SYNTHESES OF BASE AND SIDE-CHAIN MODIFIED PYRIMIDINE 1-[2-(PHOSPHONOMETHOXY)PROPYL] DERIVATIVES AS POTENT INHIBITORS OF THYMIDINE PHOSPHORYLASE (PD-ECGF) FROM SD-LYMPHOMA

Karel POMEISL[1,*], Ivan VOTRUBA[2], Antonín HOLÝ[3] and Radek POHL[4]

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, Czech Republic; e-mail: [1] pomeislk@uochb.cas.cz, [2] votruba@uochb.cas.cz, [3] holy@uochb.cas.cz, [4] pohl@uochb.cas.cz

Received January 23, 2006
Accepted March 10, 2006

In this study we synthesized a series of thymine and 5-ethyluracil acyclic nucleoside phosphonates bearing hydroxymethyl, methoxymethyl, azidomethyl, aminomethyl and (trimethylammonio)methyl group in side chain as potent inhibitors of thymidine phosphorylase. In addition, we investigated in particular the novel syntheses of fluorinated derivatives containing fluoromethyl or trifluoromethyl groups in side chain such as 5-ethyl-1-[(*S*)-3-fluoro-2-(phosphonomethoxy)propyl]uracil (**8**) or 5-ethyl-1-[3,3,3-trifluoro-2-(phosphonomethoxy)propyl]uracil and thymine derivatives **27** and **28**. Uracil acyclic nucleoside phosphonates 1-{2-[(diisopropoxyphosphoryl)methoxy]ethyl}uracil (**12**) and 1-{2-[(diisopropoxyphosphoryl)methoxy]-3,3,3-trifluoropropyl}uracil (**19**) were fluorinated to corresponding 5-fluorouracil derivatives. While the 5-fluorouracil derivatives exhibit a marginal inhibitory effect, thymine and 5-ethyluracil compound with fluorine in side chains possess considerable inhibitory potency toward thymidine phosphorylase from rat spontaneous T-cell lymphoma.

Keywords: Acyclic nucleoside phosphonates; Acyclic nucleotide analogues; Nucleotides; Thymidine phosphorylase; Fluorination; Pyrimidines; Uracil; Thymine.

Acyclic nucleoside phosphonates (ANPs) exhibit various kinds of biological activities such as antiviral, cytostatic, antiparasitic and immunomodulatory effects[1]. At present these compounds are investigated in connection with their possible use as potent inhibitors of thymidine phosphorylase[2]. Thymidine phosphorylase [dThd, EC 2.4.2.4. platelet-derived endothelial-cell growth factor (PD-ECGF)], an important salvage-pathway enzyme, catalyzes phosphorolysis of thymidine to thymine and 2′-deoxy-D-ribose 1-phosphate. The dephosphorylated product of the latter, 2′-deoxy-D-ribose, has a chemotactic activity *in vitro* and angiogenic activity *in vivo* stimulating endothelial-cell migration. This process is crucial for the formation of new

Collect. Czech. Chem. Commun. 2006, Vol. 71, No. 4, pp. 595–624

doi:10.1135/cccc20060595

blood vessels in a tumor which overexpress thymidine phosphorylase. Thus the inhibition of 2′-deoxy-D-ribose release from endothelial cells is a potential anti-angiogenic target in cancer chemotherapy.

The aim of our work has been the development of new inhibitors of thymidine phosphorylase based on the structure of specifically modified and metabolically stable pyrimidine ANPs. In this study we synthesized a series of thymine and 5-ethyluracil ANPs bearing hydroxymethyl, methoxymethyl, azidomethyl, aminomethyl and (trimethylammonio)methyl group in side chain (compounds I, Fig. 1). In addition, we investigated in particular the novel syntheses of fluorinated derivatives containing fluoromethyl (FPMP compounds) or trifluoromethyl groups in side chain because fluorine-containing substituents are often powerful modifiers of chemical and biological properties. For the same reason we fluorinated 1-[2-(phosphonomethoxy)ethyl]uracil and 1-[3,3,3-trifluoro-2-(phosphonomethoxy)propyl]-uracil in the ring position C-5 to compare the inhibitory effect of the corresponding 5-fluorouracil derivatives (compounds II, Fig. 1). The obtained compounds were tested as potent inhibitors of thymidine phosphorylase (PD-ECGF) expressed in V79 chinese hamster cells, as well as thymidine phosphorylase from SD-lymphoma and human placenta.

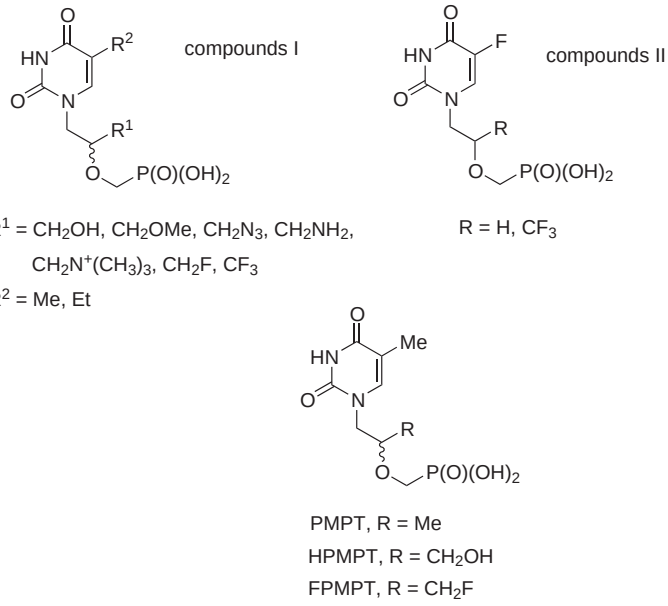
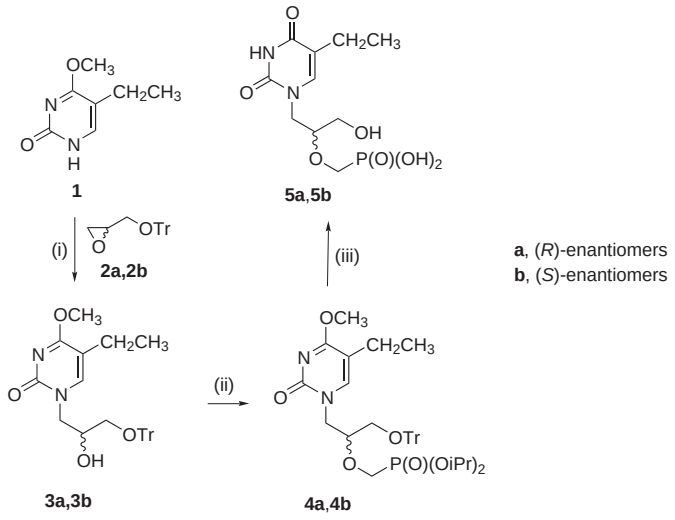


FIG. 1
Structures of compounds under study

RESULTS AND DISCUSSION

It is well known that a number of side-chain modified pyrimidine ANPs possess considerable inhibitory activity on thymidine phosphorylase isolated from rat livers[3]. This is documented by a kinetic study of several thymine ANP derivatives[4] which showed the efficient inhibitory effect of thymidine phosphorylase for 1-[2-(phosphonomethoxy)propyl]thymine (PMPT), 1-[3-hydroxy-2-(phosphonomethoxy)propyl]thymine (HPMPT) and 1-[3-fluoro-2-(phosphonomethoxy)propyl]thymine (FPMPT) (see Fig. 1).

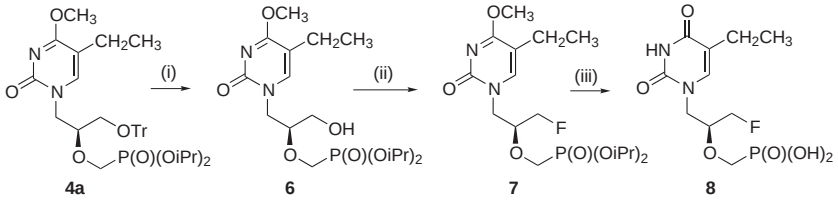
Similarly to previously reported procedure[4] developed for the preparation of HPMPT or FPMPT, we used this simple method for synthesis of compounds **5a** and **5b** to compare the influence of their bulkier alkyl at position C-5 of the uracil moiety on the bioactivity in new synthesized compounds **5a**, **5b** and **8** (Scheme 1 and 2). N^1-Alkylation[5] of protected base **1** with chiral oxiranes **2a** and **2b** takes place in dimethylformamide in the presence of cesium carbonate as catalyst affording compounds **3a** and **3b** in good preparative yields. For the preparation of **4a** and **4b** we have used a general procedure[3,5] with (diisopropoxyphosphoryl)methyl tosylate introducing a starting phosphonate block. The removal of trityl group in **4a** and the following replacement of hydroxy group with fluorine in the intermediate **6** was performed using perfluorobutane-1-sulfonyl fluoride[3] in the



(i) Cs_2CO_3, DMF, 80 °C; (ii) $TsOCH_2P(O)(OiPr)_2$, NaH, THF, -20 °C-40 °C;(iii) $(CH_3)_3SiBr$, CH_3CN, r.t.

SCHEME 1

presence of DBU to give **7** in a preparative yield (Scheme 2). Finally, the obtained phosphonates **4a**, **4b** and **7** were deprotected by treatment with bromotrimethylsilane followed by hydrolysis to give **5a**, **5b** and **8**.

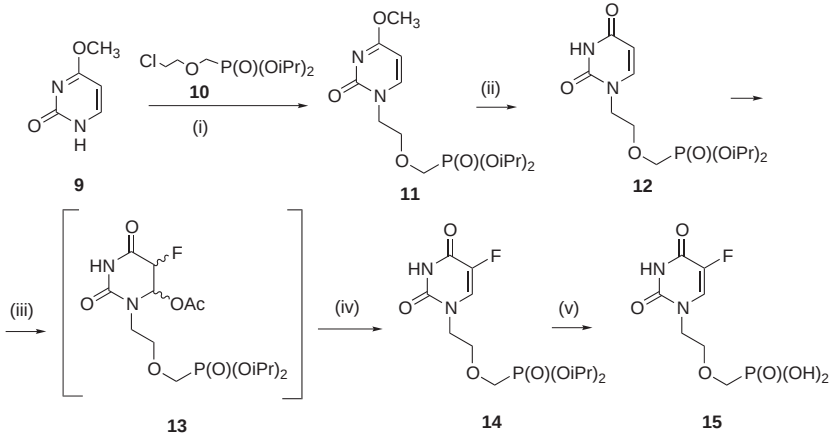


(i) H_2, Pd/C, 99% AcOH, MeOH; (ii) $CF_3(CF_2)_3SO_2F$, DBU, toluene, rt→90 °C;
(iii) $(CH_3)_3SiBr$, CH_3CN, r.t.

SCHEME 2

According to previous studies[6], a halogen atom in C-5 position of a uracil moiety can also significantly modify the inhibitory activity of thymidine phosphorylase. For example, the increased inhibition effect[2,7] of 6-amino-5-bromouracil ANPs compared with thymine derivatives can be mentioned. In addition, 5-halouracil derivatives also exhibit various kinds of biological activities[8,9]. Our effort was directed to investigate inhibitory potency toward thymidine phosphorylase of metabolically stable 5-fluorouracil ANP derivatives **15** and **21**.

Compound **15** was prepared by a multistep synthesis using direct fluorination of the uracil moiety in easily avalaible phosphonate **12** with F_2 diluted with nitrogen, in acetic acid solution (Scheme 3). The reaction course

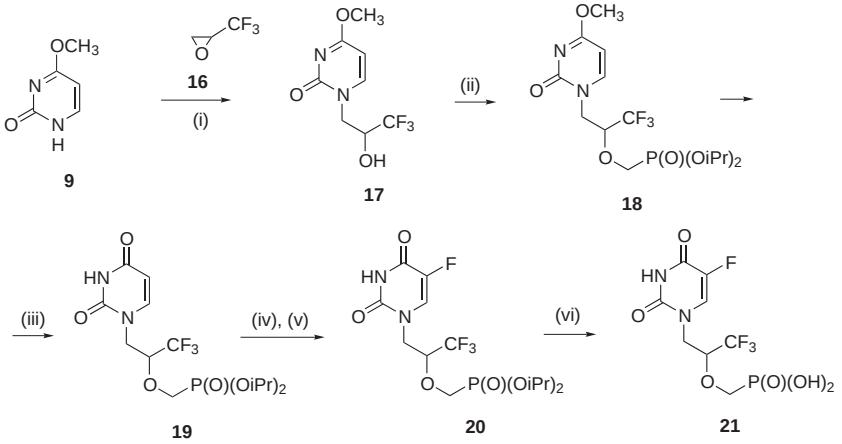


(i) NaH, DMF, 90 °C; (ii) Dowex 50 (H^+), 90% aq. MeOH, Δ;
(iii) 5-10% F_2/N_2, 99% AcOH; (iv) Et_3N, EtOH, reflux; (v) $(CH_3)_3SiBr$, CH_3CN, r.t.

SCHEME 3

may be assumed to include the primary addition of hardly formed CH_3COOF to 5,6-double bound of base[10] to form intermediate adducts **13**, which completely converted to **14** by treatment with triethylamine. The required compound **12** was obtained by reaction of 4-methoxypyrimidin-2(1*H*)-one[11] **9** with diisopropyl [(2-chloroethoxy)methyl]phosphonate (**10**) in the presence of sodium hydride followed by hydrolysis of compound **11** in aqueous methanol using Dowex 50 (H^+ form).

Fluorination of a heterocyclic base was further used in the synthesis of compound **21** bearing three additional fluorine atoms in the side chain (Scheme 4). For preliminary biological studies we developed the synthesis of only for racemic tetrafluorophosphonate **21** starting from commercially available (trifluoromethyl)oxirane (**16**). Its reaction with 4-methoxypyrimidin-2(1*H*)-one (**9**) in the presence of cesium carbonate afforded hydroxyl derivative **17** which was further alkylated with (diisopropoxyphosphoryl)methyl tosylate in the presence of sodium hydride. The methoxy group of the obtained phosphonate **18** was removed by hydrolysis on a Dowex 50 (H^+ form) to give **19** which was fluorinated under usual conditions[12].

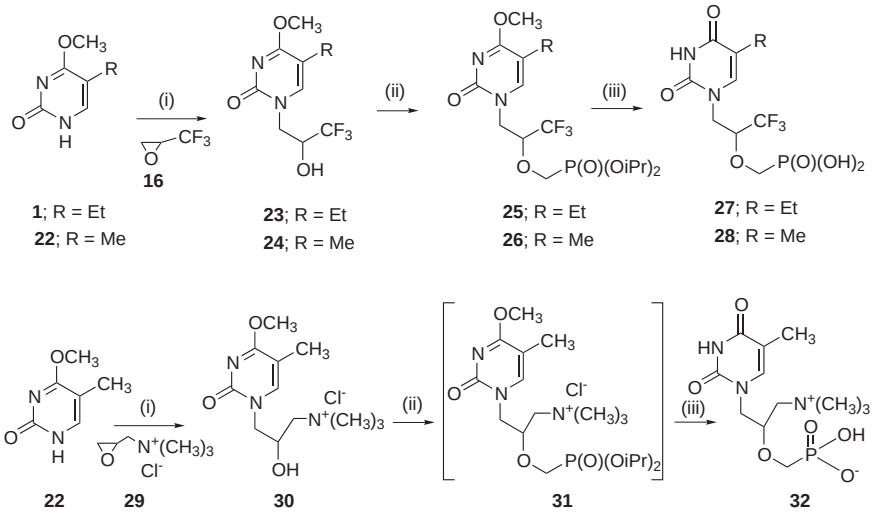


(i) Cs_2CO_3, DMF, 80 °C; (ii) $TsOCH_2P(O)(OiPr)_2$, NaH, THF, -20 °C-r.t.; (iii) Dowex 50 (H^+), 90% aq. MeOH; (iv) 5-10% F_2/N_2, 99% AcOH; (v) Et_3N, EtOH, reflux; (vi) $(CH_3)_3SiBr$, CH_3CN, r.t.

SCHEME 4

A similar procedure has used to synthesize phosphonates **27** and **28** containing thymine or 5-ethyluracil moiety (Scheme 5). The preparation of bulky quaternary (trimethylammonio)methyl derivative **32** followed the same route[13] using commercially avalaible glycidyltrimethylammonium

chloride (**29**) as a starting compound. The separation of the product from intermediate **31** by ion exchange chromatography on a Dowex 50 (H^+ form) afforded compound **32**.

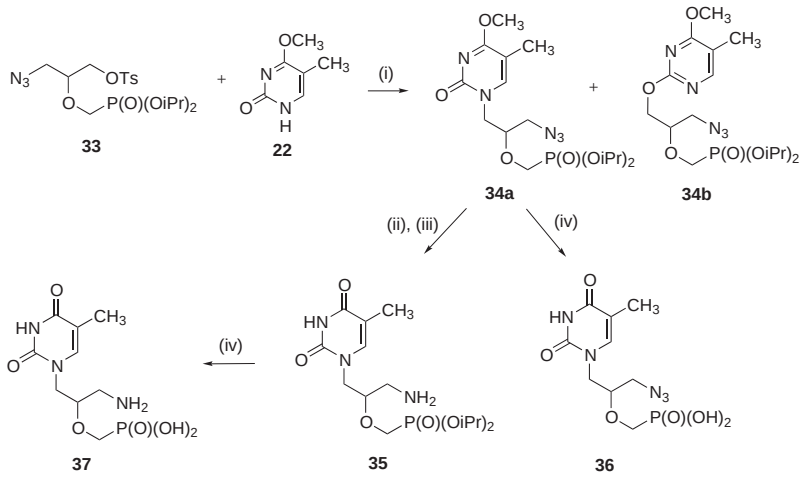


(i) Cs_2CO_3, DMF, 80 °C; (ii) $TsOCH_2P(O)(OiPr)_2$, NaH, THF, -20 °C-r.t.; (iii) $(CH_3)_3SiBr$, CH_3CN, rt

SCHEME 5

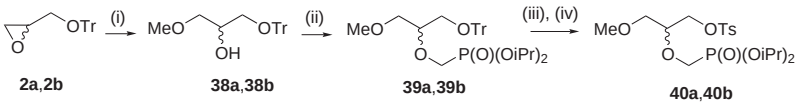
For the preparation of racemic azido and amino derivatives **36** and **37** we used phosphonate **33**, which was easily obtained by a previously reported multistep synthesis[13] starting from oxirane **2** (Scheme 6). The alkylation of 4-methyl-5-methoxypyrimidin-2(1*H*)-one (**22**) with **33** proceeded smoothly in the presence of sodium hydride. Separation of N^1- and O^2-regioisomers by column chromatography gave a mixture of **34a** and **34b** in the ratio 43:57. *N*-1-Alkylated regioisomer **34a** was then converted to the corresponding amino derivative **35** by hydrogenation in methanol over 10% palladium on charcoal followed by deprotection of the 4-OH group in aqueous methanol using ion exchanger resin Dowex 50 (H^+ form).

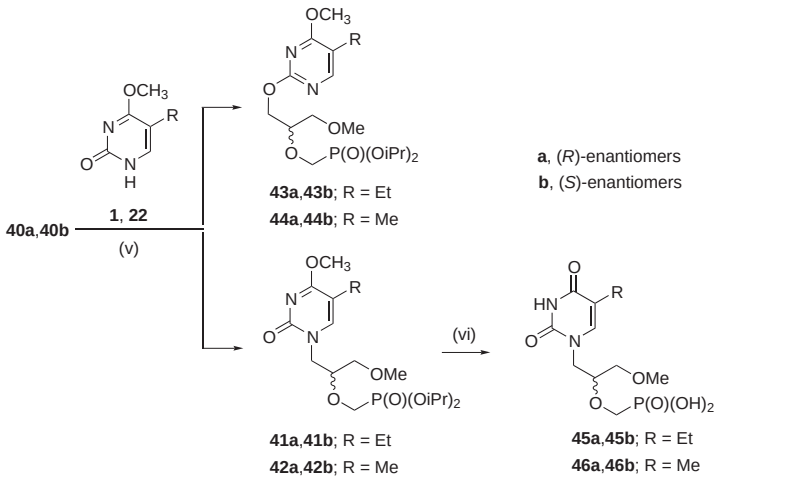
Likewise, methoxy derivatives **45a**, **45b** and **46a**, **46b** were prepared from chiral precursors **40a** and **40b** (Scheme 7). Similarly to the previously reported procedure[14] using azido derivative **33** or the other related compounds[15], we synthesized the new phosphonate building blocks from chiral oxiranes **2a** and **2b**. Their reaction with a sodium methoxide in the presence of cesium carbonate afforded quantitatively **38a** and **38b**. The alkylation of obtained derivatives proceeded by usual reaction with a



(i) NaH, DMF, 90 °C; (ii) H_2, Pd/C, MeOH, r.t.; (iii) Dowex 50 (H^+), 90% aq. MeOH; (iv) $(CH_3)_3SiBr$, CH_3CN, r.t.

SCHEME 6





(i) MeONa, Cs_2CO_3, DMF, 95 °C; (ii) NaH, $TsOCH_2P(O)(OiPr)_2$, THF, -20 °C-40 °C; (iii) Dowex 50 (H^+), MeOH, H_2O, reflux; (iv) TsCl, Et_3N, DMAP, CH_2Cl_2; 0°C-reflux; (v) NaH, DMF, 100 °C; (vi) $(CH_3)_3SiBr$, CH_3CN, r.t.

SCHEME 7

phosphonate building block to form **39a** and **39b**. Desired phosphonates **40a** and **40b** were obtained by removal of trityl group and further tosylation of HO intermediates with tosyl chloride and triethylamine in the presence of 4-(dimethylamino)pyridine in good preparative yields. Alkylation of protected pyrimidin-2(1*H*)-ones **1** and **22** in N^1- and O^2-positions with synthons **40a** and **40b** gave chiral (*R*)- and (*S*)-derivatives of **41**, **42** and **43**, **44**. After separation of both regioisomers N^1-derivatives were deprotected as well as compounds **15**, **21**, **27**, **28**, **32**, **36**, **37**, **41a**, **41b** and **42a**, **42b** with bromotrimethylsilane and hydrolysis.

The structures of all prepared compounds were confirmed by NMR spectroscopy with complete proton and carbon assignment using H,C-heterocorrelated experiments (HSQC and HMBC). The N^1- and O^2-regioisomers can be easily distinguished based on their ^{13}C and HMBC spectra. Table I shows characteristic chemical shifts for N^1- and O^2-regioisomers, wherein ^{13}C chemical shift of CH_2-1′ fragment is particularly noteworthy. H,C-HMBC spectra of N^1-isomers show crosspeaks of H-1′ to C-6 and C-2, while spectra of O^2-isomers show crosspeaks of H-1′ to only C-2. The optical purity of selected enantiomeric products was checked by ^1H NMR measurement of dynamic complexes with chiral solvating agent (–)-(*R*)-1-(9-anthryl)-2,2,2-trifluoroethan-1-ol (TFAE)[16] in $CDCl_3$ and compared with the diastereomeric mixtures of corresponding racemic compounds (Fig. 2). The optical purity was >95% ee in all cases.

In conclusion, the series of thymine and 5-ethyluracil (phosphonomethoxy)propyl derivatives substituted at the C-3′ position of the aliphatic chain with several subtituents were evaluated for their ability to inhibit thymidine phosphorylase (TP) from rat spontaneous T-cell lymphoma,

TABLE I
Characteristic chemical shifts (in ppm) for N^1- and O^2-regioisomers

Compound (isomer)	H-1′	C-1′	C-2	C-6
34a (N^1)	3.86, 4.11	50.97	156.74	145.64
41 (N^1)	3.77, 4.19	50.96	156.67	145.06
42 (N^1)	3.73, 4.20	50.84	156.77	145.92
34b (O^2)	4.39, 4.48	65.66	163.03	156.92
43 (O^2)	4.41, 4.44	65.50	163.21	156.03
44 (O^2)	4.41, 4.44	66.56	163.25	156.99

human placenta, and commercial enzymes (human TP expressed in V79 cells, *E. coli*). Table II shows that the inhibitory effect of these substituted (phosphonomethoxy)alkyl derivatives on T-cell lymphoma TP decreases according to the substitution in side chain of the (phosphonomethoxy)alkyl linker at the position C-3′ in the order: $-CH_2F > -CH_2OCH_3 > -CH_2N_3 > -CF_3 > -CH_2N^+(CH_3)_3 > -CH_2OH > -CH_2NH_2$ with one exception to this rule: Compound **5a** is the same or even stronger inhibitor of the enzyme than compound **8**, which inhibits T-cell lymphoma TP with higher efficiency than (*R*)-FPMPT ($V_i/V_0 = 0.11$)[3] and possesses also some, though low, inhibitory activity towards human enzymes ($V_i/V_0 \sim 0.77$). Very efficient inhibitors of T-cell lymphoma TP are also compounds **46a**, **45a**, **36**, **45b**, **46b** and **28**. A comparison of the inhibitory activity of compounds **46a**, **45a**, **45b**, **46b** shows the crucial effect of the side-chain substitution with $-CH_2OCH_3$ group. Substitution of the uracil ring at position 5 with methyl and/or ethyl group in both (*R*)- and (*S*)-configurations displays only a marginal effect. The inhibitory potency of 5-fluorouracil derivatives **15** and **21** is low compared with the synthesized thymine and 5-ethyluracil derivatives.

As in our previous study[3,4], the [(phosphonomethoxy)alkyl] thymines are not efficient inhibitors of *E. coli* and human TPs. These differences in the recognition of active sites of rat T-cell lymphoma by [(phosphonomethoxy)-

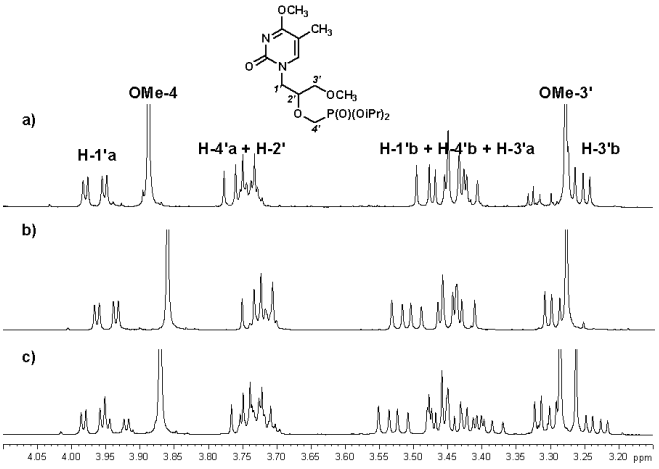


FIG. 2

Optical purity determination of the compounds **42a** and **42b**. a Part of ^{1}H NMR spectrum of the dynamic complex **42a** + TFAE; b part of ^{1}H NMR spectrum of the dynamic complex **42b** + TFAE; c part of ^{1}H NMR spectrum of the dynamic complex **42a** + **42b** + TFAE showing diastereomeric mixture (racemic mixture was obtained by mixing of **42a** and **42b** in ~2:1 ratio)

alkyl]thymines, compared with human and *E. coli* TP, could be the result of some mutation or post-translation modification.

Despite their TP inhibitory activity, any of the presented compounds do not possess at a concentration of 10 μmol l^{-1}, a significant cytostatic activity in tissue cultures estimated in mouse lymphocytic leukemia L1210 cells (ATCC CCL 219), CCRF-CEM T lymphoblastoid cells (human acute lymphoblastic leukemia, ATCC CCL 119), human promyelocytic leukemia HL-60 cells (ATCC CCL 240) and human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2).

TABLE II
Inhibition of thymidine phosphorylases by ANPs

Compound	Inhibition of thymidine phosphorylase[a], V_i/V_0			
	Escherichia coli	Human, V79 expressed	SD-Lymphoma	Human placenta
5a	0.94	0.86	0.01	0.94
5b	0.95	0.87	0.16	0.94
8	0.88	0.77	0.02	0.79
15	0.94	0.96	0.90	0.81
21	1.00	0.93	0.57	1.00
27	0.83	1.00	0.17	0.95
28	0.93	0.76	0.12	1.00
32	0.96	0.96	0.14	0.95
36	0.97	1.00	0.06	1.00
37	1.00	0.93	0.50	1.00
45a	1.00	0.96	0.04	0.99
45b	0.84	1.00	0.10	1.00
46a	1.00	0.96	0.04	1.00
46b	1.00	0.94	0.10	0.90

[a] 100 μM [^{3}H]-2′-deoxythymidine, 250 μM P_i (pH 6.7), tested compound 10 μmol l^{-1}, an appropriate amount of enzyme, 10 min incubation at 37 °C (refs[4,17]). The inhibitory efficacy is expressed by V_i/V_0 (V_i, rate of phosphorolysis in the presence of inhibitors; V_0, rate of phosphorolysis in the absence of inhibitors). P_i, inorganic phosphate

EXPERIMENTAL

Unless stated otherwise, solvents were evaporated at 40 °C/0.5–2 kPa and compounds were dried at 50 °C/13 Pa. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Autopol IV polarimeter (Rudolph Research Analytical, U.S.A.) at 25 °C; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a FTIR spectrometer Bruker IFS 55 (Equinox) in KBr, CCl_4 and $CHCl_3$. Analytical TLC was carried out on Silufol UV_{254} plates (Kavalier Votice, Czech Republic). Column chromatography was performed on neutral aluminum oxide 150 mesh 58 Å with 3% of water (Aldrich) and silica gel 60 μm (Fluka). Preparative TLC was carried out on 45 × 18 × 0.4 cm loose-layer silica gel containing UV indicator (system S1). Purification of phosphonates **5a**, **5b**, **15**, **21**, **27**, **28**, **32**, **36**, **37**, **45a**, **45b** and **46a**, **46b** was performed on Sephadex A-25-120 DEAE (Cl^- form, activated with 0.02 M triethylammonium hydrogencarbonate) and eluted in 0–0.4 M triethylammonium hydrogencarbonate buffer (TEAB) (system S2). Reversed-phase HPLC was performed on a Waters Delta 600; Xterra®, analytical RP_{18} column 5 μm 3.9 × 150 mm, 0.05 M TEAB (system S3) or 0.025 M TEAB (system S4) or 0.0025 M (system S5) and Luna Phenomenex®, preparative C_{18} column 21.20 × 250 mm, 0.025 M TEAB (system S6). NMR spectra were recorded on Bruker Avance 400, Bruker Avance 500, Varian Unity 200 or Varian Unity 500 spectrometers (^{1}H at 400 or 500, ^{13}C at 100.6 or 125.8 , ^{31}P at 162 and ^{19}F at 188.2 or 470.2 MHz) in $CDCl_3$ with internal standard tetramethylsilane , in DMSO-d_6 solutions (referenced to the solvent signal at δ 2.50) or in D_2O solutions with dioxane[18] as an internal standard (3.75 ppm for ^{1}H and and 69.3 ppm for ^{13}C). ^{31}P and ^{19}F NMR spectra were referenced to the signal of H_3PO_4 (0 ppm) and hexafluorobenzene (–163 ppm), respectively, which were used as an internal standards or as an external standards in coaxial 2 mm capillary tube. Chemical shifts (δ, ppm) and coupling constants (*J*, Hz) were obtained by first-order analysis of the spectra. The numbering system for assignment of NMR signals is outlined in Fig. 3. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (Xe ionization, accelerating voltage 8 kV, glycerol matrix) and Q-Tof micro (Waters, Milford, MA, U.S.A.) equipped with an ion source for atmospheric pressure chemical ionization (APCI probe temperature 400 °C, sample cone voltage 30 V, corona discharge current 5.0 μA).

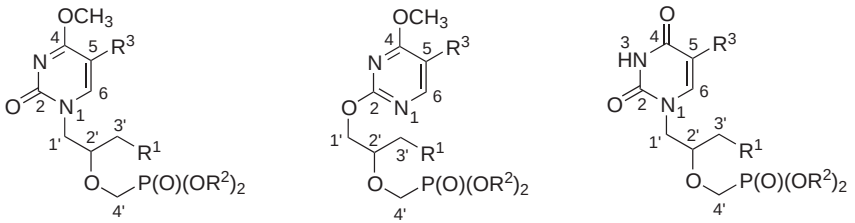


FIG. 3
General numbering scheme for assignment of NMR signals

Materials and Chemicals

Standard chemicals, ion-exchange resin (Dowex 50WX8-200) and activated charcoal were purchased from Sigma–Aldrich (Czech Republic). Ion-exchange resin (Sephadex A-25-120 DEAE) was purchased from Fluka. Perfluorobutane-1-sulfonyl fluoride was obtained from Fluorochem (Wesley Street, Old Glossop). (Trifluoromethyl)oxirane was purchased from Tokyo Kasei Kogyo Co, Ltd. (Toshima, Kita-ku Tokyo, Japan). Fluorination of uracil derivatives **12** and **19** was performed with 5–10% mixture of fluorine and nitrogen (Messer Technogas). 4-Methoxy-2-pyrimidin-2(1*H*)-ones **1**, **9** and **22** were prepared from relevant uracils as described in refs[5,11]. Oxiranes **2a** and **2b** were obtained from Daiso Co, Ltd. (Japan). (Diisopropoxyphosphoryl)methyl tosylate was synthesized as described in ref.[19], diisopropyl [(2-chloroethoxy)methyl]phosphonate (**10**) was obtained according to ref.[20]. Diisopropyl ({[1-azido-3-(tosyloxy)propan-2-yl]oxy}methyl)phosphonate (**33**) was prepared from racemic glycidyl trityl ether according to ref.[14]. Dimethylformamide, tetrahydrofuran, and acetonitrile were dried by distillation from calcium hydride and stored over molecular sieves (4Å).

Enzyme Assay

The standard reaction mixture (50 μl) contained 20 μM bis(Tris-HCl) pH 6.4, 1 mM EDTA and 2 mM DTT, 100 μM [^{3}H-methyl]thymidine, 250 μM potassium phosphate pH 6.7, tested compound 10 μmol l^{-1} and 25.5 pU of enzyme according to ref.[4]. The reaction was carried out at 37 °C for 10 min and stopped by spotting a 2 μl aliquot onto Silica gel 60 F254 plate that had been prespotted with 0.01 μmol of each thymine and thymidine. The plate was developed in the non-aqueous phase of the solvent system ethyl acetate–water–formic acid (60:35:5). The spots were visualised under UV light (254 nm) and cutted out for radioactivity determination in the toluene-based scintillation cocktail.

Synthesis of 5-Ethyl-4-methoxypyrimidin-2(1*H*)-ones **3a** and **3b**. General Procedure

A mixture of 5-ethyl-4-methoxypyrimidin-2(1*H*)-one (**1**; 500 mg, 3.2 mmol), [(trityloxy)-methyl]oxirane **2a** or **2b** (1.03 g, 3.2 mmol) and cesium carbonate (106 mg, 032 mmol) in dimethylformamide (40 ml) was heated at 90 °C for 10 h. The mixture was concentrated in vacuo to a minimum volume. The residue was codistilled with toluene (2 × 10 ml) and chromatographed on neutral aluminum oxide (toluene followed by ethyl acetate and ethyl acetate–ethanol 10:1). The fractions containing compounds **3a** or **3b** were evaporated to dryness in vacuo.

5-Ethyl-1-[(R)-2-hydroxy-3-(trityloxy)propyl]-4-methoxypyrimidin-2(1H)-one (**3a**). Yield 871 mg (59%) of a white foam. IR (CCl_4), ν_{max}: 3357, 1663, 1638, 1537, 1472, 1401, 1368, 1335, 1099, 633. $[\alpha]_D$ +43.8 (*c* 0.195 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{29}H_{31}N_2O_4$ calculated 471.2284, found 471.2270. For $C_{29}H_{30}N_2O_4$ (470.6) calculated: 74.02% C, 6.43% H, 5.95% N; found: 74.07% C, 6.49% H, 5.63% N. FAB MS, *m/z*: 471 $[MH]^+$ (4), 243 (100), 211 (15), 197 (15), 165 (38), 155 (11), 105 (10), 77 (9). ^{1}H NMR (500 MHz, DMSO-d_6): 1.00 (t, 3 H, J_{vic} = 7.4, C**H**$_3$CH$_2$); 2.23 (q, 2 H, J_{vic} = 7.4, CH$_3$C**H**$_2$CH$_3$); 2.90 (dd, 1 H, J_{gem} = 9.4, $J_{3b',2'}$ = 5.4, H-3′b); 2.97 (dd, 1 H, J_{gem} = 9.4, $J_{3'a,2'}$ = 5.0, H-3′a); 3.58 (dd, 1 H, J_{gem} = 12.9, $J_{1'b,2'}$ = 8.3, H-1′b); 3.83 (s, 3 H, OCH_3); 3.99 (m, 1 H, H-2′); 4.05 (dd, 1 H, J_{gem} = 12.9, $J_{1'a,2'}$ = 4.0, H-1′a); 5.27 (d, 1 H, $J_{OH,2'}$ = 5.9, OH-2′); 7.26 (m, 3 H, H-*p*-Ph); 7.33 (m, 6 H, H-*m*-Ph); 7.42 (m, 6 H, H-*o*-Ph); 7.60 (s, 1 H, H-6). ^{13}C NMR (125.8 MHz, DMSO-d_6): 13.28 (**C**H$_3$CH$_2$); 19.20

(CH_2CH_3); 53.16 (CH_2-1′); 53.95 (OCH_3); 66.25 (CH_2-3′); 67.08 (CH-2′); 86.03 (C-Tr); 109.60 (C-5); 127.16 (CH-*p*-Ph); 128.02 (CH-*m*-Ph); 128.42 (CH-*o*-Ph); 143.69 (C-*i*-Ph); 146.82 (CH-6); 155.56 (C-2); 169.79 (C-4).

5-Ethyl-1-[(S)-2-hydroxy-3-(trityloxy)propyl]-4-methoxypyrimidin-2(1H)-one (**3b**). Yield 944 mg (64%) of a white foam. ^{1}H, ^{13}C NMR, $[\alpha]_D$ and IR data were identical with those of compound reported in ref.[5].

Synthesis of 5-Ethyl-4-methoxypyrimidin-2(1*H*)-ones **4a** and **4b**. General Procedure

A mixture of compound **3a** or **3b** (787 mg, 1.7 mmol), (diisopropoxyphosphoryl)methyl tosylate (667 mg, 1.9 mmol) and 60% sodium hydride dispersion (103 mg, 2.6 mmol) in tetrahydrofuran (25 ml) was stirred at –20 °C. The suspension was allowed to warm to 20–40 °C for 2 h and stirred at room temperature overnight. The mixture was concentrated in vacuo and the residue was diluted with chloroform (10 ml). After cooling the mixture was evaporated to dryness. The residue was filtered on neutral aluminum oxide (ethyl acetate and followed by ethyl acetate–chloroform–methanol 21:20:1). The crude product was purified by chromatography on preparative TLC plate (S1, ethyl acetate–chloroform–methanol 21:20:1). The fractions containing product **4a** or **4b** were evaporated to dryness in vacuo.

1-{(R)-[2-(Diisopropoxyphosphoryl)methoxy]-3-(trityloxy)propyl}-5-ethyl-4-methoxypyrimidin-2(1H)-one (**4a**). Yield 363 g (32%) of a white foam. IR (CCl_4), ν_{max}: 1674, 1653, 1536, 1471, 1403, 1386, 1331, 1256, 1007, 706. $[\alpha]_D$ +35.1 (*c* 0.362 g/100 ml, $CHCl_3$). HR MS (ESI): for $C_{36}H_{46}N_2O_7P$ calculated 649.3043, found 649.3059. ESI MS, *m/z*: 649 $[MH]^+$ (7). ^{1}H NMR (400 MHz, $CDCl_3$): 1.10 (t, 3 H, J_{vic} = 7.4, C**H**$_3$CH$_2$); 1.25, 1.26, 1.28 and 1.30 (4 × d, 4 × 3 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 2.31 (q, 2 H, J_{vic} = 7.4, C**H**$_2$CH$_3$); 3.10 (dd, 1 H, J_{gem} = 10.7, $J_{3b',2'}$ = 6.5, H-3′b); 3.40 (dd, 1 H, J_{gem} = 10.7, $J_{3'a,2'}$ = 3.4, H-3′a); 3.55 (dd, 1 H, J_{gem} = 13.4, $J_{H,P}$ = 10.2, H-4′b); 3.80 (dd, 1 H, J_{gem} = 13.6, $J_{1'b,2'}$ = 8.2, H-1′b); 3.85 (dd, 1 H, J_{gem} = 13.4, $J_{H,P}$ = 8.7, H-4′a); 3.91 (dq, 1 H, $J_{2',1'}$ = 8.2, 3.7, $J_{2',3'}$ = 4.0, 3.4, H-2′); 3.96 (s, 3 H, OCH_3); 4.27 (dd, 1 H, J_{gem} = 13.6, $J_{1'a,2'}$ = 3.7, H-1′a); 4.67 and 4.70 (2 × dh, 2 × 1 H, $J_{H,P}$ = 7.7, J_{vic} = 6.2, C**H**(CH$_3$)$_2$); 7.21–7.33 (m, 10 H, H-6 + H-*m*,*p*-Ph); 7.44 (m, 6 H, H-*o*-Ph). ^{13}C NMR (100.6 MHz, $CDCl_3$): 12.96 (CH_3CH_2); 19.75 (CH_2CH_3); 23.93, 24.00 and 24.04 (d, $J_{C,P}$ = 4, $(CH_3)_2CH$); 51.55 (CH_2-1′); 54.38 (OCH_3); 62.37 (CH_2-3′); 64.90 (d, $J_{C,P}$ = 169, CH_2-4′); 70.79 and 70.97 (d, $J_{C,P}$ = 6, $CH(CH_3)_2$); 78.96 (d, $J_{C,P}$ = 13, CH-2′); 86.82 (C-Tr); 109.71 (C-5); 127.16 (CH-*p*-Ph); 127.91 (CH-*m*-Ph); 128.60 (CH-*o*-Ph); 143.54 (C-*i*-Ph); 145.17 (CH-6); 158.56 (C-2); 170.59 (C-4).

1-{(S)-[2-(Diisopropoxyphosphoryl)methoxy]-3-(trityloxy)propyl}-5-ethyl-4-methoxypyrimidin-2(1H)-one (**4b**). Yield 363 mg (32%) of a white foam. ^{1}H, ^{13}C NMR, $[\alpha]_D$ and IR data were identical with those of compound reported in ref.[5]. The procedure was repeated to obtain 8.6 g of **4a** which was further utilized for the preparation of **8**.

Synthesis of 5-Ethyluracils **5a** and **5b**. General Procedure

A mixture of compound **4a** or **4b** (302 mg, 0.5 mmol), acetonitrile (10 ml) and bromotrimethylsilane (0.7 ml) was stirred overnight at room temperature. The mixture was concentrated in vacuo and then codistilled with water (2 × 2 ml). The residue in water (10 ml) was heated with Dowex 50X8 (H^+ form; 2 ml) at 100 °C for 1 h. The mixture was cooled to room temperature and then filtered. The filtrate was concentrated in vacuo and purified on 40 ml of DEAE-Sephadex (S2) with subsequent deionization on activated charcoal. The relevant fractions were combined, evaporated in vacuo and codistilled with water (3 × 5 ml).

The residue was dissolved in water (3 ml), applied onto a column of Dowex 50X8 (Li^+ form; 30 ml) and then the column was washed with water. The appropriate UV absorbing fraction containing product **5a** or **5b** was evaporated to dryness in vacuo. The residue was dissolved in water and lyophilized. The following compounds were obtained as dilithium salts:

5-Ethyl-1-[(R)-3-hydroxy-2-(phosphonomethoxy)propyl]uracil (**5a**). Yield 129 g (87%) of a white solid, b.p. > 300 °C. IR (KBr), ν_{max}: 3418, 3260, 1684, 1471, 1462, 1440, 1076, 994, 918. $[\alpha]_D$ +31.7 (*c* 0.365 g/100 ml, $CHCl_3$). HPLC, 99% (S3). HR MS (FAB): for $C_{10}H_{16}Li_2N_2O_7P$ calculated 321.1015, found 321.1011. FAB MS, *m/z*: 321 $[MH]^+$ (9). ^{1}H NMR (400 MHz, D_2O, ref(dioxane) 3.75 ppm): 1.09 (t, 3 H, J_{vic} = 7.5, C**H**$_3$CH$_2$); 2.31 (qd, 2 H, J_{vic} = 7.5, $J_{CH2,6}$ = 1.0, CH$_3$C**H**$_2$); 3.56 (dd, 1 H, J_{gem} = 12.2, $J_{3'b,2'}$ = 4.7, H-3′b); 3.60 (dd, 1 H, J_{gem} = 12.7, $J_{H,P}$ = 9.4, H-4′b); 3.64 (dd, 1 H, J_{gem} = 12.7, $J_{H,P}$ = 9.1, H-4′a); 3.73 (m, 1 H, H-2′); 3.79 (dd, 1 H, J_{gem} = 12.2, $J_{3'a,2'}$ = 3.4, H-3′a); 3.87 (dd, 1 H, J_{gem} = 14.4, $J_{1'b,2'}$ = 6.8, H-1′b); 3.94 (dd, 1 H, J_{gem} = 14.4, $J_{1'a,2'}$ = 4.9, H-1′a); 7.49 (t, 1 H, $J_{6,CH2}$ = 1.0, H-6). ^{13}C NMR (100.6 MHz, D_2O, ref(dioxane) 69.3 ppm): 12.66 (**C**H$_3$CH$_2$); 19.97 (**C**H$_3$CH$_2$); 49.19 (CH$_2$-1′); 61.09 (CH$_2$-3′); 67.80 (d, $J_{C,P}$ = 154, CH$_2$-4′); 80.50 (d, $J_{C,P}$ = 11, CH-2′); 116.91 (C-5); 144.01 (CH-6); 153.00 (C-2); 167.29 (C-4). ^{31}P NMR (162 MHz, D_2O): 15.05 (t, *J* = 9.4, 9.1).

5-Ethyl-1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]uracil (**5b**). Yield 100 mg (67%) of a white solid, m.p. > 300 °C. HPLC, 99% (S3). $[\alpha]_D$ –30.5 (*c* 0.414 g/100 ml, H_2O). FAB MS, *m/z*: 321 $[MH]^+$ (15). ^{1}H, ^{13}C NMR, HR MS and IR data were identical with those of compound **5a**.

1-{(*S*)-2-[(Diisophosphoryl)methoxy]-3-hydroxypropyl}-5-ethyl-4-methoxypyrimidin-2(1*H*)-one (**6**)

Compound **4a** (8.6 g, 13.3 mmol) in methanol (125 ml) and glacial acetic acid (4 ml) was hydrogenated over 10% palladium on charcoal (0.7 g) at room temperature for 32 h until the conversion of starting phosphonate to **6** was complete (TLC in chloroform–methanol 25:1). The mixture was then neutralized with solid sodium hydrogencarbonate and filtered through a Celite pad. The filtrate was concentrated to a minimum volume. The residue was chromatographed on neutral aluminum oxide (chloroform followed by chloroform–methanol 25:1). Yield 2.9 g (54%) of **6** as a colorless oil. IR (CCl_4), ν_{max}: 3406, 1674, 1663, 1641, 1536, 1472, 1403, 1386, 1375, 1375, 1254, 1011, 993. $[\alpha]_D$ –7.3 (*c* 0.109 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{17}H_{32}N_2O_7P$ calculated 407.1947, found 407.1933. FAB MS, *m/z*: 407 $[MH]^+$ (100). ^{1}H NMR (400 MHz, $CDCl_3$): 1.14 (t, 3 H, J_{vic} = 7.5, C**H**$_3$CH$_2$); 1.31, 1.33 and 1.34 (3 × d, 12 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 2.37 (qd, 2 H, J_{vic} = 7.5, $J_{CH2,6}$ = 1.0, C**H**$_2$CH$_3$); 3.52–3.65 (m, 2 H, H-3′); 3.76 (dd, 1 H, J_{gem} = 14.0, $J_{H,P}$ = 8.8, H-4′b); 3.80 (dd, 1 H, J_{gem} = 14.0, $J_{H,P}$ = 7.9, H-4′a); 3.84 (m, 1 H, H-2′); 3.99 (dd, 1 H, J_{gem} = 14.0, $J_{1'b,2'}$ = 3.9, H-1′b); 4.00 (s, 3 H, OCH$_3$); 4.13 (dd, 1 H, J_{gem} = 14.0, $J_{1'a,2'}$ = 5.7, H-1′a); 4.29 (dd, 1 H, $J_{OH,3'}$ = 8.9, 5.7, OH-3′); 4.71 and 4.77 (2 × dh, 2 × 1 H, $J_{H,P}$ = 7.6, J_{vic} = 6.2, C**H**(CH$_3$)$_2$); 7.35 (t, 1 H, $J_{6,CH2}$ = 1.0, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): 13.07 (**C**H$_3$CH$_2$); 19.71 (**C**H$_2$CH$_3$); 23.99 and 24.05 (d, $J_{C,P}$ = 4, (**C**H$_3$)$_2$CH); 50.05 (CH$_2$-1′); 54.56 (OCH$_3$); 59.63 (CH$_2$-3′); 64.88 (d, $J_{C,P}$ = 170, CH$_2$-4′); 71.43 (d, $J_{C,P}$ = 7, **C**H(CH$_3$)$_2$); 80.57 (d, $J_{C,P}$ = 8, CH-2′); 110.46 (C-5); 145.18 (CH-6); 157.51 (C-2); 170.82 (C-4).

1-{(*S*)-2-[(Diisopropoxyphosphoryl)methoxy]-3-fluoropropyl}-5-ethyl-4-methoxypyrimidin-2(1*H*)-one (**7**)

A mixture of 1,8-diazabicyclo[5.4.0]undec-7-ene (1.3 g, 8.5 mmol) and 92% perfluorobutane-1-sulfonyl fluoride (3 g, 9.1 mmol) was stirred in toluene (15 ml) at room temperature for 10 min. A solution of compound **6** (2.6 g, 6.4 mmol) in toluene (30 ml) was added and the resulting mixture was stirred for 8 h at room temperature and then heated at 80 °C for 1 h until the conversion of starting phosphonate to **7** was complete (TLC in chloroform–methanol 25:1). The mixture was concentrated in vacuo and filtered over neutral aluminum oxide (chloroform followed by chloroform–methanol 25:1). After evaporation of solvents the residue was chromatographed on preparative TLC plate (S1, chloroform–methanol 15:1). Yield 792 mg (30%) of a colorless oil. IR (CCl_4), ν_{max}: 1674, 1651, 1536, 1471, 1403, 1386, 1376, 1255, 1106, 1007, 992. $[\alpha]_D$ –45.0 (*c* 0.549 g/100 ml, $CHCl_3$). For $C_{17}H_{30}FN_2O_6P$ (408.4) calculated: 50.00% C, 7.40% H, 4.65% F, 6.86% N, 7.58% P; found: 49.84% C, 7.49% H, 4.90% F, 6.69% N, 7.64% P. FAB MS, *m/z*: 409 $[MH]^+$ (100). ^{1}H NMR (500 MHz, $CDCl_3$): 1.15 (t, 3 H, J_{vic} = 7.5, C**H**$_3$CH$_2$); 1.29, 1.31 and 1.32 (4 × d, 4 × 3 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 2.37 (m, 2 H, C**H**$_2$CH$_3$); 3.71 (dd, 1 H, J_{gem} = 13.6, $J_{H,P}$ = 9.4, H-4′b); 3.81 (dd, 1 H, J_{gem} = 13.8, $J_{1'b,2'}$ = 7.5, H-1′b); 3.89 (dd, 1 H, J_{gem} = 13.6, $J_{H,P}$ = 8.8, H-4′a); 3.99 (s, 3 H, OCH_3); 4.05 (m, 1 H, H-2′); 4.20 (dd, 1 H, J_{gem} = 13.8, $J_{1'a,2'}$ = 3.8, H-1′a); 4.45 (ddd, 1 H, $J_{H,F}$ = 47.3, J_{gem} = 10.7, $J_{3'b,2'}$ = 4.6, H-3′b); 4.71 (m, 2 H, C**H**(CH$_3$)$_2$); 4.72 (ddd, 1 H, $J_{H,F}$ = 47.3, J_{gem} = 10.7, $J_{3'a,2'}$ = 2.5, H-3′a); 7.32 (t, 1 H, $J_{6,CH2}$ = 1.0, H-6). ^{13}C NMR (125.8 MHz, $CDCl_3$): 13.02 (**C**H$_3$CH$_2$); 19.73 (**C**H$_2$CH$_3$); 23.90, 23.94 and 24.03 (d, $J_{C,P}$ = 4, (**C**H$_3$)$_2$CH); 50.19 (d, $J_{C,F}$ = 9, CH$_2$-1′); 54.52 (OCH_3); 65.45 (d, $J_{C,P}$ = 169, CH$_2$-4′); 71.08 and 71.21 (d, $J_{C,P}$ = 7, **C**H(CH$_3$)$_2$); 78.42 (dd, $J_{C,F}$ = 18, $J_{C,P}$ = 11, CH-2′); 82.51 (d, $J_{C,F}$ = 173, CH$_2$-3′); 110.31 (C-5); 144.79 (CH-6); 156.60 (C-2); 170.83 (C-4). ^{19}F NMR (188.2 MHz, $CDCl_3$): –232.48 (td, $J_{F,H-3'}$ = 47.3, $J_{F,H-2'}$ = 25.1).

5-Ethyl-1-[(*S*)-3-fluoro-2-(phosphonomethoxy)propyl]uracil (**8**)

Compound **8** was obtained as dilithium salt by the same procedure reported for **5a** and **5b**.

A mixture of compound **7** (302 mg, 0.74 mmol) in acetonitrile (10 ml) and bromotrimethylsilane (1 ml) afforded 150 mg (59%) of **8** as a white solid, m.p. 249–250 °C. IR (KBr), ν_{max}: 3065, 1700, 1670, 1627, 1375, 1465, 1458, 1118, 1079, 996, 924, 583, 546. $[\alpha]_D$ –12.3 (*c* 0.141 g/100 ml, H_2O). HPLC, 99% (S3). HR MS (FAB): for $C_{10}H_{15}FLi_2N_2O_6P$ calculated 323.0971, found 323.0958. For $C_{10}H_{14}FLi_2N_2O_6P \cdot 1.3H_2O$ (345.5) calculated: 34.76% C, 4.78% H, 5.50% F, 8.12% N, 8.96% P; found: 35.03% C, 4.59% H, 5.31% F, 7.59% N, 8.61% P. FAB MS, *m/z*: 323 $[MH]^+$ (41). ^{1}H NMR (500 MHz, D_2O, ref(dioxane) 3.75 ppm): 1.09 (t, 3 H, J_{vic} = 7.5, C**H**$_3$CH$_2$); 2.31 (qd, 2 H, J_{vic} = 7.5, $J_{CH2,6}$ = 1.1, C**H**$_2$CH$_3$); 3.64 and 3.72 (2 × dd, 2 H, J_{gem} = 13.0, $J_{H,P}$ = 9.2, H-4′); 3.91-4.11 (m, 3 H, H-1′ and H-2′); 4.53 (ddd, 1 H, $J_{H,F}$ = 47.0, J_{gem} = 10.6, $J_{3'b,2'}$ = 3.6, H-3′b); 4.70 (ddd, 1 H, $J_{H,F}$ = 47.0, J_{gem} = 10.6, $J_{3'a,2'}$ = 3.2, H-3′a); 7.50 (t, 1 H, $J_{6,CH2}$ = 1.1, H-6). ^{13}C NMR (125.8 MHz, D_2O, ref(dioxane) 69.3 ppm): 12.83 (**C**H$_3$CH$_2$); 19.97 (**C**H$_2$CH$_3$); 48.49 (d, $J_{C,F}$ = 7, CH$_2$-1′); 67.64 (d, $J_{C,P}$ = 155, CH$_2$-4′); 78.31 (dd, $J_{C,F}$ = 18, $J_{C,P}$ = 11, CH-2′); 82.96 (d, $J_{C,F}$ = 168, CH$_2$-3′); 117.00 (C-5); 143.91 (CH-6); 152.97 (C-2); 167.32 (C-4). ^{19}F NMR (188.2 MHz, D_2O): –230.65 (td, $J_{F,H-3'}$ = 47.0, $J_{F,H-2'}$ = 26.6).

1-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}-4-methoxypyrimidin-2(1*H*)-one (**11**)

A mixture of compound **9** (4 g, 32 mmol) and 60% sodium hydride suspension (1.3 g, 33 mmol) in dimethylformamide (200 ml) was stirred at room temperature for 1 h. The compound **10** (10 g, 39 mmol) was added and the resulting mixture was stirred at room temperature for 2 h and then heated at 90 °C for 12 h. The mixture was evaporated and codistilled with toluene (50 ml). The residue was diluted in dichloromethane (300 ml) and filtered through a Celite pad. The residue was chromatographed on neutral aluminum oxide (ethyl acetate–methanol 20:1 followed by chloroform–methanol 25:1) and appropriate fractions were evaporated in vacuo to dryness. Yield 5.4 g (49%) of **11** as a white solid. IR (CCl_4), ν_{max}: 1678, 1638, 1543, 1483, 1415, 1386, 1369, 1257, 1011, 990. For $C_{14}H_{25}N_2O_6P$ (348.3) calculated: 48.27% C, 7.23% H, 8.04% N, 8.89% P; found: 48.05% C, 7.25% H, 7.87 N, 8.70% P. MS (FAB), *m/z*: 349 (100) $[MH]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): 1.29 and 1.32 (2 × d, 2 × 6 H, J_{vic} = 6.2, $(CH_3)_2CH$); 3.70 (d, 2 H, $J_{H,P}$ = 8.6, H-4′); 3.85 (dd, 2 H, $J_{2',1'}$ = 5.0 and 3.8, H-2′); 3.95 (s, 3 H, OCH_3); 4.05 (dd, 2 H, $J_{1',2'}$ = 5.0 and 3.8, H-1′); 4.71 (dh, 2 H, $J_{H,P}$ = 7.7, J_{vic} = 6.2, $CH(CH_3)_2$); 5.84 (d, 1 H, $J_{5,6}$ = 7.2, H-5); 7.52 (d, 1 H, $J_{6,5}$ = 7.2, H-6). ^{13}C NMR (125.8 MHz, $CDCl_3$): 23.99 and 24.04 (d, $J_{C,P}$ = 4, $(CH_3)_2CH$); 49.99 (CH_2-1′); 54.35 (OCH_3); 66.05 (d, $J_{C,P}$ = 169, CH_2-4′); 70.81 (d, $J_{C,P}$ = 12, CH_2-2′); 71.07 (d, $J_{C,P}$ = 7, $CH(CH_3)_2$); 94.97 (CH-5); 148.36 (CH-6); 156.50 (C-2); 171.90 (C-4).

1-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}uracil (**12**)

Compound **11** (5 g, 14.4 mmol) was dissolved in 90% aqueous methanol (100 ml). Dowex 50 (H^+ form; 20 ml) was added and the suspension was refluxed for 3.5 h until the conversion of starting phosphonate to **12** was complete (TLC in chloroform–methanol 25:1). The mixture was concentrated in vacuo, chromatographed on neutral aluminum oxide (chloroform–methanol 15:1) and appropriate fractions were evaporated in vacuo. The product was triturated with hexane. The solvent was decanted and the obtained product was dried in vacuo. Yield 4.4 g (91%) of **12** as a white solid. IR (CCl_4), ν_{max}: 3410, 3169, 3056, 1725, 1709, 1692, 1634, 1386, 1376, 1348, 1255, 1243, 1179, 1142, 1106, 1010, 992. For $C_{13}H_{23}N_2O_6P$ (334.1) calculated: 46.71% C, 6.93% H, 8.38% N, 9.27% P; found: 46.42% C, 7.00% H, 8.13% N, 9.34% P. FAB MS, *m/z*: 335 $[MH]^+$ (57). ^{1}H NMR (500 MHz, $CDCl_3$): 1.31 and 1.33 (2 × d, 2 × 6 H, J_{vic} = 6.2, $(CH_3)_2CH$); 3.73 (d, 2 H, $J_{H,P}$ = 8.4, H-4′); 3.82 (dd, 2 H, $J_{2',1'}$ = 5.2 and 3.9, H-2′); 3.95 (dd, 2 H, $J_{1',2'}$ = 5.2 and 3.9, H-1′); 4.73 (dh, 2 H, $J_{H,P}$ = 7.7, J_{vic} = 6.2, $CH(CH_3)_2$); 5.66 (d, 1 H, $J_{5,6}$ = 7.9, H-5); 7.35 (d, 1 H, $J_{6,5}$ = 7.9, H-6); 9.04 (bs, 1 H, NH). ^{13}C NMR (125.8 MHz, $CDCl_3$): 24.00 and 24.03 (d, $J_{C,P}$ = 4, $(CH_3)_2CH$); 48.30 (CH_2-1′); 66.07 (d, $J_{C,P}$ = 169, CH_2-4′); 70.92 (d, $J_{C,P}$ = 11, CH_2-2′); 71.16 (d, J_{CP} = 7, $CH(CH_3)_2$); 101.50 (CH-5); 145.84 (CH-6); 150.77 (C-2); 163.61 (C-4).

1-{2-[(Diisopropoxyphosphoryl)methoxy]ethyl}-5-fluorouracil (**14**)

Compound **12** (1.5 g, 4.5 mmol) was dissolved in glacial acetic acid (200 ml). A mixture of 5–10% fluorine in nitrogen was introduced to the solution until the conversion of starting phosphonate was complete (UV absorption disappears on TLC). Excess fluorine was removed by a stream of nitrogen and the mixture was concentrated in vacuo and codistilled with ethanol (2 × 50 ml). The residue was dissolved in ethanol (100 ml) and triethylamine (2 ml). The mixture was vigorously refluxed for 40 h until the conversion of intermediate dihydro adducts to **14** was complete (monitoring by ^{19}F NMR). The mixture was concentrated in

vacuo, the residue chromatographed on silica gel (chloroform–methanol 25:1 and 10:1) and appropriate fractions were evaporated in vacuo. The product was triturated with hexane. The solvent was decanted and the product dried under reduced pressure. For analytical purposes the product was crystallized in hexane–acetone. Yield 1.1 g (68%) of **14** as a white solid, m.p. 110–112 °C of crystalline product. IR (CCl_4), ν_{max}: 3403, 3146, 3078, 1729, 1713, 1670, 1386, 1378, 1356, 1255, 1239, 1178, 1142, 1105, 1010, 992. For $C_{13}H_{22}FN_2O_6P$ (352.3) calculated: 44.32% C, 6.29% H, 5.39% F, 7.95% N, 8.79% P; found: 44.33% C, 6.33% H, 5.17% F, 7.81% N, 8.69% P. FAB MS, *m/z*: 353 $[MH]^+$ (63). ^{1}H NMR (400 MHz, $CDCl_3$): 1.31 and 1.33 (2 (d, 2 (6 H, J_{vic} = 6.5, $(CH_3)_2CH$); 3.74 (d, 2 H, $J_{H,P}$ = 8.3, H-4′); 3.82 (dd, 2 H, $J_{2',1'}$ = 5.1 and 3.6, H-2′); 3.93 (dd, 2 H, $J_{1',2'}$ = 5.1 and 3.6, H-1′); 4.74 (dh, 2 H, $J_{H,P}$ = 7.7, J_{vic} = 6.5, $CH(CH_3)_2$); 7.52 (d, 1 H, $J_{H,F}$ = 5.8, H-6); 9.35 (bd, 1 H, $J_{H,F}$ = 3.4, NH). ^{13}C NMR (100.6 MHz, $CDCl_3$): 23.96 and 23.99 (d, $J_{C,P}$ = 4, $(CH_3)_2CH$); 48.40 (CH_2-1′); 66.13 (d, $J_{C,P}$ = 169, CH_2-4′); 70.95 (d, $J_{C,P}$ = 11, CH_2-2′); 71.32 (d, $J_{C,P}$ = 7, $CH(CH_3)_2$); 130.26 (d, $J_{C,F}$ = 33, CH-6); 139.93 (d, $J_{C,F}$ = 236, C-5); 149.41 (C-2); 157.18 (d, $J_{C,F}$ = 27, C-4). ^{19}F NMR (470.2 MHz, $CDCl_3$): –168.38 (t, $J_{F,H}$ = 5.8 and 3.4).

5-Fluoro-1-[2-(phosphonomethoxy)ethyl]uracil (**15**)

Compound **15** was obtained as dilithium salt by the procedure reported for **5a** and **5b**. A mixture of compound **14** (540 mg, 1.53 mmol) in acetonitrile (15 ml) and bromotrimethylsilane (2 ml) afforded 177 mg (38%) of **15** as a white solid, m.p. > 300 °C. IR (KBr), ν_{max}: 3242, 3172, 3080, 1691, 1475, 1444, 1383, 1101, 994, 925, 570, 467. HPLC, 99% (S3). For $C_7H_9FLi_2N_2O_6P{\cdot}1.2H_2O$ (301.6) calculated: 27.88% C, 3.45% H, 6.30% F, 9.29% N, 10.27% P; found: 27.62% C, 3.05% H, 6.36% F, 9.33% N, 10.55% P. FAB MS, *m/z*: 281 $[MH]^+$ (9). ^{1}H NMR (500 MHz, D_2O, ref(dioxane) 3.75 ppm): 3.58 (d, 2 H, $J_{H,P}$ = 8.6, H-4′); 3.81 (dd, 2 H, $J_{2',1'}$ = 5.3 and 4.3, H-2′); 3.97 (dd, 2 H, $J_{1',2'}$ = 5.3 and 4.3, H-1′); 7.92 (d, 1 H, $J_{H,F}$ = 6.1, H-6). ^{13}C NMR (125.8 MHz, D_2O, ref(dioxane) 69.3 ppm): 51.05 (CH_2-1′); 70.45 (d, $J_{C,P}$ = 155, CH_2-4′); 72.60 (d, $J_{C,P}$ = 11, CH_2-2′); 134.58 (d, $J_{C,F}$ = 33, CH-6); 143.04 (d, $J_{C,F}$ = 231, C-5); 154.59 (C-2); 162.92 (d, $J_{C,F}$ = 29, C-4). ^{19}F NMR (470.2 MHz, D_2O): –167.13 (d, $J_{F,H}$ = 6.1). ^{31}P NMR (162 MHz, D_2O): 15.18 (t, $J_{P,H}$ = 8.6).

4-Methoxy-1-(3,3,3-trifluoro-2-hydroxypropyl)pyrimidin-2(1*H*)-one (**17**)

A mixture of 4-methoxypyrimidin-2(1*H*)-one (**9**; 2 g, 15.9 mmol), trifluoromethyloxirane **16** (1.8 g, 16.0 mmol) and cesium carbonate (543 mg, 1.7 mmol) in dimethylformamide (140 ml) was heated at 80 °C for 8 h. The mixture was concentrated in vacuo to a minimum volume. The residue was codistilled with toluene (2 × 20 ml) and chromatographed on neutral aluminum oxide (toluene followed by ethyl acetate and ethyl acetate–methanol 75:25). The fractions containing compound **17** were evaporated in vacuo. The residue was triturated with hexane. The solvent was decanted and the obtained product dried under reduced pressure. Yield 2.6 g (69%) of **17** as a white solid. IR ($CHCl_3$), ν_{max}: 3607, 3289, 1668, 1654, 1638, 1543, 1487, 1487, 1416, 1321, 1273, 1116. For $C_8H_9F_3N_2O_3$ (238.2) calculated: 40.34% C, 3.81% H, 23.93% F, 11.76% N; found: 40.19% C, 3.72% H, 23.63% F, 11.78% N. FAB MS, *m/z*: 239 $[MH]^+$ (100). ^{1}H NMR (400 MHz, DMSO-d_6): 3.72 (dd, 1 H, J_{gem} = 13.4, $J_{1'b,2'}$ = 9.4, H-1′b); 3.82 (s, 3 H, OCH_3); 4.19 (dd, 1 H, J_{gem} = 13.4, $J_{1'a,2'}$ = 3.2, H-1′a); 4.31 (m, 1 H, H-2′); 6.02 (d, 1 H, $J_{5,6}$ = 7.2, H-5); 6.66 (d, 1 H, $J_{OH,2'}$ = 6.5, OH-2′); 7.92 (d, 1 H, $J_{6,5}$ = 7.2, H-6). ^{13}C NMR (100.6 MHz, DMSO-d_6): 45.59 (CH_2-1′); 53.97 (OCH_3); 65.99 (q, $J_{C,F}$ = 30, CH-2′); 94.21 (CH-5); 125.08 (q, $J_{C,F}$ = 283, CF_3); 150.64 (CH-6); 155.52 (C-2); 171.70 (C-4).

5-Ethyl-1-{2-[(diisopropoxyphosphoryl)methoxy]-3,3,3-trifluoropropyl}-4-methoxypyrimidin-2(1*H*)-one (**18**)

A mixture of compound **17** (2.6 g, 10.9 mmol), (diisopropoxyphosphoryl)methyl tosylate (4.6 g, 13.1 mmol) and 60% sodium hydride dispersion (655 mg, 13.4 mmol) in tetrahydrofuran (60 ml) was stirred at –20 °C. The suspension was allowed to warm to room temperature for 1 h and stirred at the same temperature overnight. The mixture was filtered through a Celite pad and concentrated in vacuo. The residue was chromatographed on neutral aluminum oxide (ethyl acetate followed by ethyl acetate–chloroform–methanol 17:10:1) and appropriate fractions containing compound **18** were evaporated in vacuo. Yield 2.5 g (55%) of crude product **18** as a slightly yellow oil. It was used for preparation of **19** without further purification. HR MS (FAB): for $C_{15}H_{25}F_3N_2O_6P$ calculated 417.3380, found 417.3383. FAB MS, *m/z*: 417 $[MH]^+$ (26). ^{1}H NMR (400 MHz, $CDCl_3$): 1.29, 1.30, 1.31 and 1.32 (4 × d, 4 × 3 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 3.60 (dd, 1 H, J_{gem} = 13.9, $J_{1'b,2'}$ = 8.9, H-1′b); 3.71 (dd, 1 H, J_{gem} = 13.3, $J_{H,P}$ = 10.8, H-4′b); 4.03 (dd, 1 H, J_{gem} = 13.3, $J_{H,P}$ = 9.6, H-4′a); 4.27 (m, 1 H, $J_{2',1'}$ = 8.9, 3.4, $J_{H,F}$ = 6.0, H-2′); 4.49 (dd, 1 H, J_{gem} = 13.9, $J_{1'a,2'}$ = 3.4, H-1′a); 4.63–4.78 (m, 2 H, C**H**(CH$_3$)$_2$); 5.90 (d, 1 H, $J_{5,6}$ = 7.2, H-5); 7.49 (d, 1 H, $J_{6,5}$ = 7.2, H-6).

1-{2-[(Diisopropoxyphosphoryl)methoxy]-3,3,3-trifluoropropyl}uracil (**19**)

Compound **18** (2.4 g, 5.8 mmol) was dissolved in 90% aqueous methanol (40 ml). Dowex 50 (H^+ form; 10 ml) was added to the solution and the suspension was heated at 100 °C for 4 h until the conversion of **18** to **19** was complete (TLC in ethyl acetate–chloroform–methanol 17:10:1). The mixture was concentrated in vacuo. The residue was chromatographed on neutral aluminum oxide (ethyl acetate–chloroform–methanol 17:10:1) and appropriate fractions containing compound **19** were evaporated in vacuo. Yield 780 mg (34%) of **19** as a colorless oil. IR (CCl_4), ν_{max}: 3407, 3161, 1719, 1691, 1634, 1433, 1276, 1356, 1255, 1177, 1147, 1017, 996. HR MS (FAB): for $C_{14}H_{23}F_3N_2O_6P$ calculated 403.1246, found 403.1260. For $C_{14}H_{22}F_3N_2O_6P$ (402.3) calculated: 41.80% C, 5.51% H, 14.17% F, 6.96% N, 7.70% P; found: 41.90% C, 5.42% H, 13.83% F, 6.77% N, 7.56% P. FAB MS, *m/z*: 403 $[MH]^+$ (36). ^{1}H NMR (400 MHz, $CDCl_3$): 1.31, 1.32, 1.33 and 1.34 (4 × d, 4 × 3 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 3.60 (dd, 1 H, J_{gem} = 14.4, $J_{1'b,2'}$ = 8.5, H-1′b); 3.82 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 9.0, H-4′b); 4.07 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 9.4, H-4′a); 4.23 (m, 1 H, H-2′); 4.35 (dd, 1 H, J_{gem} = 14.4, $J_{1'a,2'}$ = 3.3, H-1′a); 4.72 and 4.74 (2 × dh, 2 × 1 H, $J_{H,P}$ = 7.6, J_{vic} = 6.2, C**H**(CH$_3$)$_2$); 5.71 (d, 1 H, $J_{5,6}$ = 8.0, H-5); 7.29 (d, 1 H, $J_{6,5}$ = 8.0, H-6); 8.92(bs, 1 H, NH). ^{13}C NMR (100.6 MHz, $CDCl_3$): 23.91 and 24.02 (d, $J_{C,P}$ = 4, (**C**H$_3$)$_2$CH); 47.30 (CH$_2$-1′); 67.18 (d, $J_{C,P}$ = 169, CH$_2$-4′); 71.55 and 71.71 (d, $J_{C,P}$ = 7, **C**H(CH$_3$)$_2$); 77.00 (qd, $J_{C,F}$ = 30, $J_{C,P}$ = 12, CH-2′); 102.40 (CH-5); 123.77 (q, $J_{C,F}$ = 284, CF$_3$); 145.44 (CH-6); 150.59 (C-2); 163.22 (C-4).

1-{2-[(Diisopropoxyphosphoryl)methoxy]-3,3,3-trifluoropropyl}-5-fluorouracil (**20**)

Compound **19** (778 mg, 1.9 mmol) was dissolved in glacial acetic acid (90 ml). A mixture of 5–10% fluorine in nitrogen was introduced to the solution until the conversion of starting phosphonate was complete (UV absorption disappears on TLC plate). Excess fluorine was removed by a stream of nitrogen, the mixture was concentrated in vacuo and codistilled with ethanol (2 × 20 ml). The residue was dissolved in ethanol (45 ml) and triethylamine (3 ml). The mixture was vigorously refluxed for 11 h until the conversion of intermediate dihydro adducts to **20** was complete (monitoring by ^{19}F NMR). The mixture was concentrated in

vacuo and the residue was subjected to preparative TLC (S1, chloroform–methanol 20:1). Yield 365 mg (45%) of **20** as a colorless oil. IR (CCl_4), ν_{max}: 3405, 3168, 3065, 2983, 1723, 1703, 1670, 1387, 1377, 1248, 1274, 1181, 1158, 1105, 1013, 996. For $C_{14}H_{21}F_4N_2O_6P$ (420.3) calculated: 40.01% C, 5.04% H, 6.67% N, 7.37% P; found: 40.05% C, 5.17% H, 6.34% N, 7.37% P. FAB MS, *m/z*: 421 $[MH]^+$ (13). ^{1}H NMR (400 MHz, $CDCl_3$): 1.31, 1.32, 1.33 and 1.34 (4 × d, 4 × 3 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 3.51 (dd, 1 H, J_{gem} = 14.4, $J_{1'b,2'}$ = 8.6, H-1′b); 3.85 (dd, 1 H, J_{gem} = 14.1, $J_{H,P}$ = 5.7, H-4′b); 4.10 (dd, 1 H, J_{gem} = 14.1, $J_{H,P}$ = 8.8, H-4′a); 4.29 (m, 1 H, H-2′); 4.37 (dd, 1 H, J_{gem} = 14.4, $J_{1'a,2'}$ = 3.0, H-1′a); 4.73 (dh, 2 H, $J_{H,P}$ = 7.6, J_{vic} = 6.2, C**H**(CH$_3$)$_2$); 7.39 (d, 1 H, $J_{H,F}$ = 5.5, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): 23.84, 23.90 and 23.99 (d, $J_{C,P}$ = 4, (**C**H$_3$)$_2$CH); 47.45 (CH_2-1′); 67.10 (d, $J_{C,P}$ = 168, CH_2-4′); 71.76 and 71.86 (d, $J_{C,P}$ = 7, **C**H(CH$_3$)$_2$); 76.75 (qd, $J_{C,F}$ = 30, $J_{C,P}$ = 14, CH-2′); 123.75 (q, $J_{C,F}$ = 284, CF_3); 129.86 (d, $J_{C,F}$ = 33, CH-6); 140.28 (d, $J_{C,F}$ = 237, C-5); 149.35 (C-2); 157.13 (d, $J_{C,F}$ = 27, C-4). ^{19}F NMR (188.2 MHz, $CDCl_3$): –166.04 (d, $J_{F,H-6}$ = 5.5, F-5); –75.92 (dd, $J_{F,H-2'}$ = 6.0, $J_{F,H-1'a}$ = 2.0, CF_3).

5-Fluoro-1-[3,3,3-trifluoro-2-(phosphonomethoxy)propyl]uracil (**21**)

Compound **21** was obtained as dilithium salt using the same procedure as reported for **5a** and **5b**. A mixture of compound **20** (248 mg, 0.59 mmol) in acetonitrile (5 ml) and bromotrimethylsilane (0.8 ml) afforded 124 mg (59%) of **21** as a white solid, m.p. > 300 °C. IR (KBr), ν_{max}: 3250, 3080, 1701, 1669, 1482, 1442, 1378, 1252, 1179, 1154, 1136, 1079, 991, 921, 789, 759, 726, 685, 537, 469. HPLC, 99% (S3). HR MS (FAB): for $C_8H_8F_4Li_2N_2O_6P$ calculated 349.0376, found 349.0386. For $C_8H_7F_4Li_2N_2O_6P{\cdot}0.5H_2O$ (357.0) calculated: 26.91% C, 2.26% H, 22.28% F, 7.84% N, 8.68% P; found: 26.79% C, 2.42% H, 22.46% F, 7.43% N, 8.62% P. FAB MS, *m/z*: 349 $[MH]^+$ (36). ^{1}H NMR (500 MHz, D_2O, ref(dioxane) 3.75 ppm): 3.69 and 3.90 (2 × dd, 2 H, J_{gem} = 12.6, $J_{H,P}$ = 9.3, H-4′); 3.96 (dd, 1 H, J_{gem} = 14.8, $J_{1'b,2'}$ = 7.4, H-1′b); 4.25 (dd, 1 H, J_{gem} = 14.8, $J_{1'a,2'}$ = 3.7, H-1′a); 4.32 (m, 1 H, H-2′); 7.97 (d, 1 H, $J_{H,F}$ = 6.0, H-6). ^{13}C NMR (125.8 MHz, D_2O, ref(dioxane) 69.3 ppm): 49.66 (CH_2-1′); 72.61 (d, $J_{C,P}$ = 153, CH_2-4′); 79.28 (qd, $J_{C,F}$ = 30, $J_{C,P}$ = 12, CH-2′); 126.84 (q, $J_{C,F}$ = 284, CF_3); 142.18 (d, $J_{C,F}$ = 34, CH-6); 143.17 (d, $J_{C,F}$ = 232, C-5); 152.30 (d, $J_{C,F}$ = 30, C-4); 154.04 (C-2). ^{19}F NMR (188.2 MHz, D_2O): –165.22 (d, $J_{F,H-6}$ = 6.0, F-5); –73.63 (dd, $J_{F,H-2'}$ = 6.5, CF_3). ^{31}P NMR (162 MHz, D_2O): 13.36 (t, $J_{P,H-4'}$ = 9.3).

Synthesis of 1-(3,3,3-Trifluoro-2-hydroxypropyl)pyrimidin-2(1*H*)-one Derivatives **23** and **24**. General Procedure

A mixture of pyrimidin-2(1*H*)-one **1** or **22** (6.5 mmol), trifluoromethyloxirane **16** (6.5 mmol) and cesium carbonate (0.7 mmol) in dimethylformamide (70 ml) was heated at 80 °C for 15 h. The mixture was concentrated in vacuo to a minimum volume. The residue was codistilled with toluene (2 × 10 ml) and chromatographed on neutral aluminum oxide (toluene followed by ethyl acetate and ethyl acetate–methanol 60:40). The fractions containing compound **23** or **24** were evaporated to dryness in vacuo. The product was triturated with hexane and the solvent was decantated. The product was dried under reduced pressure.

5-Ethyl-4-methoxy-1-(3,3,3-trifluoro-2-hydroxypropyl)pyrimidin-2(1H)-one (**23**). Yield 951 mg (55%) of a white solid. IR (CCl_4), ν_{max}: 3606, 3280, 1663, 1630, 1537, 1475, 1402, 1271. For $C_{10}H_{13}F_3N_2O_3$ (266.2) calculated: 45.12% C, 4.92% H, 21.41% F, 10.52% N; found: 45.10% C, 4.87% H, 21.76% F, 10.47% N. FAB MS, *m/z*: 267 $[MH]^+$ (100). ^{1}H NMR (400 MHz,

DMSO-d_6): 1.06 (t, 3 H, J_{vic} = 7.4, C**H**$_3$CH$_2$); 2.30 (qd, 2 H, J_{vic} = 7.4, $J_{CH2,6}$ = 1.0, C**H**$_2$CH$_3$); 3.71 (dd, 1 H, J_{gem} = 13.4, $J_{1'b,2'}$ = 9.4, H-1′b); 3.86 (s, 3 H, OCH$_3$); 4.17 (dd, 1 H, J_{gem} = 13.4, $J_{1'a,2'}$ = 3.1, H-1′a); 4.32 (m, 1 H, H-2′); 6.62 (d, 1 H, $J_{OH,2'}$ = 6.7, OH-2′); 7.74 (t, 1 H, $J_{6,CH2}$ = 1.0, H-6). ^{13}C NMR (100.6 MHz, DMSO-d_6): 13.35 (**C**H$_3$CH$_2$); 19.20 (**C**H$_2$CH$_3$); 49.47 (CH$_2$-1′); 54.20 (OCH$_3$); 66.07 (q, $J_{C,F}$ = 30, CH-2′); 108.30 (C-5); 125.12 (q, $J_{C,F}$ = 283, CF$_3$); 146.92 (CH-6); 155.39 (C-2); 170.25 (C-4).

4-Methoxy-5-methyl-1-(3,3,3-trifluoro-2-hydroxypropyl)pyrimidin-2(1H)-one (**24**). Yield 934 mg (57%) of a white solid. IR (CCl$_4$), ν_{max}: 3623, 3273, 1671, 1650, 1542, 1478, 1399, 1339, 1270. For $C_9H_{11}F_3N_2O_3$ (252.2) calculated: 42.86% C, 4.40% H, 22.60% F, 11.11% N; found: 42.47% C, 4.33% H, 22.10% F, 10.66% N. FAB MS, *m/z*: 253 [MH]$^+$ (100). ^{1}H NMR (400 MHz, DMSO-d_6): 1.87 (d, 3 H, $J_{CH3,6}$ = 1.1, CH$_3$-5); 3.66 (dd, 1 H, J_{gem} = 13.4, $J_{1'b,2'}$ = 9.4, H-1′b); 3.85 (s, 3 H, OCH$_3$); 4.16 (dd, 1 H, J_{gem} = 13.4, $J_{1'a,2'}$ = 3.2, H-1′a); 4.31 (bm, 1 H, H-2′); 6.64 (bs, 1 H, OH-2′); 7.78 (q, 1 H, $J_{6,CH3}$ = 1.1, H-6). ^{13}C NMR (100.6 MHz, DMSO-d_6): 11.65 (CH$_3$-5); 49.45 (CH$_2$-1′); 54.22 (OCH$_3$); 65.74 (q, $J_{C,F}$ = 29, CH-2′); 102.40 (C-5); 125.09 (q, $J_{C,F}$ = 283, CF$_3$); 147.56 (CH-6); 155.49 (C-2); 170.54 (C-4).

Synthesis of 1-{2-[(Diisopropoxyphosphoryl)methoxy]-3,3,3-trifluoropropyl}-pyrimidin-2(1*H*)-one Derivatives **25** and **26**. General Procedure

A mixture of compound **23** or **24** (3.7 mmol), (diisopropoxyphosphoryl)methyl tosylate (4.1 mmol) and 60% sodium hydride dispersion (5.5 mmol) in tetrahydrofuran (20 ml) was stirred at –20 °C. The suspension was allowed to warm to room temperature for 1 h and stirred at the same temperature overnight. The mixture was filtered through a Celite pad under pressure and concentrated in vacuo to a minimum volume. The residue was chromatographed on neutral aluminum oxide (ethyl acetate followed by ethyl acetate–chloroform–methanol 17:10:1). The crude product was purified by preparative TLC (S1, ethyl acetate–chloroform–methanol 17:10:1). The fractions containing product **25** or **26** were evaporated to dryness in vacuo.

1-{2-[(Diisopropoxyphosphoryl)methoxy]-3,3,3-trifluoropropyl}-5-ethyl-4-methoxy-pyrimidin-2(1H)-one (**25**). Yield 563 mg (35%) of a white solid, m.p. > 300 °C. IR (CCl$_4$), ν_{max}: 2981, 2959, 2937, 2900, 1678, 1653, 1537, 1537, 1471, 1403, 1366, 1268, 1255, 1179, 1149, 1130, 1111, 1006, 992. For $C_{17}H_{28}F_3N_2O_6P$ (444.4) calculated: 45.95% C, 6.35% H, 12.83% F, 6.30% N, 6.97% P; found: 46.08% C, 6.43% H, 12.73% F, 6.24% N, 7.06% P. FAB MS, *m/z*: 445 [MH]$^+$ (100). ^{1}H NMR (500 MHz, CDCl$_3$): 1.16 (t, 3 H, J_{vic} = 7.5, C**H**$_3$CH$_2$); 1.27, 1.29, 1.31 and 1.32 (4 × d, 4 × 3 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 2.36 and 2.40 (2 × m, 2 H, J_{gem} = 15.1, J_{vic} = 7.5, $J_{CH2,6}$ = 1.1, C**H**$_2$CH$_3$); 3.64 (dd, 1 H, J_{gem} = 13.9, $J_{1'b,2'}$ = 8.6, H-1′b); 3.72 (dd, 1 H, J_{gem} = 13.3, $J_{H,P}$ = 10.3, H-4′b); 4.00 (s, 3 H, OCH$_3$); 4.01 (dd, 1 H, J_{gem} = 13.3, $J_{H,P}$ = 9.6, H-4′a); 4.29 (m, 1 H, H-2′); 4.43 (dd, 1 H, J_{gem} = 13.9, $J_{1'a,2'}$ = 3.7, H-1′a); 4.67 and 4.73 (2 × dh, 2 H, $J_{H,P}$ = 7.6, J_{vic} = 6.2, C**H**(CH$_3$)$_2$); 7.23 (t, 1 H, $J_{6,CH2}$ = 1.1, H-6). ^{13}C NMR (125.8 MHz, CDCl$_3$): 12.93 (**C**H$_3$CH$_2$); 19.75 (**C**H$_2$CH$_3$); 23.77, 23.85 and 23.99 (d, $J_{C,P}$ = 4, (**C**H$_3$)$_2$CH); 49.18 (CH$_2$-1′); 54.62 (OCH$_3$); 67.39 (d, $J_{C,P}$ = 170, CH$_2$-4′); 71.24 and 71.54 (d, $J_{C,P}$ = 7, **C**H(CH$_3$)$_2$); 76.54 (qd, $J_{C,F}$ = 30, $J_{C,P}$ = 14, CH-2′); 110.78 (C-5); 123.93 (q, $J_{C,F}$ = 284, CF$_3$); 144.51 (CH-6); 156.25 (C-2); 171.08 (C-4).

1-{2-[(Diisopropoxyphosphoryl)methoxy]-3,3,3-trifluoropropyl}-4-methoxy-5-methyl-pyrimidin-2(1H)-one (**26**). Yield 382 mg (24%) of a white solid, m.p. > 300 °C. IR (CCl$_4$), ν_{max}: 2982, 1679, 1654, 1541, 1476, 1398, 1386, 1376, 1366, 1337, 1267, 1255, 1013, 992. For $C_{16}H_{26}F_3N_2O_6P$ (430.4) calculated: 44.65% C, 6.09% H, 13.24% F, 6.51% N, 7.20% P; found: 44.40% C,

5.86% H, 13.06% F, 6.48% N, 7.33% P. FAB MS, *m/z*: 431 $[MH]^+$ (82). ^{1}H NMR (400 MHz, $CDCl_3$): 1.29, 1.30 and 1.31 (3 × d, 12 H, J_{vic} = 6.2, $(CH_3)_2CH$); 1.96 (d, 3 H, $J_{CH3,6}$ = 1.1, CH_3-5); 3.60 (dd, 1 H, J_{gem} = 13.9, $J_{1'b,2'}$ = 8.8, H-1′b); 3.72 (dd, 1 H, J_{gem} = 13.3, $J_{H,P}$ = 10.4, H-4′b); 4.00 (s, 3 H, OCH_3); 4.02 (dd, 1 H, J_{gem} = 13.3, $J_{H,P}$ = 9.5, H-4′a); 4.29 (m, 1 H, $J_{2',1'}$ = 8.8, 3.5, $J_{H,F}$ = 6.2, H-2′); 4.44 (dd, 1 H, J_{gem} = 13.9, $J_{1'a,2'}$ = 3.5, H-1′a); 4.68 and 4.73 (2 × dh, 2 H, $J_{H,P}$ = 7.6, J_{vic} = 6.2, $CH(CH_3)_2$); 7.27 (q, 1 H, $J_{6,CH3}$ = 1.1, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): 12.04 (CH_3-5); 23.78, 23.85 and 23.99 (d, $J_{C,P}$ = 5, $(CH_3)_2CH$); 49.04 (CH_2-1′); 54.69 (OCH_3); 67.36 (d, $J_{C,P}$ = 169, CH_2-4′); 71.27 and 71.54 (d, $J_{C,P}$ = 7, $CH(CH_3)_2$); 76.68 (qd, $J_{C,F}$ = 30, $J_{C,P}$ = 14, CH-2′); 104.80 (C-5); 123.94 (q, $J_{C,F}$ = 285, CF_3); 145.36 (CH-6); 156.35 (C-2); 171.32 (C-4).

Synthesis of 5-Ethyl-1-[3,3,3-trifluoro-2-(phosphonomethoxy)propyl]uracil and Thymine Derivatives **27** and **28**. General Procedure

Compounds **27** and **28** were obtained as dilithium salt by the same procedure reported for **5a** and **5b**.

5-Ethyl-1-[3,3,3-trifluoro-2-(phosphonomethoxy)propyl]uracil (**27**). A mixture of compound **25** (432 mg, 0.97 mmol) in acetonitrile (10 ml) and bromotrimethylsilane (1.3 ml) afforded after work-up 173 mg (48%) of **27** as a white solid, m.p. > 300 °C. IR (KBr), ν_{max}: 3254, 1685, 1474, 1459, 1440, 1375, 1354, 1266, 1167, 1137, 1223, 1087, 989, 916, 568, 542, 444. HPLC, 99% (S5). For $C_{10}H_{12}F_3Li_2N_2O_6P{\cdot}0.5H_2O$ (367.1) calculated: 32.72% C, 3.54% H, 15.52% F, 7.63% N, 8.43% P; found: 32.58% C, 3.64% H, 15.51% F, 7.34% N, 8.27% P. FAB MS, *m/z*: 359 $[MH]^+$ (4). ^{1}H NMR (400 MHz, D_2O, ref(dioxane) 3.75 ppm): 1.09 (t, 3 H, J_{vic} = 7.5, CH_3CH_2); 2.32 (qd, 2 H, J_{vic} = 7.5, $J_{CH2,6}$ = 1.1, CH_2CH_3); 3.69 (dd, 1 H, J_{gem} = 12.5, $J_{H,P}$ = 9.2, H-4′b); 3.84 (dd, 1 H, J_{gem} = 12.5, $J_{H,P}$ = 9.5, H-4′a); 4.05 (dd, 1 H, J_{gem} = 14.8, $J_{1'b,2'}$ = 6.8, H-1′b); 4.21 (dd, 1 H, J_{gem} = 14.8, $J_{1'a,2'}$ = 4.5, H-1′a); 4.32 (m, 1 H, $J_{2',1'}$ = 6.8, 4.5, $J_{H,F}$ = 6.5, H-2′); 7.56 (t, 1 H, $J_{6,CH2}$ = 1.1, H-6). ^{13}C NMR (125.8 MHz, D_2O, ref(dioxane) 69.3 ppm): 14.94 (CH_3CH_2); 22.10 (CH_2CH_3); 49.25 (CH_2-1′); 72.90 (d, $J_{C,P}$ = 152, CH_2-4′); 79.15 (qd, $J_{C,F}$ = 30, $J_{C,P}$ = 12, CH-2′); 119.29 (C-5); 126.97 (q, $J_{C,F}$ = 284, CF_3); 145.84 (CH-6); 154.90 (C-2); 169.36 (C-4). ^{19}F NMR (188.2 MHz, D_2O): –73.63 (dd, $J_{F,H-2'}$ = 6.5, CF_3). ^{31}P NMR (162 MHz, D_2O): 12.94 (t, $J_{P,H-4'}$ = 9.5, 9.2).

1-[3,3,3-Trifluoro-2-(phosphonomethoxy)propyl]thymine (**28**). A mixture of compound **26** (418 mg, 0.97 mmol) in acetonitrile (10 ml) and bromotrimethylsilane (1.3 ml) afforded after work-up 207 mg (60%) of **28** as a white solid, m.p. > 300 °C. IR (KBr), ν_{max}: 3243, 1690, 1477, 1439, 1374, 1352, 1230, 1171, 1152, 1131, 1087, 992, 916, 563, 541, 473, 455. HPLC, 99% (S3). For $C_9H_{10}F_3Li_2N_2O_6P{\cdot}0.5H_2O$ (353.0) calculated: 30.61% C, 3.14% H, 16.14% F, 7.93% N, 8.77% P; found: 30.49% C, 3.18% H, 16.07% F, 7.79% N, 9.00% P. FAB MS, *m/z*: 345 $[MH]^+$ (17). ^{1}H NMR (400 MHz, D_2O, ref(dioxane) 3.75 ppm): 1.88 (d, 3 H, $J_{CH3,6}$ = 1.2, CH_3-5); 3.68 and 3.86 (2 × dd, 2 H, J_{gem} = 12.5, $J_{H,P}$ = 9.1, H-4′); 4.01 (dd, 1 H, J_{gem} = 14.8, $J_{1'b,2'}$ = 7.0, H-1′b); 4.22 (dd, 1 H, J_{gem} = 14.8, $J_{1'a,2'}$ = 4.2, H-1′a); 4.30 (m, 1 H, $J_{2',1'}$ = 7.0, 4.2, $J_{H,F}$ = 6.0, H-2′); 7.56 (q, 1 H, $J_{6,CH3}$ = 1.2, H-6). ^{13}C NMR (125.8 MHz, D_2O, ref(dioxane) 69.3 ppm): 14.05 (CH_3-5); 49.15 (CH_2-1′); 72.84 (d, $J_{C,P}$ = 153, CH_2-4′); 79.27 (qd, $J_{C,F}$ = 30, $J_{C,P}$ = 12, CH-2′); 113.52 (C-5); 126.93 (q, $J_{C,F}$ = 284, CF_3); 146.43 (CH-6); 154.98 (C-2); 169.80 (C-4). ^{19}F NMR (188.2 MHz, D_2O): –73.58 (dd, $J_{F,H-2'}$ = 6.0, CF_3). ^{31}P NMR (162 MHz, D_2O): 13.02 (t, $J_{P,H-4'}$ = 9.1).

1-[2-Hydroxy-3-(trimethylammonio)propyl]-4-methoxy-5-methylpyrimidin-2(1*H*)-one Chloride (**30**)

A mixture of 4-methoxy-5-methylpyrimidin-2(1*H*)-one (**22**) (1 g, 7.1 mmol), trimethylglycidylammonium chloride (**29**; 1.01 g, 7.1 mmol) and cesium carbonate (273 mg, 0.8 mmol) in dimethylformamide (50 ml) was heated at 80 °C for 7.5 h. The mixture was concentrated in vacuo to a minimum volume. The residue was codistilled with toluene (20 ml) and the residue was treated with acetone (50 ml) and ethanol (5 ml). The resulting suspension was heated until a white precipitate was obtained. After cooling the precipitate was filtered off and dried under reduced pressure. Yield 661 mg (32%) of crude product **30** as a white solid which was used for preparation of **32** without further purification. HR MS (FAB): for $C_{12}H_{23}ClN_3O_3$ calculated 292.7820, found 292.7828. FAB MS, *m/z*: 292 $[MH]^+$ (71). ^{1}H NMR (500 MHz, DMSO-d_6): 1.88 (d, 3 H, $J_{CH3,6}$ = 1.0, CH_3-5); 3.13 (s, 9 H, $(CH_3)_3N$); 3.30 (dd, 1 H, J_{gem} = 13.6, $J_{3'b,2'}$ = 11.2, H-3′b); 3.45 (dd, 1 H, J_{gem} = 13.6, $J_{3'a,2'}$ = 1.9, H-3′a); 3.65 (dd, 1 H, J_{gem} = 13.4, $J_{1'b,2'}$ = 7.4, H-1′b); 3.85 (s, 3 H, OCH_3); 3.88 (dd, 1 H, J_{gem} = 13.4, $J_{1'a,2'}$ = 4.1, H-1′a); 4.38 (m, 1 H, H-2′); 6.01 (bs, 1 H, OH-2′); 7.73 (q, 1 H, $J_{6,CH3}$ = 1.0, H-6). ^{13}C NMR (100.6 MHz, DMSO-d_6): 11.77 (CH_3-5); 53.11 (CH_2-1′); 53.58 ($(CH_3)_3N$); 54.13 (OCH_3); 63.52 (CH-2′); 67.51 (CH-3′); 102.20 (C-5); 147.43 (CH-6); 155.83 (C-2); 170.31 (C-4).

1-[2-(Phosphonomethoxy)-3-(trimethylammonio)propyl]thymine (**32**)

A mixture of compound **30** (661 mg, 2.3 mmol), (diisopropoxyphosphoryl)methyl tosylate (873 mg, 2.5 mmol) and 60% sodium hydride dispersion (136 mg, 3.4 mmol) in dimethylformamide (15 ml) was stirred at –20 °C. The suspension was allowed to warm to room temperature for 1 h and stirred at the same temperature overnight. The mixture was concentrated in vacuo to a minimum volume and the residue was dissolved in water (3 ml). The mixture was applied onto a Dowex 50X8 (H^+ form; 30 ml) and the column was washed with water until the UV absoption of the eluate dropped to the original value. The product was then eluted with 2.5% aqueous ammonia. The fractions containing compound **31** were evaporated to dryness in vacuo. The residue was dissolved in acetonitrile (5 ml) and bromotrimethylsilane (0.6 ml) was added. The resulting mixture was stirred overnight at room temperature. The mixture was concentrated in vacuo and then codistilled with water (2 × 2 ml). The residue in water (20 ml) was heated with Dowex 50X8 (H^+ form; 2 ml) at 100 °C for 2 h. The suspension was applied onto a column which was then washed with 2.5% aqueous ammonia. The filtrate was evaporated to dryness in vacuo. The residue was purified on 30 ml of Sephadex (S2) with subsequent deionization on Dowex 50X8 (H^+ form; 30 ml). The relevant fractions were combined and evaporated in vacuo. The residue was dissolved in water and lyophilized. Yield 96 mg (12%) of **32** as a slightly yellow solid. IR (KBr), ν_{max}: 1685, 1605, 1524, 1175, 1477, 1436, 968, 1069, 1100, 935, 902, 540, 451. HPLC, 99% (S3). HR MS (FAB): for $C_{12}H_{23}N_3O_6P$ calculated 336.1324, found 336.1338. For $C_{12}H_{22}N_3O_6P{\cdot}1.5H_2O$ (362.3) calculated: 39.78% C, 6.96% H, 11.59% N, 8.56% P; found: 39.65% C, 6.58% H, 11.76% N, 8.17% P. FAB MS, *m/z*: 336 $[MH]^+$ (26). ^{1}H NMR (400 MHz, D_2O, ref(dioxane) 3.75 ppm): 1.89 (d, 3 H, $J_{CH3,6}$ = 1.2, CH_3-5); 3.23 (s, 9 H, $(CH_3)_3N$); 3.46 (dd, 1 H, J_{gem} = 14.2, $J_{3'b,2'}$ = 2.4, H-3′b); 3.54 (dd, 1 H, J_{gem} = 14.2, $J_{3'a,2'}$ = 9.4, H-3′a); 3.61 (dd, 1 H, J_{gem} = 12.4, $J_{H,P}$ = 10.2, H-4′b); 3.84 (dd, 1 H, J_{gem} = 12.4, $J_{H,P}$ = 9.6, H-4′a); 4.00 (dd, 1 H, J_{gem} = 15.1, $J_{1'b,2'}$ = 5.1, H-1′b); 4.12 (dd, 1 H, J_{gem} = 15.1, $J_{1'a,2'}$ = 3.6, H-1′a); 4.34 (m, 1 H, H-2′); 7.54 (q, 1 H, $J_{6,CH3}$ = 1.2, H-6). ^{13}C NMR (100.6 MHz, D_2O, ref(dioxane)

69.3 ppm): 14.05 (CH_3-5); 50.10 (CH_2-1′); 57.16 ((CH_3)$_3$N); 69.26 (d, $J_{C,P}$ = 153, CH_2-4′); 69.54 (CH-3′); 77.31 (d, $J_{C,P}$ = 13, CH-2′); 113.95 (C-5); 146.09 (CH-6); 155.32 (C-2); 169.63 (C-4). ^{31}P NMR (162 MHz, D_2O): 13.14 (t, $J_{P,H\text{-}4'}$ = 10.2, 9.6).

Synthesis of 1-{3-Azido-2-[(diisopropoxyphosphoryl)methoxy)]propyl} Derivatives **34a** and **34b**

A mixture of 4-methoxy-5-methylpyrimidin-2(1*H*)-one (**22**) (616 mg, 4.4 mmol) and 60% sodium hydride dispersion (179 mg, 4.5 mmol) in dimethylformamide (40 ml) was stirred at room temperature for 1 h. Compound **33** (1.8 g, 4.4 mmol) in dimethylformamide (25 ml) was added and the resulting mixture was heated at 90 °C for 6.5 h. The mixture was concentrated in vacuo to a minimum volume. The residue was codistilled with toluene (2 × 10 ml) The residue was chromatographed on neutral aluminum oxide (ethyl acetate followed by ethyl acetate–chloroform–methanol 11:10:1). The relevant fractions were combined and evaporated in vacuo.

1-{3-Azido-2-[(diisopropoxyphosphoryl)methoxy]propyl}-4-methoxy-5-methylpyrimidin-2(1H)-one (**34a**). Yield 662 mg (36%) of a slightly yellow oil. R_F 0.30 in ethyl acetate–chloroform–methanol 11:10:1. IR (CCl_4), ν_{max}: 2104, 1675, 1654, 1540, 1476, 1400, 1386, 1376, 1257, 1106, 1012, 992. HR MS (FAB): for $C_{16}H_{29}N_5O_6P$ calculated 418.1855, found 418.1862. FAB MS, *m/z*: 418 $[MH]^+$ (100). ^{1}H NMR (400 MHz, $CDCl_3$): 1.31, 1.32, 1.33 and 1.34 (4 × d, 4 × 3 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 1.96 (d, 3 H, $J_{CH3,6}$ = 1.1, CH_3-5); 3.28 (dd, 1 H, J_{gem} = 13.5, $J_{3'b,2'}$ = 5.3, H-3′b); 3.67 (dd, 1 H, J_{gem} = 13.5, $J_{3'a,2'}$ = 3.2, H-3′a); 3.70 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 9.4, H-4′b); 3.86 (dd, 1 H, J_{gem} = 13.6, $J_{1'b,2'}$ = 6.9, H-1′b); 3.90 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 8.5, H-4′a); 3.95 (m, 1 H, $J_{2',1'}$ = 6.9, 3.6, $J_{2',3'}$ = 5.3, 3.2, H-2′); 3.99 (s, 3 H, OCH_3); 4.11 (dd, 1 H, J_{gem} = 13.6, $J_{1'a,2'}$ = 3.6, H-1′a); 4.72 and 4.73 (2 (dh, 2 H, $J_{H,P}$ = 7.6, J_{vic} = 6.2, C**H**(CH_3)$_2$); 7.42 (q, 1 H, $J_{6,CH3}$ = 1.1, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.97 (CH_3-5); 23.92, 23.96, 24.00 and 24.03 (d, $J_{C,P}$ = 4, (**C**H$_3$)$_2$CH); 50.97 (CH_2-1′); 51.69 (CH-3′); 54.56 (OCH_3); 65.64 (d, $J_{C,P}$ = 169, CH_2-4′); 71.14 and 71.30 (d, $J_{C,P}$ = 7, **C**H(CH$_3$)$_2$); 79.14 (d, $J_{C,P}$ = 10, CH-2′); 104.34 (C-5); 145.64 (CH-6); 156.74 (C-2); 171.04 (C-4).

2-{3-Azido-2-[(diisopropoxyphosphoryl)methoxy)]propoxy}-4-methoxy-5-methylpyrimidine (**34b**). Yield 879 mg (48%) of a slightly yellow oil. R_F 0.50 in ethyl acetate–chloroform–methanol 11:10:1. IR (CCl_4), ν_{max}: 2104, 1608, 1576, 1423, 1386, 1375, 1295, 1261, 1106, 1085, 1077, 1009, 991. HR MS (FAB): for $C_{16}H_{29}N_5O_6P$ calculated 418.1855, found 418.1845. FAB MS, *m/z*: 418 $[MH]^+$ (100). ^{1}H NMR (400 MHz, $CDCl_3$): 1.33, 1.34, 1.35 and 1.36 (4 × d, 4 × 3 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 2.06 (d, 3 H, $J_{CH3,6}$ = 0.9, CH_3-5); 3.50 (dd, 1 H, J_{gem} = 13.1, $J_{3'b,2'}$ = 6.1, H-3′b); 3.59 (dd, 1 H, J_{gem} = 13.1, $J_{3'a,2'}$ = 3.9, H-3′a); 3.94 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 8.7, H-4′b); 3.98 (m, 1 H, $J_{2',1'}$ = 6.0, 5.2, $J_{2',3'}$ = 6.1, 3.9, H-2′); 3.99 (s, 3 H, OCH_3); 4.03 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 8.9, H-4′a); 4.39 (dd, 1 H, J_{gem} = 11.4, $J_{1'b,2'}$ = 6.0, H-1′b); 4.48 (dd, 1 H, J_{gem} = 11.4, $J_{1'a,2'}$ = 5.2, H-1′a); 4.76 and 4.77 (2 × dh, 2 H, $J_{H,P}$ = 7.6, J_{vic} = 6.2, C**H**(CH_3)$_2$); 7.97 (q, 1 H, $J_{6,CH3}$ = 0.9, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.82 (CH_3-5); 23.92, 24.03 and 24.05 (d, $J_{C,P}$ = 4, (**C**H$_3$)$_2$CH); 51.95 (CH-3′); 53.90 (OCH_3); 65.22 (d, $J_{C,P}$ = 168, CH_2-4′); 65.66 (CH_2-1′); 71.18 (d, $J_{C,P}$ = 7, **C**H(CH$_3$)$_2$); 78.84 (d, $J_{C,P}$ = 11, CH-2′); 111.58 (C-5); 156.92 (CH-6); 163.03 (C-2); 169.65 (C-4).

1-{3-Amino-2-[(diisopropoxyphosphoryl)methoxy]propyl}thymine (**35**)

Compound **34a** (724 mg, 1.7 mmol) in methanol (25 ml) was hydrogenated over 10% palladium on charcoal (733 mg) at room temperature for 13 h. The mixture was filtered through

a Celite pad. Water (2.5 ml) and Dowex 50X8 (H^+ form; 6 ml) were added to the filtrate and the resulting suspension was refluxed for 2 h. The suspension was applied onto a Dowex 50X8 (H^+ form; 30 ml) column and washed with 90% aqueous methanol until the UV absoption of the eluate dropped to the original value. The column was then eluted with 2.5% ammonia in 90% aqueous methanol and the fraction containing the product was evaporated in vacuo. The crude product was purified by chromatography on preparative TLC plate (S1, methanol). Yield 159 mg (24%) of **35** as a slightly yellow oil. IR (CCl_4), ν_{max}: 3411, 3172, 3060, 2981, 1693, 1608, 1424, 1255, 1010, 991. HR MS (FAB): for $C_{15}H_{29}N_3O_6P$ calculated 378.1794, found 378.1779. FAB MS, *m/z*: 378 $[MH]^+$ (58). ^{1}H NMR (400 MHz, $CDCl_3$): 1.32, 1.33 and 1.34 (3 × d, 12 H, J_{vic} = 6.2, $(CH_3)_2CH$); 1.92 (d, 3 H, $J_{CH3,6}$ = 1.2, CH_3-5); 2.75 (dd, 1 H, J_{gem} = 13.6, $J_{3'b,2'}$ = 4.9, H-3′b); 2.93 (dd, 1 H, J_{gem} = 13.6, $J_{3'a,2'}$ = 5.1, H-3′a); 3.67 (m, 1 H, H-2′); 3.74 (dd, 1 H, J_{gem} = 13.6, $J_{H,P}$ = 8.8, H-4′b); 3.85 (dd, 1 H, J_{gem} = 13.6, $J_{H,P}$ = 9.5, H-4′a); 3.86 (dd, 1 H, J_{gem} = 14.3, $J_{1'b,2'}$ = 7.6, H-1′b); 3.96 (dd, 1 H, J_{gem} = 14.3, $J_{1'a,2'}$ = 4.1, H-1′a); 4.74 (m, 2 H, $CH(CH_3)_2$); 7.25 (q, 1 H, $J_{6,CH3}$ = 1.2, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): 12.24 (CH_3-5); 23.96, 24.00 and 24.07 (d, $J_{C,P}$ = 4, $(CH_3)_2CH$); 41.54 (CH-3′); 48.84 (CH_2-1′); 64.96 (d, $J_{C,P}$ = 170, CH_2-4′); 71.14 and 71.22 (d, $J_{C,P}$ = 7, $CH(CH_3)_2$); 81.60 (d, $J_{C,P}$ = 11, CH-2′); 110.06 (C-5); 142.06 (CH-6); 151.07 (C-2); 163.89 (C-4).

Synthesis of 1-[3-Azido-2-(phosphonomethoxy)propyl]thymine (**36**) and 1-[3-Amino-2-(phosphonomethoxy)propyl]thymine (**37**). General Procedure

Compounds **36** and **37** were obtained by a procedure similar to that reported for **5a** and **5b**.

1-[3-Azido-2-(phosphonomethoxy)propyl]thymine (**36**). A mixture of compound **34a** (532 mg, 1.27 mmol) in acetonitrile (10 ml) and bromotrimethylsilane (1.8 ml) afforded 131 mg (31%) of dilithium salt of **36** as a white solid, m.p. > 300 °C. After deionization of product on activated charcoal the purification of **36** was performed by preparative HPLC (S6). IR (KBr), ν_{max}: 3242, 2106, 1684, 1475, 1437, 1386, 1351, 1225, 1177, 1139, 1077, 989, 921, 556, 470. HPLC, 99% (S4). For $C_9H_{12}Li_2N_5O_6P \cdot 1.25H_2O$ (335.6) calculated: 32.21% C, 3.75% H, 20.86% N, 9.23% P; found: 32.33% C, 3.82% H, 20.53% N, 11.26% P. FAB MS, *m/z*: 332 $[MH]^+$ (5). ^{1}H NMR (400 MHz, D_2O, ref(dioxane) 3.75 ppm): 1.88 (d, 3 H, $J_{CH3,6}$ = 1.2, CH_3-5); 3.42 (dd, 1 H, J_{gem} = 13.5, $J_{3'b,2'}$ = 4.4, H-3′b); 3.59 (dd, 1 H, J_{gem} = 12.8, $J_{H,P}$ = 9.4, H-4′b); 3.67 (dd, 1 H, J_{gem} = 13.5, $J_{3'a,2'}$ = 4.2, H-3′a); 3.71 (dd, 1 H, J_{gem} = 12.8, $J_{H,P}$ = 9.1, H-4′a); 3.88 (m, 1 H, H-2′); 3.91 (dd, 1 H, J_{gem} = 14.0, $J_{1'b,2'}$ = 6.1, H-1′b); 4.00 (dd, 1 H, J_{gem} = 14.0, $J_{1'a,2'}$ = 4.5, H-1′a); 7.57 (q, 1 H, $J_{6,CH3}$ = 1.2, H-6). ^{13}C NMR (125.8 MHz, D_2O, ref(dioxane) 69.3 ppm): 14.04 (CH_3-5); 51.63 (CH_2-1′); 53.39 (CH-3′); 69.74 (d, $J_{C,P}$ = 154, CH_2-4′); 80.64 (d, $J_{C,P}$ = 11, CH-2′); 113.29 (C-5); 146.68 (CH-6); 155.20 (C-2); 169.83 (C-4). ^{31}P NMR (162 MHz, D_2O): 14.53 (t, $J_{P,H\text{-}4'}$ = 9.4, 9.1).

1-[3-Amino-2-(phosphonomethoxy)propyl]thymine (**37**). A mixture of compound **35** (125 mg, 0.33 mmol) in acetonitrile (5 ml) and bromotrimethylsilane (0.5 ml) afforded 54 mg (56%) of phosphonic acid **37** as a white solid, m.p. 203–205 °C. The product was separated by chromatography on 40 ml of Sephadex (S2) and finally purified on Dowex 50X8 (H^+ form) column by washing with water. IR (KBr), ν_{max}: 3251, 3000, 1627, 1689, 1472, 1435, 1386, 1102, 1051, 914, 544, 459. HR MS (FAB): for $C_9H_{17}N_3O_6P$ calculated 294.0855, found 294.0841. FAB MS, *m/z*: 294 $[MH]^+$ (84). ^{1}H NMR (400 MHz, D_2O, ref(dioxane) 3.75 ppm): 1.88 (d, 3 H, $J_{CH3,6}$ = 1.1, CH_3-5); 3.02 (dd, 1 H, J_{gem} = 13.7, $J_{3'b,2'}$ = 8.9, H-3′b); 3.26 (dd, 1 H, J_{gem} = 13.7, $J_{3'a,2'}$ = 2.6, H-3′b); 3.64 and 3.86 (2 × dd, 2 H, J_{gem} = 12.8, $J_{H,P}$ = 9.6, H-4′b);

3.97–4.05 (m, 3 H, H-1′ and H-2′); 7.49 (q, 1 H, $J_{6,CH3}$ = 1.1, H-6). ^{13}C NMR (125.8 MHz, D_2O, ref(dioxane) 69.3 ppm): 14.01 (CH_3-5); 43.23 (CH-3′); 50.47 (CH_2-1′); 69.27 (d, $J_{C,P}$ = 157, CH_2-4′); 79.33 (d, $J_{C,P}$ = 12, CH-2′); 113.74 (C-5); 146.20 (CH-6); 155.25 (C-2); 169.69 (C-4). ^{31}P NMR (162 MHz, D_2O): 15.60 (t, $J_{P,H-4'}$ = 9.6).

Synthesis of 1-Methoxy-3-(trityloxy)propan-2-ols (**38a**, **38b**). General Procedure

A mixture of [(trityloxy)methyl]oxirane **2a** or **2b** (12.6 mmol) and sodium methoxide (12.7 mmol) in methanol (40 ml) was heated in an autoclave at 95 °C for 5 h. The mixture was concentrated in vacuo to a minimum volume. The residue was chromatographed on silica gel (chloroform followed by chloroform–methanol 20:1). The fractions containing product **38a** or **38b** were evaporated to dryness in vacuo.

(R)-1-Methoxy-3-(trityloxy)propan-2-ol (**38a**). Yield 3.2 g (74%) of a white solid. $[\alpha]_D$ +18.4 (*c* 0.328 g/100 ml, $CHCl_3$). HR MS (ESI): for $C_{23}H_{24}O_3Na$ calculated 371.1623, found 371.1605. For $C_{23}H_{24}O_3$ (348.4) calculated: 79.28% C, 6.94% H; found: 79.11% C, 6.68% H. ESI MS, *m/z*: 371 $[MNa]^+$ (100). ^{1}H NMR (400 MHz, $CDCl_3$): 2.42 (bd, 1 H, $J_{OH,CH}$ = 4.4, OH); 3.18 (dd, 1 H, J_{gem} = 9.4, J_{vic} = 5.3, bCH_2OTr); 3.20 (dd, 1 H, J_{gem} = 9.4, J_{vic} = 5.7, aCH_2OTr); 3.36 (s, 3 H, OCH_3); 3.43 (dd, 1 H, J_{gem} = 9.7, J_{vic} = 6.5, bCH_2OMe); 3.49 (dd, 1 H, J_{gem} = 9.7, J_{vic} = 4.1, aCH_2OMe); 3.95 (m, 1 H, J_{vic} = 6.5, 5.7, 5.3, 4.1, CH); 7.20–7.33 (m, 9 H, H-*m*,*p*-Tr); 7.41–7.46 (m, 6 H, H-*o*-Tr). ^{13}C NMR (100.6 MHz, $CDCl_3$): 59.12 (OCH_3); 64.65 (CH_2OTr); 69.81 (CH); 74.07 (CH_2OMe); 86.67 (C-Tr); 127.06 (CH-*p*-Tr); 127.83 (CH-*m*-Tr); 128.64 (CH-*o*-Tr); 143.80 (C-*i*-Tr).

(S)-1-Methoxy-3-(trityloxy)propan-2-ol (**38b**). Yield 4.1 g (93%) of a white solid. $[\alpha]_D$ –17.5 (*c* 0.192 g/100 ml, $CHCl_3$). For $C_{23}H_{24}O_3$ (348.4) calculated: 79.28% C, 6.94% H; found: 78.71% C, 6.87% H. ESI MS, *m/z*: 371 $[MNa]^+$ (100). ^{1}H, ^{13}C NMR and HR MS data were identical with compound **38a**.

Synthesis of Diisopropyl ({[1-Methoxy-3-(trityloxy)-2-propan-2-yl]oxy}methyl)-phosphonates (**39a**, **39b**). General Procedure

A mixture of compound **38a** or **38b** (11.4 mmol), (diisopropoxyphosphoryl)methyl tosylate (12.5 mmol) and 60% sodium hydride dispersion (17.1 mmol) in tetrahydrofuran (60 ml) was stirred at –20 °C. The suspension was allowed to warm to room temperature for 1 h and then stirred at the same temperature overnight. The suspension was heated at 40 °C for 8 h and the mixture was then filtered through a Celite pad. The mixture was concentrated in vacuo to a minimum volume and chromatographed on neutral aluminum oxide (chloroform). The fractions containing product **39a** or **39b** were evaporated to dryness in vacuo.

Diisopropyl ({[(R)-1-methoxy-3-(trityloxy)-2-propan-2-yl]oxy}methyl)phosphonate (**39a**). Yield 5.3 g (88%) of a slightly yellow oil. IR (CCl_4), ν_{max}: 3088, 3062, 3035, 2981, 2932, 2895, 2836, 1386, 1375, 1259, 1179, 1142, 1107, 1010, 991. HR MS (ESI): for $C_{30}H_{39}O_6NaP$ calculated 549.2382, found 549.2375. FAB MS, *m/z*: 549 $[MNa]^+$ (35). ^{1}H NMR (400 MHz, $CDCl_3$): 1.30, 1.31, 1.32 and 1.33 (4 × d, 4 × 3 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 3.22 (d, 2 H, J_{vic} = 5.1, CH_2OTr); 3.31 (s, 3 H, OCH_3); 3.50 (dd, 1 H, J_{gem} = 10.4, J_{vic} = 6.0, bCH_2OMe); 3.54 (dd, 1 H, J_{gem} = 10.4, J_{vic} = 4.2, aCH_2OMe); 3.73 (m, 1 H, J_{vic} = 6.0, 5.1, 4.2, CH); 3.91 (d, 2 H, $J_{H,P}$ = 8.8, CH_2P); 4.74 (dh, 2 H, $J_{H,P}$ = 7.5, J_{vic} = 6.2, C**H**$(CH_3)_2$); 7.20–7.33 (m, 9 H, H-*m*,*p*-Tr); 7.41–7.46 (m, 6 H, H-*o*-Tr). ^{13}C NMR (100.6 MHz, $CDCl_3$): 23.95, 23.98 and 24.11 (d, $J_{C,P}$ = 4, $(CH_3)_2$CH); 59.11 (OCH_3); 63.39 (CH_2OTr); 65.21 (d, $J_{C,P}$ = 167, CH_2P); 70.92 and 70.98

(d, $J_{C,P}$ = 6, $CH(CH_3)_2$); 73.11 (CH_2OMe); 80.33 (d, $J_{C,P}$ = 12, CH); 88.26 (C-Tr); 126.98 (CH-*p*-Tr); 127.77 (CH-*m*-Tr); 128.71 (CH-*o*-Tr); 143.90 (C-*i*-Tr).

Diisopropyl ({[(S)-1-methoxy-3-(trityloxy)-2-propan-2-yl]oxy}methyl)phosphonate (**39b**). Yield 4.9 g (82%) of a slightly yellow oil. FAB MS, *m/z*: 549 $[MNa]^+$ (100). ^1H, ^{13}C NMR, HR MS and IR data were identical with compound **39a**.

Synthesis of Diisopropyl ({[1-Methoxy-3-(tosyloxy)-2-propan-2-yl]oxy}methyl)-phosphonates (**40a**, **40b**). General Procedure

A mixture of compound **39a** or **39b** (6.6 mmol) and Dowex 50X8 (H^+ form; 15 ml) in 90% aqueous methanol (25 ml) was vigorously refluxed for 5 h until the trityl group of starting phosphonate was completely removed (TLC in chloroform). After cooling the mixture was filtered through a Celite pad and concentrated in vacuo to a minimum volume. The residue was dissolved in dichloromethane (20 ml) and triethylamine (5 ml). The solution of tosyl chloride (7.8 mmol) in dichloromethane (10 ml) was added at 0 °C and the resulting mixture was stirred at room temperarure for 2 h. 4-(Dimethylamino)pyridine (0.1 mmol) was added and the mixture was then refluxed for 10 h. The mixture was concentrated in vacuo to a minimum volume, the residue was chromatographed on silica gel (chloroform followed by chloroform–methanol 20:1) and appropriate fractions containing **40a** or **40b** were evaporated to dryness in vacuo.

Diisopropyl ({[(R)-1-Methoxy-3-(tosyloxy)-2-propan-2-yl]oxy}methyl)phosphonate (**40a**). Yield 1.3 g (46%) of a slightly yellow oil. IR (CCl_4), ν_{max}: 2981, 2880, 1386, 1375, 1258, 1190, 1179, 1142, 1121, 1106, 1100, 1020, 815, 555. $[\alpha]_D$ +3.6 (*c* 0.414 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{18}H_{32}O_8PS$ calculated 439.1556, found 439.1570. FAB MS, *m/z*: 439 $[MH]^+$ (13). ^1H NMR (500 MHz, $CDCl_3$): 1.31, 1.32 and 1.33 (3 × d, 12 H, J_{vic} = 6.2, $(CH_3)_2CH$); 2.46 (s, 3 H, CH_3-Ts); 3.29 (s, 3 H, OCH_3); 3.44 and 3.47 (2 × dd, 2 H, J_{gem} = 10.3, J_{vic} = 5.1, CH_2OMe); 3.82 (dd, 1 H, J_{gem} = 13.8, $J_{H,P}$ = 8.6, bCH_2P); 3.83 (m, 1 H, CH); 3.84 (dd, 1 H, J_{gem} = 13.8, $J_{H,P}$ = 8.6, aCH_2P); 4.06 (dd, 1 H, J_{gem} = 10.5, J_{vic} = 6.0, bCH_2OTs); 4.15 (dd, 1 H, J_{gem} = 10.5, J_{vic} = 4.2, aCH_2OTs); 4.72 (m, 2 H, C**H**$(CH_3)_2$); 7.36 (m, 2 H, H-*m*-Ts); 7.80 (m, 2 H, H-*m*-Ts). ^{13}C NMR (125.8 MHz, $CDCl_3$): 21.64 (CH_3-Ts); 23.90, 23.93 and 24.06 (d, $J_{C,P}$ = 4, $(CH_3)_2CH$); 59.22 (OCH_3); 65.10 (d, $J_{C,P}$ = 168, CH_2P); 69.06 (CH_2OTs); 70.98 (CH_2OMe); 71.16 and 71.18 (d, $J_{C,P}$ = 6, $CH(CH_3)_2$); 77.93 (d, $J_{C,P}$ = 10, CH); 127.96 (CH-*o*-Ts); 129.87 (CH-*m*-Ts); 132.48 (C-*i*-Ts); 144.93 (C-*p*-Tr).

Diisopropyl ({[(S)-1-methoxy-3-(tosyloxy)-2-propan-2-yl]oxy}methyl)phosphonate (**40b**). Yield 2.7 g (91%) of a slightly yellow oil. $[\alpha]_D$ –0.6 (*c* 0.490 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{18}H_{32}O_8PS$ calculated 439.1556, found 439.1558. FAB MS, *m/z*: 439 $[MH]^+$ (22). ^1H, ^{13}C NMR and IR data were identical with those of compound **40a**.

Synthesis of 2-[(Diisopropoxyphosphoryl)methoxy]-3-methoxypropyl Derivatives of 5-Alkyl-4-methoxypyrimidin-2(1*H*)-one and Pyrimidine **41**–**44**. General Procedure

A mixture of pyrimidin-2(1*H*)-one **1** or **22** (3 mmol) and 60% sodium hydride dispersion (3 mmol) in dimethylformamide (40 ml) was stirred at room temperature for 1 h. Compound **40a** or **40b** (3 mmol) in dimethylformamide (25 ml) was added at 80 °C and the resulting mixture was heated at 100 °C for 9 h. The mixture was concentrated in vacuo to a minimum volume. The residue was codistilled with toluene (2 × 20 ml). The residue was chromatographed on neutral aluminum oxide (ethyl acetate followed by ethyl acetate–chloroform–methanol 26:25:1). The crude product was purified by preparative TLC (S1, ethyl

acetate–chloroform–methanol 26:25:1). The relevant fractions were combined and evaporated in vacuo.

1-{(R)-2-[(Diisopropoxyphosphoryl)methoxy]-3-methoxypropyl}-5-ethyl-4-methoxypyrimidin-2(1H)-one (**41a**). Yield 441 mg (35%) of a slightly yellow oil. R_F 0.25 (ethyl acetate–chloroform–methanol 26:25:1). IR (CCl_4), ν_{max}: 2998, 2937, 2980, 1673, 1652, 1536, 1471, 1403, 1255, 1107, 1007, 991. $[\alpha]_D$ +49.0 (*c* 0.539 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{18}H_{34}N_2O_7P$ calculated 421.2104, found 421.2120. FAB MS, *m/z*: 421 $[MH]^+$ (100). ^{1}H NMR (500 MHz, $CDCl_3$): 1.16 (t, 3 H, J_{vic} = 7.5, C**H**$_3$CH$_2$); 1.28, 1.29, 1.30 and 1.31 (4 × d, 4 × 3 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 2.34 and 2.38 (2 × dqd, 2 H, J_{gem} = 17.3, J_{vic} = 7.5, $J_{CH2,6}$ = 1.0, C**H**$_2$CH$_3$); 3.36 (s, 3 H, OCH$_3$-3′); 3.44 (dd, 1 H, J_{gem} = 10.8, $J_{3'b,2'}$ = 4.7, H-3′b); 3.63 (dd, 1 H, J_{gem} = 10.8, $J_{3'a,2'}$ = 3.3, H-3′a); 3.70 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 9.2, H-4′b); 3.77 (dd, 1 H, J_{gem} = 13.8, $J_{1'b,2'}$ = 7.7, H-1′b); 3.91 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 8.6, H-4′a); 3.95 (m, 1 H, H-2′); 3.98 (s, 3 H, OCH$_3$-4); 4.19 (dd, 1 H, J_{gem} = 13.8, $J_{1'a,2'}$ = 3.7, H-1′a); 4.69 and 4.71 (2 × dh, 2 H, $J_{H,P}$ = 7.8, J_{vic} = 6.2, C**H**(CH$_3$)$_2$); 7.34 (t, 1 H, $J_{6,CH2}$ = 1.0, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): 13.03 (**C**H$_3$CH$_2$); 19.70 (**C**H$_2$CH$_3$); 23.90, 23.93, 24.02 and 24.04 (d, $J_{C,P}$ = 4, (**C**H$_3$)$_2$CH); 50.96 (CH$_2$-1′); 54.42 (OCH$_3$-4); 59.27 (OCH$_3$-3′); 65.04 (d, $J_{C,P}$ = 168, CH$_2$-4′); 70.93 and 71.02 (d, $J_{C,P}$ = 7, **C**H(CH$_3$)$_2$); 71.90 (CH-3′); 78.57 (d, $J_{C,P}$ = 11, CH-2′); 109.89 (C-5); 145.06 (CH-6); 156.67 (C-2); 170.64 (C-4).

1-{(S)-2-[(Diisopropoxyphosphoryl)methoxy]-3-methoxypropyl}-5-ethyl-4-methoxypyrimidin-2(1H)-one (**41b**). Yield 440 mg (35%) of a slightly yellow oil. R_F 0.25 in ethyl acetate–chloroform–methanol 26:25:1. $[\alpha]_D$ –45.3 (*c* 0.651 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{18}H_{34}N_2O_7P$ calculated 421.2104, found 421.2083. FAB MS, *m/z*: 421 $[MH]^+$ (100). ^{1}H, ^{13}C NMR and IR data were identical with compound **41a**.

1-{(R)-2-[(Diisopropoxyphosphoryl)methoxy]-3-methoxypropyl}-4-methoxy-5-methylpyrimidin-2(1H)-one (**42a**). Yield 304 mg (25%) of a slightly yellow oil. R_F 0.25 in ethyl acetate–chloroform–methanol 26:25:1. IR (CCl_4), ν_{max}: 2981, 2899, 2836, 1676, 1655, 1541, 1475, 1255, 1141, 1107, 1011, 991. $[\alpha]_D$ +88.8 (*c* 0.526 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{17}H_{32}N_2O_7P$ calculated 407.1947, found 407.1961. FAB MS, *m/z*: 407 $[MH]^+$ (100). ^{1}H NMR (400 MHz, $CDCl_3$): 1.29, 1.30, 1.31 and 1.32 (4 × d, 4 × 3 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 1.94 (d, 3 H, $J_{CH3,6}$ = 1.1, CH$_3$-5); 3.36 (s, 3 H, OCH$_3$-3′); 3.45 (dd, 1 H, J_{gem} = 10.8, $J_{3'b,2'}$ = 4.9, H-3′b); 3.63 (dd, 1 H, J_{gem} = 10.8, $J_{3'a,2'}$ = 3.3, H-3′a); 3.69 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 9.2, H-4′b); 3.73 (dd, 1 H, J_{gem} = 13.8, $J_{1'b,2'}$ = 7.7, H-1′b); 3.93 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 8.8, H-4′a); 3.94 (m, 1 H, H-2′); 3.98 (s, 3 H, OCH$_3$-4); 4.20 (dd, 1 H, J_{gem} = 13.8, $J_{1'a,2'}$ = 3.5, H-1′a); 4.70 (dh, 2 H, $J_{H,P}$ = 7.7, J_{vic} = 6.2, C**H**(CH$_3$)$_2$); 7.38 (q, 1 H, $J_{6,CH3}$ = 1.1, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): 12.02 (CH$_3$-5); 23.91, 23.93 and 24.04 (d, $J_{C,P}$ = 4, (**C**H$_3$)$_2$CH); 50.84 (CH$_2$-1′); 54.49 (OCH$_3$-4); 59.28 (OCH$_3$-3′); 65.05 (d, $J_{C,P}$ = 168, CH$_2$-4′); 70.90 and 71.01 (d, $J_{C,P}$ = 7, **C**H(CH$_3$)$_2$); 72.04 (CH-3′); 78.72 (d, $J_{C,P}$ = 11, CH-2′); 103.85 (C-5); 145.92 (CH-6); 156.77 (C-2); 170.88 (C-4).

1-{(S)-2-[(Diisopropoxyphosphoryl)methoxy]-3-methoxypropyl}-4-methoxy-5-methylpyrimidin-2(1H)-one (**42b**). Yield 366 mg (30%) of a slightly yellow oil. R_F 0.25 in ethyl acetate–chloroform–methanol 26:25:1. $[\alpha]_D$ –79.4 (*c* 0.487 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{17}H_{32}N_2O_7P$ calculated 407.1947, found 407.1934. FAB MS, *m/z*: 407 $[MH]^+$ (100). ^{1}H, ^{13}C NMR and IR data were identical with those of compound **42a**.

2-{(R)-2-[(Diisopropoxyphosphoryl)methoxy]-3-methoxypropoxy}-5-ethyl-4-methoxypyrimidine (**43a**). Yield 252 mg (20%) of a slightly yellow oil. R_F 0.50, ethyl acetate–chloroform–methanol 26:25:1). IR (CCl_4), ν_{max}: 2980, 1603, 1571, 1424, 1399, 1386, 1375, 1352, 1258, 1107, 1011, 991. $[\alpha]_D$ +12.8 (*c* 0.392 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{18}H_{34}N_2O_7P$ calculated

421.2104, found 421.2124. FAB MS, *m/z*: 421 $[MH]^+$ (100). ^{1}H NMR (400 MHz, $CDCl_3$): 1.16 (t, 3 H, J_{vic} = 7.5, C**H**$_3$CH$_2$); 1.31, 1.32 and 1.33 (3 × d, 12 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 2.48 (qd, 2 H, J_{vic} = 7.5, $J_{CH2,6}$ = 0.7, C**H**$_2$CH$_3$); 3.38 (s, 3 H, OCH$_3$-3′); 3.60 (dd, 1 H, J_{gem} = 10.4, $J_{3'b,2'}$ = 5.5, H-3′b); 3.64 (dd, 1 H, J_{gem} = 10.4, $J_{3'a,2'}$ = 4.5, H-3′a); 3.98 (s, 3 H, OCH$_3$-4); 3.99 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 8.8, H-4′b); 4.01 (m, 1 H, H-2′); 4.03 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 8.8, H-4′a); 4.41 (dd, 1 H, J_{gem} = 11.4, $J_{1'b,2'}$ = 5.2, H-1′b); 4.44 (dd, 1 H, J_{gem} = 11.4, $J_{1'a,2'}$ = 5.8, H-1′a); 4.75 (m, 2 H, C**H**(CH$_3$)$_2$); 7.97 (t, 1 H, $J_{6,CH2}$ = 0.7, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): 13.43 (**C**H$_3$CH$_2$); 19.79 (**C**H$_2$CH$_3$); 23.90 and 24.08 (d, $J_{C,P}$ = 4, (**C**H$_3$)$_2$CH); 53.80 (OCH$_3$-4); 59.23 (OCH$_3$-3′); 65.20 (d, $J_{C,P}$ = 167, CH$_2$-4′); 66.50 (CH$_2$-1′); 71.03 and 71.08 (d, $J_{C,P}$ = 6, **C**H(CH$_3$)$_2$); 72.48 (CH-3′); 78.83 (d, $J_{C,P}$ = 12, CH-2′); 117.07 (C-5); 156.03 (CH-6); 163.21 (C-2); 169.32 (C-4).

2-{(S)-2-[(Diisopropoxyphosphoryl)methoxy]-3-methoxypropoxy}-5-ethyl-4-methoxypyrimidine (**43b**). Yield 530 mg (42%) of a slightly yellow oil. R_F 0.50 in ethyl acetate–chloroform–methanol 26:25:1. $[\alpha]_D$ –10.2 (*c* 0.720 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{18}H_{34}N_2O_7P$ calculated 421.2104, found 421.2124. FAB MS, *m/z*: 421 $[MH]^+$ (100). ^{1}H, ^{13}C NMR and IR data were identical with those of compound **43a**.

2-{(R)-2-[(Diisopropoxyphosphoryl)methoxy]-3-methoxypropoxy}-4-methoxy-5-methylpyrimidine (**44a**). Yield 207 mg (17%) of a slightly yellow oil. R_F 0.50 in ethyl acetate–chloroform–methanol 26:25:1. IR (CCl_4), ν_{max}: 2981, 1608, 1575, 1424, 1397, 1386, 1374, 1258, 1107, 1010, 992. $[\alpha]_D$ +20.0 (*c* 0.231 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{17}H_{32}N_2O_7P$ calculated 407.1947, found 407.1936. FAB MS, *m/z*: 407 $[MH]^+$ (100). ^{1}H NMR (400 MHz, $CDCl_3$): 1.31, 1.32, 1.33 and 1.34 (4 × d, 4 × 3 H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 2.05 (d, 2 H, $J_{CH3,6}$ = 1.0, CH$_3$-5); 3.37 (s, 3 H, OCH$_3$-3′); 3.60 (dd, 1 H, J_{gem} = 10.4, $J_{3'b,2'}$ = 5.5, H-3′b); 3.63 (dd, 1 H, J_{gem} = 10.4, $J_{3'a,2'}$ = 4.6, H-3′a); 3.98 (s, 3 H, OCH$_3$-4); 3.99 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 8.8, H-4′b); 4.01 (m, 1 H, H-2′); 4.03 (dd, 1 H, J_{gem} = 13.7, $J_{H,P}$ = 8.8, H-4′a); 4.41 (dd, 1 H, J_{gem} = 11.4, $J_{1'b,2'}$ = 5.2, H-1′b); 4.44 (dd, 1 H, J_{gem} = 11.4, $J_{1'a,2'}$ = 5.8, H-1′a); 4.75 (m, 2 H, C**H**(CH$_3$)$_2$); 7.96 (q, 1 H, $J_{6,CH3}$ = 1.1, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): 11.84 (CH$_3$-5); 23.93 and 24.10 (d, $J_{C,P}$ = 4, (**C**H$_3$)$_2$CH); 53.87 (OCH$_3$-4); 59.24 (OCH$_3$-3′); 65.25 (d, $J_{C,P}$ = 167, CH$_2$-4′); 66.56 (CH$_2$-1′); 71.01 and 71.06 (d, $J_{C,P}$ = 7, **C**H(CH$_3$)$_2$); 72.50 (CH-3′); 78.84 (d, $J_{C,P}$ = 12, CH-2′); 111.23 (C-5); 156.99 (CH-6); 163.24 (C-2); 169.41 (C-4).

2-{(S)-2-[(Diisopropoxyphosphoryl)methoxy]-3-methoxypropoxy}-4-methoxy-5-methylpyrimidine (**44b**). Yield 524 mg (43%) of a slightly yellow oil. R_F 0.50 (TLC, ethyl acetate–chloroform–methanol 26:25:1). $[\alpha]_D$ –16.4 (*c* 0.268 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{17}H_{32}N_2O_7P$ calculated 407.1947, found 407.1957. FAB MS, *m/z*: 407 $[MH]^+$ (100). ^{1}H, ^{13}C NMR and IR data were identical with those of compound **44a**.

Synthesis of 1-[2-(Phosphonomethoxy)-3-methoxypropyl] Derivatives of 5-Ethyluracil and Thymine **45** and **46**. General Procedure

A mixture of compounds **41** or **42** (1 mmol) in acetonitrile (10 ml) and bromotrimethylsilane (10 mmol) afforded corresponding derivatives **45**, **46** which were obtained as dilithium salts by the same procedure as described for **5a** or **5b**.

5-Ethyl-1-[(R)-2-(Phosphonomethoxy)-3-methoxypropyl]uracil (**45a**). Yield 243 mg (69%) of a white solid, m.p. > 300 °C. $[\alpha]_D$ +22.1 (*c* 0.261 g/100 ml, H_2O). IR (KBr), ν_{max}: 3188, 3035, 2894, 2831, 1689, 1460, 1107, 1090, 991. HPLC, 99% (S3). HR MS (FAB): for $C_{11}H_{18}Li_2N_2O_7P$ calculated 335.1171, found 335.1167. For $C_{11}H_{17}Li_2N_2O_7P \cdot 1H_2O$ (352.1) calculated: 37.57% C, 5.43% H, 7.96% N, 8.80% P; found: 37.57% C, 5.54% H, 7.79% N,

8.44% P. FAB MS, *m/z*: 335 $[MH]^+$ (100). ^{1}H NMR (400 MHz, D_2O, ref(dioxane) 3.75 ppm): 1.09 (t, 3 H, J_{vic} = 7.5, C**H**$_3$CH$_2$); 2.31 (qd, 2 H, J_{vic} = 7.5, $J_{CH2,6}$ = 1.0, C**H**$_2$CH$_3$); 3.36 (OCH_3); 3.47 (dd, 1 H, J_{gem} = 11.0, $J_{3'b,2'}$ = 4.3, H-3′b); 3.58 (dd, 1 H, J_{gem} = 12.4, $J_{H,P}$ = 9.5, H-4′b); 3.62 (dd, 1 H, J_{gem} = 12.4, $J_{H,P}$ = 9.4, H-4′a); 3.67 (dd, 1 H, J_{gem} = 11.0, $J_{3'a,2'}$ = 3.8, H-3′a); 3.83 (tt, 1 H, $J_{2',1'}$ = 5.8, $J_{2',3'}$ = 4.3, 3.8, H-2′); 3.92 (dd, 1 H, J_{gem} = 14.3, $J_{1'b,2'}$ = 5.8, H-1′b); 4.01 (dd, 1 H, J_{gem} = 14.3, $J_{1'a,2'}$ = 5.8, H-1′a); 7.50 (t, 1 H, $J_{6,CH2}$ = 1.0, H-6). ^{13}C NMR (125.8 MHz, D_2O, ref(dioxane) 69.3 ppm): 15.03 (CH_3CH$_2$); 22.08 (CH_2CH$_3$); 51.15 (CH_2-1′); 61.21 (OCH_3); 70.07 (d, $J_{C,P}$ = 154, CH_2-4′); 73.48 (CH-3′); 80.23 (d, $J_{C,P}$ = 12, CH-2′); 118.96 (C-5); 146.17 (CH-6); 155.10 (C-2); 169.43 (C-4). ^{31}P NMR (162 MHz, D_2O): 14.36 (t, $J_{P,H-4'}$ = 9.5, 9.4).

5-Ethyl-1-[(S)-2-(phosphonomethoxy)-3-methoxypropyl]uracil (**45b**). Yield 239 mg (68%) of a white solid, m.p. > 300 °C. $[\alpha]_D$ –20.1 (*c* 0.250 g/100 ml, H_2O). HPLC, 99% (S3). HR MS (FAB): for $C_{11}H_{18}Li_2N_2O_7P$ calculated 335.1171, found 335.1166. For $C_{11}H_{17}Li_2N_2O_7P \cdot 1H_2O$ (352.1) calculated: 37.57% C, 5.43% H, 7.96% N, 8.80% P; found: 37.56% C, 5.36% H, 7.72% N, 8.54% P. FAB MS, *m/z*: 335 $[MH]^+$ (51). ^{1}H, ^{13}C NMR and IR data were identical with compound **45a**.

1-[(R)-2-(Phosphonomethoxy)-3-methoxypropyl]thymine (**46a**). Yield 252 mg (71%) of a white solid, m.p. > 300 °C. IR (KBr), ν_{max}: 3260, 2902, 2837, 1690, 1474, 1439, 1105, 1081, 1073, 992. $[\alpha]_D$ +27.9 (*c* 0.330 g/100 ml, H_2O). HPLC, 99% (S3). HR MS (FAB): for $C_{10}H_{16}Li_2N_2O_7P$ calculated 321.1015, found 321.1022. For $C_{10}H_{15}Li_2N_2O_7P \cdot 2H_2O$ (356.1) calculated: 33.73% C, 5.38% H, 7.86% N, 8.69% P; found: 34.07% C, 5.54% H, 7.88% N, 8.35% P. FAB MS, *m/z*: 321 $[MH]^+$ (37). ^{1}H NMR (400 MHz, D_2O, ref(dioxane) 3.75 ppm): 1.88 (d, 3 H, $J_{CH3,6}$ = 1.2, CH_3-5); 3.36 (OCH_3); 3.48 (dd, 1 H, J_{gem} = 11.0, $J_{3'b,2'}$ = 4.5, H-3′b); 3.57 (dd, 1 H, J_{gem} = 12.5, $J_{H,P}$ = 9.6, H-4′b); 3.64 (dd, 1 H, J_{gem} = 12.5, $J_{H,P}$ = 9.4, H-4′a); 3.67 (dd, 1 H, J_{gem} = 11.0, $J_{3'a,2'}$ = 3.9, H-3′a); 3.81 (bp, 1 H, $J_{2',1'}$ = 5.9, 5.1, $J_{2',3'}$ = 4.5, 3.9, H-2′); 3.93 (dd, 1 H, J_{gem} = 14.4, $J_{1'b,2'}$ = 5.1, H-1′b); 3.97 (dd, 1 H, J_{gem} = 14.4, $J_{1'a,2'}$ = 5.9, H-1′a); 7.55 (q, 1 H, $J_{6,CH3}$ = 1.2, H-6). ^{13}C NMR (125.8 MHz, D_2O, ref(dioxane) 69.3 ppm): 14.02 (CH_3-5); 51.14 (CH_2-1′); 61.23 (OCH_3); 69.87 (d, $J_{C,P}$ = 154, CH_2-4′); 73.56 (CH-3′); 80.46 (d, $J_{C,P}$ = 12, CH-2′); 113.14 (C-5); 146.78 (CH-6); 155.20 (C-2); 169.86 (C-4). ^{31}P NMR (162 MHz, D_2O): 14.67 (t, $J_{P,H-4'}$ = 9.6, 9.4).

1-[(S)-2-(Phosphonomethoxy)-3-methoxypropyl]thymine (**46b**). Yield 228 mg (64%) of a white solid, m.p. > 300 °C. $[\alpha]_D$ –23.4 (*c* 0.280 g/100 ml, H_2O). For $C_{10}H_{15}Li_2N_2O_7P \cdot 2H_2O$ (356.1) calculated: 33.73% C, 5.38% H, 7.86% N, 8.69% P; found: 34.88% C, 4.94% H, 7.52% N, 8.33% P. FAB MS, *m/z*: 321 $[MH]^+$ (27). ^{1}H, ^{13}C NMR, HR MS and IR data were identical with compound **46a**.

The study has been supported by the Grant Agency of the Czech Republic (grant No. 203/03/0089) as a part of research project Z 40550506 of the Institute of Organic Chemistry and Biochemistry, Prague and by the Descartes Prize HPAW-CT-2002-9001. The authors thank the Analytical Department of the Institute (Dr K. Ubik, Head), and Dr O. Špalek and Dr V. Jirásek (Institute of Physics, Academy of Sciences of the Czech Republic, Prague) for assistance. Excellent technical assistance of Ms J. Křelinová is also acknowledged.

REFERENCES

1. Holý A.: *Curr. Pharm. Design.* **2003**, *9*, 2567.
2. Esteban-Gamboa A., Balzarini J., Esnouf R., De Clercq E., Camarasa M.-J., Pérez-Pérez M.-J.: *J. Med. Chem.* **2000**, *43*, 971.
3. Pomeisl K., Pohl R., Holý A., Votruba I.: *Collect. Czech. Chem. Commun.* **2005**, *70*, 1465.
4. Votruba I., Pomeisl K., Tloušťová E., Holý A., Otová B.: *Biochem. Pharmacol.* **2005**, *69*, 1517.
5. Krečmerová M., Holý A., Masojídková M.: *Collect. Czech. Chem. Commun.* **2006**, *71*, 579.
6. Klein R. S., Lenzi M., Lim T. H., Hotchkiss K. A., Wilson P., Schwartz E. L.: *Biochem. Pharmacol.* **2001**, *62*, 1257.
7. Esteban-Gamboa A., Balzarini J., Esnouf R., De Clercq E., Camarasa M.-J., Pérez-Pérez M.-J.: *J. Med. Chem.* **2000**, *43*, 971.
8. Béres J., Sági G., Bentrude W. G., Balzarini J., De Clercq E., Ötvös L.: *J. Med. Chem.* **1986**, *29*, 1243.
9. Myers C. E.: *Pharmacol. Rev.* **1981**, *33*, 1.
10. Visser G. W. M., Boele S., v. Halteren B. W., Knops G. H. J. N., Herscheid J. D. M., Brinkman G. A., Hoekstra A.: *J. Org. Chem.* **1986**, *51*, 1466.
11. Holý A., Ivanova G. S.: *Nucleic Acids Res.* **1974**, *1*, 19.
12. a) Holý A., König J., Veselý J., Cech D., Votruba I., De Clercq E.: *Collect. Czech. Chem. Commun.* **1987**, *52*, 1589; b) Schwartz B., Cech D., Holý A., Škoda J.: *Collect. Czech. Chem. Commun.* **1980**, *45*, 3217.
13. Dvořáková H., Masojídková M., Holý A., Balzarini J., Andrei G., Snoeck R., De Clercq E.: *J. Med. Chem.* **1996**, *39*, 3263.
14. Holý A., Dvořáková H.: *Nucleosides Nucleotides* **1995**, *14*, 695.
15. Hocek M., Masojídková M., Holý A., Andrei G., Snoeck R., Balzarini J., De Clercq E.: *Collect. Czech. Chem. Commun.* **1996**, *61*, 1525.
16. Parker D.: *Chem. Rev.* **1991**, *91*, 1441.
17. Nencka R., Votruba I., Hřebabecký H., Tloušťová E., Horská K., Masojídková M., Holý A.: *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1335.
18. Wishart S., Bigam C. G., Abildgaard J., Yao F., Dyson H. J., Oldfield E., Markley J. L., Sykes B. D.: *Biomol. NMR* **1995**, *6*, 135.
19. Holý A.: *Collect. Czech. Chem. Commun.* **1993**, *58*, 649.
20. Holý A., Günter J., Dvořáková H., Masojídková M., Andrei G., Snoeck R., Balzarini J., De Clercq E.: *J. Med. Chem.* **1999**, *42*, 2064.