# SIMPLE TRANSFORMATION OF THYMINE 1-[3-HYDROXY-2-(PHOSPHONOMETHOXY)PROPYL] DERIVATIVES TO THEIR 1-[3-FLUORO-2-(PHOSPHONOMETHOXY)PROPYL] COUNTERPARTS

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A novel method of transformation of $HOCH_2$ group to $FCH_2$ was successfully applied to the preparation of fluorine-containing pyrimidine acyclic nucleoside phosphonates (FPMP compounds) such as (*S*)- and (*R*)-1-[3-fluoro-2-(phosphonomethoxy)propyl]thymine (**7a**, **7b**) (FPMPT). The key displacement of hydroxy group with fluorine in 1-{2-[(diisopropoxyphosphoryl)-methoxy]-3-hydroxypropyl}-4-methoxy-5-methylpyrimidin-2(1*H*)-one (**5a**, **5b**) was performed using perfluorobutane-1-sulfonyl fluoride in the presence of DBU. Novel pyrimidine acyclic nucleoside phosphonates were investigated as inhibitors of thymidine phosphorylase.

**Keywords**: Acyclic nucleoside phosphonates; Acyclic nucleotide analogues; Thymidine phosphorylase; Deoxyfluorinations; Thymine; Pyrimidines; Antivirals.

Acyclic nucleoside phosphonates[1] (ANPs) containing fluorine atom in the phosphonate-bearing side chain exhibit significant biological activity. It is documented by the antiretroviral effect of 9-[3-fluoro-2-(phosphonomethoxy)propyl]adenine[2] (FPMPA) and some of its congeners modified at the heterocyclic base. In contrast to 9-[2-(phosphonomethoxy)ethyl] (PME) or 9-[3-hydroxy-2-(phosphonomethoxy)propyl] (HPMP) purine derivatives, FPMP compounds possess no activity against DNA viruses; the hydroxy group displacement with fluorine results in the change of "quality" of antiviral effect. In analogy to PME or HPMP derivatives, also FPMP compounds are phosphorylated in the cell to give diphosphates which terminate the DNA chain[3] growth. However, the FPMP compounds are less toxic to host cells *in vitro* compared with the PME derivatives[2]. Despite their activity which was confirmed by *in vivo* experiments with animal viruses homologous to HIV, the FPMP derivatives never found the way to practical development. One of the reasons for this neglection may well be their difficult accessibility.

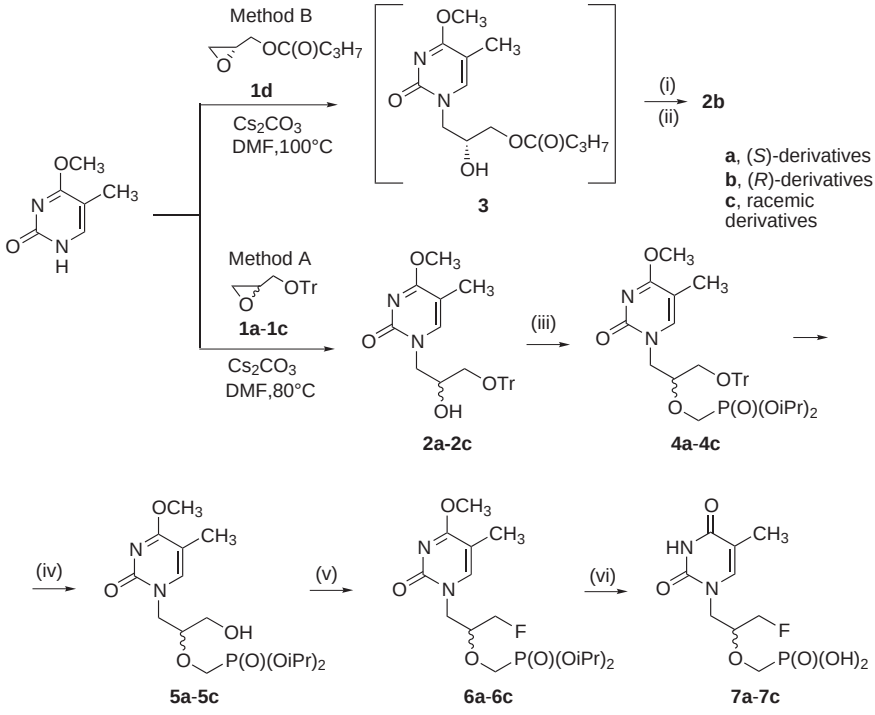
Fluorine-containing substituents are also powerful modifiers of chemical and biological properties of nucleosides (e.g. FIAU, FIAC, FMAU etc.)[4]. Their syntheses include various fluorination methods. The most useful reagents for primary or secondary hydroxy group displacement in saccharide-containing nucleosides with fluorine are DAST [5], Olah's reagent[6], fluoroalkylamine reagents[7] (FAR), etc. In addition, preformed synthons containing fluoro substituents can be used for glycosylation[8] or alkylation[2a,9] of heterocyclic bases.

Fluorination of nucleosides and ANPs [9] has been also a subject of our research. In this report, we describe the syntheses of several fluorinated side-chain modified pyrimidine ANPs which show potent *in vitro* inhibition of thymidine phosphorylase[10] (dThdPase).

In our studies, we were particularly interested in the side-chain fluorination of the hydroxy group in pyrimidine 1-[3-hydroxy-2-(phosphonomethoxy)propyl] derivatives in order to replace the existing method of FPMP synthesis by better available HPMP derivatives as starting materials. As models we were using enantiomeric thymine compounds **5a** and **5b**. For this purpose, we have elaborated an improved synthesis of starting enantiomeric non-fluorinated side-chain modified compounds **11a**, **11b** and **15a**, **15b**. The other aim of our study is search for novel efficient inhibitors of thymidine phosphorylase *in vitro*[2]; we have prepared both the optical isomers and the racemate to compare their activity.

In our previous syntheses of C-3′-fluorinated ANP derivatives (FPMP compounds), we were using fluorinated synthons obtained by multistep reactions[2a,9]. For the preparation of **7a**–**7c** we used weakly corrosive perfluorobutane-1-sulfonyl fluoride[11] as a nucleophilic fluorination agent in this study. This reagent is stable and little moisture-sensitive at room temperature, and therefore easy to handle. The key intermediates **5a**–**5c** for this kind of side-chain fluorination were obtained in several reaction steps from 4-methoxy-5-methylpyrimidin-2(1*H*)-one[12] (Scheme 1): $N^1$-alkylation of the heterocyclic base with various oxiranes[13] **1a**–**1d** takes place under the conditions used in methods *A* or *B*. Both reactions were carried out in dimethylformamide in the presence of cesium carbonate as catalyst and afforded hydroxy derivatives **2a**–**2c** in acceptable yields. Furthermore, method *B* includes even methanolysis[14] of intermediary product **3** . Subsequent tritylation proceeded by reaction with trityl chloride in pyridine in the presence of 4-(*N*,*N*-dimethylamino)pyridine.

For the preparation of **4a**–**4c** we have used general procedures reported previously[14,15]. A mixture of starting components **2a**–**2c** and [(diisopropoxyphosphoryl)methoxy]methyl tosylate in the presence of sodium



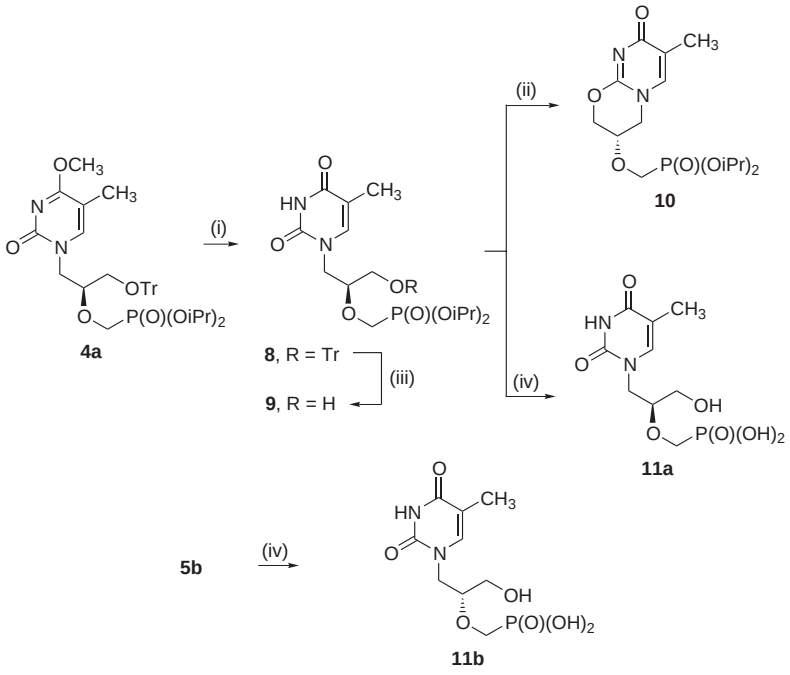

(i) MeONa, MeOH; (ii) TrCl, DMAP, Py, rt; (iii) $TsCH_2P(O)(OiPr)_2$, NaH, THF, –20°C→rt;
(iv) method C: $H_2$, Pd/C, 99% AcOH, MeOH, **5a** and **5b**; method C: 80%AcOH, **5c;**
(v) $C_4F_9SO_2F$, DBU, toluene, rt→90°C; (vi) $(CH_3)_3SiBr$, $CH_3CN$, rt

SCHEME 1

hydride was first stirred in tetrahydrofuran at –20 °C; the reaction then proceeded at 25–30 °C. The choice of adsorbent was shown to be important for chromatographic separation of reaction products. While the isolation of 3-hydroxy derivatives was successful with neutral aluminum oxide, the C-4-methoxy group of the (*S*)-enantiomer **4a** was completely removed by silica gel column chromatography (Scheme 2). On the other hand, compound **9** which resulted from this reaction sequence was applicable to side-chain fluorination. However, the displacement of the C-3′-hydroxy group with fluorine could be complicated if a heterocyclic base contains labile proton in the $N^3$-position.

Therefore we have deprotected the side chain of compounds **4a**–**4c** to give **5a**–**5c**: the trityl group was removed by mild hydrogenation in methanol over 5% palladium on charcoal in the presence of acetic

acid (method *C*). Likewise, selective deprotection of racemic derivative **4c** with excess of 80% acetic acid without hydrogenation was also tested[14] (method *D*). This reaction afforded similar results provided that the reaction mixture was performed in the temperature range of 40–70 °C.



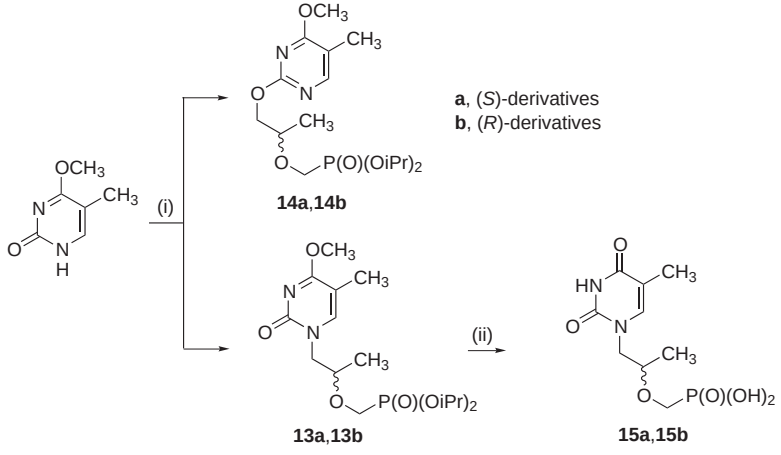

(i) $H^+$,$H_2O$, $SiO_2$; (ii) $C_4F_9SO_2F$, DBU, toluene, rt→90°C; (iii) Dowex 50 ($H^+$), MeOH, $H_2O$, reflux; (iv) $(CH_3)_3SiBr$, $CH_3CN$, rt

SCHEME 2

For nucleophilic fluorination using perfluorobutane-1-sulfonyl fluoride it is essential to find a suitable base and solvent[16]. The base has to be a good proton acceptor with a low nucleophilicity and moderately basic to avoid elimination reaction. We tested the reaction with *N*-ethylpiperidine (NEP) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), which was found useful for this type of fluorine displacement. The reaction with NEP was reported to give better fluorination results in the syntheses of saccharide-containing nucleosides[16]. However, in this case the reactions proceeded better in the presence of DBU to reach good conversion of ANP intermediates **5a**–**5c**. The reaction was carried out in toluene at room temperature. Although the fluorination afforded 49–58% yields of **6a**–**6c**, the reaction rate was slow. Signif-

icant reduction of the reaction time was achieved when the mixture was heated at 90 °C. Conversion of **9** obtained by hydrolysis of compound **8** on Dowex 50 ($H^+$ form) was not successful. Undesirable $N^3$-deprotonation of the thymine base resulted in the closure of six-membered ring under the formation of compound **10**. The obtained compounds **5b**, **6a**–**6c** and **9** were finally deprotected by the reaction with bromotrimethylsilane in acetonitrile followed by hydrolysis to give **7a**–**7c** and **11a**, **11b**.

In order to assess the effect of fluorine at the side chain of ANP on dThdPase inhibition, we also synthesized non-fluorinated ANPs **15a** and **15b** lacking fluorine in the side chain (Scheme 3). Thus, 4-methoxy-5-methylpyrimidin-2(1*H*)-one was converted into enantiomers of $N^1$-derivatives **13a** and **13b** by reaction with phosphonate synthons[15] **12a** and **12b** in the presence of sodium hydride. However, the initial alkylation reaction proceeded with low regioselectivity as was documented by $O^2$-alkyl intermediates **14a** and **14b** (see Experimental). The structures of both regioisomers were confirmed by $^{13}C$ NMR spectroscopy and H,C-HBMC experiments. $^{13}C$ NMR spectra of **13a** and **13b** exhibit characteristic chemical shifts for $CH_2N$ fragment and 4-methoxy-5-methyl-2-pyrimidin-2(1*H*)-one moiety (for numbering see Fig. 1). In addition, H,C-HMBC spectra show cross-peaks of $CH_2$-1′ (3.50 ppm) to CH-6 (145.97 ppm) and C-2 (156.79 ppm). In contrast, characteristic values for $CH_2O$ fragment and aromatic pyrimi-




(i) $TsOCH_2CH(CH_3)OCH_2P(O)(OiPr)_2$ (*S*)-**12a**, (*R*)-**12b**, NaH, DMF, rt→80-100°C;
(ii) $(CH_3)_3SiBr$, $CH_3CN$, rt

SCHEME 3

dine moiety of isomers **14a**, **14b** and cross-peaks of $CH_2$-1′ (4.23 and 4.29 ppm) to only C-2 (163.41 ppm) confirmed the structure of $O^2$-isomer. Finally, intermediates **13a** and **13b** were deprotected by reaction with bromotrimethylsilane under usual conditions.

The structures of all prepared compounds were confirmed by NMR with complete proton and carbon assignment using H,C-heterocorelated experiments (HSQC and HMBC). In addition, the optical purity of enantiomeric products **2a**, **2b**, **4a**, **4b**, **5a**, **5b**, **6a**, **6b** and **8** was checked by $^1$H NMR measurement of dynamic complex with chiral solvating agent (–)-(*R*)-1-(9-anthryl)-2,2,2-trifluoroethan-1-ol[17] in $CDCl_3$ and compared with the diastereomeric mixtures of corresponding racemic compounds. The optical purity was determined to be >98% ee in all cases. Racemates for this study were either synthesized or obtained by mixing of individual enantiomers.

TABLE I
Inhibition of thymidine phosphorylases by ANP

| Compound | Inhibition of thymidine phosphorylase[a], $V_i/V_0$ | | | |
|---|---|---|---|---|
| | *Escherichia coli* | human expressed | SD-lymphoma | human placenta |
| PMET | 1.00 | 1.02 | 0.27 | 0.85 |
| (*S*)-FPMPT (**7a**) | 0.92 | 0.91 | 0.29 | 0.83 |
| (*R*)-FPMPT (**7b**) | 0.93 | 0.82 | 0.11 | 0.56 |
| (*S*)-HPMPT (**11a**) | 0.91 | 0.95 | 0.25 | 0.75 |
| (*R*)-HPMPT (**11b**) | 0.98 | 0.84 | 0.31 | 0.84 |
| (*S*)-PMPT (**15a**) | 1.05 | 0.92 | 0.35 | 0.74 |
| (*R*)-PMPT (**15b**) | 1.00 | 0.98 | 0.47 | 0.79 |

[a] 100 μM [$^3$H]-2′-deoxythymidine, 250 μM $P_i$, tested compound 10 μmol $l^{-1}$, pH 6.7; an appropriate amount of enzyme, 10 min incubation at 37 °C.

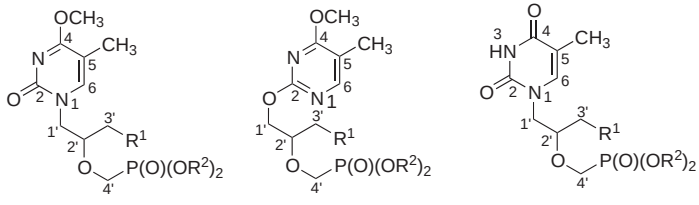



FIG. 1
General numbering scheme for assignment of NMR signals

Compounds **7a**, **7b**, **11a**, **11b**, **15a** and **15b** have showed a considerable inhibitory potency of thymidine phosphorylase. The data comprized in Table I which were taken in part from our recently published paper[18] demonstrate an interesting difference in the response to diverse inhibitors of ANP type by thymidine phosphorylases isolated from different sources of origin. The most sensitive enzyme is that of the T-cell lymphoma; the most active compounds **7b** and **7a** inhibit this enzyme with $K_i/K^{dThd}{}_m$ = 0.0026 and $K_i/K^{dThd}{}_m$ = 0.0048, respectively.

None of the compounds presented in this study possesses significant cytostatic activity *in vitro* 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) at a concentration 10 μmol $l^{-1}$.

In conclusion, the preparation of **7a** and **7b** using perfluorobutane-1-sulfonyl fluoride represents a novel direct fluorination method of thymine ANPs. In contrast to known syntheses of fluorinated building blocks[2a,9] usually using potassium fluoride this kind of fluorination requires mild conditions. However, the danger of racemization by simple displacement of hydroxy group with fluorine is not quite excluded; the exact mechanism of the reaction is not known. The described procedure opens the preparative way to FPMP derivatives of bases which do not bear amino function(s). The appropriate starting HPMP derivatives are well and reproducibly accessible, their transformation to the fluorine containing compouds is easy and the isolation is uncomplicated. However, it remains to be further searched for conditions which would make possible to transform the amino group containing HPMP derivatives, in order to give this method a general character.

## 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 $CDCl_3$. 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% water addition (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). NMR spectra were recorded on a Bruker Avance 400, Bruker Avance 500 or Varian Unity 500 spectrometers ($^1H$ at 400 or 500, $^{13}C$ at

100.6 or 125.8 and $^{19}F$ at 470.2 MHz) in $CDCl_3$ with internal standard tetramethylsilane and trichlorofluoromethane; DMSO-$d_6$ solutions (referenced to the solvent signal at δ 2.50) or in $D_2O$ solutions with dioxane as an internal standard (3.75 ppm for $^1H$ and 67.19 ppm for $^{13}C$). 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. 1. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer using FAB (ionization with xenon, accelerating voltage 8 kV, glycerol matrix). Reversed-phase HPLC was performed on a Waters Delta 600 (Xterra®, Prep $RP_{18}$ Column 10 μm, 10 × 150 mm, system S2; $RP_{18}$ Column 5 μm 3.9 × 150 mm, system S3) in 0.05 M triethylammonium hydrogencarbonate buffer (TEAB).

### 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). 4-Methoxy-5-methyl-2-pyrimidin-2(1*H*)-one and 4-methoxy-2-pyrimidin-2(1*H*)-one were prepared as described in ref.[12] Oxiranes **1a**, **1b** and **1d** were obtained from Daiso Co. Ltd. [(Trityloxy)methyl]oxirane (**1c**) was prepared by tritylation of glycidol as described in ref.[19] [(Diisopropoxyphoryl)methyl] tosylate was synthesized as described in ref.[14], (*R*)- and (*S*)-enantiomeric phosphonates **10a** and **10b** were prepared according to ref.[15] Diisopropyl [(2-chloroethoxy)methyl]phosphonate was obtained according to ref.[20] Dimethylformamide, tetrahydrofuran, and acetonitrile were dried by distillation from calcium hydride and stored over molecular sieves (4 Å).

### Synthesis of 4-Methoxy-5-pyrimidin-2(1*H*)-ones **2a**–**2c**. General Procedure

A mixture of 4-methoxy-5-methylpyrimidin-2(1*H*)-one (5.2 g, 37.2 mmol), [(trityloxy)methyl]-oxirane **1a** or **1b** or **1c** (11.8 g, 37.2 mmol) and cesium carbonate (1.2 g, 3.7 mmol) in dimethylformamide (300 ml) was heated at 70–80 °C for 19 h. The mixture was concentrated in vacuo to a minimum volume. The residue was chromatographed in the below-mentioned systems. The product **2** containing isomers were evaporated to dryness in vacuo. Compound **2c** was then crystallized from chloroform–ethyl acetate.

*(S)-1-[2-Hydroxy-3-(trityloxy)propyl]-4-methoxy-5-methylpyrimidin-2(1H)-one* (**2a**). Yield 11.4 g (70%), white sirupy product. Column chromatography was performed on silica gel (toluene–methanol–ethyl acetate–triethylamine 7:1:1.5:1). IR, $\nu_{max}$ ($CCl_4$): 3357, 1670, 1644, 1598, 1491, 1449, 1541, 1476, 1399, 1368, 1333, 1099. $[\alpha]_D$ –38.7 (*c* 0.311 g/100 ml, $CHCl_3$). For $C_{28}H_{28}N_2O_4$ (456.6) calculated: 73.66% C, 6.18% H, 6.14% N; found: 73.29% C, 6.30% H, 5.99% N. FAB MS, *m/z*: 457 $[MH]^+$ (2), 243 (100), 215 (8), 197 (10), 165 (18), 141 (7). $^1H$ NMR (400 MHz, DMSO-$d_6$): 1.81 d, 3 H, $J(CH_{3,6})$ = 1.0 ($CH_3$); 2.89 dd, 1 H, *J*(gem) = 9.5, *J*(3′b,2′) = 5.3 (H-3′b); 2.96 dd, 1 H, *J*(gem) = 9.5, *J*(3′a,2′) = 4.9 (H-3′a); 3.54 dd, 1 H, *J*(gem) = 12.9, *J*(1′b,2′) = 8.2 (H-1′b); 3.83 s, 3 H ($OCH_3$); 3.97 m, 1 H (H-2′); 4.04 dd, 1 H, *J*(gem) = 12.9, *J*(1′a,2′) = 4.1 (H-1′a); 5.26 d, 1 H, *J*(OH,2′) = 5.7 (OH); 7.26 m, 3 H (*p*-H-Ph); 7.33 m, 6 H (*m*-H-Ph); 7.41 m, 6 H (*o*-H-Ph); 7.63 q, 1 H, *J*(6,$CH_3$) = 1.0 (H-6). $^{13}C$ NMR (100.6 MHz, DMSO-$d_6$): 11.69 ($CH_3$-5); 53.08 ($CH_2$-1′); 54.02 ($OCH_3$); 66.20 ($CH_2$-3′); 67.07 (CH-2′); 86.04 (C-Tr); 101.69 (C-5); 127.20 (*p*-CH-Ph); 128.06 (*m*-CH-Ph); 128.44 (*o*-CH-Ph); 143.90 (*i*-C-Ph); 147.55 (CH-6); 155.67 (C-2); 170.09 (C-4).

*(RS)-1-[2-Hydroxy-3-(trityloxy)propyl]-4-methoxy-5-methylpyrimidin-2(1H)-one* (**2c**). Yield 10.3 g (63%), white crystals, m.p. 89–91 °C. Column chromatography was performed on neutral aluminum oxide (chloroform–methanol 25:1). For $C_{28}H_{28}N_2O_4$ (456.6) calculated: 73.66% C, 6.18% H, 6.14% N; found: 73.47% C, 6.14% H, 6.06% N. FAB MS, *m/z*: 457 $[MH]^+$ (1), 243 (100), 215 (8), 197 (18), 165 (35), 141 (14), 105 (10), 57 (8). $^1$H, $^{13}$C NMR and IR data were identical with compound **2a**.

(*R*)-1-[2-Hydroxy-3-(triphenylmethoxy)propyl]-4-methoxy-5-methylpyrimidin-2(1*H*)-one (**2b**)

A mixture of 4-methoxy-5-methylpyrimidin-2(1*H*)-one (6 g, 42.8 mmol), (*R*)-oxiranylmethyl butanoate (**1d**; 6.8 g, 42.8 mmol) and cesium carbonate (1.4 g, 4.0 mmol) in dimethylformamide (300 ml) was heated at 100 °C for 10 h. The mixture was concentrated to a minimum volume. The residue was codistilled with toluene (50 ml) and left standing with 0.05 M methanolic sodium methoxide (100 ml) for 9 h. The mixture was stirred in chloroform–methanol 25:1 and then neutralized with acetic acid. The residue was concentrated in vacuo and then dissolved in dimethylformamide (150 ml) and pyridine (70 ml). 4-(Dimethylamino)pyridine (2.9 g, 23.7 mmol) and trityl chloride (18 g, 64.2 mmol) were added to a solution and the resulting mixture was stirred at 50 °C for 28 h. The solvents were evaporated in vacuo and the residue was codistilled with toluene (2 × 50 ml). The mixture was dissolved in chloroform (200 ml) and washed with water (50 ml). The organic layer was washed with saturated sodium chloride solution (50 ml) and dried with anhydrous magnesium sulfate. After evaporation of the solvent the residue was chromatographed on neutral aluminum oxide (ether, ethyl acetate and ethyl acetate–methanol 18:1). Yield 6.5 g (30%) of **2b** as a white solid. $[\alpha]_D$ +40.2 (*c* 0.512 g/100 ml, $CHCl_3$). FAB MS, *m/z*: 457 $[MH]^+$ (2), 243 (100), 197 (6), 165 (14), 141 (6). For $C_{28}H_{28}N_2O_4 \cdot H_2O$ (474.6) calculated: 70.86% C, 6.37% H, 5.90% N; found: 70.60% C, 6.48% H, 5.70 N. $^1$H, $^{13}$C NMR and IR data were identical with compound **2a**.

Synthesis of 1-{2-[(Diisopropoxyphosphoryl)methoxy)]-3-(trityloxy)propyl}pyrimidin-2(1*H*)-one Derivatives **4a**–**4c**. General Procedure

A mixture of compound **2** (10.3 g, 22.6 mmol), [(diisopropoxyphosphoryl)methoxy]methyl tosylate (9.5 g, 27.1 mmol) and 60% sodium hydride dispersion (1.4 g, 35 mmol) in tetrahydrofuran (300 ml) was stirred at –20 °C. The suspension was allowed to warm to 20–30 °C for 1 h and stirred at room temperature overnight. The mixture was filtered through a Celite pad and concentrated in vacuo. The residue was chromatographed on neutral aluminum oxide (chloroform–methanol 25:1).

*(S)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-(trityloxy)propyl}-4-methoxy-5-methylpyrimidin-2(1H)-one* (**4a**). Yield 12.5 g (87%) of a yellowish sirupy product. IR, $\nu_{max}$ ($CCl_4$): 1676, 1656, 1599, 1541, 1491, 1475, 1450, 1399, 1332, 1259, 1106, 1009, 901. $[\alpha]_D$ –6.4 (*c* 0.202 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{35}H_{44}N_2O_7P$ found: 635.2918, calculated: 635.2886. $^1$H NMR (400 MHz, $CDCl_3$): 1.26, 1.27, 1.29 and 1.31 4 × d, 4 × 3 H, *J*(vic) = 6.0 ($CH_3$-*i*-Pr); 1.88 d, 3 H, *J*($CH_{3,6}$) = 1.1 ($CH_3$-5); 3.09 dd, 1 H, *J*(gem) = 10.7, *J*(3′b,2′) = 4.0 (H-3′b); 3.40 dd, 1 H, *J*(gem) = 10.7, *J*(3′a,2′) = 3.4 (H-3′a); 3.57 dd, 1 H, *J*(gem) = 13.5, *J*(H,P) = 10.2 (H-4′b); 3.80 dd, 1 H, *J*(gem) = 13.5, *J*(1′b,2′) = 8.0 (H-1′b); 3.87 dd, 1 H, *J*(gem) = 13.5, *J*(H,P) = 8.6 (H-4′a); 3.91 m, 1 H (H-2′); 3.96 s, 3 H ($OCH_3$); 4.26 dd, 1 H, *J*(gem) = 13.5, *J*(1′a,2′) = 3.8 (H-1′a); 4.69 m, 2 H (CH-*i*-Pr); 7.24 m, 3 H (*p*-H-Ph); 7.31 m, 7 H (H-6) and (*m*-H-Ph);

7.44 m, 6 H (*o*-H-Ph). $^{13}$C NMR (100.6, $CDCl_3$): 11.95 ($CH_3$-5); 23.90 d, *J*(C,P) = 4.7 ($CH_3$-*i*-Pr); 23.96 d, *J*(C,P) = 4.8 ($CH_3$-*i*-Pr); 23.99 d, *J*(C,P) = 4.0 ($CH_3$-*i*-Pr); 24.00 d, *J*(C,P) = 4.2 ($CH_3$-*i*-Pr); 51.18 ($CH_2$-1′); 54.40 ($OCH_3$); 62.28 ($CH_2$-3′); 64.85 d, *J*(C,P) = 169.1 ($CH_2$-4′); 70.79 d, *J*(C,P) = 6.7 (CH-*i*-Pr); 70.97 d, *J*(C,P) = 6.5 (CH-*i*-Pr); 78.87 d, *J*(C,P) = 12.6 (CH-2′); 86.78 (C-Tr); 103.66 (C-5); 127.12 (*p*-CH-Ph); 127.88 (*m*-CH-Ph); 128.55 (*o*-CH-Ph); 143.48 (*i*-C-Ph); 147.98 (CH-6); 156.62 (C-2); 170.78 (C-4).

*(R)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-(trityloxy)propyl}-4-methoxy-5-methylpyrimidin-2(1H)-one* (**4b**). Yield 7.9 g (55%) of a yellowish sirupy product. $[\alpha]_D$ +37.7 (*c* 0.136 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{35}H_{44}N_2O_7P$ found: 635.2825, calculated: 635.2886. $^1$H, $^{13}$C NMR and IR data were identical with compound **4a**.

*(RS)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-(trityloxy)propyl}-4-methoxy-5-methylpyrimidin-2(1H)-one* (**4c**). Yield 10.5 g (73%) of a white sirupy product. HR MS (FAB): for $C_{35}H_{44}N_2O_7P$ found: 635.2905, calculated: 635.2886. $^1$H, $^{13}$C NMR and IR data were identical with compound **4a**.

Synthesis of 1-[3-Hydroxy-2-(phosphonomethoxy)propyl]pyrimidin-2(1*H*)-one Derivatives **5a** and **5b**. General Procedure

Compound **4a** or **4b** (4.3 g, 6.7 mmol) in methanol (60 ml) and glacial acetic acid (1 ml) was hydrogenated over 5% palladium on charcoal (1.5 g) at room temperature for 24 h until the conversion of starting phosphonate to **5a** or **5b** 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 aluminium oxide (chloroform and chloroform-methanol 25:1).

*(S)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-hydroxypropyl}-4-methoxy-5-methyl-pyrimidin-2(1H)-one* (**5a**). Yield 1.6 g (62%) of a colorless oil. IR, $\nu_{max}$ ($CCl_4$): 3395, 1671, 1643, 1540, 1477, 1399, 1386, 1375, 1336, 1253, 1105, 1013, 994. $[\alpha]_D$ –6.4 (*c* 0.202 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{16}H_{29}N_2O_7P$ found: 393.1800, calculated: 393.1791. FAB MS, *m/z*: 393 $[MH]^+$ (100). $^1$H NMR (400 MHz, DMSO-$d_6$): 1.16, 1.19, 1.20 and 1.22 4 × d, 4 × 3 H, *J*(vic) = 6.2 ($CH_3$-*i*-Pr); 1.86 d, 3 H, *J*($CH_{3,6}$) = 1.0 ($CH_3$); 3.45 dd, 1 H, *J*(gem) = 11.9, *J*(3′b,2′) = 4.4 (H-3′b); 3.51 dd, 1 H, *J*(gem) = 11.9, *J*(3′a,2′) = 4.4 (H-3′a); 3.70 m, 3 H (H-4′ and H-1′b); 3.84 s, 3 H ($OCH_3$); 3.87 dd, 1 H, *J*(gem) = 14.0, *J*(1′a,2′) = 8.1 (H-1′a); 4.02 m, 1 H (H-2′); 4.53 dh, 2 H, *J*(H,P) = 7.7, *J*(vic) = 6.2 (CH-*i*-Pr); 4.82 bs, 1 H (OH); 7.65 q, 1 H, *J*(6,$CH_3$) = 1.0 (H-6). $^{13}$C NMR (100.6, DMSO-$d_6$): 11.75 ($CH_3$-5); 23.72, 23.82, 23.89 and 23.93 d, *J*(C,P) = 4.8 ($CH_3$-*i*-Pr); 50.24 ($CH_2$-1′); 54.03 ($OCH_3$); 62.37 ($CH_2$-3′); 63.66 d, *J*(C,P) = 164.8 ($CH_2$-4′); 70.25 d, *J*(C,P) = 6.2 (CH-*i*-Pr); 79.61 d, *J*(C,P) = 11.0 (CH-2′); 101.96 (C-5); 147.47 (CH-6); 155.71 (C-2); 170.14 (C-4).

*(R)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-hydroxypropyl}-4-methoxy-5-methylpyrimidin-2(1H)-one* (**5b**). Yield 1.3 g (51%) of a colorless oil. $[\alpha]_D$ +3.6 (*c* 0.312 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{16}H_{29}N_2O_7P$ found: 393.1781, calculated: 393.1791. FAB MS, *m/z*: 393 $[MH]^+$ (100). $^1$H, $^{13}$C NMR and IR data were identical with compound **5a**.

*(RS)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-hydroxypropyl}-4-methoxy-5-methylpyrimidin-2(1H)-one* (**5c**). A solution of compound **4c** (9.4 g, 14.8 mmol) in 80% acetic acid (40 ml) was heated at 40 °C for 2 h. The mixture was then heated to 70 °C for 4 h until the conversion of starting phosphonate **4c** to **5c** was complete (TLC in chloroform–methanol 25:1). The mixture was concentrated in vacuo and then codistilled with light petroleum. The resi-

due was chromatographed on silica gel (chloroform and chloroform–methanol 25:1). Yield 4.1 g (70%) of **5c** as a colorless oil. IR, $\nu_{max}$ ($CCl_4$): 3394, 1670, 1645, 1540, 1477, 1399, 1386, 1375, 1336, 1253, 1105, 1012, 994. HR MS (FAB): for $C_{16}H_{29}N_2O_7P$ found: 393.1748, calculated: 393.1791. FAB MS, *m/z*: 393 $[MH]^+$ (100). $^1H$, $^{13}C$ NMR and IR data were identical with compound **5a**.

Synthesis of 1-[3-Fluoro-2-(phosphonomethoxy)propyl]pyrimidin-2(1*H*)-one Derivatives **6a**–**6c**. General Procedure

A mixture of 1,8-diazabicyclo[5.4.0]undec-7-ene (0.8 g, 5.1 mmol) and 92% perfluorobutane-1-sulfonyl fluoride (1.1 g, 3.7 mmol) was stirred in toluene (3 ml) at room temperature for 10 min. A solution of compound **5** (1 g, 2.5 mmol) in toluene (7 ml) was added and the resulting mixture was stirred at room temperature overnight or heated at 90 °C for 4 h until the conversion of starting phosphonate to **6** was complete (TLC in chloroform–methanol 25:1). The mixture was concentrated in vacuo, filtered over neutral aluminum oxide (chloroform and chloroform–methanol 20:1). After evaporation of solvents the residue was chromatographed on preparative TLC (S1, chloroform–methanol 20:1).

*(S)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-fluoropropyl}-4-methoxy-5-methylpyrimidin-2(1H)-one* (**6a**). Yield 482 mg (48%) of a colorless oil. $[\alpha]_D$ –75.1 (*c* 0.865 g/100 ml, $CHCl_3$). For $C_{16}H_{28}FN_2O_6P$ (394.4) calculated: 48.73% C, 7.16% H, 4.82% F, 7.10% N, 7.85% P; found: 48.57% C, 7.38% H, 4.63% F, 6.77% N, 7.64% P. FAB MS, *m/z*: 395 $[MH]^+$ (100). $^1H$ NMR (500 MHz, $CDCl_3$): 1.30, 1.31 and 1.32 4 × d, 4 × 3 H, *J*(vic) = 6.2 ($CH_3$-*i*-Pr); 1.95 d, 3 H, $J(CH_{3,6})$ = 1.1 ($CH_3$); 3.71 dd, 1 H, *J*(gem) = 13.7, *J*(H,P) = 9.3 (H-4′b); 3.79 dd, 1 H, *J*(gem) = 13.8, *J*(1′b,2′) = 7.4 (H-1′b); 3.90 dd, 1 H, *J*(gem) = 13.7, *J*(H,P) = 8.7 (H-4′a); 3.99 s, 3 H ($OCH_3$); 4.04 m, 1 H (H-2′); 4.18 ddd, 1 H, *J*(gem) = 13.8, *J*(1′a,2′) = 4.3, *J*(H,F) = 0.6 (H-1′a); 4.45 ddd, 1 H, *J*(H,F) = 47.3, *J*(gem) = 10.7, *J*(3′b,2′) = 4.8 (H-3′b); 4.70 m, 3 H (H-3′a and CH-*i*-Pr); 7.37 q, 1 H, $J(6,CH_3)$ = 1.1 (H-6). $^{13}C$ NMR (125.8 MHz, $CDCl_3$): 11.96 ($CH_3$-5); 23.89, 23.93, 24.02 and 24.03 d, *J*(C,P) = 4.4 ($CH_3$-*i*-Pr); 50.02 d, *J*(C,F) = 9.1 ($CH_2$-1′); 54.53 ($OCH_3$); 65.47 d, *J*(C,P) = 168.6 ($CH_2$-4′); 71.08 and 71.20 d, *J*(C,P) = 6.6 (CH-*i*-Pr); 78.49 dd, *J*(C,F) = 18.3, *J*(C,P) = 10.9 (CH-2′); 82.55 d, *J*(C,F) = 173.0 ($CH_2$-3′); 104.28 (C-5); 145.62 (CH-6); 156.69 (C-2); 171.07 (C-4). $^{13}F$ NMR (470.2 MHz, $CDCl_3$): –232.72 dt, *J*(F,H-3′) = 47.3, *J*(F,H-2′) = 23.2 ($CH_2F$). IR data were identical with **6b**.

*(R)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-fluoropropyl}-4-methoxy-5-methylpyrimidin-2(1H)-one* (**6b**). Yield 362 mg (36%) of a colorless oil. IR, $\nu_{max}$ ($CCl_4$): 1676, 1655, 1540, 1476, 1399, 1386, 1376, 1335, 1255, 1106, 1010, 992. $[\alpha]_D$ +70.8 (*c* 0.237 g/100 ml, $CHCl_3$). FAB MS, *m/z*: 395 $[MH]^+$ (100). $^1H$, $^{13}C$ and $^{19}F$ NMR data were identical with compound **6a**.

*(RS)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-fluoropropyl}-4-methoxy-5-methylpyrimidin-2(1H)-one* (**6c**). Yield 583 mg (58%) of a colorless oil. HR MS (FAB): for $C_{16}H_{28}FN_2O_6P$ found: 395.1725, calculated: 394.1747. MS (FAB), *m/z*: 395 $[MH]^+$ (72). $^1H$, $^{13}C$, $^{19}F$ NMR and IR data were identical with compound **6a**. or **6b**.

Synthesis of 1-[3-Fluoro-2-(phosphonomethoxy)propyl]pyrimidin-2(1*H*)-one Derivatives **7a**–**7c**. General Procedure

A mixture of compound **6** (493 mg, 1.25 mmol), acetonitrile (10 ml) and bromotrimethylsilane (1.8 ml) was stirred at room temperature overnight. The mixture was concentrated in vacuo and then codistilled with water (2 × 5 ml). The residue in water (20 ml) was heated with Dowex 50X8 ($H^+$ form) (3 ml) at 80 °C for 3 h. The mixture was cooled to room tem-

perature and then filtered. According to paper electrophoresis of a hydrolyzed sample, the reaction was quantitative. The filtrate was concentrated in vacuo and chromatographed by preparative HPLC (S2) or purified on Sephadex (in $Cl^-$ form, activated with 0.02 M TEAB, 40 ml) with subsequent deionization on activated charcoal. The relevant fractions were combined, evaporated in vacuo and codistilled with water (3 × 10 ml). The residue was dissolved in water (3 ml), applied onto a Dowex 50X8 ($Li^+$ form, 30 ml) and then the column was washed with water. The appropriate UV absorbing fraction containing product **6** was evaporated to dryness in vacuo. The residue was dissolved in water and lyophilized. The following compounds were obtained as dilithium salts:

*(S)-1-[3-Fluoro-2-(phosphonomethoxy)propyl]thymine* (**7a**). Yield 215 mg (56%) of a white solid, m.p. 233–235 °C. IR, $\nu_{max}$ (KBr): 3199, 3058, 1691, 1469, 1438, 1385, 1352, 1230, 1182, 1098, 1074, 996, 924, 552, 455, 422. $[\alpha]_D$ –22.5 (*c* 0.561 g/100 ml, $H_2O$). HPLC: 98% (S3). For $C_9H_{12}FLi_2N_2O_6P \cdot H_2O$ (326.1) calculated: 33.15% C, 4.33% H, 5.83% F, 8.59% N, 9.50% P; found: 33.43% C, 4.74% H, 6.05% F, 8.49% N, 9.36% P. FAB MS, *m/z*: 309 $[MH]^+$ (18). $^1$H NMR (400 MHz, $D_2O$): 1.88 d, 3 H, *J*($CH_{3,6}$) = 1.2 ($CH_3$); 3.62 dd, 1 H, *J*(gem) = 12.8, *J*(H,P) = 9.2 (H-4′b); 3.71 dd, 1 H, *J*(gem) = 12.8, *J*(H,P) = 9.2 (H-4′a); 4.00 m, 3 H (H-1′ and H-2′); 4.52 ddd, 1 H, *J*(H,F) = 46.5, *J*(gem) = 10.7, *J*(3′b,2′) = 3.6 (H-3′b); 4.70 ddd, 1 H, *J*(H,F) = 47.7, *J*(gem) = 10.7, *J*(3′a,2′) = 3.0 (H-3′a); 7.57 q, 1 H, *J*(6,$CH_3$) = 1.2 (H-6). $^{13}$C NMR (100.6 MHz, $D_2O$): 11.89 ($CH_3$-5); 48.38 d, *J*(C,F) = 7.5 ($CH_2$-1′); 67.81 d, *J*(C,P) = 154.3 ($CH_2$-4′); 78.27 dd, *J*(C,F) = 18.3, *J*(C,P) = 10.9 (CH-2′); 83.00 d, *J*(C,F) = 167.6 ($CH_2$-3′); 111.23 (C-5); 144.48 (CH-6); 153.06 (C-2); 167.72 (C-4). $^{19}$F NMR (470.2 MHz, $D_2O$): –231.48 dt, *J*(F,H-3′) = 47.1, *J*(F,H-2′) = 25.9 ($CH_2F$).

*(R)-1-[3-Fluoro-2-(phosphonomethoxy)propyl]thymine* (**7b**). Yield 223 mg (58%) of a white amourphous solid. IR, $\nu_{max}$ (KBr): 3191, 3062, 1689, 1475, 1439, 1385, 1353, 1226, 1178, 1074, 1113, 995, 924,787, 715, 547, 465, 429, 418. $[\alpha]_D$ +19.3 (*c* 0.429 g/100 ml, $H_2O$). HPLC: 96% (S3). For $C_9H_{12}FLi_2N_2O_6P \cdot 3/5H_2O$ (318.9) calculated: 33.90% C, 4.14% H, 5.96% F, 8.79% N, 9.71% P; found: 33.90% C, 4.15% H, 5.87% F, 8.62% N, 9.48% P. FAB MS, *m/z*: 309 $[MH]^+$ (21). $^1$H, $^{13}$C and $^{19}$F NMR data were identical with compound **7a**.

*(RS)-1-[3-Fluoro-2-(phosphonomethoxy)propyl]thymine* (**7c**). Yield 146 mg (38%) of a white amorphous solid. HPLC: 95% (S3). HR MS (FAB): for $C_9H_{12}FN_2O_6P$ found: 309.0820, calculated: 309.0815. For $C_9H_{12}FLi_2N_2O_6P \cdot H_2O$ (326.1) calculated: 33.15% C, 4.33% H, 5.83% F, 8.59% N, 9.50% P; found: 33.42% C, 4.17% H, 6.07% F, 8.16% N, 9.77% P. FAB MS, *m/z*: 309 $[MH]^+$ (8). $^1$H, $^{13}$C, $^{19}$F NMR and IR data were identical with compound **7a**.

(*S*)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-(trityloxy)propyl}thymine (**8**)

A mixture of compound **2a** (4 g, 8.8 mmol), [(diisopropoxyphosphoryl)methoxy]methyl tosylate (4.1 g, 11.7 mmol) and 60% sodium hydride dispersion (591 mg, 14.8 mmol) in tetrahydrofuran (150 ml) was stirred at –20 °C. The suspension was let to warm to room temperature for 1 h and then stirred overnight. The mixture was filtered through a Celite pad and concentrated in vacuo. The residue was filtered on silica gel (toluene–ethyl acetate–methanol–triethylamine 7:1.5:1:1), relevant fractions were combined and evaporated to dryness in vacuo. The crude product was then chromatographed on silica gel (chloroform–triethylamine 200:1 and chloroform–methanol–triethylamine 26:1:1). Yield 4.0 g (73%) of **8** as a white sirupy product. IR, $\nu_{max}$ ($CCl_4$): 3412, 1715, 1694, 1655, 1386, 1375, 1257, 1107, 1009, 991. HR MS (FAB): for $C_{34}H_{41}N_2O_7P$ found: 620.2675, calculated: 620.2651. FAB MS, *m/z*: 379 $[M + H - Ph_3C]^+$ (16), 295 (13), 243 (100), 165 (12), 137 (25), 109 (10), 96 (6), 61

(5). $^1$H NMR (400 MHz, $CDCl_3$): 1.27, 1.29, 1.31 and 1.32 4 × d, 4 × 3 H, *J*(vic) = 6.2 ($CH_3$-*i*-Pr); 1.85 d, 3 H, *J*($CH_{3,6}$) = 1.2 ($CH_3$-5); 3.12 dd, 1 H, *J*(gem) = 10.7, *J*(3′b,2′) = 4.2 (H-3′b); 3.34 dd, 1 H, *J*(gem) = 10.7, *J*(3′a,2′) = 4.0 (H-3′a); 3.61 dd, 1 H, *J*(gem) = 13.6, *J*(H,P) = 9.5 (H-4′b); 3.72 dd, 1 H, *J*(gem) = 13.9, *J*(1′b,2′) = 7.5 (H-1′b); 3.80 dq, 1 H, *J*(2′,1′) = 7.5, 4.0, *J*(2′,3′) = 4.2, 4.0 (H-2′); 3.86 dd, 1 H, *J*(gem) = 13.6, *J*(H,P) = 8.6 (H-4′a); 4.10 dd, 1 H, *J*(gem) = 13.9, *J*(1′a,2′) = 4.0 (H-1′a); 4.71 dh, 2 H, *J*(H,P) = 7.7, *J*(vic) = 6.2 (CH-*i*-Pr); 7.14 q, 1 H, *J*(6,$CH_3$) = 1.2 (H-6); 7.25 m, 3 H (*p*-H-Ph); 7.31 m, 6 H (*m*-H-Ph); 7.43 m, 6 H (*o*-H-Ph); 7.98 bs, 1 H (NH). $^{13}$C NMR (100.6 MHz, $CDCl_3$): 12.25 ($CH_3$-5); 23.98 d, *J*(C,P) = 4.7 ($CH_3$-*i*-Pr); 24.07 d, *J*(C,P) = 4.8 ($CH_3$-*i*-Pr); 49.54 ($CH_2$-1′); 62.22 ($CH_2$-3′); 64.87 d, *J*(C,P) = 168.9 ($CH_2$-4′); 70.94 d, *J*(C,P) = 6.6 (CH-*i*-Pr); 71.06 d, *J*(C,P) = 6.6 (CH-*i*-Pr); 79.10 d, *J*(C,P) = 11.4, CH-2′); 87.02 (C-Tr); 109.68 (C-5); 127.27 (*p*-CH-Ph); 127.97 (*m*-CH-Ph); 128.54 (*o*-CH-Ph); 142.21 (CH-6); 143.42 (*i*-C-Ph); 150.58 (C-2); 163.75 (C-4).

(*S*)-1-{2-[(Diisopropoxyphosphoryl)methoxy]-3-hydroxypropyl}thymine (**9**)

A solution of compound **8** (2.4 g, 3.8 mmol) in methanol (150 ml) was heated with Dowex 50X8 ($H^+$ form, 20 ml) at 80 °C for 11 h until the conversion of starting phosphonate to **9** was complete (TLC, chloroform–methanol 12:1). The mixture was then filtered and concentrated in vacuo. The residue was chromatographed on neutral aluminum oxide (chloroform–methanol 12:1). Yield 971 mg (70%) of **9** as a colorless oil. IR, $\nu_{max}$ ($CCl_4$): 3410, 3400, 1716, 1693, 1387, 1376, 1257, 1104, 1011, 994. $[\alpha]_D$ –66.4 (*c* 0.440 g/100 ml, $CHCl_3$). For $C_{15}H_{27}N_2O_7P$ (378.4) calculated: 47.62% C, 7.19% H, 7.40% N, 8.19% P; found: 47.39% C, 7.37% H, 7.02% N, 8.50% P. FAB MS, *m/z*: 379 $[MH]^+$ (70). $^1$H NMR (400 MHz, DMSO-$d_6$): 1.19, 1.20, 1.21 and 1.23 4 × d, 4 × 3 H, *J*(vic) = 6.2 ($CH_3$-*i*-Pr); 1.74 d, 3 H, *J*($CH_{3,6}$) = 1.2 ($CH_3$-5); 3.41–3.54 m, 2 H (H-3′); 3.58 dd, 1 H, *J*(gem) = 13.8, *J*(1′b,2′) = 8.1 (H-1′b); 3.65 m, 1 H (H-2′); 3.73 dd, 1 H, *J*(gem) = 14.0, *J*(H,P) = 9.3 (H-4′b); 3.88 dd, 1 H, *J*(gem) = 13.8, *J*(1′a,2′) = 3.9 (H-1′a); 3.89 dd, 1 H, *J*(gem) = 14.0, *J*(H,P) = 8.1 (H-4′a); 4.54 m, 2 H (CH-*i*-Pr); 4.82 t, 1 H, *J*(OH,3′) = 5.6 (OH-3′); 7.37 q, 1 H, *J*(6,$CH_3$) = 1.2, H-6); 11.24 s, 1 H (NH). $^{13}$C NMR (100.6 MHz, DMSO-$d_6$): 12.21 ($CH_3$-5); 23.91 d, *J*(C,P) = 4.8 ($CH_3$-*i*-Pr); 48.66 ($CH_2$-1′); 60.27 ($CH_2$-3′); 63.62 d, *J*(C,P) = 164.6 ($CH_2$-4′); 70.28 d, *J*(C,P) = 6.6 (CH-*i*-Pr); 79.90 d, *J*(C,P) = 10.7 (CH-2′); 107.92 (C-5); 142.69 (CH-6); 151.22 (C-2); 164.53 (C-4).

(*S*)-[3-(Diisopropoxyphosphoryl)methoxy]-7-methyl-3,4-dihydro-2*H*-pyrimido[2,1-*b*][1,3]oxazin-8(9*H*)-one (**10**)

A mixture of 1,8-diazabicyclo[5.4.0]undec-7-ene (400 mg, 2.6 mmol) and 92% perfluorobutane-1-sulfonyl fluoride (800 mg, 2.6 mmol) in toluene (5 ml) was stirred at room temperature for 10 min. A solution of compound **9** (330 mg, 0.9 mmol) in toluene (10 ml) was added. The resulting mixture was stirred at room temperature overnight until the conversion of starting phosphonate to **10** was complete (TLC in chloroform–methanol 2:1). The mixture was evaporated to dryness in vacuo and separated by preparative TLC (S1, chloroform–methanol 2:1). Yield 350 mg (53%) of **10** as a white sirupy product. IR, $\nu_{max}$ ($CHCl_3$): 1668, 1648, 1629, 1527, 1496, 1388, 1377, 1255, 1103, 1018, 1000. HR MS (FAB): for $C_{15}H_{26}N_2O_6P$ found: 361.1554, calculated: 361.1529. $^1$H NMR (500 MHz, $CDCl_3$): 1.26, 1.30, 1.32 and 1.36 4 × d, 4 × 3 H, *J*(vic) = 6.2 ($CH_3$-*i*-Pr); 1.92 d, 3 H, *J*($CH_{3,6}$) = 1.2 ($CH_3$-5); 3.79 dd, 1 H, *J*(gem) = 14.0, *J*(H,P) = 8.1 (H-4′b); 3.91 dd, 1 H, *J*(gem) = 14.0, *J*(H,P) = 8.7 (H-4′a); 4.01 dt, 1 H, *J*(gem) = 12.9, *J*(1′b,2′) = 2.5, *J*(1′b,3′a) = 2.5 (H-1′b); 4.23 dd, 1 H, *J*(gem) = 12.9, *J*(1′a,2′) = 3.1 (H-1′a); 4.28 p, 1 H, *J*(2′,1′) = 3.1, 2.5, *J*(2′,3′) = 2.7, 1.9 (H-2′); 4.52 dd, 1 H,

*J*(gem) = 12.3, *J*(3′b,2′) = 1.9 (H-3′b); 4.58 dt, 1 H, *J*(gem) = 12.3, *J*(3′a,2′) = 2.7, *J*(3′a,1′b) = 2.5 (H-3′a); 4.66 and 4.72 2 ( dh, 2 ( H, *J*(H,P) = 7.6, *J*(vic) = 6.2 (CH-*i*-Pr); 6.86 q, 1 H, *J*(6,$CH_3$) = 1.2 (H-6). $^{13}$C NMR (125.8 MHz, $CDCl_3$): 13.36 ($CH_3$-5); 23.82, 23.96 and 24.00 d, *J*(C,P) = 4.8 ($CH_3$-*i*-Pr); 51.02 ($CH_2$-1′); 64.05 d, *J*(C,P) = 168.8 ($CH_2$-4′); 66.75 ($CH_2$-3′); 69.15 d, *J*(C,P) = 8.1 (CH-2′); 71.59 and 71.81 d, *J*(C,P) = 6.8 (CH-*i*-Pr); 119.93 (C-5); 137.54 (CH-6); 154.02 (C-2); 171.56 (C-4).

Dilithium Salt of (*R*)- and (*S*)-1-[3-Hydroxy-2-(phosphonomethoxy)propyl]thymine (**11a**) and (**11b**)

Compounds **11a** and **11b** were obtained as dilithium salts by the same procedure reported for **7a**–**7c**.

*(S)-1-[3-Hydroxy-2-(phosphonomethoxy)propyl]thymine* (**11a**). A mixture of compound **9** (257 mg, 0.68 mmol) in acetonitrile (10 ml) and bromotrimethylsilane (0.90 ml) afforded 123 mg (61%) of **11a** as a white solid, m.p. > 300 °C. IR, $\nu_{max}$ (KBr): 3423, 3199, 3057, 2954, 1686, 1474, 1437, 1386, 1353, 1225, 1172, 1102, 1077, 998, 916, 579, 552, 475, 425. $[\alpha]_D$ –23.0 (*c* 0.321 g/100 ml, $H_2O$). HPLC: 98% (S3). For $C_9H_{13}Li_2N_2O_7P$ (306.1) calculated: 35.32% C, 4.28% H, 9.15% N, 10.12% P; found: 35.30% C, 4.68% H, 8.84% N, 9.81% P. $^1$H NMR (500 MHz, $D_2O$): 1.82 d, 3 H, *J*($CH_{3,6}$) = 1.2 ($CH_3$-5); 3.48 dd, 1 H, *J*(gem) = 12.4, *J*(3′b,2′) = 4.8 (H-3′b); 3.50 dd, 1 H, *J*(gem) = 12.6, *J*(H,P) = 9.5 (H-4′b); 3.54 dd, 1 H, *J*(gem) = 12.6, *J*(H,P) = 9.0 (H-4′a); 3.65 dddd, 1 H, *J*(2′,3′) = 7.3, 3.4, *J*(2′,1′) = 6.7, 4.8 (H-2′); 3.72 dd, 1 H, *J*(gem) = 12.4, *J*(3′a,2′) = 3.4 (H-3′a); 3.80 dd, 1 H, *J*(gem) = 14.5, *J*(1′b,2′) = 6.7 (H-1′b); 3.89 dd, 1 H, *J*(gem) = 14.5, *J*(1′a,2′) = 4.8 (H-1′a); 7.49 q, 1 H, *J*(6,$CH_3$) = 1.2 (H-6). $^{13}$C NMR (125.8 MHz, $D_2O$): 11.92 ($CH_3$-5); 49.14 ($CH_2$-1′); 61.03 ($CH_2$-3′); 67.53 d, *J*(C,P) = 155.1 ($CH_2$-4′); 80.54 d, *J*(C,P) = 11.5 (CH-2′); 111.08 (C-5); 144.66 (CH-6); 153.09 (C-2); 167.73 (C-4).

*(R)-1-[3-Hydroxy-2-(phosphonomethoxy)propyl]thymine* (**11b**). A mixture of compound **5b** (331 mg, 0.84 mmol) in acetonitrile (15 ml) and bromotrimethylsilane (1.1 ml) afforded 136 mg (53%) of **11b** as a white solid, m.p. > 300 °C. IR: $\nu_{max}$ (KBr) 3432, 3265, 3051, 1691, 1474, 1438, 1356, 1225, 1104, 1080, 995, 912, 787, 762, 553, 463. $[\alpha]_D$ +25.2 (*c* 0.228 g/100 ml, $H_2O$). HPLC: 97% (S3). For $C_9H_{13}Li_2N_4O_7P{\cdot}0.75H_2O$ (319.6) calculated: 33.82% C, 4.54% H, 8.77% N, 9.69% P; found: 33.92% C, 4.69% H, 8.36% N, 9.31% P. FAB MS, *m/z*: 307 $[MH]^+$ (22). $^1$H, and $^{13}$C NMR data were identical with compound **11a**.

Synthesis of 1-[2-(Phosphonomethoxy)propyl]thymine Derivatives **13** and **14**. General Procedure

A mixture of 4-methoxy-5-methylpyrimidin-2(1*H*)-one (800 mg, 5.7 mmol) and 60% sodium hydride dispersion (228 mg, 5.7 mmol) in dimethylformamide (20 ml) was stirred at room temperature for 1 h. Compound **12a** or **12b** (2.3 g, 5.7 mmol) in dimethylformamide (10 ml) was added and the resulting mixture was heated at 80 °C for 4.5 h. The mixture was then heated at 100 °C for 8 h. The mixture was concentrated in vacuo and the residue was dissolved in ether (60 ml). The precipitate was filtered off through a Celite and washed with ether (60 ml). The filtrate was concentrated in vacuo and the residue was chromatographed on neutral aluminum oxide (chloroform–methanol 25:1).

*(S)-1-{2-[(Diisopropoxyphosphoryl)methoxy]propyl}-4-methoxy-5-methylpyrimidin-2(1H)-one* (**13a**). Yield 774 mg (36%) of a yellowish oil, $R_F$ 0.25. $[\alpha]_D$ +90.6 (*c* 0.587 g/100 ml, $CHCl_3$).

HR MS (FAB): for $C_{16}H_{30}N_2O_6P$ found: 377.1830, calculated: 377.1841. FAB MS, *m/z*: 377 $[MH]^+$ (78). $^1H$ NMR (400 MHz, $CDCl_3$): 1.22 d, 3 H, *J*(vic) = 6.3 ($CH_3$-2′); 1.27 d, 6 H, *J*(vic) = 6.2 (2 × $CH_3$-*i*-Pr); 1.30 d, 3 H, *J*(vic) = 6.2 ($CH_3$-*i*-Pr); 1.31 d, 3 H, *J*(vic) = 6.2 ($CH_3$-*i*-Pr); 1.94 d, 3 H, *J*($CH_{3,6}$) = 1.1 ($CH_3$-5); 3.45–3.54 m, 2 H (H-1′b and H-4′b); 3.77 dd, 1 H, *J*(gem) = 13.4, *J*(H,P) = 9.3 (H-4′a); 3.88 m, 1 H (H-2′); 3.98 s, 3 H ($OCH_3$); 4.20 dd, 1 H, *J*(gem) = 13.8, *J*(1′a,2′) = 2.6 (H-1′a); 4.68 dh, 2 H, *J*(H,P) = 7.7, *J*(vic) = 6.2 (CH-*i*-Pr); 7.37 q, 1 H, *J*(6,$CH_3$) = 1.1 (H-6). $^{13}C$ NMR (100.6 MHz, $CDCl_3$): 12.02 ($CH_3$-5); 16.52 ($CH_3$-2′); 23.93, 23.96 and 24.04 d, *J*(C,P) = 4.7 ($CH_3$-*i*-Pr); 54.49 ($OCH_3$); 54.50 ($CH_2$-1′); 63.65 d, *J*(C,P) = 170.2 ($CH_2$-4′); 70.83 and 70.96 d, *J*(C,P) = 6.6 (CH-*i*-Pr); 76.15 d, *J*(C,P) = 13.1 (CH-2′); 103.68 (C-5); 145.97 (CH-6); 156.79 (C-2); 170.84 (C-4).

*(R)-1-{2-[(Diisopropoxyphosphoryl)methoxy]propyl}-4-methoxy-5-methylpyrimidin-2(1H)-one* (**13b**). Yield, 300 mg (14%) of a yellowish oil, $R_F$ 0.25. $[\alpha]_D$ –83.4 (*c* 0.225 g/100 ml, $CHCl_3$). HR MS (FAB): for $C_{16}H_{30}N_2O_6P$ found: 377.1836, calculated: 377.1841. FAB MS, *m/z*: 377 $[MH]^+$ (100). $^1H$ and $^{13}C$ NMR data were identical with those of compound **13a**.

*(S)-2-{2-[(Diisopropoxyphosphoryl)methoxy]propoxy}-4-methoxy-5-methylpyrimidine* (**14a**). Yield 858 mg (40%) of a yellowish oil, $R_F$ 0.50. $[\alpha]_D$ +8.1 (*c* 0.744 g/100 ml, $CHCl_3$). IR, $\nu_{max}$ ($CCl_4$): 1608, 1575, 1475, 1425, 1397, 1386, 1375, 1295, 1259, 1107, 1011, 990. HR MS (FAB): for $C_{16}H_{30}N_2O_6P$ found: 377.1859, calculated: 377.1841. FAB MS, *m/z*: 377 $[MH]^+$ (76). $^1H$ NMR (400 MHz, $CDCl_3$): 1.29 d, 3 H, *J*(vic) = 6.4 ($CH_3$-2′); 1.31, 1.32, 1.33 and 1.34 4 × d, 4 × 3 H, *J*(vic) = 6.2 (4 × $CH_3$-*i*-Pr); 2.05 d, 3 H, *J*($CH_{3,6}$) = 0.9 ($CH_3$-5); 3.87 dd, 1 H, *J*(gem) = 13.5, *J*(H,P) = 9.3 (H-4′b); 3.92 dd, 1 H, *J*(gem) = 13.5, *J*(H,P) = 9.1 (H-4′a); 3.97 m, 1 H (H-2′); 3.97 s, 3 H ($OCH_3$); 4.23 dd, 1 H, *J*(gem) = 11.2, *J*(1′b,2′) = 4.7 (H-1′b); 4.39 dd, 1 H, *J*(gem) = 11.2, *J*(1′a,2′) = 6.3 (H-1′a); 4.75 dh, 2 H, *J*(H,P) = 8.0, *J*(vic) = 6.2 (CH-*i*-Pr); 7.95 q, 1 H, *J*(6,$CH_3$) = 0.9 (H-6). $^{13}C$ NMR (100.6 MHz, $CDCl_3$): 11.84 ($CH_3$-5); 16.95 ($CH_3$-2′); 23.95 d, *J*(C,P) = 4.7 ($CH_3$-*i*-Pr); 24.10 d, *J*(C,P) = 3.7 ($CH_3$-*i*-Pr); 53.84 ($OCH_3$); 64.19 d, *J*(C,P) = 168.4 ($CH_2$-4′); 70.19 ($CH_2$-1′); 70.96 and 71.03 d, *J*(C,P) = 6.8 (CH-*i*-Pr); 76.00 d, *J*(C,P) = 12.3 (CH-2′); 111.18 (C-5); 156.91 (CH-6); 163.41 (C-2); 169.58 (C-4).

*(R)-2-{2-[(Diisopropoxyphosphoryl)methoxy]propoxy}-4-methoxy-5-methylpyrimidine* (**14b**). Yield 365 mg (17%) of a yellowish oil, $R_F$ 0.50. HR MS (FAB): for $C_{16}H_{30}N_2O_6P$ found: 377.1838, calculated: 377.1841. FAB MS, *m/z*: 377 $[MH]^+$ (89). $^1H$, $^{13}C$ NMR and IR data were identical with compound **14a**.

Synthesis of 1-[2-(Phosphonomethoxy)propyl]thymine Derivatives **15a** and **15b**. General Procedure

A mixture of compounds **13a** or **13b** (2.18 mmol) in acetonitrile (25 ml) and bromotrimethylsilane (3 ml) afforded derivatives **15a** and **15b** which were obtained as dilithium salts by the same procedure as for **7a**–**7c**.

*(S)-1-[2-(phosphonomethoxy)propyl]thymine* (**15a**). Yield 164 mg (26%) of a white solid, m.p. > 300 °C. IR, $\nu_{max}$ (KBr): 3205, 3058, 2978, 1691, 1473, 1438, 1386, 1350, 1230, 1100, 1074, 995, 923, 785, 714, 579, 556, 454, 422. $[\alpha]_D$ +28.4 (*c* 0.343 g/100 ml, $H_2O$). HPLC: 99% (S3). HR MS (FAB): for $C_9H_{13}Li_2N_2O_6P$ found: 291.0903, calculated: 291.0910. For $C_9H_{13}Li_2N_2O_6P \cdot 2/5H_2O$ (297.3) calculated: 36.36% C, 4.64% H, 9.42% N, 10.41% P; found: 36.78% C, 4.88% H, 9.13% N, 10.00% P. FAB MS, *m/z*: 291 $[MH]^+$ (82). $^1H$ NMR (500 MHz, $D_2O$): 1.11 d, 3 H, *J*(vic) = 6.15 ($CH_3$-2′); 1.81 d, 3 H, *J*($CH_{3,6}$) = 1.2 ($CH_3$-5); 3.43 dd, 1 H, *J*(gem) = 13.0, *J*(H,P) = 9.4 (H-4′b); 3.58 dd, 1 H, *J*(gem) = 13.0, *J*(H,P) = 9.3 (H-4′a); 3.71 dd, 1 H, *J*(gem) = 13.8, *J*(1′b,2′) = 6.6 (H-1′b); 3.78 m, 1 H (H-2′); 3.82 dd, 1 H, *J*(gem) = 13.8,

$J$(1′a,2′) = 3.6 (H-1′a); 7.48 q, 1 H, $J$(6,$CH_3$) = 1.2 (H-6). $^{13}$C NMR (125.8 MHz, $D_2O$): 11.91 ($CH_3$-5); 16.70 ($CH_3$-2′); 52.63 ($CH_2$-1′); 66.09 d, $J$(C,P) = 156.00 ($CH_2$-4′); 76.56 d, $J$(C,P) = 11.3 (CH-2′); 110.93 (C-5); 144.79 (CH-6); 153.21 (C-2); 167.76 (C-4).

*(R)-1-[2-(Phosphonomethoxy)propyl]thymine* (**15b**). Yield 10.3 g (63%) of a white solid, m.p. > 300 °C. IR, $\nu_{max}$ (KBr): 3260, 3055, 2980, 1688, 1476, 1438, 1385, 1351, 1227, 1107, 1081, 922, 785, 716, 562, 465. $[\alpha]_D$ –27.5 (*c* 0.202 g/100 ml, $H_2O$). HPLC: 98% (S3). HR MS (FAB): for $C_9H_{13}Li_2N_2O_6P$ found: 291.0918, calculated: 291.0910. FAB MS, *m/z*: 291 $[MH]^+$ (10). $^1$H and $^{13}$C NMR data were identical with those of compound **15a**.

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## REFERENCES

1. Holý A. in: *Recent Advances in Nucleosides: Chemistry and Chemotherapy* (C. K. Chu, Ed.), p. 167. Elsevier, Amsterdam 2002.
2. a) Jindřich J., Holý A., Dvořáková H.: *Collect. Czech. Chem. Commun.* **1993**, *58*, 1645; b) Balzarini J., Holý A., Jindřich J., Dvořáková H., Hao Z., Snoeck R.: *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4961.
3. Holý A., Jindřich J., Balzarini J., De Clercq E.: *Czech. Appl.* 2047-90.
4. a) Harrada K., Matulie-Adamie J., Price R. W., Svhinazi R. F., Watanabe K. A., Fox J. J.: *J. Med. Chem.* **1987**, *30*, 226; b) Hpwell H. G., Brodfuethrer P. R., Brundige S. P., Beningi D. A., Sapino C., Jr.: *J. Org. Chem.* **1988**, *53*, 85.
5. a) Singh R. P., Shreeve J. M.: *Synthesis* **2002**, *17*, 2561; b) Haykawa H., Takai F., Tanaka H., Miyasaka T., Yamaguchi K.: *Chem. Pharm. Bull.* **1990**, *38*, 1136; c) Middleton W. J.: *J. Org. Chem.* **1975**, *40*, 547; d) Baasner B., Hagemann H., Tatlow J. C. (Eds): *Methods of Organic Chemistry, Organo-Fluorine Compounds* (Houben–Weyl), E10a. Thieme, Stuttgart, New York 1999.
6. Olah G. A., Welch J. T., Vanker Y. D., Nojima M., Kerekes I., Olah J. A.: *J. Org. Chem.* **1979**, *44*, 3872.
7. a) Yarovenko N. N., Raksha M. A.: *Zh. Obshch. Khim.* **1959**, *29*, 2159; *Chem. Abstr.* **1960**, *54*, 9724; b) Takaoka A., Iwakiri H., Ishikawa N.: *Bull. Chem. Soc. Jpn.* **1979**, *52*, 3377.
8. Novo B., Resnati G. in: *Enantiocontrolled Synthesis of Fluoro-Organic Compounds: Stereochemical Challenges and Biomedical Targets* (V. A. Soloshonok, Ed.), p. 349. John Wiley, New York 1999.
9. Jindřich J., Holý A., Dvořáková H.: *Collect. Czech. Chem. Commun.* **1992**, *57*, 1466.
10. a) Balzarini J., Degréve B., Esteban-Gamboa A., Esnouf R., De Clercq E., Engelborghs Y., Camarasa M.-J., Pérez-Pérez M.-J.: *FEBS Lett.* **2000**, *483*, 181; b) Esteban-Gamboa A., Balzarini J., Esnouf R., De Clercq E., Camarasa M.-J., Pérez-Pérez M.-J.: *J. Med. Chem.* **2000**, *43*, 971.
11. a) Beyl V., Niederprüm H., Voss P.: *Justus Liegibs Ann. Chem.* **1970**, *731*, 58; b) Bennua-Skalmowski B., Vorbrüggen H.: *Tetrahedron Lett.* **1995**, *36*, 2611.
12. Holý A., Ivanova G. S.: *Nucleic Acids Res.* **1974**, *1*, 19.

13. a) Brodfuehrer P. R., Howell H. G., Sapino C., Jr., Vemishetti P.: *Tetrahedron Lett.* **1994**, *35*, 3243; b) Holý A., Votruba I., Masojídková M.: *Collect. Czech. Chem. Commun.* **2001**, *66*, 1545.
14. Holý A.: *Collect. Czech. Chem. Commun.* **1993**, *58*, 649.
15. Holý A., Dvořáková H., Masojídková M.: *Collect. Czech. Chem. Commun.* **1995**, *60*, 1390.
16. Takamatsu S., Katayama S., Hirose N., De Cock E., Schelkens G., Demilleguand M., Brepoel J., Izawa K.: *Nucleosides Nucleotides Nucleic Acids* **2002**, *21*, 849.
17. Parker D.: *Chem. Rev.* **1991**, *91*, 1441.
18. Votruba I., Pomeisl K., Tloušťová E., Holý A., Otová B.: *Biochem. Pharmacol.* **2005**, *69*, 1517.
19. Koga I., Funakoshi K., Matsuda A., Sakai K.: *Tetrahedron: Asymmetry* **1993**, *4*, 1857.
20. Holý A., Günter J., Dvořáková H., Masojídková M., Andrei G., Snoeck R., Balzarini J., De Clercq E.: *J. Med. Chem.* **1999**, *42*, 2064.