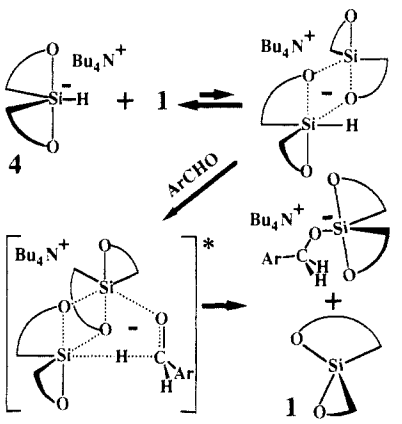
Scheme III



Reductions of carbonyl groups by hydrogen-substituted silanes were earlier found[8] to be catalyzed by nucleophiles coordinating to the silane to form a hydridosiliconate, or a 12-Si-6 species. Lewis acids[9] and protonic acids[10] were also found to catalyze reductions by hydrogen-substituted silanes, although they could not be used with hydridosiliconates. Catalysis by silane **1** as a Lewis acid, however, allows the continuing use of the hydridosiliconate (**2** or **4**) in the presence of this catalyst.

Observed pseudo-first-order kinetics for the reduction of excess aldehyde **6** (0.27 M) in CH_2Cl_2 by the measured low concentrations of hydridosiliconate **4** (initially 0.04 M) was increased linearly by added concentrations of silane **1** (0.0–0.064 M). The reduction rates are clearly third order: first order for the catalytic silane **1**, for DMAB (**6**), and for hydridosiliconate **4**. Even at 0.03 M, a low concentration, the silane provides faster rates of reductions by **4** (Figure 1), by factors of more than 250 for electron-rich aldehydes (from *p*-NMe_2 to H, $\rho^+ = -0.28$). It is clear that these aldehydes are in equilibrium, as Lewis bases, for coordination of the silane to the carbonyl oxygens (Scheme IIb) increasing the rate of hydride transfer from **4**. Although the ρ value for electron-attractive substituents (H to *p*-CN in Figure 1) is positive ($\rho = 0.87$), showing that the reaction is faster when a more electron attractive substituent makes the carbonyl carbon more electrophilic for attraction of the hydride, silane **1** still provides catalysis, although not by initial coordination to the aldehyde. We suggest another possible mechanism (Scheme III) with **1** providing catalysis by rapidly reversible coordination of **1** to the apical oxygen of hydridosiliconate **4**, and possibly coordination of one of the silane oxygens to the silicon of **4** to form a 12-Si-6 species that could provide faster hydride transfer to the carbonyl carbon. The transition state could provide simultaneous transfer of the silane catalyst to the carbonyl oxygen, as pictured in Scheme III. Small changes in the ^{19}F and ^1H NMR of **4**, upon addition of **1**, are compatible with Scheme III. The kinetics for reductions in the absence of **1** are much slower, but with $\rho = 1.71$, compatible with the mechanism of Scheme IIa. The mechanism of Scheme III provides a lower positive ρ value than that of Scheme IIb.

Acknowledgment. This research was partially funded by National Science Foundation Grant CHE-89 10896.

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Uroporphyrinogen III Methylase Catalyzes the Enzymatic Synthesis of Sirohydrochlorins II and IV by a Clockwise Mechanism

Martin J. Warren, Mario D. Gonzalez, Howard J. Williams, Neal J. Stolowich, and A. Ian Scott*

Center for Biological NMR, Department of Chemistry Texas A&M University, College Station, Texas 77843-3255

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Sirohydrochlorin (**3**), the iron-free prosthetic group of nitrite and sulfite reductases,[1-3] is normally obtained by oxidation of the vitamin B_{12} intermediate dipyrrocorphin (**2**), which is biosynthesized by C-methylation of uroporphyrinogen III (**1**) at positions 2 and 7.[4,5] Recently sirohydrochlorin I (**6**), the C-methylated isobacteriochlorin derived from uroporphyrinogen I (**4**) and a possible intermediate in the biosynthesis of the newly discovered zinc corphinate S factors,[6,7] has been synthesized enzymatically.[8] The enzyme responsible for the addition of the *S*-adenosylmethionine (SAM) derived methyl groups to the uroporphyrinogen framework, uroporphyrinogen methyl transferase (M-1), has been overexpressed as a result of the cloning of the cysG gene in *Escherichia coli*.[9] M-1 not only methylates uroporphyrinogen isomers I and III at positions 2 and 7 to yield the corresponding dipyrrocorphins (**2** = precorrin-2[10] and **5**) but also carries out a further, unexpected methylation at position 12 to yield trimethyl pyrrocorphins (Scheme I).[10] In an effort to obtain a better understanding of the regiospecificity of this enzyme, the nonphysiological uroporphyrinogen isomers, IV (**7**) and II (**10**), were

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(10) Scott, A. I.; Warren, M. J.; Roessner, C. A.; Stolowich, N. J.; Santander, P. J. *J. Chem. Soc., Chem. Commun.*, in press. The term precorrin-*n* is used to denote biosynthetic precursors of vitamin B_{12} where *n* = the number of SAM-derived C-methyl groups which are inserted into precorrin-2 (**2**) in the order C-20 (precorrin-3), C-17 (precorrin-4) > C-12 > C-1 > C-5 > C-15. See: Uzar, H. C.; Battersby, A. R.; Carpenter, T. A.; Leeper, F. J. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1689.

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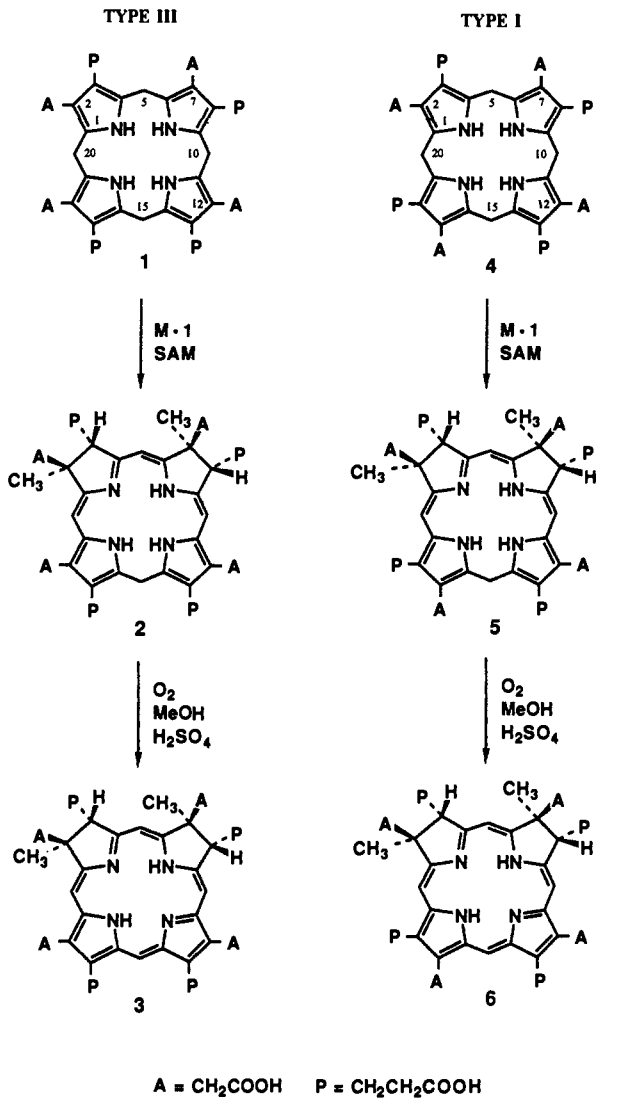
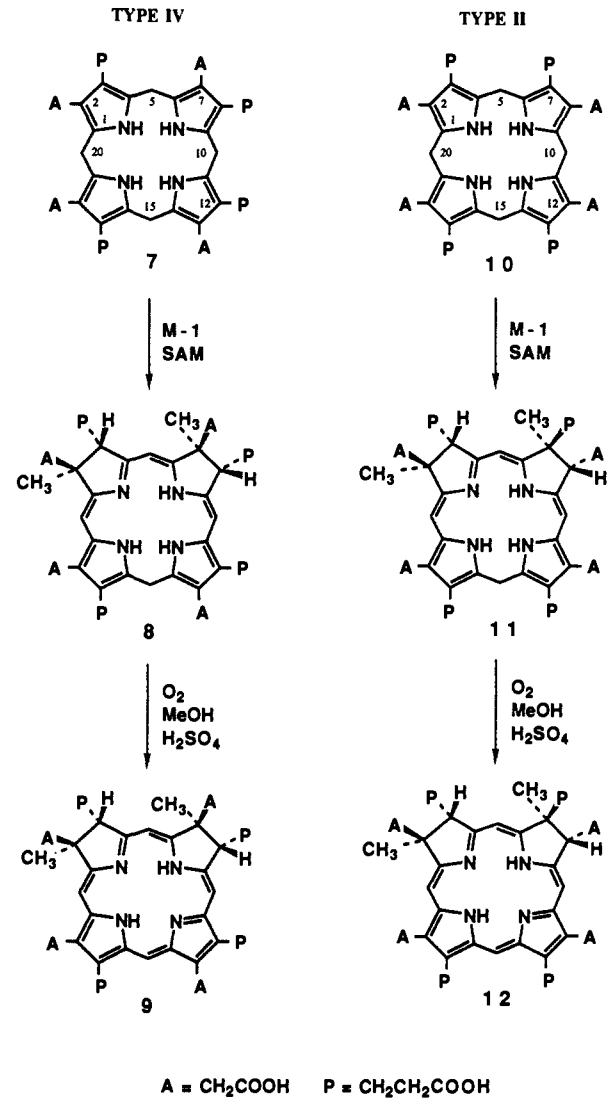
Scheme I



Scheme II



tested as substrates for M-1 in the presence of SAM.[11]

Incubation of uroporphyrinogen IV (**7**) with M-1 and SAM gave as the major product, after oxidative esterification and extraction, a dimethyl isobacteriochlorin (**9**; A = CH_2COOMe; P = CH_2CH_2COOMe) exhibiting the appropriate UV/vis and mass spectra.[14] A second, minor product was a chlorin, presumably arising from oxidation of the monomethylated product (as **8**).[15]

The proton NMR spectrum of **9** revealed two methyl groups (δ = 1.63 and 1.74 ppm) and four meso protons (C-5, 6.81 ppm; C-20, 7.55 ppm; C-10, 7.64 ppm; C-15, 8.85 ppm) characteristic of the spectral pattern of the isobacteriochlorins **3** and **6** formed from uroporphyrinogens I and III, respectively.[1,8] The NOESY spectrum allowed correlation between the C-20 meso proton and the methyl signal at δ = 1.74 ppm while the C-5 meso proton correlated with the methyl signal at δ = 1.63 ppm. The COSY and ^{13}C spectra confirmed the structure as that of sirohydrochlorin IV (**9**) bearing two methyl groups at the acetate termini at positions 2 and 7.[15] The relative stereochemistry has been assigned by analogy with that found for sirohydrochlorins I and III[1,8] but remains to be proven.

When uroporphyrinogen II (**10**) was used as a substrate, two major products were again formed, an isobacteriochlorin and a chlorin (Scheme II).[15] The proton NMR spectrum of the isobacteriochlorin methyl ester showed two methyl signals (δ = 1.79 ppm and 1.23 ppm), the latter being at much higher field than that normally observed for sirohydrochlorin, suggesting that a methyl group was in an unusual environment. Another interesting characteristic of this spectrum was that one of the two AB systems

(11) Uroporphyrins II and IV were synthesized as their octamethyl esters by respective coupling of the dipyrromethanes i with ii and ii with iii under the reaction conditions for coproporphyrin synthesis.[12] The reaction products

^{Me}A = CH_2COOCH_3
^{Me}P = $CH_2CH_2COOCH_3$

were purified to homogeneity by column chromatography, HPLC,[13] and recrystallization. The uroporphyrinogen isomers were obtained by hydrolysis of the appropriate ester with 6 N HCl and reduction with sodium amalgam. After adjustment of the pH to 8, each isomer was incubated with methylase and the reaction products were isolated as described in ref 5.

(12) Robinson, J. A.; McDonald, E.; Battersby, A. R. *J. Chem. Soc., Perkins Trans. 1* **1985**, 1699.

(13) Gonzalez, M. D.; Grant, S. K.; Williams, H. J.; Scott, A. I. *J. Chromatogr.* **1988**, *437*, 311.

(14) λ_{max} for sirohydrochlorins II and IV (rel intensity): 275 (0.155), 358 (0.442), 377 (0.650), 512 (0.064), 543 (0.120), 584 (0.180) (MeOH); identical with the spectrum of **3** (MeOH).

(15) UV/vis, NMR, and mass spectra of the chlorins (oxidized **8** and **11**) and of the isobacteriochlorins derived from uroporphyrinogens I–IV (**9** and **12**) are in full accord with the structures proposed. These chlorin structures correspond in all but regiochemistry to the chlorin Factor I, the first isolated product of the C-methylation of uroporphyrinogen III at position C-2. (a) Müller, G. In *Vitamin B_{12}*; Zagalak, B., Friedrich, W., Eds.; de Gruyter: New York, 1979; p 279. (b) Imfield, M.; Arigoni, D.; Deeg, R.; Müller, G. *Ibid.* p 315.

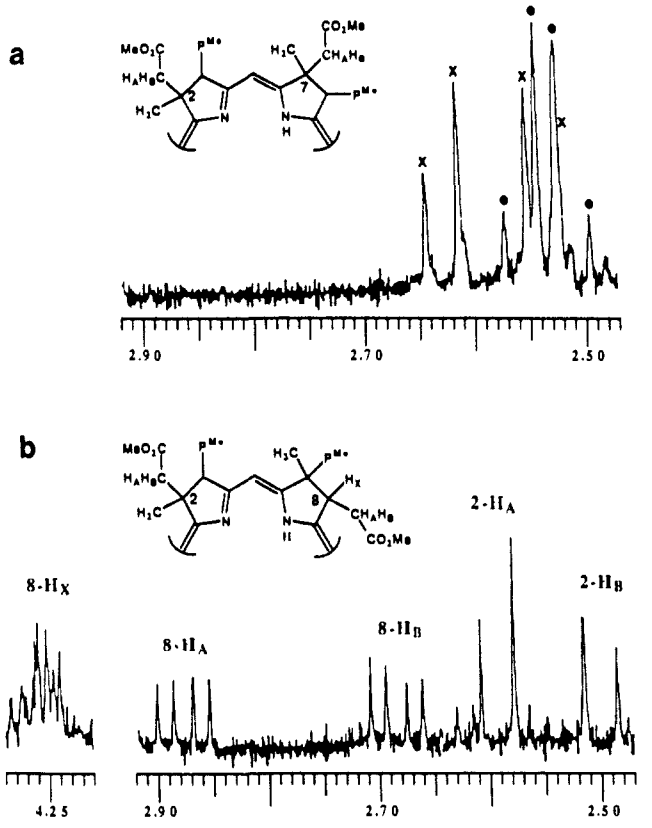
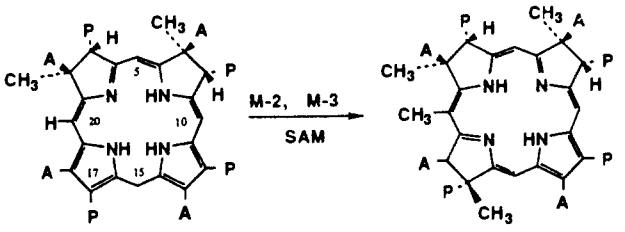


Figure 1. 500-MHz proton NMR spectra of the region 2.4–2.9 ppm of (a) sirohydrochlorin IV (**9**), showing the two AB systems (× and ●) arising from the methylene protons of the acetate side chains attached to positions 2 and 7, and (b) sirohydrochlorin II (**12**), showing an AB system (2.4–2.6 ppm) arising from the methylene protons 2-$H_{A,B}$ of the acetate side chain attached to position 2 which is clearly distinguishable from the ABX system due to the presence of the C-8 proton 8-H_X which resonates at δ = 4.25 ppm (see insert).

expected from the acetate methylene protons, as seen in sirohydrochlorin IV (**9**) (Figure 1a), occurred as an ABX system (Figure 1b). COSY and homonuclear decoupling experiments demonstrated that a proton was indeed adjacent to an acetate side chain, indicating that methylation could not have occurred at that terminus. One of the ^{13}C resonances of the two methyl groups was also shifted from the normal position between 19.5 and 20.1 ppm, lending further support for the idea that the second methyl group had been added at C-7, i.e., at a propionate- rather than an acetate-bearing carbon. This was confirmed by a NOESY experiment where correlation was observed between the C-20 meso proton (δ = 7.53 ppm) and the methyl signal at δ = 1.79 ppm and between the C-5 meso proton (δ = 6.71 ppm) and the methyl signal at δ = 1.23 ppm. Collectively these data prove that methylation had occurred at the acetate (C-2) and propionate termini (C-7), leading to structure **12** for sirohydrochlorin II, incorporating the same overall stereochemistry as **3**, **6**, and **9**.

The ramifications of these results are multiple. Firstly, the enzyme M-1 is capable of C-methylating unnatural substrates to yield nonphysiological intermediates, suggesting that, during molecular recognition, the order of the carboxylic side chains serves only to position the substrate for the first methylation event. Methylation occurs sequentially with the addition of a methyl group first to an acetate terminus,[15,16] as indicated by the isolation of monomethyl chlorins (corresponding to oxidized **8** and **11**), followed by C-methylation at the first side-chain terminus of the adjacent pyrrole ring reached in a clockwise direction in **8** and **11**, regardless of whether the side chain is acetate or propionate. Thus the pattern of C-methylation by the enzyme is identical with that observed for chemical methylation[17] ($C_2 > C_7 > C_{12}$) which identified the chromophoric array of the substrate as the major criterion for regioselectivity. Secondly, the fact that M-1 can methylate at a propionate side chain indicates that the mode of action of methylase-3, responsible for C-methylation at C-17 of precorrin-3[10] to form the hypothetical pyrrocorphin "precorrin-4", is probably similar to that of uroporphyrinogen methylase (M-1), and that the methylases used for tetrapyrrole modification may have evolved from a common ancestor. Thirdly, the products of these reactions can now be tested as substrates for a study of the further methylating enzymes required for B_{12} synthesis and of the enzymes necessary for the corrin ring contraction process. Thus the type-IV counterpart (**13** ≡ dihydro-**9**) of precorrin-2[10] (**2**) should be a substrate for the enzymes required to methylate positions 20 and 17 (M-2, M-3). The absence of an acetate group at position 12 would preclude decarboxylation and hence further methylation, yielding "precorrin-4"[10] (**14**). Similarly the type-II precorrin-2 (dihydro-**12**) should be an excellent candidate for further methylation and may possibly yield a complete type-II corrinoid.[10] These novel synthetic ideas are now under experimental test.



13 **14**

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(16) Preferentially this acetate corresponds to the second "A" of the sequence *PA*, *AP*. If such an acetate cannot be found, as in the case of uroporphyrinogen I (**4**), a random selection of acetate *AP*, *AP* is made. In each case the second methylation occurs on the C-7 position of the adjacent ring regardless of whether it is *AP* or *PA*.

(17) Biomimetic chemical methylation of hexahydroporphinoids has been described: Eschenmoser, A. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 5. See also: Leumann, C.; Hilpert, H.; Schreiber, A.; Eschenmoser, A. *J. Chem. Soc., Chem. Commun.* **1983**, 1404.

Solid-Phase-Mediated Peptide Heterodisulfide Formation[1]

Berta Ponsati, Mariano Ruiz-Gayo,[2] Ernest Giralt, Fernando Albericio,* and David Andreu*

Department of Organic Chemistry
University of Barcelona, 08028 Barcelona, Spain

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Formation of an asymmetric disulfide bridge between two peptide chains is often one of the most problematic aspects of peptide synthesis.[3] Since cooxidation of two cysteine-containing peptides gives mixtures of homo- and heterodimers, directed methods for the formation of heterodisulfides are required.[4] A

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(2) Present address: Université René Descartes (Paris V), UER des Sciences Pharmaceutiques et Biologiques, Departement de Chimie Organique; 4, Avenue de l'Observatoire, 75270 Paris, France.

(3) For reviews, see: (a) Barany, G.; Merrifield, R. B. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 2, pp 1–284. (b) Hiskey, R. G. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1981; Vol. 3, pp 137–167. (c) König, W.; Geiger, R. In *Perspectives in Peptide Chemistry*; Eberle, A., Geiger, R., Wieland, T., Eds.; Karger: Basel, 1981; pp 31–44.

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