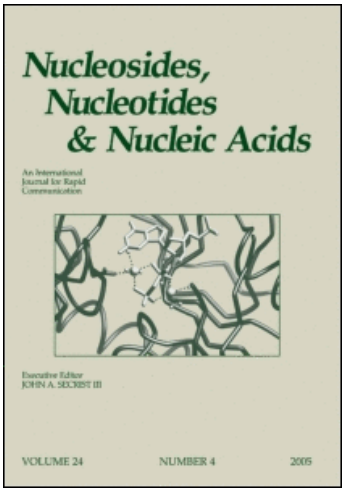

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Large-Scale Synthesis of 5'-*O*-Pixyl Protected 2'-Deoxynucleosides Useful for Oligonucleotide Synthesis

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LARGE-SCALE SYNTHESIS OF 5′-*O*-PIXYL PROTECTED 2′-DEOXYNUCLEOSIDES USEFUL FOR OLIGONUCLEOTIDE SYNTHESIS

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▫ *Synthesis of 5′-O-pixylated 2′-deoxynucleosides* **4** *has been accomplished and the products are commercially available.*

INTRODUCTION

In polyfunctional molecules such as nucleosides, selective protection becomes an issue that has been addressed by the development of a number of new reagents. Among these, the dimethoxytrityl (DMTr) ethers have proven to be one of the most commonly used protecting groups (PG) for oligonucleotide synthesis, first introduced by Khorana.[1] Chattopadhyaya and Reese developed the 9-phenyl-xanthen-9-yl **2** (Px) as an alternative for trityl group useful for oligonucleotide synthesis.[2,3] The Px group is removed by acid treatment at approximately the same rate as the DMTr group. The key attributes of the Px group are summarized below.

- The nucleosides protected with the 5′-*O*-Px group tend to crystallize easily; therefore products can be purified without expensive column chromatography.
- The Px group possesses a highly rigid and planar backbone that results in an increased stability of the Px carbocation over other analogous group currently in use such as DMTr.
- The Px group has been shown to undergo photochemically induced heterolytic carbon-oxygen bond cleavage to generate the Px cation in neutral aqueous solution.[4]
- The Px cation has a UV extinction coefficients that is ~100 times greater than for the DMTr group.

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- The pyrrole (p*K*a–0.27) has been identified as an excellent irreversible scavenger for Px cation during oligonucleotide synthesis.

Interestingly, these traits of Px group has culminated into a recent publication by Reese recommending that Px group is indeed a better choice over DMTr for the protection of the 5′-OH group during oligonucleotide synthesis.[5] For these reasons, we believe that it is important to synthesize 5′-*O*-Px protected nucleosides on large-scale and make them commercially available.

CHEMISTRY AND DISCUSSION

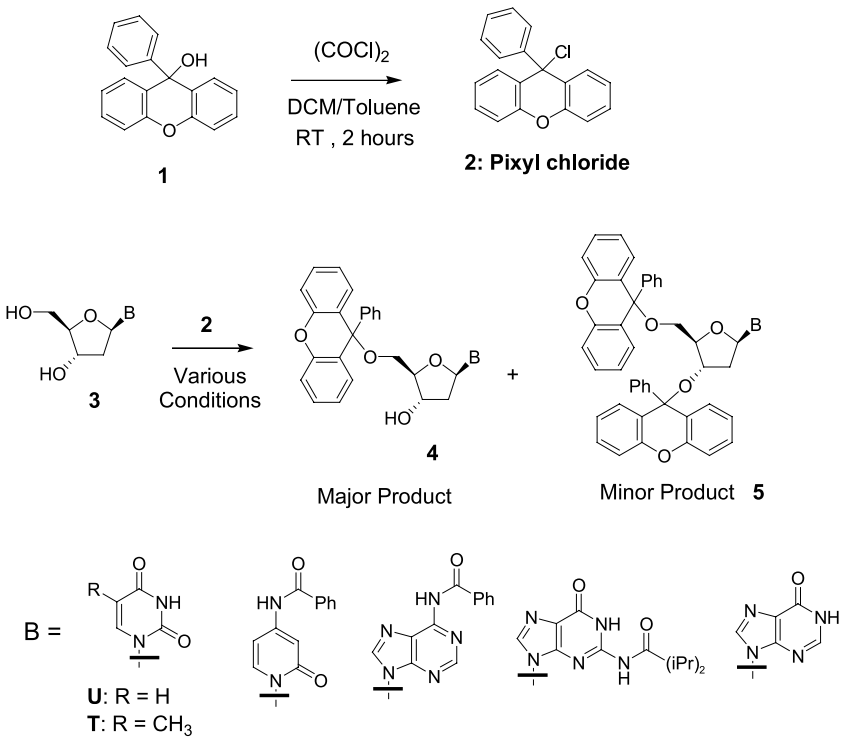
In order to synthesize 5′-*O*-pixylated nucleosides **4**, we needed a good source of Px-Cl.[2,3] Aldrich and Advanced Asymmetrics are the only two commercial suppliers of Px-Cl in the market place. Surprisingly, the cost of 1g of Px-Cl is $23, compared to $9 for 1 g of DMTr. The exorbitant price of the commercial Px-Cl from the current sources was a big roadblock in our ability to make 5′-*O*-pixylated nucleosides. As a result, we considered buying Px-OH[1] that was significantly cheaper ($6/g). The commercial **1** was chlorinated with excess of $(COCl)_2$ in DCM/toluene at RT in 2 hours to furnish **2** in quantitative yield.[6] Due to the hygroscopic nature of **2**, the crude product was used as such for pixylation studies. Recently, we have identified an Asian supplier of good quality Px-Cl at lower cost.* As a result, the chlorination step has been avoided.

The classical method for the introduction of the Px group involved the reaction of a nucleosidic primary alcoholic group with Px-Cl in pyridine. Although Px group has been extensively used for the protection of the 5′-OH of nucleosides; it has been also reported for the protection of a secondary hydroxyl group.[7] Therefore, it is not uncommon to observe the formation of 3′-*O*- and 5′-*O*-bis pixilated nucleosides **5** as a byproduct. The formation of **5** was minimized by portion-wise slow addition of Px-Cl to a solution of nucleoside in pyridine at room temperature.

The reaction of Px-Cl with nucleosides in pyridine is generally slow and may take 6–12 hours for completion. The reaction rate could be accelerated by addition of DMAP or DBU. However, this may lead to an increased amount of bis-pixylated products.

First, we repeated the synthesis of 5′-*O*-pixylated nucleosides **4** reported by Reese.[2,3] Following the literature protocol reaction of base protected nucleosides **3** with **2** furnished **4** with trace amounts of bis-pixylated products **5** (Scheme 1). Thus, the 5′-*O*-pixylated derivatives of 1) 2′-deoxyuridine, 2) thymidine, 3) N^4-Bz-2′-deoxycytidine, 4) N^6-Bz-2′-deoxyadenosine and 5) N^2-Ibu-2′-deoxyguanosine were isolated in 65, 60, 45, 57, 50% yields respectively, after crystallization from appropriate solvents. All pixylated nucleosides **4** were purified by silica gel column chromatography to obtain analytically pure samples. The structure pixylated **4** were confirmed by ^{1}H NMR and ES MS. Interestingly, pyridine was employed in

*Px-Cl is now available from Innovassynth, India (www.innovasynth.com).



SCHEME 1

this protocol that is a toxic and hazardous solvent. Although this is a common practice in academia, it is not a first choice for scale-up in the industry. Selecting a less hazardous solvent during scale-up preparation not only decreases risks to operators and community but also reduces the inconvenience and additional costs. Therefore, we selected acetonitrile as an alternative solvent of choice that is safer and cheaper than pyridine. Preliminary results indicate that the pixylation of thymidine was complete in 6–8 hours and furnished 80% isolated yield of the desired product **4** (B = T). Presently, we are optimizing the scale-up protocols for the synthesis of 5′-*O*-pixylated nucleosides.

SUMMARY

Synthesis of 5′-*O*-pixylated nucleosides has been accomplished using the literature protocols and further scale-up is in progress. A low-cost commercial source of Px-Cl has been identified in Asia.[6] With easy access to Px-Cl, we plan to make several 5′-*O*-pixylated nucleosides and make them commercially available for R&D purpose.†

†Contact RI Chemical Inc. for any 5′-*O*-pixylated nucleosides.

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