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SYNTHESIS OF GUANOSINE--3'- (5- bromo -4- chloro-3 -hyroxyindolyl)- PHOSPHATE(G- 3 '- BCIP)

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ABSTRACT

N^2-Benzoyl-5'-*O*-dimethoxytrityl-2'-*O*-tetrahydropyranyl guanosine and 1-acetyl-5-bromo-4-choroindol -3-yl-3-phosphorodichloridate were synthesized and coupled to give, after deprotection and purification, the title compound.

INTRODUCTION

Chromogenic substrates are routinely used for spectroscopic analysis of enzyme activity [1,2]. The 5-bromo-4-chloroindol-3-yl group may be a particularly useful group in identifying ribonuclease activity. Guanyl specific ribonucleases should cleave the bond between the phosphate and the chromophore.

Oxidation of the resulting 5-bromo-chloroindol-3-ol to a dimeric product results in an important absorbance increase[3] between 600 and 700 nm. For this reason a synthetic rout to the title compound has been developed.

Synthesis of G-3'-BCIP **9** was performed by coupling compounds **5** and **7**.

The N^2-benzoyl-5'-*O*-dimethoxytrityl-2'-O-tetrahydropyranylguanosine (compound **5**) was synthesized (Scheme 1)from N^2-benzoylguanosine **1** [4] by protecting the 3' and 5' hydroxyl groups using 1,3-dichloro-1,1,3,3-tetraisopropyl disiloxane[5] in anhydrous pyridine to produce, after workup and silica gel chromatography product **2** in good yield.

Compound **2** was treated with 2,3-dihydropyran (DHP) in anhydrous dioxane in the presence of p-toluenesulphonic acid [6] yielding compound **3** that was used in the next step without purification. Treatment of **3** with KF/Et_4NBr to cleave the 3', 5'-silyl protecting group as described by T.Kamimura et al [7] yielded, after workup and silica gel chromatography, compound **4** in good yield.

Compound **4** was found to be a mixture as observed by TLC of two diastereoisomers due to the tetrahydropyranyl group.

A small amount of this mixture was purified by column chromatography and the lower R_f isomer was obtained pure for ^{1}H-NMR spectra. Treatment of product **4** with 4,4' -dimethoxytritylchloride (DMTCI) in anhydrous pyridine[7] gave after purification by silica gel chromatography product **5** as a pair of diastereoisomers detected by TLC. Purification of a small amount of this mixture by column Chromatography gave the lower *Rf* isomer in pure form whose ^{1}H-NMR spectra was reported. Selective deacetylation of 3-Acetoxy-1- acetyl- 5- bromo-4-chloroindole at the 3- position[8] with 80% H_2SO_4 gave compound **6** which was phosphorylated with $POCl_3$ as described by Scheraga et al [3] yielding compound **7** (Scheme 2). Coupling of **7** with **5** in dry pyridine catalyzed by 1,2,4-triazole at room temperature was monitored by TLC and after 40 minutes quenched by triethylammonium bicarbonate (TEAB). The crude product obtained was subjected to silica gel chromatography.

It was noticed that there is a partial decomposition of the trityl group during the column chromatography, so all the fractions containing the required product were collected together, evaporated to dryness and treated with 80% AcOH to affect detritylation and to cleave the tetrahydropyranyl group[3].

Finally, the benzoyl and the acetyl groups were cleaved using concentrated ammonium hydroxide[9-11]. All the products synthesized were characterized using ^{1}H-NMR, mass spectra and ^{31}P-NMR for the final product. Compound **9** was tested against ribonuclease T_1, the details of the enzymatic study will be published elsewhere.

EXPERIMENTAL

^{1}H-NMR spectra were recorded with a Bruker AC 250 spectrometer, operating at 250 MHz. with TMS as internal standard. Chemical shift values are given in ppm. Proton decoupling ^{31}P-NMR spectra were measured with Bruker WP80 operating at 32.37 MHz. Chemical shifts are given in ppm downfield from 85% aqueous H_3PO_4 as an external standard Analytical TLC was performed on silica gel F_{254}(Merck). Detection of spots was effected by UV light and also charring with

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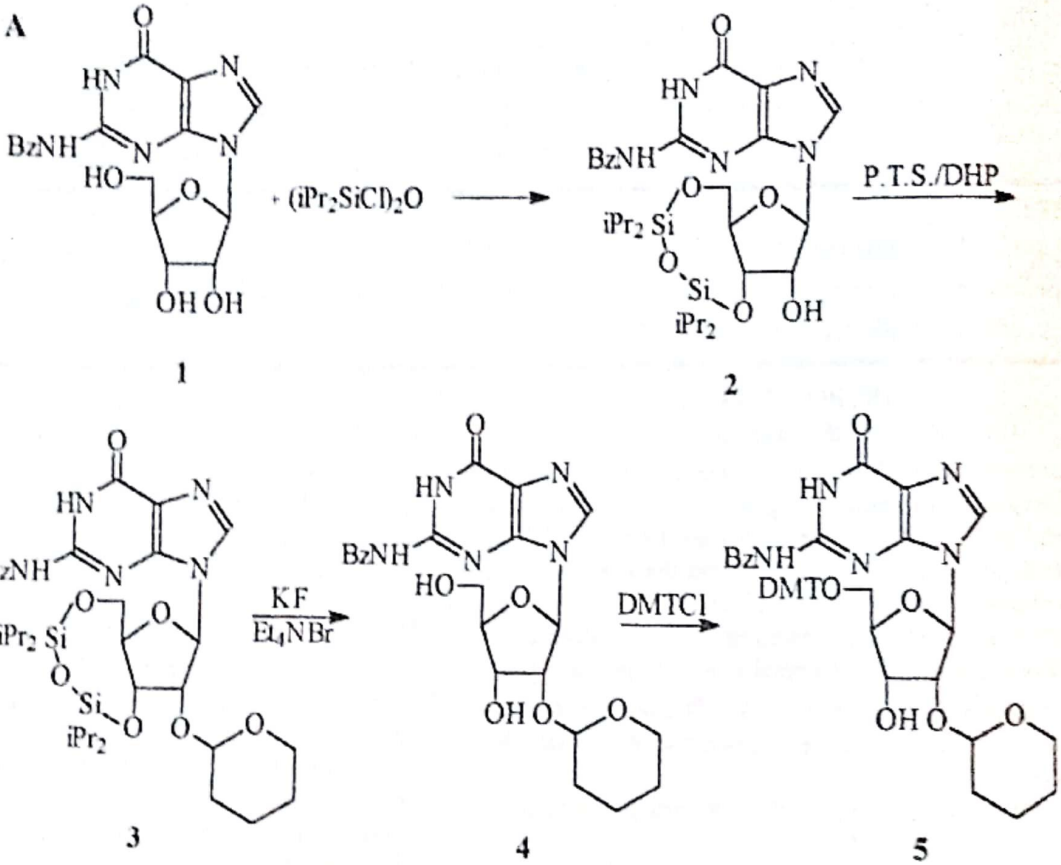
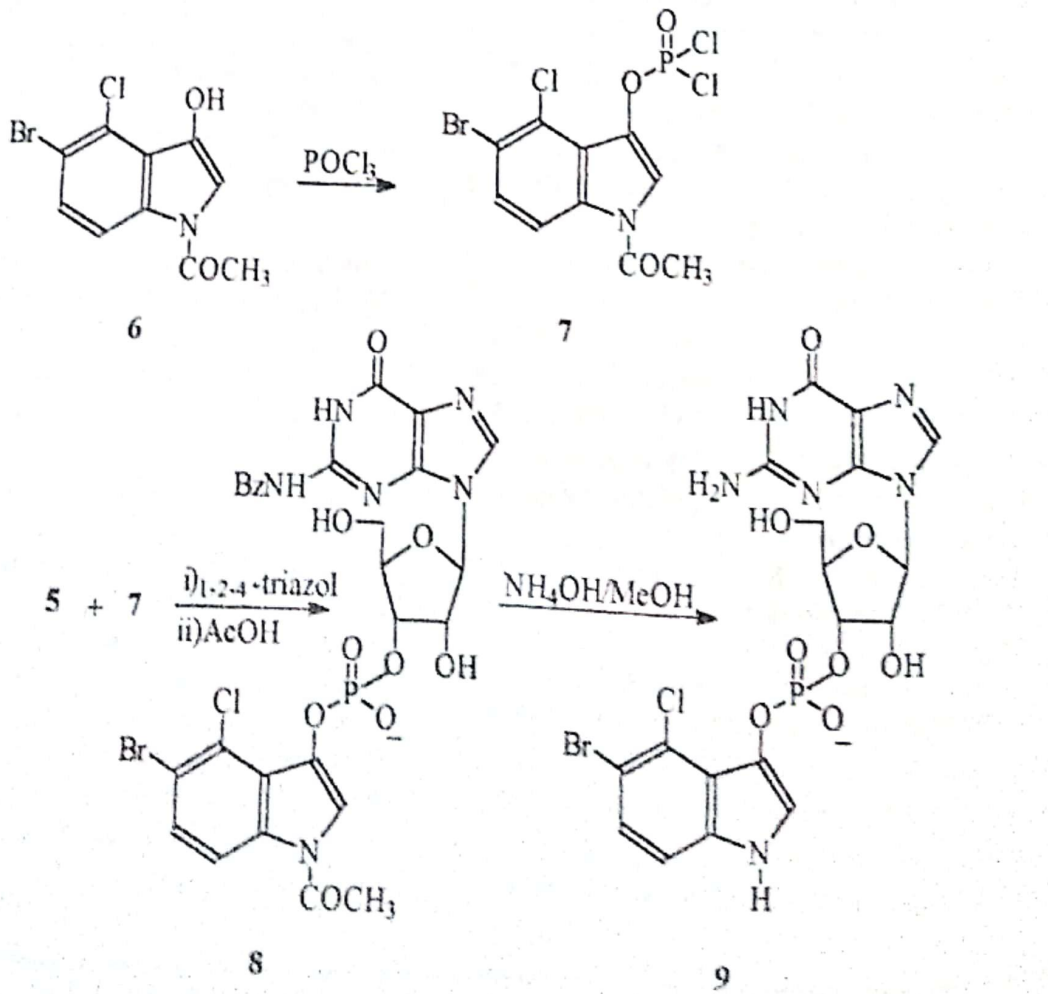
Scheme A



1 2 3 4 5

Scheme B



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10% sulfuric acid in ethanol 95%. Preparative HPLC was carried out using a VYDAC C18 column (1x25cm) particle size 10μ. Pyridine was distilled over Ca H_2 and stored over potassium hydroxide.

N^2-Benzoyl-3',5'-di-*O*- (tetra-isopropyldisiloxane-1,3-diyl)guanosine (2):

1,3 -Dichloro-1,1,3,3- tetraisopropyldisiloxane (2.71g, 8.6 mmol) was added to a solution of N^2 -benzoyl-guanosine(3g, 7.75 mmol) in anhydrous pyridine (25 mL).The reaction mixture was stirred at room temperature overnight and evaporated under reduced pressure to a small volume.

The residue was partitioned between chloroform (60 mL) and saturated aqueous sodium bicarbonate (60 mL), the chloroform layer was washed with water (2x30 mL), dried over anhydrous sodium sulphate and finally purified over silica gel column chromatography (chloroform/methanol 97:3), to obtain compound **2** (3.8 g, 78%). R_f 0.54 ($CHCl_3$ /CH_3OH 9.5:0.5); m/z=629; ^{1}H-NMR (DMSO-d_6/D_2O) δ 8.1 (s 1H; H-8), 8.06 - 7.51 (m; 6H aromatic), 5.91 (s; 1H; H-1'), 4.48 (q; 1H; H-3'), 4.33 (dd;1H; H-2'), 4.03 (m; 3H; H-4' H-5', H-5"), 1.06 (m, 28H; TIPDS protons). Elemental analysis ; calculated for $C_{29}H_{43}N_5O_7Si_2$: C 55.30; H. 6.88, N 11.12 ; Found: C 55.45, H 6.82, N 11.23.

N^2- Benzoyl -2'-*O*- tetrahydropyranylguanosine (4):

To a solution of 2 (1.5 g, 2.4 mmol) in dioxane (15mL) containing P- toluenesulphonic acid monohydrate (0.12 g), was added 2,3-dihydropyran (4.05 mL) and the reaction was stirred for 6 h at room temperature.

After neutralization with concentrated ammonium hydroxide and filtration of ammonium tosylate, the solution was washed with CH_2Cl_2 and the washings and filtrate were evaporated under reduced pressure. The residue **3** was dissolved in CH_3CN (40 mL) and KF (1.1 g, 18.7 mmol), Et_4NBr (3.9 g, 18.7 mmol) and H_2O (1 mL)were added.

The mixture was stirred for 45 minutes at 50°C and then concentrated. The successive extractive workup and silica gel chromatography ($CHCl_3$ /MeOH 9:1) gave **4** (1.02 g, 91%) as a mixture of two diastereoisomers with R_f = 0.30 and 0.40 ($CHCl_3$// .MeOH 9:1); m/z= 471. ^{1}H-NMR (DMSO-d_6/ D_2O) of the lower R_f fraction: δ 8.2 (s, 1H; H-8),7.98 - 7.47 (m; 5H aromatic), 5.99 (d; 1H; H-1'; $J_{1',2'}$ = 6.65Hz), 4.81 (m, 1H, tetrahydropyranyl acetal proton) 4.46-4.20 (m, 2H, H-3' and H-4'), 3.98 (m, 1H, H-2'), 3.64 (m, 2H, H-5' and H-5"), 3.41 (t, 2H, O-CH_2 of tetrahydropyranl group), 2.12 (m; 6H; three CH_2 of tetrahydropyranl group). Elemental analysis; calculated for $C_{22}H_{25}N_5O_7$:C 56.05, H 5.34, N 14.85. Found: C 56.25, H 5.42, N 14.73.

N^2- Benzoyl- 5'-*O*- dimethoxytrityl -2'-*O*- tetrahydro pyranylguanosine (5):

Compound 4 (0.8 g, 1.7 mmol) was coevaporated with anhydrous pyridine (3x 10 mL) and dissolved in anhydrous pyridine (15 mL). To this solution 4,4'-dimethoxytritylchroide (0.68 g, 2.03 mmol) was added and the reaction mixture was stirred at room temperature and followed by TLC. After disappearance of the starting material (one hour), water (3 mL) was added and the mixture was extracted with chloroform; the choroform layer was dried over anhydrous sodium sulfate and evaporated.

The residue was chromatographed on a silica gel column (chloroform/ methanol 9.7: 0.3) to give **5** (1.1 g, 84%) as a mixture of two diasteroisomers with R_f 0.43 and 0.33 (Chloroform/ methanol, 9.5:0.5); m/z=773; ^{1}H-NMR ($CDCl_3$/ D_2O) of the lower fraction δ 8.0 (s; 1H; H-8), 7.69-6.92 (m; 18 H aromatic), 5.91(d; 1H; H-1'; $J_{1',2'}$= 7.19Hz), 4.79 (m; 1H; tetrahydropyranyl acetal proton), (4.46-4.41 (m; 2H; H-3' and H-4'), 3.96 (m; 1H;H-2'), 3.71 (m; 2H; H-5' and H-5"), 3.40 (t; 2H; O-CH_2 groups), 3.60 (s; 6H; O-CH_3), 2.15 (m; 6H three $CH_2$of tetrahydropyranyl group). Elemental analysis; calculated for $C_{43}H_{43}N_5O_9$:C, 66.74; H, 5.60 ;N, 9.05; found C, 66.95; H, 5.43, N, 9.13.

1-Acetyl-5-bromo-4-chloro-3-hydroxy-indol (6):

80% H_2SO_4 was added dropwise into a beaker containing 3-acetoxy-1-acetyl-5- bromo-4-chloroindol (0.5g, 1.5 mmol) while stirring at room temperature for one hour and then poured onto ice water with stirring for 30 minutes.

The solid was filtered and washed with a solution of 0.1M sodium acetate until the washings were neutral, and then with water and dried over P_2O_5 giving **6** (0.4g, 92%) as a solid, m/z= 288, ^{1}H-NMR ($CDCI_3$) δ 8.38 (d, 1H),7.81(d; 1H), 4.34(s; 2H, keto-enolic protons), 2.31 (s; 3H; CH_3 acetyl).

Guanosine- 3'-(5-bromo-4-chloro-3-hydroxyindolyl)-Phosphate (G-3'- BCIP) (9):

To a solution of **5** (0.35g, 0.48 mmol) and of 1,2,4-triazole (0.164 g, 2.4 mmol) in dry pyridine (3ml), compound **7** (0.588 g, 1.43 mmol) was added and the reaction was stirred under argon for 40 minutes and quenched with 1M TEAB (2 mL) and evaporated to dryness under reduced pressure.

The residue was applied to silica gel column and eluted with ($CHCl_3$/MeOH/Et_3N 93: 4: 3). All the fractions containing the product (even partially detritylated) were collected together, evaporated and dissolved in 80% AcOH (15 mL), stirred during 1h at room temperature, evaporated to dryness and coevaporated with water (3x10 mL) to eliminate all traces of AcOH. The residue was treated with concentrated NH_4 OH (15mL) at 3°C for 15 hours and then evaporated.

The residue was purified by flash column chromatography using as eluent (n-butanol/water/methanol, 75:15:10) and repurified by preparative HPLC using a linear gradient of 1M ammonium acetate pH 6(A) and CH_3CN (B) (from 100% of A to

100% of B in 25 minutes), to give **9** (30 mg; 10%. *Rt* 8.33min.) as a white mousse ^{31}P-NMR (D_2O), δ 1.6 ; ^{1}H-NMR(D_2O), δ 7.97 (s, 1H, H-8), 7.38-7.23 (m, 3H aromatic), 5.76 (1H, H-1', $J_{1',2'}$= 3.32Hz), 4.26 (m,1H, H-3'), 4.08 (t, 1H, H-2'), 3.95 (dd, 1H, H-4'), 3.86 (dd, 2H, H-5') and H- 5'') . The triethylammonium salt of **9** (G-3'-BCIP) was exchanged by ion exchange chromatography using a column (15 mL) of Dowex 50W-X4 Na^+ form (200-400 mesh), using water (30 mL) as eluent followed by lyophylization.

HRMS (FAB) (sodium salt) m/z calculated 611.9537, found 611.9539. Compound **9** was tested against ribonuclease T1 for 3 hr giving a blue pigment as expected.

REFERENCES

1. Blake; M.S., Johnston; K.H., Russell-Jones; G.J., and Gotschlich; E.C.; **Analytical Biochem.**, 136, 175 (1984).
2. Horwitz; J.P., Chua; J., Curby; R.J., Tomson; A.J., Da Rooge; M.A., Fisher; B.E., Mauricio; J., and Klundt; I.; **J. Med. Chem.**, 7,574 (1964).
3. Witmer; M., Falcomer; C., Weine; M.R., Kay; M., Belgley; T., Ganem; B. and Scherage; H.; **Nucleic Acids Research**, 19,1 (1991).
4. Chladek; S. and Smrt; J.; **Collect. Czech. Chem. Commun.**, 29, 214 (1964).
5. Markiewiez; W.T.; **J. Chem. Research (M)**, 1181; (1979) ;(S) 24,(1979)
6. Gregoire; R.J. and Neilson T.; **Can. J. Chem.**, 56, 487 (1978).
7. Kamimura; T., Tsuchiya; M., Urakami; K., Koura; K., Sekine; M., Shinozaki; K., Miura; K. and Hata; T.; **J. Am. Chem. Soc.**, 106, 4552 (1984).
8. Holt; S.J.; Kellie; A.E., O'Sullivan; D.G. and Sadler; P.W. **J. Chem. Soc.**, 1217 (1958).
9. Rammler; D.H. and Khorana; G; **J. Am. Chem. Soc.**, 84, 3112 (1962).
10. Lapidot, Y. and Khorana, H.G.; **J. Am. Chem. Soc.**, 85, 3852 (1963).
11. Iwamoto; R.H., Acton; E.M., and Goodman L.; **J. Med. Chem.**, 6, 684 (1963).

تشييد الجوانوزين -٢' - (٥ - برومو - ٤ كلوروانديول - ٣ يل) فوسفات (G-3'-BCIP)

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تم فى هذا البحث تشييد المركبات ن-٢ بنزويل -٥ -أ - ثنائى ميثوكسى تريتايل -٢' - أ - رباعى هيدروبيرانيل جوانوزين و ٥- برومو - ٤- كلوروانديول -٣ - يل - أ - آسيتيل - ٣- فوسفورو ثنائى كلوريدات. بعد ذلك تم ازدواج هذه المركبات مع بعضها لكى تعطى الجوانوزين -٣' - (٥ - برمو -٤- كلوروانديول -٣ - يل) فوسفات بعد إزالة المجموعات الحامية.