83. Nucleotides

Part XXXV[1])

Synthesis of 3′-Deoxyadenylyl-(2′–5′)-3′-deoxyadenylyl-(2′–ω)-9-(ω-hydroxyalkyl)adenines

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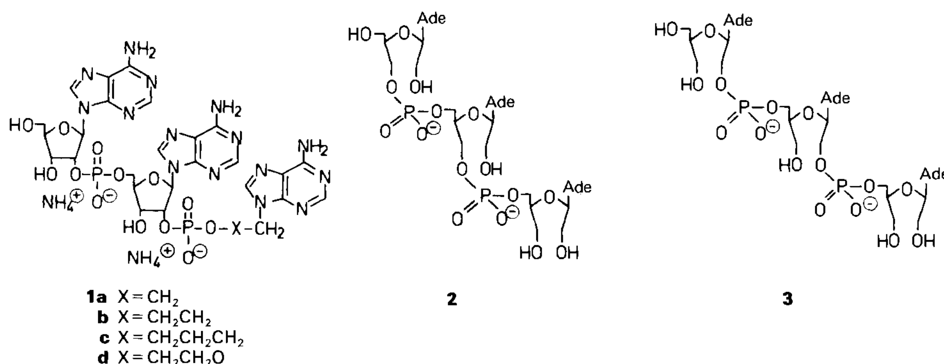
Via the phosphotriester approach, new structural analogs of (2′–5′)oligoadenylates, namely 3′-deoxyadenylyl-(2′–5′)-3′-deoxyadenylyl-(2′–ω)-9-(ω-hydroxyalkyl)adenines **18–21**, have been synthesized (see *Scheme*) which should preserve biological activity and show higher stability towards phosphodiesterases. The newly synthesized oligonucleotides **18–21** have been characterized by ^{1}H-NMR spectra, TLC, and HPLC analysis.

1. Introduction. – One mode of action of interferon in its antiviral activity is associated with the presence of 5′-triphosphates of (2′–5′)oligoadenylates (ppp5′A(2′p5′A)$_n$) ($n = 2$, 3) in cells which activate in nanomolar concentration the latent endonuclease, RNAse L [2] [3]. Its activation results in the cleavage of viral mRNA and hence in the inhibition of the protein synthesis [4–7]. The (2′–5′)A's are not readily taken up by the intact eucaryotic cells and are rapidly hydrolyzed by phosphodiesterases present in cell extracts. Hence chemical modification of the structures of (2′–5′)A's might provide better stability towards the phosphodiesterases and still possess high biological activity [8–11].

Modification of the carbohydrate moiety in the 3′-terminal nucleosides, *e.g.*, by incorporation of an acyclic nucleoside analog has been shown to decrease the rate of hydrolysis of the oligonucleotide by snake-venom phosphodiesterase [12] [13]. Since it



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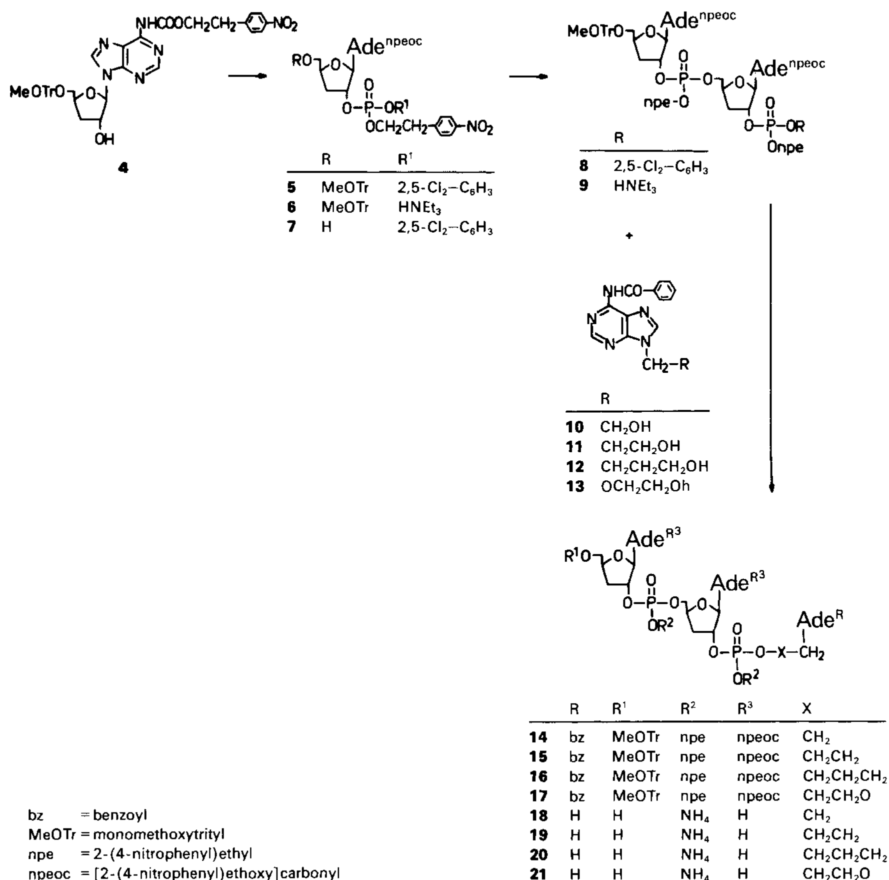
may be assumed that the properties of the (2′–5′)phosphodiesterase ((2′–5′)PDE) regarding specificity and oligonucleotide hydrolysis are similar to those of snake-venom phosphodiesterase [14] [15], stable analogues towards snake-venom phosphodiesterase presumably function even has inhibitors of (2′–5′)PDE. With this aim, we synthesized (2′–5′)A analogues **1a–d** with a 3′-terminal acyclic residue [16] as well as (2′–5′)ApApA acyclic analogues **2** and **3** [17] [18] and reported that these compounds show greater stability against the action of phosphodiesterases and high affinity for snake-venom phosphodiesterase as well as for other phosphodiesterases.

Another chemical modification starting from 3′-deoxyadenosine (cordycepin) [19] [20] was more closely related to the (2′–5′)A trimer core, which reveals almost the same biological activity as its 5′-triphosphate. The cordycepin trimer core 3′d(A2′p5′A2′p5′A) turned out to be a unique new structure analogue due to its extended metabolic stability without toxicity to cells, apart from other interesting biological activities [21–24]. These studies prompted us to synthesize a mixed combination of trimers consisting of the 3′-deoxyadenylyl-(2′–5′)-3′-deoxyadenylyl residue and different 9-(ω-hydroxyalkyl)-adenines at the 2′-terminal end [25] [26] for further biological evaluation.

Scheme



	R	R¹	R²	R³	X
14	bz	MeOTr	npe	npeoc	CH_2
15	bz	MeOTr	npe	npeoc	CH_2CH_2
16	bz	MeOTr	npe	npeoc	$CH_2CH_2CH_2$
17	bz	MeOTr	npe	npeoc	CH_2CH_2O
18	H	H	NH_4	H	CH_2
19	H	H	NH_4	H	CH_2CH_2
20	H	H	NH_4	H	$CH_2CH_2CH_2$
21	H	H	NH_4	H	CH_2CH_2O

bz = benzoyl
MeOTr = monomethoxytrityl
npe = 2-(4-nitrophenyl)ethyl
npeoc = [2-(4-nitrophenyl)ethoxy]carbonyl

2. Syntheses. – As starting material, 3′-deoxy-5′-*O*-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**4**) [20] was taken and converted into the 2′-phosphotriester **5** [20] with a 2,5-dichlorophenyl and a 2-(4-nitrophenyl)ethyl group at the phosphate function (*Scheme*). From **5**, the 2,5-dichlorophenyl group was cleaved off with 4-nitrobenzaldehyde oxime to give diester **6** [20]. On the other hand, the 5′-*O*-monomethoxytrityl group was removed by acid to give the 5′-hydroxytriester **7** [20]. These two building blocks **6** and **7** were condensed in presence of 2,4,6-triisopropylbenzenesulfonyl chloride and 1-methyl-1*H*-imidazole to yield in 88%, after purification and drying, the dimeric phosphotriester **8** [20]. Again the 2,5-dichlorophenyl group from this dimer block was cleaved off by the oximate method to give the corresponding dinucleotide monophosphodiester **9** [20].

For the syntheses of the required trimers **14**–**17**, the above dimeric phosphodiester **9** was condensed with the suitably blocked appropriate acyclic nucleosides **10**–**13** [25] [26] in presence of 2,4,6-triisopropylbenzenesulfonyl chloride and 1-methyl-1*H*-imidazole in yields of 70–75%. The purity of all products was checked by TLC, UV, and ^{1}H-NMR spectra. All trimers were deblocked sequentially, using 0.5M DBU (1,8-diazabicyclo-[5.4.0]undec-7-ene) in dry pyridine for 20 h at room temperature to cleave the 2-(4-nitrophenyl)ethyl groups, NH_3 in dioxane to remove the benzoyl groups, and finally acid treatment to deblock the 5′-*O*-monomethoxytrityl group yielding the corresponding fully deblocked trimers **18**–**21**, which were purified by *DEAE Sephadex* column chromatography and paper chromatography. The trimers were characterized by TLC, UV, HPLC, and ^{1}H-NMR spectra (*Table*).

Table. *Physical Data of Deblocked Oligonucleotides* **18**–**21**

	^{1}H-NMR Spectra (D_2O, δ [ppm])				TLC (cellulose, i-PrOH/conc. NH_3/H_2O 6:1:3)	HPLC (RP-18 column; *A*, 50 mM $(NH_4)H_2PO_4$, pH 7; *B*, MeOH/H_2O 1:1; *A*/*B* 7:3)	UV spectra (H_2O)
	H–C(8) + H–C(2)			H–C(1′)	R_f	t_R	λ_{max} [nm]
18	8.00 (*s*)	7.97 (3 H, *s*)		6.04 (*s*)	0.45	2.12 min	257
	7.83 (*s*)	7.80 (*s*)		5.65 (*d*)			
19	8.08 (*s*)	8.01 (*s*)	7.92 (*s*)	6.07 (*s*)	0.44	2.89 min	257
	7.87 (*s*)	7.81 (*s*)	7.80 (*s*)	5.79 (*d*)			
20	8.06 (*s*)	8.00 (*s*)	7.95 (*s*)	6.07 (*s*)	0.41	3.68 min	257
	7.85 (*s*)	7.82 (*s*)	7.74 (*s*)	5.82 (*d*)			
21	8.04 (*s*)	8.02 (*s*)	8.01 (*s*)	6.09 (*s*)	0.39	2.63 min	257
	7.98 (*s*)	7.83 (*s*)	7.82 (*s*)	5.81 (*d*)			

Experimental Part

General. TLC: precoated silica-gel thin-layer sheets *F 1500 LS 254* and celullose thin-layer sheets *F 1440* from *Schleicher & Schüll*. Prep. column chromatography (CC): 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*). UV/VIS: *Uvikon 820, Kontron*; λ_{max} in nm (log ε). ^{1}H-NMR: *Bruker WM 250*; δ in ppm rel. to TMS.

1. *Protected Trinucleoside Diphosphates* **14**–**17**. Dimer diester **9** [20] (1 mmol) and the appropriate acyclic nucleoside analog **10**–**13** [25] [26] (0.75 mmol) were coevaporated with dry pyridine (5 × 10 ml), and finally dissolved in pyridine (10 ml). Then 0.46 ml (6 mmol) of 1-methyl-1*H*-imidazole and 0.606 g (2 mmol) of

2,4,6-triisopropylbenzenesulfonyl chloride were added successively. The mixture was stirred at r.t. for 20 h, then diluted with $CHCl_3$ (100 ml), and washed with H_2O (2 × 50 ml). The org. phase was dried and evaporated, and after coevaporation with toluene (3 × 20 ml), the residue was purified by CC (silica gel, 10 × 2.5 cm, $CHCl_3$/MeOH 100:1): **14–17** as colorless foams in 85–90% yield.

3′-Deoxy-5′-O-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-deoxy-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(nitrophenyl)ethyl]}→2′}-N^6-benzoyl-9-(2′-hydroxyethyl)adenine (**14**): R_f (silica gel, $CHCl_3$/MeOH 95:5) 0.20. UV (MeOH): 268 (4.95). ^{1}H-NMR ($CDCl_3$): 9.43 (*m*, 1 H, NH); 9.00 (*m*, 2 H, NH); 8.12–8.48 (*m*, 3 H, H–C(8)); 7.92–8.13 (*m*, 13 H, H–C(2), H_o to NO_2, H_o to CO); 7.12–7.52 (*m*, 11 H, H_m to NO_2, C_6H_5CO); 6.71 (*d*, 2 H, H_o to OMe); 6.14, 5.91 (2*d*, 2 H, H–C(1′)); 4.30 (*m*, 14 H, CH_2, H–C(4′)); 3.72 (*s*, 3 H, MeO); 3.00 (*m*, 10 H, CH_2); 2.46 (*m*, 2 H, CH_2). Anal. calc. for $C_{88}H_{81}N_{19}O_{25}P_2$ (1866.7): C 56.62, H 4.37, N 14.25; found: C 56.82, H 4.47, N 13.55.

3′-Deoxy-5′-O-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-deoxy-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl}→3′}-N^6-benzoyl-9-(3′-hydroxypropyl)adenine (**15**): R_f (silica gel, $CHCl_3$/MeOH 95:5) 0.21. UV (MeOH): 268 (4.94). ^{1}H-NMR ($CDCl_3$): 9.41 (*m*, 1 H, NH); 8.82 (*m*, 2 H, NH); 8.52–8.77 (*m*, 3 H, H–C(8)); 7.92–8.13 (*m*, 13 H, H–C(8), H_o to NO_2, H_o to CO); 7.11–7.50 (*m*, 11 H, H_m to NO_2, C_6H_5CO); 6.74 (*d*, 2 H, H_o to OMe); 6.15, 6.07 (2*m*, 2 H, H–C(1′)); 4.01–4.54 (*m*, 14 H, CH_2, H–C(4′)); 3.71 (*s*, 3 H, MeO); 3.00 (*m*, 8 H, CH_2); 2.44 (*m*, 2 H, CH_2). Anal. calc. for $C_{89}H_{83}N_{19}O_{25}P_2$ (1880.7): C 56.89, H 4.44, N 14.15; found: C 56.59, H 4.60, N 13.96.

3′-Deoxy-5′-O-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→5′}-3′-deoxy-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]}→4′}-N^6-benzoyl-9-(4′-hydroxybutyl)adenine (**16**): R_f (silica gel, $CHCl_3$/MeOH 95:5) 0.25. UV (MeOH): 268 (4.94). ^{1}H-NMR ($CDCl_3$): 9.33 (*m*, 1 H, NH); 8.66 (*m*, 2 H, NH); 8.49–8.56 (*m*, 3 H, H–C(8)); 7.94–8.13 (*m*, 13 H, H–C(2), H_o to NO_2, H_o to CO); 7.12–7.53 (*m*, 11 H, H_m to NO_2, C_6H_5CO); 6.75 (*d*, 2 H, H_o to OMe); 6.15 (*m*, 1 H, H–C(1′)); 6.05 (*d*, 1 H, H–C(1′)); 4.46 (*m*, 16 H, CH_2, H–C(4′)); 3.72 (*s*, 3 H, MeO); 3.06 (*m*, 8 H, CH_2); 1.91 (*m*, 2 H, CH_2); 1.64 (*m*, 2 H, CH_2). Anal. calc. for $C_{89}H_{83}N_{19}O_{26}P_2$ (1896.7): C 56.35, H 4.44, N 14.03; found: C 56.35, H 4.52, N 14.52.

3′-Deoxy-5′-O-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′{O^P[2-(4-nitrophenyl)ethyl]}→5′}-3′-deoxy-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2′-{-O^P-[2-(4-nitrophenyl)ethyl]}→2″}-N^6-benzoyl-9-[(2″-hydroxyethoxy)methyl]adenine (**17**): R_f (silica gel, $CHCl_3$/MeOH 95:5) 0.22. UV (MeOH): 268 (4.93). ^{1}H-NMR ($CDCl_3$): 9.06 (*m*, 1 H, NH); 8.49–8.72 (*m*, 3 H, NH, H–C(8)); 8.00–8.19 (*m*, 15 H, H–C(2), H–C(8), H_o to NO_2, H_o to CO); 7.01–7.65 (*m*, 11 H, H_m to NO_2, C_6H_5CO); 6.80 (*d*, 2 H, H_o to OMe); 6.16 (*m*, 1 H, H–C(1′)); 6.02 (*m*, 1 H, H–C(1′)); 5.64 (*m*, 2 H, CH_2); 4.00–4.53 (3*m*, 10 H, CH_2); 3.75 (*s*, 3 H, MeO); 3.11 (*m*, 10 H, CH_2). Anal. calc. for $C_{90}H_{85}N_{19}O_{25}P_2$ (1894.7): C 57.05, H 4.52, N 14.04; found: C 57.41, H 3.98, N 13.80.

2. *3′-Deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→ω)-9-(ω-hydroxyalkyl)adenines* **18–21**. To a soln. of protected trimer **15–18** (0.028 g; 15 μmol) was added 0.5M DBU in dry pryridine (5 ml). The mixture was stirred at r.t. for 20 h, neutralized with 1M AcOH in pyridine (2.5 ml), and evaporated. The residue was stirred with conc. NH_3 (15 ml) and after stirring for 48 h, the solvent was removed *in vacuo* and the residue detritylated by treatment with 80% AcOH/H_2O (4 ml) at r.t. for 24 h. After evaporation, the residue was coevaporated several times with H_2O, taken up in H_2O and applied onto a *DEAE A-25 Sephadex* column (60 × 1 cm) and eluted with a linear gradient 0.001–0.3M (Et_3NH) HCO_3 (TBK) buffer (pH 7.5). The product fractions were evaporated and coevaporated several times with H_2O and further purified by paper chromatography (i-PrOH/conc. NH_3/H_2O 6:1:3). The product band was cut out and eluted with H_2O and lyophilized to give *3′-deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→2′)-9-(2′-hydroxyethyl)adenine* (diammonium salt; **18**), *3′-deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→3′)-9-(3′-hydroxypropyl)adenine* (diammonium salt; **19**), *3′-deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→4′)-9-(4′-hydroxybutyl)adenine* (diammonium salt; **20**), and *3′-deoxyadenylyl-(2′→5′)-3′-deoxyadenylyl-(2′→2″)-9-[(2″-hydroxyethoxy)methyl]adenine* (diammonium salt; **21**), resp., as colorless powders in 75–85% yield. *Data:* see the *Table*.

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