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SYNTHESIS OF PORPHYRIN(Fe)-INTERCALATORS WHICH CAUSE DNA SCISSION

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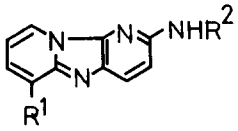
SUMMARY: New Porphyrin(Fe)-intercalators were synthesized. We chose 2-aminodipyrido[1,2-a:3',2'-d]imidazoles (Glu-P's), which were muta-carcinogens isolated from a pyrolysate of L-glutamic acid, as intercalator moieties. These porphyrin(Fe)-intercalators cause cleavage of double helical DNA.

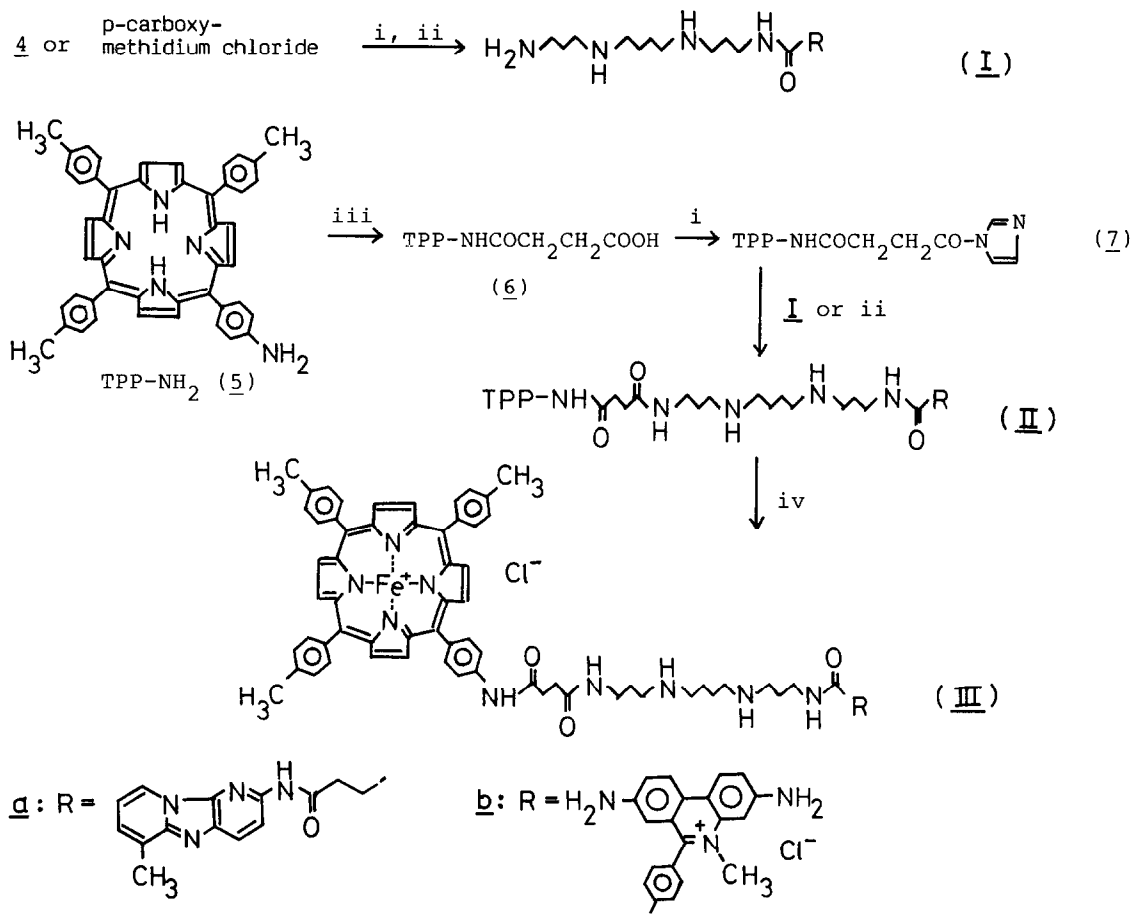
Intercalation is one of the important modes of interaction of muta-carcinogenic or antitumor agents with DNA. 2-Aminodipyrido[1,2-a:3',2'-d]imidazoles (Glu-P's) are potent muta-carcinogens isolated from a pyrolysate of L-glutamic acid.[1] We established that Glu-P's intercalate into DNA.[2] Recently, we reported that covalent connection of Glu-P-2 (1) with spermine (which is known to possess high affinity toward DNA) strongly enhances the affinity of the compound toward DNA, and that this compound (GP-SP: 3) strongly stabilizes double helical DNA from heat denaturation.[3] We also found that GP-SP (3) possesses DNA strand cleaving ability in the presence of Fe(II)-salt. We supposed that GP-SP (3) intercalate into DNA via Glu-P-2 moiety and, chelation of Fe(II) with spermine moiety and activation of oxygen causes the DNA cleavage. Such an oxygen-dependent DNA cleavage is another important mode of action of antitumor agents. Very recently, Herzberg and Dervan reported that (methidium-propyl-EDTA)-iron(II) possesses enhanced oxygen-dependent DNA cleaving ability.[4] In this communication, we describe synthesis of new porphyrin(Fe)-intercalators which possess strong DNA cleaving ability.

We chose Glu-P-1 (2) and p-carboxymethidium chloride as the intercalator moiety, which were prepared by our method described previously[5] and the method of Dervan and Becker,[6] respectively. Monoaminotetraphenylporphyrin (TPP-NH_2: 5) was synthesized by condensation of p-tolualdehyde, p-nitrobenzaldehyde and pyrrole, and successive reduction of the product with $SnCl_2$ in the yield of 2%. The structure was deduced from its ^{1}H-NMR (introduction of the p-substituent on all phenyl groups makes the complete assignment possible)

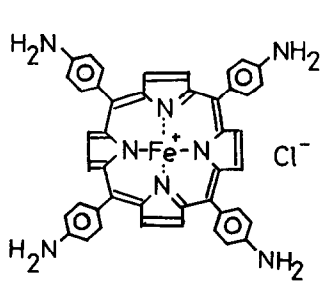


	R^1	R^2	
1:	H	H	(Glu-P-2)
2:	CH_3	H	(Glu-P-1)
3:	H	$(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	(GP-SP)
4:	H	$COCH_2CH_2COOH$	

Scheme 1



i: N,N'-carbonyldiimidazole, ii: spermine/DMF, iii: succinic anhydride, iv: $FeCl_2$/DMF



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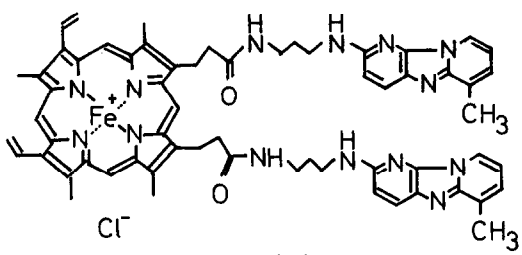

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Table 1. Percentage of closed circular DNA which was conversed to open circular DNA

drug and concentration (μM)		%[a]	drug and concentration (μM)		%[a]
none	100	<1	IIIa	1	11
$FeCl_2$	100	<1		10	39
spermine + $FeCl_2$	100	<1		100	76
GP-SP (3) + $FeCl_2$	100	56		100[c]	<1
8	100	1-2		1000	>99
IIa	100	<1	IIIb	50	45
9	100	52[b]		100	76
IIIc	100	53			

Reaction mixture (10 μl) contains final concentration of: 200 μM nucleotides of PM 2 DNA, 4 mM $Na_2S_2O_4$, 10 mM Tris-HCl (pH 7.8) and 50 mM NaCl. The reaction mixture was saturated with air and incubated at 20°C for 1 hr. Incubation at 37°C increased the percentage of the cleaved DNA. a) The percentage was calculated from the ratio of closed circular DNA against open circular DNA. b) Recovery of total DNA (calculated from ethidium bromide staining and densitometry) was low. The reason is under investigation. c) $Na_2S_2O_4$ was not added.

and elemental analysis.[7] The connection of TPP-NH_2 (5) with the intercalators was performed as shown in Scheme 1. Intercalators were derivatized to acylimidazole esters and connected with spermine (50-90%). These intercalator-spermines (I) were connected with the acylimidazole ester of succinylated TPP-NH_2 (7) (40-70%). Structure of the products were deduced from their ^{1}H-NMR and elemental analysis.[8] Iron was inserted into the products (II) by heating with $FeCl_2$ in DMF to afford IIIa-c in the yield of 40-90%. Products were purified by aluminum chromatography. Insertion of Fe(III) into the porphyrin moiety was confirmed by characteristic change on UV spectra (Q-band: 4 bands at 510-650 nm to 2 bands at 510-680 nm). We also synthesized a hemin-bis-intercalator (9) from Glu-P-1 (2) and hemin chloride. Glu-P-1 (2) was condensed with 3-bromopropylamine (50%) and the product was condensed with hemin chloride pivaloyl ester (15%). Reduction of IIIa-c, 8 and 9 with $Na_2S_2O_4$ gave Fe(II)-porphyrin derivatives which were confirmed by red shifts of their UV spectra.

The cleavage of DNA was followed by monitoring the conversion of supercoiled closed circular PM 2 DNA to open circular DNA (analyzed by agarose gel electrophoresis and quantitated by ethidium bromide staining and densitometry, Table 1). Porphyrin(Fe)-intercalators (IIIa and IIIb) possess extremely strong DNA cleaving ability in the presence of $Na_2S_2O_4$ even at 20°C. The ability of DNA cleavage with the synthesized compounds decreases in the order of: IIIa = IIIb > IIIc = 9 = GP-SP (3) >> 8. Ferrous chloride alone or spermine in the presence of $FeCl_2$ showed no effective DNA cleavage at the same concentration even in the presence of $Na_2S_2O_4$. Without $Na_2S_2O_4$ or core Fe(III) (i.e., IIa), no effective DNA cleavage were observed. Presumably, $Na_2S_2O_4$ acts as a reducing agent and regenerates Fe(II) from Fe(III) to produce continuous source of the active metal ion. A compound which lacks a DNA recognition moiety (i.e., 8) possesses almost no DNA cleaving ability. However, the introduction of spermine

moiety produces a strong DNA cleaving ability (i.e., IIIc). Effective cleavage of DNA requires an intercalator moiety and a spermine moiety which act as binding sites and as recognition sites of DNA. The compound, IIIa and IIIb, which possess strong DNA cleaving ability, bind to DNA by the intercalator moiety (and maybe via spermine moiety by ionic interaction). At the bound site of the DNA helix, activation of molecular oxygen by porphyrin(Fe) moiety results in the cleavage of the DNA, though the nature of the activated oxygen species is not yet known. The structure-activity relationships are now under investigation.

In summary, porphyrin(Fe)-intercalators, IIIa and IIIb, cleave a plasmid DNA in a reaction that is dependent on Fe(II) (i.e., in the presence of $Na_2S_2O_4$) at very low concentrations: two orders of magnitude lower than the mole P concentration of nucleotides of DNA. Porphyrin(Fe) moiety and intercalator moiety connected with spermine (spermine also possesses very high affinity toward DNA) are essential for the strong DNA cleaving ability.

Quite recently, Lown and Joshua reported that haemin-acridines also cause oxygen-dependent DNA scission similarly.[9)]

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7) Details will be published in a full. ^{1}H-NMR($CDCl_3$): 8.89(d,J=4Hz,2H), 8.82(d,J=4Hz,2H), 8.81(s,4H), 8.07(d,J=8Hz,6H), 7.52(d,J=8Hz,6H), 7.95(d,J=8Hz,2H), 7.04(d,J=8Hz,2H), 2.70 (s,9H). Anal.Calcd. C,83.95; H,5.50; N,10.44. Found. C,83.63; H,5.48; N,10.55.

8) IIIa was isolated as IIIa-$6H_2O$. Anal.Calcd. C,64.78; H,6.29; N,14.87. Found. C,65.06; H,5.97; N,14.82.
   IIIb was isolated as IIIb-$6H_2O$-6HCl. Anal.Calcd. C,60.31; H,5.37; N,10.29. Found. C,60.02; H,5.33; N,10.34.
   IIIc was isolated as IIIc-$6H_2O$. Anal.Calcd. C,73.99; H,6.37; N,10.78. Found. C,74.19; H,6.11; N,10.06.

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