ACTINOCINYL-BIS(HEMIN); DNA-INTERCALATING CLEAVAGE AGENT[1]

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Abstract——The synthesis of actinocinyl-bis(mesoporphyrinatoiron) (1) was described. The intercalating bis(hemin) (1) caused effective DNA scission in the presence of $Na_2S_2O_4$ under air.

It is well known that many antitumor and tumor agents show the biological activities by interacting with double strand DNA, such as binding to and cleaving.[2] Bleomycin, for example, is one of the most potent antitumor agents known, which strongly binds to DNA, and causes effective DNA scission.[3] In this connection, artificial DNA-cleaving agents have been extensively studied. As shown in Fig. 1, the current DNA-cleaving agents involving iron complex would be classified to four types of categories based on their characteristic functions; A) cleaving group (iron-complexing)-intercalator,[4]; B) cleaving group-nonintercalator;[5] C) bis(cleaving group)-nonintercalator;[6] D) cleaving group-bis(intercalator).[7] We now report on the synthesis and DNA scission of a new type DNA-cleaving agent, actinocinyl-bis(hemin) which should be classified to the bis(cleaving group)-intercalator(type E).[8]

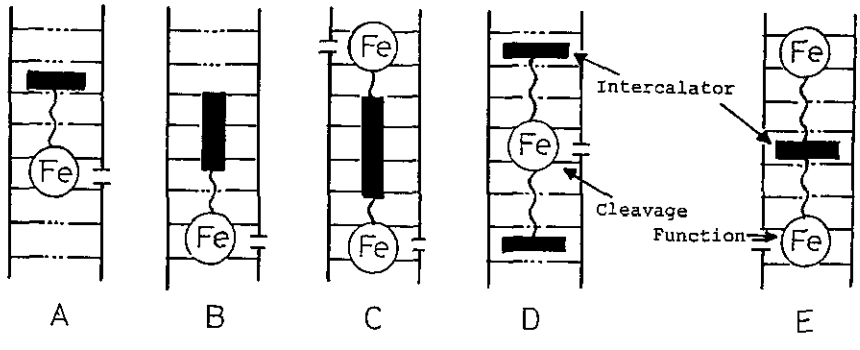
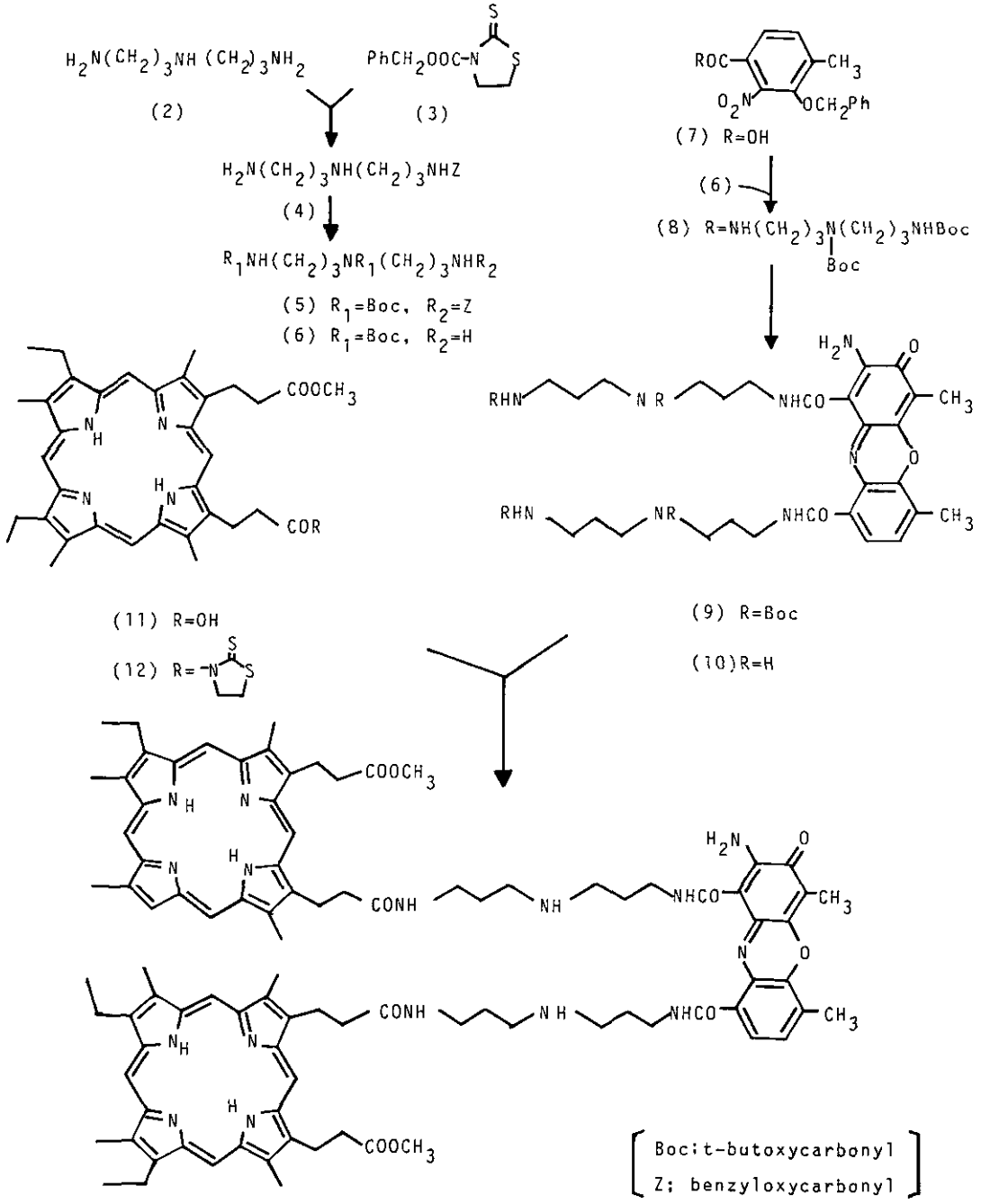


Fig. 1

Many routs have been known to the preparations of actinocin derivatives (actinomycins).[9] Both side chains of actinocin have been generally introduced prior to construction of the skeleton because actinocin, itself, is practically insoluble in most solvents. Thus, the actinocinyl-bis (hemin) (1) was synthesized according to Scheme 1. The key compound, actinocinyl-bis(triamine) (10), was prepared by utilizing a modified method of *Brockmann*.[10] *The bis(triamine)* (10) was treated with mesoporphyrin monomethyl ester monothiazolidine-2-thione (12), prepared by a condensation of mesoporphyrin monomethyl ester monoacid (11)[11] with 1,3-thiazolidine-2-thione,[12] to give actinocinyl-bis(mesoporphyrin) (13) in 38% yield. The visible spectrum of (13) shows characteristic absorption bands due to the porphyrins at 394, 498, 531, 570 and 623 nm, but does not furnish definitive absorption bands for the actinocin chromophore because of the strong bands of bis(porphyrin) chromophores. The FAB mass spectrum of (13) exhibits a molecular ion peak at m/z 1681 $(M+H)^+$ corresponding to $C_{98}H_{118}N_{16}O_{10}$.

The actinocinyl-bis(porphyrin) (13) was treated with $FeCl_3$ in AcOH to give actinocinyl-bis(mesoporphyrinatoiron) (1) in 83% yield. The visible spectrum of (1) shows characteristic absorption bands assignable to the oxidized hemin at 393 and 589 nm,[13] and the FAB mass spectrum exhibits a molecular ion peak at m/z 1790 $(M+2H-2Cl)^+$ corresponding to $C_{98}H_{114}N_{16}O_{10}Fe_2Cl_2$.

The cleavage of DNA was followed by monitoring the conversion of Col E1 supercoiled closed circular (ccc) DNA (form I) to closed circular (cc) (form II) and the recovery of total DNA of forms I and II. As shown in Table 1, protoporphyrinatoiron (Hemin) and $FeCl_3$ at $>10^{-4}$M concentration in the presence of $Na_2S_2O_4$ (5×10^{-5}M) under air caused slightly or no cleavage. In contrast, the actinocinyl-bis(hemin) (1) at $>10^{-5}$M concentration under the similar conditions caused greatly DNA scission. In view of the recovery of total DNA, it should be noted that remarkable DNA cleavage takes places at $>2.5 \times 10^{-5}$M concentration of (1).


(2)
(3)
(4)
(5) R1=Boc, R2=Z
(6) R1=Boc, R2=H
(7) R=OH
(8)
(9) R=Boc
(10) R=H
(11) R=OH
(12)
(13)
(1); Iron (III) complex of (13)
Boc; t-butoxycarbonyl
Z; benzyloxycarbonyl

Scheme 1

Table 1. Cleavage of Col E1 Plasmid DNA by (1), Hemin, and $FeCl_3$.

Run	Ligand	Concentration of Ligand ($\times 10^{-5}$M)	$Na_2S_2O_4$ ($\times 10^{-3}$M)	Ratio of Forms I : II	Recovery of[a] Forms I+II (%)
1	---	---	---	65 : 35	100
2	(1)	---	5	65 : 35	73
3	(1)	1.0	5	61 : 39	61
4	(1)	2.5	5	58 : 42	29
5	(1)	5.0	5	17 : 83	4
6	(1)	10.0	5	----	0
7	Hemin[b]	1.0	5	65 : 45	86
8	Hemin	10.0	5	60 : 40	60
9	$FeCl_3$ alone	1.0	5	61 : 39	85
10	$FeCl_3$ alone	10.0	5	61 : 39	80

a. Each recovery of total DNA (forms I and II) was calculated in terms of 100% recovery of the total DNA in run 1.
b. Hemin; protoporphyrinatoiron (III) chloride.

EXPERIMENTAL

Proton nmr spectra were recorded with JEOL-60 and GX-270, uv-visible spectra with a Hitachi 557, mass spectra with a JEOL DX-300, FAB mass spectra with a JEOL HX-100, and fluorescence densitometry with a Shimadzu CS-910 spectrometers.

N,N'-1,4-Di-t-butoxycarbonyl-1,7-diamino-4-azaheptane (6)

To a solution of 1,7-diamino-4-azaheptane (2) (8.5 g, 65 mmol) in CH_2Cl_2 (50 ml) was added dropwise a solution of 3-benzyloxycarbonyl-1,3-thiazolidine-2-thione (3)[14] at room temterature. After the mixture was stirred for 2 h, the precipitated thiazolidine-2-thione was filtered off and the filtrate was extracted with AcOEt-water. The AcOEt layer was washed with water, dried over $MgSO_4$ and concentrated in vacuo. The residue was taken on Al_2O_3 (3% water) column chromatography with CH_2Cl_2-MeOH to give 1.44 g of N-1-Z-triamine (4) in 84% yield; ms, m/z 265 $(M)^+$(0.9%).

The 1-Z-triamine (4) (0.6 g, 2.3 mmol), di-t-butyldicarbonate [$(Boc)_2O$] (1.09 g, 5 mmol) and triethylamine (0.65 g. 5 mmol) in DMF (10 ml) were stirred at room temperature for 15 h. The reaction mixture was concentrated in vacuo at 60 °C, and the residue was extracted with AcOEt. The AcOEt layer was washed with water, dried over $MgSO_4$ and concentrated in vacuo. The residue was taken on SiO_2 column chromatography with CH_2Cl_2-aceton to give 0.82 g of di-Boc-Z-

triamine (5) in 78% yield; ms, m/z 465 $(M)^+$(0.9%).

A solution of (5) (0.27 g, 0.58 mmol) in MeOH (30 ml) was hydrogenated on Pd-C to give 0.18 g of N,N'-1,4-di-Boc-triamine (6) in 94% yield; nmr ($CDCl_3$), 6.25 (m, 3H, NH), 3.4-2.8 (m, 6H, $BocNCH_2$), 2.2-2.1 (m, 6H) and 1.33 (s, 18H, CH_3).

Actinocinyl-bis(triamine) (10)

To a solution of (7) (453 mg, 1.58 mmol) in dry CH_2Cl_2-pyridine (97:3) (10 ml) was added $SOCl_2$ (3 ml) and was refluxed for 20 h. After the solution was concentrated in vacuo, to the residue was added dry benzene and the mixture was filtered off to remove the pyridinium chloride. The filtrate was concentrated in vacuo and dissolved in dry CH_2Cl_2 (10 ml). The CH_2Cl_2 solution was added to a solution of (6) (516 mg, 1.56 mmol) in CH_2Cl_2 (10 ml) contained Et_3N (0.6 ml) and stirred for 24 h. The reaction mixture was extracted with AcOEt. The ACOEt layer was washed with water, dil.HCl, water, 5% aq.$NaHCO_3$ and water, and dried over $MgSO_4$. The solution was concentrated in vacuo and the residue was taken on SiO_2 column chromatography with CH_2Cl_2-acetone to give 647 mg of (8) in 68% yield; nmr ($CDCl_3$), 7.33 (s, 4H, aromatic H), 4.93 (s, 4H, CH_2O-), 3.5-2.9 (m, 8H, $CONHCH_2CH_2$), 2.33 (s, 6H, CH_3), 2.0-1.3 (m, 4H) and 1.78, 1.75 ppm (s, 18H, t-butyl H), Anal. Calcd for $C_{21}H_{44}N_4O_8$: C, 61.98: H, 7.38; N, 9.33. Found: C, 62.35; H, 7.43; N, 9.09.

A solution of (8) (400 mg, 0.67 mmol) in MeOH (50 ml) was hydrogenated on Pd-C and filtered off. The filtrate was concentrated in vacuo and the residue was dissolved in MeOH-phosphate buffer (pH 6.0) (1:1, 50 ml). To the solution was added a solution of $K_3Fe(CN)_6$ (1.0 g) in water (2 ml) and kept at pH 7.0 by adding aq. NaOH. After stirring for 1h, the mixture was extracted three times with AcOEt (100 ml). The AcOEt layer was washed with NaCl-saturated water, dried over $MgSO_4$ and concentrated in vacuo. The residue was taken on SiO_2 column chromatography with CH_2Cl_2-MeOH to give 269 mg of actinocinyl-bis(Boc-triamine) (9) in 42% yield; nmr ($CDCl_3$), 7.45, 7.23 (dd, J=8 Hz, 2H, aromatic H), 3.5-2.8 (m, 16H, $CONHCH_2CH_2$), 2.40, 2.00 (s, 6H, CH_3), 1.9-1.2 (m, 8H, NCH_2CH_2), and 1.40 (s, 36H, t-butyl H). Anal. Calcd for $C_{48}H_{74}N_8O_{12}$: C, 60.36; H, 7.81; N, 11.73. Found: C, 60.17;H, 7.65; N, 11.65.

To a solution of (9) (119 mg, 0.125 mmol) in CH_2Cl_2 (5 ml) was added 20% CF_3COOH-CH_2Cl_2 (5 ml) at room temperature and stirred for 1.5 h. The mixture

was concentrated in vacuo and the residue was extracted waith 1% aq. HCl. The aq. solution was washed with CH_2Cl_2 and concentrated in vacuo under 45°C. The residue was recrystallized with EtOH-water to give 73 mg of actinocinyl-bis-(triamine).4HCl (10) in 84% yield: uv (EtOH), λ max; 420 (4.22) and 442 nm ($\log \varepsilon = 4.24$)[10]; Anal. Calcd for $C_{28}H_{42}N_8O_4 \cdot 4HCl$: C, 48.01; H, 6.62; N, 16.00. Found: C, 47.95; H, 6.46; N, 16.11.

Actinocinyl-bis(mesoporphyrinatoiron) (1)

A solution of mesoporphyrin monomethyl ester monoacid (11)[11] (200 mg, 0.345 mmol), 1,3-thiazolidine-2-thione,[12] (49 mg, 0.41 mmol), dicyclohexyl-carbodiimide (DCC) (93 mg, 0.45 mmol) and N,N'-dimethylaminopyridine (DMAP) (20 mg, 0.15 mmol) in dry CH_2Cl_2 (20 ml) was stirred under an argon atmosphere for 4 h. After the reaction mixture was concentrated in vacuo, the residue was taken on SiO_2 column chromatography with ethanol-free CH_2Cl_2-Et_2O under an argon atmosphere to give 164 mg of mesoporphyrin monomethyl ester monothiazolidine-2-thione (12) after the recrystallization with CH_2Cl_2-Et_2O in 70% yield.

To a solution of (10) (21 mg, 0.03 mmol) and Et_3N (40 mg, 0.39 mmol) in 20% EtOH-CH_2Cl_2 (20 ml) was added a solution of the thiazolidine (12) (46 mg, 0.067 mmol) in CH_2Cl_2 (5 ml) and stirred at room temperature for 5 h. The mixture was taken on Sephdex LH-20 column chromatography with deoxygenated MeOH to give 19 mg of actinocinyl-bis(mesoporphyrin) (13) after the recrystallization with CH_2Cl_2-hexane in 38% yield: FAB mas, m/z 1681 $(M+H)^+$; visible (EtOH), λ max 394 (5.19), 498 (4.15), 531 (3.97), 570 (3.86) and 623 nm ($\log \varepsilon$= 3.68).

A solution of (13) (6.5 mg, 0.004 mmol) and $FeCl_3$ (17 mg, 0.1 mmol) in 10% AcONa-AcOH (15 ml) was heated at 100 °C for 1 h. After cooling, the precipitate was separated by centrifugation, washed with water and dried in vacuo to give actinocinyl-bis(mesoporphyrinatoiron) (1) in 83% yield: FAB ms, m/z 1790 $(M+2H-2Cl)^+$; visible (EtOH), λ max 393 (5.31) and 589 nm ($\log \varepsilon$ =4.24).

Reactions of Col E1 Plasmid DNA with (1), Hemin and $FeCl_3$

The Col E1 DNA (purchased from Nippon Gene Co.) was dialyzed with 40 mM Tris/5 mM NaOAc buffer (pH 8.0) to remove EDTA contained in the solution of purchased DNA before using it. The reactiion mixture (15 μl) contained Col E1 DNA (0.6 μg), an iron complex and sodium dithionite (5 mM) in 40 mM Tris/ 5 mM NaOAc

buffer (pH 8.0). The reaction was carried out at 25°C for 60 min, and terminated by addition of a solution (10 µl) of 50% glycerol/25 mM EDTA/ 0.025% bromophenol blue. The reaction mixture, a final volume of 25 µl, was directly analyzed by agarose (0.9%) gel electrophoresis and fluoresence densitometry of the run gels stained by addition of ethidium bromide (0.5 µg/ml).

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