JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 847–856.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.873

Research Article

Synthesis of deuterium, tritium, and carbon-14 labeled BIRB 796, a p38 MAP kinase inhibitor

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Summary

1-(5-*tert*-Butyl-2-*p*-tolyl-2H-pyrazol-3-yl)-3-[4-(2-morpholin-4-yl-ethoxy)naphthalen-1-yl]urea (BIRB 796), currently in clinical trials for the treatment of inflammatory diseases, is a potent inhibitor of p38 MAP kinase. Labeled BIRB 796 with stable and radioactive isotopes was required for metabolism, distribution, and absorption studies. We first report the synthesis of carbon-14 labeled BIRB 796 with a specific activity of 2 GBq/mmol (54.2 mCi/mmol), using [^{14}C]-phosgene under modified Schotten–Baumann conditions; second the preparation of tritium-labeled BIRB 796 with a specific activity of 659 GBq/mmol (17.81 Ci/mmol) by reductive dehalogenation of iodo-BIRB 796 with tritium gas; and finally, the synthesis of $^{2}H_{8}$-BIRB 796 using morpholine-2,2,3,3,5,5,6,6-$^{2}H_{8}$ with isotopic enrichment of 98.9 at% ^{2}H. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: p38 MAP kinase; tritium; deuterium; carbon-14; BIRB 796

Introduction

The p38 is a member of the mitogen-activated protein (MAP) kinases that control many cellular activities.[1] It is a stress responsive, and a signal-transducing kinase.[2] Inhibition of p38 MAP kinase leads to the blockage of the synthesis of several pro-inflammatory cytokines including tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β), which mediate the inflammatory response associated with immunological recognition of infectious agents. The presence of elevated levels of pro-inflammatory cytokines is associated with several diseases.[3] Therefore inhibitors of this kinase may be used as therapeutic agents to treat chronic inflammatory diseases and offer a potential strategy to treat several other diseases including reducing HIV expression

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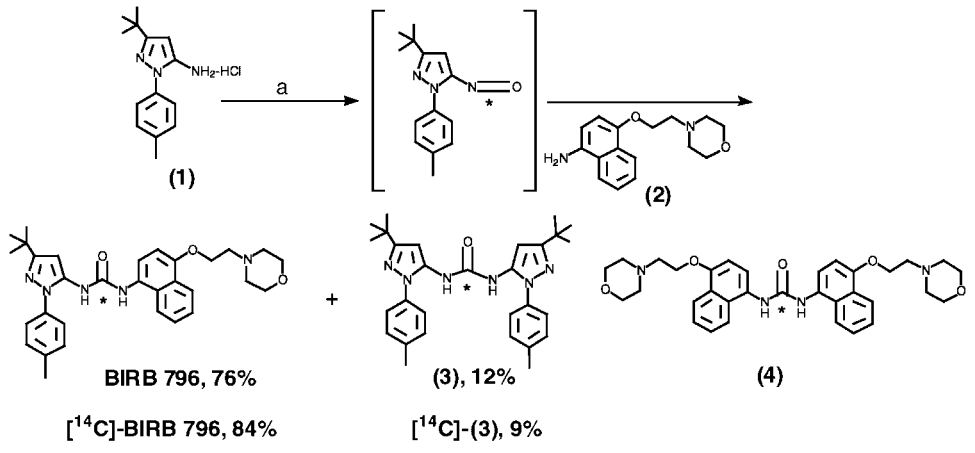
Received 20 November 2003
Revised 16 April 2004
Accepted 14 May 2004



Scheme 1. Synthesis of BIRB 796 and [^{14}C]-BIRB 796. (a) sat'd $NaHCO_3$, Cl-CO-Cl or Cl-^{14}CO-Cl, CH_2Cl_2, asterisk indicates position of isotope

when combined with standard HIV antiretroviral agents.[4] Thus, the development of p38 inhibitors is a very competitive field and the goal of many pharmaceutical companies.[5]

BIRB 796 (Scheme 1), a *N,N′*-disubstituted urea, is a potent and a selective inhibitor of human p38 MAP kinase with nanomolar inhibitory activity in cell culture. It is also one of the slowest dissociating inhibitors against human p38 MAP kinase reported so far.[6] BIRB 796 was found to inhibit p38 MAP kinase by stabilizing a conformation of a kinase that is incompatible with ATP binding.[6] BIRB 796 is currently in clinical trials for the treatment of inflammatory diseases.[7]

Results and discussion

N,N′-Disubstituted ureas are easily accessible from primary amines. The starting primary amines are first activated as isocyanates,[8] carbamates, for example from the reaction of 2,2,2-trichloroethyl chloroformate and amines (TROC-carbamates);[9] as *N,N′*-carbonyl-imidazole[10] or *N,N′*-carbonyl-1,2,4-triazole derivatives[11] using carbonyl diimidazole (CDI) or carbonyl di-1,2,4-triazole (CDT), respectively. Carbon dioxide has been used to prepare ureas,[12] and is an attractive way to introduce a carbon-14 on the carbonyl urea.[13] Another substitute for [^{14}C]-carbon dioxide is [^{14}C]-phosgene, which is available commercially as a solution in toluene. The primary disadvantages of [^{14}C]-phosgene are its instability and excess has to be used to avoid the generation of symmetrical ureas. On a small scale, excess phosgene is easily removed by evaporation. This procedure is not acceptable when using [^{14}C]-phosgene. However, under Schotten–Baumann conditions, excess phosgene is hydrolyzed to carbonic acid and trapped in a basic aqueous

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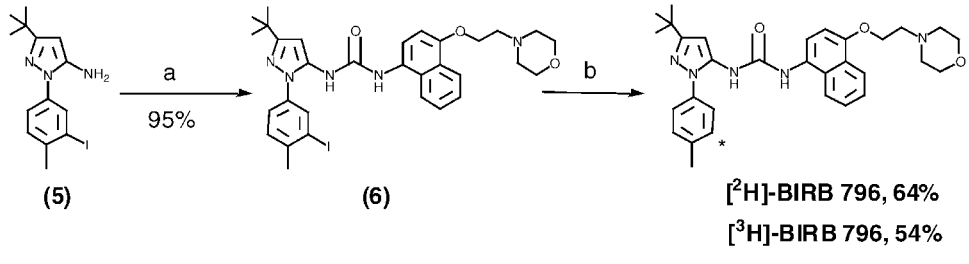
phase.[14] [^{14}C]-phosgene can also be used to prepare CDI or CDT which are easily handled as solids and stored for longer periods of time when kept away from moisture.[10,11]

In Scheme 1, good yields of the desired urea product were obtained when phosgene was used to generate the isocyanate derivative of 1-(4-methylphenyl)-3-*tert*-butyl-2*H*-aminopyrazole-HCl (**1**), which is then trapped by the addition of 4-amino-1-[(2-morpholin-yl)ethoxy]naphthalene (**2**) to produce BIRB 796 in 84% chemical yield and the undesired symmetrical pyrazole urea (**3**) in 9% chemical yield, both yields are based on pyrazole (**1**). Only 1.5 equivalent of [^{14}C]-phosgene in toluene was used to obtain the above yields. Attempts to generate the isocyanate derivative of naphthylamine (**2**) and trapping it with the pyrazolamine (**1**) gave the symmetrical naphthyl urea (**4**) as the major product. This discrepancy in reactivity is probably due to the different nucleophlicity of the pyrazole amine (**1**) and the naphthyl amine (**2**). [^{14}C]-BIRB 796 was easily separated from the symmetrical urea [^{14}C]-(**3**) by flash chromatography. Further crystallization from isopropanol gave a material with about 99% chemical and radiochemical purities and a specific activity of 54.2 mCi/mmol.

To prepare the tritium-labeled BIRB 796, Scheme 2, the iodo-precursor (**6**) was synthesized from 1-(3-iodo-4-methylphenyl)-3-*tert*-butyl-2*H*-aminopyrazole (**5**), prepared from 3-iodo-4-methylaniline according to the literature,[15] and from naphthyl amine (**2**) using phosgene as seen before. The conditions for introducing the tritium atom were first investigated using reductive dehalogenation with deuterium gas of iodo-BIRB 796 (**6**) in the presence of palladium on carbon (10%) and triethylamine in ethanol. The reduction gave the specifically labeled mono-deuterated BIRB 796. These conditions were then applied to the synthesis of tritium-labeled BIRB 796. Tritium incorporation was about 62% or 17.81 Ci/mmol.

In the synthesis of deuterium labeled BIRB 796, Scheme 3, morpholine-2,2,3,3,4,4,5,5,6,6-$^{2}H_8$ (**7**) was used to prepare the right-hand side fragment (**13**) according to the literature.[7] Thus, [$^{2}H_8$]-morpholine was refluxed with



Scheme 2. Synthesis of [^{2}H]-BIRB 796 and [^{3}H]-BIRB 796. (a) sat'd $NaHCO_3$, $COCl_2$, CH_2Cl_2, (2): (b) $^{2}H_2$ or $^{3}H_2$, EtOH, Et_3N, 10% Pd/C

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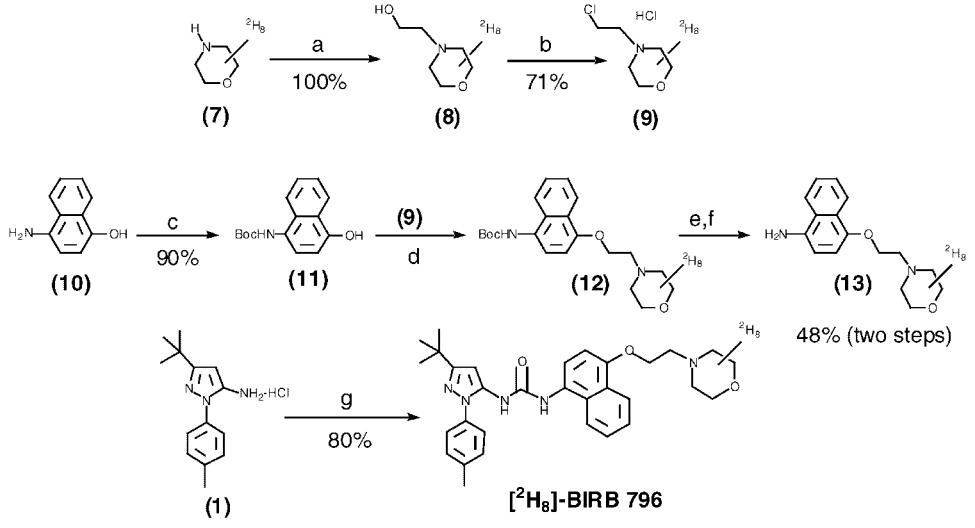

Scheme 3. Synthesis of [$^2H_8$]-BIRB 796. (a) $BrCH_2CH_2OH$, K_2CO_3, CH_3CN; (b) $SOCl_2$, $CHCl_3$; (c) *t*-BuOK, $(t\text{-Boc})_2O$, THF; (d) K_2CO_3, CH_3CN; (e) 4 N HCl, 1,4-dioxane; (f) sat'd $NaHCO_3$, EtOAc; (g) sat'd $NaHCO_3$, $COCl_2$, CH_2Cl_2, (13)

ethylene bromohydrin and potassium carbonate in acetonitrile,[16] and the product, 4-(2-hydroxyethyl) morpholine (**8**), was converted to the chloro-analog (**9**) by refluxing in thionyl chloride and chloroform. Crystallization from ethanol gave 1-chloro-2-morpholin-4-yl-ethane-HCl (**9**) in 71% overall yield in two steps.[17] 4-Amino-1-naphthol (**10**) was first protected at the amino group using di-*tert*-butyl dicarbonate, then coupled to compound (**9**). Removal of the Boc-protecting group with hydrogen chloride solution in dioxane followed by treatment with a saturated solution of sodium bicarbonate gave the free base (**13**) in 38% overall yield. Compound (**13**) was then reacted with the isocyanate of (**1**) as seen before to give [$^2H_8$]-BIRB 796.

Experimental procedures

Materials and methods

Silica gel TLC was performed for analysis with pre-coated aluminum sheets and glass plates with fluorescent indicator (EM Separating, Gibbstown, NJ, and Merck, Germany, respectively). Silica gel 60–200 Mesh (Nominal, I.D., grade 62) was obtained from EM Science (Gibbstown, NJ). Melting points (uncorrected) were recorded on MEL-TEMP® 3.0 (Laboratory Devices Inc., USA). All reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI), except for phosgene, which was purchased from Fluka (Milwaukee, WI) as a 20% solution in toluene, 4-amino-1-naphthol hydrochloride from Acros,

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morpholine-2,2,3,3,4,4,5,5,6,6-$^{2}H_8$ from CDN (Pointe-Claire, Quebec, Canada), and [^{14}C]-phosgene from ViTrax (Placentia, CA). Solvents were of HPLC grade. Reverse phase separations were accomplished at 35°C using HITACHI L-6200A Intelligent pump and Radiomatic Flo-one\Beta (Packard), and LINEAR UVIS 200 set at 254 nm and Ultima flo® AP cocktail (Packard). The HPLC columns were heated with an Eppendorf CH-30 column heater. The separation was accomplished on a 3M-Z18, 4.6 × 150 mm (Cohesive Technologies Inc.) using a gradient 40–85% methanol in water (both solvents contain 10 mM triethylamine) as the mobile phase. The flow rate was 1 ml/min. Weighing operations were performed on a Mettler MT5 microbalance or on a PG 503 Delta Range® (Mettler Toledo) for larger scale. Liquid scintillation counting was accomplished using a Beckman LS 5000 TA and Ready Safe cocktail (Beckman, Fullerton, CA). Radio-TLC imaging was carried out on a BIOSCAN System 200 Imaging Scanner using an auto-changer 1000 and winScan software V2.1a (Bioscan Inc., Washington, DC). Evaporation of solvents and volatile components was accomplished at reduced pressure using a Büchi rotary evaporator unless stated otherwise. Mass spectra were acquired by a Hewlett Packard auto sampler Series 1050, connected to a Micromass Platform LCZ in the ES mode or by a Finnigan SSQ7000 mass spectrometer in the Particle Beam-NH3-CI (PB-NH3-CI) mode. NMR spectra were recorded with a Bruker 400 MHz DPX spectrometer using deuterated chloroform as a solvent and tetramethylsilane as the internal standard. The proton NMR spectrum of [^{14}C]-BIRB 796 was recorded using a sample of 10 mg in 0.3 ml of deuterated chloroform, placed in a Teflon tube liner (Wilmad, cat No. 6005-7). The liner was capped with a Teflon plug and inserted into a screw cap NMR tube (Wilmad 507-TR-8).[18]

Non-radioactive synthesis

Synthesis of 1-(5-tert-butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[4-(2-morpholin-4-yl-ethoxy)naphthalen-1-yl]urea (BIRB 796), Scheme 1. To a suspension of 5-*tert*-butyl-2-*p*-tolyl-2*H*-pyrazol-3-ylamine-HCl (**1**) (443.4 mg, 1.67 mmol) in CH_2Cl_2 (30 ml), stirred at room temperature, was added a saturated solution of $NaHCO_3$ (30 ml). The resulting two-phase clear solution was cooled to 0°C in an ice bath. Stirring was stopped and a solution of 20% phosgene in toluene (1.43 ml, 2.5 mmol) was added via a syringe to the CH_2Cl_2 phase. The stirring was then resumed and continued for 4 h at 0°C. 4-(2-Morpholin-4-yl-ethoxy)-naphthalen-1-ylamine (**2**) (364 mg, 1.33 mmol) was added in one portion and the resulting mixture was stirred overnight as the ice melted. The organic phase was transferred via a syringe to another round bottom flask, and the remaining aqueous solution was extracted twice with methylene chloride (30 ml × 2) (stirring vigorously at room temperature with CH_2Cl_2). The combined solutions were concentrated *in vacuo* and the residue was purified by flash

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chromatography on silica gel using chloroform and methanol (1%) as eluent to give 100 mg of the symmetrical urea, 1,3-*bis*-(5-*tert*-butyl-2-*p*-tolyl-2*H*-pyrazol-3-yl)-urea (**3**) and 700 mg of the desired product as an off-white solid. The product was dissolved in 5 ml of isopropanol and about 20 mg of decolorizing charcoal was added. The mixture was heated to boiling and then filtered. The resulting colorless solution was crystallized upon standing at room temperature to give 670 mg of a white powder in 95% yield based on compound (**2**), or 76% based on the pyrazole amine (**1**). $R_f = 0.52$ in 10% MeOH/$CHCl_3$, $R_t = 14.26$ min. ^{1}H NMR ($CDCl_3$) δ: 8.26(m, 1H), 7.81(d, $J =$ 7.53 Hz, 1H), 7.52(m, 2H), 7.31(d, $J = 8.04$ Hz, 1H), 6.91(m, 4H), 6.67(d, $J = 8.54$ Hz, 1H), 6.61(s, 1H, ex.), 6.47(s, 1H), 6.42(s, 1H), 4.27(t, $J = 5.52$ Hz, 2H), 3.75(t, $J = 4.52$ Hz, 4H), 2.97(t, $J = 5.52$ Hz, 2H), 2.67(t, $J = 4.52$ Hz, 4H), 2.27(s, 3H), 1.31(s, 9H). MS-ES+: MH^+ (528, 60%), 265(100%). ES-: 526(11%). PB-NH_3-CI: 230.2 (100%), MH^+ (528.3, 20%).

Synthesis of mono-deuterated BIRB 796, Scheme 2

1-[5-tert-Butyl-2-(3-iodo-4-methylphenyl)-2H-pyrazol-3-yl]-3-[4-(2-morpholin-4-yl-ethoxy)-naphthalen-1-yl]-urea (6). To a stirred solution of (**5**) (36 mg, 0.1 mmol) in methylene chloride (2 ml) was added a saturated solution of $NaHCO_3$ (2 ml) and the mixture was cooled to 0°C in an ice bath. Stirring was stopped and a 20% solution of phosgene in toluene (100 µl) was added directly to the organic phase via a syringe. The stirring was resumed and continued for two hours at this temperature. Compound (**2**) (22 mg, 0.08 mmol) was then added and the reaction mixture was warmed to room temperature overnight. The organic phase was separated and the aqueous layer was extracted twice with methylene chloride (5 ml). The combined extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 73 mg of a solid residue. Purification by flash chromatography gave the product in 95% yield, $R_f = 0.36$ in 10% MeOH/$CHCl_3$, $R_t = 14.23$ min ($R_t = 10.52$ for BIRB 796) in HPLC. ^{1}H NMR ($CDCl_3$) δ: 8.23(d, $J = 7.53$ Hz, 1H), 7.74(m, 1H), 7.48(m, 2H), 7.33(d, $J = 8.03$ Hz, 1H), 6.92 (m, 3H), 6.69(d, $J = 8.03$ Hz, 1H), 6.60(m, 1H), 6.47(s, 1H), 6.37(s, 1H), 4.62(t, $J = 5.52$ Hz, 2H), 3.74(t, $J = 3.51$ Hz, 4H), 2.96(t, $J = 5.52$ Hz, 2H), 2.66(t, $J = 3.51$ Hz, 4H), 2.34(s, 3H), 1.28(s, 9H). MS-ES+: MH^+ (654.3, 100%), 327.9(85%).

1-[5-tert-Butyl-2-(3-deutero-4-methylphenyl)-2H-pyrazol-3-yl]-3-[4-(2-morpholin-4-yl-ethoxy)-naphthalen-1-yl]-urea [^{2}H]-BIRB 796. A mixture of the above compound (6.75 mg, 10.34 µmol), 10% Pd/C (3.5 mg), and triethylamine (2.2 µl) in absolute ethanol (0.4 ml) was freeze degassed several times before deuterium gas was introduced (8.78 µmol). The mixture was warmed to room temperature and stirred for 3 h. The reaction was then diluted with 10.0 ml of water to remove all exchangeable deuterium and then extracted with

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methylene chloride. The combined methylene chloride extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was dissolved in acetonitrile and analyzed by HPLC. The product, $R_t = 10.55$ min, 64% and starting material about 36%, $R_t = 14.23$ min, MS-ES: [^{2}H]-BIRB 796 (MH+: 529, 60%), un-reacted iodo-BIRB 796 (MH+:654.2, 20%).

Synthesis of [$^2H_8$]-BIRB 796, Scheme 3

4-(2-Hydroxyethyl)-[2,2,3,3,5,5,6,6-$^2H_8$]morpholine (8). A mixture of [2,2,3,3,5,5,6,6-$^2H_8$]morpholine (**7**) (0.5 g, 5.26 mmol), ethyl bromohydrin (0.71 g, 5.7 mmol), and potassium carbonate (0.9 g, 6.52 mmol) in acetonitrile (20 ml) was refluxed for 3 h. After cooling to room temperature, water was added (10 ml) and the aqueous phase was extracted with chloroform. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 2.0 g of product, $R_f = 0.33$ in 10% MeOH/$CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 3.65(t, $J = 5.4$ Hz, 2H), 2.58(t, $J = 5.4$ Hz, 2H). MS-ES: MH^+ (140.2, 100%), M^+ (139.2, 50%), $M^+ + 2$ (141.2, 50%).

4-(2-Chloroethyl)-[2,2,3,3,5,5,6,6-$^2H_8$]morpholine (9). The above crude product (**8**) and thionyl chloride (3 ml) in chloroform (20 ml) were refluxed for 4 h. The solution was cooled to room temperature and concentrated *in vacuo*. The resulting yellow solid residue (0.92 g) was crystallized from ethanol to give 0.72 g of a white solid as the HCl salt. Mp = 174–175°C. MS-ES: MH^+ (158.2, 100%), M^+(157.2, 50%).

(4-Hydroxy-naphthalen-1-yl)-carbamic acid tert-butyl ester (11). A suspension of 4-amino-1-naphthol hydrochloride (**10**) (2.0 g, 10.2 mmol, 90% tech) in THF (30 ml) was cooled to −70°C (dry-ice-methylene chloride bath) under a nitrogen atmosphere. Potassium-*tert*-butoxide (9 ml, 1.0 M solution in THF) was added dropwise over a 10 min period. The resulting mixture was warmed to −10°C (dry-ice- ethylene glycol bath) and stirred for 1 h. The reaction was cooled again to −70°C and di-*tert*-butyl dicarbonate (2.47 g, 11.31 mmol) was added in THF (10 ml). The reaction was warmed gradually to room temperature and stirred overnight. The mixture was concentrated *in vacuo*, dissolved in ethyl acetate (100 ml) and washed with water (3 × 30 ml), brine (3 × 30 ml), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 3.0 g of crude material. $R_f = 0.6$ in 10% MeOH/$CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 8.08(d, 1H), 7.85(d, 1H), 7.52(d, 1H), 7.42(t, 1H), 7.20(d, 1H), 7.01(brs, 1H), 6.52(s, 1H), 6.41(brs, 1H), 1.6(s, 9H). ES-, M- (258.1, 100%).

[4-(2-[2,2,3,3,5,5,6,6-$^2H_8$]Morpholin-4-yl-ethoxy)-naphthalen-1-yl]carbamic acid tert-butyl ester (12). A mixture of 4-*t*-Boc-amino-1-naphthol (0.5 g, 2.5 mmol), the above chloroethylmorpholine (**9**) (667.5 mg, 2.57 mmol) and potassium carbonate (1.3 g, 9.2 mmol) in acetonitrile (20 ml) was refluxed

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overnight. The resulting brown mixture was cooled to room temperature and water was added. The organic layer was extracted with ethyl acetate (150 ml), washed with water, brine, and then dried over $MgSO_4$. Filtration and concentration *in vacuo* gave 0.87 g of a brown solid, which was purified by flash chromatography to give 0.76 g of a brown residue, $R_f = 0.33$ in 10% MeOH/$CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 8.26(d, 1H), 7.85(d, 1H), 7.72–7.45(m, 3H), 6.75 (d, 1H), 6.64(br s, 1H), 4.28(t, $J = 5.52$ Hz, 2H), 2.91(t, $J = 5.52$ Hz, 2H), 1.51 (s, 9H). MS-ES: MH^+ (381.4, 90%), M^+ (380.3, 20%), $M^+ + 2$ (382.4, 50%).

4-(2-[2,2,3,3,5,5,6,6-$^2H_8$]Morpholin-4-yl-ethoxy)-naphthalen-1-ylamine (13). A solution of the above material (0.74 g, 1.94 mmol) in dioxane (20 ml) was stirred with a dioxane solution of HCl (4.0 M, 2 ml) for 14 h. The resulting suspension was concentrated *in vacuo* to give a cream colored solid (0.78 g), which was dissolved in a saturated solution of $NaHCO_3$ and extracted with ethyl acetate (30 ml). The ethyl acetate solution was dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 0.64 g of a brown residue. Flash chromatography gave 330 mg of a brown residue, $R_f = 0.34$ in 10% MeOH/$CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 8.25(m, 1H), 7.82(m, 1H), 7.42(m, 2H), 6.68(br s, 2H), 4.20(t, $J = 5.52$ Hz, 2H), 2.91(t, $J = 5.52$ Hz, 2H). MS-ES: MH^+ (281.3, 100%), M^+ (280.3, 25%), $M^+ + 2$ (282.3, 70%), $M^+ + 3$ (283.3, 10%).

1-(5-tert-Butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[4-(2-[2,2,3,3,4,4,5,5,6,6-$^2H_8$]-morpholin-4-yl-ethoxy)-naphthalen-1-yl]-urea, [$^2H_8$]-BIRB 796. Using the same procedure as before, compound (**1**) (390 mg, 1.47 mmol), was reacted with a 20% phosgene solution in toluene (1.2 ml, 2.1 mmol), and the isocyanate derivative was reacted with (**13**) (329 mg, 1.175 mmol) in a bi-phasic solution of methylene chloride and a saturated solution of $NaHCO_3$. The crude product (0.83 g) was purified by flash chromatography using 50% EtOAc:CH_2Cl_2 to remove the symmetrical urea (**3**) and then 15% methanol: methylene chloride to elute the desired product (0.73 g). This product was dissolved in hot isopropanol (10 ml) with decolorizing charcoal, cooled, filtered, and concentrated *in vacuo*. Crystallization from isopropanol gave 0.4 g of a white solid. Mp : 152–153°C, $R_t = 10.67$ on HPLC, $R_f = 0.47$ in 10% MeOH/$CHCl_3$. ^{1}H NMR ($CDCl_3$) δ: 8.25(d, $J = 8.54$ Hz, 1H), 7.85(d, $J = 8.54$ Hz, 1H), 7.52(m, 2H), 7.32(d, $J = 8.53$ Hz, 1H), 6.95(m, 4H), 6.66 (d, $J = 8.53$ Hz, 1H), 6.59(s, 1H), 6.41(d, $J = 3.51$ Hz, 2H), 4.31(t, $J = 5.52$ Hz, 2H), 2.98(t, $J = 5.52$ Hz, 2H), 2.27(s, 3H), 1.31(s, 9H). MS-ES: MH^+ (536.5, 50%), M^+ (535.4, 8%), $M^+ + 2$ (537.5, 20%), $M^+ + 3$ (538.5, 5%).

Radioactive synthesis

Synthesis of 1-(5-tert-butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[4-(2-morpholin-4-yl-ethoxy)naphthalen-1-yl][^{14}C]urea (BIRB 796), Scheme 1. To a suspension of

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(**1**) (531 mg, 2.0 mmol) in CH_2Cl_2 (30 ml) was added a saturated solution of $NaHCO_3$ (30 ml) and the mixture was stirred at room temperature. The resulting two-phase clear solution was cooled to 0°C in an ice bath and stirring was stopped. Once the phases were separated, [^{14}C]-phosgene (150 mCi in 2.5 ml of anhydrous toluene) was added in one portion to the methylene chloride phase via a syringe. Stirring was then resumed and continued for 4 h at 0°C. Compound (**2**) (440 mg, 1.61 mmol) was added in one portion and the resulting mixture was stirred overnight while warming to room temperature. The organic phase was removed using a syringe fitted with a long needle. The remaining aqueous phase was extracted twice with methylene chloride (30 ml × 2). The combined solutions were concentrated *in vacuo* and the residue was purified by flash silica gel chromatography using 30% ethyl acetate in methylene chloride as eluent to first separate the undesired side product [^{14}C]-(**3**), (87 mg, 6.0 mCi), $R_f = 0.85$ in 10% MeOH/$CHCl_3$. The desired [^{14}C]-BIRB 796 was obtained by eluting with methanol in chloroform (10%), $R_f = 0.52$ in 10% MeOH/$CHCl_3$, $R_t = 10.79$ min using radio-HPLC system. The purification gave 885 mg of pure product and 93 mg of 85% pure [^{14}C]-BIRB 796. Crystallization of pure [^{14}C]-BIRB 796 from 3 ml of isopropanol gave 394 mg (first crop) of a white powder. The total activity of the crystallized product was 40.5 mCi and the specific activity was determined to be 54.2 mCi/mmol.

Preparation of tritium-labeled 1-(5-tert-butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[4-(2-morpholin-4-yl-ethoxy)naphthalen-1-yl]urea, Scheme 2. Using the same procedure as in the synthesis of mono-deuterated BIRB 796, tritium (8.95 μmol, 482 mCi) was introduced and the reaction mixture was stirred at room temperature for 3 h. The reaction vessel was then frozen in liquid nitrogen and evacuated (no measurable gas was noticed). The reaction was diluted with water and extracted with methylene chloride as before. After the work-up, methanol was added to the residue and evaporated under reduced pressure to remove any exchangeable tritium. A total activity of 90 mCi was obtained. HPLC analysis showed 54% of product and 45% of starting iodo-BIRB796. No tritium exchange was observed on the iodo-BIRB796. Purification of about 10 mCi fraction of this material by HPLC gave 5.6 mCi of pure product with a specific activity of 17.81 Ci/mmol.

Conclusion

Simple and straightforward syntheses of carbon-14-, tritium-, and deuterium-labeled BIRB 796 with isotopic enrichment of 87% (2.0 GBq/mmol), 62% (659 GBq), 98.9% respectively, were accomplished. Labeled BIRB 796 was needed for ADME studies.

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Acknowledgements

My thanks to Clark Perry, and to my colleagues Jeff Song (Chemical Development Department) for providing compounds (**1**) and (**2**), Tom Gilmore for compound (**5**), John Regan, Neil Moss, Glenn Van Moffaert, Matt Hrapchack, Scott Leonard (Medicinal Chemistry Department), and Edward Harris (Mass Spec Laboratory).

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