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Synthesis of Guanosine Analogs Bearing Pendant Alkylthiol Tethers

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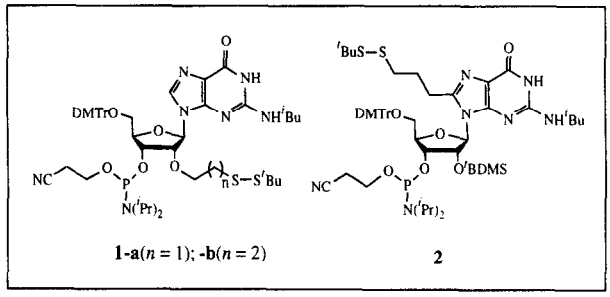
Abstract: Synthesis of three guanosine monomers substituted with alkylthiol chains at either carbon-8 or the 2'-hydroxyl is described. The ready accessibility of these monomers will facilitate the use of disulfide cross-links to study the folding and dynamics of RNA and will also provide loci for conjugation of reporter groups.

Although RNA is different from proteins with respect to monomer composition and backbone structure, these two classes of macromolecules possess several similarities. For example, both RNA and proteins display a rich diversity of tertiary structures.[2] The pathways by which RNAs fold are of considerable interest and often have been compared to protein folding pathways.[3] Similarities between proteins and RNA suggest that it should be possible to apply methods developed for studying protein folding and dynamics to RNA. One particularly useful technique for probing protein structure, folding, and dynamics is to generate mutants capable of forming disulfide cross-links between elements of secondary structure that associate in the tertiary fold.[4] While investigating disulfide cross-links as tools for probing the dynamic motion of RNA in solution, we required cross-links bridging guanosine residues. In this report, we describe the synthesis of guanosine monomers substituted with alkylthiol chains at either carbon-8 or the 2'-hydroxyl.[5] With the availability of these monomers, convenient synthetic routes for 2'-*O*-thioalkyl modifications of all four ribonucleosides are now available[6] and the ready construction of a C-8-substituted thioalkylguanosine increases the flexibility of the disulfide cross-linking approach,[7] and also provides a site for covalent attachment of reporter groups.[8]

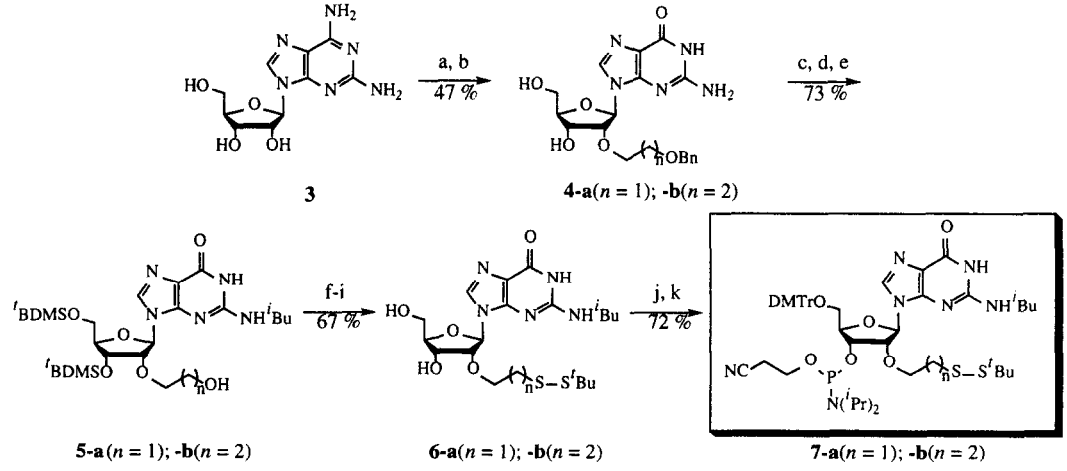


1-a(n = 1); **-b**(n = 2) **2**

The synthesis of 2'-*O*-alkylguanosines is more difficult than for any of the other standard bases because direct alkylation of guanosine is complicated by reaction at O^6.[9] Although O^6 blocking groups have been reported,[10] yields of 2'-*O*-alkyl adducts are usually low, even with highly reactive electrophiles.[9] 2'-*O*-Alkylguanosines can be synthesized by coupling protected guanine to an alkylated sugar,[11] but these procedures add to the number of steps in a synthesis and can result in undesirable stereoisomers that may be difficult to separate. Regioselective preparation of 2'-*O*-allylguanosine using the Markiewicz disiloxane reagent for simultaneous 3',5'-hydroxyl protection,[12] and a novel glyoxal protecting group[13] or TBDPS[14] for O^6

protection has been reported. However, these methods are limited to alkylation in the presence of relatively mild bases and highly reactive electrophiles. In lieu of these methods, we have used an approach described by McGee *et al.* for the preparation of a number of 2'-*O*-alkylated guanosines.[15] The key features in this approach are: alkylation of 2,6-diamino-9-ß-D-ribofuranosylpurine, chromatographic separation of the 2'-*O*- and 3'-*O*-adducts, and enzymatic conversion of the 2'-*O*-adduct to a 2'-*O*-alkylated guanosine. Using this approach to 2'-*O*-alkylated guanosines, protection of the guanosine lactam function is unnecessary because the lactam functionality is introduced after the alkylation step.

2,6-Diamino-9-ß-D-ribofuranosylpurine was prepared from guanosine,[15] and treated with NaH and either benzyl-2-bromoethylether or benzyl-3-bromopropylether in DMF (Scheme I). In both cases, the 2'-*O*-alkyl adduct was the major product (48-50%) and it was easily separated from minor amounts of the 2'-*O*-, 3'-*O*-dialkyl adduct (9-13%), and the 3'-*O*-alkyl adduct (11-12%) by flash chromatography. Regiochemical assignment of the products was performed by homonuclear decoupling NMR experiments in DMSO-d_6. Deamination was accomplished using adenosine deaminase (Sigma, Type II). In these experiments, we found that using 20% DMSO rather than 60%, as originally reported[15] results in a 3-fold increase in the rate of deamination and a nearly quantitative yield of the desired product. Following silyl protection of the ribose hydroxyls and isobutyryl protection of the 2-amino function, hydrogenolysis over $Pd(OH)_2/C$ was used to liberate the terminal hydroxyl group. Conversion of this free hydroxyl into the mixed *tert*-butyl disulfide and silyl deprotection with aqueous hydrofluoric acid was conducted as previously described.[6a] Protection of the 5'-hydroxyl as the 4,4'-dimethoxytritylether and activation of the 3'-hydroxyl as the diisopropylphosphoramidite for solid-phase RNA synthesis yielded **7** in 11 steps and 16% overall yield from 2,6-diamino-9-ß-D-ribofuranosylpurine.[16]

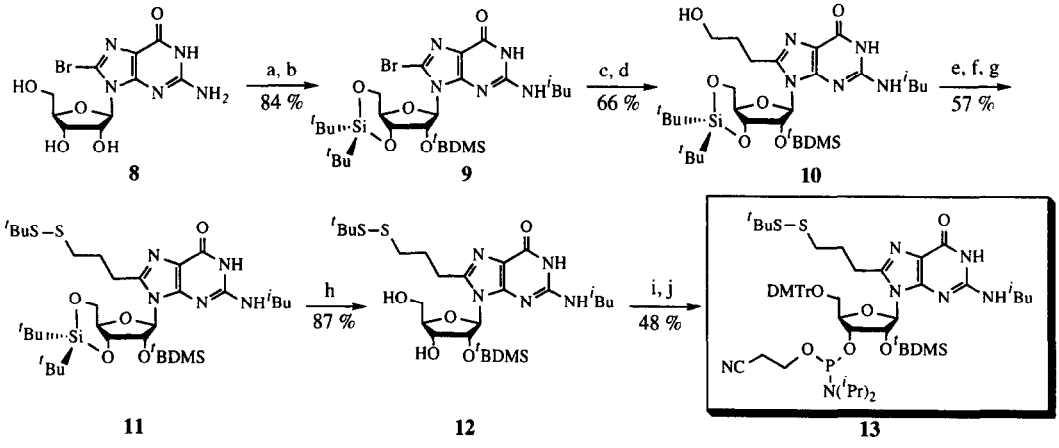


Scheme I. (a) NaH, benzyl-3-bromopropyl ether (n = 2) or benzyl-2-bromoethyl ether (n = 1), DMF; (b) adenosine deaminase, DMSO, 100 mM Tris, 100 mM sodium phosphate buffer, pH 7.4; (c) *t*-BDMSCl, imidazole, DMF; (d) isobutyryl chloride, pyridine; (e) H_2, 20% $Pd(OH)_2/C$, MeOH; (f) methanesulfonyl chloride, pyridine, $MeCl_2$; (g) thiobenzoic acid, Et_3N, DMF; (h) HF, MeCN; (i) 1-*tert*-butylthiohydrazine-1,2-dicarboxmorpholide, LiOH, THF, MeOH; (j) DMTrCl, DMAP, pyridine; (k) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, *i*-Pr_2EtN, $MeCl_2$.

In designing the synthesis of C-8-substituted thioalkylguanosines, we considered two criteria: regioselective protection of the ribose hydroxyls in such a way as to obtain solely the 2'-*O*-*tert*-butyldimethylsilyl adduct, and mild introduction of the alkyl chain at carbon-8. Hence, the di-*tert*-

butylsilylene group was used to simultaneously protect the 3'- and 5'- ribose hydroxyls because this group could be selectively cleaved in the presence of the 2'-*O*-*tert*-butyldimethylsilylether by Bu_3N•HF in THF.[17,18] For installation of the C-8 functionality, we chose to use commercially available 8-bromoguanosine in a palladium catalyzed coupling with propargyl alcohol.[19]

The ribose hydroxyls of 8-bromoguanosine were protected using a one pot procedure in which 8-bromoguanosine was first treated with di-*tert*-butyldichlorosilane and $AgNO_3$ in DMF, followed by the addition of *tert*-butyldimethylsilylchloride (Scheme II). The 2-amino function was protected with isobutyryl chloride and the product was alkynylated with propargyl alcohol, $Pd(OAc)_2$, CuI, and Ph_3P. Hydrogenation yielded the saturated primary alcohol **10** and conversion from the alcohol to the mixed *tert*-butyl disulfide was conducted as previously described.[6a] The di-*tert*-butylsilylene group was removed using Bu_3N•HF in THF, and the 3'- and 5'- hydroxyls were prepared for solid phase synthesis as described above. This sequence afforded **13** in 10 steps and 13% overall yield from 8-bromoguanosine.



Scheme II. (a) (i) $(t\text{-Bu})_2SiCl_2$, $AgNO_3$, DMF, pyridine (ii) *t*-BDMSCl; (b) isobutyryl chloride, pyridine; (c) propargyl alcohol, $Pd(OAc)_2$, CuI, PPh_3, Et_3N, DMF; (d) H_2, 10% Pd/C, MeOH; (e) methanesulfonyl chloride, pyridine, $MeCl_2$; (f) thiobenzoic acid, Et_3N, DMF; (g) 1-*tert*-butylthiohydrazine-1,2-dicarboxmorpholide, LiOH, THF, MeOH; (h) Bu_3N•HF, THF; (i) DMTrCl, pyridine; (j) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, *i*-Pr_2EtN, $MeCl_2$.

Through these straightforward procedures, we have prepared gram quantities of thiol-modified guanosines and we are currently using them to introduce guanosine-guanosine cross-links in RNA. The results of these experiments will be reported shortly.

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Abbreviations: ^{i}Bu - isobutyryl; ^{t}Bu - *tert*-butyl; DMT - 4,4'-dimethoxytrityl; ^{i}Pr - isopropyl

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