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An efficient synthesis of cytostatic mono and bis-alkynylpyrimidine derivatives by the Sonogashira cross-coupling reactions of 2,4-diamino-6-iodopyrimidine and 2-amino-4,6-dichloropyrimidine

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Abstract—A series of 6-alkynyl-2,4-diaminopyrimidine derivatives bearing various substituents at alkynyl moiety was prepared by the Sonogashira cross-coupling reaction of 2,4-diamino-6-iodopyrimidine using $Pd(PPh_3)_2Cl_2$ as catalyst. The same reaction was applied to 2-amino-4,6-dichloropyrimidine. This compound on reaction with 1 equiv. of alkyne gave 6-alkynyl-2-amino-4-chloropyrimidine derivatives as main products, while reaction with three equivalents of alkyne afforded predominantly 4,6-bis-alkynyl-2-aminopyrimidines. Some of the resulting alkynyl pyrimidines showed considerable cytostatic activity.
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1. Introduction

Alkynes are versatile intermediates in synthesis[1,2] as well as an important functional moiety in a wide range of biologically active compounds.[3] The development of methods for alkynyl group introduction into organic molecules is an important target. The widely used Sonogashira reaction typically employs a palladium catalyst and copper iodide to couple a terminal alkyne with an aryl halide.[4] Attractive features of this method include its experimental simplicity and its high atom-economy and functional-group tolerance.[5]

The application of the Sonogashira reaction to pyrimidine bases bearing amino group(s) could provide a broad spectrum of various substituted pyrimidines. Such derivatives may lead to the potential biologically active nucleoside analogues,[6,7] adenosine kinase inhibitors,[8] covalent base-pairs[9,10] or compounds related to enediyne antitumor antibiotics.[11]

2. Results and discussion

In this paper, we report on the synthesis of 2,4,6-trisubstituted pyrimidines via the Sonogashira reaction. We were particularly interested in the introduction of an alkynyl moiety into the 6-position of 2,4-diaminopyrimidine. The only known related cross-coupling is the Suzuki procedure of 2,4-diamino-6-chloropyrimidine with aryl boronic acids.[12] This type of compounds had been synthesized earlier by condensation reactions.[13,14] The purpose of our study—pyrimidines with C–C bond at position 6—required a development of cross-coupling methodology utilizing the Sonogashira reaction.

As a suitable starting material commercially available 2,4-diamino-6-chloropyrimidine was chosen; however, under the standard Sonogashira conditions (ethyne derivative, CuI, DMF, $Pd(PPh_3)_4$ or $Pd(PPh_3)_2Cl_2$, Et_3N or AcOK or Bu_4NF) this compound was unreactive. The problem was solved by conversion of 6-chloro derivative to 2,4-diamino-6-iodopyrimidine by procedure using hydroiodic acid (40%) and sodium iodide.[8] This iodo derivative reacted smoothly at room temperature with a series of substituted ethynes in dimethylformamide in the presence of triethylamine, CuI and $Pd(PPh_3)_2Cl_2$ as a catalyst to give 6-substituted pyrimidines **1a**–**1g** in good yields (Scheme 1).

Identical Sonogashira conditions were a method of choice for preparation of 2-amino-6-alkynyl-4-chloropyrimidine and 2-amino-4,6-bis(alkynyl)pyrimidine derivatives **2** and **3** (Scheme 2). In this case we used 1.1 (Method A) or 3.3 (Method B) equivalents of phenyl-, trimethylsilyl- or hydroxymethyl acetylene. The starting commercially

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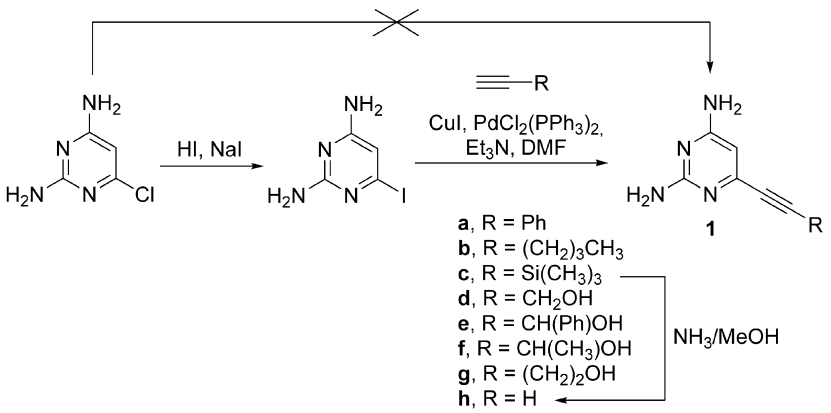
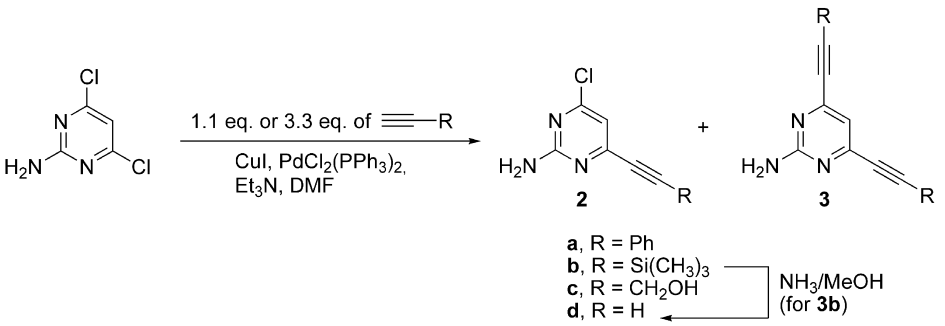


Scheme 1.



Scheme 2.

available 2-amino-4,6-dichloropyrimidine was sufficiently reactive and so the transformation to iodo derivative was not necessary, although the yields of cross-coupling were lower. The selectivity of the mono-alkynylation and bis-alkynylation reaction, taking place under conditions used in Method A and B, respectively, depends on the character of substituent at ethyne group. While the reaction with 1.1 equiv. of ethyne derivative was selective for trimethylsilylethyne and only compound **2b** was isolated, reaction with 3.3 equiv. led selectively to bis-derivatives with phenyl and hydroxymethyl groups **3a** and **3c**. In the remaining cases the both products **2** and **3** were isolated.

The stability in organic solutions of the most of thus prepared compounds was limited. In addition, while the trimethylsilyl derivatives **1c** and **3b** were deprotected under standard conditions in methanolic ammonia to form 2,4-diamino-6-ethynylpyrimidine (**1h**) and 2-amino-4,6-bis(ethynyl)pyrimidine (**3d**), respectively, the attempts to deprotect derivative **2b** under the same conditions led to a complex reaction mixture.

In conclusion, the Sonogashira cross-coupling reaction seems to be the method of choice for C–C bond formation in the positions 4 and 6 of pyrimidine to form 2,4,6-trisubstituted pyrimidines as suitable starting materials for potentially biologically active compounds. To compare the influence of various alkynes on the cross-coupling reaction, the yields of the products **1a**–**1h**, **2a**–**2c** and **3a**–**3c** are summarized in the Table 1.

The title mono and bis-alkynylpyrimidine derivatives **1**, **2** and **3** were tested on their in vitro inhibition of the cell growth in mouse leukemia L1210 cells, human T-lymphoblastoid CCRF-CEM cell line, human promyelocytic leukemia HL-60 cells and human cervix carcinoma HeLa S3 cells (for details see Section 3). The results (Table 2) indicate that compounds **2a**, **3a**, **2c** and **3c** exhibit considerable cytostatic activity (inhibition of the cell growth in vitro) towards human leukemic HL-60 and CCRF-CEM cells, while the L1210 and HeLa S3 cells are less sensitive. The most promising anti-proliferative potency is exerted by compound **3c**. Moderate effects (data not shown) were

Table 1. Summary of the Sonogashira cross-coupling yields

Compound	R	Yield (%)	Compound	Yield (%)	Compound	Yield (%)	R	Method
1a	Ph	84	**2a**	41	**3a**	15	Ph	A
1b	$(CH_2)_3CH_3$	79		—		81		B
1c	$Si(CH_3)_3$	61	**2b**	60	**3b**	—	$Si(CH_3)_3$	A
1d	CH_2OH	61		22		57		B
1e	CH(Ph)OH	66	**2c**	35	**3c**	5	CH_2OH	A
1f	$CH(CH_3)OH$	70		—		39		B
1g	$(CH_2)_2OH$	89			**3d**	36	H (2 steps)	B
1h	H (2 steps)	46						

Table 2. Inhibition of the cell growth in vitro

Compound	IC_{50}, μmol l^{-1}			
	L1210	HL-60	HeLa S3	CCRF-CEM
2a	≫10	6.6	≫10	13.3
3a	≫10	9.0	≫10	6.4
2c	10.9	4.2	10.7	3.8
3c	3.6	2.1	7.6	1.9

observed also for derivatives **1e**, **1h**, **3d** and unstable trimethylsilyl compounds **2b**, **3b**. Their activity can be explained by decomposition under the formation of **2d** and **3d**. The structure-activity relationship of the series of compounds shows that the 4-amino pyrimidines **1** were less active than 4-chloro and 4-alkynyl derivatives **2** and **3**. The active compounds bear mostly unsubstituted ethynyl as the side chain(s) or ethynyl moiety functionalised by phenyl or hydroxymethyl group.

3. Experimental

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and compounds were dried at 2 kPa over P_2O_5. Melting points were determined on a Büchi melting point apparatus. NMR spectra were measured on an FT NMR spectrometer Varian UNITY 500 (^{1}H at 500 M and ^{13}C at 125.7 M frequency) in dimethyl sulfoxide-d_6. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix). 2,4-Diamino-6-chloropyrimidine, 2-amino-4,6-dichloropyrimidine, ethyne derivatives and $Pd(PPh_3)_2Cl_2$ were obtained from Sigma–Aldrich (Praha, Czech Republic). Dimethylformamide was distilled from P_2O_5 and stored over molecular sieves (4 Å) in argon atmosphere.

3.1. 2,4-Diamino-6-ethynylpyrimidines (1a–1g). General procedure

Dimethylformamide (5 ml), the corresponding ethyne derivative (3 mmol) and Et_3N (0.15 ml) were added through septum to an argon purged flask containing 2,6-diamino-6-iodopyrimidine (240 mg, 1 mmol), CuI (10 mg), $PdCl_2$-$(PPh_3)_2$ (50 mg, 0.07 mmol) and the mixture was stirred at room temperature overnight. The solvent was evaporated in vacuo and the residue was chromatographed on a silica gel column (ethyl acetate–methanol) to give compound **1**.

3.1.1. 2,4-Diamino-6-(phenylethynyl)pyrimidine (1a). Yield 180 mg (84%) of a yellow solid, mp 200–203 °C, decomp. IR ν_{max} (KBr) 3468, 3313, 3157, 2214, 1628, 1569, 1541, 1420, 757, 689. MS (FAB) *m/z*: 211 [MH^+] (100). For $C_{12}H_{10}N_4$ (210.2) calculated 68.56% C, 4.79% H, 26.65% N; found 68.23% C, 4.91% H, 26.31% N. ^{1}H NMR (DMSO-d_6): 7.72 (brs, 2H, NH_2); 7.62 (d, 2H, arom. H); 7.53 (t, 1H, arom. H); 7.50 (t, 2H, arom. H); 7.15 (brs, 2H, NH_2); 6.16 (s, 1H, H-5). ^{13}C NMR: 164.58 (2C, C-2 and C-4); 158.16 (C-6); 132.16 (2C, arom. C); 129.24 (2C, arom. C); 120.11 arom. C); 100.03 (C-5); 93.70 and 82.21 (C-1′ and C-2′).

3.1.2. 2,4-Diamino-6-(hex-1-yn-1-yl)pyrimidine (1b). Yield 150 mg (79%) of a white solid, mp 130–133 °C (ethyl acetate). IR ν_{max} (KBr) 3369, 3319, 3166, 2931, 2871, 2232, 1647, 1587, 1525, 1437, 1420. MS (FAB) *m/z*: 191 [MH^+] (100). For $C_{10}H_{14}N_4$ (190.2) calculated 63.13% C, 7.42% H, 29.45% N; found 63.11% C, 7.53% H, 29.14% N. ^{1}H NMR (DMSO-d_6): 7.58 (brs, 2H, NH_2); 6.95 (brs, 2H, NH_2); 5.97 (s, 1H, H-5); 2.47 (t, 2H, $J_{(H\text{-}3',H\text{-}4')}$=7.1 Hz, H-3′); 1.51 and 1.40 (br pent 2H, and br pent, 2H, H-4′ and H-5′); 0.90 (t, 3H, $J_{(H\text{-}5',H\text{-}6')}$=7.36 Hz, H-6′). ^{13}C NMR: 164.67 (C-4); 158.13 (C-2); 139.15 (C-6); 99.46 (C-5); 97.09 and 75.07 (C-1′ and C-2′); 29.62, 21.51 and 18.29 (C-3′, C-4′ and C-5′); 13.54 (C-6′).

3.1.3. 2,4-Diamino-6-[(2-trimethylsilyl)ethynyl]pyrimidine (1c). Yield 125 mg (61%) of yellow foam. IR ν_{max} (KBr) 3467, 3390, 3318, 3182, 2959, 2166, 1622, 1575, 1550, 1407, 1251, 1183, 991, 848. MS (FAB) *m/z*: 207 [MH^+] (100). For $C_9H_{14}N_4Si$ (206) calculated 52.39% C, 6.84% H, 27.16% N; found 52.03% C, 6.83% H, 26.82% N. ^{1}H NMR (DMSO-d_6): 6.42 (brs, 2H, NH_2); 6.02 (brs, 2H, NH_2); 5.80 (s, 1H, H-5); 0.20 (s, 9H, $SiMe_3$). ^{13}C NMR: 164.47 (C-4); 163.56 (C-2); 147.76 (C-6); 104.38 and 92.93 (C-1′ and C-2′); 98.08 (C-5); −0.21 (3C, $SiMe_3$).

3.1.4. 2,4-Diamino-6-(3-hydroxyprop-1-yn-1-yl)pyrimidine (1d). Yield 100 mg (61%) of a white solid, mp 204–206 °C (methanol). IR ν_{max} (KBr) 3427, 3351, 3127, 2236, 2216, 1668, 1639, 1588, 1550, 1431, 1032. MS (FAB) *m/z*: 165 [MH^+] (30). For $C_7H_8N_4O.1/3H_2O$ (170.2) calculated 49.41% C, 5.13% H, 32.92% N; found 49.64% C, 4.89% H, 32.69% N. ^{1}H NMR (DMSO-d_6): 6.40 (brs, 2H, NH_2); 5.98 (brs, 2H, NH_2); 5.78 (s, 1H, H-5); 5.35 (t, 1H, $J_{(OH,\ H\text{-}3')}$= 5.0 Hz, OH); 4.24 (d, 2H, $J_{(OH,\ H\text{-}3')}$=5.0 Hz, H-3′). ^{13}C NMR: 164.48 (C-4); 163.56 (C-2); 148.33 (C-6); 97.59 (C-5); 88.68 and 83.36 (C-1′ and C-2′); 49.38 (C-3′).

3.1.5. 2,4-Diamino-6-(3-hydroxy-3-phenylprop-1-yn-1-yl)pyrimidine (1e). Yield 160 mg (66%) of a yellow amorphous solid. IR ν_{max} (KBr) 3454, 3316, 3124, 2221, 1655, 1622, 1579, 1554, 1440, 1188, 980, 700. MS (FAB) *m/z*: 241 [MH^+] (100). For $C_{13}H_{12}N_4O.2/5H_2O$ (247.5) calculated 63.10% C, 5.21% H, 22.64% N; found 63.12% C, 5.05% H, 22.25% N. ^{1}H NMR (DMSO-d_6): 7.49 (d, 2H, arom. H); 7.38 (t, 2H, arom. H); 7.31 (t, 1H, arom. H); 6.42 (brs, 2H, NH_2); 6.21 (d, 1H, $J_{(OH,\ H\text{-}3')}$=6.0 Hz, H-3′); 6.00 (brs, 2H, NH_2); 5.81 (s, 1H, H-5); 5.55 (d, 1H, $J_{(OH,\ H\text{-}3')}$= 5.0 Hz, OH). ^{13}C NMR: 164.48 (C-4); 163.57 (C-2); 148.11 (C-6); 128.48 (2C, arom. C); 127.88 (arom. C); 126.57 (2C, arom. C); 97.77 (C-5); 89.95 and 84.13 (C-1′ and C-2′).

3.1.6. 2,4-Diamino-6-(3-hydroxybut-1-yn-1-yl)pyrimidine (1f). Yield 125 mg (70%) of yellow foam. IR ν_{max} (KBr) 3401, 3205, 2233, 1622, 1581, 1548, 1418, 1203, 1093, 817. MS (FAB) *m/z*: 179 [MH^+] (100). HRMS (EI): found 178.0842, calculated for $C_8H_{10}N_4O$: 178.0855. ^{1}H NMR (DMSO-d_6): 7.02 (brs, 2H, NH_2); 6.47 (brs, 2H, NH_2); 5.88 (s, 1H, H-5); 5.59 (t, 1H, $J_{(OH,\ H\text{-}3')}$=5.0 Hz, OH); 4.56 (br pent, 1H, J=6.3 Hz, H-3′); 1.35 (d, 3H, $J_{(H\text{-}4',H\text{-}3')}$=6.6 Hz, H-4′). ^{13}C NMR: 164.52 (C-4); 160.85 (C-2); 143.81 (C-6); 98.65 (C-5); 95.22 and 79.21 (C-1′ and C-2′); 56.62 (C-3′); 24.27 (C-4′).

3.1.7. 2,4-Diamino-6-(4-hydroxybut-1-yn-1-yl)pyrimidine (1g). Yield 160 mg (89%) of a yellow solid, mp 155–158 °C, decomp. (ethyl acetate). IR ν_{max} (KBr) 3425, 3324, 3203, 2235, 1627, 1578, 1420, 1046. MS (FAB) *m/z*: 179 [MH^+] (100). For $C_8H_{10}N_4O$ (178.2) calculated 53.92% C, 5.66% H, 31.44% N; found 53.61% C, 5.73% H, 31.19% N. ^{1}H NMR (DMSO-d_6): 7.22 (brs, 2H, NH_2); 6.63 (brs, 2H, NH_2); 5.91 (s, 1H, H-5); 4.95 (br, 1H, OH); 3.56 (t, 2H, $J_{(H\text{-}3',H\text{-}4')}$=6.6 Hz, H-4′); 2.57 (t, 2H, $J_{(H\text{-}4',H\text{-}3')}$= 6.6 Hz, H-3′). ^{13}C NMR: 164.61 (C-4); 159.76 (C-2); 142.04 (C-6); 98.90 (C-5); 93.08 and 77.11 (C-1′ and C-2′); 59.31 (C-4′); 23.23 (C-3′).

3.2. 2-Amino-4-chloro-6-ethynylpyrimidines 2 and 2-amino-4,6-bis(ethynyl)pyrimidines 3. General procedure

Method A. Dimethylformamide (10 ml), the corresponding ethyne derivative (2.2 mmol) and Et_3N (0.3 ml) were added through septum to an argon purged flask containing 2-amino-4,6-dichloropyrimidine (330 mg, 2 mmol), CuI (20 mg), $PdCl_2(PPh_3)_2$ (100 mg, 0.07 mmol) and the mixture was stirred at room temperature overnight. The solvent was evaporated in vacuo and the residue was chromatographed on a silica gel column (ethyl acetate–hexane). Compounds **2** were obtained as major products, small amounts of compounds **3** were isolated as side products.

Method B. Dimethylformamide (10 ml), the corresponding ethyne derivative (6.6 mmol) and Et_3N (0.6 ml) were added through septum to an argon purged flask containing 2-amino-4,6-dichloropyrimidine (330 mg, 2 mmol), CuI (40 mg), $PdCl_2(PPh_3)_2$ (200 mg, 0.14 mmol) and the mixture was stirred at room temperature overnight. The solvent was evaporated in vacuo and the residue was chromatographed on a silica gel column (ethyl acetate–hexane). Compounds **3** were obtained as major products; in the case of **3b**, a small amount of **2b** was isolated as a by-product.

3.2.1. 2-Amino-4-chloro-6-(phenylethynyl)pyrimidine (2a) and 2-amino-4,6-bis(phenylethynyl)pyrimidine (3a).

Method A. 190 mg (41%) of **2a** and 90 mg (15%) of **3a**.

Method B. 480 mg (81%) of **3a**.

Compound **2a**. Yellow needles, mp 145–146 °C (methanol–water). IR ν_{max} (KBr) 3503, 3280, 3139, 2223, 1630, 1551, 1534, 1324, 1229, 1211, 812, 762. MS (FAB) *m/z*: 230 [MH^+] (100). For $C_{12}H_8ClN_3.1/4H_2O$ (234.2) calculated 61.55% C, 3.66% H, 17.94% N; found 61.91% C, 3.53% H, 17.71% N. ^{1}H NMR (DMSO-d_6): 7.62 (d, 2H, arom. H); 7.48 (m, 3H, arom. H); 7.33 (brs, 2H, NH_2); 6.90 (s, 1H, H-5). ^{13}C NMR: 163.50 (C-2); 160.72 (C-4); 152.07 (C-6); 132.26 (2C, arom. C); 130.46 (2C, arom. C); 120.53 (arom. C); 111.05 (C-5); 91.74 and 86.82 (C-1′ and C-2′).

Compound **3a**. Yellow solid, mp 188–189 °C (acetate–hexane). IR ν_{max} (KBr) 3304, 3186, 2216, 1558, 1533, 1522, 1372, 1218, 756, 688. MS (FAB) *m/z*: 296 [MH^+] (100). For $C_{20}H_{13}N_3$ (295.3) calculated 81.34% C, 4.44% H, 14.23% N; found 80.98% C, 4.32% H, 14.01% N. ^{1}H NMR (DMSO-d_6): 7.62 (m, 4H, arom. H); 7.53–7.46 (m, 6H, arom. H); 7.06 (brs, 2H, NH_2); 6.99 (s, 1H, H-5). ^{13}C NMR: 163.86 (C-2); 151.12 (2C, C-4 and C-6); 132.21 (4C, arom. C); 130.32 (2C, arom. C); 129.15 (4C, arom. C); 120.76 (2C, arom. C); 114.45 (C-5); 91.36 and 87.36 (2×2C, C-1′ and C-2′).

3.2.2. 2-Amino-4-chloro-6-[(2-trimethylsilyl)ethynyl]-pyrimidine (2b) and 2-amino-4,6-bis[(2-trimethylsilyl)-ethynyl]pyrimidine (3b).

Method A. 270 mg (60%) of **2b** and traces of **3b**.

Method B. 100 mg (22%) of **2b** and 330 mg (57%) of **3b**.

Compound **2b**. Yellowish solid, unstable. IR ν_{max} (KBr) 3471, 3308, 32031625, 1565, 1532, 1470, 1284, 1252, 892, 848. MS (FAB) *m/z*: 226 [MH^+] (20). HRMS (EI): found 225.0503, calculated for $C_9H_{12}ClN_3Si$: 225.0489. ^{1}H NMR (DMSO-d_6): 7.30 (brs, 2H, NH_2); 6.77 (s, 1H, H-5); 0.23 (s, 9H, $SiMe_3$). ^{13}C NMR: 163.42 (C-2); 160.83 (C-4); 151.47 (C-6); 110.97 (C-5); 101.79 and 98.22 (C-1′ and C-2′); −0.50 (3C, $SiMe_3$).

Compound **3b**. White solid, unstable. IR ν_{max} (KBr) 3501, 3430, 3304, 3194, 2961, 2167, 1620, 1557, 1524, 1335, 1251, 959, 847, 761. MS (FAB) *m/z*: 288 [MH^+] (40). HRMS (EI): found 287.1298, calculated for $C_{14}H_{21}N_3Si_2$: 287.1274. ^{1}H NMR (DMSO-d_6): 7.00 (brs, 2H, NH_2); 6.70 (s, 1H, H-5); 0.23 (s, 18H, $SiMe_3$). ^{13}C NMR: 163.72 (C-2); 150.71 (2C, C-4 and C-6); 114.19 (C-5); 102.31 and 97.78 (2×2C, C-1′ and C-2′); −0.46 (6C, $SiMe_3$).

3.2.3. 2-Amino-4-chloro-6-(3-hydroxyprop-1-yn-1-yl)-pyrimidine (2c) and 2-amino-4,6-bis(3-hydroxyprop-1-yn-1-yl)pyrimidine (3c).

Method A. 130 mg (35%) of **2c** and 20 mg (5%) of **3c**.

Method B. Traces of **2c** and 160 mg (39%) of **3c**.

Compound **2c**. White solid, mp 185–187 °C. IR ν_{max} (KBr) 3421, 3314, 2246, 2224, 1654, 1555, 1539, 1306, 1023, 812. MS (FAB) *m/z*: 184 [MH^+] (100). For $C_7H_6ClN_3O$ (183.6) calculated 45.79% C, 3.29% H, 22.89% N, 19.31% Cl; found 45.52% C, 3.21% H, 22.45% N, 19.34% Cl. ^{1}H NMR (DMSO-d_6): 7.25 (brs, 2H, NH_2); 6.72 (s, 1H, H-5); 5.50 (t, 1H, $J_{(OH,\ H\text{-}3')}$=6.1 Hz, OH); 4.31 (d, 2H, $J_{(OH,\ H\text{-}3')}$=6.1 Hz, H-3′). ^{13}C NMR: 163.44 (C-2); 160.67 (C-4); 152.15 (C-6); 110.68 (C-5); 93.32 and 81.50 (C-1′ and C-2′); 49.38 (C-3′).

Compound **3c**. Yellowish solid, unstable, >300 °C, decomp. IR ν_{max} (KBr) 3466, 3346, 2237, 1637, 1566, 1540, 1360, 1228, 1043. MS (FAB) *m/z*: 204 [MH^+] (100). For $C_{10}H_9N_3O_2$ (203.2) calculated 59.11% C, 4.46% H, 20.86% N; found 58.75% C, 4.52% H, 20.49% N. ^{1}H NMR (DMSO-d_6): 6.90 (brs, 2H, NH_2); 6.62 (s, 1H, H-5); 5.47 (t, 2H, $J_{(OH,\ H\text{-}3')}$=6.1 Hz, OH); 4.30 (d, 4H, $J_{(OH,\ H\text{-}3')}$= 6.1 Hz, H-3′). ^{13}C NMR: 163.68 (C-2); 151.11 (2C, C-4 and C-6); 113.54 (C-5); 92.68 and 81.98 (2×2C, C-1′ and C-2′); 49.38 (2C, C-3′).

3.2.4. 2,4-Diamino-6-ethynylpyrimidine (1h). Solution of 2,4-diamino-6-(2-trimethylsilylethynyl)pyrimidine (**1c**, 100 mg, 0.5 mmol) in methanolic ammonia (15 ml) was stirred at room temperature for 3 h. The solvent was evaporated and the residue subjected to preparative TLC (20% MeOH in ethyl acetate). Yield 50 mg (75%) of **1h** as an unstable yellowish solid. IR ν_{max} (KBr) 3480, 3434, 3326, 3223, 3092, 2107, 1646, 1626, 1574, 1547, 1437, 1402, 1275, 988, 829, 693. MS (FAB) *m/z*: 135 [MH^+] (40). HRMS (EI): found 134.0575, calculated for $C_6H_6N_4$: 134.0592. ^{1}H NMR (DMSO-d_6): 6.48 (brs, 2H, NH_2); 6.05 (brs, 2H, NH_2); 5.85 (s, 1H, H-5); 4.10 (s, 1H, H-2′). ^{13}C NMR: 164.46 (C-4); 163.41 (C-2); 147.40(C-6); 98.36 (C-5); 82.79 (C-1′).

3.2.5. 2-Amino-4,6-bis(ethynyl)pyrimidine (3d). Solution of 2-amino-4,6-bis(2-trimethylsilylethynyl)pyrimidine (**3b**, 220 mg, 0.76 mmol) in methanolic ammonia (20 ml) was stirred at room temperature for 2 h. Solvent was evaporated, the residue in methanol (5 ml) was filtered through a short column of silica gel and purified by preparative HPLC. Yield 90 mg (63%), unstable. IR ν_{max} (KBr) 3497, 3422, 3287, 3175, 2106, 1627, 1559, 1531, 1459, 1321, 1218, 675. MS (EI) *m/z*: 143 [MH^+] (100). HRMS (EI): found 143.0504, calculated for $C_8H_5N_3$: 143.0483. ^{1}H NMR (DMSO-d_6): 7.03 (brs, 2H, NH_2); 6.79 (s, 1H, H-5); 4.54 (s, 2H, H-2′). ^{13}C NMR: 163.72 (C-2); 150.77 (2C, C-4 and C-6); 114.66 (C-5); 81.32 (2C, C-1′); 79.27 (2C, C-2′).

Inhibition of the cell growth was estimated in mouse lymphocytic leukemia L1210 cells (ATCC CCL 219), CCRF-CEM T lymphoblastoid cells (human acute lymphoblastic leukemia, ATCC CCL 119), human promyelocytic leukemia HL-60 cells (ATCC CCL 240) and human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2).[15] L1210 cells, CCRF-CEM cells and HL-60 cells were cultivated in RPMI 1640 medium supplemented with calf foetal serum using 24-well tissue culture plates. The endpoint of the cell growth was 72 h following the drug addition. HeLa S3 cells were seeded to 24-well dishes in RPMI 1640 HEPES modification with foetal calf serum. 48 h following the drug addition the cultivation was stopped and the cell growth was evaluated. An inhibition of the cell growth was determined by cell-counting. In parallel, the cell viability was quantified using XTT (Ref. 16) standard spectrophotometric assay (Roche Molecular Biochemicals). The inhibitory potency of the compound tested was expressed as IC_{50} values.

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