A practical synthesis of 2-chloro-2′-deoxyadenosine (Cladribine) from 2′-deoxyadenosine

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A practical synthesis of 2-chloro-2′-deoxyadenosine (Cladribine) from 2′-deoxyadenosine is reported. Treatment of fully protected 2′-deoxyadenosine with 2,2,2-trifluoroacetic anhydride and tetrabutylammonium nitrate gave protected 2-nitro-2′-deoxyadenosine with high yield. 2-Chloro-2′-deoxyadenosine was synthesised in four steps and 44.8% yield after substitution by chloride and deprotection steps.

Keywords: 2-chloro-2′-deoxyadenosine (Cladribine), practical synthesis, nitration, 2′-deoxyadenosine

2-Chloro-2′-deoxyadenosine (**1**, Cladribine), an analogue of 2′-deoxyadenosine, is a deaminase inhibitor used for the treatment of hairy cell leukaemia[1] and acute leukaemia[2]. It also shows significant activity against chronic lymphocytic leukaemia[3], lymphoid neoplasms[4] and non-Hodgkin's lymphoma[5].

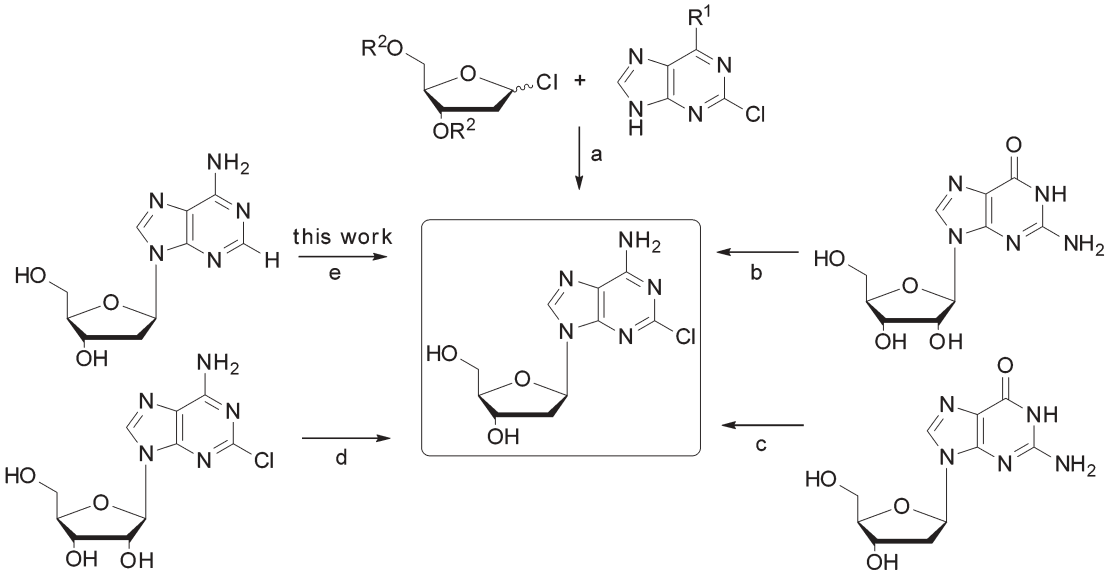
The first route to 2-chloro-2′-deoxyadenosine is through purine-sugar coupling (route a). Ikehara and Tada[6] firstly synthesised 2-chloro-2′-deoxyadenosine as an intermediate by this method. Robins[7] reported direct glycosylation with a 2-deoxy-chloro sugar of the sodium salt of 6-imidazolylpurine followed by ammonolysis, avoiding of anomeric mixtures and N7/N9 isomers successfully. The second route is through a modification of available nucleosides. Chen[8] synthesised 2-chloro-2′-deoxyadenosine from guanosine (2.8% overall yield) in eight steps (route b). Robins[9] reported a concise synthesis of 2-chloro-2′-deoxyadenosine from 2′-deoxyguanosine with 75% yield (route c). Xu[10] reported an improving method to synthesise 2-chloro-2′-deoxyadenosine from 2-chloroadenosine in five steps and 31.0% yield (route d). The third route is through an enzymatic glycosyl transfer catalysed by *trans-N*-deoxyribosylase from available 2′-deoxy nucleosides and 2-chloroadenine in low yields (< 27%)[11,12].

The disadvantages associated with these methods are the involvement of expensive starting materials, too many synthetic operations and low yields. There is a demand for a more practical method to synthesise 2-chloro-2′-deoxyadenosine. 2′-Deoxyadenosine (99% purity, 56.6$/g, Sigma-Aldrich) is much cheaper than 2-chloroadenosine (97% purity, 223$/50 mg, Sigma-Aldrich) or 2′-deoxyguanosine (99% purity, 416.7$/g, Sigma-Aldrich). It would be a practical route to synthesise 2-chloro-2′-deoxyadenosine from 2′-deoxyadenosine. We now describe the efficient and practical synthesis of 2-chloro-2′-deoxyadenosine from 2′-deoxyadenosine.

Results and discussion

Purines can be regioselectively nitrated at the 2 position under mild conditions with tetrabutylammonium nitrate and trifluoroacetic anhydride.[13,14] The nitro group was readily replaced by nucleophilic halogens (Cl, F).[15–16] This nitration/S_NAr method was successfully applied to synthesise Fludarabine and 2-fluoroadenosine.[14]

The amino group and hydroxyl groups of 2′-deoxyadenosine were protected with a benzoyl group following the reported method.[17] The substrates with activated NH or OH gave side reactions during the nitration step. *N*^{6},*N*^{6}-Dibenzoyl-3′,5′-*O*-dibenzoyl-2′-deoxyadenosine (**3**) was obtained with 95% yield. We also tried the acetyl group as protecting group, but the product was a syrup which was difficult to purify. Furthermore, the acetyl group was more easily displaced than the benzoyl group and it was not stable in the following nitration step. The nitration was realised with tetrabutylammonium nitrate and trifluoroacetic anhydride in dichloromethane which produced $F_3COO^-NO_2^+$ as an *in situ* nitration reagent. The NO_2^+ reacted at the 2-position of the purine selectively. Under the optimised conditions, 2-nitro-*N*^{6},*N*^{6}-dibenzoyl-3′,5′-*O*-dibenzoyl-2′-deoxyadenosine (**4**) was obtained in 76%



Scheme 1 The routes to 2-chloro-2′-deoxyadenosine.

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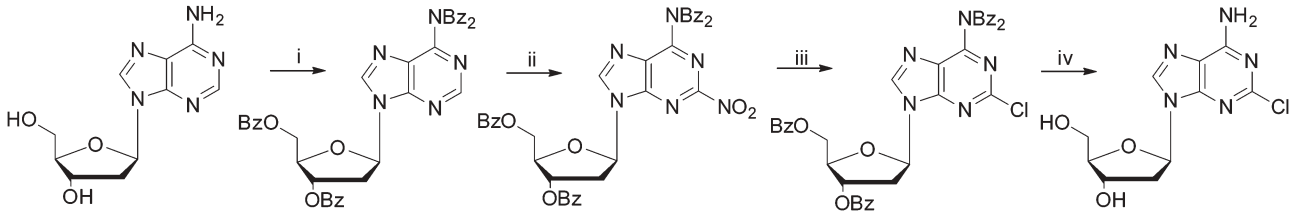

i benzoyl chloride, pyridine, 65 °C, 4 h.
ii 2,2,2-trifluoroacetic anhydride, tetrabutylammonium nitrate, CH_2Cl_2, 3 h.
iii ammonium chloride, DMF, 4 h.
iv NH_3/MeOH, 0 °C, 12 h.

Scheme 2 Synthesis of 2-chloro-2′-deoxyadenosine from 2′-deoxyadenosine.

yield. When we replaced tetrabutylammonium nitrate by the equivalent tetramethylammonium nitrate or ammonium nitrate, the yield decreased to 42% and 36% respectively. The main reason for a lower yield was the poor solubility of tetramethylammonium nitrate or ammonium nitrate in dichloromethane. The substitution of nitro group was carried out in DMF (N,N-dimethylformamide) with ammonium chloride. Other polar solvents such as acetonitrile or ethanol gave lower yields. The soluble chloride salts, such as tetrabutylammonium chloride or tetramethyl ammonium chloride, gave similar results.

Conclusion

In summary, we have described a practical and efficient method to synthesise 2-chloro-2′-deoxyadenosine from the commercially available and cheaper 2′-deoxyadenosine. The key step was the selective nitration of the purine 2 position. The nitro group was replaced by chloride through a nucleophilic aromatic substitution. 2-Chloro-2′-deoxyadenosine was synthesised in four steps and 44.8% yield. This method provided clear cost advantages over the published synthesis. The efforts to improve the batch process with respect to substrate scope are ongoing in our laboratory.

Experimental

^{1}H and ^{13}C NMR spectra were determined on a Bruker AC 400 spectrometer (Bruker, Billerica, MA, USA) as DMSO-d_6 solution. Chemical shifts were expressed in parts per million (δ) downfield from the internal standard tetramethylsilane and were reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (doublet of doublets) and coupling constants *J* were given in hertz (Hz). Mass spectra were obtained on a Waters Q-Tof MicroTM spectrometer (Waters Synapt).

N⁶,N⁶-Dibenzoyl-3′,5′-O-dibenzoyl-2′-deoxyadenosine (**3**): Benzoyl chloride (1.15 mL, 10 mmol) was slowly added to a magnetically stirred suspension of 2′-deoxyadenosine (**2**, 0.502 g, 2 mmol) in pyridine (5 mL). The mixture was stirred and heated to 65 °C, keeping at this temperature for 4 h. The solvent was removed under reduced pressure. The residue was purified by recrystallisation from acetone/EtOH to give the desire product 1.26 g, 95%. White solid, m.p. 172–174 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (s, 1H, 8-H), 8.24 (s, 1H, 2-H), 8.10 (d, *J* = 7.2 Hz, 2H, Ar), 8.04 (d, *J* = 7.2 Hz, 2H, Ar), 7.86 (d, *J* = 7.2 Hz, 4H, Ar), 7.65 (t, *J* = 7.6 Hz, 1H, Ar), 7.59 (t, *J* = 7.6 Hz, 1H, Ar), 7.51–7.41 (m, 6H, Ar), 7.37 (t, *J* = 7.6 Hz, 4H, Ar), 6.60 (t, *J* = 6.6 Hz, 1H, 1′-H), 5.86 (d, *J* = 2 Hz, 1H, 3′-H), 4.79 (dd, *J* = 4.3, 1.3 Hz, 2H, 5′-H), 4.68–4.66 (m, 1H, 4′-H), 3.26–3.20 (m, 1H, 2′-H), 2.87–2.82 (m, 1H, 2′-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.3, 165.9, 153.1, 152.4, 149.8, 140.3, 134.2, 133.0, 132.1, 130.1, 129.9, 128.9, 128.8, 128.6, 127.8, 127.5, 126.4, 92.6, 84.5, 74.6, 63.6, 36.6. HRMS calcd for $C_{38}H_{30}N_5O_7$ $[M + H]^+$ 668.2141, found 668.2141.

2-Nitro-N⁶,N⁶-dibenzoyl-3′,5′-O-dibenzoyl-2′-deoxyadenosine (**4**): The nitrating mixture was prepared by adding 2,2,2-trifluoroacetic anhydride (0.209 mL, 1.5 mmol) to a solution of tetrabutylammonium nitrate (0.457 g, 1.5 mmol) in anhydrous CH_2Cl_2 (1 mL) at 0 °C. After 20 min the nitrating mixture was added to **3** (0.668 g, 1 mmol) in anhydrous CH_2Cl_2 (1 mL) at 0 °C. After 3 h at 0 °C, the reaction mixture was poured into a cold mixture of H_2O (10 mL), aqueous $NaHCO_3$ (10 mL) and CH_2Cl_2 (5 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 5 mL). The combined organic extracts were washed with brine (2 × 10 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by chromatography on silica gel, eluting with CH_2Cl_2–MeOH (19:1), 0.542 g, 76%. Yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (s, 1H, 8-H), 8.13 (d, *J* = 7.1 Hz, 2H, Ar), 8.06 (d, *J* = 7.1 Hz, 2H, Ar), 7.82 (d, *J* = 7.1 Hz, 4H, Ar), 7.60 (t, *J* = 7.4 Hz, 1H, Ar), 7.57 (t, *J* = 7.4 Hz, 1H, Ar), 7.47–7.39 (m, 6H, Ar), 7.39 (t, *J* = 7.4 Hz, 4H, Ar), 6.64 (t, *J* = 6.6 Hz, 1H, 1′-H), 5.79 (d, *J* = 2.2 Hz, 1H, 3′-H), 4.84 (dd, *J* = 4.5, 1.4 Hz, 2H, 5′-H), 4.65–4.59 (m, 1H, 4′-H), 3.22–3.18 (m, 1H, 2′-H), 2.88–2.84 (m, 1H, 2′-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.3, 169.9, 153.1, 152.4, 149.8, 140.3, 134.2, 133.0, 132.1, 130.1, 129.9, 128.9, 128.8, 128.6, 127.8, 127.5, 126.4, 92.6, 84.5, 74.6, 64.1, 36.9. HRMS calcd for $C_{38}H_{29}N_6O_9$ $[M + H]^+$ 713.1996, found 713.1992.

2-Chloro-N⁶,N⁶-dibenzoyl-3′,5′-O-dibenzoyl-2′-deoxyadenosine (**5**): Ammonium chloride (0.053, 1 mmol) was added to a solution of **4** (0.356 g, 0.5 mmol) in DMF (2 mL) and the mixture was stirred for 4 h at ambient temperature. H_2O (2 mL) was added and the mixture was extracted with CH_2Cl_2 (2 × 2 mL). The combined organic extracts were washed with H_2O (2 mL) and brine (2 × 2 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by chromatography on silica gel, eluting with CH_2Cl_2–MeOH (19:1), 0.218 g, 62%. Light yellow solid, m.p. 190–192 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (s, 1H, 8-H), 8.09 (d, *J* = 7.1 Hz, 2H, Ar), 8.02 (d, *J* = 7.2 Hz, 2H, Ar), 7.80 (d, *J* = 7.2 Hz, 4H, Ar), 7.63 (t, *J* = 7.2 Hz, 1H, Ar), 7.58 (t, *J* = 7.2 Hz, 1H, Ar), 7.42–7.40 (m, 6H, Ar), 7.36 (t, *J* = 7.2 Hz, 4H, Ar), 6.61 (t, *J* = 6.4 Hz, 1H, 1′-H), 5.80 (d, *J* = 2.2 Hz, 1H, 3′-H), 4.81 (dd, *J* = 4.5, 1.4 Hz, 2H, 5′-H), 4. 56–4.51 (m, 1H, 4′-H), 3.20–3.17 (m, 1H, 2′-H), 2.82–2.79 (m, 1H, 2′-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.3, 167.0, 152.8, 152.0, 149.3, 140.3, 134.2, 133.0, 132.1, 130.1, 129.9, 128.9, 128.8, 126.9, 127.8, 125.7, 126.4, 92.6, 84.3, 75.1, 63.9, 36.5. HRMS calcd for $C_{38}H_{29}ClN_5O_7$ $[M + H]^+$ 702.1756, found 702.1752.

2-Chloro-2′-deoxyadenosine, Cladribine (**1**): **5** (0.351 g, 0.5 mmol) was added to saturated NH_3/MeOH solution (10 mL) at 0 °C, the reaction vessel was sealed and stirred at room temperature for 12 h. Volatiles were evaporated. The product was recrystallised from MeOH, 0.133 g, 95%. The m.p. and NMR data were in agreement with published values[9]. White solid, m.p. 306–310 °C (dec.), lit.[9] > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.36 (s, 1H, 8-H), 7.89 (brs, 2H, NH_2), 6.13 (t, *J* = 6.4 Hz, 1H, 1′-H), 5.24 (d, *J* = 4 Hz, 1H, 3′-H), 5.14 (t, *J* = 4.8 Hz, 1H, 4′-H), 4.24–4.22 (m, 2H, OH), 3.60 (dd, *J* = 3.2 Hz, 2H, 5′-H), 2.13 (dd, *J* = 3.4 Hz, 2H, 2′-H); ^{13}C NMR (100 MHz, *d*6-DMSO) δ 157.5, 153.6, 150.8, 140.5, 118.8, 88.6, 84.2, 71.4, 62.3, 38.0; HRMS calcd for $C_{10}H_{13}ClN_5O_3$ $[M + H]^+$ 286.0707, found 286.0701.

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