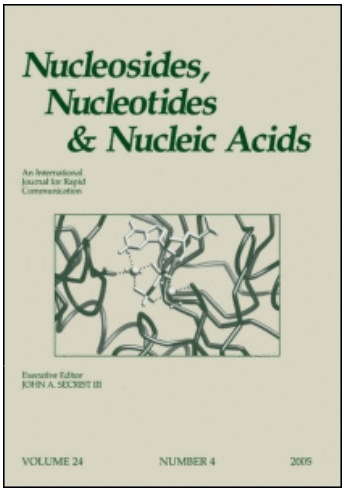

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SYNTHESES OF 1-[(2-HYDROXYETHOXY)METHYL]- AND 1-[(1,3-DIHYDROXY-2-PROPOXY)METHYL]- DERIVATIVES of 5-SUBSTITUTED-2,4-DIFLUOROBENZENE: UNNATURAL ACYCLO THYMIDINE MIMICS FOR EVALUATION AS ANTICANCER AND ANTIVIRAL AGENTS

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ABSTRACT: A group of 1-[(2-hydroxyethoxy)methyl]- (**12**) and 1-[(1,3-dihydroxy-2-propoxy)methyl]- (**13**) derivatives of 2,4-difluorobenzene possessing a variety of C-5 substituents (R = Me, H, I, NO_2) were designed with the expectation that they may serve as acyclic 5-substituted-2'-deoxyuridine (thymidine) mimics. Compounds **12** and **13** (R = Me, H, I) were inactive as anticancer agents ($CC_{50} = 10^{-3}$ to 10^{-4} M range), whereas the 5-nitro compounds (**12d**, **13d**) exhibited weak-to-moderate cytotoxicity ($CC_{50} = 10^{-5}$ to 10^{-6} M range) against a variety of cancer cell lines. All compounds prepared (**12a-d, 13a-d**) were inactive as antiviral agents in a broad-spectrum antiviral screen that also included the human immunodeficiency virus (HIV-1 and HIV-2) and herpes simplex virus (HSV-1 and HSV-2).

INTRODUCTION

Acyclic nucleosides have attracted the interest of both medicinal chemists and virologists since the potent antiherpetic agent acyclovir (ACV, **1**) was discovered.[1] The search for a new drug having superior activity to ACV subsequently resulted in the design of ganciclovir (GCV, **2**),[2] and then penciclovir (PCV, **3**),[3] which exhibit a broad spectrum of antiviral activity. These latter studies indicate that one method to improve

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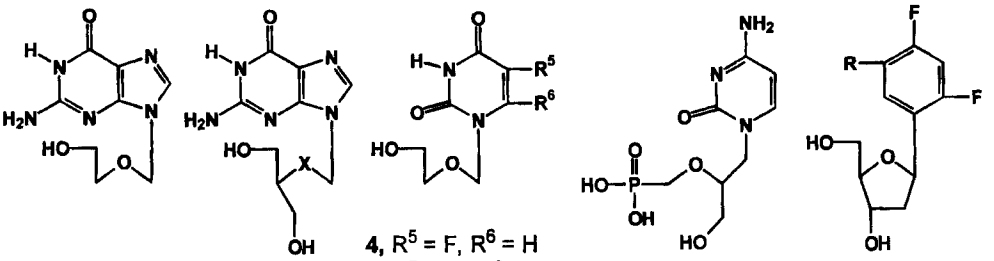
antiviral activity is to design a compound with two hydroxyl groups that mimic the 3'- and 5'-OH group of the 2'-deoxyribose ring of nucleosides which possess all (GCV), or part of (PCV), the chemical functionality of deoxyribonucleosides except for the rigid furanose ring system. It has been shown that the acyclo derivative of 5-fluorouracil (**4**) increased the survival of P388 leukemic mice by 75%,[4] and that the 5-ethyl-6-thiophenyl acyclic pyrimidine nucleoside (**5**) is a potent inhibitor of human immumodeficiency virus type 1 (HIV-1) reverse transcriptase (RT).[5] However, the 5-substituted acyclic nucleosides (**6**) exhibited little or no activity against herpes simplex virus type 1 (HSV-1), or against a range of other DNA or RNA viruses, presumably due to their inability to undergo phosphorylation to the monophosphate by HSV-1-induced thymidine kinase.[6] In contrast, cidofovir (HPMPC, **7**), which does not require activation to the monophosphate by the virus-induced kinase, is particularly effective against cytomegalovirus (CMV) infections.[7]

Recently, Kool *et al.* designed nonpolar hydrophobic isosteres of pyrimidine nucleosides which retain the closest possible structural, steric and isoelectronic relationship to the natural base that are not likely to form hydrogen bonds.[8] In this respect, the 2,4-difluoro-5-methylphenyl isostere (**8**) was designed as an unnatural mimic of thymidine. Although the 2,4-difluoro-5-methylphenyl moiety is not a nucleobase, this aromatic group is referred to as a "base" in analogy to the natural thymine base. The 5'-triphosphate of **8** (**8**-TP) was inserted into replicating DNA strands by the Klenow fragment (KF, exo mutant) of *E. coli* DNA polymerase I.[9] Steady-state measurements indicated that **8**-TP was inserted opposite adenine (A) with an efficacy (V_{max}/K_m) only 40-fold lower than the triphosphate of thymidine (dTTP). Furthermore, it was inserted opposite A (relative to C, G, or T) with a selectivity nearly as high as that for dTTP. Therefore, the 2,4-difluorotoluene moiety of **8** is a shape mimic handled by the KF polymerase almost as if it were thymine.[10-12] It was therefore anticipated that structurally related 5-substituted acyclic nucleoside mimic analogs of **8** may be cytotoxic to rapidly multiplying cancer cells (inhibit tumor growth) and/or act as antiviral agents.[13] They may also act as radiopharmaceuticals to image,[14] or chemotherapeutic agents to treat,[15] herpes simplex virus type-1 thymidine kinase positive (HSV-1 TK^+) gene-transfected tumors (gene therapy of cancer).[16] We now report the synthesis, antiviral and anticancer activities for a group of 1-[(2-hydoxyethoxy)methyl]- (**12a-d**) and 1-[(1,3-dihydroxy-2-



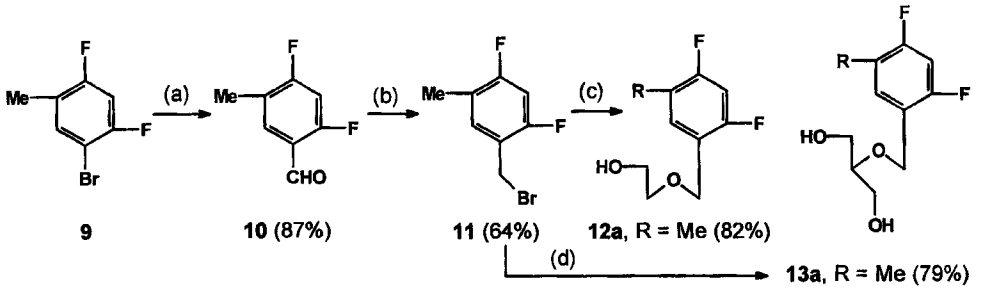
1 **2**, X = O **3**, X = CH_2 **4**, R^5 = F, R^6 = H **5**, R^5 = Et, R^6 = SPh **6**, R^5 = Me, CF_3, I R^6 = H **7** **8**, R = Me

propoxy)methyl]- (**13a-d**) derivatives of 5-substituted-2,4-difluorobenzene designed as unnatural acyclic 5-substituted-2'-deoxyuridine (thymidine) mimics.

CHEMISTRY

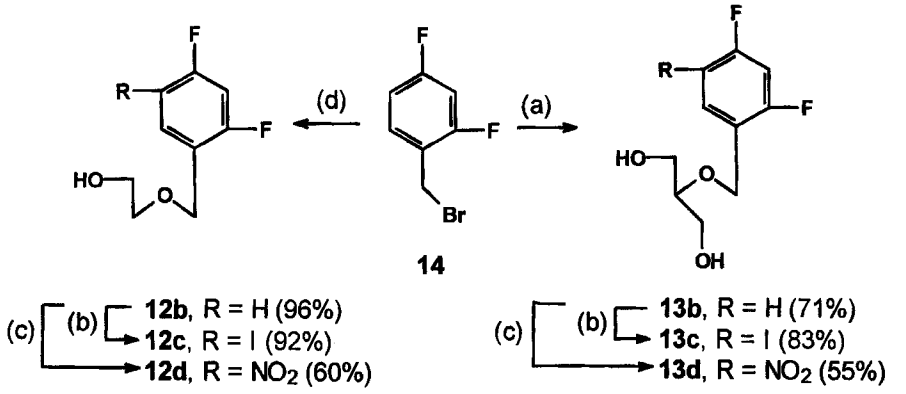
There are many methods applicable to the synthesis of 2,4-difluoro-1-[(2-hydroxyethoxy)methyl]benzenes (**12**) which are derivatives of 2-benzyloxyethanol. These include ring opening of ethylene oxide with phenylmethanol,[17] reduction of 2-phenyl-(1,3)dioxolane,[18] alkylation of phenylmethanol with 2-chloroethanol,[19] reduction of benzyloxyacetic acid,[20] and mono-alkylation of ethylene glycol with a benzyl halide in the presence of a strong base such as NaH, or KOH.[21] Among these methods, mono-benzylation of ethylene glycol using a substituted-benzyl halide is the method of choice since the starting materials are readily prepared. Formation of the bis-*O*-benzyl product can be prevented by using a large excess of ethylene glycol. The 2-*O*-benzylglycerol compounds **13** can be prepared by alkylation of a 1,3-protected glycerol followed by deprotection.[22]

Formylation of the Grignard reagent, prepared from 5-bromo-2,4-difluorotoluene (**9**), with N-formylpiperidine using Olah's procedure[23] afforded the aldehyde (**10**) in 87% yield. DIBAL-H reduction of the aldehyde **10**, and then bromination[24] with NBS/PPh_3 gave 5-methyl-2,4-difluorobenzyl bromide (**11**). Reaction of **11** with excess ethylene glycol in the presence of NaH yielded the (2-hydroxyethoxy)methyl product (**12a**). Alkylation of *cis*-1,3-*O*-benzylideneglycerol by 5-methyl-2,4-difluorobenzyl bromide (**11**) in the presence of NaH, followed by acidic hydrolysis in THF, afforded the (1,3-dihydroxy-2-propoxy)methyl derivative (**13a**) (see Scheme 1). Similar syntheses of the



SCHEME 1. Reagents: (a) Mg, THF, I_2 catalyst, reflux, 30 min; and then *N*-formylpiperidine, Et_2O, 30 min, 25°C; (b) diisobutylaluminum hydride (DIBAL-H), THF, -10°C, 1 h; and then *N*-bromosuccinimide (NBS), Ph_3P, CH_2Cl_2, 10 min at 0°C and then 30 min at 25°C; (c) NaH, excess $HOCH_2CH_2OH$, reflux, 6 h; (d) NaH, *cis*-1,3-*O*-benzylideneglycerol, 0°C for 2 h and 25°C for 1 h; and then 0.5M H_2SO_4, THF, 25°C, 30 h.

2,4-difluorobenzene analogs **12b** and **13b**, starting from 2,4-difluorobenzyl bromide (**14**), were performed as illustrated in Scheme 2. Subsequent iodination of the 2,4-difluorophenyl compound **12b**, or **13b**, with *N*-iodosuccinimide in trifluoroacetic acid[25] afforded the 2,4-difluorophenyl-5-iodo derivative **12c**, or **13c**, respectively. The 2,4-difluorophenyl-5-nitro compounds **12d** and **13d**, were prepared via nitration of **12b**, or **13b**, with NH_4NO_3 in trifluoroacetic anhydride.[26]



SCHEME 2. Reagents: (a) NaH, *cis*-1,3-*O*-benzylideneglycerol, 0°C for 2 h, and 25°C for 1 h; and then 0.5M H_2SO_4, THF, 25°C, 30 h; (b) *N*-iodosuccinimide (NIS), CF_3CO_2H, 25°C, 12 h; (c) $(CF_3CO)_2O$, NH_4NO_3, 25°, 2 h; (d) NaH, excess $HOCH_2CH_2OH$, reflux, 6 h.

BIOLOGICAL EVALUATION

Replacement of the natural guanine (**1-2**), or uracil (**4-6**), base moiety in acyclo nucleosides by an unnatural aryl isostere attracted our attention since it could bestow new properties that may be useful in the design of a novel class of third generation C-aryl acyclo nucleoside mimics for use as anticancer and/or antiviral agents.

The 1-[(2-hydroxyethoxy)methyl]- (**12a-d**) and 1-[(1,3-dihydroxy-2-propoxy)methyl]- (**13a-d**) derivatives of 5-substituted-2,4-difluorobenzenes were evaluated using the MTT cytotoxicity assay (see Table 1).[27] The 5-substituted (Me, H, I) compounds (**12a-c**, **13a-c**) exhibited negligible cytotoxicity (CC_{50} = 10^{-3} to 10^{-4} M range), even when compared to thymidine (CC_{50} = 10^{-4} to 10^{-5} M range), against KBALB, KBALB-STK, 143B and 143B-LTK cancer cell lines. In contrast, the 5-nitro derivatives (**12d**, **13d**) were more cytotoxic (CC_{50} = 10^{-5} to 10^{-6} M range). These 5-substituted compounds **12-13** exhibited similar cytotoxicity against non-transfected (KBALB, 143B), and the corresponding transfected (KBALB-STK, 143B-LTK) cancer cell lines expressing the herpes simplex virus type 1 (HSV-1) thymidine kinase gene (TK^+). The latter results show that expression of the viral TK enzyme, which would be expected to enhance phosphorylation of the terminal-OH to the monophosphate (MP), does not increase the cytotoxicity of compounds **12-13**. In contrast, acyclovir, and ganciclovir, were significantly more cytotoxic against the transfected, relative to the non-transfected, cancer cell lines.

The acyclo nucleoside mimics (**12-13**) were also evaluated for their antiviral activity in a wide variety of assay systems using previously described procedures.[28] Compounds **12a-d**, and **13a-d**, were inactive as antiviral agents at the highest non-toxic host cell concentration used (up to 400 μg/mL) against HSV-1 (KOS strain), HSV-2, vaccinia virus, vesicular stomatitis virus, and thymidine kinase-deficient (TK^-) HSV-1 (strains KOS ACV^r and VMW 1837) in E_6SM cell cultures. In addition, **12a-d**, and **13a-d**, did not reduce the cytopathogenicity induced by parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B4 virus, or Punta Toro virus, in Vero cell cultures at concentrations up to 80 μg/mL; or vesicular stomatitis virus, Coxsackie B4 virus, or respiratory syncytial virus in HeLa cell cultures at concentrations up to 400 μg/mL. Furthermore, **12a-d**, and **13a-d**, were also inactive against human immunodeficiency virus (HIV-1 and HIV-2) in human T-lymphocytes (CEM cells) at sub-toxic host cell concentrations up to 500 μg/ml,

TABLE 1. *In vitro* cell cytotoxicity of 1-[(2-hydoxyethoxy)methyl]- **(12a-d)** and 1-[(1,3-dihydroxy-2-propoxy)methyl]- **(13a-d)** derivatives of 5-substituted-2,4-difluorobenzenes determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay.

	Cellular toxicity (CC_{50}, M) toward various cell lines[a]			
Compd (R)	KBALB[b]	KBALB-STK[c]	143B[d]	143B-LTK[c]
12a, R = Me	5.5×10^{-4}	7.4×10^{-4}	3.5×10^{-4}	3.0×10^{-4}
12b, R = H	2.5×10^{-3}	—	—	—
12c, R = I	3.5×10^{-4}	8.0×10^{-4}	5.5×10^{-4}	7.5×10^{-4}
12d, R = NO_2	8.5×10^{-6}	3.0×10^{-5}	9.0×10^{-6}	2.2×10^{-5}
13a, R = Me	8.0×10^{-4}	1.8×10^{-3}	4.0×10^{-4}	7.5×10^{-4}
13b, R = H	4.0×10^{-3}	—	—	—
13c, R = I	8.5×10^{-4}	1.3×10^{-3}	8.5×10^{-4}	1.0×10^{-3}
13d, R = NO_2	2.0×10^{-5}	5.0×10^{-5}	1.3×10^{-5}	2.5×10^{-5}
Acyclovir	7.5×10^{-5}	3.5×10^{-7}	2.5×10^{-4}	8.0×10^{-6}
Ganciclovir	4.8×10^{-4}	3.0×10^{-8}	1.0×10^{-4}	6.5×10^{-8}
IUdR[e]	9.7×10^{-5}	1.0×10^{-5}	7.0×10^{-3}	7.4×10^{-3}
FUdR[f]	6.0×10^{-11}	8.8×10^{-11}	9.0×10^{-5}	1.0×10^{-4}
Thymidine	9.5×10^{-5}	1.0×10^{-4}	—	—

[a]The molar concentration of the test compound that killed 50% of the cells (or 50% cell survival) upon incubation for 3-5 days at 37°C in a humidified atmosphere of 95% air and 5% CO_2 (Mean value, n = 6). [b]Transformed fibroblast sarcoma cell line. [c]These cells were transfected by, and express, the herpes simplex virus type 1 thymidine kinase (HSV-1 TK) gene. [d]Human osteosarcoma cell line. [e]IUdR = 5-iodo-2'-deoxyuridine. [f]FUdR = 5-fluoro-2'-deoxyuridine.

and **12a-d**, and **13a-d**, did not prevent Moloney murine sarcoma virus (MSV)-induced transformation of C3H/3T3 embryo murine fibroblasts at sub-toxic host cell concentrations (20-500 μg/mL range).

There are a number of plausible reasons why these acyclic nucleoside mimics (**12-13**) are ineffective as anticancer/antiviral agents. Accordingly, compounds **12-13** may not undergo phosphorylation by host cell or viral thymidine kinase (TK) to the monophosphate (MP). Support for this explanation stems from the observation that there

were negligible differences in anticancer activity between non-transfected (KBALB, 143B) and viral TK-transfected (KBALB-STK, 143B-LTK) cancer cells; or antiviral activity between HSV-1 (KOS), and either HSV-1 TK$^-$ KOS or VMW 1837 (TK-deficient) viral strains. This explanation does not unambiguously preclude the possibility that phosphorylation of **12-13** to the MP by non-transfected and transfected cells, and their subsequent conversion to the active triphosphate (TP), are equally effective. However, this latter explanation is unlikely since incorporation of the active TP of the 2,4-difluoro-1-[(2-hydroxyethoxy)methyl]benzene derivatives (**12a-d**) into deoxyribonucleic acid (DNA) by DNA polymerase(s) would be expected to result in a significant anticancer/antiviral effect due to termination of DNA chain elongation (DNA synthesis) since **12a-d** only possess one OH group.

In conclusion, these acyclo nucleosides mimics (**12-13**) are ineffective anticancer/antiviral agents, which is most likely due to their failure to undergo phosphorylation to the MP. If this is the reason, it is possible that *O-cyclo*Sal-pronucleotide derivatives of **12-13**, based upon the efficacy of this kinase-bypass concept,[29,30] could result in the transformation of these inactive unnatural acyclo nucleoside mimics to a class of anticancer/antiviral agents like cidofovir (**7**), which does not require activation to the MP by a viral, or host cell, kinase.[7]

EXPERIMENTAL SECTION

Melting points were determined with a Thomas Hoover capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR, ^{19}F NMR) were measured on a Bruker AM-300 spectrometer. The assignment of exchangeable protons (O*H*) was confirmed by the addition of D_2O. Proton chemical shifts (δ) are given relative to internal TMS (δ 0). ^{13}C NMR spectra were acquired using the *J* modulated spin echo technique where methyl and methine carbon resonances appear as positive peaks and methylene and quaternary carbons appear as negative peaks, and carbon chemical shifts (δ) are given relative to $CDCl_3$ (δ 77). Fluorine chemical shifts (δ) are given relative to external C_6F_6 (δ 0). Infrared spectra were recorded on a Nicolet Magna 550 IR spectrometer using air as reference. Elemental analyses were performed by the MicroAnalysis Service Laboratory, Department of Chemistry, University of Alberta. Silica gel 60 (E. Merck Co.) was employed for all silica gel column flash chromatography separations. All reagents were purchased from the Aldrich Chemical Co.

2,4-Difluoro-5-methylbenzaldehyde (10). 5-Bromo-2,4-difluorotoluene (**9**, 2.07 g, 10 mmol) was added drop wise to a mixture of magnesium (0.24 g, 10 mmol) and one crystal of iodine in dry THF (15 mL) under argon, and the mixture was refluxed for 30 min. After cooling the yellow solution to 0°C using an ice-bath, a solution of *N*-formylpiperidine (1.15 g, 10 mmol) in dry diethyl ether (5 mL) was added drop wise during a period of 15 min. The reaction mixture was stirred at 25°C for 30 min, prior to quenching with 1M HCl, until the solution was acidic. After extraction with diethyl ether (3 × 20 mL), the combined organic extracts were washed with water, saturated aqueous $NaHCO_3$, and brine prior to drying (Na_2SO_4). Removal of the solvent in vacuo gave a residue that was purified via flash chromatography (hexane-ether, 10:1 v/v) to afford the aldehyde (**10**, 1.36 g, 87%) as a pale yellow liquid; IR (NaCl): 2764, 1696 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 10.26 (s, 1H), 7.73 (t, J = 8.2 Hz, 1H), 6.86 (t, J = 9.8 Hz, 1H), 2.28 (d, J = 0.9 Hz, 3H).

5-Methyl-2,4-difluorobenzyl bromide (11). DIBAL-H (1.0 M in THF, 10 mL, 10 mmol) was added drop wise to a cooled -15 °C solution of the aldehyde (**10**, 1.0 g, 6.54 mmol) in dry THF (5 mL) under argon, and the resulting solution was stirred at –10°C for 1 h. The reaction was quenched with saturated aqueous NH_4Cl (2 mL), the mixture was filtered, and the solid was washed with ether. Removal of the solvent in vacuo gave a residue that was purified via flash chromatography (hexane-ether, 3:1 v/v) to give the alcohol (0.8 g, 80%) as a colorless oil; ^1H NMR ($CDCl_3$) δ: 7.18 (dd, J = 8.8, 7.9 Hz, 1H), 6.73 (dd, J = 9.8, 9.5 Hz, 1H), 4.62 (s, 2H), 2.5 (m, 1H, OH), 2.21 (d, J = 0.6 Hz, 3H); ^{19}F NMR ($CDCl_3$) δ: 47.42 (m), 41.52 (m). *N*-Bromosuccinimide (0.98 g, 5.5 mmol) was added in aliquots to a solution of the alcohol obtained above (0.79 g, 5 mmol) and triphenylphosphine (1.45 g, 5.5 mmol) in CH_2Cl_2 (15 mL) at 0°C. The resulting solution was stirred at 0°C for 10 min, and then at 25°C for 30 min. Removal of the solvent in vacuo gave a residue which was triturated with ether/hexane (10%, v/v), and the extracts were filtered through a silica gel pad. Removal of the solvent in vacuo afforded a residue that was purified via flash column chromatography (hexane) to afford the bromide **11** (0.88 g, 80%) as a colorless liquid; ^1H NMR ($CDCl_3$) δ: 7.20 (t, J = 8.4 Hz, 1H), 6.76 (dd, J = 9.8, 9.5 Hz, 1H), 4.45 (s, 2H), 2.23 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 161.01 (dd, J = 249.4, 12.1 Hz), 158.73 (dd, J = 250.5, 12.1 Hz), 132.94 (dd, J = 6.6, 4.4 Hz), 121.15 (dd, J = 17.6, 4.4 Hz), 120.60 (dd, J = 14.3, 3.3 Hz), 103.59 (t, J = 26.4 Hz), 25.0 (d, J = 3.3 Hz), 13.71 (d, J = 3.3 Hz); ^{19}F NMR ($CDCl_3$) δ: 50.14 (m), 44.95 (m).

2,4-Difluoro-5-[(2-hydroxyethoxy)methyl]toluene (12a). Ethylene glycol (0.2 mL, 6.5 mmol) was added to a slurry of NaH (60% in mineral oil, 44 mg, 1.1 mmol) in dry THF (0.5 mL), and the mixture was heated at reflux with stirring for 15 min. 5-Methyl-2,4-difluorobenzyl bromide (**11**, 0.22 g, 1 mmol) in dry THF (0.5 mL) was added drop wise during 1 h, and the reaction mixture was stirred at reflux temperature for another 6 h. After cooling to 25°C, the reaction was quenched with saturated aqueous NH_4Cl, extracted with diethyl ether (3 × 15 mL), and the combined organic extracts were washed with saturated brine prior to drying (Na_2SO_4). Filtration, and removal of the solvent from the filtrate, gave a residue that was purified via flash chromatography (hexane-ethyl acetate, 3:1 v/v) to give **12a** (0.166 g, 82%) as a colorless liquid; ^{1}H NMR ($CDCl_3$) δ: 7.19 (dd, J = 8.5, 8.2 Hz, 1H), 6.73 (dd, J = 9.8, 9.5 Hz, 1H), 4.52 (s, 2H), 3.73 (dd, J = 4.9, 4.3 Hz, 2H), 3.58 (dd, J = 4.9, 4.3 Hz, 2H), 2.60 (br s, 1H, OH), 2.20 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 160.50 (dd, J = 247.2, 12.1 Hz), 158.84 (dd, J = 247.2, 12.1 Hz), 132.13 (dd, J = 6.6, 5.5 Hz), 120.47 (dd, J = 5.5, 3.4 Hz), 120.25 (dd, J = 4.4, 3.3 Hz), 103.04 (t, J = 26.4 Hz), 71.56, 66.16 (d, J = 2.2 Hz), 61.60, 13.66 (d, J = 2.2 Hz). Anal. calcd. for $C_{10}H_{12}F_2O_2$: C, 59.40; H, 5.98. Found: C, 59.49; H, 6.17.

2,4-Difluoro-1-[(2-hydroxyethoxy)methyl]benzene (12b). This product was prepared, using a procedure similar to that for the preparation of **12a**, via reaction of 2,4-difluorobenzyl bromide (**14**, 2.07 g, 10 mmol) with ethylene glycol (3.1 g, 50 mmol). Purification of the product via flash chromatography (hexane-ethyl acetate, 2:1 v/v) gave **12b** (1.81 g, 96%) as a colorless liquid; ^{1}H NMR ($CDCl_3$) δ: 7.34 (td, J = 8.5, 6.7 Hz, 1H), 6.85-6.70 (m, 2H), 4.54 (s, 2H), 3.70 (t, J = 4.9 Hz, 2H), 3.54 (t, J = 4.9 Hz, 2H), 2.67 (s, 1H, OH); ^{13}C NMR ($CDCl_3$) δ: 162.52 (dd, J = 249.4, 12.1 Hz), 160.75 (dd, J = 249.4 , 12.1 Hz), 130.98 (dd, J = 9.9, 6.1 Hz), 120.95 (dd, J = 15.4, 3.3 Hz), 111.15 (dd, J = 20.9, 3.3 Hz), 103.62 (dd, J = 26.4, 25.3 Hz), 71.63, 66.11 (d, J = 3.3 Hz), 61.57. Anal. calcd for $C_9H_{10}F_2O_2$: C, 57.45; H, 5.36. Found: C, 57.51; H, 5.61.

2,4-Difluoro-5-[(1,3-dihydroxy-2-propoxy)methyl]toluene (13a). *cis*-1,3-*O*-Benzylideneglycerol (0.18 g, 1 mmol) and 2,4-difluoro-5-methylbenzyl bromide (**11**, 0.22 g, 1 mmol) were added to a slurry of NaH (60% in mineral oil, 44 mg, 1.1 mmol) in dry THF (1 mL) at 0°C with stirring. The reaction was allowed to proceed at 0°C for 2 h, and then at 25°C for 1 h with stirring. The reaction was quenched with saturated aqueous NH_4Cl, and extracted with diethyl ether (3 × 20 mL). The combined organic extracts

were washed with brine and dried (Na_2SO_4). Removal of the solvent in vacuo gave a solid that was recrystallized from hexane to give white needles (0.30 g, 94%), mp 87-89 °C that were dissolved in THF (5 mL). After 0.5M aqueous H_2SO_4 (0.1 mL) was added, this solution was stirred at 25°C for 30 h, and the reaction was quenched with solid $NaHCO_3$. The solid was filtered, and the solvent from the filtrate was removed in vacuo. The residue was purified via flash chromatography (hexane-ethyl acetate, 1:2 v/v) to give a colorless syrup, which was recrystallized from hexane to give **13a** (0.165 g, 79%) as white crystals; mp 67-69°C; ^{1}H NMR ($CDCl_3$) δ: 7.18 (dd, J = 8.5, 8.2 Hz, 1H), 6.74 (dd, J = 9.7, 9.5 Hz, 1H), 4.59 (s, 2H), 3.76 (dd, J = 11.9, 4.6 Hz, 2H), 3.67 (dd, J = 11.9, 4.9 Hz, 2H), 3.53 (m, 1H), 2.85 (br, s, 2H, OH), 2.20 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 160.66 (dd, J = 248.3, 12.1 Hz), 158.97 (dd, J = 247.2, 12.1 Hz), 132.30 (dd, J = 6.6, 5.5 Hz), 120.70 (m), 120.34 (dd, J = 15.4, 4.4 Hz), 103.23 (t, J = 26.4 Hz), 79.55, 65.27 (d, J = 3.3 Hz), 62.04, 13.69 (d, J = 3.3 Hz). Anal. calcd for $C_{11}H_{14}F_2O_3$: C, 56.89; H, 6.08. Found: C, 56.52; H, 6.31.

2,4-Difluoro-1-[(1,3-dihydroxy-2-propoxy)methyl]benzene (13b). This product was prepared, using a similar reaction to that used for the preparation of **13a**, by reaction of 2,4-difluorobenzyl bromide (2.07 g, 10 mmol) with *cis*-1,3-*O*-benzylideneglycerol (1.8 g, 10 mmol) to afford **13b** as white crystals; 1.54 g, yield 71%; mp 52-54°C; ^{1}H NMR ($CDCl_3$) δ: 7.39 (td, J = 8.2, 6.7 Hz, 1H), 6.95-6.75 (m, 2H), 4.66 (s, 2H), 3.78 (dd, J = 11.7, 4.9 Hz, 2H), 3.68 (dd, J = 11.7, 4.9 Hz, 2H), 3.57 (m, 1H), 2.98 (d, J = 3.0 Hz, 2H, OH); ^{13}C NMR ($CDCl_3$) δ: 162.67 (dd, J = 249.4, 12.1 Hz), 160.86 (dd, J = 249.4, 12.1 Hz), 131.16 (dd, J = 9.9, 6.6 Hz), 121.0 (dd, J = 15.4, 4.4 Hz), 111.20 (dd, J = 20.9, 4.4 Hz), 103.76 (t, J = 25.3 Hz), 79.59, 65.17 (d, J = 3.3 Hz), 61.92. Anal. calcd for $C_{10}H_{12}F_2O_3$: C, 55.05; H, 5.54. Found: C, 54.91; H, 5.45.

5-Iodo-2,4-difluoro-1-[(2-hydroxyethoxy)methyl]benzene (12c). *N*-iodosuccinimide (0.67 g, 2.5 mmol) was added in aliquots to a solution of **12b** (0.38 g, 2 mmol) in trifluoroacetic acid at 25°C with stirring. The resulting brown solution was stirred at 25°C in the dark for 12 h. The reaction mixture was poured onto ice-water, and extracted with diethyl ether (3 × 30 mL). The combined organic extracts were washed with aqueous sodium hydrosulfite, water and aqueous $NaHCO_3$. Removal of the solvent from the organic fraction in vacuo gave a residue that was dissolved in methanol (10 mL). To this solution, $NaHCO_3$ (0.5 g) was added, and the reaction mixture was stirred at

25°C for 30 min. Removal of the solvent in vacuo gave a residue that was dissolved in ether, filtered, and washed with ether. Removal of the solvent from the filtrate in vacuo gave a residue that was purified via flash chromatography (hexane-ethyl acetate, 2.5:1 v/v) to give **12c** (0.58 g, 92%) as a colorless syrup; ^{1}H NMR ($CDCl_3$) δ: 7.77 (t, J = 7.5 Hz, 1H), 6.80 (dd, J = 9.5, 7.7 Hz, 1H), 4.51 (s, 2H), 3.73 (t, J = 4.9 Hz, 2H), 3.59 (t, J = 4.9 Hz, 2H), 2.45 (s, 1H, OH); ^{13}C NMR ($CDCl_3$) δ: 161.26 (dd, J = 247.2, 12.1 Hz), 160.79 (dd, J = 250.5, 11.0 Hz), 139.42 (dd, J = 5.5, 3.3 Hz), 123.40 (dd, J = 15.4, 4.4 Hz), 104.03 (t, J = 27.5 Hz), 74.51 (dd, J = 26.4, 4.4 Hz), 71.91, 65.61 (d, J = 3.3 Hz), 61.62. Anal. calcd for $C_9H_9F_2IO_2$: C, 34.42; H, 2.89. Found: C, 34.24; H, 2.65.

5-Iodo-2,4-difluoro-1-[(1,3-dihydroxy-2-propoxy)methyl]benzene (13c). This product was prepared, using a procedure similar to that for the preparation of **12c**, by reaction of **13b** (0.434 g, 2 mmol) with *N*-iodosuccinimide (0.67 g, 2.5 mmol) to afford **13c** as white crystals (hexane-ether); 0.57 g, yield 83%; mp 63-64.5°C; ^{1}H NMR ($CDCl_3$) δ: 7.76 (t, J = 7.5 Hz, 1H), 6.80 (dd, J = 9.8, 7.7 Hz, 1H), 4.59 (s, 2H), 3.75 (dd, J = 11.7, 4.6 Hz, 2H), 3.68 (dd, J = 11.7, 4.9 Hz, 2H), 3.54 (m, 1H), 2.95 (br s, 2H, OH); ^{13}C NMR ($CDCl_3$) δ: 161.38 (dd, J = 248.3, 12.1 Hz), 160.90 (dd, J = 4.4, 2.2 Hz), 139.57 (dd, J = 4.4, 2.2 Hz), 123.45 (dd, J = 16.5, 4.4 Hz), 104.13 (dd, J = 27.5, 16.4 Hz), 79.93, 74.55 (dd, J = 26.4, 3.3 Hz), 64.63 (d, J = 2.2 Hz), 62.05. Anal. calcd for $C_{10}H_{11}F_2IO_3$: C, 34.91; H, 3.22. Found: C, 34.69; H, 3.05.

5-Nitro-2,4-difluoro-1-[(2-hydroxyethoxy)methyl]benzene (12d). Powdered NH_4NO_3 (0.32 g, 5 mmol) was added to a solution of **12b** (0.38 g, 2 mmol) in trifluoroacetic anhydride (5 mL), at 25 °C, and the reaction was allowed to proceed with stirring at 25°C for 2 h. The reaction mixture was poured onto ice-water, and extracted with diethyl ether (3 × 30 mL). The combined organic extracts were washed with aqueous $NaHCO_3$, water, and the solvent was removed in vacuo to give a residue that was dissolved in methanol (10 mL). To this solution, $NaHCO_3$ (0.5 g) was added, and the mixture was stirred at 25°C for 30 min. Removal of the solvent in vacuo gave a residue that was dissolved in ether, filtered, and washed with ether. Removal of the solvent from the filtrate in vacuo gave a residue that was purified via flash chromatography (hexane-ethyl acetate, 2:1 v/v) to give **12d** (0.28 g, 60%) as a yellow syrup; ^{1}H NMR ($CDCl_3$) δ: 8.22 (t, J = 7.7 Hz, 1H), 7.0 (dd, J = 10.4, 9.1 Hz, 1H), 4.59 (s, 2H), 3.76 (t, J = 4.9 Hz, 2H), 3.64 (t, J = 4.9 Hz, 2H), 2.8 (br s, 1H, OH); ^{13}C NMR (CDC_3) δ: 162.80 (dd, J =

260.4, 11.0 Hz), 155.67 (dd, J = 268.1, 13.2 Hz), 133.81 (m), 127.34 (dd, J = 6.6, 2.2 Hz), 123.1 (dd, J = 16.5, 4.4 Hz), 106.17 (dd, J = 27.5, 25.3 Hz), 72.30, 65.25 (d, J = 3.3 Hz), 61.57. Anal. calcd for $C_9H_9F_2O_4N$: C, 46.36; H, 3.89; N, 6.01. Found: C, 46.60; H, 3.94; N, 5.97.

5-Nitro-2,4-difluoro-1-[(1,3-dihydroxy-2-propoxy)methyl]benzene (13d). Product **13d** was prepared, using a procedure similar to that for the preparation of **12d**, by reaction of **13b** (0.434 g, 2 mmol) with NH_4NO_3 (0.32 g, 5 mmol) in trifluoroacetic anhydride (5 mL) to give **13d** as pale yellow needles (hexane-ether); 0.29 g, 55%; mp 66-67°C; ^{1}H NMR ($CDCl_3$) δ: 8.23 (t, J = 7.7 Hz, 1H), 6.96 (dd, J = 10.1, 9.5 Hz), 4.70 (s, 2H), 3.75 (dd, J = 11.9, 4.6 Hz, 2H), 3.68 (dd, J = 11.9, 4.9 Hz, 2H), 3.56 (m, 1H), 3.23 (br s, 2H, OH); ^{13}C NMR ($CDCl_3$) δ: 162.80 (dd, J = 260.4, 11.0 Hz), 155.56 (dd, J = 268.7, 13.2 Hz), 133.83 (m), 127.47 (dd, J = 7.7, 2.2 Hz), 123.30 (dd, J = 17.6, 3.3 Hz), 106.11 (dd, J = 27.5, 25.3 Hz), 80.62, 64.13 (d, J = 3.3 Hz), 61.91. Anal. calcd. for $C_{10}H_{11}F_2O_5N$: C, 45.64; H, 4.21; N, 5.32. Found: C, 45.50; H, 4.05; N, 5.28.

***In Vitro* Cell Cytotoxicity (MTT assay).** KBALB, KBALB-STK, human 143B, and human 143B-LTK cells were cultured in complete DMEM medium supplemented with 10% fetal bovine serum (FBS). Exponentially growing cells were trypsinized, centrifuged, suspended in growth medium, and the cell number was readjusted to 8 x 10^3 cells/mL. Cells were seeded into 96-well plates at 8 x 10^2 cells/well, and incubated at 37 °C in a humidified 5% CO_2 atmosphere for 24 h.

The test compound was dissolved in DMEM medium, and 100 μL of this solution was added to cells in 96-well plates to produce the preselected test compound concentration. DMEM medium (100 μL) was added to control wells. The plates were incubated for 3-5 days at 37 °C in a humidified atmosphere consisting of 95% air and 5% CO_2. At the end of the incubation, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT, Sigma) was dissolved in phosphate-buffered saline (PBS) to produce a concentration of 5 mg/mL, filtered through a 0.45 μm membrane filter, and diluted (1:5) with pre-warmed DMEM medium. A 50 μL aliquot of this solution was added to each well, and the plates were incubated at 37 °C for 4 h. The medium was removed from the wells, dimethyl sulfoxide (150 μL) was added to each well, and the plates were placed on a shaker for 15 min to dissolve the formazan crystals. The absorbance at 540 nm (A_{540}) was measured immediately in each well using a scanning multi-well spectrophotometer

(ELISA reader). A_{540} values, corrected for the absorbance in medium blanks, reflected the concentration of viable cells. The CC_{50} values reported are the test drug concentration that reduced the A_{540} to 50% of the control value (mean value, n = 6). This assay,[27] which depends on the metabolic reduction of MTT to colored formazan, measures cytostatic and cytotoxic effects of the test drug.

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