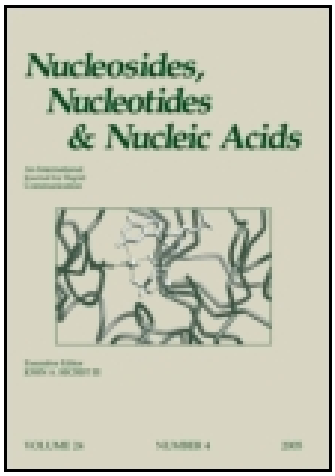

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SYNTHESIS OF UNNATURAL 7-SUBSTITUTED-1-(2-DEOXY-β-D-RIBOFURANOSYL)ISOCARBOSTYRILS†: "THYMINE REPLACEMENT" ANALOGS OF DEOXYTHYMIDINE FOR EVALUATION AS ANTIVIRAL AND ANTICANCER AGENTS

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SYNTHESIS OF UNNATURAL 7-SUBSTITUTED-1-(2-DEOXY-β-D-RIBOFURANOSYL)ISOCARBOSTYRILS†: "THYMINE REPLACEMENT" ANALOGS OF DEOXYTHYMIDINE FOR EVALUATION AS ANTIVIRAL AND ANTICANCER AGENTS

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ABSTRACT

A group of unnatural 1-(2-deoxy-β-D-ribofuranosyl)isocarbostyrils having a variety of C-7 substituents [H, 4,7-$(NO_2)_2$, I, CF_3, CN, (*E*)-CH=CH-I, -C≡CH, -C≡C-I, -C≡C-Br, -C≡C-Me], designed as nucleoside mimics, were synthesized for evaluation as anticancer and antiviral agents. This class of compounds exhibited weak cytotoxicity in a MTT assay ($CC_{50} = 10^{-3}$ to 10^{-5} M range) with the 4,7-dinitro derivative being the most cytotoxic, relative to thymidine ($CC_{50} = 10^{-3}$ to 10^{-5} M range), against a variety of cancer cell lines. The 4,7-dinitro, 7-I and 7-C≡CH compounds exhibited similar cytotoxicity against non-transfected (KBALB, 143B), and HSV-1 TK^+ gene transfected (KBALB-STK, 143B-LTK) cancer cell lines possessing the herpes simplex virus type 1 (HSV-1) thymidine kinase gene (TK^+). This observation indicates that these compounds are not substrates for HSV type-1 TK, and are therefore unlikely to be useful in gene therapy based on the HSV gene therapy paradigm.

†Nucleoside-like numbering is used by analogy to nucleoside nomenclature.

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INTRODUCTION

The specific H-bonding patterns of the Watson-Crick A-T and G-C base pairs serve an important role with respect to the storage and replication of biological information[1]. Accordingly, the design of enzymatically replicable "unnatural base pairs" may constitute a method to expand the biological and chemical genetic alphabet potential of DNA beyond the natural A-T and G-C pairs. Romesberg *et al.*[2] recently reported the unnatural hydrophobic 7-propynylisocarbostyril nucleoside mimic **1** that contains an isocarbostyril moiety, in place of the natural uracil base, that can not form H-bonds. The unnatural self-pair **1-1** is able to significantly stabilize duplex DNA relative to the natural A-T and G-C base pairs. In spite of the fact that the mimic **1** does not have shape complimentarity to any native base, the 5′-triphosphate of **1** is still incorporated opposite itself (**1-1** pair) with reasonable efficiency by the Klenow fragment of *E. coli* DNA polymerase 1 (KF) such that the **1-1** base pair is the most "orthogonal" base pair relative to the natural bases, reported to date. Although KF (DNA polymerase) inserts **1**-triphosphate opposite **1** with reasonable efficiency, continued synthesis (chain elongation) proceeds inefficiently. This is attributed to the assumption that the unnatural base pair **1-1** assumes a geometry that incorrectly positions the 3′-OH of the growing strand for nucleophilic reaction with the incoming nucleoside triphosphate[2]. These results suggest that nucleoside mimics such as **1** should be cytotoxic to rapidly multiplying cancer cells (inhibit tumor growth) and/or act as antiviral agents due to their selective phosphorylation by virus-infected cells[3]. Credence for this latter concept is provided by the discovery that the unusual bicyclic 2,3-dihydrofuro[2,3-d]pyrimidin-2-one moiety present in compounds **2b**, which are derivatives of **2a** described previously[4,5], exhibit potent and selective inhibition (300-fold greater potency than acyclovir with no detectable *in vitro* cytotoxicity) of varicella-zoster virus (VZV)[6]. (see Figure 1). This latter observation indicates that certain large bicyclic heterocyclic base moieties are tolerated with respect to retention of antiviral efficacy. It was therefore anticipated that 7-substituted nucleoside mimic derivatives of **1** may be useful as anticancer or antiviral agents, and as radiopharmaceutical agents to image[7]. or chemotherapeutic agents to treat[8], herpes simplex virus type-1 thymidine kinase positive (HSV-1 TK^+) gene-transfected tumors (gene therapy of cancer)[9]. We now report the synthesis, and antiviral-anticancer activities for a group of 1-(2-deoxy-β-D-ribofuranosyl)-7-substituted-isocarbostyrils (**1**, **7**, **9**, **11**, **13**, **15**, **18**, **20**, **23**, **24**) designed as 5-substituted-2′-deoxyuridine (thymidine) mimics.

CHEMISTRY

Nucleoside mimics are frequently synthesized by reaction of an *O*-trimethylsilylated base mimic[2], or an arylcadmium reagent[10], with the

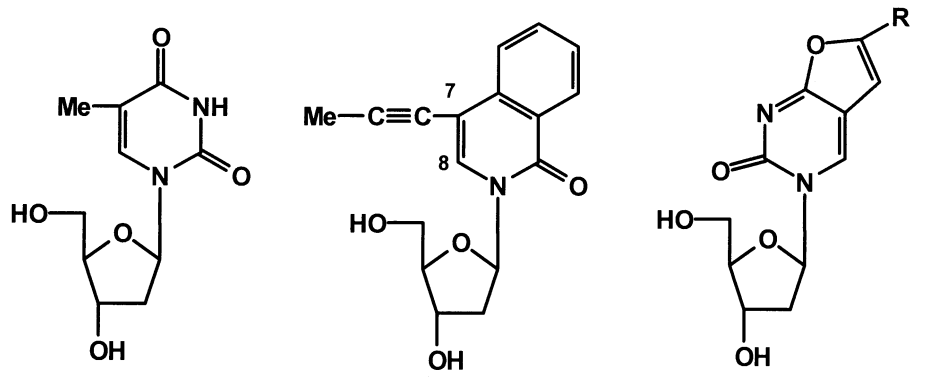
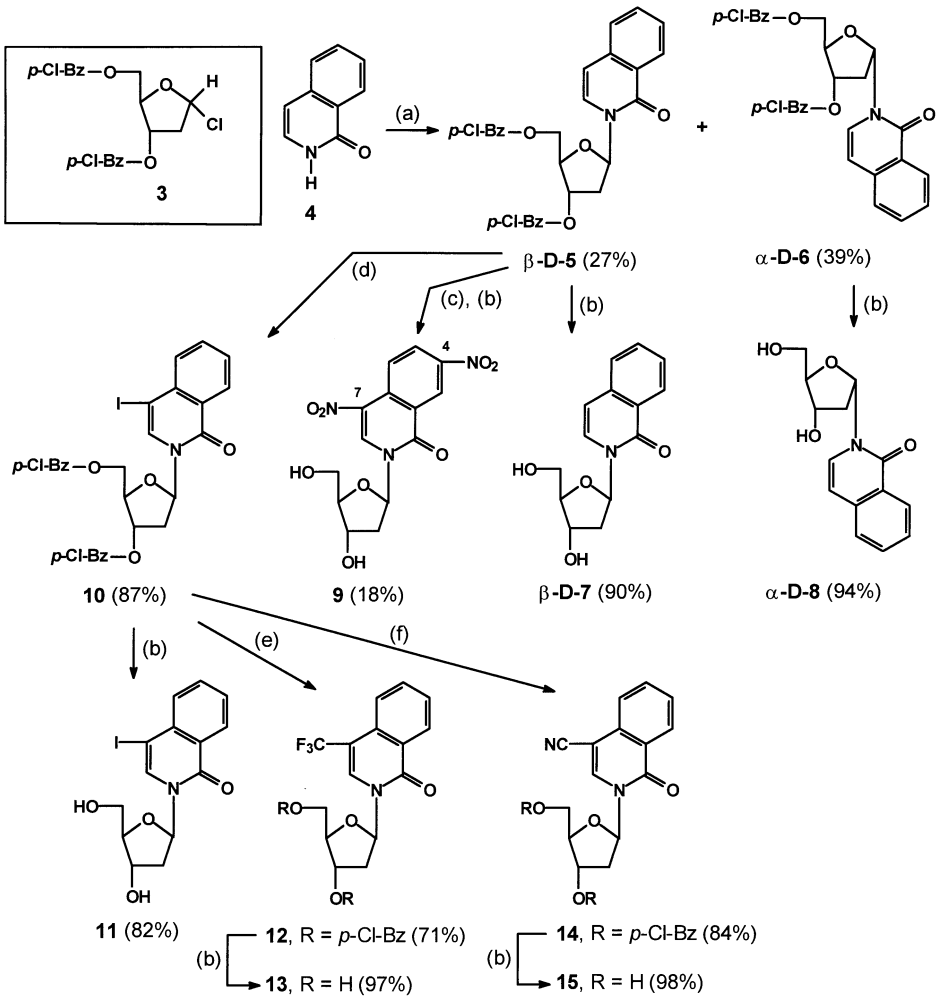


Figure 1. Structures of thymidine, 1-(2-deoxy-β-D-ribofuranosyl)-7-propynylisocarbostyril (**1**), and some unusual 3-(2-deoxy-β-D-ribofuranosyl)-6-substituted-2,3-dihydrofuro[2,3-d] pyrimido-2-one derivatives **2**.

well known Hoffer α-chloro sugar synthon [3,5-bis-*O*-(*p*-toluoyl)-2-deoxy-α-D-ribofuranosyl chloride][11]. Accordingly, 3,5-bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-α-D-ribofuranosyl chloride (**3**) was used in place of the Hoffer α-chloro sugar synthon since it is a more stable and readily accessible crystalline reagent[10]. Coupling the α-chloro sugar **3** to the 2-*O*-trimethylsilyl derivative of isocarbostyril (**4**), which was generated from the reaction of **4** with *N*,*O*-bis(trimethylsilyl)acetamide, was performed in MeCN at 25 °C (Scheme 1). As reported for a similar reaction using 3,5-bis-*O*-(*p*-toluoyl)-2-deoxy-α-D-ribofuranosyl chloride[2], this coupling reaction yielded a mixture of the 3,5-bis-*O*-(*p*-chlorobenzoyl) β-D-**5** (27%) and α-D-**6** (39%) anomers that were readily separated by flash silica gel column chromatography. Subsequent removal of the *O*-(*p*-chlorobenzoyl) protecting groups by treatment with NaOMe afforded β-D-7 (90%) and β-D-**8** (94%), respectively.

The configuration at the C-1′ carbon, and the conformation, of the β-D-**7** and α-D-**8** anomers possessing an isocarbostyril moiety was analyzed by nuclear Overhauser enhancement (NOE) ^{1}H NMR difference spectroscopy (Figure 2). Selective irradiation of the H-4′ signal (β-D-7) resulted in an enhancement of the H-1′ signal (6.8%), and irradiation of the H-2″ signal gave a 6.0% enhancement of the H-1′ signal, which support the assignment of the β-configuration[12]. The C-8 hydrogen of the isocarbostyril moiety is oriented in the direction of the sugar ring since NOE enhancements of H-8 were observed upon selective irradiation of H-2′ (6.4%) and H-3′ (3.6%). In contrast, irradiation of the H-1′ signal of α-D-**8** provided enhancements of *cis*-H-2′ (6.4%) and H-3′ (1.9%).



Scheme 1. Reagents and conditions: (a) *N*,*O*-bis(trimethylsilyl)acetamide, MeCN, 25 °C, 30 min, and then **3**, $SnCl_4$, 0 °C, 30 min; (b) NaOMe, MeOH, 25 °C, 30 min; (c) NH_4NO_3, $(CF_3CO)_2O$, 25 °C, 1 h; (d) ICl, CH_2Cl_2, reflux; (e) CuI, KF, DMF at 60 °C, and then $ClCF_2CO_2Me$, 120 °C, 6 h; (f) $Zn(CN)_2$, $(PPh_3)_4Pd$, DMF, 80–90 °C, 6 h.

The failure to observe an enhancement of H-1′ upon irradiation of H-4′ indicates these two hydrogens are on opposite faces of the sugar moiety which is indicative of the α-configuration[12].

Nitration[13] of the 3,5-bis-*O*-(*p*-chlorobenzoyl) ester *β*-D-**5** using ammonium nitrate in trifluoroacetic anhydride afforded the 4,7-dinitro derivative, which on deprotection with NaOMe in MeOH at 25 °C, gave 1-(2-deoxy-*β*-D-ribofuranosyl)-4,7-dinitroisocarbostyril (**9**, 18% overall yield from *β*-D-**5**). The ^{1}H NMR spectrum for **9** indicated that nitration occurred at the expected C-7 position since H-8 appeared as a singlet at δ 9.57, and at

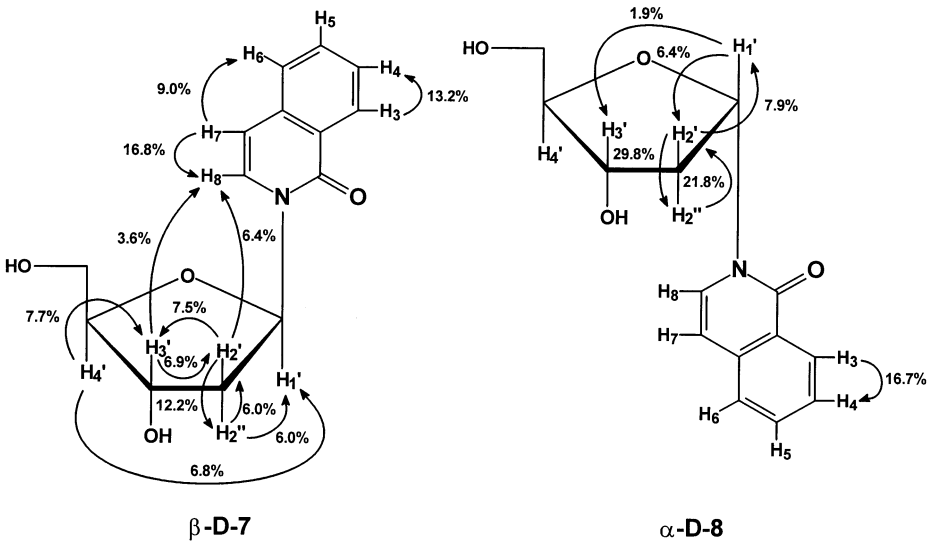
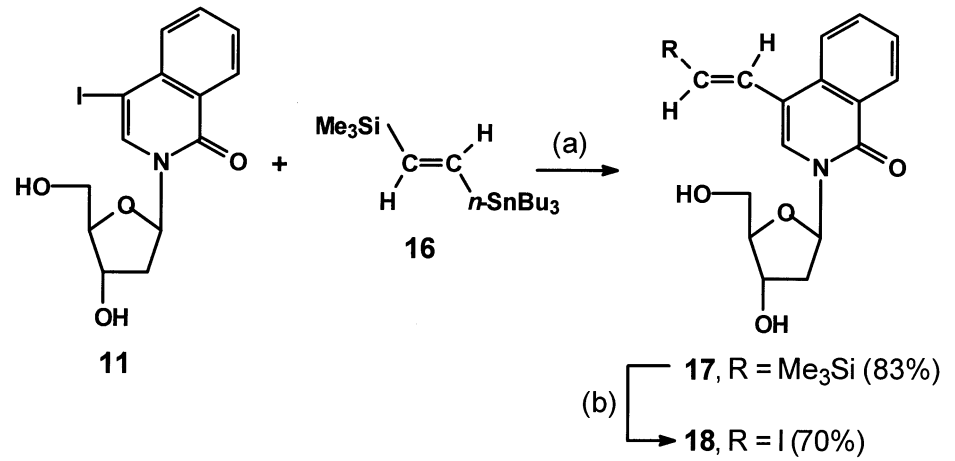


Figure 2. Some NOE studies to study the configuration and conformation of 1-(2-deoxy-β-D-ribofuranosyl)isocarbostyril (β-D-**7**), and 1-(2-deoxy-α-D-ribofuranosyl)isocarbostyril (α-D-**8**) in MeOH-d_4 at 22 °C.

either C-4 or C-5 since a doublet ($J = 2.4$ Hz), doublet ($J = 9.1$ Hz) and a doublet of doublets ($J = 9.1$, 2.4 Hz) were present at δ 8.93, 8.75 and 8.66, respectively for three phenyl protons located on the isocarbostryril ring system. A semiempirical PM3 calculation showed the electron densities in isocarbostyril (**4**) are C-3 (−0.031), C-4 (−0.129), C-5 (−0.065), C-6 (−0.117) and C-7 (−0.193). These data suggest electrophilic nitration should occur at the more electron-rich C-4 position, relative to C-5, on the phenyl moiety to give the 4,7-dinitro product.

Iodination of the 3,5-bis-*O*-(*p*-chlorobenzoyl) ester *β*-D-**5** using ICl in CH_2Cl_2 afforded the 7-iodo derivative **10** (87%), which on deprotection with NaOMe in MeOH gave 1-(2-deoxy-*β*-D-ribofuranosyl)-7-iodoisocarbostyril (**11**, 82%). Synthesis of the 7-trifluoromethyl derivative **13** was carried out using a reaction reported by Chen *et al.*[14] Thus, the protected 7-iodo compound **10** was treated with methyl chlorodifluoroacetate in the presence of CuI and KF in DMF at 120 °C to give the 3,5-bis-*O*-(*p*-chlorobenzoyl) ester **12** (71%), followed by deprotection with NaOMe in MeOH to afford 1-(2-deoxy-*β*-D-ribofuranosyl)-7-trifluoromethylisocarbostyril (**13**, 97%). The 7-cyano derivative **15** was also prepared from the protected 7-iodo derivative **10** by cyanation with zinc cyanide in the presence of $(PPh_3)_4Pd(0)$[15], and then deprotection using NaOMe in MeOH.

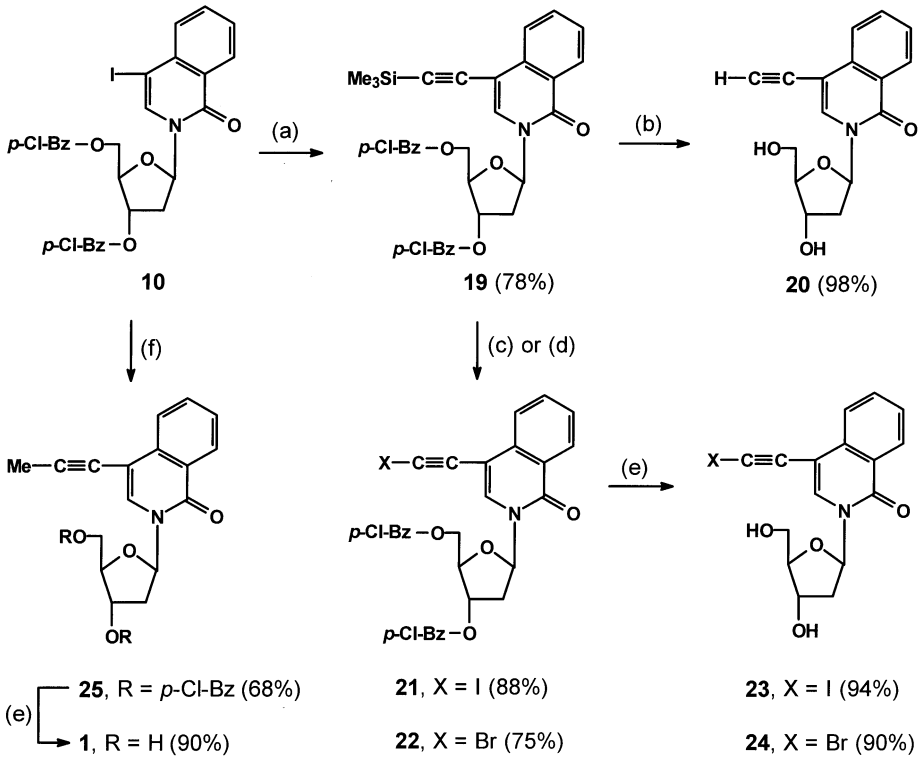
Synthesis of the (*E*)-7-(2-trimethylsilylvinyl) derivative **17** was performed using a methodology similar to that reported previously[16] for the



Scheme 2. Reagents and conditions: (a) $(Ph_3P)_2PdCl_2$, MeCN, 60 °C, 24 h; (b) ICl, MeCN, 0 °C, 15 min.

synthesis of (*E*)-5-(2-iodovinyl)-2′-fluoro-2′-deoxyuridine (IVFRU), as illustrated in Scheme 2. Accordingly, the coupling reaction of the unprotected 7-iodo compound **11** with (*E*)-1-trimethylsilyl-2-tributylstannylethene (**16**)[17] catalyzed by $(Ph_3P)_2PdCl_2$ afforded **17** (83%). The ^1H NMR spectrum for **17** showed a $J_{CH=CH} = 18.9$ Hz coupling constant that is indicative of the (*E*)-7-(-CH=CH-TMS) stereochemistry. Reaction of the (*E*)-7-(2-trimethylsilylvinyl) compound **17** with ICl in MeCN at 0 °C yielded the corresponding (*E*)-7-(2-iodovinyl) product **18** (70%). Proton NMR spectral analysis of **18** indicated that only the (*E*)-isomer was produced ($J_{CH=CH} =$ 14.7 Hz).

Reaction of 1-[3,5-bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl]-7-iodoisocarbostyril (**10**) with TMS-C≡C-H in the presence of $(Ph_3P)_2PdCl_2$ and CuI in Et_3N[18] afforded the corresponding 7-(trimethylsilyl)ethynyl derivative **19** (78%), which upon treatment with NaOMe in MeOH at 20 °C resulted in simultaneous ester cleavage and removal of the TMS group to give the 7-ethynyl product **20** (98%) as illustrated in Scheme 3. The 7-iodoethynyl **23**, or 7-bromoethynyl **24**, compounds were prepared by reaction of the 7-(trimethylsilyl)ethynyl compound **19** with either *N*-iodosuccinimide, or *N*-bromosuccinimide, in the presence of $AgNO_3$ catalyst in DMF[19], and then removal of the *p*-chlorobenzoyl protecting groups in the sugar moiety using NaOMe in MeOH. The coupling reaction of 1-[3,5-bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl]-7-iodoisocarbostyril (**10**) with Me-C≡C-H in the presence of $(Ph_3P)_2PdCl_2$, CuI and dry Et_3N, using a procedure similar to that reported by Romesberg *et al.*[2], and then ester hydrolysis using NaOMe in MeOH, afforded the target product 1-(2-deoxy-β-D-ribofuranosyl)-7-propynylisocarbostyril (**1**, 61% overall yield from **10**).



Scheme 3. Reagents and conditions: (a) H-C≡C-$SiMe_3$, $(Ph_3P)_2PdCl_2$, CuI, Et_3N, 50–60 °C, 5 h; (b) NaOMe, MeOH, 25 °C, 20 min; (c) *N*-iodosuccinimide, $AgNO_3$, DMF, 0 °C for 2 h, and then 25 °C for 1 h; (d) *N*-bromosuccinimide, $AgNO_3$, DMF, 0 °C for 2 h, and then 25 °C for 4 h; (e) NaOMe, MeOH, 25 °C, 15 min; (f) $(Ph_3P)_2PdCl_2$, CuI, Et_3N, Me-C≡C-H, 25 °C, 5 h.

BIOLOGICAL RESULTS AND DISCUSSION

Nucleoside mimics, in which the natural thymine base in thymidine is replaced by an unnatural isocarbostyril base, could induce new properties which the natural nucleosides lack thereby providing a strategy to design a new class of isocarbostyril nucleoside mimics. Isocarbostyril mimics possess a number of potentially desirable features that include i) irreversible inhibition of thymidylate synthase (TS), by the 5′-monophosphate, upon reaction of the nucleophilic -SH group of TS at the electron deficient C-8 position of the isocarbostyril ring to exhibit a cytotoxic anticancer effect, ii) utilization of the 5′-monosphosphate as a substrate for the synthesis of "false" DNA that would stop chain elongatation resulting in a cytotoxic antiviral/anticancer effect, and iii) increased lipophilicity which could enhance their ability to penetrate the blood-brain-barrier (BBB) to improve their efficacy for the treatment of brain viral infections and brain tumors. A group of 1-(2-deoxy-β-D-ribofuranosyl)-7-substituted-isocarbostyrils

having a variety C-7 substituents [H, NO_2, I, CF_3, CN, (*E*)-CH=CH-I, -CH≡CH, -C≡C-I, -C≡C-Br, -C≡C-Me] were investigated to probe the effect of size, electronic, hybridization [sp (-C≡C-), sp^2 (-CH=CH-)] and lipophilic parameters upon biological activity. Substituent selection could also influence oral bioavailability, metabolic stability and pharmacokinetic properties. All of these factors are potential determinants of antiviral/anticancer efficacy.

A group of 7-substituted [H, 4,7-$(NO_2)_2$, I, CN, (*E*)-(-CH=CH-I), -CH≡CH, -C≡C-I, -C≡C-Br, -C≡C-Me] isocarbostyril compounds, and the reference compounds 5-iodo-2′-deoxyuridine (IUDR), 5-fluoro-2′-deoxyuridine (FUDR) and thymidine, were evaluated using the MTT cytotoxicity assay[20] (see Table 1). These 7-substituted compounds exhibited weak cytotoxicity ($CC_{50} = 10^{-3}$ to 10^{-5} M range), even when compared to thymidine ($CC_{50} = 10^{-3}$ to 10^{-5} M range), against KBALB, KBALB-STK, 143B, 143B-LTK, EMT-6 and R-970-5 cancer cell lines. The 4,7-dinitro compound **9** was the most cytotoxic ($CC_{50} = 10^{-5}$ M range) agent. A comparison of the cytotoxicities against the KBALB cell line showed the cytotoxicity potency order was 4,7-dinitro **9** 7-C≡C-I **23** > 7-C≡C-Br **24** ≈ 7-C≡CH **20** ≈ 7-I **11** ≈ 7-(*E*)-CH=CH-I **18** ≈ 7-CN **15** ≈ 7-C≡C-Me **1** > 7-H **7**. The I and Me groups are potential isosteres since their van der Waal's radius are 2.15 and 2.0 Å, respectively. This concept is supported by the observation that the cytotoxicity for 7-C≡C-I **23** is greater than 7-C≡C-Me **1** toward KBALB cells. The 4,7-dinitro **9**, 7-I **11** and 7-C≡CH **20** compounds exhibited similar cytotoxicity against non-transfected (KBALB, 143B), and the corresponding transfected (KBALB-STK, 143B-LTK) cancer cell lines possessing the herpes simplex virus type 1 (HSV-1) thymidine kinase gene (TK^+). This comparison, for these compounds, indicates that expression of the viral TK enzyme did not provide a gene therapeutic effect.

The weak, or absence of, anticancer/antiviral efficacy for this novel class of 1-(2-deoxy-β-D-ribofuranosyl)-7-substituted-isocarbostyrils could be due to a number of factors. For example, it is possible that the sugar moiety does not undergo phosphorylation by thymidine kinase (TK) to the 5′-monophosphate (5′-MP). Support for this explanation is based on the observations that there are generally negligible differences in anticancer/antiviral activities between non-transfected (KBALB, 143B) and viral TK-transfected (KBALB-STK, 143B-LTK) cell lines. In this respect, one would anticipate that preferential phosphorylation to the 5′-MP by transfected cells, and subsequent transformation to the active 5′-triphosphate (5′-TP), would have resulted in a greater cytotoxic effect since the 5′-TP of **1** has been shown to deter chain elongation[2]. Alternatively, the group of nucleoside mimics evaluated may be devoid of anticancer/antiviral activity due to the fact that they are not inhibitors of thymidylate synthase (TS). Credence for this possibility is based on the belief that the anticancer activity exhibited by 5-fluoro-2′-deoxyuridine is primarily due to inhibition of DNA biosynthesis by

Table 1. *In Vitro* Cell Cytotoxicity of 1-(2-Deoxy-β-D-ribofuranosyl)-7-substituted-isocarbostyrils 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	KBALB[b]	KBALB-STK[c]	143B[d]	143B-LTK[c]	EMT-6[e]	R-970-5[f]
7	2.5×10^{-3}	–	–	–	–	–
9	2.7×10^{-5}	2.6×10^{-5}	3.2×10^{-5}	3.0×10^{-5}	2.0×10^{-5}	4.0×10^{-5}
11	3.3×10^{-4}	3.0×10^{-4}	2.6×10^{-4}	1.5×10^{-4}	2.8×10^{-4}	1.8×10^{-4}
15	5.5×10^{-4}	–	–	–	–	–
18	5.0×10^{-4}	–	–	–	–	–
20	3.1×10^{-4}	2.7×10^{-4}	2.6×10^{-4}	2.0×10^{-4}	2.3×10^{-4}	2.3×10^{-4}
23	7.5×10^{-5}	–	–	–	–	–
24	2.0×10^{-4}	–	–	–	–	–
1	5.5×10^{-4}	–	–	–	–	–
IUDR[g]	9.7×10^{-5}	1.0×10^{-5}	7.0×10^{-3}	7.4×10^{-3}	3.8×10^{-4}	6.2×10^{-4}
FUDR[h]	6.0×10^{-11}	8.8×10^{-11}	9.0×10^{-5}	1.0×10^{-4}	9.0×10^{-12}	5.5×10^{-5}
Thymidine	9.5×10^{-5}	1.0×10^{-4}	–	–	1.3×10^{-4}	2.5×10^{-3}

[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 expressed, the herpes simplex virus type 1 thymidine kinase (HSV-1 TK) gene.
[d]Human osteosarcoma cell line.
[e]Mouse mammary carcinoma cell line.
[f]Human osteosarcoma cell line.
[g]IUdR = 5-iodo-2′-deoxyuridine.
[h]FUdR = 5-fluoro-2′-deoxyuridine.

blocking TS, the enzyme which catalyzes the methylation of 2′-deoxyuridine-5′-monophosphate to thymidine-5′-monophosphate[21,22]. Furthermore, inhibition of TS is the mechanism by which certain nucleosides such as (*E*)-5-(2-iodovinyl)-2′-deoxyuridine and 5-(1-azidovinyl)-2′-deoxyuridine exhibit their cytostatic effect[23].

CONCLUSIONS

The novel class of 1-(2-deoxy-β-D-ribofuranosyl)-7-substituted-isocarbostyrils reported here lacks anticancer/antiviral activity. The results obtained in this study suggest that these isocarbostyril mimics are not suitable thymidine mimics. In the event that the failure of this class of compounds to undergo phosphorylation to the 5′-monophosphate is responsible for the lackof anticancer/antiviral activities observed, it is possible that 5′-*cyclo*Sal-pronucleotide derivatives designed to adhere to the kinase-bypass concept[24,25]

could result in the transformation of these inactive unnatural nucleoside mimics into a class of effective anticancer/antiviral agents.

EXPERIMENTAL SECTION

General Methods. Melting points were determined with a Thomas Hoover capillary apparatus and are uncorrected. ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra were measured on a Bruker AM-300 spectrometer in $CDCl_3$, MeOH-d_4, or DMSO-d_6. 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 Micro-Analysis Service Laboratory, Department of Chemistry, University of Alberta. Silica gel 60A (Silicycle Co., 230–400 mesh) was used for all silica gel column flash chromatography separations. 3,5-Bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-α-D-ribofuranosyl chloride (**3**) was prepared according to the previously reported procedure[10]. (*E*)-1-Trimethylsilyl-2-tributylstannylethene (**16**) was prepared according to the literature procedure[17]. All other reagents were purchased from the Aldrich Chemical Co. The semiempirical PM3 calculation for isocarbostyril (**4**) was determined using the Alchemy 2000 simulation program, Version 2 (SciVision, Burlington, MA, U.S.A.).

1-[3,5-Bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl]isocarbostyril (**5**) and 1-[3,5-Bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-α-D-ribofuranosyl]isocarbostyril (**6**). Dry MeCN (220 mL) and bis(trimethylsilyl)acetamide (17 mL, 68 mmol) were added to isocarbostyril (**4**, 10.0 g, 68 mmol) with stirring at 25 °C. Dissolution occurred within 10 min. Following a 30 min reaction time, additional MeCN (240 mL) and **3** (24.0 g, 56 mmol) were added. The reaction mixture was cooled to 0 °C, $SnCl_4$ (13.8 mL of a 1 M solution in CH_2Cl_2, 13.8 mmol) was added dropwise, and the reaction was allowed to proceed at 0 °C for 30 min with stirring. EtOAc (1000 mL) was added, and the resulting solution was consecutively extracted with saturated $NaHCO_3$ (3 × 200 mL) and then brine (200 mL). The organic extracts were dried (Na_2SO_4), the solvents were removed *in vacuo*, and the residue obtained was purified by flash silica gel column chromatography. Elution with CH_2Cl_2-EtOAc (40 : 040 : 1, v/v) gave the β-anomer **5** (10.1 g, 27%) as white crystals (hexane-CH_2Cl_2, mp 185–186 °C), and the α-anomer **6** (14.3 g, 39%) as white crystals (hexane-CH_2Cl_2, mp 172–173 °C), respectively.

β-Anomer 5: ^{1}H NMR ($CDCl_3$) δ 8.42 (d, $J = 7.6$ Hz, 1H, H-8), 7.95–8.04 (m, 4H, *ortho*-benzoyl hydrogens), 7.38–7.69 (complex

multiplets, 8H, H-3, H-4, H-5, H-6, *meta*-benzoyl hydrogens), 6.83 (dd, $J = 8.2$, 5.8 Hz, 1H, H-1′), 6.48 (d, $J = 7.6$ Hz, 1H, H-7), 5.64 (br d, $J = 6.7$ Hz, 1H, H-3′), 4.68–4.70 (m, 2H, H-5′), 4.55–4.63 (m, 1H, H-4′), 2.90 (ddd, $J = 14.3$, 5.8, 1.6 Hz, 1H, H-2′α), 2.34 (ddd, $J = 14.3$, 8.2, 7.0 Hz, 1H, H-2′*β*); ^{13}C NMR ($CDCl_3$) δ 165.21, 165.09, 161.67, 140.15, 139.91, 136.59, 132.61, 131.13, 130.91, 128.90, 127.81, 127.59, 127.02, 125.90, 125.81, 125.14, 106.73, 85.51, 82.43, 75.47, 64.62, 38.54. Anal. calcd. for $C_{28}H_{21}Cl_2NO_6$: C, 62.46; H, 3.93; N, 2.60. Found: C, 62.56; H, 3.87; N, 2.64.

α-Anomer 6: ^{1}H NMR ($CDCl_3$) δ 8.43 (d, $J = 8.2$ Hz, 1H, H-3), 7.20–8.20 (complex multiplets, 12H, H-4, H-5, H-6, H-8, benzoyl hydrogens), 6.65 (dd, $J = 6.7$, 1.5 Hz, 1H, H-1′), 6.57 (d, $J = 7.6$ Hz, 1H, H-7), 5.63 (d, $J = 6.1$ Hz, 1H, H-3′), 4.93–4.99 (m, 1H, H-4′), 4.64 (dd, $J = 11.9$, 4.9 Hz, 1H, H-5′a), 4.56 (dd, $J = 11.9$, 5.2 Hz, 1H, H-5′b), 3.03 (ddd, $J = 15.9$, 6.7, 6.1 Hz, 1H, H-2′*β*), 2.69 (d, $J = 15.9$ Hz, H-2′α); ^{13}C NMR ($CDCl_3$) δ 165.14, 164.69, 161.63, 139.91, 139.85, 136.78, 132.45, 130.98, 130.89, 128.87, 128.61, 127.79, 127.49, 127.37, 126.80, 125.93, 125.71, 105.62, 88.03, 84.81, 75.23, 64.45, 38.89. Anal. calcd. for $C_{28}H_{21}Cl_2NO_6$: C, 62.46; H, 3.93; N, 2.60. Found C, 62.48; H, 3.89; N, 2.68.

1-(2-Deoxy-*β*-D-ribofuranosyl)isocarbostyril (**7**). NaOMe (30 mg, 0.559 mmol) was added to a suspension of **5** (100 mg, 0.186 mmol) in MeOH (4 mL), and the mixture was stirred at 25 °C for 30 min. The reaction was quenched via addition of NH_4Cl (100 mg), and the solvent was removed *in vacuo* to give a residue that was purified via flash silica gel column chromatography using MeOH-CH_2Cl_2 (1:9, v/v) as eluent to afford **7** (43 mg, 90%) directly as white crystals: mp 109–110 °C; ^{1}H NMR (CD_3OD) δ 8.29 (d, $J = 8.2$ Hz, 1H, H-3), 7.77 (d, $J = 7.6$ Hz, 1H, H-8), 7.70 (dt, $J = 7.9$, 1.2 Hz, 1H, H-5), 7.61 (d, $J = 7.9$ Hz, 1H, H-6), 7.51 (ddd, $J = 8.2$, 7.9, 3.7 Hz, 1H, H-4), 6.72 (d, $J = 7.6$ Hz, 1H, H-7), 6.67–6.72 (m, 1H, H-1′), 4.38–4.46 (m, 1H, H-3′), 3.99 (dd, $J = 7.3$, 3.7 Hz, 1H, H-4′), 3.82 (dd, $J = 11.9$, 3.6 Hz, 1H, H-5′a), 3.75 (dd, $J = 11.9$, 3.9 Hz, 1-H, H-5′b), 2.41 (ddd, $J = 13.4$, 6.1, 3.4 Hz, 1H, H-2′α), 2.18 (ddd, $J = 13.4$, 7.0, 6.7 Hz, 1H, H-2′*β*); ^{13}C NMR (CD_3OD) δ 163.4, 138.4, 133.8, 128.1, 127.9, 127.7, 127.1, 126.4, 107.9, 88.8, 86.4, 72.3, 63.0, 41.9. Anal. calcd. for $C_{14}H_{15}NO_4$: C, 64.35; H, 5.78; N, 5.36. Found: C, 64.28; H, 5.81; N, 5.42.

1-(2-Deoxy-α-D-ribofuranosyl)isocarbostyril (**8**). NaOMe (0.15 g, 2.79 mmol) was added to a suspension of **6** (0.50 g, 0.93 mmol) in MeOH (20 mL), and the reaction was allowed to proceed at 25 °C for 30 min with stirring. The reaction was quenched via addition of NH_4Cl (300 mg), and the solvent was removed *in vacuo* to give a residue that was purified by flash silica gel column chromatography using MeOH-CH_2Cl_2 (1:9, v/v) as eluent to afford **8** (0.23 g, 94%) as a white foam: mp 86–88 °C; ^{1}H NMR (CD_3OD) δ 8.25 (d, $J = 8.2$ Hz, 1H, H-3), 7.69 (d, $J = 7.6$ Hz, 1H, H-8), 7.67 (ddd, $J = 7.6$, 7.0, 1.2 Hz, 1H, H-5), 7.59 (d, $J = 7.6$ Hz, 1H, H-6), 7.47 (ddd, $J = 8.2$, 7.0,

1.2 Hz, 1H, H-4), 6.70 (d, $J = 7.8$ Hz, 1H, H-7), 6.53 (dd, $J = 7.3$, 3.1 Hz, 1H, H-1′), 4.33–4.40 (m, 2H, H-3′, H-4′), 3.68 (dd, $J = 11.9$, 4.3 Hz, 1H, H-5′a), 3.61 (dd, $J = 11.9$, 4.3 Hz, 1H, H-5′b), 2.82 (ddd, $J = 14.3$, 7.3, 7.1 Hz, 1H, H-2′β), 2.10 (ddd, $J = 14.3$, 3.1, 3.0 Hz, 1H, H-2′α); ^{13}C NMR (CD_3OD) δ 163.4, 138.6, 133.7, 128.2, 127.9, 127.8, 127.7, 127.1, 126.4, 107.3, 90.6, 88.4, 72.5, 63.4, 42.5. Anal. calcd. for $C_{14}H_{15}NO_4$: C, 64.35; H, 5.78; N, 5.36. Found C, 64.26; H, 5.73; N, 5.30.

1-[3,5-Bis-*O*-(*p*-chlorobenzoyl)-β-D-ribofuranosyl]-4,7-dinitroisocarbostyril (**9**). Trifluoroacetic anhydride (10 mL) and ground NH_4NO_3 (0.45 g, 5.58 mmol) were added in aliquots to a solution of **5** (1.0 g, 1.86 mmol) in dry CH_2Cl_2 (10 mL), and the reaction was allowed to proceed at 25 °C for 1 h with stirring. The resultant yellow solution was poured onto cold water (100 mL), neutralized with $NaHCO_3$, and extracted with CH_2Cl_2 (3×100 mL). The combined organic extracts were washed consecutively with water (2×100 mL), and then brine (2×50 mL), prior to drying (Na_2SO_4). The solvent was removed *in vauco*, and the residue was purified via flash silica gel column chromatography using hexane-EtOAc (5:1, v/v) as eluent to give the bis-3,5-*O*-ester (0.24 g, 21%) directly as pale yellow crystals: mp 170–171 °C; ^{1}H NMR ($CDCl_3$) δ 9.25 (d, $J = 2.4$ Hz, 1H, H-3), 9.09 (s, 1H, H-8), 8.85 (d, $J = 9.2$ Hz, 1H, H-6), 8.61 (dd, $J = 9.2$, 2.4 Hz, 1H, H-5), 7.88–8.04 (m, 4H, *ortho*-benzoyl hydrogens), 7.30–7.49 (m, 4H, *meta*-benzoyl hydrogens), 6.59 (dd, $J = 7.6$, 5.8 Hz, 1H, H-1′), 5.69 (d, $J = 6.7$ Hz, 1H, H-3′), 4.83–4.89 (m, 3H, H-4′, H-5′), 3.15 (ddd, $J = 13.4$, 5.8, 1.2 Hz, 1H, H-2′α), 2.41 (ddd, $J = 13.4$, 7.6, 7.0 Hz, 1H, H-2′β); ^{13}C NMR ($CDCl_3$) δ 165.04, 165.01, 159.6, 146.8, 140.4, 140.0, 134.4, 133.1, 131.1, 130.7, 129.0, 128.8, 128.2, 127.4, 127.2, 125.6, 124.7, 124.3, 87.6, 84.0, 75.1, 64.1, 39.6. NaOMe (39 mg, 0.72 mmol) was added to a suspension of the bis-3,5-*O*-ester obtained above (0.14 g, 0.24 mmol) in MeOH (5.5 mL), and the reaction was allowed to proceed with stirring at 25 °C for 30 min. The reaction was quenched via addition of NH_4Cl (0.15 g), the solvent was removed *in vacuo*, and the residue obtained was purified via flash silica gel column chromatography using MeOH-CH_2Cl_2 (1:9, v/v) as eluent to afford **9** (70 mg, 89.5%) directly as light yellow crystals: mp 175–176 °C; ^{1}H NMR (DMSO-d_6) δ 9.57 (s, 1H, H-8), 8.93 (d, $J = 2.4$ Hz, 1H, H-3), 8.75 (d, $J = 9.1$ Hz, 1H, H-6), 8.66 (dd, $J = 9.1$, $J = 2.4$ Hz, 1H, H-5), 6.37 (t, $J = 5.9$ Hz, 1H, H-1′), 5.36 (d, $J = 4.3$ Hz, 1H, 3′-O*H*), 5.27 (t, $J = 4.5$ Hz, 1H, 5′-O*H*), 4.28–4.38 (m, 1H, H-3′), 3.96–4.03 (m, 1H, H-4′), 3.60–3.72 (m, 2H, H-5′a, H-5′b), 2.40–2.50 (m, 1H, H-2′α), 2.27 (ddd, $J = 12.0$, 6.1, 5.9 Hz, 1H, H-2′β); ^{13}C NMR (DMSO-d_6) δ 159.4, 145.8, 137.3, 133.0, 127.9, 127.7, 125.0, 124.0, 123.0, 88.3, 86.8, 69.5, 60.3, 41.0. Anal. calcd. for $C_{14}H_{13}N_3O_8$: C, 47.87; H, 3.73; N, 11.96. Found: C, 47.84; H, 3.63; N, 11.82.

1-[3,5-Bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl]-7-iodoisocarbostyril (**10**). A solution of ICl (3.04 g, 18.4 mmol) in CH_2Cl_2 (30 mL) was added dropwise to a solution of **5** (8.0 g, 14.88 mmol) in dry CH_2Cl_2 (96 mL)

with stirring, the reaction mixture was heated to reflux temperature, and then cooled to 25 °C. Saturated $NaHCO_3$ (200 mL) was added followed by the drop wise addition of saturated $Na_2S_2O_3$ until a clear solution was maintained. Extraction with CH_2Cl_2 (500 mL), washing the aqueous fraction with CH_2Cl_2 (2 × 200 mL), drying the combined CH_2Cl_2 extracts (Na_2SO_4), and removal of the solvent *in vacuo* gave a residue that was purified by flash silica gel column chromatography. Elution with hexane-EtOAc (7:1, v/v) as eluent afforded a pale yellow oil (10.8 g) which was recrystallized from CH_2Cl_2-hexane to give **10** (8.64 g, 87%) as white crystals: mp 145–146 °C; ^{1}H NMR ($CDCl_3$) δ 8.44 (dd, $J = 7.9$, 1.2 Hz, 1H, H-3), 8.01–8.05 (m, 4H, *ortho*-benzoyl hydrogens), 7.85 (s, 1H, H-8), 7.72–7.82 (m, 1H, H-5), 7.65 (dd, $J = 7.6$, 1.2 Hz, 1H, H-6), 7.57 (t, $J = 7.9$ Hz, 1H, H-4), 7.40–7.49 (m, 4H, *meta*-benzoyl hydrogens), 6.80 (dd, $J = 8.5$, 5.5 Hz, 1H, H-1′), 5.66 (d, $J = 6.7$ Hz, 1H, H-3′), 4.74–4.84 (m, 2H, H-5′), 4.60–4.66 (m, 1H, H-4′), 2.93 (ddd, $J = 14.1$, 5.5, 1.5 Hz, 1H, H-2′α), 2.36 (ddd, $J = 14.1$, 8.5, 6.7 Hz, 1H, H-2′β); ^{13}C NMR ($CDCl_3$) δ 165.1, 165.0, 160.8, 140.1, 139.8, 136.6, 133.5, 131.6, 130.9, 128.9, 127.9, 127.6, 127.4, 126.0, 123.3, 85.5, 82.8, 75.4, 72.6, 64.4, 38.8. Anal. calcd. for $C_{28}H_{20}Cl_2INO_6$: C, 50.62; H, 3.03; N, 2.10. Found: C, 50.42; H, 3.00; N, 2.14.

1-(2-Deoxy-β-D-ribofuranosyl)-7-iodoisocarbostyril (**11**). NaOMe (488 mg, 9.04 mmol) was added to a suspension of **10** (2.0 g, 3.01 mmol) in MeOH (68 mL), and reaction was allowed to proceed at 25 °C for 30 min with stirring. The reaction was quenched via addition of NH_4Cl (1.5 g), the solvent was removed *in vacuo*, and the residue obtained was purified via flash silica gel column chromatography using MeOH-CH_2Cl_2 (1:9, v/v) as eluent to yield **11** (0.95 g, 82%) directly as white crystals: mp 158–159 °C; ^{1}H NMR (CD_3OD) δ 8.30 (s, 1H, H-8), 8.25 (d, $J = 7.9$ Hz, 1H, H-3), 7.70 (dd, $J = 7.9$, 7.0 Hz, 1H, H-5), 7.69 (d, $J = 7.9$ Hz, 1H, H-6), 7.58 (dd, $J = 7.9$, 7.0 Hz, 1H, H-4), 6.62 (dd, $J = 6.7$, 6.4 Hz, 1H, H-1′), 4.40–4.48 (m, 1H, H-3′), 3.98–4.05 (m, 1H, H-4′), 3.85 (dd, $J = 11.9$, 3.3 Hz, 1H, H-5′a), 3.76 (dd, $J = 11.9$, 3.7 Hz, 1H, H-5′b), 2.40–2.50 (complex m, 1H, H-2′α), 2.20 (ddd, $J = 13.7$, 6.7, 6.4 Hz, 1H, H-2′β); ^{13}C NMR (CD_3OD) δ 162.6, 138.4, 134.7, 131.5, 128.9, 128.8, 128.7, 124.2, 89.0, 86.6, 72.8, 72.0, 62.6, 42.3. Anal. calcd. for $C_{14}H_{14}INO_4$: C, 43.43; H, 3.64; N, 3.61. Found: C, 43.32; H, 3.69; N, 3.72.

1-(3,5-Bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl]-7-trifluoromethylisocarbostyril (**12**). A mixture of **10** (1.0 g, 1.51 mmol), CuI (0.43 g, 2.26 mmol), and anhydrous KF (0.13 g, 2.26 mmol) in dry DMF (5 mL) was stirred under argon as the temperature was increased. When the reaction temperature reached 60 °C, $ClCF_2CO_2Me$ (0.32 mL, 3.01 mmol) was added, and the reaction was allowed to proceed at 120 °C for 6 h under an argon atmosphere. After cooling to 25 °C, the reaction mixture was poured onto ice-water (100 mL), the solution was filtered, and the residue was washed with CH_2Cl_2 (3 × 50 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined CH_2Cl_2 extracts were washed with water

(100 mL), the organic fraction was dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue obtained was purified via flash silica gel column chromatography using hexane-EtOAc (9:1, v/v) as eluent to give **12** (0.65 g, 71%) as a white foam: mp 85–86 °C; ^{1}H NMR ($CDCl_3$) δ 8.46 (d, $J = 7.9$ Hz, 1H, H-3), 7.90–8.04 (m, 4H, *ortho*-benzoyl hydrogens), 7.96 (s, 1H, H-8), 7.72–7.84 (m, 2H, H-5, H-6), 7.62 (ddd, $J = 8.2$, 7.9, 4.0 Hz, 1H, H-4), 7.36–7.50 (m, 4H, *meta*-benzoyl hydrogens), 6.71 (dd, $J = 8.2$, 5.5 Hz, 1H, H-1′), 5.63 (d, $J = 6.4$ Hz, 1H, H-3′), 4.73–7.85 (m, 2H, H-5′), 4.64–4.70 (m, 1H, H-4′), 2.99 (dd, $J = 14.6$, 5.5 Hz, 1H, H-2′α), 2.32 (ddd, $J = 14.6$, 8.2, 6.7 Hz, 1H, H-2′*β*); ^{19}F NMR ($CDCl_3$) δ 100.79 (s, CF_3).

1-(2-Deoxy-*β*-D-ribofuranosyl)-7-trifluoromethylisocarbostyril (**13**). A solution of NaOMe in methanol (6 mL of 0.4 M) was added to **12** (0.60 g, 1 mmol), and the reaction was allowed to proceed with stirring at 25 °C for 30 min. The reaction was quenched via addition of NH_4Cl (0.5 g), the solvent was removed *in vacuo*, and the residue was purified via flash silica gel column chromatography using MeOH-CH_2Cl_2 (1:9, v/v) as eluent to give **13** (0.32 g, 97%) directly as white crystals: mp 163–164 °C; ^{1}H NMR (CD_3OD) δ 8.53 (s, 1H, H-8), 8.35 (d, $J = 8.2$ Hz, 1H, H-3), 7.74–7.83 (m, 2H, H-5, H-6), 7.59 (ddd, $J = 8.2$, 7.9, 3.2 Hz, 1H, H-4), 6.61 (t, $J = 6.4$ Hz, 1H, H-1′), 4.42–4.50 (m, 1H, H-3′), 4.01–4.08 (m, 1H, H-4′), 3.89 (dd, $J = 11.9$, 3.0 Hz, 1H, H-5′a), 3.77 (dd, $J = 11.9$, 3.3 Hz, 1H, H-5′b), 2.50 (ddd, $J = 13.1$, 6.4, 4.0 Hz, 1H, H-2′α), 2.25 (ddd, $J = 13.1$, 6.7, 6.4 Hz, 1H, H-2′*β*); ^{13}C NMR (CD_3OD) δ 162.8, 134.5, 132.8, 130.1 (q, $J = 5.5$ Hz, *C*-8), 129.9, 129.8, 126.5, 125.6 (q, $J = 270.3$ Hz, *C*F_3), 124.2, 107.7 (q, $J =$ 31.8 Hz, *C*-7), 89.2, 87.1, 72.0, 62.4, 42.5; ^{19}F NMR (CD_3OD) δ 103.59 (s, CF_3). Anal. calcd. for $C_{15}H_{14}F_3NO_4$: C, 54.71; H, 4.28; N, 4.25. Found: C, 54.74; H, 4.20; N, 4.14.

1-[3,5-Bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-*β*-D-ribofuranosyl]-7-cyanoisocarbostyril (**14**). Compound **10** (1.0 g, 1.51 mmol) was added to $Zn(CN)_2$ (107 mg, 0.91 mmol) and $(Ph_3P)_4Pd$ (70 mg, 0.06 mmol) in dry DMF (2 mL), and the yellow slurry was heated at 80–90 °C under an argon atmosphere with stirring for 6 h. The reaction mixture was cooled to 25 °C, toluene (100 mL) was added, the mixture was washed with 2N NH_4OH (2 × 50 mL) and then brine (50 mL), and the organic fraction was dried (Na_2SO_4). Removal of the solvent *in vacuo* gave a residue that was purified via flash silica gel column chromatography using hexane-EtOAc (6:1, v/v) as eluent to give **14** (0.71 g, 84%) as white crystals (hexane-CH_2Cl_2): mp 185–186 °C; IR (KBr): 2221 (CN), 1726, 1669 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.34 (d, $J = 7.9$ Hz, 1H, H-3), 8.04 (s, 1H, H-8), 7.87–7.95 (m, 4H, *ortho*-benzoyl hydrogens), 7.68–7.78 (m, 2H, H-5, H-6), 7.55 (ddd, $J = 7.9$, 5.5, 2.7 Hz, 1H, H-4), 7.31–7.42 (m, 4H, *meta*-benzoyl hydrogens), 6.54 (dd, $J = 7.9$, 5.5 Hz, 1H, H-1′), 5.55 (dd, $J = 6.4$, 1.5 Hz, 1H, H-3′), 4.64–4.81 (m, 2H, H-5′), 4.54–4.62 (m, 1H, H-4′), 2.94 (ddd, $J = 13.1$, 5.5, 1.5 Hz, 1H, H-2′α), 2.17 (ddd, $J = 13.1$, 7.9, 6.7 Hz, 1H, H-2′*β*); ^{13}C NMR ($CDCl_3$) δ 165.1, 164.9,

160.4, 140.2, 140.0, 134.6, 133.9, 132.9, 131.0, 130.8, 130.7, 129.0, 128.8, 128.6, 128.1, 127.4, 127.3, 124.8, 124.0, 123.9, 115.3, 91.8, 86.5, 83.3, 75.1, 64.0, 39.1.

1-(2-Deoxy-β-D-ribofuranosyl)-7-cyanoisocarbostyril (**15**). NaOMe (75 mg, 1.38 mmol) was added to a suspension of **14** (0.26 g, 0.46 mmol) in MeOH (8 mL), and the mixture was stirred at 25 °C for 30 min. The reaction was quenched via addition of NH_4Cl (0.3 g) and the solvent was removed *in vacuo* to give a residue that was purified via flash silica gel column chromatography using MeOH-CH_2Cl_2 (1:19, v/v) as eluent to afford **15** (0.13 g, 98%) directly as white crystals: mp 140–141 °C; IR (KBr): 2232 (CN), 1722 (C=O) cm^{-1}; ^1H NMR (CD_3OD) δ 8.65 (s, 1H, H-8), 8.28 (dd, $J = 7.9$, 1.2 Hz, 1H, H-3), 7.86 (ddd, $J = 8.2$, 7.9, 1.5 Hz, 1H, H-5), 7.75 (dd, $J = 7.9$, 1.2 Hz, 1H, H-6), 7.60 (ddd, $J = 8.2$, 7.9, 1.2 Hz, 1H, H-4), 6.51 (dd, $J = 6.4$, 6.1 Hz, 1H, H-1′), 4.42–4.48 (m, 1H, H-3′), 4.18–4.25 (m, 1H, H-4′), 3.88 (dd, $J = 12.2$, 3.0 Hz, 1H, H-5′a), 3.77 (dd, $J = 12.2$, 3.3 Hz, 1H, H-5′b), 2.52 (ddd, $J = 13.7$, 6.1, 3.7 Hz, 1H, H-2′α), 2.20 (dt, $J = 13.7$, 6.4 Hz, 1H, H-2′β); ^{13}C NMR (CD_3OD) δ 162.1, 138.2, 135.0, 134.6, 129.4, 128.7, 125.9, 124.5, 116.7, 91.8, 89.3, 87.4, 71.7, 62.3, 42.6. Anal. calcd. for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.92; N, 9.78. Found: C, 62.87; H, 4.85; N, 9.53.

1-(2-Deoxy-β-D-ribofuranosyl)-7-(*E*)-(2-trimethylsilylvinyl)isocarbostyril (**17**). $(Ph_3P)_2PdCl_2$ (102 mg, 0.145 mmol) was added to a mixture of **11** (0.56 g, 1.45 mmol) and (*E*)-1-trimethylsilyl-2-tributylstannylethene (1.78 g, 3 mmol) in dry MeCN (22 mL) under argon, and the mixture was stirred vigorously at 60 °C for 24 h. Removal of the solvent *in vacuo* gave a residue that was purified via flash silica gel column chromatography using hexane-EtOAc (1:3, v/v) as eluent to give **17** (0.43 g, 83%) as white crystals (hexane-CH_2Cl_2): mp 132-133 °C; ^1H NMR ($CDCl_3$) δ 8.41 (d, $J = 8.2$ Hz, 1H, H-3), 7.76 (d, $J = 7.6$ Hz, 1H, H-6), 7.67 (dd, $J = 8.5, 6.7$ Hz, 1H, H-5), 7.57, (s, 1H, H-8), 7.48 (dd, $J = 8.2, 6.7$ Hz, 1H, H-4), 7.10 (d, $J = 18.9$ Hz, 1H, C*H*=CHTMS), 6.54 (dd, $J = 7.0, 6.4$ Hz, 1H, H-1′), 6.32 (d, $J = 18.9$ Hz, 1H, CH=C*H*TMS), 4.65–4.72 (m, 1H, H-3′), 4.16–4.22 (m, 1H, H-4′), 4.02–4.10 and 3.65–3.74 (two m, 1H each, 3′-O*H*, 5′-O*H*), 3.88–4.00 (m, 2H, H-5′), 2.47–2.58 (m, 2H, H-2′), 0.18 (s, 9H, Si*Me*$_3$); ^{13}C NMR ($CDCl_3$) δ 161.7, 136.9, 135.2, 132.6, 132.1, 128.2, 126.9, 125.2, 124.9, 122.5, 117.9, 87.9, 87.3, 71.7, 62.6, 40.4, −1.1.

1-(2-Deoxy-β-D-ribofuranosyl)-7-(*E*)-(2-iodovinyl)isocarbostyril (18). After dissolution of **17** (0.25 g, 0.7 mmol) in dry MeCN (12 mL), ICl (114 mg, 0.7 mmol) was added immediately, and the reaction was allowed to proceed at 0 °C for 15 min with stirring. Removal of the solvent *in vauco* gave a residue that was purified by flash silica gel column chromatography using hexane-EtOAc (1:3, v/v) as eluent to give a pale yellow oil which was recrystallized from CH_2Cl_2-hexane to yield **18** (0.20 g, 70%) directly as white crystals: mp 125–126 °C; ^1H NMR (CD_3OD) δ 8.35 (d, $J = 8.2$ Hz, 1H, H-3), 8.01 (s, 1H, H-8), 7.72–7.85 (m, 2H, H-5, H-6), 7.71 (d, $J = 14.7$ Hz,

1H, C*H*=CHI), 7.52–7.62 (m, 1H, H-4), 6.93 (d, $J = 14.7$ Hz, 1H, CH=C*H*I), 6.66 (dd, $J = 6.7, 6.4$ Hz, 1H, H-1′), 4.42–4.50 (m, 1H, H-3′), 3.97–4.02 (m, 1H, H-4′), 3.86 (dd, $J = 11.9, 3.0$ Hz, 1H, H-5 a), 3.77 (dd, $J = 11.9, 3.7$ Hz, 1H, H-5 b), 2.44 (ddd, $J = 13.4, 6.7, 6.2$ Hz, 1H, H-2′α), 2.24 (ddd, $J = 13.4, 6.7, 6.2$ Hz, 1H, H-2′*β*); ^{13}C NMR (CD_3OD + DMSO-d_6) δ 162.5, 139.9, 135.7, 134.1, 128.8, 127.9, 126.1, 126.0, 124.1, 117.8, 89.1, 86.5, 78.7, 71.8, 63.2, 42.3. Anal. calcd. for $C_{16}H_{16}INO_4$: C, 46.50; H, 3.90; N, 3.38. Found: C, 46.46; H, 3.75; N, 3.15.

1-[3,5-Bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-*β*-D-ribofuranosyl)-7-(2-trimethylsilylethynyl)isocarbostyril (**19**). A mixture of **10** (2.0 g, 3.01 mmol), CuI (90 mg), $(Ph_3P)_2PdCl_2$ (110 mg), and (trimethylsilyl)acetylene (0.85 mL, 6.02 mmol) in dry Et_3N (180 mL) was stirred at 50–60 °C for 5 h. The solvent was removed *in vacuo*, the residue was dissolved in CH_2Cl_2, washed with saturated aqueous ethylenediaminetetraacetic acid disodium salt and then brine, the organic fraction was dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue obtained was purified via flash silica gel column chromatography using hexane-EtOAc (7:1, v/v) as eluent to give **19** (1.5 g,78%) as white crystals (hexane-CH_2Cl_2): mp 102–103 °C; ^{1}H NMR ($CDCl_3$) δ 8.44 (d, $J = 7.9$ Hz, 1H, H-3), 8.00–8.05 (m, 4H, *ortho*-benzoyl hydrogens), 7.94 (d, $J = 7.6$ Hz, 1H, H-6), 7.82 (s, 1H, H-8), 7.76 (dd, $J = 7.6, 7.0$ Hz, 1H, H-5), 7.60 (dd, $J = 7.9, 7.0$ Hz, 1H, H-4), 7.41–7.49 (m, 4H, *meta*-benzoyl hydrogens), 6.80 (dd, $J = 8.2, 5.5$ Hz, 1H, H-1′), 5.64 (br d, $J = 6.7$ Hz, 1H, H-3′), 4.85 (dd, $J = 12.2, 3.7$ Hz, 1H, H-5′a), 4.72 (dd, $J = 12.2, 3.4$ Hz, 1H, H-5′b), 4.60–4.65 (m, 1H, H-4′), 2.94 (dd, $J = 14.4, 5.5$ Hz, 1H, H-2′α), 2.32 (ddd, $J = 14.4, 8.2, 7.9$ Hz, 1H, H-2′*β*), 0.26 (s, 9H, Si*Me*$_3$); ^{13}C NMR ($CDCl_3$) δ 165.1, 164.9, 160.7, 140.4, 140.0, 139.7, 135.5, 133.0, 131.0, 130.8, 130.0, 128.86, 128.82, 127.7, 127.5, 124.9, 122.1, 102.1, 98.9, 98.3, 85.6, 82.7, 75.4, 64.5, 38.7, 0.01.

1-(2-Deoxy-*β*-D-ribofuranosyl)-7-ethynylisocarbostyril (**20**). A solution of NaOMe in methanol (18.5 mL of 0.2 N) was added to **19** (0.50 g, 0.79 mmol), and the reaction was allowed to proceed with stirring at 25 °C for 20 min. The reaction was quenched via addition of NH_4Cl (0.5 g) and the solvent was removed *in vacuo*. Purification of the residue via flash silica gel column chromatography using MeOH-CH_2Cl_2 (1:19, v/v) as eluent afforded **20** (0.22 g, 98%) directly as white crystals: mp 170–171 °C; ^{1}H NMR (CD_3OD) δ 8.30 (d, $J = 8.2$ Hz, 1H, H-3), 8.15 (s, 1H, H-8), 7.94 (d, $J = 7.9$ Hz, 1H, H-6), 7.76 (ddd, $J = 8.5, 7.9, 1.5$ Hz, 1H, H-5), 7.54 (ddd, $J = 8.5, 8.2, 0.9$ Hz, 1H, H-4), 6.62 (dd, $J = 6.7, 6.4$ Hz, 1H, H-1′), 4.39–4.47 (m, 1H, H-3′), 3.98–4.02 (m, 1H, H-4′), 3.84 (dd, $J = 12.2, 3.4$ Hz, 1H, H-5′a), 3.76 (dd, $J = 12.2, 3.7$ Hz, 1H, H-5′b), 3.73 (s, 1H, *H*-C≡C), 2.41 (ddd, $J = 13.7, 6.4, 3.7$ Hz, 1H, H-2′α), 2.21 (ddd, $J = 13.7, 6.7, 6.7$ Hz, 1H, H-2′*β*); ^{13}C NMR (CD_3OD) δ 165.0, 139.7, 136.8, 135.9, 131.1, 131.0, 128.4, 128.3, 104.6, 91.6, 89.3, 84.9, 74.7, 65.3, 44.8. Anal. calcd. for $C_{16}H_{15}NO_4$: C, 67.35; H, 5.29; N, 4.90. Found: C, 67.30; H, 5.32; N, 4.91.

1-[3,5-Bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl]-7-iodoethynylisocarbostyril (**21**). *N*-Iodosuccinimide (214 mg, 0.95 mmol) and ground $AgNO_3$ (9 mg, 0.053 mmol) were added to a solution of **19** (0.50 g, 0.79 mmol) in dry DMF (5 mL) under argon at 0 °C with stirring. The reaction vessel was wrapped with aluminium foil to protect from light, and the reaction mixture was stirred at 0 °C for 2 h, and then at 25 °C for 1 h. The mixture was cooled to 0 °C, mixed with cold water (100 mL), and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic fractions were washed with water and then brine, dried (Na_2SO_4), the solvent was removed *in vacuo*, and the residue was purified via flash silica gel column chromatography using hexane-EtOAc (6:1, v/v) as eluent to give **21** (0.48 g, 88%) as white crystals (hexane-CH_2Cl_2): mp 165-166 °C; ^{1}H NMR ($CDCl_3$) δ 8.40 (d, $J = 7.7$ Hz, 1H, H-3), 7.94 (s, 1H, H-8), 7.85–7.95 (m, 4H, *ortho*-benzoyl hydrogens), 7.49–7.72 (m, 2H, H-5, H-6), 7.42–7.49 (m, 4H, *meta*-benzoyl hydrogens), 7.30–7.40 (m, 1H, H-4), 6.68–7.70 (m, 1H, H-1′), 5.52–5.61 (m, 1H, H-3′), 4.48–4.82 (m, 3H, H-5′, H-4′), 2.80–3.02 (m, 1H, H-2′α), 2.20–2.35 (m, 1H, H-2′β).

1-(2-Deoxy-β-D-ribofuranosyl)-7-iodoethynylisocarbostyril (**23**). A solution of NaOMe in MeOH (2.2 mL of 0.4 N) was added to **21** (0.25 g, 0.36 mmol) and the reaction was allowed to proceed at 25 °C for 15 min. The reaction was quenched via addition of NH_4Cl (0.30 g), the solvent was removed *in vacuo*, and the residue was purified by flash silica gel column chromatography using MeOH-CH_2Cl_2 (1:19, v/v) as eluent to give **23** (0.14 g, 94%) directly as white crystals: mp 157–158 °C; ^{1}H NMR (CD_3OD) δ 8.29 (d, $J = 8.2$ Hz, 1H, H-3), 8.07 (s, 1H, H-8), 7.84 (d, $J = 7.6$ Hz, 1H, H-6), 7.73 (ddd, $J = 8.2$, 7.9, 1.2 Hz, 1H, H-5), 7.54 (ddd, $J = 8.2$, 7.9, 1.2 Hz, 1H, H-4), 6.60 (dd, $J = 6.7$, 6.4 Hz, 1H, H-1′), 4.35–4.46 (m, 1H, H-3′), 3.98–4.01 (m, 1H, H-4′), 3.84 (dd, $J = 12.2$, 3.4 Hz, 1H, H-5′a), 3.76 (dd, $J = 12.2$, 3.9 Hz, 1H, H-5′b), 2.45 (ddd, $J = 13.4$, 6.4, 3.4 Hz, 1H, H-2′α), 2.20 (ddd, $J = 13.4$, 7.0, 6.7 Hz, 1H, H-2′β); ^{13}C NMR (CD_3OD) δ 162.3, 137.3, 134.3, 133.5, 128.6, 128.5, 125.7, 103.6, 89.0, 88.4, 86.7, 72.1, 62.7, 42.3, 14.1. Anal. calcd. for $C_{16}H_{14}INO_4$: C, 46.73; H, 3.43; N, 3.40. Found: C, 46.87; H, 3.39; N, 3.44.

1-[3,5-Bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl]-7-(2-bromoethynyl)isocarbostyril (**22**). *N*-Bromosuccinimide (0.16 g, 0.9 mmol) and ground $AgNO_3$ (9 mg, 0.053 mmol) were added to a solution of **19** (0.45 g, 0.71 mmol) in dry DMF (5 mL) under argon at 0 °C. The reaction vessel was wrapped with aluminium foil to protect from light, and the reaction mixture was stirred at 0 °C for 2 h and then at 25 °C for 4 h. The reaction mixture was cooled to 0 °C, mixed with cold-water (100 mL), and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were washed with water and then brine, the organic fraction was dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue was purified by flash silica gel column chromatography using hexane-EtOAc (6:1, v/v) as eluent to give **22** (0.34 g, 75%)

as white crystals (hexane-CH_2Cl_2): mp 151–152 °C. Product **22** was hydrolzyed to **24** as indicted below.

1-(2-Deoxy-β-D-ribofuranosyl)-7-(2-bromoethynyl)isocarbostyril (**24**). A solution of NaOMe in MeOH (1.6 mL of 0.4 N) was added to **22** (0.17 g, 0.265 mmol) with stirring and the reaction was allowed to proceed at 25 °C for 15 min. The reaction was quenched via addition of NH_4Cl (0.15 g) and the solvent was removed *in vacuo*. Purification of the residue by flash silica gel column chromatography using MeOH-CH_2Cl_2 (1:19, v/v) as eluent yielded **24** (87 mg, 90%) directly as white crystals: mp 127–128 °C; ^{1}H NMR (CD_3OD) δ 8.76 (s, 1H, H-8), 8.70 (d, $J = 8.2$ Hz, 1H, H-3), 8.27 (dd, $J = 7.9$, 1.2 Hz, 1H, H-6), 7.69 (ddd, $J = 7.9$, 7.0, 1.5 Hz, 1H, H-5), 7.48 (ddd, $J = 8.2$, 7.0, 0.9 Hz, 1H, H-4), 6.50 (dd, $J = 6.4$, 6.1 Hz, 1H, H-1′), 4.36–4.44 (m, 1H, H-3′), 3.97–4.05 (m, 1H, H-4′), 3.74–3.90 (m, 2H, H-5′), 2.47 (ddd, $J = 13.7$, 6.4, 4.0 Hz, 1H, H-2′α), 2.18 (ddd, $J = 13.7$, 6.7, 6.1 Hz, 1H, H-2′β); ^{13}C NMR (CD_3OD) δ 167.0, 136.4, 135.4, 134.3, 128.4, 128.1, 126.3, 126.2, 126.0, 108.1, 89.1, 87.2, 72.0, 62.7, 52.2, 42.4. Anal. calcd. for $C_{16}H_{14}BrNO_4$: C, 52.76; H, 3.87; N, 3.84. Found: C, 52.82; H, 3.76; N, 3.69.

1-[3,5-Bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-β-D-ribofuranosyl]-7-propynylisocarbostyril (**25**). Dry Et_3N (70 mL), $(Ph_3P)_2PdCl_2$ (53 mg, 0.075 mmol), and CuI (0.057 g, 0.3 mmol) were added to **10** (1.0 g, 1.51 mmol), the mixture was cooled to −78 °C, and propyne was added until a final volume of 93 mL was reached. The reaction vessel was sealed, and the reaction was allowed to proceed at 25 °C with stirring for 5 h. Excess propyne was allowed to escape by venting the reaction vessel, and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (200 mL) and consecutively extracted with 5% EDTA (2 × 50 mL), water (100 mL) and then brine (50 mL). The organic fraction was dried (Na_2SO_4), the solvents were removed *in vacuo*, and the residue was purified by flash silica gel column chromatography using hexane-EtOAc (7:1, v/v) as eluent to give **25** (0.59 g, 68%) as a white foam: mp 125–126 °C; ^{1}H NMR ($CDCl_3$) δ 8.42 (d, $J = 7.9$ Hz, 1H, H-3), 7.98–8.04 (m, 4H, *ortho*-benzoyl hydrogens), 7.92 (d, $J = 7.6$ Hz, 1H, H-6), 7.73 (ddd, $J = 8.2$, 7.6, 1.5 Hz, 1H, H-5), 7.66 (s, 1H, H-8), 7.54 (dd, $J = 8.2$, 7.9 Hz, 1H, H-4), 7.38–7.47 (m, 4H, *meta*-benzoyl hydrogens), 6.84 (dd, $J = 8.2$, 5.7 Hz, 1H, H-1′), 5.62–5.66 (m, 1H, H-3′), 4.79 (dd, $J = 12.2$, 3.7 Hz, 1H, H-5′a), 4.71 (dd, $J = 12.2$, 3.4 Hz, 1H, H-5′b), 4.56–4.62 (m, 1H, H-4′), 2.86 (ddd, $J = 14.3$, 5.7, 1.5 Hz, 1H, H-2′α), 2.34 (ddd, $J = 14.3$, 8.2, 7.0 Hz, 1H, H-2′β), 2.01 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 165.1, 164.9, 160.7, 140.0, 139.7, 136.0, 132.7, 131.0, 130.9, 128.8, 128.7, 127.7, 127.5, 127.3, 125.1, 125.0, 102.8, 89.0, 85.2, 82.5, 75.3, 64.5, 38.6, 4.2.

1-(2-Deoxy-β-D-ribofuranosyl)-7-propynylisocarbostyril (**1**). A solution of NaOMe in MeOH (4.2 mL of 0.4 N) was added to **25** (0.4 g, 0.69 mmol) and the reaction was allowed to proceed at 25 °C for 15 min with stirring. The reaction was quenched via addition of NH_4Cl (0.3 g) and the solvent was removed *in vacuo* to give a residue that was purified by flash silica gel column

chromatography using MeOH-CH_2Cl_2 (1:19, v/v) as eluent to afford **1** (186 mg, 90%) as a white foam: mp 145–146 °C; ^{1}H NMR (CD_3OD) δ 8.22–8.32 (m, 1H, H-3), 7.95 (s, 1H, H-8), 7.84 (d, $J = 7.6$ Hz, 1H, H-6), 7.65–7.76 (m, 1H, H-5), 7.52–7.60 (m, 1H, H-4), 6.63 (dd, $J = 7.5, 6.3$ Hz, 1H, H-1′), 4.39–4.48 (m, 1H, H-3′), 3.98–4.04 (m, 1H, H-4′), 3.83 (dd, $J = 12.1$, 3.5 Hz, 1H, H-5′a), 3.76 (dd, $J = 12.1$, 3.7 Hz, 1H, H-5′b), 2.42 (ddd, $J = 13.7$, 6.2, 3.5 Hz, 1H, H-2′α), 2.17 (ddd, $J = 13.7, 7.4, 6.5$ Hz, 1H, H-2′β), 2.10 (s, 3H, CH_3); ^{13}C NMR (CD_3OD) δ 162.4, 138.4, 137.6, 134.7, 131.5, 128.8, 128.2, 126.0, 124.2, 103.9, 90.0, 89.0, 86.7, 72.1, 62.7, 42.1, 3.9. Anal calcd. for $C_{17}H_{17}NO_4$: C, 68.21; H, 5.72; N,4.67. Found: C, 68.43; H, 5.61; N, 4.52.

In Vitro Cell Cytotoxicity (MTT assay). KBALB, KBALB-STK, human 143B, human 143B-LTK and R-970-5 cells were cultured in complete DMEM medium supplemented with 10% fetal bovine serum (FBS), and EMT-6 cells were cultured in complete WAYMOUTH medium in 10% FBS. Exponentially growing cells were trypsinized, centrifuged, resuspended in growth medium, and the cell number was readjusted to 8×10^3 cells/mL. Cells were seeded into 96-well plates at 8×10^2 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 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[20], which depends on the metabolic reduction of MTT to colored formazan, measures cytostatic and cytotoxic effects of the test drug.

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