191. Nucleotides

Part XXXII[1])

Synthesis of 2′–5′ Connected Oligonucleotides. Prodrugs for Antiviral and Antitumoral Nucleosides

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The cytotoxically and antivirally active compounds bvU_d (**1**), flU_d (**4**), acyclovir (**7**), and A_a (**12**) have chemically been combined with the appropriately protected (2′–5′)diadenylate **20** by the phosphotriester approach to give the 2′–5′ oligonucleotide trimers **21**–**24**. The deprotection of the various blocking groups by chemical means afforded the 2′–5′ trimers **25**–**28**, which can be regarded as a new type of a potential prodrug form delivering nucleotides to the targets inside cells. In an analogous series of reactions, 9-(3′-azido-3′-deoxy-β-D-xylofuranosyl)adenine was coupled with **7** to the 2′–5′ trimer **31**. The antiviral screening of the oligonucleotides **25**–**27** and **31** showed biological activities closely related to the parent nucleosides, possibly indicating their release by enzymatic cleavage of the oligomers.

1. Introduction. – The antiherpes activity of 5-[(*E*)-2-bromovinyl]-2′-deoxyuridine (= bvU_d; **1**), 9-[(2-hydroxyethoxy)methyl]guanine (= acyclovir; **7**), and 9-(β-D-arabinofuranosyl)adenine (= A_a; **12**) is highly dependent on their selective phosphorylation in infected cells [2] by virus-encoded thymidine kinase (TK). Consequently, infected cells lacking this enzyme (when they are infected by a TK^- (TK-deficient) virus strain) are resistant to the action of these drugs. The importance of intracellular phosphorylation is also demonstrated by the cytostatic activity of **1** in cells transformed by the *Herpes simplex* virus type 1 or type 2 thymidine kinase gene [3]. In these cells, 5′-*O*-monophosphate of **1** (= $p5'bvU_d$) interacts analogously to 2′-deoxy-5-fluoro-uridine 5′-*O*-monophosphate (= $p5'flU_d$) with thymidylate synthase and shuts off this key enzyme in nucleic-acid metabolism. High substrate specificities have been noted in the phosphorylation pathway, since, in contrast with the monophosphate of acyclovir, $p5'bvU_d$ is not further phosphorylated to its di- and triphosphates in *Herpes simplex* virus 2 infected cells. In general, phosphorylation to their mono-, di-, and triphosphates is crucial for the various biological effects of nucleoside analogues. A clear example is 3′-azido-3′-deoxythymidine (AZT) that needs to be phosphorylated to its 5′-*O*-triphosphate, before it can act with its target enzyme, the AIDS virus associated reverse transcriptase [4]. Unfortunately, the bad uptake of nucleotides into the cells is a serious draw-back for the

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direct use of these nucleotides, and, although many approaches have been tried to deliver nucleotides into the cells [5], only little success has been achieved.

We present here the synthesis of various trimeric oligonucleotides derived from (2′–5′)diadenylates and bearing an antitumoral or antiviral agent at the 2′-end as a new type of a potential prodrug form that should be capable to deliver nucleotides. The 5′-*O*-monophosphates of these biologically active nucleosides will be generated on enzymatic hydrolysis. It is aimed that this degradation would take place at least partially inside and not completely outside of the cell.

2. Syntheses. – The chemical synthesis of oligonucleotides *via* the phosphotriester approach [6] [7] implies the preparation of appropriately protected building blocks consisting mostly of a 3′-phosphodiester component on one hand and a 5′-OH nucleoside or nucleotide on the other hand for the buildup of a new internucleotidic linkage. Since our synthetic efforts were based on the condensation of the dimeric {N^6-benzoyl-3′-*O*-[(*tert*-butyl)dimethylsilyl]-5′-*O*-(monomethoxytrityl)adenosin}-2′-yl {2′-{O^P-[2-(4-nitrophenyl)ethyl]} → 5′}-N^6-benzoyl-3′-*O*-[(*tert*-butyl)dimethylsilyl]adenosine 2′-[2-(4-nitrophenyl)ethyl triethylammonium phosphate] (**20**) with various protected nucleosides (**3**, **6**, **11**, **15**), all these components were prepared first by conventional methods. For this purpose, bvU_d (**1**) was treated with monomethoxytrityl chloride (MeOTrCl) in pyridine to give 5-((*E*)-2-bromovinyl)-2′-deoxy-5′-*O*-(monomethoxytrityl)uridine (**2**; *Scheme 1*). Then, (*tert*-butyl)dimethylsilylation and subsequent acid-catalysed detritylation afforded 5-((*E*)-2-bromovinyl)-3′-*O*-[(*tert*-butyl)dimethylsilyl]-2′-deoxyuridine (**3**) in good yield. Starting from 2′-deoxy-5-fluorouridine (**4**), the same reaction sequence was performed to give intermediate **5** and, after acid treatment, 3′-*O*-[(*tert*-butyl)dimethylsilyl]-2′-deoxy-5′-fluorouridine (**6**) in crystalline form.

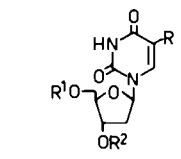
For the incorporation of acyclovir (**7**) into an oligonucleotide, the protection of the amino as well as the amide function of the guanine moiety appeared highly desirable. Acetylation of **7** with Ac_2O in pyridine gave **8** in 94% yield, and no reaction at the aglycone was detected. O^6-Alkylation under *Mitsunobu*'s conditions [8] (diethyl azodicarboxylate, triphenylphosphane, and 2-(4-nitrophenyl)ethanol) afforded 9-[(2-acetoxyethoxy)methyl]-O^6-[2-(4-nitrophenyl)ethyl]guanine (**9**) which, after acylation with 2-(4-nitrophenyl)ethyl chloroformate in CH_2Cl_2, gave almost a quantitative yield of **10**. Mild hydrolysis of **10** with ammonia led to the partially blocked modified acyclovir derivative 9-[(2-hydroxyethoxy)methyl]-N^2-[2-(4-nitrophenyl)ethoxycarbonyl]-O^6-[2-(4-nitrophenyl)ethyl]guanine (**11**).

A series of reactions was necessary to convert 9-(arabinofuranosyl)adenine **12** *via* its N^6-benzoyl derivative **13** and the N^6,N^6,2′-*O*,3′-*O*-tetrabenzoyl derivative **14** into the required appropriately protected nucleoside **15**.

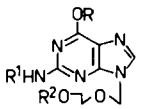
The dimeric component **20** was derived from N^6-benzoyl-3′-*O*-[(*tert*-butyl)dimethylsilyl]-5′-*O*-(monomethoxytrityl)adenosine 2′-[2,5-dichlorophenyl 2-(4-nitrophenyl)ethyl phosphate] (**16**) [9] by detritylation (→ **17**) and hydrolysis (→ **18**) [9], followed by condensation of **17** and **18** in the presence of quinoline-8-sulfonyl chloride/3-nitro-1,2,4-triazole (→ **19** in 87% yield) and treatment with 4-nitrobenzaldehyde oxime (→ **20** in 90% yield).

The coupling reaction between **20** and the partly protected nucleoside components **3**, **6**, and **11** was performed in a similar manner using 2,4,6-triisopropylbenzenesulfonyl

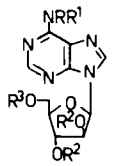
Scheme 1



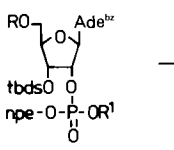
	R	R^1	R^2
1	CH=CHBr	H	H
2	CH=CHBr	MeOTr	H
3	CH=CHBr	H	tbds
4	F	H	H
5	F	MeOTr	H
6	F	H	tbds



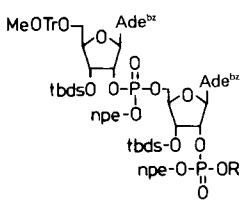
	R	R^1	R^2
7	H	H	H
8	H	H	ac
9	npe	H	ac
10	npe	npeoc	ac
11	npe	npeoc	H



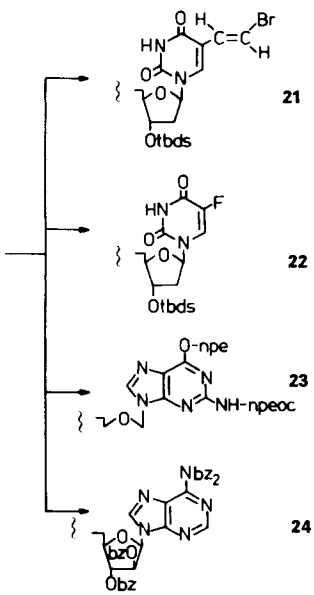
	R	R^1	R^2	R^3
12	H	H	H	H
13	bz	H	H	H
14	bz	bz	bz	MeOTr
15	bz	bz	bz	H



	R	R^1
16	MeOTr	2,5-Cl_2-C_6H_3
17	H	2,5-Cl_2-C_6H_3
18	MeOTr	Et_3NH



	R
19	2,5-Cl_2-C_6H_3
20	Et_3NH



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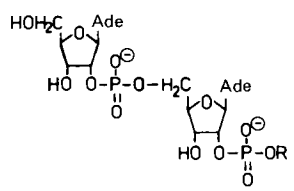
23

24



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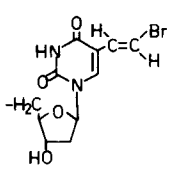

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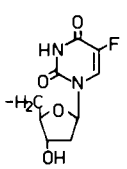
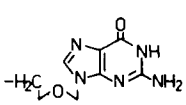
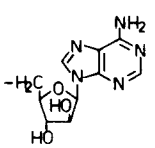


26



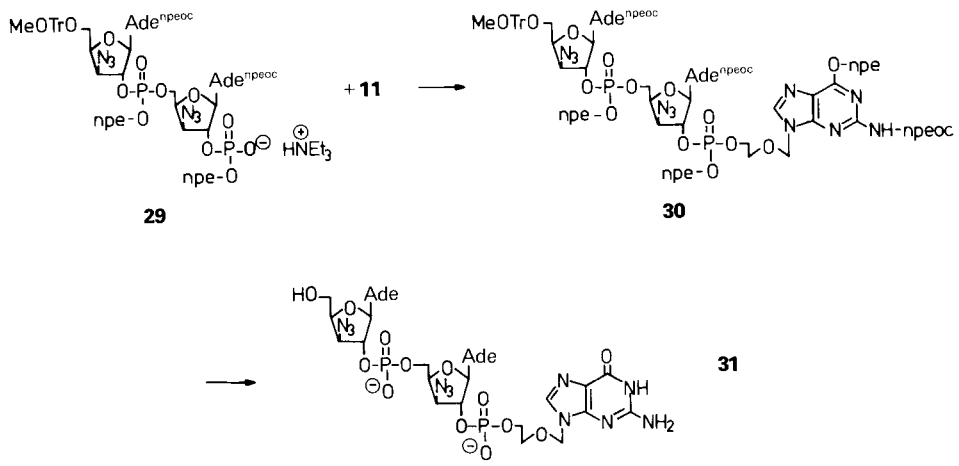
27



28

ac = acetyl; bz = benzoyl; MeOTr = monomethoxytrityl; tbds = (*tert*-butyl)dimethylsilyl; npe = 2-(4-nitrophenyl)ethyl; npeoc = 2-(4-nitrophenyl)ethoxycarbonyl

Scheme 2



chloride and *N*-methylimidazole in pyridine as condensing agents, but proceeded to **21**–**23**, respectively, with only moderate yields due to difficulties in chromatographical workup (very similar mobilities of the starting 5′-OH component and the fully protected trimer). Better results were obtained with tetrabenzoyl derivative **15** and **20** in the presence of quinoline-8-sulfonyl chloride and 3-nitro-1,2,4-triazole, affording **24** in 74% yield. Deprotection of the four trimers **21**–**24** was achieved in each case by the same reaction sequence. DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) treatment cleaved the npe and npeoc groups by β-elimination, F^- ions in THF removed the (*tert*-butyl)dimethylsilyl group, conc. ammonia the benzoyl residues, and 80% AcOH achieved the final detritylation. The free oligonucleotides **25**–**28** were isolated and purified by *DEAE-Sephadex* and paper chromatography.

Finally the combination of acyclovir (**7**) with 9-(3′-azido-3′-deoxy-β-D-xylofuranosyl)adenine to a trimeric 2′–5′ oligonucleotide followed an analogous strategy. Coupling of the dimeric phosphodiester **29** with **11** gave **30** in 79% yield *(Scheme 2)*. Its deprotection to **31** was straightforward (TsOH followed by DBU treatment). The crude trimer **31** was purified by *DEAE-Sephadex* and paper chromatography yielding an amorphous powder on lyophilisation.

3. Biological Activity. – The antiviral activity of the oligonucleotides **25**–**27** and **31** is compared with that of the parent nucleosides bvU_d (**1**), flU_d (**4**), and acyclovir (**7**) [10] [11] *(Table)*. The results clearly demonstrate that the activity of the oligonucleotides is comparable to that of the parent nucleosides, except for the activity of **31** against *Vaccinia* virus replication. The lack of activity of the oligonucleotides **25**, **27**, and **31** against the TK^- strains of *Herpes simplex* virus type 1 indicates that the oligonucleotides are hydrolyzed to yield the nucleosides (bvU_d, acyclovir) before the former can exert their antiviral activity. The anti *Vaccinia* virus activity of **31** can be attributed to the formation of 9-(3′-azido-3′-deoxy-β-D-xylofuranosyl)adenine upon enzymatic hydrolysis, since the 5′-*O*-monophosphate of the latter compound when tested as such proved active against *Vaccinia* virus at a minimum inhibitory concentration of 2 μg/ml (data not shown). Also,

Table. *Cytotoxicity and Antiviral Activity in Primary Rabbit Kidney Cell Cultures*

	Minimum cytotoxic concentration[a]) [μg/ml]	Minimum inhibitory concentration[b]) [μg/ml]									
		Herpes simplex virus 1 (KOS)	*Herpes simplex* virus 1 (F)	*Herpes simplex* virus 1 (McIntyre)	*Herpes simplex* virus 2 (G)	*Herpes simplex* virus 2 (196)	*Herpes simplex* virus 2 (Lyons)	*Vaccinia* virus	Vesicular stomatitis virus	*Herpes simplex* virus 1 TK^- B2006	*Herpes simplex* virus 1 TK^- VMW1837
25	> 100	0.07	0.07	0.07	7	> 100	70	7	> 100	> 100	> 100
26	> 100	70	100	20	70	> 100	> 100	0.2	> 100	1	2
27	> 100	0.7	0.7	1	0.2	0.7	0.7	> 100	> 100	> 100	> 100
31	> 400	0.7	2	2	0.7	1	2	7	> 400	300	70
1 (bvU_d)	> 400	0.02	0.02	0.02	7	150	20	7	> 400	150	150
4 (flU_d)	> 40	20	10	10	7	> 40	> 100	0.07	> 40	0.2	0.2
7 (Acyclovir)	> 400	0.2	0.2	0.2	0.1	0.2	0.2	150	> 400	10	20

[a]) Required to cause a microscopically detectable alteration of normal cell morphology.
[b]) Required to reduce virus-induced cytopathogenicity by 50%.

the marked activity of oligonucleotide **26** against the TK^- virus strains can readily be explained by the release of free nucleoside (flU_d), which is known to be more active against TK^- than TK^+ strains of *Herpes simplex* virus [12].

Experimental Part

General. TLC: precoated silica-gel thin-layer sheets *F 1500 LS 254* and cellulose thin-layer sheets *F 1440* from *Schleicher & Schüll.* Prep. TLC: silica gel *60 PF_{254} (Merck).* Prep. column chromatography: silica gel (*Merck 60,* 0.063–0.2 mesh). Paper chromatography (PC sheets, 58 × 60 cm) from *Schleicher & Schüll.* Ion-exchange chromatography: *DEAE Sephadex A-25 (Pharmacia):* M.p.: *Büchi* apparatus, model Dr. *Tottoli;* no corrections. HPLC: *Merck-Hitachi D 2000,* column *RP 18,* 125 × 4 mm, 5 µm, *Merck,* flow rate 1 ml/min, mobile phase 0.1M $AcONH_4/CH_3CN$ 9:1. UV/VIS: *Uvikon 820, Kontron,* and *Perkin Elmer, Lambda 5;* λ_{max} in nm (lg ε). ^{1}H-NMR: *Bruker WM-250;* in δ (ppm) relative to TMS.

1. *5-[(E)-2-Bromovinyl]-2′-deoxy-5′-O-(monomethoxytrityl)uridine* (**2**). A mixture of 333 mg (1 mmol) of 5-((*E*)-2-bromovinyl)-2′-deoxyuridine (**1**) and 386 mg (1.25 mmol) of MeOTrCl in anh. pyridine (5 ml) was kept for 24 h at r.t. MeOH (1 ml) was added, the mixture evaporated, and the residue chromatographed on silica gel (20 g) with $CHCl_3$ and $CHCl_3$/MeOH 98:2. The product was precipitated from Et_2O/hexane: 533 mg (88%) of colourless powder. UV (MeOH): 231 (4.43), 290 (4.08). ^{1}H-NMR ($CDCl_3$): 9.16 (br. *s*, NH); 7.66 (*s*, H–C(6)); 7.23–7.41 (*m*, MeO*Tr*, vinyl H); 6.86 (2 H *o* to MeO); 6.38 (*dd*, *J* = 6.0, 7.3, H–C(1′)); 5.90 (*d*, *J* = 13.7, vinyl H); 4.59 (*m*, H–C(3′)); 4.12 (*m*, H–C(4′)); 3.80 (*s*, MeO); 3.43 (*m*, CH_2(5′)); 2.27–2.54 (*m*, CH_2(2′), OH). Anal. calc. for $C_{31}H_{29}BrN_2O_6$ (605.5): C 61.49, H 4.83, N 4.63; found: C 61.77, H 4.73, N 4.46.

2. *5-[(E)-2-Bromovinyl]-3′-O-[(tert-butyl)dimethylsilyl]-2′-deoxyuridine* (**3**). A mixture of 605 mg (1 mmol) of **2**, 225 mg (1.5 mmol) of (*t*-Bu)Me_2SiCl and 204 mg (3 mmol) of imidazole in anh. pyridine (10 ml) was stirred at r.t. for 48 h. Then, the mixture was evaporated, coevaporated with toluene, and partitioned between $CHCl_3$ (10 ml) and phosphate buffer (0.1M; pH 6; 10 ml). The org. layer was washed with phosphate buffer (0.1M; pH 6; 10 ml), dried, and evaporated leaving an oil which was treated with 2% of TsOH in CH_2Cl_2/MeOH 4:1 (10 ml) for 30 min. The mixture was poured into phosphate buffer (0.1M; pH 7; 20 ml) and extracted with $CHCl_3$ (20 ml), and the org. layer was washed twice with the same buffer (2 × 20 ml), dried (Na_2SO_4), and evaporated. The residual oil was purified by column chromatography on silica gel with $CHCl_3$/MeOH 98:2 and the product crystallized from Et_2O/hexane: 313 mg (70%) of colourless crystals. M.p. 168°. UV (MeOH): 248 (4.17), 291 (4.09). ^{1}H-NMR ($CDCl_3$): 8.28 (br. *s*, NH); 7.78 (*s*, H–C(6)); 7.35 (*d*, *J* = 13.7, vinyl H); 6.17 (*t*, *J* = 6.4, H–C(1′)); 4.47 (*m*, H–C(4′)); 3.96 (*m*, 1 H–C(5′)); 3.94 (*m*, H–C(3′)); 3.79 (*m*, 1 H–C(5′)); 2.27 (*m*, CH_2(2′)); 2.06 (*t*, OH–C(5′)); 0.87 (*s*, *t*-Bu); 0.07 (*s*, Me_2Si). Anal. calc. for $C_{17}H_{27}BrN_2O_5Si$ (447.4): C 45.64, H 6.08, N 6.26; found: C 45.70, H 6.45, N 6.20.

3. *2′-Deoxy-5-fluoro-5′-O-(monomethoxytrityl)uridine* (**5**). A soln. of 246 mg (1 mmol) of 2′-deoxy-5-fluorouridine (**4**) and 386 mg (1.25 mmol) of MeOTrCl in anh. pyridine (5 ml) was kept for 24 h at r.t. MeOH (1 ml) was added, the mixture evaporated, and the residue chromatographed on silica gel (20 g) with $CHCl_3$ and $CHCl_3$/MeOH 98:2. The product was precipitated from Et_2O/hexane: 446 mg (86%) of colourless powder. UV (MeOH): 267 (3.98), 230 (4.23). ^{1}H-NMR ($CDCl_3$): 9.33 (br. *s*, NH); 7.83 (*d*, *J* = 5.6, H–C(6)); 7.20–7.45 (*m*, MeO*Tr*); 6.85 (*m*, 2 H *o* to MeO); 6.30 (*t*, *J* = 6.0, H–C(1′)); 4.57 (*m*, H–C(3′)); 4.08 (*m*, H–C(4′)); 3.79 (*s*, MeO); 3.44 (*m*, CH_2(5′)); 2.22–2.58 (*m*, CH_2(2′), OH). Anal. calc. for $C_{29}H_{27}FN_2O_6$ (518.5): C 67.17, H 5.25, N 5.40; found: C 67.21, H 5.14, N 5.30.

4. *3′-O-[(tert-Butyl)dimethylsilyl]-2′-deoxy-5-fluorouridine* (**6**). A mixture of 518 mg (1 mmol) of **5**, 225 mg (1.5 mmol) of (*t*-Bu)Me_2SiCl, and 204 mg (3 mmol) of imidazole in anh. pyridine (10 mol) was stirred at r.t. for 48 h. The mixture was evaporated, coevaporated with toluene, and partitioned between $CHCl_3$ (10 ml) and phosphate buffer (0.1M; pH 6; 10 ml). The org. layer was washed with phosphate buffer (1M; pH 6; 10 ml), dried, and evaporated leaving an oil which was treated with 2% of TsOH in CH_2Cl_2/MeOH 4:1 (10 ml) for 30 min. The mixture was poured in phosphate buffer (0.1M; pH 7; 20 ml) and extracted with $CHCl_3$ (20 ml) and the org. layer washed twice with the same buffer (2 × 20 ml), dried (Na_2SO_4), and evaporated. The residual oil was purified by column chromatography on silica gel ($CHCl_3$/MeOH 97:3) and the product crystallized from Et_2O: 253 mg (70%) of colourless crystals. M.p. 170°. UV (MeOH): 267 (4.00). ^{1}H-NMR ($CDCl_3$): 8.35 (br. *s*, NH); 7.94 (*d*, *J* = 6.4, H–C(6)); 6.20 (*dt*, *J*(1′,F) = 1.5, *J*(1′,2′) = 6.4, H–C(1′)); 4.46 (*m*, H–C(4′)); 3.95 (*m*, 1 H–C(5′)); 3.94 (*m*, H–C(3′)); 3.57 (*m*, 1 H–C(5′)); 2.26 (*m*, CH_2(2′)); 1.93 (br. *t*, OH–C(5′)); 0.87 (*s*, *t*-Bu); 0.07 (*s*, Me_2Si). Anal. calc. for $C_{15}H_{25}FN_2O_5Si$ (360.5): C 49.98, H 6.99, N 7.77; found: C 49.81, H 7.02, N 7.62.

5. *9-[(2-Acetoxyethoxy)methyl]guanine* (**8**). A suspension of 815 mg (3.3 mmol) of the sodium salt of acyclovir (**7**) in pyridine (15 ml) containing Ac_2O (2 ml) was stirred over night at r.t. The mixture was evaporated

and coevaporated 3 times with EtOH. The residue was crystallized from H_2O. The crystals were washed twice with H_2O and once with MeOH and dried *in vacuo:* 830 mg (94%) of colourless crystals. M.p. 240–242°. UV (MeOH): 253 (4.20). ^{1}H-NMR ((D_6)DMSO): 10.50 (br. *s*, NH); 7.81 (*s*, H–C(8)); 6.51 (br. *s*, NH_2); 5.34 (*s*, CH_2N); 4.06 (*m*, $AcOCH_2CH_2$); 3.64 (*m*, $AcOCH_2CH_2$); 1.95 (*s*, Ac). Anal. calc. for $C_{10}H_{13}N_5O_4$ (267.2): C 44.94, H 4.90, N 26.23; found: C 44.52, H 5.05, N 26.60.

6. *9-[(2-Acetoxyethoxy)methyl]-*O^6*-[2-(4-nitrophenyl)ethyl]guanine* (**9**). To a mixture of 535 mg (2 mmol) of **8** were added 558 mg (3.2 mmol) of diethyl azodicarboxylate, 840 mg (3.2 mmol) of triphenylphosphine, and 501 mg (3 mmol) of 2-(4-nitrophenyl)ethanol in 40 ml of abs. dioxane. The mixture was stirred at r.t. for 10 h, evaporated, and purified by column chromatography ($CHCl_3$, then $CHCl_3$/MeOH 99.5:0.5). The product was crystallized from MeOH to give 600 mg (72%) of colourless crystals. M.p. 148–149°. UV (MeOH): 278 (4.25), 250 (4.19). IR (KBr): 1735, 1510, 1335. ^{1}H-NMR ((D_6)DMSO): 8.18 (*d*, 2 H *o* to NO_2); 7.98 (*s*, H–C(8)); 7.63 (*d*, 2 H *m* to NO_2); 6.52 (br. *s*, NH_2); 5.41 (*s*, CH_2N); 4.66 (*t*, $ArCH_2CH_2$); 4.05 (*m*, $AcOCH_2CH_2$); 3.64 (*m*, $AcOCH_2CH_2$); 3.24 (*t*, $ArCH_2CH_2$); 1.92 (*s*, Ac). Anal. calc. for $C_{18}H_{20}N_6O_6$ (416.4): C 51.92, H 4.84, N 20.18; found: C 51.42, H 4.97, N 19.81.

7. *9-[(2-Acetoxyethoxy)methyl]-*N^2*-[2-(4-nitrophenyl)ethoxycarbonyl]-*O^6*-[2-(4-nitrophenyl)ethyl]guanine* (**10**). To a soln. of 416 mg (1 mmol) of **9** in 5 ml of abs. pyridine was added dropwise a soln. of 690 mg (3 mmol) of 2-(4-nitrophenyl)ethyl chloroformate in 5 ml of abs. CH_2Cl_2 at 0°. The mixture was stirred over night at r.t., diluted with H_2O (50 ml), and extracted 3 times with $CHCl_3$ (25 ml). The org. layers were washed with $NaHCO_3$ soln. (100 ml), dried, evaporated, and coevaporated with toluene. The product was obtained as a colourless foam after chromatography on silica gel ($CHCl_3$, then $CHCl_3$/MeOH 98:2): 591 mg (97%). UV (MeOH): 268 (4.51), 214 (4.63). IR (KBr): 1750 (sh), 1730, 1515, 1340. ^{1}H-NMR ($CDCl_3$): 8.15, 8.13, 8.12, 8.09 (4 H *o* to NO_2); 7.91 (*s*, H–C(8)); 7.49, 7.46, 7.41, 7.38 (4 H *m* to NO_2); 7.44 (br. *s*, NH); 5.55 (*s*, CH_2N); 4.77 (*t*), 4.44 (*t*, 2 $ArCH_2CH_2$); 4.15 (*m*, $AcOCH_2CH_2$); 3.73 (*m*, $AcOCH_2CH_2$); 3.27, 3.10 (2*t*, 2 $ArCH_2CH_2$); 1.98 (*s*, Ac). Anal. calc. for $C_{27}H_{27}N_7O_{10}$ (609.6): C 53.20, H 4.46, N 16.09; found: C 53.57, H 4.82, N 15.50.

8. *9-[(2-Hydroxyethoxy)methyl]-*N^2*-[2-(4-nitrophenyl)ethoxycarbonyl]-*O^6*-[2-(4-nitrophenyl)ethyl]guanine* (**11**). A soln. of 548 mg (0.9 mmol) of **10** in dioxane/MeOH/25% NH_3 soln. 1:1:1 (45 ml) was kept over night at 4°. The mixture was evaporated and purified by column chromatography in $CHCl_3$/MeOH 95:5. The product was crystallized from MeOH: 440 mg (86%) of colourless crystals. M.p. 111–113°. UV (MeOH): 268 (4.56), 214 (4.67). IR (KBr): 1760, 1510, 1340. ^{1}H-NMR ($CDCl_3$): 8.19, 8.17, 8.16, 8.13 (4 H_o to NO_2); 7.90 (*s*, H–C(8)); 7.50, 7.47. 7.42, 7.39 (4 H *m* to NO_2); 7.32 (br. *s*, NH); 5.71 (*s*, CH_2N); 4.78, 4.67 (2*t*, 2 $ArCH_2CH_2$); 3.79 (*m*, $HOCH_2CH_2$); 3.67 (*m*, $HOCH_2CH_2$); 3.29, 3.11 (2*t*, 2 $ArCH_2CH_2$); 2.84 (OH–C(5′)). Anal. calc. for $C_{25}H_{25}N_7O_9$ (567.5): C 52.91, H 4.44, N 17.28; found: C 52.79, H 4.47, N 17.08.

9. *9-(β-*D*-Arabinofuranosyl)-*N^6*-benzoyladenine* (**13**). In abs. pyridine (10 ml) were evaporated (3×) 0.608 g (2.28 mmol) of 9-(β-D-arabinofuranosyl)adenine (**12**). Then, the residue was suspended in abs. pyridine (12 ml) and Me_3SiCl (1.81 ml, 14 mmol) added. The mixture was stirred for 15 min, then 1.6 ml (14 mmol) of benzoyl chloride were added. After 2 h at r.t., the mixture was cooled in an ice bath, and 3 ml of H_2O were added and, after 5 min, 5.7 ml of conc. NH_3 soln. The mixture was stirred at r.t. for 0.5 h and then evaporated and the residue dissolved in 35 ml of H_2O. The soln. was shaken with AcOEt (12 ml), and shortly thereafter, the product crystallized out of the aq. layer. On cooling, more colorless crystals were obtained which were dried under high vacuum at 50°: 0.65 g (77%). UV (MeOH): 227 (4.27), 279 (4.35). ^{1}H-NMR ((D_6)DMSO): 11.20 (*s*, NH); 8.72 (*s*, H–C(8)); 8.52 (*s*, H–C(2)); 7.51–8.05 (*m*, C_6H_5CO); 6.49 (*d*, 1 H, H–C(1′)); 5.91, 5.78, 5.17 (3*m*, 3 OH); 4.17–4.25 (2*m*, H–C(2′), H–C(3′)); 3.8 (*m*, H–C(4′)); 3.45 (*m*, CH_2(5′)). Anal. calc. for $C_{17}H_{17}N_5O_5 \cdot 1\ H_2O$ (390.0): C 52.36, H 4.91, N 17.95; found: C 52.36, H 4.95, N 18.14.

10. **N^6*-Benzoyl-9-[5′-*O*-(monomethoxytrityl)-β-*D*-arabinofuranosyl]adenine and *N^6*,*N^6*-Dibenzoyl-9-[2′,3′-di-*O*-benzoyl-5′-*O*-(monomethoxytrityl)-β-*D*-arabinofuranosyl]adenine* (**14**). In abs. pyridine (10 ml), 0.57 g (1.5 mmol) of **13** were dried by 2 coevaporations. The residue was dissolved in abs. pyridine (20 ml), 0.554 g (1.8 mmol) of MeOTrCl were added, and after stirring at r.t. for 20 h, the reaction was quenched by MeOH (5 ml). After stirring for 5 min, the mixture was evaporated to a smaller volume and diluted with $CHCl_3$ (400 ml). The org. phase was washed with H_2O (2 × 100 ml), dried, and evaporated. Final coevaporation with toluene (3 × 15 ml) gave a crude product which was purified by silica-gel column chromatography (20 × 2.5 cm) using $CHCl_3$/MeOH 50:1. The product was dried under high vacuum at 40°: 0.88 g (91%) of a solid foam of *N^6*-benzoyl-9-[5′-*O*-(monomethoxytrityl)-β-D-arabinofuranosyl]adenine. UV (MeOH): 230 (4.45), 279 (4.33). ^{1}H-NMR ($CDCl_3$): 9.10 (br. *s*, NH); 8.60 (*s*, H–C(8)); 8.35 (*s*, H–C(2)); 7.16–7.98 (*m*, 17 arom. H): 6.80 (*d*, 2 H *o* to MeO); 6.38 (*d*, H–C(1′)); 4.9 (*m*, OH); 4.36–4.39 (*m*, H–C(2′), H–C(3′)); 4.07 (*m*, H–C(4′)); 3.74 (*s*, MeO); 3.37–3.59 (*m*, CH_2(5′)); 3.30 (*m*, OH). Anal. calc. for $C_{37}H_{35}N_5O_6 \cdot H_2O$ (661.7): C 67.15, H 5.63, N 10.58; found: C 67.56, H 5.71, N 9.97.

In abs. pyridine (10 ml) were coevaporated (3×) 0.68 g (1.06 mmol) of the preceding product to remove its crystal water. The residue was then dissolved in pyridine (20 ml), 0.74 ml (6.4 mmol) of benzoyl chloride were added and stirred at r.t. for 3 h. The mixture was poured onto ice and extracted with $CHCl_3$ (250 ml). The org. phase was washed with H_2O (2 × 100 ml), dried, evaporated, and coevaporated with toluene (3 × 10 ml). Column chromatography on silica gel (30 × 2 cm) with $CHCl_3$ gave 0.78 g (77%) of **14**. UV (MeOH): 232 (4.72), 270 (4.31). ^{1}H-NMR ($CDCl_3$): 8.59 (*s*, H–C(8)); 8.29 (*s*, H–C(2)); 7.16–8.09 (*m*, 27 arom. H); 6.81 (*d*, H–C(1′)); 6.70 (*d*, 2 H *o* to MeO); 5.84–5.92 (2*m*, H–C(2′), H–C(3′)); 4.46–4.49 (*m*, H–C(4′)); 3.70 (*s*, MeO); 3.62 (*m*, CH_2(5′)). Anal. calc. for $C_{58}H_{45}N_5O_9$ (956.0): C 72.86, H 4.74, N 7.32; found: C 72.37, H 4.82, N 7.19.

11. N^6,N^6-*Dibenzoyl-9-(2′,3′-di-*O*-benzoyl-β-*D*-arabinofuranosyl)adenine* (**15**). A soln. of 0.293 g (0.3 mmol) of **14** in 6 ml of 1% TsOH in CH_2Cl_2/MeOH 4:1 was stirred at r.t. for 20 min and then diluted with $CHCl_3$ (100 ml). The $CHCl_3$ phase was washed with H_2O (2 × 50 ml), dried, and evaporated. The residue was purified by silica gel column chromatography (12 × 2.5 cm) with $CHCl_3$/MeOH 99:1. The main fraction was evaporated and dried under high vacuum at 40°: 0.164 g (80%) of a solid foam. UV (MeOH): 232 (4.65), 271 (4.27). ^{1}H-NMR ($CDCl_3$): 8.58 (*s*, H–C(8)); 8.45 (*s*, H–C(2)); 7.23–8.09 (*m*, 20 arom. H); 6.77 (*d*, H–C(1′)); 6.08 (*m*, H–C(2′), H–C(3′)); 4.4 (*m*, OH); 4.15 (*dd*, CH_2(5′)). Anal. calc. for $C_{38}H_{29}N_5O_8$ (683.7): C 66.75, H 4.27, N 10.24; found: C 66.48, H 4.42, N 9.82.

12. N^6-*Benzoyl-3′-*O*-[(*tert*-butyl)dimethylsilyl]adenosine 2′-[2,5-Dichlorophenyl 2-(4-Nitrophenyl)ethyl Phosphate]* (**17**). A soln. (50 ml) of 2% TsOH in CH_2Cl_2/MeOH 4:1 was stirred together with 2.85 g (2.5 mmol) of phosphotriester **16** [9]. After 30 min, the mixture was diluted with $CHCl_3$ (500 ml) and washed with phosphate buffer (pH 7; 2 × 200 ml). The $CHCl_3$ phase was dried and evaporated and the product purified by silica-gel column chromatography (20 × 2.5 cm) with $CHCl_3$/MeOH 99:1. After evaporation, the product was purified by precipitation from $CHCl_3$ with hexane. Filtration and drying under high vacuum gave 1.96 g (92%) of amorphous powder. UV (MeOH): 277 (4.47). ^{1}H-NMR ($CDCl_3$): 8.74 (*s*, H–C(8)); 8.11 (*s*, H–C(2)); 6.91–8.09 (3*m*, arom. H); 6.13 (*d*, H–C(1′)); 5.74 (*q*, H–C(2′)); 4.66, 4.56 (2*d*, H–C(3′), diastereoisomers); 4.24 (*m*, H–C(4′), CH_2); 3.71–4.19 (*q*, CH_2(5′)); 2.78–3.00 (2*m*, 2 H + CH_2); 0.91 (*s*, *t*-Bu); 0.07, 0.10 (2*s*, Me_2Si). Anal. calc. for $C_{37}H_{41}Cl_2N_6O_{11}PSi$ (859.7): C 51.69, H 4.89, N 9.77; found: C 51.69, H 4.83, N 9.61.

13. {N^6-*Benzoyl-3′-*O*-[(*tert*-butyl)dimethylsilyl]-5′-*O*-(monomethoxytrityl)adenosin}-2′-yl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]} → 5}-N^6-*benzoyl-3′-*O*-[(*tert*-butyl)dimethylsilyl]adenosine 2′-[2,5-Dichlorophenyl 2-(4-Nitrophenyl)ethyl Phosphate]* (**19**). A mixture of 2.176 g (2 mmol) of **18** [9] and 1.288 g (1.499 mmol) of **17** was coevaporated with anh. pyridine (3 × 10 ml) and dissolved in abs. pyridine (15 ml). Then 3-nitro-1,2,4-triazole (1.368 g, 12 mmol) and quinoline-8-sulfonyl chloride (0.912 g, 4 mmol) were added and stirred at r.t. for 16 h. The mixture was diluted with $CHCl_3$ (800 ml) and washed with H_2O (2 × 300 ml), dried, and evaporated. Final coevaporation was done with toluene (2 × 50 ml). The crude dimer was chromatographed on silica gel (30 × 2.5-cm column) with $CHCl_3$ and $CHCl_3$/MeOH 99:1. The product fractions were evaporated and precipitated from $CHCl_3$ with hexane: 2.36 g (87%) of an amorphous powder. UV (MeOH): 277 (4.73), 228 (sh, 4.70). Anal. calc. for $C_{88}H_{94}Cl_2N_{12}O_{20}P_2Si_2$ (1828.8): C 57.80, H 5.18, N 9.19; found: C 57.49, H 5.14, N 9.16.

14. N^6-*Benzoyl-3′-*O*-[(*tert*-butyl)dimethylsilyl]-5′-*O*-(monomethoxytrityl)adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]} → 5′}-N^6-*benzoyl-3′-*O*-[(*tert*-butyl)dimethylsilyl]adenosine 2′-[2-(4-Nitrophenyl)ethyl Triethylammonium Phosphate]* (**20**). A soln. of 1.18 g (7.11 mmol) of 4-nitrobenzaldehyde oxime in 14 ml of H_2O/dioxane/Et_3N 1:1:1 was stirred for 30 min at r.t. Then, 1.3 g (0.711 mmol) of **19** were added and stirred for 1 h at r.t. The mixture was evaporated, then twice coevaporated with pyridine (50 ml) and twice with toluene (50 ml). The residue was dissolved in $CHCl_3$ and chromatographed on a silica-gel column (12 × 2.5 cm) with $CHCl_3$/MeOH/Et_3N 7:1:1. After evaporation and coevaporation with toluene (3 × 50 ml), the product was precipitated from $CHCl_3$ with hexane to give 1.142 g (90%) of an amorphous powder. UV (MeOH): 277 (4.78). ^{1}H-NMR ($CDCl_3$): 12.15 (*s*, NH); 8.99 (*s*, NH); 8.74, 8.62 (2*s*, 2 H, H–C(8)); 8.28, 8.18 (2*s*, 2 H, H–C(2)); 7.06–8.17 (2*m*, 24 arom. H); 6.77 (*d*, 2 H *o* to MeO); 6.06 (*m*, 2 H, H–C(1′)); 3.72 (*s*, 3 H, MeO); 0.78, 0.67 (2*s*, 18 H, *t*-Bu); 0.072, 0.023–0.13, 0.31 (5*s*, 12 H, Me_2Si). Anal. calc. for $C_{88}H_{107}N_{13}O_{20}P_2Si_2$ (1785.1): C 59.21, H 6.04, N 10.20; found: C 59.81, H 6.03, N 10.01.

15. N^6-*Benzoyl-3′-*O*-[(*tert*-butyl)dimethylsilyl]-5′-*O*-(monomethoxytrityl)adenylyl-{2′-[O^P-(2-(4-nitrophenyl)ethyl)] → 5′}-N^6-*benzoyl-3′-*O*-[(*tert*-butyl)dimethylsilyl]adenylyl-{2′-[O^P-(2-(4-nitrophenyl)ethyl)] → 5′}-5-((*E*)-2-bromovinyl)-3′-*O*-[(*tert*-butyl)dimethylsilyl]-2′-deoxyuridine* (**21**). A mixture of 330 mg (0.185 mmol) of **20** and 67 mg (0.15 mmol) of **3** was coevaporated twice with anh. pyridine and dissolved in abs. pyridine (3 ml). *N*-Methylimidazole (0.88 ml, 90 mg, 1.10 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (112 mg, 0.37 mmol) were added, and the mixture was stirred over night at r.t. The mixture was diluted with $CHCl_3$ (20 ml), washed twice with H_2O (2 × 20 ml), dried, evaporated, and coevaporated twice with toluene. The trimer was purified by prep. TLC on silica-gel plates (20 × 40 × 0.2 cm) with $CHCl_3$/MeOH 96:4 and rechromatographed

with AcOEt: 75 mg (24%) of colourless foam. UV (MeOH): 277 (4.81), 261 (sh, 4.72); 234 (4.73). ^{1}H-NMR ($CDCl_3$): 8.63 (*m*, 2 H, H–C(8)); 7.86–8.27 (*m*, 11 H); 7.01–7.56 (*m*, 23 H); 6.76 (*d*, 2 H *o* to MeO); 6.47, 6.39 (2*d*, *J* = 13.7, 1 H, vinyl H); 6.05–6.23 (*m*, 3 H, H–C(1′)); 0.83 (*m*, 27 H, *t*-Bu); 0.01 (*m*, 18 H, Me_2Si). Anal. calc. for $C_{99}H_{117}BrN_{14}O_{24}P_2Si_3$ (2113.2): C 56.27, H 5.58, N 9.28; found: C 55.79, H 5.69, N 9.14.

16. *N^6-Benzoyl-3′-O-[(*tert*-butyl)dimethylsilyl]-5′-O-(monomethoxytrityl)adenylyl-{2′-[O^P-(2-(4-nitrophenyl)ethyl)] → 5′}-N^6-benzoyl-3′-O-[(*tert*-butyl)dimethylsilyl]adenylyl-{2′-[O^P-(2-(4-nitrophenyl)ethyl)] → 5′}-3′-O-[(*tert*-butyl)dimethylsilyl]-2′-deoxy-5-fluorouridine* (**22**). A mixture of 330 mg (0.185 mmol) of **20** and 54 mg (0.15 mmol) of **6** was coevaporated twice with abs. pyridine and then dissolved in abs. pyridine (3 ml). *N*-Methylimidazole (0.088 ml, 90 mg, 1.10 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride were added and stirred over night at r.t. The mixture was diluted with $CHCl_3$ (20 ml), washed twice with H_2O (2 × 20 ml), twice with $NaHCO_3$ soln. (20 ml), dried, evaporated, and coevaporated with toluene (2×). The residual oil was purified by column chromatography ($CHCl_3$, then $CHCl_3$/MeOH 97:3) followed by prep. TLC ($CHCl_3$/MeOH 95:5; 2 developments): 92 mg (30%) of colourless amorphous solid. UV (MeOH): 275 (4.79), 233 (4.59). ^{1}H-NMR ($CDCl_3$): 9.31–9.54 (*m*, 2 H, NH); 8.63 (*m*, 2 H, H–C(8)); 7.90–8.28 (*m*, 11 H); 7.06–7.56 (*m*, 22 H); 6.76 (*d*, 2 H *o* to MeO); 5.85–6.25 (*m*, 3 H, H–C(1′)); 0.80 (*m*, 27 H, *t*-Bu); 0.10 (*m*, 18 H, Me_2Si). Anal. calc. for $C_{97}H_{115}FN_{14}O_{24}P_2Si_3$ (2026.3): C 57.50, H 5.72, N 9.86; found: C 56.86, H 6.09, N 9.67.

17. *N^6-Benzoyl-3′-O-[(*tert*-butyl)dimethylsilyl]-5′-O-(monomethoxytrityl)adenylyl-{2′-[O^P-(2-(4-nitrophenyl)ethyl)] → 5′}-N^6-benzoyl-3′-O-[(*tert*-butyl)dimethylsilyl]adenosine 2′-{2-(4-Nitrophenyl)ethyl 2-{{N^2-[2-(4-Nitrophenyl)ethoxycarbonyl]-O^6-[(4-nitrophenyl)ethyl]guanin-9-yl}methoxy}ethyl Phosphate}* (**23**). A mixture of 330 mg (0.185 mmol) of **20** and 85 mg (0.15 mmol) of **11** was coevaporated twice with pyridine and dissolved in abs. pyridine (3 ml). *N*-Methylimidazole (0.088 ml, 90 mg, 1.10 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (112 mg, 0.37 mmol) were added and stirred over night at r.t. The mixture was diluted with $CHCl_3$ (20 ml), washed twice with H_2O (2 × 20 ml), dried, evaporated, and coevaporated with toluene (3×). The residual oil was purified by column chromatography ($CHCl_3$, then $CHCl_3$/MeOH 98:2). The product fractions were evaporated, dissolved in 20 ml of $CHCl_3$, washed with H_2O (2 × 20 ml), dried, and evaporated again. The residue was purified by prep. TLC ($CHCl_3$/MeOH 95:5; 2 developments): 176 mg (53%) of an amorphous solid. UV (MeOH): 273 (4.95), 236 (4.78). ^{1}H-NMR ($CDCl_3$): 8.65 (*m*, 2 H, H–C(8)); 7.84–8.40 (*m*, 15 H); 7.08–7.58 (*m*, 26 H); 6.76 (*d*, 2 H *o* to MeO); 6.16–6.26 (*m*, 2 H, H–C(1′)); 0.80 (*m*, 18 H, *t*-Bu); 0.05 (*m*, 12 H, Me_2Si). Anal. calc. for $C_{107}H_{115}N_{19}O_{28}P_2Si_2$ (2233.3): C 57.55, H 5.19, N 11.92; found: C 56.98, H 4.99, N 11.67.

18. *N^6-Benzoyl-3′-O-[(*tert*-butyl)dimethylsilyl]-5′-O-(monomethoxytrityl)adenylyl-{2′-[O^P-(2-(4-nitrophenyl)ethyl)] → 5′}-N^6-benzoyl-3′-O-[(*tert*-butyl)dimethylsilyl]adenylyl-{2′-[O^P-(2-(4-nitrophenyl)ethyl)] → 5′}-N^6,N^6-dibenzoyl-9-(2′,3′-di-O-benzoyl-β-D-arabinofuranosyl)adenine* (**24**). A mixture of 357 mg (0.2 mmol) of **20** and 98 mg (0.15 mmol) of **15** was coevaporated with anh. pyridine (3 × 10 ml) and dissolved in pyridine (1.5 ml). Then, 3-nitro-1,2,4-triazole (136 mg, 1.2 mmol) and quinoline-8-sulfonyl chloride (91 mg, 0.4 mmol) were added and stirred at r.t. The mixture was diluted with $CHCl_3$ (100 ml), washed with H_2O (2 × 50 ml), dried, evaporated, and finally coevaporated with toluene (2 × 20 ml). The crude trimer was purified by prep. TLC with $CHCl_3$/CH_3OH 96:4 and precipitated from $CHCl_3$ with hexane: 250 mg (74%) of colourless powder. UV (MeOH): 275 (4.85), 231 (4.90). Anal. calc. for $C_{113}H_{115}N_{17}O_{26}P_2Si_2$ (2245.4): C 60.44, H 5.16, N 10.60; found: C 59.64, H 4.89, N 10.14.

19. *Adenylyl-(2′–5′)-adenylyl-(2′–5′)-5-(2-bromovinyl)-2′-deoxyuridine* (**25**), *Adenylyl-(2′–5′)-adenylyl-(2′–5′)-2′-deoxy-5-fluorouridine* (**26**), *Adenylyl-(2′–5′)-adenosine 2′-{2-[(Guanin-9-yl)methoxy]ethyl Phosphate}* (**27**), *and Adenylyl-(2′–5′)-adenylyl-(2′–5′)-9-(β-D-arabinofuranosyl)adenine* (**28**). A soln. of 0.03 mmol of **21**, **22**, **23**, or **24** in 18 ml of 0.5M DBU in pyridine was stirred for 24 h at 25–27°, then neutralized by addition of 1M AcOH and evaporated. The residue was treated with 10 ml of 1M Bu_4NF in THF and the soln., after stirring at r.t. for 24 h, evaporated. The residue was dissolved in conc. NH_3 soln. (25 ml) and stirred for 48 h at r.t. After evaporation, the resulting residue was treated with 80% AcOH soln. for 15 h. Some H_2O was added and the mixture again evaporated. It was then dissolved in H_2O (30 ml), extracted 3× with $CHCl_3$, the aq. layer evaporated, and the residue, after several coevaporations with H_2O to remove AcOH, applied on a *DEAE-Sephadex* column (60 × 1 cm) and eluted with 0.001–0.3M TEAB (triethylammonium hydrocarbonate) buffer (pH 7.5). The fractions of the main peak were evaporated and coevaporated several times with H_2O. Further purification by paper chromatography (i-PrOH/conc. NH_3 soln./H_2O 6:1:3) gave, after lyophilisation, colourless powders in 75–85% yield.

25: HPLC: 3.49 min. ^{1}H-NMR (D_2O): 8.09, 8.03 (2*s*, 2 H, H–C(8)); 7.97, 7.74 (2*s*, 2 H, H–C(2)); 6.80, 6.33 (2*d*, *J* = 6, 2 H, vinyl H); 6.11 (*t*′, 1 H, H–C(1′) of bvU_d); 6.07, 5.91 (2*d*, 2 H, H–C(1′) of A).

26: HPLC: 2.11 min. ^{1}H-NMR (D_2O): 8.11, 8.10 (2*s*, 2 H, H–C(8)); 8.06, 7.73 (2*s*, 2 H, H–C(2)); 7.5 (*s*, 1 H, H–C(6) of flU_d); 6.08 (*d*, 1 H, H–C(1′) of A); 5.94 (*t*′, 1 H, H–C(1′) of flU_d); 5.92 (*d*, 1 H, H–C(1′) of A).

27: HPLC: 2.48 min. ^{1}H-NMR (D_2O): 8.14, 8.06, 7.99 (3*s*, 3 H, H–C(8)); 7.99, 7.75 (2*s*, 2 H, H–C(2)); 6.09 (*d*, 1 H, H–C(1′)); 5.94 (*d*, 1 H, H–C(1′)); 5.19 (*s*, 2 H, Gua–CH_2–O).

28: HPLC: 2.14 min. ^{1}H-NMR (D_2O): 8.14, 8.04, 7.95 (3*s*, 3 H, H–C(8)); 7.89, 7.81 (2*s*, 3 H, H–C(2)); 6.00–5.94 (*m*, 3 H, H–C(1′)).

20. *{9-[3′-Azido-3′-deoxy-5′-O-(monomethoxytrityl)-β-*D*-xylofuranosyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenin}-2′-yl-{2′-[*O^P*-(2-(4-nitrophenyl)ethyl)] → 5′}-9-(3′-azido-3′-deoxy-β-*D*-xylofuranosyl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenin 2′-{2-(4-Nitrophenyl)ethyl 2-{{*N^2*-[2-(4-Nitrophenyl)ethoxycarbonyl]-*O^6*-[(4-nitrophenyl)ethyl]guanin-9-yl}methoxy}ethyl Phosphate}* (**30**). To a soln. of 85 mg (0.15 mmol) of **11** and 288 mg (0.16 mmol) of **29** [1] in 2 ml of pyridine were added successively 0.08 ml (1 mmol) of *N*-methylimidazole and 97 mg (0.32 mmol) of 2,4,6-triisopropylbenzenesulfonyl chloride. The mixture was stirred over night, evaporated, diluted with $CHCl_3$ (30 ml), washed twice with H_2O (2 × 30 ml), dried, evaporated, and coevaporated with toluene (2×). The product was purified by column chromatography ($CHCl_3$, then $CHCl_3$/MeOH 95:5) followed by 2 prep. TLC separations ($CHCl_3$/MeOH 94:6): 266 mg (79%) of colourless foam. UV (MeOH): 266 (5.04). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 8.62–8.66 (*m*, 2 H, H–C(8) of Ade); 8.41–8.48 (*m*, 2 H, NH); 7.98–8.18 (*m*, 14 H); 7.87–7.90 (4*s*, 1 H, H–C(8) of Gua); 7.20–7.48 (*m*, 25 H); 6.80 (*d*, 2 H *o* to MeO); 6.23, 6.09, 6.03 (*m*, 2 H, H–C(1′)); 3.75 (*s*, 3 H, MeO). Anal. calc. for $C_{99}H_{91}N_{27}O_{32}P_2$ (2232.9): C 53.25, H 4.11, N 16.94; found: C 52.61, H 3.87, N 16.41.

21. *{9-[3′-Azido-3′-deoxy-β-*D*-xylofuranosyl]adenin}-2′-yl-(2′–5′)-9-[3′-azido-3′-deoxy-β-*D*-xylofuranosyl]-adenine 2′-{2-[(Guanin-9-yl)methoxy]ethyl Phosphate}* (**31**). A 2% TsOH soln. in CH_2Cl_2/MeOH 4:1 (2 ml) and 0.11 g (0.05 mmol) of **30** were treated for 30 min at r.t. The mixture was diluted with $CHCl_3$ (50 ml) and washed with H_2O (2 × 30 ml). The $CHCl_3$ phase was dried and evaporated and the crude product purified by silica-gel column chromatography (15 × 2.5 cm; $CHCl_3$, then $CHCl_3$/MeOH 50:1 and $CHCl_3$/MeOH 25:1). The product fractions were evaporated and dried under high vacuum: 96 mg (98%) of the corresponding 5′-OH compound. Part of this material (63 mg, 0.032 mmol) was stirred with 20 ml of 0.5M DBU in pyridine for 24 h at r.t. The mixture was neutralized with 1M AcOH in pyridine (10 ml) and evaporated. The residue was taken up in H_2O (1 ml) and chromatographed on a *DEAE-Sephadex A-25* column (60 × 1 cm) using a linear gradient of 0.001–0.3M TBK buffer (pH 7.5). The main fractions were evaporated and coevaporated several times with H_2O and further purified by paper chromatography using i-PrOH/conc. NH_3 soln./H_2O 6:1:3. The product band was eluted with H_2O and lyophilised: 77% of **31** as a colourless powder. HPLC: 3.42 min. ^{1}H-NMR (D_2O): 8.20, 8.16, 8.01 (3*s*, 3 H, H–C(8)); 7.90, 7.71 (2*s*, 2 H, H–C(2)); 6.07 (*m*, 1 H, H–C(1′)); 5.83 (*d*, 1 H, H–C(1′)); 5.12 (*s*, 2 H, Gua–CH_2–O).

Comparative paper chromatography in i-PrOH/conc. NH_3 soln./H_2O 6:1:3: R_f (**25**) 0.46, R_f (**26**) 0.32, R_f (**28**) 0.51, and R_f (**31**) 0.39.

REFERENCES

[1] P. Herdewijn, R. Charubala, W. Pfleiderer, *Helv. Chim. Acta* **1989,** *72,* 1729.
[2] E. De Clercq, *Biochem. Pharmacol.* **1984,** *33,* 2159.
[3] J. Balzarini, E. De Clercq, A. Verbruggen, D. Ayuwasa, K. Shimizu, T. Seno, *Mol. Pharmacol.* **1987,** *32,* 410.
[4] P. A. Furman, J. A. Fyfe, M. H. St. Clair, K. Weinhold, J. L. Rideout, G. A. Freeman, S. Nusinoff Lehrman, D. P. Bolognesi, S. Broder, H. Mitsuya, D. W. Barry, *Proc. Natl. Acad. Sci. U.S.A.* **1986,** *83,* 8333.
[5] R. N. Hunston, A. S. Jones, C. MgGuigan, R. T. Walker, J. Balzarini, E. De Clercq, *J. Med. Chem.* **1984,** *27,* 440.
[6] C. B. Reese, *Tetrahedron* **1978,** *34,* 3143.
[7] J. H. van Boom, *Heterocycles* **1977,** *7,* 1197.
[8] F. Himmelsbach, B. S. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, *Tetrahedron* **1984,** *40,* 59.
[9] R. Charubala, E. Uhlmann, W. Pfleiderer, *Liebigs Ann. Chem.* **1981,** 2392.
[10] E. De Clercq, J. Descamps, G. Verhelst, R. T. Walker, A. S. Jones, P. F. Torrence, D. Shugar, *J. Infect. Dis.* **1980,** *141,* 563.
[11] F. Vinckier, M. Boogaerts, D. De Clerck, E. De Clercq, *J. Oral Maxillofac. Surg.* **1987,** *45,* 723.
[12] E. De Clercq, J. Bères, W. G. Bentrude, *Mol. Pharmacol.* **1987,** *32,* 286.