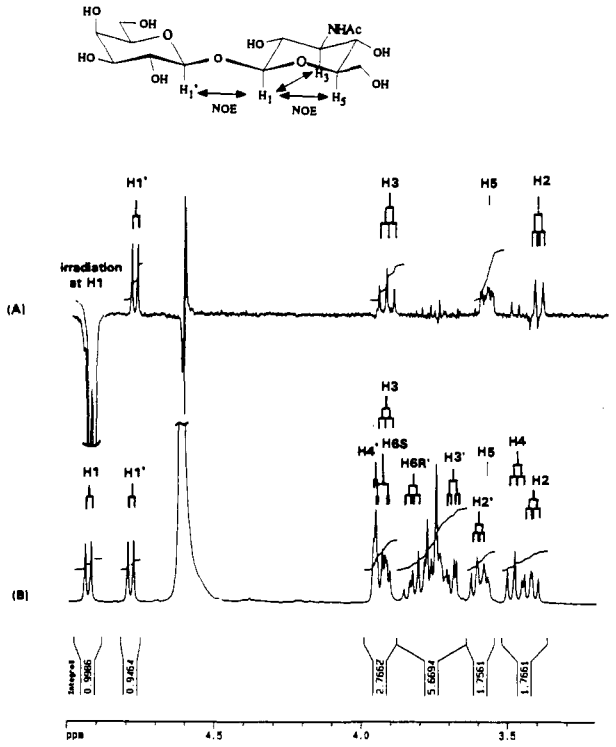

Figure 1. ^{1}H NMR spectrum (B) of βGal1,1βGlc3NAc in D_2O (50 °C) and NOE differential spectrum (A) with irradiation at the anomeric proton of the βGlc3NAc residue.

and α-lactalbumin (0.1 mg/mL)[13] in Tris-HCl buffer (pH 7.4, 12 mL). After 30 h at 30 °C, the product was isolated by gel filtration chromatography (Bio Gel P-2) as a hygroscopic solid (36 mg, 26% yield).

FAB-MS ($[M + 1]^+$ = 384, $[M + Na]^+$ = 406) was in agreement with the structure composed of galactose and Glc3NAc, while NMR showed a quite different feature from an expected βGal1,4-linked disaccharide. Only two β-anomeric protons were observed, and they were highly deshielded (4.93 and 4.78 ppm) compared with the β-anomeric proton at the βGal1,4-linkage of lactose or *N*-acetyllactosamine (ca. 4.5 ppm). The information allowed us to assign the structure as βGal1,1βGlc3NAc. In order to confirm it, NOE experiments were carried out (Figure 1). Consequently, ca. 7% NOE was detected between H-1 and H-1′ on either irradiation at H-1 or at H-1′. No NOE was observed between H-1′ and H-4 on irradiation at H-1′. These NOE data confirmed the structure with a β1,1-linkage in contrast to a β1,4-linkage.[14]

A control experiment in the absence of GalT did now show any disaccharide formation, which excluded a potential nonenzymatic pathway.

All of these results showed that GalT catalyzed the βGal transfer to the β-anomeric position of Glc3NAc, and this finding is the first regio-mistaken reaction of GalT.[15,16] This reaction may be simply rationalized when the substrate structure is compared with GlcNAc (Figure 2). Obviously, the stereochemistry of the β-anomer along C-1 to C-4 is superimposable to that of C-4 to C-1 of GlcNAc. This identity may allow GalT to transfer

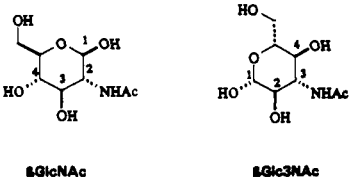

Figure 2. Stereochemical correlations between βGlcNAc and βGlc3NAc. The latter is shown from the reverse side of the ring plane.

galactose to the β-anomeric position of Glc3NAc.[17] The selective reaction of the β-anomer can be also rationalized because the stereochemistry of the α-anomer is comparable to that of GalNAc or galactose which is not accepted by GalT.

In summary, we have discovered a new type of GalT reaction with Glc3NAc, namely, βGal1,1 transfer. It provides new insights into the study of GalT, which has long been carried out based on the βGal1,4 transfer. The extension of this reaction to other acceptor substrates is in progress in our group and will be discussed in due course.

Acknowledgment. The authors are grateful to Alexander von Humboldt Stiftung for a fellowship to Y.N. and to the Deutsche Forschungsgemeinschaft, the Bundesministerium für Forschung und Technologie, and the Fonds der Chemischen Industrie for support of this study.

Supplementary Material Available: ^{1}H NMR spectra of Glc3NAc and Galβ1,1βGlc3NAc and ^{1}H-^{1}H COSY and FAB-MS spectra for Galβ1,1βGlc3NAc (7 pages). Ordering information is given on any current masthead page.

(12) GalT was purchased from Sigma Chemical Company (activity: 12 units per mg of protein; contamination: 4.4% of α-lactalbumin) and used without further purification.

(13) The reaction proceeded also in the absence, but was accelerated ca. 3 times in the presence, of α-lactalbumin.

(14) The structure was further confirmed by per-*O*-acetylation. NMR gave only two β-anomeric protons for the acetylated product. Substantial deshielding due to the geminal OAc group was not observed at H-1 (shift from 4.93 to 5.12 ppm) but at H-4 (shift from 3.44 to 4.94 ppm).

(15) Another regio-mistaken reaction by fucosyltransferase was discussed by Lemieux et al.[16]

(16) Khare, D. P.; Hindsgaul, O.; Lemieux, R. U. *Carbohydr. Res.* **1985**, *136*, 285.

(17) Glc3NAc satisfies the requirement for the acceptor substrate of GalT reported by Berliner et al.[6] for both βGal1,4 and βGal1,1 transfer. The latter reaction in the present study may be explained in the same way as the reaction of *N*-acetylmannosamine.[6]

CIDNP Detection of the 1,3-Dimethyluracil Dimer Radical Anion Splitting Sensitized by 2-Methylindole

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UV radiation (200–300 nm) causes adjacent pyrimidines in DNA to form cis, syn dimers through (2 + 2) photocycloaddition. The enzyme DNA photolyase is capable of repairing this damage by splitting the dimers back to monomers and uses visible or near UV light (300–500 nm).[1] Its activity has been attributed to dihydroflavin ($FADH_2$)[2] and either 5,10-methenyltetrahydrofolate or a deazaflavin[3] as noncovalently bound cofactors and a tryptophan residue.[1b]

It has been proposed that the dimer splitting involves electron transfer (ET) to or from the dimer.[4] Model compound studies have shown that both pathways can operate,[5] but on the basis of chemical and thermodynamic considerations, ET to the dimer

(1) (a) Sancar, A. Photolyase. In *Advances in Electron Transfer Chemistry*; Mariano, P. S.; JAI Press: London, 1992; Vol. 2, pp 215–272. (b) Kim, S.-T.; Li, Y. F.; Sancar, A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 900.

(2) Payne, G.; Heelis, P. F.; Rohrs, B. R.; Sancar, A. *Biochemistry* **1987**, *26*, 7121.

(3) (a) Jorns, M. S.; Wang, B.; Jordan, S. P.; Chanderkar, L. P. *Biochemistry* **1990**, *29*, 552. (b) Payne, G.; Wills, M.; Walsh, C.; Sancar, A. *Biochemistry* **1990**, *29*, 5706.

(4) Okamura, T.; Sancar, A.; Heelis, P. F.; Begley, T. P.; Hirata, Y.; Mataga, N. *J. Am. Chem. Soc.* **1991**, *113*, 3143.

(5) (a) Roth, H. D.; Lamola, A. A. *J. Am. Chem. Soc.* **1972**, *94*, 1013. (b) Rokita, S. E.; Walsh, C. T. *J. Am. Chem. Soc.* **1984**, *106*, 4589. (c) Jorns, M. S. *J. Am. Chem. Soc.* **1987**, *109*, 3133. (d) Hélène, C.; Charlier, M. *Photochem. Photobiol.* **1977**, *25*, 429. (e) Van Camp, J. R.; Young, T.; Hartman, R. F.; Rose, S. D. *Photochem. Photobiol.* **1987**, *45*, 365.

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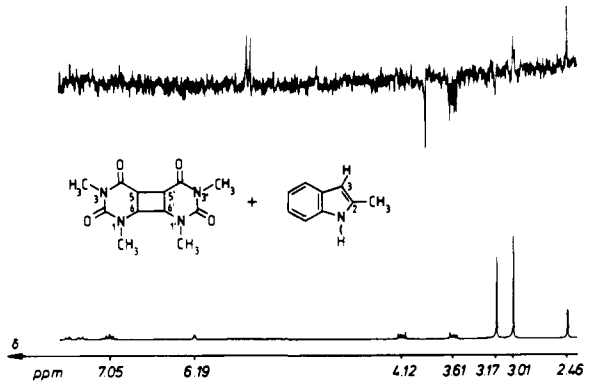


Figure 1. NMR spectra of 2MInd and DMUD in CD_3CN solution. Bottom: dark spectrum. Top: TR CIDNP spectrum 2 μs after laser flash (36 scans). The CIDNP for both methyls at N1 and N3 (3.01 and 3.17 ppm) should be emissive; the absorption at 3.01 ppm is due to incomplete presaturation. The line at 3.88 ppm is due to the spectrometer RF. The dark spectrum after photolysis contains additional lines of DMUM.

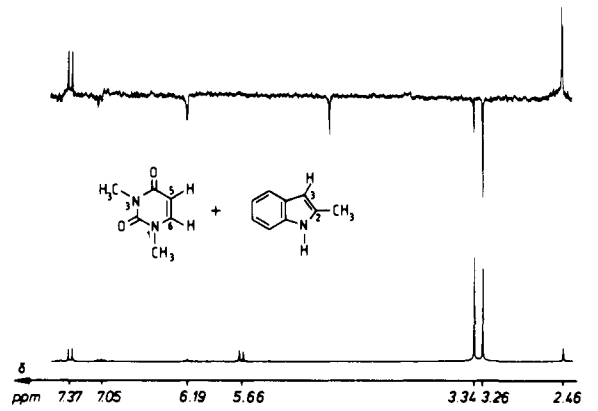


Figure 2. NMR spectra of 2MInd and DMUM in CD_3CN solution. Bottom: dark spectrum before and after photolysis. Top: TR CIDNP spectrum 2 μs after laser flash (36 scans). The line at 4.78 ppm is due to the spectrometer RF.

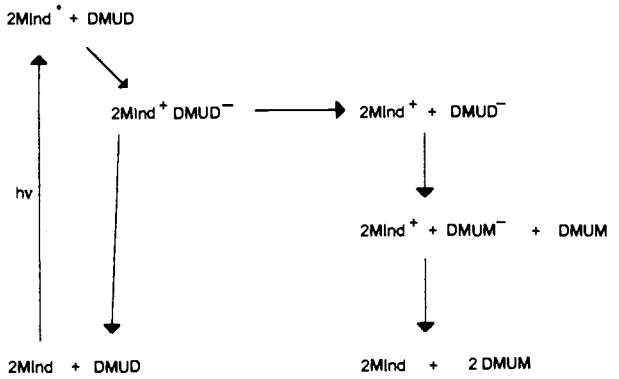
seems to be favored in vivo,[6] i.e., a dimer radical anion intermediate is involved which splits into the monomer and the monomer radical anion. The latter transfers the electron back to the enzyme chromophore, and as a result the DNA is repaired. Several model compound studies using optical measurements have supported this mechanism,[5e,7] but gave only indirect evidence for the existence of the dimer radical anion intermediate. From the appearance of the monomer radical anion a rate constant of $1.8 \times 10^6\ s^{-1}$ for the dimer anion splitting was inferred.[7a]

The ET process generates geminate radical ion pairs which through the nuclear spin sorting mechanism in a magnetic field should give rise to chemically induced nuclear spin polarization (CIDNP). This technique was applied earlier to study the pyrimidine dimer splitting via the cation mechanism,[5a,8a] and the dimer cation intermediate has been observed in experiments using anthraquinone-2-sulfonate and flavin as sensitizers.[8b,c] In this communication we report similar experiments which demonstrate the existence and the splitting of the dimer radical anion intermediate.

The model compounds used were *cis,syn*-1,3-dimethyluracil dimer (DMUD)[9] as electron acceptor and 2-methylindole (2MInd) as electron donor. Solutions of DMUD (50 mM) and 2MInd (50 mM) were prepared in acetonitrile, deoxygenated by freeze–pump–thaw cycles, and measured using a time-resolved (TR) CIDNP NMR spectrometer (308-nm, 15-ns pulse irradiation, 1.45 μs for 90° NMR pulse and complete presaturation).[10] Spectra are shown in Figure 1. The emissive CIDNP signal at 3.61 ppm is due to the cyclobutyl protons at position C5 of the dimer, and that at 3.17 ppm belongs to the methyl protons at N3. The 1,3-dimethyluracil monomer (DMUM) appears with enhanced absorptions at 5.66 ppm belonging to the C5 proton. The methyl group of the electron donor, 2MInd, shows enhanced absorption at 2.46 ppm.

The above CIDNP signal phases can be easily explained with Kaptein's rules[11] and Scheme I. From kinetic and fluorescence studies,[5e,7b] the excited singlet state ($\mu < 0$) of 2MInd is quenched by DMUD to give the radical ion pair $2MInd^+$ $DMUD^-$. Reverse

Scheme I. Dimer Splitting by 2MInd in Acetonitrile



electron transfer in the geminate ion pair regenerates 2MInd and DMUD in the singlet ground state ($\epsilon > 0$). The *g*-factor[12] of $DMUD^-$ is larger than that of $2MInd^+$, and the hyperfine coupling constants (A_i)[12b,13] for $DMUD^-$ are >0 for the C5 protons and both methyls at N1 and N3 and >0 for the methyl group of $2MInd^+$ at C2. This leads to emission for the CIDNP signals of DMUD (3.61, 3.17, and presumably, 3.01 ppm) and enhanced absorption for 2MInd at 2.46 ppm. The C5 proton of DMUM occurs in enhanced absorption (5.66 ppm) because DMUM results from the splitting of $DMUD^-$ after escape from the geminate pair ($\epsilon < 0$) and, finally, nongeminate reverse electron transfer which causes little polarization. In total, the polarizations are weak due to the small *g*-factor difference and, in addition, the existence of the two possible exit pathways, namely, singlet and triplet.[14] The emission pattern of DMUD implies that the unpaired electron resides mostly at the carbonyl group. These results correlate with MO calculations which suggested the existence of strong interactions between the SOMO in the carbonyl group and the cyclobutyl ring orbitals[15] and imply a large hyperfine coupling to

(6) (a) Kim, S. T.; Sancar, A. *Biochemistry* **1991**, *30*, 8623. (b) Heelis, P. F.; Deeble, D. J.; Kim, S. T.; Sancar, A. *Int. J. Radiat. Biol.* **1992**, *62*, 137.

(7) (a) Yeh, S.-R.; Falvey, D. E. *J. Am. Chem. Soc.* **1991**, *113*, 8557. (b) Young, T.; Kim, S.-T.; Van Camp, J. R.; Hartman, R. F.; Rose, S. D. *Photochem. Photobiol.* **1988**, *48*, 635. (c) Hélène, C.; Charlier, M. *Biochem. Biophys. Res. Commun.* **1971**, *43*, 252.

(8) (a) Kemmink, J.; Eker, A. P. M.; Kaptein, R. *Photochem. Photobiol.* **1986**, *44*, 137. (b) Young, T.; Nieman, R.; Rose, S. D. *Photochem. Photobiol.* **1990**, *52*, 661. (c) Hartman, R. F.; Rose, S. D.; Pouwels, P. J. W.; Kaptein, R. *Photochem. Photobiol.* **1992**, *56*, 305.

(9) Elad, D.; Rosenthal, I.; Sasson, S. *J. Chem. Soc. C* **1971**, 2053.

(10) Vollenweider, J.-K.; Fischer, H.; Hennig, J.; Leuschner, R. *Chem. Phys.* **1985**, *97*, 217.

(11) Kaptein, R. *J. Chem. Soc. D* **1971**, 732.

(12) For $DMUD^-$, $g = 2.0030$ based on the *g*-factor of glycine anhydride anion, which has a similar radical anion structure. (a) Sevilla, M. D.; Failor-Koszykowski, R. *J. Phys. Chem.* **1977**, *81*, 1198. For $2MInd^+$, $g = 2.0027$, based on the *g*-factor of the 1-methyl-2-phenylindole cation radical. (b) Carloni, P.; Eberson, L.; Greci, L.; Stipa, P.; Tosi, G. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1779. For $DMUM^-$, $g = 2.0030$. (c) Novais, H. M.; Steenken, S. *J. Am. Chem. Soc.* **1986**, *108*, 1.

(13) Close, D. M.; Bernhard, W. A. *J. Chem. Phys.* **1977**, *66*, 5244.

(14) The calculated ion pair energy for both DMUM and DMUD with 2MInd is higher than the triplet state of 2MInd and DMUM. (a) Yeh, S.-R.; Falvey, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 7313. (b) Merényi, G.; Lind, J.; Shen, X. *J. Phys. Chem.* **1988**, *92*, 134. (c) Kasama, K.; Takematsu, A.; Arai, S. *J. Phys. Chem.* **1982**, *86*, 2420. (d) Sasson, S.; Elad, D. *J. Org. Chem.* **1972**, *37*, 3164. If the triplet and singlet pathways are equally efficient, then no CIDNP would be observed. The CIDNP phases show that the singlet is more efficient than the triplet reaction.

(15) Hartman, R. F.; Van Camp, J. R.; Rose, S. D. *J. Org. Chem.* **1987**, *52*, 2684.

the proton at C5 and a small coupling to the proton at C6.

In a control experiment we also measured the CIDNP of deoxygenated solutions of DMUM (100 mM) and 2MInd (25 mM) in acetonitrile. The spectra are shown in Figure 2. DMUM shows emission at 3.26 and 3.34 ppm due to the two methyl groups at N3 and N1, respectively, and enhanced absorptions at 7.37 ppm for the proton at C6. 2MInd shows again enhanced absorption at 2.46 ppm and, in addition, emission at 6.19 ppm for the proton at C3, as well as weak emission and absorption for the aromatic protons. The phases can be explained again with the aid of Kaptein's rules by assuming conditions similar to those in the DMUD case for geminate recombination, but now with a large and negative coupling to the C6 and little coupling to the C5 proton (7.37 vs 5.66 ppm). This agrees with spin density calculations for DMUM radical anion which predict high spin density at C6 and small spin density at C5.[13]

There is a distinct difference between the CIDNP spectra of DMUM formed from DMUD cleavage (Figure 1) or from the purely cyclic ET process (Figure 2). This is the consequence of the difference of precursors, i.e., the parent radical anions and their spin density distributions. We did not observe any CIDNP signal due to the C6 ring proton for DMUM after DMUD cleavage which could arise from geminate[16] and from nongeminate $2MInd^+$ $DMUM^-$ pairs. This implies that the splitting of $DMUD^-$ does not compete effectively with the geminate reverse ET and that the concentration of free $DMUM^-$ is low. Further work is in progress to elucidate more kinetic information and donor and solvent dependences.

Acknowledgment. Financial support by the Swiss National Foundation for Scientific Research is gratefully acknowledged.

(16) Via the CIDNP memory effect. Den Hollander, J. A. *Chem. Phys.* **1975**, *10*, 167.

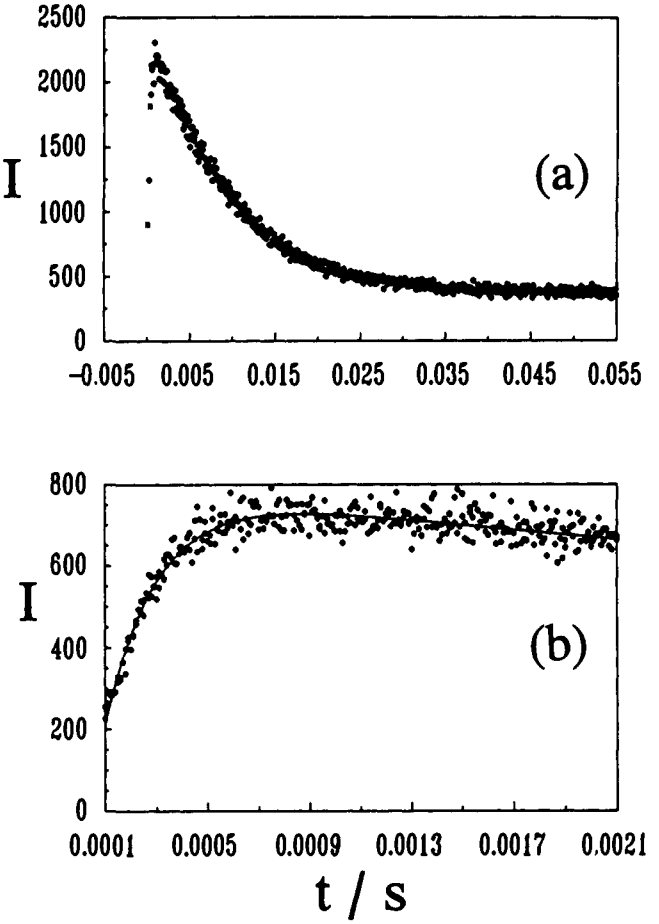


Figure 1. Plot of fluorescence intensity I (including background scattered light) vs time t obtained at (a) low and (b) high time resolutions, corresponding to the conditions of Figure 2.

Investigation of the Gas-Phase Reaction of Trimethylsilyl Radicals with HBr: Measurement of the $(CH_3)_3Si-H$ Bond Strength

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There is considerable variation between estimates of the Si–H bond dissociation enthalpy in trimethylsilane, $D_{298}((CH_3)_3Si-H)$. Walsh suggested a value equal to $D_{298}(SiH_3-H)$ mainly on the basis of iodination kinetics,[1] while McKean et al.[2] and Bernheim et al.[3] suggested that the three methyl groups weaken the Si–H bond by 24 and 57 kJ mol^{-1}, respectively, on the basis of vibrational spectroscopy. By contrast, Ding and Marshall proposed a bond strengthening of 14 kJ mol^{-1} on the basis of bromination experiments.[4] In that work the temperature dependence of the measured reverse rate constant k_{-1} for

$$(CH_3)_3Si + HBr \rightleftharpoons (CH_3)_3SiH + Br \qquad (1)$$

was combined with an *estimated* activation energy for k_1 in a second-law analysis. We have now carried out the first measurement of k_1 at room temperature to obtain the equilibrium constant $K_{eq} = k_1/k_{-1}$, and hence the thermochemistry, via a third-law method, without kinetic assumptions. The spread of ΔH implied by the range of $D_{298}((CH_3)_3Si-H)$ corresponds to a factor of 10^{12} variation in K_{eq}, so that even an approximate determination of k_1 dramatically reduces the uncertainty in the thermochemistry. We find $k_1 \approx 8 \times 10^{-11}$ cm^3 s^{-1}, which implies an Si–H bond strength of 398 ± 2 kJ mol^{-1}, about 14 kJ mol^{-1} greater than that in SiH_4.[5]

The flash photolysis apparatus has been described in detail elsewhere.[6,7] Reagents were purified by distillation and stored in the dark. Trimethylsilyl radicals were generated by pulsed photolysis of trimethylsilyl iodide (Hüls America, Inc.) with a small flash lamp through Suprasil quartz optics, and preliminary experiments[8] with resonance fluorescence detection confirmed that I atoms were formed. Halogen exchange between $(CH_3)_3SiI$ and HBr is negligible.[9] $(CH_3)_3Si$ reacted with excess HBr (Matheson Gas Products) diluted in Ar bath gas under pseudo-first-order conditions. The course of reaction was followed by monitoring the product Br by means of time-resolved atomic resonance fluorescence with pulse counting and multichannel scaling. A few millibars of H_2 was added to equilibrate $Br(^2P_{1/2})$ and $Br(^2P_{3/2})$,[10] so that effectively only $Br(^2P_{3/2})$ was detected. k_1 was found to be almost gas-kinetic; thus reaction 1 cannot be significantly endothermic. This rules out $Br(^2P_{1/2})$, excited by 44.0 kJ mol^{-1},[11]

(1) Walsh, R. In *The Chemistry of Organic Silicon Compounds*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1989; Chapter 5.
(2) McKean, D. C.; Torto, I.; Morrisson, A. R. *J. Phys. Chem.* **1982**, *86*, 307.
(3) Bernheim, R. A.; Lampe, F. W.; O'Keefe, J. F.; Qualey, J. R. III *J. Phys. Chem.* **1985**, *89*, 1087.
(4) Ding, L.; Marshall, P. *J. Am. Chem. Soc.* **1992**, *114*, 5756.
(5) Seetula, J. A.; Feng, Y.; Gutman, D.; Seakins, P. W.; Pilling, M. J. *J. Phys. Chem.* **1991**, *95*, 1658.
(6) Shi, Y.; Marshall, P. *J. Phys. Chem.* **1991**, *95*, 1654.
(7) Ding, L.; Marshall, P. *J. Phys. Chem.* **1992**, *96*, 2197.
(8) Paper to be published.
(9) Doncaster, A. M.; Walsh, R. *J. Phys. Chem.* **1979**, *83*, 3037.
(10) Nicovich, J. M.; van Dijk, C. A.; Kreutter, K. D.; Wine, P. H. *J. Phys. Chem.* **1991**, *95*, 9890.
(11) Okabe, H. *Photochemistry of Small Molecules*; Wiley: New York, 1978.

0002-7863/93/1515-2539$04.00/0