Inorganic Chemistry

Article

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Nitric Oxide Catalysis of Diazene *E*/*Z* Isomerization

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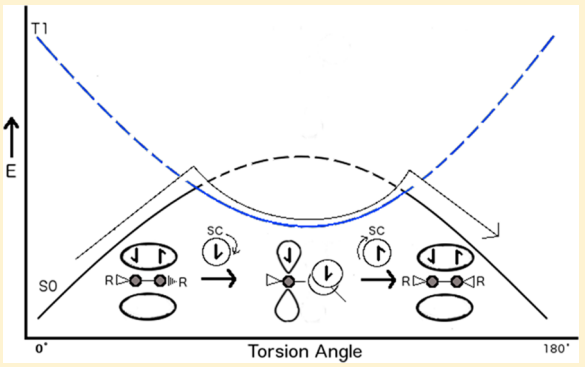
Supporting Information

ABSTRACT: Nitric oxide is an efficient catalyst for the cis-trans (*E*/*Z*) isomerization of diazenes. We compare the effect of room temperature solutions bearing low concentrations of nitric oxide, nitrogen dioxide, or oxygen on the rate of cis-trans isomerization, CTI, of the alkene bond in stilbene and on the azo double bond in azobenzene, as well as in four azo derivatives as measured by UV−vis spectroscopy. These rate enhancements can be as large as 3 orders of magnitude for azobenzene in solution. A mechanism is proposed where catalysis is promoted by the interaction of the nitric oxide with the diazene nitrogen lone pairs. Density functional theory, B3LYP/6-311++g** suggests that the binding of NO to the diazene should be weak and reversible but that its NO adduct has an E/Z isomerization barrier of 7.5 kcal/mol.



The isomerization of fatty acids by "oxides of nitrogen" was first reported by J.J.E. Poutet in 1819.[1] Subsequent investigations have shown that nitric oxide,[2,3] nitrogen dioxide,[4,5] and even oxygen itself[6] can catalyze the cis/trans isomerization (CTI) of olefins. The adventitious oxygenation of NO to NO_2 has led to some confusion as to which is the more active cis−trans catalyst, and it is noteworthy that for those working in carefully controlled, low-concentration experiments, NO is reported as the catalyst for gas-phase experiments, while researchers working in solution usually report NO_2 as the catalyst. De Maré et al. have found that photosensitized NO, in its $^4\Pi$ excited state, will catalyze CTI in 2-butene but that ground state NO on its own is not catalytically active.[7,8]

Both NO and NO_2 are stable radicals, so it is possible that they catalyze CTI by virtue of a spin exchange,[9,10] which allows access to triplet pathways, Supporting Information, Figure S1.[11,12] What makes these pathways normally inaccessible is the inability of electrons to spontaneously change spins. In most cases, intersystem crossing proceeds by spin orbital coupling, in which the interaction between the magnetic moment of an electron in a high-energy molecular orbital and the magnetic spin of that electron is sufficiently strong to effect a change in spin. This can be considered a form of "spin catalysis".[9] Another form, less well-known, involves an open-shell radical species associating with a system (such as a transition state) and exchanging its unpaired electron for an electron of opposite spin from this associated system, thus transforming a singlet to a triplet, or vice versa. Since a singlet-to-triplet transfer is usually energetically uphill, it only occurs in conformations where the triplet energy has dropped or is otherwise energetically available and the spin change remains as the sole barrier. Since the original radical is released unchanged, it qualifies as a catalyst.

Double-bond isomerization in general is an excellent candidate for spin catalysis; as the double bond rotates out of plane, the potential energy surface of the T1 state drops below that of the ground state (at 90°, the π electrons cannot be in the same orbital, and this separation is more easily achieved for two electrons of the same spin, i.e., the triplet state), Figure S1.

Low concentrations of nitric oxide are common in biological systems, so it is important to clarify whether or not it can act as a spin catalyst. As part of this investigation, we studied the interaction between nitric oxide and azo bonds (-N═N-) and compared them to the interactions with the olefin analogue (stilbene) under tightly controlled conditions. The key differences between the two systems are the energy of isomerization (azo bonds are more easily isomerizable), the presence of lone pairs on nitrogen in place of hydrogen (potentially allowing association through this lone pair), the mechanism of isomerization (azo bonds isomerize either by rotation or by inversion, where olefinic bonds can only rotate), and the geometry of the cis conformer (*cis*-stilbene is planar and somewhat more sterically hindered, whereas *cis*-azobenzene twists into a three-dimensional "palm-to-palm" formation (Figure 1)).[13] Differences in how these two systems behave when exposed to NO can therefore help illuminate the mode of interaction.

Furthermore, the isomerization of diazo bonds in the presence of NO has not been previously described. Herein we test NO as a CTI catalyst in solution at room temperature and at low concentrations, on stilbene, azobenzene, and four derivatives of azobenzene (two of which are soluble and two insoluble in water) (Figure 2). The results are then compared

Received: September 2, 2014

DOI: 10.1021/acs.inorgchem.5b00476
Inorg. Chem. XXXX, XXX, XXX−XXX

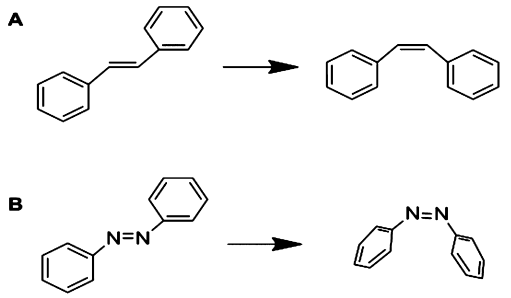


Figure 1. Isomerization of *trans*-stilbene (A) and azobenzene (B).

to DFT calculations to formulate a possible model of NO CTI catalytic behavior.

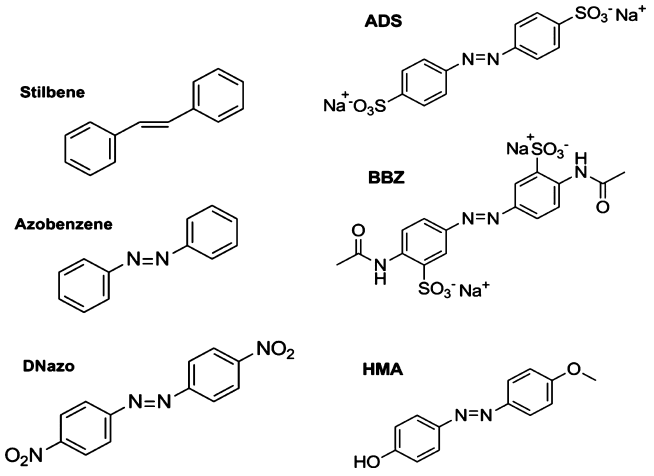


Figure 2. Diazo derivatives used in the study. (left) Stilbene, azobenzene, 4,4′-dinitroazobenzene (DNazo). (right) 4,4′-Azobenzenedisulfonate (ADS), 3,3′-bis-sulfanato-4′,4-bis-acetamidoazobenzene (BBZ), 4-hydroxy-4′-methoxyazobenzene (HMA).

EXPERIMENTAL SECTION

The five diazo compounds were first (1) prepared and dissolved in appropriate solutions, (2) then isomerized under UV light. (3) The test gases were prepared by two different methods, and (4) the solutions were treated with these test gases. Finally (5) the rate of the isomerization back to the original configuration was determined by UV−vis spectroscopy.

1. Diazo Preparation. The five diazo compounds used (Figure. 2) were all prepared in house. *cis*/*trans*-Stilbene and those reagents used to prepare the diazo substrates were purchased from Sigma-Aldrich.

Azobenzene. Method used by Hien et al. was modified.[14] Aniline and 2 equiv of sodium perborate are dissolved in acetic acid (2 g of aniline to 50 mL of acid) at 64 °C. Solution quickly darkens to black. After 30 min, adding excess hexane extracts a red-orange product. Removing hexane and residual acid from product, then redissolving in hexane and recrystallizing, gives a purified azobenzene (vivid orange, 35% yield). UV−vis (in hexane): 314 (major), 441 nm.

Azobenzenedisulfonate. Method used by Zhang et al. was modified.[15] Sulfanilic acid is dissolved in warm water with 2 equiv of Na_2CO_3 (1 g of sulfanilic acid to 12 mL of water works well). It is cooled in an ice−salt bath to below 0 °C. A 15% NaOCl solution is added dropwise, about one every 3 s. An immediate color change to deep orange should be seen. When addition is complete, the reaction vessel is left in a 4 °C refrigerator for 12 h, then filtered: the filtrate will be rich in the kinetic product (*cis*-ADS, which must be protected from ambient light if it is to be kept in this state), while the precipitate will be predominantly *trans*-ADS and side products. Dissolving in (or reducing to) the minimum water and adding ethanol or isopropanol will precipitate the inorganic salt. Two further solvent recrystallizations with water/THF or water/ethanol can separate the desired dye from the more brightly colored side product. A telltale test of purity is to leave a very dilute solution of the compound in a quartz vessel under 365 nm UV light for ∼20 min: trace impurities in this reaction will begin to visibly fluoresce (if left under UV for >1 h, photochemistry will also produce these fluorescent impurities). UV−vis: 320 (major), 433 nm. NMR: ^{1}H: 7.883 (d), 7.826 (d).

3,3′-Bis(sulfonato)-4,4′-bis(acetamido)azobenzene (BBZ). Diaminobenzenesulfonic acid is dissolved in distilled acetic acid with 1 equiv of acetic anhydride. Acetamido product precipitates and is removed by filtration. Intermediate product is treated as in the procedure for ADS to create the rich orange azo-acetamido product. UV−vis: 354 (major), 245, 445 nm.

4-Hydroxy-4′-methoxyazobenzene (HMA). The method used by Amoros et al.[16] was modified. 4-Anisidine is dissolved in 1 M HCl at 0 °C (as well as possible), and equimolar $NaNO_2$ is added as an aqueous solution. This mixture is allowed to stir for 30 min. Meanwhile, equimolar phenol is dissolved in 1 M NaOH solution (phenol to NaOH molar ratio should be 1:2), and stirred. After 30 min, it is added dropwise to the first solution, and the two are stirred at 0 °C for 1 h. Solution is neutralized with 5% HCl, and product is filtered out (adding more water can help precipitate it). Desired product can be separated with column chromatography, with dichloromethane eluent. UV−vis: 337, 355, 372 (major peak, vibronic coupling), 440 nm (shoulder).

4,4′-Dinitroazobenzene. The dinitroazobenzene is synthesized by dissolving 4-nitroaniline in ethanol, cooling to 0 °C, then adding 10% NaOCl solution dropwise and stirring for 2 h. It is separated by column chromatography. UV−vis: 336 (major), 446 nm.

2. Isomerization Protocol. All of the diazo species prepared undergo rapid isomerization from their thermodynamically favored trans form to the higher energy cis-form when exposed to low-intensity UVA light. We found that 5−10 min of 365 nm light with a 7 nm half-width (emitted by an 18.4 W handheld UV lamp) was enough to achieve a steady state of ∼70% cis (measured by NMR and confirmed by comparison to the UV spectrum of pure cis) for azobenzene, ADS, DNazo, and BBZ, and nearly 100% for HMA and stilbene. HMA, unlike the others, was found to have a profound solvent dependence; it isomerizes very slowly under 365 nm UV light in toluene, while in some polar solvents, such as ethanol, the cis-to-trans thermal return is on the millisecond scale. Ethyl acetate or tetrahydrofuran (THF) were both found to allow easy isomerization while maintaining a slow, observable thermal return rate.

3. Nitric Oxide Preparation. Nitric oxide is delivered to the solution by either one of two sources.

Method A. A dipropylammonium salt of dipropylamine diazenium diolate (DPA-NONO, which breaks apart in neutral conditions into 2 equiv of nitric oxide and two of dipropylamine), is prepared according to literature procedure.[17] Stock solutions of 10 mmol/L DPA-NONO are prepared fresh in 0.01 mol/L NaOH solution on ice and mixed as needed into a phosphate buffer (0.0029 mol/L K_2HPO_4 + 0.0073 mol/L KH_2PO_4 in water, pH ≈ 6.8) 6 min prior to use. Aliquots of this solution are then injected via syringe, thus giving controlled and relatively high concentrations of NO in solution. Since the concentration of NO in solution depends on the partial pressure of NO in the headspace, the level of dissolved nitric oxide will slowly drop as the gas diffuses out of solution into the headspace. Thus, measurements are made soon after injection, with a minimum of stirring or shaking.

Method B. A commercial sample of nitric oxide is injected with a gastight syringe into a sealed and purged cuvette (filled with solvent) and agitated to dissolve the gas. NO_2 was purged from the gas stream by a packed column of potassium hydroxide; the gas was examined by IR spectroscopy for the presence of N_2O, but this was determined to be present in only trace amounts. This method has the advantage of saturating the headspace with NO and thus maintaining a concentration over the long-term, but because the compressibility of gas allows for large differences when measuring small volumes and

DOI: 10.1021/acs.inorgchem.5b00476
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reproducible results are therefore more difficult to obtain, it was used for qualitative comparisons with experiments completed by Method A, and for the nonaqueous experiments (HMA and stilbene).

Control experiments for NO_2 and N_2O were also performed:

Nitrous oxide: The gas was obtained from a Praxair cylinder. The solution to be tested was saturated with the gas.

Nitrogen dioxide: To obtain NO_2 with a minimum of NO, lead(II) nitrate is heated over a flame to produce the gas. Calculations are adjusted to account for the 0.5 equiv of oxygen produced per 2 equiv of NO_2.[18]

4. Solution Treatment. All solutions were purged of oxygen with vigorous bubbling under argon on ice (15 min for bulk (~10 mL solutions), 5 min for small cuvette samples (<2 mL); initial tests were performed by freeze−thaw-degassing the solutions, but this yielded the same results). Since acid is also known to catalyze CTI, all aqueous solutions are adjusted to pH 6.8 with the phosphate buffer previously described; ADS and BBZ solutions are prepared in buffer only, while azobenzene is prepared in 50:50 acetone/buffer, HMA in ethyl acetate, DNAzo in 50:50 ethanol/buffer, and stilbene is dissolved in hexane. Dye solutions are placed in a gastight quartz cuvette in a dark box and irradiated for 5 min with 365 nm light. DPA-NONO is mixed with a buffer and left to incubate for 6 min (just over 4.3 half-lives, until 95% has decayed), and the desired amount of this NO solution is injected into the dye cuvette once it has been transferred to the spectrometer. The cuvette is mixed, and the rate of isomerization is measured.

5. Measurement. The sample is placed into an HP 8453 Diode-Array UV−vis spectrometer; it is protected from ambient light throughout the measurement time, and measurements are evenly spaced every 60 at 0.5 s exposures over 30 min, to minimize the effects of the measurement on the rate of isomerization. The possibility of a back reaction due to photolysis during the UV detection was found to be insignificant (the difference in isomerization rate between a sample measured 30 times and one measured twice was found to be less than the calculated measurement error). All controls were subjected to the same measurement procedure. The changing absorption at the λ_{max} is plotted against time, and the rate constant is determined by a least-squares fit to a first-order curve (catalyzed isomerization will be pseudo-first-order). Error is reported as the standard deviation of a minimum of three repeated measurements, or as the error in slope derived from the variance between the data and the first-order model, whichever error is larger.

NO_2 and N_2O control experiments were performed only in dry hexane solutions of azobenzene and stilbene, as well as ethyl acetate solutions of HMA. The gas is injected via syringe, and this is compared to equimolar injections of NO gas.

EPR measurements of the solutions after mixing were taken using a Bruker ElexSYS 580 X-band spectrometer, in an effort to detect any NO−azobenzene adducts; low-temperature measurements were performed in a Wilmad low-temperature dewar insert filled with liquid nitrogen.

To gauge the turnover capacity of NO, a 1.72 mM solution of azobenzene in toluene was purged, sealed, saturated with the test gas by injecting with 1 mL of pure gas at atmospheric pressure, and irradiated with 365 nm UV light for 17 h. This should be sufficient to completely isomerize the azobenzene from trans to cis 41.9 times, based on a rate of 1.4×10^{-7} mol/min in a 1.72 mM toluene solution. (This rate was estimated from the response of a fresh *trans*-azobenzene solution to 1 min of UV exposure, making use of Halpern's UV−vis extinction coefficients[19] of the two isomers to gauge the concentration of each). This number of turnovers corresponds to 3.5 times the amount of NO that was injected, if one molecule of NO was consumed per CTI. The sample was removed from the UV light, and the rate of its CTI was measured by UV−vis spectrometry and compared to both the baseline thermal rate and the rate prior to the 17 h of radiation.

THEORETICAL METHODS

All of the calculations were performed using Gaussian 03.[20] Computations were performed at the restricted Hartree−Fock (RHF)[21] and density functional theory (DFT) levels. DFT calculations used the hybrid B3LYP functional and triple-ζ 6-311++G** basis sets.[22,23] The calculated molecular geometries were fully optimized and correspond to minima on the potential energy surface as confirmed by the absence of imaginary vibrational frequencies. All transition states were confirmed by reaction path (IRC) following calculations.

EXPERIMENTAL RESULTS

All -N═N- bonded molecules investigated showed increases in rate of isomerization when in the presence of NO, except for DNAzo. Stilbene did not respond under these conditions (Table 1). Control tests were performed with oxygen, dipropylamine, and nitrogen dioxide. Nitrous oxide was included in the tests with azobenzene and HMA, when it was observed that solvent polarity had a measurable effect (dipole moment of NO is 0.1595 D, vs 0.166 D for N_2O and 0.289 D for NO_2).[24−26]

Table 1. Pseudo-First-Order Rate of cis to trans Isomerization of Various Species under Different Gas Exposures

species	conditions of thermal cis to trans isomerization	k of CTI,[a] s^{-1}
stilbene (28 μM)	with oxygen	$2.05(8) \times 10^{-5}$
	no oxygen	$2.05(9) \times 10^{-5}$
	dipropylamine	$1.50(4) \times 10^{-5}$
	nitric oxide (excess)	$3.0(7) \times 10^{-5}$
	nitrogen dioxide (excess)[b]	$\sim 2(1) \times 10^{-5}$
azobenzene (760 μM)	with oxygen	$1.69(6) \times 10^{-5}$
	no oxygen	$1.72(2) \times 10^{-5}$
	dipropylamine	$1.7(2) \times 10^{-5}$
	nitric oxide (NO/dye 1:2)	$1.81(7) \times 10^{-2}$
	nitrogen dioxide (NO_2/dye 1:2)	$4.23(1) \times 10^{-4}$
	nitrous oxide (large excess)	$1.72(2) \times 10^{-5}$
ADS (50 μM)	no oxygen	$1.9(2) \times 10^{-5}$
	no oxygen, in 50% ethanol	$1.68(1) \times 10^{-4}$
	nitric oxide (NO/dye 1:2)	$4.5(8) \times 10^{-4}$
	nitric oxide (NO/dye 1:2) in 50% ethanol	$1.35(1) \times 10^{-3}$
BBZ (50 μM)	no oxygen	$8.3(2) \times 10^{-5}$
	nitric oxide (NO/dye 1:2)	$2.4(9) \times 10^{-4}$
HMA (50 μM)	in ethyl acetate, no oxygen	$3.94(2) \times 10^{-4}$
	in ethyl acetate, (NO/dye 1:2)	$1.16(6) \times 10^{-2}$
	in ethyl acetate, with NO_2 (NO_2/dye 1:2)[c]	$3.29(3) \times 10^{-2}$
	in ethyl acetate, with ethanol (EtOH/dye 1:2)	$1.58(8) \times 10^{-3}$
	in ethyl acetate, with N_2O (large excess)	$3.9(2) \times 10^{-4}$
DNAzo (50 μM)	no oxygen, 50:50 ethanol/water	$1.04(1) \times 10^{-4}$
	nitric oxide (NO/dye 1:2)	$9.6(3) \times 10^{-5}$
	nitrogen dioxide (1:2 ratio), in ethyl acetate[c]	$1.7(4) \times 10^{-5}$

[a] T = 25 °C. Number in parentheses indicates the error in the last reported digit. [b] NO_2 injection confounds accurate measurement of stilbene isomerization via UV, due to overlap and possible side reaction, but the observed change is within the limits of error for the thermal rate. [c] The solubility of nitrogen dioxide in ethyl acetate was set to be that of NO in ethyl acetate, as the gases have similar solubilities in polar solvents.

DOI: 10.1021/acs.inorgchem.5b00476
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Unsubstituted azobenzene responds most dramatically to the introduction of NO and, to a lesser extent, NO_2. The substituted azo dyes appear at first glance to have had a meager response, but at slightly higher dose levels, they clearly show the same behavior, DNAzo excepted. Taking ADS as representative, we see (Figure 3) the spectrum of a 50 μM

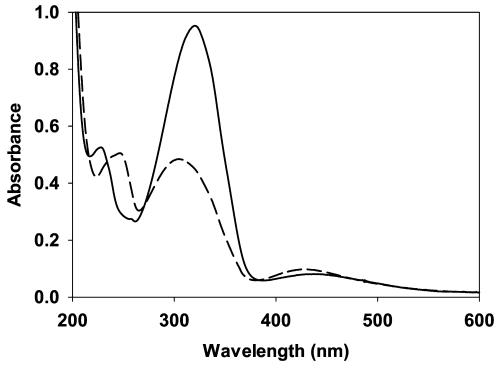

Figure 3. UV–vis spectrum of 50 μM ADS in water, before UV exposure (solid line, trans isomer) and after (dashed line, enriched in cis isomer).

sample at equilibrium (effectively 100% trans) and after 5 min of exposure to UV (69% cis), (Figure 4) the same cis-enriched ADS exposed to 100 μM NO, and (Figure 5) a kinetic trace of the peak at 320 nm when exposed to a range of solvated NO gas concentrations.

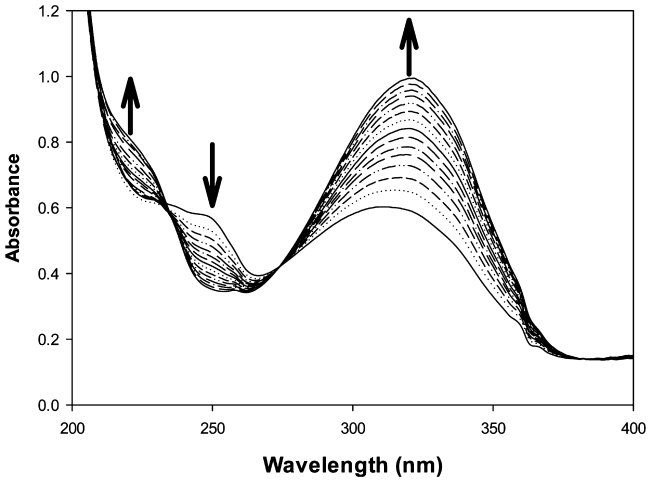

Figure 4. UV–vis of cis-enriched ADS after exposure to 100 μM NO. Measured over 15 min, every 60 s. Lowest signature at 300 nm is at time 0.

The normal CTI thermal rate for ADS is a typical first-order reaction, with $k = 1.9 \times 10^{-5}$ s^{-1}. The initial rates for the samples exposed to NO follow first-order kinetics; the overall reaction is thus pseudo-first-order. The generalized standard formula for reversible first order reactions (eq 1) was used:

$$\ln((A_t - A_\infty)/(A_0 - A_\infty)) = -(k_1 + k_{-1})t$$

Equation 1: Integrated reversible first-order rate law, where A_t is the absorbance at time t, A_0 is the absorbance initially, and A_∞ is the absorbance at equilibrium. A_o and A_∞ were determined independently from the kinetics runs, as they were found to vary slightly with extended UV exposure. The

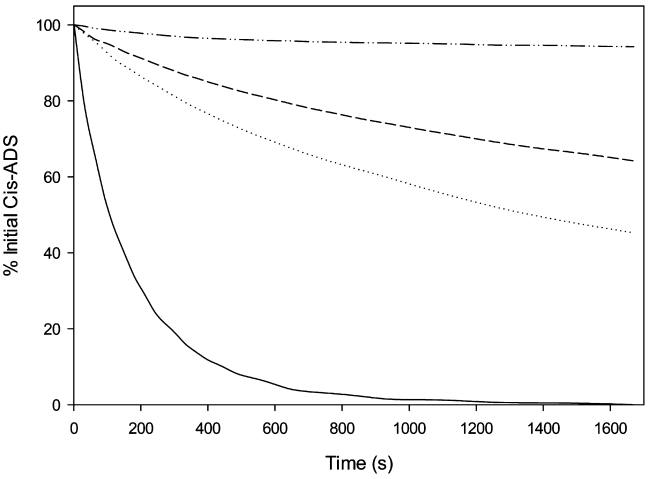

Figure 5. ADS dye response to NO exposure.

back reaction k_{-1} is small enough that it can be neglected, as evidenced by the final steady-state ratio of cis/trans, which is essentially 100% trans. The curves were fitted to this first-order model using a least-squares approach, and k was calculated at different dose levels. Plotting the logarithmic factor k versus time gives a linear dose/response curve (Figure 6).

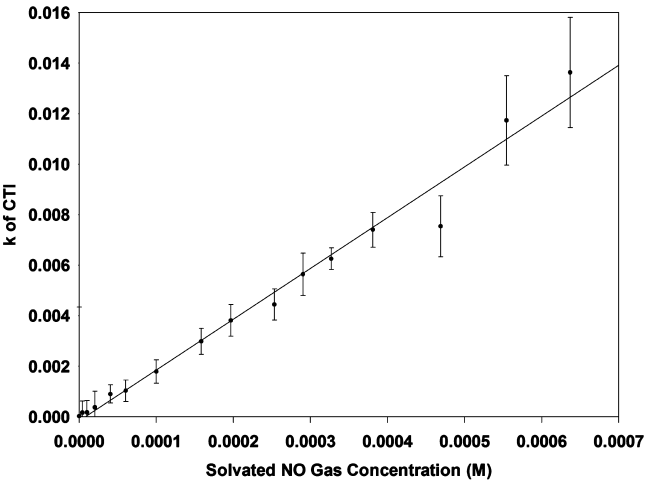

Figure 6. Dependence of first-order rate constants for cis–trans isomerization (CTI) of ADS as a function of [NO]. Error bars represent one standard deviation.

Performing this operation for the three compounds in which a significant change was found, the rate constant (k) for the relation d[cis-Dye]/dt = $-k$[NO][cis-Dye] was calculated:

azobenzene: $k_{azo} = 47(\pm 4)$ $L \cdot mol^{-1} \cdot s^{-1}$

ADS: $k_{ADS} = 18.1(\pm 0.1)$ $L \cdot mol^{-1} \cdot s^{-1}$

BBZ: $k_{BBZ} = 16.0(\pm 0.6)$ $L \cdot mol^{-1} \cdot s^{-1}$

HMA is a special case in that its isomerization can be provoked by the presence of certain solvents (ethanol, pyridine, dichloromethane, and water). Ethanol has one of the most potent effects, so it was chosen for comparison with the gases; NO_2 and NO can be seen to have a greater effect than that of ethanol, but the effect due to electronic interaction with the

DOI: 10.1021/acs.inorgchem.5b00476
Inorg. Chem. XXXX, XXX, XXX–XXX

solvent cannot be distinguished from spin catalysis, and further kinetic determinations were not performed for HMA.

When samples were irradiated for 17 h to gauge catalytic turnover, no difference was found in their rate of CTI after the long-term irradiation (i.e., after ∼41.9 turnovers). Azobenzene itself degrades with extensive UV exposure, showing the development of a broad undefined absorption band between its major ($\pi-\pi^*$) and minor ($n-\pi^*$) peaks, but a control sample demonstrated that these photoproducts do not interfere with the normal rate of CTI and, presumably, do not interfere with NO-mediated CTI either.

Electron paramagnetic resonance (EPR) measurements of azobenzene/nitric oxide solutions show a weak anisotropic signal centered at 3493 G when frozen at −196 °C, extending from 3460 to 3515 G. This shifts to an isotropic triplet at room temperature. This signal is attributable to trace amounts of NO_2 trapped in solution. No other EPR signals are detectable.

THEORETICAL RESULTS

To model the possible mechanisms for the observed spin catalysis, DFT calculations (UB3LYP/6-311++g**) were performed for the most likely ground and transition states for nitric oxide interacting with azobenzene. The absolute gas-phase energies for the stationary points are collected in Table 2

Table 2. Key Ground- and Transition-State Energies (B3LYP/6-311++G) for Azobenzene and its NO adducts[a]**

		cis		trans		Δ
GS + NO	A	−702.815283		−702.8397537	G	15.36
adducts	C	−702.816978		−702.8188314	E	1.16
T state to adducts	B	−702.810431		−702.815107	F	
T state between adducts		−702.80503	D			

[a]Energies are given in Hartrees except for the differences Δ, which are given kcal/mol. Capital letters correspond to geometries shown in Figure 7.

with letters **A** to **G** given to the successive points in the cis-to-trans isomerization. The relative energies, in kcal/mol, and their relationships are diagramed in Figure 7, and information about the key metric parameters is collected in Table 3.

DISCUSSION

That nitric oxide catalytically converts *cis*-azobenzene to *trans*-azobenzene is unequivocal. The conversion of cis to trans occurs rapidly on exposure to NO, and further UV irradiation will not produce a population of *cis*-azobenzene, even after 17 h of continuous UV light. Purging a solution with argon to remove NO restores normal functionality, and *cis*-azobenzene can develop normally. Though catalysts are typically used in much lower concentrations than their substrates, practical considerations of measurement and handling with nitric oxide made this difficult; nevertheless, it fulfills the definition of a catalyst in that it accelerates reactions without itself being consumed.

NO cis−trans catalysis functions most readily when a nitrogen lone pair is available. It does not proceed at all in stilbene and is also completely ineffective when strong electron-withdrawing groups ($-NO_2$) are present, which would pull electron density from the lone pair into the arene ring. More moderate electron-withdrawing groups ($-SO_3^-$) allow some catalysis. Weak or moderate electron-donating groups also impede the action of NO, however, and it may be that the greater electron density partly occupies the orbital through which the NO interacts (such as a π^*). Furthermore, nitric oxide is 2 orders of magnitude more catalytically active than NO_2 for azobenzene. NO has a more available unpaired electron (based on an ionization potential of 9.25 eV, compared to 11.7 eV for NO_2[27]); this electron resides in the π^* orbital and hence is delocalized over the molecule. NO_2, by contrast, has its unpaired e^- localized on the nitrogen in a nonbonding orbital. This suggests that the ability of this electron to be transferred determines the rate of catalysis. The reduced effect of NO_2 may also be ascribed to steric bulk inhibiting close association with the N atom beside an arene ring.

Careful examination of the system by UV−vis and NMR before and after short-term UV and NO exposure reveals no side products, either from organic decay or the formation of nitrites, nitrates, etc. EPR analysis of the mixtures showed no signal (which is consistent with free nitric oxide, where a bound azo-NO adduct should be visible). IR of the headspace gas

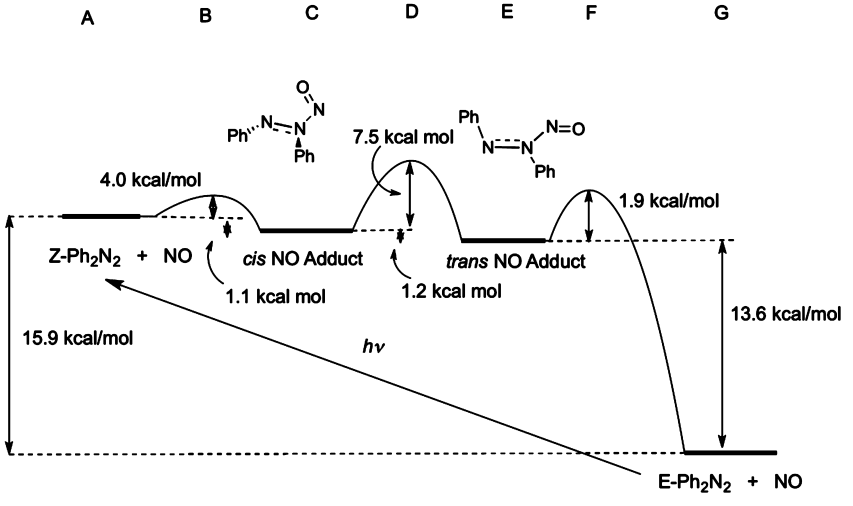

Figure 7. Relative gas-phase energies for the ground states and transition state present in the NO-catalyzed isomerization of azobenzene.

DOI: 10.1021/acs.inorgchem.5b00476
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detects no change in composition (production of N_2O_3 or NO_2, for example). Litchfield et al. suggested that the spontaneous formation of NO_2 in NO injections is the active principle for CTI catalysis, but in our case, the greatly reduced response of azo bonds to injected NO_2 rules this out.[5] Furthermore, NO_2 has a distinctive spectrum under UV, and this was not seen. We are thus confident in ruling out an interaction between NO and NO_2 as being responsible for the catalysis, and attribute it solely to NO.

All of this suggests that nitric oxide is associating with, but not permanently bonding to, the N═N moiety through the lone pairs. This may be modeled in two different ways: spin catalysis may proceed by outer-sphere associations, in a mechanism akin to solvent interactions or Eyring's sticky collisions,[28] in which transient interactions allow spin exchange to occur without the formation of chemically reactive transition states. This spin exchange allows the molecule to cross over from the S_0 to the T_1 state, which becomes the lower-energy pathway as the molecule twists. Alternatively, an inner-sphere mechanism may operate, without necessarily invoking spin catalysis, where discrete but weak bonds reversibly form between the nitric oxide and the diazene. This mechanism would be similar to how thiyl radicals are proposed to catalyze *E*/*Z* isomerizations in lipids.[29] Either mechanism must account for the dearth of side products or intermediates. Thus, any NO + diazene adduct equilibrium should favor the free NO form, be reversible, and be rapidly established. The inner-sphere mechanism is well-poised for modeling by modern density functional ab initio theory, and the results of this modeling is shown in Figure 6 (above) as well as in Table 2 and Supporting Information, Table S1.

Theoretically, both *cis*- and *trans*-azobenzenes form weakly stabilized adducts with NO, structures **C** and **E**. Both adducts offer only ∼1 kcal/mol of electronic stabilization, and the barriers that separate them from *cis*- and *trans*-azobenzene, namely, **B** and **F**, are very low (2−3 kcal/mol). The crucial features in this scheme are the final loss of NO from the trans adduct **E**, which favors the free diazene by 13.6 kcal/mol, and the highest barrier **D** in this system, 7.5 kcal/mol, is the critical *Z* to *E* isomerization in the adduct. Although these calculations are for the gas phase and are not zero-point corrected, they suggest that an inner-sphere mechanism is possible.

A complete description of this system would require an understanding of the mechanism of azobenzene isomerization; unfortunately, there is still controversy around this issue.[30−37] Like olefins they may isomerize by rotation (in which the double bond is broken), but may also do so by inversion (in which the double bond remains intact but the nitrogen rehybridizes to an sp form, putting its lone pair in a p orbital and becoming linear). It is likely that the molecule can proceed by either mechanism and uses whichever is favored by the current environment; for example, polar solvents may promote rotation by stabilizing charged intermediates, whereas inversion is preferred in a nonpolar solvent.[38] NO-catalyzed CTI proceeds most quickly in less polar solvents (a threefold increase in the rate for ADS is seen in a 50% ethanol/water mix compared to pure water), far more than could be explained by changes in NO solubility. This suggests that the isomerization may proceed by inversion or at least by an uncharged intermediate.

It has been recognized for some time[39] that olefinic bonds can isomerize through either a singlet or triplet state, and Plachkevytch[40] et al. have calculated that, in theory, nitric oxide should spin-couple to this system in the way proposed for the outer-sphere mechanism of diazene−NO catalysis. The fact that NO appears to have no effect on stilbene under these conditions suggests that the mechanism of interaction is fundamentally different and that either gas phase conditions, high temperatures, or high concentrations are needed to promote CTI catalysis. There is evidence[41] that at higher temperatures, NO catalyzes olefin isomerization by formal electron-transfer mechanisms, but this has not been reported at room temperature.

CONCLUSION

Although nitric oxide has complicated reactivity patterns that reflect its accessible redox potentials as well as its electrophilicity,[28,42] we have shown that it is a surprisingly robust cis−trans isomerization catalyst. Previous research into its catalytic quality has focused, understandably, on the gas phase, where nitric oxide is most commonly found in the laboratory and in industry. But this study has demonstrated that it maintains this behavior in solution, even in water, and can act to isomerize double bonds to a significant degree at low concentrations. Furthermore, it does so quite well with azo bonds, a previously unreported effect. Though the integrity of azo bonds specifically is not a concern in much of biochemistry, there are numerous and growing applications for diazenes in biomedical probes and materials. Any application of these materials to physiological conditions with even a minor nitric oxide flux needs to consider these findings.

Indeed, there are many other applications of diazenes, and questions about the stability of these materials in the presence of NO (as an atmospheric pollutant for example) must be considered as well. Diazenes are used extensively in organic chemistry as stereospecific electrophiles and oxidants.[43] Furthermore, the unique facile *E*/*Z* structural photoisomerism of diazenes has led to many creative uses, for example, in fuel cells,[44] fuel additives,[45] liquid crystals,[46] nonlinear optical materials,[47] anti-retroviral (HIV) drugs,[48] antibiotics,[49] and antifungal agents.[50] Diazene interactions with the GST family of enzymes[51] has led to their incorporation into other drugs, such as chemotherapy treatments.[52−55] In all of these situations, potential spin-catalyzed isomerization by low concentrations of nitric oxide will interfere or modulate their function.

A particularly intriguing biochemical consequence of these findings is that nitric oxide may qualify as a cofactor for enzymatic activity. Enzymatic transformations that generate triplet intermediates or progress through triplet excited states would also be facilitated by this type of "spin cofactor". Although applied magnetic fields are able to alter the kinetics of these types of processes,[56,57] albeit to only a modest degree, nitric oxide's reversible Lewis acidity allows it to introduce spin into enzymatic active sites to a much higher degree. When this feature is combined with its nearly universal permeability to all organelles and cells, along with its being a compact diatomic molecule, it suggests there may be a large array of possible enzymes in which it could be such a cofactor.

ASSOCIATED CONTENT

Supporting Information

Synthetic details, discussion of use of diazenium diolates as NO source, energy profile for CTI of double bonds, calculated structure for ground and transition states, calculated metrical parameters, and plots indicating dose response to NO. The

DOI: 10.1021/acs.inorgchem.5b00476
Inorg. Chem. XXXX, XXX, XXX−XXX

Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorgchem.5b00476.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors gratefully acknowledge NSERC and the CRC for support of this research.

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DOI: 10.1021/acs.inorgchem.5b00476
Inorg. Chem. XXXX, XXX, XXX−XXX