SYNTHESIS OF β-CARBOLINE DERIVATIVES AND THEIR INTERACTION WITH DUPLEX-DNA

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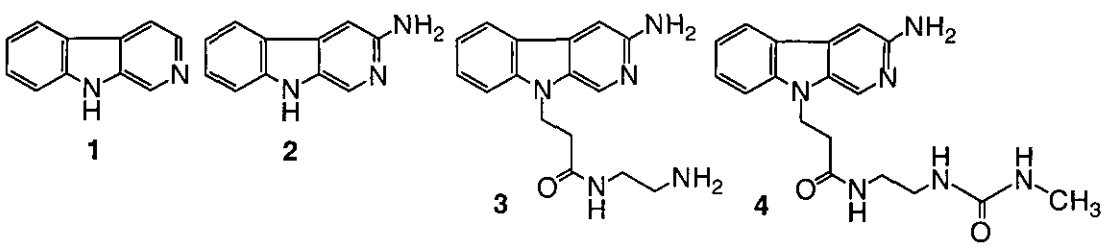
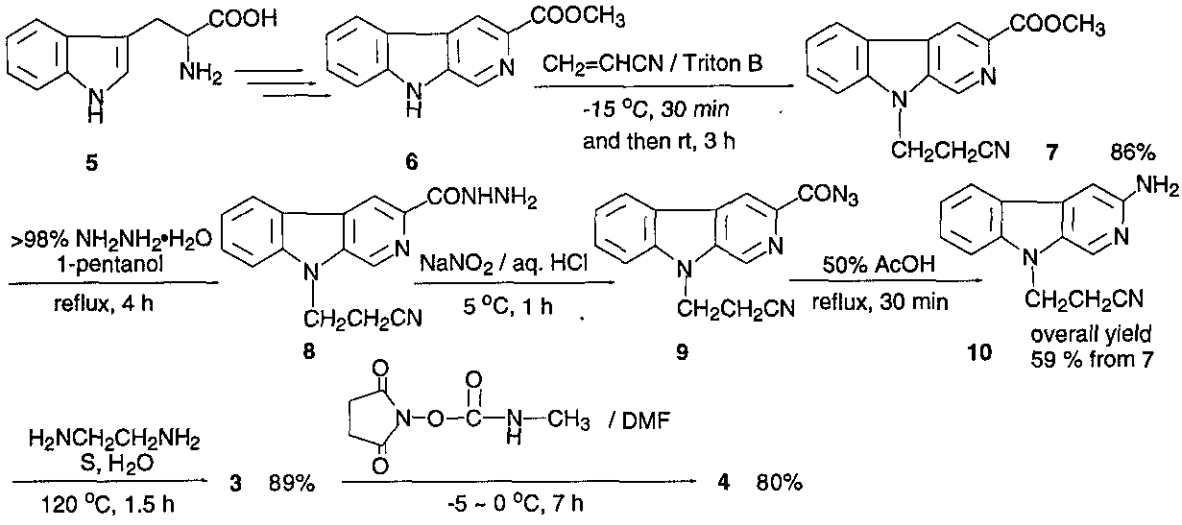
Abstract — 3-Amino-9-*N*-(2-aminoethyl)carbamoylethyl-β-carboline (**3**) was synthesized from 3-methoxycarbonyl-β-carboline through successive cyanoethylation, Curtius rearrangement, and addition reaction of ethylenediamine in 45 % overall yield. The terminal aliphatic amino group of **3** was transformed to a *N'*-methylureido group with succinimidyl *N*-methylcarbamate to afford a compound **4** in 80 % yield. Affinity of **3**, **4**, β-carboline (**1**), and 3-amino-β-carboline (**2**) to a DNA oligomer or calf thymus DNA was spectrophotometrically studied. Optical spectroscopy of these β-carbolines showed 25 ~ 65 nm bathochromic shifts of the light absorption bands upon binding to the nucleic acids, which suggests binding by intercalation. Scatchard plots, obtained by fluorescence titration, gave the corresponding binding constants K_D to the DNA oligomer; 7.45 x 10^3, 5.13 x 10^5, 7.34 x 10^5, and 7.01 x 10^5, respectively. The terminal aliphatic amino group considerably decreases the intercalating ability of **3** to DNA.

N-Alkyl-*N*-nitrosoureas alkylate DNA *via* an aliphatic diazonium ion or a solvent-separated ion pair, and this modification of DNA is associated with the mutagenic, carcinogenic, and antineoplastic activities of the drugs.[1,2] The yield and the selectivity of this alkylation, however, are extremely low. This is particularly frustrating to elucidate the mechanism of alkylation and sequence specificity. On the other hand, it has been reported that *N*-heterocyclic compounds possessing an aromatic condensed ring, such as acridines, proflavins, ellipticines, have high affinity for G-C rich sites or specific sequences of the duplex-DNA.[3] Therefore, if an alkylnitrosourea is linked to one of these intercalators, the yields and specificity of the alkylation should be accelerated by delivery of the alkylater. On the basis of this concept, we have reported a synthesis of an alkylnitrosourea linked to a DNA intercalating methidium structure, and its reaction with DNA.[4] As an extension of this study, preparation of a new intercalating molecule, 3-amino-9-*N*-(2-*N'*-methylureido-





Scheme

ethyl)carbamoylethyl-β-carboline (**4**), was planned. It has a β-carboline structure that recognizes a G-C base pair more selectively than a phenanthridine structure does.[3] In this paper, we wish to report the synthesis of 3-amino-9-*N*-(2-aminoethyl)carbamoylethyl-β-carboline (**3**) and **4** together with their interaction with calf thymus DNA or a synthesized duplex-DNA oligomer, [5'-d(CATCCCGGGATG)-3']$_2$.

Strategy for the preparation of **3** and **4** is shown in Scheme. Firstly, 3-methoxycarbonyl-β-carboline (**6**) was synthesized from *L*-tryptophan (**5**) according to a method appeared in literature.[5-7] Secondly, Triton B (a 40 % solution in methanol) was added slowly to **6** in acrylonitrile at -15 °C, then the mixture was stirred for 30 min at -15 °C and for 3 h at room temperature to give **7** (mp 213.0 ~ 213.9 °C) in 86% yield. Next, **7** was subjected to Curtius reaction in the following manner [6]: a mixture of **7** and hydrazine hydrate was heated under reflux for 4 h in 1-pentanol to give the hydrazide (**8**), which was treated with sodium nitrite / hydrochloric acid for 1 h at 5 °C to yield the azide (**9**). On refluxing for 30 min in 50 % acetic acid, **9** rearranged to 3-amino-9-cyanoethyl-β-carboline (**10**, mp 194.4 ~ 195.5 °C) in 59 % overall yield from **7** . In these successive reactions from **7** to **10**, crude products of **8** and **9** were used to the next reaction without further purification. The compound (**10**) was heated at 120 °C for 1.5 h in a mixture of ethylenediamine and H_2O in the presence of sulfur to give **3** (mp > 208 °C (decomp)) in 89 % yield. Finally, the reaction of **3** with succinimidyl *N*-methylcarbamate in dry *N,N*-dimethylformamide gave the β-carboline-*N'*-methylurea (**4**, mp > 211 °C (decomp)) in 80 % yield.[15]

The interaction of β-carboline derivatives (**1**) ~ (**4**) with the duplex-DNA oligomer was spectrophotometrically studied. Commercial product of norharman (**1**) was used without additional purification. 3-Amino-β-carboline (**2**) was synthesized from **6** according to a method appeared in literature.[7] Optical titrations of β-carboline derivatives with the duplex-DNA oligomer were shown in Figures 1 a, b. They indicate 25 ~ 65 nm bathochromic shifts of the light absorption bands upon binding to the nucleic acids, which suggest binding by intercalation.[16] Data obtained by fluorescence titration were fitted to the cooperativity equation by McGhee and von Hippel[17] (shown in Figure 2) to give the corresponding binding constants K_D, which indicate that the aliphatic amino group of **3** considerably decreases the intercalation to DNAs (Table). This suppression of intercalation ability may be owing to the hydrogen-bonding between the aliphatic amino group and anionic

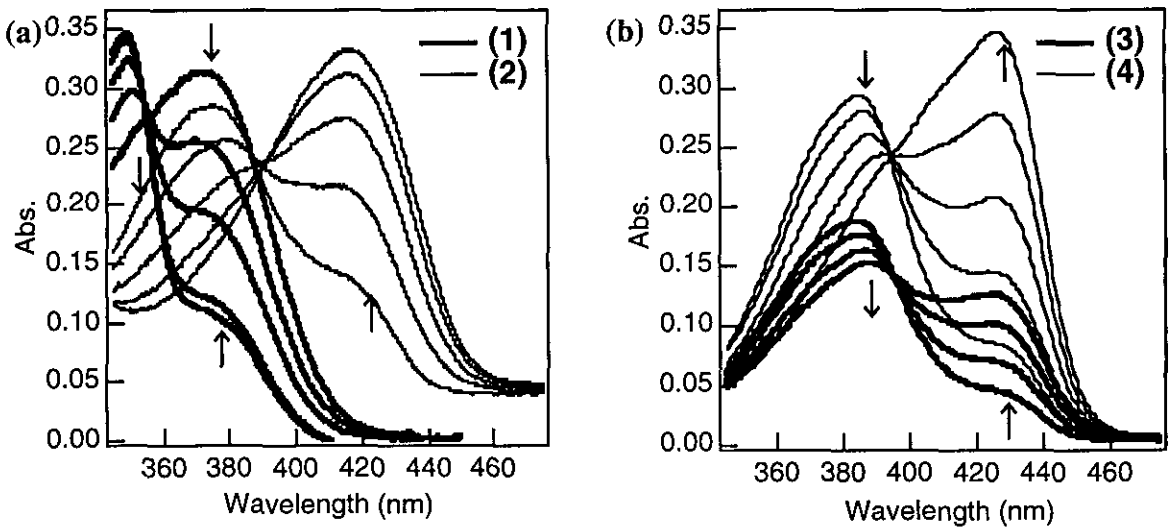


Figure 1. Optical titrations of β-carbolines with the 12 mer DNA in 1 mM sodium phosphate-EDTA·2Na buffer (pH 7.4, 25°C) ; Final volume of the DNA stock solution was 20 μL. The concentration of DNA in stock solution is 8.15×10^{-4} M (**1**, **2**) and 1.06×10^{-4} M (**3**, **4**). The concentration of **1**, **2**, **3**, and **4** in a UV cuvette was 1.25×10^{-4}, 9.83×10^{-3}, 1.28×10^{-4}, and 1.31×10^{-4} M, respectively. UV-VIS spectra were recorded on a JASCO V-550 spectrophotometer. Here, arrows in these figure show the direction of titrations, respectively.

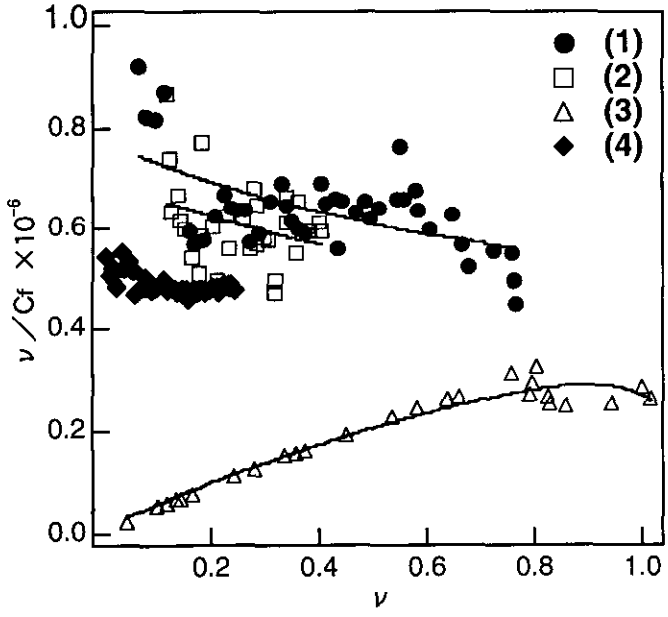


Table. Binding constants, K_D, of β-carbolines **1** ~ **4** with the duplex-DNA, obtained by Scatchard analysis.

Compound	K_D (M^{-1})	
	12 mer DNA	calf thymus DNA
1	7.75×10^5	1.63×10^{6} a)
2	7.01×10^5	———
3	7.45×10^3	4.61×10^3
4	5.13×10^5	5.91×10^5

a) lit.,[18] $K_D = 2.19 \times 10^5$

Figure 2. Scatchard plots for β-carbolines binding to the 12 mer DNA ; Fluorescence spectra were measured on a Shimadzu RF5000 spectrofluorophotometer in 1 mM sodium phosphate EDTA·2Na buffer (pH 7.4, 25°C), in which the concentration of sodium cations was adjusted to 0.05 M. The wave lengths to detect fluorescence intensities in this study were 350.4 (excitation) and 443.2 nm (emission) for **1**, 315.0 (excitation) and 454.0 nm (emission) for **2**, 376.0 (excitation) and 447.6 nm (emission) for **3**, 425.2 (excitation) and 454.0 nm (emission) for **4**, respectively, and the slit widths were 5.0 (excitation) and 1.5 nm (emission).

phosphoric acid back-bones of the nucleic acids. In fact, the curve of **3** has a different slope from the others as shown in Figure 2. It has been reported[19] that a curve has a positive slope when bound ligands of adjacent sites attract each other. The K_D value of compound **4** (5.13 x 10^5) is almost equal to that of 1 (7.34 x 10^5). Therefore it is confirmed that the binding ability of **4** to DNA is not affected by the tether linked to the 9-position. As extension of this study, methylation of DNA by the corresponding *N'*-nitroso derivative of **4** is under investigation.

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9. Spectral data for **7**; ^{1}H NMR(400 MHz, $CDCl_3$) : δ_H 3.00(t, *J*=6.8 Hz, 2H), 4.08(s, 3H), 4.85(t, *J*=6.8 Hz, 2H), 7.45(t, *J*=7.6 Hz, 1H), 7.60(d, *J*=8.4 Hz, 1H), 7.73(t, *J*=7.6 Hz, 1H), 8.25(d, *J*=8.0 Hz, 1H), 8.92(s, 1H), 9.13(s, 1H); IR(KBr) : υ_{CN} 2248 cm^{-1}, υ_{CO} 1722 cm^{-1}; MS(70 eV) : *m/z* 279(M^+).
10. IR(KBr) data for **8** : υ_{NH} 3320 cm^{-1}, υ_{CN} 2250 cm^{-1}, υ_{CO} 1660 cm^{-1}
11. IR(KBr) data for **9** : υ_{CN} 2248 cm^{-1}, υ_{N3} 2130 cm^{-1}, υ_{CO} 1690 cm^{-1}
12. Spectral data for **10**; ^{1}H NMR(400 MHz, DMSO-d_6) : δ_H 3.02(t, *J*=6.0 Hz, 2H), 4.66(t, *J*=6.0 Hz, 2H), 5.46(br, 2H), 7.10(s, 1H), 7.15(t, *J*=7.6 Hz, 1H), 7.51(t, *J*=7.6 Hz, 1H), 7.63(d, *J*=8.0 Hz, 1H), 8.05(d, *J*=7.6 Hz, 1H), 8.49(s, 1H); IR(KBr) : υ_{NH} 3420, 3325 cm^{-1}, υ_{CN} 2250 cm^{-1}; MS(70 eV) : *m/z* 236(M^+).
13. Spectral data for **3** · HCl; ^{1}H NMR(400 MHz, D_2O) : δ_H 2.56(t, *J*=6.4 Hz, 2H), 2.98(t, *J*=6.0 Hz, 2H), 3.55(t, *J*=6.0 Hz, 2H), 4.33(t, *J*=6.4 Hz, 2H), 6.82(s, 1H), 7.03(t, *J*=7.6 Hz, 1H),7.09(d, *J*=7.1 Hz, 1H), 7.49(d, *J*=7.6 Hz, 1H), 7.55(d, *J*=7.6 Hz, 1H), 7.61(s, 1H); IR(KBr) : υ_{NH} 3403, 3325 cm^{-1}, υ_{CO} 1670 cm^{-1}; MS(70 eV) : *m/z* 297(M^+).
14. Spectral data for **4**; ^{1}H NMR(300 MHz, $CD_3OD + D_2O$) : δ_H 2.62(s, 3H), 2.69(t, *J*=6.6 Hz, 2H), 2.95(t, *J*=5.7 Hz, 2H), 3.06(t, *J*=5.7 Hz, 2H), 4.62(t, *J*=6.6 Hz, 2H), 7.19(t, *J*=6.9 Hz, 1H), 7.34(s, 1H), 7.54(d, *J*=8.0 Hz, 1H), 7.58(t, *J*=6.7 Hz, 1H), 8.06(d, *J*=7.8 Hz, 1H), 8.34(s, 1H); IR(KBr) : υ_{NH} 3378 cm^{-1}, υ_{CH} 2923 cm^{-1}, υ_{CO} 1635 cm^{-1}; MS(70 eV) : *m/z* 354(M^+).
15. All new compounds (**3**, **4**,.**7**~**10**) gave satisfactory results for C, H, N analysis or HRMS.
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