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THE TETRAISOPROPYLDISILOXANE-1,3-DIYL: A VERSATILE PROTECTING GROUP FOR THE SYNTHESIS OF ADENYLYL-(2'→5')-ADENYLYL-(2'→5')-ADENOSINE (2-5A CORE).

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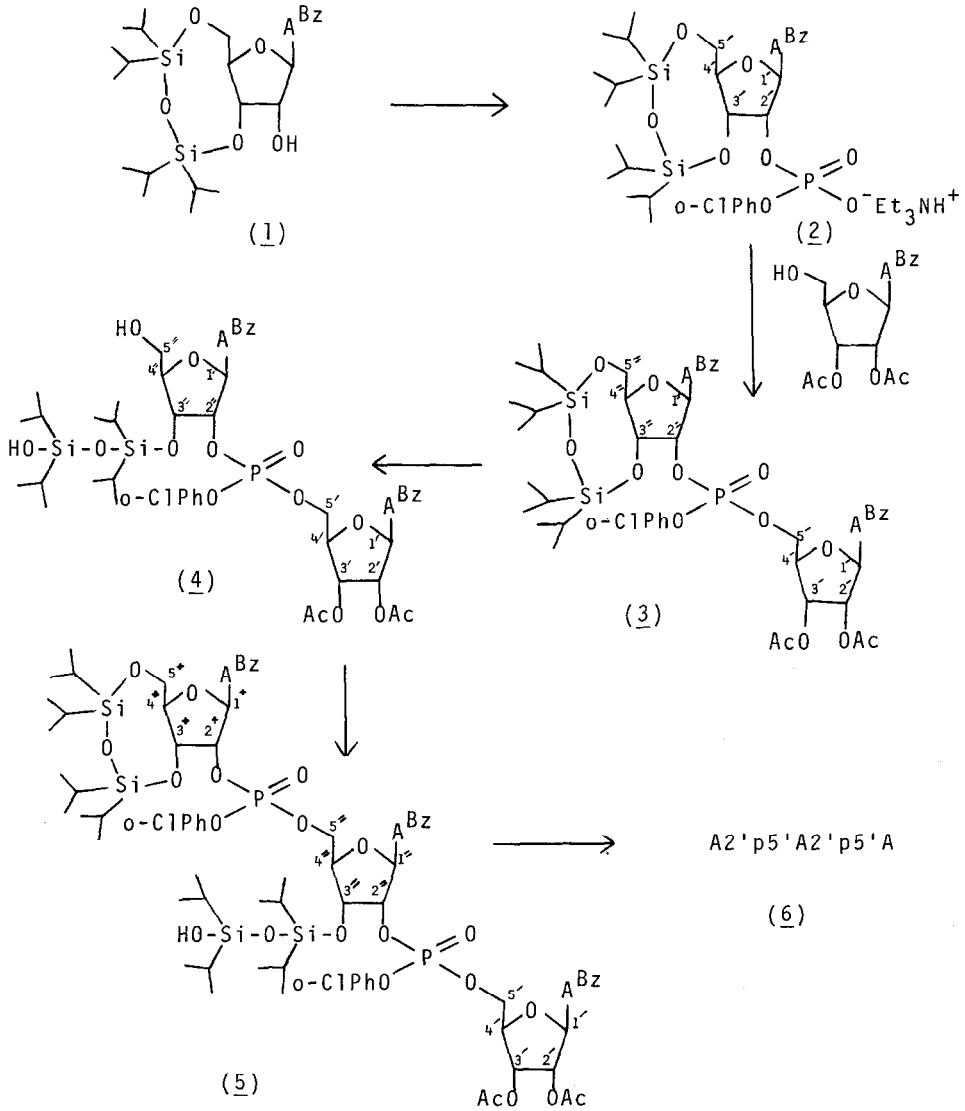
Summary: A2'p5'A2'p5'A has been synthesised in large scale using 3', 5'-tetraisopropyldisilyl-6-N-benzoyl adenosine as the starting material.

The antiviral and the antitumour activity of interferon has been directly correlated to the production of pppA2'p5'A2'p5'A in the interferon treated cells[1]. The core, A2'p5'A2'p5'A, has also been found to be remarkably active in certain biological systems[2]. These observations have triggered several chemical synthesis[3] of the core through different routes. We have recently reported[4] such a chemical synthesis using 9-phenylxanthen-9-yl and 2,2-dibromomethylbenzoyl groups for 5'- and 3'-hydroxy protection respectively. Most of the other syntheses[3] so far reported in the literature, like our own synthesis, make use of two separate protecting groups at 5'- and 3'-hydroxyls which are then removable under a different set of conditions.

We now report a chemical synthesis of the core(6) using a bifunctional protecting group, tetraisopropyldisiloxane-1,3-diyl(TIPDSi)[5], for simultanous 3'- and 5'-hydroxy protection of 6-N-benzoyl adenosine. The 3',5'-O-TIPDSi-6-N-benzoyl adenosine[16] can be obtained in 10g quantity in one step from 6-N-benzoyl adenosine. The 3',5'-O-TIPDSi protecting group has been reported to migrate[6], in presence of an acid when DMF is used as solvent, to 2',3'-O-TIPDSi derivative. However, it is stable[5] in most of the reaction conditions which are normally employed for the introduction of a phosphodiester or a triester function. Thus we introduced a phosphodiester function at 2'-position of (1) by reacting (1) with o-chlorophenylphosphorobis-(1,2,4-triazolide) under a standard condition reported in the literature[7]. That the triethylammonium phosphodiester function was specifically introduced at 2'-position in (2), was unequivocally ascertained by the characteristic downfield shift of H-2' absorption (δ5.4) in the ^{1}H-NMR spectrum of (2)[8] when compared with ^{1}H-NMR spectrum of (1)[5] (δ4.6 dd, $J_{1',2'}$=0.6Hz, $J_{2',3'}$=3HZ; H-2'). The pure phosphodiester salt (2)[8], R_f^{14}=0, (1.26 mmol) was then condensed with 6-N-benzoyl-2',3'-di-O-acetyl adenosine (0.84 mmol) in dry pyridine solution (15 ml) in presence of an excess of 1-mesitylenesulphonyl-3-nitro-1,2,4-triazole (MS-NT)[7,14] to obtain a pure, fully protected dinucleoside monophosphate (3)[9], R_f^{14}=0.66, in 87% isolated yeild. We could then selectively unmask[5,10] the 5'-hydroxyl function by treating (3) for 1 h at 20°C with 0.2M HCl in dioxane solution (10 ml).


(1)
(2)
(3)
(4)
(5)
A2'p5'A2'p5'A
(6)

The selective hydrolysis of 3',5'-O-TIPDSi derivative (3) to 5'-hydroxy dinucleoside monophosphate (4)[11] was quantitative and the product (4)[17] was homogenous on TLC, R_f[14]=0.62. This could be directly used for the second condensation reaction with a slight excess of (2) under a identical condition to that of the first condensation. The reaction resulted in a single product which was isolated in 94% yield after a usual work-up and a chromatography over a short column of silica gel. The product, R_f[14]=0.69, thus obtained, could be conveniently identified as fully protected A2'p5'A2'p5'A (5) by ^{1}H-NMR spectroscopy[12]. The (5) was deprotected to (6) in the following order: (i) 4-nitrobenzaldoximate ion in aq. dioxane[13] for 18h at 20°C, (ii) aq. NH_3(d0.9) for 50h, (iii) n-Bu_4NF (0.3M) in THF-pyridine 8:2,v/v at 20°C for 6h. The core was then purified through a DEAE-Sephadex A 25 column using $Et_3NH^+HCO_3^-$ (pH 7.6) for gradient elution (0.001M-0.6M) and was obtained in 91% isolated yield. Finally, the identity of the product was confirmed with an authentic sample[4] by ^{1}H-NMR, UV and TLC in two different solvent systems[15]:(a) R_f=0.9 in i-Pr-OH:NH_3(d0.9):H_2O (55:109:35,v/v) and (b) R_f=0.64 in i-butyric acid:NH_3(d0.9):H_2O (66:1:33,v/v). The product, A2'p5'A2'p5'A, 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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8. ^{1}H-NMR ($CDCl_3+D_2O$):δ8.75(s,1H),H-2,8.2(s,1H),H-8, 8.08(m,2H),1-&5-H of6-N-Bz, 7.28(m,4H),o-ClPh, 6.32(d,$J_{1',2'}$=2.5Hz),H-1', 5.4(m,2H),H-2'&3', 4.3(m,1H),H-4', 4.15 (m,2H),5'-CH_2, 3.05(q,6H), 2.65(m,4H) and 1.15(m,33H).^{31}P-NMR($CDCl_3$):-6.0.

9. ^{1}H-NMR ($CDCl_3+D_2O$): 8.71(s,1H),8.65(s,1H),H-2 protons, 8.45(s,1H)&8.27 (s,1H),H-8 protons, 8.02(m,4H),1-&5-H of 6-N-Bz, 7.44(m,6H),2-,3-&4-H of 6-N-Bz,7.25(m,4H),o-ClPh, 6.4(d,$J_{1',2'}$=2Hz,1H)&6.3(d,$J_{1',2'}$=2.5Hz,1H) are anomeric protons,5.75(m,3H),H-2",H-2'&H-3',5.08(m,1H),H-3",4.6(m,3H),5'-CH_2, &H-4",4.18(m,1H),H-4',4.06(m,2H),5"-CH_2,2.7(m,4H),2.06(s,3H)&1.94(s,3H),two acetate groups,1.06(m,24H).

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11. ^{1}H-NMR ($CDCl_3+D_2O$):δ8.77(s,1H),8.69(s,1H),H-2 protons,8.4(s,1H)&8.23(s,1H), H-8 protons,8.0(m,4H),1-&5-H of 6-N-Bz,7.5(m,6H),2-,3-&4-H of 6-N-Bz, 7.25(m,4H),o-ClPh,6.32(d,$J_{1',2'}$=2.5Hz,1H)&6.24(d,$J_{1',2'}$=1.8Hz,1H) are assigned to two anomeric protons,5.68(m,3H),H-2",H-2'&H-3',4.91(m,1H),H-3", 4.28(m,4H),H-4',H-4"&5'-CH_2,3.84(m,2H),5"-CH_2,2.7(m,4H),2.12&2.03(two s, 3H each),1.05(m,24H).

12. ^{1}H-NMR ($CDCl_3+D_2O$):δ8.76(s,1H),8.67(s,1H) & 8.69(s,1H),H-2 protons, 8.36(s,1H),8.33(s,1H)&8.31(s,1H),H-8 protons, 8.12(m,6H),1-&5-H of 6-N-Bz, 7.5(m,9H),2-,3-&4-H of 6-N-Bz,7.3(m,8H),o-ClPh,6.45(d,$J_{1',2'}$=3.5Hz,1H), 6.28(d,$J_{1',2'}$=3.1Hz,1H),6.22(d,$J_{1',2'}$=3.8Hz,1H) are anomeric protons, 5.7(m,4H),H-2^+,H-2"&H-2',H-3',5.1(m,2H),H-3^+&H-3",4.5(m,7H),H-4^+,H-4", H-4',5'-CH_2&5"-CH_2,4.1(m,2H),5^+-CH_2,2.6(m,4H),2.19(s,3H) & 2.13(s,3H),two acetate groups, 1.0(m,24H).

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14. Merck Silica gel 60 F_{254} pre-coated plates have been used for TLC in 10% Methanol - $CHCl_3$ solvent system.

15. DC-plasticfolien cellulose F_{254} sheets have been used.

16. HPLC analysis showed that (1) was pure and free of 2',3'-isomer (reverse phase C_{18} column: linear gradient, H_2O to 8% CH_3CN-H_2O , R_T=11.7 min for 3',5'-isomer (1) and R_T=9.8 min for 2',3'-isomer).

17. HPLC (reverse phase C_{18} column: linear gradient, H_2O to 11% CH_3CN-H_2O , R_T=14.3 min) showed a single compound and thus, it is concluded that the cleavage of (3) to (4) was virtually regiospecific.

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