Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 30 August 2007, Revised 26 September 2007, Accepted 3 October 2007 Published online 8 January 2008 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1469

Synthesis of ^{14}C-labeled and ^{13}C-,^{15}N-labeled dasatinib and its piperazine *N*-dealkyl metabolite

Alban J. Allentoff,[a]* Michael W. Lago,[a] Marc Ogan,[a] Bang-Chi Chen,[a] Rulin Zhao,[a] Ramaswarmy A. Iyer,[b] Lisa J. Christopher,[b] J. Kent Rinehart,[a] Balu Balasubramanian,[a] and Samuel J. Bonacorsi, Jr[a]

Dasatinib (SPRYCEL®) is a multiple kinase inhibitor approved for the treatment of chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia in patients with resistance to prior therapy, including imatinib mesylate (Gleevec®). Radiolabeled dasatinib and its piperazine *N*-dealkyl metabolite were synthesized to investigate absorption, distribution, metabolism, and elimination of the compounds in humans and animals. These compounds were prepared following a three-step sequence, which included thiazole carboxamide formation via cyclization of labeled thiourea with a brominated oxyacrylamide precursor. In the final step a common intermediate was converted to either [^{14}C]dasatinib or the radiolabeled piperazine *N*-dealkyl metabolite with labeling in the aminothiazole ring. Syntheses of both compounds were achieved with radiochemical purities in excess of 98%. Stable-labeled dasatinib and the piperazine *N*-dealkyl metabolite were also needed for use as mass spectral internal standards in support of bioanalytical assays. By following the same route used for the carbon-14 synthesis, [$^{13}C_4$, $^{15}N_2$]dasatinib and the [$^{13}C_4$, $^{15}N_2$]metabolite were prepared with labeling in both the dichloropyrimidine and thiazole ring systems. This convergent process introduced stable isotope labeling through (1, 2, 3-$^{13}C_3$) diethyl malonate and [^{13}C,$^{15}N_2$]thiourea.

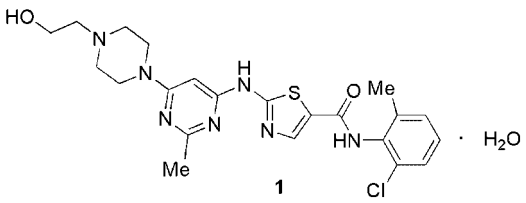
Keywords: BMS-354825; dasatinib; SPRYCEL®; carbon-14 labeling; stable labeling; thiazole; kinase inhibitor; metabolite

Introduction

Dasatinib (**1**, Figure 1), a novel 2-aminothiazole-containing compound known commercially as SPRYCEL®, is a potent inhibitor of multiple tyrosine kinases[1–3] including SRC family kinases and Bcr-Abl. It has been approved in the US and EU, for the treatment of chronic myloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia in patients with resistance to prior therapy including imatinib mesylate (Gleevec®).[4] Dasatinib is effective in tumors resistant to imatinib mesylate. X-ray data suggest that there may be differences in the mechanism of binding between these compounds.[2,5,6]

During the development of dasatinib, a radiolabeled analog was required to profile and measure the absorption, distribution, metabolism, and elimination of the compound in humans[7] and animals[8] and for cellular transporter studies. Stable-labeled dasatinib was also required for use as an internal standard in bioanalytical mass spectral analyses in support of clinical and toxicological studies.

During preliminary investigations of the cross-species biotransformation of **1**, a metabolite, **2**, resulting from *N*-dealkylation of the hydroxyethyl group attached to the piperazine ring was observed (Scheme 1).[7,8] Reaction phenotyping studies have shown that this metabolic transformation was primarily mediated by CYP3A4.[9] Recent X-ray crystallography[5] and SAR investigations[3] have suggested that when dasatinib is bound to Bcr-Abl or the ATP-binding site of SRC kinase, the hydroxyethyl group in **1** does not contribute to binding interactions. Therefore, metabolite **2** was of considerable interest since it was anticipated to exhibit kinase inhibition comparable to **1**. A radiolabeled analog of metabolite **2** was desired for *in vitro* binding studies and a stable-labeled analog of **2** was needed as an MS internal standard to monitor and quantify levels in bioanalytical assays.



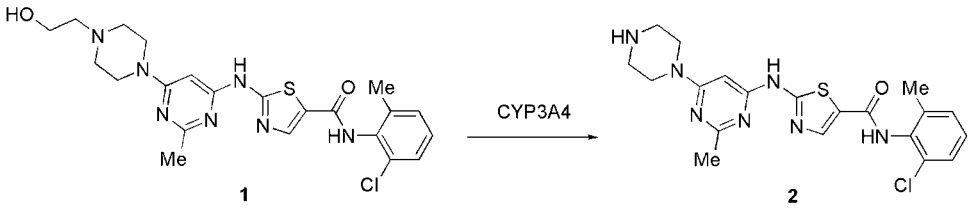
1

Figure 1. Dasatinib.

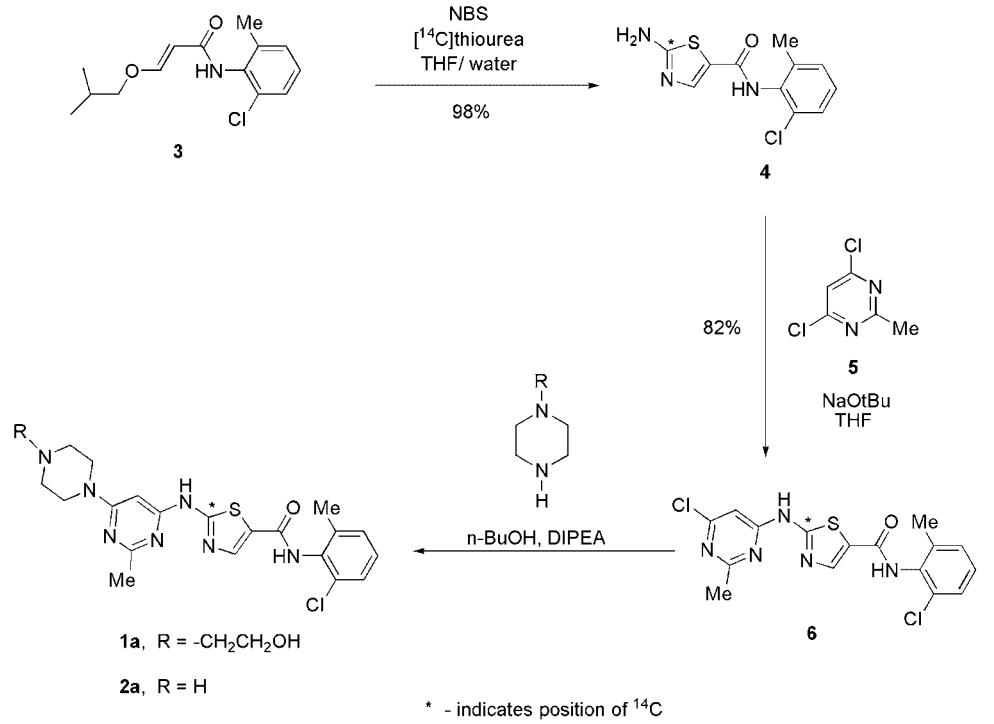
[a]*Department of Chemical Synthesis, Bristol-Myers Squibb Research and Development, One Squibb Drive, New Brunswick, NJ 08903, USA*

[b]*Department of Biotransformation, Bristol-Myers Squibb Research and Development, Route 206 and Province Line Road, Princeton, NJ 08543, USA*

**Correspondence to: Alban J. Allentoff, Department of Chemical Synthesis, Bristol-Myers Squibb Research and Development, One Squibb Drive, New Brunswick, NJ 08903, USA.*
E-mail: alban.allentoff@bms.com



Scheme 1



Scheme 2

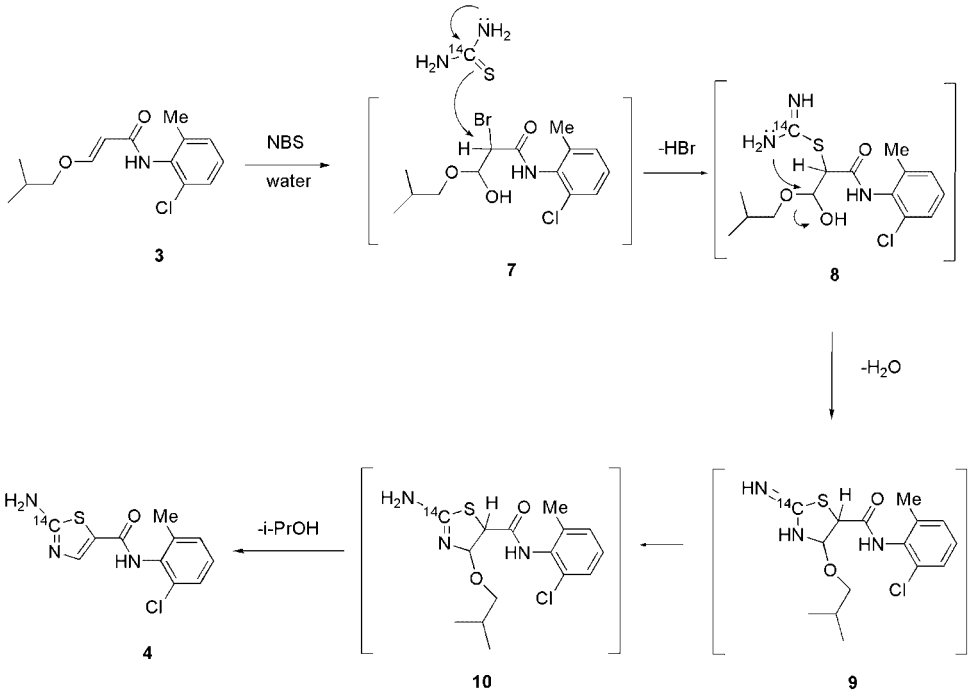
We now report on the synthesis of both ^{14}C-labeled and stable-labeled analogs of **1** and **2**. Unlabeled **1** has been previously synthesized through several different synthetic routes.[10] Preparations of labeled analogs of **1** and **2** were based on synthetic methodology used to prepare **1**. This approach allowed for the construction of a labeled penultimate intermediate via aminothiazole ring formation.[11] This intermediate was then converted in a final step to either the labeled parent **1** or metabolite **2**. Furthermore, this route allowed C-14 label introduction in the metabolically stable thiazole ring core of the molecule. The following results detail this sequence for the efficient preparation of carbon-14-labeled **1a** and **2a**. An analogous approach is also described using stable-labeled precursors to synthesize ^{13}C,^{15}N-labeled **1b** and **2b**.

Results and discussion

The synthetic route for the preparation of [^{14}C]dasatinib (**1a**) and radiolabeled piperazine *N*-dealkyl metabolite (**2a**) is shown in Scheme 2. The synthesis began with the isobutyl protected oxy-*E*-acyl benzamide **3**[11], which was treated with NBS in the presence of water in tetrahydrofuran (THF), and then reacted with [^{14}C]thiourea at 65°C to afford the labeled thiazolecarboxyamide **4** in 98% radiochemical yield.

Aminothiazole **4** (Scheme 2) reacted cleanly with 4,6-dichloro-2-methylpyrimidine in the presence of sodium *tert*-butoxide in THF at 0°C affording **6** in 82% yield. Labeled **6** was elaborated to either [^{14}C]dasatinib (**1a**) or metabolite **2a** via substitution of the pyrimidinyl chloride in **6** with the appropriately substituted piperazine. Treatment of **6** with 5 equivalents of 1-(2-hydroxyethyl)piperazine in *n*-butanol with diisopropylethylamine afforded the crude *n*-butanol solvate of **1a**. Since dasatinib was developed as a monohydrate, the labeled *n*-butanol solvate was converted to a hydrated form by crystallization from a mixture of hot ethanol and water. [^{14}C]Dasatinib (**1a**) was prepared with a radiochemical purity of 99.4% and a specific activity of 30.4 μCi/mg in 66% radiochemical yield from **6** and an overall radiochemical yield of 53% from [^{14}C]thiourea. In a similar fashion, treatment of **6** with 1 equivalent of piperazine and diisopropylethylamine in NMP afforded the labeled metabolite **2a** in 64% radiochemical yield from **6** (51% overall radiochemical yield from [^{14}C]thiourea) with a radiochemical purity of 98.3% and specific activity of 33.7 μCi/mg.

The mechanism for this transformation (Scheme 3) is proposed to be analogous to the previously reported conversion of β-ethoxyacrylates to 2-amino-thioazole-5-carboxylates.[12] The use of water in the reaction suggests the intermediacy of an



Scheme 3

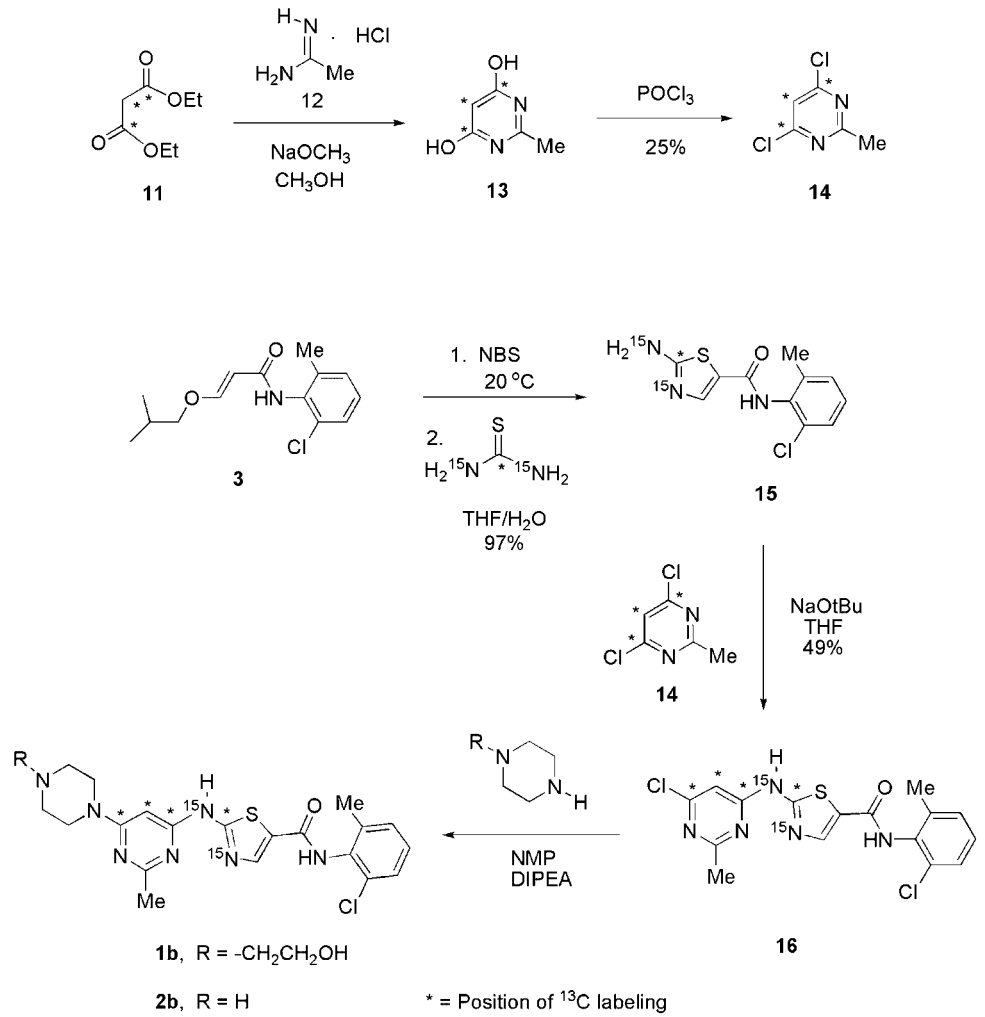
α-formyl-α-bromocarboxamide hemiacetal **7**.[12] Displacement of bromine with thiourea gives **8**, which subsequently cyclizes with the loss of water affording intermediate **9**. Internalization of the C–N double bond leads to a transient existence of **10** that rapidly aromatizes through loss of isopropanol giving the aminothiazole carboxamide product **4**.

Scheme 4 shows the synthetic route used to prepare stable-labeled dasatinib (**1b**) and its metabolite **2b**. Owing to the presence of chlorine in **1** and **2**, an MS internal standard required at least five isotopic mass units more than the parent to avoid interference with naturally abundant ^{37}Cl. The convergent synthetic paths for stable-labeled **1b** and **2b** incorporated labels in both the dichloropyrimidine and thiazole ring systems. Labeled 4,6-dihydroxypyrimidine **14** was prepared in quantitative yield from commercially available (1, 2, 3-$^{13}C_3$) diethyl malonate that was reacted with acetamidine hydrochloride in the presence of methanolic sodium methoxide.[13,14] Treatment of **13** with an excess of neat phosphorous oxychloride at reflux afforded the pyrimidine chloride **14** in 25% yield. The modest isolated yield of **14** most likely resulted from its volatility and subsequent loss during purification and removal of excess phosphorus oxychloride in the workup.[14,15] Elaboration of the stable-labeled aminothiazole portion of the molecule (15) proceeded in 97% yield via reaction of **3** with $^{13}C_2$,$^{15}N_2$-thiourea. Coupling of the dichloropyrimidine **14** and aminothiazole **15** using sodium *tert*-butoxide gave **16** in 49% yield with unreacted **15** present in the crude product. The lower yield obtained for this coupling relative to the analogous carbon-14-labeling reaction is most likely due to the presence of solvent in the crude **14** used in the reaction. Solvent impurities created difficulties in quantifying **14**. As a result, it was difficult to react **14** with **15** in an equimolar ratio required for a high yield of **16**. Finally, treatment of **16** with excess 1-(2-hydroxyethyl)piperazine gave [$^{13}C_4$, $^{15}N_2$]dasatinib (**1b**) in 80% yield after recrystallization of the crude product from aqueous ethanol. Similarly, heating **16** with excess piperazine afforded the stable-labeled metabolite **2b** in 76% yield.

In conclusion, we have developed an efficient three-step route for the preparation of both carbon-14 and stable-labeled analogs of the multiple kinase inhibitor dasatinib and its *N*-dealkyl metabolite. This process allowed elaboration of the labeled parent or metabolite from a single penultimate precursor. The sequence successfully applied a novel method for aminothiazole ring formation, allowing the facile incorporation of isotopes into the thiazole core ring system in high yield. This route incorporates labeling in a structurally common ring system that may have utility for a wider range of structurally suitable molecules.

Experimental

All unlabeled reagents were of ACS grade or better. (*E*)-*N*-(2-Chloro-6-methylphenyl)-3-[2-methylpropyl]oxyacrylamide **3** was provided by Bristol–Myers Squibb Process Research and Development. [^{14}C]Thiourea was purchased from GE Healthcare (formerly Amersham Biosciences UK Limited). (1, 2, 3-$^{13}C_3$) Diethyl malonate **11** and [^{13}C, $^{15}N_2$]thiourea were both obtained from Isotec Inc. Reactions were run under an inert atmosphere of nitrogen and magnetically stirred at a constant rate. Column chromatography was performed using a Biotage® Flash Chromatography system. Proton NMR spectra were recorded on a Bruker Spectrospin 300 MHz spectrometer. Mass spectra were obtained using a Micromass Q-Tof high-resolution mass spectrometer which was tuned to a resolution of 18 000. Radiochemical purities were determined by high-performance liquid chromatography (HPLC) (Rainin Model SD-200, Varian PDA-2 detector and Beta-Ram detector (IN/US Systems Inc.). Thin-layer chromatographic (TLC) analyses were performed using Merck 60 F_{254} silica-coated plates with radiochemical detection (QC-Scan, Bioscan Model B-QC). Specific activity was



Scheme 4

measured by gravimetric analysis using liquid scintillation counting (Wallac Model 1409). Reactions were monitored by HPLC and TLC with comparisons made to authentic unlabeled materials when available.

HPLC

HPLC methods described below were used for in-process and final product analyses.

Method 1: Column: YMC Pack Pro C18, 3 μm (4.6 × 150 mm). Mobile phase A: 90% water/10% CH_3CN with 0.075% TFA. Mobile phase B: 10% water/90% CH_3CN with 0.075% TFA. Program: gradient (0–90% B) 0–20 min; flow rate: 1 mL/min; injection volume: 10 μL; diluent: MeOH; UV detection: $\lambda = 254$ nm.

Method 2: Column: YMC Pack Pro C18, 3 μm (4.6 × 150 mm). Mobile phase A: 90% 50 mM NH_4OAc (aq, pH = 5.2)/5% CH_3CN/5% MeOH. Mobile phase B: 10% 50 mM NH_4OAc (aq, pH = 5.2)/85% CH_3CN/5% MeOH. Gradient (16–33% B) 0–18 min. Gradient (33–60% B) 18–30 min. Gradient (60–100% B) 30–40 min. Gradient (100–16% B) 40–40.5 min. Isocratic (16% B) 40.5–45 min; flow rate: 1 mL/min, injection volume: 10 μL, diluent: 50% acetic acid (0.25% aq)/50% CH_3CN acetonitrile; UV detection: $\lambda = 320$ nm.

Method 3: Column: YMC Pack Pro C18, 3 μm (4.6 × 150 mm). Mobile phase A: 90% 10 mM NH_4OAc (aq)/10% CH_3CN. Mobile phase B: 5% 10 mM NH_4OAc (aq)/95% CH_3CN. Program: Gradient (20–100% B) 0–13 min. Isocratic (100% B) 13–19 min. Isocratic (20% B) 20–25 min; flow rate: 1 mL/min, injection volume: 10 μL, diluent: 1:5 DMF:CH_3CN [v:v]; UV detection: $\lambda = 320$ nm.

*2-[^{14}C]-2-Amino-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide (**4**)*

To a round bottom flask was added THF (18 mL), water (18 mL), and (*E*)-*N*-(2-chloro-6-methylphenyl)-3-[2-methylpropyl]oxyacrylamide (**3**, 1.76 g, 6.56 mmol) and the mixture was stirred at room temperature to obtain a colorless biphasic emulsion. To this mixture was added *N*-bromosuccinimide (1.28 g, 7.22 mmol) and the resulting emulsion was stirred for 2 h at ambient temperature. The yellow emulsion turned colorless after stirring for 1 h. [^{14}C]Thiourea (0.133 g, 1.75 mmol, 100 mCi, specific activity = 57 mCi/mmol) and unlabeled thiourea (0.366 g, 4.81 mmol) were then added and the reaction mixture was heated at 65°C. The colorless, biphasic, cloudy emulsion turned to a colorless mixture upon initial heating, and then returned to a yellowish color after 20 min. TLC analysis (10:90 MeOH:CH_2Cl_2 [v:v]) of the reaction mixture after 1 h of heating showed no residual **3** remaining. The reaction mixture was cooled to room temperature. Ammonium hydroxide (3.6 mL, 28% NH_3 in water) was slowly added to adjust the pH from 2 to 11 to form a thin suspension. The volatiles were then removed with a stream of

nitrogen resulting in a heavy yellow suspension, which was stirred for 2 h at room temperature and stored at 4°C for 36 h. After carefully removing the yellow supernatant, the resulting amorphous solid residue was compacted by centrifugation and the pellet rinsed twice with water. The pellet was dissolved in THF (20 mL), concentrated to dryness under a stream of nitrogen and then dried *in vacuo* to constant weight affording **4** as an amorphous yellow solid (1.717 g, 97.6%): ^{1}H NMR (d_6-DMSO) $\delta = 2.19$ (s, 3H), 7.25 (m, 2H), 7.39 (d, 1H, $J = 7.5$ Hz), 7.61 (br s, 2H), 7.87 (br s, 1H), 9.65 (br s, 1H) the ^{1}H NMR spectrum was consistent with the published spectrum for unlabeled **4**.[11]

*2-[^{14}C]-2-(6-Chloro-2-methylpyrimidin-4-ylamino)-N-(2-chloro-6-methylphenyl) Thiazole-5-carboxamide (**6**)*

To **4** (1.66 g, 6.20 mmol, 94.2 mCi) under nitrogen in a round bottom flask was added anhydrous THF (20 mL) and 4,6-dichloro-2-methylpyrimidine (**5**, 1.21 g, 7.44 mmol). The resulting solution was stirred for 30 min at room temperature. The reaction mixture was then cooled to 0°C with an ice bath and sodium *tert*-butoxide (2.15 g, 21.7 mmol) was added in portions over 10 min. The resulting suspension was warmed to ambient temperature and was stirred for 8 h during which time it became a solution. TLC analysis (10:90 MeOH:CH_2Cl_2 [v:v]) showed clean formation of **6** ($R_f = 0.70$) and no residual **4**. Water (5.8 mL) was added and the pH was adjusted to 5–6.5 with glacial acetic acid (1.0 mL). Water (35 mL) was added resulting in the formation of a thick pale-yellow suspension. The suspension was stirred overnight at ambient temperature and the solids collected after centrifugation. After repeated rinsing with THF (7 mL), water (10 mL), and THF (7 mL) the resulting solid was dried *in vacuo* to give **6** as a pale yellow solid (1.99 g, 81.5% yield) with a radiochemical purity of 99% (HPLC method 1, $R_T = 15.1$ min): ^{1}H NMR (d_6-DMSO) $\delta = 2.25$ (s, 3H), 2.60 (s, 3H), 6.95 (s, 1H), 7.25 (m, 2H), 7.41 (d, 1H, $J = 7.5$ Hz), 8.32 (s, 1H), 7.87 (s, 1H), 10.1 (s, 1H). ^{1}H NMR spectrum was consistent with the published spectrum for unlabeled **6**.[11]

*N-(2-Chloro-6-methylphenyl)-2-(6-(4-(3-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-ylamino)-2-[^{14}C]-thiazole-5-carboxamide (**1a**)*

To a solution of **6** (1.0278 g, 2.61 mmol, 39.6 mCi) in anhydrous 1-butanol (16 mL) under nitrogen was added 1-(2-hydroxyethyl) piperazine (1.70 g, 13.03 mmol) dissolved in 16 mL of anhydrous 1-butanol. An additional 10 ml of 1-butanol and *N*, *N*-diisopropylethylamine (0.673 g, 5.21 mmol) were added and the resulting suspension was heated to 115°C for 18 h. During this period all solids dissolved affording a solution. HPLC (method 2) and TLC analyses (10:90:0.2 MeOH:CH_2Cl_2:NH_4OH [v:v:v]) showed clean formation of **1a** ($R_T = 19.3$ min, $R_f = 0.17$) with complete consumption of **6**. The reaction mixture was cooled to ambient temperature and was stirred for 2 h during which time a precipitate formed. The solids were precipitated by centrifugation, rinsed repeatedly with 1-butanol, and dried *in vacuo* to afford 1.15 g of **1a** as its butanol solvate. The beige butanol solvate (0.80 g, 1.423 mmol) was suspended in absolute ethanol (13.0 mL) and sterile water (3.40 mL). The suspension was heated to 80°C, causing the solids to slowly dissolve into a uniform solution. Additional absolute ethanol (2.0 mL) and sterile water (5.1 mL) were added and the solution was held at 75°C for 1 h. Seed crystals of the hydrated form of unlabeled **1a** (10 mg) were then added. The solution was cooled to ambient temperature over 90 min during which time a precipitate formed which was allowed to sit at ambient temperature for 18 h. This precipitate was then separated by centrifugation and the resulting solid rinsed twice with absolute ethanol:sterile water (1:1 [v:v]) and dried *in vacuo* yielding [^{14}C]dasatinib (**1a**) as an off-white solid (0.603 g, 18.3 mCi, 66% radiochemical yield from **6**) with radiochemical purity of 99.4% (HPLC method 2, $R_T = 19.2$ min) and specific activity of 30.4 μCi/mg: ^{1}H NMR (d_6-DMSO) $\delta = 2.24$ (s, 3H), 2.40 (s, 3H), 2.42 (t, 2H, $J = 6$), 2.49 (dd, 4H, $J = 6.6$ Hz), 3.52 (m, 6H), 4.45 (br t, 1H, $J = 5$ Hz), 6.05 (s, 1H), 7.28 (m, 2H), 7.42 (br d, 1H, $J = 7$ Hz), 8.23 (s, 1H), 9.88 (s, 1H). The ^{1}H NMR spectrum was consistent with the published spectrum for **1**.[11]

*N-(2-Chloro-6-methylphenyl)-2-(piperazin-1-yl)-2-(methylpyrimidin-4-ylamino)-2-[^{14}C]-thiazole-5-carboxamide (**2a**)*

To a solution of **6** (0.10 g, 0.254 mmol, 3.91 mCi) and piperazine (0.21 g, 2.54 mmol) in *N*-methylpyrrolidone (1.0 mL, anhydrous) under argon was added *N*, *N*-diisopropylethylamine (0.69 g, 0.53 mmol) dropwise. The resulting mixture was heated at 120°C for 20 min and then allowed to cool to ambient temperature. Water (5 mL) was added slowly resulting in the formation of a precipitate. The mixture was stirred for 48 h at room temperature and then subjected to centrifugation. The resulting pellet was rinsed four times with water and dried *in vacuo* affording **2a** as a white solid (72.4 mg, 2.4 mCi, 64% radiochemical yield) with radiochemical purity of 98.3% (HPLC method 3, $R_T = 11.8$ min) and specific activity = 33.7 μCi/mg: ^{1}H NMR (d_6-DMSO) $\delta = 2.25$ (s, 3H), 2.40 (s, 3H), 2.76 (m, 4H), 3.44 (m, 4H), 6.01 (s, 1H), 7.25 (m, 2H), 7.41 (d, 1H, $J =$ br d, $J = 7$ Hz), 8.21 (s, 1H), 9.87 (s, 1H).

*4, 6-Dihydroxy-2-methyl-[$^{13}C_3$]-pyrimidine (**13**)*

To a solution of (1, 2, 3-$^{13}C_3$) diethyl malonate (**11**, 1.0 g, 6.13 mmol) in anhydrous methanol (11.0 mL) under nitrogen was added sodium methoxide (36.8 mL, 0.5 M in methanol, 18.4 mmol) and acetamidine hydrochloride (**12**, 0.61 g, 6.13 mmol). The resulting mixture was stirred at ambient temperature for 48 h. After removal of the volatiles *in vacuo*, the resulting residue was dissolved in water (8.5 mL). The pH of this aqueous solution was adjusted to 2 by the addition of conc. HCl (1.30 mL) affording a thick white suspension which was stirred for 8 h at room temperature and then stored at 4°C for 18 h. The solid was collected by vacuum filtration and dried *in vacuo* giving 4, 6-dihydroxy-2-methyl-[$^{13}C_3$]-pyrimidine (**13**, 0.80 g, 100%) as a white solid with a chemical purity of 98.3% (HPLC method 1, $R_T = 2.1$ min) by relative UV peak area ratio: ^{1}H NMR (d_6-DMSO) $\delta = 2.23$ (S, 3H), 4.95 (d, 1H, $J = 166$ Hz, ^{13}C–H).

*4,6-Dichloro-2-methyl[$^{13}C_3$]-pyrimidine (**14**)*

4,6-Dihydroxy-2-methyl-[$^{13}C_3$]-pyrimidine (**13**, 0.797 g, 6.17 mmol) and phosphorous oxychloride (13.4 g, 87.4 mmol) were combined under nitrogen and the mixture heated at reflux for 2 h. The progress of the chlorination was monitored by TLC (90:10 methanol:CH_2Cl_2 [v:v]). Excess phosphorous oxychloride was then distilled off under reduced pressure. To the resulting residue was added ice water and the mixture extracted several times with ethyl acetate. The combined organic extracts were washed with brine (4 mL) and concentrated *in vacuo* yielding crude **14** (0.77 g) as a slightly viscous, yellow oil. Purification of the product was achieved by flash chromatography on silica gel (CH_2Cl_2) affording pure **14** (0.26 g, 25.4%) with chemical purity

of 98% (HPLC method 1, $R_T = 10.9$ min) by relative UV peak area ratio: ^{1}H NMR ($CDCl_3$) $\delta = 2.72$ (S, 3H), 7.23 (d, 1H, $J = 181$ Hz, ^{13}C–H).

*2-[^{13}C]-2-[^{15}N]-Amino-N-(2-chloro-6-methylphenyl)-[^{15}N]-thiazole-5-carboxamide (**15**)*

To a stirred mixture of water (17 mL) and THF (17 mL) was added (*E*)-*N*-(2-chloro-6-methylphenyl)-3-[2-methylpropyl]oxyacrylamide (1.69 g, 6.32 mmol) and *N*-bromosuccinimide (1.24 g, 6.95 mmol), and the resulting emulsion was stirred at room temperature for 2 h. [^{13}C, $^{15}N_2$]Thiourea (0.50 g, 6.32 mmol) was then added and the reaction mixture was heated at 65°C for 1 h. Ammonium hydroxide (7.0 mL) was added slowly to the mixture to adjust the pH from 2 to 11. During this addition, a cloudy suspension was formed. The mixture was then concentrated under a stream of nitrogen, resulting in the formation of more solids. After stirring the mixture at ambient temperature for 2 h, it was stored at 4°C for 18 h. The precipitate was then collected by vacuum filtration, rinsed with water (10 mL), and dried *in vacuo* affording crude **15** as a pale yellow solid (1.49 g, 87%) with chemical purity of 87% (HPLC method 1, $R_T = 8.1$ min) by relative UV peak area ratio. This material was used directly for the next step: ^{1}H NMR (d_6-DMSO) $\delta = 2.19$ (s, 3H), 7.25 (m, 2H), 7.39 (d, 1H, $J = 7.5$ Hz), 7.60 (br d, 2H, $J = 90$ Hz, H–^{15}N), 7.88 (dd, 1H, $J = 22$, 13 Hz, thiazole ring ^{13}C–H), 9.63 (br s, 1H).

*2-[^{13}C]-2-(6-Chloro-2-methylpyrimidin-4-yl-[^{15}N]-amino)-N-(2-chloro-6-methylphenyl)-[^{15}N]-thiazole-5-carboxamide (**16**)*

Carboxamide **15**, (0.43 g, 1.54 mmol) was charged to a round bottom flask under nitrogen. THF (5 mL, anhydrous) was added followed by 4,6-dichloro-2-methyl[$^{13}C_3$]-pyrimidine (**14**, 0.26 g, 1.57 mmol) and the resulting mixture was stirred for 30 min at room temperature. This mixture was then cooled to 0°C and sodium *tert*-butoxide (0.54 g, 5.50 mmol) was added portionwise over 10 min to form a suspension that was allowed to warm at room temperature. The suspension was dissolved within 8 h, giving a uniform solution. TLC analysis (10:90 MeOH:CH_2Cl_2 [v:v]) showed product formation and some residual **15** remaining. The reaction was allowed to stir at room temperature for an additional 20 h. Water (1.5 mL) was added and the pH was adjusted to 5–6.5 with glacial acetic acid (300 μL). Additional water (8.5 mL) was added, resulting in the formation of a thick pale yellow suspension. The suspension was stirred for 36 h at room temperature and then concentrated to dryness *in vacuo*. The solids were washed with THF (3 mL), water (4 mL), THF (4 mL), and dried *in vacuo* affording crude **16** (0.31 g) as a pale beige solid with some remaining **15** (HPLC method 1). This crude **16** was used directly for the next step without further purification: ^{1}H NMR (d_6-DMSO) $\delta = 2.25$ (s, 3H), 2.60 (s, 3H), 6.95 (d, 1H, $J = 179$, pyrimidine ^{13}C–H), 7.25 (m, 2H), 7.41 (d, 1H, $J = 7.5$ Hz), 8.31 (dd, 1H, $J = 22$, 13 Hz, thiazole ring ^{13}C–H), 10.0 (s, 1H).

*N-(2-Chloro-6-methylphenyl)-2-(6-(4-(3-hydroxyethyl)piperazin-1-yl)-2-methyl-[$^{13}C_3$]-pyrimidin-4-yl-[^{15}N]-amino)-2-[^{13}C]-[^{15}N]-thiazole-5-carboxamide (**1b**)*

To **16** (0.25 g, 0.625 mmol) under nitrogen was added a solution of 1-(2-hydroxyethyl) piperazine (0.41 g, 3.12 mmol) dissolved in 1-butanol (4 mL). Additional 1-butanol (2.5 mL) and *N*, *N*-diisopropylethylamine (0.161 g, 1.25 mmol) were then added. The resulting mixture was heated at 115°C with stirring for 18 h during which time the suspension dissolved affording a uniform solution. HPLC analysis (method 2, $R_T = 19.3$ min) and TLC analysis (10:90 MeOH:CH_2Cl_2 [v:v]) of the solution showed clean product formation with no residual **16**. The reaction mixture was cooled to room temperature and stirred for 2 h during which time precipitate was formed. The solids were separated by centrifugation, the pellet was rinsed twice with 1-butanol, and dried *in vacuo* giving the [$^{13}C_3$, $^{15}N_2$]dasatinib butanol solvate as a pale beige solid (0.251 g). To this butanol solvate (0.23 g, 0.40 mmol) was added absolute ethanol (3.7 mL) and water (0.96 mL). The resulting suspension was heated at 80°C and the solids gradually dissolved. Additional absolute ethanol (0.56 mL) and water (1.46 mL) were added and the solution was heated at 75°C for 1 h. Seed crystals of unlabeled **1** (approximately 1 mg) were added and the mixture was allowed to cool to room temperature over 90 min during which time a precipitate formed. The resulting suspension was maintained at room temperature overnight without stirring. The precipitate was then filtered, rinsed with a solution of 1:1 absolute ethanol:water [v:v], and dried *in vacuo* affording [$^{13}C_3$, $^{15}N_2$]dasatinib monohydrate (**1b**) as an off-white solid (0.169 g, 80%) with chemical purity of 99.5% by UV relative peak–area ratio (HPLC method 1, $R_T = 9.7$ min): ^{1}H NMR (d_6-DMSO) $\delta = 2.24$ (s, 3H), 2.40 (s, 3H), 2.42 (t, 2H, $J = 6$), 2.49 (dd, 4H, $J = 6.6$ Hz), 3.52 (m, 6H), 4.42 (br s, 1 H,O–H), 6.05 (d, 1H, $J = 162$ Hz, pyrimidine ^{13}C–H), 7.28 (m, 2 H), 7.40 (br d, 1H, $J = 7$ Hz), 8.21 (dd, 1H, $J = 20$, 9 Hz, thiazole ring C–H), 9.85 (br s, 1H); MS (positive ion) $[M+H]^+ = 494$ (96.4%, C_{18} $^{13}C_4H_{26}N_5$ $^{15}N_2O_2S_1Cl_1$), 493 (3.6%, C_{19} $^{13}C_3H_{26}N_5$ $^{15}N_2O_2S_1Cl_1$).

*N-(2-Chloro-6-methylphenyl)-2-((piperazin-1-yl)-2-methyl-[$^{13}C_3$]-pyrimidin-4-yl-[^{15}N]-amino)-2-[^{13}C]-[^{15}N]-thiazole-5-carboxamide (**2b**)*

To a solution of **16** (56 mg, 0.14 mmol) and piperazine (121 mg, 1.40 mmol, 10 eq.) in *N*-methylpyrrolidone (0.56 mL) under argon was added *N*, *N*-diisopropylethylamine (38 mg, 0.294 mmol). The reaction mixture was heated to 120°C for 15 min, and then cooled to room temperature. Water (5.4 mL) was slowly added, resulting in the formation of a precipitate which was stirred for 16 h at room temperature and separated from the mother liquor by centrifugation. The resulting pellet was rinsed three times with water (3 mL) and dried *in vacuo* yielding **2b** as a white solid (47.6 mg, 76% yield) with a chemical purity of 98.1% by UV relative peak–area ratio (HPLC method 3, $R_T = 11.8$ min): ^{1}H NMR (d_6-DMSO). $\delta = 2.25$ (s, 3H), 2.43 (s, 3H), 3.15 (m, 4H), 3.71 (m, 4H), 6.10 (d, 1H, $J = 163$, pyrimidine ^{13}C–H), 7.29 (m, 2H), 7.42 (d, 1H, $J =$ br d, $J = 7$ Hz), 8.25 (dd, 1H, $J = 20$, 9 Hz, thiazole ring ^{13}C–H), 9.91 (s, 1H); MS (positive ion) $[M+H]^+ = 450.1$ (95.01%, C_{16} $^{13}C_4H_{22}N_5$ $^{15}N_2O_1S_1Cl_1$), 449.1 (4.99%, C_{17} $^{13}C_3H_{22}N_5$ $^{15}N_2O_1S_1Cl_1$).

Acknowledgement

The authors wish to thank Richard Gedamke for providing high-resolution MS analyses of stable-labeled dasatinib and metabolite.

References

[1] L. J. Lombardo, F. Y. Lee, P. Chen, D. Norris, J. C. Barrish, K. Behnia, S. Castandea, L. A. M. Cornelius, J. Das, A. M. Doweyko, C. Fairchild,

J. T. Hunt, I. Inigo, K. Johnston, A. Kamath, D. Kan, H. Klei, P. Marathe, S. Pang, R. Peterson, S. Pitt, G. L. Schieven, R. J. Schmidt, J. Tokarski, M-L. Wen, J. Wityak, R. M. Borzilleri, *J. Med. Chem.* **2004**, *47*, 6658–6661.

[2] N. P. Shah, C. Tran, F. Y. Lee, P. Chen, D. Norris, C. L. Sawyers, *Science* **2004**, *305*, 399–401.

[3] J. Das, P. Chen, D. Norris, R. Padmanabha, J. Lin, R. V. Moquin, Z. Shen, L. S. Cook, A. M. Doweyko, S. Pitt, S. Pang, D. R. Shen, Q. Fang, H. F. de Fex, K. W. McIntyre, D. J. Shuster, K. M. Gillooly, K. Behnia, G. L. Schieven, J. Wityak, J. C. Barrish, *J. Med. Chem.* **2006**, *49*, 6819–6832.

[4] M. Talpaz, N. P. Shah, H. Kantarjia, N. Donato, J. Nicoll, R. Paquette, J. Cortes, S. O'Brien, C. Nicaise, E. Bleickardt, M. A. Blackwood-Chirchir, V. Iyer, T-T. Chen, F. Huang, A. P. Decillis, C. L. Sawyers, *N. Engl. J. Med.* **2006**, *354*, 2531–2541.

[5] J. S. Tokarski, J. A. Newitt, C. Y. J. Chang, J. D. Cheng, M. Wittekind, S. E. Kiefer, K. Kish, F. Y. F. Lee, R. Borzillerri, L. J. Lombardo, D. Xie, Y. Zhang, H. E. Klei, *Cancer Res.* **2006**, *66*(11), 5790–5797.

[6] T. O'hare, D. K. Walters, E. P. Stoffregen, T. Jia, P. W. Manley, J. Mestan, S. W. Cowan-Jacob, F. Y. Lee, M. C. Heinrich, M. W. Deininger, B. J. Druker, *Cancer Res.* **2005**, *65*, 4500–4505.

[7] D. Cui, L. J. Christopher, W. Li, D. Hawthorne, C. Wu, D. P. Reitberg, M. Lago, J. K. Rinehart, W. G. Humphreys, R. Iyer, Poster 185 Presented at the 13th National ISSX Meeting, Maui Hawaii, **2005**.

[8] L. J. Christopher, D. Cui, W. Li, F. Qiu, M. Ogan, J. K. Rinehart, W. G. Humphreys, R. Iyer, Poster 180 Presented at the 13th National ISSX Meeting, Maui Hawaii, **2005**.

[9] D. Zhang, L. Wang, D. Cui, W. Li, R. A. Iyer, W. G. Humphreys, K. He, J. A. Manning, R. Luo, B. McCann, J. Park, R. Bertz, T. Eley, E. Elefant, F. Callegari, A. Blackwood-Chirchir, L. J. Christopher. Poster 278 Presented at the 14th National ISSX Meeting, Rio Del Mar, Puerto Rico, 22–26 October **2006**.

[10] J. A. McIntyre, J. Castaner, M. Bayes, *Drugs of the Future* **2006**, *31*, 291–303.

[11] B-C. Chen, R. Droghini, J. Lajeunesse, J. D. DiMarco, M. Galella, R. Chidambaram, US patent 20060004067, **2006**.

[12] R. Zhao, S. Gove, J. E. Sundeen, B-C. Chen, *Tetrahedron Lett.* **2001**, *42*, 2101–2102.

[13] B. A. Czeskis, *J. Label. Compd. Radiopharm.* **2004**, *47*, 699–704.

[14] J. Bagli, T. Bogri, B. Palameta, S. Rakhit, S. Peseckis, J. McQuillan, D. K. H. Lee, *J. Med. Chem.* **1988**, *31*, 814–823.

[15] A. McCluskey, P. A. Keller, J. Morgan, J. Garner, *Org. Biomol. Chem.* **2003**, *1*(19), 3353–3361.