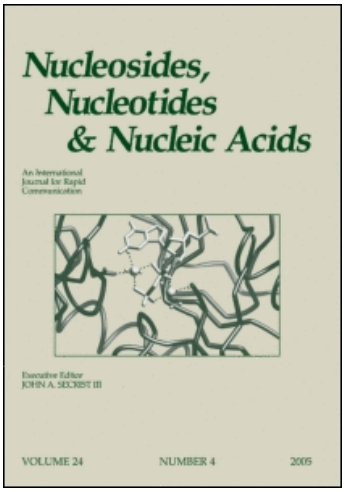


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# A One Step Synthesis of O⁶-Methyl-2'-deoxyguanosine

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# A ONE STEP SYNTHESIS OF $O^6$-METHYL-2'-DEOXYGUANOSINE

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ABSTRACT

A single step chemical synthesis of $N^7$-methyl-2'-deoxyguanosine ($m^7dG$), $N^1$-methyl-2'-deoxyguanosine ($m^1dG$) and $O^6$-methyl-2'-deoxyguanosine ($m^6dG$) is described. The products were separated on the silical gel plates and characterized by nuclear magnetic resonance and mass spectrometry.

Alkylating agents are powerful mutagens (1) and carcinogens (2) and they interact with DNA both *in vivo* and *in vitro* (3).

The $N^7$ position of the guanine is the main target of simple alkylating agents such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). However it is generally accepted that such a modification is not harmful



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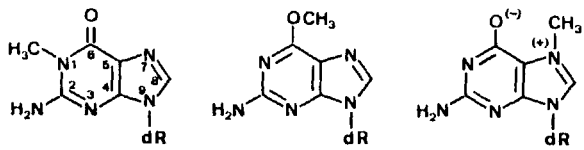

$m^1dG$ $m^6dG$ $m^7dG$

Main products obtained after methylation of dG with MNNG (dR = 2-deoxyribose)

and can persist in the cell for generations, even if it has been recently observed (4,5) that it can be removed at a very low rate. On the other hand MNNG binds also the $O^6$ position of the guanine, this lesion appears to be the major mutagenic lesion (3,6). The mode of repair of this damage ($O^6$-methylguanine) is of a particular interest because carcinogenesis induced by methylating agents is apparently correlated with defective or insufficient repair of this lesion (7,8).

In this respect, it is worthwile to note that in Escherichia coli an inducible form of repair called adaptation, is expressed after exposure of the bacteria to low concentrations of alkylating agents (9). This inducible repair results in a rapid removal of the $O^6$-methylguanine residues thereby reducing the mutations caused by the exposure of the bacteria to these agents (10).

A number of in vitro experiments have been undertaken to elucidate the role of $O^6$-methylguanine during replication (11) and transcription (12). In addition the molecular mechanism of removal of $O^6$-methylguanine residues from DNA is now beginning to be understood in vitro (13).

All these studies required enzymatic or chemical hydrolysis of DNA substrates, the resulting products being identified by chromatographic analysis (13). In order to follow these procedures, the availability of suitable markers such as $m^6dG$ in reasonable quantities is of great interest. Unfortunately the methods reporting the synthesis of $m^6dG$

are laborious and time consuming (14,15). We present here a convenient and quick method for the synthesis of methylated 2'-deoxyguanosine (dG) on $O^6$, $N^1$ and $N^7$ positions and their spectrometric identification.

## EXPERIMENTAL

N-methyl-N'-Nitro-N-Nitrosoguanidine (MNNG) was purchased from Aldrich and 2'-deoxyguanosine (dG) from Sigma.

### Synthesis

MNNG 1 g (6.8 mmoles) was cautiously added by aliquot to 3 ml KOH 40 % and 10 ml ether in an ice bath under the hood. The resulting solution of diazomethane in ether was dried over KOH pellet for 2 hours and filtered. This solution was mixed with 20 ml methanol containing 60 mg of dG (0.225 mmoles). The reaction was followed by thin layer chromatography in $CH_3OH/CHCl_3$ (3 : 7) and after 2 hours a complete disappearance of dG and three main spots corresponding to $N^1$, $N^7$, $O^6$-methyl derivatives were observed. At the end of the reaction a precipitate formed and corresponded to $m^7dG$. The resulting filtrate was evaporated to dryness under vacuum. The solid material was then dissolved in a minimun of $CH_3OH$ and chromatographied on two preparative plates of silica gel (Kieselgel Merck 60 $F_{254}$) and was then developped in $CH_3OH/CHCL_3$(3:7).

Three main bands were observed with the following Rfs 0.02, 0.52 and 0.74 corresponding to the $N^7$, $N^1$ and $O^6$- methyl derivatives. These bands were excised and eluted with $CH_3OH$ at 4°C overnight. The solvent was removed under vacuum and an aliquot of each product was chromatographied on TLC in order to check the print of each derivative. The structural characterisation of each compound was conducted with nuclear magnetic resonance and mass spectrometry. The purity of the three products dissolved in isopropyl alcohol/$H_2O$ (7:3) was also

checked by using analytical paper Whatman 1M and the following Rfs were obtained : 0.45 ($m^7$dG, 10 % yield), 0.59 ($m^1$dG, 19 % yield), 0.73 ($m^6$dG, 22 % yield). We observed that $m^7$dG and $m^6$dG were fluorescent.

Analytical Methods

Mass spectra were recorded on a Riber R10-10 with desorption chemical ionisation method and nuclear magnetic resonance spectra on a Bruker WH90 (90 MHz) in the FT mode. Chemical shifts are expressed in ppm (δ) with tetramethylsilane (TMS) as internal standard, solvent DMSO-$d^6$, concentration ~4 mg/ml (s = singlet, d = doublet, t = triplet, m = multiplet; b = broad).

NMR $m^6$dG : δ 3.55 (m, 4H, 2'-H and 5'-H), 3.84 (m, 1H, 4'-H), 3.93 (s, 3H, $CH_3$), 4.35 (m, 1H, 3'-H), 5.00 (b.l., 1H, 5'-OH), 5.30 (b.l., 1H, 3'-OH), 6.22 (t, J = 6,3 Hz, 1H, 1'-H), 6.45 (b.s., 2H, $NH_2$), 8.08 (s, 1H, 8-H).

$m^1$dG : δ 3.37 (s, 3H, $CH_3$), 3.49 (m, 4H, 2'-H and 5'H), 3.78 (m, 1H,4'H), 4.33 (m, 1H, 3'-H), 4.95 (b.s., 1H, 5'-OH), 5.29 (b.s., 1H, 3'-OH), 6.11 (t, J=6.7 Hz, 1H, 1'-H), 7.03 (b.s., 2H, $NH_2$), 7.92 (s, 1H, 8-H).

$m^7$dG : δ 3.59 (m, 4H, 2'-H and 5'-H), 3.82 (m, 1H, 4'-H), 4.00 (s, 3H, $CH_3$), 4.37 (m, 1H, 3'-H), 4.91 (b.s., 1H, 5'-OH), 5.39 (b.s., 1H, 3'-OH), 6.19 (t, J = 6,6 Hz, 1H, 1'-H), 6.55 (b.s., 2H, $NH_2$), 7.78 (s, 1H, 8-H).

MS $m^6$dG : m/e 282, 100 % ($MH^+$) ; 166, 15 % ($BH^+$) ; 134, 42 % (dR). $m^1$dG : m/e 282, 100 % ($MH^+$) ; 166, 24 % (BH+) ; 134, 25 % (dR). $m^7$dG : m/e 282, 47 % ($MH^+$) ; 166, 100 % ($BH^+$) ; 134, 84 % (dR).

Others physical constants (m.p., UV) agreed with the literature values (14).

## RESULTS (Structural Characterisation)

Mass spectrometry and nuclear magnetic resonance were used to characterize easily the three different compounds $m^7$dG, $m^1$dG and

$m^6dG$. Nuclear magnetic resonance ($^1H$) spectroscopy showed $\delta CH_3$ from $m^7dG$ and $m^6dG$ very close (4.00 and 3.93 ppm) ; they can be distinguished from $m^1dG$ for the methyl group of 3.37 ppm with chemical shift.

In the mass spectrometry of the guanosine compounds, even under good conditions, extensive pyrolysis occurs and the spectra exhibits no molecular ion. Only $(M-H_2O)^+$ is the ion of highest mass, cleavage of the glycosidic bond leads to the base and sugar ion. Desorption chemical ionisation method permitted a more satisfactory approach without using volatile derivatives. Molecular ion $MH^+$ is obtained for the three products $m^1dG$, $m^6dG$, $m^7dG$ but we observed 100 % intensity only with the first ones, and 47 % with the last one.



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REFERENCES

1. P.D. Lawley, Mut. Res. 23, 283-295 (1974).
2. P.N. Magee and J.M. Barnes, Adv. Cancer. Res. 10, 163-246 (1967).
3. P.F. Schendel and P.E. Robins, Proc. Nat. Acad. Sci. 75, 6017-6020 (1978).
4. J. Laval, J. Pienne and F. Laval, Proc. Nat. Acad. Sci. 78, 852-855 (1981).
5. B. Singer and T. Bren, Proc. Nat. Acad. Sci. 78, 856-860 (1981).
6. R. Sklar and B. Strauss, J. Mol. Biol. 143, 343-362 (1980).
7. R. Goth and M. Rajewsky, Proc. Natl. Acad. Sci. 71, 639-643 (1974).
8. J.G. Lewis and J.A. Swenberg, Nature, 288, 185-187 (1980).
9. L. Samson and J. Caimus, Nature 267, 281-283 (1977).
10. M. Olsson and T. Lindhal, J. Biol. Chem. 255, 10569-71 (1980).
11. D.B. Ludlum, J. Biol. Chem. 245, 377-382 (1970).

12. P. Abbott and R. Saffhill, Bioch. Bioph. Acta 562, 51-61 (1979).

13. S. Footer, S. Mitra and B.C. Pal, Bioch. Bioph. Res. Com. 97, 654-659 (1980).

14. P.B. Farmer, A.B. Foster, M. Jarman and J. Tisdale, Biochem. J. 135, 203-213 (1973).

15. R. Stevenson, B. Dash, G. Mahpatra and O. Friedman. Bioch. Bioph. Acta 103, 286-297 (1965).

16. C. Fenselan, M.N.N. Kan, Jr. G.H. Sack, G.W. Wood, D.E. Schmidt and P.Y. Lau, Adv. Mass Spectrom. 7B, 1572-7 (1978).

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