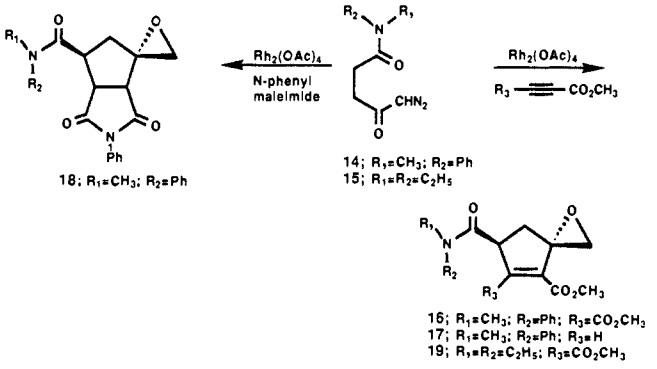
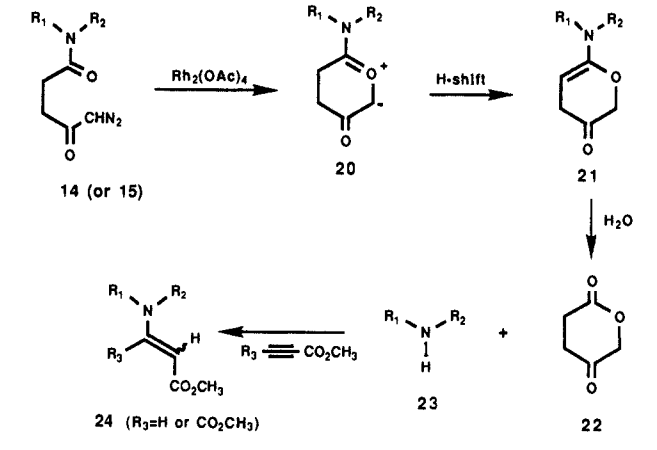
methine ylide **11a** could be detected in the crude reaction mixture. We also examined the rhodium(II) acetate catalyzed behavior of the closely related diazo keto amide **9**. Our first expectation was that the initially formed carbonyl ylide intermediate **10b** would undergo proton transfer to give the thermodynamically more stable azomethine ylide dipole.[13] We found, however, that treatment of **9** with DMAD and a catalytic amount of rhodium(II) acetate resulted in the isolation of the unexpected cycloadduct **13** in 60% yield. Similar results were obtained when diazo keto *N*-phenyl-*N*-methylamide **14** was used. Treatment of this material with rhodium(II) acetate in the presence of DMAD, methyl propiolate, or *N*-phenylmaleimide afforded cycloadducts **16** (60%), **17** (55%), and **18** (70%). Analogous products were also obtained



when diazo keto *N*,*N*-diethylamide **15** was used.[14]

A variety of reaction conditions were examined in order to maximize the yields of the cycloadducts. We found that the highest yields were obtained when the solvent (benzene or methylene chloride) was rigorously dried. Use of ordinary solvents routinely led to lower yields of cycloadducts as well as to the formation of variable amounts of enamide **24** and lactones **22** and **25** (vide infra). The reaction was also carried out in the absence of a trapping agent, and the cyclic ketene *N*,*O*-acetal **21** could be isolated in 70% yield. This material was unstable and upon standing was readily hydrolyzed to lactone **22** and amide **23**. In fact, treating a sample of **21** with "wet" benzene in the presence of DMAD (or methyl propiolate) afforded high yields of lactone **22** and enamide **24** derived from conjugate addition of the amine to the activated acetylenic π-bond.[15]



A mechanism that rationalizes the formation of the cycloadducts and that is consistent with all the data is outlined in Scheme I. The initial reaction involves generation of the expected carbonyl ylide dipole **20** by intramolecular cyclization of the keto carbenoid onto the oxygen atom of the amide group. This highly stabilized dipole does not readily undergo 1,3-dipolar cycloaddition but rather loses a proton to produce the cyclic ketene *N*,*O*-acetal **21**. In the absence of any significant amount of water, this material reacts with the activated π-bond of the dipolarophile to produce zwitterion **26**. The anionic portion of **26** adds to the adjacent carbonyl group, affording a new zwitterionic intermediate **27**. If there is some water present, this species is converted to lactone **25**. Under anhydrous conditions, however, epoxide formation occurs with charge dissipation to produce the observed cycloadduct(s) **16**.[16]

We are continuing to explore the scope and mechanistic details of these rhodium-catalyzed reactions of diazo keto amides and will report additional findings at a later date.

Acknowledgment. We gratefully acknowledge support of this work by the National Institutes of Health. Use of the high-field NMR spectrometer used in these studies was made possible through equipment grants from the NIH and NSF.

Supplementary Material Available: Experimental details of the rhodium-catalyzed reactions of **14**, of **8**, **14**, and **15** in the presence of DMAD, of **14** in the presence of methyl propiolate, and of **14** in the presence of *N*-phenylmaleimide (3 pages). Ordering information is given on any current masthead page.

(16) Treatment of a pure sample of **21** with DMAD, methyl propiolate, or *N*-phenylmaleimide under anhydrous conditions was also found to produce cycloadducts **16**-**18** in 60-80% yield.

Porphyrin Synthesis from a,c-Biladienes. Evidence for a Common Mechanistic Pathway in the Electrochemical and Chemical Routes: Formation of Novel Macrocycles Possessing the Homoporphyrin Carbon Skeleton

Paul A. Liddell, Marilyn M. Olmstead, and Kevin M. Smith*

Department of Chemistry, University of California Davis, California 95616

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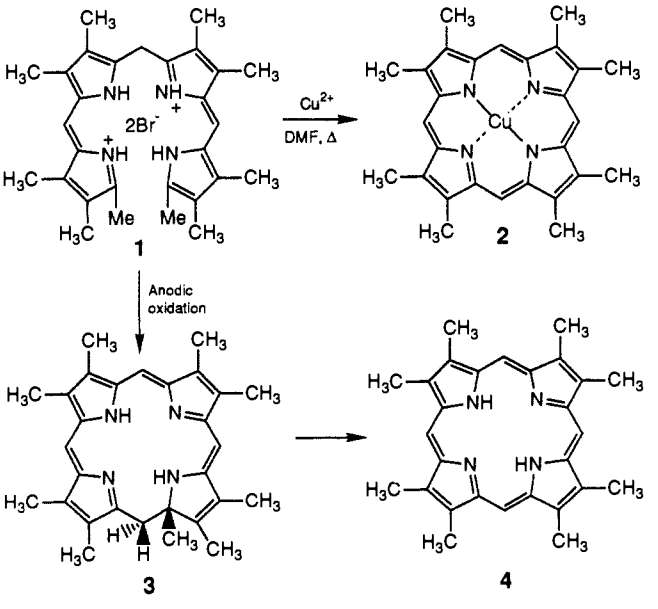
The most general synthesis of unsymmetrical porphyrins employs the copper(II)-catalyzed cyclization of 1',8'-dimethyl-a,c-biladiene salts **1** to give copper(II) porphyrins **2**. The methodology



was discovered in 1961 for symmetrical cases by Johnson and Kay[1] and has since been generalized[2-6] for preparation of completely

(14) All new compounds were characterized by ^1H and ^{13}C NMR and by high-resolution mass spectra.

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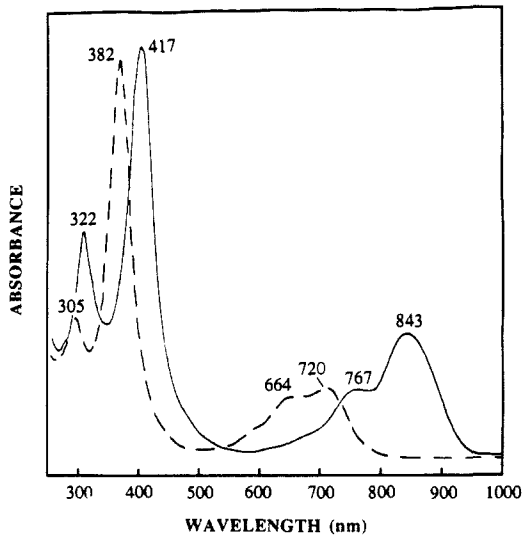


Figure 1. Optical spectra, in CH_2Cl_2, of copper(II) macrocycle **6** obtained from copper(II)-promoted cyclization of a,c-biladiene **5** (full line), and the metal-free macrocycle **7** (broken line).

unsymmetrical porphyrins. Mechanistic details of this cyclization have been investigated by using carbon-13 NMR,[7,8] and these studies established both the origin of the new meso carbon atom and a plausible pathway from 1′,8′-dimethyl-a,c-biladiene to copper(II) porphyrin.[8,9] Until the present time, no intermediates in the proposed pathway have even been isolated or characterized. We now show that use of certain terminal substituents (in place of the 1′-methyl and 8′-methyl in **1**) permits isolation of stable intermediates.

It was recently shown[10] that electrolysis of a,c-biladienes **1** gave good quantities of porphyrin. Bulk electrolysis of unsymmetrical a,c-biladiene **1** afforded a blue-green compound **3** [λ_{max} 305 (ϵ 15 000), 380 (45 000), 650 (infl. 7800), 704 nm (9000)], which was shown to be an intermediate on the pathway to porphyrin **4**. As a result, a mechanistic pathway to explain the electrochemical formation of porphyrin from **1** was put forward, the key step involving loss of the extra terminal methyl group by nucleophilic attack, followed by oxidation of the resulting phlorin to porphyrin. Isolation of a macrocycle similar to **3** from the copper(II) cyclization would serve to demonstrate similarities between the two mechanistic pathways. One way of doing this would be to synthesize an a,c-biladiene and subsequently produce a cyclized intermediate from it that was reluctant for steric and electronic reasons to undergo nucleophilic attack to form the phlorin; inhibition of the nucleophilic porphyrin-forming step should afford an "intermediate" that was more stable than **3**.

The 1′,8′-bis[2-(methoxycarbonyl)ethyl]-a,c-biladiene **5** was synthesized[11] in high yield. Heating of this compound with copper(II) acetate in dimethylformamide at 125 °C for 5 min gave a deep green solution with *no trace* of a Soret absorption band in its optical spectrum (Figure 1; full line); electrochemical cyclization (cf. ref 10) afforded no cyclized material. The single-crystal X-ray structure of the product, **6**, is shown in Figure 2. Treatment of the product with 1:1 sulfuric/trifluoroacetic acids gave the metal-free derivative **7** (54% yield) (^{1}H NMR methine protons at δ 6.14, 5.28, and 4.82 ppm) along with a small amount

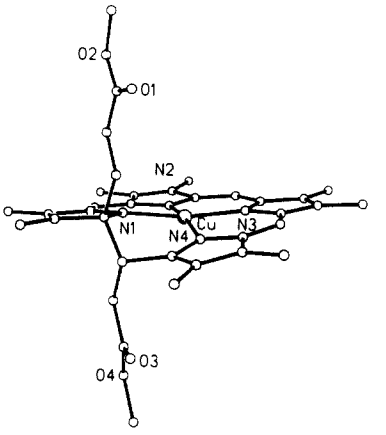


Figure 2. X-ray structure of the copper(II) complex **6**.

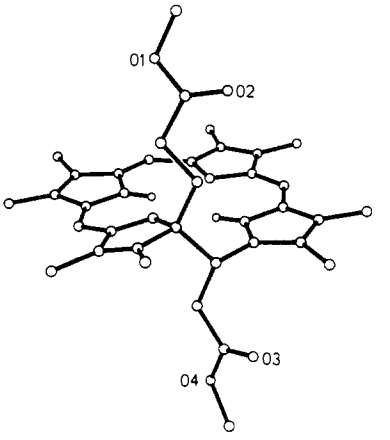
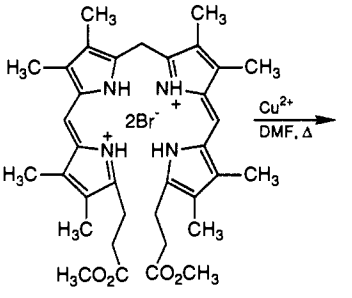
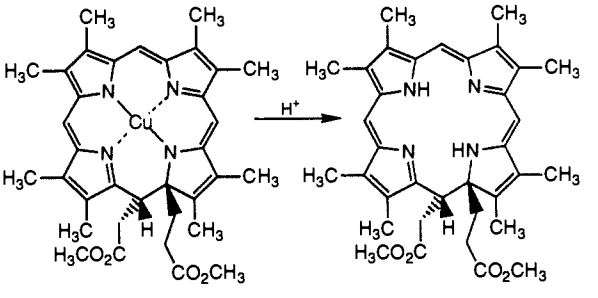


Figure 3. X-ray structure of the metal-free compound **7**.



5



6 **7**

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(10) Jeyakumar, D.; Snow, K. M.; Smith, K. M. *J. Am. Chem. Soc.* **1988**, *110*, 8562–8564.
(11) The a,c-biladiene **5** [NMR ($CDCl_3$) δ 13.53, 13.16 (each br s, 2 H, NH), 7.12 (s, 2 H, 2 × =CH), 4.86 (s, 2 H, CH_2), 3.64 (s, 6 H, OCH_3), 3.18 [t, J = 7.4 Hz, 4 H, CH_2CH_2CO], 2.83 (t, J = 7.4 Hz, 4 H, CH_2CH_2CO), 2.27, 2.21, 2.04, 1.80 (each s, 6 H, CH_3); vis (CH_2Cl_2) λ_{max} 452 (ϵ 123 000), 526 nm (82 700)] was synthesized in high yield by using standard methods[2,3] from 3,3′,4,4′-tetramethyldipyrromethane-5,5′-dicarboxylic acid and 5-[2-(methoxycarbonyl)ethyl]-2-formyl-3,4-dimethylpyrrole.

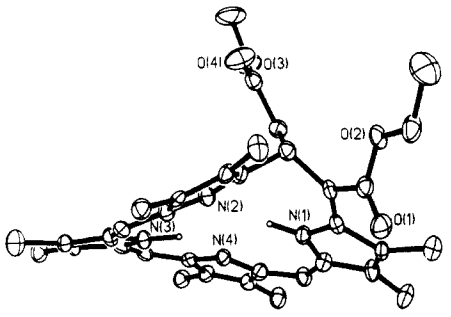
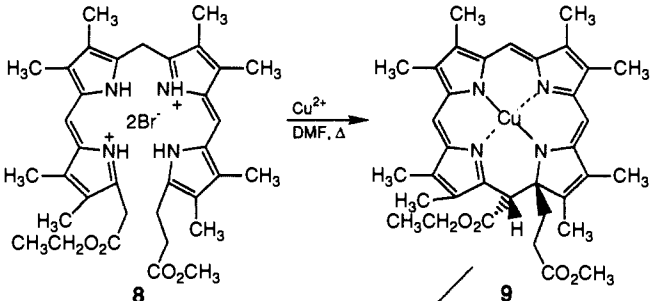
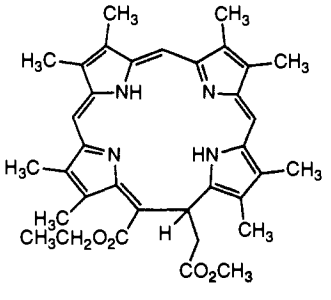


Figure 4. X-ray structure of ring-expanded macrocycle **10**.

of minor products, including a trace of porphyrin. These materials are extremely robust and readily survive even concentrated sulfuric acid without transformation into porphyrin. The optical spectrum of **7** is shown as the broken line in Figure 1, and its X-ray structure is presented in Figure 3.

Use of the unsymmetrically substituted a,c-biladiene salt[12] **8** in the copper(II)-catalyzed cyclization afforded uniquely[13,14] a 36% yield of the copper(II) macrocycle **9**, with an optical spectrum very similar to that of **6**. However, attempts to remove the





10

chelating copper with sulfuric and trifluoroacetic acids resulted in ring expansion (30%) to give the product **10** with the homoporphyrin carbon skeleton. Figure 4 presents the X-ray structure of this novel material. We presume that the ring expansion proceeds by way of initial ring opening, followed by closure in a different sense, and that the ring opening is favored in the case of **9** (compared with **6**) by the presence of the enolizable proton at the macrocyclic carbon.[14]

(12) Compound **8** was prepared via the tripyrrene route[2,3] from benzyl 5-(benzyloxycarbonyl)-3,3′,4,4′-tetramethyldipyrromethane-5′-carboxylate, 5-[2-(methoxycarbonyl)ethyl]-2-formyl-3,4-dimethylpyrrole, and 5-[2-(ethoxycarbonyl)methyl]-2-formyl-3,4-dimethylpyrrole [NMR ($CDCl_3$) δ 13.43 (br s, 2 H, NH), 13.23, 13.08 (each br s, 1 H, NH), 7.16, 7.12 (each s, 1 H, =CH), 5.19 (s, 2 H, CH_2), 4.23 (s, 2 H, CH_2CO), 4.19 (q, J = 7.2 Hz, 2 H, CH_3CH_2O), 3.66 (s, 3 H, OCH_3), 3.27 (t, J = 7.2 Hz, 2 H, CH_2CH_2CO), 2.99 (t, J = 7.2 Hz, 2 H, CH_2CH_2CO), 2.29, 2.28, 2.23, 2.21, 2.05, 1.98, 1.90, 1.88 (each s, 3 H, CH_3), 1.28 (t, J = 7.2 Hz, 3 H, OCH_2CH_3); vis (CH_2Cl_2) λ_{max} 452 (ϵ 104 200), 528 nm (105 800)].

(13) Though other isomers were possible, only the compound with structure **9** is produced. A crystal structure of this compound, which supports the structure proposed in all respects, has been obtained. See supplementary material.

(14) Complete mechanistic rationalizations will be presented in a full paper.

Acknowledgment. This research was supported by grants from the National Institutes of Health (HL 22252) and the National Science Foundation (CHE-86-19034). Part of the diffraction and computing equipment was purchased under National Science Foundation Grant CHE-88-02721.

Supplementary Material Available: Figures showing structures and tables of crystal data and data collection parameters, atomic coordinates, bond lengths, bond angles, anisotropic thermal parameters, H-atom coordinates, and isotropic displacement coefficients of compounds **6**, **7**, **9**, and **10** (28 pages). Ordering information is given on any current masthead page.

Novel General Approach for the Assay and Inhibition of Hydrolytic Enzymes Utilizing Suicide-Inhibitory Bifunctionally Linked Substrates (SIBLINKS): Exemplified by a Phospholipase A_2 Assay

William N. Washburn*,† and Edward A. Dennis

Department of Chemistry
University of California, San Diego
La Jolla, California 92093

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Kinetic studies of hydrolytic enzymes such as phospholipase A_2[1] (PLA_2) have been hampered by the lack of a versatile, sensitive, continuous spectroscopic assay. Spectroscopic assays currently employed either have low sensitivity,[2] inhibit some PLA_2's,[3] use exceedingly poor substrates,[4] require extensive synthesis,[4,5] are incompatible with free thiols,[5] or are not continuous.[6] We report herein a convenient spectrophotometric assay for PLA_2 in which the substrate closely resembles a natural phospholipid. In addition, we utilize this approach to design "suicide-inhibitory bifunctionally linked substrates" (SIBLINKS) which are specific irreversible inhibitors for PLA_2[7] as well as discuss extensions to either assay or modulate the activity of other hydrolytic enzymes.

Since direct attachment of a chromophore to the glycerol backbone is precluded by the substrate structural requirements of PLA_2,[1] we employed a dibasic acid to link the lysophospholipid moiety to a dye. Upon PLA_2-catalyzed hydrolysis of the *sn*-2 ester of 1-decanoyl-2-(*p*-nitrophenyl glutaryl)phosphatidylcholine (**1**), nucleophilic catalysis by the nascent carboxylate group of the hydrolysis product **2** releases *p*-nitrophenol via cyclization[8] (Scheme I). The cyclization of **2** should be slow relative to diffusion since $t_{1/2}$ for cyclization of **2** was found to be 140 s at 20 °C. Consequently, the concomitantly formed glutaric anhydride would be formed in bulk solution and would react with H_2O before encountering PLA_2.[9]

The spectrophotometric assay of PLA_2 from cobra venom (*Naja naja naja*[10]) using **1**[11] as substrate is linear for 2 min and is linear

* To whom correspondence should be addressed.
† Visiting Scholar UCSD (1988-1989). Permanent Address: Life Sciences Research Laboratory, Eastman Kodak Company, Rochester, NY 14650.

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(9) Inhibition of PLA_2 by exposure to anhydrides in bulk solution is very inefficient. For example, treatment of a 0.35 μM solution of PLA_2 for 5 min with 3, 0.3, and 0.03 mM glutaric anhydride resulted in 80, 14, and 0% inhibition, respectively. These values did not change with time.

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