

83. Nucleotides

Part XXXV¹⁾

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

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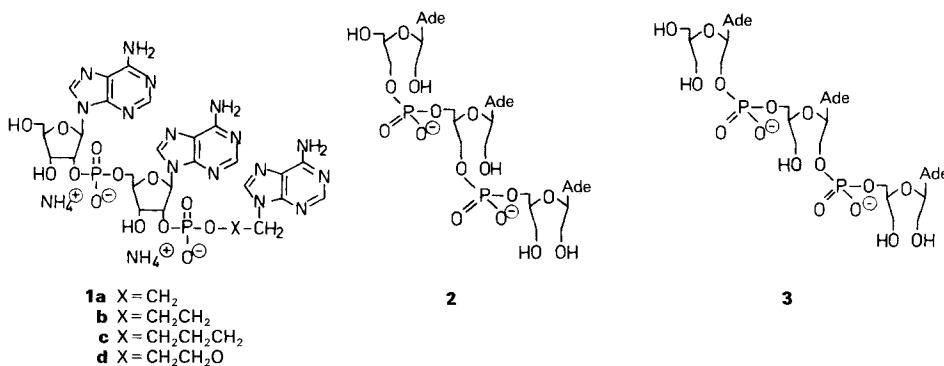
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*Via the phosphotriester approach, new structural analogs of (2'-5')oligoadenylates, namely 3'-deoxyadenylyl-(2'-5')-3'-deoxyadenylyl-(2'- ω)-9-(ω -hydroxyalkyl)adenines **18–21**, have been synthesized (see *Scheme*) which should preserve biological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

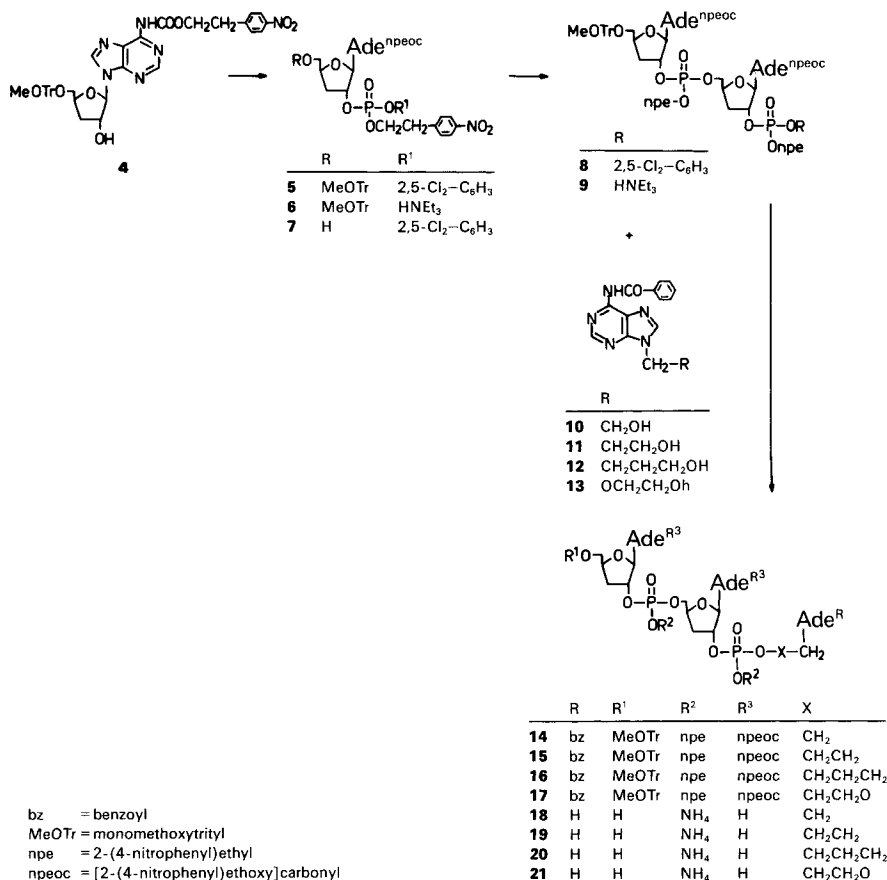


¹⁾ Part XXXIV: [1].

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



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 ¹H-NMR spectra. All trimers were deblocked sequentially, using 0.5*M* 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, NH₃ 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 ¹H-NMR spectra (*Table*).

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

	¹ H-NMR Spectra (D ₂ O, δ [ppm])			TLC (cellulose, i-PrOH/conc. NH ₃ /H ₂ O 6:1:3) <i>R</i> _F	HPLC (RP-18 column; <i>A</i> , 50 mM (NH ₄)H ₂ PO ₄ , pH 7; <i>B</i> , MeOH/H ₂ O 1:1; <i>A/B</i> 7:3) <i>t</i> _R	UV spectra (H ₂ O) <i>λ</i> _{max} [nm]
	H–C(8) +	H–C(2)	H–C(1')			
18	8.00 (<i>s</i>) 7.83 (<i>s</i>)	7.97 (3 H, <i>s</i>) 7.80 (<i>s</i>)	6.04 (<i>s</i>) 5.65 (<i>d</i>)	0.45	2.12 min	257
19	8.08 (<i>s</i>) 7.87 (<i>s</i>)	8.01 (<i>s</i>) 7.81 (<i>s</i>)	7.92 (<i>s</i>) 7.80 (<i>s</i>) 5.79 (<i>d</i>)	0.44	2.89 min	257
20	8.06 (<i>s</i>) 7.85 (<i>s</i>)	8.00 (<i>s</i>) 7.82 (<i>s</i>)	7.95 (<i>s</i>) 7.74 (<i>s</i>) 5.82 (<i>d</i>)	0.41	3.68 min	257
21	8.04 (<i>s</i>) 7.98 (<i>s</i>)	8.02 (<i>s</i>) 7.83 (<i>s</i>)	8.01 (<i>s</i>) 6.09 (<i>s</i>) 5.81 (<i>d</i>)	0.39	2.63 min	257

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. 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*). ¹H-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, $\text{CHCl}_3/\text{MeOH}$ 100:1): **14–17** as colorless foams in 85–90% yield.

3'-Deoxy-5'-O-(monomethoxytrityl)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-[O^p-[2-(4-nitrophenyl)ethyl]}→5'}-3'-deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-[O^p-[2-(4-nitrophenyl)ethyl]}→2'}-N⁶-benzoyl-9-(2'-hydroxyethyl)adenine (14**):** R_f (silica gel, $\text{CHCl}_3/\text{MeOH}$ 95:5) 0.20. UV (MeOH): 268 (4.95). ¹H-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 NO₂, H_o to CO); 7.12–7.52 (m, 11 H, H_m to NO₂, C₆H₅CO); 6.71 (d, 2 H, H_o to OMe); 6.14, 5.91 (2d, 2 H, H-C(1')); 4.30 (m, 14 H, CH₂, H-C(4')); 3.72 (s, 3 H, MeO); 3.00 (m, 10 H, CH₂); 2.46 (m, 2 H, CH₂). Anal. calc. for C₈₈H₈₁N₁₉O₂₅P₂ (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)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-[O^p-[2-(4-nitrophenyl)ethyl]}→5'}-3'-deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-[O^p-[2-(4-nitrophenyl)ethyl]}→3'}-N⁶-benzoyl-9-(3'-hydroxypropyl)adenine (15**):** R_f (silica gel, $\text{CHCl}_3/\text{MeOH}$ 95:5) 0.21. UV (MeOH): 268 (4.94). ¹H-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 NO₂, H_o to CO); 7.11–7.50 (m, 11 H, H_m to NO₂, C₆H₅CO); 6.74 (d, 2 H, H_o to OMe); 6.15, 6.07 (2m, 2 H, H-C(1')); 4.01–4.54 (m, 14 H, CH₂, H-C(4')); 3.71 (s, 3 H, MeO); 3.00 (m, 8 H, CH₂); 2.44 (m, 2 H, CH₂). Anal. calc. for C₈₉H₈₃N₁₉O₂₅P₂ (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)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-[O^p-[2-(4-nitrophenyl)ethyl]}→5'}-3'-deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-[O^p-[2-(4-nitrophenyl)ethyl]}→4'}-N⁶-benzoyl-9-(4'-hydroxybutyl)adenine (16**):** R_f (silica gel, $\text{CHCl}_3/\text{MeOH}$ 95:5) 0.25. UV (MeOH): 268 (4.94). ¹H-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 NO₂, H_o to CO); 7.12–7.53 (m, 11 H, H_m to NO₂, C₆H₅CO); 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, CH₂, H-C(4')); 3.72 (s, 3 H, MeO); 3.06 (m, 8 H, CH₂); 1.91 (m, 2 H, CH₂); 1.64 (m, 2 H, CH₂). Anal. calc. for C₈₉H₈₃N₁₉O₂₆P₂ (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)-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-[O^p-[2-(4-nitrophenyl)ethyl]}→5'}-3'-deoxy-N⁶-[2-(4-nitrophenyl)ethoxycarbonyl]adenylyl-{2'-[O^p-[2-(4-nitrophenyl)ethyl]}→2'}-N⁶-benzoyl-9-[(2'-hydroxyethoxy)methyl]adenine (17**):** R_f (silica gel, $\text{CHCl}_3/\text{MeOH}$ 95:5) 0.22. UV (MeOH): 268 (4.93). ¹H-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 NO₂, H_o to CO); 7.01–7.65 (m, 11 H, H_m to NO₂, C₆H₅CO); 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, CH₂); 4.00–4.53 (3m, 10 H, CH₂); 3.75 (s, 3 H, MeO); 3.11 (m, 10 H, CH₂). Anal. calc. for C₉₀H₈₅N₁₉O₂₅P₂ (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 pyridine (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. NH₃ (15 ml) and after stirring for 48 h, the solvent was removed *in vacuo* and the residue detritylated by treatment with 80% AcOH/H₂O (4 ml) at r.t. for 24 h. After evaporation, the residue was coevaporated several times with H₂O, taken up in H₂O and applied onto a *DEAE A-25 Sephadex* column (60 × 1 cm) and eluted with a linear gradient 0.001–0.3M (Et₃NH) HCO₃ (TBK) buffer (pH 7.5). The product fractions were evaporated and coevaporated several times with H₂O and further purified by paper chromatography (i-PrOH/conc. NH₃/H₂O 6:1:3). The product band was cut out and eluted with H₂O 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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