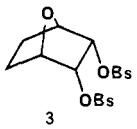
We have been able to bring about considerable enhancement of participation by double bonds and phenyl rings (π participation) through placement of an electron-withdrawing group (EWG) adjacent to the leaving group.[7] Removal of charge through π participation decreases the destabilizing interaction between the developing positive charge and the adjacent EWG. We termed this phenomenon inductive enhancement of participation.[8] Recently Wilcox and Brungardt have observed inductive enhancement of σ participation.[9] We report herein that the placement of an aptly disposed EWG in the 7-oxanorborn-2-yl system has accomplished two goals. (1) We have observed the first example of inductive enhancement of n (lone pair) participation. (2) We have brought about the participation of the 7-oxygen atom in the 2-norbornyl structure, which Martin and Bartlett[2] were unable to observe. This participation is signified by an inverse exo/endo ratio.

The systems we have studied are the 7-oxabicyclo[2.2.1]hepta-2,3-diyl dibrosylates **3** and **4**. The second brosylate group serves



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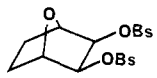

4

as the EWG that induces participation. We compare the **4**/**3** exo/endo ratio with that from the monobrosylates, **2**-OBs/**1**-OBs.[10] Rates were measured titrimetrically in buffered acetic acid at three temperatures. From the activation parameters, rates were calculated at 25, 100, and 200 °C for the purpose of computing ratios. At 25 °C, the following rates were obtained: the *endo*-brosylate **1** 2.26×10^{-11} s^{-1}, the exo brosylate **2** 1.34×10^{-8} s^{-1}, the *endo,endo*-dibrosylate (**3**) 8.10×10^{-16} s^{-1}, and the *exo,exo*-dibrosylate (**4**) 5.51×10^{-16} s^{-1}. Thus the exo/endo rate ratio for the monobrosylates is 590 and for the dibrosylates is 0.68 at 25 °C. The analogous numbers are 450 and 0.57 at 100 °C and 350 and 0.49 at 200 °C.

The inversion of the exo/endo ratio from 590 to 0.68 at 25 °C, a total factor of about 900, represents the inductive enhancement of oxygen participation. Product studies were consistent with a high level of oxygen participation in the *endo,endo*-dibrosylate **3**, which gave 90% of the retained *endo,endo*-diacetate. Most probably, each brosylate is removed with oxygen participation, one at a time, with about 95% retention. In contrast, acetolysis of the *endo*-monobrosylate **1**-OBs gave about 60% retained *endo*-acetate **1**-OAc as the main product, as well as about 10% 1-acetoxy-3-formylcyclopentane and 10% of the inverted *exo*-acetate **2**-OAc.[11]

In summary, we have induced 7-oxygen participation in the departure of a 2-brosyloxy group by the introduction of an electron-withdrawing group at the 3-position. The earlier work of Martin and Bartlett had concluded that such participation did not occur in the 2-chloro system, presumably because of relatively poor orbital overlap. In the *endo,endo*-dibrosylate, oxygen participation can reduce the unstable interaction between the developing positive charge at the 2-position and the remaining brosyloxy group at the 3-position. This inductive enhancement of oxygen participation amounts to a factor of about 900 in terms of exo/endo ratios and results in the unusual inverse ratio for the pair **4**/**3** (endo faster than exo). The observation also comprises the first example of enhanced participation by lone pair electrons through altered electron demand.

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(10) Details of the syntheses will be reported elsewhere. All new compounds gave satisfactory elementary analyses and spectral properties.
(11) The *exo*-monobrosylate **2**-OBs gave a mixture of 94% retained acetate **2**-OAc and 6% of 1-acetoxy-3-formylcyclopentane. The *exo,exo*-dibrosylate **4** gave neither the *exo,exo*- nor the *endo,endo*-diacetate. The products were converted to an unidentified, insoluble solid under reaction conditions.

Applications of Two-Dimensional NMR Methods in Photochemically Induced Dynamic Nuclear Polarization Spectroscopy

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Photochemically induced dynamic nuclear polarization (photo-CIDNP) provides a means to study surface residues of proteins[1] and single-stranded regions in nucleic acids.[2] The technique has been successful in determining the accessibility of amino acid residues to a photoexcited dye and their involvement in ligand interactions. Photo-CIDNP difference spectra, taken as "light" minus "dark" spectra, contain only resonances of the polarized residues. Although these spectra provide a great simplification with respect to normal NMR spectra of proteins, they can still be quite complex especially when they contain cross-polarization effects (transfer of polarization to nuclei close to the primarily polarized ones by dipolar cross relaxation[3,4]). A very powerful way to study transfer of magnetization in general is provided by two-dimensional (2D) NMR spectroscopy.[5] Therefore we explored the use of 2D NMR techniques in photo-CIDNP spectroscopy and present the combination of photo-CIDNP with 2D *J*-correlated spectroscopy[6] (CIDNP-COSY) and with 2D NOE spectroscopy[7] (CIDNP-NOESY).

The 2D approach normally requires that the experiment is repeated for a large number of t_1 values.[5-7] This presents a problem in photo-CIDNP experiments, because the CIDNP intensity generally decreases upon repeated irradiations due to dye exhaustion. In order to sustain the CIDNP intensity during a 2D experiment the sample was stirred after each "light" scan by increasing the sample spinning rate for a short time under computer control. This enabled us to carry out 2D experiments with 64 t_1 values in the present examples. To obtain sufficient resolution a small spectral width was chosen in the ω_1 domain covering only the aromatic region of the photo-CIDNP spectrum.

The sequences employed in the 2D CIDNP experiments are shown in Figure 1. Background magnetization is suppressed by homonuclear broad-band saturation[8] and polarization is induced photochemically during the preparation period. After frequency labeling during the evolution period t_1 this polarization is redistributed among the spins during the mixing period. Thus, in the CIDNP-COSY experiment a coherence transfer is brought about by the second 90° pulse and polarization becomes detectable during t_2 also on spins that are *J*-coupled to the originally polarized ones. In the CIDNP-NOESY experiment mixing is effectuated by cross relaxation during t_m so that spins that are close in space to an originally polarized one may receive polarization as well. The asymmetry in the mixing process causes the characteristic asymmetric appearance of 2D photo-CIDNP spectra: only those

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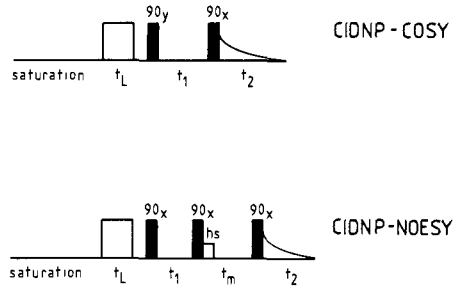

Figure 1. Pulse sequences used for the experiments described in this paper. Black boxes denote rf gating pulses. Open boxes represent other computer-controlled pulses that are used for shutter control during the illumination period t_L or for applying a homogeneity-spoil pulse (hs). During the saturation period a train of 90° pulses is applied that results in an efficient broad-band saturation of the ^1H NMR transitions.

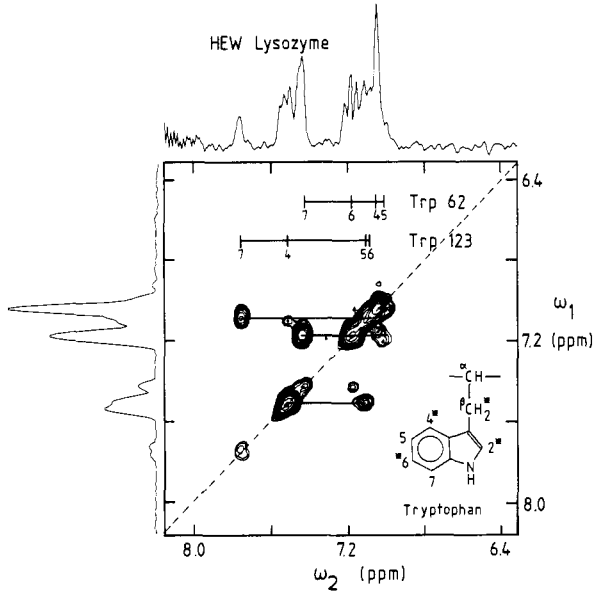

Figure 2. Aromatic region of the CIDNP-COSY spectrum of hen egg white lysozyme (1 mM) in the presence of 0.4 mM 3-*N*-(carboxymethyl)lumiflavin (a generous gift from Dr. F. Mueller, Wageningen), pH 6.0, 27 °C in ^2H_2O. Laser irradiation was during 0.1 s with 5-W argon-ion laser power, followed by a 0.05-s delay. Sixty-four experiments were performed and only one "light" free induction decay was recorded at each t_1 value with the carrier frequency at the low-field side of the aromatic region (no quadrature detection in ω_1 necessary). To suppress background magnetization (including axial peaks) a "dark" spectrum was recorded by interweaving 16 experiments without laser irradiation at each t_1 value and the difference spectrum ("light" minus "dark") was calculated after appropriate scaling. A spectral width of 714 Hz was chosen for ω_1 domain. The difference spectrum is presented in the absolute-value mode. Note the differences between the two projection spectra: the ω_1 spectrum resembles a conventional photo-CIDNP spectrum, since only resonances that were polarized during the preparation period are frequency labeled during t_1; coherent transfer of polarization, caused by the mixing pulse, is only apparent in the ω_2 projection.

polarized magnetization components that were created during the preparation period (by directly induced polarization and by cross polarization) appear as diagonal peaks. Associated cross peaks (at the same ω_1 frequency) are indicative of polarization transfer during the mixing period.

Figure 2 shows a CIDNP-COSY spectrum of the protein lysozyme from hen egg white. From earlier photo-CIDNP work on this protein[9,10] it is known that two tryptophan residues (Trp 62 and 123) are accessible to the flavin dye, and the aromatic region of the spectrum shows the primarily polarized C(2)H, C(4)H, and C(6)H resonances of these residues as diagonal peaks.

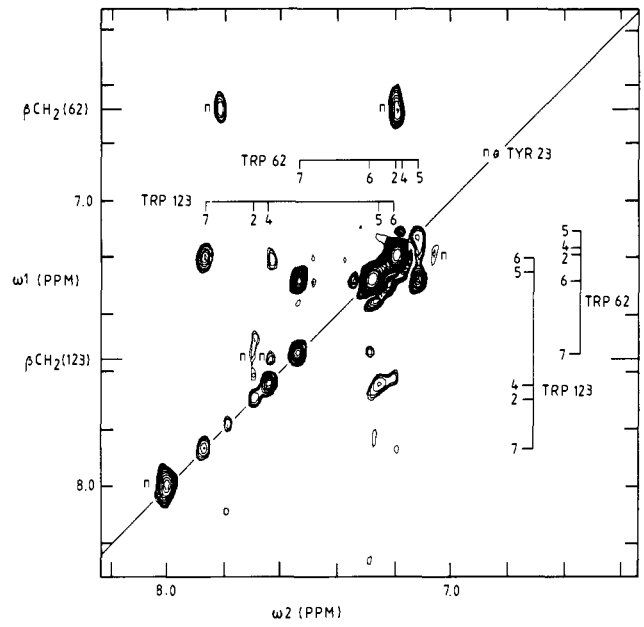

Figure 3. Aromatic region of the CIDNP-NOESY spectrum of hen egg white lysozyme. The same conditions were employed as for the CIDNP-COSY spectrum shown in Figure 2. The carrier frequency and the spectral width (714 Hz) were chosen such that cross peaks associated with polarized CH_2 resonances fold back into empty regions of the spectrum. A 30-ms homogeneity-spoil pulse was inserted at the beginning of the mixing period t_m to destroy coherences (except zero-quantum coherences). The mixing time was 0.2 s. Again, one "light" and 16 "dark" scans were accumulated at 64 t_1 values, and the difference spectrum is presented in the pure absorption mode. Negative peaks are indicated with n.

The C(5)H and C(7)H resonances are visible as polarized cross peaks, thus establishing both their chemical shifts and the through-bond connectivities. The C(5) and C(7) protons of Trp 123 could not be identified unequivocally in previous 1D photo-CIDNP experiments.[9] The emissively polarized CH_2 resonances appear as diagonal peaks (not shown), which are associated with cross peaks at the corresponding CH resonance frequencies. Thus the through-bond connectivities in the spin systems of both tryptophans can be traced in this CIDNP-COSY spectrum.

The CIDNP-NOESY spectrum of the same protein is shown in Figure 3. Apart from the through-bond contacts visible in the CIDNP-COSY spectrum, several other through-space connectivities are evident. At the (folded-in) ω_1 frequencies of the emissively polarized CH_2 resonances, negative cross peaks are visible in the aromatic region at the ω_2 frequencies of the C(2)H and C(4)H resonances of the same residue. In addition a negative cross peak is associated with the CH_2 resonance of Trp 62 at the ω_2 frequency of the C(2)H of Trp 63, which is one example of several interresidue contacts visible in this spectrum. Some resonances (like the CH resonances of tryptophans) are polarized by cross relaxation with both positively polarized (C(2)H) and negatively polarized (CH_2) protons, which causes a partial canceling in a 1D cross-polarization experiment. In the CIDNP-NOESY experiment these different cross-relaxation pathways lead to separate cross peaks of opposite signs. A more detailed analysis of these spectra will be presented elsewhere.

The experiments presented here demonstrate that CIDNP-COSY and CIDNP-NOESY are useful extensions of the photo-CIDNP technique for examining surface residues and their immediate environment in biological macromolecules.

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Registry No. Trp, 73-22-3; lysozyme, 9001-63-2.

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