# Pyrenylboronic acids as a novel entry for photochemical DNA cleavage: diradical-forming pyrene-1,6-diyldiboronic acid mimics the cleavage mechanism of enediyne antitumor antibiotics

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**Pyren-1-ylboronic acid 4 and pyrene-1,6-diyldiboronic acid 5 are bound to supercoiled double-strand circular DNA plasmid ColE1 (colDNA) and effect its efficient photocleavage under visible light irradiation. Mechanistic studies show that the cleavage occurs according to the radical mechanism. The cleavage activity of monoacid 4 could be suppressed by the addition of D-fructose because of boronic acid–saccharide complexation. Diacid 5 is bound to colDNA more strongly than is monoacid 4 and effects its efficient photocleavage to yield Form III. The results show that diradical-forming diacid 5 is able to cleave DNA at two reaction sites, mimicking the cleavage mechanism of enediyne antitumor antibiotics.**

The chemistry and biology of natural products have represented a major path to drug discovery and development. Recently, it became evident that enediyne antitumor antibiotics (the reactive site is shown as in structure **1**: typical examples are calicheamicin, esperamicin, dynemicin, *etc.*) cycloaromatize to form a substituted 1,4-didehydrobenzene diradical **2**, which can initiate efficient cleavage of duplex DNA.[1] The mechanism has been unambiguously understood to be a sort of Bergman cyclization of enediyne derivatives.[2–4] It is known, however, that in conventional enediyne derivatives this reaction scarcely proceeds under physiological conditions and can take place only at high temperature.[5] Meanwhile, the development of boronic acid receptors for saccharides has recently gained much attention.[6–11] It has been shown that this interaction is superior to the hydrogen-bonding interaction for sugar sensing in an aqueous system.[6–11] One major change induced in the boronic acid by formation of the cyclic ester with saccharides is a shift of the p$K_a$ to lower pH region.[6] Hence, one can convert neutral boronic acid $RB(OH)_2$ to anionic boronate ester $R(OH)B^-<^{O}_{O}>$Sac (Sac$<^{OH}_{OH}$ = saccharide) at constant pH simply by the addition of saccharides. This phenomenon was successfully applied to the saccharide control of the binding ability of boronic-acid-appended DNA intercalators such as the complex salt **3**.[12] During this work we came across an interesting reference which describes how the C–B bond in arylboronic acids is susceptible to photochemical cleavage.[13] It thus occurred to us that pyren-1-ylboronic acid **4**[14] would also act as a novel photochemical DNA cleavage reagent because (i) pyrene derivatives act as intercalators,[15,16] (ii) they can be photochemically excited at a wavelength longer than the absorption band of DNA[17] and (iii) the photochemical C–B bond fission should provide some species active for DNA cleavage. Furthermore, pyrene-1,6-diyldiboronic acid **5** would yield a diradical species **6** and would also cleave duplex DNA at two sites as enediyne antitumor antibiotics do. We have found that compound **4** is bound to DNA under physiological conditions and photochemically cleaves DNA according to a radical mechanism, and also that compound **5** is capable of cleaving supercoiled double-strand circular DNA (Form I) plasmid ColE1 into linear DNA (Form III) under photoirradiation.

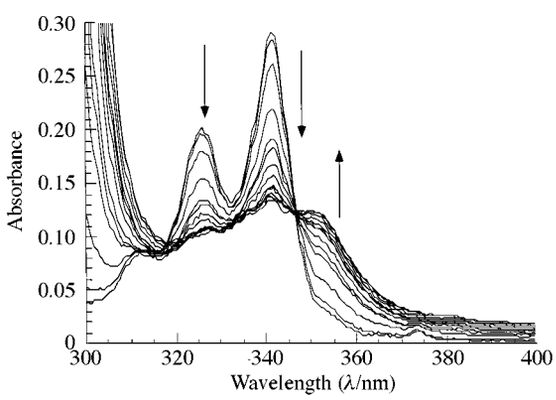
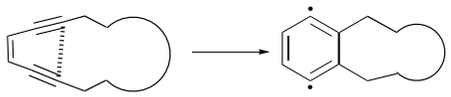



**Fig. 1** Absorption spectral change in compound **4** ($1.0 \times 10^{-5}$ mol dm$^{-3}$) induced by the addition of calf thymus DNA ($2.5 \times 10^{-4}$ to $3.0 \times 10^{-3}$ mol dm$^{-3}$): 25 °C, water–methanol (300 : 1 v/v), pH 7.50 with 10.0 mmol dm$^{-3}$ sodium hydrogen carbonate buffer

## Results and discussion

### Pyren-1-ylboronic acid 4-DNA interactions

A spectral change in compound **4** induced by the addition of calf thymus DNA is shown in Fig. 1. It is seen from Fig. 1 that with increasing DNA concentration the absorption maxima at 326 nm and 342 nm decrease while a new absorption maximum appears at 350 nm. This spectral change suggests that compound **4** is bound to calf thymus DNA mainly due to the π–π stacking interaction.[18] Although a shorter-wavelength isosbestic point at ~317 nm is disordered by the background absorption of calf thymus DNA, a longer-wavelength isosbestic point at 347 nm is very tight. This suggests that the binding of compound **4** to calf thymus DNA occurs according to a simple one-step process. The binding constant ($K$) and the mole number pyren-1-ylboronic acid **4** bound to per mole of base pairs ($n$) were estimated from a Scatchard plot[19,20] (correlation



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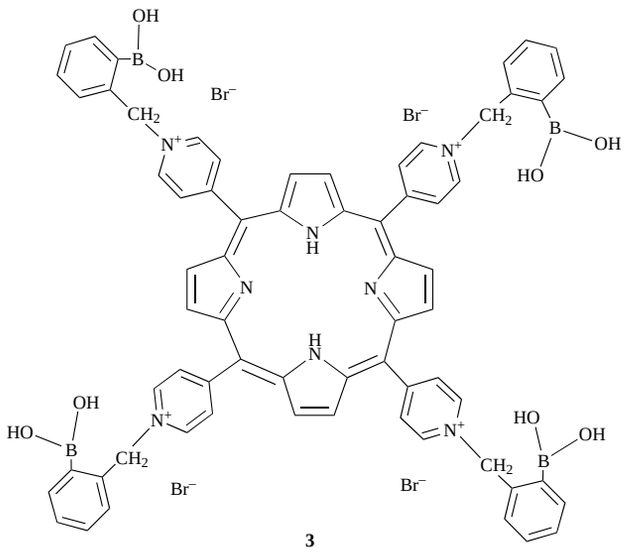

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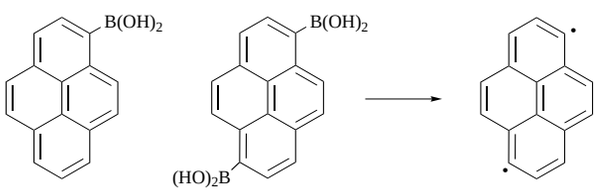

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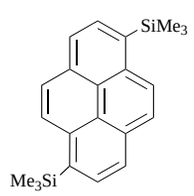

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coefficient 0.98): $K = 1.3 \times 10^5$ dm$^3$ mol$^{-1}$ and $n = 0.08$. These values are comparable with those determined for pyrene: $K = (1.6–5.1) \times 10^4$ dm$^3$ mol$^{-1}$ and $n = 0.005–0.015$.[15,16] A similar experiment was carried out for ColE1: $K = 4.0 \times 10^5$ dm$^3$ mol$^{-1}$ and $n = 0.025$. On the other hand, such a spectral change in compound **4** was scarcely induced in the presence of D-fructose (0.10 mol dm$^{-3}$). This implies that the anionic boronate ester derivative formed from neutral compound **4** and D-fructose[6,7] is scarcely bound either to calf thymus DNA or to ColE1.

Photodecomposition of compound **4** ($1.00 \times 10^{-5}$ mol dm$^{-3}$) was carried out at 30 °C in water–MeCN (3 : 7 v/v). Analysis of the product solution with an HPLC method showed that pyrene was produced in 76.2% yield. When the photochemical reaction was carried out in $D_2O$–MeCN or water–$CD_3CN$, the recovered pyrene contained 8.8% or 7.5% [$^2$H]pyrene, respectively (analyzed by EI-MS). The results show that the pyrene radical abstracts a proton nonselectively from both water and acetonitrile. The photochemical reaction of the boronic unit **4** was also carried out in aqueous solution (30 °C, pH 7.50 with 10.0 mmol dm$^{-3}$ sodium hydrogen carbonate, 0.1 vol% MeOH) in the presence of calf thymus DNA ($3.3 \times 10^{-5}$ mol dm$^{-3}$). After 30 min of photoirradiation the absorption spectrum became identical with that of calf thymus DNA plus pyrene.

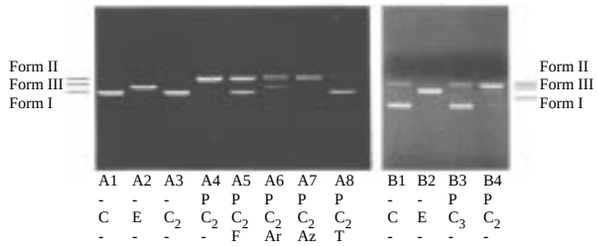


**Fig. 2** Gel electrophoresis showing results of photochemical cleavage of supercoiled circular double-strand DNA plasmid ColE1 (Form I) into nicked colDNA (Form II) and into linear DNA (Form III): Lane A1 and B1, control (C: untreated colDNA $1.8 \times 10^{-6}$ mol dm$^{-3}$); Lane A2 and B2, colDNA with restriction enzyme *Eco*RI (E: 0.1 unit/μl); Lane A3, colDNA plus compound **4** ($C_2$: $3.8 \times 10^{-6}$ mol dm$^{-3}$) in the dark; Lane A4 and B4, colDNA plus compound **4** under photoirradiation (P); Lane A5, colDNA plus compound **4** under P in the presence of D-fructose (F: $1.00 \times 10^{-2}$ mol dm$^{-3}$); Lane A6, colDNA plus compound **4** under P under anaerobic (Ar) conditions; Lane A7, colDNA plus compound **4** under P in the presence of $NaN_3$ (Az: $1.00 \times 10^{-3}$ mol dm$^{-3}$); Lane A8, colDNA plus compound **4** under P in the presence of TEMPO radical (T: $1.00 \times 10^{-3}$ mol dm$^{-3}$); Lane B3, colDNA plus pyrene ($C_3$: $3.8 \times 10^{-6}$ mol dm$^{-3}$) under P.

One can consider, therefore, that the same C–B bond scission takes place in the presence of calf thymus DNA.

The DNA photocleavage experiments were performed by illumination of an aqueous solution (pH 7.50 with 10.0 mmol dm$^{-3}$ sodium hydrogen carbonate) containing double-strand supercoiled circular DNA plasmid ColE1 (colDNA $1.8 \times 10^{-6}$ mol dm$^{-3}$) and acid **4** ($3.8 \times 10^{-6}$ mol dm$^{-3}$) for 30 min with the transilluminator at 30 °C.[20] The cleavage products were analyzed by agarose gel electrophoresis, monitoring the conversion of supercoiled colDNA (Form I) into nicked colDNA (Form II) and thence into linear DNA (Form III). Typical experimental results are shown in Fig. 2. It is seen from Lanes A1 and A2 that restriction enzyme *Eco*RI perfectly converts Form I into Form III, indicating that ColEI has been cut at one point, cleaving both strands. Comparison of Lane A3 with Lane A4 indicates that compound **4** is inactive in the dark whereas it is photoactivated to convert Form I into Form II under photoirradiation: that is, boronic acid **4** can efficiently cleave colDNA with the aid of light. As a control experiment, we used pyrene instead of acid **4** and attempted the photocleavage in the same manner as in Lane A4. Gel electrophoresis showed that colDNA is not cleaved at all, as shown in lane B3. On the other hand, the addition of D-fructose, which changes a neutral boronic acid to an anionic boronate ester,[6,7] efficiently suppresses the cleavage activity (Lane A5). The tendency is in line with the spectroscopic data that the addition of D-fructose induces the dissociation of compound **4** from colDNA.[12] This is further supported by the fact that the addition of the same amount of isopropyl alcohol (used as a non-boronic-acid-binding reference compound) does not affect the cleavage activity at all. These findings consistently establish that compound **4** serves as a novel colDNA cleavage reagent, the activity of which is controllable by two dimensional factors, *i.e.*, light and saccharides.

In general, the photochemical DNA cleavage mechanisms are classified into two categories; that is, photochemical generation of $^1O_2$ or radical species.[21–24] As shown in Lane A6, the cleavage activity of acid **4** is further enhanced under anaerobic (Ar) conditions and colDNA is more efficiently cleaved to yield Form III. The result supports the view that the activation of $O_2$ is not essential in the present system. This proposal is further corroborated by Lane A7 which shows that the addition of $NaN_3$ (as a $^1O_2$ quencher) is totally ineffective. In contrast, when 2,2,6,6-tetramethylpiperidine-*N*-oxyl radical (TEMPO) as a radical scavenger was added, the cleavage activity was reduced to 41–59% (repeated three times: Lane A8). In a separate study, we have confirmed from fluorescence spectroscopy that TEMPO does not quench the excited state of boronic acid **4**. These results now allow us to firmly propose that the C–B

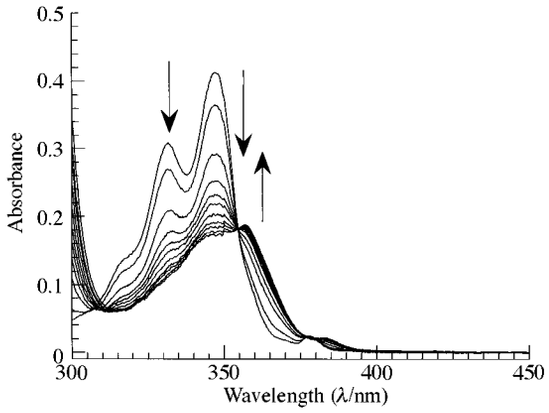


**Fig. 3** Absorption spectral change in compound **5** ($1.0 \times 10^{-6}$ mol dm$^{-3}$) induced by the addition of calf thymus DNA ($4.0 \times 10^{-5}$ to $2.8 \times 10^{-4}$ mol dm$^{-3}$): 25 °C, water–DMSO (1000 : 1 v/v), pH 6.50 with 10.0 mmol dm$^{-3}$ phosphate buffer

bond in compound **4** is photochemically cleaved to generate a radical species, which eventually cleaves colDNA.

**Pyrene-1,6-diyldiboronic acid 5–DNA interactions**

A spectra change in compound **5** induced by the addition of calf thymus DNA is shown in Fig. 3. It is seen from Fig. 3 that with increasing DNA concentration the absorption maxima at 331 nm and 347 nm decrease while a new absorption maximum appears at 357 nm. This spectral change suggests that compound **5** is also bound to calf thymus DNA mainly due to the π–π stacking interaction.[18] Although a shorter-wavelength isosbestic point at ~307 nm is disordered by the background absorption of calf thymus DNA, a longer-wavelength isosbestic point at 355 nm is very tight. This suggests that the binding of compound **5** to calf thymus DNA is also a simple one-step process as observed for the binding of compound **4**. The *K*- and the *n*-values for compound **5** were estimated from the Scatchard plot[19,20] (correlation coefficient 0.98): $K = 1.6 \times 10^6$ dm$^3$ mol$^{-1}$ and $n = 0.06$. The *K*-value is higher by about one order of magnitude than that for compound **4** ($K = 1.3 \times 10^5$ dm$^3$ mol$^{-1}$ and $n = 0.08$). Presumably, this difference is attributed to electron deficiency in the pyrene ring caused by two electron-withdrawing boronic acid groups.

Photodecomposition of compound **5** ($1.0 \times 10^{-5}$ mol dm$^{-3}$) was carried out at 30 °C in water–MeCN (3 : 7 v/v) and compared with that of compound **4** under the same reaction conditions. The analytical results showed that pyrene is produced in 65.3% yield. The photochemical reaction of compound **5** was also carried out in aqueous solution (30 °C, pH 6.50 with 10.0 mmol dm$^{-3}$ phosphate, 0.1 vol% DMSO) in the presence of calf thymus DNA ($3.3 \times 10^{-5}$ mol dm$^{-3}$). After 30 min of photoirradiation the absorption spectrum became similar to that of calf thymus DNA plus pyrene. One can consider, therefore, that the same C–B bond scission can take place in the presence of calf thymus DNA.

The DNA photocleavage experiments were performed by illumination of an aqueous solution (pH 6.50 with 10.0 mmol dm$^{-3}$ phosphate) containing colDNA ($1.0 \times 10^{-6}$ mol dm$^{-3}$) and compound **5** ($1.0 \times 10^{-6}$ mol dm$^{-3}$) for 30 min with the transilluminator at 30 °C. Typical experimental results are shown in Fig. 4. Comparison of Lane A3 with Lane A4 indicates that compound **5** is inactive in the dark whereas it is activated to convert Form I into Form II and Form III under photoirradiation: that is, compound **5** can efficiently cleave colDNA with the aid of light. On the other hand, the addition, of D-fructose, which changes a neutral boronic acid to an anionic boronate ester,[6,7] suppresses the cleavage activity (Lane A5).

To clarify the photochemical DNA cleavage mechanism with compound **5**, we carried out the following experiments. As shown in Lane A7, the addition of $NaN_3$ (as a $^1O_2$ quencher)

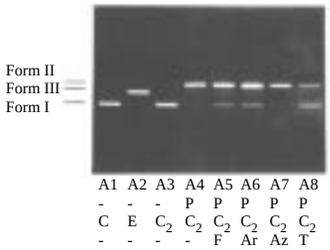


**Fig. 4** Gel electrophoresis showing results of photochemical cleavage of supercoiled circular double-strand DNA plasmid ColE1 (Form I) into nicked colDNA (Form II) and into linear DNA (Form III): Lane A1, control (C: untreated colDNA $1.0 \times 10^{-5}$ mol dm$^{-3}$); Lane A2, colDNA with restriction enzyme *Eco*RI (E: 0.1 unit/μl); Lane A3, colDNA plus compound **5** ($C_2$: $1.0 \times 10^{-6}$ mol dm$^{-3}$) in the dark; Lane A4, colDNA plus compound **5** under photoirradiation (P); Lane A5, colDNA plus compound **5** under P in the presence of D-fructose (F: $1.0 \times 10^{-2}$ mol dm$^{-3}$); Lane A6, colDNA plus compound **5** under P under anaerobic (Ar) conditions; Lane A7, colDNA plus compound **5** under P in the presence of $NaN_3$ (Az: $1.0 \times 10^{-3}$ mol dm$^{-3}$); Lane A8, colDNA plus compound **5** under P in the presence of TEMPO radical (T: $1.0 \times 10^{-3}$ mol dm$^{-3}$)

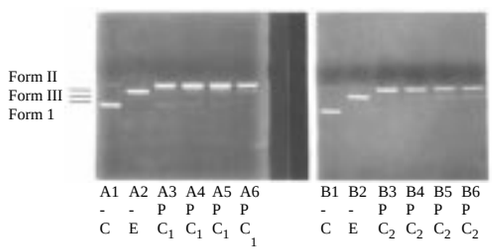


**Fig. 5** Gel electrophoresis showing results of photochemical cleavage of supercoiled circular double-strand DNA plasmid ColE1 (Form I) into nicked colDNA (Form II) and into linear DNA (Form III): Lane A1 and B1, control (C: untreated colDNA $1.0 \times 10^{-5}$ mol dm$^{-3}$); Lane A2 and B2, colDNA with restriction enzyme *Eco*RI (E: 0.1 unit/μl); Lanes A3–A6, colDNA plus compound **4** ($C_1$: 1.0–4.0 $\times 10^{-6}$ mol dm$^{-3}$) under photoirradiation (P); Lanes B3–B6, colDNA plus compound **5** ($C_2$: 1.0–4.0 $\times 10^{-6}$ mol dm$^{-3}$) under P

was totally ineffective. When 2,2,6,6-tetramethylpiperidine-*N*-oxyl radical (TEMPO) as a radical scavenger was added, the cleavage activity was reduced to 39% (repeated three times: lane A8). In a separate study, we have confirmed from fluorescence spectroscopy that TEMPO does not quench the excited state of compound **5**. These findings suggest that the DNA cleavage occurs according to the radical mechanism. The results in Lane A6 are not necessarily compatible with this proposal, however: the anaerobic (Ar) conditions, which should facilitate the radical reaction, suppressed (although weakly) this DNA cleavage reaction. We presume that the third mechanism which is effected by $O_2$ may partially contribute to this system.

The final test, which is most intriguing and directly associated with molecular design of compound **5**, was carried out to compare the activity of diradical-forming substrate **5** with that of monoradical-forming substrate **4** under identical reaction conditions (Fig. 5). In diradical-forming compound **5** the band for Form I was not detectable, whereas in monoradical-forming monoboronic acid **4** Form I was still observable (**4**; 1.0–$3.0 \times 10^{-6}$ mol dm$^{-3}$). The difference indicates that diacid **5** is more active than monoacid **4** as a photochemical DNA cleavage reagent. We had observed the time course of conversion to From II and Form III by compound **4**. According to time progress (15 min to 60 min), we found an increase of Form III and a decrease in Form II, so generation of Form III by from acid **4** from the close proximity of two separate single-stranded cleavage events. The concentrations of Form II and Form III on gel electrophoresis plates were determined by optical densitometry measurements. The ratio of FormIII/Form II is plotted in Fig. 6 as a function of boronic acid **4** or **5** concentration. It is clearly seen from Fig. 6 that compound **5** is able to yield Form

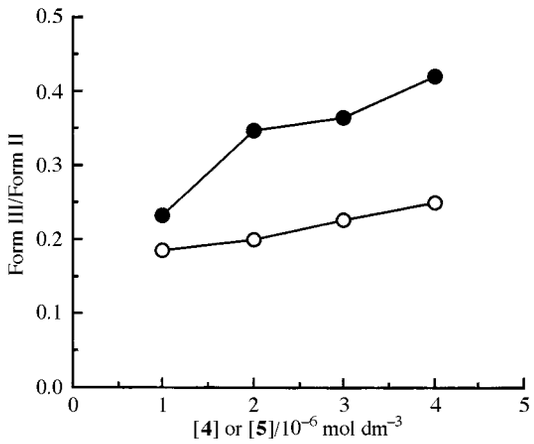


**Fig. 6** The quotient of Form III/Form II ColE1 DNA induced by photochemical cleavage in the presence of monoacid **4** (○: 1.0–4.0 × $10^{-6}$ mol $dm^{-3}$) and diacid **5** (●: 1.0–4.0 × $10^{-6}$ mol $dm^{-3}$)

III more efficiently than its analogue **4**: that is, **5** tends to cleave ColE1 at two reaction sites as enediyne antitumor antibiotics do.

## Conclusions

In conclusion, compounds **4** and **5** were bound to supercoiled double-strand circular DNA plasmid ColE1 and effected its efficient photocleavage under visible-light irradiation. Mechanistic studies showed that the cleavage occurs according to the radical mechanism. Compound **5** was bound to colDNA more strongly than was its analogue **4** and effected its efficient photocleavage to yield Form III. The present study has demonstrated that compound **5**, which was designed on the basis of the mechanistic view of enediyne antitumor antibiotics, can effect its photocleavage to yield Form III. The results imply that the mechanistic approach is an expeditious way to reproduce the basic function of these antibiotics. Further studies towards mechanistic elaboration of these reagents are currently continuing in these laboratories.

## Experimental

### Materials

Sonicated calf thymus DNA was purchased from Pharmacia Co. Inc. ColE1 DNA was purchased from Wako Pure Chemical Industries, Ltd.

### Measurements

Mps were measured on a Yanoco (HP-500D) micro melting point apparatus, and are uncorrected. UV-visible spectra were recorded at 25 °C on a Shimadzu UV-2200 UV–VIS Spectrophotometer. NMR spectra were measured on a Bruker AC-250P spectrometer. *J*-Values are in Hz.

### Pyren-1-ylboronic acid 4

1-Bromopyrene (4.22 g, 15.0 mmol) was dissolved in anhydrous diethyl ether (210 ml) and to this solution was added *n*-butyllithium (10.6 ml of a 1.60 mol $dm^{-3}$ solution in hexane, 17.0 mmol) at 0 °C. The content of the flask was poured into another flask which contained trimethyl borate (7.9 g, 0.076 mol) in 20 ml of anhydrous diethyl ether at −78 °C over a 30 min period. The mixture was stirred for 3 h at −50 to −70 °C and for 60 h at room temperature. Aq. 2 mol $dm^{-3}$ HCl (80 ml) was added and the mixture was stirred for 2 h. The ether solution was separated and washed twice with 200 ml of water. After being dried over magnesium sulfate and then treated with charcoal, the ethereal solution was concentrated to dryness. The residue was dried in a vacuum oven at 50 °C: pale yellow powder (2.25 g, 61%), mp 293.0–297.0 °C; $\nu_{max}$(KBr disk)/$cm^{-1}$ 3282 (OH); $\delta_H$[250 MHz; $CD_3OD$; TMS ($SiMe_4$); 27 °C] 8.73 (1H, d, *J* 9.1, 2-H) and 8.01–8.31 (8H, m, ArH). Elemental analysis was done on 5,5′-*dimethyl*-2-(*pyren*-1-*yl*)-1,3,2-*dioxaborinane* which was derived from compound **4** with 2,2-dimethylpropane-1,3-diol (Found: C, 80.18; H, 6.22. $C_{21}H_{19}BO_2$ requires C, 80.28; H, 6.10%).

### 1,6-Dibromopyrene 6

Compound **6** was synthesized according to the method in ref. 25. To a stirred solution of pyrene (12.2 g, 60 mmol) in $CCl_4$ (300 ml) under $N_2$ at room temperature was added dropwise a $CCl_4$ solution (50 ml) of bromine (6.32 ml, 0.126 mol) over a period of 4 h. After the mixture had been stirred for 48 h, the precipitate was collected by filtration, washed with methanol and recrystallized from toluene to afford *dibromide* **6** (8.3 g, 38.4%), mp 210–211 °C; $\delta_H$(250 MHz; [$^2H_6$]DMSO; 130 °C) 8.25 (2H, d, *J* 5.15, 3- and 8-H), 8.33 (2H, d, *J* 5.80, 4- and 9-H), 8.36 (2H, d, *J* 5.15, 2- and 7-H) and 8.42 (2H, d, *J* 5.80, 5- and 10-H) (Found: C, 53.37; H, 2.24. $C_{16}H_8Br_2$ requires C, 52.45; H, 2.24%).

### 1,6-Bis(trimethylsilyl)pyrene 7

Compounds **7** and **5** were synthesized after the method described in ref. 26. To a stirred solution of compound **6** (2.0 g, 5.6 mmol) in THF (300 ml) under $N_2$ at −78 °C was added dropwise *n*-butyllithium (8.0 ml of a 1.60 mol $dm^{-3}$ solution in hexane; 12.8 mmol) from a syringe over a period of 30 min. After 30 min, trimethylsilyl chloride (4.85 ml, 12.8 mmol) was added and the mixture was stirred further for 1 h at −78 °C. Then, it was left to reach room temperature (1 h). The reaction was quenched with water and the solution was concentrated *in vacuo*. The residue was dissolved in $CHCl_3$, washed with brine, dried over $MgSO_4$ and concentrated to dryness. Recrystallization from $CHCl_3$ and MeOH gave 1,6-*bis*(*trimethylsilyl*)*pyrene* **7** (0.98 g, 52%), mp 224–225 °C; $\delta_H$(250 MHz; $CDCl_3$; 25 °C) 0.59 (18H, s, $SiCH_3$), 8.09 (2H, d, *J* 9.12, 4- and 9-H), 8.18 (4H, m, 2-, 3-, 7- and 8-H) and 8.37 (2H, d, *J* 9.12, 5- and 10-H) (Found: C, 72.78; H, 7.21. $C_{22}H_{26}Si_2 \cdot 0.16CHCl_3$ requires C, 72.62; H, 7.25%).

### Pyrene-1,6-diyldiboronic acid dimethyl ester 5

To a stirred solution of compound **7** (960 mg, 2.77 mmol) in $CH_2Cl_2$ (30 ml) under $N_2$ at −78 °C was added $BBr_3$ (0.93 ml, 9.71 mmol) from a disposable syringe through a rubber septum. The solution was stirred at −78 °C for 1 h, allowed to reach room temperature (0.5 h) and then refluxed for 2 h. The solution was cooled to −78 °C and a solution of aq. 3.0 mol $dm^{-3}$ NaOH (5 ml) was added from the top of the condenser. The solution was allowed to reach room temperature and the free acid precipitate was collected by filtration. This precipitate was purified by reprecipitation from THF to MeOH to give the *title ester* (0.48 g, 60.0%), mp > 220 °C (decomp.); $\delta_H$(250 MHz; [$^2H_6$]DMSO; 25 °C) 3.56 (12H, s, $OCH_3$) and 8.18–8.67 (8H, m, ArH) (Found: C, 69.13; H, 5.89. $C_{20}H_{20}B_2O_4 \cdot 0.1CH_3OH$ requires C, 68.77; H, 5.46%).

### Photodecomposition

Photodecomposition of compounds **4** (1.00 × $10^{-5}$ mol $dm^{-3}$) and **5** (1.0 × $10^{-5}$ mol $dm^{-3}$) was carried out at 30 °C in water–MeCN (3 : 7 v/v). A 365 nm longwave UV tube transilluminator (Ultra Violet Products Limited, LMS-20E) was used as a light source.[20] After 30 min the solution was subjected to HPLC analysis [J′ sphere ODS-H80; water–$CH_3CN$ (3 : 7 v/v); 30 °C].

### DNA photocleavage experiments

DNA photocleavage experiments were performed by illumination of an aqueous solution (pH 6.5 or pH 7.0 with 10.0 mmol $dm^{-3}$ phosphate) containing double-strand supercoiled circular DNA plasmid ColE1 (colDNA 1.8 × $10^{-6}$ mol $dm^{-3}$ or colDNA 1.0 × $10^{-6}$ mol $dm^{-3}$) and compound **4** (3.8 × $10^{-6}$ mol

$dm^{-3}$) or diacid **5** ($1.0 \times 10^{-6}$ mol $dm^{-3}$) for 30 min with the transilluminator at 30 °C from a distance of 10 cm (4900 μW $cm^{-2}$). The cleavage products were analyzed by agarose gel electrophoresis, monitoring the conversion of supercoiled colDNA (Form I) into nicked colDNA (Form II) and into linear DNA (Form III).

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