When subjected to $H_2O$, the epoxy alcohol and (tritox)H are cleaved from the zirconium; the cleaved material was correlated with authentic $HOCMe_2\overline{CHCH_2O}$[23]via $^1H$ NMR (300 MHz) and capillary GC.

The observations above complement the proposed mechanism[20] for Sharpless' epoxidation procedure.[19] The presumed transient methylperoxy species $(tritox)_2Zr(\eta^2\text{-}OOMe)(OCMe_2CH{=}CH_2)$ (**11**) as well as the zirconium alkoxy epoxide complex **12** models intermediates in the Ti-catalyzed process. Since the epoxidation above was carried out under rigorously anhydrous conditions, the premise that a $Ti(\eta^2\text{-}OO\text{-}t\text{-}Bu)$ and *not* a bound *t*-BuOOH species is responsible for the O-atom transfer step in the Sharpless system is further substantiated. The facile dioxygen insertion reactions are of potential relevance to heterogeneous oxidation processes; the results suggest that surface alkyls may react directly with $O_2$, thus obviating the need for dissociative absorption of dioxygen.[24] Attempts to observe M–OOR complexes from the treatment of other $(tritox)_2(R'O)MR$[25] species with $O_2$, further exploitation of the oxygen transfer mediation by early metal-alkyls, and mechanistic studies of the insertion and exchange processes are currently under way.

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**Registry No. 1**, 89958-92-9; **2**, 89958-91-8; **3**, 94136-93-3; **4**, 94136-94-4; **5**, 89958-93-0; **6**, 94136-95-5; **7**, 94136-96-6; **8**, 94136-97-7; **9**, 94136-98-8; **10**, 94136-99-9; **12**, 94137-00-5; $HOCMe_2\overline{CHCH_2O}$, 19482-44-1; $LiOCMe_2CH{=}CH_2$, 94137-01-6; $(tritox)ZrCl_2$, 94137-02-7; $O_2$, 7782-44-7.

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## Cytidine-5′-triphosphate Synthetase Catalyzes the Phosphorylation of Uridine 5′-Triphosphate by Adenosine 5′-Triphosphate

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Cytidine-5′-triphosphate (CTP) synthetase from *Escherichia coli* catalyzes the following irreversible reaction:[1,2]

$$UTP + ATP + NH_3 \xrightarrow{Mg^{2+}} ADP + P_i + CTP$$

(1) Abbreviations: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; UTP, uridine 5′-triphosphate; CTP, cytidine 5′-triphosphate; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; PIX, positional isotope exchange; ●, $^{18}O$.
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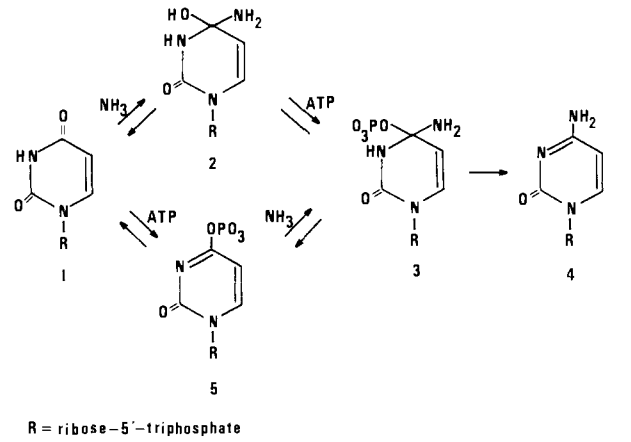
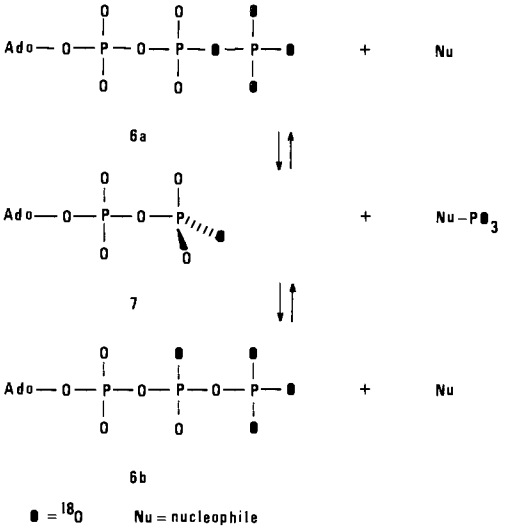
**Scheme I**




**Scheme II**




The lack of ATP/ADP exchange leads to the conclusion that ammonia attacks UTP (**1**) to form the carbinol amine **2**, which is then phosphorylated by ATP. Subsequent release of phosphate from **3** yields CTP (**4**) (Scheme I, upper pathway).[2b] We present evidence that CTP synthetase catalyzes the direct phosphorylation of UTP by ATP in the absence of ammonia (Scheme I, lower pathway).

One reason for the failure to observe ATP/ADP exchange could be that the catalytic reaction follows an ordered mechanism, in which the products (including ADP) are released only after the addition of all substrates (including ammonia). Therefore, we designed a positional isotope exchange (PIX) experiment to answer the question of whether ATP phosphorylates UTP (**1**). This experiment has an advantage over ATP/ADP-exchange experiments in that exchange of ADP between enzyme and solution is not necessary. The strategy for the PIX experiment is as follows.

During the catalytic reaction of CTP synthetase, ATP is cleaved between the $\beta$–$\gamma$-bridge oxygen atom and the $\gamma$-phosphorus atom. The nucleophile accepting the phosphate group might either be the enzyme, UTP (**1**), or the carbinol amine **2**. Cleavage in the absence of UTP and ammonia would provide evidence that a group on the enzyme becomes phosphorylated, whereas cleavage in the presence of UTP and absence of ammonia would likely result from the phosphorylation of UTP (Scheme I, lower pathway). For these experiments [$\gamma$-$^{18}O_4$]ATP (**6a**) is used and in each case leads to [$\beta$-$^{18}O$]ADP (**7**). If the terminal phosphate group of **7** rotates fast, the $^{16}O$ and $^{18}O$ atoms have equal probability to attack the phosphorylated intermediate in the back reaction to reform ATP and UTP. The result is that [$\beta$-$^{18}O$,$\gamma$-$^{18}O_3$]ATP (**6b**) is produced (Scheme II). The relative amounts of the labeled ATP molecules **6** are conveniently determined by NMR spectroscopy owing to the influence of $^{18}O$ on the chemical shifts of the $^{31}P$ nucleus.[3]

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

| compd | chem shifts, ppm | | | coupling constants, Hz | |
|---|---|---|---|---|---|
| | $\alpha$-$^{31}P$ | $\beta$-$^{31}P$ | $\gamma$-$^{31}P$ | $J_{\alpha\beta}$ | $J_{\beta\gamma}$ |
| UTP (**1**) | -13.724 | -23.966 | -8.426 | 19.5 | 19.2 |
| CTP (**4**) | -13.679 | -23.966 | -8.434 | 19.3 | 19.2 |
| [$\gamma$-$^{18}O_4$]ATP (**6a**) | -13.643 | -23.900 | -8.473 | 19.5 | 19.1 |
| [$\beta$-$^{18}O$,$\gamma$-$^{18}O_3$]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 $\mu$g 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 | | | enzyme, $\mu$g/mL | ratio of the $\gamma$-P peaks due to $P^{18}O_4$: $PO^{18}O_3$ | $V_{PIX}/V_{cat}$ |
|---|---|---|---|---|---|---|
| | UTP (**1**) | $MgCl_2$ | ADP | | | |
| 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 [$\gamma$-$^{18}O_4$]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 $\gamma$-P of **6a** was 96% ($\gamma$-$P^{18}O_4$:$\gamma$-$PO^{18}O_3$ = 80:20). The reaction was followed by measuring the $\gamma$-$^{31}P$ resonances of ATP, because the peaks are better separated than the $\beta$-$^{31}P$ resonances.[3]

[$\gamma$-$^{18}O_4$]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 $\gamma$-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 $\beta$-phosphorus atom of ATP. A small signal of that atom is initially present because of incomplete $^{18}O$ labeling of [$\gamma$-$^{18}O_4$]ATP (**6a**). (2) Positional isotope exchange of $^{18}O$ from the $\beta$-$\gamma$-bridge position into the $\beta$-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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## Inversion of the Exo/Endo Rate Ratio by Inductive Enhancement of Oxygen Participation

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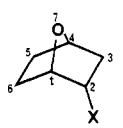
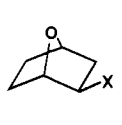
We report the previously unobserved participation of oxygen in the 7-oxanorborn-2-yl system through manipulation of electron demand at the reaction center. In 1957, Martin and Bartlett established that such an oxygen atom does not provide significant anchimeric assistance in the departure of 2-chloride.[2]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




1

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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]

(1) This work was supported by the National Science Foundation (Grant CHE83-12285).
(2) Martin, J. C.; Bartlett, P. D. *J. Am. Chem. Soc.* **1957**, *79*, 2533-2541.
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