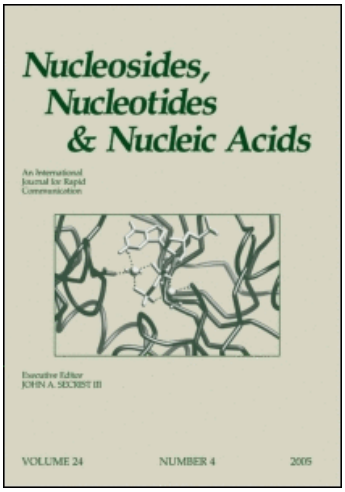


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Process Research on the Preparation of DMT Protected 2'-*O*-Methoxyethylguanosine for Oligonucleotide Synthesis in Therapeutic Applications

Shabbir Ali S. Taj[a]; S. Narayanan[a]; S. Suman Meenakshi[a]; Yogesh S. Sanghvi[b]; Bruce S. Ross[b]; Vasulinga T. Ravikumar[b]
[a] Isis Pharmaceuticals, Inc., Carlsbad, California, USA [b] Shasun Chemicals and Drugs Ltd., Chennai, India

To cite this Article Taj, Shabbir Ali S. , Narayanan, S. , Meenakshi, S. Suman , Sanghvi, Yogesh S. , Ross, Bruce S. and Ravikumar, Vasulinga T.(2008) 'Process Research on the Preparation of DMT Protected 2'-*O*-Methoxyethylguanosine for Oligonucleotide Synthesis in Therapeutic Applications', Nucleosides, Nucleotides and Nucleic Acids, 27: 9, 1024 — 1033
To link to this Article: DOI: 10.1080/15257770802271748
URL: http://dx.doi.org/10.1080/15257770802271748

Nucleosides, Nucleotides and Nucleic Acids, 27:1024–1033, 2008

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770802271748

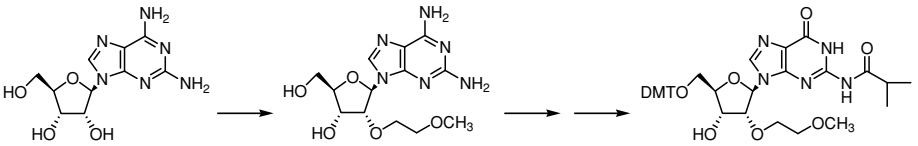
Taylor & Francis
Taylor & Francis Group

PROCESS RESEARCH ON THE PREPARATION OF DMT PROTECTED 2′-*O*-METHOXYETHYLGUANOSINE FOR OLIGONUCLEOTIDE SYNTHESIS IN THERAPEUTIC APPLICATIONS

Shabbir Ali S. Taj,[1] S. Narayanan,[1] S. Suman Meenakshi,[1] Yogesh S. Sanghvi,[2] Bruce S. Ross,[2] and Vasulinga T. Ravikumar[2]

[1]*Isis Pharmaceuticals, Inc., Carlsbad, California, USA*
[2]*Shasun Chemicals and Drugs Ltd., Chennai, India*

□ *An optimized process to synthesize DMT protected 2′-O-methoxyethylguanosine is described. A key step involves the enzymatic deamination of a mixture of alkylated products to selectively afford the desired material without resorting to chromatography for purification. This approach was scaled up to kilogram quantities for use in oligonucleotide therapeutics.*



Keywords Oligonucleotides; antisense; 2-aminoadenosine; diaminopurine riboside; modified nucleoside; 2′-*O*-methoxyethylguanosine; adenosine deaminase

INTRODUCTION

Synthetically and chemically modified oligonucleotide analogues are under going human clinical trials for the treatment of wide range of diseases such as cancers, infectious, inflammatory and cardiovascular. Antisense oligonucleotides are designed to inhibit the production of disease causing proteins. To be able to inhibit the gene expression, the oligonucleotide must reach the interior of the cell unaltered. In order to do so they should be stable toward cellular nucleases. In addition they have to hybridize with appropriate specificity and affinity to the complementary target RNA.

Received 1 February 2008; accepted 22 May 2008.
Address correspondence to Vasulinga T. Ravikumar, Shasun Chemicals and Drugs Ltd., 60 Velachery Road, Chennai 600 042, India. E-mail: vravikumar@isisph.com

Phosphorothioate oligonucleotides (ODN) developed as first generation antisense drugs represent the most extensively investigated class of modified oligonucleotides. Many of the oligonucleotides from this class have proven potent and selective inhibition of protein expression in vitro as well as in *vivo*. But, still few limitations such as lower binding affinity and some of the toxicological side effects made it necessary to investigate further structural changes to enhance the performance of these oligonucleotides. To overcome the limitations of first generation antisense drugs, 2′-*O*-alkyloligoribonucleotides have been developed recently as novel second generation analogues with properties that enhance their therapeutic value. They possess high chemical stability, resistant to degradation by nucleases and forms hybrids of high thermal stability with complimentary RNA. Among several 2′-*O*-modified oligonucleotides evaluated so far, 2′-*O*-(2-methoxyethyl) modification possesses the best properties.[1–4]

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.[5] However, synthesis of 2′-*O*-methoxyethylguanosine has not been straight forward. Direct alkylation of guanosine is not desirable since it leads to preferential alkylation of base moiety (O^6-alkylation).[6,7] Several alternative approaches for 2′-O-alkyl purines have been reported but not been scaled up so far due to various reasons.[8–19]

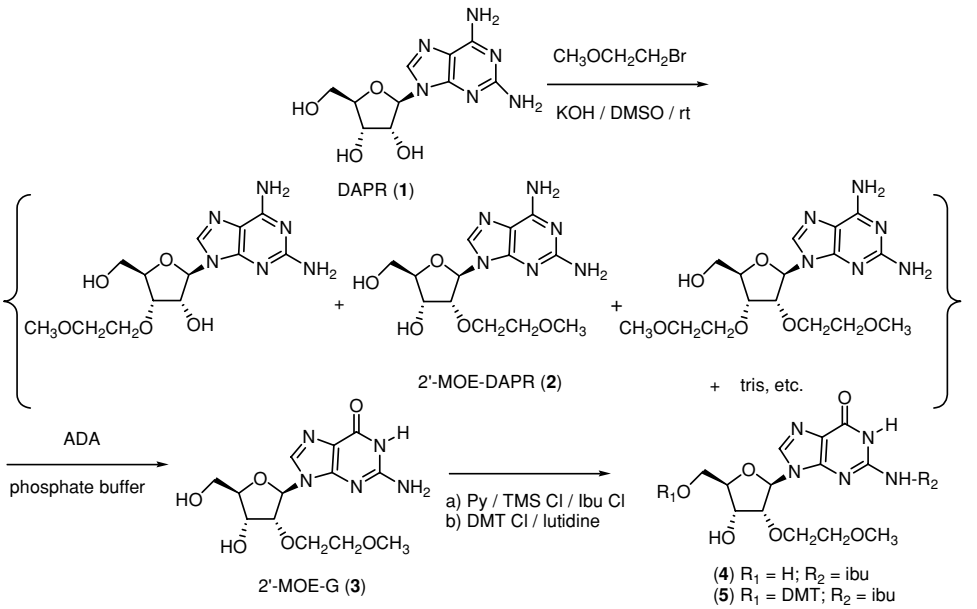
Masking of the lactam functionality is necessary and is conveniently done by converting it into an amino group viz. 2,6-diaminopurine riboside (DAPR). Recently we reported an efficient large scale synthesis of this compound.[20] This masked functionality could then be converted back after 2′-O-alkylation by treatment with adenosine deaminase enzyme (ADA).[21,22]

We report here a scalable route for synthesis of the title compound. Synthesis is shown in Scheme 1. A series of experiments were carried out to optimize various reaction parameters such as choice of solvents, base, reaction temperature for key steps involved in the process such as alkylation of DAPR and enzymatic step. The structure of 2′-MOE DAPR (**2**) was established by x-ray diffraction method. This approach was scaled up to kilogram batch size quantities for use in oligonucleotide therapeutics.

RESULTS AND DISCUSSION

Alkylation of DAPR

A key step for the preparation of title compound is the regio-selective alkylation of 2′-position of DAPR. The 2′-hydroxyl proton of DAPR is more acidic than the 3′-hydroxyl (predicted pKa value for 2′-OH is 12.9 and 3′-OH is 14.2) and we took advantage of this difference for alkylation. Further,



SCHEME 1 Synthesis of protected 2′-MOE guanosine.

DAPR is poorly soluble in many organic solvents and DMSO was chosen for alkylation studies. Several bases like sodium hydroxide, lithium hydroxide, potassium *tert*-butoxide were tried in addition to performing the reactions at various temperatures (room temperature to 50°C).

Similarly, methoxyethyl bromide, triflate, mesylate, and tosylate were tried as electrophiles. At the end of this exercise it was found that potassium hydroxide in DMSO and methoxyethyl bromide[23,24] as electrophile worked the best. Thus, dissolving DAPR (177 mmole) and KOH (194 mmole) in DMSO (1500 mL) (26 mL of DMSO per gram of DAPR) and adding methoxyethyl bromide at room temperature initiated the alkylation reaction. To push the reaction to completion, additional amounts of KOH and methoxyethyl bromide (3 × 88 mmole) were needed. At the end of 6 hours, complete disappearance of starting material was observed. Analysis by HPLC showed 55% of product formed with 11% of 3′-isomer, 24% of bis-product and rest being higher homologues of alkylated products. All volatiles were distilled out completely under high vacuum to afford the crude product as a gummy material and was used in next step without purification.

To establish the structure of 2′-MOE DAPR, a sample of pure material was obtained by crystallizing twice from hot methanol. X-ray structure of 2′-MOE DAPR is shown in Figure 1.

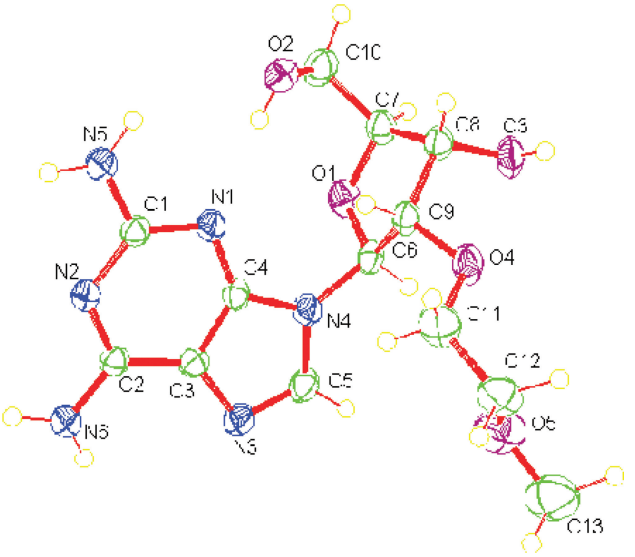


FIGURE 1 ORTEP diagram of 2′-MOE-DAPR.

Deamination of 2′-MOE DAPR

Adenosine deaminase (ADA) is known to catalyze the hydrolytic deamination of adenosine, 2-aminoadenosine, and various other purine nucleosides provided that a hydroxyl group is present at the 5′-position. ADA is commercially available and is inexpensive at large scales.[25] A systematic investigation of deamination reaction was undertaken changing variables like concentration, buffer, pH, stirring, temperature, etc. The reaction was twice fast at 50°C as compared to room temperature but enzyme was about 75% less stable. Stirring had no effect on rate but helped precipitation of product from the reaction solution. The optimal pH was 6.3–7.8. Methoxyethyl bromide, 3′-MOE DAPR and potassium bromide did not inhibit the ADA activity. However, 0.01 M zinc chloride completely killed the enzyme immediately. Also, it was observed that ammonium phosphate above 0.1 M was inhibitory. The rate of deamination of various nucleosides is shown in Figure 2.

Alkylation of DAPR leads to a variety of products viz. 2′-MOE DAPR, 3′-MOE DAPR, 2,3′-bis MOE DAPR and other polyalkylated products. Without isolation of the desired product (2′-MOE DAPR), the crude mixture was taken in phosphate buffer, pH adjusted to 7.2–7.4, adenosine deaminase enzyme added and stirred at 35°C for 70 hours. 2′-MOE guanosine crashes out of the reaction solution selectively along with a small percentage (< 1%) of 3′-MOE guanosine. Thus all other unwanted alkylated side products stays in solution and gets removed during filtration. Low levels of 3′-MOE guanosine that is carried along is removed at the next isobutyrylation step.

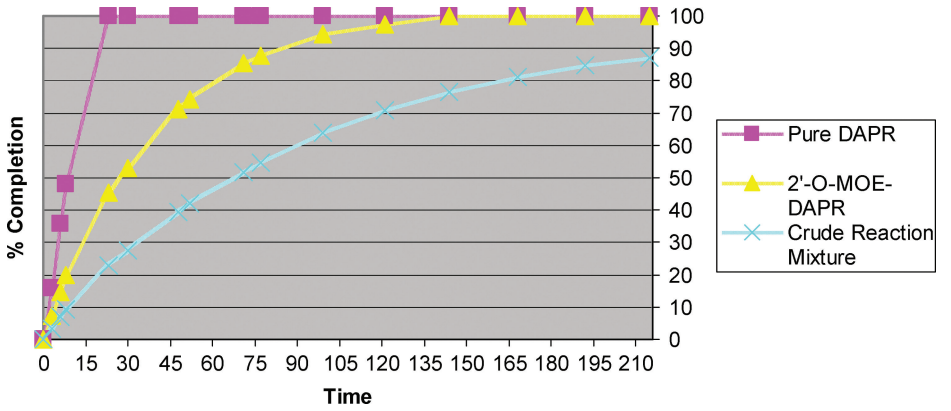


FIGURE 2 Deamination rate using adenosine deaminase enzyme (for reaction conditions, see Experimental; time in hours).

Thus the enzymatic step is a key stage where the desired product is isolated without resorting to any purification technique like chromatography.

Isobutyrylation and DMT Protection

Protection of exocyclic amino group was achieved by standard method of transient protection of hydroxyl groups with trimethylsilyl chloride followed by addition of isobutyryl chloride.[26] After workup, the product was washed with acetone to remove any undesired impurities including low levels of 3′-MOE isomer. For efficient DMT protection of 5′-hydroxyl group it was necessary to recrystallize the dimethoxytrityl chloride from hot hexane before use. Lutidine was preferred over pyridine as base since it increased the regioselectivity of the protection reaction (5′ over 3′ hydroxyl).

Thus, after optimizing all these steps, several syntheses were carried out at hundreds of grams scales. This was further scaled up to a multi-kilogram quantity to afford high quality product usable for therapeutic applications. An ion pair-HPLC-MS analysis[27,28] of a 3 Kg lot produced is shown in Figure 3 (chromatogram) and purity analysis in Table 1.

TABLE 1 IP-HPLC-MS analysis of (**5**) synthesized using optimized conditions

Quality of (**5**)	Mass	Amount present (%)
Purity	713.5	98.78
5′-DMT-N^2-ibu-2′-OMe G	669.7	0.05
5′-DMT-2′-MOE G (-ibu + N^2-DMT)	945.4	0.25
5′-DMT-N^2-ibu-3′-MOE G	713.7	0.10
Unknown impurity	903	0.25
Unknown impurity	1205	0.06

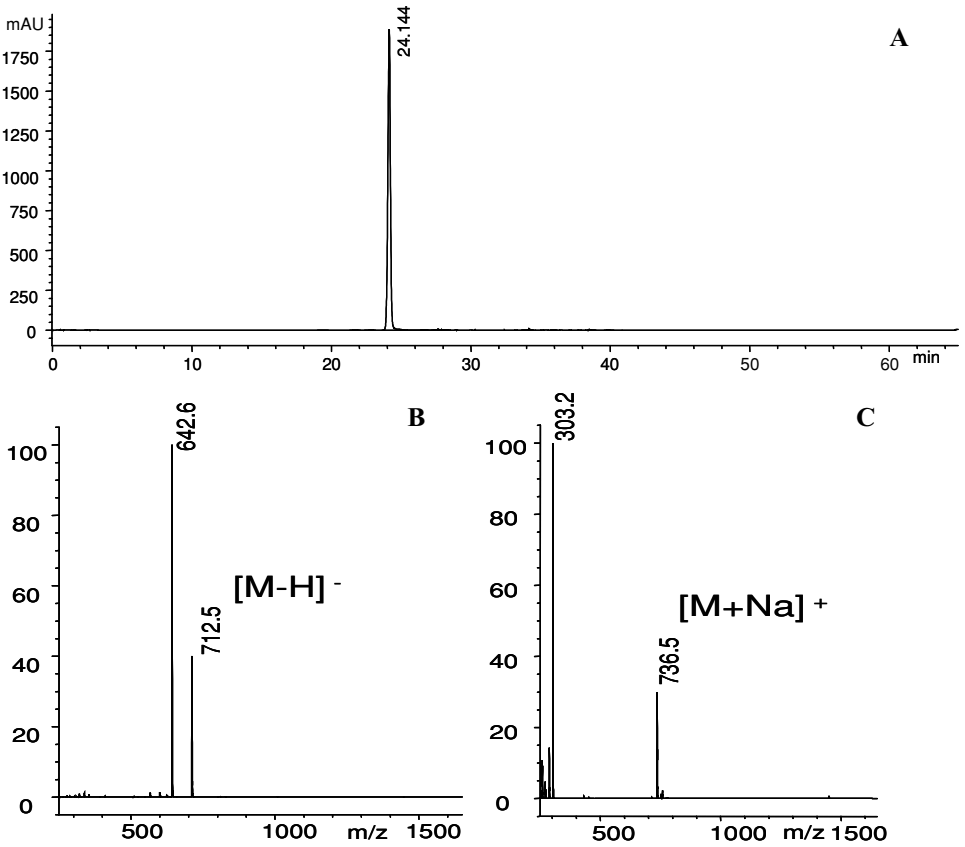


FIGURE 3 A) HPLC-UV chromatogram of (**5**) made at kilogram scale; B) average mass spectrum of $t_R = 24.144$ min component in negative mode; C): average mass spectrum of $t_R = 24.144$ min component in positive mode.

EXPERIMENTAL SECTION

Solvents and reagents were obtained from commercial sources. Reactions were monitored on Merck silica gel TLC plates (60F-254) and visualized under UV light. NMR spectra were recorded on Bruker Avanc 300 MHz spectrometer (Madras, India).

2′-*O*-(2-Methoxyethyl)-2,6-diaminopurine Riboside (2′-MOE DAPR) (2)

To a solution of KOH (10.9 g, 194 mmol) in DMSO (1500 mL) was added DAPR (**1**) (50.0 g, 177 mmol) followed by gradual addition of methoxyethyl bromide (24.6 g, 177 mmol) at ambient temperature. Three equal portions of KOH (4.95 g, 88 mmol) and methoxyethyl bromide (12.3 g, 88 mmol) were added after every hour intervals at ambient

temperature. Reaction was monitored by HPLC and at the end of 6 hours it showed complete disappearance of starting material. DMSO was distilled out completely under vacuum and the residue was extracted with acetone (2 × 500 mL). Solvents were distilled out completely under high vacuum to afford crude product (76 g, 55% pure by HPLC). Crude product was directly used for the next step without purification.

In case a pure sample of 2′-MOE-DAPR (**2**) is required, crude product was dissolved in hot methanol (200 mL), column grade silica gel (25 g) was added and stirred for 10 minutes at 60°C. The hot slurry was filtered and washed with methanol. The filtrate was stirred slowly with gradual cooling to ambient temperature and the solid formed was filtered and washed with methanol to furnish pure product (27 g, 45% yield) in 98% HPLC purity. m.p. 182.6–184.7°C; ^{1}H NMR (d_6-DMSO) δ 3.14 (s,3), 3.38 (t,2), 3.49–3.57 (m,2), 3.61–3.68 (m,2), 3.95 (d,1), 4.28 (d,1), 4.46 (t,1), 5.11 (d,1), 5.53 (t,1), 5.82 (d,1), 5.86 (s,2), 6.9 (s,2), 7.97 (s,1); ^{13}C NMR (d_6-DMSO) δ 58.06, 61.75, 68.90, 69.22, 71.1, 80.97, 85.1, 86.1, 113.5, 136.2, 151.4, 156.3,160.1; IR (KBr) 1087, 1626, 3339, 3381, 3436, 3494 cm^{-1}.

2′-*O*-(2-Methoxyethyl)guanosine (2′-MOE G) (3)

The crude reaction mass from previous step (76 g, 55% pure) was dissolved in 0.1 M sodium phosphate buffer solution of pH 7.2–7.4 (500 mL) and adenosine deaminase enzyme (75 mg) was added. The reaction mass was stirred at 35°C and the pH maintained between 7.2–7.4 by addition of 1 M solution of monosodium dihydrogen phosphate. Additional quantity of enzyme (37.5 mg each time) was added after 24 and 48 hours duration. Reaction was monitored by HPLC and at the end of 70 hours, HPLC of the reaction solution showed complete disappearance of starting material. Reaction mass was cooled to 10°C, maintained for 2 hours, filtered, washed with water (20 mL × 3) and finally with acetone. The material was dried under high vacuum to afford the desired product as colorless solid (30.1 g) in 49.9% yield. m.p. 244°C (chars); ^{1}H NMR (d_6-DMSO) δ 3.16 (s,3), 3.39–3.41 (d,2), 3.48–3.58 (m,2), 3.88 (d,1), 4.23 (t,1), 4.35 (t,1), 5.0 (d,2), 5.79 (d,1), 6.48 (s,2), 7.97 (s,1), 10.67 (s,1); ^{13}C NMR (d_6-DMSO) δ 58.35, 61.3, 61.6, 69.21, 71.42, 81.6, 84.6, 86.1, 92.3, 116.9, 135.8, 151.6, 154.0, 157.1 IR (KBr) 782.7, 1226.8, 1697.6, 3224, 3341, 3345 cm^{-1}.

N^2-Isobutyryl-2′-*O*-(2-methoxyethyl)guanosine (4)

2′-MOE-G (**3**) (25 g, 73.3 mmol) was dried by co-evaporation twice with dry pyridine (125 mL × 2). The reaction mass was dispersed in pyridine (175 mL) and treated with trimethylsilyl chloride (47.6 g, 438 mmol) at 15°C over a period of 30 minutes. The resultant slurry was stirred for 3 hours and then isobutyryl chloride (11.6 g, 108 mmol) was added neat

over a period of 30 minutes. The temperature was gradually increased to 30°C and maintained for 3 hours. The reaction was quenched by addition of methanol (5 mL), the precipitated pyridinium hydrochloride salt was filtered and washed with acetone. The filtrate was diluted with water (50 mL) and pH adjusted to 7.1–7.3 using ammonium hydroxide solution. The slurry was kept at ambient temperature for 12 hours, the solid obtained was filtered and washed with acetone. Nitrogen gas was bubbled through the filtrate for about 2 hours and concentrated at 60–65°C under vacuum. The residue was extracted with acetonitrile (200 mL) and the organic layer concentrated under vacuum and the residue was treated with 10% aqueous acetone (275 mL) at 45°C. The clear solution was cooled down to 0–5°C gradually when material starts crystallizing out. It was filtered, washed with acetone and dried under high vacuum to furnish **4** (29.2 g, 97% yield) in 99.7% purity by HPLC. m.p. 137–139.2°C; ^{1}H NMR (d_6-DMSO) δ 1.10 (d,6), 2.76 (m,1), 3.15 (s,3), 3.41 (t,4), 3.53–3.71 (m,4), 3.92 (d,1), 4.29 (d,1), 4.4 (t,1), 5.09 (m,2), 5.89 (d,1), 8.29 (s,1), 11.70 (s,1), 12.10 (s,1); ^{13}C NMR (d_6-DMSO) δ 18.89, 30.73, 34.81, 58.04, 61.25, 68.94, 71.15, 81.56, 84.49, 86.10, 120.07, 137.59, 148.3, 148.94, 154.86, 180.19, 206.6; IR (KBr) 1102, 1564, 1602, 1719, 2921, 3257, 3384.1 cm^{-1}.

5′-*O*-(4,4′-Dimethoxytrityl)-*N*^{2}-isobutyryl-2′-*O*-(2-methoxyethyl) guanosine (5)

N^2-Isobutyryl-2′-*O*-(2-methoxyethyl)guanosine (**4**) (25 g, 60.8 mmol) was dried by co-evaporation with acetonitrile (125 mL x 2) and dissolved in acetonitrile (150 mL) and 2,6-lutidine (21.5 g, 200 mmol) at 45°C under N_2 atmosphere. Freshly purified 4,4′-dimethoxytrityl chloride (20.8 g, 61.4 mmol) was divided into four equal lots and added one by one at every 30-minute interval. Reaction was monitored by TLC and at the end of 3 hours it showed complete disappearance of starting material. The reaction was quenched by addition of methanol (3 mL) and all volatiles were distilled out under vacuum. The residue was extracted with ethyl acetate (125 mL × 2) and the organic layer washed successively with saturated sodium bicarbonate solution (125 mL) and saturated brine solution (125 mL). The organic layer was dried using anhydrous sodium sulfate and concentrated to 25% volume under vacuum. The crude product was passed through a silica gel column (approximate ratio of crude mass to weight of silica gel is 1:15) and eluted with ethyl acetate:lutidine:methanol (95:1:4). The fractions containing pure product were combined and concentrated under vacuum to 25% volume and treated with 2% aqueous citric acid solution (2.0 molar equivalent of lutidine was present in solution as estimated by HPLC) at 0–5°C for 30 minutes. The organic layer was washed successively with water (75 mL), saturated sodium bicarbonate solution (75 mL), cold water (75 mL) and dried. All volatiles were removed completely under

vacuum. The viscous mass was triturated with hexane (250 mL) and stirred for 3 hours. The colorless amorphous solid obtained was filtered, washed with hexane (50 mL), and dried under high vacuum at 50°C to afford pure product (**5**), (37.7 g) in 87% yield and 99.8% product purity. m.p. 112.5–126.6°C; ^{1}H NMR (d_6-DMSO) δ 1.10–1.28 (d,6), 2.71–2.78 (m,1), 3.11 (s,3), 3.41 (t,3), 3.60–3.67 (m,1), 3.72 (s,6), 4.01 (s,1), 4.33 (s,1), 4.48 (t,1), 5.2 (s,1), 5.94 (d,1), 6.79–6.84 (m,4), 7.19–7.35 (m,9), 8.1 (s,1), 11.82 (s,2); ^{13}C NMR (d_6-DMSO) δ 12.1, 12.3, 33.0, 53.2, 56.3, 58.0, 62.2, 67.5, 68.8, 69.5, 79.2, 82.23, 83.48, 83.8, 111.3, 118.6, 124.9, 125.9, 126.0, 128.0, 133.6, 133.7, 135.9, 143.0, 146.5, 147.1, 153.1, 156.3, 178.4; IR (KBr) 1251, 1607, 1681, 2932, 3191, 3373 cm^{-1}.

REFERENCES AND NOTES

1. Crooke, S.T. Basic principles of antisense therapeutics, in *Antisense Therapeutics in Biotechnology & Genetic Engineering Reviews*, Intercept Ltd., Hampshire, UK, 1998, vol. 15, 121–157.
2. Crooke, S.T. Basic principles of antisense therapeutics, in *Handbook of Experimental Pharmacology: Antisense Research & Application*, ed. S.T. Crooke, Berlin, German, Springer-Verlag, 1998.
3. Pirollo, K. F.; Rait, A.; Sleer, L. S.; Chang, E. H. Antisense therapeutics: from theory to clinical practice. *Pharm. Ther.* **2003**, 99, 55–77.
4. Altmann, K.-H.; Dean, N.M.; Fabbro, D.; Freier, S.M.; Geiger, T.; Haner, R.; Husken, D.; Martin, P.; Monia, B.P.; Muller, M.; Natt, F.; Nicklin, P.; Phillips, J.; Pieles, U.; Sasmor, H.; Moser, H. E. Second generation of antisense oligonucleotides. From nuclease resistance to biological efficacy in animals. *Chimia*, **1996**, 50, 168–176.
5. Ross, B.S.; Song, Q.; Han, M. Kilo-scale synthesis process for 2′-*O*-(2-methoxyethyl)-pyrimidine derivatives. *Nucleosides Nucleotides Nucleic Acids* **2005**, 24, 815–818.
6. Wagner, E.; Oberhauser, B.; Holzner, A.; Brunar, H.; Issakides, G.; Schaffner, G.; Cotton, M.; Knollmuller, M.; Noe, C.R. A simple procedure for the preparation of protected 2′-O-methyl or 2′-O-ethyl ribonucleoside-3′-O-phosphoramidites. *Nucleic Acids Res.* **1991**, 19, 5965–5971.
7. Grotli, M.; Douglas, M.; Beijer, B.; Garcia, R.G.; Eritja, R.; Sproat, B. Protection of the guanine residue during synthesis of 2′-O-alkylguanosine derivatives. *J. Chem. Soc. Perkin Trans. I*, **1997**, 18, 2779–2788.
8. Wen, K.; Chow, S.; Sanghvi, Y.S.; Theodorakis, E.A. Synthesis of 2′-*O*-methoxyethyl guanosine using a novel silicon based protecting group. *J. Org. Chem.* **2002**, 67, 7887–7889.
9. Chow, S.; Wen, K.; Sanghvi, Y.S.; Theodorakis, E.A. Novel synthesis of 2′-O-methylguanosine. *Bioorg. Med. Chem. Lett.* **2003**, 13, 1631–1634.
10. Chow, S.; Wen, K.; Sanghvi, Y.S.; Theodorakis, E.A. MDPSCl2: A new protecting group for chemo selective synthesis of 2′-*O*-alkylated guanidines. *Nucleosides Nucleotides Nucleic Acids* **2003**, 22, 583–587.
11. Beagle, L.; Harebell, P.; Swindler, D.; Karpeisky, A. Improved synthetic approaches toward 2′-*O*-methyladenosine and guanosine and their N-acyl derivatives. *Tetrahedron* **2000**, 56, 1047–1056.
12. Marais, G.; Ghisalba, O. Chemo-enzymatic synthesis of 2′-O-methoxyethyl ribonucleosides using a phosphodiesterase from Serratia marcescens. *Appl. Microbiol. Biotech.* **2005**, 66, 512–519.
13. Hodge, R.P.; Sinha, N.D. Simplified s*ynthesis of 2′-O-alkyl ri*bopyrimidines. *Tetrahedron Lett.* **1995**, 36, 2933–2936.
14. Zlatev, I.; Vasseur, J-J., Morvan, F. Convenient synthesis of N^2-isobutyryl-2′-O-methyl guanosine by efficient alkylation of O^6-trimethylsilylethyl-3′,5′-di-tert-butylsilanediyl guanosine. *Tetrahedron* **2007**, 63, 11174–11178.
15. Sproat, B.S.; Beijer, B.; Iribarren, A. New synthetic routes to protected purine 2′-O-methylriboside-3′-O-phosphoramidites using a novel alkylation procedure. *Nucleic Acids Res.* **1990**, 18, 41–49.
16. Martin, P. Ein neuer Zugang zu 2′-O-(2-methoxyethyl)ribonucleosiden ausgehend von D-glucose. *Helv. Chim. Acta* **2003**, 86, 204–209.
17. Martin, P. Stereoselektive synthese von 2′-O-(2-*methoxyethyl)rib*onucleosiden: Nachbargruppenbeteiligung der methoxyethoxy-gruppe bei der ribosylierung von heterocyclen. *Helv. Chim. Acta* **2004**, 79, 1930–1938.

18. Sivets, G.G.; Vepsalainen, J.; Mikhailopulo, I.A. Synthesis of 9-[2-O-(methoxyethy)-β-D-ribofuranosyl]purines using 1-methanesulfonyloxy-2-methoxyethane. *Lett. Org. Chem.* **2005**, 2, 343–348.
19. Sivets, G.G. Synthesis study of 2′-O-(2-methoxyethyl)p*urine derivative*s. *Nucleosides Nucleotides Nucleic Acid* **2007**, 26, 1237–1240.
20. Ross, B.S.; Springer, R.H.; Ravikumar, V.T. An efficient and scalable synthesis of 2,6-diaminopurine riboside. *Nucleosides Nucleotides Nucleic Acids* **2008**, 27, 67–69, and references cited therein.
21. Santaniello, E.; Ciuffreda, P.; Alessandrini, L. Synthesis of modified purine nucleosides and related compounds mediated by adenosine deaminase (ADA) and adenylate deaminase (AMPDA). *Synthesis* **2005**, 4, 509–526, and references cited therein.
22. Zuffi, G.; Ghisotti, D.; Oliva, I.; Capra, E.; Frascotti, G.; Tonon, G.; Orsini, G. Immobilized biocatalyst for the production of nucleosides and nucleoside analogues by enzymatic transglycosylation reactions. *Biocatalysis and Biotransformation*, **2004**, 22, 25–33.
23. Dasari, V.; Srinivas, R.V.; Rao, S.R.; Chakravarthy Akula, K.; Dhamjewar, R.; Sanghvi, Y.S. A facile process for the preparation of 2-bromoethyl methyl ether. *Org. Process Res. Dev.* **2007**, 11, 468–469, and references cited therein.
24. It is essential to have goo*d quality of methoxye*thyl bromide. Generally it is contaminated with methyl bromide and this competes during the alkylation reaction to afford 2′-*O*-methyl analogue. Fresh distillation before use is preferred.
25. Even though there are many suppli*e*rs of animal-sourced origin, Biocatalytics (Pasadena, California, USA) is the major supplier of ADA of non-animal origin. At multi-kilogram scales it costs approximately $0.20 to produce one gram of the title compound under the reported overall yield. Considering the chemoselectivity of this reaction and an easily scalable reaction we feel this is a key to the success of this route.
26. Ti, G.S.; Gaffney, B.L.; Jones, R.A. Transient protection: Efficient one-flask syntheses of protected deoxynucleosides. *J. Am. Chem. Soc.* **1982**, 104, 1316–1319.
27. Kurata, C.; Bradley, K.; Gaus, H.; Luu, N.; Cedillo, I.; Ravikumar, V.T.; Van Sooy, K.; McArdle, J.V.; Capaldi, D.C. Characterization of high molecular weight impurities in synthetic phosphorothioate oligonucleotides. *Bioorg. Med. Chem. Lett.*, **2006**, 16, 607–614.
28. Rentel, C.; Wang, X.; Batt, M.; Kurata, C.; Oliver, J.; Gaus, H.; Krotz, A.H.; McArdle, J.V.; Capaldi, D.C. Formation of modified cytosine residues in the presence of depurinated DNA. *J. Org. Chem.* **2005**, 70, 7841–7845.