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SYNTHESIS OF INOSINATE TRIMER I2'p5'I2'p5'I AND TETRAMER I2'p5'I2'p5'I2'p5'I

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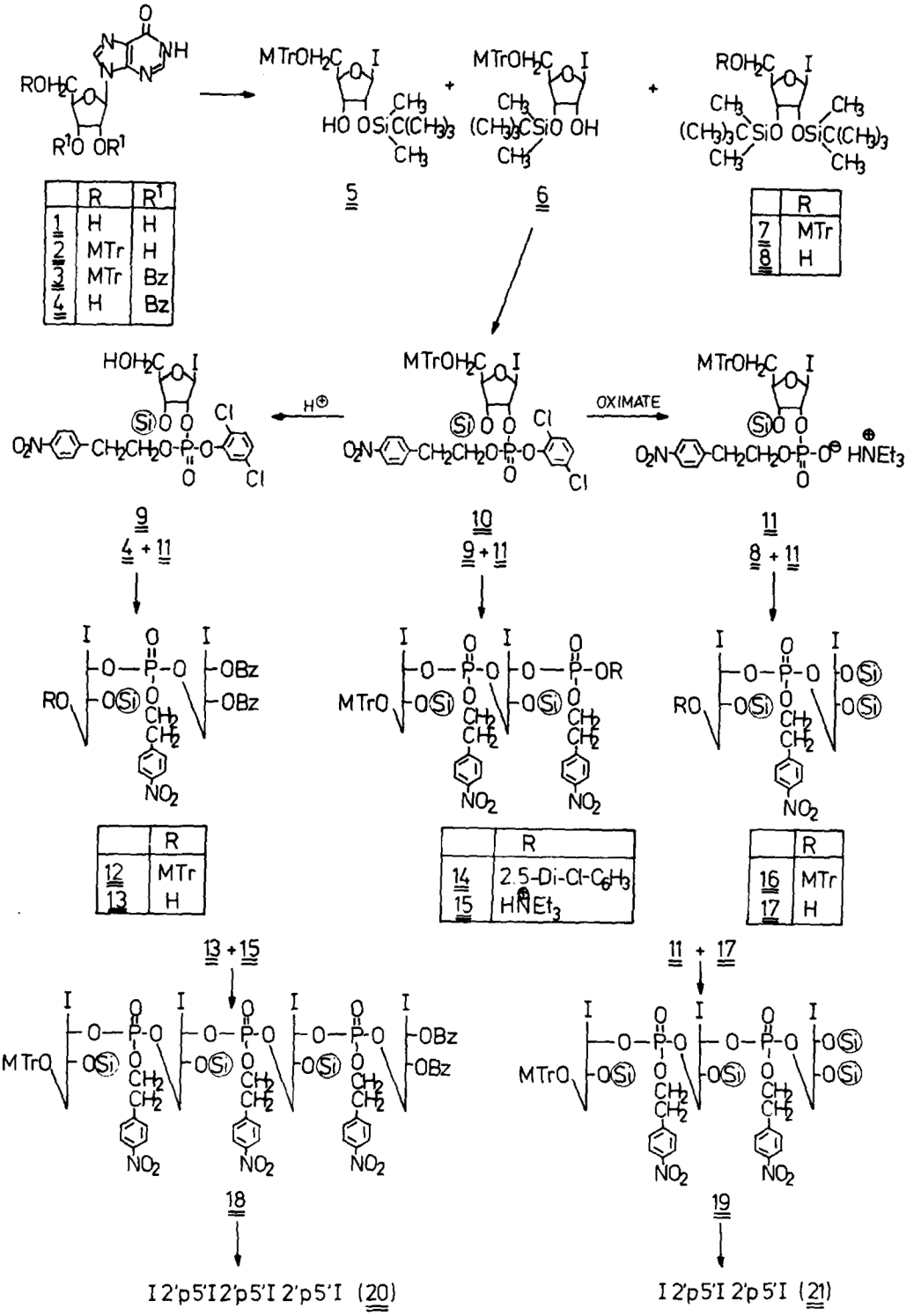
The trimeric and tetrameric inosinates with (2'-5')-linkages were synthesized via the phosphotriester approach using the p-nitrophenylethyl group for phosphate protection.

The exciting reports [1-5] 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 a strong inhibitor of cell free protein synthesis forced various research groups all over the world to synthesize this low-molecular-weight oligonucleotide [6-11] as well as its core A2'p5'-A2'p5'A [12-17] by a purely chemical approach using in general the phosphotriester method but varying the protecting group combinations at the various positions of the carbohydrate moiety and the aglycone. The corresponding 3'-deoxyadenylate trimer dA2'p5'A2'p5'A has recently been synthesized in our laboratory [18] and was found [19] to act as a substitute for human fibroblast interferon in those cells that are permeable to core nucleotides. It is further of particular interest to know that the core cordycepin trimer represents so far the most valuable candidate of the new class of (2'- 5')-oligonucleotides owing to its antiviral effect coupled with its extended metabolic stability without toxicity to cells.

The availability of various oligomers with 2'- 5' internucleotidic linkages would facilitate further studies of their biological action especially if such structural analog as the deaminated counterparts are taken into consideration. First results on these lines have been obtained recently [20] during the polymerization of inosine-5'-phosphorimidazolide in presence of Pb^{++} ions but with low yields of the trimer and tetramer. In this paper we wish to describe therefore a direct chemical synthesis of the 2'-5'-inosinate trimer and tetramer using the phosphotriester approach and the p-nitrophenylethyl group for phosphate protection [21,22] as the key features.

Inosine (1) was first blocked in 5'-position by the monomethoxytrityl group



	R	R¹
1	H	H
2	MTr	H
3	MTr	Bz
4	H	Bz

5 6

	R
7	MTr
8	H

9 = 4 + 11 10 = 9 + 11 11 = 8 + 11

	R
12	MTr
13	H

	R
14	2.5-Di-Cl-C_6H_3
15	$HNEt_3^{\oplus}$

	R
16	MTr
17	H

13 + 15 → 18 → I2'p5'I2'p5'I 2'p5'I (20)

11 + 17 → 19 → I2'p5'I 2'p5'I (21)

to give 2 in 80 % yield. Its treatment with t-butyldimethylsilylchloride and imidazole in pyridine led to a mixture of 40 % of the 2'-O-(5) and 3'-O-t-butyldimethylsilyl derivative (6) each and minor amounts (5-8 %) of the 2',3'-disilylated analog (7). Phosphorylation of 6 was carried out using 2,5-dichlorophenyl-phosphoroditriazolide in pyridine followed by p-nitrophenylethanol to give the corresponding 2'-phosphotriester 10 in 95 % yield after chromatography on a silica gel column. 10 functioned as a versatile synthon since oximate cleavage [23] deblocked quantitatively the 2,5-dichlorophenyl group to form the phosphodiester 11, whereas deprotection of the monomethoxytrityl group by 2 % p-toluene sulfonic acid in methylenechloride/methanol (7/3) gave 3'-O-t-butyldimethylsilylinosine-2'-(2,5-dichlorphenyl, p-nitrophenylethyl)-phosphate (9) in 92 % yield. 2',3'-Di-O-t-butyldimethylsilyl-(8) and 2',3'-di-O-benzoylinosine (4) were prepared from 2 by silylation and benzoylation respectively followed by detritylation.

The synthesis of the inosinate trimer 21 started from 8 and 11, which have first been coupled by quinoline-8-sulfonyl chloride (QSCl) and 3-nitro-1,2,4-triazole (NT) (1/3) in absol. pyridine at room temp. for 16 h to give the dimer 16 in 79 % isolated yield. Cleavage of the monomethoxytrityl group yielded 88 % of 17, which was then condensed in a similar manner with 11 to the fully protected inosylyl(2'- 5')inosylyl(2'- 5')inosine (19) in 88 % yield.

The preparation of the inosinate tetramer 20 was achieved by block-condensation of the two dimers 13 and 15. 13 was obtained from the condensation reaction of the phosphodiester 11 with 4 first to 12 in 76 % and subsequent detritylation, whereas 15 resulted from the reaction between 9 and 11 to 14 in 71 % yield and followed by oximate cleavage of the 2,5-dichlorophenyl group. The coupling reaction was performed under standard conditions applying QSCl and NT in pyridine to form the fully protected tetrameric inosine 18 in 81 % yield.

Both oligomers 18 and 19 were then deblocked using first 0.5 M DBU in absol. pyridine at room temp. for 6-8 h to remove the p-nitrophenylethyl group by elimination and second 0.5 M tetrabutylammonium fluoride for cleavage of the silyl groups. In 18 conc. ammonia was then applied for benzoyl deprotection, whereas the detritylation was always performed in the last step by 80 % acetic acid in 6-8 h. The resulting products were put on a DEAE-Sephadex A-25 column and were eluted with a linear gradient of 0.001M - 0.6M triethylammonium bicarbonate buffer pH 7.5. The isolation of pure inosinate trimer 21 and tetramer 20 respectively resulted from the main fractions by several coevaporations with water and final lyophylisation to stable amorphous powders in 91 % yield each. - The purity of all products has been checked by chromatographical means and the various structures have been proven by UV and NMR spectra as well as elementary analyses.

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