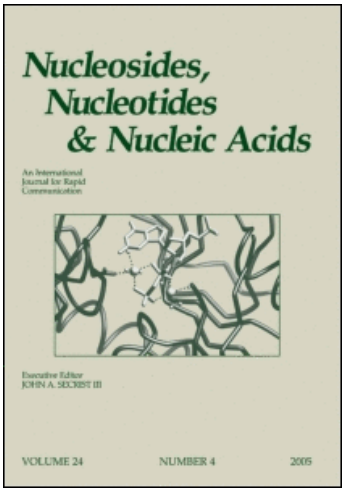

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AN EFFICIENT AND SCALABLE SYNTHESIS OF 2,6-DIAMINOPURINE RIBOSIDE

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□ *An efficient process to synthesize 2,6-diaminopurine riboside in high yield and quality is described. This inexpensive approach was scaled up to multi-hundred kilogram quantities for use in oligonucleotide therapeutics.*

Keywords 2-Aminoadenosine; antisense; 2′-*O*-methoxyethylguanosine; modified nucleoside; oligonucleotides

Phosphorothioate-linked nucleic acid analogs have found widespread application in therapeutic drug development and molecular biology.[1,2] Increased resistance to nuclease digestion displayed by phosphorothioate diester DNA and RNA analogs has prompted use of these molecules for treatment of a variety of diseases. Although phosphorothioate ODN has shown excellent promise as safe and effective therapeutic agents, their profiles are not yet ideal. Chemical modification of antisense oligonucleotides can confer additional resistance to nucleases, longer serum half-life, and reduced toxicity. Modifications can also increase affinity of an antisense oligonucleotide for its complementary target RNA, resulting in enhanced potency and specificity. One among various modifications to be largely explored is the use of 2′-*O*-methoxyethyl (MOE) group. 2′-*O*-Methoxyethyl sugar-modified oligonucleotides have shown interesting biological properties and several drugs are being evaluated in clinic to treat various acute and chronic diseases.[3]

2′-*O*-Methoxyethyl pyrimidine nucleosides have been scaled up to large quantities via a novel regioselective ring opening of *O*-2,2′-cyclo-5-methyluridine involving boron chemistry.[4] However, synthesis of 2′-*O*-methoxyethylguanosine is not straightforward and involves multiple

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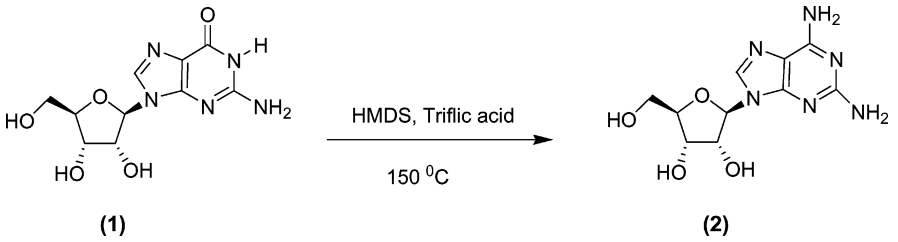

FIGURE 1 Synthesis of 2,6-diaminopurine riboside (**2**).

steps.[5] Novel alternative approaches have been reported but not been scaled up so far due to various reasons.[6–8] Direct alkylation of guanosine is not desirable since it leads to preferential alkylation of base moiety (O^6-alkylation).[9] Thus, masking of this reactive site is necessary and is conveniently done by converting it into an amino group viz. 2,6-diaminopurine riboside (DAPR). This masked functionality could be converted back after 2′-O-alkylation by treatment with adenosine deaminase enzyme (ADA).[10]

Although few enzymatic reports[11,12] are available, not much is known on chemical routes to make large quantities of DAPR. We report here a scaleable route for synthesis of the title compound. Synthesis is shown in Scheme 1. This inexpensive approach was scaled up to >100-kg batch-size quantities for use in oligonucleotide therapeutics.

EXPERIMENTAL SECTION

2,6-Diaminopurine Riboside (2)

To a 2-L stainless steel Parr bomb was added guanosine hydrate (**1**) (300 g, 1.04 mol), hexamethyldisilazane (1.1 L, 5.0 mol), and toluene (300 mL). The suspension was stirred, trimethylsilyl trifluoromethanesulfonate (30 g) was added with brief stirring, and the reaction vessel was quickly sealed to prevent the resulting exothermic reaction from spilling out.[13] One-third of the bomb was submerged in an oil bath (160°C bath, 150°C internal temperature, generating 20 atm pressure) for 5 days. The bomb was allowed to cool to room temperature and vented to allow ammonia gas to evolve in a fume hood. The reaction was poured into an open vessel containing methanol (1 L), again allowing ammonia gas to vent in a fume hood. The solution was concentrated to dark oil under reduced pressure. The oil was dissolved in a mixture of methanol (1.5 L) and water (0.5 L) and heated to reflux with mechanical stirring to hydrolyze the silyl ethers. The reaction was complete after sixteen hours (as judged by TLC) resulting in a thick suspension. The solid was collected by filtration, washed with methanol (0.4 L) and redissolved in boiling water (2 L). The solution was treated with activated carbon (20 g), filtered hot through Celite, and allowed to cool to room temperature. The resulting crystals were collected, washed

with methanol (1 L), and dried (50°C, 0.2 mmHg) for 24 hours to give 252 g (90%) of the title compound as light tan crystals, mp 240–242°C; TLC homogenous (Rf 0.50 in 2-propanol-ammonium hydroxide-water 16:3:1). ^{1}H-NMR (DMSO-d_6), 5.73 (d, 2, 2-NH_2), 5.78 (s, 1, H-1), 6.83 (br s, 2, 6-NH_2). m/z 283.

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