Research Article

Synthesis of deuterated-BCX-1777, a potent inhibitor of purine nucleoside phosphorylase

Hollis S. Kezar III[1], Tracy L. Hutchison[1], Peter C. Tyler[2] and Philip E. Morris Jr.[1,*]

[1] *BioCryst Pharmaceuticals, Inc., 2190 Parkway Lake Drive, Brimingham, Alabama 35244, USA*
[2] *Carbohydrate Chemistry, Industrial Research Limited, P.O. Box 31310, Lower Hutt, New Zealand*

Summary

BCX-1777, a novel inhibitor of the enzyme purine nucleoside phosphorylase, mimics the charged ribosyl oxocarbenium ion formed during the transition state of the enzyme-catalyzed C–N bond cleavage of nucleosides. BCX-1777 is a slow-onset, tight-binding inhibitor with a K_i^* of 23 pM and is one of the most potent inhibitors known for the enzyme. In support of our BCX-1777 program, a mass spectrometric assay has been developed utilizing 5′-[^{2}H]-BCX-1777 as an internal standard. The synthesis of 5′-[^{2}H]-BCX-1777 is described in this report. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: BCX-1777; purine nucleoside phosphorylase; T-Cell

Introduction

The enzyme purine nucleoside phosphorylase (PNP, EC 2.4.2.1) catalyzes the reversible cleavage of purine nucleosides to the corresponding purine base and sugar phosphate in the purine salvage pathway as shown.[1]

*Correspondence to: P. E. Morris, Biocryst Pharmaceuticals, Inc., 2190 Prakway Lake Drive, Birmingham, Alabama, AL 35244 USA. E-mail: pmorris@biocryst.com

In the absence of PNP, nucleoside substrates such as 2′-deoxyguanosine (dGuo) accumulate. dGuo accumulation has been observed in children with inherited PNP deficiency and as a consequence, these children exhibit severe T-cell immunodeficiency but retain normal or exaggerated B-cell function.[2] T-cell cytotoxicity is due to phosphorylation of dGuo (via 2′-deoxycytidine kinase, dCK, (EC 2.7.1.74)) to 2′-deoxyguanosine triphosphate (dGTP). dGTP allosterically inhibits the enzyme ribonucleotide diphosphate reductase (EC 1.17.4.1), preventing DNA synthesis and hence T-cell proliferation.[3] The relatively unique sensitivity of T-cells is attributed to their relatively high level of dCK compared to other cells. This observation has led to the development of PNP inhibitors for the treatment of T-cell cancers and T-cell autoimmune indications. The biochemcial basis for the use of PNP inhibitors as well as the various classes of inhibitors developed has been reviewed.[4] More recently, clinical trial experience in psoriasis with the PNP inhibitor BCX-34 has been described.[5]

Using transition state analysis, a new class of PNP inhibitors has been developed.[6] One of these inhibitors, BCX-1777 (**1**) is a clinical trial candidate. In support of our clinical program, we needed to develop a rapid and sensitive method of determining drug levels in biological matrices such as plasma and urine. One method evaluated for this purpose was an LC-MS-MS method utilizing an isotopically-labeled analog as an internal standard. In this paper, we present the synthesis of 5′-[^{2}H]-BCX-1777 (**2**), which we have used in these assays.

1   **2**

Results and discussion

We have recently described the convergent synthesis of aza-C-nucleo-sides via addition of lithiated 9-deazapurines to carbohydrate-derived cyclic imines.[7] One such aza-C-nucleoside reported by us though this route was **3**, which we have utilized as our starting material for the labeled analog as shown in Scheme 1. Treatment of **3** with 1 M tetrabutylammonium fluoride in THF afforded the corresponding alcohol **4** in excellent yield. Dess-Martin oxidation of alcohol **4** gave the corresponding aldehyde **5** in 96% isolated yield. The ^{2}H-label was introduced through reduction of aldehyde **5** with $NaB[^2H]_4$ in $CH_3O[^2H]$ as the solvent giving **6** as the product. While we have demonstrated that **6** can be converted directly to the target compound **2** by treatment with strong acid under reflux, this route tended to give product that was highly colored. Most of this color developed during the extended reflux conditions required to completely remove the *N*-9 benzyloxymethyl protecting group. We circumvented this problem by using the same chemistry we had employed previously for the non-labeled material in which the *N*-9 benzyloxymethyl protecting group was hydrogenated in the presence of Pearlman's catalyst to give **8**. After filtering the catalyst through Celite, thin layer chromatographic analysis of the filtrate indicated that in addition to **8**, a small amount of the *N*-9 CH_2OH product **7** remained. Residual **7** was conveniently converted *in situ* to the desired *N*-9 NH product **8** by treating the filtrate with a small amount of NH_4OH. In this manner, **8** was isolated in 84% yield from **6**. Final deprotection was achieved by refluxing **8** with concentrated aqueous HCl/MeOH for 2 h giving **2**, which was isolated as the hydrochloride. The overall yield of **2** was 60.4% from **3** and the final product had an isotopic purity of 98.4%. The position of the [^{2}H]-label was confirmed at the 5′-C through comparison of the 5′-CH_2 resonances of **1** (determined by a DEPT 135 experiment to be δ 58.88 for **1**) and **2**. In the ^{13}C NMR of **2**, this signal was greatly diminished in intensity and very broad due to C–[^{2}H] coupling.[†] In addition, the 5′-CH_2 resonance for **2** was shifted upfield to δ 58.47 relative to **1** consistent with a C–[^{2}H] bond.

[†]The diminution of this signal is expected and is due to loss of NOE and a longer T_1 time for C–[^{2}H] vs. C-H.

Scheme 1[a]
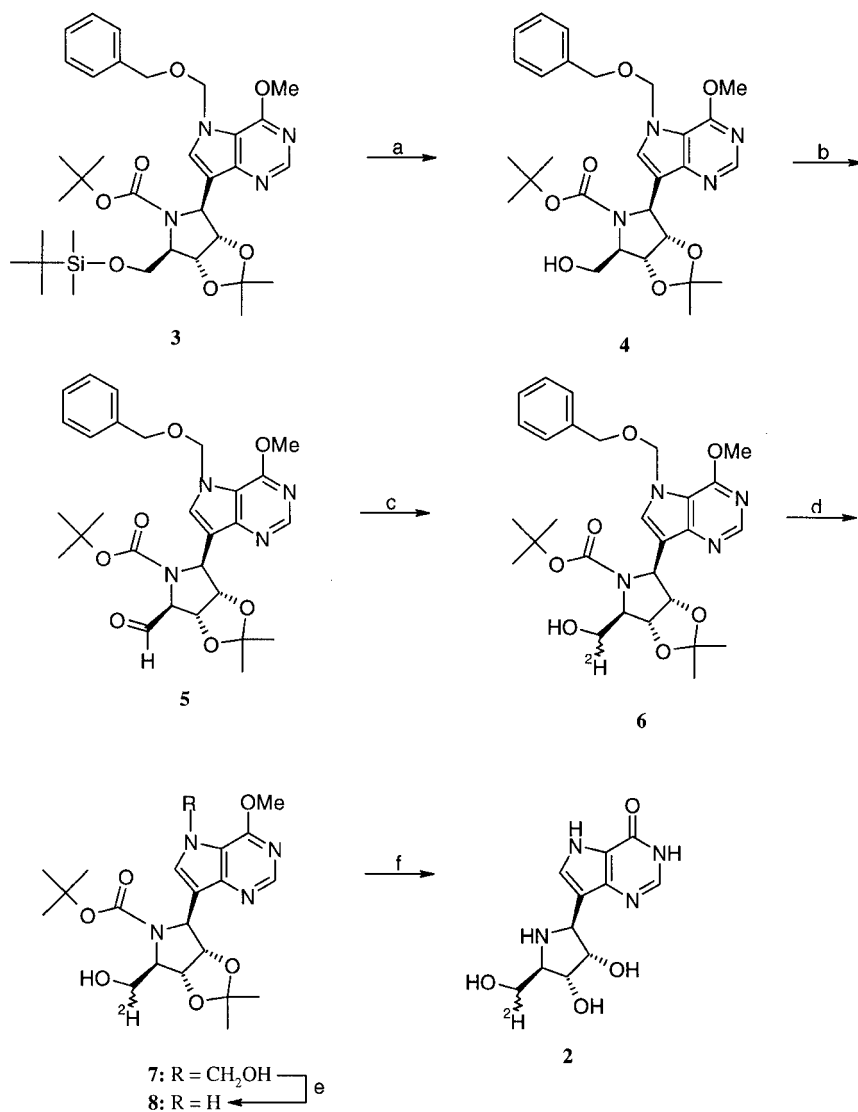
Scheme 1. Reagents and conditions: a tetrabutylammonium fluoride, THF, ice-water bath; b. Dess-Martin periodinane, CH_2Cl_2, rt; c. $NaB[^2H]_4$, $CH_3O[^2H]$; d. H_2, 20% $Pd(OH)_2/C$, EtOH; e. trace NH_4OH; f. HCl, MeOH, reflux

Experimental

General: Melting points were determined on a Meltemp II melting point apparatus and are uncorrected. The ^{1}H NMR spectra were

reported on a Bruker AMX-360 at 360 MHz or a Bruker Avance 300 at 300.13 MHz spectrometer. The ^{2}H NMR spectra were recorded on a Bruker AMX-500 spectrometer at 76.77 MHz. The ^{13}C NMR spectra were recorded on a Bruker AMX-360 at 90.56 MHz or a Bruker Avance 300 at 75.5 MHz spectrometer. Chemical shifts (ppm) are referenced to internal tertramethylsilane for ^{1}H and ^{13}C spectra and either CDCl$_3$ ($\delta = 7.27$) or DMSO-d$_6$ ($\delta = 2.52$) for ^{2}H spectra. Spectra were recorded at ambient temperature unless otherwise noted. IR spectra were obtained on a Bio-Rad FTS-7 FT-IR. Mass spectra were recorded on a Micromass ZMD in the positive electrospray mode with a scan range of 0–1000 m/z and cone voltage setting of 20 V. A solution of the sample ($\cong 100\,\mu g/ml$) in methanol (100%) was introduced into the source via a Waters 2690 autosampler. Flash chromatographic separations were performed on Whatman silica gel, 60 Å or using a Biotage Flash 40*i* or 75*i* with pre-packed silica get (60 Å) cartridges. Thin layer chromatography (TLC) was performed using aluminum backed silica gel 60 plates from E. Merck. Sodium borodeuteride (98 atom % D) was obtained from Aldrich Chemical Co. (Milwaukee, WI).

(1S)-1-C-(5-N-Benzyloxymethyl-4-methoxypyrrolo[3,2-d]pyrimidin-7-yl)-N-tert-butoxycarbonyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol (4): A sample of the silyl ether **3**[7] (15.1 g, 23.1 mmol) was dissolved in anhydrous tetrahydrofuran (450 ml) under an Ar atmosphere and cooled in an ice/water bath. To this was added tetrabutylammonium fluoride (1 M THF, 25 ml, 25 mmol, $\sim$1.1 eq.). After 30 min an additional 12.5 ml of TBAF was added followed by another 12.5 ml 30 min later ($\sim$50 ml total, $\sim$2.2 eq. total). The mixture was concentrated and the residue partitioned with CH$_2$Cl$_2$ and saturated aqueous NH$_4$Cl. The organic layer was washed with water, dried (Na$_2$SO$_4$), and concentrated to give a crude oil. The crude product was purified by chromatography (Flash 75, 200 g SiO$_2$, gradient 30 $\rightarrow$ 40% EtOAc-hexane) and the relevant fractions combined and evaporated to give 12.21 g (22.6 mmol, 98%) of **4** as a straw-colored foam. An analytical sample was obtained from a center fraction to give **4** as a white foam. IR (KBr) 3195, 2979, 2936, 1693, 1612, 1538 and 1365 cm^{-1}; MS (m/z, ES+) 541.4 (100%); ^{1}H-NMR (360 MHz, DMSO-d$_6$, 50°C) δ 8.45 (s, 1H), 7.64 (s, 1H), 7.29–7.18 (m, 5H), 5.73 (s, 2H), 5.13 (br s, 1H), 5.09–5.06 (br s, 1H, D$_2$O exchangeable), 5.05 (d, $J = 5.2$ Hz, 1H), 4.82 (d, $J = 5.5$ Hz, 1H), 4.49 (s, 2H), 4.06 (s, 3H), 4.02–3.99 (m, 2H), 3.61–3.45 (m, 2H), 1.47 (s, 3H), 1.30 (s, 12H); ^{13}C-NMR (90.56 MHz, DMSO-d$_6$, 50°C) δ 155.66, 153.52, 149.11,

147.85, 137.24, 132.20, 127.85, 127.22, 127.04, 115.73, 114.92, 110.59, 84.11, 81.67, 78.77, 76.99, 69.46, 65.93, 60.92, 60.72, 53.18, 27.76, 27.13, 25.12. Analysis calculated for $C_{28}H_{36}N_4O_7$: C, 62.21; H, 6.71; N, 10.36. Found: C, 62.32; H, 6.87; N, 10.09.

*5-Aldehydo-(1S)-1-*C-*(5-N-benzyloxymethyl-4-methoxypyrrolo[3,2-d]pyrimidin-7-yl)-N-tert-butoxycarbonyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-*D-*ribitol (5)*: A sample of alcohol **4** (11.75 g, 21.7 mmol) was dissolved in CH_2Cl_2 (300 ml) under an Ar atmosphere and Dess-Martin periodinane (18.4 g, 43.5 mmol, 2.0 eq.) added. After 3 h, the mixture was concentrated and the residue taken up in ether. The resulting organic layer was washed with $NaHCO_3/Na_2S_2O_3$ (sat aq NaHCO3 : 10% aq $Na_2S_2O_3$ – 1:1) until all solids had dissolved. The organic layer was then washed with saturated brine, dried (Na_2SO_4), and concentrated to give 11.27 g (20.9 mmol, 96%) of **5**. An analytical sample was obtained by chromatography (Flash 40, 40 g SiO_2, gradient $20 \rightarrow 30\%$ EtOAc-hexane) to give **3** as a light yellow glass. IR (KBr) 2981, 2939, 1734, 1697, 1612, 1514, 1392 and 1367 cm^{-1}; MS (*m/z*, ES+) 539.3 (100%); ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ 9.42 (d, $J = 11.5$ Hz, 1H), 8.36 (s, 1H), 7.88 (d, $J = 3.0$ Hz, 1H), 7.30–7.19 (m, 5H), 5.75 (d, $J = 12.6$ Hz, 2H), 5.30–5.22 (m, 2H), 4.86 (d, $J = 5.1$ Hz, 1H), 4.51–4.46 (m, 2H), 4.27 (d, $J = 8.7$ Hz, 1H), 4.13 (s, 3H), 1.48 (s, 3H), 1.32 (s, 9H), 1.29 (s, 3H). ^{13}C-NMR (90.56 MHz, CDCl$_3$) δ 200.77, 156.31, 153.61, 150.10, 149.89, 136.82, 132.42, 131.08, 128.46, 127.98, 127.76, 127.54, 115.04, 112.35, 84.87, 83.27, 80.84, 73.81, 70.23, 59.88, 53.61, 28.95, 28.30, 25.26. Analysis calculated for $C_{28}H_{34}N_4O_7$: C, 62.44, H, 6.36; N, 10.40. Found: C, 62.40; H, 6.41; N, 9.98.

*5-[^{2}H]-(1S)-1-*C-*(5-N-Benzyloxymethyl-4-methoxypyrrolo[3,2-d]pyrimidin-7-yl)-N-tert-butoxycarbonyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-*D-*ribitol (6)*: A sample of **5** (6.95 g, 12.8 mmol) was dissolved in $CH_3O[^2H]$ ($\sim$60 ml) under an Ar atmosphere. To this was added $NaB[^2H]_4$ (0.75 g, 17.9 mmol, 1.4 eq.). After 1 h, the volatiles were removed and the residue taken up in MeOH ($\sim$50 ml) followed by removal via rotovap ($3\times$). The crude product was purified by chromatography (Flash 40, 90 g SiO_2, gradient $25 \rightarrow 40\%$ EtOAc-hexane) and the relevant fractions combined and evaporated to give 6.32 g (11.7 mmol, 91%) of **6** as a straw-colored foam. An analytical sample was obtained from a center fraction to give **6** as a white foam. IR (KBr) 3195, 2979, 2936, 2159(w), 1693, 1612, 1538 and 1366 cm^{-1}; MS (*m/z*, ES+) 542.4 (100%); ^{1}H-NMR (360 MHz, DMSO-d$_6$, 50°C) δ 8.45

(s, 1H), 7.63 (s, 1H), 7.31–7.19 (m, 5H), 5.73 (s, 2H), 5.13 (s, 1H) 5.05–5.04 (m, 1H), 4.81 (d, $J = 5.5$ Hz, 1H), 4.49 (s, 2H), 4.06 (s, 3H), 4.00 (d, $J = 7.1$ Hz, 1H), 3.55–3.48 (m, 1H), 1.47 (s, 3H), 1.30 (s, 12H); ^{13}C-NMR (90.56 MHz, DMSO-d$_6$, 50°C) δ 155.66, 153.53, 149.11, 147.83, 137.24, 132.20, 127.86, 127.22, 127.06, 115.76, 114.92, 110.59, 84.10, 81.65, 78.77, 76.99, 69.46, 65.87, 60.70, 60.33, 53.20, 27.78, 27.14, 25.12; ^{2}H-NMR (76.77 MHz, DMSO) δ 3.50. Analysis calculated for $C_{28}H_{35}D_1N_4O_7$: Calculated: (H + D as H) C, 62.21; H, 6.71; N, 10.36. Found: C, 62.32; H, 6.87; N, 10.09.

5-[^{2}H]-(1S)-1-C-(4-Methoxypyrrolo[3,2-d]pyrimidin-7-yl)-N-tert-butoxycarbonyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-ribitol (8): A mixture of **6** (5.71 g, 10.5 mmol) and 20% Pd(OH)$_2$ on C (5.7 g) in EtOH ($\sim$75 ml) was shaken under an H$_2$ atmosphere (Parr shaker, 40 psig) for 22 h. The mixture was filtered through Celite and the Celite washed with EtOH. To the combined filtrates was added conc. aq NH$_4$OH (1 ml) and after 1 h the mixture concentrated. The crude product was purified by chromatography (Flash 40, 90 g SiO$_2$, 50% EtOAc-hexane) and the relevant fractions combined and evaporated to give 3.72 g (8.82 mmol, 84%) of **8** as a white foam. IR (KBr) 3219, 2982, 2938, 2164(w), 1664, 1630, 1541, 1400 and 1383 cm^{-1}; MS (m/z, ES+) 422.2 (100%); ^{1}H-NMR (360 MHz, DMSO-d$_6$, 50°C) δ 11.78 (br s, 1 H, D$_2$O exchangeable), 8.42 (s, 1H), 7.44 (s, 1H), 5.12 (br s, 3H), 4.83 (d, $J = 4.9$ Hz, 1H), 4.09 (s, 3H), 3.99 (d, $J = 6.2$ Hz, 1H), 3.49 (d, $J = 4.21$ Hz, 1H), 1.47 (s, 3H), 1.30 (s, 12H); ^{13}C-NMR (90.56 MHz, DMSO-d$_6$, 50°C) δ 155.31, 153.53, 148.38, 146.58, 128.42, 114.56 (coincident with another peak), 110.50, 84.16, 81.61, 78.59, 65.78, 60.85, 60.51, 52.83, 27.76, 27.16, 25.14; ^{2}H-NMR (90.56 MHz, DMSO) δ 3.52. Analysis calculated for $C_{20}H_{27}D_1N_4O_6$: Calculated: (H + D as H) C, 57.00; H, 6.70; N, 13.29. Found: C, 57.12; H, 6.64; N, 13.11.

5-[^{2}H]-(1S)-1-C-(4-hydroxypyrrolo[3,2-d]pyrimidin-7-yl)-1,4-di-deoxy-1,4-imino-D-ribitol hydrochloride (2): A mixture of **8** (3.22 g, 7.64 mmol) in MeOH (25 ml) and concentrated aqueous HCl (25 ml) was refluxed under Ar for 2 h. The solution was concentrated and the residue taken up in a small amount of water. EtOH was added and the resulting mixture concentrated and the treatment with EtOH repeated three times. The crude product was recrystallized from water/EtOH to give 1.95 g (6.42 mmol, 84%) of **6** as an off-white solid, m.p. > 250°C (dec). IR (KBr) 3413, 3087, 3042, 2945, 1693, 1661 and 1596 cm^{-1}; MS (m/z, ES+) 268.1 (100%); ^{1}H-NMR (300 MHz, DMSO-d$_6$) δ 12.38 (d, $J = 2.7$ Hz, 1H), 12.17 (s, 1H), 10.41 (br s, 1H), 8.41 (br s, 1H), 7.90

(s, 1H), 7.64 (d, $J = 3.1$ Hz, 1H), 5.60 (d, $J = 4.2$ Hz, 1H), 5.50 (d, $J = 3.1$ Hz, 1H), 5.42 (d, $J = 4.6$ Hz, 1H), 4.63 (d, $J = 7.2$ Hz, 1H), 4.44 (d, $J = 3.0$ Hz, 1H), 4.18 (d, $J = 2.6$ Hz, 1H), 3.71 (s, 1H), 3.47 (m, 1H, coincident with HDO), ^{1}H-NMR (300 MHz, DMSO-d$_6$/D$_2$O) δ 7.90 (s, 1H), 7.58 (s, 1H), 4.65 (d, $J = 8.1$ Hz, 1H), 4.48 (dd, $J = 8.1$, 4.8 Hz, 1H), 3.72–3.69 (*m*, 1H), 3.54 (dd, $J = 4.4$, 4.4 Hz, 1H); ^{13}C-NMR (75.5 MHz, DMSO-d$_6$) δ 153.52, 143.02, 142.22, 127.47, 118.09, 109.27, 74.04, 70.38, 65.12, 58.20, 56.65; ^{2}H-NMR (90.56 MHz, DMSO) δ 3.64. Analytical Calculations for $C_{11}H_{13}D_1N_4O_4HCl$: Calculated: (H + D as H) C, 43.50; H, 4.65; N, 18.45. Found: C, 43.84; H, 4.94; N, 18.21.

Acknowledgements

References

1. Stoeckler JD. *Developments in Cancer Chemotherapy*, CRC Press: Boca Raton, FL, 1984.
2. Markert ML. *Immunodeficiency Rev* 1991; **3**: 45.
3. Cory JG, Cory AH. *Inhibitors of Ribonucleotide Diphosphate Reductase Activity*, Pergamon Press: New York, 1989.
4. Morris PE, Montgomery JA. *Expert Opin Ther Patents* 1998; **8**(3): 283.
5. Morris PE, Omura GA. *Curr Pharm Des* 2000; **6**: 943.
6. Miles RW, Tyler PC, Evans GB, Furneaux RH, Parkin DW, Schramm VL. *Biochemistry*, 1999; **38**: 13147; Miles RW, Tyler PC, Evans GB, Furneaux RH, Bagdassarian CK, Schramm VL. *Biochemistry* 1998; **37**: 8615.
7. Evans GB, Furneaux RH, Hutchison TL, Kezar III HS, Morris PE, Schramm VL, Tyler PC. *J Org Chem* 2001; **66**: 5723.