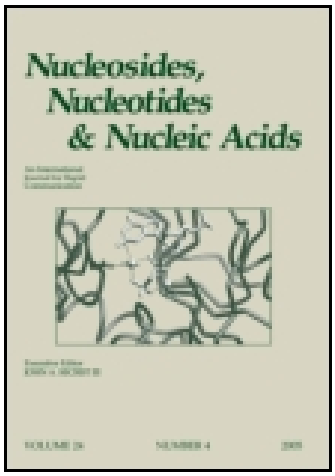

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SYNTHESIS OF 2′,3′-DIDEOXY-2′,3′-DIDEHYDRO NUCLEOSIDES VIA A SERENDIPITOUS ROUTE

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SYNTHESIS OF 2′,3′-DIDEOXY-2′,3′-DIDEHYDRO NUCLEOSIDES VIA A SERENDIPITOUS ROUTE

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ABSTRACT

This paper describes a "green" synthesis of 2′,3′-unsaturated 2′,3′-dideoxynucleosides via an electrochemical reaction. Using this approach d4T, d4U, ddA and ddI can be synthesized in high yields.

INTRODUCTION

With the growing success of clinical programs and potential market demand for oligonucleotide-based drugs, it is important that all raw materials for synthesis of these drugs are easily accessible at affordable cost (1). For the assembly of oligonucleotides, 2′-deoxynucleosides (dNs) are the key building-blocks (2). Currently all dNs are in limited supply and remain expensive due to their isolation from natural sources such as fish milt (3). Therefore, we have focused our attention on methods development for the synthesis of dNs, particularly pyrimidine dNs.

RESULTS AND DISCUSSION

Although thymidine can be synthesized (4) via direct glycosylation procedure (Scheme 1), the concomitant formation of α-anomer decreases the overall

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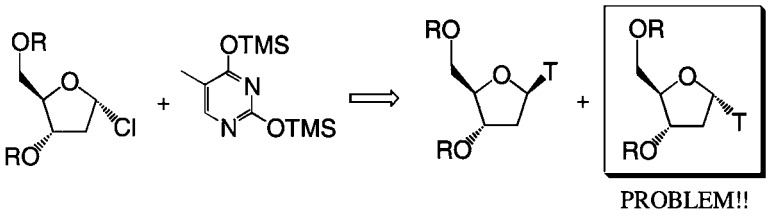
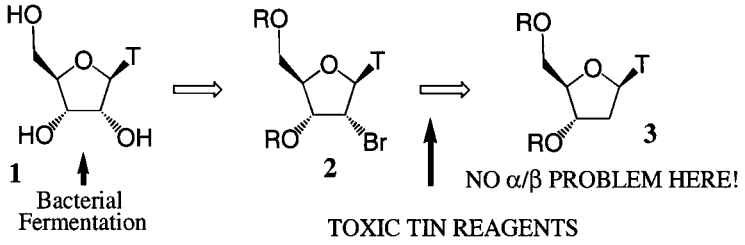


Scheme 1. Synthesis of thymidine via glycosylation procedure.

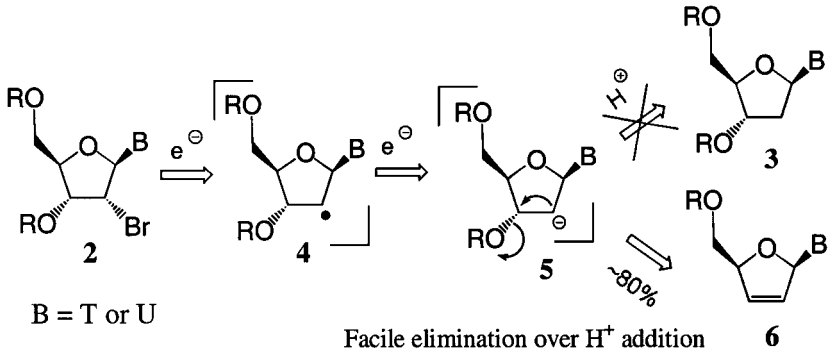
yield and increases the cost of desired β-anomer. Therefore, we elected to use ribonucleosides as starting material for synthesis of dNs due to their availability in bulk at low cost from bacterial fermentation and preformed glycosidic linkage (Scheme 2).

The Mattocks reaction of uridine analog (**1**) has been reported to provide 2′-α-bromo-3′-acetoxy nucleoside (**2**) due to anchimeric assistance of oxygen at the 2-position of the pyrimidine ring (5). Subsequent reduction of the halide with tin reagents furnished dNs (**3**, Scheme 2). Scale-up of tin mediated reduction is not practical due to the toxic nature of the reagent. In order to implement "green" chemistry in our manufacturing processes, we chose an electrochemical method that offers a versatile and nonstoichiometric alternative to metal-based reduction of the halide.

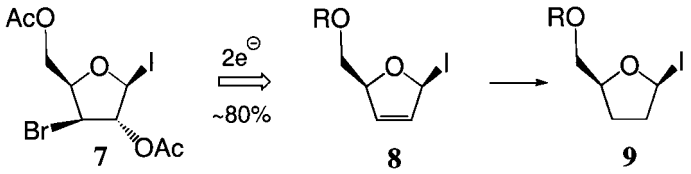
Treatment of acetyl bromide with uridine and 5-methyluridine in acetonitrile gave quantitative yields of 2′-bromo-3′,5′-diacetyluridine (**2**, B = U) and 2′-bromo-3′,5′-diacetyl-5-methyluridine (**2**, B = T), respectively. Electrochemical reduction (6) of **2** in methanol did not furnish the expected 2′-deoxy nucleoside derivatives **3**. Interestingly, both reactions gave 2′,3′-unsaturated 2′,3′-dideoxynucleosides **6** via elimination of the 3′-acetoxy group (**5**, Scheme 3). Subsequent treatment with base resulted in formation of d4T and d4U. All attempts to reduce 2′-α-bromo-3′-acetoxy nucleosides **2** via electrochemical methods have resulted in formation of elimination products only.



Scheme 2. Synthesis of thymidine via 2′-deoxygenation procedure.



Scheme 3. Serendipitous synthesis of d4T.



Scheme 4. Green synthesis of ddI.

The concept of electrochemical reduction was further extended towards synthesis of ddI (**9**). The 3′-bromo-3′-deoxy nucleoside **7** was conveniently synthesized from inosine following a literature protocol (5). Electrochemical reduction of **7** in MeOH under similar conditions furnished >80% yield of 2′,3′-unsaturated nucleoside **8** (Scheme 4). Transformation of **8** to **9** has been reported in the literature (5).

SUMMARY

Interestingly literature search revealed that electrochemical reductions of nucleosides have been tried (7). However the yields of 2′,3′-unsaturated nucleosides were somewhat lower than what we report herein (8). It seems that choice of solvent plays a major role during electrochemical reactions and we found that MeOH gave best results. Furthermore, we believe that this is a first "green" synthesis of d4T and may have potential for further scale-up. In conclusion, we have failed to accomplish our original goal of synthesis of 2′-deoxynucleosides by electrochemical reduction.

ACKNOWLEDGMENTS

We are thankful to Dr. Douglas L. Cole for his support and encouragement throughout this project.

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6. Cell assembly for electrolysis: The cell was home built using a glass beaker fitted with a compartment (fritted disk at the bottom) containing a platinum plate (2 × 2 cm, 0.1 mm thick) as anode and mercury cathode (pool at the bottom). The top was covered with a plate with an inlet and outlet for argon, a thermometer and an Ag/Ag^+ (1 mm silver wire immersed in 0.1 M $AgNO_3$ in acetonitrile) reference electrode. Cyclic and linear sweep voltammatries were conducted on the electrolysis solutions prior to the electrolysis to determine the optimum reduction potentials. Electrochemical reductions were performed in methanol or acetonitrile with tetraethylammonium *p*-toluenesulfonate (0.2 M) as electrolyte. The concentration of the electrolyte was maintained in a 5-fold excess relative to the electroactive species. The nucleosidic substrate (5 mmol) was added and the stirred solution was electrolysed under argon at an initial constant potential of -1.9 V for 1 h. The progress of the reaction was followed by TLC and most of the starting material was consumed within 1 h. Upon completion of the reaction, the solution was concentrated under vacuum and the residue purified by short silica gel column chromatography. The yield of the isolated product was always >80%. The deprotected (NH_4OH) products were identical (TLC, UV, NMR) with an authentic sample.
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