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Simple Synthesis of 4-Thiothymidine, 4-Thiouridine and 6-Thio-2'-deoxyguanosine

Yao-Zhong Xu*, Qinguo Zheng and Peter F. Swann

CRC Nitrosamine-Induced Cancer Research Group
Department of Biochemistry and Molecular Biology, University College London
Gower Street, London WC1E 6BT, England

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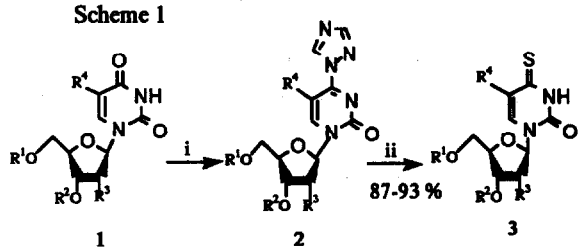
Abstract: *4-triazolo-pyrimidine nucleosides and 6-O-(mesitylenesulfonyl)-2'-deoxyguanosine, when treated with thiolacetic acid at room temperature, gave the corresponding 4-thiopyrimidine nucleosides and 6-thio-2'-deoxyguanosine with high yields (86-93%). Possible mechanisms are discussed.*

4-Thiothymidine and 4-thiouridine can be prepared by treatment of the corresponding protected pyrimidine nucleosides with phosphorus pentasulphide[1] or Lawesson's reagent [2] in appropriate solvents at reflux, but unfortunately the methods are not suitable for acid-labile and temperature-sensitive compounds. For example under these conditions the trityl group will be removed from trityl-protected nucleosides and 2'-deoxyguanosine will depurinate. For this reason, 6-thio-2'-deoxyguanosine is usually made by condensing protected 2'-deoxyribofuranosyl chloride with protected 6-chloroguanine to give a mixture of the α and β-anomers of 6-chloro-2'-deoxyguanosine. The desired β-anomer is isolated by fractional crystallization and treated with hydrogen sulfide (H_2S), or its sodium salt, to give 6-thio-2'-deoxyguanosine[3,4,5]. The method is tedious and difficult to perform, and the overall yield is poor. In the course of synthesis of oligodeoxyribonucleotides containing 4-thiothymine and 6-thioguanine[6], we have found an easy synthesis of 4-thiothymidine and 6-thio-2'-deoxguanosine and of the ribonucleoside, 4-thiouridine, in very mild conditions with high yields.

4-THIOPYRIMIDINE NUCLEOSIDES:

Protected nucleosides, with silyl, DMT (dimethoxytrityl) and acyl protecting groups on the sugar moiety (1a-c), were converted into their 4-triazolo-derivatives by reaction with $POCl_3$ and 1,2,4-triazole in quantitative yield[7]. Reaction of these with thiolacetic acid at room temperature gave the protected 4-thio-nucleosides in very

Scheme 1



1 2 3

	R^1	R^2	R^3	R^4
a.	Si ;	Si;	H;	CH_3
b.	DMT;	Si;	H;	CH_3
c.	Bz;	Bz;	BzO;	H

Si: t-butyldimethylsilyl;
DMT: dimethoxytrityl;
Bz: benzoyl;

i: $POCl_3$/ triethylamine / triazole; ii CH_3COSH;

high yields (87-93%) (Scheme 1). The thio-derivatives have been isolated and their structures confirmed by NMR, U.V. (in particular the λmax at 326 - 335nm showing the presence of sulphur) and by comparison with published data. The rate of the reaction between the 4-triazolides and thiolacetic acid increased when the acidity of the medium was increased, and reduced with basicity of the medium. The reaction took place much faster in protic solvents (e.g. CH_3OH) than in aprotic solvent (e.g. CH_3CN). Unexpectedly none of the products had the acetyl group on the 4-S position[8]. When the reaction course was followed by TLC, only 4-thiopyrimidine and no 4-*S*-acetyl-pyrimidine nucleoside was observed as an intermediate, and the same product was obtained using thiolbenzoic acid instead of thiolacetic acid. Although the mechanism has not yet been clarified, these observations suggest that protonation of the triazolo group was followed by an attack at C-4 by thio-acetate ion. Possibly the acetyl group is lost because the thio-keto form is more stable than the 4-*S*-acetyl form. However cytidine, treated with thiolacetic acid in pyridine at 50°C for 4 hrs, quantitatively gave *N*-acetylcytidine[9] rather than 4-thiouridine. A possible explanation of this observation, which offers an alternative method for selective acylation of the 4-amino group of cytidine[10], is that the amino group of cytidine is a better nucleophile and less good leaving group than the triazolo group.

It is worth noting that the very acid-labile DMT protecting group (in compounds **1-3b**), which is commonly removed by treatment with 80% acetic acid at 20°C for 20 min, is not removed by the treatment with thiolacetic acid in CH_3CN or CH_2Cl_2 even though thiolacetic acid is a stronger acid (pKa = 3.62) than acetic acid (pKa =4.76)[11]. This suggests that thiolacetic acid plays a nucleophilic rather than acidic role in these aprotic solvents[12]. The stability of the DMT protecting group to these conditions is important because it is widely used in chemical synthesis of DNA or RNA oligomers.

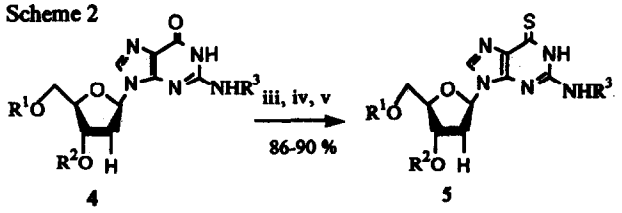
A typical thiation procedure was as follows: to a stirring solution of the protected 4-triazolo pyrimidine nucleosides **2a-c** (1 mmole) in 20 ml of CH_3CN was added 1 ml of thiolacetic acid at room temperature. The reaction was left stirring overnight and the starting material was completely converted into a new spot with higher R_f by TLC [in 5 % CH_3OH:$CHCl_3$, with visualization under UV lamp, or by treatment with anisaldehyde / conc. H_2SO_4 / ethanol (5:5:90, v/v/v) and heating]. The reaction solution was diluted with CH_2Cl_2 (50 ml), washed with saturated aqueous $NaHCO_3$ (2 x 50 ml), then with saturated aqueous NaCl (50 ml). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to give a slightly-yellow solid (one spot on TLC). Pure compounds (**3a-c**) were obtained by silica gel column chromatography and characterized by NMR and UV[13].

6-THIO-2'-DEOXYGUANOSINE:

Protected 2'-deoxyguanosines, with acyl and silyl protecting groups on the 5', 3' and N-2 position (**4d-e**), were treated with mesitylenesulphonyl chloride to form 6-*O*-mesitylenesulphonyl derivatives, then with *N*-methylpyrrolidine[7,14], followed by thiolacetic acid. This gave the protected 6-thio-2'-deoxyguanosines (**5d-e**) in high yields (86-90%) (Scheme 2). In this case also the product was the 6-thio-keto rather than the 6-*S*-acetyl derivative[8]. Presumeably the 6-thio-keto form is more stable than the 6-*S*-acetyl form. By deprotection, compound **5d** has been quantitatively converted into free 6-thio-2'-deoxyguanosine, a potential drug[15].

A typical procedure was as follows: The nucleosides were protected by published procedures. 1 ml of triethylamine, 20 mg of dimethylaminopyridine and 1.0 g of mesitylenesulphonyl chloride were sequentially

Scheme 2



4 5

	R^1	R^2	R^3
d.	i-Bu;	i-Bu;	i-Bu;
e.	Si;	Si;	PAc;

i-Bu: iso-butyryl;
Si: t-butyldimethylsilyl;
PAc: phenylacetyl

iii: mesitylenesulphonyl chloride; iv: *N*-methyl pyrrolidine v: CH_3COSH

added into the stirring CH_2Cl_2 (50 ml) solution of the protected nucleosides **4d-e** (2 mmole) at room temperature. Stirring was continued until all the nucleoside had become a new compound with higher R_f (TLC). Then the solution was cooled in an ice-bath and a mixture of *N*-methylpyrrolidine (2 ml) and CH_2Cl_2 (5 ml) was added dropwise. After TLC showed that the material had changed into a compound with very low R_f (5% CH_3OH:$CHCl_3$), a mixture of thiolacetic acid (2 ml) and CH_2Cl_2 (5 ml) was added dropwise. After 30 min, when TLC showed that the low R_f compound had completely disappeared, the reaction solution was poured into 50 ml of 0.5 M aqueous KH_2PO_4 (pH 6.3). The organic layer was washed with saturated aqueous $NaHCO_3$ (2 x 50 ml), then with saturated aqueous NaCl (50 ml), dried (Na_2SO_4), then evaporated under reduced pressure to give an oily residue which was precipitated from toluene into n-pentane to give a slightly-yellow powder. Pure compounds **5d-e** were obtained by silica gel column chromatography and characterized by NMR and UV[16].

The present method for transformation of nucleosides to thio-nucleosides has at least two advantages over phosphorus pentasulphide or Lawesson's reagent: the reactions occur at room temperature with high yields; and after reaction the reagent, thiolacetic acid, is converted into the acetate ion which is easy to remove. The method could be used for direct incorporation of radioactive ^{35}S from readily available ^{35}S-thiolacetic acid into nucleosides, nucleotides and DNA-oligomers and the method might be suitable for thiation of other temperature- and acid-sensitive heterocyclic compounds.

ACKNOWLEDGEMENTS:

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8. The NMR spectra of all products (3a-c and 5d-e) show no evidence for the presence of the acetyl group and, in addition, the imino protons of these thio-nucleosides can be detected at 1 to 1.5 ppm downfield from their oxygen-containing analogues (1a-c and 4d-e).
9. The isolated product, *N*-acetylcytidine was characterized by NMR and UV. ^{1}H NMR (δ ppm in DMSO-d_6): 10.88 (s, NH-4), 8.41 (d, H-6), 7.17 (d,H-5), 5.76 (d, H-1'), 5.47, 5.16, 5.05 (3 m, ex., OH-5', OH-3' and OH-2'), 3.97-3.93 (m, H-2' and H-3'), 3.89 (m, H-4'), 3.74-3.57 (m, H-5'), 2.08 (s, CH_3- of acetyl); UV: λmax=299 nm (in CH_3OH).
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12. It was observed that DMT group (in compound 1-3b) could be removed by prolonged exposure to thiolacetic acid when CH_3OH was used as solvent.
13. Compound 3a: 90% isolated yield from 2a, ^{1}H NMR (δ ppm in DMSO-d_6): 12.68 (s, ex., NH-3), 7.55 (d, H-6), 6.09 (t, H-1'), 4.37 (m, H-3'), 3.85-3.73 (m, H-4' and H-5'), 2.29-2.17 (m, H-2' and H-2"), 1.98 (s, 5-CH_3), 0.902, 0.869 (2s, Si-C_4H_9), 0.328, 0.202 (2s, Si-CH_3); UV: λmax=334 nm (in CH_3OH). Treatment of compound 3a with fluoride ion gave free 4-thiothymidine confirmed by NMR with following data (δ pm): 12.70 (s, ex., NH-3), 7.89 (s, H-6), 6.09 (t, H-1'), 5.27 (d, ex., OH-3'), 5.09 (t, ex., OH- 5'), 4.23 (m, H-3'), 3.78 (m, H-4'), 3.64-3.37 (m, H-5'), 2.14-2.11 (m, H-2' and H-2"), 1.95 (s, -CH_3-5).
Compound 3b: 93 % isolated yield from 2b, ^{1}H NMR (δ ppm): 12.77 (s, ex., NH-3), 7.69 (s, H-6),7.39-6.86 (m, aromatic-H of DMT), 6.07 (t, H-1'), 4.44 (m, H-3'), 3.84 (m, H-4'), 3.72 (s, O-CH_3 of DMT), 3.38-3.13 (m, H-5'), 2.39-2.15 (m, H-2' and H-2"), 1.67 (s, 5-CH_3), 0.761(s, Si-C_4H_9), -0.011, -0.071 (2s, Si-CH_3); UV: λmax=336 nm.
Compound 3c: 87 % isolated yield from 2c, ^{1}H NMR (δ ppm): 12.85 (s, ex., NH-3), 8.01-7.42 (m, H-6 and aromatic-H), 6.32 (d, H-5), 6.16 (d, H-1'), 5.98-5.91 (m, H-2' and H-3'), 4.79-4.63 (m, H-4' and H-5'); UV: λmax =326 nm.
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16. Compound 5d: 86 % isolated yield from 4d, ^{1}H NMR (δ ppm): 13.43 (s, ex., NH-1), 11.90 (s, ex., NH-2), 8.41 (s, H-8), 6.22 (t, H-1'), 5.34 (m, H-3'), 4.25-4.21 (m, H-4' and H-5'), 3.02-2.76 (m, H-2' and H-2"), 2.62-2.48 (m, -CH- of isobutyryl), 1.14-1.03 (m, -CH_3 of isobutyryl); UV: λmax = 334 nm. Treatment of compound 5d with 2 M NaOH in EtOH gave free 6-thio-2'-deoxyguanosine confirmed by NMR with following data: 11.94 (s, ex., NH-1), 8.11 (s, H-8), 6.81 (m, ex., NH-2), 6.097 (t, H-1'), 5.28 (d, ex., OH-3'), 4.94 (t, ex, OH-5'), 4.32 (m, H-3') , 3.80 (m, H-4'), 3.53 (m, H-5'), 2.51-2.17 (m, H-2' and H-2").
Compound 5e: 90 % isolated yield from 4e, ^{1}H NMR (δ ppm): 13.26 (s, ex., NH-1), 12.22 (s, ex., NH-2), 8.38 (s, H-8), 7.34-7.33 (m, aromatic-H of phenylacetyl), 6.19 (t, H-1'), 4.52-4.49 (m, H-3'), 3.86-3.84 (m, H-4'), 3.83 (s, -CH_2- of phenylacetyl), 3.74-3.63 (m, H-5'), 2.74-2.31 (m, H-2, and H-2"), 0.88, 0.85 (2s, Si-C_4H_9), 0.099-0.034 (m, Si-CH_3); UV: λmax =334 nm.

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