Torsional Effects on the Conformations of Two Diastereomeric Tetracyclic Bis(hydrazines)

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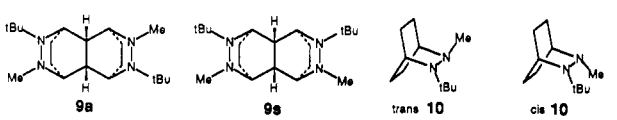
Abstract: The symmetrical tetraazatetracyclotetradecadiene (**1**) is bis(*tert*-butylated) (*tert*-butyl alcohol/H^+) and bis(methylated) (methyllithium) to a 1:1 mixture of diastereomeric bis(*tert*-butylmethylhydrazines) with like alkyl groups anti (**9a**) and syn (**9s**). **9a** has the expected C_2 symmetry on the NMR time scale (both *tert*-butyl groups are directed away from the central CC bond, conformation Oi,Oi in Scheme II). **9s** does not have the expected C_s symmetry, but has one *tert*-butyl group directed away from the central CC bond and one directed toward it (conformations Oi,oI and Io,iO in Scheme II). Both diastereomers also crystallize in these conformations. AM1 calculations carried out using VAMP both predict this result and show it to be caused by tetracyclic ring torsion (experimentally ~12.7° about the central CC bond for crystalline **9a**, ~13.2° for **9s**).

Hydrazines have unusually large internal geometry reorganization upon electron loss, leading to slow electron-transfer reactions.[1] Symmetrical bis(hydrazines), which are long-lived in the radical cation oxidation state, are desired for intramolecular electron-transfer studies. Previous work showed that the connecting linkage must prevent approach of the hydrazine units for long radical cation lifetimes to be achieved, even though radical cation lifetimes are long for compounds with a single tetraalkylhydrazine unit.[2] The connecting bridges in the compounds studied previously were too flexible to accurately define the distance between the hydrazine units. In this work, we report a practical preparation of bis(trialkyldiazenium) salts and tetraalkylhydrazines from tetracyclic bis(azo) compound **1**, which provides a symmetrical three carbon atom, four σ bond link between both ends of each dinitrogen unit, and a separation of about 4.9 Å between closest nitrogens. This paper considers the conformations of the neutral compounds, which show an interesting difference for different diastereomers.

Results: Preparation and Characterization of 9a and 9s

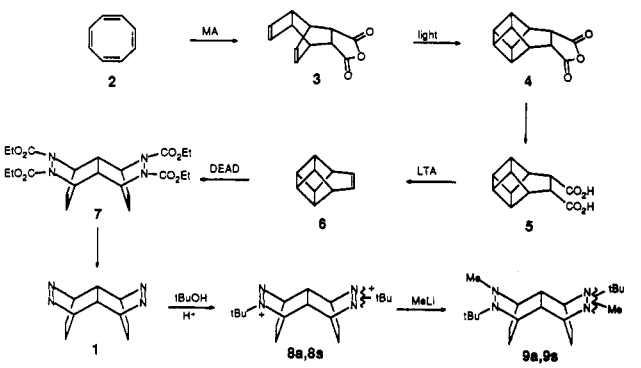
As shown in Scheme I, cyclooctatetraene (**2**) was converted in four literature steps[3] to basketene (**6**), which provided tetraurazole **7** in 63% yield upon heating with diethyl azodicarboxylate, as described by Shen.[4] Hydrogenation followed by an improved hydrolysis/oxidation procedure (see Experimental Section) gave the tetracyclic bis(azo) compound **1** in 95% yield. Bis(*tert*-butylation)[5] gives a 1:1 mixture of the diastereomeric *anti*- and *syn*-bis(trialkyldiazenium) cation salts **8a** and **8s** in 88% yield, and addition of methyllithium[6] gives **9a** and **9s** in quantitative yield. Reduced **8** and oxidized **9** are candidates for electron-transfer studies that will be reported elsewhere, and the rest of this paper is concerned with the symmetry of the neutral **9** diastereomers, which we found to be bewilderingly nonintuitive.

We expected the diastereomers of **9** to be easily identified by a count of the carbon atoms in their ^{13}C NMR spectra. The *anti-tert*-butyl isomer **9a** was expected to show eight carbons from time-averaged C_2 symmetry, and the *syn-tert*-butyl isomer **9s** to have C_s symmetry and nine carbon atoms, because its central CH carbons are different; see the projections shown below:

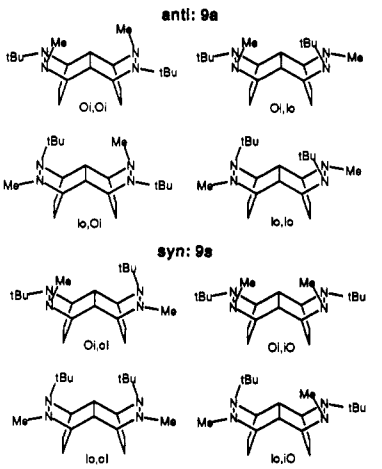


The diastereomers of **9** were separated by fractional crystallization from isooctane. **9a** does show the eight different carbons expected by ^{13}C NMR, and one *tert*-butyl group (δ 1.21) and *N*-methyl group (δ 2.62) by ^1H NMR. However, **9s** exhibits 16 different carbons by ^{13}C NMR, and 2 *tert*-butyls (δ 1.18 and 1.24) and 2 *N*-methyls (δ 2.55 and 2.63) by ^1H NMR. Examination of the monohydrazine analogue, 2-*tert*-butyl-3-methyl-2,3-diazabicyclo[2.2.2]octane (**10**) shows that double nitrogen inversion is slow

Scheme I



Scheme II



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(1) For reviews of hydrazine ET chemistry, see: (a) Nelsen, S. F. *Acc. Chem. Res.* **1981**, *14*, 131. (b) Nelsen, S. F. In *Molecular Structures and Energetics*; Liebman, J. F., Greenberg, A., Eds.; VCH Publishers, Inc.: Deerfield Beach, FL, 1986; Vol. 3, Chapter 1, pp 1-86.

(2) Nelsen, S. F.; Willi, M. R.; Mellor, J. M.; Smith, N. M. *J. Org. Chem.* **1986**, *51*, 2081.

(3) (a) Masamune, S.; Cuts, H.; Hogben, M. G. *Tetrahedron Lett.* **1966**, 1017. (b) Dauben, W. G.; Whalen, L. *Ibid.* **1966**, 3743. We employed detailed procedures of (c) Schumacher, L. *Diplomarbeit*; University of Köln, 1975. (d) Sarter, C. *Diplomarbeit*; University of Köln, 1984.

(4) Shen, K.-w. *J. Chem. Soc., Chem. Commun.* **1971**, 391; *J. Am. Chem. Soc.* **1971**, *93*, 3064.

(5) Snyder, J. P.; Heyman, M.; Gundestrup, M. *J. Org. Chem.* **1978**, *43*, 2224.

(6) Nelsen, S. F.; Parmelee, W. P. *J. Org. Chem.* **1981**, *46*, 3453. The use of TMEDA in additions of methyllithium to diazenium salts described in this reference is not necessary.

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Table I. Comparison of the X-ray Structure for **9a** and **9s** with **9** Structures Calculated by VAMP[11]

compd	alkyls[a]	rel ΔH_f, kcal/mol	dihedral ∠C–CHCH–C	dihedral ∠C–CH_2CH_2–C	dihedral ∠C–NN–C
9a	Oi,Oi (X-ray)[b]		−12.5, −12.8	−10.5, −11.0	−4.2, −3.9
	Oi,Oi (C_2)	0.0[c]	+9.8, +9.8	+8.7, +8.7	−0.1, −0.1
	Io,Io (C_2)	0.4	−9.9, −9.9	−7.4, −7.4	+1.5, +1.5
	Oi,Io, Io,Oi	1.4	+8.8, +8.8	+5.4, +7.3	+11.8, −1.0
9s	Oi,iO	1.5	+9.2, +6.8	+7.2, +5.0	−1.0, +11.9
	Io,oI	2.1	+6.5, +9.3	+4.8, +6.6	11.1, −1.3
	Oi,oI, Io,iO	0.0[d]	10.4, 10.5	+8.3, +7.9	+0.0, −0.1
	Oi,oI (X-ray)[b]		−13.1, −13.3	−10.7, −12.3	−3.8, −2.3

[a] See Scheme II for drawings. [b] Estimated error in dihedral angles: **9a**, 0.6°; **9s**, 0.4–0.5°. [c] ΔH_f = 82.97 kcal/mol. [d] ΔH_f = 82.86 kcal/mol.

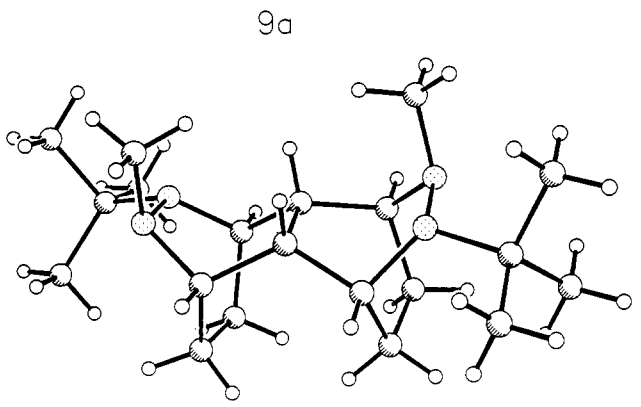


Figure 1. Ball and stick drawing of crystalline **9a**.

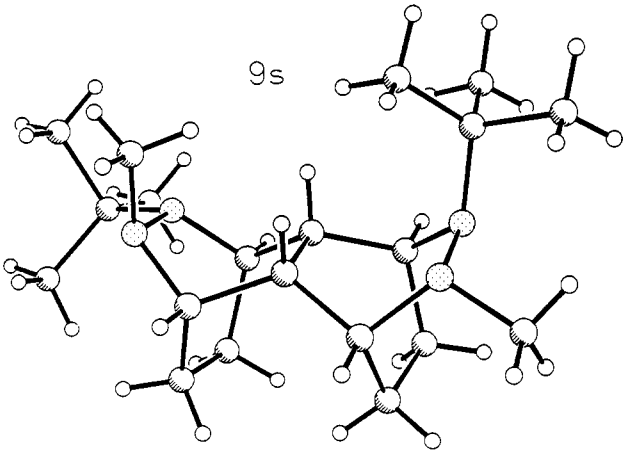


Figure 2. Ball and stick drawing of crystalline **9s**.

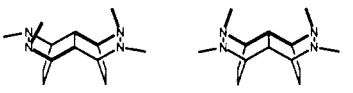
on the NMR time scale. Broadening is observed in the CH_2 carbons of **10** at 57 °C, but no CH_2 signals were observed between 87 and 147 °C. This makes it likely that $T_c \geq 117$ °C, and $\Delta G^{\ddagger}(T_c) \geq 16.8$ kcal/mol. Because *cis*-dialkyl bicyclic hydrazines are far less stable than their trans isomers (see *cis*- and *trans*-**10**), the only conformations that need be considered for **9** are those which are trans at each hydrazine unit, as shown diagrammatically in Scheme II. For convenience, the alkyl groups are designated as *o*uter or *i*nner relative to the tetracyclic system, starting at the back left hand nitrogen and proceeding counterclockwise in the view shown. Capital letters have been used for the *tert*-butyl groups, and small letters for the methyl groups. We conclude that **9a** must be almost exclusively in a single symmetrical conformation in solution, but **9s** in an unsymmetrical one, despite the C_s symmetry possible. A NOESY experiment showed that **9a** exhibits strong *N*-C*H*$_3$, central C*H* cross peaks, but only extremely weak *N*-C*H*$_3$, C*H*$_2$ cross peaks, so **9a** is present in the Oi,Oi conformation in solution. **9s** shows large chemical shift differences for the carbons most affected by one *tert*-butyl group being *I*nner (central bond *C*H's differ by δ 3.4), *C*H$_2$'s syn to *N*-alkyl by 2.7), and must be detectably only present as the mirror image Oi,oI and Io,iO conformations.[7]

X-ray crystal structures were obtained for both **9a** and **9s** (ball and stick drawings of these structures appear as Figures 1 and 2). **9a** crystallizes in the Oi,Oi conformation, and **9s** in the Oi,oI and Io,iO conformations, which the NMR work establishes are the only conformations detected in solution, as well. We were puzzled by these results. If the outer and inner faces of the bicyclic systems are slightly different in their ability to accommodate the large *tert*-butyl groups, as implied by **9a** assuming the Oi,Oi conformation both in solution and in the crystal, why would **9s** assume only conformations with one inner *tert*-butyl group in both phases?

Discussion: AM1 Calculations

Molecular mechanics would appear to be the most reasonable computational method to consider such a conformational question. Unfortunately, even MM3 is unable to handle monocyclic hydrazine relative conformational energies correctly,[8] and we need electronic as well as steric information for electron-transfer studies on these molecules. We have instead chosen to employ Dewar's AM1 semiempirical MO method,[9] which works exceptionally well for oxidized sesquibicyclic hydrazines.[10] These molecules are too large (24 heavy atoms, 38 light) for practical geometry optimization with the QCPE version of the program, but Clark's considerably faster and improved vectorized VAMP program[11] allows complete geometry optimization without imposing any symmetry. Table I shows calculated energies for the conformations of Scheme II. Many distances and bond angles of rather similar sizes are involved in causing the energy differences between different conformations, and we are unable to pick out a few that allow rationalizing the energy differences in a simple manner.

Both diastereomers of **9** are significantly twisted about the central CC bond in their more stable conformations. AM1 treats this torsion well enough to predict the observed result, that **9a** is more stable in symmetrical conformations, and **9s** in unsymmetrical ones. Despite the large difference in size between *tert*-butyl and methyl substitutents, the principal factor determining the relative energies of **9** conformations is not whether *tert*-butyl is *inner* or *outer*, but the relative distribution of *inner* substituents. The lower energy conformations of both **9a** and **9s** are those having the *inner* substituents anti (shown as iA), and



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(7) Although twisting about the central bond makes both Io,oI and Oi,iO conformations of **9s** in principle unsymmetrical, barriers to enantiomerization calculated by AM1 are under 0.5 kcal/mol. They would each show only nine carbons by ^{13}C NMR.

(8) (a) MM3: Allinger, N. L.; Yuh, Y. H.; Lii, J.-H. *J. Am. Chem. Soc.* **1989**, *111*, 8551. (b) We thank Prof. Allinger for a test version of MM3 with N parameters. The hydrazine parameters (the same as those in MM2) still get the conformations wrong for 1,2-dimethylhexahydropyridazine, obtaining diaxial as the most stable form, axial, equatorial as the next, and diequatorial highest of the three chair six-membered ring conformations in energy, which is the reverse of the proper ordering.

(9) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.

(10) Nelsen, S. F.; Frigo, T. B.; Kim, Y. *J. Am. Chem. Soc.* **1989**, *111*, 5387.

(11) Clark, T. Unpublished work. VAMP is available from Timothy Clark, Universität Erlangen-Nürnberg.

the higher energy ones those with the *inner* substituents syn (iS). Twist about the central CC bond in iA conformations decreases steric interactions for both *inner* substituents by increasing the dihedral angles highlighted, while this twist in iS increases the dihedral angle at one side and decreases it at the other side. The effects of tetracyclic ring torsion are rather large in **9**. For example, inversion of all four N atoms of partially optimized **9s** (Oi,oI) (calculated without using the PRECISE option; C–CHCH–C dihedral angles were both +7.2°, and ΔH_f was 0.80 kcal/mol higher than for the fully optimized structure in Table I) followed by partial optimization (again without using the PRECISE option) produced a **9s** (Io,iO) structure that had the wrong sense of tetracyclic ring torsion to be the mirror image (and for which the twist of +7.6 and +8.4° decreased the highlighted dihedral angles instead of increasing them); this structure was 5.8 kcal/mol higher in energy. The calculations also demonstrate that tetracyclic ring torsion is clearly the factor causing iA conformations to be strongly favored over iS conformations. Enforcing eclipsing at the central CC bond (but optimizing all other geometric parameters) maintains the same order of conformational stabilities, but greatly decreases their differences in energy: The relative heats of formation in kcal/mol are **9a**, Oi,Oi (0, ΔH_f 84.58), Io,Io (0.14), Oi,Io (0.44); **9s**, Oi,iO (0.22), Io,oI (0.56), Io,iO (0, ΔH_f 84.49). AM1 calculations do underestimate the amount of C–CHCH–C twist by ~2.8° (~20%), possibly contributing to the prediction of the energy gap between Oi,Oi and Io,Io **9a** as being too small. We only observed the Oi,Oi conformation by NMR, and but would have seen both if their energy difference were only the 0.4 kcal/mol calculated.

Conclusion

The seemingly minor torsion of about 13° about the central CC bond affects the relative energies of the conformations of **9** in a way that we did not expect, but that is in fact independently calculated by using semiempirical MO theory. We believe that the ability of MO methods to handle this rather subtle, basically steric, conformational effect on molecules as large as **9** is both noteworthy and not widely known.

Experimental Section

Tricyclo[4.2.2.0^{2,5}]deca-3,9-diene-7,8-dicarboxylic Acid Anhydride (3).[3c] Maleic anhydride (19.7 g, 201 mmol; recrystallization not necessary) and a little hydroquinone were heated under nitrogen to reflux. With stirring, cyclooctatetraene (**2**) (freshly distilled; 19.72 g, 189.3 mmol) was added dropwise within 10 min. A dark brown color developed, which faded during further 2 h of reflux. Heating was discontinued and the mixture allowed to stir overnight. The crystals thus formed were filtered and washed with ether and then pentane and then dried to yield the near to colorless anhydride: mp 164–167 °C (lit. mp 164–165, 166–168 °C); 34.45 g, 170.4 mmol, 90.0% (lit. 86%). Recrystallization from acetone yielded well-developed colorless plates: mp 165.5–166.5 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.81 (m, 2 H), 3.08 (pseudo-t, prob dd, J = 1.6 Hz, 2 H), 3.24 (m, 2 H), 5.91 (s, 2 H), 6.04 (m, 2 H).

Pentacyclo[6.2.0.0^{2,7}.0^{3,9}.0^{6,10}]decane-4,5-dicarboxylic Acid Anhydride (4).[3c] Anhydride **4** (11.99 g, 59.29 mmol) was dissolved in acetone (AR, ca. 1.5 L) and irradiated for ca. 80 h with a 450-W Hanovia Hg medium-pressure lamp (Vycor filter). The yellow solution was freed from solvent, and the remaining oil was filtered over silica gel (ca. 200 g), eluting with chloroform/hexane = 7/3. The head fraction was collected, and the solvent was evaporated. The residue was dried in an oil pump vacuum and was then mixed with an equal volume of methanol whereupon crystallization ensued. After 1 day, the crystals were collected and washed with methanol to give **4** as a colorless powder: mp 131–133 °C (lit. 104–110 °C (impure product), 127–132 °C); 3.30 g, 16.3 mmol, 27.5% (lit. 40–50% of impure product) (efficient cooling is essential during the photoclosure; further lowering of the substrate concentration seems to improve on the yield); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.93–3.38 (m, t-pattern at 2.94, J = 1.7 Hz).

***trans*-Pentacyclo[6.2.0.0^{2,7}.0^{3,9}.0^{6,10}]decane-4,5-dicarboxylic Acid (5).**[3c,d] The cage anhydride **8** (6.32 g, 31.2 mmol) was suspended in methanol (20 mL), and HCl gas was bubbled through the mixture for a short time. The anhydride dissolved, and a solution of KOH in water (4.2 g in 17 mL, 75 mmol) was added. The mixture was left at 50 °C with stirring overnight; then the pH had dropped considerably, and more KOH (2 g, 36 mmol) was added and heating continued for a further 24 h. The mixture was cooled, and concentrated HCl was added to achieve pH 1 whereupon an amorphous solid dropped out of solution. The mixture was heated for a short time and was allowed to cool. The bis(carboxylic acid) separated and was filtered, washed with water, and dried to yield a white powder: mp 212–219 °C (lit. mp 200–205, 225–228 °C); 6.76 g, 30.7 mmol, 98.3% (lit. 85%).

Pentacyclo[6.2.0.0^{2,7}.0^{3,9}.0^{6,10}]dec-4-ene (Basketene, 6).[3c,d] The bis(acid) **5** (12.87 g, 58.44 mmol) was suspended in dry acetonitrile (100 mL); dry pyridine (25 mL) was added to form a homogeneous mixture. Lead tetraacetate (35 g, Aldrich, not dried) was added all at once, and a thick yellow precipitate formed that became stirrable shortly after. The suspension was slowly heated; it became near to homogeneous around 50 °C and heterogeneous again at 54.5 °C (these temperatures were reproducible in different runs). The temperature was kept constant at 55 °C for 3 h while gas was evolved. After cooling, the mixture was poured into nitric acid (25 mL of 65% HNO_3 in 200 mL of water). Extraction with pentane (4 × 50 mL each), drying (Na_2SO_4), and removal of the solvent through a 20-cm vacuum-jacketed Vigreux column yielded a crude product that was filtered over silica gel (50 g) eluting with pentane. The solvent was removed again by distillation through a Vigreux column, and the residue was sublimed at 48 °C (15 mm) to yield basketene as a waxy solid of intense smell: 3.610 g, 27.73 mmol, 47.4% (lit. 25%); mp 58–60 °C (lit. mp 58–59, 61–62 °C); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.68 (m, 4 H), 3.28 (p, J = 2.9 Hz, 2 H), 3.70 (m, 2 H), 6.51 (m, 2 H).

4,5,9,10-Tetrakis(ethoxycarbonyl)-4,5,9,10-tetraazatetracyclo[6.2.2.2^{3,6}.0^{2,7}]tetradeca-11,13-diene (7).[4] Basketene (3.665 g, 28.15 mmol) was dissolved in carbon tetrachloride (20 mL) with diethyl azodicarboxylate (12.26 g, 70.4 mmol). The mixture was heated to reflux under nitrogen for 10 days whereupon a colorless precipitate formed. The solid was collected, washed with pentane, and recrystallized from absolute ethanol. The tetraurethane was obtained as a microcrystalline powder (longer crystallization times led to fine needles): mp 185–187 °C; 8.17 g. Workup of the mother liquors yielded an additional small crop: 0.31 g, combined yield 8.48 g, 17.1 mmol, 63.0% (lit. 80%); ^{1}H NMR ($CDCl_3$, 200 MHz, broadened by hindered rotation) δ 1.21 (m, 12 H, a t-pattern visible with J = 7 Hz), 2.94 (br s, 2 H), 4.18 (m, 8 H, a q-pattern visible with J = 7 Hz), 4.65 (br s, 2 H), 4.95 (br s, 2 H), 5.95 (br t, J = 7.0 Hz, 2 H), 6.31 (br t, J = 6.0 Hz, 2 H).

4,5,9,10-Tetrakis(ethoxycarbonyl)-4,5,9,10-tetraazatetracyclo[6.2.2.2^{3,6}.0^{2,7}]tetradecane (Catalytic Reduction of 7). The olefinic tetraurethane **7** (8.17 g, 17.1 mmol) was suspended in ethyl acetate (50 mL) and was hydrogenated over 10% Pd/C (100 mg) at atmospheric pressure and ambient temperature. The calculated amount of hydrogen was absorbed after ca. 4 h. Stirring was continued for 16 h and then the suspension was filtered; the catalyst was thoroughly washed with methylene chloride. The filtrate was freed from the solvents to yield the urethane (8.40 g, 17.4 mmol, quantitative) as an amorphous foam containing traces of solvent and with a broad mp near 85 °C. Attempts to obtain a more highly crystalline product failed: ^{1}H NMR ($CDCl_3$, 200 MHz, broadened by hindered rotation of the urethane groups) δ 1.29 (br t, J = 6.9 Hz, 12 H), 1.62–2.13 (br m, 8 H), 2.61 (br s, 2 H), 4.24 (m, 12 H); ^{13}C NMR ($CDCl_3$, 67 MHz, broadened by hindered rotation) δ 14.55, 19.74, 20.92, 22.15, 35.66, 37.06, 49.30, 50.09, 62.42, 155.60, 156.90, there are probably more signals that are not resolved; HRMS calcd for $C_{22}H_{34}N_4O_8$ 482.2377, found 482.2380 (31%).

4,5,9,10-Tetraazatetracyclo[6.2.2.2^{3,6}.0^{2,7}]tetradeca-4,9-diene (1). The tetrahydrourethane **3** (8.15 g, 16.9 mmol) was dissolved in methanol (50 mL). Water (3 mL) and KOH (25 g) were added, and the mixture was heated to reflux under nitrogen for 4 h with stirring; the solution became heterogeneous soon. The mixture was allowed to cool to rt and was then acidified with concentrated HCl to pH 1. A concentrated aqueous solution of $CuCl_2 \cdot H_2O$ (30 g) was added whereupon a brown precipitate was formed. After a further stirring of 20 min, concentrated ammonia was added until all copper ions had formed the ammine complex. The solution was extracted with methylene chloride (5 × 40 mL each); the extracts were washed with water and with saturated brine and were dried with sodium sulfate. The solvent was evaporated to leave the azo compound **1**, homogeneous by TLC: 3.07 g, 16.1 mmol, 95.5%, mp 212 °C dec.[12] Slow evaporation of a concentrated chloroform solution gave well-developed plates without altering the decomposition point: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.25 (br s 2 H), 1.30 (br m, 4 H), 2.09 (m, 4 H), 5.06 (br s, 4 H); ^{1}H NMR (CD_3CN, 200 MHz) δ 1.11 (m, 2 H), 1.15 (m, 4 H), 2.10 (m, 4 H), 4.97 (m, 4 H).

4,9-Bis(dimethylethyl)-4,9-diazonia-3,10-diazatetracyclo-

(12) This avoids the lengthy copper complex isolation and purification procedure of the organic synthesis bicyclic azo compound preparation (Gassman, P. G.; Mansfield, K. T. *Organic Synthesis*; Wiley: New York, 1973; Collect. Vol. 5, p 96), significantly increasing the yield and decreasing the time required.

[6.2.2.2^{3,6}.0^{2,7}]tetradec-4,9-diene Bis(tetrafluoroborate) (8a) and 4,10-Bis(dimethylethyl)-4,10-diazonia-5,9-diazatetracyclo[6.2.2.2^{3,6}.0^{2,7}]tetradec-4,9-diene Bis(tetrafluoroborate) (8s). To the azo compound **1** (316 mg, 1.66 mmol) in *tert*-butyl alcohol (10 mL, distilled from Na), HBF_4–etherate was added (1.10 mL of a 85% complex, d = 1.15 g/mL, 4.64 mmol), and a yellow color slowly developed. The mixture was heated to a very gentle reflux under nitrogen; the yellow color reached a maximum and then faded almost completely after 2 h. After 4 h, more *tert*-butyl alcohol (6 mL) was added and heating was continued for a total of 16 h. After cooling, the resulting precipitate was filtered, washed with ether, and dried to yield a white microcrystalline powder: 0.74 g, 1.55 mmol, 93%); mp 218–222 °C dec > 160 °C. ^{1}H NMR indicated a diastereomeric mixture in the ratio of 1:1. The spectra change with concentration, possibly a counterion effect; the *tert*-butyl signal in the ^{1}H NMR is only split in a concentrated solution. Recrystallization from hot acetonitrile gave a mixture of well-developed diamond-shaped plates (shown to be **8a**) and smaller prisms that appeared to be polycrystalline.

Mixture of 8a and 8s: ^{1}H NMR (CD_3CN, concentrated solution, 270 MHz): δ 1.698 (s), 1.702 (s), these singlets collapse into one in a dilute sample, 1.97 (br s, on dilution, a triplet at lower field is observed), 2.68 (m), 2.73 (m), 6.01 (br s), 6.11 (m), 6.16 (m); ^{13}C NMR (CD_3CN, concentrated solution, 125 MHz) [syn signal set smaller as some crystallization had taken place before the spectrum was taken; these signals are indicated by an asterisk], δ 18.37*, 18.47, 21.40, 21.59*, 26.22*, 26.30, 30.29*, 32.83, 35.38*, 65.80*, 66.21, 69.28*, 69.68, 83.32, 83.48*. Anal. Calcd for $C_{18}H_{32}B_2F_8N_4$ (478.09, mixture of diastereomers): C, 45.22; H, 6.75; N, 11.72. Found: C, 45.27; H, 6.61; N, 11.85.

8a: ^{1}H NMR (CD_3CN, dilute solution, 200 MHz) δ 1.68 (s, 18 H), 2.12 (t, J = 1.0 Hz, integrated for 8 H because of overlap with residual water, should be 6 H), 2.63 (m, 2 H), 2.71 (m, 2 H), 5.92 (m, 2 H), 6.11 (m, 2 H); ^{13}C NMR (CD_3CN, dilute solution, 125 MHz) δ 18.44, 21.49, 26.39, 32.86, 66.29, 69.27, 83.45.

4,9-Bis(dimethylethyl)-5,10-dimethyl-4,5,9,10-tetrazatetracyclo-[6.2.2.2^{3,6}.0^{2,7}]tetradecane and 4,10-Bis(dimethylethyl)-5,9-dimethyl-4,5,9,10-tetrazatetracyclo[6.2.2.2^{3,6}.0^{2,7}]tetradecane (9a and 9s). To a suspension of the mixture of fluoroborate salts **8** (200 mg, 0.42 mmol) in dry THF (10 mL) under nitrogen, methyllithium (1.2 mL of a 1.6 M solution in ether, 1.7 mmol) was added with stirring. The salt dissolved immediately. Stirring was continued for 3 h, the solvents were evaporated, and the residue was taken up in water and extracted with ether (4 × 20 mL). The ethereal layers were washed with water and brine and then dried over sodium sulfate. Evaporation of the solvent left a 1:1 mixture of hydrazines (0.15 g, 0.45 mmol, quantitative) that solidified upon standing. It was taken up in isooctane (2 mL) and cooled to −20 °C. Colorless prisms separated, which were filtered and washed with pentane to give the anti diastereomer (shown by X-ray crystallography) in 95% enriched form: 52 mg; mp 164–165 °C. A second recrystallization raised the mp to 168–169 °C; these crystals were used for the X-ray structural determination. The mother liquors of the first recrystallization were concentrated and brought to crystallization from isooctane again (1 mL); this material was then again recrystallized. The noncrystallizing fractions were evaporated to yield a slowly crystallizing oil: mp ca. 90 °C; 80% enriched in the syn diastereomer. It was taken up in isooctane. Slow evaporation of the solvent induced crystallization of the anti diastereomer first, the syn diastereomer then crystallized from the concentrated mother liquor. This material was sublimed (70 °C (0.1 mm)), yielding a sticky glass that was dissolved in isooctane. Slow evaporation of the solvent gave thin plates of the syn diastereomer of 95% purity (^{1}H NMR): mp 106–108 °C (with a small portion melting from 97 °C on); HRMS (mixture of diastereomers) calcd for $C_{20}H_{38}N_4$ 334.3096, found 334.3098 (2.0%).

9a: ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.21 (s, 18 H), 1.66–1.99 (m, 8 H), 2.62 (s, 6 H), 2.76 (br s, 2 H), 2.83 (m, 2 H), 2.07 (br s, 2 H); ^{13}C NMR (CD_2Cl_2, 125 MHz) δ 18.95 (CH_2 from DEPT), 24.52 (CH_2), 29.72 (CH_3), 34.98 (CH), 48.64 (CH_3), 49.57 (CH), 55.65 (CH), 57.82 (C_q). The only indication of a possible second conformer is a signal at 30.21, which may belong to another *tert*-butyl group (not coincident with syn diastereomer), but may also be due to a decomposed product (solution attains a violet color on prolonged standing in the NMR tube). Anal. Calcd for $C_{20}H_{38}N_4$ (334.55): C, 71.80; H, 11.45; N, 16.74. Found: C, 71.91; H, 11.58; N, 16.82.

9s: ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.18 (s, 9 H), 1.24 (s, 9 H), 1.66–2.00 (m, 8 H), 2.55 (s, 3 H), 2.63 (s, 3 H), 2.73–2.80 (m, 4 H), 2.97 (m, 1 H), 3.13 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 15.75, 18.45, 24.66, 26.08, 29.54, 29.58, 33.78, 37.20, 48.15, 48.50, 48.78, 49.60, 54.98, 55.42, 57.39, 57.63. Anal. Calcd for $C_{20}H_{38}N_4$ (334.55): C, 71.80; H, 11.45; N, 16.74. Found: C, 71.73; H, 11.50; N, 16.72.

2-(Dimethylethyl)-3-methyl-2,3-diazabicyclo[2.2.2]octane (10).[6] To a suspension of 100 mg of 2-(dimethylethyl)-2,3-diazabicyclo[2.2.2]octane tetrafluoroborate[5] in dry THF (10 mL) under nitrogen, methyllithium (0.42 mL of a 1.4 M solution in ether, 0.59 mmol) was added by syringe. The salt dissolved within 30 min. Stirring was continued for 7 h, and the solution was quenched with 10 mL of saturated ammonium chloride solution. Extraction with ether (3 × 30 mL), drying over sodium sulfate, and evaporation gave 74 mg of a clear oil, which was purified by preparative GC (column *T* 120 °C, 15% XF 1150 on Chromosorb W): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.17 (s, 9 H), 1.2–1.4 (m, 2 H), 1.6–2.4 (complex, 8 H), 2.80 (m, 1 H), 3.12 (m, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.62 (CH_2), 21.07 (CH_2), 27.30 (CH_2), 28.89 (CH_3), 29.52 (CH_2), 46.26 (CH), 47.87 (CH_3), 52.12 (CH), 57.28 (C_q); (DMSO-d_6, 125 MHz) δ 18.20, 20.61, 26.84, 28.76, 29.07, 45.69, 47.51, 51.41, 56.96.

Table II. Crystallographic Data and Refinement Parameters of **9a** and **9s**

	9a	**9s**
crystal size, mm	0.10 × 0.25 × 0.30	0.15 × 0.25 × 0.40
temperature, °C	22 (2)	−160 (2)
crystal system	orthorhombic	triclinic
space group	*Pbca*	$P\bar{1}$
a, Å	14.333 (3)	8.735 (4)
b, Å	15.165 (3)	10.524 (5)
c, Å	17.936 (4)	11.018 (5)
α, deg	90	86.46 (4)
β, deg	90	74.95 (4)
γ, deg	90	79.79 (4)
V, Å^3	3898.6 (14)	962.5 (8)
Z	8	2
D_{calcd}, g cm^{-3}	1.140	1.154
$F(000)$	1488	372
reflections collected	3001	2588
obsd reflectn [F > 4.0$\sigma(F)$]	1590	2100
parameters refined	218	218
R/R_w (obsd data), %	8.21/9.28	7.51/11.12
R/R_w (all data), %	13.16/11.26	9.21/11.56
goodness of fit	2.14	2.74
data-to-parameter ratio	7.3/1	9.6/1
largest differences, e Å^{-3}	0.27/−0.29	0.24/−0.31

X-ray Structure Analysis of 9a and 9s. Intensity data were measured on a Siemens P3f diffractometer with graphite monochromatized Cu Kα radiation (λ = 1.541 78 Å), Wyckoff scan type, 2θ 4.0–114.0°, variable scan speed 2.00–12.00°/min in ω, ω range 0.4. The solution of the structures with direct methods in full-matrix least-squares refinement of $\sum w(F_o - F_c)^2$ used Siemens SHELXTL PLUS (VMS).[13] Hydrogen atoms were treated by the riding model using isotropic U, and weighting scheme $w^{-1} = \sigma^2(F) + 0.0007F^2$ for **9a** and $w^{-1} = \sigma^2(F) + 0.0009F^2$ for **9s**. The crystallographic data and the parameters of structure refinement are given in Table II.

AM1 Calculations. Program VAMP,[11] modified for use on a Stardent 3000 computer, was obtained from Timothy Clark and used for this work. As pointed out in the text, it is absolutely necessary to use the PRECISE keyword option for these calculations, and PERSIST should also be used. Reoptimization of the **9s** (Io,iO) structure, which had the tetracyclic ring twisted the wrong way, using PRECISE required 307 cycles (1329 SCF calculations, 3.9 h of Stardent 3000 cpu time) but did successfully find the mirror image of **9s** (Oi,oI), with a calculated ΔH_f within 0.001 kcal/mol of the optimized value. Conformations within 0.01 kcal/mol of those reported in Table I, which have opposite signs for the C–CHC-H–C and C–CH_2CH_2–C dihedral angles were also found; ΔH_f is clearly not very sensitive to rather large changes in some of the heavy atom dihedral angles. **9a** (Oi,Io) structures with dihedral ∠C–CHCH–C closer to those for the other structures reported in Table I were also found, but are ca. 0.3 kcal/mol higher in energy. We know of no general method for finding global minima in quantum mechanical calculations; all we can state is that those reported are the lowest energy structures we found, using several starting structures. The same ΔH_f to within 0.005 kcal/mol results from optimizing without symmetry and from (correctly) imposing C_2 symmetry upon **9a** (Oi,Oi) and **9a** (Io,Io) structures. VAMP is fast enough and geometry optimization accurate enough that imposing symmetry to save computational time is not necessary. A message from this work is that it is also unwise to impose symmetry unless it is known that

(13) (a) Sheldrick, G. M. *SHELXTL PLUS*, Version 4.2; Siemens Analytical X-ray Instruments, Inc.: Madison, WI, 1990. (b) Complex neutral atom scattering factors from *International Tables for X-ray Crystallography*; Kynoch: Birmingham, Vol. IV, Tables 2.2b and 2.3.2, (present distributor Kluwer).

this symmetry is in fact present.

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Supplementary Material Available: X-ray crystallographic structural data (numbered thermal ellipsoid plot, atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients) for **9a** and **9s** (8 pages). Ordering information is given on any current masthead page.

^{1}H NMR Hyperfine Shift Pattern as a Probe for Ligation State in High-Spin Ferric Hemoproteins: Water Binding in Metmyoglobin Mutants

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Abstract: The ^{1}H NMR spectra of a series of high-spin ferric metmyoglobins (metMbs) have been analyzed to assess the validity of meso proton (meso-H) contact shift direction as a probe for H_2O coordination and to develop a quantitative interpretative basis of the hyperfine shift pattern as a structural probe for the heme-iron ligation state. The quantitative analyses of the hyperfine shifts are based on a comparison of the structurally characterized six-coordinate sperm whale metMbH_2O and the five-coordinate *Aplysia* metMb. The developed spectral probes are subsequently applied to elucidate the role of distal residues in modulating H_2O coordination in a series of E7 and E11 point mutants of sperm whale Mb. The elusive distal E11 residue signals are assigned in the two reference proteins on the basis of their unique relaxation properties and from the spectral characteristics of E11 sperm whale Mb point mutants. Quantitative analysis of the E11 residue dipolar shifts demonstrates that the loss of coordinated H_2O leads to a substantial increase in the zero-field splitting constant, D. The change from strongly low-field meso-H shifts in six-coordinate sperm whale metMbH_2O to strongly upfield meso-H shifts in five-coordinate *Aplysia* metMb is accompanied by predicted changes in chemical shifts for heme methyl, F8 His C_βH, and E11 Val methyl protons. The most readily recognized change is the 5-ppm low-field bias of the mean methyl shift upon loss of water coordination. The consistency in the changes of all the NMR spectral parameters supports the use of the meso-H chemical shifts as probes for ligation state but suggests that all accompanying changes should be analyzed. Substitution of E11 Val by Ile, Phe, or Ala results in minimal perturbation with full retention of coordinated water. Substitution of E7 His by Val or Phe abolishes H_2O coordination, while replacement by Gln or Gly leads to a fractional H_2O coordination. The sensitivity of the hyperfine shifts of the heme methyl protons to solvent isotope composition supports the proposed changes in H_2O ligation. The application of the heme mean methyl shifts as a probe for H_2O coordination in a series of natural genetic variants differing in E7 residue confirms previous conclusions, except for elephant metMb (E7 Gln) which is concluded here to be primarily five-coordinate rather than six-coordinate. The occupation of the sixth position by water as a function of E7 residue is found to be very similar in sperm whale E7 point mutants and natural genetic variants, and it is concluded that H bonding by E7 residue is the strongest but not the only stabilizing influence on H_2O coordination.

Introduction

Myoglobin (Mb) is a small hemoprotein whose primary function is to store molecular O_2 in skeletal muscle in its functional ferrous form.[1] This protein, in both the reduced and oxidized states, binds many other small neutral ligands and anions, and much of our understanding of the relationship between structure and function in the O_2 binding hemoproteins has been derived from the studies of these nonphysiological ligands.[2] One potential ligand which has considerable functional significance, albeit indirectly, is the H_2O molecule. While it does not directly compete with O_2 for the reduced heme-Fe binding site, H_2O is H bonded to the distal residue in a manner that requires its displacement by the ligand before it can bind to the Fe. However, this type of interaction is highly selective in that a H_2O molecule is found H bonded to the distal His E7[3] in the α subunit but not the β subunit of human deoxy hemoglobin (Hb);[4] sperm whale (SW) deoxy Mb exhibits fractional occupancy of such a site.[5] It is clear that access of H_2O to the heme cavity influences the dynamics and thermodynamics of ligation. The access of H_2O to the distal cavity, and associated dissolved anions, has also been implicated in the autoxidation of Mb and Hb,[6] where the ferrous heme is oxidized

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(1) Kagen, L. J. *Myoglobin: Biochemical, Physiological, and Clinical Aspects*; Columbia University Press: New York, 1973.
(2) Antonini, E.; Brunori, M. *Hemoglobin and Myoglobin in Their Reactions with Ligands*; North Holland Publishing Company: Amsterdam, 1971.
(3) E7, E11, and F8 are the alphanumeric codes referring to the position of the residues in the sperm whale myoglobin amino acid sequence. E7 is the seventh residue in the E helix, F8 is the eighth residue in the F helix, and so on (Edmundson, A.E. *Nature* **1965**, *205*, 883).
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