Coordination Chemistry of Lipoic Acid and Related Compounds, 2[◇]

Models for the Inhibition of Dithiol-Containing Enzymes by Organoarsenic Compounds: Synthetic Routes and the Structure of [PhAs(HlipS$_2$)] (HlipS$_2^{2-}$ = Reduced Lipoic Acid)☆

Angelika von Döllen and Henry Strasdeit*

Fachbereich Chemie, Universität Oldenburg
P. O. Box 2503, D-26111 Oldenburg, Germany
Fax: (internat.) +49(0)441/798-3329
E-mail: strasdeit@chemie.uni-oldenburg.de

Received August 15, 1997

Keywords: Arsenic / Bioinorganic chemistry / Dihydrolipoic acid / Lipoic acid / S ligands

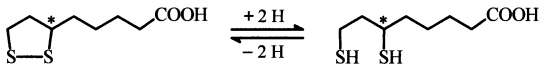
The arsenic(III) dithiolate [PhAs(HlipS$_2$)] (**1**, HlipS$_2^{2-}$ = reduced *rac*-lipoic acid) has been obtained via three different routes: (A) from (PhAsO)$_n$ and *rac*-dihydrolipoic acid, (B) from (AsPh)$_6$ and *rac*-lipoic acid, and (C) from PhAsO(OH)$_2$ and *rac*-dihydrolipoic acid. The latter method is also suitable for the preparation of [(4-NH$_2$C$_6$H$_4$)As(HlipS$_2$)] (**2**) from (4-NH$_2$C$_6$H$_4$)AsO(OH)$_2$. *rac*-Dihydrolipoic acid and Me$_2$AsO(OH) react to give [(Me$_2$As)$_2$(HlipS$_2$)] (**3**). These reactions indicate pathways by which mono- and diorganoarsenic compounds of various As oxidation states (I, III, and V) may inhibit enzymes that contain lipoic acid as a cofactor. X-ray structure analysis shows that **1** is a 2,4-disubstituted 1,3,2-dithiarsinane, i. e. a six-membered heterocycle. The phenyl group at the 2-position (As) adopts the axial orientation. (2*RS*,4*RS*)-**1**, which is the isomer found in the crystal, is thermodynamically more stable than the diastereomeric (2*SR*,4*RS*)-**1** by 4.3 ± 0.8 kJ/mol (25°C). In solution, the epimerization by inversion of configuration at the ψ-tetrahedrally coordinated As atom is faster than expected, with acid catalysis (COOH groups!) being a possible cause.

The toxicity of arsenic compounds has been known and used since classical antiquity[2]. In addition to its harmful effects on individuals, arsenic can cause severe public health problems, as a recent case of mass contamination has shown[3]. However, the underlying molecular mechanisms of action are still far from clear. It is assumed that the electronically soft species RAs^{2+} and R$_2$As$^+$ (R = e. g. alkyl, aryl) block biological sulphydryl groups; however, "the inhibition of thiol-enzymes by As^{III}, although widely quoted, is poorly understood"[4].

A probable biological target of arsenic(III) is enzyme-bound lipoic acid (α-lipoic acid, thioctic acid, 5-(1,2-dithiolan-3-yl)pentanoic acid, Hlip)[5][6]. This small biomolecule acts as a cofactor in some multienzyme complexes, namely the pyruvate dehydrogenase (and other α-keto acid dehydrogenases[7][8]) and the glycine cleavage system[9][10]. It has gained attention as a powerful biological antioxidant[11] and is approved in Germany for use as a therapeutic agent[12]. Lipoic acid can be reduced reversibly to dihydrolipoic acid (6,8-dimercaptooctanoic acid, Hlip(SH)$_2$; Scheme 1). This redox equilibrium is part of the enzymatic cycles[13].

It has repeatedly been demonstrated that lipoic acid-containing enzymes are strongly inhibited by As^{III} compounds such as arsenite[14] and organoarsoxanes (RAsO)$_n$[15]. However, only a few relevant low-molecular models have been examined; these were obtained from As^{III} starting materials in all cases[16][17][18]. In the present paper we show that such models are also accessible from compounds of *mono*- and *penta*valent arsenic related to the antimicrobial arsenicals, of which "Salvarsan" is the most prominent example[19]. In addition we report the crystal and molecular structure of [PhAs(HlipS$_2$)] (**1**), which to our knowledge is the first X-ray structure of an organoarsenic complex of a naturally occuring thiolate. A brief report on some of these results has appeared as a conference abstract[20].

Scheme 1



Hlip Hlip(SH)$_2$

Reactions

In the human body, inorganic As^V is partly methylated to MeAsO(OH)$_2$ and Me$_2$AsO(OH), and it has been suggested that the intermediate formation of As^{III} is involved in the biomethylation pathway[21]. The accompanying redox processes are probably coupled to biological thiol/disulphide equilibria. A redox reaction also occurs in the metabolism of the aryl-As^I compound Salvarsan (see below) and results in an As^{III} species, which is thought to be the active form of this formerly used medicinal drug[22]. This

[◇] Part 1: Ref.[1].

  1434−1948/98/0101−0061 $ 17.50+.50/0

prompted us to investigate the redox behaviour of some relevant organoarsenic compounds towards the dithiol dihydrolipoic acid [Hlip$(SH)_2$] and its oxidation product, the cyclic disulphide lipoic acid (Hlip, see Scheme 1).

In tetrahydrofuran (THF), phenylarsonic acid is smoothly reduced by Hlip$(SH)_2$ with the formation of the As^{III} compound [PhAs(HlipS$_2$)] (**1**) and lipoic acid (Eq. 1, method C under Experimental).

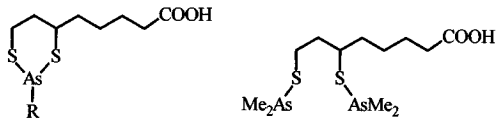
$$PhAsO(OH)_2 + 2\ Hlip(SH)_2 \xrightarrow[\text{room temp.}]{\text{THF}} \underset{\mathbf{1}}{[PhAs(HlipS_2)]} + Hlip + 3\ H_2O \quad (1)$$

1 is a disubstituted 1,3,2-dithiarsinane (Scheme 2). Its constitution was deduced from NMR data by Dill et al., who obtained **1** from $PhAsCl_2$ and Hlip$(SH)_2$ in solution but did not isolate the product[18]. The final proof of the structure of **1** comes from our crystal structure determination described below. Eq. 1 includes two reaction steps, namely the reduction of As^V and the subsequent complexation of the As^{III} formed. The second step determines the result of the overall reaction. Therefore, even with the appropriate ratio of phenylarsonic acid to Hlip$(SH)_2$, no phenylarsoxane is obtained (Eq. 2).

$$PhAsO(OH)_2 + Hlip(SH)_2 \not\longrightarrow {}^1/_n\,(PhAsO)_n + Hlip + 2\ H_2O \quad (2)$$

Instead, **1** is formed, and 50% of the $PhAsO(OH)_2$ remains unreacted.

Scheme 2



1: R = phenyl
2: R = 4-aminophenyl

3

Starting from phenylarsoxane, there is an alternative and more favourable route to **1** (Eq. 3, method A).

$${}^1/_n\,(PhAsO)_n + Hlip(SH)_2 \xrightarrow[\text{room temp.}]{\text{THF}} \underset{\mathbf{1}}{[PhAs(HlipS_2)]} + H_2O \quad (3)$$

As **1** is the only solid product of this reaction, separation problems, as in the case of method C (Eq. 1; similar solubilities of **1** and Hlip), are avoided. Triphenylarsane was also tested as a starting material. However, no reaction with dihydrolipoic acid was observed. This behaviour distinguishes $AsPh_3$ from $HgPh_2$, which under the same conditions yields the trinuclear complex $[Hg(PhHg)_2(HlipS_2)_2]$[23].

p-Aminophenylarsonic acid [arsanilic acid, (4-$NH_2C_6H_4$)$AsO(OH)_2$] was one of the first arsenicals to be used successfully. Ehrlich has already inferred from biological observations that this compound must be reduced in organisms in order to become active against pathogens[19b]. We have shown that (4-$NH_2C_6H_4$)$AsO(OH)_2$ is reduced by Hlip$(SH)_2$. The reaction, which was carried out in methanol, is analogous to that of $PhAsO(OH)_2$ (Eq. 1) and yields the crystalline As^{III} compound [(4-$NH_2C_6H_4$)As(HlipS$_2$)] (**2**). Spectroscopic data reveal that **2** is *not* zwitterionic, at least in the solid state and in $CDCl_3$, and that it has the same constitution as **1** (Scheme 2). The formation of **2** sheds light on the possible biochemical pathway of arsanilic acid and similar As^V arsenicals. It shows the facile reduction by a biological dithiol as well as the blocking of the coenzyme lipoic acid, which may be the cause or one of the causes of the antimicrobial activity.

The As^I arsenical Salvarsan is an aromatic ring-substituted (3-NH_2, 4-OH) derivative of hexaphenylcyclohexaarsane. While the parent compound is well-defined and forms cyclic $(AsPh)_6$ molecules[24], Salvarsan appears as mixtures of partly oxidized oligomeric and polymeric chains with strongly varying properties[25]. Both compounds contain As−As bonds. In Salvarsan these bonds are oxidatively cleaved under physiological conditions (see above). We used $(AsPh)_6$ as a model for Salvarsan to test whether lipoic acid acts as an oxidizing agent towards aryl-As^I and can accomplish the As−As bond cleavage. No reaction occurred in THF, toluene or diethylene glycol dimethyl ether at temperatures up to the boiling point of the respective solvent. However, when $(AsPh)_6$ and lipoic acid were melted together, compound **1** was formed quantitatively (Eq. 4, method B).

$${}^1/_6\,(AsPh)_6 + Hlip \xrightarrow[130°C]{\text{melt}} \underset{\mathbf{1}}{[PhAs(HlipS_2)]} \quad (4)$$

The need for quite drastic and thus nonphysiological conditions may be rationalized in terms of the different structures of $(AsPh)_6$ and Salvarsan, i.e. cyclic vs. open-chain. Bearing this difference in mind one can expect that in vivo, Salvarsan, after oxidation by a suitable biological disulphide (not necessarily lipoic acid), is able to inhibit lipoic acid dependent enzymes.

Finally, we studied the reaction of dihydrolipoic acid with a *di*organoarsenic(V) compound, namely dimethylarsenic acid [cacodylic acid, $Me_2AsO(OH)$]. Again reduction of As^V to As^{III}, accompanied by As^{III} complexation was observed (Eq. 5).

$$2\ Me_2AsO(OH) + 3\ Hlip(SH)_2 \xrightarrow[\text{room temp.}]{\text{THF}} \underset{\mathbf{3}}{[(Me_2As)_2(HlipS_2)]} + 2\ Hlip + 4\ H_2O \quad (5)$$

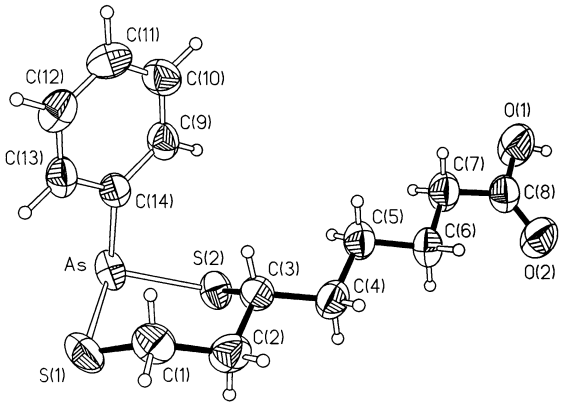
Attempted separation of $[(Me_2As)_2(HlipS_2)]$ (**3**, see Scheme 2) from the second nonvolatile product lipoic acid failed, although **3** was unambiguously identified by spectroscopic methods (^{13}C NMR, CI-MS) in the reaction mixture. The 1:2 molar ratio of **3** and Hlip expected from Eq. 5 was confirmed by ^1H-NMR spectroscopy. No unreacted starting material or other products were found. Pure **3** is accessible via reaction of Me_2AsCl with Hlip$(SH)_2$ in the presence of pyridine as a base[16]. Its formation from $Me_2AsO(OH)$ is, however, more relevant to bioinorganic and toxicological questions, because dimethylarsenic acid is a common biomethylation product of arsenate and arsenite[21]. Moreover, this compound is also a herbicide and a chemical weapon ("Agent Blue")[26].

We have shown that mono- and diorganoarsenic(V) compounds of the type $RAsO(OH)_2$ and $R_2AsO(OH)$, respectively, are easily reduced by dihydrolipoic acid. The logical extension of this, the reduction of R_3AsO by $Hlip(SH)_2$, has been studied by others for R = Me[27]. The only products observed were Me_3As and lipoic acid.

Crystal Structure of 1

Crystals of **1** consist of [PhAs(HlipS_2)] molecules (or complexes, to emphasize the semi*metallic* character of arsenic; see Figure 1). The molecules are arranged as symmetric hydrogen-bonded dimers. Each dimer is situated on a crystallographic centre of inversion and has a central eight-membered ring formed by the two carboxylic groups. The O···O distances within the two hydrogen bonds of the ring are 2.657 Å [O(1)···O(2′); 2 − *x*, 2 − *y*, 2 − *z*]. This motif is very common and has been found for the majority of monofunctional carboxylic acids in the crystalline state[28].

Figure 1. View of [PhAs(HlipS_2)] (**1**) in the solid state; non-hydrogen atoms are shown as thermal ellipsoids with 50% probability[a]



[a] Selected bond lengths [Å] and angles [°]: As−S(1) 2.230(1), As−S(2) 2.227(1), As−C(14) 1.964(4), S(1)−C(1) 1.817(5), S(2)−C(3) 1.848(4), C(1)−C(2) 1.530(6), C(2)−C(3) 1.521(5), C(3)−C(4) 1.529(6), C(4)−C(5) 1.515(6), C(5)−C(6) 1.510(6), C(6)−C(7) 1.509(6), C(7)−C(8) 1.485(6), O(1)−C(8) 1.297(5), O(2)−C(8) 1.208(5), S(1)−As−S(2) 99.41(5), S(1)−As−C(14) 99.49(11), S(2)−As−C(14) 100.51(11), As−S(1)−C(1) 102.55(14), As−S(2)−C(3) 104.56(13), S(1)−C(1)−C(2) 117.1(3), C(1)−C(2)−C(3) 115.9(3), S(2)−C(3)−C(2) 113.0(3), S(2)−C(3)−C(4) 106.5(3), C(2)−C(3)−C(4) 112.2(3), C(3)−C(4)−C(5) 113.9(3), C(4)−C(5)−C(6) 115.9(3), C(5)−C(6)−C(7) 111.1(4), C(6)−C(7)−C(8) 117.0(4), O(1)−C(8)−O(2) 122.0(4), O(1)−C(8)−C(7) 114.2(4), O(2)−C(8)−C(7) 123.8(4).

The [PhAs(HlipS_2)] molecule is a 1,3,2-dithiarsinane disubstituted at the 2-position (As) and the 4-position [C(3)], i.e. it is a 2-arsa-1,3-dithiacyclohexane derivative. The AsS_2C_3 six-membered ring adopts a chair conformation. While the 4-substituent has an equatorial orientation, the phenyl group is axially orientated. This axial conformational preference of a substituent at the As atom is not unexpected. It has been observed before in related molecules such as 2-phenyl-1,3,2-dithiarsinane (solution)[29] and 5-*tert*-butyl-2-phenyl-1,3,2-dithiaphosphinane (solution and solid state)[30] and seems to be a more general phenomenon. The consequences of the alternative equatorial conformation can be easily deduced from Figure 1. Changing the positions of the stereochemically active lone pair and the phenyl group at As [without rotation about the As−C(14) bond!] would cause severe repulsion between the phenyl *ortho*-H atoms and the four lone electron pairs at S(1) and S(2). Rotation about the As−C(14) bond would reduce this unfavourable energy contribution, but on the other hand it would move one of the *ortho*-H atoms towards the lone electron pair at As.

In the conformation of **1** that is actually adopted, *cis*-axial interactions probably occur between the phenyl group and the axial H atoms at C(1) and C(3). As suggested for substituted 1,3,2-dithiaphosphinanes and their dioxo analogues[30b][31], these van der Waals forces may be weakly attractive. In addition, hyperconjugative interactions in the S_2AsPh moiety, similar to those accepted as a basis for the anomeric effect[32], may contribute to the observed axial orientation of the phenyl group in **1**.

There are no intermolecular contacts to As smaller than the sum of the van der Waals radii. Thus the coordination number of As is three, and the geometry around this atom is pyramidal (ψ-tetrahedral). At least one related compound is known, in which, in contrast to **1**, the coordination sphere of the central atom is expanded by secondary As−S bonds. This compound is $[PhAs\{S_2P(OiPr)_2\}_2]$[33]. In **1** the metrical data of the C(14)AsS(1)S(2) part are unexceptional and fall within the ranges found for four other compounds having aryl-$As^{III}S_2$ moieties[34]: As−S 2.23−2.28, As−C 1.95−1.99 Å; S−As−S 92.7−102.1, S−As−C 94.0−101.3°. The As−S bond lengths in **1** lie marginally below the single-bond value calculated from the covalent radii and corrected for the electronegativity difference[35a].

Two chiral centres are present in [PhAs(HlipS_2)]: the one at C(3) belongs to the reduced lipoic acid and a second one at the arsenic atom. According to the numbering scheme for the 1,3,2-dithiarsinane ring, these are the 2- and the 4-position. The structure determination was performed on a crystal of the (2*RS*,4*RS*) racemate. Figure 1 shows the (2*R*,4*R*) enantiomer.

Stereoisomerism of 1

The crystalline products obtained by methods A and C (see Experimental Section) are (2*RS*,4*RS*)-**1** as found by X-ray structure analysis (see above) and spectroscopic comparison. A freshly prepared solution in $CDCl_3$ shows the expected number of 12 signals in the ^{13}C-NMR spectrum. On standing at room temp. for several days, the diastereomer (2*SR*,4*RS*)-**1** gradually forms in the solution. It gives rise to additional, weak signals in the ^1H- and ^{13}C-NMR spectra. Signals at the same positions are observed for the product prepared by method B, i.e. by melting together *rac*-lipoic acid and $(AsPh)_6$ at 130°C. A quantitative evaluation of the NMR spectra of this product gives a content of the minor isomer (2*SR*,4*RS*)-**1** of ca. 16%.

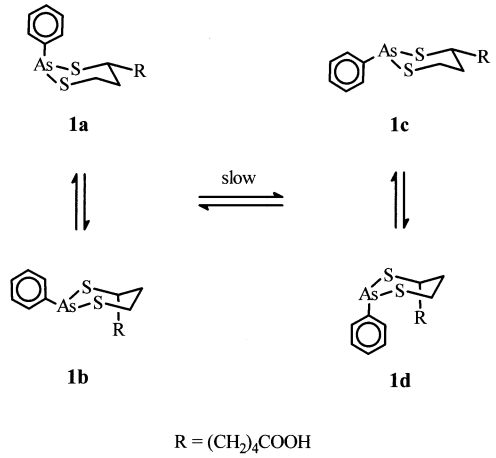
The establishment of equilibrium between the two isomers proceeds much faster in [D_4]methanol than in $CDCl_3$. Starting from pure (2*RS*,4*RS*)-**1**, it is about 90% complete after one day, and after 7 days the final ratio of ca. 85:15 is

attained. This ratio corresponds to $\Delta G = 4.3 \pm 0.8$ kJ/mol at 25°C for the equilibrium shown in Eq. 6.

$$(2RS,4RS)\text{-}\mathbf{1} \rightleftharpoons (2SR,4RS)\text{-}\mathbf{1} \qquad (6)$$

In other words, the (2*RS*,4*RS*) isomer, whose X-ray structure has been determined, is the thermodynamically more stable one. In contrast to **1**, solutions of the compound **2** in $CDCl_3$ exhibit NMR spectra compatible with the pure (2*SR*,4*RS*) isomer. Here further experiments were hampered by the low solubility.

Scheme 3



1a **1c** **1b** **1d**

The equilibrium of Eq. 6 is shown in more detail in Scheme 3. **1a** and **1b** are conformational isomers of (2*R*,4*R*)-**1**, while **1c** and **1d** correspond to (2*S*,4*R*)-**1**. The isomer **1a** is shown in Figure 1. (2*S*,4*S*)-**1** and (2*R*,4*S*)-**1** are not considered because they would merely give a mirror image of Scheme 3. In addition to the slow epimerization by inversion of configuration at the arsenic atom, two chair-chair equilibria (**1a/1b** and **1c/1d**) are included. As demonstrated above, (2*R*,4*R*)-**1** is thermodynamically favoured. Under the plausible assumptions that (i) conformations other than chair may be neglected and (ii) the $(CH_2)_4COOH$ substituent prefers the equatorial orientation, it can be concluded that **1a**, together with equal amounts of its enantiomeric form, is the major stereoisomer in solution. Thus, the conjecture that **1c** is most likely the major isomer[18] could not be confirmed.

From the early phase of the transformation of (2*RS*,4*RS*)-**1** into (2*SR*,4*RS*)-**1** in $CDCl_3$ the activation energy can be roughly estimated[35b] at ca. 110 kJ/mol at 25°C if the reaction is assumed to be monomolecular. Mislow and coworkers have found a correlation between the barriers to pyramidal inversion at arsenic and at phosphorus[36]. From this correlation and the activation energy reported for 5-*tert*-butyl-2-phenyl-1,3,2-dithiaphosphinane[30b], a $\Delta G^{\neq}$ value near 180 kJ/mol is expected for **1**. The large discrepancy of ca. 70 kJ/mol can only be accounted for by a mechanism other than a simple pyramidal inversion. Although we did not investigate this problem further, one can hypothesize that acid catalysis by the carboxyl group of **1** may be the determining step. The epimerization would then be self-catalyzed. A possible reaction mechanism involves cleavage of one of the As−S bonds accompanied by protonation of the S atom and $As-O_2C$ bond formation, rotation about the remaining As−S bond, and re-establishment of the first As−S bond by elimination of −COOH. Acid catalysis is consistent with the observed acceleration of the epimerization in [D_4]methanol. While in $CDCl_3$ hydrogen-bonded dimers like those observed in the solid state can be expected, in protic solvents monomers having more easily accessible protons should prevail.

Financial support from the *Deutsche Forschungsgemeinschaft* and the *Fonds der Chemischen Industrie* is gratefully acknowledged. We thank *ASTA Medica AG* for generous donations of *rac*-lipoic acid, Dr. *H. J. Tritschler* for providing us with copies of refs.[11][38a], and Mr. *W. Saak* for helpful discussions and for collecting the diffraction data.

Experimental Section

General: Racemic lipoic acid was obtained as a gift from ASTA Medica AG. Dihydrolipoic acid was prepared by $NaBH_4$ reduction of lipoic acid[37]. The identity of the product and its purity (≥99% based on GC) were checked by high-resolution ^{13}C-NMR spectroscopy (125.8 MHz, $CDCl_3$): δ = 22.03 (C-8), 24.02 (C-3), 26.17 (C-4), 33.67 (C-2), 38.37 (C-5), 39.03 (C-6), 42.48 (C-7), 179.8 (C-1)[38]. Dihydrolipoic acid is a colourless oil which should be stored under nitrogen. Hexaphenylcyclohexaarsane was prepared and purified according to the method described in ref.[39]. All other chemicals were purchased from commercial sources and used without further purification. The solvents were reagent grade. The compounds **1**−**3** were prepared and handled in a glove box under an atmosphere of dry nitrogen. − IR: FT-IR spectrometer Bio-Rad FTS 7PC. − ^{1}H and ^{13}C{^{1}H} NMR: Bruker AM-300, Bruker ARX-500, chemical shifts relative to TMS, solvent signal as internal reference in the ^{13}C-NMR spectra ($CDCl_3$: δ_c = 77.00, [D_4]methanol: δ_c = 49.00). Asterisked signals belong to the minor isomer (2*SR*,4*RS*)-**1**. − MS: Finnigan MAT 212, Finnigan MAT 95. − Elemental analyses: Mikroanalytisches Laboratorium Beller, D-37004 Göttingen, Germany. − *Caution!* Arsenic compounds are highly toxic, and some of them are recognized as human carcinogens. They should therefore be handled with care to avoid exposure.

5-(2-Phenyl-1,3,2-dithiarsinan-4-yl)pentanoic Acid (**1**): *Method A*: 336 mg (2.00 mmol) of phenylarsoxane was added to a solution of 416 mg (2.00 mmol) of *rac*-dihydrolipoic acid in 40 ml of tetrahydrofuran. The solution was stirred for 24 h. Subsequently its volume was reduced in vacuo until an oil remained. From this residue colourless crystals of **1** separated over a two-week period at room temp. The crystals were collected on a glass filter, washed with a small amount of toluene and dried in vacuo. A second crop of crystals was obtained from the filtrate after evaporation. Total yield: 0.43 g (60%), m.p. 97°C. − IR (KBr): $\tilde{\nu}$= 3042 cm^{-1} (w, aryl-CH), 2942 (w, alkyl-CH), 2907 (w, alkyl-CH), 1707 (s, C=O), 1429 (m), 1250 (m), 1206 (m), 745 (m, phenyl group), 370 (m, AsS[40]). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.37−1.66 (m, 6 H), 1.89 (m, 2 H), 2.34 (t, 2 H, CH_2COOH), 2.65 (m, 1 H), 2.83 (m, 2 H), 7.36 (m, 1 H, Ph-*para*), 7.46 ("t", 2 H, Ph-*meta*), 7.87 ("d", 2 H, Ph-*ortho*), 11.5 (br., 1 H, COOH). − ^{1}H NMR (500 MHz, [D_4]methanol; after keeping the solution at room temp. for ca. 7 d): δ = 1.47 (m), 1.58 (m), 1.71 (m)*, 1.82−1.95 (m), 2.21 (m)*, 2.28 (t), 2.66 (m), 2.85 (m), 3.04−3.18 (m)*, 7.40 (m), 7.44 (m)*, 7.50 ("t"), 7.82 (m)*, 7.88 ("d"). − ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 24.20, 25.07, 27.85, 33.80, 35.73, 38.75, 39.76, 128.7 (Ph-*para*), 129.2, 132.1, 139.2 (Ph-*ipso*), 179.7 (C=O). − ^{13}C NMR (125.8

MHz, [D_4]methanol; after keeping the solution at room temp. for ca. 7 d): δ = 25.58, 25.75*, 26.19, 26.98*, 28.77, 29.85*, 34.77, 36.99, 37.32*, 39.33*, 39.87, 41.01, 46.04*, 129.9, 130.3, 131.4*, 133.1, 133.6*, 140.2*, 140.5, 177.3, 177.4*. − MS (CI, isobutane); *m/z* (%): 359 (100) [MH^+], 341 (14) [$MH^+ - H_2O$], 207 (7) [$MH^+ - C_6H_5As$], 189 (35) [$MH^+ - H_2O - C_6H_5As$]. − $C_{14}H_{19}AsO_2S_2$ (358.4): calcd. C 46.92, H 5.34, As 20.91, S 17.90; found C 47.10, H 5.43, As 20.85, S 18.00.

Method B: An intimate mixture of *rac*-lipoic acid and hexaphenylcyclohexaarsane in a 6:1 molar ratio was heated to 130°C within 1 h. The mixture started melting at ca. 70°C. The clear, initially yellowish melt was kept at 130°C for 3.5 h before it was allowed to cool down to room temp. and to solidify. The reaction was quantitative and yielded a colourless product. − ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 24.18, 24.28*, 25.04, 25.78*, 27.83, 29.29*, 33.82, 35.71, 36.11*, 38.19*, 38.72, 39.74, 44.84*, 128.7, 128.8*, 129.1, 130.3*, 132.0, 132.6*, 139.2, 179.9.

Method C: *rac*-Dihydrolipoic acid and phenylarsonic acid in a 2:1 molar ratio were dissolved in tetrahydrofuran. The solution turned light yellow due to the formation of lipoic acid. After stirring for 24 h, the solvent was removed in vacuo. Colourless crystals of **1** separated from the remaining oil within 2 weeks. Their IR spectrum was identical with that of the product prepared by method A.

5-[2-(4-Aminophenyl)-1,3,2-dithiarsinan-4-yl]pentanoic Acid (**2**): 434 mg (2.00 mmol) of *p*-aminophenylarsonic acid was added to a stirred solution of 833 mg (4.00 mmol) of *rac*-dihydrolipoic acid in 60 ml of methanol. The arsenic compound slowly dissolved, and after a few hours the yellow colour of lipoic acid emerged. After stirring for 24 h, removal of the solvent in vacuo resulted in the crystallization of small colourless needles of **2**. The product was isolated on a glass filter, washed with a small amount of chloroform and dried in vacuo. Yield 0.53 g (71%), m.p. 122°C. − IR (KBr): $\tilde{\nu}$= 3376 cm^{-1} (m, NH_2), 3302 (w, NH_2), 2932 (w, alkyl-CH), 2868 (w, alkyl-CH), 1707 (s, C=O), 1591 (m), 1495 (m), 1265 (m), 1169 (m), 1074 (m), 818 (m, aryl group), 511 (m), 374 (m, AsS[40]). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.39−1.77 (m, 7 H), 2.12 (m, 1 H), 2.34 (t, 2 H, CH_2COOH), 3.00−3.19 (m, 3 H), 5.6 (br., 3 H, NH_2 and COOH), 6.67 ("d", 2 H, aromatic H), 7.59 ("d", 2 H, aromatic H). − ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 24.34, 25.71, 30.11, 33.70, 36.54, 38.50, 45.37, 115.1 (H_2NC*C*), 125.5 (AsC), 134.3 (AsC*C*), 148.7 (H_2NC), 178.9 (C=O). − MS (EI, 80 eV); *m/z* (%): 373 (1.4) [M^+], 93 (100) [$C_6H_7N^+$], 66 (50) [$C_5H_6^+$], 65 (37) [$C_5H_5^+$]. − $C_{14}H_{20}AsNO_2S_2$ (373.4): calcd. C 45.04, H 5.40, As 20.07, N 3.75, S 17.18; found C 45.14, H 5.52, As 19.56, N 3.75, S 17.44.

Formation of 6,8-Bis[(dimethylarsanyl)thio]octanoic Acid (**3**) *from Dimethylarsinic Acid and rac-Dihydrolipoic Acid:* 138 mg (1.00 mmol) of $Me_2AsO(OH)$ was added to a stirred solution of 312 mg (1.50 mmol) of *rac*-dihydrolipoic acid in 10 ml of tetrahydrofuran. After stirring overnight, the solvent was evaporated in vacuo. To remove tetrahydrofuran and water completely, the remaining oil was dissolved in dichloromethane, and the solvent was evaporated again. After drying in vacuo, a yellow oil was obtained, which consisted of **3** and lipoic acid in a 1:2 molar ratio. − ^{1}H NMR (500 MHz, $CDCl_3$): The intensities of the signals at δ = 1.24 ($AsCH_3$) and 2.27 (CH_2COOH from **3** and from lipoic acid) were used to determine the product ratio. − ^{13}C NMR (125.8 MHz, $CDCl_3$), **3**: δ = 13.88 ($AsCH_3$), 14.01 ($AsCH_3$), 24.09, 26.10, 28.78, 33.67, 37.04, 40.14, 45.30, 179.7 (C=O). The remaining signals were those of lipoic acid; no signals of unreacted dihydrolipoic acid were found (cf. ref.[38]). − MS (CI, isobutane); *m/z* (%): 416 (32) [M^+], 312 (100) [$MH^+ - As(CH_3)_2$].

X-ray Crystal Structure Analysis of **1**: A suitable single crystal of **1** was obtained from the reaction of *rac*-dihydrolipoic acid with phenylarsonic acid (similar to method C described above). $C_{14}H_{19}AsO_2S_2$, M = 358.36, colourless crystal, crystal size 0.42 × 0.29 × 0.10 mm, triclinic, space group $P\bar{1}$, a = 7.240(1), b = 8.526(1), c = 14.193(2) Å, α = 82.03(1), β = 82.84(1), γ = 67.72(1)°, V = 800.4(2) Å^3, Z = 2, $\rho_{calcd.}$ = 1.487 gcm^{-3}, $F(000)$ = 368, $\mu(MoK_\alpha)$ = 2.38 mm^{-1}. 3983 reflections were measured at 23°C on a Siemens/STOE AED2 diffractometer using graphite-monochromated MoK_α radiation. 5° ≤ 2Θ ≤ 50°, 0 ≤ h ≤ 8, −9 ≤ k ≤ 10, −16 ≤ l ≤ 16, ω/2Θ scans, empirical absorption correction, T_{min} = 0.1803, T_{max} = 0.2880. Structure solution by direct methods[41], refinement on F^2 values (full-matrix least-squares)[42], 2819 independent reflections (R_{int} = 0.0196) of which all were used in the final refinement, 173 parameters, S (on F^2) = 1.097, $R1$ (all data) = 0.0612, $wR2$ (all data) = 0.1079, $R1$ [$I > 2\sigma(I)$] = 0.0438, max. and min. electron density in final difference map: +1.13 and −0.25 eÅ^{-3}. Anisotropic thermal parameters were refined for all non-hydrogen atoms. H atoms were included on idealized positions and were given U values 1.5 (OH) and 1.2 times (CH, CH_2), respectively, the equivalent isotropic displacement factors of the atoms to which they were attached. The H atom of the OH group was allowed to rotate about the C(8)−O(1) bond (AFIX 87 instruction).

Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (deposition no. 100410). Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: int. code +44(0)1223/336-033, e-mail: deposit@chemcrys.cam.ac.uk).

[☆] Dedicated to the memory of our teacher and colleague Prof. Dr. *Siegfried Pohl*, who untimely passed away on December 18, **1996**.

[1] H. Strasdeit, A. von Döllen, A.-K. Duhme, *Z. Naturforsch.* **1997**, *52b*, 17−24.

[2] L. Lewin, *Die Gifte in der Weltgeschichte: toxikologische allgemeinverständliche Untersuchungen der historischen Quellen*, Springer, Berlin, **1920**; reprint, 3rd ed., Gerstenberg, Hildesheim, **1984**, pp. 157−166.

[3] P. Bagla, J. Kaiser, *Science* **1996**, *274*, 174−175. Although being highly toxic in larger amounts, arsenic may be an essential trace element for mammals (see ref.[5]).

[4] Quoted from ref.[5]

[5] O. M. Ni Dhubhghaill, P. J. Sadler, *Struct. Bonding (Berlin)* **1991**, *78*, 129−190.

[6] H. V. Aposhian in *Reviews in Biochemical Toxicology* (Eds.: E. Hodgson, J. R. Bend, R. M. Philpot), Vol. 10, Elsevier, New York, **1989**, pp. 265−299.

[7] T. E. Roche, M. S. Patel (Eds.), *Alpha-Keto Acid Dehydrogenase Complexes: Organization, Regulation, and Biomedical Ramifications*, New York Academy of Sciences, New York, **1989**.

[8] R. N. Perham, *Biochemistry* **1991**, *30*, 8501−8512.

[9] D. Voet, J. G. Voet, *Biochemistry*, 2nd ed., Wiley, New York, **1995**, pp. 541−548, 736−738.

[10] For relevant protein crystallographic studies see: [10a] A. Mattevi, G. Obmolova, E. Schulze, K. H. Kalk, A. H. Westphal, A. de Kok, W. G. J. Hol, *Science* **1992**, *255*, 1544−1550. − [10b] A. Mattevi, G. Obmolova, K. H. Kalk, A. Teplyakov, W. G. J. Hol, *Biochemistry* **1993**, *32*, 3887−3901. − [10c] S. Pares, C. Cohen-Addad, L. Sieker, M. Neuburger, R. Douce, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 4850−4853. − [10d] C. Cohen-Addad, S. Pares, L. Sieker, M. Neuburger, R. Douce, *Nature Struct. Biol.* **1995**, *2*, 63−68. − [10e] S. Pares, C. Cohen-Addad, L. C. Sieker, M. Neuburger, R. Douce, *Acta Crystallogr. Sect. D* **1995**, *51*, 1041−1051. − [10f] S. S. Mande, S. Sarfaty, M. D. Allen, R. N. Perham, W. G. J. Hol, *Structure* **1996**, *4*, 277−286.

[11] L. Packer, E. H. Witt, H. J. Tritschler, *Free Rad. Biol. Med.* **1995**, *19*, 227−250.
[12] Rote Liste Service GmbH (Ed.), *Rote Liste* **1997**, ECV Editio Cantor, Aulendorf, **1997**, 67001−67016.
[13] For a leading review on the chemical and biological properties of lipoic acid see: L. Teuber, *Sulfur Rep.* **1990**, *9*, 257−349.
[14] D. R. Sanadi, M. Langley, F. White, *J. Biol. Chem.* **1959**, *234*, 183−187.
[15] [15a] K. J. Stevenson, G. Hale, R. N. Perham, *Biochemistry* **1978**, *17*, 2189−2192, and literature cited therein. − [15b] S. R. Adamson, K. J. Stevenson, *Biochemistry* **1981**, *20*, 3418−3424.
[16] C. H. Banks, J. R. Daniel, R. A. Zingaro, *J. Med. Chem.* **1979**, *22*, 572−575.
[17] W. R. Cullen, B. C. McBride, J. Reglinski, *J. Inorg. Biochem.* **1984**, *21*, 179−193.
[18] K. Dill, E. R. Adams, R. J. O'Connor, E. L. McGown, *Chem. Res. Toxicol.* **1989**, *2*, 181−185.
[19] [19a] E. Schröder, C. Rufer, R. Schmiechen, *Pharmazeutische Chemie*, Thieme, Stuttgart, **1982**, pp. 837−840. − [19b] H. Hilmer in *Arzneimittel − Entwicklung, Wirkung, Darstellung* (Eds.: G. Ehrhart, H. Ruschig), Vol. 4, Verlag Chemie, Weinheim, **1972**, pp. 57−76.
[20] H. Strasdeit, A. von Döllen, A.-K. Duhme, *J. Inorg. Biochem.* **1995**, *59*, 228; presented at the 7th International Conference on Bioinorganic Chemistry, Lübeck, Germany, **1995**.
[21] M. Vahter, E. Marafante in *The Biological Alkylation of Heavy Elements* (Eds.: P. J. Craig, F. Glockling), Royal Society of Chemistry, London, **1988**, pp. 105−119.
[22] W. Schunack, K. Mayer, M. Haake, *Arzneistoffe: Lehrbuch der Pharmazeutischen Chemie*, 2nd ed., Vieweg, Braunschweig, **1983**, p. 507.
[23] A. von Döllen, H. Strasdeit, manuscript in preparation.
[24] A. L. Rheingold, P. J. Sullivan, *Organometallics* **1983**, *2*, 327−331.
[25] M. Ya. Kraft, E. N. Sytina, *Proc. Acad. Sci. USSR* **1957**, *116*, 837−840, and literature cited therein.
[26] J. B. Neilands, *Naturwissenschaften* **1973**, *60*, 177−183.
[27] W. R. Cullen, B. C. McBride, J. Reglinski, *J. Inorg. Biochem.* **1984**, *21*, 45−60.
[28] A. Gavezzotti, G. Filippini, *J. Phys. Chem.* **1994**, *98*, 4831−4837; see also: L. Leiserowitz, *Acta Crystallogr. Sect. B* **1976**, *32*, 775−802.
[29] D. W. Aksnes, *Acta Chem. Scand.* **1974**, *A28*, 1175−1179.
[30] [30a] R. O. Hutchins, B. E. Maryanoff, M. J. Castillo, K. D. Hargrave, A. T. McPhail, *J. Am. Chem. Soc.* **1979**, *101*, 1600−1602. − [30b] R. O. Hutchins, B. E. Maryanoff, *J. Am. Chem. Soc.* **1972**, *94*, 3266−3268.
[31] F. G. Riddell, *The Conformational Analysis of Heterocyclic Compounds*, Academic Press, London, **1980**, pp. 123−125.
[32] E. Juaristi, *Introduction to Stereochemistry and Conformational Analysis*, Wiley, New York, **1991**, chapter 17.
[33] R. K. Gupta, A. R. Rai, R. C. Mehrotra, V. K. Jain, B. F. Hoskins, E. R. T. Tiekink, *Inorg. Chem.* **1985**, *24*, 3280−3284.
[34] [34a] E. Adams, D. Jeter, A. W. Cordes, J. W. Kolis, *Inorg. Chem.* **1990**, *29*, 1500−1503 − [34b] A. G. Osborne, R. E. Hollands, R. F. Bryan, S. Lockhart, *J. Organomet. Chem.* **1985**, *288*, 207−217 − [34c] G. Bergerhoff, H. Namgung, *Z. Kristallogr.* **1979**, *150*, 209−213 − [34d] A. W. Cordes, P. D. Gwinup, M. C. Malmstrom, *Inorg. Chem.* **1972**, *11*, 836−838.
[35] [35a] Holleman-Wiberg: *Lehrbuch der Anorganischen Chemie*, 101st ed., de Gruyter, Berlin, **1995**, p.1842. − [35b] *ibid.*, pp. 184, 370.
[36] J. D. Andose, A. Rauk, K. Mislow, *J. Am. Chem. Soc.* **1974**, *96*, 6904−6907, and literature cited therein.
[37] A. F. Wagner, E. Walton, G. E. Boxer, M. P. Pruss, F. W. Holly, K. Folkers, *J. Am. Chem. Soc.* **1956**, *78*, 5079−5081.
[38] For assignments of NMR spectra of dihydrolipoic acid and other lipoic acid derivatives see: [38a] V. Schepkin, T. Kawabata, H. J. Tritschler, L. Packer, *Free Rad. Res.* **1996**, *25*, 195−205. − [38b] J. V. Paukstelis, E. F. Byrne, T. P. O'Connor, T. E. Roche, *J. Org. Chem.* **1977**, *42*, 3941−3944.
[39] S. Samaan in *Methoden der Organischen Chemie (Houben-Weyl)*, 4th ed., vol. XIII/8, Thieme, Stuttgart, **1978**, p. 157.
[40] Tentative assignment. For comparison see: [40a] T. B. Brill, N. C. Campbell, *Inorg. Chem.* **1973**, *12*, 1884−1888 − [40b] K. Volka, P. Adámek, H. Schulze, H. J. Barber, *J. Mol. Struct.* **1974**, *21*, 457−460.
[41] G. M. Sheldrick, *SHELXTL PLUS*, Siemens Analytical X-ray Instruments Inc., Madison, WI, USA, **1990**.
[42] G. M. Sheldrick, *SHELXL-93, Program for Crystal Structure Refinement*, University of Göttingen, Germany, **1993**.

[97195]