Synthesis and Applications of [1-^{15}N]-Labeled 4,6-Dimethyl-4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-Oxide as a Useful Tool for Mechanistic Investigations

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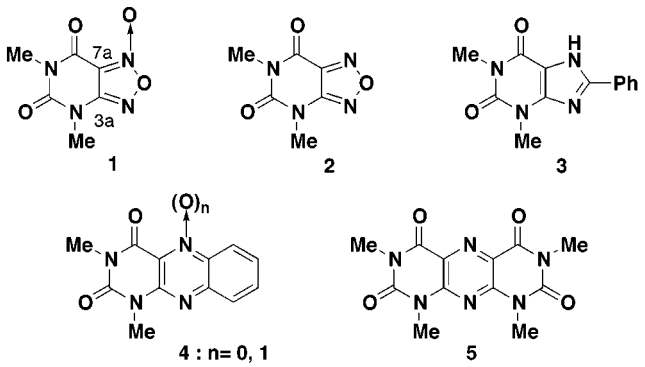
[1-^{15}N]-Labeled 4,6-dimethyl-4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxide (**1-^{15}N$_1$**) was easily prepared by nitration of commercially available 6-amino-1,3-dimethyl-1*H*-pyrimidine-2,4-dione using ^{15}N-enriched nitric acid followed by an intramolecular oxidative cyclization with iodosylbenzene diacetate under mild conditions. On the basis of the experimental results using **1-^{15}N$_1$**, the formation of 8-phenyltheophylline (**3**), the 1,3-dimethylalloxazines (**4**: $n = 0, 1$), and 1,3,7,9-tetramethyl-1*H*,9*H*-pyrimido[5,4-*g*]pteridine-2,4,6,8-tetraone (**5**) in the thermal reaction of the *N*-oxide **1** with benzylamine, aniline, or piperidine, and the generation of NO or NO-related species in the reaction with *N*-acetylcysteamine were reasonably explained by considering the initial attack of the employed nucleophiles on the 3a-position of **1**.

Introduction

4*H*-[1,2,5]Oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxides (cf. **1**) are versatile intermediates for the preparation of fused pyrimidinediones such as 3,7-dihydropurine-2,6-diones (xanthines and theophyllines),[1] 1*H*-benzo[*g*]-pteridine-2,4-diones (alloxazines),[2] and 1*H*,9*H*-pyrimido-[5,4-*g*]pteridine-2,4,6,8-tetraones [3] which are of interest from biological and pharmacological viewpoints.[4] Moreover, our recent work[5] has documented that these *N*-oxides serve as an efficient donor of nitric oxide (NO) or *S*-nitrosothiols, which are unique simple molecules with an array of signaling functions,[6] in the presence of alkanethiols under physiological conditions. The most electrophilic site of these *N*-oxides has been considered to be the 3a-position of this fused ring system.[1,5] However, detailed mechanisms for the formation of the alloxazines and pyrimidopteridinetetraones in the reactions with the anilines[2] and secondary or tertiary alkylamines[3] described above have remained incoherent or obscure.

We describe herein the first synthesis of [1-^{15}N]-labeled 4,6-dimethyl-4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxide (**1-^{15}N$_1$**) and its applications as a tool for the mechanistic investigations of the reactions with benzylamine, aniline, and piperidine leading to the formation of 8-phenyltheophylline (**3**), the 1,3-dimethylalloxazines (**4**; $n = 0, 1$), and 1,3,7,9-tetramethyl-1*H*,9*H*-pyrimido[5,4-*g*]pteridine-2,4,6,8-tetraone (**5**), and of the reaction with *N*-acetylcysteamine resulting in the generation of NO or NO-related species.



Results and Discussion

Synthesis of the ^{15}N-Labeled Oxadiazolopyrimidinedione *N*-Oxide (1-^{15}N$_1$). The conventional methods for the preparation of 4,6-disubstituted 4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxides (cf. **1**) involve (a) the thermal decomposition of the 1,3-disubstituted 6-azido-5-nitro-1*H*-pyrimidine-2,4-diones[7] and (b) the nitrosative or nitrative cyclization of the 1,3-disubstituted 6-hydroxylamino-1*H*-pyrimidine-2,4-diones in acidic media.[3,8] These synthetic methods, however, were not very effective (45−65% yields) for preparing the objective 4,6-dimethyl derivative **1**.

Recently, we have documented an efficient synthetic method for the 4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-

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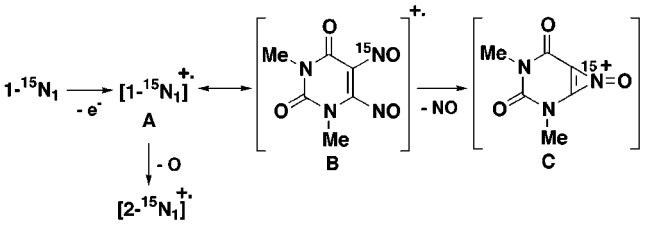
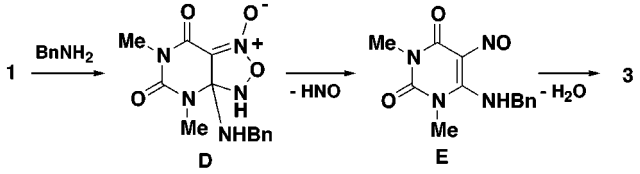
Scheme 1



Scheme 2



dione 1-oxides involving the oxidative intramolecular cyclization of the appropriate 6-amino-5-nitro-1*H*-pyrimidine-2,4-diones.[5] Therefore, the [1-^{15}N]-labeled *N*-oxide **1-^{15}N$_1$** was prepared according to the latter synthetic method. Thus, 6-amino-1,3-dimethyl-5-(^{15}N-labeled nitro)-1*H*-pyrimidine-2,4-dione, easily prepared by the nitration of commercially available 6-amino-1,3-dimethyl-1*H*-pyrimidine-2,4-dione using ^{15}N-enriched nitric acid, was treated with excess iodosylbenzene diacetate (IBD) in the presence of lithium hydride at 50 °C for 1 h. Subsequent chromatographic purification of the resulting mixture allowed the isolation of the desired **1-^{15}N$_1$** in 72% yield. The structure of the ^{15}N-labeled *N*-oxide thus obtained was confirmed by spectral comparison with the unlabeled *N*-oxide **1**;[3,5,7,8] a molecular ion peak (*m*/*z*: 199) (*m*/*z* 199.0358, Δ −0.2 mmu, in EI:HR-mass) for $C_6H_6{}^{15}N_1{}^{14}N_3O_4$ was observed in its EI-mass spectrum. A prominent loss of ^{14}NO from **1-^{15}N$_1$** under ionizing radiation to form a $[M - 30]^+$ ion corresponding to $C_6H_6{}^{15}N_1{}^{14}N_2O_3$ (*m*/z 169) was observed in its mass spectrum. This can be rationalized by assuming the oxadiazole-ring opening at the weak $^{15}N_1-O_2$ bond in the *N*-oxide cation-radical (**A**) leading to a 5,6-dinitroso tautomer (**B**) followed by breaking of the $C_6-{}^{14}NO$ bond to cause the generation of a recyclized cation (**C**) (*m*/*z* 169) and ^{14}NO as shown in Scheme 1. A very weak peak (rel intensity: 7%) for the $[M - 16]^+$ ion (*m*/*z* 183) was observed in the mass spectrum of **1-^{15}N$_1$**. This indicates that a loss of the oxygen-atom from the 4*H*-[1,2,5]-oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxide **1** leading to the cation-radical of 4,6-dimethyl-4*H*-[1,2,5]oxadiazolo-[3,4-*d*]pyrimidine-5,7-dione (**2**) is not a significant fragmentation process under the ionizing radiation, as in the cases of other types of fused oxadiazoles.[9] In the IR spectral comparison, a significant downfield shift in the peak 1645 cm^{-1} (for **1**) to 1614 cm^{-1} (for **1-^{15}N$_1$**) was observed, indicating that this peak is assignable to a stretching vibration of the $C_{7a}{=}N_1$ bond in the oxadiazole ring.

Reactions of the ^{15}N-Labeled Oxadiazolopyrimidinedione *N*-Oxide (1-^{15}N$_1$). With Benzylamine. Heating of the 4,6-dimethylated *N*-oxide **1** with a slight excess of benzylamine in hexamethylphosphoramide at 170 °C or in *N*,*N*-dimethylformamide under reflux for 4 h allows the formation of 8-phenyltheophylline (**3**) in 55% and 38% yields, respectively.[1] The theophylline formation under these reaction conditions has been proposed to involve the initial nucleophilic attack of benzylamine on the 3a-position of **1** leading to the corresponding adduct (**D**) followed by the elimination of hyponitrous acid to form 6-benzylamino-1,3-dimethyl-5-nitroso-1*H*-pyrimidine-2,4-dione (**E**) as a transient intermediate for the formation of the theophylline **3** as shown in Scheme 2. This mechanism has been strongly supported by the isolation of the key intermediate, 6-benzylamino-3-methyl-5-nitroso-1*H*-pyrimidine-2,4-dione, in the thermal reaction of the 4-unsubstituted 6-methyl-4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxide (cf. **1**) with benzylamine under milder conditions.[1]

The treatment of the labeled *N*-oxide **1-^{15}N$_1$** with excess benzylamine in dioxane at 95 °C overnight under an argon atmosphere afforded the anticipated [7-^{15}N]-labeled theophylline **3-^{15}N$_7$** in 33% yield as a crystalline product, though the unchanged **1-^{15}N$_1$** was recovered in 43% yield. A molecular ion peak (*m*/*z* 257) (*m*/*z* 257.0933, Δ +0.3 mmu, in EI:HR-mass) for $C_{13}H_{12}{}^{15}N_1{}^{14}N_3O_2$ was observed in the EI-mass spectrum of **3-^{15}N$_7$**,[10] obviously indicating the retention of the N_1-nitrogen atom in the starting *N*-oxide **1-^{15}N$_1$** during the theophylline formation in this reaction. This provides further evidence supporting the mechanism previously proposed for the formation of the 8-substituted theophyllines (cf. **3**) in the reactions of the *N*-oxide **1** with primary alkylamines.[1] In the ^{1}H NMR spectrum of the ^{15}N-labeled product **3-^{15}N$_7$**, a broad doublet signal with a large ^{15}N−^{1}H coupling constant (*J* = 94 Hz) at 13.86 ppm was observed, clearly indicating that the 3,7-dihydro form (cf. **3**) predominates over the tautomeric 3,9-dihydro form for the structure of theophylline in a polar solvent such as dimethyl sulfoxide.

With Aniline. Thermal condensation of the *N*-oxide **1** with N-unsubstituted anilines is a convenient method for the preparation of 1,3-dimethylalloxazines (cf. **4**; *n* = 0) and their 5-oxides (cf. **4**; *n* = 1).[2] The construction of the alloxazine-ring in this reaction has been explained by considering the initial isomerization of **1** to the corresponding N_3-oxide (**6**) under the employed conditions and subsequent nucleophilic attack of the anilines as an enamine at the N_1-position of the isomeric **6**.[11] The proposed mechanism, involving the elimination of the amino group in the employed anilines during the con-

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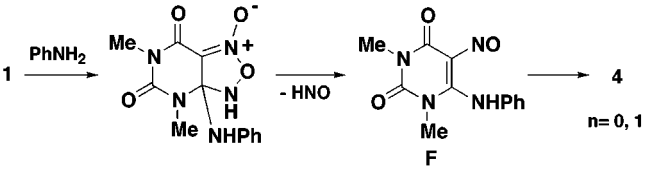
(11) This mechanism relied upon the experimental observations that the same alloxazine **4** was isolated in both of the reactions of the *N*-oxide **1** with aniline and *N*-monomethylaniline. These erroneous observations have perplexed us quite a while for elucidating the mechanism of the alloxazine formation in the reactions of **1** with anilines.

Scheme 3

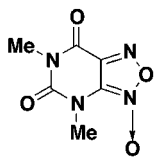


struction of the alloxazine-ring, was not consistent with that for the theophylline formation described above and led us to reinvestigate the reactions with the N-unsubstituted and N-substituted anilines using the labeled *N*-oxides **1-$^{15}N_1$**.

Treatment of the *N*-oxide **1-$^{15}N_1$** with excess aniline in dioxane at 95 °C overnight under an argon atmosphere gave [5-^{15}N]-labeled 1,3-dimethylalloxazine (**4-$^{15}N_5$**; n = 0) as the major product together with small amounts of the expected [5-^{15}N]-labeled alloxazine 5-oxide **4-$^{15}N_5$** (n = 1) and the deoxygenated oxadiazolopyrimidinedione **2-$^{15}N_1$**.[12] A molecular ion peak (m/z 243) (m/z 243.0780, Δ +0.6 mmu, in EI:HR-mass) for $C_{12}H_{10}{}^{15}N_1{}^{14}N_3O_2$ was observed in the EI-mass spectrum of the major product, indicating the retention of the labeled nitrogen-atom in the starting *N*-oxide **1-$^{15}N_1$** during the construction of the alloxazine-ring. On the other hand, the employment of *N*-monomethylaniline or *N*,*N*-dimethylaniline in place of aniline in this reaction resulted in no formation of the alloxazines **4-$^{15}N_5$** (n = 0, 1) and recovery of the starting **1-$^{15}N_1$**. These facts were different from the previously reported results.[2,11] To confirm the insertion of the anilino nitrogen-atom into the alloxazine-ring, the thermal reaction of the unlabeled *N*-oxide **1** with the ^{15}N-enriched aniline was carried out. In this experiment, the insertion of the ^{15}N-labeled nitrogen-atom into the alloxazine ring was proved; a molecular ion peak (m/z 243) (m/z 243.0772, Δ −0.2 mmu, in EI:HR-mass) for $C_{12}H_{10}{}^{15}N_1{}^{14}N_3O_2$ was observed in the EI-mass spectrum of the isolated major product **4-$^{15}N_{10}$** (n = 0).

On the basis of these facts, the alloxazine-ring formation during the reaction of the *N*-oxide **1** with aniline can be reasonably explained by the revised sequence involving the initial nucleophilic attack of aniline, not as an enamine, on the 3a-position in the fused ring system to form an intermediary 6-anilino-5-nitrosopyrimidinedione (**F**) as shown in Scheme 3. Analogous formation of the alloxazines (cf. **4**: n = 0, 1) proceeding via the transient formation of the corresponding 6-anilino-5-nitroso derivatives (cf. **F**) has been demonstrated in the nitrosation of 6-anilino-1,3-dimethyl-1*H*-pyrimidine-2,4-diones.[13]

With Piperidine. Refluxing a solution of the *N*-oxide **1** in dioxane including excess piperidine or in *N*,*N*-dimethylaniline for 8 h has been shown to afford a mixture of 1,3,7,9-tetramethyl-1*H*,9*H*-pyrimido[5,4-*g*]-pteridine-2,4,6,8-tetraone (**5**) and isomeric 1,3,5,7-tetramethyl-1,5-dihydro-1,3,5,7,9,10-hexaaza-anthracene-2,4,6,8-tetraone (**8**), but without descriptions of their detailed mechanisms and other products.[3]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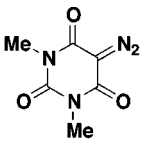
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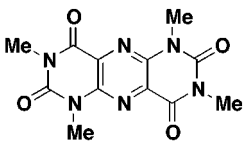
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When the labeled *N*-oxide **1-$^{15}N_1$** was treated with two equimolar amounts of piperidine in dioxane at 95 °C overnight, [5-^{15}N]-labeled 1,3,7,9-tetramethyl-1*H*,9*H*-pyrimido[5,4-*g*]pteridine-2,4,6,8-tetraone (**5-$^{15}N_5$**) (m/z 305.0885, Δ −0.5 mmu for $C_{12}H_{12}{}^{15}N_1{}^{14}N_5O_4$, in EI:HR-mass) was isolated in 20% yield after the column chromatographic separation of the resulting complex mixtures, together with the deoxygenated oxadiazolopyrimidinedione **2-$^{15}N_1$** (8%) and unexpected ^{15}N-labeled 5-diazo-1,3-dimethylpyrimidine-2,4,6-trione (**7-$^{15}N_5$**) (7%). The isomeric hexaaza-anthracenetetraone **8-^{15}N** was not isolated at least as the main product under the employed conditions as previously reported.[3] The structure of the obtained **5-$^{15}N_5$** was confirmed by spectral comparison with the unlabeled authentic compound **5** independently prepared by the thermal condensation of 6-chloro-1,3-dimethyl-5-nitroso-1*H*-pyrimidine-2,4-dione with 6-amino-1,3-dimethyl-1*H*-pyrimidine-2,4-dione in *N*,*N*-dimethylformamide followed by the deoxygenation with sodium hydrosulfite;[14] the UV spectrum of this product clearly contained the pyrimido[5,4-*g*]pteridinetetraone ring system (362, 265, and 232 nm for **5**), not the isomeric hexaaza-anthracenetetraone ring system (427 and 405 nm for **8**).[3,10a,14b,c,15] The structure of the 5-diazopyrimidinetrione **7-$^{15}N_5$** was assigned on the basis of its ^{1}H NMR, IR, and mass spectral data and confirmed by spectral comparison with the unlabeled authentic compound previously reported;[16] two molecular ion peaks of m/z 184 (m/z 184.0373, Δ −0.8 mmu, in EI:HR-mass) for $C_6H_6{}^{15}N_2{}^{14}N_2O_3$ and m/z 183 (m/z 183.0403, Δ −0.7 mmu, in EI:HR-mass) for $C_6H_6{}^{15}N_1{}^{14}N_3O_3$ were observed in the EI-mass spectrum of this product, indicating that the

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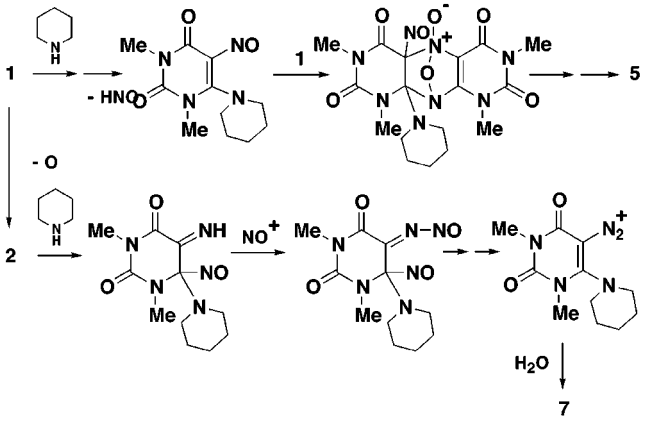
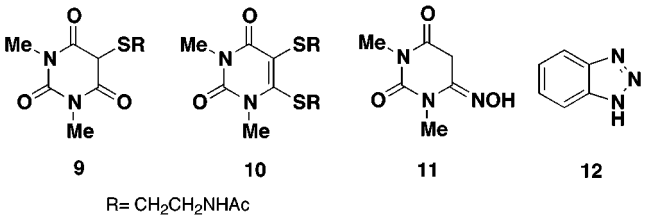
Scheme 4



product **5-$^{15}N_5$** is a mixture of compounds bearing the ^{15}N-mono-labeled and ^{15}N,^{15}N-dilabeled diazo groups.

Taking the chemical reactivity of the *N*-oxide **1** demonstrated above into consideration, the formation of the pyrimidopteridinetetraone **5** and 5-diazopyrimidinetrione **7** during the thermal reaction of **1** with piperidine can be explained by plausible mechanisms involving the initial nucleophilic attack of piperidine on the 3a-position of the *N*-oxide **1** or the deoxygenated product **2** as shown in Scheme 4.[17]

With *N*-Acetylcysteamine. Treatment of the *N*-oxide **1** with excess *N*-acetylcysteamine in a pH 7.5 phosphate buffer solution at 37 °C has been proved to generate NO or S-nitroso-*N*-acetylcysteamine, accompanied by the formation of 5-(2-acetylaminoethyl)thio-1,3-dimethylpyrimidine-2,4,6-trione (**9**), 5,6-bis(2-acetylaminoethylthio)-1,3-dimethyl-1*H*-pyrimidine-2,4-dione (**10**), and 1,3-dimethylpyrimidine-2,4,6-trione 4-oxime (**11**).[5] The generation of NO or *S*-nitrosothiol has been explained by the initial attack of the thiol to both the 3a- and 7a-positions of **1** and subsequent ring-opening to produce the key intermediary adduct (**G**), followed by the further attack of another thiol on the 5-nitroso group in the adduct **G** to release *S*-nitroso-*N*-acetylcysteamine as a NO precursor.

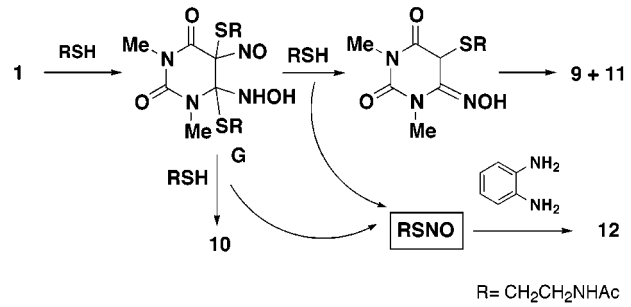


9 **10** **11** **12**

R= CH_2CH_2NHAc

When the reaction of the labeled *N*-oxide **1-$^{15}N_1$** with *N*-acetylcysteamine was carried in the presence of 1,2-phenylenediamine,[18] [2-^{15}N]-labeled benzo-1,2,3-triazole (**12-$^{15}N_2$**) (*m*/*z* 120.0452, Δ −0.2 mmu, for $C_6H_5{}^{15}N_1{}^{14}N_2$, in EI:HR-mass) was isolated in 25% yield, together with the anticipated pyrimidine derivatives **9**−**11**. No formation of the benzotriazole **12-$^{15}N_2$** was observed in the reaction which was carried out in the absence of *N*-acetylcysteamine under similar conditions, indicating that *N*-acetylcysteamine is required for the formation of **12-$^{15}N_2$** in this reaction. These facts strongly support the mechanism described above, suggesting that the NO and NO-related species are from the N_1-nitrogen atom of the *N*-oxide **1** as shown in Scheme 5. When the phenylenediamine was added to the reaction mixture of **1-$^{15}N_1$** with *N*-acetylcysteamine, which was carried out under the similar conditions, the 5% formation of **12-$^{15}N_2$** was observed, indicating that the generated NO and NO-related species are fairly stable under the employed conditions.

Scheme 5



R= CH_2CH_2NHAc

Conclusion

The objective 1,3-dimethyl-4*H*-[1,2,5]oxadiazolo[3,4-*d*]-pyrimidine-5,7-dione 1-oxide (**1-$^{15}N_1$**) bearing ^{15}N-labeled *N*-oxide was successfully prepared by the IBD-oxidation of 6-amino-1,3-dimethyl-(5-^{15}N-labeled nitro)-1*H*-pyrimidine-2,4-dione in the presence of lithium hydride. The experimental results using the labeled *N*-oxide **1-$^{15}N_1$** provided further evidence supporting the mechanisms previously proposed for the theophylline formation and the generation of NO or NO-related species in the reactions of the *N*-oxide **1** with benzylamine or *N*-acetylcysteamine. The mechanism previously proposed for the alloxazine formation in the reaction with aniline was revised from the experimental results using **1-$^{15}N_1$** and further experiments using ^{15}N-enriched aniline or N-substituted anilines. Plausible sequences for the complex reactions of **1** with piperidine were proposed on the basis of the isolation of [5-^{15}N]-labeled 1,3,7,9-tetramethyl-1*H*,9*H*-pyrimido[5,4-*g*]pteridine-2,4,6,8-tetraone (**5-$^{15}N_5$**) as the major product and [5-^{15}N]-labeled 5-diazopyrimidinetrione (**7-$^{15}N_5$**) as the byproduct in the reaction using **1-$^{15}N_1$**.

Experimental Section

^1H NMR and ^{13}C NMR spectra were recorded at 400 and 75 MHz, respectively, using DMSO-d_6 (unless otherwise noted) as the solvent. Mass spectra were determined at an ionizing voltage of 70 eV. For thin-layer chromatographic (TLC) analyses, Merck precoated TLC plates (Merck No. 5715; silica gel 60-F_{254}) were used. Column chromatography was performed on silica gel (Merck No. 9385-5B; silica gel 60). Unless otherwise noted, materials obtained from commercial suppliers were used without further purification.

Preparation of [1-^{15}N]-Labeled 4,6-Dimethyl-4*H*-[1,2,5]-oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-Oxide (1-$^{15}N_1$). To a vigorously stirred solution of 6-amino-1,3-dimethyl-1*H*-pyrimidine-2,4-dione (Aldrich, 98% purity) (155.2 mg, 1.0 mmol) in concentrated sulfuric acid (1.0 mL) was added

(17) In this reaction, a volatile product derived from piperidine also was isolated after the column chromatographic separation of the complex mixtures. The structure of this product could not be determined in this stage due to its high volatility. The mass, ^1H NMR, and ^{13}C NMR spectral data (see the Experimental Section) of this product, however, suggested the occurrence of a redox reaction between the *N*-oxide **1** and piperidine leading to the oxadiazolopyrimidinedione **2** and the oxygenated piperidine (and subsequent nitrosation).

(18) Uppu, R. M.; Pryor, W. A. *J. Am. Chem. Soc.* **1999**, *121*, 9738−9739.

dropwise 10 M ^{15}N-enriched nitric acid (C/D/N isotopes, 99.3 at. % ^{15}N) (1.0 mL, 10.0 mmol) at 0 °C, and then the stirring was continued at room temperature for 1 h. An ice block was added to the reaction mixture during the continuous stirring. The resulting precipitate was collected, washed with cold water (1.0 mL), and then recrystallized from methanol to give the desired labeling compound, 6-amino-1,3-dimethyl-5-(^{15}N-labeled nitro)-1*H*-pyrimidine-2,4-dione (164.0 mg, 82%): mass *m*/*z* (rel intensity) 201 (M^+, 100), 185 ($M^+ - O$, 18), 183 ($M^+ - H_2O$, 52), 170 (10), 149 (8), 140 (4), 128 (10), 111 (10); IR (KBr) 3526, 3449, 3367, 3302, 1720, 1625, 1522 cm^{-1}. Anal. Calcd for $C_6H_8{}^{15}N_1{}^{14}N_3O_4$: *m*/*z* 201.0516. Found: *m*/*z* 201.0509.

To a stirred suspension of the 6-amino-5-(^{15}N-labeled nitro)-pyrimidinedione (100.5 mg, 0.5 mmol) thus obtained in dry *N*,*N*-dimethylformamide (5.0 mL) was added lithium hydride (Aldrich, 95% purity) (8.5 mg, 1.0 mmol) in one portion. After stirring at ambient temperature for 0.5 h, iodosylbenzene diacetate (Aldrich, 98% purity) (410.8 mg, 1.25 mmol) was added to the mixture, and the solution was heated at 50 °C with stirring for 1 h. The reaction mixture was diluted with water (25 mL), neutralized with dilute hydrochloric acid, and extracted with ethyl acetate (30 mL, three times). The collected extract was washed with brine (30 mL), dried over anhydrous magnesium sulfate, and evaporated to dryness. The resulting residue was purified by column chromatography eluting with chloroform−acetone (100/1) to isolate the ^{15}N-labeled *N*-oxide **1-^{15}N$_1$** (71.4 mg, 72%): mass *m*/*z* (rel intensity) 199 (M^+, 100), 183 ($M^+ - O$, 7), 169 ($M^+ - {}^{14}NO$, 32), 149 (3), 142 (4), 112 (5), 84 (70), 81 (96); IR (KBr) 1741, 1686, 1614 cm^{-1}. Anal. Calcd for $C_6H_6{}^{15}N_1{}^{14}N_3O_4$: *m*/*z* 199.0360. Found: *m*/*z* 199.0358.

Reaction of the ^{15}N-Labeled *N*-Oxide (1-^{15}N$_1$) with Benzylamine. A mixture of the *N*-oxide **1-^{15}N$_1$** (20.0 mg, 0.1 mmol) and benzylamine (32.8 μL, 0.3 mmol) in dioxane (1.0 mL) was heated at 95 °C overnight with stirring under an argon atmosphere. The resulting precipitate was collected by filtration and washed with dioxane (1.0 mL) to isolate the [7-^{15}N]-labeled 1,3-dimethyl-8-phenylpurine-2,4-dione (**3-^{15}N$_7$**) (7.5 mg, 29%) as colorless crystals: mass *m*/*z* (rel intensity) 257 (M^+, 100,) 228 ($M^+ -$ NMe, 5), 200 ($M^+ -$ MeNCO, 5), 171 (3), 104 (21), 69 (23); IR (KBr) 3147, 1688, 1646 cm^{-1}; UV (MeOH) λ_{max} 307, 236 nm; ^{1}H NMR δ 3.27 (3H, s, NMe), 3.51 (3H, s, NMe), 7.60−7.65 (3H, m, ArH), 8.24−8.27 (2H, m, ArH), 13.86 (1H, br d, *J* 94 Hz, NH). Anal. Calcd for $C_{13}H_{12}{}^{15}N_1{}^{14}N_3O_2$ *m*/*z* 257.0930. Found: *m*/*z* 257.0933. Column chromatographic separation of the filtrate using chloroform−acetone (100/1 to 10/1) as the eluant allowed the isolation of the starting *N*-oxide **1-^{15}N$_1$** (8.6 mg, 43%) and the labeled theophylline **3-^{15}N$_7$** (1.0 mg, 4%). TLC analyses of the reaction mixture showed the presence of small amounts of other undetermined products including fluorescent compounds.

Reaction of the ^{15}N-Labeled N-Oxide (1-^{15}N$_1$) with Aniline. A solution of the *N*-oxide **1-^{15}N$_1$** (20.0 mg, 0.1 mmol) in dioxane (1.0 mL) containing aniline (27.5 μL, 0.3 mmol) was heated at 95 °C overnight under an argon atmosphere. The mixture was diluted with ethyl acetate (30 mL), washed with dilute hydrochloric acid (10 mL) and then brine (10 mL, two times), and evaporated to dryness. The residual oil was subjected to column chromatography eluting with chloroform−acetone (100/1) to isolate a 19:1 (by ^{1}H NMR) mixture (14.2 mg, 58%) of ^{15}N-labeled 1,3-dimethylalloxazine (**4-^{15}N$_5$**; *n* = 0) [mass *m*/*z* (rel intensity) 243 (M^+, 100), 214 ($M^+ -$ NMe, 8), 200, 186 ($M^+ -$ MeNCO, 7), 158 (40), 131 (58); IR (KBr) 1722, 1676, 1559 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.62 (3H, s, NMe), 3.84 (3H, s, NMe), 7.77 (1H, t, ArH), 7.91 (1H, t, ArH), 8.05 (1H, d, ArH), 8.36 (1H, d, ArH). Anal. Calcd for $C_{12}H_{10}{}^{15}N_1{}^{14}N_3O_2$: *m*/*z* 243.0774. Found: *m*/*z* 243.0780] and its *N*-oxide (**4-^{15}N$_5$**; *n* = 1) [FAB-MS: *m*/*z* 260 $(M + 1)^+$; ^{1}H NMR ($CDCl_3$) δ 3.51 (3H, s, NMe), 3.80 (3H, s, NMe)], together with the recovered **1-^{15}N$_1$** (6.0 mg, 30%) and the deoxygenated 4,6-dimethyl-4*H*-[1,2,5]-oxadiazolo[3,4-*d*]pyrimidine-5,7-dione (**2-^{15}N$_1$**) (0.6 mg, 3%) [mass *m*/*z* (rel intensity) 183 (M^+, 100), 153 ($M^+ -$ NO, 36), 126 ($M^+ -$ MeNCO, 14), 112 (10), 68 (90); UV (in MeOH) 284, 245 nm; ^{1}H NMR ($CDCl_3$) δ 3.48 (3H, s, NMe), 3.63 (3H, s, NMe). Anal. Calcd for $C_6H_6{}^{15}N_1{}^{14}N_3O_3$: *m*/*z* 183.0410. Found: *m*/*z* 183.0409].

Reaction of the *N*-Oxides (1 and 1-^{15}N$_1$) with ^{15}N-Enriched Aniline or N-Substituted Anilines. A solution of the unlabeled *N*-oxide **1** (19.9 mg, 0.1 mmol) in dioxane (1.0 mL) containing ^{15}N-enriched aniline (Aldrich, 99% at. % ^{15}N) (27.5 μL, 0.3 mmol) was heated at 95 °C overnight under an argon atmosphere. After treatment similar to the case described above, column chromatographic separation allowed the isolation of ^{15}N-labeled 1,3-dimethylalloxazine (**4-^{15}N$_{10}$**; *n* = 0) [mass *m*/*z* (rel intensity) 243 (M^+, 100), 214 ($M^+ -$ NMe, 8), 199, 186 ($M^+ -$ MeNCO, 6), 158 (38), 131 (54); IR (KBr) 1721, 1675, 1556 cm^{-1}; Anal. Calcd for $C_{12}H_{10}{}^{15}N_1{}^{14}N_3O_2$: *m*/*z* 243.0774. Found: *m*/*z* 243.0772] and its *N*-oxide **4-^{15}N$_{10}$** (*n* = 1) [FAB-MS: *m*/*z* 260 $(M + 1)^+$] (14.6 mg, as a 20:1 mixture, by ^{1}H NMR) together with the recovered **1** (6.0 mg, 30%) and a trace amount of the deoxygenated oxadiazolopyrimidinedione **2** (detected by TLC and ^{1}H NMR analyses).

No formation of the alloxazine **4-^{15}N$_5$** (*n* = 0) and its *N*-oxide **4-^{15}N$_5$** (*n* = 1) were observed in the reactions of the *N*-oxide **1-^{15}N$_1$** (10.0 mg, 0.05 mmol) with aniline (13.8 μL, 0.15 mmol) in dioxane (1.0 mL) at room temperature even after a prolonged reaction time (e.g., after 2 days) and with *N*-monomethylaniline (16.2 μL, 0.15 mmol) or *N*,*N*-dimethylaniline (19.0 μL, 0.15 mmol) in dioxane (1.0 mL) at 95 °C overnight.

Reaction of the ^{15}N$_1$-Labeled *N*-Oxide (1-^{15}N$_1$) with Piperidine. A solution of the *N*-oxide **1-^{15}N$_1$** (40.7 mg, 0.2 mmol) in dioxane (1.0 mL) containing piperidine (40 μL. 0.4 mmol) was heated at 95 °C overnight under an argon atmosphere. TLC analyses of the resulting dark-brown solution showed the formation of complex mixtures in this reaction. After removal of the solvent, the residual oil was subjected to column chromatography eluting with toluene−ethyl acetate (10/1) to isolate the oxadiazolopyrimidinedione **2-^{15}N$_1$** (3.0 mg, 8%), ^{15}N-labeled 5-diazo-1,3-dimethylpyrimidine-2,4,6-trione (**7-^{15}N$_5$**) (6.3 mg, 17%) [mass *m*/*z* (rel intensity) 184 (M^+ for the ^{15}N,^{15}N-dilabeled form, 80), 183 (M^+ for the ^{15}N-monolabeled form, 83), 154 ($M^+ - {}^{15}N_2$ or $^{15}N^{14}N$, 39), 97 (55), 69 (100); IR (KBr) 2149, 2090, 1720, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.35 (6H, s, 2 NMe); ^{13}C NMR ($CDCl_3$) δ 28.6, 71.5, 150.5, 158.2; UV (MeOH) 262, 209 nm. Anal. Calcd for $C_6H_6{}^{15}N_2{}^{14}N_2O_3$: *m*/*z* 184.0381. Found: *m*/*z* 184.0373. Anal. Calcd for $C_6H_6{}^{15}N_1{}^{14}N_3O_3$: *m*/*z* 183.0410. Found: *m*/*z* 183.0403], and an undetermined volatile product [mass *m*/*z* (rel intensity) 142 ($C_5H_{10}N_4O$, 100), 114 ($C_5H_{10}N_2O$, 10), 58 (34), 56 (25); UV (MeOH) 229 nm; ^{1}H NMR ($CDCl_3$) δ 1.5−1.7 (2H, m), 1.7−1.9 (4H, m), 3.78 (2H, t, *J* = 6 Hz), 4.19 (2H, t, *J* = 6 Hz); ^{13}C NMR ($CDCl_3$) δ 24.2, 24.8, 26.4, 39.8, 50.9]. Subsequent elution with chloroform−methanol (100/1) allowed the isolation of [5-^{15}N]-labeled 1,3,7,9-tetramethyl-1*H*,9*H*-pyrimido[5,4-*g*]-pteridine-2,4,6,8-tetraone (**5-^{15}N$_5$**) (6.0 mg, 20%) [mass *m*/*z* (rel intensity) 305 (M^+, 100), 276 ($M^+ -$ NMe, 12), 248 ($M^+ -$ MeNCO, 13), 222 (14), 220 (14), 193 (20), 108 (17); IR (KBr) 1716, 1679 cm^{-1}; UV (MeOH) 361, 260, 233 nm; ^{1}H NMR δ 3.33 (6H, s, 2 NMe), 3.58 (6H, s, 2 NMe)]. Anal. Calcd for $C_{12}H_{12}{}^{15}N_1{}^{14}N_5O_4$: *m*/*z* 305.0890. Found: *m*/*z* 305.0885.

Reaction of the ^{15}N$_1$-Labeled *N*-Oxide (1-^{15}N$_1$) with N−Acetylcysteamine in the Presence of 1,2-Phenylenediamine. To a solution of the *N*-oxide **1-^{15}N$_1$** (19.9 mg, 0.1 mmol) and *N*-acetylcysteamine (Aldrich, 95% purity) (22.4 μL, 0.2 mmol) in 0.1 Mol K_2HPO_4−KH_2PO_4 buffer (pH 7.5) − acetonitrile (1/1) (2.0 mL) was added 1,2-phenylenediamine (10.9 mg, 0.1 mmol). The mixture was stirred at 37 °C under an argon atmosphere for 1 h. After removal of the solvent under reduced pressure, the residue was subjected to column chromatography eluting with chloroform-ethyl acetate (10/1 to 5/1) to isolate the [2-^{15}N]-labeled benzo-1,2,3-triazole (**12-^{15}N$_2$**) (3.0 mg, 25%) [mass *m*/*z* (rel intensity) 120 (M^+, 100), 91 ($M^+ - {}^{15}N^{14}N$, 67); ^{1}H NMR ($CDCl_3$) δ 7.45−7.50 (2H, m, Ar−H), 7.89 (3H, m, Ar−H), 12.0 (1H, br, NH). Anal. Calcd for $C_6H_5{}^{15}N_1{}^{14}N_2$: *m*/*z* 120.0454. Found: *m*/*z* 120.0452], together with the recovered **1-^{15}N$_1$** (5.0 mg, 25%). The formation of 5-(2-acetylaminoethyl)thio-1,3-dimethylpyrimidine-2,4,6-trione (**9**), 5,6-bis(2-acetylaminoethylthio)-1,3-dimethyl-1*H*-pyrimidine-2,4-dione (**10**), and 1,3-dimethylpyrimidine-2,4,6-trione 4-oxime (**11**) in this reaction was confirmed by TLC analyses of the

reaction mixture. TLC and ^{1}H NMR spectral analyses of the reaction mixtures showed that the product distributions of these pyrimidine derivatives in this reaction were not markedly affected by the addition of phenylenediamine. When the phenylenediamine (10.9 mg, 0.1 mmol) was added to the reaction mixture of **1-^{15}N$_1$** (19.9 mg, 0.1 mmol) and *N*-acetylcysteamine (22.4 μL, 0.2 mmol), which was treated in the buffer−acetonitrile solution at 37 °C under an argon atmosphere for 1 h with continuous stirring, the 5% formation of the benzotriazole **12-^{15}N$_2$** in this reaction was shown by TLC-densitometric analysis of the resulting mixture.

Supporting Information Available: MS, IR, NMR, and UV spectra for the ^{15}N-labeled compounds prepared or isolated. This material is available free of charge via the Internet at http://pubs.acs.org.

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