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# Mutarotation of Tetramethylglucose Catalyzed by Ribonucleosides

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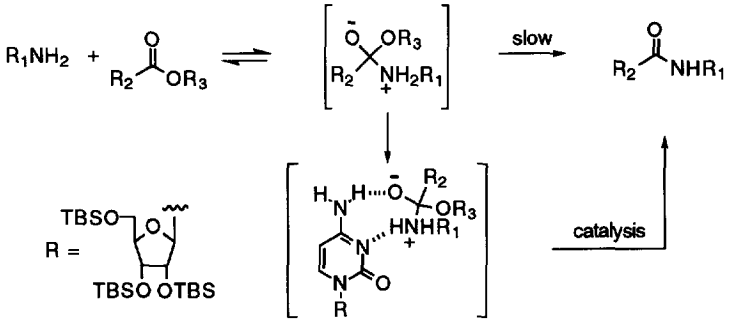
***Abstract:*** The inherent catalytic ability of various 2', 3', 5' tri-OTBS protected RNA nucleosides in facilitating mutarotation of tetramethylglucose in benzene was investigated. Nucleosides A, G, and Ψ showed modest rate enhancements (3-5 fold) over the uncatalyzed rate whereas C demonstrated a rate increase of over 20-fold. The relatively large rate increase for C points to a bifunctional mode of catalysis wherein the amidine functionality of C provides simultaneous acceptance and deliverance of protons in the catalytic mechanism.

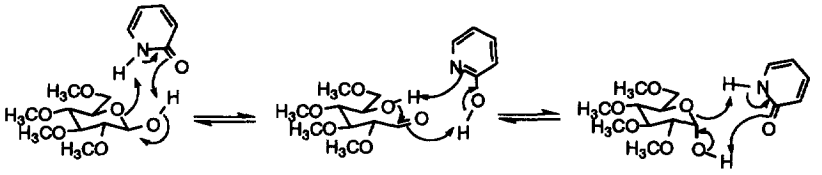

The ability of RNA to catalyze various chemical reactions has advanced rapidly during the past several years due to powerful selection and amplification techniques that have been made available through molecular biology.[1] While the repertoire of reactions that RNA catalyzes continues to increase, the individual components that comprise the overall catalysis remain obscure. In this regard, we recently explored the inherent catalytic properties of the functional groups on individual nucleoside bases toward ester aminolysis.[2,3] This process lies at the core of protein synthesis.[4] It was found that in a non-polar medium, individual nucleosides possess the ability to catalyze amide bond formation and that cytidine (C) was far superior in its catalytic ability over the other nucleosides, adenosine (A), guanosine(G), uridine (U), and pseudouridine (Ψ). This result was attributed to C's ability to function as a bifunctional catalyst in facilitating breakdown of the tetrahedral intermediate (Scheme 1).[2,3] Since the notion that C can act as a bifunctional catalyst is new, we decided to explore other chemical processes in which the bifunctional catalytic properties of C would manifest itself. In this communication, the inherent catalytic properties of nucleosides toward facilitating the mutarotation of tetramethylglucose are reported.

**Scheme 1.** Amide bond catalysis in chloroform.



Bifunctional catalysis was first demonstrated by Swain and Brown for the mutarotation of tetramethylglucose by 2-pyridone (Scheme 2).[4] Simultaneous delivery and acceptance of a proton by 2-pyridone facilitates the interconversion of α and β anomers of tetramethylglucose to a much greater extent than monofunctional donor and acceptor catalysts with similar $pk_a$ values. Similarly, nucleosides employing a general base or acid mechanism would show only modest rate accelerations while ones proceeding through a bifunctional mechanism would exhibit similar enhanced accelerations comparable to that of 2-pyridone.

**Scheme 2**

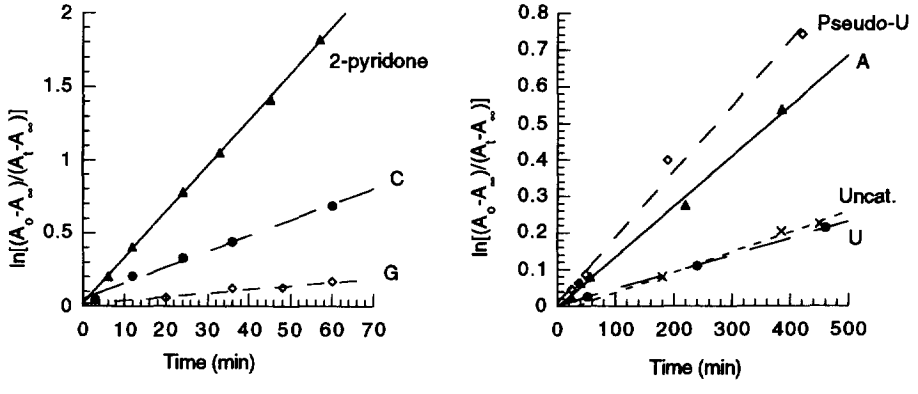


Along these lines, tetramethylglucose[5] and the tri-TBS protected ribonucleosides[6] were synthesized as previously described. For determining the rate of mutarotation, a typical run contained 6.0 mM of catalyst and 30.0 mM of tetramethylglucose in benzene. Polarimeter readings were taken on a Jasco DIP-1000 polarimeter in a temperature regulated polarimeter tube (T=20 °C) and readings were taken periodically. Rate constants were determined as described by Swain and Brown[7] using the rate equation:

$$k = (\ln [(A_0-A_\infty)/(A_t-A_\infty)])/t$$

where t is time, $A_0$ is the reading at t=0 and $A_\infty$ is the reading after negligible change in optical rotation. Figure 1 shows the extent of the catalysis by the various nucleosides. These results are summarized in Table 1.

**Figure 1**

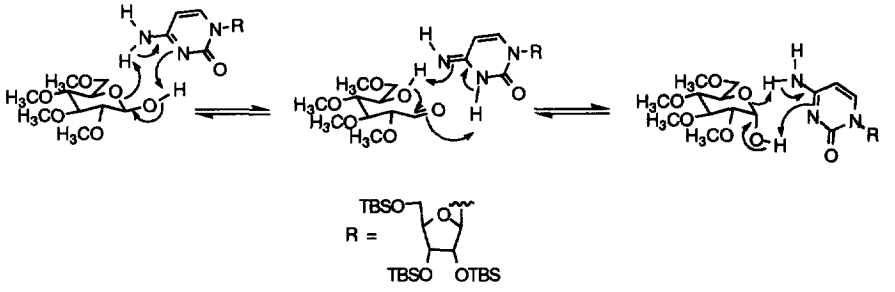


As expected, 2-pyridone shows a relatively large rate enhancement with respect to the uncatalyzed rate. C also shows significant rate enhancement which suggests its mode of catalysis may be bifunctional (Scheme 3). Additional support for this mode of catalysis is provided by the result that the N,N-dimethyl C analog is catalytically inactive. The amidine functionality of C is ideally suited to simultaneously deliver and accept a proton from tetramethylglucose, thus allowing it to interconvert from its α to β anomer presumable through an uncharged intermediate. The other nucleosides A, Ψ, and G all result in slight rate enhancements, which is indicative of a general base or acid mechanism.

**Table 1. Summary of Catalysts**

| Catalyst | Rate (x100 M/min) | | Relative Rate |
|---|---|---|---|
| 2-pyridone | 31.0 | ± 1.8 | 62 |
| C | 11.0 | ± 0.8 | 22 |
| G | 2.5 | ± 0.2 | 5.0 |
| Ψ | 1.8 | ± 0.2 | 3.6 |
| A | 1.4 | ± 0.1 | 2.8 |
| N,N-dimethyl C | 0.5 | ± .06 | 1.0 |
| U | 0.5 | ± .06 | 1.0 |
| Uncat. | 0.5 | ± .06 | 1.0 |

**Scheme 3**




Although C does not quite display the catalytic performance of 2-pyridone with respect to the mutarotation of tetramethylglucose, one cannot strictly conclude C is an *inherently* inferior catalyst. As discussed by Swain and Brown,[4] the catalytic nature of these molecules (bifunctional catalysts) is believed to be manifested in their monomeric forms. It has been shown that nucleosides,[8] as well as 2-pyridone,[9-12] dimerize in solution, although the exact extent of nucleoside dimerization under the present conditions is unknown. Experiments to determine this are currently in progress.

The various contributions to ribozyme catalysis have been difficult to sort, due to the complexity of the systems being studied. Virtually all of the examples of catalysis by nucleic acids have dealt with relatively complicated 3-dimensional structures. In this communication, further evidence has been presented that the functional groups of RNA nucleosides exhibit catalytic properties. Finally, as seen in the aminolysis studies,[2,3] C surpasses the catalytic ability of the other nucleosides adenosine, guanosine, uridine, and pseudouridine. This catalytic effect is attributed to C's ability to function as a bifunctional catalyst.

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