STERICAL PROPERTIES OF *N,N'*-DIMETHYLUREA MOIETY ENHANCE FORMATION OF TRIPLY HYDROGEN-BONDED COMPLEXES

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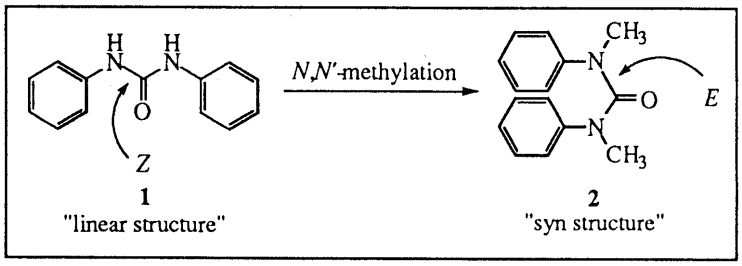
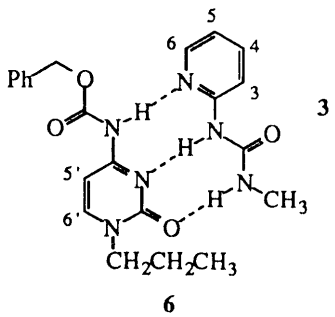
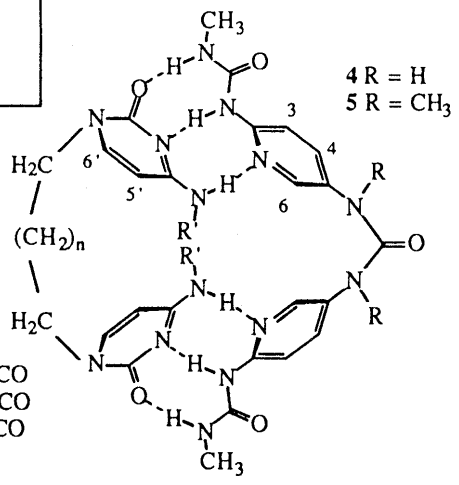
N,N'-Dimethyl-*N,N'*-[2-(3-methylureido)pyrid-5-yl]urea (**5**) was designed and synthesized as a guanylyl-guanosine equivalent molecule. (*E*)-Preference of the urea bond of **5** enhanced the formation of highly associated complexes of **5** with bis(cytosyl) derivatives.

KEY WORDS *N*-methylamide; *cis*-urea; hydrogen-bonding complex

Conformational alteration by *N*-methylation of aromatic amides and ureas is an intrinsic property of these molecules,[1-3] and often affects their biological properties.[4] The *N*-methyl group in an amide or urea favors *cis* orientation to the carbonyl group. Thus, *N*-methylated amides have folded structures, while the corresponding secondary amides are elongated. When two *N*-methylanilino moieties are attached to the carbonyl group (i.e. *N,N'*-dimethyl-*N,N'*-diphenylurea, **2**), the compound has a conformation in which the two phenyl groups lie facing to each other,[2,3] while *N,N'*-diphenylurea (**1**) always exists in a linear (*trans*) conformation.[5] Such (*E*)-preference (*syn* structure) of aromatic *N,N'*-dimethylurea allows it to be a unit for the construction of multi-layered aromatic molecules,[3] which can be functionalized, for example, by the introduction of hydrogen-bonding ability into the aromatic rings. In this paper, we describe the design of an aromatic molecule that interacts with dinucleotide analogs,[6] to form highly associated complexes, based on the (*E*)-preference of the urea bond.







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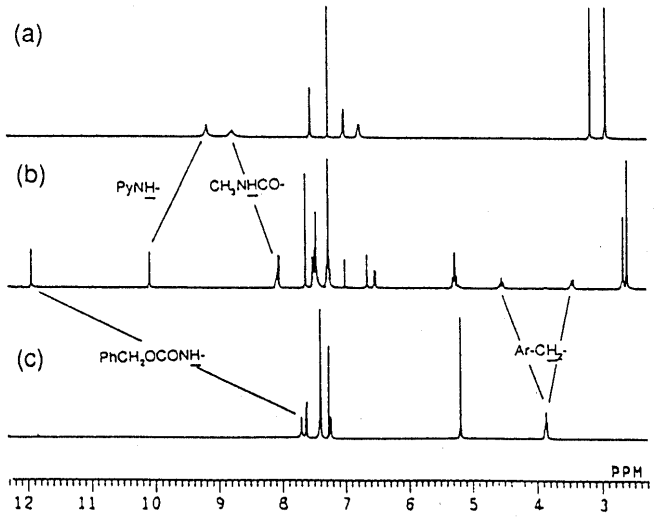
2-(3-Methylureido)pyridine (**3**) was designed as a guanine-equivalent molecule that is able to form triple hydrogen bonds with a cytosine derivative. Two 2-(3-methylureido)pyridines are linked by a urea bond: a secondary urea connects **4** and an *N,N'*-dimethylurea connects **5**. ^{1}H-NMR studies indicated that **4** exists in *Z,Z*-conformation, while the introduction of the *N,N'*-dimethyl group resulted in the *E,E*-conformation of **5** in solution. Thus, the signals of the aromatic protons of **5** were shifted to a higher field than those of **4** ($\Delta\delta$ 0.54 ppm for H_4 and 0.58 ppm for H_6 in DMSO-d_6 at 30 °C), with a similar magnitude of shift difference to that between **1** and **2**. As expected, **5** has a crystal structure in which the two pyridyl rings lie in a face-to-face position.[7] Formation of a triply hydrogen-bonded system between these pyridine derivatives and the cytosine derivatives was examined by ^{1}H-NMR.

The proton signals of the complex (**3·6**) formed from **3** and a monocytosyl compound **6** could be observed at -60 °C in $CDCl_3$. Triple hydrogen bonding in the complex was suggested by the observation that H_3 of **3** and $H_{5'}$ of **6** are shifted to lower field ($\Delta\delta$ 1.0 ppm and 1.4 ppm, respectively) due to the anisotropic effects of the carbonyl groups.[8] The association constant of **3·6** is 3.0×10^3 M^{-1} ($CDCl_3$) at -60 °C, as calculated from ^{1}H-NMR integration data. This is comparable to the reported values for several complexes between hydrogen bond donor-donor-acceptor (DDA) molecules and the complementary (AAD) molecules.[9]

When the *N,N'*-dimethyldimer **5** interacted with a bis(cytosyl) derivative **7a** having a linear alkyl chain in methanol or in chloroform at higher concentrations, a colorless powder (mp 222-223°C) was precipitated, and a 1:1 composition ratio was deduced from the elemental analysis. Though the resultant powder was poorly soluble in a variety of solvents, the complexation between **5** and **7a** could be observed in $CDCl_3$ at low concentrations (ca. 10^{-4} M) even at 0 °C by ^{1}H-NMR.. The chemical shift differences for the aromatic protons and amide protons between the complex and the free molecules correlate well with those of **3** and **6**. The conformational rigidity of the **5·7a** complex was suggested by the observation that the methylene protons attached to the cytosine ring become nonequivalent with a large chemical shift difference (δ 4.57 triplet-like, J = 11.5 Hz, and 3.45 doublet, J = 13 Hz). Since less than 1 % of the free compounds was detected in a 0.3 mM $CDCl_3$ solution of the 1:1 mixture, the association constant of the complex **5·7a** is above 5×10^6 M^{-1} (Fig. 1). Similar complex formation with an extremely high association constant was observed between **5** and **7b** or **7c**, regardless of the length of the linking alkyl chain. Unfortunately we could not determine the association constant between **4** and **7a** because of the extremely poor solubility of **4**.

Fig. 1 ^{1}H-NMR (Region of 2.3 – 12.3 ppm) of (a) **5**, (c) **7a**, and (b) 1:1 Mixture of **5** and **7a** (0.3 mM in $CDCl_3$, -60 °C)



UV spectral studies revealed hypochromicity at 270 nm of 10–14 % in a 0.04 mM 1:1 chloroform solution of **5** and **7a–7c** at 0 °C and at 20 °C (Table 1). No significant hypochromicity was observed (less than 3 %) when a monomer derivative (**3** or **7**) or a secondary urea **4** was mixed with the complementary bis-derivative. The secondary urea did not show any observable hypochromic interaction at 10^{-4} – 10^{-5} M (the association constant is around 10^3 M^{-1}). Significant hypochromicity (maximum at 270 nm) was observed only when **5** was mixed with **7a**, **b** or **c** in chloroform. The UV-mixing curve at 270 nm, where the maximum hypochromicity was observed at 1:1 ratio,

reflects the components of the complex. This hypocromicity also suggests that the face-to-face aromatic conformation of **5** has a significant role in the formation of the highly associated complex.

Structurally, there are two possible dimeric layered structures of **5** and **7**, that is, C_2 and C_v symmetrical (or related) structures, according to the directional relationships of the two planes of the hydrogen-bonded aromatics. These structures could not be distinguished from each other by ^{1}H-NMR, and determination of the structures of the complexes will require X-ray crystallographic or/and computational investigations.

Table 1. Hypochromicities of Complexes of Methylureidopyridines and Cytosine Derivatives

Entry	Compound[a]		Solvent	Temp	Hypochromicity
	Pyridine	Cytosine		°C	(270 nm), %
1	**3**	**7a**	$CHCl_3$	0	2.2
2	**4**	**7a**	5%DMSO-$CHCl_3$[b]	0	0.4
3	**5**	**7a**	$CHCl_3$	0	11.4
4	**5**	**7a**	$CHCl_3$	20	10.1
5	**5**	**7a**	5%DMSO-$CHCl_3$	0	7.1
6	**5**	**7b**	$CHCl_3$	0	13.1
7	**5**	**7c**	$CHCl_3$	0	13.6
8	**5**	**7c**	$CHCl_3$	20	13.3
9	**5**	**6**	$CHCl_3$	0	2.1

[a] Total concentrations are 0.04 mM (1:1 ratio), except entry 1 and 9 in which 2 equivalent molar of monomers (**3** or **6**) were used.
[b] **4** is insoluble to $CHCl_3$.

In conclusion, **5** has a high association ability with bis(cytosyl) derivatives **7a–7c**. Since the distances between the two aromatic rings in *N,N'*-dimethylureas are close to those of the nucleic acid base pairs in a double helix,[3,5] **5** might be a useful tool as a guanylyl-guanosine equivalent molecule. Since the (*E*)-preference of aromatic *N,N'*-dimethylureas has wide generality, various chemical modifications of the aromatic rings or even of the *N*-methyl groups should be able to endow the molecules with chemical functions and physicochemical properties which favor interactions with macromolecules. The *N,N'*-dimethylurea group may become a key linking group for aromatic architectures in the field of molecular recognition, supramolecular chemistry, and medicinal molecular design.

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7) Crystal data of **5**: $C_{17}H_{22}N_8O_3$, *Mr* = 386.41, monoclinic, $P2_1/c$, a = 7.167(2), b = 31.127(2), c = 10.6222(9) Å, β = 107.5920°, V = 2258.9(5) Å^3, Z = 4, Dx = 1.136 g/cm^{-3}, λ(CuKα) = 1.54178 Å, μ = 6.81 cm^{-1}, F(000) = 816, R = 0.091.
8) In the ^{1}H NMR of the complex **3**·**6**, the signal of one amide proton attached to the benzyloxycarbonyl group among the three amide protons shifts markedly to lower field ($\Delta\delta$ 5.3 ppm). The pyridyl amide proton signal also shifts to lower field by 0.75 ppm, while the signal of the terminal methylamide proton of **3** shifts to higher field. The changes of the chemical shifts of these two protons on complexation with **6** are owing to the destruction of the cyclic intramolecular hydrogen bonding between the methylureido group and the pyridyl nitrogen atom of free **3**. Such intramolecular hydrogen bonding of the methylureido group at the 2 position of the pyridine nucleus can be observed in the crystal structure of **5**.
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