Chemistry of *N*-Hydroxyguanidines: Photo-Sensitized Oxygenation and Reaction with Nitric Oxide

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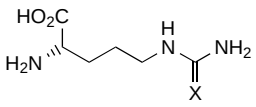
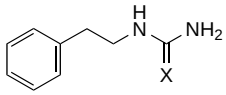
The chemistry of *N*-hydroxyguanidines, focusing on photo-sensitized oxygenation and reaction with nitric oxide (NO), was examined. In the former reaction, a urea derivative was obtained as the main product together with the generation of NO. On the other hand, in the latter case, oxidative nitrosation occurred under aerobic conditions to give a nitrosourea derivative, which was also obtained from urea by the same treatment.

1. Introduction. – It is well-known that nitric oxide (NO) *in vivo* plays important roles in the cardiovascular and central and peripheral nervous systems, and modulates immunological responses [1], in spite of its being one of the air-polluting nitrogen oxides. NO is biosynthesized during the conversion of L-arginine (**1**) to L-citrulline (**3**) through *N*-hydroxy-L-arginine (NOHA; **2**) [2]. We have examined the photo-sensitized oxygenation of *N*-hydroxyguanidine derivatives as a chemical model for the biological NO generation and preliminarily reported that 2-hydroxy-1-(2-phenylethyl)guanidine (**4**) was photochemically oxidized to produce (2-phenylethyl)urea (**5**) and (2-phenylethyl)cyanamide (**6**) with the evolution of NO [3].

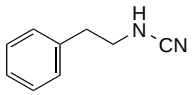
On the other hand, *Zembowicz et al.* [4] proposed that an alternative active principle, derived from a further reaction between NOHA (**2**) and NO, was possibly responsible for biological activity based on the observation that a product resulting



1 X=NH
2 X=NOH
3 X=O



4 X=NOH
5 X=O
7 X=NH



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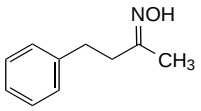

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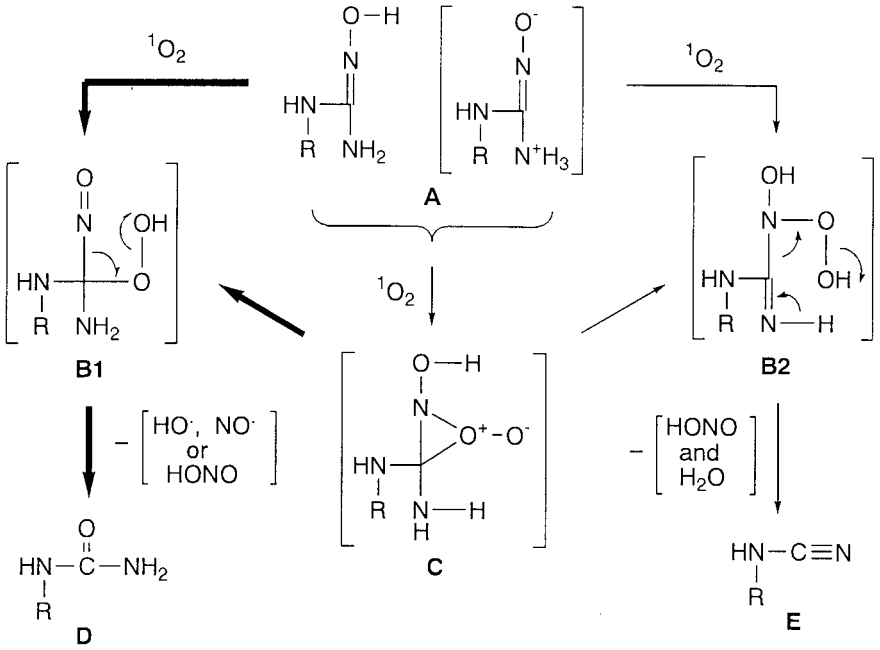
from reaction of hydroxyguanidine with HONO had caused vasorelaxation. However, our trials for the reaction of **4** with aqueous HONO according to the reported procedure [4] resulted only in formation of a complex mixture[1]). Instead, reaction with NO gas in place of HONO under aerobic conditions led unexpectedly to the production of an *N*-nitrosourea derivative **12** (see the *Table*).

In this paper, we present further photo-sensitized oxygenation with the NOHA derivative **9** as a substrate, in addition to experimental details of the preliminary reactions of 2-hydroxy-1-(2-phenylethyl)guanidine **4**, and the results of reactions of **4** and its related compounds with NO gas.

2. Results and Discussion. – 2.1. *Photo-Sensitized Oxygenation*. – The singlet-oxygen ($^1O_2$) ene reaction [6] has been shown to be a powerful method for allylic oxyfunctionalization of cyclic and acyclic olefin systems. We assumed that, when a 2-hydroxyguanidine was used as a substrate in the $^1O_2$ ene reaction, the oxime bond (C=N–OH) could act as an ene to give a nitrosohydroperoxide (like **B1** in *Scheme 1*), structurally related to a peroxyheme complex [7], which may spontaneously be degraded to a urea derivative with the evolution of NO or its equivalent. As stated in a preliminary communication [3], the photo-sensitized oxygenation of 2-hydroxy-1-(2-phenylethyl)guanidine (**4**) in AcOEt for 1 h with a high-pressure Hg lamp (400 W) as a

Scheme 1. *Proposed Mechanisms for the Photo-Sensitized Oxygenation of 2-Hydroxyguanidines*



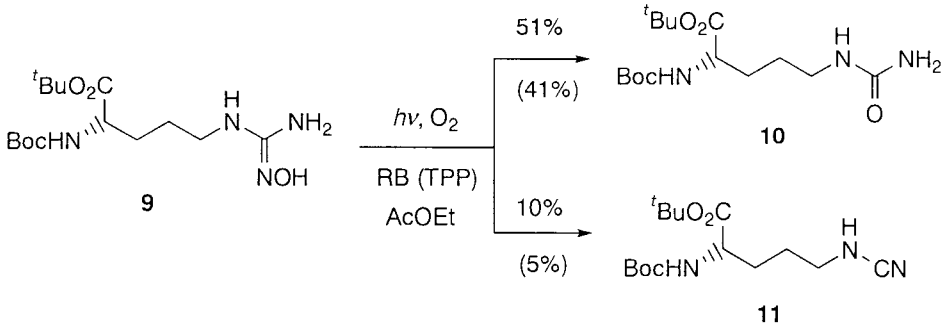
[1]) During our study, *Southan et al.* [5] reported the successful isolation of zwitterionic diazenium diolates as reaction products between hydroxyguanidines and HONO, supporting *Zembowicz*'s proposal [4].

light source through a *Pyrex* filter in the presence of a catalytic amount of rose bengal (RB) as a sensitizer with bubbling O_2 under water-cooling smoothly gave the expected (2-phenylethyl)urea (**5**) in 58% isolated yield together with (2-phenylethyl)cyanamide (**6**) as a minor oxidation product (4% yield). Nearly the same product distribution had been observed when tetraphenylporphine (TPP) was used instead of RB as a sensitizer.

The failure of photo-oxygenation with (2-phenylethyl)guanidine (**7**) or 4-phenylbutan-2-one oxime (**8**) indicated that the presence of a whole 2-hydroxyguanidine function [$RNHC(NH_2)=NOH$], not the limited functionalities such as C=N–OH or N=C–NH, was essential for the photo-oxidative production of not only a urea but also a cyanamide. Analysis of the reaction mixture obtained in the RB-sensitized photoreaction of 2-hydroxy-1-(2-phenylethyl)guanidine (**4**) by application of the *Griess* reaction [8] after irradiation for 1.5 h, followed by extraction with H_2O, led to identification of NO_2^- and NO_3^-, albeit in low 4 and 9% yields, respectively, strongly indicating that NO should be generated during the reaction. Based on these experimental observations, we proposed a plausible mechanism for the photo-oxygenation of 2-hydroxyguanidines **A** to yield ureas **D** and cyanamides **E** with evolution of NO or its equivalent through *C*- and *N*-hydroperoxides **B1** and **B2** (or endoperoxides **C**) as shown in *Scheme 1*.

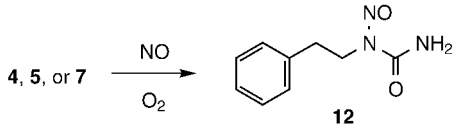
To further confirm the photoreactivity of an 2-hydroxyguanidyl function, we next examined the photoreaction with *tert*-butyl N^{α}-Boc-N^{ω}-hydroxy-L-arginate (**9**), an *N*-Boc-protected NOHA ester, which was prepared according to the method described in [9], with some modifications [10], as a more structurally-closed model substrate for the biological oxidation of NOHA (**2**). The photo-irradiation of the protected NOHA **9** for 2 h in the presence of RB under the same conditions mentioned above, as expected, afforded the corresponding urea **10** and cyanamide **11** in 51 and 10% isolated yields, respectively (*Scheme 2*). The use of TPP in place of RB as a sensitizer also led to effective formation of both products (**10**: 41%; **11**: 5%). Thus, it was clear that the C=N–OH group of an 2-hydroxyguanidine function was smoothly cleaved by an activated O_2[2]) to effectively yield a urea derivative as a main product.

Scheme 2. *The Photo-Sensitized Reaction of NOHA Derivative* **9**



[2]) During our study, the chemical oxidation of 2-hydroxy-1-arylguanidines with a superoxide was reported [11]. However, no mechanistic consideration was given.

Table. *Reactions of 2-Hydroxy-1-(2-phenylethyl)guanidine* (**4**), *(2-Phenylethyl)urea* (**5**), *and 1-(2-Phenylethyl)guanidine* (**7**) *with NO under Various Conditions*



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Entry	Substrate	Time [h]	Workup[a])	Product
1[b])	**4**	3	*A*	**12**: 64%
2[c])	**4**	4	*B*	**12**: 36%
3[c])	**4**	0.3	*C*	Recovery of **4**
4[b])	**5**	0.8	*B*	**12**: 100%
5[c])	**5**	5.7	*B*	**12**: 100%
6[b])	**7**	7.6	*B*	**12**: 9%

[a]) *A:* Evaporation after washing with H_2O, *B:* passing Ar before treatment of *A*, *C:* evaporation after passing Ar without washing with H_2O. [b]) Commercially available NO gas was used. [c]) NO gas was purified through 30% aq. NaOH soln.

2.2. *Reactions with NO.* It is known that some N-containing compounds such as amines[3]) react with NO to give various types of product depending on the conditions used. *N*-Nitrosation has occurred in the cases of amides [15]; however, there have been no reports on 2-hydroxyguanidines. We tried reactions of 2-hydroxy-1-(2-phenylethyl)guanidine (**4**) and its related compounds with NO for chemical approaches (*Table*) to the possible production of the vasorelaxation-active principle [4] in the reaction of NOHA (**2**) with NO *in vivo*.

An ice-cooled solution of **4** in AcOEt was saturated with NO through bubbling commercially available NO gas for 5 min after passing Ar for 10 min, and then the mixture was stirred for 3 h under ice-cooling. After removal of the excess NO by passing Ar (10 min), the solution was washed with H_2O, dried, and evaporated. An *N*-nitrosated urea **12** was obtained in 64% yield as a crystalline product (*Entry 1*). Its structure was confirmed by spectroscopic means, including ^1H,^{15}N-HMQC and ^1H,^{15}N-HMBC experiments. In the HMQC experiment, cross-peaks due to an NH_2 group appeared between a N-atom at δ −296 ppm and two exchangeable H-atoms (δ 7.80 and 8.12 ppm). In the HMBC experiment, cross-peaks between a N-atom at δ −106 ppm and two methylene H-atoms (δ 2.62 and 3.94 ppm) were observed through three- and two-bonds correlation. Among them, the CH_2 signal at lower field additionally showed a cross-peak between a N-atom at δ 184 ppm through three-bonds correlation, indicating the presence of a partial structure of $CH_2CH_2N(N)CNH_2$ in its molecule.

We carried out the reaction with NO gas purified by passing through 30% aqueous NaOH solution under the same conditions mentioned above (*Entry 2*). The same **12** was obtained in a lower 36% yield. On tracing this reaction, the appearance of a new

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[3]) Aromatic primary amines were either reductively deaminated or diazotized by NO in the absence [12] or in the presence [13] of O_2, respectively, whereas aromatic secondary amines afforded *N*-nitroso derivatives under aerobic conditions [13]. Recently, it was reported that Li salts of secondary amines could be reacted with NO to give *N*-nitroso derivatives [14].

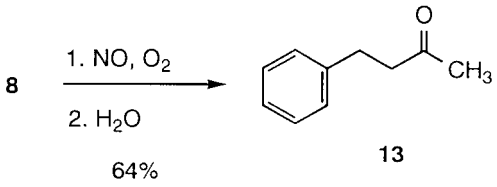
spot on TLC was noted after disappearance of the starting **4** (*ca.* 15 min). The intermediate was different from the urea derivative **5**, an expected precursor for **12** (*vide infra*); however, it was converted to **12** after further reaction for 2 h. Attempts at isolation of the intermediate after a short reaction (20 min) by bubbling Ar into the reaction mixture, followed by evaporation of the solvent without washing with H_2O, resulted in the recovery of the starting **4** (*Entry 3*). On the other hand, an *N*-nitrosourea **12** was produced in the case of omission of the Ar treatment in the above reaction, whereas decomposition of the reaction products was observed when the reaction mixture was bubbled with O_2 after the Ar treatment. Furthermore, the starting **4** was found to be quite stable under the conditions in the absence of NO. These results allowed us to interpret the reaction of **4** with NO as follows: *1*) the intermediate may be in equilibrium with the starting 2-hydroxyguanidine **4** under anaerobic conditions, and *2*) O_2, even in a catalytic amount, in addition to NO should be needed for the conversion of the intermediate to an *N*-nitrosourea **12** (*Scheme 3*).

Scheme 3. *Reaction of 2-Hydroxy-1-(2-phenylethyl)guanidine* (**4**) *with NO*

Next, we examined the reaction using (2-phenylethyl)urea (**5**) as a starting substrate under the conditions described in *Entries 1* and *2* (*Entries 4* and *5*, respectively). In both reactions, a nitrosated product **12** was, as expected, formed in quantitative yields; however, a longer reaction time (5.7 h) was needed when degassing was performed with purified NO (*Entry 5*), indicating that O_2 dissolved in the reaction system plays an important role for this nitrosation reaction. On the other hand, the reaction of 1-(2-phenylethyl)guanidine (**7**) with NO under the conditions of *Entry 1* resulted in low conversion to the nitrosourea **12** (9%) (*Entry 6*).

In the case of the oxime **8**, the corresponding ketone **13** was smoothly obtained in 64% yield (*Scheme 4*). Thus, it was found that an *N*-hydroxy imine (oxime) function is susceptible to being converted to the corresponding carbonyl function by NO gas under aerobic conditions.

Scheme 4. *Reaction of 4-Phenylbutan-2-one Oxime* (**8**) *with NO under Aerobic Conditions*



It is known that NO is a less-reactive nitrosation agent than other nitrogen oxides such as NO_2, N_2O_3, and N_2O_4, among which NO_2 is easily generated from NO with O_2 [16]. Therefore, it could be reasonably assumed that NO_2 is the actual nitrosating

agent[4]) because O_2 dissolved in the solvent clearly plays an important role in these reactions mentioned above.

3. Conclusions. – We examined two types of reactions of 2-hydroxyguanidines, photo-sensitized oxygenation and reaction with NO gas, as chemical models for biological NO generation from NOHA (**2**) and for chemical approaches to the possible production of an alternative, biologically active principle by the reaction of **2** with NO. The former photo-sensitized oxygenations are often used as chemical mimics for oxygenase-catalyzed biological oxidations (see, *e.g.*, [18]). The smooth conversion of an 2-hydroxyguanidine to urea and NO (or its equivalent) in the photo-sensitized O_2 oxidation may allow us to consider the oxygenation reaction as a possible chemical model[5]) for the NOS-catalyzed oxidation of NOHA (**2**) to citrulline (**3**).

In the latter reaction, an 2-hydroxyguanidyl residue is oxidatively nitrosated with NO gas under aerobic conditions to give an *N*-nitrosourea function, which can be smoothly obtained by treatment of a urea with NO under the same conditions. However, detection of a new spot on TLC, but not that of urea during the reaction, suggested that this oxidative nitrosation is not composed of simple stepwise reactions of oxidation of the hydroxy imine function to a C=O function, followed by *N*-nitrosation of the formed ureido function, or even smooth conversion of an oxime group into the corresponding ketone function by action of NO. Biological tests of vasorelaxation on *N*-nitrosourea derivatives are planned to be performed in the future.

Experimental Part

General. Reactions were monitored by TLC on *Kieselgel 60 F254* (*Merck*, 5715). Column chromatography (CC): silica gel (*Fuji Silysia*, *FL100D*). In the photoreaction, *Toshiba HLS-4002* (400 W) was used as a light source. Both ions of NO_2^- and NO_3^- were analyzed by *TCI-NOX 5000S* (*Tokyo Kasei Kogyo Co.*, Ltd). M.p.: a micro melting-point hot stage (*Yanagimoto*), uncorrected. IR Spectra: *JASCO FT/IR-300E* spectrophotometer. ^{1}H-, ^{13}C-, and ^{15}N-NMR spectra: in $CDCl_3$ on a *JEOL JNM-LA500*; in the former two cases, TMS (0.00 ppm) and the middle resonance of $CDCl_3$ (77.0 ppm) were used as an internal standard, respectively, whereas ^{15}N chemical shifts were referenced to 0 ppm for $MeNO_2$ as an external standard. EI-MS (MS) and HR-FAB-MS (HR-MS): *JEOL Automass 20* and *JMS-HX110* with *m*-nitrobenzyl alcohol as a matrix, respectively.

(2-Phenylethyl)cyanamide (**6**). A soln. of bromocyan (95%, 1.84 g, 16.5 mM) in dry MeOH (6 ml) was added to a mixture of 2-phenylethylamine (1.95 g, 16.1 mM) and AcONa (1.65 g, 20.2 mM) in dry MeOH (12 ml) at $-10°$, and the whole was stirred at the same temp. for 1 h. After evaporation of MeOH, the residue was partitioned with AcOEt and H_2O. The org. soln. was washed with H_2O and brine, dried (K_2CO_3), and evaporated to give **6** (1.74 g, 74%) as a yellow oil, which was used in the next reaction without further purification. IR (neat): 3404, 2224. ^{1}H-NMR: 2.89 (*t*, *J* = 6.9, CH_2(2)); 3.30 (*q*, *J* = 6.9, CH_2(1)); 3.99 (br. *s*, NH); 7.21 (*d*, *J* = 7.0, H–C(2′)); 7.25 (*t*, *J* = 7.4, H–C(4′)); 7.32 (*t*, *J* = 7.0, H–C(3′)). MS: 146 (53, M^+).

2-Hydroxy-1-(2-phenylethyl)guanidine (**4**). A mixture of **6** (0.805 g, 5.51 mM), $NH_2OH \cdot HCl$ (0.460 g, 6.61 mM), and Et_3N (1 ml, 7.17 mM) in EtOH (6.4 ml) was stirred at r.t. for 3.5 h. After evaporation of EtOH, the residue was adjusted to pH 8–9 with sat. aq. Na_2CO_3 soln. and extracted with AcOEt. After workup, recrystallization of the crude product with AcOEt afforded **4** (0.435 g, 44%). Colorless powder. M.p. 106–110. IR (nujol): 3418, 3354. ^{1}H-NMR (CD_3OD): 2.80 (*t*, *J* = 7.2, CH_2(2)); 3.26 (*t*, *J* = 7.2, CH_2(1)); 7.15–7.30

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[4]) During our study, *Collet et al.* [17] reported the decarbamoylation of monoalkylurea derivatives with NO and O_2 under aqueous conditions, in which *N*-nitroso derivatives were supposed to be key intermediates.

[5]) During preparation of this paper, *Oecal* and *Erden* [19] reported the successful oxygenation of the C=N bond in amidoximates by $^1O_2$. Unfortunately, they had not seen our paper [3].

(*m*, ArH). ^{13}C-NMR (CD_3OD): 36.9; 43.7; 127.3; 129.5; 129.8; 140.7; 158.9. HR-MS: 180.1156 ($C_9H_{14}N_3O^+$; calc. 180.1137). Anal. calc. for $C_9H_{14}N_3O$: C 60.32, H 7.31, N 23.45; found: C 60.08, H 7.24, N 23.38.

Photo-Sensitized Oxygenation of **4**: *Isolation of (2-Phenylethyl)urea* (**5**) *and (2-Phenylethyl)cyanamide* (**6**). A mixture of RB (2.4 mg) in AcOEt (100 ml) was stirred at 75° (bath temp.) for 1 h. Compound **4** (0.018 g, 0.1 mM) was added to the supernatant soln. (6 ml, RB < 0.018 mol/l) prepared above, and the whole was stirred at 75° for 1 h. After cooling, a part of the mixture (5 ml) was subjected to photo-irradiation for 1 h through a *Pyrex* filter under ice-cooling. During the photoreaction, O_2 was bubbled into the reaction mixture. After evaporation of the solvent, the residue was dissolved in excess MeOH, and *Norit* (0.014 g) was added. The mixture was stirred at r.t. for 10 min, and the insoluble materials were filtered off. After evaporation of the filtrate, purification of the residue by prep. TLC ($CHCl_3$/MeOH 10:1) afforded **5** (R_f 0.45; 0.008 g, 58%). The less polar product ($R_f > 0.45$, 0.0041 g) was further purified by prep. TLC (hexane/AcOEt 2:1) to give **6** (0.0005 g, 4%), which was identified with authentic sample prepared above.

Photo-Sensitized Oxygenation of **4**: *Identification of NO by the* Griess *Reagent*. The same photoreaction described above with **4** (0.012 g, 0.07 mM) was carried out for 1.5 h, and the mixture was extracted twice with H_2O. Each aq. soln. was analyzed with the *Griess* reagent, a soln. of sulfanilamide and *N*-(naphthalen-1-yl)ethylenediamine in HCl, after passing through a Cd–Cu column. The first extract showed the presence of NO_2^- (1.955 μmol) and NO_3^- (5.750 μmol), and the second extract the presence of NO_2^- (0.465 μmol) and NO_3^- (0.265 mmol). Thus, total nitrogen oxide ions was 8.43 μmol.

tert-*Butyl* N$^{\alpha}$-*Boc*-L-*citrullinate* (**10**). A mixture of $TsOH \cdot H_2O$ (0.0069 g, 0.0036 mM) in dry toluene (5 ml) was refluxed in a *Dean-Stark* apparatus. To the soln. were added a soln. of *tert*-butyl N$^{\alpha}$-Boc-L-ornithinate [9] (0.0915 g, 0.32 mM) in toluene (2.5 ml) and then a soln. of Me_3SiNCO in dry toluene (0.2 ml, 0.52 mM as Me_3SiNCO), which was prepared from Me_3SiNCO (0.35 ml, 2.6 mM) and toluene (1.0 ml). The soln. was stirred at r.t. for 4.8 h and extracted with AcOEt after addition of sat. $NaHCO_3$ (pH 9). The org. soln. was washed with brine, dried (K_2CO_3), and evaporated to dryness. Purification of the residual oil by CC ($CHCl_3$/MeOH 25:1) afforded **10** (0.0892 g, 85%). Yellow oil. IR (neat): 1679. ^{1}H-NMR (CD_3OD): 1.44 (*s*, *t*-Bu); 1.47 (*s*, *t*-Bu); 1.56–1.69 (br., CH_2(3)); 1.70–1.87 (*m*, CH_2(4)); 3.20 (*t*, *J* = 5.9, CH_2(5)); 4.14 (br., H–C(2)); 4.62 (*s*, $CONH_2$); 5.26 (*s*, 2 CONH). ^{13}C-NMR: 25.64; 28.0; 28.4; 30.7; 40.1; 53.5; 80.0; 82.2; 155.76, 159.0, 171.8. HR-MS: 322.2180 ($C_{15}H_{29}N_3O_5^+$; calc. 332.2180).

Photo-Sensitized Oxygenation of the Protected NOHA **9**. A mixture of RB (2.1 mg) in AcOEt (100 ml) was stirred at 75° (bath temp.) for 1 h and filtered. Compound **9** (0.0193 g, 0.06 mM), which was prepared according to the method described in [9] with some modification [10], was dissolved in the filtrate (6 ml) with heating at 75° (bath temp.). A part of the soln. (5 ml) was photo-irradiated for 2 h under the conditions described above and worked up. Purification of crude products by prep. TLC ($CHCl_3$/MeOH 10:1) afforded the urea **10** (0.008 g, 51%) as a more-polar product (R_f 0.30). Further purification of the less-polar fraction (R_f 0.66) by prep. TLC (hexane/AcOEt 2:1) gave the cyanamide **11** (0.002 g, 10%). These products were identified with authentic samples.

Reaction with NO. A soln. of the starting material in AcOEt was saturated with NO by bubbling for 5 min under ice-cooling after pre-bubbling with Ar for 10–15 min. The NO-saturated soln. was stirred for an appropriate time under the same conditions, and Ar was passed through for 20 min in order to expel the remaining NO. The mixture was washed with H_2O and brine, dried ($MgSO_4$), and evaporated. Purification of the residue afforded the product.

Reaction of **4** *with NO: 1-(2-Phenylethyl)-1-nitrosourea* (**12**). A soln. of **4** (0.050 g, 0.28 mM) in AcOEt (30 ml) was treated for 40 min and worked up. Purification of the residue by prep. TLC (AcOEt/hexane 1:2), followed by recrystallization from Et_2O/hexane, afforded **12** (0.026 g, 48%). Yellow prisms. M.p. 101–102°. IR (Nujol): 3529, 3412, 1740. ^{1}H-NMR ((D_6)DMSO): 2.62 (*t*, *J* = 7.6, CH_2(1)); 3.94 (*t*, *J* = 7.6, CH_2(2)); 7.15 (*d*, *J* = 7.3, H–C(2′)); 7.21 (*d*, *J* = 7.3, H–C(4′)); 7.28 (*t*, *J* = 7.6, H–C(3′)); 7.80, 8.12 (2*s*, NH_2). ^{13}C-NMR ((D_6)DMSO): 32.8; 39.9; 30.2; 126.5; 128.4; 128.6; 137.9; 158.8. ^{15}N-NMR (50.55 MHz, (D_6)DMSO): −296 (N_AH_2), −106 (N_B), 184 (NO). HR-MS: 194.0931 ($C_9H_{12}N_3O_2^+$; calc. 194.0929). Anal. calc. for $C_9H_{11}N_3O_2$: C 55.96, H 5.70, N 21.76; found: C 55.79, H 5.69, N 21.76.

Reaction of **5** *with NO:* **12**. A soln. of **5** (0.050 g, 0.31 mM) in AcOEt (50 ml) was treated for 50 min to give **12** (0.061 g, quant.).

Reaction of 1-(2-Phenylethyl)guanidine (**7**) *with NO*: **12**. A soln. of **7** (0.050 g, 0.24 mM) in AcOEt (50 ml) was treated for 7.5 h to give **12** (0.004 g, 9%) after purification by prep. TLC (AcOEt/hexane 1:2).

Reaction of 4-Phenylbutan-2-one Oxime (**8**) *with NO*: *4-Phenylbutan-2-one* (**13**). A soln. of **8** (0.040 g, 0.24 mM) in AcOEt (10 ml) was treated for 3 h to give **13** (0.023 g, 64%) after purification by prep. TLC (AcOEt/hexane 1:4).

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