ORGANIC LETTERS

2011
Vol. 13, No. 5
1178–1181

O^2-(*N*-Hydroxy(methoxy)-2-ethanesulfonamido) Protected Diazen-1-ium-1,2-diolates: Nitric Oxide Release via a Base-Induced β-Elimination Cleavage

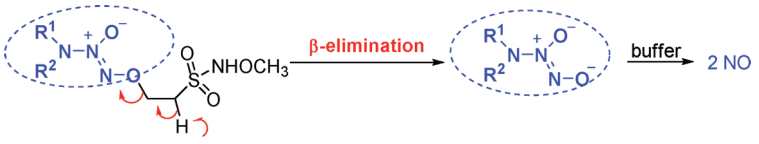
Zhangjian Huang and Edward E. Knaus*

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, T6G 2N8, Canada

eknaus@pharmacy.ualberta.ca

Received January 7, 2011

ABSTRACT



O^2-(Ethanesulfohydroxamic acid) and O^2-(*N*-methoxy-2-ethanesulfonylamido) diazen-1-ium-1,2-diolates (4−7), a novel type of O^2-(protected) diazeniumdiolate, were synthesized using a key thioacetate oxidation reaction. Nitric oxide release studies showed that O^2-(*N*-methoxy-2-ethanesulfonylamido) diazeniumdiolates 5 and 7 released NO in a nonphysiological alkaline buffer, in the presence of bases such as the basic natural amino acids Arg and His, or the non-nucleophilic organic base DBU in PBS at pH 7.4, via a β-elimination cleavage reaction.

Diazeniumdiolates, which can spontaneously release nitric oxide (NO) under physiological conditions (pH 7.4, 37 °C) with a range of half-lives from a few seconds to several days,[1] display vasorelaxant,[2] antithrombotic,[3] cytostatic,[4] and genotoxic[5] activities. Selective protection at the terminal oxygen atom (O^2) of the diazen-1-ium-1,2-diolate offers a method to facilitate delivery of the NO-donor to the target tissue site and release of NO to induce the desired therapeutic effect. A number of different O^2-protected diazeniumdiolates have been reported that include O^2-vinyl,[6] O^2-acetoxymethyl,[7] O^2-glycosylated,[8] and O^2-(2,4-dinitrophenyl) diazeniumdiolates.[9] These O^2-protected compounds, which are metabolized by enzymes such as hepatic cytochrome P450,[6] esterase,[7] certain glycosidases,[8] and glutathione/glutathione S-transferase (GSH/GST),[10] have been studied extensively. Although there have been many attempts directed toward the development of O^2-protected diazeniumdiolates as potential therapeutic candidates, no compound has achieved clinical approval to date. As part of our ongoing program to investigate the application of NO-donors as potential therapeutic agents, we now report a new type of O^2-ethanesulfohydroxamic acid and O^2-(*N*-methoxy-2-ethanesulfonylamido) protected diazen-1-ium-1,2-diolates. These compounds are NO donors which can release NO in the presence of a base, including the basic natural amino acids Arg and His, via a β-elimination cleavage reaction.

(1) (a) Keefer, L. K.; Nims, R. W.; Davies, K. M.; Wink, D. A. *Methods Enzymol.* **1996**, *268*, 281–293. (b) Fitzhugh, A. L.; Keefer, L. K. *Free Radical Biol. Med.* **2000**, *28*, 1463–1469.

(2) Morley, D.; Maragos, C. M.; Zhang, X. Y.; Boignon, M.; Wink, D. A.; Keefer, L. K. *J. Cardiovasc. Pharmacol.* **1993**, *21*, 670–676.

(3) Diodati, J. G.; Quyyumi, A. A.; Hussain, N.; Keefer, L. K. *Thromb. Haemostasis* **1993**, *70*, 654–658.

(4) Mooradian, D. L.; Hutsell, T. C.; Keefer, L. K. *J. Cardiovasc. Pharmacol.* **1995**, *25*, 674–678.

(5) Routledge, M. N.; Wink, D. A.; Keefer, L. K.; Dipple, A. *Chem. Res. Toxicol.* **1994**, *7*, 628–632.

(6) Saavedra, J. E.; Billiar, T. R.; Williams, D. L.; Kim, Y.; Watkins, S. C.; Keefer, L. K. *J. Med. Chem.* **1997**, *40*, 1947–1954.

(7) Saavedra, J. E.; Shami, P. J.; Wang, L. Y.; Davies, K. M.; Booth, M. N.; Citro, M. L.; Keefer, L. K. *J. Med. Chem.* **2000**, *43*, 261–269.

(8) Wu, X.; Tang, X.; Xian, M.; Wang, P. G. *Tetrahedron Lett.* **2001**, *42*, 3779–3782.

(9) Saavedra, J. E.; Srinivasan, A.; Bonifant, C. L.; Chu, J.; Shanklin, A. P.; Flippen-Anderson, J. L.; Rice, W. G.; Turpin, J. A.; Davies, K. M.; Keefer, L. K. *J. Org. Chem.* **2001**, *66*, 3090–3098.

(10) Shami, P. J.; Saavedra, J. E.; Wang, L. Y.; Bonifant, C. L.; Diwan, B. A.; Singh, S. V.; Gu, Y.; Fox, S. D.; Buzard, G. S.; Citro, M. L.; Waterhouse, D. J.; Davies, K. M.; Ji, X.; Keefer, L. K. *Mol. Cancer Ther.* **2003**, *2*, 409–417.

10.1021/ol200053z
Published on Web 02/09/2011

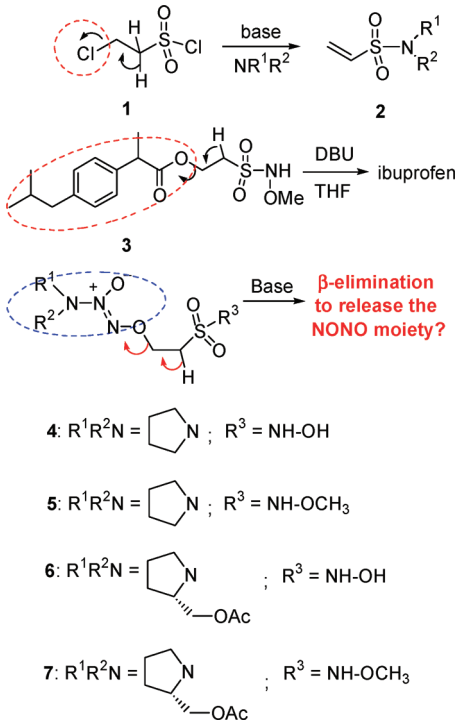
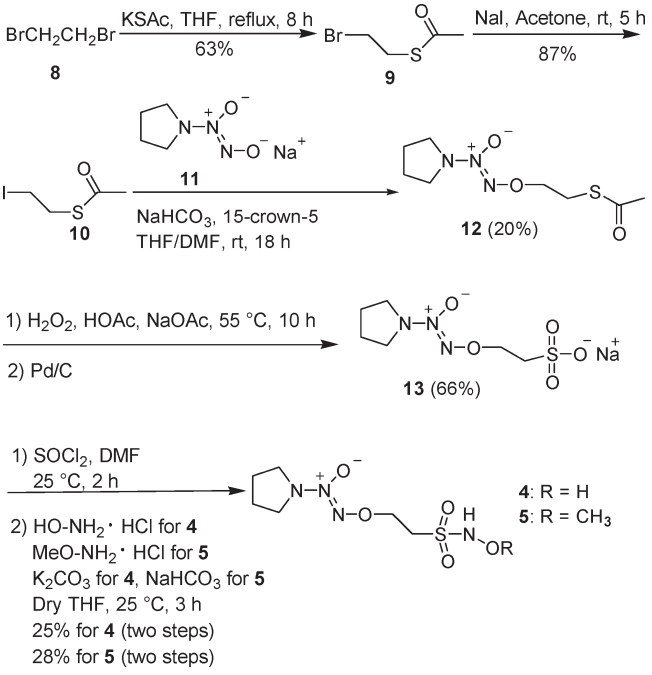


Figure 1. Rationale for the design of novel base-sensitive O^2-(ethanesulfonyl) diazen-1-ium-1,2-diolates.

The concept that O^2-(ethanesulfonyl) diazen-1-ium-1,2-diolates will undergo a base-induced β-elimination reaction is based on the knowledge that (i) 2-chloroethanesulfonyl chloride (**1**), upon reaction with an amine, is reported to release HCl (dehydrochlorination) to form a vinylsulfonylamide product **2** (Figure 1)[11] and (ii) the *N*-methoxyethanesulfonylamide ester of ibuprofen (**3**), which was developed by our group and shown to exhibit more potent anti-inflammatory activity (AI) than the parent drug ibuprofen,[12] released ibuprofen in the presence of the non-nucleophilic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (Figure 1). Accordingly, it was of interest to investigate whether coupling a diazen-1-ium-1,2-diolate (NONOate) moiety to the β-position of an ethanesulfonyl group would provide a hitherto-unknown class of O^2-protected diazen-1-ium-1,2-diaolates in which the NONOate moiety acts initially as a leaving group via a base-induced β-elimination which would subsequently release NO. Credence for this objective is consistent with previous data showing that (i) alkylsulfohydroxamic acids release NO in PBS at pH 7.4, and nitroxyl (HNO) in the presence of base, to act as NO/HNO donors[12] and (ii) *N*-methoxysulfonylamides, which cannot act as NO/HNO donors, are useful model compounds to study the potential β-elimination reaction in the absence of NO release. We now describe the synthesis, β-elimination, and NO release properties for the O^2-(ethanesulfohydroxamic acid) and O^2-(*N*-methoxy-2-ethanesulfonylamido) derivatives of the pyrrolidine and prolinol diazen-1-ium-1,2-diolate compounds **4**–**7** illustrated in Figure 1.

Scheme 1. Synthesis of the Pyrrolidine Analogs **4** and **5**



According to previous reports,[11] it is likely that the PYRRO/NO compound **11** could act as a base to induce β-elimination (−HCl) from a 2-chloroenthanesulfonyl compound to give a vinyl product and release of NO from the PYRRO/NO reactant **11**, rather than condensation with the chloro compound to form the desired O^2-alkylation product. Consequently, an alternative synthetic strategy was required. It is reported that oxidation of a thioacetate using aqueous hydrogen peroxide (H_2O_2) and sodium acetate affords a sodium sulfonate.[13] It was anticipated that β-elimination would not occur when O^2-alkylation of a diazen-1-ium-1,2-diolate was performed since the thioacetate group is a weak electron-withdrawing moiety compared to a sulfohydroxamic group. The synthetic methods used to prepare the target sulfohydroxamic products **4** and **5** are depicted in Scheme 1. Thus, reaction of 1,2-dibromoethane **8** with potassium thioacetate furnished the monosubstituted 2-bromoethyl thioacetate (**9**). The 2-bromoethyl compound **9** was transformed by reaction with sodium iodide in acetone to the more reactive 2-iodoethyl thioacetate (**10**) to improve the yield in the subsequent O^2-alkylation reaction. In this regard, O^2-alkylation of PYRRO/NO **11** using

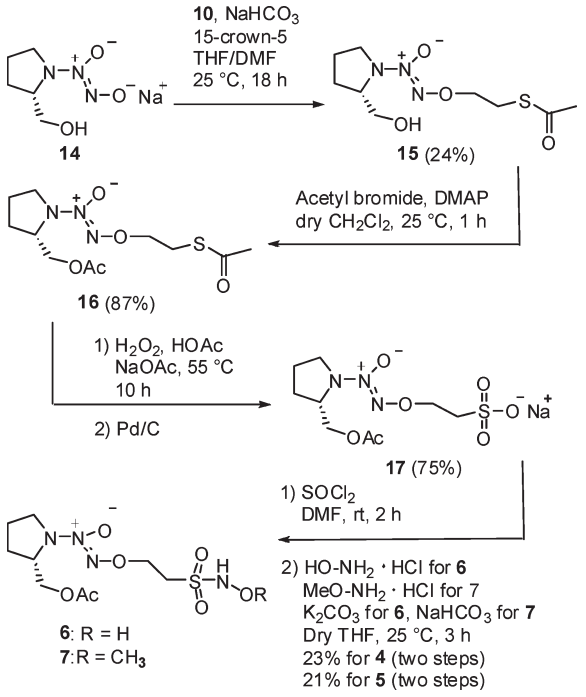
(11) (a) Davies, W. G.; Hardisty, E. W.; Nevell, T. P.; Peters, R. H. *J. Chem. Soc. B* **1970**, *5*, 998–1004. (b) Hromatka, O.; Binder, D.; Knollmueller, M. *Monatsh. Chem.* **1968**, *99*, 1062–1067. (c) Johnson, O. H.; Holum, J. R. *J. Org. Chem.* **1958**, *23*, 738–740.

(12) Huang, Z.; Velázquez, C. A.; Abdellatif, K. A.; Chowdhury, M. A.; Reisz, J. A.; DuMond, J. F.; King, S. B.; Knaus, E. E. *J. Med. Chem.* **2010**, dx.doi.org/10.1021/jm101403g.

(13) (a) Showell, J. S.; Russell, J. R.; Swern, D. *J. Org. Chem.* **1962**, *27*, 2853–2858. (b) Brouwer, A. J.; Ceylan, T.; Linden, T. V. D.; Liskamp, R. M. *Tetrahedron Lett.* **2009**, *50*, 3391–3393.

10 furnished the O^2-(2-acetthioethyl) 1-(pyrrolidin-1-yl)-diazen-1-ium-1,2-diolate (**12**) in 20% yield. Although the NONO moiety is quite sensitive to an acidic medium, fortunately the thioacetate moiety in the O^2-alkylated diazeniumdiolate **12** can be converted to the sodium sulfonate analog **13** in the presence of H_2O_2 and sodium acetate in a solution of acetic acid at 55 °C with a reaction time of 10 h in a 66% yield without decomposition of the NONO moiety. Reaction of the sodium sulfonate **13** with thionyl chloride in DMF afforded the corresponding relatively unstable sulfonyl chloride product which was used immediately in the subsequent reaction without further purification. Thus, reaction of this sulfonyl chloride with hydroxylamine hydrochloride in the presence of potassium carbonate in dry THF afforded the target sulfohydroxamic product **4**. A similar reaction of the sulfonyl chloride with methoxylamine hydrochloride in the presence of sodium bicarbonate in dry THF furnished the *N*-methoxysulfonylamide product **5**.

Scheme 2. Synthesis of the Prolinol Analogs **6** and **7**



Given that prolinol may have a more favorable toxicological profile compared to pyrrolidine, the prolinol diazeniumdiolate analogues **6** and **7** were synthesized as illustrated in Scheme 2. Condensation of sodium 1-[2-hydroxymethyl]-pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (**14**) with 2-iodoethyl thioacetate (**10**) furnished the O^2-(2-acetthioethyl) prolinoldiazeniumdiolate **15** in 24% yield. Since the hydroxyl group present in the prolinol derviative **15** will be sensitive to thionyl chloride during a subsequent step to prepare the sulfonyl chloride, this hydroxyl group was acetylated using acetyl bromide to protect the hydroxyl group as the acetate **16**. Oxidation of the thioacetate moiety present in **16** upon treatment with H_2O_2 and sodium acetate furnished the sodium sulfonate **17** in 75% yield. Transformation of the sodium sulfonate **17** to the sulfonyl chloride by reaction with thioyl chloride and the subsequent reaction of sulfonyl chloride with hydroxylamine hydrochloride or methoxylamine hydrochloride in the presence of potassium carbonate or sodium bicarbonate in dry THF afforded the respective target products **6** and **7**.

Table 1. Amount of Nitric Oxide (nmol) Released from **4**−**7**, **11** (120 nmol) in PBS at Different pH Values[a]

compd	PBS 4.0[b]	PBS 7.4[b]	PBS 10.0[b]	PBS 7.4 + Serum[c]
4	0	86	0.74	10
5	0	43	94	8.6
6	3.4	36	1.1	1.6
7	3.1	26	41	2.6
11	—	223	—	—

[a] The amount of NO_2^- (nmol) arising from 120 nmol test compound. It is assumed that NO_2^- formation is an equimolar reflection of the reaction of NO with H_2O to produce nitrite. The result is the mean value of three measurements (n = 3) where the variation from the mean value was ≤2%; [b] A solution of the test compound (120 nmol) in phosphate buffer (2.4 mL) at various different pH values (4.0, 7.4, or 10.0) was incubated at 37 °C for 1.5 h. [c] A solution of the test compound (120 nmol) in phosphate buffer (2.4 mL) at pH 7.4, to which 90 μL of rat serum had been added, was incubated at 37 °C for 1.5 h.

The amount (nmol) of NO released from the O^2-[*N*-hydroxy(methoxy)-2-ethanesulfonamido]diazen-1-ium-1,2-diolates **4**−**7** and PYRRO/NO **11** (120 nmol) upon incubation in phosphate-buffered-solution (PBS) at pH 4.0, PBS at pH 7.4, PBS at pH 10.0, or PBS at pH 7.4 containing rat serum were measured by quantitation of nitrite using the Griess reaction (see data in Table 1). Compounds **4** and **6** can theoretically release 3 equiv of NO (1 equiv from the SO_2NHOH moiety and 2 equiv from the diazeniumdiolate moiety). Compounds **5**, **7**, and **11** can theoretically release 2 equiv of NO from the diazeniumdiolate moiety. In PBS pH 4.0, all target compounds released a very low amount of NO (0−3.4 nmol of NO_2^- were detected from 120 nmol of the test compound), showing a good stability in an acidic medium. In PBS pH 7.4 sulfohydroxamic acids **4** and **6** released more NO relative to their *O*-methyl counterparts **5** and **7** (86 > 43 nmol, for **4** and **5**, 36 > 26 nmol, for **6** and **7**, respectively), which can be attributed to the alkylsulfohydroxamic acid moiety acting as a NO donor in PBS at pH 7.4.[12] It is interesting that the *N*-methoxy compounds **5** and **7** release more NO at pH 10.0 than at pH 7.4 (94 and 43 nmol for **5** at pH 10.0 and 7.4; 41 and 26 nmol for **7** at pH 10.0 and 7.4). Since the *N*-methoxysulfonylamide moiety cannot act as a NO donor, the higher NO release at pH 10.0 likely arises from the NONO moiety after its base-induced β-elimination. Furthermore, in PBS at pH 10.0, NO release from the sulfohydroxamic acids **4** and **6** was suppressed (0.74−1.1 nmol). One plausible explanation for this reduction in NO release could be due to the fact that the sulfohydroxamic acid moiety, at nonphysiological alkaline pH, decomposes to release HNO (rather than NO) and sodium sulfinate[14]

Table 2. Amount of Nitric Oxide (nmol) Released from **5** and **7** (120 nmol) in the Presence of Basic Compounds at Physiological pH[a]

compd	PBS + DBU[b]	PBS + Arg[b]	PBS + His[b]	H_2O[c]	H_2O + DBU[d]
5	98	96	89	36	209
7	34	34	29	—	—

[a] The amount of NO_2^- (nmol) arising from 120 nmol test compound. The result is the mean value of three measurements ($n = 3$) where variation from the mean value was ≤2%. [b] A solution of the test compound (120 nmol) in phosphate buffer (2.4 mL) at pH 7.4 containing basic compound DBU, Arg, or His (240 nMol) was incubated at 37 °C for 1.5 h. [c] A solution of the test compound (120 nmol) in ultrapure water (2.4 mL) was incubated at 37 °C for 1.5 h. [d] A solution of the test compound (120 nmol) in ultrapure water (2.4 mL) containing DBU (240 nmol) was incubated at 37 °C for 1.5 h.

which would prevent any subsequent β-elimination from occurring that is necessary to release the NONO moiety. On the other hand, NO release from **4**−**7** in PBS containing rat serum was suppressed (1.6−10 nmol) possibly due to strong serum protein binidng, which is consistent with a similar serum effect previously observed for aryl and alkyl sulfohydroxamic acids.[12,15]

These preliminary NO release studies indicate that *N*-methoxysulfonylamides **5** and **7** can decompose to release NO via a nonphysiological alkaline-induced β-elimination process. It was also of interest to investigate whether this β-elimination process could be induced in the presence of basic natural amino acids [arginine (Arg), histidine (His)] at physiological pH. Therefore, NO release from **5** and **7** upon incubation in PBS at pH 7.4 containing DBU, Arg, or His was investigated (see data in Table 2).

The amounts of NO_2^- (nmol) produced from **5** (120 nmol) in the presence of DBU, Arg, or His are 98, 96, and 89 nmol, respectively, which are similar to that arising from **5** in PBS at pH 10.0 (94 nmol, in Table 1). Similar comparisons were observed for compound **7**. Furthermore, the amounts of NO released from **5** (120 nmol) in H_2O alone and in H_2O containing DBU (240 nMol) were 36 and 209 nmol, respectively. In this regard, the basic amino acids Arg and His, and the organic non-nucleophilic base DBU, at physiological pH appear to function similar to that observed at a non-physiological alkaline pH of 10 with respect to inducing a β-elimination process for these O^2-((*N*-methoxy)ethanesulfonylamido) diazen-1-ium-1,2-diolates to free the NONO moiety which then acts as a NO donor to release NO.

In summary, the stability data for the new O^2-(ethanesulfohydroxamic acid) protected diazeniumdiolates (**4**, **6**) in the presence of serum at physiological pH, and the capability of the O^2-((*N*-methoxy)ethanesulfonylamido) diazeniumdiolates (**5**, **7**) to release NO in the presence of basic compounds, particularly basic amino acids, at physiological pH has been acquired. These data provide insight into their relevant pharmaceutical properties that are useful for developing drug design strategies pertaining to NONOate donors.

Acknowledgment. We are also grateful to the Canadian Institutes of Health Research (Grant No. MOP-14712) for financial support of this research.

Supporting Information Available. Experimental procedures and compound characterization for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

(14) (a) Bonner, F. T.; Ko, Y. *Inorg. Chem.* **1992**, *31*, 2514–2519. (b) Fukuto, J. M.; Hszieh, R.; Gulati, P.; Chiang, K. T.; Nagasawa, H. T. *Biochem. Biophys. Res. Commun.* **1992**, *187*, 1367–1373.

(15) Huang, Z.; Velázquez, C. A.; Abdellatif, K. A.; Chowdhury, M. A.; Jain, S.; Reisz, J. A.; DuMond, J. F.; King, S. B.; Knaus, E. E. *Org. Biomol. Chem.* **2010**, *8*, 4124–4130.