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Process Development for the Synthesis of 5′-*O*-(4,4′-Dimethoxytrityl)-N^2-isobutyryl-2′-*O*-(2-methoxyethyl)-guanosine—A Potential Precursor for the Second Generation Antisense Oligonucleotides: An Improved Process for the Preparation of 2′-*O*-Alkyl-2,6-diaminopurine Riboside

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ABSTRACT

An efficient four step process for the preparation of 5′-*O*-(4,4′-dimethoxytrityl)-N^2-isobutyryl-2′-*O*-(2-methoxyethyl)-guanosine **1** was developed. Direct 2′-*O*-alkylation of 2,6-diaminopurine riboside **2** was accomplished via inexpensive and commercially available reagents such as KOH, DMSO and alkyl halides at room temperature in 4–6 hrs. Pure 2′-*O*-(2-methoxyethyl)-DAPR **3** was isolated by crystallization from methanol. Enzymatic deamination of **3** followed by selective N^2-isobutyrylation and 5′-*O*-dimethoxytritylation furnished desired **1** in high yield and purity. Fully optimized four step synthetic process has been scaled up to the pilot plant level.

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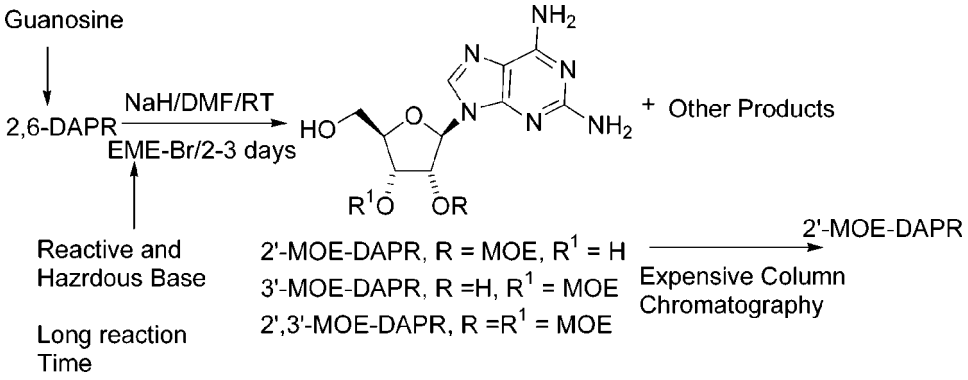
INTRODUCTION

Antisense technology is a novel drug discovery method. Understanding the genetic basis of complex disease process using antisense may open the doors to creating safer and more efficient drugs. In addition to the first generation phosphorothioate oligonucleotides, the 2′-*O*-alkylribonucleotides have been recently developed as second-generation oligonucleotide analogues with improved antisense properties.[1] Therefore, it is increasingly important to develop an efficient, cost effective, non-hazardous, industrially feasible and eco-friendly process for the synthesis of various modified nucleosides required to construct these oligonucleotides. Among these 5′-*O*-(4,4′-dimethoxytrityl)-N^2-isobutyryl-2′-*O*-(2-methoxyethyl)-guanosine (5′-*O*-DMT-N^2-Ibu-2′-MOE-G) **1** serves as one of the key building blocks required for the synthesis of second generation oligonucleotides.[2]

RESULTS AND DISCUSSION

In order to synthesize nucleoside **1**, regioselective alkylation at the 2′-OH position of guanosine is essential. Literature survey revealed variety of methods available but none of them appear to be suitable from industrial perspective and commercial scale-up. For example, 2′-*O*-alkylguanosine can be synthesized via glycosylation of D-ribose.[3] This method involves too many steps and formation of undesired α-anomer resulted in low overall yield. Selective alkylation at 2′-OH group of guanosine can also be effected by simultaneous protection of 3′- and 5′-OH groups using expensive protecting group such as TIPDS-Cl followed by alkylation using non-practical bases such as NaH[4] or BEMP.[5] Direct alkylation of 2,6-diaminopurine riboside using NaH/DMF followed by expensive column chromatographic purification[6] is reported but not feasible for large-scale production (Sch. 1).



Scheme 1. Direct alkylation of DAPR, literature protocols.

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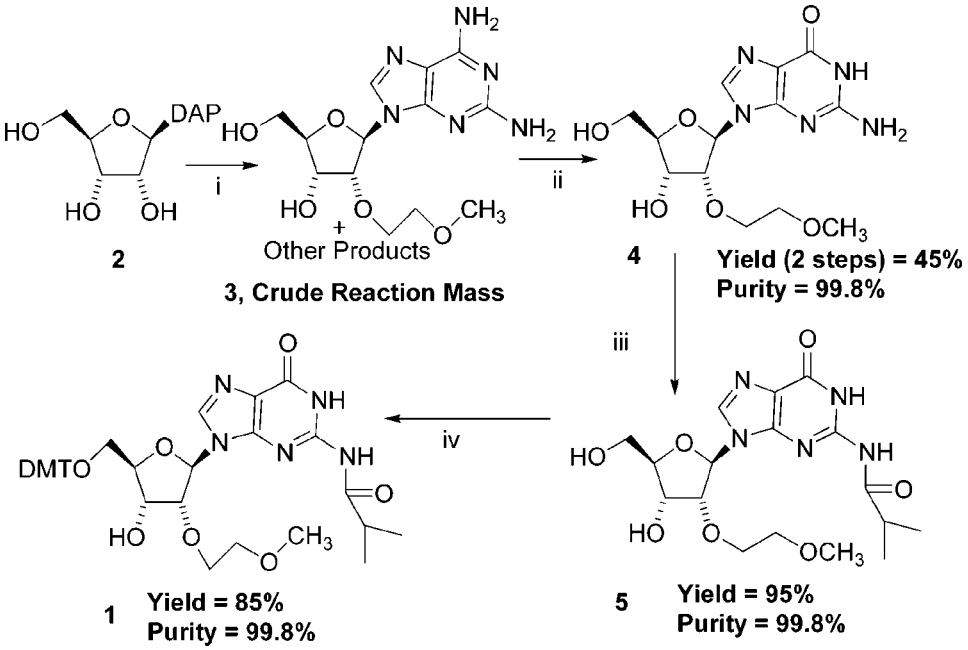

Scheme 2. i) KOH (2.6 equiv), DMSO (25 volumes), 2-methoxyethyl bromide (2.5 equiv), r.t., 7h; ii) adenosine deaminase, sodium phosphate buffer, pH 7.2; iii) acetonitrile/TMS-Cl (6.0 equiv)/r.t./3h, Ibu-Cl (1.5 equiv)/r.t./3h, aq. ammonia (pH 6.4), acetonitrile/aq. acetone; iv) acetonitrile, 2,6-lutidine (3.3 equiv), DMT-Cl (1.01 equiv)/45°C/3h, EtOAc.

In the wake of these shortcomings, we have developed a suitable process applicable on industrial scale for the synthesis of **1** (Sch. 2). Direct 2′-*O*-alkylation of 2,6-diaminopurineriboside **2** was efficiently performed using inexpensive reagents such as KOH, DMSO and 2-methoxyethyl bromide at room temperature in 4–6 hrs. Changing various reaction parameters, high 2′-regioselectivity was obtained furnishing 62–67% of the desired product **3** in the crude reaction mixture. Pure 2′-*O*-(2-methoxyethyl)-2,6-DAPR **3** could be isolated from a complex mixture of other by-products by simple crystallization from methanol in 40% yield. Structures of 2′- and 3′-regioisomers were established by X-ray diffraction analysis. Further improvements in the yield of **1** were realized when crude reaction mass after alkylation was directly subjected to deamination using ADA to furnish 2′-MOE-G **4** in 45% overall yield and 99.8% product purity. Silylation of **4** using TMS-Cl and isobutyryl chloride followed by de-protection provided crude product **5**. Crystallization of crude **5** from acetonitrile and aqueous acetone furnished 99.8% product purity in 95% yield. Treatment of **5** with freshly crystallized 4,4′-dimethoxytrityl chloride in acetonitrile and 2,6-lutidine afforded **1** in 85% yield and 99.8% product purity. Suitable HPLC conditions were also developed which enabled us to separate and evaluate all intermediates, final product and impurities during synthesis of **1**. The improved protocol has been successfully scaled-up on the pilot plant scale ($>$1Kg).

SUMMARY

An efficient process for the preparation of **1** in high purity and yield has been developed using simple reaction conditions and inexpensive reagents. Purification of intermediates and final product has been achieved by crystallization avoiding column chromatography. Improved process has been successfully implemented in pilot plant with reproducible results. Structures of 2′ and 3′-MOE-DAPR has been confirmed by single crystal X-ray diffraction method. Suitable HPLC methods have been developed to monitor the reactions and estimate the product purities.

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REFERENCES

1. Altmann, K-H.; Martin, P.; Dean, N.M.; Monia, B.P. Nucleos. & Nucleot. **1997**, *16*, 917–926.
2. Sanghvi, Y.S.; Andrade, M.; Deshmukh, R.R.; Holmburg, L.; Scozzari, A.N.; Cole, D.L. *Manual of Antisense Methodology*; Hartmann, G., Endres, S., Eds.; Kluwer Academic Publishers, 1999; 3–23.
3. Martin, V.P. Helv. Chim. Acta. **1995**, *78*, 486–504.
4. Grotli, M.; Douglas, M.; Beijer, B.; Garcia, R.G.; Eritja, R.; Sproat, B. J. Chem. Soc., Perkin Trans. 1 **1997**, 2779–2788.
5. Beigelman, L.; Haeberli, P.; Sweedler, D.; Karpeisky, A. Tetrahedron **2000**, *56*, 1047–1056.
6. McGee, D.P.C. U.S. Patent 5,506,351, April 9, 1996.

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