# Secondary Amine/Nitric Oxide Complex Ions, $R_2N[N(O)NO]^-$ O-Functionalization Chemistry

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Alkylation of the $R_2N[N(O)NO]^-$ anion has been studied with the aim of extending the reaction's scope, probing its stereochemistry, and exploring the reactivity of its variously functionalized products. Using the sodium salt of the R = Et ion (1) as the standard starting material, numerous novel products having the structure $Et_2N[N_2O_2]R'$ (2) were isolated from its reaction with alkyl halides, sulfate esters, and oxiranes. In addition to previously described examples in which R' is a simple straight-chain alkyl or benzyl group, new compound types in which R' is hydroxylated, halide-containing, α-methoxylated, and olefinic were prepared. The 2-bromoethyl derivative could be dehydrohalogenated to the *O*-vinyl compound or further reacted with other nucleophiles such as amines, water, or a second mole of 1 to produce additional new compound types. Ethylation of 1 appeared to occur exclusively at the terminal oxygen to give $Et_2NN(O)=NOEt$ as the only isomer detected; this conclusion regarding the regiochemistry of the reaction conflicts with that found in the previous literature, but its generality was supported by X-ray crystallographic analysis of the $Et_2NN(O)=NOCH_2CH_2(NC_5H_5)^+Br^-$ analogue. Hydrolytic decomposition of 2 was slow, even for the *O*-vinyl and -methoxymethyl derivatives, as reflected in the sluggish loss of the intense chromophore these compounds characteristically show at 225–245 nm ($\epsilon$ (6.5–9) × $10^3$ $M^{-1}$ $cm^{-1}$); half-lives at 37 °C for the latter two compounds were 12 h in 0.1 M HCl and 9 h in 1 M HCl, respectively. *N*-Nitrosodiethylamine was a frequent byproduct of both the synthesis and hydrolysis of 2. The results should aid the effort to design prodrug derivatives of 1, a compound type which has recently been shown to exhibit useful pharmacological effects.

## Introduction

Ions of structure 1 formed by reacting secondary amines with nitric oxide (NO)[1,2] are of current interest as pharmacological probes[3,4] by virtue of their ability to regenerate NO, a multifaceted bioregulatory agent,[5] nonenzymatically on dissolution in physiological media. Their decomposition to NO has been shown to be a spontaneous, first-order reaction.[3] This characteristic has proven advantageous in research applications, but it can be a liability in the pharmaceutical realm. For example, one might expect all cells in the vicinity of a group of spontaneously decomposing $R_2N[N_2O_2]^-$ ions to be exposed to NO more or less indiscriminately, making it difficult to target its effects selectively to any specific cell type. Considering the surprising variety of organs now known to be affected by NO's action and the impressive number of biological phenomena it mediates,[5] such targeting would appear to be an important requisite for most pharmaceutical applications.

For this reason, we have been interested in designing prodrug derivatives of 1 that cannot release NO until they are metabolically reconverted to 1 by enzymes specific to the desired target cell type. A potentially useful means of masking 1 toward this end could be to react it with an alkylating agent that might be removed by oxidative or other enzymes.

$$\underset{1}{R_2N[N_2O_2]^-} \xrightarrow{R'X} \underset{2}{R_2N[N_2O_2]R'} \qquad (1)$$

That such an alkylation is possible has been demonstrated by Reilly[2] and by Longhi and Drago,[6] who prepared several such alkylation products via eq 1 where R' was ethyl, *n*-butyl, or benzyl. Aside from the syntheses of these simple derivatives, however, we have found no basis in the prior literature for inferring how generally O-functionalized derivatives of 1 might be accessible synthetically or how versatilely they might be additionally manipulated.

We address these issues in the present report. Several examples of eq 1 products (2) are described in which R' is an alkyl group substituted with hydroxy, amino, halo, olefinic, or alkoxy residues that can be used as points of

**Table I. Alkylation Products $[Et_2N(N_2O_2)R']$ Synthesized by Reacting $Et_2N(N_2O_2)Na$ with Electrophiles**

| product | R' | electrophile | solvent | isolated yield (%) |
|---|---|---|---|---|
| **2a** | Et | $Et_2SO_4$ | MeOH | 33 |
| **2a** | Et | EtI | DMF | 23 |
| **2b** | Me | $Me_2SO_4$ | MeOH | 23 |
| **2c** | *n*-Pr | *n*-PrI | DMF | 33 |
| **2d** | $CH_2$=$CHCH_2$ | allyl bromide | DMF | 36 |
| **2e** | $BrCH_2CH_2$ | $BrCH_2CH_2Br$ | DMF–THF | 17 |
| **2f** | $MeCHOHCH_2$ | propylene oxide | THF | 6[a] |
| **2f'** | $HOCH_2CH(CH_3)$ | $HOCH_2CH(CH_3)Br$ | THF | 20[b] |
| **2g** | $HOCH_2CH_2$ | $BrCH_2CH_2OH$ | THF | 33 |
| **2h** | 2-hydroxycyclohexyl | cyclohexene oxide | THF | 7 |
| **2i** | $MeOCH_2$ | $ClCH_2OMe$ | THF | 41 |

[a] Crude product contained 9% **2f'**. The yield given is that of pure **2f** after chromatographic purification. [b] Since the starting bromohydrin was a mixture of $HOCH_2CH(CH_3)Br$ and $HOCH(CH_3)CH_2Br$, the product contained both **2f'** and **2f**. The yield given represents the total for the two isomers.

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Table II. $Et_2N(N_2O_2)R'$ Derivatives Prepared by Further Reaction of 2e

| product | R′ | produced from 2e by | yield (%) |
|---|---|---|---|
| 2j | $CH_2$=CH | dehydrohalogenation | 70 |
| 2k | $Et_2N(N_2O_2)$-$CH_2CH_2$ | further reaction with 1 | 21 |
| 2l | $C_5H_5N^+CH_2CH_2$ | N-alkylation of pyridine | 92 |
| 2m | $MeNHCH_2CH_2$ | reaction with $MeNH_2$ | 71 |

entry for further synthetic (or biological) transformations. We have also investigated the regioselectivity of electrophilic attack by alkylating agents on 1, showing that the previous conclusion regarding the structure of the products was in need of revision. Finally, we have collected information on the stability of these compounds in the absence of enzymes that is relevant to their possible utility in pharmacology.

## Results and Discussion

**Preparative Chemistry.** Anions of structure 1 proved reactive toward a variety of electrophiles, including alkyl halides,[2] sulfate esters,[2,6] and epoxides. Compounds that we have synthesized via eq 1 thus far using the sodium salt of the $Et_2N(N_2O_2)^-$ ion (1, R = Et) as the standard starting material are listed in Table I.

A noteworthy byproduct was found in the reaction of 1 with ethylene dibromide. Removal of the principal product, 2e, by column chromatography left a more polar residue that was identified as the bis-alkylation product, 2k, suggesting that 2e might itself serve as a useful alkylating agent. This possibility was examined by reacting 2e not only with a second molar equivalent of 1 in a synthesis yielding 2k as the major product, but also with methylamine and pyridine. Moreover, 2e could be hydrolyzed to 2g and dehydrobrominated to the *O*-vinyl derivative, 2j. Syntheses we performed that have provided structurally diverse prodrug candidates by further reaction of 2e are listed in Table II.

In general, the O-functionalization products of 1 were easily isolated by preparative chromatography, distillation, and/or crystallization. Given the facility of the synthesis and isolation procedures employed in these transformations, we conclude that the $R_2N[N_2O_2]^-$ ions are reasonable substrates for alkylation reactions which should allow the preparation and pharmacological testing of a wide variety of compounds 2.

**Reactivity.** With potential prodrug applications in mind, we explored the stability of the products. As predicted, the alkylated derivatives, 2, showed considerably more stability toward hydrolysis than the underivatized 1 ion. Whereas 1 (R = Et) has a half-life of 2 min at 37 °C in pH 7.4 buffer,[3] 2a was indefinitely stable at this pH and higher. Loss of the strong chromophore these compounds characteristically display ($\lambda_{max}$ 225–245 nm, $\epsilon$ (6.5–9.0) × $10^3$ $M^{-1}$ $cm^{-1}$) was very slow for 2a even in 0.01 M HCl at room temperature, 18 h at reflux being required to decompose 32% of the starting material in this solvent.

Of particular interest was the behavior of the acetal derivative 2i, whose half-lives at 37 °C were >20 days at pH 7.4 and 9 h in 1 M HCl. Even the *O*-vinyl compound (2j) tenaciously retained its ultraviolet spectrum, the half-life for chromophore loss being 12 h in 0.1 M HCl at 37 °C. That this outcome was not attributable to anti-Markovnikov addition across the double bond (which would not change the ultraviolet spectrum) was established by following the reaction using NMR spectrometry. The β-hydroxyethyl derivative 2g, which was separately ascertained to be stable under these conditions, was not observed in the reaction mixture's NMR spectrum. Instead, the major product was the $Et_2NH_2^+$ ion, consistent with the initial formation of the hemiacetal (or the corresponding α-chloro derivative) followed by hydrolytic loss of acetaldehyde; this would leave the $Et_2N(N_2O_2)^-$ ion, already known to fragment to the amine in acidic solution with loss of two NO molecules.[3]

Other significant byproducts were sometimes observed in these reactions. One of the more important from the pharmacological point of view was *N*-nitrosodiethylamine (NDEA). This potent carcinogen is a known decomposition product of the $Et_2N(N_2O_2)^-$ ion on standing in air,[7] and it was more abundant than the $Et_2NH_2^+$ ion in the 32% decomposed solution of 2a in 0.01 M HCl (11 versus 8% yields, respectively). Moreover, when 2a was heated in air at 100 °C for 24 h, NDEA was produced in 1.1% yield. We conclude that conversion to the nitrosamine is a significant component of the reaction course during both the alkylation of 1 and the thermal or hydrolytic decomposition of 2 under these conditions.

**Stereochemistry.** When the first O-alkylated 1 derivatives were described three decades ago, the assumption was made that the attacking electrophiles became attached to the interior oxygen of the 1 ion to form *N*-nitroso derivatives of structure $R_2NN(OR')NO$ as the major product. This structural assignment was consistent with all the physicochemical data then at hand, including NMR, IR, and elemental analysis.[2]

Since electrophilic attack at other positions of the 1 ion seemed conceivable, however, we reinvestigated the regiochemistry of 1 alkylation using additional approaches not available to the previous investigators. For example, we prepared a sample of a reportedly isomeric structure first synthesized in 1987 by oxidizing ethoxyamine to the *N*-nitrene and condensing it with NDEA,[8] as in eq 2. Our

$$\underset{\text{NDEA}}{Et_2NNO} + EtONH_2 \xrightarrow{Pb(OAc)_4} Et_2NN(O)=NOEt \qquad (2)$$

intent was to examine the gas chromatograms of the eq 1 (R = Et = R′) reaction mixture for traces of the eq 2 product. The pure standards produced in the two different reactions failed to separate on any of the three columns examined, however. Nor were their proton NMR spectra distinguishable, a mixture of the eq 1 and eq 2 products showing only two ethyl groups, rather than the expected four. Since additional GC, GC/MS, and NMR scrutiny of this and the other O-functionalization reactions studied here also failed to reveal more than one product with the required $R_2N(N_2O_2)R$ structure, we were forced to conclude that eqs 1 and 2 both yield the same product.

But which isomer was it? To answer this question, we turned to X-ray crystallographic analysis of the highest melting product we isolated, 2l. The X-ray analysis quickly provided an unambiguous description of the chemical connectivity and relative stereochemistry of the compound, showing that the terminal (rather than interior) oxygen was the site of alkylation. The molecule is made up of three planar segments. The central plane (to within ±0.08 Å) consists of the eight-atom chain from C17 through C7. Both the second ethyl group and the six-membered ring are approximately perpendicular (with interplanar angles of 88.2 and 77.9°, respectively) to, but on opposite sides of, this plane. The X-ray results are shown in Figure

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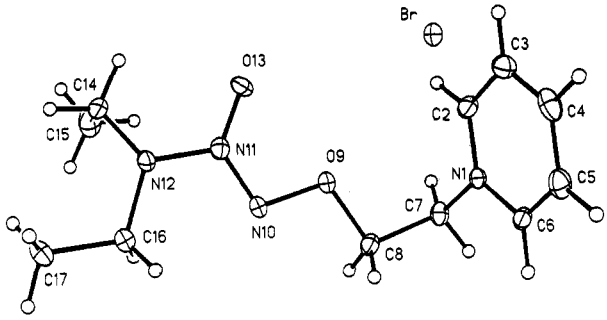


**Figure 1.** The molecular structure and numbering scheme for 1-(2-((3,3-diethyl-2-oxo-1-triazen-1-yl)oxy)ethyl)pyridinium bromide (**21**). The figure is drawn using the experimentally determined coordinates with the thermal ellipsoids at the 20% probability level.

1. This compound clearly has the $R_2NN(O)=NOR'$ structure. Therefore, we rewrite eq 1 as

$$\underset{\mathbf{1}}{R_2N[N_2O_2]^-} \xrightarrow{R'X} \underset{\mathbf{2}}{R_2NN(O)=NOR'} \qquad (1')$$

We cannot for the moment exclude arrival at this stereochemistry by isomerization of some initially formed kinetic product, but it seems most unlikely that electrophilic attack occurs on nitrogen in the above examples. Attachment of an electrophilic carbon to the nitroso nitrogen would yield an intermediate $[R_2NN(O)=N(O)R']$ that, by analogy to the structurally similar $RN(O)=N(O)R$ dimers,[9] might be expected to fragment to a nitrosamine and a *C*-nitroso compound; the latter agent should in turn isomerize to an oxime, but no oximes were detected in the reactions described in Table I. Quaternization of the $R_2N$ group, on the other hand, should lead to a zwitterionic $R_3N(N_2O_2)$ species, but neither this nor the tertiary amine it would be expected[1,3] to produce on loss of NO was detected in any reaction mixture, either.

As to the possibility of forming the $R_2NN(OR')N=O$ isomer, additional evidence against this formulation was found in the electronic spectra. As an *N*-nitroso compound, such a derivative might be expected to show an $n \rightarrow \pi^*$ transition in the 330–400-nm range;[10] the only times such a band was seen, however, occurred when NDEA was produced. This interpretation is consistent with the results for alkylation of *N*-nitroso(*cis*-4-methylcyclohexyl)-hydroxylamine silver salt, an $R[N_2O_2]^-$ derivative that forms both $RN(OMe)N=O$ and $RN(O)=NOMe$ on reaction with MeI; both isomers show a strong $\pi \rightarrow \pi^*$ band at 235 nm, while only the former has an $n \rightarrow \pi^*$ absorption (355 nm).[11]

We tentatively conclude, pending confirmation via further studies including additional X-ray structural analyses, that alkylation of **1** is indeed regiospecific but that the stereochemistry is as shown in Figure 1 rather than as previously assigned.

**Conclusion.** The data indicate that a wide variety of potential prodrugs should be available by O-derivatization of the anionic functional group formed by electrophilic attack of nitric oxide on secondary amines. The products of these alkylations appear generally to have the $R_2NN(O)=NOR'$ structure, which is remarkably resistant to hydrolysis even when R′ is vinyl or alkoxymethyl. These results suggest that cell types capable of accelerating the required hydrolysis (or oxidation, etc.) reactions might respond usefully to the nitric oxide generated in the process. Preliminary data with a few of these O-functionalized **1** derivatives indicate that they can be used to lower the blood pressure of anesthetized rats, an effect consistent with the release in vivo of the established vasorelaxant,[5] NO. Future studies will be aimed at characterizing in detail the full potential of compounds **2** in a variety of pharmacological applications.

## Experimental Section

Unless otherwise indicated, nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ and ultraviolet (UV) data were collected for aqueous solutions. Gas chromatography (GC) was performed on columns of either 10% Carbowax 20 M containing 2% KOH on 80/100 Gaschrom Q or 3% OV-210 (or OV-17) on 80/100 Supelcoport with flame ionization detection. Elemental analyses were performed by Galbraith Laboratories or Atlantic Microlab, Inc. Mass spectra (MS) were collected in the electron impact mode with sample introduction via direct probe.

**Reaction of $Et_2N(N_2O_2)Na$[1b] with Electrophiles To Produce 2a–i.** In general, reactions were conducted as summarized in Table I according to the methods of Reilly[2] and Longhi and Drago[6] or suitable adaptations thereof. Except as otherwise indicated, reaction mixtures were neither heated nor cooled, progress was monitored by GC, and product was isolated by diluting the reaction mixture with water, extracting with ether or $CH_2Cl_2$, drying the organic phase with $MgSO_4$ or $Na_2SO_4$, evaporating the solvent at reduced pressure, and distilling the product under vacuum. When the reaction was conducted in methanol or tetrahydrofuran (THF), the solvent was removed by concentrating the reaction mixture in vacuo before extraction or chromatography.

**1-Ethoxy-2-oxo-3,3-diethyl-1-triazene (2a).** Prepared by reacting $Et_2N(N_2O_2)Na$ with $Et_2SO_4$ as described in refs 2 and 6, the product (**2a**) had the following properties: bp 52 °C (0.7 mmHg) (lit.[2] bp 52–55 °C (0.5 mmHg)); UV $\lambda_{max}$ 235 nm ($\epsilon$ 6.7 × $10^3$ $M^{-1}$ $cm^{-1}$); NMR[6] $\delta$ 1.10 (6 H, t, $J$ = 7.1 Hz), 1.39 (3 H, t, $J$ = 7.1 Hz), 3.09 (4 H, q, $J$ = 7.1 Hz), 4.34 (2 H, q, $J$ = 7.1 Hz); MS $m/z$ (relative intensity) 162 ($MH^+$, 100), 161 ($M^+$, 7), 145 (30), 131 (16), 127 (12), 103 (34), 99 (33), 73 (21), 72 (96), 44 (44). The same product was also prepared by treating 1.92 g (0.012 mol) of $Et_2N(N_2O_2)Na$ with 3.2 mL (0.04 mol) of EtI in 12 mL of dimethylformamide (DMF) for 72 h.

**1-Methoxy-2-oxo-3,3-diethyl-1-triazene (2b).** Mixing 2.0 mL (0.021 mol) of $Me_2SO_4$ with 2.24 g (0.014 mol) of $Et_2N(N_2O_2)Na$ in 14 mL of absolute MeOH was distinctly exothermic, requiring dropwise addition of the alkylating agent. **2b** was isolated in 23% yield (0.47 g): bp 44 °C (0.4 mmHg); UV $\lambda_{max}$ 234 nm ($\epsilon$ 7.9 × $10^3$ $M^{-1}$ $cm^{-1}$); NMR $\delta$ 1.10 (6 H, t, $J$ = 7.2 Hz), 3.10 (4 H, q, $J$ = 7.1 Hz), 4.06 (3 H, s); MS $m/z$ (relative intensity) 147 ($M^+$, 3), 102 (100), 87 (27), 57 (32), 56 (33), 54 (92), 42 (44); exact mass calcd for $C_5H_{14}N_3O_2$ ($MH^+$) 148.1086, found 148.1109. Anal. Calcd for $C_5H_{13}N_3O_2$: C, 40.82; H, 8.84; N, 28.57. Found: C, 40.81; H, 8.86; N, 28.62.

**1-*n*-Propoxy-2-oxo-3,3-diethyl-1-triazene (2c).** A slurry of 1.55 g (0.010 mol) of $Et_2N(N_2O_2)Na$ in 10 mL of DMF became homogeneous within 5 min of mixing with 1.46 mL (0.015 mol) of *n*-PrI and then gradually formed a precipitate (NaI). After the mixture was stirred overnight, 0.60 g (33%) of the product (**2c**) was isolated: bp 70–72 °C (0.5 mmHg); UV $\lambda_{max}$ 237 nm ($\epsilon$ 8.0 × $10^3$ $M^{-1}$ $cm^{-1}$); NMR $\delta$ 0.97 (3 H, t, $J$ = 7.4 Hz), 1.09 (6 H, t, $J$ = 7.1 Hz), 1.79 (2 H, m), 3.08 (4 H, q, $J$ = 7.1 Hz), 4.24 (2 H, t, $J$ = 6.9 Hz); MS $m/z$ (relative intensity) 176 ($MH^+$, 1), 103 (100), 75 (38), 44 (32), 43 (62), 42 (22), 41 (27); exact mass calcd for $C_7H_{18}N_3O_2$ ($MH^+$) 176.1499, found 176.1410. Anal. Calcd for $C_7H_{17}N_3O_2$: C, 48.00; H, 9.71; N, 24.00. Found: C, 47.80; H, 9.39; N, 23.57.

**1-(Allyloxy)-2-oxo-3,3-diethyl-1-triazene (2d).** Allyl bromide (1.73 mL, 0.020 mol) was mixed in an ice bath with 2.48 g (0.016 mol) of $Et_2N(N_2O_2)Na$ in DMF, allowed to warm to room temperature, and stirred overnight. **2d** was isolated in 36% yield (1.01 g): bp 76–77 °C (0.6 mmHg); UV $\lambda_{max}$ 243 nm ($\epsilon$ 8.9 × $10^3$ $M^{-1}$

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$cm^{-1}$); NMR δ 1.09 (6 H, t, $J$ = 7.1 Hz), 3.09 (4 H, q, $J$ = 7.1 Hz), 4.73 (2 H, m), 4.76 (1 H, m), 5.33 (2 H, m), 6.02 (1 H, m); MS $m/z$ (relative intensity) 174 ($MH^+$, 3), 143 (40), 132 (26), 103 (25), 102 (100), 98 (30), 57 (20), 56 (36), 44 (82), 42 (83); exact mass calcd for $C_7H_{15}N_3O_2$ ($M^+$) 173.1164, found 173.1135. Anal. Calcd for $C_7H_{15}N_3O_2$: C, 48.55; H, 8.67; N, 24.28. Found: C, 48.78; H, 8.67; N, 23.93.

**1-(2-Bromoethoxy)-2-oxo-3,3-diethyl-1-triazene (2e).** A slurry of 4.7 g (0.017 mol) of $Et_2N(N_2O_2)Na$ in DMF was treated under a stream of $N_2$ with 1.4 mL (0.016 mol) of 1,2-dibromoethane in ice-cold THF. After being allowed to warm to room temperature and stand overnight, the crude product was isolated and purified by chromatography on a silica gel column eluted with $CH_2Cl_2$. Pure **2e** (0.70 g, 17% yield): UV $\lambda_{max}$ 233 nm (ε 9.0 × $10^3$ $M^{-1}$ $cm^{-1}$); NMR δ 1.10 (6 H, t, $J$ = 7.1 Hz), 3.12 (4 H, q, $J$ = 7.1 Hz), 3.58 (2 H, m), 4.52 (2 H, m); MS $m/z$ (relative intensity) 242 ($MH^+$, $^{81}Br$, 8), 240 ($MH^+$, $^{79}Br$, 9), 211 (37), 209 (36), 132 (41), 109 (56), 107 (56), 102 (100), 101 (64), 84 (32), 56 (36); exact masses calcd for $C_6H_{14}{}^{81}BrN_3O_2$ and $C_6H_{14}{}^{79}BrN_3O_2$ ($M^+$) 242.0327 and 240.0347, respectively, found 242.0356 and 240.0412, respectively. Anal. Calcd for $C_6H_{14}BrN_3O_2$: C, 30.13; H, 5.86; N, 17.57. Found: C, 30.33; H, 6.01; N, 17.72.

**1-(2-Hydroxypropoxy)-2-oxo-3,3-diethyl-1-triazene (2f).** A slurry of 4.2 g (0.027 mol) of $Et_2N(N_2O_2)Na$ in THF was refluxed with 2.1 mL (0.03 mol) of propylene oxide for 20 h. The crude product could be freed from an isomeric contaminant (see next paragraph) by column chromatography. Pure **2f** was isolated in 6% yield (0.30 g): bp 140 °C (0.7 mmHg); UV $\lambda_{max}$ 236 nm (ε 7.2 × $10^3$ $M^{-1}$ $cm^{-1}$); NMR δ 1.10 (6 H, t, $J$ = 7.1 Hz), 1.22 (3 H, d, $J$ = 5.0 Hz), 3.12 (4 H, q, $J$ = 7.1 Hz), 4.1–4.3 (3 H, m); MS $m/z$ (relative intensity) 192 ($MH^+$, 7), 164 (14), 103 (100), 102 (66), 86 (22), 84 (34), 75 (53), 59 (53), 56 (26), 49 (52), 45 (69), 44 (75), 43 (23), 42 (44), 41 (39); exact mass calcd for $C_7H_{18}N_3O_3$ ($MH^+$) 192.1348, found 192.1417. Anal. Calcd for $C_7H_{17}N_3O_3$: C, 43.98; H, 8.90; N, 21.99. Found: C, 43.93; H, 8.98; N, 21.91.

**1-(1-Methyl-2-hydroxyethoxy)-2-oxo-3,3-diethyl-1-triazene (2f′).** NMR revealed the presence of this compound as a 9% contaminant of the **2f** preparation above: NMR δ 1.10 (6 H, t, $J$ = 7.1 Hz), 1.36 (3 H, d, $J$ = 6.6 Hz), 3.11 (4 H, q, $J$ = 7.1 Hz), 3.78 (2 H, d, $J$ = 5.2 Hz), 4.57 (1 H, m). A 1:1 mixture of **2f** and **2f′** was independently prepared as follows. Eastman technical-grade 1-bromo-2-propanol, whose label indicated that it contained 20% 2-bromo-1-propanol, was distilled, concentrating the latter isomer. A solution of 1.34 mL of this distillate in 30 mL of THF containing a suspension of 2.3 g (0.015 mol) of 1 was refluxed for 24 h and cooled. Solvent was removed in vacuo, and the residue was taken up in $CH_2Cl_2$, washed with sodium metabisulfite, dried, and chromatographed on silica gel eluted with 1:1 $CH_2Cl_2$–EtOAc. A distinct GC peak for **2f′** was observed at a slightly longer retention time than that of **2f** when the purified product was run on OV-17. This peak could also be detected in the tail of **2f** when the 11:1 isomer mixture produced on reacting $Et_2N(N_2O_2)Na$ with propylene oxide was chromatographed, as described in the previous paragraph.

**1-(2-Hydroxyethoxy)-2-oxo-3,3-diethyl-1-triazene (2g).** A mixture of 2.54 g (16.4 mmol) of $Et_2N(N_2O_2)Na$ and 1.13 mL (16 mmol) of freshly distilled 2-bromoethanol in 20 mL of THF was refluxed under $N_2$ overnight. The evaporated reaction mixture was eluted from a silica gel column with 1:1 $CH_2Cl_2$–EtOAc. Fractions containing the product were combined and evaporated to give 0.94 g (33% yield) of pure **2g**: UV $\lambda_{max}$ 234 nm (ε 7.1 × $10^3$ $M^{-1}$ $cm^{-1}$); NMR δ 1.11 (6 H, t, $J$ = 7.1 Hz), 3.13 (4 H, q, $J$ = 7.1 Hz), 3.92 (2 H, m), 4.39 (2 H, m); MS $m/z$ (relative intensity) 178 ($MH^+$, 3), 103 (100), 102 (56), 75 (29), 56 (20); exact mass calcd for $C_6H_{16}N_3O_3$ ($MH^+$) 178.1191, found 178.1188. Anal. Calcd for $C_6H_{15}N_3O_3$: C, 40.67; H, 8.47; N, 23.72. Found: C, 40.44; H, 8.57; N, 23.52.

**1-((2-Hydroxycyclohexyl)oxy)-2-oxo-3,3-diethyl-1-triazene (2h).** Heating a slurry of 3.64 g (0.023 mol) of $Et_2N(N_2O_2)Na$ with 2.94 g (0.03 mol) of cyclohexene oxide in 50 mL of THF produced a homogeneous solution which was refluxed for 24 h. The crude product was eluted from a silica gel column with 5:1 $CH_2Cl_2$–EtOAc to give 0.40 g (7%) of pure **2h**: UV $\lambda_{max}$ 237 nm (ε 6.7 × $10^3$ $M^{-1}$ $cm^{-1}$); NMR δ 1.09 (6 H, t, $J$ = 7.1 Hz), 1.31 (2 H, m), 1.74 (2 H, m), 2.12 (2 H, m), 3.10 (4 H, q, $J$ = 7.1 Hz), 3.77 (1 H, m), 4.13 (1 H, m); MS (fast atom bombardment) $m/z$ (relative intensity) 232 ($MH^+$, 17), 103 (100), 81 (54), 72 (13); exact mass calcd for $C_{10}H_{22}N_3O_3$ ($MH^+$) 232.1660, found 232.1676. Anal. Calcd for $C_{10}H_{21}N_3O_3$: C, 51.95; H, 9.09; N, 18.18. Found: C, 51.91; H, 9.11; N, 18.05.

**1-((Methoxymethyl)oxy)-2-oxo-3,3-diethyl-1-triazene (2i).** A slurry of 3.5 g (0.023 mol) of $Et_2N(N_2O_2)Na$ and 3 g of $Na_2CO_3$ in 40 mL of THF was treated in an ice bath with 2.43 g (0.03 mol) of $ClCH_2OMe$. After warming to room temperature and stirring under Ar for 72 h, 1.68 g (41% yield) of pure **2i** was isolated: bp 67–68 °C (1.2 mmHg); UV $\lambda_{max}$ 227 nm (ε 6.5 × $10^3$ $M^{-1}$ $cm^{-1}$); NMR δ 1.11 (6 H, t, $J$ = 7.1 Hz), 3.16 (4 H, q, $J$ = 7.1 Hz), 3.50 (3 H, s), 5.26 (2 H, s); MS $m/z$ (relative intensity) 147 ($M^+$ – 30, 5), 177 (100), 102 (48), 86 (42), 72 (21), 71 (20), 58 (63), 57 (72), 56 (99). Anal. Calcd for $C_6H_{15}N_3O_3$: C, 40.68; H, 8.47; N, 23.73. Found: C, 40.69; H, 8.65; N, 23.90.

**1-(Vinyloxy)-2-oxo-3,3-diethyl-1-triazene (2j).** A solution of **2e** (1.04 g, 4.3 mmol) in 20 mL of THF was refluxed for 24 h with 2 g of powdered NaOH. The reaction mixture was cooled, filtered, evaporated in vacuo, and distilled to give 0.48 g of pure **2j** (70% yield): bp 63–64 °C (2 mmHg); UV $\lambda_{max}$ 243 nm (7.2 × $10^3$ $M^{-1}$ $cm^{-1}$); NMR δ 1.13 (6 H, t, $J$ = 7.1 Hz), 3.22 (4 H, q, $J$ = 7.1 Hz), 4.44 (1 H, m), 4.90 (1 H, m), 6.91 (1 H, m); MS (chemical ionization positive ion spectrum, $NH_3$) $m/z$ (relative intensity) 160 ($MH^+$, 75), 103 (90), 102 (30), 74 (75), 73 (30), 72 (100), 71 (20), 58 (35), 44 (40); exact mass calcd for $C_6H_{14}N_3O_2$ ($MH^+$) 160.1085, found 160.1097. Anal. Calcd for $C_6H_{13}N_3O_2$: C, 45.28; H, 8.17; N, 26.42. Found: C, 45.18; H, 8.05; N, 26.13.

**1,2-Bis((3,3-diethyl-2-oxo-1-triazen-1-yl)oxy)ethane (2k).** **2e** (425 mg, 1.76 mmol) in 5 mL of THF was mixed with 6.35 mg (4.1 mmol) of $Et_2N(N_2O_2)Na$ in 5 mL of DMF and stirred overnight at 25 °C. The reaction mixture was diluted with 100 mL of distilled water and extracted with pentane. The organic layer was dried with $Na_2SO_4$ and evaporated. The crude product was eluted from a dry-packed silica gel (activity III) column with 5:1 $CH_2Cl_2$–EtOAc to give 0.11 g (21% yield) of pure **2k**: UV $\lambda_{max}$ 243 nm (ε 15.1 × $10^3$ $M^{-1}$ $cm^{-1}$); NMR δ 1.10 (12 H, t, $J$ = 7.1 Hz), 3.11 (8 H, q, $J$ = 7.1 Hz), 4.5 (4 H, s); MS $m/z$ (relative intensity) 292 ($M^+$, 0.4), 132 (15), 103 (30), 102 (100), 56 (20), 44 (87), 42 (25); exact mass calcd for $C_{10}H_{24}N_6O_4$ ($M^+$) 292.1854, found 292.1911. Anal. Calcd for $C_{10}H_{24}N_6O_4$: C, 41.09; H, 8.22; N, 28.77. Found: C, 40.96; H, 8.32; N, 28.63.

**1-(2-((3,3-Diethyl-2-oxo-1-triazen-1-yl)oxy)ethyl)-pyridinium Bromide (2l).** A solution of 408 mg (1.7 mmol) of **2e** and 10 mL of pyridine in 15 mL of absolute ethanol was refluxed for 20 h. Cooling and evaporation left 0.5 g of crude oil that was adsorbed on 5 g of silica gel in a short column and eluted with pentane, ether, $CH_2Cl_2$, EtOAc, acetone, and MeOH. Only the MeOH eluate contained **2l**, which was recrystallized from EtOH to give 0.50 g (92% yield) of product: mp 114–115 °C; UV $\lambda_{max}$ 258 (7.2 × $10^3$ $M^{-1}$ $cm^{-1}$) and 233 nm (9.1 × $10^3$ $M^{-1}$ $cm^{-1}$); NMR δ 1.07 (6 H, t, $J$ = 7.1 Hz), 3.29 (4 H, q, $J$ = 7.1 Hz), 4.90 (2 H, m), 5.57 (2 H, m), 8.07 (2 H, m), 8.49 (1 H, m), 9.54 (2 H, m); MS (fast atom bombardment) $m/z$ (relative intensity) 239 (100, $MH^+$ – Br), 124 (49), 122 (20), 109 (36), 108 (17). Anal. Calcd for $C_{11}H_{19}N_4O_2Br$: C, 41.25; H, 5.94; N, 17.50; Br, 25.00. Found: C, 41.12; H, 6.01; N, 17.46; Br, 25.15.

**1-(*N*-Methyl-2-aminoethoxy)-2-oxo-3,3-diethyl-1-triazene (2m).** A solution of 308 mg (1.3 mmol) of **2e** in 3 mL of 95% ethanol was added dropwise to 10 mL of 33% ethanolic $MeNH_2$ (obtained from Fluka AG) in an ice bath. The reaction mixture was warmed to room temperature, stirred for 72 h, concentrated under vacuum, dissolved in 10% HCl, and washed with $CH_2Cl_2$. The aqueous layer was neutralized with NaOH and extracted with $CH_2Cl_2$. The $CH_2Cl_2$ layer was dried with $Na_2SO_4$ and evaporated to give 0.17 g (7% yield) of **2m**: UV $\lambda_{max}$ 234 nm (ε 8.8 × $10^3$ $M^{-1}$ $cm^{-1}$); NMR δ 1.10 (6 H, t, $J$ = 7.1 Hz), 2.50 (3 H, s), 2.99 (2 H, m), 3.12 (4 H, q, $J$ = 7.1 Hz), 4.42 (2 H, m); MS $m/z$ (relative intensity) 191 ($MH^+$, 1) 117 (16), 103 (35), 102 (20), 59 (27), 58 (100), 57 (36), 56 (67). Anal. Calcd for $C_7H_{19}N_4O_2Cl$ (the hydrochloride of **2m**): C, 37.08; H, 8.39; N, 24.72. Found: C, 36.87; H, 8.41; N, 24.63.

**X-ray diffraction analysis of 1-(2-((3,3-diethyl-2-oxo-1-triazen-1-yl)oxy)ethyl)pyridinium bromide (2l):** $C_{11}H_{19}N_4O_2Br$, FW = 319.2, monoclinic space group $P2_1/c$, $a$ = 10.847 (2) Å, $b$ = 10.989 (3) Å, $c$ = 12.501 (3) Å, β = 104.35 (2)°, $V$ = 1443.6 (6) Å$^3$, $Z$ = 4, $\rho_{calc}$ = 1.469 mg $mm^{-3}$, λ(Mo Kα) = 0.710 73 Å, $\mu$

= 2.85 mm$^{-1}$, $F(000)$ = 656, $T$ = 22 °C.

A clear colorless 0.18- × 0.32- × 0.60-mm crystal, in the shape of an irregular prism, was used for data collection on an automated Siemens R3m/V diffractometer equipped with an incident beam monochromator. Lattice parameters were determined from 25 centered reflections within $27.0 \leq 2\theta \leq 38.5°$. The data collection range of $hkl$ was: $-14 \leq h \leq 0$, $0 \leq k \leq 14$, $-15 \leq l \leq 16$, with $[(\sin\theta)/\lambda]_{max} = 0.59$. Three standards, monitored after every 97 reflections, exhibited random variations with deviations up to ±2.5% during the data collection. A set of 3736 reflections was collected in the $\theta/2\theta$ scan mode, with scan width $[2\theta(K_{\alpha 1}) - 1.2]$ to $[2\theta(K_{\alpha 2}) + 1.2]°$ and $\omega$ scan rate (a function of count rate) from 7.5 to 30.0 deg/min. For this compound, there were 3262 unique reflections, and 2020 were observed with $F_o > 3\sigma(F_o)$. Data were corrected for Lorentz and polarization effects. An empirical absorption correction was also applied (maximum and minimum transmission factors were 0.964 and 0.637, respectively). The structure was solved and refined with the aid of the SHELXTL system of programs.[12] The full-matrix least-squares refinement varied 163 parameters, including atom coordinates and anisotropic thermal parameters for all non-H atoms. H atoms were included using a riding model [coordinate shifts of C applied to attached H atoms, C–H distance set to 0.96 Å, H angles idealized, constant $U_{iso}$]. Final residuals were $R$ = 0.050 and $R_w$ = 0.041 with final difference Fourier excursions of 0.44 and −0.47 e Å$^{-3}$ in the vicinity of the Br atom.

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**Registry No.** **2a**, 91725-44-9; **2b**, 112753-63-6; **2c**, 112753-64-7; **2d**, 143706-20-1; **2e**, 143706-21-2; **2f**, 143706-22-3; **2f'**, 143706-19-8; **2g**, 143706-23-4; **2h**, 143706-24-5; **2i**, 143706-25-6; **2j**, 143706-26-7; **2k**, 143706-27-8; **2l**, 143706-28-9; **2m**, 143706-29-0; $Et_2N(N_2O_2)Na$, 92382-74-6.

**Supplementary Material Available:** Complete crystal structure parameters for **2l**, including atomic positional and thermal parameters (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

# Facilitated Intramolecular Conjugate Addition of *N*-(*p*-Methoxyphenyl)-3-(3′,6′-dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionamide. 1. Product Characterization

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*N*-(*p*-Methoxyphenyl)-3-(3′,6′-dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionamide (1), a pro-prodrug model, was chosen to study the reaction of quinone propionic amides in mildly acidic aqueous solution. The quinone propionic amide 1 equilibrates rapidly with its hydroxy dienone 6 and undergoes a much slower 1,4 conjugate addition to form its enol spirolactam 4. The enol spirolactam 4 then tautomerizes to give the keto spirolactam 5. Spectroscopic evidence suggests that the keto spirolactam 5 is present as a diastereomeric mixture, wherein the preferred diastereomer has the 2′-methyl and the lactam nitrogen anti to each other.

## Introduction

Bioreversible derivatives of drugs, commonly referred to as prodrugs, have been shown to improve the physicochemical (e.g., solubility, lipophilicity) and biological (e.g., membrane permeability, metabolic stability) properties of many compounds.[1-4] Whereas there is considerable published research on ester prodrugs, less is available on prodrugs of amines.[1-4] One approach to development of amine prodrugs is acylation; however, amides are typically too stable in vivo to be useful prodrug forms for amines.

Recently, our laboratory described the synthesis of a highly chemically reactive hydroxy amide, which lactonized with a half-life of approximately 1 min at near physiological pH and temperature.[5] In order to transform this hydroxy amide into useful prodrug model systems for amines, methods were developed for converting this hydroxy amide into chemically stable yet enzymatically labile pro-prodrugs.[6,7] Using the hydroxy amide template,[5] ester-sensitive[7] and redox-sensitive[6] pro-prodrug systems for amines were developed by our laboratory. The redox-sensitive pro-prodrug system consists of amides of 3-(3′,6′-dioxo-2′,4′-dimethylcyclohexa-1′,4′-dienyl)-3,3-dimethylpropionic acid (e.g., **1**, Figure 1). The chemical reduction of the quinone moiety of **1** was shown to generate the intermediate hydroquinone **2**, which rapidly lactonizes, yielding the lactone **3** and the amine (e.g., *p*-anisidine).[6] A similar redox-sensitive system has been described by Carpino et al.[8]

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