SYNTHESIS OF OLIGO-N-METHYLPYRROLECARBOXAMIDE DERIVATIVES AND THEIR PHOTOCHEMICAL DNA CLEAVING ACTIVITIES
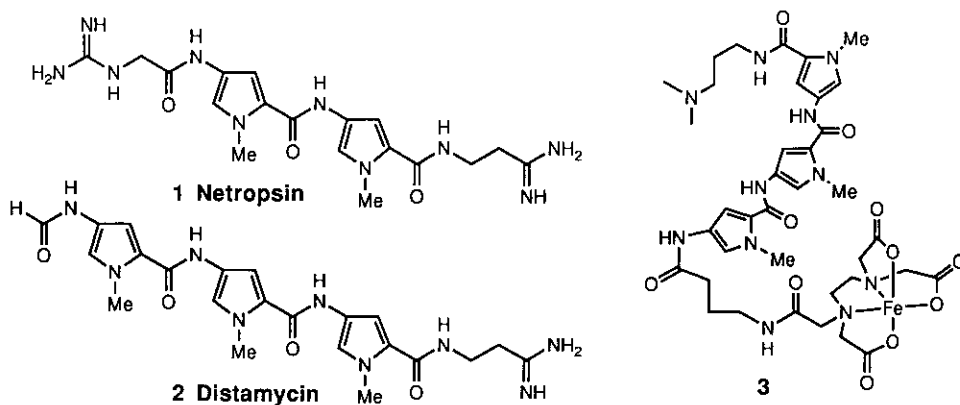
Eiji Nishiwaki, Hirofumi Nakagawa, Michika Takasaki, Toyomi Matsumoto, Hiromu Sakurai,
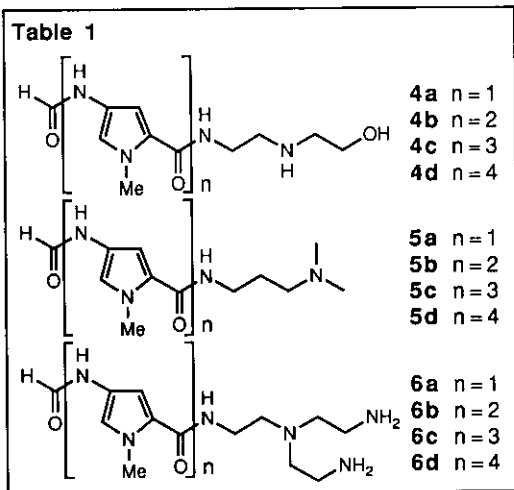and Masayuki Shibuya*
Faculty of Pharmaceutical Sciences, University of Tokushima,
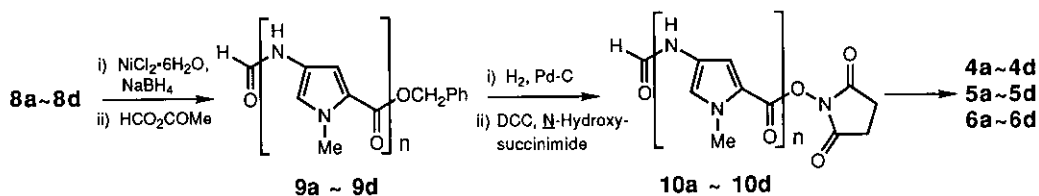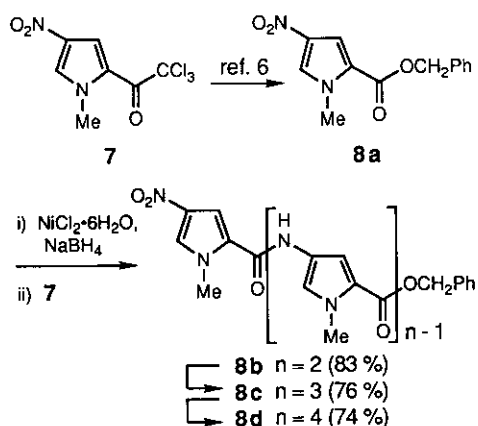Sho-machi 1, Tokushima 770, Japan

Abstract – Synthesis of various oligo-N-methylpyrrolecarboxamide derivatives and their DNA cleaving activities under UV-A irradiation were described.

Low molecular weight substances which possess both sequence specific DNA binding and cleaving activities are of considerable interest in the field of fundamental molecular biology.[1]   There are a number of substances that bind to DNA in the minor groove.[2]   Among them, oligo-N-methylpyrrolecarboxamides such as Netropsin (1) and Distamycin (2) and their analogues have attracted attention because of their strong minor groove nonintercalative binding ability to double-stranded B-DNA at specific AT rich region.[3]   Dervan and co-workers designed and synthesized distamycin-EDTA-Fe(II) (3) and its analogues which were shown to cleave double helical nucleotide at specific AT recognition sites.[4]   In a previous work, we have reported the synthesis of nitrated oligo-N-methylpyrrolecarboxamides and proposed the mechanism in which both hydroxyl radicals and nitro anion radicals participate in their photochemical DNA cleaving activities.[5]   Here we report the synthesis of various oligo-N-methylpyrrolecarboxamides which do not possess special side groups, such as nitro group, sensitive to uv light and their DNA cleaving activities under near uv light (UV-A) irradiation.

Table 1

4a n = 1
4b n = 2
4c n = 3
4d n = 4

5a n = 1
5b n = 2
5c n = 3
5d n = 4

6a n = 1
6b n = 2
6c n = 3
6d n = 4

Scheme 1[9]

7 → ref. 6 → 8a

i) NiCl$_2$·6H$_2$O, NaBH$_4$
ii) 7

8b n = 2 (83 %)
8c n = 3 (76 %)
8d n = 4 (74 %)

8a~8d
i) NiCl$_2$·6H$_2$O, NaBH$_4$
ii) HCO$_2$COMe
→ 9a ~ 9d

i) H$_2$, Pd-C
ii) DCC, N-Hydroxy-succinimide
→ 10a ~ 10d

4a~4d
5a~5d
6a~6d

Various oligo-N-methylpyrrolecarboxamides as well as the monomers listed in Table 1 were synthesized by the way summarized in Scheme 1. Benzyl 1-methyl-4-nitropyrrole-2-carboxylate (8a),[6] which is readily available in large scale from 7,[6] was reduced using nickel boride according to the Kudo method[7] and the resulting 4-amino compound was condensed with 7 to afford 8b. The reduction-condensation process was repeated, leading to the oligopeptides (8c) and (8d), respectively. The 4-amino compounds obtained as above as well as the amine obtained from 8d were formylated to give 9a ~ 9d, respectively. The carboxylic acids obtained by the hydrogenolysis of 9a ~ 9d were transformed into the N-succinimidyl esters (10a ~ 10d), respectively. The esters (10a ~ 10d) were condensed with 2-(2-aminoethylamino)ethanol, 3,3-dimethylaminopropylamine, and tris(2-aminoethyl)amine to afford the corresponding amides (4, 5, and 6).

DNA cleaving activities of the peptides (4 ~ 6) were assayed with supercoiled plasmid Col E1 (ca. 40 µg/ml) under UV-A light (360 nm maximum, 13 J·m^{-2}·sec^{-1}) irradiation at 20°C. DNA single strand cleavage was estimated on agarose gels by conversion of the covalently closed circular (ccc) DNA to the open circular (oc) form. After electrophoresis each DNA was quantitated by ethidium bromide staining and densitometry. All compounds tested exhibited activities, depending on the drug concentrations (0.1, 1, 10, and 100 µM final concentrations). Activities of compounds [4d ~ 6d (n=4)] are typically shown in Figure 1, comparing with that of Netropsin. Table 1 summarizes the relative cleavage efficiency of compounds (4 ~ 6) by comparing the activities at 10 µM drug concentrations. Single strand cleavage was predominant in all experiments, and a remarkable correlation between the peptide chain lengths and the activities was observed in each series of the compound.
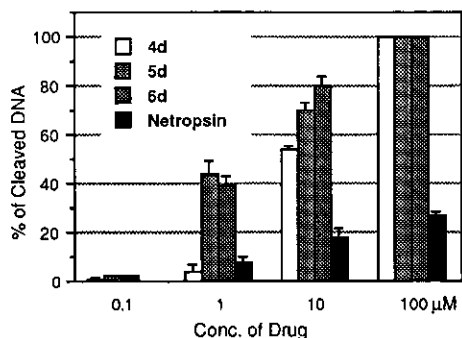
	% of ccc DNA converted into oc DNA			
compound	a (n = 1)	b (n = 2)	c (n = 3)	d (n = 4)
4	3	4	15	54
5	4	8	26	70
6	2	21	49	80

Figure 1. Photoinduced DNA-cleavage. Col E1 was incubated in 20 μl of Tris-acetate (TAE) buffer (pH 7.8) with various amount of compounds and irradiated for 2 h. Results presented are mean value ±SD 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.

Table 2. Photoinduced DNA-cleavage at 10μM drug concentrations. The reaction mixture containing 10 μM drug was irradiated for 2 h. Values were obtained from mean values of three runs.

In order to investigate the mechanism of DNA cleavage by these compounds, UV-A irradiations of Col E1 DNA with **4d**, **5d**, or **6d** were carried out in the presence of various active oxygen scavengers. In the experiments at two different drug concentrations, SOD (O_2^- scavenger) and catalase (H_2O_2 scavenger) had little effect on the extent of photo-nicking activities (data not shown). On the other hand, hydroxyl radical scavengers such as sodium benzoate, phenol, and potassium iodide revealed concentration-dependent inhibition of strand breakage (e. g., Figure 2). Although the effects of above scavengers were found to be weaker than those of the analogous nitro compounds which we reported previously,[5] they suggest the participation of hydroxyl radicals in the reaction process. UV-A irradiation for the mixtures of compounds (**4d**, **5d**, or **6d**) and DMPO led to the generation of esr signals due to spin adducts. (e. g. Figure 3) In these signals, the characteristic 1:2:2:1 quartet (a^N = 15.0, a^H = 15.2 G) of the DMPO-OH adduct, the triplet of triplet (a^N = 16.5, a^H(2H) = 22.7 G) of the DMPO-H adduct, and an unidentified six lines (a^N = 15.6, a^H = 22.6 G) were observed. When molecular dioxygen was removed from the reaction mixtures by N_2 bubbling, the extent of DNA cleavage was definitely reduced, indicating that the molecular dioxygen is related to the present reactions.
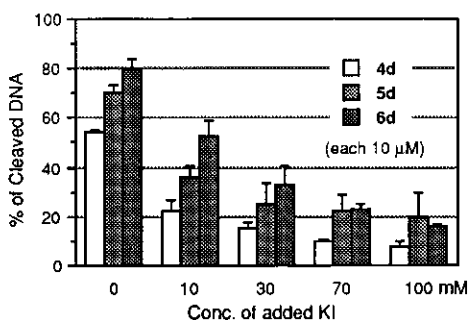

Figure 2. Effect of KI as hydroxyl radical scavenger on photocleavage of Col E1 DNA by compounds (**4d**, **5d**, and **6d**). Results presented are mean values ±SD of three runs.
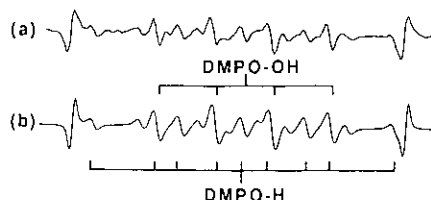

Figure 3. Spin trapping by DMPO under UV-A (573·m^{-2}· sec^{-1}) irradiation at pH 6.98. (a) Compound (**5d**) (10mM) in TAE Buffer. Gain, 1x10^3; mod. amp., 2.0 G; 10mw microwave power. (b) Computer simulation of DMPO-OH, DMPO-H, and unidentified six lines.

Although our studies do not provide unequivocal mechanism for the DNA-cleavage by these compounds, the mechanism in which participation of both hydroxyl radical and molecular dioxygen is probable. Several reports on the photoinduced DNA-cleavage by phenothiazine derivatives (PZD) have been reported.[8] They have proposed the mechanisms in which PZD cation radicals, solvated electrons, and hydroxyl radicals are included. Further investigations on the precise mechanisms of DNA cleavage by our compounds and relationship between the mechanisms for the DNA-cleavage by PZD and our compounds are now in progress.

REFERENCES AND NOTES

1. L. A. Basile and J. K. Barton, *J. Amer. Chem. Soc.*, 1987, **109**, 7548; S. E. Sherman and S. J. Lippard, *Chem. Rev.*, 1987, **87**, 1153; P. E. Nielsen, C. Jeppesen, M. Egholm, and O. Buchardt, *Nucleic Acids Res.*, 1988, **16**, 3877; L. H. Hurley, *J. Med. Chem.*, 1989, **32**, 2027; A. M. Pyle, J. P. Rehmann, R. Meshoyrer,C. V. Kumar, N. J. Turro, and J. K. Barton, *J. Amer. Chem. Soc.*, 1989, **111**, 3051.

2. J. Stubbe and J. W. Kozarich, *Chem. Rev.*, 1987, **87**, 1107; N. Zein, M. Poncin, R. Nilakantan, and G. A. Ellestad, *Science*, 1989, **244**, 696; T. A. Scahill, R. M. Jensen, D. H. Swenson, N. T. Hatzenbuhler, G. Petzold, W. Wierenga, and N. D. Brahme, *Biochem.*, 1990, **29**, 2852.

3. C. Zimmer and U. Wähnert, *Prog. Biophys. Molec. Biol.*, 1986, **47**, 31; F. Debart, C. Perigaud, G. Gosselin, D. Mrani, B. Rayner, P. Le Ber, C. Auclair, J. Balzarini,E. De Clercq, C. Paoletti, and J. L. Imbach, *J. Med. Chem.*, 1989, **32**, 1074; F. M. Arcamone, F. Animati, B. Barbieri, E. Configliacchi, R. D'Alessio, C. Geroni, F. C. Giuliani, E. Lazzari,M. Menozzi, N. Mongelli, S. Penco, and M. A. Verini, *J. Med. Chem.*, 1989, **32**, 774;J. W. Lown, K. Krowicki, J. Balzarini, R. A. Newman, and E. De Clercq, *J. Med. Chem.*, 1989, **32**, 2368; M. Lee, R. G. Shea, J. A. Hartley, K. Kissinger, R. T. Pon, G. Vesnaver,K. J. Breslauer, J. C. Dabrowiak, and J. W. Lown, *J. Amer. Chem. Soc.*, 1989, **111**, 345; M. Otsuka, T. Masuda, A. Haupt, M. Ohno, T. Shiraki, Y. Sugiura, and K. Maeda, *J. Amer. Chem. Soc.*, 1990, **112**, 838; T. Matsumoto, K. Toyooka, E. Nishiwaki, and M. Shibuya, *Heterocycles*, 1990, **31**,1629.

4. P. B. Dervan, *Science*, 1986, **232**, 464.

5. E. Nishiwaki, H. Lee, T. Matsumoto, K. Toyooka, H. Sakurai, and M. Shibuya, *Tetrahedron Letters*, 1990, **31**, 1299 .

6. E. Nishiwaki, S. Tanaka, H. Lee, and M. Shibuya, *Heterocycles*, 1988, **27**, 1945.

7. A. Nose and T. Kudo, *Chem. Pharm. Bull.*, 1988, **36**, 1529.

8. J. Decuyper, J. Piette, M. Lopez, M. P. Merville, and A. Vorst, *Biochem. Pharmacol.*, 1984, **33**, 4025; A. G. Motten, G. R. Buettner, and C. F. Chignell, *Photochem. Photobiol.*, 1985, **42**, 9; H. Fujita and I. Matsuo, *Chem. Biol. Interac.*, 1988, **66**, 27.

9. Selected data, **4c**: mp 163~164°C (decomp); uv (EtOH) 303 nm (log ε=4.32); ir (KBr) 3285 and 1641cm^{-1}; ^{1}H nmr (200 MHz, CD$_3$OD): 2.76~2.88 (4H, m), 3.47 (2H, t, J=6.0), 3.69 (2H, t, J=5.4), 3.89 (3H, s), 3.92

(6H, s), 6.80 (1H, d, J=1.8), 6.86 (1H, d, J=1.8), 6.90 (1H, d, J=2.0), 7.19 (3H, m), and 8.14 (1H, s). **4d**: mp 87~88°C (decomp.); uv (EtOH) 303 nm (logε=4.56); ir (KBr) 3306 and 1646cm^{-1}; ^{1}H nmr (200 MHz, CD$_3$OD) 2.85~2.71 (4H, m), 3.45 (2H, t, J=6.2), 3.66 (2H, t, J=5.4), 3.85 (3H, s), 3.89 (9H, s), 6.81 (1H, d, J=2.0), 6.86 (1H, d, J=2.0), 6.92 (2H, m), 7.17 (4H, m), and 8.12 (1H, s). **5c**: mp 138~140°C (decomp.); uv (EtOH) 303 nm (log ε=4.68); ir (KBr) 3306, and 1641cm^{-1}; ^{1}H nmr (200 MHz, CD$_3$OD): 1.78 (2H, m), 2.27 (6H, s), 2.42 (2H, t, J=7.7), 3.33 (2H, t, J=7.7), 3.87 (3H, s), 3.91 (6H, s), 6.79 (1H, d, J=2.0), 6.88 (1H, d, J=2.0), 6.93 (1H, d, J=2.0), 7.17 (3H, m), and 8.14 (1H, s). **5d**: mp 165~166°C; uv (EtOH) 307nm (log ε = 4.62); ir (KBr) 3286 and 1641cm^{-1}; ^{1}H nmr (200 MHz, CD$_3$OD):1.77 (2H, m), 2.28 (6H, s), 2.42 (2H, t, J=7.7), 3.33 (2H, t, J=7.7), 3.86 (3H, s), 3.90 (9H, s), 6.78 (1H, d, J=2.0), 6.85 (1H, d, J=2.0), 6.92 (2H, m), 7.17 (4H, m), and 8.13 (1H, s). **6c**: mp 147~150°C; uv (EtOH) 305 nm (log ε=4.18); ir (KBr) 3284 and 1641cm^{-1}; ^{1}H nmr (200 MHz, CD$_3$OD) 2.55~2.75 (10H, m), 3.39 (2H, t, J=6.1), 3.87 (3H, s), 3.90 (6H, s), 6.81 (1H, d, J=2.0), 6.86 (1H, d, J=2.0), 6.92 (1H, d, J=2.0), 7.17 (3H, m), and 8.12 (1H, s). **6d**: mp 136~137°C; uv (EtOH) 303 nm (log ε=4.58); ir (KBr) 3280 and 1641cm^{-1}; ^{1}H nmr (200 MHz, CD$_3$OD): 2.55~2.74 (10H, m), 3.39 (2H, t, J=6.1), 3.87 (3H, s), 3.90 (9H, s), 6.81 (1H, d, J=2.0), 6.87 (1H, d, J=2.0), 6.94 (2H, m), 7.20 (4H, m), and 8.13 (1H, s).