Deutsche Ausgabe: DOI: 10.1002/ange.201504413
Internationale Ausgabe: DOI: 10.1002/anie.201504413

Chemical Detoxification of Organomercurials**

*Mainak Banerjee, Ramesh Karri, Kuber Singh Rawat, Karthick Muthuvel, Biswarup Pathak, and Gouriprasanna Roy**

Abstract: *Organomercurials including methylmercury are ubiquitous environmental pollutants and highly toxic to humans. Now it could be shown that N-methylimidazole based thiones/selones having an N-CH_2CH_2OH substituent are remarkably effective in detoxifying various organomercurials to produce less toxic HgE (E = S, Se) nanoparticles. Compounds lacking the N-CH_2CH_2OH substituent failed to produce HgE nanoparticles upon treatment with organomercurials, suggesting that this moiety plays a crucial role in the detoxification by facilitating the desulfurization and deselenization processes. This novel way of detoxifying organomercurials may lead to the discovery of new compounds to treat patients suffering from methylmercury poisoning.*

Organomercurials, especially methylmercury ($MeHg^+$), are highly toxic due to their lipophilic nature and high affinity toward thiol or selenol residues found in cellular systems.[1] The cysteine complex MeHgCys can easily cross cell membranes including the blood–brain barrier causing irreversible damage to the nervous system.[2] Currently, thiol-based chelating agents such as sodium 2,3-dimercaptopropanesulfate (DMPS) and *meso*-2,3-dimercaptosuccinic acid (DMSA) are widely used as drugs for the treatment of mercury poisoning.[3–5] However, evidences suggest that these two clinically used drugs are not very effective as chelators and fail to remove mercury (Hg) from the brain, and are themselves known to exhibit toxic effects. Thus, there is an urgent need to develop effective and safe compounds that can be used for the detoxification of organomercurials.[5,6]

Microorganisms have evolved resistance mechanisms to deal with Hg compounds[7,8] and are known to play a crucial role in detoxifying $MeHg^+$ by forming mercury sulfide (HgS).[9–11] The detoxification of organic or inorganic forms of mercury occurs in natural systems and the formation of HgSe has been observed in tissues of animals and in organs of mercury-mine workers.[12] Few years ago, HgSe was detected in the brains of humans exposed to $MeHg^+$.[13] Insoluble HgE (E = S, Se) particles are considered to be much less toxic than soluble $MeHg^+$ species such as MeHgCys.[10,13] Thus, efficient detoxification of organomercurials including methylmercury species by forming insoluble HgE particles at physiologically relevant conditions may offer a novel approach to treat patients suffering from methylmercury poisoning. Herein, we report that the *N*-methylimidazole based thione **1** and its selenium analogue **2** having a 2-hydroxyethyl substituent are remarkably effective in the detoxification of various organomercurials such as RHgOH (R = Me, Ar; Ar = -$C_6H_4CO_2Na$) and RHgCl (R = Me, Et) by producing insoluble HgS and HgSe nanoparticles (NPs) as the end products at 35 °C (Figure 1).

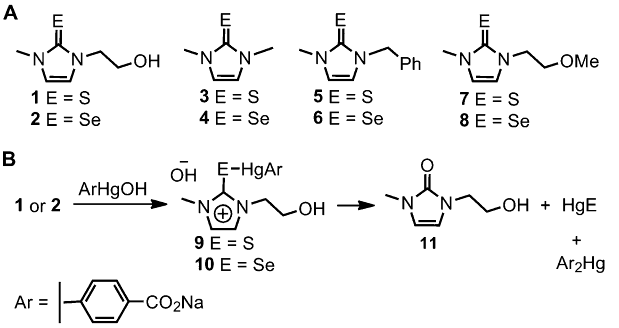


Figure 1. A) Chemical structures of some imidazole based thiones and selones. B) Formation of HgS and HgSe NPs in the reactions of ArHgOH with **1** and **2**, respectively.

When a solution of **1** (15 mM) in phosphate buffer (pH 7.4), or in water, was treated with one equivalent of ArHgOH at 35 °C, the immediate formation of the corresponding 1:1 ArHgOH conjugated (with compound **1**) complex **9** (*m*/*z* 481.0506) was observed (Figure 1 and Figure 2A,C). Interestingly, after one hour, we detected a white precipitate, which gradually turned into a black precipitate of HgS. Furthermore, the gradual formation of a new product with an LC/MS retention time of 1.8 min (Figure 2A) was observed, which was isolated and identified as ketone **11** by various characterization techniques (*m*/*z* 143.0822; Figure 2D). The amount of **11** and HgS NPs increased with a decrease in the amount of **9** during the course of the reaction. Complete degradation of **9** was observed in 6–7 days at 35 °C (Figure 2B). The grayish-black precipitate of HgS, which is insoluble in water and

---

[*] M. Banerjee, R. Karri, K. Muthuvel, Dr. G. Roy
Department of Chemistry, School of Natural Sciences
Shiv Nadar University
NH91, Dadri, Gautam Buddha Nagar, UP 201314 (India)
E-mail: gouriprasanna.roy@snu.edu.in
K. S. Rawat, Dr. B. Pathak
Discipline of Chemistry and Center for Material Science & Engineering
Indian Institute of Technology (IIT) Indore (India)

[**] This work was supported by SNU, IIT Indore, SERB (SB/S1/IC-44/2013), CSIR [01(2723)/13/EMR(II)], and the Government of India. We are grateful to Prof. Rupamanjari Ghosh (Director, SNS, SNU) for encouragement and support. M.B. contributed significantly to this study and both M.B. and R.K. thank SNU for fellowships. We thank AIRF, JNU for TEM, SEM, and NMR facilities, and IIT Roorkee for a TEM facility.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201504413.

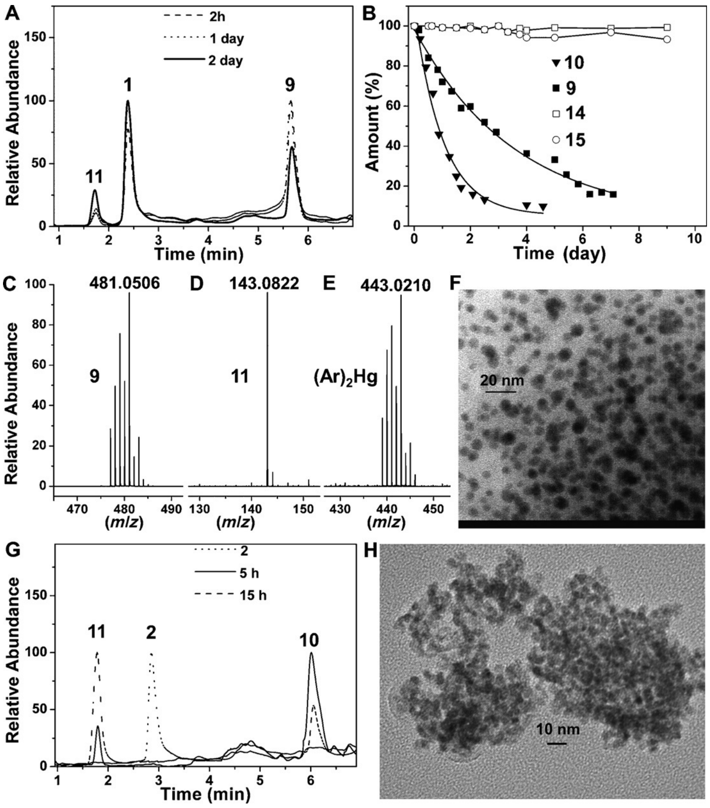


Figure 2. A) LC/MS chromatograms of **9** and **11**, obtained by treating **1** with ArHgOH at 35 °C, after different reaction times. B) Degradation of the complexes **9**, **10**, **14**, and **15** over time. Mass spectra of **9** (C), **11** (D), and Ar_2Hg (E). F) TEM image of HgS NPs obtained by treating **1** with ArHgOH. G) LC/MS chromatograms of **10** and **11**, obtained from the reaction of **2** with ArHgOH, after different reaction times. H) TEM image of HgSe NPs obtained from the reaction of **2** with ArHgOH.

common organic solvents, was characterized thoroughly by various techniques. TEM images showed that the HgS NPs were spherical and almost monodisperse with an average size of 7 nm (Figure 2F). Furthermore, both products $(Ar)_2Hg$ and ArH were identified by mass spectrometry (Figure 2E, Figure S8 in the Supporting Information).

Selones are expected to be more reactive toward organomercurials because of the soft and nucleophilic nature of the selenium atom. When **2** (15 mM) was treated with one equivalent of ArHgOH in phosphate buffer, or in water, at 35 °C, the formation of the corresponding 1:1 complex **10** with ArHgOH was observed by LC/MS at a retention time of 6.2 min (Figure 2G). A black precipitate of HgSe started to appear within a few minutes and complete degradation of **10** was observed in 2–3 days (Figure 2B). The HgSe NPs were isolated from the above reaction mixture after completion of the reaction and characterized thoroughly by various techniques. They were spherical with an average size of 6 nm (Figure 2H). Ketone **11**, $(Ar)_2Hg$, and ArH were also confirmed as byproducts in this reaction.

The toxic effects of mercury depend on its chemical form and the route of exposure. While $MeHg^+$ is the most common toxic form, the toxicity levels of various chemical forms of $MeHg^+$ detected in tissues of high-trophic-level animals are still not clear, although studies have shown that $MeHg^+$ in fish as well as in humans is likely to exist as the cysteine complex, that is, MeHgCys. On the other hand, under acidic high Cl^- conditions in the human stomach MeHgCys may convert to MeHgCl. The Hg−Cl bond in MeHgCl is highly covalent in nature, and presumably it remains intact in dilute aqueous solution.[14] Therefore, we examined the detoxification of RHgCl (R = Me, Et) with **1** and **2**. Surprisingly, when **1** or **2** (15 mM) was treated with one equivalent of the organomercurial in water/acetonitrile (1:1) at 35 °C, no HgE NPs were formed.[15] Instead, very small amounts of chloro adducts **16** and **17** (4–6 % based on **12**–**15**) were detected by mass spectrometry after 2–3 days along with **12**–**15** as major products (Figure 3 and Figures S9–S15 in the Supporting Information).

The identification of chloro adducts **16** and **17** by mass spectrometry suggests the presence of a strong Hg−Cl covalent bond in these two compounds. In general, organomercurials of the type R−Hg−X are remarkably inert toward Hg−C cleavage under physiological conditions. However, thiones and selones have been shown to mediate the protolytic cleavage of Hg−C bonds.[16,17] Interestingly, the decomposition of **12**–**15** and the subsequent formation of ketone **11** and the corresponding HgE NPs were observed under alkaline conditions. When, after treating **1** with RHgCl (R = Me, Et), one equivalent of hard bases such as KOH and sodium methoxide (NaOMe) was added, HgS NPs were formed. Similar to **9** and **10**, product **14** was completely degradated in the presence of one equivalent of KOH in 6–7 days (initial rate: $2.96\times10^{-4}\,\mathrm{M\,h^{-1}}$). A TEM image of well-dispersed crystalline HgS NPs obtained in the reaction of **1** with EtHgCl in the presence of NaOMe is shown in Figure 3C. When a similar reaction was performed with the selenium analogue **2**, it resulted in the formation of triangularly shaped HgSe NPs with an average size of 35 nm

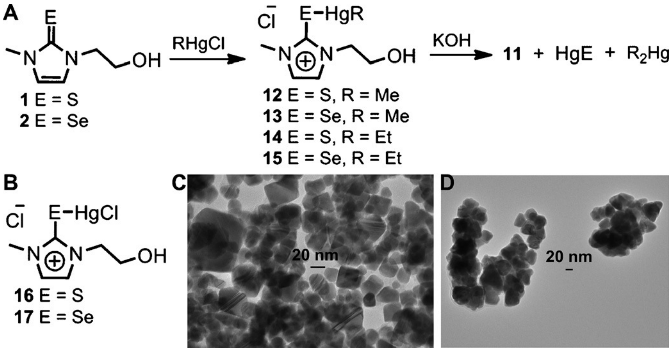


Figure 3. A) Synthetic route to HgS and HgSe NPs in the reaction of RHgCl with **1** and **2**, respectively, in the presence of a base. B) Chemical structures of chloro adducts **16** and **17**. C) TEM image of HgS NPs obtained in the reaction of **1** with EtHgCl in the presence of NaOMe. D) TEM image of HgSe NPs obtained in the reaction of **2** with EtHgCl in the presence of KOH.

(Figure 3D). A complete degradation of **15** was observed in the presence of one equivalent of KOH in 2 days (initial rate: $9.17\times10^{-4}\,\text{M}\,\text{h}^{-1}$), as shown in Figure 4. To investigate the effect of **1** and **2** on MeHgOH, we synthesized MeHgOH following the literature procedure.[18] The reaction of MeHgOH with **1** and **2** resulted, like the reaction of ArHgOH, in the formation of the corresponding HgE NPs (Figures S4 and S5 in the Supporting Information).[19]

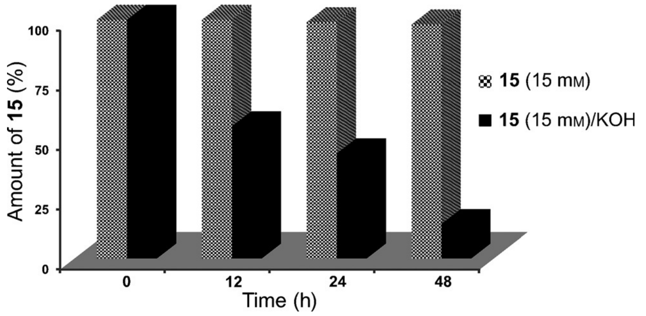


Figure 4. Degradation of compound **15** in the presence and absence of one equivalent of KOH.

The high reactivity of compounds **1** and **2** may be attributed to the ability of the OH group of their *N*-CH_2CH_2OH moiety to participate in the desulfurization and deselenization process. To understand this pathway, the CH_2CH_2OH group was replaced with a CH_3 (compounds **3** and **4**) and a CH_2Ph group (compounds **5** and **6**; Figure 1). Although the corresponding 1:1 complexes with ArHgOH were produced when **3** and **4** were treated with one equivalent of ArHgOH, the formation of HgE and the corresponding ketone was not observed under identical reaction condition even after 15 days of stirring at 35 °C. Similarly, the reactions of **5** and **6** with ArHgOH did not result in the formation of the corresponding HgE NPs. Moreover, to ascertain the role of the OH group in **1** and **2** we synthesized **7** and **8** (Figure 1), where the OH group is replaced by an OMe group. Interestingly, both these compounds failed to produce any HgS or HgSe upon treatment with ArHgOH or RHgCl (R = Me, Et) in the presence or absence of KOH. All the reactions were monitored for 15 days at 35 °C unless otherwise mentioned. These observations clearly suggest that the OH group in compounds **1** and **2** plays a crucial role in detoxifying organomercurials by producing insoluble HgE NPs.

The crystal structures of **1** (CCDC-1056210)[20] and **2** and the optimized geometries of the 1:1 RHgOH conjugated complexes with **1** and **2** (R = Me, $C_6H_4CO_2H$ (Ar1); Figures S21 and S22 in the Supporting Information) suggest that the OH group of the *N*-CH_2CH_2OH moiety is oriented towards the C2 atom of the imidazole ring (the carbon atom of the C=E bond). The deprotonation of the OH group in **9, 10**, and **12–15** by a strong base favors an attack at the positively charged C2 atom to form intermediate **18** (Figure 5 A). A subsequent reaction of **18** with OH^- (or any other nucleophile) produces the unstable fused hemiketal **19**, which is eventually converted into stable ketone **11**. DFT calculations showed that hemiketal **19** is energetically highly

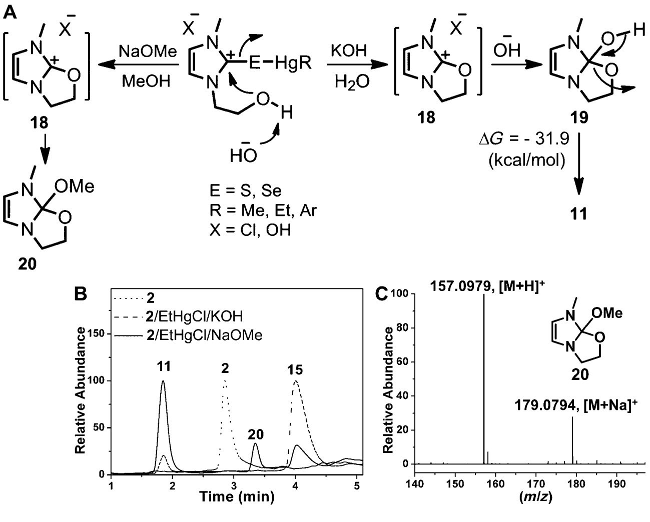
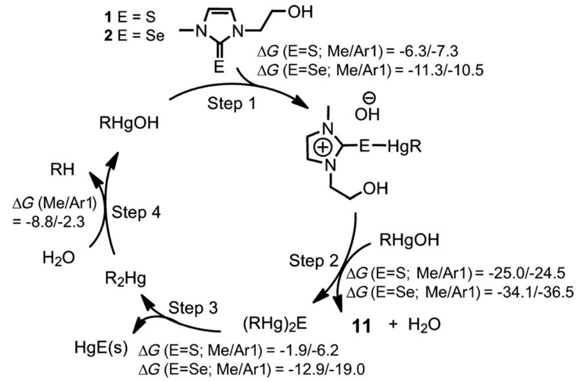


Figure 5. A) Mechanism for the formation of ketone **11** and ketal **20**. B) LC/MS chromatograms of ketal **20** formed in the reaction of **2** with EtHgCl in the presence of NaOMe/MeOH. C) Mass spectrum of **20**.

unstable by 31 kcal mol^{-1} compared to its keto form **11**. To support our proposed mechanism, compound **2** was treated with EtHgCl followed by the addition of one equivalent of NaOMe in methanol. Interestingly, under these reaction conditions, we observed the fused imidazolone ketal **20**, a methyl derivative of compound **19** (Figure 5). Ketal **20**[21] was isolated from the above reaction mixture and characterized thoroughly. In addition, the positively charged intermediate **18** was detected by mass spectrometry when the same reaction was performed in dichloromethane/acetonitrile. However, the positively charged intermediate **18** was gradually converted into **11** upon treatment with KOH.

Our observations suggest that **1** and **2** react with RHgOH to produce the corresponding 1:1 RHgOH conjugated complexes, for example **9** and **10**, which in turn react with another molecule of RHgOH to produce ketone **11** and the corresponding HgE. The addition of one more equivalent of RHgOH ([RHgOH]/[**2**] ratio 2:1) drove the reaction (step 2, Scheme 1 in the forward direction and, consequently, the formation of ketone **11** was significantly increased (Figure S17 in the Supporting Information). According to the FT-IR spectrum of the HgSe NPs a certain amount of ketone **11** and RH formed in the reaction is bound to the NPs (Figure S6 in the Supporting Information). Theoretical calculations showed that the formation of the corresponding 1:1 complexes (step 1, Scheme 1) and their subsequent decomposition upon addition of one equivalent of RHgOH (R = Me, Ar1; step 2) are more favorable with **2** than with its sulfur analogue **1**, which is in good agreement with our experimental results, where the formation of **11** and the corresponding HgE NPs was observed at a significantly faster rate with **2** than with **1**.

In the reaction of MeHgCl with H_2S, the formation of a white-color precipitate of $(MeHg)_2S$ as an intermediate was observed previously; this intermediate gradually decomposed into a black precipitate of β-HgS and Me_2Hg.[9,22,23] The



Scheme 1. Proposed mechanism for the detoxification of organomercurials by compounds **1** and **2**. The calculated Gibbs energies (ΔG, kcal mol^{-1}) for each step are given for both substituents R (=Me, Ar1).

selenium analogue of $(MeHg)_2S$ was identified in the blood of rabbits when MeHgCl and sodium selenite were added to it.[24] Furthermore, the formation of the $(MeHg)_2Se$ intermediate from MeHg seleno amino acid complexes was also observed in the reaction of MeHgOH with seleno amino acids.[25] The half-life of $(MeHg)_2Se$ was reported to be only in the order of 1 h and thus it readily decomposed to HgSe and Me_2Hg. As the Gibbs energies for step 2 are thermodynamically highly favorable for both the sulfur and selenium compounds it is more likely that the 1:1 RHgOH conjugated complexes with **1** and **2** decompose into ketone **11** and the corresponding unstable intermediates $(RHg)_2E$, which subsequently decompose to the corresponding insoluble HgE NPs and R_2Hg, as shown in step 3 (Scheme 1). The mass spectrometric analysis confirmed the formation of Ar_2Hg in the reactions of ArHgOH with compounds **1** and **2** (Figure 2E). Finally, the dialkyl/diaryl mercury compounds R_2Hg formed in step 3 further react with water to yield RHgOH and RH and thereby complete the cycle.[25,26] Overall, in every cycle two molecules of RHgOH react with one molecule of **1** or **2** to form one molecule of insoluble HgS or HgSe NPs.

In conclusion, we have described a novel pathway for the detoxification of organomercurials by thiones/selones having an *N*-CH_2CH_2OH substituent under physiologically and environmentally relevant conditions. The reactions produce less toxic insoluble HgS and HgSe NPs. The OH group of the *N*-CH_2CH_2OH substituent plays a crucial role in the detoxification by facilitating the desulfurization and deselenization processes. Organomercurials are highly toxic to humans both at high levels through poisoning in the environment and at lower levels through consumption of seafood. Thus, the efficient detoxification of organomercurials presented herein is of great importance and this study may lead to the development of compounds to treat patients suffering from organomercury poisoning. Such compounds may also be useful in detoxifying other compounds based on toxic heavy metals such as Cu, Pb, Cd, and Sn.

CAUTION! Organomercurials are highly toxic to humans, and thus appropriate safety precautions must be taken in handling these toxic chemicals.

Keywords: detoxification · mercury · organomercurials · selones · thiones

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 9323–9327
Angew. Chem. **2015**, *127*, 9455–9459

---

[1] a) T. W. Clarkson, L. Magos, *Crit. Rev. Toxicol.* **2006**, *36*, 609–662; b) J. Mutter, J. Naumann, C. Guethlin, *Crit. Rev. Toxicol.* **2007**, *37*, 537–549; c) T. W. Clarkson, *Crit. Rev. Clin. Lab. Sci.* **1997**, *34*, 369–403; d) V. Branco, J. Canário, A. Holmgren, C. Carvalho, *Toxicol. Appl. Pharmacol.* **2011**, *251*, 95–103; e) V. Branco, J. Canário, J. Lu, A. Holmgren, C. Carvalho, *Free Radical Biol. Med.* **2012**, *52*, 781–793.

[2] a) C. M. L. Carvalho, E. H. Chew, S. I. Hashemy, J. Lu, A. Holmgren, *J. Biol. Chem.* **2008**, *283*, 11913–11923; b) Z. Yin, H. Jiang, T. Syversen, J. B. T. Rocha, M. Farina, *J. Neurochem.* **2008**, *107*, 1083–1090; c) M. Aschner, J. L. Aschner, *Neurosci. Biobehav. Rev.* **1990**, *14*, 169–176.

[3] a) N. Ballatori, T. W. Clarkson, *Fundam. Appl. Toxicol.* **1985**, *5*, 816–831; b) T. W. Clarkson, *Environ. Health Perspect.* **2002**, *110*, 11–23.

[4] C. M. L. Carvalho, J. Lu, X. Zhang, E. S. J. Arnér, A. Holmgren, *FASEB J.* **2011**, *25*, 370–381.

[5] J. P. K. Rooney, *Toxicology* **2007**, *234*, 145–156.

[6] a) G. N. George, R. C. Prince, J. Gailer, G. A. Buttigieg, M. B. Denton, H. H. Harris, I. J. Pickering, *Chem. Res. Toxicol.* **2004**, *17*, 999–1006; b) H. V. Aposhian, D. L. Morgan, H. L. Queen, R. M. Maiorino, M. M. Aposhian, *J. Toxicol. Clin. Toxicol.* **2003**, *41*, 339–347; c) J. P. Buchet, R. R. Lauwerys, *Toxicology* **1989**, *54*, 323–333.

[7] a) M. J. Moore, M. D. Distefano, L. D. Zydowsky, R. T. Cummings, C. T. Walsh, *Acc. Chem. Res.* **1990**, *23*, 301–308; b) C. T. Walsh, M. D. Distefano, M. J. Moore, L. M. Shewchuk, G. L. Verdine, *FASEB J.* **1988**, *2*, 124–130.

[8] W. F. Fitzgerald, C. H. Lamborg, C. R. Hammerschmidt, *Chem. Rev.* **2007**, *107*, 641–662.

[9] F. Baldi, M. Pepi, M. Filippelli, *Appl. Environ. Microbiol.* **1993**, *59*, 2479–2485.

[10] H. S. K. Pan-Hou, N. Imura, *Arch. Microbiol.* **1981**, *129*, 49–52.

[11] a) M. Marvin-Diapsquale, J. Agee, C. McGowan, R. S. Oremland, M. Thomas, D. Krabbenhoft, C. C. Gilmour, *Environ. Sci. Technol.* **2000**, *34*, 4904–4917; b) T. Barkay, S. M. Miller, A. O. Summers, *FEMS Microbiol. Rev.* **2003**, *27*, 355–384; c) K. J. Glendinning, L. E. Macaskie, N. L. Brown, *Biotechnol. Lett.* **2005**, *27*, 1657–1662; d) A. M. M. Essa, L. E. Macaskie, N. L. Brown, *Biotechnol. Lett.* **2005**, *27*, 1649–1655.

[12] a) M. A. K. Khan, F. Wang, *Environ. Toxicol. Chem.* **2009**, *28*, 1567–1577; b) M. Nigro, C. Leonzio, *Mar. Ecol. Prog. Ser.* **1996**, *135*, 137–143; c) L. Magos, T. W. Clarkson, A. R. Hudson, *J. Pharmacol. Exp. Ther.* **1984**, *228*, 478–483; d) E. Nakazawa, T. Ikemoto, A. Hokura, Y. Terada, T. Kunito, S. Tanabe, I. Nakai, *Metallomics* **2011**, *3*, 719–725; e) P.-S. Ng, H. Li, K. Matsumoto, S. Yamazaki, T. Kogure, T. Tagai, H. Nagasawa, *Proc. Jpn. Acad. Ser. B* **2001**, *77*, 178–183; f) L. Kosta, A. R. Byrne, V. Zelenko, *Nature* **1975**, *254*, 238–239.

[13] M. Korbas, J. L. O'Donoghue, G. E. Watson, I. J. Pickering, S. P. Singh, G. J. Myers, T. W. Clarkson, G. N. George, *ACS Chem. Neurosci.* **2010**, *1*, 810–818.

[14] a) H. H. Harris, I. J. Pickering, G. N. George, *Science* **2003**, *301*, 1203; b) A. H. Stern, *Science* **2003**, *299-302*, 763; c) R. J. M. Hudson, C. W. Shade, *Science* **2003**, *299-302*, 763.

[15] Only 6–8% degradation of compounds **13** and **15** was observed after stirring 7–8 days in water/acetonitrile mixture in the absence of any strong base, and neither the degradation of **13** or **15** nor the formation of HgSe was increased significantly even after 15 days of stirring. The small amounts of HgSe observed in

this case are due to cleavage of the labile C–Se bond in **13** and **15** (Figure S25 in the Supporting Information).

[16] J. H. Palmer, G. Parkin, *J. Am. Chem. Soc.* **2015**, *137*, 4503 – 4516.

[17] a) J. G. Melnick, G. Parkin, *Science* **2007**, *317*, 225 – 227; b) J. G. Melnick, K. Yurkerwich, G. Parkin, *Inorg. Chem.* **2009**, *48*, 6763 – 6772; c) J. G. Melnick, K. Yurkerwich, G. Parkin, *J. Am. Chem. Soc.* **2010**, *132*, 647 – 655.

[18] D. K. Breitinger in *Synthetic Methods of Organometallic and Inorganic Chemistry*, *Vol. 5* (Ed.: W. A. Herrmann), Thieme, Stuttgart, New York, **1999**, p. 214.

[19] Detailed detoxification studies with other organomercurials such as RHgCys (R = Me, Et) are in progress.

[20] CCDC 1056210 (**1**) and 1056211 (**8**) contain the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre.

[21] The synthesis of similar fused imidazolone ketals has been reported recently: A. J. Arduengo III, G. Gurau, S. P. Kelley, W. J. Marshall, J. W. Runyon, *Angew. Chem. Int. Ed.* **2013**, *52*, 10871 – 10873; *Angew. Chem.* **2013**, *125*, 11071 – 11073.

[22] a) P. J. Craig, P. D. Bartlett, *Nature* **1978**, *275*, 635 – 637; b) I. R. Rowland, M. J. Davies, P. Grasso, *Nature* **1977**, *265*, 718 – 719.

[23] E. Yoshida, T. Toyama, Y. Shinkai, T. Sawa, T. Akaike, Y. Kumagai, *Chem. Res. Toxicol.* **2011**, *24*, 1633 – 1635.

[24] A. Naganuma, N. Imura, *Res. Commun. Chem. Pathol. Pharmacol.* **1980**, *27*, 163 – 173.

[25] a) M. A. K. Khan, F. Wang, *Chem. Res. Toxicol.* **2010**, *23*, 1202 – 1206; b) M. A. K. Khan, F. Wang, *Chem. Res. Toxicol.* **2009**, *22*, 1827 – 1832; c) M. A. K. Khan, A. M. Asaduzzaman, G. Schreckenbach, F. Wang, *Dalton Trans.* **2009**, 5766 – 5772.

[26] A. M. Asaduzzaman, G. Schreckenbach, *Inorg. Chem.* **2011**, *50*, 2366 – 2372.

Received: May 15, 2015