

THE SYNTHESIS AND RADIOLABELING OF NOVEL MARKERS OF TISSUE HYPOXIA OF THE IODINATED AZOMYCIN NUCLEOSIDE CLASS

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SUMMARY

Seven second-generation hypoxic markers of the iodinated azomycin nucleoside class have been synthesized and tested for hypoxia marking activity with tumor cells *in vitro* and *in vivo*. β -D-Iodoazomycin galactoside (IAZG) and β -D-Iodoazomycin xylopyranoside (IAZXP) demonstrated superior hypoxia marking properties relative to IAZA because of their higher water solubilities, rapid plasma clearance rates from tumor-bearing mice and maximum tumor/blood (T/B) and tumor/muscle (T/M) ratios. Our studies with animal tumor models show that T/B or T/M ratios of these markers determined by scintigraphy or planar imaging can predict for the relative degree of tumor hypoxia and for tumor radioresistance.

KEYWORDS: hypoxic markers, tumor oxygenation, azomycin nucleosides, tumor radioresistance, bioreducible drug.

INTRODUCTION

Viable tumor cells which reside in hypoxic microenvironments are 2.5 - 3.0 X more resistant to inactivation by ionizing radiation than are oxygenated tumor cells (1). There is no standard procedure in clinical use today for measuring this tumor property. The presence of hypoxic cells in solid tumors has been assumed and was the basis for extensive clinical trials using physical (neutrons) and chemical (hyperbaric oxygen (HBO) and hypoxic sensitizers) techniques for overcoming their radioresistance. Several novel techniques for measuring tumor oxygenation status have been proposed (2). A non-invasive method which could quantify tumor hypoxia and predict for radioresistance using equipment available in modern cancer centers would have several practical advantages.

Chapman (3) proposed that the bioreductive linkage of hypoxic radiosensitizers to cellular biomolecules, an oxygen dependent process, might be exploited for the detection of treatment resistant (hypoxic) cells within solid human tumors. Azomycin derivatives are enzymatically reduced within cells (4), primarily by P450 cytochrome C reductase (5). The one-electron reduction product of azomycin, its radical anion, will rapidly transfer its unpaired electron to O_2 which is more electron affinic. These superoxide anions will be converted to hydrogen peroxide and less toxic chemical species. Consequently, the intracellular level of molecular oxygen becomes an important regulator of the extent of azomycin bioreduction. Under hypoxic

conditions, additional reductions can lead to the nitroso and hydroxylamino products which are known to be highly reactive and can covalently link to cellular molecules (6). If trace quantities of labeled marker selectively bound to hypoxic cells can be measured non-invasively, an acceptable procedure for the routine measurement of this tumor property might be possible.

Of the iodinated azomycin nucleosides investigated to date, 1-(5-Iodo-5-deoxy- β -D-arabinofuranosyl)-2-nitroimidazole (IAZA) was selected for clinical testing (7,8). Its uptake into human tumors was found to be heterogeneous both within a specific tumor class and between different tumor classes. Its plasma clearance half-life (~10 hr) was relatively long. Hepatobiliary excretion produced non-hypoxic-specific signal throughout the abdomen and dehalogenation resulted in excessive thyroid labeling (7). The objective of our research was to design, synthesize and evaluate second-generation hypoxic markers of the iodinated azomycin nucleoside class with higher water solubility to facilitate the renal excretion of unbound marker. A plasma clearance half-life in humans of 2-4 hr with the renal excretion of >99% of unmetabolized marker would be ideal.

MATERIALS

Chemicals and reagents were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin and SIGMA Chemical Co., St. Louis, Missouri and used as received. Preparative flash chromatography was carried out using E. Merck silica gel 60 (200-400 mesh). TLC was performed on E. Merck silica gel 60 fluorescent plates, using a $\text{CHCl}_3/\text{MeOH}$ (77.5:22.5) elution system. Autoradiographs were prepared on Kodak X-OMAT-AR scientific imaging film and analyzed using a UMAX Hi Resolution scanner with NIH Image Software. Iodine-125 (NaI), 1 mCi, Carrier Free, 17Ci/mg, was obtained from DuPont, Billerica, MA in a pH 8.0-9.0 solution. Iodine-123 (NaI), 10 mCi, 48.94 mCi/mg, was obtained from MediPhysics, Philadelphia, PA as a solution in 0.1N NaOH. Melting points were determined with a Laboratory Devices Melt Temp II and are uncorrected. Infrared spectra were determined using KBr pellets with a Nicolet 205 FTIR. UV/Visible spectra were obtained with a Beckman Model DU70. HPLC analyses were carried out on a Beckman System Gold equipped with a 3.9 X 300 mm Millipore Delta Pak 15 μm C18 column. Unless stated otherwise, the solvent system was methanol/water (1:1) at 0.7 ml/min. The detector was set at 308 nm. NMR (δ ppm from Me_4Si) spectra were determined with a Brücker AM-300-WB. Gamma counting was performed with a Packard Cobra II Auto Gamma spectrometer. For Iodine-125 a 15-75 KeV window was used with a %E of 81.5% and 4 π geometry and for Iodine-123 a 50-200 KeV window was used with a %E of 80.3 and 4 π geometry. Planar imaging studies were carried out with a Picker 2000XP-Double Head imaging system using a 20% Iodine-123 window and a low energy/high resolution collimator.

EXPERIMENTAL

Chemical Syntheses

1-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)-2-nitroimidazole (1). Anhydrous CH_3CN (330 ml), 3.29 g (8 mmol) acetobromo- α -D-galactose, 1.00 g (8.85 mmol) azomycin and 5.03 g (20 mmol) $\text{Hg}(\text{CN})_2$ were stirred at 40°C for 23.0 hr. The solvent was removed and the residue taken up in 350 ml CH_2Cl_2 . The solution was filtered and extracted two times each with 75 ml portions of sat NaHCO_3 , 25% NaI and water. After drying with MgSO_4 , CH_2Cl_2 was removed and the residue loaded onto a 5 X 25 cm flash chromatography column, eluted with EtOAc /toluene (2:3) and collected in 50 ml fractions. Fractions 11-19 contained the product. Evaporation of the solvent gave 3.12 g (88%) of 1 as a pale yellow syrup, which was used as is.

1-(β -D-galactopyranosyl)-2-nitroimidazole (2). 1 (3.121g (7.04 mmol)), 51 ml of anhydrous MeOH and 4.67 ml (0.3 mmol) of 0.0645M MeOH/MeONa were stirred at ambient temperature for 1.5 hr. The reaction mixture was quenched by the addition of three drops of glacial AcOH . The product was collected and dried under vacuum to give 2. Yield 1.14 g (58.9%), mp 213-214°C (Lit: 220-221°C (9)). HPLC 100%, RT=3.68 min.

1-(6-Deoxy-6-iodo- β -D-galactopyranosyl)-2-nitroimidazole (3). A solution consisting of 50 ml anhydrous pyridine, 1.06 g (4 mmol) triphenylphosphine and 1.01 g (4 mmol) iodine was prepared. To this was added 0.55 g (2 mmol) of 2. The mixture was stirred at 60°C for 7.0 hr. The progress of the reaction was followed by HPLC. The yield of product reached a maximum of 66% in about 6 hr. MeOH (5.0 ml) was added and stirred at ambient temperature overnight. The solvent was removed and the residue dissolved in 100 ml anhydrous EtOH and re-evaporated. The residue was loaded onto a 5 X 31 cm flash chromatography column. The column was eluted first with CHCl_3 to remove any unreacted triphenylphosphine, triphenylphosphine oxide and iodine. The eluant was changed to $\text{CHCl}_3/\text{MeOH}$ (9:1) and collected in 20 ml fractions. The progress of the elution was followed via HPLC. Evaporation of the combined product fractions gave 0.420 g of crude 3. Recrystallization from anhydrous $\text{MeOH}/\text{CHCl}_3$ yielded 0.217 g (28.2%), mp 184-185°C (Lit: 187-188°C (9)). HPLC 100%, RT=7.70 min. NMR- CD_3OD - 6.103 (C_1H - d, J=8.97 Hz).

1-(2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl)-2-nitroimidazole (4). Anhydrous CH_3CN (200 ml), 0.738 g (6.53 mmol) azomycin, 3.72g (14.7 mmol) $\text{Hg}(\text{CN})_2$ and 2.425 g (5.9 mmol) acetobromo- α -D-glucose were stirred at ambient temperature for 24 hr. The solvent was removed and the residue taken up in 200 ml CH_2Cl_2 . After filtration, the CH_2Cl_2 solution was extracted with 75 ml portions of saturated NaHCO_3 , 25% KI and water. After drying with MgSO_4 the solvent was removed and the residue loaded onto a 5.0 X 17.0 cm flash chromatography column. The eluate was toluene/ EtOAc (3:2) and 18 X 50 ml fractions were collected with the product appearing in fractions 10-17. Evaporation of the combined fractions gave 1.81 g of 4 (69.3%) as a sticky solid which was used as is.

1-(β -D-glucopyranosyl)-2-nitroimidazole (5). 4 (1.812 g (4.09 mmol)), 30 ml anhydrous MeOH and 0.3 ml of 0.0645 M MeOH/MeONa were stirred at ambient temperature for 2.7 hr after which an additional 0.3 ml 0.0645 M MeOH/MeONa was added and stirred for an additional 3.3 hr. One drop of glacial AcOH was added and the mixture evaporated to dryness. The gummy residue was taken up in about 5 ml 95%

ethanol and after a few minutes the product began to crystallize. After 16 hr in the freezer, the precipitate was collected, washed with cold 95% EtOH and dried in vacuum to give 0.95 g (84.4%) **5**, mp 104-111°C. HPLC 97.8%, RT=3.78 min. NMR - CD₃OD - 6.27 (C₁H - d, J = 8.5 Hz).

1-(6-deoxy-6-iodo-β-D-glucopyranosyl)-2-nitroimidazole (6). A mixture consisting of 50 ml anhydrous pyridine, 1.06 g (4 mmol) triphenylphosphine and 1.01 g (4 mmol) iodine was prepared and stirred at 60°C for 3 min. Then 0.550 g (2 mmol) of **5** was added and the reaction mixture stirred at 60-62°C for 6.3 hr. HPLC indicated that the reaction was 88% complete in less than 15 min. The reaction was terminated by the addition of 5 ml anhydrous MeOH and stirring for 1/2 hr. The solvent was removed and the residue taken up with 200 ml anhydrous EtOH and re-evaporated. The residue was loaded onto a 5 X 31 cm flash chromatography column and eluted with 2 L of CHCl₃ followed by CHCl₃/MeOH (90:10) collected in 20 ml fractions. The product was found in fractions 66-88. The solvent was removed and the product, after drying under vacuum for 16 hr at 75°C, was collected. Yield 0.174 g (22.6%), mp 95-105°C (gel). TLC single spot R_f=0.58. HPLC >95% RT=7.51 min. NMR - CD₃OD - 6.191 (C₁H - d, J = 8.57 Hz). UV/VIS (H₂O) λ_{max} 317nm (E=6533M⁻¹).

1-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-4-nitroimidazole (7). Anhydrous CH₃CN (330 ml), 1.00 g (8.86 mmol) azomycin, 5.03 g (20 mmol) Hg(CN)₂ and 3.29 g (8 mmol) acetobromo-α-D-galactose were stirred at 50°C for 24 hr. The solvent was removed and the residue taken up with 300 ml CH₂Cl₂ and extracted two times each with 75 ml portions of saturated NaHCO₃, 25% KI and water. The CH₂Cl₂ solution was dried over MgSO₄. Removal of the solvent gave 2.58 g of a solid which HPLC showed contained 91% of **7**. The solid was loaded onto a 5 X 25 cm flash chromatography column which was eluted with EtOAc/toluene (2:3). Fractions (20 ml) were collected with the product recovered in fractions 133-193. Removal of the solvent gave 1.433 g (40.4%) **1**, mp 179-180°C. HPLC 100%, RT=9.79 min. NMR - CDCl₃ - (7.6262 C₃H - d, J = 1.58 Hz), (7.9909 C₄H - d, J = 1.56 Hz), (5.3443 C₁H - d, J=9.14 Hz).

1-(β-D-galactopyranosyl)-4-nitroimidazole (8). Anhydrous MeOH (25 ml), 1.433 g (3.23 mmol) of **7** and 2.77 ml of 0.05 M MeOH/MeONa were stirred at ambient temperature. The reaction was followed by HPLC. After 2.5 hr an additional 0.25 ml of 0.05 M MeOH/MeONa was added. After stirring for an additional 20 hr the reaction mixture was filtered to yield 0.465 g (52.3%) of **8**. The filtrate was air evaporated to dryness and the residue taken up with anhydrous EtOH. Filtration yielded an additional 0.122 g (13.7%) of **8**. The combined precipitates had a mp 251-252°C. HPLC 99.1%, RT=3.69 min. TLC EtOAc/toluene (4:6) single spot, origin. The starting material, **7**, gives a single spot at R_f = 0.13.

1-(6-deoxy-6-iodo-β-D-galactopyranosyl)-4-nitroimidazole (9). Anhydrous pyridine (53 ml), 1.131 g (4.31 mmol) triphenylphosphine and 0.593 g (4.67 mmol) iodine were brought into solution. **8** (0.587 g (2.13 mmol)) was added and stirred at 60°C. An additional 0.23 g (1.81 mmol) of iodine was added after 4.5 hr. The reaction mixture was stirred an additional 18 hr after which HPLC indicated only 7.8% of **8** remained. Anhydrous MeOH (5 ml) was added and stirred for 0.5 hr. The solvent was removed and the residue treated twice with anhydrous EtOH and re-evaporated. The residue was loaded onto a 5 X 31 cm flash chromatography column. The column was eluted first with 1600 ml CHCl₃ followed by CHCl₃/MeOH (9:1). Fractions (20 ml) were collected with the product found in fractions 83-120. Removal of the solvent

gave 0.216 g of **9** which assayed 94% pure by HPLC. The impurities were removed using preparative HPLC with a C18 column and MeOH/H₂O (1:1). The final product 0.087 g (10.5%), HPLC 100%, RT 6.92 min. TLC single spot at R_f=0.55, mp 95-105°C gel-resolidifies, melts 170°C. NMR-CD₃OD - 8.3796 (C'₅H - d, J = 1.49 Hz), 7.9663 (C'₄H - d, J = 1.48 Hz), 5.2461 (C₁H - d, J = 8.97 Hz).

Tetra-O-acetyl-β-D-arabinopyranoside (10). Anhydrous NaOAc (12.0 g (0.146 mol)) and 160 ml (173.12 g (1.696 mol)) AcOAc were placed into a 500 ml round bottom flask. The mixture was heated to gentle reflux and 20.0 g (0.133 mol) D-arabinose was added in portions sufficient to maintain reflux (about 15 minutes is required for this step). The reaction mixture was refluxed for an additional 5 min and poured into 500 ml of crushed ice. The mixture was stirred for 3 hr and extracted with three 100 ml portions of CHCl₃. The combined extracts were washed with sat NaHCO₃ followed by water and dried over MgSO₄. Removal of the CHCl₃ yielded (91.1%) 38.6 g **10** as a syrup which was used as is.

2,3,4-tri-O-acetyl-β-D-arabinopyranosyl bromide (11). A solution of 38.58 g (0.121 mol) of **10** in 97 ml CH₂Cl₂ was chilled to 0°C. A prechilled (0-5°C) 30-32% v/v solution of HBr in AcOH (115.7 ml) was added in one lot. The mixture was stirred at 0°C for 2.0 hr. CH₂Cl₂ (700 ml) was added and the mixture extracted successively with water, sat NaHCO₃, water and dried with Na₂SO₄. Removal of the solvent gave 23.9 g of **11** as a syrup. The syrup was dissolved in EtOAc/hexane and carbon treated. Chilling in the freezer produced 7.43 g (18.1%) of **11**. The filtrate was evaporated to a syrup to which was added 15 ml EtOAc. After 16 hr in the freezer an additional 4.06 g (9.9%) **11** was recovered. The melting point of the combined samples was 134-137°C (Lit: 138-140°C (10)). NMR - CDCl₃ - 6.6764 (C₁H - d, J=3.8 Hz).

1-(2,3,4-tri-O-acetyl-α-D-arabinopyranosyl)-2-nitroimidazole (12). Anhydrous CH₃CN (200 ml), 0.738 g (6.53 mmol) azomycin, 3.72 g (14.7 mmol) Hg(CN)₂ and 2.0 g (5.90 mmol) of **11** were stirred at ambient temperature for 12.5 hr. The solvent was removed and the residue taken up in 200 ml CH₂Cl₂. The mixture was filtered and washed twice with 50 ml sat NaHCO₃, 25% KI and water. After drying with MgSO₄ the solvent was removed and the residue stirred with approximately 5 ml EtOAc/toluene (2:3). A pale yellow solid separated which was recovered, washed well with ice cold solvent and dried to give 1.496 g (68.3%) **12**, mp 181-184°C (Lit: 184-185 (11)).

1-(α-D-arabinopyranosyl)-2-nitroimidazole (13). Anhydrous MeOH (8.5 ml), 0.4345 g (1.17 mmol) **12** and 0.82 ml (0.0645 mol) MeOH/MeONa were stirred at ambient temperature for 2.0 hr. Filtration and drying gave 0.153 g (53.3%) **13**, mp 194.5-195.0°C (Lit: 196-197 (11)). HPLC 100%, RT=4.07 min. UV/VIS (EtOH) λ_{max} 312 nm (E=6702 M⁻¹).

1-(4-deoxy-4-iodo-α-L-xylopyranosyl)-2-nitroimidazole (14). Anhydrous pyridine (50 ml), 1.06 g (4 mmol) triphenylphosphine, 0.505 g (2.0 mmol) iodine and 0.500 g (2 mmol) **13** were heated for 23 hr at 60-61°C. The reaction was quenched with 5 ml MeOH. Solvent was removed and the residue taken up with 100 ml anhydrous MeOH and re-evaporated. The residue was loaded onto a 5 X 31 cm flash chromatography column and eluted with 1 L of CHCl₃. The eluent was changed to CHCl₃/MeOH (19:1) and collected in 50 ml fractions. HPLC indicated that fractions 18-32 contained the product. The combined fractions were evaporated to give 1.111 g of crude **14**. The crude material was loaded onto a 3 X 30 cm silica gel (200-400 mesh) flash chromatography column and eluted with CHCl₃/MeOH (19:1). Fractions (20 ml)

were collected and the product located in fractions 27-40. Evaporation gave 0.1062 g of **14** which recrystallized from anhydrous MeOH to give 59.9 mg (8.5%), mp 175.5-176°C (Lit: 175-176°C (11)). HPLC 98.9%, RT=9.96 min. NMR - CD₃OD - 6.126 (C,H - d, J = 8.91 Hz).

Tetra-0-acetyl- α -L-arabinopyranoside (15). Prepared as per **10**. Yielded 40.05 g (94.6%) as a syrup.

2,3,4-tri-0-acetyl- α -L-arabinopyranosyl bromide (16). Prepared as per **11** except the syrup crystallized on standing. Recrystallization from EtOAc yielded 16.16 g (37.8%) **16**, mp 136-138°C (Lit: 138-140 (10)). NMR-CDCl₃-6.78 (C,H - d, J = 3.94 Hz).

1-(2,3,4-tri-0-acetyl- β -L-arabinopyranosyl)-2-nitroimidazole (17). Anhydrous CH₃CN (300ml), 1.107 g (9.79 mmol) azomycin, 5.58 g Hg(CN)₂ and 3.0 g (8.85 mmol) of **16** were stirred at ambient temperature for 24 hr. The solvent was removed and the residue taken up in 300 ml CH₂Cl₂. The mixture was filtered and the filtrate was successively extracted with two 100 ml portions of sat NaHCO₃, 25% KI and water. After drying with MgSO₄ and evaporation of the solvent, a residue was obtained which was stirred with EtOAc/toluene (4:6). A precipitate formed which after 16 hr in the freezer was removed, washed with cold solvent and dried under vacuum to give 1.678 g (51.1%) **17**, mp 181-185°C (Lit: 184-185°C for D-enantiomer (11)).

1-(β -L-arabinopyranosyl)-2-nitroimidazole (18). **17** (1.678 g (4.52 mmol)), 33 ml anhydrous MeOH and 3.0 ml of 0.0645 M MeOH/MeONa were stirred at ambient temperature for 3.0 hr. The solvent was removed and the sticky residue stirred with about 10 ml anhydrous EtOH. A solid precipitated which was removed, washed with cold anhydrous EtOH and dried to yield 0.9472 g (85-5%) **18**, mp 193-194°C (dec) (Lit: 196-197°C for D-enantiomer (11)).

1-(4-deoxy-4-iodo- β -D-xylopyranosyl)-2-nitroimidazole (19). Anhydrous pyridine (50 ml), 1.06 g (4 mmol) triphenylphosphine, 0.505 g (2.0 mmol) iodine and 0.5 g (2.0 mmol) of **17** were stirred at 60-62°C for 22 hr. The reaction was quenched by cooling, adding 5 ml anhydrous MeOH and stirring at ambient temperature for 0.5 hr. The solvent was removed and the residue dissolved in 100 ml anhydrous EtOH and re-evaporated. The residue was loaded onto a 5 X 32 cm flash chromatography column and eluted with 700 ml CHCl₃ followed by elution with CHCl₃/MeOH (19:1). Fractions (20 ml) were collected with fractions 65-85 containing the product. The combined fractions were evaporated to a residue which recrystallized from anhydrous MeOH to give 0.2348 g (33.1%) **19**, mp 175-176°C (Lit: - 175-176°C (for L-enantiomer (11)). NMR - CD₃OD - 6.144 (C,H - d, J=8.83 Hz).

2,3,4-tri-0-acetyl- β -D-ribose bromide (20). Tetra-0-acetylribose (5.0 g (15.7 mmol)) and 32 ml of 30-32% HBr in acetic acid were stirred for 2.0 hr at ambient temperature. HBr was removed at 35-40°C on a rotary evaporator. Toluene (100 ml) was added and re-evaporated to an oil. Evaporations were repeated twice each with 50 ml portions of toluene followed by 50 ml of benzene. The residue was placed under vacuum (1 mmHg) at 35°C for 1.0 hr. The resulting syrup was dissolved in 25 ml anhydrous EtOEt and carbon treated. The colorless filtrate was treated with petroleum ether until incipient crystallization occurred. The precipitate was collected and dried under vacuum to give 3.63 g (68.2%) **20**, mp 93-94°C (Lit: ~93°C (10)).

1-(2,3,4-tri-O-acetyl-β-D-ribofuranosyl)-2-nitroimidazole (21). Anhydrous CH₃CN (300 ml), 1.107 g (9.79 mmol) azomycin, 5.58 g Hg(CN)₂ and 3.0 g (8.85 mmol) of **20** were stirred at room temperature for 24.0 hr. The solvent was removed and the residue taken up with 300 ml CH₂Cl₂ which was successively extracted with sat NaHCO₃, 25% KI and water. After drying with MgSO₄, the solvent was removed and the yellowish syrup loaded onto a 5 X 31 cm flash chromatography column and eluted with EtOAc/toluene (4:6). Fractions (20 ml) were collected with the product found in fractions 42-69. Removal of the solvent gave 2.567 g (78.1%) **21**, as a syrup which was used as is. HPLC 99.9%, RT=12.3 min.

1-(β-D-ribofuranosyl)-2-nitroimidazole (22). **21** (2.567 g (6.91 mmol)), 51 ml anhydrous EtOH and 5.9 ml of 0.05 M MeOH/MeONa were stirred at ambient temperature for 70 min. Two drops of glacial AcOH were added and the solvent removed. The residue was dissolved in about 35 ml anhydrous EtOH. Chilling in the freezer gave a precipitate of 0.924 g (54.5%) **22**, mp 177-178°C. HPLC 100%, RT=4.08 min.

1-(4-deoxy-4-iodo-β-L-xylofuranosyl)-2-nitroimidazole (23). Anhydrous pyridine (50 ml), 1.06 g (4 mmol) triphenylphosphine, 0.505 g (2 mmol) iodine and 0.490 g (2 mmol) **22** were stirred at 63°C for 40 hr. An additional 0.256 g (1 mmol) of iodine was then added and stirred for 2.0 hr. The reaction was quenched by cooling to 50° C and adding 10 ml anhydrous MeOH. After removal of the solvent the residue was treated with 75 ml anhydrous MeOH and re-evaporated to give a residue which was loaded onto a 5 X 31 cm flash chromatography column. The column was eluted first with 1650 ml CHCl₃, then CHCl₃/MeOH (9:1) and collected in 20 ml fractions. Fractions 55-77 contained the product. Evaporation of the solvent gave a brownish residue which was dissolved in hot water, carbon treated and air evaporated to give a white solid. Recrystallization from hot water gave 0.112 g (15.8%) **23**, mp 177-178.5°C (dec). HPLC 99.8%, RT=5.01 min. TLC single spot at R_f=0.74. NMR - DMSO - D₆- 6.097 (C₁H - d, J = 7.49 Hz).

Methyl-α-D-arabinofuranoside tribenzoate (24). Anhydrous MeOH (400 ml), 20.0 g (0.133 mol) D-arabinose and 126 ml of freshly prepared 1.06 N HCl/MeOH were stirred at ambient temperature until complete solution was obtained. The progress of the reaction was followed until a negative reaction was obtained with Fehlings solution after a total reaction time was about 4.0 hr. The reaction was terminated by the addition of 74 ml anhydrous pyridine. The solvent was removed at 70° C on a rotary evaporator. Anhydrous pyridine (60 ml) was added to the residue and re-evaporated. The residue was then dissolved in 150 ml anhydrous pyridine, chilled to 0° and 62 ml BzOCl was added over 60 min. The mixture was stirred at 0°C for an additional 60 min, then warmed to 55°C for 30 min. H₂O (3.0 ml) was added to destroy any excess BzOCl. CH₂Cl₂ (300 ml) was added and the mixture extracted successively with 125 ml H₂O, twice each with 100 ml 3N H₂SO₄ and 100 ml sat NaHCO₃. After drying with Na₂SO₄, the solvent was removed to give a syrup which was dissolved in 200 ml anhydrous EtOH. Upon stirring at ambient temperature for 16 hr followed by 1 hr in the freezer, crystals formed. Recrystallization from anhydrous EtOH gave 32.44 g (51.1%) **24**, mp 99-100°C (Lit. 100-101.5°C (12)). TLC n-butyl chloride/acetonitrile (95:5) single spot R_f = 0.41.

Tri-O-benzoyl-α-D-arabinopyranosyl bromide (25). Finely ground **24** (9.23 g (19.37 mmol)) was added to 46.2 ml glacial AcOH. The mixture was stirred at ambient temperature for 30 min. 30-32% HBr (46.2 ml) in acetic acid was added and the mixture stirred at ambient temperature until a homogeneous solution

was obtained and then for an additional 30 min. The reaction mixture was diluted with 300 ml CH_2Cl_2 and then poured into 1.0 L of ice-water mixture which was quickly extracted with sat NaHCO_3 (check for a $\text{pH} > 7.0$ by paper). After drying with Na_2SO_4 , the CHCl_3 was removed and the resulting syrup placed into a freezer. Crystals which began to form in about 72 hr were removed to room temperature to complete the crystallization. Recrystallization from ether/pentane (2:1) gave 2.74 g (27.4%) **25**, mp 100.5-102°C (Lit. 103-104°C (12)). TLC hexane/ethyl acetate (70:30) single spot $R_f = 0.38$.

1-(Tri-*o*-benzoyl- α -D-arabinofuranosyl)-2-nitroimidazole (26). Anhydrous CH_3CN (320 ml), 1.00 g (8.86 mmol) azomycin, 5.03 g (20 mmol) $\text{Hg}(\text{CN})_2$ and 4.202 g (8 mmol) of **25** were stirred at 40°C for 5 hr and then at ambient temperature for 16 hr. The solvent was removed and the residue taken up with 300 ml CH_2Cl_2 and filtered. The filtrate was washed twice each with 100 ml sat NaHCO_3 , 50 ml 25% KI and 100 ml water. After drying with MgSO_4 and removal of the solvent, a yellowish solid residue remained which was loaded onto a 5 X 32 cm flash chromatography column and eluted with hexane/EtOAc (70:30). Fractions (20 ml) were collected. Fractions 62-79 contained the β -anomer and 89-120 contained the α -anomer. The fractions were evaporated. TLC hexane/EtOAc (70:30) α -anomer single spot at $R_f = 0.26$ while the β -anomer gave a single spot at $R_f = 0.34$. The yield of the α -anomer was 1.013 g (22.7%) while that of the β -anomer was 0.959 g (21.5%). A small portion of the α -anomer was recrystallized from ether/hexane to give a melting point sample. The melting point was 71-77°C (Lit: 82°C (13)).

1-(2-benzoyl- α -D-arabinofuranosyl)-2-nitroimidazole (27). MeOH/MeONa (28.4 ml of 0.05 M) was chilled to 0°C. **26** (α -anomer) (3.167 g (5.69 mmol)) was added and stirred at 0°C for 2.5 hr. Any excess methoxide was destroyed by the addition of a few drops of glacial AcOH. The mixture was filtered and the precipitate recrystallized from anhydrous MeOH to give 0.490 g (35.2%) **27**, mp 196-197°C (dec). IR -KBr - 3300 (OH), 1716 (benzoyl ester), 1470 ($-\text{NO}_2$), 1200 (C-O) cm^{-1} . NMR- CD_3OD -7.3194 (benzoyl H-m), 6.5067 (C,H-d, $J = 1.28$ Hz).

1-(α -D-arabinofuranosyl)-2-nitroimidazole (28). **27** (0.317 g (0.91 mmol)) and 32 ml NH_3 saturated MeOH were placed into a 0-4°C refrigerator for 50 hr. Nitrogen gas was used to remove some of the NH_3 and the solvent was removed on a rotary evaporator, keeping the temperature of the bath at $\leq 40^\circ\text{C}$. The residue was taken up with 5-10 ml CHCl_3 , stirred for 10 min and filtered to give 0.190 g (85.4) **28**, mp 153-155°C (Lit: 160°C (13)). TLC single spot $R_f = 0.51$ (starting material had $R_f = 0.78$). HPLC 100%, RT=3.92 min. NMR - DMSO - D_6 -6.2976 (C,H-d, $J = 1.47$ Hz).

1-(5-deoxy-5-iodo- α -D-arabinofuranosyl)-2-nitroimidazole (29). To a solution containing 0.247 g (1.01 mmol) of **28** dissolved in 25.2 ml anhydrous pyridine were added 0.534 g (2.04 mmol) triphenylphosphine and 0.256 g (1.00 mmol) iodine. The solution was stirred for 2.0 hr at 60°C. The reaction was quenched by cooling to 50°C and adding 2.5 ml anhydrous MeOH. The solvent was removed, 25 ml additional anhydrous MeOH was added to the residue and re-evaporated. The residue was loaded onto a 4 X 34 cm flash chromatography column and eluted first with 660 ml CHCl_3 , then with $\text{CHCl}_3/\text{MeOH}$ (9:1) and collected in 15 ml fractions. Fractions 66-80 were collected and evaporated to a sticky residue to which ether was added. Rotary evaporation yielded a foamy solid. Drying at 1 mmHg for 1 hr at 50°C and 16 hr at ambient temperature yielded 0.1891 g (52.8%) **29**. HPLC assay indicated that the sample contained 92% of

the expected product and 8% starting material. Preparative HPLC with a C18 Magnum Column and methanol/water (1:1) at 1.0 ml/min yielded after evaporation 47.1 mg (13.2%) **29**. HPLC 100%, RT=9.87 min. TLC single spot Rf=0.74. NMR-DMSO-D₆-6.38899 (C,H - d, J=1.48 Hz).

Radiolabelling Procedures

Na¹²⁵I (1 mCi) was transferred to a 0.1 ml reactival and evaporated to dryness under a stream of dry nitrogen. Anhydrous ethanol (100 µl) was added and re-evaporated to dryness.

The marker (1 mg) was dissolved in 20 µl anhydrous DMF, added to the reactival, sealed and placed in a heated oil bath. Compounds **3**, **6**, **9**, **23** and **29** were heated at 80-82°C for 5-6 hr while **14** and **19** were heated at 110°C for 6.0 hr. The vial was cooled and the DMF removed under a stream of dry nitrogen. The labeled marker was purified by passing it through a mini-column containing (from bottom to top) 40 mg BioRad AG1XB anion exchange resin (200-400 mesh), chloride form and 50 mg AgCl impregnated celite (56 mg AgCl/100 mg). The solvent was 2.5 ml water for compounds **3**, **6**, **9** and 2.5 ml methanol/water (1:1) for compounds **14**, **19**, **23**, **29**. The markers that were dissolved in methanol/water were evaporated to dryness with a stream of dry nitrogen and reconstituted with 2.5 ml water. This class of marker was warmed to 40-50° prior to further use. For labelling with I-123, the procedure was identical to that used for I-125 except that the initial I-123 (10 mCi/0.2 ml) solution as received from MediPhysics was adjusted to pH 6.5-7.5 with 1N sulfuric acid prior to evaporation. The final solutions were assayed by spotting 1 µl on a TLC plate and developing a distance of 5 cm. The dried plates were autoradiographed and spots quantified by densitometry.

Octanol-Buffer Distribution Coefficient

Octanol-saturated sodium phosphate buffer (0.05M, pH 7.4) and buffer-saturated octanol (2.0 ml each) were placed into a stoppered tube with 10⁵-10⁶ dpm of the labeled marker. The stoppered tube was vigorously hand shaken for 1.0 min, allowed to settle for 1.0 min then reshaken for an additional 1.0 min. The phases were allowed to separate for 2.0 min. Aliquots (50 µl) of each phase were counted for radioactivity. The ratio of the dpm-octanol to dpm-buffer gave the distribution coefficient, Kd.

Cell Uptake Studies

The uptake of the radiolabeled markers into EMT-6 tumor cells was determined according to the method previously described by Chapman et al. (14). The amount of radioactivity which precipitated with the TCA extracted macromolecular fractions was determined at various times. The initial binding rate of marker to cells was defined by a linear regression to the linked radioactivity versus time (in units of pmole/10⁶ cell/hr).

Drug Tissue Distribution - Mice

Female C.B17/lcr *scid* mice were implanted with EMT-6 tumors by subcutaneous injection of a cell suspension containing 4 X 10⁶ cells in 0.02 ml. The tumors were allowed to grow to a diameter of approximately 10 mm (12-14 days). Radiolabelled hypoxic marker (60-70 kBq) was administered by either tail vein or intraperitoneal injection. After the desired time period the animals were anesthetized with metophane and sacrificed by decapitation. The tissues to be analyzed were excized, weighed and their radioactivity content determined with a gamma counter.

Mouse imaging: Mice that had been implanted with EMT-6 tumors as previously described were given 0.2 to 0.3 mCi ¹²⁵I labeled **3** via intraperitoneal injection. SPECT images were taken at 1 and 10 hr post injection. The mice were anesthetized with metophane just prior to imaging.

RESULTS

Synthesis: The synthetic pathways leading to compounds **3**, **6**, **9**, **14**, **19**, **23**, **29** are shown in Figures 1, 2, 3. All were prepared according to procedures described in the literature although sometimes in modified form. The position of the nitro substituent of **9** was established by NMR. According to Raju et al. (15), the 4-nitro

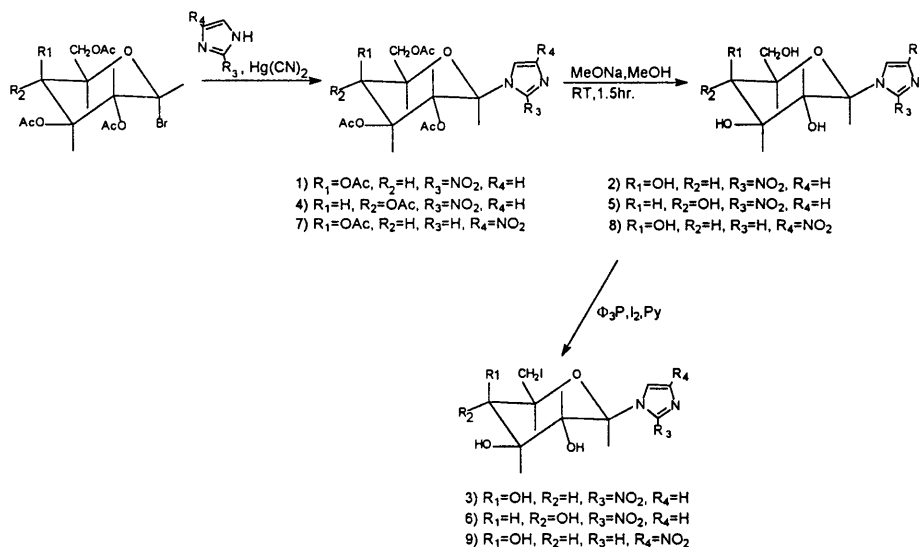


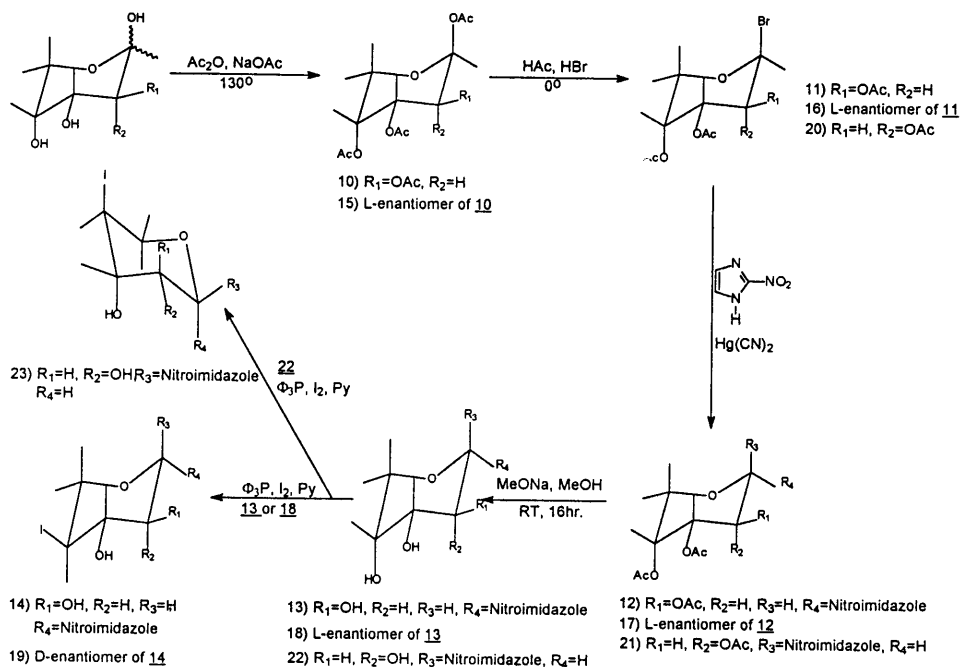
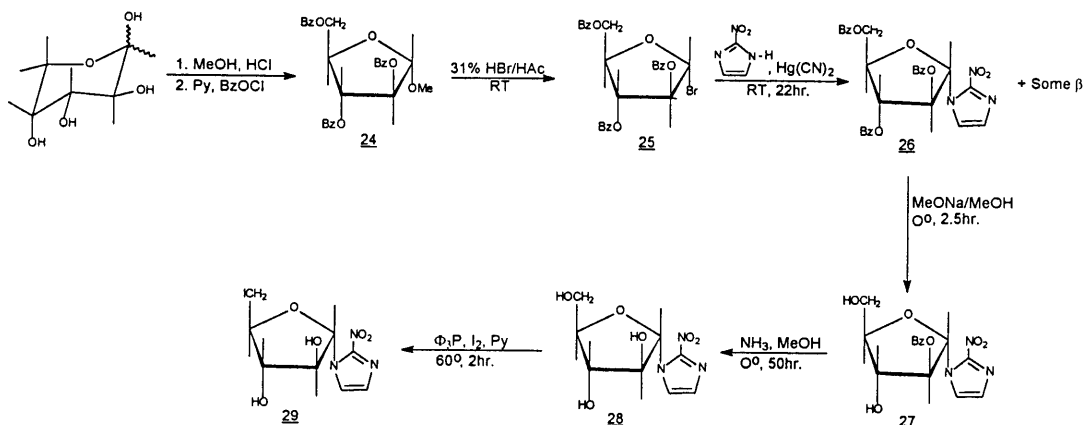
Figure 1: Synthesis of **3, **6**, **9**.**

isomer gives rise to a pair of doublets while the 5-nitro isomer gives rise to a single doublet. Compound **9** has two sets of doublets at 8.3796 and 7.9663 ppm and on this basis was identified as the 4-isomer.

The α -anomeric configuration for **14** was confirmed by NMR spectroscopy. The coupling constant for the C_1 anomeric proton and the C_2 proton was found to be 8.91 and is consistent with a coupling constant range of 8-14 as predicted by the Karplus equation for vicinal protons transaxially located on a six membered ring in the chair form (16).

Compound **14** was compared via melting point, HPLC and NMR to a sample of a compound reported by Mannan et al. (17) as the β -anomer of **14**. Their compound was identical in all respects to **14** which leads us to conclude that Mannan's compound is the α -anomer and not the reported β -anomeric form.

The previously unreported compound **19** is the enantiomeric form of **14** and with the exception of optical rotation is identical in melting point, HPLC retention time and NMR spectra.

Figure 2: Synthesis of 14, 19, 23.Figure 3: Synthesis of 29.

In the synthesis of 23, the conversion of the bromopyranoside to the azomycin derivative is of special interest. The nucleophilic displacement of bromine by azomycin is an S_N1 type reaction assisted by $Hg(CN)_2$, with the more stable α -anomer as the expected product. However, the position of the acetyl group

on C₂ is such that it can anchimerically assist the removal of the bromine, leaving a stabilized cyclic carbonium ion in its place. Under these conditions normal nucleophilic attack is blocked by the cyclic carbonium ion and the azomycin nucleophile attacks from the same side as the departing bromide, generating the β rather than the expected α-anomer (18).

The synthesis of **29** was carried out according to published procedures (17,13). The preparation of intermediate **26** gave rise to two anomers with the α slightly predominate, by the same mechanism previously described for the formation of **21**.

The debenzoylation of **26** presented some difficulties. The use of sodium methoxide failed to completely debenzoylate **26** leaving the benzoylated hydroxyl group on C₂ intact. Changing the reaction conditions resulted in mixtures of products and low yields. It was found that the remaining benzoyl group could be removed by treating with ammonia/methanol at 0°C for 50 hr. The final product, **28**, was identical to that reported by Sakaguchi et al. (13) for the α-anomer. The conversion of **28** to **29** gave a crude product which contained about 8% starting material. In this case preparative HPLC was used to isolate pure **29**. NMR spectroscopy confirmed **29** as the α-anomer (13,19,20).

Radiolabeling: The Na¹²⁵I used to prepare radiolabeled marker for imaging studies came as a solution in 0.1 N NaOH. Exchange labeling at high pH gave little product and extensive degradation. It was necessary to lower the pH to 6.0-7.0 by the addition of 1.0 N sulfuric acid. After evaporation, the labeling step can be carried out in normal fashion. The sodium sulfate which is formed does not hinder the radiolabeling and is removed when the drug is passed through the minicolumn for final purification. The radiolabeling results with several markers are shown in Table 1.

When radiolabeled **14** and **19** are subjected to thin layer chromatography, they yield a spot at Rf=0.72, as expected, along with an additional product at Rf=0.68 (see Table 1 for all TLC data). The iodine

TABLE I: RESULTS OF RADIOLABELING OF CHEMICAL MARKERS

Compounds	Iodine Isotope	Chemical yield (%)	Labeling efficiency (%)	Specific activity GBq/mM	TLC* Rf labeled	TLC* Rf labeled	Rad. Chem. purity* (%)
3	125	86.8	61.4	10.2	0.58	0.60	100
3	123	82.3	52.7	94.0	0.58	0.57	100
6	125	85.3	45.5	7.6	0.58	0.54	100
9	125	83.8	77.0	13.5	0.55	0.52	100
14	125	78.0	45.7	7.2	0.74	0.68, 0.72 ^b	15.3, 84.7
19	125	89.9	59.9	9.73	0.73	0.68, 0.72 ^b	30.0, 70.0
23	125	92.3	39.6	5.2	0.74	0.61, 0.72, 0.76 ^b	46.9, 14.3, 38.8
29	125	88.7	38.6	5.3	0.74	0.77	

*SG60F with mobile phase of CHCl₃/MeOH (77.5:22.5)

^bsee text for discussion of multiple spots

exchange reaction, whose mechanism at the tracer level is not well understood could proceed through an Sn2 type transition state in which the incoming iodine nucleophile is positioned on the side opposite the departing iodide. Decomposition to the ground state could then yield an axial or equatorial iodinated product with the axial to equatorial ratio being determined by their relative stabilities (21). Compound **23** also yields two closely spaced spots on radio TLC, the expected one at Rf=0.76 and an additional one at Rf=0.72 which could also be explained by the above mechanism. Compound **23** also gave an additional spot at Rf=0.61. The iodine at C-4 is transaxial to the hydroxyl group on C-3 and the hydroxyl group can assist the leaving of the iodine via backside attack with subsequent formation of an oxonium ion, which upon reaction with iodide, could either regenerate **23** or form the axial 3-iodoisomer, 1-(3-deoxy-3-iodo- β -L-xylopyranosyl)-2-nitroimidazole. More work will be required to confirm this mechanism.

Octanol/buffer partition coefficients: Some of the methods described in the literature for determining partition coefficient involve longer shaking times than those used in this study. We performed comparative studies with these markers and found that the shorter mixing times yielded identical values to those produced by the longer mixing times. The partition coefficients of the novel markers are presented in Table II.

Hypoxic Tumor Cell Marking: Marker binding to hypoxic cells was measured as a function of drug and oxygen concentration according to procedures described by Chapman et al. (14). The data shown in Figures 4, 5 and 6 were generated with **19** and are typical of the other markers. The oxygen concentration dependency (Figures 4&5) is similar for all the markers and is consistent with azomycin being the common bioreducible substituent. These data clearly show the strong oxygen dependency of marker linkage to cells which provides the basis for the measurement of tumor hypoxia. The rate of marker linkage to hypoxic cells

TABLE II: CHEMICAL AND BIOLOGICAL PROPERTIES OF NOVEL HYPOXIC MARKERS

Marker	P Octanol/water	<i>In vitro</i> binding rate at 10 μ M (pmol/10 ⁶ cells hr)	Plasma T _{1/2} (hr)	T/B ^b 8-24 hr
3	0.63	13.6	0.46	11.1
6	1.07	20.9	0.52	7.9
9	0.37	1.55 ^a	---	---
14	1.29	65.5	1.51	11.7
19	1.26	133	0.46	9.9
23	1.00	51.5	0.79	7.3
29	3.85	76.0	0.87	8.3

^alow binding rate results from lower reduction potential of 4-nitroimidazole.

^bT/B = tumor to blood ratio

in vitro increases with marker concentration (Figure 6) according to 1/2-order to 1-order kinetics (14). The relevance of these *in vitro* binding kinetics to *in vivo* tumor labeling is not known. The rates of marker binding

to hypoxic cells *in vitro* at a concentration of 10 μM are presented in Table II. All of the compounds exhibited a low level of metabolism-independent binding as indicated by the cell associated radioactivity at zero time (Figures 4&6). Non-specific binding of markers will contribute to the background over which the desired hypoxic-specific signal must be detected.

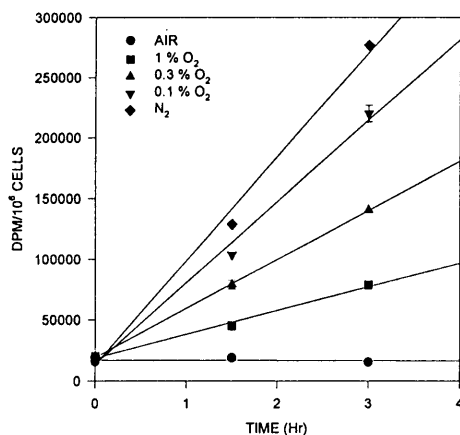


Figure 4: Cell binding of ¹²⁵I labeled 19 (20 μM) at various O₂ concentrations.

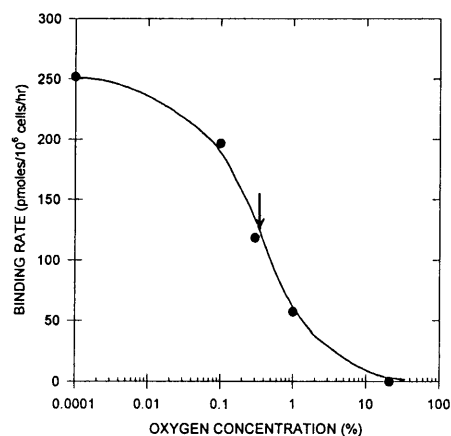


Figure 5: Absolute binding rates of 19 versus O₂ concentration.

In vivo biodistribution studies of the markers were performed with EMT-6 tumor-bearing mice. The measured plasma half-life of unmetabolized markers and average maximal tumor to blood (T/B) ratios between 10-24 hr are summarized in Table II. Examples of the biodistribution data generated with 19 and 3 are shown in Tables III and IV, respectively.

Neither the octanol/water partition nor the *in vitro* binding rates of markers strictly predict for their *in vivo* biodistributions or their tumor marking potential. The biodistribution data indicates a considerable hepatobiliary excretion which could interfere with the imaging of tumors in the abdomen. The range of octanol/water partition coefficients for these markers was quite small and more hydrophilic markers are required to assess the role of this parameter in tumor marking activity.

The blood levels of 19 and 3 are shown in Figure 7. The curves for these and the other markers are biphasic with the rapid renal clearance of unmetabolized marker complete within 2-3 hr followed by a more gradual clearance associated with hepatobiliary excretion of metabolized drug.

Imaging: For imaging studies we chose compound 3 labeled with Iodine-123 because of its low P, short plasma half-life and high T/B ratio. A short plasma half-life suggests a rapid clearance of the unmetabolized background marker while a high T/B ratio indicates good contrast between hypoxic tumor tissue and other

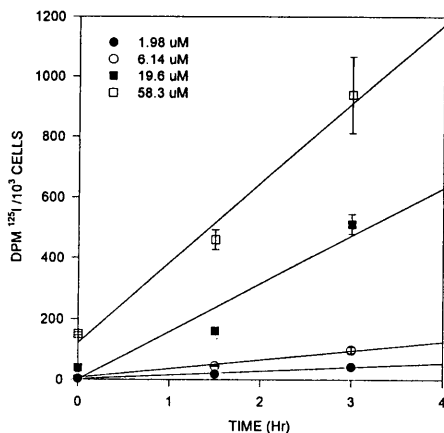


Figure 6: Binding of ^{125}I labelled 19 to EMT-6 cells at different marker concentrations.

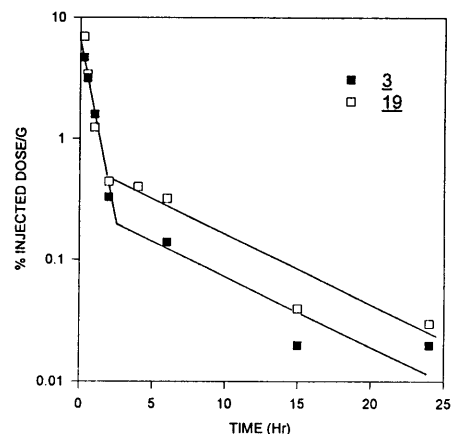


Figure 7: Levels of 19 and 3 in mouse blood at various times after i.v. administration.

TABLE III: MOUSE DISTRIBUTION DATA FOR COMPOUND 19^{a,b}

Tissue	Time (hrs)							
	0.25	0.50	1.00	2.00	4.00	8.00	15.00	24.00
Blood	6.96±27 ^c (1.00)	3.41±22 (1.00)	1.24±11 (1.00)	0.44±03 (1.00)	0.40±02 (1.00)	0.32±07 (1.00)	0.04±005 (1.00)	0.034±002 (1.00)
Liver	18.66±58 (2.68)	8.88±1.04 (2.60)	2.65±23 (2.14)	0.98±07 (2.23)	1.18±49 (2.95)	0.55±04 (1.72)	0.41±05 (10.25)	0.23±003 (6.76)
Kidney	61.89±2.39 (8.89)	45.23±2.62 (13.26)	11.80±1.13 (9.52)	1.46±13 (3.32)	0.75±04 (1.88)	0.56±05 (1.75)	0.27±02 (6.75)	0.21±02 (6.18)
Spleen	4.26±15 (0.61)	2.90±62 (0.85)	0.92±10 (0.74)	0.39±03 (0.89)	0.35±02 (0.88)	0.27±04 (0.84)	0.07±005 (1.75)	0.07±006 (2.06)
Lungs	7.29±28 (1.05)	4.08±13 (1.20)	1.59±29 (1.28)	0.67±09 (1.52)	0.63±12 (1.58)	0.42±05 (1.31)	0.16±02 (4.00)	0.10±005 (2.94)
Muscle	4.52±16 (0.65)	2.78±53 (0.82)	0.76±16 (0.61)	0.32±04 (0.73)	0.16±01 (0.40)	0.11±02 (0.34)	0.03±007 (0.75)	0.023±001 (0.68)
Brain	0.90±09 (0.13)	0.75±04 (0.22)	0.53±08 (0.43)	0.28±03 (0.64)	0.11±004 (0.28)	0.08±01 (0.25)	0.04±002 (1.00)	0.027±001 (0.79)
Tumor	5.34±45 (0.77)	5.54±19 (1.62)	4.46±07 (3.60)	3.27±16 (7.43)	2.71±05 (6.78)	1.85±38 (5.78)	0.61±09 (15.20)	0.35±02 (10.3)

^aValues represent the % injected dose per gram tissue

^bNumbers in parentheses are the tissue to blood ratio

^cmean ± S.D., n=5

tissues. Figs. 8A and 8B show planar images of the same mice at 1.0 and 10 hr post marker injection, respectively. The early image shows a general distribution of the marker with intense marker activity in the bladder and liver while the 10 hr image shows marker retained in tumors (T) against an intense background of marker undergoing GI excretion. The thyroids were weakly labelled in some animals at this later time. These data correlate well with the biodistribution data for 3 shown in Table IV.

Summary and Conclusion: We have synthesized and radioiodinated seven azomycin nucleosides with lower partition coefficients than IAZA. Binding to viable hypoxic cells *in vitro* has been demonstrated and planar

images of hypoxic tumors in mice have been obtained. The optimal marker, **3**, has the lowest partition coefficient, the fastest plasma clearance rate and the maximal T/B and T/M ratios at 6-24 hr after administration to EMT-6 tumor-bearing mice.

These data suggest that markers of this class with even greater water solubility (lower P) could have superior hypoxic marking potential. Additional water soluble hypoxic markers of the azomycin-nucleoside, azomycin-aromatic and azomycin-chelate classes are being synthesized for testing as nuclear medicine markers of this tumor property.

TABLE IV: MOUSE BIODISTRIBUTION DATA FOR COMPOUND 3^a

	Time(hrs)						
Tissue	0.25	0.50	1.00	2.00	6.00	15.00	24.00
Blood	4.70±.30 ^c	3.17±.09	1.60±.16	.033±.08	0.14±.01	0.02±.003	0.02±.002
	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
Liver	10.89±1.18	6.73±.43	3.63±.33	0.77±.22	0.19±.03	0.05±.01	0.06±.007
	(2.32)	(2.12)	(2.27)	(2.33)	(1.36)	(2.50)	(3.00)
Kidney	11.14±.89	15.97±5.10	5.06±.43	0.79±.16	0.18±.02	0.03±.006	0.03±.005
	(2.37)	(5.04)	(3.16)	(2.39)	(1.29)	(1.50)	(1.50)
Spleen	8.84±1.30	3.54±.16	1.55±.13	0.34±.06	0.10±.02	0.02±.002	0.02±.003
	(1.88)	(1.12)	(0.97)	(1.03)	(0.71)	(1.00)	(1.00)
Lungs	5.10±.41	3.48±.09	1.86±.18	0.39±.10	0.14±.02	0.02±.004	0.02±.003
	(1.09)	(1.10)	(1.16)	(1.18)	(1.00)	(1.00)	(1.00)
Muscle	2.34±.05	2.42±.10	1.75±.17	0.35±.07	0.06±.005	0.01±.002	0.01±.001
	(0.50)	(0.76)	(1.09)	(1.06)	(0.43)	(0.50)	(0.50)
Brain	0.25±.02	0.20±.04	0.14±.01	0.05±.01	0.01±.002	.0018±.0004	.002±.0004
	(0.05)	(0.06)	(0.09)	(0.15)	(0.07)	(0.09)	(0.01)
Tumor	2.80±.14	3.27±.08	2.78±.16	1.54±.19	1.38±.22	0.19±.04	0.21±.03
	(0.56)	(1.03)	(1.74)	(4.67)	(9.86)	(9.50)	(10.50)

^aValues represent the % injected dose per gram tissue

^bValues in parentheses are the tissue to blood ratio

^cmean ± S.D., n=5

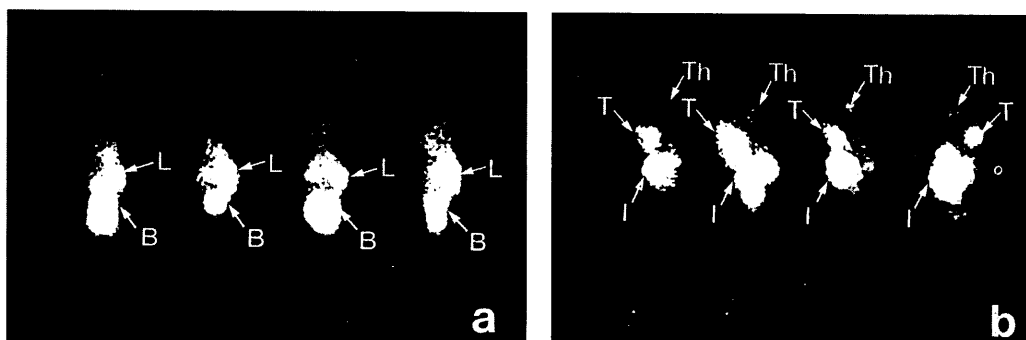


Figure 8: Planar nuclear medicine images of EMT-6 tumor-bearing mice at 1 hr (a) and 10 hr (b) after administration of hypoxic marker, **3. B=bladder, I=intestine, L=liver, T=tumor and Th=thyroid.**

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