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Thiol-Activated *gem*-Dithiols: A New Class of Controllable Hydrogen Sulfide Donors

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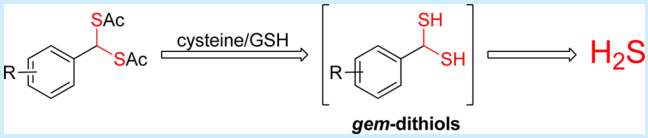
Supporting Information

ABSTRACT: A class of novel thiol-activated H_2S donors has been developed on the basis of the *gem*-dithiol template. These donors release H_2S in the presence of cysteine or GSH in aqueous solutions as well as in cellular environments.



Hydrogen sulfide (H_2S) has been recently recognized as a new member of the family of gasotransmitters, along with nitric oxide (NO), carbon monoxide (CO), and dioxygen (O_2).[1−5] Biosynthesis of H_2S has been attributed to at least three enzymes: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfur-transferase (MPST).[6−8] These enzymes convert cysteine or cysteine derivatives to H_2S in different tissues and organs. It is believed that H_2S-induced biological actions, such as anti-inflammation, vasodilation, and cardioprotection, are related to some specific reactions of H_2S in living systems.[9−12] For example, H_2S can interact with methemoglobin to form sulfhemoglobin, and this reaction might act as a metabolic sink for H_2S.[13] H_2S has been reported to cause protein *S*-sulfhydration to form protein-S-SH, while how this reaction occurs is still under debate.[14−16] Nevertheless, this process is potentially significant as it provides a possible route by which H_2S can alter functions of a wide range of cellular proteins and enzymes.[17,18] As a potential reducing agent, H_2S can rapidly scavenge reactive oxygen species, such as hydrogen peroxide, superoxide, and peroxynitrite. These reactions account for H_2S's protective functions in cardiovascular systems.[19−22] In addition, the reaction between H_2S and nitrosothiols could result in the formation of thionitrous acid (HSNO), the smallest *S*-nitrosothiol. HSNO possibly serves as a cell-permeable nitrosylating agent.[23] All of these findings suggest that regulation of endogenous H_2S formation and exogenous H_2S administration may have therapeutic benefits.

In this field, H_2S releasing agents (also known as H_2S donors) are important tools.[24,25] Currently, sulfide salts (i.e., sodium sulfide Na_2S and sodium hydrogen sulfide NaHS) are still the most often used H_2S donors in this field. Although these salts have the advantage of boosting H_2S concentration fast, the uncontrollable H_2S release makes them not ideal to mimic slow and controllable H_2S release in living systems. In addition, H_2S can quickly escape from solution due to volatilization under laboratory conditions.[26] The effective residence time of sulfide salts in testing samples, therefore, is very short. It should also be noted that commercially available sulfide salts, especially NaHS, always contain a significant amount of impurities. Recent studies revealed that polysulfides rapidly form in NaHS solution.[27] All of these problems may lead to disparate results when using sulfide salts as H_2S precursors. Considering these drawbacks, researchers have started to use organic molecules as H_2S donors. Several types of synthetic H_2S donors have been developed and used in studies. Representative donors include GYY4137, dithiolethiones, *N*-mercapto-based molecules, perthiol-based molecules, geminal-dithiol (*gem*-dithiol) species, and thioamino acids (Figure 1). These compounds release H_2S under different conditions, and their H_2S-related biological actions have been explored.[28−35]

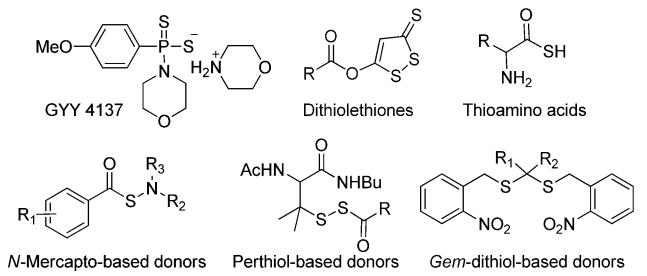


Figure 1. Representative synthetic H_2S donors.

It should be noted that although a number of H_2S donors have been reported, donors with controllable H_2S release capability are still very limited and under high demand. The research in our group focuses on the development of controllable H_2S donors. We have reported two types of thiol-activated donors based on *N*-mercapto and perthiol templates (Figure 1).[31,33] These molecules do not release H_2S without the interaction with thiols (i.e., cysteine and glutathione). Recently, we also reported a series of *gem*-dithiol-based H_2S donors (GDDs), which were activated by UV irradiation.[34] Based on these results, we envisioned that *gem*-dithiols are valuable templates for the design of H_2S donors and decided to explore new donors using this structure. Herein, we reported a new class of thiol-activated *gem*-dithiol-based H_2S

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donors (TAGDDs). H_2S release from these TAGDDs can be triggered by cellular thiols.

It is known that *gem*-dithiols (**1**) are unstable species in aqueous environments and the decomposition of **1** should lead to H_2S release.[36,37] In the development of TAGDDs, an acetyl group was selected to stabilize **1**. More importantly, this protecting group would be selectively removed in the presence of thiols to retrieve **1**, therefore achieving controllable H_2S release (Figure 2).

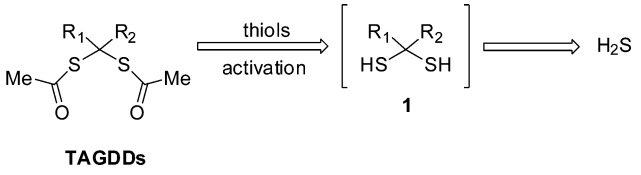

Figure 2. Design of TAGDDs.

With this idea in mind, a series of TAGDDs were synthesized from the substituted benzaldehydes (Figure 3). Briefly,

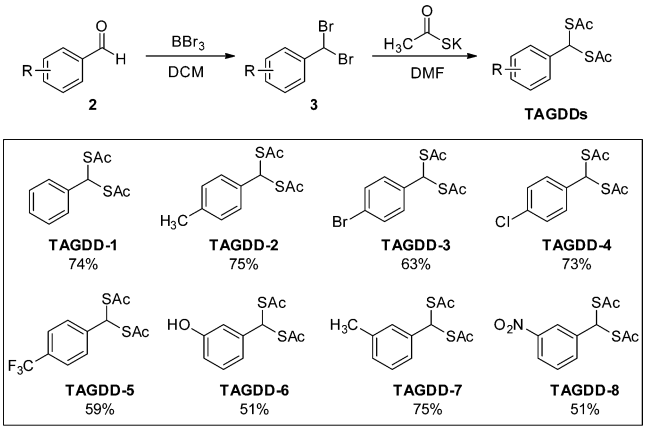

Figure 3. Chemical synthesis of TAGDDs.

benzaldehyde derivatives **2** reacted with boron tribromide (BBr_3) to form the corresponding dibromide intermediates **3**. Then compounds **3** were treated with potassium thioacetate to provide the desired TAGDDs. In this study, eight TAGDDs were synthesized in yields of 51−75%.

Unlike hydrolysis-based H_2S donors (i.e., GYY4137 and Na_2S/NaHS), TAGDDs were stable in aqueous solutions. They did not release H_2S upon hydrolysis. Cellular nucleophiles, such as lysine and serine, did not trigger H_2S release, either. However, a time-dependent H_2S generation was observed in the presence of cysteine, indicating thiols were essential to trigger H_2S release. In order to systematically compare H_2S generation capability of these donors we studied the effects of donor concentrations, cysteine concentrations, solvent systems, as well as reaction time/temperatures. H_2S release was monitored at room temperature for 2 h. The standard methylene blue (MB) method was used to measure H_2S generation. Eventually the optimized conditions were found to be 100 μM donors in PBS buffer (pH 7.4, 50 mM) containing 10% THF. Varied cysteine concentrations caused different H_2S release profiles (Figure 4). Taking **TAGDD-1** as the example, a maximum of 93 μM of H_2S (peak H_2S concentration) at 25 min (peak time) was detected from 100 μM of the donor in the presence of 1000 μM cysteine. H_2S concentrations started to

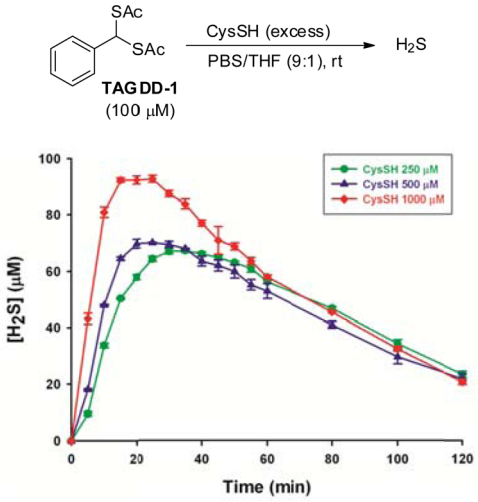

Figure 4. H_2S release from **TAGDD-1** in the presence of cysteine.

drop after peak time probably due to volatilization. In our following studies we decided to use 500 μM cysteine to trigger H_2S release from TAGDDs.

In addition to cysteine, GSH's capability in promoting H_2S release from TAGDDs was also evaluated. As shown in Figure 5, GSH (500 μM) successfully triggered H_2S release, but at a

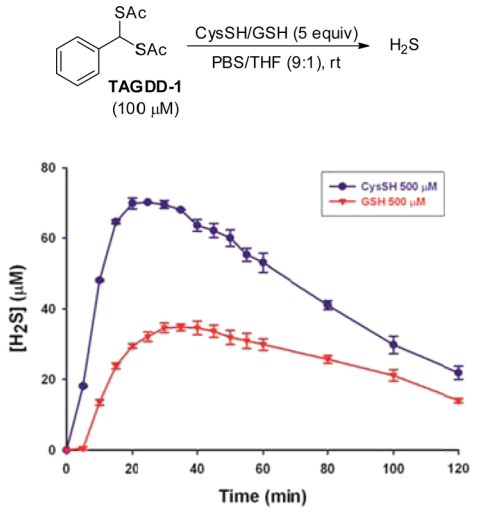

Figure 5. H_2S release from **TAGDD-1** in the presence of cysteine and GSH.

relatively lower level. This is presumably due to increased steric hindrance of GSH, therefore leading to a slower reaction to liberate H_2S. Homocysteine showed similar effects as GSH (data shown in Figure S1, Supporting Information).

H_2S-releasing profiles of all 8 TAGDDs were determined under the optimized conditions. Briefly, a solution of donor (100 μM) and cysteine (500 μM) was prepared in a mixed PBS (pH 7.4, 50 mM)/THF (9:1, v:v) solvent. Reaction aliquots (1.0 mL) were taken to MB cocktail (0.5 mL) at different reaction times. After 15 min, UV absorbance at 670 nm was measured. H_2S concentrations were calculated by using a standard curve generated by Na_2S. Each donor was tested three times, and their average results are summarized in Table 1. The results showed that peak times of TAGDDs ranged from 29 to 38 min with peak H_2S concentrations of 25.3−94.3 μM. The

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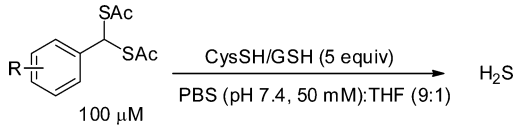
Table 1. H_2S Release from TAGDDs[a]



		CysSH		GSH	
TAGDDs	R	T_{peak} (min)	$[H_2S]_{peak}$ (μM)	T_{peak} (min)	$[H_2S]_{peak}$ (μM)
1	H	30	69.5	40	34.6
2	4-CH_3	37	94.0	43	24.6
3	4-Br	38	25.3	48	10.1
4	4-Cl	34	35.8	53	16.8
5	4-CF_3	37	27.2	N/A	N/A
6	3-OH	34	94.3	49	52.2
7	3-CH_3	29	70.6	39	24.8
8	3-NO_2	35	36.1	47	17.6

[a]Data were reported as the average value of three measurements.

profiles in the presence of GSH were also measured. In general, GSH led to slower and much decreased H_2S release from these donors. These results demonstrated that TAGDDs are potent H_2S donors and structure modifications could regulate H_2S release ability.

The mechanism of H_2S release is proposed as follows (Figure 6): the reaction is initiated by a reversible thiol exchange

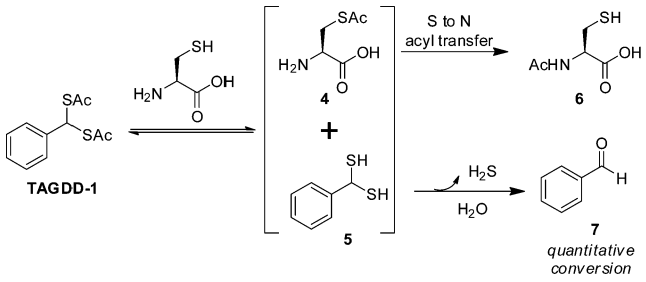


Figure 6. Proposed mechanism for H_2S release from TAGDD-1.

between **TAGDD-1** and cysteine to generate *S*-acetyl cysteine (**4**) and *gem*-dithiol (**5**). Compound **4** should undergo a fast S-to-N acyl transfer to form *N*-acetylcysteine (**6**) and drive the equilibrium.[31] Meanwhile, **5** should release H_2S spontaneously in aqueous solution to yield benzaldehyde (**7**). To prove the mechanism, we analyzed the reaction between **TAGDD-1** and cysteine (5 equiv) by HPLC equipped with a UV detector. Indeed, the formation of benzaldehyde **7** and 2-phenylthiazolidine-4-carboxylic acid, a product from benzaldehyde and cysteine, was observed in high yields by using authentic samples (see the Supporting Information). The mechanism, when activated by GSH, should be similar to the mechanism when activated by cysteine. However, GSH, compared with cysteine, is more bulky. In addition, there is no S-to-N acyl transfer in GSH-involved reactions. Therefore, the initial equilibrium might be slow. The consumption of the final product benzaldehyde by cysteine is also expected to be faster than GSH due to the formation of 2-phenylthiazolidine-4-carboxylic acid. Because of these reasons, it is expectable the reactions between TAGDDs and GSH are slower and less effective. The GSH experiments proved our hypothesis.

Considering significant amounts of free cysteine and GSH in living systems,[38−41] we envisioned that TAGDDs could achieve intracellular H_2S release. Before conducting experiments to test this hypothesis, we evaluated cytotoxicity of a representative donor, **TAGDD-1**, in HeLa cells. A cell counter kit (CCK-8) assay was used to detect cell viability (Figure 7). The results

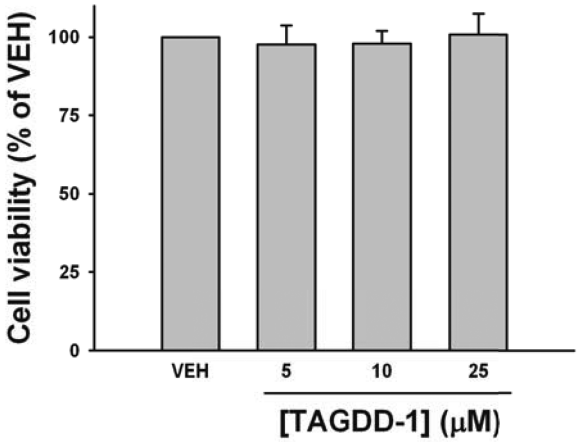


Figure 7. Effects of **TAGDD-1** on cell viability. HeLa cells were treated with different concentrations of **TAGDD-1** (5−25 μM) for 1 h. The cell counter kit (CCK)-8 assay was performed to measure cell viability. Data were shown as the mean ± SD (n = 4).

showed that 1 h exposure of HeLa cells to **TAGDD-1** at varied concentrations (5−25 μM) did not decrease cell viability, indicating that TAGDDs do not induce cytotoxicity to HeLa cells at doses used (cytotoxicity data of other TAGDDs are shown in Figure S3, Supporting Information).

Experiments were then conducted to test whether TAGDDs could release H_2S in cells. As shown in Figure 8, HeLa cells

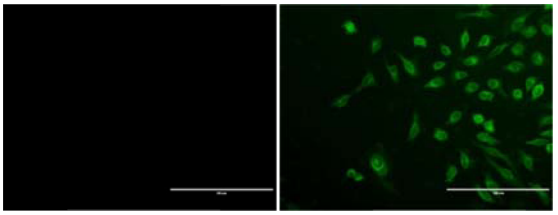


Figure 8. H_2S production from **TAGDD-1** in HeLa cells. Cells were incubated with vehicle (left) and **TAGDD-1** (25 μM) (right) for 30 min. After removal of excess **TAGDD-1**, a H_2S fluorescent probe (WSP-4) was added. Images were taken after 30 min.

were incubated with **TAGDD-1** (25 μM) for 30 min. Then cells were washed by PBS twice to remove extracellular **TAGDD-1**. A selective H_2S fluorescent probe, WSP-4,[42] was then applied to detect H_2S generation. As expected, donor-treated cells exhibited significantly enhanced fluorescent signals compared to vehicle-treated group, demonstrating that TAGDDs can release H_2S in cells.

In conclusion, a series of thiol-activated H_2S donors have been developed on the basis of *gem*-dithiol structures. These donors are stable in aqueous solutions. However, a time-dependent H_2S generation was observed in the presence of thiols. In addition, H_2S release of TAGDDs in cells was also proved. Further development of these donors and evaluation of their H_2S-related biological activities are currently ongoing in our laboratory.

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■ ASSOCIATED CONTENT

Supporting Information

Detailed synthetic procedures, characteristic data, and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Li, L.; Moore, P. K. *Annu. Rev. Pharmacol. Toxicol.* **2011**, *51*, 169.
(2) Fukuto, J. M.; Carrington, S. J.; Tantillo, D. J.; Harrison, J. G.; Ignarro, L. J.; Freeman, B. A.; Chen, A.; Wink, D. A. *Chem. Res. Toxicol.* **2012**, *25*, 769.
(3) Vandiver, M. S.; Snyder, S. H. *J. Mol. Med.* **2012**, *90*, 255.
(4) Kolluru, G. K.; Shen, X.; Bir, S. C.; Kevil, C. G. *Nitric Oxide* **2013**, *35*, 5.
(5) Wang, R. *Physiol. Rev.* **2012**, *92*, 791.
(6) Kabil, O.; Banerjee, R. *J. Biol. Chem.* **2010**, *285*, 21903.
(7) Kabil, O.; Banerjee, R. *Antioxid. Redox. Signal.* **2014**, *20*, 770.
(8) Kimura, H. *Amino Acids* **2011**, *41*, 113.
(9) Ariyaratnam, P.; Loubani, M.; Morice, A. H. *Microvasc. Res.* **2013**, *90*, 135.
(10) Wallace, L.; Vong, L.; Mcknight, W.; Dicay, M.; Martin, G. R. *Gastroenterology* **2009**, *137*, 569.
(11) Predmore, B. L.; Lefer, D. J.; Gojon, G. *Antioxid. Redox. Signal.* **2012**, *17*, 119.
(12) Lefer, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 17907.
(13) Haouzi, P.; Bell, H.; Philmon, M. *Respir. Physiol. Neurobiol.* **2011**, *177*, 273.
(14) Paulsen, C. E.; Carroll, K. S. *Chem. Rev.* **2013**, *113*, 4633.
(15) Pan, J.; Carroll, K. S. *ACS Chem. Biol.* **2013**, *8*, 1110.
(16) Zhang, D.; Macinkovic, I.; Devarie-Baez, N. O.; Pan, J.; Park, C. M.; Carroll, K. S.; Filipovic, M. R.; Xian, M. *Angew. Chem., Int. Ed.* **2014**, *53*, 575.
(17) Paul, B. D.; Snyder, S. H. *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 499.
(18) Krishnan, N.; Fu, C.; Pappin, D. J.; Tonks, N. K. *Sci. Signaling* **2011**, *4*, ra86.
(19) Filipovic, M. R.; Miljkovic, J.; Allgauer, A.; Chaurio, R.; Shubina, T.; Herrmann, M.; Ivanovic-Burmazovic, I. *Biochem. J.* **2012**, *441*, 609.
(20) Jones, C. M.; Lawrence, A.; Wardman, P.; Burkitt, M. J. *Free Radic. Biol. Med.* **2002**, *32*, 982.
(21) Carballal, S.; Trujillo, M.; Cuevasanta, E.; Bartesaghi, S.; M?ller, M. N.; Folkes, L. K.; Garcia-Bereguian, M. A.; Gutierrez-Merino, C.; Wardmann, P.; Denicola, A.; Radi, R.; Alvarez, B. *Free Radic. Biol. Med.* **2011**, *50*, 196.
(22) Calvert, J. W.; Jha, S.; Gundewar, S.; Elrod, J. W.; Ramachandran, A.; Pattillo, C. B.; Kevil, C. G.; Lefer, D. J. *Circ. Res.* **2009**, *105*, 365.
(23) Filipovic, M. R.; Miljkovic, J. Lj.; Nauser, T.; Royzen, M.; Klos, K.; Shubina, T.; Joppenol, W. H.; Lippard, S. J.; Ivanvic-Burmazovic, I. *J. Am. Chem. Soc.* **2012**, *134*, 12016.
(24) Caliendo, G.; Cirino, G.; Santagada, V.; Wallace, J. L. *J. Med. Chem.* **2010**, *53*, 6275.
(25) Zhao, Y.; Biggs, T. D.; Xian, M. *Chem. Commum.* **2014**, DOI: 10.1039/c4cc00968a.
(26) DeLeon, E. R.; Stoy, G. F.; Olson, K. R. *Anal. Biochem.* **2012**, *421*, 203.
(27) Greiner, R.; Palinkas, Z.; Basell, K.; Becher, D.; Antelmann, H.; Nagy, P.; Dick, T. P. *Antioxid. Redox. Signal.* **2013**, *19*, 1749.
(28) Kashfi, K.; Olson, K. R. *Biochem. Pharmacol.* **2013**, *85*, 689.
(29) Song, Z.; Ng, M.; Lee, Z.; Dai, W.; Hagen, T.; Moore, P. K.; Huang, D.; Deng, L.; Tan, C. *MedChemComm* **2014**, *5*, 557.
(30) Li, L.; Whiteman, M.; Guan, Y. Y.; Neo, K. L.; Cheng, Y.; Lee, S. W.; Zhao, Y.; Baskar, R.; Tan, C. H.; Moore, P. K. *Circulation* **2008**, *117*, 2351.
(31) Zhao, Y.; Wang, H.; Xian, M. *J. Am. Chem. Soc.* **2011**, *133*, 15.
(32) Foster, J. C.; Powell, C. R.; Radzinski, S. C.; Matson, J. B. *Org. Lett.* **2014**, *16*, 1558.
(33) Zhao, Y.; Bhushan, S.; Yang, C.; Otsuka, H.; Stein, J. D.; Pacheco, A.; Peng, B.; Devarie-Baez, N. O.; Aguilar, H. C.; Lefer, D. J.; Xian, M. *ACS Chem. Biol.* **2013**, *8*, 1283.
(34) Devarie-Baez, N. O.; Bagdon, P. W.; Peng, B.; Zhao, Y.; Park, C. M.; Xian, M. *Org. Lett.* **2013**, *15*, 2786.
(35) Zhou, Z.; von Wantoch Rekowski, M.; Coletta, C.; Szabo, C.; Bucci, M.; Cirino, G.; Topouzis, S.; Papapetropoulos, A.; Giannis, A. *Bioorg. Med. Chem.* **2012**, *20*, 2675.
(36) Cairns, T. L.; Evans, G. L.; Larchar, A. W.; Mckusick, B. C. *J. Am. Chem. Soc.* **1952**, *74*, 3982.
(37) Berchtold, G. A.; Edwards, B. E.; Campaigne, E.; Carmack, M. *J. Am. Chem. Soc.* **1959**, *81*, 3148.
(38) Wu, G.; Fang, Y.; Yang, S.; Lupton, J. R.; Turner, N. D. *J. Nutr.* **2004**, *134*, 489.
(39) Lu, S. C. *Curr. Top. Cell Regul.* **2000**, *36*, 95.
(40) Dominy, J. E., Jr.; Hirschberger, L. L.; Coloso, R. M.; Stipanuk, M. H. *Biochem. J.* **2006**, *394*, 267.
(41) Stipanuk, M. H.; Dominy, J. E., Jr.; Lee, J.; Coloso, R. M. *J. Nutr.* **2006**, *136*, 1652S.
(42) Peng, B.; Chen, W.; Liu, C.; Rosser, E. W.; Pacheco, A.; Zhao, Y.; Aguilar, H. C.; Xian, M. *Chem.—Eur. J.* **2014**, *20*, 1010.