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SYNTHESIS OF A MODIFIED 2',5'-ADENYLATE TRIMER WITH A 2',3'-DI-O-(2-CARBOXYETHYL)-ETHYLIDENE TERMINAL GROUP

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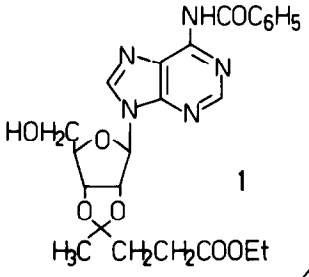
The trimer of 2',5'-oligoadenylic acid with a (2-carboxyethyl)ethylidene group (16) at the 2'-terminal adenosine moiety and its 3'-deoxyadenosine analog (17) have been synthesized by the phosphotriester method.

One mode of action of interferone in its antiviral activity is associated with the appearance of 5'-triphosphates of 2',5'-oligoadenylates [pppA2'-(p5'A)$_n$, n 2] in cells which activate in nanomolar concentration the latent endonuclease L [1,2]. Its activation results in cleavage of virus mRNA and, hence, in inhibition of the protein synthesis [3-6]. In order to prepare the affinity sorbents suitable for isolation of endonuclease L, we have undertaken the synthesis of the basic fragment 2',5'-adenylate trimer 16 bearing at the 2'-terminal adenosine moiety a (2-carboxyethyl)ethylidene group which has been employed in analogous cases [7-9] with promising success.

The synthesis of the 2'-terminal fragment 2',3'-di-O-(2-ethoxycarbonylethyl)ethylidene-N^6-benzoyladenosine (1) was achieved according to the data of [10] in 79 % yield. The second component 8 was obtained from N^6-benzoyl-5'-O-monomethoxytrityladenosine (2) [11] in a series of reactions starting with benzoyl cyanide [12]-treatment in acetonitrile at 20^{o}C in the presence of triethylamine to afford a mixture of the three benzoates 4 - 6. Separation into the individual compounds using silica gel column chromatography yielded 37 % 4, 5 % 5 and 22 % 6 respectively.



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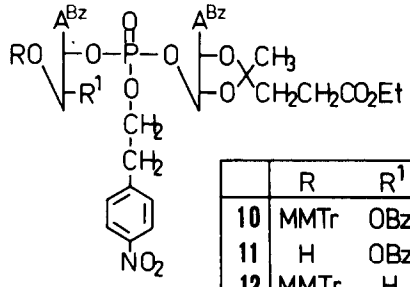
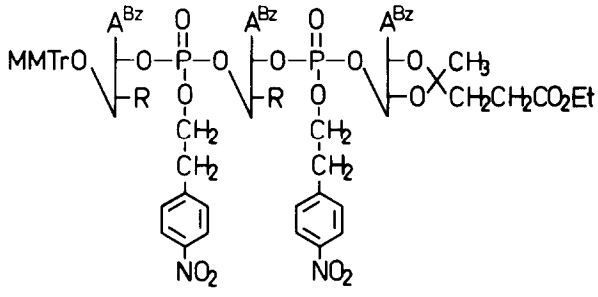
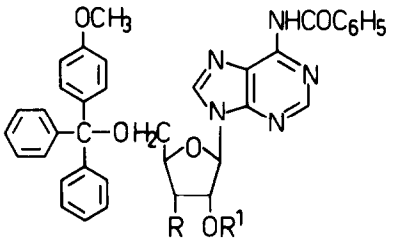
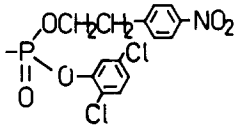
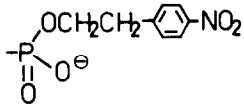
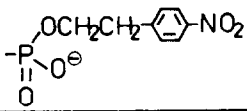


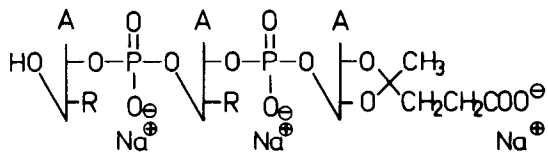
	R	R^1
10	MMTr	OBz
11	H	OBz
12	MMTr	H
13	H	H



	R
14	OBz
15	H



	R	R^1
2	OH	H
3	H	H
4	OBz	H
5	OH	Bz
6	OBz	Bz
7	OBz	
8	OBz	
9	H	



	R
16	OH
17	H

The phosphorylation of 4 was accomplished under the action of 2,5-dichlorphenyl phosphorodichloridate in presence of 1,2,4-triazole and subsequent addition of 2-(p-nitrophenyl)ethanol to form the fully blocked phosphortriester N^6,3'-dibenzoyl-5'-O-monomethoxytrityladenosine-2'-(2,5-dichlorophenyl, p-nitrophenylethyl) phosphate (7) in 89 % yield. Selective removal of the 2,5-dichlorophenyl protecting group in 7 by the oximate method [13] using p-nitrobenzaldoxime/triethylamine led to a 90 % yield of the corresponding phosphodiester 8 after short column chromatography on silica gel.

The synthesis of the fully protected dinucleoside monophosphotriester 10 resulted from the condensation of 1 and 8 under the activation of a mixture of quinoline-8-sulfonyl chloride (QsCl) and 3-nitro-1,2,4-triazole (NT) to yield 88 % pure material. Detritylation of 11 worked best with 2 % p-toluenesulfonic acid in a methylene chloride/methanol (7/3) mixture and gave on chromatographical separation and purification an 80 % yield. The second condensation step between 8 and 11 was performed again by QsCl/NT-activation and led to a 71 % isolated yield of the fully protected trinucleoside diphosphotriester 14.

Deblocking of the latter compound was effected via a successive action of 1) p-toluenesulfonic acid, 2) 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [14], 3) saturated methanolic ammonia at $0^{o}C$, and finally 4) short (2-5 min.) treatment by 0.5 N sodium hydroxide in ethanol/water (1/1). Purification of the fully deblocked adenylyl-2',5'.adenylyl-2',5'-[2',3'-di-O-(2-carboxyethyl)ethylidene]-adenosine (16) was carried out on a DEAE-Sephadex A-25 (HCO_3^--form) column with a linear gradient of 0.001-0.4 M TEAB buffer pH 7 to give 46 % yield of the lyophilized material.

Analogously, the synthesis of 3'-deoxy-adenylyl-2',5'-3'-deoxyadenylyl-2',5'-[2',3'-di-O-(2-carboxyethyl)ethylidene]-adenosine (17) was carried out starting from 1 and cordycepin [15], which was first converted into N^6-benzoyl-5'-O-monomethoxytrityl-3'-deoxyadenosine (3) and then followed by the same sequence of reactions involving phosphorylation, partial deblocking (9), condensations (12,15), and final deprotection (17).

The preparation of the affinity sorbent loaded with 16 and 17 respectively is under investigation and will be described elsewhere in detail.

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