Synthesis and Base-Pairing of Self-Complementary Mesomeric Betaines of Guanine

Andreas Schmidt* and Nikoloz Kobakhidze
Technische Universität Clausthal, Institut für Organische Chemie, Leibnizstrasse 6, D-38678 Clausthal-Zellerfeld, Germany

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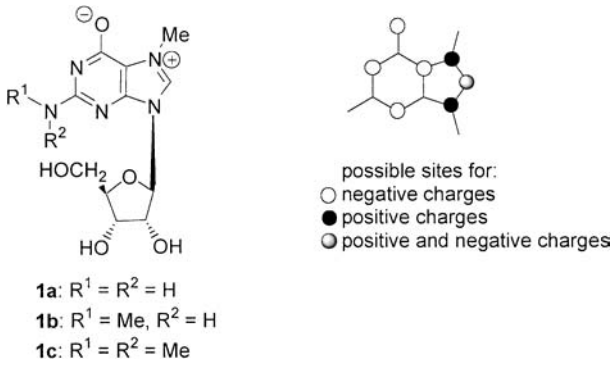
Substitution of 8-H of guanine-N-oxide was accomplished by pyridine, 4-dimethylaminopyridine and acetyl chloride, respectively, to give a cationic purine base after hydrolysis. Deprotonation formed mesomeric betaines of guanine. ESI mass spectrometry and ^{1}H NMR revealed their self-complementarity. Base-pairing to cytosine was observed with a fully protected DNA model compound.

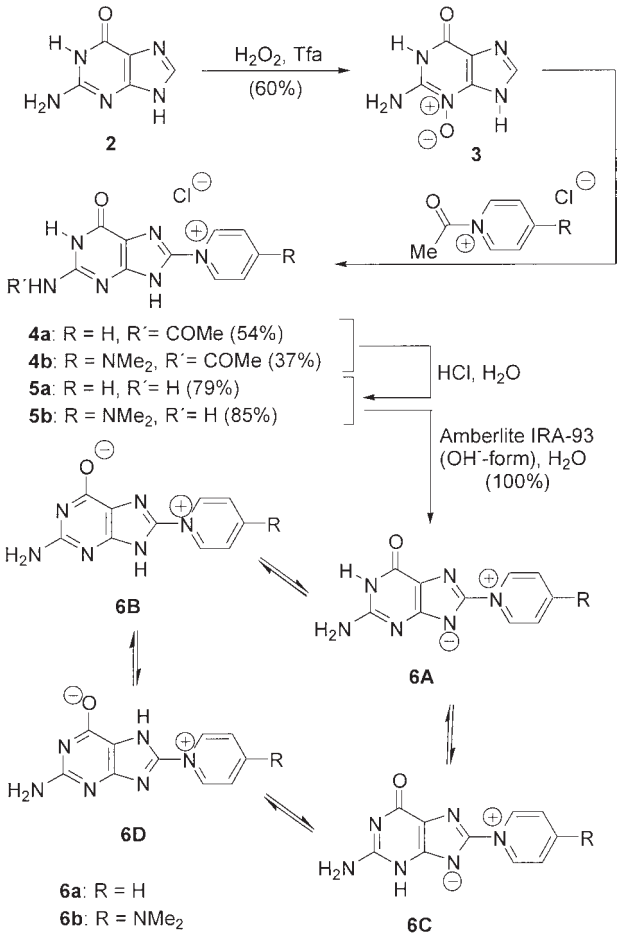
Posttranscriptional modification of RNA yields an exceptional number and structural diversity of modified nucleobases.[1] Among these, 7-methylguanosine **1a** (m^7G), 2,7-dimethylguanosine **1b** ($m^{2,7}$G), and 2,2,7-trimethylguanosine **1c** ($m^{2,2,7}$G) are mesomeric betaines (MB). They were isolated from distinct types of RNA molecules such as t, m, r, archaea, bacterial, and eucaryotic RNA.[2] 7-Methylguanosine was furthermore identified as 5′-terminal cap-structure of eucaryotic mRNA. Presumably it is responsible for the molecular recognition of the 5′-terminal cap of mRNA to a binding protein on the surface of the ribosomes prior to the initiation of protein biosynthesis.[3] The guanines **1** are members of the class of conjugated mesomeric betaines (CMB) which is one of four distinct classes (conjugated, cross-conjugated, pseudo-cross-conjugated and ylidic).[4] In general, in conjugated MB the positive and negative charges are in mutual conjugation and common atoms for either charge exist in the canonical formulae (Scheme 1).



Scheme 1.

As part of an ongoing project we are interested in synthesizing and studying mesomeric betaines of nucleobases.[5] Currently we investigate the dependence of the type of conjugation on the base pairing properties at the Watson-Crick- and Hoogsteen-binding sites. We want to report here our first results on novel betainic guanines, their self-complementarity and base-pairing.

Treatment of guanine **2** with hydrogen peroxide in trifluoroacetic acid resulted in the formation of guanine-3-N-oxide **3**.[6] Reaction of **3** with acetyl chloride and pyridine or 4-dimethylaminopyridine in dimethylformamide at room temperature formed the 1-[2-acetylamino-6(1*H*)oxopurin-8-yl]pyridinium chlorides **4a**,**b** which were deacylated to the 1-[2-amino-6(1*H*)-oxopurin-8-yl]pyridinium chlorides **5a**,**b** using hydrochloric acid at 90 °C. Deprotonations of aqueous solutions of **5a**,**b** to the 2-amino-8-pyridiniopurin-6-olates **6a**,**b** were finally accomplished by the anion exchange resin Amberlite® IRA-93 in its hydroxy form in nearly quantitative yields. In DMSO-d_6 solution at room temperature, only one set of resonance frequencies is detectable in ^{1}H NMR spectroscopy, respectively, although four tautomeric forms **6A-D** can be formulated.

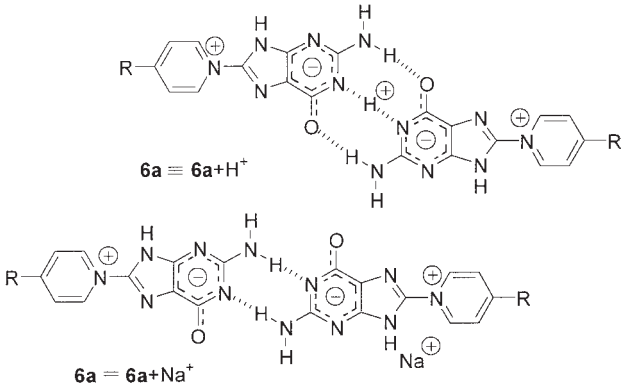


Scheme 2.

6A-C are conjugated mesomeric betaines (CMB), whereas **6D** is a cross-conjugated system (CCMB). In contrast to CMB, in CCMB the positive and negative charges are restricted to separate parts of the common π-electron system. Thus, in **6D** the positive charge is exclusively delocalized in the pyridinium substituent, whereas the negative charge is exclusively delocalized in the purin ring. According to a semiempirical calculation, **6A** is the

most stable, and **6D** the most unstable monomeric tautomer.[7]

In order to elucidate the base-pairing properties of these betainic nucleobases, we performed electrospray ionization mass spectrometry (ESIMS) and ^{1}H NMR titrations. Spraying **6a,b** from acetonitrile at 0 V fragmentor voltage and 140 °C desolvating gas temperature, the monomeric cationic systems **5a,b** as well as semiprotonated dimers **6a,b** ≡ **6a,b** + H^+ and sodium adducts **6a,b** = **6a,b** + Na^+ were detectable.[8] In accordance to the self-complementary structures (Scheme 3), which resembles dimeric uracilyl-betaines[9] and m^{7}g derivatives,[10] ^{1}H NMR signals shifted typically upfield on dilution.[11]



Scheme 3.

Equimolar solutions of **5a,b** and **6a,b**, respectively, and their complementary nucleobases cytosine and cytidine, respectively, sprayed from acetonitrile solutions gave additional peaks which correspond to 1 : 1 base-pairs.[8] No associates were detected with guanine under analogous conditions. NMR titrations in DMSO-d_6 at rt were performed at total nucleobase concentration, and the mole fraction of cytidine was increased from 0 to 0.99. The chemical shifts of the N(1)H and NH_2 group of *e.g.* **5a** (Scheme 4) shifted steadily downfield with increasing concentration of cytidine, thus indicating horizontal interactions. As expected for a Watson-Crick-type base-pairing, $\Delta\delta$[N(1)-H] was essentially twice as large as $\Delta\delta[NH_2]$.

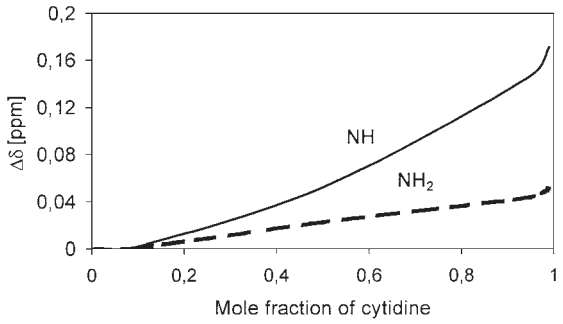
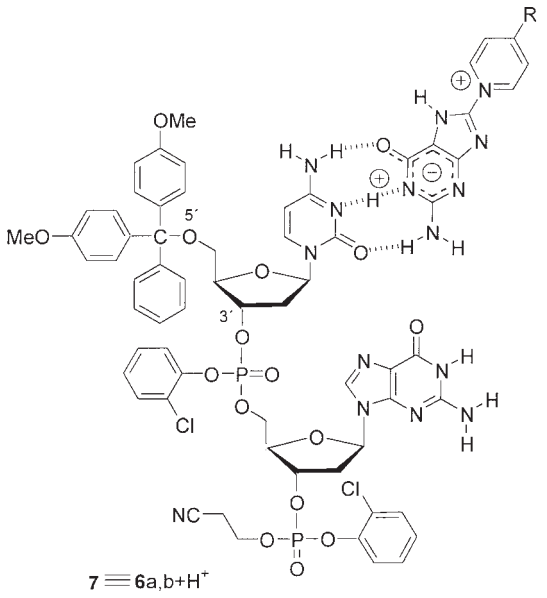


Figure 1. Variations in ^{1}H NMR chemical shifts in N(1)H and NH_2 of **5a** on addition of cytidine.

Spraying acetonitrile solutions of the self-complementary phosphoric acid 5-[2-amino-6(1*H*)-oxopurin-9-yl]-3-[2-chlorophenoxy(2-cyanoethoxy)phosphinoyloxy]tetrahydrofuran-2-ylmethyl 5-[4-amino-2(1*H*)-oxopyrimidin-1-yl]-2-[phenybis(4-methoxyphenyl)methoxymethyl]tetrahydrofuran-3-yl 2-chlorophenyl phosphate d(CpGp) **7** and the betaines **6a,b** gave peaks



Scheme 4.

of the monomeric **7** + Na^+, of 1 : 1 associates such as **7** ≡ **6a,b** + H^+ (Scheme 4), and of $\mathbf{7}_2$ + Na^+ in ESIMS.

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References and Notes

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7 **6A**: ΔH_f(PM3) = 249.20 KJ/mol; **6D**: ΔH_f = 356.75 KJ/mol.
8 *e.g.* **6b**: m/z = 272.1, 543.2, 565.2 amu; *e.g.* **6b** + cytosine: m/z = 383.2, 405.1 amu.
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11 *e.g.* for **6b** in DMSO-d_6 at rt from 20 mM to 6 mM: $\Delta\delta$ = −0.15 ppm (NH), −0.22 ppm (NH_2), −0.03 ppm (α-H).
12 *e.g.* for **6b**: m/z = 1410.2, 1659.2, 2797.4 amu.