Tetrahedron Letters 55 (2014) 2146–2149

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis of ^{15}N-labeled 4-oxo-2,2,6,6-tetraethylpiperidine nitroxide for EPR brain imaging

Xiaolei Wang [a], Miho Emoto [b], Atsushi Sugimoto [a], Yusuke Miyake [c], Kaori Itto [a], Mitsuo Amasaka [c], Shu Xu [a], Hiroshi Hirata [c], Hirotada Fujii [b], Hirokazu Arimoto [a,*]

[a] Department of Biomolecular Sciences, Graduate School of Life Sciences, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan
[b] Department of Arts and Sciences, Center for Medical Education, Sapporo Medical University, South 1, West 17, Chuo-ku, Sapporo 060-8556, Japan
[c] Division of Bioengineering and Bioinformatics, Graduate School of Information Science and Technology, Hokkaido University, North 14, West 9, Kita-ku, Sapporo 060-0814, Japan

ARTICLE INFO

Article history:
Received 22 December 2013
Revised 11 February 2014
Accepted 18 February 2014
Available online 25 February 2014

Keywords:
Nitroxide radical
EPR imaging
Synthesis
^{15}N-labeling

ABSTRACT

A scalable synthetic route for ^{15}N-labeled 4-oxo-2,2,6,6-tetraethylpiperidine nitroxide (^{15}N-TEEPONE) is described. This ^{15}N-labeled nitroxide is suitable for electron paramagnetic resonance imaging of brain, and its higher sensitivity compared with that of its ^{14}N-counterpart is an important advantage of the labeled derivative.

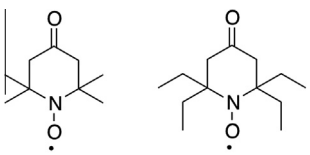
Introduction

Electron paramagnetic resonance (EPR) imaging is a powerful tool for investigating aspects of cellular and tissue environments, such as the partial oxygen pressure, redox status, and pH. Nitroxide radicals[1,2] are often used as redox-sensitive contrast agents for non-invasive EPR imaging of the redox status of tumors because of their paramagnetic properties and interesting redox properties.[3,4]

In vivo EPR imaging of the brain is also a promising area of research. However, spin probes must be blood–brain barrier (BBB) permeable for this application, and only a few simple nitroxides (e.g., 3-hydroxymethyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (HMP), and methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (MCP)) have been employed.[5–7] An additional challenge in EPR brain imaging is the rapid reduction of these nitroxide radicals to the corresponding hydroxylamines by reductants such as ascorbic acid (AsA).[8,9]

Many efforts have been made to improve the stability of nitroxides toward bioreduction by varying the steric and electronic environments around the N—O moiety.[10,11] For example, 4-oxo-2,2,6,6-tetraethylpiperidine nitroxide (TEEPONE) is the most promising probe with respect to its resistance to AsA degradation in comparison to other spin probes such as 4-oxo-2,2,6,6-tetramethylpiperidine nitroxide (TEMPONE). In EPR and magnetic resonance imaging of mouse brains, TEEPONE showed a remarkably long in vivo half life of greater than 80 min.[12,13] While TEEPONE exhibits good resistivity to bioreduction in in vivo systems, a higher signal intensity is desirable for EPR imaging with a better signal-to-noise (S/N) ratio. Therefore, to fulfill this condition, ^{15}N-labeling of TEEPONE was performed in this study.

In EPR spectroscopy, the two isotopes of nitrogen (^{14}N and ^{15}N) give rise to different splitting patterns in an absorption spectrum because of the differences in their nuclear spins. Thus, the signal for a nitroxide radical containing ^{15}N is a doublet, while that for the ^{14}N analogue is a triplet. The reduction in the spectral multiplicity of ^{15}N derivatives results in higher sensitivity, and thus, ^{15}N-labeled compounds are more useful for in vivo EPR imaging.



TEMPONE TEEPONE

* Corresponding author. Tel.: +81 22 217 6201; fax: +81 22 217 6204.
E-mail address: arimoto@biochem.tohoku.ac.jp (H. Arimoto).

http://dx.doi.org/10.1016/j.tetlet.2014.02.063
0040-4039/

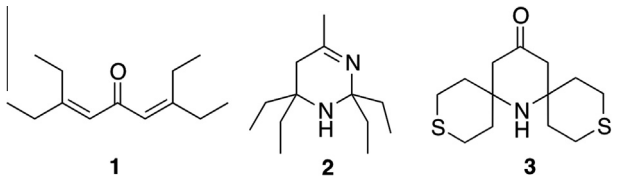
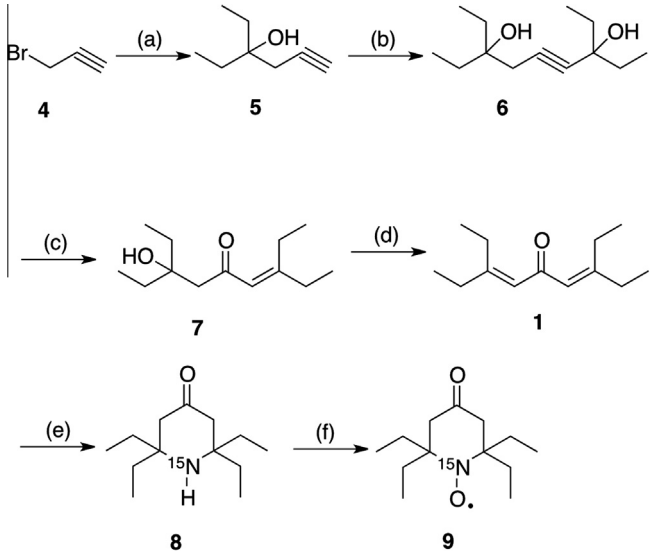


Figure 1. Previously reported intermediates for the preparation of ^{14}N-TEEPONE.



Scheme 1. Synthesis of ^{15}N-TEEPONE. Reagents and conditions: (a) 3-pentanone (1.1 equiv), Zn (1.5 equiv), I_2 (cat.), Et_2O/PhH (3:1), rt, 30 min; (b) 3-pentanone (1.1 equiv), NaH (1.2 equiv), Et_2O, rt, overnight, 52% over two steps; (c) AgOTf (0.1 equiv), DIPEA (1.0 equiv), CO_2 (3 atm), CH_2Cl_2, rt, 24 h, 83%; (d) concd H_2SO_4 (cat.), $CHCl_3$, 60 °C, 1 h, 80%; (e) $^{15}NH_4Cl$ (6.5 equiv), KOH (6.5 equiv), EtOH/H_2O (3:4), sealed tube, 3 days, 40% (73% brsm); (f) $Na_2WO_4{\cdot}2H_2O$ (0.29 equiv), H_2O_2 (6.0 equiv), MeOH/H_2O (3:1), 80%. cat. = catalytic, rt = room temperature, Tf = trifluoromethanesulfonyl, DIPEA = diisopropylethylamine, brsm = based on recovered starting material.

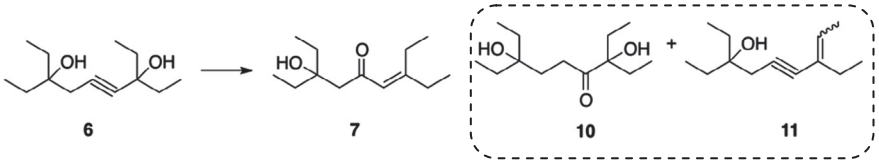
Although several different protocols have been developed for the synthesis of ^{14}N-TEEPONE, none are suitable for the gram-scale preparation of ^{15}N-TEEPONE for imaging applications, because ^{15}N sources are very expensive; thus, an effective route must involve introduction of the labeled nitrogen at the latest possible stage in the synthesis. In fact, the preparation of ^{15}N-TEEPONE has not been previously described in the literature. The Studer group used symmetric ketone **1** (Fig. 1) as an intermediate for ^{14}N-TEEPONE, and initially, we thought that this route may be a good method for the preparation of ^{15}N-TEEPONE.[14] However, in a later paper, Studer abandoned this method and disclosed that only small amounts of product were obtained.[15] His group then presented a 6-step scalable route using compound **2** as a key intermediate instead of **1**. Recently, the Yamada and Rajca groups reported a series of synthetic routes (4–6 steps)[11,16–18] for the synthesis of TEEPONE using compound **3** as an intermediate. However, these methods based on compounds **2** and **3** are inappropriate for the preparation of ^{15}N-TEEPONE because the nitrogen atom is introduced at the very beginning of the syntheses, which would result in significant loss of the expensive ^{15}N source during the later steps, making the desired product very costly.

Regardless of the negative results obtained by the Studer group, we believed that the use of intermediate **1** was essential for implementation of our late-stage nitrogen installation strategy for the gram-scale preparation of ^{15}N-TEEPONE. Herein, we report a scalable procedure for ^{15}N-TEEPONE that involves an improvement in the synthesis of TEEPONE via intermediate **1**. Furthermore, EPR imaging of a mouse head using both ^{15}N-TEEPONE and ^{14}N-TEEPONE was performed to demonstrate the advantages of labeling.

Results and discussion

Our ^{15}N-labeled TEEPONE synthesis is presented in Scheme 1. First, compound **5** was prepared according to a literature procedure starting from commercially available 3-bromo-1-propyne.[19] Compound **6** was then obtained by reacting **5** with 3-pentanone in the presence of NaH. The Meyer–Schuster rearrangement[20] (Table 1) of this propargyl alcohol to α,β-unsaturated carbonyl compound **7** via a formal 1,3-hydroxyl shift and tautomerization using either sulfuric acid in 80% acetic acid (entry 1) or silver carbonate in 80% acetic acid (entry 2) was unsuccessful.[21] It has been reported that the reaction of propargyl alcohols with carbon dioxide catalyzed by silver salts in polar solvents affords the corresponding allene-enolates.[22] This reaction was thus attempted by using compound **6**, and in the presence of 10 mol % $AgNO_3$ and 1.0 equiv of *N,N*-diisopropylethylamine under 1 atm CO_2 at room temperature, only a trace amount of the desired product was detected (entry 3). However, the use of silver triflate gave compound **7** in modest yield (entry 4), and an increased CO_2 pressure (3 atm, entry 5) further improved the yield to 83%. Next, elimination of the hydroxyl group in **7** under acidic conditions gave a mixture of desired ketone **1** and isomerized β,γ-unsaturated ketones. Heating this mixture in an $^{15}NH_4OH$ aqueous solution at 60 °C afforded piperdinone **8**. The desired ^{15}N-TEEPONE was then obtained via the oxidation of **8** with Na_2WO_4/H_2O_2. This synthetic procedure

Table 1
Preparation of compound **7** using different reaction conditions[a]



Entry	Reagents	Temp (°C)	Time (h)	Yield of **7**
1	H_2SO_4 (cat.), 80% HOAc (aq)	95	20	None
2	Ag_2CO_3 (0.05 equiv), K_2CO_3 (0.3 equiv), 80% HOAc (aq)	100	3.5	None (**6**:**10**:**11** = 1:3.7:1.5)
3	$AgNO_3$ (0.05 equiv), DIPEA (1 equiv), CH_2Cl_2, CO_2 (1 atm)	rt	20	Trace
4	AgOTf (0.1 equiv), DIPEA (1 equiv), CH_2Cl_2, CO_2 (1 atm)	rt	55	47%
5	AgOTf (0.1 equiv), DIPEA (1 equiv), CH_2Cl_2, CO_2 (3 atm)	rt	24	83%

[a] aq. = aqueous.

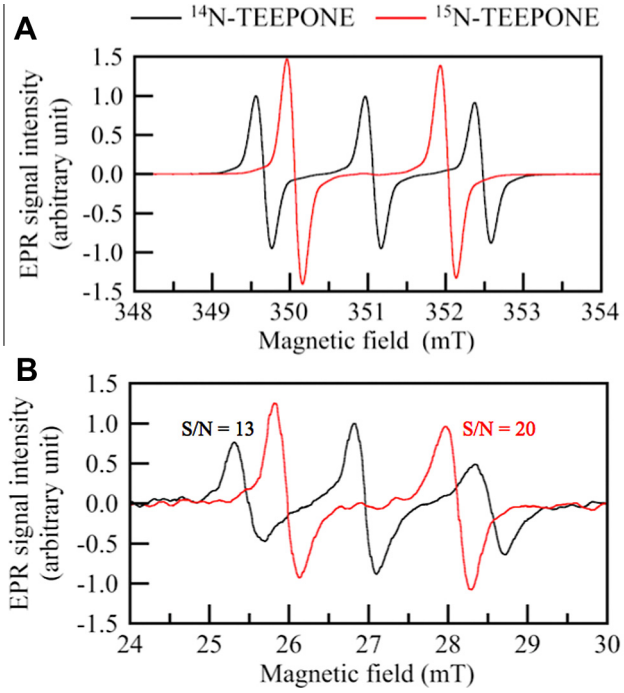


Figure 2. (A) X-band EPR spectra of 1 mM ^{14}N-TEEPONE (black line) and ^{15}N-TEEPONE (red line) in dimethyl sulfoxide in quartz tubes; (B) 750 MHz EPR spectra of ^{14}N-TEEPONE and ^{15}N-TEEPONE in mouse heads and a comparison of signal-to-noise (S/N) ratio.

was employed for the successful preparation of more than 2 g of ^{15}N-TEEPONE for use in EPR studies.

Both ^{14}N-TEEPONE and ^{15}N-TEEPONE were examined by X-band EPR spectroscopy (9.8 GHz, Fig. 2A), and it was found that the amplitude of the EPR signal for ^{15}N-TEEPONE was approximately 1.5 times greater than that of ^{14}N-TEEPONE.

Next, in vivo EPR imaging of mice was performed to demonstrate the potential of ^{15}N-TEEPONE as a spin probe. An in-house built 750 MHz CW-EPR imager was used for this experiment.[23] Both ^{15}N-TEEPONE and ^{14}N-TEEPONE were emulsified by using 10% INTRAFAT, and each probe was injected into mice via the tail vein (1.5 μmol/g weight of body). As shown in Figure 2B, ^{15}N-TEEPONE exhibited an in vivo spectrum with a better S/N ratio in a mouse head than ^{14}N-TEEPONE. The distributions of the spin probes in mouse heads were also observed via 3-dimensional (3D)-EPR imaging and the 2D-slice-selective images are depicted in Figure 3C. These images show that TEEPONE was distributed in the mouse brain, and that the in vivo half lives of ^{15}N-TEEPONE and ^{14}N-TEEPONE were both approximately 80 min (data not shown). However, the sensitivity of the instrument did not allow the monitoring of the ^{14}N-TEEPONE signals for such a long period under these conditions (see the single time-point images in Fig. 3C). In contrast, ^{15}N-TEEPONE signals were successfully observed even at 1 h after the injection (Fig. 3C). In fact, the accumulation of images over a certain time period improved their quality (Fig. 3). However, such processing prevents observation of the dynamic changes in the abundance and localization of the nitroxide. Notably, however, these results demonstrate that ^{15}N-TEEPONE signals can be monitored for longer than 1 h, and thus the use of ^{15}N-TEEPONE is quite advantageous compared to its ^{14}N

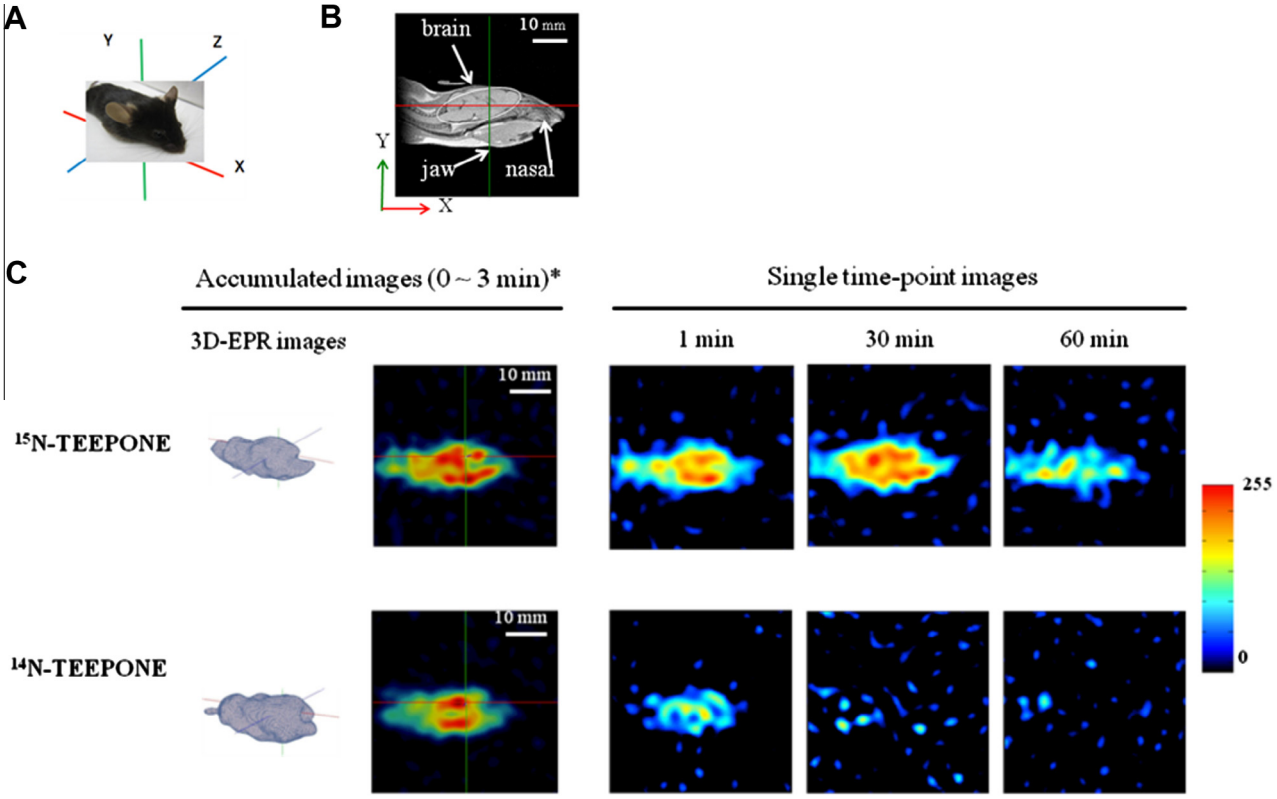


Figure 3. EPR imaging of mouse heads with injected ^{15}N-TEEPONE and ^{14}N-TEEPONE. The mice were anesthetized with isoflurane (1.5%). The EPR spectra were collected after probe injection, and the images were reconstructed from the spectra using the filtered back-projection method. (A) Photograph of a mouse head. (B) MRI image of an examined mouse head. (C) 3D-EPR images and 2D-slice-selective images of mouse heads. EPR images were obtained at the indicated time after the injection. ^{15}N-TEEPONE (upper) and ^{14}N-TEEPONE (lower). *12 sets of EPR images were accumulated.

counterpart. Moreover, ^{15}N-TEEPONE can potentially be used as a labeling agent for biologically active molecules, and thus may enable the 3D imaging of dynamic changes in the localization of intriguing molecules in the brain. In these cases, the higher sensitivity of ^{15}N-labeled probes would not only serve to enhance the quality of the images, but also lead to reduction of the injection dose. Consequently, the use of ^{15}N-TEEPONE as a labeling agent reduces the risk of undesired toxic effects in laboratory animals.

Another potential use of ^{15}N-TEEPONE may be in our recently developed 'simultaneous 3D-EPR imaging of enantiomer pairs' analytical method.[24] In this advanced technique, a pair of nitroxide radical enantiomers is labeled by using isotopic nitrogen. Because the distributions of the ^{14}N- and ^{15}N-nitroxides can be independently and simultaneously monitored by using our EPR imaging system, the difference in the pharmacokinetics of chiral molecules and their interactions in biological systems can be investigated. Because TEEPONE is one of the most stable BBB-permeable nitroxides in the brain, an ^{15}N-TEEPONE and ^{14}N-TEEPONE pair would have significant potential as labeling agents for the detailed pharmacological investigation of chiral drug candidates.

Conclusion

A scalable synthetic procedure for the preparation of ^{15}N-TEEPONE was developed, and a comparison of ^{15}N-TEEPONE and ^{14}N-TEEPONE was performed for mouse brain imaging for the first time. Notably, ^{15}N-TEEPONE provided 50% greater signal intensity than ^{14}N-TEEPONE. As a result, ^{15}N-TEEPONE was detectable for a longer time during in vivo EPR analysis, even though ^{15}N-TEEPONE and ^{14}N-TEEPONE have similar half lives. In addition, ^{15}N-TEEPONE in combination with ^{14}N-TEEPONE has great potential as a spin label precursor for use in simultaneous EPR imaging techniques.

Acknowledgments

X. W. wishes to thank the G-COE program and Campus Asia at Tohoku University for support. This study was also supported by a Grant from the Japan Society for the Promotion of Science (NEXT Program Grant LR002 to H.H.)

Supplementary data

Supplementary data (details of experimental procedures) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.02.063.

References and notes

1. Soule, B. P.; Hyodo, F.; Matsumoto, K.; Simone, N. L.; Cook, J. A.; Krishna, M. C.; Mitchell, J. B. *Free Radical Biol. Med.* **2007**, *42*, 1632–1650.
2. Hicks, R. G. *Stable Radicals: Fundamentals and Applied Aspects of Odd-Electron Compounds*; John Wiley & Sons Ltd: Chichester, 2010.
3. Hyodo, F.; Matsumoto, K.; Matsumoto, A.; Mitchell, J. B.; Krishina, M. C. *Cancer Res.* **2006**, *66*, 9921–9928.
4. Kulkarni, A. C.; Bratasz, A.; Rivera, B.; Krishna, M. C.; Kuppusamy, P. *Isr. J. Chem.* **2008**, *48*, 27–31.
5. Hyodo, F.; Chuang, K.-H.; Goloshevsky, A. G.; Sulima, A.; Griffiths, G. L.; Mitchell, J. B.; Koretsky, A. P.; Krishna, M. C. *J. Cereb. Blood Flow Metab.* **2008**, *28*, 1165–1174.
6. Yokoyama, H.; Itoh, O.; Aoyama, M.; Obara, H.; Ohya, H.; Kamada, H. *Magn. Reson. Imaging* **2002**, *20*, 277–284.
7. Shen, J.; Liu, S.; Miyake, M.; Liu, W.; Pritchard, A.; Kao, J. P. Y.; Rosen, G. M.; Tong, Y.; Liu, K. J. *Magn. Reson. Med.* **2006**, *55*, 1433–1440.
8. Kocherginsky, N.; Swartz, H. M. In *Nitroxide Spin Labels: Reactions in Biology and Chemistry*; Kocherginsky, N., Swartz, H. M., Eds.; CRC press: Boca Raton, 1995; pp 27–66.
9. Couet, W. R.; Brasch, R. C.; Sosnovsky, G.; Tozer, T. N. *Magn. Reson. Imaging* **1985**, *3*, 83–88.
10. Yamasaki, T.; Mito, F.; Ito, Y.; Pandian, S.; Kinoshita, Y.; Nakano, K.; Murugesan, R.; Sakai, K.; Utsumi, H.; Yamada, K. *J. Org. Chem.* **2011**, *76*, 435–440.
11. Paletta, J. T.; Pink, M.; Foley, B.; Rajca, S.; Rajca, A. *Org. Lett.* **2012**, *14*, 5322–5325.
12. Emoto, M.; Mito, F.; Yamasaki, T.; Yamada, K.; Sato-Akaba, H.; Hirata, H.; Fujii, H. *Free Radical Res.* **2011**, *45*, 1325–1332.
13. Emoto, M. C.; Yamada, K.; Yamato, M.; Fujii, H. G. *Neurosci. Lett.* **2013**, *546*, 11–15.
14. Wetter, C.; Gierlich, J.; Knoop, C. A.; Müller, C.; Schulte, T.; Studer, A. *Chem. Eur. J.* **2004**, *10*, 1156–1166.
15. Schulte, T.; Siegenthaler, K. O.; Luftmann, H.; Letzel, M.; Studer, A. *Macromolecules* **2005**, *38*, 6833–6840.
16. Kinoshita, Y.; Yamada, K.; Yamasaki, T.; Sadasue, H.; Sakai, K.; Utsumi, H. *Free Radical Res.* **2009**, *43*, 565–571.
17. Sakai, K.; Yamada, K.; Yamasaki, T.; Kinoshita, Y.; Mito, F.; Utsumi, H. *Tetrahedron* **2010**, *66*, 2311–2315.
18. Kinoshita, Y.; Yamada, K.; Yamasaki, T.; Mito, F.; Yamato, M.; Kosem, N.; Deguchi, H.; Shirahama, C.; Ito, Y.; Kitagawa, K.; Okukado, N.; Sakai, K.; Utsumi, H. *Free Radical Biol. Med.* **2010**, *49*, 1703–1709.
19. Gutmann, H.; Isler, O.; Ryser, G.; Zeller, P.; Pellmone, B. *Helv. Chim. Acta* **1959**, *42*, 719–728.
20. Engel, D. A.; Dudley, G. B. *Org. Biomol. Chem.* **2009**, *7*, 4149–4158.
21. Bushby, R. J.; Jarecki, C. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2215–2222.
22. Sugawara, Y.; Yamada, W.; Yoshida, S.; Ikeno, T.; Yamada, T. *J. Am. Chem. Soc.* **2007**, *129*, 12902–12903.
23. Sato-Akaba, H.; Kuwahara, Y.; Fujii, H.; Hirata, H. *Anal. Chem.* **2009**, *81*, 7501–7506.
24. (a) Miyake, Y.; Wang, X.; Amasaka, M.; Itto, K.; Xu, S.; Arimoto, H.; Fujii, H.; Hirata, H. *Anal. Chem.* **2013**, *85*, 985–990; (b) Pawlak, A.; Ito, R.; Fujii, H.; Hirata, H. *Chem. Commun.* **2011**, 3245–3247.