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Novel protection–deprotection strategies in diazeniumdiolate chemistry: synthesis of V-IPA/NO[†]

Rahul S. Nandurdikar^a, Larry K. Keefer^a, Joseph E. Saavedra^b

^aChemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute at Frederick, Frederick, MD 21702, USA.

^bBasic Science Program, SAIC-Frederick Inc., National Cancer Institute at Frederick, Frederick, MD 21702, USA.

Abstract

Synthesis of previously inaccessible, potentially liver selective HNO donor V-IPA/NO ([iPrHN³–N¹(O¹)=N²–O²–R], where R = vinyl) is reported here. A novel fluoride-labile TOM group at O-2 in conjunction with MOM protection at N-3 in IPA/NO is employed. The strategy developed is also extended to synthesis of other NO-releasing prodrugs and has applications in diversity-oriented synthesis of HNO- and NO-prodrugs.

Nitric oxide (NO) and nitroxyl (HNO) chemically differ by one hydrogen atom, but they have different biological properties. NO plays a critical role in a variety of bioregulatory processes like normal physiological control of blood pressure, neurotransmission, and macrophage-induced cytostasis and cytotoxicity.¹ On the other hand, HNO has been implicated in the mechanism of the inhibitory effect of cyanamide on aldehyde dehydrogenase in treating alcohol abuse² and has been demonstrated to enhance contractility in experimental models of heart failure.³ Secondary amine diazeniumdiolate prodrugs are considered reliable sources of NO (Fig. 1a, left arrow).⁴ More recently, primary amine diazeniumdiolate IPA/NO (**2**, Fig. 1b) and its prodrug AcOM–IPA/NO (**3**, Fig. 1b) were found to be sources of HNO.⁵

IPA/NO (**2**) as a potential HNO donor has inclined us to synthesize several additional IPA/NO prodrugs that can be metabolically triggered to release HNO. For example, vinyl protection of a diazeniumdiolate was designed to release NO upon activation by cytochrome P450. Thus, V-PYRRO/NO (**4**, Fig. 1b) is activated by cytochrome P450 to release NO, and shows hepatoprotective properties in relevant animal models against a variety of life-threatening situations, including the ischemia-reperfusion injury associated with organ transplantation and the toxicity of acetaminophen and other agents.⁶ We envisioned that such vinyl protection for IPA/NO as in **5** (V-IPA/NO) might lead to a liver selective HNO donor. The conventional synthesis of such vinyl diazeniumdiolate prodrugs involves alkylation of a diazeniumdiolate sodium salt to form a bromoethyl diazeniumdiolate, followed by

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nandurdikar@mail.nih.gov; Fax: +1 301-846-5946; Tel: +1 301-846-7145.

elimination of HBr.^{6a} However, when IPA/NO sodium salt (**2**) was reacted with 1,2-dibromoethane, the expected bromide **6** was not isolated (Scheme 1). The free N–H group in **6** interfered to form *E*-diazoniumdiolate adduct **7** (Scheme 1).⁷

This highlighted the need for temporary protections at O-2 and N-3 in IPA/NO, followed by O-2-alkylation (see **2**, Fig. 1b; for atom numbering system), to avoid the undesired *N*-alkylation in the bromide **6**. We planned to design a synthetic protecting group at O-2 that can be stable in standard reaction conditions for functional group transformations, and can be converted easily to the required NO-prodrug of biological significance. However, diazeniumdiolate anions are highly sensitive to low pH, and often decompose to release NO and the corresponding amine. Hence, an acid-labile protecting group for O-2 of the diazeniumdiolate is to be avoided. We decided to explore a fluoride-labile synthetic protecting group at the O-2 position of IPA/NO. Diazeniumdiolate salts, when reacted with trialkylsilyl chlorides, give a complex mixture of products. However, diazeniumdiolate salts are known to readily react with primary alkyl chlorides and bromides, hence we decided to explore the reaction with (triisopropylsilyloxy)-methyl chloride (TOMCl). In recent times, TOM has become a popular protecting group in nucleoside chemistry, and has been advantageous over other classical protecting groups.^{8,9}

IPA/NO sodium salt **2** was transformed into TOM-ylated IPA/NO **8** by reaction of IPA/NO with TOMCl, DIPEA (*N,N*-diisopropylethylamine) in DMSO in 69% yield (Scheme 2). Compound **8** on treatment with *n*BuLi and MOMCl (chloromethyl methyl ether) in THF gave compound **9** (59% yield). Now with the absence of the interfering N–H group in **9** (unlike **2** or **6**), the TOM group was removed using tetrabutylammonium fluoride (TBAF) in the presence of a 4-fold excess of 2-bromo-1-[(trifluoromethane)sulfonyl]-oxy]ethane and triethylamine (TEA) to obtain bromide **10** in 62% yield. The elimination of HBr from bromide **10** was carried out using Verkade's superbase (**11**) in acetonitrile followed by an acid-induced removal of the MOM group to obtain V-IPA/NO (**5**) (71% for 2 steps).

The reaction conditions enabling temporary protection of the N–H group in IPA/NO as in compound **9** would allow one to tag a polymer, peptide or fluorescent group directly to the N-3 of IPA/NO. Efforts are underway to synthesize such IPA/NO derivatives, measure their NO- and HNO-releasing profiles and evaluate their biological properties.

TOM-ylated diazeniumdiolates can have further applications in making various NO-prodrugs. To explore this chemistry, carboethoxy piperazine diazeniumdiolate **12**¹⁰ was reacted with TOMCl, DIPEA and 15-crown-5 in THF, to afford TOM-ylated diazeniumdiolate **13** in good yield (Table 1). TBAF in THF was added to compound **13** in THF, readily inducing removal of the TOM group, and forming anionic **12** as the tetra-*n*-butylammonium salt, which was found to be highly soluble in THF, unlike its sodium counterpart.¹¹ When this reaction was carried out in the presence of an electrophile RX, the desired NO-prodrugs were achieved in 59–92% yields (Table 1). Syntheses of prodrugs **14**,¹⁰ **15**,¹⁰ and JS-K (**18**)¹² are reported in the literature, and the method developed provides an alternate route for their synthesis. The method developed also allowed synthesis of novel compounds **16** and **17**, and is useful in structure activity relationship (SAR) studies of

diazeniumdiolate prodrugs, where one TOM-ylated diazeniumdiolate derivative can be transformed into several NO-prodrugs, leading to diversity-oriented synthesis.

In conclusion, we have developed a method for the synthesis of the TOM-protected diazeniumdiolates. These TOM-protected diazeniumdiolates were transformed into different biologically significant NO-releasing prodrugs. The TOM-protection in conjunction with the MOM protecting group was utilized in the synthesis of V-IPA/NO (**5**), which could not be synthesized by conventional procedures. Efforts are underway to study the cytochrome P450 activation of V-IPA/NO to release HNO. The protection–deprotection strategy developed has potential applications in SAR studies of diazeniumdiolate prodrugs and may enable a TOM-derivative to be transformed into several prodrugs activated by different mechanisms to release NO and/or HNO.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

(a) Activation of diazeniumdiolate prodrugs to release NO and HNO. (b) Structures of IPA/NO (**2**), AcOM-IPA/NO (**3**), V-PYRRO/NO (**4**), and V-IPA/NO (**5**).

Scheme 1. Formation of *E*-diazeniumdiolate adduct (**7**) during attempted synthesis of **6** [ref. 7].

Scheme 2.
Synthesis of V-IPA/NO (**5**).

Table 1

Transformation of a TOM-diazeniumdiolate into different prodrugs

Entry	RX	Product	% Yield
1	(CH ₃) ₂ SO ₄	14	78
2	TfOCH ₂ CH ₂ Br	15 ^a	65
3	AcOCH ₂ Br	16	74
4	1-Chloro- <i>N</i> -acetyl-glucosamine triacetate	17	59
5	2,4-Dinitrophenyl fluorobenzene	18 (JS-K)	92

^aTriflate is the leaving group in this reaction to form bromide **15**.