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V-PROLI/NO, a Prodrug of the Nitric Oxide Donor, PROLI/NO

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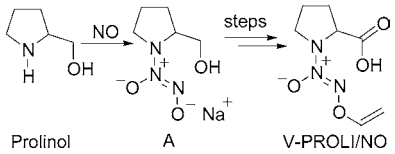
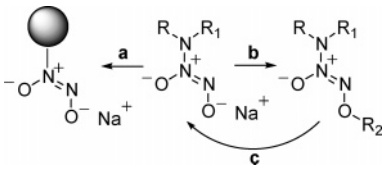
ABSTRACT



The sensitivity to decomposition of the nitric oxide (NO) donor ion, 1-[2-(carboxylato)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (PROLI/NO), complicates direct electrophilic substitution to form useful prodrug derivatives. A modified general synthetic approach involving 1-[2-(hydroxymethyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate ion (structure A, above) was used to prepare several PROLI/NO prodrugs including the previously inaccessible *O*^{2}-vinyl derivative, V-PROLI/NO. Metabolism of V-PROLI/NO by liver microsomes enriched in human cytochrome P450 isoforms was demonstrated.

Secondary diazeniumdiolate ions (general structure at the center of Scheme 1) are routinely used as reliable sources of nitric oxide (NO) in chemical and biological studies.[1] Such NO donors have well-defined half-lives for NO release from a few seconds to several hours to suit the duration of the experiment. Localization of therapeutic effects and site-directed delivery of NO are highly desirable in clinical settings and broadly, two strategies are employed (Scheme 1). An example of the first approach is incorporation of the diazeniumdiolate into insoluble polymeric material, then placing the NO donor in close proximity to the target tissue (path a, Scheme 1).[1c,2] When noninvasive access to certain tissues or cell types is not possible, an alternate approach is to protect the *O*^{2}-position of the diazeniumdiolate (path b, Scheme 1) with groups that can be selectively removed under specific conditions.[1b,d,3] Nitric oxide release from such

Scheme 1. Strategies for Site-Specific Delivery of NO[a]



[a] The solid sphere = polymer, microparticle, etc.; R, R_1 = alkyl groups; R_2 = protective group. Path a yields a material that concentrates NO release at its surfaces; path b depicts conversion of the starting ion into a neutral prodrug form that can circulate freely in the blood stream but (path c) be metabolically converted to the spontaneously NO-generating ionic form in the target organ.

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prodrugs will ideally not occur until the substituent at the O^2-position is cleaved in the target tissue to generate the free ionic diazeniumdiolate (path c, Scheme 1). A specific metabolic trigger resulting in selective removal of the O^2-protective group ensures site-directed delivery of therapeutic NO.

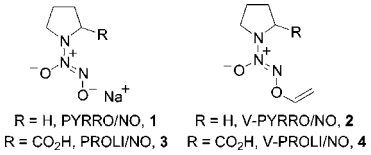


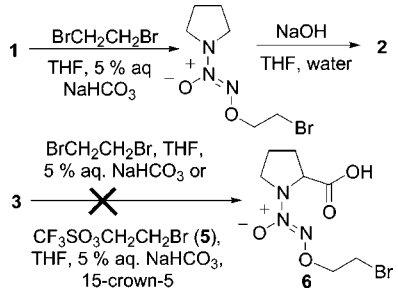
Figure 1. PYRRO/NO, PROLI/NO, and their prodrug forms.

An agent designed for this latter approach is the O^2-vinyl derivative of PYRRO/NO (**1**), V-PYRRO/NO (**2**), which is a NO prodrug that was shown in relevant animal models to protect the liver in a variety of life-threatening situations, including the ischemia-reperfusion injury associated with organ transplantation and the toxicity of acetaminophen and other agents (Figure 1).[4]

It would be desirable to modify its structure to improve water solubility for formulation and site-directed delivery while retaining the beneficial effects of rapid liver metabolism to NO-releasing form. The diazeniumdiolate of L-proline, PROLI/NO (**3**),[5] is structurally similar to PYRRO/NO, but has an additional carboxylic acid that may improve solubility for its corresponding O^2-protected prodrug forms (Figure 1). Furthermore, this additional carboxyl functionality serves as a synthetic handle for further derivatization by using a peptide or polymer that potentially improves efficacy, bioavailability, and/or site-directed delivery. Finally, the products of decomposition of PROLI/NO are all naturally occurring metabolites, suggesting a favorable toxicological profile.[5b] Hence, V-PROLI/NO (**4**) may be an ideal candidate for liver-specific delivery of therapeutic NO (Figure 2).

Unfortunately, the convenient synthetic strategy of O^2-bromoethylation/dehydrohalogenation used for the synthesis

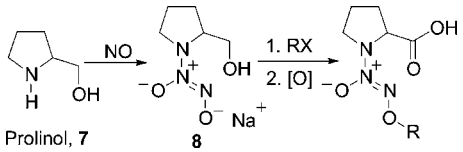
Scheme 2. Synthesis of **2** and Attempted Synthesis of **6**



of V-PYRRO/NO in good yield[4a] failed completely when attempted with PROLI/NO (Scheme 2). The reaction of PROLI/NO in the presence of 15-crown-5 with 2-bromo-1-(trifluoromethanesulfonyloxy)ethane (**5**) also failed to produce the desired intermediate **6**, necessitating a revised strategy for the synthesis of **4** and other O^2-derivatized PROLI/NO prodrug forms.

We postulated that, by diazeniumdiolating prolinol (**7**) instead of proline (Scheme 3) to produce **8** as a starting

Scheme 3. Proposed General Synthetic Route to V-PROLI/NO (**4**) and other O^2-Substituted PROLI/NO Derivatives



material, absence of the carboxyl group might allow substitution at the O^2-position to occur without significant competing decomposition. Subsequent oxidation of the primary alcohol functionality might then be used to regenerate the carboxyl group and produce the desired PROLI/NO derivative (Scheme 3).

As predicted, **4** could be prepared by this route. Treatment of diazeniumdiolate **8** with **5** in the presence of 15-crown-5 afforded bromoethyl derivative **9** in 48% yield. Mild oxidation with a modified Sharpless protocol (sodium periodate and ruthenium trichloride)[6] led to carboxylate **6** in 19% yield. Dehydrobromination of **6** by treatment with sodium hydroxide generated V-PROLI/NO in 19% yield (Scheme 4).

To examine the generality of this procedure, we reacted **8** with 2,4-dinitrofluorobenzene (FDNB). This produced **10a** in 54% yield (Table 1, entry 1), an outcome that contrasted sharply with that seen when FDNB was reacted directly with **3**. In the latter case, no combination of reaction conditions (solvent, temperature, crown ether, or other additives) was found that generated the desired product, **11a**. Instead, the major products isolated were *N*-nitrosoproline (**12**) and *N*-(2,4-dinitrophenyl)proline (Scheme 5). Conversion of **10a**

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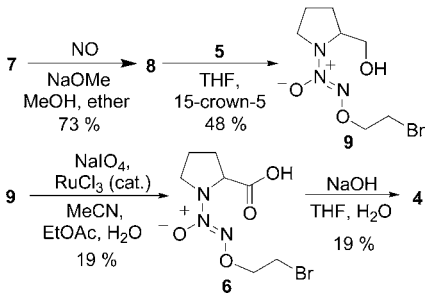
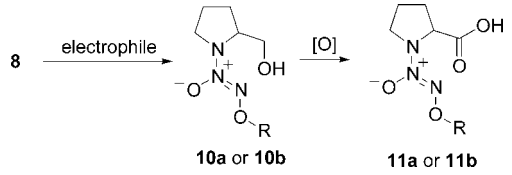
Scheme 4. Successful Preparation of V-PROLI/NO (**4**) by the Procedure Outlined in Scheme 4



to **11a** proceeded satisfactorily in 74% yield (Table 1, entry 3). Similarily, **8** was converted to **10b**, which, after extensive chromatography, was isolated in 10% yield (Table 1, entry 2); subsequent oxidation proceeded without affecting the acetylated glucosyl group to afford **11b** in 46% yield (Table 1, entry 4).

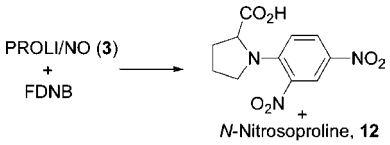
Table 1. Synthesis of Some O^2-Substituted Derivatives of **3**



entry	R	compd	yield, %
1	2,4-dinitrophenyl[a]	**10a**	54
2	2,3,4,6-tetra-*O*-acetyl-β-D-(glucopyranosyl)[b]	**10b**	10
3	2,4-dinitrophenyl[c]	**11a**	74
4	2,3,4,6-tetra-*O*-acetyl-β-D-(glucopyranosyl)[c]	**11b**	46

[a] FDNB, 5% aq $NaHCO_3$, *t*-BuOH. [b] 1-Bromo-2,3,4,6-tetraacetoxyglucose, 5% aq $NaHCO_3$, acetone. [c] $NaIO_4$, $RuCl_3$ (cat.), MeCN, EtOAc, H_2O.

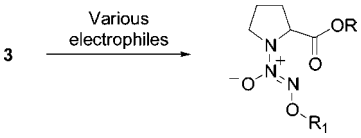
Scheme 5. Attempted Arylation of PROLI/NO



It might be noted that direct reaction of PROLI/NO dianion with strong electrophiles did in some cases lead to isolable O^2-derivatives in reasonable yield. Thus, dimethyl sulfate gave dimethylated product **13** in 18% yield, while diethyl sulfate and *N*,*N*-dimethylsulfamoyl chloride led to O^2-substituted PROLI/NO derivatives in yields of 13% and 57%, respectively (Table 2).

The synthetic methodology described in this paper provides access to several derivatives of PROLI/NO, enabling further expansion of PROLI/NO-based nitric oxide prodrugs. Certain O^2-2,4-dinitroaryl derivatives of diazeniumdiolates are potent antitumor agents that are reported to operate through activation by glutathione-*S*-transferase, which is frequently overexpressed in tumor tissue.[7] It will be of interest to explore the anticancer activity of **11a** and its derivatives.

Table 2. Reaction of PROLI/NO with Some Electrophiles



entry	electrophile	R	R_1	compd	yield, %
1	Me_2SO_4	Me	Me	**13**	18
2	Et_2SO_4	H	Et	**14**	13
3	$ClSO_2NMe_2$	H	SO_2NMe_2	**15**	57

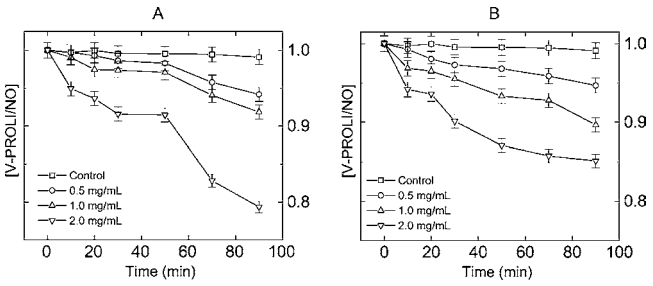


Figure 2. Metabolism of V-PROLI/NO by microsomes enriched in human cytochrome P450 isoforms 2E1 (A) and 3A4 (B). No significant decomposition of V-PROLI/NO was observed in the presence of the NADPH-regenerating system alone during a 90-min incubation, but the rate of metabolism increased progressively as the enzyme was added at increasing levels (0.5, 1.0, and 2.0 mg/mL). All solutions contained NADPH-regenerating system (1.3 mM $NADP^+$, 3.3 mM glucose-6-phosphate, 3.3 mM magnesium chloride, and 0.4 U/mL glucose-6-phosphate dehydrogenase) plus 100 μM V-PROLI/NO in 0.1 M phosphate buffer (pH 7.4). Samples were incubated for 90 min at 37 °C.

Nitric oxide release from the O^2-glucosylated derivative of PYRRO/NO upon treatment with β-D-glucosidase was recently demonstrated.[8] Here again, the presence of the carboxyl group may lead to improved functional characteristics relative to the V-PYRRO/NO-based materials.[8]

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It seems possible that these PROLI/NO derivatives could be therapeutically promising. With respect to V-PROLI/NO, preliminary studies were conducted to determine whether it can be metabolized by cytochrome P450 isoforms 2E1 and 3A4. Like V-PYRRO/NO, V-PROLI/NO was consumed in the presence of an NADPH-generating system at rates that increased with the concentration of enzyme (Figure 2). Future work will focus on the pharmacological profiles of these compounds and their analogues.

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Supporting Information Available: Preparative procedures, analytical data for new compounds, and details of the metabolism study. This material is available free of charge via the Internet at http://pubs.acs.org.

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