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SYNTHESIS OF NEW MODIFIED 2′,5′-ADENYLATE TRIMERS CARRYING 3'-AMINO-3'-DEOXYADENOSINE AT THE 2'-TERMINUS

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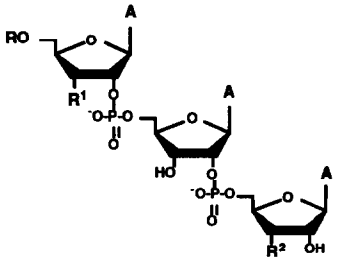
Abstract : The trimeric 2′,5′-linked adenylates **2** and **3** constituting 3′-amino-3′-deoxyadenosine at the 2′-terminal end have been prepared by the phosphotriester and phosphoramidite method. Amino protection was achieved by the p-nitrophenylethoxycarbonyl residue which forms stable carbamate functions cleavable, however, by ß-elimination using DBU. The new trimers were characterized by physical means and their biological activity screened regarding antiviral and antitumoral effects.

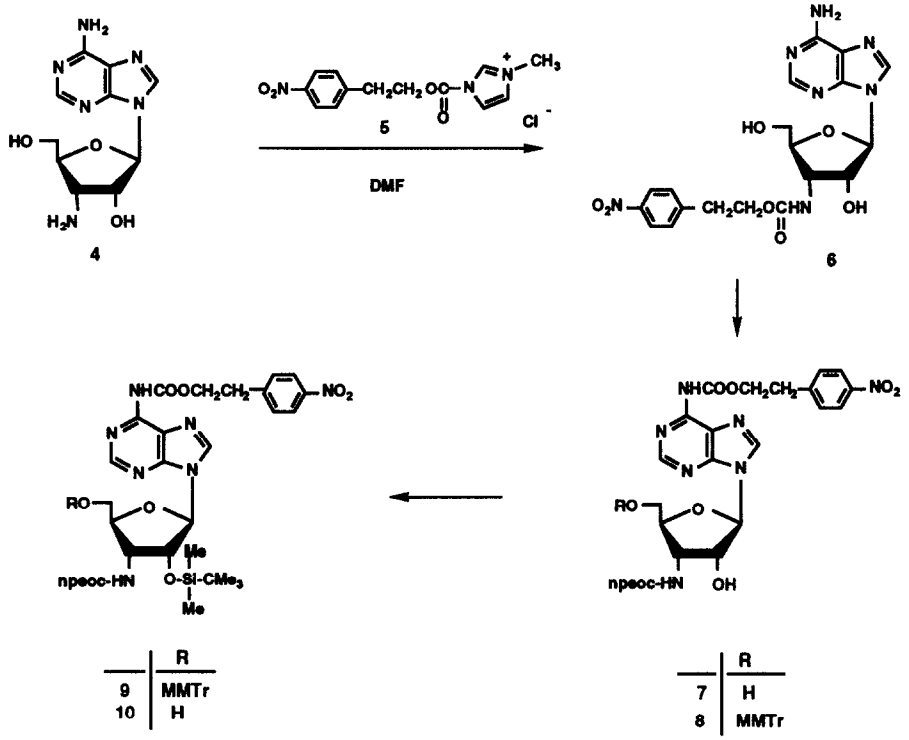
The unique 2′,5′-linked oligoadenylate (2-5A) **1**, a product of the action of the interferon-induced enzyme 2-5 A synthetase, interacts with a latent endoribonuclease (RNase L) which can lead to RNA degradation and inhibition of translation [1].It appears that each individual nucleotide moiety of 2-5 A3 may assume a fundamentally different role in binding to and activation of RNase L [2]. The 2-5 A molecules are not readily taken up in cell extracts, by the intact eucaryotic cells and are rapidly hydrolysed by an exonucleolytic 2′,5′-phosphodiesterase [3] and thus their ability to inhibit protein synthesis is only transitory.

In order to study the interesting 2-5 A system, several analogs of 2-5 A containing modifications primarily on the sugar moiety, on the base and on the internucleotide linkages, respectively, have been synthesised and investigated[4]. Pronounced enzymic stability of the 2-5 A molecules can be achieved by the introduction of either 2-5 phosphorothioate functions [5] or the use of cordycepin (3′-deoxyadenosine) as a monomeric building block [6].Another approach to counteract the enzymic digestion of the 2-5 A oligonucleotides can be seen in the synthesis of adenylyl-(2′-5′)-adenylyl-(2′-5′)-3′-amino-3′-deoxyadenosine (**2**) and cordycepinyl-(2′-5′)-adenylyl-(2′-5′)-3′-amino-3′-deoxyadenosine (**3**).This molecule bears at the 2′-terminus a 3′-amino group, which is present at neutral pH in its ammonium state preventing degradation by phosphodiesterases. Compound **2** was first synthesised applying the phosphotriester approach using p-nitrophenylethyl (NPE) and p-nitrophenylethoxycarbonyl (NPEPOC) groups for phosphate and amino protection[7].

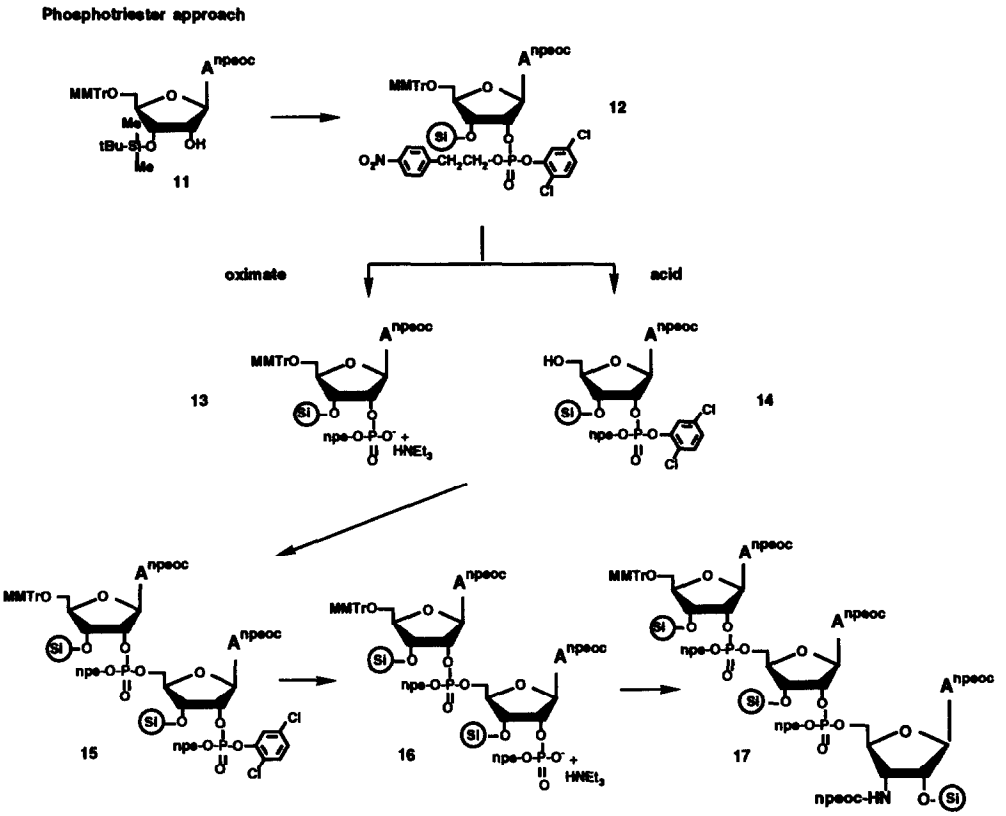


	R	R¹	R²
1	ppp	OH	OH
2	H	OH	$\overset{+}{N}H_3$
3	H	H	$\overset{+}{N}H_3$

The synthetic approach to the trimers 2 and 3 afforded 3'-amino-3'-deoxyadenosine (**4**)[8] as a 2'-terminal building block which shows interesting biological activity [9] in itself and which had to be suitably protected at its reactive functions. Acetyl, trifluoroacetyl [10] as well as tert-butoxycarbonyl groups [11] were used in already to block the 3'-amino function but we chose the p-nitrophenylethoxycarbonyl protection for the 3'-amino group since a selective reaction took place on treatment of 3'-amino-3'-deoxyadenosine (**4**) with the acylating agent **5**[12] in dry dimethylformamide due to the high nucleophilicity of the 3'- amino function. Simple dilution of the reaction mixture led to precipitation of the carbamate **6** in almost quantitative yield. For the protection of the 6-amino function the sugar hydroxyls were first silylated with trimethylsilyl imidazole, then treatedwith the acylating agent **5** in dichloromethane to introduce the p-nitrophenylethoxycarbonyl group atthe N^6 position followed by the removal of the silyl groups with pyridine/ water to give crystalline compound **7** in 81 % overall yield.In subsequent reactions the 5'-hydroxyl group was first selectively monomethoxytritylated in 88 % yield by monomethoxytritylchloride in pyridine, then the tert-butyldimethylsilyl group was introduced at the 2'-hydroxyl position to give compound **9**, which on treatment with acid gave finally the required monomeric building block **10** in high overall yield.

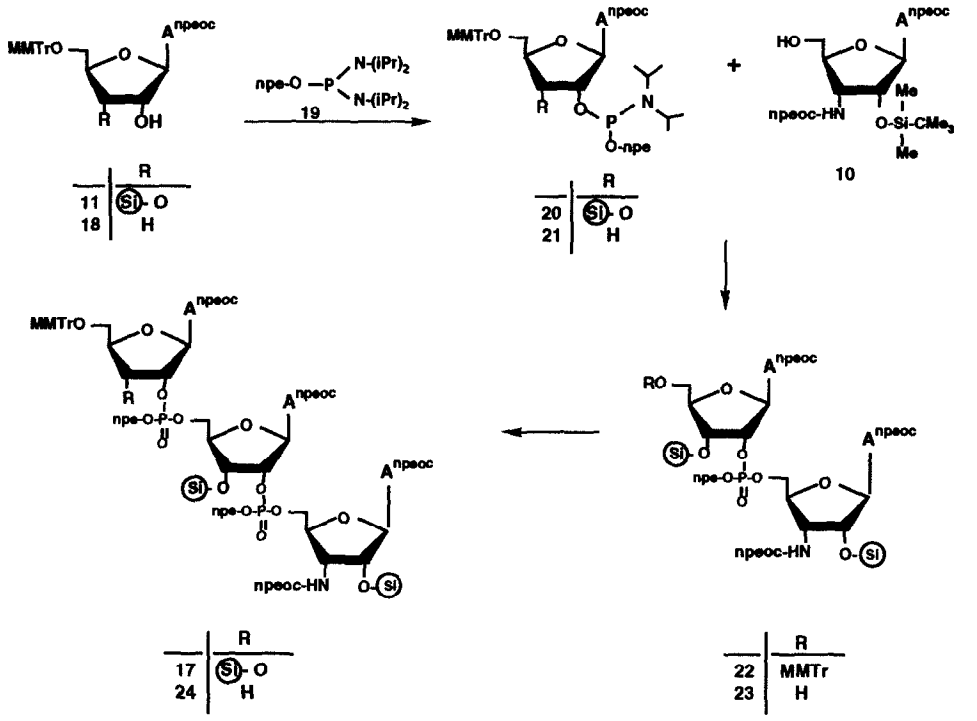


The second synthetic component for the formation of **2** was derived from 3′-O-tert-butyldimethylsilyl-5′-O-monomethoxytrityl-N^6-p-nitrophenylethoxycarbonyl-adenosine (**11**)[7,13] which was converted into the corresponding phosphotriester **12** in 85% yield by first phosphorylating with 2,5-dichlorophenylphosphoditriazolide in pyridine and subsequent addition of p-nitrophenylethanol followed by silica gel column chromatography for purification. The fully protected phosphotriester **12** was used as common precursor either for selective deprotection to the corresponding phosphodiester **13** in 92% yield using the oximate method [14] or by treatment with p-toluenesulfonic acid in dichloromethane and methanol (4/1) to give the 5′-hydroxy phosphotriester **14** in 84 % yield.Both these components **13** and **14** formed on coupling using 2,4,6-triisopropylbenzenesulfonyl chloride and N-methylimidazole in pyridine the fully blocked dimer **15** in 84 % yield which was then transferred by the oximate method into the 2′-terminal phosphodiester **16** also in good yield prone for condensation with the 5'-OH monomer **10** in an analogous manner to achieve 85% of the fully protected trimer **17**.



The same trimer **17** as well as the trimer **24** containing cordycepin at the 5′end and 3′-amino-3′-deoxy-adenosine at the 2' terminus were synthesized via the phosphoramidite approach [15] since phosphoramidites are more reactive. Compound **11** was converted into the corresponding phosphoramidate **20** by treatment with p-nitrophenylethoxy-bis-N,N-diisopropylaminophosphane **19**[16] in presence of tetrazole as catalyst and acetonitrile as solvent at room temperature for 20 h yielding 80% after silica gel column chromatography. Condensation of **20** with 2′-O-tert-butyldimethylsilyl-N^6,3′-di-p-nitrophenylethoxycarbonyl-3′-amino-3′-deoxyadenosine (**10**) under tetrazole catalysis in dichloromethane for 3h and subsequent oxidation of the phosphitetriester intermediate with iodine in pyridine/water gave the protected dimer **22** in 85 % yield after column chromatographical purification. The 5′-monomethoxytrityl group was then removed with acid to give **23** in 88 % isolated yield and the final conversion into the trimer **17** consisted of another condensation between **20** and **23** followed by oxidation and chromatographic isolation of pure material in 90% yield.

Phosphoramidite approach



The synthesis of fully protected cordycepinyl-(2'-5')-adenylyl-(2'-5')-3'amino-3'-deoxydenosine **24** was achieved from 5'-monomethoxytrityl-N^6-p-nitrophenylethoxycarbonyl-3'-deoxyadenosine **18** [17] as starting material which was converted into the 2'-phosphoramidite derivate **21** in the usual manner using the phosphitylating agent **19** and tetrazole and had been isolated as a diastereomeric mixture in 89% yield.The chain elongation to the trimer **24** resulted from a condensation reaction of **21** and the dimer **23** forming the intermediary phosphitetrister function which was subsequently oxidized by iodine in pyridine / water to the corresponding phosphotriester. As catalyst for phosporamidite activation either tetrazole or 3-nitro-1,2,4-triazole were applied with the same success leading to a final yield of the fully protected trimer **24** in 88% yield after chromatographic isolation and purification.

Both trimers **17** and **24**, respectively, were deblocked by the same subsequent sequence of reactions consisting first of acid treatment to liberate the 5'-OH group second by removal of the p-nitrophenylethyl and p-nitrophenylethoxycarbonyl groups in a ß-elimation reaction using 0.5 M DBU in pyridine and third by the cleavage of the tert-butyldimethylsilyl groups in 1M tetrabutylammonium fluoride in tetrahydrofurane. Isolation of the fully deblocked trimers **2** and **3** was performed by ion exchange chromatography on DEAE Sephadex A-25 with triethylammonium bicarbonate as the eluent and followed by evaporation and further purification by paperchromatography using the system isopropanol / ammonia / water (6/1/3), elution with water and final lyophilization gave colorless powders in 80 - 85% yield.
The purity of these trimers was checked by TLC and HPLC and their structural characterizations have been done by UV and NMR spectra.

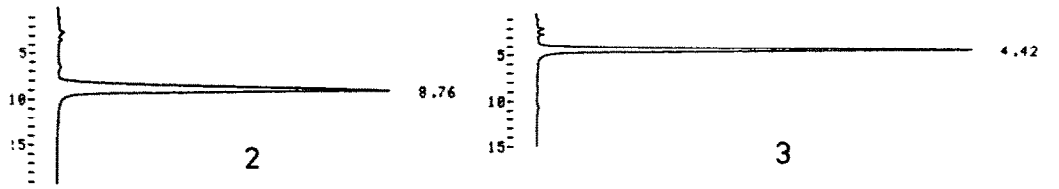


Fig.1. Purity control of **2** and **3** by HPLC on RP-18 in 0.1 M NH_4OAc / CH_3CN 95 : 5

Preliminary biological screening experiments have indicated the expected high enzymatic stability of these structures towards phosphodiesterase and an antiviral effect against HIV-1 in peripheral blood mononuclear cells in culture. More detailled studies are in progress.

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