Table I. ^{31}P NMR Chemical Shifts (145.61 MHz) and Coupling Constants (Hz)[a]

compd	chem shifts, ppm α-^{31}P	β-^{31}P	γ-^{31}P	coupling constants, Hz $J_{\alpha\beta}$	$J_{\beta\gamma}$
UTP (1)	-13.724	-23.966	-8.426	19.5	19.2
CTP (4)	-13.679	-23.966	-8.434	19.3	19.2
[γ-$^{18}O_4$]ATP (6a)	-13.643	-23.900	-8.473	19.5	19.1
[β-^{18}O,γ-$^{18}O_3$]ATP (6b)	-13.643	-23.911	-8.457	19.5	19.1

[a] A 1-mL solution of 1.7 mM [$\gamma^{18}O_4$]ATP (6a), 2 mM UTP (1), 5 mM $MgCl_2$, and 0.5 mM $(NH_4)_2SO_4$ in 50 mM Hepes buffer, pH 8.0, was incubated for 3 h at 38 °C in the presence of 75 μg of CTP synthetase (protein determined according to Bradford[5]). The reaction was stopped by the addition of 0.5 mL of 0.2 M EDTA in 1 M Tris buffer, pH 8.5, and 4 drops of CCl_4. After vortexing and centrifuging, the solution was filtered into the NMR tube. 2000 scans were accumulated with an acquisition time of 1.6 s and a sweep width of 5 kHz. Zero filling to 64K was applied prior to Fourier transformation. All chemical shifts are relative to phosphate = 0 ppm.

Table II. Positional Isotope Exchange Experiments[a]

expt	concentration, mM UTP (1)	$MgCl_2$	ADP	enzyme, μg/mL	ratio of the γ-P peaks due to $P^{18}O_4$:$PO^{18}O_3$	V_{PIX}/V_{cat}
1	2.0	30	0	0	80:20	0
2	2.0	0	0	60	80:20	0
3	0	30	0	60	80:20	0
4	2.0	30	0	60	53:47	0.03
5	2.0	30	2.0	60	54:46	0.03

[a] One milliliter of a solution containing 1.4 mg/mL CTP synthetase, 2.5 mM ATP, 2.5 mM UTP, 10 mM $MgCl_2$, 1 mM EDTA, 0.2 M Hepes buffer, pH 8.0, and 20% glycerol was passed through a small Sephadex G-25 column equilibrated with 60 mM Hepes buffer, pH 8.0, and 1 mM EDTA.[2a] One-milliliter solutions containing 2.0 mM [γ-$^{18}O_4$]ATP (6a) and the substrates listed in the table in 50 mM Hepes buffer, pH 8.0, were incubated for 2 h at 38 °C. The reaction was stopped and the NMR spectra taken as described in Table I. V_{PIX} was determined according to Rose,[6] V_{cat} according to Anderson.[2a] The initial ^{18}O content in each of the four positions at the γ-P of 6a was 96% (γ-$P^{18}O_4$:γ-$PO^{18}O_3$ = 80:20). The reaction was followed by measuring the γ-^{31}P resonances of ATP, because the peaks are better separated than the β-^{31}P resonances.[3]

[γ-$^{18}O_4$]ATP (6a) was synthesized by the method of Midelfort and Rose.[4] A preliminary experiment (Table I) showed that it is possible to determine the concentrations of both labeled ATP's (6a and 6b) in the presence of UTP by measuring the integrals of the ^{31}P NMR signals of the γ-P atoms.

The results (Table II) clearly showed the following: (1) There is no ATP/ADP exchange in the presence of UTP (experiment 5). This exchange would have increased the ^{31}P NMR signal of the unlabeled β-phosphorus atom of ATP. A small signal of that atom is initially present because of incomplete ^{18}O labeling of [γ-$^{18}O_4$]ATP (6a). (2) Positional isotope exchange of ^{18}O from the β-γ-bridge position into the β-nonbridge position of ATP is observed only if UTP, Mg^{2+}, and CTP synthetase are present (experiment 4). This is consistent with the lower pathway of Scheme I. To be meaningful, the PIX rate has to be at least 50% as fast as V_{max} in the slower direction of catalysis.[6] Because the back reaction (deamination of CTP) has never been observed with CTP synthetase, the PIX rate, which is a minimum rate for the first partial reaction, is a meaningful measure of the phosphorylation of UTP by ATP.

Thus, our results provide the first experimental evidence for the stepwise nature of the reactions catalyzed by CTP synthetase, a mechanism entertained by Westheimer[7] several years ago. The mechanism proceeds by formation of a phosphorylated pyrimidinone, and compounds like 5 are easily attacked by nucleophiles even nonenzymatically.[8]

Acknowledgment. We gratefully acknowledge support for this work from the National Science Foundation (PCM-8409737, J.J.V.) and the National Institutes of Health (GM-22434, P. M.A.).

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(4) Midelfort, C. F.; Rose, I. A. *J. Biol. Chem.* **1976**, *251*, 5881-5887.
(5) Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248-254.
(6) Rose, I. A. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1979**, *50*, 361-395.
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Inversion of the Exo/Endo Rate Ratio by Inductive Enhancement of Oxygen Participation

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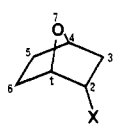
We report the previously unobserved participation of oxygen in the 7-oxanorborn-2-yl system through manipulation of electron demand at the reaction center. In 1957, Martin and Bartlett established that such an oxygen atom does not provide significant anchimeric assistance in the departure of 2-chloride.[2] The anticipated participation would have occurred in the endo isomer (1-Cl) and would have been signified by a reduced or even an



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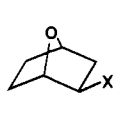

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inverse exo/endo ratio, i.e., a ratio less than unity. Martin and Bartlett observed a relatively normal exo/endo ratio of 310 at 25 °C (2-Cl/1-Cl). Moreover, the product in both exo and endo cases was 3-formylcyclopentanol, the expected result of rearrangement of a localized carbocation. The electron-withdrawing nature of oxygen, its low polarizability, and an imperfect antiperiplanar arrangement between O and X in 1 contribute to the absence of participation in this system.[3]

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(3) Heteroatom participation has been observed, however, in some related cases. Location of the oxygen atom at the 6-position (see 1) gives rise to anchimeric assistance in the exo isomer, presumably because of a more nearly antiperiplanar relationship between O and *exo*-X.[4] Oxygen at the bridging 7-position in the less rigid oxabicyclo[4.2.1]non-2-yl system participates for similar reasons.[5] The much more highly polarizable sulfur atom participates very strongly even at the 7-position of *endo*-2-norbornyl systems and gives rise to inverse exo/endo ratios.[6]
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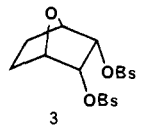
We have been able to bring about considerable enhancement of participation by double bonds and phenyl rings (π participation) through placement of an electron-withdrawing group (EWG) adjacent to the leaving group.[7] Removal of charge through π participation decreases the destabilizing interaction between 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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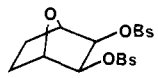

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as the EWG that induces participation. We compare the **4**/**3** exo/endo ratio with that from the monobrosylates, **2**-OBs/**1**-OBs.[10] Rates were measured titrimetrically in buffered acetic acid at three temperatures. From the activation parameters, 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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