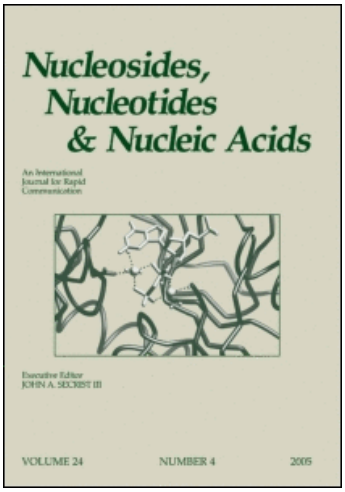

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PYRROLO[2,3-*d*]PYRIMIDINE NUCLEOSIDES: SYNTHESIS AND ANTITUMOR ACTIVITY OF 7-SUBSTITUTED 7-DEAZA-2'-DEOXYADENOSINES

Frank Seela*[,a], Matthias Zulauf[a] and Shih-Fong Chen[b]

Dedicate to the memory of Dr. Gertrude B. Elion

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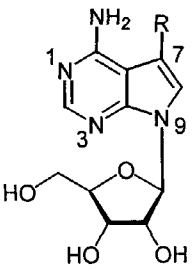
ABSTRACT: A one step synthesis, using the nucleoside 7-iodo-2'-deoxytubercidin (**2b**) in a Pd(0)/Cu(I)-catalyzed cross coupling reaction furnished a series of 7-alkynyl-2'-deoxytubercidin derivatives. The 7-iodo-, 7-chloro- or 7-bromo 2'-deoxytubercidins **2b-d** as well as certain 7-alkynyl derivatives show significant activity against several tumor cell lines, with 7-iodo-2'-deoxytubercidin (**2b**) as the most effective compound.

Introduction

The biological activity of 7-deazapurine (pyrrolo[2,3-*d*]pyrimidine) nucleosides[1,2] has prompted us to evaluate the antitumor activity of 7-substituted 7-deaza-2'-deoxyadenosine (2'-deoxytubercidin) derivatives (purine numbering is used throughout the general section). The ribonucleosides tubercidin (**1a**), toyocamycin (**1b**) and sangivamycin (**1c**) are naturally-occurring antibiotics. Also 7-substituted derivatives such as the 7-iodo-7-deaza-5'-deoxytubercidin - isolated from the red alga Hypnea valendiae - and the 7-bromo-7-deazaadenine base - formed by a marine sponge - are of natural origin.[3] The ribonucleosides **1a-c** exhibit significant antitumor activity.[1,2,4-7] The 2'-deoxynucleoside 2'-deoxytubercidin (**2a**) shows reduced activity, but the introduction of a 7-cyano group (2'-deoxytoyocamycin; **2e**) or a 7-carboxamido function (2'-deoxy-

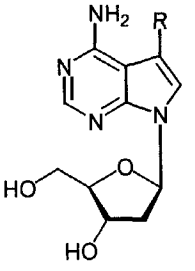
sangivamycin; **2f**) increases the inhibitory effect strongly.[6] The 7-deazaadenine nucleosides including the 7-substituted derivatives are neither deaminated by adenosine deaminase nor cleaved at the glycosylic bond by adenosine phosphorylase.[8] As it is also well known that the introduction of alkenyl and alkynyl residues into pyrimidine or purine nucleosides results in cytostatic, antiviral or other biological activities,[9-14] the synthesis and biological evaluation of 7-substituted 7-deazapurine (pyrrolo[2,3-*d*]-pyrimidine) nucleoside derivatives containing such residues is of considerable interest.

The present study describes the synthesis of 7-alkynylated 7-deaza-2'-deoxyadenosines and the antineoplastic activity of 7-substituted 7-deaza-2'-deoxyadenosines against three different cell lines.

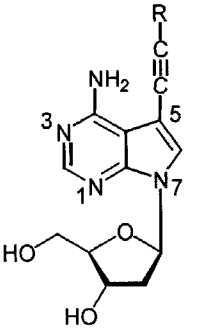


1a R = H
b R = CN
c R = $CONH_2$
d R = I

purine numbering



2a R = H
b R = I
c R = Br
d R = Cl
e R = CN
f R = $CONH_2$
g R = CH_3



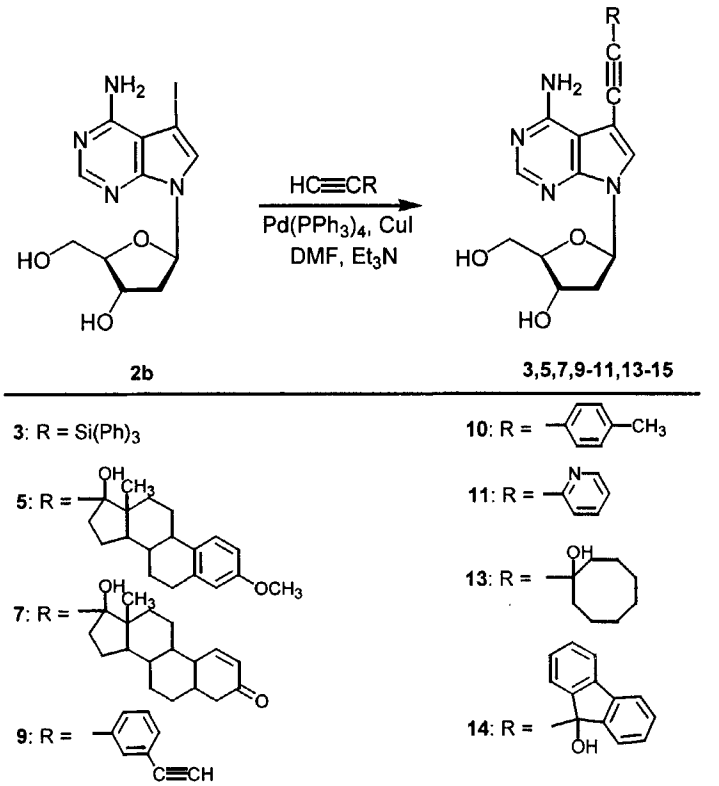
3-15 R = alkyl,aryl

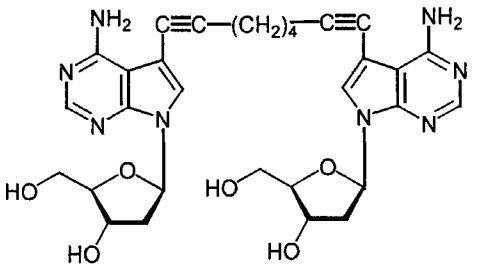
systematic numbering

Results and Discussion

1. Chemistry.- 2'-Deoxytoyocamycin (**2e**) and 2'-deoxytubercidin (**2a**) were prepared as described in [15]. The synthesis of several 7-alkynylated 7-deaza-2'-deoxyadenosines has been reported recently.[16] For the synthesis of the 7-alkynyl derivatives **3,5,7,9-11,13-15** 2'-deoxy-7-iodotubercidin (**2b**)[16] was employed as precursor. Compound **2b** was linked to various terminal alkynes in a Pd(0)-catalyzed cross-coupling reaction.[16,17] The

reaction was performed in DMF with tetrakis(triphenylphosphine)palladium(0), copper(I)iodide, and triethylamine. The reaction was allowed to proceed until TLC indicated that all of the starting material was consumed. The desired 7-alkynyl-2'-deoxy-tubercidins **3,5,7,9-11,13** and **15** were purified by flash-chromatography and isolated as solids or foams.





15

All new compounds were characterized by elemental analyses and/or FAB-mass spectra, ^{1}H- and ^{13}C-NMR (Table 1) spectra. For the assignment of ^{13}C-NMR signals

TABLE 1. ^{13}C-NMR Chemical Shifts of **2b,e,f** and 7-Alkynylated 7-Deazadenine 2'-Deoxy-ß-D-ribofuranosides; Measured in (D_6)DMSO at 25°C.[a]

[b]	C(2)	C(4)	C(4a)	C(5)	C(6)	C(7a)	C(1')	C(3')	C(4')	C(5')
[c]	C(2)	C(6)	C(5)	C(7)	C(8)	C(4)				
2b[16]	152.0	157.3	103.2	51.9	126.9	149.8	83.0	71.0	87.5	62.0
2e[15]	153.0	157.0	101.2	82.9	132.1	149.7	83.8	70.6	87.8	61.6
2f[15]	152.8	158.0	100.9	110.9	125.0	150.5	82.9	70.8	87.5	62.1
3	153.0	157.5	102.4	93.8	128.5	149.3	83.3	70.8	87.6	61.8
5	152.6	157.5	102.3	95.0	125.5	149.1	83.1	70.9	87.5	61.8
7	152.6	157.6	102.3	95.0	125.5	149.1	83.1	70.9	87.5	61.8
9	152.9	157.6	102.2	94.5	127.4	149.6	83.3	71.0	87.7	62.0
10	152.7	157.5	102.1	94.8	126.5	149.3	83.2	70.9	87.5	61.8
11	152.9	157.5	102.2	93.7	127.5	149.5	83.3	70.8	87.6	61.8
13	152.6	157.5	102.3	94.8	125.4	149.1	83.1	70.9	87.5	61.8
14	152.7	157.4	102.3	94.0	126.3	149.2	83.0	70.9	87.5	61.8
15	152.5	157.5	102.3	95.4	125.5	149.0	83.1	70.9	87.4	61.9

[a] C(2') is superimposed by DMSO. [b] Systematic numbering. [c] Purine numbering.

gated-decoupled as well as heteronuclear correlation spectra were used. By attaching an alkynyl group at C-7 the ^{13}C-NMR signal is shifted 40 ppm downfield compared to the 7-iodo compound **2b**. This is due to a unexpected mesomeric effect of the triple bond.

2. Antitumor Evaluation. The growth inhibitory activities of 2'-deoxytubercidin (**2a**) and the 7-substituted derivatives **2b-e**[15,16,19], the 7-methylated **2g**[19] and the 7-alkynylated ß-D-2'-deoxynucleosides **3-15** against three cultured human and murine tumor cell lines were evaluated (Table 2). The 2-chloro-2'-deoxyadenosine ($\mathbf{Cl^2A_d}$)[2], the 2-chloro-7-deaza- 2'-deoxyadenosine ($\mathbf{Cl^2c^7A_d}$)[20], and the ribonucleoside tubercidin (**1a**) were included as reference agents. In general, these compounds demonstrate similar growth inhibitory activity against these three different cell lines.

Tubercidin (**1a**) shows the most potent growth inhibitory activity against the 293 transformed human embryonic kidney cell line with an IC_{50} value of < 0.05 μM. It was interesting to note that 2'-deoxytubercidin (**2a**) shows very little growth inhibitory activity against the 293 cell line with an IC_{50} value of > 2000 μM. However, the growth inhibitory activity of compounds **2b-d** with a halogeno substitution at the 7-position is enhanced significantly. The order of growth inhibitory activity is I > Br > Cl >> H. The growth inhibitory activity of the 7-iodo compound **2b** (IC_{50} values range from 0.88 to 1 μM) is similar to that of the nucleoside 2-chloro-2'-deoxyadenosine ($\mathbf{Cl^2A_d}$) (IC_{50} values range from 0.67-1.7 μM). 2'-Deoxytoyocamycin (**2e**) and compound **2g** with 7-cyano and 7-methyl substitution were not as active as the compounds with 7-halogeno substitution (**2b-d**). The silicon-containing compound **3** - which might be hydrolyzed in the reaction medium to the corresponding acetylene - shows good to modest growth inhibitory activity (IC_{50} values range 2-35 μM). In the series of the alkynyl derivatives, the compounds with an aromatic ring system (**8-10**) show considerable growth inhibitory activity with IC_{50} values ranging from 6.9-21 μM; whereas compound **11** is approximately one order of magnitude less active (IC_{50} values ranging from 37-342 μM). The compounds with a steroid skeleton are also active. Herewith, the estradiol-derivatives **4** and **5** had similar growth inhibitory activity, whereas the testosterone-containing derivative **6** was one order less cytotoxic and compound **7** was two orders less potent than **4** or **5**. The nucleosides **2g, 12** and **13** with non-aromatic alkyl or alkynyl side chains were two orders less active than **4** or **5**. The "bis-tubercidin" derivative **15** exhibits no significant activity.

Regarding the antitumor action of the 2-chloro derivatives of 2'-deoxyadenosine and 7-deaza-2'-deoxyadenosine, the absence of nitrogen-7 abolishes the antineoplastic activity almost completely. This is shown in the three cell lines of Table 2 but has been also demonstrated earlier in the case of acute T-cell leukemia, T-lymphoblastic or myeloma cell lines.[21,22] Interestingly, the incorporation of a halogeno substituent into the position-7 of the rather inactive 7-deaza-2'-deoxyadenosine increases the antitumor activity strongly (Table 2).

Conclusion

The biological activity of 7-deazapurines (pyrrolo[2,3-*d*]pyrimidines) as well as of their nucleosides and nucleotides is well documented.[1,2,4-7] The bases can act as receptor

Table 2. Inhibitory Effects of 7-Substituted 7-Deaza-2'-deoxyadenosines on the Growth of Three Different Cells in vitro

Compd	R	IC_{50}[a] [μM] 293[b]	HT29	P388
1a[c]		<0.05	NT[d]	NT[d]
2a	H	2121	583	NT[d]
2b	I	0.85	0.88	1.0
2c	Br	3.5	2.9	4.8
2d	Cl	12	16	5.2
2e	CN	66	186	154
2g	CH_3	410	377	520
3	2-(triphenylsilyl)ethynyl	2.0	32	35
4	2-(17ß-hydroxy-1,3,5[10]-estratriene-17α-yl)ethynyl	3.6	15	7.0
5	2-(17ß-hydroxy-3-methoxy-1,3,5[10]-estratriene-17α-yl)ethynyl	4.9	18	15
6	2-(17ß-hydroxy-4-androsten-3-one)-17α-yl)ethynyl	49	98	42
7	2-(17ß-hydroxy-19-norandrost-4-en-3-one-17α-yl)ethynyl	498	168	316
8	2-(phenyl)ethynyl	9.3	6.9	10
9	2-(m-ethynylphenyl)ethynyl	10	20	13
10	2-(p-tolyl)ethynyl	21	12	18
11	2-(2-pyridyl)ethynyl	37	342	193
12	hex-1-ynyl	247	699	292
13	2-(1-hydroxy-cyclooctyl)ethynyl	203	249	264
14	2-(9-fluoren-9-oyl)ethynyl	162	462	392
15	1,7-octadiinyl-8-(7-deaza-2'-deoxyadenosine)	1274	332	332
Cl^2A_d		0.67	0.67	1.7
$Cl^2c^7A_d$		977	525	84

[a] Inhibitory dose (IC_{50}) is the concentration of the compound in the culture media that produced 50% inhibition of the tumor cell growth as compared to the untreated controls. [b] Abbreviations are as follows: 293, transformed human embryonic cells; HT29, human colon carcinoma; P388, murine leukemia cells. [c] **1a** = 7-Deazaadenosine (tubercidin). [d] Not tested.

antagonists.[23,24] The nucleosides are inhibitors of kinases and develop antiviral and anticancer activity.[8,25,26] The compounds related to adenine or adenosine are generally more active than those related to guanine or guanosine. The 2'-deoxynucleosides are less active than the ribonucleosides.[7] In many cases the 7-substituents increase the activity.[7] The incorporation of the ribonucleoside tubercidin as well as related 7-substituted derivatives into RNA in vitro has been demonstrated.[7,27-31] Therefore, it can be assumed that the cytotoxicity of these molecules is associated with the RNA incorporation.[7,29] Also 2'-deoxytubercidin and its 7-substituted derivatives are incorporated in high-molecular- weight DNA by various polymerases on a DNA template.[32] From DNA model building it is obvious that the 7-substituents of 7-deazapurines have similar steric requirements as the 5-substituents of pyrimidines; both are well accommodated in the major groove of DNA.[18,33-39] Oligonucleotides which were synthesized chemically become stabilized within the duplex structure.[18,33-36] As a consequence of this, the cytotoxicity of the 7-substituted 7-deaza-2'-deoxyadenosine derivatives might be the result of their incorporation in DNA.

With regard to the anticancer activity the most potent derivatives are the halogeno derivatives **2b-e**. The 7-methyl compound **2g** is significantly less active but shows a somewhat higher activity as the parent 2'-deoxytubercidin (**2a**). The alkynyl compounds show in general a higher activity as the alkyl derivatives. From these observations it can be concluded that the electron-withdrawing character of the 7-substituent plays a significant role in structure-activity relationship. The σ_{para}-Hammett constants of selected substituents are the following: methyl = -0.17 < $C{\equiv}CH_3$ = 0.03 < $C{\equiv}CC_6H_5$ < iodo = 0.18 < bromo, chloro, $C{\equiv}CH$ = 0.23 < carbamoyl < cyano = 0.66.[40]

From conformational studies in solution it is apparent that the electronic properties of 7-substituents have a strong impact on the conformation on the sugar moiety. The higher the electron-withdrawing character of the 7-substituents, the more is the change of the sugar conformer population from S to N.[41] According to the low energy barrier of the conformational changes one cannot expect a steroelectronic influence of the substituents on a chemical reaction. However, when a nucleoside substrate is bound to an enzyme or is incorporated into an oligonucleotide these phenomena become important. The situation

is even more complex as the analogs shown in Table 2 must be converted to their triphosphates, because the cytoxicity is developed at the nucleic acid level Ribonucleosides, such as tubercidin, toyocamycin and sangivamycin are known to be converted to their 5'-triphosphates.[7,27-31] The enzymatic conversion of the 2'-deoxy-nucleosides into monophosphates is documented.[42] From the present study one can conclude that the nature of the 7-substituents of 7-deaza-2'-deoxyadenosine has a definite effect on the antitumor activitity. Least active are the alkyl compounds, alkynyl compounds show an increased activity while the 7-halogenated derivatives, in particular the iodo compound **2b**, shows an activity similar to 2-chloro-2'-deoxyadenosine.

Experimental

General preparations. All chemicals were supplied by Aldrich, Sigma or Fluka (*Sigma-Aldrich Chemie GmbH; Deisenhofen, Germany*). Solvents were of laboratory grade. CHN analyses were performed by Mikroanalytisches Labor Beller (*Göttingen, Germany*). NMR-Spectra were measured on AC 250 or AMX 500 spectrometers (*Bruker, Germany*) operating at proton resonance frequencies of 250.13 MHz and 500.14 MHz (125.13 MHz for ^{13}C), respectively. Chemical shifts are in ppm relative to TMS as internal standard. *J* values are given in Hz. Mps were measured with a Büchi SMP-20 apparatus (*Büchi, Switzerland*); uncorrected. Positive ion Fast Atom Bombardment (*FAB*) mass spectra were provided by Dr. Markus Sauer (*University of Heidelberg, Germany*) with 3-nitrobenzyl alcohol as matrix. UV-spectra were recorded on a U 3200 spectrometer (*Hitachi, Japan*). Thin-layer chromatography (TLC) was performed on aluminium sheets, silica gel 60 F_{254}, 0.2 mm layer (*Merck, Germany*) and column chromatography (*FC*) on silica gel 60 (*Merck, Germany*) at 0.4 bar (4×10^4 Pa) using the following solvent system: (A) CH_2Cl_2-MeOH (9:1, v/v).

Pd-Catalyzed Cross-Coupling; General Procedure. A suspension of 4-amino-7-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine[16] (**2b**) (100 mg, 0.27 mmol) and CuI (10.1 mg, 0.05 mmol) in anh. DMF (3 ml) was treated with the alkyne (10-20 eq.), Et_3N (54 mg, 0.53 mmol), and $Pd(PPh_3)_4$ (31 mg, 0.027 mmol). The mixture was stirred under Ar at r.t. After the reaction was complete (TLC-monitoring),

the mixture was diluted with MeOH/CH_2Cl_2 (20 ml, 1:1) and Dowex 1X8 (100-200 mesh; 500 mg, bicarbonate form) was introduced. After stirring for 45 min the mixture was filtered and the resin was washed twice with MeOH/CH_2Cl_2 (100 ml, 1:1). The combined filtrates were evaporated, and the residue subjected to a silica gel column (12 x 3 cm) using CH_2Cl_2 with an increasing amount of MeOH (2-10%). The main zone afforded the nucleoside derivative upon evaporation.

4-Amino-7-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-5-[2-(triphenylsilyl)ethynyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (3). General procedure with triphenylsilylacetylene (1g, 3.52 mmol): the reaction time was 7 h; slightly yellowish foam (65 mg, 46%): R_f (A) 0.53; UV (MeOH): 284 (18800), 255 (15300), 241 (13300); ^{1}H-NMR (DMSO-d_6): δ 2.22 (m, 1H, H_α-C(2')), 2.48 (m, 1H, $H_ß$-C(2')), 3.56 (m, 2H, H-C(5'$_{\alpha,ß}$)), 3.85 (m, 1H, H-C(4')), 4.36 (m, 1H, H-C(3')), 5.02 (t, J = 5.4, 1H, OH-C(5')), 5.26 (d, J = 3.8, 1H, OH-C(3')), 6.51 ('t', J = 6.9, 1H, H-C(1')), 6.68 (br s, 2H, NH_2); 7.51 (m, H-arom.), 7.65 (m, H-arom.), 8.05 (s, 1H, H-C(6)), 8.17 (s, 1H, H-C(2)). Anal. Calcd for $C_{31}H_{28}N_4O_3Si$ (532.69): C 69.90, H 5.30, N 10.52; Found C 70.04, H 5.39, N 10.59.

4-Amino-7-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-5-[2-(17ß-hydroxy-3-methoxy-1,3,5[10]-estratriene)-17α-yl)ethynyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (5). General procedure with 17α-ethynyl-3-OMe-estradiol (800 mg, 2.58 mmol): the reaction time was 5 h; colourless crystals (MeOH/H_2O) (80 mg, 54%): mp 151-153°C; R_f(A) 0.41; UV (MeOH): 279 (12700); ^{1}H-NMR (DMSO-d_6): δ 0.82 (s, 3H, CH_3), 1.33-2.76 (several m, 11H, H_α-C(2'), $H_ß$-C(2'), H-$C_{steroid}$(6,7,8,9,11,12,14,15,16)), 3.55 (m, 2H, H-C(5'$_{\alpha,ß}$)), 3.67 (s, 3H, OMe), 3.80 (m, 1H, H-C(4')), 4.32 (m, 1H, H-C(3')), 5.05 (t, J = 5.5, 1H, OH-C(5')), 5.24 (d, J = 3.9, 1H, OH-C(3')), 5.66 (s, 1H, OH), 6.46 ('t', J = 7.0, 1H, H-C(1')), 6.59 (br s, 1H, H-$C_{Steroid}$4), 6.65 (d, J = 8.6, 1H, H-C_S2), 6.72 (br s, 2H, NH_2), 7.15 (d, J = 8.6, 1H, H-$C_{Steroid}$1), 7.69 (s, 1H, H-C(6)), 8.10 (s, 1H, H-C(2)). Anal. Calcd for $C_{32}H_{38}N_4O_5$·¼ H_2O (563.18): C 68.25, H 6.89, N 9.95; Found C 67.96, H 6.99, N 9.77.

4-Amino-7-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-5-[2-(17ß-hydroxy-4-estren-3-one)-17α-yl)ethynyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (7). General procedure with 19-Norethisteron (800 mg, 2.68 mmol): the reaction time was 4 h; colourless crystals (MeOH) (77 mg, 53%): mp 168-170°C; R_f (A) 0.32; UV (MeOH): 280 (11000), 240

(28000); ^{1}H-NMR (DMSO-d_6): δ 0.84 (s, 3H, CH_3), 0.96-2.49 (several m, 15H, H_α-C(2'), H_β-C(2'), H-$C_{steroid}$(1,2,4,6,7,8,9,10,11,12,14,15,16)), 3.55 (m, 2H, H-C(5'$_{\alpha,\beta}$)), 3.97 (m, 1H, H-C(4')), 4.31 (m, 1H, H-C(3')), 5.05 (t, J = 5.5, 1H, OH-C(5')), 5.24 (d, J = 3.9, 1H, OH-C(3')), 6.46 ('t', J = 6.7, 1H, H-C(1')), 6.70 (br s, 2H, NH_2), 7.66 (s, 1H, H-C(6)), 8.10 (s, 1H, H-C(2)). Anal. Calcd for $C_{31}H_{38}N_4O_5$ (546.66): C 68.11, H 7.01, N 10.25; Found C 68.34, H 7.21, N 10.63.

4-Amino-7-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-5-[2-(m-ethynylphenyl)-ethynyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (9). General procedure with m-diethynylbenzene (700 mg, 5.55 mmol): the reaction time was 4 h; colourless solid (53 mg, 53%): R_f (A) 0.46; UV (MeOH): 300 (22600), 248 (24100), ^{1}H-NMR (DMSO-d_6): δ 2.22 (m, 1H, H_α-C(2')), 2.48 (m, 1H, H_β-C(2')), 3.57 (m, 2H, H-C(5'$_{\alpha,\beta}$)), 3.86 (m, 1H, H-C(4')), 4.27 (s, 1H, C≡CH), 4.37 (m, 1H, H-C(3')), 5.06 (t, J = 5.5, 1H, OH-C(5')), 5.27 (d, J = 3.9, 1H, OH-C(3')), 6.53 ('t', J = 6.9, 1H, H-C(1')), 6.73 (br s, 2H, NH_2), 7.43-7.63 (m, 3H, H-arom.), 7.73 (s, 1H, H-arom.), 7.91 (s, 1H, H-C(6)), 8.16 (s, 1H, H-C(2)). Anal. Calcd for $C_{21}H_{18}N_4O_3$ (374.4): C 67.37, H 4.85, N 14.96; Found C 67.39, H 4.90, N 14.94.

4-Amino-7-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-5-[2-(p-tolyl)ethynyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (10). General procedure with p-tolylacetylene (350 mg, 3.01 mmol): the reaction time was 4 h; colourless foam (62 mg, 64%): R_f (A) 0.46; UV (MeOH): 295 (19600); ^{1}H-NMR (DMSO-d_6): δ 2.22 (m, 1H, H_α-C(2')), 2.34 (s, 3H, CH_3), 2.48 (m, 1H, H_β-C(2')), 3.58 (m, 2H, H-C(5'$_{\alpha,\beta}$)), 3.86 (m, 1H, H-C(4')), 4.37 (m, 1H, H-C(3')), 5.06 (t, J = 5.3, 1H, OH-C(5')), 5.28 (d, J = 3.9, 1H, OH-C(3')), 6.53 ('t', J = 6.9, 1H, H-C(1')), 6.69 (br s, 2H, NH_2), 7.24 (d, J = 7.8, 2H, H-arom.), 7.48 (d, J = 7.8, 2H, H-arom.), 7.86 (s, 1H, H-C(6)), 8.16 (s, 1H, H-C(2)). Anal. Calcd for $C_{20}H_{20}N_4O_3$ (364.40): C 65.92, H 5.53, N 15.38; Found C 65.04, H 5.41, N 15.51. FAB-MS (3-NOBA): 365.2 $[M+H]^+$.

4-Amino-7-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-5-[2-(2-pyridyl)ethynyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (11). General procedure with 2-ethynylpyridine (300 mg, 2.91 mmol): the reaction time was 4 h; colourless crystals (MeOH) (52 mg, 56%): mp 214-216°C. R_f (A) 0.28; UV (MeOH): 320 (20800), 258 (12700); ^{1}H-NMR (DMSO-d_6): δ 2.20 (m, 1H, H_α-C(2')), 2.48 (m, 1H, H_β-C(2')), 3.54 (m, 2H, H-C(5'$_{\alpha,\beta}$)), 3.82 (m, 1H,

H-C(4')), 4.34 (m, 1H, H-C(3')), 5.03 (t, J = 5.4, 1H, OH-C(5')), 5.23 (d, J = 3.9, 1H, OH-C(3')), 6.49 ('t', J = 6.9, 1H, H-C(1')), 6.74 (br s, 2H, NH_2), 7.35 (m, 1H, H-arom.), 7.63 (d, J = 7.80, 1H, H-arom.), 7.80 (m, 1H, H-arom.), 7.94 (s, 1H, H-C(6)), 8.14 (s, 1H, H-C(2)), 8.56 (d, J = 4.3, 1H, H-arom.). Anal. Calcd for $C_{18}H_{17}N_5O_3$ (351.36): C 61.52, H 4.88, N 19.93; Found C 61.61, H 4.88, N 19.86.

4-Amino-7-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-5-[2-(1-hydroxycyclooctyl)-ethynyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (13). General procedure with 1-ethynylcyclooctanol (500 mg, 3.28 mmol): the reaction time was 4 h; colourless solid (64 mg, 60%). R_f (A) 0.29; UV (MeOH): 279 (11700), 241 (13800); ^{1}H-NMR (DMSO-d_6): δ 1.48 (m, 4H, 2 CH_2), 1.59 (m, 6H, 3 CH_2), 1.91 (m, 4H, 2 CH_2), 2.18 (m, 1H, H_α-C(2')), 2.47 (m, 1H, $H_ß$-C(2')), 3.55 (m, 2H, H-C(5'$_{\alpha,ß}$)), 3.84 (m, 1H, H-C(4')), 4.35 (m, 1H, H-C(3')), 5.04 (t, J = 5.5, 1H, OH-C(5')), 5.24 (d, J = 3.9, 1H, OH-C(3')), 5.38 (s, 1H, OH), 6.49 ('t', J = 7.0, 1H, H-C(1')), 6.71 (br s, 2H, NH_2), 7.68 (s, 1H, H-C(6)), 8.12 (s, 1H, H-C(2)). Anal. Calcd for $C_{21}H_{28}N_4O_4$ (400.48): C 62.98, H 7.05, N 13.99; Found C 62.98, H 7.08, N 13.86.

4-Amino-7-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-5-[2-(9-fluoren-9-oyl)ethynyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (14). General procedure with 9-ethynyl-9-fluorenol (700 mg, 3.39 mmol): the reaction time was 4 h; colourless solid (55 mg, 45%): R_f (A) 0.35; UV (MeOH): 279 (22600), 236 (30700), 230 (31300). ^{1}H-NMR (DMSO-d_6): δ 2.12 (m, 1H, H_α-C(2')), 2.40 (m, 1H, $H_ß$-C(2')), 3.42 (m, 2H, H-C(5'$_{\alpha,ß}$)), 3.77 (m, 1H, H-C(4')), 4.27 (m, 1H, H-C(3')), 4.97 (t, J = 5.1, 1H, OH-C(5')), 5.22 (d, J = 3.6, 1H, OH-C(3')), 6.43 ('t', J = 6.9, 1H, H-C(1')), 6.67 (br s, 2H, NH_2), 6.94 (s, 1H, OH), 7.40 (m, H-arom.), 7.67 (s, 1H, H-C(6)), 7.70 (d, J = 7.0, H-arom.), 7.80 (d, J = 7.0, H-arom.), 8.11 (s, 1H, H-C(2)). Anal. Calcd for $C_{26}H_{22}N_4O_4$ (454.48): C 68.71, H 4.88, N 12.33; Found C 68.85, H 4.76, N 12.44.

Bis{[4-amino-7-(2-deoxy-ß-D-*erythro*-pentofuranosyl)]-7*H*-pyrrolo[2,3-*d*]pyrimidin-5,5-yl}-1,8-(1,7-octa-diyne) (15). General procedure with 4-amino-7-(2-deoxy-ß-D-*erythro*-pentofuranosyl)-5-[1-(octa-1,7-diyne)]-7*H*-pyrrolo[2,3-*d*]-pyrimidine[16] (200 mg, 0.56 mmol) and **2b** (100 mg, 0.266 mmol) as coupling agents; the reaction time was 5 h; colourless crystals (MeOH) (105 mg, 31%): mp 200-202°C. R_f (A)

0.13; UV (MeOH): 280 (19200), 239 (26100); ^{1}H-NMR (DMSO-d_6): δ 1.72 (m, 4H, $CH_2CH_2CH_2$-$CH_2C{\equiv}CH$), 2.18 (m, 2H,2 H_α-C(2')), 2.47 (m, 2H, 2 H_β-C(2')), 2.55 (m, 4H, 2 $CH_2C{\equiv}C$), 3.55 (m, 4H, 2 H-C(5'$_{\alpha,\beta}$)), 3.83 (m, 2H, 2 H-C(4')), 4.35 (m, 2H, 2 H-C(3')), 5.07 (t, J = 5.4, 2H, 2 OH-C(5')), 5.27 (d, J = 3.9, 2H, 2 OH-C(3')), 6.48 ('t', J = 6.9, 2H, 2 H-C(1')), 6.67 (br s, 4H, 2 NH_2), 7.66 (s, 2H, 2 H-C(6)), 8.11 (s, 2H, 2 H-C(2)). Anal. Calcd for $C_{30}H_{34}N_8O_6$ (602.65): C 59.79, H 5.69, N 18.59; Found C 59.70, H 5.84, N 18.50.

Cell Culture, Culture Media and Supplement. MTT (3-[4,5-Dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide) was obtained from Sigma Chemical Co. RPMI-1640 medium and Joklik medium, and fetal bovine medium were obtained from GibcoBRL. The complement in fetal bovine serum was inactivated at 56°C for 30 min. Murine leukemia P388 cells and human colon adenocarcinoma HT29 cells were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum. Human transformed embryonic kidney 293 cells were maintained in MEM Joklik modified medium supplemented with 5% fetal bovine serum.

Growth Inhibitory Assay. The anti-proliferative activity of pyrrolo[2,3-*d*]pyrimidine nucleosides against the murine leukemia P388, human colon adenocarcinoma HT29, and human transformed embryonic kidney 293 cell lines was evaluated by the MTT assay.[43,44] Exponentially growing cells (1-2 x 10^3 cells, unless specified otherwise) were seeded in a 96-well micro culture plate in a total volume of 100 µl/well. After overnight incubation in a humidified incubator at 37 °C with 5% CO_2 – 95% air, pyrrolo[2,3-*d*]pyrimidine nucleoside solutions dissolved in culture medium at various concentrations were added in the amount of 100 µl to each well. The plates were placed in a humidified incubator at 37°C for three days (P388) or 6 days (HT29 and 293). The plates were then centrifuged briefly and 100 µl of the growth medium was removed. Cell cultures were incubated with 50 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide [MTT, 1 mg/ml in Dulbecco's phosphate buffered saline (PBS)] for 4 h at 37°C. The resulting purple formazan precipitate was solubilized with 200 µl of 0.04N HCl in isopropyl alcohol. Absorbance was monitored in a BioRad Model 3550 Microplate Reader at a test wavelength of 570 nm and a reference wavelength at 630 nm. The IC_{50} values were

determined by a computer program (EZ-ED50, Perrella Scientific, Inc. NH, USA) that fits all of the data to the following four parameter logistic equation:

$$Y = \frac{A_{max} - A_{min}}{1 + \left(X/IC_{50}\right)^{n}} + A_{min}$$

where A_{max} is the absorbance of the control cells, A_{min} is the absorbance of the cells in the presence of highest agent concentration, Y is the observed absorbance, X is the agent concentration, IC_{50} is the concentration of agent that inhibits the cell growth by 50% compared to the control cells (based on the absorbance), and n is the slope of the curve.

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