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RECOGNITION OF NUCLEOTIDES BY MELAMINE DERIVATIVES BEARING A GUANIDINIUM ION THROUGH HYDROGEN BONDINGS

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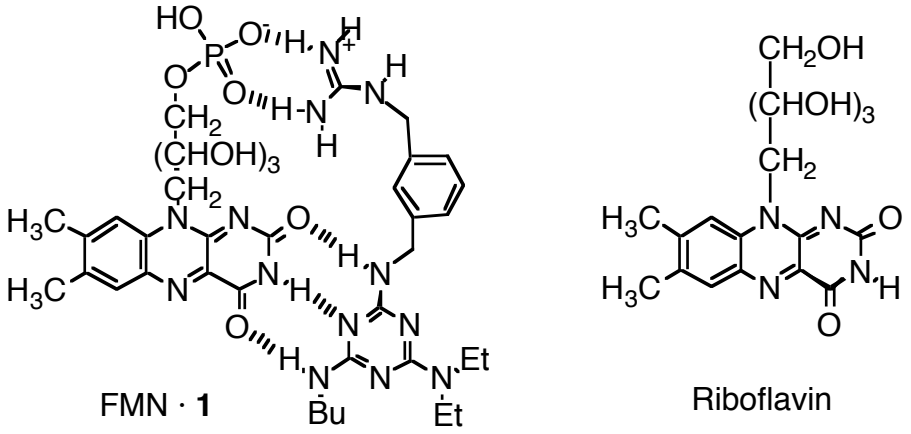
Abstract – Melamine derivatives bearing a guanidinium ion were found to extract uridine-5'-monophosphate (UMP) and guanosine-5'-monophosphate (GMP) in water into chloroform layer, whereas not the corresponding nucleosides under the same conditions.

Although some nucleotide analogues are known to exhibit anti-viral activity, they are inactive *in vivo* due to their inability to pass through lipophilic cell membranes.[1] Thus, it would be useful to investigate recognition of nucleotides by receptor molecules to form lipophilic complexes from a viewpoint of the therapeutic agents. Meanwhile, for the design of effective receptors using H-bonds, the number and the directionality of H-bonds must be taken into consideration.[2] Effective binding of a nucleotide monophosphate, for example, would be achieved by a ditopic receptor which possesses both binding sites for the nucleoside and the phosphate anion. Sessler *et al*. have reported that cytosine derivatives bearing a phosphate-binding site such as protonated tertiary amines and a sapphyrin moiety bind GMP *via* base-pairing and phosphate-binding through H-bonds.[1b,3] Kimura *et al*. have reported that Zn(II)-cyclen and bis(Zn(II)-cyclen) complexes act as efficient carriers for imide-containing nucleosides and nucleotides *via* both metal-coordinate and hydrogen bonds in aqueous solution.[4] Since a guanidinium ion is known to form H-bonds with carboxylate and phosphate anions,[5] ditopic receptors using H-bonds of nucleobase-pairing and guanidinium-phosphate interactions have been reported as a nucleotide carrier.[6]

During the course of our investigations on flavin receptors,[7] we have found that a melamine derivative bearing a guanidinium ion (**1**) extracts flavin mononucleotide (FMN) into chloroform layer by forming a

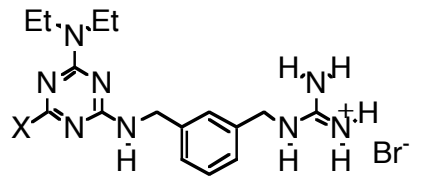
Figure 1 Complex structure of FMN•**1** and riboflavin



1 : 1 H-bonded complex as shown in Figure 1, whereas **1** is unable to extract riboflavin free from a phosphate moiety.[8] We noticed a structural analogy between FMN and a nucleotide in a sense that both possess a nucleobase skeleton and a phosphate moiety as the binding sites. This led us to expect that the similar H-bonded complexes are formed with nucleotides such as UMP and GMP in the presence of **1**.

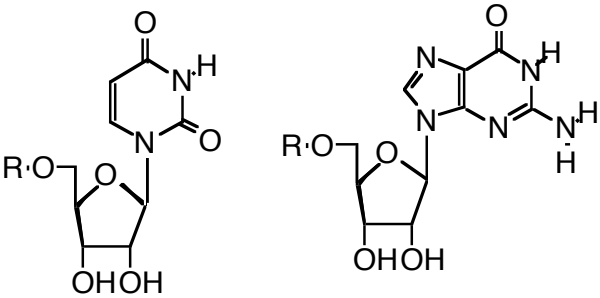
In this paper, we wish to report that melamine derivatives bearing a guanidinium ion (**2** and **3**) act as the extraction carriers for UMP and GMP in aqueous layer into chloroform layer.[9] The compounds employed are shown in Figure 2. Extraction of a water-soluble substance in water into an organic solvent takes place through the solubility difference toward both the solvents. Thus, it is important to make water-soluble nucleotides to be lipophilic by forming H-bonded complexes accompanying charge

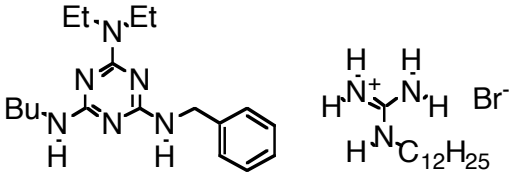
Figure 2. Receptors, nucleosides, and nucletides



1: X = BuNH **2**: X = $C_{12}H_{25}NH$

3: X = $C_{12}H_{25}O$





4 **5**

neutralization. A dodecyl group and a guanidinium moiety of **2** and **3** are expected to increase the lipophilicity of the H-bonded complexes.

The extraction experiment was performed as follows: A two-phase mixture of H_2O (3 mL)[10] containing a nucleotide (5.0×10^{-5}M) and $CHCl_3$ (3 mL) containing a receptor (1.0×10^{-4}M) was stirred for 1 h at 25 °C.[10] The concentration of the nucleotide in the H_2O phase was determined spectrophotometrically by monitoring the absorption decreases at 265 nm for UMP and 275 nm for GMP.[11] The results are shown

Table 1 Extractability of nucleotides, nucleosides, and FMN

Receptor	Extractability (%)[a]				
	UMP	U	GMP	G	FMN
1	—[b]	0	—[b]	0	41± 1
2	84 ± 2	0	78 ± 1	0	90 ± 1
3	44 ± 0	-	88 ± 0	0	66 ± 2
4	0	0	0	0	0
5	0	0	7.3 ± 0.3	0	8.8 ± 0.6
4 + **5**	0	0	7.8 ± 0.5	0	8.7 ± 0.3
CTABr[c]	0	0	3.0 ± 0.5	0	6.0 ± 0.5
none	0	0	0	0	0

[Nucleotide] = [Nucleoside] = [FMN] = 5.0×10^{-5}M, [Receptor] = 1.0×10^{-4}M.

a) Extractability(%) = ($[Nucleotide]_o$-$[Nucleotide]_{aq}$)/$[Nucleotide]_o$ x 100.

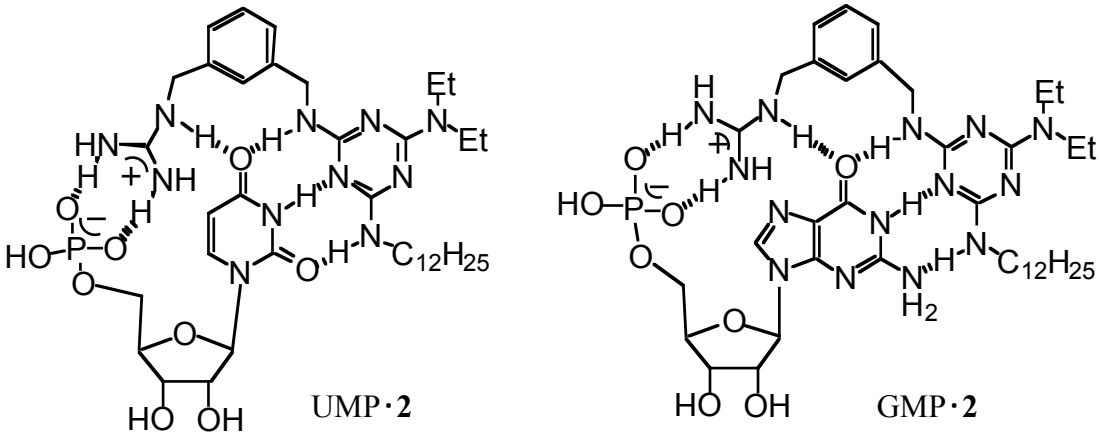
b) Not determined due to turbidity. c) Cetyltrimethylammonium bromide

in Table 1 together with those for FMN. The extractabilities of **1** for UMP and GMP were not determined due to turbidity of the H_2O layer. A larger extractability of **2** for FMN than that of **1** indicates that the lipophilicity of the H-bonded complex is effective for the present extraction. The larger extractability of **3** for GMP than those of UMP and FMN suggests that the oxygen atom in **3** forms an H-bond with 2-NH_2 of GMP, whereas no H-donors to the oxygen atom in cases of UMP and FMN.

Much lager extractability of **2** than that of a mixture of a monotopic melamine derivative and guanidinium ion (**4** + **5**) indicates the importance of the ditopic receptor to achieve the effective binding.

For **4** and **5**, no extraction ability of **4** implies that two H-bonds of a guanidinium ion with a phosphate anion accompanying charge neutralization is more important than three H-bonds between the melamine and the nucleobase moieties for the complex lipophilicity. The extractability of CTABr may support this. It should be noted that the nucleosides (U and G) and riboflavin are unable to be extracted al all under the same conditions. It was also confirmed that the stoichiometry of the complex formation is 1 : 1 for UMP•**2** and GMP•**2** with ESI-MS.[12] These results allow us to depict the complex structures of UMP•**2** and GMP•**2** as shown in Figure 3.

Figure 3. Plausible complex structures of UMP•**2** and GMP•**2**



The present study demonstrates that a melamine derivative bearing a guanidinium ion acts as the receptor for nucleotides such as UMP and GMP via multi-site hydrogen bonds. The binding for both the nucleotides should lead to potential applications such as separation of the nucleotides and the corresponding nucleosides. Furthermore, conversion of hydrophilic nucleotides to lipophilic complexes by a receptor molecule would provide a possibility as an anti-viral agent of nucleotides derivatives.

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9 Receptors (**2** and **3**) were prepared from 2,4-dichloro-6-diethyl-*s*-triazine according to the essentially similar procedures for **1** (ref. 7). Compounds (**4** and **5**) were supplied from our previous study (ref.7).

10 Distilled water containing the nucleotide (UMP or GMP, mono sodium salts) was used (pH = ~6.5).

11 For FMN, the absorption at 445 nm was used.

12 Electron spray ionization (ESI) mass spectra of the $CHCl_3$ layers showed 1 : 1 complex formation; m/z 836.7 ($[M + H]^+$), M; calcd for UMP•**2** ($C_{37}H_{62}N_{11}O_6P$). m/z 875.7 ($[M + H]^+$). M; calcd for GMP•**2** ($C_{38}H_{63}N_{14}O_8P$).