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by NMR spectroscopy and X-ray diffraction**

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Originally published:

March 2022

Environmental Toxicology and Pharmacology 92(2022), 103837-103844

DOI: <https://doi.org/10.1016/j.etap.2022.103837>

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1 Phenylarsonic acid–DMPS redox reaction and conjugation investi-
2 gated by NMR spectroscopy and X-ray diffraction

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6 **Abstract**

7 The reaction between 2,3-dimercaptopropane-1-sulfonate (DMPS, unithiol) and four phenylar-
8 sonic(V) acids, *i.e.* phenylarsonic acid (PAA), 4-hydroxy-3-nitrophenylarsonic acid (HNPA),
9 2-aminophenylarsonic acid (*o*-APAA) and 4-aminophenylarsonic acid (*p*-APAA), is investi-
10 gated in aqueous solution. The pentavalent arsenic compounds are reduced by DMPS to their
11 trivalent analogs and instantly chelated by the *vicinal* dithiol, forming covalent As–S bonds
12 within a five-membered chelate ring. The different types and positions of polar substituents at
13 the aromatic ring of the arsonic acids influence the reaction rates in the same way as observed
14 for reaction with glutathione (GSH), as well as the *syn* : *anti* molar ratio of the diastereomeric
15 products, which was analyzed using time- and temperature-dependent nuclear magnetic reso-
16 nance (NMR) spectroscopy. Addition of DMPS to the conjugate formed by a phenylarsonic(V)
17 acid and the biologically relevant tripeptide GSH showed the immediate replacement of GSH
18 by chelating DMPS, underlining the importance of dithiols as detoxifying agent.

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22 **Keywords**

23 Arsenic; Unithiol; GSH; Molecular Structure; Kinetics; Toxicology.

24 **Abbreviations**

25 DMPS, 2,3-dimercaptopropane-1-sulfonate; GSH, glutathione, γ -L-glutamyl-L-cysteinyl-gly-
26 cine; PAA, phenylarsonic acid; HNPA, 4-hydroxy-3-nitrophenylarsonic acid; *o*-APAA, 2-
27 aminophenylarsonic acid; *p*-APAA, 4-aminophenylarsonic acid; BAL, British Anti-Lewisite,
28 2,3-dimercaptopropane-1-ol; PAO, phenylarsine oxide; DMSA, meso-2,3-dimercaptosuccinic
29 acid; PDA, phenyldichloroarsine; PDT, 1,2-dimercaptopropane; NMR, Nuclear Magnetic
30 Resonance; XRD, X-ray diffraction; NOESY, Nuclear Overhauser effect spectroscopy;
31 EXSY, exchange spectroscopy; COSY, correlation spectroscopy; HSQC, heteronuclear sin-
32 gle-quantum coherence; HMBC, heteronuclear multiple-bond correlation.

33

34 **1. Introduction**

35 Organoarsenic compounds play a crucial role in environmental contaminations caused by
36 residues of arsenic containing chemical warfare agents (Leermakers et al., 2006; Niemikoski
37 et al., 2020; Pitten et al., 1999; Stock, 1996; Stock and Lohs, 1997; Tørnes et al., 2006). Di-
38 phenylarsine chloride and cyanide, phenarsazine chloride, and phenylarsine dichloride were
39 produced in large scale during the First and Second World War (Chauhan et al., 2008; Stock,
40 1996). At former military sites hydrolysis and oxidation products contaminate soils and wa-
41 ters resulting in high arsenic levels (Pitten et al., 1999). Other phenylarsenic compounds, es-
42 pecially 4-hydroxy-3-nitrophenylarsonic acid (HNPA, roxarsone) and 4-aminophenylar-
43 sonic acid (*p*-APAA) have been used as growth promoters and veterinary drugs in poultry
44 and swine feed additives (Anderson, 1983; Czarnecki et al., 1984; D'Angelo et al., 2012;
45 Pergantis et al., 1997; Sun et al., 2002). Additionally, various inorganic and organic arseni-
46 cals have been used for centuries or are still used in human medicine to treat diseases such as
47 human trypanosomiasis (sleeping sickness), syphilis, or promyelocytic leukemia (Chen et al.,
48 2015; Dilda and Hogg, 2007; Miller Jr et al., 2002).

49 Arsenic metabolism is of paramount interest and hence subject of current and ongoing re-
50 search. Depending on speciation, tri- and pentavalent organic and inorganic arsenicals are as-
51 sociated with severe health effects, including the gastrointestinal as well as the central nerv-
52 ous system (Dringen et al., 2016; McCann and Maguire-Zeiss, 2021; Ray et al., 2020; Sattar
53 et al., 2016; Wisessaowapak et al., 2021).

54 Treatment of arsenic poisoning basically means the administration of *vicinal* dithiols
55 (Bjørklund et al., 2020) such as the British Anti-Lewisite (BAL), 2,3-dimercaptopropane-1-
56 ol, the archetype of arsenic antidotes. However, for BAL the disadvantages prevail: dreadful
57 stench, low water solubility affording for a painful deep intramuscular injection as well as se-
58 vere side-effects as it is toxic itself (Adams et al., 1990; Andersen, 1999). Hence, BAL was
59 replaced by more water soluble and less toxic chelating agents such as sodium 2,3-dimercap-
60 topropane-1-sulfonate (DMPS, unithiol) (Bjørklund et al., 2017; Lu et al., 2017; Nurchi et al.,
61 2020; Petrunkin, 1956) or meso-2,3-dimercaptosuccinic acid (DMSA), the latter primarily
62 developed for better antimony uptake in schistosomiasis treatment (Friedheim et al., 1954),
63 and later also for heavy metal chelation (Liang et al., 1980). Their efficacy in heavy metal
64 and metalloid detoxification has been widely proven and the underlying chemistry has been
65 investigated (Aaseth et al., 2018; Andersen, 2004; Blanusa et al., 2005; Cavanillas et al.,
66 2012; Gong et al., 2002; Guha Mazumder, 2003; Heinrich-Ramm et al., 2003; Kaviani et al.,
67 2019; Suzuki et al., 2012).

68 As early as in the 1930s Cohen and co-workers (Cohen et al., 1931a, b, 1932a, b) con-
69 ducted extensive studies regarding the chemistry and the concomitant therapeutic action of
70 various arylarsenic compounds. They report that variation in the efficacy of pentavalent ar-
71 senicals is (in part) due to different rates of As(V) reduction. However, no straightforward
72 correlation between redox potential and toxicity of the respective arsenicals could be found.
73 Contributions by Cullen and co-workers regarding both arsenic in the environment (Cullen
74 and Reimer, 1989) and mechanistic studies (Cullen et al., 1984a, b), the latter focusing on the
75 reaction of methylarsenicals with various thiols, considerably improved the understanding of
76 arsenic–thiol interactions. So far, the structure of (di)thiol conjugates of (substituted) phe-
77 nylarsenicals have only scarcely been investigated by NMR and XRD, among them the un-
78 substituted, trivalent phenylarsine oxide (PAO) together with its interchangeably used di-
79 chloro analog (Aksnes and Bjorøy, 1975; O'Connor et al., 1989), and the *p*-APAA conjugate

80 of 6,8-dimercaptooctanoic acid (von Döllén and Strasdeit, 1998). Very recent work on the in-
81 teraction of arsenous acid with BAL and the resulting products illustrate the ongoing interest
82 in and importance of understanding the chemistry behind these detoxification processes
83 (López-Moreno et al., 2018).

84 The related reaction of pentavalent phenylarsonic acids with (di)thiols is more complex as it
85 is, in general, a two-stage process. Initially, the thiol is oxidized while the pentavalent arseni-
86 cal is reduced to its trivalent analog and, subsequently, the latter is covalently bound to (re-
87 maining) thiol molecules (Cohen et al., 1931b; Cullen et al., 1984b; Kretzschmar et al.,
88 2014). Serum and intracellular thiols such as the tripeptide glutathione (GSH) are the first tar-
89 get molecules for incorporated arsenicals (Delnomdedieu et al., 1993; Doerge et al., 2020) to
90 react with *in vivo* and, hence, become reduced and conjugated. But for the success of the de-
91 toxification therapy the chelation by administered dithiols is crucial and therefore in this
92 work the combination of redox and conjugation reactions between DMPS and pentavalent
93 phenylarsenicals is investigated. For a better understanding of the thiol-arsenic reactions as
94 well as structural impacts on reactivity and stability, we performed fundamental research fo-
95 cusing on the chelation agent DMPS and four phenylarsonic(V) acids, possessing different
96 types and positions of polar substituents (Scheme 1), *i.e.*, phenylarsonic acid (PAA), 4-hy-
97 droxy-3-nitrophenylarsonic acid (HNPA), 2-aminophenylarsonic acid (*o*-APAA) and 4-
98 aminophenylarsonic acid (*p*-APAA). This comprises the elucidation of both the solution and
99 the crystal structures of trivalent conjugates formed by the reaction of *RS*-DMPS with the
100 pentavalent organoarsenic compounds by one- and two-dimensional NMR spectroscopic
101 methods and single-crystal X-ray diffraction. Furthermore, the dynamic behavior regarding
102 the interconversion of the formed diastereomers, and the replacement of GSH by DMPS are
103 investigated.

104

105 **2. Materials and methods**

106 **2.1 Materials**

107 *Caution!* The arsenic compounds used in this study are toxic and should be handled with care.
108 All arsenic compounds used were of analytical grade and purchased from Acros Organics.
109 Glutathione (>99%, Sigma-Aldrich) and *RS*-DMPS (95%, Alfa-Aesar) were used as obtained
110 without further purification to prepare stock solutions in deuterium oxide (D₂O, 99.98%, Armar

111 Chemicals) for NMR experiments and in H₂O for single crystal preparation. For pH adjustment
112 and pH dependent measurements, diluted deuteriochloric acid (DCl) made from concentrated
113 DCl (35 wt.-% in D₂O, 99 atom-% D, Sigma-Aldrich) or diluted sodium deuterioxide (NaOD)
114 made from concentrated NaOD (40 wt.-% in D₂O, 99 atom-% D, Sigma-Aldrich) in D₂O were
115 added, respectively. The use of buffers was avoided; see considerations stated with Fig. S13).

116

117 **2.2 Sample preparation for reaction monitoring**

118 The samples were degassed to prevent autoxidation by dissolved oxygen and used immedi-
119 ately after preparation. Reaction samples were prepared by diluting appropriate volumes of
120 stock solutions to obtain reaction mixtures of 5 mM arsenical(V) and 15 mM DMPS. pH was
121 adjusted to 6.0 ± 0.5 with pH meter reading uncorrected for deuterium, and was stable within
122 ± 0.2 units during the reaction. This pH was chosen according to species distribution calcula-
123 tions performed for mixtures of the arylarsenic compounds with GSH or DMPS (Figs. S14 and
124 S15), showing that at pH 6.0 ± 0.5 speciation undergoes only minor changes with pH.

125

126 **2.3 NMR spectroscopy**

127 All experiments were performed on a Bruker Avance III 500 spectrometer with a magnetic
128 field of 11.75 T corresponding to resonance frequencies of 500.13 and 125.76 MHz for ¹H and
129 ¹³C, respectively, using a 5 mm direct detection probe with z-gradient. For structure elucidation,
130 2D-NMR, *i.e.* H,H-COSY, H,H-NOESY, H,C-HSQC and H,C-HMBC spectra were recorded.
131 All spectra were referenced relative to 3-(trimethylsilyl)-propionic acid sodium salt
132 (TSP, 98%, Sigma-Aldrich) with $\delta_{\text{H}} = 0$ ppm and $\delta_{\text{C}} = 0$ ppm. All measurements were carried
133 out at (22 ± 0.1) °C and in particular cases additionally at 5 °C to reduce the reaction rate, with
134 temperature stability achieved by the spectrometer's temperature control combined with a
135 BCU05 cooling unit (Bruker).

136 NMR signal assignment was carried out by means of H,H- and H,C-correlation spectra (*cf.*
137 Supporting Information), scalar H,H coupling constants (*vide supra*) as well as integration of
138 ¹H signal areas in order to discriminate the *syn* and the *anti* products' signals.

139

140 **2.3 X-ray crystallography**

141 In order to obtain single-crystals, aqueous solutions containing 20 mM of the phenylar-
142 senic(V) acids and 80 mM of DMPS were allowed to react at room temperature in a desiccator
143 over silica gel.

144 Crystals suitable for X-ray diffraction were selected under a microscope with polarized light
145 and mounted on a glass capillary with a thin film of silicon grease. The data sets were collected
146 on a IPDS-2T diffractometer (Stoe) using Mo K α radiation ($\lambda = 0.71073 \text{ \AA}$) at 150 K. The
147 structures were solved by direct methods with SHELXS and all non-hydrogen atoms were an-
148 isotropically refined in full-matrix least-squares cycles against $|F^2|$ with SHELXL-2018/3
149 (Sheldrick, 2008; Sheldrick). Carbon-bound hydrogen atoms were placed in idealized positions
150 and refined isotropically. In particular cases, *i.e.*, NH groups, the hydrogen coordinates were
151 obtained directly from differential-Fourier-syntheses. Graphics of the molecular structures
152 were generated with ORTEP32 (Farrugia, 1997) and POV-RAY (Persistence of Vision Pty.
153 Ltd. Persistence of Vision Raytracer (Version 3.6). Retrieved from
154 <http://www.povray.org/download/>, 2004).

155 Crystallographic data for the structures reported in this paper have been deposited with the
156 Cambridge Crystallographic Data Centre under CCDC numbers 1019299, 1019300, and
157 1019298 for PAA–DMPS, HNPA–DMPS, and *p*-APAA–DMPS, respectively. Copies of the
158 data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge, CB2
159 1EZ, UK (Fax: + 44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

160

161 **2.4 Elemental analyses**

162 For the elemental analyses the obtained crystalline products were used and carried out with
163 a Vario Micro Cube analyzer (Elementar, Hanau, Germany).

164

165 **2.5 Redox potentials**

166 Aqueous solutions of the reactants without further pH-adjustment were used to determine
167 half-cell potentials by means of high ohmic multimeter reading of the potential difference be-
168 tween two chambers, one containing 5 mM arsenical(V) and 15 mM together with a Pt working
169 electrode, and the other containing a saturated KCl solution together with a Ag/AgCl electrode
170 as reference, both connected by a glass tube salt bridge filled with 1 M NH₄NO₃ solution.

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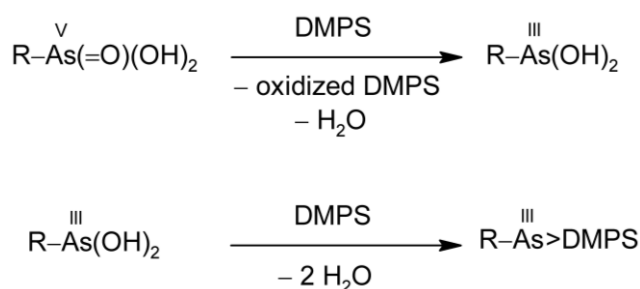
172 **3. Results and Discussion**

173

174 For all investigated phenylarsonic(V) acids the formation of trivalent phenylarsenic–DMPS
175 conjugates, comprising five-membered chelate rings, alongside with oxidized DMPS di- and
176 oligomers were obtained (Scheme 1). Since the chelating agent DMPS was used in its racemic

177 form, formation of product compounds with four different configurations is expected, that is
 178 (*R,R*) and (*S,S*) as well as (*R,S*) and (*S,R*) isomers. The trivalent arsenic atom and one DMPS
 179 carbon (C8 in Scheme 2) are chiral, resulting in two diastereomeric pairs of enantiomers
 180 (Fig. S1). For simplicity, in the following the isomers will be denoted as the *syn* and the *anti*
 181 products (Scheme 2), as we do not differentiate between their enantiomers. These terms (*syn*
 182 and *anti*) consider the orientation of the arsenic bound aryl residue relative to the DMPS CH₂-
 183 SO₃⁻ residue within the conjugate.

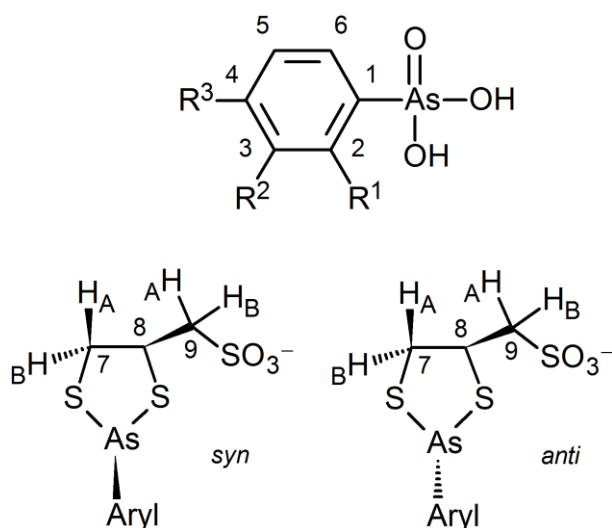
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186 **Scheme 1.** Schematic reaction of pentavalent phenylarsonic acids with DMPS: redox reaction
 187 (upper trace) and conjugation (lower trace); R denoting (substituted) phenyl residues.

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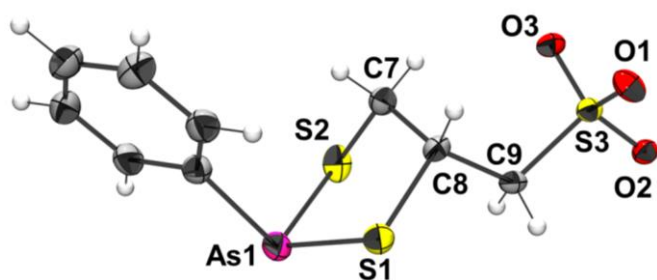
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190 **Scheme 2.** Generic structures and labeling of the investigated pentavalent phenylarsonic acids
 191 (top) and the stereoisomers of their trivalent DMPS conjugates (bottom). R¹, R², R³ = H: phe-
 192 nylarsonic acid (PAA); R¹ = NH₂, R², R³ = H: 2-aminophenylarsonic acid (*o*-APAA); R¹, R² =
 193 H, R³ = NH₂: 4-aminophenylarsonic acid (*p*-APAA); R¹ = H, R² = NO₂, R³ = OH: 4-hydroxy-
 194 3-nitrophenylarsonic acid (HNPAAs); A and B denoting diastereotopic H atoms.

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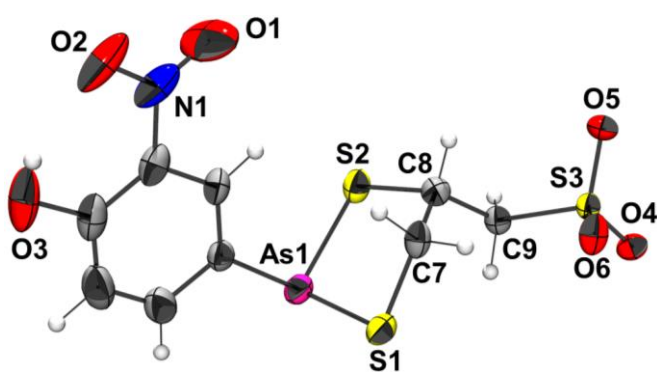
3.1 Crystal structures of phenylarsenic(III)–DMPS conjugates

In the course of our study we succeeded in growing crystals of the DMPS conjugates of three phenylarsenicals out of the four under investigation. Crystallographic parameters of data collection and structure refinement for the crystal structures of conjugates of DMPS with PAA, HNPA, and *p*-APAA are summarized in Table S1. Whereas the arsadithiolanes obtained from PAA (Fig. 1) and from HNPA (Fig. 2) crystallized as sodium salts, the corresponding product obtained from *p*-APAA (Fig. 3) crystallized as a zwitterionic compound with the protonated *p*-amino group serving as the cationic component. In contrast to the DMPS conjugates obtained from PAA and HNPA, which crystallize as the *anti* isomers in high purity (which will be made use of for purification of the *anti* isomer and its utilization in isomerization studies, *vide infra*), the product obtained from *p*-APAA crystallized as a mixture of enantiomers of the *anti* product and an enantiomer of the *syn* product on the same site of the asymmetric unit in a disordered manner. As the HNPA–DMPS conjugate also exhibits disorders in the solid state structure (in this case the phenyl groups are disordered), only one of the three crystal structures is basically free of severe disorders, and therefore we cannot discuss substituent effects on bond lengths in a comparative way. A structural feature of greater importance for the further discussion, *i.e.* characterization in solution and the effects of $^3J(\text{H,H})$ coupling, however, can be extracted even from the disordered structures. That is, the H-C(8)-C(7)-H torsion angles of the *anti* products were found in the range 61–71° (for the *exo* H atom at C7) and 47–58° (for the *endo* H atom at C7). In the *syn* product, which is contained in the structure of the *p*-APAA–DMPS conjugate (Fig. 3, bottom) there is a significantly larger difference between the torsion angles, *i.e.*, 179° for the *endo* H atom at C7 and 58° for the *exo* H atom. Even though the hydrogen atoms in the crystal structures had been placed in idealized positions, the H-C-C-H torsion angles should be representative of notable differences between the *anti* and *syn* isomers. Although two out of the three crystal structures suffer disorder effects, it appears that for all conjugates the two As–S bonds within one molecule show systematically different bond lengths. In fact, the bonds between the arsenic and that sulfur adjacent to the chiral carbon atom are shorter by ca. 0.01–0.03 Å. This is similar to the situation in the lipoic acid conjugate of PAO (0.03 Å)³⁵ but in contrast to the BAL adduct of tolyldichloroarsine with the latter bond being longer by 0.05 Å (Adams et al., 1990).



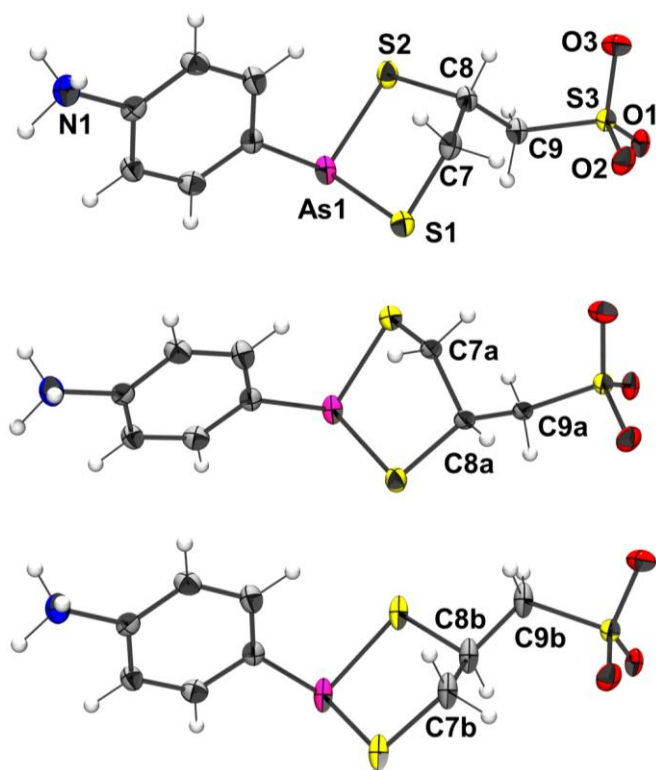
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229 **Fig. 1.** Molecular structure of the anion of the PAA–DMPS conjugate sodium salt $\text{Na}_2(\text{PAA-}$
 230 $\text{DMPS})_2(\text{H}_2\text{O})_3$ in the crystal (one of the two crystallographically independent anions is shown,
 231 ellipsoids set at 50% probability).



232

233 **Fig. 2.** Molecular structure of the anion of the HNPAAs–DMPS conjugate sodium salt
 234 $\text{Na}_2(\text{HNPAAs-DMPS})_2(\text{H}_2\text{O})_3$ in the crystal (one of the two crystallographically independent
 235 anions is shown, ellipsoids set at 50% probability).



236

237 **Fig. 3.** Molecular structure of the *p*-APAA–DMPS conjugate in the crystal (ellipsoids set at
 238 50% probability). Two enantiomers of the *anti*-product (top and middle) and one enantiomer
 239 of the *syn*-product (bottom) occupy the same site in approximate ratio 8:1:1. Whereas the po-
 240 sition of the aryl group is identical for the three isomers, the arsacyclic part of the molecules
 241 (incl. the sulfonate group) occupies alternative positions.

242

243 3.2 Solution structures of phenylarsenic(III)–DMPS conjugates

244

245 Comprehensive ^1H and ^{13}C NMR data of the obtained conjugates as well as representative
 246 two-dimensional NMR spectra is given as Supplementary material. Coupling constants (J) and
 247 chemical shifts (δ) can be used to assign the stereoisomers as follows.

248 The protons of the arsadithiolane ring of the *syn* products are, in general, more shielded (show
 249 smaller δ) than the corresponding protons in the *anti* products. Particularly H7A and H8 in the
 250 *o*-APAA–DMPS *syn* conjugate are shielded by 0.14 and 0.31 ppm, respectively. This can be
 251 attributed to an increased steric demand in the *syn* configuration. Since the C7 methylene group
 252 is structurally fixed in the five-membered ring, owing to their diastereotopicity the protons in
 253 both the *anti* and *syn* product show *geminal* coupling constants $^2J(7A,7B)$ of ≈ 13 Hz, and sig-
 254 nificant δ_{H} differences.

255 The C9 methylene protons in the *syn* products are virtually equivalent, with the same δ_{H} and
256 no 2J observable. The *vicinal* coupling constants (3J) show values of ≈ 6.7 Hz, which is com-
257 parable to averaged values in alkyl chains. In the *anti* products $^2J(\text{H9A,B})$ were determined to
258 be 14.4 Hz. According to the Karplus relationship (Karplus, 1963), with $^3J(\text{H8,H9A})$ and
259 $^3J(\text{H8,H9B})$ being 8.3 and 5.3 Hz, the couplings exhibit an angle-dependence attributed to *trans*
260 and *gauche* patterns, respectively, suggesting a reduced flexibility of the $\text{CH}_2\text{-SO}_3^-$ residue
261 (rotation about the C8–C9 axis is slow on the NMR time scale).

262 Whereas $^3J(\text{H8,H7A})$ and $^3J(\text{H8,H7B})$ in the *anti* products are equal (HNPAAs: 4.1 Hz, *p*-
263 APAA: 4.4 Hz) or at least very similar (*o*-APAA: 4.3 and 3.8 Hz), the *syn* products exhibit
264 remarkable differences (9.2 vs. 3.9, 9.0 vs. 3.9, and 9.7 vs. 4.1 Hz for HNPAAs, *p*-APAA, and
265 *o*-APAA, respectively). This is in total agreement with observations in the crystal structures
266 (*vide supra*). They reveal only small differences in the torsion angles between H8 and the *endo*
267 and *exo* H7 for the *anti* products (corresponding to similar 3J), but reveal large differences and
268 thus varying 3J values in the *syn* products. Consequently, H7A can be denoted as the *endo* and
269 H7B as the *exo* hydrogen. This assignment is corroborated by NOESY (Fig. S6), showing di-
270 polar interaction between H2 (*ortho*) and H7A but not H7B.

271 For the trivalent arsenic–DMPS conjugates the ^{13}C NMR signals are shifted downfield
272 (larger δ_{C}) by about 1.3 ppm (C9), 1.5 ppm (C8), and 1.1 ppm (C7) compared to free DMPS.
273 The most profound structural and hence spectral changes occur at the asymmetric carbon, C8
274 and, to some extent, for those in their direct vicinity. $\Delta\delta_{\text{C}}$ between *syn* and *anti* products are
275 -0.97 , -0.93 , and -0.85 ppm (C9), 2.21, 2.19, and 2.07 ppm (C8), and 0.40, 0.39, and 0.28 ppm
276 (C7) for the conjugates of HNPAAs, *o*-APAA, and *p*-APAA, respectively. Even the aryl carbons
277 are sensitive to configuration. Significant $\Delta\delta_{\text{C}}$ can be found in the HNPAAs conjugate for C3
278 (0.63 ppm), in the *p*-APAA conjugate for C2 (-0.84 ppm) and C3 (0.35 ppm) as well as in the
279 *o*-APAA conjugate for C1 (0.58 ppm) and C6 (0.18 ppm).

280

281 3.3 Monitoring the phenylarsenic(V)–DMPS reaction

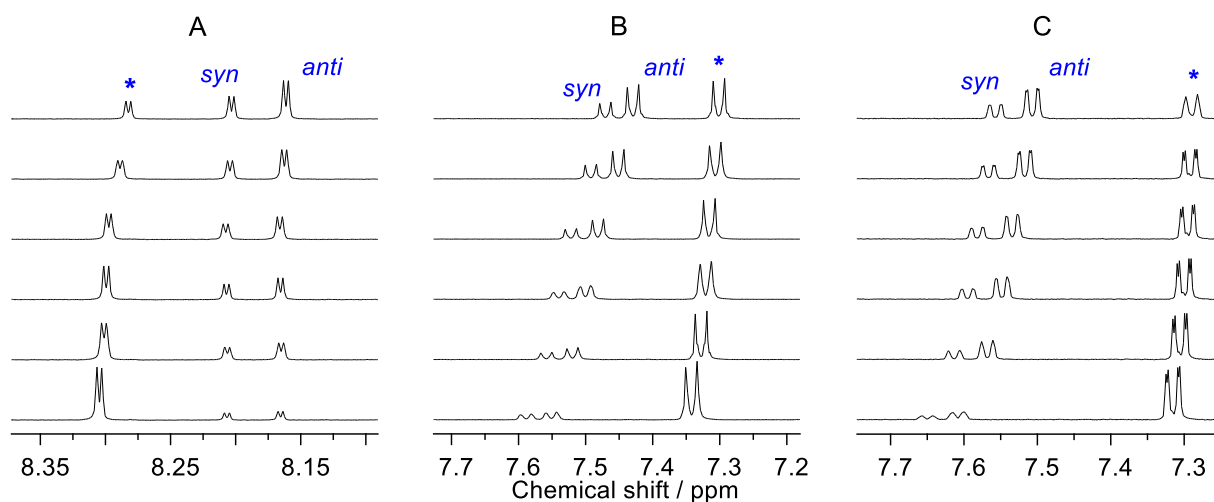
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283 By simply mixing aqueous solutions of both DMPS and phenylarsonic(V) acids at room
284 temperature the reaction starts instantly.

285 The reaction progress is slow enough to be monitored by ^1H NMR at room temperature.
286 Fig. 4 shows exemplary time-dependent spectra of DMPS systems of HNPAAs as well as *o*-
287 and *p*-APAA. In the course of the reaction the pentavalent arsenicals' signals (indicated by

288 asterisks) decrease while the two sets of signals for the *syn* and *anti* isomers appear and
 289 increase. According to the reaction of pentavalent methyl- and arylarsenic compounds as well as
 290 inorganic arsenate with several other thiols (Cullen et al., 1984b; Delnomdedieu et al., 1993;
 291 Kretzschmar et al., 2014) the reaction occurs *via* two reaction steps: (1) redox reaction with
 292 oxidation of DMPS and concomitant reduction of the pentavalent arylarsenic acid to its triva-
 293 lent analog (arylarsonite), and (2) the subsequent chelation of the latter by further (free) DMPS,
 294 *cf.* Scheme 1. Since the trivalent analog R–As(OH)₂ cannot be observed by ¹H NMR (Cullen
 295 et al., 1984a; Kretzschmar et al., 2014), the second reaction step is considered to be signifi-
 296 cantly faster and the redox reaction to be rate determining. For further information on the nature
 297 of the oxidized DMPS see Fig. S7 and Scheme S2.

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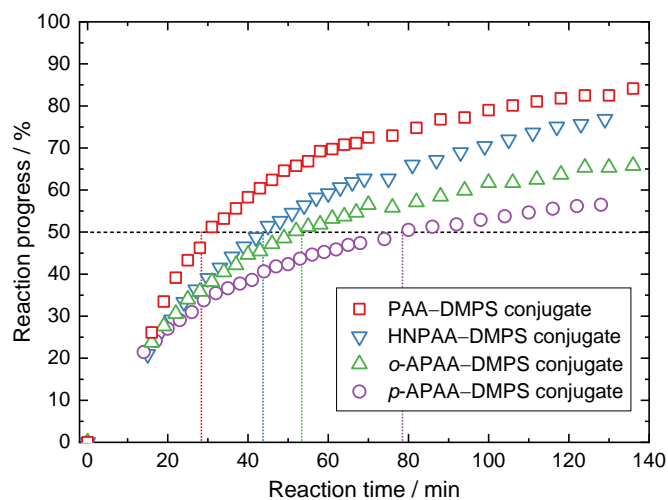


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300

301 **Fig. 4.** Section of the aromatic *ortho* ¹H signals of time-dependent ¹H NMR spectra for DMPS
 302 systems of HNpAA (A), *p*-APAA (B), and *o*-APAA (C). Signals of the formed trivalent dia-
 303 stereomeric conjugates are denoted *syn* and *anti*, and those of the pentavalent precursors are
 304 indicated by asterisks. From bottom to top: 15, 30, 45, 60, 90, and 120 min after mixing. Signals
 305 of the aliphatic protons are shown in Figs. S8–S11.

306 As already observed by Cullen et al. (1984b) for the reaction of trimethylarsine with mercap-
 307 toethanol, but in contrast to our own results (Kretzschmar et al., 2014) for the reaction of pen-
 308 tavalent phenylarsenic compounds with GSH, the obtained curves did not fit any simple rate
 309 expression. This is likely due to the variety of possible di- and oligomeric oxidation products
 310 of DMPS (Fig. S7 and Scheme S2) involved in the reaction equilibrium. In order to evaluate
 311 the reaction progress and allowing for comparison between the systems, the half-lives (*t*_{1/2}),

312 indicating that point of time when 50% of the initial As(V) is converted to As(III), were deter-
313 mined. Therefore, in the time-dependent spectra (Fig. 4) the phenyl signals of both the As(V)
314 reactant and the As(III) conjugate were integrated and the obtained molar fractions plotted
315 *versus* time, giving $t_{1/2}$ as the intercept of both curves (Scheme S1).
316



317
318 **Fig. 5.** Time-dependent formation of As(III)-DMPS conjugates of different phenylarsonic(V)
319 acids for pH 6 solutions initially 5 mM in the arsenic(V) component and 15 mM in DPMS at
320 5 °C. The reaction progress is expressed in terms of reacted arsenic(V) starting material as
321 determined from mole fractions obtained from NMR signal integration. Colored dotted lines
322 indicate the respective half-lives.

323
324 For a convenient and reliable observation of the reaction progress, the sample series were
325 measured at 3 : 1 molar ratio (DMPS : As) and at 5 °C, see Figs. 5 and S16. The obtained $t_{1/2}$
326 values are 29, 43, 53, and 79 minutes for PAA, HNPAAs, *o*-APAA, and *p*-APAA, respectively,
327 at pH = 6 ± 0.5. The relative order of $t_{1/2}$ values of the DMPS reactions is similar to that of
328 kinetic investigations of these arylarsenicals with GSH (Kretzschmar et al., 2014). In principle,
329 at comparable conditions the reaction of phenylarsonic(V) acids is considerably faster with
330 DMPS than with GSH, with $t_{1/2}$ values being 2-3 times shorter than for the GSH reactions.

331 Although not investigated in detail the reaction monitoring at different pH showed that in
332 solutions of DMPS and HNPAAs at pH 1, the latter is quantitatively converted within 90
333 minutes, whereas at pH 10 no reaction can be observed even after 8 days. According to the pK_a
334 values of the reactants (Arnold et al., 1985; Huckerby et al., 1985; Nualláin and Cinnéide, 1973;
335 Roerdink and Aldstadt, 2005) (*cf.* Tables S2-S3 and Figs. S14-S15), the reaction kinetics are
336 mainly attributed to the arsenicals' speciation. The amino derivatives (*o*- and *p*- APAA) are

337 able to form cations at very low pH by protonation of the amino group. At $1 < \text{pH} \leq 3.5$ –4
338 (depending on the individual arsenical), but prior to the deprotonation of the As-bound OH
339 groups, the latter exist as neutral molecules. Upon increasing the pH, in general at the latest at
340 pH 4.5, only negatively charged arsenic(V) species exist. Owing to the deprotonation of the
341 SO_3H group, DMPS is considered to be negatively charged even at low pH values. Deprotona-
342 tion of the SH groups starts above pH 8. Consequently, the lower the pH, the faster the reaction,
343 since the total number of negative charges and, hence, repulsion is decreased. A similar behav-
344 ior and interpretation was recently reported for the reaction of arsenite and methylarsenicals
345 with GSH by Doerge et al. (2020) and, earlier, it was already suggested for the reaction of
346 methylarsenicals with (di)thiols that the reaction involves the unionized thiol group (Cullen et
347 al., 1984a, b). Accordingly, the pH-dependent rate differences are primarily impacted by spe-
348 ciation of the arsenicals.

349 Additionally, the relative order of the reaction rates is apparently influenced by the half-cell
350 potentials of the reactants (Table S4). It has to be considered that these potentials are also pH-
351 dependent. Although the reduction ability of DMPS increases with increasing pH, the specia-
352 tion (anionic nature of both reactants) hampers the interaction due to electrostatic repulsion at
353 higher pH values.

354

355 **3.4 Interconversion between *anti* and *syn* isomer**

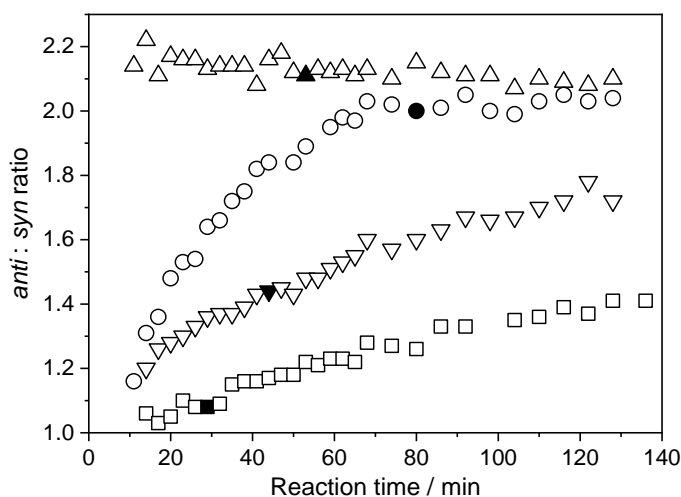
356

357 Considering the *anti* : *syn* ratio, literature reveals a dependence on the nature of the chelator
358 only (Aksnes and Holak, 1982; Dill et al., 1987; Dill et al., 1991; O'Connor et al., 1989). For
359 the reaction of DMPS with both the *trans*-2-chlorovinylarsine oxide (Lewisite oxide) and phe-
360 nyldichloroarsine (PDA, trivalent analog of PAA) O'Connor et al. (1989) found an *anti* : *syn*
361 ratio of 2.0 whereas the reaction with BAL (OH instead of the SO_3^- group) yields a ratio ≥ 4 ,
362 whereupon they state that the organic group on the arsenical does not appear to affect the ratio
363 appreciably.

364 In the systems investigated here it appeared that the *anti* : *syn* ratio depends on both the re-
365 action time and the nature of the arsenic-bound aryl residue. The *anti* : *syn* ratio increases with
366 time for PAA, HNPAA, and *p*-APAA up to an equilibrium value of 2.0 ± 0.1 , whereas in the
367 case of *o*-APAA the *anti* : *syn* ratio amounts ≈ 2.2 already at the beginning and decreases
368 slightly with time (Fig. 5). Dill et al. (1991) investigated the dynamics of the adduct yielded by
369 the reaction of PDA with 1,2-dithiopropene (PDT, forming an analogous five-membered ar-

370 sadithiolane ring with a methyl group instead of the methylenesulfonate as in DMPS) and de-
371 termined the temperature-dependent rates of interconversion between the isomers. Accord-
372 ingly, by increasing the temperature from 288 to 310 K the *anti*→*syn* conversion, k_1 , and the
373 *syn*→*anti* conversion, k_2 , increase from 0.55 to 2.93 and from 1.30 to 6.68 1/s, respectively,
374 corresponding to very similar activation energies of 57.2 and 55.9 kJ/mol, respectively. Addi-
375 tionally, they found the conversion rate to be dependent on the adduct concentration, but not
376 on that of the ligand though ascribing the interconversion to a ligand exchange reaction.

377 As seen from the presented NMR spectra, and qualitatively shown by exchange spectroscopy
378 (EXSY, Fig. S12) the interconversion rate is slow on the NMR time scale, hence allowing for
379 the observation of two sets of well-resolved signals, one for each isomer, which is in full agree-
380 ment with the small k values reported by Dill et al. (1991). Regarding their PDA–PDT adduct,
381 calculation of the $k_2 : k_1$ ratio yields 2.3 which is equal to the *anti* : *syn* ratio, since $k_2 : k_1$ is not
382 determined by the magnitude of the activation energies (of either conversion step) but their
383 difference, ΔE_a . The *anti* : *syn* ratios obtained in our experiments suggest ΔE_a values of
384 ≈ 1 kJ/mol, thus being very similar to ΔE_a of 1.3 kJ/mol found by Dill et al. (1991). Further-
385 more, for the interconversion between the PDA–1,3-dimercaptopropane-2-ol adduct isomers
386 (forming a six-membered ring) the activation energies of 42.9 and 37 kJ/mol yield ΔE_a of
387 5.9 kJ/mol, corresponding to $k_2 : k_1 = 4$ equivalent to an *anti* : *syn* ratio of 4 (Dill et al., 1991).
388



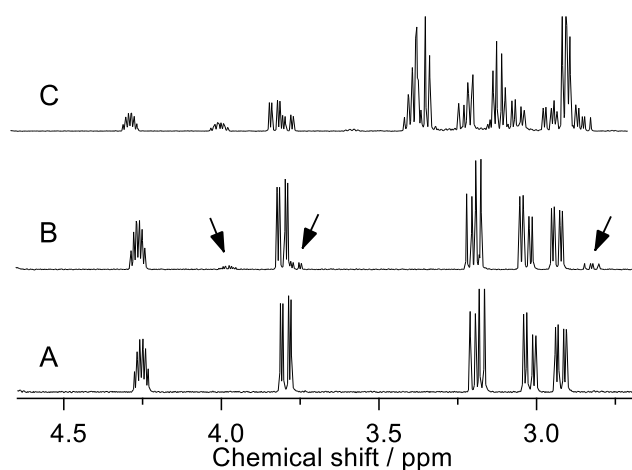
389
390 **Fig. 6.** Time-dependent *anti* : *syn* ratios of DMPS conjugates of PAA (□), HNPPAA (▽), *p*-
391 APAA (○), and *o*-APAA (△). Filled symbols indicate $t_{1/2}$.

392 At the beginning, the redox reaction determines the overall reaction behavior. Once reduced,
393 for the reaction (conjugation) of racemic DMPS and (prochiral) As(III) the sterics impact the

394 kinetics of the diastereomer formation, favoring the *anti* product after equilibration. As can be
395 derived from Fig. 6, two different types of rate constants have to be considered – one for the
396 conjugate formation and one for the interconversion. Each type exhibits individual rates for
397 either diastereomer, whereas the former are, in principle, higher than the latter. Consequently,
398 the *ortho*-position of the amino group in *o*-APAA impacts the isomeric ratio already during the
399 conjugate formation, being attributed to a strong steric influence of the amino group. It can be
400 further concluded that the energy barriers for the interconversion are high, since no significant
401 ratio changes occur. In the case of PAA, with the smallest steric hindrance, the ratio increases
402 slowly with time, indicating only narrow differences in the energy barriers with the equilibrium
403 being more depending on the interconversion rate rather than individual formation rates.

404 In contrast to the observations of Dill *et al.* we found a strong dependence of the ligand
405 concentration on the interconversion rates. Moreover, by re-dissolution of a single-crystal con-
406 taining the *anti* isomer only, Fig. 7, even 10 days later we found the *syn* isomer to be less than
407 7 % of the total amount of conjugate, implying a very small interconversion rate. However, 12
408 hours after addition of two equivalents of free DMPS to this solution we observed the usual
409 *anti* : *syn* ratio of 2.0 ± 0.1 . We therefore conclude that free DMPS accelerates remarkably the
410 interconversion of the *anti* and *syn* products, according to a bimolecular reaction (*cf.* Scheme
411 S3).

412



413

414

415 **Fig. 7.** Aliphatic region of ^1H NMR spectra of a single-crystal obtained from the reaction of
416 PAA with DMPS immediately (A) and 10 days (B) after re-dissolution in D_2O , and 12 hours
417 after addition of two equivalents of DMPS (C). The arrows indicate the appearing signals as
418 compared to the bottom trace.

419

420 3.5 Replacement of the monothiol GSH by chelating dithiol DMPS

421

422 Since GSH is an important intracellular reducing and detoxification agent for heavy metals
423 and metalloids, it is likely that, *e.g.*, incorporated arsenic is bound primarily to GSH. Addition
424 of DMPS to a solution containing the conjugate of, for instance, HNPAA and GSH, revealed
425 an instant replacement of the monothiol from the trivalent arsenic and a quantitative chelation
426 of the latter by DMPS (see Fig. S17 for ^1H spectra and generic structures). Similar behavior
427 has been observed by Delnomdedieu et al. (1993) for arsenate (AsO_4^{3-}). Both the yield and the
428 rate of this reaction are remarkable, the former being attributed to the stability of these com-
429 pounds, and the latter confirming the redox reaction (upper trace in Scheme 1) to be rate deter-
430 mining. Since HNPAA was already reduced (and conjugated) by GSH to an arsenic(III) com-
431 pound, DMPS does not need to undergo the significantly slower redox reaction, but is able to
432 act instantaneously as the chelator. Promptly after the admixture of DMPS the *anti* : *syn* ratio
433 is 1 : 1, whereas 12 hours after addition of free DMPS an *anti* : *syn* ratio of 2 : 1 was observed.
434 These results are comparable to those of the reaction monitoring. Prior to the *anti*–*syn* inter-
435 conversion, the 1 : 1 ratio of these isomers is due to the use of racemic DMPS with equal prob-
436 ability of the formation of either isomer. Subsequently, with increasing time, interconversion
437 proceeds and converges to the usual ratio favoring the *anti* isomer.

438

439 4. Conclusion

440

441 By means of both the ^1H NMR signals of oxidized DMPS and the trigonal pyramidal coor-
442 dination geometry of the conjugates' arsenic atom in the crystal structures, it can be unambig-
443 uously proven that DMPS reduces the studied arsenic(V) compounds to their arsenic(III) ana-
444 logs in addition to the conjugation as bidentate chelating agent. Particularly the dependence of
445 the *vicinal* coupling constant on the torsion angle allows a good perception of the solution
446 structures which enables the assignment of the products and the comparability with the solid
447 structures.

448 Among the investigated phenylarsonic acids, the presence and nature of the substituents, in-
449 cluding OH, NO_2 , and NH_2 (in *ortho* or *para* position) impacts not only the arrangement within
450 the crystalline products but in particular the reaction behavior. The latter is illustrated by the

451 similar succession of $t_{1/2}$ values for GSH (Kretzschmar et al., 2014) and DMPS conjugate for-
452 mation depending on the individual phenyl residues. Analogous to the correlation between tox-
453 icity of organoarsenic(V) compounds and reaction rate for their GSH conjugation – the latter
454 occurring *in vivo* for arsenic detoxification –, the reaction of DMPS again is fastest for the most
455 toxic among the phenylarsonic(V) acids studied. Based on comparison of determined half-lives
456 and velocities, the overall reaction rates further depend (beside temperature and concentration)
457 on pH, the latter influencing both the reduction potentials and the speciation of the reactants.
458 Since electrostatic repulsion between the reactants is able to prevent the reaction, the pH-de-
459 pendent speciation has to be considered as a prerequisite to initiate the reaction, together with
460 sufficient differences in the redox potentials. Protonated thiol groups are favorable for the re-
461 action progress and because they are stable beyond the physiological pH for DMPS this under-
462 lines its relevance for detoxification.

463 As for other As(V)–thiol systems, the redox reaction is the rate determining step. Signifi-
464 cantly faster is the promptly occurring chelation step once the arsenic is reduced to its trivalent
465 form. This could be proven additionally by the immediate replacement of the intracellular
466 tripeptide GSH bound to arsenic, emphasizing the suitability of DMPS in arsenic detoxifica-
467 tion. Conjugates of DMPS derivatives are thus much more stable than those of GSH, which is
468 supported by the 2-3 times smaller magnitude of $t_{1/2}$ values for the reaction of DMPS with
469 arylarsenic(V) compounds compared to GSH. The ability to reduce As(V) together with the
470 fast reaction as well as the high stability of the yielded products gives a better understanding
471 why the use of DMPS is conceivable in arsenic detoxification and for replacement, but also for
472 the protection of thiol containing molecules, beyond biological relevance.

473 Although the *anti* : *syn* ratio of the isomers formed is mainly influenced by the dithiol rather
474 than the aryl residue, showing values of 2.0 ± 0.1 in equilibrium, the sterics exert influence on
475 the ratio particularly at the beginning of the reaction progress. The difference in energy barriers
476 for the conversion of the isomers was estimated to be less than 2 kJ/mol, whereas the energy
477 barriers themselves are assumed to be in the order of 60 kJ/mol. Furthermore, the interconver-
478 sion is dependent on the concentration of free ligand, implying a bimolecular mechanism. To-
479 gether with the solutions' isomeric ratio, the crystal structures reveal that the *anti* isomer crys-
480 tallises preferably, either due to higher solubility of the *syn* isomer or as a result of preferred
481 seed crystal formation of the *anti* isomer (which is present in higher concentration) and contin-
482 uous transformation of *syn* into *anti* isomer in the course of crystallization.

483

484 **Declaration of Competing Interest**

485 The authors report no declarations of interest.

486 **Appendix A. Supplementary data**

487 Supplementary material related to this article (Generic stereostructures; comprehensive NMR
488 spectroscopic characterization of the compounds investigated, crystallographic information
489 files (cif); NMR spectra showing the replacement of GSH from its roxarsone conjugate by
490 DMPS; schemes visualizing the half-life determination, and the bimolecular reaction of
491 *syn/anti* interconversion; pK_a values of the arsenicals investigated; half-cell potentials of the
492 reactants; species distribution calculations) can be found, in the online version, at doi:
493 <https://doi.org...>

494 **CRedit authorship contribution statement**

495 The manuscript was written through contributions of all authors. All authors have given ap-
496 proval to the final version of the manuscript.

497 **Acknowledgement**

498 We thank Regina Hüttl, Institute of Physical Chemistry, TU Bergakademie Freiberg, for her
499 support with the redox potential measurements, Philipp Rathsack and Klara Sander, Institute
500 of Analytical Chemistry, TU Bergakademie Freiberg, for performing the species distribution
501 calculations and for the support in the literature search, respectively.

502

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