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Porphyrins Coupled with Nucleoside Bases. Synthesis and Characterization of Ether-Linked Adenine-Thymine and Guanine-Cytosine Derivatives

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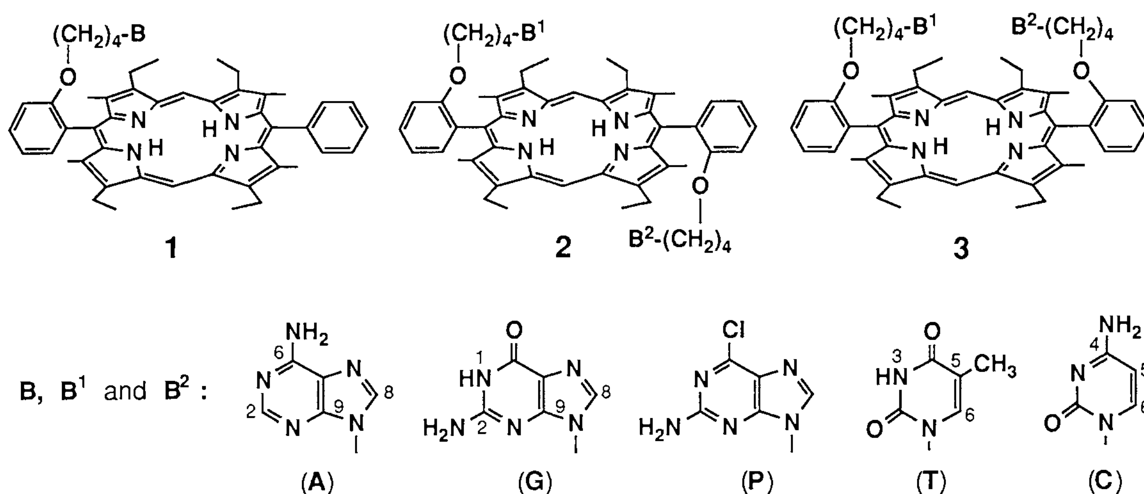
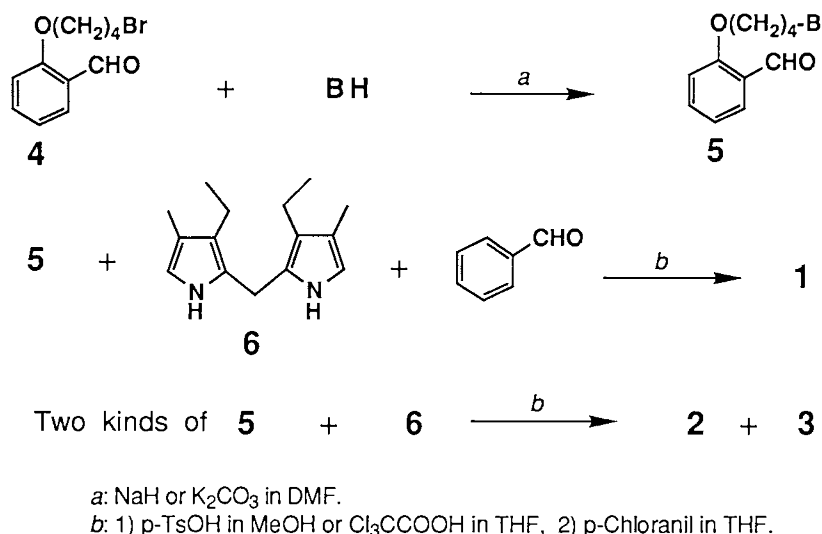
Porphyrins having an adenine-thymine or guanine-cytosine pair have been synthesized. Spectroscopic study has revealed that the two nucleobases form inter- and intramolecular base pairs in *anti* and *syn* atropisomers, respectively, and that the guanine-cytosine pair is more close to the porphyrin ring than the adenine-thymine pair.

Porphyrin-DNA interaction has been of interest in connection with photodynamic therapy, antiviral and anticancer activity,[1] and some evidences that porphyrins can intercalate between the base pairs of nucleic acids were shown by spectroscopic examinations of mixing systems of porphyrin derivatives with DNA or poly-nucleotides.[2] Porphyrin derivatives covalently bonded to nucleobases with a favorable mode for stacking each other would be available as a model system for investigation on a situation that a porphyrin intercalates into the space between base pairs in nucleic acids. On the basis of such a view we previously synthesized several nucleobase-porphyrin derivatives linked by amide bonds between the two moieties,[3] but there were some synthetic and spectrometric limitations in the amide system. Preparation of amide-linked porphyrins with both guanine and cytosine has not been achieved,[3b] and some compounds could not be characterized by ^{1}H NMR spectroscopy due to broadening of their spectra in $CDCl_3$.[3b] In this letter, we describe a ready method for synthesis of porphyrin derivatives (**1**-**3**) connecting a nucleobase or a base pair with alkyl ether chains and discuss their spectroscopic properties from the standpoint of porphyrin-nucleobase interaction.



Some porphyrins bonded to nucleobases or a nucleoside have been reported,[4)] but their structural features and the purpose of preparation are different from our compounds.



Scheme 1.

Nucleobase-salicylaldehydes (**5**) except for guanine were prepared by reaction of bromobutoxybenzaldehyde (**4**) with nucleobases. Guanine derivative (**5-G**) was obtained by hydrolysis of chloropurine derivative (**5-P**). Coupling reaction of **5** with dipyrrylmethane (**6**) in the presence of benzaldehyde followed by oxidation with *p*-chloranil gave the corresponding nucleobase-porphyrins (**1**)[5)] in 14-21%. Reaction in the coexistence of **5-A**, **5-T** and **6** afforded *anti* (**2-AT**) and *syn* (**3-AT**) isomers[5,6)] (11 and 9%, respectively). Synthesis of guanine-cytosine porphyrins was achieved by coupling of chloropurine- and cytosine-salicylaldehydes (**5-P** and **5-C**) followed by hydrolysis with hydrochloric acid (**2-GC**, 13%; **3-GC**,14%).[5,6)]. *Anti* and *syn* derivatives having the two same bases (B^1=B^2 in **2** and **3**)[7)] were also produced in the porphyrin formation reactions.

The ^{1}H NMR spectral data of the base moieties of **1**-**3** are described in the chemical shift differences of the proton signals from those of the corresponding base-salicylaldehydes (**5**) in Table 1. All signals of the base moieties except for the amino and NH protons appear at remarkably high fields compared with those of the original nucleobases (**5**). The high field shifts are apparently due to the ring current effect of the porphyrin ring and demonstrate their conformational features that the base moieties are located at the upper zone of the porphyrin ring. The amino and NH protons of *anti* dinucleobase derivatives (**2**) shift to fairly lower fields than those of mononucleobases (**1**), and base-pairing between the two bases accompanied with intermolecular association of **2** are indicated (Fig. 1, a). The high field shifts of **2-AT** are smaller than those of mononucleobases (**1**), but the shifts of **2-GC** are only slightly different from **1**. That is, the adenine and thymine deviate a little from the upper side of the porphyrin ring with intermolecular hydrogen bond formation, whereas the locations of the guanine and cytosine to the porphyrin are almost not influenced by hydrogen bonding.

The behavior of the proton signals in **3-GC** is very interesting. The two protons of the amino and methylene groups appear as nonequivalent signals each other, and the high field shifts of the base protons are remarkably large compared with those of **1** and **2**. Splitting of the amino protons and the extraordinarily high field shift of one of them suggest that the intramolecular hydrogen bond between guanine and cytosine (Fig. 1,b)

is fixed on the upper side of the porphyrin ring in the NMR time scale at room temperature. The magnetic nonequivalence of the methylene protons should arise from the fixing of hydrogen bonding. The two meso protons of the porphyrin were also split into two signals. This conformational feature of **3-GC** is supported by observation of correlation peaks between the 4-NH_2 and 1-NH, and between the inner NH of the porphyrin and the 5-H, 4-NH_2 and 1-NH in the NOESY. The splitting pattern in the ^{1}H NMR of **3-GC** coalesced at 60 °C.

The ^{1}H NMR spectra of **2** and **3** generally show that a guanine-cytosine pair is closer to the center of porphyrin ring than an adenine-thymine pair. This result implies that the affinity of the former to porphyrin than the latter. The unusual access of cytosine to the porphyrin is paticularly noticeable.

Table 1. Chemical Shift Differences ($\Delta\delta$ in ppm) of the Base Moiety Protons in the ^{1}H NMR Spectra[a] of **1-3** from Those of the Corresponding Base-salicylaldehydes (**5**) in $CDCl_3$

Compd.	Purine moiety					Pyrimidine moiety					
	1-NH	2-H	-NH_2	8-H	9-CH_2	1-CH_2	3-NH	4-NH_2	5-H	6-CH_3	6-H
1-A		-0.46	-0.52	-2.23	-1.79						
1-G[b]	-1.35		-0.72	-1.11	-1.73						
1-T						-1.95	-0.79			-1.94	-3.57
1-C						-1.73		c)	-6.33		-4.85
2-AT		-0.08	+1.51	-1.64	-1.14	-1.35	+4.75			-0.13	-0.92
3-AT		-0.45	-0.57	-2.09	-1.72	-2.12	-0.77			-1.97	-3.59
2-GC[b]	+1.20		+0.76	-1.45	-1.56	-1.96		c)	-5.86		-5.05
3-GC[b]	-0.17		-0.10	-2.66	-2.58	-1.62		-6.49	-9.04		-6.11
			+1.18		-2.33	-3.17		+0.23			

a) The spectra were measured on solutions in concentration of $0.9–1.1X10^{-3}$ mol m^{-3} at room temperature at 500 MHz. The signal assignments were confirmed by two dimensional spectrometry.
b) The shift differences of the guanine protons were estimated by comparison with ethyl 9-guaninebutanoate,[3b] since **5-G** could not be soluble in deuteriochloroform.
c) The signal was not observed in deuteriochloroform, possibly, due to broadening.

The Soret bands of the porphyrin in the electronic spectra of **1-3** are shown in Table 2. The absorption bands are almost unchanged from the reference compound in wavelength but obviously changed in intensity. The hypochromicities are summarized as follows: Purines>pyrimidines, **G>A**, **C>T**, **G-C>A-T** and *syn>anti*. Preponderance of purine bases to pyrimidines would indicate that the hypochromism depends on the size of the chromophore and on features of stacking to the porphyrin ring. By considering the behavior of the proton shifts in the ^{1}H NMR, we presume that the stacking feature of purines and pyrimidines in **1** are a face-to-face and edge-to-face modes, respectively. The behavior of the hypochromic effect in **2** and **3** is compatible with the diamagnetic shift values of the base protons, and indicates that the effects arise from the proximity of the base to the porphyrin ring. Pasternack et al.[2a] observed 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), and Marzilli et al.[2b] suggested that porphyrin intercalation occurred in GC-rich regions in DNA and outside binding occurred in AT-rich regions. The spectral data in this study are comparable with their observation and would support the suggestion that the

hypochromism is due to intercalation of porphyrin into DNA, although it is necessary to take into account our results in organic solvents. Further experiments are in progress.

Table 2. Soret Band of the Porphyrin Ring in Electronic Absorption Spectra of **1-3** in CH_2Cl_2

Compd.	λ_{max} (nm)	ε (x10^5)	Hypochromicity (%)
Reference[a)]	408	2.13	0
1-A	409	1.84	14
1-G	410	1.18	45
1-T	408	1.99	7
1-C	409	1.90	11
2-AT	410	1.67	22
3-AT	410	1.37	36
2-GC	411	1.31	38
3-GC	409	1.11	48

a) 5,15,-Bis(o-propoxy)phenyl-2,8,12,18-tetra-ethyl-3,7,13,17-tetramethylporphyrin.

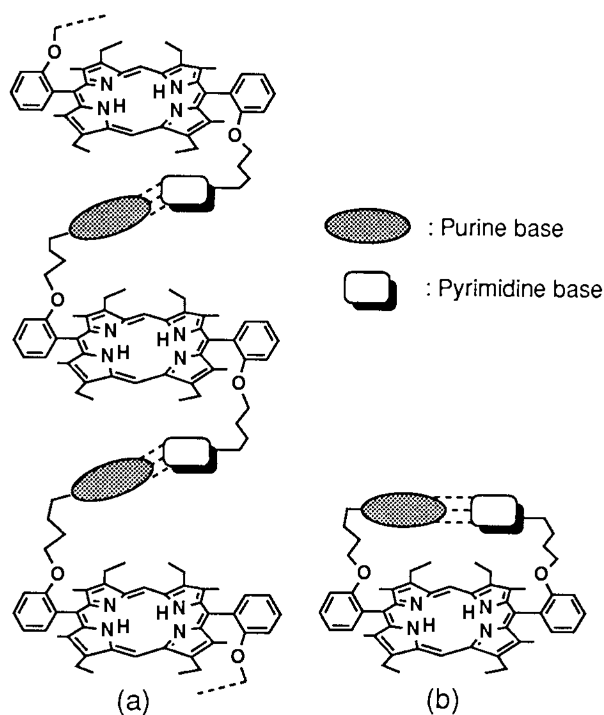


Fig. 1. Inter- and intramolecular base pairing in *anti* (a) and *syn* (b) isomers, respectively.

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5) The field desorption mass spectra of all base-porphyrins (**1-3**) showed the corresponding molecular ion peak.
6) The assignments of *anti* and *syn* atropisomers were supported by ^{1}H NMR spectroscopy.
7) These compounds (**2** and **3**, $B^1 = B^2$) were afforded in fairly good yields in the reaction of **6** with one of **5** without benzaldehyde. Isolation and characterization of those will be published elsewhere.

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