Pergamon

Tetrahedron 56 (2000) 1047–1056

TETRAHEDRON

Improved Synthetic Approaches Toward 2′-*O*-Methyl-Adenosine and Guanosine and Their *N*-Acyl Derivatives

Leonid Beigelman,* Peter Haeberli, David Sweedler and Alexander Karpeisky

Department of Organic Chemistry, Ribozyme Pharmaceuticals Inc., 2950 Wilderness Place, Boulder, CO 80301, USA

Received 21 September 1999; accepted 22 December 1999

Abstract—We developed several improved approaches toward 2′-*O*-methyl adenosine and guanosine and their *N*-acyl derivatives. (a) Transglycosylation of N^4-acetyl-5′, 3′-di-*O*-acetyl-2′-*O*-methyl cytidine with N^6-Bz-adenine provided N^6-benzoyl-5′3′-di-*O*-acetyl-2′-*O*-methyl adenosine in 50% yield. (b) Regioselective methylation of 2-amino-6-chloro purine riboside with MeI/NaH followed by hydrolysis provided 2′-*O*-Me-guanosine in high yield. The same 2′-*O*-Me-precursor was transformed into 2′-*O*-Me-adenosine in 58% yield. (c) Very efficient transformation of 2,6-diamino-purine riboside into N^2-isobutyryl (isopropylphenoxyacetyl) 2′-*O*-Me-guanosine through methylation of 5′,3′-*O*-TIPDSi derivative followed by selective N^2-acylation, deamination and desylilation provided target compounds in 70% combined yield. (d) Mg^{2+} and Ag^{+} directed methylation of N^1-Bzl-guanosine proceeded in >80% yield with ratio of 2′-*O*-Me/3′-*O*-Me=9:1. The same methylation of adenosine with Ag^{+} and Sr^{2+} acetylacetonates provided 2′-*O*-Me-adenosine in 75–80% yield.

Introduction

2′-*O*-Methylribonucleosides are widely distributed in RNA[1] and since the late 1950s have been the focus of considerable synthetic efforts. These synthetic efforts were further stimulated by recent applications of 2′-*O*-methyloligoribonucleotides in studying pre-mRNA splicing[2] and the structure of splicesomes[3] as well as in the preparation of nuclease-resistant hammerhead ribozymes.[4] 2′-*O*-Alkyl substituted oligonucleotides are also emerging as a second generation antisense constructs with improved hybridization properties and favorable nuclease resistance and pharmacological profiles.[5] In light of this interest in oligonucleotide based therapeutics, it is increasingly important to develop cost effective synthesis of 2′-*O*-alkyl nucleosides that serves as a key raw material. Previously developed methods for the preparation of 2′-*O*-Me nucleosides can be divided into five categories.

Synthesis from carbohydrate precursors

This approach was pioneered by Haines[6] and Imbach.[7] Recent improvements involved optimization of the large scale synthesis of the 2-*O*-Me ribofuranose precursor,[8] synthesis of an alternately protected 2-*O*-Me-synthon[9] and the utilization of D-glucose as a starting material.[10] In general, this approach requires the multistep synthesis of carbohydrate precursors for glycosylation. The critical glycosylation step typically provides a mixture of α and β anomers and is especially problematic for adenosine and guanosine nucleosides.

Direct methylation of ribonucleosides

The observation by Broom and Robins, in 1965, that diazomethane in DME directed methylation of adenosine preferentially to the *cis*-diol system[11] resulted in the first practical synthesis of 2′-*O*-Me-adenosine,[11,14] 2′-*O*-Me-cytidine[13] and 2′-*O*-Me-uridine[13] and the indirect synthesis of 2′-*O*-Me-guanosine[12] through methylation of 2-amino-6-chloro purine riboside. Shugar et al. described alkylation of cytidine[15] and adenosine[16] by methyl(ethyl)sulfate resulting in the preparation of all possible alkylated products. Direct methylation of uridine and guanosine by NaH/MeI reagent causes undesired base methylation, but provide satisfactory yields of 2′-*O*-Me-Adenosine.[17] This procedure was utilized for the preparation of all four 2′-*O*-Me(Et) ribonucleoside phosphoramidites.[18] In the case of uridine and guanosine, protection of N^3 and O^6 of the base prior to alkylation was necessary. Methylation of 5′-*O*-DMT-N^4-*t*-butylphenoxyacetyl(or benzoyl)cytidines by Ag_2O/MeI in the presence of a trace amount of pyridine resulted in exclusive 2′-*O*-methylation in 65–95% yield.[19] In general, the above methodology usually requires extensive chromatographic separation of the 2′ and 3′-*O*-Me isomers.

Metal-directed methylation of the activated *cis*-diol group

Diazomethane mediated methylation of the *cis*-diol group

Keywords: ribozymes; oligonucleotides; 2′-*O*-Me-nucleosides; methylation.

* Corresponding author. E-mail: lnb@rpi.com

0040–4020/00/$ - see front matter
PII: S0040-4020(00)00002-8

using $SnCl_2 \cdot 2H_2O$ activation is the most popular method of synthesis of all four 2′-*O*-Me ribonucleosides. These procedures typically produce mixtures of 2′ and 3′-*O*-Me isomers under mild conditions.[20] Direct methylation of guanosine by this method provided 2′-*O*-Me-guanosine in only a 15% yield. Therefore, a better method was developed using the same technique, but with 2,6-diaminopurine riboside as a starting material, providing 2′-*O*-Me-guanosine in 40% yield.[21] Application of this method for methylation of 5′-*O*-TBDPSi[22], 5′-*O*-Tr[23] and 5′-*O*-DMT-*N*-NPEOC[24] protected ribonucleosides resulted in preferential 2′-*O*-methylation. Recently, $TMSiCHN_2$ substituted the hazardous reagent diazomethane[25] for the methylation of base protected guanosine.

The activation of *cis*-diol groups by derivatization with Bu_2SnO followed by methylation with MeI provided 2′ and 3′-*O*-Me derivatives in the case of uridine but not for other nucleosides.[26] The combination of dibutylstannylene activation followed by methylation with diazomethane allowed to obtain 2′-*O*-Me adenosine are uridine, but failed in the case of cytidine and guanosine.[27] Several metal acetylacetonates used together with trimethylsulfonium hydroxide directed methylation primarily to 2′ and 3′-hydroxyls for uridine, adenosine are cytidine, but guanosine was methylated at 2′ and 3′-hydroxyls and N^1 of the base.[28] Once again, the above methodology usually requires extensive chromatographic separation of 2′ and 3′-*O*-Me isomers.

Methylation of 3′, 5′-*O*-TIPDSi-protected ribonucleosides

Introduction of selective and simultaneous protection of 3′- and 5′-hydroxyl groups in ribonucleosides through a 5′, 3′-*O*-triisopropyldisiloxane bridge, simplified direct selective alkylation of 2′-hydroxyls in such compounds. It is notable that mostly MeI/Ag_2O reagent was used so far. Convenient preparation of 2′-*O*-Me-uridine and cytidine through the common precursor 5′,3′-*O*-TIPDSi-4-(2-nitrophenyl)-2-pyrimidinone using MeI/Ag_2O was described by Nyilas and Chattopadhyaya.[29] A comprehensive investigation of the methylation of 5′,3′-*O*-TIPDSi-protected ribonucleosides by MeI/Ag_2O reagent was described by Inoue and Otsuka.[30] 5′,3′-*O*-TIPDSi N^4-Bz-Cyd was methylated directly in 70% yield. The uridine derivative required N^3-Bz protection and the adenosine derivative was prepared through methylation of the 6-*Cl*-purine intermidiate. Attempts to methylate 5′,3′-*O*-TIPDSi-N^2-ibu-Guo or its N-protected derivative were not successful and guanosine synthone was prepared by methylation with diazomethane. Optimized large scale preparation of N^4-ibu-2′-*O*-Me-cytidine using this method was also reported.[31] Sproat and co-workers concentrated on development of a convenient route to guanosine derivatives utilizing 5′,3′-*O*-TIPDSi protected purine ribonucleosides. The best synthesis of 2′-*O*-Me guanosine was first achieved from 5′,3′-*O*-TIPDSi-2,6-dicloropurine precursor using new methylation system MeI/BDDDP.[32] This procedure was later extended to the direct methylation of 5′,3′-*O*-TIPDSi-O^6-TBDPSi-Guanosine with MeI/BEMP.[33] High cost of the BEMP reagent, relative instability of 5′,3′-*O*-TIPDSi-O^6-TBDPSi-Guanosine intermediate and modest overall yield (50%) warrant further improvements in the synthesis of this compound.

Miscellaneous

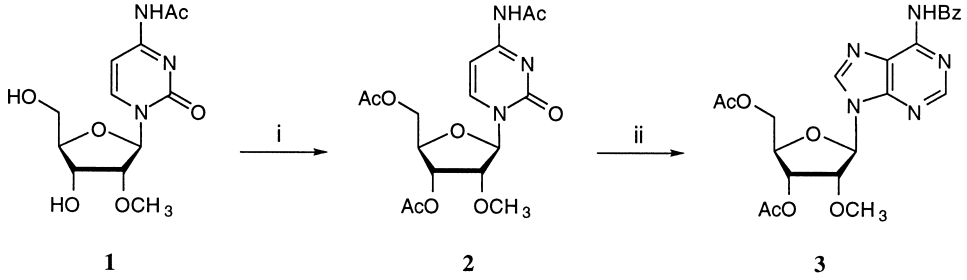
Since the direct methylation of uridine and guanosine with NaH or Ag_2O/MeI requires the protection of acidic N^3 and N^1 atoms of the pyrimidine and purine bases, it was logical to develop new approaches which introduce the precursors of the methyl group without protection of acidic functions on the base. Sekine and Hata[34] reported the introduction of a 1,3-benzodithiol-2-yl group into 5′,3′-*O*-TIPDSi-uridine without protection of N^3 nitrogen; the 2′-*O*-methyl group was regenerated after desulfurization with Raney Ni. Methylthiomethyl (MTM)[35] or methylthiophenyl (MTPh)[10] ethers were introduced at 2′-OH of 5′,3′-*O*-TIPDSi-uridine without base protection. Desulfurization with Raney Ni (MTM) or radical reduction (MTPh) regenerated the methyl group.

Pyrimidine 2,2′-anhydronucleosides are very attractive starting materials for the synthesis of 2′-*O*-methyl nucleosides because of their ease of synthesis, scalability and susceptibility to the 2,2′-anhydro ring opening by many nucleophiles. It was demonstrated recently that Mg^{2+} and Ca^{2+} alkoxides can open 5′-*O*-protected pyrimidine 2,2′-anhydronucleosides providing 2′-*O*-alkyl ribonucleosides in 50–90% yield.[36] Opening of unprotected 2,2′-anhydro pyrimidine nucleosides by action of $B(OMe)_3$ at high temperature resulted in the preparation of 2′-*O*-Me-uridine (>90% yield).[37] In addition, Mg $(OMe)_2$ was also effective in this reaction.[38]

Results and Discussion

Synthesis of 2′-*O*-Me-adenosine through transglycosylation (Scheme 1)

In 1982 Imbach et al.[7] demonstrated the utilization of 2-*O*-methyl-1,3,5-tri-*O*-benzoyl-α-D-ribofuranose for the



1 2 3

Scheme 1. Synthesis of 2′-*O*-Me-adenosine by transglycosylation: (i) Ac_2O, pyridine/DMF; (ii) N^6-benzoylaminopurine, BSA, TMStriflate, MeCN, 75°C, 16h.

stereospecific synthesis of 2′-*O*-Me pyrimidine-β-D-ribonucleosides through glycosylation of silylate bases in the presence of Lewis acids as catalysts. This procedure was optimized for a large scale preparation of 2′-*O*-methyl-pyrimidine ribonucleosides by Ross et al.[8] Even after optimization, this procedure required methylation of 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose with a large excess of diazomethane which is potentially explosive. To overcome this problem, we decided to utilize transglycosylation of a suitably protected 2′-*O*-Me-pyrimidine ribonucleosides that were obtained through $B(OMe)_3$ mediated opening of 2,2′-anhydronucleosides.

The transglycosylation reaction proceed with high β-selectivity if carbohydrate donor contains 2′-*O*-acyl group capable to stabilize postulated C-1 carboxonium ion for exclusive β-attack by an incoming silylated base. The stereochemical outcome of transglycosylation reactions with a carbohydrate donor without participating group at 2′-OH (such as 2′-*O*-Me) usually resulted in a mixture of α,β nucleosides as documented for the synthesis of 2′-azido purine ribonucleoside.[39]

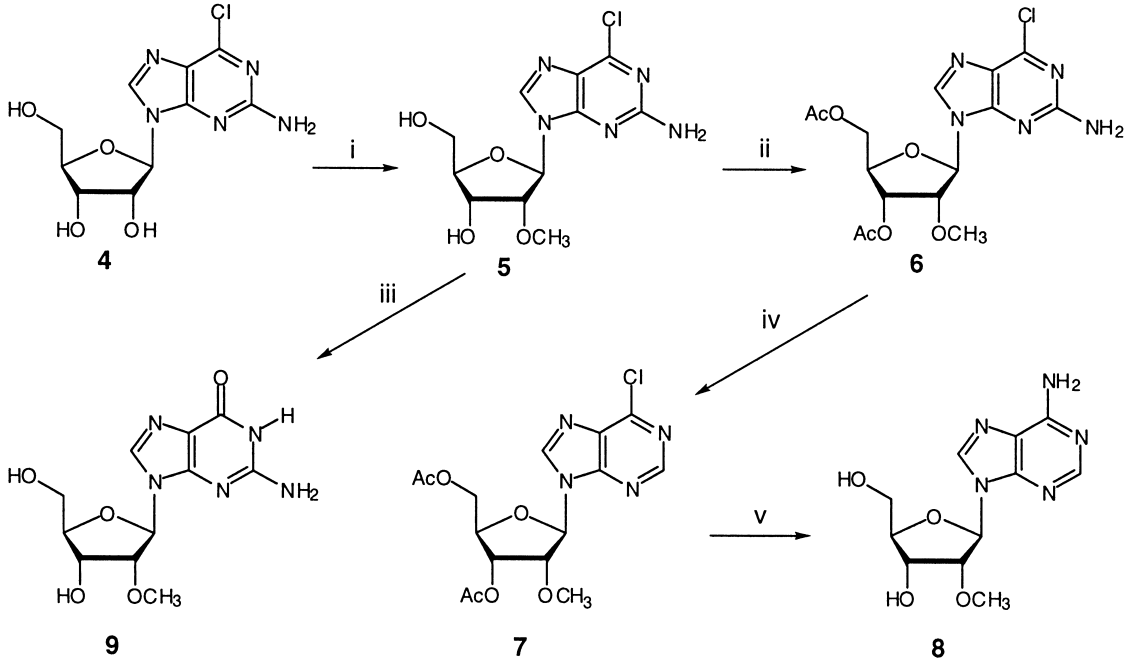
We first investigated the transglycosylation of 5′,3′-di-*O*-acetyl-2′-*O*-Me uridine obtained by acylation of 2′-*O*-Me-uridine. The transglycosylation of 5′,3′-di-*O*-acetyl-2′-*O*-Me uridine with 3 equiv. of N^6-benzoylaminopurine and 3 equiv. TMStriflate in CH_3CN at 75°C for 16 h resulted in an inseparable mixture of α and β isomers of N^6-benzoyl-5′,3′-di-*O*-acetyl-2′-*O*-methyl adenosine in a 60% yield and a 1:1 ratio (α/β). Since the nature of the aglycone can also influence the product distribution in transglycosylation reactions,[40] we decided to try N^4-acetyl-5′,3′-di-*O*-acetyl-2′-*O*-methyl cytidine (**2**) as a carbohydrate donor. Utilization of this donor under the same conditions as for the 2′-*O*-Me uridine derivative resulted in predominant formation of the β anomer of N^6-benzoyl-5′,3′-di-*O*-acetyl-2′-*O*-methyl adenosine (**3**) that can be isolated in 50% yield. Several other products were also formed along with α-isomer of **3** that contributed to the overall moderate yield. When N^6-phenoxyacetyl-aminopurine was used instead of N^6-benzoyladenine under the same conditions extensive decomposition of initially formed adenosine derivative was observed and target nucleoside was not isolated.

The described procedure for transglycosylation is a useful alternative in the synthesis of 2′-*O*-Me adenosine derivative since the starting cytidine synthone can be easily prepared on large scale by modified procedure based on $B(OMe)_3$[37] mediated opening of 2,2′-*O*-anhydro-Cyd followed by acylation.

Synthesis of 2′-*O*-Me-guanosine and adenosine from 2-amino-6-chloropurine riboside (Scheme 2)

High regioselectivity in the methylation of 2-amino-6-choloropurine riboside by diazomethane was reported by Robins in 1966.[12] Despite exclusive methylation of the 2′-OH, the desired 2′-*O*-Me product was not isolated. Subsequent transformations of this key intermediate resulted in the preparation of 2′-*O*-Me-guanosine in 15% yield. Since diazomethane is not practical for large scale preparations, it was reasonable to investigate other methylation reagents.

Methylation of 2-amino-6-chloropurine riboside with a small excess of NaH/MeI reagent in DMF at −20°C resulted in 2′-*O*-Me nucleoside (**5**) in a 65% isolated yield together with the formation of 2′,3′ bis-*O*-Methyl derivative in 15% yield; no 3′-methylation was observed under these conditions. Several procedures were tried for the transformation of intermediate (**5**) into 2′-*O*-Me-guanosine (**9**), the best result was obtained then intermediate (**5**) was treated with



Scheme 2. Synthesis of 2′-*O*-methyl-adenosine and guanosine from 2-amino-6-chloropurine riboside: (i) NaH/MeI/DMF; (ii) Ac_2O,DMAP,Et_3N/CH_3CN; (iii) DABCO/H_2O; (iv) isoamyl nitrile/THF; (v) NH_3/MeOH.

1,4-diazabicyclo[2.2.2]octane (1 equiv.) and water at 90°C for 45 min with subsequent hydrolysis by 2 M NaOH at pH 12.[41] The target 2′-*O*-Me-guanosine was obtained in 65% yield.

The high regioselectivity in the methylation of 2-amino-6-chloropurine riboside allows one to obtain gram quantities of 2′-*O*-Me-synthone (**5**) which can serve as a key intermediate in the preparation of not only 2′-*O*-Me-guanosine but also 2′-*O*-Me adenosine. This latter transformation was achieved through radical deamination[42,43] of 3′,5′-di-*O*-acetyl-2′-*O*-methyl-6-*Cl*-2-aminopurine riboside (**6**) which provided 3′,5′-di-*O*-acetyl-2′-*O*-methyl-6-chloropurine riboside **7** in 72% yield from **5**. Subsequent amination of **7** with methanolic ammonia at 125°C for 4 h provided 2′-*O*-Me-adenosine (**8**) in 80% yield.

The convergent synthesis of both 2′-*O*-Me-Ado and Guo from the same precursor is attractive for scale up since the starting 2-amino-6-chloropurine riboside is available from guanosine in three steps without chromatographic separations in 75% yield.[44]

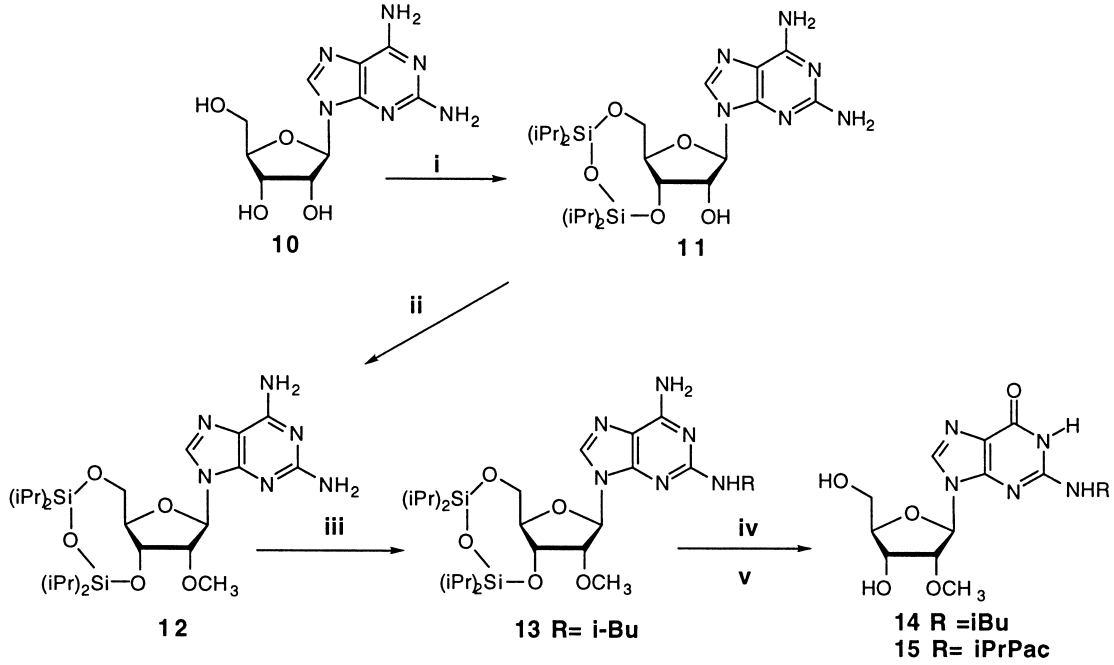
Synthesis of N^2-acyl(isobutyryl or isopropylphenoxyacetyl)-2′-*O*-methylguanosine from 2,6-diamino-β-D-ribofuranosylpurine (Scheme 3)

It was reported that the diazomethane methylation of 2,6-diamino-β-D-ribofuranosylpurine in the presence of $SnCl_2 \cdot 2H_2O$ provided a 1:1 mixture of 2′ and 3′-*O*-methylated derivatives in a quantitative yield. These compounds can be separated on a Dowex 1 $(OH)^-$ column and deaminated to the corresponding guanosine derivatives with adenosine deaminase.[21] Literature analysis also indicated that direct methylation of guanosine (NaH/MeI or diazomethane) usually resulted in a preferential methylation at N^1 or/and N^7 position of the base.[17,18]

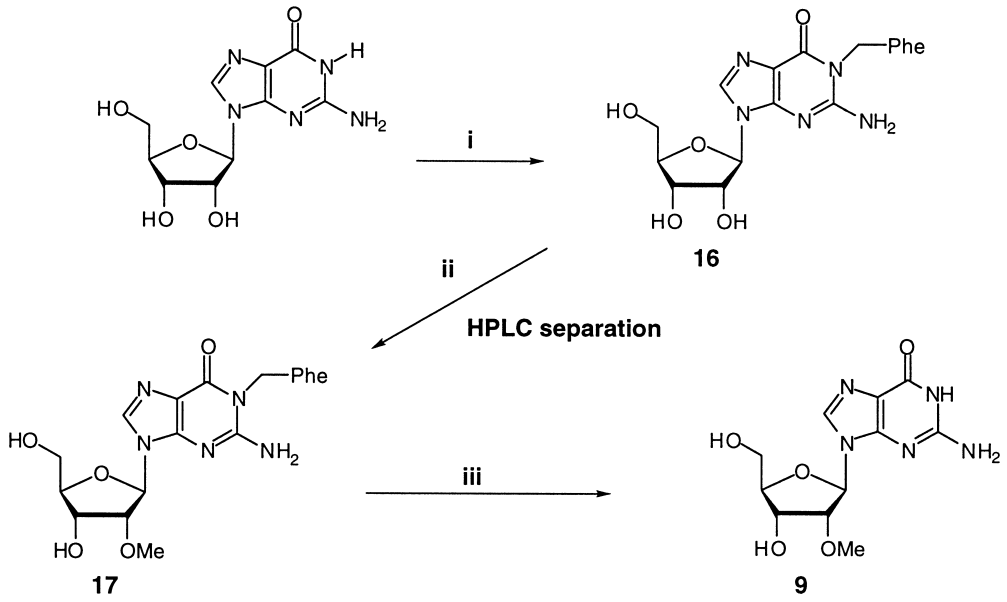
In 2,6-diamino-β-D-ribofuranosylpurine the guanosine C_6-N_1 acidic amide function is replaced by weekly basic amidine function. Therefore, one would expect that such replacement should reduce the extent of methylation at N^1 under basic conditions (i.e. NaH/MeI). In order to increase regioselectivity we decided to use 5′,3′-*O*-tetraisopropyldisiloxane-1,3-diyl protection, which was reported to be relatively stable under NaH/MeI methylation conditions.[9] The 5′,3′-*O*-tetraisopropyldisiloxane-1,3-diyl protection was introduced by a standard procedure; utilization of ethylacetate–water extraction allowed the isolation of pure protected derivative (**11**) in 90% yield in a crystalline form due to its low solubility in the above system.

We investigated several methylation procedures for the NaH/MeI system, varying the amount and type of solvent and equivalents of NaH and MeI. The best results were obtained when methylation was performed at 0°C in DMF with 1.5 equiv. of NaH and 3 equiv. of MeI. If the reaction was performed at higher temperature or in a more concentrated solution, extensive hypermethylation occurred. Utilization of a lower amount of MeI required longer reaction time and opening of the cyclic silyl group was observed with simultaneous methylation of both 2 and 3′-hydroxyls. The 2′-*O*-Me derivative (**12**) can be isolated in 90% yield without column chromatography by crystallization from ethanol–water.

In order to synthesize N^2-acyl-2′-*O*-methylguanosine derivatives (**14**) and (**15**) we decided to explore selective acylation of the 2-amino group of intermediate (**12**) followed by chemical deamination of the 6-amino group. Two factors are critical for the success of this approach: the degree of selectivity in acylation of the N-2 amino group vs. the 6-amino group in the intermediate (**12**); and the stability of *N*-2-protection to acidic conditions of deamination.[45] We found that when acylation of diaminopurine (**12**) was



Scheme 3. Synthesis of N^2-acyl derivatives of 2′-*O*-methyl-guanosine from 2,6-diaminopurine riboside: (i) TIPDSiCl/pyridine; (ii) MeI, NaH/DMF 0°C; (iii) iPrPacCl/pyridine; (iv) $HOAc/NaNO_2/H_2O$; (v) TEA·3HF.



Scheme 4. N^1-Benzyl guanosine route: (i) N,N-dimethylformamide dibenzyl acetal, 80°C, 18h; 2N NaOH; (ii) Metal acetylacetonate, Me_3SOH, 80°C, 2h.; (iii) Na^+ naphthalene.

performed at −10°C with 1.1 equiv. of acyl chloride, the exclusive acylation of 2-amino group in (**12**) occurred: for example, the N^2-isobutyryl intermediate (**13**) was isolated in 97% yield. The structure of (**13**) was confirmed by NMR data and its deamination to N^2-isobutylryl-2′-*O*-methylguanosine (**14**) by $NaNO_2/CH_3COOH$ followed by desilylation with TEA·3HF. We also observed that during deamination with $NaNO_2/CH_3COOH$ the 3′,5′-*O*-cyclic silyl group partially opened, presumably at the 5′ end. Therefore, this intermediate was not isolated but desilylated directly with TEA·3HF. It is worth noting that the N^2-isobutyryl group was completely stable during deamination and that the presence of the hydrophobic silyl group in intermediate allowed for easy purification by extraction from an excess of inorganic salts. This allowed subsequent desilylation without isolation in direct preparation of (**14**) from (**13**).

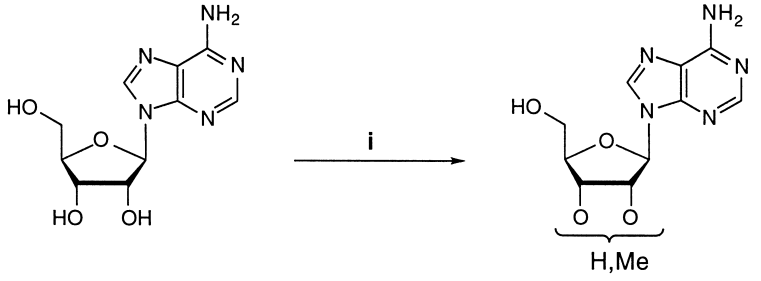
The high yield on all steps of transformation from (**12**) to (**14**) prompted us to combine all reactions into ‘one pot’ procedure without isolation of intermediate (**13**). Selective acylation of (**12**) with isobutyryl chloride followed by deamination with $NaNO_2/CH_3COOH$ and desilylation with TEA·3HF resulted in the preparation of N^2-isobutyryl-2′-*O*-methylguanosine (**14**) in 88% yield.

We also applied this procedure to the synthesis of N^2-isopropylphenoxyacetyl-2′-*O*-methylguanosine on 50 g scale. The N^2-isopropylphenoxyacetyl protecting group was sufficiently stable for acidic diazotization conditions, although minor loss of this group was observed resulting in 83% overall yield from (**12**). To the best of our knowledge this is the most efficient synthesis of N^2-acyl derivatives of 2′-*O*-Me-Guanosine (**13**) and (**14**) (70% overall yield from 2,6-diaminopurine riboside).

Synthesis of 2′-*O*-Me-guanosine and 2′-*O*-Me-adenosine via metal-directed methylation (Scheme 4)

It was demonstrated that metal acetylacetonates direct the methylation of ribonucleosides with trimethylsulfonium hydroxide mostly to 2′ and 3′ hydroxyls in the case of uridine, cytidine and adenosine.[28] Application of this procedure to guanosine resulted in the isolation of six compounds including products with methylation in both the base and ribose moieties. When N^1-methyl-guanosine was subjected to the same methylation conditions 1,2 and 1,3′ di-N,O methyl guanosines were isolated in 82% yield.[28]

We decided to investigate metal-directed methylation of N^1-protected guanosine with trimethylsulfonium hydroxide



Scheme 5. Metal directed methylation of Adenosine: (i) Metal acetylacetonate, Me_3SOH, 80°C, 2h.

to optimize the 2′:3′ ratio of methylated products with subsequent separation and deblocking in order to obtain 2′-*O*-Me guanosine.

The protection of N^1 in guanosine was achieved using benzylation with *N*,*N*-dimethylformamide dibenzyl acetal. We observed, however, that the complete cleavage of 2′,3′-orthoamide required more drastic condition than reported (2 N NaOH vs MeOH/NH_3)[46]. The desired compound (**16**) was isolated in 80% yield after crystallization.

We investigated several metal acetylacetonates in the methylation reaction. Whereas Cu^{2+} acetylacetonate mediated methylation provided a 1:1 ratio of 2′- and 3′-*O*-methylated products, Mg^{2+} and Ag^{+} directed methylation improved the ratio to 9:1. With Ag^{+} the overall conversion was higher than with Mg^{2+}, resulting in a 70% isolated yield of 2′-*O*-Me-N^1-Bzl-guanosine. The separation of 2′-*O*-and 3′-*O*-Me-N^1-Bzl-guanosine derivative was achieved on a preparative scale using a Waters Delta-Pak ODS 50×300 mm HPLC column. Removal of N^1-Bzl protection with Na^{+} naphthalene provided 2′-*O*-Me-guanosine in 90% yield.

Encouraged by results obtained with N^1-Bzl-guanosine, we investigated several different acetylacetonate in direct methylation of adenosine (Scheme 5).

Whereas Fe and Cu acetylacetonates provided 1:1 and 2:1 ration of 2′- and 3′-*O*-Me isomers, we discovered that Ag^{+} and St^{2+} both shifted the equilibrium toward 2′-*O*-Me isomer, providing 4:1 and 8:1 ratio, respectively. This allows isolation of 2′-*O*-Me adenosine in 75–80% yield. It is also helpful for the process scale-up that St^{2+} acethylacetonate is eight times cheaper than Ag^{+} derivative.

Conclusion

In this communication we disclose several improved synthetic methods toward 2′-*O*-methyl adenosine (guanosine) and their *N*-acyl derivatives. We demostrated that: (1) transglycosylation of N^4-acetyl-5′,3′-di-*O*-acetyl-2′-*O*-methyl cytidine with N^6-Bz-adenine provided N^6-benzoyl-5′,3′-di-*O*-acetyl-2′-*O*-methyl adenosine in 50% yield. (2) Regioselective methylation of 2-amino-6-chloropurine riboside with MeI/NaH followed by hydrolysis resulted in 2′-*O*-Me-guanosine in high yield. The same 2′-*O*-Me-precursor can be transformed into 2′-*O*-Me-adenosine in 58% yield. (3) Very efficient transformation of 2,6-diamino-purine riboside into N^2-isobutyryl (isopropylphenoxyacetyl) 2′-*O*-Me-guanosine can be achieved through methylation of 5′,3′-*O*-TIPDSi derivative followed by selective N^2-acylation, deamination and desilylation providing target compounds in 70% combined yield. (4) Mg^{2+} and Ag^{+} directed methylation of N^1-Bzl-guanosine with trimethylsulfonium hydroxide proceeded in >80% yield with ratio of 2′-*O*-Me/3′-*O*-Me=9:1. The same methylation of adenosine with Ag^{+} and Sr^{2} acetylacetonates provided 2′-*O*-Me-adenosine in 75–80% yield.

Experimental

NMR spectra were recorded on a Varian Gemini 400 spectrometer operating at 400.075 MHz for proton and 161.947 MHz for phosphorus. Chemical shifts in ppm refer to TMS and H_3PO_4, respectively. Analytical thin-layer chromatography (TLC) was performed with Whatman MK6F silica gel 60 Å F_{254} plates and column chromatography using Merck 0.040–0.063 mm Silica gel 60.

***N*⁴-Acetyl-2′-*O*-methyl cytidine (1).** To an oven baked stainless steel bomb (300 mL), equipped with magnetic stirrer and purged with argon, 50 mL anhydrous methanol was added followed by the addition of commercially available 1 g 2,2′-Anhydro-1-(β-D-arabinofuranosyl)cytosine-acetate (Aldrich) (3.5 mmol). To the resulting slurry, 8 mL trimethylborate (70 mmol) was added in the presence of boron trifluoride-methanol (50%) (1.5 mL, 8.84 mmol). The bomb was sealed and then heated in an oil bath at 130°C for 38–48 h. Upon cooling, the resulting clear, slightly colored reaction mixture was evaporated in vacuo to afford an off white foam. After drying in vacuo, the crude foam was dissolved in anhydrous DMF (50 mL) in the presence of acetic anhydride (0.36 mL, 3.85 mmol) which was added drop-wise to the reaction mixture. The resulting clear, light yellow solution was stirred overnight at room temperature. The reaction mixture was evaporated in vacuo. Crystallization of the crude product from methanol–ethyacetate gave a pure compound **1** (0.94 g, 90%). Mp 220–223°C ^{1}H NMR DMSO-d_6: δ 10.89 (exch. s, 1H NH), 8.46 (d, $J_{6,5}$=7.4 Hz, 1H, H6), 7.18 (d, $J_{5,6}$=7.4 Hz, 1H, H5), 5.83 (d, $J_{1',2'}$=2.5 Hz, 1H, H1′), 5.18 (exch. t, $J_{OH,5'}$=4.6 Hz, $J_{OH,5''}$=4.9 Hz, 1H, 5′OH), 5.08 (exch. d, $J_{OH,3'}$=6.7 Hz, 1H, 3′OH), 4.04 (t, $J_{3',2'}$=4.9 Hz, $J_{3',4'}$=6.8 Hz, 1H, H3′), 3.88 (m, 1H, H4′), 3.75 (dd, $J_{5',4'}$=2.3 Hz, $J_{5',5''}$=12.2 Hz, 1H, H5′), 3.59 (dd, $J_{5'',4'}$=2.5 Hz, $J_{5'',5'}$=12.2 Hz, 1H, H5″), 3.45 (s, 3H, OCH_3), 2.10 (s, 3H, CH_3).

***N*⁶-Benzoyl-5′,3′-di-*O*-acetyl-2′-*O*-methyl adenosine (3).** To a solution of N^4-acetyl-2′-*O*-methyl cytidine(**1**) (1.87 g, 6.25 mmol) stirring at room temperature under argon in DMF–pyridine (20 mL, 20 mL) was added acetic anhydride (1.76 mL, 18.75 mmol). The reaction mixture was stirred at room temperature for 1 h then quenched with EtOH (2 mL). The reaction mixture was evaporated to dryness in vacuo and partitioned between dichloromethane (50 mL) and saturated $NaHCO_3$ (20 mL). The aqueous layer was extracted with additional dichloromethane (50 mL) and the combined organics dried over Na_2SO_4. The filtrate was evaporated in vacuo to afford a white foam.

A stirred solution of N^6-benzoylaminopurine (Lancaster) (1.23 g, 5.16 mmol) in anhydrous acetonitrile (50 mL) under an argon atmosphere was treated with BSA (3.82 mL, 15.48 mmol) at reflux for 3 h. Upon cooling, a solution of N^4-acetyl-5′,3′-di-*O*-acetyl-2′-*O*-methyl cytidine (**2**) (see above) (0.66 g, 1.72 mmol) in 20 mL anhydrous acetonitrile was added to the reaction mixture followed by TMStriflate (1.03 mL, 5.16 mmol). The reaction mixture was then heated to 75°C for 16 h under positive pressure of argon. Upon cooling, an additional 1.03 mL (5.1 mmol) of TMStriflate was added, and the reaction

mixture heated to 75°C for an additional 20 h. Once cool, the reaction mixture was diluted with two volumes of dichloromethane and washed with saturated $NaHCO_3$. The organic layer was then dried over Na_2SO_4 and evaporated in vacuo. Flash chromatography employing a gradient of 10–80% ethyl acetate–hexanes afforded (**3**) as a white foam; 0.403 g, 50% yield. Anal. Calcd for $C_{22}H_{23}N_5O_7$ (469.45): C, 56.29; H, 4.94; N, 14.92. Found: C, 56.10; H, 5.15; N, 14.69; ^{1}H NMR ($CDCl_3$): δ 8.88 (br s, 1H, NH), 8.81 (s, 1H, H8), 8.31 (s, 1H, H2), 8.11–7.53 (m, 5H, benzoyl), 6.18 (d, $J_{1',2'}$=4.8 Hz, 1H, H1′), 5.41 (t, $J_{3',2'}$= 4.8 Hz, $J_{3',4'}$=4.8 Hz, 1H, H3′), 4.75 (t, $J_{2',1'}$=4.8 Hz, $J_{2',3'}$=4.8 Hz, 1H, H2′), 4.50–4.34 (m, 3H, H4′, H5′, H5″), 3.44 (s, 3H, OCH_3), 2.19 (s, 3H, OAc), 2.14 (s, 3H, OAc).

2-Amino-6-chloro-9-[2′-*O*-methyl-β-D-ribofuranosyl] purine (5). Sodium hydride (0.44 g, 18.2 mmol) was added to the cooled (−20°C) solution of 2-amino-6-chloropurine riboside[4] **4** (5 g, 16.6 mmol) in dry dimethylformamide (100 mL) under stirring. After 1 h the solution of CH_3I (1.24 mL, 19.9 mmol) in dry dichloromethane (10 mL) was added dropwise to the reaction mixture during 1 h. The resulting yellow solution was stirred at −20°C for additional 2 h until TLC (methylene chloride–methanol 9:1) showed complete dissappearance of the starting material. Reaction mixture was quenched with methanol (20 mL) warmed to the room temperature and evaporated to dryness in vacuo. The residue was dissolved in water (200 mL) and extracted with methylene chloride (3×200 mL), organic layer was back extracted with water (100 mL). Combined aqueous phase was evaporated to dryness and the residue was purified by flash chromatography on silica using gradient of MeOH (7–10%) in methylene chloride to give 3.4 g (65%) of the compound **5**. Flash chromatography purification can be substituted by several crystallization from acetonitrile, mp 216–217°C Anal. Calcd for $C_{11}H_{14}N_5O_4Cl$ (331.10): C, 41.85; H, 4.47; N, 20.27. Found: C, 41.63; H, 4.35; N, 20.19. ^{1}H NMR (DMSO-d_6 δ 3.32 (3H, s, 2′-OMe); 3.58 (1H, dd, 5′-H, $J_{4',5'}$=4.0 Hz, $J_{5',5''}$=12.0 Hz); 3.66 (1H, dd, 5″-H, $J_{4',5''}$=4.0 Hz, $J_{5',5''}$=12.0 Hz); 3.95 (1H, dd, 4′-H, $J_{3',4'}$=3.6 Hz); 4.52 (1H, t, 2′-H, $J_{2',3'}$=4.0 Hz); 4.31 (1H, m, 3′-H); 5.07 (1H, bs, 3′-OH exchangeable); 5.24 (1H, bs, 5′-OH, exchangeable); 5.91 (1H, d, 1′-H, $J_{1',2'}$=6.0 Hz); 6.96 (2H, bs, 2-NH_2); 8.3 (1H, s, 8-H).

2′-*O*-Methyl guanosine (9). A mixture of **5** (2.05 g, 6.5 mmol), 1,4-diazabicyclo[2.2.2]octane (1 equiv.) and water (30 mL) was heated to 90°C for 45 min. The solution was then cooled to ambient temperature, adjusted to pH 12 with 2 M NaOH, and washed with methylene chloride (3×60 mL). The aqueous phase was acidified to pH 6 with 6 M HCl and left at refrigerator (0°C) overnight. Precipitate was filtered off, the mother liquor was evaporated to dryness and residue was dissolved is water and applied on the short column with RP-18 silica gel. Solid phase was washed with water and remaining product was eluted with 5% aqueous methanol. Appropriate fractions were combined, evaporated to dryness and recrystallize from water to provide 1.25 g (65%) of 2′-*O*-methyl guanosine **9**. Analytical sample was recrystallized from methanol mp 234–236°C. Lit.[21] mp 235–237°C (methanol). Anal. Calcd for $C_{11}H_{15}N_5O_5$ (297.27): C. 44.44; H, 5.09; N 23.56. Found: C, 44.34; H, 5.03; N, 23.36. The product was identical to authentic sample by HPLC, UV, ^{1}H NMR spectroscopy.

6-Chloro-9-[3′,5′-di-*O*-acetyl-2′-*O*-methyl-β-D-ribofuranosyl] purine (7). To the solution of compound **5** (1.25 g, 3.96 mmol), 4-dimethylaminopyridine (39 mg, 0.32 mmol) and triethylamine (0.37 mL, 2.64 mmol) in dry acetonitrile was added acetic anhydride (0.9 mL, 9.5 mmol) and the reaction mixture was left at room temperature for 40 min. It was then quenched with MeOH (10 mL) and evaporated to dryness. The residue was dissolved in methylene chloride and washed with 1% aqueous acetic acid, saturated aqueous sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated to dryness yielding diacetate **6**. The residue was additionally dried in vacuo for 3 h, dissolved in dry THF and degassed with dry argon. To the above boiling solution under positive pressure of argon, isoamylnitrite (10 equiv.) was added dropwise. After 2 h the solvent was removed in vacuo and the residue was dissolved in methylene chloride, washed with saturated aqueous $NaHCO_3$ and brine. After evaporation of organic phase the residue was purified by flash chromatography on silica gel. Elution with hexanes–ethyl acetate (1:1) mixture provided 1.1 g (72%) of compound **7** as yellow oil. Anal. Calcd for $C_{15}H_{17}N_4O_6Cl$ (384.78): C, 46.82; H, 4.45; N, 14.56. Found: C, 46.76; H, 4,56; N, 14.43. ^{1}H NMR ($CDCl_3$): δ d 2.13 (3H, s, 3′-OAc or 5′-OAc); 2.18 (3H, s, 3′-OAc or 5′-OAc); 3.44 (3H, s, 2′-OCH_3); 4.43 (3H, m, 4′-H, 5′-CH_2); 4.69 (1H, 2′-H, $J_{2',3'}$=5.04 Hz); 5.36 (1H, t, 3′-H, $J_{3',4'}$=4.28 Hz); 6.13 (1H, d, 1′-H, $J_{1',2'}$=4.88 Hz); 8.31 (1H, s, 8-H); 8.77 (1H, s, 2-H).

2′-*O*-Methyl adenosine (8). Solution of compound **7** (0.45 g, 1.17 mmol) in saturated methanolic ammonia (20 mL) was autoclaved at 125°C for 4 h. The solvent was removed in vacuo and the remaining residue was purified by flash chromatography on silica gel. Elution with methylene chloride–methanol (9:1) mixture provided 0.25 g (80%) of 2′-*O*-methyl adenosine **8** as a white solid. The analytical sample was recrystallized from abs. EtOH. mp 203–204°C. Lit.[20] mp 202–203.5°C (EtOH). Anal. Calcd for $C_{11}H_{15}N_5O_4$ (384.78): C, 47.00; H, 5.30; N, 24.90. Found: C, 47.03; H, 5.28; N, 24.85. The product was identical to authentic sample by HPLC, UV, ^{1}H NMR-spectroscopy.

2,6-Diamino-9-[3′,5′-*O*-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]purine (11). To an oven dried 500 mL three neck round bottom flask equipped with mechanical stirrer, positive pressure argon and rubber septum was added 2,6-diamino-9-(β-D-ribofuranosyl)-purine[21] (**10**) (10.0 g, 35.4 mmol), anhydrous DMF (100 mL), and anhydrous pyridine (200 mL). The resulting light brown suspension was cooled to 0°C in an ice–water bath while stirring. $TIPSDSiCl_2$ (42.48 mmol, 13.6 mL) was added dropwise to the stirred reaction mixture via syringe over a 20 min period, mantaining temperature at 0°C. The reaction mixture was then warmed to rt, resulting in a homogenious solution. TLC indicated complete reaction after 3 h at room temperature, at which time the reaction was quenched by addition of ethanol (20 mL). The reaction mixture was then evaporated in vacuo and the resulting residue partitioned between ethyl acetate and saturated

aqueous $NaHCO_3$ at which time (**11**) precipitated from the organics layer. The aqueous layer was then extracted back with ethyl acetate and the combined orgainics cooled to 0°C. The precipitate was filtered and washed with ethyl acetate to afford (**11**) as beige solid; 16.5 g, 89% yield. The analytical sample was recrystallized from ethanol–water (2:1). Mp 174–176°C. Anal. Calcd for $H_{22}H_{40}N_6O_5Si_2$ (524.77): C, 50.35; H, 7.68; N, 16.01. Found: C, 49.94; H, 7.79; N, 15.63; ^{1}H NMR (DMSO-d$_6$): δ 7.77 (s, 1H, H8), 6.75 (s, exch., 2H, N^6-NH_2), 5.74 (s, exch., 2H, N^2-NH_2), 5.71 (s, 1H, H1′), 5.56 (d, $J_{OH,2'}$=5.0 Hz, 1H, 2′-OH), 4.43 (dd, $J_{3',2'}$=4.5 Hz, $J_{3',4'}$=7.8 Hz, 1H, H3′) 4.29 (m, $J_{2',OH}$= 5.0 Hz), 4.06–3.08 (m, 3H, H4′, H5′, H5″), 1.04 (m, 28H, TIPDSi).

2,6-Diamino-9-[3′,5′-*O*-tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-methyl-β-D-ribofuranosyl]purine (12). To an oven baked 500 mL three neck round bottom flask equipped with mechanical stirrer and positive pressure argon was added (**11**) (15.6 g, 29.7 mmol) followed by anhydrous DMF (300 mL) and methyl iodide (5.55 mL, 89.2 mmol). The reaction mixture was cooled to 0°C in an ice–water bath and 60% sodium hydride in oil (1.78 g, 4.46 mmol) added slowly. A temperature of 0°C was maintained for 35 min at which time the reaction was quenched with anhydrous ethanol and diluted into two volumes of cold dichloromethane. The dilute reaction mixture was washed two times with saturated NH_4Cl, the aqueous layer was extracted with dicloromethane, and the combined organics dried over Na_2SO_4, filtered and evaporated to dryness in vacuo. Crystallization from ethanol–water 1:1 afforded 14.7 g of (**12**), 92% yield, mp 156–158°C. Anal. Calcd for $C_{23}H_{42}N_6O_5Si_2$ (538.80): C, 51.27; H, 7.86; N, 15.60. Found: C, 51.19; H, 7.86; N, 15.39; ^{1}H NMR ($CHCl_3$-d$_3$): δ 7.75 (s, 1H, H8), 6.76 (s, exch., 2H, N^6-NH_2), 5.78 (s, 1H, H1′), 5.73 (s, exch., 2H, N^2-NH_2) 4.58 (dd, $J_{3',2'}$=4.8 Hz, $J_{3',4'}$=4.8 Hz, 1H, H3′), 4.12 (d, $J_{2',3'}$=4.8 Hz, 1H, H2′), 4.09–3.91 (m, 3H, H4′, H5′, H5′′), 3.54 (s, 3H, OCH_3), 1.03 (m, 28H, TIPDSi).

2,6-Diamino-N^2-isobutyryl-9-[3′5′-*O*-tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-methyl-β-D-ribofuranosyl]purine (13). A solution of (**12**) (0.5 g, 0.93 mmol) in anhydrous pyridine (20 mL) was cooled to −10°C in an ice–ethanol bath while stirring under argon. Isobutyryl chloride (0.11 mL, 1.02 mmol) was added dropwise to the stirred (−10°C) solution over a period of 5 min. The reaction mixture was stirred at −10°C for 2 h followed by 1 h at room temperature then quenched with ethanol (2 mL). After evaporating the reaction mixture to dryness in vacuo, the resulting residue was partitioned between dichloromethane and saturated aqueous $NaHCO_3$. The aqueous layer was extracted with dichloromethane (50 mL) and the combined organic layer dried over Na_2SO_4. Filtration and evaporation in vacuo afforded beige foam which was purified by flash chromatography using a gradient of 2–4% ethanol in dichloromethane and afforded (**13**) as a white foam; 0.55 g, 97% yield. Anal. Calcd for $C_{27}H_{48}N_6O_6Si_2$ (609.25): C, 53.23; H, 7.94; N, 13.85. Found: C, 53.09; H, 8.01; N, 13.76; ^{1}H NMR (DMSO-d$_6$): δ 9.76 (s, exch., 1H, N^2-NH), 8.04 (s, 1H, H8), 7.20 (s, exch., 2H, N^6-NH_2) 5.88 (s, 1H, H1′), 4.71 (dd, $J_{3',2'}$=5.2 Hz, $J_{3',4'}$=5.2 Hz, 1H, H3′), 4.26 (d, $J_{2',3'}$=5.2 Hz, 1H, H2′), 4.15–3.91 (m, 3H, H4′, H5′, H5″), 3.55 (s, 3H, OCH_3), 2.87 (m, 1H, iBu-CH), 1.06–0.96 (m, 34H, TIPDSi, iBu-$(CH_3)_2$).

N^2-Isobutylryl-2′-*O*-methylguanosine (14). A solution of (**12**) (5.0 g, 9.28 mmol) in anhydrous pyridine (100 mL) was cooled to −10°C in an ice–ethanol bath while stirred under argon. Isobutyryl chloride (10.21 mmol, 1.07 mL) was added dropwise to the stirred −10°C solution over a period of 30 min. The reaction mixture was stirred at −10°C for 2 h followed by 1 h at room temperature then quenched with ethanol (20 mL). After evaporating the reaction mixture to dryness in vacuo, the resulting residue was partitioned between dichloromethane and saturated $NaHCO_3$. The aqueous layer was extracted back with dichloromethane and the combined organics dried over Na_2SO_4. Filtration and evaporation of the filtrate in vacuo afforded a beige foam which was dissolved in glacial acetic acid (80 mL). To the stirred acetic acid solution was added water (40 mL) followed by $NaNO_2$ (74.24 mmol, 5.12 g). After 30 min another portion of $NaNO_2$ (5.12 g, 74.24 mmol) was added and the reaction stirred at room temperature for 48 h. The reaction mixture was diluted with one volume of *n*-butanol and evaporated in vacuo to 50% of the original volume. Co-evaporation with *n*-butanol (3×50 mL) was followed by partitioning the crude syrup between ethyl acetate and saturated aqueous $NaHCO_3$. After extracting back the aqueous layer with ethyl acetate, the combined organics were evaporated to dryness in vacuo. The crude residue was then dissolved in anhydrous dichloromethane (50 mL) and treated with a solution of TEA·3HF (27.84 mmol, 4.54 mL) and TEA (8.17 mL), in dichloromethane (20 mL). The reaction mixture was evaporated to dryness in vacuo and subsequently dissolved in additional dichloromethane (20 mL). Evaporation followed by dilution was repeated three times, and the crude product purified by flash chromatography. A gradient of 2–10% ethanol in dichloromethane afforded (**14**) as light yellow foam; 3.02 g, 88% yield. Anal. Calcd for $C_{15}H_{21}N_5O_6$ (367.66): C, 49.00; H, 5.77; N, 19.13. Found C, 49.05; H, 5,83; N, 19.20. ^{1}H NMR (DMSO-d$_6$): δ 12.08 (s, exch., 1H, NH), 11.63 (s, exch., 1H, NH), 8.29 (s, 1H, H8), 5.90 (d, $J_{1'2'}$=6.3 Hz, 1H, H1′), 5.23 (d, $J_{OH,3'}$=4.9 Hz, 1H, 3′-OH), 5.07 (t, $J_{OH,5''}$=5.3 Hz, $J_{OH,5''}$=5.3 Hz, 1H, 5-OH), 4.30 (m, $J_{3',2'}$=4.8 Hz, $J_{3'4'}$=3.3 Hz, 1H, H3′), 4.22 (t, $J_{2',1'}$=6.3 Hz, $J_{2',3'}$=4.8 Hz, 1H, H2′), 3.93 (m, $J_{4',3'}$=3.3 Hz, $J_{4',5'}$= 3.9 Hz, $J_{4',5''}$=3.9 Hz, 1H, H4′) 3.65–3.53 (m, 2H, H5′, H5″), 3.33 (s, 3H, OCH_3), 2.78 (m, 1H, iBu-CH), 1.12 (d, 6H, iBu-$(CH_3)_2$).

N^2-Isopropylphenoxyacetyl-2′-*O*-methylguanosine (15). A solution of (**12**) (47.4 g, 88 mmol) in anhydrous pyridine (500 mL) was cooled to −10°C in an ice–ethanol bath while stirred under argon. Isopropylphenoxyacetyl chloride (20.6 mL, 96.8 mmol) was added dropwise to the stirred −10°C solution over a period of 5 min. The reaction mixture was stirred at −10°C for 2 h followed by 1 h at room temperature then quenched with ethanol (20 mL). After evaporating the reaction mixture to dryness in vacuo, the resulting residue was partitioned between dichloromethane and saturated aqueous $NaHCO_3$. The aqueous layer was extracted back with dichloromethane and the combined organics dried over Na_2SO_4. Filtration

and evaporation of the filtrate in vacuo afforded a beige foam which was dissolved in glacial acetic acid (1000 mL). To the stirred acetic acid solution was added water (400 mL) followed by $NaNO_2$ (742.4 mmol, 51.2 g). Another portion of $NaNO_2$ (742.4 mmol, 51.2 g) was added after 30 min and the reaction stirred at room temperature for 48 h. The reaction mixture was diluted with one volume of *n*-butanol and evaporated in vacuo to 50% of the original volume. Co-evaporation with *n*-butanol (3×) was followed by partitioning the crude syrup between ethyl acetate and saturated aqueous $NaHCO_3$. After extracting back the aqueous layer with ethyl acetate, the combined organics were evaporated to dryness in vacuo. The crude residue was then dissolved in anhydrous dichloromethane (500 mL) and treated with a solution of TEA·3HF (278.4 mmol, 45.4 mL) and TEA (81.7 mL), in dichloromethane (200 mL). The reaction mixture was evaporated to dryness in vacuo and subsequently dissolved in additional dichloromethane (200 mL). Evaporation followed by dilution was repeated 3 times, and the crude product purified by flash chromatography. A gradient of 2–10% ethanol in dichloromethane afforded (**5**) as light yellow foam; 29.2 g, 85% yield. Anal. Calcd for $C_{22}H_{27}N_5O_7$ (473.79): C, 55.84; H, 5.74; N, 14.84. Found C, 55.57; H, 5.78; N, 14.72. ^{1}H NMR (DMSO-d_6): δ 11.65 (s, exch., 2H, NH, NH), 8.30 (s, 1H, H8), 7.18–6.88 (dd, 4H, phenoxy), 5.91 (d, $J_{1'2'}$=6.0 Hz, 1H, H1′), 5.24 (d, $J_{OH,3'}$=4.8 Hz, 1H, 3′-OH), 5.09 (t, $J_{OH,5'}$=5.6 Hz, $J_{OH,5''}$=5.2 Hz, 1H, 5′-OH), 4.82 (s, 2H, CH_2), 4.31 (m, $J_{3',2'}$=4.8 Hz, $J_{3'4'}$=3.6 Hz, 1H, H3′), 4.23 (t, $J_{2',1'}$=6.0 Hz, $J_{2',3'}$=4.8 Hz, 1H, H2′), 3.93 (m, $J_{4',3'}$=3.6 Hz, $J_{4',5'}$=4.0 Hz, $J_{4',5''}$=3.9 Hz, 1H, H4′) 3.65–3.53 (m, 2H, H5′, H5″), 3.35 (s, 3H, OCH_3), 2.84 (m, 1H, *i*Pr-CH), 1.17 (d, 6H, *i*Pr-$(CH_3)_2$).

***N*^{1}-Benzyl guanosine (16).** Guanosine hydrate (50 g, 177 mmol) was co-evaporated with dimethylformamide (2×250 mL) and dissolved in dry DMF (400 mL). *N*,*N*-dimethylformamide dibenzyl acetal was added (230 mL, 885 mmol) and the solution was heated with stirring to 80°C for 18 h. The excess of acetal was removed by steam distillation on a rotary evaporator. The product was recovered without chromatography by washing with dichloromethane–hexanes (1:1 v/v) to yield 8 g of the *ortho*-amide intermediate. The *ortho*-amide was cleaved by treatment with 2 N NaOH (133 mL) at room temperature for 4 h. The product was recrystallized from boiling water to yield 54 g, 82% yield of pure (**16**). Mp 148–150°C (Lit.[46] mp 149–150°C). Anal. Calcd for $C_{17}H_{19}N_5O_5$ (373.37): C, 54.69; H, 5.13; N, 21.43; Found: C, 54.75 H, 5.07; N, 21.29. ^{1}H NMR (DMSO-d_6): δ 7.97 (s, 1H, H8), 7.29 (m, 5H, Bz), 7.02 (bs, 2H, $2NH_2$), 5.70 (d, $J_{1'2'}$=5.6 Hz, 1H, H1′), 5.42 (bs, 1H, 2′OH) 5.23 (s, 1H, CH_2-Bz), 5.15 (bs, 1H, 3′OH), 5.00 (bs, 1H, 5′-OH), 4.41 (t, $J_{3',2'}$=5.6 Hz, $J_{3'4'}$=4.0 Hz, 1H, H3′), 4.08 (t, $J_{2',1'}$=6.3 Hz, $J_{2',3'}$= 4.8 Hz, 1H, H2′), 3.85 (m, $J_{4',3'}$=3.5 Hz, $J_{4',5'}$=4.0 Hz, 1H, H4′) 3.58–3.49 (m, 2H, H5′, H5″).

***N*^{1}-Benzyl-2′-*O*-methyl guanosine (17).** To suspension of **16** (50 g, 134 mmol) and silver acetylacetonate (41 g 200 mmol) in dimethylformamide (400 mL) TMSH (200 mL of 1 N solution in methanol) was added and reaction mixture was heated to 70°C for 2 h. The solution was cooled to ambient temperature, neutralized to pH 7 with 1 M HCl, and dried to a solid tar. The tar was dissolved in water (500 mL) and filtered through a sintered glass funnel to remove silver salts. The filtrate was evaporated to dryness and a portion [10 g (26 mmol)] was purified on a Waters Delta-Pak ODS 50×300 mm HPLC column in water. The N^1-Bz1-2′-*O*-Me guanosine(**17**) eluted first (7 g, 70%). Anal. Calcd for $C_{18}H_{21}N_5O_5$ (387.40): C, 55.81; H, 5.46 5; N, 18.08. Found C, 55.67; H, 5.61; N, 17.96. ^{1}H NMR (DMSO-d_6): δ 8.02 (s, 1H, H8), 7.26 (m, 5H, Ph), 7.02 (bs, 2H, $2NH_2$), 5.82 (d, $J_{1'2'}$=6.4 Hz, 1H, H1′), 5.23 (s, 2H, CH_2-Bzl), 5.18 (d, $J_{OH,3'}$=4.8 Hz, 1H, 3′-OH), 5.04 (t, $J_{OH,5'}$=5.2 Hz, $J_{OH,5''}$=5.6 Hz, 1H, 5′-OH), 4.28 (m, $J_{3',2'}$= 4.8 Hz, $J_{3'4'}$=3.2 Hz, 1H, H3′), 4.20 (t, $J_{2',1'}$=6.4 Hz, $J_{2',3'}$=4.8 Hz, 1H, H2′), 3.90 (m, $J_{4',3'}$=3.6 Hz, $J_{4',5'}$= 4.0 Hz, 1H, H4′), 3.65–3.53 (m, 2H, $J_{OH,5'}$=5.2 Hz, $J_{5',5'}$=11.6 Hz, H5′, H5″), 3.33 (s, 3H, OCH_3).

2′-*O*-Methyl guanosine (9). The 0.6 M sodium naphthalene solution was prepared from sodium (3.5 g, 0.152 M) and naphthalene (21.2 g, 0.16 M) in dry THF (210 mL). This solution (50 mmol, 90 mL) was added to N^1-Bzl-2′-*O*-methyl guanosine (**17**) (2 g, 5.0 mm) and reaction mixture was stirred overnight. The reaction was quenched with 10 mL of methanol and all solvent were removed in vacuo. Water (100 mL) was added and resulted solution was neutralized by 1 N HCl to pH 7. Naphthalene was removed by extraction with toluene (3×100 mL) and aqueous solution was purified on ODS Delta Pak column to recover 2′-*O*-Me guanosine nucleoside (**9**). The product (1.3 g, 4.5 mmol, 90%) was identical to an authentic sample by HPLC, UV–Vis and ^{1}H NMR spectroscopy.

Trimethylsulfonium hydroxide (TMSH) solution in methanol. A 0.2 M solution of trimethyl sulfonium iodide (TMSI, 102 g, 0.5 mol), in 2.5 L of methanol and water (9:1 v/v) was prepared by heating to 40°C and mixing continuously for 30 min. The clear, colorless solution was allowed to cool to room temperature. A glass chromatography column (Ace #50 thread; 75×300 mm) containing Duolite 147 anion exchange resin (900 g) in the hydroxide form was previously packed and used for the conversion of TMSI to TMSH. The solution was pumped over the column using a gear pump at 100 mL per minute. The resin was washed with an additional 1 L of 90% methanol and the total 3.5 L was reduced in volume to 500 mL using a rotary evaporator with the bath set at 20°C. The solution was checked for the presence of iodide ion using acidified silver nitrate solution and found to be negative. The solution was not characterized further and was stored in a teflon bottle with a gas vent at 5°C.

2′-*O*-Methyl adenosine (8). To a solution of adenosine (50 g, 187 mmol) in dimethylformamide (400 mL) silver acetylacetonate (58 g, 28 mmol) and TMSH (280 mL of a 1 M) solution was added and the mixture was heated to 75°C with stirring for 45 min. A sample of the mixture showed no starting adenosine and two spots of which the predominant one comigrated with an authentic sample of 2′-OMe adenosine. An HPLC assay showed a 9:1 ratio for 2′ to 3′-OMe adenosine. The solvent was removed in vacuo. Residue dissolved in 500 mL of water and neutralized by addition 250 mL of 1 N HCl to pH 7. The mixture was purified on ODS 25×300 mm Delta Pak HPLC column using water as

the eluant. Overall recovery was 42 g of (**8**) for a 75% yield from adenosine. The product was identical to a authentic sample by HPLC, UV, ^{1}H NMR spectroscopy.

References

1. Hall, R. H. *The Modified Nucleosides in Nucleic Acids*, Columbia University Press: New York, 1971.
2. Lamond, A. I.; Sproat, B. S.; Ryder, U. *Cell* **1989**, *58*, 383–390.
3. Blencowe, B. J.; Sproat, B. S.; Ryder, U.; Barbino, S.; Lamond, A. I. *Cell* **1989**, *59*, 531–539.
4. Beigelman, L.; McSwiggen, J. A.; Draper, K. G.; Gonzalez, C.; Jensen, K.; Karpeisky, A. M.; Modak, A. S.; Matulic-Adamic, J.; DiRenzo, A. B.; Haeberli, P.; Sweedler, D.; Tracz, D.; Grimm, S.; Wincott, F. E.; Thackray, V. G.; Usman, N. *J. Biol. Chem.* **1995**, *270*, 25702–25708.
5. Altman, K.-H.; Dean, N. M.; Fabbro, D.; Frier, S. M.; Geiger, T.; Haner, R.; Husken, D.; Martin, P.; Monia, B. P.; Muler, M.; Natt, F.; Nicklin, P.; Phillips, J.; Pieles, U.; Sasmor, H.; Moser, H. H. *Chimia* **1995**, *50*, 168–176.
6. Haines, A. H. *Tetrahedron* **1973**, *29*, 2807–2810.
7. Chavis, C.; Dumont, F.; Wightman, R. H.; Zeigler, J. C.; Imbach, J. L. *J. Org. Chem.* **1982**, *47*, 202–206.
8. Ross, B. S.; Springer, R. H.; Vasquez, G.; Andrews, R. S.; Cook, P. D.; Acevedo, O. L. *J. Heterocycl. Chem.* **1994**, *31*, 765–769.
9. Parmentier, G.; Schmitt, G.; Dolle, F.; Luu, B. *Tetrahedron* **1994**, *50*, 5361–5368.
10. Beigelman, L.; Karpeisky, A.; Matulic-Adamic, J.; Usman, N. *Nucleosides & Nucleotides* **1995**, *14*, 421–424.
11. Broom, A. D.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, *87*, 1145–1151.
12. Khwaija, T. A.; Robins, R. K. *J. Am. Chem. Soc.* **1966**, *88*, 3640–3643.
13. Martin, D. M. G.; Reese, C. B.; Stephenson, G. F. *Biochemistry* **1968**, *7*, 1406–1412.
14. Gin, J. B.; Dekker, C. A. *Biochemistry* **1968**, *7*, 1413–1419.
15. Kusmeirek, J. T.; Giziewicz, J.; Shugar, D. *Biochemistry* **1973**, *12*, 194–200.
16. Kusmeirek, J. T.; Darzynkiewicz, E.; Shugar, D. *Biochemistry* **1976**, *15*, 2735–2740.
17. Yano, J.; Kan, L. S.; Ts'O, P. O. P. *Biochim Biophys. Acta* **1980**, *629*, 178–183.
18. Wagner, E.; Oberhauser, B.; Holzner, A.; Brunar, H.; Issakides, G.; Schaffner, G. F.; Cotten, M.; Knoll Muller, M.; Noe, R. C. *Nucleic Acids Res.* **1987**, *15*, 6131–6148.
19. Hodge, R. P.; Sinha, N. D. *Tetrahedron Lett.* **1995**, *36*, 2933–2936.
20. Robins, M. J.; Naik, S. R.; Lee, A. S. K. *J. Org. Chem.* **1974**, *39*, 1891–1899.
21. Robins, M. J.; Hansske, F.; Bernier, S. E. *Can. J. Chem.* **1981**, *59*, 3360–3364.
22. Heikkila, J.; Bjorkman, S.; Oberg, B.; Chattopadhyaya, J. *Acta Chem. Scand.* **1982**, *B36*, 715–717.
23. Ekborg, G.; Garegg, P. J. *J. of Carbohydrates, Nucleosides & Nucleotides* **1980**, *7*, 57–60.
24. Cramer, H.; Pfleiderer, W. *Helv. Chim. Acta* **1996**, *79*, 2114–2136.
25. Leonard, T. E.; Bhan, P.; Miller, P. S. *Nucleosides & Nucleotides* **1992**, *11*, 1201–1204.
26. Wagner, D.; Verhyden, J. P. H.; Moffat, J. G. *J. Org. Chem.* **1974**, *39*, 24–30.
27. Pathak, T.; Chattopadhyaya, J. *Chem. Scr.* **1986**, *26*, 135–139.
28. Yamauchi, K.; Nakagima, T.; Kinoshita, M. *J. Org. Chem.* **1980**, *45*, 3865–3868.
29. Nyilas, A.; Chattopadhyaya, J. *Acta Chem. Scand.* **1986**, *B40*, 826–830.
30. Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S.; Miura, K.; Ohtsuka, E. *Nucleic Acids Res.* **1987**, *15*, 6131–6148.
31. Vaghefi, M. M.; Hogrefe, R. I. *Nucleosides & Nucleotides* **1993**, *12*, 1007–1013.
32. Sproat, B. S.; Beijer, B.; Iribarren, A. *Nucleic Acids Res.* **1990**, *18*, 41–49.
33. Grotli, M.; Douglas, M.; Beijer, B.; Eritja, R.; Sproat, B. S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 425–428.
34. Sekine, M.; Peshakova, L. S.; Hata, T.; Yokoyama, S.; Miyazawa, Y. *J. Org. Chem.* **1974**, *52*, 5060–5061.
35. Zavgorodny, S.; Polanski, M.; Besidsky, E.; Kriukov, V.; Sanin, A.; Pokrovskaya, M.; Gurskay, G.; Lonnberg, H.; Azhayev, A. *Tetrahedron Lett.* **1991**, *31*, 7593–7596.
36. McGee, D. P. C.; Zhai, Y. *Nucleosides & Nucleotides* **1996**, *15*, 1797–1803.
37. Ross, B. S.; Springer, R. H.; Tortorici, Z.; Dimock, S. *Nucleosides & Nucleotides* **1997**, *16*, 1641–1644.
38. Roy, S.; Tang, J. T. US Patent 5,739,314.
39. Imazawa, M.; Eckstein, F. *J. Org. Chem.* **1979**, *44*, 2039–2041.
40. Azuma, K.; Isono, K. *Chem. Pharm. Bull.* **1977**, *25*, 3347–3353.
41. Linn, J. A.; McLean, Ed. W.; Kelley, J. L. *J. Chem. Soc., Chem. Commun.* **1994**, 1913–1914.
42. Nair, V.; Richardson, S. G. *Synthesis* **1982**, 670–672.
43. Nair, V.; Richardson, S. G. *J. Org. Chem.* **1980**, *45*, 3969–3974.
44. Gerster, J. F.; Jones, J. W.; Robins, R. K. *J. Org. Chem.* **1963**, *28*, 945–948.
45. Davoll, J.; Lowy, B. A. *J. Am. Chem. Soc.* **1951**, *73*, 1650–1655.
46. Philips, K. D.; Horwitz, J. P. *J. Org. Chem.* **1975**, *40*, 1856–1858.