Facile Synthesis and NO-Generating Property of 4*H*-[1,2,5]Oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-Oxides

Magoichi Sako,* Souichi Oda, Seiji Ohara, Kosaku Hirota, and Yoshifumi Maki

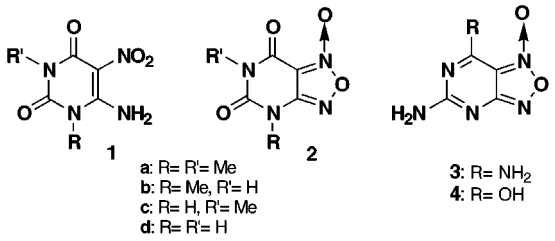
Gifu Pharmaceutical University, 5-6-1, Mitahora-higashi, Gifu 502-8585, Japan

Received April 20, 1998

4*H*-[1,2,5]Oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxides (**2**) are conveniently prepared in high yields by the oxidative intramolecular cyclization of 6-amino-5-nitro-1*H*-pyrimidine-2,4-diones (**1**) employing iodosylbenzene diacetate as an oxidant in the presence of lithium hydride. The generation of nitric oxide (NO) and NO-related species from **2** occurs in the presence of thiols such as *N*-acetylcysteamine, cysteine, and glutathione under physiological conditions. The evidence for the NO generation derives from mechanistic interpretations for the reaction of **2** with thiols and other chemical observations.

Nitric oxide (NO) generates in vivo in the process of the oxygenative conversion of L-arginine to L-citrulline, catalyzed by a heme-containing NO synthase,[1] and has been recognized to have a number of important physiological roles in systems as varied as the transmission of nerve impulses,[2] regulation of blood flow,[3] synergistic inhibition of platelet aggregation,[4] and nonspecific immune response to bacterial infection.[5] NO has limited solubility (1.9 mM/L at 25 °C) in aqueous solutions[6] and is highly sensitive to autoxidation ($-d[O_2]/dt = k_1[NO]^2[O_2]$ with $k_1 = 2.9 \times 10^6$ M^{-2} s^{-1} at 22 °C)[7] to convert into the nitrite ion (NO_2^-) together with a slight amount of the nitrate ion (NO_3^-). In view of physiological significance and transient physicochemical property of NO, much attention has been paid to the compounds that serve as a NO donor with well-controlled releasing property under physiological conditions and have the potential pharmacological effects.[8]

During the course of our investigations on syntheses, chemical reactivities, and biological activities of novel nitrogen-containing heterocyclic *N*-oxides,[9] we have found that 4,6-dimethyl-4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxide (**2a**)[10] causes relaxation of vascular smooth muscles and inhibition of platelet aggregation in guinea pig.[11] This fact implies the release of NO from **2a** in the biological systems and has directed our attention to the chemistry of this heterocycle having a structurally unique fused ring system. In this paper, we describe a new and facile synthetic method for 4,6-unsubstituted or 4- and/or 6-substituted 4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxides **2** and demonstrate the generation of NO and NO-related species from **2** under physiological conditions in order to substantiate our assumption on the biological activity of **2a**.



Results and Discussion

Synthesis. [1,2,5]Oxadiazolo[3,4-*d*]pyrimidine 1-oxides (furazano[3,4-*d*]pyrimidine 1-oxides; pyrimidofuroxans) have been used as versatile intermediates for the preparation of biologically interesting fused pyrimidines such as 8-substituted 9*H*-purines,[10] 4-aminopteridine 5,8-dioxides,[12] and 1*H*-benzo[*g*]pteridine-2,4-dione 5-oxides.[13] There have been two primary methods for the construction of this fused ring system: (a) the thermal decom-

(1) Butler, A. R.; Williams, D. L. H. *Chem Soc. Rev*. **1993**, 233−241. Anbar, M. *Experientia* **1995**, *51*, 545−550. Kerwin, J. F., Jr.; Lancaster, J. R., Jr.; Feldman, P. L. *J. Med. Chem*. **1995**, *38*, 4343−4362. Williams, R. J. P. *Chem. Soc. Rev*. **1996**, 77−83. Wiseman, H.; Halliwell, B. *Biochem. J*. **1996**, *313*, 17−29.

(2) Wang, T.; Xie, Z.; Lu, B. *Nature* **1995**, *374*, 262−266.

(3) Huang, P. L.; Huang, Z.; Mashimo, H.; Bloch, K. D.; Moskowitz, M. A.; Bevan, J. A.; Fishman, M. C. *Nature* **1995**, *377*, 239−242; Stamler, J. S. *Nature* **1996**, *380*, 108−111; Perutz, M. F. *Nature* **1996**, *380*, 205−206; Jia, L.; Bonaventura, C.; Bonaventura, J.; Stamler, J. S. *Nature* **1996**, *380*, 221−226; Hoshino, M.; Maeda, M.; Konishi, R.; Seki, H.; Ford, P. C. *J. Am. Chem. Soc*. **1996**, *118*, 5702−5707.

(4) Katzenschlager, R.; Weiss, K.; Rogatti, W.; Peskar, B. A.; Sinzinger, H. *Prostaglandins Leukot. Essent. Fatty Acids* **1992**, *45*, 207−210.

(5) Groves, J. T.; Marla, S. S. *J. Am. Chem. Soc*. **1995**, *117*, 9578−9579. Suzuki, T.; Yamaoka, R.; Nishi, M.; Ide, H.; Makino, K. *J. Am. Chem. Soc*. **1996**, *118*, 2515−2516. Fukuto, J. M.; Ignarro, L. J. *Acc. Chem. Res*. **1997**, *30*, 149−152.

(6) Shaw, A. W.; Vosper, A. J. *J. Chem. Soc., Faraday Trans. 1* **1977**, *73*, 1239−1244. NO is much more soluble in apolar solvents such as *n*-hexane, with a concentration at saturation of 0.13 M.

(7) Goldstein, S.; Czapski, G. *J. Am. Chem. Soc*. **1995**, *117*, 12078−12084.

(8) Wink, D. A.; Darbyshire, J. F.; Nims, R. W.; Saavedra, J. E.; Ford, P. C. *Chem. Res. Toxicol*. **1993**, *6*, 23−27. Ignarro, L. J.; Fukuto, J. M.; Griscavage, J. M.; Rogers, N. E.; Byrns, R. E. *Proc. Natl. Acad. Sci. U.S.A*. **1993**, *90*, 8103−8107. Lefer, A. M.; Lefer, D. J. *Drugs Future* **1994**, *19*, 665−672. 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.

(9) Maki, Y.; Sako, M. *J. Synth. Org. Chem. Jpn*. **1994**, *52*, 149−160.

(10) Yoneda, F.; Tachibana, T.; Tanoue, J.; Yano, T.; Sakuma, Y. *Heterocycles* **1981**, *15*, 341−344.

(11) Unpublished results. During the course of our investigation, 7-morpholino-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine 1-oxide has been documented to relax potassium-depolarized guinea pig pulmonary arteries, which implies a NO-mediated mechanism, without any chemical evidence for the NO generation (cf. Brendel, J.; Schoenafinger, K.; Bohn, H. Eur. Pat. Appl. EP 573829, 1993; *Chem. Abstr*. **1994**, *120*, 217724v).

(12) Binder, D.; Noe, C. R.; Prager, B. C.; Turnowsky, F. *Arzneim.-Forsch*. **1983**, *33*, 803−805.

(13) Yoneda, F.; Sakuma, Y.; Matsumoto, S. *Heterocycles* **1975**, *3*, 113−116.

S0022-3263(98)00732-4 CCC: $15.00
Published on Web 09/05/1998

position of 6-azido-5-nitropyrimidines, which are prepared by treatment of 6-chloro-5-nitropyrimidines with sodium azide or by the nitrosation of 6-hydrazino-5-nitropyrimidines, with accompanying loss of nitrogen;[14] (b) the nitrosative or nitrative cyclization of 6-hydroxylamino-1*H*-pyrimidine-2,4-diones (cf. pyrimidine-2,4,6-trione 4-oximes **8**)[15] in acidic media. These synthetic methods are not so effective (29−65% yield) to prepare the desired 4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxides **2** because of concurrent occurrence of undesirable side reactions during the intramolecular cyclization, i.e., tetrazole-ring formation without loss of nitrogen in the former case and dehydrative dimerization of the starting pyrimidine-2,4,6-trione 4-oximes leading to the corresponding 1*H*,9*H*-pyrimido[5,4-*g*]pteridine-2,4,6,8-tetrones in the latter case.

Our recent work has developed a facile method for the preparation of 4,6-dimethyl-4*H*-[1,2,5]oxadiazolo[3,4-*d*]-pyrimidine-5,7-dione, the deoxygenated compound of **2a**, involving an oxidative intramolecular cyclization of 6-amino-1,3-dimethyl-5-nitroso-1*H*-pyrimidine-2,4-dione with iodosylbenzene diacetate (IBD) after treatment with lithium hydride (LiH).[16] From a mechanistic point of view, this method appears to be applicable to the synthesis of **2** by using appropriate 6-amino-5-nitro-1*H*-pyrimidine-2,4-diones **1** as starting materials.[17] Along this line, the IBD oxidation of 6-amino-1,3-dimethyl-5-nitro-1*H*-pyrimidine-2,4-dione (**1a**) was examined.

To a solution of **1a** pretreated with two equimolar amounts of LiH in DMF for 0.5 h, slightly excess IBD was added. Warming the mixture at 50 °C with stirring caused smooth consumption of **1a** to give the corresponding 4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxide **2a** almost quantitatively after 1 h. The structure of **2a** was confirmed both by its microanalytical results and spectral data and by independent synthesis starting from 1,3-dimethylpyrimidine-2,4,6-trione 4-oxime (**8a**) as previously reported.[15] Analogous results were obtained in the IBD oxidations of 6-amino-1(or 3)-methyl-5-nitro-1*H*-pyrimidine-2,4-dione (**1b** or **1c**) and 1,3-unsubstituted 6-amino-5-nitro-1*H*-pyrimidine-2,4-dione (**1d**) leading to the corresponding oxadiazolopyrimidinedione 1-oxides **2b**−**d**. The pretreatment with LiH was required for the smooth conversion of **1a** to **2a** in this synthetic method, i.e., the IBD oxidation of **1a** without the LiH treatment under similar conditions caused a significant delay in the formation of **2a** (the yield of **2a**: 28% after 1 h; 61% after 3 h). The formation of **2a** was also observed by employment of sodium hydride in place of LiH or *N*-halogenosuccinimides in place of IBD. Their efficiency, however, was lower than that of the IBD oxidation assisted with LiH.

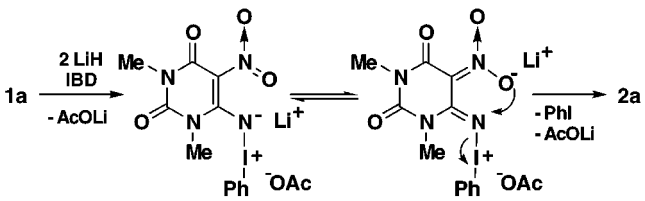


Figure 1.

Taking the chemical reactivities of these oxidants into consideration, a most plausible reaction sequence for the oxidative formation of the oxadiazolopyrimidinedione 1-oxides **2** from the 6-amino-5-nitropyrimidinediones **1** is depicted in Figure 1 for the case of **1a** with IBD.

The reaction is initiated by activation of the C_6-amino group in **1a** with IBD after treatment with LiH to form a *N*-halogenated intermediate. The transient intermediate undergoes a neighboring group participation by the C_5-nitro group assisted by LiH to afford the ultimate product **2a**, accompanied with the release of iodobenzene and lithium acetate.[18] Characteristics of this synthetic method are (a) the use of 6-amino-5-nitro-1*H*-pyrimidine-2,4-diones **1**, which can be prepared from readily available 6-amino-1*H*-pyrimidine-2,4-diones in high yields, as the starting material, (b) the use of IBD which is a commercially available mild oxidant, and (c) the reaction proceeding smoothly even under mild conditions without accompanying undesired side reactions. The present synthetic method was applicable to the syntheses of other oxadiazolopyrimidines, e.g., [1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-diamine 1-oxide (**3**)[14] and 5-amino-6*H*-[1,2,5]-oxadiazolo[3,4-*d*]pyrimidin-7-one 1-oxide (**4**).[14]

Generation of NO and NO-Related Species. Chemical reactivities of the [1,2,5]oxadiazolo[3,4-*d*]pyrimidine 1-oxides (cf. **2**) have been investigated on the behavior toward primary amines,[10,13,15b,19] alcohols,[20] and active methylene compounds[12] under thermal conditions. The most electrophilic site of these heterocyclic *N*-oxides has been consequently demonstrated to be significantly dependent on the nature of substituents on the pyrimidine ring and, in the case of the oxadiazolopyrimidinedione 1-oxides **2**, to be the 3a-position of the fused ring system.[21] The chemical reactivities of **2a** to the primary alkyl- or arylamines were successfully utilized for the syntheses of 8-substituted 1,3-dimethyl-3,9-dihydropurine-2,6-diones (theophyllines)[10] and 1,3-dimethyl-1*H*-benzo[*g*]-pteridine-2,4-dione 5-oxides[13] as described above. The chemical behavior of this compound **2a** toward other

(14) (a) Temple, C., Jr.; Kussner, C. L.; Montgomery, J. A. *J. Org. Chem.* **1968**, *33*, 2086−2089. (b) Nutiu, R.; Boulton, A. J. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1327−1331. (c) Dang, V. T.; Stadlbauer, W. *Molecules* **1996**, *1*, 201−206.

(15) (a) Yoneda, F.; Sakuma, Y.; Ueno, M. *J. Heterocycl. Chem.* **1973**, *10*, 415. (b) Yoneda, F.; Sakuma, Y. *J. Heterocycl. Chem.* **1973**, *10*, 993−996. The 6-hydroxyimino-1*H*-pyrimidine-2,4-diones possess exclusively, so far as can be judged by those spectral data, an oxime form (cf. **8**) in a solution.[14b]

(16) Sako, M.; Oda, S.; Hirota, K.; Beardsley, G. P. *Synthesis* **1997**, 1255−1257.

(17) In fact, benzo[1,2,5]oxadiazoles are prepared by means of the oxidative cyclization of appropriate *o*-aminonitrobenzenes with IBD or alkaline hypochloride. Cf. Boulton, A. J.; Ghosh, P. B. In *Advances in Heterocyclic Chemistry*, Vol. 10; Katritzky, A. R., Boulton, A. J., Eds. Academic Press: New York, 1969; pp 1−41. Gasco, A.; Boulton, A. J. In *Advances in Heterocyclic Chemistry*, Vol. 29; Katritzky, A. R., Boulton, A. J., Eds.; Academic Press: New York, 1981; pp 251−340.

(18) Analogous reaction sequence has been proposed for the oxidative cyclization of *o*-aminonitrobenzenes leading to benzo[1,2,5]-oxadiazoles. Cf. Dyall, L. K.; Kemp, J. E. *Aust. J. Chem.* **1973**, *26*, 1969−1976.

(19) Tennant, G.; Yacomeni, C. W. *J. Chem. Soc., Chem. Commun.* **1982**, 60−62. Tennant, G.; Wallece, G. M. *J. Chem. Soc., Chem. Commun.* **1982**, 267−268.

(20) Remennikov, G. Y.; Pirozhenko, V. V.; Dyachenko, I. A. *Khim. Geterosikl. Soedin.* **1992**, 101−106.

(21) Previously, Yoneda et al. have demonstrated that the initial attack of anilines occurs at the N_1 position of the oxadiazolopyrimidinedione ring after isomerization on the basis of the structural consideration of products, 1,3-dimethyl-1*H*-benzo[*g*]pteridine-2,4-dione 5-oxides.[13] Our recent results of experiments using ^{15}N-labeled compound of **2a** as a starting material have indicated that the formation of the benzopteridinedione 5-oxides also can be reasonably explained by the initial attack of the anilines to the 3a position of this fused ring. These results will be published elsewhere.

nucleophiles such as thiols and hydroxide ion, however, is hitherto unknown.

The *N*-oxide **2a**, being very stable in organic solvents such as acetonitrile, was fairly unstable in aqueous solution to undergo gradually an alkaline hydrolysis to give 4-methylaminofurazan-3-carboxylic acid methylamide 2-oxide (**5**), e.g., the 15% conversion of **2a** leading to **5** was observed after heating in a pH 7.5 phosphate buffer solution at 37 °C for 5 h. In sharp contrast, **2a** was very unstable in the pH 7.5 buffer solution containing two equimolar amounts of *N*-acetylcysteamine to convert into 5-(2-acetylaminoethyl)thio-1,3-dimethylpyrimidine-2,4,6-trione (**6a**) (64%), 5,6-bis[(2-acetylaminoethyl)thio]-1,3-dimethyl-1*H*-pyrimidine-2,4-dione (**7a**) (3%), and 1,3-dimethylpyrimidine-2,4,6-trione 4-oxime (**8a**) (8%), together with the formation of bis(2-acetylaminoethyl) disulfide. No formation of detectable amounts of **5** was observed in the reaction with *N*-acetylcysteamine.[22] The structures of **6a** and **7a** were assigned on the basis of these microanalytical results and spectral data, and that of **8a** was confirmed by spectral comparison with the authentic compound prepared by the reaction of 6-chloro-1,3-dimethyl-1*H*-pyrimidine-2,4-dione with hydroxylamine.[15] The formation of these products led us to expect the generation of NO or *S*-nitroso-*N*-acetylcysteamine during the reaction. Thionitrites, however, have been demonstrated to be unstable to undergo homolytic cleavage of the S−N bond even at ambient temperature and physiological pH to give the corresponding disulfides, accompanied with the generation of NO.[23] Furthermore, the generated NO is very sensitive to air oxidation to convert to NO_2^- as described above. Therefore, the total amounts of NO and NO-related species generated during the reaction of **2a** with *N*-acetylcysteamine were evaluated by the detection of NO_2^-, which was formed after autoxidation of the reaction mixture, using the Griess reagent (see Experimental Section). For example, the evaluated NO_2^- was 28% yield in the 25 μM scale experiment of **2a**. In this reaction, the consumption of **2a** and the generation of NO and NO-related species were significantly dependent on pH in the media and the concentration of the thiol employed as shown in Tables 1 and 2. No formation of NO_2^- was observed in the reaction which was carried out in the absence of *N*-acetylcysteamine, even on exposure of the reaction mixture to air. The reaction using two equimolar amounts of thiols in pH 7.5 buffer solution was most effective for the generation of the NO-related species. Analogous results were obtained by the use of cysteine and glutathione in place of *N*-acetylcysteamine and by the use of **2b**−**d** in place of **2a** as shown in Table 2, although their efficiency in the generation of NO and NO-related species was lower than that in the case of **2a** and *N*-acetylcysteamine. Thus, a substituent in the N_4-position of the *N*-oxides **2** seems to enhance their NO-generating capacity.

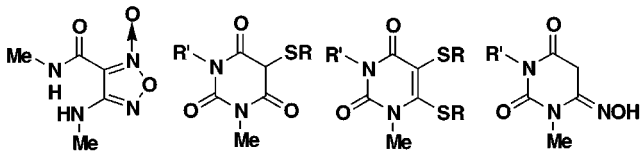


Table 1. pH Dependency in the Reaction of the *N*-Oxide 2a with *N*-Acetylcysteamine[a]

pH	remaining **2a** (%)	**6** (%)	**8** (%)
5.91	73	21	3
6.45	50	41	5
6.98	25	63	7
7.55	17	64	8
8.04	10	70	9

[a] Reaction conditions: **2a** (0.025 mmol) in buffer−MeCN (1:1) (1.5 mL) containing *N*-acetylcysteamine (2 equiv), at 37 °C for 1 h.

On the basis of the above and precedent facts on the chemical reactivities of **2**, reasonable mechanisms for the present reactions resulting in the NO generation are outlined as shown in Figure 2 for the case of **2a**. The attack of thiols to both of the 3a and 7a positions in **2a** and the subsequent ring opening produce the key intermediary adduct **A**. Further attack of another thiol to the *C*-5 nitroso group of **A** releases thionitrite, a NO precursor, together with the formation of **6a**, **7a**, and **8a**.[24]

It should be noted that no formation of the NO or NO-related species was observed in reactions of the deoxygenated compound of **2a** and 6-amino-1,3-dimethyl-5-nitroso(or nitro)-1*H*-pyrimidine-2,4-dione with *N*-acetylcysteamine under similar conditions.

Conclusion

The IBD oxidation of 6-amino-5-nitro-1*H*-pyrimidine-2,4-diones **1** in the presence of LiH afforded the corresponding 4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxides **2** in high yields. The present synthetic method is widely applicable for the preparation of other 4*H*-[1,2,5]-oxadiazolo[3,4-*d*]pyrimidine 1-oxides. The generation of NO and NO-related species from **2a**,**b** in the presence of thiols such as *N*-acetylcysteamine under physiological conditions was strongly supported on the basis of the structural elucidation of the reaction products and other chemical evidence. The present results point out that the 4-substituted 4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxides, e.g., **2a**,**b**, behave as useful NO donors[8] by the action of thiol cofactors.

Experimental Section

Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 75 MHz, respectively, by using DMSO-d_6 (unless otherwise noted) as a solvent and TMS

(22) Previously, Hecht et al. have documented that the deoxygenation of heterocyclic *N*-oxides proceeds under analogous conditions, accompanied with the generation of a hydroxyl radical. However, no formation of detectable amounts of the deoxygenated product of **2a** was observed in this reaction, indicating no generation of hydroxyl radicals under these reaction conditions.

(23) Oae, S.; Shinhama, K. *Org. Prep. Proc. Int.* **1983**, *15*, 167−198. Williams, D. L. H. *Chem. Soc. Rev.* **1985**, *14*, 171−196. Roy, B.; d'Hardemare, A. du M.; Fontecave, M. *J. Org. Chem.* **1994**, *59*, 7019−7026. Williams, D. L. H. *J. Chem. Soc., Chem. Commun.* **1996**, 1085−1091. Beloso, P. H.; Williams, D. L. H. *J. Chem. Soc., Chem. Commun.* **1997**, 89−90.

(24) Analogous reaction sequence has been proposed for the generation of NO from [1,2,5]oxadiazoles in the presence of thiol cofactors. Cf. Medana, C.; Ermondi, G.; Fruttero, R.; Stilo, A. D.; Ferretti, C.; Gasco, A. *J. Med. Chem.* **1994**, *37*, 4412−4416. In sharp contrast to their results, the product **5** was very stable under the conditions employed even in the presence of *N*-acetylcysteamine. See: Sorba, G.; Medana, C.; Fruttero, R.; Cena, C.; Stilo, A. D.; Galli, U.; Gasco, A. *J. Med. Chem.* **1997**, *40*, 463−469 and references therein.

Table 2. Generation of NO and NO-Related Species in the Reaction of the *N*-Oxides 2 with Thiols[a]

N-oxides	thiols	generated NO and NO-related species		
		after 1 h (%)	after 2 h (%)	after 3 h (%)
2a	without	0 (92)[b]	0 (86)	
2a	*N*-acetylcysteamine (1 equiv)	23 (69)		
2a	*N*-acetylcysteamine (2 equiv)	28 (23)	21 (6)	21 (ND)
2a	*N*-acetylcysteamine (3 equiv)	25 (7)	22 (trace)	22 (ND)
2a	cysteine (2 equiv)	4 (91)	3 (87)	
2a	glutathione (2 equiv)	2 (86)	4 (84)	
2b	*N*-acetylcysteamine (2 equiv)	13 (66)		
2c	*N*-acetylcysteamine (2 equiv)	7 (90)		
2d	*N*-acetylcysteamine (2 equiv)	7 (89)		

[a] Reaction conditions: a solution of **2** (0.025 mmol) and an appropriate thiol in 0.1 M phosphate buffer (pH 7.5)−MeCN (1:1) (1.5 mL) was stirred at 37 °C in test tube equipped with balloon containing argon atmosphere. At hourly intervals, TLC analyses were done to determine the consumption of **2** and then quantitative analyses were carried to evaluate nitrite ion generated in these reactions after air treatment of the mixture. [b] Values in parentheses are the yields of **2** that remained in these reactions.

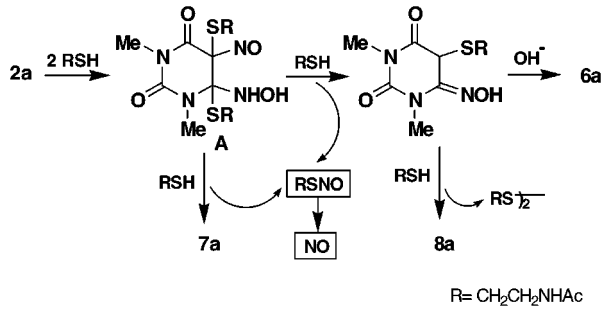


Figure 2.

as an internal standard. Mass spectra were determined at an ionizing voltage of 70 eV. Elemental analyses were performed by the microanalytical laboratory of our university. 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. 7734-5B; silica gel 60). Anhydrous solvents were distilled and stored over activated 4 Å sieves before use. Unless otherwise noted, materials obtained from commercial suppliers were used without further purification. Starting materials, 6-amino-1,3-dimethyl-5-nitro-1*H*-pyrimidine-2,4-dione (**1a**),[25] 6-amino-1(or 3)-methyl-5-nitro-1*H*-pyrimidine-2,4-dione (**1b** or **1c**),[26] 6-amino-5-nitro-1*H*-pyrimidine-2,4-dione (**1d**),[27] 5-nitropyrimidine-2,4,6-triamine,[28] and 2,6-diamino-5-nitro-3*H*-pyrimidin-4-one,[28] were prepared by the nitration of the corresponding 6-aminopyrimidines (Aldrich or Tokyo Kasei; 95−98% purity) in 88−99% yields.

Preparation of 4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-Oxides (2) Using Iodosylbenzene Diacetate; General Procedure. To a stirred suspension of the appropriate 6-amino-5-nitropyrimidinedione (**1**) (1.0 mmol) in dry DMF (3.0 mL) was added LiH (Aldrich, 95% purity) [17.7 mg (2.1 mmol) for **2a**; 26.0 mg (3.1 mmol) for **2b**−**d**] in one portion. After stirring for 0.5 h at ambient temperature, iodosylbenzene diacetate (Aldrich, 98% purity) (483.1 mg, 1.5 mmol) was added to the mixture and the solution was heated with stirring at 50 °C for 1 h. The mixture was decolorized gradually from pale yellow during the reaction. After ensuring complete consumption of the starting 6-amino-5-nitropyrimidinedione **1** by TLC analysis [developing solvent: chloroform−MeOH−acetic acid (40/8/1)], the reaction mixture was diluted with water (50 mL), neutralized with dilute hydrochloride, and extracted with ethyl acetate (3 × 50 mL). The extract was washed with brine (50 mL), dried over anhydrous magnesium sulfate, and evaporated to dryness. After trituration of the residue with chloroform, the resulting precipitate was collected by filtration and washed with chloroform to isolate the corresponding 4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxides (**2**) in almost pure state.

4,6-Dimethyl-4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxide (2a): 95%; mp 243 °C (lit.[10,14b,15] mp 245 °C dec]; mass *m*/*z* (rel intensity) 198 (M^+, 100), 182 ($M^+ - O$, 5), 168 ($M^+ - NO$, 36), 149 (14), 83, 81; IR (KBr) 1740, 1689, 1640 cm^{-1}; UV (MeOH, ϵ) λ_{max} 312 (2.1×10^3), 268 (1.7×10^4) nm; ^1H NMR δ 3.49 (3H, s), 3.57 (3H, s); ^{13}C NMR δ 151.9, 151.8, 149.6, 101.2, 29.4, 28.1. This product was identical in every respect with the authentic compound prepared independently by the reaction of 6-chloro-1,3-dimethyl-1*H*-pyrimidine-2,4-dione with hydroxylamine and subsequent nitrosation.[10,14b,15]

4-Methyl-4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxide (2b): 72%; mp 203 °C; mass *m*/*z* (rel intensity) 184 (M^+, 100), 168 ($M^+ - O$, 8), 154 ($M^+ - NO$, 44), 149 (9) 81 (69); IR (KBr) 3160, 1741, 1688, 1642 cm^{-1}; UV (MeOH, ϵ) λ_{max} 311 (1.5×10^3), 266 (1.4×10^4) nm; ^1H NMR δ 3.41 (3H, s), 12.1 (1H, br s); ^{13}C NMR δ 153.2, 151.9, 149.5, 101.6, 28.3. Anal. Calcd for $C_5H_4N_4O_4$: C, 32.62; H, 2.19; N, 30.43. Found: C, 32.60; H, 2.27; N, 30.35.

6-Methyl-4*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxide (2c): 85%; mp 212 °C (lit.[10,14b,15a] mp 210−211 °C); mass *m*/*z* (rel intensity) 184 (M^+, 100), 168 ($M^+ - O$, 26), 154 ($M^+ - NO$, 36); IR (KBr) 3108, 1738, 1680, 1639 cm^{-1}; UV (MeOH, ϵ) λ_{max} 302 (1.5×10^3), 266 (1.2×10^4) nm; ^1H NMR δ 3.23 (3H, s), 12.8 (1H, br s); ^{13}C NMR δ 152.4, 150.8, 149.7, 100.8, 27.4.

4*H*-[1,2,5]Oxadiazolo[3,4-*d*]pyrimidine-5,7-dione 1-oxide (2d): 97%; mp 257 °C (lit.[14a] mp 260 °C dec); mass *m*/*z* (rel intensity) 170 (M^+, 100), 154 ($M^+ - O$, 13), 140 ($M^+ - NO$, 70), 127 (2) 111 (3); IR (KBr) 3305, 3190, 3100, 1743 (sh), 1714, 1643 cm^{-1}; UV (MeOH, ϵ) λ_{max} 302 (2.0×10^3), 264 (1.6×10^4) nm; ^1H NMR δ 11.7 (1H, br), 12.3 (1H, br); ^{13}C NMR δ 152.5, 152.3, 149.8, 101.2.

In an analogous manner, 5-amino[1,2,5]oxadiazolo[3,4-*d*]-pyrimidine 1-oxides **3** and **4** were prepared by using appropriate 2,6-diamino-5-nitropyrimidines as starting materials.

[1,2,5]Oxadiazolo[3,4-*d*]pyrimidine-5,7-diamine 1-oxide (3): 57%; mp >300 °C (lit.[14a] mp >264 °C); mass *m*/*z* (rel intensity) 168 (M^+, 100), 152 ($M^+ - O$, 13), 138 ($M^+ - NO$, 7), 122 (19); IR (KBr) 3428, 3163, 1640 cm^{-1}; UV (MeOH, ϵ) λ_{max} 371 (2.6×10^3), 290 (1.3×10^4), 230 (1.4×10^4) nm; ^1H NMR δ 8.58 (1H, br), 7.67 (1H, br), 7.15 (1H, br), 7.07 (1H, br); ^{13}C NMR δ 164.5, 162,2, 152.3, 98.7.

5-Amino-6*H*-[1,2,5]oxadiazolo[3,4-*d*]pyrimidin-7-one 1-oxide (4): 71%; mp >300 °C (lit.[14a] mp >264 °C); mass *m*/*z* (rel intensity) 169 (M^+, 100), 153 ($M^+ - O$, 8), 139 ($M^+ - NO$, 33), 123 (33); IR (KBr) 3367, 1725, 1631 cm^{-1}; UV (MeOH, ϵ) λ_{max} 329 (2.0×10^3), 275 (1.0×10^4) 227 (2.0×10^4) nm; ^1H NMR δ 11.3 (1H, br), 7.5 (1H, br), 6.7 (1H, br); ^{13}C NMR δ 160.2, 155.2, 152,7, 101.1.

In the cases of **2b**, **3**, and **4**, small amounts of the corresponding starting materials were recovered under the conditions described above, respectively.

(25) Taylor, E. C.; McKillop, A. *J. Org. Chem*. **1965**, *30*, 3153−3155.
(26) Pfleiderer, W.; Hutzenlaub, W. *Chem. Ber*. **1973**, *106*, 3149−3174.
(27) Bitterli, P.; Erlenmeyer, H. *Helv. Chim. Acta* **1951**, *34*, 835−840.
(28) Carbon, J. A. *J. Org. Chem*. **1961**, *26*, 455−461.

The oxidation of **1a** (12.5 mg, 0.063 mmol) with IBD (30.6 mg, 0.093 mmol), which was carried out without the LiH treatment or after treatment with sodium hydride in place of LiH, was followed by TLC densitometry [chloroform−MeOH (20/1)] at hourly intervals. The yields of **2a** in these reactions were as follows: 28% after 1 h, 51% after 2 h, and 61% after 3 h in the former case; 89% after 1 h in the latter case, accompanied with recovery of the starting **1a**.

Oxidation of the 6-Amino-5-nitropyrimidinedione 1d with *N*-Halogenosuccinimides. Addition of *N*-bromosuccinimide (Aldrich; 95% purity) (11.6 mg, 0.062 mmol) to the solution of **1d** (86.0 mg, 0.05 mmol) in dry DMF (1.0 mL) containing LiH (1.3 mg, 0.15 mmol) and subsequent treatment of the mixture at 50 °C for 1 h with stirring afforded **2d** in 19% yield (by TLC densitometry) after 1 h, with recovery of **1d**. Employment of *N*-iodosuccinimide (NIS) or *N*-chlorosuccinimide (NCS) in place of NBS in this reaction did not effect increase in the formation of **1d**, i.e., the yields of **2d** were 5% in the case of NIS and 7% in the case of NCS.

Alkaline Hydrolysis of 2a. A solution of **2a** (198 mg, 1.0 mmol) in acetonitrile (12 mL) containing 1 N NaOH (5 mL) was stirred at ambient temperature for 0.5 h. After neutralization with diluted hydrochloride followed by concentration under reduced pressure, the resulting precipitate was collected by suction and was recrystallized from acetone to give 4-methylaminofurazan-3-carboxylic acid methylamide 2-oxide (**5**) (126 mg, 73%): mp 279 °C; mass *m*/*z* (rel intensity) 172 (M^+, 100), 155 ($M^+ - OH$, 67), 142 ($M^+ - NO$, 87), 126 (8) 83 (19); IR (KBr) 3236, 1749, 1647 cm^{-1}; UV (MeOH) λ_{max} 287, 250 nm; ^1H NMR δ 3.01 (3H, s), 3.49 (3H, s), 11.02 (1H, br s), 11.34 (1H, br s). Anal. Calcd for $C_5H_8N_4O_3$: C, 34.89; H, 4.68; N, 32.55. Found: C, 34.91; H, 4.62; N, 32.45. The reaction was carried out in the acetonitrile solution containing 0.1 M pH 7.0 (or pH 7.5) phosphate buffer and was followed by TLC densitometry. The analyses of the reaction mixtures showed the formation of **5** in 8% (at pH 7.0) and 15% (at pH 7.5) yields, respectively, with recovery of **2a** after stirring at 37 °C for 5 h.

Reactions of 2a and 2b with *N*-Acetylcysteamine. A solution of **2a** (99.1 mg, 0.5 mmol) in 0.1 M K_2HPO_4−KH_2PO_4 buffer (pH 7.5)−acetonitrile (1/1) (10.0 mL) containing *N*-acetylcysteamine (Aldrich, 95% purity) (112.0 μL, 1.0 mmol) was stirred at 37 °C under argon for 1 h. After removal of the solvent under reduced pressure, the resulting residue was subjected to column chromatography and was eluted with chloroform−MeOH (10/1 to 4/1) to separate 5-(2-acetylaminoethyl)thio-1,3-dimethylpyrimidine-2,4,6-trione (**6a**) [99.6 mg (64% as a potassium salt); mp >300 °C; mass *m*/*z* (rel intensity) 213 ($M^+ - AcNH_2 - 1$, 16), 156 ($M^+ - AcNHCH_2CH_2S + 1$, 8), 86 (18), 44 (100); IR (KBr) 3388, 1676, 1652, 1562 cm^{-1}; UV (MeOH, ϵ) λ_{max} 260 (1.7×10^4) nm; ^1H NMR δ 1.81 (3H, s), 2.34 (2H, t), 3.08 (2H, q), 3.10 (6H, s), 8.48 (1H, br t); ^{13}C NMR δ 168.7, 163.9, 152.6 (2C), 77.0, 38.1, 35.6, 27.7 (2C), 22.7. Anal. Calcd for $C_{10}H_{14}N_3O_4SK$: C, 38.57; H, 4.53; N, 13.49. Found: C, 38.44; H, 4.49; N, 13.65], 5,6-bis[(2-acetylaminoethyl)thio]-1,3-dimethyl-1*H*-pyrimidine-2,4-dione (**7a**) [5.8 mg (3%); mp 140−141 °C; mass *m*/*z* (rel intensity) 374 (M^+, 7), 315 ($M^+ - AcNH_2$, 8), 86 (100); IR (KBr) 3281, 1699, 1642 cm^{-1}; UV (MeOH, ϵ) λ_{max} 300 (1.5×10^4) nm; ^1H NMR ($CDCl_3$) δ 2.00 (3H, s), 2.02 (3H, s), 2.96 (2H, t), 3.18 (2H, t), 3.39 (3H, s), 3.40 (2H, q), 3.48 (2H, q), 3.73 (3H, s), 6.42 (1H, br t), 6.82 (1H, br t). Anal. Calcd for $C_{14}H_{22}N_4O_4S_2$: C, 44.90; H, 5.92; N, 14.96. Found: C, 44.74; H, 5.92; N, 14.79], 1,3-dimethylpyrimidine-2,4,6-trione 4-oxime (**8a**) (6.4 mg, 8%), and bis(2-acetylaminoethyl) disulfide (34.3 mg, 27%), together with recovered **2a** (16.8 mg, 17%) and *N*-acetylcysteamine (40.9 mg, 34%). The product **8a** was identical in every respect with the authentic compound prepared independently by the reaction of 6-chloro-1,3-dimethyl-1*H*-pyrimidine-2,4-dione with hydroxylamine.

Under analogous conditions, the reaction of **2b** (18.4 mg, 0.1 mmol) with *N*-acetylcysteamine (21.3 μL, 0.2 mmol) was carried out to give 5-(2-acetylaminoethyl)thio-1-methylpyrimidine-2,4,6-trione (**6b**) [20.9 mg (70% as a potassium salt); mp >300 °C; mass *m*/*z* (rel intensity) 259 ($M^+ - 1$, 1), 200 ($M^+ - AcNH_2$, 2), 142 ($M^+ - AcNHCH_2CH_2S + 1$, 95), 118 (47), 86 (100); IR (KBr) 3424, 1686, 1637, 1562 cm^{-1}; UV (MeOH, ϵ) λ_{max} 259 (1.9×10^4) nm; ^1H NMR δ 1.81 (3H, s), 2.32 (2H, t), 3.03 (3H, s), 3.08 (2H, q), 8.56 (1H, br t), 9.53 (1H, br s); ^{13}C NMR δ 168.5, 165.2, 164.5, 152.0, 76.4, 37.9, 35.6, 26.8, 22.7] and 5,6-bis[(2-acetylaminoethyl)thio-1-methyl-1*H*-pyrimidine-2,4-dione (**7b**) [4.1 mg (11%); mass *m*/*z* (rel intensity) 360 (M^+, 7), 301 ($M^+ - AcNH_2$, 8), 86 (100); IR (KBr) 3308, 1676, 1645 cm^{-1}; UV (MeOH) λ_{max} 302 nm; ^1H NMR ($CDCl_3$) δ 2.01 (3H, s), 2.02 (3H, s), 2.98 (2H, t), 3.21 (2H, t), 3.41 (2H, q), 3.50 (2H, q), 3.69 (3H, s), 6.37 (1H, br t), 6.69 (1H, br t), 8.89 (1H, br). Anal. Calcd for $C_{14}H_{22}N_4O_4S_2$: *m*/*z* 360.0938. Found: *m*/*z* 360.0932], accompanying with bis(2-acetylaminoethyl) disulfide.

Quantitative Analyses of Nitrite Ion. The appropriate oxadiazolopyrimidinedione **2** (0.025 mmol) was added to a mixed solution of 0.1 M phosphate buffer (pH 5.91−8.04) and acetonitrile (1:1) (1.5 mL) containing the appropriate thiol (*N*-acetylcysteamine, cysteine, or glutathione) or without, with stirring. After continuation of stirring at 37 °C for 1 h in a test tube equipped with an argon balloon followed by air treatment for 10 min, 10 μL of the reaction mixture was diluted with 1.0 mL of water and was treated with 100 μL of the Griess reagent [sulfanilamide (4 g), *N*-naphthylenediamine dihydrochloride (0.2 g), and 85% phosphoric acid (10 mL) in distilled water (final volume: 100 mL)] for 10 min at room temperature. After dilution of the solution with water (3.1 mL), the absorbance was measured at 540 nm; 2−10 nmol/mL sodium nitrite standard solutions were used for the calibration curve. The results estimated were shown in Table 2.

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research (C) (No. 08672564) from the Ministry of Education, Science, Sports and Culture in Japan.

Supporting Information Available: Spectral data and spectra for compounds prepared (12 pages). This material is contained in 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.

JO980732Y