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Oxidative Conversion of *N*-Dimethylformamidine Nucleosides to *N*-Cyano Nucleosides

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Abstract: Reaction of the *N*-dimethylformamidine (dmf) derivatives of 2'-deoxyguanosine, guanosine, and 2'-deoxyadenosine with iodine and aqueous ammonia gives the corresponding *N*-cyano nucleosides. This reaction occurs in oligonucleotides under conditions where iodine is retained on the solid support, or in the synthesis column, prior to cleavage with aqueous ammonia. This base modification can be eliminated with lower iodine concentration in the oxidation reagent.

The *N*-dimethylformamidine (dmf) group was first reported for exocyclic amine protection of nucleosides by Zemlicka and Holy and has been used effectively in oligonucleotide synthesis.[1-8] The dmf group can be introduced selectively in near quantitative yield, and it stabilizes the glycosidic linkage of deoxyadenosine. Because of instability of the adenine and cytosine derivatives to hydrolysis, recent work has focused on its use for guanine protection.[9] We now report that our two groups each observed heterogeneity in oligonucleotides synthesized with *N*-2-dimethylformamidine-dG using both phosphoramidite[10] and H-phosphonate[11] methods.

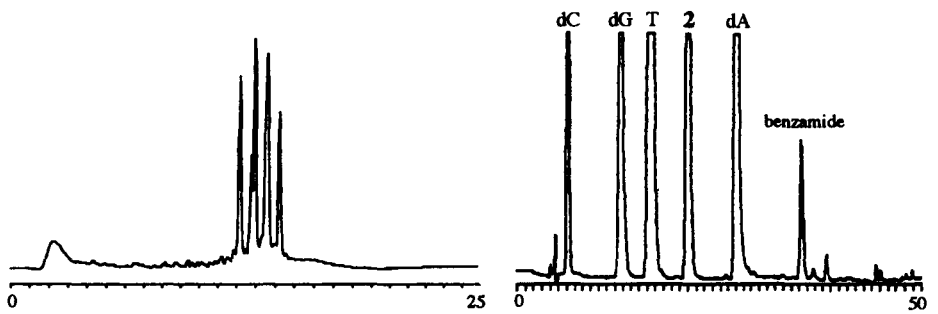
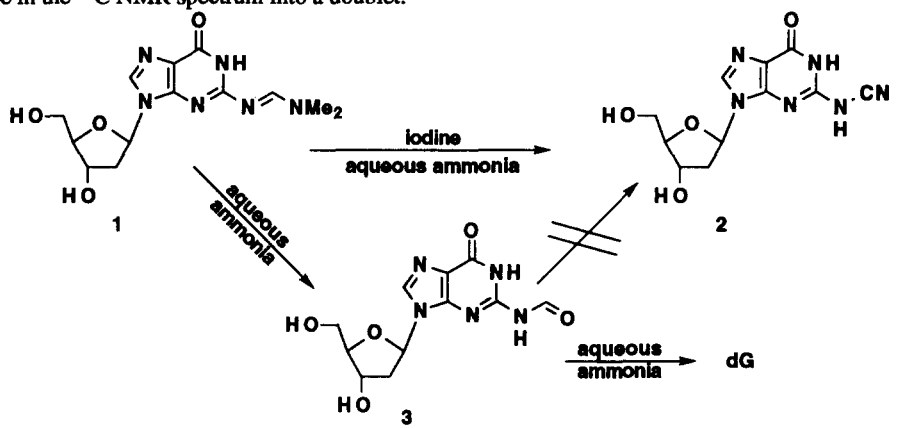


Figure 1. Anion-exchange HPLC (left) and RP HPLC of enzymatic digest (right) of 18mer 5' TCA CAG TCT GAT CTC GAT 3' made with 0.1M iodine oxidation reagent

We[10] have reported recently a new synthesis column design with large porous frits to occupy dead volume and minimize reagent consumption.[12] During experiments on further optimizing this system,[13] we found that the use of iodine at concentrations greater than 0.05 M (THF:H_2O:pyr / 7:1:2) correlated with the heterogeneity observed when the *N*-2 dimethylformamidine (dmf) group was used for dG protection. Enzymatic degradation[14,15] of crude oligonucleotide mixtures revealed the presence of a modified nucleoside, that was found to comigrate with material obtained by treatment of **1** with 0.1 M iodine in THF/water/pyridine (7/1/2)[10] or by addition of **1** and 1 eq of iodine to aqueous ammonia.[11] The IR spectrum of **2** has a characteristic nitrile

stretch at 2170 cm^{-1}, while the ^{13}C NMR of **2** differs significantly from that of the deoxyguanosine only by the presence of an additional resonance at 121 ppm.[16] Moreover, use of ^{15}N-ammonia in the conversion of **1** to **2** resulted in incorporation of ^{15}N into the cyano group, as shown by ^{15}N NMR and MS, and split the 121 resonance in the ^{13}C NMR spectrum into a doublet.[17]



The *N*-6-dimethylformamidine derivatives of deoxyadenosine,[18] and guanosine,[19] react similarly, while unprotected nucleosides (G, dG, dA) do not react. It is well known that iodine and aqueous ammonia form nitrogen triiodide, a black solid that is an unpredictable contact explosive and oxidant.[20] In the reactions using 1 eq of iodine, no black precipitate was formed. In contrast, when iodine and aqueous ammonia were allowed to react for several hours before adding **1** (1 eq), a black insoluble material was present, and there was limited formation of **2**, along with *N*-2-formyl-2'-deoxyguanosine **3** and dG. A portion of the insoluble material was removed and allowed to dry on a filter paper. Contact with a spatula then caused it to detonate, indicating the presence of nitrogen triiodide. Thus, it appears that nitrogen triiodide is not involved in the conversion of **1** to **2**.

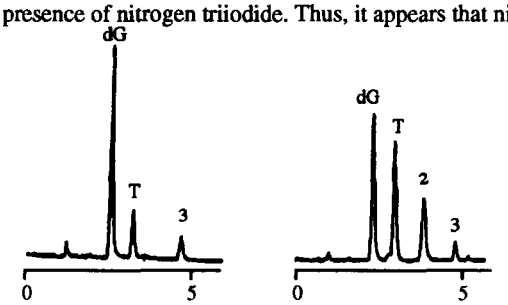


Figure 2. RP HPLC of the mixtures obtained upon enzymatic degradation (nuclease P1 and calf alkaline phosphatase)[15] of crude d[GgT] after deprotection and cleavage from the support by treatment with conc. aq. NH_3 at 50 °C for 6h. (left) with no iodine present, and (right) with traces of iodine present.[21]

Enzymatic degradation and HPLC analyses of crude d(GgT), prepared by an H-phosphonate method, showed, in addition to **2**, *N*-2-formyl-2'-deoxyguanosine (**3**, Fig 2).[11] Treatment of **1** with aqueous ammonia for three hours gives **3** in an isolated yield of about 20%.[22] HPLC during the reaction shows several intermediates, but **3** appears to be the only isolable compound. Further treatment of **3** with aqueous ammonia and iodine gives only hydrolysis to dG, with no detectable formation of **2**. Thus, although **3** is an intermediate in the hydrolysis of **1**, it is not an intermediate in the conversion of **1** to **2**. The presence of **3** in d(GgT) presumably is due to some secondary structure, or aggregation, of this molecule which slows further hydrolysis, since there was no trace of **3** found in many other syntheses of oligomers containing mixed sequences.[10]

The conversion of dmf protected G bases in oligonucleotides to the *N*-2-cyano derivatives is completely banished by the use of a lower concentration (0.02M) iodine oxidation reagent. This reagent provides rapid and complete oxidation of the internucleotide phosphite to phosphotriester during oligonucleotide synthesis with any nucleobase protecting group, any scale, DNA or RNA. The presence of 10% water was also helpful in washing residual iodine from the solid support, frit, and synthesis column compartment. When the same 18mer shown in Figure 1 was prepared using 0.02M iodine in the oxidation reagent, and analyzed, high purity and yield (Figure

3) was obtained. No *N*-2 cyano deoxyguanosine was detected by digestion analysis of this sample. Rapid deprotection of unmodified oligonucleotides can be attained with *N*-2 dimethylformamidine protection of G under these conditions.

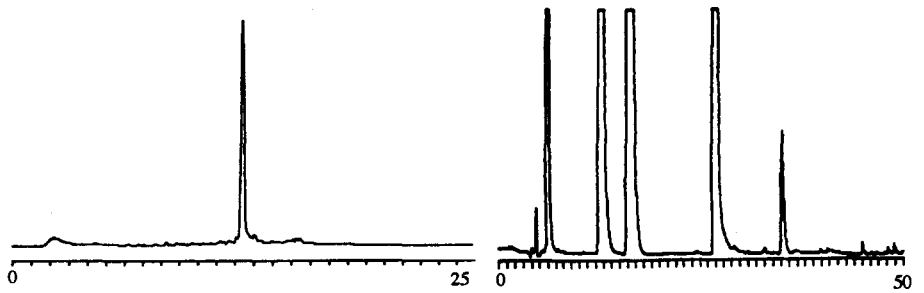


Figure 3. Anion-exchange HPLC (left) and RP HPLC of enzymatic digest (right) of 18mer 5' TCA CAG TCT GAT CTC GAT 3' made with 0.02M iodine oxidation reagent

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REFERENCES AND NOTES

1. Zemlicka, J.; Chládek, S.; Holy, A.; Smrt, J. *Collect. Czech. Chem. Commun.* **1966,** *31*, 3198-3212.
2. Holy, A.; Zemlicka, J. *Collect. Czech. Chem. Commun.* **1968,** *34*, 2449-2458.
3. Arnold, L.; Tocik, Z.; Bradkova, E.; Hostomsky, Z.; Paces, V.; Smrt, J. *Collect. Czech. Chem. Commun.* **1989,** *54*, 523-532.
4. Froehler, B. C.; Matteucci, M. D. *Nucleic Acids Research* **1983,** *11*, 8031-8036.
5. McBride, L. J.; Kierzek, R.; Beaucage, S. L.; Caruthers, M. H. *Journal of the American Chemical Society* **1986,** *108*, 2040-2048.
6. Vu, H.; McCollum, C.; Jacobson, K.; Theisen, P.; Vinayak, R.; Speiss, E.; Andrus, A. *Tetrahedron Letters* **1990,** *31*, 7269-7272.
7. Arnold, L.; Smrt, J.; Zajícek, J.; Ott, G.; Schiesswohl, M.; Sprinzl, M. *Collect. Czech. Chem. Comm.* **1991,** *56*, 1948-1956.
8. Andrus, A.; Theisen, P. D.; McCollum, C. *Nucleic Acids Symposium Series* **1993,** *29*, 5-6.
9. The set of A^{bz}, G^{dmf}, C^{bz}, and T provide a good balance of stability, during preparation of the nucleoside monomers and oligonucleotide synthesis, and rapid deprotection. After synthesis and cleavage, deprotection of this set is complete within 1 hour at 65 °C in concentrated aqueous ammonia. Theisen, P.; McCollum, C. *Nucleic Acids Symposium Series* **1993**, *29*, 5-6.
10. Mullah, B.; Andrus, A., Applied Biosystems Division of Perkin-Elmer Co.
11. Zhao, H.; Jones, R.A., Rutgers University
12. McCollum, C.; Chakerian, V.; Kaufman, J.; Wenz, M.; Andrus, A. *Biomedical Peptides, Proteins & Nucleic Acids* **1995,** *1*, 25-30.
13. Oligonucleotide synthesis was conducted on Applied Biosystems 392 and 394 DNA/RNA Synthesizers at 40 nmole scale using the recommended reagents and protocols, including version 2.01 software. Average step-wise yields were calculated by AutoAnalysis - real-time, conductivity trityl monitoring (Kaufman, J.; Le, M.; Ross, G.; Hing, P.; Budiansky, M.; Yu, E.; Campbell, E.; Yoshimura, V.; Fitzpatrick, V.; Nadimi, K.; Andrus, A. *Biotechniques* **1993**, *14*, 834-839).
14. Appendix 1, *Evaluating and Isolating Synthetic Oligonucleotides,* (1992) Applied Biosystems Division of Perkin-Elmer Co., available upon request.
15. Gao, H.; Fathi, R.; Gaffney, B. L.; Goswami, B.; Kung, P.-P.; Rhee, Y.; Jin, R.; Jones, R. A. *Journal of Organic Chemistry* **1992,** *57*, 6954-6959.
16. *N*-2-Cyano-2'-deoxyguanosine (**2**). To 322 mg (1 mmol) of *N*-2-dimethylformamidine-2'-deoxyguanosine and 279 mg (1.1 mmol) of iodine was added 80 mL of concentrated aqueous ammonia. The mixture was stirred at room temperature for one hour, evaporated to about 10 mL, and applied to a 40 mm x 300 mm C18 Delta-Pak HPLC column.Elution using a gradient of 0 to 5 % acetonitrile to 0.1 M

NH_4HCO_3 in 30 min at a flow rate of 24 mL/min gave 22,500 OD_{270} (0.86 mmol, 86%) of **2** as the ammonium salt. UV (H_2O) max 270 nm (ε_{270}=2.6 x 10^4 $cm^{-1}mol^{-1}L$); min 234 nm (ε_{234}=6.8 x 10^3 $cm^{-1}mol^{-1}L$). IR (KBr) 3499, 3120, 2168, 1673 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ (ppm) 10.41 (b, 1, H_1), 8.03 (s, 1, H_8), 7.34, 7.08 & 7.83 ("t", 4, J_{app}=51.1 Hz, NH_4^+), 6.16 ("t", 1, J_{app}=6.9 Hz, $H_{1'}$), 5.28 (m, 1, 3'-OH), 4.35 (m, 1, $H_{3'}$), 3.82 (m, 1, $H_{4'}$), 3.54 (m, 2, $H_{5',5''}$), 2.56 & 2.28 (m & m, 1 & 1, $H_{2'}$ & $H_{2''}$); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ (ppm) 159.9 (C_6=O), 158.3 (C_2), 151.7 (C_4), 145.0 (C_8), 120.9 (C≡N), 116.0 (C_5), 87.7 ($C_{1'}$), 82.4 ($C_{4'}$), 71.1 ($C_{2'}$), 62.0 ($C_{3'}$), 39.6 ($C_{5'}$); CIMS (FAB+) m/z: 293(100, M+1); EIMS m/z: 176(71), 151(94), 81(98). A portion of this material was converted to the sodium form using a Bio-Rad 50W-X4 column. ^1H NMR (200 MHz, DMSO-d_6) δ (ppm) 10.20 (s, 1, H1), 8.03 (s, 1, H8), 6.13 ("t", 1, J_{app}=6.3 Hz, H1'), 5.28 & 5.26 (d, 1, Japp=2.5 Hz, 3'-OH), 4.86 (m, 1, 5'-OH), 4.33 (m, 1, H3'), 3.79 (m, 1, H4'), 3.51 (m, 2, H5',5"), 2.48 & 2.19 (m & m, 1 & 1, H2' & H2"). Anal. calcd. for $C_{11}H_{11}N_6O_4Na•H_2O$: C 39.76, H 3.94, N 25.30. Found: C 39.55, H 4.06, N 25.03.

17. *N*-2-[^{15}N]-cyano-2'-deoxyguanosine. To 545 mg (10 mmol) of $^{15}NH_4Cl$, 400 mg (10 mmol) of NaOH and 279 mg (1.1 mmole) of iodine were added 100 mL of water and 322 mg (1 mmol) of *N*-2-dimethylformamidine-2'-deoxyguanosine (**1**). The mixture was maintained at room temperature for one day, concentrated to about 10 mL, and applied to the above preparative reversed-phase HPLC column. Elution as above gave *N*-2-[^{15}N]-cyano-2'-deoxyguanosine as the sodium salt. ^1H NMR (200 MHz, DMSO-d_6) δ (ppm) 10.16 (b, 1, H_1), 7.78 (s, 1, H_8), 6.13 ("t", 1, J_{app}=6.9 Hz, $H_{1'}$), 5.27 & 5.25 (d, 1, J_{app}=3.9 Hz, 3'-OH), 4.85 (m, 1, 5'-OH), 4.32 (m, 1, $H_{3'}$), 3.79 (m, 1, $H_{4'}$), 3.51 (m, 2, $H_{5',5''}$), 2.48 & 2.19 (m & m, 1 & 1, $H_{2'}$ & $H_{2''}$); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ (ppm) 159.9 (C_6=O), 158.1 (C_2), 151.6 (C_4), 134.8 (C_8), 121.0 & 120.7 (d, C≡^{15}N, J=16.7 Hz), 116.0 (C_5), 87.6 ($C_{1'}$), 82.2 ($C_{4'}$), 71.0 ($C_{2'}$), 61.9 ($C_{3'}$), 34.5 ($C_{5'}$); ^{15}N NMR (40.5 MHz, H_2O) δ (ppm) 173.9; ^{15}N NMR (40.5 MHz, DMSO-d_6) δ (ppm) 190.4; CIMS (FAB-) m/z: 292.
18. *N*-6-cyano-2'-deoxyadenosine. To 306 mg (1 mmol) of *N*-2-dimethylformamidine-2'-deoxyadenosine and 0.279 g (1.1 mmol) iodine was added 80 mL of concentrated aqueous ammonia. The mixture was stirred at room temperature for one hour, evaporated to about 10 mL, and applied to the above preparative HPLC column. Elution using a gradient of 0 to 10 % acetonitrile in 0.1 M TEAA buffer in 30 min at a flow rate of 24 mL/min gave the triethylammonium salt of *N*-6-cyano-2'-deoxyadenosine. This material was desalted on the same column using water in place of TEAA, and converted to the sodium form as above to give 27,800 OD_{289} (0.93 mmol, 93%). UV (H_2O) max_1 289 nm (ε_{289}=3.0 x 10^4 $cm^{-1}mol^{-1}L$); max_2 220 nm (ε_{220}=1.4 x 10^4 $cm^{-1}mol^{-1}L$); min 239 nm (ε_{239}=1.8 x 10^3 $cm^{-1}mol^{-1}L$). IR (mineral oil) 2144, 1588, 1460, 1376 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ (ppm) 8.14 (s, 1, H_8), 8.02 (s, 1, H_2), 6.28 ("t", 1, J_{app}=7.0 Hz, $H_{1'}$), 5.43 (m, 1, 3'-OH), 5.26 (m, 1, 5'-OH), 4.37 (m, 1, $H_{3'}$), 3.86 (m, 1, $H_{4'}$), 3.57(m, 2, $H_{5',5''}$), 2.68 & 2.22 (m & m, 1 & 1, $H_{2'}$ & $H_{2''}$); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ (ppm) 161.4 (C_6=O), 150.7 (C_2), 148.3 (C_4), 138.5 (C_8), 123.7 (C_5),120.9 (C≡N), 88.1 ($C_{1'}$), 84.2 ($C_{4'}$), 71.1 ($C_{2'}$), 62.0 ($C_{3'}$); CIMS (FAB-) m/z: 275(100, M-1). Anal. calcd. for $C_{11}H_{11}N_6O_3Na•H_2O$: C 41.77, H 4.14, N 26.58. Found: C 42.08, H 4.26, N 26.54.
19. *N*-2-cyano-guanosine. IR (cm^{-1}) 2175, 1670
20. Jander, J. *Adv. Inorg. Chem. Radiochem.* **1976**, *19*, 2.
21. The gradient is 2 to 15% CH_3CN:0.1M TEAA in 5 min at 2 mL/min on a 5mm x 10cm Nova-Pak cartridge.
22. *N*-2-Formyl-2'-deoxyguanosine (**3**). To 322 mg (1 mmol) of *N*-2-dimethylformamidine-2'-deoxyguanosine was added 80 mL of concentrated aqueous ammonia. The solution was maintained at room temperature for three hours, evaporated to about 10 mL, and applied to the above preparative HPLC column. Elution using a gradient of 0 to 10 % acetonitrile in 0.1 M NH_4HCO_3 in 30 min at a flow rate of 24 mL/min gave 4580 OD_{261} (0.22 mmol, 22%) of **3**. UV (H_2O) max 261 nm (ε_{261}=2.1 x 10^4 $cm^{-1}mol^{-1}L$); min 236 nm (ε_{236}=8.1 x 10^3 $cm^{-1}mol^{-1}L$). IR (KBr) 3375, 1691, 1603 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ (ppm) 10.17 (b, 2, H_1 & N_2H), 9.13 (b, 1, O=C—H), 8.12 (s, 1, H_8), 6.22 ("t", 1, J_{app}=6.6Hz, $H_{1'}$), 5.35 (m, 1, 3'-OH), 4.95 (m, 1, 5'-OH), 4.37 (m, 1, $H_{3'}$), 3.82 (m, 1, $H_{4'}$), 3.53 (m, 2, $H_{5',5''}$), 2.62 & 2.28 (m & m, 1 & 1, $H_{2'}$ & $H_{2''}$); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ (ppm) 163.2 (HC=O), 157.8 (C_6=O), 149.4 (C_2), 148.9 (C_4), 137.5 (C_8), 120.5 (C_5), 87.7 ($C_{1'}$), 83.2 ($C_{4'}$), 70.8 ($C_{2'}$), 61.7 ($C_{3'}$); CIMS (FAB+) m/z: 296(52, M+1), 180(100). Anal. calcd. for $C_{11}H_{13}N_5O_5•1/2H_2O$: C 43.57, H 4.77, N 22.89. Found: C 43.34, H 4.63, N 23.02.

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