JOC The Journal of Organic Chemistry

Article

Calcium Dialkylamine Diazeniumdiolates: Synthesis, Stability, and Nitric Oxide Generation

Nabeelah Kauser, Mark Weisel, Yong-Li Zhong, Michael Man-Chu Lo, and Amjad Ali

J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.joc.0c00020 • Publication Date (Web): 13 Mar 2020

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Calcium Dialkylamine Diazeniumdiolates: Synthesis, Stability, and Nitric Oxide Generation

Nabeelah I. Kauser,† Mark Weisel, ‡ Yong-Li Zhong,‡ Michael Man-Chu Lo,† and Amjad Ali,*,†

Departments of † Discovery Chemistry, ‡ Process Research and Development, Merck & Co., Inc, Kenilworth, New Jersey 07033, United States

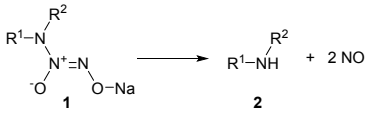
KEYWORDS Diazeniumdiolate, nitric oxide, NO donors, calcium diazeniumdiolate

ABSTRACT: The therapeutic application of nitric oxide, an endogenous cellular signaling molecule, has been limited due to the difficulty of developing stable pro-drugs with slow kinetics of NO release. Diazeniumdiolates are valuable NO donors however synthetic challenges have hampered their use. *O*^{2}-alkylation or –arylation of diazeniumdiolates form stable pro-drugs which have found application in hypertension, cancer and as anti-microbial agents. The synthesis of sodium diazeniumdiolates proved challenging due to hazardous reaction conditions, (high N_2O concentrations, and flammable solvents) which can lead to detonation and suffered from limited scope. We have previously disclosed that synthesis of calcium diazeniumdiolate salts are a safer and more scalable alternative. Herein, we report the expanded scope of calcium diazeniumdiolates from benzylic amines, amides, and sterically bulky amines hitherto inaccessible, and a comparison of their reactivity in comparison to sodium diazeniumdiolate.

Introduction

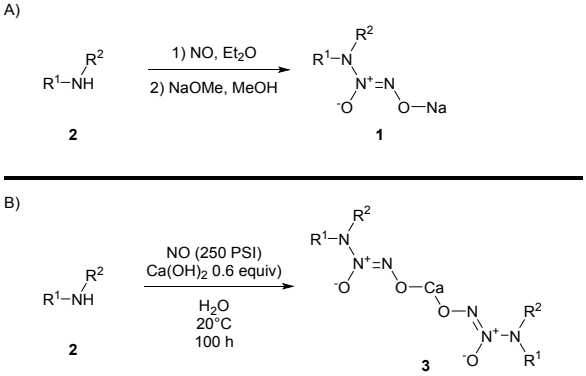
Nitric oxide, heralded as molecule of the year in 1992[1], acts as a crucial signaling molecule for vasodilation,[2] cellular signaling in the brain,[3] and defense system against pathogens.[4] First discovered in 1847, organic nitrates are effective for vasodilation and treatment of angina but suffer from uncontrollable NO release profiles, often resulting in unintended side effects such as headaches,[5] and hypotension.[6]

Diazeniumdiolates, (DAZD), have shown viability as NO donors and recently, a representative from this class of NO donors has advanced into human clinical trials.[6,7,8] The sodium diazeniumdiolate **1** are stable in the solid form but will release 2 moles of NO theoretically and the corresponding amine upon dissolution in an aqueous solution under physiological conditions. (Scheme 1). The rate of NO release is affected by pH. However, the rate can also be conveniently varied by modifying the R groups. Numerous diazeniumdiolates, over the past half century, have been synthesized with half-lives varying from 2 s to 20 h.[7]



Scheme 1. Decomposition of diazeniumdiolate to amine and NO

The synthesis of the diazeniumdiolate **1** was first reported by Drago et al, in 1961, through the reaction of nitic oxide with various amines to form white solids with the structure R^1R^2N-N(O)=NO$^-$ $R^1R^2N^+$.[9] (Scheme 2a). These solids were found to be unstable and converted to the corresponding nitrosamine overnight.[10] In 2001, Keefer *et al.* reported that converting the unstable solids to the sodium diazeniumdiolate salts **1** improved shelf-life.[11] However, this method of synthesizing the diazeniumdiolate would in our hands result in the occasional detonation due to the nature of the flammable solvents used during the synthesis, high nitrous oxide content and use of a strong alkoxide base.[12]



Scheme 2. Synthesis of sodium[11] and calcium[13] diazeniumdiolates.

The use of sterically bulky amines was often unsuccessful under these conditions leading to little or even no product in some cases. The failures to apply sterically bulky or electronically-varied amines implied that these diazeniumdiolates were either too unstable and underwent decomposition too rapid for isolation or were not applicable to the synthesis of diazeniumdiolates. While various amines were utilized for the synthesis of diazeniumdiolates, the challenges of low yields, instability of the product, or a lack of chemical matter greatly limited the scope of diazeniumdiolates and their subsequent research into biological or pharmaceutical applications.

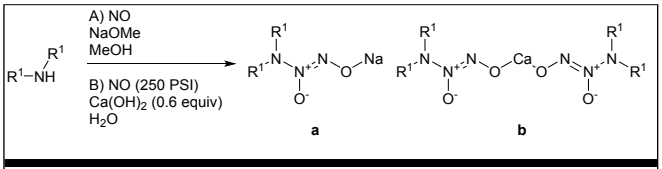
Recently studies reported by colleagues in our Process Research and Development department at Merck & Co., Inc., Kenilworth, NJ, USA have reported a safer and scalable method for the synthesis of the calcium diazeniumdiolates **3** on gram- and multikilogram-scale.[13] (Scheme 2b). These reported conditions enable the reaction to occur at room temperature in the absence of a flammable solvent or strong base.[13] Another challenge associated with the sodium diazeniumdiolates, as reported by Drago[9] and Andrei[14], was that storage of quantities above 250 mg were not recommended due to a detonation risk and stability issues.

Differential scanning calorimetry, DSC analysis showed that the calcium diazeniumdiolates were more thermally stable compared to the sodium diazeniumdiolates.[13,15,16]

Results and Discussion

Herein, we examine the advantages of the calcium diazeniumdiolate method towards the synthesis of simple dialkylamines, benzylic amines with various electronic natures and other various amine functionalities and their physiochemical properties. We began our investigation by analyzing the synthesis of simple dialkylamines.[13] Some of the sodium dialkylamine salts have been previously synthesized by Drago and others.[9] However, the yields are generally low and/or the reactions required careful control of temperature to prevent decomposition.

Table 1. The synthesis of dialkylamine DAZD calcium salt

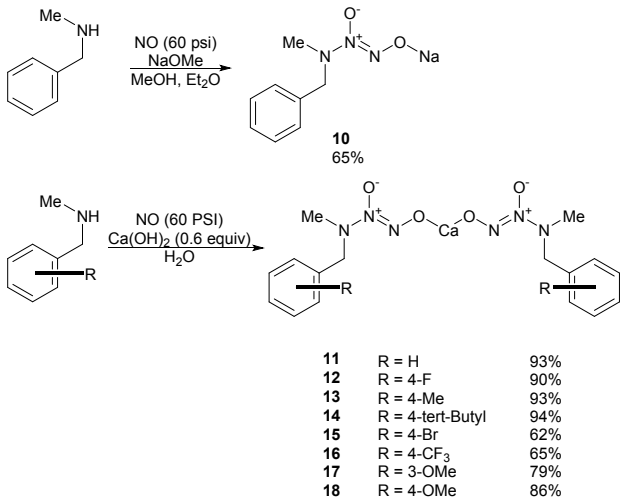


R^1	R^2	DAZD	Yield of Sodium DAZD Salt[a]	Yield of Calcium DAZD Salt[b]
$(n\text{-Bu})_2N$	$(n\text{-Bu})_2N$	**4**	80%[9]	98%[17]
$(i\text{-Bu})_2N$	$(i\text{-Bu})_2N$	**5**	N. R.	41%
sec-Butyl	*sec*-Butyl	**6**	N.R.[c]	38%[d]
Cy	Cy	**7**	N. R.	28%[d]
Cy	*tert*-Butyl	**8**	N. R.	0%[d]
Boc	*tert*-Butyl	**9**	N. R.	0%[d]

[a]Reported isolated yields of sodium DAZD salt. [b]Procedure b: All of the reactions were run on a 5.2 mmol scale for 100 h. All yields are isolated yields. [c] Synthesized but no yield reported. [d] 350 psi. N.R. not reported.

The method developed by Drago[9] or the modification by Keefer[11] provided little or no yield of the desired diazeniumdiolate from sterically hindered amines. Drago's attempt to synthesize the di-*sec*-butylamine DAZD **6a** resulted only in a low yield of ammonium nitrate whereas the method provided herein gave the diazeniumdiolate **6b** in 38%.[9] Dicyclohexylamine was able to undergo the reaction in 28% yield. (Table 1) The *t*-Butyl cyclohexylamine diazeniumdiolate **8b** was not synthesized as the steric bulk around the nitrogen prevented the reaction from occurring. Also, the electron-poor amine, N-Boc N-*tert*-Butylamine diazeniumdiolate **9b** failed to be synthesized as the nitrogen was most likely not sufficiently electron-rich enough to act as a nucleophile.[18]

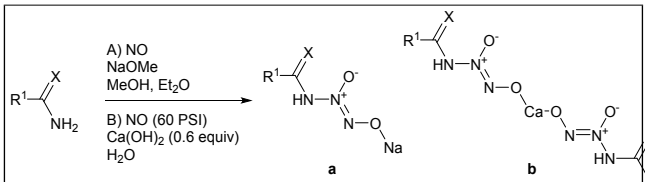
With the success of the dialkylamines, we turned our attention towards benzylic amines, such that the electronic nature of the amine could be altered through functionalization of the arene. The rate-limiting step in the mechanism to synthesize diazeniumdiolates is reported to be nucleophilic attack of the amine on the nitric oxide to form the nitrosamine which is then further reacted to form the diazeniumdiolate.[18] Accordingly, the more nucleophilic benzylic amines demonstrated that 60 psi of nitric oxide was sufficient pressure to provide high yields of the corresponding calcium diazeniumdiolate salt. (Scheme 3).



Scheme 3. The synthesis of benzylic amine DAZD calcium salt

The benzyl methyl amine sodium diazeniumdiolate **10** has been previously synthesized in 65% yield. [19,20] With our improved calcium salt method, we were able to synthesize the calcium diazeniumdiolate **11** in 93%. The more electron rich arenes, such as 4-*tert*-butylbenzylamine were also more nucleophilic and provided the corresponding calcium diazeniumdiolate salts in high yields. Our method also showed tolerance for aryl halides such as aryl fluorides or even aryl bromides, while the sodium salts of these diazeniumdiolates have not been reported.

Table 2. The synthesis of amide and amidine DAZD calcium salt



X	R^1	DAZD	Yield of Sodium DAZD Salt[a]	Yield of Calcium DAZD Salt[b]
N	$N(Me)_2$	**19**	N. R.	92%
N	Ph	**20**	39%[21]	83%
O	Me	**21**	10%[22]	32%
O	2-pyridyl	**22**	N.R.	70%

[a] Reported yields of sodium DAZD salt. [b] Procedure b: All of the reactions were run on a 5.2 mmol scale for 100 h. All yields are isolated yields. N.R. not reported.

Other amine functional groups such as guanidines and amidines were also found to be viable substrates, forming the DAZD salts **19** and **20** in 92% and 83% yield, respectively. Primary amides, acetamide and picolinamide formed the DAZD **21** and **22** in 32% and 70%, respectively, compared to the previous reports of 10% for acetamide.[22,23] (Table 2). The lower yield for the acetamide diazeniumdiolate is attributed to the difference in nucleophilic nature between acetamide, a weak nucleophile and picolinamide, a stronger nucleophile. The nitrogen on the pyridyl ring of the picolinamide diazeniumdiolate salt may also engage in H-bonding with the amide moiety to help stabilize the diazeniumdiolate. This H-bonding event is weaker for the acetamide diazeniumdiolate.[22]

Table 3. NO Release Rates of Calcium Diazeniumdiolates

DAZD Calcium Salt (R^1R^2N-N(O)=N(O))$_2$Ca			k X 10^4 (s^{-1})	Half-life ($t_{1/2}$)[a]
#	R^1	R^2		
4	*n*Bu	*n*Bu	90	77 s
5	*iso*-butyl	*iso*-butyl	85	82 s
6	*sec*-butyl	*sec*-butyl	77	90 s
7	cyclohexyl	cyclohexyl	40	2.9 min
11	Bn	Me	122	56 s[24]
12	(*p*-FC_6H_4)CH_2	Me	85	85 s
13	(*p*-$CH_3C_6H_4$)CH_2	Me	158	44 s
14	(*p*-*tert*-butyl-C_6H_4)CH_2	Me	126	50 s
15	(*p*-BrC_6H_4)CH_2	Me	84	84 s
16	(*p*-$F_3CC_6H_4$)CH_2	Me	29	4.0 min
17	(*m*-$CH_3OC_6H_4$)CH_2	Me	81	81 s
18	(*p*-$CH_3OC_6H_4$)CH_2	Me	124	56 s
19	$(CH_3)_2NC(N)$	H	267	26 s

[a]Decomposition of DAZD calcium salt in 0.1 M pH 7.4 potassium phosphate buffer at 37 °C was measured by UV/Vis analysis and detected by 250 nm wavelength.[27] Data supported first-order decomposition.

The decomposition of the sodium and calcium diazeniumdiolates is reported to occur at pH 7.4 and 37 °C, which can be monitored by the decrease in the absorbance at 250 nm wavelength which represents the diazeniumdiolate chromophore.[27] Decomposition rates for the sodium and calcium diazeniumdiolates have been previously reported to be comparable.[26] The half-lives of the calcium diazeniumdiolate salts were found to be affected by the alkyl chain branching.[25] (Table 3). The di-*n*-butylamine diazeniumdiolate **4b** had a half-life of 1.28 min, while branching the alkyl chain to di-*sec*-butylamine **6b** increased the half-life by 13 s.

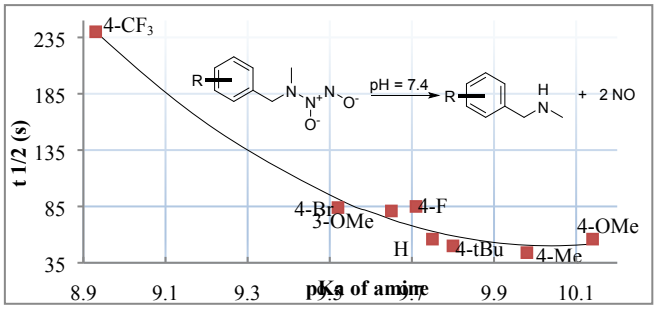
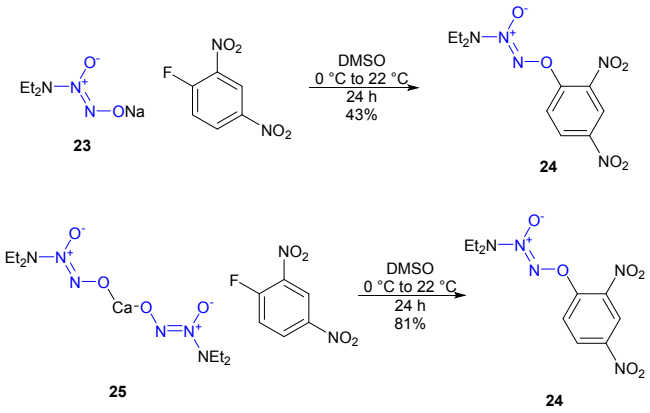


Figure 1. Comparison of pKa to half-lives of benzylic amines.

By examining the electronic effects of the aromatic ring on the half-lives of the diazeniumdiolate, a clear relationship between the pka and the half-lives was established. As the electron-withdrawing nature of the substituents on the aromatic ring increases, the pKa decreases. Thus, acidic amines undergo the protonation and subsequent decomposition to the amine and 2 equivalence of NO much slower than the more basic amines.[26] (Figure 1).

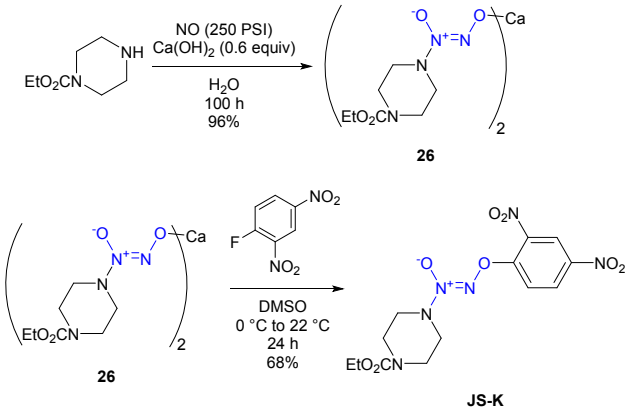
The alkylation of the calcium diazeniumdiolates was previously reported by Zhong *et al*.[13] Using previously reported conditions for the arylation of the sodium diazeniumdiolate **23**, the calcium diazeniumdiolate **25** was converted to the O^2-arylated product in 81% yield compared to 43% for the sodium diazeniumdiolate **23**.[19] (Scheme 4).

Scheme 4. Application of the sodium and calcium diazeniumdiolates towards sNAr coupling.



We applied the improved calcium diazeniumdiolate salt method of synthesizing O^2-arylated DAZD to the synthesis of JS-K, a tool molecule described previously for its anti-cancer activity.[27] The calcium diazeniumdiolate **26** was provided in 96%, compared to previously reports of 54%.[28] The sodium diazeniumdiolate of **26** can be converted to JS-K in one step through the sNAr reaction or be converted to the O^2-(triisopropylsilyloxy) methylated-diazeniumdiolate which is more stable; this in turn is converted to JS-K upon addition of TBAF and the aryl fluoride in 70% over two steps. In comparison, our calcium diazeniumdiolate **26** undergoes the sNAr in 68% yield for an overall yield of 65% compared to 38% for the sodium DAZD. (Scheme 5).

Scheme 5. Application of the Calcium diazeniumdiolate **26** towards the synthesis of JS-K.



In summary, we have expanded the scope and demonstrated the steric and electronic effects of the amine component on the synthesis of diazeniumdiolates and the reactivity of calcium diazeniumdiolate salts. In comparison to the reported method developed by Drago and Keefer, we have shown that sterically-hindered amines and electronically diverse amines are well-

tolerated by using our reaction conditions. Application of this method improved the synthesis of JS-K to an overall yield of 68%.

EXPERIMENTAL SECTION

General. Unless otherwise mentioned, all chemicals were purchased from commercial sources and used without further purification. Ultraviolet (UV) spectra were recorded on a diode array spectrophotometer. Nuclear magnetic resonance (NMR) spectra were collected with a 400 MHz spectrometer using appropriate deuterated solvents with chemical shifts reported in parts per million downfield from tetramethylsilane or 2,2,3,3-tetradeuterotrimethylsilylpropionic acid.

General Procedure for the Synthesis of Diazeniumdiolate: An 8 mL test-tube was charged with dialkylamine (5.2 mmol, 1.0 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and degassed H2O (3.5 mL). (Note: DI water was degassed by bubbling nitrogen for 2h). The mixture was then degassed under vacuum and then pressurized by nitrogen gas. The process of degassing/pressurizing was repeated 3 times. Then the reaction mixture was pressurized with NO gas. The reaction mixture was stirred at 800 rpm at 20 ºC.

After the reaction was complete, then the system was depressurized by releasing the NO gas under nitrogen gas. The solid was then collected by filtration and washed with 20 mL Et_2O. Then, it was dried under vacuum with nitrogen sweep to give the desired calcium salt.

Calcium 1-(di-*n*-butylamino)diazen-1-ium-1,2-diolate (4). A solution of dipropylamine (0.88 mL, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 250 psi of nitric oxide and stirred under pressure at room temperature to give 1.07 (98%) of the corresponding calcium salt as a white solid. UV (in 0.01 M NaOH) λmax 250 nm (3 mM^{-1} cm^{-1}); ^1H NMR (500 MHz,CD_3OD): 2.92 (t, J = 8.5 Hz, 8H), 1.38 (m, 16H), 0.90 (t, J = 8.7 Hz, 12H). $^{13}C\{1H\}$ NMR (CD_3OD): 54.1, 28.6, 19.7, 12.9. HRMS (ESI-TOF): [M + OH] calcd for $C_{16}H_{37}CaN_6O_5$ 433.2451, found 433.2464.

Calcium 1-(di-*i*sobutylamino)diazen-1-ium-1,2-diolate (5). A solution of di-isobutylamine (0.91 mL, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 250 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 446 mg (41%) of the corresponding calcium salt as a white solid. ^1H NMR (500 MHz,CD_3OD): 2.70 (d, J = 10 Hz, 8H), 1.53 (m, J = 10 Hz, 4H), 0.94 (d, J = 10 Hz, 24 H). $^{13}C\{1H\}$ NMR (125 MHz,CD_3OD): 62.2, 25.7, 19.5. HRMS (ESI-TOF): [M + OH] calcd for $C_{16}H_{37}CaN_6O_5$ 433.2451, found 433.2475.

Calcium 1-(di-*sec*-butylamino)diazen-1-ium-1,2-diolate (6). A solution of di-sec-butylamine (0.89 mL, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 350 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 411 mg (38%) of the corresponding calcium salt as a white solid. UV (in 0.01 M NaOH) λmax 250 nm (1.4 mM^{-1} cm^{-1}) ^1H NMR (500 MHz,CD_3OD): 3.32 (sextet, J = 8.5 Hz, 4H), 1.62 (m, 4H), 1.35 (m, 4H), 1.13 (dd, J = 8.5 Hz, 4.8 Hz, 12H), 0.92 (dt, J = 8.5 Hz, 4.8 Hz, 12H). $^{13}C\{1H\}$ NMR (125 MHz,CD_3OD): 55.3, 25.4, 15.0, 9.5. HRMS (ESI-TOF): [M + OH] calcd for $C_{16}H_{37}CaN_6O_5$ 433.2451, found 433.2467.

Calcium 1-(di-cyclohexylamino)diazen-1-ium-1,2-diolate (7). A solution of dicyclohexylamine (943 mg, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 350 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 380 mg (28%) of the corresponding calcium salt as a white solid. UV (in 0.01 M NaOH) λmax 250 nm (2.5 mM^{-1} cm^{-1}). ^1H NMR (500 MHz,CD_3OD): 3.23 (quintet, J = 9.5 Hz, 4H), 1.91-1.70 (m, 16H), 1.63 (m, 4H), 1.41-1.12 (m, 20H). $^{13}C\{1H\}$ NMR (125 MHz,CD_3OD): 56.9, 29.2, 25.8, 25.1. HRMS (ESI-TOF): [M + OH] calcd for $C_{24}H_{45}CaN_6O_5$ 537.3077, found 537.3078.

Calcium 1-(N-methylbenzylamino)diazen-1-ium-1,2-diolate (11). A solution of N-methyl-1-phenylmethanamine (0.67 mL, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 60 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 964.7 mg (93%) of the corresponding calcium salt as a white solid. Spectral data matches with the reported data.[19,20] ^1H NMR (500 MHz,CD_3OD): 7.18-7.42 (m, 10 H), 4.15 (s, 4H), 2.83 (s, 6H). $^{13}C\{1H\}$ NMR (125 MHz,CD_3OD): 129.1, 127.8, 127.3, 126.8, 59.1, 41.7. HRMS (ESI-TOF): [M + OH] calcd for $C_{16}H_{21}CaN_6O_5$ 417.1199, found 417.1219.

Calcium 1-(-(4-fluorophenyl)-N-methylmethanamino)diazen-1-ium-1,2-diolate (12). A solution of 1-(4-fluorophenyl)-N-methylmethanamine (0.69 mL, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 60 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 1.029 g (90%) of the corresponding calcium salt as a white solid. UV (in 0.01 M NaOH) λmax 250 nm (0.67 mM^{-1} cm^{-1}). ^1H NMR (500 MHz, CD_3OD): 7.38 (dt, J = 10 Hz, 3Hz, 4H), 6.99 (dt, J = 10 Hz, 3Hz, 4H), 4.12 (s, 4H), 2.83 (t, J = 2.3Hz, 6H). $^{13}C\{1H\}$ NMR (125 MHz, CD_3OD): 131.9, 131.1, 129.9, 114.3, 58.2, 41.7. ^{19}F NMR (470 MHz, CD_3OD): -117.2. HRMS (ESI-TOF): [M + OH] calcd for $C_{16}H_{19}CaF_2N_6O_5$ 453.1011, found 453.1049.

Calcium 1-(-(4-methylphenyl)-N-methylmethanamino)diazen-1-ium-1,2-diolate (13). A solution of 1-(4-methylphenyl)-N-methylmethanamine (0.77 mL, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI HvO was charged with 60 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 1.036 g (93%) of the corresponding calcium salt as a white solid. UV (in 0.01 M NaOH) λmax 250 nm (3.7 mM^{-1} cm^{-1}). ^1H NMR (500 MHz,CD_3OD): 7.25 (d, J = 10 Hz, 4H), 7.08 (d, J =10 Hz, 4H), 4.10 (s, 4H), 2.80 (s, 6H), 2.28 (s, 6H). $^{13}C\{1H\}$ NMR (125 MHz,CD_3OD): 137.0, 132. 7, 129.1, 128.5, 59.0, 41.5, 19.7. HRMS (ESI-TOF): [M + OH] calcd for $C_{18}H_{25}CaN_6O_5$ 445.1512, found 445.1534.

Calcium 1-(-(4-tert-butylphenyl)-N-methylmethanamino)diazen-1-ium-1,2-diolate (14). A solution of 1-(4-(tert-butyl)phenyl)-N-methylmethanamine (1.02 mL, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 60 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 1.243 g (94%) of the corresponding calcium salt as a white solid. UV (in 0.01 M NaOH) λmax 250 nm (1.65 mM^{-1} cm^{-1}). ^1H NMR (500 MHz,CD_3OD): 7.31 (m, 10H), 4.12 (s, 4H), 2.82 (s, 6H), 1.28 (s, 18H). $^{13}C\{1H\}$ NMR (125 MHz,CD_3OD): 128.8, 127.9,

124.9, 124.6, 58.8, 41.7, 30.3. HRMS (ESI-TOF): [M + OH] calcd for $C_{24}H_{37}CaN_6O_5$ 529.2451, found 529.2469.

Calcium 1-(-(4-bromophenyl)-N-methylmethanamino)diazen-1-ium-1,2-diolate (15). A solution of 1-(4-bromophenyl)-N-methylmethanamine (1.04 mL, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 60 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 902 mg (62%) of the corresponding calcium salt as a white solid. UV (in 0.01 M NaOH) λmax 250 nm (3.65 mM^{-1} cm^{-1}). ^1H NMR (500 MHz,CD_3OD): 7.42 (d, J = 10 Hz, 4H), 7.29 (d, J = 10 Hz, 4H), 4.11 (s, 4H), 2.86 (s, 6H). $^{13}C\{1H\}$ NMR (125 MHz,CD_3OD): 131.1, 131.0, 130.9, 130.1, 54.2, 34.0. HRMS (ESI-TOF): [M + OH] calcd for $C_{16}H_{19}Br_2CaN_6O_5$ 572.9410, found 572.9441.

Calcium 1-(-(4-trifluoromethylphenyl)-N-methylmethanamino)diazen-1-ium-1,2-diolate (16). A solution of 1-(4-trifluoromethylphenyl)-N-methylmethanamine (0.86 mL, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 60 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 912 mg (65%) of the corresponding calcium salt as a white solid. UV (in 0.01 M NaOH) λmax 250 nm (1.48 mM^{-1} cm^{-1}). ^1H NMR (500 MHz,CD_3OD): 7.56 (m,10H), 4.22 (s, 4H), 2.87 (s, 6H). $^{13}C\{1H\}$ NMR (125 MHz,CD_3OD): 14.0, 65.5, 113.5, 124.6, 129.5, 129.6, 134.9. ^{19}F NMR (470 MHz, CD_3OD): -64.1. HRMS (ESI-TOF): [M + OH] calcd for $C_{18}H_{19}CaF_6N_6O_5$ 553.0947, found 553.0930.

Calcium 1-(-(3-methoxyphenyl)-N-methylmethanamino)diazen-1-ium-1,2-diolate (17). A solution of 1-(3-methoxyphenyl)-N-methylmethanamine (0.78 mL, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 60 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 953 mg (79%) of the corresponding calcium salt as a white solid. UV (in 0.01 M NaOH) λmax 250 nm (2.5 mM^{-1} cm^{-1}). ^1H NMR (500 MHz,CD_3OD): 7.17 (t, J = 10 Hz, 2H), 6.98 (t, J = 2.7 Hz, 2H), 6.93 (d, J = 10 Hz, 2H), 6.79 (dd, J = 10 Hz, 2.6 Hz, 2H), 4.12 (s, 4H), 3.76 (s, 6H), 2.82 (s, 6H). $^{13}C\{1H\}$ NMR (125 MHz,CD_3OD): 159.7, 137.4, 128.8, 121.1, 114.3, 112.9, 59.1, 54.2, 41.7. HRMS (ESI-TOF): [M + OH] calcd for $C_{18}H_{25}CaN_6O_7$ 477.1411, found 477.1443.

Calcium 1-(-(4-methoxyphenyl)-N-methylmethanamino)diazen-1-ium-1,2-diolate (18). A solution of 1-(4-methoxyphenyl)-N-methylmethanamine (0.78 mL, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 60 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 1.028 g (86%) of the corresponding calcium salt as a white solid. UV (in 0.01 M NaOH) λmax 250 nm (2.65 mM^{-1} cm^{-1}). ^1H NMR (500 MHz,CD_3OD): 7.28 (d, J = 10 Hz, 2H), 6.81 (d, J = 10 Hz, 2H), 4.07 (s, 4H), 3.74 (s, 6H), 2.79 (s, 6H). $^{13}C\{1H\}$ NMR (125 MHz,CD_3OD): 158.3, 130.4, 127.8, 113.2, 58.6, 54.2, 41.4. HRMS (ESI-TOF): [M + OH] calcd for $C_{18}H_{25}CaN_6O_7$ 477.1411, found 477.1344.

Calcium (guanidinium)diazen-1-ium-1,2-diolate (19). A solution of 1,1- dimethylguanidine sulfate (983mg, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (616 mg, 8.32 mmol, 1.6 equiv) and 3.5 mL degassed DI H_2O was charged with 60 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 798 mg (92%) of the corresponding calcium salt as a white solid. UV (in 0.01 M NaOH) λmax 250 nm (2.1 mM^{-1} cm^{-1}). ^1H NMR (500 MHz,CD_3OD): 3.02 (s, 12 H). $^{13}C\{1H\}$ NMR (125 MHz,CD_3OD): 158.0, 36.8. HRMS (ESI-TOF): [M + OH] calcd for $C_6H_{17}CaN_{10}O_5$ 349.1009, found 349.1041.

Calcium (benzamidinium)diazen-1-ium-1,2-diolate (20). A solution of benzamidine (624.8 mg, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 60 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 855 mg (83%) of the corresponding calcium salt as a white solid. Spectral data matches with the reported data.[21] ^1H NMR (500 MHz,CD_3OD): 7.82 (d, J = 9.0 Hz, 4H), 7.53 (t, J = 9.0 Hz, 2H), 7.46 (t, J = 9.0 Hz, 4H). $^{13}C\{1H\}$ NMR (125 MHz,CD_3OD): 159.4, 133.4, 130.7, 128.1, 128.1, 126.9. HRMS (ESI-TOF): [M - H] calcd for $C_{14}H_{13}CaN_8O_4$ 397.0691, found 397.0718.

Calcium N-(acetamide)diazen-1-ium-1,2-diolate (21). A solution of acetamidine (307 mg, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 60 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 226.4 mg (32%) of the corresponding calcium salt as a white solid. Spectral data matches with the reported data.[22] ^1H NMR (500 MHz,D_2O): 1.68 (s, 6H). $^{13}C\{1H\}$ NMR (125 MHz,D_2O): 177.1, 20.8. HRMS (ESI-TOF): [M + OH] calcd for $C_4H_9CaN_6O_7$ 293.0159, found 293.0179.

Calcium N-(picolinamide)diazen-1-ium-1,2-diolate (22). A solution of picolinamide (635 mg, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 60 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 728.1 mg (70%) of the corresponding calcium salt as a white solid. UV (in 0.01 M NaOH) λmax 250 nm (0.85 mM^{-1} cm^{-1}) ^1H NMR (500 MHz,$(CD_3)_2O$): 8.57 (d, J = 5.1 Hz, 1H), 7.95 (d, J = 9.7 Hz, 2H), 7.52 (t, J = 9.7 Hz, 1H). $^{13}C\{1H\}$ NMR (125 MHz,D_2O):152.2, 148.3, 138.4, 126.1, 123.9. HRMS (ESI-TOF): [M + OH] calcd for $C_{12}H_{11}CaN_8O_7$ 419.0377, found 419.0384.

1-(2,4-dinitrophenoxy)-3,3-diethyltriaz-1-ene 2-oxide (24). In a 24 mL round bottom flask, a solution of Diethyl amino calcium DAZD **24** (183 mg, 0.6 mmol, 1.25 equiv) in 0.6 mL DMSO was cooled to 0°C under nitrogen. Then 1-fluoro-2,4-dinitrobenzotrifluoride (0.127 mL, 1.0 mmol, 1 equiv) in 0.9 mL DMSO was added dropwise. The mixture was warmed naturally to room temperature and stirred for 18 hours. The reaction is quenched with 5 mL H_2O and extracted with Et_2O (20 mL x 3). Then the organic layers were combined and then dried with sodium sulfate and concentrated under vacuum. The crude oil product was chromatographed on a 12 g silica gel column and eluted with a gradient from 100% hexanes to 70:30 hexanes/ethyl acetate to give 238 mg (80% yield) of 1-(2,4-dinitrophenoxy)-3,3-diethyltriaz-1-ene 2-oxide as an orange solid. Spectral data matches with the reported data.[19]

Calcium (Ethyl piperazine-1-carboxylate)diazen-1-ium-1,2-diolate (26). A solution of ethyl piperazine-1-carboxylate (0.77 mL, 5.2 mmol, 1 equiv), $Ca(OH)_2$ (231 mg, 3.12 mmol, 0.6 equiv) and 3.5 mL degassed DI H_2O was charged with 250 psi of nitric oxide and stirred under pressure at room temperature for 100 h to give 712 mg (96%) of the corresponding calcium salt as a white solid. ^1H NMR (500 MHz,CD_3OD): 4.13 (t, J = 5 Hz, 4H), 3.65 (m, 4H), 3.42 (m, 4H), 3.17 (t, J = 5Hz, 4H), 2.75 (t, J = 5Hz, 4H), 1.26 (q, J = 5Hz, 6H). $^{13}C\{1H\}$ NMR (125 MHz,CD_3OD): 155.5, 61.6, 51.7, 44.8, 13.5. HRMS (ESI-TOF): [M + OH] calcd for $C_{14}H_{27}CaN_8O_9$ 491.1527, found 491.1541.

JS-K. In a 24 mL round bottom flask, a solution of piperidine calcium DAZD **25** (178 mg, 0.375 mmol, 1.25 equiv) in 0.3 mL DMSO was cooled to 0°C under nitrogen. Then 1-fluoro-2,4-dinitrobenzotrifluoride (0.076 mL, 0.6 mmol, 1 equiv) in 0.5 mL DMSO was added dropwise. The mixture was warmed naturally to room temperature and stirred for 18 hours. The reaction is quenched with 5 mL H_2O and extracted with Et_2O (20 mL x 3). Then the organic layers were combined and then dried with sodium sulfate and concentrated under vacuum. The crude oil product was chromatographed on a 12 g silica gel column and eluted with a gradient from 100% hexanes to 50%:50% hexanes/ethyl acetate to give 157 mg (68% yield) of 1-(2,4-dinitrophenoxy)-3,3-diethyltriaz-1-ene 2-oxide was isolated as a yellow solid. Spectral data matches with the reported data.[27]

Kinetic Studies. Kinetic experiments were performed at 37 °C using a standard UV−visible spectrophotometer. Reactions were initiated by addition of substrate to a preheated cuvette containing the buffer. Analyte concentration was measured in 100 μM 0.1M phosphate buffer, pH 7.4. In each experiment, the data were analyzed at the λ_{max} and the rate was derived by fitting the data to an exponential curve typical for first-order processes.

ASSOCIATED CONTENT

Supporting Information.
This material is available free of charge via the Internet at http://pubs.acs.org.

- ^{1}H, ^{13}C, and ^{19}F NMR spectra of new compounds

AUTHOR INFORMATION

Corresponding Author

*** Email:** amjad_ali@merck.com

Notes
The authors declare no competing financial interest.

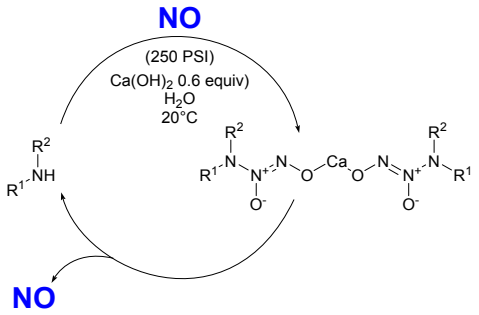
ACKNOWLDEGMENT

The authors acknowledge support from the MRL Postdoctoral Research Program.

REFERENCES

[1] Koshland, D.E. The molecule of the year. *Science*. **1992,** *258*, 1861.
[2] Moncada, S.; Higgs, E.A. Nitric oxide and the vascular endothelium. *Handbook Exp. Pharmacol*. **2006**, *176*, 213–254
[3] a) Garthwaite, J.; Boulton, C. L. Nitric Oxide Signaling in the Central Nervous System. *Annu. Rev. Physiol*. **1995**, *57*, 683-706. b) Faraci, F. M.; Brian, J. E. Nitric Oxide and the Cerebral Circulation. *Stroke*. **1994**, *25*, 692–703.
[4] A) Fang F. C. Mechanisms of Nitric Oxide–related Antimicrobial Activity. *J. Clin. Invest.* **1994**, 2818-2825. B) Groote, M. A.; Fang, F. C. NO Inhibitions: Antimicrobial Properties of Nitric Oxide. *Clin. Infect. Dis.* **1995**, *21*, S162–S165
[5] Bagdy, G.; Riba, P.; Kecskeméti, V.; Chase, D.; Juhász, G.; Headache-type adverse effects of NO donors: vasodilation and beyond. *Br. J. Pharmacol.* **2010**, *160,* 20–35.
[6] Gross, S. S. Nitric Oxide: Pathophysiological Mechanisms. *Annu. Rev. Physiol*. **1995**. *57*, 737-769.
[7] Keefer, L. R. Nitric Oxide (NO)- and Nitroxyl (HNO)-Generating Diazeniumdiolates (NONOates): Emerging Commercial Opportunities. *Curr. Top. Med. Chem.* **2005**, *5,* 625-636.
[8] Ali, A.; Knox, C.; Jan de Kam, P.; Azer, K.; Wong, P.; Ederveen, A. G.; Shevell, D.; Morabito, C.; Meehan, A.; Liu, W.; Reynders, T.; Denef, J.F.; Mitselos, A.; Jonathan, D.; Gutstein, D.; Mitra, K.; Sun, S.Y.; Lo, M.; Cully, D. The Discovery and Clinical Evaluation of MK-8150, A Novel Nitric Oxide Donor With A Unique Mechanism of Action. *J. Am. Heart Assoc*. **2016** *25*: 5-17.
[9] Drago, R. S.; Karstetter, B. R. The Reaction of Nitrogen(II) oxide with various primary and secondary amines. *J. Am. Chem. Soc.*, **1961**, *83*, 1819–1822.
[10] Ragsdale, R. O.; Karstetter, B. R.; Drago, R. S. Decomposition of the adducts of diethylamine and isopropylamine with nitrogen(II) oxide. *Inorg. Chem*. **1965**, *4*, 420–422
[11] Keefer, L. K.; Flippen-Anderson, J. L.; George, C.; Shanklin, A. P.; Dunams, T. M.; Christodoulou, D.; Saavedra, J. E.; Sagan, E. S.; Bohle, D. S. Chemistry of the diazeniumdiolates. 1. Structural and spectral characteristics of the [N(O)NO] functional group. *Nitric Oxide*. **2001**, *5*, 377–394.
[12] Muzzio, D.; Bassan, E.; Dienemann, E.; Weisel, M.; Cowden, C.; Hoerrner, S.; Olsen, W.; Man-Chu Lo, M.; Ali, A. Chemical Safety: Nitric Oxide At High Pressure. *Chem. Eng. News Lett.* **2012**, *90, 6.*
[13] Zhong, Y.-L.; Weisel, M.; Humphrey, G. R.; Muzzio, D. J.; Zhang, L.; Huffman, M. A.; Zhong, W.; Maloney, K. M.; Campos, K. R. *Org. Lett.*, **2019**, 4210-4214.
[14] Andrei, D.; Puglisi, M. P.; Bradaric, M. J.; Pontikis, J.; Cabai, J.; Weyna, T.; Tednes, P.; Schretzman, R.; Rickert, K.; Cao, Z.; Novel primary amine diazeniumdiolates—Chemical and biological characterization *Drug Dev. Res*. **2018,** *79*, 136–143.
[15] See DSC Results: Zhong, Y.-L.; Weisel, M.; Humphrey, G. R.; Muzzio, D. J.; Zhang, L.; Huffman, M. A.; Zhong, W.; Maloney, K. M.; Campos, K. R. *Org. Lett.*, **2019**, *21*, 4210-4214.
[16] Clas, S. D.; Dalton, C.R.; Hancock, B.R. Differential scanning calorimetry: applications in drug development. *Pharm. Sci. Tech. Today.* **1999.** *2*, 311-320.
[17] At 40 psi, the Calcium 1-(di-*n*-butylamino)diazen-1-ium-1,2-diolate was isolated in 84% yield.
[18] Drago, R.S.; Ragsdale, R.O.; Eyman, D. P. A Mechanism for the Reaction of Diethylamine with Nitric Oxide. *J. Am. Chem. Soc.* **1961**, *83*, 4337-4339.
[19] Saavedra, J. E.; Srinivasan, A.; Challice, L.; Bonifant, J. C.; Shanklin, A. P.; Flippen-Anderson, J.L.; Rice, W. G.; Turpin J. A.; Davies, K. M.; Keefer, L. K. The Secondary Amine/Nitric Oxide Complex Ion $R_2N[N(O)NO]^-$ as Nucleophile and Leaving Group in S_NAr Reactions.

J. Org. Chem. **2001**, *66*, 3090-3098.
[20] Smirnov, G. A.; Gordeev, P. B.; Nikitin, S. V.; Pokhvisneva, G. V.; Ternikova, T. V.; Luk´yanov, O. A. Synthesis of methylenebis(1-oxy-3,3-dialkyl-1-triazene-2-oxides) and their analogs. *Russ. Chem. Bull., Int. Ed*. **2015,** *64*, 1057-1061.
[21]Biswas, D.; Deschamps, J.R.; Keefer, L.K.; Hrabie, J.A. Nitrogen-bound diazeniumdiolated amidines. *Chem. Commun*. **2010**, *46*, 5799–5801.
[22]Holland, R.J.; Klose, J.R.; Deschamps, J.R.; Cao, Z.; Keefer, L.K.; Saavedra, J.E. Direct Reaction of Amides with Nitric Oxide To Form Diazeniumdiolates. *J. Org. Chem*. **2014**, *79*, 9389−9393
[23] The acetamide diazeniumdiolate was difficult to separate from the unreacted acetamide.
[24] Konter, J.; Abuo-Rahma, G. E. A. A.; El-Emam, A.; Lehmann, J. Synthesis of Diazen-1-ium-1,2-diolates Monitored by the "NOtizer" Apparatus: Relationship between Formation Rates, Molecular Structure and the Release of Nitric Oxide. *Eur. J. Org. Chem.* **2007**, 616–624.
[25] Not enough data of decomposition rates of the sodium DAZD salts has been reported to make the comparison for the sodium salts as well.
[26] Andrei, D.; Salmon, D. J.; Donzelli, S.; Wahab, A.; Klose, J. R.; Citro, M. L.; Keefer, L. K. Dual mechanisms of HNO generation by a nitroxyl prodrug of the diazeniumdiolate (NONOate) class. *J. Am. Chem. Soc.*, **2010**, *132*, 16526–16532.
[27] 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. JS-K, a glutathione/glutathione S-transferase-activated nitric oxide donor of the diazeniumdiolate class with potent antineoplastic activity. *Mol. Cancer Ther.* **2003**, *2*, 409-417.
[28] Nandurdikar,R. S.; Keefer, L. K.; Saavendra, J. A. Novel protection–deprotection strategies in diazeniumdiolate chemistry: synthesis of V-IPA/NO. *Chem. Commun.* **2011**, *47*, 6710–6712.



ToC graphic