Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 4, pp. 425-428, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00024-3

A Simple Method for the Synthesis of 2'-*O*-Alkylguanosine Derivatives

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Abstract: A new synthetic route has been devised for the preparation of 2'-*O*-alkyl guanosine derivatives. Utilizing a strategy of minimal protection, the alkylation was performed on partly protected guanosine using an alkyl halide and a sterically hindered strong organic base.

2'-*O*-Alkyloligoribonucleotides possess properties that make them interesting for *in vivo* gene expression studies and therapeutic applications. These oligonucleotide analogues exhibit high resistance to degradation by either RNA or DNA specific nucleases and form hybrids of high thermal stability with complementary RNA.[2] 2'-*O*-Allyl and 2'-*O*-methyl modified oligoribonucleotides have been used for studying *in vitro* RNA processing.[2,3] Moreover, multiple 2'-*O*-allylation or methylation of hammerhead ribozymes results in high activity and greatly enhanced nuclease resistance.[4,5]

Considerable effort has been directed towards developing efficient alkylation reactions that yield 2'-*O*-alkylribonucleoside building blocks. Early methods of methylation using diazomethane on unprotected or partially protected nucleosides[6] suffered from low yields and the presence of the undesired 3'-*O*-methyl isomer complicated the purification. The use of sodium hydride and an alkyl iodide on partially protected ribonucleosides enhanced the selectivity for alkylation of the 2'-hydroxyl function.[7] However, the separation of the 2'-*O*-methyl from the 3'-*O*-methyl ribonucleosides required subsequent protection of the exocyclic amino function and 5'-hydroxyl moiety.[7] In an elegant procedure developed by Chanteloup et al.[8] 2'-*O*-alkyl derivatives were prepared by a glycosylation reaction using an activated 2'-*O*-alkyl ribose derivative and a per-silylated base. However, the method is a multistep procedure and result in low overall yield. The procedures originally developed by us for preparing 2'-*O*-methylribonucleoside phosphoramidite monomers require the use of expensive starting materials.[9] The latter route has also been adapted to palladium(0) catalysed allylation[10] and as described recently, modified to give a simple and more cost effective synthesis of 2'-*O*-allylribonucleosides.[11] Lately, Hodge et al.[12] published a simple synthesis of 2'-*O*-alkylribopyrimidines , using partially protected cytidine and silver oxide/alkyl iodide. The 2'-*O*-alkylcytidine

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products could be converted to 2'-*O*-alkyluridines in one step. However, the silver oxide catalysed alkylation procedure cannot be transferred to purine ribosides.[9]

In this paper we describe a simple and efficient procedure for the preparation of 2'-*O*-alkylguanosine derivatives using inexpensive guanosine as starting material. The key feature is the use of a silyl protected intermediate, where the selective alkylation is performed with an alkyl halide in the presence of a sterically hindered strong organic base.

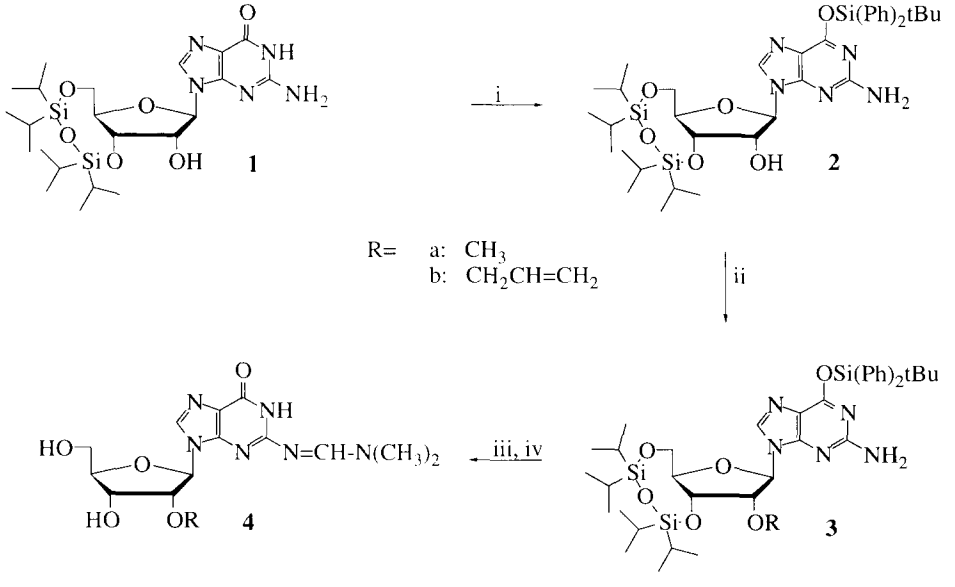

Figure 1 Reaction scheme for the synthesis of 2'-*O*-alkyl guanosine derivatives. Reagents: i, 1.2 eq *tert.*-butyldiphenylchlorosilane, 0.2 eq 4-dimethylaminopyridine, 5 eq triethylamine in dichloromethane; ii, 2.5 eq 2-*tert.*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine , 5 eq of alkyl halide in acetonitrile; iii, 1 eq tetrabutylammonium fluoride in tetrahydrofuran; iv, 5 eq N,N-dimethylformamide dimethyl acetal in methanol.

Protection of the guanine ring is essential to prevent heterocyclic alkylation and so we based our protection of the guanine lactam function on the work of Daskalov et al.[13], who reported O^6-silylation of 2',3',5'-tri-*O*-acetyl-N^2-tritylguanosine using 4-dimethylaminopyridine (DMAP) and *tert.*-butyldiphenylchlorosilane (TBDPSCl). The use of the Markiewicz reagent for the protection of the 5'- and 3'-hydroxyls[14] allows exclusive alkylation of the 2'-hydroxyl group. Further, the disiloxane bridge and the TBDPS-group can be removed simultaneously under mild conditions[14] after the alkylation. Thus, guanosine was first protected with the Markiewicz disiloxane reagent giving compound **1**. This compound was then converted into the highly versatile O^6-TBDPS derivative **2**[15], using TBDPSCl, DMAP and triethylamine in dichloromethane at room temperature. The reaction gave selectively O^6-silylation without any observable 2'-*O*-silylation. The O^6-silylated product was found to be formed essentially quantitatively by monitoring the reaction on tlc. TBDPSCl/ DMAP/triethylamine with pyridine or dimethylformamide as solvent did not produce any silylated products. However, compound **2** was only partly stable to chromatography on silica gel

and was therefore used further without any purification. Other reagents like *tert.*-butoxydiphenylchlorosilane and *tert.*-butyldimethylchlorosilane also yielded O^6-silylated products (data not shown), but were excluded from further use due to their low stability. It is worth noting that the IR spectrum of compound **2** lacked the usual carbonyl band at 1700 cm^{-1} thereby indicating that the lactam function was in the enol form and that the TBDPS-group was attached to the O^6.

It should be noted that the presence of small amounts of imidazole from the first reaction changed the selectivity almost completely from O^6-silylation to 2'-*O*-silylation. It is likely that the imidazole is assisting the removal of the OH proton at position 2' and this results in the formation of the 2'-*O*-silylated derivative. It is therefore essential to remove all the imidazole.

It was found that compound **2** is readily alkylated on the 2'-hydroxyl function using an alkyl halide and the sterically hindered strong organic base, 2-*tert.*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP). No N-2 alkylation was observed and ring opening of the disiloxane bridge was minimal. After 2-3 h some degradation started to occur. The degradation was mainly due to loss of the TBDPS-group. In complete contrast the silver oxide catalysed alkylation[12] route, when applied to compound **2**, resulted only in small amounts of N7-alkylation.

Silylation Procedure

Guanosine (35 mmol) in DMF was treated with 1.1 eq 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane and 5 eq imidazole[14] under anhydrous conditions at room temperature for 1 h. (Alternatively, 7 eq pyridine was used as base[10] and the reaction was left at 50°C for 1 h. This excluded the necessity of silica gel chromatography before the next step). The solvent was evaporated *in vacuo* and the residue coevaporated with toluene to leave a white foam. Residual imidazole was removed by silica gel chromatography eluting with a gradient of ethanol (5-10%) in dichloromethane. Compound **1** was then treated with 1.2 equivalents of TBDPSCl and 5 equivalents of triethylamine in the presence of 0.2 equivalents of DMAP, in dichloromethane at room temperature for 8h. The reaction mixture was poured into vigorously stirred phosphate buffer. The organic layer was then separated, washed with saturated NaCl (aq) solution, dried (Na_2SO_4), filtered and evaporated *in vacuo*.

General Alkylation Procedure

Compound **2** (10 mmol) was dried by evaporation of anhydrous acetonitrile and dissolved in anhydrous acetonitrile under argon. The solution was cooled in an ice bath and 2.5 equivalents of BEMP and 5 equivalents of alkyl halide were added with stirring and exclusion of moisture.[9] The solution was kept for 10 min at 0°C and then stirred at room temperature. Reactions were considered complete when less than 20% starting material remained, or when degradation started to become significant, usually after 2-3h. The reaction time was dependent on alkylating agent; 1.5 h for methyl iodide and 2 h for allyl bromide. The solvent was evaporated *in vacuo*. Ethyl acetate was added to the residue and the mixture washed with phosphate buffer (pH 7) and saturated NaCl solution. The organic layer was then dried over sodium sulphate, filtered and evaporated *in vacuo*.

Without any further purification the products **3a-b** were subsequently desilylated[13] followed by introduction of dimethylaminomethylidene[16] protection on the exocyclic amino function to give compounds

4a-b. Products **4a-b**[17] were finally purified on silica gel using a gradient of ethanol (5-20%) in dichloromethane. The overall yield of compounds, **4a-b** based on guanosine were 48 and 51%, respectively. This is a substantial improvement over the previous rather complicated 8 step procedure which has an overall yield of about 40% after 4 chromatographic purifications.[11]

In summary we have developed a versatile synthetic route enabling the preparation of 2'-*O*-alkyl guanosine derivatives in only 5 steps starting from inexpensive guanosine. The route can easily be scaled up, it requires a minimum of purification and gives a good overall yield. The combination of the silyl protected intermediate and the highly selective alkylation procedure will enable us to prepare a variety of 2'-*O*-alkylguanosine derivatives and work in this direction is currently in progress.

Acknowledgements

We thank Dr. S. Wagner for recording the infrared spectra and for performing the elemental analyses.

References and notes

1) Current address: Zeneca CRB, Gadbrook Park, Northwich, Cheshire, CW9 7RA, UK.

2) Sproat, B.S.; Lamond, A.I. : 2'-*O*-Alkyloligoribonucleotides. In *Antisense Research and Applications*, Eds.: Crooke, S.T.; Lebleu, B.; CRC Press Inc. 1993; pp. 351-362.

3) Cotten, M.; Oberhauser, B.; Bruner H., Holzner, A.; Issakides, G.; Noe, C.; Schaffner, G.; Wagner, E.; Birnstiel, M. *Nucl. Acids Res.*, **1993**, *19*, 2629.

4) Paolella, G.; Sproat, B.S.; Lamond, A.I. *EMBO J.*, **1992**, *11*, 1913.

5) Beigelman, L.; McSwiggen, J.L.; 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. Bio. Chem.* **1995**, *270*, 25702.

6) Robins, M.; Naik, S.; Lee,S. *J. Org. Chem.*, **1986**, *39*, 1891.

7) Wagner, E.; Oberhauser, B.; Holzner, A.; Bruner, H.; Issakides, G.; Schaffner, G.; Cotten, M., Knollmuller, M.; Noe, C. *Nucl. Acids Res.*, **1991**, *19*, 5965.

8) Chanteloup, L.; Thuong, N.T. *Tetrahedron Lett.* **1994**, 877.

9) Sproat, B.S.; Beijer, B. ; Iribarren, A. *Nucl. Acids Res.*, **1990**, *18*, 41.

10) Sproat, B.S.; Iribarren, A.; Garcia, R.G.; Beijer, B. *Nucl. Acids Res.*, **1991**, *19*, 733.

11) Beijer, B.; Grøtli, M.; Douglas, M.E. ; Sproat, B.S. *Nucleosides & Nucleotides* **1994**, *13*, 1905.

12) Hodge, R.P.; Sinha, N.D. *Tetrahedron Lett.* **1995**, *36*, 2933.

13) Daskalov, H.P.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1980**, *21*, 3899.

14) Markiewicz, W.T. *J. Chem. Res.* **1979**, 24.

15) IR(KBr) cm^{-1}: 3500, 3400 (OH, NH_2 st), 2950, 2870 (CH st), 1610, 1580 (ar C=C, C=N st), 1460, 1250, 1090, 700. Analysis $C_{38}H_{57}N_5O_6Si_3$ requires C, 59.72; H, 7.53; N, 9.16; found C, 60.03; H, 7.57; N, 9.20. ^{13}C NMR ($CHCl_3$); 159.31 (C-6), 158.86 (C-2), 153.35 (C-4), 137.43 (C-8), 135.24 (phenyl, C-2, C-6), 132.54 (phenyl, C-1), 129.37 (phenyl, C-4), 127.11 (phenyl, C-3, C-5), 117.06 (C-5), 88.90 (C-1'), 81.27 (C-4'), 74.58 (C-2'), 70.07 (C-3'), 60.93 (C-5'), 26.86 (tBu, CH_3's), 19.30 (tBu, quart. C), 17.13-16.77 (isopropyl, CH_3's) and 13.13-12.07 (isopropyl, CH's).

16) McBride, L.H.; Kierzek,R.; Beaucage, S.L.; Caruthers, M.H. *J. Am. Chem. Soc.* **1986**, *108*, 2040.

17) The spectroscopic data were in agreement with previously published data; 8) and 9).

(*Received in Belgium* 9 *October* 1996; *accepted* 9 *January* 1997)