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SYNTHESIS AND PROPERTIES OF ADENYLATE TRIMERS A2'p5'A2'p5'A, A2'p5'A3'p5'A and A3'p5'A2'p5'A

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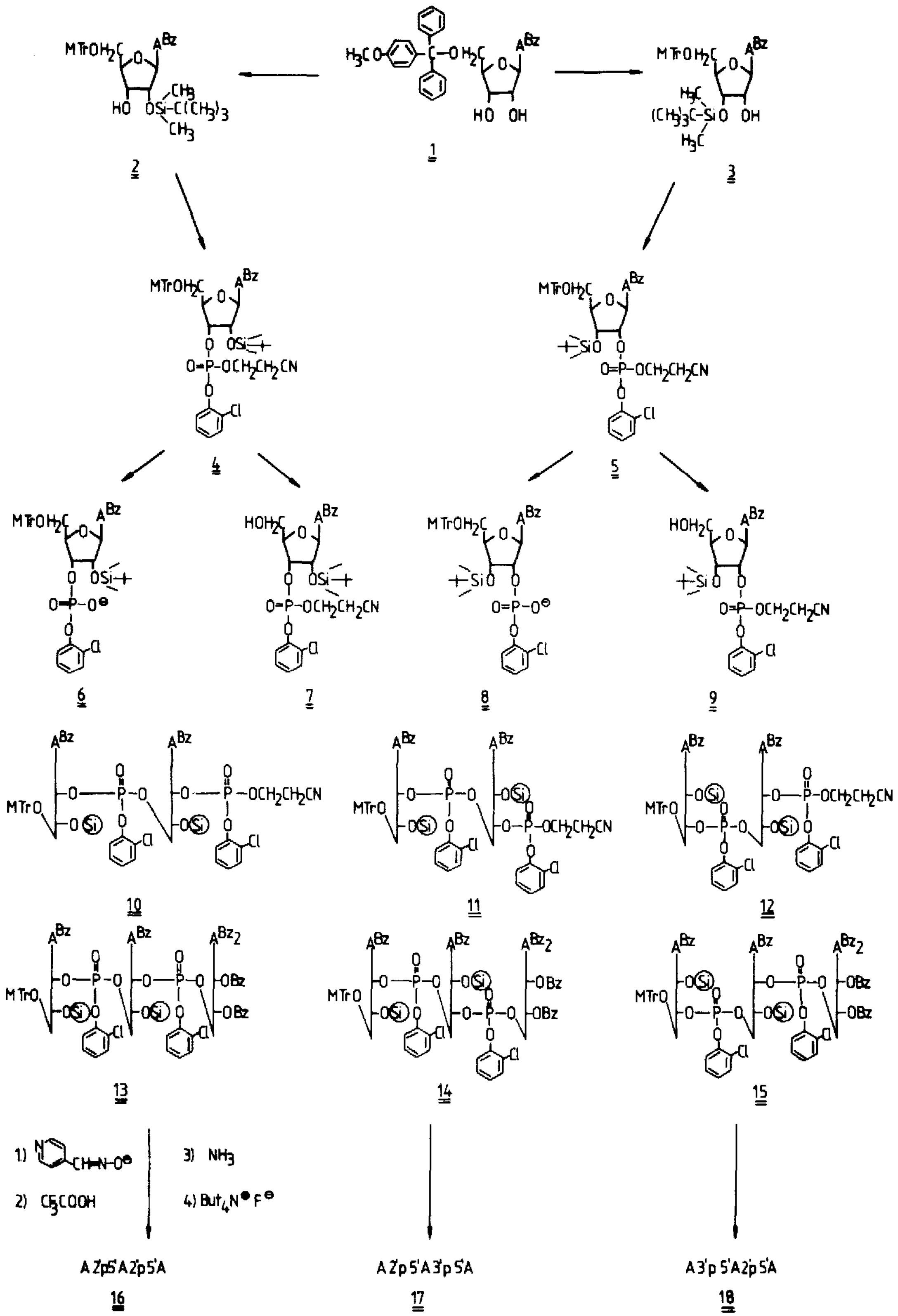
Triadenylates with (2'-5')(2'-5') and mixed (2'-5')(3'-5') and (3'-5')(2'-5') linkages respectively were synthesized via the phosphotriester approach followed by deblocking of the fully protected intermediates. The isomeric trimers were characterized by NMR- and CD-spectra and show very similar hypochromicity effects.

Recent reports [1-11] have demonstrated that a very effective low-molecular-weight inhibitor of cell-free protein synthesis is formed on incubation of cytoplasmic extracts from interferon-treated cells with double-stranded RNA and ATP. Its structure turned out to be 5'-O-triphosphoryl-adenylyl(2'→5')-adenylyl(2'→5')adenosine (pppA2'p5'A2'p5'A) [10] showing the so far unnatural 2'→5' internucleotidic linkage as the most striking structural feature of chemical interest.

Various chemical syntheses of this exciting small oligonucleotide [12-15] as well as of its also active core A2'p5'A2'p5'A [16-18] have recently been published by several groups applying in general the phosphotriester approach but using different blocking group combinations.

In this paper we wish to report a further synthesis of A2'p5'A2'p5'A (16) and its two structural isomers A2'p5'A3'p5'A (17) and A3'p5'A2'p5'A (18) respectively. N^6-Benzoyl-5'-O-monomethoxytrityladenosine (1) was chosen as starting material and converted by treatment with t-butyldimethylsilyl chloride and imidazole in pyridine to a mixture of the corresponding 2'-O-(2) and 3'-O-t-butyldimethylsilyl derivatives (3) [19] in 36 and 42 % yields respectively. Phosphorylations of 2 and 3 were carried out with o-chlorophenyl phosphoroditriazolide in pyridine and subsequent treatment with cyanoethanol leading to the phosphotriesters 4 and 5 which after column chromatography on silica gel were isolated in 78-85 % yields. Quantitative cleavage of the cyanoethyl groups to the phosphodiesters 6 and 8 was achieved by triethylamine/pyridine at room temperature whereas deblocking of the monomethoxytrityl group to the phosphotriesters 7 and 9 worked best with 2 % trifluoroacetic acid [20] in chloroform.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14
15
1)
CH=N-O
2) CF3COOH
3) NH3
4) But4N F
A 2'p5'A 2'p5'A
16
A 2'p 5'A 3'p 5'A
17
A 3'p 5'A 2'p 5'A
18

The monomeric building blocks 8 and 9, 8 and 7 and 6 and 9 respectively were assembled by condensation with triisopropylbenzenesulfonyl nitrotriazolide (TPSNT) to give the fully protected dinucleosidediphosphotriesters A2'p5'A2'p (10), A2'p5'A3'p (11) and A3'p5'A2'p (12) in 85-95 % yield. Removal of the terminal cyanoethyl group was done again by triethylamine/pyridine forming the corresponding phosphodiesters which were then condensed with N^6,N^6,2',3'-tetrabenzoyl adenosine in presence of TPSNT to the fully blocked trinucleoside-diphosphoditriesters 13, 14 and 15 in 78-90 % yields after purification by silica gel column chromatography.

The three trimers 13, 14 and 15 were deprotected using known procedures of first 0.3 N N^1,N^1,N^2,N^2-tetramethylguanidinium pyridine-4-carboxaldoximate in aqueous dioxane (1/1) at room temperature (4-6 h) to cleave the o-chlorophenyl groups [21], second 2 % trifluoroacetic acid in chloroform (30 min) to split off the monomethoxytrityl group followed by conc. ammonia in dioxane (2 days) to hydrolyse the benzoyl groups and finally 0.5 N tetrabutylammonium fluoride in tetrahydrofurane/pyridine (7/3) (4 days) to remove the silyl groups.

Purification was carried out by DEAE Sephadex A-25 chromatography with triethylammonium bicarbonate (pH 7.5; linear gradient 0.001-0.5 M) as the eluting buffer yielding 68 % 16, 65 % 17, and 70 % 18. The purity of these ApApA trimers was checked by TLC and paper chromatography (Tab. 1) and the characterization based on ^{1}H-NMR-spectra in D_2O/dioxane as well as CD-spectra in phosphate buffer pH 7.

Tab. 1 - Physical Data of ApApA-Trimers

	A2'p5'A2'p5'A	A2'p5'A3'p5'A	A3'p5'A2'p5'A	A3'p5'A3'p5'A
Chromatography				
Rf[a)]	0.96	0.93	0.93	0.93
Rf[b)]	1.44	1.27	1.25	1.02
NMR-Spectra[c)]				
1'-H (d)	5.80 5.90 6.02	5.62 5.95 6.10	5.69 5.77 6.10	5.83 5.87 5.92
2-H; 8-H	7.76 7.91 7.95 7.96 8.10 8.16	7.72 7.83 8.00 8.10 8.12 8.23	7.86 8.00 8.04 8.08 8.13 8.16	7.93 8.02 8.12 8.19 8.20 8.23
CD-Spectra[d)]				
λ	251 271	250 270	252 273	250 268
Θ	-41400 +46400	-46100 +43600	-49600 +37000	-64100 +59700
Hypochromicity[e)]				
257 nm	22 %	24 %	22 %	19 %

a) Cellulose i-PrOH/conc. NH_3/H_2O (50/10/35)) Reference: Ap = 1.00;
b) PEI-Cellulose 0.2 M ammonium bicarbonate)
c) 90 MHz-NMR-Spectra in D_2O/dioxane (δ = 3.71 ppm); d) Phosphate buffer pH 7;
e) Alkaline hydrolysis; calculated from ε of pA assumed to be 15400.

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