Demethylation of 7-Methylguanosine with Lithium 2-Methylpropane-2-thiolate

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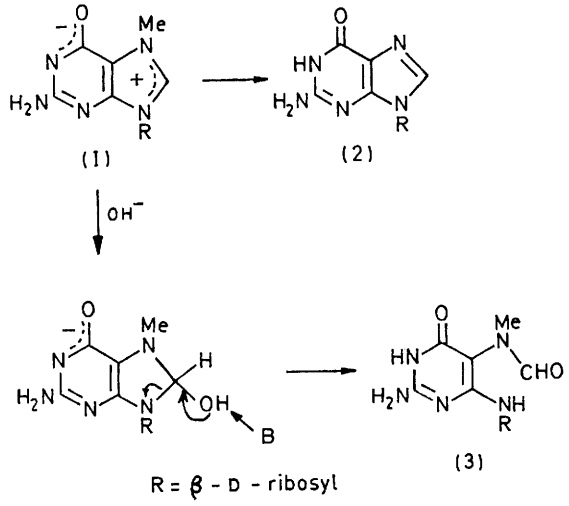
Summary 7-Methylguanosine, a component of transfer ribonucleic acid, is demethylated to guanosine, its presumed biochemical precursor, by a powerful new nucleophilic reagent, lithium 2-methylpropane-2-thiolate in hexamethylphosphoramide.

OF the substantial number of modified nucleoside species in transfer RNA,[1] 7-methylguanosine is unusual in its zwitterionic character at physiological pH. Our interest in the function of this nucleoside at the tRNA level as well as in its biosynthetic origin, prompted us to examine chemical methods directed towards the synthesis of tRNA analogues in which the 7-methylguanylic acid units have been formally replaced by guanylic acid, the presumed biochemical precursor. We now report the demethylation of 7-methylguanosine (**1**) to guanosine (**2**).

Although procedures have been developed for the demethylation of amines, *e.g.* photolytic[2] and catalytic[3] methods, the demethylation of 7-methylguanosine by a procedure applicable to tRNA must be highly selective, since tRNA species contain at least eight other *N*-methylated nucleosides. Among these nucleosides, only in 7-methylguanosine does the methyl group reside on a nitrogen atom with substantial quaternary character. This methyl group would therefore be expected to be more susceptible to displacement by nucleophilic attack than those in the other ribonucleosides. However, strong bases add readily to C-8 of the purine, deactivating the imidazolium ring and, in aqueous medium, ultimately affording the pyrimidine (**3**).[4] Of several reported methods for effecting demethylations,[5–12] none was effective in the conversion (**1**) → (**2**) with the exception of treatment with lithium propane-1-thiolate in hexamethylphosphoramide, which, at 75 °C for 4 h, afforded trace amounts of guanosine and caused complete decomposition of 7-methylguanosine to other products. These products were not identified, but were presumed to be derived from initial nucleophilic attack on C-8.



(1) (2) (3)

The glycosyl torsion angle[13] defines two basic types of conformational isomers of ribonucleosides, possessing substantially different steric features in the region of C-8. If conformers of the *anti*-type were to contribute significantly to the solution chemistry of 7-methylguanosine, a more hindered nucleophile might exhibit greater selectivity for attack on the 7-methyl group than C-8. We therefor

prepared lithium 2-methylpropane-2-thiolate, a sterically hindered compound expected to have nucleophilic properties superior to lithium propane-1-thiolate.† The reagent was prepared by treatment of 2-methylpropane-2-thiolate with lithium hydride in hexamethylphosphoramide at room temperature. Conversion into guanosine in quantitative yield was achieved by successive 4 h treatments of the nucleoside (**1**) with several 25-equiv. portions of the thiolate at 70 °C.‡ The product was isolated by precipitation with ether and purified chromatographically.

The tRNA itself is sufficiently inert to the reaction conditions described here to allow demethylation of the nucleic acid itself. None of the other known methylated ribonucleosides in tRNA was demethylated by this reagent. Therefore, selective conversion of 7-methylguanosine into guanosine at the tRNA level, as well as the selective demethylation of other sensitive compounds,[14] should now be possible.

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† During this work we learned of the simultaneous development of this reagent in the laboratory of Professor G. Büchi. The 2-methylpropane-2-thiolate was found to be superior to the propane-1- thiolate analogue for conversion of Aflatoxin B_1 into Aflatoxin P_1.[14] We thank Professor Büchi and R. L. Garnick for discussions.

‡ Essentially no conversion was observed when dimethylformamide was substituted for hexamethylphosphoramide.

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