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Note

Catalytic monomethylation of the *cis*-glycol system by diazomethane. Effects of novel inorganic catalysts on isomer ratios*

Morris J Robins, Alan S K Lee, and Frank A Norris†
Department of Chemistry, The University of Alberta, Edmonton, Alberta T6G 2G2 (Canada)
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Nucleoside 2′-methyl ethers are ubiquitous "minor components" of ribonucleic acids (RNAs)[1] These component 2′-*O*-methyl-nucleosides and -nucleotides are resistant to certain enzymic reactions common to the corresponding unmethylated products[2] Synthetic polynucleotides containing these monomers have been prepared and studied to evaluate aspects of this structural feature *vs* a free 2′-OH on macromolecular properties and biochemical responses[3–5] Further background information and reasons for employing these features in the design of nucleoside "drugs" resistant to certain enzymic degradations have been discussed[6]

Studies involving uncatalyzed methylation of adenosine by diazomethane gave overall yields of methylated products in the 50% range[7–9] with 2′-*O*-methyladenosine as the major (of at least five[9]) product Other routes to *O*-methylnucleosides are often rather extensive multistep procedures or lead to complex mixtures[6] Complexing of copper ions by the *cis*-glycol-containing D-ribose moiety of adenosine (but not by 2′-deoxyadenosine), reported[10] during our investigation of methylated pyrimidine nucleosides[11], prompted our study of the influence of metal salts on the diazomethane reaction with nucleosides Stannous chloride or aluminum chloride in methanolic solution was found to catalyze quantitative monomethylation of adenosine by diazomethane with the formation of the 3′-isomer predominating[12] Further work has extended this convenient procedure to all the major nucleosides[6] This catalyst also extends access to 2′-*O*- and 3′-*O*-benzyl compounds by use of phenyldiazomethane[13] Analogous catalysis by tin (but reportedly not by aluminum) chloride was observed in methylations of glucopyranosides[14]

Other Lewis acids were evaluated[12] and we now report several novel catalysts for monomethylation of the *cis*-glycol system of nucleosides (and other diols) using

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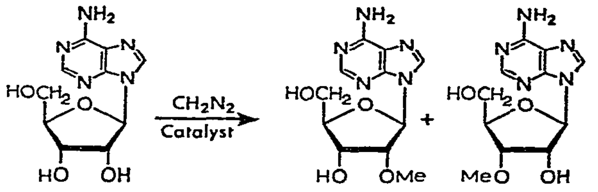
†Postdoctoral fellow 1971–1972

diazomethane The inorganic compounds were tested for catalytic activity at ~1 mM concentration in methanol or as "saturated solutions", if insoluble (marked with an asterisk *) at that concentration Adenosine (**1**) was added and the resulting mixture was stirred at room temperature while a solution of diazomethane in Glyme[6] was added In the case of colorless solutions, the addition was continued until the yellow color of diazomethane persisted A maximum of 20 ml (22 equivalents) of diazomethane solution was used for other evaluations Catalytic activity was indicated by t l c analysis of the reaction mixtures, adenosine (**1**), 2'-*O*-methyladenosine[6] (**2**), and 3'-*O*-methyladenosine[6] (**3**) being used as standards for comparison



1 **2** **3**

Essentially complete conversion (t l c) of **1** into **2** and **3** was observed with $SnCl_2$ $2H_2O$, $SnCl_4$, $SnSO_4$*, $SnBr_2$*, $AlCl_3$ $6H_2O$, $FeCl_2$, $FeCl_3$, and $TiCl_4$

A second group of compounds had moderate catalytic activity, but some starting material remained untransformed under these conditions $CeCl_3$ $7H_2O$, MoO_2Cl_2, $(NH_4)_6Mo_7O_{24}$ $4H_2O$*, and $MnCl_2$ $4H_2O$

Trace amounts of methylated products were formed in the presence of B_2O_3, $Na_2B_4O_7$ $10H_2O$, $MoCl_5$, and $VOSO_4$*

No detectable methylation of **1** under the conditions used was observed with $MgCl_2$ $6H_2O$, $MgSO_4$*, $Ca(NO_3)_2$ $4H_2O$, $CrCl_3$, $Cr_2(SO_4)_3$ nH_2O*, $MnSO_4$ H_2O*, $CoCl_2$ $2H_2O$, $NiCl_2$ $6H_2O$, $PdCl_2$ $2H_2O$*, $PtCl_4$, Cu_2Cl_2*, $CuCl_2$ $2H_2O$, $ZnCl_2$, Hg_2Cl_2*, $HgCl_2$, H_3BO_3, BF_3 $O(Et)_2$, $PbCl_2$*, $Pb(OAc)_2$ $3H_2O$, SeO_2, CH_3CO_2H, H_3PO_4, HCl, and H_2SO_4

The qualitative t l c evaluations indicated that the ratio of formation of **2** and **3** was similar (**3** preponderating slightly) for all but four of the active catalysts Samples (1 millimole) of **1** were subjected to the treatment just described with these catalysts, followed by quantitative ion-exchange chromatographic separation[6] The following isomer distributions were found

Catalyst	*Yield* (%)		
	2	**3**	*Overall*
$SnCl_2$ $2H_2O$	44	56	100
$TiCl_4$	23	63	86
MoO_2Cl_2	50	18	68
$CeCl_3 \cdot 7H_2O$	60	15	75

Stannous chloride dihydrate is convenient since it is a stable, innocuous, and colorless solid. It provided quantitative monomethylation yields (ratios of isomers vary slightly with reaction conditions) rapidly and cleanly. The readily available iron chlorides were equally effective, but gave yellow solutions, which precluded observation of the persistence of the diazomethane color, and methylation proceeded more slowly. There was no observable difference due to the oxidation state of the metal when ferrous or ferric chloride or stannous or stannic chloride was used. Also, the anion appeared to exert little influence (except solubility) on the activity of stannous chloride, sulfate, or bromide. It is important to note that organic or mineral acids produced no catalysis and, thus, simple solvolysis of the inorganic compounds to give HX is not sufficient to explain the effect. Boron trifluoride etherate is a standard catalyst for methylation of alcohols in aprotic solvents[15]. It is ineffective in this case, presumably owing to methylation of the solvent methanol (the diazomethane color was very rapidly discharged). However, $\sim$1mM boron trifluoride etherate in *N,N*-dimethylformamide also failed to promote this methylation. These results stand in contrast to the reported methylations of various glycosides by use of boron trifluoride in nonpolar aprotic solvents[16]. It is not known whether this failure to react is a function of the basic heterocycle of the nucleoside system, which might effectively complex the boron trifluoride reagent, or of the *N,N*-dimethylformamide solvent.

Boric acid does not catalyze the methylation, but commercial samples of boron oxide and sodium tetraborate (borax) gave rise to observable product. Cyclic borate complexes of *cis*-glycols are well studied systems[17], but the presence of iron (catalytically active) or other active trace contaminants was not excluded. Since a free diol system of restricted steric dimensions appears to be required (as shown by studies with model diol, sugar, and nucleoside systems, to be published), an intramolecular, bidentate complex involving the two hydroxyl groups is indicated.

The 3′-isomer selectivity shown by titanium tetrachloride (proportion of **2** to **3** $\sim$1:3) is seen to be reversed to 2′-isomer predominance by molybdenum dioxydichloride (proportion of **2** to **3** $\sim$3:1) and cerium trichloride heptahydrate ($\sim$4:1). The rather insoluble ammonium molybdate compound also favored formation of **2**. Formation of further methylated by-products was observed during the longer reaction times required for the sluggish catalysis by cerium trichloride. However, since adenosine (**1**) can be eluted from the Dowex column after **3**, recycling of the starting material with the titanium tetrachloride or molybdenum dioxydichloride catalysts provides a high-yield procedure with good isomeric selectivity. Preliminary experiments with uridine indicated enhancement of the formation of the 3′-isomer with a 1·1 ratio of 3′- to 2′-isomer where titanium tetrachloride was used, as compared to a 1:2 ratio where stannous chloride was used[6].

EXPERIMENTAL

The appropriate amount of each inorganic compound (all commercially available products) was added to methanol (50 ml) to give $\sim$1mM solutions. The compounds marked with an asterisk were not readily soluble at that concentration and were evaluated as "saturated solutions" after stirring for ~1 h (the undissolved solid was not filtered) Adenosine (**1**) (0 1 g, 0 37 mmol) was added and the resulting mixture was stirred at room temperature Slow addition of ~0 4M diazomethane in Glyme[6] was continued until the yellow color persisted (with colorless inorganic compounds), or until a maximum of 20 ml (8 mmol) of diazomethane solution was added Catalytic activity was evaluated qualitatively by t l c comparison of the reaction mixtures with authentic markers of **1**, **2**, and **3** on Eastman Silica Gel sheets no 13181 in 9 1 (v/v) chloroform–methanol as developing solvent The relative R_F values, when spotted directly from the reaction mixture, were R_2 R_1 ~2 5 and R_3 R_1 ~2 2 The pure markers had R_2 R_1 ~3 5 and R_3·R_1 ~2 3, when spotted independently

A sample of **1** (0 27 g, 1 mmol) was treated by the just described general reaction procedure in the presence of $SnCl_2$ $2H_2O$, $TiCl_4$, MoO_2Cl_2, or $CeCl_3$ $7H_2O$, until the yellow color persisted and t l c indicated no further significant increase of **2** and **3** The resulting solution was evaporated to dryness, and the residue was dissolved in 2 1 (v/v) ethanol–water (7 ml) The solution was applied to a column (2×50 cm, 110 ml) of Dowex 1-X2 (OH^-) ion-exchange resin[9] packed in the same solvent, and elution with the same solvent gave clean resolution of **2** and **3** as previously described[6] Evaporation of the appropriately pooled fractions *in vacuo* using a Büchler rotary evaporator gave a colorless solid, which was dried by additions and evaporations of absolute ethanol The resulting crystalline compounds **2** and **3** were dried *in vacuo* before weighing for yields

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