Table I. Ratio of N_2O-45 (Obtained from Nitrosation with $^{14}N_3^-$) and N_2O-46 (from Denitrification) as a Function of $^{15}NO_2^-$ Concentration

$[^{15}NO_2^-]$, mM	N_2O-45/ N_2O-46	$[^{15}NO_2^-]$, mM	N_2O-45/ N_2O-46
0.05[a]	0.79 ± 0.07	0.1[b]	0.24 ± 0.01
0.10[a]	0.44 ± 0.01	0.2[b]	0.22 ± 0.02
1.0[a]	0.17 ± 0.03	0.5[b]	0.086 ± 0.001
		1.0[b]	0.052 ± 0.005

[a] 50 mM succinate as reductant. [b] Luria broth medium as reductant.

by incubating cell-free extracts with varying amounts of $^{15}NO_2^-$ in the presence of 50 mM $^{14}N_3^-$. Trapping the $E{\cdot}^{15}NO^+$ intermediate with $^{14}N_3^-$ (nitrosation) produces $^{14}N^{15}NO$ (mass 45), while denitrification produces $^{15}N_2O$ (mass 46). The results clearly show that NO_2^- competes with N_3^- for the $E{\cdot}NO^+$ intermediate.

Previous work in our laboratories[9] with whole cells of *P. stutzeri* has shown that the extent of $H_2{}^{18}O$ exchange with the $E{\cdot}NO^+$ intermediate, as determined by the ^{18}O content of product N_2O, decreases with increasing nitrite concentration, which indicates that $H_2{}^{18}O$ and NO_2^- compete for the same intermediate, $E{\cdot}NO^+$. We have observed similar behavior for cell-free extracts (data not shown). In order to examine this point more carefully, we have performed an isotope dilution experiment, in which cell-free extracts are incubated with 1 mM $^{15}NO_2^-$, 50 mM $^{14}N_3^-$, and 9% $H_2{}^{18}O$, and the ^{18}O content of N_2O originating from denitrification (N_2O-48/(N_2O-46 + N_2O-48)) is compared to that of N_2O originating from nitrosation (N_2O-47/(N_2O-45 + N_2O-47)). Nitrite is a "sticky" substrate for *P. stutzeri* nitrite reductase,[13,14] and the free NO_2^- pool does not equilibrate rapidly with $H_2{}^{18}O$. Consequently, the ^{18}O content of $^{15}N_2O$ produced by denitrification should be the same as (eq 2) or approximately one-half that of (eq 3) the $E{-}^{15}NO^+$ pool, as monitored by trapping with $^{14}N_3^-$. We find experimentally that $^{15}N_2O$ from denitrification is 52.7 ± 2% equilibrated[15] with the $H_2{}^{18}O$, while $^{14}N^{15}NO$ from nitrosation is 80.5 ± 2.4% equilibrated with the $H_2{}^{18}O$ solvent, consistent with eq 3. (Even at high $[NO_2^-]$ in the absence of N_3^-, we always observe ca. 8% equilibration of N_2O with $H_2{}^{18}O$, possibly indicating that some ^{18}O exchange occurs via an intermediate containing an N–N bond, as has been observed for nitrosation with NH_2OH.[9])

These results demonstrate that H_2O, N_3^-, and *nitrite* compete for a common intermediate, $E{\cdot}NO^+$. This provides the first direct evidence that unambiguously distinguishes between the mechanisms of eq 2 and 3 and indicates that denitrification occurs by *sequential* reaction of two nitrite ions with the enzyme, as first proposed by us.[8] This conclusion is supported by recent isotope effect studies.[16,17] Further mechanistic studies are in progress to define the second nitrite binding site and the factors which cause purified nitrite reductase to produce primarily NO rather than N_2O.[1,3]

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Photochemical Dehydrofragmentation Reactions: Importance of Donor and Acceptor Structure in Determination of Reactivity in Radical Ion Pairs Formed in Electron-Transfer Photoreactions

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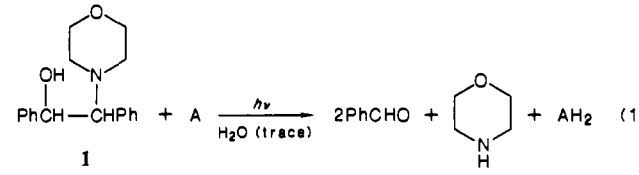
Photoinduced electron-transfer reactions are the subject of considerable interest due both to the versatility of net chemical reactions which can be achieved as well as to the variety of intermediates and mechanistic complexities encountered in their study.[1-8] For initially neutral donors and acceptors quenching of excited singlet states is frequently dominated by formation of a geminate ion pair, A^-/D^+, which can subsequently decay by back electron transfer or, in moderately polar to polar solvents, by cage escape to form free ions.[7-10] In nonpolar solvents the back electron transfer process acts as an effective clock to limit the lifetime for reaction of the geminate pair to the ns time scale; consequently only relatively rapid reactions can occur efficiently from the geminate pair and these frequently involve participation of the solvent or other reagents present in high concentration.[11-14] We have previously reported the chemically clean and moderately efficient C–C bond photofragmentation reactions of β-amino-alcohols initiated by electron transfer to excited acceptors (eq 1).[15,16] We report here a mechanistic study where this reaction



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is restricted to the geminate pair and which emphasizes the complimentary roles of reduced acceptor and oxidized donor in facilitating chemical reaction in competition with fast back electron

(12) Experimental conditions: 50 mM $Na^{14}N_3$; reductant was 50 mM succinate or Luria broth medium; cell-free extracts of *P. stutzeri*[9] prepared by French press (12 000 psi, 2 passages); all reagents in 25 mM HEPES buffer, pH 7.3; samples incubated at 25 °C overnight, 2–3 replicates per $[^{15}NO_2^-]$, 2 injections per sample, 2 integrations per peak. Sterile controls (autoclaved for 20 min at 200 °C) gave no detectable N_2O. Gas samples were analyzed with an HP 5985 GC/MS system equipped with a Porapak Q column. The mass spectrometer was operated in EI mode with selective ion monitoring. System temperatures were as follows: injector, 80 °C; column, 55 °C; ion source, 100 °C. The electron multiplier voltage was 1400–3000 MeV, and the autotune value was 2000 MeV. The amount of extract added was such that the extent of reaction for the incubation period and for the lowest $[NO_2^-]$ did not exceed 20%; thus, the $[NO_2^-]$ and $[N_3^-]$ did not change significantly during the course of the experiments.

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(16) In this study with the acceptors listed in Table I the (acceptor) was between 5×10^{-5} and 1×10^{-4} M. For degassed solutions the reaction could be carried out until the acceptor was completely consumed. For all four acceptors the only products observed from **1** are those in eq 1.

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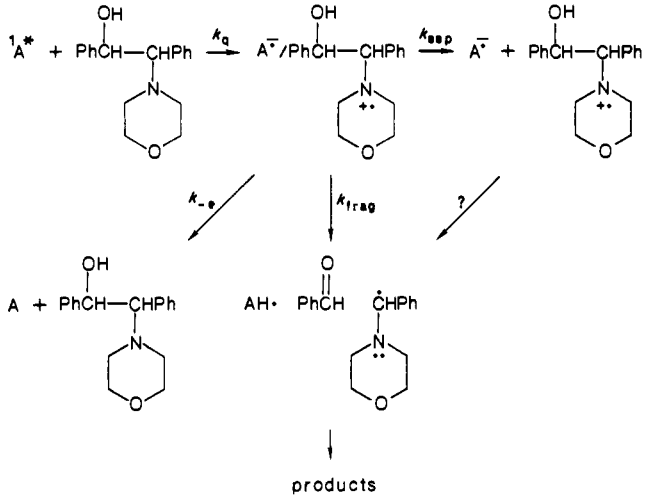
Table I. Relative Quantum Efficiencies for Reduction of Photoexcited Acceptors ($A \rightarrow AH_2$) by Aminoalcohol-**1**[a]

	TI[d]			DCA[d]		TCA[d]	DCN[d]
	C_6H_6[c]	CH_2Cl_2[c]	CH_3CN[c]	C_6H_6[c]	CH_3CN[c]	C_6H_6[c]	C_6H_6[c]
$\Phi^{\text{erythro-1}}$	1.0	0.24	6.7×10^{-4}	0.25	2.7×10^{-3}	0.05	2.4×10^{-3}
$\Phi^{\text{threo-1}}$	0.17	0.026	3.4×10^{-5}	0.012	8.7×10^{-5}	1.9×10^{-3}	3.7×10^{-5}
erythro/threo	5.8	9.4	19.3	19	31	27	62
$\Phi^{H}/\Phi^{D(\text{erythro})}$ [b]	1.3			1.7		2.1	3.7
$\Phi^{H}/\Phi^{D(\text{threo})}$ [b]	1.3			2.5		3.3	4.0

[a] Irradiations of vacuum degassed solutions in a merry-go-round apparatus using appropriate filters to isolate the exciting wavelength so that only the acceptor is excited; the reaction is monitored by measuring the disappearance of acceptor.[16] For TI, DCA, and TCA the products and stoichiometry have been established to be as given in eq 1; the same pattern of oxidation of **1** has been demonstrated for DCN. All quantum yields relative to $\Phi^{\text{erythro-1}} = 0.027$ in benzene for TI. Ferrioxalate actinometry used for solutions of DCA, TCA, and DCN: T = 23 °C. [b] Water (or D_2O) (5%) added to benzene solutions; conversion to **1**-(OD) verified by NMR. [c] Solvent. [d] Acceptor.

Scheme I



transfer. A key result is that rapid fragmentation is coincident with acceptor radical anion induced deprotonation of the donor cation radical and thus strongly dependent on acceptor structure.

The dehydrofragmentation in eq 1 can be carried out for either diastereomer of **1** and with a variety of acceptors including thioindigo (TI), $Ru(bpy)_3^{2+}$, quinones, and cyanoaromatics.[15,16] Here we focus on reaction initiated by quenching of excited singlet acceptors such as TI and the cyanoaromatics in solvents of low-to-moderate polarity; under these conditions the most likely mechanism should involve formation and subsequent reaction of the geminate pair as outlined in Scheme I. In fact, for both **1**-TI and **1**-9,10-dicyanoanthracene (DCA) the efficiency of reaction is substantially higher in nonpolar solvents such as benzene or CH_2Cl_2 than in the more polar acetonitrile (Table I).[17] Values for k_{sep} for the monovalent ions formed from reaction of neutral donors and acceptors have been determined to be 5×10^8 s^{-1} for acetonitrile and 2×10^8 s^{-1} for methanol;[7-9] considerably slower rates should be anticipated for CH_2Cl_2 and benzene. Elegant recent work by Farid et al. has shown that for cyanoaromatics k_{-e} varies with reaction exothermicity and structure showing a "Marcus-inverted" relationship where $-\Delta G = 1$–3 eV;[9,10] from calculated exothermicities (1.6–2.6 eV) k_{-e} can be estimated for the eight D,A pairs studied here to be between 1×10^9 and 2×10^{10} s^{-1}. Thus for reaction of **1** with singlet acceptors in the nonpolar solvents fragmentation must be occurring within the geminate pair on a time scale governed by k_{-e}.

With regards to the magnitude of k_{frag}, the use of a thermochemical cycle calculation shows that there should be an ca. 90% reduction in the gas-phase C–C bond energy of β-aminoalcohols upon oxidation of the neutral compound to give an energy in the range 4–8 kcal/mol.[18,19] Assuming a preexponential factor of ca. 10^{13}, an activation energy in this range should give a rate constant for fragmentation of ca. 10^7–10^{10} s^{-1} at room temperature which would be at least moderately competitive with k_{-e}.[20] As reported earlier, the photoreaction of TI and **1** at room temperature is characterized by a moderate diastereomeric preference in the reactivity of erythro over threo following the quenching process; this has been attributed to a preference for an anticoplanar arrangement of the C–OH, C–C, and C–N bonds during the cleavage.[15] A study of the photoreaction in toluene (Table II) shows that reaction efficiency increases while the erythro/threo selectivity decreases with increase in temperature. If it is assumed that the only temperature-dependent process in reaction of the geminate pair is k_{frag}, an activation energy may be obtained from Arrhenius plots of the data from Table II. These plots shows good linearity and provide values of E_a for threo-**1**-TI and erythro-**1**-TI of 4.9 and 2.8 kcal/mol, respectively.

Table II. Temperature Effect on the Photochemical Dehydrofragmentation of **1** with Thioindigo in Toluene[a]

temp, °C	Φ^{erythro}	Φ^{threo}	erythro/threo
75	0.081	0.071	1.1
35	0.047	0.028	1.7
0	0.020	0.0025	8.2
−20	0.012	0.00074	15.4
−63	0.0053	0.00013	42.1

[a] Vacuum degassed solutions, irradiated at 540 nm with use of Corning 3-73 and 4-69 filters; reaction followed by monitoring ΔA of the TI absorption (546 nm) to less than 10% conversion. The stoichiometry for this reaction is according to eq 1, and the quantum yields are corrected for differential quenching of the excited TI.

Although the above calculated values suggest that unassisted fragmentation of the donor cation radical could be competitive with k_{-e}, a pronounced effect on reactivity is observed by systematically varying the acceptor used to mediate the reaction. The three cyano aromatics are very strong oxidants in their fluorescent singlets,[9,23,24] and measured values of k_q are large in each case and show little erythro/threo sensitivity. In contrast the quantum efficiencies for fragmentation with the cyanoaromatics are lower than for TI, and they show a marked decrease in the series DCA > 2,6,9,10-tetracyanoanthracene (TCA) > 1,4-dicyanonaphthalene (DCN). The erythro/threo selectivity increases as the overall quantum yields decrease similar to the increase in selectivity for TI as T decreases (vide supra). The pronounced variation in quantum efficiency with acceptor must derive mostly from large decreases in k_{frag} in the series TI > DCA > TCA > DCN since k_{sep} should show little variation (and should be slow

(17) The rather striking retardation of the reaction in acetonitrile compared to benzene may reflect greater donor–acceptor separation in the geminate pair in the former solvent and a greater probability of back electron transfer over the acceptor ion radical induced fragmentation which requires contact.

(18) Dinnocenzo, J. P., unpublished results.

(19) Lowered C–C bond energies for other cation radicals have been calculated by the same method. Okamoto, A.; Snow, M. S.; Arnold, D. R. *Tetrahedron* **1986**, *42*, 6175.

(20) Studies[21] with **1** and an equivalent of the strong oxidant O_2^+ in CHF_2Cl[22] indicate unassisted fragmentation of $\mathbf{1}^+$ to benzaldehyde and iminium ion occurs, perhaps even at −125 °C; however, the rate of the unassisted fragmentation has not yet been measured.

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compared to k_{-e} in all cases), while k_{-e} should follow the order TI ~ TCA > DCA ≥ DCN.[9,10,25] The most plausible explanation for the variation in k_{frag} is that proton loss and fragmentation are synchronous, and thus basicity of the acceptor radical anion plays a major role.[26] The spread of k_{frag} for these acceptors estimated from Φ_{-A} and k_{-e} values spans a range of nearly three orders of magnitude from 3×10^8 s^{-1} (erythro-**1**/TI) to 7×10^5 s^{-1} (erythro-**1**/DCN) for a common donor.

Further evidence supporting the role of acceptor anion radical assisted deprotonation in the fragmentation is the finding of significant deuterium isotope effects when the -OH of the aminoalcohol is replaced by -OD (Table I). In line with the trends in overall reactivity, the isotope increases from 1.3 for TI to ca. 4 with DCN. In summary, the results obtained in this study provide a clear demonstration that radical ion pairs formed by electron transfer in low-to-moderately polar media have properties much like electronically excited states or diradicals such that only relatively rapid reactions can compete with "unimolecular" decay. In the present case the critical matching of reactivity of both acceptor and donor ion radicals allows a rapid and yet highly specific reaction to occur in the relatively narrow time window between formation and decay of the geminate pair.[28]

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(25) Values of k_{-e} show dependence upon both D and A structure as well as exothermicity; thus although exothermicity for DCN/erythro-**1** (2.4 eV) is greater than for DCA/erythro-**1** (2.0 eV), data for the two acceptors fall on displaced parabolic plots (Farid, S., private communication).

(26) Basicity of acceptor anion radicals clearly decreases in the series TI > DCA > TCA. The question of basicity of $DCN^{\bullet-}$ relative to the others is apparently unsettled.[9,27]

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(28) We have found electron-transfer dehydrofragmentation for molecules having the structure H-Z-C-C-Y, where Z = O, N and Y = O, N, or S, to be fairly general provided the acceptor used to mediate the photolysis is strong enough to oxidize the heteroatom Y *and* the corresponding anion radical is basic.[29]

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Coexistence of Conformations in a DNA Heteroduplex Revealed by Site Specific Labeling with ^{13}C-Labeled Nucleotides

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The current bias regarding the conformation of a DNA duplex is that under a given set of environmental conditions each nucleotide unit will assume a rapidly averaged conformation within one of the possible structural families (e.g., A, B, or Z). Time-averaged variations within families have been directly observed by X-ray crystallography, NMR spectroscopy, circular dichroism, and vibrational spectroscopy. However, little information is actually available regarding the rates and mechanisms of interconversion of conformations lying within the same or even different families. In this communication we report the first use of DNA fragments specifically labeled with ^{13}C in a single nucleotide unit to assist the study of duplex conformations by ^1H and ^{13}C NMR spectroscopy; such labeling can be expected to yield unambiguous resonance assignments as well as higher resolution in congested spectral regions. We focus on a comparison of two heteroduplexes labeled with [1′,3′-$^{13}C_2$]deoxyuridine: d(CGCACGC) paired with d(GCGUGCG) [AU_I] and d(GGCACGG) paired with d(CCGUGCC) [AU_{II}].[2,3] In low salt, two slowly interconverting conformations of the deoxyuridine are observed for AU_{II} but not AU_I. The populations of the conformers are temperature dependent and approximately equal at 37 °C, demonstrating a previously unrecognized potential for coexistence of multiple, slowly interconverting conformations of a DNA sequence under biological conditions.

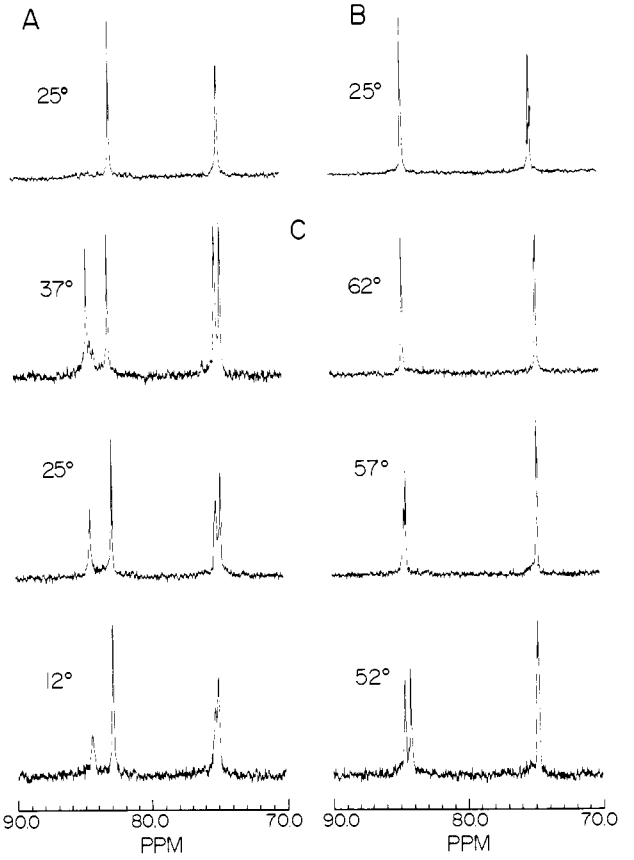

Figure 1. Comparisons of the 100-MHz ^{13}C NMR spectra of AU_I at 25 °C (panel A), of d(CCGUGCC) [the labeled single strand in AU_{II}] at 25 °C (panel B), and of AU_{II} at the indicated temperatures (panel C).

Characterization of AU_I and AU_{II} by conventional one-dimensional ^{13}C NMR spectroscopy at 100 MHz revealed unanticipated spectral differences. In low salt[12] at 25 °C, the spectrum

(1) (a) Leukemia Society of America Fellow, 1986-1989. (b) Alfred P. Sloan Fellow, 1983-1987.

(2) The choice of heteroduplexes containing an A-U base pair was influenced by our interest in the conformational properties and enzymatic processing of DNA duplexes containing structural lesions such as uracil and baseless sugar residues.

(3) [1,3-$^{13}C_2$]Ribose (Omicron Biochemicals) was converted to [1′,3′-$^{13}C_2$]deoxyuridine by using standard literature procedures.[4-6] The labeled deoxyuridine was converted to 5′-dimethoxytrityl 3′-*o*-chlorophenylphosphate deoxyuridine[7,8] and incorporated with solution phase phosphotriester chemistry[9,10] into single strands for the duplexes.[11]

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