JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Article
pubs.acs.org/JACS

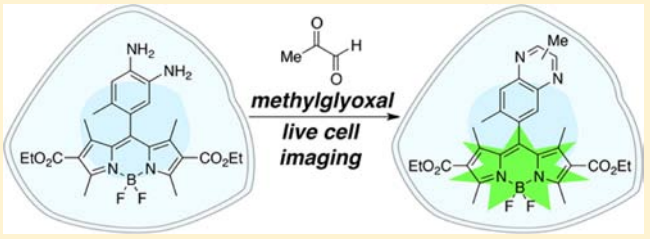
A "Turn-On" Fluorescent Sensor for Methylglyoxal

Tina Wang,[†] Eugene F. Douglass, Jr.,[†] Kelly J. Fitzgerald,[‡] and David A. Spiegel*[,†,‡]

[†]Department of Chemistry, Yale University, 225 Prospect Street, New Haven, Connecticut 06511, United States
[‡]Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510, United States

Supporting Information

ABSTRACT: Methylglyoxal (MGO), a dicarbonyl metabolite produced by all living cells, has been associated with a number of human diseases. However, studies of the role(s) MGO plays biologically have been handicapped by a lack of direct methods for its monitoring and detection. To address this limitation, we have developed a fluorescent sensor (methyl diaminobenzene-BODIPY, or "MBo") that can detect MGO under physiological conditions. We show that MBo is selective for MGO over other biologically relevant dicarbonyls and is suitable for detecting MGO in complex environments, including that of living cells. In addition, we demonstrate MBo's utility in estimating plasma concentrations of MGO. The results reported herein have the potential to advance both clinical and basic science research and practice.



INTRODUCTION

Methylglyoxal (MGO) is a reactive dicarbonyl produced by all living cells during glucose, fatty acid, and amino acid metabolism.[1,2] It is a potent glycating agent, capable of nonenzymatically modifying both proteins and DNA.[2] Reactions of biological macromolecules with MGO can also lead to the formation of advanced glycation end-products (AGEs), which have been shown to cause protein dysfunction,[3] activate membrane receptors and trigger pro-inflamatory signaling, and have been linked to aging disorders, diabetic complications, and chronic inflammation.[4,5] Interestingly, elevated levels of MGO have also been associated with pathologies such as diabetes,[6,7] cardiovascular disease,[8] hyperalgesia,[9,10] and kidney disease.[11,12] MGO's connection with diabetes is particularly notable; levels of MGO have consistently been reported to increase in the plasma of diabetics.[7] Furthermore, MGO by itself has been shown to induce a number of deleterious effects in cellular systems, including oxidative stress,[13,14] inflammation,[13,15,16] defects in cell adhesion,[17] and endothelial cell dysfunction.[18−20] Thus, MGO has been hypothesized to contribute directly to the pathophysiology of diabetic complications.

Despite its links to disease, MGO's roles in cellular processes and pathogenesis remain poorly understood.[21] This limitation is partly due to a lack of straightforward methods to monitor this small molecule in complex systems, and no method exists for visualizing it in live biological samples. Available techniques for measuring MGO levels include electrochemical and absorbance-based approaches, but these are either not directly applicable to living systems (the former),[22] or too insensitive to be of use (the latter).[23] Due to the reactivity of MGO, most methods for monitoring its levels involve derivatization with *o*-phenylenediamine (OPD) or similar molecules to form stable adducts, followed by HPLC or LC−MS analysis.[24−27] These methods require cell lysis followed by extensive processing and deproteinization under harsh conditions. As a result, estimates of cellular MGO levels using this method have been extremely variable, spanning 3 orders of magnitude.[26,28]

Here we present the first fluorescent "turn-on" sensor for MGO detection, called methyl diaminobenzene-BODIPY or MBo, that can be used in living systems. We provide data showing that MBo is selective for MGO over other biological dicarbonyls and also can be used to detect changes in MGO levels in live cells by fluorescence microscopy. In addition, MBo can be used to directly estimate MGO concentrations in plasma or serum. The facility with which MBo can be used to both measure MGO levels and image its presence in live cells has the potential to represent an enabling advance both in clinical diagnosis and basic science research.

RESULTS AND DISCUSSION

Existing methods for detection of MGO and other 1,2-dicarbonyls involve the use of *o*-phenylenediamine (OPD) (**1**). OPD reacts quickly with these metabolites to form the stable, strongly UV-absorbing 2-methylquinoxaline (**2**), which is detectable using HPLC or LC−MS (Figure 1a). We sought a way to take advantage of this reactivity to develop "turn-on" sensors for MGO. Along these lines, the Nagano group has previously reported that DAF-2 (**3**), an agent based on an OPD scaffold, could be used to detect nitric oxide (NO) (Scheme S2 in Supporting Information [SI]).[29,30] In this sensor, fluorescence is quenched via an intramolecular photoinduced electron transfer from the OPD functionality to the acceptor

Received: June 19, 2013
Published: August 9, 2013

dx.doi.org/10.1021/ja406077j | J. Am. Chem. Soc. 2013, 135, 12429−12433

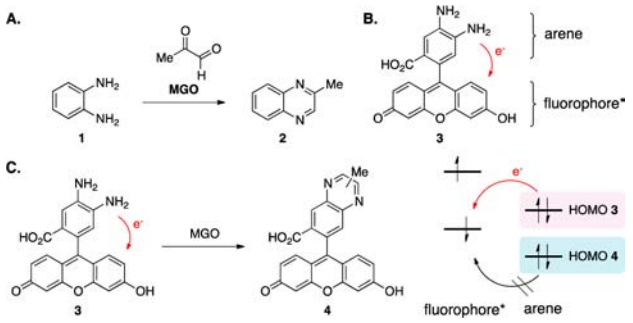


Figure 1. (a) *o*-Phenylenediamine (OPD, **1**) traps MGO to form stable 2-methylquinoxaline adduct **2**. (b) OPD, an electron-rich arene, quenches fluorescence in **3** by a-PeT. (c) Conversion of the pendant OPD in **3** to a quinoxaline (**4**) lowers its E_{HOMO}, preventing quenching by a-PeT.

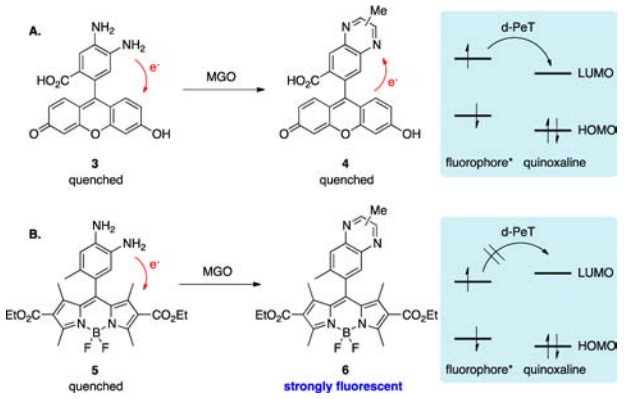


Figure 2. (a) Fluorescence of **4** is quenched by donor-excited photoinduced electron transfer (d-PeT) due to the lower E_{LUMO} of the electron-poor quinoxaline. (b) Addition of electron-donating groups raises the quinoxaline E_{LUMO} in **6**, preventing quenching and allowing fluorescence to occur.

fluorophore, a process known as acceptor-excited photoinduced electron transfer (a-PeT, Figure 1b).[31,32]

Reaction of the pendant OPD in **3** with NO affords a triazole (**S5**, Scheme S2, SI). Because of its relatively low HOMO energy, the triazole in **S5** does not serve as an a-PeT donor, rendering **S5** highly fluorescent.[31] By analogy, we hypothesized that reaction with MGO would relieve acceptor-excited photoinduced electron transfer, and "turn on" fluorescence in compound **4**. As a first step toward testing this hypothesis, we compared the HOMO energy values between the 2-methylquinoxaline and the benzotriazole moieties found in **4** and **S5**, respectively (Table S1, SI). The similarity between these energy values suggested that reaction of **3** with a dicarbonyl should relieve autoinhibitory effects on fluorescence emission. We therefore decided to evaluate **3** as an MGO sensor.

Treatment of **3** with either MGO or *S*-nitroso-*N*-acetylpenicillamine (SNAP) (an NO donor) led to mixed results. While **3** responded vigorously to the presence of NO, as reported previously, the compound exhibited virtually no response to MGO (Figure S1a, SI). LC–MS analysis confirmed formation of quinoxaline product (Figure S1b, SI), which led us to conclude that the quinoxaline adduct of **3** must lack fluorescence. We speculated that perhaps the LUMO of quinoxaline was sufficiently low in energy to accept an electron donated from the excited fluorophore, thus quenching fluorescence emission through donor-excited photoinduced electron transfer (d-PeT) (Figure 2a).[32] Indeed, calculations indicated that quinoxaline **S6** has a relatively low-lying LUMO compared to that of triazole **S11** (Table S1, SI).

These data suggested that careful tuning of the energetic matching between quencher and fluorophore would be necessary for sensor development. Thus, we reasoned that the *o*-arene-diamine functionality in an MGO sensor must give rise to a quinoxaline with a sufficiently high LUMO energy to avoid a d-PeT process; yet simultaneously, we must also avoid raising the quinoxaline's HOMO energy so much that it can quench fluorescence by an a-PeT mechanism. We further reasoned that lowering the HOMO/LUMO energies of the fluorophore component would widen the energy gap between the fluorophore's excited state and the LUMO energy of the quinoxaline acceptor, thus decreasing d-PeT quenching. With these considerations in mind, we pursued a sensor containing both an electron-deficient fluorophore and an electron-rich OPD derivative.

To this end, we chose to adopt a BODIPY scaffold, as it is easily derivatizable at both the aryl moiety and the fluorophore.[33] Gaussian calculations performed on various 2-methylquinoxalines led us to select a 6-methyl substituent as ideal for raising the LUMO energy without forcing simultaneous increases in E_{HOMO} (**S9**, Table S1, SI). Thus, we synthesized methyl diaminobenzene-BODIPY derivative (**5**, "MBo") in four steps and in 13.5% overall yield starting from *N*-(4-formyl-5-methyl-2-nitrophenyl)acetamide,[34] as detailed in the SI (Scheme S1).

Although a modified version of this compound (diaminobenzene-BODIPY ethyl ester, DAMBO-CO_2Et, **7**), has previously been employed as a sensor for NO,[30] we reasoned that **5** would not respond to NO under the conditions of our experiments; as the pK_a of DAMBO-triazole (**S15**) is approximately 7.7, its deprotonation at physiological pH results in the formation of an electron-rich triazolate. This electron-rich system induces fluorescence quenching through an a-PeT mechanism. (**S16**, Figure S2, SI).[30] Due to the lack of acidic protons in quinoxaline **S9**, on the other hand, we reasoned that the MBo–MGO adduct should not experience similar quenching, and therefore, MBo should exhibit a significantly stronger fluorescence response to MGO over NO under physiological conditions (pH 7.4). For purposes of comparison, we also synthesized diaminobenzene-BODIPY ethyl ester (DAMBO-CO_2Et, **7**) and diaminobenzene-BODIPY (DAMBO, **8**).[30] Electronic calculations led us to predict that **5** should have the greatest fluorescence increase upon reaction with MGO, followed by **7**, while **8** should exhibit the smallest change in signal.

The ability of dyes **5**, **7**, and **8** to detect MGO in an *in vitro* assay was then tested. As anticipated, compound **5** displayed over a 10-fold increase in fluorescence upon reaction with MGO, making it the most sensitive MGO detector among the compounds synthesized. The quantum yield of the MBo–MGO adduct **6** was found to be 0.326, compared to 0.003 for MBo (Table S2, SI). Compounds **3** and **8** exhibited no response to MGO, while **7** showed a modest increase in fluorescence (Figure 3).

As a next step, we went on to characterize MBo's dicarbonyl selectivity and reactivity in more detail. We therefore compared MBo-induced fluorescence upon reaction with a variety of α-dicarbonyl species (Figure 4), including MGO, glyoxal (GO),

dx.doi.org/10.1021/ja406077j | J. Am. Chem. Soc. 2013, 135, 12429–12433

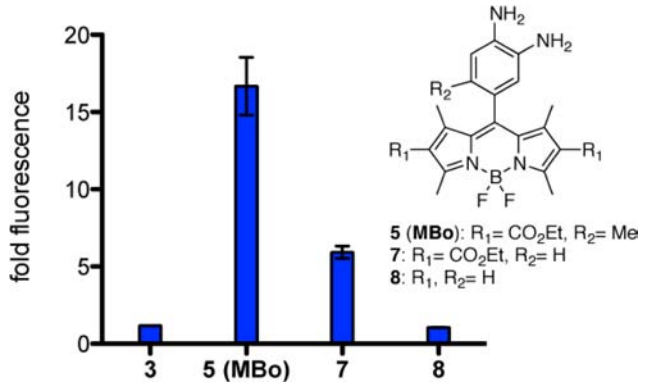


Figure 3. Response of fluorophores to MGO. Fluorophore (10 μM) was incubated with 50 μM MGO for 1 h at 37 °C in PBS (pH 7.4) before fluorescence was read by plate reader. Fold fluorescence represents fluorescence in the presence of MGO divided by the fluorescence of the blank control (fluorophore only). Error bars represent standard deviation (n = 3).

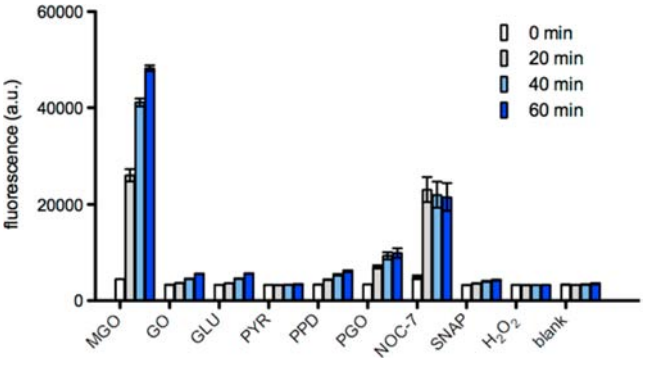


Figure 4. Fluorescence response of MBo (**5**) upon reaction with various dicarbonyls and reactive oxygen species. MBo (10 μM) was incubated with 50 μM substrate for the indicated times at 37 °C in PBS (pH 7.4) before fluorescence was read by plate reader. MGO: methylglyoxal; GO: glyoxal; GLU: glucosone; PYR: pyruvate; PPD: 1-phenyl-1,2-propanedione; PGO: phenylglyoxal; NOC-7: nitric oxide donor, $t_{1/2} \approx 1.7$ min;[35] SNAP: nitric oxide donor, $t_{1/2} \approx 4.6$ h;[36] H_2O_2: hydrogen peroxide; blank: PBS only. Error bars represent standard deviation (n = 3).

glucosone (GLU), pyruvate (PYR), 1-phenyl-1,2-propanedione (PPD), and phenylglyoxal (PGO). Following incubations of various dicarbonyls with MBo for 20−60 min, only MGO led to significant fluorescence increases versus baseline (Figure 4). Selectivity for MGO over GO is particularly noteworthy, given their structural similarity, and we hypothesize that our observations result from the lower LUMO energy of the GO-derived quinoxaline (**S13**, Table S1, SI), which can quench fluorescence by d-PeT. Other dicarbonyls are less reactive toward MBo than MGO likely because they are less electrophilic and/or more sterically encumbered (PPD), or present mostly as the hemiacetal tautomer (GLU).

Since MBo is based on a scaffold initially designed to detect NO, we wished to determine our fluorophore's cross-reactivity with this species. At 50 μM, MBo shows some fluorescence upon addition of NOC-7, an NO donor with a half-life of 1.7 min (Figure 4).[35] However, the estimated physiological concentration of NO has been estimated at low to mid-nanomolar range.[37] By comparison, values of intracellular MGO are at least 2 orders of magnitude larger,[2] and human plasma MGO concentrations have also been estimated to be as high as single-digit micromolar.[7,38] In addition to its low steady-state concentration, NO is also likely to have a much shorter half-life than MGO due its reactivity with cellular antioxidants such as glutathione.[39] Taken together, these observations suggested that NO would not interfere with MGO detection in cellular settings.

We also evaluated the sensitivity and kinetic properties for the MBo−MGO reaction. The detection limit of MBo for MGO by plate reader after 1 h of incubation in PBS at 37 °C is 50−100 nM (Figure S4a, SI); furthermore, the sensitivity improves with longer incubation times (Figure S4b, SI). This implies that reaction under these concentrations is not complete at 1 h; indeed, the pseudo-first-order rate constant was estimated to be 4.56×10^{-3} s^{-1} (Figure S5, SI), giving a half-life of ∼2.5 h for a concentration of 100 nM MGO and 10 μM MBo. Assuming bimolecular kinetics, this rate is comparable to what has been reported for other useful fluorescent probes.[40] Taken together, these results suggested that MBo would be suitable for detecting MGO in biological milieu.

After confirming that MBo could detect MGO under physiological conditions, we examined its capacity for MGO imaging in live cells. HeLa cells were loaded for 1 h with 10 μM MBo before being treated with MGO at three separate concentrations (0, 5, or 10 μM) for an additional hour. These concentrations are close to the physiologically relevant range, which has been estimated to be 1−5 μM.[2] Cells loaded with MBo were also tested for cross-reactivity with nitric oxide by treatment with NOC-7 for 1 h. Application of 5 or 10 μM MGO was found to cause a significant fluorescence increase in MBo-treated cells (Figure 5, Figure S7, SI). This fluorescent

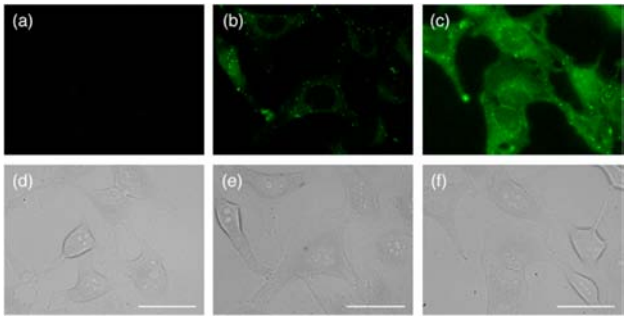


Figure 5. Fluorescence microscopy detection of MGO in live HeLa cells. Cells were loaded with 10 μM MBo in HEPES/Krebs/Ringer's (HKR) buffer for 1 h, then washed once and treated with HKR buffer containing (a) 0 μM, (b) 5 μM, or (c) 10 μM MGO for an additional 1 h. (d−f) Corresponding phase contrast images. Scale bars represent 40 μm.

signal appears to be cytosolic, with no distinct compartmentalization.[41] This is consistent with the observation that MGO is membrane permeable.[42] In contrast, cells pretreated with the nitric oxide donor NOC-7 exhibited only a minimal increase in fluorescence in the presence of MBo (Figure S8, SI). Overall, these experiments indicate that MBo is both sufficiently selective over NO and sensitive enough to image MGO in live cells.

MBo's selectivity for MGO under physiological conditions suggested that it might also be applicable to the estimation of MGO levels in biological samples. Therefore, we first evaluated the relationship between MBo fluorescence and MGO

dx.doi.org/10.1021/ja406077j | J. Am. Chem. Soc. 2013, 135, 12429−12433

concentration in both buffer and serum. The fluorescence response of MBo to MGO at various concentrations was linear in both PBS and fetal bovine serum (FBS), suggesting that MBo is suitable for use in MGO quantification in both buffer and serum (Figure S9, SI).

Next, we developed a method to measure MGO concentrations using a standard addition procedure (SI). The average percent recovery of our method (defined as $([MGO_{estimated}]/[MGO_{actual}]) \times 100$) was obtained by spiking known amounts of MGO into PBS or FBS. These recoveries were 76% in PBS and 64% in FBS (Figure S10, SI), values that are comparable to the existing "gold standard" strategy for determining MGO levels (the OPD derivatization method).[24,27] Next, we evaluated the MGO concentration of pooled mouse serum using MBo and compared these values to the OPD derivatization strategy. Analysis of pooled BALB/c mouse serum using both protocols gave similar values of estimated MGO concentrations (Figure S12, SI). The interday coefficient of variance was 28% for the MBo method, and 47% for the OPD dervatization method (Figure S12, SI).

We then compared MBo- and OPD-based protocols to measure MGO concentrations in the plasma of several B6 mice (Figure S13, SI, and Table 1). The average MGO concentration

Table 1. MGO Concentrations in Mouse Plasma Estimated by MBo

mouse	[MGO], μM
1 and 3[a]	0.99
2	0.59
4 and 5[a]	0.54
6	0.51
7 and 8[a]	0.68
average	0.66 ± 0.20

[a]Pooled to obtain sufficient material for analysis.

in these mice was found to be 0.66 ± 0.20 μM using MBo and 0.63 ± 0.07 μM using the OPD method (Figure S14a, SI). Taking into account interday variance, these values do not differ significantly between the two methods (Figure S14b, SI) and are comparable to MGO levels reported in the literature for mice of the same genetic background.[43] Taken together, the results of these experiments indicate that MBo is effective for estimating MGO concentrations in biological samples and provides results that are comparable to those obtained by OPD derivatization.

Notably, the MBo method holds some significant advantages versus OPD derivatization. First, sample processing using MBo is operationally more simple than using OPD because the protein precipitation step can be skipped. Also, the MBo method requires a shorter incubation time (3 h vs 24 h). Finally, the use of a plate reader in lieu of analytical HPLC allows for the simultaneous analysis of many samples, thus increasing the efficiency of the MBo method.

CONCLUSIONS

In summary, by relating calculated HOMO and LUMO energies to experimental observations, we have been able to rationally design MBo, a BODIPY-based probe for MGO. MBo takes advantage of the reactivity of OPD toward MGO, while overcoming the electron-deficient nature of quinoxalines by tuning the electronics of both the fluorophore and pendant aryl moiety. To the best of our knowledge, MBo is the first compound reported capable of visualizing MGO in living cells. In addition, MBo can be applied to quantifying MGO levels in serum or plasma samples as a faster, more time-efficient alternative to existing HPLC-based methods. *In vitro* experiments have shown that MBo is sensitive even to low concentrations of MGO (50 nM) and is selective over other dicarbonyls. We anticipate that this compound will be a useful tool for clarifying MGO's roles in various diseases and cellular processes.

ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures and compound characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

david.spiegel.@yale.edu

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Takuji Shoda for invaluable discussions. We also thank Dr. Thihan Padukkavidana for his assistance with animal work. This work was supported by the SENS Foundation.

REFERENCES

(1) Kalapos, M. P. *Toxicol. Lett.* **1999**, *110*, 145.
(2) Thornalley, P. J. *Drug Metab. Drug Interact.* **2008**, *23*, 125.
(3) Matafome, P.; Sena, C.; Seica, R. *Endocrine* **2013**, *43*, 472.
(4) Singh, R.; Barden, A.; Mori, T.; Beilin, L. *Diabetologia* **2001**, *44*, 129.
(5) Rabbani, N.; Thornalley, P. J. *Amino Acids* **2012**, *42*, 1133.
(6) Beisswenger, P. J.; Howell, S. K.; Touchette, A. D.; Lal, S.; Szwergold, B. S. *Diabetes* **1999**, *48*, 198.
(7) Kalapos, M. *Diabetes Res. Clin. Pract.* **2013**, *99*, 260.
(8) Kilhovd, B. K.; Juutilainen, A.; Lehto, S.; Ronnemaa, T.; Torjesen, P. A.; Hanssen, K. F.; Laakso, M. *Atherosclerosis* **2009**, *205*, 590.
(9) Bierhaus, A.; Fleming, T.; Stoyanov, S.; Leffler, A.; Babes, A.; Neacsu, C.; Sauer, S. K.; Eberhardt, M.; Schnolzer, M.; Lasitschka, F.; Neuhuber, W. L.; Kichko, T. I.; Konrade, I.; Elvert, R.; Mier, W.; Pirags, V.; Lukic, I. K.; Morcos, M.; Dehmer, T.; Rabbani, N.; Thornalley, P. J.; Edelstein, D.; Nau, C.; Forbes, J.; Humpert, P. M.; Schwaninger, M.; Ziegler, D.; Stern, D. M.; Cooper, M. E.; Haberkorn, U.; Brownlee, M.; Reeh, P. W.; Nawroth, P. P. *Nat. Med.* **2012**, *18*, 926.
(10) Eberhardt, M. J.; Filipovic, M. R.; Leffler, A.; de la Roche, J.; Kistner, K.; Fischer, M. J.; Fleming, T.; Zimmermann, K.; Ivanovic-Burmazovic, I.; Nawroth, P. P.; Bierhaus, A.; Reeh, P. W.; Sauer, S. K. *J. Biol. Chem.* **2012**, *287*, 28291.
(11) Odani, H.; Shinzato, T.; Matsumoto, Y.; Usami, J.; Maeda, K. *Biochem. Biophys. Res. Commun.* **1999**, *256*, 89.
(12) Nakayama, K.; Nakayama, M.; Iwabuchi, M.; Terawaki, H.; Sato, T.; Kohno, M.; Ito, S. *Am. J. Nephrol.* **2008**, *28*, 871.
(13) Wu, L.; Juurlink, B. H. *Hypertension* **2002**, *39*, 809.
(14) Kalapos, M. P. *Chem.-Biol. Interact.* **2008**, *171*, 251.
(15) Wang, H.; Meng, Q. H.; Gordon, J. R.; Khandwala, H.; Wu, L. *Clin. Biochem.* **2007**, *40*, 1232.
(16) Welten, A. G.; Schalkwijk, C. G.; ter Wee, P. M.; Meijer, S.; van den Born, J.; Beelen, R. J. *Peritoneal Dial. Int.* **2003**, *23*, 213.
(17) Dobler, D.; Ahmed, N.; Song, L.; Eboigbodin, K. E.; Thornalley, P. J. *Diabetes* **2006**, *55*, 1961.
(18) Chan, W. H.; Wu, H. J. *J. Cell. Biochem.* **2008**, *103*, 1144.

(19) Brouwers, O.; Niessen, P. M.; Haenen, G.; Miyata, T.; Brownlee, M.; Stehouwer, C. D.; De Mey, J. G.; Schalkwijk, C. G. *Diabetologia* **2010**, *53*, 989.
(20) Dhar, A.; Dhar, I.; Desai, K. M.; Wu, L. *Br. J. Pharmacol.* **2010**, *161*, 1843.
(21) Nemet, I.; Varga-Defterdarovic, L.; Turk, Z. *Mol. Nutr. Food Res.* **2006**, *50*, 1105.
(22) Chatterjee, S.; Chen, A. C. *Anal. Chim. Acta* **2012**, *751*, 66.
(23) Wild, R.; Ooi, L.; Srikanth, V.; Munch, G. *Anal. Bioanal. Chem.* **2012**, *403*, 2577.
(24) McLellan, A. C.; Phillips, S. A.; Thornalley, P. J. *Anal. Biochem.* **1992**, *206*, 17.
(25) Chaplen, F. W.; Fahl, W. E.; Cameron, D. C. *Anal. Biochem.* **1996**, *238*, 171.
(26) Dhar, A.; Desai, K.; Liu, J.; Wu, L. *J. Chromatogr., B* **2009**, *877*, 1093.
(27) Nemet, I.; Varga-Defterdarovic, L.; Turk, Z. *Clin. Biochem.* **2004**, *37*, 875.
(28) Chaplen, F. W.; Fahl, W. E.; Cameron, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5533.
(29) Kojima, H.; Nakatsubo, N.; Kikuchi, K.; Kawahara, S.; Kirino, Y.; Nagoshi, H.; Hirata, Y.; Nagano, T. *Anal. Chem.* **1998**, *70*, 2446.
(30) Gabe, Y.; Urano, Y.; Kikuchi, K.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc.* **2004**, *126*, 3357.
(31) Tanaka, K.; Miura, T.; Umezawa, N.; Urano, Y.; Kikuchi, K.; Higuchi, T.; Nagano, T. *J. Am. Chem. Soc.* **2001**, *123*, 2530.
(32) Ueno, T.; Urano, Y.; Setsukinai, K.; Takakusa, H.; Kojima, H.; Kikuchi, K.; Ohkubo, K.; Fukuzumi, S.; Nagano, T. *J. Am. Chem. Soc.* **2004**, *126*, 14079.
(33) Boens, N.; Leen, V.; Dehaen, W. *Chem. Soc. Rev.* **2012**, *41*, 1130.
(34) Tanaka, A.; Ito, K.; Nishino, S.; Motoyama, Y.; Takasugi, H. *Chem. Pharm. Bull.* **1994**, *42*, 560.
(35) Zhang, P.; Ohara, A.; Mashimo, T.; Sun, J.; Shibuta, S.; Takada, K.; Kosaka, H.; Terada, M.; Yoshiya, I. *Circulation* **1996**, *94*, 2235.
(36) Ignarro, L. J.; Lippton, H.; Edwards, J. C.; Baricos, W. H.; Hyman, A. L.; Kadowitz, P. J.; Gruetter, C. A. *J. Pharmacol. Exp. Ther.* **1981**, *218*, 739.
(37) Hall, C. N.; Garthwaite, J. *Nitric Oxide* **2009**, *21*, 92.
(38) We have observed that, at these relative concentrations, the presence of NO does not impact MGO sensing by MBo (Figure S6).
(39) Sies, H. *Eur. J. Biochem.* **1993**, *215*, 213.
(40) Miller, E. W.; Albers, A. E.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 16652.
(41) We cannot rule out that this staining is due solely to the distribution of the MBo−MGO adduct. The slight punctate staining observed may be due to aggregation of small amounts of insoluble MBo or MBo−MGO adduct.
(42) Thornalley, P. J. *Biochem. J.* **1988**, *254*, 751.
(43) Watson, A. M.; Soro-Paavonen, A.; Sheehy, K.; Li, J.; Calkin, A. C.; Koitka, A.; Rajan, S. N.; Brasacchio, D.; Allen, T. J.; Cooper, M. E.; Thomas, M. C.; Jandeleit-Dahm, K. J. *Diabetologia* **2011**, *54*, 681.