New Azomycin Acyclonucleosides. Synthesis and Biodistribution of Radiohalogenated Analogues in Tumor-bearing Mice [1]

Ahmad Hasan* and Furn F. Knapp, Jr.

Nuclear Medicine Group, Health Sciences
Research Division, Oak Ridge National Laboratory,
Oak Ridge, Tennessee 37831-6229

Michael R. Kilbourn

Department of Internal Medicine,
University of Michigan Medical Center,
Ann Arbor, Michigan 48109-0028

Donald J. Buchsbaum

Department of Radiation Oncology, University of
Alabama at Birmingham, 1824 6th Avenue S,
Birmingham, Alabama 35233

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Dedicated to the memory of Dr. Roland K. Robins

The design, synthesis and biological activities of several acyclonucleoside analogues related to misonidazole are described. The hydroxy-**5**, bromo-**6**, iodo-**7**, and fluoro-**8** derivatives of ethoxymethylazomycin and iodopropenyloxymethylazomycin (**12**) have been prepared. Alkylation of silylated azomycin with haloethoxymethylene chloride gave the corresponding acyclonucleosides. Similarly, propargyloxymethylene chloride gave propargyloxymethylazomycin (**10**), which after hydrostannylation and subsequent iododestannylation yielded iodopropenyloxymethylazomycin (**12**). The radiolabeled [^{125}I] or [^{18}F] compounds were prepared from the corresponding substrates. Biodistribution results of the radiolabeled analogues in mice showed that compound **7** had good tumor uptake (2.0% injected dose/g at 1 hour). The high radioactive levels in blood and stomach, however, were perhaps due to *in vivo* deiodination or metabolism. Compound [^{125}I]-**12** showed the highest tumor uptake (4.8 and 3.6% injected dose/g at 1 and 4 hours respectively) of all of the compounds tested. Relatively low thyroid uptake of radioactivity in mice dosed with compound [^{125}I]-**12** indicates significantly reduced *in vivo* deiodination in comparison to compound [^{125}I]-**7**.

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Introduction.

Most solid tumors, unlike normal tissues, contain hypoxic cells due to an insufficient blood supply. A variety of 2-nitroimidazole (azomycin) derivatives have been shown to selectively sensitize hypoxic cells, present in solid tumors, toward the lethal effect of ionizing radiation [2,3]. The radiosensitizing property of these agents appears to correlate with their ability to penetrate tumors which contain hypoxic cells and necrotic areas [4,5]. Among the various nitroimidazole analogues evaluated thus far, misonidazole (1 [2-hydroxy-3-methoxypropyl]-2-nitroimidazole), (Chart 1) has been found to be an effective radiosensitizer of hypoxic cells [3] and has been evaluated clinically [6,7]. However, at safe clinical doses [4,8], little or no radiosensitization has been observed. Increased doses may not be safe, however, since the major limiting host toxicities of misonidazole are neurotoxicity [9], peripheral neuropathy [10] and mutagenicity [11]. The sugar-substituted azomycin analogues iodoazomycin riboside [12], and iodoazomycin arabinoside [13] (Chart 1) have recently been reported to exhibit hypoxia-dependent binding greater than misonidazole, but iodoazomycin arabinoside showed very little uptake of activity in a normoxic environment compared to a hypoxic environment. Furthermore, the iodoazomycin riboside is a more potent cytotoxic agent and also binds selectively to the hypoxic tumor cells *in vitro* than azomycin or misonidazole.

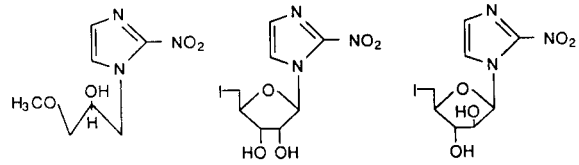


Chart 1

The potent chemotherapeutic activity exhibited by certain acyclonucleosides [14,15] in contrast to their inactive ribonucleoside natural congeners led us to investigate the synthesis and biodistribution of azomycin acyclonucleosides that would selectively bind to hypoxic cells within tumors. Such agents may potentially be useful as radiolabeled tracers for detection of hypoxic regions in solid tumors. In this paper, we describe the synthesis, radiolabeling, and biodistribution of 2-nitro-1-[2-iodoethoxy-

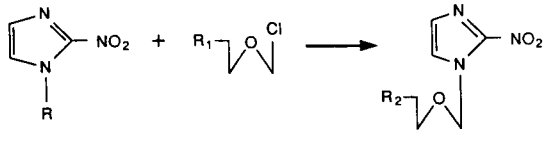
methyl]imidazole (**7**), 2-nitro-1-[2-fluoroethoxymethyl]imidazole (**8**), and 2-nitro-1-[2-iodopropenyloxymethyl]imidazole (**12**).

Results and Discussion.

Chemistry.

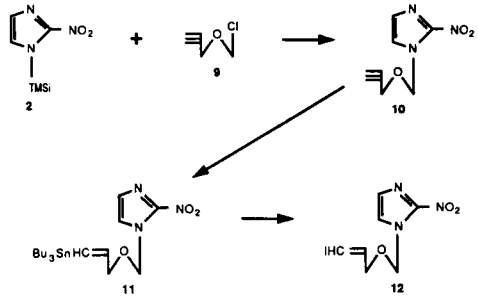
The synthesis of 2-nitro-1-[2-hydroxyethoxymethyl]imidazole (azomycin acyclonucleoside, **5**) has recently been reported [16]. The halogenated nucleosides are useful precursors for exchange labeling [12] with $Na^{125}I$ and were therefore selected for this study. Since compound **5** provided 2-nitro-1-[2-iodoethoxymethyl]imidazole (**7**) in poor yield when treated with methyltriphenoxyphosphonium iodide (Rydon Reagent) [17], we investigated an alternative route outlined (Scheme I). Alkylation of the silyl derivtive of azomycin (**1**) with 2-iodoethoxymethylene chloride (**4**) in the presence of triethylamine gave compound **7** in 69% yield. The analytical and spectral data were analogous to the compound **7** synthesized using the Rydon reagent. Similarly, compound **6** was prepared as a precursor for exchange reaction with silver fluoride (AgF). Upon refluxing with silver fluoride in toluene, compound **6** yielded 2-nitro-1-(2-fluoroethoxymethyl)imidazole (**8**). The [^{18}F] radiolabeled analogue of compound **8** was prepared by [^{18}F]fluoride substitution of compound **7**.

Scheme I



Primary iodides are reported to be more susceptible to *in vivo* deiodination than vinyl or aryl iodides [18]. In an attempt to optimize the *in vivo* stability and tumor specificity of azomycin acyclonucleoside, we synthesized an iodovinyl analogue of azomycin acyclonucleoside (Scheme II). Treatment of compound **2** with propargyloxymethylene chloride (**9**) [19] in the presence of triethylamine in dichloroethane provided 2-nitro-1-(propargyloxymethyl)-imidazole (**10**) in 76% yield. We have previously studied

Scheme II

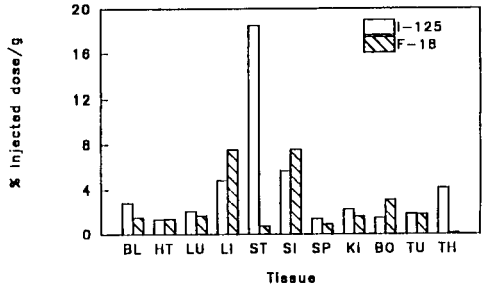


in some detail the synthesis of vinyl iodides from the corresponding alkenyl substrates utilizing various hydrometalation techniques [19-24]. The optimal conditions determined for the synthesis of **12** were *via* the formation of 2-nitro-1-(*E*-1-tributylstannyl-1-propenyloxymethyl)imidazole (**11**) by the reaction of compound **10** with excess of tributyltin hydride [25] followed by iododestannylation with sodium iodide in the presence of *N*-chlorosuccinimide. The *E*-isomeric configuration of compounds **11** and **12** was assigned on the basis of nmr spectroscopy [26]. In the ^{1}H nmr of compound **11**, the signal for vinyl protons appeared as a multiplet at 6.95-6.75 ppm and the observed chemical shift for vinyl protons of compound **12** appeared at 7.2 (dd, J = 14.8 and 8.0 Hz) ppm and at 5.9 (d, J = 16.5 Hz) ppm which are consistent with the literature reports for an *E*-isomer [25-27]. The ^{125}I-radiolabeled analogues of compound **7** and **12** were prepared similarly using $Na^{125}I$ for tissue distribution studies.

Tissue Distribution Studies.

Both [^{125}I]iodo- and [^{18}F]fluoroazomycin acyclonucleosides **7** and **8** were evaluated in nude mice bearing LS174T human colon cancer xenografts. The results of a dual labeled biodistribution experiment are shown in Figure 1. Both compounds [^{125}I]-**7** and [^{18}F]-**8** showed similar tumor uptake of about 2% injected dose/g at 1 hour following intraperitoneal injection. Major differences observed in the level of tissue uptake were in the stomach and thyroid, perhaps due to *in vivo* deiodination or metabolism. In these organs, the uptake of the ^{18}F congeners is significantly lower as compared to the ^{125}I congeners which would suggest deiodination.

Tissue Distribution At 1Hour Following Intraperitoneal Administration Of 2-Nitro-1-(2-[^{125}I]-Iodoethoxymethyl) Imidazole (7) And 2-Nitro-1-(2-[^{18}F]-fluoroethoxymethyl) Imidazole (8) In Nude Mice Bearing LS174T Human Colon Cancer Xenografts



BL=Blood, HT=Heart, LU=Lung, LI=Liver, ST=Stomach SI=Small Intestine, SP=Spleen, KI=Kidney, BO=Bone, TU=Tumor, TH=Thyroid

Figure 1

In order to stabilize radioactive iodine and therefore reduce *in vivo* deiodination and to optimize the tumor uptake relative to the normal organs, we have also synthesized a vinylic iodide derivative **12** of azomycin acyclonu-

cleoside. Compound **12** was evaluated after intravenous administration to tumor-bearing mice to determine tumor specificity and *in vivo* stability of radioiodine. The tissue distribution data are presented (Table 1). Compound [^{125}I]-**12** showed the highest tumor uptake (4.8% and 3.6% injected dose/g at 1 and 4 hours respectively) of all compounds tested. The relatively low thyroid uptake of radioactivity in mice indicates significantly reduced *in vivo* deiodination of compound [^{125}I]-**12** as compared to compound [^{125}I]-**7**.

Table 1

Tissue Distribution Following Intraperitoneal Administration of 1-(E-1-[^{125}I]iodopropenyloxymethyl)azomycin (12) in Nude Mice Bearing LS174T Human Colon Cancer Xenografts [a,b]

Mean % dose/g (range)				
TIME AFTER INJECTION	TUMOR	BLOOD	LIVER	THYROID [c]
1 hour	4.8 (3.1-6.9)	7.2 (6.3-7.9)	6.4 (4.9-7.1)	0.4 (0.2-0.6)
4 hour	3.6 (1.1-4.7)	6.0 (1.6-8.6)	4.5 (2.0-6.0)	0.2 (0.2-0.4)
24 hour	0.3 (0.2-0.4)	0.2 (0.2-0.3)	0.9 (0.8-1.0)	0.3 (0.2-0.4)

[a] Six animals per group were used.

[b] Other organs evaluated included heart, lungs, small intestine, spleen, kidneys, skin, bone, and muscle

[c] Values as % dose/thyroid

Conclusions.

The objective of these studies was to synthesize acyclonucleoside anaolgues and determine biodistribution and tumor uptake of their radiolabeled [^{125}I] and [^{18}F] congeners. The biodistribution results of compounds [^{125}I]-**7** and [^{18}F]-**8** in animal tumor models showed similar tumor uptake, but compound [^{125}I]-**7** also showed high levels of radioactivity in other normal organs including stomach and thyroid (Figure 1) perhaps due to *in vivo* deiodination. The biodistribution results of the compound [^{125}I]-**12** showed reasonably good tumor uptake at the early times after injection, with relatively low uptake in thyroid. The low thyroid uptake indicates reduced *in vivo* deiodination as compared to [^{125}I]-iodoazomycin acyclonucleoside (**7**). The results indicate the potential for the development of similar [^{18}F] and [^{125}I] compounds which may have relatively high tumor uptake compared to the other organs for study of hypoxic regions of tumor by position emission computed tomography (PET) or single photon emission computed tomography (SPECT), respectively.

EXPERIMENTAL

General Methods.

Chemistry.

All solvents, chemicals, and reagents were analytical grade and were used without further purification unless otherwise indicated. The ^{1}H nmr spectra were recorded on a Varian Gemini-200 spectrometer and are reported in ppm downfield from the internal tetramethylsilane (TMS = 0 ppm). The thin-layer chromatographic (tlc) analyses were performed using precoated 250 nm layers of silica gel glass plates (Analtech, Inc.). The R_f values provided relate to specific experiments and the absolute values may vary depending upon the experimental conditions. The elemental analyses were determined by Galbraith Laboratories, Knoxville, TN. Sodium [^{125}I]iodide was purchased from New England Nuclear, Inc., North Billerica, MA.

Biology.

Athymic nude female Balb-C mice, 4-5 weeks old, were obtained from Charles River, Inc. (Wilmington, MA). Mice were kept under sterile conditions in a laminar flow room in cages with filter bonnets and were fed sterilized mouse diet and sterilized tap water. The LS174T tumor cells were suspended in RPMI 1640 medium at a concentration of 25 x 10^6 cells/ml. Cell viability was determined by trypan blue dye exclusion. Cells (5 x 10^6) in sterile medium were injected subcutaneously into the flank of nude mice. When the tumors were approximately 0.6 cm in diameter, about 10 days after tumor cell injection, the animals were injected intraperitoneally with radiolabeled nucleoside analogue and the biodistribution was determined. Leith *et al* [28] reported a hypoxic fraction of 18.5% for LS174T tumor xenografts in athymic mice.

In vivo tissue distribution was evaluated in nude mice bearing LS174T tumors following intraperitoneal administration of 1-5 μCi ^{125}I or ^{18}F labeled compounds **7**, **8** or **12** in 0.2 ml of sterile saline. The animals were allowed food and water *ad libitum* prior to and during the experiment. Groups of 4 to 6 animals were bled, sacrificed and dissected at 1, 4 and 24 hours after intraperitioneal injection. The tumor and other desired organs were excised, rinsed with saline solution, blotted dry, placed in tared vials, and weighed. The samples were counted for radioactive contents in a Packard Minaxi 5000 sodium iodide auto gamma counter. Results are expressed as % injected dose/g of tissue, except the thyroid, for which the results are expressed as % injected dose/tissue.

1-Bromo-2-chloromethoxyethane (**3**).

Anhydrous hydrogen chloride gas was bubbled into a mixture of 2-bromoethanol (5 g, 40 mmoles) and paraformaldehyde (1.5 g) in methylene chloride (10 ml) for 3 hours at $-15°$ to $-10°$. The solvent was removed under reduced pressure at room temperature. The two layers from the crude reaction mixture were separated and the lower layer (4.3 g, 66% yield) was characterized as compound **3**, which was used without further purification; ^{1}H nmr (deuteriochloroform): δ 5.6 (s, 2H, $-H_2CCl$), 4.2-3.9 (t, J = 5.5 Hz, 2H, $-CH_2O$), 3.70-3.40 (t, J = 5.8 Hz, 2H, $BrCH_2-$).

2-Nitro-1-(2-bromoethoxymethyl)imidazole (**6**).

A mixture of 2-nitroimidazole (**1**, 500 mg, 4.4 mmoles), and ammonium sulfate (100 mg) in 1,1,1,3,3,3-hexamethyldisilazane (40 ml) was refluxed under anhydrous condition until a clear solution was obtained. The solvent was removed under reduced pressure and the residue was dissolved in dichloroethane (40 ml) under an argon atmosphere. The solution was stirred and cooled to 0-5° and anhydrous triethylamine (1.5 ml) was added followed by dropwise addition of a solution of compound **3** (1.7 g, 9.8 mmoles)

in dichloroethane (20 ml). The solution was then allowed to warm to room temperature and stirring continued for 16 hours. The solution was filtered and the solvent removed under reduced pressure. The residue was dissolved in chloroform and washed with water (5 x 30 ml). The organic phase was dried (sodium sulfate), filtered, and the solvent removed to yield (1.1 g) of the crude product which was purified on a silica gel column, slurried in petroleum ether. Elution of the column with methylene chloride:petroleum ether (1:1 v/v) yielded compound **6** (800 mg, 73%) as an oil; ^{1}H nmr (deuteriochloroform): δ 7.5 (s, 1H, H-5), 7.3 (s, 1H, H-4), 6.0 (s, 2H, H-1'), 4.2-3.5 (m, 4H, H-4' and H-5'), and analysed as the corresponding iodinated product.

2-Nitro-1-(2-iodoethoxymethyl)imidazole (**7**).

The silylated derivative of 2-nitroimidazole (**1**, 1.0 g, 8.8 mmoles) was dissolved in dichloroethane (60 ml) under argon and anhydrous triethylamine (3.0 ml) was added. A solution of compound **4**, (4.0 g, prepared similarly as **3**) in dichloroethane (15 ml) was added slowly during cooling. The cooling bath was removed and stirring was continued for 16 hours. The solvent was removed *in vacuo* and the residue was dissolved in chloroform and washed with a saturated solution of sodium thiosulphate followed by water. The organic phase was separated and dried (sodium sulfate), filtered and the solvent removed. The residue was then purified on a silica gel column, slurried in hexane. Elution of the column with 40% methylene chloride in hexane afforded compound **7** (1.8 g, 69% yield) as an oil. An analytical sample was purified by preparative tlc (solvent 20% ethyl acetate in chloroform) and the band corresponding to the authentic sample was scraped and eluted with 10% methanol in chloroform. Evaporation of the solvent yielded **7** as an oil; ^{1}H nmr (deuteriochloroform): δ 7.47 (s, 1H, H-5), 7.35 (s, 1H, H-4), 5.95 (s, 2H, H-1'), 4.09-3.8 (t, J = 6.0 Hz, 2H, H-3'), 3.40-3.15 (t, J = 6.0 Hz, 2H, H-4').

Anal. Calcd. for $C_6H_8IN_3O_3 \cdot 0.5CH_3OH$: C, 24.92; H, 3.19; I, 40.57. Found: C, 24.91; H, 2.90; I, 40.29.

2-Nitro-1-(2-Fluoroethoxymethyl)imidazole (**8**).

Compound **6**, (250 mg, 1 mmole) was dissolved in anhydrous toluene (3 ml) and silver fluoride (381 mg, 3.0 mmoles) was added. The mixture was heated at refluxed temperature for 18 hours, filtered and the solvent removed under reduced pressure. The crude product was dissolved in ethyl acetate and washed with water. The organic phase was dried (sodium sulfate), filtered and the solvent was removed *in vacuo*. The crude product was then purified by preparative tlc (10% ethyl acetate in chloroform) and the band (R_f = 0.55) less polar than the starting material (**6**, R_f = 0.58) was scraped and eluted with chloroform. Evaporation of the solvent yielded **8** (35 mg, 46%) as an oil; ^{1}H nmr (deuteriochloroform): δ 7.48 (s, 1H, H-5), 7.3 (s, 1H, H-4), 5.95 (s, 2H, H-1'), 4.7-4.4 (dt, J = 47.6, J = 3.9, 2H, $-CH_2-$), 3.9-3.7 (dt, J = 4.0, J = 27.8, 2H, $-CH_2-$).

Anal. Calcd. for $C_6H_8FN_3O_3$: C, 38.09; H, 4.23; F, 10.05. Found: C, 38.18; H, 4.51; F, 10.05.

2-Nitro-1-(propargyloxymethyl)imidazole (**10**).

The freshly prepared silyl derivative of 2-nitroimidazole (**2**, 2.0 g, 17.7 mmoles) was taken up in dichloroethane (70 ml) under argon and triethylamine (2.67 g, 26.4 mmoles) was added followed by a solution of compound **9** (2.75 g, 26.5 mmoles) in dichloroethane (30 ml). The solution was stirred for 15 hours at room temperature. The suspension was filtered and solvent was removed from the filtrate under reduced pressure. The residue was dissolved in chloroform (60 ml), washed with water (3 x 50 ml), and the organic layer separated, dried (sodium sulfate) and filtered. Removal of the solvent *in vacuo* afforded the crude product which was purified on a silica gel column, slurried in chloroform. Elution of the column with chloroform yielded pure compound **10** (2.2 g, 76%) as an oil; ^{1}H nmr (deuteriochloroform): δ 7.4 (s, 1H, H-5), 7.3 (s, 1H, H-4), 6.0 (s, 2H, H-1'), 4.3 (d, J = 1.5 Hz, 2H, H-3'), 2.5 (t, J = 2.0 Hz, 1H, H-5').

Anal. Calcd. for $C_7H_7N_3O_3$: C, 46.41; H, 3.87; N, 23.2. Found: C, 46.49; H, 4.09; N, 23.45.

2-Nitro-1-(*E*-1-tributyltinpropenyloxymethyl)imidazole (**11**).

Compound **10** (131 mg, 0.72 mmole) was added into a two neck round bottom flask containing a magnetic stirring bar and equipped with a septum and argon gas inlet. Anhydrous toluene (3 ml) and 2,2'-azobisisobutyronitrile (5 mg) were added. The mixture was stirred at room temperature for 5 minutes, and tributyltin hydride (212.4 mg, 0.73 mmole) was added through a syringe. The mixture was heated at 60-65° for 5 hours and the solvent was then removed and the crude product was applied on a preparative tlc plate (solvent chloroform). The spots were located under uv light and the bands were scraped and eluted with chloroform. Evaporation of the solvent yielded compound **12** (48 mg, 24%) as an oil; ^{1}H nmr (deuteriochloroform): δ 7.2 and 7.1 (two s, 2H, H-5 and H-4), 6.95-6.75 (m, 2H, vinyl proton), 5.65 (s, 2H, H-1'), 4.1 (m, 2H, H-3') and analysed as the corresponding iododestannylated product.

2-Nitro-1-(*E*-1-iodopropenyloxymethyl)imidazole (**12**).

Compound **12** (155 mg, 0.33 mmole) was dissolved in anhydrous tetrahydrofuran (2 ml) and a solution of iodine monochloride (81.25 mg, 0.5 mmole) in tetrahydrofuran (2 ml) was added in the dark. The reaction mixture was stirred at room temperature for 2 hours, the solvent was removed *in vacuo* and the crude product taken up in ethyl acetate. A saturated solution of sodium thiosulphate was added with stirring until the purple color disappeared. The mixture was extracted with ethyl acetate and washed with water. The organic phase was dried (sodium sulfate), filtered and the solvent was removed. The crude product was purified on a silica gel column slurried in hexane. Elution of the column with 50% hexane in chloroform gave compound **12** (90 mg, 90% yield). The analytical sample was purified on preparative tlc (solvent chloroform). The major band was located under uv light, scraped and eluted with chloroform. The solvent was removed to yield compound **12** as an oil; ^{1}H nmr (deuteriochloroform): δ 7.30 (s, 1H, H-5), 7.29 (s, 1H, H-4), 7.2 (dd, J = 14.8 and 8.0 Hz, 1H, $-CH=CH-$), 5.9 (s, 2H, $-CH_2-N$), 5.8 (d, J = 16.5 Hz, 1H, $HC=CH$), 4.3 (s, 2H, $-CH_2O$).

Anal. Calcd. for $C_7H_8IN_3O_3$: C, 27.18; H, 2.59; I, 41.10. Found: C, 26.93; H, 2.16; I, 41.13.

Radiolabeling Experiments.

Radiolabeled [^{125}I]-**12**.

A mixture of sodium iodide (1.5 mg, 0.01 mmole) and Na[^{125}I] (2.0 mCi) in anhydrous tetrahydrofuran (0.2 ml) was added to a solution of compound **11** (0.01 mmole) in tetrahydrofuran (0.5 ml). A solution of *N*-chlorosuccinimide (3.0 mg) in tetrahydrofuran (0.2 ml) was added and the reaction mixture was stirred in the dark for 4 hours at room temperature. The solvent was evapo-

rated under the stream of argon to remove tetrahydrofuran and the residue was diluted with water 0.5 ml. A crystal of sodium thiosulphate was added and the mixture was stirred for 5 minutes. The solution was extracted with ethyl acetate (2 x 2.0 ml). The ethyl acetate portion was dried (sodium sulfate), filtered and the volume of the solvent was reduced to (0.1 ml) by evaporation under a stream of argon and the residue was applied onto a 20 x 20 cm (250 μm) tlc plate. The plate was developed in 20% hexane in ether and the band corresponding to unlabeled authentic **12** was then scraped and eluted with chloroform. Evaporation of solvent under argon provided 0.64 mCi (31% radiochemical yield) of radiolabeled **12**. The radiolabeled compound **12** moved identical to the unlabeled standard and was homogenous on tlc when examined under uv light, and by radioactive scanning. The radiolabeled compounds were stored in sealed vials in the freezer for animal studies.

Radiolabeled [^{125}I]-**7**.

A mixture of compound **7** (2.97 mg, 0.01 mmole), sodium iodide (0.5 mg, 3.3 μmoles) and Na[^{125}I] (2.5 mCi) in methyl ethyl ketone (2.0 ml) was refluxed for 16 hours, cooled, and the solvent was removed under a stream of argon. The residue was dissolved in ethyl acetate (2.0 ml) was washed with saturated sodium thiosulphate solution followed by water. The organic phase was separated and dried (sodium sulfate). The crude mixture was purified on a preparative tlc plate. The plate was developed in 20% ethyl acetate in chloroform. The band corresponding to the unlabeled authentic was scraped and eluted with chloroform. The solvent was removed to yield 1.0 mCi (40% radiochemical yield) of radiolabeled [^{125}I]-**7**. The sample was stored in a sealed vial in the freezer for biological studies.

Radiolabeled [^{18}F]-**8**.

Aqueous [^{18}F]fluoride ion (27 mCi; obtained by proton irradiation of a [^{18}O]water target) [29] was placed in a glass tube. After addition of potassium carbonate (2 mg) and kryptofix-222 (10 mg) the mixture was evaporated to dryness. Acetonitrile (100 μl) was added and the solution was evaporated again to dryness. A solution of iodoazomycin acyclonucleoside (**7**, 1 mg) in dimethyl sulfate (150 μl) was then added to the residue and the solution was heated at 100° for 45 minutes. The solution was cooled, diluted with water and extracted with diethyl ether to give 2.53 mCi of crude product. The ether extract was dried (magnesium sulfate) and passed through a 1 cm x 5 cm silica gel column, which yielded 1.25 mCi (6.6% corrected for decay) of purified product free of [^{18}F]fluoride ion. The total synthesis and purification time was 60 minutes. A portion of this product (270 μCi) was evaporated to dryness and the residue was redissolved in isotonic saline for use in animal experiments. The tlc analysis (silica gel, 20% ethyl acetate in chloroform) showed one radioactive product with R_f = 0.20 which was identical to an authentic sample of unlabeled fluoroazomycin acyclonucleoside.

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* Address correspondence to this author at Biology Division, P. O. Box 2009, Building 9220, Oak Ridge National Laboratory, Oak Ridge, TN 37831-8077.

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