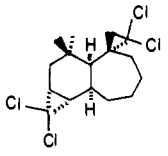
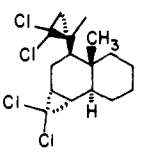
confirmed the structures of **9** and **10** and, by extrapolation, the structures of **6** and **8**.

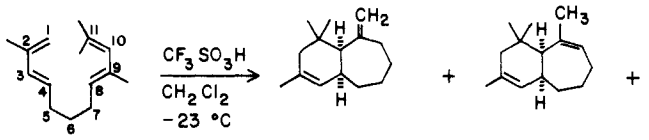


9

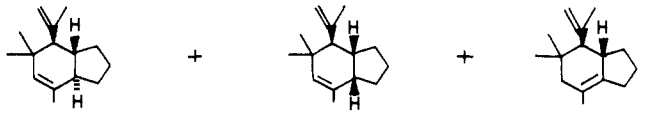


10

The addition of another methyl group of **4** resulted in major changes. When **11** was treated with 10 mol % of triflic acid in methylene chloride at −23 °C for 2 min, we obtained 43% of **12**, 5% of **13**, 15% of **14**, 15% of **15**, and 14% of **16**.[12,13] When the reaction was carried out at −78 °C for 1.5 h, the yields were 21% of **12**, 15% of **13**, 12% of **14**, 12% of **15**, and less than 1% of **16**. This indicated that **12** and **16** were secondary products that resulted from the acid-catalyzed isomerization of **13** and of **14** and **15**, respectively. This was confirmed by isomerization studies on **13**, **14**, and **15**.

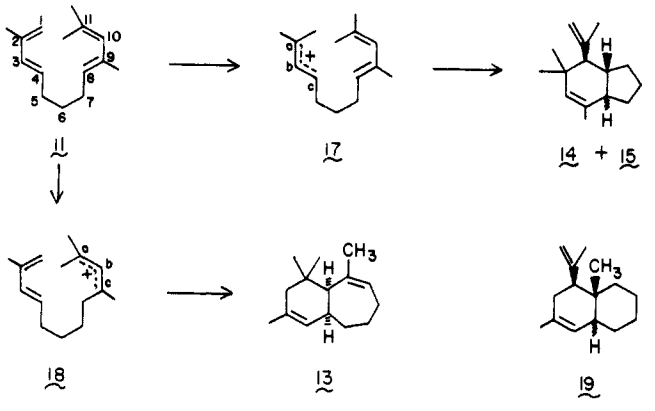


11 **12** **13**



14 **15** **16**

Treatment of **11** with 40 mol % of *p*-toluenesulfonic acid in methylene chloride at 23 °C for 1 h gave 4% of **12**, 2% of **13**, 20% of **14**, 20% of **15**, and 30% of **16**.[12,13] Again, a change in acid and temperature resulted in a major change in product ratio. These changes are best understood through examination of the mechanistic pathways involved in the formation of **13**, **14**, and **15**. Product structures dictate that the allyl cation **17** must be



involved in the formation of **14** and **15**. This requires protonation of C-1 of **11** to produce the trisubstituted allyl cation **17**, followed by exclusive *formal* cycloaddition of the b–c portion of the allyl cation to the diene moiety of **17**. In order to form **13**, **11** must be protonated at C-8 to give the tetrasubstituted allyl cation **18**. The complete absence of **19** shows that, in contrast to the reactions of **5**, **18** underwent *formal* intramolecular cycloaddition of only the a–b portion of the allyl cation **18**.

In summary, the bicyclo[4.3.0]nonyl, bicyclo[4.4.0]decyl, or bicyclo[5.4.0]undecyl ring systems can be produced from 1,3,8,10-undecatetraene through a judicious choice of methyl substitution, acid catalyst, and temperature. This is particularly useful in the case of the bicyclo[5.4.0]undecanes, which are not readily available.[14]

Acknowledgment. We are indebted to the National Institute of General Medical Sciences of the National Institutes of Health for a grant that supported this investigation. We thank Professor D. Britton for his assistance in the structure determination.

Supplementary Material Available: Spectral and other analytical data for **2**, **6–10**, and **12–16** and crystallographic experimental details, ORTEP drawings, and tables of positional and thermal parameters and significant distances and angles for **9** and **10** (20 pages). Ordering information is given on any current masthead page.

(12) Two additional products were detectable by GLC in 5–7% combined yield.

(13) Under the reaction conditions, **12** and **13** were not interconverted with **14**, **15**, and **16**.

(14) For previous syntheses of the bicyclo[5.4.0]undecane skeleton via intramolecular Diels–Alder reactions, see: Wenkert, E.; Naemura, K. *Synth. Commun.* **1973**, *3*, 45. Oppolzer, W.; Snowden, R. L. *Helv. Chim. Acta* **1981**, *64*, 2592.

Stepwise Mechanism for the Formation of 2π + 4π Cycloadducts in the Ionic Diels–Alder Reaction

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Received August 16, 1990

The term Diels–Alder reaction[2] has long been used to describe 2π + 4π cycloaddition reactions in which cyclohexene derivatives are formed. The mechanistic details of these cycloadditions have been much discussed and widely debated.[3,4] Theoretical studies have suggested that transition states for various Diels–Alder reactions range from a concerted synchronous process for the addition of ethylene to 1,3-butadiene[3,4] to very asynchronous pathways for the reactions of acrolein or cyanoalkenes with butadiene[4] and for certain Lewis acid catalyzed cycloadditions.[5] Completely stepwise "formal" 2π + 4π cycloaddition processes have been suggested in a few cases for the "classical" Diels–Alder reaction.[6–8] Since we have extensively investigated "ionic" Diels–Alder reactions in recent years,[9,10] we became intrigued with the mech-

(1) Rohm and Haas Fellow, 1988–1989.

(2) Diels, O.; Alder, K. *Justus Liebigs Ann. Chem.* **1928**, *460*, 98.

(3) Sauer, J.; Sustmann, R. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 779. Fukui, K. *Acc. Chem. Res.* **1971**, *4*, 57. Fleming, I. *Frontier Orbitals and Organic Chemical Reactions*; Wiley and Sons: New York, 1976. Woodward, R. B.; Hoffman, R. *The Conservation of Orbital Symmetry*; Verlag Chemie: Weinheim/Berlin, Germany; Academic Press: New York, 1970; p 145.

(4) For recent leading references, see: Back, R. D.; McDonall, J. J. W.; Schlegel, H. B. *J. Org. Chem.* **1989**, *54*, 2931. Burke, L. A. *Int. J. Quantum Chem.* **1986**, *29*, 511. Houk, K. N.; Lin, Y.-T.; Brown, F. K. *J. Am. Chem. Soc.* **1986**, *108*, 554. Dewar, M. J. S. *J. Am. Chem. Soc.* **1984**, *106*, 209. Dewar, M. J. S.; Olivella, S.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1986**, *108*, 5771. Loncharich, R. J.; Brown, F. K.; Houk, K. N. *J. Org. Chem.* **1989**, *54*, 1129. Houk, K. N.; Loncharich, R. J.; Blake, J. F.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1989**, *111*, 9172. Gajewski, J. J.; Peterson, K. B.; Kagel, J. R.; Huang, Y. C. J. *J. Am. Chem. Soc.* **1989**, *111*, 9078.

(5) Houk, K. N.; Strozier, R. W. *J. Am. Chem. Soc.* **1973**, *95*, 4094. See also: Gompper, R. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 312.

(6) Gupta, R. B.; Frank, R. W. *J. Am. Chem. Soc.* **1987**, *109*, 5393.

(7) Jensen, F.; Foote, C. S. *J. Am. Chem. Soc.* **1987**, *109*, 6376.

(8) Baran, J.; Mayr, H.; Ruster, V.; Klarner, F.-G. *J. Org. Chem.* **1989**, *54*, 5016.

(9) Gassman, P. G.; Singleton, D. A. *J. Am. Chem. Soc.* **1984**, *106*, 6085. Gassman, P. G.; Singleton, D. A. *J. Org. Chem.* **1986**, *51*, 3075. Gassman, P. G.; Singleton, D. A. *J. Am. Chem. Soc.* **1984**, *106*, 7993. Gassman, P. G.; Singleton, D. A.; Wilwerding, J. J.; Chavan, S. P. *J. Am. Chem. Soc.* **1987**, *109*, 2182.

(10) Gassman, P. G.; Gorman, D. B. *J. Am. Chem. Soc.*, preceding paper in this issue.

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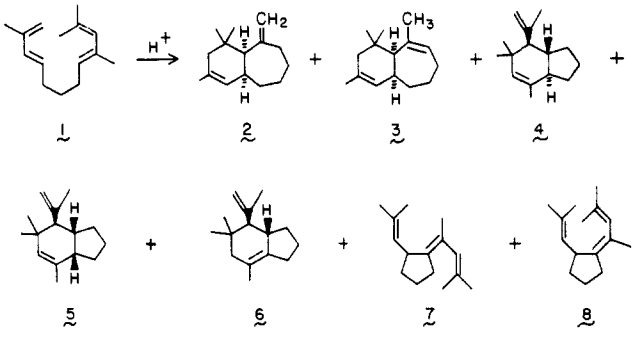
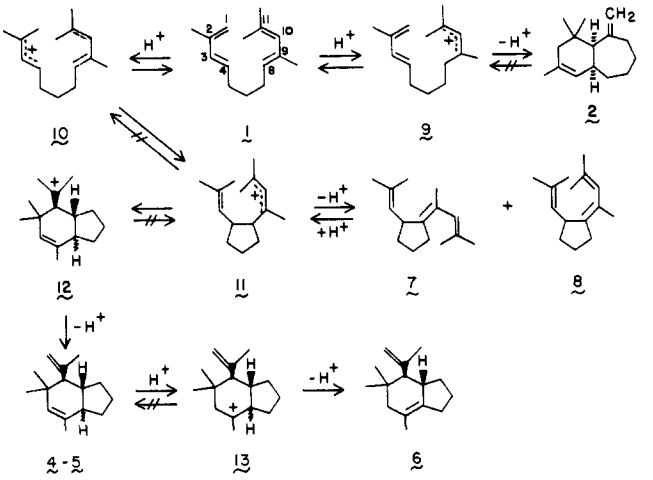
anistic details of this cyclohexene synthesis. We now report the existence of a stepwise mechanism for an intramolecular ionic Diels–Alder reaction.

In the preceding communication,[10] we showed that treatment of **1** with various acids gave mixtures of **2**–**6** and two minor products (in 5–7% yield under the conditions described). It was demonstrated that **2** and **6** were secondary products derived from the acid-catalyzed isomerization of **3** and of **4** and **5**, respectively. Because of the impressive dependency of product yields and ratios on both the acid catalyst and temperature, the detailed nature of the mechanism was investigated. Identification of the two minor products showed that these were the monocyclic trienes **7** and **8**.[11] When **7** and **8** were individually subjected to the reaction conditions (40 mol % *p*-toluenesulfonic acid, 23 °C, 1 h, methylene chloride), **7** gave 21% of **4** and **5** (1:1 mixture), 58% of **6**, and 6% of **8**, while **8** gave 41% of **4** and **5** (1:1 mixture), 33% of **6**, 3% of **7**, and 9% of **8**.[12] This established that **7** and **8** served as precursors of **4**–**6**, but not of **2** or **3**.[13]



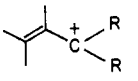
Since **7** and **8** were converted into **4** and **5** (and subsequently into **6**) at observable rates under the reaction conditions, and since control reactions showed that these conversions were not reversible, it was reasonable to expect that **4** and **5** were derived primarily from **7** and **8**. If this were true, decreased acid concentrations and shorter reaction times should lead to increased yields of **7** and **8**. Treatment of **1** with 10 mol % of *p*-toluenesulfonic acid at 23 °C for 30 min in methylene chloride gave 1% of **2**, 2% of **3**, 12% of **4**, 12% of **5**, 1% of **6**, 27% of **7**, and 34% of **8**.[14] *Under these conditions, the combined yield of 7 and 8 was 61%.* Control reactions established that **7** and **8** were converted into **4**–**6** during the course of the reaction. Thus, the actual amount of **7** and **8** that was formed in the reaction was much larger than 61%. By extrapolation, the vast majority (if not all) of **4**, **5**, and **6** must be derived from **7** and **8** via protonation of **7** and **8** or from a monocyclic protonated precursor of **7** and **8**. Thus, the formation of **4** and **5**, which initially appeared to arise from a normal intramolecular Diels–Alder reaction, has been shown to occur via a stepwise process.

It is now possible to construct a detailed mechanistic picture of the overall pathway which eventually leads from **1** to **6** as the major end product. Initial protonation of **1** can occur reversibly[15] at C-8 to produce **9**, which eventually yields **2**,[16] or reversibly[15] at C-1 to produce the trisubstituted allyl cation **10**. Cyclization



of **10** to **11** occurs in a nonreversible process. The allyl cation can either cyclize directly to **12** or deprotonate to produce a mixture of **7** and **8**. The formation of approximately equimolar amounts of **4** and **5** suggests that deprotonation–reprotonation may be the prevailing process because the stereochemistry of the ring fusion would then be determined by the direction from which **7** and **8** were protonated rather than by the stereochemistry of bond formation in the conversion of **10** to **11**.[18] Deprotonation of **12** would yield **4** and **5** which, on reprotonation, produces **6** via **13**. Since the reprotonation of **7** and **8** fails to produce **2**, the formation of **11** from **10** must not be reversible. Similarly, the failure of **4**, **5**, or **6** to give **7** or **8** indicates that the formation of **12** from **11** is not reversible. These and other examples from our laboratory[9,10] suggest that protonation–deprotonation reactions are reversible in most (but not all) cases while cyclization reactions are not reversible.

When the allyl cation is viewed in terms of the resonance contributor **14**, it becomes obvious that the double bond will be extremely electron deficient and highly polarized. A carbocation



is the strongest carbon-based electron-withdrawing group known. Thus, the allyl cation represents one extreme in the spectrum of polarities to be found in Diels–Alder dienophiles. It seems reasonable that, in this extreme case, the formal Diels–Alder reaction should have reached the point where it is so asynchronous as to be stepwise.[19]

Acknowledgment. We are indebted to the National Institute of General Medical Sciences of the National Institutes of Health for a grant that supported this investigation.

(11) Satisfactory elemental analyses and exact mass molecular weights were obtained for all new compounds. All compounds had ^{13}C NMR, ^{1}H NMR, and IR spectra that were consistent with the assigned structures. Spectral data for **7** and **8** are included in the supplementary material. The stereochemical assignments for **7** and **8** were based on ^{13}C NMR and ^{1}H NMR spectral comparisons to known analogues.

(12) Prolonged treatment with acid led to the slow decomposition of **6**.

(13) GLC analysis indicated the complete absence of **2** and **3** in the reaction mixtures derived from **7** and **8**.

(14) Yields were determined by GLC using undecane as an internal standard.

(15) Treatment of **1** with 40 mol % of *p*-toluenesulfonic acid-*O*-*d* at 23 °C for 1 min in methylene chloride, followed by isolation of "unreacted" **1**, showed that **1** had incorporated deuterium at C-1, C-4, and C-8 and at all four methyl groups to varying degrees. Thus, **1** was involved in a complicated protonation–deprotonation process prior to any cyclization. This study indicated that extensive protonation–deprotonation of **1** occurred at C-8 and C-1 to produce **9** and **10**, respectively.

(16) While we suspect that **2** is also formed in a stepwise process, starting with **9**, we have not been able to isolate intermediate materials which would establish a stepwise mechanism for the formation of **2**. A stepwise route would require the formation of i from **9**. Precedent for a cyclization of this type exists.[17] Subsequent conversion of i to ii followed by deprotonation would produce **2**.

(17) Gassman, P. G.; Riehle, R. J. *J. Am. Chem. Soc.* **1989**, *111*, 2319.

(18) In certain examples where less stable allyl cations are involved, only trans-fused bicyclo[4.3.0]nonenes were produced.[9]

(19) For a related discussion, see: Hoffmann, H. M. R.; Vathke-Ernst, H. *Chem. Ber.* **1981**, *114*, 1464, 2208.

Supplementary Material Available: Spectral and other analytical data for **7** and **8** and a listing of spectral data for related compounds (3 pages). Ordering information is given on any current masthead page.

An Affinity Label of Absolute Peptidic Origin

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A large number of peptide-based active site directed irreversible inhibitors (affinity labels) have been reported for enzymes that act upon protein substrates.[1] These inhibitors are an amalgam of two components. The peptidic portion is designed to resemble the substrate and therefore specifically binds to the active site. It serves as the carrier for highly reactive non-proteinoid electrophilic appendages, such as chloromethyl ketones. These electrophilic groups irreversibly modify an active site nucleophile resulting in the concomitant inactivation of the target enzyme. In this communication, we describe a purely peptidic affinity label for the cAMP-dependent protein kinase ("A-kinase"). *In contrast to previously described peptide-based inhibitors, this species contains only functionality present in naturally occurring proteins.*

The A-kinase catalyzes phosphoryl transfer from MgATP to the hydroxyl groups of serine and threonine residues in a vast array of proteins.[2] In addition, a number of peptide-based substrates have been reported for this enzyme, including kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly).[3] The arginine dyad is known to be crucial for substrate recognition and therefore would, by necessity, comprise an essential portion of any active site directed inhibitor of the A-kinase. We synthesized[4] the heptapeptide Leu-Arg-Arg-Cys-Cys-Leu-Gly and subsequently oxidized[5] it to the intramolecular disulfide analogue, Leu-Arg-Arg-Cys⇔Cys-Leu-Gly (**1**)[6] (where Cys⇔Cys represents a Cys-Cys dyad connected via both a peptide and disulfide bond). The intramolecular disulfide is a potent electrophile, resulting in the rapid inactivation of the cAMP-dependent protein kinase.[7]

Incubation of the A-kinase[4] with Leu-Arg-Arg-Cys⇔Cys-Leu-Gly under standard conditions[8] resulted in a time-dependent

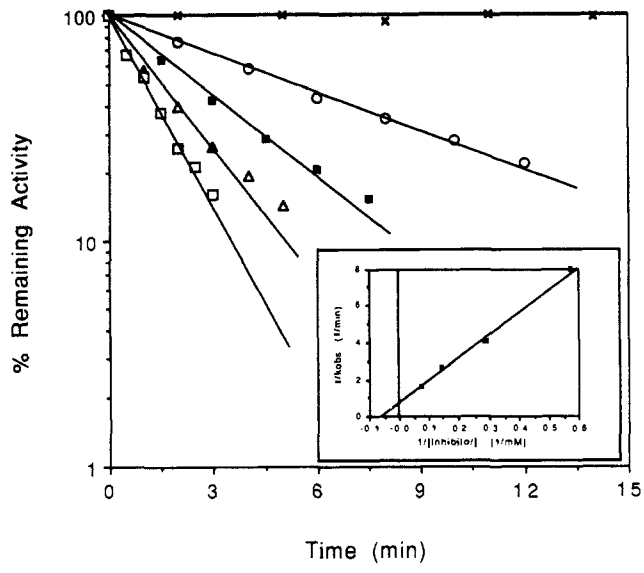
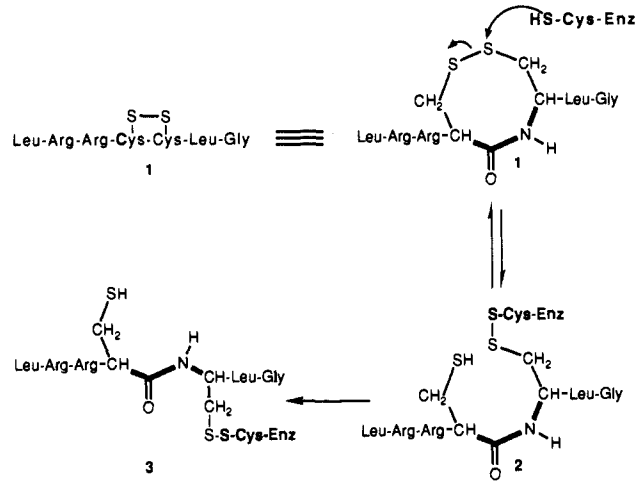


Figure 1. Time-dependent inactivation of the cAMP-dependent protein kinase by **1** at the following concentrations: 14 mM (□), 7 mM (△), 3.5 mM (■), 1.75 mM (○), and 0 mM (×). A double reciprocal plot of rate constant versus inhibitor concentration yields K_i = 17.7 ± 0.9 mM and k_2 = 1.42 ± 0.1 min^{-1}.

Scheme I



pseudo-first-order inactivation of kinase activity (Figure 1). Saturation kinetics is observed, suggesting that the affinity label is active site directed. The double reciprocal plot of $1/k_{obs}$ versus $1/[I]$ (Figure 1, inset) yields a K_i = 17.7 ± 0.9 mM and a k_2 = 1.42 ± 0.1 min^{-1} (unimolecular rate constant for modification). Modification is covalent, since dialysis of the inactivated enzyme against buffer did not restore activity. However, treatment of the covalently modified enzyme with dithiothreitol did reestablish its ability to catalyze phosphoryl transfer, suggesting that it is an active site cysteine that has undergone modification (the A-kinase contains a cysteine residue in the active site[9,10]). In addition, MgATP (150 μM) completely protected the enzyme against inactivation by the affinity label.[11] It has been previously proposed

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(1) *Methods Enzymol.* **1977**, *46*.

(2) Carlson, G. M.; Bechtel, P. J.; Graves, D. J. *Adv. Enzymol.* **1979**, *50*, 41.

(3) Bramson, H. N.; Kaiser, E. T.; Mildvan, A. S. *CRC Crit. Rev. Biochem.* **1983**, *15*, 93.

(4) The peptides were synthesized and the cAMP-dependent protein kinase was purified as previously described: Prorok, M.; Lawrence, D. S. *J. Biochem. Biophys. Methods* **1989**, *18*, 167.

(5) Zhang, R.; Snyder, G. H. *J. Biol. Chem.* **1989**, *264*, 18472.

(6) The structure **1** was confirmed by fast atom bombardment mass spectrometry and two-dimensional correlated and nuclear Overhauser enhancement NMR spectroscopies.

(7) Peptide-based enzyme inhibitors containing disulfide groups composed of nonpeptidic functionality have been described. For example, see: (a) Bramson, H. N.; Thomas, N.; Matsueda, R.; Nelson, N. C.; Taylor, S. S.; Kaiser, E. T. *J. Biol. Chem.* **1982**, *257*, 10575. (b) Evans, B.; Shaw, E. *J. Biol. Chem.* **1983**, *257*, 10227.

(8) Inactivation reactions (total volume 60 μL, pH 7.1, 150 mM KCl, 100 mM MOPS, and 0.125 mg/mL BSA) were carried out by incubating the A-kinase (100 nM) with peptide **1** (0–14 mM) at 6 °C. At selected times, aliquots were removed and diluted 20-, 25-, or 30-fold into an ice-cold assay mixture containing 100 mM MOPS, 150 μM [γ-^{32}P]ATP (200 cpm/pmol), 12.5 mM $MgCl_2$, 150 mM KCl, and 0.125 mg/mL BSA (total volume 100 μL, pH 7.1). No further inactivation occurred after dilution of the enzyme-inactivator solution. After incubation at 30 °C, the kinase reactions were initiated by addition of kemptide to a final concentration of 50 μM. After 1.5 min, the assays were quenched by spotting 25-μL aliquots onto phosphocellulose paper followed quickly by immersion into 10% acetic acid. After exhaustive washings with 5 mM H_3PO_4, the disks were dried and scintillation counted for radioactivity.

(9) First, E. A.; Johnson, D. A.; Taylor, S. S. *Biochemistry* **1989**, *28*, 3606 and references cited therein.

(10) The A-kinase contains two cysteine residues, one of which is in the active site. We have found that [1-^{14}C]Ac-Leu-Arg-Arg-Cys⇔Cys-Leu-Gly labels the enzyme only once (1.02 ± 0.04 equiv of label/mol of enzyme; performed in triplicate). Modification results in complete enzymatic inactivation. In marked contrast, a nonselective reagent, such as Ellman's reagent, labels both cysteine residues. See: Armstrong, R. N.; Kaiser, E. T. *Biochemistry* **1978**, *17*, 2840.

(11) Kemptide (5.0 mM), in the absence of ATP, confers approximately 65% protection against inactivation.

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