acetyl group into the polymerizable vinyl group, the compound **1** was reduced with $NaBH_4$ and the resulting alcohol was dehydrated by $KHSO_4$ to obtain the desired UV stabilizing functional monomer, 2-(2H-benzotriazol-2-yl)-4-methyl-6-vinyl phenol (4M-6VP) in 70% yield (Scheme 2).[7]

Attempt of homopolymerization of 4M-6VP using 0.5 mole% of AIBN as an initiator was unsuccessful. The starting 4M-6VP was recovered even after 24 hr of polymerization at 90℃. But copolymerization of 4M-6VP with 99 mole% of MMA and styrene proceeded smoothly to produce the corresponding copolymers in 40 and 59% yields. There is a report that the polymerization of 2-(2H-benzotriazol-2-yl)-4-vinyl phenol, which had vinyl group at the 4-position of the phenyl ring of the BT molecule, proceeded smoothly.[8] However, this is the first report on the polymerizable BT's in which polymerizable group is attached at the 6- position of the phenyl ring of BT.

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Inhibition and Active-Site Requirements of 5-Aminolevulinic Acid Dehydratase

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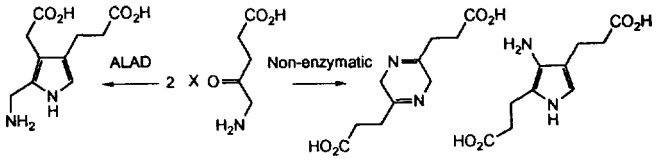
5-Aminolevulinic acid dehydratase[1] (ALAD, porphobilinogen synthase EC 4.2.1.24), found in both plants and animals, catalyzes the transformation of two 5-aminolevulinic acid (ALA, **1**) molecules into porphobilinogen (PBG, **2**), one pyrrole unit of the tetrapyrrolic pigments such as chlorophylls, hemes, phycobilins, and cobalamins.[2]



2 **1** **3** **4**

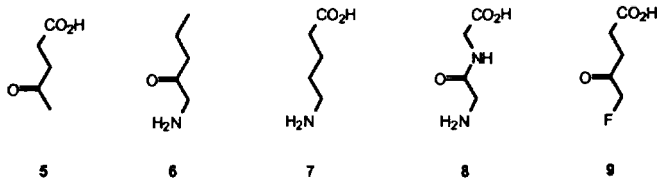
Scheme 1.

This enzyme requires an exogeneous thiol such as mercaptoethanol, dithioerythritol or dithiothreitol and metal ion Zn^{2+}, for the full catalytic activity.[1,3] Though the molecular weights[4] and amino acid sequences[5] of this enzyme from several sources were known, no X-ray picture of this enzyme has yet been revealed. Furthermore, no systematic study to find out the structural requirements for binding to ALAD has been undertaken.[6] The importance of proper binding of two substrates at the active site of ALAD is realized by comparison of enzymatic and non-enzymatic reaction of ALA (Scheme 1). PBG has never been formed by a chemical method from ALA.[7] Instead two moles of ALA are condensed in acid and/or base to yield 2,5-(β-carboxyethyl)dihydropyrazine (**3**) as the major product with a relatively small amount of pseudo-PBG (**4**). The structural requirements of ALAD enzyme can be assessed by inhibition studies of some simple substrate analogues (**5-9**).

Compounds **5-7** were designed to find out the relative importance of the three functional groups attached to the natural substrate, amine, ketone and carboxylate. Compounds **8** and **9** can give an insight into the electronic environment required for proper binding. We report herein the results of the inhibition of the enzyme isolated from bovine liver[8] by simple substrate analogues.

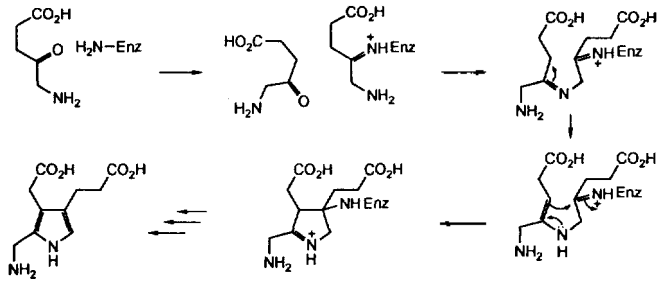
Compounds **5**, **7** and **8** were purchased from Aldrich and purified prior to use. 1-Amino-2-pentanone (**6**) was prepared by bromination[9] and amination[10] of 2-pentanone. 5-Fluorolevulinic acid (**9**) was obtained by the reported method.[11]

ALAD was assayed by a modified literature procedure.[12] The enzyme ALAD from bovine liver, purchased from SIGMA (1.5 unit/mg protein), was diluted with 0.10 M sodium phosphate buffer containing 0.1 mM $ZnSO_4$ and 20 mM of dithiothreitol, pH 6.8. This enzyme solution (0.2-0.3 unit/ as stable enough to keep in refrigerator for a few weeks. The standard enzyme assay contained 0.15 m*l* of 0.10 M sodium phosphate buffer containing 0.1 mM $ZnSO_4$ and 20 mM of dithiothreitol, pH 6.8, 0.15 m*l* of a solution of crystalline 5-aminolevulinate hydrochloride in distilled water, 0.10 m*l* of buffer or inhibitor solution, and 0.10 m*l* of enzyme solution. The blank was prepared by using boiled enzyme solution instead of the active one. After incubation for 30 min at 37℃, the reaction was terminated by addition of 0.5 m*l* of 0.1 M $HgCl_2$ in 10% trichloroacetic acid and added 0.5 m*l* of modified Ehrlich's reagent, which was prepared by dissolving 4-dimethyl aminobenzaldehyde (2 g) in a mixture of 80 m*l* glacial acetic acid and 20 m*l* 60-62% perchloric acid. After 15 min at room temperature the resultant precipi-



5	6	7	8	9
8.3	20	231	>600	0.03

Chart 1. Inhibitors of ALAD with K_i/K_m values.



Scheme 2.

tate was pelleted by centrifugation at 2,000×g for 10 min. The intensity of the color in the supernatant, due to condensation of the enzymatically produced porphobilinogen with the modified Ehrlich's reagent, was quantitated at 555 nm in a spectrophotometer based on $\varepsilon_{555}=6.02\times10^4$ $M^{-1}cm^{-1}$. Inhibition studies were conducted as above with substrate at K_m (290 mM) or at various substrate and inhibitor concentration (Hanes-Woolf analysis and least squares by KINFIT, a program kindly provided by Prof. V. Anderson).

The reaction obeyed Michaelis-Menten kinetics. All tested compounds were competitive inhibitors[13] and the inhibition activities were evaluated by K_i/K_m values shown in Chart 1. The K_i/K_m values of compounds **5** (8.3)[14], **6** (20), **7** (231) missing one of three distinctive functional groups showed that all three functional groups are important for initial recognition at the active-site of ALAD. The great difference in the values, from 8.3 to 231, is due to the relative importance of functional groups. Among the three compounds **5**, **6** and **7**, **5** was the best and **6** was the second best inhibitor. Compound **5** and **6** bear a ketone in common. The worst inhibition was observed with compound **7**, possessing amine and carboxylate but without a ketone group. Therefore we can draw the conclusion that the ketone is the most important among three distinctive functional groups of amine, ketone and carboxylate.

The importance of the ketone can be realized by the mechanism of the reaction, one version of which is shown in Scheme 2.[15] The reaction is known to involve formation of a Schiff base linkage with the bovine liver ALAD enzyme through the ε-amino group of lysine residue.[8,16] An interesting point can be found on the K_i/K_m value of glycylglycine **8** (>600) bearing all three required functional groups except that the carbon atom at the 3 position in the natural substrate is changed to a nitrogen atom, giving an amide. The extremely poor inhibition by **8** reveals the subtlety of the recognition of this enzyme for its substrate due to the change of the electronic environment around the ketone. To our surprise 5-fluorolevulinic acid (**9**), having the amine of the natural substrate replaced by fluoride, gives a K_i/K_m value of 0.03.[17] This result suggests that the electronic environment around ketone is more important than the functional group itself.[18] Further efforts are in progress to draw a better picture of ALAD enzyme action by preparation and testing of some other inhibitors.

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15. Three different reaction mechanisms have been proposed according to the order of binding two substrates and the sequence of aldol condensation and Schiff base formation. see, reference 2 and P. M. Jordan, in "Biosynthesis of Tetrapyrroles," P. M. Jordan, Ed., Elsevier, Amsterdam (1991), pp. 1-66. Scheme 2 is the mechanism based on the recent finding by Jaffe. see, E. K. Jaffe, W. R. Abrams, H. X. Kaempfen, and K. A. Harris, Jr., *Biochemistry.*, **31**, 2113 (1992).
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17. Prof. Neier reports[6] that 5-cyanolevulinic acid is a good inhibitor ($K_i/K_m=0.19$) of the *Rhodopseudomonas spheroides* enzyme. He explained this result by the preferred formation of the enamine in the "wrong" position of carbon 4 and 5 instead of 3 and 4 at the active-site of the enzyme. 5-Fluorolevulinic acid may act in the same way with bovine liver enzyme as 5-cyanolevulinic acid with the *R. spheroides* enzyme. However, the question is still open that K_i/K_m value of 5-fluorolevulinic acid is much smaller than that of 5-cyanolevulinic acid while tendency for 5-cyanolevulinic acid to make the enamine in the "wrong" position is much greater.
18. Further efforts are in progress to find out the correlation between binding and the electronic environment by the calculation of the atomic charges of the substrate and its analogues using MOPAC.

Facile Nucleophilic Substitution of α-Alkoxysulfonium Salts

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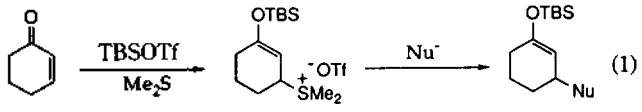
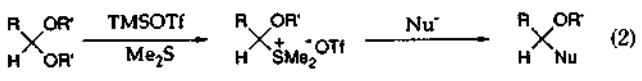
Sulfonium salts have been mainly utilized in the preparation of sulfur ylides[1] and the synthetic application of sulfonium salts as leaving groups has been limited due to their relatively poor leaving group ability.[2] During the study of selective protection of ketones in the presence of aldehydes,[3] we have experienced the unusually high reactivity of α-silyloxysulfonium salts toward several nucleophiles. Furthermore, we have reported that 3-trialkylsilyloxy-2-alkenylenesulfonium salts, derived from sulfoniosilylation of α,β-enones, are highly reactive species and undergo facile nucleophilic substitution reactions to give the products bearing the silyl enol ether group (Eq. 1).[4] In this paper we wish to report (i) the formation of α-alkoxysulfonium salts from acetals and (ii) their reactivity toward nucleophiles (Eq. 2).



(1)



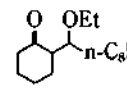
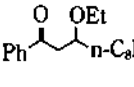
(2)

1 : R=n-C_3H_7; R'=Me
2 : R=n-C_8H_{17}; R'=Et

3 4

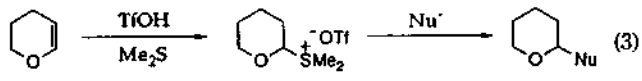
Reaction of **1** with trimethylsilyl triflate (TMSOTf, 1.1 equiv) and dimethyl sulfide (1.2 equiv) in tetrahydrofuran, ether, or dichloromethane at −78℃ afforded **3**, which was characterized by the low temperature ^{1}H NMR spectroscopy in $CDCl_3$ at −40℃.[5] The reaction proceeded cleanly and almost instantly. Simple dialkyl sulfide and tetrahydrothiophene could be successfully employed but diphenyl sulfide failed to form the corresponding sulfonium salts due to its low nucleophilicity. The sulfonium salts were thermally unstable and decomposed above −20℃. Futhermore, it is noteworthy that the present reaction did not work with the ketals due to steric reasons.

Table 1. Nucleophilic Substitution of α-Alkoxysulfonium Salt (**3**) with Nucleophiles

Nucleophile[a]	Temp, ℃	Time, h	Product	Yield, %[b]
RMgBr[c]				
R=Me	−78	3	R=Me	82
=Ph	−78	1	=Ph	95
=C≡C-Ph	−78→0	1	=C≡C-Ph	77
=CH=CH_2[d]	−78	2	=CH=CH_2	95
	−30	5	=CH_2CH=CH_2	96
	−78	1	=CH_2CH=CH_2	95
PhS-$SiMe_3$	−78	3	=SPh	82
PhSLi	−78	1	=SPh	91
	−30	1		73
	−30	3		82

[a] 1.2 equiv of the nucleophile was used. [b] The yield refers to the isolated yield. [c] A tetrahydrofuran solution of nucleophiles was added. [d] 0.1 equiv of CuBr-SMe_2 was added.

Nucleophilic substitution reactions of α-alkoxysulfonium salts (**3**) were generally carried out in dichloromethane using several nucleophiles by one-pot procedure as shown in Table 1. Reaction of **3** in dichloromethane with tetrahydrofuran solution of methylmagnesium bromide proceeded cleanly at −78℃, yielding the desired product in high yield. The use of phenylethynylmagnesium bromide required higher temperature and vinylmagnesium bromide failed to react with **3**. However, in the presence of 0.1 equiv of cuprous bromide, the reaction occurred smoothly. **3** was also reactive toward allyltrimethylsilane, silyl enol ethers, and phenylthiotrimethylsilane. Among three different types of silyl related functional groups, phenylthiotrimethylsilane was the most reactive, yielding the synthetically useful O,S-acetals[6] which could be also prepared by treatment of **3** with lithium thiophenoxide.



(3)

5 6

Nu⁻ : PhMgBr (91%); Ph-C(OTMS)=CH_2 (70%); PhSTMS (85%)

In order to study the possibility of extending the present