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The ^{14}C, ^{13}C, and ^{15}N syntheses of a potent VEGFR-2 kinase inhibitor, Brivanib, and its prodrug, Brivanib Alaninate

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The interruption of tyrosine kinase vascular endothelial growth factor receptor-2 (VEGFR-2) signaling by the binding of a small molecule inhibitor, for example, Brivanib, to VEGFR-2 kinase domain has been shown as an effective method of slowing angiogenesis and tumor progression. [^{14}C]Brivanib, 13 and its prodrug [^{14}C]Brivanib Alaninate, 15 were prepared to support preclinical and clinical studies. Their respective stable isotope-labeled versions, [$^{13}C_3^{15}N_2$]Brivanib, 21 and [$^{13}C_3^{15}N_2$]Brivanib Alaninate, 28, were also prepared to support bioanalytical LC-MS analyses of clinical samples. All of the four title compounds were synthetically derived from the respective isotopically labeled common pyrrolotriazinone intermediate, 6 or 16. This labeled central core pyrrolotriazinone was also conveniently used to synthesize other structurally related drug discovery candidates.

Keywords: carbon-14; carbon-13; nitrogen-15; Brivanib; Vegfr-2

Introduction

Isoforms of signaling protein vascular endothelial growth factor (VEGF) and their corresponding tyrosine kinase receptors (vascular endothelial growth factor receptors (VEGFRs)) have been connected to angiogenesis that is essential for maintaining the nutrients and oxygen needed to support solid tumor growth and metastasis. In particularly, VEGFR-2, a tyrosine kinase receptor, appears to account for most of the mitogenic and chemotactic effects of VEGF on adult endothelial cells.[1] Because the process of solid tumor growth is angiogenesis dependent, one of several strategies that has been developed is the discovery of low molecular weight inhibitors of the specific tyrosine kinase domain of VEGFR-2, suitable for chronic oral administration and continual reduction of tumor angiogenic growth. The drug candidate (*R*)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[1,2-f][1,2,4]triazin-6-yloxy)propan-2-ol, Brivanib,[2] has emerged as a potent and selective VEGFR-2 inhibitor, demonstrating promising preclinical *in vivo* antitumor activity in human tumor xenograft models. The L-alanine ester prodrug of Brivanib, Brivanib Alaninate, is currently under evaluation in clinical trials for the treatment of solid tumors. [^{14}C]Brivanib, **13**, and prodrug [^{14}C]Brivanib Alaninate, **15**, were prepared to support preclinical and clinical metabolism and pharmacokinetic studies. Their respective stable isotope-labeled versions, [$^{13}C_3^{15}N_2$]Brivanib, **21** and [$^{13}C_3^{15}N_2$]Brivanib Alaninate, **28**, were also prepared to serve as internal standards for liquid chromatography–mass spectrometry quantitative analyses of parent and prodrug found in biological samples such as blood, plasma, serum, urine, or tissues. We report here the details of the preparations and characterizations of the four title isotopically labeled molecules, **13**, **15**, **21**, and **28** (Figure 1).

Experimental

General

Radioactivity was measured with a Wallac Model 1409 liquid scintillation counter (Wallac-LKB instruments, Inc.). Counting efficiency was determined by the channels ratio method. Mass spectra were obtained with a Finnigan TSQ or a Finnigan LCQ mass spectrometer. Proton NMR spectra were recorded on a Bruker Advance Ultrashield 300 MHz or a Jeol EC+500 MHz. For stable isotope labels, only carbon-13-enriched positions are reported for ^{13}C-NMR characterization purposes. UV and radiochemical purities were determined by High-Performance Liquid Chromatography (HPLC) (Shimadzu SCL-10A VP/UV-1 detector or Varian Prostar 330 PDA detector with an IN/US System β-Ram radiometric flow detector with a 0.5 mL flow cell). TLC was performed on 60 F_{254} silica gel plates (Merck). Flash chromatography was conducted on KP-Sil silica gel (Biotage). Radiolabeled and stable isotope-labeled products were compared with

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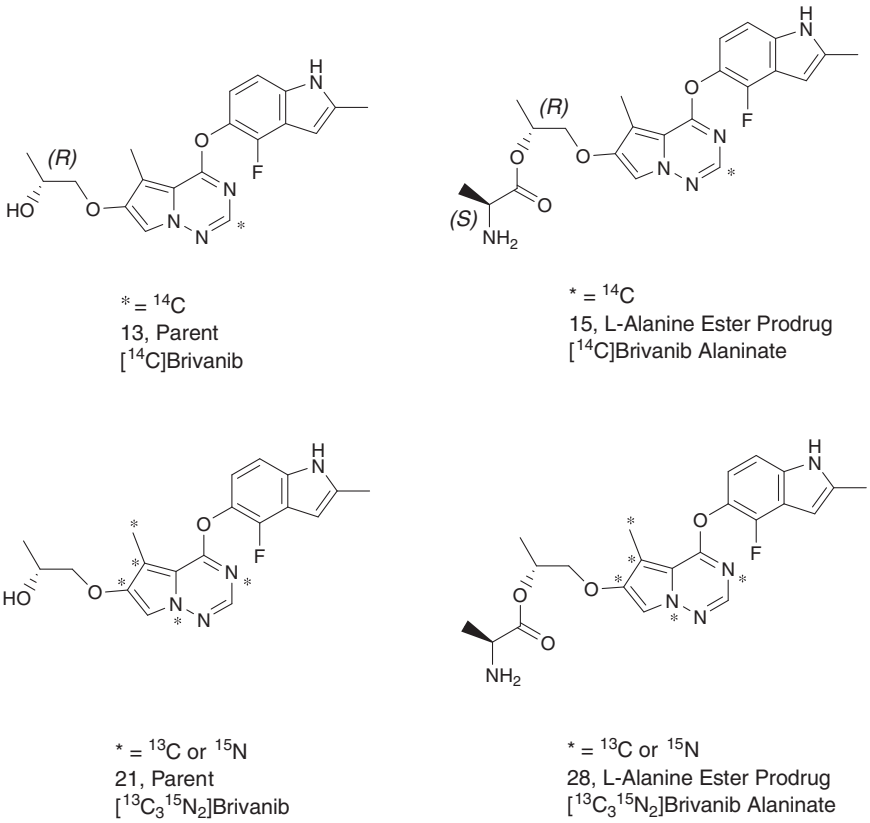


Figure 1. Four isotopically labeled Brivanib molecules.

authentic standards when possible. All reagents and solvents were ACS grade or better.

Specific activities were determined gravimetrically by dissolution of the weighed sample (ca 0.5 mg) in a 10 mL volumetric flask and diluted to the mark with DMF. Aliquots of 25 and 50 μL of the solution were diluted in 10 mL Ecolite cocktail and counted by liquid scintillation counting. HPLC methods described below were used for in process and final product analyses. Co-injections with authentic samples were used when possible. All HPLC purities were measurements of UV chemical and radiochemical purities.

Method 1: YMC-ODS-AQ C18, 3 μm, 4.6 × 150 mm, detected at 230 nm. Mobile phase A: 0.05% acetic acid in water, B: 0.05% acetic acid in acetonitrile. Gradient: 0 min 5% B, 5 min 5% B, 18 min 95% B, 23 min 95% B, 25 min 5%B. Flowrate = 1.0 mL/min. Method 2: Zorbax SB-Aq, 3.5 μm, 4.6 × 150 mm, detected at 237 nm. Mobile phase A: 10% Methanol, 90% water, 0.05% TFA, B: 10% Methanol, 90% acetonitrile, 0.05% TFA. Gradient: 0 min 20% B, 2 min 20%B, 47 min 25% B, 62 min 80% B, 63 min 80% B, 70 min 80% B, 75 min 20% B. Flowrate = 2 mL/min. Method 3: YMC-ODS-AQ C18, 3 μm, 4.6 × 150 mm, detected at 215 nm. Mobile phase A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile. Gradient: 0 min 10% B, 5 min 10% B, 25 min 100% B, 30 min 10% B, 32 min 10%B. Flowrate = 1.0 mL/min.

6-(2-Hydroxypropan-2-yl)-5-methylpyrrolo[1,2-f][1,2,4]triazin-4(3H)-one-[^{14}C], 7

Ethyl 5-methyl-4-oxo-3,4-dihydropyrrolo[1,2-f][1,2,4]triazine-6-carboxylate-[^{14}C], **6** (345.7 mg, 1.54 mmol, 258.0 μCi/mg, SA = 57.1 mCi/mmol, 89.2 mCi) and unlabeled **6** (660 mg, 3.0 mmol) were weighed into a dry 100 mL round bottom flask under nitrogen. THF (50 mL) was added and the solution was reduced by rotovap to a volume of 13 mL resulting to a white suspension. The suspension was cooled to −5°C and MeMgCl (9.0 mL, 27.0 mmol, 3 M in THF) was added over a period of 20 min. The reaction was stirred at 0°C for 0.5 h and at 47°C for 6 h with monitoring by TLC (40% EtOAc/hexane, product R_f = 0.3) and HPLC (Method 1, product retention time = 9.62 min). It was cooled to 0°C, aqueous NH_4Cl (1.6 g, 29.9 mmol, dissolved in 15 mL water) was added slowly and extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated to dryness. The crude product was purified by silica gel chromatography by eluting with a gradient 25–100% EtOAc/Hexane then 5% MeOH/EtOAc to give a white solid (865 mg, 92% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.91 (1H, s), 7.83 (1 H, s), 4.82 (1 H, bs), 2.60 (3 H, s), 1.44 (6 H, m). MS ESI$^+$ $[M+H]^+$ = 208.0.

6-Hydroxy-5-methylpyrrolo[1,2-f][1,2,4]triazin-4(3H)-one-[^{14}C], 8

Compound **7** (0.84 g, 4.0 mmol) was dissolved in THF (9.7 mL) in a 100 mL round bottom flask. The solution was cooled to −8°C and charged with cold H_2O_2 (1.36 g, 40 mmol, 2.73 mL, 50 weight% in water). Ice-cold $MeSO_3H$ (3.86 g, 40 mmol) diluted with water (1.20 mL) was added dropwise over a period of 50 min and the solution was stirred at −8°C for 2 h while being monitored by TLC (50% EtOAc/hexane, product R_f = 0.3) and HPLC (Method 1, product retention time = 7.69 min). The reaction was quenched slowly at 0°C with a cold mixture of $NaHSO_3$ (58.5% SO_2, 4.83 g, 44.2 mmol) and 28% NH_3 (5.8 mL, 92.5 mmol) in water (10 mL). The reaction was extracted with

ethyl acetate (4 × 25 mL). After the layers were separated, the combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* affording the desired crude product as a light-gray solid (0.70 g, quantitative yield). This crude product was used in the next reaction without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 11.29 (1 H, br. s.), 8.80 (1 H, s), 7.61 (1 H, d, J = 4.02 Hz), 6.94 (1 H, s), 2.23 (3 H, s). MS ESI$^+$ $[M+H]^+$ = 166.1.

5-Methyl-4-oxo-3,4-dihydropyrrolo[1,2-f][1,2,4]triazin-6-yl pivalate-[^{14}C], 9

Compound **8** (0.70 g, 4.23 mmol) was suspended in THF (8.0 mL). The suspension was cooled to 0°C, *N,N*-diisopropylethylamine (0.66 g, 5.08 mmol) was added followed by the addition of trimethylacetyl chloride (0.61 g, 5.08 mmol) over 30 min. The reaction was stirred at 0°C for 30 min and at rt for 2 h while being monitored by TLC (50% EtOAc/hexane, product R_f = 0.6) and HPLC (Method 1, product retention time = 17.03 min). The reaction was cooled to 0°C, water (20 mL) was added, and the solution was extracted with EtOAc (3 × 25 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated to dryness. The crude product was purified by silica gel flash chromatography eluting with 5-100% EtOAc/hexane to afford a white solid (0.49 g, 46% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 9.82 (1 H, br. s.), 7.50 (1 H, s), 7.47 (1 H, d, J = 3.89 Hz), 2.39 (3 H, s), 1.38 (9 H). MS ESI$^+$ $[M+H]^+$ = 250.2.

4-Chloro-5-methylpyrrolo[1,2-f][1,2,4]triazin-6-yl pivalate-[^{14}C], 10

Compound **9** (0.78 g, 3.13 mmol) was suspended in acetonitrile (9.5 mL). *N,N*-diisopropylethylamine (0.47 g, 3.6 mmol) was added under nitrogen to the suspension followed by $POCl_3$ (1.3 g, 8.48 mmol). The cloudy white suspension turned to a clear solution. The reaction was stirred at 82°C for 3 h and at 60°C for 17 h while being monitored by TLC (30% EtOAc/hexane, product R_f = 0.8) and HPLC (Method 1, product retention time = 20.09 - min). The reaction was warmed to rt and the solvent was removed by rotovap. The residue was washed with K_2HPO_4 (3.3 g, 18.8 mmol) dissolved in water (30 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated to dryness. The crude product was dried under vacuum to give 1.05 g (quantitative yield) as an off-white solid. The crude product was used in the next reaction without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.51 (1 H, s), 7.48 (1 H, d, J = 3.89 Hz), 2.40 (3 H, s), 1.38 (9 H). MS ESI$^+$ $[M+H]^+$ = 268.2.

4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[1,2-f][1,2,4]triazin-6-yl pivalate-[^{14}C], 12

Compounds **10** (1.0 g, 3.88 mmol) and **11** (0.71 g, 4.27 mmol) were dissolved in acetonitrile (10.0 mL) under nitrogen. 1,4-Diazabicyclo[2.2.2]octane (0.48 g, 4.27 mmol) was added slowly to the solution. The reaction became a suspension. It was stirred at 48°C for 1.5 h. The reaction was monitored by TLC (50% EtOAc/hexane, product R_f = 0.5) and HPLC (Method 1, product retention time = 21.70 min). The acetonitrile was removed by rotovap. The mixture was washed with water (20 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated to dryness. The crude product was purified by triturating in ether (4 mL) with stirring at rt for 1 h. The product was collected by vacuum filtration to give a white fluffy solid (0.94 g, 75% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 11.37 (1 H, br. s.), 8.08 (1 H, s), 8.03 (1 H, s), 7.15 (1 H, d, J = 8.53 Hz), 7.01 (1 H, t, J = 8.53 Hz), 6.25 (1 H, s), 2.41 (3 H, s), 2.38 (3 H, s), 1.36 (9 H, s). MS ESI$^+$ $[M+H]^+$ = 397.3.

(*R*)-1-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[1,2-f][1,2,4]triazin-6-yloxy)propan-2-ol-[^{14}C], 13

Sodium methoxide (25% in MeOH, 0.49 mL, 2.27 mmol) was added slowly to a suspension of compound **12** (0.90 g, 2.27 mmol) in acetonitrile (1.8 mL) under nitrogen at 10°C. The mixture was stirred at rt for 1 h. The progress of the reaction was monitored by HPLC (Method 1) to indicate complete formation of the intermediary alcohol, 4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[1,2-f][1,2,4]triazin-6-ol (R_t = 15.70 min). Water (4.6 mL) was added to the reaction followed by (*R*)-(+)-propylene oxide (233.2 mg, 4.01 mmol). The mixture was stirred at 28°C for 24 h. The suspension was cooled to 10°C, filtered to collect the solid, and the filtrate was rinsed with water/acetonitrile (80:20, 2 mL). The crude solid was recrystallized in acetone/water (1:1, 8 mL) at 50°C. The product was collected by vacuum filtration. The mother liquor was also collected and purified by flash chromatography eluting with 10–60% EtOAc/hexane then 3% MeOH/CH_2Cl_2 to afford a combined 0.62 g (74% yield) of a white solid. TLC (50% EtOAc/hexane, product R_f = 0.2) and HPLC (Method 1, product retention time = 16.60 min). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 11.34 (1 H, br. s.), 7.90 (2 H, d, J = 6.27 Hz), 7.14 (1 H, d, J = 8.78 Hz), 7.04−6.92 (1H, m), 6.24 (1 H, s), 4.90 (1 H, d, J = 4.77 Hz), 4.08−3.95 (1 H, m), 3.93−3.81 (2 H, m), 2.45−2.36 (6 H, m), 1.18 (3 H, m). MS ESI$^+$ $[M+H]^+$ = 371.2.

(*S*)-((*R*)-1-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[1,2-f][1,2,4]triazin-6-yloxy)propan-2-yl) 2-(benzyloxycarbonylamino)propanoate-[^{14}C], 14

Compound **13** (0.62 g, 1.7 mmol) was dissolved in THF/DMF (4:1) (6 mL) under nitrogen. The solution was cooled to 0°C and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.55 g, 2.89 mmol), 4-dimethylaminopyridine (8 mg, 0.07 mmol), and Cbz-L-alanine (0.56 g, 2.91 mmol) were added. The mixture was stirred at 0°C for 4.5 h while monitoring by TLC (50% EtOAc/hexane, product R_f = 0.7) and HPLC (Method 1, product retention time = 20.09 min). The reaction mixture was cooled to 10°C, washed with water (5 mL) and extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified by silica gel flash chromatography eluting with 0–50% EtOAc/hexane to afford a white solid (0.92 g, 96% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.01 (1 H, br. s.), 7.84 (1 H, s), 7.40 (1 H, s), 7.35−7.26 (5 H, m), 7.08 (1 H, d, J = 8.80 Hz), 6.99 (1 H, t, J = 7.14 Hz), 6.34 (1 H, s), 5.30−5.20 (2 H, m), 5.11 (2 H, m), 4.42 (1 H, m), 4.15 (2 H, m), 2.46−2.45 (6 H, m), 1.45–1.38 (6 H, dd, J = 7.15, 26.94 Hz). MS ESI$^+$ $[M+H]^+$ = 576.2.

(*S*)-((*R*)-1-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[1,2-f][1,2,4]triazin-6-yloxy)propan-2-yl) 2-aminopropanoate-[^{14}C], 15

To a solution of compound **14** (0.31 g, 0.54 mmol) in EtOAc (3 mL) was added K_2CO_3 (50 mg, 0.36 mmol). The mixture was placed under vacuum at 170 mBar for 5 min and nitrogen (g)

was introduced. Wet (50%) palladium (5%) on activated carbon (150 mg) was quickly added to the mixture. The mixture was placed under vacuum and purged with hydrogen (g). This step was repeated three times. The mixture was reacted under hydrogen (g) for 2 h while monitoring by HPLC (Method 2, product retention time = 45.83 min) and TLC (50% EtOAc/Hexane, product $R_f = 0.7$ or 20% iPA/EtOAc, product $R_f = 0.2$). The reaction was filtered through a bed of celite and rinsed with EtOAc (50 mL). The EtOAc was removed by rotovap to dryness and the crude product was purified by flash chromatography. It was incrementally eluted with 10% EtOAc/hexane to EtOAc and then with 10% iPA/EtOAc. The pure fractions were collected and rotovapped to dryness to give the product as a white foam (206 mg, radiochemical purity = 92.71%). This material was further purified by recrystallization. In a 25 mL pear shaped-flask was added the impure material (206 mg, 83% yield, with a radiochemical purity = 92.71%) and EtOAc (1.1 mL). The clear solution was heated slowly to 50°C and heptane (2.1 mL) was added over 15 min. Cloudiness was observed at the end of the addition, but gradually all the solid dissolved. It was seeded with pure unlabeled **15** (0.5 mg). The solution was cooled slowly. It was stirred at rt for 16 h. The crystallized product was collected by vacuum filtration as a white solid (114.5 mg, 56% yield, SA = 23.24 μCi/mg, 10.30 mCi/mmol, chemical purity = 99.20%, radiochemical purity = 99.23%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.95 (1 H, br. s.), 7.78 (1 H, s), 7.35 (1 H, s), 7.08 (1 H, d, $J = 8.80$ Hz), 6.90 (1 H, m), 6.27 (1 H, s), 5.32−5.20 (1 H, m), 4.04 (2 H, m), 3.56 (1 H, m), 2.47 (3 H, s), 2.39 (3 H, s), 1.64 (2 H, br. s.), 1.36 (6 H, m). MS ESI$^+$ $[M+H]^+ = 442.2$.

Ethyl 4-chloro-5-(methyl-^{13}C)methyl(5,6-$^{13}C_2$,1,8-$^{15}C_2$)pyrrolo[1,2-f][1,2,4]triazine-6-(carbonyl-^{13}C)carboxylate, 17

Compound **16**[5] (0.60 g, 2.64 mmol) was suspended in dry toluene (15 mL) under a nitrogen atmosphere. To the flask was added phosphorus oxychloride (0.61 g, 3.96 mmol) and *N,N*-diisopropylethyl amine (0.44 g, 3.43 mmol). The mixture was heated to 110°C for 18 h as the reaction became clear and brownish. The reaction was monitored by TLC (50% EtOAc/Hexane, product $R_f = 0.75$). Upon completion, the reaction was rotovapped to dryness. To the crude solid was added EtOAc (30 mL), cooled to 0°C, washed with ice-cold K_2HPO_4 (1 M) until pH reached 8, then extracted further with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was dried under vacuum for 16 h to give an off-white solid (677 mg, quantitative yield). This product was used crude in the next reaction without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.23 (1 H, t, $J = 5.60$ Hz), 8.12 (1 H, q, $J = 6.28$ Hz), 4.38 (2 H, m, dq, $J = 3.14$ Hz), 2.87 (3 H, dm, $J = 130$ Hz), 1.40 (3 H, t, $J = 7.12$ Hz). ^{13}C NMR ($CDCl_3$) δ ppm 164.69 (m), 121−118 (m), 12.39 (m). MS ESI$^+$ $[M+H]^+ = 246.2$.

Ethyl 4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-(methyl-^{13}C)-methyl(5,6-$^{13}C_2$,1,8-$^{15}C_2$)pyrrolo[1,2-f][1,2,4]triazine-6-(carbonyl-^{13}C)carboxylate, 18

Compound **17** (674 mg, 2.74 mmol) was suspended in DMF (10 mL), K_2CO_3 (1.13 g, 8.22 mmol) and 4-fluoro-2-methyl-1H-indol-5-ol (452 mg, 2.74 mmol) were added. The reaction was stirred at rt for 21 h. The reaction was cooled in an ice bath, washed with ice-cold water (30 mL) and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and rotovapped to dryness. MeOH (7 mL) was used to triturate the crude product. The pure product (578 mg) was collected by vacuum filtration. The mother liquor was also collected, concentrated, and chromatographed on silica gel eluting with 25% EtOAc/hexane to give an additional 95 mg of white solid (673 mg, 66% yield). TLC (40% EtOAc/Hexane, product $R_f = 0.5$). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.15 (1 H, t, $J = 5.50$ Hz), 8.04 (1 H, s), 7.89 (1 H, q, $J = 8.24$ Hz), 7.10 (1 H, d, $J = 8.25$ Hz), 6.98 (1 H, t, $J = 7.15$ Hz), 6.35 (1 H, s), 4.38 (2 H, dq, $J = 3.14$ Hz), 2.86 (3 H, dm, $J = 129.2$ Hz), (3 H, s), 1.40 (3 H, t, $J = 7.14$ Hz). ^{13}C NMR ($CDCl_3$) δ ppm 164.84 (d, $J = 83.93$ Hz), 119.12 (t, $J = 56.17$ Hz), 116.83 (m), 12.02 (d, $J = 48.32$ Hz). MS ESI$^+$ $[M+H]^+ = 374.9$.

2-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-(methyl-^{13}C)-methyl(5,6-$^{13}C_2$,1,8-$^{15}C_2$)pyrrolo[1,2-f][1,2,4]triazin-6-yl)propan-2(^{13}C)-ol, 19

Compound **18** (665 mg, 1.78 mmol) was dissolved in THF/toluene (1:1) (10 mL). To the solution was added LiCl (1.03 g, 24.2 mmol) and it was cooled to 0°C under nitrogen. To the mixture was slowly added CH_3MgBr (1.4 M in toluene/THF, 75:25) (8.07 mL, 5.76 mmol). The reaction was stirred at 0°C for 1 h and at 15°C for 3 h. The reaction was cooled to 0°C and an ice-cold 15% NH_4Cl (30 mL) was added. The aqueous layer was extracted with EtOAc (4 × 30 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and rotovapped to dryness. The crude product was chromatographed on silica gel eluting with 10–100% EtOAc/hexane to afford 360 mg (56% yield) of a white solid. TLC (1% MeOH/CH_2Cl_2, product $R_f = 0.5$) and HPLC (Method 3, product, retention time = 19.64 min). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.01 (1 H, s), 7.85−7.81 (1 H, m), 7.68 (1 H, m), 7.09 (1 H, d, $J = 8.24$ Hz), 6.97 (1 H, q, $J = 6.60$ Hz), 6.34 (1 H, s), 5.23 (1 H, m), 2.72 (3 H, m), 2.46 (3 H, s), 1.71 (6 H, q, $J = 3.85$ Hz). ^{13}C NMR ($CDCl_3$) δ ppm 134.14 (t, $J = 55.95$ Hz), 112.85 (q, $J = 50.87$ Hz), 70.67 (d, $J = 53.41$ Hz), 12.27 (t, $J = 40.69$ Hz). MS ESI$^+$ $[M+H]^+ = 361.16$.

4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-(methyl-^{13}C)-methyl(5,6-$^{13}C_2$,1,8-$^{15}C_2$)pyrrolo[1,2-f][1,2,4]triazin-6-ol, 20

Hydrogen peroxide (46.91 mg, 1.38 mmol) (50%) at 0°C was added to cold CH_2Cl_2 (2.0 mL) followed by BF_3OEt_2 (2.38 g, 16.74 mmol). The solution was stirred at 0°C for 20 min. To a separate flask containing compound **19** (355 mg, 0.99 mmol) dissolved in CH_2Cl_2 (8.0 mL) and cooled to 15°C was added over 20 min the above prepared solution. At the end of the addition, the reaction became cloudy. The reaction was stirred at −15°C for 1 h while being monitored by TLC (50% EtOAc/hexane or 1% MeOH/CH_2Cl_2, both, product $R_f = 0.5$) and HPLC (Method 3, product, retention time = 18.49 min). The reaction was then washed with ice-cold Na_2SO_3 (10%) (25 mL) and ethanolamine (33%) (25 mL) and extracted with EtOAc (4 × 34 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified by silica gel flash chromatography eluting with 10–60% EtOAc/hexane to afford a white solid (212.9 mg, 68% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.01 (1 H, s), 7.87–7.83 (1 H, q, $J = 8.25$ Hz), 7.43 (1 H, t, $J = 4.95$ Hz), 7.08 (1 H, d, $J = 8.79$ Hz), 6.98 (1 H, t, $J = 8.25$ Hz), 6.33 (1 H, s), 2.55 (3 H, dm, $J = 128.1$ Hz), 2.45 (3 H, s), 2.37 (1.5 H, m). ^{13}C NMR ($CDCl_3$) δ ppm 144.48 (dd, $J = 7.63$, 66.11 Hz), 101.12 (m), 8.38 (d, $J = 48.32$ Hz). MS ESI$^+$ $[M+H]^+ = 317.9$.

(*R*)-1-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-(methyl-^{13}C)-methyl(5,6-$^{13}C_2$,1,8-$^{15}C_2$)pyrrolo[1,2-f][1,2,4]triazin-6-yloxy)-propan-2-ol, 21

Compound **20** (205 mg, 0.65 mmol) was dissolved in ethanol (3 mL) in a pressure tube. To the solution was added triethylamine (1 drop), lithium chloride (109.5 mg, 2.58 mmol), followed by (*R*)-(+)-propylene oxide (233.2 mg, 4.01 mmol). The mixture was heated to 70°C for 3 h while monitoring by TLC (50% EtOAc/hexane, product $R_f = 0.3$) and HPLC (Method 3, product, retention time = 19.41 min). The reaction was cooled and rotovapped to dryness. To the residue was added water (20 mL) and extracted with EtOAc (4 × 30 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified on silica gel flash chromatography eluting with 10–60% EtOAc/hexane to afford a white solid (140 mg, 58% yield). Product HPLC purity = 98.0%. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.02 (1 H, s), 7.87–7.83 (1 H, q, J = 8.25 Hz), 7.42 (1 H, t, J = 4.95 Hz), 7.09 (1 H, d, J = 8.80 Hz), 6.98 (1 H, t, J = 8.25 Hz), 6.34 (1 H, s), 4.26 (1 H, m), 3.97 (1 H, m), 3.85 (1 H, m), 2.50 (3 H, dm, J = 128.9 Hz), 1.32 (3 H, d, J = 6.60 Hz). ^{13}C NMR ($CDCl_3$) δ ppm 148.05 (dd, J = 7.63, 68.67 Hz), 101.37 (m), 8.67 (dd, J = 17.8, 48.32 Hz). MS ESI$^+$ $[M+H]^+$ = 375.9, mass isotopic distribution m/z 375.9 = 94.1%, 374.9 = 5.3%, 373.9 = 0.6%, 370.9 = undetectable.

(*S*)-((*R*)-1-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-(methyl-^{13}C) methyl(5,6-$^{13}C_2$,1,8-$^{15}C_2$)pyrrolo[1,2-f][1,2,4]triazin-6-yloxy)propan-2-yl) 2-(benzyloxycarbonylamino)propanoate, 27

Prepared using same procedure as in preparation of **14**. Product **27** (off-white foam, 45.5 mg, 92% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.01 (1 H, s), 7.85 (1 H, m), 7.40 (1 H, m), 7.35–7.26 (5 H, m), 7.10 (1 H, d, J = 8.79 Hz), 6.99 (1 H, t, J = 7.14 Hz), 6.34 (1 H, s), 5.25–5.20 (2 H, m), 5.11 (2 H, s), 4.42 (1 H, m), 4.15 (2 H, m), 2.45 (3 H, s), 2.10 (3 H, dm, J = 128.0 Hz), 1.45 (3 H, m). ^{13}C NMR ($CDCl_3$) δ ppm 148.01 (dd, J = 7.63, 68.67 Hz), 101.45 (m), 8.55 (dd, J = 17.8, 48.32 Hz). MS ESI$^+$ $[M+H]^+$ = 580.9.

(*S*)-((*R*)-1-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-(methyl-^{13}C) methyl(5,6-$^{13}C_2$,1,8-$^{15}C_2$)pyrrolo[1,2-f][1,2,4]triazin-6-yloxy)propan-2-yl) 2-aminopropanoate, 28

To a solution of compound **27** (45.5 mg, 0.08 mmol) in EtOAc (0.9 mL) under nitrogen was added ground K_2CO_3 (6.8 mg, 0.05 mmol) in a 2 mL V shaped vial. Wet (50%) palladium (5%) on activated carbon (6.80 mg) was quickly added to the mixture. The reaction vial was placed under vacuum and flushed with hydrogen. This step was repeated three times. It was charged with hydrogen to 20 psi for 2.5 h. The reaction was monitored by HPLC (Method 2, product retention time = 45.83 min) and TLC (50% EtOAc/Hexane, product $R_f = 0.7$). The reaction was filtered through a syringe filter (0.45 μm nylon membrane) then rinsed with EtOAc (2 mL). The solvent was removed by rotovap leaving a solid. The solid product was dried under vacuum for 18 h to give 29.6 mg of glassy solid (84% yield). HPLC chemical purity = 96.50%. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.35 (1 H, br. s.), 8.05–7.95 (2 H, m), 7.14 (1 H, d, J = 8.25 Hz), 6.98 (1 H, t, J = 7.97 Hz), 6.24 (1 H, s), 5.19 (1 H, td, J = 6.60, 3.85 Hz), 4.35–4.10 (2 H, m), 3.50–3.40 (1 H, m), 2.45–2.20 (6 H, m), 1.40–1.34 (3 H, d, J = 6.59 Hz), 1.24 (3 H, d, J = 7.14 Hz).^{13}C NMR (DMSO-d_6) δ ppm 147.01 (m), 101.45 (m), 8.29 (m). MS ESI$^+$ $[M+H]^+$ = 447.19, mass isotopic distribution m/z 447.19 = 94.8%, 446.19 = 5.1%, 442.19 = undetectable.

6-(2-Hydroxy(^{13}C)propan-2-yl)-5-(methyl-^{13}C)methyl(5,6-$^{13}C_2$,1,8-$^{15}C_2$)pyrrolo[1,2-f][1,2,4]triazin-4(3H)-one, 22

Prepared using same procedure as in preparation of **7**. Product **22** (off-white solid, 637 mg, 77% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.27 (1 H, br. s.), 7.64 (1 H, dd, J = 8.80, 6.05 Hz), 7.28 (1 H, t, J = 6.60 Hz), 4.82 (1 H, br. s.), 2.52 (3 H, dm, J = 130.0 Hz), 1.40–1.48 (6 H, m). ^{13}C NMR (DMSO-d_6) δ ppm 132.69 (t, J = 52.92 Hz), 117.42 (t, J = 54.18 Hz), 68.39 (d, J = 54.18 Hz), 11.54 (d, J = 45.36 Hz). MS ESI$^+$ $[M+H]^+$ = 214.19.

6-Hydroxy-5-(methyl-^{13}C)methyl(5,6-$^{13}C_2$,1,8-$^{15}C_2$)pyrrolo[1,2-f][1,2,4]triazin-4(3H)-one, 23

Prepared using same procedure as in preparation of **8**. Product **23** (light yellow solid, 535 mg, 97% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.38 (0.5 H, br. s.), 11.20 (0.5 H, br. s.), 8.80 (1 H, s), 7.60 (1 H, m), 6.94 (1 H, t, J = 5.0 Hz), 2.23 (3 H, dm, J = 130.0 Hz). ^{13}C NMR (DMSO-d_6) δ ppm 143.41 (dd, J = 8.82, 64.26 Hz), 107.05–106.18 (m), 8.27 (d, J = 49.14 Hz). MS ESI$^+$ $[M+H]^+$ = 171.12.

5-(Methyl-^{13}C)methyl-4-oxo-3,4-dihydro(5,6-$^{13}C_2$,1,8-$^{15}C_2$)-pyrrolo[1,2-f][1,2,4]triazin-6-yl pivalate, 24

Prepared using same procedure as in preparation of **9**. Product **24** (white solid, 487 mg, 61% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 10.16 (0.5 H, br. s.), 9.98 (0.5 H, br. s.), 7.49–7.47 (2 H, m), 2.39 (3 H, dm, J = 130.0 Hz), 1.37 (9 H, s). ^{13}C NMR ($CDCl_3$) δ ppm 137.20 (d, J = 76.44 Hz), 113.16–112.59 (m), 8.63 (d, J = 48.41 Hz).

4-Chloro-5-(methyl-^{13}C)methyl(5,6-$^{13}C_2$,1,8-$^{15}C_2$)pyrrolo[1,2-f][1,2,4]triazin-6-yl pivalate, 25

Prepared using same procedure as in preparation of **10**. Product **25** (white solid, 239 mg, 89% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 8.16–8.11 (1 H, m), 8.0 (1 H, m), 2.49 (3 H, dm, J = 128.1 Hz), 1.41 (9 H, s). ^{13}C NMR ($CDCl_3$) δ ppm 140.09 (d, J = 76.86 Hz), 107.15–106.18 (m), 9.14 (d, J = 49.14 Hz). MS ESI$^+$ $[M+H]^+$ = 273.08.

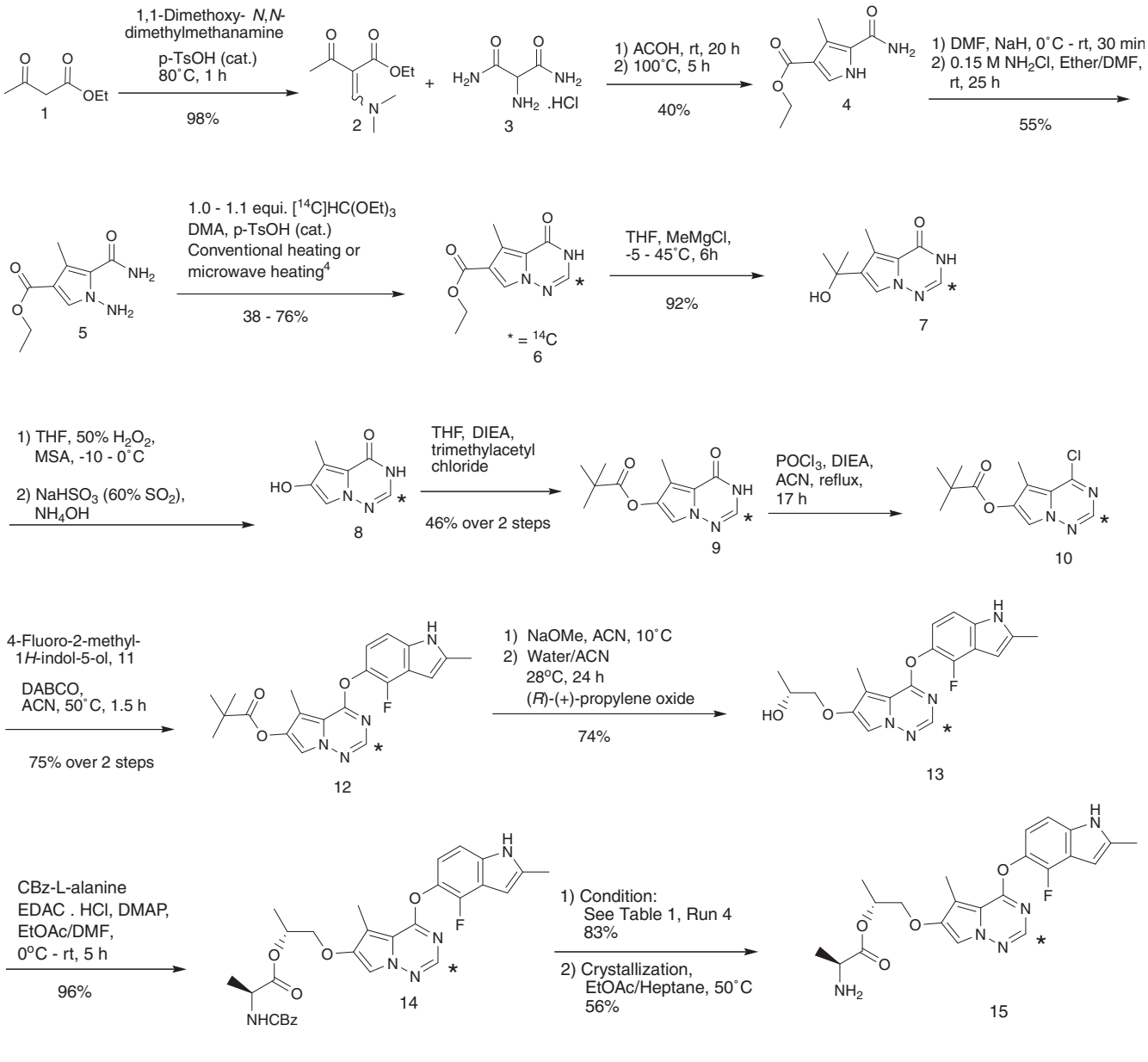
4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-(methyl-^{13}C)methyl-(5,6-$^{13}C_2$,1,8-$^{15}C_2$)pyrrolo[1,2-f][1,2,4]triazin-6-yl pivalate, 26

Prepared using same procedure as in preparation of **12**. Product **26** (white solid, 335 mg, 82% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.36 (1 H, s), 8.08–8.00 (2 H, m), 7.16 (1 H, d, J = 10.0 Hz), 7.01 (1 H, t, J = 10.0 Hz), 6.25 (1 H, s), 2.41 (3 H, s), 2.37 (3 H, dm, J = 129.0 Hz), 1.35 (9 H, s). ^{13}C NMR (DMSO-d_6) δ ppm 138.07 (d, J = 69.30 Hz), 104.84 (m), 8.51 (d, J = 50.40 Hz). MS ESI$^+$ $[M+H]^+$ = 402.0.

Results and discussion

Synthesis of [^{14}C]Brivanib, **13** was completed, via a ring cyclization reaction, by reacting 1.0–1.1 eq [^{14}C]$HC(OEt)_3$ with ethyl 1-amino-5-carbamoyl-4-methyl-1H-pyrrole-3-carboxylate, **5**,[3] to give [^{14}C]ethyl 5-methyl-4-oxo-3,4-dihydropyrrolo[1,2-f][1,2,4]triazine-6-carboxylate, **6**, in 38% yield by conventional heating or in 76% yield by microwave at 150°C and 200 W power.[4] The ethyl ester at the C-6 position was converted to an hydroxyl group, **8** via a sequence of reduction, oxidation, and Baeyer–Villiger rearrangement. Subsequent chemical steps, involving alcohol protection with trimethylacetyl chloride,

chlorination with phosphorus oxychloride, coupling with 4-fluoro-2-methyl-1*H*-indol-5-ol, **11**, afforded pyrrolotriazine-aryl ether, **12** in an overall yield of 23% from **6**. The pivaloyl group was removed by deprotecting with NaOMe followed by selective O-alkylation with (*R*)-(+)-propylene oxide preferably at the site of the unhindered carbon of the epoxide to produce 17.2 mCi of the desired parent drug, **13**, with a 98.1% radiochemical purity in an overall yield of 34% (Scheme 1).



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Scheme 1. Synthesis of parent drug candidate [^{14}C]Brivanib, **13** and prodrug [^{14}C]Brivanib Alaninate, **15**.

Table 1. Investigation of balloon hydrogenation to remove CBz group

Run	Unlabeled Compound 14(mg)	5% Pd/C, wet 50/50 (mg)	K_2CO_3 (mg)	EtOAc (mL)	Time (h)	HPLC Yield (%) of 15
1	300	36	50	3	32	15
2	300	36[a]	50	3	32	15
3	300	250	50	3	6	42
4	300	150	50	3	2	77
5	300	100	50	3	1.5	49

[a]Run 2, 10% Pd/C was used.

Radiochemical stability of **13** as a solid at a specific activity of 24.3 μC/mg (10.3 mCi/mmol) was shown to decrease by 0.7% after 3 years of storage at −78°C.

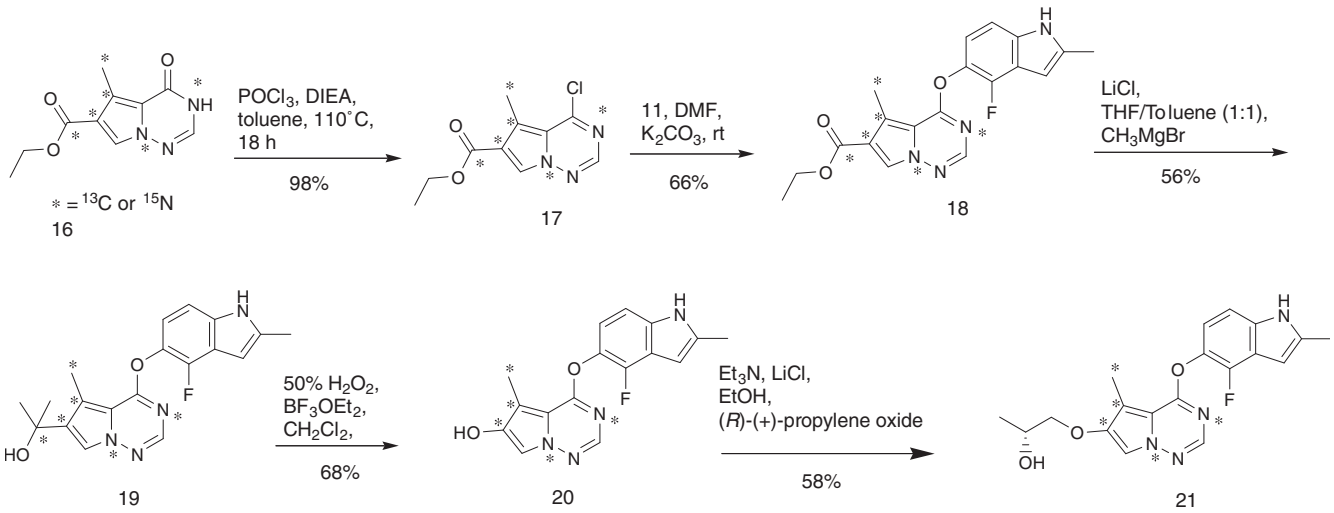
The pharmaceutic and pharmacokinetic properties of the parent were improved by preparing the Alaninate prodrug. Balloon hydrogenation of the penultimate, **14**, removed the CBz group and generated the prodrug, Brivanib Alaninate. Results of various conditions for CBz group deprotection are summarized in Table 1. Reaction conditions shown in Run 4 were applied in the radioactive synthesis to produce an 83% yield of the desired product. Recrystallization of the final product gave 2.7 mCi of [^{14}C]Brivanib Alaninate, **15**, with a chemical purity of 99.2%, a specific activity of 23.2 μCi/mg (10.3 mCi/mmol) and a radiochemical purity of 99.2%.

The stable isotope-labeled central core pyrrolotriazinone **16**, prepared from suitably labeled materials using the same route as in the [^{14}C]synthesis, was reported by Ogan *et al.*[5] Initially, the medicinal chemistry synthetic route was used to produce 140 mg of [$^{13}C_3^{15}N_2$]Brivanib, **21**, with a chemical purity of 98.6%. Mass spectrometric analysis of [$^{13}C_3^{15}N_2$]Brivanib was performed using positive ion electrospray on a Q-Tof Ultima mass spectrometer giving a molecular ion $[M+H]^+$ at 375.9 Da. This is consistent with the molecular formula $^{13}C_3C_{16}H_{19}F^{15}N_2N_2O_3$. Isotopic purity was calculated to be 94.1%. A molecular ion of 374.9 Da, corresponding to the empirical formula $^{13}C_2C_{17}H_{19}F^{15}N_2N_2O_3$, was 5.3% (Scheme 2).

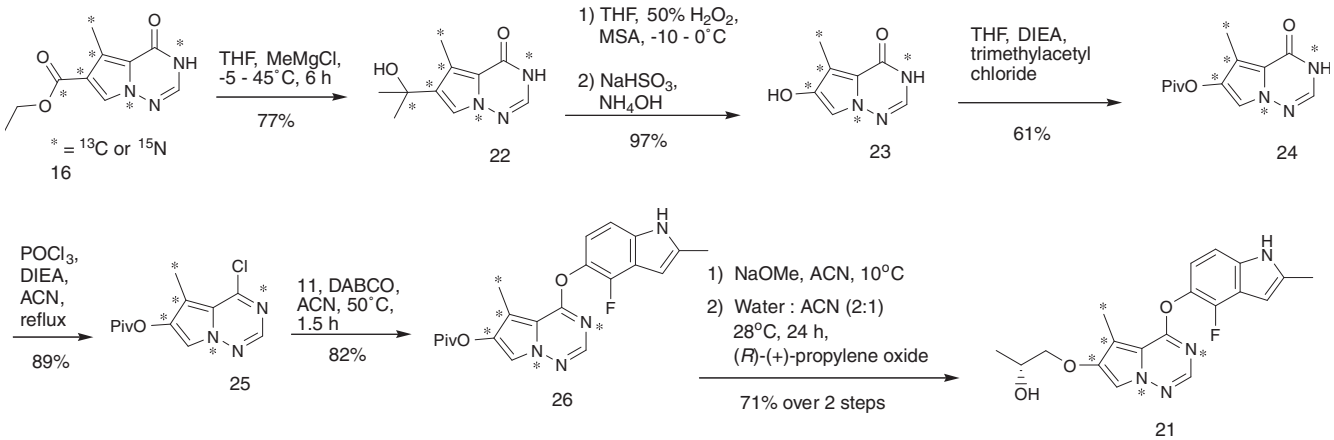
Subsequently, a second lot of 215 mg at 98.0% chemical purity was produced using the later established Process Research and Development (PR&D) synthetic route[6] (Scheme 3). The removal of the CBz group on a small scale required high pressure hydrogenation using this route. The [$^{13}C_3^{15}N_2$]prodrug penultimate, **27** (45 mg, 0.08 mmol), dissolved in 0.9 mL EtOAc, conditioned with ground K_2CO_3 (6.8 mg), Pd-C 5% (6.8 mg) was hydrogenated at 20 psi for 2.5 h to give 29.6 mg, 84% yield of the prodrug [$^{13}C_3^{15}N_2$]Brivanib Alaninate, **28** at 96.5% chemical purity. This represents a reduction of 70% less 5% Pd-C than in the previous balloon hydrogenation (Scheme 4). Both [$^{13}C_3^{15}N_2$]Brivanib, **21** and [$^{13}C_3^{15}N_2$]Brivanib Alaninate, **28** had undetectable levels of [M+0] unlabeled products by LC-MS and were suitable for use as internal standards to support bioanalytical LC-MS analyses of biological samples.

Conclusion

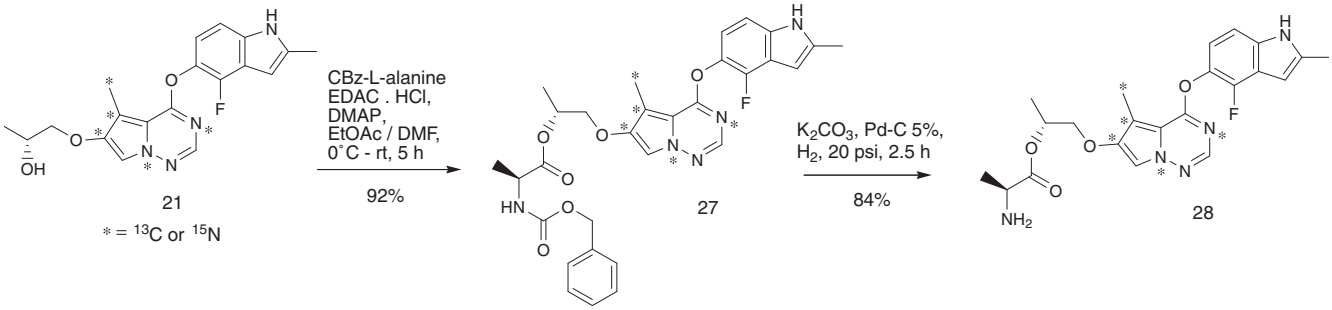
The synthesis of [^{14}C]Brivanib was completed to produce 17.2 mCi of the desired parent drug, **13** with a 98.1% radiochemical purity. Balloon hydrogenation of the penultimate,



Scheme 2. Synthesis of stable isotope labeled parent drug candidate [$^{13}C_3^{15}N_2$]Brivanib, **21** prepared by the medicinal chemistry route.



Scheme 3. Synthesis of stable isotope-labeled parent drug candidate [$^{13}C_3^{15}N_2$]Brivanib, **21** prepared by the PR&D route.



Scheme 4. Synthesis of stable isotope labeled prodrug candidate [$^{13}C_3^{15}N_2$]Brivanib Alaninate, **28**.

14 to generate the final prodrug Brivanib Alaninate was investigated and the best conditions were applied in the radioactive synthesis to produce 2.7 mCi of [^{14}C]Brivanib Alaninate, **15** with a specific activity of 23.2 μCi/mg (10.3 mCi/mmol) and a radiochemical purity of 99.2%.

For stable isotope labels, the medicinal chemistry synthetic route was used to produce 140 mg of [$^{13}C_3^{15}N_2$]Brivanib, **21** with a chemical purity of 98.6%. Subsequently, a second lot of 215 mg at 98.0% chemical purity was produced using the later established PR&D synthetic route. A small scale high-pressure hydrogenation reaction using the [$^{13}C_3^{15}N_2$]prodrug penultimate was completed to produce 29.6 mg of the prodrug [$^{13}C_3^{15}N_2$]Brivanib Alaninate, **28**. The stable isotope-labeled products had undetectable levels of [M+0] unlabeled products by LC-MS.

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