NUCLEOTIDES, XXXIII[1]: SYNTHESIS AND PROPERTIES OF INOSINATE TRIMER I2'p5'I2'p5'I AND INOSINATE TETRAMER I2'p5'I2'p5'I2'p5'I.

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Abstract - The chemical synthesis of 2'-5' inosinate trimer and tetramer was achieved by the phosphotriester method using the 2-(p-nitrophenyl)ethyl group for phosphate protection. The sugar hydroxyl groups have either been protected by the (tert-butyl)-dimethylsilyl or the benzoyl group. Final deprotection to the unblocked oligomers was achieved in over 85 % yield. The inosinate oligomers show some antiviral activity against TMV.

INTRODUCTION

The exciting reports[2-4] on the unusual structure of the oligonucleotide 5'-O-triphosphoryladenylyl-(2'-5')-adenylyl-(2'-5')-adenosine (pppA2'p5'A2'p5'A) and its biological activity as strong inhibitor of cell-free protein synthesis forced various research groups to synthesize the low-molecular weight oligomer[5-8] and its core A2'p5'A2'p5'A[9-14] by chemical approach using in general the phosphotriester method but varying the protective group combinations at various positions of the carbohydrate moiety and the aglycone. The rapid degradation of 2'-5' adenylates in cell cultures by phosphodiesterase activity[15] prompted us to synthesize various 2'-5'-adenylate analogues modified at the sugar moiety, the internucleotidic linkage as well as at the aglycone part. The availability of various oligomers with 2'-5'-internucleotidic linkages would facilitate further studies of their biological activity, especially if such structural analogues as the deaminated counterparts are taken into consideration. First results on these lines have been obtained[16] during the polymerisation of inosine 5'-phosphoroimidazolide in presence of Pb^{++} ions to yield the trimer and tetramer. Our approach describes the direct chemical synthesis of 2'-5'-inosinate trimer and tetramer using the phosphotriester method[17] and the 2-(p-nitrophenyl)ethyl group for phosphate protection[18,19] as the key features.

SYNTHESES

Inosine (1) was first blocked in the 5'-position by the monomethoxytrityl group to give 2, treatment with (tert-butyl)dimethylsilyl chloride and imidazole in pyridine led to a mixture of 40 % of each of the 2'-O- (5) and 3'-O-(tert-butyl)-dimethylsilyl derivative (6) as well as of 3 % of 2',3'-disilylated analogue (7). The structural assignments of 5 and 6 were based upon nmr-data in analogy to former results.[21] Phosphorylation of 6 was carried out using 2,5-dichlorphenylphosphoroditriazolide in pyridine, followed by p-nitrophenylethanol to give the corresponding 2'-phosphotriester 10 in 95 % yield. The latter compound functioned as a versatile synthon, since oximate cleavage deblocked the 2,5-dichlorophenyl group to the phosphodiester 11 and deprotection of monomethoxytrityl group by 2% p-toluenesulfonic acid in CH_2Cl_2/CH_3OH (8:3) gave 3'-O-(tert-butyl)dimethylsilyl-inosine-2'-[2,5-dichlorophenyl-2-(p-nitrophenyl)ethyl phosphate] (9) in 92 % yield. 2',3'-Di-O-benzoylinosine (4) was prepared from 2 by benzoylation and subsequent detritylation. The synthesis of the inosine trimer 21 was done starting from 8 and 11, which were condensed in presence of quinoline-8-sulfonyl chloride (QSCl) and 3-nitro-1,2,4-triazole (NT) in dry pyridine at room temperature for 16 h to give the dimer 16 in 79 % yield. Cleavage of the monomethoxytrityl group yielded 88 % of 17, which was again condensed with 11 to the fully protected inosinate trimer 19 in 88 % yield. The synthesis of the inosinate tetramer 20 was achieved by block condensation of the two dimers 13 and 15. The former compound was obtained from the condensation reaction of phosphodiester 11 with 4 to give 12 in 76 % yield and subsequent detritylation. On the other hand, 15 resulted from the reaction between 9 and 11 to form first 14 and followed by cleavage of the 2,5-dichlorophenyl group by the oximate method. These two components (13+15) were condensed in presence of QSCl and NT in pyridine to give the fully protected tetrameric inosine 18 in 81 % yield. The oligomers 18 and 19 were then deblocked subsequently first by treatment with 0.5 M 1,5-diazabicyclo-(5.4.0)-undec-5-ene (DBU) in pyridine for 6-8 h to remove the 2-(p-nitrophenyl)-ethyl group, second by 0.5 M Bu_4NF to cleave the silyl groups and then in the case of 18 by conc. ammonia to achieve deprotection of the benzoyl groups. Finally detritylation was performed with 80 % acetic acid and the crude products were purified by DEAE Sephadex chromatography in $Et_3NH^+HCO_3^-$ buffer (pH 7) applying a linear gradient and giving a 90 % yield of the oligomers.

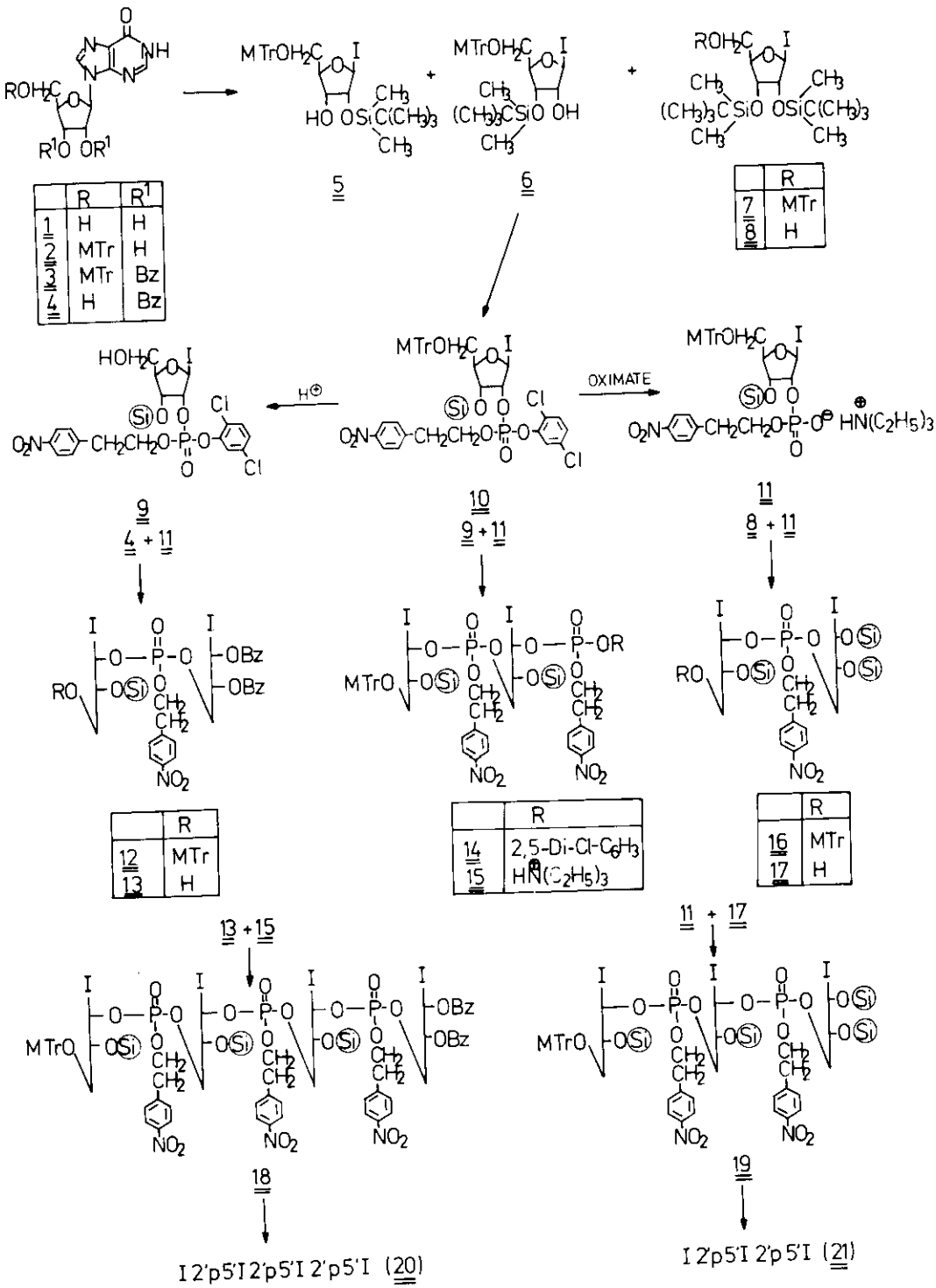
R R1
1 H H
2 MTr H
3 MTr Bz
4 H Bz
5
6
R
7 MTr
8 H
OXIMATE
9
4 + 11
10
9 + 11
11
8 + 11
R
12 MTr
13 H
R
14 2,5-Di-Cl-C6H3
15 HN(C2H5)3
R
16 MTr
17 H
13 + 15
11 + 17
18
19
I 2'p5'I 2'p5'I 2'p5'I (20)
I 2'p5'I 2'p5'I (21)

Table - Uv - Absorption Spectra of Nucleotides and Oligoriboncuelotides in MeOH and H_2O*

	λ_{max} (nm) / (lg ε)				
2	235 (4.32)	250 (sh 4.15)	274 (sh 3.72)	281 (sh 3.55)	
3	231 (4.66)	250 (sh 4.24)	270 (sh 3.90)	280 (sh 3.75)	
4	232 (4.49)	250 (sh 4.16)	270 (sh 3.78)	280 (sh 3.61)	
5	235 (4.33)	250 (sh 4.15)	275 (sh 3.71)	281 (sh 3.56)	
6	235 (4.32)	250 (sh 4.15)	274 (sh 3.73)	281 (sh 3.57)	
7	235 (4.32)	250 (sh 4.14)	274 (sh 3.70)	281 (sh 3.54)	
8	244 (4.06)	249 (4.06)	270 (sh 3.63)		
9	224 (sh 4.22)	229 (sh 4.20)	244 (sh 4.15)	251 (4.19)	266 (4.15)
10	229 (sh 4.46)	250 (sh 4.27)	266 (4.23)	273 (sh 4.22)	280 (sh 4.13)
11	235 (4.35)	250 (sh 4.25)	267 (4.17)		
12	233 (4.73)	250 (sh 4.50)	270 (sh 4.30)	280 (sh 4.17)	
13	235 (4.62)	250 (sh 4.48)	264 (4.31)		
14	228 (4.62)	242 (4.46)	250 (4.56)	264 (4.51)	
15	238 (4.52)	243 (4.52)	250 (4.51)	266 (4.45)	
16	237 (4.50)	243 (sh 4.49)	250 (sh 4.45)	270 (sh 4.28)	
17		245 (sh 4.41)	250 (4.43)	270 (sh 4.26)	
18	235 (4.85)	250 (4.78)	264 (4.69)		
19	236 (sh 4.59)	244 (4.63)	250 (4.63)	266 (sh 4.51)	
20*	246	250	270 (sh)		
21*	246	250	270 (sh)		

sh = shoulder

The protected intermediates were purified by silica gel chromatography and analysed for C,H,N 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 are very complicated in most cases showing many overlapping signals due to the presence of diastereomeric mixtures in the protected phosphotriesters. The nmr method is therefore significantly limited for structural proofs of

such type of complex molecules. Some characteristic signals like the one of OCH_3 of monomethoxytrityl group help in the isolation procedure to detect the right reaction product.

From various in vitro tests of tobacco mosaic virus (TMV) infected Nicotiana glutinosa can be seen that the inosinate trimer core is able to inhibit to some extent TMV replication.[20]

EXPERIMENTAL

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 preparative column chromatography on silica gel (Merck 60, 0.063-0.2 mesh). Ion exchange chromatography on DEAE-Sephadex A-25 (Pharmacia). Uv/Vis: Cary Recording spectrometer, model 118, Applied Phys. Corp. and Uvikon 820, Kontron: λ_{max} nm (lg ε). ^{1}H-Nmr: Bruker WM 250; δ (ppm) relative to TMS.

5'-O-(Monomethoxytrityl)inosine (2). - 6.7 g (25 mmol) of inosine (1) were made anhydrous by repeated coevaporation with dry pyridine and finally suspended in 85 ml of dry DMSO/pyridine (1:1), 9 g (30 mmol) of monomethoxytrityl chloride were then added and the reaction mixture was stirred at room temperature for 18 h. Thereafter 10 ml of MeOH were added and the product was extracted with $CHCl_3$ (1000 ml). The organic phase was washed with H_2O (2x500 ml), dried over Na_2SO_4, filtered and evaporated to a gum. Final evaporation was done with toluene (2x50 ml). The residue was dissolved in 220 ml of $CHCl_3$ and chromatographed on a silica gel column (35x2.5 cm) first with $CHCl_3$ and then $CHCl_3/CH_3OH$ (98:2). Evaporation of the main fraction yielded 10.8 g (80 %) of 2 as a colourless solid, mp: 219-220°C. ^{1}H-Nmr (DMSO-d_6): 12.39 (bs, 1H, NH); 8.20 (s, 1H, H-2); 7.99 (s, 1H, H-8); 7.19-7.36 (m, 12H, aryl); 6.83 (d, J = 8.85 Hz, 2H, o-OMe); 5.89 (d, J = 4.57 Hz, 1H, H-1'); 3.72 (s, 3H, oMe). Anal. Calcd for $C_{30}H_{28}N_4O_6$: C, 66.65; H, 5.22; N, 10.36. Found: C, 66.60; H, 5.29; N, 10.09.

2',3'-Di-O-benzoyl-5'-O-(monomethoxytrityl)inosine (3). - To a solution of 8.0 g (1.48 mmol) of 5'-O-(monomethoxytrityl)inosine (2) in dry pyridine (50 ml) was added under cooling in an ice-bath benzoyl chloride (15 ml). After stirring for 3 h at room temperature the reaction mixture was poured onto ice. The product was

extracted with $CHCl_3$ (800 ml) and the $CHCl_3$ phase was washed with H_2O, dried over Na_2SO_4 and evaporated to dryness. Final coevaporation was done with toluene (2x50 ml). The residue was dissolved in $CHCl_3$ (20 ml) and added dropwise to n-hexane (600 ml) with stirring to give 3 as an amorphous powder (10 g; 91 %). ^{1}H-Nmr ($CDCl_3$): 12.84 (bs, 1H, NH); 7.10-8.36 (m, 24 H, H-2, H-8, aryl); 6.81 (d, J = 8.8 Hz, 2H, o-OMe); 6.43 (d, J = 6.4 Hz, 1H, H-1'); 3.74 (s, 3H, OCH_3). Anal. Calcd for $C_{44}H_{36}N_4O_8$: C, 70.57; H, 4.84; N, 7.48. Found: C, 69.99; H, 4.93; N, 7.27.

2',3'-Di-O-benzoylinosine (4). - To 50 ml of 2 % p-TsOH (1 g) in $CHCl_3/CH_3OH$ (7:3) were added 3.0 g (4 mmol) of 3 and the mixture was stirred for 30 min at room temperature. 0.045 M phosphate buffer pH 7 (20 ml) was added and the product was extracted with $CHCl_3$ (600 ml). The organic phase was washed with H_2O (2x200 ml), dried over Na_2SO_4 and evaporated to dryness. Purification was done using silica gel column (15x2.5 cm) with $CHCl_3$, $CHCl_3/CH_3OH$ (97:3). The product fractions were collected and evaporated to give 1.71 g (90 %) of 4 as a colourless solid of mp 142°C (decomp.). ^{1}H-Nmr ($CDCl_3$): 12.25 (bs, 1H, NH); 8.46 (s, 1H, H-2); 7.26-8.07 (m, 11H, aryl, + H-8); 6.32 (d, 1H, J = 2.2 Hz, H-1'); 5.79 (m, 1H, OH). Anal. Calcd for $C_{24}H_{20}N_4O_7$: C, 60.50; H, 4.23; N, 11.76. Found: C, 60.10; H, 4.33; N, 11.57.

2'-O-(tert-Butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosine (5), 3'-O-(tert-Butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosine (6), and 2',3'-Di-O-(tert-butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosine (7). - a) 5.92 g (10.35 mmol) of 5'-O-(monomethoxytrityl)inosine (2) were made anhydrous by coevaporation with dry pyridine (3x10 ml) and taken up in 48 ml of dry pyridine, (tert-butyl)-dimethylsilyl chloride (1.982 g; 13.15 mmol) and imidazole (1.78 g; 26.3 mmol) were added and the mixture was stirred at room temperature for 24 h. CH_3OH (6 ml) was added and the solution was evaporated to 3/4 of its volume and extracted with $CHCl_3$ (2x500 ml). The organic phase was washed with H_2O (2x200 ml), dried over Na_2SO_4 and evaporated again to dryness. Final evaporation was done with toluene and the residue was allowed to stand in EtOAc. Part of 2'-O-(tert-butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosine (5) crystallized out from EtOAc in colourless needles (1.5 g; 22 %), mp 219-221°C. The filtrate was concentrated, applied to a silica gel column (30x2.5 cm) and chromatographed with toluene/C_2H_5OAc (90:1)

to elute first the 2',3'-disilyl product 7, which was obtained in 3 % yield (0.238 g) as a solid foam. ^{1}H-Nmr ($CDCl_3$): 13.14 (s, 1H, NH); 8.02 and 7.89 (1H each, s, purine protons); 7.14-7.47 (m, 12H, aryl); 6.82 (d, J = 8.8 Hz, 2H, o-OMe); 5.95 (d, J = 5.8 Hz, 1H, H-1'); 4.70 (t, 1H, H-2'); 4.19-4.24 (m, 2H, H-3', H-4'); 3.77 (s, 1H, OCH_3); 0.84 and 0.76 (2s, 18H, 2x$C(CH_3)_3$; -0.03, -0.04, -0.06, -0.27 (4s, 12H, 2x$Si(CH_3)_3$). Anal. Calcd for $C_{42}H_{56}N_4O_6Si_2$: C, 65.59; H, 7.34; H, 7.28. Found: C, 65.75; H, 7.56; N, 6.97.

b) Continuation of the chromatography with toluene/C_2H_5OAc (4:1) gave 1.23 g (18 %) 2'-O-(tert-butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosine (5) as a solid foam. ^{1}H-Nmr ($CDCl_3$): 13.33 (s, 1H, NH); 8.13 (s, 1H, H-2); 8.03 (s, 1H, H-8); 7.47-7.22 (m, 12H, aryl); 6.82 (d, J = 8.5 Hz, 2H, o-OMe); 6.02 (d, J = 5.5 Hz, 1H, H-1'); 4.89 (t, 1H, H-2'); 4.26-4.34 (m, 2H, H-3', H-4'); 3.77 (s, 3H, OCH_3); 0.85 (s, 9H, $C(CH_3)_3$); 0.01, -0.12 (2s, 6H, $Si(CH_3)_2$). Anal. Calcd for $C_{36}H_{42}N_4O_6Si$: C, 66.03; H, 6.46; N, 8.55. Found: C, 65.78; H, 6.54; N, 8.59.

c) Finally the 3'-O-(tert-butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosine (6) was eluted with toluene/C_2H_5OAc (2:3) to give 2.71 g (40 %) of a colourless solid. ^{1}H-Nmr ($CDCl_3$): 13.30 (s, 1H, NH); 8.17 (s, 1H, H-2); 8.05 (s, 1H, H-8); 7.14-7.44 (m, 12H, aryl); 6.75 (d, J = 8.85 Hz, 2H, o-OMe); 6.00 (d, J = 4.8 Hz, 1H, H-1'); 4.64 (t, 1H, H-2'); 4.51 (t, 1H, H-3'); 4-20 (m, 1H, H-4'); 3.75 (s, 3H, OCH_3); 0.88 (s, 9H, $C(CH_3)_3$); 0.08, 0.01 (2s, 6H, $Si(CH_3)_2$). Anal. Calcd for $C_{36}H_{42}N_4O_6Si$: C, 66.03; H, 6.46; N, 8.55. Found: C, 66.31; H, 6.65; N, 8.27.

2',3'-Di-O-(tert-Butyl)dimetyhlsilylinosine (8). - A solution of 0.966 g (1.256 mmol) of 7 in 25 ml of 2 % p-TsOH (500 mg) in CH_2Cl_2/CH_3OH (7:3) was stirred at room temperature for 20 min. 15 ml of 0.045 M phosphate buffer oH 7 were added and then the solution was extracted with $CHCl_3$ (3x150 ml). The organic phase was dried over Na_2SO_4, filtered and evaporated to give a gum. The residue was dissolved in 5 ml of $CHCl_3$ and chromatographed on a silica gel column (10x2.5 cm) with $CHCl_3$, $CHCl_3/CH_3OH$ (98:2). The main fractions were collected and evaporated to give 0.54 g (86 %) of 8 as a colourless foam. ^{1}H-Nmr (DMSO-d_6): 12.42 (bs, 1H, NH); 8.38 (s, 1H, H-2); 8.09 (s, 1H, H-8); 5.88 (d, J = 6.7 Hz, 1H, H-1'); 5.24 (t, 1H,

5'-OH); 0.90 and 0.69 (2s, 18H, 2xC(CH_3)$_3$); 0.10, 0.89, -0.11, -0.38 (4s, 12H), 2xSi(CH_3)$_2$). Anal. Calcd for $C_{22}H_{40}N_4O_5Si_2$: C, 53.19; H, 8.11; N, 11.27. Found: C, 53.43; H, 8,31; N, 11.25.

3'-O-(tert-Butyl)dimethylsilylinosine-2'-[2,5-dichlorphenyl 2-(p-nitrophenyl)-ethyl phosphate] (9). - 1.028 g (1 mmol) of the fully protected phosphotriester 10 were stirred with 20 ml of 2 % p-TsOH (400 mg) in $CHCl_3$/CH_3OH (4:1) for 30 min at room temperature. 0.045 M Phosphate buffer pH 7 (20 ml) was added and the product was extracted with $CHCl_3$ (500 ml), dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column (15x2.5 cm) chromatography using $CHCl_3$, $CHCl_3$/CH_3OH (96:4). The pure product fractions were collected and evaporated. The residue was dissolved in $CHCl_3$ (5 ml) and added dropwise to n-hexane (200 ml). The white powder was filtered and dried in vacuum to give 0.696 g (92 %) of 9 as an amorphous powder. Anal. Calcd for $C_{30}H_{36}Cl_2N_5O_{10}P$ Si; C, 47.62; H, 4.79; N, 9.25. Found: C, 47.53; H, 4.89; N, 9.27.

3'-O-(tert-Butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosine-2'-[2,5-dichlorophenyl 2-(p-nitrophenyl)ethyl phosphate] (10). - 1.26 g (4.5 mmol) of 2,5-dichlorophenylphosphorodichloridate and 0.683 g (9.9 mmol) of 1,2,4-triazole were stirred in pyridine (7.4 ml) in an ice-bath for 15 min. Compound 6 (1.96 g; 3 mmol) was added, the mixture was stirred for 30 min till tlc showed complete conversion, and then (2-(p-nitrophenyl)ethanol (1 g; 6 mmol) was added and stirring was continued for 6 h. The product was extracted with $CHCl_3$ (2x250 ml), the $CHCl_3$-phase was washed with H_2O (2x100 ml), dried over Na_2SO_4 and evaporated to dryness. Final evaporation was done with toluene (2x50 ml) and purification by using silica gel chromatography (20x2.5 cm) in $CHCl_3$/CH_3OH (97:3). The product fractions were collected and evaporated to give 2.92 g (95 %) of 10 as a colourless foam. Anal. Calcd for $C_{50}H_{52}Cl_2N_5O_{11}P$ Si: C, 58.36; H, 5.09; N, 6.80. Found: C, 58.66; H, 5.07; N, 6.76.

2'-O-(tert-Butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosinyl-[2-(p-nitrophenyl)-ethyltriethylammonium phosphate] (11). - A solution of 3.35 g (20 mmol) of p-nitrobenzaldoxime in 20 ml of $(C_2H_5)_3N$/dioxane/H_2O (1:1:1) was stirred at room temperature for 20 min. Then 2.05 g (2 mmol) of 10 were added and stirring was continued for 3 h. The solution was evaporated after addition of 100 ml of pyridine.

The residue was coevaporated twice with toluene (2x50 ml) and dissolved in $CHCl_3$ and chromatographed on a silica gel column (10x2.5 cm) with 500 ml $CHCl_3$, 1000 ml $CHCl_3/CH_3OH$ (97:3), and 1000 ml $CHCl_3/CH_3OH/(C_2H_5)_3N$ (8:1:1). Evaporation of the main fraction yielded a solid residue, which was dissolved in 6 ml of $CHCl_3$ and added dropwise to a stirred solution of 500 ml of n-hexane. The precipitate was collected by suction and dried in vacuum at 40°C to yield 1.88 g (95 %) of a colourless powder. Anal. Calcd for $C_{50}H_{65}N_6O_{11}P$ Si: C, 60.65; H, 6.61; N, 8.48. Found: C, 60.71; H, 7.02; N, 8.34.

2'-O-(tert-Butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosinyl{2'-[O^P-(2-(p-Nitrophenyl)ethyl)]→5'}-2',3'-di-O-benzoylinosine (12). - A mixture of 2.277 g (2.3 mmol) of the phosphodiester 11 and 0.952 g (2 mmol) of 4 was coevaporated three times with dry pyridine (3x5 ml). The residue was then dissolved in 20 ml of pyridine, 2-nitro-1,2,4-triazole (1.57 g; 13.8 mmol), and QSCl (1.04 g; 4.6 mmol) were added and the mixture was stirred at room temperature for 16 h. The mixture was quenched by 0.045 M phosphate buffer pH 7 (10 ml) and the crude product was extracted with $CHCl_3$ (800 ml). After evaporation of the solvent, the product was purified by silica gel column chromatography (20x2.5 cm) using $CHCl_3$, $CHCl_3/CH_3OH$ (95:5) to give 2.0 g (76 %) of 12 as a solid foam. Anal. Calcd for $C_{68}H_{68}N_9O_{17}P$ Si.1 H_2O: C, 60.03; H, 5.18; N, 9.26. Found: C, 59.53; H, 5.16; N, 9.25.

2'-O-(tert-Butyl)dimethylsilylinosinyl{2'-[O^P-(2-(p-nitrophenyl)ethyl)]→5'}-2',3'-di-O-benzoylinosine (13). - 1.358 g (1 mmol) of 12 were stirred for 30 min with *20 ml of 2 % p-TsOH (400 mg) in CH_2Cl_2/CH_3OH(4:1). 0.045 M Phosphate buffer pH 7* (20 ml) was added and the product was extracted with $CHCl_3$ (500 ml). The organic phase was dried over Na_2SO_4 and evaporated to dryness. For purification, silica gel column chromatography (15x2.5 cm) was used and eluted with $CHCl_3$, $CHCl_3/CH_3OH$ (9:1) to give 0.906 g (83 %) of 13 as a colourless solid. Anal. Calcd for $C_{48}H_{52}N_9O_{16}P$ Si: C, 53.88; H, 4.89; N, 11.79. Found: C, 53.36; H, 5.00; N, 11.81.

2'-O-(tert-Butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosinyl{2'-[O^P-(2-(p-nitrophenyl)ethyl)]→5'}-2'-O-(tert-butyl)dimethylsilylinosine-2'-[2,5-dichlorophenyl 1-(p-nitrophenyl)ethyl phosphate] (14). - A mixture of 0.84 g (0.85 mmol)

of 11 and 0.49 g (0.65 mmol) of 9 was coevaporated three times with 3 ml of dry pyridine and then the residue was dissolved in 7.5 ml of pyridine. 2-Nitro-1,2,4-triazole (0.580 g; 5.1 mmol) and QSCl (0.387 g; 1.7 mmol) were added and then the solution was stirred at room temperature for 48 h. The reaction mixture was then extracted with $CHCl_3$ (800 ml), washed with H_2O (2x100 ml), dried over Na_2SO_4 and evaporated. After coevaporation with toluene (3x20 ml) the crude product was purified by silica gel column chromatography (35x2.5 cm) using $CHCl_3$ and $CHCl_3$/CH_3OH (98:2). The main fractions were collected and after evaporation dissolved in 5 ml of $CHCl_3$ and then the solution was added dropwise to n-hexane (250 ml) to give 0.74 g (71 %) of 14 as an amorphous powder. Anal. Calcd for $C_{74}H_{82}Cl_2N_{10}O_{20}P_2Si_2$: C, 54.77; H, 5.22; N, 8.63. Found: C, 54.76; H, 5.15; N, 9.09.

2'-O-(tert-Butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosinyl{2'-[O^P-(2-(p-nitrophenyl)ethyl)]→5'}-2'-O-(tert-butyl)dimethylsilylinosine-2'-[2-(p-nitrophenyl)ethyl triethylammonium phosphate] (15). - A solution of 0.416 g (2.5 mmol) of p-nitrobenzaldoxime in 15 ml of $(C_2H_5)_3N$/dioxane/H_2O (1:1:1) was stirred at room temperature for 20 min. Then 0.409 g (0.25 mmol) of 14 were added and stirring continued for 4 h. The solution was evaporation after addition of 30 ml of dry pyridine and then coevaporated with toluene (2x50 ml). The residue was dissolved in $CHCl_3$ and applied to a silica gel column (10x2.5 cm) and eluted with $CHCl_3$, $CHCl_3$/CH_3OH (95:5) and $CHCl_3$/CH_3OH/$(C_2H_5)_3N$ (7:1:1). The main fraction was evaporated and finally coevaporated with toluene to give 0.374 g (95 %) of 15 as a foam. Anal. Calcd for $C_{74}H_{79}N_{11}O_{20}P_2Si_2$: C, 56.30; H, 6.19; N, 9.76. Found: C, 56.38; H, 6.26; N, 9.60.

2'-O-(tert-Butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosinyl{2'-[O^P-(2-(p-nitrophenyl)ethyl)]→5'}-2',3'-bis-O-(tert-butyl)dimethylsilylinosine (16). - A mixture of 0.99 g (1 mmol) of 11 and 0.425 g (0.8 mmol) of 8 was coevaporated with dry pyridine and finally dissolved in pyridine (10 ml). 2-Nitro-1,2,4-triazole (0.684 g; 6 mmol) and QSCl (0.455 g; 2 mmol) were added and the solution stirred at room temperature for 23 h. 0.045 M Phosphate buffer pH 7 (100 ml) was added and the reaction mixture was extracted with $CHCl_3$ (500 ml), the $CHCl_3$ phase was dried over Na_2SO_4 and evaporated to dryness and finally coevaporation was done with toluene (2x20 ml). Purification was achieved by silica gel column chromato-

graphy using $CHCl_3$ and $CHCl_3/CH_3OH$ (96:4). The product fractions were collected and evaporated to give 0.868 g (79 %) of a colourless foam. Anal. Calcd for $C_{66}H_{88}N_9O_{15}P\ Si_3$: C, 58.17; H, 6.51; N, 9.25. Found: C, 57.71; H, 6.47; N, 9.17.

2'-O-(tert-Butyl)dimethylsilylinosinyl-{2'-[O^P-(2-(p-nitrophenyl)ethyl)]→5'}-2',3'-bis-O-(tert-butyl)dimethylsilylinosine (17). - 0.641 g (0.46 mmol) of 16 was treated with 10 ml of 2 % p-TsOH (200 mg) in $CHCl_3/CH_3OH$ (7:3) at room temperature for 30 min. After addition of phosphate buffer pH 7 the product was extracted with $CHCl_3$ (500 ml). The organic phase was washed with H_2O (2x50 ml) and evaporated to dryness. Purification on a silica gel column (13x25 cm) with $CHCl_3$ and $CHCl_3/CH_3OH$ (97:3) gave 0.447 g (88 %) of 17 as a solid foam. Anal. Calcd for $C_{46}H_{72}N_9O_{14}P\ Si_3$: C, 50.62; H, 6.65; N, 11.52. Found: C, 50.37; H, 6.73; N, 11.40.

2'-O-(tert-Butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosinyl{2'-[O^P-(2-(p-nitrophenyl)ethyl)]→5'}-2'-O-(tert-butyl)dimethylsilylinosinyl{2'-[O^P-(2-(p-nitrophenyl)ethyl)]→5'}-2'-O-(tert-butyl)dimethylsilylinosinyl{2'-[O^P-(2-(p-nitrophenyl)ethyl)]→5'}-2',3'-di-O-benzoylinosine (18). - A mixture of 0.207 g (0.13 mmol) of 15 and 0.130 g (0.12 mmol) of 13 was coevaporated three times with 3 ml of dry pyridine. The residue was dissolved in 1 ml of pyridine, 2-nitro-1,2,4-triazole (0.083 g; 0.79 mmol) and QSCl (0.059 g; 0.26 mmol) were added and after stirring at room temperature for 24 h the product was extracted with $CHCl_3$ (50 ml). The $CHCl_3$ phase was wshed with H_2O (2x20 ml), evaporated to dryness and coevaporated with toluene. Purification was done on preparative silica gel plates (20x20x0.2 cm) developing first with toluene/C_2H_5OAc/CH_3OH (5:4:1) and secondly with $CHCl_3/CH_3OH$ (9:1). The product band was cut and eluted with $CHCl_3/CH_3OH$ (4:1) to give 0.25 g (81 %) of pure tetramer 18 as a solid foam. Anal. Calcd for $C_{116}H_{132}N_{19}O_{34}P_3Si_3$: C, 54.56; H, 5.21; N, 10.42. Found: C, 54.02; H, 5.01; N, 10.12.

2'-O-(tert-Butyl)dimethylsilyl-5'-O-(monomethoxytrityl)inosinyl{2'-[O^P-(2-(p-nitrophenyl)ethyl)]→5'}-2'-O-(tert-butyl)dimethylsilylinosinyl{2'-[O^P-(2-(p-nitrophenyl)ethyl)]→5'}-2',3'-bis-O-(tert-butyl)dimethylsilylinosine (19). - A mixture of 0.247 g (0.20 mmol) of 11 and 0.22 g (0.2 mmol) of 17 was coevaporated three times with 3 ml of dry pyridine. The mixture was then dissolved in 2.5 ml of dry

pyridine, 2-nitro-1,2,4-triazole (0.17 g; 1.5 mmol) and QSCl (0.154 g; 0.5 mmol) were added and the mixture was stirred at room temperature for 20 h. The mixture was then extracted with 500 ml of $CHCl_3$. The organic phase was washed with 0.045 M phosphate buffer pH 7, dried over Na_2SO_4 and evaporated to dryness. Final coevaporation with done with toluene and purified by silica gel column chromatography (13x2.5 cm) using $CHCl_3$ and $CHCl_3/CH_3OH$ (96:4) to yield 0.348 g (88 %) of pure trimer 19 as an amorphous powder. Anal. Calcd for $C_{90}H_{120}N_{14}O_{24}P_2Si_4$: C, 55.25; H, 6.18; N, 10.02. Found: C, 55.37; H, 6.43; N, 9.85.

Inosinyl-(2'-5')-inosinyl-(2'-5')-inosinyl-(2'-5')-inosine (20). - A solution of 13.3 mg (5.2 mmol) of 18 in 4 ml of 0.5 M DBU in dry pyridine was stirred at room temperature for 18 h. The mixture was then neutralized with 2 ml of 1 M AcOH in pyridine, evaporated and coevaporated twice with 3 ml of dry pyridine. The residue was then treated with 3 ml of 0.5 M Bu_4NF in pyridine and stirred at room temperature for 24 h. The mixture was evaporated and treated with conc. ammonia (6 ml) and stirred at room temperature for 48 h. The final deblocking of the 5'-monomethoxytrityl group was done with 5 ml of 80% AcOH at room temperature for 18 h. The solution was again evaporated and coevaporated several times with H_2O to remove AcOH and finally taken up in H_2O (10 ml) and then was applied to a DEAE-Sephadex A-25 column (60x1 cm) using a linear gradient of 0.001 M-0.8 M Et_3NH^+-HCO_3^- buffer. The product fractions were collected and evaporated to dryness, coevaporated several times with H_2O and lyophilized to give 225 OD (90 %) of the pure tetramer. R_f on cellulose in i-C_3H_7OH/conc. NH_3/H_2O (55:10:35) = 0.67 (AMP = 0.70). R_f on PEI-cellulose in 0.2 M NH_4HCO_3 = 0.25 (AMP = 0.52). The hypochromicity of the inosine-2',5'-tetramer obtained by alkaline hydrolysis was calculated to 9.0 % considering an extinction coefficient of ε = 12200 for pI.

Inosinyl-(2'-5')-inosinyl-(2'-5')-inosine (21). - A solution of 19.8 mg (10 mmol) of 19 in 5 ml of 0.5 M DBU in dry pyridine was stirred at room temperature for 6 h. The mixture was then neutralized with 2.5 ml of 1 M AcOH in pyridine, evaporated and the residue was treated with 5 ml of 0.5 M Bu_4NF. After stirring for 48 h it was evaporated and the residue was treated with 6 ml of 80% AcOH and kept at 10°C for 20 h. The solution was again evaporated and coevaporated several times with H_2O. The purification was done using a DEAE-Sephadex A-25 column (60x1 cm) with $(C_2H_5)_3NH^+HCO_3^-$ buffer (linear gradient 0.001-0.5 M). The product fractions were

collected, evaporated and finally coevaporated several times with H_2O to give 333 OD (91 %) of **21**. R_f on cellulose in i-C_3H_7OH/conc. NH_3/H_2O (55:10:35) = 0.70 (AMP = 0.70); R_f on PEI-cellulose in 0.2 M NH_4HCO_3 = 0.45 (AMP = 0.52).
The hypochromicity of the inosine-2',5'-trimer obtained by alkaline hydrolysis was calculated to 8.5 % considering an extinction coefficient of ε = 12200 for pI.

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