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An improved synthesis of V-PROLI/NO, a cytochrome P450-activated nitric oxide prodrug

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ABSTRACT

An improved synthesis of V-PROLI/NO, a cytochrome P450-activated nitric oxide (NO) prodrug, in an overall yield of 26% in four steps from prolinol is reported; the previously published yield of this transformation was 1%. Using this revised strategy, the sarcosine analogue (**14**) of V-PROLI/NO was prepared. Finally, the methyl ester of V-PROLI/NO (**15**) was found to be an esterase-activated prodrug form of V-PROLI/NO.

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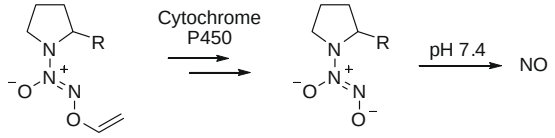
O^2-(Vinyl) diazeniumdiolates are prodrugs that are designed to be activated by cytochrome P450 to release nitric oxide (NO). O^2-Vinyl 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (V-PYRRO/NO, **1**), a member of this class of prodrugs, was shown to be a hepatoprotective agent against a variety of toxins in several animal models (Scheme 1).[1–18]

Earlier, we reported that O^2-vinyl [2-carboxylato)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (V-PROLI/NO, **2**), the L-proline analogue of V-PYRRO/NO, was metabolized by two isoforms of cytochrome P450 (Scheme 1). The nitric oxide prodrug PROLI/NO (**4**) may have a favorable toxicological profile as the expected products of decomposition are L-proline and NO, both naturally occurring metabolites.[19] Upon treatment of human liver HepG2 cells with V-PROLI/NO, the formation of nitrite, a product of aerobic oxidation of NO, in a time- and concentration-dependent manner, was reported.[20] Finally, V-PROLI/NO enhanced arsenite's chemotherapeutic efficacy in a HepG2 liver cell model.[20]



Scheme 1. The nitric oxide prodrugs V-PYRRO/NO (**1**) and V-PROLI/NO (**2**) and their corresponding devinylated metabolites, the spontaneously NO-releasing anions PYRRO/NO (**3**) and V-PROLI/NO (**4**).

V-PROLI/NO was previously synthesized in four steps from prolinol in an overall yield of roughly 1% (Scheme 2).[19] Diazeniumdiolation of prolinol provided **5** (73%), which was then subsequently treated with 2-bromo-1-(trifluoromethanesulfonyloxy)ethane to afford **6** in 48% yield (Scheme 2).[19] Oxidation of **6** formed **7** in 19% yield; and dehydrohalogenation of **7** afforded V-PROLI/NO in 19% yield.[19]

At the outset, our goal was to improve the overall yield of V-PROLI/NO. Towards this aim, first, the diazeniumdiolation of prolinol was carried out numerous times and the yield of **5** varied from 73% to 82%. Next, the alkylation of **5** to form **6** was optimized and the yield obtained was 78%.[21] Recently, we reported the improved synthesis of V-PYRRO/NO using an alkylation followed by a dehydrohalogenation of **8** by Verkade's SuperBase (**9**) (Scheme 3).[18,22]

Under similar conditions, when **7** was treated with **9**, we observed complete disappearance of starting material but without any trace of the desired product, V-PROLI/NO.[18]

Instead, an inseparable mixture of presumably intra- and intermolecular substitution products resulted. This observation is consistent with a previous study by Arumugam and Verkade who reported that 1-bromo-propanoic acid failed to produce the dehydrohalogenation product, acrylic acid, but instead formed the nucleophilic substitution product, β-propiolactone, in a nearly quantitative yield.[22] Other bases such as DBU or proton sponge failed to induce elimination of **7** and resulted in the recovery of the starting material.

Thus, a revised strategy to synthesize V-PROLI/NO was necessary. Instead of oxidizing **6** to **7** and then attempting to dehydrohalogenate **7** to form V-PROLI/NO, a reversal of the order of the aforementioned reactions was envisaged (Scheme 4). Dehydrohalogenation of **6** is expected to produce **10** and subsequent oxidation of **10** should produce V-PROLI/NO.

Indeed this strategy to synthesize V-PROLI/NO was successful (Scheme 5). The alcohol **10** was isolated in 83% yield from the

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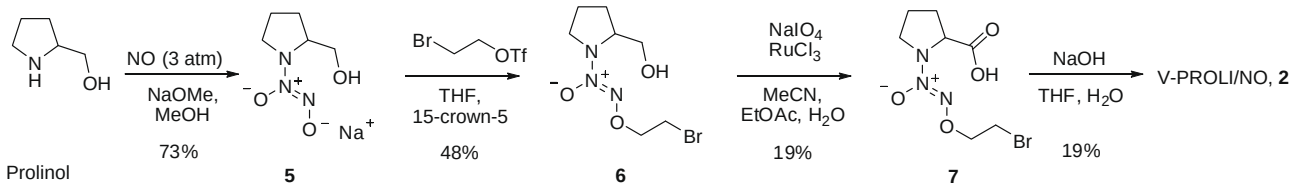
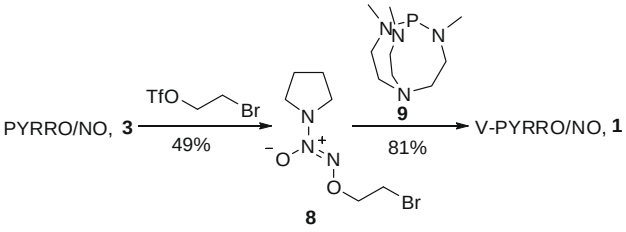
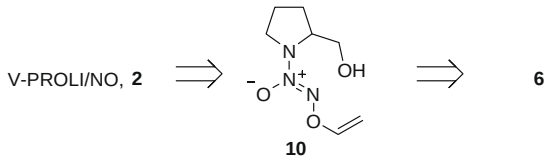


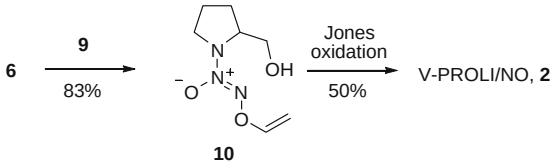
Scheme 2. Reported synthesis of V-PROLI/NO (**2**).[19]



Scheme 3. Improved synthesis of V-PYRRO/NO (**1**).[18]



Scheme 4. Revised retrosynthetic analysis of V-PROLI/NO (**2**).

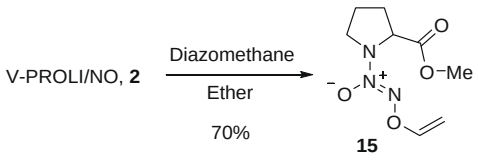


Scheme 5. Improved synthesis of V-PROLI/NO from **6**.

treatment of **6** with **9**.[23] Next, oxidation of **10** was carried out using the Jones oxidation method to produce V-PROLI/NO in a yield of 50%; this step was also convenient as no chromatography was required during isolation of the product.[24] Thus, starting from prolinol, the overall yield of V-PROLI/NO was 26% in four steps.

This improved strategy was applied to the synthesis of the sarcosine analogue of V-PROLI/NO (Scheme 6). A reported method was used to prepare the diazeniumdiolate salt **11**.[25] Next, **11** was alkylated to afford **12** in 71% yield.[26] Dehydrohalogenation of **12** produced **13** in 55% yield;[27] and finally, the alcohol **13** was oxidized to form **14** in 57% yield.[28] The overall yield of **14** from the commercially available 2-(methylamino)ethanol was 16% (four steps).

Next, the methyl ester of V-PROLI/NO was prepared in 70% yield by reacting V-PROLI/NO with diazomethane in ether (Scheme 7).[29]



Scheme 7. Synthesis of **15**.

The methyl ester **15** was found to be a prodrug form of V-PROLI/NO. In pH 7.4 buffer at 37 °C, gradual disappearance of **15** (Fig. 1A) over several days (Fig. 1B) with concomitant formation of V-PROLI/NO (Fig. 1C) was observed.[30] A time course of this ester hydrolysis to form V-PROLI/NO is shown in Figure 2. Finally, treatment of **15** with esterase formed V-PROLI/NO in a nearly quantitative yield (by HPLC).[31] Taken together, these results indicate that **15** is a prodrug for V-PROLI/NO.

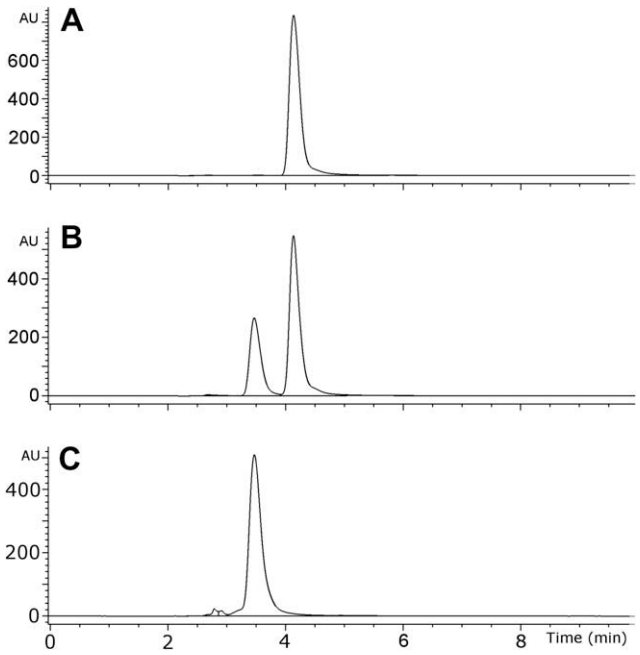
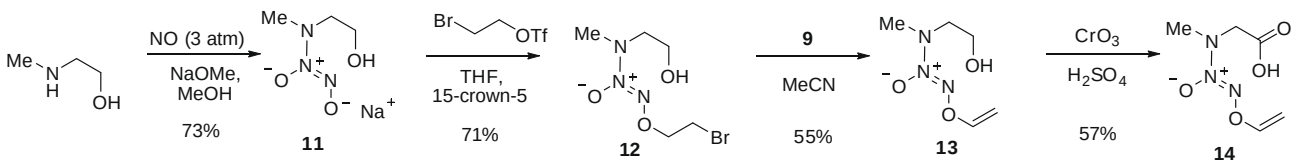


Figure 1. HPLC traces of: (A) **15** in pH 7.4 phosphate buffered saline (PBS) at time = 0; (B) **15** and its hydrolysis product in pH 7.4 PBS at time = 14 days, the hydrolysis product was identified as V-PROLI/NO; (C) V-PROLI/NO in pH 7.4 PBS.



Scheme 6. Synthesis of **14** in 4 steps from *N*-methyl-*N*-(2-hydroxyethyl)amine.

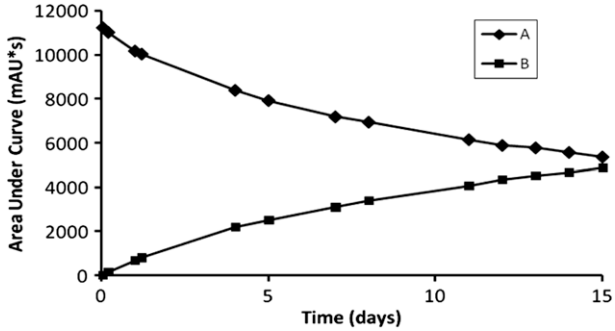


Figure 2. Time course of ester hydrolysis of **15** (A) forming V-PROLI/NO (B) in pH 7.4 Hank's Balanced Salt Solution (HBSS) at 37 °C during 15 days.

Carboxylic acid esters are reported to have improved bioavailability in comparison with their carboxylic acid counterparts.[32] Recently, we reported that ester derivatives of PROLI/NO were superior cell penetrators and inhibitors of proliferation of HL-60 leukemia cells than their corresponding free carboxylic acid counterparts.[33] Studies on the cell permeability and the efficacy of **14** and **15** as hepatoprotective agents will be reported in due course.

Acknowledgments

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References and notes

1. Saavedra, J. E.; Billiar, T. R.; Williams, D. L.; Kim, Y.-M.; Watkins, S. C.; Keefer, L. K. *J. Med. Chem.* **1997**, *40*, 1947–1954.
2. Kim, Y.-M.; Kim, T.-H.; Chung, H.-T.; Talanian, R. V.; Yin, X.-M.; Billiar, T. R. *Hepatology* **2000**, *32*, 770–778.
3. Ricciardi, R.; Foley, D. P.; Quarfordt, S. H.; Saavedra, J. E.; Keefer, L. K.; Wheeler, S. M.; Donohue, S. E.; Callery, M. P.; Meyers, W. C. *Transplantation* **2001**, *71*, 193–198.
4. Shokolenko, I.; Oberyszyn, T. M.; D'Ambrosio, S. M.; Saavedra, J. E.; Keefer, L. K.; LeDoux, S. P.; Wilson, G. L.; Robertson, F. M. *Nitric Oxide Biol. Chem.* **2001**, *5*, 555–560.
5. Liu, J.; Saavedra, J. E.; Lu, T.; Song, J.-G.; Clark, J.; Waalkes, M. P.; Keefer, L. K. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 18–25.
6. Stinson, S. F.; House, T.; Bramhall, C.; Saavedra, J. E.; Keefer, L. K.; Nims, R. W. *Xenobiotica* **2002**, *32*, 339–347.
7. Li, C.; Liu, J.; Saavedra, J. E.; Keefer, L. K.; Waalkes, M. P. *Toxicology* **2003**, *189*, 173–180.
8. Liu, J.; Li, C.; Waalkes, M. P.; Clark, J.; Myers, P.; Saavedra, J. E.; Keefer, L. K. *Hepatology* **2003**, *37*, 324–333.
9. Gong, P.; Cederbaum, A. I.; Nieto, N. *Mol. Pharmacol.* **2004**, *65*, 130–138.
10. Liu, J.; Qu, W.; Saavedra, J. E.; Waalkes, M. P. *J. Pharmacol. Exp. Ther.* **2004**, *310*, 18–24.
11. Balogh, G. T.; Dalmadi, B.; Bielik, A.; Keserü, G. M. *Comb. Chem. High Throughput Screening* **2005**, *8*, 347–352.
12. Liu, J.; He, Y.-Y.; Chignell, C. F.; Clark, J.; Myers, P.; Saavedra, J. E.; Waalkes, M. P. *Biochem. Pharmacol.* **2005**, *70*, 144–151.
13. Liu, J.; Waalkes, M. P. *Toxicology* **2005**, *208*, 289–297.
14. Qu, W.; Liu, J.; Fuquay, R.; Shimoda, R.; Sakurai, T.; Saavedra, J. E.; Keefer, L. K.; Waalkes, M. P. *Nitric Oxide Biol. Chem.* **2005**, *12*, 114–120.
15. Inami, K.; Nims, R. W.; Srinivasan, A.; Citro, M. L.; Saavedra, J. E.; Cederbaum, A. I.; Keefer, L. K. *Nitric Oxide Biol. Chem.* **2006**, *14*, 309–315.
16. Qu, W.; Liu, J.; Fuquay, R.; Saavedra, J. E.; Keefer, L. K.; Waalkes, M. P. *Cancer Lett.* **2007**, *256*, 238–245.
17. Edwards, C.; Feng, H.-Q.; Reynolds, C.; Mao, L.; Rockey, D. C. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2008**, *294*, G1311–G1317.
18. Hong, S. Y.; Saavedra, J. E.; Keefer, L. K.; Chakrapani, H. *Tetrahedron Lett.* **2009**, *50*, 2069–2071.
19. Chakrapani, H.; Showalter, B. M.; Kong, L.; Keefer, L. K.; Saavedra, J. E. *Org. Lett.* **2007**, *9*, 3409–3412.
20. Qu, W.; Liu, J.; Dill, A. L.; Saavedra, J. E.; Keefer, L. K.; Waalkes, M. P. *Cancer Sci.* **2009**, *100*, 382–383.
21. The reaction was carried out in the same way as reported earlier (Ref. 19) but was initiated at room temperature and not on ice. Detailed procedure: To a slurry of **5** (4.00 g, 21.8 mmol) and 15-crown-5 (100 μL) in THF (44 mL), 2-bromo-1-(trifluoromethanesulfonyloxy)ethane (6.18 g, 24.0 mmol, 1.1 equiv) was added and the mixture was stirred overnight at room temperature. The solution was washed with 10% NaOH and the organic layer was separated, dried (Na_2SO_4) and filtered through a plug of $MgSO_4$. The filtrate was concentrated under reduced pressure and the resulting oil was then purified by silica gel flash chromatography using a mixture of 12→75% EtOAc/hexanes to afford **6** (4.40 g, 16.4 mmol, 75%) as an oil. The spectral features of this material were consistent with the previously published values (Ref. 19).
22. Arumugam, S.; Verkade, J. G. *J. Org. Chem.* **1997**, *62*, 4827–4828.
23. *O^2-Vinyl 1-[2-(hydroxymethyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate* (**10**): To a solution of **6** (1.70 g, 6.3 mmol) in anhydrous acetonitrile (64 mL), **9** (2.74 g, 2.27 mmol) was added and the resulting mixture was stirred overnight at room temperature. The solvent was removed and the resulting oil was purified using silica gel flash chromatography with a mixture of 12→75% EtOAc/hexanes to afford **10** (0.99 g, 5.27 mmol, 83%) as an oil: UV (EtOH) λ_{max} (ε) 267 nm (8.84 mM^{-1} cm^{-1}); ^1H NMR (400 MHz, $CDCl_3$) δ 6.80 (dd, *J* = 14.1, 6.7 Hz, 1H), 4.84 (dd, *J* = 14.1, 2.5 Hz, 1H), 4.39 (dd, *J* = 6.7, 2.5 Hz, 1H), 4.24–4.09 (m, 1H), 3.89–3.48 (m, 4H), 2.66 (t, *J* = 5.9 Hz, 1H), 2.25–1.80 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.5, 91.9, 65.1, 64.0, 52.8, 26.8, 23.1. Anal. Calcd for $C_7H_{13}N_3O_3$·0.1 EtOAc: C, 45.35; H, 7.10; N, 21.44. Found: C, 45.35, H, 6.93; N, 21.08.
24. *Oxidation of* **10**: To **10** (100 mg, 0.53 mmol) in acetone (5 mL) at rt, Jones reagent (40 g CrO_3 + 32 mL 98% sulfuric acid + 75 mL H_2O) was added dropwise until TLC showed a complete disappearance of starting material. The solution was then quenched with 2-propanol (1 mL) and concentrated. The ensuing residue was taken up in water (5 mL) and washed with DCM (4 × 4 mL). The organic fractions were collected and concentrated. The pale yellow oil was dissolved in a NaOH solution (1.5 M, 3 mL) and washed with DCM (3 × 2 mL). The aqueous phase was then acidified with HCl (6 M) until pH 2.0 was reached. The aqueous phase was washed with DCM (5 × 4 mL). The organic fractions were collected and concentrated under reduced pressure affording **2** (54 mg, 0.267 mmol, 50% yield) as a waxy brown solid. The spectral features of this solid matched with previously reported values (Ref. 19). Note: to address any possible chromium impurities, additional purification by silica gel flash column chromatography (9:1 CH_2Cl_2/ethyl acetate), was conducted; the yield of **2** in such a case was 21%.
25. Nguyen, J.-T.; Velazquez, C. A.; Knaus, E. E. *Bioorg. Med. Chem.* **2005**, *13*, 1725–1738.
26. *O^2-(2-Bromoethyl) 1-[N-(2-hydroxyethyl)-N-methylamino]diazen-1-ium-1,2-diolate* (**12**): To a slurry of **11** (100 mg, 0.64 mmol) and 15-crown-5 (100 μL) in THF (7 mL), 2-bromo-1-(trifluoromethanesulfonyloxy)ethane (213 mg, 0.83 mmol, 1.3 equiv) was added and the resulting reaction mixture was stirred overnight at room temperature. The solution was washed with 10% NaOH and the organic layer was separated, dried (Na_2SO_4) and filtered through a plug of $MgSO_4$. The filtrate was concentrated under reduced pressure and the resulting oil was then purified by silica gel flash chromatography using a mixture of 12→75% EtOAc/hexanes to afford **12** (110 mg, 0.45 mmol, 71%) as an oil: UV (MeCN) λ_{max} (ε) 261 nm (7.00 mM^{-1} cm^{-1}); ^1H NMR (400 MHz, $CDCl_3$) δ 4.50 (t, *J* = 8.0 Hz, 2H), 3.79–3.74 (m, 2H), 3.58 (t, *J* = 8.0 Hz, 2H), 3.45–3.42 (m, 2H), 3.05 (s, 3H), 2.14 (t, *J* = 5.8 Hz, 1H);^{13}C NMR (100 MHz, $CDCl_3$) δ 72.7, 59.6, 57.1, 42.0, 27.8. Anal. Calcd for $C_5H_{12}BrN_3O_3$·0.1 EtOAc: C, 25.85; H, 5.14; N, 16.75; Br, 31.85. Found: C, 25.97; H, 5.14; N, 16.48; Br, 31.76.
27. *O^2-Vinyl 1-[N-(2-hydroxyethyl)-N-methylamino]diazen-1-ium-1,2-diolate* (**13**): To a solution of **12** (2.20 g, 9.1 mmol) in anhydrous acetonitrile (90 mL), **9** (3.93 g, 18.18 mmol) was added and the mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the resulting oil was purified by silica gel flash chromatography with a mixture of 12→75% EtOAc/hexanes to afford **13** (0.80 g, 4.98 mmol, 55%) as an oil: UV (EtOH) λ_{max} (ε) 264 nm (8.23 mM^{-1} cm^{-1}); ^1H NMR (400 MHz, $CDCl_3$) δ 6.83 (dd, *J* = 14.1, 6.7 Hz, 1H), 4.87 (dd, *J* = 14.1, 2.5 Hz, 1H), 4.42 (dd, *J* = 6.7, 2.5 Hz, 1H), 3.81 (dt, *J* = 5.6, 4.8 Hz, 2H), 3.53 (t, *J* = 4.8 Hz, 2H), 3.14 (s, 3H), 2.13 (t, *J* = 5.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.4, 92.4, 59.7, 56.7, 41.6. Anal. Calcd for $C_5H_{11}N_3O_3$: C, 37.26; H, 6.88; N, 26.07. Found: C, 37.34, H, 6.99; N, 25.91.
28. *O^2-Vinyl 1-[N-(carboxymethyl)-N-methylamino]diazen-1-ium-1,2-diolate* (**14**): To a vigorously stirred solution of **13** (800 mg, 4.96 mmol) at 0 °C in acetone (50 mL), Jones reagent (40 g CrO_3 + 32 mL 98% sulfuric acid + 75 mL H_2O) was added dropwise; TLC showed a complete disappearance of starting material. The reaction was quenched with 2-propanol (1 mL). The organic solvent was removed using a rotary evaporator and the aqueous residue was extracted with DCM. The crude product was purified by dissolving in 1.5 M NaOH and washing with DCM. The aqueous layer was acidified using 6 M HCl until the pH reached 3. The product was collected after DCM extraction followed by removal of the organic layer under reduced pressure affording **14** (499 mg, 2.85 mmol, 57%) as an oil: UV (EtOAc) λ_{max} (ε) 261 nm (9.2 mM^{-1} cm^{-1}); ^1H NMR (400 MHz, $CDCl_3$) δ 8.43 (s, 1H), 6.79 (dd, *J* = 14.0, 6.7 Hz, 1H), 4.82 (dd, *J* = 14.0, 2.4 Hz, 1H), 4.39 (dd, *J* = 6.7, 2.4 Hz, 1H), 4.30 (s, 2H), 3.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.6, 91.9, 53.0, 40.6. Anal. Calcd for $C_5H_9N_3O_4$·0.1 EtOAc: C, 35.17; H, 5.48; N, 22.60. Found: C, 35.39, H, 5.48; N, 22.60.
29. *O^2-vinyl [2−carboxylato)pyrrolidin−1−yl]diazen-1-ium-1,2-diolate methyl ester* (**15**): To a solution of **2** (100 mg, 0.57 mmol) in methanol (4 mL), a solution of diazomethane in ether (1.5 mL) was added. The ensuing reaction mixture was stirred at room temperature for 30 min and then concentrated under reduced pressure. The crude product was purified by silica gel flash column chromatography (9:1 CH_2Cl_2/ethyl acetate) to afford **15** (76 mg, 0.40 mmol, 71%) as a yellow oil: UV (EtOH) λ_{max} (ε) 266 nm (8.70 mM^{-1} cm^{-1}); ^1H NMR

(400 MHz, $CDCl_3$) δ 6.74 (dd, J = 14.1, 6.7 Hz, 1H), 4.78 (dd, J = 14.1, 2.4 Hz, 1H), 4.60 (dd, J = 8.5, 3.8 Hz, 1H), 4.34 (dd, J = 6.7, 2.4 Hz, 1H), 3.96–3.87 (m, 1H), 3.76 (s, 3H), 3.76–3.68 (m, 1H), 2.40–2.26 (m, 1H), 2.19–2.00 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 148.6, 91.4, 61.8, 52.4, 50.7, 27.9, 22.2. Anal. Calcd for $C_8H_{13}N_3O_4$: C, 44.65; H, 6.09; N, 19.54. Found: C, 44.74; H, 6.10; N, 19.43.

30. Stock solutions (10 mM) of the compounds in DMSO were prepared and diluted in 1 × pH 7.4 phosphate buffered saline (PBS) to a final concentration of 100 μM (10 μL stock solution in DMSO + 990 μL PBS) and incubated at 37 °C in the dark. An Agilent 1100 series HPLC fitted with a C-18 reverse-phase column (Phenomenex Luna 250 × 4.60 mm) operating at 262 nm and run isocratically with acetonitrile:water (75%, v/v) was used to analyze the reaction course. Injections (20 μL) of these solutions were carried out once per day. The data plotted were a mean of three independent experiments.
31. In Hank's Balanced Salt Solution (HBSS, 3 mL), **15** (30 μL, 10 mM DMSO stock solution) was added. Porcine liver esterase (5 μL, 14 units) was then added and the resulting solution was incubated overnight at 37 °C. HPLC analysis was carried out according to Ref. 30.
32. (a) Rautio, J.; Kumpulainen, H.; Heimbach, T.; Oliyai, R.; Oh, D.; Jarvinen, T.; Savolainen, J. *Nat. Rev. Drug Disc.* **2008**, *7*, 255–270; (b) Liderer, B. M.; Borchardt, R. T. *J. Pharm. Sci.* **2006**, *95*, 1177–1195; (c) Potter, P. M.; Wadkins, R. M. *Curr. Med. Chem.* **2006**, *13*, 1045–1054; (d) Beaumont, K.; Webster, R.; Gardner, I.; Dack, I. *Curr. Drug Metabol.* **2003**, *4*, 461–485.
33. Chakrapani, H.; Maciag, A. E.; Citro, M. L.; Keefer, L. K.; Saavedra, J. E. *Org. Lett.* **2008**, *10*, 5155–5158.