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Reactions of a Copper(II) Superoxo Complex Lead to C–H and O–H Substrate Oxygenation: Modeling Copper-Monooxygenase C–H Hydroxylation**

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Mononuclear species derived from copper(I) and dioxygen such as cupric superoxide $Cu^{II}(O_2^{\bullet -})$, cupric hydroperoxide $Cu^{II}(OOH^-)$, or even high-valent copper oxo species have all been considered as possible active-site reactive intermediates in the copper monooxygenases dopamine β-monooxygenase (DβM) and peptidylglycine α-hydroxylating monooxygenase (PHM).[1] These enzymes effect neurohormone and neurotransmitter biosynthesis through active-site substrate C–H hydroxylation, which involves H-atom abstraction. However, synthetic investigations have to date revealed only very limited substrate reactivity with $Cu^{II}(O_2^{\bullet -})$ or $Cu^{II}(OOH^-)$ complexes,[2] especially with C–H-containing substrates. There are as yet no discrete examples of, nor evidence for, high-valent copper oxo species $Cu^{II}O^{\bullet -}$ ($\leftrightarrow Cu^{III}{=}O$)[3] or $Cu^{III}O^{\bullet -}$ (i.e. $\{CuO\}^{2+}$).[1e] On the basis of a recent X-ray structure of PHM,[4] an entity with η^1-coordination (end-on) of a superoxo ligand to copper(II) is formulated and suggested to also apply to DβM. Such a cupric superoxo complex capable of effecting an enzymatic substrate hydroxylation by H-atom abstraction has drawn experimental[5] and theoretical[1d,6] support, although, as mentioned, higher-valent CuO species are predicted to be the hydroxylating agent by some researchers.[1f,g]

Synthetically derived 1:1 dioxygen–copper(I) adducts are best described as superoxo copper(II) or peroxo copper(III) complexes, with the O_2 moiety bound either in an η^1 (end-on) or η^2 (side-on) metal coordination mode.[1e,7] Recent efforts employing nitrogenous N_4 tripodal tetradentate ligands have led to the crystallographic characterization of the mononuclear $Cu^{II}(O_2^{\bullet -})$ complex with an end-on superoxo ligand $[Cu^{II}(TMG_3tren)(\eta^1\text{-}O_2^{\bullet -})]^+$ (**1**, TMG_3tren = tris(2-(N-tetramethylguanidyl)ethyl)amine; $\sphericalangle$(Cu-O-O) = 123.5°, d(Cu–O) = 1.927 Å, d(O–O) = 1.280 Å, and $\tilde{\nu}_{O\text{-}O} = 1118\ cm^{-1}$ (resonance Raman spectroscopy)), which is formed reversibly from the corresponding cuprous analogue.[8] Spectroscopic analysis showed that a related dioxygen–copper adduct, $[Cu^{II}(NMe_2\text{-}tmpa)(\eta^1\text{-}O_2^{\bullet -})]^+$ (**2**, NMe_2-tmpa = tris(4-dimethylaminopyrid-2-ylmethyl)amine), also possesses an end-on superoxo ligand; the chemistry of **2** provided the first clear demonstration of $Cu^{II}(O_2^{\bullet -})$ oxidative reactivity with exogenous substrates (substituted phenols), resulting in their oxidation, oxygenation, or hydroperoxylation.[2a] Herein, we report our initial findings concerning the reactivity of **1**. It also oxygenates or oxidizes phenols in a manner similar to that found for **2**. Of greater interest and importance is our demonstration that starting with the cupric superoxide complex **1**, addition of a H-atom ($H^\bullet$) donor leads to C–H activation, O-atom insertion into an *N*-methyl group on the TMG_3tren ligand, and formation of a copper(II) alkoxide product.

When 4-MeO-2,6-tBu$_2$-phenol was added to a solution of a newly synthesized tetraarylborate salt $[Cu^{II}(TMG_3tren)(O_2^{\bullet -})]B(C_6F_5)_4$ (**1**, excess O_2 removed)[9] at −80 °C in 2-methyltetrahydrofuran (MeTHF) and the mixture kept cold for 48 h, the color changed from the initial light green to bright green. A sharp, strong peak was observed at 407 nm in the absorption spectrum of the reaction solution, and EPR spectroscopy revealed a $g \approx 2$ signal, both of which indicate the formation of the corresponding stabilized phenoxyl radical (**B**, Scheme 1) formed in approximately 37 % yield. Other products identified in this reaction of **1** and 4-MeO-2,6-tBu$_2$-phenol are the 2,6-tBu$_2$-benzoquinone (**A**, ca. 22 % yield) and the aryl hydroperoxide (**C**). More importantly, a crystalline, green copper complex was also isolated in approximately 80 % yield; the product is the alkoxide complex $[Cu^{II}(TMG_3trenO^-)]B(C_6F_5)_4$ (**3**; ESI-MS: m/z 518.19, $[Cu^{II}(TMG_3trenO^-)]^+$). Its X-ray structure[10] reveals that hydroxylation of a ligand methyl group has

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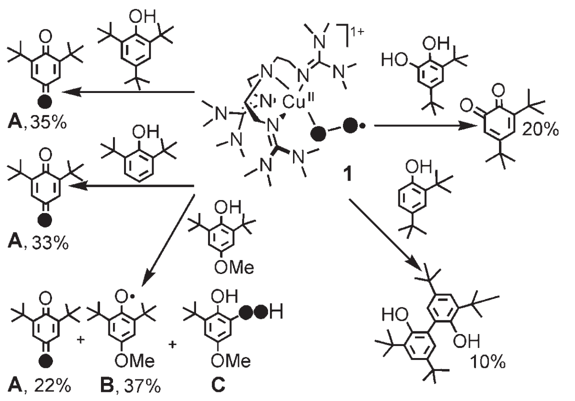


Scheme 1. Reactivity of **1** with exogenous phenolic substrates. Dark circles represent ^{18}O.

occurred, with the result that the cupric ion is now coordinated by the inserted O atom of the alkoxide (Figure 1).[11] With an $^{18}O_2$ source used in the generation of **1** and subsequent addition of 4-MeO-2,6-*t*Bu$_2$-phenol, GC-MS reveals ^{18}O incorporation into **A** and **C** (68% and 85%, respectively). For product cupric complex **3**, the ESI-MS positive ion parent peak (which shows the expected ^{63,65}Cu isotope pattern) shifts to *m/z* 520.27 (99% incorporation), thus indicating that $[Cu^{II}(TMG_3tren^{18}O^-)]^+$ has formed; the alkoxide O atom is derived from dioxygen.[9]

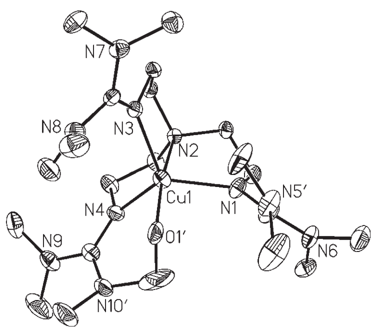


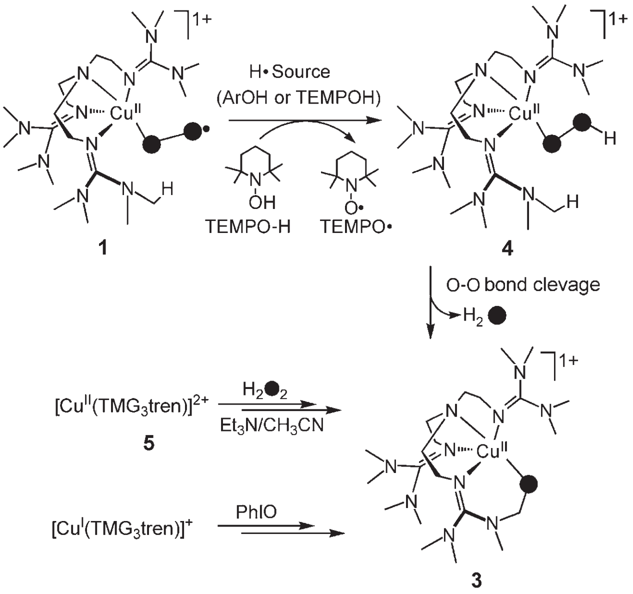
Figure 1. X-ray structure of $[Cu^{II}(TMG_3trenO^-)]B(C_6F_5)_4$ (**3**) with near trigonal-bipyramidal copper-ion coordination. Selected bond lengths [Å] and angles [°]: Cu1–O1′ 1.972(5), Cu1–N2 2.091(2), Cu1–(N1,N3,N4) 2.053–2.117; O1′-Cu1-N2 170.01(16).

The observed oxidative reactivity of $[Cu^{II}(TMG_3tren)(O_2^{\bullet -})]^+$ (**1**) is similar to the Cu$_M$-centered action at the PHM active site (Cu$_M$ has His$_2$Met coordination), in which oxygenation of a prohormone peptide substrate C–H group adjacent to an amide nitrogen atom occurs.[1c] Furthermore, our cupric alkoxide complex **3** mimics the "product complex" discussed for both PHM and DβM,[1c] which is formed in the enzyme in the step just prior to product release. When **1** is warmed from −80 °C to room temperature, O$_2$ is released, giving the cuprous compound $[Cu^I(TMG_3tren)]^+$.[12] Thus, the cupric superoxo complex itself is not capable of the observed hydroxylation reaction. As described above, reaction of **1** with a phenol is required.

Superoxo complex **1** also reacts with other phenols to give oxidation products similar to those seen for **2** (Scheme 1).[2a] As determined by low-temperature reactions and subsequent warming and workup, 2,6-*t*Bu$_2$-phenol and 2,4,6-*t*Bu$_3$-phenol produce benzoquinone **A** (Scheme 1, 33 and 35%, respectively). With $^{18}O_2$-labeled **1**, approximately 65% ^{18}O-atom incorporation into **A** occurs for the reaction of **1** and 2,4,6-*t*Bu$_3$-phenol. With 2,4-*t*Bu$_2$-phenol, the typical[13] oxidative coupling product 4,4′,6,6′-*t*Bu$_4$-2,2′-biphenol (10%) is observed. Reaction of 3,5-*t*Bu$_2$-catechol with **1** leads to the corresponding benzoquinone (20%, Scheme 1).[9]

In fact, all of the reactions of **1** with these other phenols also lead to substantial yields (65% or more)[9] of ligand-hydroxylated copper(II) alkoxide $[Cu^{II}(TMG_3trenO^-)]^+$ (**3**), as determined by comparison of authentic **3** (see above) ESI-MS and EPR spectroscopic signatures of the dark green crystalline solids obtained. As we concluded for the chemistry of **2** with phenols,[2a] the products observed with **1** (Scheme 1) can be explained by initial phenol-substrate H-atom abstraction and subsequent addition of the superoxo complex to the ArO$^\bullet$ species. We suggest that alkoxide complex **3** arises from the course of reaction $\{Cu^{II}(O_2^{\bullet -})\} + ArOH \rightarrow \{Cu^{II}(OOH^-)\} + ArO^\bullet$, wherein a highly reactive cupric hydroperoxo species is produced in close proximity to the TMG$_3$tren *N*-methyl group.

To further test this hypothesis, we used a H-atom donor that might not itself react further. Reaction of **1** with TEMPO-H (Scheme 2) at −80 °C or below (i.e. to −120 °C) in MeTHF over one hour produces a dark green solution. This process leads to disappearance of the characteristic bands ascribed to **1** (447, 680, 780 nm; Figure 2a),[8b] while a new absorption at approximately 350 nm (sh) appears. The presence of an isosbestic point suggests that **1** directly converts to the new complex formulated as hydroperoxo complex $[Cu^{II}(TMG_3tren)(OOH^-)]^+$ (**4**, Scheme 2). Support for this supposition comes from EPR spectroscopy. During



Scheme 2. Varying pathways to produce hydroxylated product **3**.

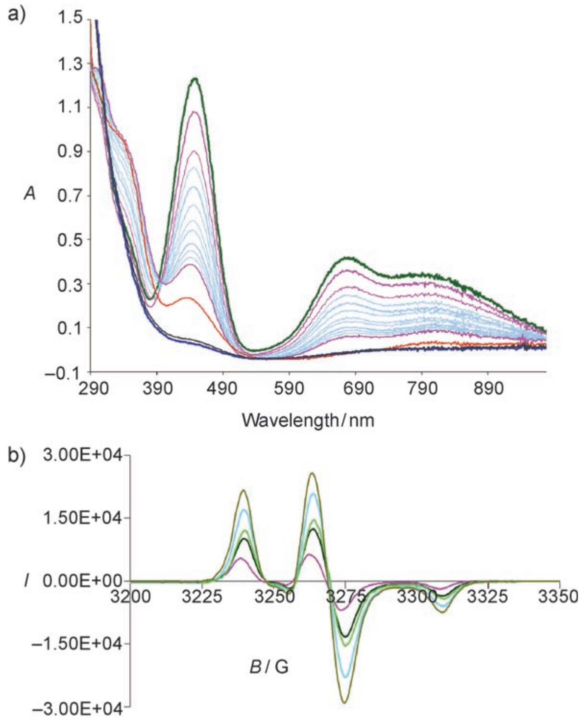


Figure 2. a) UV/Vis and b) EPR spectroscopic monitoring of the reaction of [Cu^{II}(TMG$_3$tren)(O$_2^{\bullet-}$)]$^+$ (**1**) with TEMPO-H. In (a), the starting spectrum is green and the final spectrum is dark blue; in (b), the starting spectrum is pink and the final spectrum in light green.

the UV/Vis monitoring at −80 °C, aliquots of the solution were transferred to EPR sample tubes and immediately frozen at 77 K. The EPR spectra thus obtained (as a function of time) confirm the increasing intensity of the signal arising from TEMPO$^\bullet$ as the reaction progresses (Figure 2b). Comparison of EPR instensity to authentic TEMPO$^\bullet$ suggests the reaction yield to be approximately 90%. Using ESI-MS or EPR spectroscopy to characterize reaction solutions from which the TEMPO$^\bullet$ product has been separated by extraction,[9] the formation of [Cu^{II}(TMG$_3$trenO$^-$)]$^+$ (**3**, Scheme 2) was confirmed by comparison of data to the crystallographically characterized **3** (see above). The results thus strongly support the view that a hydroperoxo complex derived from **1** forms and is active in the hydroxylation of the *N*-methyl ligand.

More support comes from the finding that the same chemistry occurs by treating a copper(II) analogue with hydrogen peroxide (Scheme 2). The complex [Cu^{II}(TMG$_3$tren)]$^{2+}$ (**5**) was synthesized as the bis-perchlorate salt and isolated after reaction of Cu^{II}(ClO$_4$)$_2$·6H$_2$O with the ligand TMG$_3$tren in acetonitrile. Addition of a small excess of H$_2$O$_2$/Et$_3$N to a greenish-blue acetonitrile solution of **5** at −40 °C rapidly leads to a dark green product solution, which, after workup, was confirmed to be authentic **3**. The formation of ^{18}O-labeled **3** (as determined by ESI-MS) when H$_2{}^{18}$O$_2$ was employed in this reaction[9] further implicates formation of a Cu^{II}(OOH$^-$) species that leads to the hydroxylation.

In light of the possibility that high-valent copper oxo species may be involved in copper-mediated O$_2$ activation (see above), we carried out the reaction of iodosylbenzene with the cuprous complex [Cu^{I}(TMG$_3$tren)]$^+$.[12] PhIO and its analogues have been extensively employed as oxo-transfer reagents to generate high-valent metal oxo complexes through reactions with reduced species (i.e. PhIO + [M^{n+}-(ligand)]→[M^{n+2}=(O^{2-})(ligand)] + PhI; M = heme, non-heme Fe, Mn, etc.).[14] Interestingly, [Cu^{I}(TMG$_3$tren)]$^+$ [12] also produces alkoxo species **3** in near quantitative yields upon reaction with PhIO (Scheme 2).[9,15] This observation suggests the possibility that a high-valent Cu oxo (or perhaps a Cu(OIPh) species)[16] forms during the hydroxylation reaction.

In summary, we have described a novel example of a copper–dioxygen adduct that undergoes a reaction reminiscent of certain copper monooxygenases, that is, O-atom transfer from a dioxygen-derived species to the *N*-methyl group of the ligand in [Cu^{II}(TMG$_3$tren)(O$_2^{\bullet-}$)]$^+$ (**1**). The hydroxylated product has been captured as the alkoxide complex [Cu^{II}(TMG$_3$trenO$^-$)]$^+$ (**3**). The Cu^{II}(O$_2^{\bullet-}$) moiety in **1** is not able to effect this reaction, but when a hydrogen-atom donor (i.e. a phenol or TEMPO-H) is added, the hydroxylation reaction occurs. A hydroperoxo complex [Cu^{II}-(TMG$_3$tren)(OOH$^-$)]$^+$ (**4**) is thus implicated as the active species formed by such a reaction,[11] as further supported by the observation that ligand hydroxylation occurs when [Cu^{II}-(TMG$_3$tren)]$^{2+}$ (**5**) is subjected to basic hydrogen peroxide.

Thus, while these [Cu^{II}(ligand)(O$_2^{\bullet-}$)]$^+$ complexes (**1** and **2**) can effect phenolic H-atom abstractions and subsequent phenol oxidation or oxygenation, a copper(II) hydroperoxo complex (but not an analogue with an end-on superoxo ligand) can effect C−H activation, that is, O$_2$-derived hydroxylation of a methyl group. As we have suggested in reports concerning the chemistry of mono- and dinuclear copper(II) hydroperoxo complexes,[2b,c,17] the true oxidant may be the (Cu^{II})$_n$OOH moiety or a species derived from it, such as a high-valent copper oxo moiety (see above).[1e,3] This possibility is hinted at but is certainly not proven by the results of reactions using PhIO. Additional experimental and theoretical research is required to provide deeper insights into the dioxygen activation chemistry described.

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[1] a) J. P. Klinman, *Chem. Rev.* **1996**, *96*, 2541–2561; b) M. A. McGuirl, D. M. Dooley in *Encyclopedia of Inorganic Chemistry, Vol. II*, 2nd ed. (Ed.: R. B. King), Wiley, Chichester, **2005**, pp. 1201–1225; c) J. P. Klinman, *J. Biol. Chem.* **2005**, *281*, 3013–3016; d) P. Chen, E. I. Solomon, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 13105–13110; e) S. Itoh, *Curr. Opin. Chem. Biol.* **2006**, *10*, 115–122; f) K. Yoshizawa, N. Kihara, T. Kamachi, Y. Shiota, *Inorg. Chem.* **2006**, *45*, 3034–3041; g) A. Crespo, M. A. Marti, A. E. Roitberg, L. M. Amzel, D. A. Estrin, *J. Am. Chem. Soc.* **2006**, *128*, 12817–12828.

[2] a) D. Maiti, H. C. Fry, J. S. Woertink, M. A. Vance, E. I. Solomon, K. D. Karlin, *J. Am. Chem. Soc.* **2007**, *129*, 264–265; b) D. Maiti, H. R. Lucas, A. A. N. Sarjeant, K. D. Karlin, *J. Am. Chem.*

Soc. **2007**, *129*, 6998 – 6999; c) D. Maiti, A. A. Narducci Sarjeant, K. D. Karlin, *J. Am. Chem. Soc.* **2007**, *129*, 6720 – 6721; d) M. Mizuno, K. Honda, J. Cho, H. Furutachi, T. Tosha, T. Matsumoto, S. Fujinami, T. Kitagawa, M. Suzuki, *Angew. Chem.* **2006**, *118*, 7065 – 7068; *Angew. Chem. Int. Ed.* **2006**, *45*, 6911 – 6914; e) T. Fujii, S. Yamaguchi, Y. Funahashi, T. Ozawa, T. Tosha, T. Kitagawa, H. Masuda, *Chem. Commun.* **2006**, 4428 – 4430; f) S. Yamaguchi, H. Masuda, *Sci. Technol. Adv. Mater.* **2005**, *6*, 34 – 47; g) T. Fujii, A. Naito, S. Yamaguchi, A. Wada, Y. Funahashi, K. Jitsukawa, S. Nagatomo, T. Kitagawa, H. Masuda, *Chem. Commun.* **2003**, 2700 – 2701.

[3] a) A. Decker, E. I. Solomon, *Curr. Opin. Chem. Biol.* **2005**, *9*, 152 – 163; b) D. Schröder, M. C. Holthausen, H. Schwarz, *J. Phys. Chem. B* **2004**, *108*, 14407 – 14416; c) Y. Nakao, K. Hirao, T. Taketsugu, *J. Chem. Phys.* **2001**, *114*, 7935 – 7940.

[4] S. T. Prigge, B. Eipper, R. Mains, L. M. Amzel, *Science* **2004**, *304*, 864 – 867.

[5] a) J. P. Evans, K. Ahn, J. P. Klinman, *J. Biol. Chem.* **2003**, *278*, 49691 – 49698; b) A. T. Bauman, E. T. Yukl, K. Alkevich, A. L. McCormack, N. J. Blackburn, *J. Biol. Chem.* **2006**, *281*, 4190 – 4198.

[6] a) P. Chen, K. Fujisawa, E. I. Solomon, *J. Am. Chem. Soc.* **2000**, *122*, 10177 – 10193; b) B. Gherman, D. Heppner, W. Tolman, C. Cramer, *J. Biol. Inorg. Chem.* **2006**, *11*, 197.

[7] C. Cramer, W. Tolman, *Acc. Chem. Res.* **2007**, *40*, 601 – 608.

[8] a) C. Würtele, E. Gaoutchenova, K. Harms, M. C. Holthausen, J. Sundermeyer, S. Schindler, *Angew. Chem.* **2006**, *118*, 3951 – 3954; *Angew. Chem. Int. Ed.* **2006**, *45*, 3867 – 3869; b) M. Schatz, V. Raab, S. P. Foxon, G. Brehm, S. Schneider, M. Reiher, M. C. Holthausen, J. Sundermeyer, S. Schindler, *Angew. Chem.* **2004**, *116*, 4460 – 4464; *Angew. Chem. Int. Ed.* **2004**, *43*, 4360 – 4363.

[9] See the Supporting Information.

[10] Experimental details of the structure determination can be found in the Supporting Information. CCDC-661204 (**3**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[11] Henkel and co-workers have also recently reported an example of O_2 activation and methyl-group hydroxylation in binculear copper complexes with bis(guanidine) ligands: a) S. Herres, A. J. Heuwing, U. Florke, J. Schneider, G. Henkel, *Inorg. Chim. Acta* **2005**, *358*, 1089 – 1095; b) S. Herres-Pawlis, U. Florke, G. Henkel, *Eur. J. Inorg. Chem.* **2005**, 3815 – 3824.

[12] V. Raab, J. Kipke, O. Burghaus, J. Sundermeyer, *Inorg. Chem.* **2001**, *40*, 6964 – 6971.

[13] L. Quant Hatcher, K. D. Karlin, *J. Biol. Inorg. Chem.* **2004**, *9*, 669 – 683.

[14] a) J. L. McLain, J. Lee, J. T. Groves, *Biomimetic Oxid. Catal. Transition Met. Complexes* **2000**, 91 – 169; b) L. Que, Jr., *Acc. Chem. Res.* **2007**, *40*, 493 – 500; c) W. J. Song, M. S. Seo, S. DeBeer George, T. Ohta, R. Song, M. J. Kang, T. Tosha, T. Kitagawa, E. I. Solomon, W. Nam, *J. Am. Chem. Soc.* **2007**, *129*, 1268 – 1277; d) K. Qin, C. D. Incarvito, A. L. Rheingold, K. H. Theopold, *J. Am. Chem. Soc.* **2002**, *124*, 14008 – 14009.

[15] E. V Gauchenova, Dissertation, Philipps-Universität Marburg, Germany, **2006**; accessible online at http://archiv.ub.uni-marburg.de/diss/z2006/0155/pdf/devg.pdf.

[16] D. P. Goldberg, *Acc. Chem. Res.* **2007**, *40*, 626 – 634.

[17] a) L. Li, A. A. N. Sarjeant, M. A. Vance, L. N. Zakharov, A. L. Rheingold, E. I. Solomon, K. D. Karlin, *J. Am. Chem. Soc.* **2005**, *127*, 15360 – 15361; b) K. Itoh, H. Hayashi, H. Furutachi, T. Matsumoto, S. Nagatomo, T. Tosha, S. Terada, S. Fujinami, M. Suzuki, T. Kitagawa, *J. Am. Chem. Soc.* **2005**, *127*, 5212 – 5223.