

π-Interactions of Modified Nucleobases. On Mesomeric Purine Betaines with Inversed Charge Properties.

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Intermolecular interactions of modified nucleobases with altered charge properties in relation to natural systems are studied. We prepared conjugated mesomeric betaines of purines and examined their properties by semiempirical calculations, ^{1}H NMR titrations and ESI mass spectrometry. Thus, nucleophilic substitutions of 4-(dimethylamino)pyridine and pyridine, respectively, on 2-amino-6-chloropurine **1** and 2,6-dichloropurine **2** resulted in the formation of purin-6-yl hetarenium salts **3**–**6**. These were converted into the conjugated mesomeric betaines **7**–**10**, respectively, on treatment with the anion exchange resin Amberlite® IRA-400 in its hydroxy form. The betaines **7**–**10** possess umpoled purine rings in relation to 7-methylguanine that forms the betainic 5′-cap structure of *m*-RNA. We studied the π-stacking interactions of the conjugated mesomeric betaine **8** and of its cationic precursor **4** with L-tryptophan, adenine, adenosine, and guanosine in deuterated water.

During recent decades, several mesomeric betaines have been isolated from natural sources,[1] including biologically highly active alkaloids such as *norzooanemonine*,[2] *matadine*,[3] *serpentine*,[4] and *ungerimine*.[5] Among the modified nucleobases, the mesomeric betaine 7-methylguanine (m^{7}G) is known to be present in tRNA molecules and has been identified as 5′-terminal *cap*-structure of eucaryotic viral and cellular mRNA.[6] Numerous efforts have been devoted to the elucidation of its biological function. Stabilizing effects as well as betaine–protein molecular recognition to enable the binding of the mRNA molecules to proteins on the surface of the ribosomes prior to the initiation of translation have been discussed.[7] In general, to explain the chemical specifity that guarantees molecular recognition, the influence of π–π stacking by frontier orbital interactions,[8] π–σ attractions that overcome π–π attractions,[9] as well as electrostatic interactions involving the out-of-plane π-electron density[10] have been presented. Base-mispairing induced by 7-methylguanine causes its mutagenicity,[11] and it was surprisingly identified as one of the main metabolites on treatment of DNA with carcinogens such as hydrazine.[12] Furthermore, model systems of m^{7}G are self-complementary at physiological pH. The biological role of this phenomenon still remains uncertain.[13]

We currently are interested in studying mesomeric betaines with different types of conjugation and isoconjugate relationships within a given nucleobase skeleton in order to gain insights into the role of charge distribution on molecular recognition. Therefore, we prepared guanine derivatives with inversed charged properties for comparison to the naturally occurring m^{7}G that possesses positive substituents at C-4 instead of the 4-olate moiety, and a negative charge delocalized in the purin ring system. In view of their interesting biological properties, pyridinium rings seemed to be suitable cationic partial structures of the target nucleobase betaines. In continuation of our interest in the chemistry of nucleobases[14] and betaine derivatives[15] we report here the syntheses and the intermolecular interactions of those systems.

Results and Discussion

Syntheses. We chose 2-amino-6-chloropurine **1**, the isomer of the cytotoxic natural alkaloid *trachycladine A* from *Trachycladus laevispirulifer*,[16] and 2,6-dichloropurine **2** as suitable starting materials for betaine formation. The betaines were smoothly formed by a two-step procedure. Thus, nucleophilic displacement on the purines, respectively, by pyridine or 4-(dimethylamino)pyridine in chlorobenzene gave the 1-(purin-6-yl)pyridinium salts **3**–**6** in high yields and as single tautomers, respectively. Aqueous solutions of **3**–**6** were passed through the anion exchange resin Amberlite® IRA-400 in its hydroxy form. Concentration of the elutes at ambient temperature in vacuo yielded the (6-pyridinio)purinates **7**–**10**, respectively, as intensely orange colored compounds which could be protonated to the starting materials by hydrochloric acid.[17] For the case of **3**, attempts to deprotonate with hydroxide gave guanine **11** and glutaconaldehyde **12**, as a result of pericyclic ring cleavage at C-2 of the pyridinium ring and subsequent substitution of the resulting penta-1,3-dienal derivative (Scheme 1). In contrast to this, the 4-(dimethylamino)pyridinium derivative **4** proved to be stable towards ring opening and gave the betaine **8** in nearly quantitative yield on treatment

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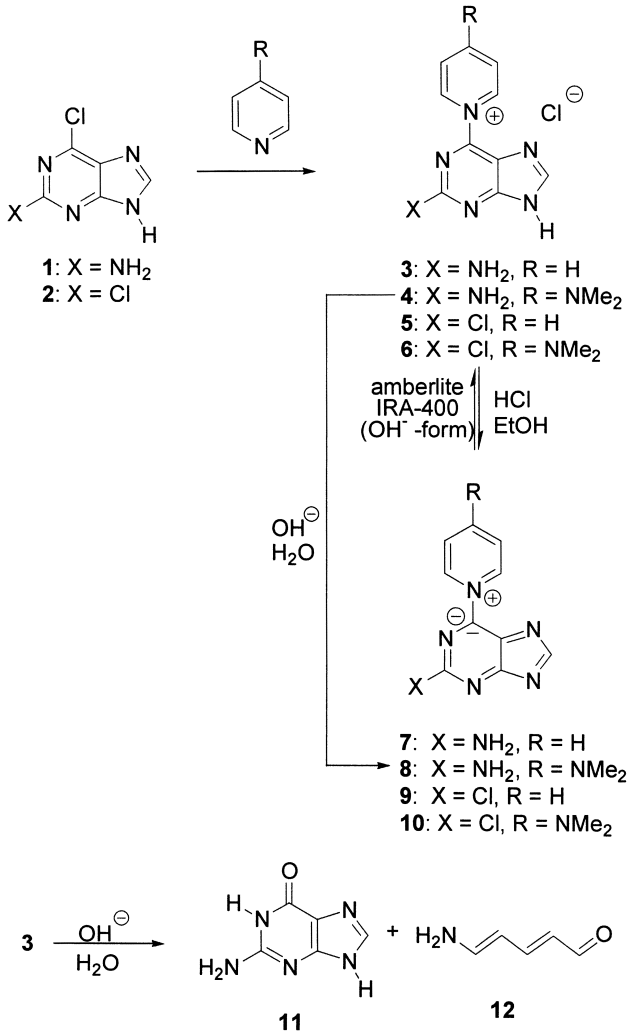


Scheme 1.

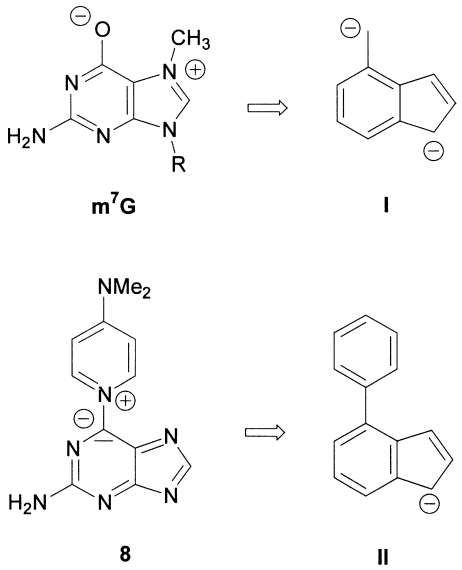


Fig. 1.

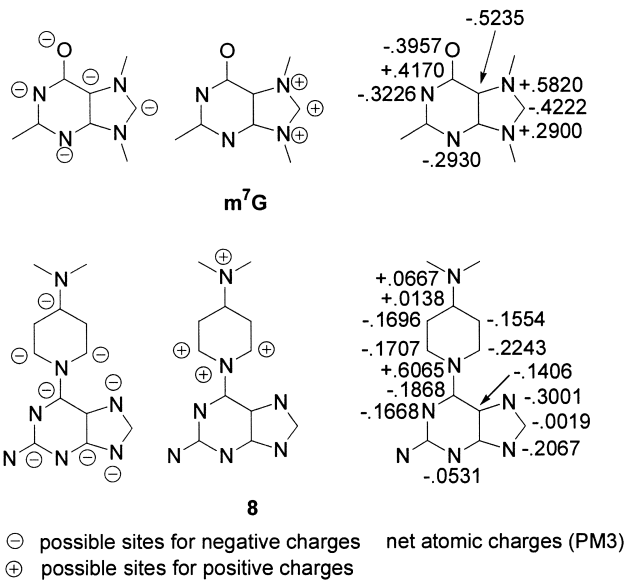


Fig. 2.

with OH^-. Under the applied reaction conditions, no disubstitution of 2,6-dichloropurine **2** with the heteroaromatics to dicationic species was observed.

The mRNA nucleobase m^7G as well as **7**–**10** belong to the class of conjugated heterocyclic betaines (CMB). Characteristically, the charges are in mutual conjugation, so that common sites for either positive and negative charge exist in the canonical formulae. However, the molecules are members of different subclasses of conjugated mesomeric betaines, sixteen of which can be distinguished by their isoconjugate relationships.[18] Whereas m^7G is isoconjugate with the even non-alternant hydrocarbon dianion I and thus belongs to class 4 of mesomeric betaines, **3**–**6** are heterocyclic *N*-ylides isoconjugate to the odd non-alternant hydrocarbon anion II (class 6) (Fig. 1). As outlined in Fig. 2, the canonical formulae as well as the PM3 calculation predict inversed atomic charges of the purine moieties in m^7G and **8** and the umpolung of C(6), N(7), and N(9).

The frontier orbital profile of the m^7G/L-trp system seems to be responsible for the interaction of the 5′-cap structure to the binding protein of the ribosom prior to the initiation of translation. L-tryptophan, which is preferentially involved in π–π-stacking interactions of proteins with nucleic acids such as between phenylalanyl-tRNA and synthetase-tRNA[19] or glutamate dehydrogenase and ADP,[20] is known to have the best π-donating character among the aromatic amino acids.[21] Therefore, the examination of electronically inversed guanine betaines could provide valuable insights into the role of charge distribution on mutual recognition of molecules. Semiempirical calculations applying PM3 parameters predict profound differences in the frontier orbitals.[22] The HOMO [IP(PM3) = −8.18 eV] of the planar m^7G is essentially located at N(1), N(3), C(5), and O(13), i.e. in the pyrimidine moiety of the purine skeleton, whereas the LUMO [IP(PM3) = −0.92 eV] has its largest coefficients at N(7), C(8), and N(9) in the imidazole ring. In contrast to this, the HOMO of the betaine **8** [IP(PM3) = −7.77 eV] has its largest coefficients at the exocyclic amino nitrogen atom, at C(6) and at N(7), whereas the LUMO [IP(PM3) = −1.97 eV] is essentially located at the nitrogen

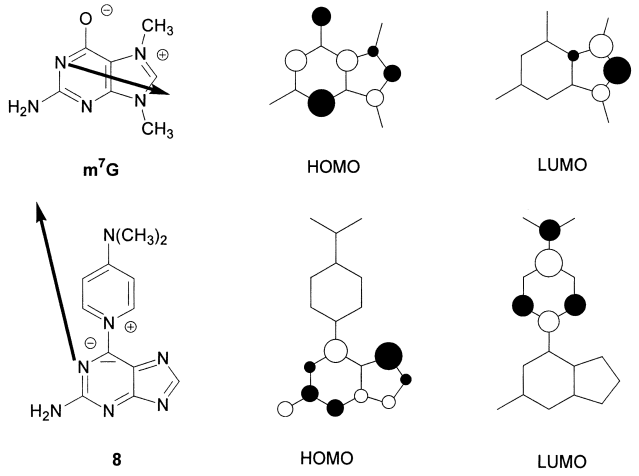


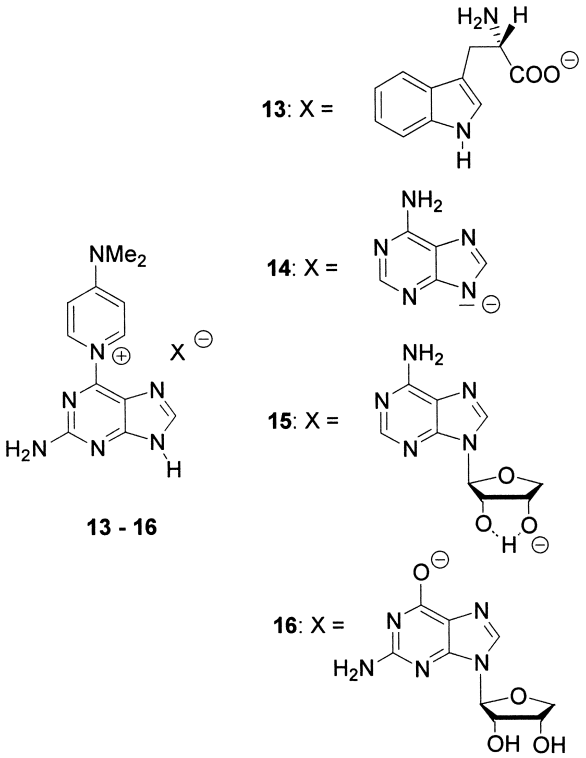
Fig. 3.

atoms and the α- and γ-carbon atoms of the pyridinium ring (Fig. 3). According to the PM3 calculation and in agreement with the classification of **8** as a conjugated mesomeric betaine (CMB), the most stable conformation is the planar one [ΔH_f = 488.89 kJ]. The purine and pyridinium rings are joined through a σ-bond at an active position of the HOMO. The semiempirically calculated permanent dipole moments in the ground states of m^7G and **8**[25] are presented in Fig. 3 in correct size and direction. Dipole–dipole interactions are known to contribute to specifying the mutual orientation of molecules in stacks.[26]

π-Stacking Interactions in D_2O. At first, we examined the interactions of cationic purine derivative **4** and of the betainic purine derivative **8** as model substances with L-tryptophan in deuterated water by means of ^1H NMR spectroscopy. In view of the expected inversed π-electron donor–acceptor capabilities of the purine in relation to the natural system, and the biological importance of charge-transfer interactions of π-donor molecules to pyridinium coenzymes,[27] we performed additional measurements with adenine, adenosine, and guanosine. Due to the limited solubility of guanine in water at pH 7 we were prevented from measurements using this nucleobase. The chemical shift changes are presented in Table 1.

On addition of L-tryptophan, adenine, adenosine, and guanosine to a solution of the mesomeric betaine **8** in D_2O, considerable downfield shifts of the pyridiniopurinate protons can be observed, while the substrate's protons shift upfield. In D_2O, quantitative salt formation of **8** to **13**–**16** unambigously occurs (Scheme 2), each salt consisting of the cationic part of **4** and the monoanions of L-tryptophan, adenine, adenosine, and guanosine, respectively. Adenine is known to be deprotonated at N(9), whereas deprotonation of guanosine occurs at N(1) and of adenosine at the secondary hydroxyl group of the ribose moiety.[28] In agreement with the salt formation, on addition of the substrates, decolorization of the betaine **8** from intense orange-yellow to pale yellow (**4**) is observable.

Thus, the effects of possible π-stacking interactions of the betaine **8** and the substrates on the ^1H NMR spectra were superseded and could not be estimated for that reason. Therefore, we undertook measurements in 0.1 M NaOD in D_2O to avoid salt formation; however, the variety of substrates was to



Scheme 2.

be reduced due to the instability of adenosine and guanosine toward bases. But, upfield shifts of the ^1H NMR signals attributable to π-stacking interactions between the betaine **4** and the anions of L-tryptophan and adenine are observable. The chemical shift changes can be explained by ring-current effects that exert a shielding of the protons in vertical position in relation to the aromatic system.[28]

In agreement with the expectation of better π-acceptor capabilities due to a decreased LUMO energy and in accord with observations with adenine,[26] stronger interactions were observed with the cationic purine nucleobase **4**. Thus, on treatment of a 10 mM solution of the cation **4** with a 10 mM solution of L-trp and adenine in D_2O all the resonance frequencies shift characteristically upfield, consistent with the formation of π-stacked associates (Table 1). It is apparent that the chemical shift changes of tryptophan on addition of guanine are 2.0 to 2.4-fold larger than those documented for the natural m^7G–L-trp π-interaction, which is presumably responsible for the binding of the mRNA to the ribosome.[8] On the other hand, the purine **4**/L-trp system is an intermolecular stacking model for π-electron donor/pyridinium coenzyme interactions.[27]

We performed NMR titrations in order to elucidate the possible geometries of the π-stacked associates (Fig. 4). In agreement with the formation of π-complexes, the indole protons of the L-trp are shifted to a larger extent than the amino acid residue, indicative of a stacking of the whole aromatic system not involving the side chain. Indeeed, the largest differences in proton chemical shifts of the cation **4** are observed in the α-positions of the pyridinium moiety. At these positions, the PM3-calculated LUMO has its largest coefficients.

π-Interactions were also observed on addition of adenine to **4** (Table 1.). Quaternization of adenine to adeninium is essen-

Table 1. Chemical Shifts Changes of the Cationic Guanine **4** and the Mesomeric Betaine **8** on Addition of L-Tryptophan (trp), Adenine (ade), Adenosine (ado), and Guanosine (guo) in D_2O, and of **8** in 0.1 M NaOD in D_2O, at 300 MHz and 25 °C, Respectively. Chemical Shift Changes $\Delta\delta$ are Given in ppm. Concentration of **4** and **8** in D_2O: 10 mM

		$\Delta\delta$ of the guanines			$\Delta\delta$ of the substrates					
Guanine	Substr.	α-H	β-H	8-H	2-H	4-H	5-H	6-H	7-H	8-H
	trp	−0.11	−0.04	−0.01	−0.14	−0.17	−0.15	−0.14	−0.17	—
4	ade	−0.13	−0.11	−0.10	−0.15	—	—	—	—	−0.15
in D_2O	ado	−0.02	−0.01	0	−0.13	—	—	—	—	−0.07
	guo	+0.07[a]	+0.05[a]	+0.04[a]	—	—	—	—	—	−0.09
	trp	+0.27	+0.45	+0.18	−0.05	−0.04	−0.05	−0.05	−0.06	—
8	ade	+0.44	+0.71	n.d.[b]	+0.06	—	—	—	—	+0.08
in D_2O	ado	+0.23	+0.41	+0.11	−0.15	—	—	—	—	−0.08
	guo	+0.43	+0.71	+0.16	—	—	—	—	—	−0.12
8[c]	trp	−0.06	−0.08	−0.02	−0.01	−0.01	−0.01	−0.01	−0.01	—
in NaOD	ade	−0.35	−0.20	−0.07	−0.01	—	—	—	—	−0.01

n.d.: not detectable. a) broadened signals due to precipitation during the NMR measurement. b) overlapped. c) measured in 0.1 M NaOD in D_2O; ado and guo are not stable toward NaOD, **4** gives **8**.

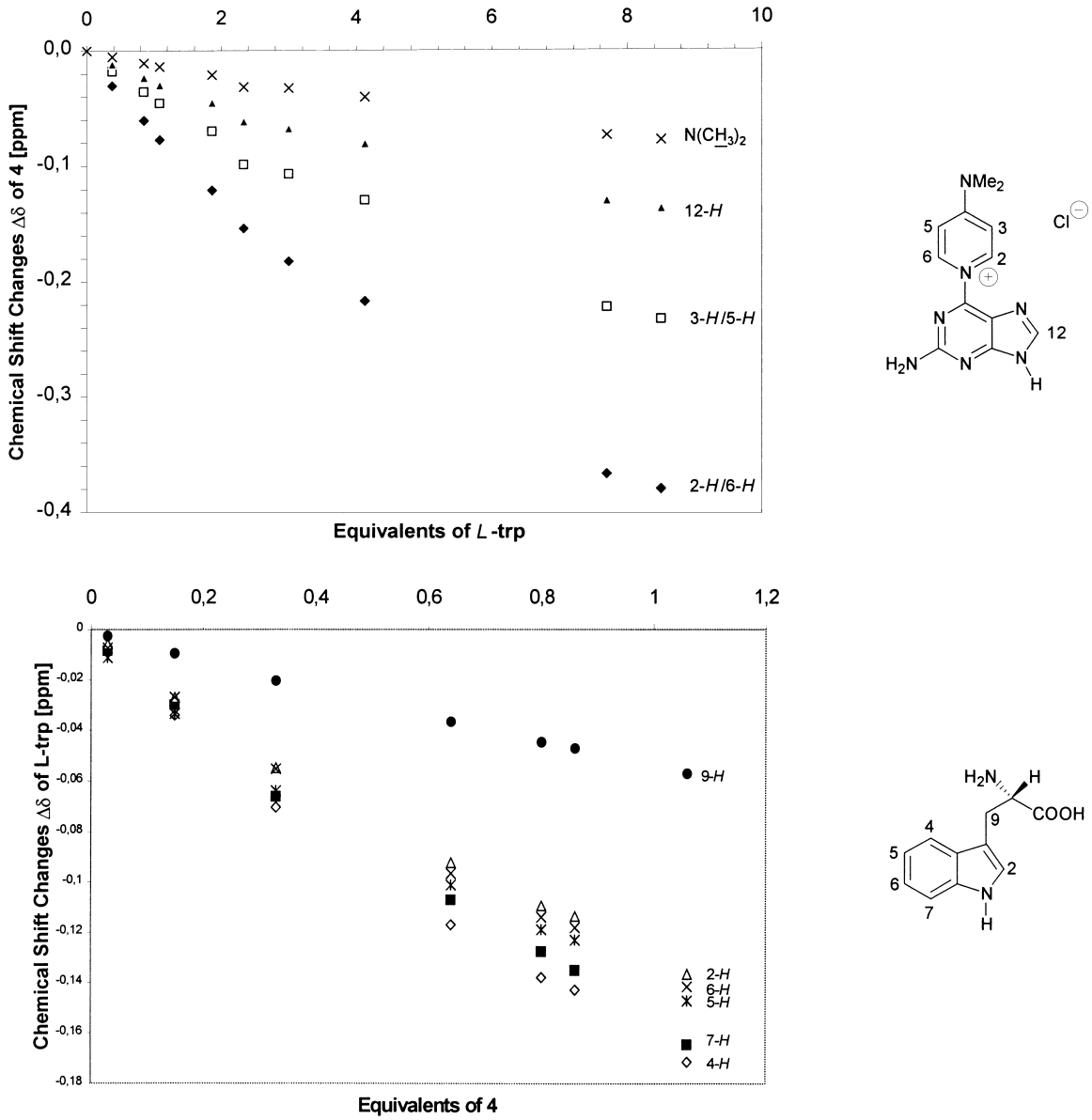


Fig. 4. ^{1}H NMR titrations of cation **4** with L-tryptophan.

tial for the formation of π-stacks with indole, due to a considerable lowering of the LUMO energy.[26,28] As all resonance frequencies shift upfield on addition of **4** to adenine and vice versa, no acid-base reaction such as **4** + **ade** → **8** + **adeH**$^+$ takes place. Thus, in contrast to observed stacking interactions to indole derivatives,[27] the adenine apparently is the π-donor molecule of the interaction. On titrating the cation **4** to a 10 mM solution of adenine in D_2O at rt, the resonance frequencies of 2-H are shifted to a larger extent than those at the 8-H position.

Chemical shift changes on treatment the solutions of **4** with adenosine and guanosine, respectively, are small.

The specific architecture prevents **4** and **8** from forming *Watson–Crick* or wobble base pairs. In principle, only *Hoogsteen*-type base pairing is possible, due to steric reasons. However, in DMSO-d_6-solution, no hydrogen bonds of the mesomeric betaine **8** to adenine, adenosine, guanine, or guanosine could be observed. The resonance frequencies of 1 : 1 mixtures correspond to the chemical shifts of the pure compounds under analogous reaction conditions. Obviously, substitution of the olate group by an hetarenium substituent and concomitant umpolung of the purine skeleton causes a complete loss of hydrogen-bonding capabilities. In accordance with the NMR measurements and in contrast to our earlier results obtained with uracil betaines,[14] no hydrogen bonded *homo*-intermolecular associates were detectable by electrospray ionization mass spectrometry (ESIMS) in anhydrous MeCN. In contrast to the 5′-cap structure of mRNA, derivative **8** is obviously not self-complementary. The reason for that is the direct consequence of the altered charge properties in comparison to the natural analogue m^7G, as no coupling of the permanent dipole moments by forming a centrosymmetric hydrogen-bonded dimer is possible.

Experimental

General Methods. The NMR spectra were acquired on a Bruker ARX 300. Me_4Si was used as internal standard. IR spectra were obtained on a Nicolet 205 and were determined on pellets (2.5% in KBr). FAB mass spectra were recorded on an AMD M-40 (AMD Intectra GmbH Harbstedt) in positive ion detection mode in mNBA. ESI mass spectra were taken on a Hewlett-Packard Series 1100 LC/MSD. All samples were dried for at least 24 h at 80 °C. However, in accordance with known hetarenium compounds and X-ray crystallographic results,[14,15] some of the compounds crystallize with varying amounts of water. Therefore, the UV spectra were measured qualitatively with a Perkin Elmer UV/VIS/NIR spectrometer Lambda 19, because the water of hydration causes some changes in solvent polarity. All melting points were determined on a Boëtius melting apparatus; the values reported are uncorrected.

1-(2-Amino-purin-6-yl)pyridinium Chloride 3. 0.85 g (5.0 mmol) 2-amino-6-chloropurine **1** were suspended in 50 mL of pyridine and were heated at reflux temperature for 5 h. After cooling, the resulting yellow-brownish precipitate was filtered off, washed with ethyl acetate and recrystallized (0.86 g, 81%), mp > 300 °C (from EtOH–H_2O) Found: C, 48.35; H, 4.08; N, 33.61%. Calcd for $C_{10}H_9N_6{\cdot}2H_2O$: C, 48.19; H, 5.26; N, 33.72%; UV: compd is not soluble in dichloromethane; UV λ_{max}(MeCN)/nm 383.7 and 266.4; λ_{max}(MeOH)/nm 393.0, 267.6, and 228.4; IR 3421, 3311, 3200, 3059, 2897, 2758, 1638, 1572, 1556, 1519, 1470, 1368, 1299, 1204, 1152, 933, 903, 777, 675, 626, and 552 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6; Me_4Si) δ 7.21 (2H, s), 8.44 (2H, m), 8.48 (1H, s), 8.91 (1H, tt, J = 6.3 and 5.7 Hz), 10.10 (2H, d, J = 5.7 Hz), and 13.54 (1H, s); ^{13}C NMR (75 MHz, DMSO-d_6; Me_4Si) δ 116.83, 127.87, 142.44, 144.44, 145.70, 149.42, 158.80, and 159.48; MS *m*/*z* (FAB, mNBA) 213.9 (M^+, 41) and 77.3 (100).

1-(2-Amino-purin-6-yl)-4-dimethylaminopyridinium Chloride 4. 0.85 g (5.0 mmol) of 2-amino-6-chloropurine **1** were suspended in 50 mL of chlorobenzene. Then, 0.61 g (5.0 mmol) of 4-(dimethylamino)pyridine was added and the reaction mixture was heated at reflux temperature over a period of 24 h (tlc). After cooling, the yellow precipitate was filtered off, washed with ethyl acetate and recrystallized (0.99 g, 69%), mp > 300 °C (from ethanol–water) Found: C, 49.40; H, 5.06; N, 33.36%. Calcd for $C_{12}H_{14}ClN_7$: C, 49.40; H, 4.83; N, 33.61%. UV: compd is not soluble in CH_2Cl_2; UV λ_{max}(MeCN)/nm 350.8, 313.6, and 220.1 nm; λ_{max}(MeOH)/nm 352.9, 307.9, and 220.6; IR 3422, 3309, 3195, 3073, 2995, 1661, 1637, 1591, 1567, 1518, 1473, 1401, 1375, 1305, 1211, 1158, 816, and 748 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6; Me_4Si) δ 3.35 (6H, s), 6.93 (2H, s), 7.31 (2H, d, J = 8.4 Hz), 8.29 (1H, s), 9.47 (2H, d, J = 8.4 Hz); ^{13}C NMR (75 MHz, DMSO-d_6; Me_4Si) δ 40.31, 107.61, 115.41, 137.91, 142.42, 145.32, 156.97, 157.86, and 159.36; MS *m*/*z* (FAB, mNBA) 257.2 (M^++1, 100).

1-(2-Chloro-purin-6-yl)pyridinium Chloride 5. A suspension of 0.94 g (5.0 mmol) 2,6-dichloropurine **2** in 50 mL of anhydrous pyridine was heated at reflux temperature for 3 h. After cooling, the precipitated solid was filtered off and recrystallized (0.76 g, 57%), mp > 300 °C (from EtOH) Found: C, 44.34; H, 3.31; N, 25.14%. Calcd for $C_{10}H_7Cl_2N_5$: C, 44.79; H, 2.63; N, 26.12%; UV λ_{max}(MeOH)/nm 451.4. 316.14. 270.0, and 221.5; IR 3049, 2868, 2747, 1633, 1617, 1574, 1561, 1499, 1361, 1318, 1187, 992, 923, 881, and 671 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6; Me_4Si) δ 8.48 (2H, dd, J = 7.5 Hz), 8.99 (1H, tt, J = 7.8 and 1.2 Hz), 9.09 (1H, s), 10.14 (2H, d, J = 5.7 Hz).

1-(2-Chloro-purin-6-yl)4-dimethylaminopyridinium Chloride 6. A suspension of 0.94 g (5.0 mmol) of 2,6-dichloropurine **2** in 50 mL of ethyl acetate was treated with 0.61 g (5.0 mmol) of 4-(dimethylamino)pyridine and heated at reflux temperature for 7 h. After cooling, the resulting precipitate was filtered off, washed with ethyl acetate and recrystallized (0.92 g, 62%), mp > 300 °C (from EtOH) Found: C, 44.91; H, 4.42; N, 25.69%. Calcd for $C_{12}H_{12}N_5Cl_2{\cdot}H_2O$: C, 45.73; H, 4.47; N, 22.22%; UV λ_{max}(MeCN)/nm 351.1, 338.0, and 279.1; IR 3056, 1656, 1590, 1562, 1377, 1312, 1228, 1179, 1141, 1125, and 985 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6; Me_4Si) δ 7.34 (2H, d, J = 8.2 Hz), 8.65 (1H, s), 9.56 (2H, d, J = 8.2 Hz).

General Procedure for the Synthesis of the Mesomeric Betaines 7–10. 50 mL of the anion exchange resin Amberlite® IRA-400 were washed with 2 L of water and then filled into a column without frit (height: 16 cm; diameter: 3 cm). Then, the resin was treated with 10% aqueous sodium hydroxide over a period of 45 min. Then, the base was rinsed out by water to pH 7 of the elute and a sample of the purinyl salts in 20 mL of water were given on the resin. Each aqueous elute were concentrated in vacuo to yield its analytically pure betaine.

2-Amino-6-(1-pyridinio)purinate 7. 0.15 g (0.60 mmol) of **3** were used to give an intensely orange solid (0.12 g, 98%), mp > 300 °C (from EtOH) Found: C, 45.28; H, 4.87; N, 31.07%. Calcd for $C_{10}H_8N_6{\cdot}3H_2O$: C, 45.11; H, 5.30; N, 31.56%; UV λ_{max}(CH_2Cl_2)/nm 469.0; λ_{max}(MeCN)/nm 338.7 and 221.1; λ_{max}(MeOH)/nm 397.5 and 224.9; IR 3320, 1623, 1538, 1493,

1434, 1299, 877, 656, and 630 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6; Me_4Si) δ 6.02 (2H, s), 8.03 (1H, s), 8.34 (2H, t, J = 7.2 Hz), 8.73 (1H, dd, J = 7.2 and 6.9 Hz), 10.47 (2H, dd, J = 6.9 and 1.2 Hz).

2-Amino-6-(4-dimethylaminopyridinio)purinate 8. 0.15 g (0.51 mmol) of cation **3** were given on the resin to give a lemon-yellow solid (0.12 g, 98%) Found: C, 50.92; H, 5.82; N, 34.16%. Calcd for $C_{12}H_{13}N_7 \cdot 1.5H_2O$: C, 51.06; H, 5.71; N, 34.73%; UV λ_{max}(CH_2Cl_2)/nm 385.8 and 300.8; λ_{max}(MeCN)/nm 352.9, 305.1, and 219.9; λ_{max}(MeOH)/nm 353.4, 307.4, and 220.7; IR 3313, 3178, 1658, 1583, 1536, 1490, 1398, 1304, 1217, and 1157 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6; Me_4Si) δ 3.32 (6H, s), 5.90 (2H, s), 7.29 (2H, d, J = 8.2 Hz), 7.89 (1H, s), 9.77 (2H, d, J = 8.2 Hz); MS *m/z* (FAB, mNBA) 257.3 (M^++2, 91) and 40.1 (100).

2-Chloro-6-(1-pyridinio)purinate 9. 0.15 g (0.55 mmol) of cation **5** were used to give a yellow solid (0.12 g, 95%), mp > 300 °C (from EtOH) Found C, 39.94; H, 4.65; N, 22.89%. Calcd for $C_{10}H_6ClN_5$: C, 39.54; H, 4.65; N, 23.05%; UV λ_{max}(MeCN)/nm 374.2; IR 1605, 1526, 1479, 1376, 1306, and 1183 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6; Me_4Si) δ 8.38 (2H, m), 8.42 (1H, s), 8.82 (1H, tt, J = 7.8 Hz), 10.43 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6; Me_4Si) δ 125.64, 127.80, 140.95, 142.43, 146.26, 148.24, 163.05, and 170.85; MS *m/z* (FAB, mNBA) 232.4.

2-Chloro-6-(4-dimethylpyridinio)purinate 10. 0.15 g (0.50 mmol) of the cation **6** were given on the resin to give a yellow solid (0.12 g, 95%), mp > 300 °C (from water). Found: C, 36.51; H, 4.36; N, 18.79%. Calcd for $C_{12}H_{11}ClN_6 \cdot 6.5H_2O$: C, 36.78; H, 6.17; N, 21.44%; UV λ_{max}(MeCN)/nm 344.1, 287.3, and 257.4; IR 1654, 1585, 1527, 1445, 1388, 1295, 1228, 1181, 1142, and 987 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6; Me_4Si) δ 7.29 (2H, d, J = 8.4 Hz), 8.23 (1H, s), 9.73 (2H, d, J = 8.4 Hz); MS *m/z* (FAB, mNBA) 275.6.

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