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Efficient synthesis and in vitro cytostatic activity of 4-substituted triazolyl-nucleosides

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Abstract—We report herein an efficient synthesis of 4-substituted triazolyl-nucleosides and their in vitro cytostatic activity. The synthesis is based on a straightforward 1,3-dipolar cycloaddition between 1-azido-ribose **2** and terminal alkynes under a cooperative effect of microwave activation and copper (I) catalysis. All cycloadducts were obtained in nearly quantitative yield after a short reaction time (1 to 2 min). After removal of acetyl protecting groups, the free nucleosides were evaluated against L1210, Molt4/C8, and CEM tumor cell lines. Structure–activity relationship study shows that the substituent on the triazole ring has a major effect since nucleosides **4c** and **4g,** containing, respectively, a long alkyl chain and an aryl donor group are the most active compounds in this series.

Nucleosides, which are the genomic building blocks, interact with major constituents of living cells such as nucleic acids, enzymes, and proteins. Naturally occurring and synthetic analogues of nucleosides have been the cornerstone of antiviral therapy over the last decades. The growing interest in such analogues arises from their high potential value not only as therapeutic agents but also as biochemical probes and as building blocks in oligonucleotide synthesis following the well-known phosphoramidite chemistry.[1]

Among antitumor nucleosides, those anchoring a five-membered heterocyclic ring are of great interest. Thus, tiazofurin (Fig. 1) is a synthetic C-nucleoside recently approved as orphan drug for treatment of chronic myelogenous leukemia in accelerated phase or blast crisis. It is a potent inhibitor of inosine monophosphate dehydrogenase (IMPDH), a rate-limiting enzyme of the de novo guanylate pool synthesis.[2]

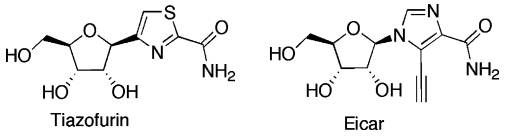

Figure 1. Examples of five-membered antitumor nucleosides, tiazofurin and Eicar.

Eicar is an other five-membered N-nucleoside with a potent antiviral and antitumor activity (Fig. 1).[3] These encouraging results prompted us to investigate the synthesis of new five-membered nucleosides in the triazolyl series.

We recently found that azido-deoxyribose and terminal alkynes undergo fast 1,3-dipolar cycloaddition under the cooperative effect of microwave activation and cooper(I) catalysis to give high yield of 2′-deoxy-triazolyl-nucleosides cycloadducts.[4] To get further insight into such a process, we describe herein a straightforward two-step's synthesis of 4-substituted triazolyl-nucleosides **4**, in ribo series, and their cytostatic activity on L1210, Molt4/C8, and CEM cell lines. The role of SiO_2 support or

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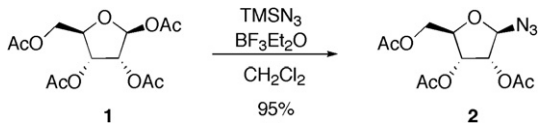


Scheme 1. Synthesis of azido-tri-*O*-acetylribose **2**.

Brønsted acid in the activation of the key 1,3-dipolar cycloaddition step will also be discussed.

First, the protected azido-ribose starting material (**2**) was obtained in high yield from commercially available tetra-*O*-acetylribose by using $TMSN_3$ and BF_3Et_2O as a catalyst (Scheme 1). The reaction was completely stereoselective since only the β-anomer **2** was obtained.[5]

The survey of the 1,3-dipolar cycloaddition conditions was next examined by using azide **2** and ethyl propiolic ester as a model (Table 1). We found that the reaction, when performed under solvent free microwave activation, Cu(I) catalysis and supported on SiO_2, provided the desired cycloadduct **3a** stereo- and regioselectively and almost quantitatively (entry 1). In this cycloaddition, only the β-anomer-4-substituted-triazole **3a** was obtained within a very short reaction time (1 min).[6]

The reaction proceeded also efficiently in solution since high yields were obtained in CH_2Cl_2 or toluene at reflux (entry 3). However, poor yields were obtained at room temperature even with increased reaction times (entry 2).

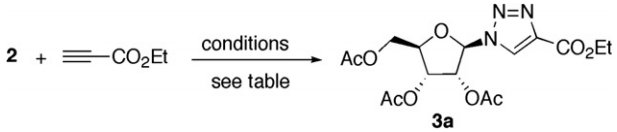
To get more information about the role of silica gel, we tested Al_2O_3 and sand,[7] a poorly hydroxylated silicate, as support. When the reaction was performed directly on Al_2O_3 or sand and under microwave, **3a** was obtained with yields comparable to those obtained with SiO_2 as support (entries 4 and 5). By contrast, in solution, the best results were obtained with SiO_2 (entries 6 and 7) instead of Al_2O_3 (entry 8) or sand (entry 9). Moreover, the reaction proceeded in solution more slowly when carried out with Al_2O_3 or sand (entries 8 and 9, respectively) than with SiO_2.

These differences are likely attributable to an acid catalytic role of the SiO_2 support for this 1,3-dipolar cycloaddition.[8] The importance of this acid catalytic property was unambiguously proven by the use of acetic acid (1 equiv/mmol of alkyne) instead of SiO_2, which resulted in a fast reaction and high cycloaddition yield (20 min, 95%, entry 10). This cycloaddition could also be carried out efficiently in protic solvents and aqueous media as shown in entries 11 and 12, respectively. To our knowledge, this is the first report of such acid catalysis in azide-alkyne 1,3-dipolar cycloaddition. It offers further new perspectives in the well-known click chemistry field. However, the mechanism by which Brønsted acid is involved in the catalytic cycloaddition process is not very clear and further experiments are required for its elucidation.

The synthesis of the various protected triazolyl-nucleosides **3** listed in Table 2 was performed applying the most efficient procedure indicated in entry 1 of Table 1.[9] It should be noted that all these nucleosides were obtained with high yields whatever the chemical nature of the starting alkyne. The different alkynes were chosen in order to increase the molecular diversity around the triazole nucleobase (long chain, polar, and aromatic alkynes).

The acetyl groups of the protected nucleosides **3a**–**i** were then cleaved using NH_3/MeOH solution to afford in high yields the corresponding free analogues **4a**–**i**, respectively (Table 2).

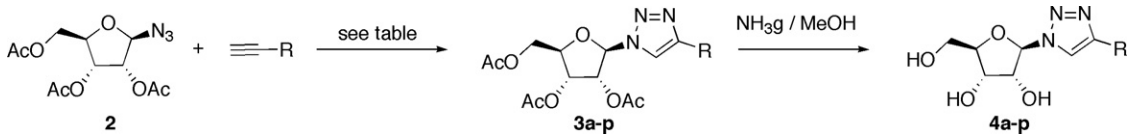
Table 1. Survey of cycloaddition reaction and SiO_2 effect

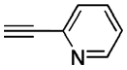
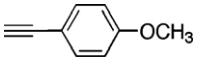
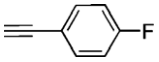
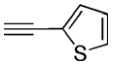


Entry[a]	Additives	Activation	Time	*T* (°C)	Yield[b] (%)
1	SiO_2	MW	1 min	110	96
2	No	CH_2Cl_2 or toluene	24 h	rt	20[c]
3	No	CH_2Cl_2 or toluene	16 h	Reflux	85
4	Sand	MW	1 min	110	95
5	Al_2O_3	MW	1 min	110	95
6	SiO_2	CH_2Cl_2	16 h	rt	90
7	SiO_2	Toluene	236 h	rt	65[c]
8	Al_2O_3	CH_2Cl_2	16 h	rt	37[c]
9	Sand	CH_2Cl_2	16 h	rt	35[c]
10	AcOH	CH_2Cl_2	20 min	rt	95
11	AcOH[d]	EtOH	8 h	rt	82
12	AcOH[d]	H_2O/*t*-BuOH	8 h	rt	79

[a] Azide (1 mmol), alkyne (1.2 equiv), CuI (2 equiv), DIEA (5 equiv), support (100 mg/mmol of azide).
[b] Yield based on the isolated product.
[c] The starting material **2** was recovered.
[d] 1 equiv/mmol of alkyne.

Table 2. Two-steps synthesis of free nucleosides **4a**–**i**



Entry[a]	Alkyne	Time (min)	Products **3a**–**p** (yield[b] %)	Products **4a**–**p** (yield[b] %)
1	$-CO_2Et$	1	**3a** (97)	**4a** (85) (R = $CONH_2$)
2	$-(CH_2)_5CH_3$	2	**3b** (95)	**4b** (87)
3	$-(CH_2)_7CH_3$	2	**3c** (95)	**4c** (90)
4	$-CH_2OH$	1	**3d** (93)	**4d** (94)
5	$-CH(OH)CH_3$	1	**3e** (96)	**4e** (92)
6		2	**3f** (93)	**4f** (90)
7		2	**3g** (95)	**4g** (88)
8		2	**3h** (94)	**4h** (95)
9		2	**3i** (93)	**4i** (92)

[a] Conditions: azide (1 mmol), alkyne (2 equiv), CuI (2 equiv), and DIEA (5 equiv) were adsorbed on silica gel (1 g/mmol of azide) and irradiated (95 °C < T < 115 °C, reaction temperature was digitally measured).
[b] Yields of isolated products.

The newly synthesized free nucleosides **4a**–**i** were evaluated for their in vitro inhibitory effects on the proliferation of murine leukemia cells (L1210) and human T-lymphocyte cells (Molt4/C8 and CEM) as described elsewhere.[10] 5-Fluorouracil (5FU) was used as a control. The results are listed in Table 3.

Among all the nucleosides tested, **4c** with a C8 alkyl chain was the most active one on the three cell lines though its activity was much lower than that of 5-FU. It is surprising that its structurally close analogue **4b** with a C6 alkyl chain did not show any cytostatic activity (at least at a concentration below 200 μM). Furthermore, the aromatic substituted triazoles **4f**–**i** showed moderate to low activities, the most active one on the three cell lines being *p*-methoxyphenyltriazolyl-nucleoside **4g**.

Table 3. Inhibitory effects of triazolyl-nucleosides **4a**–**i** on L1210, Molt4/C8, and CEM cells

Compound	R	IC_{50} (μM)		
		L1210	Molt4/C8	CEM
4a	$-CONH_2$	>200	>200	>200
4b	$-(CH_2)_5-CH_3$	>200	>200	>200
4c	$-(CH_2)_7-CH_3$	56	50	44
4d	$-CH_2OH$	>200	>200	>200
4e	$-CH(OH)CH_3$	>200	>200	>200
4f	–2-Pyridine	236	170	245
4g	–*p*-Ph–OMe	152	36	73
4h	–*p*-Ph–F	209	171	217
4i	–3-Thiophenyl	182	126	139
5-Fluorouracil		0.28	23	9.0

In summary, we reported an efficient microwave-assisted eco-friendly synthesis of 4-substituted triazolyl-nucleosides together with their cytostatic activity on L1210, Mol4/C8, and CEM tumor cell lines. All cycloadducts were obtained in high yields by using 1,3-dipolar cycloaddition between azido-ribose and a range of terminal alkynes when proceeded in the presence of a Brønsted acid such as SiO_2 and AcOH. Of the various target nucleosides tested, we found that nucleosides **4c** and **4g** bearing a C8 alkyl chain or *p*-methoxyphenyl on the triazolyl ring are the most active compounds. They can be used as lead compounds in the search for more active and potent nucleosides to be applied in cancer chemotherapy.

Acknowledgments

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5. *1-Azido-tri-O-acetylribose*(**2**), selected spectral data: ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.99 (s, 3H, Ac), 2.04 (s, 6H, 2× Ac), 4.06 (dd, 1H, J = 11.6 and 2.1 Hz, H-5), 4.24–4.40 (m, 2H, H-4 and H-5), 4.25 (m, 1H, H-4), 5.05 (dd, 1H, J = 4.8 and 2.0 Hz, H-2), 5.25 (dd, 1H, J = 6.6 and 4.7 Hz, H-3), 5.27 (d, 1H, J = 2.0 Hz, H-1). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 20.27, 20.32, 20.48, 62.85, 70.32, 74.31, 79.23, 92.51, 169.30, 169.45, 170.41. MS (ESI^+) m/z = 324 $(MNa)^+$. The β-configuration of azide **2** was attested by ^{1}H 2D NOESY experiment and by comparison to the previously reported data Stimac, A.; Kobe, J. *Carbohydr. Res.* **1992**, *232*, 359; Camarasa, M.-J.; Alonso, R.; De Las Heras, F. G. *Carbohydr. Res.* **1980**, *83*, 152.
6. The stereo- and regio-chemistry of **3a** (β-configuration and phenyl in position 4) was unambiguously attested by NOESY and HMBC experiments. Indeed, the ^{1}H 2D NOESY spectrum shows correlations between $H_{1'}$–$H_{4'}$ and the ^{1}H–^{13}C HMBC experiment shows $C_{1'}$–H_5 cross coupling, in accordance with the proposed structure for **3a**.
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9. Analytical and spectral data for selected active nucleosides. **3a** : ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.34 (t, 3H, J = 7.1, CH_3), 2.05 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.07 (s, 3H, Ac), 4.19 (dd, 1H, J = 12.6 and 3.5 Hz, H-5′), 4.37 (q, J = 7.2 Hz, CH_2 ester), 4.30–4.50 (m, 2H, H-4′ and H-5′), 5.49 (t, J = 5.5 Hz, H-3′), 5.74 (dd, J = 5.1 and 3.6 Hz, H-2′), 6.16 (d, 1H, J = 3.6, H-1′), 8.29 (s, 1H, H-5 triazole). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 14.37, 20.45, 20.53, 20.77, 61.57, 62.51, 70.29, 74.54, 81.14, 90.52, 126.77, 140.65, 160.41, 169.27, 169.51, 170.36. MS (ESI^+) m/z = 423 $(MNa)^+$; **4a** ^{1}H NMR (DMSO-d_6, 200 MHz) δ (ppm) 3.45–3.70 (m, 2H, 2× H-5′), 3.98 (m, 1H, H-4′), 4.12 (dd, 1H, J = 9.6 and 4.8 Hz, H-3′), 4.37 (dd, 1H, J = 10.3 and 4.9 Hz, H-2′), 5.07 (t, 1H, J = 5.3 Hz, OH-5′), 5.27 (d, 1H, J = 5.3 Hz, OH-3′), 5.65 (d, 1H, J = 5.9 Hz, OH-2′), 5.98 (d, 1H, J = 4.5 Hz, H-1′), 7.52 (br s, 1H, NH), 7.92 (br s, 1H, NH), 8.76 (s, 1H, H-5). ^{13}C NMR (DMSO-d_6, 50 MHz) δ (ppm) 70.56, 79.70, 84.89, 95.53, 101.93, 134.53, 152.72, 170.93. MS (ESI^+) m/z = 267 $(MNa)^+$. Anal. Calcd for $C_8H_{12}N_4O_5$: C, 39.35; H, 4.95. Found: C, 39.56; H, 4.73; **3c** ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 0.80 (t, 3H, J = 7.1 Hz, CH_3), 1.10–1.40 (br m, 8H, $(CH_2)_4$), 1.62 (br m, 2H, CH_2), 2.00 (s, 3H, Ac), 2.10 (s, 6H, 2× Ac), 2.62 (t, 2H, J = 7.3 Hz, CH_2), 4.15 (dd, 1H, J = 12.2 and 4.4 Hz, H-5′), 4.35 (dd, 1H, J = 12.7 and 3.2 Hz, H-5′), 4.40 (m, 1H, H-4′), 5.55 (t, 1H, J = 5.3 Hz, H-3′), 5.74 (dd, 1H, J = 5.2 and 3.8 Hz, H-2′), 6.05 (d, 1H, J = 3.8 Hz, H-1′), 7.37 (s, 1H, H-5); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 14.15, 20.48, 20.55, 20.73, 22.70, 25.66, 29.24, 29.34, 3.89, 62.97, 70.80, 74.34, 80.87, 119.80, 149.00, 158.15, 169.39, 169.54, 170.44. MS (ESI^+) m/z = 440 $(MH)^+$; **4c** ^{1}H NMR (CD_3OD, 200 MHz) δ (ppm) 0.80 (t, 3H, J = 6.6 Hz, CH_3), 1.10–1.40 (br m, 8H, $(CH_2)_4$), 1.52 (m, 2H, CH_2), 2.59 (t, 2H, J = 7.8 Hz, CH_2), 3.58 (dd, 1H, J = 12.2 and 4.2 Hz, H-5′), 3.72 (dd, 1H, J = 12.1 and 3.2 Hz, H-5′), 4.01 (dd, 1H, J = 4.2 and 3.3 Hz, H-4′), 4.23 (t, 1H, J = 5 Hz, H-3′), 4.37 (t, 1H, J = 4.2 Hz, H-2′), 5.90 (d, 1H, J = 4.0 Hz, H-1′), 7.91 (s, 1H, H-5).^{1}H NMR (acetone-d_6, 50 MHz) δ (ppm) 14.01, 22.92, 25.80, 32.20, 62.28, 71.31, 76.44, 86.44, 93.30, 120.63, 148.21, 158.62. MS (ESI^+) m/z = 314 $(MH)^+$, 336 $(MNa)^+$, 649 $(2M+Na)^+$. Anal. Calcd for $C_{15}H_{27}N_3O_4$: C, 57.49; H, 8.68; **3g** ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 1.94 (s, 3H, Ac), 2.02 (s, 6H, 2× Ac), 3.72 (s, 3H, OMe), 4.14 (dd, 1H, J = 12.1 and 4.2 Hz, H-5′), 4.34 (dd, 1H, J = 12.2 and 3.2 Hz, H-5′), 4.41 (m, 1H, H-4′), 5.56 (t, 1H, J = 5.3 Hz, H-3′), 5.82 (dd, 1H, J = 3.6 and 3.4 Hz, H-2′), 6.11 (d, 1H, J = 3.3 Hz, H-1′), 6.85 (d, 2H, J = 8.8 Hz, Ph), 7.65 (d, 2H, J = 8.8 Hz, Ph), 7.83 (s, 1H, H-5). ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 20.32, 20.37, 20.56, 55.21, 62.77, 70.63, 74.15, 80.74, 89.93, 113.94, 114.23, 118.11, 122.62, 127.02, 128.89, 147.88, 159.72, 169.26, 169.42, 170.31. MS (ESI^+) m/z = 456 $(MNa)^+$; **4g** ^{1}H NMR (CD_3OD, 200 MHz) δ (ppm) 3.75 (dd, 1H, J = 12.2 and 4.2 Hz, H-5′), 3.84 (s, 3H, OMe), 3.88 (dd, 1H, J = 12.1 and 3.1 Hz, H-5′), 4.19 (br dd, 1H, J = 4.6 and 4.0 Hz, H-4′), 4.39 (t, 1H, J = 5.0 Hz, H-3′), 4.58 (t, 1H, J = 4.2 Hz, H-2′), 6.10 (d, 1H, J = 4.0 Hz, H-1′), 7.00 (d, 2H, J = 8.8 Hz, Ph), 7.75 (d, 2H, J = 8.8 Hz, Ph), 8.49 (s, 1H, H-5). ^{13}C NMR (CD_3OD, 50 MHz) δ (ppm) 55.77, 62.82, 71.87, 77.03, 87.14, 94.42, 115.02, 115.34, 119.98, 124.02, 128.05, 130.19, 148.90, 159.70. MS (ESI^+) m/z = 30 $(MNa)^+$. Anal. Calcd for $C_{14}H_{17}N_3O_5$: C, 54.72; H, 5.58. Found: C, 54.85; H, 5.67.
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