severe steric repulsion between Bu_3Sn and TBDPS groups, whereas such steric hindrance is significantly diminished in **13**.

It was seemed likely that the steric size of the 2-(hydroxymethyl) substituent on the tetrahydropyran ring would play an important role in the asymmetric induction of these allylstannane–aldehyde condensations. To test this hypothesis, we prepared the corresponding OCH_3 (**15**, 2-[(methoxymethyl)oxy] THP) and CH_3 (**16**, 2-methyl THP) derivatives of the γ-alkoxyallylstannane. The Lewis acid-mediated reaction of **15** and **16** with benzaldehyde gave the syn adducts in good yields, but the diastereomer ratios of *RR*:*RS* were 2.5:1 and 1:1, respectively. Therefore, it is now clear that steric bulk of the alkoxy group is important to obtain high enantio- and diastereoselection.

Synthesis of **2a** is representative (entry 3 of Table I). In a 50-mL two-necked flask under Ar were placed dry CH_2Cl_2 (1 mL) and anhyd $AlCl_3$ (35 mg, 0.26 mmol), purified by sublimation of commercially available material. Benzaldehyde (45 μL, 0.44 mmol) was slowly added at −78 °C, and the resulting mixture was stirred for 30 min. To the resulting homogeneous solution was added slowly a dry CH_2Cl_2 (1 mL) solution of **1** (158 mg, 0.22 mmol), cooled at −78 °C. The reaction was continued for 10 min and quenched with MeOH. The mixture was allowed to warm to rt. Extraction with ether, concentration in vacuo, treatment with aqueous KF solution at rt for 1 h, extraction with ether, washing with brine, drying ($MgSO_4$), concentration in vacuo, and purification by flash column chromatography 15 cm × 17 mm; hexane, 50 mL and then hexane:AcOEt = 10:1) gave **2a** (58.9 mg, 0.114 mmol) in 52% yield.

Supplementary Material Available: Synthetic methods, characterization data, and NMR spectra (16 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Synthesis of an Acylphosphate Driven by a Proton Gradient. A Model for H^+-ATPase

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Summary: We describe the first model for a proton pump, H^+-ATPase. This model uses the energy from an indirect transfer of two protons from a solution at pH 0.3 to a solution at pH 10 to drive the synthesis of a high-energy phosphate, citraconyl phosphate.

H^+-ATPases link proton transfer across cell membranes to the synthesis or hydrolysis of ATP. Some H^+-ATPases synthesize ATP using a proton gradient as the driving force (e.g., F_oF_1-ATPase in mitochondria); others create a proton gradient using the hydrolysis of ATP as the driving force (e.g., H^+/K^+-ATPase in the mucosa of the stomach).[1] While some mechanistic features of these enzymes are known,[2] the molecular-level mechanism of coupling is not. Other models of active transport have shown how proton transport can drive the transport of other cations;[3] this is the first model that shows how proton transport can drive the synthesis of a high-energy phosphate, citraconyl phosphate.

To model a proton gradient across a lipid bilayer, we separated solutions of pH 0.3 and pH 10 with a layer of chloroform in a concentric ring cell, Figure 1a. When the acidic compartment contained citraconic acid (1.0 M), protons (detected by pH stat) and citraconate dianion (detected by ^{1}H-NMR) were transferred to the basic compartment.

Citraconic acid did not pass directly from the acidic to the basic compartment. Instead, citraconic acid dehydrated to the anhydride, which then diffused to the basic compartment, hydrolyzed, and generated two protons, Figure 1b. We call this mechanism of proton transfer indirect because the protons that appeared in the basic compartment upon hydrolysis of anhydride came from water, not from the acidic compartment. It is a true proton transfer since a molecule of citraconic acid has been removed from the acidic compartment and the citraconate dianion and two protons generated in the basic compartment. In support of this mechanism, citraconic anhydride (0.6 mol %) was detected in an acidic aqueous solution of citraconic acid by ^{1}H-NMR. This facile formation of anhydride is due to the high effective molarity of the neighboring carboxylic acid group.[4] The equilibrium constant for dehydration of citraconic acid is larger than that for maleic acid (<0.2 mol %) and succinic acid (10^{-4} mol %) but smaller than that for dimethylmaleic acid (84 mol %).[5] In a parallel experiment involving only two phases, a ^{1}H-NMR of a $CDCl_3$ phase, equilibrated with acidic aqueous citraconic acid (1.0 M, 0.5 M HCl), showed only citraconic anhydride (65 ± 10 mM, <2 mM citraconic acid). More anhydride formed in the chloroform phase because there was less water in the chloroform (50 mM H_2O) and because the anhydride was more soluble in the chloroform than the acid. Thus, protons were indirectly transferred across the chloroform layer via citraconic anhydride.

Transfer of two protons from pH 0.3 to pH 10 released 26.5 kcal, while formation of citraconic anhydride at pH 10 ($-\Delta G°_{hyd}$) required 18.8 kcal/mol, based on K_{hyd} = 167 for uncharged citraconic acid and pK_1 = 2.29 and pK_2 = 6.15.[6] Thus, indirect proton transfer provided a thermodynamic driving force of 7.7 kcal/mol for the formation of citraconic anhydride.

The high free energy of hydrolysis of citraconic anhydride indicates that it is thermodynamically capable of making high-energy phosphates such as acyl phosphates.

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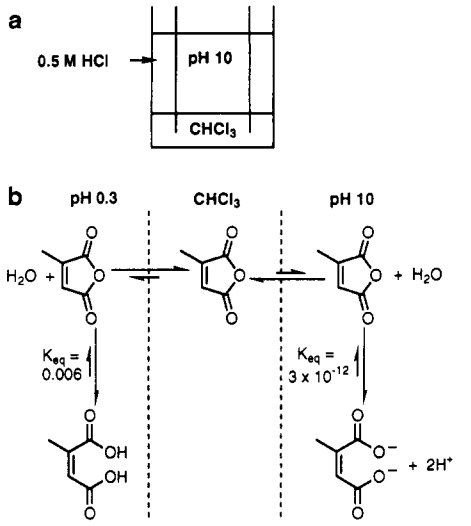
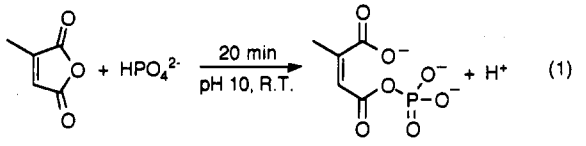


Figure 1. Indirect proton transfer through a chloroform liquid membrane involved citraconic anhydride. (a) Aqueous solutions with pH 0.3 and pH 10 were separated by a layer of chloroform in a concentric ring cell (a tube within a beaker) to model a proton gradient across a cell membrane. (b) When citraconic acid (1.0 M) was added to the acidic compartment (40 mL containing 0.5 M HCl), citraconic anhydride formed and diffused to the basic compartment where it hydrolyzed generating two protons. Transfer was monitored by pHstat of the basic layer (25 mL, 50 mM carbonate buffer) using KOH (5.0 M). With slow stirring of the chloroform layer, the initial rate of indirect proton transfer was 0.27 mmol H^+/h.

The free energy of hydrolysis of acetyl phosphate is 13.8 kcal/mol at pH 10.[5] Consistent with this expectation, citraconic anhydride reacted with aqueous phosphate at pH 10 to give citraconyl phosphate (18% yield) and generate one proton, eq 1.[7] Citraconyl phosphate was



(1)

unstable ($t_{1/2}$ = 4.2 ± 0.2 h at pH 10), but could be characterized in solution: ^{13}C-NMR (75 MHz, ^{1}H decoupled) δ 178.9 (s); 164.4 (d, $^2J_{C-P}$ = 7.2 Hz); 156.8 (s); 113.9 (d, $^3J_{C-P}$ = 7.0 Hz); 20.7 (s); ^{1}H-NMR (200 MHz) δ 5.4 (m); 1.8 (d, $^4J_{H-P}$ = 1.3 Hz). Acetic, succinic, and maleic anhydrides also reacted with aqueous phosphate to give the corresponding acyl phosphate[8,9] which showed similar carbonyl ^{13}C–^{31}P coupling: 8.1, 8.6, 7.2 Hz.

To demonstrate synthesis of citraconyl phosphate using proton transfer as the driving force, we added dipotassium phosphate (1.0 M) to the basic compartment of the experiment described in Figure 1. The ^{31}P-NMR, Figure 2, showed the formation of citraconyl phosphate: 5.7 mM,

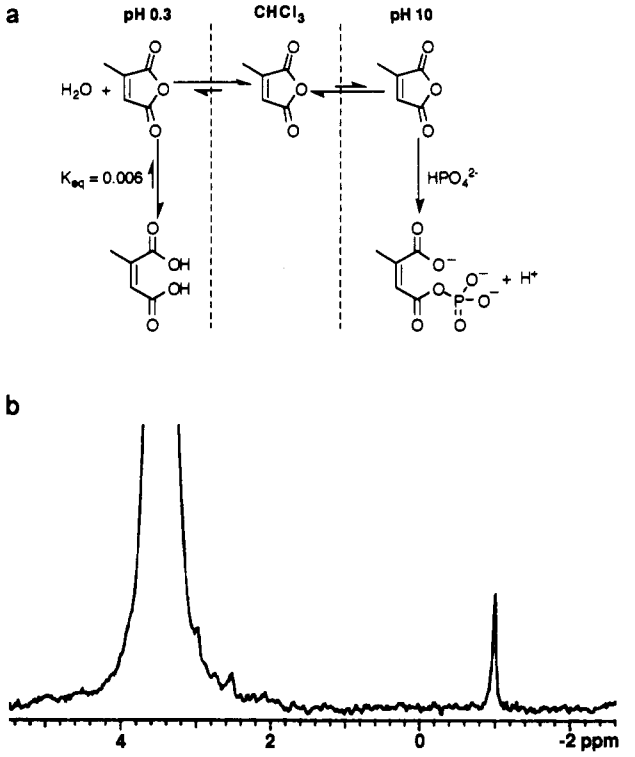


Figure 2. Indirect proton transfer-driven formation of citraconyl phosphate. (a) Citraconic anhydride reacted with phosphate (1.0 M) added to the basic compartment to form citraconyl phosphate. (b) ^{31}P-NMR of the basic compartment showed citraconyl phosphate (δ −1.0, 5.7 mM, 0.15 mmol) after 7.4% (3.0 mmol) of citraconic acid had been transferred from the acidic compartment. Since only one resonance was observed for citraconyl phosphate, we believe that a single regioisomer forms. When methyl succinic anhydride reacted with aqueous phosphate, two resonances were seen in the ^{31}P-NMR at δ −1.1 and −1.3.

5.0% yield based on the amount of citraconic acid transferred. In five similar experiments the yield ranged from 0.6% to 7.0%, based on the amount of citraconic acid transferred.

These experiments show that citraconic acid can couple proton transfer to the synthesis of an acylphosphate. The P-type ATPases involve a phosphoenzyme intermediate, and our model suggests that carboxylic acid anhydride and acyl phosphate intermediates may be involved. While there is no evidence for or against a carboxylic acid anhydride intermediate, an acyl phosphate intermediate, β-aspartyl phosphate, has been identified in three H^+-ATPases.[10] On the other hand, the F_1F_o-ATP-synthetase is believed to act without an acyl phosphate intermediate;[1] thus, our model does not apply to this enzyme.

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Supplementary Material Available: Experimental procedures and NMR data (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(7) Dimethylmaleic acid could not be used to create a model of the H^+-ATPase because the free energy of hydrolysis was only 12.5 kcal/mol at pH 10, 1.3 kcal less than the estimated 13.8 kcal/mol needed to form an acyl phosphate. Consistent with this expectation, dimethylmaleic anhydride did not react with aqueous phosphate to form an acyl phosphate.

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