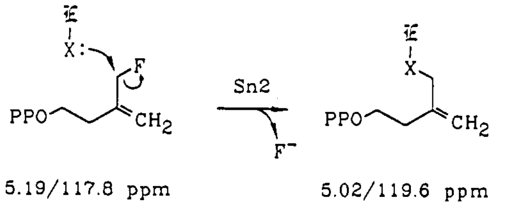
Scheme III. Mechanism for Inactivation of Isomerase by [4-^{13}C]-3



placement is an S_N1 process. Alternatively, we propose an S_N2 reaction (see Scheme III) involving a nucleophile in the active site of isomerase. Although the nucleophile has not yet been identified, a carboxylate oxygen or a thioether sulfur are unlikely candidates. We have shown that the isomerase–**3** adduct is stable to treatment with base under conditions where an ester linkage should be hydrolyzed.[2] Furthermore, we do not favor the sulfur in methoinine because the resulting allylic sulfonium salt should be unstable toward solvolysis when the inhibited protein is denatured.[8] At the present time our NMR experiments do not permit us to distinguish among hydroxyl, sulfhydryl, and amine nucleophiles, and studies to address this question are now in progress.

Experimental Section

General. [1-^{14}C]Isopentenyl diphosphate for assays was purchased from Amersham and used directly or diluted to a specific activity to 10 μCi/μmol with synthetic material. All other materials were obtained from Sigma or Aldrich Chemical Co. and were of reagent grade or higher. Instafluor was purchased from Packard Instrument Co., and liquid scintillation spectrometry was on a Packard Tricarb Model 4530 spectrometer.

All steps in the purification of isomerase were at 4 °C except for HPLC which was at room temperature. Plasticware was used exclusively. Dialysis was performed in Spectrapor dialysis bags (25.5 mm, M_r cutoff 6000–8000) or in a Micro-ProDicon Model 115 forced dialysis concentrator using ProDimem PA-10 membranes (M_r cutoff 10000). SDS–polyacrylamide gel electrophoresis of proteins employed the Laemli discontinuous buffer system. Gels were stained with Coomassie Brilliant Blue R or silver nitrate. Enzyme activity was determined according to the acid-lability assay.[2]

Isopentenyldiphosphate:dimethylallyldiphosphate isomerase was purified from fresh baker's yeast (*Saccharomyces cerevisiae*, Westco Distributing Co., Salt Lake City, UT) by combination of previously reported procedures. Four 1.5-kg batches were carried through ammonium sulfate precipitation and DE-52 chromatography, combined, and then carried through subsequent steps (chromatography on butyl-Sepharose, chromatofocusing, and chromatography on Sephacryl-200 columns) to yield 7.5 mg (sp act. 4.9 μmol mg^{-1} min^{-1}) of enzyme for NMR experiments.[2,3]

NMR Spectroscopy. NMR spectra were recorded on a Varian VXR 500-MHz spectrometer. A 5-mm broad-band probe was used for all experiments with ^{1}H and ^{19}F observation through the ^{1}H-decoupling coil. This permitted ^{1}H, ^{13}C, and ^{19}F spectra to be acquired during the course of the enzymatic reaction without removing the sample from the insert. ^{1}H and ^{13}C spectra were referenced downfield to internal DSS, ^{19}F upfield to external CFCl$_3$, and ^{31}P downfield to external 85% phosphoric acid.

^{13}C spectra were collected with a spectral width of 25 kHz into 32K complex points which were zero-filled to 64K prior to transformation. Approximately 2 W of radio-frequency power was used with WALTZ-16 modulation to decouple protons. The ^{13}C 90° pulse width was 11 μs.

^{19}F spectra were collected with a spectral width of 10 kHz into 8K points which were zero-filled to 16K points prior to transformation. A relatively narrow sweep width was used to observe fluorine bound to carbon or free fluoride since it was not possible to cover the entire range in a single experiment. The ^{19}F 90° pulse width was 30 μs.

^{1}H and ^{13}C difference decoupled spectra were obtained by collection of 14000 transients with single-frequency ^{13}C decoupling at 119.6 ppm (on-resonance) and 14000 transients with the ^{13}C-decoupling field 75 kHz off-resonance. Spectra were collected in blocks of 64 transients in an interleaved fashion and then subtracted to produce the difference spectrum. The residual HDO peak and the most intense protein peaks did not cleanly cancel. The ^{1}H 90° pulse width was 20 μs.

Synthesis of [4-^{13}C]-3-(Fluoromethyl)-3-butenyl Diphosphate ([4-^{13}C]-3). [4-^{13}C]-3-(Fluoromethyl)-3-butenyl diphosphate ([4-^{13}C]-**3**) was synthesized from [^{13}C]triphenylmethylphosphonium iodide according to the procedure developed by Muehlbacher and Poulter[2] for unlabeled material. The phosphonium salt was prepared from [^{13}C]methyl iodide (Sigma) by the following procedure. Triphenylphosphine (1.83 g, 7 mmol) was dissolved in 5 mL of ether in a heavy-walled ampule. The vial containing methyl iodide (1.00 g, 7 mmol) was opened, 1 mL of chilled ether was added, and the contents were transferred to the ampule. The procedure was repeated twice with 1-mL portions of ether. The resulting solution was cooled to −78 °C, and the ampule was sealed. The reaction mixture was allowed to stand for 3 days at room temperature before the ampule was opened, and the resulting white suspension was filtered. The residue was washed with 4:1 hexane/ethyl acetate and dried under vacuum for 6 h to yield 2.33 g (82%) of [^{13}C]triphenylmethylphosphonium iodide, which was used in the subsequent reactions to give 200 mg (21% based on [^{13}C]methyl iodide) of a white powder; ^{1}H NMR (300 MHz, D$_2$O) 2.24 (2 H, q, J = 6.1 Hz, H at C2), 3.85 (2 H, q, J = 6.1 Hz, H at C1), 4.71 (2 H, dd, $J_{^1\mathrm{H}^{13}\mathrm{C}}$ = 5.7 Hz, $J_{^1\mathrm{H}^{19}\mathrm{F}}$ = 46.1 Hz, fluoromethyl H), 4.96 (1 H, d, $J_{^1\mathrm{H}^{13}\mathrm{C}}$ = 157.9 Hz, H at C4), and 5.01 ppm (1 H, d, $J_{^1\mathrm{H}^{13}\mathrm{C}}$ = 157.2 Hz, at C4); ^{13}C NMR (75 MHz, D$_2$O, ^{1}H decoupled) 117.72 ppm ($J_{^{13}\mathrm{C}^{19}\mathrm{F}}$ = 10.8 Hz); ^{19}F NMR (470 MHz, D$_2$O) 213.52 ppm (dt, $J_{^1\mathrm{H}^{19}\mathrm{F}}$ = 46.1 Hz, $J_{^1\mathrm{H}^{19}\mathrm{F}}$ = 9.3 Hz); ^{31}P NMR (121 MHz, D$_2$O, ^{1}H decoupled) −7.02 (1 P, d, J = 21.2 Hz) and −9.96 ppm (1 P, d, J = 21.2 Hz).

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Communications to the Editor

Solid-State Chemistry in Antimony Pentafluoride Matrices. Infrared Spectra of Reactive Intermediates[†]

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On the basis of Olah's fundamental work[1] which demonstrated the advantage of superacidic media for the study of carbocations we developed a method for matrix deposition of suitable precursors in SbF$_5$ and subsequent IR analysis of some carbocations and carbocation-like species in the solid state. Our technique is a combination of Saunders' "molecular beam" method[2] and the cold window codeposition technique of Craig et al.[3] Using a liquid

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[†] Dedicated to the memory of Dr. Zdenko Majerski (1937–1988).

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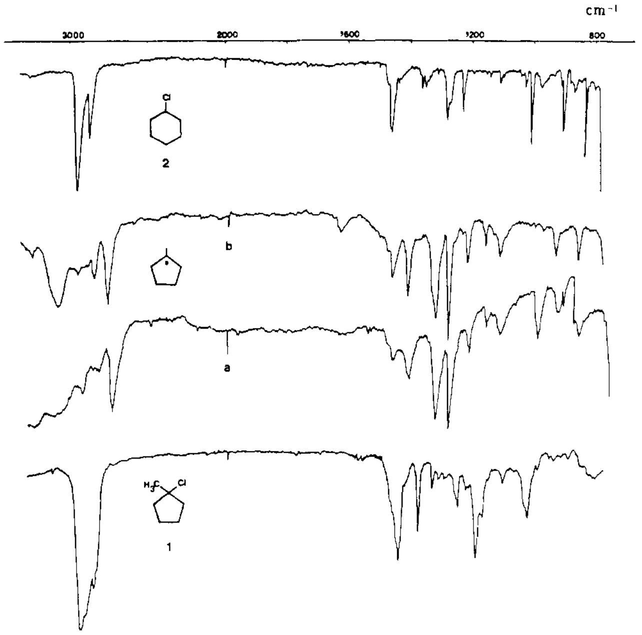

Figure 1. IR spectra of the 1-methylcyclopentyl cation (a and b), compared with those of precursors **1** and **2**. The spectra were recorded between 77 and 150 K.

nitrogen cooled cryostat,[4] SbF_5 and the respective chloride or ketone were deposited in vacuo on a liquid nitrogen cooled CsI window at a ratio of 5:1. By gradually warming the probe to 150 K all changes in the spectrum of the sample can be observed by continuous recording. All IR spectra were taken on Perkin-Elmer 167 and 783 spectrometers. In this account only the gross features of the spectra will be commented upon, postponing detailed vibrational assignments to subsequent papers.

1-Methylcyclopentyl Cation. 1-Chloro-1-methylcyclopentane (**1**) and SbF_5 were codeposited on the CsI window at 77 K, and the spectrum was recorded. By gradually warming the probe the signals of the starting material decreased in intensity and at approximately 100 K the shape of the spectrum drastically changed. By further raising of the temperature to about 150 K the base line resumed the original position and remained stable (Figure 1).

There is a marked difference in the IR spectrum (a) of the matrix-generated cation in comparison with the spectrum of the precursor chloride (**1**). The contour and positions of the signals in the C–H stretching and CH_3 and CH_2 deformation regions differ from any known IR spectra of hydrocarbons. It is difficult to assign the two broad bands centered around 3275 and 3100 cm^{-1} which could be caused by some moisture, but the most intensive absorption at 2775 cm^{-1} can be assigned to the stretching vibration of (weak) C–H bonds adjacent to the cationic center. These bonds are well positioned for hyperconjugative interaction with the empty p-orbital, and the shift to lower frequencies is a direct measurement of this interaction. However, we are not aware of any report of a direct observation of the hyperconjugative weakening of the C–H bond which in this case amounts to about 220 cm^{-1}. The change in bond strengths is also manifested on the deformation vibrations. In this region new absorption bands appear at 1463, 1408, and 1320 cm^{-1}. Note that the corresponding CH_3 deformation in chloride **1** appears at 1385 cm^{-1}. The strongest peak in the spectrum is at 1280 cm^{-1}. From previous work in superacid solutions,[1a,c] signals in this spectral region have been assigned to the asymmetric stretching of the carbon–carbon bonds around the cationic center ($C–C^+–C$).

Since it is known[5] that upon ionization in superacids cyclohexyl chloride (**2**) rearranges to the 1-methylcyclopentyl cation, the experiment was repeated using **2** as a precursor. The resulting spectrum is shown in (b). A comparison of the spectra of cations generated from **1** and **2** shows that the rearrangement of the respective cations occurs also in the solid state below 150 K. The mechanism of this rearrangement has never been thoroughly investigated. However, it is believed that it involves the formation of an intermediate pentacoordinated carbon species, i.e., a protonated cyclopropane which subsequently undergoes rearrangement and intramolecular hydrogen shifts.[6]

Adamantyl Cations.[7] 1,2-Hydride shifts, usually facile processes in carbonium ions, are known not to occur in adamantyl cations under solvolytic or dilute superacid conditions.[8] This observation was rationalized by considering the relative orientations of the C–H bonds with respect to the adjacent p-orbital at C_1 and C_2, respectively. Sometimes under different conditions, hydride transfers have been observed, but they are thought to be intermolecular.[8b] When deposited on a SbF_5 matrix at 77 K the tertiary chloride **3** gives the spectrum of the respective cation. The same spectrum is obtained by using the secondary chloride **4** as precursor and warming the matrix to 150 K. Since 1,2-hydride shifts in the solid state are more likely to be intramolecular, it is difficult to rule out this possibility solely on the basis of orbital considerations.

Carbocation-like Species. Experiments in superacid media always produce the thermodynamically most stable cation. Some cations have never been isolated and studied spectroscopically because they rapidly rearrange.[5,9] One way to circumvent this problem would be the application of the matrix isolation technique to species which have a high density of the positive charge on carbon and behave like carbonium ions. Such species are complexes formed by reacting Lewis acids with aldehydes or ketones.[10] Recently, Laube studied complexes of 5-phenyladamantan-2-one and $SbCl_5$ by X-ray diffraction methods.[11] As predicted, the carbon–oxygen bond was found to be 0.045 Å longer than the normal carbonyl bond.

In a typical experiment, cyclohexanone was codeposited with SbF_5 on a CsI window at 77 K. By warming the probe to 150 K the carbonyl stretching frequency shifted from 1708 to 1585 cm^{-1} with a concurrent increase in the intensity. Other signals show some changes in the contours of the bands but are not markedly shifted. Interesting is the appearance of a medium strong band at 2640 cm^{-1} which can be associated with a hyperconjugative weakening of the β-CH bonds in the complex.

An important advantage of this technique is that direct measurements of bond strengths, i.e., frequencies and force constants, are possible which when combined with X-ray structure determination[11-13] and theoretical calculations[14,15] could yield fundamental data on structures of transient cationic intermediates.

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Systematic studies along these lines are under way in our laboratory.

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Supplementary Material Available: Drawings of the cryostat, description of the matrix deposition procedure, and IR spectra of 1-adamantyl cation, the two precursor chlorides **3** and **4**, and cyclohexanone–pentafluoroantimony (4 pages). Ordering information is given on any current masthead page.

A Bisubstrate Reaction Template

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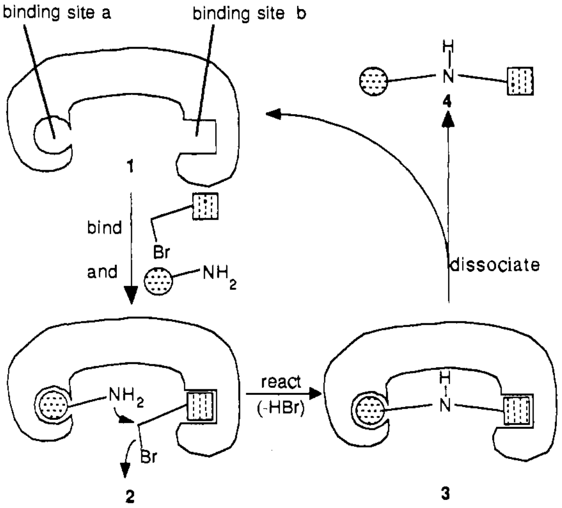
The ability to construct artificial "enzymes" for which there are no natural counterparts would render possible innumerable chemical transformations that are beyond the reach of current methodology.[1] Natural enzymes in part[2] exploit the kinetic advantage[5] of converting normally intermolecular reactions into intramolecular ones by binding substrate(s) prior to the commencement of bond reorganization. To date,[6] studies in the area of artificial enzymes have focussed almost exclusively on processes involving a single substrate, with bond cleavage being the dominant theme; the serine protease mimics of Cram[6c,7b] and Breslow[6d,7a] are prominent examples.

Scheme I



We now report the first[8] example of a fully synthetic system wherein *two* organic substrates are bound simultaneously—but temporarily—by a designed[8b] receptor possessing two binding sites, and reaction *between* the two substrates is accelerated because of this transient intramolecularity.[5] The system is rudimentary at present, but it demonstrates the validity of the basic concept.

The mechanistically straightforward S_N2 alkylation of an amine by an alkyl halide was selected for initial study. The overall process is represented in general terms in Scheme I: the ditopic receptor **1** binds the two substrates, giving the ternary complex **2** and placing the two potentially interacting functional groups in relative proximity to each other. Bond formation (→ **3**) followed by dissociation of the template-product complex (**3**) completes the process. Scheme II supplies molecular detail. The specifics of **5–8** were designed using CPK models, taking into account synthetic accessibility and solubility in nonpolar organic solvents (which would not interfere with the requisite hydrogen bonding[10] between template and substrates). For initial simplicity the binding sites a and b of **1** are identical in **5**, but such identity is not required (nor, ultimately, desirable). It was hoped that **5** (and **8**) possessed a satisfactory balance between conformational flexibility and preorganization[11] such that any imprecisions in design, although perhaps debilitating, would not be fatal. The synthesis of **5** relies heavily on recent developments in organopalladium chemistry[12,13,15] and is outlined in Scheme III; the two substrates were prepared from **11**[16] as indicated.

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