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Selective Cleavage of the O^6-Diphenylcarbamoyl Group from Sugar-modified Guanosines for Incorporation into Oligo-RNA

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Abstract: A facile conversion of 2',3',5'-*O*-tri-(Bz, Tol or Ac)-N^2-(Ac or iBu)-O^6-DPC-guanosine to N^2-(Ac or iBu)-guanosine has been achieved in 89-98% yield by short treatment with 90% aqueous TFA for smooth cleavage of the DPC group followed by treatment with sodium ethoxide in pyridine-ethanol mixture at room temperature.

At the early stage of development of the solid-phase oligo-DNA or -RNA synthesis, side reactions at the lactam function of the guanine moiety was a major concern, but it was soon found to be unnecessary to protect the O^6 or N^1 position during the preparation of phosphoramidite precursors as well as during the assemblage of oligomers.[1] On the other hand, as the preparation of ^{2}H-,[2] ^{13}C-,[3] ^{2}H/^{13}C labelled sugars,[4] as well as α-anomers,[5] L-ribo[6], carbocyclic[7] or carboacyclic[8] derivatives of guanin-9-yl block is gaining importance for incorporation into oligo-DNA or RNA for structural studies by NMR, as well as a monomer block, for development of pharmaceutically active products, it is mandatory to employ an appropriate O^6-protecting group such as the 2-(4-nitrophenyl)ethyl (NPE)[7] or diphenylcarbamoyl (DPC)[9] for the control of N^9 versus N^7 regioselectivity during glycosylation. Clearly, the DPC group is the O^6-protecting group of choice over the NPE group because of two reasons: use of basic conditions for deprotection is essentially avoided which otherwise may cause degradation of RNA (even 2'-*O*-TBDMS protected RNA is gradually degraded under basic conditions!), and the second reason is that the DPC group is known to stabilise the glycosyl bond in DNA synthesis. The main disadvantage of using the bulky O^6-DPC protected guanosine block lies in the fact that the separation of its 2'-*O*-*tert*butyldimethylsilyl (TBDMS) regioisomer (commonly used for large scale production of oligo-RNA on the solid-phase[10]) from the corresponding 3'-*O*-isomer is tedious or even impossible.[2b,3b] In attempts to selectively remove the DPC group, the use of F^- ion[5] resulted in moderate yield of the N^2 protected nucleoside (~66%) whereas deblocking with methanolic ammonia followed by reprotection with an N^2-isobutyryl (iBu) group[2b,3b] is laborious and time-consuming. We herein report a simple deprotection procedure of the O^6-DPC group of guanosine under mild acidic conditions in order to remedy some of the above problems.

The O^6-DPC group was reported to be stable under the acidic conditions normally used for the synthesis of oligo-RNA.[11] During deacetonation of 2',3'-*O*-isopropylidene-N^2-acetyl(Ac)-O^6-DPC-guanosine-5'-$^2H_1$-1',2',3',4',5'-$^{13}C_5$ by 90% aqueous trifluoroacetic acid (TFA) we observed the rapid formation of a crystalline material which proved to be the corresponding N^2-acetylguanosine derivative. To probe the synthetic utility of this finding, compounds **1** and **2** (Bz = benzoyl, Tol = 4-toluoyl), which are the standard glycosylation products obtained in the synthesis of guanosine nucleosides, were treated with 90% aqueous TFA at room temperature for 15 min.[12] This resulted in quantitative formation of compounds **3** and **4** by thin layer chromatography (95-96% after short column chromatography). The structural integrity of all products **3**-**4** was verified by their ^{1}H- and ^{13}C-NMR spectra as well as by high-resolution mass spectrometry measurements. In an attempt to follow the kinetics of the reaction, we found that only a trace of the sharp H-8 signal (δ 8.06 ppm) of **2c** was present in

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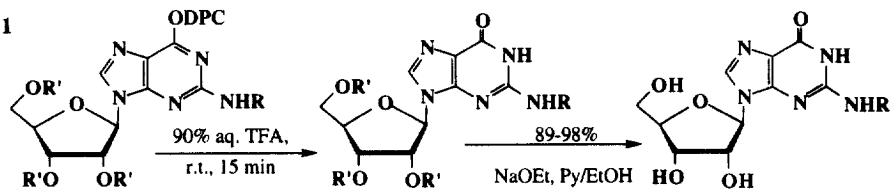
Scheme 1



1a: R = R' = Ac
1b: R = Ac; R' = Bz
1c: R = Ac; R' = Tol
2a: R = iBu; R' = Ac
2b: R = iBu; R' = Bz
2c: R = iBu; R' = Tol

3a: R = R' = Ac (96%)
3b: R = Ac; R' = Bz (96%)
3c: R = Ac; R' = Tol (95%)
4a: R = iBu; R' = Ac (94%)
4b: R = iBu; R' = Bz (98%)
4c: R = iBu; R' = Tol (96%)

5: R = Ac
6: R = iBu

the ^{1}H-NMR spectrum of the crude reaction mixture after 2 min. In a large scale (3 mmol) deprotection study, we found that the rate of **2c** going into solution in 90% aqueous TFA at room temperature was the rate determining factor for the DPC deprotection. From compounds **3** and **4**, the *O*-acyl protections were removed with sodium ethoxide (1 *N*, 8 ml/mmol substrate) in pyridine-ethanol (2:3 ml/mmol substrate) for 5 min at room temperature followed by neutralisation with Dowex 50 WX (pyridinium form)[2a,13] to afford compounds **5** and **6** (89-98%) after work-up, which involves filtration of the resin, evaporation of the volatile matter and coevaporation with toluene. In the case of the N^2-iBu derivatives **4**, the residue is dissolved in water, extracted with ethyl acetate and evaporation of the aqueous phase gives the product **6**, whereas in case of the N^2-Ac derivatives **3**, the residue is triturated twice with diethyl ether, followed by addition of methanol, which induces immediate crystallisation of compound **5**.

The same deblocking sequence was carried out in a one-pot fashion excluding the chromatographic purification of intermediates **3** and **4**. From the N^2-acetyl derivatives **1**, the removal of the *O* -protecting groups to crystalline **5** was carried out in 94-96% yield. Taking either **2a**, **2b** or **2c** as a starting material, the desired guanosine derivative **6** was easily obtained in 93-97% yield. It is noteworthy that **6** is perhaps the most widely used guanosine building block for oligo-RNA synthesis.

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12. Preparation of **3c**: 90% Aqueous TFA (10 ml) was added to compound **1** (2.62 g, 3 mmol) and the resulting mixture was stirred at r.t. for 15 min. Volatile matter are evaporated and the residue was coevaporated with toluene (3x). After work-up with sat. $NaHCO_3$ solution, short column chromatography on silica gel yielded compound **3c** (1.94 g, 2.85 mmol, 95%) as a foam.
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