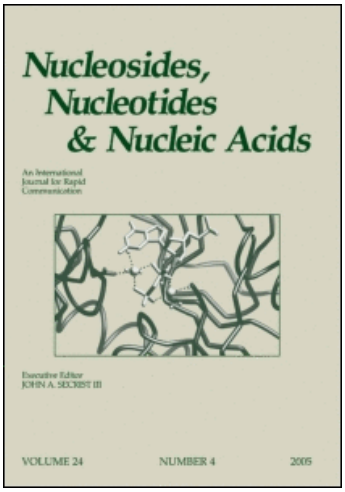

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An Efficient and Scalable Synthesis of Arabinosylguanine and 2'-Deoxy-2'-Fluoro-guanosine

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AN EFFICIENT AND SCALABLE SYNTHESIS OF ARABINOSYLGUANINE AND 2'-DEOXY-2'-FLUORO-GUANOSINE

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Abstract: An efficient conversion from commercially available 2,6-diaminopurine-2',3',5'-tri-O-benzyl arabinoside to arabinosylguanine and its further transformation to 2'-deoxy-2'-fluoro-guanosine is outlined. This process has been used to produce more than one hundred grams of final product.

When incorporated into oligonucleotides, 2'-fluoro substituted 2'-deoxy-ribonucleosides have a higher affinity towards RNA complement (as measured by melting temperature) than 2'-O-methyl ribonucleosides.[1] Furthermore, some of these oligonucleotides have demonstrated increased biological activities for antisense and ribozyme applications.[2] 2'-Deoxy-2'-fluoro-guanosine nucleoside (**7**) has also been reported[3] to possess antiviral and antiprotozoal activity. In order to investigate this substitution, larger quantities of the fluorinated nucleosides are needed. The synthesis of the 2'-deoxy-2'-fluoro-pyrimidines is straightforward.[4] The synthesis of the purines is more difficult.[1,5,6] Enzymatic transglycosylation[3] from the pyrimidines to the purines is attractive but it requires enzymes that are not readily available. One route to the purines involves displacement of the triflate of the ara 2'-hydroxyl with fluoride. Arabinosyladenine is commercially available in bulk lots via fermentation. Arabinosylguanine is not and we required several hundred grams to complete the synthesis. In this report we describe an efficient, larger scale conversion from arabinosyl nucleoside derivative **1** to arabinosylguanine (**3**) and its further transformation to 2'-deoxy-2'-fluoro-guanosine (**7**).

Guanosine has been converted to arabinosylguanine[7] by first blocking the 3' and 5' hydroxyls with the Markiewicz disiloxane reagent, then oxidation and reduction of the 2' oxygen, followed by tedious chromatographic separation of the isomers. Alternatively, the 2' hydroxyl of a suitable derivative has been displaced with acetate[8] to give the arabino configuration. Neither of these possibilities seemed attractive for larger scale work.

Our route took advantage of a more advanced, commercially available intermediate[9] (**1**, 2,6-diaminopurine-2',3',5'-tri-O-benzyl arabinoside) used for the production of fludarabine phosphate which is efficiently made in multi-kilogram lots from protected diaminopurine and a commercially available arabinose derivative. Removal of the benzyl protecting groups could be accomplished in several ways. Birch reduction conditions worked well for smaller scales but was limited due to large volumes of liquid ammonia needed to solubilize partially deprotected species. Catalytic hydrogenation required acyl protection on the exocyclic amines and this was complicated by the lability of the N-6 acyl. For our purpose of converting a kilogram of material, boron trichloride gas in dichloromethane proved best by far (see Figure 1). The product (**2**) precipitated

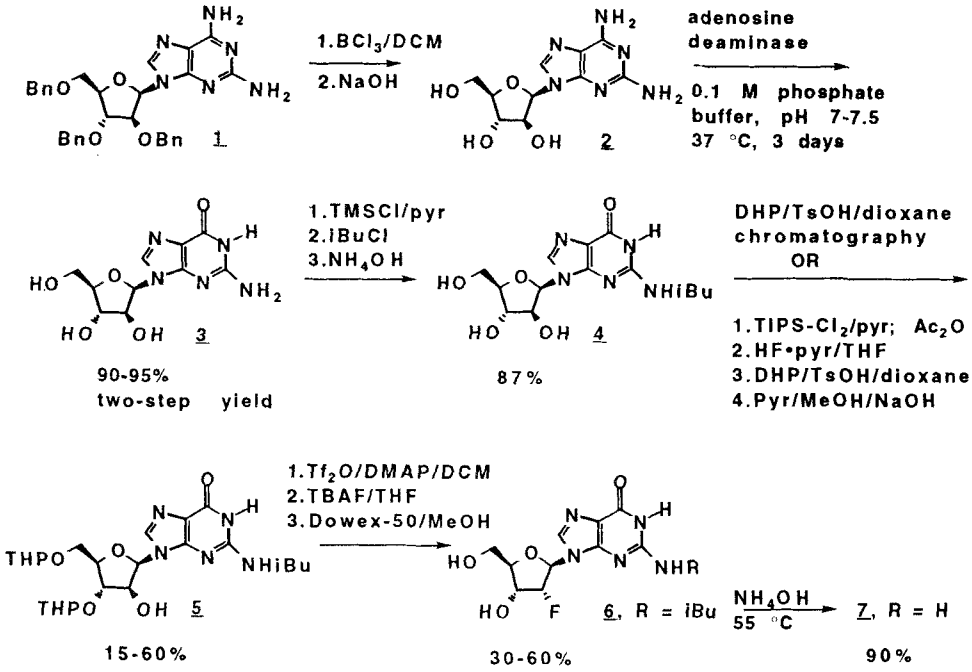

FIGURE 1

out of solution upon quenching the reaction with sodium hydroxide solution. The collected solid was used as is and suspended in phosphate buffer in the presence of adenosine deaminase. The even less soluble product, arabinosylguanine (**3**), formed a new suspension. This was collected and dried to give clean **3** in 90-95% two-step yield. The exocyclic amine was protected with isobutyryl using standard methods to afford **4** (87%).

Transformation to the fluoro derivative **6** first required blocking the 3' and 5' hydroxyls with a non-silyl based group such as tetrahydropyranyl. This could be accomplished in two ways. The first more direct method paralleled work reported for the adenosine analog[4] in which dihydropyran was slowly added to an acidic solution of the nucleoside. The primary 5'-hydroxyl reacted first and then a competition was seen between the remaining positions with the 3' hydroxyl favored about four to one. The correct diastereomer set was then carefully separated by chromatography. The separation was more difficult than for the adenosine analog and the isolated yields of **5** were low and variable (10-25%). This route might be more appropriate for smaller scale work. The second method was longer but more efficient. The 3' and 5' hydroxyls of **4** were blocked with Markiewicz reagent first followed by protection of the 2' hydroxyl with acetate in the same pot to give the fully blocked derivative which was isolated after workup by precipitation. The disiloxane group was removed by treatment with hydrogen fluoride-pyridine complex and after workup, the solid product was isolated by trituration. The free hydroxyls were blocked with tetrahydropyranyls and the resulting oil was treated with base to remove the 2' acetate. After workup, most of the product crystallized from ethyl acetate. The mother liquor was stripped and the residue was purified by

chromatography to afford tetrahydropyranyl product **5** (60% from **4**, 48% overall from **1**) with only one chromatographic separation on a portion of the last step.

Conversion of **5** to **6** followed the literature route[1,6] of displacing the triflate with tetrabutylammonium fluoride. On a gram scale, for the three steps, the high yield in our hands was 60% but more typically 30 to 40% for larger runs (100+ g of **5**) with the material balance recovered of about 10-15% composed in order of isobutyrylguanine, guanine and a small amount of **7**. Several other fluorinating reagents were tried but none proved better. A 5 g sample of **6** was deprotected in concentrated ammonium hydroxide at 55 °C for 24 h to 3.6 g of **7** (90%, recrystallized from methanol). Even with a 30% fluorination sequence, the overall yield was 13%. Subsequent conversion to the dimethoxytrityl and phosphoramidite derivatives went well.

As an alternative, 2,6-diaminopurine arabinoside was carried through the same sequence, but the yields suffered due to the lability of the isobutyryl on the N-6. A small portion of the fluorinated nucleoside was converted to **7** via adenosine deaminase and the remainder was carried on to its phosphoramidite.

In summary, a new starting material has been identified and a very efficient and scalable route for its conversion to arabinosylguanine (**3**) has been developed. A method to transform this to the known key intermediate **5** which used little or no chromatography was also developed. Conversion of **5** to the 2'-fluoro derivative **6** did not work as well as desired but still, to our knowledge, the overall yield (**1** to **7**) of 13-22% is far more efficient and described in far larger scale than any other literature route. More than 100 g of nucleoside **6** has been produced by this route and converted to its phosphoramidite for biological testing.

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