A particularly instructive example is the thermolysis of (*Z*)-1,3,8-nonatriene [(*Z*)-**10**],[21] in which an intramolecular Diels–Alder reaction leading to *cis*-**12** competes with a sigmatropic [1,5]-hydrogen shift leading to (1,5*E*,7*Z*)-nonatriene (**13**, Scheme 3). The use of high pressure here enables a reversal of the selectivity. At 150 °C and 1 bar the 1,5-hydrogen shift is preferred to the monocyclic transition state **14**‡. At 7.7 bar, on the other hand, the intramolecular Diels–Alder reaction is preferred to the bicyclic transition state **15**‡.

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[14] The investigation of the pressure effect in the rearrangement of 2,5-diphenylhexa-1,5-diene, for which a 1,4-diphenylcyclohexa-1,4-diyl intermediate is assumed [8], is under way.

[15] a) The following Arhenius equations were calculated from the temperature dependence of the rate constants k, measured in hexane at five temperatures between 133 and 174 °C: $k(meso\text{-}\mathbf{3} \rightarrow (E,Z)\text{-}\mathbf{6}) = 4.68 \times 10^{10}\ s^{-1} \exp[-(28.54 \pm 0.33)\ kcal\,mol^{-1}/RT^{-1}]$, from which the following parameters were derived (153.2 °C): $\Delta H^{\ddagger} = (27.69 \pm 0.33)\ kcal\,mol^{-1}$; $\Delta S^{\ddagger} = -(12.43 \pm 0.71)\ cal\,mol^{-1}\,K^{-1}$. $k(meso\text{-}\mathbf{3} \rightarrow (E,E)\text{-}\mathbf{6}) = 1.58 \times 10^{11}\ s^{-1} \exp[-(29.85 \pm 0.30)\ kcal\,mol^{-1}/RT]$, from which the following parameters were derived (153.2 °C): $\Delta H^{\ddagger} = (29.01 \pm 0.30)\ kcal\,mol^{-1}$; $\Delta S^{\ddagger} = -(9.99 \pm 0.70)\ cal\,mol^{-1}\,K^{-1}$. The activation enthalpies of the rearrangements of *meso*-**3** to (*E,Z*)-**6** and (*E,E*)-**6** are, respectively, 3.7 and 5.0 kcal mol^{-1} larger than those of the Cope rearrangement of *rac*-**3** to (*E,E*)-**6** ($\Delta H^{\ddagger} = 24.0\ kcal\,mol^{-1}$; $\Delta S^{\ddagger} = -12.4\ cal\,mol^{-1}\,K^{-1}$) [12], a fact that is certainly due to steric effects in the *cis*-configured transition state. From the activation enthalpies and the enthalpies of formation calculated with the force field MM2ERW for *meso*-**3** and *rac*-**3** ($\Delta H_f = 73.8$ and 73.5 kcal mol^{-1} respectively)[8], the enthalpies of formation of the transition states for the reactions *meso*-**3** → (*E,Z*)-**6** ($\Delta H_f^{\circ} = 101.5$ kcal mol^{-1}), *meso*-**3** → (*E,E*)-**6** ($\Delta H_f^{\circ} = 102.8\ kcal\,mol^{-1}$) and *rac*-**3** → (*E,E*)-**6** ($\Delta H_f = 97.5\ kcal\,mol^{-1}$) could be calculated. The enthalpies of formation thus obtained for the transition states are, without exception, 12 to 17 kcal mol^{-1} lower than the enthalpies of formation, also calculated with the MM2ERW force field, for the potential diradical intermediates *cis*- and *trans*-2,3-diphenylcyclohexa-1,4-diyl (*cis*-chair: $\Delta H_f^{\circ} = 118.2\ kcal\,mol^{-1}$; twisted *cis*-boat (twist-boat [8]): $\Delta H_f^{\circ} = 120.1\ kcal\,mol^{-1}$; *trans*-chair: $\Delta H_f^{\circ} = 113.8\ kcal\,mol^{-1}$) or those of the free radicals resulting from the homolysis of the C-3–C-4 bond (2 × 1-phenylallyl: $\Delta H_f^{\circ} = 2 \times 57.2 = 114.4\ kcal\,mol^{-1}$) [8]. It is, therefore, unlikely that a diradical rearrangement *meso*-**3** → (*E,E*)-**6** occurs as proposed by one of the referees. b) W. von E. Doering, W. R. Roth, R. Breuckmann, L. Figge, H.-W. Lennartz, W.-D. Fessner, H. Prinzbach, *Chem. Ber.* **1988**, *121*, 1–9; c) W. R. Roth, O. Adamczak, R. Breuckmann, H.-W. Lennartz, R. Boese, *ibid.* **1991**, *124*, 2499–2521. We thank Prof. Roth for providing us with a copy of the MM2ERW force field program.

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[20] From the activation enthalpies of the reactions (*E*)-**10** → *cis*-**12** and (*E*)-**10** → *trans*-**12** as well as the enthalpy of formation calculated with the force field MM2ERW [15b, c] for (*E*)-**10** ($\Delta H_f^{\circ} = 25.9\ kcal\,mol^{-1}$), enthalpies of formation for the transition states were calculated: $\Delta H_f^{\circ} = 51.7$ and 53.0 kcal mol^{-1} respectively. Comparison with the enthalpy of formation of the energetically favored, potential diradical intermediate cyclonon-2-ene-1,6-diyl ($\Delta H_f^{\circ} = 67.6\ kcal\,mol^{-1}$) also calculated with the MM2ERW force field yielded values of about −16 and −15 kcal mol^{-1} for the transition state resonance energy (TS-RE, energy of concert); thus the two intramolecular Diels–Alder reactions certainly take place by pericyclic processes.

[21] From a mixture of (*Z*)- and (*E*)-**10**, (about 1:1) prepared in a Wittig reaction of (2-propenylidene)triphenylphosphorane [22] and 5-hexenal [23], isomerically pure (*Z*)-**10** was obtained by allowing the (*E*)-**10** to react with maleic anhydride in a selective Diels–Alder reaction and separating the unconverted (*Z*)-**10** from the adduct by column chromatography.

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Polymorphism and C=N–N=C Conformational Isomers of Azines: X-ray Crystal and Ab Initio Structures of Two Rotational Isomers of Methyl (*para*-Tolyl) Ketone Azine**

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Azines are important substrates for crisscross additions and their organometallic variants,[2, 3] they react as the "ene" component in [3+2] additions,[4] and they are becoming increasingly important for C–C bond forming reactions.[5] Azines also are receiving increasing attention for their biological,[6] chemical,[7] and physical properties.[8] The characterization of the electronic structure of azines is fundamental to the understanding and the advancement of their chemistry. We have been studying the stereochemistry and stereoelectronics of azines in a systematic fashion,[1] and we report here on the rotamers of methyl (*para*-tolyl) ketone azine, **I**.

Compound **I** was prepared from methyl (*para*-tolyl) ketone and hydrazine hydrate in acidic ethanol.[9] Yellow single

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crystals[10] suitable for X-ray crystallography were formed from a solution of **I** in methylene chloride by vapor diffusion of *n*-hexane. **I** cocrystallizes in two monoclinic racemic modifications,[11] space groups $P2_1/n$ and $P2_1/c$. The two independent molecules **A** and **B** are shown in Figure 1. Both assume the (*E*,*E*) configuration in which the large groups attached to the C=N bonds are *trans*. In general, the configurations of azines are governed by steric factors and result in "*trans*-*trans* structures" which, depending on the priorities of the attached groups, are (*E*,*E*)[12] or (*Z*,*Z*) configured.[1a, 13] The stereochemistry of the azines with regard to the N–N single bond is the focus of the present study.

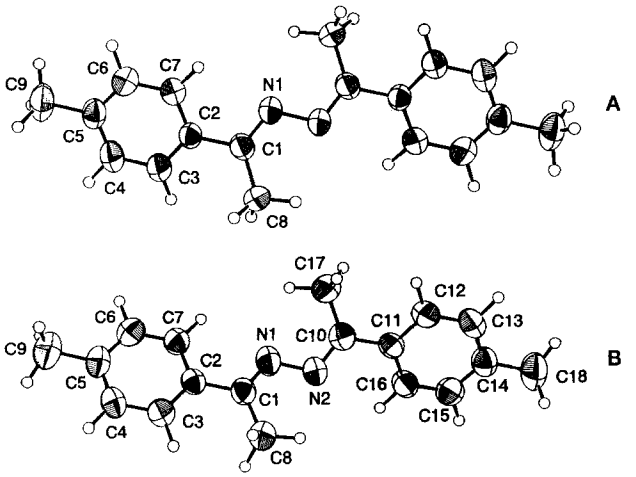
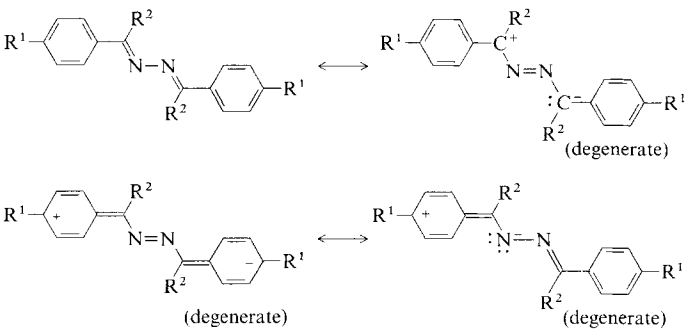


Fig. 1. ORTEPII drawings of the azines **A** (top) and **B** (bottom) with the numbering scheme.

The crystal structures of azines reported previously all show either N–N s-*trans* or *gauche* conformations, and only two cases are known in which *both* conformations occur in polymorphous crystals.[14] In the case of **I** molecule **A** exhibits a *trans* conformation with a C-N-N-C torsion angle τ of 180°, while **B** shows a *gauche* conformation with $\tau = 142.8°$. Similarly, for the azine of 3-acetyl-4-(2-chlorophenyl)-4-hydroxy-2-methoxycrotonic acid lactone, **II**,[15] the *trans* and *gauche* conformers crystallize in different modifications. Our study is of particular significance, as **I** contains phenyl substituents and allows for the first experimental examination of conjugation effects in azines as a function of the N–N conformation. The importance of such solid-state studies has been emphasized by the recent studies by the research groups led by Brock, Grützmacher, and Gompper.[16]

Molecule **A** has a center of inversion, and the phenyl rings are twisted out of the best molecular plane by $\theta_1 = \theta_2 = 23.7°$ (see Table 1 for a definition of the angles). These phenyl conformations would allow for only attenuated conjugation with the azine fragment even though the N–N s-*trans* conformation is best suited for conjugation through the entire azine system (Scheme 1). The C–N bond lengths are 1.279 Å, similar to that in formaldoxime (1.276 Å).[17] The N–N bond lengths of 1.405 Å in **A** are shorter than the generally accepted N–N single bond length of 1.47 Å,[18] but this shortening is expected for N–N bonds between sp^2-hybridized N atoms and cannot be interpreted as an indication of conjugation. Such hybridization effects are also found for the C–C bonds between the azine C and the aromatic C atom; the bond lengths of 1.482 Å agree with the normal $C_{sp^2}-C_{sp^2}$ single bond length of 1.48 Å.[19]



Scheme 1. Is conjugation with the azine fragment (top) or within the Ar-C-N fragment (bottom) important? Not all resonance forms are shown, and only one form is shown for degenerate structures $R^1 = R^2 = CH_3$.

Thus, neither the C–N and N–N bonds nor the $C_{ipso}-C$ bonds show clear structural indications of phenyl conjugation. This result supports the conclusion we made previously by comparative analysis of related phenyl-substituted azines.[1a, 20]

The N–N and C–N bonds in **A** and **B** differ by no more than 0.002 Å, which leads us to the important conclusion that they are essentially independent of the conformation. Thus, conjugation within the azine functional group cannot be significant for **I**.

In **B** one phenyl ring is twisted only slightly ($\theta_1 = 0.5°$), while the torsion angle of the other is more significant ($\theta_2 = 19.9°$). Structure **B** gives us the unique opportunity of examining the possibility of partial conjugation over the fragment Ar–C–N (Scheme 1, bottom) with experimental data. Surprisingly, the C–N bond in the fragment with the N-C-C_{ipso}-C_{ortho} dihedral angle of 0.5° is 0.005 Å *shorter* than that in the fragment with the more marked torsion angle (19.9°). Moreover, the C–C_{ipso} bond in the first fragment is 0.012 Å *longer* than the analogous bond in the second. The latter C–C_{ipso} bond is virtually identical to those in **A**. These structural data provide strong evidence against significant conjugative interactions in the fragment Ar–C–N. We have shown for Ph(HOOC)C=N–N=CH_2[1a] that the barrier to rotation about the Ph–C bond is less than 5 kcal mol^{-1}, and the present analysis corroborates the conclusions of the theoretical study.

Based on the *gauche* preference of formaldazine, according to CNDO/2 calculations, Ishida et al. concluded that *gauche*-**II** would be thermodynamically preferred over *trans*-**II**. However, higher level ab initio studies of the N–N rotational profile of formaldazine[21] show that the *gauche*-**II** structure is over 2 kcal mol^{-1} less stable than *trans*-**II**. Hence, Ishida's conclusion requires revision. We have investigated the energetic preferences of **I** with all-electron ab initio calculations at the RHF/6-31G* level.[22] We completely optimized **I** (C_1) at the RHF/3-21G level, and the de facto D_{2h}-symmetric N–N s-*trans* structure **1** resulted (Fig. 2 and Table 1). Since no *gauche* conformer exists on the potential energy surface, a model for the *gauche* structure, **2**, was obtained by optimizing **I** with the single constraint of $\tau = 142.8$ (τ angle in **B**). The s-*trans* structure **1** is

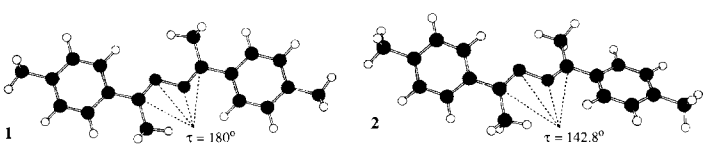


Fig. 2. RHF/3-21G-optimized structures of methyl (*para*-methylphenyl) ketone azine **I**. The planar N–N s-*trans* structure **1** is the minimum; the *gauche* structure **2** was obtained with the constraint C-N-N-C = 142.8°.

Table 1. Selected bond lengths and angles of **A**, **B** [a], **1**, and **2** [b].

Parameter [c, d]	A	B	1	2
N1-N2	1.405(5)	1.407(3)	1.431	1.430
N1-C1	1.279(4)	1.277(4)	1.268	1.267
C1-C2	1.482(4)	1.493(4)	1.491	1.490
C1-C8	1.493(5)	1.494(4)	1.511	1.511
C5-C9	1.509(5)	1.511(4)	1.516	1.516
N2-C10	[e]	1.282(4)	[e]	[e]
C10-C11	[e]	1.481(4)	[e]	[e]
C10-C17	[e]	1.496(4)	[e]	[e]
C14-C18	[e]	1.520(4)	[e]	[e]
N1-N2-C10	[e]	114.17(24)	[e]	[e]
N1-C1-C2	116.0(3)	116.1(3)	117.1	117.2
N1-C1-C8	124.9(3)	123.9(3)	125.3	124.9
N2-N1-C1	114.1(3)	114.97(24)	115.7	116.5
N2-C10-C11	[e]	116.6(3)	[e]	[e]
N2-C10-C17	[e]	123.5(3)	[e]	[e]
C1-N1-N2-C10 = τ	180.0	142.8(3)	180.0	142.8
N2-N1-C1-C8 = ϕ_1	0.5	2.5(2)	0.0	2.2
N1-C1-C2-C7 = θ_1	23.7(2)	0.5(2)	0.0	9.7
N1-N2-C10-C17 = ϕ_2	[e]	3.6(2)	[e]	[e]
N2-C10-C11-C16 = θ_2	[e]	19.9(2)	[e]	[e]
E/E_{rel}(RHF/3-21G): **1**	−797.673044/0.00		**2**: −797.670982/1.29	
E/E_{rel}(RHF/6-31G*): **1**	−802.148751/0.00		**2**: −802.148182/0.36	

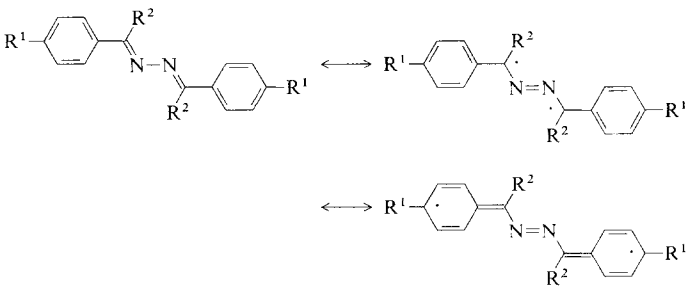
[a] Lengths in Å and angles in degrees. The standard deviations given in parentheses refer to the last digit(s). [b] Structures **1** (optimized in C_1 symmetry, de facto D_{2h} symmetry) and **2** (optimized in C_1 symmetry with C-N-N-C fixed to 142.8°) calculated at the RHF/3-21G level. [c] For atom numbering see Fig. 1. [d] Total and relative energies in Hartrees and kcal mol^{-1}, respectively. Vibrational zero-point energy for the minimum **1** is 227.21 kcal mol^{-1} (RHF/3-21G). [e] Symmetry equivalent to another structural parameter.

preferred over structure **2** by 0.36 kcal mol^{-1} at the RHF/6-31G*//RHF/3-21G level. This theoretical result demonstrates in a compelling fashion that the activation energy required for τ variations between the N–N s-*trans* and *gauche* structures is very small. Hence, small differences in packing interactions in polymorphous crystals suffice to overcome the intrinsic *trans* preference, and solid-state structures with *gauche* molecules also are realized.

Molecule **1** is inversion symmetric and planar, and **2** is C_2-symmetric with azine-phenyl and azine-methyl group twists of 9.7° and 2.2°, respectively. The N–N bonds are about 0.025 Å longer than in the solid-state *trans* and *gauche* conformers, while the C–N bonds are shorter by 0.011 Å.[23] The C–C_{ipso} bond lengths in the gas phase are the same as the corresponding bond in the fragment with $\theta_1 = 0.5°$ in **B** and slightly longer than the others. As was found for the solid-state structures, the important conclusion here is that the conformation dependence of the main structural parameters is minor: for **1** and **2**, all bond lengths are almost the same and the angles differ by less than 1°.

The ^{1}H, ^{13}C, and ^{15}N NMR spectra recorded for **I** show the presence of one isomer in solution. Similarly, the two modifications of **II** give the same ^{1}H NMR spectrum. Therefore, either one centrosymmetric isomer exists in solution or *gauche* enantiomers are found in a fast equilibrium. Our ab initio results favor the former alternative.

Sinha suggested, "there is thus no doubt that ... an excited [diradical] structure ... must also contribute ..." to the ground state of benzaldazine.[24] If the diradical resonance form (Scheme 2) were to contribute to the ground states of phenyl-substituted azines, one would predict that its importance would increase if radical-stabilizing groups were placed in the *para* positions. We examined this prediction by comparison of **1** to the *trans* conformer of unsubstituted methyl phenyl ketone azine[1b] and found that the lengths of the calculated C–N, N–N, and C_{ipso}–C bonds of two D_{2h} structures *are the same.*



Scheme 2. Diradical resonance forms for aryl-substituted azines as suggested by Sinha.

Our analysis thus demonstrates that the resonance form suggested by Sinha plays no major role for the ground state, although other resonance forms may be important for the description of electronically excited states and in discussions of the UV/VIS spectroscopic properties of azines.

Experimental Procedure

Hydrazine hydrate (8 mmol) was added dropwise to a solution of methyl (*para*-tolyl) ketone (10 mmol) in 5 mL of ethanol, and 1 drop of concentrated HCl was then added. The solution was refluxed for 40 min and yellow crystals formed after cooling. M.p. 134–135 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.29 (s, 6H; CH_3), 2.38 (s, 6H; CH_3), 7.21 (d, J = 8.1 Hz, 4H; ArH), 7.80 (d, J = 8.1 Hz, 4H; ArH); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.8, 21.3, 126.6, 129.0, 135.7, 139.6, 157.6; ^{15}N NMR (51 MHz, CD_2Cl_2): δ = 29.6 [25].

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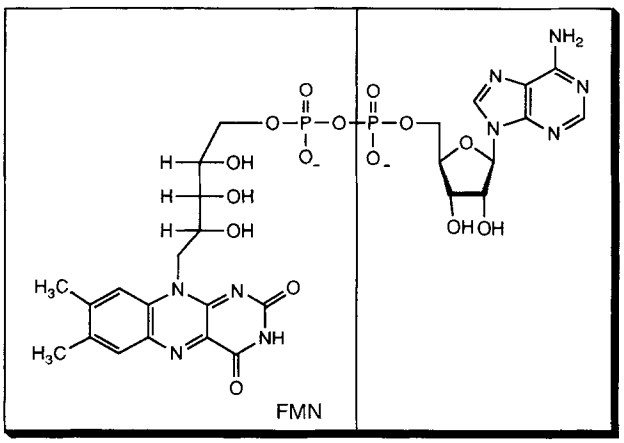
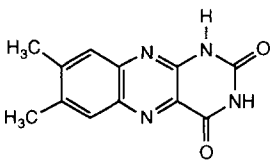
Isolation of RNA Aptamers for Biological Cofactors by In Vitro Selection**

Petra Burgstaller and Michael Famulok*

In vitro selection enables the simultaneous screening of a large number ($\geq 10^{15}$) of different DNA or RNA sequences for certain functionalities. In vitro selection experiments encompass a number of sequential steps, in which the first is always the synthesis of a library of random DNA sequences. After amplification of the DNA by polymerase chain reaction (PCR), an RNA pool is produced by transcription in vitro. Those RNAs (aptamers) that specifically bind to the target molecule are selected from this RNA pool, for example, by affinity chromatography.[1, 2] Such specific ligand-binding nucleic acids not only can have use as potential lead structures for protein inhibitors,[3] but can also help to increase our understanding of the biochemically important recognition processes that involve the interaction of RNA with substrates such as nucleotides, proteins, peptides, amino acids, metal ions, and antibiotics. Furthermore, detailed knowledge of this kind could facilitate the development of new ribozymes.

We report here on the isolation of RNA motifs that bind to the flavin portion of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). In addition, selection experiments were performed in order to isolate RNA aptamers for nicotinamide adenine dinucleotide (NAD^+) and nicotinamide mononucleotide (NMN^+) (Scheme 1).





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Scheme 1. The FMN, NAD^+, and NMN^+ ligands employed in the selection, and 7,8-dimethylalloxazine which was employed for investigating the binding specificity.

For the selections we used a pool of ^{32}P-labeled RNA 113-mers with a complexity of 10^{15} different sequences which consisted of a random sequence of 74 nucleotides flanked by two defined primer binding sites. By means of affinity chromatography with agarose derivatized with the given cofactor, those RNA sequences were enriched that were bound to agarose and eluted with a solution containing the appropriate ligand (Scheme 2, Table 1).

After six cycles significant amounts of the RNA were bound to the FMN and NAD^+ columns. Binding to the FAD matrix could be detected after four cycles, whereas enrichment of

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