6.30–6.46 (q, 2 H), 7.28 (d, 2 H, J = 7.8 Hz); 7.64 (d, 2 H, J = 7.8 Hz): IR 1630, 1590, 1360, 1180, 960 cm^{-1}; MS m/e 223 (M^+).

X-ray Crystallographic Analysis of the 13-$NaClO_4$ Complex. Experimental details are in the supplementary material.

log β Determinations. Binding constants for the interaction of ligands **3**, **4**, **6**, **9**, and **10** with protons and selected metal cations were determined potentiometrically using an Orion-Ross double junction semimicro combination glass electrode. The semimicro potentiometric titrations were carried out in a sealed, thermostated vessel (5 mL, 25.0 ● 0.1 °C) under a CO_2-free N_2 atmosphere. During each titration experiment, the emf values of the glass electrode, which are linearly related to p[H] under constant ionic strength, were recorded as a function of the amount of titrant added.

Standard electrode potential, ϵ^0 (462.0 mV), and the ion products of water, pK'_w (13.76), at 0.1 M ionic strength were determined by titrating a standardized $(CH_3)_4NOH$ solution into a HNO_3 solution.

The proton binding constants for the ligands were computed from data obtained by titrating acidified ligand solutions with the $(CH_3)_4NOH$ solution. Binding constants for the ligands with metal ions were calculated from data obtained from titrating acidified ligand solution in the presence of the guest ions.

The ionic strength of the solutions was maintained at 0.1 M with $(CH_3)_4NNO_3$. The electrode filling solution was a saturated $(CH_3)_4NNO_3$ solution. Program SUPERQUAD[36] was used for all the calculations.

Acknowledgment. This work was supported by the Department of Energy (Basic Energy Sciences, Grant No. DE-FG02-86ER134463).

Supplementary Material Available: Experimental details for the X-ray structural study, tables of X-ray structural data, and ^1H NMR spectra for compounds **7**, **8**, **22**, **23**, and **35** (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.

(36) Gans, P.; Sabatini, A.; Vacca, A. *J. Chem. Soc., Dalton Trans.* 1985, 1195.

Assessment of the Active-Site Requirements of 5-Aminolaevulinic Acid Dehydratase: Evaluation of Substrate and Product Analogues as Competitive Inhibitors

Rainer M. Lüönd, Josef Walker, and Reinhard W. Neier*

Department of Organic Chemistry, University of Neuchâtel, Av. de Bellevaux 51, CH-2000 Neuchâtel, Switzerland

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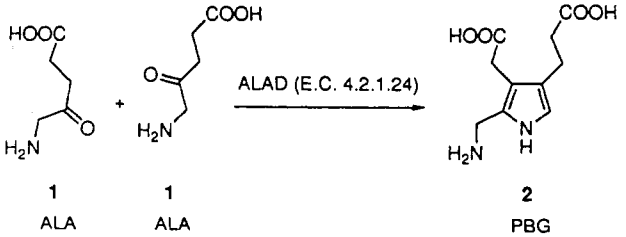
The enzyme 5-aminolaevulinic acid dehydratase (ALAD) is responsible for the synthesis of porphobilinogen (PBG) from two molecules of 5-aminolaevulinic acid (ALA). Porphobilinogen is an important committed intermediate in the biosynthesis of tetrapyrroles. The inhibition of ALAD from the purple bacterium *Rhodopseudomonas sphaeroides* was tested with various substrate and product analogues. Excellent inhibition was observed with the nitro analogue **5** (K_i = 0.018 mM) of laevulinic acid (**10**) (K_i = 1 mM), *rac*-2-hydroxy- (**7**) (K_i = 0.43 mM), *rac*-3-hydroxy- (**8**) (K_i = 1.2 mM), 5-hydroxy- (**11**) (K_i = 0.25 mM), and 5-nitrilolaevulinic acid (**12**) (K_i = 0.060 mM). The sulfonic acid **3** and the phosphonic acid analogue **4** did not inhibit the enzyme. The product analogues **15–18** only showed a moderate inhibition (K_i = 10–15 mM) whereas the pyrazole **19**, a close analogue of porphobilinogen, did not inhibit the enzyme at all (K_i = 32 mM). Comparison of the K_i values for the substrate analogues indicated the ALAD active site to be sensitive to the hybridization and charge at position 1, to be insensitive to polar and neutral substituents at position 5 unless they are negatively charged (**14**) or too bulky (**13**), and to require flexibility of the carbon chain of the substrate, since stiff molecules like β-acetylacrylic acid **6** showed no affinity. The product analogues **15–19** indicated that the active site of ALAD was not inhibited by its direct product PBG.

Introduction

5-Aminolaevulinic acid dehydratase[1,2] (ALAD, porphobilinogen synthetase, EC 4.2.1.24) catalyzes the condensation of two molecules of 5-aminolaevulinic acid (ALA) to produce porphobilinogen (PBG) which is a committed intermediate in the biosynthesis of tetrapyrrolic natural products like porphyrins, chlorophylls, and corrins[3] (Scheme I). 5-Aminolaevulinic acid dehydratase has been purified to homogeneity from a wide variety of sources, including bovine liver,[4] photosynthetic bacteria,[5,6] and human erythrocytes.[7] The dehydratases from all sources studied so far require the presence of an exogenous thiol such as 2-mercaptoethanol or dithioerythritol and of a metal ion (K^+, Mg^{2+}, Zn^{2+})[4,6,8] to maintain the catalytic

Scheme I. Enzymic Formation of Porphobilinogen from Two Molecules of 5-Aminolaevulinic Acid



(1) Shemin, D. *Enzymes*; 3rd ed.; Academic Press: New York, 1972; pp 323–337.
(2) Cheh, A. M.; Neilands, J. B. *Structure and Bonding*; Springer Verlag: Berlin, 1976; Vol. 29, pp 123–169.
(3) Leeper, F. J. *Nat. Prod. Rep.* **1989**, 171–203.
(4) Tsukamoto, I.; Yoshinaga, T.; Sano, S. *Biochim. Biophys. Acta* **1979**, *570*, 167–178.
(5) van Heyningen, S.; Shemin, D. *Biochemistry* **1971**, *10* (25), 4676–4682.
(6) Nandi, D. L.; Baker-Cohen, K. F.; Shemin, D. *J. Biol. Chem.* **1968**, *243* (6), 1224–1230.
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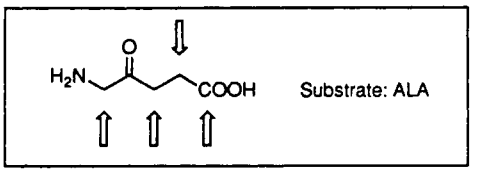
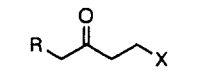
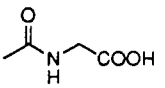
0022-3263/92/1957-5005$03.00/0

Scheme II. Substrate Analogues of ALA

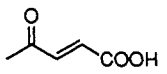




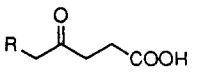
3: R = NH_2, X = SO_3H
4: R = H, X = $PO(OH)_2$
5: R = H, X = NO_2



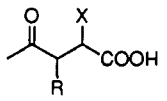
9



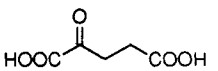
6



10: R = H
11: R = OH
12: R = CN
13: R = NHCO(2-$C_6H_4CO_2H$)

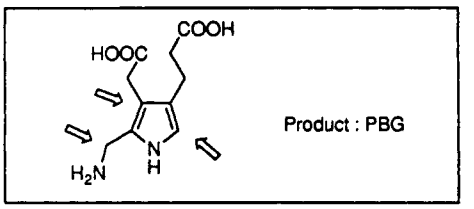


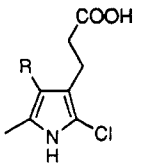
rac-7: R = H, X = OH
rac-8: R = OH, X = H



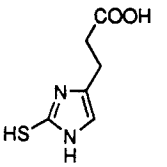
14

Scheme III. Product Analogues of PBG

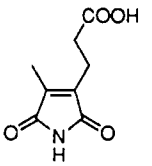




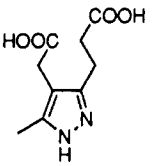
15: R = $COCH_3$
16: R = CO_2CH_3



18



17



19

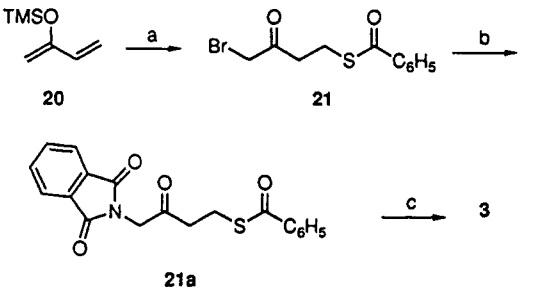
activity.[1] The eukaryotic dehydratases have a molecular weight of about 280000 and are composed of eight subunits of molecular weight 35000.[9] Only four of the eight subunits react with ALA to produce a Schiff base.[9] The active site lysine has been identified in the human, the rat, and the *E. coli* enzyme.[10] The sequences of the ALAD's from *E. coli*,[11] yeast,[12] and human liver[13] are known. No X-ray structure of ALAD, which could lead to a better insight into the mechanism, has been published.

A few years ago we became interested in the function and mechanism of the ALAD from the photosynthetic bacteria *Rhodopseudomonas spheroides*. We planned to screen substrate as well as product analogues as potential competitive inhibitors. Except the early investigations in Shemin's laboratories,[14] no systematic research has been undertaken so far with ALAD. We herein report the synthesis and the results of the incubation of the enzyme with various ALA and PBG analogues.

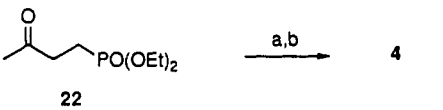
Synthesis

Schemes II and III present a list of substrate and product analogues which were tested as potential competitive inhibitors of the ALAD. The amino sulfonic acid **3** (Scheme IV) already described in the literature[15] was synthesized by an alternative route. Bromo ketone **21** could easily be obtained in large quantities by a one-pot procedure. Silyl enol ether[16] **20** in THF was brominated with NBS followed by the addition of thiobenzoic acid. The pure product was obtained in 42% yield by crystallization. Finally the amino sulfonic acid **3** was obtained after Gabriel reaction, oxidation with H_2O_2, and hydrolysis in 6 N HCl according to the literature procedure.[15] The known phosphonic acid **4** was synthesized by applying a general protocol for the hydrolysis of phosphonic esters.[17] Diethyl phosphonate[18] **22** and sodium iodide in acetonitrile

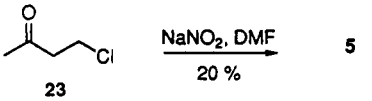
Scheme IV. Synthesis of Carboxylic Acid Analogues 3–5



(a) NBS, THF, 0 °C to rt; C_6H_5COSH, 0 °C to rt (41%); (b) $C_6H_4(CO_2)NK$, DMF, −5 °C to rt (44%); (c) H_2O_2, HOAc, 100 °C; 6 N HCl, reflux (20%).



(a) NaI, $(CH_3)_3SiCl$, CH_3CN, 60 °C (91%); (b) H_2O, rt, lyophil (50%).



(8) Prasad, D. D. K.; Singh, N. K.; Datta, K.; Prasad, A. R. K. *Biochem. Int.* **1988**, *17* (1), 87–102.
(9) Shemin, D. *Phil. Trans. R. Soc. Lond. B* **1976**, *273*, 109–115.
(10) Gibbs, P. N. B.; Jordan, P. M. *Biochem. J.* **1986**, *236*, 447–451.
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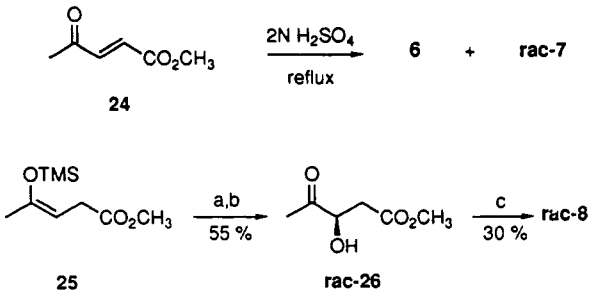
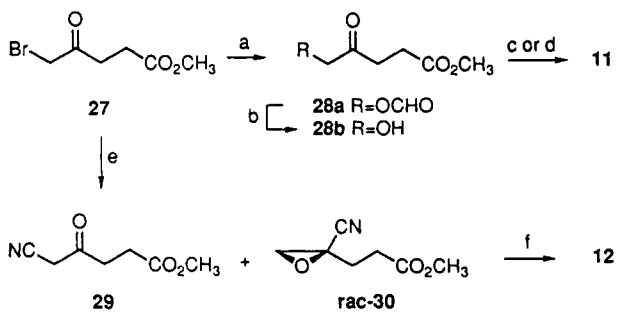
(12) Myers, A. M.; Crivellone, M. D.; Koerner, T. J.; Tzagaloff, A. *J. Biol. Chem.* **1987**, *262* (35), 16822–16829.
(13) Wetmur, J. G.; Bishop, D. F.; Cantelmo, C.; Desnick, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 7703–7707.
(14) Nandi, D. L.; Shemin, D. *J. Biol. Chem.* **1968**, *243* (6), 1236–1242.
(15) Daeniker, H. U.; Druey, J. *Helv. Chim. Acta* **1957**, 2148.
(16) Jung, M. E.; McCombs, C. A. *Org. Synth.* **1978**, *58*, 163–169.
(17) Morita, T.; Okamoto, Y.; Sakurai, H. *Tetrahedron Lett.* **1978**, 2523–2526.
(18) Hall, L. A. R.; Gordon, D. A. Ger. Offen. 2727266; U.S. Appl. 698623, 1991 (Abstract).

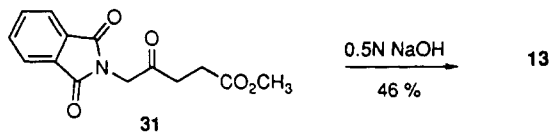
Scheme V. Synthesis of Analogues 6–8 and 11–13: Variations at Position C2, C3, and C5



(a) *m*-CPBA, hexane, 0 °C; (b) Et_2O/1 N HCl, rt; (c) PLE, 30 °C, pH 7.5.

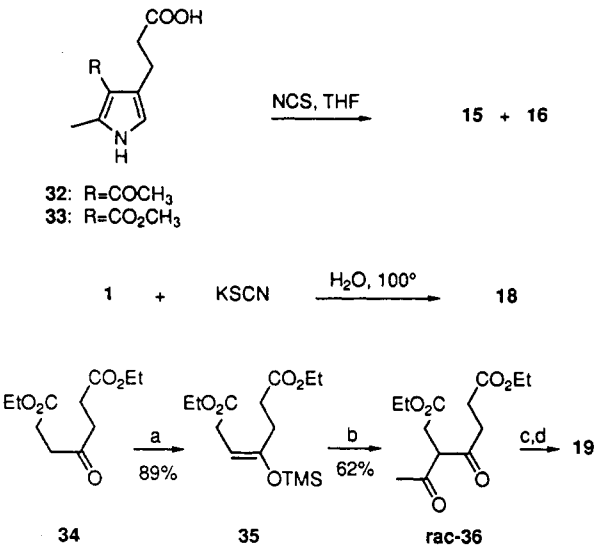


(a) HCOOH, DBU, C_6H_6, rt (70%); (b) Alumina N-super I (60%); (c) THF/4 N HCl, 90 °C (33%); (d) PLE, 30 °C, pH 7.5 (60–70%); (e) KCN, THF/H_2O; (f) PLE, pH 7.5 (51%).



were treated with trimethylchlorosilane at 60 °C. The resulting trimethylsilyl phosphonate was isolated by distillation and hydrolysed with water at rt. Lyophilization and crystallization afforded analytically pure phosphonic acid **4**. The synthesis of nitro ketone[19] **5** was already reported in 1957. A *Kornblum* reaction in DMF with 4-chloro-2-butanone[20] afforded the important nitro analogue of laevulinic acid (**10**), a potent inhibitor[14] of ALAD. Acidic hydrolysis of methyl 4-oxopentenoate (**24**) in 2 N sulfuric acid led to the corresponding carboxylic acid **6** and the racemic 2-hydroxy acid **7** (Scheme V). Racemic 3-hydroxylaevulinic acid (**8**) was obtained from the corresponding methyl ester **26** by an enzymatic hydrolysis[21] with PLE in a 0.05 M phosphate buffer pH 7.5 at 30 °C. Ester **26** was synthesized through the oxidation of the silyl enol ether[22] **25** with *m*-CPBA followed by a hydrolytic cleavage of the trimethylsilyl-protected secondary alcohol. *N*-Acetylglycin as a 3-aza analogue of laevulinic acid was obtained commercially as well as laevulinic (**10**) and 2-oxoglutaric acid (**14**). 5-Hydroxy acid **11** was prepared in three steps starting from the 5-bromo compound[23] **27**. S_N2 reaction with formic acid under basic conditions in benzene yielded the formyloxy compound which was easily hydrolyzed to the 5-hydroxy methyl ester **28** by passing through a column with neutral alumina. The free acid **11**

Scheme VI. Synthesis of Product Analogues



(a) $CF_3SO_3Si(CH_3)_3$, Et_3N, Et_2O; (b) CH_3COCl, $ZnCl_2$, CH_2Cl_2/Et_2O; (c) $H_2NNH_2 \cdot H_2O$, EtOH, 100%; (d) 15% NaOH, EtOH, reflux, 81%.

was obtained after hydrolysis with 4 N HCl in the presence of THF. Better results were obtained with an enzymatic ester cleavage using PLE. Nitrile **12** was obtained by the same strategy. S_N2 displacement of the bromide by cyanide followed by an enzymatic ester cleavage afforded compound **12**. The oxirane **30** was always observed as a byproduct but could easily be separated by chromatography. Mild basic hydrolysis of compound **31**, a well-known intermediate in the synthesis of ALA,[24] afforded the amido acid **13**.

Chloropyrroles **15** and **16** (Scheme VI) were readily prepared by an NCS chlorination in THF of the corresponding α-free pyrroles[25] **32** and **33**. Hematinic acid[26] (**17**) as well as the thioimidazole[27] **18** were prepared according to the literature. Pyrazole **19** was obtained from the commercially available diethyl 4-oxopimelate (**34**). The silyl enol ether **35** was acylated with acetyl chloride in the presence of $ZnCl_2$. The resulting diketone **36** was treated with hydrazine hydrate affording the pyrazole ring. The ester hydrolysis was best done in 15% refluxing sodium hydroxide. Upon acidification pyrazole **19** precipitated and was isolated by filtration.

Enzyme Studies and Discussion

ALAD from *R. spheroides* was isolated as described by Shemin.[28] The ultracentrifugation step could be replaced by an easy and very efficient precipitation with polymin-P. The inhibition of the compounds described in this study were evaluated by a UV/vis assay of the product.[25,28] K_i values were determined using Lineweaver–Burk analysis. The results are shown in Tables I and II. The Lineweaver–Burk plot of all inhibitors was typical for a reversible competitive inhibition.

Replacing the carboxylic acid of the substrate by a sulfonic acid resulted in an analogue **3** with no inhibition and therefore indicating no affinity for the enzyme. The phosphonic acid analogue **4** showed only a negligible inhibition (K_i = 25 mM). The most powerful inhibitor **5** (K_i

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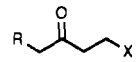
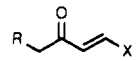
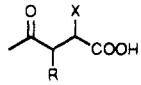
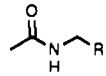
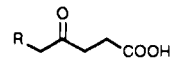
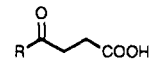
(24) Benedikt, E. M.; Köst, H. P. *Z. Naturforsch.* **1986**, *41b*, 138.
(25) Mauzerall, D.; Granick, S. *J. Biol. Chem.* **1956**, *219*, 435–446.
(26) Wittenberg, J.; Shemin, D. *J. Biol. Chem.* **1950**, *103*, 185.
(27) Wynn, R. W.; Corwin, A. H. *J. Org. Chem.* **1950**, *15*, 203.
(28) Colowich, S. P.; Kaplan, N. O. *Methods in Enzymology*; Academic Press: New York, 1970; Vol. XVII, Part A, pp 205–211.

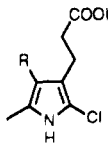
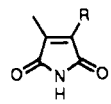
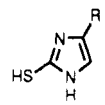
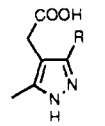
Table I. K_i Values for Substrate Analogues 3–14[a]

structure	R, X	no.	K_i
	R = NH_2, X = SO_3H	3	no inhibition
	R = H, X = $PO(OH)_2$	4	25 ± 3 mM
	R = H, X = NO_2	5	18 ± 3 μM
	R = H, X = COOH	6	-
	R = H, X = OH	*rac*-7	0.43 ± 0.13 mM
	R = OH, X = H	*rac*-8	1.2 ± 0.4 mM

structure	R	no.	K_i
	$R = CO_2H$	9	28 ± 2 mM
	R = H	10	1 ± 0.15 mM
	R = OH	11	0.25 ± 0.05 mM
	R = CN	12	60 ± 15 μM
	R = NHCO(2-$C_6H_4CO_2H$)	13	27 ± 4 mM
	$R = CO_2H$	14	19 ± 2 mM

[a] Substrate: K_M = 0.32 ± 0.05 mM.

Table II. K_i Values for Product Analogues 15–19

structure	R	no.	K_i
	$R = COCH_3$	15	13 ± 2 mM
	$R = CO_2CH_3$	16	15 ± 0.5 mM
	$R = (CH_2)_2CO_2H$	17	12 ± 2 mM
	$R = (CH_2)_2CO_2H$	18	20 ● 5 mM
	$R = (CH_2)_2CO_2H$	19	32 ± 4 mM

= 0.018 mM) was obtained when the carboxylic acid was replaced by the isosteric nitro group. These results show clearly that the ALAD is sensitive to variations at position C1. A possible explanation for this observation would be that the enzyme can distinguish between planar (sp^2-hybridized) and tetrahedral (sp^3-hybridized) groups at position 1 with a strong preference for a planar arrangement. Furthermore the delocalized negative charge on the two oxygen atoms of the carboxylic acid or the nitro group seems to be necessary for a good recognition. The methylesters of the corresponding acids 8 and 11 were 5–10 times weaker inhibitors. The nitronate analogue of isocitrate was used with great success by Schloss et al.[29] as a transition state analogue to inhibit the enzyme aconitase. The nitro analogue 5 cannot function as a transition-state analogue for ALAD from *R. spheroides*. The nitro group in our case simply acts as an isosteric replacement of the carboxylic acid.

Interestingly, no inhibition of the ALAD was observed with compound 6 up to a concentration of 13 mM. However when the inhibitor concentration was greater than 13 mM no PBG formation was observed, and therefore, it was not possible to determine the type of inhibition. The difference between laevulinic acid (10), a good competitive inhibitor, and compound 6 is the double bond between positions 2 and 3. The conjugation reduces the rotational freedom of compound 6 considerably. The result is a rigid skeleton which has lost its affinity for the enzyme. *N*-

(29) Schloss, J. V.; Porter, D. J. T.; Bright, H. J.; Cleland, W. W. *Biochemistry* 1980, *19*, 2358.

Scheme VII.[a] Enamine or Enol Formation

natural substrate substrate analogue 12

[a] X = O or NH.

Acetylglycine (9) shows a very low affinity for the active site. The amide function of 9 is a bad substitute for the carbonyl group of the natural substrate.

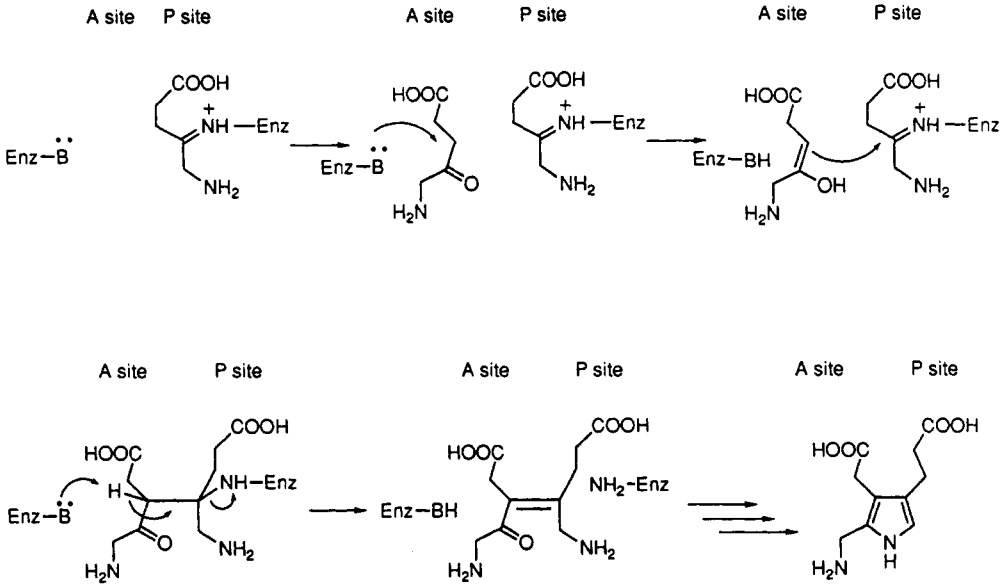
Both racemic hydroxy acids 7 and 8 were shown to be good inhibitors. Analysis of the enzymic transformation reveals that position 3 of the substrate molecule which forms the acetic acid side chain of PBG is going to be bonded to the carbonyl atom of the second molecule ALA (Scheme I). Therefore extra space should be available for substituents at position 3 (e.g. OH in 8) as long as the second subtrate has not yet been bound to the active site.

5-Substituted analogues 10–12 were good to excellent inhibitors (K_i < 1 mM) as long as the substituent was not too bulky (e.g. 13). The ammonium group of the substrate seems not to be of great importance, since laevulinic acid 10 itself is a good inhibitor.[14] 2-Oxoglutaric acid (14) was shown to be only a weaker inhibitor (K_i = 19 mM). Formally the positively charged ammonium group has been replaced by the negatively charged carboxylate. The nitrile 12 was a very strong inhibitor (K_i = 0.06 mM). The methylene group at position C5 of this compound was shown to be quite acidic. We observed complete proton deuterium exchange when running a ^1H NMR spectrum in D_2O. The excellent inhibition of nitrile 12 might arise from the preferred formation of the enamine into the "wrong" position at the active site of the enzyme (Scheme VII).

The product analogues 15–18 (Table II) inhibited ALAD only to a moderate extent. The steric and electronic differences to PBG are big. This may explain the low affinity for the enzyme. The structures of the analogues 15–18 were chosen not to interfere with the enzyme assay. Pyrazole 19, a close analogue of PBG, showed an extremely small inhibition (K_i = 32 mM). We believe therefore that ALAD is not sensitive to product inhibition and releases PBG quite easily from the active site. This also implicitly rules out a regulation of the enzyme's activity by its product. However, it is known that hemin and protoporphyrin are potent feedback inhibitors of ALAD.[14]

ALAD is an unusual enzyme. It catalyzes a reaction between two identical substrate molecules. Analysis of the possible mechanisms for PBG formation reveals that such an enzyme should not give normal Michaelis–Menten kinetics. Under our experimental conditions the reaction

Scheme VIII. Mechanism for 5-Aminolaevulinic Acid Dehydratase According to Jordan



catalyzed by ALAD obeys Michaelis–Menton[30] kinetics and can be analyzed as a one-substrate reaction. If the formation of the pyrrole as well as the Schiff base formation with the substrate molecule are irreversible steps under the reaction conditions (substituted mechanism), the analysis of the kinetic expression using the method of King and Altman[31] shows a rate law that can be reduced to the Michaelis–Menten kinetics. As a consequence the calculated K_i values are no longer absolute inhibition constants but *apparent* values. This means that only K_i values of structurally related compounds or analogues can be compared.

Conclusion

There have been three different mechanisms proposed for the formation of PBG.[14,32,33] The mechanism recently postulated by Jordan[33] combines two major aspects of the older proposals (Scheme VIII). The order of binding the two substrates to the enzyme is as follows: binding of ALA to the future propionic acid side ("P" site) first, binding to the future acetic acid side ("A" site) second. For stereoelectronic reasons the sequence aldol condensation first and Schiff base formation second seems to be more attractive. The discussion of our results will be based on the scheme recently published by Jordan.[33]

Our results indicate that ALAD can be inhibited very effectively using substrate analogues. Comparing the inhibitory strength the following conclusions can be drawn. At position 1 of the substrate the enzyme accepts only planar, sp^2-hybridized groups (COO^-, NO_2). A change in the hybridization to sp^3 (SO_3^-, PO_3^{2-}) resulted in a considerably lower inhibition. A negative charge at this position is preferred since the methyl ester analogues had lower affinities. Substitutions in either the position C2 or C3 are accepted as long as the inhibitor keeps its flexibility around the C2–C3 bond. The major problem for the interpretation of the inhibition data is to know which site is blocked by the inhibitor: the "A" site or the "P" site or both. If the studied inhibitors block only the "P" site the interpretation of our studies is delicate as long as no X-ray structure of the enzyme is known. If we assume that the "A" site or both sites can be blocked, the interpretation is easier. If the product of the aldol condensation is an early intermediate in the enzyme mechanism as proposed by Shemin[14] and recently by Jordan,[33] there should be space available for additional substituents at C3 of a substrate fixed at the "A" site. The observation that substrate analogues with an additional substituent at C3 are good inhibitors is in agreement with the proposed mechanistic ideas. The methylene group at C2 is not involved in any of the proposed mechanistic schemes. Therefore a reasonable interpretation of our results will only be possible if the three-dimensional structure of the enzyme ALAD will be known. Variations of the substituent at position 5 are tolerated for polar or neutral substituents. However, switching to a negatively charged substituent (CO_2^-) or to a sterically demanding group resulted in a low affinity for the enzyme. That the amino or ammonium group at C5 can be replaced by other polar or even neutral substituents is rather surprising. It had been assumed that the ammonium group at C5 is one of the sites of substrate recognition on the enzyme surface.[1]

All of our product analogues were weak inhibitors. As we have pointed out **15** to **18** are not close analogues to the product PBG, so the weak inhibition could be due to the structural differences. In contrast to these compounds pyrazole **19** is closely related to PBG. To our surprise even compound **19** was only a very weak inhibitor. Our inhibition studies with product analogues do not hint at an essential product inhibition of the ALAD, at least not at low in vivo concentrations of PBG.

Experimental Section

General. Melting points are uncorrected. Thin-layer chromatography was conducted on aluminum sheets precoated with silica gel $60F_{254}$ (E. Merck, Darmstadt). All flash column chromatography was performed on silica gel 60 (230–400 mesh, Merck, Darmstadt) as described by Still.[34] THF was freshly distilled from potassium benzophenone ketyl. Diisopropylamine, triethylamine, benzene, and dichloromethane were distilled from CaH_2. Elemental analyses were performed in the microanalytical laboratories of Ciba-Geigy AG, plastic and additive division,

(30) Michaelis, S.; Menten, M. L. *Biochem. Z.* **1913**, *49*, 333.
(31) King, E. L.; Altmann, C. *J. Phys. Chem.* **1956**, *60*, 1375.
(32) Jordan, P. M.; Seehra, J. S. *J. Chem. Soc., Chem. Commun.* **1980**, 240–242.
(33) Jordan, P. M. In *Biosynthesis of Tetrapyrroles*; Jordan, P. M., Ed.; Elsevier: Amsterdam, 1991; pp 1–66.
(34) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

Marly/Fribourg. All chemicals or biochemicals were from either Fluka/Buchs, Merck/Darmstadt or Biorad/Munich.

Isolation and Purification of ALAD. Until otherwise stated the purification of the ALAD was performed at 4 °C. A unit of enzyme activity[28] is that amount of enzyme which produces 1 μmol of porphobilinogen in 60 min at 37 °C. Specific activity is defined as units of enzyme activity per mg of protein. **Growth of Bacteria.** Rhodopseudomonas spheroides was grown as described by Lascelles.[35] **Crude Extract.**[28] To 364 g of wet cells were added 0.1 M phosphate buffer pH 7.6 and 10 mM mercaptoethanol up to a total weight of 1456 g, and the suspension was then treated with a french pressure cell at 18000 psig to break the bacterial cells. The broken cell suspension was centrifuged at 40000*g* for 30 min. The supernatant (1180 mL) had a specific activity of 0.3 units. **Heat Treatment.** Aliquots (150 mL) of the crude extracts were stirred and heated in a period of about 5 min to 55 °C, and stirring was continued for another 5 min. The flask was then immediately cooled in an ice bath. The extracts were combined, and the precipitate was removed by centrifugation (30 min, 40000*g*), 960 mL, specific activity 0.4 units. **Polymin-P Treatment.** To the above supernatant at 4 °C was added slowly 9.6 mL of a 10% polymin-P solution (10 μL/mL extract) and gently stirred for 30 min. The precipitate was removed by centrifugation (30 min, 40000*g*), 930 mL, specific activity 0.5 units. **First Fractionation with Ammonium Sulfate.** Solid ammonium sulfate was added to the supernatant solution until its concentration reached 40% of saturation. The pH was adjusted to 6.0 with 2 N acetic acid, and the solution was centrifuged (30 min, 40000*g*) after gentle stirring for 30 min. The precipitate was dissolved in 140 mL of the above phosphate mercaptoethanol buffer and stirred for 15 min. The solution was clarified by centrifugation (10 min, 3000*g*), 140 mL, specific activity 2.0 units. **pH 5 Precipitation.** The supernatant solution was adjusted to pH 5 with 2 N acetic acid, stirred for 30 min and centrifuged for 20 min at 30000*g*. The precipitate was dissolved in 72 mL of the above buffer and stirred for 20 min. The solution was then clarified by centrifugation (15 min, 10000*g*), 72 mL, specific activity 7.5 units. **Second Fractionation with Ammonium Sulfate.** Solid ammonium sulfate was added to the gently stirred solution until the concentration of the salt reached 35% of saturation. The pH of the solution was adjusted to 6.0 with 2 N acetic acid, and the ammonium sulfate concentration was readjusted to 35% of saturation. The mixture was stirred gently for another 30 min, and the precipitate, collected by centrifugation (30 min, 30000*g*), was dissolved in 35 mL of 1 mM sodium phosphate buffer pH 6.8 and dialyzed against the same buffer overnight. The dialyzed solution was then clarified by centrifugation (15 min, 10000*g*), 33 mL, specific activity 11 units. **Hydroxylapatite Filtration.**[36] The above solution was applied to a hydroxylapatite column (1.6 × 17 cm) which had been equilibrated with 1 mM sodium phosphate buffer pH 6.8, 50 mM KCl, 5 mM mercaptoethanol. The column was washed with 30 mM sodium phosphate buffer pH 6.8 until the eluate was protein-free. The enzyme was eluted (23 mL/h) with 90 mM sodium phosphate buffer pH 6.8, 50 mM KCl, 5 mM mercaptoethanol, and the most active tubes were pooled, 19 mL, specific activity 26 units. **Protein Determination.** Protein was estimated by the method of Bradford[37] using the Bio-Rad protein assay from Bio-Rad laboratories. Bovin serum albumin was taken as a standard.

Assay of ALAD Activity and Incubation. ALAD was assayed by a modified literature procedure.[25,28] The final volume was 1.5 mL of 0.1 M Tris buffer pH 8.6 containing 0.05 M KCl, 0.005 M mercaptoethanol, and 0.005 M glutathione. All incubation flasks contained an equal amount of dehydratase (0.3 units), and the substrate concentrations varied from 0.4 to 2.8 mM in 0.4 mM steps. The inhibitor concentrations were chosen so as to achieve easily detectable decreases in enzyme activity. Values for K_m, K_i, and v_{max} were determined by using double-reciprocal analysis (Lineweaver–Burk analysis). Typically data are accurate to within 10–15% of the reported values.

The incubations were performed at 37 °C. The enzyme-containing flasks were preincubated at this temperature for 1 h followed by the addition of the inhibitor to equilibrate the system for a further 30 min. The incubation was then started by the addition of δ-aminolaevulinic acid as substrate. After 20 min the reaction was stopped by the addition of 500 μL of 20% CCl_3CO_2H, 0.1 M $HgCl_2$. The incubation flasks were then cleared by centrifugation (4000*g*). To 1 mL of the resulting supernatant was added 1 mL of modified Ehrlich's reagent, and the absorption was read after 10 min at 555 nm. For reproducibility of the experiment it was necessary to centrifuge (2000*g*) the cuvettes prior to the optical density reading. The amount of PBG was determined applying the known ε value[25] of 62000.

4-Amino-3-oxobutanesulfonic Acid (3). To NBS (70.5 g, 0.4 mol) in 220 mL of dry THF under N_2 was added slowly at 0 °C 2-[(trimethylsilyl)oxy]-1,3-butadiene[16] (**20**) (45 g, 0.32 mol). After 2 h at rt thiobenzoic acid (39.4 g, 0.29 mol) was added at 4 °C. After 2 h at rt the solution was cooled and filtered. The filtrate was diluted with 600 mL of ether and washed with 500 mL of water, and the water phase was back-extracted. The combined organic layer was washed with 200 mL of 0.1 N $NaHCO_3$ and 200 mL of water and dried over sodium sulfate. Double crystallization afforded bromide **21** (37 g, 41%): mp 67 °C (ether); R_f (toluene/ether, 9:1) 0.42; IR (KBr) 3050, 2990, 2940, 1720, 1655, 1595, 1580, 1450, 1410, 1390, 1350, 1315, 1265, 1235, 1210, 1180, 1075, 1010, 910, 770, 685, 645; ^{1}H NMR ($CDCl_3$) δ 7.94 (m, 2 H, H-C(2′), H-C(6′)); 7.58 (m, 1 H, H-C(4′)); 7.45 (m, 2 H, H-C(3′), H-C(5′)), 3.90 (s, 2 H, CH_2(4)), 3.30 (t, J = 6.6, 2 H, CH_2(2)), 3.10 (t, J = 6.6, 2 H, CH_2(1)); ^{13}C NMR ($CDCl_3$) δ 200.0 (s, C(3)), 191.5 (s, COS), 136.6 (s, C(1′)), 133.5 (d, C(4′)), 128.6 (d, C(3′), C(5′)), 127.1 (d, C(2′), C(6′)), 39.8 (t, C(4)), 33.8 (t, C(2)), 22.9 (t, C(1)); EI-MS 290 (0.3, $M^{\cdot+}$ + 2), 288, 287 (6, $M^{\cdot+}$), 207 (16), 123, 121 (15), 105 (100), 95, 93 (20), 77 (100), 51 (100), 42 (21). Anal. Calcd for $C_{11}H_{11}BrO_2S$ (287): C, 45.98; H, 3.83. Found: C, 46.02; H, 3.91.

To bromide **21** (11 g, 38 mmol) in 40 mL of dry DMF was added potassium phthalimide (7 g, 38 mmol) at rt. After 2 h the suspension was cooled and filtered. The filter cake was washed DMF and made salt free with a lot of water. The crude compound was dried azeotropically with acetonitrile on the rotary evaporator. Crystallization from chloroform afforded the pure Gabriel product **21a** (6 g, 44%): mp 172 °C (chloroform); IR (KBr) 2930, 1775, 1720, 1660 s, 1580, 1465, 1415, 1210, 1090, 920, 730, 715, 685, 645; ^{1}H NMR ($CDCl_3$) δ 7.95 (dd, J = 8.7, J = 1.4, 2 H, H-C(3′), H-C(5′)), 7.86 (m, 2 H, H-C(3″), H-C(4″)), 7.75 (m, 2 H, H-C(2″), H-C(5″), 7.58 (tt, J = 7.4, J = 1.4, 1 H, H-C(4′)), 7.44 (tt, J = 7.5, J = 1.4, 2 H, H-C(2′), H-C(6′)), 4.50 (s, 2 H, CH_2(4)), 3.31 (t, J = 6.7, 2 H, CH_2(2)), 2.98 (t, J = 6.7, 2 H, CH_2(1)); ^{13}C NMR ($CDCl_3$) δ 200.2 (s, C(3)), 191.6 (s, COS), 167.5 (s, CON), 136.7 (s, C(1′)), 134.1 (d, C(3″), C(4″)), 133.4 (d, C(4′)), 132.0 (s, C(1″), C(6″)), 128.6 (d, C(3′), C(5′)), 127.2 (d, C(2′), C(6′)), 123.5 (d, C(2″), C(5″)), 46.5 (t, C(4)), 40.0 (t, C(2)), 22.4 (t, C(1)); FAB-MS (glycerol) 354 (2, $[M + H]^+$), 216 (12), 193 (24), 160 (71), 105 (100), 77 (100), 51 (80). Anal. Calcd for $C_{19}H_{15}NO_4S$ (353): C, 64.59; H, 4.25; N, 3.97. Found: C, 64.60; H, 4.16; N, 4.09.

A mixture of compound **21a** (12 g, 34 mmol), 12 mL of H_2O_2 (30%), and 120 mL of acetic acid was gently heated to 100 °C. After 90 min a further 7 mL of H_2O_2 was added, and heating was continued for 4.5 h. After cooling the solution was brought to dryness, and to the white residue was added 120 mL of 6 N HCl. Insoluble benzoic acid was filtered, and the filtrate was refluxed for 20 h. After cooling phthalic acid was filtered off, and the filtrate was brought to dryness. Crystallization from MeOH/H_2O afforded sulfonic acid **3** (1 g, 20%): mp 232 °C dec (water/methanol); IR (KBr) 3300–2500, 1730, 1510, 1440, 1430, 1390, 1350, 1290, 1250, 1220–1120, 1095, 1080, 1040, 1000, 980, 900, 820, 795, 755, 665, 580, 540; ^{1}H NMR (D_2O) δ 4.14 (s, 2 H, H_2C(4)), 3.24 (t, J = 7.0, 2 H, H_2C(2)), 3.02 (t, J = 7.0, 2 H, H_2C(1)); ^{13}C NMR (D_2O) δ 203.4 (s, C(3)), 48.3 (t, C(4)), 45.9 (t, C(2)), 35.7 (t, C(1)); FAB-MS 168 (100, $[M + H]^+$).

3-Oxobutanephosphonic Acid (4). To a mixture of diethyl (3-oxobutyl)phosphonate[18] (**22**) (10 g, 48 mmol) and sodium iodide (15.1 g, 0.1 mol) in 60 mL of dry acetonitrile was added TMS chloride (12.75 ml, 0.1 mol) at rt. The reaction mixture was heated at 60 °C for 30 min. After filtration and evaporation the resulting TMS phosphonate was distilled at 90 °C (0.02 Torr), affording 8 g which was immediately hydrolyzed at rt in 60 mL of water. Lyophilization and crystallization yielded pure phosphonic acid

(35) Lascelles, J. *Biochem. J.* **1956**, *62*, 78.
(36) Jordan, P. M.; Shemin, D. *J. Biol. Chem.* **1973**, *248* (3), 1019–1024.
(37) Bradford, M. *Anal. Biochem.* **1976**, *72*, 248.

4 (3.6 g, 50%): mp 81–82 °C; IR (KBr) 3700–2000, 2240, 1710, 1430, 1410, 1350, 1280, 1240, 1200, 1170, 1150, 1065, 1020, 985, 950, 835, 780, 740, 715; ^{1}H NMR (D_2O) δ 2.68–2.60 (m, J(2,P) = 11.9, J = 7.8, 2 H, CH_2(2)), 2.05 (s, 3 H, CH_3(4)), 1.82–1.73 (m, J(1,P) = 18.0, J = 7.8, 2 H, CH_2(1)); ^{13}C NMR (D_2O, dioxane) 213.7 (d, J(3,P) = 15, C(3)), 37.2 (t, C(2)), 30.0 (q, C(4)), 21.6 (dt, J(1,P) = 38, C(1)); FAB-MS (glycerol) 153 (100, $[M + H]^+$), 135 (38), 71 (26), 43 (15). Anal. Calcd for $C_4H_9O_4P$ (152): C, 31.58; H, 5.92. Found: C, 31.70; H, 5.93.

4-Nitro-2-butanone (5). 4-Chloro-2-butanone[20] (**23**) (3.2 g, 30 mmol) and sodium nitrite (4.2 g, 60 mmol) were mixed in 40 mL of DMF at rt. After 17 h 100 mL of ether was added, and the etheral solution was washed 6× with 100 mL of water, dried ($MgSO_4$), and concentrated in vacuo. Distillation at 150 °C (30 Torr) afforded pure nitro ketone **5** (710 mg, 20%): R_f (hexane-/EtOAc, 3:2) 0.20; IR (CCl_4) 2970, 2930, 1735, 1565, 1425, 1410, 1380, 1295, 1250, 1210, 1175, 1125, 1090, 1025, 905; ^{1}H NMR ($CDCl_3$) δ 4.63 (t, J = 6.0, 2 H, CH_2(4)), 3.09 (t, J = 6.0, 2 H, CH_2(3)), 2.27 (s, 3 H, CH_3(1)); ^{13}C NMR ($CDCl_3$) δ 203.2 (s, C(2)), 68.9 (t, C(4)), 39.1 (t, C(3)), 29.8 (q, C(1)); GC-MS t_R 2.17 min; 118 (0.5), 117 (2, $M^{\bullet+}$), 102 (1), 89 (10), 71 (1), 55 (15), 43 (100).

4-Oxo-2-pentenoic Acid (6) and *rac*-2-Hydroxy-4-oxopentanoic Acid (7). Methyl 4-oxopentenoate (3 g, 29 mmol) was refluxed fort 4 h in 30 mL of 2 N sulfuric acid. The NaCl saturated solution was then extracted continuously with diethyl ether for 5 h. The organic layer was dried over sodium sulfate and concentrated in vacuo. Flash chromatography on silica (2.3 EtOAc/hexane) yielded **6** (440 mg, 10%) and **7** (325 mg, 11%). **6**: mp 121 °C (EtOAc/hexane); R_f (EtOAc/hexane, 1:1) 0.39; IR (KBr) 3650–2300, 1800–1550, 1620, 1440, 1410, 1360, 1305, 1295, 1275, 1260, 1240, 1225, 1170, 1110, 1030, 1005, 980, 930, 895, 680; ^{1}H NMR (CD_3OD, TMS) δ 6.93, 6.69 (2 d, J = 16.1, 2 H, H-C(2), H-C(3)), 2.35 (s, 3 H, CH_3(5)); ^{13}C NMR (CD_3OD, TMS) δ 200.4 (s, C(4)), 168.7 (s, COO), 141.3, 133.5 (d, 2 C, C(2), C(3)), 27.9 (q, C(5)); EI-MS 115 (3), 114 (23, $M^{\bullet+}$), 100 (4), 99 (94), 97 (6), 87 (8), 81 (15), 69 (8), 43 (100). Anal. Calcd for $C_5H_6O_3$ (114): C, 52.63; H, 5.26. Found: C, 52.74; H, 5.42. **7**: mp 25 °C (THF/hexane); R_f (methanol) 0.61; IR (KBr) 3700–2300, 1700, 1630, 1415, 1360, 1255, 1205, 1165, 1100; ^{1}H NMR (CD_3OD, TMS) δ 4.50 (X, J(2,3) = 7.6, J(2,3) = 4.3, 1 H, H-C(2)), 2.92 (AB, J = 16.7, J(2,3) = 4.3, 1 H, H-C(3)), 2.83 (AB, J = 16.7, J(2,3) = 7.6, 1 H, H-C(3)), 2.18 (s, 3 H, CH_3(5)); ^{13}C NMR (CD_3OD, TMS) δ 208.5 (s, C(4)), 177.0 (s, COO), 68.0 (d, C(2)), 48.4 (t, C(3)), 30.5 (q, C(5)); EI-MS 133 (0.5), 132 (0.3, $M^{\bullet+}$), 115 (1.3), 114 (12), 99 (53), 87 (54), 71 (19), 61 (10), 58 (19), 55 (15), 45 (22), 43 (100), 42 (21). Anal. Calcd for $C_5H_8O_4 \cdot 0.124H_2O$ (134.23): C, 44.70; H, 6.15. Found: C, 45.10; H, 6.68.

***rac*-3-Hydroxy-4-oxopentanoic Acid (8).** To *m*-CPBA (3.75 g, 22 mmol) under N_2 in 60 mL of hexane was added at 4 °C silyl enol ether[22] **25** (4 g, 20 mmol) in 10 mL of hexane. After 3 h at rt the reaction mixture was filtered and concentrated in vacuo. Excess *m*-CPBA was precipitated by the addition of pentane and filtered off. The resulting protected alcohol was isolated by bulb to bulb distillation (95 °C/0.04 Torr) and then deprotected at rt in 20 mL of ether/30 mL of 1 N HCl. After 4 h the reaction mixture was extracted 3× with 50 mL of ether, dried over sodium sulfate, and concentrated in vacuo. Flash chromatography on silica (EtOAc/hexane, 2:3) and distillation (70 °C/0.01 Torr) afforded pure racemic hydroxy ester **26** (1.43 g, 55%): R_f (Hexane/EtOAc, 2:3) 0.35; IR (CCl_4) 3480, 3000, 2960 m, 2900, 2850, 1745, 1720, 1440, 1410, 1360, 1300, 1270, 1250, 1200, 1170, 1105, 1055, 1040, 1010, 975, 900, 845, 625; ^{1}H NMR ($CDCl_3$) δ 4.36 (m, J = 10.8, J, = 4.7, 1 H, H-C(3)), 3.75 (d, J = 5.0, 1 H, OH), 3.70 (s, 3 H, CH_3O), 2.86 (dd, J = 16.4, J(2,3) = 4.2, 1 H, H-C(2)), 2.74 (dd, J = 16.4, J(2,3) = 6.3, 1 H, H-C(2)), 2.27 (s, 3 H, CH_3(5)); ^{13}C NMR ($CDCl_3$) δ 208.3 (s, C(4)), 171.1 (s, COO), 76.6 (d, C(3)), 51.9 (q, CH_3O), 37.8 (t, C(2)), 25.2 (q, C(5)), EI-MS 147 (7, $[M^{\bullet+}]$ + 1), 115 (18), 103 (66), 97 (28), 71 (52), 61 (23), 59 (10), 43 (100). Anal. Calcd for $C_6H_{10}O_4$ (146): C, 49.32; H, 6.85. Found: C, 49.20; H, 7.03.

Hydroxy ester **26** (2 g, 13.7 mmol) was hydrolyzed in 25 mL of 0.05 M phosphate buffer pH 7.5 at 30 °C using commercially available pig liver esterase (PLE, 300 units). The pH was kept constant by the addition of 0.5 N NaOH with a pH-stat apparatus. After the addition of 1 equiv of base the solution was extracted twice with 60 mL of chloroform. The aqueous phase was saturated with sodium chloride, acidified to pH 1 with 4 N HCl, and extracted 3× with 80 mL of THF. Drying (Na_2SO_4), filtration, and evaporation afforded 2.5 g of crude product. Flash chromatography on silica (EtOAc/hexane, 1:1) and distillation (170 °C/0.02 Torr) afforded 830 mg of product **8** which was crystallized from EtOAc/hexane (525 mg, 29%): mp 66–67 °C; R_f (EtOAc/MeOH, 10:1) 0.26; IR (KBr) 3700–2600, 1715, 1400, 1360, 1260, 1195, 1170, 1100, 970, 915; ^{1}H NMR ($CDCl_3$) δ 5.0–4.4 (br s, 2 H, COOH, OH), 4.40 (X, J(2,3) = 6.4, J(2,3) = 4.2, 1 H, H-C(3)), 2.93 (AB, J = 16.6, J(2,3) = 4.2, 1 H, H-C(2)), 2.79 (AB, J = 16.6, J(2,3) = 6.4, 1 H, H-C(2)), 2.28 (s, 3 H, CH_3(5)); ^{13}C NMR (CD_3OD, TMS) δ 211.9 (s, C(4)), 174.3 (s, COO), 74.8 (d, C(3)), 39.1 (t, C(2)), 25.9 (q, C(5)); FAB-MS (glycerol) 134 (6), 133 (100, $[M + H]^+$), 116 (15), 115 (90), 98 (10), 97 (60). Anal. Calcd for $C_5H_8O_4$ (132): C, 45.45; H, 6.10. Found: C, 45.54; H, 6.08.

5-Hydroxy-4-oxopentanoic Acid (11). DBU (6.85 g, 45 mmol) was added slowly at 6 °C to a mixture of methyl 5-bromolaevulinate[23] (**27**) (7.5 g, 35.8 mmol) and formic acid (2 g, 43.4 mmol) in 50 mL of benzene. After 2 h at rt water (100 mL) was added, and the mixture was extracted 3× with dichloromethane (70 mL). The organic layer was washed with 0.1 N HCl and the neutralized with water. Drying (Na_2SO_4), filtration, and evaporation of the solvent afforded crude formyloxy compound (5.35 g). Distillation (115 °C/0.03 Torr) yielded pure compound (4.3 g, 70%): R_f (EtOAc/hexane, 1:1) 0.28; IR (KBr, film) 3640, 3545, 3460, 3000, 2960, 2860, 1725, 1440, 1425, 1370, 1320, 1275, 1175, 1105, 1060, 1035, 1025, 990, 975, 945, 900, 850, 825, 790, 760; ^{1}H NMR ($CDCl_3$, 90 MHz) δ 8.1 (s, 1 H, HCO), 4.75 (s, 2 H, CH_2(5)), 3.65 (s, 3 H, CH_3O), 2.75–2.55 (m, 4 H, CH_2(2), CH_2(3)); ^{13}C NMR ($CDCl_3$) δ 201.3 (s, C(4)), 172.6 (s, COOMe), 159.7 (d, CHO), 67.1 (t, C(5)), 51.8 (q, CH_3O), 33.3 (t, C(3)), 27.3 (t, C(2)); EI-MS 143 (3), 129 (10), 128 (40), 114 (12), 113 (100), 97 (72), 85 (27), 82 (12), 69 (28), 59 (40), 54 (15), 53 (23), 43 (100), 42 (11), 41 (11), 39 (11). Anal. Calcd for $C_7H_{10}O_5$ (174): C, 48.27; H, 5.74. Found: C, 48.38; H, 5.98.

The formyloxy compound (8.84 g, 50 mmol) was dissolved in little dichloromethane and applied to a column of neutral Alox (400 g, activity I). 5-Hydroxy ester **28** was eluted with CH_2Cl_2/methanol, 1:1, evaporated, and distiled (130 °C/0.07 Torr) (4.3 g, 60%): R_f (Et_2O) 0.25; IR (KBr, film) 3510, 2960, 2910, 1745, 1730, 1440, 1415, 1355, 1320, 1280, 1250, 1210, 1175, 1125, 1080, 1020, 960; ^{1}H NMR ($CDCl_3$) δ 4.32 (s, 2 H, CH_2(5)), 3.69 (s, 3 H, CH_3O); 2.71 (s, 4 H, CH_2(2), CH_2(3)); ^{13}C NMR ($CDCl_3$) δ 208.3 (s, C(4)), 172.9 (s, COOMe), 68.2 (t, C(5)), 51.9 (q, CH_3O), 32.9 (t, C(3)), 27.5 (t, C(2)); EI-MS 116 (8), 115 (100), 88 (10), 87 (15), 59 (48), 56 (9), 55 (72), 31 (46). Anal. Calcd for $C_6H_{10}O_4$ (146): C, 49.31; H, 6.85. Found: C, 49.18; H, 7.00.

Compound **28** (1.5 g, 10.3 mmol) was hydrolyzed at 90 °C for 6 h in 15 mL of THF/40 mL of 4 N HCl. Then the solution was continuously extracted with diethyl ether for 6 h after saturation with sodium chloride. The organic layer was dried (Na_2SO_4) filtered, concentrated in vacuo, and crystallized from THF/hexane, yielding pure **11** (440 mg, 33%). Better yields were obtained with an enzymic hydrolysis using PLE (60–70%, see compound **8**): mp 96–97 °C (THF/hexane); R_f (methanol/EtOAc, 5:1) 0.47; IR (KBr) 3420, 3370, 2920, 1715, 1690, 1445 m, 1430, 1405, 1310, 1225, 1205, 1085, 1005, 930, 735; ^{1}H NMR (CD_3OD, TMS) δ 4.24 (s, 2 H, CH_2(5)), 2.70 (t, J = 6.6, 2 H, CH_2(3)), 2.59 (t, J = 6.6, 2 H, CH_2(2)); ^{13}C NMR (CD_3OD, TMS) δ 211.2 (s, C(4)), 176.5 (s, COO), 68.8 (t, C(5)), 33.9 (t, C(3)), 28.5 (t, C(2)); EI-MS 133 (3.1), 132 (0.3 $M^{\bullet+}$), 115 (49), 114 (9), 102 (28), 101 (100), 85 (20), 74 (48), 73 (79), 57 (11), 56 (27), 55 (82), 45 (75), 43 (58), 42 (45), 41 (14), 39 (14), 31 (81). Anal. Calcd for $C_5H_8O_4$ (132): C, 45.45; H, 6.06. Found: C, 45.52; H, 6.07.

5-Nitrilo-4-oxopentanoic Acid (12). Methyl 5-bromolevulinate[23] (**27**) (5 g, 24 mmol) in 5 mL of THF was added slowly at rt to potassium cyanide (3.1 g, 48 mmol) dissolved in 10 mL of water and 20 mL of THF. Normal workup afforded crude compounds **29** and **30** which were destiled (155 °C/0.04 Torr) and flash chromatographed on silica (EtOAc/hexane, 1:2). **29** (920 mg, 25%): R_f (hexane/EtOAc, 1:1) 0.25; bp 130 °C (0.02 Torr); IR (CCl_4, NaCl) 2960, 2920, 2260, 1735, 1440, 1410, 1400, 1355, 1330, 1300, 1260, 1240, 1200, 1180, 1120, 1090, 1000, 965, 925, 875; ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.7 (s, 3 H, CH_3O), 3.6 (s, 2 H, CH_2(5)), 2.9–2.7, 2.7–2.5 (m, 4 H, CH_2CH_2COOMe); ^{13}C NMR ($CDCl_3$) δ 196.9 (s, C(4)), 172.1 (s, COOMe), 113.8 (s, CN), 51.4

(q, CH_3O), 36.0 (t, C(5)), 31.6 (t, CH_2CH_2COOMe), 27.2 (t, CH_2CH_2COOMe); FAB-MS (ONPOE) 156 ($[M + H]^+$); GC–MS (T-program, HP-1, EI, 15 eV) t_R 4.5 min; 125 (2), 124 (28), 116 (5), 115 (100), 96 (3), 87 (13), 68 (4), 59 (10), 55 (24). Anal. Calcd for $C_7H_9NO_3$ (155): C, 54.19; H, 5.81; N, 9.03. Found: C, 54.03; H, 5.84; N, 8.85.

30 (272 mg, 8%): R_f (EtOAc/hexane, 1:1) 0.46; IR (CCl_4, NaCl) δ 3000, 2950, 1740, 1440, 1420, 1350, 1265, 1200, 1180, 1155, 1125, 995, 930, 920, 885, 835; ^1H NMR ($CDCl_3$, 90 MHz) δ 3.7 (s, 3 H, CH_3O), 3.2 (d, J = 5.4, 1 H, H-C(3)), 2.9 (d, J = 5.4, 1 H, H-C(3)), 2.7–2.4, 2.2–2.0 (m, 4 H, CH_2CH_2COOMe); ^{13}C NMR ($CDCl_3$) δ 171.3 (s, COOMe), 117.2 (s, CN), 52.1 (t, C(3)), 51.4 (q, CH_3O), 47.1 (s, C(2)), 28.6, 28.3 (2 t, CH_2CH_2COOMe); FAB-MS (ONPOE) 157, 156 ($[M + H]^+$); GC–MS (T-program, HP-1, EI, 15 eV) t_R 3.83 min; 140 (10), 125 (63), 124 (21), 115 (78), 113 (14), 110 (47), 97 (76), 96 (61), 95 (35), 94 (27), 87 (19), 83 (27), 82 (33), 79 (19), 74 (31), 68 (39), 59 (47), 55 (100), 45 (96), 42 (11).

Nitrile **29** (1.2 g, 7.74 mmol) was emzymatically hydrolyzed with PLE (see compound **8**) and extracted continously for 24 h with diethyl ether. Crystallization (THF/hexane, 4:1) yielded pure nitrilo acid **12** (552 mg, 51%): mp 85 °C (THF/hexane, 4:1); R_f (MeOH/EtOAc, 1:1) 0.32. IR (KBr) δ 3600–2300, 3450, 2950, 2920, 2260, 1725, 1700, 1435, 1405, 1390, 1375, 1325, 1300, 1220, 1155, 1100, 1050, 940, 915, 875, 745, 720, 640; ^1H NMR ($CDCl_3$, 3 Tr.CD_3CN)) δ 3.49 (s, 2 H, CH_2(5)), 2.70–2.55 (m, 2 H, CH_2(3)), 2.46–2.42 (m, 2 H, CH_2(2)), [in CD_3OD or deuterium oxide a fast deuterium exchange takes place in position 5!]; ^{13}C NMR (CD_3OD) δ 199.4 (s, C(4)), 175.8 (s, COO), 115.6 (s, CN), 37.4 (t, C(3)), 32.4 (t, C(5), plus signal of the monodeuterated species; 28.5 (t, C(2)); EI-MS 124 (10), 102 (4), 101 (100), 73 (21), 68 (11), 55 (12), 45 (17), 41 (4), 40 (9). Anal. Calcd for $C_6H_7NO_3$ (141): C, 51.06; H, 4.96; N, 9.93. Found: C, 51.18; H, 4.80; N, 9.77.

***N*-(2-Carboxybenzoyl)-5-amino-4-oxopentanoic Acid (13).** Imide[24] **31** (1 g, 3.6 mmol) was hydrolyzed at rt for 4 h in 20 mL of 0.5 N NaOH. The solution was once extracted with 50 mL of chloroform, acidified to pH 2 with 4 N HCl, saturated with sodium chloride, and continuously extracted for 2 d with diethyl ether. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Crystallization afforded pure **13** (466 mg, 46%): mp 141–142 °C (THF/hexane); IR (KBr) 3360, 3700–200, 1730, 1720, 1705, 1625, 1600, 1580, 1550, 1505, 1465 m, 1455, 1430, 1410, 1385, 1365, 1325, 1280, 1250, 1235, 1225, 1170, 1135, 1125, 1100, 1080, 1050, 1040, 1030, 1010, 925, 895, 860, 835, 805, 800, 785, 750, 715, 670, 640; ^1H NMR (CD_3OD) δ 7.98–7.93 (m, 1 H, Ar), 7.68–7.50 (m, 3 H, Ar), 4.22 (s, 2 H, CH_2(5)), 2.86 (t, J = 6.3, 2 H, CH_2(3)), 2.61 (t, J = 6.3, CH_2(2)); ^{13}C NMR (CD_3OD) δ 206.7 (s, C(4)), 176.3 (s, C(1)), 172.9, 169.4 (2 s, COO-C(2'), CO-N), 139.2 (s C(1')), 133.0 (d, C(5')), 131.2 (d, C(3')), 131.0 (s, C(2')), 130.8 (d, C(4')), 129.0 (d, C(6')), 50.3 (t, C(5)), 35.4 (t, C(3)), 28.6 (t, C(2)); FAB-MS (NBA) 303 (10), 302 (40, $[M + Na]^+$), 282 (5), 281 (20), 280 (100, $[M + H]^+$), 262 (10), 216 (10), 149 (12), 132 (43), 114 (10), 90 (20), 88 (15), 77 (8), 63 (7). Anal. Calcd for $C_{13}H_{13}N_6 \cdot 0.24H_2O$ (283.4): C, 55.05; H, 5.34; N, 4.94. Found: C, 55.25; H, 5.29; N, 4.75.

α-Chlorination of Pyrroles 32 and 33. General procedure: To pyrrole[25] **32** (1.5 g, 7.7 mmol) in 30 mL of THF was added NCS (1.03 g, 7.7 mmol) at rt. After 1 h the reaction mixture was diluted with 150 mL of EtOAc and washed 3× with 50 mL of 0.5 N HCl. The organic layer was washed with brine, dried over cotton wool, and concentrated in vacuo. Filtration through a short column charged with silica afforded the crude product. Crystallization yielded pure pyrrole **15** (520 mg, 30%): mp 181 °C (EtOAc/hexane) dec; R_f (EtOAc) 0.31; IR (KBr) 3190, 3170, 3100–2400, 1705, 1620, 1585, 1525, 1480, 1455, 1440, 1410, 1390, 1365, 1340, 1310, 1255, 1245, 1220, 1175, 1095, 1075, 1050, 1000, 960, 910, 820, 795; ^1H NMR (CD_3OD, TMS) δ 2.93–2.89 (m, 2 H, CH_2CH_2COO), 2.47, 2.41 (2 s, 6 H, CH_3CO, CH_3-C(5)), 2.46–2.42 (m, 2 H, CH_2CH_2COO); ^{13}C NMR (CD_3OD, TMS) δ 196.9 (s, CH_3CO), 177.4 (s, COO), 136.5 (s, C(5)), 121.6 (s, C(4)), 120.3 (s, C(2)), 113.6 (s, C(3)), 35.4 (t, CH_2CH_2COO), 30.7 (q, CH_3CO), 22.1 (t, CH_2CH_2COO), 15.1 (q, CH_3-C(5)); EI-MS 231 (19), 230 (6), 229 (57, $M^{\bullet+}$), 194 (8), 188 (10), 186 (37), 185 (12), 184 (10), 183 (35), 182 (10), 174 (23), 172 (84), 171 (16), 170 (69), 169 (40), 168 (59), 156 (14), 154 (11), 142 (13), 141 (11), 134 (22), 128 (11), 122 (12), 106 (10), 92 (15), 77 (27), 65 (28), 51 (22), 43 (100), 28 (42). Anal. Calcd for $C_{10}H_{12}ClNO_3$ (229.45): C, 52.30; H, 5.23; N, 6.10. Found: C, 52.34; H, 5.17; N, 5.84.

16: (1.2 g, 70%): mp 170 °C (EtOAc/hexane) dec; R_f (EtOAc) 0.43; IR (KBr) 3260, 3180, 3100, 3000, 2960, 2940, 2860, 2760, 2700, 2660, 1715, 1700, 1660, 1585, 1525, 1470, 1455, 1445, 1430, 1415, 1370, 1350, 1320, 1305, 1290, 1265, 1230, 1195, 1160, 1125, 1080, 1040, 995, 970, 940, 830, 795, 735; ^1H NMR (CD_3OD, TMS) δ 3.77 (s, 3 H, CH_3O), 2.92–2.87 (m, 2 H, CH_2CH_2COO), 2.45–2.43 (m, 2 H, CH_2CH_2COO), 2.40 (s, 3 H, CH_3-C(5)); ^{13}C NMR (CD_3OD, TMS) δ 177.4 (s, COO), 167.6 (s, COOMe), 136.8 (s, C(5)), 120.0 (s, C(2)), 112.5 (s, C(3)), 110.9 (s, C(4)), 51.1 (q, CH_3O), 35.7 (t, CH_2CH_2COO), 21.9 (t, CH_2CH_2COO), 13.6 (q, CH_3-C(5)); EI-MS 247 (26), 245 (90, $M^{\bullet+}$), 215 (5), 213 (16), 210 (7), 201 (13), 200 (6), 199 (71), 188 (23), 187 (28), 186 (87), 185 (100), 174 (15), 172 (48), 171 (16), 170 (10), 169 (10), 168 (34), 167 (25), 158 (18), 156 (58), 77 (17), 65 (16), 59 (6), 51 (14), 43 (17), 42 (9). Anal. Calcd for $C_{10}H_{12}ClNO_4$ (245.45): C, 48.89; H, 4.89; N, 5.70. Found: C, 48.96; H, 4.84; N, 5.54.

3-(2-Carboxyethyl)-4-methylmaleimide (17) was prepared according to Wittenberg and Shemin:[26] mp 114 °C; R_f ($CHCl_3$/EtOAc/cyclohexane, 6:3:1) 0.12; ^1H NMR (D_2O) δ 2.51–2.45 (m, 4 H, CH_2CH_2COO), 1.78 (s, 3 H, CH_3); ^{13}C NMR (D_2O) δ 176.8 (CH_2COO), 174.8, 174.3 (C(2), C(5)) 140.0, 139.5 (C(3), C(4)), 31.7 (CH_2COO), 18.5 (CH_2CH_2COO), 7.8 (CH_3); EI-MS 184 (5), 183 (1, $M^{\bullet+}$), 165 (95), 137 (100), 124 (20), 119 (20), 109 (55), 94 (50), 83 (20), 66 (90), 53 (60), 45 (35), 41 (75), 39 (80).

3-(2-Mercaptoimidazol-4-yl)propanoic acid (18) was prepared according Wynn and Corwin:[27] mp 201–203 °C (H_2O); R_f (EtOAc/hexane, 1:1) 0.35; IR (KBr) 3400, 3300–2000, 1715, 1680, 1635, 1580, 1510, 1445, 1430, 1400, 1365, 1320, 1280, 1235, 1210, 1185, 1150, 1110, 1060, 1030, 1010, 975, 895, 795, 770s, 740, 680, 650; ^1H NMR (DMSO-d_6) δ 11.84 (s, 1 H, SH or NH), 11.65 (s, 1 H, NH or SH), 6.49 (s, 1 H, CH(5')), 2.57–2.45 (m, 4 H, CH_2CH_2COO); ^{13}C NMR (DMSO-d_6) δ 173.5 (s, COO), 160.3 (s, C(2')), 128.5 (s, C(4')), 111.4 (d, C(5')), 32.3 (t, CH_2CH_2COO), 20.0 (t, CH_2CH_2COO); EI-MS 174 (5), 173 (10), 172 (100, $M^{\bullet+}$), 156 (2), 155 (5), 154 (57), 127 (7), 126 (9), 113 (23), 112 (26), 99 (6), 86 (6). Anal. Calcd for $C_6H_8N_2O_2S$ (172): C, 41.86; H, 4.65; N, 16.27. Found: C, 41.84; H, 4.79; N, 16.12.

3-(2-Carboxyethyl)-4-(carboxymethyl)-5-methylpyrazole (19). In a dry, three-necked round-bottomed flask under inert atmosphere was placed 50 mL of absolute diethyl ether and diethyl 4-oxopimelate (10.7 mL, 0.05 mol). Below 3 °C triethylamine (7.7 mL, 0.055 mol) was added followed by TMS triflate (10 ml, 0.055 mol). Then the mixture was kept at rt for 28 h and a further 0.8 mL of triethylamine and 1 mL of TMS triflate were added for completion. After 12 h at rt the ethereal solution was separated and distilled (105 °C/0.1 Torr). **35** (13.4 g, 89%) E/Z 3:7: bp 105 °C (0.1 Torr). E isomer: ^1H NMR ($CDCl_3$) δ 4.70 (t, J = 7.4 1 H, HC(3)), 4.07 (q, J = 7.1, 2 H, CH_3CH_2O), 2.97–2.94 (m, 2 H, $H_2C(2)$); 2.44–2.29 (m, 4 H, CH_2CH_2COO), 1.19 (t, J = 7.1, 3 H, CH_3CH_2O), 0.14 (s, 9 H, $(CH_3)_3Si$); ^{13}C NMR ($CDCl_3$) δ 172.9, 172.5 (C(1), C(7)), 152.4 (C(4)), 99.5 (C(3)), 60.5 (CH_3CH_2O), 32.4, 31.4, 26.6 (C(2), C(5), C(6)), 14.3 (CH_3CH_2O), 0.1 $(CH_3)_3Si$); EI-MS 302 (2, $M^{\bullet+}$), 257 (11), 256 (27), 155 (18), 111 (100). Z isomer: ^1H NMR ($CDCl_3$) δ 4.66 (t, J = 7.4, 1 H, HC(3)), 4.06 (q, J = 7.1, 2 H, CH_3CH_2O), 2.97–2.94 (m, 2 H, $H_2C(2)$), 2.44–2.29 (m, 4 H, CH_2CH_2COO), 1.19 (t, J = 7.1, 3 H, CH_3CH_2O), 0.14 (s, 9 H, $(CH_3)_3Si$); ^{13}C NMR ($CDCl_3$) δ 172.7, 172.2 (C(1), C(7)), 152.4, (C(4)), 100.4 (C(3)), 60.3 (CH_3CH_2O), 31.8, 31.6, 31.2 (C(2), C(5), C(6)), 14.2 (CH_3CH_2O), 0.5 $(CH_3)_3Si$); EI-MS 302 (2, $M^{\bullet+}$), 257 (11), 256 (27, 183 (11), 169 (14), 155 (18), 111 (100).

To a mixture of dry $ZnCl_2$ (0.67 g, 4.9 mmol) and acetyl chloride (0.35 mL, 4.9 mmol) in 5 mL of dichloromethane and 1 mL of diethyl ether under N_2 was added silyl enol ether **35** (1.48 g, 4.9 mmol) at 0 °C. After 2 h at this temperature the reaction was quenched with 9 mL of water. The solution was extracted with dichloromethane, washed with saturated $NaHCO_3$, dried (Na_2SO_4), and concentrated in vacuo. Distillation yielded *rac*-**36** (0.83 g, 62%): bp 148 °C (0.01 Torr); R_f (ether/hexane, 2:1) 0.27; IR (film) 3435, 2980, 2900, 2880, 1730, 1600; EI-MS 272 (1, $M^{\bullet+}$), 185 (18), 184 (16), 180 (18), 156 (17). 1,3-Diketone: ^1H NMR ($CDCl_3$) δ 4.13 (t, J = 7.3, 1 H, HC(3)); 4.06 (q, J = 7.1, 4 H, CH_3CH_2O), 2.84 (d, J = 7.3, 2 H, $H_2C(2)$), 2.91–2.47 (m, 4 H, CH_2CH_2COO), 2.22 (s, 3 H, CH_3CO), 1.19, 1.18 (t, J = 7.1, 6 H, CH_3CH_2O); ^{13}C NMR ($CDCl_3$) δ 203.1, 201.9 (C(4), CH_3CO), 172.1, 171.1 (C(1), C(7)), 62.6 (C(3)), 61.0, 60.7 (CH_3CH_2O), 37.1, 32.6, 27.8 (C(2), C(5), C(6)), 29.4 (CH_3CO), 14.1 (CH_3CH_2O). Enol: ^1H NMR

($CDCl_3$) δ 8.68 (s, OH), 4.11 (q, J = 7.1, 4 H, CH_3CH_2O), 3.25 (s, 2 H, $H_2C(2)$), 2.91–2.47 (m, 4 H, CH_2CH_2COO), 2.07 (s, 3 H, CH_3CO), 1.22 (t, J = 7.1, 6 H, CH_3CH_2O). Anal. Calcd for $C_{13}H_{20}O_6$ (272): C, 57.34; H, 7.40. Found: C, 57.38; H, 7.57.

To diketone **36** (1.52 g, 5.5 mmol) in 0.6 mL of ethanol was added carefully hydrazine hydrate (0.33 g, 6.6 mmol) at 5 °C. A further 0.4 mL of ethanol was added, and the reaction mixture was kept at rt for 4.5 h. Evaporation of the solvents yielded pure pyrazole diester (1.49 g, 100%): R_f (ether) 0.2, n 1.4837; UV 205 (4.2); IR (film) 3580, 3340, 3200, 3140, 3080, 2980, 2940, 1730, 1590, 1260, 1170; ^1H NMR ($CDCl_3$) δ 10.20 (s, NH), 4.02 (q J = 7.1, 4 H, CH_3CH_2O), 3.29 (s, 2 H, CH_2-C(4)), 2.82, 2.57 (t, 4 H, CH_2CH_2COO), 2.12 (s, 3 H, CH_3-C(5)), 1.15, 1.13 (t, J = 7.1, 6 H, CH_3CH_2O); ^{13}C NMR ($CDCl_3$) δ 172.7, 171.1 (COO), 145.5 (C(5)), 141.9 (C(3)), 107.8 (C(4)), 60.3, 60.1 (CH_3CH_2O), 33.1 (CH_2CH_2COO), 28.9 (CH_2-C(4)), 20.5 (CH_2CH_2COO); 13.7, 10.2 (CH_3CH_2O); EI-MS 269 (4), 268 (9, $M^{\bullet+}$), 223 (14), 222 (36), 195 (64), 194 (11), 151 (17), 150 (12), 149 (27), 123 (11), 122 (15), 121 (100). Anal. Calcd for $C_{13}H_{20}N_2O_4$ (268): C, 58.19; H, 7.52; N, 10.44. Found: C, 57.45; H, 7.89; N, 10.56.

The pyrazole diester (0.5 g, 1.9 mmol) was refluxed for 10 h in 1 mL of ethanol containing 1.84 g of a 15% potassium hydroxide solution. The cold solution was diluted with water (2 mL) and acidified to pH 3.5 with 2 M HCl. The precipitate was filtered and washed with cold water and diethyl ether. Crystallization from water/ethanol yielded pure **19** (0.32 g, 81%): mp 225 °C; UV 222 (4.0); IR (KBr) 3260, 3100, 2980, 2940, 1710, 1580, 1520, 1220; ^1H NMR (DMSO-d_6) δ 3.43 (s, 2 H, CH_2-C(4)), 2.92–2.88, 2.66–2.62 (m, 4 H, CH_2CH_2COO), 2.28 (s, 3 H, CH_3-C(5)); ^{13}C NMR (DMSO-d_6) δ 174.2, 173.2 (COO), 145.4 (C(5)), 140.8 (C(3)), 108.5 (C(4)), 33.6 (CH_2CH_2COO), 29.2 (CH_2-C(4)), 21.1 (CH_2CH_2COO), 10.4 (CH_3-C(5)); EI-MS 213 (3), 212 (23, $M^{\bullet+}$), 168 (14), 167 (63), 166 (15), 150 (20), 149 (32), 125 (20), 123 (19), 122 (16), 121 (100). Anal. Calcd for $C_9H_{12}N_2O_4$ (212): C, 50.94; H, 5.70; N, 13.20. Found: C, 50.70; H, 5.69; N, 13.15.

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Notes

A Highly Selective Methodology for the Direct Conversion of Acetals to Esters

N. Chidambaram, Shridhar Bhat, and S. Chandrasekaran*

Department of Organic Chemistry, Indian Institute of Science, Bangalore 560 012, India

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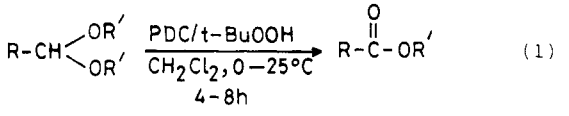
The acetal funtionality is the most commonly used protecting group for aldehydes,[1] and the direct conversion of cyclic and acyclic acetals to the corresponding esters is a useful synthetic methodology in organic synthesis. Systems utilizing molecular oxygen,[2] ozone,[2,3] dinitrogen tetroxide,[2] alkylhydroperoxides[2,4,5] halogen-based reagents,[6] *tert*-butyl hydroperoxide in the presence of palladium(II) catalyst,[7] potassium permanganate under phase-transfer conditions,[8] and electrochemical oxidation[9] have all been shown to bring about this transformation with varying degrees of flexibility.

Ozonolysis appears to be superior to all other methods in this category if yield and generality of the reaction are singled out as the important criteria. However, even this methodology is not effective in cases where the acetals contain carbon–carbon multiple bonds, and none of the above-mentioned procedures has been successfully used for acetals derived from α,β-unsaturated carbonyl compounds.

We now report that the 1:1.5 molar mixture of PDC and *tert*-butyl hydroperoxide that we had shown earlier to be an effective reagent for allylic and benzylic oxidations[10] as well as oxidative rearrangement of enol ethers[11] is also a superior reagent for the direct conversion of acetals to the corresponding esters.

Treatment of a wide variety of acetals with PDC/*t*-BuOOH (1:1.5) in dichloromethane led to the formation of the corresponding esters in high yield under mild reaction conditions (0–25 °C; 4–8 h) (eq 1). The results of this oxidation are summarized in Table I.



(1)

Unlike other reported procedures[2,9] acyclic acetals reacted with equal facility as the cyclic acetals. Five-membered ring acetals **1**, **3**, **5**, and **7** bearing alkyl and aromatic substituents gave the monoesters of ethylene glycol in good yields (entries 1–4). Similarly, six-membered cyclic acetals **9** and **11** gave good yields of the corresponding monoesters of propane-1,3-diols (entries 5 and 6).

It is interesting to note that the cyclic acetals (entries 1–7) gave the corresponding hydroxy esters without accompanying oxidation of the hydroxy groups. Cyclic acetal

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