PORPHYRINS COUPLED WITH NUCLEOSIDE BASES. SYNTHESIS AND SOME PROPERTIES OF GUANINE, CYTOSINE AND ADENINE-THYMINE DERIVATIVES

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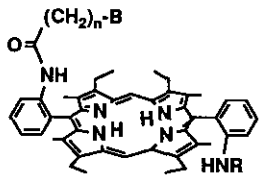
Abstract- Synthesis of several porphyrin derivatives having a guanine, cytosine or adenine-thymine pair is described. Diamagnetic shift behaviors of the base proton signals in the ^{1}H-nmr spectra of the derivatives and hypochromism of the Soret band in the electronic spectra are briefly discussed.

Recently, various kinds of compounds linked with nucleoside bases have been of interest in connection with base pairing, molecular aggregation, molecular recognition, anticancer agent, and so on.[1] Porphyrin derivatives covalently bonded to nucleoside bases may be important as a synthetic model system which is available for investigation on an affinity between porphyrins and DNA.[2] We previously reported preparation and characterization of adenine- and thymine-porphyrin derivatives (**4**, **5**).[3] This paper mentions synthesis of guanine- and cytosine-porphyrins (**1**-**3**) and two isomeric derivatives (**6**, **7**) having both adenine and thymine, and briefly describes their spectroscopic properties.

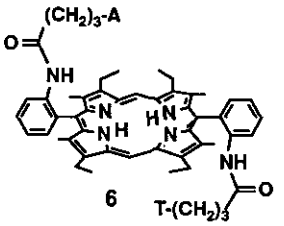
9-Guaninealkanoic acids (**14**) were prepared by reaction of 2-amino-6-chloropurine with ethyl bromoalkanoate by using K_2CO_3 in dimethylformamide (DMF) followed by hydrolysis with aq. HCl (**14a**: 71%; **14b**: 77%). The reaction of cytosine with ethyl 4-bromobutanoate in the presence of NaH in DMF gave 1-alkylcytosine (60%) and *O*-alkylcytosine (12%). 1-Cytosinepentanoate was obtained (44%) by the reaction of cytosine with ethyl 5-bromopentanoate. Those esters were converted to the corresponding carboxylic acid (**15a**, **15b** and **16a**) by hydrolysis. Condensation reactions between *o*-aminophenylporphyrins and base-alkanoic acids were carried out under the same conditions as those described in the previous paper[3] [the reaction conditions: carboethoxy (CET) chloride or carbobenzyloxy (CBZ) chloride/$(i\text{-}Pr)_2NEt$/pyridinium chloride in DMF/THF]. The reaction of

mono-CBZ or CET derivative (**8**-CBZ or **8**-CET) of *anti* bis(*o*-aminophenyl)porphyrin (**8**, R^1=R^2=H)[4] with guanine- or cytosinealkanoic acids (**14**-**16**) gave the corresponding nucleobase-porphyrin (**1**-**3**, 5-35%).



1: B=G
2: B=C
3: B=C'
4: B=A
5: B=T

a: n=3
b: n=4



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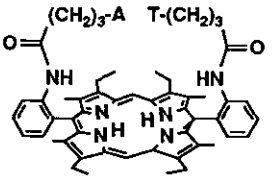

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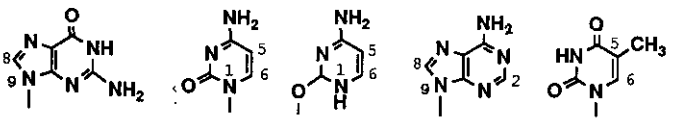
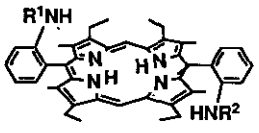
B:



R, R^1, R^2 = -H, $-CO_2CH_2C_6H_5$ (CBZ) or $-CO_2C_2H_5$ (CET)



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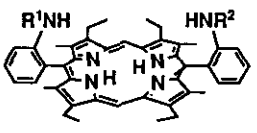

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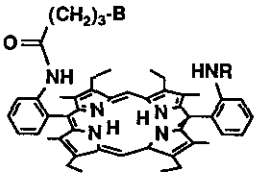

10: B=G
11: B=C
12: B=A
13: B=T

B-$(CH_2)_nCO_2H$
14: B=G, **15**: B=C, **16**: B=C', **17**: B=A, **18**: B=T

Preparation of adenine-thymine derivatives was achieved by stepwise condensation of porphyrins with two base-butanoic acids. *Anti* isomer (**6**) was formed by removal of the protecting group of **4a**-CBZ[3] with BBr_3 (90%) followed by reaction with 4-(thymin-1-yl)butanoic acid (**18a**) (21%). *Syn* isomeric porphyrin (**9**-CBZ) derived from **9**-H,H (R^1=R^2=H)[4] was condensed with 4-(adenin-9-yl)butanoic acid (**17a**) to give **12**-CBZ (21%), which was converted to **12**-H by removal of the CBZ group with BBr_3 (76%). Condensation of **12**-H with 1-thyminebutanoic acid (**18a**) yielded adenine-porphyrin-thymine derivative (**7**, 12%). Its structure was chemically proved by preparation *via* an alternate route, in which coupling of porphyrin with thymine moiety (**18a**) was carried out prior to that with adenine (**9**-CBZ→**13**-CBZ→**7**). The structures of the all nucleobase-porphyrin derivatives were confirmed by measurements of mass spectra by field desorption ionization (FDms) and ^{1}H-nmr spectra in dimethylsufoxide-d_6 (DMSO-d_6). Synthesis of *anti* and *syn* derivatives having both guanine and

cytosine was also attempted according to the same procedure as that of adenine-thymine derivatives. However, isolation of the expected compounds has been unsuccessful.

Table 1. ^{1}H-Nmr (in $CDCl_3$) and Electronic (in CH_2Cl_2) Spectral Data of Nucleoside Base-Porphyrins.

	^{1}H-nmr spectrum[a]			Electronic spectrum[d]		
	Purine	Pyrimidine		λmax	ε	Hypochromic
Compound	2-H[b] and 8-H	5-H or 5-CH_3[c]	6-H	(nm)	(X10^5)	effect (%)
8 (R^1=R^2=H)				408	2.12	0
1a (R=CET)	3.90 (3.71)			408	1.12	47
1a (R=CBZ)	3.94 (3.67)			408	1.18	44
1b (R=CBZ)	6.39 (1.22)			408	1.32	38
2a (R=CBZ)		4.03 (1.68)	5.06 (2.22)	408	1.46	31
2b (R=CBZ)		3.13 (2.58)	5.00 (2.28)	408	1.37	35
3a (R=CBZ)		5.28 (0.81)	7.32 (0.69)	408	1.63	23
6	6.46 (1.35)	1.27 (0.66)	6.12 (0.90)	411	1.65	22
	6.58 (1.78)					
7				416	1.27	40

a In δ (ppm) at 500 MHz. Values in parentheses are chemical shift differences (-Δδ) from the corresponding reference compound (ethyl ester of **14**, **15**, **16**, **17** or **18**). *b* Adenine proton of **6**. *c* Thymine protons of **6**. *d* The Soret band of porphyrin ring.

In the ^{1}H-nmr spectra (Table 1) of guanine and thymine derivaives (**1-3**) in $CDCl_3$, the signals of the base moiety appear at remarkably high fields compared with those of base-alkanoates (ethyl esters of **14-16**). The high field shifts due to the ring current effect of the porphyrin ring demonstrate their conformational features in which the base moieties are located at the upper zone of the porphyrin ring. The shift behaviors of the signals are summarized as follows: 1) The high field shifts (-Δδ) of the 8-proton of the guanine and the 6-proton of the cytosine are larger than those of the corresponding protons in adenine and thymine derivatives (-Δδ values of **4a** and **4b**: 1.64 and 0.40 ppm; -Δδ values of **5a** and **5b**: 1.19 and 1.27 ppm).[3] 2) An increase of the methylene side-chain in length results in decrease of the high field shift in guanine system, while the shift behavior of cytosine system is contrary to the guanines. 3) The shielding effect on the protons of *O*-alkylcytosine derivative (**3a**) is smaller than that of *N*-alkyl compound (**2a**).

The spectrum of *anti* isomer (**6**) in $CDCl_3$ showed a resoluble pattern and their all signals could be assigned to

the corresponding protons, but that of *syn* isomer (**7**) was so extremely broadened that it could not be analyzed. The spectral pattern of **7** in $CDCl_3$ was unchanged even by measurement under degassed conditions at 60°C. The reason for the unusual broadening has not yet been found. The proton signals of *anti* isomer (**6**) are comparable to those of **4a** and **5a**[3] in chemical shift. Therefore, the two base of **6** are independent of each other and in a situation similar to the porphyrin coupled with each base.

The electronic spectra of the base-porphyrin derivatives involving those described in the previous report[3] were measured in CH_2Cl_2. The Soret band of the porphyrin ring is shown in Table 1. The absorption bands are almost unchanged from the reference compound (**8**, R^1=R^2=H) in wavelength but fairly decrease in intensity. The magnitudes of the hypochromic effect are generally compatible with the diamagnetic shift values in the ^{1}H-nmr spectra mentioned above. Therefore, the effect is possibly brought about by approach of the base to the porphyrin ring. Pronouncedly large hypochromic effects of guanine and cytosine compared with adenine and thymine are of interest in connection with the Pasternack's results[2b] that mixing of a porphyrin derivative with poly(dG-dC) lead to a larger hypochromicity of the Soret band than that with poly(dA-dT). The intensity of the band in *syn* isomer (**7**) extremely decreases compared with *anti* isomer (**6**). This behavior may be due to approach of the base to the porphyrin ring by intramolecular base-pairing.

Further experiments are in progress.

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