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Reductive Metalation of Cyclic and Acyclic Pseudopeptidic Bis-Disulfides and Back Conversion of the Resulting Diamidato/Dithiolato Complexes to Bis-Disulfides

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Cyclic and acyclic pseudopeptidic bis-disulfides built on an *o*-phenylene diamine scaffold were prepared: $(N_2H_2S_2)_2$, **1a**, $N_2H_2(S\text{-}SCH_3)_2$, **1b**, and $N_2H_2(S{-}S\mathit{t}Bu)_2$, **1c**. Reductive metalation of these disulfides with $(PF_6)[Cu(CH_3CN)_4]$ in the presence of Et_4NOH as a base, or with $(Et_4N)[Fe(SEt)_4]$ and Et_4NCl, yields the corresponding diamidato/dithiolato copper(III) or iron(III) complex, $(Et_4N)[Cu(N_2S_2)]$, **2**, or $(Et_4N)_2[Fe(N_2S_2)Cl]$, **5**. These complexes display characteristics similar to those previously described in the literature. The mechanism of the metalation with copper has been investigated by X-band electron paramagnetic resonance (EPR) spectroscopy at 10 K. After metalation of the bis-disulfide **1c** and deprotonation of the amide nitrogens, the reductive cleavage of the S−S bonds occurs by two one-electron transfers leading to the intermediate formation of a copper(II) complex and a thyil radical. Complexes **2** and **5** can be converted back to the cyclic bis-disulfide **1a** with iodine in an 80% yield. Reaction of **5** with iodine in the presence of $CH_3S\text{-}SCH_3$ affords a 1/1 mixture of the acyclic $N_2H_2(S\text{-}SCH_3)_2$ disulfide **1b** and cyclic bis-disulfide **1a**. From **2**, the reaction was monitored by ^1H NMR and gives **1b** as major product. While there is no reaction of **2** or **5** with *t*BuS-S*t*Bu and iodine, reaction with an excess of *t*BuSI affords quantitatively the di-*tert*-butyl disulfide **1c**. To assess the role of the Cu^{III} oxidation state, control experiments were carried out under strictly anaerobic conditions with the copper(II) complex, $(Et_4N)_2[Cu(N_2S_2)]$, **6**. Complex **6** is oxidized to **2** by iodine, and it reacts with an excess of *t*BuSI, yielding **1c** as final product, through the intermediate formation of complex **2**.

Introduction

The Cys-X-Cys tripeptide motif provides, through the deprotonated peptide nitrogens and the thiolate sulfurs, the N_2S_2 donor set to the metal cation at the active site of nitrile hydratase (NHase)[1] and acetyl coenzyme A synthase/carbon monoxide dehydrogenase (ACS/CODH).[2] The reactivity of these two metalloproteins are finely tuned by specific metal coordination and post-translational modifications of the cysteines thiolates. Oxygenation to sulfinate and sulfenate is required for activity of iron and cobalt NHase[1] and while a first nickel cation is found at the N_2S_2 site, incorporation of a second nickel cation is required for activation of ACS/CODH.[2] In this latter case, the two cysteine thiolates are bridging ligands between the two Ni atoms. Very recently, hepcidin, a 25 amino acid, cysteine rich peptide was found to act as the central regulator of body iron metabolism.[3] Hepcidin binds to the iron exporter ferroportin leading to its internalization and degradation, thus regulating the export of iron from cells to plasma.[4] This peptide contains eight cysteines involved in four disulfide bridges, the connectivities of which were assigned by NMR experiments.[5] With so many cysteines, hepcidin is proposed to bind iron and to be an iron sensor. A first hypothesis is the formation of an iron sulfur center. Indeed, a proportion of urine purified hepcidin retains iron, and electron paramagnetic resonance (EPR) spectroscopy analysis suggests

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[1] (a) Nagashima, S.; Nakasako, M.; Dohmae, N.; Tsujimura, M.; Takio, K.; Odaka, M.; Yohda, M.; Kamiya, N.; Endo, I. *Nat. Struct. Biol.* **1998**, *5*, 347–351. (b) Miyanago, A.; Fushinobu, S.; Ito, K.; Wakagi, T. *Biochem. Biophys. Res. Commun.* **2001**, *288*, 1169–1174.

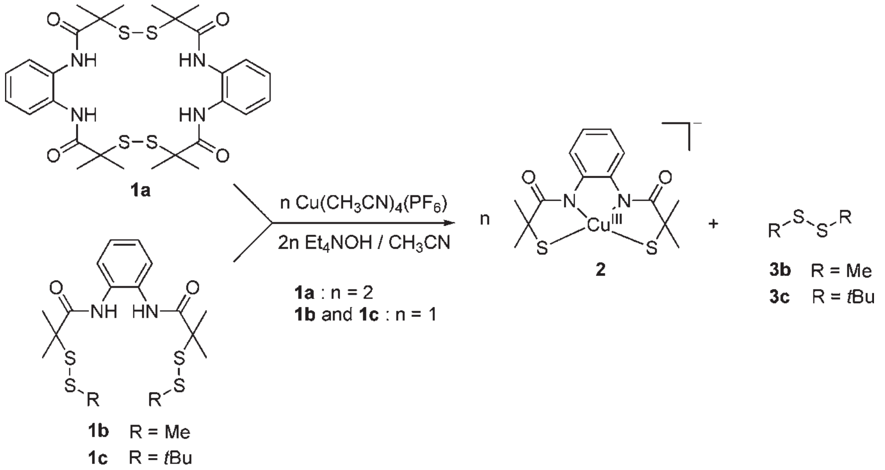
[2] (a) Darnault, C.; Volbeda, A.; Kim, E. J.; Legrand, P.; de Verne, X.; Lindahl, P. A.; Fontecilla-Camps, J. C. *Nat. Struct. Biol.* **2003**, *10*, 271–279. (b) Doukov, T. I.; Iverson, T. M.; Seravalli, J.; Ragsdale, S. W.; Drennan, C. L. *Science* **2002**, *298*, 567–572.

[3] Ganz, T.; Nemeth, E. *Biochim. Biophys. Acta* **2006**, *1663*, 690–699.

[4] Nemeth, E.; Tuttle, M. S.; Powelson, J.; Vaughn, M. B.; Donovan, A.; Ward, D. M.; Ganz, T.; Kaplan, J. *Science* **2004**, *306*, 2090–2093.

[5] (a) Jordan, J. B.; Poppe, L.; Haniu, M.; Arvedson, T.; Syed, R.; Li, V.; Kohno, H.; Helen Kim, H.; Schnier, P. D.; Miranda, L. P.; Cheetham, J.; Sasu, B. J. *J. Biol. Chem.* **2009**, *284*, 24155–24167. (b) Hunter, H. N.; Fulton, D. B.; Ganz, T.; Vogel, H. J. *J. Biol. Chem.* **2002**, *277*, 37597–37603.

Scheme 1. Reductive Metalation of the Bis-Disulfides with $(PF_6)[Cu^I(CH_3CN)_4]$



a rubredoxin-like iron sulfur site.[6] An iron sulfur cluster has also been previously postulated.[7] Nevertheless, all the characterizations are very difficult because purified hepcidin is only available in a very low amount. Whatever the iron sulfur center, presently its formation always involves metalation of the reduced form of hepcidin. Moreover, when looking at the sequence of hepcidins, we can notice another potential metal binding site defined by a Cys-X-Cys tripeptide, in which the two cysteines are post-translationally oxidized into disulfides. Why not consider that this motif defines a cavity able to trap a metal cation such as iron or copper? Actually, some urine purified hepcidin was also found to retain copper ion.[6b] We have studied this hypothesis using pseudopeptidic cyclic $(N_2H_2S_2)_2$ and acyclic bis-disulfide $N_2H_2(S\text{-}SR)_2$ synthetic analogues, and we have shown that direct metalation of such proligands under reductive conditions using Fe_3CO_{12} or $(Et_4N)[Fe^{III}(SEt)_4]$ and $(PF_6)[Cu^I(CH_3CN)_4]$ leads to the formation of the well-known $(Et_4N)_2[Fe^{III}(N_2S_2)(Cl)]$ and $(Et_4N)[Cu^{III}(N_2S_2)]$ complexes with the typical $[N_2S_2]^{4-}$ donors. Interestingly upon oxidation with iodine, the iron and copper complexes alone or in the presence of an exogenous linear disulfide RSSR, revert back to the cyclic and acyclic disulfide ligands respectively, while the iron and copper ions are ejected.

Results and Discussion

The cyclic bis-disulfide, **1a** (Scheme 1), was selectively prepared, as previously described,[8] by iodine oxidation of the Ni complex derived from the dithiol, *N*,*N*′-(1,2-phenylene)bis-(2-mercapto-2-methylpropanamide), $N_2S_2H_4$. The acyclic bis-disulfides, **1b** and **1c**, (Scheme 1) were synthesized by reaction of the dithiol, $N_2S_2H_4$, with *S*-methyl methane thiosulfonate and *S*-*tert*-butyl methane thiosulfonate, respectively, following a procedure described by Grayson et al.[9]

Reductive Metalation of the Bis-Disulfides 1a-1c with Tetrakis(acetonitrile)copper Hexafluorophosphate, $(PF_6)[Cu^I(CH_3CN)_4]$. Reaction of the cyclic bis-disulfide, **1a**, in acetonitrile, with 2 equiv of the copper(I) salt, $(PF_6)[Cu^I(CH_3CN)_4]$ and 4 equiv of Et_4NOH in methanol solution, as a base, affords cleanly the diamidato/dithiolato copper(III) complex $(Et_4N)[Cu(N_2S_2)]$ **2** (Scheme 1), previously prepared by Hanss and Krüger[10] upon electrochemical oxidation of the corresponding diamidato/dithiolato copper(II) complex. While there is no reaction without adding a base, upon addition of Et_4NOH to deprotonate the amide nitrogens, the solution turns instantaneously greenish yellow, exhibiting an absorption at 417 nm in electronic spectroscopy in CH_3CN. After removing the solvents, complex **2** was precipitated in cold diethyl ether and characterized by mass spectrometry, UV−vis spectroscopy, electrochemistry, and ^1H NMR. It displays the same characteristics as those previously reported in the literature.[10,11] Complex **2** was also quantitatively obtained starting from the acyclic bis-disulfides **1b** and **1c**, after addition of 1 equiv of the copper(I) salt and 2 equiv of Et_4NOH. This reaction also leads to the formation of 1,2-dimethyl disulfide from **1b** and 1,2-di-*tert*-butyl disulfide from **1c**, respectively, that were identified by GC and ^1H NMR, respectively.

Copper(I) is well-known to coordinate to sulfur-containing groups, including organic disulfides. Several dinuclear copper(I) complexes have been isolated and characterized by X-ray crystallography, such as those derived from bis[2,(*N*,*N*′-dimethylamino)ethyl]disulfide[12] or bis-{2-[(alkyl)(2-pyridylethyl)amino]ethyl} disulfide.[13] In these complexes the two Cu^I ions are bridged by the disulfide. In the pyridyl series, changing the spacer from ethyl to methyl, as in bis{2-[(benzyl)(2-pyridylmethyl)amino]ethyl} disulfide, promotes the reductive cleavage of the S−S bond and the conversion of the disulfide-dicopper(I) complex into a bis(μ-thiolato)dicopper(II) complex.[13] This effect

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[6] (a) Farnaud, S.; Rapisarda, C.; Bui, T.; Drake, A.; Cammak, R.; Evans, R. W. *Biochem. J.* **2008**, *413*, 553–557. (b) Farnaud, S.; Patel, A.; Evans, R. W. *Biometals* **2006**, *19*, 527–533.

[7] Gerardi, G.; Biasiotto, G.; Santambrogio, P.; Zanella, I.; Ingrassia, R.; Corrado, M.; Cavadini, P.; Derosas, M.; Levi, S.; Aorosio, P. *Blood Cells Mol. Dis.* **2005**, *35*, 177–191.

[8] Bourlès, E.; Alves de Sousa, R.; Selkti, M.; Tomas, A.; Artaud, I. *Tetrahedron* **2007**, *63*, 2466–2471.

[9] Grayson, E. J.; Ward, S. J.; Hall, A. L.; Rendle, P. M.; Gamblin, D. P.; Andrei, S.; Batsanov, A. S.; Davis, B. G. *J. Org. Chem.* **2005**, *70*, 9740–9754.

[10] Hanss, J.; Krüger, H.-J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2827–2830.

[11] Desbenoit, N.; Galardon, E.; Roussel, P.; Artaud, I.; Tomas, A. *J. Coord. Chem.* **2009**, *62*, 2472–2479.

[12] Ottersen, T.; Warner, L. G.; Seff, K. *Inorg. Chem.* **1974**, *13*, 1904–1911.

[13] (a) Itoh, S.; Nagagawa, M.; Fukuzumi, S. *J. Am. Chem. Soc.* **2001**, *123*, 4087–4088. (b) Osako, T.; Ueno, Y.; Itoh, S. *Inorg. Chem.* **2003**, *42*, 8087–8097.

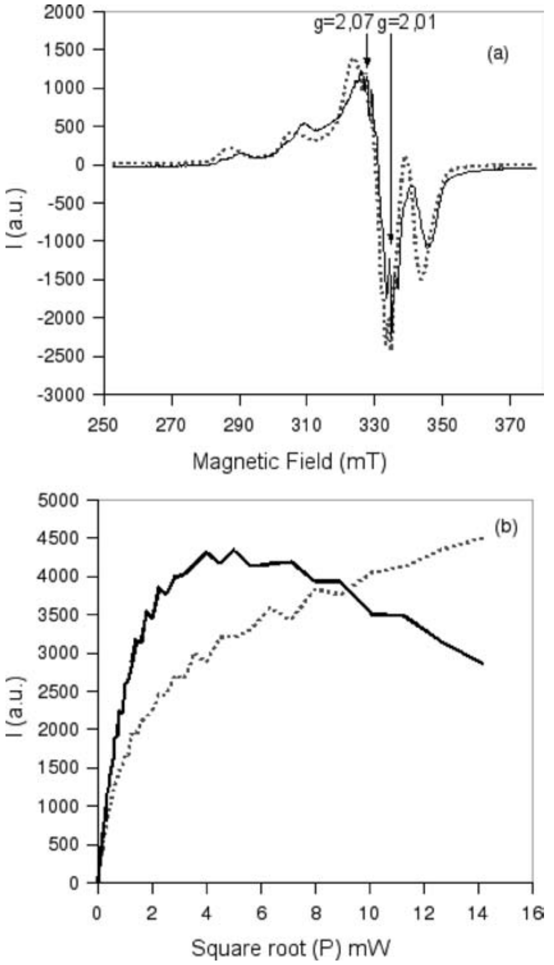

Figure 1. (a) EPR spectrum of complex **6** in dashed line and of species **4** in black solid line; (b) Intensity of the EPR signal versus the square root of the microwave power for the $\mathrm{Cu^{II}}$ complex of **4** at $g = 2.07$ (dotted gray line) and *t*BuS• at $g = 2.01$ (solid black line), the g value of the power saturation study are indicated by arrows.

has been attributed to the stronger donor ability of the pyridine nitrogen in the latter ligand that enhances the electron transfer from copper(I) to the disulfide leading to the S−S bond scission.[13b] In our case, deprotonation of the amide nitrogens is likely to promote the reductive cleavage of the S−S bonds by enhancing the electron density on the copper center, and to stabilize the copper(III) dithiolato complex. This reduction can occur by a two-electron transfer from $\mathrm{Cu^{I}}$, or by two one-electron transfers leading to the intermediate formation of a copper(II) complex. To discriminate between these two processes, a mixture of **1c** and $(\mathrm{PF_6})[\mathrm{Cu^{I}(CH_3CN)_4}]$, in acetonitrile was trapped at liquid nitrogen temperature after the addition of 2 equiv of $\mathrm{Et_4NOH}$, and was analyzed by X-band EPR spectroscopy at 10 K. The EPR spectrum, shown in Figure 1a, exhibits the typical pattern of a copper(II) complex in a tetragonal geometry with bands at $g_{\perp} = 2.03$ and $g_{\|} = 2.12$ and a hyperfine coupling constant $A_{\|} = 181$ G as the best fit of the g-values, and an additional signal at $g = 2.01$ attributed to the thyil radical *t*BuS•. The superimposition of the two paramagnetic entities has been confirmed by recording the variation of the intensities of the signals related to the two species with the microwave power (P) between 0 and 200 mW and between 4 and 30 K, at $g = 2.07$ for the copper(II) complex, and $g = 2.01$ for the organic radical species. As expected, and as shown in Figure 1b from the plot of I versus $P^{1/2}$ in mW at 30 K, the intensity of the radical signal is easily saturated and further decreases, while the intensity of the copper(II) signal slowly increases and reaches a plateau. Such an experiment enables to separate radical and metal spectra.[14] The variation of the intensity versus the power can be modeled from eq 1 derived from that of Innes and Brudvig[15] that has been modified to take in account the presence of two different magnetic species.[16]

$$\frac{C_{\mathrm{copper}} \times \sqrt{P}}{\left(1 + \dfrac{P}{P_{1/2\mathrm{copper}}}\right)^{b_{\mathrm{copper}}/2}} + \frac{C_{\mathrm{radical}} \times \sqrt{P}}{\left(1 + \dfrac{P}{P_{1/2\mathrm{radical}}}\right)^{b_{\mathrm{radical}}/2}} \tag{1}$$

In this equation, C_{i} is a proportionality factor, $P_{1/2\mathrm{i}}$ the microwave power at half saturation, and b the inhomogeneity factor taking in account the different type of spin packets. Using this equation we fitted the saturation curves obtained at $g = 2.07$ ($C_{\mathrm{copper}} = 391$, $P_{1/2\mathrm{copper}} = 11.9897$, $b_{\mathrm{copper}} = 1$ and $C_{\mathrm{radical}} = 72$, $P_{1/2\mathrm{radical}} = 1.4483$, $b_{\mathrm{radical}} = 3$) and 2.01 ($C_{\mathrm{copper}} = 156$, $P_{1/2\mathrm{copper}} = 11{,}99$, $b_{\mathrm{copper}} = 1$ and $C_{\mathrm{radical}} = 185{,}6$, $P_{1/2\mathrm{radical}} = 1.45$, $b_{\mathrm{radical}} = 3$). This result is coherent with a $S = 1/2$ copper(II) and a radical.

This result supports a reductive cleavage of the S−S bond of the cyclic disulfide **1c** by two successive one-electron transfers, as proposed in Scheme 2. In a first step, incorporation of copper(I) into **1c** should lead to a bis-imine/bis-disulfide copper(I) complex, in which the $\mathrm{Cu^{I}}$ ion is coordinated to the amide nitrogens under their iminol forms and to the internal sulfur atom of each disulfide unit. Deprotonation of the amides promotes the first electron-transfer, yielding species **4**, which can be described as a bis-amidato/thiolato/disulfide copper(II) complex, and a thyil radical. The second electron transfer from the copper(II) results in the formation of the isolated reaction products, the copper(III) complex **2** and the di-*tert*-butyl disulfide.

Similarly incorporation of two Cu(I) ions in the cyclic bis-disulfide **1a** in $\mathrm{CH_3CN}$ allows to detect by EPR at liquid nitrogen temperature, only after addition of base (4 equiv of $\mathrm{Et_4NOH}$/**1a**), a Cu(II) signal (Figure S1 in Supporting Information). Using $\mathrm{Cu(ClO_4)_2}$ as an internal standard, we evaluated the Cu(II)/**1a** ratio to be 2. The EPR pattern is very similar to that of the copper(II) complex in species **4** (Figure 1a). Such observations are consistent with a binuclear Cu(II) complex **1a′** (Scheme 3) in which the two copper cations are in a similar $\mathrm{N_2SS^*}$ tetragonal environment and bridged by one disulfide. The cleavage of one disulfide bond of **1a** should result from a one-electron transfer from each Cu(I) cation. Allowing the temperature to reach room temperature, leads to the formation of the copper(III) complex **2**, after reductive cleavage of the second S−S bridge in **1a′**. Such a binuclear Cu(II) complex, in which the two copper(II) sites are connected

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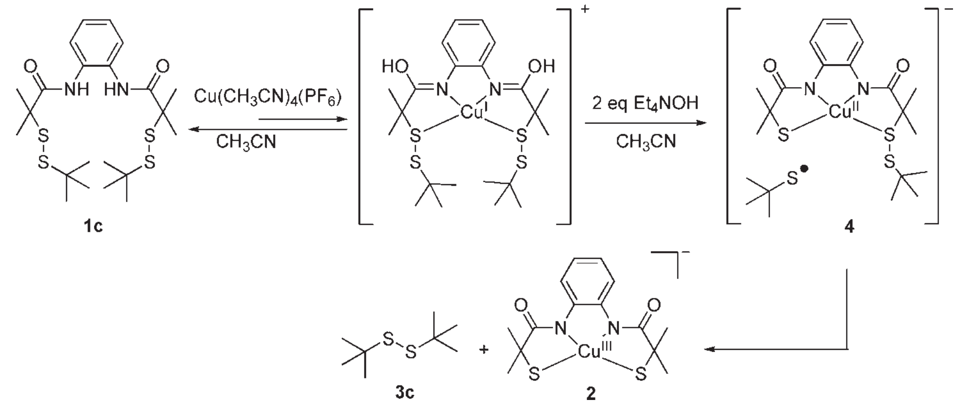
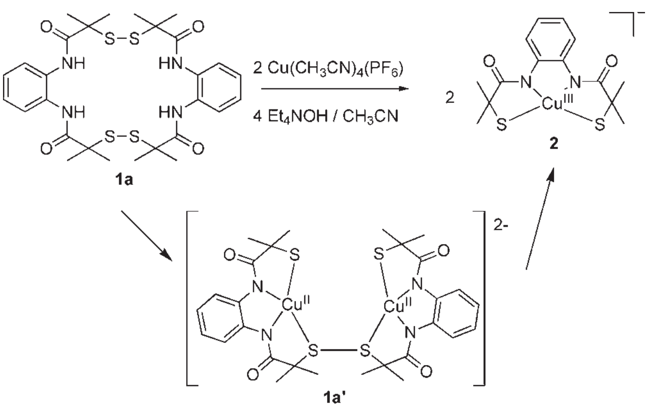
[14] Frapart, Y.-M.; Boussac, A.; Anxolabehere-Mallard, E.; Delroissse, M.; Verlhac, J.-B.; Blondin, G.; Girerd, J.-J.; Guilhem, J.; cesario, M.; Rutherford, A. W.; Lexa, D. *J. Am. Chem. Soc.* **1996**, *118*, 2669–2678.

[15] Innes, J. B.; G. W. Brudvig, G. W. *Biochemistry* **1989**, *28*, 1116–1125.

[16] Merdy, P.; Guillon, E.; Frapart, Y.-M.; Aplincourt, M. *New J. Chem.* **2003**, *27*, 577–582.



Scheme 2. Proposed Mechanism for the Reductive Metalation of the *tert*-Butyl Bis-Disulfide **1c** with $(PF_6)[Cu^I(CH_3CN)_4]$



Scheme 3. Proposed Structure of the Intermediate Trapped by EPR during Copper Insertion in **1a**

through a disulfide bridge, has been previously prepared from oxidized glutathione and crystallized. The magnetic susceptibility and the EPR showed that the Cu(II)—Cu(II) interaction was negligibly small.[17]

Reductive Metalation of the Bis-Disulfides 1a-1c with Triiron Dodecacarbonyl, Fe_3CO_{12}, or Iron(tetraethylthiolate) Tetraethylammonium, $(Et_4N)[Fe(SEt)_4]$. Since simple iron(I) complexes are not easily accessible, we first tried the metalation experiments of the bis-disulfides with an iron(0) species, Fe_3CO_{12}. These reactions were performed at 60 °C in DMF under argon, with **1a** and **1b**, and Fe_3CO_{12} (2/3 equiv versus **1a** and 1/3 equiv versus **1b**) in the presence of sodium methanolate as a base to deprotonate the amides (4 equiv relative to **1a** and 2 equiv relative to **1b**). After adding Et_4NCl (4 equiv relative to **1a** and 2 equiv relative to **1b**), the solution was opened to the air. While there was no reaction from **1a**, the diamidato/dithiolato complex **5**, $[(Et_4N)_2][Fe(N_2S_2)Cl]$, was isolated from **1b** in a 33% yield after purification over Sephadex LH20 (Scheme 4). The iron complex **5** was characterized by mass spectrometry, UV−vis spectroscopy, and EPR. Typically, it exhibits an absorption at 475 nm in DMF solution, and its X-band EPR spectrum in frozen DMF at 5 K displays the two typical bands at $g = 4.52$ and $g = 2.05$ corresponding to an iron(III) center with a $S = 3/2$ ground state. These characteristics are similar to those of an authentic sample prepared as previously detailed by iron(III) insertion in the dithiol $N_2S_2H_4$.[18]

To incorporate iron in **1a**, we turned to the tetraethylammonium iron(III) tetraethylthiolate $(Et_4N)[Fe(SEt)_4]$[19] (Scheme 4). The bis-disulfide **1a** was readily and quantitatively converted into **5** upon adding 2 equiv of $(Et_4N)[Fe(SEt)_4]$ in DMF and 2 equiv of Et_4NCl, as indicated by the X-band EPR spectrum recorded at 5 K, which shows the disappearance of the signals attributed to the HS ferric state of $(Et_4N)[Fe(SEt)_4]$ at $g = 4.25$[20] and the appearance of the signals characteristic of the $S = 3/2$ state of complex **5** (Figure S2, Supporting Information). Similarly the reaction was monitored by electronic spectroscopy (Figure S3, Supporting Information). For a set point of comparison, we checked that reacting **1b** with $(Et_4N)[Fe(SEt)_4]$ (1 equiv) in DMF also yields complex **5** (data not shown).

With Fe_3CO_{12}, the reductive metalation of **1b** is likely to involve an electron transfer from the iron(0) to the disulfide, promoting the scission of the S−S bonds when deprotonating the amides with NaOMe. This probably leads to the formation of an iron(II) complex equivalent to **5** (that has not been isolated), along with dimethyl disulfide. Complex **5** is then formed upon air oxidation. As previously detailed, the five-coordinate chloro species is air stable.[18,21] It is reduced at a very low potential explaining why the iron(II) form is so unstable and is readily oxidized to the iron(III) state.[18] The steric hindrance of the bis-disulfide **1a** probably disfavors the reaction with Fe_3CO_{12}.

When using either **1a** or **1b** and $(Et_4N)[Fe(SEt)_4]$, addition of base is not necessary. In this reaction the ethyl thiolates ligands are both the reducing agent able to trigger the S−S bond cleavage through several successive thiolate/disulfide exchanges, and the base able to deprotonate the amides. By forcing the cleavage of the S−S bonds, they drive the reaction toward the formation of the stable $[(Et_4N)][Fe^{III}(N_2S_2)Cl]$, complex **5**. Such thiolate/disulfide exchange has been previously reported to induce the interconversion of iron dinitrosyl complexes by reacting

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[17] Miyoshi, K.; Sugiura, Y.; Ishizu, K.; Iitaka, Y.; Nakamura, H. *J. Am. Chem. Soc.* **1980**, *102*, 6130–6136.

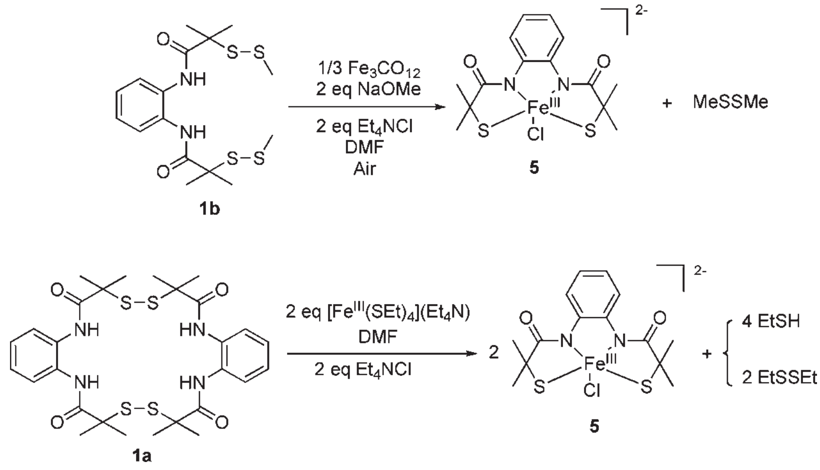
[18] Chatel, S.; Chauvin, A.-S.; Tuchagues, J.-P.; Leduc, P.; Bill, E.; Chottard, J.-C; Mansuy, D.; Artaud, I. *Inorg. Chim. Acta* **2002**, *336*, 19–28.

[19] Maelia, L.; Millar, M.; Koch, S. A. *Inorg. Chem.* **1992**, *31*, 4594–4600.

[20] Millar, M.; Lee, J. F.; O'Sullivan, T.; Koch, S. A.; Fikar, R. *Inorg. Chim. Acta* **1996**, *243*, 333–343.

[21] Galardon, E.; Giorgi, M.; Artaud, I. *Chem; Commun.* **2004**, 286–287.

Scheme 4. Reductive Metalation of Bis-Disulfides with Iron Species



R′S-SR′ with $[Fe(SR)_2(NO)_2]^-$ to yield $[Fe(SR')_2(NO)_2]^-$ and RS-SR.[22]

Reoxidation of the Copper and Iron Complexes 2 and 5 into Cyclic and Acyclic Bis-Disulfides. Some years ago, Fox et al.[23] described the synthesis of macrocyclic bis(disulfide)tetramine ligands by oxidative coupling of the corresponding bis-imine/bis-thiolate Ni complex $[Ni^{II}(N_2S_2)]$ or copper complex $[Cu^{II}(N_2S_2)]$ with iodine. We used a similar procedure to prepare **1a** from the diamidato/dithiolato nickel complex, $[(Et_4N)_2][Ni(N_2S_2)]$[8], and here we similarly submitted complexes **2** and **5** to iodine oxidation in CH_3CN at room temperature. Oxidation of the iron complex **5** with 1 equiv of iodine affords, after purification over silica gel, the cyclic bis-disulfide **1a** in an 80% yield. Since iodine[24] and bromine[25] adducts of nickel thiolate as well as nickel sulfenyl bromide[25] were reported in the literature, we suspected the intermediate formation of an iron(III) sulfenyl iodide during the course of iodine oxidation of **5**, but unfortunately we did not succeed in isolating any intermediate.

With **2**, for unclear reasons which are very likely due to a change in the redox state of the copper ion, the reaction requires an excess of iodine for completion (3 equiv). After purification, **1a** was isolated with a yield similar to that obtained from the iron complex **5**. In contrast to Fox et al.,[23] we did not isolate any dinuclear complexes derived from the cyclic bis-disulfide ligand but directly the free ligand, indicating that this iodine oxidation of diamidato/dithiolato complexes is associated to protonation of the amidates, probably with the solvent, and to release of iron or copper ions in solution. We have already noticed that N_2S_2 complexes with carboxamido nitrogen donors tend to lose their metal cation when the donating properties of the sulfur atoms are too decreased upon oxidation.[26]

More interestingly, complexes **2** and **5** can also be converted back to the acyclic bis-disulfides $N_2H_2(S{-}S{-}CH_3)_2$ **1b** and $N_2H_2(S{-}S{-}t\text{Bu})_2$ **1c**. For this, **2** and **5** were first treated with iodine in the presence of the dimethyl disulfide, **3b** (Scheme 5). In both cases, no reaction takes place in the absence of iodine. Oxidation of **5** with 1 equiv of I_2 and 1 equiv of **3b** affords a 1/1 mixture of **1a** and **1b** as determined by ^{1}H NMR analysis of the material isolated after precipitation in cold diethyl ether (see Figure S4, Supporting Information). Products **1a** and **1b** were isolated in a 50 and 45% yield, respectively.

The reaction of **2** with **3b** and iodine was monitored by ^{1}H NMR in CD_3CN. Figure 2 displays the aromatic region of a typical spectrum obtained after addition of iodine (2 equiv) and **3b** (1 equiv relative to **2**). It clearly shows a mixture of three main products: the starting complex **2**, along with a mixture of the two disulfides **1a** and **1b** in a 20/80 ratio. For an equimolar ratio of **3b** and **2**, when increasing the I_2/**2** ratio from 1 to 3 the conversion yield (see the Experimental Section) determined after a reaction time of 22 h at room temperature, increases from 45 to 100% (I_2/**2**: 1, 1.5, 2, and 3, conversion yield: 45, 60, 80, and 100%). In a preparative experiment with 1 equiv of **3b** and 3 equiv of iodine, **1b** and **1a** were isolated in a 50% and 8% yield after purification over silica gel, respectively. No other product has been identified by ^{1}H NMR. Using a **3b**/**2** ratio of 2 neither significantly increases the conversion yield of **2**, nor changes the selectivity of the reaction.

Despite several attempts, we were unable to define the final oxidation state of the copper at the end of the reaction or to trap and identify at low temperature any intermediate complexes. Nevertheless, it is reasonable to postulate the involvement of a dimethyl disulfide iodo sulfonium species,[27] resulting from the reaction of **3b** with iodine. On the basis of the known reactivity of disulfide halo sulfonium,[28] it could react with a thiolate of **2** to give the methyl sulfenyl iodide (CH_3SI) and of a mono *S*-methyldisulfide species. Then, the second thiolate should be

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[22] Tsai, F.-T.; Chiou, S.-J.; Tsai, M.-C.; Huang, H.-W.; Chang, M.-H.; Liaw, W.-F. *Inorg. Chem.* **2005**, *44*, 5872–5881.

[23] Fox, S.; Stibrany, R. T.; Potenza, J. A.; Knapp, S.; Schugar, H. J. *Inorg. Chem.* **2000**, *39*, 4950–4961.

[24] Lyon, E. J.; Musie, G.; Reibenspies, J. H.; Darensbourg, M. Y. *Inorg. Chem.* **1998**, *37*, 6942–6946.

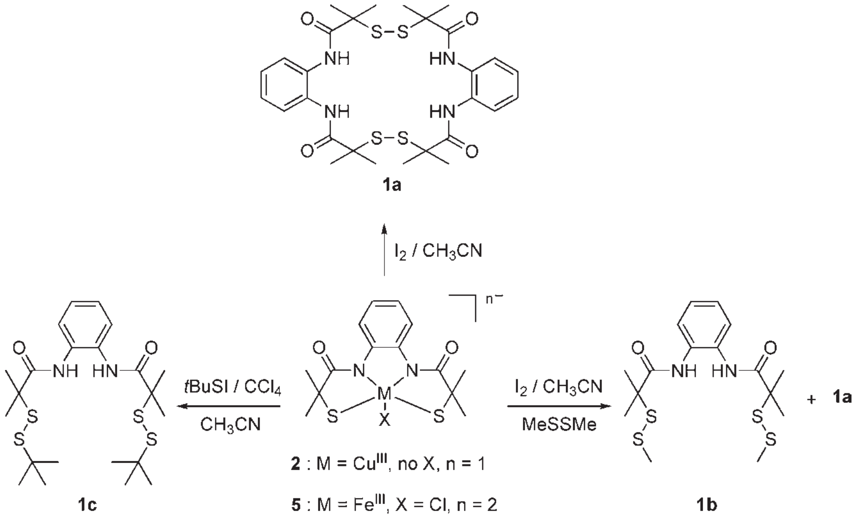
[25] Steinfeld, G.; Lozan, V.; Krüger, H.-J.; Kersting, B. *Angew. Chem., Int. Ed.* **2009**, *48*, 1954–1957.

[26] Alves de Sousa, R.; Galardon, E.; Rat, M.; Giorgi, M.; Artaud, I. *J. Inorg. Biochem.* **2005**, *99*, 690–697.

[27] Gooda, M.; Major, A.; Nag-Chaughuri, J.; McGlynn, S. P. *J. Am. Chem. Soc.* **1961**, *83*, 4329–4333.

[28] (a) Billard, T.; Langlois, B. R.; Large, S.; Anker, D.; Roidot, N.; Roure, P. *J. Org. Chem.* **1996**, *61*, 7545–7550. (b) Doi, J. T.; Luehr, G. W.; Musker, W. K. *J. Org. Chem.* **1985**, *50*, 5716–5719.

Scheme 5. Conversion of the Iron and Copper Diamidato/Dithiolato Complexes Back to the Bis-Disulfide Ligands



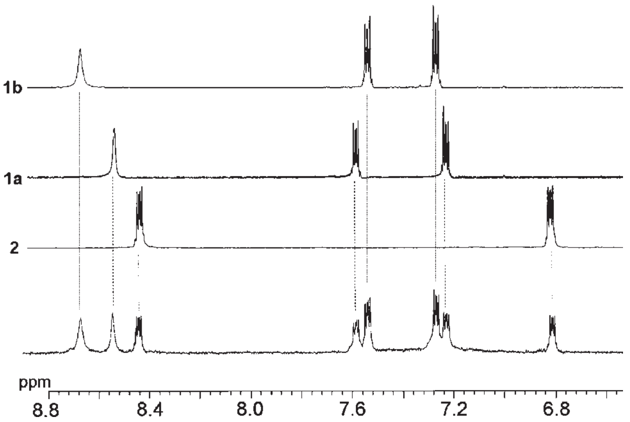


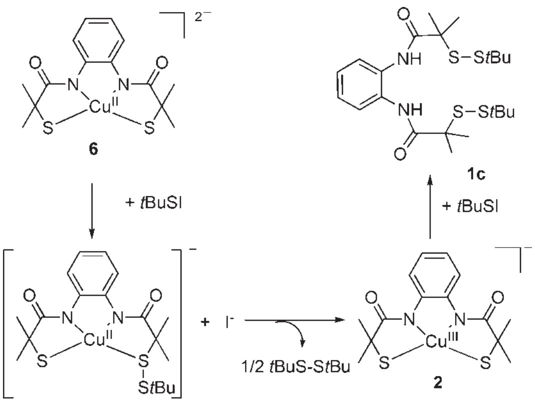
Figure 2. Typical ^{1}H NMR spectra, recorded at 500 MHz, obtained after addition of 1 equiv of dimethyl disulfide and 2 equiv of iodine to a 0.04 M solution of complex **2** in CD_3CN (bottom), along with the spectra of the authentic compounds **1a**, **1b** and **2** for comparison (top).

thionylated by reaction with CH_3SI, leading to the formation of the observed bis-*S*-methyl disulfide **1b** (see Scheme S1 in the Supporting Information).

All attempts to convert back the copper or iron complexes **2** and **5** into **1c** by an analogous reaction with *t*BuS-S*t*Bu **3c** and iodine have failed, and we only observed the cyclic bis-disulfide **1a**. The di-*tert*-butyl disulfide iodo sulfonium is probably too sterically hindered, and we turned to the reaction with *tert*-butylsulfenyl iodide. Hence, reaction of an excess of *t*BuSI (10 equiv) with **2** or **5** in CH_3CN affords quantitatively and selectively the di-*tert*-butyl disulfide **1c**.

Finally, to assess the role of the Cu^{III} oxidation state, we carried out some control experiments with the copper(II) complex **6**, $[(Et_4N)_2][Cu(N_2S_2)]$, prepared as previously described.[10] All the experiments were monitored by ^{1}H NMR in CD_3CN. The NMR tubes were prepared in a glovebox under strictly anaerobic conditions to avoid any air oxidation of **6** into **2**. The copper(II) complex **6** is oxidized with 1 equiv of iodine into the copper(III) species **2**, as expected from the low Cu^{II}/Cu^{III} redox potential.[10] Like **2**, the Cu(II) derivative **6** does not react with dimethyl or di-*tert*-butyl disulfide in the absence of iodine. However, its reaction with *t*BuSI (10 equiv) selectively yields the bis-disulfide **1c** (Scheme 6). It is noteworthy that lowering the amount of *t*BuSI allows the detection in the reaction mixture of the copper(III) derivative **2** along with **1c** (Figure S5, Supporting Information). This suggests that thionylation of **6** with *t*BuSI probably promotes, as shown in Scheme 6, the intermediate formation of a Cu^{II} diamidato/thiolato/*S*-disulfide and further the reductive cleavage of the S−S bond and the formation of the Cu^{III} complex **2** along with a thyil radical that dimerizes into disulfide. Further reaction of **2** with *t*BuSI yields as previously detailed the acyclic bis-disulfide **1c**.

Scheme 6. Proposed Mechanism for the Reaction of the Copper(II) Complex **6** with *tert*-Butyl Sulfenyl Iodide



Conclusion

In this paper, we have shown that pseudopeptidic bis-disulfides built on an *o*-phenylene diamide scaffold, mimicking a Cys*-X-Cys* tripeptide motif containing two cysteines modified into disulfides, can be easily and directly metalated under reductive conditions to produce the iron or copper diamidato/dithiolato species $(Et_4N)_2[Fe^{III}(N_2S_2)Cl]$ or $(Et_4N)[Cu^{III}(N_2S_2)]$. Interestingly, these complexes revert back to the starting cyclic bis-disulfide or acyclic bis-disulfide $N_2H_2(SSR)_2$ when exposed to iodine or to a mixture of an exogenous disulfide RSSR and iodine, when this disulfide is not too sterically hindered. With bulky R groups such as *t*Bu, the corresponding sulfenyl iodide, *t*BuSI, appears as an alternative to turn back to the bis-disulfide N_2H_2(S-S-*t*Bu)$_2$. Even though iodine is not a biologically relevant oxidant, the reversibility of the overall process, metalation/reoxidation, might be of interest for the investigation of the reactivity of disulfides on a peptide backbone with metal cations. Moreover, it is an additional example of the reactivity of sulfur oxidized species toward metal cations that we study from a general point of view and that includes persulfides,[29] pseudopeptidic sulfinates,[30] and pseudopeptidic cyclic thiosulfinates.[31]

Experimental Section

Physical Measurements. IR spectra were obtained with a Perkin-Elmer Spectrum One FT-IR spectrometer equipped with a MIRacleTM single reflection horizontal ATR unit (zirconium−selenium crystal). ESI-MS mass spectra were recorded on a Thermo Finnigan LCD Advantage spectrometer. UV spectra were recorded on a SAFAS mc^2 spectrometer. Elemental analyses and high resolution ESI-MS mass analyses were carried out in CNRS at Gif-sur-Yvette. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker ARX-250 spectrometer or AVANCEII-500, with chemical shifts reported in parts per million (ppm) relative to the residual solvent (CH_3CN, $\delta_H = 1.96$ ppm). Cyclic voltammograms were obtained in deaerated CH_3CN solution at room temperature using an EGGR-PAR model 173 potentiostat and model interface instruments with a three electrode system which consists of a NaCl saturated calomel electrode (SCE), a platinum auxiliary electrode, and a glassy carbon working electrode. Tetrabutylammonium hexafluorophosphate (NBu_4PF_6, 0.1 M in CH_3CN) was used as the supporting electrolyte. The potential sweep rate was 50 mV.s^{-1}, and under our conditions the potential of the ferrocene/ferrocenium ion couple was 0.38 V. EPR spectra were recorded on a Bruker Elexsys 500 EPR spectrometer operating at X-band frequency (9.44 GHz) equipped with a shq0011 cavity fitted with an Oxford Instrument liquid helium probe. The following instrument settings were used: field modulation amplitude frequency of 100 kHz, field modulation amplitude of 0.1 mT, time constant of 0.04 s, sampling time 41 ms, 8192 sampling points, field sweep 1 T, microwave power of 10 mW. Xsophe has been used for EPR spectra simulation of the non saturated experimental spectrum after subtraction of a solvent tube spectrum. The g, D (E/D), and A values are those found to reasonably fit the experimental spectrum with the calculated one using a g and A strain line width mode.

Materials. CH_3CN, DMF, and MeOH were dried and distilled before use, following standard procedures. Anhydrous diethyl ether was purchased from Riedel-de-Haën.

Synthesis. The bis-disulfide **1a** was synthesized according to Bourlès et al.[8] *N,N''*-1,2-phenylenebis(2-mercapto-2-methylpropanamide) was prepared according to Chatel et al.[18] and complexes **2**, **6**, and **5** according to Hanss and Krüger,[10] and Chatel et al.,[18] respectively. *tert*-Butyl sulfenyl iodide in solution in CCl_4 was prepared as described by Field et al.[32] The concentration of the solution was estimated by ^{1}H NMR using dioxane ($\delta = 3.57$ ppm) as an internal standard ($\delta = 1.45$ ppm for *t*BuSI).

Synthesis of *S-tert*-butyl methane Thiosulfonate. To a solution of *t*BuSH (100 μL, 1.58 mmol) in dry CH_2Cl_2 was added triethylamine (1.1 equiv, 244 μL, 1.74 mmol); then the reaction mixture was cooled to 0 °C, and a solution of methane sulfonyl chloride, CH_3SO_2Cl, (1.1 equiv, 1.74 mmol, 134 μL) in 3 mL of CH_2Cl_2 was added over 20 min. After stirring for 16 h, the solution was successively washed with water, saturated aqueous $NaHCO_3$ solution, then water, and dried over $MgSO_4$. After evaporating the solvents, CH_3SO_2StBu was isolated as a white oil (186 mg, 70% yield) and characterized by its ^{1}H NMR as previously described: ^{1}H NMR (δ, $CDCl_3$): 3.37 (s, 3H), 1.63 (s, 18H).[33]

General Procedure for the Synthesis of the Acyclic Bis-Disulfide Ligands. One equivalent of triethylamine (99 μL, 0.71 mmol) was added to a CH_2Cl_2 solution (5 mL) of the alkyl methane thiosulfonate (0.71 mmol, 72 μL of *S*-methyl methanethiosulfonate or 119 mg of *S-tert*-butyl methanethiosulfonate). After cooling the solution to 0 °C, a solution of 0.45 equiv of *N,N'*-1,2-phenylenebis(2-mercapto-2-methylpropanamide) (100 mg, 0.32 mmol) in CH_2Cl_2 (8 mL) was added dropwise over 30 min, and the reaction mixture was allowed to warm to room temperature and was stirred for 2 h. Volatiles were then removed under vacuo. The residue was purified by column chromatography (silica gel, 90/10 (v:v) CH_2Cl_2/EtOAc mixture) and was precipitated in diethyl ether yielding the pure product as a white powder.

2-Methyl-*N*-(2-(3-methyl-3-(methyldisulfanyl)butan-2-ylamino)-phenyl)-2-(methyldisulfanyl)propanamide (1b). Yield: 103 mg (80%). Anal. Calcd (found) for $C_{16}H_{24}N_2O_2S_4$: C, 47.37 (47.51); H, 5.98 (5.84); N, 6.92 (7.01). HRMS (CI$^+$, CH_4, *m*/*z*): Calcd (found) for [$C_{16}H_{24}N_2O_2S_4$ + Na^+]: 427.0618 (427.0621). ^{1}H NMR (δ, CD_3CN): 8.67 (s, 2H), 7.53 (m, 2H), 7.27 (m, 2H), 2.43 (s, 6H), 1.66 (s, 12). ^{13}C NMR (CD_3CN): 173.2, 131.3, 126.4, 125.7, 54.0, 25.3, 25.1. FT-IR (ATR, cm^{-1}): 1666.

2-(*tert*-Butyldisulfanyl)-*N*-(2-(3-(*tert*-butyldisulfanyl)-3-methyl-butan-2-ylamino)phenyl)-2-methylpropanamide (1c). 125 mg, (80%). HRMS (CI$^+$, CH_4, *m*/*z*): Calcd (found) for [$C_{22}H_{36}N_2O_2S_4$ + Na^+]: 511.1557 (511.1571). ^{1}H NMR (δ, CD_3CN): 8.80 (s, 2H), 7.54 (m, 2H), 7.25 (m, 2H), 1.65 (s, 12), 1.32 (s, 18H). ^{13}C NMR (CD_3CN): 173.4, 131.3, 126.2, 125.6, 56.1, 47.7, 30.2, 25.8. FT-IR (ATR, cm^{-1}): 1663.

Reductive Metalation of the Bis-disulfide 1a-1c with (PF_6)[Cu(CH_3CN)$_4$]. From 1a. In an acetonitrile solution (5 mL) of bis-disulfide (310 mg, 0.5 mmol) were added successively under argon tetrakis(acetonitrile)copper(I)hexafluorophosphate (PF_6)[Cu(CH_3CN)$_4$] (372 mg, 1 mmol) and tetraethylammonium hydroxide in methanol (2.0 mmol, 1.43 mL of a 1.4 M solution in methanol). After stirring for 2 h at room temperature, the solvent was removed. The crude product was dissolved in acetone (2 mL), filtered over Celite, and the solvent evaporated to dryness. After dissolving the product in the minimum amount of acetonitrile (1 mL), the complex was precipitated several times in cold diethyl ether, and pure compound **2** was isolated as a greenish yellow powder in a quantitative yield. Analyses were identical to those reported in the literature:[10] UV (CH_3CN) λ_{max}: 417 nm (ε_M = 6130 M^{-1} cm^{-1}); ^{1}H NMR(δ, CD_3CN): 8.48 (dd, 2H, $^3J_{H-H} = 6.3$ Hz and $^4J_{H-H} = 3.5$ Hz), 6.84 (dd, 2H, $^3J_{H-H} = 6.3$ Hz and $^4J_{H-H} = 3.5$ Hz), 3.18 (q, 8H, $^3J_{H-H} = 7.3$ Hz), 1.51 (s, 12H), 1.23 (t, 12H, $^3J_{H-H} = 7.3$ Hz); MS(ESI-): 371 (100%, [**2**-Et_4N]$^-$); $E_{1/2}$ (CH_3CN): −1.16 V versus the ferrocene/ferrocenium (Fc/Fc$^+$) redox couple.

From 1b or 1c. Using a similar procedure, 250 mg of **2** (100%) were obtained starting from **1b** (202 mg, 0.5 mmol) or **1c** (244 mg, 0.5 mmol) upon reaction with (PF_6)[Cu(CH_3CN)$_4$] (1 equiv, 184 mg, 0.5 mmol) and Et_4NOH (1.4 M in methanol, 2 equiv, 1.0 mmol, 707 μL). From **1b** the dimethyl disulfide **3b** also formed and was identified by GC, and from **1c**, the di-*tert*-butyl disulfide **3c** also formed and was identified by ^{1}H NMR (*t*Bu: 1.34 ppm in CD_3CN).

Reductive Metalation of 1b with Triiron Dodecacarbonyl. To a solution of **1b** (100 mg, 0.25 mmol) in 2 mL of DMF were successively added under argon Fe_3CO_{12} (43 mg, 0.08 mmol),

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[29] Galardon, E.; Tomas, A.; Selkti, M.; Roussel, P.; Artaud, I. *Inorg. Chem.* **2009**, *48*, 5921–5927.

[30] Alves de Sousa, R.; Artaud, I. *Tetrahedron* **2008**, *64*, 2198–2206.

[31] (a) Bourlès, E.; Alves de Sousa, R.; Galardon, E.; Artaud, I. *Angew. Chem., Int. Ed.* **2005**, *44*, 6162–6165. (b) Galardon, E.; Bourlès, E.; Artaud, I.; Daran, J.-C.; Roussel, P.; Tomas, A. *Inorg. Chem.* **2007**, *46*, 4515–4522.

[32] Field, L.; Vanhorne, J. L.; Cunningham, L. W. *J. Org. Chem.* **1970**, *35*, 3267–3273.

[33] Block, E.; Bentley, M. D.; Davis, F. A.; Douglass, I. B.; Lacadielb, J. A. *J. Org. Chem.* **1975**, *40*, 2770–2773.

sodium methoxide (1.0 M in methanol, 0.50 mmol, 500 μL), and Et_4NCl (82 mg, 0.50 mmol). After stirring for 4 h at 60 °C, the solvents were removed, and the crude product purified by exclusion chromatography (Sephadex LH-20 eluted with MeOH). The brown fraction was collected and found to have the same spectroscopic properties as **5**[18] (55 mg, 33% yield).

Reductive Metalation of 1a with (Et_4N)[Fe(SEt)$_4$]. To a solution of **1a** (20 mg, 0.032 mmol) in DMF (1 mL) was added under argon [(Et_4N)][Fe(SEt)$_4$] (27.5 mg, 0.064 mmol) followed by tetraethylammonium chloride (10.6 mg, 0.064 mmol). After stirring for 5 h, the solvent was removed, and the resulting orange powder analyzed as **5** (49 mg, quantitative yield).

Synthesis of the Bis-disulfides 1a and 1b by Reaction of Complex 5 with Iodine or Iodine and Dimethyl Disulfide. To a solution of complex **5** (100 mg, 0.15 mmol) in CH_3CN (2 mL) were successively added under argon iodine alone (38 mg, 0.15 mmol) or dimethyl disulfide (266 μL, 3 mmol) and iodine (38 mg, 0.15 mmol). The mixture was stirred for 16 h at room temperature, and the solvent removed. The crude product was dissolved in dichloromethane and washed abundantly with water, aqueous sodium thiosulfate (0.05 M), and water, and then was dried over $MgSO_4$. After removing the solvent to dryness, the products were purified over column chromatography and identified by comparing their ^{1}H NMR data with those of authentic samples prepared in this work (**1b**) or previously published (**1a**).[8] Treatment of **5** with I_2 afforded only **1a**, that was purified over chromatography (silica gel, 80/20 (v:v) CH_2Cl_2/EtOAc mixture) and isolated as a white powder (38 mg, 80% yield). Treatment of **5** with dimethyl disulfide **3b** and iodine afforded a mixture of **1b** and **1a** that was separated by elution over silica gel with a 90/10 (v:v) mixture of CH_2Cl_2/EtOAc for **1b** and a 80/20 mixture for **1a**. After removal of the solvents, **1b** (30 mg, 50% yield) and **1a** (22 mg, 45% yield) were isolated as white powders.

Synthesis of the Bis-disulfide 1c by Reaction of Complexes 2 or 5, with *tert*-Butyl Sulfenyl Iodide. To an acetonitrile solution (3 mL) of complexes **2** (100 mg, 0.2 mmol) or **5** (100 mg, 0.15 mmol) was added under argon an excess (10 equiv) of a 0.13 M solution of *tert*-butyl sulfenyl iodide in CCl_4 (2 mmol, 15 mL for **2** and 1,5 mmol, 11 mL for **5**). The resulting mixture was stirred for 16 h at room temperature in a dark room. The solvent was removed, and after dissolving the crude product in CH_2Cl_2, the solution was washed abundantly with water, sodium thiosulfate (0.05 M), and water, and was then dried over $MgSO_4$. Evaporation of the solvent to dryness gave **1c** as a white powder in a quantitative yield (98 mg). ^{1}H NMR and mass spectra of **1c** were identical to those of an authentic sample prepared in this work.

NMR Studies. Reactions from Complex 2. In a deuterated acetonitrile solution (0.5 mL) of complex **2** (10 mg, 0.02 mmol) were added 1 or 2 equiv of methyl disulfide as well as 1, 1.5, 2, or 3 equiv of iodine. The conversion yield of complex **2** was determined after 22 h by ^{1}H NMR by comparing the integration ratio of the CH_2 signal at 3.24 ppm of the countercation of **2**, Et_4N^+, used as an internal standard, and the aromatic signals of complex **2** at 6.82 ppm.

Reaction from Complex 6. All these experiments were performed in NMR tubes prepared in a glovebox under strictly anaerobic conditions. Ten milligrams of the paramagnetic complex **6** (0.016 mmol) were dissolved in 0.5 mL of CD_3CN, and 1 equiv of iodine or 1 equiv of dimethyl disulfide **3b** or 4 equiv of *t*BuSI in solution in CCl_4 were added.

Supporting Information Available: Synthesis of (Et_4N)[Fe(SEt)$_4$], Figures S1−S5 and Scheme S1 as a PDF file. This material is available free of charge via the Internet at http://pubs.acs.org.