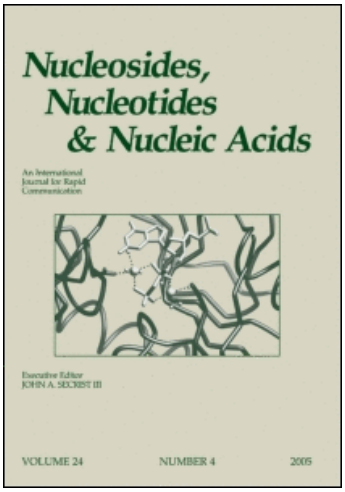

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UNEXPECTED RESULTS AND RECOURSE IN PROCESS OPTIMIZATION OF NUCLEOSIDE 3'-*O*-SUCCINATES

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UNEXPECTED RESULTS AND RECOURSE IN PROCESS OPTIMIZATION OF NUCLEOSIDE 3′-*O*-SUCCINATES

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ABSTRACT

An improved and scalable protocol for the synthesis of 3′-*O*-succinates of nucleosides has been developed using succinic anhydride. As a result, formation of unwanted dimer has been completely eliminated and use of toxic and smelly reagents have been avoided during synthesis of nucleoside succinates. All succinates were isolated in pure state without silica gel column chromatography.

INTRODUCTION

Automated synthesis on solid-supports has been the most successful method for the large-scale synthesis of oligonucleotide-based drugs (1). As a rule, the first nucleoside is always attached to the solid-support via a dicarboxylic acid, such as succinic acid (2) or hydroquinone-*O*, *O*-diacetic acid (3) (Q-linker). These acids are connected to the 3′-end of the nucleoside via an ester linkage and to the solid-support via an amide linkage. Currently, the succinyl group is the most commonly used linker due to its low cost and ease of incorporation. On the other hand, Q-linker appears to be a very good linker for reusable solid-support chemistry (4). Therefore, we have focused our attention on the large-scale synthesis of nucleosidic hemiesters **2** and **4** containing a variety of linkers.

Although there are numerous methods reported in the literature for the synthesis of 3′-*O*-succinyl linked 2′-deoxynucleosides, none appear to be suitable for further scale-up (5). For example, literature protocols describe product isolation

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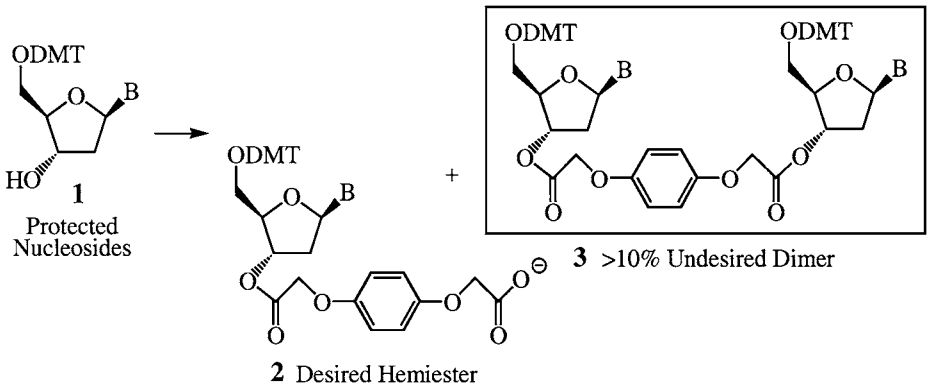
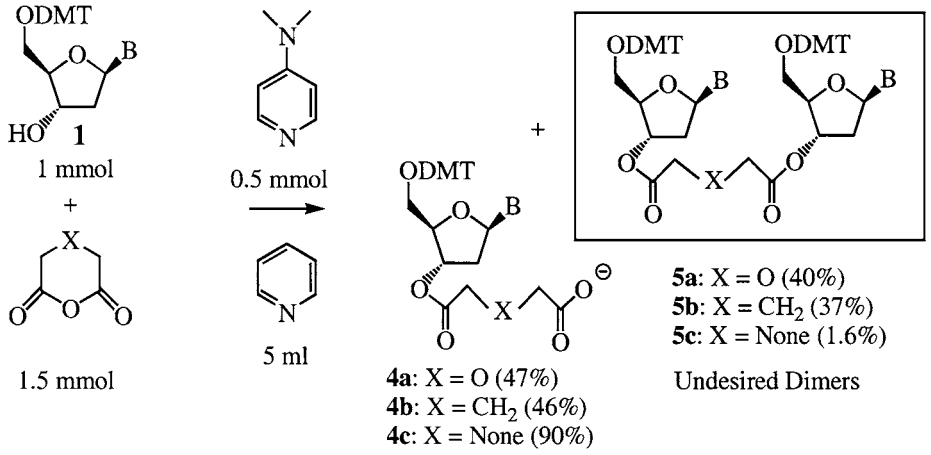


Scheme 1. Synthesis of Q-linked nucleosides.

by chromatographic methods, this increases the overall cost and reduces the yield. Most of these reactions were performed in pyridine, a solvent that is both unpleasant and expensive. Lastly, DMAP a toxic reagent is often used as an activator. Large-scale synthesis of 3′-*O*-hydroquinone-*O*, *O*′-diacetyl linked 2′-deoxynucleosides using HBTU/DMAP mediated coupling was hampered with the formation of an undesired dimer (>10%, Scheme 1) and difficult isolation of the desired product via column chromatography (6).

Chemistry. The protected nucleosides **1** readily reacted with succinic anhydride in pyridine in presence of DMAP to furnish hemiesters **4c** in good yields. However, the products had to be isolated by expensive chromatographic purification and choice of pyridine as a solvent for scale-up work was not convenient. Furthermore, upon careful investigation we found that the crude product was always



Scheme 2. Synthesis of nucleosides hemiester under normal conditions.

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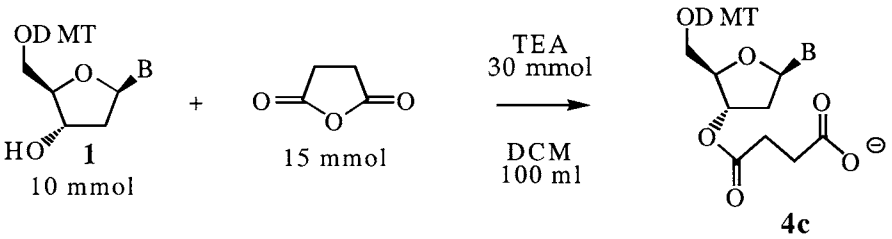

Scheme 3. Synthesis of nucleoside succinates under new conditions.

contaminated with an impurity (1.6%, Scheme 2) characterized as a nucleosidic dimer **5c**. Surprisingly, treatment of **1** with diglycolic anhydride furnished even more (40%, Scheme 2) of the dimeric product **5a**. Similarly, treatment of **1** with glutaric anhydride gave 46% of the desired product **4b**; however contaminated with 37% of undesired dimer **5b**.

As a result, we have done an extensive process optimization of these reactions and developed a new and improved protocol that avoids the formation of undesired dimers **5**. In a typical procedure, protected nucleoside **1** was stirred with succinic anhydride in dichloromethane with an excess of triethylamine (Scheme 3) at room temperature for 3 hours. Upon completion of the reaction (judged by TLC) the contents were poured into triethylammonium phosphate buffer (0.5 M soln.; pH 7.4) and the aqueous layer extracted with dichloromethane. The combined extracts were evaporated to furnish nucleosidic hemiesters **4c** as white foam in high yields (see Scheme 3). All succinates (**4c**; B = T, C^{Bz}, A^{Bz} and G^{Ibu}) were identified by ^{1}H NMR and MS analysis. In addition, HPLC analysis confirmed that all four succinates were >98% pure and free of dimeric products.

CONCLUSIONS

An efficient synthesis of 3′-*O*-succinates of 2′-deoxynucleosides has been developed. The new method is easily scalable and avoids the use of toxic reagents. A simplified aqueous work-up allows easy isolation of products without expensive chromatography.

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