Journal of Medicinal Chemistry

Article

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Design, Synthesis, and Preliminary Biological Evaluation of 6-*O*-Glucose−Azomycin Adducts for Diagnosis and Therapy of Hypoxic Tumors

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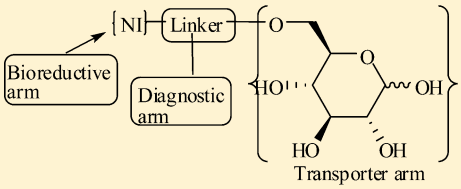
Supporting Information

ABSTRACT: Several 2-nitroimidazole-based molecules (NIs) are used as clinical hypoxic tumor radiodiagnostics, but they are not effective as radiosensitizers/radiochemotherapeutics. These NIs permeate tumor cells nonselectively via diffusion, and in therapy, where high doses are required, their dose limiting toxicities preclude success. The synthesis and preliminary in vitro evaluations of three glucoazomycins, members of a novel class of C6-*O*-glucose-linked-azomycin conjugates that are putative substrates of glucose transport proteins (GLUTs) and possess hypoxia-selective radiosensitization features, are now reported. The hypoxia-dependent upregulation of several GLUTs provides a rational basis to develop these glucoazomycins because more selective uptake in hypoxic cells would decrease systemic toxicities at effective doses. Calculated partition coefficients (ClogP, −1.70 to −2.99) predict rapid in vivo clearance for low systemic toxicity. In vitro experimental data show that glucoazomycins are radiosensitizers and that they competitively inhibit glucose uptake.



Glucoazomycins 8, 11, 13

INTRODUCTION

Hypoxia was identified over 50 years ago as an important factor in biological responsiveness to ionizing radiation[1] and as a complicating phenomenon in the radiotherapy of solid tumors.[2−4] Hypoxia has been recognized as a principle determinant of the microenvironment of a number of pathological conditions including stroke, myocardial infarction, diabetes, arthritis, and transplant rejection; it is the direct consequence of regional ischemia arising from poor vasculature and compression of blood and lymphatic vessels in tumors. Ischemia reduces the delivery of nutrients and oxygen to affected areas.[5] Hypoxia gives rise to an aggressive cancer cell phenotype that promotes resistance to drugs and low linear energy transfer (LET) ionizing radiation, elevates metastatic potential, and upregulates glycolysis, transport mechanisms, and an almost unlimited array of intermediary and effector proteins.[6,7]

Many classes of compounds have been tested as hypoxic cell theranostics, that is, as radiosensitizers (therapeutics) and as radiopharmaceuticals (diagnostics) of hypoxic tumor.[8] The main approach has been to mimic the effects of oxygen, i.e., to trap radiation damage to vital cellular macromolecules, thereby mimicking the oxygen effect. Most hypoxic mammalian cells are 2.5−3 times more radioresistant than normally oxygenated cells (the oxygen enhancement ratio; OER). Adams postulated that the oxygen effect could be mimicked by electron-affinic compounds (oxygen mimetics) in the absence of oxygen,[9] an effect quantified as the sensitizer enhancement ratio (SER). A number of oxygen mimetic sensitizers with acceptable SERs have been synthesized and tested,[10,11] and among these, 2-nitroimidazoles (azomycins) with single electron reduction potentials (E^1_7) of about −390 mV have offered the best balance between cytotoxicity and radiosensitizing efficacy.[12] Bioreductive activation of the nitro group in these sensitizers leads to their covalent binding with the intracellular macromolecules in hypoxic cells, resulting in their selective and enhanced accumulation therein. The lipophilic/hydrophilic balance provided by the azomycin N-1 "tail" plays a pivotal role in trans-membrane diffusion, tissue biodistribution, and clearance from the body, with optimal partition coefficients (log *P*) below 1 to avoid deposition into lipoidal tissue (i.e., brain) and peripheral neurotoxicity.[13,14] Among the many 2-nitroimidazole-based radiosensitizers, misonidazole (Ro-07−0582, 1-(2-nitro-1-imidazolyl-1)-3-methoxypropanol, MISO) appeared to be one of the most efficacious azomycin radiosensitizers in preclinical tests[15] but eventually failed because of dose-limiting toxicities at clinically effective concentrations.[16]

Several radiohalogenated azomycins (Figure 1), including ^{123}IAZA,[17,18] [^{18}F]FMISO,[19] and [^{18}F]FAZA,[20−22] are used clinically for the diagnosis of hypoxic tumors,[23] hypoxia-selective mapping,[24] in staging and restaging of the disease, and

Received: December 22, 2011
Published: June 18, 2012

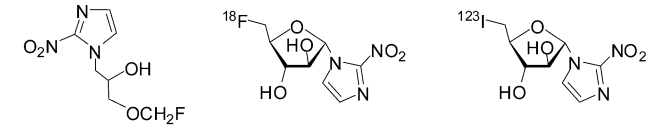


Figure 1. Structure of clinically used azomycin-based hypoxia-selective radiopharmaceuticals and their ClogP values. FMISO and FAZA are used in positron emission tomographic imaging (PET), whereas IAZA is used for single photon emission computed tomography (SPECT).

the therapy planning for patients.[25] The challenge has been, and remains, to design an electron-affinic radiosensitizer that will attain efficacious concentrations in target (i.e., hypoxic tumor), yet not reach toxic levels in other tissues.[26−30] Until recently, no mechanism was known that could be exploited to achieve this balance, as existing radiosensitizers nonselectively permeate cells via diffusion. The discovery that a number of transport elements, particularly the facilitative glucose transport proteins (GLUTs), are upregulated by hypoxia[31] has created an opportunity to conceptualize and design new azomycin adducts that may exploit this hypoxia-upregulated transport while retaining optimal E^1_7 and hypoxia-dependent adduct forming properties of the azomycins. Glucose-radiosensitizer adducts, specifically glucose−azomycin (glucoazomycin) conjugates, are logical choices for this design, but limitations to the nature and site of substitution[32] on glucose invoke substantial constraints and challenges to harnessing such molecules effectively. The synthesis and preliminary in vitro radiosensitization studies of novel 2-nitroimidazolyl−glucose conjugates coupled at C6-*O*- via a 3-carbon propyl linker (glucoazomycins, Figure 2) that are putative substrates of GLUTs are now reported. Preliminary biological data and design considerations for this new class of molecules are discussed.

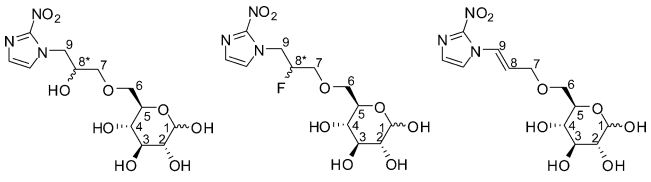


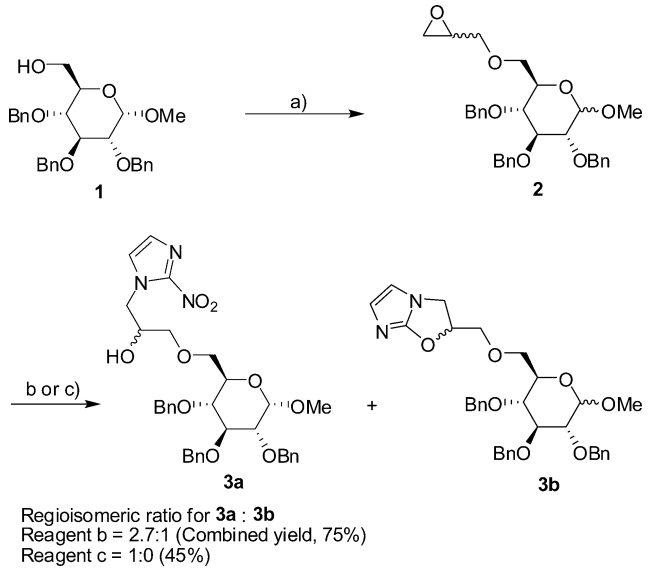
Figure 2. Structures and the ClogP values of **8**, **11**, and **13**.

RESULTS AND DISCUSSION

The principle objectives of this work were to develop a new class of bioreductively activated, hypoxia-selective molecules that are substrates for transport by hypoxia-upregulated GLUTs in solid tumors. GLUTs-facilitated permeation would offer increased concentrations of these molecules in the target tissues, leading to enhanced hypoxia-selective binding by the tumor cell and resulting in improved hypoxia-specific theranostic properties. The chemistry and preliminary biological evaluations of 1-α/β-D-(6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8-hydroxypropyl)glucopyranose (glucoazomycin, GAZ, **8**), 1-α/β-D-(6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8-fluoropropyl)-glucopyranose (fluoroglucoazomycin, F-GAZ, **11**), and 1-α/β-D-(6-(((8,9*E*)-9-(2-nitro-1*H*-imidazol-1-yl)allyloxy)methyl)-glucopyranose (allylglucoazomycin, A-GAZ, **13**) are now reported.

Chemistry. C6-*O*-linked glucose−azomycin adducts were designed so that the C1 −OH group remains unsubstituted and the GLUT interaction is not compromised, since it is reported that C1 glycosides are not substrates for GLUTs.[32] 1-α-D-*O*-Methyl-2,3,4-tri-*O*-benzyl-6-*O*-((oxiran-2-yl)methyl)-glucopyranose **2** was obtained in almost quantitative yield by base-catalyzed reaction of 1-α-D-*O*-methyl-2,3,4-tri-*O*-benzyl-glucopyranose **1** with epibromohydrin in DMF. The use of racemic epibromohydrin led to the formation of a diastereomeric mixture of **2**. Proton spectroscopy of **2** demonstrated two sets of magnetic resonances, confirming the formation of the *H8R* and *H8S* diastereomers. No attempt was made to separate the diastereomers of the intermediate products. Coupling of **2** with 2-nitroimidazole in the presence of Na_2CO_3 in refluxing ethanol yielded methyl-1-α-D-(2,3,4-tri-*O*-benzyl)-6-*O*-(3-[2-nitro-1*H*-imidazolyl]-2-hydroxypropyl)glucopyranose **3a** along with the bicyclic derivative **3b** (Scheme 1) that formed as a

Scheme 1. Preparation of Glucose-6-*O*-Linked Azomycin Conjugates[a]



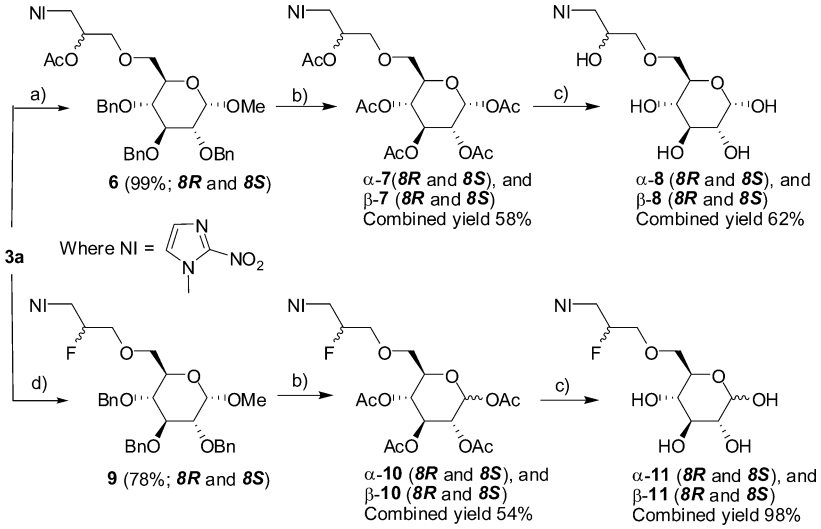
[a]Reagents and conditions: (a) NaH/DMF, 0−22 °C, 1 h, epibromohydrin addition, 5 → 22 °C, 20 h; (b) Na_2CO_3, EtOH, 2-nitroimidazole, reflux, 15 h; (c) 2-nitroimidazole, Cs_2CO_3, EtOH, reflux, 27 h.

result of the intramolecular substitution of the nitro group in **3a** (combined yield 78%, ratio **3a**:**3b** is 2.7:1). Compounds **3a** and **3b** were separated by column chromatography and both were identified as the diastereomeric mixtures, with each product displaying the signals for two diastereoisomers in its ^{1}H NMR spectrum. HPLC analysis confirmed 98% purity for the diastereomers of **3a**, the desired product that separated as yellow solid.

It was also observed that when 2-nitroimidazole was coupled with **2** in the presence of cesium carbonate, a milder base, the reaction yielded **3a** exclusively (45% yield) and no traces of **3b** were found. The ^{1}H NMR spectrum of **3a** verified the presence of two diastereomers (8*R* and 8*S* generated from the diastereomeric mixture of **2**) because it displayed dual resonances for several protons. The ^{13}C NMR spectrum also demonstrated dual resonances for several carbons, specifically for C9 (δ 52.16 and 52.24), C8 (δ 69.24 and 69.34), C5 (δ 70.00 and 70.04), C4 (δ 77.19 and 77.27), and for C1 (δ 98.05

dx.doi.org/10.1021/jm2017336 | J. Med. Chem. 2012, 55, 6033−6046

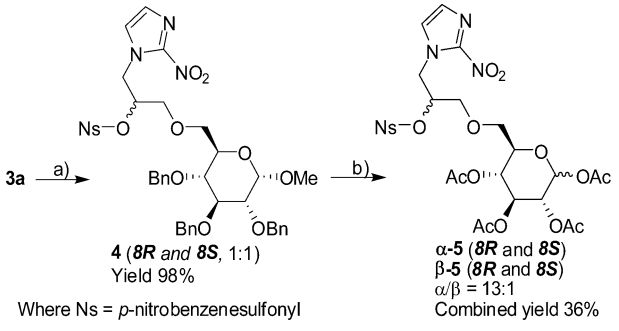
Scheme 2. Preparation of Compounds 8 and 11[a]



[a]Reagents and conditions: (a) Ac_2O, pyridine, 22 °C, 20 h; (b) trimethylsilyl triflate, Ac_2O, −40 °C → 22 °C, 18 h; (c) 0.1N NaOH, MeOH, 22 °C, 20 min; (d) DAST, THF, −20 °C → 22 °C, 20 h.

and 98.11 ppm). The mass spectrum (MS) showed the molecular ion for **3a**, providing additional evidence for its formation. This compound was the key intermediate to synthesize the target molecules **8**, **11**, and **13**; the removal of protecting groups in **3a** led to the formation of **8**, while its C8 fluorination transformed **3a** into the protected fluoroglucoazomycin, **9**, that was used for the synthesis of **11** (Scheme 2). Moreover, the introduction of a nosyl group at C8 −OH in **3a** gave methyl-1-*α*-D-(2,3,4-tri-*O*-benzyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8-(4-nitrobenzenesulfonyloxy)propyl)glucopyranose **4** (Scheme 3), a precursor of **13**. Acetylation of **4** to the corresponding pentaacetate 1-*α*/*β*-D-(1,2,3,4-tetra-*O*-acetyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8-*O*-(4-nitrobenzenesulfonyloxy)propyl)glucopyranose **5** provided the optimal precursor for nucleophilic radiofluorination to prepare the positron-emitting radiofluorinated ^{18}**F-11**.

Scheme 3. Preparation of Compound 5, a Useful Intermediate for Synthesizing F-18 Labeled 11[a]



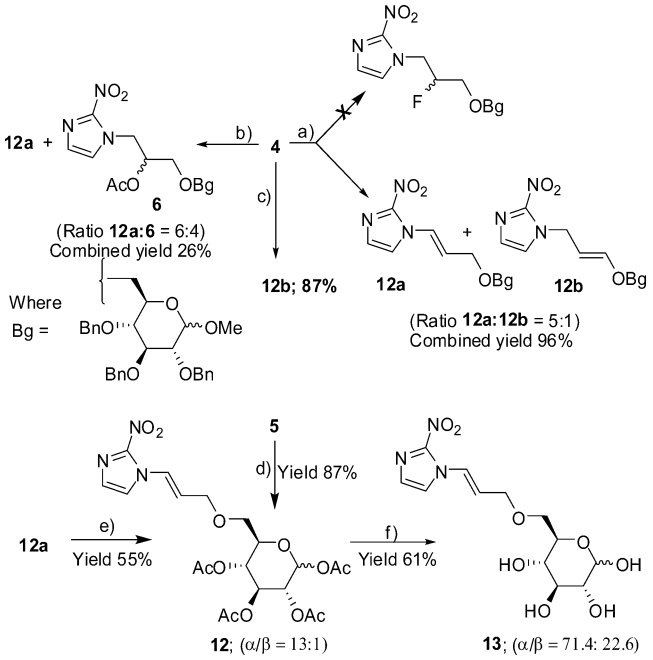
[a]Reagents and conditions: (a) nosyl chloride, Et_3N, DMAP, CH_2Cl_2, −10 °C, 14 h; (b) trimethylsilyl triflate, Ac_2O, −40 °C → 14 °C, 4 h.

The synthesis of **8** was initially attempted by boron trichloride-assisted debenzylation of **3a**, followed by the removal of the 1-*O*-methyl group using 1 N HCl at 80 °C, but this led to a very complicated mixture of the products. The MS analysis of this mixture, however, indicated the formation of the desired product, albeit in low yields. An alternate approach to synthesize **8** therefore involved the replacement of protective ether (benzyl) groups in the sugar moiety of **3a** by ester (acetyl) groups so that mild alkaline conditions could be applied to hydrolyze the latter. This route involved the acetylation of **3a** using acetic anhydride/pyridine that converted **3a** to the corresponding C8-*O*-acetate derivative, the 8*R* and 8*S* isomers of methyl-1-*α*-D-(2,3,4-tri-*O*-benzyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8-*O*-(acetyl)propyl)glucopyranose **6** in 99% yield. Appearance of two distinct s at *δ* 1.91 and 1.96 in the ^{1}H NMR spectrum of **6** indicated the incorporation of acetyl function at C8−OH in **3a**. Alternate procedures to synthesize **6** involved the reaction of **4** with tetrabutylammonium acetate or cesium acetate in anhydrous toluene, but these reactions, in addition to **6**, always led to a secondary olefinic product methyl-1-*α*-D-(2,3,4-tri-*O*-benzyl)-6-(((8,9*E*)-9-(2-nitro-1*H*-imidazol-1-yl)allyloxy)methyl)glucopyranose **12a** (Scheme 4).

When the diastereomeric mixture of **6** was treated with excess acetic anhydride and trimethylsilyl triflate at 0 °C, all benzyl groups in the glucose moiety, as well as the methyl group at the anomeric C1 position, were replaced with acetate groups. Acetylation at C1 was not stereoselective and therefore led to the formation of C1*α*- and C1*β*-acetates of **7**, each with 8*R* and 8*S* diastereomers (**6** being a mixture of 8*R* and 8*S* stereomers). The total isolated yield (both *α*- and *β*- anomers) of 1-D-(1,2,3,4-tetra-*O*-acetyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8-*O*-(acetyl)propyl)glucopyranose **7** was 58%. The ^{1}H NMR spectrum of **7** revealed the *α*/*β* diastereomeric ratio to be 84:12.5. The appearance of a d ($J_{2,1}$ = 3.6 Hz) at *δ* 6.32 and a dd at *δ* 5.09 ($J_{1,2}$ = 3.6 Hz, $J_{3,2}$ = 10.0 Hz) in the ^{1}H NMR spectrum reflected the formation of the ***α***-**7** isomer (*R* and *S* mixture), while these protons for the corresponding ***β***-**7** isomer appeared at *δ* 5.68 ($J_{2,1}$ = 8.0 Hz) and 5.14 ppm, respectively. The presence of dual resonances for C2 at *δ* 71.13 and 71.16 for the ***α***-**7** isomer, and at *δ* 74.09 and 74.16 for ***β***-**7**, respectively, in the C-13 NMR spectrum of **7** further confirmed the presence of ***α***-**7**[8*S*], ***α***-**7**[8*R*], ***β***-**7**[8*S*], and ***β***-**7**[8*R*] diastereomers as the products. A few other protons and carbons also displayed dual resonances, of which the signals for acetyl and imidazole protons and carbons were clearly distinct.

dx.doi.org/10.1021/jm2017336 | J. Med. Chem. 2012, 55, 6033−6046

Scheme 4. Preparation of Compound 13[a]



[a]Reagents and conditions: (a) TBAF (1 M in THF), 22 °C, 17 h; (b) DBU·AcOH, DMF, 22 °C, 24 h; (c) CsF, DMF, 120 °C, 17.5 h; (d) DBU, MeCN, 22 °C, 14 h; (e) trimethylsilyl triflate, Ac_2O, −40 to 22 °C, 14 h; (f) 0.1 M NaOH, MeOH, 22 °C, 20 min.

The two diastereomers of ***α*-7** and ***β*-7** were separated by HPLC, where the 8*R* and 8*S* stereoisomers for ***α*-7** (isomeric composition 83.9%) appeared at retention time of 11.02 min and for ***β*-7** (isomeric composition 14%) at retention time of 12.44 min, respectively. Methanolic NaOH-based (0.1 N) hydrolysis easily removed the protective acetyl groups from **7** (four diastereomers) and afforded a mixture of ***α*-8** and ***β*-8** anomers in 62% yield, again each containing a mixture of 8*R* and 8*S* stereoisomers. Proton and carbon resonances for ***α*-8** displayed a d ($J_{2,1}$ = 3.6 Hz) at δ 5.10 for H1 proton and a signal at δ 92.87 for C1, respectively, while ***β*-7** demonstrated the resonances for H1 proton at δ 4.48 (dd, $J_{2,1}$ = 8.0 Hz, $J_{3,2}$ = 9.2 Hz) and for C1 carbon at δ 97.09 ppm, respectively. HPLC-based retention times were 7.03 and 6.97 min for ***α*-8** and ***β*-8**, respectively (Figure 3).

3a was also the key intermediate for synthesizing **5**, the nosylate precursor of ^{18}F-**11**. Thus, nosylation of **3a** in the presence of dimethylaminopyridine (DMAP) and triethylamine (Et_3N) afforded an 8*R* and 8*S* diastereomeric mixture of **4** (because **3a** was mixture of diastereomers) in 98% yield (Scheme 3). The ^{1}H NMR spectrum confirmed the formation of this product as H8 displayed a multiplet (m) at δ 5.02 that was shifted downfield by 0.94 ppm in comparison to the unsubstituted **3a** (Table 1). The dual resonances for several protons and carbons due to the presence of 8*R* and 8*S* stereomers of **3a** were observed in both ^{1}H and ^{13}C NMR spectra. Because time is of the essence in developing short-lived positron-emitting radiopharmaceuticals (PERs), and because acetylated precursors can be rapidly deacetylated under mild alkaline conditions, **4** was converted to the corresponding tetraacetyl derivative **5** using trimethylsilyl triflate (9 mol equiv) and an excess of acetic anhydride at −40 °C (Scheme 3). The disappearance of benzylic methylene and aromatic protons and the appearance of proton signals for acetyl groups in the ^{1}H NMR spectrum of **5** confirmed the replacement of benzyl protective groups with acetyl functions at the glucose −OHs. In addition, the 1-*O*-methyl group in **4** was also replaced by acetyl, although not stereoselectively. As a result, the formation of ***α*-5** and ***β*-5** anomers (anomeric ratio, 13:1 by ^{1}H NMR) was seen during this debenzylative-acetylation process, with each anomer containing 8*R* and 8*S* diastereomers (94% combined yield, purity >99% as determined by HPLC).

Attempts to synthesize **11** from nosylate **4** using *n*-Bu_4NF as the fluorinating agent were unsuccessful. The presence of neighboring protons at C7 and C9 led to intramolecular elimination under basic conditions, resulting in the formation of a mixture of olefins **12a** and methyl-1-*α*-D-(2,3,4-tri-*O*-benzyl)-6-(((7,8*E*)-9-(2-nitro-1*H*-imidazol-1-yl)allyloxy)methyl)-glucopyranose **12b** (5:1 ratio by NMR). Such competitive elimination is known to occur in the presence of the strongly basic fluoride ion if the rate of fluorination is slow.[33,34] Because these efforts to synthesize **11** from the nosylates **4** and **5** were not successful, the DAST-assisted fluorination of **3a** was pursued (Scheme 2). This afforded the desired fluorinated product, methyl-1-*α*-D-(2,3,4-tri-*O*-benzyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8-fluoropropyl)glucopyranose, **9**, as a mixture of 8*R* and 8*S* fluorinated diastereomers in 78% yield with ∼98% combined chemical purity. The incorporation of fluorine creates strong spin−spin interactions with the protons and the carbons at the site of attachment as well as with neighboring nuclei.[35] The H8 resonance for the compound **9** appeared at δ 4.81 ppm, shifted downfield by 0.73 ppm in comparison to the corresponding nonfluorinated precursor **3a**. It displayed a complex splitting pattern due to its coupling with the neighboring protons at C7 and C9 and also with fluorine that was introduced at C8 ($J_{F,H9}$ = 47 Hz). The F−H coupling constants with H7 ($J_{F,H7}$ = 31 Hz) and H9 (J_{F-H9} = 20 Hz) are in accordance with the coupling constants reported for similar environments.[36] The ^{19}F NMR spectrum of **9** further confirmed the incorporation of fluorine at C8, and the fluorine signal (δ −191.88 ppm) demonstrated the spin−spin couplings with the protons substituted at C7, C8, and C9 as one would expect based on the ^{1}H NMR spectrum.

Compound **9**, on treatment with the Lewis acid TMS-triflate as described earlier, afforded 1-*α*/*β*-D-(1,2,3,4-tetra-*O*-acetyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8-fluoropropyl)glucopyranose **10** in satisfactory (54%) yield. The ^{1}H NMR spectrum of **10** revealed the presence of both ***α*-10** and ***β*-10** anomers (isomeric ratio; 91:9), and each anomer displayed resonances for the 8*R* and 8*S* stereoisomers. HPLC analysis of this mixture resolved ***α*-10** (retention time 11.08 min, 74.5% and 12.57 min, 12%) and ***β*-10** diastereomers (retention time 9.19 min, 2% and 9.34 min, 9.5%), respectively. ^{1}H, ^{13}C, and ^{19}F NMR spectra, ^{1}H spin−spin decoupling, NOE experiments, and the chemical shift values for each nucleus all provided structural verification for **10** and confirmed the presence of four diastereomers. The strongest proof for the formation of ***α***-[8*R*]-**10**, ***α***-[8*S*]-**10**, ***β***-[8*R*]-**10**, and ***β***-[8*S*]-**10** diastereomers came from dual resonances for several protons and carbons in the ^{1}H and ^{13}C NMR spectra, respectively. In addition, the ratios for the α/β anomers determined from the ^{1}H NMR spectra (91:9) corresponded closely with the composition determined by HPLC (87:11.5). Fluorine at C8 was not affected during the transformation of **9** to **10**, and the corresponding F−H spin−spin split patterns were visible in H8, H7, H7′, H9, and H9′ protons. The ^{13}C NMR spectrum of **10** also displayed two d at δ 90.31 (J_{F-C} = 179.2 Hz) and δ 90.60 (J_{F-C} = 177.3 Hz) for the

dx.doi.org/10.1021/jm2017336 | J. Med. Chem. 2012, 55, 6033−6046

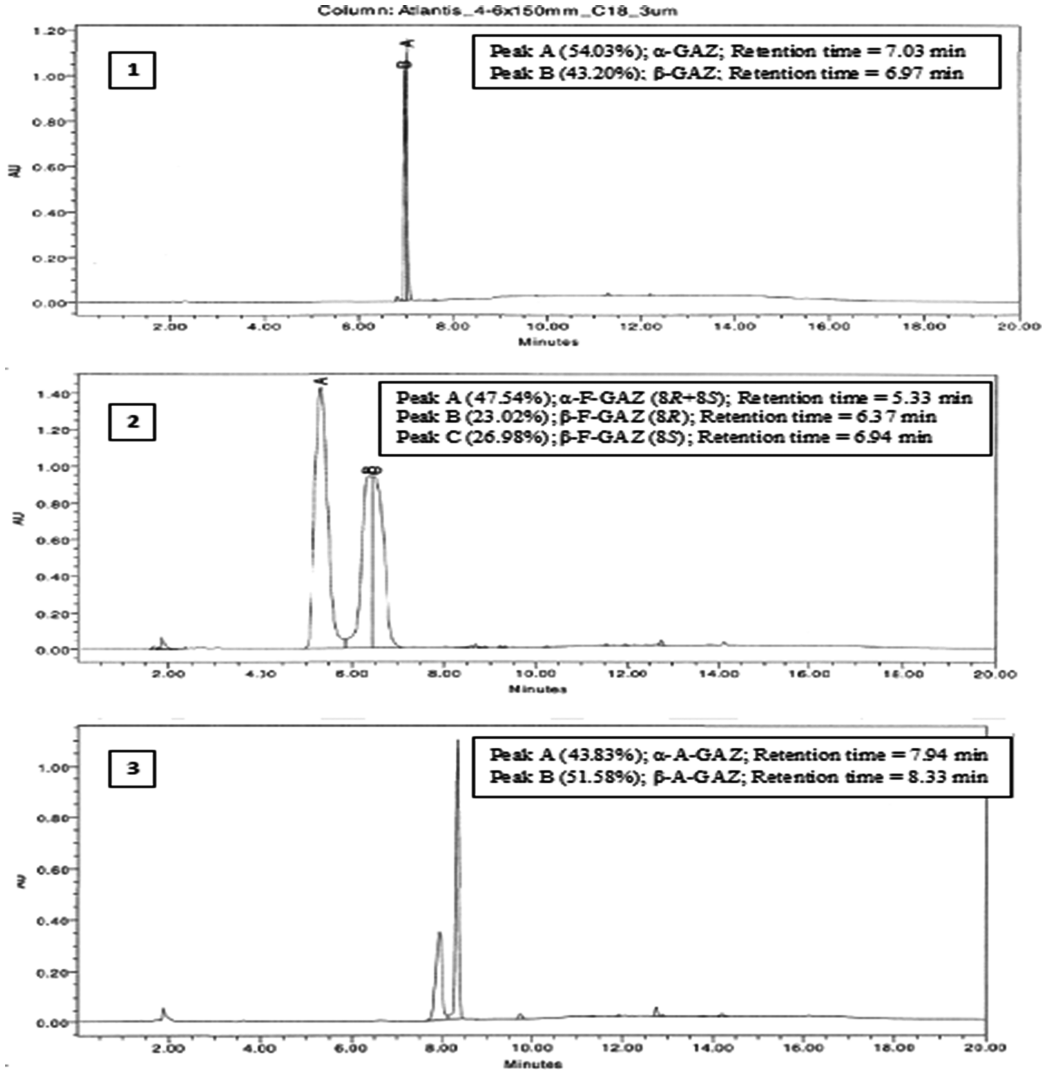


Figure 3. HPLC chromatograms of glucoazomycins **8**, **11**, and **13** indicating their purity and the retention times (Ace C18, 5 μm, 150 mm × 4.6 mm column [Agilent]; mobile phase, gradients of aqueous 0.1% H_3PO_4 solution and acetonitrile, flow rate 1.0 mL/min).

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts and Respective Coupling Constants for α- and β-Isomers of 8, 11, and 13

	chemical shifts in δ ppm and related coupling constants J (Hz)								
	proton			carbon					
compd	H-7, H-7′	H-8	H-9, H-9′	C7	C8	C9	C2-Im	C4-Im	C5-Im
***α*-8** (*R* and *S*)	3.44, 3.54 (two dd)	4.07 (m)	4.48, 4.74 (two ddd)	52.14, 52.19	68.83	68.78	145.33	126.90	128.09
***β*-8** (*R* and *S*)	3.54, 3.71 (two dd)	3.86 (m)	4.44, 4.70 (two ddd)	52.24, 52.26	68.86	68.78	145.33	126.90	128.20
***α*-11** (*R* and *S*)	3.78, 3.81 (two ddd, $J_{F,7} = J_{F,7'} =$ 29.7)	5.05 (dddd, $J_{F,8} =$ 48.0)	4.74 (ddd, $J_{F,9} =$ 16.0) 4.86 (ddd, $J_{F,9} =$ 16.0)	54.09 (d, $J_{F,C} =$ 23.5)	94.61 (d, $J_{F,C} =$ 176.1)	74.15 (d, $J_{F,C} =$ 21.4)	149.06	131.25	131.93
***β*-11** (*R* and *S*)	3.78, 3.87 (two ddd, $J_{F,7} =$ 16.0, $J_{F,7'} =$ 27.5)	4.90 (dddd, $J_{F,8} =$ 48.0)	4.75 (ddd, $J_{F,9} =$ 22.8) 4.83 (ddd, $J_{F,9'} =$ 16.0)	54.18 (d, $J_{F,C} =$ 22.7)	94.57 (d, $J_{F,C} =$ 176.9)	74.29 (d, $J_{F,C} =$ 23.5)	149.06	131.25	132.04
***α*-13**	4.26 (m)	6.28 (dd)	7.62 (d, $J_{8,9} =$ 14)	69.96	123.69	124.06	143.97	125.68	127.98
***β*-13**	4.26 (m, *H*7 and *H*7′)	6.24 (dd, $J_{7,8} =$ 5.6, $J_{9,8} =$ 14.0)	7.58 (d, $J_{8,9} =$ 14)	69.96	123.78	124.13	buried	125.70	buried

8*R* and 8*S* diastereomers of the α-anomer; the signals for the corresponding ***β*-10** carbons were of very low intensity, and thus not marked. The ^{19}F NMR spectrum confirmed these interpretations with two m signals at δ −192.33 and −193.91 ppm, which showed the presence of four diastereomers (8*R* and 8*S*, for each α- and β-isomer). In ^{1}H-NOE equilibrium experiments on **10**, irradiation of H1α enhanced H2α resonance by 12%, H8α irradiation enhanced the signals for H9, H9′, H7, and H7′ (7.5% combined), while the irradiation of H9 proton affected H9′ (27%), H8, and surprisingly also imidazolyl H5 (4% and 2.9%, two stereoisomeric signals) indicating that H9′ and H5 imidazole protons are spatially

dx.doi.org/10.1021/jm2017336 | *J. Med. Chem.* 2012, 55, 6033−6046

oriented close enough to interact with each other. This interaction, which could influence the electronegativity (reducibility) of the imidazole-NO_2 function, was not seen in the corresponding deacetylated compound **11**.

Compound **10** was deacetylated using 0.1 N NaOH to afford four diastereomers (α-8*R*, α-8*S*, β-8*R*, and β-8*S*) of **11** in >98% yield (combined for all diastereomers). An HPLC chromatogram of this mixture demonstrated the presence of four diastereomers for **11**, which were not completely separated (Figure 3). The MS of this mixture displayed the molecular ion ($M + 1^+$ 100%) peak, validating the formation of **11**. The ^1H NMR spectrum also revealed dual resonances (chiral C8) for most of the protons for both α- and β-**11**. Distinct F−H and F−C coupling patterns were also visible in both ^1H and ^{13}C NMR spectra, indicating that the fluorine was not lost during the alkali-based deacetylation. Proton−proton decoupling spectra and NOE experiments confirmed the assignment of the protons in **11**.

Separation of the diastereomeric mixture of **10** and **11** was attempted by normal silica gel chromatography as well as by HPLC chromatography using C-18 reverse phase and columns with chiral stationary phase, varying gradients of the eluents (hexanes−isoPrOH), and changes in the flow rate (0.4−1.0 mL/min), but no separation of the diastereomers could be achieved. The independent preparation of pure diastereomers of **8** and **11**, or determination of their absolute configuration at C8 via stereoselective synthesis was considered, but given that the compounds **8** and **11** demonstrated only moderate radiosensitization in vitro, it was not deemed practical. No further isolation of the diastereomers was pursued.

For the synthesis of **13**, treatment of benzylated compound **4** with TBAF or CsF led to the formation of two olefinic regioisomers in varied reagent-dependent ratios. Thus, the reaction of **4** with TBAF led to the formation of both **12a** and **12b** (isomeric ratio 5:1) while the use of CsF afforded the isomer **12b** exclusively. The treatment of **4** with DBU−acetic acid led to the regioselective formation of **12a**, a C8−C9 olefin product, along with **6** (ratio of **12a**:**6** = 6:4; Scheme 4). Because the use of DBU afforded the C8−C9 olefin product **12a** selectively in mild conditions, this method was used for converting **5** into the corresponding olefinic compound **12**, which was obtained as a mixture of ***α*-12** and ***β*-12** anomers (α/β ratio, 13:1) in 28% yield (Scheme 4). The ^1H NMR spectrum of the isomeric mixture of **12** revealed that the olefin bond was generated exclusively between C8 and C9, and the coupling constants for H8 and H9 ($J_{8,9}$ = 14 Hz) confirmed that they were in the trans orientation. H8 appeared as a doublet of triplet (dt) due to its coupling with H7 and H7′ protons ($J_{8,7}$ = $J_{8,7'}$ = 5.6 Hz). Deacetylation of **12** using 0.1 M aqueous NaOH followed by its treatment with Dowex 50W × 4−200 (H+) resin gave a pure mixture of ***α*-13** and ***β*-13** (isomeric ratio, 71.4: 22.6 by HPLC). Besides shielding H1−H4 protons in **13**, H8 and H9 protons resonances moved downfield in comparison to the acetylated product **13** and appeared at δ 6.28 (dd, $J_{8,9}$ = 14 Hz) and at δ 7.62 (d, $J_{8,9}$ = 14 Hz), respectively. Proton−proton decoupling and NOE experiments were performed to confirm the assignment of the ^1H NMR signals. NOE irradiation of H8 affected the signals for H7 and H7′ (3.6%) and H9 protons (1.3%) and reaffirmed these assignments. The C-13 NMR spectrum, elemental composition, and mass spectral (ES^+) analyses provided additional structural confirmation for this product. A comparison of α/β ratio for the final products to their acetylated derivatives indicated that a restrictive stereochemistry due to an olefinic bond in **13** has a "retaining" impact on the α/β ratio during the deacetylation step because this ratio changed much less (71.4% α-isomers in **13** and 91% α- in **12**) in comparison to the compounds **8** (50.4% α-isomers in comparison to 84% in **7**) and **11** (53.3% α-isomers in comparison to 74.5% in **10**).

Biological Testing In Vitro. Bioreductive activation is the critical step leading to adduct formation,[37] which in turn is the basis for the diagnosis (imaging) and radiosensitization (as adjunct therapy to XRT) of hypoxic tumor.[38] Compounds **8** and **11** demonstrated moderate hypoxia-selective biological potential, which suggested that the stereochemistry at C8 was expected to have a minimal influence on cytotoxicity, radiosensitization, and GLUT-1-facilitated transmembrane transport. Consequently, only the stereomeric mixtures (α/β/8*R*/8*S*) of each compound were tested.

Cytotoxicity (IC_{50}). This study included evaluation of **8** and **11**, against EMT-6, HeLa, MOO6X, KBALB, and KBALB-STK cells, and of **13** against EMT-6 cells in cell culture using an MTT assay, which was compared to IAZA, a known nitroimidazole-based radiosensitizer. With the exception of **8** against MOO6X cells, in which it was about 10 times more toxic than IAZA, all compounds had cytotoxic effects in the 0.5−1 mM range, similar to IAZA (Table 2).

Table 2. Estimated Cytotoxicities (IC50, mM) of Compounds 8, 11, 13, and IAZA in Selected Murine and Human Cell Lines

	cell line IC_{50}[a] (mM)				
compd	MOO6X	HeLa	KBALB	KBALB-STK	EMT-6
IAZA (std)	>1	>1	0.5	0.5	>1
8	0.05	0.5	0.5	0.5	0.6
11	>1	>1	>1	>1	>1
13	ND[b]	ND	ND	ND	>1

[a]IC_{50}s were estimated from survival plots, the maximum concentration tested for each compound was 1 mM. [b]ND = not done.

Radiosensitization. HeLa, EMT-6, and MOO6X cancer cell lines were irradiated using radiation doses up to 18 Gy with compounds **8**, **11**, and **13**, at concentrations ranging from 0.5 to 1 mM, under air (normoxic) and nitrogen (hypoxic) conditions. SERs (surviving fraction under nitrogen/surviving fraction under nitrogen plus sensitizer) were calculated for a radiation dose (18 Gy) which produced OERs (surviving fraction under nitrogen/surviving fraction under air) ranging from >2.0 to 4.3. Under these conditions, **13** was consistently the most effective (SER 1.3−1.7) and **8** was the least effective (SER 1−1.2) in the three cell lines used (Table 3). SERs for well-characterized radiosensitizers such as IAZA and MISO have been reported to be around 1.5, depending on cell line and radiosensitizer concentrations used.[39]

Transport Studies. In vitro ^{14}C-glucose influx experiments showed moderate inhibition of glucose uptake by *Xenopus* oocytes when challenged by compounds **8**, **11**, or **13**. It appears that glucoazomycins most likely bind strongly to the transporter and therefore are poorly transported just like 6-NBDG that has high-affinity for GLUT-1, but it is not readily translocated via GLUT-1.[40] Of the three glucoazomycins, **13** was the most potent inhibitor of ^{14}C-glucose accumulation by *Xenopus* oocytes (Figure 4). Further analysis of data for **13**

dx.doi.org/10.1021/jm2017336 | J. Med. Chem. 2012, 55, 6033−6046

Table 3. In Vitro Cell Survival Following a Single Radiation Dose of 18 Gy (Maximum Dose) Using 0.5 mM Concentrations of Compounds 8, 11, and 13

	in vitro radiosensitization								
	MOO6X			EMT-6			HeLa		
compd (0.5 mM)	surviving fraction (N_2)[a]	OER	SER	surviving fraction (N_2)[a]	OER	SER	surviving fraction (N_2)[a]	OER	SER
8	0.15	2.8	1	0.05	3.8	1	0.06	3.6	1.2
11	0.15	3.3	1.3	0.2	3.8	1.2	0.05	3.0	1.3
13	0.08	2.4	1.30	0.2	4.3	1.4	0.05	2.8	1.7

[a]OER and SER values were estimated from plots of surviving fraction as a function of radiation dose. OER and SER values quoted are based on the survival fractions observed following a single 18 Gy X-ray dose.

(Figure 5) indicated an effective inhibitor dose (ED_{50}) of approximately 0.5 mM.

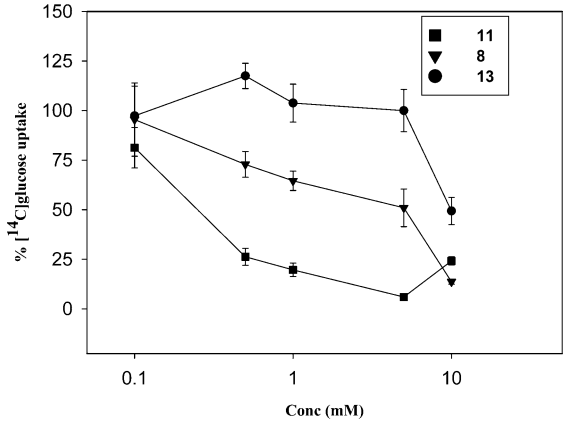


Figure 4. Competitive inhibition of [^{14}C]glucose uptake by oocytes expressing GLUT-1 in the presence of **8**, **11**, and **13**.

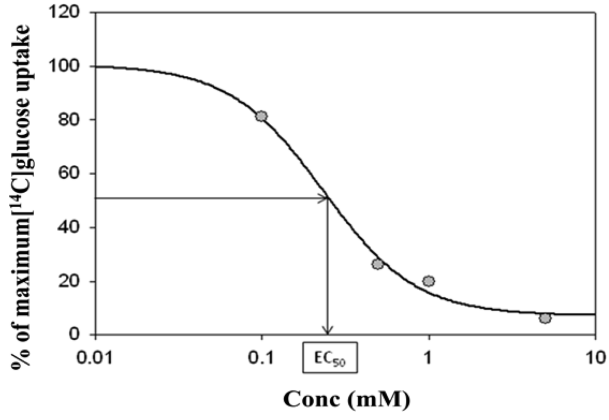


Figure 5. Estimation of the median effective concentration (EC_{50}) for the inhibition of [^{14}C]glucose uptake by uptake by oocytes expressing GLUT-2 in the presence of **13**.

CONCLUSIONS

Three representatives of the novel 6-*O*-glucoazomycin class of bioreductive agents, **8**, **11**, and **13**, were synthesized and chemically characterized. The synthesis of **8** and **11** led to the formation of four diastereomers due to α- and β-anomers at C1 and the chiralitiy of the C8 carbon. Consequently, α- and β-anomers of 8*R* and 8*S* stereomers were obtained but not isolated. Moderate radiosensitization and glucose inhibition properties for 8*R*/*S* pairs in **8** and **11** (chiral compounds) indicated that stereochemistry at C8 would exert little or no impact on either nitro reduction or on GLUT-1-facilitated transmembrane transport. Consequently, only limited efforts to separate these diastereomers were considered appropriate, which did not lead to pure diastereomers.

ClogPs of these glucoazomycins (**11** = −1.70, **13** = −2.20, **8** = −2.99) indicated that they are more hydrophilic than current clinically used azomycin radiopharmaceuticals (ClogP, FAZA = −1.23, IAZA = −0.36) (Figures 1 and 2). Preliminary in vitro evaluations indicated that **13** demonstrates moderate hypoxia-selective radiosensitization of MOO6X, EMT-6, and HeLa cancer cells (SER 1.3−1.7, Table 3), while **8** and **11** were weaker radiosensitizers than literature compounds like IAZA. These differences in radiosensitization potency (SERs) were small and most apparent at high μM to low mM concentrations as is typical for other 2-nitroimidazoles. However, the determination of the effects of the C8 substituent itself (i.e., I vs F vs OH vs others) on bioreduction requires additional study, including the determination of enzyme-catalyzed reduction kinetics and/or determination of their E^1_7 and correlation of such data with in vitro adduct formation experiments. The cytotoxicities of these glucoazomycins toward MOO6X, HeLa, EMT-6, K-Balb, and K-Balb-STK cancer cell lines were similar to IAZA, a known radiosensitizer and clinically used hypoxia-selective radiopharmaceutical for diagnosing and staging of a variety of solid tumors.

Glucose uptake inhibition studies were indicative of interaction with GLUT-1 (Figure 4), but the only dose−response relationship data that could be modeled was for the inhibition of glucose uptake by **13** in the GLUT-2 expressing oocyte model (Figure 5). Importantly, GLUTs affinity and transport are not synonymous as reported for 6-NBDG. 6-NBDG was initially thought to be transported by GLUT-1 but has more recently been found to be a very strong binder that impedes glucose uptake by blocking the transporter rather than by competing for transport. Thus, 6-NBDG has high-affinity for GLUT-1, but it is not readily transported via GLUT-1 because of the large substituent at glucose C6.[40] The glucose challenge data for **8**, **11**, and **13** demonstrate rather weak inhibition of glucose uptake in the oocytes model and low in vitro cytotoxicity. In summary, the introduction of the glucose substituent at *N*1 of azomycin appeared to have only small impact on electron densities around the nitro substituent (the bioreductive arm). Current GLUT-1 data indicate that the glucoazomycins may bind to the transporter as is seen with 6-NBDG, but its transport via GLUT-1 has not been ascertained. It is possible that without facilitated transport, diffusion of these very hydrophilic glucoazomycins across the phospholipid cell membrane may be very slow, thereby precluding localized intracellular concentrations required for effective oxygen-mimetic radiosensitization.

dx.doi.org/10.1021/jm2017336 | J. Med. Chem. 2012, 55, 6033−6046

EXPERIMENTAL SECTION

Chemistry: General. All reagents were purchased from commercial suppliers (Sigma-Aldrich, USA; Fluka, USA) and were of reagent grade. Anhydrous solvents were used as obtained from the suppliers. Sodium hydride (60% in mineral oil) was prewashed by the hexanes prior to use. The progress of synthetic reactions was monitored by thin layer chromatography (TLC) in a suitable solvent system (system A, 5:95, v/v, CH_3OH:CH_2Cl_2; system B, 10:90, v/v, CH_3OH:CH_2Cl_2; system C, hexanes:EtOAc, 2:1, v/v; system D, hexanes:EtOAc, 1:2, v/v; and system E, hexanes:EtOAc 1:1, v/v). Column chromatography was performed on Merck silica gel 60 (70−200 and 230−400 mesh ASTM). Dry chromatography, wherever needed, was performed using TLC grade silica gel 5−15 μm from Sorbent Technologies Inc. Preparative HPLC purification used a reverse phase ACE C18 (5 μm, 150 mm × 4.6 mm) column (Agilent) and gradients of aqueous 0.1% H_3PO_4 solution and acetonitrile (mobile phase A, gradient elution) as mobile phase at a flow rate of 1.0 mL/min that enabled the collection of pure diastereomeric mixtures of the glucoazomycins. The attempts to separate the diastereomers included reversed phase columns (Agilent, 150 mm × 4.6 mm) and the columns with chiral stationary phases (ChiralpakAD 150 mm × 4.6 mm 10 μm containing amylase carbamate and ChiralcelOJ-H 150 mm × 4.6 mm, cellulose tris [4-methyl benzoate] coated on 5 μm silica gel, Daicel, USA) using hexanes:iso-PrOH (9:1, v/v) at a flow rate of 1.0 mL/ min or hexanes:iso-PrOH 1:1 (v/v) at a flow rate of 0.4 mL/min as eluents. Synthesized compounds were isolated as diastereomeric mixtures, and therefore the melting points were not determined. ^{1}H, ^{19}F, and ^{13}C NMR spectra, NOE studies were acquired using a Bruker AM-300 spectrometer or a Varian Mercury 400 MHz NMR spectrophotometer (Naeja) in deuterated chloroform ($CDCl_3$) or methanol (CD_3OD), depending on the solubility of the product. Chemical shifts are reported in δ ppm downfield with respect to tetramethylsilane as an internal standard in ^{1}H and ^{13}C NMR spectra and trichlorofluoromethane as an external standard for ^{19}F NMR spectra. Being sugar conjugates, the nomenclature of these molecules uses glucose as the "base" moiety for denoting the positions of the carbons, and this is extended to the linker; nitroimidazole protons are represented by the prefix "Im". The elemental composition of final products was confirmed by C, H, and N elemental analyses and/or by HRMS (ES^+) spectra recorded using an AEI-MS-12 mass spectrometer. Low resolution mass spectra were measured on a Waters 2795 separation module using either ES^+ or ES^- ionization modes. No attempt was made to resolve the diastereomers formed by creation of an asymmetric center at C8 in the three-carbon linker that couples the glucosyl and nitroimidazolyl moieties. HPLC analyses confirmed >95% chemical purity for the final target compounds.

Synthesis of 1-α-D-*O*-Methyl-2,3,4-tri-*O*-benzyl-6-*O*-((oxiran-2-yl)methyl)-glucopyranose (2). A precooled (0 to 5 °C) solution of **1** (35.0 g, 0.0754 mol) in anhydrous DMF (60 mL) was added dropwise to a stirred cooled (ca. 0 °C) suspension of NaH (4.53 g, 0.113 mol) in anhydrous DMF (75 mL). The resultant mixture was allowed to warm up to 22 °C (1 h) and then cooled down to +5 °C. Epibromohydrin (15.5 g, 0.113 mol) was added dropwise to this reaction mixture under the stirring at +5 to 10 °C. The mixture was allowed to warm to 22 °C, and stirring was continued for an additional 20 h to the complete reaction. EtOAc (300 mL) was added to the reaction mixture, and the resultant mixture was washed with cold water (3 × 150 mL), citric acid (0.5M, 2 × 100 mL), saturated aqueous solution of $NaHCO_3$ (100 mL), brine (200 mL), and dried over anhydrous $MgSO_4$. Filtration and removal of the solvent from the filtrate under reduced pressure provided yellow oil (41.1 g). Dry chromatography (5% EtOAc−hexanes → 30% EtOAc−hexanes) of this material afforded a mixture of 8*R* and 8*S* diastereomers (due to the chirality at C8, diastereomers ratio 1:1) of product **2** as a colorless oil, which were not separated. Combined yield 31.3 g (85%). ^{1}H NMR ($CDCl_3$): δ 2.56 (dd, $J_{9',9}$ = 9.2 Hz, $J_{8,9}$ = 4.9 Hz for 8*S* and $J_{8,9}$ = 2.8 Hz for 8*R* diastereomers, oxirane H9 for both diastereomers), 2.76 (dd, $J_{8,9'}$ = 4.2 Hz, $J_{9,9'}$ = 9.4 Hz, oxirane H9′ for both diastereomers), 3.13 (dddd, $J_{9,8}$ = 4.9 Hz, $J_{9',8}$ = 4.2 Hz, $J_{7,8}$ = 4.9 Hz, $J_{7',8}$ = 3.2 Hz, oxirane H8 for both diastereomers, 3.31 (dd, $J_{6',6}$ = 11.6 Hz, $J_{6,5}$ = 4.6 Hz, H6 for two diastereomers), 3.36 and 3.37 (two s, each for 3H, OCH_3 for two diastereomers), 3.42 (dd, $J_{6,6'}$ = 11.6 Hz, $J_{5,6'}$ = 5.6 Hz, H6′ for two diastereomers), 3.54 (dd, $J_{1,2}$ = 3.6 Hz, $J_{3,2}$ = 9.2 Hz, 1H, H2 for two diastereomers), 3.60 (dd, $J_{3,4}$ = 9.2 Hz, $J_{5,4}$ = 9.6 Hz, H4 for two diastereomers), 3.66 (dd, $J_{6,5}$ = 4.0 Hz, $J_{4,5}$ = 10.4 Hz, 1H, H5, 8*S* diastereomer), 3.68 (dd, $J_{7',7}$ = 11.2 Hz, $J_{8,7}$ = 4.9 Hz,, H-7 for two diastereomers), 3.72 (ddd, $J_{4,5}$ = 9.6 Hz, $J_{6,5}$ = 4.6 Hz, $J_{6',5}$ = 5.6 Hz, 1H, H5 for 8*R* diastereomer), 3.79 (dd, $J_{8,7'}$ = 3.2 Hz, $J_{7,7'}$ = 11.2 Hz, H7′ for two diastereomers), 3.99 (dd, $J_{2,3}$ = $J_{4,3}$ = 9.2 Hz, H3 for two diastereomers), 4.60 (d, $J_{2,1}$ = 3.6 Hz, H1 for two diastereomers), 4.61 (d, $J_{4a,b}$ = 11.2 Hz, 1H, H4a of 8*S* diastereomer, Bn), 4.64 (d, $J_{4a,b}$ = 11.2 Hz, 1H, H4a of 8*R* diastereomer, Bn), 4.66 (d, $J_{3a,3b}$ = 12.0. Hz, 1H, H3a Bn, both diastereomers), 4.79 (d, $J_{3a,3b}$ = 12.0 Hz, 1H, H3b Bn, both diastereomers), 4.82 and 4.83 (d, $J_{4a,b}$ = 11.0 Hz, 1H, H4b Bn, each d denotes one diastereomer), 4.88 and 4.90 (two d, $J_{2a,2b}$ = 11.2 Hz, 1H, H2a, each d for one diastereomer), 4.98 (d, $J_{2a,2b}$ = 11.2 Hz, 1H, H2b Bn, both diastereomers), 7.28−7.37 (15H, 3 phenyl moieties for both diastereomers). ^{13}C NMR ($CDCl_3$): δ 50.60 and 50.71 (oxirane C8, both diastereomers), 55.16 (Me), 69.78 and 69.87 (oxirane C9, both diastereomers), 69.99 and 70.04 (C5), 72.03 and 72.24 (C6), 72.30 and 72.56 (C7), 73.39 (CH_2 at C4), 75.00 and 75.05 (CH_2 at C2), 75.72 (CH_2 at C3), 77.54 (C4), 79.72 and 79.84 (C2), 81.98 and 82.08 (C3), 98.21 (C1), 127.51−138.75 (phenyl Cs). ESI-MS ($C_{31}H_{36}NaO_7$): *m*/*z* 543.2348 $(M + Na)^+$.

Synthesis of Methyl-1-α-D-(2,3,4-tri-*O*-benzyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*S*-hydroxypropyl)glucopyranose and Methyl-1-α-D-(2,3,4-tri-*O*-benzyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*R*-hydroxypropyl)glucopyranose (3a). 2-Nitroimidazole (3.62 g, 32 mmol) and Cs_2CO_3 (1.04 g, 3.2 mmol) were added to a solution of **2** (16.66 g, 32 mmol) in ethanol (310 mL) under stirring, and the suspension was heated to reflux (80 °C) for 27 h. The resulting clear yellow solution was cooled to 22 °C, the solvent was removed under reduced pressure, and the yellow semisolid residue was redissolved in EtOAc (300 mL) and then filtered through a pad of silica gel (for TLC). The filtrate was collected and the solvent was removed under reduced pressure to afford a yellow semisolid (19.7 g) that was subjected to dry chromatography using EtOAc−hexanes (20% → 80%) as the eluent to afford pure product **3a** as a mixture of two diastereomers with ratio 1:1. Combined yield: 15.9 g (78%). ^{1}H NMR ($CDCl_3$): δ 2.74 and 2.83 (two d, $J_{8\text{-OH}}$ = 5.2 Hz, OH for 8*S* and 8*R* diastereomers), 3.37 and 3.38 (two s, each for 3H, OCH_3 for 8*S* and 8*R* diastereomers), 3.43 and 3.44 (two dd, $J_{3,4}$ = 9.6 Hz, $J_{5,4}$ = 8.4 Hz, each representing 1H, H4 for 8*S* and 8*R* diastereomers), 3.49 and 3.51 (two dd, $J_{3,2}$ = 9.6 Hz, $J_{1,2}$ = 3.6 Hz, each denoting 1H, H2, 8*S* and 8*R* diastereomers), 3.54 (two dd, $J_{5,6}$ = 4.4 Hz, $J_{6',6}$ = 12.2 Hz, H6 for both diastereomers), 3.62 (dd, $J_{5,6'}$ = 3.2 Hz, $J_{6',6}$ = 12.0 Hz, H6′, both diastereomers), 3.69 (dd, $J_{8,7}$ = 3.2 Hz, $J_{7',7}$ = 12.0 Hz, H7, both diastereomers), 3.72 (ddd, $J_{4,5}$ = 8.0 Hz, $J_{6,5}$ = 4.4 Hz, $J_{6',5}$ = 3.2 Hz, denoting H5, two diastereomers), 3.73 (dd, $J_{8,7'}$ = 4.8 Hz, $J_{7,7'}$ = 12.0 Hz, H7′, two diastereomers), 3.99 (dd, $J_{2,3}$ = $J_{4,3}$ = 8.8 Hz, H3, both diastereomers), 4.08 (m, 1H, H8, both diastereomers), 4.33 (two dd, $J_{8,9}$ = 6.8 Hz, $J_{9,9'}$ = 14.0 Hz, each representing one diastereomer, H9), 4.57 (dd, $J_{2,1}$ = 4.0 Hz, H1, two diastereomers), 4.58 (d, $J_{2a,b}$ = 11.8 Hz, H2a, Bn), 4.60 (broad, 1H, H9′, both diastereomers), 4.67 (d, $J_{3a,3b}$ = 12.0 Hz, H3a, Bn), 4.79 (d, $J_{3a,3b}$ = 12.0 Hz, H3b, Bn), 4.81 (d, $J_{4a,4b}$ = 11.0 Hz, H4a, Bn), 4.90 (d, $J_{4a,b}$ = 11.0 Hz, H4b, Bn), 4.99 (d, $J_{2a,2\,b}$ = 11.8 Hz, H2b, Bn), 7.09 and 7.10 (two s, representing 8*S* and 8*R* diastereomer, H4, Im), 7.14 and 7.15 (two s, H5, 8*S* and 8*R* diastereomers, Im), 7.28−7.38 (15H, 3 phenyls). ^{13}C NMR ($CDCl_3$): δ 52.16 and 52.24 (C9 for 8*S* and 8*R* diastereomers), 55.29 (Me), 69.24 and 69.34 (C8, 8*S* and 8*R* diastereomers), 70.00 and 70.04 (C5), 70.55 (C6), 72.60 (C7), 73.34, (CH_2 at C4), 74.87 and 74.97 (CH_2 at C2), 75.76 (CH_2 at C2), 77.19 and 77.27 (C4), 79.90 (C2), 81.89 (C3), 98.05 and 98.11 (C1), 127.61−128.93 (phenyl Cs). ESI-MS ($C_{34}H_{39}N_9O_9$): *m*/*z* 634 $(M + 1)^+$.

Synthesis of Methyl-1-α-D-(2,3,4-tri-*O*-benzyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*S*-*O*-(4-nitrobenzenesulfonyloxy)-propyl)glucopyranose and Methyl-1-α-D-(2,3,4-tri-*O*-benzyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*R*-*O*-(4-

dx.doi.org/10.1021/jm2017336 | J. Med. Chem. 2012, 55, 6033−6046

nitrobenzenesulfonyloxy)propyl)glucopyranose (4). A solution of 4-(dimethylamino)pyridine (2.69 g, 22 mmol) and triethylamine (4.45 g, 44 mmol) in CH_2Cl_2 (60 mL) was added dropwise to a cold stirred (ca. −10 °C) solution of compound **3a** (13.93 g, 22 mmol) and 4-nitrobenzenesulfonyl chloride (5.85 g, 26.4 mmol) in anhydrous CH_2Cl_2 (60 mL), and the reaction mixture was kept at this temperature. The reaction was complete after 14 h. The mixture was warmed to 22 °C and then washed sequentially with water (3 × 100 mL) followed by 1N HCl (100 mL). The organic phase was separated, dried over $MgSO_4$, and then filtered through a pad of silica gel (for TLC). The solvents were removed from the organic filtrate under reduced pressure to afford pure 8*R* and 8*S* diastereomers (ratio 1:1) of product **4** as a yellowish solid. Yield: 17.7 g (98%). ^{1}H NMR ($CDCl_3$): δ 3.37 and 3.38 (two s, each for 3H, OCH_3 for 8*S* and 8*R* diastereomers), 3.41 (dd, $J_{3,4}$ = 9.6 Hz, $J_{5,4}$ = 9.2 Hz, 1H, H4 for 8*S* diastereomer) and 3.50 (dd, $J_{3,4}$ = 10.0 Hz, $J_{5,4}$ = 9.2 Hz, 1H, H4 for 8*R* diastereomer), 3.51 (dd, $J_{3,2}$ = 9.2 Hz, $J_{1,2}$ = 3.2 Hz, representing H2, 8*S* diastereomer), 3.58 (two dd, $J_{5,6}$ = 2.0 Hz, $J_{6',6}$ = 11.2 Hz, H6, 8*S* and 8*R* diastereomers), 3.62 (dd, $J_{5,6'}$ = 2.8 Hz, $J_{6',6}$ = 11.2 Hz, representing H6′, 8*S* and 8*R* diastereomers), 3.68 (dd, $J_{8,7}$ = 3.6 Hz, $J_{7',7}$ = 11.2 Hz, H7, 8*S* and 8*R* diastereomers), 3.71 (dd, $J_{4,5}$ = 9.2 Hz, $J_{6,5}$ = 2.0 Hz, $J_{6',5}$ = 2.8 Hz, denoting H5 for two diastereomers), 3.74 (dd, $J_{8,7'}$ = 4.4 Hz, $J_{7,7'}$ = 11.2 Hz, H7′ for two diastereomers), 3.99 (dd, $J_{2,3}$ = 9.2 Hz, $J_{4,3}$ = 9.6 Hz, H3 for 8*S* and 8*R* diastereomers), 4.13 and 4.46 (two dd, each for one diastereomer, $J_{8,9}$ = 8.4 Hz, $J_{9,9'}$ = 13.8 Hz, H9, 8*S* and 8*R* diastereomers), 4.55 (broad dd, $J_{8,9'}$ = 8.4 Hz, $J_{9,9'}$ = 13.8 Hz, H9′, 8*S* and 8*R* diastereomers), 4.58 (dd, $J_{2,1}$ = 4.0 Hz, representing H1 for two diastereomers), 4.68 (d, $J_{2a,b}$ = 12.0 Hz, 1H, H2a, Bn), 4.73 (d, $J_{3a,3b}$ = 12.0 Hz, 1H, H3a, Bn), 4.81 (d, $J_{4a,4b}$ = 11.2 Hz, 2H, H4a and H4b, Bn), 4.90 (d, $J_{3a,3b}$ = 12.0 Hz, 1H, H3b, Bn), 4.99 (d, $J_{2a,2b}$ = 11.2 Hz, 1H, H2b, Bn), 5.02 (m, $J_{8,9}$ = 8.4 Hz, $J_{8,9'}$ = 8.0 Hz, H8 for two diastereomers), 6.99 and 7.01 (two s, each 1H, 8*S* and 8*R* diastereomers, H4, Im), 7.04 (s, represents two diastereomers, H5, Im), 7.26−7.39 (m, 3 phenyls from two diastereomers), 7.82 and 7.83 (two d, *J* = 8.8 Hz, for two diastereomers each denoting 1H, H3, nosyl), 7.86 (two d, *J* = 8.8 Hz, each for 1H of diastereomers, H5, nosyl), 8.21 (two d, *J* = 8.4 Hz, 1H of each diastereomer, H2, nosyl), 8.23 (two d, *J* = 8.4 Hz, 1H of each diastereomer H6, nosyl). ^{13}C NMR ($CDCl_3$): δ 49.94 (C9), 55.35 (Me), 69.24 (C8), 70.44 (C5), 70.70 (C6), 72.65 (C7), 73.44 (CH_2 at C4), 74.94 (CH_2 at C2), 75.76 (CH_2 at C2), 78.88 (C4), 79.95 (C2), 81.86 (C3), 98.15 (C1), 114.57 (C4, Im), 124.52 (C5, Im), 127.68−128.85 (phenyl Cs), C2 imidazole and C4 nosyl (buried in the baseline). ESI-MS ($C_{40}H_{42}N_4O_{13}S$): *m*/*z* 841.2350 $(M + Na)^+$.

Synthesis of 1-α/β-D-(1,2,3,4-Tetra-*O*-acetyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*S*-*O*-(4-nitrobenzenesulfonyloxy)propyl)-glucopyranose and 1-α/β-D-(1,2,3,4-Tetra-*O*-acetyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*R*-*O*-(4-nitrobenzenesulfonyloxy)-propyl)glucopyranose (5). Trimethylsilyl triflate (4.0 g, 18 mmol) was added dropwise to a stirred cooled (ca. −40 °C) solution of compound **4** (1.64 g, 2 mmol) in acetic anhydride (20 mL), allowed to warm up to +14 °C, and stirred for an additional 4 h at this temperature. The resulting dark-colored reaction solution was cooled to −5 °C, diluted with cold EtOAc (100 mL), and then poured into a cold (0 °C) saturated aqueous $NaHCO_3$ solution (100 mL). The organic phase was separated off and washed with saturated aqueous $NaHCO_3$ (5 × 100 mL), followed by brine (100 mL), and then dried over $MgSO_4$. Filtration of the contents through a pad of silica gel and removal of the solvent under reduced pressure gave 2.2 g of red oil, that was subjected to dry chromatography using EtOAc−hexanes (20% → 70%) to afford product **5** (1.32 g) as a yellow solid. An additional purification by dry chromatography using toluene followed by EtOAc−toluene (40%) afforded 1.32 g of **5**. A final purification of this product using reversed phase preparative HPLC (eluent; MeCN−gradient 0.1% CF_3CO_2H in H_2O) eluted pure diastereomeric mixture of **α**- and **β**-**5** anomers at a retention time of 11.8 min. Combined yield for the diastereomeric mixture: 0.512 g (36%). ESI-MS: *m*/*z* 703 $(M + 1)^+$. Anal. Calcd ($C_{26}H_{30}N_4O_{17}S$): C, 44.45; H, 4.30; N, 7.97. Found: C, 44.17; H, 4.39; N, 7.75. HPLC retention time using conditions as described in Chemistry: General, 11.81 min for all isomers. HPLC: 99.16% (combined chemical purity for all diastereomers).

***α*-5** *(8R and 8S).* ^{1}H NMR ($CDCl_3$): δ 2.03, 2.05, 2.13, 2.19 (4 s for 8*S* diastereomer) and 2.04, 2.05, 2.07 and 2.20 (4 s, for 8*R* diastereomer, each for 3H, CH_3), 3.50 (dd, $J_{6',6}$ = 11.2 Hz, $J_{5'6'}$ = 4.0 Hz, 1H, H6 for 8*S* diastereomer), 3.61 (dd, $J_{6',6}$ = 11.2 Hz, $J_{5,6'}$ = 2.0 Hz, 1H, H6′ for 8*S* diastereomer), 3.66 (dd, $J_{5,6}$ = 2.0 Hz, $J_{6',6}$ = 8.8 Hz, 1H, H6, 8*R* diastereomer), 3.70 (dd, $J_{5,6'}$ = 2.8 Hz, $J_{6',6}$ = 8.8 Hz, 1H, H6′, 8*R* diastereomer), 3.73 (d, $J_{7,7'}$ = 11.2 Hz, $J_{8,7}$ = 3.2 Hz, 1H, H7, 8*S* diastereomer), 3.80 (dd, $J_{7',7}$ = 12.0 Hz, $J_{8,7}$ = 3.6 Hz, 2H, H7 and H7′ for 8*R* diastereomer), 3.88 (dd, $J_{7',8}$ = 2.8 Hz, $J_{7',7}$ = 12.0 Hz, 1H, H7′, 8*S* diastereomer), 4.02 (m, $J_{4,5}$ = 10.4 Hz, $J_{6'5}$ = 2.0 Hz, $J_{6,5}$ = 3.6 Hz, H5, both diastereomers), 4.55 (dd, $J_{8,9}$ = 8.4 Hz, $J_{9',9}$ = 14.4 Hz, 1H, H9, 8*S* diastereomer), 4.60 (dd, $J_{8,9'}$ = 3.2 Hz, $J_{9',9}$ = 14.4 Hz, 1H, H9′, 8*S* diastereomer), 4.71 (dd, $J_{8,9}$ = 8.4 Hz, $J_{9',9}$ = 14.4 Hz, 1H, H9, 8*R* diastereomer), 4.78 (dd, $J_{8,9'}$ = 3.6 Hz, $J_{9',9}$ = 14.4 Hz, 1H, H9, 8*R* diastereomer), 4.96 (dddd, $J_{9,8}$ = 8.4 Hz, $J_{9',8}$ = 3.2 Hz, $J_{7,8}$ = 3.6 Hz, $J_{7',8}$ = 2.8 Hz, 1H, H8 of 8*S* diastereomer), 5.05 (dddd, $J_{9',8}$ = 8.4 Hz, $J_{9,8}$ = 3.6 Hz, $J_{7,8}$ = 3.6 Hz, $J_{7',8}$ = 2.8 Hz, 1H, H8 of 8*R* diastereomer), 5.08 (two dd, merged, $J_{1,2}$ = 4.0 Hz, $J_{3,2}$ = 10.0 Hz, H2, both diastereomers), 5.17 (dd, $J_{5,4}$ = 10.4 Hz, $J_{3,4}$ = 9.6 Hz, 1H, H4, 8*S* diastereomer), 5.26 (dd, $J_{5,4}$ = 10.4 Hz, $J_{3,4}$ = 9.6 Hz, 1H, H4, 8*R* diastereomer), 5.48 (dd, $J_{2,3}$ = 10.0 Hz, $J_{4,3}$ = 9.6 Hz, 1H, H3, 8*S* diastereomer), 5.49 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 9.6 Hz, 1H, H3, 8*R* diastereomer), 6.29 (d, $J_{2,1}$ = 4.0 Hz, 1H, H1, 8*S* diastereomer), 6.31 (d, $J_{2,1}$ = 4.0 Hz, 1H, H1, 8*R* diastereomer), 7.05 (s, H4, Im, 8*S* diastereomer), 7.08 (s, H4, Im, 8*R* diastereomer), 7.15 (s, H5, Im, 8*S* diastereomer) and 7.26 (s, H5, Im, 8*R* diastereomer), 7.85 and 7.95 (two dd, $J_{6,5}$ = $J_{2,3}$ = 9.2 Hz, 4H, H2, H3, H5 and H6 of Ns, 8*S* diastereomer), 8.32 and 8.34 (two d, $J_{5,6}$ = $J_{3,2}$ = 9.2 Hz, 4H, H2, H3, H5, and H6 of Ns, 8*R* diastereomer).

***β*-5** *(8R and 8S).* ^{1}H NMR ($CDCl_3$): The signals for most of the protons for this isomer appeared as buried under the corresponding signals for the α-isomer. Only the signals that appeared separate from **α**-**5** and were identifiable in this complex mixture of diastereomers are being provided. δ 2.03, 2.06, 2.11 and 2.12 (4 s, each representing 3H of CH_3), 3.80 (m, H7), 3.85 (dd, $J_{8,7}$ = 4.0, $J_{7,7'}$ = 12 Hz, 1H, H7′), 4.60 (H9), 4.78 (H9′), 5.65, and 5.68 (two d, $J_{2,1}$ = 8.4 Hz, H1 for two diastereomers).

Synthesis of Methyl-1-α-D-(2,3,4-tri-*O*-benzyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*S*-*O*-(acetyl)propyl)glucopyranose and Methyl-1-α-D-(2,3,4-tri-*O*-benzyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*R*-*O*-(acetyl)propyl)glucopyranose (6). Acetic anhydride (3.75 g, 36.7 mmol) was added dropwise at +3° to +5 °C to a stirred solution of **3a** (15.51 g, 24.5 mmol) in anhydrous pyridine (60 mL). The yellow reaction solution was allowed to warm up to 22 °C and was stirred for 20 h. EtOAc (300 mL) was added to the reaction solution. The resultant mixture was washed with water (3 × 100 mL), 2 N HCl (2 × 100 mL), saturated aqueous $NaHCO_3$ (100 mL), brine (150 mL), and dried over $MgSO_4$. The solvent from the filtrate was removed to provide a diastereomeric mixture of **6** as yellow semisolid. Yield: 16.4 g (99%). ^{1}H NMR ($CDCl_3$): δ 1.91 (s, 3H of $COCH_3$, 8*S* diastereomer) and 1.96 (s, 3H of $COCH_3$, 8*R* diastereomer), 3.39 (s, 3H, CH_3, 8*S* diastereomer) and 3.40 (s, 3H of CH_3, 8*R* diastereomer), 3.48 and 3.50 (two dd, $J_{3,2}$ = 8.8 Hz, $J_{1,2}$ = 3.5 Hz, 1H, H2, 8*S* and 8*R* diastereomers), 3.53 (dd, $J_{6,5}$ = 4.4 Hz, $J_{6,6'}$ = 10.8 Hz, 1H, H6, both diastereomers), 3.55 (dd, $J_{6',5}$ = 4.4 Hz, $J_{6,6'}$ = 10.8 Hz, 1H, H6′ for both diastereomers), 3.59 (dd, $J_{3,4}$ = 9.6 Hz, $J_{5,4}$ = 8.4 Hz, 1H, H4, 8*S* diastereomer) and 3.61 (two dd, $J_{3,4}$ = 9.6 Hz, $J_{5,4}$ = 8.4 Hz, 1H, H4, 8*R* diastereomer), 3.62 (dd, $J_{4,5}$ = 10.4 Hz, $J_{6,5}$ = $J_{6'5}$ = 4.4 Hz, 1H, H5, 8*S* diastereomer) and 3.64 (dd, $J_{4,5}$ = 10.4 Hz, $J_{6,5}$ = $J_{6'5}$ = 4.4 Hz, 1H, H5, 8*R* diastereomer), 3.67 (dd, $J_{7',7}$ = 11.0 Hz, $J_{8,7}$ = 7.6 Hz, H7, both diastereomers), 3.75 (dd, $J_{7,7'}$ = 11.0 Hz, $J_{8,7'}$ = 7.6 Hz, H7′, both diastereomers), 4.00 (dd, $J_{2,3}$ = $J_{4,3}$ = 8.8 Hz, 1H, H3), 4.45 (two dd, each denoting one diastereomer, $J_{9',9}$ = 11.2 Hz, $J_{8,9}$ = 8.4 Hz, H9, both diastereomers), 4.59 (two dd, each for one diastereomer, $J_{9,9'}$ = 11.2 Hz, $J_{8,9'}$ = 8.0 Hz, H9′, both diastereomers), 4.61 (d, $J_{2,1}$ = 3.6 Hz, H1, both diastereomers), 4.68 (two d, each denoting one diastereomer, $J_{2a,b}$ = 12.0 Hz, 1H, H2a, Bn), 4.82 (d, $J_{3a,3b}$ = 10.8 Hz, H3a, Bn, both diastereomers), 4.83 (d, $J_{4a,4b}$ = 10.8 Hz, H4a, both diastereomers), 4.86 (two d, each for one diastereomer, $J_{4a,4b}$ = 10.8 Hz, 1H, H4b, Bn),

dx.doi.org/10.1021/jm2017336 | J. Med. Chem. 2012, 55, 6033−6046

4.90 (two d, each representing one diastereomer, $J_{3a,3b}$ = 10.8 Hz, H3b, Bn), 4.99 (d, $J_{2a,2b}$ = 10.0 Hz, H2b, both diastereomers, Bn), 5.02 (m, $J_{8,9}$ = 8.4 Hz, $J_{8,9'}$ = 8.0 Hz, representing H8 for two diastereomers), 7.07 (d, $J_{5,4}$ = 1.2 Hz, 8*S* diastereomer, H4, Im) and 7.08 (d, $J_{5,4}$ = 1.2 Hz, 8*R* diastereomer, H4, Im), 7.08 (d, $J_{5,4}$ = 1.2 Hz, 8*S* diastereomer, H5, NI) and 7.09 (d, $J_{5,4}$ = 1.2 Hz, 8*R* diastereomer, H5, NI), 7.27–7.39 (15H for each diastereomer, 3 phenyls).

Synthesis of 1-α/β-D-(1,2,3,4-Tetra-*O*-acetyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*S*-*O*-(acetyl)propyl)glucopyranose and 1-α/β-D-(1,2,3,4-Tetra-*O*-acetyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*R*-*O*-(acetyl)propyl)glucopyranose (7). Trimethylsilyl triflate (59.3 g, 267 mmol) was added dropwise to a stirred cooled (ca. −40 °C) solution of **6** (16.4 g, 24.3 mmol) in acetic anhydride (243 mL). The reaction solution was allowed to warm up to 22 °C and stirred for an additional 18 h. After cooling to −10 °C, the dark reaction solution was diluted with cold EtOAc (700 mL) and poured in a cold (0 °C) saturated aqueous $NaHCO_3$ (500 mL). The resultant mixture was stirred for 0.5 h at room temperature. The organic phase was separated off and washed with saturated aqueous $NaHCO_3$ (10 × 300 mL) and brine (500 mL) and dried over $MgSO_4$. Filtration through a pad of silica gel (for TLC) and removal of the solvent from the filtrate under reduced pressure provided 23.3 g of dark oil, which was subjected to dry chromatography (30% EtOAc–hexanes → 80% EtOAc–hexanes) to afford 11.09 g of product **7** as a yellow solid. An additional purification of **7** by dry chromatography (5% MeCN–toluene → 35% MeCN–toluene) provided **7**, a yellowish solid, as an *R* and *S* stereoisomeric mixture of α- and β-anomers (α/β anomeric ratio ~83.9:14.0), which were not separated. Yield: 7.91 g (58%). ESI-MS: m/z 560 $(M + 1)^+$. Anal. ($C_{22}H_{29}N_3O_{14}$) CHN. HPLC: 97.9% (combined chemical purity for all diastereomers).

***α*-7** *(8R and 8S).* Retention time, 11.02 min. ^{1}H NMR ($CDCl_3$): δ 2.00, 2.01, 2.07 (s, 12H of CH_3, 8*S* stereomer) and 2.03, and 2.04 (s, 12H, of 4 CH_3, for 8*R* stereomer), 2.20 (s, 3H, $COCH_3$ at C8), 3.44 (dd, $J_{6,5}$ = 5.2 Hz, $J_{6',6}$ = 11.2 Hz, H6, both 8*S* and 8*R* diastereomers), 3.56 (dd, $J_{6,6'}$ = 11.2 Hz, $J_{6',5}$ = 4.0 Hz, H6′, both diastereomers), 3.57 (dd, $J_{8,7}$ = 5.6 Hz, $J_{7',7}$ = 10.8 Hz, H7, both diastereomers), 3.64 (dd, $J_{7,7'}$ = 10.8 Hz, $J_{8,7'}$ = 4.0 Hz, H7′, both diastereomers), 4.03 (dd, $J_{6,5}$ = 5.2 Hz, $J_{6',5}$ = 4.0 Hz, $J_{4,5}$ = 9.6 Hz, H5 for both diastereomers), 4.53 (dd, $J_{8,9}$ = 6.8 Hz, $J_{9',9}$ = 14.2 Hz, 2H, H9 and H9′ for 8*S* and 8*R* diastereomers), 4.90 (dd, $J_{9',9}$ = 14.2 Hz, $J_{8,9'}$ = 3.6 Hz, 2H, H9′ and H9 for 8*S* and 8*R* diastereomers), 5.09 (two merged dd, $J_{1,2}$ = 3.6 Hz, $J_{3,2}$ = 10.0 Hz, each for 1H, H2 for both diastereomers), 5.19 (dd, $J_{5,4}$ = 9.6 Hz, $J_{3,4}$ = 9.2 Hz, 1H, H4, 8S diastereomer) and 5.22 (dd, $J_{5,4}$ = 9.6 Hz, $J_{3,4}$ = 9.2 Hz, 1H, H4, 8*R* diastereomer), 5.28 (ddd, $J_{9,8}$ = 6.8 Hz, $J_{9',8}$ = 3.6 Hz, $J_{7',8}$ = 4.0 Hz, $J_{7,8}$ = 5.6 Hz, H8, both diastereomers), 5.48 (dd, $J_{2,3}$ = 10.0 Hz, $J_{4,3}$ = 9.2 Hz, 1H, H3, 8*S* diastereomer) and 5.49 (dd, $J_{2,3}$ = 10.0 Hz, $J_{4,3}$ = 9.2 Hz, 1H, H3, 8*R* diastereomer), 6.32 (d, $J_{2,1}$ = 3.6 Hz, H1 for both diastereomers), 7.14 (s, 1H, H4, Im, 8*S* diastereomer) and 7.19 (s, 1H, H4, Im, 8*R* diastereomer), 7.20 (s, 1H, H5, Im, 8*S* diastereomer) and 7.27 (s, 1H, H5, Im, 8*R* diastereomer). ^{13}C NMR ($CDCl_3$): δ 20.71–21.13 (CH_3S), 49.67 and 49.72 (C7, two diastereomers), 68.18 and 68.24 (C6, two diastereomers), 69.40 and 69.42 (C9, for two diastereomers), 69.60 and 69.63 (C4, two diastereomers), 69.77 (C8), 70.13 and 70.19 (C5 for two diastereomers), 70.30 and 70.39 (C2, two diastereomers), 71.13 and 71.16 (C3 for two diastereomers), 89.30 (C1), 127.08 and 127.16 (C4 Im for two diastereomers), 128.40 and 128.45 (C5 Im for two diastereomers), 145.67 (C2, Im), 169.14, 169.66, 169.73, 169.91, 169.96, 169.98, 170.43, 170.45 (8 s, representing 4 C=O for two diastereomers).

***β*-7** *(8R and 8S).* Retention time, 11.02 min. The presence of these diastereomers was detected in ~13% (calculated by NMR integration and HPLC chromatographic analysis). Proton signals that appeared at identifiable, and separate chemical shifts from the α-anomer are reported. ^{1}H NMR ($CDCl_3$): δ 1.98, 2.03, 2.06 and 2.12 (four s, 4 × CH_3), 5.14 (H2), 5.23 (H8), 5.68 (d, $J_{2,1}$ = 8.0 Hz, H1), 7.11 and 7.15 (H4, Im), 7.23 and 7.25 (H5, Im). ^{13}C NMR ($CDCl_3$) δ: 21.04–21.13 (signals for CH_3s), 49.55 (C6, two diastereomers), 73.04 and 73.10 (C3, two diastereomers), 74.09 and 74.16 (C5 for two diastereomers), 91.99 (C1), 128. 08 (C4, Im), 129.26 (C5 Im for two diastereomers), other carbon signals were buried under the signals for α-anomer.

Synthesis of 1-α/β-D-(6-*O*-(9-[2-Nitro-1*H*-imidazolyl]-8*S*-hydroxypropyl)glucopyranose and 1-α/β-D-(6-*O*-(9-[2-Nitro-1*H*-imidazolyl]-8*R*-hydroxypropyl)glucopyranose (8). Compound **7** (5.595 g, 10 mmol) was dissolved in a solution of 0.1 M NaOH in methanol (600 mL). After 20 min of stirring at 22 °C, the reaction solution was treated with Dowex 50W × 4–200 (H^+, 18.6 g) resin to adjust the pH of the solution to 5 and then filtered. The filtrate was evaporated under reduced pressure to dryness to provide 2.46 g of a yellow solid that was subjected to dry chromatography (5% MeOH–$CHCl_3$ → 30% MeOH–$CHCl_3$) to afford **8** as a yellow solid. Yield: 2.18 g (62%). HPLC: 97.23% (combined chemical purity for all diastereomers). ESI-MS: m/z 350 $(M + 1)^+$. Anal. Calcd ($C_{12}H_{19}N_3O_9 \cdot 1H_2O$): C, 39.24; H, 5.76; N, 11.44. Found: C, 38.90; H, 5.85; N, 11.16.

***α*-8** *(8R and 8S).* Retention time 7.03 min. Isomeric composition 50.4% by HPLC). ^{1}H NMR (CD_3OD): δ 3.34 (dd, $J_{5,4}$ = $J_{3,4}$ = 10.0 Hz, 1H, H4), 3.38 (ddd, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 10.0 Hz, 1H, H2), 3.44 and 3.54 (two dd, merged, $J_{7',7}$ = 9.6 Hz, $J_{8,7}$ = 4.8 Hz, 2H, H7 and H7′), 3.67 (dd, $J_{4,3}$ = $J_{2,3}$ = 10.0 Hz, 1H, H3), 3.66 and 3.68 (two dd, $J_{6,5}$ = 2.8 Hz, $J_{5,6'}$ = 2.0 Hz, $J_{6,6'}$ = 11.2 Hz, 2H, H6 and H6′), 3.70 (dd, $J_{6',5}$ = 3.6 Hz, $J_{4,5}$ = 10.0 Hz, 1H, H5), 4.07 (m, 1H, H8), 4.48 (ddd, $J_{9',9}$ = 14.0 Hz, $J_{8,9}$ = 4.0 Hz, $J_{OH\text{-}9}$ = 2.4 Hz, 1H, H9), 4.74 (ddd, $J_{9',9}$ = 14.0 Hz, $J_{8,9'}$ = 4.0 Hz, $J_{OH\text{-}9'}$ = 2.4 Hz, 1H, H9′), 5.10 (d, $J_{2,1}$ = 3.6 Hz, 1H, H1), 7.12 (d, $J_{5,4}$ = 1.2 Hz, 1H, H4 Im), 7.50 (d, $J_{4,5}$ = 1.2 Hz, 1H, H5, Im). ^{13}C NMR ($CDCl_3$): δ 52.14 and 52.19 (C7, two diastereomers), 68.78 (C9), 68.83 (C8), 70.33 and 70.38 (C6, dual resonances due to diastereomers), 70.73 (C4), 72.62 (C5), 72.67 (C2), 73.62 and 73.65 (C3, dual resonances due to diastereomers), 92.87 (C1), 126.90 (C4 Im), 128.09 (C5 Im), 145.33 (C2, Im).

***β*-8** *(8R and 8S).* Retention time 6.97 min. Isomeric composition 47.6% by HPLC. ^{1}H NMR (CD_3OD): δ 3.14 (dd, $J_{1,2}$ = 7.6 Hz, $J_{3,2}$ = 9.2 Hz, 1H, H2), 3.28–3.34 (m, $J_{5,4}$ = $J_{3,4}$ = 10.0 Hz, 2H, H3 and H4), 3.40 (m, 1H, H5), 3.54 (dd, $J_{7',7}$ = 9.6 Hz, $J_{8,7}$ = 4.0 Hz, 1H, H7), 3.66 (two dd, merged, $J_{6,5}$ = 3.2 Hz, $J_{5,6'}$ = 2.4 Hz, $J_{6',6}$ = 9.6 Hz, 2H, H6 and H6′), 3.71 (dd, $J_{7,7'}$ = 9.6 Hz, $J_{8,7'}$ = 4.0 Hz, 1H, H7′), 3.86 (m, 1H, H8), 4.44 (ddd, $J_{OH,9}$ = 3.2 Hz, $J_{8,9}$ = 4.8 Hz, $J_{9,9'}$ = 14.6 Hz, 1H, H9), 4.48 (dd, $J_{2,1}$ = 8.0 Hz, 1H, H1), 4.70 (ddd, $J_{OH,9'}$ = 3.2 Hz, $J_{8,9'}$ = 5.6 Hz, $J_{9,9'}$ = 14.6 Hz, 1H, H9′), 7.11 (d, $J_{5,4}$ = 1.2 Hz, 1H, H4 Im), 7.46 (d, $J_{4,5}$ = 1.2 Hz, 1H, H5, Im). ^{13}C NMR (CD_3OD): δ 52.24 and 52.26 (C7, two diastereomers), 68.78 (C9, dual resonances due to diastereomers), 68.86 (C8), 70.54 and 70.63 (C6, two diastereomers), 70.78 (C4), 75.07 and 75.09 (C2, dual resonances due to diastereomers), 75.58 (C3), 76.81 and 76.85 (C5), 97.09 (C1), 126.90 (C4 Im), 128.20 (C5 Im), 145.33 (C2, Im).

Synthesis of Methyl-1-α-D-(2,3,4-tri-*O*-benzyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*S*-fluoropropyl)glucopyranose, and Methyl-1-α-D-(2,3,4-tri-*O*-benzyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*R*-fluoropropyl)glucopyranose (9). DAST (3.24 g, 20.1 mmol) was added dropwise to a cooled (ca. −20 °C) stirred solution of **3** (8.50 g, 13.4 mmol) in anhydrous THF (250 mL). The reaction mixture was allowed to warm up to 22 °C and stirred for an additional 20 h. After cooling to −10 °C, the reaction mixture was quenched by MeOH (20 mL), the solvent was removed, and the residual red oil was redissolved in EtOAc (250 mL). The resultant solution was washed with saturated aqueous $NaHCO_3$ (250 mL) and brine (250 mL) and dried over $MgSO_4$. Filtration of the solution through a pad of silica gel (for TLC), followed by removal of the solvent from the filtrate under reduced pressure afforded red oil (8.9 g) that upon dry silica gel chromatography (EtOAc–hexanes 20%→ 50%) afforded pure diastereomeric mixture of **9** as yellow oil. Yield: 6.62 g (78%). HPLC: 97.89% (combined chemical purity for two diastereomers). ^{1}H NMR ($CDCl_3$): δ 3.38 (s, 3H, OCH_3, both diastereomers), 3.50 and 3.54 (two dd, $J_{5,4}$ = 9.6 Hz, $J_{3,4}$ = 9.6 Hz, 1H each, H4, 8*S* and 8*R* diastereomers), 3.52 and 3.55 (two dd, $J_{1,2}$ = 3.6 Hz, $J_{3,2}$ = 10.0 Hz, 1H each, H2, 8*S* and 8*R* diastereomers), 3.63 (dd, $J_{5,6}$ = 4.4 Hz, $J_{6,6'}$ = 11.2 Hz, H6, both diastereomers), 3.68 (dd, $J_{5,6'}$ = 2.8 Hz, $J_{6,6'}$ = 11.2 Hz, H6′, both diastereomers), 3.73 (ddd, $J_{7',7}$ = 11.4 Hz, $J_{8,7}$ = 6.4 Hz, $J_{F,7}$ = 31.2 Hz, H7, both diastereomers), 3.75 (ddd, $J_{4,5}$ = 9.6 Hz, $J_{6,5}$ = 4.4

dx.doi.org/10.1021/jm2017336 | J. Med. Chem. 2012, 55, 6033–6046

Hz, $J_{6',5}$ = 2.8 Hz, H5, both diastereomers), 3.79 (ddd, $J_{7',7}$ = 11.4 Hz, $J_{8,7'}$ = 6.4 Hz, $J_{F,7'}$ = 31.0 Hz, H7′, both diastereomers), 4.00 (dd, $J_{4,3}$ = 9.2 Hz, $J_{2,3}$ = 10.0 Hz, H3, both diastereomers), 4.53 and 4.58 ($J_{9',9}$ = 14.4 Hz, $J_{8,9}$ = 6.8 Hz, $J_{8,9'}$ = 8.4 Hz, $J_{F,H}$ = 20.0 Hz, H9 and H9′, both diastereomers), 4.59 (dd, $J_{2,1}$ = 3.6 Hz, H1, both diastereomers), 4.60 (d, $J_{2a,b}$ = 11.2 Hz, H2a, Bn, both diastereomers), 4.67 and 4.69 (d, $J_{3a,3b}$ = 12.0 Hz, diastereomeric H3a, Bn, both diastereomers), 4.75–4.88 (complex m, H4a, H4b of Bn, and H8, both diastereomers), 4.91 (d, $J_{3a,3b}$ = 12.0 Hz, H3b, Bn, both diastereomers), 4.99 (d, $J_{2a,2b}$ = 11.2 Hz, 1H, H2b, Bn, both diastereomers), 7.11 (s, H4, Im, 8*S* diastereomer) and 7.12 (s, H4, Im, 8*R* diastereomer), 7.13 (s, 1H, H4, Im, 8*S* diastereomer) and 7.14 (s, 1H, H4, Im, 8*R* diastereomers) and 7.27–7.38 (m, 30H, each diastereomer, 3 × Ph). ^{19}F NMR ($CDCl_3$): δ −191.88 (m). ESI-MS ($C_{34}H_{38}FN_3O_8$): m/z 635 $(M + 1)^+$.

Synthesis of 1-α/β-D-(1,2,3,4-Tetra-*O*-acetyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*S*-fluoropropyl)glucopyranose and 1-α/β-D-(1,2,3,4-Tetra-*O*-acetyl)-6-*O*-(9-[2-nitro-1*H*-imidazolyl]-8*R*-fluoropropyl)glucopyranose (10; fluoroglucoazomycin acetate). Trimethylsilyl triflate (25.5 g, 114.6 mmol) was added dropwise to a stirred cooled (ca. −40 °C) solution of **9** (6.62 g, 10.4 mmol) in acetic anhydride (117 mL). The reaction solution was allowed to warm up to 22 °C and stirred for additional 14 h. After cooling to −10 °C, the dark reaction solution was diluted with cold EtOAc (400 mL) and poured in a cold (0 °C) saturated aqueous $NaHCO_3$ (500 mL). The resultant mixture was stirred for 0.5 h at room temperature. The organic phase was separated off and washed with saturated aqueous $NaHCO_3$ (10 × 200 mL) and brine (200 mL) and dried over $MgSO_4$. Filtration through a pad of silica gel (for TLC) and removal of the solvent from the filtrate provided 8.9 g of dark oil, which was subjected to dry chromatography (30% EtOAc–hexanes → 80% EtOAc–hexanes) to afford 3.69 g of **7** as a yellow solid. Another purification of this material by dry chromatography (5% MeCN–toluene → 30% MeCN–toluene) provided diastereomeric (*R* and *S*) mixture of pure α/β-isomers of **10**. α/β ratio 91:9 by ^{1}H NMR analyses. Yield: 2.92 g (54%). HPLC: 95.9% (combined chemical purity for all diastereomers). MS (ES^+): m/z 520 $(M + 1)^+$. Anal. ($C_{20}H_{26}FN_3O_{12}$) CHN.

***α*-10** *(8R and 8S).* Retention times for two diastereomers 11.08 (74.5%) and 12.57 (12%) min. ^{1}H NMR ($CDCl_3$): δ 2.02, 2.06, 2.06, and 2.19 (4 s, each for 3H of 4 CH_3s, 8*S* diastereomer) and 2.03, 2.04, 2.08 and 2.20 (4 s, each for 3H of 4 CH_3s, 8*R* diastereomer), 3.55 (dd, $J_{6',6}$ = 11.6 Hz, $J_{5,6}$ = 4.4 Hz, 1H, H6, 8*S* diastereomer), 3.64 (dd, $J_{6',6}$= 11.6 Hz, $J_{5,6}$ = 3.6 Hz, 1H, H6, 8*R* diastereomer), 3.66 (dd, $J_{5,6}$ = 2.0 Hz, $J_{6,6'}$ = 8.8 Hz, 1H, H6′, 8*S* diastereomer), 3.72 (dd, $J_{5,6'}$ = 3.2 Hz, $J_{6',6}$ = 8.8 Hz, 1H, H6′, 8*R* diastereomer), 3.80 (ddd, $J_{7,7'}$ = 11.2 Hz, $J_{8,7}$ = 3.6 Hz, $J_{F,H}$ = 30.4 Hz, 1H, H7, both diastereomers), 3.86 (ddd, $J_{7',7}$ = 11.2 Hz, $J_{8,7'}$ = 2.8 Hz, $J_{F,H}$ = 31.2 Hz, 1H, H7′, both diastereomers), 4.04 (complex ddd, $J_{4,5}$ = 10.4 Hz, H5, both diastereomers), 4.63 (ddd, $J_{8,9}$ = 5.6 Hz, $J_{9',9}$ = 14.4 Hz, $J_{F,9}$ = 16.2 Hz, 1H, H9, 8*S* diastereomer), 4.67 (ddd, $J_{8,9}$ = 8.0 Hz, $J_{9',9}$ = 14.4 Hz, $J_{F,9}$ = 16.2 Hz, 1H, H9, 8*R* diastereomer), 4.81 (ddd, $J_{8,9'}$ = 7.6 Hz, $J_{9,9'}$ = 14.4 Hz, $J_{F,9'}$ = 16.2 Hz, 1H, H9′,8*S* diastereomer), 4.88 (ddd, $J_{8,9'}$ = 4.4 Hz, $J_{9',9}$ = 14.4 Hz, $J_{F\text{-}9'}$ = 16.2 Hz, 1H, H9′, 8*R* diastereomers), 4.92 (dm, $J_{F,9}$ = 47.0 Hz, H8, both diastereomers), 5.09 (dd, $J_{1,2}$ = 3.6 Hz, $J_{3,2}$ = 10.0 Hz, 1H, H2, 8*S* diastereomer) and 5.11 (dd, $J_{1,2}$ = 3.2 Hz, $J_{3,2}$ = 10.0 Hz, 1H, H2, 8*R* diastereomer), 5.19 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, 1H, H3, 8*S* diastereomer), 5.26 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 9.6 Hz, 1H, H3, 8*R* diastereomer), 5.49 (dd, $J_{3,4}$ = $J_{5,4}$ = 10.0 Hz, 1H, H4, 8*S* diastereomer), 5.50 (dd, $J_{3,4}$ = $J_{5,4}$ = 10.0 Hz, 1H, H4, 8*R* diastereomer), 6.32 (two d merged, $J_{2,1}$ = 3.2 Hz, 1H, H1, 8*S* diastereomer), 6.34 (d, $J_{2,1}$ = 4.0 Hz, 1H, H1, 8*R* diastereomer), 7.17 (s, H4 Im, 8*S* diastereomer) and 7.18 (s, H4 Im, 8*R* diastereomer), 7.24 (s, H5 of Im, 8*S* diastereomer) and 7.35 (s, H5 of Im, 8*R* diastereomer). ^{13}C NMR ($CDCl_3$): δ 20.69, 20.91 and 21.11 ($COCH_3$s), 50.67 and 50.73 ($J_{F,7}$ = 23 Hz, C7 for two diastereomers), 68.17 and 68.36 (C6 for two diastereomers), 69.40 and 69.44 (C4), 69.95 and 70.09 (C5), 70.21 and 70.26 (C2, dual resonances), 70.46 and 70.61 (C9, two d, $J_{F,9}$ = 20 Hz, dual resonances), 71.17 and 71.24 (C3, dual resonances), 89.29 and 89.35 (C1, dual resonances), 90.31 and 90.88 (C8, two d, $J_{F,8}$ = 177 Hz), 127.60 and 127.89 (C4 Im, two resonances), 128.68 and 128.72 (C5 Im, dual resonances), 145.00 (C2 Im), 169.08–170.47 ($COCH_3$). ^{19}F NMR ($CDCl_3$): δ 192.33 (dtt, $J_{F,7}$ = 31.2 Hz, $J_{F,9}$ = 16.2 Hz, $J_{F,8}$ = 47.5 Hz) for 8*R* diastereomer, and 194.06 (dtt, $J_{F,7}$ = 31.2 Hz, $J_{F,9}$ = 16.2 Hz, $J_{F,8}$ = 47.0 Hz) for 8*S* diastereomer.

***β*-10** *(8R and 8S).* Retention times for two diastereomers 9.19 (2.0%) and 9.34 (9.5%) min. ^{1}H NMR ($CDCl_3$): NMR signals for both *R* and *S* diastereomers are observed in the spectrum, but being very low in the composition most of the signals were of very low intensity and buried in the baseline. Clearly identified signals are indicated; δ 4.66 (H8), 4.84 (H9), 4.87 (H9′), 5.70 (d, $J_{2,1}$ = 8.4 Hz, 1H, H1). ^{13}C NMR ($CDCl_3$): δ 20.79 and 21.03 ($COCH_3$s), 68.02 (C7), 73.05 and 73.22 (C3), 74.05 and 74.42 (C5), 92.02 and 92.05 (C1), 128.42 (C4 Im), 129.5 (C5 Im). Other carbon signals appeared to be buried in the baseline due to low amounts of this isomer. ^{19}F NMR ($CDCl_3$): δ 192.33 ((dtt, $J_{F,7}$ = 31.2 Hz, $J_{F,9}$ = 16.2 Hz, $J_{F,8}$ = 47.5 Hz) for 8*R* diastereomer, and −194.06 (dtt, $J_{F,7}$ = 31.2 Hz, $J_{F,9}$ = 16.2 Hz, $J_{F,8}$ = 47.0 Hz) for 8*S* diastereomer.

Synthesis of 1-α/β-D-(6-*O*-(9-[2-Nitro-1*H*-imidazolyl]-8*S*-fluoropropyl)glucopyranose and 1-α/β-D-(6-*O*-(9-[2-Nitro-1*H*-imidazolyl]-8*R*-fluoropropyl)glucopyranose (11). Compound **10** (2.66 g, 5.12 mmol) was dissolved in 0.1 M NaOH in methanol (256 mL). After 20 min of stirring at room temperature, the reaction solution was treated with Dowex 50W × 4–200 (H^+, 20 g) to adjust the pH of the solution to a value of 5. The resin was filtered off, and the filtrate was evaporated under reduced pressure to dryness to provide 1.43 g of a yellowish solid, which was subjected to dry chromatography (2% MeOH–$CHCl_3$ → 25% MeOH–$CHCl_3$) to afford pure isomeric mixture of **11** as a pale solid (α/β ratio, 10:9 by HPLC and ^{1}H NMR). Yield: 1.26 g (70%). HPLC: 98.4% (combined chemical purity for all diastereomers). ESI-MS: m/z 352 $(M + 1)^+$. Anal. Calcd ($C_{12}H_{18}FN_3O_8{\cdot}0.5H_2O$): C, 41.03; H, 5.41; N, 11.96. Found: C, 41.03; H, 5.16; N, 11.96.

***α*-11** *(8R and 8S).* Retention times 6.37 and 6.46 min (isomeric composition by HPLC, 53.3% for α*R* and α*S* diastereomers; mobile phase A). ^{1}H NMR (CD_3OD): δ 3.31 (dd, $J_{5,4}$ = $J_{3,4}$ = 10.0 Hz, 1H, H4), 3.38 (dd, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 8.4 Hz, 1H, H2), 3.67 (dd, $J_{4,3}$ = $J_{2,3}$ = 8.4 Hz, 1H, H3), 3.73 (dd, $J_{6,5}$ = 2.8 Hz, $J_{6,6'}$ = 11.2 Hz, 1H, H6), 3.76 (dd, $J_{6,5}$ = 2.8 Hz, $J_{6,6'}$ = 11.2 Hz, 1H, H6′), 3.78 (ddd, $J_{7',7}$ = 10.0 Hz, $J_{8,7}$ = 8.0 Hz, $J_{F,H}$ = 29.7 Hz, 1H, H7), 3.81 (ddd, $J_{7,7'}$ = 10.0 Hz, $J_{8,7'}$ = 8.0 Hz, $J_{F,H}$ = 29.7 Hz, 1H, H7′), 3.89 (ddd, $J_{6,5}$ = 2.8 Hz, $J_{4,5}$ = 10.0 Hz, 1H, H5), 4.74 (ddd, $J_{8,9}$ = 7.6 Hz, $J_{9,9'}$ = 11.2 Hz, $J_{F,9}$ = 16.0 Hz, 1H, H9, both diastereomers), 4.86 (ddd, $J_{9',9}$ = 11.2 Hz, $J_{8,9'}$ = 3.6 Hz, $J_{F\text{-}9'}$ = 16.0 Hz, 1H, H9′, both diastereomers), 5.05 (dddd, $J_{8,7}$ = $J_{8,7'}$ = 8.0 Hz, $J_{8,9}$ = 7.6 Hz, $J_{8,9'}$ = 3.6 Hz, $J_{F\text{-}8}$ = 48.0 Hz, H8, both diastereomers), 5.10 (d, $J_{2,1}$ = 3.6 Hz, H1, both diastereomers), 7.16 (s, H4 Im, both diastereomers), 7.52 (s, H5 Im, both diastereomers). ^{13}C NMR (CD_3OD): δ 54.09 (C7, d, $J_{F\text{-}C}$ = 23.5 Hz), 74.15 (C9, d, $J_{F\text{-}C}$ = 21.4 Hz), 74.46 and 74.53 (C6, two diastereomers), 74.97 (C4), 76.55 (C5), 77.64 (C2), 79.00 (C3), 94.61 (C8, d, $J_{F,C}$ = 176.1 Hz), 96.76 (C1), 131.25 (C4 Im), 131.93 (C5 Im), 149.06 (C2, Im). ^{19}F NMR (CD_3OD): δ −194.26 (dtt, $J_{F,8}$ = 47.0 Hz, $J_{7,F}$ = 29.7 Hz, $J_{F,9}$ = 16.0 Hz).

***β*-11** *(8R and 8S).* Retention time 5.33 min (for both β*R* and β*S* diastereomers; mobile phase A). ^{1}H NMR (CD_3OD): δ 3.14 (dd, $J_{1,2}$ = 6.0 Hz, $J_{3,2}$ = 8.4 Hz, 1H, H2), 3.33–3.37 (m, 2H, H3 and H4), 3.36 (ddd, $J_{6,5}$ = 4.0 Hz, $J_{6',5}$ = 1.2 Hz, $J_{4,5}$ = 9.2 Hz, 1H, H5), 3.68 (dd, $J_{6,5}$ = 4.0. Hz, $J_{6',6}$ = 9.2 Hz, 1H, H6), 3.71 (dd, $J_{5,6'}$ = 2.4 Hz, $J_{6',6}$ = 9.2 Hz, 1H, H6′), 3.78 (ddd, merged, $J_{7',7}$ = 9.6 Hz, $J_{8,7}$ = 8.0 Hz, $J_{7,F}$ = 16.0 Hz, 1H, H7), 3.87 (ddd, $J_{7',7}$ = 9.6 Hz, $J_{8,7'}$ = 8.0 Hz, $J_{7',F}$ = 27.5 Hz, 1H, H7′), 4.47 (d, $J_{2,1}$ = 6.0 Hz, 1H, H1), 4.75 (ddd, $J_{8,9}$ = 7.6 Hz, $J_{9,9'}$ = 11.2 Hz, $J_{F,9}$ = 22.8 Hz, 1H, H9, both diastereomers), 4.83 (ddd, $J_{9',9}$ = 11.2 Hz, $J_{8,9'}$ = 3.6 Hz, $J_{F\text{-}9'}$ = 16.0 Hz, 1H, H9′, both diastereomers), 4.90 (dddd, $J_{8,7}$ = $J_{8,7'}$ = 8.0 Hz, $J_{8,9}$ = 7.6 Hz, $J_{8,9'}$ = 3.6 Hz, $J_{F\text{-}8}$ = 48.0 Hz, 1H, H8 for both diastereomers), 7.15 (d, $J_{5,4}$ = 1.2 Hz, 1H, H4 Im), 7.50 (d, $J_{4,5}$ = 1.2 Hz, 1H, H5, Im). ^{13}C NMR (CD_3OD): δ 54.18 (C7, d, $J_{F\text{-}C}$ = 22.7 Hz), 74.27 (C9, d, $J_{F\text{-}C}$ = 23.5 Hz), 74.53 and 74.89 (C6, two diastereomers), 75.13 (C4), 79.63 (C2), 79.69 (C3), 80.85 (C5), 94.57 (C8, d, $J_{F,C}$ = 176.9 Hz), 100.99 (C1), 131.25 (C4 Im), 132.04 (C5 Im), 149.06 (C2, Im). ^{19}F NMR (CD_3OD): δ −194.26

dx.doi.org/10.1021/jm2017336 | J. Med. Chem. 2012, 55, 6033–6046

(dtt, for both diastereomers, $J_{7,F}$ = 27.5 Hz, $J_{F,8}$ = 47.0 Hz, $J_{F,9}$ = 16.0 Hz).

Synthesis of 1-α-D-(1,2,3,4-Tetra-*O*-acetyl)-6-(((8,9*E*)-9-(2-nitro-1*H*-imidazol-1-yl)allyloxy)methyl)glucopyranose and 1-β-D-(1,2,3,4-Tetra-*O*-acetyl)-6-(((8,9*E*)-9-(2-nitro-1*H*-imidazol-1-yl)allyloxy)methyl)glucopyranose (12; allylglucoazomyin acetate). DBU (3.14 g, 20.6 mmol) was added dropwise to a stirred solution of nosylate **5** (7.25 g 10.3 mmol) in anhydrous acetonitrile (50 mL) at 22 °C. The dark reaction solution was stirred for 22.5 h and then concentrated under reduced pressure. The dark oily residue was treated with EtOAc (200 mL) and water (100 mL). The organic phase was separated off and washed with water (2 × 100 mL), 1N HCl (50 mL), saturated aqueous $NaHCO_3$ (100 mL), and brine (100 mL). After drying over $MgSO_4$, the resultant solution was filtered through a pad of silica gel (for TLC) and the solvent was removed under reduced pressure to provide 3.8 g of a yellowish solid, which was subjected to dry chromatography (5% MeCN−toluene → 30% MeCN−toluene) to afford a mixture of α- and β-isomers (α/β isomeric ratio 13:1 by ^{1}H NMR spectral analysis) of **12** (87%) as yellow solid. Yield: 1.425 g (28%).

***α*-12**. ^{1}H NMR ($CDCl_3$): δ 2.02, 2.03, 2.04 and 2.18 (s, 12H, 4 × CH_3), 3.57 (dd, $J_{6,5}$ = 4.4 Hz, J_{gem} = 10.8 Hz, 1H, H6), 3.66 (dd, $J_{6',5}$ = 2.4 Hz, J_{gem} = 10.8 Hz, 1H, H6′), 4.07 (ddd, $J_{6,5}$ = 4.4 Hz, $J_{6',5}$ = 2.2 Hz, $J_{4,5}$ = 10 Hz, 1H, H5), 4.16 (dd, $J_{7',7}$ = 13.2 Hz, $J_{8,7}$ = 6.0 Hz, 1H, H7), 4.23 (dd, $J_{7,7'}$ = 13.2 Hz, $J_{8,7'}$ = 6.0 Hz, 1H, H7′), 5.09 (dd, $J_{1,2}$ = 3.2 Hz, $J_{3,2}$ = 10.0 Hz, 1H, H2), 5.22 (dd, $J_{2,3}$ = 10 Hz, $J_{4,3}$ = 9.2 Hz, 1H, H4), 5.49 (dd, $J_{3,4}$ = 9.2 Hz, $J_{5,4}$ = 10.0 Hz, 1H, H3), 6.02 (dt, $J_{7,8}$ = $J_{7',8}$ = 6.0 Hz, $J_{9,8}$ = 14.0 Hz, 1H, H8), 6.33 (d, $J_{2,1}$ = 3.2 Hz, 1H, H1), 7.19 (s, 1H, H4, Im), 7.33 (s, 1H, H5, Im), 7.58 (d, J_{vic} = 14.0 Hz, 1H, H9).

***β*-12**. ^{1}H NMR ($CDCl_3$): Only the specific proton signals visible in the spectrum of the isomeric mixture are being described, the signals for remaining carbons in the molecule were buried under the signals belonging to the ***α*-12**; δ 2.03, 2.11 and 2.36 (s, 12H, 4 × CH_3), H6 (buried along with the signal for α-isomer), 3.7 (dd, unresolved, H6′), 5.14 (dd, $J_{1,2}$ = 8.0 Hz, $J_{3,2}$ = 9.2 Hz, 1H, H2), 5.28 (dd, $J_{2,3}$ = $J_{4,3}$ = 9.2 Hz, 1H, H3), H4 (buried under the signal for α-isomer), 5.70 (d, $J_{2,1}$ = 8.0 Hz, 1H, H1), 6.04 (dt, $J_{9,8}$ = 14.0 Hz, $J_{7,8}$ = 6.0 Hz, 1H, H8), 7.17 (s, 1H, H4, Im), 7.24 (s, 1H, H5 Im), 7.56 (d, 1H, $J_{9,8}$ = 14.0 Hz, 1H, H9).

1-α-D-(6-(((8,9*E*)-9-(2-Nitro-1*H*-imidazol-1-yl)allyloxy)-methyl)glucopyranose and 1-β-D-(6-(((8,9*E*)-9-(2-Nitro-1*H*-imidazol-1-yl)allyloxy)methyl)glucopyranose (13). Compound **12** (1.358 g, 2.72 mmol) was dissolved in 0.1 M NaOH in methanol (136 mL). After 20 min of stirring at 22 °C, the reaction solution was treated with Dowex 50W × 4−200 (H^+, 5 g) to adjust the pH of the solution to 5. The resin was filtered off, and the filtrate was evaporated under reduced pressure to dryness to provide 0.716 g of a yellowish solid that was subjected to column chromatography (2% MeOH−$CHCl_3$ → 30% MeOH−$CHCl_3$) to afford pure isomeric mixture of **13** as a pale solid (α and β, isomeric ratio 71.4:22.6 determined by ^{1}H NMR). HPLC purity: 95.4% (combined chemical purity for all diastereomers). Yield: 0.55 g (61%); mp (anomeric mixture) 156−158 °C; λ_{max} 324 nm. ESI-MS: *m*/*z* 331.00 $(M + 1)^+$. Anal. Calcd ($C_{12}H_{17}N_3O_8 \cdot 4/5H_2O$): C 41.69, H 5.42, N 12.16. Found: C 41.71, H 5.12, N 12.02.

***α*-13**. Retention time, 8.33 min (mobile phase A). Isomeric composition 71.4% by HPLC. ^{1}H NMR (CD_3OD): δ 3.32 (dd, $J_{5,4}$ = $J_{3,4}$ = 10.0 Hz, 1H, H4), 3.34 (dd, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$ = 3.7 Hz, 1H, H2), 3.67 (dd, $J_{4,3}$ = $J_{2,3}$ = 9.8 Hz, 1H, H3), 3.77 (dd, $J_{4,5}$ = 10.0 Hz, $J_{6,5}$ = 5.5 Hz, 1H, H5), 3.73 (dd, $J_{6,5}$ = 2.4 Hz, $J_{6',6}$ = 10.9 Hz, 1H, H6), 3.76 (dd, $J_{6',5}$ = 2.7 Hz, $J_{6,6'}$ = 10.9 Hz, 1H, H6′), 4.26 (m, 2H, H7 and H7′), 5.10 (d, $J_{2,1}$ = 3.7 Hz, 1H, H1), 6.28 (dd, $J_{7,8}$ = 3.6 Hz, $J_{9,8}$ = 14.0 Hz, 1H, H8), 7.18 (s, 1H, H4, Im), 7.62 (d, $J_{8,9}$ = 14.0 Hz 1H, H9), 7.71 (s, 1H, H5, Im). ^{13}C NMR (CD_3OD): δ 68.31 (C6), 69.96 (C7), 70.47 (C4), 70.88 (C5), 72.64 (C2), 73.69 (C3), 92.84 (C1), 123.69 (C8), 124.06 (C9), 125.68 (C4, Im), 127.98 (C5, Im) and 143.97 (C2, Im).

***β*-13**. Retention time, 7.94 min (mobile phase A). Isomeric composition 22.6% by HPLC. ^{1}H NMR (CD_3OD): δ 3.14 (dd, $J_{1,2}$ = 8.8 Hz. $J_{3,2}$ = 10 Hz. 1H, H2), 3.30 (m, 1H, H3), 3.32 (dd, $J_{5,4}$ = $J_{3,4}$ = 10.0 Hz, 1H, H4), 3.42 (ddd, $J_{6,5}$ = 5.5 Hz, $J_{6',5}$ = 2.9 Hz, $J_{4,5}$ = 10.0 Hz, 1H, H5), 3.84 (dd, $J_{6,5}$ = 5.5 Hz, $J_{6',6}$ = 10.9 Hz, 1H, H6), 3.92 (dd, $J_{6',5}$ = 2.9 Hz, $J_{6,6'}$ = 10.9 Hz, 1H, H6′), 4.26 (m, 2H, H7 and H7′), 4.46 (d, $J_{2,1}$ = 8.8 Hz, 1H, H1), 6.24 (dd, $J_{7,8}$ = 5.6 Hz, $J_{9,8}$ = 14.0 Hz. 1H, H8), 7.17 (s, 1H, H4, Im), 7.58 (d, $J_{8,9}$ = 14.0 Hz, 1H, H9), 7.72 (s, 1H, H5, Im). ^{13}C NMR (CD_3OD): δ 68.39 (C6), 69.96 (C7), 70.68 (C4), 75.05 (C2), 75.82 (C3), 76.93 (C5), 97.07 (C1), 123.78 (C8), 124.13 (C9), 125.70 (C4, Im), 127.98 (C5, Im).

Lipophilicity. Estimated lipophilicity for the compounds, expressed as ClogP, was calculated using ChemDraw (CambridgeSoft/Perkin-Elmer).

Biological Evaluations. Human mammary MOO6X, cervical HeLa, and murine mammary EMT6, KBALB, and KBALB-STK cancer cells were used for the biological studies. DMEM/F12 medium and Garth medium (depending on the study) were used for cell work. Test compounds were dissolved in sterile water and were serially diluted to generate appropriate test concentrations. High purity gases were used for radiosensitization studies.

Cytotoxicity. The MTT-assay was used to estimate the toxicity of **8**, **11**, and **13** to human HeLa, MOO6X, K-BALB, and K-BALB-STK cancer cells and murine EMT-6 cell lines in cell culture.[21] Exponentially growing cells were trypsinized, centrifuged, and suspended in growth medium and the cell counts were readjusted to 8×10^3 cells/mL. The cells were seeded into 96-well plates at a count of 8×10^2 cells/well and incubated in 37 °C, 5% CO_2 for 24 h. The test compounds (stock solution 2 mM in 95% ethanol) were added to the growth medium and exposed to the cells in 96-well plates at a final volume of 300 μL to produce the required dilution of the experimental design. Control wells were filled with 100 μL of medium. These plate groups were incubated for 3 days at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2. At the end of incubation, 50 μL solution of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H* tetrazolium bromide (MTT), predissolved in PBS (5 mg/mL), filtered through a 0.45 μm membrane, and diluted 1:5 in prewarmed medium, was added to each well. The plates were incubated at 37 °C for 4 h. The medium was then removed from the wells and 150 μL of dimethylsulfoxide was added to each well. The plates were placed on a shaker for 15 min to dissolve the formazan crystal and then read immediately at 540 nm on a scanning multiwall spectrophotometer. Data are summarized in Table 2. Data were plotted on Microsoft Excel.

Radiosensitization. Cell radiosensitization was determined using a ^{60}Co X-ray source together with a clonogenic survival assay[22] using HeLa, EMT-6, and MOO6X cancer cell lines. Cells (300000 in 3 mL of DMEM/F12 medium per T60 Petri dish) were individually incubated under 5% CO_2 in air at 37 °C for 24 h. The dishes were assigned to either the control (normoxic) or hypoxic groups, the test substance (stock solution 10 mM in 95% ethanol) was added to these groups to achieve a concentration of 100 μM, and the incubation was allowed for 30 min. Those in the hypoxic group were degassed to hypoxia by six consecutive vacuum and nitrogen fill cycles in a vacuum chamber. The Petri dishes (hypoxic and normoxic controls) were incubated for 30 min on an oscillating shaker at $R/T \times 60$ cycles per min and then irradiated in a ^{60}Co γ-irradiator at 0 (control), 4, 8, 12, 16, and 20 Gy in N_2 (hypoxic subgroup) and air chambers (normoxic subgroup). The cells were then recovered from each dish by two consecutive washes with PBS followed by the addition of trypsin (500 μL) and quenching with fresh medium (4.5 mL). Cells were then plated at densities from 100 to 15000 cells/5 mL of medium for normoxic conditions and 100 and 5000 cells/5 mL of medium for hypoxic conditions. The cells were incubated for 10−14 days at 37 °C under 5% CO_2 and then stained with methylene blue or crystal violet in ethanol, clones counted, and surviving fractions calculated. Tests were done in triplicate. SERs, presented in Table 3, were derived from cell survival plots on Microsoft Excel.

Transmembrane Transport Studies. *Xenopus* oocytes expressing recombinant GLUT-1 or GLUT-2 were prepared as reported elsewhere.[41] Oocytes were injected with either water (20 nL), containing RNA transcript (1 ng/nL) encoding the required GLUT, or water (10 nL) alone (control). Injected oocytes were then incubated (4 days; 18 °C) in modified Barth's medium (MBM)

dx.doi.org/10.1021/jm2017336 | *J. Med. Chem.* 2012, 55, 6033−6046

(changed daily) (88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 10 mM Hepes, 2.5 mM sodium pyruvate, 0.1 mg/mL penicillin, and 0.05 mg/mL gentamycin sulfate; pH 7.5) prior to the assay. Radioisotope influx and electrophysiology experiments were performed in Na^+-containing transport medium (100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MGAZl_2$, 10 mM Hepes; pH 7.5). For radioisotope influx studies, uptake in *Xenopus* oocytes was traced using radiolabeled permeant ([^{14}C]glucose; 1 μCi/mL), each assay using an incubation period of 20 min at 20 °C on groups of 10−12 oocytes in transport medium. At the end of the incubation period, extracellular label was removed by six rapid washes in ice-cold transport medium, and individual oocytes were dissolved in 1% (w/v) SDS for quantifying oocyte-associated radioactivity by liquid scintillation counting (LS 6000 IC, Beckman, Fullerton, CA, USA). Data plots, showing the inhibition of [^{14}C]glucose uptake as a function of inhibitor concentration for GLUT-1, are shown (Figure 4). The median effective concentration (EC_{50}) for F-GAZ, defined as the concentration of inhibitor that can be expected to cause a 50% reduction in [^{14}C]glucose uptake in oocytes expressing GLUT-2, was estimated using the four-parameter logistic analysis and plotted on a log[conc] axis (Figure 5).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Data pertaining to the elemental analysis, ^{1}H-, ^{13}C-, ^{19}F-NMR, and mass spectra of the final and other relevant compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by grants from the Canadian Institutes of Health Research (CIHR POP program), CIHR-ACF Partner Bridge grant, the Canadian Association of Radiation Oncologists (CARO), and the Alberta Cancer Foundation (ACF Pilot). Additional equipment and resource support by Naeja Inc., Edmonton, to facilitate chiral chromatography experiments are sincerely appreciated.

■ ABBREVIATIONS USED

A-GAZ, allylglucoazomycin; DAST, diethylaminosulfurtrifluoride; DBU, 1,8-diazabicyclo(5,4,9)-undec-7-ene; d, doublet; dd, doublet of doublet; DMAP, dimethylaminopyridine; DMF, dimethylformamide; dt, doublet of triplet; EtOH, ethanol; EtOAc, ethyl acetate; E^1_7, single electron reduction potential; F-GAZ, fluoroglucoazomycin; FAZA, 1-α-D-5-fluoro-5-deoxyarabinofuranose-2-nitroimidazole; FMISO, fluoromisonidazole; GAZ, glucoazomycin; GLUTs, glucose transport proteins; HPLC, high performance liquid chromatography; IAZA, 1-α-D-5-iodo-5-deoxyarabinofuranose-2-nitroimidazole; iso-PrOH, isopropyl alcohol; m, multiplet; MeCN, acetonitrile; MBM, modified Barth's medium; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide; NaOH, sodium hydroxide; $NaHCO_3$, sodium bicarbonate; Na_2CO_3, sodium carbonate; NOE, nuclear Overhauser effect; OER, oxygen enhancement ratio; PBS, phosphate buffer saline; s, singlet; SER, sensitization enhancement ratio; TMS-triflate, trimethylsilyltrifluoromethanesulfonate; t, triplet; tlc, thin layer chromatography

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