4,4-Disubstituted 1,2-Dithiolanes as Simple Models for Enzyme-bound Lipoic Acid

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Two substituents on C-4 drastically reduce the tendency of 1,2-dithiolane to polymerize, whereas they do not significantly alter the reactivity of the remote disulfide; the dithiolanes **1a–e** show high reactivity towards the carbon nucleophile EtMgBr as expected for the enzyme-bound lipoic acid.

Coenzyme lipoic acid is covalently bound to pyruvate dehydrogenase complex and mediates acetyl transfer from pyruvate to CoA at the start of the Krebs cycle.[1] The proposed mechanism[2] involves S–S bond cleavage of the 1,2-dithiolane moiety in the enzyme-bound lipoic acid (Lip-E_2) by a carbanion of hydroxyethylthiamine pyrophosphate (HET) producing mono-acetyl dihydrolipoyl-E_2[3] as delineated in Scheme 1. However, direct observation of this enzymic process[3] or mimicking the process using model compounds[4] has been unsuccessful.

Owing to the mutual repulsion of the lone electron pairs on its two sulfur atoms, 1,2-dithiolane is strained[5,6] and highly polymerizable, and the expected high reactivity towards nucleophiles was observed only with thiolates,[6,7] which can act as a polymerization regulator for the 1,2-dithiolane in solution. We recently found that 4,4-diethyl-1,2-dithiolane **1a** is nonpolymerizable and reacts quantitatively with Grignard reagents[8] to which some lipoyl derivatives were completely unreactive.[9] Thus, we examined the effect of the substituents remote from the disulfide moiety on the polymerizability of the 1,2-dithiolane moiety and its reactivity towards the carbon nucleophile EtMgBr in diethyl ether.

Various 4,4-disubstituted 1,2-dithiolanes **1a–e** were prepared by a known method[10] from the corresponding 2,2-disubstituted propane-1,3-diols *via* tosylation and subsequent treatment with Na_2S_4. Dithiolanes **1a** and **1b** are virtually nonpolymerizable, whereas **1c–e** become viscious liquids within a day and **1e** solidifies after several days. 4,4-Dimethyl-1,2-dithiolane **1f** is highly polymerizable,[11] and could not be prepared as a monomeric material, since it polymerized during distillation. A subtle change in the structure of the substituents obviously greatly affects the polymerization tendency of the dithiolane nucleus.

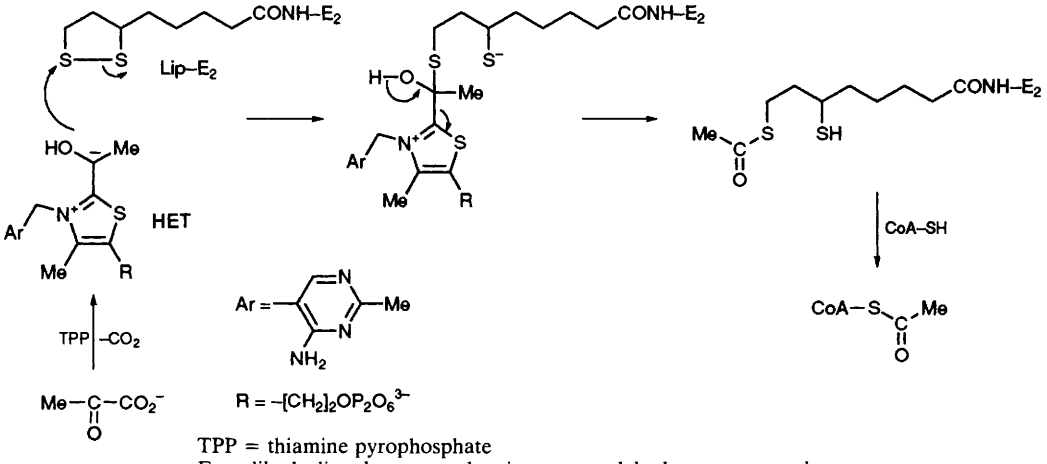
All the dithiolanes except for **1f** reacted with various Grignard reagents (0.1 mol l^{-1} in ether) in a quantitative manner as briefly reported[8] for **1a**, if they were distilled just before use. Selected results are in Table 1. The products **2a–e** are the simple products of ring opening and were identified by ^1H and ^{13}C NMR, MS and IR spectra. No side reactions were detectable by direct GLC analysis of the reaction mixture after acid hydrolysis, and the products were isolated pure by simple distillation. These results are in line with the mechanism proposed for Lip-E_2,[2] but are in contrast with the results using lipoyl derivatives whose ring opening by carbon nucleophiles was performed only in 12.2% yield or less.[9]

The relative reactivities of the dithiolanes **1a–e** and related compounds were examined by a competition method, and the results are included in Table 1. The reactivity of **1a–e** to EtMgBr increased slightly with increase in the poly-

Table 1 Reaction of 1,2-dithiolanes **1a–e** with ethylmagnesium bromide

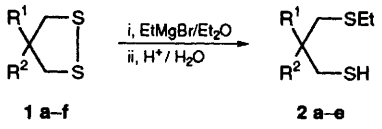
1,2-Dithiolane	Product[b]	Yield (%)[a,c]	Reactivity[d]
1a	**2a**	94	1.00
1b	**2b**	96	1.03
1c	**2c**	96	1.26
1d	**2d**	93	1.26
1e	**2e**	97	2.3
1,2-Dithiane	$HS[CH_2]_4SEt$	94[e,f]	0.0009
BnSSBn	BnSEt	—	0.00012
BuSSBu	BuSEt	—	0.00006

[a] Reaction with ice–water cooling for 30 min. [1,2-Dithiolane]$_0$ 0.10 mol l^{-1}, [EtMgBr]$_0$ 0.15 mol l^{-1}, solvent: diethyl ether (10 ml). [b] The products were fully characterized by IR, NMR and mass spectra. [c] Yield isolated by kugelrohr distillation. Purity of products was >99% unless otherwise noted. [d] Relative rate of the reaction estimated from the competitive method. [1,2-Dithiolane]$_0$ 0.1 mol l^{-1} each. Reaction in ether at 30 °C. [e] Enforced conditions: [EtMgBr]$_0$ 0.2 mol dm^{-3}, reaction at 30 °C for 2 h. [f] Product contained 1.5% of starting dithiane.



TPP = thiamine pyrophosphate
E_2 = dihydrolipoyl transacetylase in pyruvate dehydrogenase complex
CoA-SH = coenzyme A

Scheme 1



1 a–f **2 a–e**

a; $R^1 = R^2 = Et$
b; $R^1R^2 = [CH_2]_5$
c; $R^1 = Me$, $R^2 = Pr$
d; $R^1 = Me$, $R^2 = Et$
e; $R^1R^2 = [CH_2]_4$
f; $R^1 = R^2 = Me$

Scheme 2

merizability of **1a–e**, but the effect is minute, *i.e.* the 4,4-disubstituents had virtually no effect on the reactivity of the 1,2-dithiolanes **1a–e**. The results clearly show that the dithiolanes **1a–e** are about 10^4 times more reactive than linear disulfides BuSSBu and $PhCH_2SSCH_2Ph$. These results are comparable to or exceed the 5000-fold activation of 1,2-dithiolane observed in cleavage by thiolate anions.[6] The six-membered cyclic disulfide 1,2-dithiane reacted about 10 times faster than the linear disulfides. The order of the reactivity, *i.e.* 1,2-dithiolanes >> 1,2-dithane > linear disulfides, is in good accord with the order of the strain around the disulfide bonds, showing the major factor of this activation can be attributed to the ring strain of 1,2-dithiolane.

Since direct observations on the enzyme bound lipoic acid are difficult, a suitable model compound would be required to clarify its chemical behaviour towards carbon nucleophiles. Such a model must have the following characteristics. (*i*) It should have no polymerizing tendency since the enzyme-bound lipoic acid cannot polymerize. (*ii*) It must react with carbon nucleophiles to give ring-opened products without any side reactions, since the enzymic reaction is absolutely chemoselective. (*iii*) It must have high reactivity to carbon nucleophiles as expected from the intrinsic ring strain.

The present dithiolanes having two appropriate substituents on C-4 fulfil the above requisites, and are well suited to a simple model for studying the chemical behaviour of the enzyme-bound lipoic acid. On the other hand, lipoic acid and its low molecular mass derivatives are not suitable as models, since they are highly polymerizable in solution and resistant to carbon nucleophiles.

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