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Tetrahedron Letters 47 (2006) 4201–4203
Tetrahedron Letters

Electron-deficient benzotriazoles for the selective N-acetylation of nucleosides

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Received 13 March 2006; revised 3 April 2006; accepted 10 April 2006

Abstract—The use of an acetylated benzotriazole for the selective protection of the amino groups of cytidine and 2′-deoxycytidine is reported. The use of the acetyl group is of considerable interest industrially in this role, and a single-step protection strategy advantageous in bulk production. 1-Acetyl-4-nitrobenzotriazole was found to readily acetylate the amine of cytidine preferentially over the exposed alcohol functionalities. With adaptation of the protocol, 2′-deoxycytidine was protected using the same reagent. A similar approach was attempted for the benzoylation of adenosine but was found to be unsuitable.

In accordance with the increase in research, based upon oligonucleotides and their analogues[1] including such topics as antisense therapeutics and the development of synthetic ribozymes,[2] there has been a concomitant rise in the manufacture of synthetic oligonucleotides to implement this research. Although the synthetic routes employed to produce sequence specific oligonucleotides (and a variety of analogues, depending on their intended application) are well-established and are robust methods,[3] they are open to adaptation and, where possible, improvement.[4,5]

In the final step of the phosphoramidite method of DNA synthesis, the nucleoside base protection is removed by heating at 55 °C in the presence of 28% ammonium hydroxide for 12–14 h.[6] Faster deprotection times, in the order of 2 h, can be obtained by the use of a methylamine/ammonia mixture,[7] although under these conditions the hydrolysis of the *N*4-benzoyl 2′-deoxycytidine (dC) competes with a methylamine exchange reaction, and to counter this, the *N*4-acetyl group, a more labile protecting group, is used instead.[8]

In 1995, Katritzky et al.[9] demonstrated that *N*-formylbenzotriazole acted as an effective O- and N-formylating agent. This research was later developed into a method for the introduction of a 'masked' formyl group into an organic framework by the reaction of an N-substituted benzotriazole with a nucleophilic organometallic species.[10] More generally, Katritzky et al. developed the use of *N*-acylbenzotriazoles as acylating reagents for the preparation of primary, secondary and tertiary amides,[11] eventually combining this application with solid-phase methods.[12]

During the development of this method it was noted that the use of an electron-deficient acyl group (trifluoroacetyl) gave selective acylation of an amino group in the presence of an alcohol.[13] This result suggests that a more general method of selective amine acylation could be obtained by use of an electron-deficient N-substituted benzotriazole, as the reactivity of the acylating reagent would be less dependent on the electronic nature of the acyl group: a condition well suited to the selective protection of the amino functionality of dC in the presence of the free hydroxyl groups.

This idea was supported by the work of Himmelsbach, et al.[14] who utilised an N-substituted benzotriazole species to protect selectively the N4 position of dC using an electron-deficient *para*-nitrophenylethoxycarbonyl species.

The synthesis of electron-deficient benzotriazoles has been investigated previously by us[15] and the literature precedent set out by Katritzky and Himmelsbach

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doi:10.1016/j.tetlet.2006.04.079

strongly suggests that these compounds could prove to be a useful addition to the toolkit of available protection chemistries.

Studies of several electron-deficient benzotriazole species showed that the N-acetylated derivative of 7-nitrobenzotriazole, 1-acetyl-4-nitrobenzotriazole (**1**, Fig. 1) was sufficiently stable to handle in large amounts and could be stored with relative ease. More reactive species, such as 1-acetyl-5,6-dinitrobenzotriazole (**2**, Fig. 1) were prone to decomposition within a matter of hours.

The synthesis of **1** was achieved by nitration of benzotriazole with potassium nitrate and sulfuric acid to form 7-nitrobenzotriazole, which was then refluxed in acetic anhydride to produce **1** (Scheme 1). The overall yield from benzotriazole was 84%.

Direct addition of a slight excess of **1** to a solution of cytidine at room temperature resulted in the formation of *N*-acetyl cytidine, **3**, in 89% yield. Similar reaction of **1** with 2′-deoxycytidine resulted in the formation of a mixture of N- and O-acetylated products (Scheme 2).

Using a slow addition protocol, wherein a solution of **1** was added to the reaction mixture over the course of 1 h, the technique was adapted to produce *N*-acetyl 2′-deoxycytidine, **4**, initially in 73% yield. Repeated on batches of nucleoside on gram scale, the reaction has been found regularly to achieve yields greater than 85%.

Purification of the reactions was carried out by column chromatography. Attempts at recrystallisation under a range of solvent conditions resulted in the co-crystallisation of 7-nitrobenzotriazole with both **3** and **4**.

An attempt was made to synthesise the benzoyl analogue of **1** with a view to adapt the method for the protection of adenosine and 2′-deoxyadenosine. Direct reaction of benzoic anhydride with 7-nitrobenzotriazole was unsuccessful, and N-benzoylation with benzoyl chloride resulted in the formation of a pale oil in the crude mixture which quickly degraded during attempted purification (Scheme 3).

Although the 7-nitrobenzotriazole precursor is not directly suited to adaptation for benzoyl protection, its use as an amine-selective acetylation reagent for the cytidine species shows that there is fertile ground for improvement and advancement in the use of softer acylating reagents to effect a one-step protection of nucleosides for DNA synthesis.

7-Nitrobenzotriazole: Benzotriazole (2 g, 16.8 mmol, 1 equiv) was dissolved in concentrated sulfuric acid (70 ml) and cooled to 0 °C. To this was added potassium nitrate (3.44 g, 34 mmol, 2 equiv) in small portions over 30 min. Once this had been completed, the reaction mixture was heated to 60 °C for 3 h. After cooling, the reaction mixture was poured slowly onto ice. The resultant suspension was filtered to remove the precipitate and washed thoroughly with water until the washings were consistently pH 7. After drying, 7-nitrobenzotriazole was isolated as a yellow powder, 2.39 g (87%), mp 218 °C (decomp). ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm): 15.59 (br s, <1H, N–H), 8.55 (m, 1H), 8.38 (m, 1H), 7.69 (m, 1H), *m*/*z* (FAB): 165.04 (MH^+) CHN: (exp) found: C (43.91) 43.73; H (2.46) 2.48; N (34.14) 34.34.

1-Acetyl-4-nitrobenzotriazole, **1**: 7-Nitrobenzotriazole (1 g, 6.1 mmol, 1 equiv) was suspended in excess acetic anhydride (50 ml) and heated to 80 °C for 5 h. After cooling, the excess acetic anhydride was removed in vacuo and the product was left as a pale orange solid, 1.21 g (96%), mp 143 °C. ^{1}H NMR (400 MHz, acet-

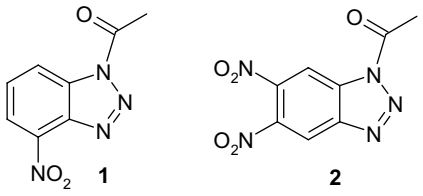
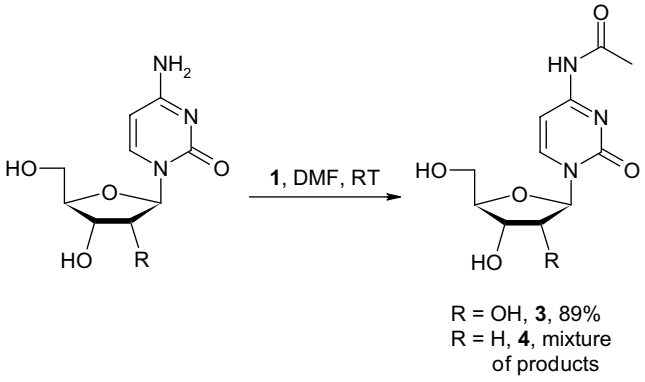
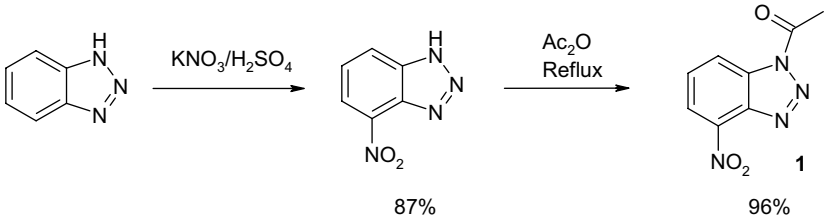


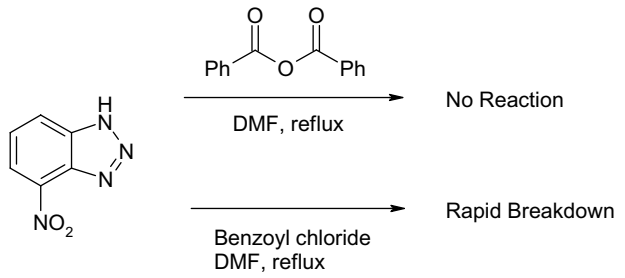
Figure 1.



Scheme 2. Direct N-acetylation of C and dC.



Scheme 1. Synthesis of **1**.



Scheme 3. Attempted syntheses of a benzoyl analogue of **1**.

one-d_6) δ ppm: 8.71 (dd, $J = 8.3$, 0.7 Hz, 1H), 8.43 (dd, $J = 7.8$, 0.7 Hz, 1H), 8.02 (m, 1H), 3.05 (s, 3H, $-CH_3$). ^{13}C NMR (100 MHz, DMSO) δ ppm: 170.0, 138.6, 138.2, 133.6, 131.1, 123.3, 121.5, 23.7, *m*/*z* (FAB): 207.1 (MH^+), CHN (exp) found: C (46.61) 46.61; H (2.93) 2.84: N (27.18) 27.08.

N-Acetyl-cytidine, **3**: Into a flame-dried flask under N_2 was weighed cytidine (0.1 g, 0.41 mmol, 1 equiv), and this was dissolved in anhydrous DMF (5 ml). Compound **1** (0.085 g, 0.41 mmol, 1 equiv) was added in small portions over 5 min, and the resultant yellow solution was left to stir at room temperature overnight. After this time, the solvent was removed in vacuo and the yellow residue loaded onto silica and purified by flash column chromatography, eluting with a gradient system of 0–5% methanol in dichloromethane. The product was obtained as a yellow solid, 0.09 g (89%). mp 123–124 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.85 (s, <1H, N–H), 8.40 (d, $J = 7.2$ Hz, 1H, 6 C–H), 7.17 (d, $J = 7.4$ Hz, 1H, 5 C–H), 5.78 (m, 1H, 1′C–H), 5.43 (m, 1H, 2′O–H), 5.13 (m, 1H, 5′O–H), 5.02 (m, 1H, 3′O–H), 3.95 (m, 2H, 5′C–H), 3.90 (m, 1H, 4′C–H), 3.72 (m, 1H, 2′C–H), 3.59 (m, 1H, 3′C–H), 2.1 (s, 3H, $-CH_3$), *m*/*z* (FAB): 286.104 (MH^+).

N-Acetyl-2′-deoxycytidine, **4**: In a flame-dried flask under N_2, 2′-deoxycytidine (0.1 g, 0.44 mmol, 1 equiv) was dissolved in anhydrous DMF (5 ml). To this was added **1** (0.091 g, 0.44 mmol, 1 equiv) as a solution in DMF (5 ml) over 1 h using a syringe pump. The reaction mixture was left to stir for 1 h, before removing the solvent in vacuo. The crude mixture was purified by column chromatography on silica, eluting with 0–10% methanol in DCM to yield 0.086 g (73%) of the desired product. Mp 150–152 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.83 (s, <1H, N–H), 8.31 (d, $J = 7.48$ Hz, 1H, 6 C–H), 7.18 (d, $J = 7.44$ z, 1H, 5 C–H), 6.10 (m, 1H, 1′C–H), 5.24 (m, <1H, 3′O–H), 5.01 (m, <1H, 5′O–H), 4.21 (m, 1H, 3′C–H), 3.84 (m, 1H, 4′C–H), 3.57 (m, 2H, 5′C–H), 2.30 (m, 1H, 2′C–H), 2.09 (s, 3H, $-CH_3$), 2.02 (m, 1H, 2′C–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.92 (amide C=O), 163.20, 155.39, 145.91, 96.15, 88.84, 87.08, 61.9, 25.28 (acetyl $-CH_3$), *m*/*z* (FAB): 270.11 (MH+). CHN (exp) found: C (49.07) 48.46; H (5.62) 5.65; N: (15.61) 15.34.

Acknowledgement

The authors would like to acknowledge Avecia for funding the supply of raw materials, and for their valuable input.

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