

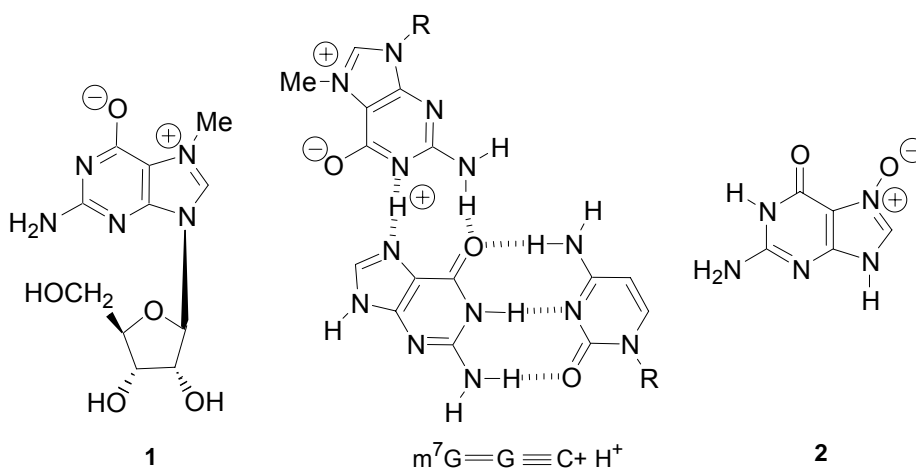
## SYNTHESIS, TAUTOMERISM AND CALCULATIONS OF MESOMERIC BETAINES OF GUANINE

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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**).

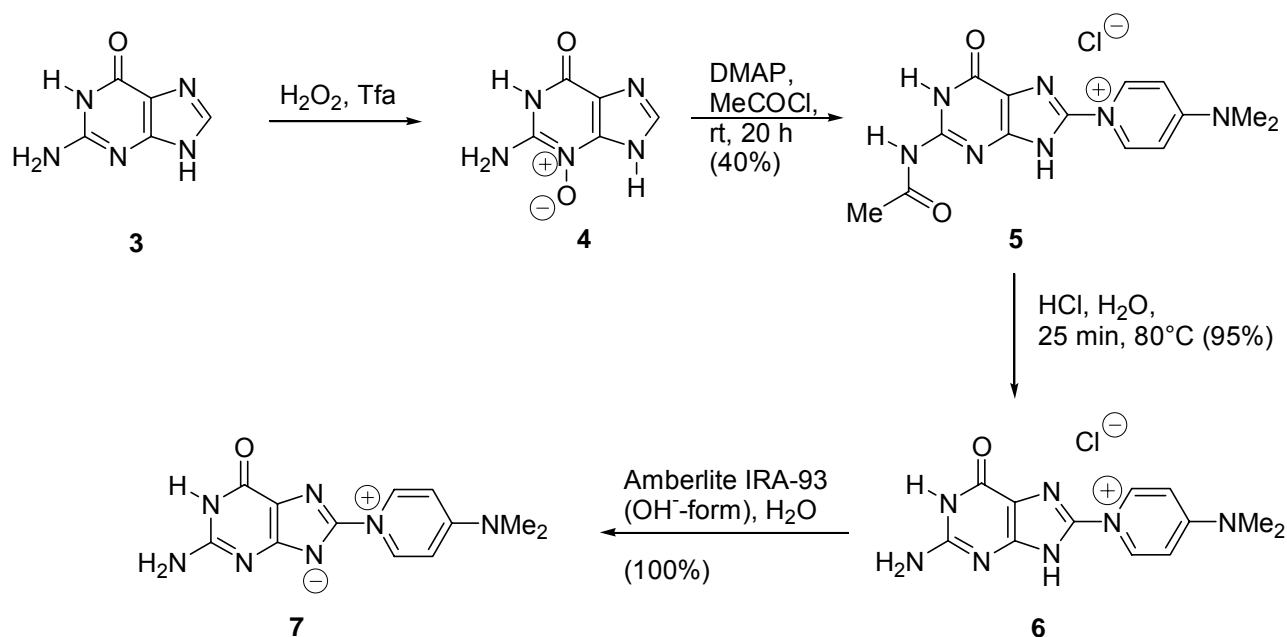
Base-mispairings of nucleobases due to the formation of tautomers are of interest since the discovery of the *Watson-Crick* base-pairs in DNA.<sup>1</sup> Their role in mutations, cancer development and other diseases has been discussed intensively.<sup>2</sup> An additional impetus was the discovery 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<sup>3</sup>) and identified as 5'-terminal cap structure of *messenger*-RNA.<sup>4</sup> 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.<sup>5</sup> Obviously, on converting this nucleobase into a mesomeric betaine, biologically important horizontal (*Watson-Crick* and *Hoogsteen* base pairing) as well as vertical interactions ( $\pi$ -stacking) change. The cancerostatic, antimicrobial and antiviral guanine-7-oxide (**2**), produced by *Streptomyces* species,<sup>6</sup> is an additional example of a betainic nucleobase.



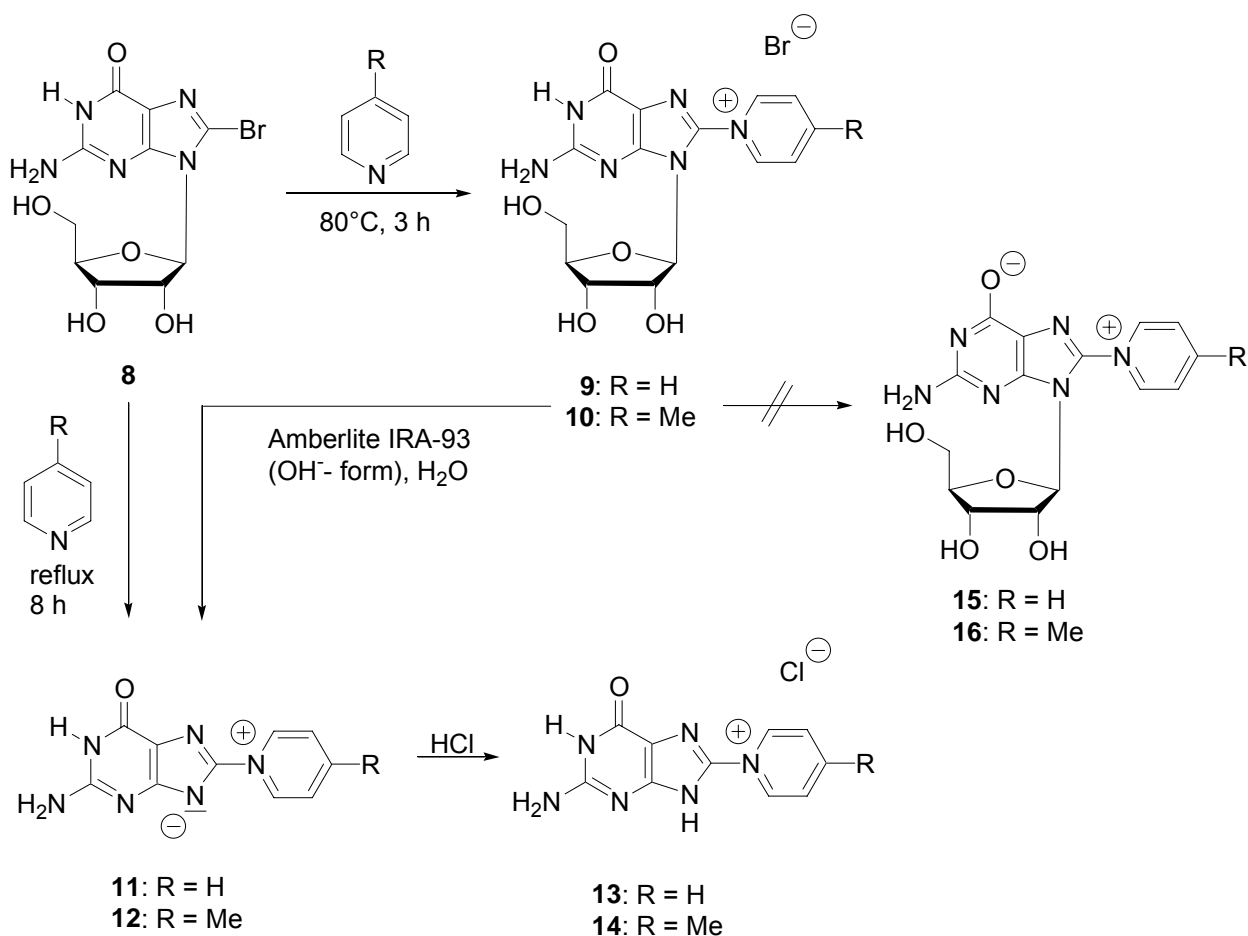
Scheme 1

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.<sup>7</sup> In continuation of our work on mesomeric betaines and betainic nucleobases<sup>8</sup> 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**).<sup>9</sup> 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-acetyl-amino-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-(dimethylamino)pyridinium chloride (**6**) on treatment with 1*N* 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.



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).<sup>10</sup> 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 sparsely soluble compounds (55 and 25%, respectively). On treatment of aqueous solutions of **9**<sup>11</sup> and **10**<sup>12</sup> with the anion exchange resin Amberlite IRA-93 in its hydroxy form, these cationic systems were quantitatively converted into the orange mesomeric betaines (**11**)<sup>13</sup> and (**12**)<sup>14</sup>; 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 1*N* hydrochloric acid at room temperature and evaporation gave the yellow colored chlorides (**13**) and (**14**) in 94 and 96% yield, respectively.

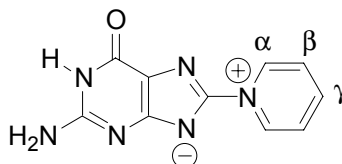


**Scheme 3**

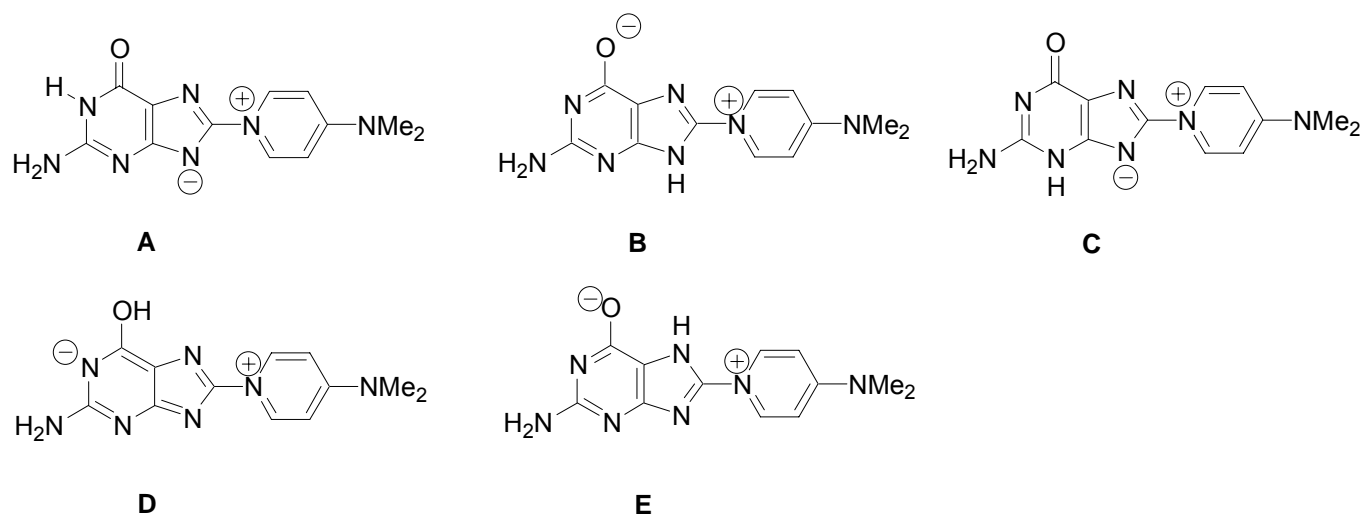
On converting the betaines into the cations all  $^1\text{H}$  NMR resonance frequencies except for the  $\alpha$ -position of the pyridinium rings shift considerably downfield. As a representative example, the chemical shift changes of the pyridinium substituted derivatives (**11**) and (**13**) are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

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

	<b>11</b>	<b>13</b>	$\Delta\delta$
NH	10.40	11.82	+1.42
NH <sub>2</sub>	6.03	7.91	+1.87
$\alpha$ -H	9.71	9.68	-0.03
$\beta$ -H	8.10	8.20	+0.10
$\gamma$ -H	8.48	8.65	+0.17

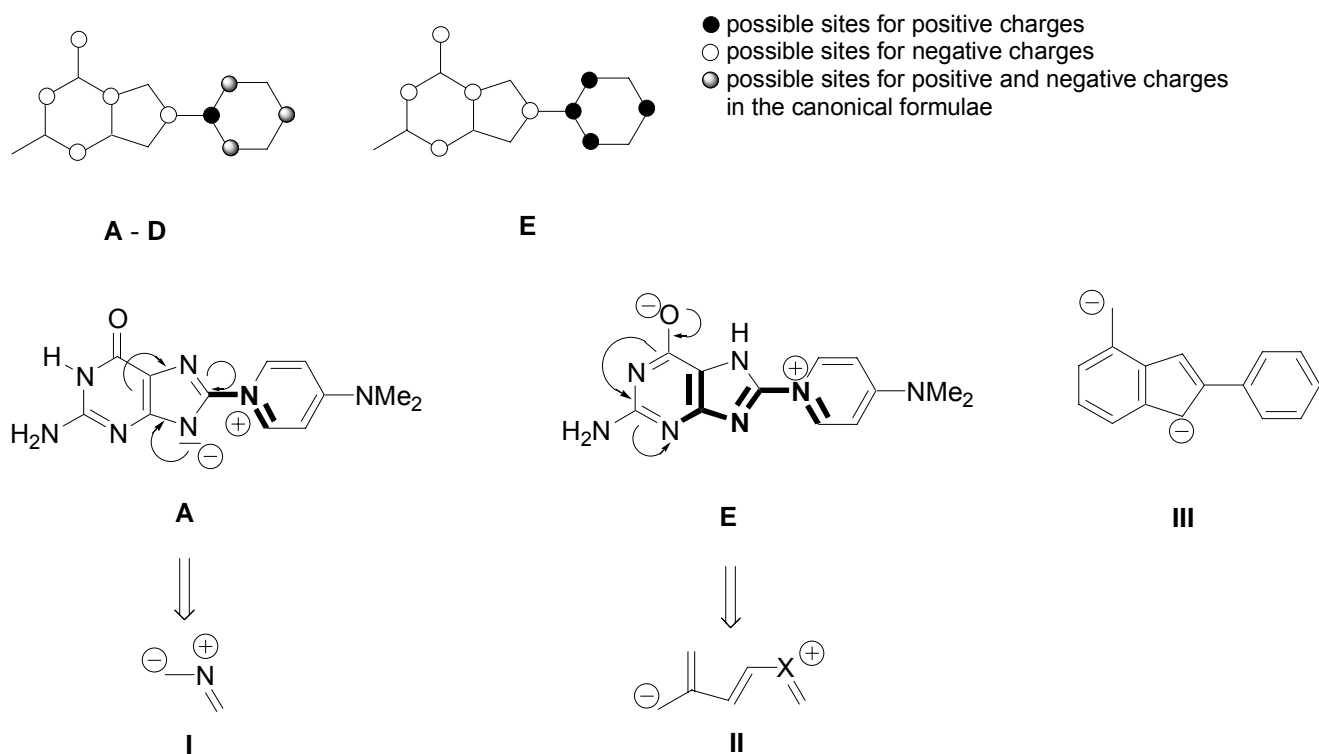


Although five tautomeric forms (**A-E**) of the mesomeric betaines (**7**, **11**, and **12**) can be formulated (Scheme 4), the  $^1\text{H}$  NMR spectra in DMSO- $d_6$  at room temperature display only one tautomer.



**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  $\pi$ -electron system. There are specific associations of various types of dipoles with the types of conjugation in heterocyclic mesomeric betaines.<sup>7</sup> Characteristically for the class of conjugated mesomeric betaines, the dipole (**I**) can be dissected from the canonical formulae of tautomer **A**, whereas the vinylog 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.<sup>7</sup>



**Figure 1**

According to a PM3 calculation,<sup>15</sup> **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 [ $\tau = 0.19^\circ$ ]. 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  $\alpha$ - and  $\gamma$ -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.

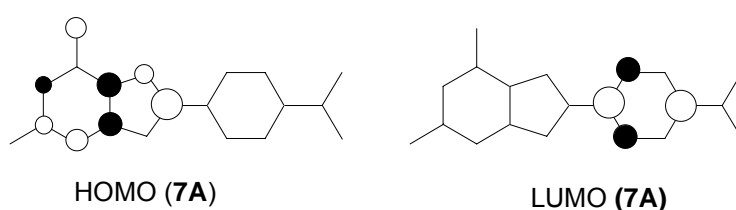


Figure 2

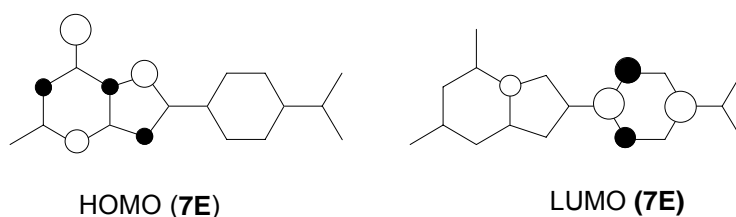


Figure 3

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

Tautomer	$\Delta H_f(\text{PM3})$ [KJ/mol]	Calcd dipole moment $\mu$ [D]
<b>7A</b>	249.20	9.86
<b>7B</b>	300.60	13.40
<b>7C</b>	261.28	12.79
<b>7D</b>	268.51	11.64
<b>7E</b>	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. <sup>1</sup>H NMR:  $\delta$  = 10.62 (s, 1H; NH), 9.68 (m, 2H;  $\alpha$ -H), 8.48 (m, 1H;  $\gamma$ -H), 8.09 (m, 2H;  $\beta$ -H), 6.08 (s, 2H; NH<sub>2</sub>), 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<sup>-1</sup>; UV (MeOH):  $\lambda_{\max}$  = 656, 598, 422 nm.

12.  $^1\text{H}$  NMR:  $\delta = 11.09$  (s, 1H; NH), 9.57 (m, 2H;  $\alpha$ -H), 7.94 (m, 2H;  $\beta$ -H), 6.60 (s, 2H;  $\text{NH}_2$ ), 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  $\text{cm}^{-1}$ ; UV (MeOH):  $\lambda_{\text{max}} = 656, 576, 412, 248$  nm.
13.  $^1\text{H}$  NMR:  $\delta = 10.40$  (s, 1H; NH), 9.71 (m, 2H;  $\alpha$ -H), 8.48 (m, 1H;  $\gamma$ -H), 8.10 (m, 2H;  $\beta$ -H), 6.03 (s, 2H;  $\text{NH}_2$ );  $^{13}\text{C}$  NMR:  $\delta = 191.77, 161.57, 158.10, 151.97, 144.36, 138.10, 128.00, 120.53$  ppm; IR: 3417, 3132, 1668.0, 1482.5, 1371.3  $\text{cm}^{-1}$ ; UV (MeOH):  $\lambda_{\text{max}} = 658, 600, 420$  nm.
14.  $^1\text{H}$  NMR:  $\delta = 10.53$  (s, 1H; NH), 9.56 (m, 2H;  $\alpha$ -H), 7.93 (m, 2H;  $\beta$ -H), 5.87 (s, 2H;  $\text{NH}_2$ ), 2.62 (s, 3H; Me) ppm; UV (MeOH):  $\lambda_{\text{max}} = 408, 272, 246$  nm.
15. Semiempirical calculations were carried out using MOPAC 6.0<sup>16</sup> 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 =  $1 \times 10^{-10}$ . The absolute minima were proved by a force calculation.
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