SYNTHESIS OF HALOGENATED THIAZOLE DERIVATIVES OF OLIGO-N-METHYLPYRROLECARBOXAMIDE AND THEIR PHOTOCHEMICAL DNA CLEAVING ACTIVITIES

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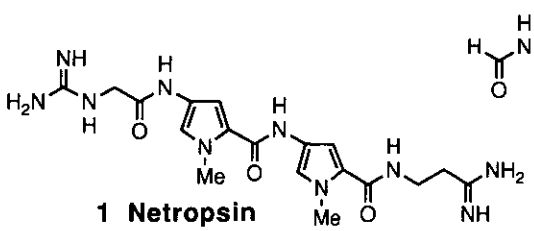
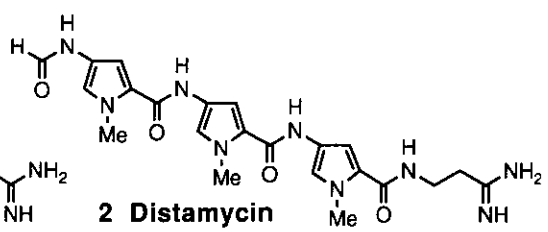
Abstract – Synthesis of various halogenated thiazole derivatives of oligo-N-methylpyrrolecarboxamide and their DNA cleaving activities under UV-A irradiation were described.

The design of compounds which cleave DNA under photo-irradiation is of great importance not only from a fundamental biological point of view but also in a photodynamic therapeutic approach as antitumor agents.[1] On the other hand, 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.[2] We have reported the synthesis of several oligo-N-methylpyrrolecarboxamide derivatives aimed at the development of the molecules which possess both DNA binding abilities and photo-induced DNA cleaving activities.[3] In our recent communication, we reported the synthesis of several halogenated oligo-N-methylpyrrolecarboxamide derivatives and their photochemical DNA cleaving activities.[4] Our recent results prompted us to search for other more active DNA cleaving substances. Here we report the synthesis of 2-halothiazole-5-carboxamido derivatives of oligo-N-methylpyrrolecarboxamide and their potent DNA cleaving activities under UV-A irradiation.

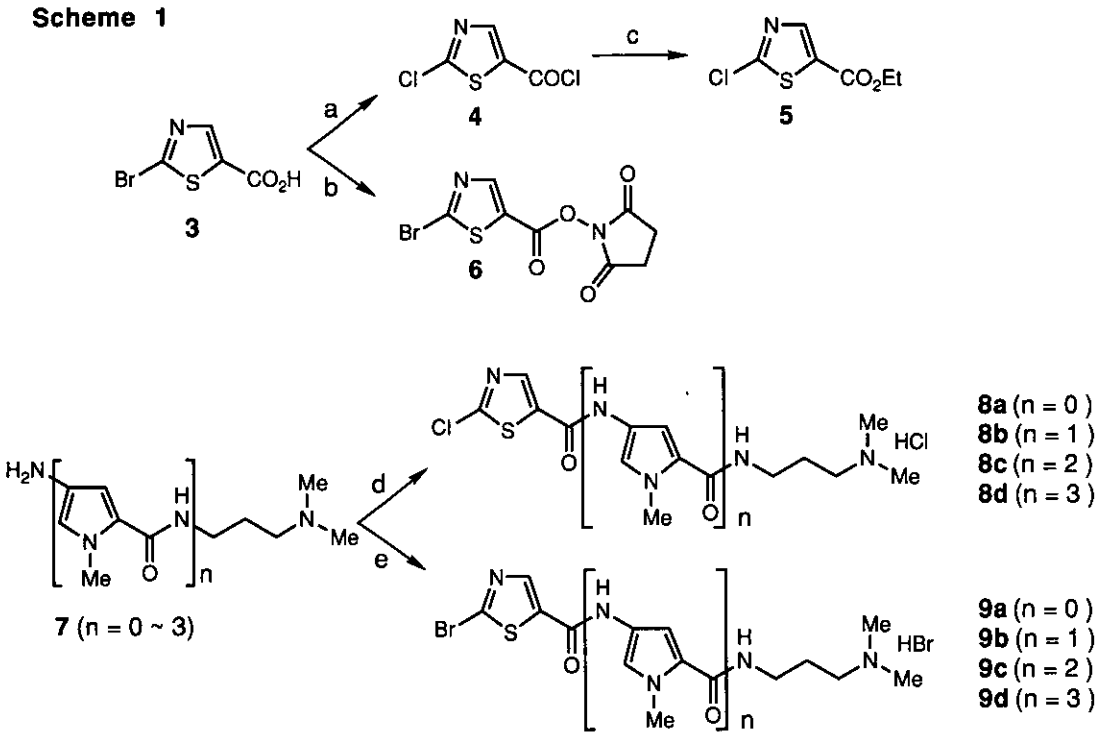


1 Netropsin



2 Distamycin

Scheme 1



Reagents: (a) $SOCl_2$, 65°C, 2 h (b) N-hydroxysuccinimide, DCC, room temperature, 12 h (c) EtOH,pyridine, room temperature, 3 h (d) **4**, -70~0°C, 1~3 h(e) **6**, -70°C~room temperature, 2~13 h

The synthesis of 2-halothiazole-5-carboxamido derivatives (**8**a~d) and (**9**a~d) were completed as outlined in Scheme 1. Reaction of the carboxylic acid (**3**)[5] with thionyl chloride in ethyl acetate afforded the acid chloride (**4**). Complete halogen exchange at C-2 position was confirmed unequivocally by mass spectrum measurement of the ethyl ester (**5**) obtained from **4**. The acid chloride (**4**) was condensed with **7** (n=0~3)[3a,c] to afford the appropriate peptides (**8**a~d). For the preparation of the bromo analogues of **8**, an alternative procedure was adopted employing succinimide ester (**6**) to give **9**a~d in good to moderate yields, respectively. All compounds here synthesized were found decompose slowly under room light.

DNA cleaving activities of the peptides (**8** and **9**) were assayed with supercoiled plasmid Col E1 (ca. 13 μg/ml) under UV-A light (365 nm maximum, 13 $J \cdot m^{-2} \cdot sec^{-1}$) irradiation at 20°C. A single-strand break converted covalently closed circular DNA (form I) into the open circular DNA (form II). A double-strand break of close-spaced single-strand breaks changed form I DNA into linear DNA

(form III). After electrophoresis each DNA was quantitated by ethidium bromide staining and densitometry. Activities of compounds (**8**a~d and **9**a~d) are shown in Figure 1.

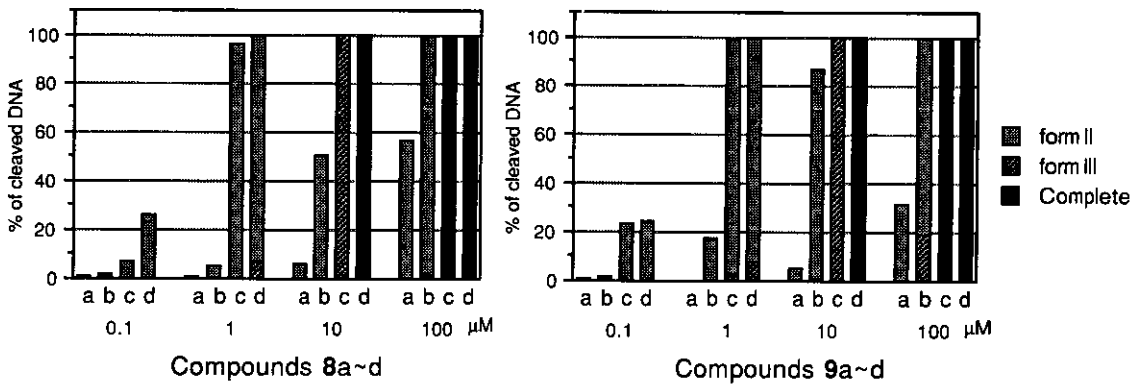
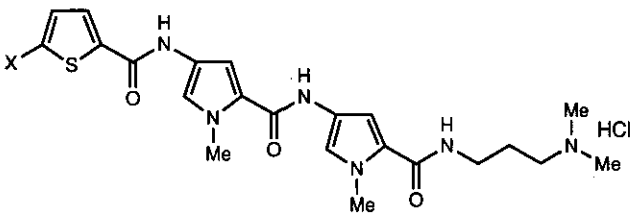


Figure 1. Photo-induced DNA-cleavage by compounds (**8**) and (**9**). Col E1 was incubated in 20 μl of Tris-acetate (TAE) buffer (pH 7.8) with various amount of compounds and irradiated for 2 h. Results presented are mean value of three runs. A control reaction mixture without the addition of drug was irradiated and used as the background to be subtracted from the obtained values. Complete means complete fragmentation of DNA.

All compounds tested exhibited potent activities, depending on the drug concentrations (0.1, 1, 10, and 100 μM final concentrations) and peptide chain length. The DNA cleaving activities of (**8**a~c) and (**9**a~c) were more potent or approximately the same comparing with those of halogenated pyrrole-, thiophene-, or furan-analogues which we reported previously.[4] Compounds (**8**d) and (**9**d) were found to possess much more potent activities comparing with the oligo-N-methylpyrrolecarboxamide derivatives which we hitherto reported.[3,4] Compounds (**8**c,d) and (**9**c,d) exhibited conversion of form I DNA into form II DNA at 10 μM drug concentrations for 2 h, respectively, even under room light. Table 1 summarizes the relative cleavage efficiency of compounds (**8**c,d), (**9**c,d), (**10**)[4] and (**11**)[4] by comparing the activities at 10 μM drug concentrations under room light.

	% of form I DNA converted into form II DNA					
compound	**8c**	**9c**	**8d**	**9d**	**10**	**11**
(halogen)	(Cl)	(Br)	(Cl)	(Br)	(Cl)	(Br)
n = 2	13±6	25±6			2±2	8±2
n = 3			41±4	83±2		



10: X = Cl
11: X = Br
(ref. 4)

Table 1. *Photoinduced DNA-cleavage at 10 μM drug concentrations.* The reaction mixture containing 10 μM drug was irradiated for 2 h under room light. Values were obtained from mean values ±SD of three runs.

We have previously shown that several non-halogenated hetero-aromatic derivatives of oligo-N-methylpyrrolecarboxamides exhibit DNA cleaving activities but much less potent than the halogenated derivatives.[3c,4] Motten *et al.* reported the the production of promazine radical (P•) from chloropromazine under UV-A irradiation and found that the reactivity of P• was similar to that of hydroxyl or phenyl radical in its capability to abstract hydrogen atoms from a variety of substrates.[6] It is probable that similar aryl radical was produced by a photo-homolysis of carbon-halogen bond of our compounds (**8** and **9**). Generally, as the carbon-halogen bond dissociation energy increases, the photolysis is expected to proceed with increasing difficulty and the facility order of photolytic cleavage has been reported as C-I > C-Br > C-Cl > C-F.[7] Actually the bromo derivatives (**9**c,d and **11**) possess more potent DNA cleaving avtivities than those of the chloro derivatives (**8**c,d and **10**) under the room light conditions (Table 1). Further investigations will be necessary in establishing the mechanism of DNA cleavage by our compounds, which are now in progress in our laboratory.

EXPERIMENTAL

Melting points were determined by the capillary method and were uncorrected. 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. Mass spectra were recorded on a JEOL D-300 instrument. Column chromatography was performed on silica gel (K-100-S, from Katayama Chemicals).

2-Chlorothiazole-5-carbonyl chloride (4) A solution of the carboxylic acid (**3**)[5] (300 mg, 1.44 mmol), DMF (0.04 ml), and $SOCl_2$ (0.26 ml, 1.59 mmol) in AcOEt (10 ml) was heated at 65°C with stirring for 2 h and the excess reagent was evaporated to give the amorphous solid. The acid chloride (4) obtained was used for the following condensation reactions without purification.

Ethyl 2-chlorothiazole-5-carboxylate (5) To a solution of the acid chloride (**4**) prepared as above in THF (8 ml), pyridine (0.12 ml, 1.44 mmol) and EtOH (8 ml) were added successively at 0°C. The solution was stirred at 0°C for 1 h and at room temperature for 2 h and then evaporated. The residue was dissolved in AcOEt (50 ml) and washed with brine, then dried over $MgSO_4$, filtered, concentrated, and chromatographed on silica gel with mixed solvent of hexane and AcOEt (4 : 1) as eluent to give the ester (**5**) (101 mg, 37% yield) as a colorless oil. Ir (neat) 1719 cm^{-1}. Nmr ($CDCl_3$) δ 1.38 (3H, t, J=7.1), 4.37 (3H, q, J=7.1), 8.15 (1H, s) ppm. Ms(EI) 190.9813 (M^+), Calcd 190.9808.

N-Succinimidyl 2-bromothiazole-5-carboxylate (6) To a solution of **3** (500 mg, 2.40 mmol) and N-hydroxysuccinimide (300 mg, 2.64 mmol) in DMF (5 ml), DCC (0.52 g, 2.52 mmol) was added gradually at 0°C. The solution was stirred for 12 h at room temperature and then filtered. The filtrate was evaporated to dryness, and the residue was dissolved in $CHCl_3$ and filtered through a short column of silica gel. Evaporation of the solvent gave unstable and colorless solid of **6** (0.72 g, 99% yield) which was used for the following condensation reactions without further purification.

3-(2-Chlorothiazole-5-carboxamido)dimethylaminopropane hydrochloride (8a) A solution of 3-aminopropyldimethylamine (0.20 ml, 1.59 mmol) in THF (5 ml) was added dropwise to a stirred solution of **4** (prepared from 300 mg of **3**) in THF (10 ml) at -70°C and stirring was

continued for 1 h. The mixture was warmed up to room temperature and condensed under reduced pressure. The residue was purified by column chromatography on silica gel with mixed solvent of $CHCl_3$, MeOH, and 28% aq. NH_4OH (400:100:3) as eluent. To a solution of the product in MeOH (5 ml), 1N hydrochloric acid (1.73 ml, 1.73 mmol) was added at 0 °C. After stirring at the same temperature for 5 min, the mixture was evaporated to dryness to give the hydrochloride (**8a**) (409 mg, 100% yield) as a pale yellow oil. Ir (KBr) 1641, 3262 cm^{-1}. Nmr (DMSO-d_6) δ 1.90 (2H, m), 2.73 (3H, s), 2.75 (3H, s), 3.10 (2H, m), 8.40 (1H, s), 9.27 (1H, br), 10.49 (1H, br) ppm. Ms(FAB) 248 (M^+).

3-[1-Methyl-4-(2-chlorothiazole-5-carboxamido)pyrrole-5-carboxamido]dimethylaminopropane hydrochloride (8b) A solution of **7** (n=1) [prepared from 403 mg (1.59 mmol) of appropriate nitro derivative as described in the literature[3a,c]] in THF (10 ml) was added dropwise to a stirred solution of **4** (prepared from 300 mg of **3**) in THF (10 ml) at 0°C and stirring was continued for 2 h. The mixture was warmed up to room temperature and condensed under reduced pressure. The residue was purified by column chromatography on silica gel with mixed solvent of $CHCl_3$, MeOH, and 28% aq. NH_4OH (400:100:3) as eluent. The product was treated with hydrochloric acid as above to give the hydrochloride (**8b**) (404 mg, 69% yield) as a pale yellow powder; mp 135-138°C. Ir (KBr) 1642, 3324, 3449 cm^{-1}. Nmr (DMSO-d_6) δ 1.89(2H, m), 2.72 (3H, s), 2.74 (3H, s), 3.02 (2H, m), 3.22 (2H, m), 3.82 (3H, s), 6.95 (1H, d, J=1.6), 7.25 (1H, d, J=1.6), 8.30 (1H, br), 8.56 (1H, s), 10.58 (1H, br), 10.99 (1H, s) ppm. Ms(FAB) 370 (M^+).

3-{1-Methyl-4-[1-methyl-4-(2-chlorothiazole-5-carboxamido)pyrrole-5-carboxamido]pyrrole-5-carboxamido}dimethylaminopropane hydrochloride (8c) By the same procedure as that described for **8b**, **8c** was obtained from **7** (n=2) [prepared from 597 mg (1.59 mmol) of appropriate nitro derivative as described in the literature[3a,c]] and **4** (prepared from 300 mg of **3**) as a pale yellow powder. Yield, 586 mg (77% yield); mp 165-170°C. Ir (KBr) 1641, 3281 cm^{-1}. Nmr (DMSO-d_6) δ 1.90 (2H, m), 2.72 (3H, s), 2.74 (3H, s), 3.05 (2H, m), 3.28 (2H, m), 3.82 (3H, s), 3.86 (3H, s), 6.94 (1H, d, J=1.8), 7.13 (1H, d, J=1.6), 7.22 (1H, d, J=1.8), 7.30 (1H, d, J=1.6), 8.21 (1H, br), 8.65 (1H, s), 10.03 (1H, s), 10.80 (1H, br), 11.13 (1H, s) ppm. Ms(FAB) 492 (M^+).

3-{1-Methyl-4-[1-methyl-4-(1-methyl-4-(2-chlorothiazole-5-carboxamido)pyrrole-5-carboxamido)pyrrole-5-carboxamido]pyrrole-5-carboxamido}dimethylaminopropane hydrochloride (8d) By the same procedure as that described for **8**b, **8**d was obtained from **7** (n=3) [prepared from 527 mg (1.06 mmol) of appropriate nitro derivative as described in the literature[3a,c]] and **4** (prepared from 200mg of **3**) as a pale yellow powder. Yield, 275 mg (44% yield); mp 190-195°C. Ir (KBr) 1641, 3278 cm^{-1}. Nmr (DMSO-d_6) δ 1.87 (2H, m), 2.73 (3H, s), 2.76 (3H, s), 3.05 (2H, m), 3.27 (2H, m), 3.82 (3H, s), 3.85 (3H, s), 3.88 (3H, s), 6.93 (1H, d, J=1.7), 7.07 (1H, d, J=1.7), 7.11 (1H, d, J=1.6), 7.20 (1H, d, J=1.6), 7.26 (1H, d, J=1.7), 7.29 (1H, d, J=1.6), 8.19 (1H, br), 8.52 (1H, s), 9.94 (1H, s), 10.04 (1H, s), 10.34 (1H, br), 10.92 (1H, s) ppm. Ms(FAB) 614 (M^+).

3-(2-Bromothiazole-5-carboxamido)dimethylaminopropane hydrobromide (9a) A solution of 3-aminopropyldimethylamine (0.14 ml, 1.08 mmol) in THF (5 ml) was added dropwise to a stirred solution of **6** (300 mg, 0.98 mmol) in DMF (10 ml) at -70°C and stirring was continued for 1 h. The mixture was warmed up to room temperature and stirred for further 1 h, and then condensed under reduced pressure. The residue was purified by column chromatography on silica gel with mixed solvent of $CHCl_3$, MeOH, and 28% aq. NH_4OH (400:100:3) as eluent. To a solution of the product in MeOH (5 ml), 1N hydrobromic acid (0.87 ml, 0.87 mmol) was added at -70°C. After stirring at the same temperature for 5 min, the mixture was evaporated to dryness to give the hydrobromide **9**a (290 mg, 79% yield) as a pale yellow oil. Ir (KBr) 1636, 3060, 3275 cm^{-1}. Nmr (DMSO-d_6) δ 1.87 (2H, m), 2.76 (3H, s), 2.79 (3H, s), 3.01 (2H, m), 3.28 (2H, m), 8.30 (1H, s), 9.00 (1H, br), 9.48 (1H, br) ppm. Ms(FAB) 292 (M^+), 294 (M^++2).

3-[1-Methyl-4-(2-bromothiazole-5-carboxamido)pyrrole-5-carboxamido]dimethylaminopropane hydrobromide (9b) A solution of **7** (n=1) [prepared from 458 mg (1.80 mmol) of appropriate nitro derivative as described in the literature[3a,c]] in DMF (5 ml) was added dropwise to a stirred solution of **6** (500 mg, 1.64 mmol) in DMF (10 ml) at -70°C and stirring was continued for 1 h. The mixture was warmed up to room temperature and stirred for further 12 h, and then condensed under reduced pressure. The residue was purified by column chromatography on silica gel with mixed solvent of $CHCl_3$, MeOH, and 28% aq. NH_4OH (400:100:3) as eluent. The product was treated with hydrobromic acid as above to give the hydrobromide (**9**b) (325 mg, 40%

yield) as a brown oil. Ir (KBr) 1636, 3393 cm^{-1}. Nmr (DMSO-d_6) δ 1.94 (2H, m), 2.77 (3H, s), 2.80 (3H, s), 3.07 (2H, m), 3.23 (2H, m), 3.83 (3H, s), 6.92 (1H, d, J=2.1), 7.22 (1H, d, J=2.1), 8.24 (1H, br), 9.46 (1H, br), 10.69 (1H, br) ppm. Ms(FAB) 414 (M^+), 416.(M^++2).

3-{1-Methyl-4-[1-methyl-4-(2-bromothiazole-5-carboxamido)pyrrole-5-carboxamido]-pyrrole-5-carboxamido}dimethylaminopropane hydrobromide (9c) By the same procedure as that described for **9b**, **9c** was obtained from **7** (n=2) [prepared from 407 mg (1.08 mmol) of appropriate nitro derivative as described in the literature[3a,c]] and **6** (300 mg, 0.98 mmol) as an amorphous pale yellow powder. Yield, 324 mg (54% yield); mp 62-73°C. Ir (KBr) 1641, 3304 cm^{-1}. Nmr (DMSO-d_6) δ 1.86 (2H, m), 2.77 (3H, s), 2.80 (3H, s), 3.06 (2H, m), 3.26 (2H, m), 3.82 (3H, s), 3.87 (3H, s), 6.93 (1H, d, J=1.8), 7.06 (1H, d, J=1.8), 7.20 (1H, d, J=1.8), 7.27 (1H, d, J=1.8), 8.18 (1H, br), 8.41 (1H, s), 9.45 (1H, br), 9.98 (1H, s), 10.71 (1H, s) ppm. Ms(FAB) 536 (M^+), 538 (M^++2).

3-{1-Methyl-4-[1-methyl-4-(1-methyl-4-(2-bromothiazole-5-carboxamido)pyrrole-5-carboxamido)pyrrole-5-carboxamido]pyrrole-5-carboxamido}dimethylaminopropane hydrobromide (9d) By the same procedure as that described for **9b**, **9d** was obtained from **7** (n=3) [prepared from 324 mg (0.19 mmol) of appropriate nitro derivative as described in the literature[3a,c]] and **6** (180 mg, 0.59 mmol) as a pale yellow powder. Yield, 116 mg (83% yield); mp 75-87°C. Ir (KBr) 1641, 3304 cm^{-1}. Nmr (DMSO-d_6) δ 1.86 (2H, m), 2.78 (3H, s), 2.80 (3H, s), 3.08 (2H, m), 3.27 (2H, m), 3.83 (3H, s), 3.86 (3H, s), 3.88 (3H, s), 6.94 (1H, d, J=1.5), 7.07 (1H, d, J=1.8), 7.08 (1H, d, J=1.6), 7.20 (1H, d, J=1.5), 7.26 (1H, d, J=1.6), 7.28 (1H, d, J=1.5), 8.18 (1H, br), 8.43 (1H, s), 9.50 (1H, br), 9.94 (1H, s), 10.02 (1H, s), 10.73 (1H, s) ppm. Ms(FAB) 658 (M^+), 660 (M^++2).

Plasmid relaxation 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 0.1 ~ 100 μM compound was irradiated in Eppendorf tubes from above at 20°C using a Vilber Lourmat VL-30L (2x15W, 365 nm maximum, 13 $J \cdot m^{-2} \cdot sec^{-1}$) fluorescent light at a distance of 8.6 cm for 2 h. Immediately following irradiation, 15 μl samples were loaded into 1% agarose gels. The running buffer was 20 mM TAE, pH 7.8. Electrophoresis was at 50 V for 8 h. After

electrophoresis, gels were 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-900) using fluorescence mode.

REFERENCES

1. A. MacRobert and D. Phillips, *Chem. Ind.,* 1992, 17.
2. M. Otsuka, T. Masuda, A. Haupt, M. Ohno, T. Shiraki, Y. Sugiura, and K. Maeda, *J. Am. Chem. Soc.*, 1990, **112**, 838; E. J. Verner, B. J. Oliver, L. Schlicksupp, and N. R. Natale, *Heterocycles*, 1990, **31**, 327; T. Matsumoto, K. Toyooka, E. Nishiwaki, and M. Shibuya, *Heterocycles*, 1990, **31**, 1629; K. E. Rao, Y. Bathini, and J. W. Lown, *J. Org. Chem.,* 1990, **55**, 728; J. L. Aubagnac, F. Debart, D. Mrani, G. Gosselin, B. Rayner, and J.-L. Imbach, *J. Heterocycl. Chem.,* 1991, **28**, 145; K. E. Rao, K. Krowicki, G. Burckhardt, C. Zimmer, and J. W. Lown, *Chem. Res. Toxicol.,* 1991, **4**, 241; F. Subra, S. Carteau, J. Pager, J. Paoletti, C. Paoletti, C. Auclair, D. Mrani, G. Gosselin, and J.-L. Imbach, *Biochem.,* 1991, **30**, 1642; K. E. Rao, J. Zimmermann, and J. W. Lown, *J. Org. Chem.,* 1991, **56**, 786; S. L. Grokhovsky and V. E. Zubarev, *Nucleic Acids Res.,* 1991, **19**, 257; B. Plouvier, C. Bailly, R. Houssin, K. E. Rao, W. J. Lown, J-P. Hénichart, and M. J. Waring, *Nucleic Acids Res.,* 1991, **19**, 5821.
3. a) E. Nishiwaki, H. Lee, T. Matsumoto, K. Toyooka, H. Sakurai, and M. Shibuya, *Tetrahedron Lett.*, 1990, **31**, 1299; b) T. Matsumoto, K. Toyooka, E. Nishiwaki, and M. Shibuya, *Heterocycles*, 1990, **31**, 1629; c) E. Nishiwaki, H. Nakagawa, M. Takasaki, T. Matsumoto, H. Sakurai, and M. Shibuya, *Heterocycles*, 1990, **31**, 1763; d) T. Matsumoto, Y. Sakai, K. Toyooka, and M. Shibuya, *Heterocycles*, 1992, **33**, 135.
4. T. Matsumoto, Y. Utsumi, Y. Sakai, K. Toyooka, and M. Shibuya, *Heterocycles*, 1992, **34**, 1697.
5. J. A. Edwards, *U.S.Patent*, **1977**, 4,001,421 (*Chem. Abstr.*, 1977, **86**, 171436b).
6. A. G. Motten, G. R. Buettner, and C. F. Chignell, *Photochem. Photobiol.*, 1985, **42**, 9.
7. R. K. Sharma and N. Kharasch, *Angew. Che. internat. Edit.*, 1968, **7**, 36; J. Grimshaw, *Chem. Soc. Rev.*, 1981, **10**, 181.

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