Molecular recognition of modified nucleobases. Self-complementarity and base-pairing of betainic guanine model compounds †

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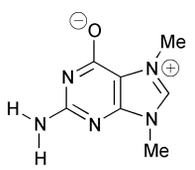
Cross-conjugated models **11**–**14** of the natural modified RNA-nucleobase 7-methylguanosine **2**, which is a conjugated mesomeric betaine, were synthesized and their self-complementarity and base-pairing properties were studied. Nucleophilic substitution of heteroaromatics with 2-amino-6-chloropyrimidin-4-ol **6** in 1,2-dichlorobenzene and subsequent treatment of the resulting pyrimidinylheteroarenium salts **7**–**10** with the anion exchange resin Amberlite IRA-93 in its hydroxy form gave the title compounds **11**–**14** almost quantitatively. Electrospray ionization mass spectrometry (ESIMS) and ^{1}H NMR titrations reveal that **11**–**14** are self-complementary molecules which form *homo*-intermolecular dimers. Semiempirical as well as *ab initio* calculations predict analogous geometries of the dimers and lead to considerable stabilization energies in comparison to the monomeric species. In ESI mass spectrometry, the molecular masses of noncovalent 1 : 1 associates between the model compounds and the complementary nucleobase cytosine and the nucleoside cytidine can be detected. An *ab initio* calculation leads to a stabilization energy of 71.3 kJ mol^{-1} on base-pairing of the mesomeric betaine **11** with cytosine. Additionally, dimeric 1 : 1 associates can be detected between **11** and the self-complementary d(CpGp) as a DNA model compound.

Introduction

Since the first isolation of the betainic alkaloid herbipoline **1** (Scheme 1) from the marine sponge *geodia gigas*,[1] the discovery of new betainic nucleobases in nature continued in parallel with the steadily increasing knowledge of the array of functional roles of posttranscriptional modification of RNA. Among the exceptional number and structural diversity of modified and hypermodified nucleosides thus formed,[2] the conjugated mesomeric betaine 7-methylguanosine (m^{7}G) **2** was isolated from ribosomal-RNA[3] as well as from distinct types of transfer-RNA (archaea, bacterial, eukaryotic[4]). In addition, its derivatives 2,7-dimethylguanosine (m^{2,7}G) **3** and 2,2,7-trimethylguanosine (m^{2,2,7}G) **4** were identified (*sn*-[5] and viral RNA[6]). On converting guanosine into these mesomeric betaines, biologically important horizontal as well as vertical interactions change. Thus, due to blocked Hoogsteen and electronically disturbed Watson–Crick binding sites, 7-methylguanosine, which in pure form is unstable at neutral and basic pH, is involved in nonstandard base-triplets such as m^{7}G=G≡C and m^{7}G=A and stabilizes the tertiary structure of the RNA polynucleotide chains.[7] These m^{7}G interactions may be parallel or antiparallel. Unusual stacking patterns are due to base-intercalation of *e.g.* adenine into m^{7}G and G where three strands meet in t-RNA.[8] Presumably the most important biological role plays 7-methylguanosine as 5′-terminal *cap*-structure of eukaryotic m-RNA **5**,[9] although Nature's selection of a mesomeric betaine at this position is still somewhat enigmatic. Stabilizing effects as well as betaine–protein molecular recognition to enable the binding of the m-RNA molecules to proteins on the surface of the ribosomes prior to the initiation of translation have been discussed.[10] Base-mispairing induced by 7-methylguanine **1** causes its biological activity,[11] and it was surprisingly identified as one of the main metabolites on treatment of DNA with carcinogens such as hydrazine.[12]



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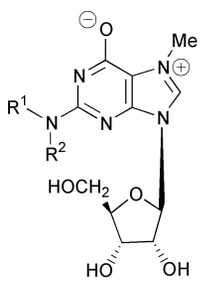
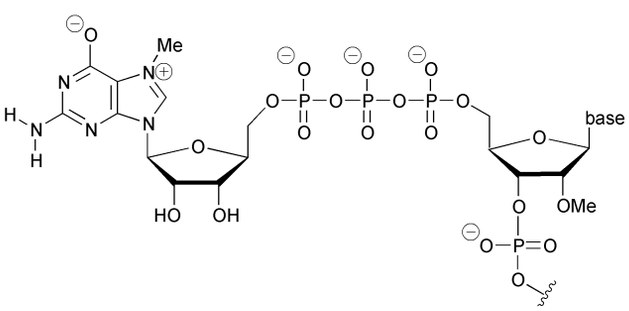


2: $R^1 = R^2 = H$
3: R^1 = Me, R^2 = H
4: $R^1 = R^2$ = Me



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Scheme 1

† Electronic supplementary information (ESI) available: electrospray ionization mass spectra. See http://www.rsc.org/suppdata/p1/b1/b110318k/

 DOI: 10.1039/b110318k

Very recently, betaines of nucleic acid bases have found use as fluorescent probes.[13]

Mesomeric betaines are neutral conjugated molecules which can be represented only by dipolar structures in which both the positive and the negative charges are delocalised within a common π-electron system. In general, mesomeric betaines are divided into four major classes (conjugated, cross-conjugated, pseudo-cross-conjugated, ylidic) and 16 subclasses on the basis of their isoconjugate equivalency to odd and even alternant or nonalternant hydrocarbons, respectively.[14] Surprisingly, nothing is known to date about the influence of the type of conjugation on molecular recognition and biological activity. In continuation of our interest in mesomeric betaines[15] and nucleobases,[16] this publication presents an experimental and theoretical investigation of the role of charge distribution in betainic guanine model compounds. We examined the base-pairing properties of complementary and non-complementary nucleobases, nucleosides and a DNA model compound.

Results and discussion

As shown in Fig. 1, the natural guanines **1**–**5** belong to the class

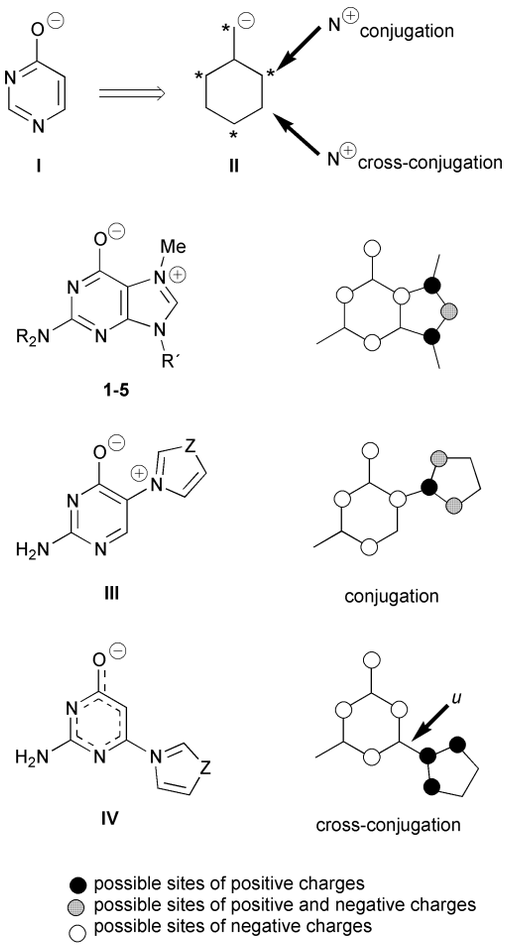
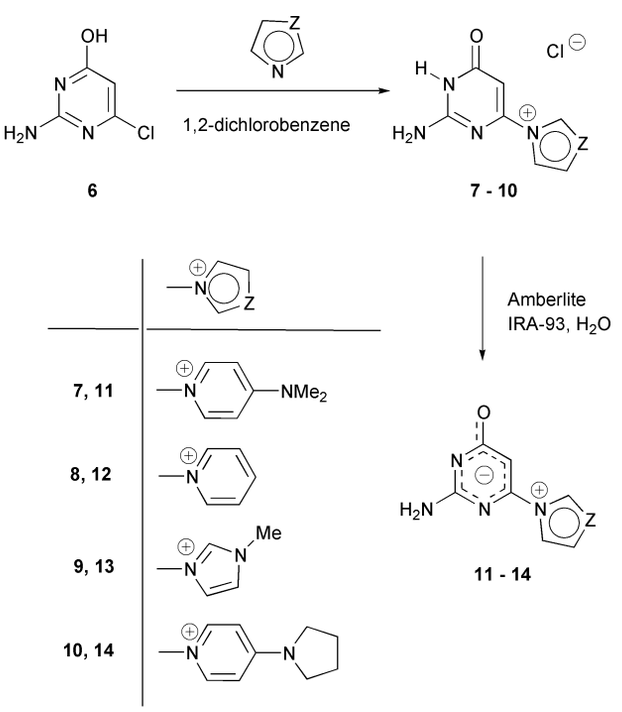


Fig. 1

of conjugated mesomeric betaines (CMB). The positive and negative charges are in mutual conjugation and are therefore *not* restricted to separate parts of the π-electron system of the molecule. This is the result of the π-connection of the negative to the positive fragment through an "active" atom, *i.e.* one of the starred positions of the isoconjugated benzyl anion **II** of the pyrimidin-4-olate moiety **I** of **1**–**5**. In contrast to the C(5)–N^+ bond in **III**, the C(6)–N^+ bond in **IV** is a *union* ("*u*") in the sense of Dewar,[17] so that substitution of the "inactive" C(6) by a cationic substituent forms a cross-conjugated mesomeric betaine (CCMB) without changing the Watson–Crick binding site of guanine. In **IV** the positive and negative charges are *exclusively* restricted to separate parts of the π-electron system of the molecule. Thus, on changing a conjugated into a cross-conjugated system, larger permanent dipole moments of **IV** and altered hydrogen bond donor and acceptor capabilities in relation to **1**–**5** can be predicted.

Syntheses of the model compounds

Although several methods exist for the synthesis of pyridinium substituents (Ortoleva–King reaction,[18] nucleophilic ring transformations,[19] TMSOTf-[20] and $NaBPh_4$-assisted anion interceptions,[15e] Menschutkin-[21] and Finkelstein-type reactions[15e]), approaches to conjugated or cross-conjugated heteroarenium compounds are very limited in number as they afford a vinyl halide substrate. Best results were achieved by noncatalyzed nucleophilic substitution of 4-dimethylaminopyridine, pyridine, 1-methylimidazole, and 4-pyrrolidinopyridine, respectively, on 2-amino-4-hydroxy-6-chloropyrimidine **6** in 1,2-dichlorobenzene at reflux temperature which resulted in the smooth formation of the corresponding (2-amino-6-oxopyrimidin-4-yl)heteroarenium chlorides **7**–**10** in moderate to high yields (Scheme 2). Conducting the reaction in boiling chlorobenzene



Scheme 2

and interception of the leaving chloride as the volatile TMSCl through the presence of equimolar amounts of TMSOTf gave only low yields of the desired molecules. Finkelstein reaction conditions failed. The anion exchange resin Amberlite® IRA-93 in its hydroxy form proved to be the most suited medium to convert the monocationic systems **7**–**10** in almost quantitative yields into the mesomeric betaines **11**–**14** which are yellow in color. The deprotonation can easily be monitored by the ^1H NMR shift changes. Thus, on conversion to the betaine, the resonance frequencies of the 5-H of the pyrimidine moiety of a concentrated solution in DMSO-d_6 shifts characteristically from $\delta = 6.15$ (**7**) to 5.87 ppm (**11**), while the NH-signal at 11.66 ppm disappears. According to the NMR spectra, the salts **7**–**10** exist as single NH-tautomers.

Classification and calculations of the monomeric species

The 7-methylguanines **1**–**5** are members of class 4 of the mesomeric betaines due to their isoconjugate equivalency with

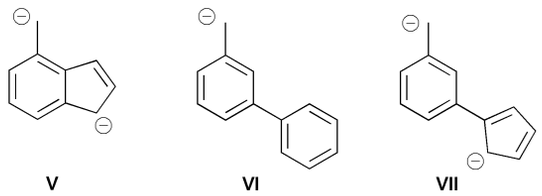


Fig. 2

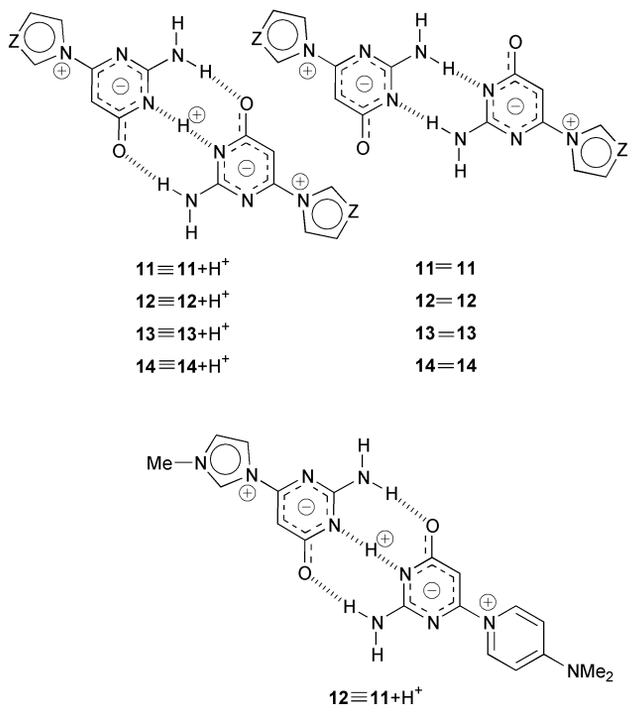
the even nonalternant hydrocarbon dianion **V** shown in Fig. 2.[14] In contrast, the cross-conjugated mesomeric betaines **11**, **12**, and **14** are isoconjugate with the odd alternant hydrocarbon anion **VI** and thus are members of class 9. In **13** the six-membered cationic substituent is changed for a five-membered ring, and thus it is isoconjugated with the even nonalternant hydrocarbon dianion **VII**, which represents class 12. To gain insight into the electronic differences between conjugated and cross-conjugated systems, we performed first semiempirical[22–24] and then *ab initio* calculations[25] on 7,9-dimethylguanine **1** as well as **11** as the cross-conjugated model compound. As presented in Fig. 3, the HOMO [IP(PM3) = +8.19 eV] of the planar 7,9-dimethylguanine is essentially located at N(1), N(3), C(5) and O(13) of the pyrimidine moiety and in the imidazole ring, whereas the LUMO [IP(PM3) = +0.92 eV] has its largest coefficients at N(7), C(8), and N(9) in the imidazole ring and at C(5) of the pyrimidine.

In contrast to the conjugated **1**, the calculation reflects the cross-conjugation of **11**. Characteristically, the HOMO(PM3) [IP: +7.64 eV] and LUMO(PM3) [IP: +2.19 eV] shown in Fig. 3 are essentially located in different parts of the π-electron system. As a confirmation of our theoretical approach in Fig. 1, the positive partial structure is joined to the negative one at atoms which are nodal positions of the HOMO. As a consequence, the permanent dipole moment of **11** is by far larger than those of the conjugated mesomeric betaines **1**–**5**, and they differ (as shown by the arrows in Fig. 3) in size and direction.[29] In addition, the differences in the net atomic charges of **1** and **11** at the Watson–Crick binding site are very much in accord with the corresponding type of conjugation, resulting in a higher electron density at oxygen and a lower density at the exocyclic amino nitrogen atom.

Molecular recognition: self-complementarity

In view of the interesting base-pairing properties of m^7G in RNA and in order to compare a conjugated with a cross-conjugated system, we studied the base-pairing properties of the betaines **11**–**14** and of their cationic precursors **7**–**10** by electrospray ionization mass spectrometry (ESIMS), ^1H NMR titrations and an *ab initio* calculation of **11** as the model compound.

The extremely mild electrospray ionization technique enables the detection of charged species and their hydrogen-bonded associates by mass spectrometry and it proved to be extremely valuable for the detection of oligonucleotides, proteins, enzyme–substrate and enzyme–product complexes.[30] Under ESI conditions, the self-complementarity of the modified guanines is unambiguous. At 0–20 V fragmentor voltage the *homo*-intermolecular associate between two semiprotonated betaines **11**≡**11** + H^+ forms the base peak of the spectrum at 463.3 amu (Scheme 3) spraying a solution from 90% aqueous



Scheme 3

acetonitrile. A dimeric, non-protonated betaine **11**═**11** was detected as sodium adduct at *m*/*z* = 485.2 amu. In addition, the spectrum of **11** displays peaks of the monomeric species at 232.1 amu (**11** + H^+) and 254.1 amu (**11** + Na^+) at fragmentor voltages between 10 and 50 V. As shown in Scheme 3, three hydrogen bonds are possible in **11**≡**11** + H^+ forming a centrosymmetric dimer, whereas the betaine can dimerize to **11**═**11** through two hydrogen bonds. Analogous results were obtained in ESIMS experiments of the derivatives **12**–**14** and its precursors **8**–**10** (Table 1). It is noteworthy that *all* base-pairing combinations of mixed solutions between **11**, **12**, **13**, and **14** appear in the ESIMS. Thus, an equimolar aqueous acetonitrile

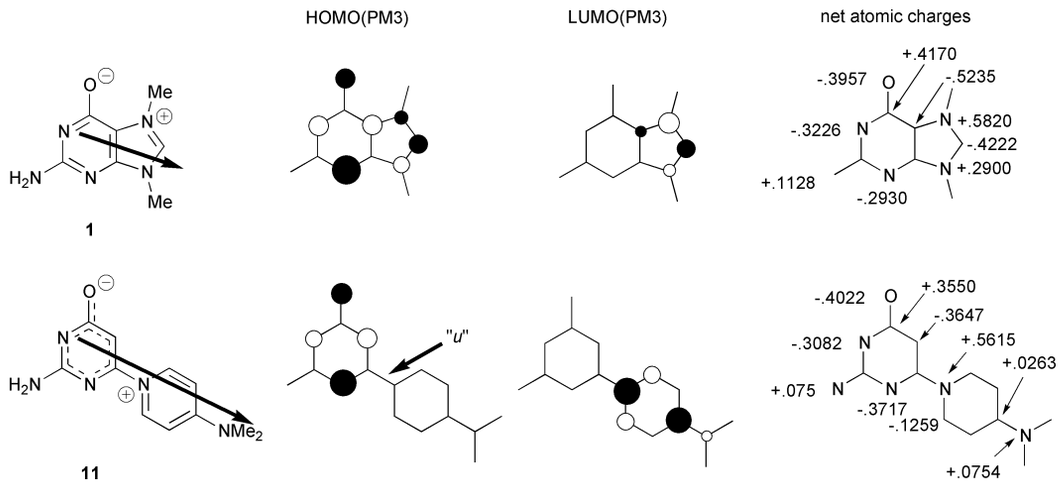


Fig. 3

Table 1 Results of electrospray ionization mass spectrometry (ESIMS) of the pure compounds **11**–**14** sprayed from 90% aqueous acetonitrile depending on the fragmentor voltage

	m/*z* at 0 V (%)	*m*/*z* at 20 V (%)
11	463.3 (**11**≡**11** + H^+; 100)	232.1 (**11** + H^+, 100)
	485.2 (**11**=**11** + Na^+; 21)	254.1 (**11** + Na^+, 12)
12	377.1 (**12**≡**12** + H^+; 100)	189.1 (**12** + H^+; 100)
	399.0 (**12**=**12** + Na^+; 19)	377.1 (**12**≡**12** + H^+; 18)
13	383.1 (**13**≡**13** + H^+, 100)	192.1 (**13** + H^+, 100)
	405.2 (**13**=**13** + Na^+; 16)	214.0 (**13** + Na^+, 9)
14	515.2 (**14**≡**14** + H^+; 100)	258.0 (**14** + H^+, 100)
	537.2 (**14**≡**14** + Na^+; 6)	280.1 (**14** + Na^+; 7)

solution of all four betaines **11**–**14** gives peaks of all eight betaine–betaine + H^+ and betaine–betaine + Na^+ combinations. As an example, a possible structure of **12**≡**11** + H^+ is presented in Scheme 3.

Next, we performed ^{1}H NMR titrations in DMSO-d_6.[31,32] On concentrating a diluted solution of **7**–**10** and **11**–**14**, respectively, the amino group signals shift considerably to lower fields, thus indicating horizontal interactions. Although the chemical shift changes of the NH and the NH_2 group are larger because they are involved in hydrogen bonding at the Watson–Crick binding site, the chemical shift of 12-H of the pyrimidine ring is the most reliable for such an observation. It forms a sharp nonoverlapping singlet that is not deuterium-exchangeable at δ = 5.8–6.2 ppm depending on the concentration. Fig. 4

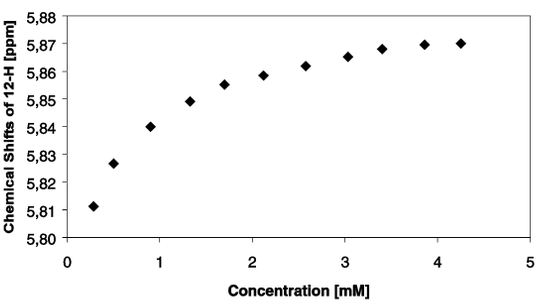


Fig. 4 Chemical shift changes of 12-H of **11** with concentration in DMSO-d_6 at rt; slope characteristic of hydrogen bonding.

presents the typical dependence of ^{1}H NMR chemical shifts on the concentration of self-complementary molecules **7** and **11** in DMSO-d_6. As confirmation, the slope of the line is opposite in D_2O due to competition with water molecules at the Watson–Crick binding site. Instead, vertical interactions, *i.e.* π-stacking, are observable.[16c] This plot is shown in Fig. 5. The results of our NMR studies are summarized in Table 2.

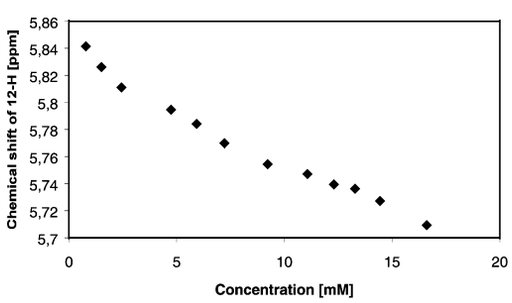


Fig. 5 Chemical shift changes of 12-H of **11** in D_2O at rt with concentration; slope characteristic of π-stacking interactions.

No associates between guanosine and the betaines **11**–**14** were detectable under analogous conditions by ESI mass spectrometry. No base-pairing was observable by ^{1}H NMR spectroscopy in DMSO-d_6 at room temperature.

Then we focussed our interest on a theoretical investigation of the *homo*-intermolecular dimers and performed a semiempirical as well as an *ab-initio* calculation to study the possible geometries of **11**=**11**. The PM3 calculation led to a "U"-like arrangement of the individual betaines in the dimer; they are hydrogen-bonded through N(3) as the acceptor and C(2)–NH_2 as the donor. The PM3 method calculates the dimer to be 23.7 kJ mol^{-1} more stable than the monomeric species. The *ab initio* RHF/6-31G(d) calculation led to a very similar result which is presented in Fig. 6. In the first approach, the two monomers

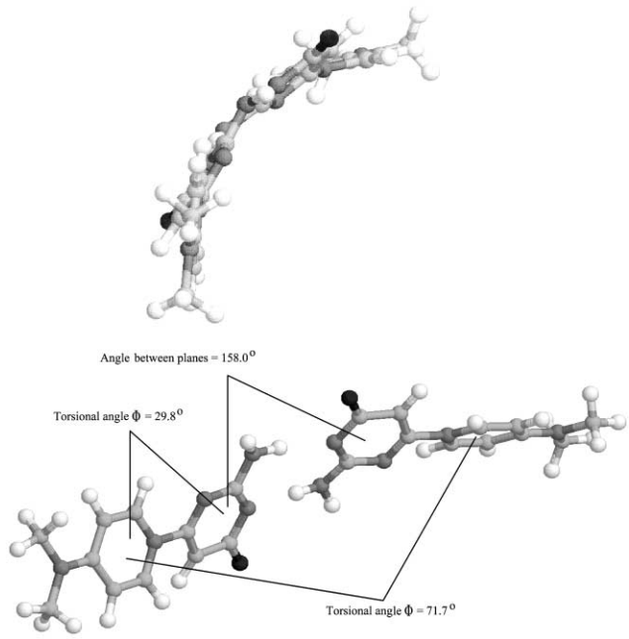


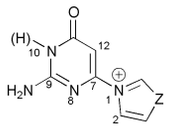
Fig. 6 Results of the *ab initio* calculation of **11**=**11**. Top: "U"-like shape of the dimer. Bottom: view of the helical arrangement of the *homo*-intermolecular dimer and torsional angles.

were combined linearly, in the second they were twisted by approximately 180° about the planes of the two pyrimidine rings. Either *ab initio* calculation led to a "U"-like arrangement with the two essentially planar pyrimidine rings twisted about the hydrogen bonds by 22° from planarity. In the dimers, the individual betaines adopt a nonplanar conformation around the N(1)$^+$–C(7) bond with a torsion angle of 29.8°. In order to elucidate the influence of conformational changes on the base-pairing properties, we performed an *ab initio* calculation on the monomeric molecule. In good agreement with the facts that the conformation is influenced by i) p-overlap between the positive and negative fragment of the cross-conjugated system, ii) steric repulsion between 12-H and the α-H of the heteroaromatic ring, and iii) attracting forces between the N(1) lone pair and the α-H atoms,[16d] the calculation produced a most stable conformation in which the pyrimidine and the pyridinium rings are twisted by 31.2° from planarity. This conformation leads to an interesting helical arrangement of the dimerized species with the two pyridinium rings twisted by 71.7°. The *ab initio* calculation resulted in a stabilization energy of 47.6 kJ mol^{-1} on dimerization of the betaines, which is obviously not influenced by conformational changes, as demonstrated by the geometries of the monomeric species. Furthermore, it is evident that the geometry of the dimer leads to a coupling of the individual permanent dipole moments.[29] This phenomenon, which stands in contrast to the known dimerization of nucleobases such as the reversed Hoogsteen homopurine base-pair of adenine,[33] has already been observed by us with cross-conjugated uracilyl-betaines.[16b]

Base-pairings

Next, we turned our attention to the intermolecular interactions of **11**–**14** with complementary and noncomplementary

Table 2 Selected ^{1}H NMR resonance frequencies of associated and monomeric cations **7**–**10** and betaines **11**–**14** in DMSO–d_6 at 400 MHz and 25 °C, respectively (numbering as shown below)[a]

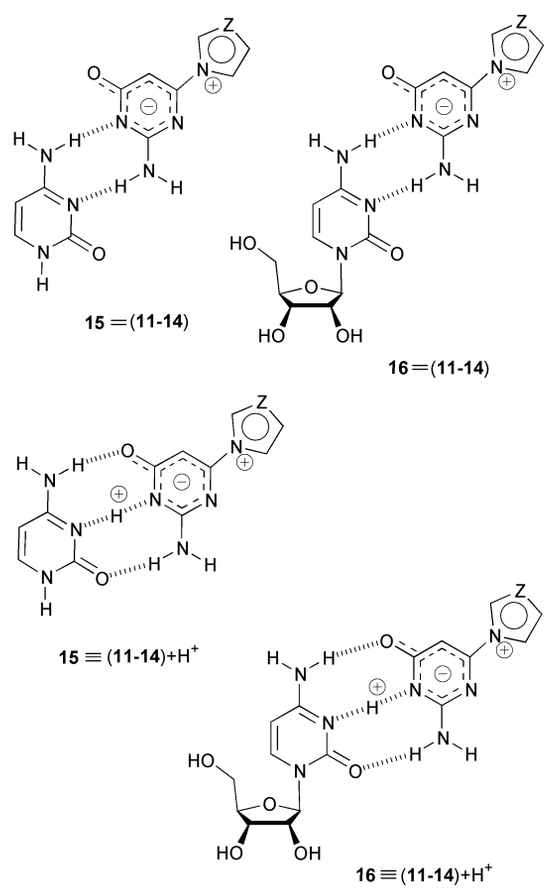


Compound		δ (ppm)			$\Delta\delta$ (ppm)		
		NH_2	NH	12-H	NH_2	NH	12-H
7	c[a]	7.527	11.661	6.155	0.229	0.165	0.028
	d[a]	7.298	11.496	6.127			
11	c	7.286	—	5.879	0.267	n.d.	0.041
	d	7.019	—	5.838			
8	c	7.626	11.856	6.360	0.022	0.336	0.013
	d	7.604	11.520	6.347			
12	c	7.304	—	6.112	0.253	—	0.034
	d	7.051	—	6.078			
9	c	7.509	11.705	6.175	0.137	0.123	0.005
	d	7.372	11.582	6.170			
13	c	7.200	—	5.892	0.108	—	0.015
	d	7.092	—	5.877			
10	c	4.369	7.600	6.048	n.d.	0.235	0.059
	d	n.d.	7.365	5.989			
14	c	7.598	—	5.907	0.194	—	0.013
	d	7.404	—	5.894			

[a] c: concentrated solution in DMSO-d_6 (**7**–**10**: 7 mM; **11**–**14**: 10 mM). d: 50% dilution of the concentrated solution with DMSO-d_6. n.d.: not detectable; errors of the NMR values: 0.0015 ppm; DMSO (δ = 2.500 ppm) as internal standard.

nucleobases. First, we studied the molecular recognition between **11**–**14** and cytosine, cytidine, and guanosine.[34] By means of ESI mass spectrometry, noncovalent associates between the betaines **11**–**14** and cytosine **15** were detectable as sodium adducts and as protonated associates spraying 1 : 1 mixtures from 90% aqueous acetonitrile at 0 V fragmentor voltage.[35] The latter species forms the base peaks of the spectra under the conditions applied. With increasing fragmentor voltage, the ratio of associated species to monomers decreases. In general, at voltages > 30 V, no noncovalent associates can be detected. Analogously, by spraying 1 : 1 solutions of **11**–**14**, respectively, with cytidine **16** from 90% aqueous acetonitrile, all base-paired betaines and cations were detectable.[36] Possible structures of the noncovalent associates are shown in Scheme 4. Whereas structures such as the **15**≡**11** + H^+ and **16**≡**11** + H^+ pairs are formulated in analogy to the Watson–Crick G≡C base pair, the geometries of the associates such as **15**=**11** and **16**=**11** are related to results of X-ray analyses: the O(6) of naturally occurring betainic guanine such as m^7G is not involved in hydrogen bonding, unless N(1) is protonated.[7,8,16a] Similarly, cross-conjugated betainic uracils do not dimerize through C(4)=O.[16b]

We next performed NMR titrations and calculations to gain additional knowledge about the base-pairs. NMR spectra of **7** and **11**, respectively, containing varying mole fractions of cytosine **15** or cytidine **16** in DMSO-d_6 were measured.[31] The total nucleobase concentration was constant during the titration in order to rule out concentration effects, and the mole fraction of **15** and **16**, respectively, was increased from zero to 0.8. In general, three effects have to be considered: i) as our model compounds are self-complementary, dilution results in *upfield* shifts of resonance frequencies (*cf.* Fig. 4 and Table 2). ii) Acid–base equilibria such as between **7** + **15** and **11** + **15** + H^+ would cause *upfield* shifts of the signals of **7**. iii) Base-pairing results in *downfield* shifts, especially of the signals near the Watson–Crick binding sites of the molecules.[37] Our results for **7** and cytosine **15** are presented in Fig. 7. On addition of **7** to cytosine **15**, the N(10)-H of **7** moved steadily downfield (for numbering, see Table 2). Likewise, 12-H is deshielded [$\Delta\delta$ = −20 ppb] although the concentration of the dimer **7**=**7** decreases on dilution, *i.e.* with increasing mole fraction of cytosine **15** at constant total



Scheme 4

nucleobase concentration. The maximum concentration is limited to 10 mM because of the solubility of **7** (*cf.* Fig. 4). As the ratio of the integrals of 12-H and N(10)-H remains constant during the measurement, acid–base equilibria between cation **7** and betaine **11** were eliminated from consideration.[38] All the resonance frequencies of the pyridinium ring remained virtually unchanged. In the same samples, all the signals of

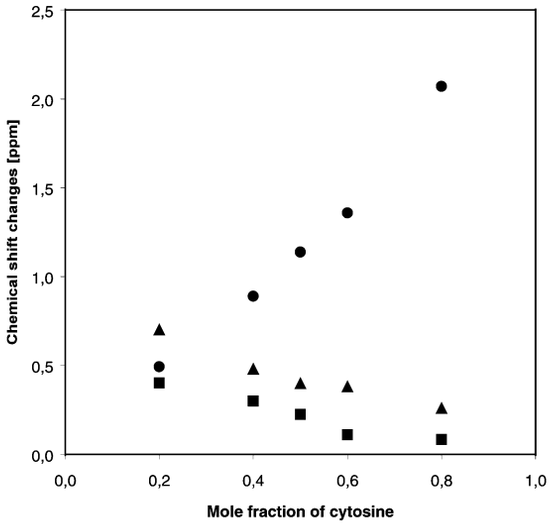


Fig. 7 Variations in ^{1}H NMR chemical shifts in N(10)-H of **7** with added cytosine **15** in DMSO-d_6 at rt. Total concentration of nucleobases was maintained at 0.01 M. ● N(10)-H of **7**, ▲ NH of **15**, ■ NH_2 of **15**.

cytosine **15** moved downfield with increasing mole fraction of **7** [$\Delta\delta$ (5-H) = −10 ppb; $\Delta\delta$ (6-H) = −20 ppb] (Fig. 7). These results are in accord with hydrogen bonding at the Watson–Crick binding sites. Cytosine **15**, however, obviously has additional noncovalent interactions *via* N(1)-H in DMSO-d_6 at rt. Very similar results were obtained by recording ^{1}H NMR spectra of **7** with varying mole fractions of cytidine **16** between 0 and 0.8 at 10 mM total concentration of the nucleobases in DMSO-d_6 at rt. Again, the signals of N(10)-H and 12-H of **7** shift steadily downfield [$\Delta\delta$ = −45 and −10 ppb, respectively], whereas the pyridinium signals remain virtually unchanged. In the same samples, the resonance frequencies of the amino group, 5-H and 6-H of cytidine **16** shift downfield with increasing concentration of **7** [$\Delta\delta$ = −40, −20, −20 ppb].

Due to the rather limited solubility of the betaine **11** in all common solvents, the total concentration of the solutions was only 7 mM in DMSO-d_6 at rt. In a similar fashion to the NMR studies mentioned above, during NMR titrations of the betaine **11** with cytosine **15** at constant total concentrations of the solutions with varying mole fractions between zero and 0.8, the resonance frequencies of the NH group of **15** shift steadily from 10.29 to 10.56 ppm. Likewise, 5-H and 6-H of **15** move slightly downfield [$\Delta\delta$ = −5 and −2 ppb, respectively]. The resonance frequencies of the betaine **11** move steadily upfield with increasing mole fraction of cytidine **15**. As already presented in Fig. 4, dilution of the self-complementary betaine results in an upfield shift due to depairing of the dimers **11**=**11** to the "monomeric" **11**. The NMR titration with cytidine **16**, however, displays a considerably smaller effect than predicted by the results presented in Fig. 4. The observed chemical shift is obviously the result of competing shielding and deshielding effects. Analogous results were obtained by varying the mole fraction of cytidine **16** in DMSO-d_6 solutions of **11** at constant total nucleobase concentration (7 mM).

We then focused our attention on a theoretical investigation of the base-pairs and performed an *ab initio* calculation.[25] Thus, the base-pairing **11**=**15** presented in Scheme 4 leads to a considerable stabilization energy of 71.3 kJ mol^{-1} (PM3: 38.1 kJ mol^{-1}). Again, the betaine **11** adopts a nonplanar conformation with a torsion angle of 30.6° between the pyrimidine and pyridinium ring so that the distance between 2-H and 12-H is 219.3 pm. As presented in Fig. 8, the hydrogen bonds have different lengths. It is apparent that the base-pairing between betaine **11** and cytosine **15** is an energetically favored process that is able to overcome the self-complementarity of **11**.

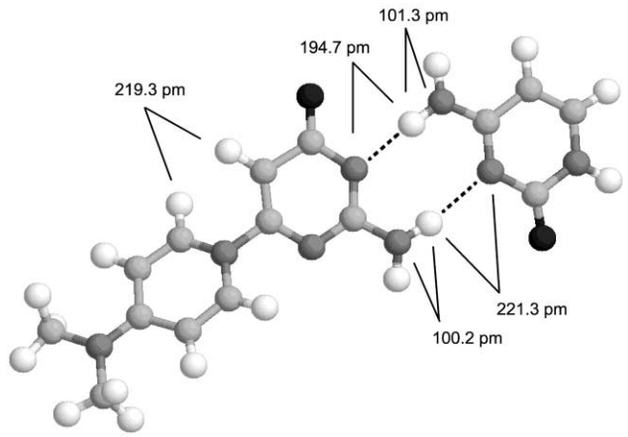
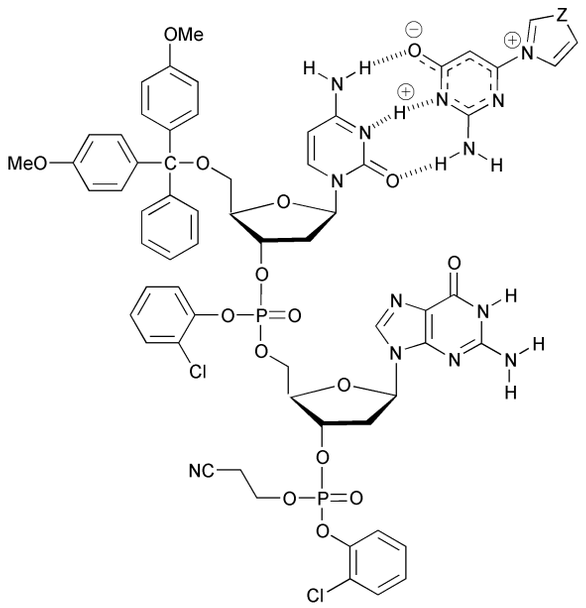


Fig. 8 Results of the *ab initio* calculation of the **11**=**15** base-pair. Hydrogen bond lengths and 2-H–12-H distance.

The results mentioned above encouraged an examination of a DNA model compound which contains the complementary nucleobase cytosine as well as unmodified guanine. We chose the fully protected and self-complementary (*N*-blocked-5′-*O*-DMT-3′-(2-chlorophenyl)-2′-deoxynucleotidylyl-[3′⟶5]-*N*-blocked-2′-deoxynucleoside 3′-(2-chlorophenyl-2-cyanoethyl)-phosphate d(CpGp) **17** as the DNA model compound. Indeed, 1 : 1 associates of **11**–**14** and d(CpGp) were unambiguously detectable. On addition of the betainic nucleobases **11**–**14** to a solution of d(CpGp) in 90% aqueous acetonitrile immediate precipitation of a yellowish solid occurred. In the case of **11**, a monocationic hydrogen-bonded dimer between d(CpGp) **17** and the mesomeric betaine forms an intense peak at *m*/*z* = 1619.3 amu. In addition, this solution sprayed from 90% aqueous acetonitrile shows the molecular peaks of the protonated mesomeric betaine (*m*/*z* = 232.1), the monomeric d(CpGp) as the sodium adduct (**17** + Na)$^+$ at *m*/*z* = 1410.1 amu, and the dimeric DNA d(CpGp)$_2$ which forms a sodium adduct at *m*/*z* = 2797.3 amu. Analogous results were obtained on mixing d(CpGp) with **12**–**14**.[39] 1 : 1 : 1 Associates between two betaine molecules and the DNA model compound were not detectable. However, addition of one equivalent of cytosine **15** to the 1 : 1 mixture of d(CpGp) **17** and **11** gives additional peaks at 1498.2 amu [**17** + **15**] and 1730.3 amu [**17** + **15** + **11**].[40] Analogous results were achieved spraying 1 : 1 : 1 solutions of **12**, **13**, and **14**, d(CpGp) **17**, and cytosine **15** under identical reaction conditions.[41] A structure can be proposed with the protonated betaines **11**–**14** bound through three hydrogen bonds to the C moiety of d(CpGp) **17**, respectively, and the cytosine bound through three hydrogen bonds to the G moiety of d(CpGp) **17**.

We performed an HH-COSY spectrum in order to unambiguously assign the resonance frequencies of **17**. ^{1}H NMR spectroscopy showed that the resonance frequency of the amino group of **11** shifts considerably downfield ($\Delta\delta$ = 0.21 ppm) on treatment with d(CpGp), while the signals of 12-H and 2-H remain unchanged. Chemical shift changes are therefore unambiguously due to horizontal interactions and not to protonation of **11**–**14** to **7**–**10** (*cf.* Table 2). The aforementioned detection of base-pairings of **11**–**14** with the complementary nucleobases cytosine and cytidine, and the absence of base-mispairings with guanosine lend support to the formulation of associate **17**≡(**11**–**14**) + H$^+$ as shown in Scheme 5. To the best of our knowledge, this is the first reported base-pairing of mesomeric betaines with DNA model compounds.

In conclusion, converting the nucleobase guanine into a cross-conjugated mesomeric betaine without changing the geometry of the Watson–Crick binding site results in self-complementary molecules which can dimerize in betainic (two hydrogen bonds) as well as in semiprotonated form (three hydrogen bonds). Electrospray mass spectrometry, ^{1}H NMR titrations, and *ab initio* calculations lend support to



17≡(11-14)+H$^+$

Scheme 5

complementary base-pairing of **11**–**14** with cytosine, cytidine and to the cytosine in d(CpGp) as a DNA model compound. No base-mispairing to the nonmodified guanosine could be observed.

Experimental

Chemical shift data for ^{1}H and ^{13}C NMR spectra were measured relative to tetramethylsilane (TMS) standard in DMSO-d_6 unless otherwise noted. IR spectra were taken of KBr pellets with 2.5% substance. All melting points are uncorrected. ESIMS parameters were as follows: ionization mode: API-ES positive, drying gas temperature: 140 °C, drying gas flow: 10 L min^{-1}, nebulizer pressure: 50 psig, capillary voltage: 3500 V, fragmentor voltage: 0 V (unless otherwise noted), quadrupole temperature: 99 °C, solvent flow rate: 0.8 mL min^{-1} of 90% aqueous acetonitrile, mass range 120–3000 amu. All compounds and compound mixtures were dissolved in 90% aqueous acetonitrile, respectively (1 mg in 8 mL), prior to direct injection. All compounds were vigorously dried prior to NMR titrations and were used immediately to avoid the incorporation of water in the NMR samples. In all NMR titrations, the water content of the solutions is less than 0.5%. In accord with previously reported nucleobases and mesomeric betaines,[14–16] all of the compounds pick up water during weighing and on storage, and crystallize with various amounts of water of crystallization which were found in the elemental analyses.

1-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-yl)-4-(dimethylamino)pyridinium chloride (7)

0.49 g (3 mmol) of 2-amino-6-chloro-4-hydroxypyrimidine hydrate **6** and 0.366 g (3 mmol) of 4-dimethylaminopyridine in 40 mL of *o*-dichlorobenzene were heated at reflux temperature over a period of 1.5 hours. After cooling, the precipitate was filtered off, washed twice with ethyl acetate and recrystallized from ethanol–water in the presence of charcoal, yield 95%; mp 300–302 °C (decomp.) (Found: C, 45.91, H, 4.86; N, 23.56. $C_{11}H_{14}ClN_5O$ requires: C, 45.91; H, 4.87; N, 24.35%). δ_H (200 MHz, DMSO-d_6; concentrated solution): 11.58 (1H, s, broad), 8.77 (2H, d, *J* 8.08), 7.37 (2H, s, broad), 7.15 (2H, d, *J* 8.22), 6.15 (1H, s), 3.02 (6H, s); δ_C (20 MHz, DMSO-d_6) 84.1, 89.5, 107.5, 137.3, 157.1, 167.1; ν_{max}/cm^{-1} 3416, 3143, 2934, 1645, 1219, 1157.

1-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-yl)pyridinium chloride (8)

0.49 g (3 mmol) of 2-amino-6-chloro-4-hydroxypyrimidine hydrate **6** in 15 mL of pyridine were heated at reflux temperature over a period of 40 minutes. After cooling, the precipitate was filtered off, washed with ethyl acetate, and recrystallized from ethanol–water in the presence of charcoal, yield 60%; mp > 350 °C (decomp.) (Found: C, 44.73; H, 4.64; N, 23.21, $C_9H_9ClN_4O{\cdot}H_2O$ requires: C, 44.57; H, 4.57; N, 23.10%). δ_H (200 MHz, DMSO-d_6) 11.57 (1H, s, broad), 9.46 (2H, d, *J* 6.96 Hz), 8.80 (1H, m), 8.29 (2H, m), 7.60 (2H, s, broad), 6.34 (1H, s); ν_{max}/cm^{-1} 3352, 2926, 1684, 1643, 1228.

1-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-yl)-3-methylimidazolium chloride (9)

0.49 g (3 mmol) of 2-amino-6-chloro-4-hydroxypyrimidine hydrate **6** and 2.29 ml (30 mmol) of *N*-methylimidazole in 30 mL of *o*-dichlorobenzene were heated at reflux temperature over a period of 1.5 hours. After cooling, the precipitate was filtered off, washed with ethyl acetate and recrystallized from ethanol–water in the presence of charcoal, yield 70%; mp 288–289 °C (decomp.) (Found: C, 40.87; H, 4.54; N, 29.09; Cl, 15.45. $C_8H_{10}ClN_5O{\cdot}0.5\ H_2O$ requires: C, 40.63; H, 4.69; N, 29.61; Cl, 15.00%). δ_H (D_2O) 7.94 (1H, d, *J* 2.44), 7.54 (1H, d, *J* 2.16), 5.98 (1H, s), 3.93 (3H, s); δ_C (D_2O) 34.8, 87.4, 117.3, 123.1, 152.7, 154.2, 164.9; ν_{max}/cm^{-1} 3361, 2919, 1666, 1637, 1207.

1-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-yl)-4-(pyrrolidin-1-yl)pyridinium chloride (10)

0.49 g (3 mmol) of 2-amino-6-chloro-4-hydroxypyrimidine hydrate **6** and 0.44 g (3 mmol) of 4-(pyrrolidin-1-yl)pyridine in 50 mL of *o*-dichlorobenzene were heated at reflux temperature over a period of 30 minutes. After cooling, the precipitate was filtered off, washed with ethyl acetate and recrystallized from ethanol–water in the presence of charcoal, yield 100%; mp 293–294 °C (decomp.) (Found: C, 53.89; H, 6.34; N, 23.07. $C_{13}H_{16}ClN_5O$ requires C, 53.16; H, 5.45, N, 23.85%). δ_H (D_2O) 8.37 (2H, d, *J* 8.08), 6.72 (2H, d, *J* 7.94), 5.82 (1H, s), 3.51 (4H, m), 2.01 (4H, m); δ_C (D_2O) 23.0, 47.6, 87.1, 106.4, 135.0, 153.8, 157.0, 165.0; ν_{max}/cm^{-1} 3426, 3113, 1636, 1213, 1157.

General procedure for the preparation of the cross-conjugated mesomeric betaines 11–14

150 mL of the anion exchange resin Amberlite IRA-93 were placed in a column and washed with 2 L of water. Then, 150 mL of an 8% aqueous sodium hydroxide solution were added and left in the column for 45 min. The sodium hydroxide was then rinsed out with water to pH 7. The samples of the chlorides **7**–**10** were dissolved in water. Then, the solutions were passed through the column and eluted with water. The flow rate was adjusted to one drop per second. Then the eluates were evaporated *in vacuo* to dryness. In all cases the yields were nearly quantitative. Details are given below.

2-Amino-6-(4-dimethylaminopyridinio)pyrimidin-4-olate (11)

A sample of 267 mg (1.0 mmol) of **7** was dissolved in 30 mL of water and passed through the ion exchange resin. Evaporation of the eluate to dryness gave **11**, yield: 98%; decomp. 285–287 °C (Found: C, 47.28; H, 6.30; N, 24.78. $C_{11}H_{13}N_5O{\cdot}2.5\ H_2O$ requires: C, 47.83; H, 6.56; N, 25.35%). δ_H (DMSO-d_6) 8.79 (2H, d, *J* 8.08), 7.28 (2H, s, broad), 7.12 (2H, d, *J* 8.2), 5.87 (1H, s), 3.28 (6H, s); ν_{max}/cm^{-1} 3382, 3200, 1651, 1210, 1128.

2-Amino-6-pyridiniopyrimidin-4-olate (12)

A sample of 224 mg (1.0 mmol) of **8** was dissolved in 200 mL of water and was passed through the ion exchange resin. Evaporation of the eluate to dryness gave **12**, yield: 85%; decomp.

> 350 °C (Found: C, 57.33; H, 4.03; N, 29.54. $C_9H_8N_4O$ requires: C, 57.44; H, 4.25; N, 29.87%). δ_H (DMSO-d_6) 9.47 (2H, d, *J* 6.96), 8.80 (1H, m), 8.29 (2H, m), 7.05 (2H, s, broad), 6.11 (1H, s); ν_{max}/cm^{-1}: 3393, 3117, 1700, 1617, 1189.

2-Amino-6-(3-methylimidazolio)pyrimidin-4-olate (13)

A sample of 227 mg (1.0 mmol) of **9** was dissolved in 30 mL of water and passed through the ion exchange resin. Evaporation of the eluate to dryness gave **13**, yield: 90%; mp 257–259 °C (Found: C, 41.97; H, 4.81; N, 30.34. $C_8H_9N_5O\cdot 2\ H_2O$ requires: C, 42.30; H, 5.76; N, 30.82%). δ_H (D_2O) 7.86 (2H, d, *J* 2.16), 7.47 (2H, d, *J* 2.16), 5.84 (1H, s), 3.89 (3H, s); δ_C (D_2O) 34.6, 87.5, 117.2, 122.7, 132.9, 133.3, 152.1, 158.9, 171.2; ν_{max}/cm^{-1} 3402, 3200, 1610, 1107, 1043.

2-Amino-6-[4-(pyrrolidin-1-yl)pyridinio]pyrimidin-4-olate (14)

A sample of 293 mg (1.0 mmol) of **10** was dissolved in 30 mL of water and passed through the ion exchange resin. Evaporation of the eluate to dryness gave **14**, yield: 98%; mp 293–294 °C (decomp.) (Found: C, 49.05; H, 6.81; N, 27.47. $C_{13}H_{15}N_5O\cdot 3.5\ H_2O$ requires: C, 48.47; H, 6.92; N, 27.22%). δ_H (D_2O) 8.25 (2H, d, *J* 7.96), 6.63 (2H, d, *J* 7.96), 5.68 (1H, s), 3.45 (4H, m), 1.99 (4H, m); δ_C (D_2O) 23.0, 47.3, 87.8, 106.2, 135.2, 152.8, 156.7, 160.2, 173.4; ν_{max}/cm^{-1} 3425, 3100, 1647, 1206, 1120.

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40 Although we varied the spraying solvent as well as the desolvation gas temperature (140–300 °C), on spraying 1 : 1 : 1 mixtures of d(CpGp), cytidine **16** and the betaines **11**–**14** from 90% aqueous acetonitrile no peaks of 1 : 1 : 1 adducts [**17** + **16** + (**11**–**14**)] were detectable under analogous reaction conditions. Instead, very intense peaks of **11**–**14** + H^+ (80%), (**11**–**14**) = **16** (100%), and **17** = (**11**–**14**) (10%) were found (140 °C drying gas temperature).
41 Analogous results were obtained with **12**–**14**: *m*/*z* = 1688.8 amu (**17** + **15** + **12** + H^+), 1692.3 amu (**17** + **15** + **13** + H^+), and 1756.2 amu (**17** + **15** + **14** + H^+), respectively, were detected in addition to the peaks of **15** = (**11**–**14**) + H^+ **17**≡**16** **17**≡(**11**–**14**) + H^+, and $\mathbf{17}_2$ as described before.