188. Nucleotides

Part XXV[1])

Chemical Synthesis of the Hexaribonucleotide C-A-A-C-C-A

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The chemical synthesis of the ribo-hexamer C-A-A-C-C-A has been achieved by two different routes applying the phosphotriester approach and using the (*p*-nitrophenyl)ethyl group for phosphate protection. The 2'-hydroxy groups as well as the terminal 2',3'-diol function have been blocked by the (*tert*-butyl)dimethylsilyl group as permanent protection throughout all the synthetic steps with great success. Final deblocking of all protective groups could be performed with 80% yield to give the hexamer **23**. The various protected intermediates have been characterized by elemental analysis and UV spectra.

1. Introduction. – In recent years, the chemical synthesis of oligodeoxyribonucleotides has been improved tremendously due to the advantages of the phosphotriester [2] [3] and the phosphoramidite approach [4] over the older phosphodiester method. Analogous good progress could not be achieved in the ribonucleotide area largely because of the difficulties of finding suitable procedures for protection of the additional 2'-hydroxy group. The problem of protection of the various functionalities is the crucial point in this series and has been modified very widely [5–7]. Many efforts have been made, especially towards the synthesis of partial structures of tRNA's using a single addition procedure [8] [9] as well as a block-assembly phosphotriester methodology [10–17].

In order to test the (*p*-nitrophenyl)ethyl-phosphate protection [18] in an oligoribonucleotide synthesis, we have chosen the hexaribonucleotide sequence C-A-A-C-C-A corresponding to the 3'-terminus of a series of methionine-initiator tRNA's [19] as a model. The strategy accounts for the phosphotriester approach applying two series of block condensations [20] in comparison to the stepwise chain elongation of the corresponding heptaribonucleotide G-C-A-A-C-C-A [9]. The permanent OH protection of the sugar moieties was performed by the (*tert*-butyl)dimethylsilyl group due to its striking chemical features [21] [22] and high stability.

2. Syntheses. – The anticipated syntheses afforded three starting materials, the N^4-benzoyl-2'-*O*-[(*tert*-butyl)dimethylsilyl]-5'-*O*-(monomethoxytrityl)cytidine 3'-[2,5-dichlorophenyl 2-(*p*-nitrophenyl)ethyl phosphate] (**1**), the N^6-benzoyl-2'-*O*[(*tert*-butyl)-dimethylsilyl]-5'-*O*-(monomethoxytrityl)adenosine 3'-[2,5-dichlorophenyl 2-(*p*-nitrophenyl)ethyl phosphate] (**2**), and the N^6-benzoyl-2',3'-di-*O*-[(*tert*-butyl)dimethyl-

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silyl]adenosine (**3**), which have been described already earlier [22] [23]. The oximate cleavage [24] of **1** and **2** led to the phosphodiesters **4** and **5**, respectively, which were isolated as their triethylammonium salts, whereas detritylation of **1** and **2** afforded the 5'-OH components **6** and **7**, respectively [23]. The various condensations to the fully protected dimers **8**, **10**, **12**, and **14** were achieved by 2,4,6-triisopropylbenzenesulfonyl nitrotriazolide (TPSNT) as well as by *p*-toluenesulfonyl chloride/*N*-methylimidazole in abs. pyridine with isolated yields ranging from 67–91%. These dimer blocks function then as intermediates in the chain-elongation process to the hexamer **22**, which was performed by two different sequences of reactions according to the schemes 2 + 1→3, 3 + 3→6 units and 2 + 2→4, 4 + 2→6 units, respectively.

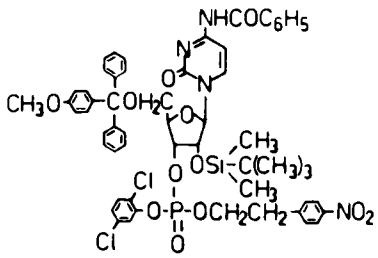
The first route included oximate treatment of **8** and **10** with *p*-nitrobenzaldoxime in Et_3N/H_2O/dioxane to convert the terminal phosphotriester group into the corresponding phosphodiester (→**9**, **11**). Then, **9** was condensed with **7** to give the trimer **16** and **10** with **3** to form the trimer **18** in 75 and 72% yield. The second approach afforded again oximate cleavage of **12** to the triethylammonium phosphodiester **13** and detritylation of **14** to the terminal dimer **15** by use of the $ZnBr_2$ procedure [25]. The 2 + 2 block condensation of the two components **13** and **15** was achieved under similar reaction conditions using two different condensing agents and yielded 65 and 75%, respectively, of **20**.

The final steps of the two synthetic pathways consisted of analogous procedures for the partial deblocking of the appropriate protecting groups forming **17** from **16**, **19** from **18**, and **21** from **20** followed by the condensations of the two trimers **17** and **19** and of the dimer **9** with tetramer **21** in the usual manner using TPSNT for the coupling reaction to form the fully protected hexamer **22** in 62 and 69% yield.

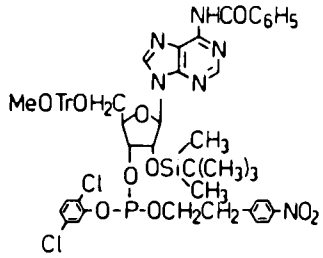
The deprotection of **22** to the free hexaribonucleotide C-A-A-C-C-A (**23**) has been achieved by subsequent treatment by 1) 0.5M 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) for 15 h to eliminate the (*p*-nitrophenyl)ethyl groups, 2) 0.25M Bu_4NF for 20 h to cleave the (*tert*-butyl)dimethylsilyl groups, 3) conc. NH_3 for 24 h to remove the *N*-benzoyl groups, and 4) 80% AcOH for another 24 h to split off finally the monomethoxytrityl residue. The crude reaction product was then purified by *DEAE-Sephadex* chromatography in $Et_3NH^+HCO_3^-$ buffer (pH 7) applying a linear gradient and giving 71% yield of the hexamer **23**. Spleen phosphodiesterase affected further digestion to give Cp, Ap and A in a 3:2:1 ratio after 16 h incubation at 37°.

3. Physical Data. – The characterization of nucleotides and oligonucleotides is still a difficult problem due to the limitations of the analytical methods, from which usually more qualitative than quantitative measurements can be derived. The protected intermediates **1**–**22** were purified by silica-gel chromatography and then applied to C,H,N-elementary analysis to get the correct compositions. The molar extinction coefficients of the UV spectra account then as a characteristic feature of every compound in a quantitative sense *(Table)*.

The ^{1}H-NMR spectra (high resolution) are very complicated and not too conclusive regarding the structures. Too many overlapping signals and the fact that the protected phosphotriesters always consist of diastereoisomeric mixtures limits the NMR method significantly for structural proofs of such types of complex molecules. Some characteristic signals like the one of CH_3O of the monomethoxytrityl group help in the isolation procedures to find and detect the right reaction product.



1



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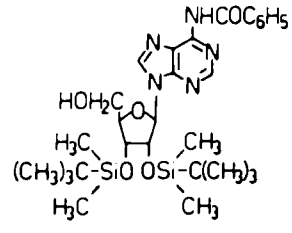

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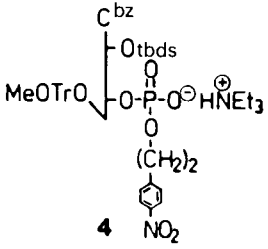

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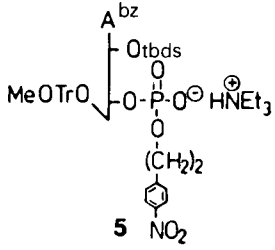

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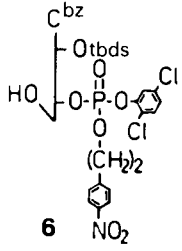

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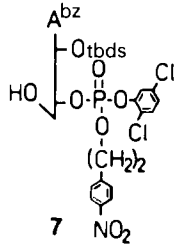

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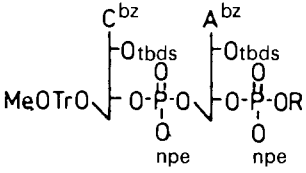
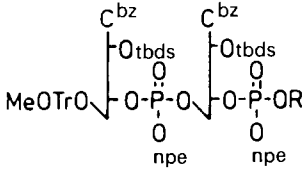
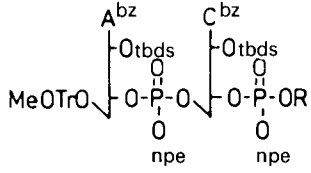


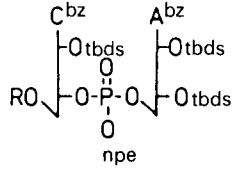
	R
8	2,5-Di-Cl-C_6H_4
9	$HNEt_3$



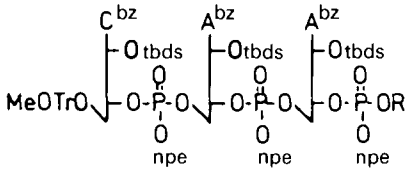
	R
10	2,5-Di-Cl-C_6H_4
11	$HNEt_3$



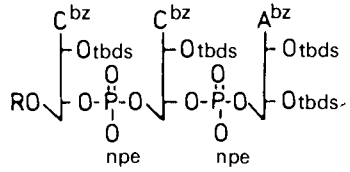
	R
12	2,5-Di-Cl-C_6H_4
13	$HNEt_3$



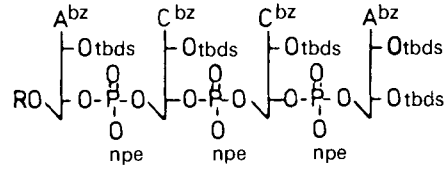
	R
14	MeOTr
15	H



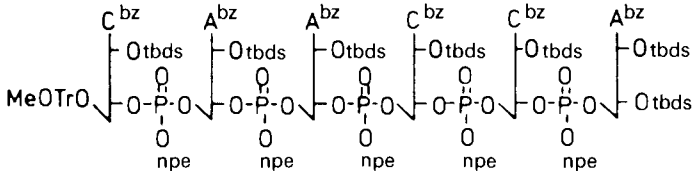
	R
16	2,5-Di-Cl-C_6H_4
17	$HNEt_3$



	R
18	MeOTr
19	H



	R
20	MeOTr
21	H



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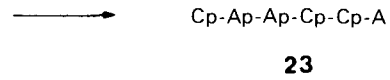

23

MeOTr = monomethoxytrityl bz = benzoyl tbds = (*tert*–butyl)dimethylsilyl npe = 2-(*p*-nitrophenyl)ethyl

Table. *UV Absorption Spectra of Nucleotides and Oligoribonucleotides in MeOH*

	λ_{max} [nm] (lg ε)		λ_{max} [nm] (lg ε)
1	230 (sh, 4.54), 263 (4.51), 304 (4.09)	**12**	230 (sh, 4.75), 268 (4.78), 315 (sh, 4.03)
2	227 (sh, 4.61), 260 (sh, 3.68), 278 (4.39)	**13**	230 (sh, 4.63), 268 (4.71), 315 (sh, 3.99)
3	227 (4.18), 280 (4.36)	**14**	230 (4.66), 270 (4.62), 315 (sh, 4.03)
4	232 (sh, 4.44), 262 (4.51), 300 (sh, 4.14)	**15**	230 (sh, 4.48), 268 (4.62), 315 (sh, 3.87)
5	278 (4.51)	**16**	228 (sh, 4.92), 270 (4.94), 315 (sh, 4.05)
6	230 (sh, 4.32), 262 (4.54), 300 (sh, 4.10)	**17**	230 (sh, 4.79), 270 (4.86), 315 (sh, 4.05)
7	278 (4.50)	**18**	230 (4.80), 262 (4.81), 317 (sh, 4.09)
8	228 (sh, 4.74), 268 (4.73), 315 (sh, 4.09)	**19**	230 (sh, 4.67), 262 (4.71), 317 (sh, 3.99)
9	230 (sh, 4.67), 265 (4.71), 315 (sh, 4.02)	**20**	232 (sh, 4.89), 267 (4.88), 315 (sh, 4.18)
10	230 (sh, 4.68), 262 (4.81), 300 (sh, 4.36)	**21**	233 (sh, 4.84), 267 (4.95), 315 (sh, 4.20)
11	230 (4.55), 260 (4.73), 300 (4.35)	**22**	235 (5.00), 266 (5.16), 315 (sh, 4.50)

4. Conclusion. – Solution chemistry to oligoribonucleotides can be achieved in a preparative scale applying the phosphotriester approach in a stepwise manner or by a block-condensation procedure. The appropriate choice of the various protecting groups account to a large extent for the obtained yields in the synthetic as well as the deblocking steps.

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 on silica gel *60 PF_{254} (Merck)* and prep. column chromatography on silica gel (*Merck 60*, 0.063–0.2 mesh). M.p.: *Büchi* apparatus, model Dr. *Tottoli;* no corrections. UV/VIS: *Cary Recording* spectrometer, model *118, Applied Phys. Corp.*, and *Uvikon 820, Kontron;* λ_{max} in nm (lg ε).

1. N^6-*Benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]adenosine 3'-[2,5-Dichlorophenyl 2-(*p-*Nitrophenyl)ethyl Phosphate]* (**7**) [22]. For 2 h, 2.19 g (4.4 mmol) of $ZnBr_2$ are dried in high vacuum at 140°. The salt is suspended in 25 ml of abs. nitromethane, 0.78 g (0.69 mmol) of **2** added, and the mixture stirred for 5 min at r.t. The mixture is treated twice with 50 ml of ice/H_2O each and the org. layer dried over Na_2SO_4 and evaporated. The residue (0.73 g) is dissolved in 5 ml of CH_2Cl_2, put on a silica-gel column (7 × 3 cm) and chromatographed subsequently with 200 ml of CH_2Cl_2, 200 ml of CH_2Cl_2/MeOH 100:1, and 100 ml of CH_2Cl_2/MeOH 97:3. The main fraction yields 0.5 g (85%) of **7** as amorphous solid. Reprecipitation from 3 ml of $CHCl_3$ into 200 ml of hexane with stirring gives an amorphous colourless powder. UV (MeOH): 278 (4.50).

2. N^4-*Benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]-5'*-O-*(monomethoxytrityl)cytidylyl-{3'-[*O^P-*(2-(*p-*nitrophenyl)ethyl)]→5'}-*N^6-*benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]adenosine 3'-[2,5-Dichlorophenyl 2-(*p-*Nitrophenyl)ethyl Phosphate]* (**8**). Twice, 0.515 g (0.48 mmol) of triethylammonium N^4-benzoyl-2'-*O*-[(*tert*-butyl)dimethylsilyl]-5'-*O*-(monomethoxytrityl)cytidine 3'-[2-(*p*-nitrophenyl)ethyl phosphate] (**4**) and 0.344 g (0.4 mmol) of **7** are coevaporated with 2 ml of abs. pyridine and then dissolved in the same amount of abs. pyridine. After addition of 0.17 g of 2,4,6-triisopropylbenzenesulfonyl nitrotriazolide (TPSNT), the mixture is stirred for 6 h at r.t. and then kept over night in the ice box. The mixture is then diluted with 100 ml of $CHCl_3$ and treated once with 100 ml of phosphate buffer (pH 7) and 3 times with 100 ml of H_2O. The org. phase is dried over Na_2SO_4, filtered, and evaporated to give a foam. The residue is dissolved in 2 ml of CH_2Cl_2 and chromatographed on a silica-gel column (5 × 1 cm) with 600 ml of $CHCl_3$/MeOH 98:2. The main fraction is evaporated and the solid further purified by another column chromatography with $CHCl_3$/MeOH 99.5:0.5. Evaporation of the main fraction yields 0.24 g (59%) of **8a** as a colourless amorphous solid. Anal. calc. for $C_{87}H_{94}Cl_2N_{10}O_{21}P_2Si_2$ (1804.8): C 57.90, H 5.25, N 7.76; found: C 57.88, H 5.30, N 7.67.

3. N^4-*Benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]-5'*-O-*(monomethoxytrityl)cytidylyl-{3'-[*O^P-*(2-(*p-*nitrophenyl)ethyl)]→5'}-*N^6-*benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]adenosine 3'-[2-(*p-*Nitrophenyl)ethyl Triethylammonium Phosphate]* (**9**). A soln. of 0.67 g (4 mmol) of *p*-nitrobenzaldoxime in 24 ml of Et_3N/dioxane/H_2O 1:1:1 is

stirred at r.t. for 20 min. Then, 0.722 g (0.4 mmol) of **8** are added, and stirring is continued for 2 h. The soln. is evaporated after addition of 30 ml of abs. pyridine. The residue is coevaporated twice with abs. pyridine and twice with toluene, dissolved in $CHCl_3$, and chromatographed on a silica-gel column (5 × 2.5 cm) with 200 ml of $CHCl_3$, 200 ml of $CHCl_3$/MeOH 98:2, and a gradient of $CHCl_3$/MeOH/Et_3N 90:5:5→70:20:10. Evaporation of the main fraction yields a solid residue which is dissolved in 6 ml of $CHCl_3$ and added dropwise to a stirred soln. of 50 ml of hexane/Et_2O 4:1 with separation of an amorphous powder. The precipitate is collected by suction and then dried in vacuum at 50° to yield 0.643 g (91%) of a colourless powder. Anal. calc. for $C_{87}H_{107}N_{11}O_{21}P_2Si_2 \cdot H_2O$ (1779.0): C 58.74, H 6.18, N 8.66; found: C 58.74, H 5.75, N 8.56.

4. N^4-*Benzoyl-2'*-O-*[(*tert*-butyl)dimethylsilyl]-5'*-O-*(monomethoxytrityl)cytidylyl-{3'-[*O^P-*(2-(*p*-nitrophenyl)ethyl)]→5'}*-N^4-*benzoyl-2'*-O-*[(*tert*-butyl)dimethylsilyl]cytidine 3'-[2,5-Dichlorophenyl 2-(*p*-Nitrophenyl)-ethyl Phosphate]* (**10**). At 40°, 0.383 g (0.36 mmol) of **4** [22] and 0.251 g (0.3 mmol) of N^4-benzoyl-2'-*O*-[(*tert*-butyl)dimethylsilyl]cytidine 3'-[2,5-dichlorophenyl 2-(*p*-nitrophenyl)ethyl phosphate] (**6**) [22] are evaporated twice with 2 ml of abs. pyridine. The mixture is dissolved in 2 ml of abs. pyridine, 0.25 g of TPSNT are added and stirred at r.t. for 6 h. After storage over night in the ice box, the mixture is diluted with 100 ml of $CHCl_3$ and then washed once with 100 ml of phosphate buffer (pH 7) and 3 times with H_2O. The org. layer is dried over Na_2SO_4, filtered, and evaporated to a solid foam. The material is dissolved in little $CHCl_3$, put on a silica-gel column (5 × 1 cm) and then developed with 500 ml of $CHCl_3$/MeOH 98:2. The main fraction is evaporated: 0.486 g (91%) of **10** as a colourless amorphous solid. Anal. calc. for $C_{86}H_{94}Cl_2N_8O_{22}P_2Si_2$ (1780.8): C 58.00, H 5.32, N 6.29; found: C 58.00, H 5.45, N 6.34.

5. N^4-*Benzoyl-2'*-O-*[(*tert*-butyl)dimethylsilyl]-5'*-O-*(monomethoxytrityl)cytidylyl-{3'-[*O^P-*(2-(*p*-nitrophenyl)ethyl)]→5'}*-N^4-*benzoyl-2'*-O-*[(*tert*-butyl)dimethylsilyl]cytidine 3'-[2-(*p*-Nitrophenyl)ethyl Triethylammonium Phosphate]* (**11**). To a mixture of 1.5 g (10 mmol) of *p*-nitrobenzaldoxime in 33 ml of Et_3N/H_2O/dioxane 1:1:1 is added 1.0 g (0.56 mmol) of **10**. The mixture is stirred for 2.5 h at r.t., then evaporated, and twice coevaporated with 5 ml of abs. pyridine as well as 10 ml of toluene. The residue is dissolved in $CHCl_3$ and chromatographed on a silica-gel column (60 × 3 cm) with 800 ml of $CHCl_3$, 300 ml of $CHCl_3$/MeOH 98:2, and 500 ml of $CHCl_3$/MeOH/Et_3N 98:2:0.5. The product fraction yields 0.85 g (85%) of **11** as a colourless amorphous solid. Anal. calc. for $C_{86}H_{106}N_6O_{22}P_2Si_2$ (1736.0): C 59.50, H 6.15, N 7.26; found: C 59.22, H 6.09, N 6.99.

6. N^6-*Benzoyl-2'*-O-*[(*tert*-butyl)dimethylsilyl]-5'*-O-*(monomethoxytrityl)adenylyl-{3'-[*O^P-*(2-(*p*-nitrophenyl)ethyl)]→5'}*-N^4-*benzoyl-2'*-O-*[(*tert*-butyl)dimethylsilyl]cytidine 3'-[2,5-Dichlorophenyl 2-(*p*-Nitrophenyl)-ethyl Phosphate]* (**12**). 6.1. Three times, 0.55 g (0.5 mmol) of **5** and 0.35 g (0.42 mmol) of **6** are coevaporated with 3 ml of abs. pyridine. The residue is dissolved in 4 ml of abs. pyridine, and 0.32 g of TPSNT are added and stirred over night at r.t. The mixture is extracted twice with 25 ml of $CHCl_3$, washed twice with 25 ml of phosphate buffer (pH 7) and 50 ml of cold H_2O. The org. phase is dried over Na_2SO_4, evaporated, and coevaporated twice with 10 ml of toluene. The residue is dissolved in 5 ml of $CHCl_3$ and chromatographed on a silica-gel column (30 × 3 cm) with 1.2 l of $CHCl_3$. The main fraction yields 0.50 g (67%) of **12** as a colourless amorphous solid.

6.2. Three times, 0.55 g (0.5 mmol) of **5** and 0.35 g (0.42 mmol) of **6** are coevaporated with 3 ml of abs. pyridine. The mixture is then dissolved in 4 ml of abs. pyridine, 0.31 g (1 mmol) of 2,4,6-triisopropylbenzenesulfonyl chloride and 0.25 ml (3 mmol) of *N*-methylimidazole are added and then stirred for 20 h at r.t. Workup and purification as in *6.1*. Anal. calc. for $C_{87}H_{94}Cl_2N_{10}O_{21}P_2Si_2 \cdot H_2O$ (1822.8): C 57.32, H 5.28, N 7.64; found: C 57.19, H 5.27, N 7.45.

7. N^6-*Benzoyl-2'*-O-*[(*tert*-butyl)dimethylsilyl]-5'*-O-*(monomethoxytrityl)adenylyl-{3'-[*O^P-*(2-(*p*-nitrophenyl)ethyl)]→5'}*-N^4-*benzoyl-2'*-O-*[(*tert*-butyl)dimethylsilyl]cytidine 3'-[2-(*p*-Nitrophenyl)ethyl Triethylammonium Phosphate]* (**13**). A soln. of 0.584 g (3.5 mmol) of *p*-nitrobenzaldoxime in 15 ml of H_2O/dioxane/Et_3N 1:1:1 is stirred for 30 min at r.t. Then 0.631 g (0.35 mmol) of **12** is added and the mixture stirred for 30 min at r.t. The mixture is evaporated to dryness, then twice coevaporated with 5 ml of pyridine and twice with 10 ml of toluene. The residue is dissolved in 100 ml of $CHCl_3$ and chromatographed on a silica-gel column (15 × 3 cm) with 500 ml of $CHCl_3$, 500 ml of $CHCl_3$/MeOH 98:2 and 800 ml of $CHCl_3$/MeOH/Et_3N 97:3:0.5. The main fraction is collected and evaporated to yield 0.58 g (92%) of **13** as an amorphous solid. Anal. calc. for $C_{87}H_{107}N_{11}O_{21}P_2Si_2 \cdot 3\ H_2O$ (1815.0): C 57.57, H 6.28, N 8.29; found: C 57.46, H 5.91, N 7.96.

8. N^4-*Benzoyl-2'*-O-*[(*tert*-butyl)dimethylsilyl]-5'*-O-*(monomethoxytrityl)cytidylyl-{3'-[*O^P-*(2-(*p*-nitrophenyl)ethyl)]→5'}*-N^6-*benzoyl-2',3'-bis-*O-*[(*tert*-butyl)dimethylsilyl]adenosine* (**14**). A mixture of 0.257 g (0.24 mmol) of **4** [22] and 0.12 g (0.2 mmol) of N^6-benzoyl-2',3'-bis-*O*-[(*tert*-butyl)dimethylsilyl]adenosine (**3**) [23] is evaporated twice with 2 ml of pyridine at 40° and the residue dissolved in 2 ml of abs. pyridine. Then, 0.17 g of

TPSNT are added, and the mixture is stirred for 7 h at r.t. before keeping it over night in the ice box. The mixture is extracted twice with 100 ml of $CHCl_3$, the extract washed with 100 ml of phosphate buffer (pH 7) and 3 times with H_2O. The org. layer is dried over Na_2SO_4, filtered, and evaporated in vacuum to give a solid foam. The residue is dissolved in 5 ml of $CHCl_3$ and chromatographed on a silica-gel column (7.5 × 1 cm) with 600 ml of $CHCl_3$/MeOH 98:2. The residue of the main fraction is dissolved in $CHCl_3$ and rechromatographed on a silica-gel column (15 × 2 cm) with $CHCl_3$/MeOH 99.5:0.5. The main fraction yields 0.23 g (75%) of **14** as a colourless amorphous foam. Anal. calc. for $C_{79}H_{98}N_9O_{16}PSi_3$ (1545.0): C 61.41, H 6.39, N 8.15; found: C 61.58, H 6.13, N 8.15.

9. N^4-*Benzoyl-2'*-O-*[(* tert-*butyl)dimethylsilyl]cytidylyl-{3'-[*O^P-*(2-(* p-*nitrophenyl)ethyl)]→5'}*-N^6-*benzoyl-2',3'-bis*-O-*[(* tert-*butyl)dimethylsilyl]adenosine* (**15**). For 2 h, 1.1 g (4.4 mmol) of $ZnBr_2$ are dried at 140° under high vacuum. The salt is then suspended in 20 ml of dry nitromethane, 0.7 g (0.45 mmol) of **14** added, and the mixture stirred at r.t. for 10 min. The mixture is then treated twice with 20 ml of ice H_2O and the org. layer dried over Na_2SO_4 and evaporated. The residue is dissolved in CH_2Cl_2 and chromatographed on a silica-gel column (7 × 3 cm) with 200 ml of CH_2Cl_2, 250 ml of CH_2Cl_2/MeOH 100:1 and 200 ml of $CHCl_2$/MeOH 97:3. The main fraction yields 0.50 g (88%) of **15** as a colourless amorphous solid. Anal. calc. for $C_{58}H_{82}N_9O_{15}PSi_3$ (1260.6): C 54.49, H 6.62, N 9.86; found: C 54.39, H 6.68, N 9.95.

10. N^4-*Benzoyl-2'*-O-*[(* tert-*butyl)dimethylsilyl]-5'*-O-*(monomethoxytrityl)cytidylyl-{3'-[*O^P-*(2-(* p-*nitrophenyl)ethyl)]→5'}*-N^6-*benzoyl-2'*-O-*[(* tert-*butyl)dimethylsilyl]adenylyl-{3'-[*O^P-*(2-(* p-*nitrophenyl)ethyl)]→5'}*-N^6-*benzoyl-2'*-O-*[(* tert-*butyl)dimethylsilyl]adenosine 3'-[2,5-Dichlorophenyl 2-(* p-*Nitrophenyl)ethyl Phosphate]* (**16**). Twice, 1.1 g (0.63 mmol) of **9** and 0.36 g (0.42 mmol) of **6** [22] are coevaporated with 5 ml of abs. pyridine. The mixture is dissolved in 3 ml of abs. pyridine, then 0.52 g of TPSNT are added and stirred overnight at r.t. The mixture is treated with 50 ml of $CHCl_3$ and washed with 50 ml of phosphate buffer (pH 7) and 50 ml of ice/H_2O. The org. layer is dried over Na_2SO_4, evaporated to dryness, and then coevaporated twice with 10 ml of toluene. The residue is dissolved in 3 ml of CH_2Cl_2 and chromatographed on a silica-gel column (14 × 3 cm) with 500 ml of CH_2Cl_2, 200 ml of CH_2Cl_2/$CHCl_3$ 1:1, 500 ml of $CHCl_3$, and 500 ml of $CHCl_3$/MeOH 99:1. The main fraction yields 0.78 g (75%) of **16** as a colourless amorphous foam. Anal. calc. for $C_{118}H_{131}Cl_2N_{16}O_{30}P_3Si_3$ (2501.5): C 56.66, H 5.28, N 8.98; found: C 56.49, H 5.42, N 8.83.

11. N^4-*Benzoyl-2'*-O-*[(* tert-*butyl)dimethylsilyl]-5'*-O-*(monomethoxytrityl)cytidylyl-{3'-[*O^P-*(2-(* p-*nitrophenyl)ethyl)]→5'}*-N^6-*benzoyl-2'*-O-*[(* tert-*butyl)dimethylsilyl]adenylyl-{3'-[*O^P-*(2-(* p-*nitrophenyl)ethyl)]→5'}*-N^6-*benzoyl-2'*-O-*[(* tert-*butyl)dimethylsilyl]adenosine 3'-[2-(* p-*Nitrophenyl)ethyl Triethylammonium Phosphate]* (**17**). A soln. of 0.7 g (4.9 mmol) of *p*-nitrobenzaldoxime in 16 ml of H_2O/dioxane/Et_3N 1:1:1 is stirred for 20 min at r.t. Then 0.7 g (0.28 mmol) of **16** is added and stirred for 1.5 h at r.t. The mixture is evaporated, coevaporated twice with 3 ml of pyridine and twice with 10 ml of toluene, and the residue dissolved in 80 ml of $CHCl_3$. This soln. is chromatographed on a silica-gel column (15 × 3 cm) with 800 ml of $CHCl_3$, 200 ml of $CHCl_3$/MeOH 98:2 and 800 ml of $CHCl_3$/MeOH/Et_3N 98:2:0.5. The main fraction yielded 0.58 g (85%) of **17** as an amorphous colourless solid. Anal. calc. for $C_{118}H_{144}N_{17}O_{30}P_3Si_3 \cdot 2\ H_2O$ (2493.7): C 56.83, H 5.98, N 9.55; found: C 56.89, H 5.73, N 9.29.

12. N^4-*Benzoyl-2'*-O-*[(* tert-*butyl)dimethylsilyl]-5'*-O-*(monomethoxytrityl)cytidylyl-{3'-[*O^P-*(2-(* p-*nitrophenyl)ethyl)]→5'}*-N^4-*benzoyl-2'*-O-*[(* tert-*butyl)dimethylsilyl]cytidylyl-{3'-[*O^P-*(2-(* p-*nitrophenyl)ethyl)]→5'}*-N^6-*benzoyl-2',3'-bis*-O-*[(* tert-*butyl)dimethylsilyl]adenosine* (**18**). Three times, 1.1 g (0.63 mmol) of **11** and 0.24 g (0.4 mmol) of **3** [23] were coevaporated with 3 ml of abs. pyridine. The residue is dissolved in 2.5 ml of abs. pyridine, 0.46 g (1.2 mmol) of TPSNT are added, and the mixture is stirred over night at r.t. The mixture is then extracted twice with 25 ml of $CHCl_3$, washed with 50 ml of phosphate buffer (pH 7) and 50 ml of ice/H_2O. The org. layer is dried over Na_2SO_4, evaporated, and then coevaporated twice with 10 ml of toluene. The residue is dissolved in 5 ml of CH_2Cl_2 and chromatographed on a silica-gel column (13 × 3 cm) with 800 ml of CH_2Cl_2, 1000 ml of CH_2Cl_2/$CHCl_3$ 1:1, 400 ml of $CHCl_3$, and 500 ml of $CHCl_3$/MeOH 100:0.5. The main fraction yields 0.64 g (72%) of **18** as an amorphous colourless solid foam. Anal. calc. for $C_{109}H_{135}N_{13}O_{26}P_2Si_4$ (2217.7): C 59.04, H 6.14, N 8.21; found: C 58.77, H 6.12, N 7.56.

13. N^4-*Benzoyl-2'*-O-*[(* tert-*butyl)dimethylsilyl]cytidylyl-{3'-[*O^P-*(2-(* p-*nitrophenyl)ethyl)]→5'}*-N^4-*benzoyl-2'*-O-*[(* tert-*butyl)dimethylsilyl]cytidylyl-{3'-[*O^P-*(2-(* p-*nitrophenyl)ethyl)]→5'}*-N^6-*benzoyl-2',3'-bis*-O-*[(* tert-*butyl)dimethylsilyl]adenosine* (**19**). For 2 h, 0.5 g (1 mmol) of $ZnBr_2$ are dried at 140° under high vacuum. The salt is then suspended in 5 ml of abs. nitromethane, 0.4 g (0.18 mmol) of **18** are added and stirred for 1.5 h. The mixture is extracted twice with 5 ml of $CHCl_3$ and then washed with 25 ml of phosphate buffer (pH 7) and 50 ml of ice/H_2O. The org. layer is dried over Na_2SO_4 and evaporated and the residue dissolved in 3 ml of CH_2Cl_2 and chromatographed on a silica-gel column (10 × 3 cm) with 500 ml of CH_2Cl_2 and 800 ml of CH_2Cl_2/MeOH 98:2.

Evaporation of the main fraction yields 0.29 g (82%) of **19** as a colourless amorphous solid. Anal. calc. for $C_{89}H_{119}N_{13}O_{25}P_2Si_4$ (1945.3): C 54.90, H 6.17, N 9.36; found: C 54.71, H 6.12, N 9.11.

14. N^6-*Benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]-5'*-O-*(monomethoxytrityl)adenylyl-{3'-[*O^P-*(2-(*p-*nitrophenyl)ethyl)]→5'}*-N^4-*benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]cytidylyl-{3'-[*O^P-*(2-(*p-*nitrophenyl)ethyl)]→5'}*-N^4-*benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]cytidylyl-{3'-[*O^P-*(2-(*p-*nitrophenyl)ethyl)]→5'}*-N^6-*benzoyl-2',3'-bis-*O-*[(*tert-*butyl)dimethylsilyl]adenosine* (**20**). 14.1. Three times, 0.42 g (0.23 mmol) of **13** and 0.19 g (0.15 mmol) of **15** are coevaporated with 3 ml of abs. pyridine. The residue is dissolved in 2 ml of abs. pyridine, 0.18 g (0.46 mmol) of TPSNT are added and stirred over night at r.t. The mixture is extracted with 20 ml of $CHCl_3$, washed twice with 15 ml of phosphate buffer (pH 7) and once with 25 ml of ice/H_2O. The org. layer is dried over Na_2SO_4, filtered, evaporated, and coevaporated twice with 10 ml of toluene. The residue is dissolved in 3 ml of $CHCl_3$ and chromatographed on a silica-gel column (17 × 3 cm) with 600 ml of $CHCl_3$ and then with 1 l of $CHCl_3$/MeOH 100:1. The main fraction yields 0.28 g (65%) of **20** as a colourless amorphous solid.

14.2. Three times, 0.85 g (0.46 mmol) of **13** and 0.272 g (0.22 mmol) of **15** are coevaporated with 3 ml of abs. pyridine. The residue is dissolved in 2.9 ml of abs. pyridine, and 0.3 g of 2,4,6-triisopropylbenzenesulfonyl chloride and 0.25 ml of *N*-methylimidazole are added and stirred for 20 h at r.t. The mixture is treated with 50 ml of $CHCl_3$ and then washed twice with 25 ml of H_2O. The org. layer is dried over Na_2SO_4, evaporated to dryness, and coevaporated twice with 10 ml of toluene. The residue is dissolved in 10 ml of $CHCl_3$ and chromatographed on a silica-gel column (20 × 3 cm) with 500 ml of $CHCl_3$ and 800 ml of $CHCl_3$/MeOH 100:1. Evaporation of the main fraction gives 0.48 g (75%) of **20** as a colourless amorphous solid. Anal. calc. for $C_{140}H_{172}N_{19}O_{35}P_3Si_5 \cdot H_2O$ (2952.3): C 56.95, H 5.94, N 9.01; found: C 57.19, H 6.04, N 8.89.

15. N^6-*Benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]adenylyl-{3'-[*O^P-*(2-(*p-*nitrophenyl)ethyl)]→5'}*-N^4-*benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]cytidylyl-{3'-[*O^P-*(2-(*p-*nitrophenyl)ethyl)]→5'}*-N^4-*benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]cytidylyl-{3'-[*O^P-*(2-(*p-*nitrophenyl)ethyl)]→5'}*-N^6-*benzoyl-2',3'-bis-*O-*[(*tert-*butyl)dimethylsilyl]adenosine* (**21**). 15.1. A soln. of 0.437 g (0.15 mmol) of **20** in 5 ml of 2% TsOH in CH_2Cl_2/MeOH 4:1 is stirred at r.t. for 50 min. The mixture is diluted with 40 ml of $CHCl_3$ and then extracted 3 times with 25 ml of H_2O. The org. layer is dried over Na_2SO_4, filtered, and evaporated to dryness. The residue is chromatographed on a silica-gel column (15 × 3 cm) with 400 ml of $CHCl_3$ and 600 ml of $CHCl_3$/MeOH 100:3. The main fraction is collected and evaporated to give 0.34 g (85%) of **21** as a colourless amorphous solid.

15.2. A soln. of 0.2 g (0.07 mmol) of **20** in 2 ml of 2% CF_3COOH in $CHCl_3$ is stirred for 30 min at r.t. The mixture is diluted with 10 ml of $CHCl_3$, washed first with 15 ml of buffer (pH 7) and secondly with 20 ml of ice/H_2O. The org. layer is dried over Na_2SO_4, evaporated, and the residue purified by prep. TLC (40 × 20 × 0.2 cm) on silica gel with $CHCl_3$/MeOH 96:4. The main band is extracted by $CHCl_3$ to yield 0.11 g (61%) of **21** as an amorphous colourless powder. Anal. calc. for $C_{120}H_{156}N_{19}O_{34}P_3Si_5$ (2642.1): C 54.54, H 5.95, N 10.07; found: C 54.09, H 5.83, N 9.70.

16. N^4-*Benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]-5'*-O-*(monomethoxytrityl)cytidylyl-{3'-[*O^P-*(2-(*p-*nitrophenyl)ethyl)]→5'}*-N^6-*benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]adenylyl-{3'-[*O^P-*(2-(*p-*nitrophenyl)ethyl)]→5'}*-N^6-*benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]adenylyl-{3'-[*O^P-*(2-(*p-*nitrophenyl)ethyl)]→5'}*-N^4-*benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]cytidylyl-{3'-[*O^P-*(2-(*p-*nitrophenyl)ethyl)]→5'}*-N^4-*benzoyl-2'*-O-*[(*tert-*butyl)dimethylsilyl]cytidylyl-{3'-[*O^P-*(2-(*p-*nitrophenyl)ethyl)]→5'}*-N^6-*benzoyl-2',3'-bis-*O-*[(*tert-*butyl)dimethylsilyl]adenosine* (**22**). 16.1. Three times, 0.15 g (0.085 mmol) of **9** and 0.15 g (0.057 mmol) of **21** are coevaporated with 3 ml of abs. pyridine. The mixture is then dissolved in 0.75 ml of abs. pyridine, 65 mg of TPSNT are added and stirred over night at r.t. The mixture is then extracted twice with 30 ml of $CHCl_3$, washed twice with 20 ml of H_2O, the org. layer dried over Na_2SO_4 and evaporated followed by coevaporation twice with 10 ml of toluene. The residue is dissolved in 5 ml of CH_2Cl_2 and chromatographed on a silica-gel column (10 × 3 cm) with 500 ml of CH_2Cl_2, 400 ml of CH_2Cl_2/$CHCl_3$ 1:1, 300 ml of $CHCl_3$, and 600 ml of $CHCl_3$/MeOH 100:1.5. The residue of the main fraction is dissolved in 3 ml of $CHCl_3$ and then added dropwise to 100 ml of hexane with stirring. The amorphous solid is dried under vacuum: 0.17 g (69%) of **22** as a colourless solid.

16.2. Three times, 0.22 g (0.11 mmol) of **19** and 0.42 g (0.17 mmol) of **17** are coevaporated with 3 ml of abs. pyridine. The residue is then dissolved in 1.5 ml of abs. pyridine, 0.13 g of 2,4,6-triisopropylbenzenesulfonyl nitrotriazolide (TPSNT) are added and stirred at r.t. over night. The mixture is extracted twice with 10 ml of $CHCl_3$ and then washed with 25 ml of buffer (pH 7) and 25 ml of ice/H_2O. The org. layer is dried over Na_2SO_4, evaporated to dryness, and twice coevaporated with 10 ml of toluene. The residue is dissolved in 5 ml of CH_2Cl_2 and chromatographed on a silica-gel column (10 × 3 cm) with 500 ml of CH_2Cl_2, 400 ml of CH_2Cl_2/$CHCl_3$ 1:1, 400 ml of $CHCl_3$ and 1 l of $CHCl_3$/MeOH 100:1.5. The main fraction yields on evaporation and drying under vacuum 0.27

g (62%) of **22** as an amorphous colourless solid. Anal. calc. for $C_{197}H_{246}N_{28}O_{54}P_5Si_7 \cdot 2\ H_2O$ (4299.1): C 55.81, H 5.86, N 9.44; found: C 55.42, H 5.46, N 9.36.

16.3. Three times, 0.111 g (0.042 mmol) of **21** and 0.111 g (0.063 mmol) of **9** are coevaporated with 3 ml of abs. pyridine. The residue is dissolved in 0.55 ml of abs. pyridine, 38 mg of 2,4,6-triisopropylbenzenesulfonyl chloride and 30 µl of *N*-methylimidazole are added and stirred at r.t. for 20 h. The mixture is extracted with 40 ml of $CHCl_3$, the org. layer washed twice with 20 ml of H_2O dried over Na_2SO_4, and evaporated. The residue is coevaporated twice with 10 ml of toluene, dissolved in CH_2Cl_2 and chromatographed on a silica-gel column (10 × 3 cm) with 400 ml of CH_2Cl_2, 300 ml of $CH_2Cl_2/CHCl_3$ 1:1, 250 ml of $CHCl_3$, and 600 ml of $CHCl_3$/MeOH 100:1.5. The main fraction is evaporated, the residue dissolved in 2 ml of $CHCl_3$ and added dropwise to 50 ml of hexane with stirring. The amorphous solid is collected by centrifugation. Drying under high vacuum at 40° yields 0.125 g (70%) of **22** as an amorphous colourless powder.

17. *Cytidylyl-(3'–5')-adenylyl-(3'–5')-adenylyl-(3'–5')-cytidylyl-(3'–5')-cytidylyl-(3'–5')-adenosine* (**23**). A soln. of 13 mg (3 µmol) of **22** in 4 ml of 0.5M DBU in abs. pyridine is stirred at r.t. for 18 h. The mixture is then neutralized with 2 ml of 1M AcOH in pyridine, evaporated, and coevaporated twice with 3 ml of abs. pyridine. The residue is dissolved in 5 ml of a pyridine soln., which has been prepared from 0.8 g of Bu_4NF and 0.11 ml of HF by 3 times dissolving in dry pyridine and evaporation under vacuum. After stirring for 20 h, 3 ml of H_2O are added, the mixture is evaporated and then this process repeated twice. Deacylation is achieved by treatment with 8 ml of conc. NH_3 for 24 h followed by evaporation and coevaporation twice with H_2O. The final deblocking step to remove the monomethoxytrityl group is done with 10 ml of 80% AcOH at 0° for 20 h. The soln. is again evaporated, coevaporated 3 times with EtOH, dissolved in 2 ml of H_2O, and extracted several times with $CHCl_3$. The crude mixture was purified by paper and ion-exchange chromatography, respectively. a) The crude aq. reaction soln. is concentrated to a small volume and put on 2 large sheets of paper for chromatography with i-PrOH/conc. NH_3/H_2O 50:10:35. The main band is eluted with 150 ml of H_2O, concentrated, and lyophylized to give 63 *OD* units (30%) of **23**. b) The crude aq. reaction soln. is applied onto a *DEAE-Sephadex A-25* column (60 × 1 cm) for elution with a linear gradient of 0.001–0.75M $Me_3NH^+HCO_3^-$ buffer. The product fraction is evaporated, then 10 times coevaporated with each 10 ml of H_2O to give finally 149 *OD* units (71%) of **23**.

The hypochromicity of **23** is determined by alkaline hydrolysis in 0.3N NaOH at 37° for 24 h. There is a 9.2% decrease of absorbance in the hexamer due to base-stacking and giving a total calculated yield of 80% of **23**.

18. *Enzymic Hydrolysis of C-A-A-C-C-A.* A soln. of 63 *OD* units of **23** in 80 µl of H_2O is mixed with 20 µl of a cocktail prepared from 30 µl of 1M K_3PO_4 (pH 6.2), 25 µl of 0.1M EDTA-sodium salt (pH 7), 30 µl of 1% aq. *Tween 80*, and 110 µl of H_2O and then incubated for 16 h at 37°. The mixture is applied onto a sheet of paper for chromatographical separation in i-PrOH/conc. aq. NH_3/H_2O 50:10:35. The 3 bands are cut out, eluted with H_2O, and the ratio calculated from the UV spectrometric determinations to be Cp/Ap/A = 3:2.08:1.15.

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