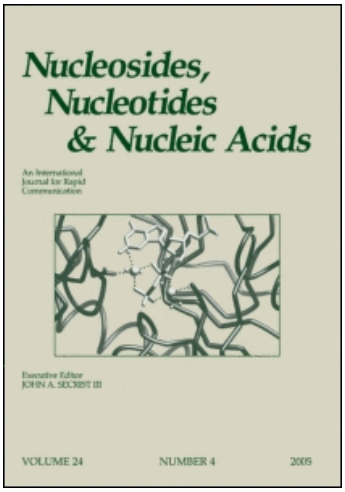

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A Convenient Preparation of Protected 2'-0-Methylguanosine

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A CONVENIENT PREPARATION OF PROTECTED 2'-O-METHYLGUANOSINE

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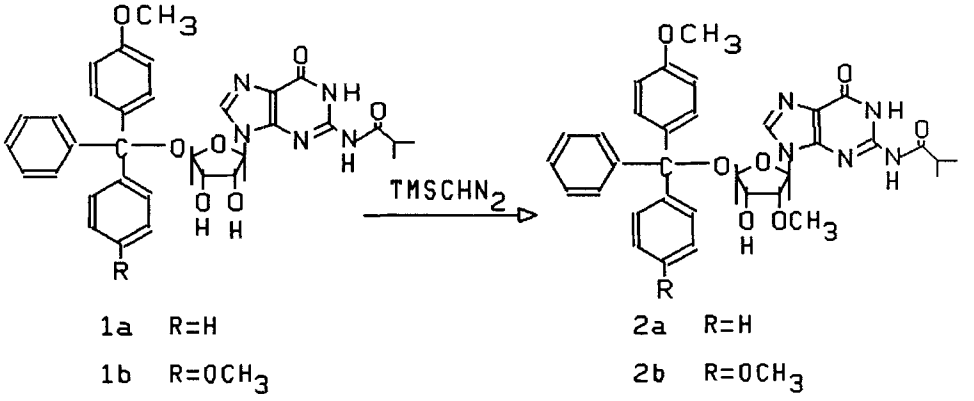
Abstract: *Protected guanosine nucleosides can be converted directly to their 2'-O-methyl derivatives by methylation with trimethylsilyldiazomethane in the presence of stannous chloride. This procedure circumvents the need to use the potentially hazardous reagent, diazomethane.*

Recent reports have described the syntheses and properties of oligo-2'-O-methylribonucleotides and oligo-2'-O-methylribonucleoside methylphosphonates[1-4]. These oligonucleotide analogs show increased resistance to nuclease hydrolysis and a high affinity for complementary RNA sequences and thus they are attractive candidates for use as antisense reagents. The oligomers are prepared from suitably protected 2'-O-methylribonucleoside-3'-O-N,N-*bis*-(diisopropyl)amino-ß-cyanoethylphosphoramidite or methylphosphonamidite synthons. These in turn are prepared from suitably protected 2'-O-methylribonucleosides.

The 2'-O-methyl derivatives of some common ribonucleosides have been synthesized from their appropriately protected precursors using methyl iodide in the presence of either silver oxide[5] or sodium hydride[6]. The more difficult to synthesize guanosine derivatives have been prepared in a six step procedure starting from the 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-2-amino-6-chloropurine riboside using methyl iodide and the sterically hindered base, 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1.3.2-diazaphosphorin[7]. Alternatively 2'-O-methylguanosine can be obtained directly by methylation with diazomethane at low temperature in dimethylformamide in the presence of the Lewis acid catalyst, stannous chloride[8]. Although the latter method is more direct, it does employ the hazardous and potentially explosive reagent, diazomethane.



1a R=H
1b R=OCH_3

2a R=H
2b R=OCH_3

Scheme 1

Recently, a generealized procedure employing trimethylsilyldiazomethane ($TMSCHN_2$) for the methylation of alcohols has been published[9]. We wish to report here, the use of trimethylsilyldiazomethane as a suitable substitute for diazomethane in the methylation of N^2-isobutyryl-5'-O-(4-methoxytrityl)-guanosine (1a) and N^2-isobutyryl-5'-O-(4,4'-dimethoxytrityl)-guanosine (1b).

In 130 mL of dichloromethane was dissolved 3.12 g (5 mmol) of N^2-isobutyryl-5'-O-(4-methoxytrityl)-guanosine[10] (1a). A solution of stannous chloride was prepared by dissolving 230 mg of stannous chloride (prepared by drying the dihydrate at room temperature, in vacuo over phosphorous pentoxide) in 10 mL of dimethylformamide. This was added to the first solution and the entire mixture cooled to 0°C. Then, 2.8 mL of a 2.0 M solution of $TMSCHN_2$ in hexane was added dropwise over 30 minutes. The reaction was brought to room temperature and stirred for 18 hours. Silica gel TLC in 94:6 dichloromethane/methanol showed spots corresponding to dimethylated, monomethylated, and unmethylated material. The reaction mixture was washed first with bicarbonate, then with brine and finally evaporated to dryness. The residue was taken up in dichloromethane, applied to a silica gel column (4cm X 30cm), and eluted with 94:6 dichloromethane/methanol to give a product, which by proton NMR, was revealed to be a mixture of the 2' and 3'-O-methyl isomers in approximately a 3 to 1 ratio. The regioisomers were

separated as previously described[5] to give 675 mg (1.06mmoles, 21% overall yield) of the desired N^2-isobutyryl-2'-O-methyl-5'-O-(4-methoxytrityl)-guanosine (**2a**).

The spectral data were consistent with the structure of **2a**, a compound previously reported by Inoue[5]. The FAB mass spectrum indicated one degree of methylation (MH^+ = 640) and the BH_2^+ at m/z 240 indicated sugar versus base methylation. The proton NMR spectrum in $CDCl_3$ further supported 2'-O-methylation. ^1H NMR (in ppm, $CHCl_3$ internal std.) 8.98 (1,s,N^1-H), 7.88 (1,s,H-8), 6.78-7.49 (14,m,MMTr), 5.90 (1,d,H-1'), 4.57 (2,d,H-5'), 3.75 (3,s,MMTrOMe), 3.45 (3,s,2'-OCH_3).

For the purposes of preparing oligonucleotides, it is desirable to protect the 5'-OH group of the nucleotide synthons with the more acid labile 4,4'-dimethoxytrityl group. Methylation of 1.96 g (3 mmoles) of the dimethoxytriyl derivative, **1b**, under the conditions described above gave results identical to those obtained with **1a** as evidenced by the proton NMR spectrum. As in the case of the monomethoxytrityl compound, initial separation on silica gel using 94:6 dichloromethane/methanol as solvent, afforded material which displayed two singlets at 3.43 and 3.41 ppm in a 3:1 ratio corresponding to the 2'- and 3'-O-methyl isomers respectively. The product, **2b**, also reported by Inoue[5], was isolated in 19% overall yield (381 mg, 0.57 mmoles) by flash chromatography on silica gel using 60:24:1 chloroform/acetonitrile/methanol[5] (v/v) as solvent.

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