Tetrahedron Letters Vol. 21, pp 4077 - 4080
0040-4039/80/1015-4077$02.00/0

SYNTHESIS AND PROPERTIES OF 3'-DEOXYADENYLATE TRIMER dA2'p5'A2'p5'A

RAMAMURTHY CHARUBALA, WOLFGANG PFLEIDERER

Fakultät für Chemie, Universität Konstanz, Postfach 5560, D-7750 Konstanz/West Germany

The trimeric 3'-deoxyadenylyl-(2'→5')-3'-deoxyadenylyl-(2'→5')-3'-deoxyadenosine (12) was synthesized via the phosphotriester approach starting from cordycepine (1). Various physical data have been determined and compared with those of the ribo-A2'p5'A2'p5'A analog.

It has recently been reported [1-7] that the oligonucleotide 5'-O-triphosphoryladenylyl-(2'→5')-adenylyl-(2'→5')-adenosine (pppA2'p5'A2'p5'A) is synthesized by reticulyte lysates and extracts from interferon-treated cells in the presence of double-stranded RNA and is acting as a strong inhibitor of cell-free protein synthesis. Various groups have synthesized this low-molecular-weight inhibitor [8-11] as well as its core A2'p5'A2'p5'A [12-14] by chemical means using in general the triester approach but varying the blocking group combinations.

Part of our program in oligonucleotide chemistry is the synthesis of various structurally modified adenylate trimers [15] including the internucleotidic linkages as well as the sugar moiety.

In this paper we wish to report the synthesis of the corresponding 3'-deoxyadenylate trimer dA2'p5'A2'p5'A (12) as a closely related structural analog of the naturally occurring inhibitor. 3'-Deoxyadenosine (Cordycepine) (1) was treated in the first step by benzoylchloride in pyridine yielding the tetrabenzoyl derivative 2 [16] which on selective hydrolysis with 1N NaOH resulted in the formation of N^6-benzoyl-3'-deoxyadenosine (3). Monomethoxytritylation at the 5'-position proceeded in quantitative yield to N^6-benzoyl-5'-O-monomethoxytrityl-cordycepine (4) as a key intermediate. When 4 was reacted with a small excess of o-chlorophenylphosphoro ditriazolide in pyridine followed by subsequent treatment with cyanoethanol phosphorylation to the phosphotriester 5 took place homogeneously and led to an isolated yield of 79 % after column chromatography on silica gel. Quantitative deblocking of the cyanoethyl group was done by triethylamine/pyridine at room temp. yielding the phosphodiester 7 in form of its triethylammonium salt and cleavage of the

monomethoxytrityl group to the 5'-OH-phosphotriester 8 was achieved by 2 % trifluoroacetic acid in chloroform [17]. The third component for the condensation steps was also prepared from 4 by further benzoylation to N^6,N^6,2'-O-tribenzoyl-5'-O-monomethoxytrityl-3'-deoxyadenosine (6) and its detritylation to N^6,N^6,2'-O-cordycepine (9).

A solution of the phosphotriester 8 and a slight excess (1.1 mol equiv.) of the phosphodiester 7 in anhydrous pyridine was then condensed in presence of triisopropylbenzenesulfonyl-nitro-1,2,4-triazolide (TPSNT) [10] to give after 20 h and the usual work-up including separation and purification by column chromatography on silica gel 58 % yield of the fully protected dinucleoside diphosphotriester 10. The cyanoethyl group was removed from this material by treatment again with triethylamine/pyridine forming the corresponding terminal phosphodiester which on further condensation with N^6,N^6,2'-O-tribenzoyl-3'-deoxyadenosine (9) and TPSNT reacted to the fully blocked trinucleosidediphosphotriester 11 in 79 % isolated yields after purification by silica gel column chromatography.

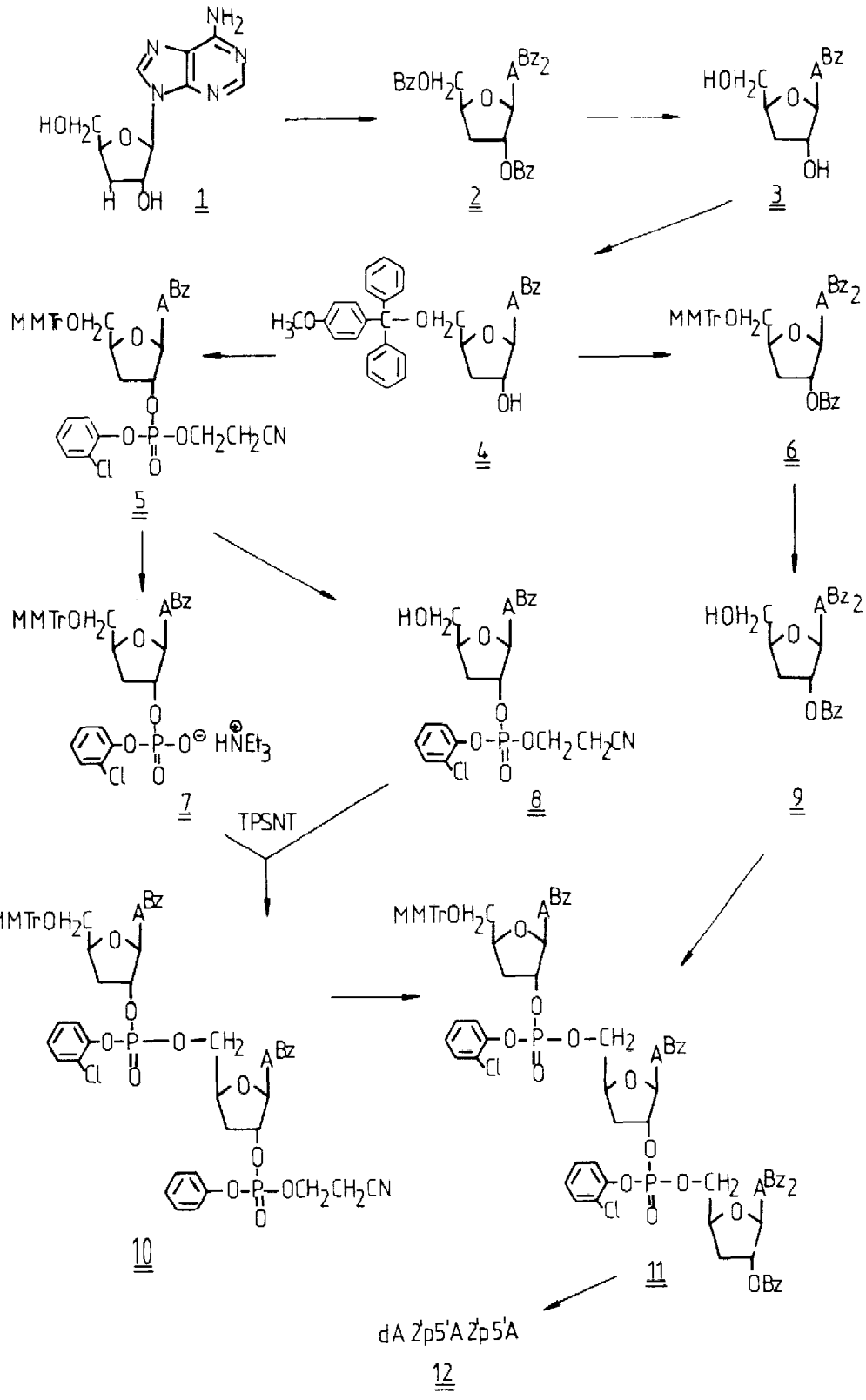
The trimer 11 was deprotected using first 0.3 N N^1,N^1,N^2,N^2-tetramethylguanidinium pyridine-4-carboxaldoximate in aqueous dioxane (1/1) at room temp. for 5 h to cleave the o-chlorophenyl groups [18], second with conc. ammonia for 42 h to remove the benzoyl groups and finally with 80 % acetic acid for 30 min to split off the monomethoxytrityl group. Then the unblocked product thereby obtained was purified by chromatography on DEAE-Sephadex A 25 with a linear gradient of 0.001 - 0.5 M triethylammonium bicarbonate at pH 7.5 3'-deoxyadenylyl-(2'→5')-3'-deoxyadenylyl-(2'→5')-3'-deoxyadenosine (12) accounted for 80 % yield calculated on the extinction coefficient of cordycepine (ε = 15200) and considering a hypochromicity of 27 %.

The purity of 12 was checked by TLC and paper chromatography as well as a complete hydrolysis by snake venom phosphodiesterase to cordycepine (1) and cordycepine-5'-monophosphate in a 1/2 ratio. Further physical data (Tab. 1) such as the CD-values and the hypochromicity have been determined and show in comparison with the ribo-trimer A2'p5'A2'p5'A significant differences the origin of which will be studied in more details.

Tab. 1 - Physical Data of ApApA-Trimers

	dA2'p5'A2'p5'A		A2'p5'A2'p5'A	
Chromatography[a]				
Cellulose i-PrOH/conc. NH_3/H_2O (11/2/7)	1.18		0.96	
PEI-Cellulose 0.2 M ammonium bicarbonate	1.52		1.44	
CD-Spektrum λ (nm)	252	272	251	271
Phosphate buffer pH 7 Θ	-54200	+61900	-41400	+46400
Hypochromicity[b]				
257 nm	27 %		22 %	

a) Rf-values calculated for Ap = 1.00;
b) Enzymic hydrolysis; calculated from ε of cordycepine assumed to be 15200.


TPSNT
dA 2'p5'A 2'p 5'A

REFERENCES

1) A.G. HOVANESSIAN, R.E. BROWN and I.M. KERR, Nature (London) 268, 537 (1977).

2) I.M. KERR, R.E. BROWN and A.G. HOVANESSIAN, Nature (London) 268, 540 (1977).

3) I.M. KERR and R.E. BROWN, Proc.Natl.Acad.Sci. USA 75, 256 (1978).

4) B.R.G. WILLIAMS and I.M. KERR, Nature (London) 276, 88 (1978).

5) E.M. MARTIN, J.M. BIRDSALL, R.E. BROWN and I.M. KERR, Eur.J.Biochem. 95, 295 (1979).

6) M.J. CLEMENS and B.R.G. WILLIAMS, Cell 13, 565 (1978).

7) C.M. VAQUERO and M.J. CLEMENS, Eur.J.Biochem. 98, 245 (1978).

8) M. IKEHARA, K. OSHIE and E. OHTSUKA, Tetrahedron Lett. 1979, 3677.

9) H. SAWAI, T. SHIBATA and M. OHNO, Tetrahedron Lett. 1979, 4573.

10) J.F.M. DE ROOIJI, G.W. HAZELEGER, P.H. VAN DEURSEN, J. SERDJIN and J.H. VAN BOOM, Recl.Trav.Chim. Pays-Bas 98, 537 (1979).

11) S.S. JONES and C.B. REESE, J.Am.Chem.Soc. 101, 7399 (1979).

12) K.K. OGILVIE and N.Y. THERIAULT, Tetrahedron Lett. 1979, 2111.

13) A.K. MARKHAM, R.A. PORTER, M.J. GAIT, R.C. SHEPPARD and I.M. KERR, Nucleic Acid Res. 6, 2569 (1979).

14) J. ENGELS and U. KRAHMER, Angew. Chem. 91, 1007 (1979).

15) R. CHARUBALA and W. PFLEIDERER, Tetrahedron Lett. 1980,

16) R.J. SUHADOLNIC and T. UEMATSU, Carbohydr.Res. 61, 545 (1978).

17) R.W. ADAMIAK, E. BIALA, K. GRZESKOWIAK, R. KIERCEK, A. KRASZEWSKI, W.T. MARKIEWICZ, J. OKUPNIAK, J. STAWINSKI and M. WIEWIOROWSKI, Nucleic Acid Res. 5, 1889 (1978).

18) C.B. REESE, R.C. TITMAS and L. YAU, Tetrahedron Lett. 1978, 2727.

(Received in Germany 2 August 1980)