SHORT PAPER

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An improved synthesis of guanosine 5′-monothiophosphate†

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A synthesis of guanosine 5′-monothiophosphate which requires no chromatographic purification is described.

A number of important RNA species have a phosphorylated guanosine residue at the 5′-terminus. These include the majority of t-RNAs, the catalytic subunit of *E.coli* RNase P, and the Rev response element of HIV RNA. Macosko *et al.*[1] substituted guanosine 5′-monothiophosphate (Fig. 1) for guanosine 5′-phosphate in the Rev response element, attached a spin-label to the modified RNA, and showed that this was a useful tool for monitoring the interactions of the RNA with associated protein. We are interested in studying the RNA-protein interaction of bacterial RNase P in the same fashion[2] and report here an easy route to the thiophosphate using thiophosphoryl chloride. It is interesting to contrast the difficulties encountered in phosphorylations of guanosine using phosphorus oxychloride.[3]

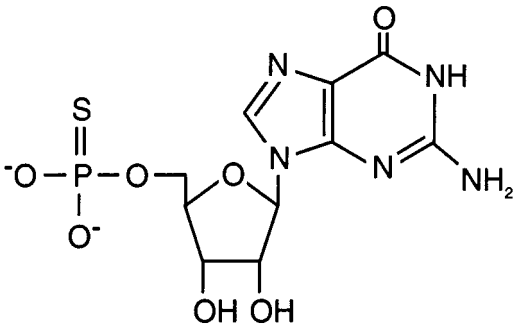

Fig. 1 Guanosine 5′-Monothiophosphate

Guanosine 5′-thiophosphate has been synthesized many times but always by procedures requiring chromatographic purification. Fischer *et al.*[4], working with adenosine derivatives, have recently shown that the principal complication in these syntheses lay in the use of trialkyl phosphates as solvent. Thiophosphoryl chloride and trialkyl phosphates undergo oxygen/sulfur exchange thus leading to mixtures of the ordinary phosphate with the thiophosphate. Another complication inherent in the use of unprotected guanosine, as has been common practice, is reaction at the secondary hydroxyl groups. We therefore used 2′,3′-isopropylidene guanosine which is easy to prepare by the method of Tomasz.[5] This derivative also has increased solubility in pyridine as compared with guanosine. Removal of the protecting group following thiophosphorylation is a more delicate operation than usual as Lönnberg[6] has shown that the thiophosphoryl group is much more labile to acid-catalysed hydrolysis than is the ordinary phosphate. We were, however, successful in finding conditions for removal of the isopropylidene group without significant hydrolysis of the thiophosphate functionality (see experimental).

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Experimental

2′, 3′-isopropylidene guanosine 5′-thiophosphate, barium salt: 1 mmol of 2′,3′-isopropylidene guanosine (Sigma or ref. 5), 0.32 g, was partially dissolved in 6 ml dry pyridine, 0.5 g of 3A sieves was added, and the mixture cooled on an ice bath. Then, 0.4 ml (4 equiv.) $PSCl_3$ was added dropwise with stirring. After 30 min, 8 g of ice was added together with 1.6 g of $Ba(OH)_2 \cdot 8H_2O$. The mixture was stirred on ice for 30 min. The final pH is about 6.5. The second pKa of the thiophosphoryl group is about 5.5.[7] The heavy precipitate which formed (largely $BaPSO_3H$) was removed by centrifugation or suction-filtered through a Celite pad. The supernatant fraction was treated with twice its volume of 95% ethanol to give a precipitate of the product. This was collected by centrifugation, washed twice with 95% ethanol, and then acetone to yield 220 mg of product. Another 100 mg was recovered by cooling the supernatant fraction and from the washings of the $BaPSO_3H$ precipitate. Total yield 57% based on the anhydrous M.W. The reaction has been scaled up to 6 mmol without difficulty. IR (Nujol): 1676, 1625, 1592, 1531, 1211, 1116, 1084, 1017, 966, 855, 816, 798, 784 cm^{-1}. The crude sodium salt gave a ^{31}P NMR shift at 44.6 in D_2O. NMR, D_2O, pH 7: ^1H, δ 8.09 (s,1), 6.01 (d,1), 5.28 (dd, 1), 5.18 (dd,1), 4.52 (m,1), 3.96 (m,2) 1.58 (s,3), 1.37 (s,3). ^{13}C, δ 159.1, 153.9, 151.4, 138.1, 116.2, 114.7, 89.9, 85.3 (d, *J*=9.5), 83.9, 81.7, 64.2 (d, *J*=3.3), 26.2, 24.4. Inorganic thiophosphate showed IR absorption at 1039 and 943 cm^{-1}.

Guanosine 5′-thiophosphate, barium, sodium, and lithium salts: 110 mg (0.2 mmol) of the above crude barium salt was dissolved in 4 ml of 0.4 M HCl and kept at 23–25°C for 10–12 h. The solution was neutralized with a few drops of conc. ammonia to about pH 6.5. A precipitate appears. Two volumes of 95% ethanol were added with stirring. The precipitated barium salt of the product was collected by centrifugation, washed twice with 95% ethanol and once with acetone. This material was resuspended in acetone and air-dried to yield 93 mg of a white powder (0.18 mmol, 90%, based on the anhydrous M.W.). IR(Nujol): 1693, 1642, 1534, 1113, 1012, 800, 779, cm^{-1}. Guanosine 5′-phosphate, barium salt; 1678, 1641, 1600, 1109, 978, 801, 782 cm^{-1}.

This material was suspended in 3 ml water containing 28 mg of sodium sulfate. The suspension was stirred for about 1 h. Barium sulfate was removed by centrifugation. The solution of the sodium salt of the product was treated with 4–5 volumes of acetone to yield a precipitate of the sodium salt. Centrifugation and air drying yielded a free-flowing off-white powder. (66mg, 0.16 mmol, 87% based on the anhydrous molecular weight). The proton and ^{13}C NMR spectra were almost indistinguishable from those of guanosine 5'-phosphate, but the ^{31}P spectrum in D_2O gave a singlet at 44.5 ppm (H_3PO_4 external reference); Eckstein[8] reports 42.9. An ordinary phosphate has a shift near zero. Any impurity of inorganic thiophosphate appears at about δ=55. The ^{31}P resonance of phosphate and thiophosphate groups shift in opposite directions as a function of pH[7]. The oxidized (disulfide) dimer of the product has a ^{31}P shift of 18.2.

This method of preparing the sodium salt yields a product containing a variable amount of sulfate. A sulfate-free product may be prepared by treating the barium salt in suspension with Dowex 50 in the sodium form, however, the yield is poor probably due to hydrophobic adsorption on the resin. Crystallisation of the sodium salt was unsuccessful. The dilithium salt was prepared using lithium sulfate. This crystallised from hot acetone – water (2:1) to yield the trihydrate.

Calcd for $C_{10}H_{12}N_5O_7PSLi_2 \cdot 3H_2O$: C, 26.97; H, 4.07; N, 15.73: Found. C, 26.83; H, 4.20; N, 16.19. IR: 1692, 1651, 1612, 1580, 1533, 1116, 1016, 801, 779.

The molar extinction coefficient for guanosine derivatives has been well established as 13 650 $M^{-1}cm^{-1}$ at pH 7 252.5 nm[9]. However, the thiophosphoryl group also has significant absorption in this region: inorganic thiophosphate trianion (made by hydrolysis of

Table 1 Optical properties of GMP and GMPS[a]

	$Na_2GMP{\cdot}2H_2O$	$Li_2GMPS{\cdot}3H_2O$
λ_{max}	252.5 nm	252.5 nm
ε_{max}	13,650 $M^{-1}cm^{-1}$	14,300 M^{-1} cm^{-1} (±500)
λ_{min}	224 nm	228 nm
ε_{min}	3300 $M^{-1}cm^{-1}$	7500 $M^{-1}cm^{-1}$
Ratio, $\varepsilon_{max}/\varepsilon_{min}$	4.1	1.9
$[M]_D^{25,b}$	–176 (2×10^{-2}M, H_2O)	–141 (5.6×10^{-3}M, H_2O)

[a]GMP is guanosine 5′-phosphate; GMPS is guanosine 5′-thiophosphate. All solutions are in water, pH ~7.

[b]The corresponding values for guanosine and 2′, 3′-isopropylidene guanosine, determined on saturated aqueous solutions, 24°C, are –133 and –121 respectively using the value 13 650 for $\varepsilon_{252.5}$ taken from ref. 9. $[M]_D$ values are good to about ±5%. The units are 10^{-1} deg cm^2 mol^{-1}.

thiophosphoryl chloride in dilute base) shows λ_{max} at 236 nm with ε=2100 $M^{-1}cm^{-1}$; the extinction coefficient at 252.5 nm is 1100 $M^{-1}cm^{-1}$. At pH 7, the dianion gives ε_{236} = 1500 and $\varepsilon_{252.5}$ = 500 M^{-1} cm^{-1}. On this basis, guanosine 5′-monothiophosphate should have $\varepsilon_{252.5}$ = 14,200; we find 14,300 M^{-1} cm^{-1}.

This UV absorption of the thiophosphoryl group does not appear to be widely known; Katchalski *et al*. published the UV spectra of inorganic thiophosphate and its oxidised dimer in 1965.[10] Shagidullin and his colleagues described the spectra of a number of other thiophosphates in 1975[11] without reference to Katchalski. The resultant correction at the wavelength maximum of guanosine and its derivatives is small but there is a large effect on the minimum (Table 1).

R_f values for TLC on silica using 2-propanol/water/NH_4OH: 17/5/1 are: isopropylidene guanosine, 0.95; guanine, 0.75; guanosine, 0.65; isopropylidene guanosine 5′-thiophosphate, 0.35; guanosine 5′-thiophosphate, 0. Guanosine 5′-thiophosphate moves as a single UV-absorbing spot upon paper electrophoresis at pH 7 with $R_{picrate}$ = 1.1

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