

Synthesis of an *N*-Alkyl Derivative of 2'-Deoxyisoguanosine

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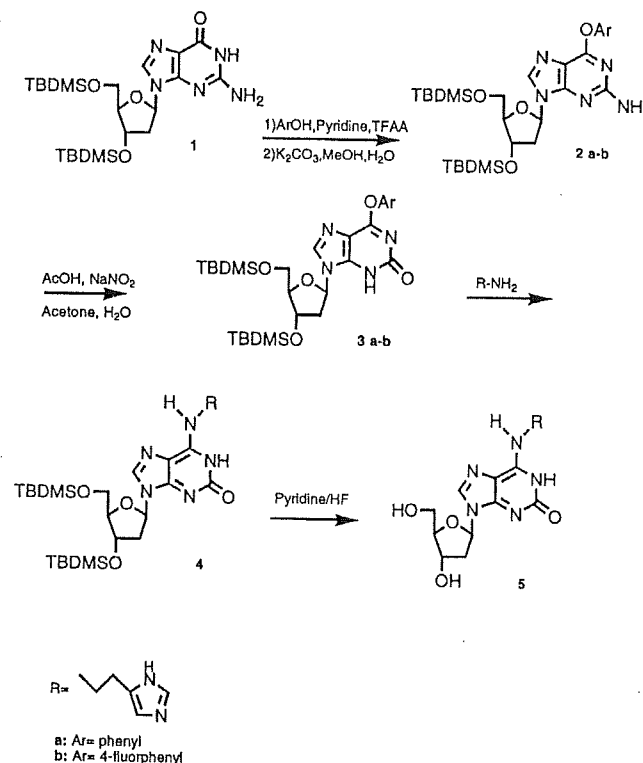
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Abstract: 3',5'-*O*-bis-*tert*-butyldimethylsilyl-2'-deoxyguanosine is converted in two steps to 3',5'-*O*-*tert*-butyldimethylsilyl-6-*O*-aryl-2'-deoxyxanthosine. This compound is used to make a 2'-deoxyisoguanosine analog with a functionalized side chain.

Although oligonucleotides are known to catalyze chemical reactions,^{1,2} and oligonucleotide catalysts can be obtained by combinatorial methods,^{3,4} their catalytic activities are normally many orders of magnitude below that of proteins of comparable size, and appear to be far more limited in scope as well.⁵ This has generated substantial interest in new synthetic methods for preparing oligonucleotides that carry functionality similar to that found in proteins,⁶ and increasing the number of replicatable letters in the oligonucleotide alphabet to carry this functionality.^{7,8} These might generate a new type of biopolymer with the replicatability of DNA and the functionality of proteins. We describe here the synthesis of 2'-deoxyisoguanosine, an extra letter in the genetic alphabet,⁷ carrying on its exocyclic nitrogen an imidazole side chain, the functional group from histidine, which plays important catalytic roles in many enzymes.

Several synthetic procedures for making unsubstituted 2'-deoxyisoguanosine have been described. Photolysis of 2'-deoxyadenosine-*N*-oxide⁹ or 2-chloro-2'-deoxyadenosine¹⁰ can yield functionalized *isoG*, but are not readily applied to larger scale syntheses. A chemical route from 6-amino-1-(2'-deoxy-beta-D-erythropentofuranosyl)-1H-imidazol-4-carbonitrile (AICA 2'-deoxynucleoside)¹¹ is more suitable to large scale synthesis, but is not readily adapted to make *N*-6 alkylated 2'-isoguanosine precursors, and starts from a precursor that is not commercially available as the 2'-deoxyribose. Likewise, deamination of 2'-deoxydiaminopurine, synthesized from 2'-deoxyguanosine¹², does not lend itself readily to the preparation of 6-substituted 2'-deoxyisoguanosine derivatives.

To develop a single compound that can serve as a synthon for different kinds of *N*-6 alkylated 2'-deoxyisoguanosine derivatives, we sought a 2'-deoxyxanthosine derivative with a substitutable group in the C-6 position. The 6-*O*-aryl derivative of xanthosine appeared appropriate. This might be prepared by nitrosative 6-*O*-phenyl derivatives of 2'-deoxyguanosine. 6-*O*-*p*-nitrophenyl-*N*-2-trifluoroacetyl-2'-deoxyguanosine has been reported by Fathi *et al.*¹⁴ We reproduced this procedure, but were unable to deaminate this compound using aq. HNO₂. We could, however, use their procedure to obtain 6-*O*-phenyl- and 6-*O*-*p*-fluorophenyl-2-*N*-trifluoroacetyl-2'-deoxyguanosine. The synthesis proceeded more satisfactorily for these compounds with the sugar fully protected, however. Accordingly, 3',5'-*O*-bis-*O*-*tert*-butyldimethylsilyl-2'-deoxy-guanosine **1** was synthesized according to the general procedure of Ogilvie¹³. Compound **1** was reacted with trifluoroacetic anhydride in pyridine and then phenol or *p*-fluorophenol following the procedure of Fathi *et al.*¹⁴ The *N*-2-trifluoroacetyl protecting group was removed by hydrolysis with water / methanol / K₂CO₃ to yield 6-*O*-phenyl-3',5'-*O*-*tert*-butyldimethylsilyl-2'-deoxyguanosine **2a** and 6-*O*-*p*-fluorophenyl-3',5'-*O*-*tert*-butyldimethyl-silyl-2'-deoxyguanosine **2b** in 61%¹⁵ and 80% respectively¹⁹.



Both **2a** and **2b** were deaminated in CH₃COOH/NaNO₂/water using acetone as co-solvent, to give the corresponding 2'-deoxyxanthosine derivatives **3 a** and **3 b** in 71%¹⁶ and 60%¹⁹ yields. The deamination was complete in 3 h at r.t. Upon longer reaction times, some loss of the sugar protecting groups would be found to occur.

Both **3 a** and **3 b** were shown to react readily with histamine to give the corresponding 3',5'-*O*-bis-*tert*-butyldimethylsilyl-*N*-6-alkylated 2'-deoxyisoguanosine **4**¹⁷. The silyl protecting groups were then removed with 5% HF in pyridine (stirring for 24 h at r.t.) to give 6-*N*-[2-(1H-imidazol-4-yl)ethyl]-2'-deoxyisoguanosine **5**¹⁸ in 98% yield.

6-*N*-[2-(1H-Imidazol-4-yl)ethyl]-2'-deoxyisoguanosine is the first *N*-6 alkylated 2'-deoxyisoguanosine derivative to be reported, and is an interesting building block for designing novel oligonucleotide catalysts. The 6-*O*-aryl-2'-deoxyxanthosine may prove to be a general useful synthon for 2'-deoxyisoguanosine and derivatives. This is currently under investigation.

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- (15) **2-amino-6-phenoxy-9-[2'-deoxy-3,5-bis-O-(tert-butylidimethylsilyl)-β-D-erythro-pentofuranosyl]-purine (2a)**. **1** (241 mg, 0.49 mmol) was evaporated twice from dry pyridine, and then re-dissolved in dry pyridine (7.35 mL). The solution was cooled in an ice bath and trifluoroacetic anhydride (0.42 mL, 2.92 mmol) was added dropwise (15 min) while stirring under Ar. Stirring was continued for additional 10 min at 0 °C. Phenol (900 mg, 9.6 mmol), evaporated twice from dry pyridine, was re-dissolved in dry pyridine (24 mL). The resulting solution was added to the reaction mixture, which was stirred at 0 °C for 2 hours, and then an additional 48 hours at r.t. under Ar. The mixture was concentrated to 1/3 the volume and poured into water (74 mL). The mixture was extracted with EtOAc (3 x 25 mL each). The combined organic layers were washed with aq NaOH (1 M, 3x 10 mL), and then water (10 mL). The organic phase was concentrated by rotary evaporation to a gum (dark brown), evaporated several times with toluene, and re-dissolved in MeOH (4 mL) containing K₂CO₃ (50 mg). Water (0.5 mL) was added, and the mixture was stirred for 3 h at r.t., and poured into EtOAc (25 mL). The mixture was extracted with water (2 x 25 mL) and the water extracted with EtOAc (25 mL). The organic phases were collected, dried (MgSO₄), concentrated *in vacuo* and then transferred to a silica column. The product was eluted with EtOAc/petroleum ether (1:1 v/v). Pooling and evaporation of the fractions containing product yielded **2a** (170 mg, 61%) as a light yellow foam. ¹H NMR (CDCl₃) (ppm) 8.00 (s, 1, H₈), 7.18-7.45 (m, 5, C₆H₅O), 6.35 ("t", 1, J_{app}=6 Hz, H_{1'}), 4.85 (br s, 2, NH₂), 4.58 (m, 1, H_{3'}), 3.97 (m, 1, H_{4'}), 3.79 (m, 2, H_{5',5''}), 2.58 & 2.37 (m, 1 & 1, H_{2'} & H_{2''}), 0.89 & 0.08 (m, 30 H, TBDMS).
- (16) **2-Hydroxy-6-phenoxy-9-[2'-deoxy-3,5-bis-O-(tert-butylidimethylsilyl)-β-D-erythro-pentofuranosyl]-6-purine (3a)**: **2** (170 mg, 0.3 mmol) was dissolved in acetone (1.5 mL). Water (1 mL), acetic acid (1 mL) and NaNO₂ (435 mg) was added. This mixture was stirred for 3 hours at r.t. and then quenched with aq NaHCO₃ (20 mL). The aqueous phase was extracted with EtOAc (3x 50 mL), and then re-extracted with water (20 mL). The organic phase was collected, dried (MgSO₄), concentrated *in vacuo*, and then transferred to a silica column. The product eluted with CH₂Cl₂/MeOH 95:5 v/v. Pooling and evaporation of the fractions containing product yielded **3a** (120 mg, 71%) as a light brown foam. ¹H NMR (CDCl₃) 8.06 (s, 1, H₈), 7.18-7.40 (m, 5, C₆H₅O), 6.38 ("t", 1, J_{app}=6 Hz, H_{1'}), 4.57 (m, 1, H_{3'}), 3.92 (m, 1, H_{4'}), 3.80 (m, 2, H_{5',5''}), 2.40 (m, 2, H_{2',2''}), 0.89 (d, 18, *t*-butyl), 0.08 (d, 12, Si-CH₃). MS (FAB-Pos; m/z, relative intensity) 573 (M⁺ 100), 343 (23), 324 (32), 229 (72).
- (17) **2-Hydroxy-6-N-[2-(1H,imidazol-4-yl)-ethyl]-9-[2'-deoxy-3,5-bis-O-(tert-butylidimethylsilyl)-β-D-erythro-pentofuranosyl]-6-aminopurine (4)**: **3** (224 mg, 0.4 mmol) was evaporated twice from toluene, re-dissolved in dry DMF (3 mL). Histamine base (450 mg, 4 mmol) was added, the reaction solution was warmed to 50 °C and stirred under Ar for 24 h, and then transferred to a silica column. The product eluted with CH₂Cl₂/MeOH 85:15 v/v as eluent. Pooling and evaporation of the fraction containing the product yielded a white powder which was separated between ether/water, the ether phase was dried (MgSO₄) evaporated to yield **4** (128 mg, 56%) as a white powder, which was recrystallized from Ethanol/water. mp 196-199 °C, UV_{max}(EtOH) 303, 251, 221 nm UV_{min} 268, 238 nm, ¹H-NMR (CDCl₃) 7.91 (s, 1, H₈), 7.61 (s, 1, H-C(2-Im)), 6.86 (s, 1, H-C(4-Im)), 6.31 ("t", 1, J_{app}=6 Hz, H_{1'}), 4.57 (m, 1, H_{3'}), 3.98 (m, 1, H_{4'}), 3.84 (m, 2, H_{5',5''}), 3.03 (t, 2, CH₂), 2.41 (m, 2, CH₂) 1.80-2.20 (m, 2, H_{2',2''}), 0.91 (d, 18, *t*-butyl), 0.12 (d, 12, Si-CH₃). MS (FAB-POS; m/z, relative intensity) 590 (M⁺ 52), 246 (100).
- (18) **2-Hydroxy-6-N-[2-(1H,imidazol-4-yl)-ethyl]-9-(2'-deoxy-β-D-erythro-pentofuranosyl)-6-aminopurine (5) via 2a**: **4** was evaporated twice from pyridine, placed in a plastic container and re-dissolved in dry pyridine (3.5 mL). 70 % HF in pyridine (0.25 mL) was added and the reaction solution was stirred at r.t. under argon for 24 h. Cooled with an ice bath and methoxy-trimethylsilane (5 mL) was added slowly. The ice bath was removed and the reaction solution was stirred for 2 h at r.t., concentrated *in vacuo* and washed several times with CH₂Cl₂, to give **5** (72 mg, 98 %) as a slightly yellow powder. mp 205-208 °C, UV_{max}(H₂O) 293, 249 nm UV_{min} 267, 237 nm, ¹H-NMR (d₆-DMSO) 8.00 (s, 1, H₈), 7.61 (s, 1, H-C(2-Im)), 6.86 (s, 1, H-C(4-Im)), 6.18 ("t", 1, J_{app}=6.6 Hz, H_{1'}), 4.37 (m, 1, H_{3'}), 3.88 (m, 1, H_{4'}), 3.33-3.67 (m, 2, H_{5',5''}), 2.84 (t, 2, J_{app}= 6.6 Hz, CH₂), 2.53 (m, 2, CH₂) 2.64 & 2.23 (m, 2, H_{2',2''}).
- (19) **2-Hydroxy-6-N-[2-(1H,imidazol-4-yl)-ethyl]-9-(2'-deoxy-β-D-erythro-pentofuranosyl)-6-aminopurine (5) via 2b**: The synthesis of **2b** to **3b** with 4-fluorophenol replacing phenol. The deamination procedure was identical.