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# Synthesis of pppA2'p5'A2'p5'A γ-amidates by one pot procedure from A2'p5'A2'p5'A

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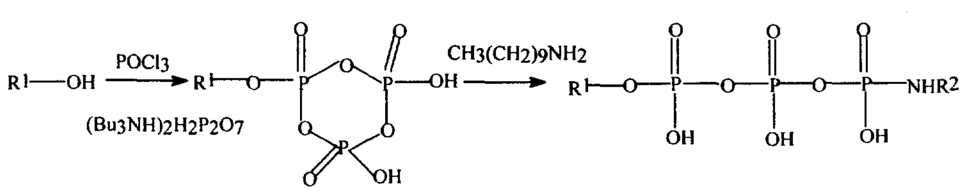
**Abstract**: One pot synthesis of γ-amidate of pppA2'p5'A2'p5'A described here using phosphoroxychloride and bis-tri-n-butylammonium pyrophosphate for preparing cyclic trimetaphosphate intermediate opening up with n-decylamin. © 1997 Elsevier Science Ltd.

The 5'-phosphorylation of 2',5'A analogues helps them to bind to endoribonuclease[1], but a phosphatase degrades it. Therefore it is important to synthesize phosphatase resistant analogues[2]. Altough ATP γ-derivatives[3], and adenosine-(β,γ-*N*-methylimido)triphosphate[4] derivatives are known, few phosphatase-resistant 2',5'A analogues have been synthesized so far. $pCH_2$ppA2'p5'A2'p5'A[5], and A5'pppA2'p5'A2'p5'A[6] were without translational inhibitory activity and lost their ability to activate RNase L. The 5'-γ-phosphorothioate $(2'\text{-}5')(A)_4$, β,γ-difluoromethylene $(2'\text{-}5')(A)_4$ failed to induce an antiviral response after microinjection in HeLa cells[7]. Uronic acid derivatives[8] were substituted the 5'-terminal adenosine residue of 2',5'A and unable to activate the mouse RNaseL, but could activate human RNaseL at concentration 100-fold greater than that required for the parent 2',5'A.

In our synthetic strategy we prepared 2',5'A with 2-nitrobenzyl (2-NBz)[9] on 3'-OH, monomethoxytrityl on 5'-OH, benzoyl for exocyclic amino and 2-chlorophenyl for the phosphate protection with phosphotriester method. The partially deprotected, 2-NBz protected 2',5'A was the starting material for the triphosphate synthesis which was carried out, as in earlier publications[10,11]. The mechanism of triphosphate synthesis gave us the idea for the preparation of γ-amidate, since the cyclic trimetaphosphate can be opened up with different amines (FIG 1).




The general procedure for the one pot synthesis of γ-amidate is the following: 2-NBz protected 2',5'A (15.3 mg, 10 μmol) was dried overnight in dessicator and dissolved in 0.5 ml $(MeO)_3P=O$. After 30 min cooling on ice 31 μl $POCl_3$ was added and the reaction mixture was stirred for 3 h at 0 °C. 2 ml Bis-tri-n-butylammonium pyrophosphate and 200 μl tributylamine was added and the reaction mixture was poured into 0.1 M triethylammonium bicarbonate (TEAB) and loaded on DEAE Sephadex A25 column which was equilibrated with 0.2 M TEAB in 50 % ethanol. Ethanol was necessary to keep the product soluble on the column. The gradient was 600-600 ml 0.2-0.6 M TEAB. The γ-amidate with 2-NBz protection was eluted between 0.3-0.4 M TEAB. The proper fractions were collected, combined and evaporated, and lyophilised from water. The yield was 75 %, $A_{260}$ units.

The deprotection of 2-NBz has been carried out 4×1 μmol scale at 360 nm of UV lamp in 60 ml $EtOH/H_2O$ = 1/1 (v/v) for 2 h and the reaction mixture was loaded on DEAE Sephadex A 25 coloumn and eluted with 0.25-0.55 M TEAB. The yield of this step was 75 %, $A_{260}$ units, $R_f$ in n-propanol/$NH_4OH/H_2O$ = 11/7/2 (v/v/v): 0.6; in 1 M NaCl: 0.15. $^{31}$P-NMR (DMSO-$d_6/D_2O$ = 1/1): -1.3; -1.5 (internucleotide phosphate); -1.8 (γ-P); -11.2 (α-P); -21.2 (β-P).

The γ-amidate was resistant to phosphatase and converted into pppA2'p5'A2'p5'A with 80 % aqueous acetic acid treatment for 2 h.

The n-decyl analogue gives similar rRNA (28S and 18S) degradation to smaller specific cleavage products in interferon-treated HeLa cell extracts - even at concentration of $10^{-9}$M - as the natural compound does[12], showing the RNaseL activating ability of the molecule.

In summary pppA2'p5'A2'p5'A γ-amidate can be synthesized by n-decylamine nucleophile, opening up the cyclic trimetaphosphate of 2',5'A. This reaction demonstrates that the procedure Ludwig[10] originally reported for the synthesis of ATP[10] and later for the synthesis of cordicepin and aracordicepin trimer triphosphates[11] can be extended to include a terminal phosphoramidate.

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