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SYNTHESIS OF ADENYLYL-(2'→5')-ADENYLYL-(2'→5')-ADENOSINE (2-5A CORE)

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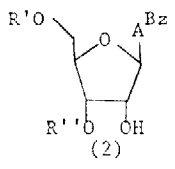
Summary. A convenient procedure to synthesise the title compound in large quantity has been described using acid and acyl protecting groups at the 5'- and 3'-positions respectively.

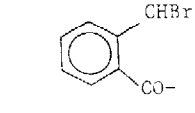
2'-5'-oligoisoadenylate Synthetase E, an enzyme produced in interferon treated cells, polymerizes ATP into a series of oligonucleotides of general structure ppp $(A_{2'p})_{n5'}A$. Of these, ppp $A_{2'p5'}A_{2'p5'}A$ was directly correlated with the antiviral activity of interferon.[1-3]. This highly charged nucleotide could not penetrate intact cells. Therefore, the action of $A_{2'p5'}A_{2'p5'}A$ (1), without 5'-phosphates, has been investigated and it has been found to be an effective inhibitor of concanavalin A stimulated DNA synthesis in mouse spleen cells[4].

The chemical routes to introduce a 2'→5' linkage for the synthesis of the core (1) involves the preparation of the building blocks (2) and (3). The protecting groups on these blocks have to be appropriately chosen to be able to operate on one functionality at a time.

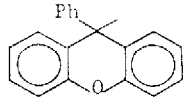


(2)

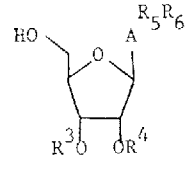
(2A), R'=OH, R''=DBMB.
(2B), R'=Pixyl, R''=DBMB.



=DBMB



=Pixyl

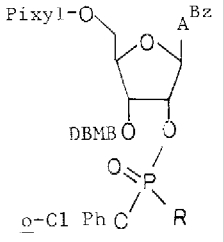


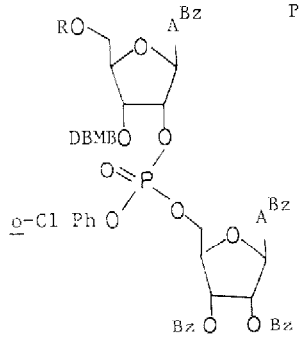
(3)

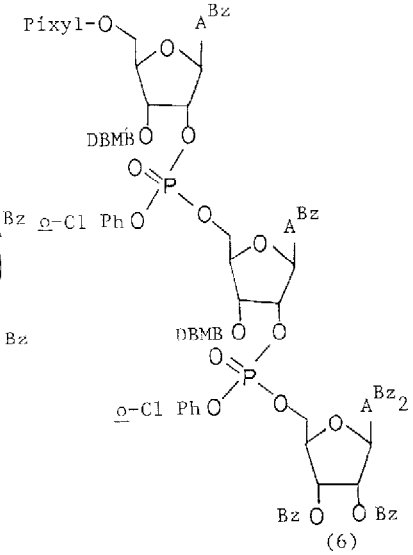
(3A), $R_3=R_4=R_5=R_6=Bz$.

Earlier workers have used (i) the combination of a base labile protecting group at 5' and an acid labile group at 3'[5], (ii) an acid labile protecting group at 5' and a neutral group at 3'-(t-butyldimethylsilyl-), removable with fluoride ion after converting internucleotide phosphotriester function to the phosphodiester level[6]. The use of an acyl protecting group has not yet been reported for the synthesis of the title compound because of the strong tendency of these acyl groups to migrate (benzoyl>acetyl>formyl) from 3' to 2' and vice versa to generate an equilibrium mixture of 2'/3' monoacylated derivatives in any protic (faster rate) or aprotic solvent. This isomerization is base catalysed[7]. Thus a commonly available acyl group at 3' position would migrate partially to 2' under a standard phosphorylating condition and this would result in an introduction of a phosphodiester or a phosphotriester function unspecifically. Now, we wish to report that a crystalline 2-dibromomethylbenzoyl (DBMB) group at 3' position has a much slower aptitude to migrate to a vicinal 2'-OH function. Thus 3'-O-2-dibromomethylbenzoyl adenosine and its 6-N-benzoyl derivative [8] were stable over eleven days in dry pyridine solution but isomerizes slowly to an equilibrium 2'/3' mixture (Ca. 0.8:3.2) in 117h. at 20°C in 2% aqueous pyridine (0.4 ml) solution[9].

This slower migration of the DBMB group than any other acyl group[7] so far reported may be due to the destabilization of the oxonium-carbonium ion intermediate by the bulky 2-dibromomethyl group. Further advantage of the 3'-O-DBMB group is that it is removable under a neutral condition[10,17] and therefore an acid labile group could be introduced at 3' before







(4A), R= 

(4B), R=$-O^{-}Et_3NH^{+}$

(5A), R=Pixyl

(5B), R=H

(6)

the deprotection of the final product (6) to (1) to minimize any neighbouring group participation. The 5'-OH function of (2A) was protected with an acid labile protecting group, (9-phenyl-9-xanthyl-(Pixyl))[11,17] by treating (2A) with a slight excess of Pixyl chloride in dry DMF - $CHCl_3$ (1:5 v/v, 20 ml/m.mol.) solution, in presence of finely powdered 4Å molecular sieves (3.5 g/m.mol) as a neutral acid scavenger[12], to give its 5'-O- Pixyl derivative (2B) as a powder (79%) after usual work up and column chromatography (2% ethanol-$CHCl_3$ was used as eluent). ^{1}H-NMR confirmed[13] that no isomerization took place under the above reaction condition. Having prepared such a building block, R_f=0.43 in solvent: A[14], with free 2'-OH group (2B), the phosphodiester function was introduced under a virtual neutral condition[15] to obtain (4B). Thus, when a solution of (2B) (1.5 g, 1.66 m.mol.) in dry acetonitrile (5 ml) was added to a solution of o-chlorophenylphosphorochloridate (815 mg, 3.33 m.mol.), 1,2,4-traizole (462 mg, 6.7 m.mol.) and Et_3N (674 mg, 6.66 m.mol.) in dry acetonitrile (50 ml) containing 4Å molecular sieves (powder, 23.5 g) and stirred at 20°C for 25 min. a product with R_f=0 in solvent: A was formed which gave positive test for Pixylated derivatives[14a]. The monotriazolide (4A), R_f=0.31 in solvent:B, formed could, within 10 min., be hydrolyzed to (4B) by the addition of a mixture of Et_3N (6.66 m.mol.) and water (10 m.mol). The reaction was filtered and the residue was washed with $CHCl_3$ (6x25 ml) and was then worked up following published[17] procedure to obtain pure (4B), R_f=0.21 in solvent: B, as white powder (1.84 g, 92%). The building block (4B)[16] (781 mg, 0.653 m.mol.) was then condensed with N^6, N^6,$O^{2'}$,$^{3'}$-tetrabenzoyl adenosine (3A) (369 mg, 0.54 m.mol) in dry pyridine (0.5 ml) using 1-mesitylenesulphonyl-3-nitro-1,2,4-triazole(MST-NT)[17] (1.48 g, 5 m.mol.) at 20°C for 70 min. Following a standard work up[17] and column chromatogrpahy (4.5% ethanol-$CHCl_3$), the fully protected dinucleoside monophosphate (5A), R_f=0.36 in solvent: A, was obtained (831 mg, 87%). This material was depixylated[17] to unmask the 5'-OH function using 4-toluenesulphonic acid. H_2O

in 2% ethanol-$CHCl_3$ at 20°C to obtain (5B) (643 mg, 91%), R_f=0.27 in solvent:A. This was further condensed with (4B) (561 mg, 0.47 m.mol.) in dry pyridine (0.4 ml) solution using MST-NT (1.39g, 4.7 m.mol.) as condensing agent in the above reaction condition. Thus the fully protected trinucleoside diphosphate (6) was obtained (781 mg, 71%), R_f= 0.41 in solvent:A, after usual work up and column chromatography (5.4% ethanol-$CHCl_3$). The protecting groups from (6) (22mg) were removed[17] in the following order to obtain (1):(i) DBMB group was removed using $AgClO_4$ (16 parts) 2,4,6-collidine (9 parts) in 98% aq.acetone, followed by treatment with morpholine, (ii) 3'-OH was then blocked reacting this with Pixyl chloride in pyridine solution, (iii) treatment with N^1,N^1, N^2,N^2-tetramethylguanidinium-syn-4-nitrobenzaldoximate in dioxane-water (1:1 v/v) at 20°C for 19h., (iv)aq. NH_3 (d0.88) at 20°C for 31h., (v) 80% aq. CH_3COOH for 15 min at 20°C (vi) aq NH_3(d0.88) at 20°C for 5 min. The (1), thus obtained, was purified through a DEAE-Sephadex A25 column eluting with $Et_3NH^+HCO_3$ buffer linear gradient (pH 7.6, 0.001-0.5M, 200 ml each), 5.7 mg, 79.6%, R_f=0.9 (solvent:B), 0.64 (solvent:C), ^{1}H-NMR (DMSO-d_6:D_2O::1:1 v/v)[6]: 7.7,7.85,7.96,7.83,7.97,8.03 (aromatic protons, H-2 and H-8), 5.76(d,J=3.5Hz), 5.9(d,J=4Hz) and 6.04(d,J=4.3Hz). UV(pH7.6):λ_{max}258.5 (ε32.362).

The trimer(1) was completely digested by Crotalus adamantus snake venom phosphodiesterase and was totally resistant to T_2 RNase which cleaves only 3'→5' diester bond.

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3'-O-DMB-adenosine and its 6-N-benzoyl derivative were prepared by the reaction of 2',3'-O-dibutylstannylene derivatives (20 mmol) of adenosine and 6-N-benzoyl adenosine in methanol (200 ml) solution with DBMB-Cl (100 mmol) and Et_3N (100 mmol) at room temp. The crystals of the title compounds separate out from the reaction mixture on standing in 72 and 83% yield respectively. Correct microanalytical data have been obtained for these compounds.3'-O-2-dibromomethylbenzoyl adenosine, mp 197° ^{1}H-NMR(DMSO-d_6+D_2O):3.72 (br.s, 2H,$C_{5'}H_2$), 4.4(m,1H,$C_{4'}H$),5.09(dd,1H,$J_{1',2'}$=8Hz,$J_{2',3'}$=5.5Hz,$C_{2'}H$, collapsed to a doublet when $C_{1'}H$ was irradiated), 5.68(dd,1H,$C_{3'}H$), 6.1(d,1H,$C_{1'}H$),7.4-8.18(m,total 5 protons of DBMB group), 8.23 and 8.4 (s,1H each, C_2H and C_8H).
3'-O-2-dibromomethylbenzoyl-6-N-benzoyl adenosine, mp 153° ^{1}H-NMR(DMSO-d_6+D_2O): 3.81(br.s, 2H,$C_{5'}H_2$), 4.45(m,1H,$C_4'H$),5.16(dd,1H,$J_{1',2'}$=6.5Hz,$J_{2',3'}$=5Hz,$C_{2'}H$, collapsed

to a doublet when C_1'H was irradiated), 5,58(dd,1H,C_3'H), 6.2(d,1H,C_1'H), 7.4-8.2 (m, total 10 aromatic protons from 3'-O-DBMB and 6-N-benzoyl group) 8.74 and 8.77 (s,1H each, C_2H and C_8H).

9. The ratio of the isomers in the equilibrium mixture was determined by comparison of the integration of NMR absorption of the anomeric protons in Pyridine-d_5 with 2% of D_2O solution:(i)3'-O-DBMB adenosine (6.89,J=5Hz for 2' isomer, 6.6,J=7.5Hz for 3' isomer), (ii)3'-O-DBMB-6-N-Bz adenosine (6.64,J=4.8Hz for 2' isomer, 6.34, J=8Hz for 3' isomer). See Fromageot et al., Tetrahedron, 22, 705 (1966) for the rule of ^{1}H-NMR characterization of 2' and 3' isomer.
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13. ^{1}H-NMR($CDCl_3$+CD_3OD): 3.38(br.s, 2H, C_5'H_2), 4.53(br.d, 1H,$J_{3',4'}$=3Hz,C_4'H), 5.12(dd,1H,$J_{1',2'}$=5Hz$J_{2',3'}$=5Hz,C_2'H, collapsed to a a doublet on irridiation at C_1'H), 5.65(dd,1H,C_3'H), 6,18(d,1H,C_1'H), 6.8-8.24 (m, total 23 protons). When this compound was allowed to equilibrate in Pyridine-d_5+2% D_2O for 102h., it gave two sets of anomeric protons (i) (6.54,d,J=3Hz for 2' isomer), (ii) (6.37,d,J=6Hz for 3' isomer) in ratio of Ca. 0.95:3.05.
14. TLC was carried out on Merck silica gel 60 F_{254} plates in solvent system A ($CHCl_3$: methanol(9:1 v/v) and on DC-plasticfolien cellulose F_{254} in solvent system B (i-PrOH:-NH_3 (d0.88): water (55:109:35 v/v)) and C (i-butyric acid:NH_3 (d0.88):water (66:1:33 v/v)).

14a No hydrolysis of the Pixyl group could be detected during the preparation of the phosphodiester salt. 9-hydroxy-9-phenylxanthen and its derivatives could be detected on TLC, both on cellulose and on silica gel upto 10^{-9}M concentration under a 366 nm UV lamp after spraying the plate with 10% H_2SO_4 and warming it slightly.

15. Pyridine(pK_a5.25) is normally employed as a solvent or a part of the mixed solvent system used in general phoisphorylation methods[17]. Alternatively, 2,6-lutidine or a derivative of imidazole is used in a solvent like CH_3CN, dioxan or CH_2Cl_2 (see the Tetrahedron report number 26 (C.B. Reese, Tetrahedron, 34, 3143 (1978) for an extensive review on phosphorylation methods and conditions).
16. That the phosphodiester group has been specifically introduced at 2' position of (4B) has been determined by isolation and characterization of 6-N-benzoyl adenosine-2'-O-2-chlorophenyl phosphate by removing Pixyl group from 5' position (with 4-toluenesulphonic acid. H_2O in 2% ethanol-$CHCl_3$) and the 3'-O-DBMB group (with $AgClO_4$ (16 parts) 2,4,6-collidine (9 parts) in 98% aq.acetone, followed by morpholine treatment)[17]. ^{1}H-NMR (DMSO-d_6+CD_3OD): 4.4(m, 1H,C_4'H), 4.91(dd,1H,C_3'H), 5.29(ddd,1H,C_2'H collapsed to a double doublet (due to coupling with phosphorus) when C_1 H was irridiated), 6.36 (d,1H,C_1'H), 6.8-7.48(m, 5 aromatic protons of o-chlorophenyl group), 7.5-8.06 (m, 5 aromatic protons of 6-N-benzoyl group).
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