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Research Article

Synthesis of deuterium-, tritium-, and carbon-14-labeled BILN2061, a potent hepatitis C virus protease inhibitor

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Summary

Hepatitis C virus (HCV) serine protease is a target for antiviral therapy against HCV infection, a leading cause of liver transplantation in the US. BILN2061, (1*S*, 4*R*, 6*S*, 7*Z*, 14*S*, 18*R*)-14-cyclopentyloxycarbonylamino-18-[2-(2-isopropylamino-thiazol-4-yl)-7-methoxyquinolin-4-yloxy]-2,15-dioxo-3,16-diazatricyclo[14.3.0.0^{4,6}]nonadec-7-ene-4-carboxylic acid, is a potent inhibitor of HCV and the first compound in this class of cyclic peptides in human trials. Here, we report the synthesis of deuterium-labeled BILN2061 with isotopic enrichment of 99%, tritium-labeled BILN2061 with a specific activity of 17.1 GBq/mmol, and carbon-14-labeled BILN2061 with a specific activity of 1.83 GBq/mmol. The isotopes were incorporated via a Hantzsch thiazole synthesis of labeled isopropyl thiourea and α-bromoketone intermediate. The preparation of labeled isopropyl thiourea is reported. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: HCV; BILN2061; deuterium; tritium; carbon-14

Introduction

Since it was identified in 1989, hepatitis C virus (HCV) has been viewed as the major cause of chronic hepatitis in humans.[1] Globally, 3% of the world population is infected with this virus. Interferon or a combination of interferon and ribavirin, are the only approved therapies for treatment of HCV infections. However, these therapies suffer from low efficacy and various side effects.[2] The

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other treatment is liver transplantation for a few lucky patients. Therefore a lot of effort has been concentrated on the discovery and development of new and selective drugs to prevent and treat HCV-related diseases.

The HCV-encoded NS3 serine protease is essential for viral replication *in vivo*, and consequently, it is a good target for antiviral therapy against HCV.[3–5] An extensive search for potent inhibitors of this protease led to the discovery of novel macro-cyclic tripeptides with nanomolar cellular potency and an adequate oral pharmacokinetic profile in animals.

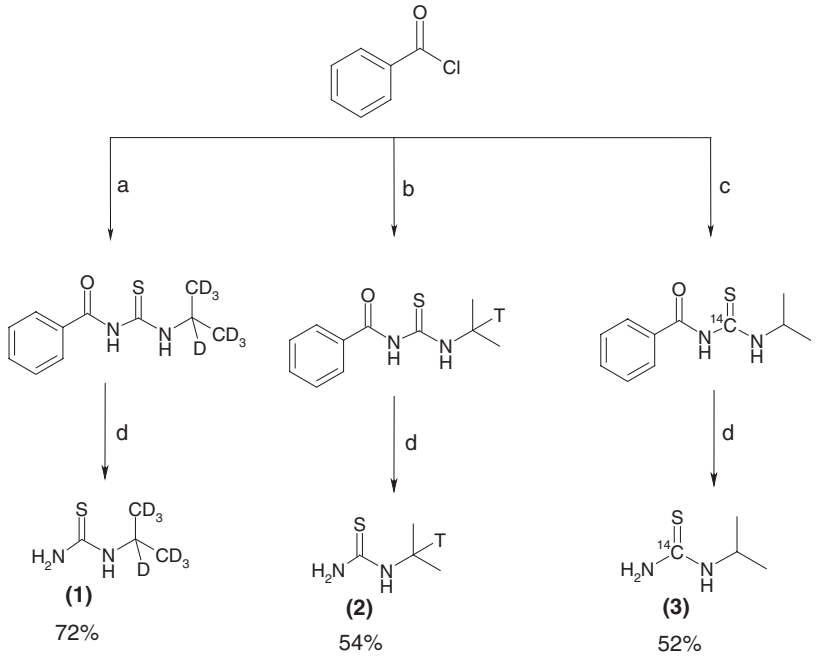
BILN2061 is the first of this class of compounds to reach human trials. Administration of BILN2061 to patients infected with HCV genotype 1 for 2 days resulted in an impressive reduction of HCV RNA plasma levels.[6–9] The goal of this work was to prepare labeled BILN2061 with stable and radioactive isotopes to support research, drug metabolism and pharmacokinetics studies.

Results and discussion

BILN2061 is a tripeptide molecule, with a 15-member ring, five stereo centers, and a cyclic *cis*-double bond. The synthesis of this compound included (among many manipulations), preparation of a substituted cyclopropyl-amino acid, a quinoline-thiazole moiety, and ring closure metathesis.[10] The quinoline-thiazole moiety was considered to introduce stable and radioactive isotopes on BILN2061 to simplify the synthesis. The classical Hantzsch thiazole synthesis has been used extensively to prepare substituted thiazoles from the condensation of α-halo-ketones and *N*-monosubstituted thioureas.[11] The mechanisms of this reaction have been studied in detail.[12] For example, under neutral conditions, the 2-(*N*-substituted amine)thiazoles are exclusively formed. The regioselectivity can be altered to give both the 2-substituted and the 3-substituted-2-imino-2,3-dihydrothiazoles under acidic conditions.[13]

As shown in Scheme 1, the preparation of [$^2H_7$]-isopropylthiourea was accomplished by first treating benzoyl chloride with potassium thiocyanate in acetone. The isothiocyanate thus produced was then treated *in situ* with [$^2H_7$]-isopropylamine (99.2 at% D) to give the benzoylthiourea intermediate in 80% yield. Hydrolysis of this benzoylthiourea with aqueous sodium hydroxide gave [$^2H_7$]-isopropylthiourea in 72% yield after crystallization from hexane: ethyl acetate.[14,15] Deuterated isopropylthiourea was then condensed with the α-bromoketone (**5**) ((1*S*, 4*R*, 6*S*, 14*S*, 18*R*)-18-[2-(2-bromoacetyl)-7-methoxyquinolin-4-yloxy]-14-cyclopentyloxycarbonylamino-2,15-dioxo-3,16-diaza-tricyclo[14.3.0.0^{4,6}]nonadec-7-ene-4-carboxylic acid methyl ester) at 70°C in isopropanol to give the substituted thiazole in 98% yield. This material was then hydrolyzed with a solution of LiOH in THF/methanol at room temperature to give [$^2H_7$]-BILN2061 in 91% yield (Scheme 2).

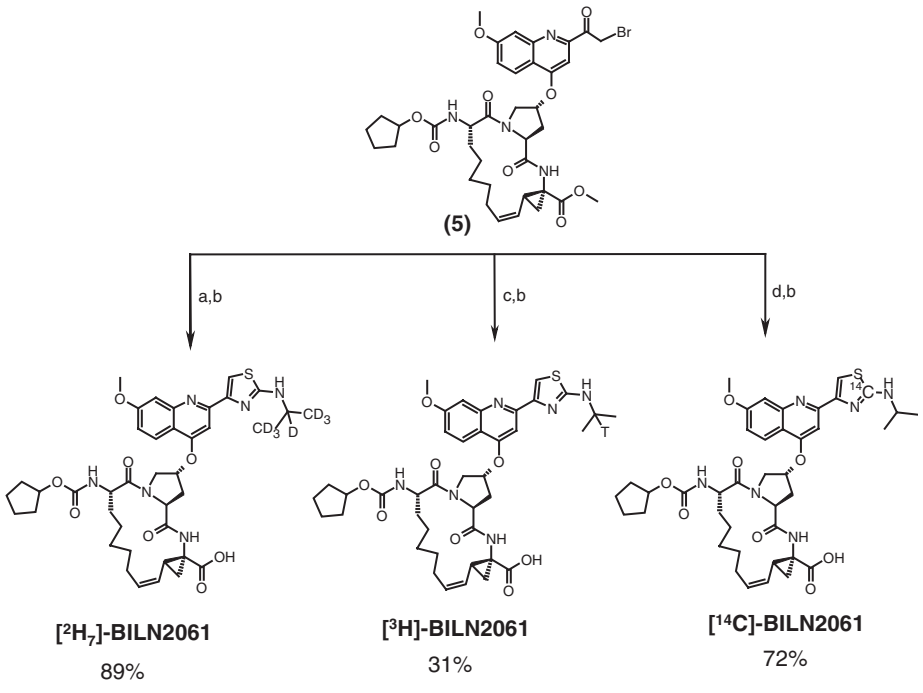
The same approach was used to prepare tritium-labeled BILN2061 using tritium-labeled isopropylamine ([2-^{3}H]-isopropylamine, prepared by ARC by



Scheme 1. Synthesis of labeled isopropylthiourea. (a) KSCN, acetone [$^2H_7$]-isopropylamine; (b) KSCN, acetone, [2-^{3}H]-isopropylamine; (c) KS^{14}CN, acetone, isopropylamine; (d) aqueous 10% NaOH

tritium gas reduction of acetone oxime) in 17% overall yield. Thus, tritium-labeled isopropylamine with a specific activity of 25 Ci/mmol was diluted with unlabeled isopropylamine and used to prepare tritium-labeled isopropyl thiourea in 54% radiochemical yield. Coupling as seen before to the α-bromoketone (**5**) followed by ester hydrolysis gave [^{3}H]-BILN2061 with a specific activity of 462 mCi/mmol.

For the synthesis of [^{14}C]-BILN2061, initial consideration was given to the carbamate-capping group. Carbon-14 atom may be introduced into this moiety if [^{14}C]-phosgene is used to prepare the cyclopentyl-carbamoyl chloride. However, concerns over the stability of the carbamates *in vivo* prevented the pursuit of this route. The focus was then shifted to parts of the molecule which are less susceptible to metabolism. As seen before in the synthesis of [$^2H_7$]- and [^{3}H]-BILN2061, the quinoline-thiazole part was built into the molecule from a reaction of α-bromoketone-quinoline intermediate (**5**) and isopropylthiourea. The radiosynthesis required the preparation of [^{14}C]-isopropylthiourea, with the carbon-14 atom preferably in the urea and not on the isopropyl side chain, due to the fact that 2-alkylaminothiazoles are prone to metabolic cleavage to aminothiazoles. So to prepare [^{14}C]-labeled isopropylthiourea, [^{14}C]-potassium thiocyanate with a specific activity of



Scheme 2. Synthesis of labeled BILN2061. (a) isopropanol, (1), 70°C; (b) LiOH, THF/water/MeOH, rt; (c) isopropanol, (2), 70°C; (d) isopropanol, (3), 70°C; (e) isopropanol, (4), 70°C

50 mCi/mmol was reacted with benzoyl chloride giving benzoyl isothiocyanate, which was trapped with isopropylamine in acetone to give *N*-benzoyl-*N*′-isopropylthiourea as reported above. Hydrolysis with a 10% aqueous sodium hydroxide solution gave the desired [^{14}C]-labeled isopropylthiourea in more than 50% radiochemical yield (Scheme 1). Reaction of [^{14}C]-isopropylthiourea with the α-bromoketone (**5**) in isopropanol with heating at 70°C gave the quinoline-thiazole derivative (referred to as BILN2061 methyl ester) in 76% radiochemical yield. Simple hydrolysis of the methyl ester to the acid using lithium hydroxide in a mixture of methanol, tetrahydrofuran and water gave [^{14}C]-BILN2061 in 95% yield and with a specific activity of 49.58 mCi/mmol (Scheme 2). [^{14}C]-BILN2061 was stored neat at −80°C or as a dilute ethanolic solution at −20°C for up to 3 months with little decomposition.

Experimental procedures

Materials and methods

All radioactive experiments were carried out in the radiosynthesis laboratory in hoods dedicated to carbon-14 or tritium syntheses only. Weighing operations were performed on a Mettler MTS microbalance (calibrated

and checked for accuracy every 6 months). Liquid scintillation counting was accomplished using a Beckman LS5000TA and ready safe™ cocktail (Beckman, Fullerton, CA). Radio-TLC was carried out on a BIOSCAN System 200 imaging scanner using an auto-changer 1000 and WinScan software version 2.1a (Bioscan Inc., Washington, DC). The quantification of the HPLC chromatograms was carried out using an HPLC system comprised of a radiomatic A515 Flo-one\Beta radioactivity flow detector (Packard Instrument Company, Meriden, CT), two pumps (HITACHI L-6200A intelligent pump), a linear UVIS 200, Ultima Flo™ AP cocktail (Packard, Meriden, CT), and radiomatic 500TR V 3.60 for data evaluation. The analytical HPLC purity verification was carried out on a Zorbax SB-C18 column, particle size 5 μm, 4.6 × 150 mm, and the column was fitted with an OPTI-Guard C18 column guard (1.0 mm). UV detection was at 220 nm. Mobile phase: A (water), B (acetonitrile), both solvents contain TFA (10 mM). Gradient: 20–100% B in 30 min, then back to 20% B in 5 min and hold for 2 min at 20% B. Evaporation of solvents and non-radioactive volatile components was accomplished at reduced pressure using Büchi rotary evaporator unless stated otherwise. Mass spectra for non-radioactive compounds were acquired by a Hewlett-Packard auto sampler Series 1100, connected to a Micromass Platform LCZ in the ES mode. NMR spectra were recorded with a Bruker 400 MHz DPX spectrometer using deuterated chloroform as a solvent and tetramethyl silane as the internal standard unless stated otherwise. Melting points are uncorrected and were obtained using MEL-TEMP® 3.0 (Laboratory Devices, Inc., USA). Pre-coated TLC sheets (silica gel 60 F_{254}) and silica gel 60–200 Mesh (Nominal, I.D., grade 62) for flash chromatography, were obtained from EM Science (Gibbstown, NJ). [$^2H_7$]-isopropylamine with 99.2at% D, was from CDN, Pointe Claire, Que., Canada. Tritium-labeled isopropylamine was purchased from ARC, Saint Louis, MO with a specific activity of 25 Ci/mmol. [^{14}C]-Potassium thiocyanate was obtained from Moravek Biochemicals & Radiochemicals Inc. with a specific activity of 50 mCi/mmol. Isopropylthiourea was purchased from Lancaster Synthesis. The rest of the reagents were purchased from Aldrich Chemicals Company.

Non-radioactive synthesis

Synthesis of [$^2H_7$]-isopropylthiourea (1). To a solution of potassium thiocyanate (1.47 g, 15.13 mmol) in acetone (30 ml) was added benzoyl chloride (1.67 ml, 14.37 mmol) at 0°C under nitrogen atmosphere. The resulting cloudy solution was stirred for 90 min at this temperature before the ice-bath was removed and [$^2H_7$]-isopropylamine (99.2at% D, 1.0 g, 15.13 mmol) was added via syringe. The mixture was further stirred at room

temperature for another 90 min, and then poured into an Erlenmeyer flask and water was added to give a fluffy white solid. This solid was filtered, washed with water and dried under reduced pressure to give 2.618 g of the *N*-benzoyl-*N'*-isopropylthiourea as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 10.60 (brs, 1H), 8.91 (brs, 1H), 7.83 (d, $J = 8.20$ Hz, 2H), 7.62 (dt, $J = 8.20$, 1.14 Hz, 1H), 7.51 (t, $J = 8.20$ Hz, 2H). MS-ES+: *m/z* 230.1 (MH^+, 50%), MS-ES-: *m/z* 228 (M^-, 20%). A mixture of this derivative (2.6 g, 11.34 mmol) in 10% aqueous NaOH (40 ml), was heated to reflux for 30 min to give a colorless solution. After cooling to 60°C, it was extracted with ethyl acetate. The combined extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to remove most of the solvent. Hexane was then added to give a fluffy white precipitate, which was filtered, washed with hexane, and dried under reduced pressure to give 1.02 g of product in 72% yield. ^{13}C-NMR (DMSO-d_6) δ: 181.82, 44.64 (m), 20.96 (m). MS-ES+: *m/z* 126 (MH^+, 100%). Mp = 161–163°C.

Synthesis of [$^2H_7$]-BILN2061 methyl ester. A solution of [$^2H_7$]-isopropylthiourea (50 mg, 0.4 mmol), (**5**) (252.5 mg, 0.33 mmol) in isopropyl alcohol (4 ml) was stirred at 70°C in a preheated oil bath for 3 h. After cooling to room temperature, the solution was concentrated *in vacuo*. The residue was dissolved in chloroform and washed with a saturated aqueous $NaHCO_3$ (2 × 60 ml), brine (100 ml), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The yellow residue was purified by flash column chromatography to give 310 mg of a white solid. ^{1}H-NMR ($CDCl_3$) δ: 7.98 (d, $J = 9.10$ Hz, 1H), 7.47 (s, 1H), 7.38 (brs, 2H), 7.05 (dd, $J = 9.10$, 2.43 Hz, 1H), 6.99 (s, 1H), 5.54 (m, 1H), 5.39 (m, 2H), 5.24 (t, $J = 9.60$ Hz, 1H), 5.18 (s, 1H), 5.03 (m, 1H), 4.90 (m, 1H), 4.62 (t, $J = 7.34$ Hz, 1H), 4.31 (d, $J = 11.45$ Hz, 1H), 4.06 (m, 1H), 3.94 (s, 3 H), 3.68 (s, 3 H), 3.06 (m, 1H), 2.39 (m, 1H), 2.16–2.24 (m, 3 H), 1.85–1.93 (m, 2H), 1.60–1.84 (m, 11H), 1.56 (m, 2H), 1.42 (m, 3 H). MS-ES+: *m/z* 796.5 (MH^+, 100%), MS-ES-: *m/z* 794.3 (M-, 100%).

Synthesis of [$^2H_7$]-BILN2061. A solution of [$^2H_7$]-BILN2061 methyl ester (270 mg, 0.34 mmol) in THF (8 ml) and methanol (4 ml), was added a solution of lithium monohydrate in water (114 mg, 4 ml). The resulting mixture was stirred at room temperature overnight. Then, most of the solvents were removed under reduced pressure to obtain a white paste. To this, were added water (5 ml) and brine (2 ml) followed by aqueous 1 N HCl solution until pH = 6. The solution was extracted with ethyl acetate and the combined extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 241 mg of a yellow solid. ^{1}H-NMR ($CDCl_3$) δ: 10.01 (brs , 1H), 7.96 (d, J= 9.08 Hz, 1H), 7.48 (s, 1H), 7.42 (s, 1H), 7.31 (s, 1H), 7.11 (d, J= 9.08 Hz, 1H), 6.39 (s, 1H), 5.48–5.60 (m, 2H), 5.11 (m, 1H), 5.09 (m, 1H), 4.78 (d, J= 7.87 Hz, 1H), 4.65 (m, 1H), 4.28 (m, 1H), 3.95 (s, 3 H), 3.76 (m, 1H), 2.90

(m, 1H), 2.45 (m, 1H), 2.30 (m, 1H), 2.14 (m, 1H), 1.75–2.00 (m, 9H), 1.60–1.75 (m, 4 H), 1.41–1.60 (m, 4 H), 1.25–140 (m, 4 H). MS-ES: m/z 782.4 (MH^+, 100%), MS-ES-: m/z 780.2 (M-, 100%). HPLC, $R_t = 16.00$, 99.3%.

Radiosynthesis

Synthesis of [^{3}H]-BILN2061. A batch of [^{3}H]-BILN2061 was prepared in an analogous fashion to the deuterium-labeled material starting from [^{3}H]-isopropylamine. To a solution of KSCN in acetone (0.5 ml) was added benzoyl chloride (4.8 µl, 41.1 µmol) at 0°C. After stirring at this temperature for 2 h, the ice-bath was removed and a solution of [^{3}H]-isopropylamine (70 mCi, 25 Ci/mmol, 2.8 µmol) in toluene (1 ml) was added. The resulting mixture was stirred at room temperature for 3 h before unlabeled isopropylamine (3.25 µl, 38.3 µmol) was added and the mixture was stirred overnight. Most of the solvents were removed under reduced pressure and the residue was dissolved in 10% aqueous NaOH (1 ml) and heated to 100–110°C for 30 min. The solution was cooled to 60–70°C and extracted with ethyl acetate (3×2 ml). The combined extracts were concentrated under a stream of nitrogen then under reduced pressure for 3 h. The residue was dissolved in ethanol (2 ml) to afford a stock solution of [^{3}H]-isopropylthiourea (2) (39 mCi, 56% radiochemical yield). [^{3}H]-Isopropylthiourea (38 mCi) was taken on to afford a total of 11.9 mCi of [^{3}H]-BILN2061 with a specific activity of 462 mCi/mmol. Radio-HPLC: $R_t = 16.20$ (97.46%).

Synthesis of [^{14}C]-BILN2061. Acetone (2 ml) was added to a vial containing [^{14}C]-potassium thiocyanate (50 mCi, specific activity = 50 mCi/mmol, 1.0 mmol). The resulting solution was cooled to 0°C in an ice-bath before benzoyl chloride (110 µl, 0.95 mmol) was added in one portion via syringe. The cloudy mixture was further stirred at this temperature for 1.5 h, then the ice-bath was removed and isopropylamine (85.2 µl, 1.0 mmol) was added quickly. The mixture was stirred at room temperature for 1.5 h. Water (10 ml) was added to give a fluffy white solid. The solid was filtered and transferred to a new reaction vial using acetone. Acetone was removed under reduced pressure and the residue was treated with 10% aqueous NaOH (3 ml) and heated to 110°C for 30 min. The clear solution was cooled to 40°C and extracted with ethyl acetate. The combined extracts were concentrated under a stream of nitrogen to about 1.0 ml, and then *n*-hexane was added slowly to give a fluffy white solid. Most of the solvents were removed and the remaining solid was dried *in vacuo* to give 62 mg of [^{14}C]-isopropylthiourea (3) as a white solid in 52% chemical yield. [^{14}C]-BILN2061 methyl ester was obtained as described in the non-radioactive synthesis from [^{14}C]-isopropylthiourea (62 mg, 0.52 mmol) and (**5**) (384.5 mg, 0.5 mmol). The product was isolated by flash chromatography purification as a white solid (319 mg) with a specific activity of

49.58 mCi/mmol and a total radioactivity of 20 mCi. The product elutes with cold BILN2061 methyl ester on TLC (10% MeOH/$CHCl_3$) and HPLC: Rad-detection, $R_t = 17.90$ min (99%); UV detection, $R_t = 17.55$ min (99%). Hydrolysis of this methyl ester (315 mg, 0.4 mmol) as seen before, gave 309 mg of a yellow solid in 95% chemical yield or 19.0 mCi with a specific activity of 49.58 mCi/mmol. The product elutes with cold BILN2061 on HPLC: Rad-detection, $R_t = 16.00$ min (99%); UV detection, $R_t = 15.65$ min (99%). ^{1}H-NMR using double encapsulation of both this material and a non-radioactive sample of BILN2061 (similar concentrations in $CDCl_3$) were identical.

Conclusion

Deuterium- and tritium-labeled BILN2061 was prepared in 64 and 17% overall yield, respectively, from the commercially available [$^2H_7$]-isopropylamine or [2-^{3}H]-isopropylamine. Carbon-14-labeled BILN2061 was prepared in 37% overall radiochemical yield starting from commercially available [^{14}C]-potassium thiocyanate to incorporate the radioactive carbon in the thiazole ring. The syntheses were efficient and straightforward.

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