Research article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 30 January 2016, Revised 22 February 2016, Accepted 8 March 2016 Published online 13 April 2016 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3398

A potent IκB kinase-β inhibitor labeled with carbon-14 and deuterium

Bachir Latli,* Magnus Eriksson, Matt Hrapchak, Carl A. Busacca, and Chris H. Senanayake

3-Amino-4-(1,1-difluoro-propyl)-6-(4-methanesulfonyl-piperidin-1-yl)-thieno[2,3-*b*]pyridine-2-carboxylic acid amide (1) is a potent IκB Kinase-β (IKK-β) inhibitor. The efficient preparations of this compound labeled with carbon-14 and deuterium are described. The carbon-14 synthesis was accomplished in six radiochemical steps in 25% overall yield. The key transformations were the modified Guareschi–Thorpe condensation of 2-cyano-^{14}*C*-acetamide and a keto-ester followed by chlorination to 2,6-dichloropyridine derivative in one pot. The isolated dichloropyridine was then converted in three steps in one pot to [^{14}C]-(1). The carbon-14 labeled (1) was isolated with a specific activity of 54.3 mCi/mmol and radiochemical purity of 99.8%. The deuterium labeled (1) was obtained in eight steps and in 57% overall chemical yield using 4-hydroxypiperidine-2,2,3,3,4,5,5,6,6-^{2}H$_9$. The final three steps of this synthesis were run in one pot.

Keywords: IKK-β; thienopyridines; carbon-14; deuterium; radiosynthesis

Introduction

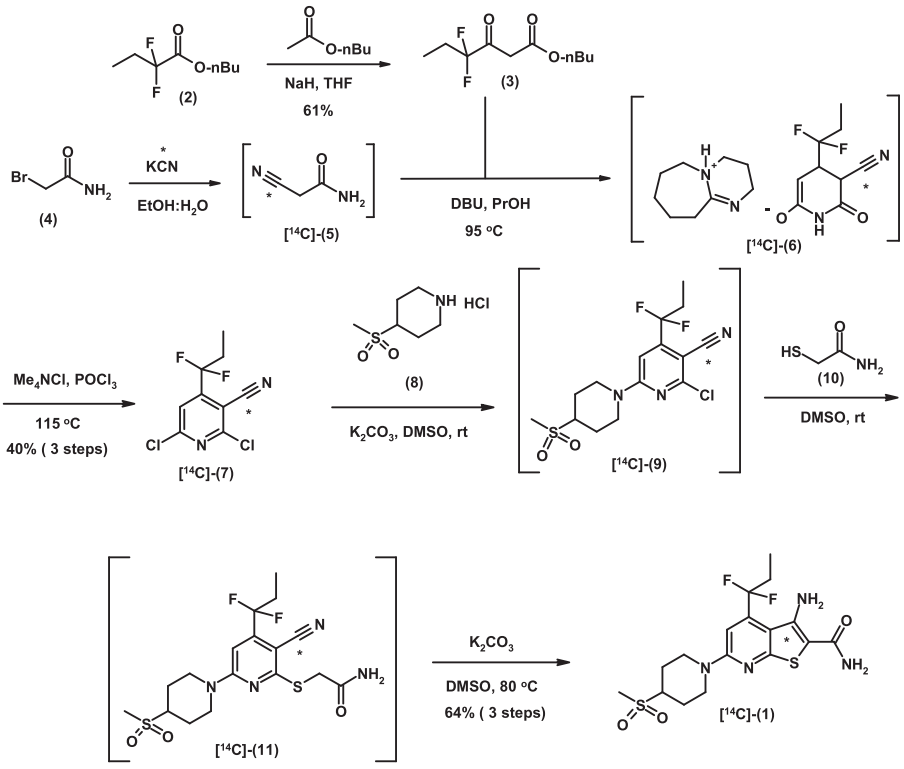
Thienopyridines are a class of compounds with a broad range of biological activity. They have been reported as hepatitis C virus (HCV) inhibitors,[1] as ligands for the dopamine D_2 receptor,[2] as allosteric modulator of the M_4 muscarinic receptor,[3] as inhibitors of *N*-methyl-D-aspartate (NMDA) receptors,[4] as potential antipsychotics,[5] as antagonists of cell adhesion molecule (ICAM-1) expression,[6,7] as SrC kinase,[8] Janus kinase-2[9] and as sirtuins inhibitors.[10] Thienopyridine analogs were also identified as potent inhibitors of IκB kinase-β (IKKB), a key regulatory enzyme in the nuclear factor-kB (NF-kB) pathway.[11] This enzyme is considered a potential target for the treatment of inflammatory and autoimmune diseases.[11] 3-Amino-4-(1,1-difluoro-propyl)-6-(4-methanesulfonyl-piperidin-1-yl)-thieno[2,3-*b*]pyridine-2-carboxylic acid amide (**1**) was found to have superior oral activity in a rat model of collagen induced arthritis (CIA) and good pharmacokinetics properties.[12] Herein, we describe an efficient preparation of this compound labeled with carbon-14 and deuterium to advance its development as a drug for treatment of inflammatory diseases.

Results and discussion

To prepare carbon-14 labeled (**1**), Scheme 1, we envisioned the synthesis of the dihydroxypyridine derivative using a Guareschi-Thorpe condensation of 2-cyanoacetamide-[2-^{14}C] and a difluoroketo ester.[13] Butyl 4,4-difluoro-3-oxohexanoate (**3**) was prepared from the corresponding butyl 2,2-difluorobutyrate (**2**) and butyl acetate. The butyl group was chosen to minimize the volatility of product (**3**).[14] Compound (**2**) is either prepared as described in the literature[14] or from a simple esterification of the commercially available 2,2-diflurobutyric acid and *n*-butanol. Reaction of carbon-14 potassium cyanide and 2-bromoacetamide (**4**) in mixture of ethanol and water gave 2-cyanoacetamide-[2-^{14}C] ([^{14}C]-(**5**)) which was concentrated to remove the solvents and used as a solid mixture with potassium bromide in the following step.[15] Condensation of [^{14}C]-(**5**) and (**3**) in propanol in the presence of DBU (diazobicyclo-[5,4,0]undece-7-ene) as a base at 95 °C gave the pyridine-DBU salt [^{14}C]-(**6**) via a modified Guareschi-Thorpe condensation.[16] This salt was suspended with tetramethylammonium chloride in phosphorus oxychloride and heated at 115 °C to give 2,6-dichloronicotinonitrile derivative [^{14}C]-(**7**) after an aqueous work up and column chromatography purification in 40% radiochemical yield over three steps. The dichloronicotinonitrile [^{14}C]-(**7**) was then reacted with 4-methanesulfonyl piperidine HCl salt (**8**) in DMSO in the presence of potassium carbonate at room temperature to give [^{14}C]-(**9**). 2-Mercaptoacetamide (**10**) was prepared in one step from methyl thioglycolate and ammonia in methanol, and added to the flask containing [^{14}C]-(**9**). The reaction was stirred at room temperature for 15 h. It is worth mentioning that (**10**) is not stable for long-term storage and has to be kept under an inert atmosphere to minimize dimerization and air oxidations. Finally, the reaction suspension was heated to 80 °C for 8 h to cyclize [^{14}C]-(**11**) to [^{14}C]-(**1**). The last three steps were all run in one pot and gave carbon-14 labeled (**1**) in 64% radiochemical yield. The pure product, 51 mCi, was isolated by column chromatography purification with a specific activity of 54.3 mCi/mmol and 99.8% radiochemical purity.

Chemical Development, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, P.O. Box 368, Ridgefield, CT 06877-0368, USA

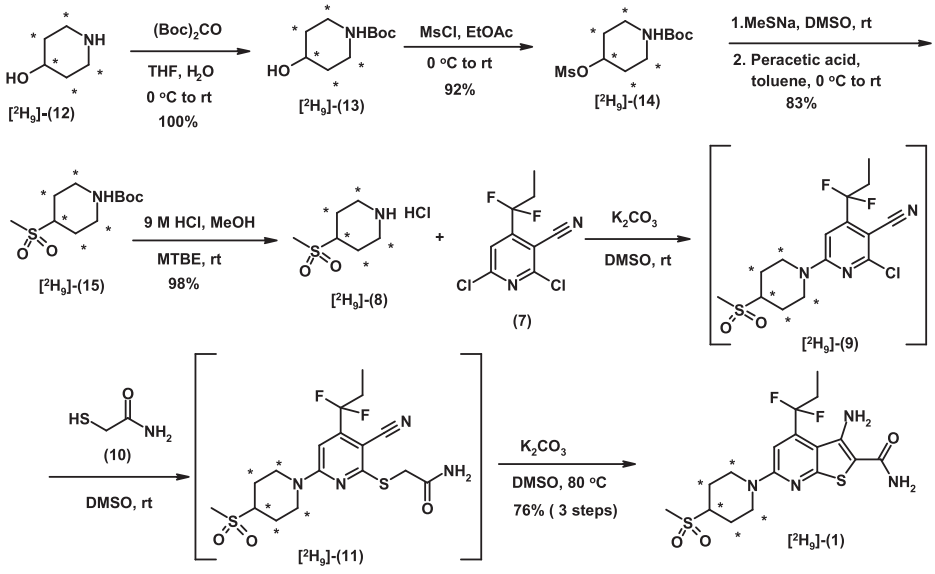
**Correspondence to: Bachir Latli, Chemical Development, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, P.O. Box 368, Ridgefield, CT 06877-0368, USA.*
E-mail: bachir.latli@boehringer-ingelheim.com



[ ] denotes not isolated
Asterisk indicates position of C14.

Scheme 1. Synthesis of [^{14}C]-(1).

With the availability of the dichloronicotinonitrile (**7**) from in house synthesis;[16] we focused on the synthesis of deuterium labeled 4-methanesulfonylpiperidine (**8**) to prepare deuterium labeled (**1**), Scheme 2. Commercially available 4-hydroxypiperidine-2,2,3,3,4,5,5,6,6-$^{2}H_9$ ([$^{2}H_9$]-(**12**)) was first protected at the amino group then converted to the mesylate derivative [$^{2}H_9$]-(**14**) in 92%.[17] The mesylate group was displaced by sodium thiomethoxide. The elimination product (up to 10%) observed[16] when displacing the mesylate group in the synthesis of unlabeled (**8**) was totally suppressed when using [$^{2}H_9$]-(**14**)



[ ] denotes not isolated
asteriskes show positions of deuterium atoms

Scheme 2. Synthesis of [$^{2}H_9$]-(1).

due to deuterium isotope effect. Oxidation of sulfur atom with peracetic acid in toluene, followed by removal of the protecting group, gave sulfonylpiperidine hydrochloride salt [$^2H_9$]-(**8**) in 83% and 98% yield respectively. This salt was then reacted with the dichloronicotinonitrile (**7**) in the presence of potassium carbonate in DMSO to give [$^2H_9$]-(**9**). As described in the carbon-14 synthesis, this intermediate was not isolated and reacted in the same pot with 2-mercaptoacetamide (**10**) at room temperature to give [$^2H_9$]-(**11**). Ring closure to the desired [$^2H_9$]-(**1**) was accomplished in the same reaction flask as well by adding more potassium carbonate and heating to 80 °C for 12 h. This three-step-one-pot transformation gave deuterium labeled (**1**) in 76% yield after crystallization from acetic acid and heptane.

Conclusion

3-Amino-4-(1,1-difluoro-propyl)-6-(4-methanesulfonyl-piperidin-1-yl)-thieno[2,3-*b*]pyridine-2-carboxylic acid amide (**1**) is a potent IκB Kinase-β Inhibitor. It was prepared efficiently labeled with carbon-14 in six radiochemical steps in 25% radiochemical yield using a modified Guareschi-Thorpe reaction followed by a three-step-one-pot reaction that included an S_NAr, a condensation with thioacetamide, and a cyclization. The deuterium labeled (**1**) was obtained in eight steps and in 58% overall chemical yield starting from 4-hydroxypiperidine-2,2,3,3,4,5,5,6,6-$^2H_9$ ([$^2H_9$]-(**12**)). Transformation of this material to 4-methanesulfonyl-piperidine-2,2,3,3,4,5,5,6,6-$^2H_9$-HCl ([$^2H_9$]-(**8**)) was accomplished in five steps in 75% yield. Then, a three-step-one-pot reaction with dichloronicotinonitrile derivative (**7**) gave [$^2H_9$]-(**1**) in 76% yield. These labeled materials were utilized in drug metabolism, pharmacokinetics, bioanalytical and in other studies.

Experimental procedures

Materials and methods

NMR spectra of radioactive compounds were recorded on a Bruker 500-MHz spectrometer using double encapsulated NMR tubes in deuterated dimethyl sulfoxide. ^{1}H NMR, ^{19}F NMR and ^{13}C NMR spectra of non-radioactive compounds were acquired using Bruker 400 MHz. Liquid scintillation counting was accomplished using a Beckman LS6500TA and UltimaGold™ cocktail (PerkinElmer, Boston, MA, USA). HPLC analysis was performed on an Agilent 1200 instrument. HPLC Conditions: Mobile Phase gradient 20 to 100% (MeCN in H_2O both 10 mM TFA) over 20 min, column: Eclipse XDB C8 (5.0 μm, 4.5 mm × 150 mm), injection volume 10 μL. LCMS were acquired using Waters Acquity™ Ultra Performance LC (Milford, MA, USA) and either a fast medium polar method: run time 2.0 min, gradient 95% water (0.1% TFA) and 5% MeCN (0.1%TFA) to 5% water in 1.7 min, hold to 2 min at 5% water, flow 2.5 mL/min; column: Zorbax C18-SB (3.5 μm, 4.6 mm × 30 mm), or a long medium polar method, run time 9.0 min, gradient 95% water (0.1% TFA) and 5% MeCN (0.1%TFA) to 5% water in 7 min, hold to 9 min at 5% water, flow 1.5 mL/min; column: Zorbax Eclipse XDB-C8 (5 μm, 4.6 mm × 150 mm). The radiochemical purity was measured using a radio-HPLC detector β-Ram model 3 or model 4 (LabLogic systems, Inc. Brandon, FL, USA) connected to Agilent HPLC instrument using IN-FLOW™ 2:1 liquid scintillation (LabLogic systems, Inc. Brandon, FL, USA). Potassium-[^{14}C]cyanide was purchased from ViTrax (Placentia, CA, USA). 4-Hydroxypiperidine-2,2,3,3,4,5,5,6,6-$^2H_9$ with 98.9 atom % ^{2}H was purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). 2,2-Difluorobutyric acid was purchased from Oakwood Chemicals (West Columbia, SC, USA). The rest of the reagents were obtained from Sigma-Aldrich Company.

Synthesis of [^{14}C]-(1)

Butyl 4,4-difluoro-3-oxohexanoate (3)

Sodium hydride (4.7 g, 117 mmol, 60% oil dispersion) was suspended in anhydrous THF (136 mL) and stirred at room temperature under argon. Butyl 2,2-difluorobutyrate (**2**) (22 g, 117 mmol) was added, and the suspension was stirred at ambient temperature for 14 h. Butyl acetate (18.62 mL, 140.6 mmol) was dissolved in anhydrous THF (20 mL) and added via addition funnel over 45–60 min. The funnel was rinsed with THF (5 mL). The suspension became yellow toward the end of the addition. The yellow suspension was stirred at ambient temperature for 26 h. The reaction was quenched by a saturated aqueous solution of NH_4Cl (50 mL) and diluted with water (50 mL). The mixture was acidified to pH ca. 5 using concentrated. HCl and the layers separated. The aqueous layer was extracted with EtOAc (100 mL ×2), and the combined organic layers were washed with water (100 mL) and concentrated to 21 g of orange oil. The crude product contains ca. 8–10% starting material. This crude product was purified by vacuum distillation at *ca.* 0.3 torr/30–35 °C with oil bath at 55–65 °C to give 15.8-g colorless (95% pure) in 61% yield. ^{1}H NMR in $CDCl_3$ indicated a mixture of about 1:1 of the keto-ester and the enol-ester.[16] LCMS (fast medium polar method), t_R = 1.08 min, MH^+ (100%) = 223.0. ^{1}HNMR ($CDCl_3$) δ: 11.98(s, 1H, OH enol form), 5.50(s, 1H, =CH enol form), 4.19(t, *J* = 6.75 Hz, 2H), 3.70(s, 3H), 2.01–2.18(m, 2H), 1.6–1.71(m, 2H), 1.32–1.45(m, 2H), 1.01(t, *J* = 7.69 Hz, 3H), 0.95(t, *J* = 7.48 Hz, 2H). ^{13}CNMR ($CDCl_3$) δ: 193.62, 172.30, 167.47 (m), 165.91, (121.26, 120.74), (118.84, 118.24), (116.43, 115.74), 90.33(t, *J* = 5.21 Hz), (65.64, 64.82), 43.45, (30.63, 30.43), 27.99(t, *J* = 25.4 Hz), 25.75(t, *J* = 23.2 Hz), (19.09, 18.95), 13.62, 6.08(t, *J* = 5.03 Hz), 5.39(t, *J* = 5.51 Hz). ^{19}FNMR ($CDCl_3$) δ: −108.23, −108.32.

2-Cyano-^{14}C-acetamide, [^{14}C]-(5)

To a solution of 2-bromoacetamide (**4**) (0.51 g, 3.63 mmol) in ethanol (5 mL) was added a solution of potassium-[^{14}C]cyanide (200 mCi, 55 mCi/mmol, 3.63 mmol) in water (5 mL) dropwise at room temperature. The resulting was stirred for 24 h to give a pale yellow solution. TLC (20% MeOH/$CHCl_3$) showed the starting bromide was consumed and the presence of the product which was visualized in an iodine chamber and co-eluted with unlabeled sample. The solution was transferred to a 100-mL round bottom flask, and the reaction flask was rinsed with methanol. Concentration *in vacuo* gave a cream colored solid. Methanol (10 mL) was added, and the mixture was concentrated again to remove water. This procedure was repeated again with 10 mL of methanol to give 758 mg of mixture of 2-cyano-^{14}C-acetamide and potassium bromide. This mixture was used as it is in the following step.

5-Cyano-^{14}C-4-(1,1-difluoro-propyl)-6-oxo-1,6-dihydro-pyridin-2-ol-DBU salt, [^{14}C]-(6)

To **[^{14}C]-(5)** (3.63 mmol) in *n*-propanol (8 mL) was added (**3**) (0.85 g, 3.63 mmol) followed by DBU (608 μL, 4 mmol). The resulting was heated to 95 °C and stirred for 20 h. After cooling to room temperature, the dark mixture was concentrated under reduced pressure to dryness. Heptane (10 mL) was added and the mixture was concentrated again to give 2.1 g of a brown solid, which was used as it is in the next step.

2,6-Dichloro-4-(1,1-difluoro-propyl)-nicotinonitrile-^{14}C, ([^{14}C]-(7)

The DBU-salt **[^{14}C]-(6)** was mixed with phosphorus oxychloride (8 mL) and tetramethylammonium chloride (440 mg, 3.9 mmol) and heated to 115 °C for 16 h. After cooling to 60 °C, toluene (20 mL) was added followed by water (15 mL) dropwise (exotherm). When the heat subsided, more water was added (20 mL) and toluene (20 mL). The resulting mixture was filtered through a short pad of Celite® and washed with toluene (20 mL ×2). The aqueous phase was removed and the organic layer was washed with water (30 mL), brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give viscous oil, which was purified on a 40-g disposable silica gel column and methylene chloride as eluent. A total of 396 mg of pure product was isolated or 80 mCi in 40% yield

staring from [^{14}C]-potassium cyanide. HPLC, t_R = 22.6 min, identical to an unlabeled sample.

2-Mercaptoacetamide, (10)

Methyl thioglycolate (3.6 g, 34 mmol) was added to a solution of ammonia in methanol (7.0 M, 45 mL) and stirred at room temperature for 14 h under argon atmosphere. The solution was concentrated under reduced pressure to give 3.1 g of a white solid in quantitative yield. ^{1}H NMR (DMSO-d_6) δ: 7.43(brs, 1H), 7.02(brs, 1H), 3.07(s, 2H), 1.51(brs, 1H). ^{13}C NMR (DMSO-d_6) δ: 173.87, 29.36.

3-Amino-4-(1,1-difluoro-propyl)-6-(4-methanesulfonyl-piperidin-1-yl)-thieno[2,3-b]pyridine-2-carboxylic acid amide-3-^{14}C, ([^{14}C]-(1))

A mixture of **[^{14}C]-(7)** (396 mg, 1.55 mmol), (**8**) (315 mg, 1.55 mmol) and K_2CO_3 (654 mg, 4.64 mmol) in DMSO (7 mL) was stirred under nitrogen atmosphere for 16 h. HPLC showed the presence of a new product at t_R = 19.97 min similar to (**9**) and no starting material was observed. 2-Thioacetamide (**10**) (214 mg, 1.64 mmol) containing about 20% of the oxidized disulfide compound was added followed by K_2CO_3 (216 mg, 1.53 mmol) and DMSO (3 mL). The resulting thick mixture was stirred at room temperature for 16 h. HPLC showed the formation of a new product, t_R = 16.08 min, similar to (**11**). The mixture was then heated to 80 °C and stirred for 16 h. HPLC showed a new product with R_t similar to a standard sample of (**1**). The mixture was cooled to 60 °C and water (20 mL) was added, cooled to room temperature and stirred for 14 h. The yellow solid was filtered washed with water and dried under reduced pressure to give 540 mg of a yellow solid. Purification on a 40-g disposable silica gel column gave 464 mg of the desired product in 64% radiochemical yield or 51.2 mCi with a specific activity of 54.3 mCi/mmol and 99.8% radiochemical purity. HPLC: t_R = 8.34 min (100%, UV detection at 230 nm), t_R = 8.35 (99.8%, rad detection). ^{1}HNMR (DMSO-d_6) δ:7.05(s, 2H), 6.98(s, 1H), 6.66(s, 2H), 4.57(d, J = 8 Hz, 2H), 3.45(t, J = 5 Hz, 1H), 3.05(t, J = 5 Hz, 2H), 2.95(s, 3H), 2.46(m, 2H), 2.15(d, J = 9 Hz, 2H), 1.64(m, 2H), 1.03(t, J = 7 Hz, 3H), identical to a reference unlabeled sample.

Synthesis of [^{2}H$_9$]-(1)

tert-Butyl-4-hydroxypiperidine-2,2,3,3,4,5,5,6,6-$^2H_9$-1-carboxylate, [$^2H_9$]-(13)

To a solution of **[^{2}H$_9$]-(12)** (2.0 g, 18.2 mmol) in THF (20 mL) and water (20 mL) was added at 0 °C a solution of di-*tert*-butyl dicarbonate (4.1 g, 18.2 mmol) in THF (20 mL). The reaction was warmed to room temperature and stirred for 16 h. Water (50 mL) was added to the reaction and extracted with methylene chloride (50 mL ×3). The combined extracts were washed with water (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 4.2 g of colorless oil. LCMS-ESI$^+$: t_R = 5.56 min, long medium polar method, MH$^+$ = 211.4 (100%). ^{1}HNMR ($CDCl_3$) δ: 4.62(s, 1H), 1.45 (s, 9H).

tert-Butyl-4-methanesulfonyloxy-piperidine-2,2,3,3,4,5,5,6,6-$^2H_9$-1-carboxylate, [$^2H_9$]-(14)

To a solution of **[^{2}H$_9$]-(13)** (4.2 g, 18.07 mmol) in ethyl acetate (35 mL) was added *N*-methylmorpholine (2.4 mL, 21.2 mmol). The solution was cooled in an ice-bath and methanesulfonyl chloride (1.4 mL, 18.1 mmol) was added dropwise in a 3-h period. The resulting suspension was then warmed to room temperature and stirred for 16 h. Water (30 mL) was added, and the organic phase was extracted with ethyl acetate (30 mL ×3). The combined extracts were washed with water (50 mL), brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 5.1 g of viscous colorless oil. This oil was dissolved in ethyl acetate (6 mL), and heptane (50 mL) was added while stirring to give a cloudy solution. The product precipitated as a white solid. After stirring at room temperature for 26 h, the product was filtered and dried in the air to give 4.77 g of a white solid in 92% yield. LCMS-ESI$^+$: t_R = 1.56 min, fast medium polar method, MH$^+$ = 233.55 (100%). ^{1}H NMR ($CDCl_3$) δ: 3.08(s, 3H), 1.46(s, 9H). ^{13}C NMR ($CDCl_3$) δ:.154.6, 80.41, 56.3(m), 40.1(m), 27.6, 37.5, 23.8(m).

tert-Butyl-4-methanesulfonyl-piperidine-2,2,3,3,4,5,5,6,6-$^2H_9$-1-carboxylate, [$^2H_9$]-(15)

To a solution of **[^{2}H$_9$]-(14)** (2.7 g, 9.5 mmol) in dimethylacetamide (DMAC) (20 mL) was added sodium methanethiolate (sodium thiomethoxide) (0.92 g, 11.8 mmol) in one portion and the mixture was stirred for 2 h. Toluene (30 mL) was added followed by water (20 mL), and the mixture was stirred for 20 min. After the layers were settled, the aqueous was removed, and the toluene layer was washed with water (20 mL). The aqueous layer was removed and the combined aqueous layers were extracted with toluene (20 mL ×2). The combined toluene extracts were used in the next reaction without further purification. LCMS, fast medium polar method: Product: t_R = 1.81 min, MH$^+$ = 241 (100%). The toluene solution was cooled in an ice-bath under nitrogen. Peracetic acid (32% solution in acetic acid, 4.0 mL) was added dropwise. After the addition was completed, the ice-bath was removed, and the solution was stirred at room temperature for 1 h. LCMS showed the presence of new product. The reaction was treated with water (60 mL). The aqueous was removed, and the organic phase was washed with water again (60 mL). The organic phase was concentrated *in vacuo*. The resulting white residue was dissolved in toluene (6 mL) and heptane (50 mL) was added slowly. The resulting mixture was stirred overnight. The product was filtered and washed with heptane, dried under reduced pressure to give 2.15 g of a white solid in 83% yield. LCMS: t_R = 1.35 min in fast medium polar method, MH$^+$ = 217.51 (100%). ^{1}H NMR ($CDCl_3$) δ: 2.84(s, 3H), 1.46(s, 9H). ^{13}C NMR ($CDCl_3$) δ: 154.33, 80.42, 54.68(m), 39.98(m), 37.33, 28.38, 23.92(m).

4-Methanesulfonyl-piperidine-2,2,3,3,4,5,5,6,6-$^2H_9$-HCl, [$^2H_9$]-(8)

To a suspension of **[^{2}H$_9$]-(15)** (1.71 g, 6.3 mmol) in methyl-*tert*-butyl ether (MTBE) (25 mL) was added a solution of HCl in methanol (9 M, 3.6 mL, 32.4 mmol). The suspension became homogenous for few seconds before a white solid started to precipitate. The mixture was stirred at room temperature for 16 h, then filtered, washed with MTBE (10 mL) and dried on the frit for 4 h to give 1.3 g of a white solid in 98.5% yield. LCMS: fast medium polar method, MH$^+$ = 173.71 (100%). ^{1}H NMR (DMSO-d_6) δ: 9.84(s, 1H), 9.21(s, 1H), 2.96(s, 3H). ^{13}C NMR (DMSO-d_6) δ: 54.97(m), 40.03(m), 37.66, 20.19(m).

3-Amino-4-(1,1-difluoro-propyl)-6-(4-methanesulfonyl-piperidin-1-yl-2,2,3,3,4,5,5,6,6-$^2H_9$)-thieno[2,3-b]pyridine-2-carboxylic acid amide ([$^2H_9$]-(1)

A mixture of (**7**) (1.44 g, 5.74 mmol), 4-methenasulfonyl piperidine-$^2H_9$ hydrochloride salt ([$^2H_9$]-(**8**)) (1.2 g, 5.75 mmol) and K_2CO_3 (2.4 g, 17 mmol) in DMSO (16 mL) was stirred under nitrogen atmosphere for 16 h. HPLC showed the presence of a new product with a t_R = 19.97 min similar to (**9**), and no starting material was observed. 2-Mercaptocetamide (**10**) (0.79 g, 8.63 mmol) containing about 20% of the disulfide impurity was added followed by K_2CO_3 (0.8 g, 5.8 mmol) and DMSO (8 mL). The resulting thick mixture was stirred at room temperature for 16 h. HPLC showed the formation of a new product, t_R = 16.1 min, co-eluted with (**11**). The mixture was then heated to 80 °C and stirred for 16 h. HPLC showed a new product with retention time (t_R) similar to a reference sample of (**1**). The mixture was cooled to 60 °C and water (50 mL) was then added, cooled to room temperature and stirred for 24 h. The yellow solid was filtered washed with water and dried under reduced pressure to give 2.3 g of a yellow solid. Acetic acid (18 mL) was added to this solid, and the mixture was heated to 78 °C. The resulting clear solution was then cooled to 48 °C with stirring to develop a seed bed, then heated to 53 °C and heptane (9 mL) was added dropwise via addition funnel. The mixture was cooled to room temperature in 3-h period and then stirred for another 16 h. The yellow solid was filtered and washed with 1:1 acetic acid-heptane (15 mL), and dried for 4 h. The product was further dried in a vacuum oven at 65–70 °C for 24 h to give 1.9 g of product in 76% yield. LCMS (fast medium polar method): t_R = 1.47 min, MH$^+$ = 442.73 (100%). HRMS: MH$^+$: $[C_{17}H^2_{14}H_9F_2N_4O_3S_2]^+$, calculated 442.17391, found 442.17426. ^{1}HNMR (DMSO-d_6) δ:7.06(s, 2H), 6.97(s, 1H), 6.64(s, 2H), 2.95 (s, 3H), 2.41(m, 2H), 1.01(t, J = 7 Hz, 3H). ^{13}CNMR (DMSO-d_6) δ: 169.54,

163.28, 159.34, 148.16, 143.50(t, J = 28.93 Hz), 127.50, 125.57, 123.65, 113.99, 104.62(t, J = 20.25 Hz), 97.26, 60.29(m), 45.04(m), 39.76, 32.69(t, J = 25.47 Hz), 25.22(m), 8.51.

References

[1] N.-Y. Wang, W.-Q. Zuo, X. Ying, G. Chao, X.-X. Zeng, L.-D. Zhang, X.-Y. You, C.-T. Peng, Y. Shen, S.-Y. Yang, Y.-Q. Wei, L.-T. Yu, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1581–1588.
[2] M. Szabo, C. K. Herenbrink, A. Christopoulos, J. R. Lane, B. Capuano, *J. Med. Chem.* **2014**, *57*, 4924–4939.
[3] T. Huynh, C. Valant, I. T. Crosby, P. M. Sexton, A. Christopoulos, B. Capuano, *J. Med. Chem.* **2013**, *56*, 8196–8200.
[4] H.-P. Buchstaller, C. D. Siebert, R. Steinmetz, I. Frank, M. L. Berger, R. Gottschlich, J. Leibrock, M. Krug, D. Steinhilber, C. R. Noe, *J. Med. Chem.* **2006**, *49*, 864–871.
[5] J. S. New, W. L. Christopher, J. P. Yevich, R. Butler, R. F. Schlemmer, Jr., C. P. VanderMaelen, J. A. Cipollina, *J. Med. Chem.* **1989**, *32*, 1147–1156.
[6] G.-D. Zhu, D. L. Arendsen, I. W. Gunawardana, S. A. Boyd, A. O. Stewart, D. G. Fry, B. L. Cool, L. Kifle, V. Schaefer, J. Meuth, K. C. Marsh, A. J. Kempf-Grote, P. Kilgannon, W. M. Gallatin, G. F. Okasinski, *J. Med. Chem.* **2001**, *44*, 3469–3487.
[7] A. O. Stewart, P. A. Bhatia, C. M. McCarty, M. V. Patel, M. A. Staeger, D. L. Arendsen, I. W. Gunawardana, L. M. Melcher, G.-D. Zhu, S. A. Boyd, D. G. Fry, B. L. Cool, L. Kifle, K. Lartey, K. C. Marsh, A. J. Kempf-Grote, P. Kilgannon, W. Wisdom, J. Meyer, W. M. Gallatin, G. F. Okasinski, *J. Med. Chem.* **2001**, *44*, 988–1002.
[8] D. H. Boschelli, B. Wu, A. C. Barrios Sosa, H. Durutlic, F. Ye, Y. Raifeld, J. M. Golas, F. Boschelli, *J. Med. Chem.* **2004**, *47*, 6666–6668.
[9] L. B. Schenkel, X. Huang, A. Cheng, H. L. Deak, E. Doherty, R. Emkey, Y. Gu, H. Gunaydin, J. L. Kim, J. Lee, R. Loberg, P. Olivieri, J. Pistillo, J. Tang, Q. Wan, H.-L. Wang, S.-W. Wang, M. C. Wells, B. Wu, V. Yu, L. Liu, S. Geuns-Meyer, *J. Med. Chem.* **2011**, *54*, 8440–8450.
[10] J. S. Disch, G. Evindar, C. H. Chiu, C. A. Blum, H. Dai, L. Jin, E. Schuman, K. E. Lind, S. L. Belyanskaya, J. Deng, F. Coppo, L. Aquilani, T. L. Graybill, J. W. Cuozzo, S. Lavu, C. Mao, G. P. Vlasuk, R. B. Perni, *J. Med. Chem.* **2013**, *56*, 3666–3679.
[11] T. Morwick, A. Berry, J. Brickwood, M. Cardozo, K. Catron, M. DeTuri, J. Emeigh, C. Hamon, M. Hrapchak, S. Jacober, S. Jakes, P. Kaplita, T. A. Kelly, J. Ksiazek, M. Liuzzi, R. Magolda, C. Mao, D. Marshall, D. McNeil, A. Prokopowicz, C. Sarko, E. Scouten, C. Sledziona, S. Sun, J. Watrous, J. P. Wu, C. L. Cywin, *J. Med. Chem.* **2006**, *49*, 2898–2908.
[12] J. D. Ginn, R. J. Sorcek, M. R. Turner, E. R. R. Young, 3-Amino-thieno[2,3-B]pyridine-2-carboxylic acid amide compounds and processes for preparing and their uses. U.S. Patent US0293533A1, **2007**.
[13] (a) I. Guareschi, *Berichte* **1896**, *A29*, 654–656; (b) F. B. Thole, J. F. Thorpe, *J. Chem. Soc., Trans.* **1911**, *99*, 422–448; (c) H. Baron, F. G. P. Remfry, J. F. Thrope, *J. Chem. Soc.* **1904**, *85*, 1726–1961; (d) P. Galatsis, Guareschi-thorpe pyridine synthesis. In Name Reactions in Heterocyclic Chemistry (Eds.: J. J. Li, E. J. Corey), Wiley & Sons: Hoboken, NJ, **2005**, 307–308; (e) A. O. Fitton, R. K. Smalley, Practical Heterocyclic Chemistry, Academic Press: London, **1968**, 71–72; (f) M. Mišić-Vuković, M. Radojković-Veličković, *J. Chem. Soc. Perkin Trans.* **1965–1970**, *2*, 1992.
[14] M. F. Parisi, G. Gattuso, A. Notti, F. M. Raymo, R. H. Abeles, *J. Org. Chem.* **1995**, *60*, 5174–5179.
[15] J. Zhingang, C. Filer, T. P. Kelly, *J. Labelled. Compd. Radiopharm.* **1998**, *41*, 781–784.
[16] M. C. Eriksson, X. Zeng, J. Xu, D. Reeves, C. A. Busacca, V. Farina, C. H. Senanayake, Manuscript in preparation, **2016**.
[17] R. N. Waterhouse, T. L. Collier, J. C. O'Brien, *J. Labelled. Compd. Radiopharm.* **1996**, *38*, 215–226.