Inhibitory potencies against related enzymes were determined in assays similar to that described above,[23] 15-lipoxygenase from soybean (Sigma) or cycloxygenase from sheep seminal vesicles in place of the RBL-1 preparation.

Rat PMNL 5-Lipoxygenase Assay. Rat PMNL were obtained from the pleural cavity of nonfasted male rats (Sprague–Dawley, 200–250 g) injected intrapleurally with 200 μL of 0.05% (w/v) carrageenan. Cells were pooled and centrifuged for 15 min at 500*g*. The cell pellet was resuspended in PBS-H, washed by centrifugation, and then briefly resuspended in water for 25 s to lyse contaminating erythrocytes. The cells were washed two more times with PBS-H and suspended at a concentration of 2×10^7 cells/mL in 20 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (Sigma Chemical Co., St. Louis, MO) in Earle's balanced salts (GIBCO Laboratories, Grand Island, NY), pH 7.0, containing 1 mg/mL BSA (EH-BSA). About 7.5×10^7 cells were recovered per rat and greater than 90% of the cells were polymorphonuclear leukocytes (PMNL) as determined by differential counting using Wright's stain. Test compounds or DMSO vehicle (final concentration 2%) were preincubated with cell suspensions (5×10^6 cells/250 μL) for 15 min at 37 °C. Cellular arachidonate metabolism was initiated by adding calcium ionophore A23187 (final concentration 8.3 μM) and terminated after 10 min by rapid cooling in an ice bath. Cell supernatants were extracted with two volumes of methanol and analyzed for LTB_4 content by HPLC.

Human Whole Blood 5-Lipoxygenase Assay. Heparinized (20 units/mL) human blood (0.7 mL) was preincubated with test compounds or vehicle for 15 min at 37 °C. Eicosanoid biosynthesis was initiated by adding calcium ionophore A23187 in DMSO (final concentration 50 μM) and terminated after 30 min by rapid cooling of the blood in an ice bath and centrifuging at 3 °C for 10 min at 2500*g*. The plasma was mixed with 4 volumes of methanol and allowed to stand for at least 2 h at 3 °C prior to centrifuging at 1800*g* for 30 min. The level of LTB_4 in aliquots of the methanol–plasma extract was analyzed by RIA (Amersham Corp., Arlington Heights, IL) or by EIA (Cayman Chemical Co., Ann Arbor, MI).

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Acknowledgment. We wish to thank E. Otis, D. Bornemeier, and T. Clark for technical assistance.

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Synthesis of an (Iodovinyl)misonidazole Derivative for Hypoxia Imaging

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Nitroimidazoles undergo a bioreduction in viable hypoxic tissue, resulting in trapping within these tissues, as demonstrated by misonidazole. A radioiodinated analogue of misonidazole (IVM, (*E*)-5-(2-Nitroimidazolyl)-4-hydroxy-1-iodopent-1-ene, 3) has been synthesized by halodestannylation, for evaluation as an imaging agent for hypoxia. A key step in the synthetic sequence involves the use of the Lewis acid $BF_3 \cdot Et_2O$ to promote the nucleophilic ring opening of glycidyl tosylate with (*E*)-1-lithio-2-(tributylstannyl)ethylene. Direct comparison of IVM versus F-MISO (2) another misonidazole type hypoxic cell marker, in several in vitro cell culture studies, indicates that IVM behaves in analogous fashion to F-MISO and has promise as a hypoxia imaging agent for SPECT.

Our interest in misonidazole (MISO, 1) and its derivatives is due to the ability of viable hypoxic tissues, as might be found in ischemic heart and brain and the central regions of tumors, to metabolically trap these compounds following a bioreduction reaction.[1,2] This property was the impetus for the synthesis of ^{18}F-labeled fluoromisonidazole (F-MISO, 2) for in vivo nuclear imaging of hypoxic tissues by positron emission tomography (PET).[3-5] We now report the synthesis of an (iodovinyl)misonidazole derivative (IVM, 3) which has been labeled with ^{131}I and is being evaluated biologically as a hypoxia imaging agent.

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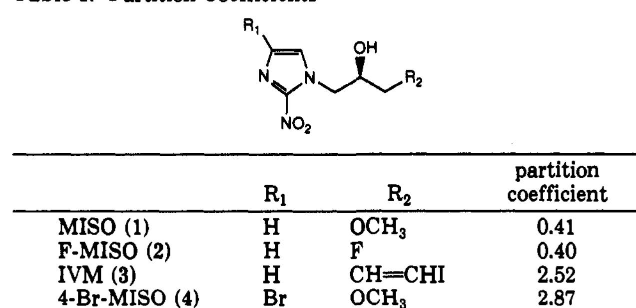
Table I. Partition Coefficients



	R_1	R_2	partition coefficient
MISO (1)	H	OCH_3	0.41
F-MISO (2)	H	F	0.40
IVM (3)	H	CH=CHI	2.52
4-Br-MISO (4)	Br	OCH_3	2.87

The success of these efforts will directly lead to radiolabeling with ^{123}I, useful with more common nuclear imaging instrumentation, including single photon emission computed tomography (SPECT).

Chemistry Results and Discussion

In addition to the synthesis of F-MISO, other work in our laboratory involved the synthesis of [^{82}Br]-4-Br-MISO

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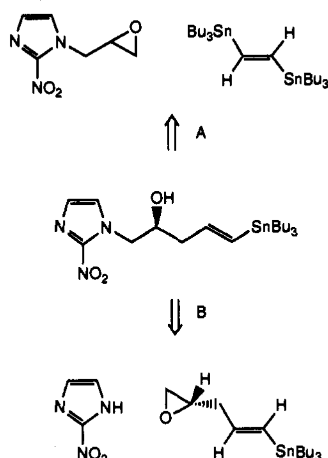
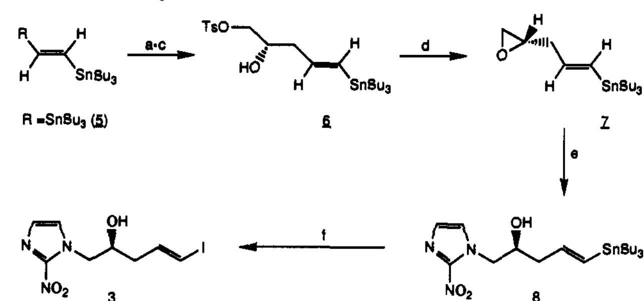
Scheme I. Retrosynthetic Scheme



Scheme II.[a] Synthetic Scheme



[a] (a) BuLi (1 equiv), R = LI; (b) $BF_3{\cdot}Et_2O$; (c) 2S-(+)-glycidyl tosylate; (d) NaOH(s); (e) 2-nitroimidazole, KOH, DMF/CH_3CN; (f) NaOAc buffer, pH 4.5, NaI, H_2O_2/HOAc.

(4). The compound possesses a favorable octanol/water partition coefficient of 2.87 (Table I); however, it undergoes debromination in vivo, resulting in a blood background of radiobromine that is not suitable for imaging.[6] We therefore felt that a compound with a similar partition coefficient that did not suffer from loss of label in vivo would be highly desirable. An (iodovinyl)misonidazole derivative (3) appeared to be an appropriate radiosynthetic target, due to the demonstrated metabolic stability of the iodovinyl moiety.[7] Further, the ease of radioiodination of a suitable precursor (a vinyltributylstannane, 8) via halodestannylation permitted the synthesis of IVM at the no-carrier-added level using commercially available [^{131}I]NaI of high specific activity. This chemistry is similar to that reported for radioiodination of 17α-(*E*)-(iodovinyl)estradiol.[8] Also, to avoid having a potentially reactive allylic hydroxyl group present in **3**, a methylene spacer between the hydroxyl and the vinyl groups was deemed necessary.

Retrosynthetic analysis (Scheme I) indicated two routes to the desired labeling precursor. Route A involved ring opening of the epoxide by the anion of bis(tributylstannyl)ethylene. Attempts to effect this conversion with the lithium anion and with the mixed cuprate from lithium 2-thienylcyanocuprate failed. Route B required that the anion from 2-nitroimidazole facilitate the ring opening of the epoxide in analogous fashion to the chemistry utilized in the synthesis of F-MISO.[3] The requisite epoxide was envisioned as being obtainable from either epichlorohydrin and TMS acetylene or glycidyl tosylate and (*E*)-1,2-bis(tributylstannyl)ethylene. The epoxide ring opening of epichlorohydrin with the lithium anion of TMS acetylene in the presence of $BF_3{\cdot}Et_2O$ has been previously reported.[9] We believed that $BF_3{\cdot}Et_2O$ could be utilized to promote the ring opening of glycidyl tosylate with the lithium anion of (*E*)-1,2-bis(tributylstannyl)ethylene. This latter route to the epoxide proved to be more efficient and is reported here.

The synthesis of IVM (3) has been accomplished in five steps in a 10% overall yield, from (*E*)-1,2-bis(tributylstannyl)ethylene (5, Scheme II). The key step in the synthetic sequence is the $BF_3{\cdot}Et_2O$-promoted nucleophilic ring opening of the oxirane glycidyl tosylate with (*E*)-1-lithio-2-(tributylstannyl)ethylene. It has been reported that $BF_3{\cdot}Et_2O$ promotes the nucleophilic addition of simply substituted alkyl-, allyl-, alkynyl-, alkenyl-, and aryllithiums to epoxides and oxetanes.[9-11] As for functionalized vinylic carbanions, the method has been limited to the use of various di- and (trifluorovinyl)lithium reagents as well as α-(alkoxyvinyl)lithiums with oxiranes.[12] In these reactions evidence indicates organolithiums and BF_3 are reasonably stable at low temperature (<−78 °C), reacting independently as potent nucleophile and strong Lewis acid, respectively.[11] We report an application of this synthetic method where both the alkene and oxirane fragments are unusually functionalized. The presence of this Lewis acid is crucial to the success of the reaction. In the absence of $BF_3{\cdot}Et_2O$, the reaction does not occur. Further, the cuprate reagent derived from (*E*)-1-lithio-2-(tributylstannyl)ethylene and lithium 2-thienylcyanocuprate[13] does not react with glycidyl tosylate.

The lithium anion of 5 (Scheme II) was prepared by reaction of 5 with *n*-BuLi, followed by addition of $BF_3{\cdot}Et_2O$ in THF at −78 °C. This reagent was then treated with glycidyl tosylate under the same conditions to afford (*E*)-1-(tributylstannyl)-5-tosyl-4-hydroxypent-1-ene (6) in 50% yield after chromatography (silica gel, 2:8 EtOAc/hexanes). Treatment of 6 with 7.5 equiv of powdered NaOH in monoglyme[14] at room temperature for 1 h gave (*E*)-4,5-epoxy-1-(tributylstannyl)pent-1-ene (7, yield 76%) after chromatography (silica gel, 1:9 EtOAc/hexanes). Epoxide 7 was converted to (*E*)-5-(2-nitroimidazolyl)-4-hydroxy-1-(tributylstannyl)pent-1-ene (8, yield 50%) by the nucleophilic ring opening of 7 with 2-nitroimidazole (2-NIM) in analogous fashion to the synthesis of F-MISO from 2-NIM and epifluorohydrin.[3]

(Iodovinyl)misonidazole (IVM, 3) and its iodine-131-labeled analogue were prepared by halodestannylation.[8] Thus the (tributylstannyl)misonidazole derivative (8) was treated with sodium iodide (specific activity 1280 Ci/mmol) in the presence of a hydrogen peroxide/acetic acid (2:1) mixture to afford IVM (3) in 50–60% radiochemical yield (specific activity ca. 400 Ci/mmol, radiochemical purity > 98.5%) after reverse-phase HPLC purification. Radiochemical purity was determined by TLC of the radioactive sample cospotted with cold material by eluting with 40% $CH_3CN/CHCl_3$, sectioning the developed plate,

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Table II. Anoxic:Oxic Uptake Ratios[a]

cell line	F-MISO	IVM
RIF-1	28.5 ± 10.3	23.2 ± 3.4
EMT-6	12.6 ± 0.5	11.0 ± 0.8
V-79	27.3 ± 6.6	12.5 ± 3.0
CaOs-1	18.0 ± 8.2	10.2 ± 1.4
rat myocytes	20.3	22.0

[a] Anoxic:oxic uptake ratio at 3 h: mean ± SD for two or three determinations. If no SD is given, values are for a single determination.

and counting for radioactivity.

Biological Testing Results and Discussion

Initial biological evaluation of IVM (3) indicates that it has potential as an imaging agent for hypoxic tissue. Protein binding of [^{131}I]IVM (3) does not appear to be significant relative to [^{99m}Tc]sodium pertechnetate. Percent protein binding for [^{131}I]IVM in human serum albumin (HSA) was 5.7% as compared to 7.9% for [^{99m}Tc]sodium pertechnetate. Similar results were obtained (i.e. low protein binding for [^{131}I]IVM) in serum from mice, rats, and dogs (results not shown). The anoxic:oxic drug uptake ratios for [^{131}I]IVM (3) in rat myocytes and in tumor cells were determined after incubation in 50 μM IVM (values are from a single experiment and given as representative values). The anoxic:oxic uptake ratio in rat myocytes was 9.6 at 1 h (anoxic 16210 ± 4440, oxic 1690 ± 350 dpm/10^5 cells, standard deviation from eight plates) and reached 22.0 at 3 h (anoxic 57490 ± 7340, oxic 2610 ± 300). The uptake of [^{131}I]IVM in four tumor cell lines at 3 h was also calculated as pmol/10^5 cells with a standard deviation from three plates. Typical uptake ratios for the individual experiments were as follows: V-79, 13.7 (anoxic 265.3 ± 34.2, oxic 19.34 ± 5.9); RIF-1, 25.6 (anoxic 611.5 ± 35.4, oxic 23.91 ± 1.2); EMT-6, 10.4 (anoxic 136.7 ± 25.1, oxic 13.12 ± 2.3); CaOs-1, 9.1 (anoxic 352.04 ± 22.2, oxic 38.54 ± 3.0). Table II compares the anoxic:oxic uptake ratios for F-MISO (50 μM)[15,16] and IVM in these cell lines at 3 h.

Conclusion

The data presented illustrate the similar oxygen dependency between F-MISO and IVM and are consistent with IVM being suitable as an imaging agent for hypoxia in vivo. Nonspecific protein binding for IVM was negligible inspite of its increased lipophilicity (octanol/water partition coefficient of 2.52, versus 0.40 for F-MISO[4]), and therefore does not adversely affect the uptake ratio. Electron affinity, i.e. the reduction potential of the nitro group, of the nitroimidazoles is another important feature of molecules that exhibit enhanced anoxic:oxic uptake ratios. It is not expected to be significantly different between IVM and F-MISO, and therefore the O_2 level which inhibits binding should be similar for the two drugs. Further biological studies are ongoing to evaluate IVM as a marker for hypoxia.

Experimental Section

^{1}H and ^{13}C NMR spectral data were obtained on a General Electric GN Omega 300 NMR spectrometer. All NMR data are reported in δ (ppm) values downfield from TMS, using either TMS, $CDCl_3$, or CD_3OD as an internal reference. UV spectra were obtained with a Beckman DU-64 spectrometer. Mass spectra were obtained on a HP 5985 GC/MS spectrometer (EI mode) or a VG 70SEQ spectrometer (FAB mode). Elemental analyses were determined by Galbraith Laboratories, Inc., Knoxville, TN. Column chromatography separations were performed with E. Merck silica gel 60 (230–400 mesh). Thin-layer chromatography separations were carried out on E. Merck aluminum-supported silica gel 60 (0.2 mm, F-254). TLC plates were visualized by short-wavelength light or charring with molybdic acid. HPLC separations were performed with a C18 semipreparative column (Whatman M9 ODS3, 250 × 9.0 mm, 10 mm) using 1:1 EtOH/H_2O at a flow rate of 1.5 mL/min, monitoring of the eluent at 280 nm (Beckman UV detector Model 153, fixed wavelength) and a NaI(Tl) detector for radioactivity. Iodine-131 activity was measured on a Packard Minaxi γ 5000 series γ-counter.

(*E*)-1,2-Bis(tributylstannyl)ethylene (5) was synthesized by a literature procedure.[17] THF was distilled from Na/benzophenone. $BF_3 \cdot Et_2O$, (2*S*)-(+)-glycidyl tosylate, and 2-nitroimidazole were purchased from Aldrich Chemical Co. and used without purification. *n*-BuLi was also purchased from Aldrich Chemical Co. and handled under an argon atmosphere. [^{131}I]NaI in 0.1 N NaOH (0.01 mL, specific activity 1300 Ci/mmol) was purchased from Du Pont NEN as a labeling-grade reagent. Human serum albumin (HSA) was purchased from Armour Pharmaceutical Co. (Albuminar-25) as a 25% solution in H_2O (w:v).

(*E*)-1-(Tributylstannyl)-5-tosyl-4-hydroxypent-1-ene (6). A cold (−78 °C), stirred solution of (*E*)-1,2-bis(tributylstannyl)ethylene (5) (8.05 g, 13.3 mmol) in dry THF (50 mL) under argon was treated with a solution of *n*-butyllithium (2.5 M in hexane, 5.8 mL, 14.5 mmol). Upon the addition of *n*-BuLi the initial color of the reaction mixture changed to an intense yellow-green. After stirring for 1.5 h at −78 °C, the resultant solution was treated by the dropwise addition of $BF_3 \cdot Et_2O$ (1.63 mL, 13.3 mmol) followed, after 20 min, by a solution of (2*S*)-(+)-glycidyl tosylate (2.76 g, 12.1 mmol) in THF (5 mL). The addition of the latter solution changed the color of the mixture to orange. After 2 h the reaction was warmed to 0 °C and followed, after 1 h, by addition of the reaction mixture to 50 mL of saturated NH_4Cl (aqueous). The resultant mixture was extracted with Et_2O (3 × 80 mL). Concentration of the combined and dried ($MgSO_4$) extracts afforded 11.22 g of a yellow oil. Column chromatography of this residual material (silica gel, 2:8 EtOAc/hexanes) afforded 3.6 g (50%) of 6 as a clear oil. TLC (silica gel, 2:8 EtOAc/hexanes) indicated a single component (R_f 0.20). ^{1}H NMR ($CDCl_3$): δ 7.78 (d, 2 H, J = 8.3 Hz), 7.33 (d, 2 H, J = 8.3 Hz), 5.93 (m, 2 H), 4.02 (m, 1 H), 3.91 (m, 2 H), 2.43 (s, 3 H), 2.30 (m, 2 H). ^{13}C NMR ($CDCl_3$): δ 145.0 (s), 142.7 (d), 134.0 (d), 132.7 (s), 129.9 (d), 127.9 (d), 73.1 (t), 68.5 (d), 41.4 (t), 29.1 (t), 27.2 (t), 21.6 (q), 13.7 (q), 9.4 (t). FABMS: found m/e 547 (MH^+), calcd for $C_{24}H_{42}O_4SSn$ 546.1826. Anal. ($C_{24}H_{42}O_4SSn$): C, H.

(*E*)-4,5-Epoxy-1-(tributylstannyl)pent-1-ene (7). A stirred solution of compound 6 (3.4 g, 6.24 mmol) in monoglyme (100 mL) was treated for 2 h with powdered NaOH (1.87 g, 46.8 mmol). The mixture was filtered, and the collected solids were washed with ether. Concentration of the combined filtrates afforded 2.70 g of a light brown residual oil. This material was purified by column chromatography (silica gel, 1:9 EtOAc/hexanes) to afford 1.77 g (76%) of 7 as a clear oil. TLC (silica gel, 2:8 EtOAc/hexanes) indicated a single component (R_f 0.62). ^{1}H NMR ($CDCl_3$): δ 5.99 (m, 2 H), 2.99 (m, 1 H), 2.75 (m, 1 H), 2.50 (m, 1 H), 2.38 (m, 2 H), 1.48 (m, 6 H), 1.28 (m, 6 H), 0.87 (m, 15 H). ^{13}C NMR ($CDCl_3$): δ 142.8 (d), 131.9 (d), 51.4 (d), 46.6 (t), 40.4 (t), 29.1 (t), 27.2 (t), 13.7 (q), 9.4 (t). EIMS: found m/e 373 (M^+), calcd for $C_{17}H_{34}OSn$ 374.1631. Anal. ($C_{17}H_{34}OSn$): C, H.

(*E*)-5-(2-Nitroimidazolyl)-4-hydroxy-1-(tributylstannyl)pent-1-ene (8). 2-Nitroimidazole (0.44 g, 3.91 mmol) was dissolved in warm DMF (2.5 mL) and treated with a concentrated aqueous solution of KOH (0.146 mL, 26.7 M) followed by heating (95 °C) until solution was achieved. While warm, this solution was added, with stirring, to a solution of epoxide 7 (1.46 g, 3.91 mmol) in CH_3CN (10 mL). The resultant mixture was heated (95 °C) and stirred under argon for 24 h. The volatile materials were removed in vacuo, and the residual oil was purified by column chromatography (silica gel, 1:1 EtOAc/hexanes) to afford 0.72 g of 8 (50%) as a clear oil. TLC (1:1 EtOAc/hexanes)

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indicated a single component (R_f 0.28). ^{1}H NMR ($CDCl_3$): δ 7.14 (d, 1 H, J = 1.0 Hz), 7.00 (d, 1 H, J = 1.0 Hz), 5.97 (m, 2 H), 4.68 (m, 1 H), 4.13 (m, 1 H), 4.09 (m, 1 H), 2.81 (br d, 1 H), 2.50 (m, 1 H), 2.33 (m, 1 H), 1.47 (m, 6 H), 1.26 (m, 6 H), 0.84 (m, 15 H). ^{13}C NMR ($CDCl_3$): δ 142.5, 134.7, 127.7, 127.4, 68.9, 55.1, 43.3, 29.0, 27.2, 13.6, 9.4. FABMS: found m/e 488 (MH^+). Calcd for $C_{20}H_{37}N_3O_3Sn$ 487.1857. Anal. ($C_{20}H_{37}N_3O_3Sn$): C, H, N.

(Iodovinyl)misonidazole (IVM, (*E*)-5-(2-Nitroimidazolyl)-4-hydroxy-1-iodopent-1-ene, 3). A stirred solution of compound 8 (19.3 mg, 0.0526 mmol) in THF (0.5 mL) was diluted with 0.1 mL of 0.61 M aqueous NaOAc buffer (pH 4.5) and 0.07 mL of 0.79 M (0.056 mmol) NaI (aqueous). The resultant solution was treated with 0.2 mL of H_2O_2/HOAc (2:1) and, after 30 min, poured into 20 mL of a 5% $Na_2S_2O_3$ (aqueous) solution, followed by extraction of the mixture with EtOAc (3 × 20 mL). Concentration of the combined and dried ($MgSO_4$) extracts afforded a residual oil which was chromatographed (silica gel, 2:3 $CH_3CN/CHCl_3$) to afford 9.2 mg (54.2%) of IVM (3) as a clear oil. TLC (silica gel, 2:3 $CH_3CN/CHCl_3$) indicated a single component (R_f 0.41). UV: (EtOH) λ_{max} 318 nm (ϵ 6680). Partition coefficient was determined by the ratio of the absorbance at 318 nm for water-saturated octanol (24 h prior to study) and octanol-saturated water (24 h prior to study) and was 2.52. ^{1}H NMR (CD_3OD): δ 7.57 (d, 1 H, J = 1.2 Hz), 7.26 (d, 1 H, J = 1.2 Hz), 6.76 (m, 1 H), 6.45 (dt, 1 H, J = 14.6, 1.2 Hz), 4.76 (dd, 1 H, J = 14.0, 3.0 Hz), 4.39 (m, 1 H), 4.10 (m, 1 H), 2.44 (m, 2 H). ^{13}C NMR (CD_3OD): δ 142.9 (d), 129.2 (d), 128.1 (d), 78.2 (d), 69.9 (d), 55.9 (t), 42.1 (t). FABMS: found m/e 324 (MH^+), calcd for $C_8H_{10}IN_3O_3$ 323.0917. Anal. Calcd ($C_8H_{10}IN_3O_3$): C, 29.74; H, 3.12; I, 39.28; N, 13.01. Found: C, 32.12; H, 3.59; I, 36.84; N, 11.93.

[^{131}I]IVM Radioiodination. For the sake of limiting exposure to potentially volatile radioiodine, the radioiodination procedure was conducted within a well-ventilated fume hood and the original septum-sealed shipping vial for the [^{131}I]NaI, equipped with a Teflon-coated microstir bar, served as a reaction vessel for radiolabeling. To the commercial solution of [^{131}I]NaI in 0.1 N NaOH (0.010 mL, 6.62 mCi, specific activity 1280 Ci/mmol) were successively added compound 8 (0.2 mg, 0.042 mmol) in THF (0.1 mL) and NaOAc buffer (0.61 M, pH 4.5, 0.1 mL). The resultant stirred mixture was treated with a solution of H_2O_2 (30% aqueous)/HOAc (2:1) (0.025 mL) within the sealed vial. After 30 min, the reaction was quenched by the addition of 5% aqueous $Na_2S_2O_3$ solution (0.1 mL). The entire crude reaction mixture (0.335 mL) was loaded onto a Whatman ODS-3 reverse-phase column, followed by elution with 1:1 EtOH/water. [^{131}I]IVM (3) (3.82 mCi, 58% radiochemical yield) was isolated after 11 min, in ca. 4 mL of solution. Postpurification analysis of the isolated solution by TLC (silica gel, 2:3 $CH_3CN/CHCl_3$, R_f 0.41) and HPLC revealed chromatographic characteristics identical with that of unlabeled IVM. The specific activity of the isolated material was determined (UV absorbance at 280 nm, ϵ 3230) to be 450 Ci/mmol. The radiochemical purity of the sample was >98.5%. The solution of the labeled material may be conveniently stored at 5 °C in the dark (no significant loss of label detected after 2 weeks).

Protein Binding. To 0.10 mL of HSA containing either 0.002 mL of an [^{131}I]IVM solution or 0.002 mL of a [^{99m}Tc]sodium pertechnetate solution was added 0.90 mL of a $MeOH/CH_3CN$ solution (v:v). The resulting mixture was sonicated for 3 min followed by centrifugation for 3 min. The protein pellet was separated from the supernate and both were assayed for radioactivity. Results are expressed as percent protein binding (radioactivity in pellet/total radioactivity × 100).

Myocyte Cell Culture. Isolated, calcium-tolerant adult rat myocytes were obtained by collagenase dissociation.[18] After overnight incubation at 37 °C in 95% air/5% CO_2 with calcium-free and insulin-, taurine-, and creatine-supplemented, modified minimum essential medium (MEM, Gibco Laboratories, Grand Island, NY) containing 100 mg/mL bovine serum albumin, the cells were rinsed and covered with a thin layer of medium (1 mL per 60-mm dish) containing [^{131}I]IVM (50 μM). The cells were exposed to the desired oxygen conditions using the thin film culture techniques of Koch.[19] Each chamber was equilibrated with either 95% air/5% CO_2 (oxic) or 95% N_2/5% CO_2 (anoxic) and kept at 37 °C for 1 or 3 h. Anoxia was verified at the end of incubation by testing the maintenance of reducing capacity of a dish of sodium dithionite indicator solution in the incubation chamber by its ability to rapidly decolorize methylene blue. The medium was removed and the cells were rinsed twice with cold albumin-free medium to remove unbound labeled drug and then fixed with 70% EtOH. Cell numbers per dish were determined by counting 13 premarked, high-power fields and correcting for cell attachment area. The plates were scraped and the cell material counted for iodine-131 activity. The uptake of [^{131}I]IVM at 1 and 3 h was calculated as dpm/10^5 cells.

Tumor Cell Culture.[14] Four cell lines were used to examine uptake of [^{131}I]IVM in immortal cells. V-79-171B Chinese hamster lung fibroblasts were maintained in Eagles MEM (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS, Flow Laboratories, McLean VA). CaOs-1, a spontaneous dog osteosarcoma, was grown in BioRich medium (Flow Laboratories) with 10% FBS. Two mouse tumor lines, EMT-6(UW), a sarcoma of BALB/c mice, and RIF-1, a sarcoma of C3H/Km mice, were grown in Waymouth's MB752/1 medium with 15% FBS. All media contained penicillin/streptomycin and for hypoxia experiments contained 25 mM HEPES buffer. Cells were used while growing exponentially or were allowed to reach an unfed plateau phase of growth.

To assay for uptake of [^{131}I]IVM, culture dishes were removed from the incubator, growth medium was aspirated, and 1 mL of medium containing 50 μM [^{131}I]IVM was used to rinse the culture medium from the monolayer disk of cells. This medium was aspirated and replaced with 1 mL of fresh labeled medium for the incubation under the desired gas mixture. The cells were then exposed to the desired oxygen conditions for 3 h by using the thin film culture techniques described above. Cells were harvested by trypsinization and a proportion of the total sample was counted for iodine-131 activity. The uptake of [^{131}I]IVM at 3 h was calculated as pmol/10^5 cells, for each cell type.

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Registry No. 3, 133933-39-8; [^{131}I]-3, 133933-40-1; 5, 14275-61-7; 6, 133933-36-5; 7, 133933-37-6; 8, 133933-38-7; (2*S*)-(+)-glycidyl tosylate, 70987-78-9; 2-nitroimidazole, 527-73-1.

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