Tetrahedron Vol. 46, No. 9, pp 3211-3232, 1990
Printed in Great Britain

0040-4020/90 $3 00+.00

BIOMIMETIC, CHEMICAL, AND SPECTROSCOPIC EVALUATIONS FOR THE RADIOSENSITIZING POTENTIAL OF N1- AND N2-SUBSTITUTED DERIVATIVES OF 3-NITRO-1, 2, 4-TRIAZOLE TOWARD HYPOXIC CELLS IN THE RADIOTHERAPY: REMARKABLY DIFFERENT SUBSTITUTION EFFECT†

Yoshimitsu Nagao*, Shigeki Sano, and Masahito Ochiai
Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan

Eiichi Fujita*
Osaka University of Pharmaceutical Sciences, 10-65 Kawai 2-Chome, Matsubara 580, Japan

(*Received in Japan* 8 *November* 1989)

Abstract: N1- and N2-derivatives of 3-nitro-1, 2, 4-triazole (3-NTR) were subjected to the non-biological evaluation methods involving biomimetic, chemical, and spectroscopic procedures for the radiosensitizing potential. Consequently, the N1-derivatives of 3-NTR were suggested to be more promising radiosensitizers to hypoxic cells *in vivo* than the N2-derivatives.

In order to develop new biologically active agents, it should be very important to establish the suitable evaluation methods for the desired biological activity. In the preliminary research for drug discovery, the *in vitro* evaluation can provide useful and suggestive information for the direction of the subsequent *in vivo* evaluation and it is certainly economic for screening of biologically active compounds from various test samples. However, we often encounter the undesired cases where there is no mutual relationship between the *in vitro* data and the *in vivo* data. In these cases, the metabolic pathway and/or the pharmacokinetics of the *in vitro* active compounds will be carefully investigated using animals. Recently, we are interested in the establishment of non-biological evaluation methods which would be useful for the discrimination between the *in vitro* active (or inactive) compounds and the *in vivo* inactive (or active) ones.[1-3] We wish to report, herein, dramatical differences of chemical behavior and spectroscopic phenomena between N1-substituted derivatives 1a-f of 3-nitro-1, 2, 4-triazole (3-NTR) and N2-substituted ones 2a-f of 3-NTR, based on their biomimetic, chemical, and spectroscopic evaluations. These data should be efficient for understanding of the radiosensitizing

† This paper is dedicated to Professor Yu Wang on the occasion of his 80th birthday.

potential of 3-NTR derivatives toward hypoxic cancer cells *in vivo* and/or *in vitro*.

Instead of 2-nitroimidazoles having potent radiosensitizing ability to hypoxic cancer cells in the radiotherapy but exhibiting serious neurotoxicity to the cancer patients,[4] much attention is paid toward 3-NTR derivatives which can be expected as less neurotoxic radiosensitizers.[5] Recently, regioisomeric derivatives of 3-NTR, 1a-f and 2a-f, were synthesized from the corresponding alcohols 3 and 4, respectively, as hopeful radiosensitization agents.[6] The 3-NTR N2-derivatives (N2-derivs) 2a-f proved to show stronger radiosensitizing ability to hypoxic cells *in vitro* (Chinese hamster V79 cells under irradiation with ^{60}Co γ-rays) than misonidazole (5) and the 3-NTR N1-derivatives (N1-derivs) 1a-f.[6] However, in the *in vivo* assay (SCCVII carcinoma cells inoculated into C3H/He mouse under irradiation with 10 MV X-rays of a linear accelerator), radiosensitization effect of these 3-NTR N2-derivs onto the carcinoma cells was shown to be less than that of the 3-NTR N1-derivs.[6] The stronger radiosensitization effect of the N2-derivs onto the *in vitro* hypoxic cells can be rationalized in terms of the electron affinity expressed as the one-electron reduction potentials [See $E^{Red}_{1/2}$ (V) value in Table I]. The N2-derivs having higher electron affinity in comparison with the corresponding N1-derivs must be stronger radiosensitizers. On the other hand, it was speculated that strong oxidizing ability of the N2-derivs might disappear by reaction with the reducing substances such as non-protein SH compounds and/or enzymes involving NADH or NADPH in the living system. Thus, N1- and N2-derivs of 3-NTR were subjected to the non-biological evaluation methods (biomimetic, chemical and spectroscopic ones), in order to rationalize remarkably different aspect on the radiosensitization to hypoxic cells *in vitro* or *in vivo*.

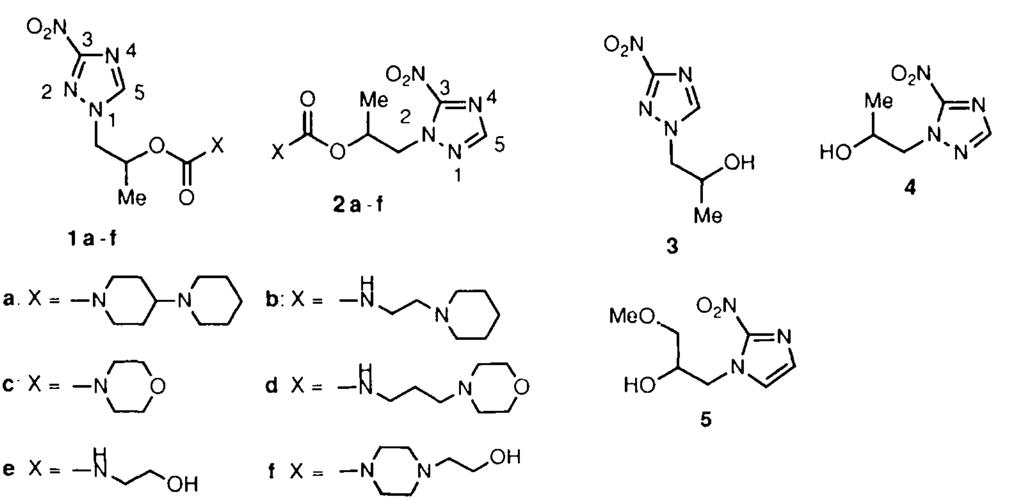


Table I. Reduction Potential of N1- and N2-Derivatives of 3-NTR

N1-derivs	$E^{Red}_{1/2}$ (V)	N2-derivs	$E^{Red}_{1/2}$ (V)
1a	-1.050	**2a**	-0.840
1b	-1.030	**2b**	-0 760
1c	-1.060	**2c**	-0.780
1d	-1.060	**2d**	-0.785
1e	-1.090	**2e**	-0.775
1f	-1.065	**2f**	-0 785
3	-1.120	**4**[a]	-0 800
		misonidazole **5**	-1.040

[a] An inseparable isomer (17%) was contained.

Biomimetic Evaluation. On the basis of our speculation that the N2-derivs 2a-f may be susceptible to non-protein SH compounds,[7] 2c was treated with L-cysteine or glutathione in 0.1M phosphate buffer solution (pH 7.40) at room temperature. The compound 2c gave 3-thio derivative 6 with elimination of NO_2 group in 69% yield after 1 h reaction with L cysteine. The yield after 19 h reaction was 73%. The similar mild reaction of 2c with glutathione was also shown to afford 3-thio derivative 7 in 83% yield (Scheme I). The structures of 6 and 7 were readily confirmed by comparison of their C5-H chemical shift (δ : 8.05 or 8.08 in D_2O) with that (δ : 8.08 in D_2O) of 1-(2'-hydroxypropyl)-1,2,4-triazole (29) (See experimental section). However, the similar reaction of an N1-deriv 1c with L-cysteine did not proceed at all and 93% recovery of 1c was observed even after treatment for 25 h. Treatment of 2c with 1-benzyl-1,4-dihydronicotinamide (8)[8] as a model compound of NADH or with L-lysine resulted in recovery of 2c in both cases (Scheme I).

This remarkably easy reaction of N2-deriv 2c with L-cysteine and glutathione (9) under the neutral conditions can be rationalized in terms of an ionic mechanism illustrated in Figure 1, instead of a radical one under the basic conditions.[7c,9] Namely, addition of thiol to the electron-deficient C3-atom of 2c resulted in formation of a conjugated anion 10, so called, a Meisenheimer type complex.[10] The thiol adduct 10 should be converted to the corresponding C3 thiol substituted product 6 or 7 releasing nitrous acid.

Thus, extremely smooth reactivity of 2c toward thiol compounds compared with that of 1c can be understood as follows. Because the anion-delocalized σ-complex 10 should be more stable than the corresponding anion-located σ-complex 11 to be obtained from 1c, addition of R'SH to 2c can be more readily promoted

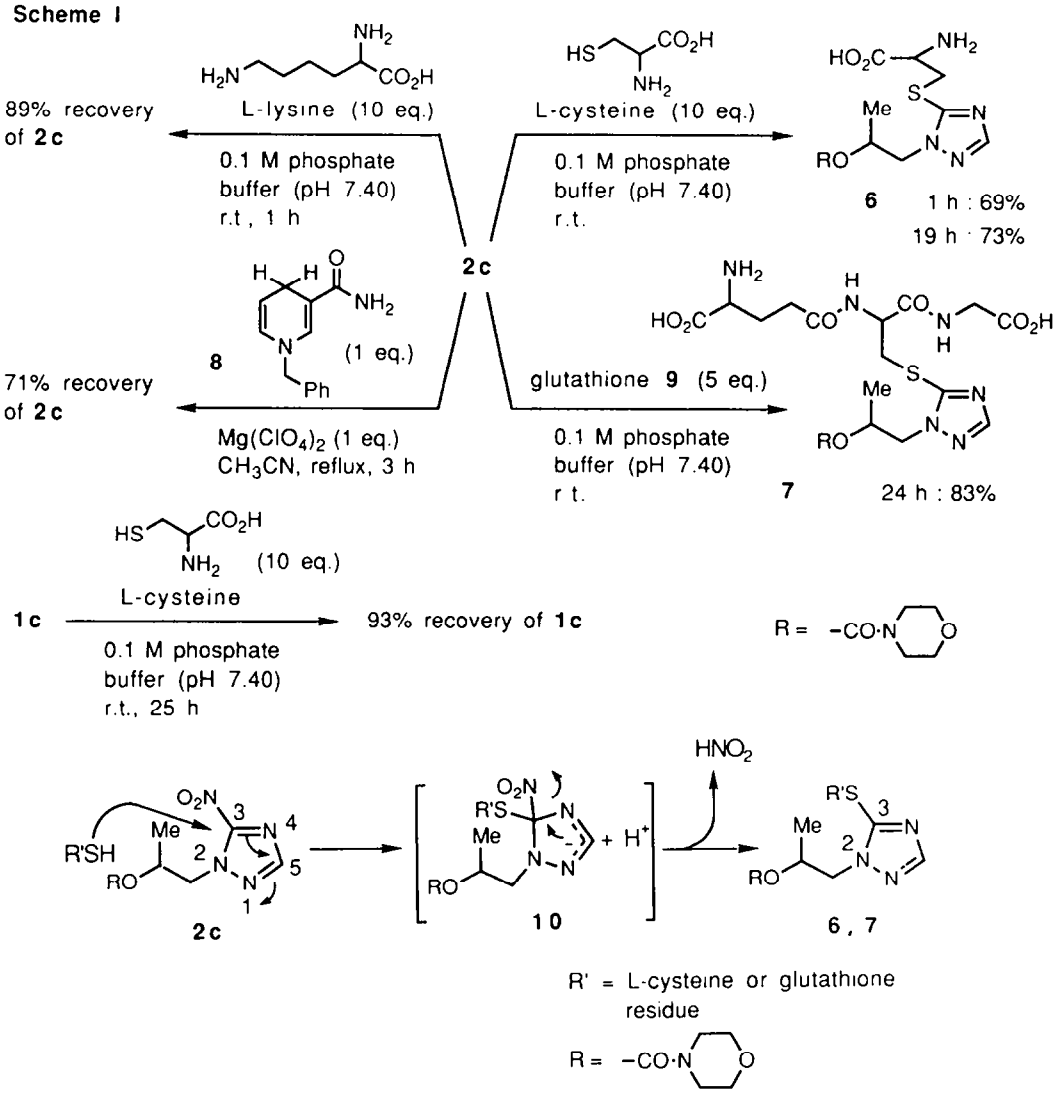
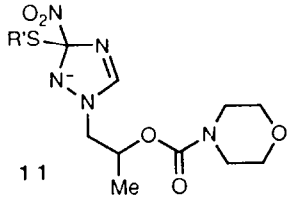


Figure 1. A plausible pathway for thiol substitution reaction



than addition to 1c. Thiol (R'SH) may much more readily attack the remarkably electron-deficient C3 atom caused by minus inductive (-I) effect of NO_2 group and double minus mesomeric (-M) effects in the conjugated bis-imine system A than the C3 atom in system B (Figure 2). This anionic mechanism (Figure 1) is supported by the electron density distribution (A and B in Figure 3) obtained from the simple Hückel LCAO-MO calculation.[11]

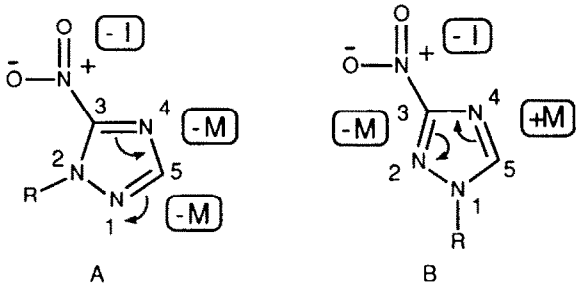


Figure 2. Inductive and mesomeric effects on the C3 atom

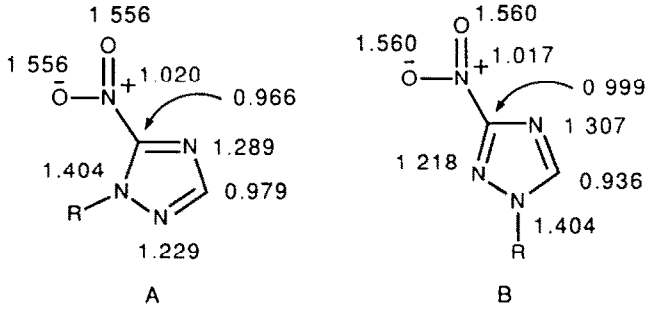
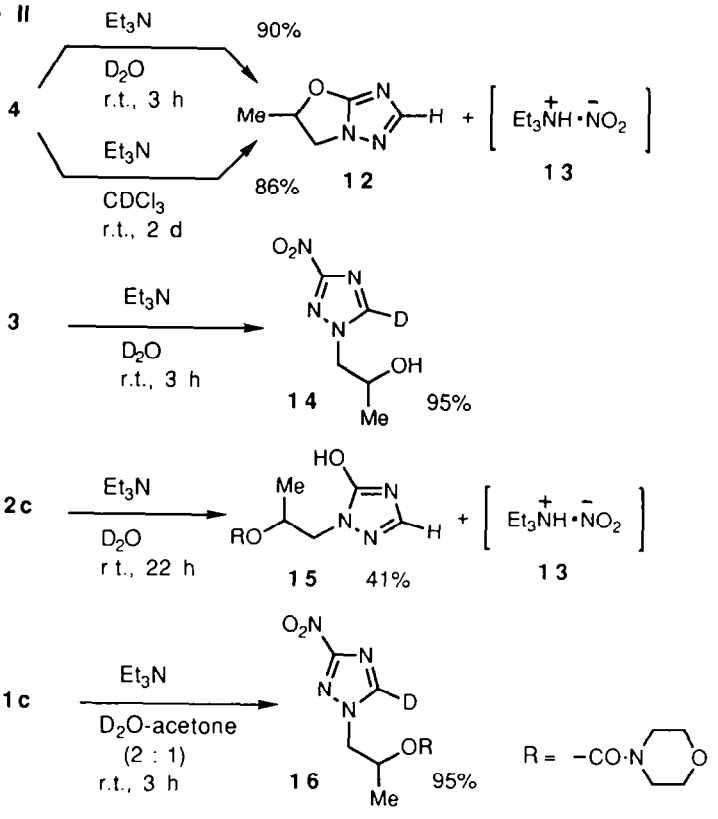


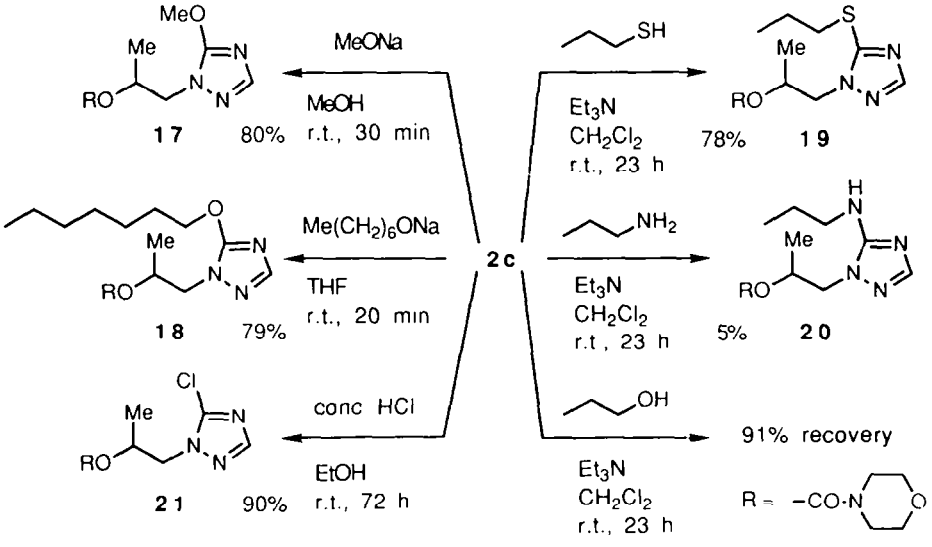
Figure 3. Electron density distribution

Chemical Evaluation. In order to inspect the reactivity of the N1-derivs and the N2-derivs toward nucleophiles, the following tests were carried out. First of all, alcohol 4 was kept in D_2O in the presence of Et_3N (1 mol equiv) monitoring the reaction with ^1H-NMR spectroscopic method. We recognized easy intramolecular addition-elimination reaction forming an oxazolidine derivative 12. Simultaneous formation of triethylammonium nitrite (13) was strongly suggested from the observation of lower magnetic field shift of the ethyl protons signals of Et_3N in proportion to the formation of 12. Compound 12 was preparatively obtained by treatment of 4 with Et_3N in D_2O (3 h, 90% yield) or in $CDCl_3$ (3 d, 86% yield) (Scheme II). Similar Et_3N-treatment of 2c in D_2O gave a hydrolysis product 15 in 41% yield eliminating the NO_2 group. However, the N1-derivs 3 and 1c, on treatment with Et_3N in D_2O or in D_2O-acetone (2 : 1), gave C5-H deuteriated products, 14 (ca. 85% of D-content, checked by 100 MHz ^1H-NMR analysis) and 16 (ca. 76% of D-content) in 95% yield, respectively.

Scheme II



Scheme III



Surprisingly, any deuteriated product of 12 and 15 has never been obtained despite the reactions for the long time (3 d and 22 h). Therefore, deuteriation of C5-H seems to be difficult even in the case of NO_2-surviving system, compounds **4** and 2c themselves.

Compound 2c was allowed to react with some nucleophiles such as MeONa, sodium *n*-heptanoate, *n*-propanethiol with Et_3N, *n*-propylamine with Et_3N, and Cl^- (Scheme Ⅲ). The corresponding substitution products 17-21 were formed, whereas similar treatment of 1c with MeONa or n-propanethiol resulted in 91% or 94% recovery of the starting compound. It is notable that 1c is remarkably stable even toward such a strong nucleophile as MeO^- anion.

Thus, we demonstrated that N2-substituted 3-nitro-1,2,4-triazoles 22 can be useful as a synthetically equivalent synthon, C3-cation 23 which should be efficiently available for the reaction with nucleophiles to give the 24 type compounds (Figure 4). This methodology should promise to develop new triazole analogs 25 of prostaglandins.[12]

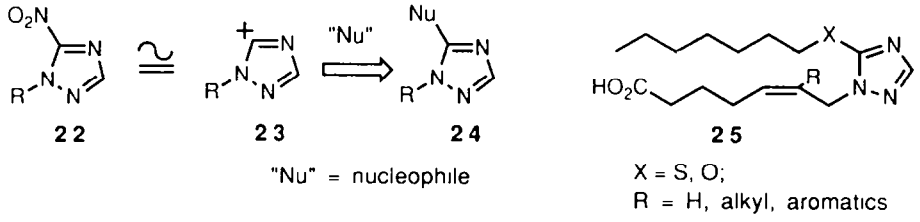
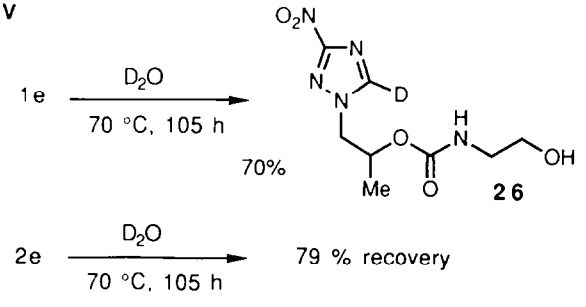


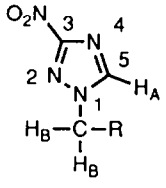
Figure 4. Synthetically equivalent 22 to cation 23

From the reactions described above, we were very much interested in evaluation of the acidity of C5-H of the N1-derivs and C5-H of the N2-derivs. Thus, detailed deuteriation experiments for the triazole-related compounds were systematically investigated under the various conditions. Surprisingly, C5-H of compound 1e was deuteriated only by heating 1e in D_2O at 70 ℃ for 105 h to give 26 (ca. 96% of D-content) in 70% yield. Similar treatment of 2e, however, resulted in the recovery of the original compound in 79% (Scheme Ⅳ). Hence, the C5-H of N1-derivs seems to be more acidic than the C5-H of N2-derivs.

Scheme IV



Deuteriation of compounds 3, 27, and 28 was attempted under three reaction conditions, A-C (see the footnote in Table II and experimental section). All results are shown in Table II. The proton (H_A) at C5 in the compound 3 was readily deuteriated in D_2O in the presence of Et_3N (B) or of NaOD (C). In the case of ester 27 under the condition A [treated with Et_3N in d6-acetone-D_2O (8 : 1)], the protons (2 × H_B) of N1-substituent group (CH_2CO_2Et) were shown to be very rapidly deuteriated without deuteriation of C5-H. In the case of carboxylic acid 28, three protons (H_A and 2 × H_B) were simultaneously deuteriated under the conditions B and C but in a rather rapid manner at C5 under the condition B. Thus, the C5-H (H_A) of the N1-substituted 3-NTR derivs proved to be quite acidic except for that of N1-CH_2CO_2Et derivative 27.



3 : R = —CH(OH)Me

27 : R = —CO_2Et

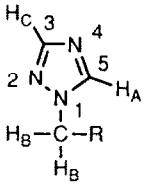
28 : R = —CO_2H

Table II. Deuteriation of N1-Substituted 3-NTR Derivatives

compound	condition[a]	time	deuteriation (%)[b]	
			H_A	H_B
3	A	5 d	None	None
〃	B	20 min	30	〃
〃	〃	26 h	100	〃
〃	C	5 min	93	〃
〃	〃	27 h	97	〃
27[c]	A	2 min	None	40
〃	〃	20 min	〃	94
〃	〃	2 h	〃	100
28	A	4 d	〃	None
〃	B	14 h	88	40
〃	〃	38 h	100	76
〃	〃	3 d	100	85
〃	C	30 min	100	100

[a] Stirred at room temperature (A) in Et_3N (2 mol equiv) and d_6-acetone-D_2O (8 : 1), (B) in Et_3N (2 mol equiv) and D_2O, and (C) in NaH (2 mol equiv) and D_2O. [b] Determined by 100 MHz ^{1}H-NMR analysis. [c] Not examined under the conditions B and C because of hard solubility of **27** in D_2O.

We checked the acidity of C3-H (H_C), C5-H (H_A), and N1-substituted CH_2 (H_B) of 1,2,4-triazole derivatives 29-32 in the deuteriation manner. All results under the same conditions (A-C) as in the cases of 3-NTR derivs are summarized in Table Ⅲ. Although significant deuteriation onto CH_2 (H_B) of 30 was observed, no deuteriation occurred in compounds 29, 31, and 32 under the condition A. Interestingly, only C5-H (H_A) among two sp_2-protons (H_A and H_C) was exclusively deuteriated in compounds 29, 31, and 32 under the conditions B and C. Deuteriation of C3-H (H_C) was not recognized at all. This specific deuteriation of C5-H (H_A) cannot be rationalized in terms of a neighboring group



29 : R = —CHOH (Me)

30 : R = —CO_2Et

31 : R = —CO_2H

32 : R = —H

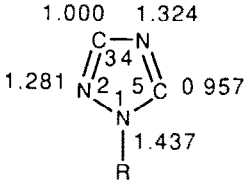


Figure 5. Electron density distribution

Table III. Deuteriation of N1-Substituted 1,2,4-Triazole Derivatives

compound	condition[a]	time	deuteriation (%)[b]		
			H_A	H_B	H_C
29	A	4.5 d	None	None	None
〃	B	27 h	19	〃	〃
〃	〃	4.5 d	66	〃	〃
〃	C	15 h	81	〃	〃
〃	〃	4.5 d	100	〃	〃
30[c]	A	23 h	None	85	〃
〃	〃	53 h	〃	100	〃
31	A	5 d	〃	None	〃
〃	B	46 h	32	〃	〃
〃	〃	5 d	63	〃	〃
〃	C	20 h	86	100	〃
〃	〃	47 h	100	100	〃
32	A	3 d	None	None	〃
〃	B	20 h	21	〃	〃
〃	〃	3 d	53	〃	〃
〃	C	20 h	98	〃	〃
〃	〃	3 d	100	〃	〃

[a] See foot note a in Table II. [b] See foot note b in Table II. [c] See foot note c in Table II.

participation and/or general basic catalysis because of the same deuteriation behavior of N1-methyl triazole 32 as that of 29-31. Thus, it should be concluded that the C5-H (H_A) is far more acidic than the C3-H (H_C) in the N1-substituted 1,2,4-triazoles. This conclusion can be understood by the electron density distribution (Figure 5) obtained from the simple Huckel LCAO-MO calculation.[11] Based on the deuteriation experiments, the C3 position in the N1-substituted 1,2,4-triazoles can be regarded as the more stable one for the NO_2 location rather than C5. This evaluation is directly supported by electron impact toward N1-derivs and N2-derivs of 3-NTR in their mass spectral analysis (*vide infla*). Fairly rapid anion formation at C5-H in the 3-NTR N1-derivs under the basic conditions in the animal organs may furthermore increase the stability of C3-NO_2 group against nucleophiles such as non-protein thiols and water. However, there is no possibility of such kind of assistance in the case of the 3-NTR N2-derivs.

Spectroscopic Evaluation (High Resolution Mass Spectrometry). Fragmentation in the high resolution electron impact (EI)-mass spectrometry (MS) of N1-derivs and N2-derivs of 3-NTR should be remarkable. Although, in all cases of

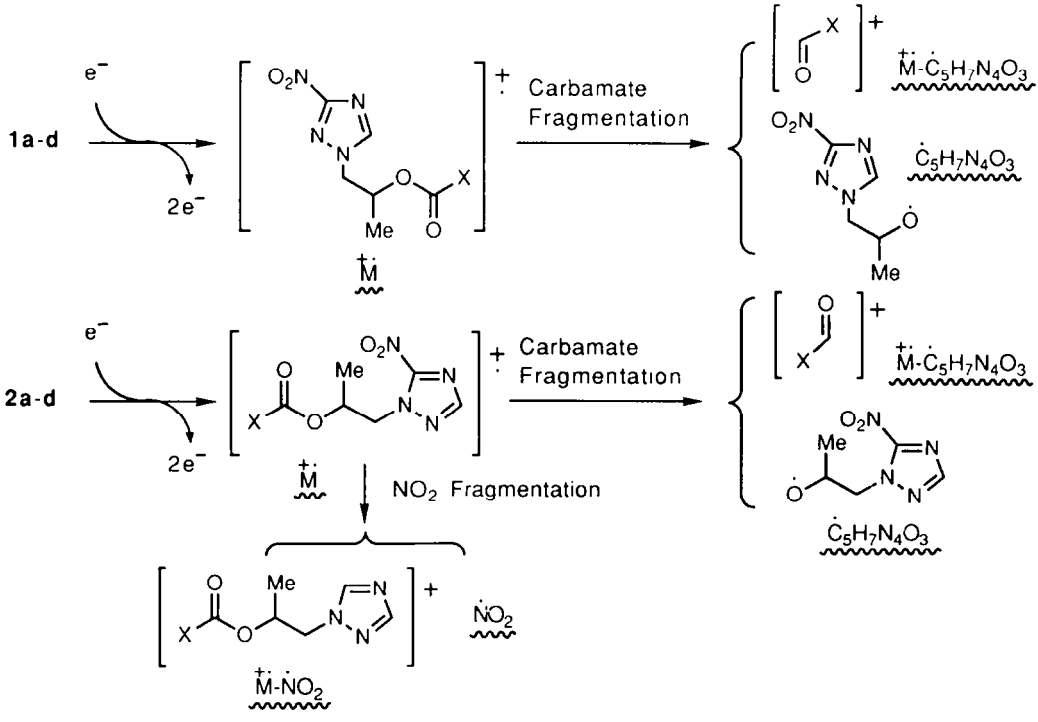


Figure 6. Fragmentation of the $\overset{+\cdot}{M}$ ion in the high resolution EI-MS of compounds 1a-d and 2a-d

nitrotriazoles having a carbamate moiety (1a-f and 2a-f), a common fragment ion peak ($X\text{-}\overset{+}{C}\text{=}O$) due to $\overset{+\cdot}{M}\text{-}\dot{C}_2H_2N_4O_3$ in their EI-MS is always observed, the particular fragment ion peak ($\overset{+}{M}\text{-}NO_2$) caused by the "$NO_2$"-fragmentation can be recognized only in the case of N2-derivs 2a-d except for hydroxyl derivatives 2e,f (See experimental section and Figure 6). This easy "NO_2"-fragmentation aspect coincides with the easy NO_2-substitution reactions in the N2-derivs of 3-NTR. Thus, it has been also revealed by the EI MS that considerable weakness of the C3-NO_2 bond must be native to the N2-substituted C3-nitro-1,2,4-triazoles. This EI-MS observation method may be strongly helpful for screening of useful radiosensitizers because the NO_2 group of radiosensitizers should be stepwise reduced capturing electrons without releasing the NO_2 group from the heterocycles under electron exposure.

Conclusions. Oxidizing ability of the 3-NTR derivatives directly exerts a great influence on their radiosensitization onto hypoxic cells *in vitro*. Fairly long survival of the NO_2 group bound to the triazole moiety in the living system should be essential for the effective radiosensitization *in vivo*. Nitro group of the N2-derivs of 3-NTR could be readily eliminated by thiols (L-cysteine and glutathione) under the neutral conditions, by water under the basic conditions, and by electron impact under the EI-MS conditions. In the N1-derivs of 3-NTR, however, their NO_2 groups turn out to be intact under the similar biomimetic and chemical evaluation conditions. In the EI-MS of the N1-derivs, the direct "NO_2"-fragmentation from the $\overset{+\cdot}{M}$ ion observed in that of the N2-derivs has never been recognized. Instability of the NO_2 group bound to the 1,2,4-triazoles could be rationalized in terms of deuteriation experiments and electron density distribution calculated by the Huckel LCAO-MO method. Thus, we have demonstrated that the N1-derivs of 3-NTR should be more promising radiosensitizers to hypoxic cells *in vivo* than the N2-derivs, on the basis of the non-biological evaluation methods described above.

Experimental Section

Melting points (mp) were determined with a YANAGIMOTO microapparatus and are uncorrected. Infrared (IR) spectra were obtained on JASCO A-202 and JASCO IR-810 spectrophotometers. ^1H- and ^{13}C-Nuclear magnetic resonance (^1H and ^{13}C-NMR) spectra were recorded on JEOL JNM-FX100, Varian Gemini 200, and JEOL JNM-GX400 spectrometers in the indicated solvents; chemical shifts are expressed in part per million (ppm) relative to tetramethylsilane (TMS) or 3-(trimethylsilyl) propionic acid-d_4 sodium salt (TSP) as internal standard. Low-resolution mass spectra (MS), high resolution mass spectra (HRMS), and fast atom bombardment mass spectra (FABMS) were recorded on a JEOL JMS-DX300 mass spectrometer. Combustion analyses were performed by YANACO CHN CORDER MT-3. Cyclic voltammograms for determination of the reduction potential were measured with a Hokuto

Denki HB-104 voltage scanner and a Hokuto Denki HA-305 potentiostat (Platinum wire and coil were employed as the working auxiliary electrodes, respectively).

All reactions were monitored by thin layer chromatography (TLC) employing 0.25 mm silica gel plates (E. Merk; 60 F_{254}) with UV light irradiation and 10% ethanolic phosphomolybdic acid-heat as detecting methods. Preparative thin layer chromatography (PTLC) was performed on 0.5 mm silica gel plates (E. Merk; 60 F_{254}). Column chromatography and flash column chromatography were carried out on silica gel (Wako; Wakogel C-200E 100-200 mesh, E. Merk; Kieselgel 60 230-400 mesh). Sephadex LH-20 (Pharmacia Fine Chemicals) was used for gel filtration. Usual workup means washing an organic portion with brine, drying over anhydrous Na_2SO_4, filtration, and concentration in vacuo. THF was distilled from sodium benzophenone ketyl under N_2. Pyridine and CH_2Cl_2 were distilled from CaH_2. All other solvents were distilled prior to use. All reagents were used as purchased. 1-Benzyl-1,4-dihydronicotinamide was kindly gifted by Professor A. Ohno (Kyoto University).

General Procedure for the Measurement of Reduction Potentials. The reduction potential of each compound was evaluated with a Ag / AgCl (saturated) / 3.5 M KCl electrode using cyclic volutammetry for an Ar-purged *N,N*-dimethylformamide solution (0.01 M) containing 0.1 M tetra-*N*-butylammonium perchlorate as a supporting electrode.

Treatment of 2-(2'-Morpholinocarbonyloxy-propyl)-3-nitro-1,2,4-triazole (2c) with L-Cysteine. To a solution of 2c (50.0 mg, 0.175 mmol) in 0.1 M phosphate buffer (pH 7.40, 1 mL) was added a solution of L-cysteine (212.4 mg, 1.753 mmol) in 0.1 M phosphate buffer (pH 7.40, 2 mL). The mixture was stirred at room temperature for 1 h under nitrogen and then the precipitation was filtered off. The filtrate was concentrated in vacuo to give an oily residue. The residue was purified by PTLC with isopropanol-ammonium hydroxide-H_2O (9 : 1 : 2)] and then by gel filtration (Sephadex LH-20 with MeOH) to afford 2-(2'-morpholinocarbonyloxy-propyl)-3-cystein-*S*-yl-1,2,4-triazole (6) (43.6 mg, 69%) as white powder: ^{1}H NMR (400 MHz, D_2O) δ 1.350 (3 H × 1/2, d, J = 6.6 Hz), 1.356 (3 H × 1/2, d, J = 6.2 Hz), 3.30-3.60 (4 H, m), 3.60-3.82 (6 H, m), 4.15 (1 H, dd, J = 6.2, 4.0 Hz), 4.29-4.47 (2 H, m), 5.16-5.25 (1 H, m), 8.05 (1 H, s); IR (KBr) 3400, 3000, 1700, 1630, 1430, 1280, 1240, 1110 cm^{-1}; FABMS calcd for $C_{13}H_{22}N_6O_5S$ M$^+$ + H 360, found *m/e* 360 (M$^+$ + H). The similar treatment of 2c with L-cysteine was also carried out for 19 h to give compound 6 in 73% yield.

Treatment of 2c with Glutathione (9). To a solution of 2c (46.0 mg, 0.161 mmol) in 0.1 M phosphate buffer (pH 7.40, 1 mL) was added a solution of glutathione (9) (247.8 mg, 0.806 mmol) in 0.1 M phosphate buffer (pH 7.40, 3 mL). The mixture was stirred at room temperature for 24 h under nitrogen and then concentrated in vacuo to give an oily residue. Purification of the residue utilizing PTLC [isopropanol-ammonium hydroxide-water (9 : 1 : 2)] and gel filtration (Sephadex LH-20 with MeOH) afforded 2-(2'-morpholinocarbonyloxy-

propyl)-3-glutathion-*S*-yl-1,2,4-triazole (7) (73.1 mg, 83%) as white powder: ^{1}H NMR (400 MHz, D_2O) δ 1.325 (3 H × 1/2, d, J = 6.6 Hz), 1.335 (3 H × 1/2, d, J = 6.2 Hz) 2.05-2.20 (2 H, m), 2.45 (2 H, t, J = 7.5 Hz), 3.30-3.60 (4 H, m), 3.60-3.80 (8 H, m), 4.28-4.43 (2 H, m), 5.13-5.21 (1 H, m), 8.065 (1 H × 1/2, s), 8.068 (1 H × 1/2, s), 4.79 (2 H, overlap with solvent); IR (KBr) 3600-2800, 1700-1500, 1400, 1270, 1240, 1110 cm^{-1}; FABMS calcd for $C_{20}H_{32}N_7O_9S$ M$^+$ + H 546, found *m/e* 546 (M$^+$ + H).

Treatment of 2c with L-Lysine. Compound 2c (53.0 mg, 0.186 mmol) in 0.1 M phosphate buffer (pH 7.40, 1 mL) was added to a solution of L-lysine (271.6 mg, 1.858 mmol) in 0.1 M phosphate buffer (pH 7.40, 2 mL). The mixture was stirred at room temperature for 1 h under nitrogen and extracted with CH_2Cl_2. The CH_2Cl_2 extract was subjected to the usual workup to give an oily residue. PTLC of the residue using hexane-AcOEt-acetone (3 : 1 : 2) as eluent recovered the starting compound 2c (46.9 mg, 89%).

Treatment of 2c with 1-Benzyl-1,4-dihydronicotinamide (8). A solution of 2c (47.0 mg, 0.165 mmol), 8 (35.3 mg, 0.165 mmol), and magnesium perchlorate (36.8 mg, 0.165 mmol) in CH_3CN (8 mL) was stirred at room temperature for 5 h under nitrogen in the dark. After refluxing for 3 h, the reaction mixture was poured into some water and extracted with CH_2Cl_2. The usual workup of the extract followed by PTLC [CH_2Cl_2-acetone (9 : 1)] resulted in recovery (33.5 mg, 71%) of the starting compound 2c. The reaction of 2c with compound 8 was also attempted as follows A solution of 2c (49.0 mg, 0.172 mmol), 8 (73.6 mg, 0.344 mmol), and 2,2'-azobisisobutyronitrile (2.8 mg, 0.017 mmol) in CH_3CN (10 mL) was refluxed for 3 h and some CH_2Cl_2 was added. After washing with an aqueous solution saturated with NH_4Cl, the organic portion was subjected to the usual workup to give an oily crude substance, whose PTLC using CH_2Cl_2-acetone (9: 1) recovered 2c (27.4 mg, 56%).

Treatment of 1-(2'-Morpholinocarbonyloxy-propyl)-3-nitro-1,2,4-triazole (1c) with L-Cysteine. To a solution of 1c (45.0 mg, 0.158 mmol) in 0.1 M phosphate buffer (pH 7.40, 1 mL) was added a solution of L-cysteine (191.1 mg, 1.578 mmol) in 0.1 M phosphate buffer (pH 7.40, 2 mL). The mixture was stirred at room temperature for 25 h under nitrogen and extracted with CH_2Cl_2. After evaporation in vacuo, the residue was chromatographed on a silica gel plate using hexane-AcOEt-Acetone (3 : 1 : 2) to recover the starting compound 1c (41.8mg, 93%).

2,3-Dihydro-2-methyl-4,5,7-triazo[5,1-b]oxazole (12). To a solution of 1-(2'-hydroxypropyl)-5-nitro-1,2,4-triazole (4) (50.0 mg, 0.290 mmol, a 5 : 1 mixture of 4 and another inseparable isomer) in D_2O (1 mL) was added Et_3N (40.5 μL, 0.290 mmol). The reaction mixture was stirred at room temperature for 3 h and extracted with CH_2Cl_2. The usual workup of the extract gave a crude product, which was subjected to PTLC [hexane-AcOEt (1 : 9)] to give compound 12

(27.4 mg, 90%): mp 77.0-78.5℃, colorless plates from isopropyl ether; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.68 (3 H, d, J = 6.3 Hz), 3.88 (1 H, dd, J = 9.4, 7.4 Hz), 4.42 (1 H, dd, J = 8.9, 7.4 Hz), 5.40-5.75 (1 H, m), 7.57 (1 H, s); IR ($CHCl_3$) 3000, 1570, 1540, 1420, 1140 cm^{-1}; HRMS calcd for $C_5H_7N_3O$ MW 125.0588, found *m/e* 125.0587 (M$^+$); Anal. Calcd for $C_5H_7N_3O$: C, 48.00; H, 5.64; N, 33.58. Found: C, 47.88; H, 5.55; N, 33.75. The similar reaction was also carried out in the following manner. A solution of 4 (50.0mg, 0.290 mmol, a 5 : 1 mixture of 4 and another inseparable isomer) and Et_3N (40.5 μL, 0.290 mmol) in $CDCl_3$ (1 mL) was stirred at room temperature for 3 days and then evaporation in vacuo gave an oily residue, which was purified on a silica gel column to afford compound 12 in 86% yield.

2-(2'-Morpholinocarbonyloxy-propyl)-3-hydroxy-1, 2, 4-triazole (15). To a solution of 2c (50.0 mg, 0.175 mmol) in D_2O (1 mL) was added Et_3N (24.4 μL, 0.175 mmol). After being stirred at room temperature for 22 h, the reaction mixture was concentrated in vacuo to give an oily residue. PTLC of the residue using hexane-AcOEt-acetone (3 : 1 : 2) afforded compound 15 (18.3 mg, 41%) and the starting compound 2c (26.7 mg, 53% recovery). Compound 15: mp 98.5-100.5 ℃, colorless needles from CH_2Cl_2-hexane; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.32 (3 H, d, J = 6.8 Hz), 3.35-3.70 (8 H, m), 3.93 (2 H, d, J = 5.9 Hz), 5.00-5.30 (1 H, m), 7.44 (1 H, s), 11.95 (1 H, brs); IR ($CHCl_3$) 3000, 1700, 1435, 1280, 1245, 1120 cm^{-1}; HRMS calcd for $C_{10}H_{16}N_4O_4$ MW 256.1120, found *m/e* 256.1166 (M$^+$); Anal. Calcd for $C_{10}H_{16}N_4O_4$: C, 46.87; H, 6.29; N, 21.86. Found: C, 46.67; H, 6.28; N, 21.74.

Deuteriation of 1-(2'-Hydroxypropyl)-3-nitro-1, 2, 4-triazole (3) by Triethylamine in D_2O. To a solution of compound 3 (50.0 mg, 0.290 mmol) in D_2O (1 mL) was added Et_3N (40.5 μL, 0.290 mmol). The mixture was stirred at room temperature for 3 h and concentrated in vacuo to give a crude product, which was purified by PTLC using CH_2Cl_2-acetone (4 : 1) to afford C5-deuteriated triazole 14 (48.0 mg, 95%). D-content of C5-D in compound 14 was determined to be ca. 85% by the ^{1}H NMR (100 MHz) analysis.

Deuteriation of 1c by Triethylamine in D_2O-Acetone. To a solution of 1c (50.0 mg, 0.175 mmol) in 33.3% acetone in D_2O (1.5 mL) was added Et_3N (24.4 μL, 0.175 mmol). After being stirred at room temperature for 3 h, the reaction mixture was concentrated in vacuo to give an oily residue. Its chromatographic purification afforded C5-deuteriated triazole 16 (47.7 mg, 95%). D-content of C5-D in compound 16 was determined to be ca. 76% by the ^{1}H NMR (100 MHz) analysis.

2-(2'-Morpholinocarbonyloxy-propyl)-3-methoxy-1, 2, 4-triazole (17). Sodium methoxide powder (9.1 mg, 0.168 mmol) was added to a solution of 2c (31.9 mg, 0.112 mmol) in methanol (1 mL) with stirring. After being stirred at room temperature for 30 min under nitrogen, the reaction mixture was treated as usual

to give a crude product, which was subjected to PTLC using hexane-AcOEt-acetone (3 : 1 : 2) to afford methyl ether 17 (24.2 mg, 80%) as a colorless oil: ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.29 (3 H, d, J = 6.9 Hz), 3.35-3.50 (4 H, m), 3.55-3.70 (4 H, m), 4.00-4 10 (2 H, m), 4.08 (3 H, s), 5.00-5.25 (1 H, m), 7.50 (1 H, s); IR ($CHCl_3$) 1690, 1560, 1240, 1130, 1110 cm^{-1}; HRMS calcd for $C_{11}H_{18}N_4O_4$ MW 270.1327, found m/e 270.1311 (M^+); Anal. Calcd for $C_{11}H_{18}N_4O_4$: C, 48.88; H, 6 71; N, 20.73. Found: C, 48.59; H, 6.62; N, 20.92.

2-(2'-Morpholinocarbonyloxy-propyl)-3-heptyloxy-1,2,4-triazole (18). To a suspension of sodium hydride (6.1 mg, 0.252 mmol) in THF (1 mL) was added 1-heptanol (63.6 μL, 0.505 mmol) at 0℃ and then the mixture was stirred at room temperature under nitrogen for 100 min. To the reaction mixture was added a solution of 2c (48.0 mg, 0.168 mmol) in THF (1 mL) at 0 ℃. After being stirred at 0 ℃ for 5 min and at room temperature for 20 min, the reaction mixture was poured into some water and extracted with CH_2Cl_2. The usual workup of the CH_2Cl_2 extract gave a crude product. PTLC of the crude product using CH_2Cl_2-acetone (4 : 1) afforded compound 18 (46 8 mg, 79%) as a colorless oil: ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.89 (3 H, brm), 1.29 (3 H, d, J = 6.4 Hz), 1.10-1.55 (8 H, m), 1.65-1.90 (2 H, m), 3.35-3.50 (4 H, m), 3.55-3.70 (4 H, m), 4.05 (2 H, d, J = 5.4 Hz), 4.38 (2 H, t, J = 6.6 Hz), 4.95-5.20 (1 H, m), 7.49 (1 H, s), IR ($CHCl_3$) 1695, 1550, 1240, 1130, 1110 cm^{-1}; HRMS calcd for $C_{17}H_{30}N_4O_4$ MW 354.2267, found m/e 354.2295 (M^+); Anal. Calcd for $C_{17}H_{30}N_4O_4$: C, 57.61; H, 8.53; N, 15.81. Found: C, 57.45; H, 8 42; N, 15.95.

2-(2'-Morpholinocarbonyloxy-propyl)-3-propylthio-1,2,4-triazole (19). To a solution of 2c (44.5 mg, 0.156 mmol) in CH_2Cl_2 (1 mL) was added 1-propanethiol (141.3 μL, 1.560 mmol) and Et_3N (21.7 μL, 0.156 mmol), successively. The mixture was stirred at room temperature for 23 hr under nitrogen and the solvent was removed in vacuo to leave an oily residue. PTLC of the residue using CH_2Cl_2-acetone (9 · 1) afforded thiol adduct 19 (38.2 mg, 78%) as a colorless oil and the starting compound 2c (8.6 mg, 19% recovery). Compound 19: ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.03 (3 H, t, J = 7.2 Hz), 1.31 (3 H, d, J = 6.4 Hz), 1.60-1.90 (2 H, m), 3.20 (3 H, t, J = 7.1 Hz), 3.30-3.50 (4 H, m), 3.50-3.70 (4 H, m), 4.22 (2 H, d, J = 5.4 Hz), 5.00-5.30 (1 H, m), 7.85 (1 H, s); IR ($CHCl_3$) 1690, 1430, 1240, 1115 cm^{-1}; HRMS calcd for $C_{13}H_{22}N_4O_3S$ MW 314.1412, found m/e 314.1413 (M^+); Anal. Calcd for $C_{13}H_{22}N_4O_3S$: C, 49.66; H, 7.05; N, 17.82. Found: C, 49.53; H, 7.08, N, 18.01

2-(2'-Morpholinocarbonyloxy-propyl)-3-propylamino-1,2,4-triazole (20). To a solution of 2c (54.0 mg, 0 189 mmol) in CH_2Cl_2 (1 mL) was added Et_3N (26.4 μL, 0.189 mmol) and 1-propylamine (155.6 μL, 1.893 mmol) The mixture was stirred at room temperature for 23 hr under nitrogen and the solvent was removed in vacuo to leave an oily residue PTLC of the residue using CH_2Cl_2-methanol (19 : 1) afforded amine adduct 20 (2.6 mg, 5%) as a colorless oil and the starting compound 2c (46.0 mg, 85% recovery) Compound 20: ^{1}H NMR (100MHz, $CDCl_3$) δ

0.99 (3 H, t, J = 7.4 Hz), 1.38 (3 H, d, J = 6.3 Hz), 1.50-1.90 (2 H, m), 1.85 (1 H, brs, 1/2 H_2O), 3.25-3.55 (6 H, m), 3.60-3.80 (4 H, m), 4.60-4.90 (1 H, m), 5.15-5.35 (1 H, brs), 7.46 (1 H, s); IR ($CHCl_3$) 1690, 1595, 1245, 1120 cm^{-1}; HRMS calcd for $C_{13}H_{23}N_5O_3$ MW 297.1800, found *m/e* 297.1783 (M^+); Anal. Calcd for $C_{13}H_{23}N_5O_3$ + 0.5 H_2O: C, 50.97; H, 7.90; N, 22.86. Found: C, 51.40; H, 7.91; N, 22.47.

Treatment of 2c with 1-Propanol. A solution of 2c (52.5 mg, 0.184 mmol), 1-propanol (137.6 μL, 1.840 mmol), and Et_3N (25.7 μL, 0.184 mmol) in CH_2Cl_2 (1 mL) was stirred at room temperature for 23 h and evaporated in vacuo to recover the starting compound 2c (47.8 mg, 91%).

2-(2'-Morpholinocarbonyloxy-propyl)-3-chloro-1,2,4-triazole (21). 37% Hydrochloric acid (104.5 μL, 1.262 mmol) was added to a solution of 2c (72.0 mg, 0.252 mmol) in ethanol (2 mL). After being stirred at room temperature for 72 hr, the solvent was removed in vacuo to leave an oily residue. PTLC of the residue using CH_2Cl_2-acetone (9 : 1) afforded chloride 21 (62.1 mg, 90%) as colorless needles from ether-hexane: mp 60.0-61.0 ℃; ^1H NMR (100 MHz, $CDCl_3$) δ 1.34 (3 H, d, J = 6.4 Hz), 3.30-3.50 (4 H, m), 3.55-3.70 (4 H, m), 4.28 (1 H, d, J = 1.0 Hz), 4.33 (1 H, s), 5.05-5.40 (1 H, m), 7.85 (1 H, s); IR ($CHCl_3$) 1700, 1240, 1115 cm^{-1}; HRMS calcd for $C_{10}H_{15}N_4O_3Cl$ MW 274.0833, found *m/e* 274.0835 (M^+); Anal. Calcd for $C_{10}H_{15}N_4O_3Cl$: C, 43.72; H, 5.50; N, 20.40. Found: C, 43.73; H, 5.44; N, 20.49.

Treatment of 1c with NaOMe. To a solution of 1c (38.0 mg, 0.133 mmol) in MeOH (1 mL) was added NaOMe powder (10.8 mg, 0.200 mmol) with stirreing. After being stirred at room temperature for 2 h under nitrogen, the reaction mixture was treated as usual to give a crude substance, which was subjected to PTLC using hexane-AcOEt-acetone (3 : 1 : 2) to recover the starting compound 1c (34.4 mg, 91%).

Treatment of 1c with 1-Propanethiol. To a solution of 1c (45.0 mg, 0.158 mmol) in CH_2Cl_2 (1 mL) was added 1-propanethiol (142.9 μL, 1.578 mmol) and Et_3N (22.0 μL, 0.158 mmol). The mixture was stirred at room temperature for 23 h under nitrogen and the solvent was removed in vacuo to leave an oily residue. PTLC of the residue using hexane-AcOEt-acetone (3 : 1 : 2) recovered the starting compound 1c (42.5 mg, 94%).

Deuteriation of 1-(2'-Hydroxyethylcarbamoyloxy-propyl)-3-nitro-1,2,4-triazole (1e) by Heating in D_2O. A solution of 1e (50.9 mg, 0.196 mmol) in D_2O (0.7 mL) was heated at 70 ℃ for 105 h and concentrated in vacuo to give an oily residue. PTLC of the residue [CH_2Cl_2-methanol (9 : 1)] afforded deuteriated compound 26 (35.8 mg, 70%), whose C5-D content was determined by the ^1H NMR (100 MHz) analysis to be 96%.

Heating of 2-(2'-Hydroxyethylcarbamoyloxy-propyl)-3-nitro-1, 2, 4-triazole (2e) in D_2O. A solution of 2e (52.1 mg, 0.201 mmol) in D_2O (0.7 mL) was heated at 70 ℃ for 105 h and similarly treated to recover the starting compound 2e (41.1 mg, 79%).

Ethyl 3-Nitro-1, 2, 4-triazolylacetate (27). To a solution of 3-nitro-1, 2, 4-triazole (4.00 g, 35.07 mmol) in dry EtOH (230 mL) was added a solution of NaOEt (2.86 g, 42.08 mmol) in dry EtOH (23.0 mL) and ethyl chloroacetate (4.49 mL, 42.08 mmol). The mixture was heated at 120 ℃ for 30 h in a sealed tube. The solvent was removed in vacuo to give an oily residue. After adding some water, the residue was extracted with CH_2Cl_2. The organic layer was washed with 1% hydrochloric acid and an aqueous solution saturated with $NaHCO_3$, and the usual workup gave a crude product. The crude product was purified by flash column chromatography (elution with 40% hexane in AcOEt) to afford compound 27 (6.35 g, 91%) as colorless plates from ethanol: mp 64.5-65.5℃ ; ^1H NMR (100 MHz, $CDCl_3$) δ 1.32 (3 H, t, J = 7.1 Hz), 4.31 (2 H, q, J = 7.0 Hz), 5.12 (2 H, s), 8.39 (1 H, s); IR ($CHCl_3$) 1760, 1565, 1310, 840 cm^{-1}; HRMS calcd for $C_6H_8N_4O_4$ MW 200.0545, found m/e 200.0566 (M$^+$). Anal. Calcd for $C_6H_8N_4O_4$: C, 36.01; H, 4.03; N, 27.99. Found: C, 36.03; H, 3.93; N, 28.19.

3-Nitro-1, 2, 4-triazolylacetic Acid (28). To a solution of 27 (1.26 g, 6.31 mmol) in MeOH (10 mL) was added 1 N NaOH (6.94 mL, 6.94 mmol). The mixture was stirred at room temperature for 20 min and the solvent was removed in vacuo to leave an oily residue. To the residue was added 1% hydrochloric acid under ice-cooling and extracted with AcOEt. The usual workup gave compound 28 (1.024 g, 94%) as colorless plates from hexane-AcOEt: mp 189-192 ℃ ; ^1H NMR (100 MHz, D_2O) δ 5.29 (2 H, s), 8.70 (1 H, s); IR (KBr) 1720, 1560, 1530, 1320, 1220, 840 cm^{-1}; HRMS calcd for $C_4H_4N_4O_4$ MW 172.0233, found m/e 172.0258 (M$^+$); Anal. Calcd for $C_4H_4N_4O_4$: C, 27.91; H, 2.34; N, 32.56. Found: C, 28.12; H, 2.47; N, 32.63.

1-(2'-Hydroxypropyl)-1, 2, 4-triazole (29). To a solution of 1, 2, 4-triazole (2.00 g, 29.0 mmol) in dry EtOH (60 mL) was added propylene oxide (19.6 mL, 290.0 mmol). The mixture was stirred at room temperature for 50 h, an additional propylene oxide (78.3 mL, 1.16 mol) was furthermore added, and the mixture was stirred at room temperature for 33 h. The solvent was removed in vacuo to leave an oily residue. The residue was subjected to column chromatography (elution with 10% methanol in CH_2Cl_2) to afford 29 (2.80 g, 76%) as a colorless oil and 4-(2'-hydroxypropyl)-1, 2, 4-triazole (0.46 g, 13%). Compound 29: ^1H NMR (400 MHz, $CDCl_3$) δ 1.26 (3 H, d, J = 5.8 Hz), 2.38 (1 H, brs), 4.00-4.07 (1 H, dd, J = 14.7, 8.3 Hz), 4.15-4.25 (2 H, m), 7.84 (1 H, s), 8.08 (1 H, s); ^1H NMR (100 MHz, D_2O) δ 1.24 (3 H, d, J = 6.4 Hz), 4.10-4.45 (3 H, m), 8.08 (1 H, s), 8.45 (1 H, s); Nuclear overhauser effect (NOE) (400 MHz, $CDCl_3$) was observed between H_A and H_B [2.9% (H_A - $H_{B,1}$) and ca. 0% (H_A - $H_{B,2}$)]; IR ($CDCl_3$) 3400, 3000, 1510, 1275, 1140 cm^{-1}; HRMS calcd for $C_5H_9N_3O$ MW 127.0745, found m/e 127.0720 (M$^+$). Anal. Calcd for $C_5H_9N_3O$: C, 47.24; H, 7.13; N, 33.05. Found: C,

46.82; H, 7.17; N, 32.98. 1-(2'-Hydroxypropyl)-1,3,4-triazole: colorless columns from CH_2Cl_2: mp 93.5-94.5℃; ^1H NMR (100 MHz, D_2O) δ 1.20 (3 H, d, J = 6.0 Hz), 3.95-4.35 (3 H, m), 8.48 (2 H, s); IR (KBr) 3300, 1535, 1190, 1140, 1070, 840 cm^{-1}; HRMS calcd for $C_5H_9N_3O$ MW 127.0745, found m/e 127.0727 (M^+); Anal. Calcd for $C_5H_9N_3O$: C, 47.24; H, 7.13; N, 33.05. Found: C, 47.01; H, 7.08; N, 33.21.

Ethyl 1,2,4-Triazolylacetate (30). To a solution of 1,2,4-triazole (1.00 g, 14.48 mmol) in dry EtOH (30 mL) was added NaOEt (1.18 g, 17.37 mmol) and ethyl chloroacetate (1.85 mL, 17.37 mmol). The mixture was heated at 70 ℃ for 12 h in a sealed tube. The solvent was concentrated in vacuo, some water was added, and the mixture was extracted with CH_2Cl_2. The extract was washed with 1% hydrochloric acid and an aqueous solution saturated with $NaHCO_3$, and the usual workup gave an oily product, whose purification by flash column chromatography (elution with 5% hexane in ethyl acetate) afforded 30 (1.54 g, 69%) as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 1.30 (3 H, d, J = 7.1 Hz), 4.26 (2 H, q, J = 7.3 Hz), 4.98 (2 H, s), 7.97 (1 H, s), 8.21 (1 H, s); NOE (400 MHz, $CDCl_3$) was observed between H_A and H_B, 3.5% (H_A - H_B); IR ($CDCl_3$) 3000, 1750, 1270, 1140, 1010 cm^{-1}; HRMS calcd for $C_6H_9N_3O_2$ MW 155.0695, found m/e 155.0708 (M^+); Anal. Calcd for $C_6H_9N_3O_2$: C, 46.45; H, 5.85; N, 27.08. Found: C, 45.98; H, 5.84; N, 27.35.

1,2,4-Triazolylacetic Acid (31). To a solution of 30 (1.014 g, 6.535 mmol) in MeOH (10 mL) was added 1 N NaOH aqueous solution (7.189 mL, 7.189 mmol). The mixture was stirred at room temperature for 15 min and the solvent was removed in vacuo to leave an oily residue. To the residue was added 1% hydrochloric acid and extracted with AcOEt. Usual workup gave compound 31 (524.0 mg, 63%) as colorless plates from EtOH: mp 199-202℃; ^1H NMR (400 MHz, D_2O) δ 5.17 (2 H, s), 8.31 (1 H, s), 8.86 (1 H, s); NOE (400 MHz, D_2O) was observed between H_A and H_B, 5.6% (H_A - H_B); IR (KBr) 1710, 1520, 1360, 1290, 1240, 1130, 1010, 800 cm^{-1}; HRMS calcd for $C_4H_5N_3O_2$ MW 127.0380, found m/e 127.0350 (M^+); Anal. Calcd for $C_4H_5N_3O_2$: C, 37.80; H, 3.97; N, 33.06. Found: C, 37.76; H, 3.98; N, 33.11.

1-Methyl-1,2,4-triazole (32). To a solution of 1,2,4-triazole (1.00 g, 14.48 mmol) in MeOH (40 mL) was added NaOMe (860.3 mg, 15.93 mmol) and MeI (0.99 mL, 15.93 mmol). The mixture was heated at 120 ℃ for 1.5 h in a sealed tube. The solvent was removed in vacuo, some water was added, and the mixture was extracted with CH_2Cl_2. The extract was washed with water and treated as usual to give an oily product, which was purified by flash column chromatography (elution with 8% methanol in CH_2Cl_2) to furnish compound 32 (0.16 g, 13%) as a colorless oil: ^1H NMR (100 MHz, $CDCl_3$) δ 3.95 (3 H, s), 7.94 (1 H, s), 8.05 (1 H, s); ^1H NMR (100 MHz, D_2O) δ 3.95 (3 H, s), 8.03 (1 H, s), 8.38 (1 H, s); IR ($CDCl_3$) 3000, 1515, 1270, 1140 cm^{-1}; HRMS calcd for $C_3H_5N_3$ MW 83.0484, found m/e 83.0501 (M^+); Anal. Calcd for $C_3H_5N_3$: C, 43.36; H, 6.07; N, 50.57. Found: C, 43.01; H, 6.09; N, 50.46.

General Procedure for Deuteriation of N1-Substituted 3-NTR Derivatives 3, 27, and 28 and N1-Substituted 1,2,4-Triazole Derivatives 29, 30, 31, and 32. Condition A. To a solution of each compound (ca. 15 mg) in d_6-acetone-D_2O (8 : 1) (450 μL) was added 2 mol equiv of Et_3N. The mixture was stirred at room temperature for a time indicated on Tables II and III and then the D-content of C5-D in each sample was determined by the 100 MHz ^{1}H-NMR analysis.

Condition B. Carried out similarly to the condition A employing 2 mol equiv of Et_3N and D_2O (400 μL).

Condition C Carried out similarly to the condition A employing 2 mol equiv of NaH and D_2O (400 μL).

High Resolution Mass Spectrometry (HRMS) for Compounds 1a-f and 2a-f. 1-[2'-(4"-Piperidinopiperidinylcarbonyloxy)-propyl]-3-nitro-1,2,4-triazole (1a). Chamber temperature (C.T.) = 140 ℃, probe temperature (P.T.) = 90-110 ℃; HRMS calcd for $C_{16}H_{26}N_6O_4$ MW 366.2015, found *m/e* 366.2012; calcd for $C_{11}H_{19}N_2O$ M^+ - $C_3H_7N_4O_3$ 195.1497, found *m/e* 195.1524.

1-(2'-Piperidinoethylcarbamoyloxy-propyl)-3-nitro-1,2,4-triazole (1b). C.T. = 50 ℃, P.T. = 270-280 ℃; HRMS calcd for $C_{13}H_{22}N_6O_4$ MW 326.1703, found *m/e* 326.1717; calcd for $C_8H_{15}N_2O$ M^+ - $C_5H_7N_4O_3$ 155.1183, found *m/e* 155.1165.

1-(2'-Morpholinocarbonyloxy-propyl)-3-nitro-1,2,4-triazole (1c). C.T. = 120 ℃, P.T. = 80-100 ℃; HRMS calcd for $C_{10}H_{15}N_5O_5$ MW 285.1073, found *m/e* 285.1116; calcd for $C_{10}H_{13}N_5O_4$ M^+ - H_2O 267.0967, found *m/e* 267.0970; calcd for $C_5H_8NO_2$ M^+ - $C_5H_7N_4O_3$ 114.0554, found *m/e* 114.0553.

1-(2'-Morpholinopropylcarbamoyloxy-propyl)-3-nitro-1,2,4-triazole (1d). C.T. = 140 ℃, P.T. = 100-130 ℃; HRMS calcd for $C_{13}H_{22}N_6O_5$ MW 342.1651, found *m/e* 342.1684; calcd for $C_{14}H_{21}N_6O_4$ M^+ - OH 325 1623, found *m/e* 325.1599; calcd for $C_8H_{15}N_2O_2$ M^+ - $C_5H_7N_4O_3$ 171.1134, found *m/e* 171.1142.

1-(2'-Hydroxyethylcarbamoyloxy-propyl)-3-nitro-1,2,4-triazole (1e). C.T. = 110 ℃, P.T. = 200-280 ℃; HRMS calcd for $C_8H_{14}N_5O_5$ M^+ + H 260.0994, found *m/e* 260.0988; calcd for $C_8H_{13}N_5O_4$ M^+ - H_2O 241.0810, found *m/e* 241.0790; calcd for $C_7H_{10}N_5O_4$ M^+ - CH_2OH 228.0733, found *m/e* 228.0735; calcd for $C_3H_6NO_2$ M^+ - $C_5H_7N_4O_3$ 88.0397, found *m/e* 88.0396.

1-[2'-(4"-Hydroxyethylpiperazinylcarbonyloxy)-propyl]-3-nitro-1,2,4-triazole (1f). C.T. = 140 ℃, P.T. = 100-200 ℃; HRMS calcd for $C_{12}H_{20}N_6O_5$ MW 328.1494, found *m/e* 328.1489; calcd for $C_{12}H_{19}N_6O_4$ M^+ - OH 311.1466, found *m/e* 311.1461; calcd for $C_{11}H_{17}N_6O_3$ M^+ - CH_2OH 297.1310, found *m/e* 297.1288; calcd for $C_7H_{14}N_2O_2$ M^+ - $C_5H_7N_4O_3$ 157.0976, found *m/e* 157.0953.

2-[2'-(4"-Piperidinopiperidinylcarbonyloxy)-propyl]-3-nitro-1,2,4-triazole (2a). C.T. = 140 ℃, P.T = 80-90 ℃; HRMS calcd for $C_{16}H_{26}N_6O_4$ MW 366.2015, found *m/e* 366.2023; calcd for $C_{16}H_{25}N_6O_3$ M^+ - OH 349.1987, found *m/e* 349.1956;

calcd for $C_{16}H_{26}N_5O_2$ M$^+$ - NO_2 320.2086, found *m/e* 320.2119; calcd for $C_{11}H_{19}N_2O$ M$^+$ - $C_5H_7N_4O_3$ 195.1497, found *m/e* 195.1518.

2-(2'-Piperidinoethylcarbamoyloxy-propyl)-3-nitro-1, 2, 4-triazole (2b). C.T. = 120 ℃, P.T. = 100-130 ℃; HRMS calcd for $C_{13}H_{22}N_6O_4$ MW 326.1702, found *m/e* 326.1722; calcd for $C_{13}H_{22}N_5O_2$ M$^+$ - NO_2 280.1773, found *m/e* 280.1782; calcd for $C_8H_{15}N_2O$ M$^+$ - $C_5H_7N_4O_3$ 155.1184, found *m/e* 155.1185.

2-(2'-Morpholinocarbonyloxy-propyl)-3-nitro-1, 2, 4-triazole (2c). C.T. = 130 ℃, P.T. = 20-70 ℃; HRMS calcd for $C_{10}H_{15}N_5O_5$ MW 285.1074, found *m/e* 285.1105; calcd for $C_{10}H_{13}N_5O_4$ M$^+$ - H_2O 267.0966, found *m/e* 267.0924; calcd for $C_{10}H_{15}N_4O_4$ M$^+$ - NO_2 239.1143, found *m/e* 239.1141; calcd for $C_5H_8NO_2$ M$^+$ - $C_5H_7N_4O_3$ 114.0554, found *m/e* 114.0554.

2-(2'-Morpholinopropylcarbamoyloxy-propyl)-3-nitro-1, 2, 4-triazole (2d). C.T. = 140 ℃, P.T. = 70-90 ℃; HRMS calcd for $C_{13}H_{22}N_6O_5$ MW 342.1652, found *m/e* 342.1671; calcd for $C_{13}H_{21}N_6O_4$ M$^+$ - OH 325.1624, found *m/e* 325.1647; calcd for $C_{13}H_{22}N_5O_3$ M$^+$ - NO_2 296.1723, found *m/e* 296.1732; calcd for $C_8H_{16}N_2O_2$ M$^+$ - $C_5H_7N_4O_3$ 171.1133, found *m/e* 171.1126.

2-(2'-Hydroxyethylcarbamoyloxy-propyl)-3-nitro-1, 2, 4-triazole (2e). C.T. = 50 ℃, P.T. = 150-220 ℃; HRMS calcd for $C_8H_{14}N_5O_5$ M$^+$ + H 260.0994, found *m/e* 260.0986; calcd for $C_8H_{12}N_5O_4$ M$^+$ - OH 242.0888, found *m/e* 242.0881; calcd for $C_7H_{10}N_5O_4$ M$^+$ - CH_2OH 228.0732, found *m/e* 228.0750; calcd for $C_3H_6NO_2$ M$^+$ - $C_5H_7N_4O_3$ 88.0397, found *m/e* 88.0389.

2-[2'-(4"-Hydroxyethylpiperazinylcarbonyloxy)-propyl]-3-nitro-1, 2, 4-triazole (2f). C.T. = 140 ℃, P.T. = 60-110 ℃; HRMS calcd for $C_{12}H_{20}N_6O_5$ MW 328.1495, found *m/e* 328.1532; calcd for $C_{12}H_{19}N_6O_4$ M$^+$ - OH 311.1468, found *m/e* 311.1506; calcd for $C_{12}H_{18}N_6O_4$ M$^+$ - H_2O 310.1344, found *m/e* 310.1390; calcd for $C_{11}H_{17}N_6O_4$ M$^+$ - CH_2OH 297.1311, found *m/e* 297.1315; calcd for $C_7H_{13}N_2O_2$ M$^+$ - $C_5H_7N_4O_3$ 157.0976, found *m/e* 157.0938.

Acknowledgments

Part of this work was supported by Grants-in-Aid for Cancer Research and for Developmental Scientific Research from the Ministry of Education, Science and Culture, Japan. We are grateful to Professor Tsutomu Kagiya, Department of Hydrocarbon Chemistry, Faculty of Engineering, Kyoto University, for the reduction potential measurement and his useful advice. We also thank Professor Hiroaki Oya, Department of Chemistry, Faculty of Science, Kyoto University, for his suggestion and discussion about the Huckel LCAO-MO calculation.

References and notes:

(1) Fujita, E. ; Nagao, Y. *Bioorg. Chem.* 1977, *6*, 287.

(2) Nagao, Y. ; Ochiai, M. *Bull. Inst. Chem. Res., Kyoto Univ.* 1985, *63*, 132.

(3) Nagao, Y. ; Sano, S. ; Miyasaka, T. ; Ochiai, M. ; Fuji, K. ; Fujita, E. ; Ishii, H. *Nucleic Acid Res., Symposium Series* 1985, 37.

(4) (a) Monney, H. ; Parrick, J. ; Wallaoe, R. G. *Pharmac. Ther.* 1981, *14*, 197 and references cited therein. (b) Narayanan, V. L. ; Lee, W. W. *Advances Pharmacol. Chemother.* 1982, *19*, 155 and references cited therein. (c) Adams, G. E. *Strahlentherapie* 1984, *160*, 688 and references cited therein. (d) Sehgal, R. K. ; Webb, M. W. ; Agrawal, K. C. *J. Med. Chem.* 1981, *24*, 601. (e) Murayama, C. ; Hori, H. ; Inayama, S. ; Mori, T. *Jpn. J. Cancer Res. (Gann)* 1982, *73*, 588. (f) Urtasun, R. C. ; Coleman, C. N. ; Wasserman, T. H. ; Phillips, T. L. *Int. Radiat. Oncol. Biol. Phys.* 1984, *10*, 1691. (g) Adams, G. E. ; Ahmed, I. ; Sheldon, P. W. ; Stratford, I *J. Br J. Cancer* 1984, *41*, 571. (h) Coleman, C. N *Int. J. Radiat. Oncol. Biol. Phys.* 1985, *11*, 323. (i) Dennis, M. F. ; Strafork, M R. L. ; Wardman, P. ; Watts, M. E. *Int. J. Radiat. Biol* 1985, *47*, 629. (j) Watts, M. E. ; Jones, N. R. *Int. J Radiat. Biol.* 1985, *47*, 645. (k) Brown, J. M. ; Lee, W. W. *Radiation Sensitizers' Their Use in Clinical Management of Cancer*, Ed by Brady, L. W., Masson, New York, 1980, p. 2. (l) Workman, P. *Cancer Clin. Trials* 1984, *3*, 237. (m) Wardman, P. *Radiat. Phys. Chem.* 1984, *24*, 293. (n) Murayama, C. ; Nagao, Y. ; Sano, S. ; Ochiai, M. ; Fuji, K. ; Fujita, E. ; Mori, T *Experientia* 1987, *43*, 1221.

(5) (a) Astor, M. B. ; Parham, J. C. ; Hall, E. J. ; Templeton, M. A. ; Hartog, B. *Br. J. Cancer* 1983, *47*, 155. (b) Shibamoto, Y. ; Sakano, K. ; Kimura, R. ; Nishida, T. ; Nishimoto, S. ; Ono, K. ; Kagiya, T. ; Abe, M. *Int. J. Radiat. Oncol. Biol. Phys.* 1986, *12*, 1063. (c) Shibamoto, Y. ; Nishimoto, S. ; Mi, F ; Sasai, K. ; Kagiya, T. ; Abe, M. *Int. J. Radiat. Biol.* 1987, *52*, 347.

(6) Nagao, Y ; Sano, S. ; Ochiai, M. ; Fuji, K. ; Nishimoto, S. ; Kagiya, T. ; Murayama, C ; Mori, T ; Shibamoto, Y. ; Sasai, K. ; Abe, M. *Chem. Pharm. Bull.* 1989, *37*, 1951; The synthesis of compounds 3, 4, 1a-f, and 2a-f will be soon reported elsewhere together with the full details of their radiosensitizing effect on hypoxic cells

(7) (a) For Michael type addition reactions of thiol, see: ref. (1) and Fujita, E , Nagao, Y., Kaneko, K. ; Nakazawa, S. ; Kuroda, H. *Chem. Pharm. Bull.* 1976, *24*, 2118. (b) For substitution reaction of 2-ethoxycarbonyl-nitrobenzene with thiolate anion, see: Knudsen, R. D. ; Synder, H. R. *J. Org. Chem.* 1974, *39*, 3343. (c) Nucleophilic substitution of the NO_2 group of nitroimidazoles with 2-aminoethanethiol at pH 5.0 (H_2O, 37℃, 120 h or 100 ℃, 1.5 h) or at pH 9.5 (H_2O, 37℃, 24 h) was reported. See: Goldman, P. ; Wuest, J. D. *J. Am. Chem. Soc.* 1981, *103*, 6224

(8) (a) Ohno, A ; Shio, T ; Yamamoto, H ; Oka, S *J. Am. Chem. Soc.* 1981, *103*, 2045. (b) Ono, N. ; Tamura, R ; Kaji, A. *J Am Chem. Soc.* 1983, *105*, 4017.

(9) Recently, radical-anionic mechanism for the transition state in the Michael

addition reaction of CN onto substituted 1,1-diaryl-2-nitroethylenes has been proposed. See: Gross, Z.; Hoz, S. *J. Am. Chem. Soc.* 1988, *110*, 7489.

(10) Strauss, M. J. *Chem. Rev.* 1970, *70*, 667.

(11) (a) Huckel, E. *Z. Phys.* 1930, *60*, 423. (b) Pauling, L.; Wilson, E. B. *Introduction to Quantum Mechanics with Applications to Chemistry*, McGraw-Hill, 1935. (c) Pople, J. A.; Beveridge, D. L. *Approximate Molecular Orbital Theory*, McGraw-Hill, 1979.

(12) For recent publication of development of an imidazole analog of prostaglandin, see: Amino, Y.; Eto, H.; Eguchi, C. *Chem. Pharm. Bull.* 1989, *37*, 1481 and references cited therein.