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8-Aza-7,9-dideazaxanthine acyclic nucleoside phosphonate inhibitors of thymidine phosphorylase

David Mařák, Miroslav Otmar *, Ivan Votruba, Martin Dračínský, Marcela Krečmerová

Institute of Organic Chemistry and Biochemistry, v.v.i., Gilead Sciences & IOCB Research Center, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic

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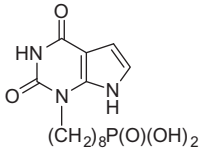
ABSTRACT

3- and 8-(8-phosphonooctyl)-8-aza-7,9-dideazaxanthine, and 1,8-bis(8-aza-7,9-dideazaxanthin-8-yl) octane were prepared and found to inhibit thymidine phosphorylase from *Escherichia coli*, human recombinant TP expressed in V79, and TP purified from human placenta. The IC_{50} values ranged from 3.5 to 27 μM.

Thymidine phosphorylase (TP, EC 2.4.2.4) catalyzes a reversible phosphorolysis of thymidine and other pyrimidine 2′-deoxynucleosides, except for those bearing an amino group in position 4. Its main metabolic function appears to be catabolic, although some bacteria and tumors utilize the reverse reaction anabolically under the stress of certain gene damage or a dietary deficiency. Thymidine phosphorylase (TP) is identical to the human angiogenic factor PD-ECGF, which is clearly involved in tumor dependent angiogenesis. Its high levels correlate with neoplastic growth. Therefore TP inhibitors are of permanent interest as potential antitumor agents. Particular attention has been paid to the multisubstrate inhibitors of TP binding to both the nucleoside and the phosphate binding sites. Their advantage consists mainly in having more points of interaction with the receptor than single-substrate inhibitors.[1] In a report published a decade ago, a series of acyclic phosphonate derivatives of thymine was tested as multisubstrate analog inhibitors of *Escherichia coli* TP, among which 3-(8-phosphonooctyl)-7-deazaxanthine (**1**, Chart 1) was shown to have a remarkable inhibitory potency.[2]

Our aim[3] was to design further multisubstrate inhibitors of TP by a structural modification of the parent compound **1**. At first we tried to make a slight structural change consisting in a shift of the pyrrole nitrogen atom from position 9 to position 8, which led us to the synthesis of 3-(8-phosphonooctyl)-8-aza-7,9-dideazaxanthine (**2**).

Compound **2** was synthesized in six steps from uracil as it is outlined in Scheme 1. The Michaelis–Arbuzov reaction of



1

Chart 1.

1-(8-bromooctyluracil) (**3**), obtained by the alkylation of uracil by 1,8-dibromooctane, afforded the phosphonate **4**. The BOM-protected phosphonate **5** was treated with toluenesulfonylmethyl isocyanide (TosMIC) under conditions of a van Leusen pyrrole synthesis[4] to yield the protected 8-aza-7,9-dideazaxanthine **6**. A catalytic hydrogenation of **6** produced compound **7**. Finally, the phosphonate esters of **7** were removed with TMSBr under a formation of the acyclic 8-aza-7,9-dideazaxanthine nucleotide **2**.[5]

Considering the promising inhibition capability of compound **2** (see below), we decided to extend the SAR study by a preparation of its positional isomer **8** (Scheme 2). Compound **8** was synthesized in a manner analogous to that for phosphonate **2**. 1,3-Bis(benzyloxymethyl)uracil[6] was treated with TosMIC[4] to yield the protected 8-aza-7,9-dideazaxanthine **9**. Alkylation of **9** with 1,8-dibromooctane afforded compound **10** together with a small amount of the disubstituted product **11**. When the protected nucleobase **9** was taken into the reaction in a 2:1 ratio to 1,8-dibromooctane, the yield of **11** amounted to 73%. Compound **11** was deprotected to the 'double-headed' acyclic nucleoside **12**. The Michaelis–Arbuzov reaction of the bromide **10** afforded the protected nucleotide **13**.

* Corresponding author. Tel.: +420 220183375.
E-mail address: otmar@uochb.cas.cz (M. Otmar).

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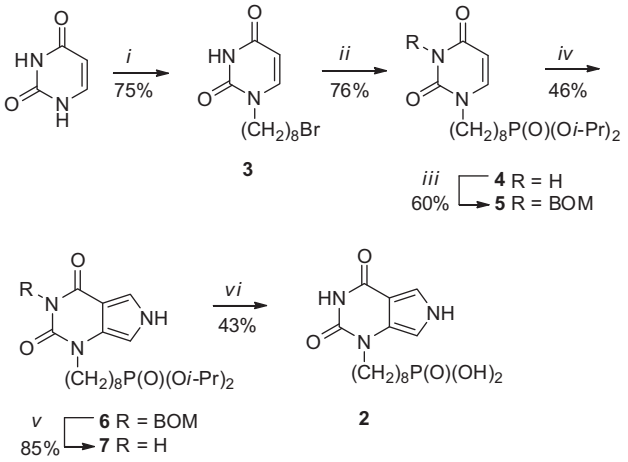


Scheme 1. Reagents and conditions: (i) BSA, 1,8-dibromooctane, CH_3CN, 75 °C, 3 h; (ii) P(O*i*-Pr)$_3$, 160 °C, 6 h; (iii) NaH, BOM-Cl, DMF, 75 °C, 5 h; (iv) TosMIC, DMSO–dioxane (1:4), 75 °C, 6 h; (v) H_2/Pd, MeOH, overnight; (vi) (1) Me_3SiBr, CH_3CN, overnight; (2) NH_3/H_2O.

The final deprotection of **13** by catalytic hydrogenation followed by a treatment with TMSBr afforded the acyclic nucleoside phosphonate **8**.

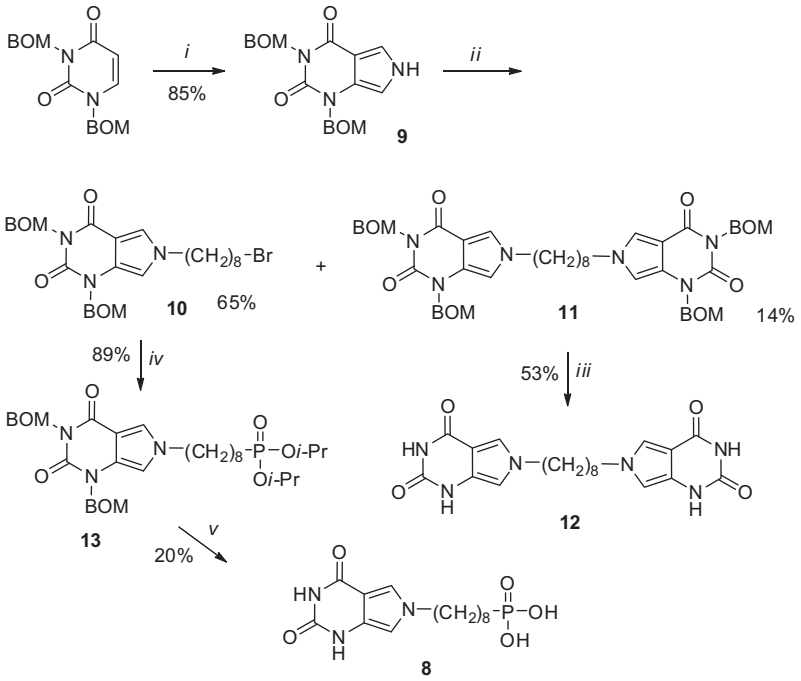
All of the new compounds were fully characterized on the basis of their ^1H and ^{13}C NMR spectra and further spectroscopic and analytical data.

The inhibitory potency of 8-aza-7,9-dideazaxanthine acyclic nucleoside phosphonates **2** and **8**, 'double-headed' nucleoside **12**, and the parent compound **1** on the phosphorolysis of thymidine was evaluated using two recombinant TPs of a commercial origin (TP from *E. coli* and human TP expressed in V79 Chinese hamster cells) and the enzyme isolated from human placenta. The TP from the human placenta was purified according to Yoshimura et al.[7] with a minor modification (additional chromatography on Q Sepharose) and the hydroxyapatite fraction was used for the inhibition experiments. The standard reaction mixture (50 μL) contained 100 μL [^{3}H-methyl]thymidine, 200 μM potassium phosphate buffer (pH 6.7), and enzyme (6.7 mU TP from *E. coli*, Sigma T 2807; 0.41 mU human TP, expressed in V79 Chinese hamster cells, Sigma T 9319; 0.45 mU TP purified from human placenta[7] in 20 mM bis Tris–HCl, pH 6.4, 1 mM EDTA, and 2 mM DTT). The reaction was carried out at 37 °C for 10 min and stopped by spotting a 2 μL aliquot onto a Silica Gel 60 F_{254} plate that had been prespoted with 0.01 μmol of each thymine and thymidine. The plate was developed in the solvent system ethyl acetate/water/formic acid (60:35:5). The spots were visualized under UV light (254 nm) and cut out for a radioactivity determination in the toluene-based scintillation cocktail.

The results show that all of the above-mentioned compounds efficiently inhibit at 0.1 mM thymidine and 0.2 mM potassium phosphate buffer bacterial and/or human TPs with IC_{50} values in the concentration range from 3.5 to 27 μM (Table 1). At a higher phosphate concentration (1 mmol/L) in the reaction mixture the IC_{50} are about 50% higher (data not shown).

In the literature,[2] the capability of the parent compound **1** to inhibit TP from *E. coli* is reported as a 97%, 83%, and 48% inhibition at 1000, 100, and 20 μM concentrations in 20 min of incubation time. The values roughly correspond to the IC_{50} for compound **1** given in Table 1. The inhibition of TP from other sources was not reported. Table 1 shows that, on TP from *E. coli*, the inhibition capability of the new compounds **2**, **8**, and **12** is quite comparable with that for the parent compound **1**. On human enzymes, the IC_{50} values of compound **2** are still comparable with those for **1**, but the highly modified derivatives **8** and **12** are less active.

In conclusion, the modification of the parent compound **1** leading to the acyclic nucleoside **2**, which differs by the position of the pyrrole nitrogen, only slightly influences the degree of TP inhibition. When the above-mentioned structural change is accompanied by a different substitution of the nucleobase with the 8-phosphononooctyl chain (compound **8**), the inhibition potency on the TP from *E. coli* still remains, but on the human enzymes it decreases.



Scheme 2. Reagents and conditions: (i) TosMIC, NaH, DMSO–Et_2O (1:4), 6 h; (ii) NaH, 1,8-dibromooctane, DMF, 95 °C, 3 h; (iii) H_2/Pd, MeOH, overnight; (iv) P(O*i*-Pr)$_3$, 160 °C, 6 h; (iv) (1) H_2/Pd, MeOH, overnight; (2) Me_3SiBr, CH_3CN, overnight; (3) NH_3/H_2O.

Table 1
The inhibition of thymidine phosphorylase from different sources by test compounds

	Inhibition of TPase (IC_{50} [μM])[a] Compound			
	1[b]	**2**	**8**	**12**
TP from *Escherichia coli*	11.4	6.8	12	3.5
Human recombinant TP expressed in V79	7.3	27	ND[c]	23
TP purified from human placenta	15.1	17	ND[c]	ND[c]

[a] The inhibition data represent the mean of at least three independent experiments.
[b] Parent compound.[2]
[c] Not determined. If the compound in a concentration of 10 μM inhibits the enzyme from less than 20% (V/V_0), the IC_{50} value was not determined.

Paradoxically, when the third modification—replacement of the phosphonate function by a further 8-aza-7,9-dideazaxanthine nucleobase—is made (compound **12**), the inhibitory capability increases again. The results revealed that there is a considerable scope for the further development of new multisubstrate inhibitors of TP possessing an 8-aza-7,9-dideazaxanthine nucleobase.

The in vitro cell growth inhibition by compounds **1**, **2**, **8**, and **12** was evaluated in the following cell lines: CCRF-CEM T-lymphoblastoid cells (human acute lymphoblastic leukemia, ATCC CCL 119), human promyelocytic leukemia HL-60 cells (ATCC CCL 240), human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2), and mouse lymphocytic leukemia L1010 cells (ATCC CCL 219); no antitumor activity was found.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.027.

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5. ^{1}H NMR (D_2O) 7.42 (d, 1H, *J*(7,5) = 1.8 Hz, H-7), 6.67 (d, 1H, *J*(5,7) = 1.8 Hz, H-5), 3.70 (t, 2H, *J*(1,2) = 7.3 Hz, H-1′), 1.44–1.62 and 1.20–1.34 (m, H-2′–H-8′). ^{13}C NMR (APT, D_2O) 165.8 (C-4), 154.8 (C-2), 131.6 (C-4b), 121.30 (C-7), 107.2 (C-4a), 104.5 (C-5), 47. 7 (C-1′), 32.8 (d, *J*(7,P) = 16.2 Hz, C-7′), 31.1, 31.0, 30.9 (C-2′, C-3′, C-4′, C-5′), 29.5 (*J*(8′,P) = 132.9 Hz, C-8′), 25.8 (C-6′). ^{31}P NMR (D_2O): 27.7. HRMS (ESI) calcd for $C_{14}H_{22}N_3O_5P$ (M+H): 344.1375, found: 344.1375.
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