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Research Article

Synthesis of the stable isotope labeled antiviral nucleoside analog [8-^{13}C–7,9-$^{15}N_2$]-ganciclovir

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Summary

Ganciclovir, a nucleoside analog of 2′-deoxyguanosine, is a drug used in suicide gene therapy for the treatment of mesothelioma. We required a stable isotope analog of ganciclovir for use in pharmacokinetic studies in order to monitor the systemic exposure of patients to the drug. Therefore, a facile and efficient synthesis of [8-^{13}C–7,9-$^{15}N_2$]-ganciclovir, was devised. The synthesis was achieved in 4 steps with 25% total yield using commercially-available [8-^{13}C–7,9-$^{15}N_2$]-guanine, without the need for purification of intermediates. The key step of the synthesis involved the coupling of [8-^{13}C–7,9-$^{15}N_2$]-guanine with 3-propionyloxy-2-propionyloxy-methoxypropyl propionate. The latter was synthesized from a commercially available dichlorohydrin. Each step of the reaction could be easily monitored by liquid chromatography–mass spectrometry. The structure of the labeled ganciclovir was confirmed using ^{1}H, ^{13}C, and ^{15}N nuclear magnetic resonance spectroscopy. Copyright © 2006 John Wiley & Sons, Ltd.

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Key Words: ganciclovir; stable isotopes; nucleoside; liquid chromatography/mass spectrometry; nuclear magnetic resonance spectroscopy

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Introduction

Ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl) guanine] is a clinically effective antiviral drug known as Cytovene® that has been used for the treatment of viral diseases caused by herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus (VZV) and the human cytomagalovirus (HCMV).[1] Ganciclovir is monophosphorylated by viral-specific kinases, then converted to its triphosphate form by cellular kinases.[2] Ganciclovir triphosphate is a competitive inhibitor of DNA polymerase,[3] and it can also be incorporated into viral DNA as a substrate.[4] The antiviral specificity of ganciclovir is attributed to its being an excellent substrate for viral kinases and a poor substrate for endogeneous mammalian kinases.[5] The ability of ganciclovir triphosphate to inhibit replication has been used in suicide gene therapy for the treatment of human tumors, such as malignant mesothelioma,[6] in which cancer cells are transfected with recombinant adenovirus containing the herpes simplex virus thymidine kinase (HSV-tk) gene. Tumors regress when patients are treated with intravenous ganciclovir after they are transfected with the HSV-tk gene.[7]

In support of our clinical mesothelioma program, we required a rapid, sensitive, and specific method to monitor plasma levels of ganciclovir.[7] This would then enable a pharmacokinetically based dosing strategy to be used in combination with suicide gene therapy to provide optimal treatment of mesothelioma patients. Maximal specificity for analysis of ganciclovir could only be obtained with stable isotope dilution liquid chromatography–tandem mass spectrometry (LC–MS/MS). A [^{15}N,^{13}C]-analog was required for use as an internal standard because a deuterium-containing analog would separate from the unlabeled ganciclovir during LC–MS analysis as described in the quantitative analysis of peptides.[8] This could compromise precision and accuracy as a result in differential suppression of analyte and internal standard signals by co-eluting contaminants from the biological matrix.[9] The synthesis of guanine derivatives such as acyclovir and ganciclovir, is challenging due to the nature of guanine as a polyfunctional, amphoteric and poorly soluble compound. Previous syntheses of unlabeled ganciclovir have employed purines, furazanopyridines, or imidazoles as starting materials.[10] The most commonly employed route involves regioselective N-9 alkylation of guanine precursors.[11] A scheme based on the use of a labeled purine derivative ([8-^{13}C–7,9-$^{15}N_2$]-guanine) was chosen for the preparation of labeled ganciclovir because it avoids many of the cumbersome steps involved in isolating and purifying the intermediates required by other routes.[12] We report the synthesis of [8-^{13}C–7,9-$^{15}N_2$]-ganciclovir for use in pharmacokinetic studies.[7]

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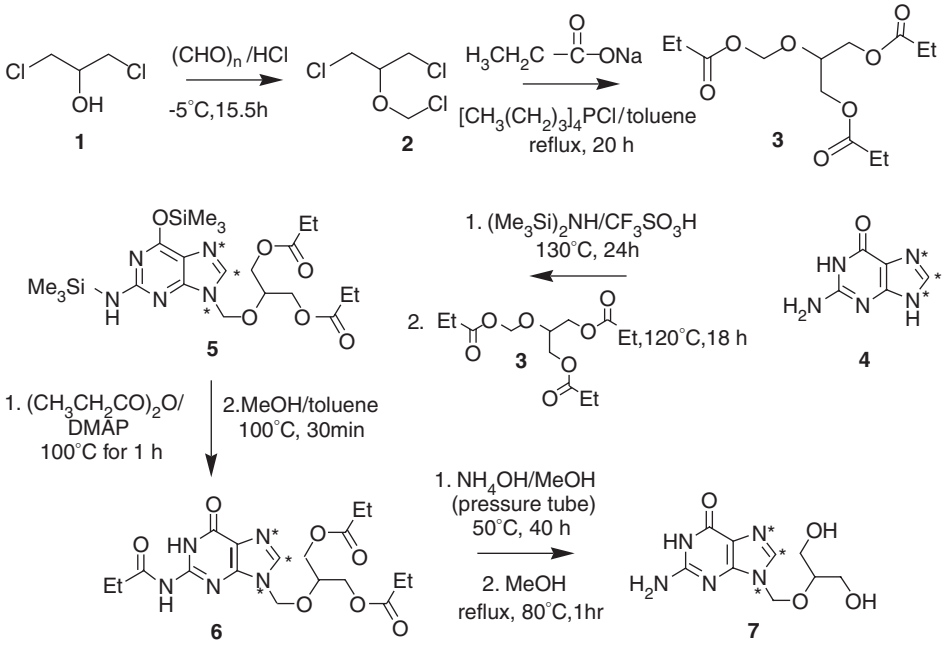
Results and discussion

Commercially available dichlorohydrin (**1**) was converted to the corresponding chloromethyl ether (**2**) by reaction with anhydrous HCl in a suspension of paraformaldehyde at −5 °C (Scheme 1).[12,13] The resulting trichloride was reacted with sodium propionate in the presence of a phase transfer catalyst, tetrabutylphosphonium chloride, to give 3-propionyloxy-2-propionyloxymethoxypropyl-propionate (**3**). Product **3** eluted at 13.7 min on LC–MS analysis and gave a negative ion spectrum with a major ion corresponding to the loss of a proton from the molecule ([M-H]$^-$) at $m/z = 289$, together with two adduct ions at $m/z = 325$ and $m/z = 363$, corresponding to [M + ^{35}Cl]$^-$ and [M + EtCOO]$^-$, respectively. In addition, a fragment ion was observed at $m/z = 185$, which resulted from cleavage at the C–O ether bond. The yield of compound **3** after distillation was 93%.

[8-^{13}C–7,9-^{15}N$_2$]-Guanine (**4**) was condensed with hexamethyldisilazane (HMDS) in the presence of trifuoromethanesulfonic acid to give tri(trimethylsilyl) (TMS) derivative, which was then alkylated with 3-propionyloxy-2-propionyloxy-methoxypropyl propionate (**3**) to give **5**. The structure of **5** was confirmed through analysis by LC–MS. There was an intense peak at 13.4 min, with an ion at $m/z = 515$ expected for the protonated molecule (MH$^+$) of **5**, a major product formed by alkylation of **4** at the N-9 position.



Scheme 1. Synthesis of [8-^{13}C–7,9-^{15}N$_2$]-ganciclovir (* = ^{15}N or ^{13}C-labeled atom)

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The reconstructed ion chromatogram at $m/z = 515$ also showed a smaller peak at 12.3 min, which was due to an isomeric product formed by alkylation of **4** at the N-7 position. The area ratio of the N-9 (**5**) product to the N-7 isomer was 6:1, indicating that the N-9 isomer (**5**) was the major product. The mass spectrum of the LC peak at 13.4 min showed a MH^+ at $m/z = 515$, a fragment $[MH\text{-}SiMe_3]^+$ at $m/z = 443$ and another major fragment at $m/z = 187$, which was due to cleavage in the side chain attached to N-9. The N-9 isomer **5** was used without further purification for the next reaction.

The TMS protecting groups of **5** were removed by acylation with an acylating agent, propionic anhydride, in the presence of 4-(dimethylamino)-pyridine (DMAP) as a catalyst to give mostly the N-9 substituted guanine (**6**). The structure of **6** was confirmed by mass spectral analysis. LC–MS analysis showed an intense peak at 15.1 min, with a MH^+ at $m/z = 427$ as expected for **6**. The reconstructed ion chromatogram at $m/z = 427$ also showed a smaller peak at 14.0 min, which was due to an N-7 isomer. The area ratio of N-9 product (**6**) to N-7 product was 4:1.

The crude product **6** was hydrolyzed by treatment with ammonium hydroxide at 50 °C in a pressure tube for 40 h to give the desired N-9 substituted guanine, ganciclovir (**7**). For the small-scale reaction, the pressure

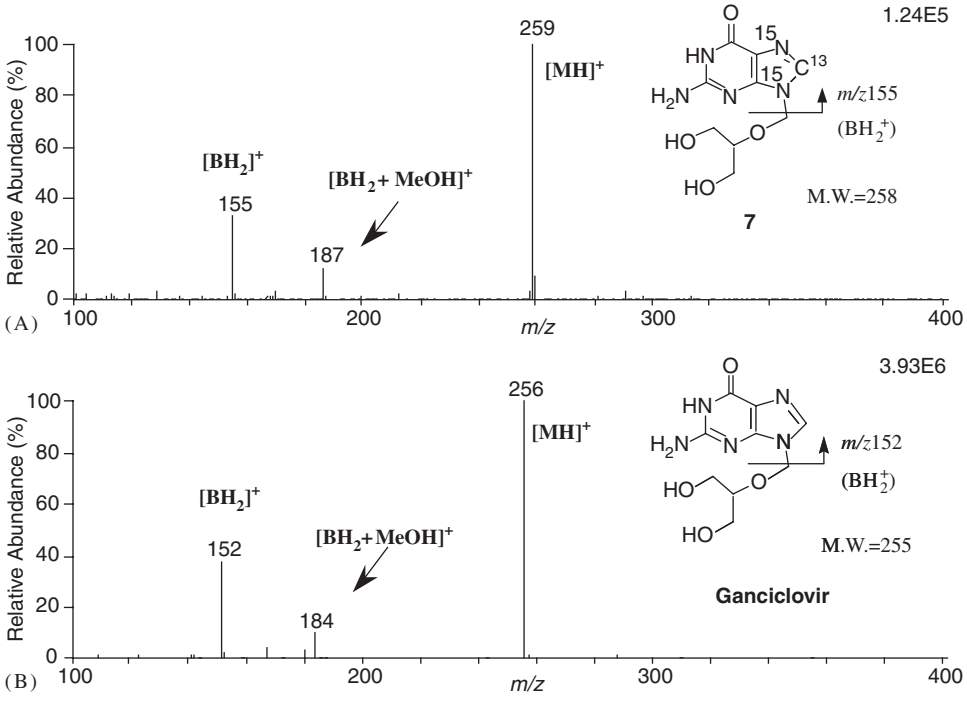

Figure 1. Full scan ESI/MS mass spectra: (A) [8-^{13}C–7,9-$^{15}N_2$]-Ganciclovir (7); (B) Authentic ganciclovir

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tube was found to be very efficient. Final step, HPLC purification gave pure [8-^{13}C–7,9-$^{15}N_2$]-ganciclovir with a 25% overall yield. Purified **7** showed only one peak on LC–MS analysis at 8.2 min, the area ratio of unlabeled ganciclovir to **7** was 0.2%, indicating the isotope purity of **7** was 99.8%. The full scan electrospray ionization (ESI) mass spectrum of **7** (Figure 1(A)) showed an intense MH^+ at $m/z = 259$, a characteristic depurination fragment ion BH_2^+ at $m/z = 155$ and a methanol adduct of BH_2^+ at $m/z = 187$. The fragment ions were identical to those observed in authentic unlabeled ganciclovir as shown in Figure 1(B).

^{1}H-NMR analysis of **7** indicated the characteristic ddd couplings of 8-H at 7.76 ppm. The distinct coupling constants ($J = 212.5$, 11.9, 8.7 Hz) were consistent with those expected from the couplings between H-8 and ^{13}C-8, H-8 and ^{15}N-7, and H-8 and ^{15}N-9, respectively. In addition, the signal of H-1′ at 5.41 ppm showed a doublet ($J = 3.9$ Hz) that was due to the coupling between H-1′ and ^{15}N-9. The signals of the remaining protons in the ^{1}H NMR spectrum were consistent with authentic unlabeled ganciclovir.[12] The ^{13}C NMR spectrum of **7** showed the ^{13}C enrichment at C8 (140.18 ppm, dd). The ^{15}N-NMR analysis of **7** displayed a single peak at 214.85 and a doublet at 150.42 ppm, respectively, which was consistent with the introduction of ^{15}N only at the N-7 and N-9 positions.

Experimental

Chemicals and Materials

Toluene, methanol, methylene chloride, ammonium acetate (glacial) and ammonium hydroxide were purchased from Fisher Scientific (Pittsburgh, PA). The solvents used for HPLC were Optima grade. [8-^{13}C–7,9-$^{15}N_2$]-Guanine was purchased from Cambridge Isotope Laboratory (Andover, MA). 1,3-Dichloro-2-propanol, paraformaldehyde, hexamethyldisilazane (HMDS), trifluoromethanesulfonic acid, sodium propionate, tetrabutylphosphonium chloride, 4-(dimethylamino)-pyridine (DMAP), propionic anhydride, anhydrous pyridine and anhydrous toluene were obtained from Sigma (St. Louis, MO). Costar Spin X nylon centrifuge filters (0.22 μm) were purchased from VWR Scientific Products (Bridgeport, NJ).

Instrumentation and Equipment

All HPLC-UV analysis was performed using a Hitachi L-6200 intelligent pump equipped with Hitachi L-400 UV detector and Hitachi D-2500 Chromato-Integrator (Hitachi, San Jose, CA). Ultraviolet spectra were recorded with a Beckman 550 instrument (Beckman, Palo Alto, CA). Centrifuges (Juoan, Winchester, VA, or Beckman, Columbia, MD) were used for the purification. LC–MS was performed using a Waters 2690 Alliance LC

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(Milford, MA) interfaced to a TSQ7000 triple quadruple mass spectrometer (Thermo Electron Co., San Jose, CA) equipped with an ESI or an atmospheric pressure chemical ionization source (APCI). Preparative HPLC was performed on a Beckman C_{18} preparative column (250 × 10 mm i.d., 5 μm, 120 Å, Alltech Associates, Deerfield, IL). Reversed-phase analytical HPLC was performed on YMC-AQ ODS analytical columns (150 × 2.0 mm i.d., 3 μm, 120 Å, or 250 × 4.6 mm i.d., 5 μm, 120 Å, YMC, Wilmington, NC). Normal phase analytical HPLC was performed on an Econosil silica analytical column (250 × 10 mm i.d., 5 μm, Alltech Associates, Deerfield, IL). A pressure tube with threaded type A plug (15 ml) was purchased from Aldrich (Milwaukee, WI). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker (Billerica, MA) DMX-400 NMR spectrometer at 400 and 100 MHz, respectively. The ^{15}N-NMR spectrum was recorded at 40.55 MHz using $CH_3COO^{15}NH_4$ as internal standard (0 ppm for $^{15}NH_4^+$).

Mass Spectrometry

ESI was performed in positive or negative mode using N_2 as both sheath (pressure, 70 psi) and auxiliary (30 units) gas in order to assist with nebulization. The source was maintained at 245 °C, and the needle potential at 5.5 kV. The multiplier voltage was set at 1340 V, and argon was used as the collision gas at 2.80 mTorr. Atmospheric pressure chemical ionization (APCI) was also performed using N_2 as both sheath (pressure, 30 psi) and auxiliary (10 units) gas. The vaporizer temperature and heated capillary temperature were set at 500 and 250 °C, respectively. The corona discharge was set to be 10 μA for positive mode or 16 μA for negative mode. The multiplier voltage was set at 1340 V.

Synthesis of [8-^{13}C–7,9-$^{15}N_2$]-Ganciclovir

*3-Propionyloxy-2-propionyloxy-methoxypropylpropionate (***3***).* A mixture of 1,3-dichloro-2-propanol (**1**, 10 g, 77.5 mmol) and paraformaldehyde (3.02 g, 104 mmol) in CH_2Cl_2 (100 ml) was cooled down to −5 °C, then gaseous HCl was bubbled into the mixture at −5 °C over 1.5 h. The resulting mixture was kept at −5 °C for 14 h. Sodium sulfate (3 g) was added, and the mixture was stirred at room temperature for 2 h. The solid was filtered off, and the filtrate was washed with aqueous sodium hydroxide (20%, 2 × 20 ml). The organic phase was dried over anhydrous sodium sulfate (3 g), and the solvent was removed under reduced pressure, giving 2-(chloromethoxy)-1,3-dichloropropane (**2**) as a colorless liquid (13 g, 94.5% yield). Compound **2** (2.5 g, 14.1 mmol) and a solution of tetrabutyphosphonium chloride (0.42 g, 3.4 mmol) in toluene (4 ml) were added slowly to sodium propionate (4.74 g, 49.3 mmol) that was already evaporated with anhydrous toluene to azeotrope

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off water. After refluxing for 20 h, the mixture was cooled to room temperature. An aqueous solution of Na_2CO_3 (1.25%, 15 ml) was added. The mixture was stirred for 15 min, the aqueous layer was separated, and the toluene layer washed with water (10 ml). The combined aqueous extracts were washed with toluene (15 ml), and the combined toluene extracts were dried over anhydrous Na_2SO_4 (1.5 g). The solid in toluene extracts was filtered off, and the solvents in the filtrate were removed under reduced pressure. The resulting liquid was distilled at 120 °C under reduced pressure, giving 3-propionyloxy-2-propionyloxy-methoxypropyl propionate **3** (3.8 g, 93.0% yield).

[8-^{13}C-7,9-$^{15}N_2$]-Ganciclovir (**7**). [8-^{13}C–7,9-$^{15}N_2$]-Guanine (**4**, 100 mg, 0.645 mmol) was evaporated with anhydrous toluene (3 × 1 ml) to azeotrope off the water prior to use. After adding hexamethyldisilazane (HMDS, 2 ml, 9.5 mmole) and trifluoromethanesulfonic acid (4 µl, 0.045 mmol), the reaction mixture was refluxed at 130 °C for 24 h until the suspended solid dissolved completely. The excess HMDS in the reaction mixture was removed under reduced pressure. The residue as a slurry was heated to 105 °C, then cooled to 100 °C. Compound **3** (500 µl, ~1.7 mmol) was added. The reaction mixture was stirred at 120 °C for 18 h (monitored by LC–MS analysis), then evaporated to remove low boiling point materials to give [8-^{13}C–7,9-$^{15}N_2$]-*N*,*O*-*bis*-trimethylsilyl-9-(1,3-dipropionoxy-2-propoxymethyl)-guanine (**5**) as a solution, which was cooled to 100 °C and used directly for the next reaction without further purification. The crude compound **5** was analyzed using LC/positive APCI/MS on an Econosil silica column (250 × 10 mm i.d., 5 µm). The mobile phases consisted of 1% 2-propanol in hexane as solvent A and 90% 2-propanol in hexane as solvent B. HPLC separation was performed by a linear gradient elution at a flow rate of 0.7 ml/min as follows: 0 min, 10% B; 4 min, 10% B; 8 min, 100% B; 12 min, 100% B; 14 min, 10% B; 30 min, 10% B.

To the solution of **5** at 100 °C, 4-dimethylaminopyridine (DMAP, 8 mg, 0.0065 mmol) and propionic anhydride (300 µl, 2.3 mmol) were added. After stirring at 100 °C for 1 h, a mixture of MeOH (80 µl) and toluene (2 ml) was added to quench the reaction. The reaction mixture was stirred at 100 °C for 0.5 h, then transferred to a 15 ml pressure tube, and evaporated under a nitrogen stream to produce an oil, [8-^{13}C–7,9-$^{15}N_2$]-*N*,*O*-*bis*-trimethylsilyl-9-(1,3-propionoxy-2-propoxymethyl)-N^2-propionylguanine (**6**). Compound **6** was used for the next reaction without further purification. The crude compound **6** was analyzed using LC/Positive APCI/MS on an Econosil silica column (250 × 10 mm i.d., 5 µm). The mobile phases consisted of 1% 2-propanol in hexane as solvent A and 90% 2-propanol in hexane as solvent B. HPLC separation was performed by a linear gradient elution at a flow rate of

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0.7 ml/min as follows: 0 min, 10% B; 4 min, 10% B; 9 min, 100% B; 20 min, 100% B; 22 min, 10% B; and 30 min, 10% B.

To the compound **6** in the pressure tube, a mixture of MeOH (960 µl) and concentrated NH_4OH (840 µl) was added. The reaction mixture was stirred at 50 °C for 40 h (monitored by LC–MS analysis), then concentrated under a nitrogen stream. To the residue, MeOH (1.5 ml) was added. The mixture was refluxed at 80 °C for 1 h and kept on ice for 1 h. The precipitate thus obtained was centrifuged, and the supernatant was discarded. The precipitate was washed with MeOH (400 µl), then vacuum dried. The residue was dissolved in hot water and filtered through a Costar Spin X filter. The filtrate was purified by reversed-phase HPLC using a Beckman Preparative C18 column (10 × 250 mm). The mobile phases consisted of 2% MeOH as solvent A and 100% MeOH as solvent B. HPLC separation was performed by a linear gradient elution at a flow rate of 2.0 ml/min as follows: 0 min, 0% B; 3 min, 0% B; 20 min, 30% B; 22 min, 100% B; 24 min, 100% B; 26 min, 0% B; and 36 min, 0% B. The UV detector was set at $\lambda = 254$ nm for analytical runs and $\lambda = 300$ nm for preparative runs. Compound **7**, [8-^{13}C–7,9-$^{15}N_2$]-ganciclovir, had a retention time of 16.8 min. HPLC purification gave the pure compound **7** (42 mg, 25% yield for the four steps combined). Pure **7** was analyzed using LC/Positive APCI/MS on a YMC ODS AQ column (150 × 2 mm i.d., 3 µm, 120 Å). The mobile phases consisted of 2% MeOH in water containing 5 mM NH_4OAc and 0.1% HOAc as solvent A, and 100% MeOH containing 5 mM NH_4OAc and 0.1% HOAc as solvent B. HPLC separation was performed by a linear gradient elution at a flow rate of 0.2 ml/min as follows: 0 min, 2% B; 3 min, 2% B; 14 min, 30% B; 16 min, 100% B; 18 min, 100% B; 20 min, 2% B, and 30 min, 2% B. Pure MeOH (0.8 ml/min) was added as a post-column addition before entering the APCI source.

The structure of **7** was further confirmed by NMR analysis. ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.59 (1H, s, –NHCO), 7.76 (1H, ddd, $-^{15}N = {}^{13}CH-{}^{15}N$, $J = 212.5$ Hz, $J = 11.9$ Hz, $J = 8.7$ Hz), 6.45 (2H, br.s, $-NH_2$), 5.41 (2H, d, $^{15}N-CH_2-O$, $J = 3.9$ Hz), 4.57 (2H, 2t, 2 C–OH, $J = 5.5$ Hz), 3.51 (1H, m, –CH–O), 3.45–3.20 (4H, m, 2 CH_2–O). ^{13}C NMR: enrichment at 140.18 ppm (dd, C-8, $^{13}C-{}^{15}N$ coupling). ^{15}N-NMR (calibrated using ^{15}N-ammonium acetate): enrichment at 150.42 ppm (d, ^{15}N-9, $^{15}N-{}^{13}C$ coupling) and 214.85 ppm (^{15}N-7), respectively.

Conclusion

In summary, we have demonstrated the facile synthesis of [8-^{13}C–7,9-$^{15}N_2$]-ganciclovir from commercially available [8-^{13}C–7,9-$^{15}N_2$]-guanine in 4 steps with a 25% overall yield, without the need to purify intermediates. This compound was isotopically pure enough to allow the development of a stable

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isotope dilution LC–MS method for pharmacokinetic studies of ganciclovir used in the treatment of mesothelioma.[7]

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References

1. Hirsch MS, Kaplan JC. In *Virology*, vol 1. Fields BN, Knipe DM, Chanock RM, Hirsch MS, Melnick JL, Monath TP, Roizman B (eds). Raven: New York, 1990; 441–468.
2. Cheng YC, Grill SP, Dutschman GE, Nakayama K, Bastow KF. *J Biol Chem* 1983; **258**: 12460–12464.
3. (a) Frank KB, Chiou JF, Cheng YC. *J Biol Chem* 1984; **259**: 1566–1569; (b) Mar EC, Chiou J, Cheng YC, Huang ES. *J Virol* 1985; **53**: 776–780.
4. (a) Hamzeh FM, Lietman PS, Gibson W, Hayward GS. *J Virol* 1990; **64**: 6184–6195; (b) Hamzeh FM, Lietman PS. *Antimicrob Agents Chemother* 1991; **35**: 1818–1823.
5. (a) Crumpacker CS. *New Engl J Med* 1996; **335**: 721–729; (b) Freeman S, Gardiner JM. *Mol Biotechnol* 1996; **5**: 125–137.
6. Banerjee D. *Curr Opin Mol Ther* 1999; **1**: 517–520.
7. Sterman DH, Recio A, Vachani A, Sun J, Cheung L, DeLong P, Amin KM, Litzky LA, Wilson JM, Kaiser LR, Albelda SM. *Clin Cancer Res* 2005; **11**: 7444–7453.
8. Zhang R, Sioma CS, Wang S, Regnier FE. *Anal Chem* 2001; **73**: 5142–5149.
9. (a) Bonfiglio R, King RC, Olah TV, Merkle K. *Rapid Commun Mass Spectrom* 1999; **13**: 1175–1185; (b) King R, Bonfiglio R, Fernandez-Metzler C, Miller-Stein C, Olah T. *J Am Soc Mass Spectrom* 2000; **11**: 942–950.
10. Gao H, Mitra AK. *Synthesis* 2000; **3**: 329–351.
11. (a) Ogilvie KK, Cheiyan UO, Radatus BK, Smith KO, Galloway KS, Kennell WL. *Can J Chem* 1982; **60**: 3005–3010; (b) Ashton WT, Karkas JD, Field AK, Tolman RL. *Biochem Biophys Res Commun* 1982; **108**: 1716–1721; (c) Martin JC, Dvorak CA, Smee DF, Mathews TR, Verheyden JPH. *J Med Chem* 1983; **26**: 759–761.
12. Lodewijk E, Han YK, Schloemer GC. European Patent, EP 0704445 B1, 1996.
13. Janicki SZ, Fairgrieve JM, Petillo PA. *J Org Chem* 1998; **63**: 3694–3700.

DOI: 10.1002/jlcr