Efficient Syntheses of 2-Chloro-2′-deoxyadenosine (Cladribine) from 2′-Deoxyguanosine

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We report efficient syntheses of the clinical agent cladribine (2-chloro-2′-deoxyadenosine, Clad), which is the drug of choice against hairy-cell leukemia and other neoplasms, from 2′-deoxyguanosine. Treatment of 3′,5′-di-O-acetyl- or benzoyl-2′-deoxyguanosine (1) with 2,4,6-trisopropyl- or 4-methylbenzenesulfonyl chloride gave high yields of the 6-O-arylsulfonyl derivatives 2 or 2b. Deoxyclorination at C6 of 1 also proceeded to give the 2-amino-6-chloropurine derivative 5 in excellent yields. The nonaqueous diazotization/chloro dedaa formulation (acetyl chloride/benzylthiethyl-ammonium nitrite) of 2, 2b, and 5 gave the 2-chloropurine derivatives 3, 3b, and 6, respectively. The selective ammonolysis at C6 (arylsulfonate with 3 or chloride with 6) and accompanying deprotection of the sugar moiety gave Clad (64−75% overall yield from 1).

Introduction

The lymphoselective toxicity of 2-chloro-2′-deoxyadenosine (Clad, cladribine) and its potential as a chemotherapeutic agent against lymphoid neoplasms were reported by Carson et al.2 This potent, deaminase-resistant analogue of 2′-deoxyadenosine (dAdo) is currently the drug of choice for hairy-cell leukemia.3,4 It also shows significant activity against chronic lymphocytic leukemia,5,6 indolent non-Hodgkin’s lymphoma,7 and Waldenström’s macroglobulinemia.8 It also shows significant activity against chronic lymphocytic leukemia,9,6 indolent non-Hodgkin’s lymphoma,7 and systemic lupus erythematosus-associated glomerulonephritis,10 and other rheumatoid and immune disorders are in progress. Cladribine is a nucleoside prodrug, which is phosphorylated by deoxycytidine kinase to CladAMP and then sequentially to CladADP and the active CladATP.2b,11a Cladribine also is a good substrate for mitochondrial 2′-deoxyguanosine (dGuo) kinase,11 and induction of programmed cell death by direct effects on mitochondria has been implicated in its potent activity against indolent lymphoid malignancies (via apoptosis) as well as in proliferating cells.12,13 Venner reported the Fischer–Helfrich syntheses of naturally occurring 2′-deoxynucleosides in 196014 and employed Clad as an intermediate for 2′-deoxyguanosine and -inosine. Ikehara and Tada also synthesized dAdo with Clad as an intermediate [obtained by the desulfurization of 8,2′-anhydro-9-(β-d-arabinofuranosyl)-2-chloro-8-thioadene].15 The syntheses of Clad as a target compound have exploited the greater reactivity of leaving groups at C6 relative to those at C2 of the purine ring in S₃Ar displacement reactions. Robins and Robins16 employed the fusion coupling of 2,6-dichloropurine with 1,3,5-tri-O-acetyl-2-deoxy-α-d-ribofuranose. The 9-(3,5-di-O-acetyl-2-deoxy-α-d-erythro-pentofuranosyl)-2,6-dichloropurine anomer was obtained by fractional crystallization. The selective ammonolysis at C6 and accompanying deprotection gave 6-amino-2-chloro-9-(2-deoxy-α-d-erythro-pentofuranosyl)purine. The pharmacologically active β anomer (cladribine) was prepared by an analogous coupling, chromatographic separation of anomers, and ammonolysis.17


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The stereoselective glycosylation of the sodium salts of halopurines and analogues with 2-deoxy-3,5-di-O-p-toloyl-α,β-erythro-pentofuranosyl chloride gave β-nucleoside anomers via predominantly Walden inversion,18,19 and ammonolysis/deprotection gave CldAdo.20 Although the sodium salt glycosylation procedure usually gave good anomeric stereoselectivity, minor quantities of α anomers and >10% of the N7 regioisomers were usually formed.21,22 This requires separations and results in diminished yields of the desired N9 product. Carson et al.23 had reported an enzymatic transfer of the 2-deoxy sugar from thymidine to 2-chloroadenine (ClAde). Holy and co-workers noted that cells of a strain of Escherichia coli performed glycosyl transfer from 2-deoxyuridine to 2-chloro-6-(dimethylaminomethylene)aminopurine to give a derivative of CldAdo.24 Very recently Barai and co-workers described an E. coli-mediated glycosyl transfer synthesis of 2,6-diamino-9-(3-deoxy-β,β-erythro-pentofuranosyl)purine25 and its enzymatic deamination to 3′-deoxyguanosine.26 They reported glycosyl transfer from 2′-deoxyguanosine to ClAde and claimed an 81% yield of CldAdo (on the basis of ClAde).26 However, a 3:1 ratio of CldAdo with the retention of both β-anomeric stereoisomer and N9-isomeric purity.

We have recently reported improved methods for the replacement of an amino group on purine nucleoside derivatives with chlorine, bromine, or iodine under nonaqueous conditions.1,2 These mild diazotization/halo dediazotiation methods were found to be applicable at nonaqueous conditions.1,28 These mild diazotization/chloro dediazoniation of the 2-amino function of dGuo into the appropriate leaving groups, dGuo required the efficient transformation of the 6-oxo function into good leaving groups without protection of the 6-oxo moiety, chloro dediazotiation at C2, and selective ammonolysis at C6 with accompanying sugar deprotection. Two approaches we evaluated for functionalization at C6 were arylsulfonylation of O6 and chloro-deoxygenation at C6.

Our chemical approach for the synthesis of regio- and stereochemically pure CldAdo, which avoids the separation of mixtures with fusion and sodium salt glycosylation procedures, employs the transformation of naturally occurring mixtures with fusion and sodium salt glycosylation procedures, employs the transformation of naturally occurring nucleosides.27 The conversion of the 6-oxo function of dGuo into the appropriate leaving groups, dGuo required the efficient transformation of the 6-oxo function into good leaving groups without protection of the 6-oxo moiety, chloro dediazotiation at C2, and selective ammonolysis at C6 with accompanying sugar deprotection. Two approaches we evaluated for functionalization at C6 were arylsulfonylation of O6 and chloro-deoxygenation at C6.

Our projected synthesis of CldAdo (Scheme 1) from dGuo required the efficient transformation of the 6-oxo function into good leaving groups without protection of the 6-oxo moiety, chloro dediazotiation at C2, and selective ammonolysis at C6 with accompanying sugar deprotection. Two approaches we evaluated for functionalization at C6 were arylsulfonylation of O6 and chloro-deoxygenation at C6.

Several acyl-protected 6-O-sulfonyl derivatives of dGuo are readily available.29–31 The treatment of 3′,5′-di-O-acetyl-3′-deoxyguanosine32 (1a) or its 3′,5′-di-O-benzoyl analogue 1b32 with TiPBS–Cl/Et3N/DMAP/CHCl3 by a modification of the method of Hata et al.30 gave the 6-O-TiPBS derivatives 2a31 (91%) and 2b31 (86%), respectively. The similar treatment of 1b with TsCl/Et3N/DMAP/CHCl3 gave the 6-O-Ts derivative 2b (89%). Efficient displacement of sulfonate from C6 required a sterically hindered arylsulfonyl derivative. Our attempts with a more economical 6-O-Ts derivative gave poor yields at

![Scheme 1](image)

Scheme 1

(a) TiPBS–Cl/Et3N/DMAP/CHCl3. (b) AcCl/BTEA-NO2/CH2Cl2, -5 to 0 °C. (c) NH3/MeOH/CH2Cl2/Δ. (d) POCl3/BTEA-Cl/N,N-dimethylaniline/Mecn/Δ.

benzyltriethylammonium nitrite (BTEA-NO2)-mediated chloro dediazotiation of 6-O,2,4,6-trisopropylbenzenesulfonyl (TiPBS) or 6-chloro derivatives that are readily obtained from dGuo.

**Results and Discussion**

Our projected synthesis of CldAdo (4; Scheme 1) from dGuo required the efficient transformation of the 6-oxo function into good leaving groups without protection of the 6-oxo moiety, chloro dediazotiation at C2, and selective ammonolysis at C6 with accompanying sugar deprotection. Two approaches we evaluated for functionalization at C6 were arylsulfonylation of O6 and chloro-deoxygenation at C6.


the final stage owing to attack of ammonia at both the sulfonyl sulfur and C6 (3b) gave 4 in 43% yield. By contrast, ammonolysis of the 6-O-TIPBS derivatives proceeded efficiently at C6 with minimal attack at the hindered sulfur atom. Both types of the arylsulfonate derivatives, and especially the 6-OTs, underwent increased nucelophilic attack at the sulfur with lower temperatures (−20 to 0 °C) to give 6-oxopurine derivatives. However, treatment of the 6-O-TIPBS compounds with NH3/MeOH/CH2Cl2 in a pressure tube at 80 °C strongly favored nucelophilic attack at C6 to give good yields of Cl-Ado. This suggests a crossover of the activation-energy profiles for nucleophilic attack at the sulfonyl sulfur versus C6 between 0 and 80 °C.

Chlorine has been the most frequently used leaving group in sulfonyl chemistry. Chlorination of the acid-labile 2-amino group of purines, such as 2-amino-9-(3,5-di-tert-butylbenzenesulfonyl)purine (2a), proceeded efficiently at C6 with minimal attack at the other sulfonate sulfur atoms for formation of the 6-oxopurine derivative (3a) with 95% yield. The replacement of the 2-amino group of 2a with other nucleosides gave the indicated products and quantities.

**Experimental Section**

The melting points for 4 were determined with a hot-stage apparatus. UV spectra were recorded with solutions in MeOH. 1H NMR spectra were recorded at 300 MHz with solutions in CDC13 unless otherwise indicated. "Apparent" peak shapes are in quotation marks when the first-order splitting should be more complex or when the peaks were poorly resolved. Mass spectra were determined with FAB (glycerol) unless otherwise indicated. The chemicals and solvents were of reagent quality. CH2Cl2 and MeCN were distilled over P2O5 and distilled. AcCl, POCl3, and N,N-dimethylaniline were freshly distilled before use. BTEA-NO2 was prepared from BTEA-Cl by ion exchange (Dowex 1×2 (N2+)). Column chromatography (silica gel, 230–400 mesh) was performed with CH2Cl2/MEOH. Compounds 1a and 1b were prepared as described.32

Method 1 (nucleoside/TIPBS–Cl/DMAP/ Et3N/CHCl3) is described for 1a → 2a, method 2 (nucleoside/ACl2/TEA-NO2/ CH2Cl2) for 2a → 3a, method 3 (nucleoside/N,N-dimethylaniline/ POCl3) for 3a → 2a, and method 4 (nucleoside/ NH3/MeOH/CH2Cl2/Δ) for 3a → 4. Analogous reactions with equivalent molar proportions of other nucleosides gave the indicated products and quantities.

9-(3,5-Di-O-acetyl-2-deoxy-β-D-erythro-pentofuranosyl)-2-amino-6-O-(2,4,6-triisopropylbenzenesulfonyl)purine (2a).

Method 1. Et3N (1.25 mL, 910 mg, 9.0 mmol) was added to a solution of 1a (1.67 g, 4.8 mmol), TipPBS–Cl (2.73 g, 9.0 mmol), and DMAP (72 mg, 0.6 mmol) in dry CHCl3 (70 mL) under N2. Stirring was continued for 24 h, and volatiles were evaporated. The orange residue was chromatographed (CH2Cl2/MeOH) to give 2a (2.67 g, 91%) as a slightly yellow foam: UV λmax 238, 291 nm, λmin 264 nm; 1HNMR (500 MHz) δ 1.26–1.32 (m, 13H), 2.08 (s, 3H), 2.14 (s, 3H), 2.54 (dd, J = 4.7, 9.0, 14.0 Hz, 1H), 2.91–2.99 (m, 2H), 4.22–4.37 (m, 3H), 4.43–4.47 (m, 2H), 4.97 (br s, 2H), 5.41–5.42 (“d”, 1H), 6.26–6.29 (m, 1H), 7.21 (s, 2H), 7.84 (s, 1H); LRMS m/z 618 (MH+ [C39H43N5O8SNa]+) = 618; HRMS m/z 640.4213 (MNa+ [C39H44N5O8SNa]+ = 640.2147).

2-Amino-9-(3,5-di-O-benzoyl-2-deoxy-β-D-erythro-pentofuranosyl)-6-O-(2,4,6-triisopropylbenzenesulfonyl)purine (2b).

Method 1 (nucleoside/TeA-NO2/CH2Cl2) was performed with 2a (1.68 g, 89%) as a white solid foam: UV λmax 289 nm, λmin 264 nm; 1HNMR (500 MHz) δ 1.29–1.32 (m, 18H), 2.76 (d, δ J = 2.1, 6.0, 14.3 Hz, 1H), 2.96 (“quint”, δ J = 6.8 Hz, 1H), 3.15–3.25 (m, 1H), 4.34 (“quint”, δ J = 6.8 Hz, 2H), 4.65–4.74 (m, 2H), 4.85–4.90 (m, 1H), 5.00 (br s, 2H), 5.62–5.63 (“d”, 1H), 6.38–6.43 (m, 1H), 7.30 (s, 2H), 7.44–7.55 (m, 4H), 7.58–7.69 (m, 2H), 7.85 (s, 1H), 8.04 (d, δ J = 7.1 Hz, 2H), 8.11 (d, δ J = 7.1 Hz, 2H); LRMS m/z 742 (MH+ [C39H42N5O8SNa]+) = 742, (MNa+ [C39H42N5O8Na]+) = 764; HRMS m/z 764.2730 (MNa+ [C39H43N5O8SNa]+ = 764.2730).

2-Amino-9-(3,5-di-O-benzoyl-2-deoxy-β-D-erythro-pentofuranosyl)-6-O-(4-methylbenzenesulfonyl)purine (2b).

Method 2. A solution of 2b (1.27 g, 86%) as a white solid foam: UV λmax 238 nm; 1HNMR (500 MHz) δ 2.43 (s, 3H), 2.73 (d, δ J = 2.1, 8.4, 14.4 Hz, 1H), 3.17–3.27 (“quint”, δ J = 7.2 Hz, 1H), 4.52–4.65 (m, 3H), 5.76–5.78 (“d”, 1H), 6.37–6.41 (m, 1H), 6.95 (br s, 2H), 7.47–7.73 (m, 8H), 7.95 (d, δ J = 7.8 Hz, 2H), 8.03–8.11 (m, 4H), 8.30 (s, 1H); LRMS m/z 630 (M+ [C39H25N5O8S]+ = 630), 652 (MNa+ [C39H26N5O8SNa]+ = 652); HRMS m/z 652.1467 (MNa+ [C39H26N5O8Na]+ = 652.1478).

9-(3,5-Di-O-acetyl-2-deoxy-β-D-erythro-pentofuranosyl)-2-chloro-6-O-(2,4,6-triisopropylbenzenesulfonyl)purine (3a).

Method 2. A solution of 2b (1.68 g, 89%) as a white solid foam: UV λmax 289 nm; 1HNMR (500 MHz) δ 2.43 (s, 3H), 2.73 (d, δ J = 2.1, 8.4, 14.4 Hz, 1H), 3.17–3.27 (“quint”, δ J = 7.2 Hz, 1H), 4.52–4.65 (m, 3H), 5.76–5.78 (“d”, 1H), 6.37–6.41 (m, 1H), 6.95 (br s, 2H), 7.47–7.73 (m, 8H), 7.95 (d, δ J = 7.8 Hz, 2H), 8.03–8.11 (m, 4H), 8.30 (s, 1H); LRMS m/z 630 (M+ [C39H25N5O8S]+ = 630), 652 (MNa+ [C39H26N5O8SNa]+ = 652); HRMS m/z 652.1467 (MNa+ [C39H26N5O8Na]+ = 652.1478).
H₂O (100 mL) / CH₂Cl₂ (100 mL). The layers were separated, and the organic phase was washed with cold (0 °C) H₂O (2 × 100 mL) and dried (MgSO₄) for 1 h. Volatiles were evaporated, and the residue was chromatographed (CH₂Cl₂ / MeOH) to give 3a (267 mg, 89%) as a white solid foam: UV λ_max 230, 266 nm, λ_min 255 nm; ¹H NMR δ 1.22–1.34 (m, 18H), 2.92–3.00 (m, 3H), 4.30–4.34 (m, 2H), 6.85–7.72 (m, 1H), 8.00 (d, J = 8.4 Hz, 2H), 8.10 (d, J = 8.4 Hz, 2H), 8.26 (s, 1H); HRMS m/z 783.2224 (MNa⁺ [C₃₀H₂₈ClN₄O₈S] = 783.2231).

9-(3,5-Di-O-benzoyl-2-deoxy-β-D-erythro-pentofuranosyl)-2-chloro-6-O(2,4,6-triisopropylbenzenesulfonyl)purine (3b). The treatment of 2b (1.45 g, 2.3 mmol) by method 2 gave 3b (1.30 g, 87%) as a slightly yellow foam: UV λ_max 267 nm, λ_min 255 nm; ¹H NMR (DMSO-d₆) δ 2.45 (s, 3H), 2.86 (ddd, J = 3.4, 6.2, 14.0 Hz, 1H), 3.17–3.20 (dd, J = 7.0 Hz, 1H), 4.73–4.87 (m, 1H), 5.64–5.84 (m, 1H), 7.42–7.72 (m, 1H), 7.88 (d, J = 7.8 Hz, 2H), 8.04–8.07 (m, 1H), 8.14 (s, 1H); HRMS m/z 671.0983 (MNa⁺ [C₁₉H₁₆Cl₂N₄O₅Na] = 671.0979).

9-(3,5-Di-O-acetyl-2-deoxy-β-D-erythro-pentofuranosyl)-2-amino-6-chloropurine (5a). Method 3. A mixture of 1a (540 mg, 1.54 mmol), BTEA·CI (710 mg, 3.1 mmol), N,N-dimethylamine (215 µL, 206 mg, 1.7 mmol), and POCI₃ (720 µL, 1.2 g, 7.7 mmol) in MeCN (6 mL) was stirred in a preheated oil bath (85 °C) for 10 min. Volatiles were flash evaporated immediately (in vacuo), and the yellow foam was dissolved (CHCl₃, 15 mL) and stirred vigorously with crushed ice for 15 min. The layers were separated, and the aqueous phase was extracted (CHCl₃, 3 × 5 mL). Crushed ice was frequently added to the combined organic phase, which was washed [ice H₂O (3 × 5 mL), 5% NaHCO₃ / H₂O (to pH ~7)] and dried (MgSO₄). Volatiles were evaporated, and the residue was chromatographed (CH₂Cl₂ / MeOH) to give 5a (517 mg, 90%) as a white solid foam: UV λ_max 230, 268, 310 nm, λ_min 268 nm; ¹H NMR (DMSO-d₆) δ 2.02 (s, 3H), 2.08 (s, 3H), 2.49–2.52 (m, 1H), 3.04–3.06 (m, 1H), 4.20–4.29 (m, 3H), 5.32–5.34 (dd, 1H, 6.23–6.26 (m, 1H), 7.03 (br s, 2H), 8.35 (s, 1H); HRMS m/z 535.0552 (MNa⁺ [C₁₉H₁₂O₂ClN₄O₆S] = 535.0552).

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Supporting Information Available: ¹H NMR spectra of compounds 2a, 2b, 2b, 3a, 3b, 3b, 5b, and 6b. This material is available free of charge via the Internet at http://pubs.acs.org.

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