aqueous solution (10 vol %) was used as solvent for **1** and DNA. When DNA was added to **1** (3.0×10^{-5} M), the spectra change was recorded.
12. *Fluorescence measurements of* **3** *and DNA*: Fluorescence spectra were measured using Perkin–Elmer LS40B Fluorescence Spectrophotometer. The excitation wavelength of the fluorescence spectra was 494 nm and excitation and emission slit were 5 nm. Compound **3**, of which item code is 50512 and concentration is ×10,000) was purchased from TAKARA BIO INC. For measurement of fluorescence spectra, **3** was diluted a 10,000 with a 10 vol % DMSO aqueous solution. When DNA solution added to the **3** solution, the spectra change was recorded.
13. *Preparation of* **4**: To solution of 6^{A}, 6^{C}-di(thioacetic acid) γ-CyD (0.2569 g, 0.172 mmol), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM, 0.5367 g, 1.72 mmol) and 8-(1-pyrenemethoxy)-3,6-(dioxa)octa-1-amine (0.6224 g, 1.72 mmol) in dry-DMF (50 mL). The reaction mixture was stirred for 2.5 h at room temperature. The mixture was concentrated in vacuo which was treated with 200 mL of acetone. The resulting precipitates were filtered and dried up to give crude product (0.3398 g). The crude product was dissolved in a mixture of 5 mL of water and 5 mL of DMF. The soluble fraction was applied to a reversed-phase column (Lobar column LiChroprep RP-18, Merck Ltd., 310 mm × 10 mm). Stepwise elution with 80–100% aqueous methanol gave pure **4**, which was collected and dried up in vacuo to give pure **4** (0.0336 g, yield; 8.87%). R_f: 0.59 (1-BuOH/EtOH/H_2O = 5:4:3, TLC; silica gel 60F_{254}). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.49–4.70 (6H, m, OH of C^6 of CyD), 4.87 (8H, s, H of C^1 of CyD), 5.19 (4H, s, CH_2 of pyrenemethoxy), 5.75–5.98 (16H, s, OH of C^2 and C^3 of CyD), 8.05–8.40 (20H, m, aromatic H of pyrene and amide), FAB MS *m*/*z* 2136 [M+H]$^+$.
14. *Preparation of* **5**: Compound **5** was prepared by same procedure as Compound **4** (yield; 8.90%). R_f: 0.60 (1-BuOH/EtOH/H_2O = 5:4:3, TLC; silica gel 60F_{254}). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.3–4.6 (5H, m, OH of C6 of CyD), 4.7–4.9 (7H, m, C^{1}H of CyD), 5.1–5.3 (4H, s, CH_2 of pyrenemethoxy), 5.5–6.0 (14H, m, OH of C^2 and C^3 of CyD), 7.9–8.0 (2H, m, –NH), 8.0–8.4 (18H, m, aromatic H of pyrene) FAB MS *m*/*z* 1974 [M+H]$^+$.