190. Nucleotides

Part XXXI[1])

Modified Oligomeric 2′–5′A Analogues: Synthesis of 2′–5′ Oligonucleotides with 9-(3′-Azido-3′-deoxy-β-D-xylofuranosyl)adenine and 9-(3′-Amino-3′-deoxy-β-D-xylofuranosyl)adenine as Modified Nucleosides

by **Piet Herdewijn**[a]), **Ramamurthy Charubala**[b]), and **Wolfgang Pfleiderer**[b])*

[a]) Rega Institute for Medical Research, Katholieke Universiteit Leuven, 10 Minderbroedersstraat, B-3000 Leuven

[b]) Fakultät für Chemie der Universität Konstanz, Universitätsstrasse 10, D-7750 Konstanz

(3.X.89)

A series of new 2′–5′ oligonucleotides carrying the 9-(3′-azido-3′-deoxy-β-D-xylofuranosyl)adenine moiety as a building block has been synthesized *via* the phosphotriester method. The use of the 2-(4-nitrophenyl)ethyl (npe) and 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) blocking groups for phosphate, amino, and hydroxy protection guaranteed straightforward syntheses in high yields and easy deblocking to form the 2′–5′ trimers **21**, **22**, and **25** and the tetramer **23**. Catalytic reduction of the azido groups in [9-(3′-azido-3′-deoxy-β-D-xylofuranosyl)adenin]-2′-yl-[2′-(O^P-ammonio)→5′]-[9-(3′-azido-3′-deoxy-β-D-xylofuranosyl)adenin]-2′-yl-[2′-(O^P-ammonio)→5′]-9-(3′-azido-3′-deoxy-β-D-xylofuranosyl)adenine (**21**) led to the corresponding 9-(3′-amino-3′-deoxy-β-D-xylofuranosyl)-adenine 2′–5′ trimer **26** in which the two internucleotidic linkages are formally neutralized by intramolecular betaine formation.

1. Introduction. – Ever since the isolation and characterization of 5′-*O*-triphosphoryl-adenylyl(2′–5′)adenylyl(2′–5′)adenosine [2] as a very potent low-molecular-weight inhibitor of cell-free protein synthesis was achieved, oligomeric (2′–5′)adenylates and structural analogues [3] have attracted much attention. Since the natural oligomeric (2′–5′) adenylates loose rapidly their biological activity due to cleavage of the internucleotidic bond by a specific phosphodiesterase, much efforts have been directed towards the synthesis of base-, sugar-, and phosphate-modified analogues to improve enzymatic stability and to increase the poor uptake of the polar molecules into intact cells [3]. A supplementary problem to preserve the antiviral and antineoplastic activity is the design of such 2′–5′A analogues which not only will be potentially resistant to enzymic degradation but still have the ability to bind and activate the 2′–5′A dependent endonuclease [4–8]. We present here the synthesis of different oligonucleotides containing 9-(3′-azido-3′-deoxy-β-D-xylofuranosyl)adenine (**1**) as the modified adenosine analogue. Reduction of the azido functions will, furthermore, allow the development of a formally neutral molecule at physiological pH by inner-salt formation of the betaine type.

[1]) Part XXX: [1].

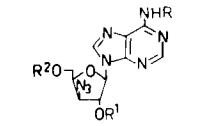
2. Syntheses. – The chemical syntheses of the new 2′–5′ oligonucleotides were achieved in a stepwise approach from the appropriately protected monomeric building blocks *via* the phosphotriester method [9–11]. Starting from 9-(3′-azido-3′-deoxy-β-D-xylofuranosyl)adenine (**1**) [12], transient trimethylsilyl protection at the sugar moiety [13] was first performed and then the NH_2 group blocked by the 2-(4-nitrophenyl)ethoxycarbonyl (npeoc) residue [14] to give **2** in almost quantitative yield. Monomethoxytritylation to **3** proceeded also very well, and subsequent acylation of the 2′-OH group using 1-methyl-3-[2-(4-nitrophenyl)ethoxycarbonyl]imidazolium chloride [14] gave **4** in 93% yield. Detritylation of **4** to N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-9-{2′-*O*-[2-(4-nitrophenyl)ethoxycarbonyl]-3′-azido-3′-deoxy-β-D-xylofuranosyl}adenine (**5**) worked best (90% yield) with *p*-toluenesulfonic acid in CH_2Cl_2/MeOH. In the next step, **3** was converted into the mixed phosphotriester **6** by phosphorylation using a mixture of 2,5-dichlorophenyl dichlorophosphate and 1,2,4-triazole and followed by 2-(4-nitrophenyl)ethanol treatment. Phosphotriester **6** was then transformed by detritylation into **7** in 96% yield and, by oximate treatment, into the corresponding phosphodiester **8** in 93% yield. The 5′-phosphotriester **9** resulted from **5** on treatment with bis[2-(4-nitrophenyl)ethyl] chlorophosphate [15], and the building block **10** was obtained from N^6-[2-(4-nitrophenyl)ethoxy-carbonyl]adenosine [14] by *Markiewicz*'s protecting procedure [16].

The dinucleoside phosphotriester **11** was formed in excellent yield from **5** and **8** using 2,4,6-triisopropylbenzenesulfonyl chloride and *N*-methylimidazole as condensing agents. Detritylation gave a 95% yield of **12**, the good yield being mainly due to the stable amino protection by the npeoc group. Another dimer synthesis was successfully achieved with the components **7** and **8** yielding 88% of **13** under the usual reaction conditions. Selective cleavage of the 2′-terminal 2,5-dichlorophenyl phosphate protecting group by the oximate method led to the anticipated dimeric phosphodiester **14**. The introduction of a mixed phosphotriester function into **12** giving **15** proceeded also very well according to standard procedures. The 5′-terminal dimeric phosphodiester **16** was obtained from **15** by oximate treatment. The purification of this triethylammonium salt **16** caused difficulties so that no accurate elemental analysis was obtained as it is often the case with this type of compounds.

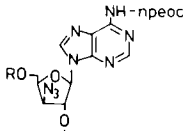
The buildup of the various trimers and the tetramer was achieved by the same phosphotriester methodology and led again to excellent yields in the condensation steps. The trinucleoside (2′–5′)-diphosphoditriester **17** was obtained from two different routes condensing either **8** and **12** or **5** and **14**. Detritylation of **17** afforded **18** in 93% yield, and the phosphorylation led to the trinucleoside triphosphotriester **19**. The tetramer **20** resulted from a block condensation using the 5′-OH free dimer **12** and the dimeric 2′-terminal phosphodiester **14**.

The deprotection of **18**–**20** to the free oligonucleotides **21**–**23**, respectively, turned out to be a straightforward process due to the uniform type of blocking groups. Thus, the npe and npeoc groups of **18** and **19** were cleaved in only one step by DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) with β-elimination, and in the case of **20**, only one additional acid treatment was necessary to free the tetramer. Isolation and purification were performed by *DEAE-Sephadex* and paper chromatography to give the ammonium salts **21**–**23** as lyophilized powders.

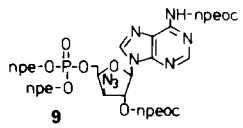
Furthermore, the phosphodiester **16** was condensed with N^6-[2-(4-nitrophenyl) ethoxycarbonyl]-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)adenosine (**10**) in the



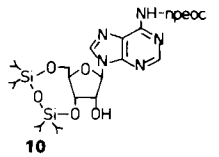
	R	R^1	R^2
1	H	H	H
2	npeoc	H	H
3	npeoc	H	MeOTr
4	npeoc	npeoc	MeOTr
5	npeoc	npeoc	H



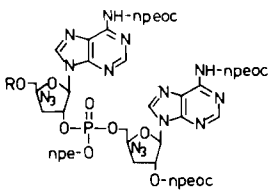
	R	R^1
6	MeOTr	2,5-Cl_2-C_6H_3
7	H	2,5-Cl_2-C_6H_3
8	MeOTr	$HNEt_3$



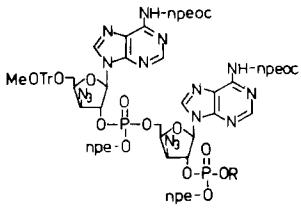
9



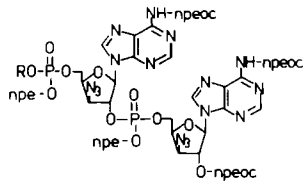
10



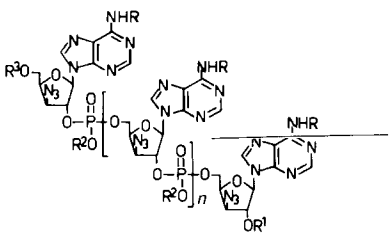
	R
11	MeOTr
12	H



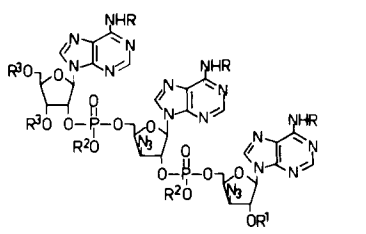
	R
13	2,5-Cl_2-C_6H_3
14	$HNEt_3$



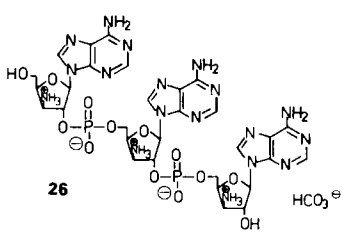
	R
15	2,5-Cl_2-C_6H_3
16	$HNEt_3$



	R	R^1	R^2	R^3	*n*
17	npeoc	npeoc	npe	MeOTr	1
18	npeoc	npeoc	npe	H	1
19	npeoc	npeoc	npe	$(npeO)_2PO$	1
20	npeoc	npeoc	npe	MeOTr	2
21	H	H	NH_4	H	1
22	H	H	NH_4	$PO_3H^{\ominus}\ ^{\oplus}NH_4$	1
23	H	H	NH_4	H	2



	R	R^1	R^2	R^3
24	npeoc	npeoc	npe	-Si(i-Pr)$_2$OSi(i-Pr)$_2$-
25	H	H	NH_4	H



26

MeOTr = monomethoxytrityl; npe = 2-(4-nitrophenyl)ethyl;
npeoc = [2-(4-nitrophenyl)ethoxy]carbonyl; $-Si(i\text{-}Pr)_2OSi(i\text{-}Pr)_2-$ = 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl

usual manner applying 2,4,6-triisopropylbenzenesulfonyl chloride and either *N*-methylimidazole or 4-(dimethylamino)pyridine *N*-oxide, but the 30% isolated yield of **24** was unexpectedly low. Deblocking in two steps using DBU/pyridine followed by Et_3NHF in THF gave, after purification, a 58% yield of **25**.

In a final experiment, the 2′–5′ trimer **21** was catalytically reduced with Pd/C and H_2 at normal pressure to the corresponding 3′-amino-3′-deoxy-xylonucleotide trimer **26**.

Studies on the chemical and enzymatic stability of the free oligonucleotides **21**–**23** and **26** revealed complete resistance towards 0.33N NaOH for 24 h at 37° due to the absence of any 3′-neighbouring group that could catalyse the internucleotidic hydrolysis, but with snake-venom phosphodiesterase, a complete digestion from the 2′-end took place.

We thank the *Alexander von Humboldt Foundation* for a research fellowship and the *Fonds der Chemischen Industrie* for financial support.

Experimental Part

General. TLC: Precoated silica-gel TLC sheets *F 1500 LS 254* and cellulose TLC sheets *F 1140* 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).* HPLC: *Merck-Hitachi D 2000;* column *RP 18,* 125 × 4 mm, 5 μm, *Merck;* flow rate 1 ml/min, mobile phase 0.1M NH_4OAc/CH_3CN 9:1. M.p.: *Büchi* apparatus, model Dr. *Tottoli;* no corrections. UV/VIS: *Uvikon 820, Kontron,* and *Perkin Elmer, Lambda 5;* λ_{max} in nm (lg ε). ^{1}H-NMR: *Bruker WM-250;* δ in ppm rel. to TMS.

1. *9-(3′-Azido-3′-deoxy-β-*D*-xylofuranosyl)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenine* (**2**). A suspension of 2.92 g (10 mmol) of 9-(3′-azido-3′-deoxy-β-D-xylofuranosyl)adenine [12] (**1**) in dioxane (3 ml), hexamethyldisilazane (3 ml), and a catalytic amount of $(NH_4)_2SO_4$ were refluxed for 3 h. The mixture was cooled to r.t., evaporated, and treated with 12 ml of abs. toluene. The suspension was filtered and the filtrate evaporated. To the oily residue in anh. CH_2Cl_2 (40 ml), 6.23 g (20 mmol) of 1-methyl-3-[2-(4-nitrophenyl)ethoxycarbonyl]imidazolium chloride were added. The suspension was stirred for 36 h at r.t., the mixture filtered and evaporated, and the residue dissolved in MeOH (100 ml). After addition of 16% aq. NH_3 soln. (15 ml), the mixture was kept at r.t. for 45 min and then evaporated. The residue was chromatographed on silica gel with $CHCl_3$/MeOH 98:2 and 95:5. The main fraction gave, on recrystallization from i-PrOH, 4.76 g (96%). M.p. 102° (soften). UV (MeOH): 267 (4.46). ^{1}H-NMR ((D_6)DMSO): 10.6 (br. *s*, NH); 8.63 (*s*, H–C(8)); 8.59 (*s*, H–C(2)); 8.16, 7.61 (2*d*, $NO_2C_6H_4$); 6.26 (*d*, OH–C(2′)); 5.96 (*d*, *J* = 4.9, H–C(1′)); 5.15 (*t*, OH–C(5′)); 4.79 (*m*, H–C(2′)); 4.39 (*m*, H–C(3′), H–C(4′), CH_2CH_2O); 3.69 (*m*, 2 H–C(5′)); 3.11 (*t*, CH_2CH_2O). Anal. calc. for $C_{19}H_{19}O_7N_9 \cdot \frac{1}{2} H_2O$ (494.4): C 46.16, H 4.08, N 25.50; found: C 46.29, H 3.85, N 25.34.

2. *9-[3′-Azido-3′-deoxy-5′-*O*-(monomethoxytrityl)-β-*D*-xylofuranosyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenine* (**3**). To a soln. of 825 mg (1.67 mmol) of **2** (which was first coevaporated twice with pyridine) in anh. pyridine (20 ml) were added 660 mg (2.13 mmol) of monomethoxytrityl chloride (MeOTrCl) and kept at r.t. for 48 h. MeOH (1 ml) was added and the mixture evaporated and coevaporated 3 times with toluene. Purification by silica-gel column chromatography ($CHCl_3$, then $CHCl_3$/MeOH 99:1) followed by precipitation by Et_2O gave 1.19 g (94%) of an amorphous powder. UV (MeOH): 267 (4.50), 234 (4.35). ^{1}H-NMR ($CDCl_3$): 8.69 (*s*, H–C(8)); 8.16, 7.41 (2*s*, $NO_2C_6H_4$); 8.07 (*s*, H–C(2)); 8.05 (br. *s*, NH); 7.15–7.33 (*m*, 12 H, MeO*Tr*); 6.75 (*d*, 2 H *o* to MeO); 5.92 (*d*, *J* = 3.4, H–C(1′)); 5.0 (*d*, OH–C(2′)); 4.91 (*m*, H–C(2′)); 4.57 (*m*, H–C(4′)); 4.52 (*t*, CH_2CH_2O); 4.37 (*dd*, H–C(3′)); 3.76 (*s*, MeO); 3.42 (*dd*, 1 H–C(5′)); 3.31 (*dd*, 1 H–C(5′)); 3.13 (*t*, CH_2CH_2O). Anal. calc. for $C_{39}H_{35}N_9O_8$ (757.8): C 61.82, H 4.66, N 16.64; found: C 61.56, H 4.74, N 16.45.

3. *9-{3′-Azido-3′-deoxy-5′-*O*-(monomethoxytrityl)-2′-*O*-[2-(4-nitrophenyl)ethoxycarbonyl]-β-*D*-xylofuranosyl}-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenine* (**4**). A suspension of 748 mg (2.4 mmol) of 1-methyl-3-[2-(4-nitrophenyl)ethoxycarbonyl]imidazolium chloride, 909 mg (1.2 mmol) of **3** and 43 mg (0.36 mmol) of 4-(dimethylamino)pyridine in CH_2Cl_2 (10 ml) was stirred at r.t. for 15 h. CH_2Cl_2 (10 ml) was added and the mixture washed with H_2O (20 ml), dried, and evaporated, leaving an oil which was purified by silica-gel column chromatography ($CH_2Cl_2/CHCl_3$). The crude product was dissolved in little $CHCl_3$ and precipitated by Et_2O/hexane: 1.06 g (93%) of an amorphous powder. UV (MeOH): 267 (4.61), 234 (4.43). ^{1}H-NMR ($CDCl_3$): 8.68 (*s*, H–C(8)); 8.43 (*s*, NH); 8.12–8.19 (*m*, H–C(2), H *o* to NO_2); 7.19–7.44 (*m*, 16 H, MeO*Tr*, H *m* to NO_2); 6.82 (*d*, 2 H *o* to MeO); 6.25 (*d*, *J* = 1.8, H–C(1′)); 5.36 (*m*, H–C(2′)); 4.31–4.53 (*m*, H–C(3′), H–C(4′), 2 CH_2CH_2O); 3.78 (*s*, MeO); 3.63 (*dd*, 1 H–C(5′)); 3.36 (*dd*, 1 H–C(5′)); 3.11, 3.12 (2*t*, 2 CH_2CH_2O). Anal. calc. for $C_{48}H_{42}N_{10}O_{12}$ (950.9): C 60.63, H 4.45, N 14.73; found: C 60.25, H 4.18, N 14.40.

4. *9-{3′-Azido-3′-deoxy-2′-*O*-[2-(4-nitrophenyl)ethoxycarbonyl]-β-*D*-xylofuranosyl}-*N^6*-[2-(4-nitrophenyl) ethoxycarbonyl]adenine* (**5**). a) A soln. of 951 mg (1 mmol) of **4** in CH_2Cl_2/MeOH 4:1 (40 ml) containing

TsOH·H_2O (800 mg) was kept at r.t. for 15 min. The mixture was diluted with $CHCl_3$ (60 ml), washed with phosphate buffer pH 7 (0.15M, 3 × 100 ml), dried, and evaporated. The colourless foam was further purified by silica-gel column chromatography ($CHCl_3$, then $CHCl_3$/MeOH 98:2): 614 mg (90%) of colourless solid foam. UV (MeOH): 267 (4.57). ^{1}H-NMR ($CDCl_3$): 9.04 (*s*, NH); 8.65 (*s*, H–C(8)); 8.07–8.14 (*m*, H–C(2), 4 H *o* to NO_2); 7.30–7.37 (2*d*, 4 H *m* to NO_2); 6.04 (*d*, *J* = 5.2, H–C(1′)); 5.80 (*dd*, H–C(2′)); 4.75 (*t*, OH–C(5′)); 4.51 (*t*, CH_2CH_2O); 4.44 (*m*, H–C(3′), H–C(4′)); 4.29, 4.30 (2*t*, CH_2CH_2O); 3.91 (*m*, 2 H–C(5′)); 2.99, 3.11 (2*t*, 2 CH_2CH_2O). Anal. calc. for $C_{28}H_{26}N_{10}O_{11}$ (678.6): C 49.56, H 3.88, N 20.64; found: C 49.43, H 4.08, N 20.28.

b) A mixture of 1.52 g (2 mmol) of **3**, 72 mg (0.6 mmol) of 4-(dimethylamino)pyridine, and 1.25 g (4 mmol) of 1-methyl-3-[2-(4-nitrophenyl)ethoxycarbonyl]imidazolium chloride in CH_2Cl_2 (20 ml) was stirred at r.t. for 20 h. CH_2Cl_2 (20 ml) was added and the mixture washed 3 times with H_2O (40 ml), dried, and evaporated. The resulting light-yellow foam was dissolved in CH_2Cl_2/MeOH 4:1 (40 ml) containing 800 mg of TsOH·H_2O and kept at r.t. for 30 min. The mixture was diluted with $CHCl_3$ (60 ml), washed 3 times with phosphate buffer pH 7.0 (0.15M; 3 × 100 ml), dried, and evaporated. The resulting foam was purified by silica-gel column chromatography with AcOEt: 1.8 g (88%) of amorphous solid.

5. *9-[3′-Azido-3′-deoxy-5′-*O*-(monomethoxytrityl)-β-*D*-xylofuranosyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenin 2′-[2,5-Dichlorophenyl 2-(4-Nitrophenyl)ethyl Phosphate]* (**6**). A mixture of 1.68 g (6 mmol) of 2,5-dichlorophenyl dichlorophosphate and 0.90 g (13 mmol) of 1,2,4-triazole were stirred for 20 min at r.t. in abs. pyridine (16 ml). The suspension was cooled in an ice-bath and a soln. of 3.03 g (4 mmol) of **3** in abs. pyridine (24 ml) added dropwise. The mixture was stirred for 30 min and then warmed to r.t. After addition of 1.34 g (8 mmol) of 2-(4-nitrophenyl)ethanol, the mixture was stirred over night at r.t., evaporated, diluted with H_2O (100 ml), and extracted with $CHCl_3$ (2 × 100 ml). The org. layer was washed with H_2O (100 ml), dried, evaporated, and coevaporated with toluene. Purification of **6** by silica-gel column chromatography (CH_2Cl_2/$CHCl_3$ 7:3, then $CHCl_3$/MeOH 99.5:0.5) gave 3.63 g (80%) of colourless foam. UV (MeOH): 267 (4.62). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 8.64 (*s*, H–C(8)); 8.04–8.19 (*m*, H–C(2), NH, 4 H *o* to NO_2); 7.02–7.48 (*m*, 19 H, MeO*Tr*, 4 H *m* to NO_2, $Cl_2C_6H_3$); 6.85 (*d*, 2 H *o* to MeO); 6.28 (*s*, H–C(1′)); 5.28 (*m*, H–C(2′)); 4.54 (*m*, H–C(3′), H–C(4′), 2 CH_2CH_2O); 3.80 (*s*, MeO); 3.64–3.72 (*m*, 1 H–C(5′)); 3.35–3.47 (*m*, 1 H–C(5′)); 3.16 (*m*, 4 H, 2 CH_2CH_2O). Anal. calc. for $C_{53}H_{45}Cl_2N_{10}O_{13}P$ (1131.9): C 56.24, H 4.01, N 12.28; found: C 56.59, H 4.05, N 11.80.

6. *9-[3′-Azido-3′-deoxy-β-*D*-xylofuranosyl]-*N^6*-[(4-nitrophenyl)ethoxycarbonyl]adenin 2′-[2,5-Dichlorophenyl 2-(4-Nitrophenyl)ethyl Phosphate]* (**7**). A soln. of 1.13 g (1 mmol) of **6** in 20 ml of CH_2Cl_2/MeOH 4:1 containing 2% of TsOH·H_2O was kept at r.t. for 30 min. Then, $CHCl_3$ (20 ml) was added and the soln. washed with phosphate buffer 0.15M (3 × 40 ml), dried, and evaporated. Purification by silica-gel column chromatography ($CHCl_3$, then $CHCl_3$/MeOH 95:5) gave 830 mg (96%) of an amorphous solid. UV (MeOH): 266 (4.59). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 8.70, 8.68 (2*s*, H–C(8)); 7.97–8.18 (*m*, NH, H–C(2), 4 H *o* to NO_2); 7.42 (*d*, 2 H *m* to NO_2); 6.96–7.28 (*m*, 5 arom. H); 6.02 (2*d*, H–C(1′)); 5.76 (*q*, H–C(2′)); 5.07, 5.21 (OH); 4.23–4.57 (*m*, 6 H); 3.92 (*m*, 2 H–C(5′)); 3.15 (*t*, CH_2CH_2O); 2.96, 3.02 (2*t*, CH_2CH_2O). Anal. calc. for $C_{33}H_{29}Cl_2N_{10}O_{12}P$ (859.5): C 46.11, H 3.40, N 16.30; found: C 45.84, H 3.14, N 15.96.

7. *9-[3′-Azido-3′-deoxy-5′-*O*-(monomethoxytrityl)-β-*D*-xylofuranosyl]-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenin 2′-[2-(4-Nitrophenyl)ethyl Triethylammonium Phosphate]* (**8**). To a soln. of 1.66 g (10 mmol) of 4-nitrobenzaldehyde oxime in dioxane/Et_3N/H_2O 1:1:1, which has been stirred for 20 min at r.t., were added 1.13 g (1 mmol) of **6**. The mixture was evaporated after 40 min, coevaporated once with pyridine and 3 times with toluene, and chromatographed on a silica-gel column ($CHCl_3$/MeOH 97:3, then $CHCl_3$/MeOH/Et_3N 90:5:5): 1.03 g (93%) of **8** as colourless foam. UV (MeOH): 266 (4.60), 234 (4.40). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 8.62 (*s*, H–C(8)); 7.98–8.18 (*m*, NH, H–C(2), 4 H *o* to NO_2); 7.14–7.44 (*m*, 14 arom. H); 6.81 (*d*, 2 H *m* to NO_2); 6.28 (*d*, *J* = 1.2, H–C(1′)); 4.86 (*m*, H–C(2′)); 4.66 (*m*, H–C(4′)); 4.51 (*m*, H–C(3′), CH_2CH_2O); 4.19 (*m*, CH_2CH_2O); 3.78 (*s*, MeO); 3.58 (*q*, 1 H–C(5′)); 3.30 (*q*, 1 H–C(5′)); 3.14 (*t*, CH_2CH_2O); 3.00 (*m*, CH_2CH_2O, 3 CH_3CH_2); 1.25 (*t*, 3 CH_3CH_2). Anal. calc. for $C_{53}H_{58}N_{11}O_{13}P \cdot 2\,H_2O$ (1124.1): C 56.63, H 5.56, N 13.70; found: C 56.99, H 5.40, N 13.47.

8. *9-[3′-Azido-3′-deoxy-2′-*O*-[2-(4-nitrophenyl)ethoxycarbonyl]-β-*D*-xylofuranosyl]-*N^6*-[(4-nitrophenyl)-ethoxycarbonyl]adenin 5′-{Bis[2-(4-nitrophenyl)ethyl] Phosphate}* (**9**). A soln. of 122 mg (0.78 mmol) of **5** and 150 mg (0.36 mmol) of bis[(4-nitrophenyl)ethyl] chlorophosphate [15] in abs. pyridine (2 ml) was kept over night at 4°. The mixture was evaporated, diluted with 0.15M phosphate buffer pH 7.0 (20 ml), and extracted with $CHCl_3$ (2 × 20 ml). The org. layer was washed with H_2O (20 ml), dried, evaporated, and coevaporated with toluene. Silica-gel column chromatography (AcOEt, then AcOEt/acetone 7:3) gave 153 mg (80%) of amorphous solid. UV (MeOH): 267 (4.76). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 8.72 (*s*, H–C(8)); 8.14 (*m*, H–C(2), 8 H *o* to NO_2); 7.94 (*s*, NH); 7.34 (*m*, 8 H *m* to NO_2); 6.20 (*d*, *J* = 2.75, H–C(1′)); 5.51 (*t*, H–C(2′)); 4.54 (*t*, CH_2CH_2O); 4.41 (*t*, CH_2CH_2O); 4.06–4.39 (*m*, H–C(3′), H–C(4′), 2 H–C(5′), 2 CH_2CH_2O); 3.16 (*t*, CH_2CH_2O); 3.09 (*t*, CH_2CH_2O);

3.02 (*t*, 2 CH_2CH_2O). Anal. calc. for $C_{44}H_{41}N_{12}O_{18}P$ (1056.9): C 50.01, H 3.91, N 15.90; found: C 50.17, H 3.85, N 15.38.

9. N^6-*[2-(4-Nitrophenyl)ethoxycarbonyl]-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine* (**10**). A soln. of 2.76 g (6 mmol) of **2** [14] and 2 ml of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in abs. pyridine (50 ml) was stirred at r.t. over night. The mixture was evaporated, diluted with AcOEt (100 ml), and washed with H_2O (100 ml), 1N HCl (2 × 100 ml), H_2O (100 ml), 10% $NaHCO_3$ soln. (100 ml), and H_2O (100 ml). The org. soln. was dried, evaporated, and purified on a silica-gel column ($CHCl_3$/MeOH 98:2). Recrystallization from Et_2O/hexane and drying at 60° *in vacuo* gave 3.6 g (85%) of colourless crystals. M.p. 148.5–150°. UV (MeOH): 266 (4.48). ^{1}H-NMR ($CDCl_3$): 9.06 (*s*, NH); 8.64 (*s*, H–C(8)); 8.09 (*m*, H–C(2), 2 H *o* to NO_2); 7.37 (*d*, 2 H *m* to NO_2); 5.97 (*s*, H–C(1′)); 5.00 (*dd*, H–C(3′)); 4.55 (*dd*, H–C(2′)); 4.49 (*t*, CH_2CH_2O); 4.07 (*m*, H–C(4′), 2 H–C(5′)); 3.51 (*d*, OH–C(2′)); 3.10 (*t*, CH_2CH_2O); 1.00 (*m*, 4 i-Pr). Anal. calc. for $C_{31}H_{46}N_6O_9Si_2$ (702.9): C 52.97, H 6.60, N 11.96; found: C 52.93, H 6.82, N 11.98.

10. {*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-2′-O-[2-(4-nitrophenyl)ethoxycarbonyl]-β-*D*-xylofuranosyl*}*-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenine* (**11**). A mixture of 365 mg (0.33 mmol) of **8** and 195 mg (0.29 mmol) of **5** was coevaporated twice with pyridine and then dissolved in abs. pyridine (3 ml). *N*-Methylimidazole (162 mg, 1.98 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (200 mg, 0.66 mmol) were added. The mixture was kept at r.t. over night and then diluted with $CHCl_3$ (20 ml). The org. phase was washed with H_2O (2 × 20 ml), dried, evaporated, and coevaporated with toluene. Purification by silica-gel column chromatography ($CHCl_3$, then $CHCl_3$/MeOH 98:2) gave 444 mg (94%) of an amorphous solid. UV (MeOH): 266 (4.88). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 8.68, 8.67, 8.62, 8.61 (4*s*, H–C(8)); 8.32 (*m*, 2 H, NH); 8.01–8.15 (*m*, 10 H, H–C(2), H *o* to NO_2); 7.24–7.44 (*m*, 20 H, MeO*Tr*, H *m* to NO_2); 6.84 (*d*, 2 H *o* to MeO); 6.21, 6.17, 6.12 (*m*, 2 H, H–C(1′)); 5.59, 5.54 (*m*, 1 H, H–C(2′)); 5.16, 5.10 (*m*, 1 H, H–C(2′)); 4.26–4.54 (*m*, 14 H, H–C(3′), H–C(4′), CH_2(5′), CH_2CH_2); 3.77, 3.76 (2*s*, 3 H, MeO); 3.63, 3.37 (*m*, 2 H, CH_2(5′)); 2.99–3.15 (*m*, 8 H, 4 CH_2CH_2O). Anal. calc. for $C_{75}H_{67}N_{20}O_{23}P$ (1647.5): C 54.68, H 4.10, N 17.00; found: C 54.77, H 4.16, N 17.19.

11. {*9-(3′-Azido-3′-deoxy-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-2′-O-[2-(4-nitrophenyl)ethoxycarbonyl]-β-*D*-xylofuranosyl*}*-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenine* (**12**). A soln. of 330 mg (0.2 mmol) of **11** in 5 ml of CH_2Cl_2/MeOH 4:1 containing 2% of $TsOH \cdot H_2O$ was kept at r.t. for 30 min. The mixture was diluted with $CHCl_3$ (20 ml), washed with 0.15M phosphate buffer pH 7.0 (2 × 20 ml), dried, evaporated, and chromatographed on a silica-gel column ($CHCl_3$, then $CHCl_3$/MeOH 95:5) to give 261 mg (95%) of an amorphous solid. UV (MeOH): 266 (4.87). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 9.01 (*m*, 2 H, NH); 8.63 (*m*, 2 H, H–C(8)); 7.99–8.19 (*m*, 10 H, H–C(2), H *o* to NO_2); 7.19–7.39 (*m*, 8 H, H *m* to NO_2); 6.19, 6.02 (*m*, 2 H, H–C(1′)); 5.55 (*m*, 2 H, H–C(2′)); 5.18 (*t*, 1 H, OH); 4.07–4.50 (*m*, 14 H, H–C(3′), H–C(4′), CH_2(5′), CH_2CH_2O); 3.87 (*m*, 2 H, CH_2(5′)); 2.89, 3.10 (2*m*, 8 H, CH_2CH_2O). Anal. calc. for $C_{55}H_{51}N_{20}O_{22}P \cdot 1.5\ H_2O$ (1402.1): C 47.15, H 3.88, N 19.97; found: C 47.23, H 3.75, N 19.72.

12. {*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]adenine) 2′-[2,5-Dichlorophenyl 2-(4-Nitrophenyl)ethyl Phosphate]* (**13**). To a mixture of 995 mg (0.9 mmol) of **8** and 688 mg (0.8 mmol) of **7** in abs. pyridine (10 ml) were added successively 0.43 ml (54 mmol) of *N*-methylimidazole and 545 mg (1.8 mmol) of 2,4,6-triisopropylbenzenesulfonyl chloride. The mixture was stirred over night, evaporated, diluted with $CHCl_3$ (50 ml), washed twice with H_2O (50 ml), dried, evaporated, and coevaporated with toluene (2×). Chromatography on a silica-gel column ($CHCl_3$, then $CHCl_3$/MeOH 98:2) followed by prep. TLC ($CHCl_3$/MeOH 95:5) gave 1.29 g (88%) of amorphous solid. UV ($CHCl_3$): 266 (4.89). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 8.62 (*s*, 2 H, H–C(8)); 8.32 (2 H, NH); 8.00–8.15 (*m*, 10 H, H–C(2), H *o* to NO_2); 6.98–7.44 (*m*, 23 H, arom. H); 6.81 (*d*, 2 H *o* to MeO); 6.10–6.25 (*m*, 2 H, H–C(1′)); 5.53 (*m*, 1 H, H–C(2′)); 5.12 (*m*, 1 H, H–C(2′)); 4.27–4.53 (*m*, 14 H, H–C(3′), H–C(4′), CH_2(5′), CH_2CH_2O); 3.77 (*s*, 3 H, MeO); 3.37, 3.64 (*m*, 2 H, CH_2(5′)); 3.02–3.15 (*m*, 8 H, 4 CH_2CH_2O). Anal. calc. for $C_{80}H_{70}Cl_2N_{20}O_{24}P_2 \cdot 0.3\ CHCl_3$ (1864.2): C 51.74, H 3.80, N 15.02; found: C 51.87, H 3.57, N 14.86.

13. {*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]adenine 2′-[2-(4-Nitrophenyl)ethyl Triethylammonium Phosphate]* (**14**). A soln. of 166 mg (1 mmol) of 4-nitrobenzaldehyde oxime in dioxane (2 ml), Et_3N (2 ml), and H_2O (2 ml) was stirred for 20 min. Then, 183 mg (0.1 mmol) of **13** were added and stirred for another 40 min at r.t. The mixture was evaporated, coevaporated with pyridine followed by toluene, and chromatographed on a silica-gel column ($CHCl_3$/MeOH 97:3, then $CHCl_3$/MeOH/Et_3N 90:5:5). From the main fraction resulted an oil which was coevaporated with

toluene (2×), CCl_4 (1×), $CHCl_3$ (2×), and CH_2Cl_2 giving 165 mg (92%) of colourless solid foam. UV (MeOH): 266 (4.87). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 8.62–8.68 (*m*, 2 H, H–C(8)); 8.01–8.33 (*m*, 12 H, H–C(2), H *o* to NO_2); 7.16–7.44 (*m*, 20 H, arom. H); 6.86 (*d*, 2 H, H *o* to MeO); 6.14–6.22 (*m*, 2 H, H–C(1')); 5.00–5.18 (*m*, 2 H, H–C(2')); 4.10–4.53 (*m*, 14 H, H–C(3'), H–C(4'), CH_2(5'), CH_2CH_2O); 3.77 (*s*, 3 H, MeO); 3.62 (*m*, 1 H, H–C(5')); 3.36 (*m*, 1 H, H–C(5')); 3.14 (*m*, 4 H, CH_2CH_2O); 2.97 (*m*, 10 H, CH_2CH_2O, CH_3CH_2); 1.25 (*m*, 9 H, CH_3CH_2).

14. *{9-{3'-Azido-3'-deoxy-5'-O-{(2,5-dichlorophenoxy)[2-(4-nitrophenyl)ethoxy]phosphoryl}-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-2'-O-[2-(4-nitrophenyl)ethoxycarbonyl]-β-*D*-xylofuranosyl}-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenine*. (**15**). To a mixture of 210 mg (0.75 mmol) of 2,5-dichlorphenyl dichlorophosphate and 110 mg (1.6 mmol) of 1,2,4-triazole in pyridine (2 ml), which was stirred for 20 min at r.t. and then cooled in an ice-bath, was added dropwise a soln. of 688 mg (0.5 mmol) of **12** in abs. pyridine (3 ml). The mixture was stirred for 25 min and warmed up to r.t. Then, 0.2 g (1.2 mmol) of 2-(4-nitrophenyl)ethanol was added. After stirring over night, the mixture was evaporated, diluted with $CHCl_3$ (30 ml), washed with H_2O (2 × 30 ml), dried (Na_2SO_4), evaporated, and coevaporated with toluene (3×). The resulting light-yellow foam was purified on a silica-gel column (CH_2Cl_2/$CHCl_3$ 7:3, then $CHCl_3$/ MeOH 97:3) and then by prep. TLC ($CHCl_3$/MeOH 96:4) to give 690 mg (79%) of amorphous foam. UV ($CHCl_3$): 266 (4.95). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 9.34 (1 H, NH); 9.17 (1 NH, NH); 8.56 (2 H, H–C(8)); 8.20 (2 H, H–C(2)); 8.02 (*m*, 10 H, H *o* to NO_2); 6.93–7.35 (*m*, 13 H, arom. H); 6.17 (*m*, 2 H, H–C(1')); 5.37 (*m*, 1 H, H–C(2')); 5.57 (*m*, 1 H, H–C(2')); 4.22–4.61 (*m*, 18 H, H–C(3'), H–C(4'), CH_2(5'), CH_2CH_2O); 2.90–3.09 (*m*, 10 H, CH_2CH_2O). Anal. calc. for $C_{29}H_{61}Cl_2N_{21}O_{27}P_2$ (1749.2): C 47.38, H 3.51, N 16.82; found: C 47.24, H 3.29, N 16.42.

15. *{9-{3'-Azido-3'-deoxy-5'-O-{[2-(4-nitrophenyl)ethyl](triethylammonio)phosphonato}-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-2'-O-[2-(4-nitrophenyl)ethoxycarbonyl]-β-*D*-xylofuranosyl}-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-adenine* (**16**). To a soln. of 332 mg (2 mmol) of 4-nitrobenzaldehyde oxime in dioxane/Et_3N/H_2O 1:1:1 (12 ml), which was stirred for 20 min, were added 350 mg (6.2 mmol) of **15**. The mixture was stirred for 60 min, evaporated, and coevaporated with pyridine and toluene. The oily residue was chromatographed on a silica-gel column ($CHCl_3$/MeOH 97:3, then $CHCl_3$/MeOH/Et_3N 86:7:7). The main fraction was evaporated and coevaporated with toluene (2×), $CHCl_3$ (2×), and CH_2Cl_2 (2×) to give 331 mg (89%) of amorphous foam containing 1.5 equiv. of Et_3N according to ^{1}H-NMR. UV (MeOH): 266 (4.92). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 8.59 (2 H, H–C(8)); 8.16–8.41 (2 H, H–C(2)); 7.96–8.05 (*m*, 10 H, H *o* to NO_2); 7.16–7.37 (*m*, 10 H, H *m* to NO_2); 6.11–6.26 (*m*, 2 H, H–C(1')); 5.25–5.55 (*m*, 2 H, H–C(2')); 3.97–4.67 (*m*, 18 H, H–C(3'), H–C(4'), CH_2(5'), CH_2CH_2O); 2.95 (*m*, CH_2CH_2O, CH_3CH_2); 1.24 (*m*, CH_3CH_2).

16. *{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'-yl-{2'-{O^P-[2-(4-nitrophenyl)ethyl]}→5'}-9-{3'azido-3'-deoxy-2'-O-[2-(4-nitrophenyl)ethoxycarbonyl]-β-*D*-xylofuranosyl}-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenine* (**17**). a) To a mixture of 1.24 g (1.14 mmol) of **8** and 1.50 g (1.09 mmol) of **12** in abs. pyridine (12 ml) was added successively 0.57 ml (7.2 mmol) of *N*-methylimidazole and 726 mg (2.4 mmol) of 2,4,6-triisopropylbenzenesulfonyl chloride. The mixture was stirred over night, evaporated, diluted with $CHCl_3$ (100 ml), washed with H_2O (2 × 100 ml), dried (Na_2SO_4), evaporated, and coevaporated twice with toluene. The residue was purified by silica-gel column chromatography ($CHCl_3$, then $CHCl_3$/MeOH 97:3) and prep. TLC ($CHCl_3$/MeOH 95:5): 2.35 g (92%) of amorphous solid.

b) To a mixture of 92 mg (0.05 mmol) of **14** and 31 mg (0.045 mmol) of **5** in abs. pyridine (1 ml) were added successively 24 µl (0.3 mmol) of *N*-methylimidazole and 30 mg (0.1 mmol) of 2,4,6-triisopropylbenzenesulfonyl chloride. The mixture was stirred over night, evaporated, diluted with $CHCl_3$ (10 ml), washed twice with H_2O (10 ml), dried, evaporated, and coevaporated with toluene. Purification by prep. TLC ($CHCl_3$/MeOH 95:5) gave 90 mg (85%) of amorphous solid. UV ($CHCl_3$): 266 (5.03). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 8.71–8.92 (3 H, NH); 8.58–8.64 (3 H, H–C(8)); 7.98–8.22 (*m*, 15 H, H–C(2), arom. H); 7.24–7.44 (*m*, 24 H, arom. H); 6.80 (*d*, 2 H *o* to MeO); 6.03–6.23 (*m*, 3 H, H–C(1')); 5.15–5.57 (*m*, 3 H, H–C(2')); 4.24–4.52 (*m*, 22 H, H–C(3'), H–C(4'), CH_2(5'), CH_2CH_2O); 3.76 (*m*, 1 H, H–C(5')); 3.75 (*s*, 3 H, MeO); 3.62 (*m*, 1 H, H–C(5')); 2.92–3.10 (*m*, 12 H, CH_2CH_2O). Anal. calc. for $C_{102}H_{92}N_{30}O_{34}P_2 \cdot 1/3\ CHCl_3$ (2403.8): C 51.22, H 3.88, N 17.47; found: C 51.53, H 3.71, N 17.05.

17. *{9-(3'-Azido-3'-deoxy-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'-yl-{2'-{O^P-[2-(4-nitrophenyl)ethyl]}→5'}-9-{3'azido-3'-deoxy-2'-O-[2-(4-nitrophenyl)ethoxycarbonyl]-β-*D*-xylofuranosyl}-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenine* (**18**). A soln. of 1.172 g (0.5 mmol) of **17** in

CH_2Cl_2/MeOH 4:1 (10 ml) containing 2% of TsOH·H_2O was kept at r.t. for 45 min. $CHCl_3$ (30 ml) was added, the mixture washed 3 times with 0.15M phosphate buffer pH 7 (30 ml), dried (Na_2SO_4), evaporated, and purified by silica-gel column chromatography ($CHCl_3$/MeOH 99:1, then $CHCl_3$/MeOH 95:5): 970 mg (93%) of amorphous solid. UV (MeOH): 266 (5.06). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 8.62–8.67 (3 H, H–C(8)); 8.03–8.29 (*m*, 15 H, H–C(2), H *o* to NO_2); 7.24–7.44 (*m*, 12 H, H *m* to NO_2); 5.98–6.22 (3 H, H–C(1')); 5.26, 5.61 (3 H, H–C(2')); 5.43 (*t*, 1 H, OH–C(5')); 4.10–4.53 (*m*, 22 H); 3.88 (*m*, 2 H); 2.92–3.14 (*m*, 12 H). Anal. calc. for $C_{82}H_{76}N_{30}O_{33}P_2 \cdot 1\frac{1}{2}\ H_2O$ (2098.7): C 46.93, H 3.79, N 20.02; found: C 47.06, H 3.84, N 19.76.

18. *{9-{3'-Azido-5'-O-{bis[2-(4-nitrophenyl)ethoxy]phosphoryl}-3'-deoxy-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'-yl-{2'-{O^P-[2-(4-nitrophenyl)ethyl]}→5'}-9-{3'azido-3'-deoxy-2'-O-[2-(4-nitrophenyl)ethoxycarbonyl]-β-D-xylofuranosyl}-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]-adenine* (**19**). A mixture of 0.58 g (0.28 mmol) of **18**, 464 mg (1.12 mmol) of bis[(4-nitrophenyl)ethyl] chlorophosphate [15] and 0.178 ml (2.24 mmol) of *N*-methylimidazole in abs. pyridine (2 ml) was kept at r.t. for 24 h. The mixture was diluted with $CHCl_3$ (50 ml), washed with H_2O (3 × 50 ml), dried, evaporated, and coevaporated with toluene. Purification was achieved by prep. TLC ($CHCl_3$/MeOH 96:4, two developments); 580 mg (84%) of an amorphous solid. UV ($CHCl_3$): 266 (5.12). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 9.12–9.30 (3 H, NH); 8.57 (3 H, H–C(8)); 7.93–8.24 (*m*, 19 H); 7.18–7.38 (*m*, 16 H); 6.07–6.20 (*m*, 3 H, H–C(1')); 5.33 (*m*, 2 H, H–C(2')); 5.57 (*m*, 1 H, H–C(2')); 4.19–4.47 (*m*, 28 H); 2.92–3.41 (*m*, 16 H). Anal. calc. for $C_{98}H_{91}N_{32}O_{40}P_3$ (2450.0): C 47.10, H 3.74, N 18.29; found: C 47.31, H 3.44, N 18.00.

19. *{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'-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'-yl-{2'-{O^P-[2-(4-nitrophenyl)ethyl]}→5'}-9-{3'-azido-3'-deoxy-2'-O-[2-(4-nitrophenyl)ethoxycarbonyl]-β-D-xylofuranosyl}-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenine* (**20**). To a mixture of 206 mg (0.75 mmol) of **12** and 288 mg (0.16 mmol) of **14**, which was coevaporated twice with abs. pyridine and dissolved in abs. pyridine (2 ml), were added 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 with H_2O (2 × 30 ml), dried, evaporated, coevaporated with toluene (2×), and purified by silica-gel column chromatography ($CHCl_3$, then $CHCl_3$/MeOH 95:5) followed by prep. TLC ($CHCl_3$/MeOH 94:6, two developments): 345 mg (75%) of a colourless foam. UV ($CHCl_3$): 266 (5.18). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 8.83–9.10 (4 H, NH); 8.60 (4 H, H–C(8)); 7.96–8.25 (*m*, 20 H); 7.20–7.43 (*m*, 28 H); 6.79 (*d*, 2 H *o* to MeO); 6.05–6.24 (*m*, 4 H, H–C(1')); 5.60, 5.32, 5.17 (*m*, 4 H, H–C(2')); 4.12–4.49 (*m*, 30 H); 3.75 (*s*, MeO); 3.62 (*q*, 1 H, H–C(5')); 3.37 (*q*, 1 H, H–C(5')); 2.91–3.09 (*m*, 16 H). Anal. calc. for $C_{129}H_{117}N_{40}O_{45}P_3 \cdot CHCl_3$ (3159.0): C 49.41, H 3.76, N 17.73; found: C 49.34, H 3.56, N 17.75.

20. *[9-(3'-Azido-3'-deoxy-β-D-xylofuranosyl)adenin]-2'-yl-[2'-(O^P-ammonio) → 5']-[9-(3'-azido-3'-deoxy-β-D-xylofuranosyl)adenin]-2'-yl-[2'-(O^P-ammonio) → 5']-9-(3'-azido-3'-deoxy-β-D-xylofuranosyl)adenine* (**21**). A soln. of **18** (104 mg, 50 μmol) in pyridine (12 ml) containing DBU (0.9 ml) was kept at r.t. for 2 days. The mixture was evaporated, dissolved in 1M NH_3 (10 ml), washed with $CHCl_3$ (4 × 10 ml), evaporated, and applied onto a *DEAE* cellulose column. The column was washed with H_2O and eluted with a NH_4HCO_3 gradient 0 → 0.3M yielding **21** with 0.175M NH_4HCO_3. The fractions were evaporated, coevaporated 3 times with MeOH and once with H_2O, filtered, and freeze-dried till constant weight: 47.5 mg (92%). Stable in 0.33N NaOH for 24 h at 37°. TLC (silica gel, i-PrOH/NH_3 (25%)/H_2O 8:1:1): R_f 0.58. Electrophoresis (acrylamide gel, ref., bromophenyl blue): R_f 0.76. HPLC: R_t 220 s. UV (H_2O): 258; hypochromicity by phosphodiesterase digestion: 8.6%. IR (KBr): 2110. ^{1}H-NMR (D_2O): 7.97 (*s*); 7.84 (*s*); 7.81 (*s*); 7.74 (*s*); 7.72 (*s*); 5.86 (*d*, J = 3.0, H–C(1')); 5.59 (*d*, J = 3.0, H–C(1')); 5.49 (*d*, J = 5.2, H–C(1')); 3.50–4.86 (*m*).

21. *{9-[5'-O-(Ammoniophosphonato)-3'-azido-3'-deoxy-β-D-xylofuranosyl]adenin}-2'-yl-[2'-(O^P-ammonio) → 5']-[9-(3'-azido-3'-deoxy-β-D-xylofuranosyl)adenin]-2'-yl-[2'-(O^P-ammonio) → 5']-9-(3'azido-3'-deoxy-β-D-xylofuranosyl)adenine* (**22**). A soln. of **19** (70 mg, 28.5 μmol) in pyridine (50 ml) containing DBU (3.8 ml) was stirred at r.t. for 2 days and then neutralized with 1M AcOH (25 ml) and evaporated. The residue was taken up in H_2O (50 ml) and washed with $CHCl_3$ (2 × 20 ml). The H_2O phase was concentrated to a small volume and chromatographed on a *DEAE Sephadex* column (60 × 1 cm) with 0.001–0.5M TBK buffer (pH 7.5). The product fractions were evaporated, coevaporated several times with H_2O, and further purified by paper chromatography using i-PrOH/conc. NH_3/H_2O 55:10:35. The product band was eluted with H_2O and lyophilised: 77% of colourless powder. TLC (cellulose, i-PrOH/conc. NH_3/H_2O 6:1:3): R_f 0.24. HPLC: R_t 108 s. ^{1}H-NMR (D_2O): 8.27 (*s*, 1 H); 8.23 (*s*, 1 H); 8.08 (*s*, 2 H); 8.00 (*s*, 1 H); 7.97 (*s*, 1 H); 6.13 (*d*, 1 H, H–C(1')); 5.81 (*d*, 1 H, H–C(1')); 5.74 (*d*, 1 H, H–C(1')).

22. *[9-(3'-Azido-3'-deoxy-β-D-xylofuranosyl)adenin]-2'-yl-[2'-(O^P-ammonio)* → *5']-[9-(3'-azido-3'-deoxy-β-D-xylofuranosyl)adenin]-2'-yl-[2'-(O^P-ammonio)* → *5']-[9-(3'azido-3'-deoxy-β-D-xylofuranosyl)adenin]-2'-yl-[2'-(O^P-ammonio)* → *5']-9-(3'-azido-3'-deoxy-β-D-xylofuranosyl)adenine* (**23**). A soln. of **20** (45 mg, 15 μmol) in pyridine (9 ml) containing DBU (0.684 ml) was stirred at r.t. for 2 days. After neutralization with 1M AcOH in pyridine (3.5 ml), the mixture was evaporated, the residue taken up in 80% AcOH (10 ml), stirred at r.t. for 24 h, and then again evaporated. The remaining solid was dissolved in H_2O (50 ml), the soln. washed with $CHCl_3$ (2 × 25 ml), and the aq. layer evaporated and coevaporated several times with H_2O to remove the acid. After chromatography on a *DEAE-Sephadex A-25* column (60 × 1 cm) with a linear gradient of 0.001–0.4M TBK buffer (pH 7.5), the product fractions were evaporated and coevaporated with H_2O. The tetramer was further purified by paper chromatography using i-PrOH/conc. NH_3/H_2O 7:1:2. The main bands were cut out, eluted by H_2O, and lyophilised: 71% of colourless powder. TLC (cellulose, i-PrOH/conc. NH_3/H_2O 7:1:2): R_f 0.31. HPLC: R_t 240. ^{1}H-NMR (D_2O): 8.17 (*s*, 1 H); 8.15 (*s*, 1 H); 8.02 (*s*, 1 H); 8.00 (*s*, 1 H); 7.94 (*s*, 2 H); 7.91 (*s*, 1 H); 7.89 (*s*, 1 H); 6.02 (*d*, 1 H, H–C(1')); 5.77 (*d*, 1 H, H–C(1')); 5.73 (*d*, 1 H, H–C(1')); 5.68 (*d*, 1 H, H–C(1')).

23. *{N^6-[2-(4-Nitrophenyl)ethoxycarbonyl]-3',5',O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosin}-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'-yl-{2'-{O^P-[2-(4-nitrophenyl)ethyl]}* → *5'}-9-{3'-azido-3'-deoxy-2'-O-[2-(4-nitrophenyl)ethoxycarbonyl]-β-D-xylofuranosyl}-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenine* (**24**). a) To a soln. of 260 mg (0.75 mmol) of **16** and 95 mg (0.135 mmol) of **10** in abs. pyridine (2 ml) were added 6.07 ml (0.9 mmol) of *N*-methylimidazole and 91 mg (0.3 mmol) of 2,4,6-triisopropylbenzenesulfonyl chloride. The mixture was stirred at r.t. for 40 h, evaporated, diluted with $CHCl_3$ (10 ml), washed twice with H_2O (2 × 10 ml), dried, evaporated, and coevaporated with toluene. The mixture was purified by silica-gel column chromatography ($CHCl_3$ then $CHCl_3$/MeOH 96:4). Since the main fraction contained *N*-methylimidazole (^{1}H-NMR), further purification was necessary. The product was dissolved in $CHCl_3$ (5 ml), washed twice with H_2O (2 × 5 ml), dried, evaporated, and chromatographed on a silica-gel column with $CHCl_3$/MeOH 97:3 to give 96 mg (31%) of an amorphous solid.

b) To a soln. of 230 mg (0.135 mmol) of **16**, 141 mg (0.2 mmol) of **10**, and 124 mg (0.9 mmol) of 4-(dimethylamino)pyridine *N*-oxide in pyridine (2 ml) were added 91 mg (0.3 mmol) of 2,4,6-triisopropylbenzene-sulfonyl chloride. The mixture was stirred for 40 h at r.t., evaporated, dissolved in $CHCl_3$ (10 ml), washed 3 times with H_2O (10 ml), dried, evaporated, and coevaporated with toluene. Purification was achieved by silica-gel column chromatography ($CHCl_3$, then $CHCl_3$/MeOH 97:3). The main fraction was further purified by prep. TLC ($CHCl_3$/MeOH 95:5) and coevaporation with Et_2O to give 90 mg (29%) of a solid foam. UV ($CHCl_3$): 266 (5.06). IR (KBr): 2100. ^{1}H-NMR ($CDCl_3$): 7.98–8.96 (*m*, 21 H); 7.20–7.41 (*m*, 12 H); 6.19 (*m*, 3 H, H–C(1')); 3.86–5.72 (*m*, 27 H); 2.84–3.14 (*m*, 12 H); 1.05 (*m*, 28 H). Anal. calc. for $C_{94}H_{103}N_{27}O_{35}P_2Si_2 \cdot Et_2O$ (2363.2): C 49.80, H 4.82, N 15.99; found: C 49.68, H 4.84, N 15.65.

24. *(Adenosin)-2'-yl-[2'-(O^P-ammonio)* → *5']-[9-(3'-azido-3'-deoxy-β-D-xylofuranosyl)adenin]-2'-yl-[2'-(O^P-ammonio)* → *5']-9-(3'-azido-3'-deoxy-β-D-xylofuranosyl)adenine* (**25**). A soln. of 41 mg (0.018 mmol) of **24** in 0.5M DBU/pyridine (10 ml) was stirred at r.t. for 48 h. The mixture was neutralized with 1M AcOH/pyridine (5 ml) and then evaporated. The residue in 1M Bu_4NF/THF (2 ml) was stirred for 48 h at r.t. and then the soln. evaporated. The residue was dissolved in H_2O (50 ml) and washed with $CHCl_3$ (2 × 20 ml). The aq. phase was chromatographed on a *DEAE-Sephadex A-25* column with a linear gradient of 0.001–0.3M Et_3NHCO_3 buffer pH 7.5. The product was eluted at 0.13–0.17M: 492 *OD* (76%) of **25**. Further purification was done by paper chromatography (i-PrOH/conc. NH_3/H_2O 6:1:3). The main band was eluted by H_2O and lyophilized: colourless powder of the ammonium salt in 58% (378 *OD*) yield. TLC (cellulose, i-PrOH/conc. NH_3/H_2O 6:1:3): R_f 0.54. HPLC: R_t 170. UV (H_2O): 258. ^{1}H-NMR (D_2O): 8.17 (*s*); 8.15 (*s*); 8.05 (*s*); 8.01 (*s*); 7.88 (*s*); 7.83 (*s*); 6.11 (*d*, 1 H, H–C(1')); 5.82 (*d*, 1 H, H–C(1')); 5.69 (*d*, 1 H, H–C(1')).

25. *[9-(3'-Ammonio-3'-deoxy-β-D-xylofuranosyl)adenin]-2'-yl-[2'p$^-$5']-[9-(3'-ammonio-3'-deoxy-β-D-xylofuranosyl)adenin]-2'-yl-[2'p$^-$5']-9-(3'-ammonio-3'-deoxy-β-D-xylofuranosyl)adenine Hydrocarbonate* (**26**). A soln. of 540 *OD* of **21** in EtOH/H_2O 1:1 (10 ml) containing 20 mg of 10% Pd/C was hydrogenated at 1 atm for 65 h while stirring. The mixture was filtered, the filter washed with H_2O (5 × 1 ml), and the filtrate evaporated. The mixture was applied on a *DEAE* cellulose column which was washed with H_2O and eluted with a linear gradient of Et_3NHHCO_3 (0 → 0.4M). The product was eluted with 0.125M Et_3NHHCO_3, coevaporated twice with MeOH and twice with H_2O, dissolved in H_2O (5 ml), filtered, and freeze-dried to yield 418 *OD* (77%). Completely stable in 0.33N NaOH, for 24 h at 37°. TLC (silica gel, i-PrOH/NH_3 (25%)/H_2O 7:1:2): R_f 0.67. Electrophoresis (acrylamide gel, ref., bromophenyl blue): R_f 0.76. HPLC: R_t 115 s. UV (H_2O): 258; hypochromicity on digestion with phosphodiesterase: 11%. ^{1}H-NMR (D_2O): 7.93 (*s*); 7.85 (*s*); 7.80 (*s*); 7.78 (*s*); 7.73 (*s*); 7.69 (*s*); 5.83 (*d*, J = 2.7, H–C(1')); 5.70 (*d*, J = 2.7, H–C(1')); 5.53 (*d*, J = 4.3, H–C(1')); 3.31–4.68 (*m*).

REFERENCES

[1] R. Charubala, W. Pfleiderer, R. Suhadolnik, R.W. Sobol, S.W. Li, N.L. Reichenbach, *Helv. Chim. Acta* **1989,** *72,* 1354.

[2] A.G. Hovanessian, R.E. Brown, I.M. Kerr, *Nature (London)* **1977,** *268,* 537; I.M. Kerr, R.E. Brown, A.G. Hovanessian, *ibid.* **1977,** *268,* 540; I.M. Kerr, R.E. Brown, *Proc. Natl. Acad. Sci. U.S.A.* **1978,** *75,* 256; E.M. Martin, N.J.M. Birdsall, R.E. Brown, I.M. Kerr, *Eur. J. Biochem.* **1979,** *95,* 295.

[3] R. Charubala, E. Uhlmann, F. Himmelsbach, W. Pfleiderer, *Helv. Chim. Acta* **1987,** *70,* 2028, and ref. cit. therein.

[4] D. Alster, D. Brozda, Y. Kitade, A. Wong, R. Charubala, W. Pfleiderer, P.F. Torrence, *Biochem. Biophys. Res. Commun.* **1986,** *141,* 555.

[5] K. Kariko, S.W. Li, R.W. Sobol, L. Suhadolnik, N.L. Reichenbach, R.J. Suhadolnik, R. Charubala, W. Pfleiderer, *Nucleos. Nucleot.* **1987,** *6,* 173; *Biochemistry* **1987,** *26,* 7127.

[6] K. Kariko, S.W. Li, R.W. Sobol, R.J. Suhadolnik, R. Charubala, W. Pfleiderer, *Biochemistry* **1987,** *26,* 7136.

[7] R.J. Suhadolnik, C. Lee, K. Kariko, S.W. Li, *Biochemistry* **1987,** *26,* 7143.

[8] P.F. Torrence, D. Brozda, D. Alster, R. Charubala, W. Pfleiderer, *J. Biol. Chem.* **1988,** *263,* 1131.

[9] C.B. Reese, *Phosphorus Sulfur* **1976,** *1,* 245.

[10] J.H. van Boom, *Heterocycles* **1977,** *7,* 1197.

[11] C.B. Reese, *Tetrahedron* **1978,** *34,* 3143.

[12] M.J. Robins, Y. Fouron, R. Mengel, *J. Org. Chem.* **1974,** *39,* 1564.

[13] G.S. Ti, B.C. Gaffney, R.A. Jones, *J. Am. Chem. Soc.* **1982,** *104,* 1316.

[14] F. Himmelsbach, B.S. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, *Tetrahedron* **1984,** *40,* 59.

[15] F. Himmelsbach, R. Charubala, W. Pfleiderer, *Helv. Chim. Acta* **1987,** *70,* 1286.

[16] W.T. Markiewicz, *J. Chem. Res. (S)* **1979,** 24.