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Regio- and stereoselective modification of cytosine with cyanopropargylic alcohols

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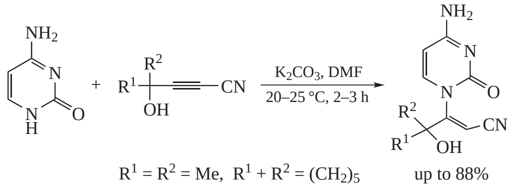
Cyanopropargylic alcohols (4-hydroxyalk-2-ynenitriles) add cytosine at the triple bond under mild conditions (3–5 mol% K_2CO_3, DMF, 20–25 °C, 2–3 h) to afford regio- and stereoselectively the Michael adducts, (*Z*)-3-[4-amino-2-oxo-1(2*H*)-pyrimidinyl]-4-hydroxy-4-alkylalk-2-enenitriles, in 80–88% yield.

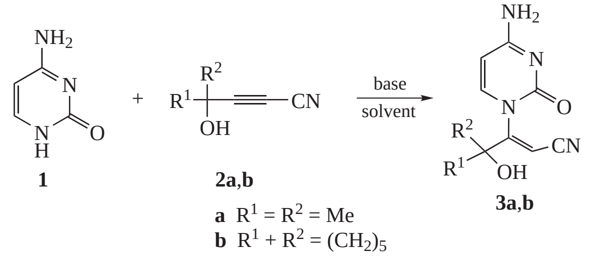


Cytosine is an essential nucleic base with respect to DNA structure.[1] Its scaffold is a key structural unit of various important modern drugs. Its derivative Cidofovir is effective against herpes-, papilloma-, polyoma-, adeno- and pox-virus infections, and suppresses cytomegalovirus (CMV) replication by selective inhibition of viral DNA synthesis.[2] Another congener of cytosine-based drugs,[3] Cytarabine, is used for the treatment of myeloid leukemia and non-Hodgkin's lymphoma.[2(a)] Cytosine-related nucleosides, Zalcitabine[2(a)] and Lamivudine,[4] are reverse transcriptase inhibitors (anti-HIV drugs).[2(a),5] The known cerebrostimulant, antiparkinsonian, lipometabolism coenzyme (lecithin- and plasmalogen biosynthesis), Citicoline, represents a combination of the cytosine core with the functionalized ribose moiety.[2(a)] Densely functionalized cytosine modifications, Ancitabine and Capecitabine, are commercial anticancer modern drugs.[2(a)] The carbocyclic analogue of cytidine weakly inhibits the replication of the HCV replicon in Hua-7 cells.[6] All the mentioned drugs contain the cytosine scaffold modified at the N^1-position.

In light of the above achievements, it is no wonder that efforts to find new modifications of cytosine, particularly onto the N^1-position, do not stop, but rather continue to increase. For instance, *N*^{1}-vinylcytosine was synthesized *via* selective alkylation of the heterocyclic ring with 1,2-dibromoethane.[3(c)] *N*^{1}-[2′-(Hydroxymethyl)allyloxymethyl]cytosine was obtained by multistep reaction from ethyl 2-(hydroxymethyl)acrylate and protected *N*^{4}-benzoylcytosine.[3(g)] Other N^1-modifications[3(e),(h)] of cytosine were also performed using protection of the NH_2 function.

Non-stereoselective addition of cytosine to diethyl acetylenedicarboxylate (50 mol% K_2CO_3, DMF, 25 °C, 1 h) led to a mixture (1.5 : 1) of *E*- and *Z*-adducts in 70% yield. To our knowledge, this is the only example of cytosine addition to the C≡C bond.[3(f)]

Here we describe a new group of polyfunctional N^1-modifications of cytosine **1** originated from its base-catalyzed regio- and stereoselective addition to available[7] cyanopropargylic alcohols **2a**,**b** (Table 1).† In the presence of base (K_2CO_3 or DABCO), cytosine **1** reacts with cyanopropargylic alcohols **2a**,**b** at 20–25 °C in polar non-hydroxylic solvents (DMF, MeCN) to regio- and stereoselectively give the Michael adducts, (*Z*)-3-[4-amino-2-oxo-1(2*H*)-pyrimidinyl]-4-hydroxy-4-alkylalk-2-enenitriles **3a**,**b**, in up to 88% yield (Scheme 1, Table 1). The optimum **1** : **2** molar ratio was 1 : 1–1.1. The yield depended on the nature of base and its amount. The best results were attained with K_2CO_3 (3–5 mol%) in DMF (20–25 °C).



Scheme 1

Table 1 The influence of conditions on the yield of adducts **3a**,**b** (loading of cytosine **1**, 1 mmol).

Acetylene	**1** : **2** molar ratio	K_2CO_3 (mol%)	Solvent (ml)	*T* / °C	*t* / h	Product	Yield (%)[a]	Conversion of cytosine (%)
2a	1:1	3	DMF (5)	20–25	3.0	**3a**	80	
2a	1:1.1	5	DMF (5)	20–25	1.5	**3a**	69	
2a	1:1.4	10	DMF (3)	20–25	1.5	**3a**	56	
2a	1:1	13[b]	MeCN (4)	20–25	18 days	**3a**	50	51
2b	1:1.1	5	DMF (5)	20–25	2.0	**3b**	88	
2b	1:1	5	MeCN (5)	20–25	20.0	**3b**	66	44
2b	1:1	20	MeCN (16)	30–35	7.0	**3b**	69	88
2b	1:1	30	MeCN (5)	40–45	2.0	**3b**	74	75

[a] Taking into account the conversion. [b] DABCO instead of K_2CO_3.

The reaction completion has been controlled by IR spectroscopy following the disappearance of the bands at 2295–2279 cm^{-1} (–C≡C–CN) and arising of the bands at 2228–2223 cm^{-1} (–C=C–CN) associated with the starting and target compounds, respectively.

Multinuclear ^{1}H, ^{13}C, ^{15}N, and 2D (NOESY, HMBC, HSQC) NMR and IR spectroscopy data of adducts **3a**,**b** are in agreement with their structure. In the ^{1}H NMR spectra of adducts **3a**,**b** there is a =CHCN signal at 6.28 ppm, that is indicative of the formation of only one isomer. In the ^{13}C NMR spectra of compounds **3a**,**b**, the olefinic carbon (=CHCN) resonates in the region of 99.9–100.0 ppm, and the signal of the N^{1}–C= group carbon appears at 167.5–168.2 ppm. In the ^{15}N NMR spectra of products **3a**,**b** two nitrogen signals of heterocyclic fragment are observed at –229.2 and –230.2 (N^{1}), –168.9 and –170.6 (N^{3}) ppm, while nitrogen signals of CN and NH_2 groups are present in the region of –115.4 and –115.5 ppm, as well as –283.7 and –281.7, respectively. The cross-peaks between proton of C^{6} and α-C of cyanoethenyl fragment in HMBC (^{1}H–^{13}C) spectra as well as olefinic proton and N^{1} of the pyrimidine ring in HMBC (^{1}H–^{15}N) spectra are observed, thus unambiguously supporting the formation of the N^{1} adducts. The *Z*-configuration of adducts **3a**,**b** follows from the NOESY spectra having cross-peaks between olefinic proton and protons of the hydroxyalkyl substituents (see Online Supplementary Materials).

The advantageous features of cytosine modification here developed are regio- and stereoselectivity of the reaction and avoiding protection of NH_2-substitutent. The regio- and stereoselectivity of the reaction is determined by the presence of strong electron-withdrawing CN group (EWG) at the triple bond. Such groups are commonly known[8] to control the better direction (relative to the EWG substitutent and *Z*-configuration of the adduct) of the nucleophilic addition to the acetylenic bond. Obviously, the hydroxyl substituents in cyanopropargylic alcohols are not the cause of the regio- and stereoselectivity observed. A novel combination of promising functionalities (OH, CN and vinyl groups) in the cytosine modifications obtained represents another benefit of the methodology. Since the starting cyanopropargylic alcohols are now accessible,[7] scope of the methodology is not limited to the examples given in this communication.

In summary, the results obtained allow one to synthesize novel families of acyclic nucleosides with biologically important functionalities (OH, CN and vinyl groups). Such compounds can be potential pharmaceuticals and promising building blocks for drug design.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2017.01.003.

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[†] *(Z)-3-[4-Amino-2-oxo-1(2H)-pyrimidin-1-yl]-4-hydroxy-4-methylpent-2-enenitrile* **3a**. Cyanopropargylic alcohol **1a** (0.120 g, 1.1 mmol) in DMF (2 ml) was added dropwise to a mixture of cytosine **1** (0.111 g, 1.0 mmol) and K_2CO_3 (0.04 g, 0.03 mmol) in DMF (3 ml) over 30 min. The mixture was stirred at 20–25 °C for 3 h. The mixture was passed through Al_2O_3 (1 cm) using Schott funnel, eluents: DMF (5 ml) and Me_2CO (5 ml). The solvents were removed *in vacuo*. The residue was washed with small portions of the mixture of Et_2O/EtOH (1 : 1, 5 ml) and hexane, dried *in vacuo* to afford compound **3a** (0.176 g, 80%) as a beige powder, mp 197–199 °C (decomp). ^{1}H NMR (400.13 MHz, DMSO-d_6) δ: 7.41, 7.33 (br. s, 2 H, NH_2), 7.38 (d, 1H, H^{6}, $^{3}J_{H^6H^5}$ 7.2 Hz), 6.28 (s, 1 H, =CHCN), 5.78 (d, 1 H, H^{5}, $^{3}J_{H^5H^6}$ 7.3 Hz), 5.48 (s, 1H, OH), 1.30, 1.21 (s, 6 H, 2 Me). ^{13}C NMR (100.62 MHz, DMSO-d_6) δ: 167.5 (N*C*=CCN), 166.4 (O=C), 154.0 (C^{4}), 145.0 (C^{6}), 115.4 (CN), 99.9 (=*C*HCN), 93.8 (C^{5}), 72.4 (Me_2*C*O), 28.5, 28.2 (Me). ^{15}N NMR (40.56 MHz, DMSO-d_6) δ: –283.7 (NH_2), –230.2 (N^{1}), –168.9 (N^{3}), –113.3 (CN). IR (KBr, ν/cm^{-1}): 3412, 3339, 3250, 3210, 3076, 2982, 2942, 2881, 2228, 1649, 1600, 1567, 1511, 1490, 1445, 1369, 1299, 1251, 1222, 1197, 1173, 1137, 1115, 976, 948, 856, 830, 785, 708, 676, 595, 566, 532. Found (%): C, 54.26; H, 5.63; N, 25.10. Calc. for $C_{10}H_{12}N_4O_2$ (%): C, 54.54; H, 5.49; N, 25.44.

(Z)-3-[4-Amino-2-oxo-1(2H)-pyrimidinyl]-3-(1-hydroxycyclohexyl)prop-2-enenitrile **3b**. Cyanopropargylic alcohol **1b** (0.164 g, 1.1 mmol) in DMF (2 ml) was added dropwise to a mixture of cytosine **1** (0.111 g, 1.0 mmol) and K_2CO_3 (0.007 g, 0.05 mmol) in DMF (3 ml) over 30 min. The mixture was stirred at 20–25 °C for 2 h. The precipitate was filtered, washed with EtOH (5 ml) and dried *in vacuo* to afford compound **3b** (0.142 g). The DMF solution was passed through Al_2O_3 (1 cm) using Schott funnel, eluents: DMF (5 ml) and Me_2CO (5 ml). The solvents were removed *in vacuo*. The residue was washed with small portions of the mixture of Et_2O/EtOH (1 : 1, 5 ml) and hexane, and dried *in vacuo* to afford compound **3b** (0.088 g). The total yield of **3b** was 0.230 g (88%) as a white powder, mp 209–212 °C. ^{1}H NMR (400.13 MHz, DMSO-d_6) δ: 7.40, 7.33 (br. s, 2 H, NH_2), 7.38 (d, 1H, H^{6}, $^{3}J_{H^6H^5}$ 7.2 Hz), 6.28 (s, 1H, =CHCN), 5.78 (d, 1H, H^{5}, $^{3}J_{H^5H^6}$ 7.3 Hz), 5.25 (s, 1H, OH), 1.66–1.07 (m, 10 H, CH_2). ^{13}C NMR (100.62 MHz, DMSO-d_6) δ: 168.2 (N*C*=CCN), 166.4 (C=O), 154.2 (C^{4}), 145.1 (C^{6}), 115.5 (CN), 100.0 (=*C*HCN), 93.8 (C^{5}), 73.4 (C$^{1'}$), 34.9, 34.7 (C$^{2'}$), 24.8 (C$^{4'}$), 21.0, 20.8 (C$^{3'}$). ^{15}N NMR (40.56 MHz, DMSO-d_6) δ: –281.7 (NH_2), –229.2 (N^{1}), –170.6 (N^{3}), -112.3 (CN). IR (KBr, ν/cm^{-1}): 3299, 3211, 3083, 3063, 2948, 2938, 2863, 2223, 1672, 1655, 1638, 1613, 1505, 1453, 1438, 1378, 1316, 1302, 1255, 1213, 1154, 1138, 1101, 1083, 1067, 964, 855, 820, 784, 705, 666, 594. Found (%): C, 59.61; H, 6.36; N, 21.65. Calc. for $C_{13}H_{16}N_4O_2$ (%): C, 59.99; H, 6.20; N, 21.52.