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SYNTHESIS, TAUTOMERISM AND CALCULATIONS OF MESOMERIC BETAINES OF GUANINE

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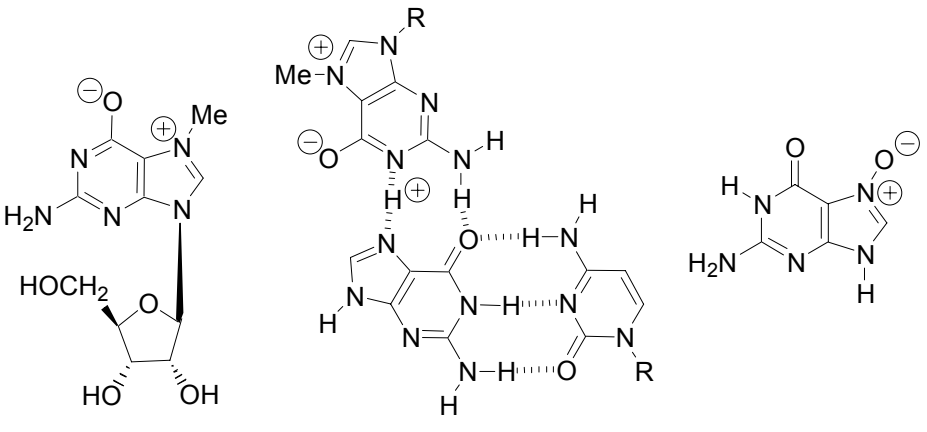
Abstract - Reaction of purine-*N*-oxide (**4**) with 4-(dimethylamino)pyridine and acetyl chloride, followed by the treatment with hydrochloric acid gave the purine-pyridinium salt (**6**) which was deprotonated to the mesomeric betaine (**7**). Depending on the reaction conditions, 4-methylpyridine and pyridine, respectively, converted the nucleoside (**8**) into the pyridinium salts (**9**) and (**10**), or into the mesomeric betaines (**11**) and (**12**). According to calculations, the conjugated tautomers (**A-D**) of betaine (**7**) are more stable than the cross-conjugated tautomer (**7E**).

Base-mispairings of nucleobases due to the formation of tautomers are of interest since the discovery of the *Watson-Crick* base-pairs in DNA.[1] Their role in mutations, cancer development and other diseases has been discussed intensively.[2] An additional impetus was the discovery of the posttranscriptionally modified, mutagenic and self-complementary nucleoside 7-methylguanosine (**1**), isolated from distinct types of RNA (*r*-RNA, archaea, bacterial, and eucaryotic *t*-RNA[3]) and identified as 5′-terminal cap structure of *messenger*-RNA.[4] In *t*-RNAs, 7-methylguanosine (**1**) forms nonstandard base-triplets and base-mispairs. Examples are $m^7G{=}G{\equiv}C$ and $m^7G{=}A$ which stabilize the tertiary structure of the polynucleotide chains.[5] Obviously, on converting this nucleobase into a mesomeric betaine, biologically important horizontal (*Watson-Crick* and *Hoogsteen* base pairing) as well as vertical interactions (π-stacking) change. The cancerostatic, antimicrobial and antiviral guanin-7-oxide (**2**), produced by *Streptomyces* species,[6] is an additional example of a betainic nucleobase.

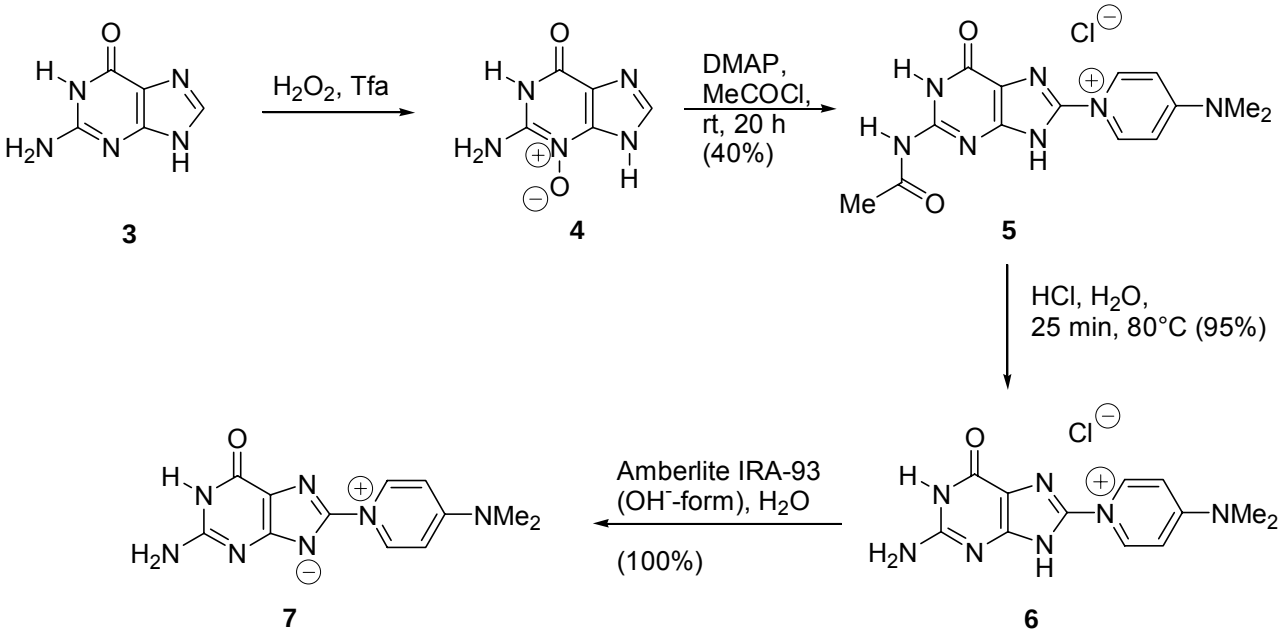


1 **2**

Scheme 1

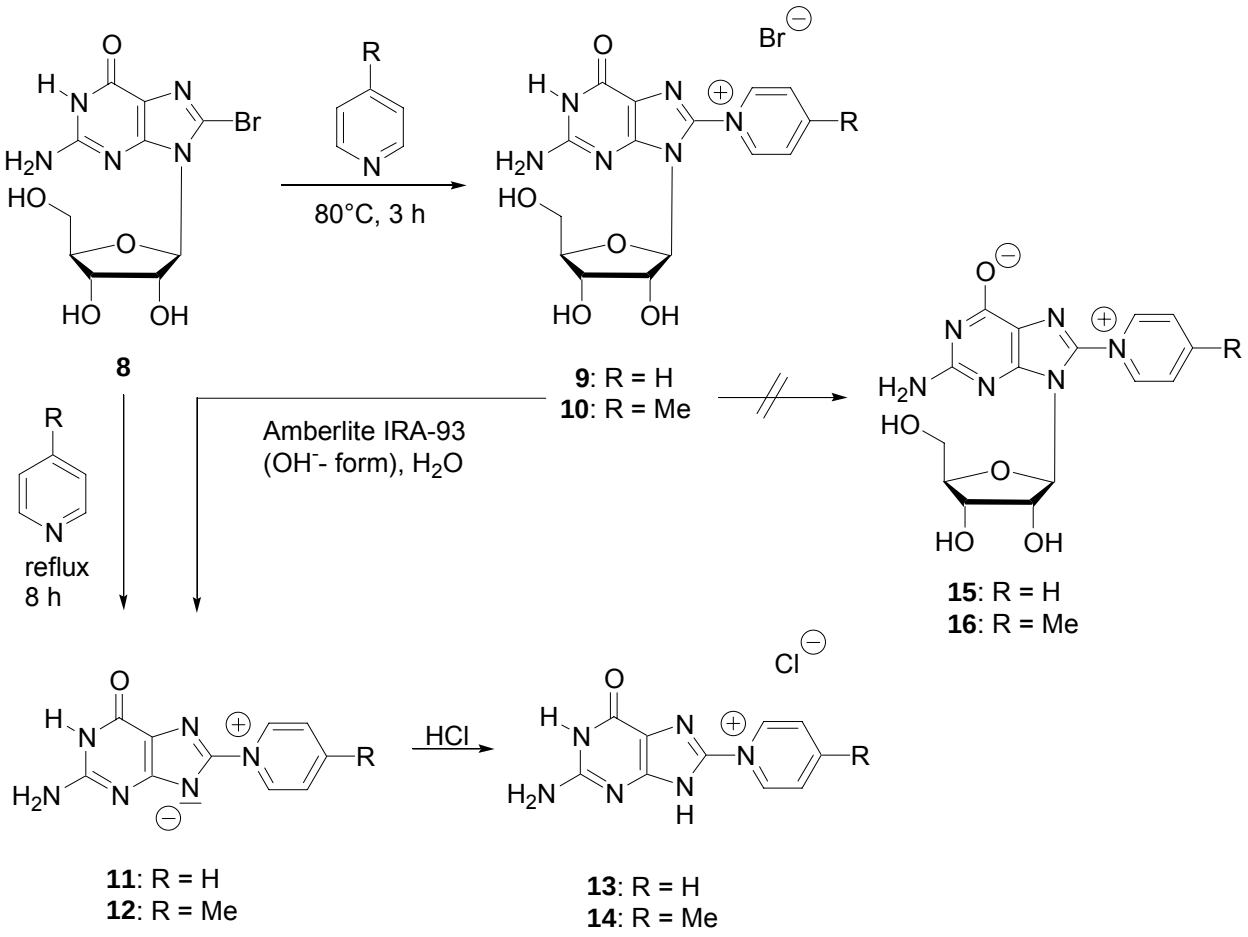
The purines (**1**) and (**2**) belong to the class of conjugated mesomeric betaines (CMB) which contrasts to cross-conjugated (CCMB) and pseudo-cross-conjugated (PCCMB) systems known in heterocyclic chemistry.[7] In continuation of our work on mesomeric betaines and betainic nucleobases[8] we became interested in studying a model compound of 7-methylguanosine which in principle could adopt different tautomers and types of conjugation. We report here the syntheses and the results of semiempirical calculations of such novel betainic purine derivatives.

Guanine (**3**) was oxidized at N-3 by hydrogen peroxide in the presence of trifluoroacetic acid to yield the conjugated heterocyclic *N*-ylide (**4**).[9] Acetylation of **4** with acetyl chloride in the presence of 4-(dimethylamino)pyridine over a period of 20 h at room temperature forms 1-(2-acetylamino-6-oxo-6,9-dihydro-1*H*-purin-8-yl)-4-(dimethylamino)pyridinium chloride (**5**) in satisfactory yield. The chloride **5** was almost quantitatively converted into 1-(2-amino-6-oxo-6,9-dihydro-1*H*-purin-8-yl)-4-(dimethyl-amino)pyridinium chloride (**6**) on treatment with 1N aqueous hydrochloric acid at 80°C. Deprotonation of aqueous solutions of **6** was accomplished by Amberlite IRA-93 in its hydroxy form to give the slightly yellow mesomeric betaine 8-[4-(dimethylamino)pyridinio]-2-aminopurin-6-olate (**7**) in quantitative yield.



Scheme 2

This procedure could not be applied to heteroaromatics that are less basic than 4-(dimethylamino)-pyridine. Pyridine and 4-methylpyridine, however, are able to substitute C(8) of 8-bromoguanosine which is - in contrast to the corresponding guanine - readily available by bromination of guanosine in water (Scheme 3).[10] The reactions afford pure anhydrous solvents and prolonged reaction times. At moderate temperatures, the 1-(guanosin-8-yl)-pyridinium salts (**9**) and (**10**) were formed as intensely red and very sparcely soluble compounds (55 and 25%, respectively). On treatment of aqueous solutions of **9**[11] and **10**[12] with the anion exchange resin Amberlite IRA-93 in its hydroxy form, these cationic systems were quantitatively converted into the orange mesomeric betaines (**11**)[13] and (**12**)[14]; no traces of the betainic guanosines (**15**) and (**16**) were isolated. Reaction of **8** with pyridine and 4-methylpyridine, respectively, at reflux temperature over a period of 8 h gave the mesomeric betaines (**11**) and (**12**) in one step after column chromatography (silica gel, 1. ethyl acetate, 2. MeOH). Treatment of **11** and **12** with 1N hydrochloric acid at room temperature and evaporation gave the yellow colored chlorides (**13**) and (**14**) in 94 and 96% yield, respectively.

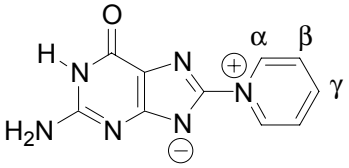


Scheme 3

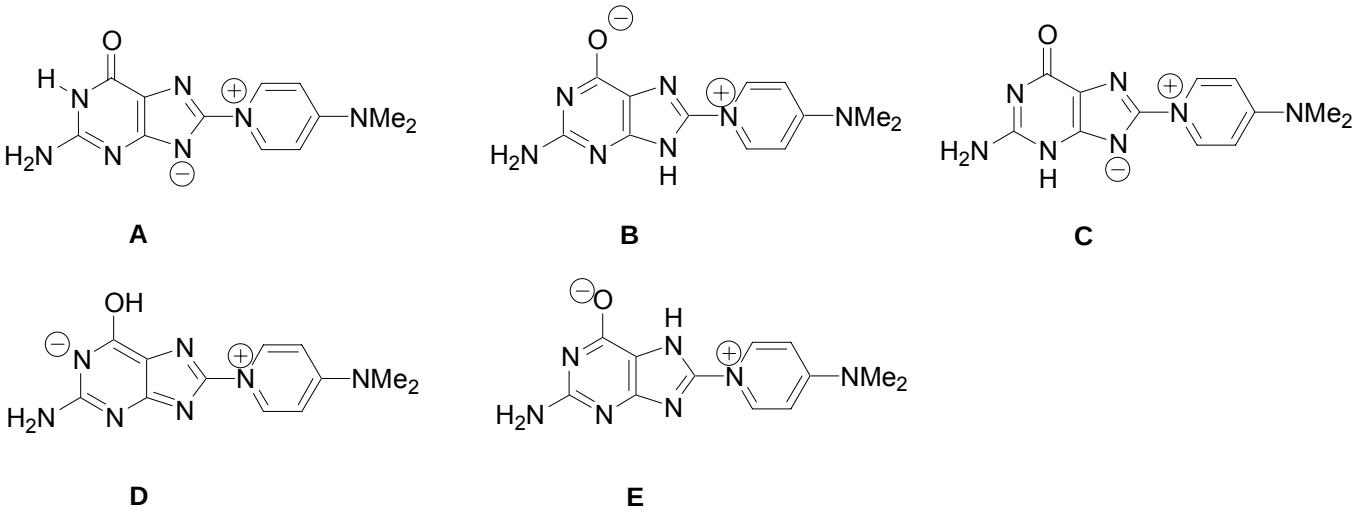
On converting the betaines into the cations all ^{1}H NMR resonance frequencies except for the α-position of the pyridinium rings shift considerably downfield. As a representative example, the chemical shift changes of the pyridinium substituted derivatives (**11**) and (**13**) are given in Table 1.

Table 1. ^{1}H NMR chemical shift changes [ppm] in DMSO-d_6 at rt on conversion of the mesomeric betaine (**11**) into cation (**13**).

	11	**13**	Δδ
NH	10.40	11.82	+1.42
NH_2	6.03	7.91	+1.87
α-H	9.71	9.68	-0.03
β-H	8.10	8.20	+0.10
γ-H	8.48	8.65	+0.17



Although five tautomeric forms (**A-E**) of the mesomeric betaines (**7**, **11**, and **12**) can be formulated (Scheme 4), the ^{1}H NMR spectra in DMSO-d_6 at room temperature display only one tautomer.



Scheme 4

Interestingly, the structures (**A – D**) are conjugated mesomeric betaines, whereas **E** belongs to the class of cross-conjugated mesomeric betaines. Thus, a closer inspection of the canonical formulae reveal common atoms for either positive and negative charge in the CMBs (**A** – **D**), whereas in the CCMB (**E**) the charges are exclusively delocalized in separated parts of the common π-electron system. There are specific associations of various types of dipoles with the types of conjugation in heterocyclic mesomeric betaines.[7] Characteristically for the class of conjugated mesomeric betaines, the dipole (**I**) can be dissected from the canonical formulae of tautomer A, whereas the vinyloge of a characteristic dipole increment of cross-conjugated mesomeric betaines (**II**) can be found in tautomer (**E**). **A** is a CMB isoconjugate to the even nonalternant hydrocarbon 4-methyl-2-phenyl-1*H*-indene dianion (**III**) and thus belongs to class 4, whereas the CCMB (**E**) is a member of class 12 of heterocyclic mesomeric betaines.[7]

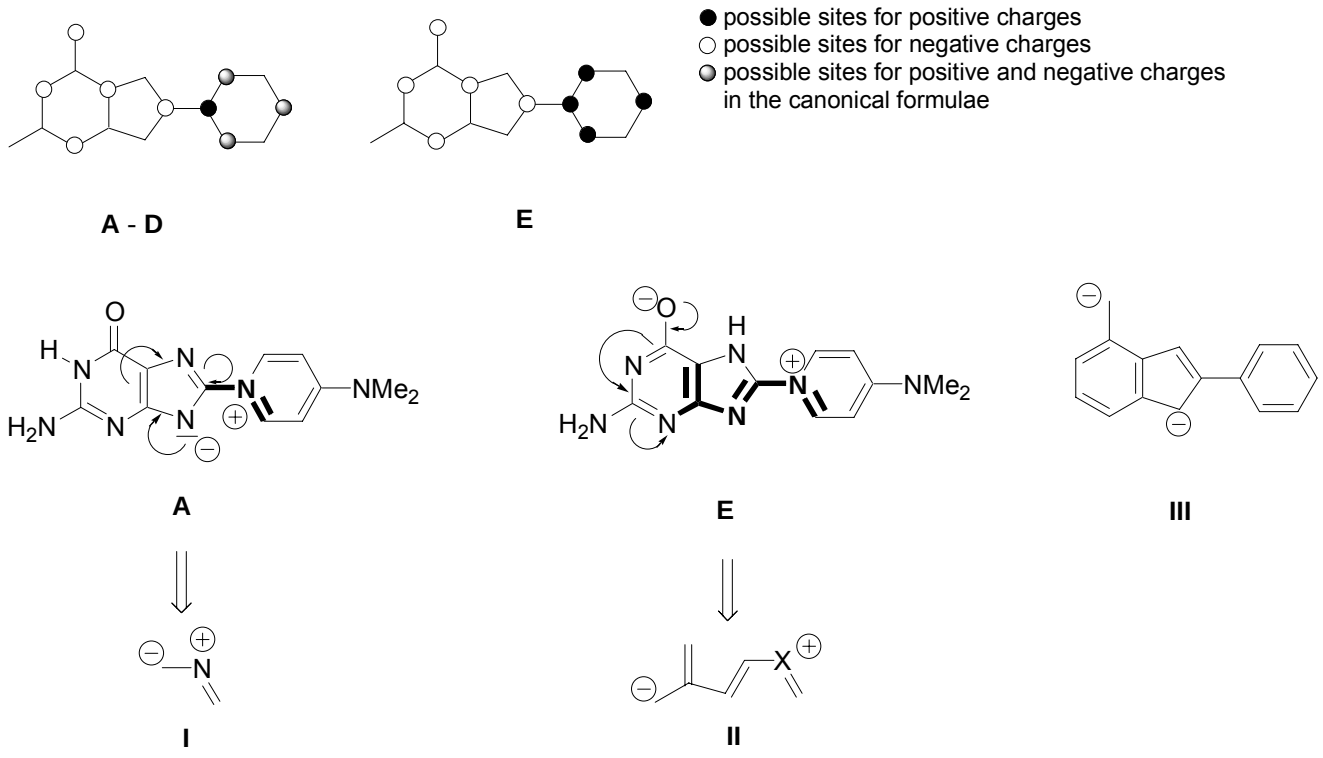
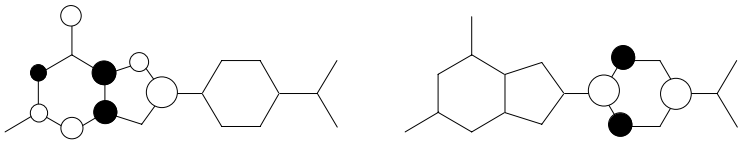


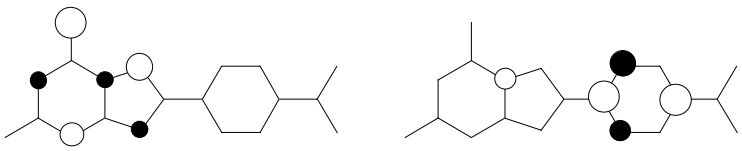
Figure 1

According to a PM3 calculation,[15] **A** is the most stable and **E** the most unstable tautomer which explains the finding that the betaines (**15**) and (**16**) were not formed (Table 2). As to be expected, the calculation leads to essentially planar molecules in either case as the most stable conformers [τ = 0.19°]. The calculated frontier orbital profiles reflect the distinct types of conjugation. The LUMOs of the tautomers (**7A**) and (**7E**) are essentially located at the nitrogen atom and the α- and γ-atoms of the pyridinium rings. In the CCMB (**7E**), however, the positive moiety of the molecule is joined to a nodal position of the HOMO which characteristically is located in the anionic portion of the betaine (Figure 3). As a consequence, C(8) serves as an isolator and the positive and the negative charges are separated in the ground state of the molecule. Correspondingly, the permanent dipole moment of **7E** is the largest of all tautomers (Table 2). In contrast to that, C(8) of **7A** is an active position of the HOMO, so that the charges are in mutual conjugation (Figure 2). Consequently, the permanent dipole moment is considerably smaller.



HOMO (**7A**) LUMO **(7A)**

Figure 2



HOMO (**7E**) LUMO **(7E)**

Figure 3

Table 2. Heats of formation of the tautomers (**7A-E**) according to a PM3 calculation. Permanent dipole moments.

Tautomer	ΔH_f(PM3) [KJ/mol]	Calcd dipole moment μ [D]
7A	249.20	9.86
7B	300.60	13.40
7C	261.28	12.79
7D	268.51	11.64
7E	356.75	20.79

As a conclusion, similar to the biologically important mesomeric betaine 7-methylguanosine m^7G isolated from RNA the model compounds described here adopt tautomers which are conjugated systems. The most stable tautomer is a conjugated mesomeric betaine with the *Watson-Crick* binding site of unmodified guanine. In contrast to this, the most unstable tautomer has the binding site of m^7G, but is a cross-conjugated mesomeric betaine.

ACKNOWLEDGMENTS.

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11. ^{1}H NMR: δ = 10.62 (s, 1H; NH), 9.68 (m, 2H; α-H), 8.48 (m, 1H; γ-H), 8.09 (m, 2H; β-H), 6.08 (s, 2H; NH_2), 5.68 (d, J = 8.0 Hz, 1H; CH), 5.46, (m, 1H; OH), 5.14 (m, 1H; OH), 4.40 (m, 1H; OH), 4.12 (m, 1H; CH), 3.90 (m, 1H; CH), 3.64 (m, 1H; CH), 3.54 (m, 1H; CH) ppm; IR: 3414, 3100, 1688, 1480, 1373, 1234 cm^{-1}; UV (MeOH): λ_{max} = 656, 598, 422 nm.

12. ^{1}H NMR: δ = 11.09 (s, 1H; NH), 9.57 (m, 2H; α-H), 7.94 (m, 2H; β-H), 6.60 (s, 2H; NH_2), 5.68 (d, J = 7.9 Hz, 1H; CH), 5.48 (m, 1H; OH), 5.13 (m, 1H; OH), 4.99 (m, 1H; OH), 4.11 (m, 1H; CH), 3.86 (m, 1H; CH), 3.62 (m, 1H; CH), 3.62 (m, 1H; CH), 3.53 (m, 1H; CH), 2.62 (s, 3H; Me); IR: 3391, 1676, 1465, 1223 cm^{-1}; UV (MeOH): λ_{max} = 656, 576, 412, 248 nm.
13. ^{1}H NMR: δ = 10.40 (s, 1H; NH), 9.71 (m, 2H; α-H), 8.48 (m, 1H; γ-H), 8.10 (m, 2H; β-H), 6.03 (s, 2H; NH_2); ^{13}C NMR: δ = 191.77, 161.57, 158.10, 151.97, 144.36, 138.10, 128.00, 120.53 ppm; IR: 3417, 3132, 1668.0, 1482.5, 1371.3 cm^{-1}; UV (MeOH): λ_{max} = 658, 600, 420 nm.
14. ^{1}H NMR: δ = 10.53 (s, 1H; NH), 9.56 (m, 2H; α-H), 7.93 (m, 2H; β-H), 5.87 (s, 2H; NH_2), 2.62 (s, 3H; Me) ppm; UV (MeOH): λ_{max} = 408, 272, 246 nm.
15. Semiempirical calculations were carried out using MOPAC 6.0[16] on a IBM workstation RS/6000, AIX 4.3 to perform the PM3 calculations. The structures were first optimized with the default gradient requirements and subsequently refined with the options GNORM = 0.01, SCFCRT = 1 x 10^{-10}. The absolute minima were proved by a force calculation.
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