PHOTOCLEAVAGE OF DNA BY THE HALO BENZAMIDE DERIVATIVES OF OLIGO-*N*-METHYLPYRROLECARBOXAMIDES

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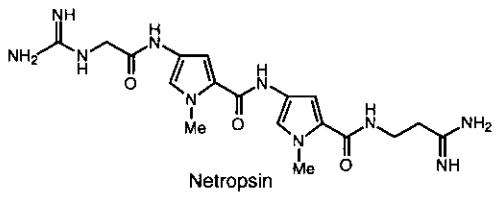
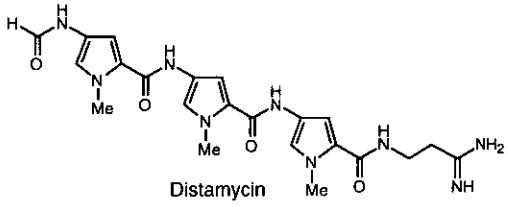
Abstract - The preparation of halo benzamide photonuclease containing DNA minor groove binding units of varying lengths is described. These molecules have been assayed for DNA-cleaving ability, and bromo and iodo derivatives were found to be very effective. The reaction mechanism was also investigated and a benzenoid carbon radical was shown to be responsible for the reaction.

Low molecular weight substances which possess both sequence-specific DNA binding and cleaving activities have generated considerable interest in the field of medicinal and bioorganic chemistry.[1] Among them, oligo-*N*-methylpyrrolecarboxamides such as Netropsin and Distamycin and their analogues have



Netropsin

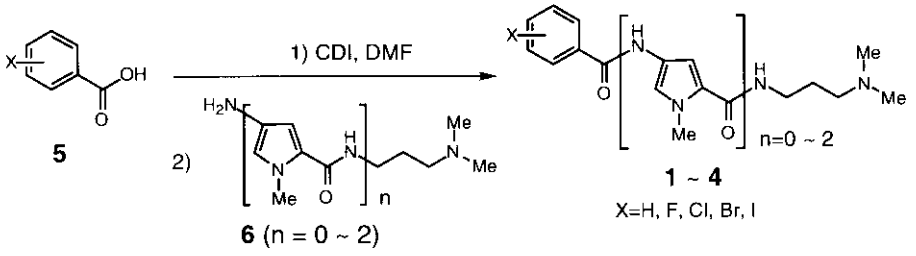


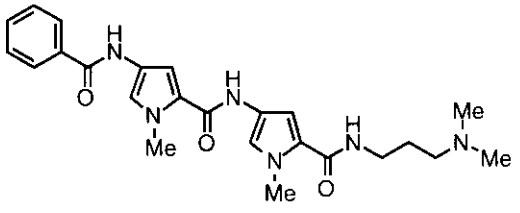
Distamycin

attracted attention because of their strong minor groove nonintercalative binding ability to double-stranded B-DNA at specific AT rich region.[2] Recently, a major advance has been represented by the development of 'hairpin' oligomers of imidazole- and pyrrolecarboxamides that allowed recognition of any four natural Watson-Crick base pairs.[3] On the other hand, the rational design of compounds which cleave DNA under photo-irradiation is of great importance not only from a bioorganic point of view but also in a photodynamic therapeutic approach as antitumor agents.[4] We have reported the synthesis of several oligo-*N*-methylpyrrolecarboxamide derivatives which have been shown to efficiently cleave DNA upon photochemical activation.[5] As it has been reported for the UV-induced DNA cleavage by various halogenated compounds,[6] including oligo-*N*-methylpyrrolecarboxamide derivatives prepared by us,[5a,b,d,f] halogenated aromatic compounds are generally expected to cleave DNA under photo-irradiation *via* carbon radical formation. Herein we describe the synthesis of novel and simple halogenated benzamide

derivatives of oligo-*N*-methylpyrrolecarboxamide and the study on their potent DNA cleaving activities under UV-A irradiation.

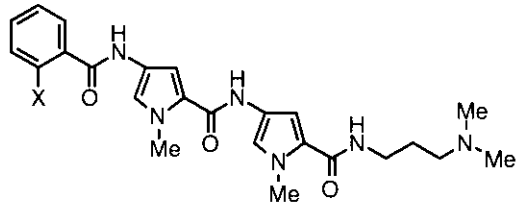
Compounds (**2** ~ **4**), which contain *ortho-*, *meta-*, or *para*-substituted halogenated benzamide terminals, were prepared. For reference of the activity, benzamide derivative (**1c**) was synthesized, as well. Since it has been reported that increasing the number of *N*-methylpyrrole units increases the binding affinity with DNA,[1c] compounds having zero, one, and two pyrrole units were prepared for the *para*-substituted benzamide derivatives to explore the effect on cleavage efficiency. Commercially available benzoic acid derivatives (**5**) were activated by using *N,N'*-carbonyldiimidazole, and then condensed with aminopyrroles (**6**, n = 1, 2)[7] or dimethylaminopropylamine (**6**, n = 0) to afford compounds (**1** ~ **4**) in the yields summarized in Table 1.





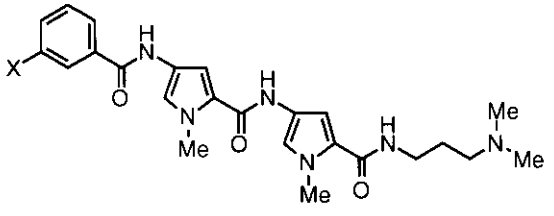
1c

2c (*ortho*-X)



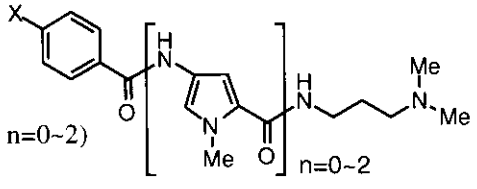
2c-F : X = F
2c-Cl : X = Cl
2c-Br : X = Br
2c-I: X = I

3c (*meta*-X)



3c-F : X = F
3c-Cl : X = Cl
3c-Br : X = Br
3c-I: X = I

4a~c (*para*-X, n=0~2)



4a-F :	X = F,	n = 0	**4a-Br** :	X = Br,	n = 0
4b-F :	X = F,	n = 1	**4b-Br** :	X = Br,	n = 1
4c-F :	X = F,	n = 2	**4c-Br** :	X = Br,	n = 2
4a-Cl :	X = Cl,	n = 0	**4a-I** :	X = I,	n = 0
4b-Cl :	X = Cl,	n = 1	**4b-I** :	X = I,	n = 1
4c-Cl :	X = Cl,	n = 2	**4c-I** :	X = I,	n = 2

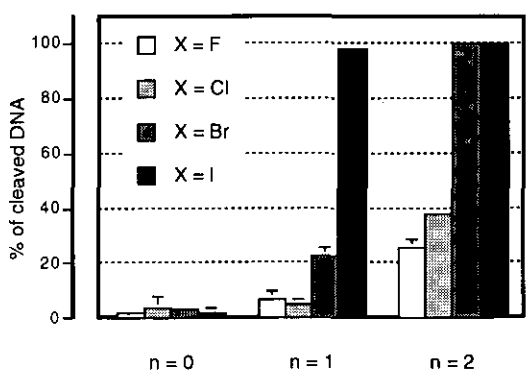
Table 1. Synthesized compounds and yields

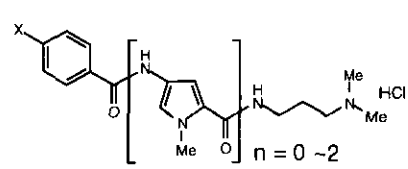
Compound	X	Yield (%)		
		n = 0 (a)	n = 1 (b)	n = 2 (c)
1c	H	-	-	74
2c-F	*o*-F	-	-	44
3c-F	*m*-F	-	-	65
4a~c-F	*p*-F	100	50	55
2c-Cl	*o*-Cl	-	-	31
3c-Cl	*m*-Cl	-	-	53
4a~c-Cl	*p*-Cl	99	49	51
2c-Br	*o*-Br	-	-	32
3c-Br	*m*-Br	-	-	41
4a~c-Br	*p*-Br	93	61	41
2c-I	*o*-I	-	-	36
3c-I	*m*-I	-	-	30
4a~c-I	*p*-I	95	64	42

The DNA cleaving activity of *para*-halo derivatives (**4a~c**) was initially tested by monitoring the conversion of closed circular (form I) to open circular (form II) and linear (form III) DNA. Compounds (10 μM) were irradiated with UV-A light (365 nm maximum, 13 $J \cdot m^{-2} \cdot sec^{-1}$) in the presence of plasmid Col E1 (*ca.* 40 μg/ml) and the results are shown in Figure 1. In all cases, single-strand cleavage (from form I to form II) was predominant. Form III was observed (less than 5%, data not shown) with bromo and iodo derivatives (**4b-Br**, **4c-Br**, **4b-I**, **4c-I**). The cleaving activity increased with increasing number of *N*-methylpyrrole units, as was expected from the enhanced association of the compound with the DNA minor groove.

The cleavage efficiency of various halogen compounds was next investigated by a similar method. Figure 2 summarizes the relative cleavage efficiency of compounds (**2c** ~ **4c**) by comparing the activities at 0.1 ~ 100 μM drug concentrations. As the strength of the carbon-halogen bond increases, the photolysis is expected to proceed with increasing difficulty. In fact, the potency of activities was in order of F- < Cl- « Br- < I- derivative. Figure 3 presents a graphical representation of DNA-cleaving activities plotted against

Figure 1. Photo-induced DNA-cleavage by compounds (**4a~c**) (10 μM concentrations).





4a: n = 0, **4b**: n = 1, **4c**: n = 2

X = F, Cl, Br, I

Figure 2. Photo-induced DNA-cleavage by halogenated benzamide derivatives (**2c** ~ **4c**).

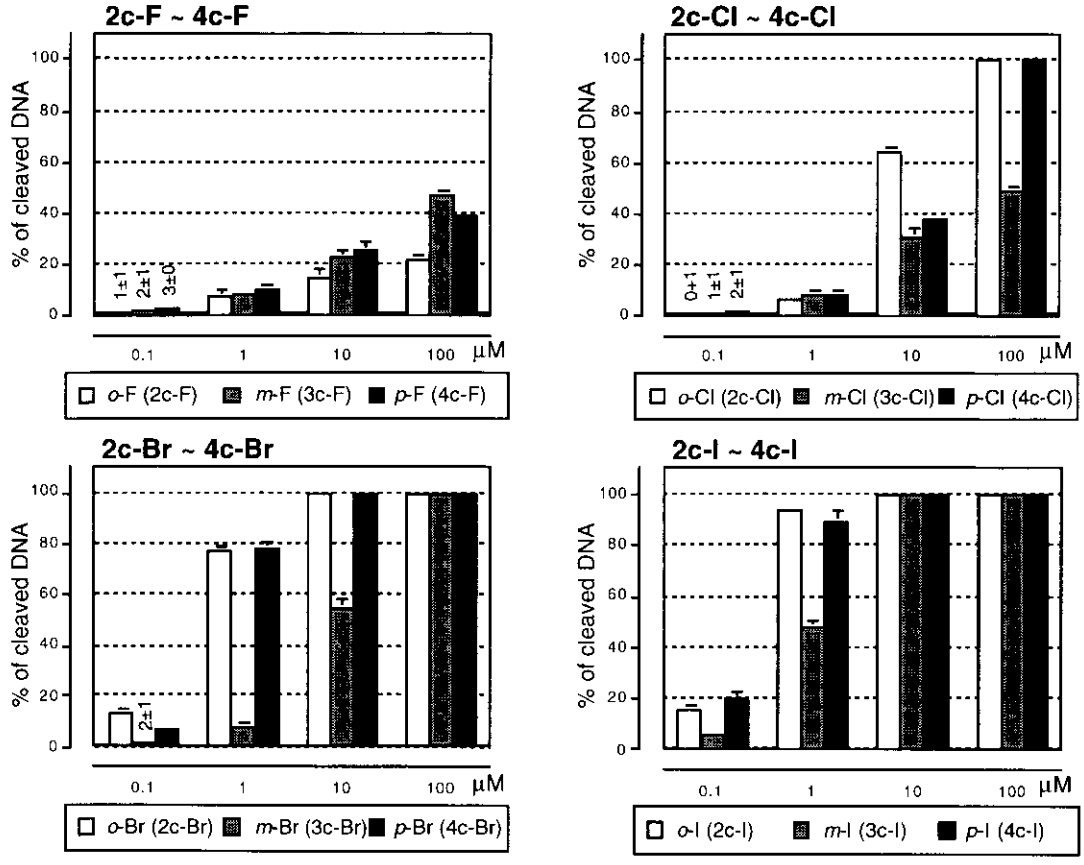


Figure 3.
Graphical representation of DNA-cleaving activities plotted against C-X bond dissociation energies calculated for a model structure (**5**).

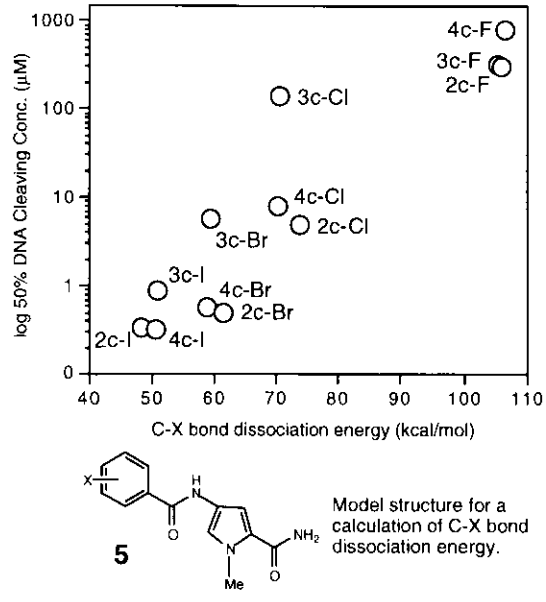
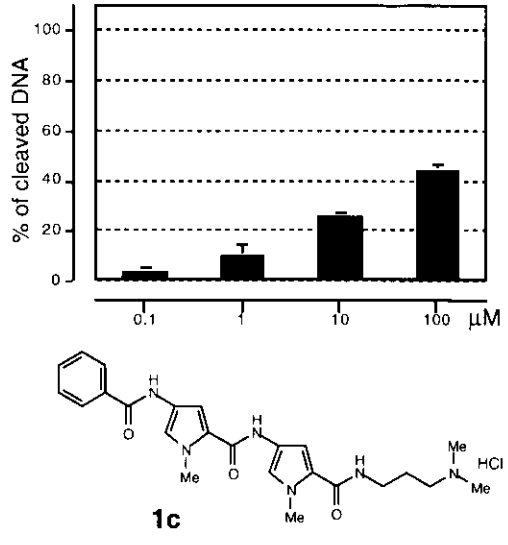


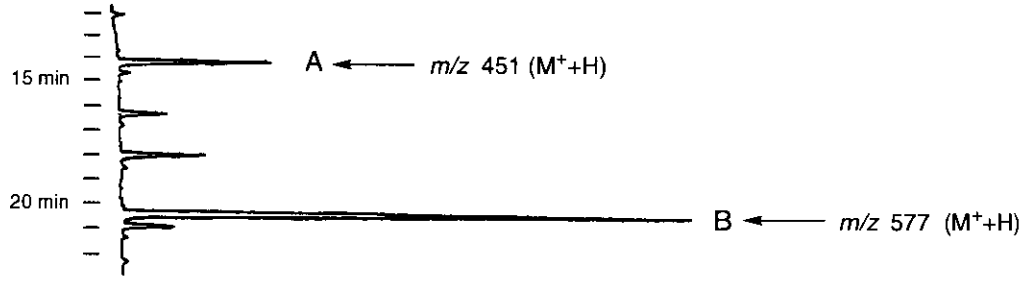
Figure 4.
Photo-induced DNA-cleavage by benzamide derivative (**1c**).

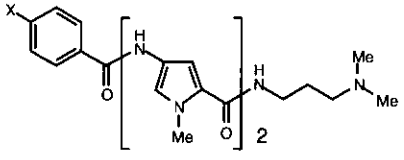


C-X bond dissociation energies calculated by the Sony-Tektronix CAChe-MOPAC program for a model structure (**5**). The DNA cleavage ability of non-halogenated compound (**1c**) was tested to compare with that of the corresponding halogenated compounds and was found to be quite similar to that of fluoro derivatives (Figure 4). We previously reported the synthesis of various oligo-*N*-methylpyrrolecarboxsamides which do not possess special side groups sensitive to UV light.[5c,e] Their DNA-cleaving activities under UV-A irradiation were demonstrated and the mechanism in which a hydroxyl radical (•OH) participates was proposed. To investigate the participation of the hydroxyl radical in the reaction of **1c**, the experiment was carried out in the presence of hydroxyl radical scavengers such as phenol, potassium iodide, sodium formate, and sodium benzoate. Tested at two different concentrations, these four scavengers partially inhibited the nicking reactions (data not shown). Therefore, the relatively lower cleavage ability of **1c** and **2c-F** ~ **4c-F** must be due to hydroxyl radical generation under photo-irradiation. On the other hand, the major participation of an aryl radical, produced by photo-homolysis of the carbon-halogen bond, is probable for the action of chloro, bromo, and iodo derivatives. Among them, *ortho*- and *para*-substituted benzamide derivatives showed relatively higher activities than *meta*-substituted derivatives (Figure 2).

To confirm the carbon radical formation under photo-irradiation, a solution containing **4c-I** and 1,2-(1,4-cyclohexadiene)dimethanol[8] (hydrogen radical-donor) in water was irradiated with UV-A lamp at 25°C for 24 h. HPLC analysis of the reaction mixture was conducted and the result is shown in Figure 5. Product A was isolated and identified as **1c** by ^{1}H NMR and FABMS (m/z 451, M$^+$+H), while a major product B was

Figure 5. HPLC profiles of the UV-irradiation products of **4c-I**. A solution containing **4c-I** and 1,2-(1,4-cyclohexadiene)dimethanol in MeCN was irradiated with UV-A lamp at 25°C for 24 h. The reaction mixture was analyzed on YMC R-ODS-5 column (6x250 mm); eluated with 0.1% aqueous trifluoroacetic acid, 20 ~ 80% MeCN, linear gradient, 40 min, at a flow rate of 1 mL/min.





1c (X = H): MW = 450.54
4c-I (X = I) : MW = 576.44

identified as unreacted **4c-I**. This result suggests the carbon radical formation under the DNA-cleaving reaction, although in a quite low concentration.

The above studies describe the preparation of halogenated benzamide photonuclease containing DNA minor groove binding units of varying lengths. These molecules have been assayed for DNA-cleaving ability, and bromo and iodo derivatives were found to be very effective. The DNA base pair selectivity of these molecules is under investigation.

EXPERIMENTAL

Melting points were determined by the capillary method and are uncorrected. Column chromatography was performed on silica gel (60 K-620, from Katayama Chemicals) at 0.5 kg/cm^2 pressure. IR spectra were recorded on a Perkin-Elmer 1720 Infrared Fourier Transform Spectrophotometer. ^{1}H-NMR spectra were measured with a JEOL JMS FX-200 spectrometer using tetramethylsilane as an internal reference. UV spectra were recorded on HITACHI 330 spectrophotometer. MS spectra were recorded on a JEOL D-300, JMS-DX303, or JMS-SX102A instrument. High-performance liquid chromatography was performed on a HITACHI L-6200 model. UV irradiation for DNA-cleavage assays was performed with Vilber Lourmat VL-215L (365 nm maximum) fluorescent light. Relative amounts of DNA in form I ~ III were determined with Shimadzu CS-9000 densitometer.

General Procedure for the Synthesis of 4a (n = 0, X = F, Cl, Br, I).

To a stirred solution of *p*-halogenobenzoic acid (**5**) (1.4 mmol) in DMF (3 ml), 1,1'-carbonyldiimidazole (340 mg, 2.1 mmol) was added at 0°C, and then the mixture was allowed to warm to rt. After being stirred for 1 h at this temperature, 3-dimethylaminopropylamine (0.21 mL, 1.7 mmol) was added dropwise to the mixture, and the reaction mixture was stirred for 30 min and then evaporated. The residue was dissolved in $CHCl_3$ and washed with brine, then the organic layer was dried over $MgSO_4$, filtered, concentrated, and chromatographed on silica gel (28% aqueous NH_4OH and MeOH, 1:99 v/v) to give the amide as a pale yellow oil. A solution of hydrogen chloride in AcOEt (4N, 0.36 mL, 1.4 mmol) was added to a solution of the product in MeOH (2 mL) at 0°C and then evaporated. The residue was recrystallized from EtOH to afford the hydrochloride.

3-(4-Fluorobenzamido)dimethylaminopropane hydrochloride (4a-F). Pale yellow needles, mp 124-130 °C. FT-IR (KBr) : 3312, 1665, and 1505 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.80 (2H, m), 2.44 (6H, s), 2.66 (2H, m), 3.33 (2H, m), 5.18 (1H, br s), 7.24 (2H, m), 7.94 (2H, m), and 8.64 (1H, m). UV (H_2O) λmax nm (log ε) : 228 (4.08). EI-MS : *m/z* 224 (M^+). HRMS : *m/z* calcd for $C_{12}H_{17}N_2OF$ 224.1325, Found 224.1339.

3-(4-Chlorobenzamido)dimethylaminopropane hydrochloride (4a-Cl). Pale yellow amorphous solid. FT-IR (KBr) : 3436, 1640, and 1544 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.94 (2H, m), 2.73 (3H, s), 2.75 (3H, s), 3.09 (2H, m), 3.34 (2H, m), 7.52 (2H, d, J=8.1 Hz), 7.92 (2H, d, J=8.1 Hz), 8.83 (1H, m), and

10.53 (1H, br s). UV (H_2O) λmax nm (log ε) : 239 (4.17). EI-MS : *m/z* 240 (M^+). HRMS : *m/z* calcd for $C_{12}H_{17}N_2OCl$ 240.1029, Found 240.1037.

3-(4-Bromobenzamido)dimethylaminopropane hydrochloride (4a-Br). Pale yellow powder, mp 128-130 °C. FT-IR (KBr) : 3465, 3324, 2686, 1630, and 1545 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.94 (2H, m), 2.72 (3H, s), 2.75 (3H, s), 3.08 (2H, m), 3.34 (2H, m), 7.66 (2H, d, J=8.1 Hz), 7.85 (2H, d, J=8.1 Hz), 8.85 (1H, m), and 10.61 (1H, br s). UV (H_2O) λmax nm (log ε) : 243(4.17). EI-MS : *m/z* 284 (M^+). HRMS : *m/z* calcd for $C_{12}H_{17}N_2OBr$ 284.0524, Found 284.0529.

3-(4-Iodobenzamido)dimethylaminopropane hydrochloride (4a-I). Pale yellow powder, mp 158-160 °C. FT-IR (KBr) : 3339, 1636, 1589, and 1544 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.94 (2H, m), 2.75 (3H, s), 2.77 (3H, s), 3.09 (2H, m), 3.35 (2H, m), 7.67 (2H, d, J=7.6 Hz), 7.84 (2H, d, J=7.6 Hz), 8.76 (1H, br m), and 10.28 (1H, br s). UV (H_2O) λmax nm (log ε) : 253 (4.17). EI-MS : *m/z* 332 (M^+). HRMS : *m/z* calcd for $C_{12}H_{17}N_2OI$ 332.0386, Found 332.0384.

General Procedure for the Synthesis of 4b (n = 1, X = F, Cl, Br, I).

A suspension of PtO_2 (20 mg) in a solution of 3-(1-methyl-4-nitropyrrole-2-carboxamido)dimethylaminopropane[7] (204 mg, 0.8 mmol) in MeOH (10 mL) was stirred for 2 h under a current of H_2 at rt, and then filtered. The residual catalyst was washed with MeOH and the combined filtrate and washings were evaporated to give the crude amine (**6**, n=1), which was used for further reaction without purification. To a stirred solution of *p*-halogenobenzoic acid (**5**) (1.2 mmol) in DMF (3 mL), 1,1'-carbonyldiimidazole (234 mg, 1.4 mmol) was added at 0°C, and then the mixture was allowed to warm to rt. After beeng stirred for 1 h at this temperature, a solution of crude amine (**6**, n=1) in DMF (3 mL) was added dropwise to the mixture, and the reaction mixture was stirred for 4 h and then evaporated. The residue was dissolved in $CHCl_3$ and the organic layer was washed with brine, then dried over $MgSO_4$, filtered, concentrated, and chromatographed on silica gel (28% aqueous NH_4OH, MeOH, and $CHCl_3$, 0.3:9.7:90 v/v) to give the amorphous product. By the same procedure as that described for **4a**, the product was converted into hydrochloride.

3-[1-Methyl-4-(4-fluorobenzamido)pyrrole-2-carboxamido]dimethylaminopropane hydrochloride (4b-F). Pale red amorphous solid; FT-IR (KBr) : 3423, 1640, and 1528 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.88 (2H, m), 2.73 (3H, s), 2.76 (3H, s), 3.06 (2H, m), 3.18 (2H, m), 3.84 (3H, s), 6.96 (1H, s), 7.28~7.36 (3H, m), 7.99~8.06 (2H, m), 8.23 (1H, m), 10.34 (1H, br s), and 10.38 (1H, s). UV (H_2O) λmax nm (log ε) : 237 (4.17), 280 (4.13). EI-MS : *m/z* 346 (M^+). HRMS : *m/z* calcd for $C_{18}H_{23}N_4O_2F$ 346.1805, Found 346.1812.

3-[1-Methyl-4-(4-chlorobenzamido)pyrrole-2-carboxamido]dimethylaminopropane hydrochloride (4b-Cl). Pale red amorphous solid; FT-IR (KBr) : 3424, 1640, and 1528 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.89 (2H, m), 2.73 (3H, s), 2.76 (3H, s), 3.06 (2H, m), 3.25 (2H, m), 3.84 (3H, s), 6.97 (1H, d, J=1.5 Hz), 7.30 (1H, d, J=1.5 Hz), 7.57 (2H, d, J=8.4 Hz), 7.99 (2H, d, J=8.4 Hz), 8.25 (1H, m), 10.45 (1H, br s),

and 10.46 (1H, s). UV (H_2O) λmax nm (log ε) : 241 (4.27), 280 (4.16). EI-MS : *m/z* 362 (M^+). HRMS : *m/z* calcd for $C_{18}H_{23}N_4O_2Cl$ 362.1510, Found 362.1519.

3-[1-Methyl-4-(4-bromobenzamido)pyrrole-2-carboxamido]dimethylaminopropane hydrochloride (4b-Br). Pale yellow amorphous solid; FT-IR (KBr) : 3424, 1639, and 1528 cm^{-1}. ^{1}H-NMR (DMSO-d6, 200 MHz) : δ 1.88 (2H, m), 2.74 (3H, s), 2.77 (3H, s), 3.06 (2H, m), 3.28 (2H, m), 3.84 (3H, s), 6.96 (1H, s), 7.29 (1H, s), 7.71 (2H, d, J=8.4 Hz), 7.91 (2H, d, J=8.4 Hz), 8.23 (1H, m), 10.22 (1H, br s), and 10.42 (1H, s). UV (H_2O) λmax nm (log ε) : 244 (4.29), 282 (4.17). EI-MS : *m/z* 406 (M^+). HRMS : *m/z* calcd for $C_{18}H_{23}N_4O_2Br$ 406.1004, Found 406.0988.

3-[1-Methyl-4-(4-iodobenzenecarboxamido)pyrrole-2-carboxamido]dimethylaminopropane hydrochloride (4b-I). Pale yellow amorphous solid; FT-IR (KBr) : 3415, 1639, and 1543 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.91 (2H, m), 2.74 (3H, s), 2.76 (3H, s), 3.06 (2H, m), 3.27 (2H, m), 3.84 (3H, s), 6.97 (1H, s), 7.30 (1H, s), 7.75 (2H, d, J=8.1 Hz), 7.89 (2H, d, J=8.1 Hz), 8.24 (1H, m), and 10.43 (2H, s). UV (H_2O) λmax nm (log ε) : 254 (4.22). EI-MS : *m/z* 454 (M^+). HRMS : *m/z* calcd for $C_{18}H_{23}N_4O_2I$ 454.0866, Found 454.0867.

General Procedure for the Synthesis of 1c and 2c ~ 4c (n = 2, X = H, F, Cl, Br, I).

A suspension of PtO_2 (20 mg) in a solution of 3-[1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxamido]dimethylaminopropane[7] (301 mg, 0.8 mmol) in MeOH (10 mL) was stirred for 2 h under a current of H_2 at rt, and then filtered. The residual catalyst was washed with MeOH and the combined filtrate and washings were evaporated to give the crude amine (**6**, n=2), which was used for further reaction without purification. To a stirred solution of benzoic acid or *p*-halogenobenzoic acid (**5**) (1.2 mmol) in DMF (3 mL), 1,1'-carbonyldiimidazole (234 mg, 1.4 mmol) was added at 0°C, and then allowed to warm to rt. After beeng stirred for 1 h at this temperature, a solution of crude amine (**6**, n=2) in DMF (3 ml) was added dropwise to the mixture, and the reaction mixture was stirred for 6 h and then the mixture was evaporated. The residue was dissolved in $CHCl_3$ and the solution was washed with brine, then dried over $MgSO_4$, filtered, concentrated, and chromatographed on silica gel (28% aqueous NH_4OH, MeOH, and $CHCl_3$, 0.3:9.7:90 v/v) to give the amorphous product. By the same procedure as that described for **4a**, the product was converted into hydrochloride.

3-{1-Methyl-4-[1-methyl-4-(benzamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}dimethylaminopropane hydrochloride (1c). Pale yellow amorphous solid; FT-IR (KBr) : 3294, 1641, and 1534 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.90 (2H, m), 2.73 (3H, s), 2.76 (3H, s), 3.06 (2H, m), 3.26 (2H, m), 3.83 (3H, s), 3.88 (3H, s), 6.94 (1H, s), 7.13 (1H, s), 7.22 (1H, s), 7.53 (1H, s), 7.45~7.56 (3H, m), 7.98 (2H, d, J=8.1 Hz), 8.20 (1H, m), 9.98 (1H, s), 10.41 (1H, s), and 10.60 (1H, br s). UV (H_2O) λmax nm (log ε) : 298 (4.34). FAB-MS : *m/z* 451 (M^++H). HRMS : *m/z* calcd for $C_{24}H_{31}N_6O_3$ 451.2458, Found 451.2468.

3-{1-Methyl-4-[1-methyl-4-(2-fluorobenzamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-dimethylaminopropane hydrochloride (2c-F). FT-IR (KBr) : 3288, 1641, and 1534 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.89 (2H, m), 2.74 (3H, s), 2.76 (3H, s), 3.06 (2H, m), 3.26 (2H, m), 3.82 (3H, s), 3.87 (3H, s), 6.93 (1H, s), 7.03 (1H, s), 7.19 (1H, s), 7.27~7.35 (3H, m), 7.50~7.68 (2H, m), 8.18 (1H, m), 9.95 (1H, s), 10.28 (1H, br s), and 10.35 (1H, s). UV (H_2O) λmax nm (log ε) : 238 (4.30), 298 (4.36). FAB-MS : *m/z* 469 (M^++H). HRMS : *m/z* calcd for $C_{24}H_{30}N_6O_3F$ 469.2363, Found 469.2362.

3-{1-Methyl-4-[1-methyl-4-(3-fluorobenzamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-dimethylaminopropane hydrochloride (3c-F). Pale yellow amorphous solid; FT-IR (KBr) : 3400, 1640, and 1585 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.89 (2H, m), 2.75 (3H, s), 2.77 (3H, s), 3.07 (2H, m), 3.26 (2H, m), 3.83 (3H, s), 3.88 (3H, s), 6.94 (1H, s), 7.12 (1H, s), 7.20 (1H, s), 7.34 (1H, s), 7.38~7.61 (2H, m), 7.74~7.85 (2H, m), 8.18 (1H, m), 9.97 (1H, s), 10.25 (1H, br s), and 10.46 (1H, s). UV (H_2O) λmax nm (log ε) : 237 (4.29), 300 (4.36). FAB-MS : *m/z* 469 (M^++H). HRMS : *m/z* calcd for $C_{24}H_{30}N_6O_3F$ 469.2363, Found 469.2364.

3-{1-Methyl-4-[1-methyl-4-(4-fluorobenzamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-dimethylaminopropane hydrochloride (4c-F). Pale yellow amorphous solid; FT-IR (KBr) : 3423, and 1639 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.89 (2H, m), 2.73 (3H, s), 2.75 (3H, s), 3.05 (2H, m), 3.25 (2H, m), 3.82 (3H, s), 3.87 (3H, s), 6.93 (1H, d, J=1.5 Hz), 7.12 (1H, d, J=1.5 Hz), 7.20 (1H, s), 7.29~7.37 (3H, m), 8.02~8.09 (2H, m), 8.19 (1H, m), 9.98 (1H, s), 10.44 (1H, s), and 10.51 (1H, br s). UV (H_2O) λmax nm (log ε) : 237 (4.29), 299 (4.32). FAB-MS : *m/z* 469 (M^++H). HRMS : *m/z* calcd for $C_{24}H_{30}N_6O_3F$ 469.2363, Found 469.2364.

3-{1-Methyl-4-[1-methyl-4-(2-chlorobenzamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-dimethylaminopropane hydrochloride (2c-Cl). Pale yellow amorphous solid; FT-IR (KBr) : 3411, 1640, and 1528 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.89 (2H, m), 2.74 (3H, s), 2.76 (3H, s), 3.05 (2H, m), 3.26 (2H, m), 3.82 (3H, s), 3.87 (3H, s), 6.93 (1H, d, J=1.5 Hz), 7.02 (1H, d, J=1.5 Hz), 7.19 (1H, s), 7.27 (1H, s), 7.37~7.58 (4H, m), 8.17 (1H, m), 9.95 (1H, s), 10.35 (1H, br s), and 10.45 (1H, s). UV (H_2O) λmax nm (log ε) : 297 (4.31). FAB-MS : *m/z* 485 (M^++H). HRMS : *m/z* calcd for $C_{24}H_{30}N_6O_3Cl$ 485.2068, Found 485.2041.

3-{1-Methyl-4-[1-methyl-4-(3-chlorobenzamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-dimethylaminopropane hydrochloride (3c-Cl). Pale yellow amorphous solid; FT-IR (KBr) : 3401, 1641, and 1535 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.90 (2H, m), 2.74 (3H, s), 2.76 (3H, s), 3.06 (2H, m), 3.26 (2H, m), 3.83 (3H, s), 3.89 (3H, s), 6.94 (1H, s), 7.14 (1H, s), 7.22 (1H, s), 7.35 (1H, s), 7.54 (1H, t, J=7.3 Hz), 7.59 (1H, d, J=7.3 Hz), 7.96 (1H, d, J=7.3 Hz), 8.02 (1H, m), 10.00 (1H, s), and 10.54 (2H, br s). UV (H_2O) λmax nm (log ε) : 299 (4.37). FAB-MS : *m/z* 485 (M^++H). HRMS : *m/z* calcd for $C_{24}H_{30}N_6O_3Cl$ 485.2068, Found 485.2059.

3-{1-Methyl-4-[1-methyl-4-(4-chlorobenzamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-dimethylaminopropane hydrochloride (4c-Cl). Pale yellow amorphous solid; FT-IR (KBr) : 3424,

1640, and 1528 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.90 (2H, m), 2.74 (3H, s), 2.76 (3H, s), 3.06 (2H, m), 3.26 (2H, m), 3.83 (3H, s), 3.88 (3H, s), 6.94 (1H, s), 7.12 (1H, s), 7.21 (1H, s), 7.34 (1H, s), 7.57 (2H, d, J=7.7 Hz), 8.01 (2H, d, J=7.7 Hz), 8.19 (1H, m), 9.98 (1H, s), 10.46 (1H, br s), and 10.49 (1H, s). UV (H_2O) λmax nm (log ε) : 240 (4.38), 300 (4.37). FAB-MS : *m/z* 485 (M^++H). HRMS : *m/z* calcd for $C_{24}H_{30}N_6O_3Cl$ 485.2068, Found 485.2083.

3-{1-Methyl-4-[1-methyl-4-(2-bromobenzamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-dimethylaminopropane hydrochloride (2c-Br). Pale yellow amorphous solid; FT-IR (KBr) : 3414, 1640, and 1528 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.89 (2H, m), 2.73 (3H, s), 2.76 (3H, s), 3.05 (2H, m), 3.25 (2H, m), 3.82 (3H, s), 3.88 (3H, s), 6.93 (1H, d, J=1.5 Hz), 7.02 (1H, d, J=1.5 Hz), 7.20 (1H, d, J=1.5 Hz), 7.27 (1H, d, J=1.5 Hz), 7.35~7.49 (3H, m), 7.69 (1H, d, J=7.7 Hz), 8.18 (1H, m), 9.97 (1H, s), 10.34 (1H, br s), and 10.44 (1H, s). UV (H_2O) λmax nm (log ε) : 297 (4.36). FAB-MS : *m/z* 529 (M^++H). HRMS : *m/z* calcd for $C_{24}H_{30}N_6O_3Br$ 529.1563, Found 529.1546.

3-{1-Methyl-4-[1-methyl-4-(3-bromobenzamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-dimethylaminopropane hydrochloride (3c-Br). Pale yellow amorphous solid; FT-IR (KBr) : 3423, 1639, 1562, and 1528 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.89 (2H, m), 2.73 (3H, s), 2.76 (3H, s), 3.06 (2H, m), 3.27 (2H, m), 3.82 (3H, s), 3.88 (3H, s), 6.93 (1H, d, J=1.5 Hz), 7.12 (1H, d, J=1.5 Hz), 7.20 (1H, s), 7.33 (1H, s), 7.49 (1H, dd, J=7.7 and 8.1 Hz), 7.76 (1H, d, J=8.1 Hz), 7.98 (1H, d, J=7.7 Hz), 8.14 (1H, s), 8.18 (1H, m), 9.98 (1H, s), 10.38 (1H, br s), and 10.51 (1H, s). UV (H_2O) λmax nm (log ε) : 299 (4.27). EI-MS : *m/z* 528 (M^+). HRMS : *m/z* calcd for $C_{24}H_{29}N_6O_3Br$ 528.1485, Found 528.1478.

3-{1-Methyl-4-[1-methyl-4-(4-bromobenzamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-dimethylaminopropane hydrochloride (4c-Br). Pale yellow amorphous solid; FT-IR (KBr) : 3402, 1640, and 1529 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.92 (2H, m), 2.77 (3H, s), 2.79 (3H, s), 3.09 (2H, m), 3.30 (2H, m), 3.84 (3H, s), 3.89 (3H, s), 6.96 (1H, s), 7.13 (1H, s), 7.20 (1H, s), 7.33 (1H, s), 7.68 (2H, d, J=7.7 Hz), 7.93 (2H, d, J=7.7 Hz), 8.17 (1H, m), 9.94 (1H, s), 10.26 (1H, br s), and 10.43 (1H, s). UV (H_2O) λmax nm (log ε) : 244 (4.37), 300 (4.35). FAB-MS : *m/z* 529 (M^++H). HRMS : *m/z* calcd for $C_{24}H_{30}N_6O_3Br$ 529.1563, Found 529.1516.

3-{1-Methyl-4-[1-methyl-4-(2-iodobenzamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-dimethylaminopropane hydrochloride (2c-I). Pale yellow amorphous solid; FT-IR (KBr) : 3399, 1640, and 1528 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.89 (2H, m), 2.72 (3H, s), 2.75 (3H, s), 3.05 (2H, m), 3.25 (2H, m), 3.81 (3H, s), 3.87 (3H, s), 6.92 (1H, s), 7.01 (1H, s), 7.19 (2H, m), 7.27 (1H, s), 7.41 (1H, d, J=7.3 Hz), 7.48 (1H, dd, J=7.3 and 7.9 Hz), 7.91 (1H, d, J=7.9 Hz), 8.17 (1H, m), 9.97 (1H, s), 10.38 (1H, s), and 10.40 (1H, br s). UV (H_2O) λmax nm (log ε) : 307 (4.36). FAB-MS : *m/z* 577 (M^++H). HRMS : *m/z* calcd for $C_{24}H_{30}N_6O_3I$ 577.1424, Found 577.1409.

3-{1-Methyl-4-[1-methyl-4-(3-iodobenzamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-dimethylaminopropane hydrochloride (3c-I). Pale yellow amorphous solid; FT-IR (KBr) : 3278, 1641, and 1534 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.89 (2H, m), 2.74 (3H, s), 2.76 (3H, s), 3.06 (2H, m),

3.26 (2H, m), 3.82 (3H, s), 3.87 (3H, s), 6.93 (1H, s), 7.11 (1H, s), 7.20 (1H, s), 7.32 (1H, s), 7.32 (1H, t, J=7.9 Hz), 7.92 (1H, d, J=7.9 Hz), 7.97 (1H, d, J=7.9 Hz), 8.18 (1H, m), 8.30 (1H, s), 9.97 (1H, s), 10.28 (1H, br-s), and 10.46 (1H, s). UV (H_2O) λmax nm (log ε) : 298 (4.36). FAB-MS : *m/z* 577 (M^++H). HRMS : m/z calcd for $C_{24}H_{30}N_6O_3I$ 577.1424, Found 577.1398.

3-{1-Methyl-4-[1-methyl-4-(4-iodobenzamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido}-dimethylaminopropane hydrochloride (4c-I) Pale yellow amorphous solid; FT-IR (KBr) : 3285, 1641, and 1529 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 200 MHz) : δ 1.90 (2H, m), 2.73 (3H, s), 2.75 (3H, s), 3.06 (2H, m), 3.26 (2H, m), 3.82 (3H, s), 3.87 (3H, s), 6.93 (1H, s), 7.12 (1H, s), 7.21 (1H, s), 7.34 (1H, s), 7.77 (2H, d, J=8.1 Hz), 7.89 (2H, d, J=8.1 Hz), 8.20 (1H, m), 9.98 (1H, s), 10.48 (1H, s), and 10.52 (1H, br s). UV (H_2O) λmax nm (log ε) : 252 (4.39), 300 (4.41). EI-MS : *m/z* 576 (M^+). HRMS : *m/z* calcd for $C_{24}H_{29}N_6O_3I$ 576.1346, Found 576.1324.

DNA-cleavage assay.

In a typical experiment, 0.25 μg Col E1 DNA (Wako Pure Chemical Industries, Ltd.) in 20 μL Tris acetate (TAE) buffer (20 mM Tris-AcOH, 2 mM EDTA, pH 7.8) containing compound (0.1 ~ 100 μM) was irradiated in Eppendorf tube from above at 20°C using a Vilber Lourmat VL-215L (2x15W, 365 nm maximum, 13 $J•m^{-2}•sec^{-1}$) fluorescent light at a distance of 8.6 cm for 2 h. Immediately following irradiation, 15 μL samples were loaded into 1% agarose gel. The running buffer was 20 mM TAE, pH 7.8. Electrophoresis was at 50 V for 8 h. After electrophoresis, gel was stained for 1 h in ethidium bromide (1 μg/mL), and de-stained for 5 min in water. Relative amounts of DNA in form I, form II, form III were determined by densitometer; Dual-wavelengths Flying-spot Scanner (Shimadzu CS-9000) using fluorescence mode. Results presented are mean value of three runs. A control reaction mixture without the addition of drug was irradiated and used as the background to be subtracted from the obtained values.

Calculation of the C-X bond dissociation energies for a model structure (5) (Table 1).

To obtain the initial coordinate, grand state conformation of each compound was calculated by the Sony-Tekronix CAChe-MOPAC (PM3 with geometrical optimization). In each compound, direction of the C-X bond was fixed and the length of C-X bond was constrained (from 1Å to 5Å in units of 0.1Å), and then optimized by the same calculation method. The gap between the energy value of ground state and that of the highest energy state conformation was calculated as C-X bond dissociation energy.

HPLC analysis of the UV-irradiation products of 4c-I (Figure 5).

A solution containing **4c-I** (20 mg, 32.6 μmol) and aqueous 1 M solution of 1,2-(1,4-cyclohexadiene) dimethanol[8] (1.3 mL, 40 equiv) in MeCN (20 mL) was irradiated with UV-A light (365 nm maximum, 13 $J•m^{-2}•sec^{-1}$) at 25°C for 24 h. The reaction mixture was evaporated and the residue was dissolved in AcOEt and the solution was washed with aqueous $NaHCO_3$ and brine, successively, then dried over

$MgSO_4$, filtered, and concentrated. After removing the nonpolar substances with column chromatography on silica gel, the polar substances were analyzed on YMC R-ODS-5 column (6x250 mm). Elution was performed with 0.1% aqueous trifluoroacetic acid and 20 ~ 80% acetonitrile (linear gradient, 40 min) at a flow rate of 1 mL/min. A small peak at 14.4 min was separated (0.6 mg, 3.3%) and identified as **1c** by NMR and FABMS. A major peak at 20.2 min was ascribed to that of unreacted **4c-I** as judged by FABMS.

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