Nucleotides

Part LXVI[1])

β-Heteroarylethyl Groups – New Phosphate-Protecting Groups for Phosphotriester Chemistry

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The β-heteroaryl-substituted ethanols **6**–**10** were synthesized and, together with pyridine-2-ethanols and pyridine-4-ethanols, were tested as a new type of phosphate-protecting groups in the synthesis of oligonucleotides by the phosphotriester approach. The synthesis of 5′-*O*-(monomethoxytrityl)thymidine 3′-(β-heteroarylethyl 2,5-dichlorophenyl phosphates) **13**–**17** and **21** provided useful monomeric building blocks in which the various blocking groups could be removed selectively by acid (MeOTr), oximate (2,5-dichlorophenyl phosphate), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (heteroarylethyl phosphate) treatment. The new, fully blocked dimers **38**–**41**, with β-heteroarylethyl protecting groups in the phosphate moiety, were synthesized. The β-heteroarylethyl groups show a broad range of stability towards base treatment in aprotic solvents depending upon the activation of the H–C(β) atoms by the heterocyclic moiety.

1. Introduction. – The use of β-eliminating phosphate-protecting groups can be regarded as an important improvement in phosphotriester chemistry [2] since cleavage of the phosphotri- to the phosphodiester stage will proceed without nucleophilic attack at the P-atom, avoiding a potential breakdown of the internucleotidic linkage. Among those protecting groups are the β-cyanoethyl (ce) group [3], the methyl-substituted β-cyanoethyl groups [4], and the β-(alkylsulfonyl)- and β-(arylsulfonyl)ethyl groups [5]. Since these blocking groups are relatively sensitive to base treatment and eliminate already with ammonia and amines, a more stable type was found in the 2-(4-nitrophenyl)ethyl (npe) group [2a], which is inert towards amines but will be cleaved by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a much stronger base in a clean β-eliminating process. The npe group has proven its advantages in a series of oligonucleotide syntheses [6], which could be extended to a unified blocking-group strategy by introduction of the 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) group for base protection [7] and the 2-(2,4-dinitrophenyl)ethoxycarbonyl (dnpeoc) [8], the 2-(4-nitrophenyl)ethylsulfonyl (npes) [9], and the 2-(dansylethoxycarbonyl) (dnseoc) [10] groups for sugar-OH blocking. To broaden the spectrum of β-eliminating protecting groups for oligonucleotide chemistry, the use of N-containing heteroaryl residues has been tested as activators in β-elimination reactions [11]. Since an sp^2-hybridized ring N-atom reveals a similar activation power as a NO_2 group, the pyridinyl, pyrimidinyl, and pyrazinyl moieties were chosen as new entities in these investigations.

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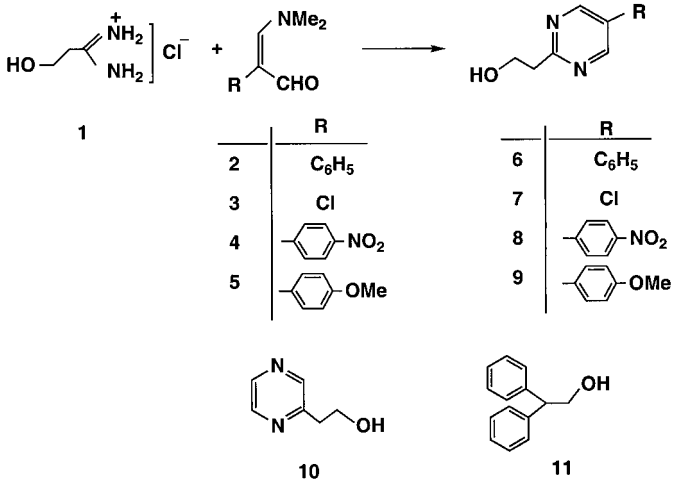
[1]) Part LXV: [1].

2. Syntheses. – Various 5-substituted pyrimidine-2-ethanols were synthesized in analogy to 5-phenylpyrimidine-2-ethanol [12] from 3-hydroxypropanamidine hydrochloride [13] and the 2-substituted 3-(dimethylamino)acrylaldehydes in a base-catalyzed 3 + 3 cyclocondensation reaction (*Scheme 1*); the pyrimidine derivatives **6** – **9** were obtained in moderate to good yields without optimizing the reaction conditions. The 3-(dimethylamino)-2-phenyl- (**2**) and 2-chloro-3-(dimethylamino)acrylaldehyde (**3**) were prepared according to *Arnold* [14], and the 3-(dimethylamino)-2-(4-nitrophenyl)- (**4**) and 3-(dimethylamino)-2-(4-methoxyphenyl)acrylaldehyde (**5**) resulted from an analogous *Vilsmeier* reaction with the corresponding AcOH derivatives.

Scheme 1

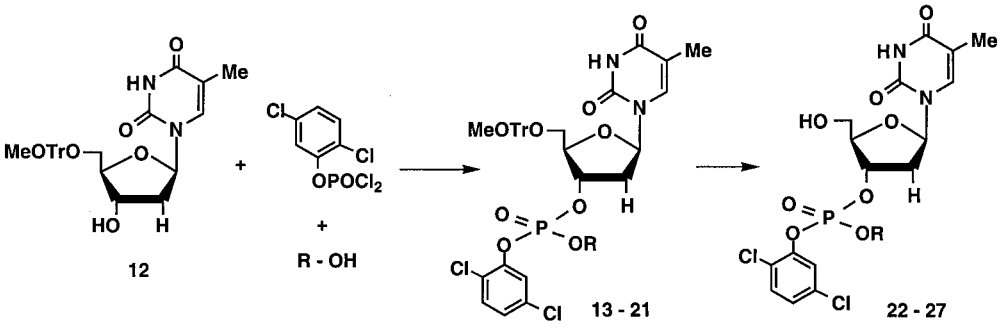


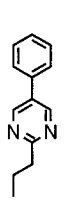
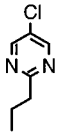
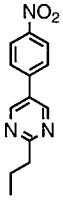
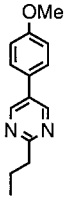
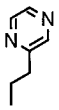
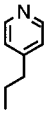
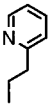
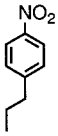
The synthesis of pyrazine-2-ethanol (**10**) was achieved from 2-methylpyrazine and formaldehyde in a hydroxymethylation reaction [15] in relatively low yield, and 2,2-diphenylethanol (**11**) resulted from the reaction of phenyloxirane with phenylmagnesium bromide [16] according to literature procedures.

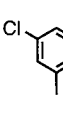
The syntheses of the new modified thymidine phosphotriesters were performed from 5′-*O*-(monomethoxytrityl)thymidine (**12**) by the triazolide method [2a][17] with 2,5-dichlorophenyl phosphorodichloridate [18] and 1,2,4-1*H*-triazole in pyridine to give first the intermediate 3′-phosphodiester triazolides, which reacted subsequently with the β-substituted ethanols to the corresponding 5′-*O*-(monomethoxytrityl)thymidine 3′-phosphotriesters **13** – **20** in 56 – 94% yield, respectively (*Scheme 2*).

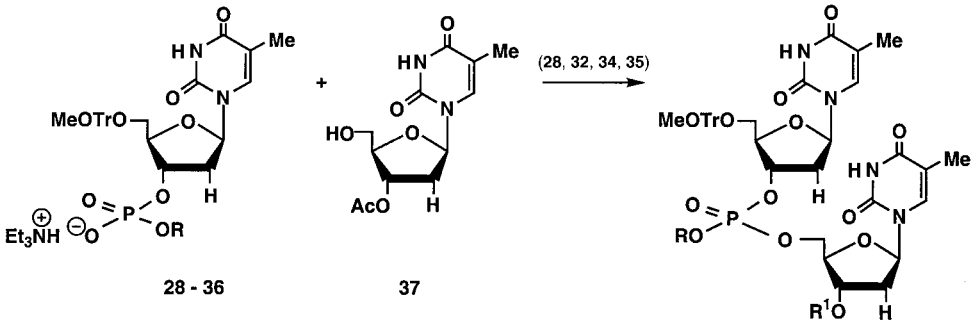
Detritylation of **13** – **18** was achieved in the usual manner by treatment with *p*-toluenesulfonic acid to give **22** – **27** in high yields. The 2,5-dichlorophenyl group could also be cleaved selectively by the oximate method [19] with *p*-nitrobenzaldehyde oxime/Et_3N in dioxane/H_2O, and the resulting phosphodiesters **28** – **35** were isolated by silica-gel column chromatography as their triethyl ammonium salts. Finally, the chemical stability of the β-heteroarylethyl protecting groups was established to be sufficient for the formation of internucleotidic bonds by the normal phosphotriester approach. The phosphodiesters **28**, **32**, **34**, and **35** were coupled with 3′-*O*-acetyl-

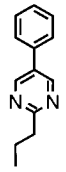
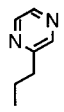
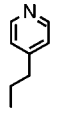
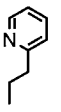
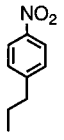
Scheme 2



	13 / 22	14 / 23	15 / 24	16 / 25	17 / 26	18 / 27	19	20	21
R									

	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
R											



	38	39	40	41	42
R					
R^1	Ac	Ac	Ac	Ac	tbdms

thymidine (**37**) to form, under 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-Cl)/1-methyl-1*H*-imidazole activation [20], the dinucleoside phosphotriesters **38**–**41** in 67, 68, 77, and 74% yields, respectively. The corresponding dithymidinyl npe phosphotriester **42** was prepared according to [21] for comparative studies.

3. Stability of the Phosphate-Protecting Groups. – The phosphotriesters **13**–**21** were treated with various bases under aprotic conditions to study their stabilities towards 0.5M DBU or 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in MeCN and pyridine, respectively, as well as towards Et_3N/solvent 1:1. The removal of the base-labile protecting groups was analyzed quantitatively by reversed-phase HPLC, in comparison to the npe phosphotriester **21** as the standard (see *Figure*). The reactions were monitored by aliquots quenched with dilute AcOH at distinct time intervals. The arithmetical evaluation of the data obtained showed that all reactions obey a pseudo-first-order rate law. The HPLCs established that the cleavage of the protecting groups occurred by a β-elimination reaction, as shown by the detection of the corresponding styrene derivatives.

Various conclusions can be drawn from the kinetic data (*Table* and *Fig.*) showing that the β-elimination process is fastest in MeCN with DBU as base. DBN does not show much dependence on the solvent, and Et_3N, even in very high concentration, is much too weak for this type of cleavage reaction.

The activation of the H-atoms at C(β) by the NO_2 group in the npe residue is superseded by the 2-(pyrimidin-2-yl)ethyl group, which can be tuned in its stability by electron-attracting and -donating groups in the usual manner. Therefore, the 2-[5-(4-nitrophenyl)pyrimidin-2-yl]ethyl residue is the most-labile protecting group in this series, whereas its structural analog, the 2-[5-(methoxyphenyl)pyrimidin-2-yl]ethyl group meets the stability of the npe residue. The 2-(pyrazin-2-yl)ethyl residue in **17** is much more stable than the pyrimidinylethyl groups since the second ring N-atom located in *meta* position to the ethyl side-chain activates only by the inductive effect of this heteroatom. The benzhydryl group on **18** is absolutely stable under the applied reaction conditions and is, therefore, useless in approaches towards the synthesis of oligonucleotides.

In the series of the 2-(pyridinyl)ethyl deriatives **19**, **20**, **40**, and **41**, the 2-(pyridin-2-yl)ethyl blocking group in **20** and **41** is more stable than the 2-(pyridin-4-yl)ethyl group in triesters **19** and **40**. In the reaction of the phosphotriesters **19** and **20** with 0.5M DBU/MeCN solution, we observed also the diester **36** as a by-product. We assume that the formation of this phosphodiester took place because of the presence of H_2O in the starting materials. This assumption was confirmed by the special experiments in which the triesters **19** and **20** were treated with 0.5M DBU solution in MeCN/H_2O 9:1; the diester **36** was the only product of these reactions. Therefore, the use of 2-(pyridin-4-yl)ethyl and 2-(pyridin-2-yl)ethyl blocking groups in the synthesis of oligonucleotides is problematic and can not be recommended.

Experimental Part

General. TLC: Precoated silica-gel thin-layer sheets *F 1500 LS254* from *Schleicher & Schüll*. Prep. TLC: silica gel *Merck 60 PF 254*. Column chromatography (CC): silica gel *Merck 60* (0.063–0.2 mesh). HPLC for compounds **13**–**17**, **21**, **38**, and **42**: *Spectra Physics*, *SP8000 B*; column *RP 18* (*LiChrosorb* 250 × 4.6 mm, 7 mm,

Table. *Kinetic Data of Phosphotriester Cleavage by β-Elimination*

	Base/Solvent	$t_{1/2}$	t_∞ [a])	Retention time t_R [s]		
				triester	styrene	diester
13	0.5M DBU/MeCN	1.1 min	13 min	370, 392	215	86
	0.5M DBU/pyridine	2.6 min	24 min			
	0.5M DBU/MeCN	4.2 min	42 min			
	0.5M DBN/pyridine	4.2 min	44 min			
	Et_3N/MeCN 1:1	20 h	194 h			
14	0.5M DBU/MeCN	0.4 min	4 min	330, 345	195	90
	0.5M DBU/pyridine	0.5 min	5 min			
	0.5M DBN/MeCN	1.5 min	14 min			
	0.5M DBN/pyridine	1.7 min	15 min			
	Et_3N/MeCN 1:1	6.1 h	58 h			
15	0.5M DBU/MeCN	0.15 min	1.6 min	320, 342	190	90
	0.5M DBU/pyridine	0.17 min	1.6 min			
	0.5M DBN/MeCN	0.41 min	3.8 min			
	0.5M DBN/pyridine	0.41 min	3.8 min			
	Et_3N/MeCN 1:1	2.73 h	20 h			
16	0.5M DBU/MeCN	2.8 min	27 min	352, 372	215	90
	0.5M DBU/pyridine	6.2 min	54 min			
	0.5M DBN/MeCN	7.4 min	70 min			
	0.5M DBN/pyridine	11.2 min	107 min			
	Et_3N/MeCN 1:1	34 h	334 h			
17	0.5M DBU/MeCN	18.8 min	152 min	1250	1020	700[b])
19	0.5M DBU/MeCN	1.16 h	10 h	1118, 1130		863
20	0.5M DBU/MeCN	8.25 h	80 h	1161, 1175		863
21	0.5M DBU/MeCN	2.8 min	26 min	312, 330	190	87
	0.5M DBU/pyridine	2.8 min	28 min			
	0.5M DBN/MeCN	6.6 min	61 min			
	0.5M DBN/pyridine	5.7 min	52 min			
	Et_3N/MeCN 1:1	31 h	276 h			
	Et_3N/pyridine 1:1	53 h	490 h			
38	0.5M DBU/MeCN	4 min	38 min	411, 435	87	367[c])
40	0.5M DBU/MeCN	6.25 h	55 h	860		728
41	0.5M DBU/MeCN	114 h		874		728
42	0.5M DBU/MeCN	5.4 min	51 min	443	175	59[d])

[a]) Calculated for 0.5%. [b]) *LiChrosphere 100-RP18*; gradient: MeCN/0.1M (Et_3NH)OAc (pH 7) 100:0 → 1:1 in 7 min and 1:1 → 0:100 in 15 min. [c]) Mobile phase: H_2O/MeCN 1:1. [d]) Mobile phase: H_2O/MeCN 1:3.

Merck); flow rate 1.3 ml/min; elution: MeOH/H_2O 89:11. HPLC for compounds **19**, **20**, **40**, and **41**: *Merck-Hitachi, L-6200-Intelligent* pump, *D-2000* chromatointegrator, detection at 260 nm (*Uvicon 730 SLC*, Fa. *Kontron*); column *RP 18* (*LiChrospher* 125 × 4 mm, 5 mm, *Merck 50943*); flow rate 1 ml/min: *A* = 0.1M aq. (Et_3NH)OAc buffer (pH 7.0), *B* = 0.1M aq. (Et_3NH)OAc buffer (pH 7.0)/MeCN 1:1, *C* = MeCN; gradient: *A* in 3 min, from *A* to *B* in 7 min, from *B* to *C* in 15 min. M.p.: *Büchi* apparatus, model Dr. *Tottoli*; no corrections. UV/VIS: *Kontron*, *Uvikon 820*, and *Perkin-Elmer*, *Lambda 15*; λ_{max} in nm (log ε). ^{1}H-NMR: *Bruker WM 250*; δ in ppm rel. to $SiMe_4$ as internal standard.

1. *3-(Dimethylamino)-2-(4-nitrophenyl)prop-2-enal* (**4**). To a cold mixture (0°) of $POCl_3$ (63 g) and of DMF (53 ml), (4-nitrophenyl)acetic acid (25 g, 138 mmol) was added and then heated in an oil bath to 90° for 7 h with stirring. After cooling, the mixture was poured slowly on ice and then neutralized by solid Na_2CO_3. Toluene (300 ml) was added and the mixture heated on a boiling-water bath for 30 min. The aq. phase was additionally extracted twice with CH_2Cl_2 (2 l) and then combined with the toluene extract. After drying (Na_2SO_4) and evaporation, the remaining DMF was distilled off under vacuum, and the resulting residue was recrystallized

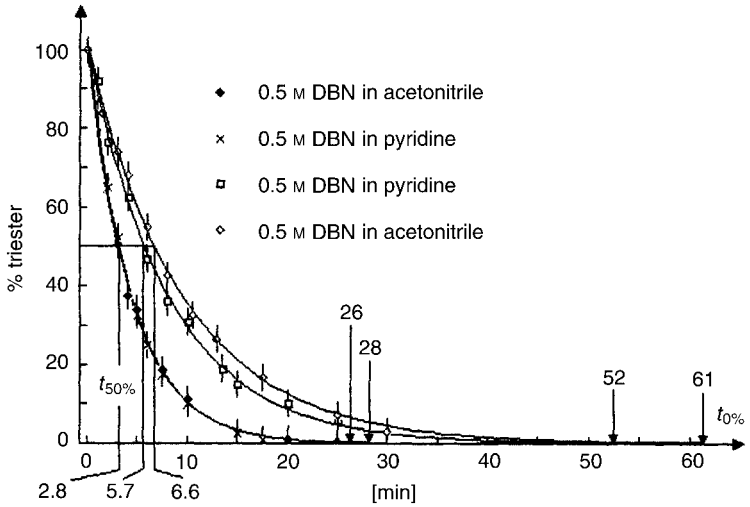


Figure. *Kinetics of npe-cleavage in* **21** *by base-catalyzed β-elimination*

from $CHCl_3$ with charcoal: 21.5 g (71%) of **4**. Yellowish crystals. M.p. 105–110°. UV (MeOH): 287 (4.44), 349 (3.70). ^{1}H-NMR ($CDCl_3$): 9.1 (2*s*, CHO); 8.17–8.34 (2*d*, 2 H *o* to NO_2); 7.30–7.55 (2*d*, 2 H *m* to NO_2); 7.00 (br. *s*, CH); 2.4–3.5 (*m*, Me_2N). Anal. calc. for $C_{11}H_{12}N_2O_3$ (220.2): C 59.99, H 5.49, N 12.72; found: C 60.11, H 5.54, N 12.93.

2. *3-(Dimethylamino)-2-(4-methoxyphenyl)prop-2-enal* (**5**). Analogously to *Exper. 1*, with $POCl_3$ (55 g), DMF (46 ml), and (4-methoxyphenyl)acetic acid (20 g, 0.12 mol) for 5 h. Extraction with CH_2Cl_2 (2.5 l) gave a red-brown sirup. On treatment with Et_2O and stirring, a solid resulted, which was dried in a vacuum desiccator: 13.8 g (54%) of **5**. Deliquescent crystals. The crude material was used for the condensation reaction. UV (MeOH): 221 (3.92), 250 (3.58), 314 (4.32).

3. *2-(5-Substituted Pyrimidine-2)-ethanols: General Procedure.* To a soln. of Na (1.1 g, 48 mmol) in abs. EtOH (90 ml), 3-hydroxypropanamidine hydrochloride [13] (8.3 g, 67 mmol) was added and stirred for 30 min. The precipitate of NaCl was filtered off, then 2-substituted 3-(dimethylamino)prop-2-enal (48 mmol) added to the filtrate, and the mixture heated under reflux for 6 h. After evaporation, the residue was dissolved in $CHCl_3$ and the soln. washed with Na_2SO_4 soln. and H_2O, dried (Na_2SO_4), and again evaporated.

5-Chloropyrimidine-2-ethanol (**7**). From 2-chloro-3-(dimethylamino)prop-2-enal (**3**) (5.32 g, 47 mmol) [14]. The resulting solid was recrystallized from AcOEt (21 ml) and hexane (2 ml): 2.8 g (38%) of **7**. Brownish crystals. M.p. 68°. UV (MeOH): 257 (sh, 3.34), 262 (3.42), 294 (2.71). ^{1}H-NMR ($CDCl_3$): 8.65 (*s*, H–C(4), H–C(6)); 4.1 (*m*, CH_2CH_2O); 3.46 (*s*, OH); 3.22 (*t*, CH_2CH_2O). Anal. calc. for $C_6H_7ClN_2O$ (158.6): C 45.45, H 4.44, N 17.66; found: C 45.36, H 4.26, N 17.53.

5-(4-Nitrophenyl)pyrimidine-2-ethanol (**8**). From **4** (10.1 g, 46 mmol). Recrystallization from $CHCl_3$ (50 ml) and EtOH (50 ml) with charcoal gave 8.5 g (76%) of **8**. Yellow crystals. M.p. 176–178°. UV (MeOH): 213 (4.13), 288 (4.24). ^{1}H-NMR ($CDCl_3$): 8.90 (*s*, H–C(4), H–C(6)); 8.45 (*m*, 2 H *o* to NO_2); 7.75 (*m*, 2 H *m* to NO_2); 4.10 (*m*, 2 H, CH_2 CH_2O); 3.65 (br. *s*, OH); 3.30 (*t*, CH_2CH_2O). Anal. calc. for $C_{12}H_{13}N_3O_3$ (245.2): C 58.77, H 4.52, N 17.13; found: C 58.47, H 4.47, N 17.04.

5-(4-Methoxyphenyl)pyrimidine-2-ethanol (**9**). From **5** (10 g, 48 mmol). The resulting dark red residue was purified by CC (10 × 4.5 cm, $CHCl_3$) and recrystallized from AcOEt (20 ml) and pentane (1 ml): 11.9 g (64%) of **9**. Colorless crystals. M.p. 100–102°. UV (MeOH): 207 (4.25), 271 (4.33). ^{1}H-NMR ($CHCl_3$): 8.85 (*s*, H–C(4), H–C(6)); 7.5 (*m*, 2 H *o* to MeO); 7.05 (*s*, 2 H *m* to MeO); 4.12 (*m*, CH_2CH_2O, OH); 3.90 (*s*, MeO); 3.27 (*t*, CH_2CH_2O). Anal. calc. for $C_{13}H_{14}N_2O_2$ (230.2): C 67.81, H 6.13, N 12.17; found: C 68.06, H 6.47, N 12.22.

4. *5′-O-(4-Methoxytrityl)thymidine 3′-Phosphotriester: General Procedure.* A soln. of 1,2,4-1*H*-triazole (0.414 g, 6 mmol) in abs. pyridine (4.5 ml) was treated with 2,5-dichlorophenyl phosphorodichloridate (0.732 g, 2.6 mmol) and stirred at 0° for 30 min. Then a soln. of 5′-*O*-(monomethoxytrityl)thymidine (**12**; 1.03 g, 2 mmol) in abs. pyridine (10 ml) was added dropwise. Stirring was continued for 1 h. Finally, the 2-substituted ethanol

derivative (4 mmol) was added and the mixture stirred for 1 d. To protect unreacted **12**, the mixture was treated with Ac_2O (1.5 ml) for 2 h, evaporated, and co-evaporated with toluene (3×15 ml). The residue was dissolved in $CHCl_3$, the soln. washed with H_2O, dried (Na_2SO_4), then concentrated to a small volume, and submitted to CC (silica gel, 20×3 cm, 0–5% MeOH/$CHCl_3$). The product was co-evaporated with CH_2Cl_2: colorless amorphous solid, which was dried under vacuum at r.t.

5′-O-(Monomethoxytrityl)thymidine 3′-[2,5-Dichlorophenyl 2-(5-Phenylpyrimidin-2-yl)ethyl Phosphate] (**13**). With 5-phenylpyrimidine-2-ethanol (**6**) [24] (0.8 g): 1.68 g (91%) of **13**. Colorless amorphous foam. UV (MeOH): 228 (sh, 4.58), 249 (4.45). ^{1}H-NMR ($CDCl_3$): 8.85 (2*s*, 2 H (pyr)); 8.2 (*s*, H–N(3)); 7.65 (*m*, 21 arom. H); 6.82 (*d*, 2 H *o* to MeO); 6.46 (*m*, H–C(1′)); 5.35 (*m*, H–C(3′)); 4.77 (*m*, CH_2CH_2O); 4.32 (*m*, H–C(4′)); 3.78 (*s*, MeO); 3.56–3.33 (*m*, CH_2CH_2O, 2 H–C(5′)); 2.70 (*m*, 1 H–C(2′)); 2.40 (*m*, 1 H–C(2′)); 1.35 (*s*, Me). Anal. calc. for $C_{48}H_{43}Cl_2N_4O_9P$ (926.3): C 62.22, H 4.68, N 6.05; found: C 61.85, H 4.74, N 5.92.

5′-O-(Monomethoxytrityl)thymidine 3′-[2-(5-Chloropyrimidin-2-yl)ethyl 2,5-Dichlorophenyl Phosphate] (**14**). With **7** (0.634 g): 1.55 g (88%) of **14**. Colorless foam. UV (MeOH): 227 (sh, 4.42), 264 (4.10), 281 (sh, 3.79). ^{1}H-NMR ($CDCl_3$): 8.58 (*d*, 2 H (pyr)); 8.34 (*s*, H–N(3)); 7.56 (*s*, H–C(6)); 7.43–7.08 (*m*, 15 arom. H); 6.82 (*m*, 2 H *o* to MeO); 6.45 (*m*, H–C(1′)); 5.32 (*m*, H–C(3′)); 4.70 (*m*, CH_2CH_2O); 4.30 (*m*, H–C(4′)); 3.78 (*s*, MeO); 3.60–3.30 (*m*, CH_2CH_2O, 2 H–C(5′)); 2.70 (*m*, 1 H–C(2′)); 2.40 (*m*, 1 H–C(2′)); 1.37 (*s*, Me)). Anal. calc. for $C_{42}H_{38}Cl_3N_4O_9P$ (880.0): C 57.32, H 4.35, N 6.37; found: C 57.48, H 4.35, H 6.22.

5′-O-(Monomethoxytrityl)thymidine 3′-[2,5-Dichlorophenyl 2-[5-(4-Nitrophenyl)pyrimidin-2-yl]ethyl Phosphate] (**15**). With **8** (0.98 g): 1.08 g (56%) of **15**. Colorless foam. UV (MeOH): 223 (sh, 4.55), 273 (4.35), 279 (sh, 4.34), 304 (sh, 4.18). ^{1}H-NMR ($CDCl_3$): 8.90 (*s*, 2 H (pyr)); 8.35 (*m*, 2 H *o* to NO_2); 8.20 (*d*, NH); 7.73 (*m*, 2 H *m* to NO_2); 7.55 (*d*, H–C(6)); 7.5–7.0 (*m*, 15 arom. H); 6.80 (*d*, 2 H *m* to MeO); 6.47 (*m*, H–C(1′)); 5.37 (*m*, H–C(3′)); 4.80 (*m*, CH_2CH_2O); 4.32 (*m*, H–C(4′)); 3.80 (*s*, MeO); 3.55 (*m*, CH_2CH_2O, 2 H–C(5′)); 2.75 (*m*, 1 H–C(2′)); 2.40 (*m*, 1 H–C(2′)); 1.40 (*s*, Me). Anal. calc. for $C_{48}H_{42}Cl_2N_5O_{11}P$ (966.7): C 59.63, H 4.37, N 7.24; found: C 59.33, H 4.61, N 7.22.

5′-O-(Monomethoxytrityl)thymidine 3′-[2,5-Dichlorophenyl 2-[5-(4-Methoxyphenyl)pyrimidin-2-yl]ethyl Phosphate] (**16**). With **9** (0.92 g): 1.29 g (68%) of **16**. Colorless foam. UV (MeOH): 270 (4.43), 280 (sh, 4.37). ^{1}H-NMR ($CDCl_3$): 8.78 (*d*, 2 H (pyr)); 8.30 (*s*, H–N(3)); 7.53 (*s*, H–C(6)); 7.45 (*m*, 2 H *o* to MeO); 7.39–7.09 (*m*, 15 arom. H); 7.00 (*m*, 2 H *m* to MeO); 6.79 (*m*, 2 H *m* to MeO (MeOTr)); 6.43 (*m*, H–C(1′)); 5.32 (*m*, H–C(3′)); 4.75 (*m*, CH_2CH_2O); 4.32–4.24 (*m*, H–C(4′)); 3.84 (*s*, MeO); 3.75 (*s*, MeO); 3.51–3.33 (*m*, CH_2CH_2O, 2 H–C(5′)); 2.65 (*m*, 1 H–C(2′)); 2.40 (*m*, 1 H–C(2′)); 1.43 (*s*, Me). Anal. calc. for $C_{49}H_{45}Cl_2N_4O_{10}P$ (951.8): C 61.83, H 4.72, N 5.89; found: C 61.76, H 4.65, N 5.85.

5′-O-(Monomethoxytrityl)thymidine 3′-[2,5-Dichlorophenyl 2-(Pyrazin-2-yl)ethyl Phosphate] (**17**). With pyrazine-2-ethanol (**10**) [15] (0.5 g): 1.15 g (68%) of **17**. Colorless foam. UV (MeOH): 227 (sh, 4.43), 258 (sh, 4.18), 265 (4.24), 281 (3.81). ^{1}H-NMR ($CDCl_3$): 8.5–8.3 (*m*, H–C(5)(Pyr), H–C(6)(pyr), H–N(3)); 8.05 (*s*, H–C(3)(pyr)); 7.50 (*s*, H–C(6)); 7.4–7.0 (*m*, 15 arom. H); 6.80 (*d*, 2 H *m* to MeO); 6.43 (*m*, H–C(1′)); 5.27 (*m*, H–C(3′)); 4.63 (*m*, CH_2CH_2O); 4.25 (*m*, H–C(4′)); 3.78 (*s*, MeO); 3.45 (*m*, CH_2CH_2O); 3.16 (*dd*, 2 H–C(5′)); 2.60 (*m*, 1 H–C(2′)); 2.38 (*m*, 1 H–C(2′)); 1.35 (*s*, Me). Anal. calc. for $C_{42}H_{39}Cl_2N_4O_9P$ (845.7): C 59.65, H 4.65, N 6.63; found: C 59.66, H 4.66, N 6.55.

5′-O-(Monomethoxytrityl)thymidine 3′-(2,5-Dichlorophenyl 2,2-Diphenylethyl Phosphate) (**18**). With 2,2-diphenylethanol (**11**) [16] (0.79 g): 1.73 g (94%) of **18**. Colorless amorphous solid. UV (MeOH): 230 (sh, 4.42), 259 (sh, 4.03), 265 (4.06), 280 (sh, 3.85). ^{1}H-NMR ($CDCl_3$): 8.45 (*s*, H–N(3)); 7.50 (*s*, H–C(6)); 7.45–7.0 (*m*, 25 arom. H); 6.83 (*d*, 2 H *m* to MeO); 6.40 (*m*, H–C(1′)); 5.17 (*m*, H–C(3′)); 4.70 (*m*, $CHCH_2O$); 4.38 (*t*, $CHCH_2O$); 4.10 (*m*, H–C(4′)); 3.80 (*s*, MeO); 3.33 (*m*, 2 H–C(5′)); 2.50 (*m*, 1 H–C(2′)); 2.20 (*m*, 1 H–C(2′)); 1.52 (*s*, Me). Anal. calc. for $C_{50}H_{45}Cl_2N_2O_9P$ (919.8): C 65.29, H 4.93, N 3.04; found: C 64.71, H 4.98, N 2.93.

5′-O-(Monomethoxytrityl)thymidine 3′-[2,5-Dichlorophenyl 2-(Pyridin-4-yl)ethyl Phosphate] (**19**). With pyridine-4-ethanol (200 mg, 1.6 mmol): 470 mg (54%) of **19**. Solid foam. UV (MeOH): 263 (4.09), 228 (sh, 4.42). ^{1}H-NMR ((D_6)DMSO): 11.41 (*s*, NH); 8.40 (*d*, 2 H–C(2)(py), H–C(6)(py)); 7.62, 7.58 (2*s*, H–C(6)($Cl_2C_6H_3$)); 7.47 (*s*, H–C(6)(T)); 7.40–6.82 (*m*, 18 arom. H, H–C(3)(py), H–C(5)(py)); 6.20 (*dd*, H–C(1′)); 5.14 (*m*, H–C(3′)); 4.43 (*m*, $POCH_2CH_2$); 4.13 (*m*, H–C(4′)); 3.70 (*s*, MeO); 3.20 (*m*, 2 H–C(5′)); 2.93 (*t*, $POCH_2CH_2$); 2.43 (*m*, 2 H–C(2′)); 1.48 (*s*, Me). Anal. calc. for $C_{43}H_{40}Cl_2N_3O_9P \cdot H_2O$ (862.7): C 59.86, H 4.90, N 4.87; found: C 60.45, H 5.01, N 4.52.

5′-O-(Monomethoxytrityl)thymidine [2,5-Dichlorophenyl 2-(Pyridin-2-yl)ethyl Phosphate] (**20**). With pyridine-2-ethanol (200 mg, 1.6 mmol): 640 mg (74%) of **20**. Solid foam. UV (MeOH): 261 (4.10), 228

(sh, 4.40). ^{1}H-NMR ((D_6)DMSO): 11.40 (*s*, NH); 8.43 (*m*, H–C(6)(py)); 7.68 (*m*, H–C(5)(py)); 7.59 (*m*, H–C(6)($Cl_2C_6H_3$)); 7.47 (*s*, H–C(6)(T)); 7.37–6.85 (*m*, 18 arom. H, 2 H–C(3)(py), H–C(4)(py)); 6.19 (*dd*, H–C(1′)); 5.14 (*m*, H–C(3′)); 4.53 (*m*, POCH_2CH_2); 4.14 (*m*, H–C(4′)); 3.70 (*s*, MeO); 3.22 (*m*, 2 H–C(5′)); 3.07 (*t*, POCH_2CH_2); 2.43 (*m*, 2 H–C(2′)); 1.48 (*s*, Me(T)). Anal. calc. for $C_{43}H_{40}Cl_2N_3O_9P \cdot H_2O$ (862.7): C 59.86, H 4.90, N 4.87; found: C 60.27, H 4.97, N 4.57.

5. *Thymidine 3′-Phosphotriesters* **22**–**27**: *General Procedure.* The 5′-O-(monomethoxytrityl)thymidine 3′-phosphotriester **13**–**18** (0.35 mmol) was dissolved in a soln. of 1% TsOH in $CHCl_3$/MeOH 4:1 and stirred at r.t. for 1.5 h. After dilution with $CHCl_3$ (100 ml) and extraction with phosphate buffer (pH 7; 2 × 35 mmol) followed by H_2O, the org. layer was dried (Na_2SO_4), evaporated to a small volume, and purified by prep. TLC (silica gel, 40 × 20 × 0.2 cm, $CHCl_3$/MeOH 19:1). The product band was eluted with $CHCl_3$/MeOH 4:1, the eluate evaporated, the residue redissolved in CH_2Cl_2, and the soln. filtered through cotton, and again evaporated: colorless amorphous foam.

Thymidine 3′-[2,5-Dichlorophenyl 2-(5-Phenylpyrimidin-2-yl)ethyl Phosphate] (**22**). From **13** (0.324 g): 0.209 g (92%) of **20**. UV (MeOH): 223 (4.34). 228 (4.32), 254 (4.35). ^{1}H-NMR ($CDCl_3$): 8.88 (*d*, 2 H (pyr)); 8.20 (br. *s*, H–N(3)); 7.65–7.06 (*m*, 9 arom. H); 6.13 (‘*t*’, H–C(1′)); 5.25 (*m*, H–C(3′)); 4.80 (*m*, CH_2CH_2O); 4.21 (*m*, H–C(4′)); 3.87 (br. *s*, 2 H–C(5′)); 3.43 (*t*, CH_2CH_2O); 3.37 (br. *s*, OH); 2.50 (*m*, 2 H–C(2′)); 1.89 (*s*, Me). Anal. calc. for $C_{28}H_{27}Cl_2N_4O_8P$ (649.4): C 51.78, H 4.19, N 8.63; found: C 51.61, H 4.21, N 8.45.

Thymidine 3′-[2,5-Dichlorophenyl 2-(5-Chloropyrimidin-2-yl)ethyl Phosphate] (**23**). From **14** (0.308 g): 0.172 g (81%) of **23**. UV (MeOH): 210 (sh, 4.45), 263 (4.10), 268 (sh, 4.08), 280 (sh, 3.79). ^{1}H-NMR ($CDCl_3$): 9.05 (*s*, H–N(3)); 8.65 (*s*, 2 H (pyr)); 7.45 (*s*, H–C(6)); 7.4–7.1 (*m*, 3 arom. H); 6.20 (‘*t*’, H–C(1′)); 5.28 (*m*, H–C(3′)); 4.76 (*m*, CH_2CH_2O); 4.23 (*m*, H–C(4′)); 3.87 (*m*, 2 H–C(5′)); 3.40 (*t*, CH_2CH_2O); 3.23 (*m*, OH); 2.50 (*m*, 2 H–C(2′)); 1.90 (*s*, Me). Anal. calc. for $C_{22}H_{22}Cl_3N_4O_8P \cdot 1.5\ H_2O$ (634.7): C 42.84, H 3.76, N 9.08; found: C 42.95, H 3.98, N 9.14.

Thymidine 3′-[2,5-Dichlorophenyl 2-[5-(4-Nitrophenyl)pyrimidin-2-yl]ethyl Phosphate] (**24**). From **15** (0.34 g): 0.177 g (73%) of **24**. UV (MeOH): 218 (sh, 4.46), 273 (4.37), 280 (sh, 4.35), 302 (sh, 4.19). ^{1}H-NMR ($CDCl_3$): 11.3 (*s*, H–N(3)); 9.20 (*s*, 2 H(pyr)); 8.35 (*d*, 2 H *o* to NO_2); 8.15 (*d*, 2 H *m* to NO_2); 7.70–7.25 (*m*, H–C(6), 3 arom. H); 6.15 (*m*, H–C(1′)); 5.25 (*t*, OH); 5.10 (*m*, H–C(3′)); 4.75 (*m*, CH_2CH_2O); 4.05 (*m*, H–C(4′)); 3.57 (*m*, 2 H–C(5′)); 3.40 (*t*, CH_2CH_2O); 2.3 (*m*, 2 H–C(2′)); 1.75 (*s*, Me). Anal. calc. for $C_{28}H_{26}Cl_2N_5O_{10}P \cdot 0.5\ H_2O$ (702.4): C 47.81, H 3.86, N 9.96; found: C 47.89, H 3.68, N 9.92.

Thymidine 3′-[2,5-Dichlorophenyl 2-[5-(4-Methoxyphenyl)pyrimidin-2-yl]ethyl Phosphate] (**25**). From **16** (0.333 g): 0.2 g (85%) of **25**. UV (MeOH): 223 (sh, 4.38), 267 (4.43). ^{1}H-NMR ($CDCl_3$): 8.85 (*m*, H–N(3), 2 H, (pyr)); 7.55–7.30 (*m*, H–C(6), 4 arom. H); 7.05 (*d*, 2 H *o* to MeO); 6.17 (‘*t*’, H–C(1′)); 5.30 (*m*, H–C(3′)); 4.80 (*m*, CH_2CH_2O); 4.22 (*m*, H–C(4′)); 3.87 (*m*, MeO, 2 H–C(5′)); 3.56 (*m*, OH); 3.43 (*t*, CH_2CH_2O); 2.52 (*m*, 2 H–C(2′)); 1.90 (*s*, Me). Anal. calc. for $C_{29}H_{29}Cl_2N_4O_9P$ (679.4): C 51.26, H 4.30, N 8.24; found: C 50.95, H 4.26, N 8.07.

Thymidine 3′-[2,5-Dichlorophenyl 2-(Pyrazin-2-yl)ethyl Phosphate] (**26**). From **17** (0.296 g): 0.182 g (91%) of **26**. UV (MeOH): 221 (sh, 4.23), 264 (4.24), 270 (sh, 4.19), 307 (3.02). ^{1}H-NMR ($CDCl_3$): 8.66 (*m*, H–N(3), H–C(5)(pyr), H–C(6)(pyr)); 8.45 (*s*, H–C(3)(pyr)); 7.43 (*s*, H–C(6)); 7.36–7.10 (*m*, 3 arom. H); 6.15 (‘*t*’, H–C(1′)); 5.24 (*m*, H–C(3′)); 4.69 (*m*, CH_2CH_2O); 4.20 (*m*, H–C(4′)); 3.82 (*m*, 2 H–C(5′)); 3.25 (*t*, CH_2CH_2O); 2.90 (*m*, OH); 2.49 (*m*, 2 H–C(2′)); 1.82 (*s*, Me). Anal. calc. for $C_{22}H_{23}Cl_2N_4O_8P$ (573.3): C 46.08, H 4.04, N 9.77; found: C 45.98, H 3.98, N 9.67.

Thymidine 3′-[2,5-Dichlorophenyl 2,2-Diphenylethyl Phosphate] (**27**). From **18** (0.322 g): 0.206 g (91%) of **27**. UV (MeOH): 218 (sh, 4.43), 264 (4.04). ^{1}H-NMR ($CDCl_3$): 8.33 (*s*, H–N(3)); 7.45–7.07 (*m*, H–C(6), 12 arom. H); 6.08 (*dd*, H–C(1′)); 5.08 (*m*, H–C(3′)); 4.70 (*m*, $CHCH_2O$); 4.42 (*t*, $CHCH_2$); 4.05 (*m*, H–C(4′)); 3.70 (*m*, 2 H–C(5′)); 2.56 (br. *s*, OH); 2.32 (*m*, 2 H–C(2′)); 1.80 (*s*, Me). Anal. calc. for $C_{30}H_{29}Cl_2N_2O_8P$ (647.4): C 55.65, H 4.51, N 4.32; found: C 55.44, H 5.01, N 4.19.

6. *5′-O-(Monomethoxytrityl)thymidine 3′-(2-Heteroarylethyl Triethylammonium Phosphates)* **28**–**35**: *General Procedure.* At r.t. 4-nitrobenzaldehyde oxime (0.83 g, 5 mmol) was dissolved with stirring in dioxane (10 ml), Et_3N (10 ml), and H_2O (10 ml). After 10 min, the 5′-*O*-(monomethoxytrityl)thymidine 3′-phosphotriester **13**–**20** (0.5 mmol) was added, and stirring was continued for 2.5 h. The mixture was then evaporated, co-evaporated with pyridine (4 × 10 ml) and toluene (4 × 10 ml), and then submitted to CC (silica gel, 15 × 2.5 cm, $CHCl_3$/MeOH 95:5 (→ excess oxime and 4-nitrobenzonitrile), then $CHCl_3$/MeOH/Et_3N 100:10:3 (→ product)). The product was co-evaporated with CH_2Cl_2, taken up in CH_2Cl_2, filtered through cotton, and then evaporated again: colorless foam, which was dried at 40° high vacuum.

5′-O-(Monomethoxytrityl)thymidine 3′-[2-(5-Phenylpyrimidin-2-yl)ethyl Triethylammonium Phosphate (**28**). From **13** (0.463 g): 0.435 g (98%) of **28**. ^{1}H-NMR ($CDCl_3$): 12.2 (br. *s*, Et_3NH^+); 8.80 (*s*, 2 H (pyr));

8.37 (*s*, H–N(3)); 7.60 (*s*, H–C(6)); 7.55–7.12 (*m*, 17 arom. H); 6.80 (*d*, 2 H *o* to MeO); 6.48 (*dd*, H–C(1′)); 5.07 (*m*, H–C(3′)); 4.45–4.33 (*m*, CH_2O, H–C(4′)); 3.78 (*s*, MeO); 3.45 (*dd*, 2 H–C(5′)); 3.32 (*t*, CH_2CH_2O); 3.05 (*q*, 3 MeCH_2N); 2.68 (*m*, 1 H–C(2′)); 2.35 (*m*, 1 H–C(2′)); 1.34 (*m*, 3 *Me*CH_2N, Me).

*5′-*O*-(Monomethoxytrityl)thymidine 3′-[2-(5-Chloropyrimidin-2-yl)ethyl Triethylammonium Phosphate]* (**29**). From **14** (0.44 g): 0.287 g (70%) of **29**. ^{1}H-NMR ($CDCl_3$): 12.5 (br. *s*, Et_3NH^+); 8.40 (br. *s*, H–N(3), 2 H(pyr)); 7.61 (*s*, H–C(6)); 7.55–7.22 (*m*, 16 arom. H); 6.80 (*d*, 2 H *o* to MeO); 6.51 (*m*, H–C(1′)); 5.03 (*m*, H–C(3′)); 4.35 (*m*, CH_2O, H–C(4′)); 3.79 (*s*, MeO); 3.45 (*m*, 2 H–C(5′)); 3.25 (*t*, CH_2CH_2O); 3.05 (*m*, 3 MeCH_2N); 2.70 (*m*, 1 H–C(2′)); 2.35 (*m*, 1 H–C(2′)); 1.30 (*m*, 3 *Me*CH_2N, Me).

*5′-*O*-(Monomethoxytrityl)thymidine 3′-[2-[5-(4-Nitrophenyl)pyrimidin-2-yl]ethyl Triethylammonium Phosphate]* (**30**). From **15** (0.483 g): 0.41 g (90%) of **30**. ^{1}H-NMR ($CDCl_3$): 12.6 (br. *s*, Et_3NH^+); 8.90 (*s*, 2 H (pyr)); 8.64 (br. *s*, H–N(3)); 8.33 (*d*, 2 H *o* to NO_2); 7.8–7.5 (*m*, H–C(6), 2 H *m* to NO_2); 7.42–7.20 (*m*, 14 arom. H); 6.82 (*d*, 2 H *o* to MeO); 6.51 (*m*, H–C(1′)); 5.15 (*m*, H–C(3′)); 4.39 (*m*, CH_2O, H–C(4′)); 3.80 (*s*, MeO); 3.70–3.30 (*m*, CH_2CH_2O, 2 H–C(5′)); 3.09 (*m*, 3 MeCH_2N); 2.75 (*m*, 1 H–C(2′)); 2.40 (*m*, 1 H–C(2′)); 1.40 (*m*, 3 *Me*CH_2N, Me).

*5′-*O*-(Monomethoxytrityl)thymidine 3′-[2-[5-(4-Methoxyphenyl)pyrimidin-2-yl]ethyl Triethylammonium Phosphate]* (**31**). From **16** (0.476 g): 0.393 g (88%) of **31**. ^{1}H-NMR ($CDCl_3$): 12.2 (br. *s*, Et_3NH^+); 8.77 (*s*, 2 H (pyr)); 8.58 (br. *s*, H–N(3)); 7.71 (*s*, H–C(6)); 7.45–7.15 (*m*, 16 H, H *m* to MeO, arom. H); 7.00 (*d*, 2 H *o* to MeO); 6.81 (*d*, 2 H *o* to MeO (MeOTr)); 6.51 (*dd*, H–C(1′)); 5.06 (*m*, H–C(3′)); 4.39 (*m*, CH_2O, H–C(4′)); 3.87 (*s*, MeO); 3.77 (*s*, MeO); 3.45 (*m*, 2 H–C(5′)); 3.29 (*t*, CH_2CH_2O); 3.10 (*q*, 3 MeCH_2N); 2.70 (*m*, 1 H–C(2′)); 2.35 (*m*, H–C(2′)); 1.3 (*m*, 3 *Me*CH_2N, Me).

*5′-*O*-(Monomethoxytrityl)thymidine 3′-[2-(Pyrazin-2-yl)ethyl Triethylammonium Phosphate]* (**32**). From **17** (0.422 g): 0.372 g (93%) of **32**. UV (MeOH): 205 (4.75), 230 (sh, 4.17), 265 (4.14), 310 (2.96). ^{1}H-NMR ($CDCl_3$): 12.2 (br. *s*, Et_3NH^+); 8.55–8.35 (*m*, H–N(3), 2 H (pyr)); 8.25 (*s*, 1 H (pyr)); 7.61 (*s*, H–C(6)); 7.45–7.17 (*m*, 12 arom. H); 6.83 (*d*, 2 H *o* to MeO); 6.45 (*m*, H–C(1′)); 4.98 (*m*, H–C(3′)); 4.33–4.18 (*m*, CH_2CH_2O, H–C(4′)); 3.78 (*s*, MeO); 3.40 (*dd*, 2 H–C(5′)); 3.05 (*m*, CH_2CH_2O; 3 MeCH_2N); 2.65 (*m*, 1 H–C(2′)); 2.34 (*m*, 1 H–C(2′)); 1.36 (*m*, 3 *Me*CH_2N, Me).

*5′-*O*-(Monomethoxytrityl)thymidine 3′-[2,2-Diphenylethyl Triethylammonium Phosphate]* (**33**). From **18** (0.46 g): 0.346 g (79%) of **33**. ^{1}H-NMR ($CDCl_3$): 12.2 (br. *s*, Et_3NH); 8.17 (*s*, H–N(3)); 7.54 (*s*, H–C(6)); 7.4–7.05 (*m*, 22 arom. H); 6.81 (*d*, 2 H *o* to MeO); 6.40 (*m*, H–C(1′)); 4.91 (*m*, H–C(3′)); 4.41 (*t*, $CHCH_2O$); 4.31 (*d*, $CHCH_2O$); 4.17 (*m*, H–C(4′)); 3.78 (*s*, MeO); 3.30 (*dd*, 2 H–C(5′)); 2.83 (*m*, 3 MeCH_2N); 2.48 (*m*, 1 H–C(5′)); 2.15 (*m*, 1 H–C(5′)); 1.31 (*s*, Me); 1.15 (*m*, 3 *Me*CH_2N).

*5′-*O*-(Monomethoxytrityl)thymidine 3′-[2-(Pyridin-4-yl)ethyl Triethylammonium Phosphate]* (**34**). From **20** (410 mg, 0.46 mmol): 320 mg (88%) of **34**. Solid foam. UV (MeOH): 262 (4.04), 230 (sh, 4.21). ^{1}H-NMR ((D_6)DMSO): 11.37 (*s*, H–N(3)); 10.58 (br. *s*, Et_3NH^+); 8.33 (*d*, H–C(3)(py), H–C(5)(py)); 7.46 (*s*, H–C(6)(T)); 7.38–6.87 (*m*, 14 arom. H, H–C(2)(py), H–C(6)(py)); 6.17 (*dd*, H–C(1′)); 4.60 (*m*, H–C(3′)); 4.05 (*m*, H–C(4′)); 3.82 (*m*, $POCH_2CH_2$); 3.72 (*s*, MeO); 3.17 (*m*, 2 H–C(5′)); 2.94 (*q*, 3 MeCH_2N); 2.74 (*t*, $POCH_2CH_2$); 2.19 (*m*, 2 H–C(2′)); 1.36 (*s*, Me); 1.11 (*t*, 3 *Me*CH_2N). Anal. calc. for $C_{43}H_{53}N_4O_9P \cdot H_2O$ (818.9): C 63.07, H 6.76, N 6.84; found: C 63.15, H 6.76, N 6.25.

*5′-*O*-(Monomethoxytrityl)thymidine 3′-[2-(Pyridin-2-yl)ethyl Triethylammonium Phosphate]* (**35**). From **21** (570 mg, 0.66 mmol): 470 mg (89%) of **35**. Solid foam. UV (MeOH): 267 (sh, 4.11), 261 (4.12), 230 (sh, 4.23). ^{1}H-NMR ((D_6)DMSO): 11.35 (*s*, H–N(3)); 10.77 (br. *s*, Et_3NH^+); 8.41 (*d*, H–C(6)(py)); 7.62 (*s*, H–C(5)(T)); 7.46 (*s*, H–C(6)(T)); 7.37–6.86 (*m*, 14 arom. H, H–C(3)(py), H–C(4)(py)); 6.17 (*dd*, H–C(1′)); 4.61 (*m*, H–C(3′)); 4.06 (*m*, H–C(4′)); 3.93 (*m*, $POCH_2CH_2$); 3.70 (*s*, MeO); 3.18 (*m*, 2 H–C(5′)); 2.92 (*m*, $POCH_2CH_2$, 3 MeCH_2N); 2.24 (*m*, 2 H–C(2′)); 1.36 (*s*, Me(T)); 1.11 (*t*, 3 *Me*CH_2N). Anal. calc. for $C_{43}H_{53}N_4O_9P \cdot 1.5\ H_2O$ (827.91): C 62.38, H 6.81, N 6.76; found: C 62.44, H 6.64, N 6.40.

7. *5′-*O*-(Monomethoxytrityl)thymidylyl-{3′-{O^P-[2-(5-phenylpyrimidin-2-yl)ethyl]}-5′}-3′-*O*-acetylthymidine* (**38**). A mixture of **28** (0.281 g, 0.32 mmol) and 3′-*O*-acetylthymidine (**37**) (0.07 g, 0.25 mmol) was twice co-evaporated with abs. pyridine. The residue was dissolved in abs. pyridine (2.5 ml), 1-methyl-1*H*-imidazole (0.15 ml, 1.9 mmol) and TPS-Cl (0.194 g, 0.64 mmol) were added, and the mixture was stirred at r.t. for 24 h. The soln. was then treated with H_2O (2.5 ml) and evaporated, the residue dissolved in $CHCl_3$ (40 ml), the soln. washed twice with ice-water, dried (Na_2SO_4) and evaporated, and the residue co-evaporated with toluene, dissolved in a little $CHCl_3$, and submitted to prep. TLC (silica gel, 2 plates 40 × 20 × 0.2 cm, $CHCl_3$/MeOH 95:5). The main band was eluted with $CHCl_3$/MeOH 4:1 and the eluate rechromatographed by the same procedure. The resulting foam was dissolved in dioxane (8 ml) and lyophilized: 0.175 g (67%) of **38**. Amorphous solid. UV (MeOH): 236 (4.45), 257 (4.47). ^{1}H-NMR ($CDCl_3$): 8.86, 8.83 (2*s*, 2 H (pyr)); 8.67, 8.57 (2*s*, 2 NH); 7.6–7.1 (*m*, 19 arom. H); 6.83 (*d*, 2 H *o* to MeO); 6.46–6.27 (*m*, 2 H, H–C(1′)); 5.30 (*m*, 2 H, H–C(3′));

4.72–4.52 (*m*, OCH_2CH_2); 4.36–4.05 (*m*, 4 H, 2 H–C(4′), 2 H–C(5′)); 3.78 (*s*, MeO); 3.6–3.27 (*m*, 4 H, OCH_2CH_2, 2 H–C(5′)); 2.67–2.12 (*m*, 4 H, 2 H–C(2′)); 2.10 (*s*, AcO); 1.87 (*s*, 3 H, Me–C(5)); 1.40 (*s*, 3 H, Me–C(5)). Anal. calc. for $C_{54}H_{55}N_6O_{15}P \cdot H_2O$ (1077.0): C 60.21, H 5.34, N 7.80; found: C 60.02, H 5.45, N 7.54.

8. *5′*-O-*(Monomethoxytrityl)thymidine-{3′-{O*^{P}*-[2-(pyrazin-2-yl)ethyl]}-5′}-3′*-O-*acetylthymidine* (**39**). Analogously to *Exper. 7* from **32** (0.206 g, 0.24 mmol), **37** (56 mg, 0.2 mmol), pyridine (2 ml), 1-methyl-1*H*-imidazole (0.11 ml, 0.14 mmol), and TPS-Cl (0.15 g, 0.48 mmol): 0.134 g (68%) of **39**. Amorphous powder. UV (MeOH): 230 (sh, 4.28), 265 (4.39), 272 (sh, 4.34), 309 (2.86). ^{1}H-NMR ($CDCl_3$): 8.80 (br. *s*, 2 NH); 8.54–8.30 (*m*, 3 H(pyr)); 7.56, 7.52 (2*s*, 2 H, H–C(6)); 7.42–7.20 (*m*, 12 arom. H); 6.85 (*d*, 2 H *o* to MeO); 6.44–6.26 (*m*, 2 H, H–C(1′)); 5.27–5.08 (*m*, 2 H, H–C(3′)); 4.56–4.35 (*m*, OCH_2CH_2); 4.30–4.04 (*m*, 4 H, 2 H–C(4′), 2 H–C(5′)); 3.78 (*s*, MeO); 3.42 (*dd*, 2 H–C(5′)); 3.24–3.05 (*m*, OCH_2CH_2); 2.65–2.12 (*m*, 4 H, 2 H–C(2′)); 2.10 (2*s*, AcO); 1.87 (2*s*, 3 H, Me–C(5)); 1.40 (2*s*, 3 H, Me–C(5)). Anal. calc. for $C_{48}H_{51}N_6O_{15}P \cdot H_2O$ (1001.0): C 57.93, H 5.34, N 8.40; found: C 57.83, H 5.55, N 8.27.

9. *5′*-O-*(Monomethoxytrityl)thymidine-{3′-{O*^{P}*-[2-(pyridin-4-yl)ethyl]}-5′}-3′*-O-*acetylthymidine* (**40**). A mixture of **34** (310 mg, 0.387 mmol) and **37** (99 mg, 0.35 mmol) was co-evaporated with pyridine (4 × 10 ml). The residue was dissolved in pyridine (5 ml), 1-methyl-1*H*-imidazole (190 mg, 2.32 mmol) and TPS-Cl (234 mg, 0.774 mmol) were added, and the mixture was stirred at r.t. for 24 h. The soln. was then treated with H_2O (0.5 ml) and evaporated. The residue was dissolved in AcOEt (200 ml), washed with phosphate buffer pH 7 (50 ml) and H_2O (50 ml), the org. layer dried (Na_2SO_4) and evaporated, and the residue co-evaporated with toluene and purified by CC (silica gel, 3.5 × 12 cm, CH_2Cl_2, then CH_2Cl_2/MeOH 50:1 and 20:1): 270 mg (77%) of **40**. Solid foam. UV (MeOH): 263 (4.27), 232 (sh, 4.29). ^{1}H-NMR ((D_6)DMSO): 11.39, 11.36 (2*s*, 2 H–N(3)); 8.41, 8.39 (2*d*, H–C(2)(py), H–C(6)(py)); 7.45 (*s*, 2 H–C(6)(T)); 7.37–6.88 (*m*, 16 arom. H), H–C(3)(py), H–C(5)(py)); 6.15 (*m*, H–C(1′)); 5.12, 4.97 (2*m*, 2 H–C(3′)); 4.21 (*m*, $POCH_2$); 4.16 (*m*, 2 H–C(4′), MeOTrOCH_2); 3.71 (*s*, MeO); 3.20 (*m*, 2 H–C(5′)); 2.87 (*m*, $POCH_2CH_2$); 2.40, 2.24 (2*m*, 4 H–C(2′)); 2.04, 2.03 (2*s*, 2 AcO (2 diastereoisomers)); 1.70, 1.69, 1.44, 1.43 (4*s*, 2 Me (T) (2 diastereoisomers)). Anal. calc. for $C_{49}H_{52}N_5O_{14}P \cdot 2\,H_2O$ (1002.0): C 58.73, H 5.63, N 6.98; found: C 58.67, H 5.64, N 6.87.

10. *5′*-O-*(Monomethoxytrityl)thymidine-{3′-{O*^{P}*-[2-(pyridin-2-yl)ethyl]}-5′}-3′*-O-*acetylthymidine* (**41**). A mixture of **35** (440 mg, 0.55 mmol) and **37** (128 mg, 0.45 mmol) was co-evaporated with pyridine (4 × 10 ml). The residue was dissolved in pyridine (4 ml), 1*H*-tetrazole (230 mg, 3.3 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (333 mg, 1.1 mmol) were added, and the mixture was stirred at r.t. for 20 h. The soln. was diluted with $CHCl_3$ (100 ml) and washed with phosphate buffer pH 7 (50 ml) and H_2O (50 ml). The org. layer was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (20 ml). The residue was purified by CC (silica gel, 3.5 × 10 cm, $CHCl_3$, then $CHCl_3$/MeOH 30:1): 326 mg (74%) of **41**. UV (MeOH): 261 (4.43), 232 (sh, 4.39). ^{1}H-NMR ((D_6)DMSO): 11.39, 11.36 (2*s*, 2 H–N(3)); 8.42 (*d*, H–C(6)(py)); 7.63 (*dd*, H–C(5)(py)); 7.46 (*s*, 2 H–C(6)(T)); 7.37–6.86 (*m*, 16 H, arom. H, H–C(3)(py), H–C(4)(py)); 6.13 (*dd*, H–C(1′)); 5.11, 4.97 (2*m*, 2 H–C(3′)); 4.33 (*m*, $POCH_2CH_2$); 4.07 (*m*, 2 H–C(4′), MeOTrOCH_2); 3.71 (*s*, MeO); 3.20 (*m*, 2 H–C(5′)); 3.01 (*m*, $POCH_2CH_2$); 2.40, 2.24 (2*m*, 4 H–C(2′)); 2.04, 2.03 (2*s*, 2 AcO); 1.68, 1.42 (2*s*, 2 Me (T)). Anal. calc. for $C_{49}H_{52}N_5O_{14}P \cdot 2\,H_2O$ (1002.0): C 58.73, H 5.63, N 6.98; found: C 58.78, H 5.66, N 6.61.

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