preparation, and IC_{50} values of the compounds being tested were normalized according to a published procedure.[32] K_e values for naloxone as antagonist were determined from the ratio of IC_{50} values obtained in the presence and absence of a fixed naloxone concentration.[33]

Molecular Dynamics Studies. Dyamics simulations were carried out by using the software package SYBYL (Tripos Associates, St. Louis, MO) on a VAXstation 3500. Molecules were viewed on an Evans & Sutherland PS330 computer graphics display terminal and a Hewlett-Packard HP7475 plotter was used for the preparation of the figures. The simulations were carried out with a step size of 1 fs and data were recorded for analysis every 50 fs. Starting conformations were low-energy conformers obtained in a molecular mechanics study (Wilkes, B. C.; Schiller, P. W., manuscript in preparation). After an equilibration period of 2 ps the simulations were carried out for 100 ps at 600 K. The elevated temperature of 600 K was chosen in order to obtain better insight into the accessible conformational space, since conformational transitions are expected to be more frequent at 600 K than at 300 K. Each dynamics trajectory was analyzed for torsion angles (Figures 2 and 3), and conformations were sampled every 5 ps to generate Figure 4.

Acknowledgment. This work was supported by grants from the Medical Research Council of Canada (Grants MT-5655 and MT-10131) and the National Institute on Drug Abuse (Grant DA-04443). Naloxone hydrochloride was a gift from Endo Laboratories, Inc. We thank Drs. M. Evans and M. Bertrand, Department of Chemistry, University of Montreal, for the performance of the FAB mass spectroscopic determinations. Thanks are also due to H. Zalatan for typing the manuscript.

Registry No. 1, 135943-98-5; 2, 136030-31-4; 3, 135943-99-6; (L,D,L,L)-4, 136031-52-2; (L,D,D,L)-4, 135944-04-6; 5, 135972-37-1; (L,D,L,L)-6, 135972-38-2; (L,D,D,L)-6, 136031-53-3; 8, 135944-00-2; 9, 136030-32-5; 10, 135944-01-3; 11, 135944-02-4; 12, 135944-03-5; (±)-AcNHCH(CN)COOEt, 90877-70-6; 2-$MeC_6H_4CH_2Cl$, 552-45-4; H-Phe-OH, 63-91-2; H-DL-oMePhe-OH, 22888-51-3; H-Aic-OH, 27473-62-7; H-DL-Atc-OH, 6331-63-1; H-Tic-OH, 74163-81-8; H-Hfe-OH, 943-73-7; H-D-Hfe-OH, 82795-51-5; H-Nal-OH, 55516-54-6; H-N^{α}MePhe-OH, 2566-30-5; Fmoc-C^{α}MePhe-OH, 135944-05-7; Fmoc-DL-oMePhe-OH, 135944-06-8; Fmoc-Aic-OH, 135944-07-9; Fmoc-DL-Atc-OH, 135944-08-0; Fmoc-Hfe-OH, 132684-59-4; Fmoc-D-Hfe-OH, 135944-09-1; Fmoc-Nal-OH, 96402-49-2; Fmoc-N^{α}MePhe-OH, 77128-73-5; Fmoc-Tic-OH, 136030-33-6; Fmoc-Phe-OH, 35661-40-6; Fmoc-D-Phe-OH, 86123-10-6; Fmoc-Glu(OBu-t)-OH, 71989-18-9; Fmoc-D-Orn(Boc)-OH, 118476-89-4; Boc-Tyr(Boc)-OH, 20866-48-2; H-C^{α}MePhe-OH, 23239-35-2; 2-indanone, 615-13-4; 2-spirohydantoinindan, 27473-61-6; (±)-2-spirohydantointetralin, 6270-37-7.

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Synthesis and Evaluation of Some Nitrobenzenesulfonamides Containing Nitroisopropyl and (Ureidooxy)methyl Groups as Novel Hypoxic Cell Selective Cytotoxic Agents

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Basic nitrobenzenesulfonamides containing nitroisopropyl and (ureidooxy)methyl groups were prepared and evaluated as novel hypoxic cell selective cytotoxic agents. In vitro, *N*-(2-aminoethyl)-*N*-methyl-3-nitro-4-(1-methyl-1-nitroethyl)benzenesulfonamide hydrochloride (11) proved to be preferentially toxic to hypoxic EMT6 mammary carcinoma cells. At 1 mM concentration in vitro, 11 reduced the surviving fraction of these hypoxic cells to 3×10^{-3} with no effect on aerobic cells. In radiation experiments, 11 appeared to function as a hypoxic cell radiosensitizer as well as a selective cytotoxic agent. However, administration of 11 at 200 mg/kg ip or 100 mg/kg iv to BALB/c mice implanted with solid EMT6 tumors produced no evidence of significant in vivo cytotoxic or radiosensitizing activity. *N*-Methyl-*N*-[2-(methylamino)ethyl]-3-nitro-4-[(ureidooxy)methyl]benzenesulfonamide hydrochloride (20) showed slight differential toxicity toward EMT6 cells at 3 mM concentration and radiosensitizing activity comparable to misonidazole at 1 mM concentration.

Introduction

Convincing evidence is now available that solid tumors possess significant numbers of hypoxic cells[1] which adversely affect the efficacy of radiotherapy[2] and chemotherapy[3] regimens. However, from a medicinal chemistry perspective, this apparent obstacle to successful therapy can conceivably be exploited to provide novel approaches for the design of hypoxia selective agents. For example, reduction of nitro groups by the reducing environment of hypoxic cells is one strategy which has been utilized for the activation of latent cytotoxic compounds.[4-6]

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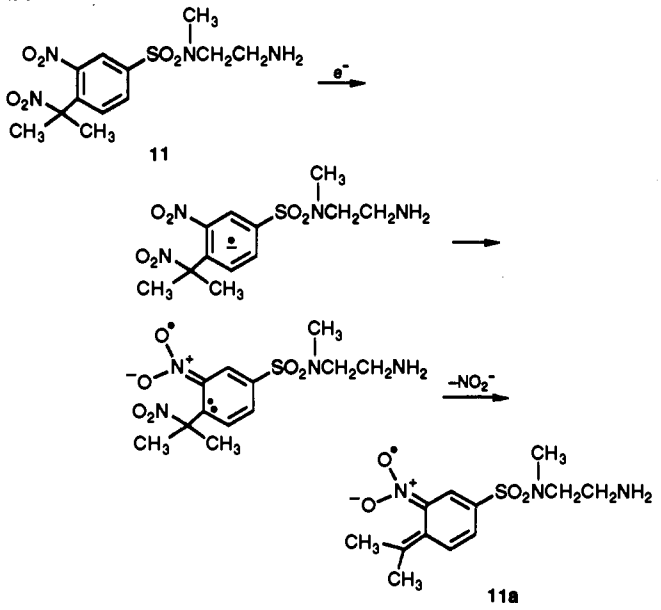
Scheme I

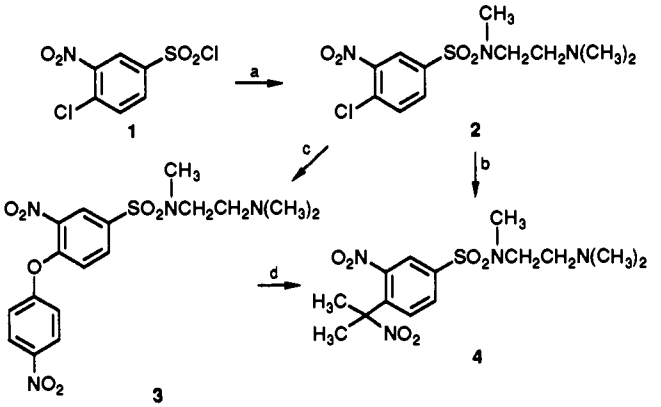


The pioneering work of Kornblum[7] on $S_{RN}1$ substitution reactions of nitroaromatics suggested that this mechanism might be applied to the selective generation of cytotoxic species in hypoxic cells. Particularly appealing are the observations that $S_{RN}1$-mediated reactions proceed through a radical chain mechanism initiated by the generation of a nitro radical anion and that the $S_{RN}1$ mechanism is completely inhibited by molecular oxygen. This latter property offers unique opportunities for the selective release of toxic agents in hypoxic vs oxic environments.

The weakly basic nitrobenzenesulfonamide series of hypoxic cell radiation sensitizers[8] appears to be an ideal format for exploring applications of the $S_{RN}1$ mechanism to selective hypoxic cell therapy. Several members of this series were found to have one-electron reduction potentials in the -320 to -350 mV range and to be effective radiation sensitizers in vitro. For example, generation of the radical anion of 11 in a reducing environment could lead to the intramolecular expulsion of nitrite ion by an $S_{RN}1$ mechanism as depicted in Scheme I. In addition, radical 11a formed in this process is a reactive species and might also contribute to cytotoxic reactions.

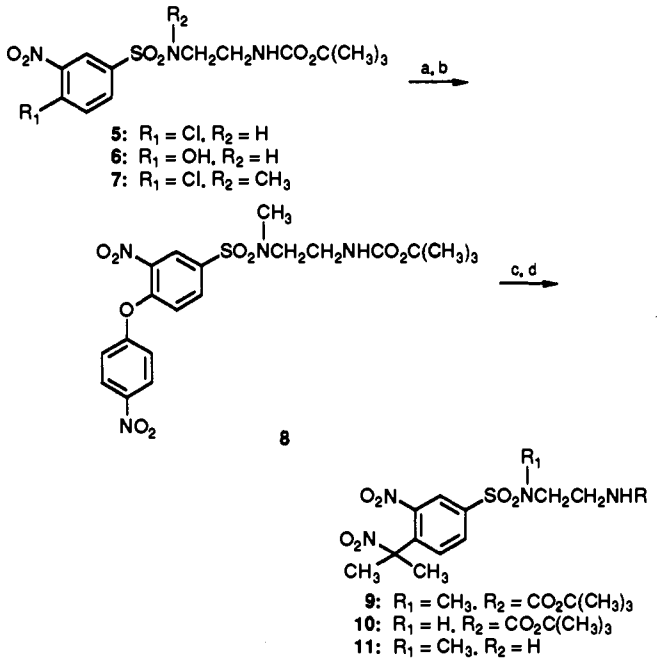
In this report, we describe the syntheses and evaluation of some nitrobenzenesulfonamide derivatives with the potential of releasing nitrite ion and hydroxyurea, a DNA repair and synthesis inhibitor,[9] selectively within hypoxic cells by a $S_{RN}1$ mechanism.

Scheme II[a]



[a] (a) $Me_2NCH_2CH_2NHMe$, $(i\text{-}Pr)_2NEt$, THF, 0 °C for 1 h, 25 °C for 24 h, 77.8%; (b) $LiMe_2CNO_2$, DMSO, 25 °C, 3 days, 10.6%; (c) $NaOC_6H_4NO_2(p)$, DMF, 60 °C, 20 h, 97%; (d) $LiMe_2CNO_2$, DMSO, 25 °C, 3 h, 49.6%.

Scheme III[a]



[a] (a) Me_2SO_4, aqueous NaOH, toluene, 0 °C for 40 min, 25 °C for 21 h, 74.8%; (b) $LiOC_6H_4NO_2(p)$, hexane, 60 °C, 25 h, 86.2%; (c) $LiMe_2CNO_2$, HMPA, 25 °C, 3 days, 39.6%; (d) HCl(g), EtOAc, 0 °C for 30 min, 25 °C for 30 min, 97.4%.

Chemistry

Commercially available sulfonyl chloride 1 served as starting material for the syntheses of dinitro compounds 4 (Scheme II) and 11 (Scheme III). Condensation of 1 with *N,N,N'*-trimethylethylenediamine gave sulfonamide 2, which could be converted to 4 in 10–16% yield by reaction with the lithium salt of 2-nitropropane.[10] A higher overall yield of C-alkylation product 4 (48%) was obtained upon conversion of 2 to *p*-nitrophenyl ether 3 by reaction with sodium *p*-nitrophenoxide and subsequent displacement of *p*-nitrophenoxide with lithio-2-nitropropane.

Synthesis of 11 required protection of the primary amine with a blocking group which would prevent self condensation with the *o*-chloronitro function and which could

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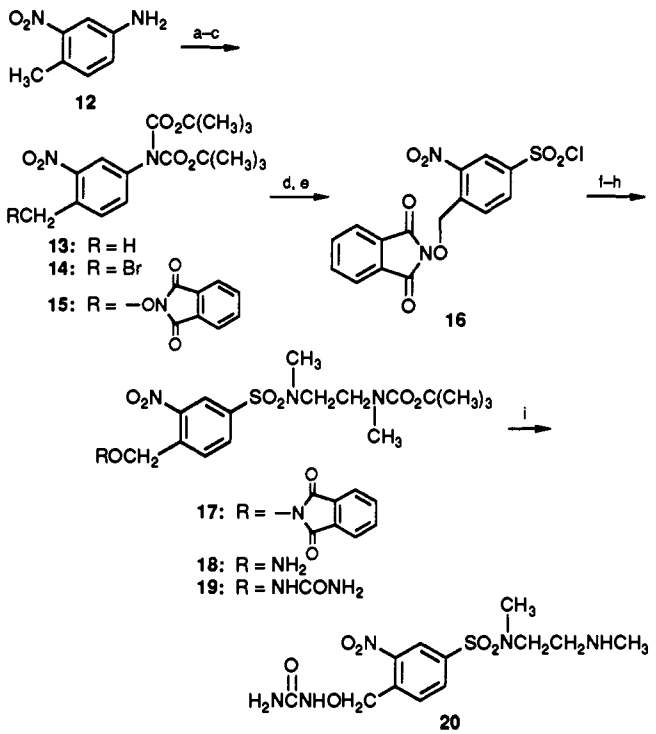
Scheme IV[a]



[a] (a) $(t\text{-BuOCO})_2O$, DMAP, MeCN, 80 °C, 25 °C for 18 h, 95%; (b) NBS, CCl_4, reflux, 20 h, 69%; (c) PhthOH, Et_3N, MeCN, reflux, 6 h, 57%; (d) TFA, CH_2Cl_2; (e) aqueous $NaNO_2$, concentrated HCl, HOAc, –5 °C, 25 min, SO_2, $CuCl_2$, 0 °C for 1 h, 25 °C for 2 h, 90.4%; (f) $MeNHCH_2CH_2NMeBoc$, $(i\text{-Pr})_2NEt$, THF, 0 °C for 45 min, 25 °C for 3 h, 81.9%; (g) NH_2NH_2, EtOH, reflux, 3 h, 46%; (h) Me_3SiNCO, toluene, 80 °C, 18 h, 38%; (i) HCl(g), EtOAc, 0 °C for 20 min, 25 °C for 1 h, 83%.

eventually be removed in the presence of nitro groups. The *tert*-butoxycarbonyl (Boc) moiety appeared to satisfy these conditions.

Condensation of sulfonyl chloride 1 with the mono-Boc derivative of ethylenediamine[11] gave sulfonamide 5 in high yield (Scheme III). However, reaction of 5 with lithio-2-nitropropane under a variety of conditions gave mainly the O-alkylation phenol product 6 (42%) after workup instead of the desired C-alkylation product 10. It is suspected that competing O-alkylation also contributes to the low yield of 4 obtained upon reaction of 2 with lithio-2-nitropropane.

Since *p*-nitrophenoxide displacement proved to be advantageous in the preparation of 4, a similar strategy was applied to the synthesis of 11. Alkylation of 5 with dimethyl sulfate gave 7 which was converted to *p*-nitrophenyl ether 8 by reaction with sodium *p*-nitrophenoxide. Displacement of *p*-nitrophenoxide from 8 with nitropropane anion then afforded dinitro derivative 9 in 34% overall yield. The relative efficiency of C- and O-alkylation is known to be dependent upon the nature of the leaving group in displacement reactions of nitro anions.[7] Removal of the Boc protective group under acidic conditions completed the synthesis of 11.

Synthesis of hydroxyurea derivative 20 required a different approach and began with nitrotoluidine 12 (Scheme IV). Protection of the amino function of 12 as the di-Boc derivative 13[12] followed by NBS radical bromination gave 14, which was condensed with hydroxyphthalimide to give 15 in 37.5% overall yield. Introduction of the chlorosulfonyl function was accomplished in high yield by diazotization of the deprotected amine corresponding to 15 and reaction with SO_2.

Condensation of sulfonyl chloride 16 with the mono-Boc derivative of *N,N'*-dimethylethylenediamine[11] and removal of the phthalimide protective group with hydrazine led to hydroxyamine 18. After introducing the urea function with trimethylsilyl isocyanate, the Boc group was removed with anhydrous HCl to give 20.

Table I. Partition Coefficients and pK_a Values of 4, 11, 20, and Pimonidazole

compd	part. coeff[a]	pK_a
4	7.4	7.83
11	0.37	8.45
20	0.28	8.39
pimonidazole[b]	0.40[c]	8.8[d]

[a] *n*-Octanol/pH 7.4 buffer. [b] α-[(2-Nitro-1*H*-imidazol-1-yl)-methyl]-1-piperidineethanol. [c] Reference 13. [d] Reference 14.

Results and Discussion

Evidence that nitrite ion could be generated from the nitrobenzenesulfonamides under reducing conditions was obtained upon electrolytic reduction of 4 in DMF. When carried out under an atmosphere of argon, 92.9% of the theoretically available nitrite was released in 4–5 min while none was formed under oxic conditions. These results are consistent with elimination of nitrite by an $S_{RN}1$ mechanism.

The tertiary nature of the aliphatic nitro group in these derivatives is especially advantageous for $S_{RN}1$ reactions. Loss of nitrite by S_N2 displacement is difficult due to the hindered nitroisopropyl group, and the electronegative nature of the aromatic ring discourages S_N1 reaction. Loss of nitrite then is only possible through the internal $S_{RN}1$ mechanism. In addition, dimethyl substitution on the C atom bearing the aliphatic nitro group eliminates the problem of having strongly acidic C–H bonds at this position.

Determination of partition coefficients and pK_a values for 4 and 11 (Table I) revealed that in these respects, primary amine 11 is quite similar to the clinically effective hypoxic cell radiosensitizer pimonidazole.[13,14] Since pimonidazole is known to have significant tumor penetration in vivo,[15] it appeared that primary amine 11, rather than 4, would have better transport properties in possible future animal studies. Therefore, primary amine 11 was selected for in vitro assessment of cytotoxic potential.

Studies were performed with exponentially growing cultures of EMT6 mouse mammary tumor cells incubated with the test compound for 2 h under aerobic and hypoxic conditions using methodology previously described.[16,17] In vitro, 11 showed a striking toxicity to hypoxic EMT6 cells,

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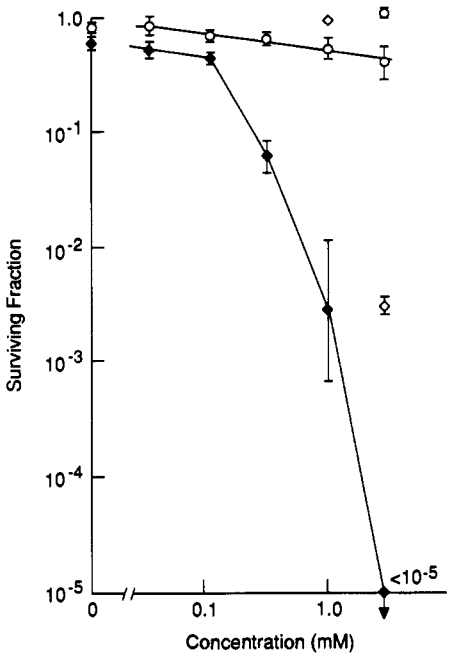

Figure 1. Cytotoxicity of a 2 h treatment with different dose of 11 or **20** in air or hypoxia: **20**, hypoxia (●); **20**, air (○); 11, hypoxia (♦); 11, air (◇).

(Figure 1). At 1 mM concentration, this compound reduced the survival of hypoxic EMT6 cells to 3×10^{-3}, while survival of aerobic cells was virtually unchanged. A higher concentration, 3 mM, essentially abolished colony formation in hypoxia (only an upper estimate of the surviving fraction is given in Figure 1, as many platings produced no colonies). However, this concentration was also toxic to aerobic cells.

In radiation experiments, 11 appeared to function as a hypoxic cell radiosensitizer as well as a selective cytotoxic agent. However, the complexity of the data makes unambiguous identification of the processes and mechanisms involved problematic. The survival curve for aerobic cells treated with 1 nM 11 (Figure 2) suggests a possible small sensitizing or cytotoxic effect in air in that it differs slightly from that for radiation alone (the difference in elevation is statically significant; the difference in D_0 is not).[18]

In contrast, regimens combining 1 mM concentrations of 11 and radiation under hypoxic conditions were extremely toxic (Figure 3). The data suggest a steepening of the dose–response curve, and therefore radiosensitization, but large error limits on the survival data preclude rigorous demonstration of sensitization, i.e., of a statistically significant change in D_0. The large experimental error in this data is probably a reflection of the nature of the cytotoxicity. Small run to run variations in the speed with which the cultures became hypoxic, the temperature of the X-ray room, etc. will all markedly affect the cytotoxicity of the drug and thereby introduce error into replicate experiments. Moreover, measurements of cell viability become increasingly difficult and imprecise at very low cell survivals, such as those observed with this compound in these studies. The large effect seen with 1 mM 11 in hypoxia [extensive cytotoxicity (Figures 1 and 3), plus an SER of 2.9 (Figure 3)] contrasts markedly with the limited cytotoxicity and statistically insignificant SER of 1.1 seen

(18) D_0 is defined as the dose necessary to reduce survival by a factor of $1/e$ on the exponential portion of the dose–response curve. The extrapolation number, n, is the value at which the linear portion of the survival curve would intercept the abscissa if extrapolated back to zero dose.

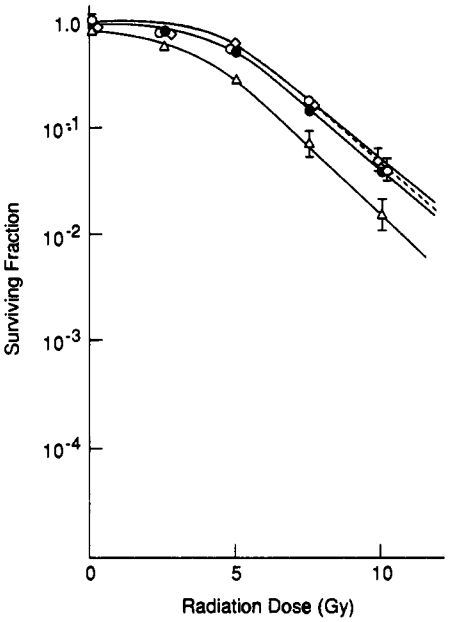

Figure 2. Radiation dose–response curves for aerobic cells treated with 11, **20**, or misonidazole for 75 min before and during irradiation: no drug, $D_0 = 1.9$, $n = 7.0$ (●); 1 mM misonidazole, $D_0 = 2.0$, $n = 7.3$ (○); 1 mM 11, $D_0 = 1.7$, $n = 5.1$ (△); 1 mM **20**, $D_0 = 2.0$, $n = 8.0$ (◇).

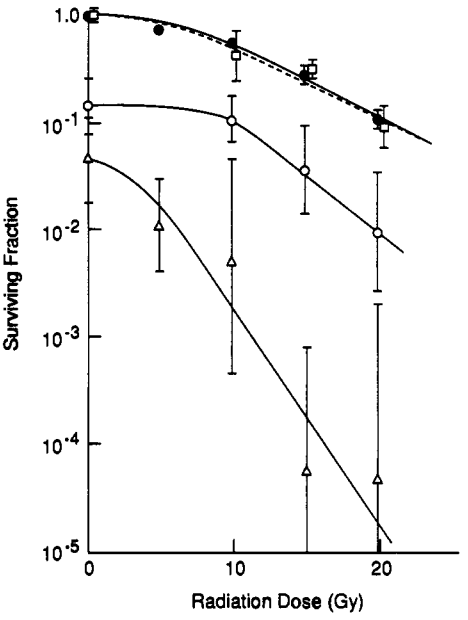

Figure 3. Radiation dose–response curves for cells treated with 11 for 75 min before and during irradiation in hypoxia: radiation only, $D_0 = 6.1$, $n = 2.9$ (●); 0.1 mM 11, $D_0 = 6.3$, $n = 2.4$ (□); 0.3 mM 11, $D_0 = 4.1$, $n = 1.2$ (○); 1 mM 11, $D_0 = 2.1$, $n = 0.21$ (△).

in air (Figures 1 and 2), indicating that the effect is produced selectively under hypoxic conditions. A small radiosensitizing effect, as well as a cytotoxic effect, was seen in hypoxia at a concentration of 0.3 mM, while no significant cytotoxic or radiosensitizing effects were seen at 0.1 mM.

Since 11 appeared to be selectively toxic to hypoxic cells and also to have radiopotentiating activity under hypoxic conditions, this compound was examined for its effects, alone and in combination with X-rays, on solid EMT6 tumors in BALB/c mice. In these experiments, 11 was injected either at a dose of 200 mg/kg ip or 100 mg/kg iv. In the radiation studies, radiation was given 30 min after injection of drug, and surviving fractions were measured

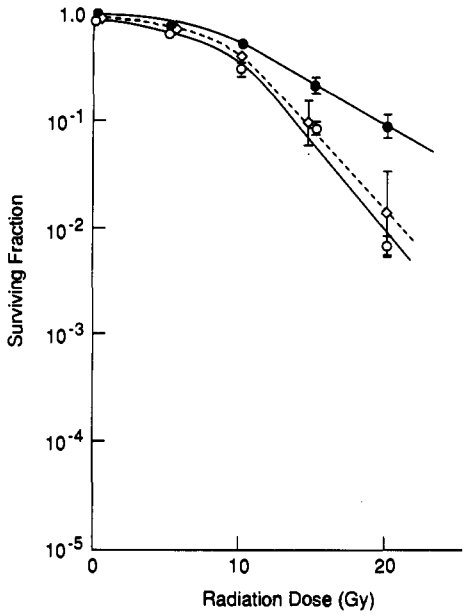

Figure 4. Radiation dose–response curves for cells treated with **20** or misonidazole for 75 min before and during irradiation in hypoxia: radiation only, $D_0 = 5.6$, $n = 3.2$ (●); 1 mM misonidazole, $D_0 = 2.6$, $n = 12$ (○); 1 mM **20**, $D_0 = 3.0$, $n = 11$ (◇).

2 h after drug treatment. Two individual surviving fraction determinations for drug given by either route, alone or with 15 Gy of X-rays, were performed. In all cases the surviving fractions were not significantly different from the respective control values. These measurements provided no evidence for significant cytotoxic or radiosensitizing activity of **11** in this in vivo solid tumor system.

This finding was disappointing, as the cytotoxicity and radiosensitization seen in vitro raised the possibility of significant and therapeutically useful activity at doses achievable in solid tumors. The reasons for this dichotomy between the in vitro and in vivo data for **11** are not clear. The compound is stable under oxic conditions at pH 7.4 and in rat plasma at 37 °C and is not extensively bound to bovine serum albumin, $K_D = 1.09 \times 10^{-4}$. Reasonable speculative causes may involve metabolism, pharmacokinetics, or intratumoral drug distribution.

Hydroxyurea derivative **20** showed only a modest differential toxicity toward hypoxic EMT6 cells in vitro. Survival was reduced to about 50% of that seen with the sham-treated controls after a 2-h incubation of **20** at 3 mM concentration, a concentration approaching the solubility limit; this treatment was not toxic to aerobic cells (Figure 1). At a concentration of 1 mM, **20** was an effective hypoxic cell radiosensitizer, comparable to misonidazole at the same concentration (Figure 4). No sensitization of aerobic cells was observed (Figure 2).

In summary, nitrite-releasing compounds such as **11** may have potential as hypoxic cell selective cytotoxic and radiosensitizing agents if the problem limiting in vivo activity can be determined and circumvented. Extension of this concept to other structural types, such as the nitroimidazoles, should be considered.

Experimental Section

All melting points were obtained on a Thomas-Hoover Unimelt capillary melting point apparatus using open capillaries and are uncorrected. Analytical results are indicated by atom symbols and are within 0.4% of theoretical values. ^{1}H NMR spectra were recorded for all intermediates and final products on either a Varian XL-300 or a GE NT-360 instrument using Me_4Si as internal standard and are consistent with assigned structures. E. Merck silica gel, 200–400 mesh, was used for the flash chromatographies.

***N*-[2-(Dimethylamino)ethyl]-*N*-methyl-4-chloro-3-nitrobenzenesulfonamide Hydrochloride (2).** A solution of commercial 93% *N,N,N'*-trimethylethylenediamine (21.4 mL, 0.157 mol) and *N,N*-diisopropylethylamine (28 mL, 0.161 mol) in THF (150 mL) was added over 1 h to a stirred, cooled solution of 4-chloro-3-nitrobenzenesulfonyl chloride (40 g, 0.156 mol) in THF (250 mL). After addition was complete, the reaction mixture was stirred in the ice bath for 1 h, at 20–5 °C for 25 h and then concentrated under reduced pressure. After partitioning between EtOAc and H_2O, the organic extract was washed with a saturated aqueous solution of NaCl, dried (Na_2SO_4), filtered, and concentrated. The residue was treated with anhydrous EtOH–HCl and the salt recrystallized from EtOH–EtOAc to give 43.5 g (77.8%) of product, mp 220–4 °C dec. An analytical sample, mp 227–30 °C dec, was obtained upon further recrystallization from MeOH–EtOAc. Anal. ($C_{11}H_{16}ClN_3O_4S$·HCl) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-*N*-methyl-4-(1-methyl-1-nitro-1-ethyl)-3-nitrobenzenesulfonamide Hydrochloride (4) Method A. By Displacement of Chloride.** A solution of *N*-[2-(dimethylamino)ethyl]-*N*-methyl-4-chloro-3-nitrobenzenesulfonamide base (15.65 g, 49 mmol) and the Li salt of 2-nitropropane[10] (4.66 g, 49 mmol) in DMSO (100 mL) was stirred under N_2 at 20–5 °C for 3 days. An additional 0.35 g of the Li salt was then added and stirring continued for 3 days more. After pouring the reaction mixture on ice, product was extracted into a 1:1 mixture of EtOAc and toluene which was washed with water, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography of the residue over silica gel and elution with 3% MeOH–97% CH_2Cl_2 afforded pure product. Treatment with anhydrous EtOH–HCl and recrystallization from MeOH–EtOAc–hexane gave the HCl salt (2.13 g, 10.6%): mp 231–2.5 °C dec; ^{1}H NMR (D_2O) δ = 2.13 (6 H, s), 2.88 (3 H, s), 3.03 (6 H, s), 3.46 (2 H, m), 3.53 (2 H, m), 8.17 (1 H, d), 8.28 (1 H, d *of* d), 8.54 (1 H, d). Anal. ($C_{14}H_{22}N_4O_6S$·HCl) C, H, N.

Method B. By Displacement of *p*-Nitrophenoxide. To a solution of *N*-[2-(dimethylamino)ethyl]-*N*-methyl-4-chloro-3-nitrobenzenesulfonamide (11.7 g, 36 mmol) and 4-nitrophenol (5.07 g, 36 mmol) in DMF (156 mL) under N_2 was added over 15 min 60% NaH–mineral oil suspension (1.46 g, 36 mmol). The resulting solution was stirred at 60 °C for 20 h under N_2. After concentrating under reduced pressure at 50 °C, the residue was partitioned between EtOAc and water. The organic extract was washed with brine, dried (Na_2SO_4), filtered, and concentrated. The residue was flash chromatographed over silica gel and 15 g of oily nitrophenyl ether **3** eluted with 3% MeOH–97% $CHCl_3$: ^{1}H NMR ($CDCl_3$) δ = 2.27 (6 H, s), 2.54 (2 H, t), 2.90 (3 H, s), 3.49 (2 H, t), 7.16 (2 H, d), 7.25 (1 H, d), 8.04 (1 H, d), 8.32 (2 H, d), 8.55 (1 H, s).

A solution of *p*-nitrophenyl ether **3** (15 g, 35 mmol) and the Li salt of 2-nitropropane[10] (5.03 g, 52.5 mmol) in DMSO (200 mL) was stirred under N_2 for 1 day. Additional Li salt (3.36 g, 35 mmol) was added and the reaction stirred for 6 h more. After pouring of the reaction mixture onto ice, product was extracted into EtOAc, which was washed with brine, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography over silica gel and elution with 5% MeOH–95% $CHCl_3$ gave 6.5 g (48.2% overall yield) of product identical with that prepared by method A.

***N*-[2-[[(1-Dimethylethoxy)carbonyl]amino]ethyl]-4-chloro-3-nitrobenzenesulfonamide (5).** Mono-Boc-ethylenediamine[11] (14.7 g, 92 mmol) and *N,N*-diisopropylethylamine (18 mL, 101 mmol) were dissolved in THF (200 mL). The solution was stirred in an ice bath under N_2 while a solution of 4-chloro-3-nitrobenzenesulfonyl chloride (24 g, 92 mmol) in THF (130 mL) was added dropwise over 2 h. After standing overnight, water (2 mL) was added and THF removed under vacuum. The residue was dissolved in EtOAc (250 mL) and the solution washed with brine, dried (Na_2SO_4), filtered, and concentrated to about 100 mL. Diluting with hexane (150 mL) and cooling gave 23.5 g (67.2%) of product, mp 129–31 °C. An analytical sample, mp 130–2 °C, was obtained upon recrystallization from EtOAc–hexane. Anal. ($C_{13}H_{18}N_3ClO_6S$) C, H, N.

***N*-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]-4-hydroxy-3-nitrobenzenesulfonamide (6).** A solution of chloro derivative **5** (0.50 g, 1.32 mmol) and lithio-2-nitropropane[10] (375 mg, 3.95 mmol) in DMSO (10 mL) was stirred at room temperature under N_2 for three days and poured onto ice. After acidifying

with citric acid, product was extracted into EtOAc. The organic extract was washed with saturated $NaHCO_3$ solution and brine and dried (Na_2SO_4). The filtered solution was concentrated under reduced pressure and the residue flash chromatographed over silica gel. Elution with 3% MeOH–97% $CHCl_3$ gave 200 mg (42%) of phenol 6. Recrystallization from EtOAc–hexane gave an analytical sample: mp 147–50 °C; ^1H NMR ($CDCl_3$) δ = 1.41 (9 H, s), 3.10 (2 H, m), 3.25 (2 H, m), 4.81 (1 H, br s), 5.55 (1 H, br s), 7.30 (1 H, d), 8.02 (1 H, d), 8.63 (1 H, s). Anal. ($C_{13}H_{19}N_3O_7S$) C, H, N.

***N*-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]-*N*-methyl-4-chloro-3-nitrobenzenesulfonamide (7).** Sulfonamide 5 (15.2 g, 40 mmol) was added to an ice-cold solution of NaOH (8.0 g, 200 mmol) in water (200 mL). Toluene (200 mL) was added and the mixture stirred vigorously in an ice bath while a solution of dimethyl sulfate (10.1 g, 80 mL) in toluene (40 mL) was added dropwise over 40 min. The reaction mixture was allowed to warm to room temperature over 4 h and an additional portion of dimethyl sulfate (7.3 mL) in toluene (10 mL) was added. After stirring at room temperature for 17 h, toluene (200 mL) was added. The organic layer was washed with 1 N NaOH and water and then dried (Na_2SO_4). Removal of toluene gave 11.8 g (74.8%) of product, mp 118–9 °C. An analytical sample, mp 119.5–21.5 °C, was obtained upon recrystallization from EtOAc. Anal. ($C_{14}H_{20}N_3ClO_6S$) C, H, N.

***N*-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]-*N*-methyl-3-nitro-4-(4-nitrophenoxy)benzenesulfonamide (8).** A solution of 4-nitrophenol (3.12 g, 22.4 mmol) was added gradually to a stirred slurry of LiH (178 mg, 22.4 mmol) and hexane (10 mL). When H_2 evolution was complete, chloro compound 7 (4.26 g, 10.8 mmol) was added. After stirring at 60 °C for 25 h, hexane was removed under a stream of N_2 and the residue partitioned between toluene and 1 N NaOH. The organic extract was washed with brine, dried (Na_2SO_4), filtered, and concentrated. Recrystallization from EtOAc–hexane gave 4.63 g (86.2%) of product, mp 110–1 °C. Anal. ($C_{20}H_{24}N_4O_9S$) C, H, N.

***N*-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]ethyl]-*N*-methyl-3-nitro-4-(1-methyl-1-nitroethyl)benzenesulfonamide (9).** Lithio-2-nitropropane[10] (1.77 g, 18.6 mmol) was added to a cooled solution of nitrophenyl ether 8 (4.63 g, 9.33 mmol) in HMPA (28 mL). After stirring in an ice bath for 1 h, the solution was stirred at room temperature for 3 days. Equal volumes of toluene and EtOAc were added, and the mixture was washed with 1 N NaOH and brine. The organic extract was dried (Na_2SO_4), filtered, and concentrated. Recrystallization from EtOAc gave 1.65 g (39.6%) of product: mp 146–50 °C; ^1H NMR ($CDCl_3$) δ = 1.45 (9 H, s), 2.10 (6 H, s), 2.90 (3 H, s), 3.23 (2 H, m), 3.37 (2 H, m), 4.86 (1 H, br s), 7.79 (1 H, d), 8.06 (1 H, d of d), 8.32 (1 H, d). Anal. ($C_{17}H_{28}N_4O_8S$) C, H, N.

***N*-(2-Aminoethyl)-*N*-methyl-3-nitro-4-(1-methyl-1-nitroethyl)benzenesulfonamide Hydrochloride Hemihydrate (11).** A solution of Boc-protected amine 9 (2.50 g, 5.60 mmol) in EtOAc (80 mL) was cooled in an ice bath and saturated with HCl gas for 15 min. After stirring at ice-bath temperature for 15 min more and then at room temperature for 30 min, the precipitated solid was removed, washed with EtOAc, and dried to give 2.14 g (97.4%) of product. A 5.0-g portion of blended products from several runs was dissolved in water (67 mL) at 80 °C. The solution was filtered hot and the filtrate diluted with concentrated HCl. After cooling, product was filtered off, washed with 1 N HCl, and dried to give analytically pure material: mp 210–1 °C dec; ^1H NMR (D_2O) δ = 2.13 (6 H, s), 2.90 (3 H, s), 3.27 (2 H, m), 3.43 (2 H, m), 8.15 (1 H, d), 8.27 (1 H, d of d), 8.53 (1 H, d). Anal. ($C_{12}H_{18}N_4O_6S\cdot HCl\cdot 0.5H_2O$) C, H, N.

***N*,*N*-Bis[(*tert*-butyloxy)carbonyl]-3-nitro-4-[(phthalimidooxy)methyl]aniline (15).** A solution of di-*tert*-butyl dicarbonate (45.9 g, 0.21 mol) in MeCN (75 mL) was added to a stirred solution of 4-methyl-3-nitroaniline (15 g, 0.099 mol) and 4-(dimethylamino)pyridine (1.2 g, 9.9 mmol) in MeCN (150 mL) over 15 min and the mixture warmed at 80 °C until solution was complete. After stirring at room temperature for 18 h, solvents were removed under reduced pressure, and the residue was partitioned between EtOAc and 10% citric acid. The organic layer was washed with water and then brine and dried (Na_2SO_4). The filtered solution was concentrated under reduced pressure and the residue flash chromatographed over silica gel. Elution with 1:1 hexane–$CHCl_3$ gave 33.0 g (95%) of 13 as a yellow oil.

A mixture of *N*,*N*-bis[(*tert*-butyloxy)carbonyl]-4-methyl-3-nitroaniline (13; 33 g, 93.6 mmol), *N*-bromosuccinimide (21.4 g, 120 mol), and dibenzoyl peroxide (50 mg) in CCl_4 (500 mL) was stirred at reflux under a 100-W bulb for 20 h. After cooling, succinimide was removed by filtration, and solvents were removed under reduced pressure. This residue, containing 69% bromomethyl derivative 14, was dissolved in MeCN (500 mL). *N*-Hydroxyphthalimide (13.1 g, 80 mmol) and Et_3N (11.2 mL) were added, and the solution was stirred at reflux for 6 h under a Drierite tube. After concentrating under reduced pressure, the residue was partitioned between EtOAc and water. The water extract was reextracted two times more with fresh EtOAc, and the organic extracts were combined. The extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography over silica gel gave 19 g (39.5%) of 15 upon elution with CH_2Cl_2. An analytical sample, mp 112–22 °C was obtained upon recrystallization from EtOAc–hexane: ^1H NMR ($CDCl_3$) δ = 1.45 (18 H, s), 5.68 (2 H, s), 7.51 (1 H, d of d), 7.78 (2 H, m) 7.82 (2 H, m), 7.98 (1 H, d), 8.02 (1 H, d). Anal. ($C_{25}H_{27}N_3O_9$) C, H, N.

***N*-[2-[*N*-[(*tert*-Butyloxy)carbonyl]-*N*-methylamino]ethyl]-*N*-methyl-3-nitro-4-[(phthalimidooxy)methyl]benzenesulfonamide (17).** A solution of aniline derivative 15 (4.3 g, 8.37 mmol) in CH_2Cl_2 (100 mL) was cooled in an ice bath and CF_3CO_2H (12 mL) added. The reaction mixture was stirred in the ice bath under a Drierite tube for 6 h and then at 6 °C overnight. Solvents were removed under vacuum. A cold mixture of AcOH (40 mL) and concentrated HCl (40 mL) was added and the mixture cooled to −10 °C. A solution of $NaNO_2$ (640 mg, 9.3 mmol) in water (6 mL) was added over 10 min. After addition was complete, the mixture was stirred at −5–0 °C for an additional 15 min and added in a stream to a stirred mixture of SO_2 (13 g) in AcOH (40 mL) and $CuCl_2\cdot 2H_2O$ (0.9 g) in water (3 mL). After stirring at 0 °C for 1 h and then at room temperature for 2 h, the reaction mixture was poured onto ice. The precipitated tan solid was removed by filtration, washed with water, and dissolved in EtOAc. After drying and filtering of the EtOAc solution, solvent was removed under reduced pressure to give 3.0 (90.4%) of sulfonyl chloride 16, mp 138–48 °C slow dec.

A solution of *tert*-butyl *N*-methyl-*N*-[2-(methylamino)ethyl]-carbamate[11] (1.43 g, 7.56 mmol) and *N*,*N*-diisopropylethylamine (1.32 mL, 7.56 mmol) in THF (40 mL) was added over 15 min to a stirred, cooled solution of sulfonyl chloride 16 (3.0 g, 7.56 mmol) in THF (75 mL). After addition was complete, the reaction mixture was stirred at ice-bath temperature for 30 min and then at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure and the residue partitioned between EtOAc and brine. The EtOAc extract was dried (Na_2SO_4), filtered, and concentrated. Flash chromatography of the residue over silica gel and elution with 2% MeOH–98% $CHCl_3$ gave 3.4 g (81.9%) of 17. An analytical sample, mp 114.5–17.5 °C, was obtained upon recrystallization from EtOAc–hexane. Anal. ($C_{24}H_{28}N_4O_9S$) C, H, N.

***N*-[2-[*N*-[(*tert*-Butyloxy)carbonyl]-*N*-methylamino]ethyl]-*N*-methyl-3-nitro-4-[(ureidooxy)methyl]benzenesulfonamide (19).** A solution of phthalimide 17 (1.0 g, 1.82 mmol) and 55% hydrazine (116 mg, 2.0 mmol) in EtOH (20 mL) was stirred at 80 °C for 3 h. After filtering and concentrating, the residue was flash chromatographed over silica gel. Elution with 1% MeOH–99% $CHCl_3$ gave 0.35 g (46%) of hydroxylamine 18.

A solution of 18 (0.35 g, 0.84 mmol) and 85% trimethylsilyl isocyanate (0.27 mL, 1.68 mmol) in toluene (15 mL) was stirred at 80 °C for 18 h. After cooling, 10% NH_4Cl (15 mL) was added and the mixture stirred at room temperature for 30 min. EtOAc was added and the organic extract was washed with brine, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography of the residue over silica gel and elution with 3% MeOH–97% $CHCl_3$ gave 0.15 g (38%) of product. An analytical sample, mp 165.0–5.5 °C, was obtained upon recrystallization from MeOH–EtOAc–hexane. Anal. ($C_{17}H_{27}N_5O_8S$) C, H, N.

***N*-Methyl-*N*-[2-(methylamino)ethyl]-3-nitro-4-[(ureidooxy)methyl]benzenesulfonamide Hydrochloride (20).** A mixture of Boc-protected amine 19 (0.58 g, 1.26 mmol) and EtOAc (200 mL) was cooled in an ice bath and saturated with HCl gas for 5 min. After stirring at ice-bath temperature for 15 min and

then at room temperature for 60 min, solvent was removed under reduced pressure and the residue recrystallized from MeOH–EtOAc–hexane to give 0.41 g (83%) of analytically pure product: mp 195–7 °C; ^{1}H NMR (D_2O) δ = 2.82 (3 H, s), 2.88 (3 H, s), 3.32 (2 H, m), 3.45 (2 H, m), 5.34 (2 H, s), 8.05 (1 H, d), 8.23 (1 H, d of d), 8.57 (1 H, d). Anal. ($C_{12}H_{19}N_5O_6S$·HCl) C, H, N.

Nitrite Release from Electrolytic Reduction of 4. A 0.01 mM solution of 4 in 0.1 M tetrabutylammonium perchlorate–DMF solution (5 mL) was electrolyzed with dc polarography at a Hg electrode (vs Ag/AgCl) under Ar. Immediately after electrolysis for 4–5 min, 1 mL of the resulting solution was added to the supporting electrolyte, 4 mL of an aqueous solution (pH 2–3) of diphenylamine, NaSCN, and $HClO_4$. Released nitrite was determined by differential pulse polarography under conditions where the test compound did not release nitrite. Nitrite concentration was determined by comparison of peak current with calibration curves. The method of standard additions was also used to insure accuracy.

Biological Assays. In vitro studies were performed with exponentially growing cultures of EMT6 mouse mammary tumor cells incubated under aerobic and hypoxic conditions. The methodology used was essentially the same as that previously reported.[16] In cytotoxicity studies (Figure 1) cells were treated with drug for 2 h under fully hypoxic conditions. In radiation/drug interaction studies, cells were treated with drug for a total of 75 min and were irradiated (or sham irradiated) during the final minutes of drug treatment with 250-kV X-rays at a dose rate of ~1 Gy/min. In all cases, points shown are geometric mean ± SEM's of survivals determined in three or more independent experiments. Survival curves, D_0's, and n's were fitted by regression analyses.[1,17,18] Cells treated with radiation alone and with radiation plus misonidazole were included in the studies, as were drug- and solvent-treated controls. The oxygen enhancement ratios, calculated from the ratios of the D_0's for cells irradiated in hypoxia and air, were 3.2 and 2.9 for the experiments shown in Figures 3 and 4, respectively, showing that good, radiobiological hypoxia was achieved. Sensitizer enhancement ratios (SERs) were calculated analogously, as the ratios of the D_0's in the absence and presence of drug.

In vivo experiments were performed with EMT6 mouse mammary tumors in BALB/c mice. In these studies, the response of the tumors to treatment was assessed by euthanizing the animals after treatment, removing the tumors, preparing a single cell suspension from the tumors, and assaying the ability of the suspended cells to form colonies in vitro.[16]

Stability of 11 at pH 7.4 and in Rat Plasma. Stability of 11 in both pH 7.4 buffer at 23 °C and rat plasma at pH 7.4 and 37 °C was studied. A 10.5 mM solution of the compound in 0.085% H_3PO_4 was prepared. One hundred microliters of this solution was added to 1600 μL of rat plasma and 400 μL of pH 7.4 phosphate buffer to yield an assay concentration of 0.5 mM. The pH was monitored with an Orion Research pH meter and maintained by the addition of 0.1 N HCl. Temperature was maintained by using a Haake circulating water bath.

For the plasma sample, 50-μL aliquots were removed periodically and quenched in 950 μL of 7% $HClO_4$. The quenched solutions were centrifuged (Beckman Microfuge) and the supernate analyzed on a Hewlett-Packard HPLC (50-μL injection, 280 nm, gradient elution of 5–95% MeCN with 0.085% H_3PO_4 over 10 min at a flow rate of 3 mL/min, 10-cm VYDAC C18 protein and peptide column).

The pH 7.4 buffer experiment was prepared by using the same 10.5 mM stock prepared previously and diluting 20-fold into pH 7.4 buffer. Five-microliter aliquots were taken directly by the HPLC autosampler without dilution (the temperature of the autosampler was 23 °C).

For the plasma sample, 100% of the starting material was recovered after 2 h. For the pH 7.4 buffer sample 82.2% of the starting material was recovered after 96 h.

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Communications to the Editor

Monovalent Sialosides That Bind Tightly to Influenza A Virus

The first step in the cascade of events that leads to infection by influenza virus is adsorption of virus particles to the surface of susceptible cells.[1] This process occurs through recognition and binding of cell-surface oligosaccharides that terminate in sialic acid (SA) residues.[2] Previous studies have shown that SA is the only component of these carbohydrate chains that is recognized by the virus[3] and that the viral entity responsible for this interaction is the protein hemagglutinin (HA).[4] One approach to combating cellular infection may be to inhibit this adsorption process by using SA analogues that compete with the cell surface sialyl oligosaccharides, for viral HA. However, the binding affinity for HA of all known monovalent α-sialosides, such as *N*-acetyl-2-*O*-methyl-α-D-neuraminic acid (Neu5Acα2Me, 1) is weak ($K_d \approx 2$ mM),[5] and the virus presumably attaches to cells at many

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