Aqueous methods for the preparation of 5′-substituted guanosine derivatives†

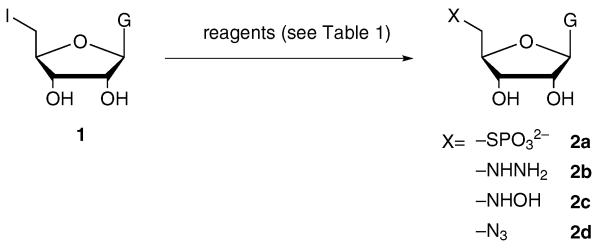
Paul Brear, Gemma R. Freeman, Mark C. Shankey, Milena Trmčić and David R. W. Hodgson*

Received (in Cambridge, UK) 5th May 2009, Accepted 19th June 2009
First published as an Advance Article on the web 6th July 2009
DOI: 10.1039/b908727c

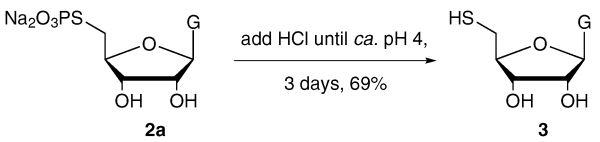
We have developed simple, aqueous strategies, that avoid the use of protecting groups and chromatography, for the preparation of a series of 5′-substituted guanosine derivatives.

Guanosine derivatives with modified 5′-functionalities have been used in the preparation of modified RNA molecules *via* phosphoramidite-mediated oligonucleotide synthesis[1] and *in vitro* transcription.[2–5] In addition, they are required for the preparation of nucleic acid analogues with non-phosphate-based linkages.[6] Thus, easy access to this class of molecules is essential. Guanosine derivatives often present a greater synthetic challenge than the other nucleosides owing to their poor solubility properties, which limit reactivity in solution. Chromatographic purification procedures are also hampered by poor solubility, thus, methodologies that overcome solubility problems and afford cleanly converted products that do not require chromatography offer a significant advance. We now describe simple, aqueous procedures, that avoid the use of protecting groups and chromatography, for the preparation of 5′-*S*-bridging thiophosphate, hydrazine, hydroxylamine and azide derivatives **2a–d** from 5′-deoxy-5′-iodoguanosine **1**[7] (Scheme 1). In addition, further aqueous transformations performed on thiophosphate **2a** and azide **2d** permitted the facile preparation of thiol **3** and amino **4b** derivatives, respectively (Schemes 2 and 3).

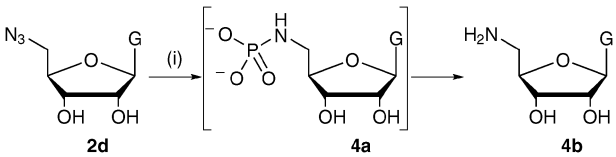
We recently took advantage of the fact that guanosine derivatives become highly soluble in aqueous solution when dissolved in alkali, owing to the removal of the *N*-1 proton. We have used this approach to facilitate the synthesis of thiophosphate derivative **2a** through solubilisation of iodoguanosine starting material **1a**. To improve the kinetics of the bimolecular displacement process, a large excess of thiophosphate nucleophile was used and initial concentrations of both reagent and substrate were high (0.1 M and 2 M, respectively). Progress of the displacement process was monitored by ^{31}P NMR spectroscopy. Excess thiophosphate was removed simply by selective precipitation with methanol (60% MeOH final volume), and the crude product was isolated by evaporation followed by freeze-drying. Finally, sodium iodide from the displacement process was removed by washing the lyophilised solid with acetone. Whilst the material that was isolated was not analytically pure (Table 1, ESI†), the only detectable contaminants were the thiol **2e** and inorganic phosphate. A previous approach to thiophosphate **2a** followed a four step route with HPLC purification after the final step,[6] rather than our simple two step approach that avoids chromatography.



Scheme 1 Transformations of 5′-deoxy-5′-iodoguanosine **1**.



Scheme 2 Acid-catalysed phosphate ester hydrolysis.



Scheme 3 Proposed mechanism of reaction of thiophosphate ion with azide **2d**. (i) Na_3PSO_3, water, 110 °C, 1 h, 70%.

Initial experiments towards the preparation of hydrazine **2b** focused on the use of alkali for the solubilisation of iodide **1**, however, we found that 50% aqueous hydrazine solution proved to be a remarkably good solvent for **1** in the absence of alkali. We used ^{13}C NMR spectroscopy to monitor reaction progress at the 5′-methylene centre, and initially we found that a solution of iodide **1** was attained, which was subsequently converted to **2b** over the course of 16 h. Again, the use of a highly concentrated reagent solution enhanced the rate of reaction significantly. The hydrazine **2b** was isolated by simple precipitation from the reaction mixture with methanol, followed by recrystallisation from methanol, which also allowed for simple removal of the excess reagent and the hydrazinium iodide by-product. Although the isolated yield was modest, owing to the partial solubility of hydrazine **2b** in

Department of Chemistry, Durham University, Science Laboratories, South Road, Durham, UK DH1 3LE.
E-mail: d.r.w.hodgson@durham.ac.uk

† Electronic supplementary information (ESI) available: Experimental details, ^{1}H, ^{13}C and (where appropriate) ^{31}P NMR spectra of compounds **2a–d**, **3** and **4b**. See DOI: 10.1039/b908727c

Table 1 Reagents and conditions

Reagent	Temp./°C	Time/h	Product	Purification method	Purity (%)	Yield (%)
Na_3SPO_3 and NaOH	50	2.5	**2a**	Precipitation of excess reagent with MeOH, removal of solvent, washed with acetone	99[a], 95[b]	72
H_2NNH_2	40	16	**2b**	Precipitation with methanol followed by recrystallisation from methanol	99[b]	40
H_2NOH	60	72	**2c**	Removal of solvent/reagent followed by recrystallisation from water	95[b]	63
NaN_3	Reflux	18	**2d**	Crystallisation from reaction mixture	99[b]	42

[a] Determined by ^{31}P NMR spectroscopy. [b] Determined by ^{1}H NMR spectroscopy.

methanol, the approach provided easy access to the hydrazine, which, as far as we are aware, has not been reported elsewhere.

Preliminary experiments towards hydroxylamine **2c** revolved around the use of basic conditions with hydroxylamine hydrochloride as the source of hydroxylamine, however, owing to the relatively low concentrations of hydroxylamine that are attainable using this approach, reaction rates were exceedingly low. We therefore moved to the use of 50% aqueous hydroxylamine solution as the source of nucleophile, again employing basic conditions in order to solubilise iodide **1**. Unfortunately, hydroxylamine is an ambident nucleophile, with *O*-nucleophilicity being enhanced under basic conditions,[8] therefore, we omitted base and we found that the iodide **1** slowly dissolved. ^{13}C NMR spectroscopy, performed directly on the reaction mixture, was used to give details of reaction progress at the 5′-methylene centre. In the absence of base, *O*-alkylation was minimised (<2.5%) and the *N*-alkylated product **2c** was isolated by removal of the reagent/solvent mixture on the rotary evaporator followed by recrystallisation from water. The isolated yield was modest because hydroxylamine **2c** showed appreciable solubility in water, however, in comparison to an earlier six step route,[9] which used several protecting groups, our new route offers a much more concise alternative.

A convenient, organic solvent-based approach for the preparation of azide **2d** has already been reported by Dean,[7] however, the use of dry dimethylformamide as the solvent may be considered undesirable in some situations. Thus, we explored the use of an aqueous system for the preparation of the azide. Using relatively high concentrations of the starting iodide **1** and a large excess of NaN_3, we were able to obtain moderate yields of the azide directly through crystallisation on cooling of the reaction mixture. The large excess of azide improves the kinetics of the displacement process, which are hampered by solvation of the azide anion by water molecules.

The thiol **3** was readily prepared through hydrolysis of thiophosphate ester **2a** under mildly acidic conditions (Scheme 2). Fortunately, the thiol product **3** displays poor solubility in water, and precipitates from the reaction mixture. The solid can be isolated *via* filtration and washing to afford material that is contaminated with some disulfide oxidation product (20%), in a reasonable overall yield. Thiol **3** has been prepared in the past using a novel protected thiolate nucleophile,[10] which has to be prepared in-house, and a protected guanosine precursor. Thus, our three step method from guanosine, which avoids chromatographic steps, represents a much simpler alternative.

The reactions between thio acids and azides have received much attention in recent times for the formation of amides[11] and sulfonamides.[12] In analogy with these thiocarboxylic acid methods we hoped to extend this approach towards the use of thiophosphoric acids with the intention of preparing the phosphoramidate **4a**, however, there was no observable reaction at room temperature. We therefore heated the reaction mixture, and, on cooling, we isolated the amine **4b**. Although the oxidation–reduction properties of the thiophosphate ion have been reported,[13] we believe that this is the first synthetic usage of the thiophosphate ion as a reducing agent. The reaction likely proceeds *via* the original target phosphoramidate **4a**, but this hydrolyses rapidly under the reaction conditions.

In conclusion, we have developed a suite of reactions that allow for the simple, facile, aqueous preparation of several guanosine derivatives, where protecting groups and time-consuming chromatography steps are avoided.

This work was supported by Durham University.

Notes and references

1 S. R. Das and J. A. Piccirilli, *Nat. Chem. Biol.*, 2005, **1**, 45–52.
2 D. Williamson, M. J. Cann and D. R. W. Hodgson, *Chem. Commun.*, 2007, 5096–5098.
3 D. Williamson and D. R. W. Hodgson, *Org. Biomol. Chem.*, 2008, **6**, 1056–1062.
4 R. Fiammengo, K. Musilek and A. Jaschke, *J. Am. Chem. Soc.*, 2005, **127**, 9271–9276.
5 B. L. Zhang, Z. Y. Cui and L. L. Sun, *Org. Lett.*, 2001, **3**, 275–278.
6 H. Li and M. J. Miller, *Tetrahedron Lett.*, 2000, **41**, 4323–4327.
7 D. K. Dean, *Synth. Commun.*, 2002, **32**, 1517–1521.
8 A. J. Kirby, J. E. Davies, T. A. S. Brandao, P. F. Da Silva, W. R. Rocha and F. Nome, *J. Am. Chem. Soc.*, 2006, **128**, 12374–12375; A. J. Kirby, B. S. Souza, M. Medeiros, J. P. Priebe, A. M. Manfredi and F. Nome, *Chem. Commun.*, 2008, 4428–4429.
9 H. Li and M. J. Miller, *J. Org. Chem.*, 1999, **64**, 9289–9293.
10 J. H. Marriott, M. Mottahedeh and C. B. Reese, *Tetrahedron Lett.*, 1990, **31**, 7485–7488.
11 R. V. Kolakowski, S. G. Ning and L. J. Williams, *Tetrahedron Lett.*, 2006, **47**, 1163–1166; R. V. Kolakowski, N. Shangguan, R. R. Sauers and L. J. Williams, *J. Am. Chem. Soc.*, 2006, **128**, 5695–5702.
12 K. N. Barlett, R. V. Kolakowski, S. Katukojvala and L. J. Williams, *Org. Lett.*, 2006, **8**, 823–826; X. Zhang, F. Li, X. W. Lu and C. F. Liu, *Biomacromolecules*, 2009, **20**, 197–200.
13 H. Neumann, I. Z. Steinberg and E. Katchalski, *J. Am. Chem. Soc.*, 1965, **87**, 3841–3848.