Tetrahedron Letters,Vol.31,No.3,pp.319-322,1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

SYNTHESIS OF 6-SUBSTITUTED 2'-DEOXYGUANOSINE DERIVATIVES USING TRIFLUOROACETIC ANHYDRIDE IN PYRIDINE

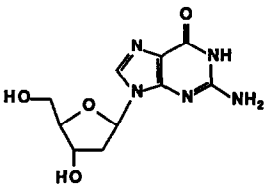
Reza Fathi, Bhaswati Goswami, Pei-Pei Kung, Barbara L. Gaffney, and Roger A. Jones*

Department of Chemistry
Rutgers, The State University of New Jersey
Piscataway, NJ 08855

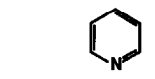
Summary: Trifluoroacetic anhydride at 0° C reacts with a pyridine suspension of deoxyguanosine to generate a polar intermediate, presumably the corresponding 6-pyridyl derivative. The reaction is complete in less than 15 minutes, and is not accompanied by degradation. From this intermediate a variety of 6-substituted deoxyguanosine derivatives can be obtained, some in excellent yields.

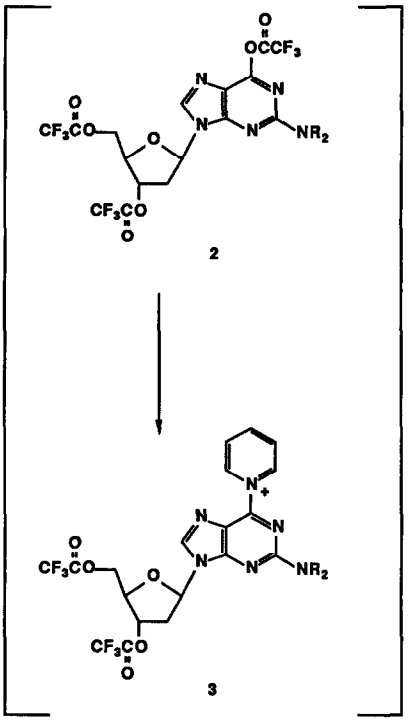
Guanine nucleosides are known to be susceptible to reaction at the 6-oxygen with a variety of acylating,[1] sulfonylating,[2-5] and phosphorylating[6-8] agents. This susceptibility, while problematic for oligonucleotide synthesis,[9] has provided access to many guanine 6-*O*, *N*, and *S* derivatives. Because of our work on the effect of O^6-methyldeoxyguanosine on DNA structure and stability, we had developed a route to d(O^6Me)G and other 6-substituted deoxyguanosine compounds *via* sulfonylation of a triacyldeoxyguanosine derivative.[5,10,11] This procedure, although generally satisfactory, does not give high enough yields for our present work, which involves the use of ^{15}N labeled deoxyguanosine. At this time we report a simple route for synthesis of 6-substituted deoxyguanosines *via* a putative 6-pyridyl intermediate generated using trifluoroacetic anhydride in pyridine.

The 6-pyridyl derivative of inosine had been obtained by Adamiak, using the reaction of 2',3',5'-tri-*O*-acetylinosine with 4-chlorophenylphosphorodichloridate in pyridine solution.[12] This reaction was later extended to guanosine by these same workers,[7,8] and to deoxyguanosine by Chollet.[13] The reaction requires approximately 24 hours and, at least in our hands, gives darkly colored mixtures of the 6-pyridyl compound along with unknown degradation products. A further disadvantage of both this procedure and our sulfonylation procedure is that a fully *O*, *N*-protected deoxyguanosine derivative must be employed. Triisobutyryl deoxyguanosine can be prepared in high yield, but the isobutyryl group is quite difficult to remove from the N^2 position of most 6-substituted deoxyguanosines, particularly after incorporation into oligonucleotides. The acetyl group is a better choice for protection, but triacetyl deoxyguanosine generally is obtained in only moderate yields. We investigated the reaction of deoxyguanosine with trifluoroacetic anhydride in pyridine as an alternative means of generating a 6-pyridyl intermediate. In this case prior protection would not be required since *O*-trifluoroacetyl groups are generally quite labile. We found that, using either deoxyguanosine itself (**1**) or its 3',5'-bis-*O*-(*tert*-butyldimethylsilyl) derivative (**5**), the corresponding 6-pyridyl derivative is formed rapidly at 0°, as evidenced by the formation of the polar, fluorescent (tlc) material, which is characteristic of 6-pyridyl guanine nucleosides. Reaction of **1** under these conditions most likely leads first to the 6-trifluoroacyl compound **2**, with the trifluoroacetyl group subsequently displaced by pyridine to give **3**. We have not been able to isolate and characterize either **2** or **3**, however.



1

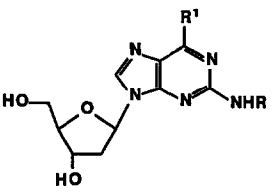




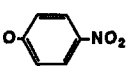
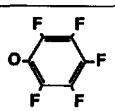
2

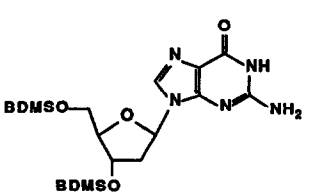
3

2,3; R = H or CF_3CO

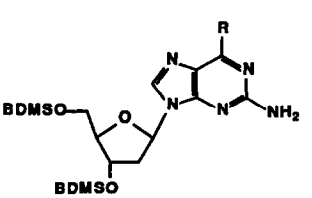


4a-d

	R	R^1
a	H	NH_2
b	H	OCH_3
c	CF_3CO	
d	CF_3CO	



5



6a-b

a, R = OCH_3
b, R = OCH_2CH_3

Conversion to the 6-*O*-methyl (**4b**[14] or **6a**[15]) or ethyl (**6b**[16]) derivatives is effected by dilution of the reaction mixture with the corresponding alcohol and addition of the sodium alkoxide. Although the trifluoroacetic anhydride reaction is complete in minutes, displacement of the 6-pyridyl moiety requires 1-2 days, in most cases. This, in part, is due to the fact that the concentration of alkoxide must be kept below 0.13*M* for sodium methoxide or 0.015*M* for sodium ethoxide. If higher concentrations of alkoxide are used, the solution changes from a pale yellow to a red-brown color and the yield is reduced drastically. Using these low alkoxide concentrations, **4b** is obtained after a 24 hour reaction in 60 % yield, **6a** after 20 hours in 80 % yield, and **6b** after 60 hours in 51 % yield. Treatment of **3** with aqueous ammonia for 1 1/2 hours gives 2-amino-2′-deoxyadenosine (**4a**), but in only 34 % yield.[17]

The basic conditions needed for preparation of **4a-b/6a-b** result in cleavage of all of the trifluoroacyl groups so that the free deoxynucleosides (**4a-b**) or the 3′,5′-bis-*O*-(TBDMS) derivatives (**6a-b**) are obtained exclusively. In contrast, the 6-*O*-nitrophenyl (**4c**[18]) and pentafluorophenyl (**4d**[19]) derivatives are prepared under much milder conditions, simply by addition of 4-nitrophenol or pentafluorophenol to the pyridine solution of **3**, allowing an N^2- trifluoroacyl group to be retained. The reaction times are similar but the yields are somewhat better : 48 hours for **4c** (67 %) and 24 hours for **4d** (95 %).

This trifluoroacetic anhydride/pyridine reaction provides an effective route for the synthesis of 6-substituted deoxyguanosine derivatives, presumably via the 6-pyridyl compound. Although our main interest is in the deoxy series, in the one preliminary reaction that we have carried out on guanosine, the 2-*N*-trifluoroacetyl-6-*O*-(pentafluorophenyl) derivative was obtained in good yield. These 6-*O*-pentafluorophenyl compounds are prepared easily and in high yields, and may prove useful as general synthons for 6-substitution.[20]

Acknowledgements: This work was supported by a Faculty Research Award to RAJ from the American Cancer Society and by grants from the National Institutes of Health (GM31483) and the Busch Memorial Fund.

References:

1. Bridson, P. K.; Markiewicz, W. T.; Reese, C. B. *J. C. S. Chem. Comm.* **1977**, 791.
2. Bridson, P. K.; Markiewicz, W.; Reese, C. B. *J. C. S. Chem. Comm.* **1977**, 447.
3. Reese, C. B.; Ubasawa, A. *Tetrahedron Lett.* **1980**, *21*, 2265.
4. Daskalov, H. P.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1980**, *21*, 3899.
5. Gaffney, B. L.; Jones, R. A. *Tetrahedron Lett.* **1982**, *23*, 2253.
6. Reese, C. B.; Richards, K. H. *Tetrahedron Lett.* **1985**, *26*, 2245.
7. Adamiak, R. W.; Biala, Z. G.; Gdaniec, Z.; Mielewczyk, S.; Skalski, B. *Chemica Scripta* **1986**, *26*, 3.
8. Adamiak, R. W.; Biala, Z. G.; Gdaniec, Z.; Mielewczyk, S.; Skalski, B. *Chemica Scripta* **1986**, *26*, 7.
9. Reese, C. B.; Skone, P. A. *J. Chem. Soc.,Perkin Trans. 1* **1984**, 1263.
10. Gaffney, B. L.; Marky, L. A.; Jones, R. A. *Biochemistry* **1984**, *23*, 5686.
11. Gaffney, B. L.; Jones, R. A. *Biochemistry* **1989**, *28*, 5881.
12. Adamiak, R. W.; Biala, E.; Skalski, B. *Nucleic Acids Res.* **1985**, *13*, 2989.
13. Chollet, A.; Chollet-Damerius, A.; Kawashima, E. H. *Chemica Scripta* **1986**, *26*, 37.
14. **2-amino-6-methoxy-9-(2-deoxy-ß-D-*erythro*-pentofuranosyl)purine (6-*O*-methyl-2′-deoxyguanosine) (4b).** To 2 mmol of deoxyguanosine (**1**), dried by evaporation of pyridine and suspended in 10 mL of dry pyridine was added dropwise 2.3 mL (16 mmol) of trifluoroacetic anhydride, with cooling in an ice bath. After ten minutes a suspension of 4.3 g of sodium methoxide in 300 mL of methanol was added dropwise. After a further 24 hrs, the mixture was treated with a solution of pyridine hydrochloride (12 mL of pyridine/ 4 mL conc HCl). The excess acid was destroyed by addition of 2 g of sodium bicarbonate and the mixture was evaporated to dryness. The residue was dissolved in water and purified on a Dynamax reversed-phase hplc column using a gradient of 2 → 5% acetonitrile in water in 45 min at a flow rate of 6 mL/min. Evaporation of appropriate fractions gave 0.36 g (60%) of pure **4b**. A crystalline sample was obtained by crystallization from water, mp 155-7°. UV_{max} (MeOH) 248 nm, UV_{min} 281 nm; ^1H NMR (DMSO-d_6) δ (ppm) 8.07 (s, 1,H_8), 6.43 (br s, 2, NH_2), 6.20("t", 1, J_{app}=6.9 Hz, $H_{1'}$), 5.27 (d, 1, J=3.8 Hz, 3'-OH), 4.98 (t, 1, J=5.5 Hz, 5'-OH), 4.35 (m, 1, $H_{3'}$), 4.00 (s, 3, OCH_3), 3.82 (m, 1, $H_{4'}$), 3.53 (m, 2, $H_{5',5''}$), 2.57 & 2.22 (m & m, 1 & 1, $H_{2'}$ & $H_{2''}$). Anal. Calcd. for $C_{11}H_{15}N_5O_4{\cdot}H_2O$: C, 44.15; H, 5.72; N, 23.40. Found: C, 44.15; H, 5.83; N, 23.43.

15. **2-amino-6-methoxy-9-[2-deoxy-3,5-bis-*O*-(*tert*-butyldimethylsilyl)-ß-D-*erythro*-pentofuranosyl]-purine (6a).** To 6 mmol of **5**, dried by evaporation of pyridine and dissolved in 60 mL of dry pyridine was added dropwise 3.0 mL (21 mmol) of trifluoroacetic anhydride, with cooling in an ice bath. After stirring for 15 minutes a suspension of 5.7 gm of sodium methoxide in 610 mL of methanol was added in portions. After a further 20 hrs the reaction mixture was poured into 500 mL of water. The mixture was partitioned using four 200 mL portions of petroleum ether. The combined organic layers were evaporated, traces of pyridine were removed by evaporation of toluene and the resulting foam was dissolved in 50 mL of petroleum ether and placed in the cold. Crystallization gave, after filtration, 2.4 gm (80%) of **6a**, mp 101-105°d. UV_{max} (MeOH) 250 nm, UV_{min} 283 nm; ^1H NMR ($CDCl_3$) δ (ppm) 7.90 (s, 1, H_8), 6.30 ("t", 1, J_{app}=6.6 Hz, $H_{1'}$), 4.81 (s, 2, NH_2), 4.57 (m, 1, $H_{3'}$), 4.01 (s, 3, OCH_3), 3.96 (m, 1, $H_{4'}$), 3.77 (m, 2, $H_{5',5''}$), 2.55 & 2.38 (m & m, 1 & 1, $H_{2'}$ & $H_{2''}$), 0.89 & 0.06 (m & m, 9 & 9, Me_3CSi). Anal. Calcd. for $C_{23}H_{43}N_5Si_2O_4$: C, 54.18; H, 8.50; N, 13.73; Si, 11.01. Found: C, 54.05; H, 8.63; N, 13.80; Si, 10.69.

16. **2-amino-6-ethoxy-9-[2-deoxy-3,5-bis-*O*-(*tert*-butyldimethylsilyl)-ß-D-*erythro*-pentofuranosyl]purine (6b).** To 2 mmol of **5**, dried by evaporation of pyridine and suspended in 50 mL of dry pyridine was added dropwise 1.0 mL (7 mmol) of trifluoroacetic anhydride, with cooling in an ice bath. After 15 minutes a solution of 2.3 gm of sodium ethoxide in 1.75 L of absolute ethanol was added in portions. After a further 60 hours the mixture was concentrated to about 800 mL and poured into 600 mL of cold water. The mixture was filtered and partitioned using five 200 mL portions of petroleum ether. Crystallization from petroleum ether as described above for **6a**, gave 540 mg (51%) of **6b**, mp 120-124°d. UV_{max} (MeOH) 250 nm, UV_{min} 283 nm; ^1H NMR ($CDCl_3$) δ (ppm) 7.9 (s, 1, H_8), 6.33 ("t", 1, J_{app}=6.6 Hz, $H_{1'}$), 4.80 (s, 2, NH_2), 4.59 (m, 1, $H_{3'}$), 4.54 (q, 2, J=7, CH_2), 3.87 (m, 1, $H_{4'}$), 3.79 (m, 2, $H_{5',5''}$), 1.46 (t, 3, CH_3), 2.55 & 2.38 (m & m, 1 & 1, $H_{2'}$ & $H_{2''}$), 0.91 & 0.06 (m & m, 9 & 9, Me_3CSi). Anal. Calcd. for $C_{24}H_{45}N_5Si_2O_4$: C, 55.03; H, 8.65; N, 13.36; Si, 10.72. Found: C, 54.97; H, 8.83; N, 13.36; Si, 11.28.

17. **2,6-diamino-9-(2-deoxy-ß-D-*erythro*-pentofuranosyl)purine (2-amino-2'-deoxyadenosine) (4a).** To 2 mmol of **1**, dried by evaporation of pyridine and suspended in 20 mL of dry pyridine was added dropwise 2.3 mL (16 mmol) of trifluoroacetic anhydride, with cooling in an ice bath. After ten minutes 20 mL of cold, concentrated aqueous ammonia was added. After a further 1 1/2 hrs the mixture was evaporated to dryness, the residue dissolved in water and the solution filtered through a 100 mL portion of Bio Rad AG 1-X2, hydroxide form resin to remove colored impurities. The resin was washed with 40% methanol in water to elute crude **4a**, which was further purified on the reversed-phase column described above using a gradient of 2 → 15 % acetonitrile in water. Evaporation of appropriate fractions gave 192 mg (34%) of **4a**, a sample crystallized from water gave a mp of 148°. UV_{max} (MeOH) 282 nm, UV_{min} 256 nm; ^1H NMR (DMSO-d_6) δ (ppm) 7.91 (s, 1,H_8), 6.73 (br s, 2, NH_2), 6.17 ("t", 1, J_{app}=6 Hz, $H_{1'}$), 5.73 (br s, 2, NH_2), 5.25 (m, 2, 3'-OH & 5'-OH), 4.35 (m, 1, $H_{3'}$), 3.84 (m, 1, $H_{4'}$), 3.54 (m, 2, $H_{5',5''}$), 2.59 & 2.17 (m & m, 1 & 1, $H_{2'}$ & $H_{2''}$). Anal. Calcd. for $C_{10}H_{14}N_6O_3 \cdot H_2O$: C, 42.25; H, 5.67; N, 29.56. Found: C, 42.54; H, 5.33; N, 29.56.

18. **2-*N*-trifluoroacetamido-6-(4-nitrophenoxy)-9-(2-deoxy-ß-D-*erythro*-pentofuranosyl)purine (4c).** To 4 mmol of **1**, dried by evaporation of pyridine and suspended in 60 mL of dry pyridine was added dropwise 3.4 mL (24 mmol) of trifluoroacetic anhydride, with cooling in an ice bath. After 15 minutes a solution of 11.1 g (80 mmol) of 4-nitrophenol in 200 mL of pyridine was added. After a further 48 hours the mixture was concentrated to about 150 mL and poured into 600 mL of water. The mixture was partitioned using four 200 mL portions of ethyl acetate. The combined organic layers were backwashed with three 50 mL portions of water, concentrated to about 15 mL, toluene was added and the mixture was concentrated to a gum which was dissolved in ethyl acetate, filtered and added dropwise to a 400 mL portion of toluene. Filtration and washing with petroleum ether gave 1.3 g (67 %) of **4c**. UV_{max} (MeOH) 271; ^1H NMR (DMSO-d_6) δ (ppm) 12.12 (br s, 1, NH), 8.72 (s, 1,H_8), 8.34 & 7.69 (dd, 4, J=9.1 Hz, $C_6H_4NO_2$), 6.41 ("t", 1, J_{app}=6.7 Hz, $H_{1'}$), 5.35 (d, 1, J=4.2 Hz, 3'-OH), 4.91 (t, 1, J=5.5 Hz, 5'-OH), 4.50 (m, 1, $H_{3'}$), 3.88 (m, 1, $H_{4'}$), 3.57 (m, 2, $H_{5',5''}$), 2.78 & 2.40 (m & m, 1 & 1, $H_{2'}$ & $H_{2''}$). Anal. Calcd. for $C_{18}H_{15}N_6F_3O_7$: C, 44.63; H, 3.12; N, 17.35; F, 11.77. Found: C, 44.80; H, 3.13; N, 17.20; F, 11.30.

19. **2-*N*-trifluoroacetamido-6-pentafluorophenoxy-9-(2-deoxy-ß-D-*erythro*-pentofuranosyl)purine (4d).** To 4 mmol of **1**, dried by evaporation of pyridine and suspended in 60 mL of dry pyridine was added dropwise 3.4 mL (24 mmol) of trifluoroacetic anhydride, with cooling in an ice bath. After 15 minutes a solution of 9.6 g (52 mmol) of pentafluorophenol in 200 mL of pyridine was added. After a further 24 hrs the mixture was concentrated to about 150 mL and poured into 500 mL of water. The mixture was partitioned using four 200 mL portions of ethyl acetate. The combined organic layers were washed with three 50 mL portions of water, concentrated to a gum, which was dissolved in about 15 mL of ethyl acetate, and the product precipitated by dropwise addition of this solution to 700 mL of petroleum ether. Filtration gave 2.0 g (95%) of **4d**. An analytical sample of **4d** was obtained by treatment with carbon and reprecipitation. UV_{max} (MeOH) 268 nm; ^1H NMR (DMSO-d_6) δ (ppm) 12.1 (br s, 1, NH), 8.80 (s, 1,H_8), 6.42 ("t", 1, J_{app}=6.7 Hz, $H_{1'}$), 5.38 (m, 1, 3'-OH), 4.90 (m, 1, 5'-OH), 4.20 (m, 1, $H_{3'}$), 3.90 (m, 1, $H_{4'}$), 3.55 (m, 2, $H_{5',5''}$), 2.80 & 2.38 (m & m, 1 & 1, $H_{2'}$ & $H_{2''}$). Anal. Calcd. for $C_{18}H_{11}N_5F_8O_5 \cdot 3/4\ H_2O$: C, 39.83; H, 2.32; N, 12.90; F, 28.00. Found: C, 39.38; H, 2.03; N, 12.60; F, 28.56.

20. Preliminary experiments indicate that both **4c** and **4d** are readily transformed into other 6-derivatives.

(Received in USA 25 May 1989)