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A Convenient Preparation of Protected 3'-Deoxyguanosine from Guanosine

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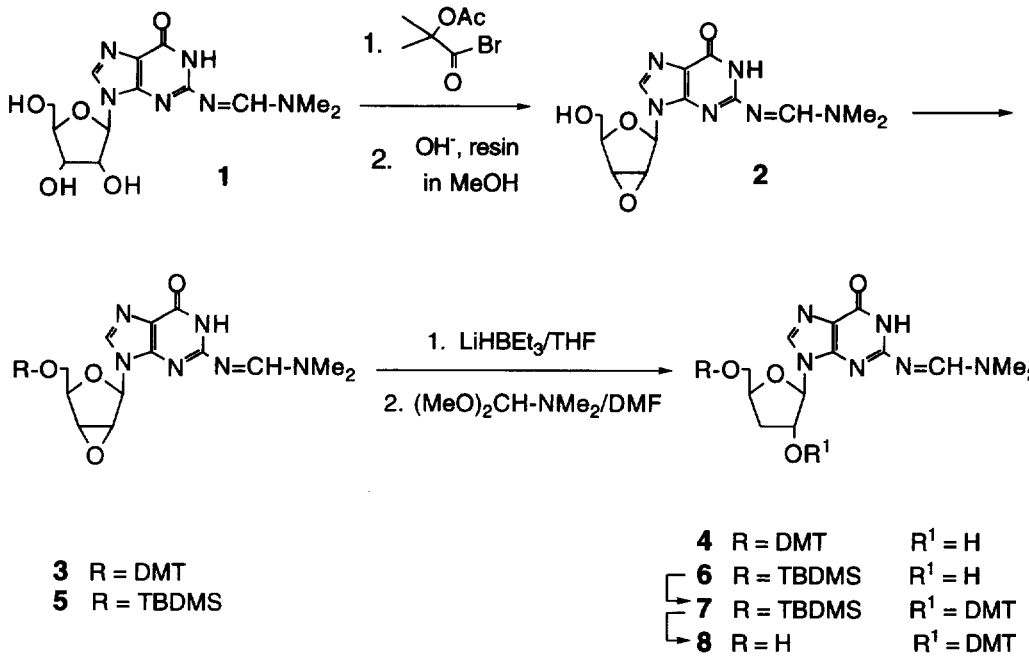
Abstract: Protected 3'-deoxyguanosine was synthesized from guanosine in five steps and 50% overall yield through reaction of the 2',3'-diol with α-acetoxyisobutyryl bromide followed by base treatment to form 2',3'-anhydroguanosine and subsequent selective reduction of the epoxide with $LiHBEt_3$.

3'-Deoxyribonucleosides have been studied for their potential antiviral, antiparasitic, and antitumor activities.[1] 3'-Deoxyadenosine, known as cordycepin, was the first reported nucleoside antibiotic.[2] Interest in this class of compounds also stems from their application in the sequencing of RNA[3] and as probes for mechanism and structural studies.[4] More recently, 3'-deoxynucleosides have been incorporated into oligonucleotides to study the effects of 2',5' linkages on hybridization with complementary oligonucleotides.[5] It was found that 2',5' linked oligodeoxynucleotides associate through complementary base pairing, suggesting that 2',5' linked DNA comprises an alternative genetic material to 3',5' linked DNA. As part of a program to explore the structure-activity relationship of an oligodeoxynucleotide thrombin inhibitor,[6] we required the preparation of 3'-deoxyguanosine on a preparative scale. A survey of the literature revealed that current methods are cumbersome and require difficult separations with low overall yields.[7] Approaches which have been described for 3'-deoxyribonucleosides include attachment of the heterocyclic base to a suitably protected 3'-deoxyribose unit.[8] This method requires a protected 3'-deoxyribose which has to be prepared in many steps. Another approach to 3'-deoxyribonucleosides involves the conversion of the corresponding ribonucleosides to the 3'-O-thiocarbonates[9] or the 3'-deoxy-3'-halo derivatives[10] followed by reduction. However, synthesis of 3'-deoxyguanosine by this method results in a mixture of 2' and 3'-deoxy isomers in low yield.[9,10] Cordycepin can be synthesized from adenosine in high yield using α-acetoxyisobutyryl bromide or similar reagents.[11,12] However, in our hands, guanosine itself did not react with α-acetoxyisobutyryl bromide in acetonitrile, presumably because of its low solubility, while N^2-isobutyrylguanosine resulted in a mixture of products. In this paper, we wish to report that by using dimethylaminomethylene protecting group for the N^2-



3 R = DMT
5 R = TBDMS

4 R = DMT R^1 = H
6 R = TBDMS R^1 = H
7 R = TBDMS R^1 = DMT
8 R = H R^1 = DMT

amino group of guanosine, a modified procedure can successfully be used to synthesize 3'-deoxyguanosine in high yield.

The starting material N^2-(dimethylaminomethylene)guanosine (**1**) was readily prepared from guanosine and dimethylformamide dimethyl acetal.[7] To a solution of **1** (5.5 g, 16 mmol) and water (0.3 g, 17 mmol) in acetonitrile (200 mL), α-acetoxyisobutyryl bromide (13 g, 64 mmol) was added and the mixture was stirred at room temperature for 2 h resulting in a clear solution. The solution was poured into saturated $NaHCO_3$ (100 mL) and extracted with CH_2Cl_2 (4 x 200 mL), and the organic layers were dried (Na_2SO_4) and concentrated to give a white solid, which was a 1:1 mixture of N^2-(dimethylaminomethylene)-2'-O-acetyl-3'-bromo-3'-deoxy-5'-O-(2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl)guanosine and N^2-(dimethylaminomethylene)-2'-O-acetyl-3'-bromo-3'-deoxyguanosine as indicated by TLC and ^{1}H-NMR. The solid was dissolved in methanol (200 mL) and stirred with 10 eq of basic ion-exchange resin [Amberlite, IRA-400(OH)]. After 2 h, TLC indicated complete consumption of starting materials. The resin was filtered off and washed with 10% HOAc in methanol (5 x 100 mL). The filtrate was neutralized with triethylamine and concentrated, yielding **2** (4 g) as a white solid.[13] Reaction of **2** with DMT-Cl in dry pyridine gave **3** (7 g) as a pale yellow foam after column chromatography purification (SiO_2/CH_2Cl_2:CH_3OH:Et_3N =

100:5:1, 69% from **1**). A solution of **3** in dry THF (400 mL) was treated with $LiHBEt_3$ (60 mmol, 1 M solution in THF) at 0°C and subsequently at room temperature for 10 h. After quenching with CH_3OH and 5% aqueous $NaHCO_3$, the mixture was concentrated. To the residue, 5% $NaHCO_3$ aqueous solution (200 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 200 mL). The combined organic layers were dried (Na_2SO_4) and concentrated resulting in a crude product. ^{1}H-NMR indicated selective epoxide opening with the 3'-deoxy isomer being formed. At the same time, however, the dimethylaminomethylene protecting group had been removed. Thus, the crude product was re-dissolved in dry DMF (100 mL) and reacted with dimethylformamide dimethyl acetal (6 g, 50 mmol) in the presence of CH_3OH (0.5 mL) at room temperature for 10 h. After work-up and column chromatography (SiO_2/CH_2Cl_2:CH_3OH:Et_3N = 100:10:1), pure **4**[14] was obtained as a white foam (5 g, 50% from **1**).

A similar procedure was also used in the preparation of 2'-O-DMT protected 3'-deoxyguanosine **8**. **5** was prepared from **2** in 70% yield using TBDMS-Cl/imidazole/DMF. Reduction of **5** with $LiHBEt_3$ followed by reprotecting the 2-amino group as described above gave **6** in 61% yield. Treatment of **6** with DMT-Cl/pyridine/DMAP followed by removal of the TBDMS-group (TBAF/THF) yielded **8**[15] (overall 40% yield from **2**). The protected 3'-deoxyguanosine derivatives **4** and **8** obtained above can be easily converted to the corresponding H-phosphonates or amidite derivatives for incorporation into oligonucleotides by solid phase oligonucleotide synthesis using standard H-phosphonate[16] or amidite coupling protocols.[7,17]

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13. Compound **2**: ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.32(br., NH, 1H), 8.61(s, =CH-, 1H), 8.00(s, 8-H, 1H), 6.12(s, 1'-H, 1H), 5.02(br., OH, 1H), 4.38(d, J = 2.5 Hz, 2'-H, 1H), 4.21(d, J = 2.5 Hz, 3'-H, 1H), 4.15(t, J = 5.5 Hz, 4'-H, 1H), 3.53(br, 5', 5"-H, 2H), 3.16(s, CH_3, 3H), 3.03(s, CH_3, 3H).
14. Compound **4**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.48(br., NH, 1H), 8.45(s, =CH-, 1H), 7.61(s, 8-H, 1H), 7.16-7.41(m, Ar-H, 11H), 6.78(d, J = 9 Hz, Ar-H, 4H), 5.87(d, 3 Hz, 1'-H, 1H), 5.22(br., OH, 1H), 4.86(br., 2'-H, 1H), 4.60(br., 4'-H, 1H), 3.76(s, OCH_3, 6H), 3.32(br., 5', 5"-H, 2H), 3.07(s, CH_3, 3H), 3.01(s, CH_3, 3H), 2.10-2.30(m, 3', 3"-H, 2H). The ribo configuration of the 2'-hydroxyl was confirmed by 2D NMR techniques (HMBC and HMQC).
15. Compound **8**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.92(br., NH, 1H), 8.25(s, =CH-, 1H), 7.47(s, 8-H, 1H), 7.12-7.32(m, Ar-H, 11H), 6.70(d, J = 9 Hz, Ar-H, 2H), 6.65(d, 9 Hz, Ar-H, 2H), 5.65(d, 5.5 Hz, 1'-H, 1H), 4.85(dd, J = 7, 12.5 Hz, 2'-H, 1H), 4.45(br., 4'-H, 1H), 4.27(d, J = 12 Hz, OH, 1H), 3.80(d, J = 12 Hz, 5'-H, 1H), 3.76(s, OCH_3, 3H), 3.73(s, OCH_3, 3H), 3.32(t, J = 12 Hz, 5"-H, 1H), 3.16(s, CH_3, 3H), 3.06(s, CH_3, 3H), 1.93-2.06(m, 3', 3"-H, 2H).
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