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Original article

Inhibition of human thymidine phosphorylase by conformationally constrained pyrimidine nucleoside phosphonic acids and their "open-structure" isosteres

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ABSTRACT

A series of conformationally constrained uridine-based nucleoside phosphonic acids containing annealed 1,3-dioxolane and 1,4-dioxane rings and their "open-structure" isosteres were synthesized and evaluated as potential multisubstrate-like inhibitors of the human recombinant thymidine phosphorylase (TP, EC 2.4.2.4) and TP obtained from peripheral blood mononuclear cells (PBMC). From a large set of tested nucleoside phosphonic acids, several potent compounds were identified that exhibited K_i values in the range of 0.048–1 μM. The inhibition potency of the studied compounds strongly depended on the degree of conformational flexibility of the phosphonate moiety, the stereochemical arrangement of the sugar-phosphonate component, and the substituent at position 5 of the pyrimidine nucleobase.

1. Introduction

Thymidine phosphorylase (TP), the enzyme identical to platelet-derived endothelial cell growth factor [1–5], catalyzes the reversible phosphorolysis of thymidine to thymine and 2-deoxy-α-D-ribofuranosylphosphate (Fig. 1).

In contrast to non-neoplastic tissues, the elevated levels of TP have been found in colorectal, ovarian, pancreatic, and breast tumors [6,7], and in other hyperproliferative disease states such as rheumatoid arthritis [8] and psoriasis [9]. The inhibition of TP may result in the reduction of tumor growth and metastasis [10–17]. To date, a number of TP inhibitors of human TP based on the analogues of uracil and thymine nucleobases and nucleosides have been reported [18–32]. The most potent and therapeutically promising inhibitor of human TP is 5-chloro-6-[1-(2-iminopyrrolidinyl)methyl]uracil hydrochloride (**1**, TPI [13], Taiho Pharmaceutical Company) with a K_i of 17 nM [33] which, in a combination with the anticancer drug trifluorothymidine, is currently in clinical trials (Fig. 2) [34,35].

In addition to these mono-substrate-like inhibitors, there is a class of compounds exists in which both nucleoside and phosphoryl moieties are combined in one molecule that can bind simultaneously to both nucleobase and phosphate binding sites, respectively. Balzarini et al. [36,37] and Votruba et al. [38] reported the inhibition of *Escherichia coli* TP with structurally diverse acyclic nucleoside phosphonic acids (Fig. 2, structures **2**–**4**), and Lequeux et al. [39] recently published a thorough structural study on the inhibition of the enzyme by various α,α-difluoromethyl-moiety-containing 1-thyminylalkylphosphonic acids **4a**. Comparative study on the inhibition of *E. coli* and human TPs with 3-pyrimidinylalkylphosphonic acids was recently reported by Pomeisl et al. [40]. Irrespective of the structural and genetic similarities among thymidine phosphorylases from various sources including *E. coli* and human recombinant TPs, the sensitivity of the two enzymes toward inhibitors based on nucleoside phosphonic acids appears to differ. Recently, we described the potent bi-substrate-like inhibitors **5** and **6a**–**b** of TP isolated from T-cell lymphomas of the Sprague–Dawley rat strain; the inhibitors were based on pyrrolidine nucleoside phosphonic acids [41], with IC_{50} values in the range of 11–45 nM at a thymidine concentration of 100 μM. Interestingly, these compounds did not inhibit healthy rat liver, *E. coli*, or human TPs.

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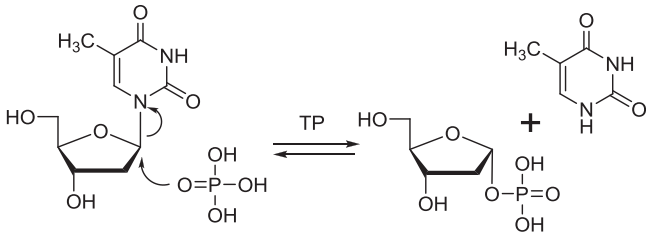


Fig. 1. TP-catalyzed thymidine cleavage.

In contrast to guanine-based pyrrolidine nucleoside phosphonic acids, recently reported as potent bi-substrate-like inhibitors [42] of human purine nucleoside phosphorylase, the similar thymine-based compounds did not inhibit the human TP.

The first nucleoside phosphonic acid inhibiting human TP in the submicromolar range, 5-methyl-2′,3′-*O*-(2-phosphono-1,1-ethylidene)uridine (**50**, Fig. 1), a conformationally constrained bi-substrate-like inhibitor with a K_i value of 236 nM, was reported by Li [43]. Recently described [44] carba analogues of **50**, i.e., nucleoside phosphonic acids **7a** and **7b**, inhibited the human TP in the high micromolar range.

In this paper, we describe the synthesis of two new series of pyrimidine nucleoside phosphonic acids, conformationally constrained and flexible ("open-structure"), respectively, and describe their ability to inhibit human TP. The target compounds were designed to offer a variety of structural types believed to cover a range of potential candidates that could provide significant data on the structure–activity relationship.

2. Results and discussion

2.1. Chemistry

2.1.1. Nucleosid-2′,3′-di-O-ylmethanephosphonic acids (Scheme 1)

Conformationally constrained pyrimidine nucleotide analogs **12a**,**b**–**14a**,**b**, the isopolar phosphonate analogs of pyrimidine nucleoside 2′(3′)-phosphates, were synthesized from the appropriate nucleosides **8** and **9** by several step syntheses (Scheme 1) [45]. These compounds were obtained as epimeric mixtures that provided individual epimers after separation by RP HPLC. 5′-Chlorouridine phosphonates **13a** and **13b** were prepared from the protected 5-chlorouridine **9** which was easily obtained by chlorination of the uridine derivative **8** with *N*-chlorosuccinimide (NCS) according to the described procedure [46]. Protected nucleosides **8** and **9** were transformed into the 2′,3′-*O*-orthoester derivatives **10** and **11**, respectively, by the reaction with trimethyl orthoformate in the presence of anhydrous HCl in DMF. Subsequent reaction of **10** and **11** with two equivalents of dimethyl chloro phosphite gave, after removal of the silyl protecting group with TBAF and the following treatment with bromotrimethylsilane in a CH_3CN–2,6-lutidine mixture (to remove the phosphoester groups), a mixture of **13a** (*endo*) and **13b** (*exo*) epimers (25:75). The epimers were separated by RP HPLC. In contrast to the synthesis of **13a**,**b**, the 5-iodo derivatives **14a** and **14b** were prepared by a direct iodination of pure epimers **12a** and **12b** with iodine in diluted nitric acid [47]. Compounds **12c** and **12d** were synthesized according to the method described in the literature [45].

2.1.2. Nucleosid-2′(3′)-O-ylmethanephosphonic acids (Scheme 2)

The regioisomeric 3′- (**23a**–**26a**) and 2′-*O*-methanephosphonic acids (**23b** and **24b**), which are conformationally flexible congeners related to the constrained phosphonates **12** and **13**, were prepared from the corresponding protected nucleosides **15a**–**18a**, **15b** and **16b** in three steps (Scheme 2). The alkylation of these nucleosides with methyl tosyloxymethanephosphonate [48] provided phosphonate methyl esters **19a**–**22a**, **19b** and **20b**, respectively. Detritylation of these compounds using 80% acetic acid followed by removal of the ester groups by bromotrimethylsilane treatment afforded the final 3′- and 2′-*O*-methanephosphonic acids **23a**–**26a** and **23b**, **24b**, respectively. The synthesis of **23a** was already reported [49].

2.1.3. 2-(Nucleosid-3′(2′)-O-yl)-ethanephosphonic acids (Schemes 3 and 4)

For the synthesis of nucleoside 3′- and 2′-*O*-(hydroxyethane) phosphonic acids (**31a** and **31b**, respectively), and 3′- and 2′-*O*-ethanephosphonic acids (**33a** and **33b**, respectively) which are one methylene group longer congeners of compounds **23a** and **23b** with an additional methylene group, we used the following synthetic strategy. The *O*-allylation of 5′-*O*-dimethoxytrityl nucleosides **15a** and **15b** with allyl bromide [50] afforded the allyl derivatives **27a** and **27b**. Treatment of these compounds with osmium tetroxide in the presence of *N*-methylmorpholine-*N*-oxide provided, after silica gel chromatography, pure *O*-(2,3-dihydroxypropyl) derivatives **28a** and

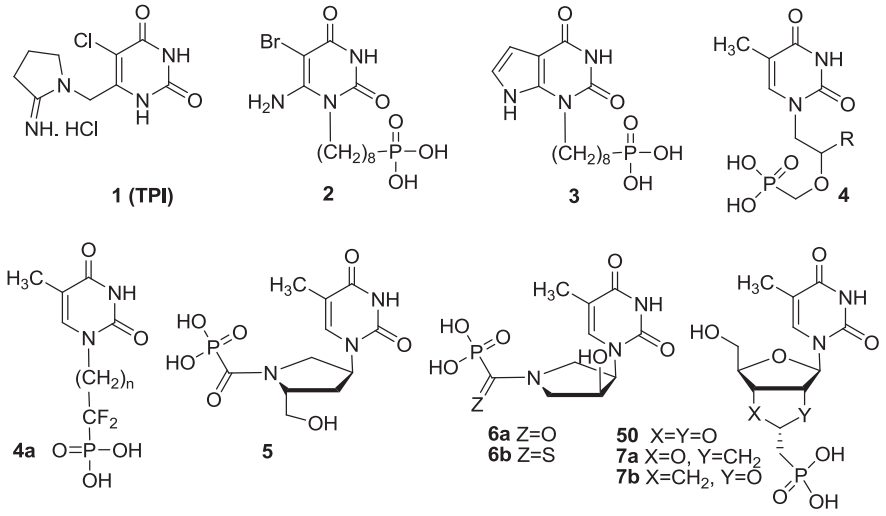
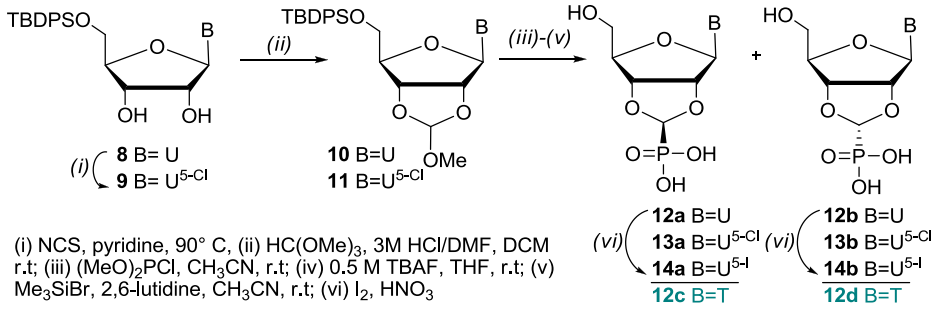
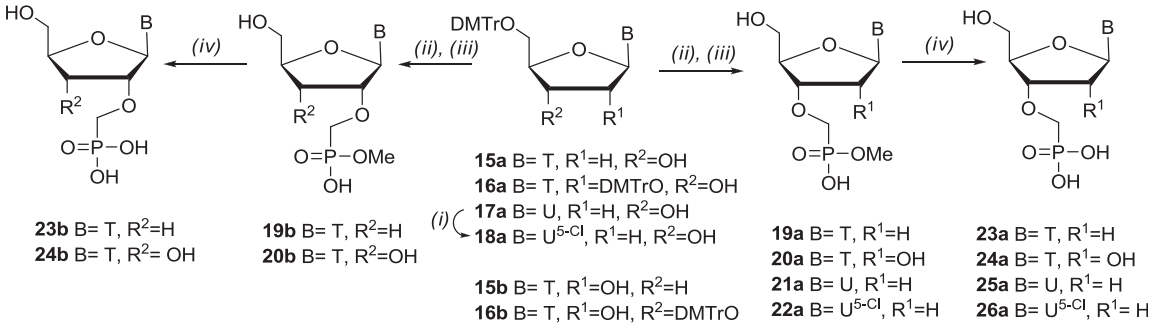


Fig. 2. TPI and bi-substrate like nucleoside phosphonic acid-based TP inhibitors.



Scheme 1. Synthesis of nucleosid-2′,3′-di-*O*-ylmethanephosphonic acids.

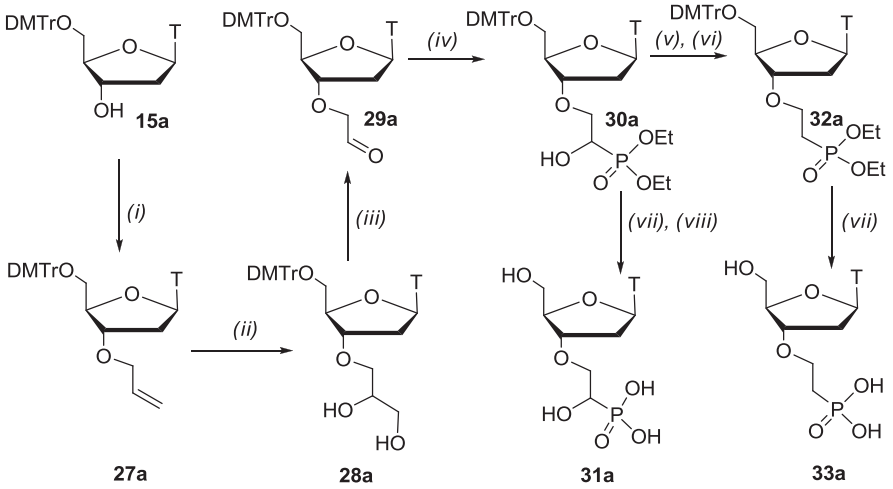


(i) NCS, pyridine, 90 °C; (ii) $TsOCH_2P(O)(OH)(OCH_3)$, NaH, DMF, rt; (iii) 80% AcOH, rt, 30 min; (iv) Me_3SiBr, 2,6-lutidine, CH_3CN;

Scheme 2. Synthesis of nucleosid-2′(3′)-*O*-ylmethanephosphonic acids.

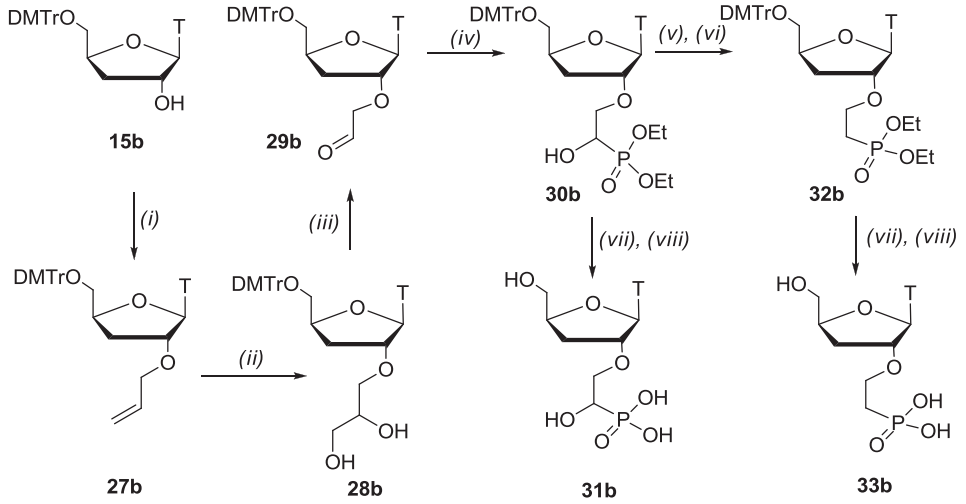
28b which were subsequently cleaved with sodium periodate to provide the aldehydes **29a** and **29b**, respectively. To obtain aldehydes **29a** and **29b** in one step from the allyl derivatives **27a** and **27b**, we also examined the OsO_4/$NaIO_4$ system. To our surprise, the starting nucleosides **15a** and **15b** were isolated from the reaction mixture as the main products. We concluded that the formed aldehyde **29a**,**b** is enolized, the resulting enol form is hydroxylated, and the dihydroxy derivative is cleaved with periodate to produce the unstable 2′(3′)-*O*-formyl derivative of nucleosides **15a** and **15b**.

The prepared aldehydes **29a** and **29b** were subjected to the Abramov reaction [51] with diethyl phosphite in the presence of triethylamine, which provided the respective epimeric hydroxyphosphonate derivatives **30a** and **30b**, respectively. Removal of the dimethoxytrityl group using 80% acetic acid and subsequent treatment with bromotrimethylsilane afforded the free phosphonates **31a** and **31b**, respectively. Compounds **30a** and **30b** were converted into the respective deoxygenated products **32a** and **32b**, respectively, upon treatment with thiocarbonyldiimidazole to



(i) NaH, allyl bromide, THF, rt; (ii) OsO_4, *N*-methylmorpholine-*N*-oxide, THF-water (2:1), rt; (iii) $NaIO_4$, acetone-water (1:1), rt; (iv) diethyl phosphite, TEA, DCM, rt; (v) 1,1'-thiocarbonyldiimidazole, DCE, rt; (vi) tributyltin hydride, AIBN, toluene, reflux; (vii) 80% aq. CH_3COOH, rt; (viii) BSA, Me_3SiBr, CH_3CN;

Scheme 3. Synthesis of 2-(thymidin-3′-*O*-yl)-ethanephosphonic acid and 1-hydroxy-2-(thymidin-3′-*O*-yl)-ethanephosphonic acid.



(i) NaH, allyl bromide, THF, rt; (ii) OsO_4, 4-methylmorpholine N-oxide, THF-water (2:1), rt; (iii) $NaIO_4$, acetone-water (1:1), rt; (iv) diethyl phosphite, TEA, DCM, rt; (v) 1,1'-thiocarbonyldiimidazole, DCE, rt; (vi) tributyltin hydride, AIBN, toluene, reflux; (vii) 80% aq. CH_3COOH, rt; (viii) BSA, Me_3SiBr, CH_3CN

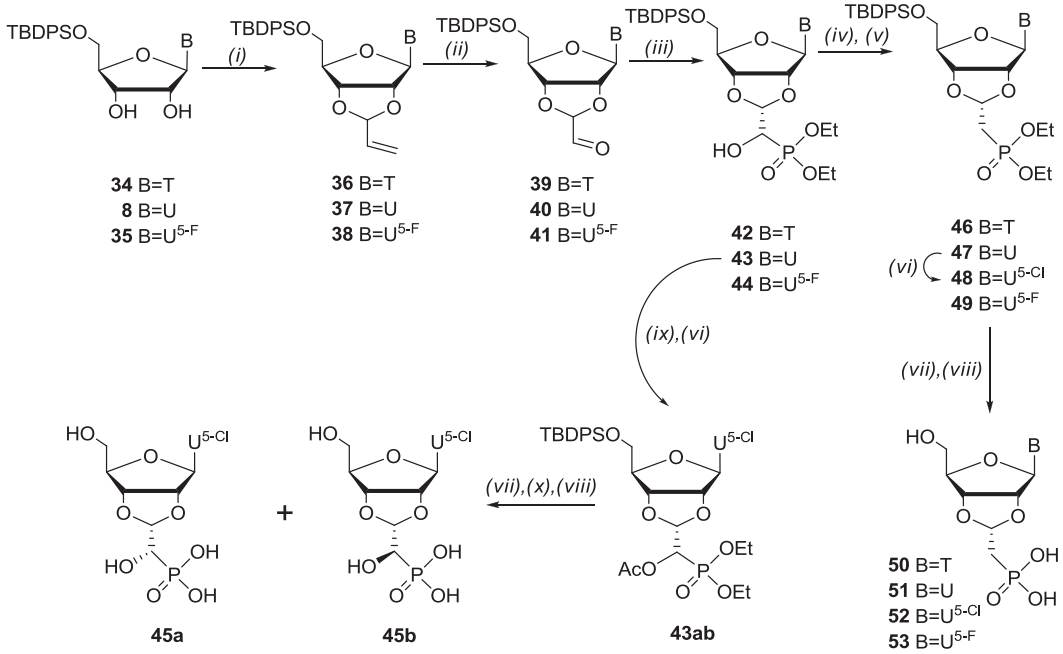
Scheme 4. Synthesis of 2-(3′-deoxy-5-methyluridin-2′-*O*-yl)-ethanephosphonic acid and 2-(3′-deoxy-5-methyluridin-2′-*O*-yl)-1-hydroxyethanephosphonic acid.

provide the corresponding imidazoylthiocarbonyl derivatives, which were subsequently reacted with tributyltin hydride and AIBN. Subsequent treatment of **32b** with bromotrimethylsilane afforded the free phosphonic acid **33b**, whereas the deprotection of **32a** under the same conditions described for **32b** led to complete glycosidic bond cleavage. However, the deprotection of **32a** using bromotrimethylsilane in a *N,O*-bis-(trimethylsilyl)acetamide–acetonitrile mixture provided the desired phosphonate **33a**.

2.1.4. 2-(Nucleosid-2′,3′-di-O-yl)-ethanephosphonic acid (Scheme 5)

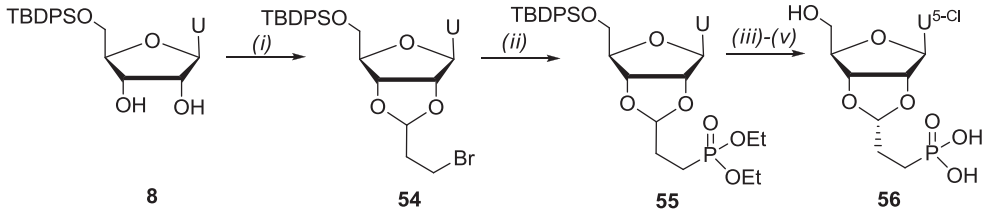
The conformationally constrained nucleotide analogs **45a**, **45b**, and **50**–**53**, which are related to compounds **12**–**14** but contain one additional methylene group on their side chains, were prepared by the reaction sequence shown in Scheme 5.

The starting 5′-*O*-silylated nucleosides **34**, **8**, and **35** were transformed into the 2′,3′-*O*-allylidene derivatives **36**–**38**, respectively, by treatment with acrolein dimethyl acetal in



(i) acrolein dimethyl acetal, HCl/DMF, DCM, rt; (ii) OsO_4, $NaIO_4$, THF-water (2:1), rt; (iii) diethyl phosphite, TEA, DCM, rt; (iv) 1,1'-thiocarbonyldiimidazole, DCE. rt; (v) tributyltin hydride, AIBN, toluene, reflux; (vi) *N*-chlorosuccinimide, pyridine, 90 °C (vii) 0.5M TBAF, THF, rt; (viii) Me_3SiBr, 2,6-lutidine, CH_3CN; (ix) acetic anhydride, pyridine, rt; (x) NH_3, EtOH, rt.

Scheme 5. Synthesis of 2-(nucleosid-2′,3′-di-O-yl)-ethanephosphonic acid and 1-hydroxy-2-(nucleosid-2′,3′-di-O-yl)-ethanephosphonic acid.



(i) bromopropionaldehyde diethyl acetal, HCl/DMF, DCM, rt; (ii) diethyl phosphite, NaH, THF, rt; (iii) *N*-chlorosuccinimide, pyridine, 90 °C; (iv) 0.5M TBAF, THF, rt; (v) BSA, Me_3SiBr, CH_3CN

Scheme 6. Synthesis of 3-(5-chlorouridin-2′,3′-di-*O*-yl)-propanephosphonic acid.

dichloromethane under hydrogen chloride catalysis. The obtained allylidene derivatives **36**–**38** were treated with OsO_4 and $NaIO_4$ to provide the corresponding aldehydes **39**–**41** which subsequently afforded the hydroxyphosphonate diesters **42**–**44**, respectively (single epimers on C2 of 1,3-dioxolane ring). The final α-hydroxyphosphonic acids **45a** and **45b** were prepared starting from the uracil compound **43**. It was first *O*-acetylated using acetic anhydride and the obtained acetyl derivative was chlorinated with NCS in pyridine to give the fully protected 5-chlorouridine derivative **43ab** as a mixture of epimers. Their separation on silica gel followed by successive treatment with TBAF and ethanolic ammonia to remove the silyl and acetyl protecting groups, respectively, and final treatment with bromotrimethylsilane to remove the phosphonate ester protecting groups afforded the desired nucleoside phosphonic acids **45a** and **45b**.

The hydroxyphosphonates **42**–**44** were transformed into imidazoylthiocarbonyl derivatives by reaction with 1,1′-thiocarbonyldiimidazole in DCE, and these compounds were reduced with tributyltin hydride in toluene in the presence of AIBN to afford the phosphonoethylidene derivatives **46**, **47**, and **49**, respectively, in good yields. Derivative **48** was prepared from compound **47** by a direct chlorination with NCS in pyridine. Removal of the silyl protecting group with TBAF and subsequent treatment with bromotrimethylsilane afforded the free phosphonic acids **50**–**53**, respectively.

2.1.5. 3-(5-Chlorouridin-2′,3′-di-O-yl)-propanephosphonic acid (Scheme 6)

Another type of the conformationally constrained nucleoside phosphonic acids, the derivative **56** was prepared in five steps. The reaction of protected nucleoside **8** with bromopropionaldehyde diethylacetal smoothly provided the bromo derivative **54**.

The reaction of compound **54** with the sodium salt of diethyl phosphite in THF, followed by chlorination of the uracil moiety with NCS, provided the fully protected nucleotide, which was successively treated with a mixture of TBAF and BSA–bromotrimethylsilane to remove the silyl and phosphonate diester groups, respectively, thus yielding the phosphonate derivative **56** as a single epimer in moderate yield.

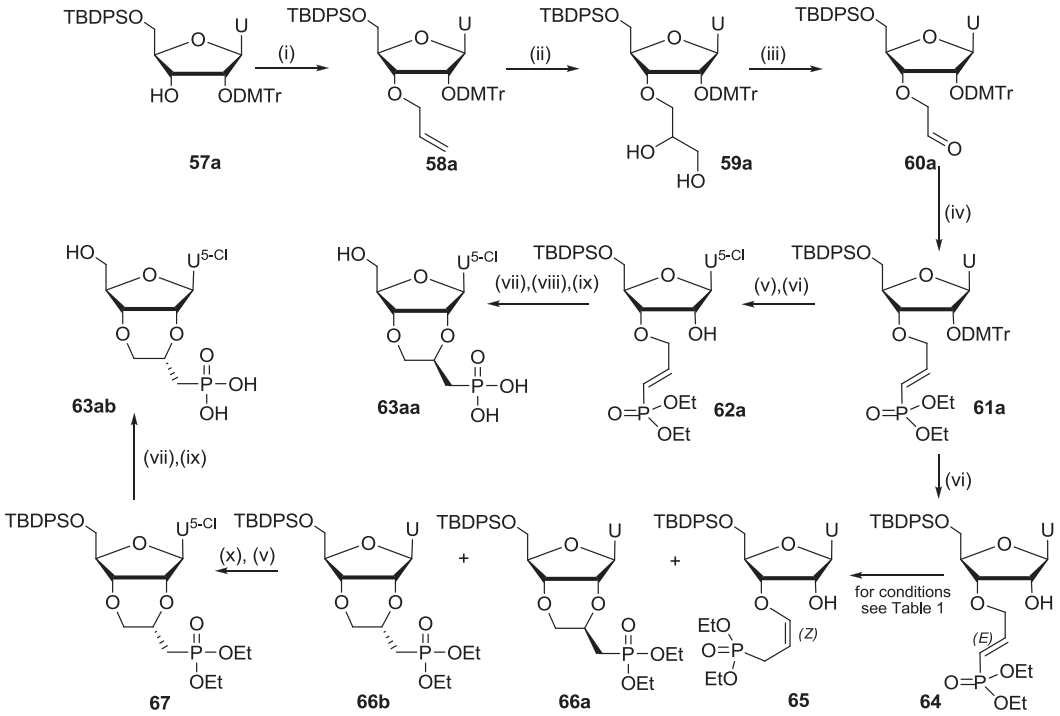
2.1.6. Epimeric 2,3-(5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→2; (3′-O)→3]- and 2,3-(5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→3; (3′-O)→2]-propanephosphonic acids (Schemes 7 and 8)

The synthesis of the regioisomeric pairs of nucleoside phosphonic acids **63aa**, **63ab** and **63ba**, **63bb** is depicted in Schemes 7 and 8. The Horner–Emmons–Wadsworth reaction of aldehydes **60a** and **60b**, which are easily prepared in three steps from the regioisomeric 3′- and 2′-*O*-DMTr derivatives **57a** and **57b**, respectively, with tetraethyl methylenediphosphonate smoothly provided the fully protected vinylphosphonates **61a** and **61b**. Subsequent chlorination with NCS and removal of the *O*-dimethoxytrityl and *O*-silyl protecting groups with 80% acetic acid and TBAF, respectively, afforded the vinylphosphonate diesters **62a** and **62b**.

These compounds underwent, upon treatment with sodium methoxide in methanol, an intramolecular Michael addition of the 2′- and 3′-hydroxyl to the vinylphosphonate moiety resulting in the formation of the diethyl esters of **63** with anneled 1,4-dioxane rings. Whereas the cyclization of **62b** under sodium methoxide catalysis provided the epimers **63ba** and **63bb** in a 3:2 ratio, the cyclization of **62a** proceeded stereoselectively, giving a single epimer **63aa** in a yield greater than 95%. To obtain the second epimer **63ab**, we examined various conditions for the cyclization reaction of the vinylphosphonate **64** (Scheme 7). The results of the study are summarized in Table 1. Careful RP HPLC analysis of the products revealed that the ratio of epimers **66a** and **66b** could be dramatically influenced by the solvent and base used. The best result regarding the content of the desired epimer **66b** in the reaction mixture was achieved with *t*-BuOH–Cs_2CO_3 and pinacol–Cs_2CO_3 (Entries 3, 4, and 6). In the latter case, however, the conversion of the starting vinylphosphonate **64** was only 60%. In contrast to the successful use of the *t*-BuOH–Cs_2CO_3 system to increase the amount of epimer **66b**, the application of use DMF–Cs_2CO_3 led to an almost exclusive formation of a novel product, *cis*–allylphosphonate **65** (Entry 10, 11). Interestingly, when any reaction mixture (Entry 1–4) was evaporated to dryness and then analysed by RP HPLC, the presence of ~90% of *cis*–allylphosphonate **65** in the mixture was detected. This phenomenon could be explained by a reverse Michael addition–decyclization of **66a** and **66b** providing the *cis*–allylphosphonate **65**. The mechanism of the reaction in this particular case is unclear and will be studied further. In view of these findings, the workup of the reaction mixtures (Entries 3 and 4) was performed; desalting under the use of Dowex 50 (Et_3NH^+ form) was performed first, and only then could the solution be safely evaporated to dryness. Mixtures of epimers **66a** and **66b**, as well as the *cis*-compound **65**, were successfully separated by silica gel chromatography. The desired epimer **66b** was chlorinated by NCS in pyridine, giving the 5-chlorouracil derivative **67**. Its deprotection with TBAF, followed by bromotrimethylsilane treatment, provided the desired, epimerically pure phosphonic acid **63ab**.

2.1.7. Epimeric pairs 1,2-(5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→1; (3′-O)→2]-ethanephosphonic acids 1,2-(5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→2; (3′-O)→1]-ethanephosphonic acids (Schemes 9 and 10)

The synthesis of the regioisomeric pairs of these compounds **71aa**, **71ab** and **71ba**, **71bb** started from the aldehydes **60a** and **60b**, respectively. Removal of the DMTr group followed by acetylation of the formed hemiacetal hydroxyl led to the acetates **69a** and **69b**



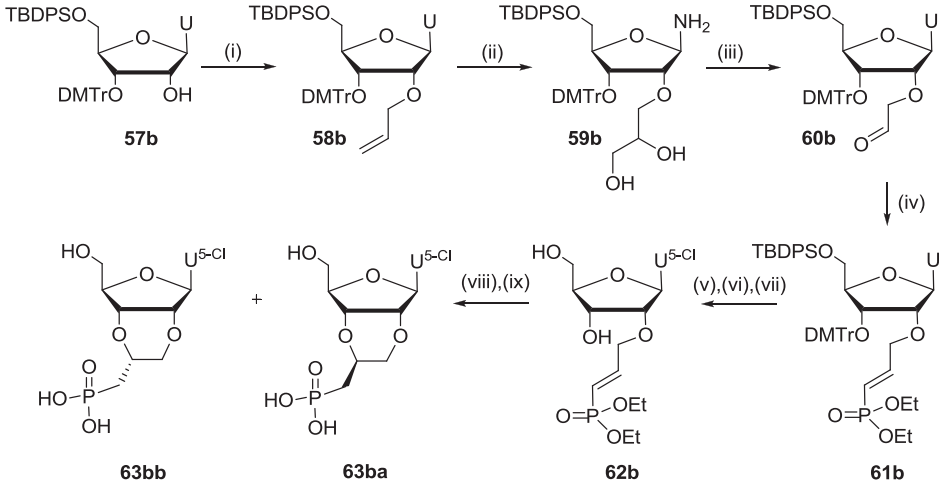
(i) NaH, allyl bromide, THF, rt; (ii) OsO_4, 4-methylmorpholine N-oxide, THF-water (2:1), rt; (iii) $NaIO_4$, acetone-water (1:1), rt; (iv) NaH, $CH_2[P(O)(OEt)_2]_2$, THF; (v) NCS, pyridine, 90 °C; (vi) 80% aq. CH_3COOH; (vii) TBAF, THF, rt; (viii) NaOMe, MeOH; (ix) BSA, Me_3SiBr, CH_3CN; (x) silica gel separation

Scheme 7. Synthesis of epimeric 2,3-(5-chlorouridin-2′,3′-di-*O*-yl)-[(2′-*O*)→2; (3′-*O*)→3]-propanephosphonic acids.

which were chlorinated with NCS in pyridine. Subsequent treatment with diethyl trimethylsilyl phosphite and TMSOTf in acetonitrile provided the fully protected regioisomeric pairs of epimeric phosphonates **70aa**, **70ab** and **70ba**, **70bb**. The two-step deprotection of these compounds afforded the phosphonic acids **71aa**, **71ab** and **71ba**, **71bb**.

2.2. NMR structure determination

The structures of all important products were determined using ^1H and ^{13}C NMR spectra. The NMR data are summarized in Tables 5 and 6 The epimers **13a** and **13b** were distinguished by the observed NOE contacts of the >C**H**–P proton. The compound with >C**H**–P



(i) NaH, allyl bromide, THF, rt; (ii) OsO_4, *N*-methylmorpholine-*N*-oxide, THF-water (2:1), rt; (iii) $NaIO_4$, acetone-water (1:1), rt; (iv) NaH, $CH_2[P(O)(OEt)_2]_2$, THF; (v) NCS, pyridine, 90 °C; (vi) TBAF, THF; (vii) 80% aq. CH_3COOH, rt; (viii) NaOMe, MeOH; (ix) BSA, Me_3SiBr, CH_3CN

Scheme 8. Synthesis of the epimeric 2,3-(5-chlorouridin-2′,3′-di-*O*-yl)-[(2′-*O*)→3; (3′-*O*)→2]-propanephosphonic acids.

Table 1
Study of the intramolecular Michael addition reaction of vinylphosphonate **64** (Scheme 7).

Entry	Solvent [0.5 mL/ 5 μmol of **64**]	Base	Base [equiv]	Products [ratio]			Conversion of **64** [%]
				65	**66a**	**66b**	
1	MeOH	MeONa	6	5	90	5	100
2	MeOH	Cs_2CO_3	6	5	80	15	100
3	*t*-BuOH		6	16	39	45	100
4	*t*-BuOH		1	15	45	40	100
5	Cyclohexanol		6	5	90	5	30 (and by-products)
6	Pinacol		6	12	42	46	60
7	Pyridine		6	40	18	42	100
8	Pyridine		1	43	20	37	30 (and by-products)
9	2,6-(di-*t*-Bu) pyridine		6	5	90	5	20 (and by-products)
10	DMF		6	90	5	5	100
11	DMF	K_2CO_3	6	90	5	5	100
12	*t*-BuOH[a]	CsF	6	40	16	44	50 (and by-products)
13	*t*-BuOH		6	0	0	0	0
14	*t*-BuOH	$Pd(OAc)_2$	0.1	0	0	0	0
15	*t*-BuOH	$(PPh_3)_4Pd$	0.1	0	0	0	0
16	Phenol	Cs_2CO_3	6	0	0	0	Decomp.
17	DBU		6	0	0	0	0

[a] Microwave irradiation 81 °C, 200 W, 10 min.

contacts to hydrogens H-1′ and H-4′ was assigned as *endo* epimer **13a**, whereas the compound with >C**H**—P contacts to hydrogen H-2′ and H-3′ was assigned as *exo* epimer **13b**. The application of the PSEUROT program [52] for the observed vicinal coupling constants in the ribofuranose ring conformation gave an equilibrium of two significantly flattened (max. pucker 20°) ^4E and E_4 forms in the ratio 43:57 in **13a** and in the ratio 66:34 in **13b**. The position of the substituent in the 3′-*O*-methylphosphonates (**23a**, **24a**, **25a**, and **26a**) and 2′-*O*-methyl-phosphonates (**23b**, **24b**) is determined by the splitting of the C-3′ or C-3′ carbon signal due to ^3J(C,P) coupling. The presence of a CH_2 group (manifested by upfield shifts of proton and carbon signals) in position 2′ or 3′ of the ribofuranose ring clearly distinguishes phosphonates **31a**, **33a** from **31b**, **33b**.

The *R*-configuration at the O—**C**H(R)—O carbon atom was determined from the observed NOE contacts of O—C**H**(R)—O to the H-2′ and H-3′ hydrogen for compounds **45a**, **45b**, **50**–**53**, and **56**. The configuration at the neighboring carbon atom (—**C**H(OH)—P) in epimers **45a** and **45b** could not be reliably determined from the NMR spectra. The interesting conformation effects were observed in the series of nucleosides with a six-membered dioxane ring annealed in positions 2′,3′ (compounds **63**, **67**, **69**, **70**, and **71**). In each of 12 compounds (**63aa**, **63ab**, **63ba**, **67**, **70aa**, **70ab**, **70ba**, **70bb**, **71aa**, **71ab**, **71ba**, and **71bb**), the substituent **Rp** in the dioxane ring always adopts the equatorial position, as follows from the vicinal coupling constants of protons *J*(Ha,Hb) and *J*(Ha,Hc) (see Fig. 3 and Table 2) and this equatorial position of **Rp** in a chair conformation of the dioxane ring determines the conformation of the ribofuranose ring either in the ^3E form (in compounds **63ab**, **67**, **70ab**, **71ab**, **63ba**, **70ba** and **71ba**) or in the ^2E form (in compounds **63aa**, **70aa**, **71aa**, **63bb**, **70bb**, and **71bb**), as indicated by the vicinal coupling constants *J*(H1′,H2′), *J*(H2′,H3′), and *J*(H3′,H4′) (see Fig. 3, Table 2 and Refs. [52,53]).

2.3. Biochemistry

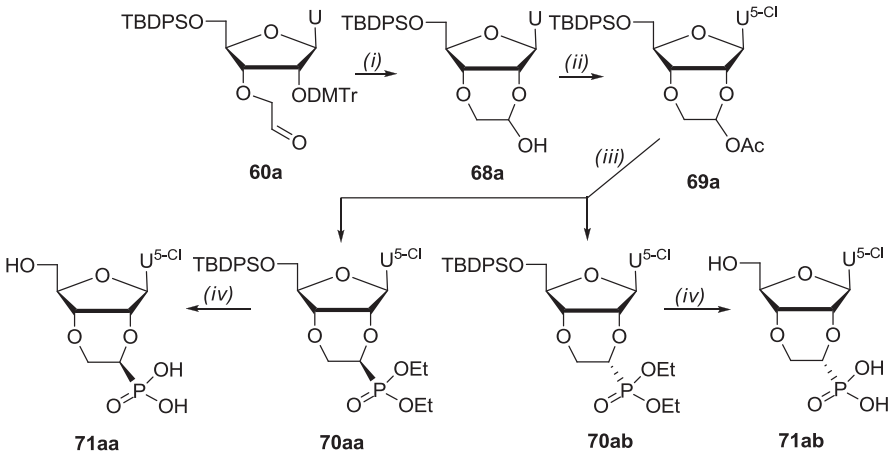
The main focus of this study was to assess the inhibitory potential of novel conformationally constrained pyrimidine nucleoside phosphonic acids and their corresponding "open-structure" analogs toward human recombinant thymidine phosphorylases hrTP-1 (Sigma) and hrTP-2 (kindly provided by Dr. J. Brynda of the Institute) and isolated enzyme from the human PBMC (buffy coats) of healthy donors. To achieve this, we (i) optimized a two-step partial purification of TP to remove a large excess of low-molecular-weight compounds and the substantial part of ballast proteins and (ii) performed the inhibition assays to select potent bi-substrate-like inhibitors of human TP.

2.3.1. Partial purification of hpTP from human PBMC

Partially purified TP suitable for kinetic and inhibition studies was obtained in three steps from a 10^5 *g* supernatants of the cell homogenates, including (i) ammonium sulfate precipitation, (ii) dialysis, and (iii) subsequent fast anion-exchange chromatography on a HiTrap Q column. Protein with TP activity was eluted with 150–200 mM NaCl with minority of other proteins (purification factor ~20).

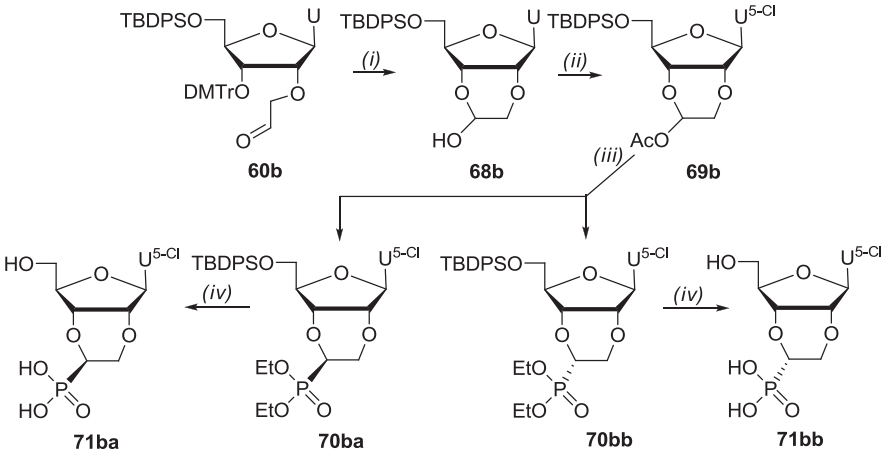
2.3.2. Determination of TP activity and kinetic parameters

TP activity and inhibition assays were based on the phosphorolysis of 2′-deoxy-5-nitrouridine (NdU) to 5-nitrouracil [54]. This



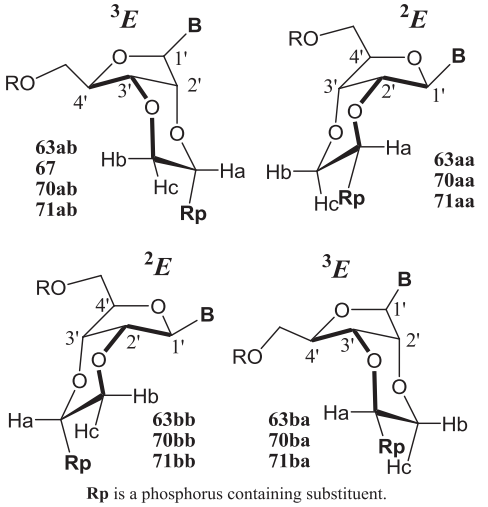
(i) 80% aq. CH_3COOH, r.t; (ii) *a* acetic anhydride, pyridine, r.t, *b* N-chlorosuccinimide, pyridine, 90 °C; (iii) *a* $(Me_3SiO)P(OEt)_2$, TMSOTf, acetonitrile; *b* separation of epimers on silica gel column (iv) *a* 1M TBAF-THF; *b* Me_3SiBr, BSA, acetonitrile, rt

Scheme 9. Synthesis of the epimeric 1,2-(5-chlorouridin-2′,3′-di-*O*-yl)-[(2′-*O*)→1; (3′-*O*)→2]-ethanephosphonic acids.



(i) 80% aq. CH_3COOH, r.t; (ii) *a* acetic anhydride, pyridine, r.t, *b* N-chlorosuccinimide, pyridine, 90 °C; (iii) *a* $(Me_3SiO)P(OEt)_2$, TMSOTf, acetonitrile; *b* separation of epimers on silica gel column (iv) *a* 1M TBAF-THF; *b* Me_3SiBr, BSA, acetonitrile, rt

Scheme 10. Synthesis of epimeric 1,2-(5-chlorouridin-2′,3′-di-*O*-yl)-[(2′-*O*)→2; (3′-*O*)→1]-ethanephosphonic acids.



Rp is a phosphorus containing substituent.

Fig. 3. Preferred conformations of compounds with an annealed 1,4-dioxane ring.

conversion resulted in a significant spectrophotometric shift from 340 nm to 314 nm ($\Delta\varepsilon_{340} = 9200\ M^{-1}\ cm^{-1}$ at pH 6.5). The K_m and V values for 2′-deoxy-5-nitrouridine (NdU) and inorganic phosphate were determined with human TPs of different origins. The cleavage was measured for 5–7 min in 96-well plates at 37 °C using an Infinite IF500 reader (Tecan), and the K_m and V values were calculated using GraphPad Prism 4 (average of three measurements). Michalis-Menten and Lineweaver-Burk plots are available in the Supporting Information. All data are summarized in Table 3. Interestingly, there is one order of magnitude difference between $^{Pi}K_m$ values for hrTP-1 (Sigma) and hpTP (and hrTP-2).

2.3.3. Inhibition assays – determination of K_i values using 2′-deoxy-5-nitrouridine (NdU) as a substrate

The initial inhibition experiments with compounds depicted in Fig. 4 were conducted with human recombinant hrTP-1 (Sigma) at concentrations of 50, 10, and 1 μM for the inhibitor (NdU = 300 μM; P_i = 5 mM) to select the most potent inhibitors for determining K_i values. When a compound exhibited no inhibition (v_i/v_0) at an inhibitor concentration of 50 μM (6:1 NdU–inhibitor ratio), further evaluation was not performed. The compounds that demonstrated modest and high inhibitory activities were subjected to the determination of K_i values from Dixon and Hanes plots (available in the Supporting Information) using the SigmaPlot software. The K_i values were calculated from the equations of linear regressions on the basis

Table 2
Selected vicinal proton couplings in ribofuranose and annealed 1,4-dioxane ring.

Compound	Solvent	*J* (H1′, H2′)	*J* (H2′, H3′)	*J* (H3′, H4′)	Ribofuranose conformation	*J* (Ha, Hb)	*J* (Ha, Hc)
63ab	D_2O	<1	4.5	9.6	^3E	10.2	2.6
67	$CDCl_3$	1.2	4.6	8.8	^3E	9.6	2.7
70ab	$CDCl_3$	1.0	4.7	9.1	^3E	10.7	3.1
71ab	D_2O	<1	*a*	9.0	^3E	11.3	2.7
63aa	D_2O	7.9	4.5	1.2	^2E	9.9	2.6
71aa	D_2O	8.2	4.4	<1	^2E	11.2	2.8
70aa	$CDCl_3$	8.3	4.3	<1	^2E	11.3	2.7
63bb	D_2O	8.2	4.4	<1	^2E	10.2	2.6
70bb	$CDCl_3$	8.4	4.3	<1	^2E	[a]	[a]
71bb	D_2O	8.2	4.4	0.6	^2E	11.5	2.6
63ba	D_2O	1.2	4.5	9.0	^3E	9.5	2.7
70ba	$CDCl_3$	2.8	4.7	7.0	^3E	[a]	[a]
71ba	D_2O	0.5	4.6	9.6	^3E	11.1	2.8

The superscript "a" denotes the *J*-values that could not be determined due to the overlap of signals.

Table 3
Comparison of K_m and V values for NdU and inorganic phosphate (P_i) for three TPs.

TP	$^{NdU}K_m$ [μM]	^{NdU}V [nmol/min]	$^{Pi}K_m$ [μM]	^{Pi}V [nmol/min]
hrTP-1[a]	53 ± 7	1.10 ± 0.05	140 ± 10	1.50 ± 0.01
hpTP[b]	80 ± 20	0.55 ± 0.01	1330 ± 100	0.52 ± 0.01
hrTP-2[c]	85 ± 20	0.92 ± 0.05	1500 ± 300	0.80 ± 0.07

[a] Human recombinant TP expressed in Chinese hamster ovarian cells (Sigma; the enzyme is not further available).
[b] Partially purified TP from human PBMC (buffy coat).
[c] Human recombinant TP expressed in *E. coli*.

of three independent measurements. These data for the most potent compounds are summarized in Table 4 and are also depicted in Fig. 4.

2.4. Inhibitory properties of prepared compounds

Four series of conformationally constrained nucleoside phosphonic acids derived from uridine and 5-substituted uridines were synthesized (Table 4, Fig. 4).

The first series consists of the derivatives **12**–**14** with an annealed 1,3-dioxolane ring substituted in the C2 position with a phosphoryl group. This substitution resulted in chirality on the C2-atom and, thus, resulted in the formation of pairs of *endo* and *exo* epimers (**12a**–**12b**, **12c**–**12d**, **13a**–**13b**, and **14a**–**14b**). Whereas the *endo* epimers were found to be low micromolar and submicromolar inhibitors that exhibited a decrease in K_i values in the order **12a**, **12c**, **13a**, **14a** (B = U ≈ T » U^{5-Cl} ≈ U^{5-I}) with a single order of magnitude K_i increase between **12c** (B = T) and **13a** (B = U^{5-Cl}), the *exo* epimers **12b** and **12d** (B = U and T) were inactive. The compound **13b** (B = U^{5-Cl}) showed, surprisingly, a K_i value only three times lower than its *endo* congener **13a**, which suggests that a different binding mode between the *endo* and *exo* epimers led to a similar efficiency. Moreover, the introduction of an electron-withdrawing substituent at the C5 position (Cl, I) of the uracil nucleobase led to a significant increase in the inhibitory effect. These data are in agreement with those reported previously and suggest that the 5-halo group may improve the active site–ligand interaction in the lipophilic pocket of the enzyme [55] and may

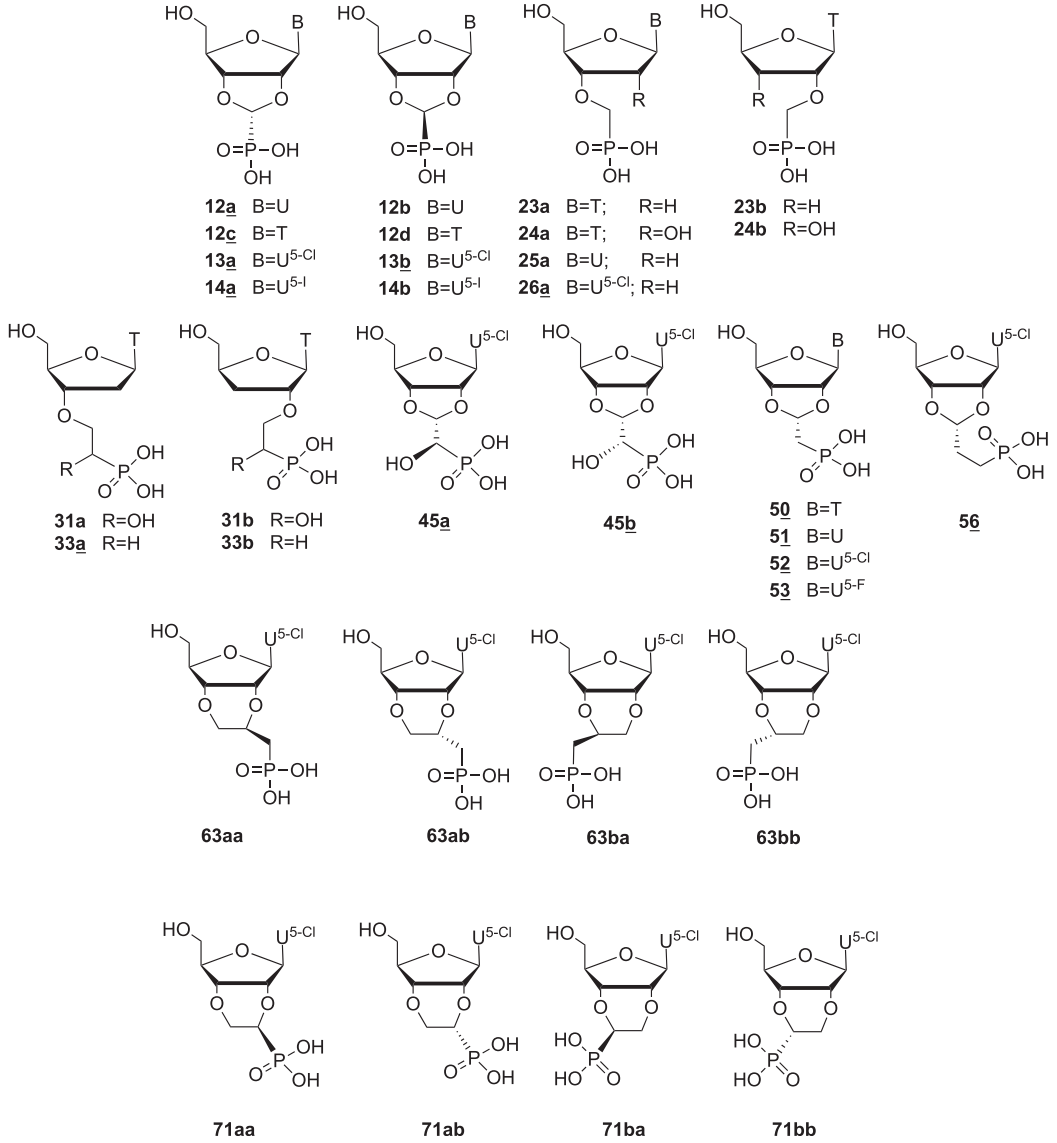
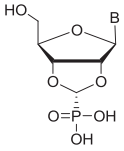
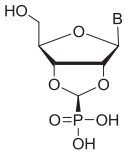
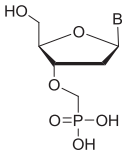
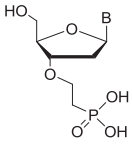
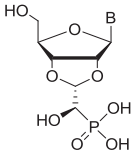
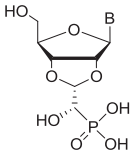
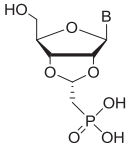
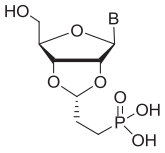


Fig. 4. Overview of the prepared nucleoside phosphonic acids as potential TP inhibitors (for inhibitory data see Table 4); compounds with underlined numbers exhibited K_i values equal or below 15 μM; remaining compounds did not pass the selection assay (no inhibition at ratio of substrate to inhibitor 6:1).

Table 4
The K_i values for the most potent inhibitors of human thymidine phosphorylase hrTP-1 and hpTP.

Inhibitor	B	Structure	hrTP-1[a]		hpTP[b]	
			K_i [μM]	$K_i/^{hrTP\text{-}1}K_m$	K_i [μM]	$K_i/^{hp}TPK_m$
12a	U		11.0	0.207	nd	nd
12c[c]	T		8.80	0.160	nd	nd
13a	U^5—Cl		0.95 (0.77)[g]	0.018	1.12	0.0140
14a	U^5—I		0.75	0.014	nd	nd
13b	U^5—Cl		2.90	0.054	nd	nd
26a	U^5—Cl		11.0	0.207	8.68	0.109
33a	T		nd	nd	3.90[e]	0.049
45a	U^5—Cl		nd	nd	0.306	0.0038
45b	U^5—Cl		nd	nd	1.67	0.0210
50[d]	T		0.201	0.0038	0.148	0.0019
51	U		0.893[e]	0.0169	0.840	0.0105
52	U^5—Cl		0.066	0.0012	0.048 (0.030)[g]	0.0006
53	U^5—F		0.460[f]	0.0086	0.277	0.0035
56	U^5—Cl		14.5[f]	0.270	18.2	0.22

[a] Human recombinant TP expressed in Chinese hamster ovarian cells (Sigma; the enzyme supply was disconnected).
[b] Partially purified TP from human PBMC (buffy coat).
[c] Ref. [45].
[d] Ref. [43,44].
[e] The K_i values for **33a** and **51** were calculated from IC_{50} values according to the equation $K_i = (IC_{50}/(1 + [S]/K_m$ which is valid for competitive inhibition (K_m = 53 μM, [S] = 125 μM, IC_{50} = 10 and 3 μM, respectively).
[f] Human recombinant TP expressed in *E. coli* (hrTP-2).
[g] K_i regarding to inorganic phosphate; nd – not determined.; K_i values represent the average value of three experiments; standard deviation varied from 7 to 10%.

increase the acidity of the 3-NH moiety [56] to strengthen the hydrogen bonding between the uracil nucleobase and serine 217. In addition, the presence of an electronegative substituent at the C5 position of the uracil nucleobase may increase the electropositivity of the O4′–C1′ atoms; thus, such a compound should more effectively mimic the transition state of the substrate → product conversion.

On the basis of these results, we synthesized conformationally flexible, regioisomeric 3′-*O*- and 2′-*O*-methylphosphonic acids **23a**–**26a** and **23b**–**24b**, respectively, that are isosteric with their cyclic congeners **12**–**14**, to achieve better conformational accommodation in TP binding sites. These "open-structures", however, were inactive, with the exception of 3′-*O*-methanephosphonic acid **26a** (B = U^{5}–^{Cl}), which exhibited a K_i value identical to that of cyclic compound **12a** (B = U) but more than one order of magnitude greater than that of **13a** (B = U^{5}–^{Cl}). This result suggests that **26a** possesses a higher binding entropy of due to the flexibility of the phosphonate chain.

The second series consists of derivatives **45a**, **45b**, and **50**–**53** with an annealed 1,3-dioxolane ring substituted with a phosphonomethyl moiety in the 2-position. The synthesis of compound **50** (B = T) and its submicromolar inhibition effect on hrTP-1 (K_i = 236 nM) have been previously reported [43,44]; however, we were unable to reproduce the published synthesis of **50**. Therefore, we devised a new synthetic route that provided **50** as a single epimer in good yield and found, for hrTP-1 and hpTP, K_i values of 201 and 148 nM, respectively. On the basis of these results, we synthesized additional compounds **51**–**53**, which differ in the nucleobase (B = U, U^{5}–^{Cl}, or U^{5}–^{F}, respectively); among these compounds, we selected the nucleoside phosphonic acid **52** (B = U^{5}–^{Cl}) as the most potent, potentially bi-substrate-like inhibitor of hrTP-1 and hpTP developed thus far, with K_i values of 66 and 48 nM, respectively. Our assumption that **52** could be a bi-substrate-like inhibitor was supported by the determination of the K_i value relative to inorganic phosphate as the second substrate of TP; the K_i values were determined with various concentrations of inorganic phosphate (from 1 to 5 mM). The determined value of 30 nM (the Dixon plot is available in the Supporting Information) strongly supported the hypothesis that **52** is a bi-substrate-like inhibitor of human TP. Further extension of the phosphonoalkyl chain (phosphonate **56**) led to a decrease in the affinity to hrTP-1 and hpTP by factors of 220 and 380, respectively.

In general, bi-substrate-like inhibitors can be expected to exhibit lower binding entropies than either of the individual substrates. In addition, bi-substrate-like inhibitors may also improve the binding enthalpy through an additional interaction in the binding site. Following this idea, we synthesized epimeric compounds **45a** and **45b** (B = U^{5}–^{Cl} in both cases) bearing an additional hydroxy group in the alpha position to the phosphoryl group. The hydroxyl could serve both as the donor and the acceptor of the hydrogen bond in the TP binding site. However, in the case of compounds **45a** and **45b**, their affinity to hpTP was decreased by factors of 6 and 35, respectively, in comparison with that of **52**. The "open-structures", i.e., the regioisomeric 3′-*O*- (**31a** and **33a**) and 2′-*O*-(2-phosphonoethyl) (**31b** and **33b**) derivatives, did not exert any significant inhibition effect. Only compound **33a** (B = T) exhibited a weak activity (decreasing the affinity to hpTP by a factor of 81) compared to that of **52**.

The third series consists of conformationally constrained compounds, the vicinal 2′,3′-*O*-phosphonopropylidene derivatives **63** (all compounds with B = B = U^{5}–^{Cl}) with annealed 1,4-dioxane ring, related to **52**. In fact, the phosphonic acids **63** are isosteric to that of **52**. Two possible size-positionings of the phosphonomethyl moiety on the 1,4-dioxane ring gave rise to two regioisomers and each of them in the form of epimers **63aa**–**63ab** and **63ba**–**63bb**. The enlargement of the anneled ring from a 1,3-dioxolane to a 1,4-dioxane ring led to a loss of inhibitory activity of compounds **63**. An NMR spectroscopy-based conformational study of compounds **63** revealed (similar to the case of **71**) interesting conformational changes of the pentofuranose part of the molecule, which is dependent on the configuration of the carbon atom bearing the phosphomethyl residue (for more details, see chapter *NMR structure determination*). The conformational changes, the spatial requirements of compounds **63**, and the positions of nucleobase and phosphoryl groups may explain the inactivity of these compounds.

The fourth series consists of two epimeric pairs, **71aa**–**71ab** and **71ba**–**71bb** (all four compounds with B = U^{5}–^{Cl}), which are isosteric to **13a**. Similar to the case of compounds **63**, we did not observe any inhibitory activity, most likely due to the inappropriate stereochemical arrangement of the molecule.

3. Conclusions

We synthesized a series of new conformationally constrained uracil-based nucleoside phosphonic acids containing annealed 1,3-dioxolane and 1,4-dioxane rings bearing a phosphonate moiety and their "open-structure" isosters. These compounds were designed to provide a variety of structural types believed to cover a range of potential candidates that could provide sufficient data on the structure–activity relationship. Inhibition study with human recombinant and native TPs revealed that compounds with an annealed ring are much more potent inhibitors of TPs than their flexible isosters. Of 33 tested nucleoside phosphonic acids, we selected 6 compounds that exhibited the K_i values in the range of 0.048–1 μM. These compounds exhibited a competitive type of inhibition relative to NdU as obvious from Dixon–Webb plots (see Supporting Information). The most potent inhibitor **52** exhibited K_i values 48 and 30 nM with 2′-deoxy-5-nitrouridine and inorganic phosphate as substrates, respectively. These values suggest that the phosphonic acid **52** is a bi-substrate-like inhibitor of human TP. The bi-substrate-like binding mode of **52** to human TP should be confirmed unambiguously through resolution of the crystal structure of human TP with inhibitor **52**. Interestingly, no crystal structure of human TP with a nucleoside phosphonic acid has thus far been reported.

4. Experimental

4.1. General

The course of the reactions was followed by TLC on Merck Silica gel 60 F_{254} aluminum sheets and the products were visualized either by UV monitoring (254 nm), or by spraying with 1% ethanolic solution of 4-(4-nitrobenzyl)pyridine (PNBP) which, after short heating and exposing the sheet to ammonia vapors, showed the phosphonate esters as blue spots, or by spraying with 5% ethanolic solution of sulfuric acid and subsequent heating (detection of *O*-DMTr derivatives, deep orange spots). Preparative column chromatography (PLC) was performed on silica gel (40–60 μm, Fluka), with elution at the rate of 40 mL/min. PLC and TLC were carried out with the following solvent systems (v/v): chloroform–ethanol 9:1 (C-1), ethyl acetate–toluene 1:1 (T-1), ethyl acetate–acetone–ethanol–water 4:1:1:1 (H-1), ethyl acetate–acetone–ethanol–water 12:2:2:1 (H-3), and 2-propanol-conc. aqueous ammonia–water 7:1:2 (IPAW).

HPLC analyses were performed on an Alliance (Waters) instrument using Luna C18 column (5 μm, 4.6 × 150 mm, Phenomenex) under gradient elution with acetonitrile in 0.1 M-triethylammonium acetate buffer (TEAA). The preparative reversed-phase HPLC

was performed on an Axia column (20 × 200 mm, Luna C18(2), 5 μm, Phenomenex), using a linear gradient of methanol in 0.1 M triethylammonium hydrogencarbonate buffer (TEAB) or in water.

The MS HR-ESI spectra (*m*/*z*) were recorded on an LTQ Orbitrap XL (Thermo Fischer Scientific) instrument. The IR spectra were recorded on FTIR spectrometer (Bruker Equinox 55, Germany). The ^{1}H and ^{13}C NMR spectra were measured on a Bruker AVANCE-600 spectrometer (^{1}H at 600.13 MHz, ^{13}C at 150.9 MHz) using cryoprobe (5 mm CPTCI $^{1}H-^{13}C/^{15}N/D$ Z-GRD) in D_2O or $CDCl_3$ at 300 K. Structural assignment of proton and carbon signals was achieved using 2D-H,H-COSY, 2D-H,H-ROESY, 2D-H,C-HSQC and 2D-H,C-HMBC spectra. NMR data are presented in Tables 5 and 6.

All compounds used in biochemical testing were of proved purity of over 95% as determined by RP HPLC.

The instruments used in the work on enzyme purification involved Beckman Coulter Allegra X-22R centrifuge, Beckman Coulter Optima L-100 XP ultracentrifuge, and Cell ultrasound disintegrator Soniprep 150 (Sanyo) and, in enzyme assays, the Infinite F500 Tecan reader was used. The protease inhibitor cocktail was bought from Sigma. The human recombinant thymidine phosphorylase hrTP-1 expressed in hamster ovarian cells was obtained from Sigma (this enzyme is not further available), and human recombinant thymidine phosphorylase expressed in *E. coli* (hrTP-2) was kindly provided by Dr. Jiří Brynda of this Institute.

4.2. Biochemistry

The human recombinant thymidine phosphorylase (hrTP-1) and protease inhibitor cocktail were obtained from Sigma (hrTP-1 is no longer available). Peripheral blood mononuclear cells (PBMC) were isolated from the buffy coat of healthy donors provided by the 1st Department of Internal Medicine, Department of Hematooncology, General Teaching Hospital and by the 1st Faculty of Medicine, Charles University, Prague.

4.2.1. Partial purification of thymidine phosphorylase (hpTP)

All steps were conducted at 4 °C. Human peripheral blood mononuclear cells (PBMC) of healthy donors (2 packages, ~300 mL) were sedimented at 4000×*g*, washed (2 × 50 mL) with buffer A (20 mM Tris–HCl, 15 mM sodium citrate, 135 mM sodium chloride, 2.5 mM EDTA, 5.5 mM glucose, pH 7.5), and stored at −70 °C.

The frozen blood cells were disrupted by three thawing–freezing cycles, and the homogenate was suspended in 50 mM Tris–HCl buffer (pH 7.5; 15 mL) containing a protease inhibitor cocktail (0.1 mL). The suspension was sonicated three times for 10 s. The crude lysate was centrifuged at 11 000 × *g* for 30 min to remove cell debris and then at 100 000 × *g* for 1 h. The resulting supernatant (15 mL) was saturated with ammonium sulfate (3.5 g) to 40%. After 30 min of standing, the precipitate was collected by centrifugation at 9000×*g* for 40 min and dissolved in 50 mM Tris–HCl buffer (pH 7.5; 7.5 mL). The enzyme solution was dialyzed overnight against the same buffer (1 L) containing 25 mM DTT and 20% glycerol. The desalted solution was loaded onto a HiTrap Q column (GE Healthcare) equilibrated with 50 mM Tris–HCl buffer (pH 6.5; 20 mL). The column was eluted with a linear gradient (0–1 M) of NaCl in the same buffer at a flow rate of 1 mL/min. Twenty fractions (1 mL each) were collected and analyzed for TP activity and protein content. The fractions containing TP activity were used immediately for assays. The two-step purification process resulted in a partially purified enzyme with a specific activity of approximately 13 $\mu mol\,h^{-1}\,mg^{-1}$. The protein concentration was determined by the Bradford method [57] using bovine serum albumin as a standard.

4.2.2. TP activity assay and determination of $^{NdU}K_m$ and $^{Pi}K_m$ values for TP (Table 3)

A partially purified thymidine phosphorylase (hpTP), hrTP-2, or hrTP-1 was assayed in 96-well plates filled with 200 μL of 50 mM Tris–HCl buffer (pH 6.5) containing 300 μM 2′-deoxy-5-nitrouridine (NdU) and 5 mM potassium phosphate. The phosphorolysis of NdU was initiated at 37 °C by the addition of TP solution (10 μL ≈ 2–3 μg of protein) and was monitored at 340 nm for 5–7 min using the Infinite F500 Tecan reader. The initial steady-state rates were calculated from a linear portion of the phosphorolytic patterns of NdU over its wide concentration range (10–500 μM) at a constant 5 mM concentration of inorganic phosphate (for hrTP-1, 300 μM phosphate was used), using the extinction coefficient $\varepsilon_{340} = 9200\ M^{-1}\,cm^{-1}$. The determination of $^{Pi}K_m$ values was performed at a constant 300 μM concentration of NdU and at variable concentrations of inorganic phosphate (from 150 μM to 5 mM). The obtained data fit the Michaelis–Menten equation. For calculations of $^{NdU}K_m$ and $^{Pi}K_m$ values, the GraphPad Prism 4 and SigmaPlot software packages were used.

4.2.3. Inhibition study

Initially, all synthesized compounds (Fig. 4) were tested against hrTP-1 (Sigma) at concentrations of 1, 10, and 50 μM. The reaction mixture (200 μL) was composed of 300 μM NdU and 300 μM potassium phosphate, with variable concentrations of inhibitor (1, 10, and 50 μM) and 50 mM Tris buffer (pH 6.5). The cleavage was started by the addition of ~1.2 mU of hrTP-1 (5–10 μL), and the absorbance change was monitored within 5–7 min at 37 °C. Compounds which demonstrated inhibitory activity at concentrations less than 50 μM were subjected to the determination of $^{NdU}K_i$ and $^{Pi}K_i$ values (the latter only for the most potent inhibitors). For the determination of $^{NdU}K_i$, the reaction mixture (200 μL) was composed of 50 mM Tris buffer (pH 6.5), 150 or 300 μM NdU, and 300 μM potassium phosphate. For the determination of $^{Pi}K_i$, the reaction mixture (200 μL) was composed of 50 mM Tris buffer (pH 6.5), 300 μM NdU, and 150 or 300 μM phosphate. In both cases, the concentrations of the inhibitors were varied from 50 nM to 10 μM. The phosphorolysis of NdU was initiated at 37 °C by the addition of hrTP-1 (~1.2 mU), hrTP-2, and/or hpTP (approximately 1–3 μg of protein in the latter two cases) and was monitored within 5–7 min at 37 °C, as previously described. The type of inhibition and K_i values were determined from a Dixon plot ($1/v$ versus $[I]$) and a Hanes plot, respectively, using the SigmaPlot software. The K_i values were calculated on the basis of three independent measurements.

4.3. Chemistry

Unless stated otherwise, all phosphonic acids were lyophylized from water as triethylammonium salts and dried at 13 Pa over phosphorus pentoxide for 48 h.

4.3.1. (R)-(uridin-2′,3′-di-O-Yl)-methanephosphonic acid (**12a**) and (S)-(uridin-2′,3′-di-O-yl)-methanephosphonic acid (**12b**) (Scheme 1)

The suspension of 5′-*O*-*tert*-butyldiphenylsilyluridine **8** (5.0 g, 10.4 mmol) in dichloromethane (100 mL) and trimethyl orthoformate (3.4 mL, 31.2 mmol) was acidified with 4 M HCl in DMF (slightly red color on a wet pH paper), and the resulting mixture was stirred at rt overnight (TLC in C-1). Triethylamine was added, and the clear solution was concentrated under reduced pressure. The product was purified by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform. The obtained nucleoside orthoester **10** (4.9 g, 9.4 mmol) was treated with dimethyl chlorophosphite (2.4 g, 18.8 mmol) in dry acetonitrile

(90 mL) at rt overnight (TLC in C-1). The reaction mixture was quenched by addition of 1M TEAB in 50% aqueous ethanol (30 mL) at 0 °C, and concentrated under reduced pressure. The residue was co-evaporated with ethanol (2 × 50 mL), and the phosphonate diesters of compounds **12a**,**b** were purified as a mixture by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform. The obtained mixture of protected diesters of compounds **12a**,**b** (4.4 g, 7.3 mmol) was co-evaporated with dry acetonitrile and treated with bromotrimethylsilane (3.8 mL, 29.2 mmol) and 2,6-lutidine (6.9 mL, 58.4 mmol) in acetonitrile (70 mL). The resulting solution was left aside at rt overnight and then concentrated under reduced pressure. The residue was treated with 0.5 M TBAF in THF (50 mL) at rt for 30 min, the solution was concentrated under reduced pressure, and the mixture of epimers **12a**,**b** was separated by RP HPLC. Overall yield: 1.4 g (4.3 mmol, 31%), out of which 0.95 g (68%) of (*R*)-epimer **12a** and 0.45 g (32%) of (*S*)-epimer **12b**. The ^{1}H and ^{13}C NMR spectra were identical with those reported earlier [45].

4.3.2. (*R*)-(5-Chlorouridin-2′,3′-di-O-Yl)-methanephosphonic acid (**13a**) and (*S*)-(5-chlorouridin-2′,3′-di-O-yl)-methanephosphonic acid (**13b**) (Scheme 1)

5′-*O*-*tert*-Butyldiphenylsilyluridine **8** (5.0 g, 10.4 mmol) was treated with *N*-chlorosuccinimide (2.1 g, 15.6 mmol) in pyridine (100 mL) at 90 °C for 45 min. The solution was concentrated under reduced pressure, and the product was purified by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform. The obtained derivative **9** (4.1 g, 7.9 mmol) was suspended in dichloromethane (80 mL) under stirring, trimethylorthoformate (2.6 mL, 23.7 mmol) was added, the suspension was acidified with 4 M HCl in DMF (slightly red color on a wet pH paper), and the resulting mixture was stirred at rt overnight (TLC in C-1). Triethylamine was added, and the clear solution was concentrated under reduced pressure. The product was purified by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform. The obtained orthoester **11** (3.3 g, 6.4 mmol) was used directly for the synthesis of phophonates **13a**,**b** by the procedure described for compounds **12a** and **12b**.

Overall yield of **13a** and **13b**: 0.77 g (2.1 mmol, 33%). (*R*)-epimer **13a**: 0.56 g (71%); HRMS $(M-H)^-$ for $C_{10}H_{11}N_2O_9P$: calcd *m*/*z* 369.0016, found 368.9900; IR (KBr, cm^{-1}): 3422, 3074, 2966, 1699, 1646, 1541, 1111, 1007, 976, 780, 593. NMR data – see Tables 5 and 6

(*S*)-epimer **13b**: 0.21 g (29%); HRMS $(M-H)^-$ for $C_{10}H_{11}N_2O_9P$: calcd *m*/*z* 369.0017, found 368.9896; IR (KBr, cm^{-1}): 3383, 2965, 2877, 1700, 1645, 1447,1541, 1447, 1109, 1008, 975, 780, 758, 591. NMR data – see Tables 5 and 6

4.3.3. (*R*)-(5-Iodouridin-2′,3′-di-O-Diyl)-methanephosphonic acid (**14a**) and (*S*)-(5-iodouridin-2′,3′-di-O-diyl)-methanephosphonic acid (**14b**) (Scheme 1)

The solution of epimeric mixture of **12a**,**b** [45] (34 mg; 0.10 mmol) and iodine (25 mg; 0.10 mmol) in 1.5% aq. nitric acid (5 mL) was stirred at rt for 24 h. The reaction mixture was neutralized with triethylamine and applied on Axia C18 column. The elution with a linear gradient of methanol in water (0 → 50%) yielded single epimers **14a** (29 mg; 63%) and **14b** (11 mg; 24%). UV spectrum, λ_{max} = 289.5 nm (in water). HRMS $(M-H)^-$ for $C_{10}H_{12}N_2O_9INaP$: calcd *m*/*z* 484.92228, found 484.92173;

4.3.4. (Thymidin-3′-O-yl)-methanephosphonic acid (**23a**) (Scheme 2)

Sodium hydride (0.6 g, 15.2 mmol) was added at 0 °C to a stirred solution of 5′-*O*-dimethoxytrityl thymidine (**15a**) (2.0 g, 3.8 mmol) and methyl tosyloxymethanephosphonate [48] (2.1 g, 7.6 mmol) in DMF (30 mL). The resulting mixture was left to warm gradually to rt, stirred overnight (TLC in H-3), then quenched by the addition of glacial acetic acid (0.4 mL; 7.6 mmol) at 0 °C, and concentrated under reduced pressure. The product was purified by silica gel chromatography using a linear gradient of H-1 (0–100%) in ethyl acetate. This product (2.1 g, 3.2 mmol) was treated with 80% aq. acetic acid (30 mL) at rt for 30 min (TLC in C-1). The reaction mixture was concentrated at reduced pressure, the residue was diluted with chloroform (100 mL), and the solution was washed with water (3 × 50 mL). Water layers were combined, evaporated, and the pure product **19a** was co-distilled subsequently with ethanol and acetonitrile, diluted in dry acetonitrile (10 mL), and treated with bromotrimethylsilane (1.7 mL, 12.8 mmol) and 2,6-lutidine (2.9 mL, 25.6 mmol). The resulting mixture was stirred at rt overnight and concentrated under reduced pressure. The crude product **23a** was purified by RP HPLC.

Overall yield of **23a**: 0.41 g (1.2 mmol, 32%). HRMS $(M-H)^-$ for $C_{11}H_{16}N_2O_8P$: calcd *m*/*z* 335.0645, found 335.0641; IR (KBr, cm^{-1}): 3501, 3404, 3355, 3282, 3065, 1697, 1685, 1064, 915, 563. NMR data – see Tables 5 and 6

4.3.5. (3′-Deoxy-5-methyluridin-2′-O-yl)-methanephosphonic acid (**23b**) (Scheme 2)

Compound **23b** was prepared from **15b** (1.2 g, 1.4 mmol) by the procedure described for compound **23a.** Overall yield: 0.219 g (0.65 mmol, 46%). HRMS $(M-H)^-$ for $C_{11}H_{16}N_2O_8P$: calcd *m*/*z* 335.0645, found 335.0652. IR (KBr, cm^{-1}): 3425, 2930, 1697, 1476, 1444, 1271, 1116, 1076, 973, 913, 768, 588. NMR data – see Tables 5 and 6

4.3.6. (5-Methyluridin-3′-O-yl)-methanephosphonic acid (**24a**) (Scheme 2)

Compound **24a** was prepared from **16a** (1.0 g, 1.2 mmol) by the procedure described for compound **23a**. Overall yield: 0.197 g (0.56 mmol, 47%). HRMS $(M-H)^-$ for $C_{11}H_{16}N_2O_9P$: calcd *m*/*z* 351.0594, found 351.0591; IR (KBr, cm^{-1}): 3418, 3251, 3062, 2985, 2929, 2680, 2490. 1693, 1472, 1393, 1264, 1233, 1136, 1114, 1067, 914, 839, 790, 767, 575, 550, 490, 464. NMR data – see Tables 5 and 6

4.3.7. (5-Methyluridin-2′-O-yl)-methanephosphonic acid (**24b**) (Scheme 2)

Compound **24b** was prepared from **16b** (1.0 g, 1.8 mmol) by the procedure described for compound **23a**. Overall yield: 0.352 g (1.0 mmol, 55%). HRMS $(M-H)^-$ for $C_{11}H_{16}N_2O_9P$: calcd *m*/*z* 351.0594, found 351.0596; IR (KBr, cm^{-1}): 3420, 3069, 2940, 2909, 2828, 2361, 1696, 1474, 1270, 1093, 1067, 919, 792, 770, 591. NMR data – see Tables 5 and 6

4.3.8. (2′-Deoxyuridin-3′-O-yl)-methanephosphonic acid (**25a**) (Scheme 2)

Compound **25a** was prepared from **17a** (3.6 g, 6.8 mmol) by the procedure described for compound **23a**. Overall yield: 0.64 g (1.9 mmol, 28%). HRMS $(M-H)^-$ for $C_{10}H_{14}N_2O_8P$: calcd *m*/*z* 321.0488, found 321.0487; IR (KBr, cm^{-1}): 3193, 3057, 2991, 2946, 2834, 2694, 2475, 2361, 1692, 1463, 1384, 1276, 1145, 1121, 1098, 1063, 916, 811, 765, 559, 538, 470, 420. NMR data – see Tables 5 and 6

4.3.9. (5-Chloro-2′-deoxyuridin-3′-O-yl)-methanephosphonic acid (**26a**) (Scheme 2)

N-chlorosuccinimide (1.8 g, 13.5 mmol) was added under stirring to a solution of 2′-deoxy-5′-*O*-dimethoxytrityluridine **17a** (4.8 g, 9.0 mmol) in pyridine (90 mL). The resulting mixture was heated at 90 °C for 45 min and then concentrated under reduced pressure. The separation on silica gel column using a linear gradient of ethanol (0 → 10%) in chloroform afforded the product **18a** (4.5 g,

Table 5
Proton NMR data.

Compound	Solv.	H-1′	H-2′	H-2″/H-3″	H-3′	H-4′	H-5′a	H-5′b	Base	Substituents
13a	D_2O	5.95 d (2.5)	5.06 ddd (2.5; 6.5; 0.5)	–	4.84 ddd (6.5; 3.5; 0.5)	4.50 m (3.5; 3.7; 5.5)	3.86 dd (3.7; 12.4)	3.79 dd (5.5; 12.4)	H-6: 8.12 s	P–CH<: 5.12 d (20.8)
13b	D_2O	5.88 d (2.9)	4.98 dd (2.9; 6.5)	–	4.86 dd (6.5; 4.7)	4.26 ddd (4.7; 5.8; 3.7)	3.90 dd (12.3; 3.7)	3.83 dd (12.3; 5.8)	H-6: 8.02 s	P–CH<: 5.08 d (16.0)
23a	D_2O	6.29 dd (7.7; 6.3)	2.32 ddd (14.2; 7.7; 6.5)	2.52 ddd (14.2; 6.3; 3.1)	4.28 m (6.5; 3.2; 3.1)	4.17 ddd (3.2; 3.9; 5.0)	3.83 dd (12.4; 3.9)	3.78 (12.4; 5.0)	H-6: 7.65 q (1.2); 5-CH_3: 1.89 d (1.2)	P–CH_2–O: 3.63 dd (12.9; 9.4) and 3.59 dd (12.9; 9.2)
23b	D_2O	5.96 d (1.3)	4.28 dt (1.3; 1.4; 5.7)	2.18 ddd (14.0; 1.4; 5.4)	2.03 ddd (14.0; 5.7; 11.0)	4.49 m (11.0; 5.4; 2.8; 4.4)	3.96 dd (12.9; 2.8)	3.76 dd (12.9; 4.4)	H-6: 7.78 q (1.3); 5-CH_3: 1.87 d (1.3)	P–CH_2–O: 3.71 dd (12.7; 9.5) and 3.64 dd (12.7; 9.4)
24a	D_2O	5.94 d (4.9)	4.46 dd (4.9; 5.3)	–	4.08 t (5.3; 5.2)	4.23 ddd (5.2; 3.2; 4.1)	3.90 dd (12.8; 3.2)	3.83 dd (12.8; 4.1)	H-6: 7.69 q (1.2); 5-CH_3: 1.88 d (1.2)	P–CH_2–O: 3.76 dd (13.0; 9.1) and 3.72 dd (13.0; 9.0)
24b	D_2O	5.99 d (3.7)	4.18 dd (3.7; 5.3)	–	4.33 dd (5.3; 6.4)	4.12 ddd (6.4; 2.8; 4.1)	3.93 dd (12.9; 2.8)	3.82 dd (12.9; 4.1)	H-6: 7.77 q (1.3); 5-CH_3: 1.88 d (1.3)	P–CH_2–O: 3.88 dd (13.0; 9.4) and 3.75 dd (13.0; 9.3)
25a	D_2O	6.27 dd (7.7; 6.2)	2.31 ddd (7.7; 14.3; 6.3)	2.56 ddd (6.2; 14.3; 2.9)	4.27 m (6.3; 2.9; 3.0)	4.20 ddd (3.0; 3.8; 5.0)	3.82 dd (12.4; 3.8)	3.76 dd (12.4; 5.0)	H-5: 5.88 d (8.1); H-6: 7.88 d (8.1)	P–CH_2–O: 3.69 dd (13.1; 9.4) and 3.64 dd (13.1; 9.4)
26a	D_2O	6.26 dd (7.4; 6.1)	2.31 ddd (7.4; 14.3; 6.4)	2.58 ddd (6.1; 14.3; 3.1)	4.28 m (6.4; 3.1; 3.2)	4.21 ddd (3.2; 3.7; 4.8)	3.84 dd (3.7; 12.5)	3.79 dd (4.8; 12.5)	H-6: 8.16 s	P–CH_2–O: 3.65 dd (12.9; 9.3) and 3.60 dd (12.9; 9.3)
31a	D_2O	6.26 dd (7.5; 2.2)	2.70 m (7.5; 15.2; 6.2)	2.34 dt (15.2; 2.2)	4.24 m (6.2; 2.2; 1.8)	4.58 m (1.8; 4.2; 5.5)	3.71 dd (4.2; 12.3)	3.64 dd (5.5; 12.3)	H-6: 7.76 q (1.2); 5-CH_3: 1.91 d (1.2)	P–CH(OH)–CH_2–O: 3.80–3.88 m and 3.60 m
		6.25 dd (7.5; 2.2)		2.31 dt (15.2; 2.2)	4.22 (6.2; 2.2; 1.8)	4.61 m (1.8; 4.2; 5.5)	3.70 dd (4.2; 12.3)			
31b	D_2O	5.953 d (1.5)	4.317 m (1.5; 5.7; 1.6)	2.071 ddd (5.7; 14.0; 10.8)	2.156 ddd (1.6; 14.0; 5.6)	4.477 m (10.8; 5.6; 2.8; 4.4)	3.966 dd (2.8; 12.8)	3.76 dd (4.4; 12.8)	H-6: 7.783 q (1.2); 5-CH_3: 1.87 d (1.2)	P–CH(OH)–CH_2–O: ~3.94 m, 1H; 3.996 ddd and 3.694 ddd, 2H
		5.961 d (1.4)	4.319 m (1.4; 5.7; 1.6)	2.066 ddd (5.7; 14.0; 10.8)	2.173 ddd (1.6; 14.0; 5.6)	4.479 m (10.8; 5.6; 2.8; 4.4)	3.969 dd (2.8; 12.8)	3.76 dd (4.4; 12.8)	H-6: 7.786 q (1.2); 5-CH_3: 1.87 d (1.2)	P–CH(OH)–CH_2–O: ~3.94 m, 1H; 3.91 m and 3.752 m, 2H
33a	D_2O	6.21 dd (7.2; 2.4)	2.68 ddd (7.2; 15.2; 6.2)	2.29 ddd (2.4; 15.2; 2.0)	4.22 dt (6.2; 2.0; 1.9)	4.58 ddd (1.9; 4.2; 5.5)	3.70 dd (4.2; 12.4)	3.64 dd (5.5; 12.4)	H-6: 7.69 q (1.2); 5-CH_3: 1.91 d (1.2)	P–CH_2–CH_2–O: 1.82–1.92 m (2H); 3.71 m (2H)
		6.27 dd (7.4; 6.5)	2.36 ddd (7.4; 14.4; 6.6)	2.49 ddd (6.5; 14.4; 3.5)	4.26 dt (6.6; 3.5; 3.7)	4.11 td (3.7; 3.7; 5.0)	3.83 dd (3.7; 12.4)	3.77 dd (5.0; 12.4)	H-6: 7.64 q (1.2); 5-CH_3: 1.89 d (1.2)	P–CH_2–CH_2–O: 1.82–1.92 m (2H); 3.97 m (2H)
33b	D_2O	5.93 d (1.5)	4.30 ddd (1.5; 5.6; 1.7)	2.13 ddd (1.7; 14.1; 5.7)	2.07 ddd (5.6; 14.1; 10.7)	4.44 m (10.7; 5.7; 2.8; 4.5)	3.96 dd (2.8; 12.8)	3.75 dd (4.5; 12.8)	H-6: 7.77 q (1.2); 5-CH_3: 1.87 d (1.2)	P–CH_2–CH_2–O: 1.87 m (2H); 3.84 m and 3.79 m
45a	D_2O	5.94 d (2.7)	5.00 dd (2.7; 6.5)	–	4.83 dd (6.5; 3.0)	4.54 ddd (3.0; 3.7; 5.4)	3.84 dd (3.7; 12.3)	3.79 dd (5.4; 12.3)	H-6: 8.10 s	P–CH(OH)–CH<: 3.80 dd (2.1; 14.0); 5.40 d (2.1)
45b	D_2O	5.94 d (2.6)	5.00 dd (2.6; 6.5)	–	4.79 dd (6.5; 3.1)	4.50 m (3.1; 3.6; 5.4)	3.86 dd (3.6; 12.3)	3.78 dd (5.4; 12.3)	H-6: 8.05 s	P–CH(OH)–CH<: 3.78 dd (1.6; 14.0); 5.42 d (1.6)
50	D_2O	5.90 d (2.7)	5.01 dd (2.7; 6.7)	–	4.78 dd (6.7; 3.6)	4.38 m (3.6; 3.9; 5.8)	3.83 dd (3.9; 12.2)	3.77 dd (5.8; 12.2)	H-6: 7.59 q (1.2); 5-CH_3: 1.88 d (1.2)	P–CH_2–CH<: 2.11 dd (5.5; 17.4); 5.34 td (5.5; 5.5; 3.9)
51	D_2O	5.88 d (2.6)	5.01 dd (2.6; 6.6)	–	4.77 dd (6.6; 3.3)	4.43 m (3.3; 3.8; 5.8)	3.83 dd (3.8; 12.3)	3.77 dd (5.8; 12.3)	H-5: 5.84 d (8.1); H-6: 7.78 d (8.1)	P–CH_2–CH<: 2.11 dd (5.5; 17.4); 5.33 td (5.5; 5.5; 3.9)
52	D_2O	5.87 d (2.6)	4.96 dd (2.6; 6.6)	–	4.77 dd (6.6; 3.0)	4.48 m (3.0; 3.7; 5.5)	3.86 dd (3.7; 12.3)	3.78 dd (5.5; 12.3)	H-6: 8.08 s	P–CH_2–CH<: 2.10 dd (5.5; 17.3); 5.33 td (5.5; 5.5; 3.8)

53	D_2O	5.85 ss (2.6; 0.8)	4.94 dd (2.6; 6.8)	–	4.74 dd (6.8; 3.5)	4.38 m (3.5; 3.8; 5.6)	3.84 dd (3.8; 12.2)	3.78 dd (5.6; 12.2)	H-6: 7.74 d (6.2)	P–CH_2–CH<: 2.08 dd (5.4; 16.8); 5.32 td (5.4; 5.4; 3.8)
56	D_2O	5.85 d (2.5)	5.02 dd (2.5; 6.6)	–	4.80 dd (6.6; 3.8)	4.38 td (3.8; 3.8; 5.6)	3.84 dd (3.8; 12.4)	3.77 dd (5.6; 12.4)	H-6: 7.89 s	P–CH_2–CH_2–CH<: 1.51 m, 2H; 1.98 m, 2H; 5.23 t (4.5; 4.5)
60a[a]	$CDCl_3$	6.36 d (7.6)	4.33 dd (7.6; 5.0)	–	3.06 dd (5.0; 1.4)	4.05 m (1.4; 4.0; 2.4)	3.76 dd (4.0; 11.6)	3.60 dd (2.4; 11.6)	NH: 8.38 bd (2.4); H-5: 5.24 dd (8.1; 2.4); H-6: 7.25 d (8.1);	O–CH_2–CH=O: 3.65 dd (17.2; 0.9); 3.50 dd (17.2; 0.9); 9.57 t (0.9; 0.9)
60b[b]	$CDCl_3$	6.09 d (3.9)	3.04 dd (3.9; 4.5)	–	4.15 dd (4.5; 5.5)	3.92 dt (5.5; 2.1; 2.2)	3.94 dd (2.1; 11.8)	3.25 dd (2.2; 11.8)	NH: 8.37 bd (2.2); H-5: 5.16 dd (8.1; 2.2); H-6: 7.68 d (8.1);	O–CH_2–CH=O: 4.12 dd (17.7; 0.8); 3.89 dd (17.7; 0.8); 9.67 t (0.8; 0.8)
63aa	D_2O	6.58 d (7.9)	4.45 dd (7.9; 4.5)	–	4.18 dd (4.5; 1.2)	4.24 dt (1.2; 3.6; 3.6)	3.80 d, 2H (3.6)		H-6: 8.14 s	P–CH_2–CH(O)–CH_2–O: 1.82 ddd (4.9; 14.8; 19.2); 1.74 ddd (9.0; 14.8; 17.0); 4.30 m (2.6; 9.9; 4.9; 9.0; 7.8); 4.17 ddd (12.4; 2.6; 0.9); 3.51 dd (12.4; 9.9)
63ab	D_2O	5.79 s	4.39 d (4.5)	–	4.32 dd (4.5; 9.6)	4.64 ddd (9.6; 2.4; 3.5)	4.03 dd (2.4: 13.1)	3.84 dd (3.5; 13.1)	H-6: 8.28 s	P–CH_2–CH(O)–CH_2–O: 1.88 ddd (6.1; 15.0; 18.2); 1.75 ddd (7.5; 15.0; 17.6); 3.98 m (2.6; 10.2; 6.1; 7.5; 8.1); 3.84 dd (12.0; 2.6); 3.62 dd (12.0; 10.2)
63ba	D_2O	5.85 d (1.2)	4.24 dd (1.2; 4.5)	–	4.37 dd (4.5; 9.0)	4.66 ddd (9.0; 2.4; 3.6)	4.03 dd (2.4; 13.2)	3.88 dd (3.6; 13.2)	H-6: 8.26 s	P–CH_2–CH(O)–CH_2–O: 1.81 ddd (14.8; 5.4; 18.7); 1.72 ddd (14.8; 8.3; 17.1); 4.21 m (2.7; 9.5; 5.4; 8.3; 9.7); 4.13 dd (12.2; 2.7); 3.47 dd (12.2; 9.5);
63bb	D_2O	6.58 (8.2)	4.37 dd (8.2; 4.4)	–	4.32 bd (4.4; <0.5)	4.23 bt (<0.5; 3.7; 3.4)	3.82 dd (3.7; 12.6)	3.79 dd (3.4; 12.6)	H-6: 8.14 s	P–CH_2–CH(O)–CH_2–O: 1.85 ddd (14.8; 5.8; 18.3); 1.73 ddd (14.8; 7.9; 17.3); 4.00 m (2.6; 10.2; 5.8; 7.9; 8.0); 3.91 dd (12.0; 2.6); 3.69 dd (12.2; 10.2);
67[c]	$CDCl_3$	5.83 d (1.2)	4.21 dd (1.2; 4.6)	–	4.36 dd (4.6; 8.8)	4.47 ddd (8.8; 1.9; 3.1)	4.12 dd (1.9; 12.3	3.85 dd (3.1; 12.3)	NH: 8.75 bs; H-6: 7.85 s	P–CH_2–CH(O)–CH_2–O: 2.11 ddd (5.7; 15.3; 19.5); 1.94 ddd (7.6; 15.3; 19.6); 4.01 m (2.7; 9.6; 5.7; 7.6; 9.9); 3.76 dd (11.8; 2.7); 3.49 dd (11.8; 9.6); $P(OEt)_2$: 4.10–4.16 m; 1.33 t (7.1)
69a[d]	$CDCl_3$	6.70 d (8.3)	4.24 dd (8.3; 4.6)	–	4.01 d (4.6)	4.19 t (1.9; 1.5)	4.00 dd (1.9; 12.0)	3.78 dd (1.5; 12.0)	NH: 8.38 bs; H-6: 7.91 s	CH_3–CO–O–CH(O)–CH_2–O: 2.25 s; 6.17 dd (4.1; 4.9); 4.00 dd (12.2; 4.1); 3.50 dd (12.2; 4.9)
		6.15 d (4.7)	4.24 t (4.7; 5.0)	–	4.30 t (5.0; 4.6)	4.25 m (4.6; 2.0; 2.8)	4.03 dd (2.0; 12.0)	3.81 dd (2.8; 12.0)	NH: 8.49 bs; H-6: 7.78 s	CH_3–CO–O–CH(O)–CH_2–O: 2.12 s; 5.90 d (1.5); 3.93 d (12.7); 3.78 dd (12.7; 1.5)
70aa[e]	$CDCl_3$	6.61 d (8.3)	4.30 dd (8.3; 4.3)	–	3.97 d (4.3)	4.10 t (2.0; 1.9)	3.95 dd (2.0; 12.0)	3.75 dd (1.9; 12.0)	NH: 8.45 bs; H-6: 7.92 s	P–CH(O)–CH_2–O: 4.42 dt (2.7; 11.3; 11.3);

(*continued on next page*)

Table 5 (*continued*)

Compound	Solv.	H-1′	H-2′	H-2″/H-3″	H-3′	H-4′	H-5′a	H-5′b	Base	Substituents
										4.09 ddd (12.0; 2.7; 0.7); 3.77 ddd (12.0; 11.3; 3.6); P(OEt)$_2$: 4.20 m; 1.36 t (7.1)
70 ab[f]	$CDCl_3$	5.81 d (1.0)	4.21 dd (1.0; 4.7)	–	4.43 ddd (4.7; 9.1; 2.3)	4.51 ddd (9.1; 1.9; 3.2)	4.12 dd (1.9; 12.2)	3.86 dd (3.2; 12.2)	NH: 8.75 bs; H-6: 7.81 s	P–CH(O)–CH$_2$–O: 4.01 ddd (10.7; 3.1; 12.3); 3.87 ddd (12.0; 10.7; 4.7); 3.78 ddd (12.0; 3.1; 1.6); P(OEt)$_2$: 4.23 m; 1.35 m
71aa	D_2O	6.37 d (8.2)	4.26 dd (8.2; 4.4)	–	4.00 d (4.4)	3.98 t (3.4; 3.4)	3.61 dd (3.4; 12.6)	3.58 dd (3.4; 12.6)	H-6: 8.05 s	P–CH(O)–CH$_2$–O: 3.85 ddd (2.8; 11.2; 10.9); 3.90 ddd (12.1; 2.8; 0.9); 3.59 ddd (12.1; 11.2; 3.8)
71ab	D_2O	5.83 s	4.27 m	–	4.28 m	4.69 m (9.0; 2.4; 3.7)	4.04 dd (2.4; 13.2)	3.84 dd (3.7; 13.2)	H-6: 8.26 s	P–CH(O)–CH$_2$–O: 3.74 ddd (11.3; 2.7; 11.7); 3.91 ddd (11.9; 11.3; 3.8); 3.82 dd (11.9; 2.7)
69b[g]	$CDCl_3$	6.56 d (7.7)	4.28 ddd (7.7; 4.7; 0.8)	–	4.31 dd (4.7; 1.1)	4.15 td (1.1; 2.2; 2.1)	3.99 dd (2.2; 12.0)	3.76 dd (2.1; 12.0)	NH: 8.97 bs; H-6: 7.92 s	O–CH$_2$–CH<: 4.15 dd (12.8; 2.9); 3.61 dd (12.8; 2.4); 6.06 bt (2.9; 2.4; 0.8); OAc: 2.17 s
		6.28 d (4.6)	4.21 t (4.6; 4.5)	–	4.38 dd (4.5; 5.0)	4.40 ddd (5.0; 1.8; 2.3)	4.05 dd (1.8; 12.1)	3.80 dd (2.3; 12.1)	NH: 8.95 bs; H-6: 7.88 s	O–CH$_2$–CH<: 3.90 dd (12.2; 5.0); 3.77 dd (12.2; 2.3); 5.88 dd (5.0; 2.3); OAc: 2.08 s
70bb[h]	$CDCl_3$	6.66 d (8.4)	4.25 dd (8.4; 4.3)	–	4.06 d (4.3)	4.19 t (1.9; 1.9)	3.95 dd (1.9; 12.0)	3.76 dd (1.9; 12.0)	NH: 8.94 bs; H-6: 7.94 s	P–CH(O)–CH$_2$–O: 4.07 m (1H); 4.10 m (1H) and 3.84 m (1H); P(OEt)$_2$: 4.19 m and 4.23 m; 1.37 t (7.2) and 1.35 t (7.2)
70ba[i]	$CDCl_3$	6.02 d (2.8)	4.20 dd (2.8; 4.7)	–	4.65 dd (4.7; 7.0)	4.37 ddd (7.0; 1.8; 2.8)	4.08 dd (1.8; 12.2)	3.83 dd (2.8; 12.2)	NH: 8.58 bs; H-6: 7.86 s	P–CH(O)–CH$_2$–O: 4.13 m (2H); 3.86 m (1H); P(OEt)$_2$: 4.19 m and 4.17 m; 1.32t (7.2)
71ba	D_2O	5.79 d (0.5)	4.25 dd (0.5; 4.6)	–	4.36 dd (4.6; 9.6)	4.65 ddd (9.6; 2.4; 3.5)	4.05 dd (2.4; 13.2)	3.90 dd (3.5; 13.2)	H-6: 8.30 s	P–CH(O)–CH$_2$–O: 4.01 td (2.8; 11.1; 11.1); 4.08 ddd (2.8; 12.1; <1); 3.74 ddd (11.1; 12.1; 3.6)
71bb	D_2O	6.63 d (8.2)	4.34 ddd (8.2; 4.4; 2.1)	–	4.19 bd (4.4; 0.6)	4.29 m (0.6; 3.8; 3.5)	3.83 dd (3.8; 12.6)	3.80 dd (3.5; 12.6)	H-6: 8.15 s	P–CH(O)–CH$_2$–O: 3.79 ddd (11.5; 2.6; 12.0); 4.01 ddd (11.5; 11.9; 3.7); 3.87 ddd (11.9; 2.6)

[a] ODMTr: 7.30 m, 2H (*ortho*-ArH); 7.28 m, 2H (*ortho*-ArH); 6.76 m, 2H (*meta*-ArH); 6.74 m, 2H (*meta*-ArH); 7.40–7.23 m, 5H (C_6H_5); 3.755 s, 3H (OCH_3); 3.750 s, 3H (OCH_3); TBDPS: 1.01 s, 9H (*tert*-Bu); 7.52 m, 2H (*ortho*-ArH); 7.48 m, 2H (*ortho*-ArH); 7.36 m, 2H (*meta*-ArH); 7.31 m, 2H (*meta*-ArH); 7.45 m, 1H (*ipso*-ArH); 7.43 m, 1H (*ipso*-ArH).

[b] ODMTr: 7.31 m, 4H (*ortho*-ArH); 6.73 m, 4H (*meta*-ArH); 7.33 m, 2H (*ortho*-ArH); 7.45 m, 2H (*meta*-ArH); 7.21 m, 1H (*ipso*-ArH); 3.75 s, 3H (OCH_3); 3.74 s, 3H (OCH_3); TBDPS: 0.98 s, 9H (*tert*-Bu); 7.51 m, 4H (*ortho*-ArH); 7.21 m, 4H (*meta*-ArH); 7.43 m, 2H (*ipso*-ArH).

[c] TBDPS: 1.10 s (*tert*-Bu); 7.67 m, 4H (*ortho*-ArH); 7.39 m, 4H (*meta*-ArH); 7.45 m, 2H (*ipso*-ArH).

[d] TBDPS: 1.12 s and 1.11 s, (*tert*-Bu); 7.64–7.66 m (*ortho*-ArH); 7.40–7.43 m (*meta*-ArH); 7.46–7.49 m (*ipso*-ArH).

[e] TBDPS: 1.11 s (*tert*-Bu); 7.65 m, 4H (*ortho*-ArH); 7.43 m, 4H (*meta*-ArH); 7.48 m, 2H (*ipso*-ArH).

[f] TBDPS: 1.10 s (*tert*-Bu); 7.66 m, 4H (*ortho*-ArH); 7.39 m, 4H (*meta*-ArH); 7.45 m, 2H (*ipso*-ArH).

[g] TBDPS: 1.12 s and 1.10 s (*tert*-Bu); ~7.66 m, 4H (*ortho*-ArH); ~7.42 m, 4H (*meta*-ArH); ~7.47 m, 2H (*ipso*-ArH).

[h] TBDPS: 1.10 s (*tert*-Bu); 7.64 m, 4H (*ortho*-ArH); 7.43 m, 4H (*meta*-ArH); 7.48 m, 2H (*ipso*-ArH).

[i] TBDPS: 1.10 s (*tert*-Bu); 7.66 m, 4H (*ortho*-ArH); 7.40 m, 4H (*meta*-ArH); 7.45 m, 2H (*ipso*-ArH).

Table 6
Carbon-13 NMR data. Coupling constants *J*(C,P) are given in brackets.

Compound	Solvent	C-1′	C-2′	C-3′	C-4′	C-5′a	Base	Substituents
13a	D_2O	95.64	88.17 d (9.2)	84.79 (9.2)	89.02	64.01	C-2: 153.22; C-4: 164.74; C-5: 111.14; C-6: 143.02	P–CH < O: 106.73 d (181.2)
13b	D_2O	95.13	85.56 d (8.1)	83.15 d (8.3)	86.89	64.34	C-2: 154.66; C-4: 157.01; C-5: 111.53; C-6: 142.86	P–CH < O: 104.49 d (172.9)
23a	D_2O	87.95	38.55	83.50 d (11.8)	87.35	64.31	C-2: 154.47; C-4: 169.32; C-5: 114.20; C-6: 140.33; 5-CH_3: 14.26	P–CH_2–O: 68.67 d (155.1)
23b	D_2O	92.84	88.17 d (12.4)	33.32	84.35	64.40	C-2: 154.16; C-4: 169.44; C-5: 113.50; C-6: 140.32; 5-CH_3: 14.28	P–CH_2–O: 68.94 d (155.1)
24a	D_2O	91.64	75.28	81.79 d (11.3)	85.06	63.47	C-2: 154.52; C-4: 169.26; C-5: 114.21; C-6: 140.05; 5-CH_3: 14.26	P–CH_2–O: 69.51 d (155.8)
24b	D_2O	90.24	85.78 (12.3)	71.16	86.46	62.98	C-2: 154.29; C-4: 169.25; C-5: 114.12; C-6: 140.18; 5-CH_3: 14.30	P–CH_2–O: 69.46 d (156.7)
25a	D_2O	88.42	38.74	83.82d (12.2)	87.51	64.33	C-2: 154.33; C-4: 169.05; C-5: 104.95; C-6: 144.78	P–CH_2–O: 68.00 d (157.4)
26a	D_2O	88.72	39.03	83.45d (12.0)	87.71	64.18	C-2: 153.52; C-4: 164.72; C-5: 111.54; C-6: 141.48	P–CH_2–O: 68.46 d (156.0)
31a 2 diastereomers	D_2O	89.46	39.74	82.01	89.21	64.48	C-2: 154.45; C-4: 169.55; C-5: 113.32; C-6: 141.06; 5-CH_3: 14.36	P–CH(OH)–CH_2–O: 72.03 d (148.9); 73.13 d (11.3)
		89.51	39.89	82.07	89.44	64.54	C-2: 154.38; C-4: 169.52; C-5: 113.26; C-6: 141.02; 5-CH_3: 14.37	P–CH(OH)–CH_2–O: 71.99 d (149.2); 73.07 d (11.3)
31b 2 diastereomers	D_2O	93.01	86.54	33.48	84.32	64.44	C-2: 154.14; C-4: 169.40; C-5: 113.50; C-6: 140.24; 5-CH_3: 14.29	P–CH(OH)–CH_2–O: 71.82 d (149.9); 73.78 d (11.1)
		92.95	86.47	33.40	84.31	64.41	C-2: 154.14; C-4: 169.40; C-5: 113.50; C-6: 140.22; 5-CH_3: 14.29	P–CH(OH)–CH_2–O: 71.73 d (149.9); 73.78 d (11.1)
33a mixture of C-1 epimers (70:30)	D_2O	89.70	39.97	81.52	89.35	64.50	C-2: 154.31; C-4: 169.53; C-5: 113.06; C-6: 140.87; 5-CH_3: 14.38	P–CH_2–CH_2–O: 32.22 d (128.0); 68.00 d (2.9)
		87.88	38.82	81.28	87.42	64.16	C-2: 154.45; C-4: 169.28; C-5: 114.20; C-6: 140.26; 5-CH_3: 14.27	P–CH_2–CH_2–O: 32.22 d (128.0); 68.65 d (2.2)
33b	D_2O	93.00	85.94	33.52	84.27	64.46	C-2: 154.16; C-4: 169.42; C-5: 113.52; C-6: 140.22; 5-CH_3: 14.29	P–CH_2–CH_2–O: 32.72 d (125.8); 69.49 d (3.3)
45a	D_2O	95.64	86.76	84.50	88.93	64.10	C-2: 154.33; C-4: 166.09; C-5: 111.23; C-6: 142.51	P–CH(OH)–CH<: 71.48 d (144.3); 110.00 d (7.7)
45b	D_2O	95.69	88.11	83.47	88.84	64.15	C-2: 156.40; C-4: 168.50; C-5: 111.37; C-6: 141.97	P–CH(OH)–CH<: 71.42 d (143.0); 110.10 d (7.3)
50	D_2O	95.32	86.97	83.59	89.12	64.10	C-2: 154.18; C-4: 169.52; C-5: 113.67; 5-CH_3: 14.17; C-6: 141.99	P–CH_2–CH<: 36.76 d (127.8); 108.64
51	D_2O	96.02	87.25	83.80	86.49	64.12	C-2: 154.10; C-4: 169.29; C-5: 104.32; C-6: 146.23	P–CH_2–CH<: 36.71 d (128.0); 108.45
52	D_2O	96.09	87.47	83.88	89.53	64.07	C-2: 154.32; C-4: 166.03; C-5: 111.11; C-6: 142.89	P–CH_2–CH<: 36.84 d (126.9); 108.63
53	D_2O	95.78	87.40	83.53	88.99	64.23	C-2: 159.79; C-4: n.d.; C-5: n.d; C-6:128.31	P–CH_2–CH<: 37.30 d (125.1); 109.29
56	D_2O	96.00	87.51	83.65	89.04	64.19	C-2: 159.83; C-4: 163.46; C-5: 111.77; C-6: 141.89	P–CH_2–CH_2–CH<: 25.45 d (132.8); 31.04 d (2.9); 111.51 d (19.0)
60a[a]	$CDCl_3$	87.03	75.17	79.20	83.22	64.38	C-2: 150.28; C-4: 162.68; C-5: 102.61; C-6: 140.45	O–CH_2–CH=O: 75.15; 200.18
60b[b]	$CDCl_3$	86.81	82.56	70.90	83.20	62.96	C-2: 149.85; C-4: 162.66; C-5: 102.12; C-6: 139.46	O–CH_2–CH=O: 75.72; 199.00
63aa	D_2O	84.89	78.39	75.74	86.62	64.08	C-2: 154.12; C-4: 164.74; C-5: 112.32; C-6: 140.91	P–CH_2–CH(O)–CH_2–O: 33.70 d (130.2); 70.01 d (2.1); 71.64 d (5.4)
63ab	D_2O	92.29	79.77	71.22	79.20	62.18	C-2: 153.39; C-4: 165.03; C-5: 111.15; C-6: 141.42	P–CH_2–CH(O)–CH_2–O: 33.93 d (131.1); 73.80; 67.44 d (9.4)
63ba	D_2O	91.34	78.45	72.53	80.20	62.31	C-2: 153.67; C-4: 165.35; C-5: 111.25; C-6: 141.14	P–CH_2–CH(O)–CH_2–O: 33.54 d (130.2); 69.49; 71.48 d (11.3)
63bb	D_2O	84.04	77.25	77.23	87.31	64.20	C-2: 154.56; C-4: 165.23; C-5: 112.42; C-6: 140.74	P–CH_2–CH(O)–CH_2–O: 34.21 d (130.2); 74.14; 68.21 d (8.7)
67[c]	$CDCl_3$	89.11	77.48	68.71	~77.0	61.95	C-2: 148.72; C-4: 158.86; C-5: 109.40; C-6: 136.19	P–CH_2–CH(O)–CH_2–O: 29.24 d (141.9); 69.04; 65.00 d (9.4); $P(OEt)_2$: 62.23 s (6.3); 61.89 d (6.4); 16.44 d (6.2); 16.40 d (6.2)

(*continued on next page*)

Table 6 (*continued*)

Compound	Solvent	C-1′	C-2′	C-3′	C-4′	C-5′a	Base	Substituents
69a[d] 2 diastereomers	$CDCl_3$	84.89	74.84	74.18	83.98	64.42	C-2: 149.17; C-4: 158.32; C-5: 110.15; C-6: 135.94	CH_3–CO–O–CH(O)–CH_2–O: 21.36; 170.54; 87.71; 64.57
		87.50	74.57	72.06	81.81	63.21	C-2: 148.82; C-4: 158.36; C-5: 109.86; C-6: 136.61	CH_3–CO–O–CH(O)–CH_2–O: 20.96; 169.14; 85.94; 66.18
70aa[e]	$CDCl_3$	80.30	76.67 d (10.5)	73.59	84.25	64.22	C-2: 149.25; C-4: 158.35; C-5: 110.02; C-6: 135.89	P–CH(O)–CH_2–O: 65.70 d (167.2); 64.67 d (4.7); $P(OEt)_2$: 63.24 d (6.8); 63.03 d (6.1); 16.40 d (5.7); 16.37 d (5.8)
70ab[f]	$CDCl_3$	89.76	77.44 d (11.7)	69.05	76.82	61.90	C-2: 148.60; C-4: 158.65; C-5: 109.36; C-6: 136.63	P–CH(O)–CH_2–O: 70.39 d (169.7); 60.30 d (5.9); $P(OEt)_2$: 63.59 d (6.6); 63.06 d (6.6); 16.48 d (~6.0)
71aa	D_2O	84.07	77.82 d (9.2)	76.35	87.09	64.31	C-2: 154.63; C-4: 164.78; C-5: 112.13; C-6: 140.94	P–CH(O)–CH_2–O: 70.76 d (152.0); 68.64 d (5.5)
71ab	D_2O	92.55	79.59 d (10.3)	71.50	79.22	62.21	C-2: 153.33; C-4: 164.96; C-5: 111.08; C-6: 141.43	P–CH(O)–CH_2–O: 75.54 d (150.5); 64.23 d (6.8)
69b[g]	$CDCl_3$	81.82	76.64	68.59	83.79	64.07	C-2: 149.23; C-4: 158.52; C-5: 110.19; C-6: 135.87	O–CH_2–CH<: 62.02; 88.13; OAc: 169.46; 21.00
		84.17	76.16	71.51	82.36	62.66	C-2: 149.53; C-4: 158.57; C-5: 109.88; C-6: 136.00	O–CH_2–CH<: 63.89; 87.84; OAc: 169.04; 21.03
70bb[h]	$CDCl_3$	87.36	76.76	69.33 d (7.6)	79.02	62.41	C-2: 148.86; C-4: 158.41; C-5: 109.65; C-6: 136.14	P–CH(O)–CH_2–O: 66.71 d (165.5); 63.02 d (4.2); $P(OEt)_2$: 63.23 d (6.6); 62.74 d (6.6); 16.52 d (5.6); 16.45 d (5.6)
70ba[i]	$CDCl_3$	80.06	76.18	74.99 d (12.0)	84.05	64.24	C-2: 149.49; C-4: 158.60; C-5: 110.08; C-6: 135.88	P–CH(O)–CH_2–O: 70.98 d (170.3); 60.63 d (5.3); $P(OEt)_2$: 63.53 d (6.4); 62.92 d (6.7); 16.50 d (5.0); 16.43 d (5.7)
71ba	D_2O	91.80	78.75	72.02 d (9.3)	79.57	62.14	C-2: 153.18; C-4: 164.80; C-5: 111.10; C-6: 141.22	P–CH(O)–CH_2–O: 70.10 d (152.5); 68.54 d (5.9)
71bb	D_2O	84.26	77.64	77.06 d (10.3)	87.50	64.24	C-2: 154.04; C-4: 164.68; C-5: 112.29; C-6: 140.93	P–CH(O)–CH_2–O: 75.65 d (162.7); 64.91 d (7.1)

[a] ODMTr: 130.40 (4× *ortho*-ArC); 128.36 (2× *ortho*-ArC); 129.12 (2× *meta*-ArC); 113.24 (2× *meta*-ArC); 113.21 (2× *meta*-ArC); 158.86 (2× *ipso*-ArC); 127.27 (*ipso*-ArC); 135.45 (*ipso*-ArC); 135.34 (*ipso*-ArC); 144.41 (*ipso*-ArC); 86.92 (>C<); 55.24 (2× OCH_3); TBDPS: 26.99 and 19.22 (*t*-Bu); 135.37 (2× *ortho*-ArC); 135.25 (2× *ortho*-ArC); 128.01 (2× *meta*-ArC); 128.05 (2× *meta*-ArC); 130.23 and 130.15 (2× *ipso*-ArC); 132.38 and 132.09 (2× *ipso*-ArC).

[b] ODMTr: 130.54 (2× *ortho*-ArC); 130.42 (2× *ortho*-ArC); 127.94 (2× *ortho*-ArC); 128.50 (2× *meta*-ArC); 113.17 (2× *meta*-ArC); 113.13 (2× *meta*-ArC); 158.89 (*ipso*-ArC); 158.86 (*ipso*-ArC); 127.28 (*ipso*-ArC); 135.93 (*ipso*-ArC); 135.75 (*ipso*-ArC); 144.85 (*ipso*-ArC); 87.03 (>C<); 55.25 and 55.23 (2× OCH_3); TBDPS: 27.02 and 19.38 (*t*-Bu); 135.54 (2× *ortho*-ArC); 135.25 (2× *ortho*-ArC); 127.92 (2× *meta*-ArC); 127.86 (2× *meta*-ArC); 130.20 and 130.15 (2× *ipso*-ArC); 132.74 and 132.21 (2× *ipso*-ArC).

[c] TBDPS: 27.03 and 19.34 (*t*-Bu); 135.41 and 135.61 (*ortho*-ArC); 127.88 (*meta*-ArC); 129.99 (*ipso*-ArC); 132.74 and 132.35 (*ipso*-ArC).

[d] TBDPS: 27.11, 27.03 and 19.27, 19.23 (*t*-Bu); 135.66, 135.62, 135.39 and 135.36 (*ortho*-ArC); 128.18, 128.10, 127.98 and 127.97 (*meta*-ArC); 130.41, 130.29, 130.17 and 130.12 (*ipso*-ArC); 132.33, 132.19, 131.85 and 131.83 (*ipso*-ArC).

[e] TBDPS: 27.08 and 19.19 (*t*-Bu); 135.62 and 135.38 (*ortho*-ArC); 128.14 and 128.11 (*meta*-ArC); 130.36 and 130.26 (*ipso*-ArC); 131.85 and 131.83 (*ipso*-ArC).

[f] TBDPS: 27.00 and 19.33 (*t*-Bu); 135.60 and 135.42 (*ortho*-ArC); 127.87 and 127.86 (*meta*-ArC); 129.99 (*ipso*-ArC); 132.73 and 132.41 (*ipso*-ArC).

[g] TBDPS – major: 27.11 and 19.24 (*t*-Bu); 135.61 (2× *ortho*-ArC); 135.42 (2× *ortho*-ArC); 128.07 (2× *meta*-ArC); 128.06 (2× *meta*-ArC); 130.34 and 130.26 (2× *ipso*-ArC); 132.03 and 132.00 (2× *ipso*-ArC); TBDPS – minor: 27.01 and 19.26 (*t*-Bu); 135.61 (2× *ortho*-ArC); 135.36 (2× *ortho*-ArC); 128.05 (2× *meta*-ArC); 128.02 (2× *meta*-ArC); 130.22 and 130.14 (2× *ipso*-ArC); 132.26 and 131.94 (2× *ipso*-ArC).

[h] TBDPS: 27.01 and 19.31 (*t*-Bu); 135.57 and 135.37 (*ortho*-ArC); 127.97 and 127.95 (*meta*-ArC); 130.08 (*ipso*-ArC); 132.46 and 132.16 (*ipso*-ArC).

[i] TBDPS: 27.06 and 19.18 (*t*-Bu); 135.62 and 135.36 (*ortho*-ArC); 128.17 and 128.10 (*meta*-ArC); 130.40 and 130.27 (*ipso*-ArC); 131.83 and 131.69 (*ipso*-ArC).

8.0 mmol) which was transformed into the title compound **26a** by the procedure described for compound **23a**. Overall yield: 0.5 g (1.4 mmol, 16%). HRMS $(M-H)^-$ for $C_{10}H_{13}ClN_2O_8P$: calcd *m*/*z* 355.0098, found 355.0103. IR (KBr, cm^{-1}): 3432, 2924, 2837, 1699, 1631, 1449, 1274, 1063, 916, 558, 454. NMR data – see Tables 5 and 6

*4.3.10. (1RS)-1-hydroxy-2-(thymidin-3′-O-yl)-ethanephosphonic acid (**31a**) (Scheme 3)*

Sodium hydride (1.1 g, 27.6 mmol) was added to a solution of 5′-*O*-dimethoxytritylthymidine (**15a**, 5.0 g, 9.2 mmol) in THF (90 mL) and the mixture was stirred at room temperature (rt) for 15 min. Then allyl bromide (1.2 mL, 13.8 mmol) was added dropwise to the stirred solution. The resulting mixture was stirred at room temperature overnight (TLC in C-1), quenched with acetic acid at 0 °C and concentrated under reduced pressure. The residue was diluted with chloroform (100 mL), and the solution was washed with water (3 × 50 mL). The organic layer was dried over anhydrous sodium sulfate, evaporated, and the allyl derivative **27a** was treated with a catalytic amount of osmium tetroxide (0.1 mL, 2.5% solution in *tert*-butanol) and *N*-methylmorpholine-*N*-oxide (1.08 g, 9.2 mmol) in a 2:1 (v/v) THF–water mixture at rt overnight (TLC in C-1). The reaction mixture was diluted with chloroform (250 mL); the organic layer was washed with water (3 × 100 mL), dried over anhydrous sodium sulfate and evaporated. The crude vicinal diol **28a** was dissolved in a 1:1 acetone–water mixture (100 mL), then sodium periodate (3.9 g, 18.4 mmol) was added, and the resulting mixture was stirred at room temperature for 1 h (TLC in C-1). Chloroform (100 mL) was added, the organic layer was washed with water (3 × 50 mL), dried over anhydrous sodium sulfate and evaporated. Purification of the residue by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform afforded 79% (4.3 g, 7.3 mmol) of the aldehyde **29a** which was immediately reacted with diethyl phosphite (2.8 mL, 21.9 mmol) in dichloromethane (50 mL) and in the presence of a 10 mol% of triethylamine (0.73 mL, 5.7 mmol). The resulting mixture was stirred at room temperature overnight (TLC in C-1) and then concentrated under reduced pressure. Oily residue was purified by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform. The obtained epimeric mixture **30a** (5.1 g, 7.0 mmol, 76%) was detritylated in 80% acetic acid (70 mL) for 30 min (TLC in C-1), then

the solution was concentrated under reduced pressure, and the residue was co-distilled sever times with water, alkalified with triethylamine, and finally dried by co-evaporation with ethanol and toluene. The residue was then dissolved in acetonitrile (40 mL), 2,6-lutidine (2.9 mL, 25.6 mmol) followed with bromotrimethylsilane (1.7 mL, 12.8 mmol) were added and the solution was stirred at rt overnight and then concentrated under reduced pressure. The crude **31a** was purified by an RP HPLC.

Overall yield of **31a**: 0.269 g (0.736 mmol, 8%). HRMS $(M-H)^-$for $C_{12}H_{18}N_2O_9P$: calcd *m*/*z* 365.0750, found 365.0745; IR (KBr, cm^{-1}): 3194, 3065, 2928, 2824, 1699, 1475, 1465, 1277, 1059, 970, 913, 768, 570, 493, 419. NMR data – see Tables 5 and 6

4.3.11. (1RS)-2-(3′-deoxy-5-methyluridin-2′-O-yl)-1-hydroxyethanephosphonic acid (**31b**) (Scheme 3)

3′-Deoxy-5′-*O*-dimethoxytrityl-5-methyluridine **15b** (2.0 g, 3.7 mmol) was transformed in four steps (→**27b**→**28b**→**29b**→), according to the procedure described above for **31a**, into hydroxyphosphonate **30b** in 81% yield (2.2 g, 3.0 mmol). This compound was subsequently detritylated and finally treated with bromotrimethylsilane (followed by the procedure for **31a**) to afford the phosphonic acid **31b**.

Overall yield of **31b**: 0.6 g (1.6 mmol, 44%). HRMS $(M-H)^-$ for $C_{12}H_{18}N_2O_9P$: calcd *m*/*z* 365.0750, found 365.0748; IR (KBr, cm^{-1}): 3210, 3061, 2984, 2946, 2820, 2683, 2483, 1696, 1471, 1391, 1266, 1122, 1108, 1082, 974, 911, 838, 795, 768, 585, 565, 492, 416. NMR data – see Tables 5 and 6

4.3.12. 2-(Thymin-3′-O-yl)-ethanephosphonic acid (**33a**) (Scheme 3)

The protected hydroxyphosphonate **30a** (3.6 g, 5.0 mmol), prepared according to the procedure described in **31a**, was treated with 1,1′-thiocarbonyldiimidazole (1.98 g, 11.0 mmol) in 1,2-dichloroethane (50 mL) at rt overnight (TLC in T-1 and C-1). The solution was concentrated under reduced pressure, and the residue was purified by silica gel chromatography using a linear gradient of ethyl acetate (0–50%) in toluene to afford the corresponding imidazoylthiocarbonyl derivative which was immediately deoxygenated. This compound in toluene (50 mL) containing AIBN (33 mg, 0.2 mmol) was heated under stirring at 120 °C, then tributyltinhydride (2.7 mL, 10 mmol) was added in one portion, the resulting mixture was heated for 1 h (TLC in T-1), and evaporated. The residue was purified by silica gel chromatography using a linear gradient of ethyl acetate (0–50%) in toluene affording **32a** (2.5 g, 3.5 mmol). This compound was detritylated in 80% acetic acid (30 mL) for 30 min (TLC in C-1), then the solution was concentrated under reduced pressure, and the residue was co-evaporated several times with water, alkalified with trietylamine, and finally dried by co-evaporation with ethanol and toluene. The residue was then dissolved in acetonitrile (40 mL), *N*,*O*-bis(trimethylsilyl)acetamide (8.7 mL, 35 mmol) followed with bromotrimethylsilane (1.9 mL, 14.0 mmol) were added and the solution was stirred at rt overnight, and then concentrated under reduced pressure. The crude **33a** was purified by an RP HPLC.

Yield: 0.343 g (0.98 mmol, 28%). HRMS $(M-H)^-$ for $C_{12}H_{18}N_2O_8P$: calcd *m*/*z* 349.0801, found 349.0802; IR (KBr, cm^{-1}): 3432, 1695, 1476, 1278, 1072, 1057, 975, 901, 769. NMR data – see Tables 5 and 6

4.3.13. 2-(3′-Deoxy-5-methyluridin-2′-O-yl)-ethanephosphonic acid (**33b**) (Scheme 4)

Compound **33b** was prepared from **30b** (0.44 g, 0.6 mmol) by the procedure described for compound **33a**.

Yield: 0.094 g (0.26 mmol, 43%). HRMS $(M-H)^-$ for $C_{12}H_{18}N_2O_8P$: calcd *m*/*z* 349.0801, found 349.0801; IR (KBr, cm^{-1}): 3262, 3071, 2928, 2820, 2360, 1697, 1518, 1477, 1444, 1389, 1272, 1110, 1079, 973, 903, 789, 769, 587, 566, 495, 421. NMR data – see Tables 5 and 6

4.3.14. 2(R)-(5-chlorouridin-2′,3′-di-O-yl)-1(S)-hydroxyethanephosphonic acid (**45a**) and 2(R)-(5-chlorouridin-2′,3′-di-O-yl)-1(R)-hydroxyethanephosphonic acid (**45b**) (Scheme 5)

To a stirred suspension of 5′-*O*-*tert*-butyldiphenylsilyluridine (**8**) (8.0 g, 16.6 mmol) in dichloromethane (150 mL) was added acrolein dimethylacetal (5.1 g, 49.8 mmol) and the mixture was acidified by 4 M HCl in DMF (pH on wet pH paper slightly red). After stirring at rt (TLC in C-1) for 24 h and neutralization with triethylamine, the clear reaction mixture was concentrated under reduced pressure. The obtained acetal **37** was treated with a catalytic amount of osmium tetroxide (0.1 mL, 2.5% solution in *tert*-butanol) and sodium periodate (7.1 g, 33.2 mmol) in THF–water mixture (7:3, 100 mL) at rt overnight (TLC in C-1). Then the mixture was diluted with chloroform (150 mL), and the whole was washed with water (3 × 50 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The aldehyde **40** (6.2 g, 11.8 mmol) obtained by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform, was treated with diethyl phosphite (4.5 mL, 35.4 mmol) and triethylamine (0.14 mL, 1 mmol) in dichloromethane (100 mL) at rt overnight (TLC in C-1). The solution was concentrated under reduced pressure and the residue was purified by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform to afford pure hydroxyphosphonate **43** (5.0 g, 7.57 mmol). This compound was treated with acetic anhydride (1.43 mL, 15.14 mmol) in pyridine (70 mL) at rt overnight (TLC in C-1). The reaction was quenched with water at 0 °C, and the solution was concentrated under reduced pressure. The residue was diluted with chloroform (100 mL), the solution was washed with water (3 × 50 mL), and the organic layer was dried over anhydrous sodium sulphate and evaporated. The obtained *O*-acetyl derivative of compound **43** was heated with *N*-chlorosuccinimide (5.24 g, 39.30 mmol) in dry pyridine (70 mL) at 90 °C for 45 min and the solution was concentrated under reduced pressure. The crude product was purified by silica gel chromatography using a linear gradient of ethyl acetate (0–50%) in toluene to afford TLC pure 5-chlorouracil derivative **43a**,**b** (2.76 g, 3.87 mmol) as an epimeric mixture. This product was treated with a 50% concentrated aq. NH_3 in ethanol (100 mL) at rt overnight (TLC in C-1). The solution was concentrated at reduced pressure, the residue was co-distilled with ethanol and toluene and the residue was treated with bromotrimethylsilane (1.45 mL, 11.06 mmol) and 2,6-lutidine (2.6 mL, 22.12 mmol) in acetonitrile (10 mL) at rt overnight. The mixture was concentrated *in vacuo*, the residue was treated with 0.5 M TBAF in THF (40 mL) at rt for 30 min and the solution was concentrated under reduced pressure. The oily residue was partitioned between water and diethyl ether, aqueous layer was washed with ether and evaporated *in vacuo*. The product **45a**,**b** was separated by an RP HPLC to afford both epimers in a pure form.

Yield **45a** 0.44 g (1.1 mmol, 7%, R,S diastereomer); HRMS $(M-H)^-$ for $C_{11}H_{13}ClN_2O_{10}P$: calcd *m*/*z* 398.9997, found 399.0002; IR (KBr, cm^{-1}): 3419, 1696, 1447, 1269, 1137, 1082, 976, 781, 587. NMR data – see Tables 5 and 6

Yield **45b** 0.50 g (1.25 mmol, 8%, R,R diastereomer); HRMS $(M-H)^-$ for $C_{11}H_{13}ClN_2O_{10}P$: calcd *m*/*z* 398.9997, found 399.0001; IR (KBr, cm^{-1}): 3396, 2948, 1698, 1448, 1273, 1140, 1074, 973, 905, 759, 668, 579. NMR data – see Tables 5 and 6

4.3.15. 2(R)-(5-methyluridin-2′,3′-di-O-yl)-ethanephosphonic acid (**50**) (Scheme 5)

The 4 M HCl in DMF was carefully added to a stirred suspension of 5′-*O*-*tert*-butyldiphenylsilyl-5-methyluridine (**34**, 6.0 g,

12 mmol) and acrolein dimethylacetal (3.8 g, 37.4 mmol) in dichloromethane (100 mL) until pH reached slightly red color using a wet pH paper. The resulting mixture was stirred at rt overnight (TLC in C-1), neutralized by addition of triethylamine at 0 °C, and concentrated under reduced pressure. The obtained acetal **36** was treated with a catalytic amount of osmium tetroxide (0.2 mL, 2.5% solution in *tert*-butanol) and sodium periodate (7.1 g, 33.2 mmol) in THF–water mixture (7:3, 100 mL) at rt overnight (TLC in C-1). Then the mixture was diluted with chloroform (150 mL), and the whole was washed with water (3 × 50 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The aldehyde **39** (4.3 g, 9.0 mmol) obtained by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform, was treated with diethyl phosphite (4.5 mL, 35.4 mmol) and triethylamine (0.14 mL, 1 mmol) in dichloromethane (100 mL) at rt overnight (TLC in C-1). The solution was concentrated under reduced pressure and the residue was purified by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform to afford TLC pure hydroxyphosphonate **42** (4.4 g, 6.5 mmol) which was treated with 1,1′-thiocarbonyldiimidazole (3.0 g, 16.9 mmol) in 1,2-dichloroethane (60 mL) at rt overnight (TLC in T-1 and C-1), and the solution was concentrated under reduced pressure. The residue was purified by silica gel chromatography using a linear gradient of ethyl acetate (0–50%) in toluene to afford the corresponding thioester. The solution of the thioester and AIBN (45 mg, 0.26 mmol) in toluene (60 mL) was heated under stirring at 120 °C, and then tributyltin hydride (3.62 mL, 13.46 mmol) was added and heating continued for the next 1 h (TLC in T-1). Diester **46** (3.5 g, 5.1 mmol) was obtained by silica gel chromatography using a linear gradient of ethyl acetate (0–50%) in toluene. This compound was treated with bromotrimethylsilane (2.8 mL, 20.41 mmol) and 2,6-lutidine (4.6 mL, 40.8 mmol) in acetonitrile (50 mL) at rt overnight and then concentrated under reduced pressure. The residue was treated with 0.5 M TBAF in THF (20 mL), the resulting mixture was stirred at room temperature for 30 min, and concentrated under reduced pressure. The oily residue was partitioned between water and diethyl ether, aqueous layer was washed with ether and evaporated *in vacuo*. The product **50** was separated by an RP HPLC. Yield: 1.34 g (3.7 mmol, 73%). HRMS $(M-H)^-$ for $C_{12}H_{16}N_2O_9P$: calcd *m*/*z* 363.0594, found 363.0587; IR (KBr, cm^{-1}): 3190, 2933, 1697, 1472, 1278, 1122, 1066, 974, 769, 582. NMR data – see Tables 5 and 6

4.3.16. (2R)-(uridin-2′,3′-di-O-yl)-ethanephosphonic acid (**51**) (Scheme 5)

Title compound **51** was prepared by the two step procedure including the deoxygenation of **43** (3.0 g, 4.5 mmol) by the procedure described for compound **46**, followed by deprotection of the obtained diester **47** by the procedure described for compound **50**.

Yield of **51**: 0.84 g (2.4 mmol, 53%). HRMS $(M-H)^-$ for $C_{11}H_{14}N_2O_9P$: calcd *m*/*z* 349.0437, found 349.0427; IR (KBr, cm^{-1}): 3419, 2923, 1694, 1454, 1276, 1122, 1065, 974, 572, 551. NMR data – see Tables 5 and 6

4.3.17. (2R)-(5-chlorouridin-2′,3′-di-O-yl)-ethanephosphonic acid (**52**) (Scheme 5)

The deoxygenation of **43** (1.5 g, 2.1 mmol) by the procedure described for compound **46** afforded diester **47** (1.08 g, 1.7 mmol) which was treated with *N*-chlorosuccinimide (1.8 g, 13.5 mmol) in pyridine (20 mL) at 90 °C for 45 min. The solution was then concentrated under reduced pressure and the 5-chlorouracil derivative **48** (1.5 g, 2.1 mmol) obtained by silica gel chromatography using a linear gradient of ethanol (0–10%) was deprotected by the procedure described for compound **50**.

Yield of **52**: 0.26 g (0.69 mmol, 33%). HRMS $(M-H)^-$ for $C_{11}H_{13}ClN_2O_9P$: calcd *m*/*z* 383.0047, found 383.0044; IR (KBr, cm^{-1}): 3406, 2360, 1697, 1644, 1583, 1445, 1270, 1240, 1125, 2064, 977, 576. NMR data – see Tables 5 and 6

4.3.18. (2R)-(5-fluorouridin-2′,3′-di-O-yl)-ethanephosphonic acid (**53**) (Scheme 5)

The conversion of 5′-*O*-tert-butyldiphenylsilyl-5-fluorouridine (**35**) (1.9 g, 3.8 mmol) into the title phosphonate **53** was performed as follows. The compound **35** was converted into **49** by the procedure described for 5-methyluracil derivative **46**. The fully protected phosphonate **49** was deprotected as described for phosphonate **50**.

Yield of **53**: 0.097 g (0.2 mmol, 5%); HRMS $(M-H)^-$ for $C_{11}H_{13}FN_2O_9P$: calcd *m*/*z* 367.0343, found 367.0345; IR (KBr, cm^{-1}): 3398, 1740, 1672, 1628, 1456, 1395, 1360, 1085, 1051, 977, 834, 701, 685, 577. NMR data – see Tables 5 and 6

4.3.19. (3R)-(5-ChloroUridin-2′,3′-di-O-yl)-propanephosphonic acid (**56**) (Scheme 6)

The stirred suspension of 5′-*O*-*tert*-butyldiphenylsilyluridine (**8**) (1.1 g, 2.4 mmol) and bromopropionaldehyde diethyl acetal (1.0 g, 4.8 mmol) in dichloromethane (20 mL) was acidified with 4 M HCl in DMF until pH reached slightly red color using a wet pH paper, and stirred at rt overnight (TLC in C-1). The clear solution was neutralized with triethylamine and concentrated under reduced pressure. The obtained product **54** was purified by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform.

Sodium salt of diethyl phosphite prepared from sodium hydride (0.13 g, 3.3 mmol) and diethyl phosphite (0.42 mL, 3.3 mmol) in THF (10 mL) under 20 min of stirring was added to the bromo derivative **54** (0.66 g, 1.1 mmol), and the resulting mixture was stirred at room temperature overnight (TLC in C-1). Then the homogeneous solution was quenched with 2 M TEAB (1 mL), and concentrated under reduced pressure. The residue was purified by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform to afford the diethyl ester **55**.

This compound **55** (0.23 g, 0.35 mmol) was treated with *N*-chlorosuccinimide (0.052 g, 0.385 mmol) in pyridine (5 mL) under heating at 90 °C for 45 min, and the solution was concentrated under reduced pressure. The residue was co-evaporated with acetonitrile (3 × 5 mL), dissolved in the same solvent (5 mL), and treated with bromotrimethylsilane (0.184 mL, 1.4 mmol) and 2,6-lutidine (0.326 mL, 2.8 mmol) at room temperature overnight. The solution was concentrated under reduced pressure, the residue was treated with 0.5 M TBAF in THF (10 mL) at rt for 30 min and the mixture was concentrated under reduced pressure. The obtained crude product **56** was purified by an RP HPLC.

Yield of **56**: 0.106 g (0.26 mmol, 11%); HRMS $(M-H)^-$ for $C_{12}H_{15}ClN_2O_9P$: calcd *m*/*z* 397.0204, found 397.0211; IR (KBr, cm^{-1}): 3422, 2932, 1697, 1646, 1626, 1540, 1449, 1398, 1537, 1276, 1084, 1049, 995, 834, 705, 689, 522. NMR data – see Tables 5 and 6

4.3.20. (5′-O-tert-Butyldiphenylsilyl-2′-O-dimethoxytrityluridin-3′-O-yl)-acetaldehyde (**60a**) (Scheme 7)

Sodium hydride (0.9 g, 22.9 mmol) was added to a stirred solution of 5′-*O*-*t*-butyldiphenylsilyl-2′-*O*-dimethoxytrityluridine (**57a**, 6.0 g, 7.6 mmol) in THF (70 mL), and the resulting mixture was heated at 48 °C for 45 min. Allyl bromide (0.86 mL, 9.9 mmol) was added to the stirred suspension, and the stirring was continued at rt overnight (TLC in C-1). On quenching the reaction with acetic acid (0.9 mL, 15 mmol) at 0 °C, the mixture was concentrated under reduced pressure, and the residue was dissolved in chloroform (100 mL) and washed with water (3 × 50 mL). The organic phase was dried over anhydrous sodium sulfate and evaporated. The

crude product **58a** was used to the next reaction without characterization.

The product **58a** was treated with a catalytic amount of osmium tetroxide (0.1 mL, 2.5% solution in *tert*-butanol) in a 2:1 THF/water mixture (60 mL) and *N*-methylmorpholine-*N*-oxide (1.0 g, 8.6 mmol). The resulting mixture was stirred at rt overnight (TLC in C-1), diluted with chloroform (250 mL), and the whole was washed with water (3 × 100 mL). Organic layer was dried over anhydrous sodium sulfate and evaporated. The obtained dihydroxy derivative **59a** and sodium periodate (2.2 g, 10.6 mmol) was stirred in 50% aqueous acetone (40 mL) at room temperature for 1 h (TLC in C-1). The mixture was diluted with chloroform (250 mL) and washed with water (3 × 100 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude product **60**a was purified by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform).

Yield of **60a**: 3.8 g (4.6 mmol, 60%); HRMS $(M-H)^-$ for $C_{48}H_{49}N_2O_9Si$: calcd *m*/*z* 825.3208, found 825.3228; IR ($CHCl_3$, cm^{-1}): 3391, 3012, 2933, 2861, 1716, 1694, 1608, 1510, 1463, 1254, 1114, 1106, 1035, 831, 703, 602, 517, 505. NMR data – see Tables 5 and 6

*4.3.21. (5′-O-tert-Butyldiphenylsilyl-3′-O-dimethoxytrityluridin-2′-O-yl)acetaldehyde (**60b**) (Scheme 8)*

The title compound **60b** was prepared from **57b** (6.0 g, 7.6 mmol) by the procedure as described for **60a**.

Yield of **60b**: 4.2 g (5.1 mmol, 67%); HRMS $(M-H)^-$ for $C_{48}H_{49}N_2O_9Si$: calcd *m*/*z* 825.3208, found 825.3217; IR ($CHCl_3$, cm^{-1}): 3392, 3012, 2962, 2934, 1737, 1692, 1608, 1509, 1463, 1463, 1429, 1392, 1302, 1253, 1179, 1114, 1082, 1036, 986, 830, 788, 783, 738, 704, 602, 585, 506. NMR data – see Tables 5 and 6

*4.3.22. 2(R),3-(5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→2; (3′-O)→3]-propanephosphonic acid (**63aa**) (Scheme 7)*

Tetraethylmethylenediphosphonate (1.4 mL, 5.7 mmol) was added dropwise to a stirred mixture of sodium hydride (0.23 g, 5.7 mmol) in dry THF (10 mL) at 0 °C. The stirring was continued at 0 °C for 10 min and then at room temperature for 30 min. Afterwards, the mixture was cooled to 0 °C again and the solution of aldehyde **60a** (2.6 g, 3.1 mmol) in dry THF (10 mL) was added to the stirred reaction mixture dropwise. The resulting mixture was stirred at room temperature for 3 h (TLC in C-1), and concentrated under reduced pressure. The phosphonate **61a** (2.43 g, 2.5 mmol), obtained by purification by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform, was treated with *N*-chlorosuccinimide (0.37 g, 2.78 mmol) in pyridine (25 mL) at 90 °C for 45 min, and the mixture was concentrated under reduced pressure. The residue was dissolved in 80% acetic acid (40 mL), the resulting mixture was stirred at room temperature for 30 min (TLC in C-1) and concentrated under reduced pressure. The residue was co-evaporated several times with water, neutralised with triethylamine, and finally co-evaporated with ethanol and toluene. The residue was treated with 0.5 M TBAF in THF (30 mL), the resulting mixture was stirred for 30 min at rt and concentrated under reduced pressure. The product **62a** (0.86 g, 1.9 mmol), obtained by silica gel chromatography using a linear gradient of ethyl acetate (0–50%) in toluene, was cyclized with sodium methoxide (0.13 g, 2.5 mmol) in dry methanol (20 mL). The mixture was stirred at room temperature for 6 h (TLC in C-1), neutralized with acetic acid at 0 °C, and concentrated under reduced pressure. The residue was purified by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform to afford diester of compound **63aa** (0.71 g, 1.6 mmol). This diester was treated with *N*,*O*-bis(trimethylsilyl)acetamide (4.0 mL, 16.0 mmol) in dry acetonitrile (12 mL) at rt for 1 h. Then bromotrimethylsilane (0.84 mL, 6.4 mmol) was added, and the mixture was stirred at rt overnight and finally concentrated under reduced pressure. The obtained crude product **63aa** was purified by an RP HPLC.

Yield of **63aa**: 0.44 g (1.09 mmol, 19%), *R* epimer; HRMS $(M-H)^-$ for $C_{12}H_{15}ClN_2O_9P$: calcd *m*/*z* 368.0204, found 397.0211; IR (KBr, cm^{-1}): 3407, 2892, 2362, 1697, 1632, 1451, 1380, 1279, 1130, 1090, 1050, 803, 780, 754, 667, 572, 555. NMR data – see Tables 5 and 6

*4.3.23. (2S),3-(5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→2; (3′-O)→3]-propanephosphonic acid (**63ab**) (Scheme 7)*

Compound **67** (25 mg; 0.036 mmol) was treated with TBAF (23 mg; 72 μmol) in THF (5 mL) under stirring at rt for 3 h (TLC in C-1). Reaction mixture was diluted with chloroform (20 mL) and poured directly onto a silica gel column. The product was eluted with a linear gradient of ethanol (0–10%) in chloroform yielding 12 mg (0.026 mmol, 75%) of product which was converted into **63ab** according to the procedure described for **63aa**. Overall yield, 9 mg (23 μmol, 86%). HRMS $(M-H)^-$ for $C_{12}H_{15}O_9N_2ClP$: calcd *m*/*z* 397.02092, found 397.02087; IR (KBr, cm^{-1}): 3433, 3253, 3060, 2805, 1698, 1627, 1450, 1417, 1366, 1345, 1345, 1270, 1147, 1111, 1062, 957, 924, 860, 783, 760, 735. NMR data – see Tables 5 and 6

*4.3.24. (2S),3-(5-chlorouridin-2′,3′-di-O-yl)-[(3′-O)→2; (2′-O)→3]-propanephosphonic acid (**63ba**) and (2R)-2,3-(5-chlorouridin-2′,3′-di-O-yl)-[(3′-O)→2; (2′-O)→3]-propanephosphonic acid (**63bb**) (Scheme 8)*

Compounds **63ba** and **63bb** were prepared from **60b** (1.2 g, 1.45 mmol) by the procedure described for compound **63aa**. The obtained epimeric mixture of **63ba** and **63bb** was resolved by an RP HPLC.

Overall yield: 0.055 g (0.014 mmol, ~1%). **63ba** (*S*-epimer): 0.035g (0.09 mmol); HRMS $(M-H)^-$ for $C_{12}H_{15}ClN_2O_9P$: calcd *m*/*z* 397.0204, found 397.0207; IR (KBr, cm^{-1}): 3404, 2931, 2902, 1700, 1628, 1447, 1383, 1274, 1256, 1117, 1091, 1051, 907, 782, 756, 681, 559. **63bb** (*R*-epimer): 0.020 g (0.05 mmol); HRMS $(M-H)^-$ for $C_{12}H_{15}ClN_2O_9P$: calcd *m*/*z* 397.0204, found 397.0213; IR (KBr, cm^{-1}): 3432, 3067, 2361, 1702, 1634, 1449, 1363, 1276, 1257, 1143, 1117, 1061, 960, 895, 782, 759, 661, 599, 569. NMR data – see Tables 5 and 6

*4.3.25. Diethyl 3-(5′-O-tert-butyldiphenylsilyluridin-2′-O-yl)-trans-2-propenephosphonate (**65**) and diethyl (2R)-3-(5′-O-tert-butyldiphenylsilyluridin-2′,3′-di-O-yl)-[(2′-O)→2; (3′-O)→3]-propanephosphonate (**66a**), and diethyl (2S)-3-(5′-O-tert-butyldiphenylsilyluridin-2′,3′-di-O-yl)-[(2′-O)→2; (3′-O)→3]-propanephosphonate (**66b**) (Scheme 7)*

The vinylphosphonate **61a** (300 mg, 0.3 mmol) was detritylated in 80% aq. acetic acid (20 mL) at rt overnight (TLC in C-2). The solution was concentrated under reduced pressure, the residue was co-evaporated several times with water to remove acetic acid, and the compound **64** was purified by a silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform. The obtained compound **64** was treated with Cs_2CO_3 (469 mg, 1.44 mmol) in *tert*-butanol (20 mL) under stirring at rt for 3 h. The progress of the reaction was monitored by LC–MS analysis. The reaction was quenched by DOWEX 50 in H^+ cycle, the suspension was filtered, and the filtrate was concentrated under reduced pressure. The residue was separated by an RP HPLC. Yield: **66a**, 54 mg (0.08 mmol, 30%); **66b**, 72 mg (0.10 mmol, 40%); and **65**, 18 mg (0.03 mmol, 10%). NMR data – see Tables 5 and 6

*4.3.26. Diethyl (2S)-3-(5′-O-terc-butyldiphenylsilyl-5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→2; (3′-O)→3]-propanephosphonate (**67**) (Scheme 7)*

Compound **66b** (29 mg, 0.04 mmol) in dry pyridine (2 mL) was treated with *N*-chlorosuccinimide (8 mg, 0.05 mmol) under stirring

at 90 °C for 30 min (TLC in C-1). The reaction mixture was diluted with $CHCl_3$ (50 mL) and the solution was washed with brine (2 × 50 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated. The crude product **67** was purified by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform. Yield: 24 mg (0.03 mmol, 80%). HRMS $(M-H)^-$ for $C_{32}H_{43}ClN_2O_9PSi$: calcd *m/z* 693.21585, found 693.21632; IR ($CHCl_3$, cm^{-1}): 3382, 3177, 3074, 3054, 3000, 2962, 1720, 1701, 1622, 1590, 1487, 1472, 1447, 1429, 1393, 1363, 1344, 1283, 1269, 1160, 1131, 1115, 1106, 1053, 1029, 998, 962, 864, 709, 703, 489. NMR data – see Tables 5 and 6

*4.3.27. (1RS)-2-(5′-O-tert-butyldiphenylsilyl-5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→1; (3′-O)→2]-ethyliden-1-ylacetate (**69a**) (Scheme 9)*

The solution of **60a** (1.2 g, 1.4 mmol) in 80% aq. acetic acid (20 mL) was set aside at rt for 30 min (TLC in C-1) and concentrated under reduced pressure. The compound **68a** (0.58 g, 1.1 mmol), obtained by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform, was treated with acetic anhydride (0.21 mL, 2.2 mmol) in pyridine (20 mL). The resulting mixture was stirred at rt overnight (TLC in C-1), quenched by addition of water (5 mL) at 0 °C, and concentrated under reduced pressure. The residue was diluted with chloroform (50 mL), the organic layer was washed with water (3 × 20 mL), dried over anhydrous sodium sulfate and evaporated. The crude product was treated with *N*-chlorosuccinimide (0.19 g, 1.4 mmol) in dry pyridine (10 mL) at 90 °C for 45 min (analysis by RP HPLC). The solution was concentrated under reduced pressure, and the crude product **69a** was purified by silica gel chromatography using a linear gradient of ethyl acetate (0–50%) in toluene.

Yield of **69a**: 0.65 g (1.08 mmol, 77%). HRMS $(M-H)^-$ for $C_{29}H_{33}ClN_2O_8SiNa$: calcd *m/z* 623.1587, found 623.1587; IR ($CHCl_3$, cm^{-1}): 3383, 3187, 3074, 3054, 3027, 2963, 1745, 1724, 1703, 1632, 1590, 1488, 1471, 1450, 1429, 1393, 1376, 1365, 1348, 1280, 1230, 1139, 1114, 1106, 1088, 1046, 1023, 999, 964, 862, 710, 703, 623, 616, 602, 505, 489. NMR data – see Tables 5 and 6

*4.3.28. (1RS),2-(5′-O-tert-butyldiphenylsilyl-5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→2; (3′-O)→1]-ethyliden-1-ylacetate (**69b**) (Scheme 10)*

Compound **69b** was prepared from **60b** (2.0 g, 2.4 mmol) by the same procedure as compound **69a**.

Overall yield: 0.88 g (1.46 mmol, 61%); HRMS $(M-H)^-$ for $C_{29}H_{32}ClN_2O_8Si$: calcd *m/z* 599.1622, found 599.1628; IR ($CHCl_3$, cm^{-1}): 3382, 3074, 3029, 2932, 2861, 1722, 1702, 1631, 1451, 1429, 1231, 1227, 1163, 1137, 1113, 1074, 1035, 964, 941, 879, 823, 807, 789, 783, 703, 667, 616, 568, 505. NMR data – see Tables 5 and 6

*4.3.29. Diethyl (1S)-2-(5′-O-tert-butyldiphenylsilyl-5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→1; (3′-O)→2]-ethanephosphonate (**70aa**) and diethyl (1R)-2-(5′-O-tert-butyldiphenylsilyl-5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→1; (3′-O)→2]-ethanephosphonate (**70ab**) (Scheme 9)*

Compound **69a** (150 mg, 0.25 mmol), dried by co-evaporation with acetonitrile (2 × 5 mL), was treated in dry acetonitrile (5 mL) with diethyl trimethylsilyl phosphite (164 μL, 0.75 mmol) and trimethylsilyl triflate (67 μL, 0.38 mmol) under argon atmosphere at rt for 12 h (TLC in C-2). The reaction was quenched by addition of 1 M TEAB in 50% aqueous ethanol (10 mL) at 0 °C, and the solution was concentrated under reduced pressure. The residue was co-evaporated with ethanol (2 × 50 mL) and finally with toluene (1 × 50 mL). The epimeric phosphonate diesters were resolved by silica gel chromatography using a linear gradient of ethanol (0–10%) in chloroform. Yield: 75 mg (0.11 mmol, 44%); 27 mg of **70aa**, 48 mg of **70ab**. For **70aa** HRMS $(M-H)^-$ for $C_{31}H_{40}ClN_2O_9PSiNa$: calcd *m/z* 701.1821, found 701.1820; IR ($CHCl_3$, cm^{-1}):3383, 3181, 3074, 3054, 2990, 2963, 2932, 2860, 1722, 1701, 1632, 1590, 1566, 1488, 1471, 1465, 1429, 1393, 1370, 1364, 1346, 1329, 1305, 1247, 1162, 1114, 1104, 1084, 1042, 1025, 978, 956, 877, 844, 704, 616, 489. For **70ab** HRMS $(M-H)^-$ for $C_{31}H_{40}ClN_2O_9PSiNa$: calcd *m/z* 701.1821, found 701.1819; IR ($CHCl_3$, cm^{-1}): 3381, 3175, 3074, 3053, 3028, 2995, 2930, 2857, 1720, 1702, 1631, 1620, 1590, 1487, 1471, 1465, 1455, 1446, 1429, 1392, 1368, 1270, 1245, 1162, 1114, 1105, 1080, 1045, 1025, 973, 950, 880, 703, 645, 489. NMR data – see Tables 5 and 6

*4.3.30. (1S),2-(5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→1; (3′-O)→2]-ethanephosphonic acid (**71aa**) (Scheme 9)*

Compound **70aa** (54 mg; 80 μmol) was treated with TBAF (51 mg; 0.16 mmol) in THF (5 mL) under stirring at rt for 10 min (TLC in C-2). The reaction mixture was diluted with chloroform (20 mL), the solution was then poured directly onto a silica gel column, and the product was eluted with a linear gradient of ethanol (0–10%) in chloroform. The obtained product (desilylated derivative of **70aa**) was subsequently treated with *N,O*-bis(trimethylsilyl)acetamide (100 μl, 0.4 mmol) in acetonitrile (5 mL) under argon atmosphere at rt for 12 h. Then bromotrimethylsilane (21 μl, 0.16 mmol) was added and the mixture was stirred overnight (TLC in IPAW). The resulting clear solution was concentrated under reduced pressure, the residue was treated shortly with 2 M triethylammonium hydrogen carbonate buffer (1 mL) and the obtained solution was evaporated to dryness. The residue was co-evaporated with ethanol (2 × 50 mL) and purified by an RP HPLC. The product was converted into sodium salt on Dowex 50 (Na^+). Yield of **71aa**: 9 mg (0.02 mmol, 64%). HRMS $(M-H)^-$ for $C_{11}H_{14}ClN_2O_9P$: calcd *m/z* 383.0053, found 383.0054; IR (KBr, cm^{-1}): 1697, 1632, 1537, 1452, 1278, 1129, 1110, 1085, 1071, 1050, 925, 782, 760. NMR data – see Tables 5 and 6

*4.3.31. (1R)-2-(5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→1; (3′-O)→2]-ethanephosphonic acid (**71ab**) (Scheme 9)*

Compound **70ab** (48 mg, 0.07 mmol) was converted into **71ab** according to the procedure described for **71aa**. Yield, 12 mg (0.02 mmol, 44%). HRMS $(M-H)^-$ for $C_{11}H_{14}ClN_2O_9P$: calcd *m/z* 383.0053, found 383.0048; IR (KBr, cm^{-1}): 1697, 1643, 1624, 1561, 1537, 1449, 1271, 1142, 1111, 1092, 1069, 1052, 928, 783, 760. NMR data – see Tables 5 and 6

*4.3.32. Diethyl (1S)-2-(5′-O-tert-butyldiphenylsilyl-5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→2; (3′-O)→1]-ethanephosphonate (**70ba**) and diethyl (1R)-1,2-(5′-O-tert-butyldiphenylsilyl-5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→2; (3′-O)→1]-ethanephosphonate (**70bb**) (Scheme 10)*

Compound **69b** (200 mg, 0.33 mmol) was converted into **70ba** and **70bb** according to the procedure described for **70aa** and **70ab**. Overall yield: 85 mg (0.13 mmol, 38%), 54 mg of **70bb**, 31 mg of **70ba**. For **70bb**, HRMS $(M-H)^-$ for $C_{31}H_{40}ClN_2O_9PSiNa$: calcd *m/z* 701.1821, found 701.1821; IR ($CHCl_3$, cm^{-1}): 3409, 3207, 3074, 2962, 2932, 2860, 1722, 1711, 1632, 1590, 1487, 1471, 1465, 1453, 1431, 1392, 1363, 1328, 1303, 1113, 1051, 1025, 970, 917, 849, 703, 618, 489. For **70ba**, HRMS $(M-H)^-$ for $C_{31}H_{40}ClN_2O_9PSiNa$: calcd *m/z* 701.1821, found 701.1819; IR ($CHCl_3$, cm^{-1}): 3381, 3177, 3074, 3054, 2961, 1720, 1702, 1631, 1620, 1590, 1566, 1487, 1471, 1463, 1452, 1429, 1393, 1364, 1329, 1305, 1252, 1161, 1115, 1106, 1049, 1028, 977, 917, 846, 703, 613, 489. NMR data – see Tables 5 and 6

*4.3.33. (1R)-2-(5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→2; (3′-O)→1]-ethanephosphonic acid (**71ba**) (Scheme 10)*

Compound **70ba** (16 mg, 0.04 mmol) was converted into **71ba** according to the procedure described for **71aa**. Overall yield, 8 mg

(0.02 mmol, 57%). HRMS $(M-H)^-$ for $C_{11}H_{14}ClN_2O_9P$: calcd *m/z* 383.0053, found 383.0050; IR (KBr, cm^{-1}): 1699, 1628, 1540, 1455, 1273, 1112, 1071, 908, 782, 761. NMR data – see Tables 5 and 6

*4.3.34. (1S)-2-(5-chlorouridin-2′,3′-di-O-yl)-[(2′-O)→2; (3′-O)→1]-ethanephosphonic acid (**71bb**) (Scheme 10)*

Compound **70bb** (25 mg, 0.06 mmol) was converted into **71bb** according to the procedure described for **71aa**. Overall yield, 10 mg (0.03 mmol, 45%). HRMS $(M-H)^-$ for $C_{11}H_{14}ClN_2O_9P$: calcd *m/z* 383.0053, found 383.0055; IR (KBr, cm^{-1}): 1698, 1634, 1539, 1450, 1266, 1137, 1099, 1070, 917, 781, 761. NMR data – see Tables 5 and 6

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.12.026.

References

[1] R. Nencka, in: F.R.S. Atta-ur-Rahma, M. Iqbal Choudhary (Eds.), Thymidine Phosphorylase Inhibitors in Anti-angiogenesis Drug Discovery and Development, Bentham Science Publishers, 2011, pp. 116–147.
[2] A. Moghaddam, R. Bicknell, Expression of platelet-derived endothelial cell growth factor in *Escherichia coli* and confirmation of its thymidine phosphorylase activity, Biochemistry 31 (1992) 12141–12146.
[3] T. Furukawa, A. Yoshimura, T. Sumizawa, M. Haraguchi, S.-I. Akiyama, K. Fukui, M. Ishizawa, Y. Yamada, Angiogenetic factor, Nature 356 (1992) 668.
[4] N.S. Brown, A. Jones, C. Fujiyama, A.L. Harris, R. Bicknell, Thymidine phosphorylase induces carcinoma cell oxidative stress and promotes secretion of angiogenic factors, Cancer Res. 60 (2000) 6298–6302.
[5] K. Usuki, J. Saras, J. Waltenberger, K. Miyazono, G. Pierce, A. Thomason, C.H. Heldin, Platelet-derived endothelial cell growth factor has thymidine phosphorylase activity, Biochem. Biophys. Res. Commun. 184 (1992) 1311–1316.
[6] M.J. Pérez-Pérez, E.M. Priego, A.I. Hernández, M.J. Camarasa, J. Balzarini, S. Liekens, Thymidine phosphorylase inhibitors: recent developments and potential therapeutic applications, Mini-Rev. Med. Chem. 5 (2005) 1113–1123.
[7] A. Moghaddam, H.T. Zhang, T.P. Fan, D.E. Hu, V.C. Lees, H. Turley, S.B. Fox, K.C. Gatter, A.L. Harris, R. Bicknell, Thymidine phosphorylase is angiogenic and promotes tumor growth, Proc. Nat. Acad. Sci. U.S.A 92 (1995) 998–1002.
[8] M. Takeuchi, T. Otsuka, N. Matsui, K. Asai, T. Hirano, A. Moriyama, I. Isobe, Y.Z. Eksioglu, K. Matsukawa, T. Kato, T. Tada, Aberrant production of gliostatin/platelet-derived endothelial cell growth factor in rheumatoid synovium, Arthritis Rheum. 37 (1994) 662–672.
[9] D. Creamer, R. Jaggar, M. Allen, R. Bicknell, J. Barker, Overexpression of the angiogenic factor platelet-derived endothelial cell growth factor/thymidine phosphorylase in psoriatic epidermis, Brit. J. Dermatol. 137 (1997) 851–855.
[10] J. Folkman, Tumor angiogenesis: therapeutic implications, New. Engl. J. Med. 285 (1971) 1182–1186. http://www.scopus.com/scopus/search/submit/citedby.url?eid=2-s2.0-33845944644&refeid=2-s2.0-0015231516&src=s&origin=reflist&refstat=core.
[11] J. Folkman, Tumor angiogenesis, in: fourth ed., in: J.F. Holland, R.C. Bast, D.L. Morton, E. Frei, D.W. Kufe, R.R. Weichselbaum (Eds.), Cancer Medicine, vol. 1, Williams and Wilkens, Baltimore, MD, 1996, p. 181.
[12] J. Folkman, What is the evidence that tumors are angiogenesis dependent? J. Natl. Cancer Inst. 82 (1990) 4–6.
[13] S. Matsushita, T. Nitanda, T. Furukawa, T. Sumizawa, A. Tani, K. Nishimoto, S. Akiba, K. Miyadera, M. Fukushima, Y. Yamada, H. Yoshida, T. Kanzaki, S. Akiyama, The effect of a thymidine phosphorylase inhibitor on angiogenesis and apoptosis in tumors, Cancer Res. 59 (1999) 1911–1916.
[14] F. Focher, S. Spadari, Thymidine phosphorylase: a two-face Janus in anticancer chemotherapy, Curr. Cancer Drug. Targets 1 (2001) 141–153.
[15] A. Matter, Tumor angiogenesis as a therapeutic target, Drug. Discov. Today 6 (2001) 1005–1024.
[16] M. Cristofanilli, C. Charnsangavej, G.N. Hortobagyi, Angiogenesis modulation in cancer research: novel clinical approaches, Nat. Rev. Drug. Discov. 1 (2002) 415–426. http://www.scopus.com/scopus/search/submit/citedby.url?eid=2-s2.0-33845944644&refeid=2-s2.0-0036593385&src=s&origin=reflist&refstat=core.
[17] D.W. Davis, D.J. McConkey, W. Zhang, R.S. Herbst, Antiangiogenic tumor therapy, BioTechniques 34 (2003) 1048–1063.
[18] J.G. Niedzwicki, M.H. El Kouni, S.H. Chu, S. Cha, Structure-activity relationship of ligands of the pyrimidine nucleoside phosphorylases, Biochem. Pharmacol. 32 (1983) 399–415.
[19] K. Grancharov, J. Mladenova, E. Golovinsky, Inhibition of uridine phosphorylase by some pyrimidine derivatives, Biochem. Pharmacol. 41 (1991) 1769–1772.
[20] F. Focher, D. Ubiali, M. Pregnolato, C. Zhi, J. Gambino, G.E. Wright, S. padari, Novel nonsubstrate inhibitors of human thymidine phosphorylase, a potential target for tumor-dependent angiogenesis, J. Med. Chem. 43 (2000) 2601–2607.
[21] P.E. Murray, V.A. McNally, S.D. Lockyer, K.J. Williams, I.J. Stratford, M. Jaffar, S. Freeman, Synthesis and enzymatic evaluation of pyridinium-substituted uracil derivatives as novel inhibitors of thymidine phosphorylase, Bioorg. Med. Chem. 10 (2002) 525–530.
[22] C. Cole, P. Reigan, A. Gbaj, P.N. Edwards, K.T. Douglas, I.J. Stratford, S. Freeman, M. Jaffar, Potential tumor-selective nitroimidazolylmethyluracil prodrug derivatives: inhibitors of the angiogenic enzyme thymidine phosphorylase, J. Med. Chem. 46 (2003) 207–209.
[23] P. Langen, G. Etzold, D. Bärwolff, B. Preussel, Inhibition of thymidine phosphorylase by 6-aminothymine and derivatives of 6-aminouracil, Biochem. Pharmacol. 16 (1967) 1833–1837.
[24] R.S. Klein, M. Lenzi, T.H. Lim, K.A. Hotchkiss, P. Wilson, E.L. Schwartz, Novel 6-substituted uracil analogs as inhibitors of the angiogenic actions of thymidine phosphorylase, Biochem. Pharmacol. 62 (2001) 1257–1263.
[25] P.W. Woodman, A.M. Sarrif, C. Heidelberger, Inhibition of nucleoside phosphorylase cleavage of 5-fluoro-2′-deoxyuridine by 2,4-pyrimidinedione derivatives, Biochem. Pharmacol. 29 (1980) 1059–1063.
[26] M.L.P. Price, W.C. Guida, T.E. Jackson, J.A. Nydick, P.L. Gladstone, J.C. Juarez, F. Doñate, R.J. Ternansky, Design of novel N-(2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidin-7-yl)-guanidines as thymidine phosphorylase inhibitors, and flexible docking to a homology model, Bioorg. Med. Chem. Lett. 13 (2003) 107–110.
[27] K. Hirota, M. Sawada, H. Sajiki, M. Sako, Synthesis of 6-aminouracils and pyrrolo[2,3-d]pyrimidine-2,4-diones and their inhibitory effect on thymidine phosphorylase, Nucl. Acids Symp. Ser. 37 (1997) 59–60.
[28] M. Fukushima, N. Suzuki, T. Emura, S. Yano, H. Kazuno, Y. Tada, Y. Yamada, T. Asao, Structure and activity of specific inhibitors of thymidine phosphorylase to potentiate the function of antitumor 2′-deoxyribonucleosides, Biochem. Pharmacol. 59 (2000) 1227–1236.
[29] S. Yano, Y. Tada, H. Kuzuno, T. Suto, J. Tamashita, N. Suzuki, T. Emura, T. Asao, Int. Patent Appl. 1996, WO96/3034.
[30] P. Reigan, P.N. Edwards, A. Gbaj, C. Cole, S.T. Barry, K.M. Page, S.E. Ashton, R.W.A. Luke, K.T. Douglas, I.J. Stratford, M. Jaffar, R.A. Bryce, S. Freeman, Aminoimidazolylmethyluracil analogues as potent inhibitors of thymidine phosphorylase and their bioreductive nitroimidazolyl prodrugs, J. Med. Chem. 48 (2005) 392–402.
[31] R. Nencka, I. Votruba, H. Hřebabecký, E. Tloušťová, K. Horská, M. Masojídková, A. Holý, Design and synthesis of novel 5,6-disubstituted uracil derivatives as potent inhibitors of thymidine phosphorylase, Bioorg. Med. Chem. Lett. 16 (2006) 1335–1337.
[32] R. Nencka, I. Votruba, H. Hřebabecký, P. Jansa, E. Tloušťová, K. Horská, M. Masojídková, A. Holý, Discovery of 5-substituted-6-chlorouracils as efficient inhibitors of human thymidine phosphorylase, J. Med. Chem. 50 (2007) 6016–6023.
[33] S. Liekens, E. De Clercq, J. Neyts, Angiogenesis: regulators and clinical applications, Biochem. Pharmacol. 61 (2001) 253–270.
[34] M.B. Thomas, P.M. Hoff, S. Carter, G. Bland, Y. Lassere, R. Wolff, H. Xiong, J. Abbruzzese, A dose-finding, safety and pharmacokinetics study of TAS-102, an antitumor/antiangiogenic agent given orally on a once-daily schedule for five-days every three weeks in patients with solid tumors, Proc. Am. Assoc. Cancer Res. 43 (2002) 554.
[35] S. Yano, H. Kazuno, T. Sato, N. Suzuki, T. Emura, K. Wierzba, J.I. Yamashita, T. Asao, Synthesis and evaluation of 6-methylene-bridged uracil derivatives. Part 2: optimization of inhibitors of human thymidine phosphorylase and their selectivity with uridine phosphorylase, Bioorg. Med. Chem. 12 (2004) 3443–3450.
[36] J. Balzarini, A.E. Gamboa, R. Esnouf, S. Liekens, J. Neyts, E. De Clercq, M.J. Camarasa, M.J. Pérez-Pérez, 7-Deazaxanthine, a novel prototype inhibitor of thymidine phosphorylase, FEBS Lett. 438 (1998) 91–95.
[37] A. Esteban-Gamboa, J. Balzarini, R. Esnouf, E. De Clercq, M.J. Camarasa, M.J. Pérez-Pérez, Design, synthesis, and enzymatic evaluation of multi-substrate analogue inhibitors of *Escherichia coli* thymidine phosphorylase, J. Med. Chem. 43 (2000) 971–983.
[38] I. Votruba, K. Pomeisl, E. Tloušťová, A. Holý, B. Otová, Inhibition of thymidine phosphorylase (PD-ECGF) from SD-lymphoma by phosphonomethoxyalkyl thymines, Biochem. Pharmacol. 69 (2005) 1517–1521.

[39] S.A. Diab, C. De Schutter, M. Muzard, R. Plantier-Royon, E. Pfund, T. Lequeux, Fluoro-phosphonylated nucleoside derivatives as new series of thymidine phosphorylase multisubstrate inhibitors, J. Med. Chem. 55 (2012) 2758–2768.
[40] K. Pomeisl, A. Holý, I. Votruba, R. Pohl, Syntheses of N3-substituted thymine acyclic nucleoside phosphonates and a comparison of their inhibitory effect towards thymidine phosphorylase, Bioorg. Med. Chem. Lett. 18 (2008) 1364–1367.
[41] P. Kočalka, D. Rejman, V. Vaněk, M. Rinnová, I. Tomečková, Š. Králíková, M. Petrová, O. Páv, R. Pohl, M. Budešínský, R. Liboska, Z. Točík, N. Panova, I. Votruba, I. Rosenberg, Structural diversity of nucleoside phosphonic acids as a key factor in the discovery of potent inhibitors of rat T-cell lymphoma thymidine phosphorylase, Bioorg. Med. Chem. Lett. 20 (2010) 862–865.
[42] D. Rejman, N. Panova, P. Klener, B. Maswabi, R. Pohl, I. Rosenberg, N-Phosphonocarbonylpyrrolidine derivatives of guanine: a new class of bi-substrate inhibitors of human purine nucleoside phosphorylase, J. Med. Chem. 55 (2012) 1612–1621.
[43] Y. Li, Studies in Nucleoside Chemistry. Synthesis of Purine and Pyrimidine Nucleoside Phosphorylase Inhibitors. Total Synthesis of the Sulfated Nucleoside Glycoside HF-7, Ph.D. Dissertation, Cornell University, Ithaca, NY, 2000.
[44] A.L. Allan, P.L. Gladstone, M.L.P. Price, S.A. Hopkins, J.C. Juarez, F. Doñate, R.J. Ternansky, D.E. Shaw, B. Ganem, Y. Li, W. Wang, S. Ealick, Synthesis and evaluation of multisubstrate bicyclic pyrimidine nucleoside inhibitors of human thymidine phosphorylase, J. Med. Chem. 49 (2006) 7807–7815.
[45] M. Endová, M. Masojídková, M. Budešínský, I. Rosenberg, 2′,3′-O-phosphonoalkylidene derivatives of ribonucleosides: synthesis and reactivity, Tetrahedron 54 (1998) 11151–11186.
[46] D. Rai, M. Johar, N.C. Srivastav, T. Manning, B. Agrawal, D.Y. Kunimoto, R. Kumar, Inhibition of *Mycobacterium tuberculosis*, *Mycobacterium bovis*, and *Mycobacterium avium* by novel dideoxy nucleosides, J. Med. Chem. 50 (2007) 4766–4774.
[47] N.S. Mourier, A. Eleuteri, S.J. Hurwitz, P.M. Tharnish, R.F. Schinazi, Enantioselective synthesis and biological evaluation of 5-O-carboranyl pyrimidine nucleosides, Bioorg. Med. Chem. 7 (1999) 2759–2766.
[48] I. Kóšiová, Z. Točík, M. Buděšínský, O. Šimák, R. Liboska, D. Rejman, O. Pačes, I. Rosenberg, Methyl 4-toluenesulfonyloxymethylphosphonate, a new and versatile reagent for the convenient synthesis of phosphonate-containing compounds, Tetrahedron Lett. 50 (2009) 6745–6747.
[49] I.A. Mikhailopulo, T.I. Kulak, O.V. Tkachenko, S.L. Sentyureva, L.S. Victorova, H. Rosenmeyer, F. Seela, Nucleosides Nucleotides 19 (2000) 1885–1910.
[50] S. Manfredini, P.G. Baraldi, R. Bazzanini, D. Simoni, J. Balzarini, E. De Clercq, Synthesis and antiproliferative activity of 2′-*O*-allyl-1-β-d-arabinofuranosyl-uracil, -cytosine and -adenine, Bioorg. Med. Chem. Lett. 7 (1997) 473–478.
[51] V.S. Abramov, Dokl. Akad. Nauk. SSSR 95 (1954) 991–992.
[52] J. van Wijk, C.A.G. Haasnoot, F.A.A.M. de Leeuw, B.D. Huckried, W.A. Hoekzema, C. Altona, PSEUROT 6.3, Leiden Institute of Chemistry, Leiden University, 1999.
[53] F.A.A.M. de Leeuw, C. Altona, Conformational analysis of β-d-ribo-, β-d-deoxyribo-, β-d-arabino- β-d-xylo-, and β-ᴅ-lyxo-nucleosides from proton–proton coupling constants, J. Chem. Soc. Perkin II (1982) 375–384.
[54] Y. Wataya, D.W. Santi, Continuous spectrophotometric assay of thymidine phosphorylase using 5-nitro-2′-deoxyuridine as substrate, Anal. Biochem. 112 (1981) 96–98.
[55] R.A. Norman, S.T. Berry, M. Bate, J. Breed, J.G. Colls, R.J. Ernill, R.W.A. Luke, C.A. Mishull, M.S.B. McAlister, E.J. McCall, H.H.J. McMiken, D.S. Patterson, D. Timms, J.A. Tucker, R.A. Pauptit, Crystal structure of human thymidine phosphorylase in complex with a small molecular inhibitor, Structure 12 (2004) 75–84.
[56] V.A. McNally, M. Rajabi, A. Gbaj, I.J. Stradford, P.N. Edwards, K.T. Douglas, R.A. Bryce, M. Jaffar, S. Freeman, Design, synthesis and enzymatic evaluation of 6-bridged imidazolyluracil derivatives as inhibitors of human thymidine phosphorylase, J. Pharm. Pharmacol. 59 (2007) 537–547.
[57] M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding, Anal. Biochem. 27 (1976) 248–254.