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Synthesis of lead LFA-1 antagonist [^{14}C]spyrocyclic hydantoin

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Radiolabelled drug lead candidate leukocyte function-associated antigen 1 antagonist [^{14}C]spyrocyclic hydantoin: 5-(((5*S*,9*R*)-9-(4-[^{14}C]-cyanophenyl)-3-(3,5-dichlorophenyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)methyl)thiophene-3-carboxylic acid, 12, was conveniently prepared in three radiochemical steps from (5*S*,9*R*)-*tert*-butyl 9-(4-bromophenyl)-3-(3,5-dichlorophenyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate 9. The radiochemical yield of 12 was 28.5% from the resolved bromide 9. The preparation of the racemic spyrocyclic hydantoin 3 was obtained via a [3+2]dipolar cycloaddition reaction between 2 and *N*-benzyl-*N*-(methoxymethyl)trimethylsilylmethylamine. The introduction of [^{14}C] cyanide was completed via a palladium (0) catalyzed reaction by the addition of Zn(^{14}CN)$_2$ to aryl bromide 9. The radiochemical and chiral purities of 12 determined by high-performance liquid chromatography were 98.7 and 99.7%, respectively. The specific activity of 12 was 87.5 μCi/mg (48.6 mCi/mmol).

Keywords: Zn(^{14}CN)$_2$; leukocyte function-associated antigen 1 antagonist; palladium (0) catalyzed cyanation

Introduction

The CD11a/CD18 (also known as leukocyte function-associated antigen 1 or LFA-1) leukocyte integrin is expressed at high levels on the cell surface of T lymphocytes and macrophages, where it mediates homotypic and heterotypic adherence between leukocytes and other cell types by binding to intracellular adhesion molecules (ICAM) 1–3 on the conjugate cell.[1] Monoclonal antibodies have been used to demonstrate the critical role of the LFA-1/ICAM interaction in models of autoimmune and inflammatory diseases,[2] and some have gained regulatory approvals. There has been an intense effort to take on the more challenging task of identifying small, biologically active, and orally bioavailable molecule inhibitors of this LFA-1/ICAM interaction.[3–5] The preparation of [^{14}C]spyrocyclic hydantoin 12 (Figure 1) was requested for use in pre/clinical absorption, distribution, metabolism, and elimination studies. This report details the preparation and characterization of radiolabelled drug lead candidate LFA-1 antagonist [^{14}C]spyrocyclic hydantoin 12.

Results and discussion

The synthesis of [^{14}C]spyrocyclic hydantoin 12 began with a Knoevenagel reaction[6] of 4-bromobenzaldehyde and an active methylene compound 1 (Scheme 1). The reaction produced two isomeric products. The trans olefin was the desired and more thermodynamically stable product. The *E*/*Z* ratio by high-performance liquid chromatography (HPLC) (Method A) was 95:5. The purification to isolate the trans olefin was completed by slowly adding THF (30 v/v%) at 55°C with stirring for 24 h, which afforded compound 2 in pure form. Compound 2 was reacted in a [3+2]dipolar cycloaddition with the intermediary iminium ylide formed from *N*-benzyl-*N*-(methoxymethyl)trimethylsilylmethylamine[7] to generate racemic compound 3. The benzyl protection group was removed to improve the solubility of 4 in MeOH for supercritical fluid chromatography (SFC). Reaction of 3 with 1-chloroethyl chloroformate[8] followed by treatment with MeOH and 6 N HCl gave racemic freebase 4. Racemic 4 analyzed by chiral HPLC (Method B) indicated two enantiomers to be present with product 4a, retention time (R_t) = 9.07 min (57.6%), and product 4b, R_t = 15.65 min (42.2%). To identify the biologically active and optically desired enantiomer, optical resolution of the racemate via diastereoisomeric salt formation[9] was completed to prepare each enantiomer. Small-scale fractional crystallization of 4 was attempted with various chiral acids to form the corresponding diastereoisomeric salt pairs. The results showed that the chiral acid (1*S*)-(+) camphor sulfonic acid when used with 0.25–0.5 equivalent to the base produced higher % ee than di-*p*-toluoyl-D-tartaric acid, (2*S*,3*S*)-2,3-dihydroxysuccinic acid, or (1*R*)-(−) camphor sulfonic acid. Freebasing of each diastereoisomeric salt pair with saturated $NaHCO_3$ gave two separate enantiomers identified as 4a and 4b (Scheme 2). At this stage, the two stereo centers at positions 5 and 9 were not known. Each of the obtained optically enriched materials was sequentially Boc

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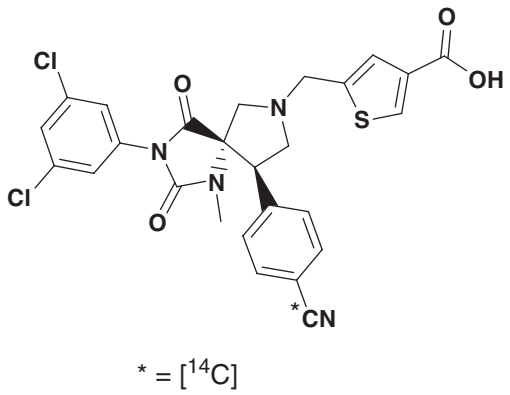
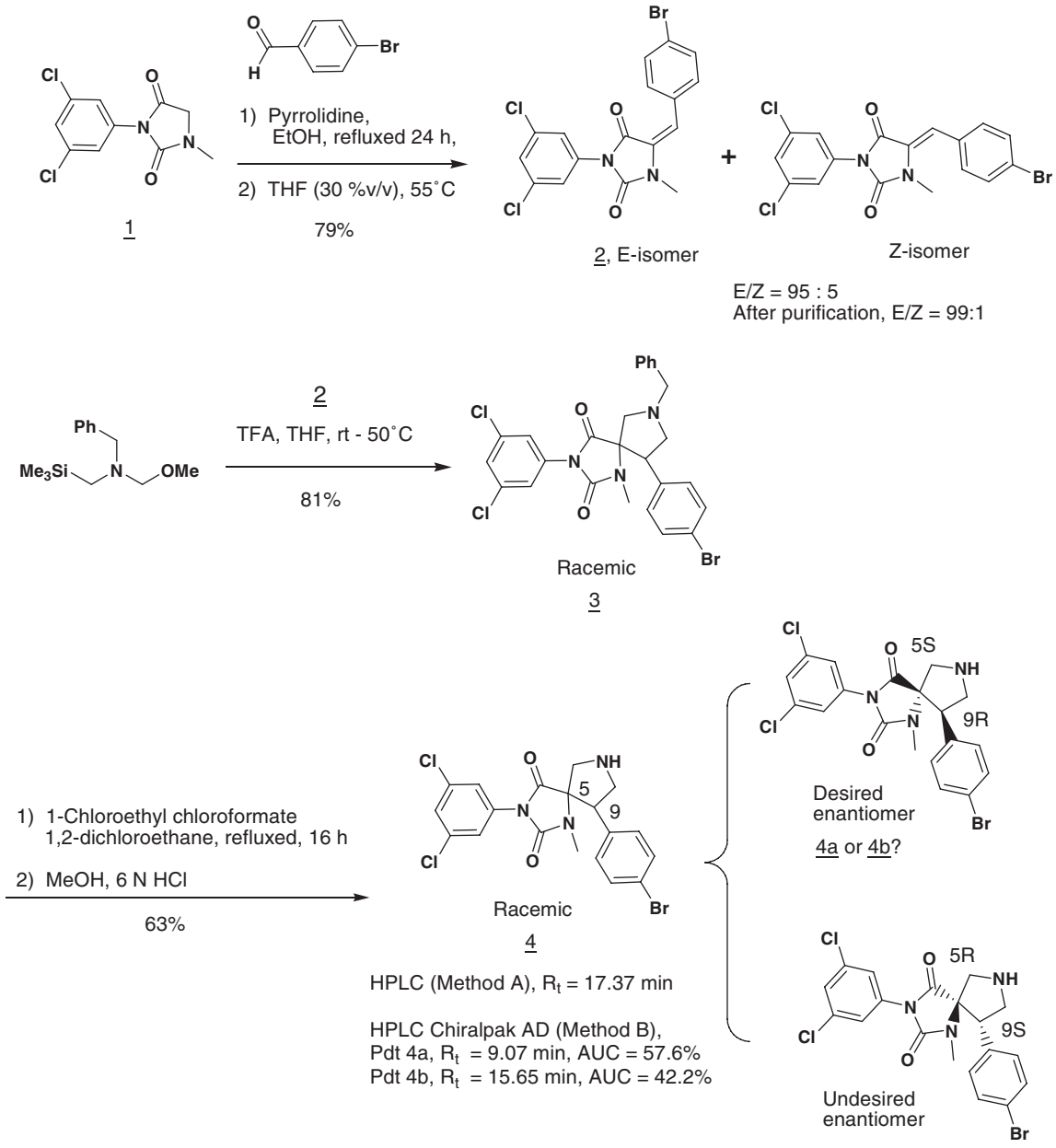


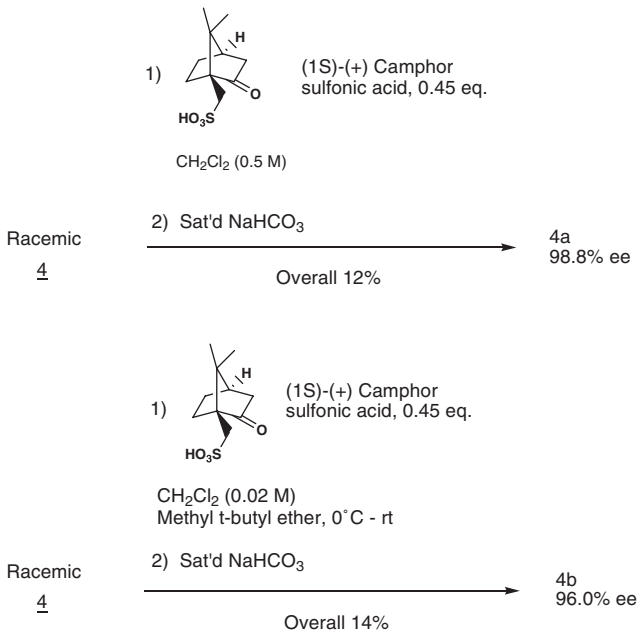
Figure 1. [^{14}C]12.

protected, cyanated, and Boc deprotected to produce 7 and 8 (Scheme 3). The cyanated compounds 7 and 8 were compared with the cyanated authentic reference standard (5*S*,9*R*; R_t = 33.1 min) by chiral HPLC (Method B). It was determined that 7 (R_t = 20.0 min) did not match the authentic desired material (R_t = 33.1 min) concluding that compound 4a was the undesired enantiomer. Compound 8 (R_t = 33.1 min), which coeluted with the authentic material, indicated that compound 4b was the desired enantiomer.

Large-scale enantiomeric separation of racemic 4 was completed by SFC to produce the desired enantiomer 4b with 99.4% ee in 23% yield. Compound 4b was Boc protected with Boc anhydride to produce 9 in 98% yield. In the presence of a catalytic amount of $Pd(PPh_3)_4$, compound 9 was cyanated with freshly prepared $Zn(^{14}CN)_2$ to give radioactive 10 in 50% yield.



Scheme 1. Synthesis of the racemic base 4.



Scheme 2. Enantioenrichment by chiral crystallization.

The Boc-protecting group on product 10 was removed to give 11 in 92% yield. Then 11 was reacted via a reductive amination reaction with 5-formyl-3-thiophenecarboxylic acid and sodium triacetoxyborohydride to give the desired product [^{14}C]spyrocyclic hydantoin 12 in an overall yield of 62% (Scheme 4).

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. UV and radiochemical purities were determined by HPLC (Shimadzu SCL-10A VP/UV-1 detector or Varian Prostar 330 PDA detector and IN/US System β-Ram radiometric flow detector with 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). Radiolabelled products were compared with authentic standards when possible. All reagents and solvents were ACS grade or better.

Specific activity was determined gravimetrically by dissolution of the weighed sample (ca 0.5 mg) into DMF in a 10-mL volumetric flask. Aliquots of 25 and 50 μL of the solution sample were diluted into 10-mL Ecolite cocktail and counted.

High-performance liquid chromatography: 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 and radiochemical purities.

Method A: Used for compounds 2–4 and 10. YMC-ODS-AQ C18, 3 μm, 4.6 × 150 mm, detected at 254 nm. Mobile phase A: 0.05% trifluoroacetic acid (TFA) in water, B: 0.05% TFA in acetonitrile. Gradient: 0 min 25% B, 5 min 55% B, 15 min 95% B, 30 min 95% B, 32 min 25%B. Flowrate = 1 mL/min.

Method B: Used for compounds 4, 4a, 4b, and 7–9. Chiralpak AD, Daicel, 5 μm, 4.6 × 250 mm, detected at 254 nm. Mobile phase: *n*-hexane:isopropyl alcohol:triethyl amine:TFA (93:7:1:1). Isocratic, flowrate = 1.5 mL/min.

Method C: Used for compound 12. YMC-ODS-AQ C18, 4 μm, 4.6 × 150 mm, detected at 220 nm. Mobile phase A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile. Gradient: 0–25 min 35%B, 35 min 98%B, 47 min 35%B. Flowrate = 1 mL/min.

Method D: Used for compound 12. Chiralcel OJ-RH, 4.6 × 150 mm, 5 μm, Chiral Technologies, detected at 220 nm. Mobile phase: methanol:acetonitrile:0.2% aq. phosphoric acid (30:30:40). Isocratic, flowrate = 1.0 mL/min.

(E)-5-(4-bromobenzylidene)-3-(3,5-dichlorophenyl)-1-methylimidazolidine-2,4-dione, 2

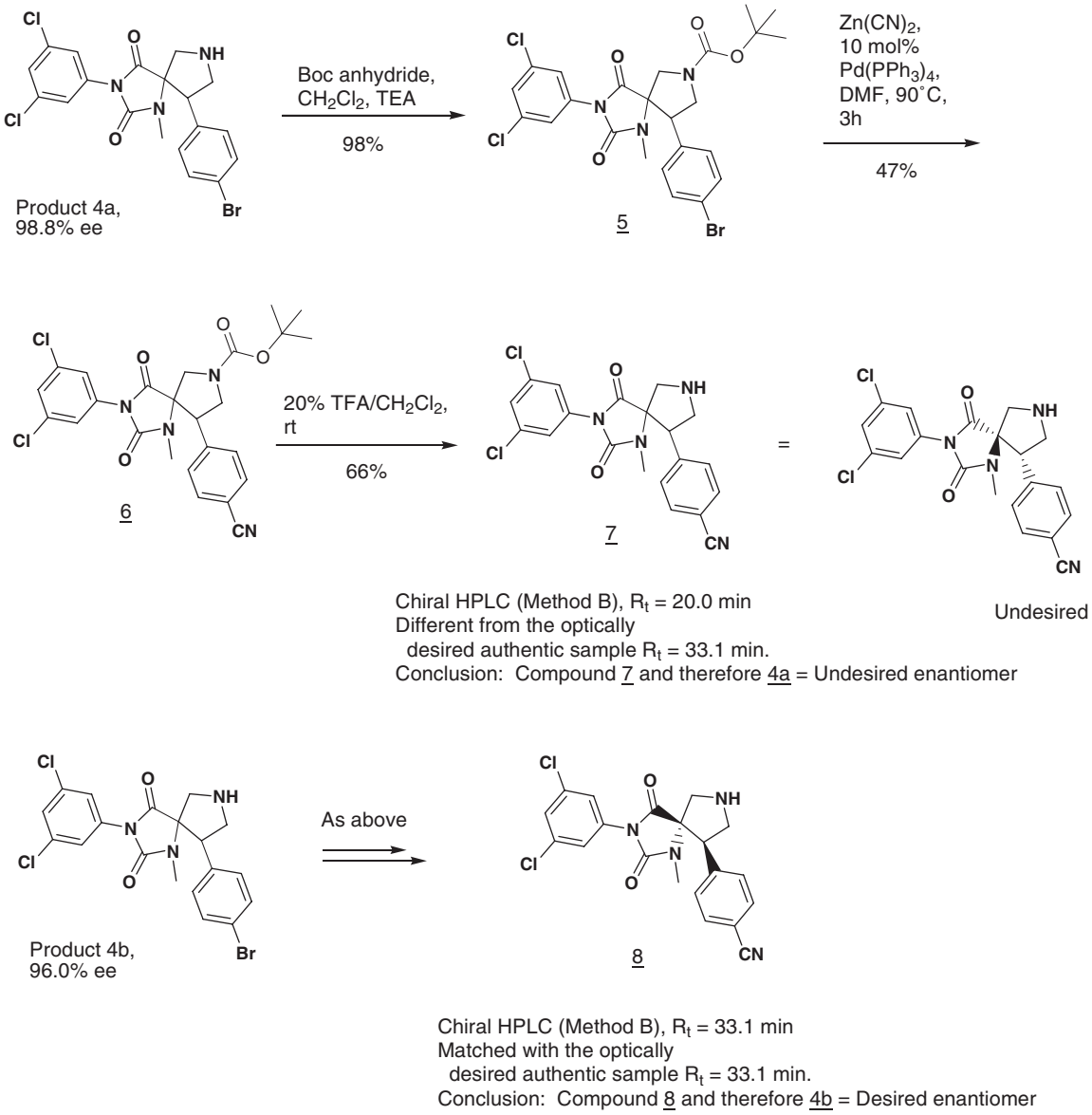
A reaction flask was charged with 3-(3,5-dichlorophenyl)-1-methylimidazolidine-2,4-dione 1 (15.1 g, 58.3 mmol), 4-bromobenzaldehyde (12.7 g, 68.8 mmol), ethanol (210 mL), and pyrrolidine (3.9 g, 55.4 mmol). The mixture was heated to reflux for 24 h. The reaction was monitored by TLC (20% EtOAc/hexane, product $R_f = 0.7$, SM $R_f = 0.3$), HPLC (Method A, product $R_t = 14.0$ min), and ^{1}H NMR for the completion of reaction. The resulting clear solution was cooled slowly to 55°C and the desired E-isomer began to crystallize. At 55°C, THF (70 mL) was added. The thick mixture was stirred at 55°C for 24 h. The product was collected by vacuum filtration as a white solid (19.6 g, 79%, $E/Z > 99\%$). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.88 (d, 2H, $J = 8.3$ Hz), 7.70 (d, 1H, $J = 1.6$ Hz), 7.69–7.57 (m, 4H), 6.63 (s, 1H), 3.21 (s, 3H). MS (ESI$^+$ and ESI$^-$) *m/z* not detectable.

Racemic 7-benzyl-9-(4-bromophenyl)-3-(3,5-dichlorophenyl)-1-methyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione, 3

Compound 2 (3.0 g, 7.0 mmol) was dissolved in THF (60 mL). The solution was cooled to 0°C and *N*-methoxymethyl-*N*-trimethylsilylmethyl benzylamine (1.92 g, 8.1 mmol) was added. TFA (50 μL, 0.63 mmol) was added slowly. The reaction was stirred at 0°C for 2.5 h and at rt for 40 h. After this period, additional *N*-methoxymethyl-*N*-trimethylsilylmethyl benzylamine (0.6 g, 2.5 mmol) and TFA (50 μL, 0.63 mmol) were added. The reaction was heated to 50°C for 2.5 h to drive the reaction to completion. The solvent was evaporated to dryness. The dry residue was diluted with EtOAc (30 mL), washed with water (30 mL), and backextracted with EtOAc (2 × 30 mL). After separation, the organic layer was washed with brine, dried over $MgSO_4$, and evaporated to dryness. The crude product was triturated in diethyl ether to give a white solid (3.2 g, 81%). TLC (20% EtOAc/hexane, product $R_f = 0.3$) and HPLC (Method A, $R_t = 18.6$ min). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.60 (d, 1H, $J = 1.6$ Hz), 7.51 (d, 2H, $J = 8.8$ Hz), 7.40–7.25 (m, 5H), 7.12 (d, 2H, $J = 8.3$ Hz), 6.70 (d, 2H, $J = 1.9$ Hz), 3.90–3.80 (m, 2H), 3.69 (d, 1H, $J = 13.2$ Hz), 3.30–3.18 (m, 2H), 3.12 (s, 3H), 3.01 (t, 1H, $J = 11.6$ Hz), 2.82 (d, 1H, $J = 11.6$ Hz). MS ESI$^+$ $[M+H]^+ = 560.03$.

Racemic 9-(4-bromophenyl)-3-(3,5-dichlorophenyl)-1-methyl-1,3,7-triazaspiro[4.4]nonane-2,4-dione, 4

A solution of racemic 3 (2.0 g, 3.6 mmol) in 1,2-dichloroethane (30 mL) under a nitrogen atmosphere at 5°C was added dropwise over 15 min 1-chloroethyl chloroformate (1.5 g, 10.5 mmol). The white suspension was stirred at 5°C for 1 h and heated to reflux at 80°C for 18 h. The reaction was cooled to rt and the solution was evaporated to dryness to yield a light yellow oil. This oil was further concentrated using a

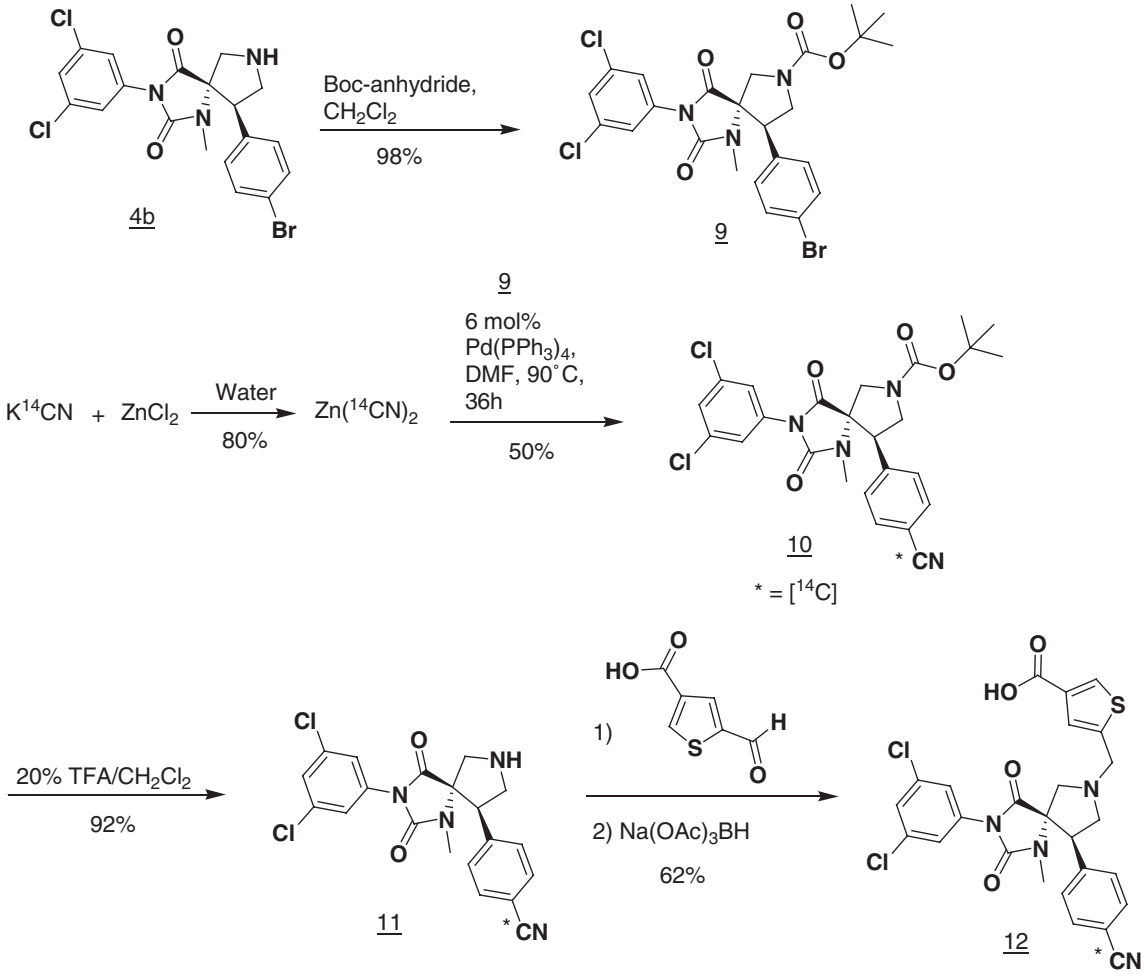


Scheme 3. Synthesis and chiral HPLC analyses to determine which enantiomer was the desired product.

high-vacuum pump to remove residual benzyl chloride that was formed in the first step. An amorphous solid was obtained. The solid was suspended in MeOH (30 mL) and 6 N HCl (1.3 mL). The suspension was heated to 50°C for 1.5 h and then evaporated to dryness. The residue was suspended in dichloromethane (90 mL) and water (15 mL) and neutralized to pH = 8.5 with 2 N NaOH (~7 mL). The layers were separated and the organic layer was washed with water, brine, dried over sodium sulfate, and concentrated to a foam. It was purified by silica gel flash chromatography by eluting with 0–5% MeOH/CH_2Cl_2 to give a white solid as a racemic mixture (1.1 g, 63%). TLC (30% EtOAc/hexane, product R_f = 0.3), HPLC (Method A, R_t = 17.37 min), and chiral HPLC (Method B, R_t = 9.07 min, 57.6%, and R_t = 15.65 min, 42.2%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.60 (d, 1H, J = 2.2 Hz), 7.53 (d, 2H, J = 8.8 Hz), 7.10 (d, 2H, J = 8.3 Hz), 6.70 (d, 2H, J = 1.7 Hz), 3.50 (m, 1H), 3.35–3.30 (m, 2H), 3.30–3.20 (m, 2H), 3.09 (s, 3H), 3.05 (bs, 1H). MS ESI$^+$ $[M+H]^+$ = 470.2.

Fractional chiral crystallization, 4a

The pyrrolidine racemic mixture 4 (0.5 g, 1.06 mmol) was dissolved in dichloromethane (2.0 mL). To this solution was added (1*S*)-(+) camphor sulfonic acid (0.124 g, 0.53 mmol). Scratching the sides of the flask with a spatula facilitated the crystallization. Over 10 min the solution became cloudy. It was left at rt for 30 min. The crystallized diastereoisomeric salt was collected by vacuum filtration to give a white solid (147 mg). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.45 (s, 2H), 7.67 (t, 1H, J = 2.2 Hz), 7.62 (d, 2H, J = 8.8 Hz), 7.17 (d, 2H, J = 8.3 Hz), 6.67 (d, 2H, J = 1.3 Hz), 3.99–3.95 (t, 1H, J = 10.4 Hz), 3.83–3.81 (m, 2H), 3.73 (m, 2H), 3.14 (s, 3H), 2.88 (d, 1H, J = 14.3 Hz), 2.69–2.62 (m, 1H), 2.39 (d, 1H, J = 14.8 Hz), 2.20 (1H, tt), 1.93 (t, 1H, J = 4.4 Hz), 1.91–1.80 (m, 1H), 1.80 (d, 1H, J = 18.1 Hz), 1.30–1.20 (m, 2H), 1.03 (s, 3H), 0.73 (s, 3H). MS ESI$^+$ $[M+H]^+$ = 470.0. This salt was freebased with saturated sodium bicarbonate (25 mL) and extracted with dichloromethane (3 × 20 mL) to give the optically pure enantiomer as a white foam (60 mg, yield = 12%). Chiral HPLC



Scheme 4. Incorporation of [^{14}C].

(Method B, R_t = 9.07 min, ee = 98.8%). $[\alpha]_D$ = 20.7–76.08°, conc. = 0.700 w/v% in methanol. ^{1}H NMR (500 MHz, CD_3OD) δ 7.41–7.38 (dd, 2H, *J* = 2.3, 9.1 Hz), 7.28 (t, 1H, *J* = 1.9 Hz), 7.04 (d, 2H, *J* = 8.5 Hz), 6.60 (d, 2H, *J* = 1.9 Hz), 3.61–3.56 (m, 1H), 3.51 (t, 1H, *J* = 9.1 Hz), 3.28–3.19 (m, 3H), 3.07 (s, 3H). MS ES$^+$ $[M+H]^+$ = 470.2.

Fractional chiral crystallization, 4b

The pyrrolidine racemic mixture 4 (0.4 g, 0.85 mmol) was dissolved in dichloromethane (45 mL). To this solution was added (1*S*)-(+) camphor sulfonic acid (80.0 mg, 0.34 mmol). To the stirred solution was added *t*-butyl methyl ether (5 mL) dropwise. It was left unstirred at rt for 2 h for the precipitation to occur. The crystallized diastereoisomeric salt was collected by vacuum filtration to give a white solid (88 mg). This salt was freebased with saturated sodium bicarbonate (20 mL) and extracted with dichloromethane (3 × 15 mL). The organic layer was concentrated under reduced pressure to give the optically pure enantiomer as a white thin oil (54.2 mg, yield = 13.6%). Chiral HPLC (Method B, R_t = 15.65 min, ee = 95.8%). ^{1}H NMR: Same as compound 4a. MS ES$^+$ $[M+H]^+$ = 470.2.

Preparation of 5

To a solution of compound 4a (99.2 mg, 0.21 mmol) in dichloromethane (2 mL) was added di-*tert*-butyl dicarbonate (55.8 mg, 0.26 mmol). The reaction was stirred at rt for 3 h. It was diluted with dichloromethane (5 mL), washed with water (20 mL), and extracted with dichloromethane (2 × 20 mL). The layers were separated and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated to dryness to give a white solid (119 mg, 98%). TLC (5% MeOH/dichloromethane, R_f = 0.8). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.65 (t, 1H, *J* = 1.7 Hz), 7.57 (d, 2H, *J* = 7.7 Hz), 7.15 (d, 2H, *J* = 6.0 Hz), 6.80 (d, 2H, *J* = 9.3 Hz), 4.07–4.05 (m, 1H), 3.93–3.84 (m, 3H), 3.70 (d, 1H, *J* = 12.0 Hz), 3.10 (s, 3H), 1.48 (s, 9H). MS (ESI$^+$ and ESI$^-$) *m/z* not detectable.

Preparation of 6

In a pressure tube under argon was added compound 5 (119 mg, 0.21 mmol) and DMF (2.0 mL). The solution was purged with argon for 10 min. To this was quickly added $Zn(CN)_2$ (12.3 mg, 0.11 mmol) and tetrakis(triphenylphosphine)palladium (0) (24.3 mg, 0.021 mmol). The reaction mixture was heated to 90°C for 3 h. It was cooled to rt, washed with 10% LiCl (5 mL), and extracted with EtOAc (3 × 10 mL). After the layers were separated, the organic layer was washed with water, brine, dried over $MgSO_4$, and concentrated to dryness. The crude product was purified by silica gel chromatography by eluting with a gradient 0–50% EtOAc/hexane to give a white solid (50.6 mg,

47%). TLC (40% EtOAc/hexane, $R_f = 0.3$). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.92 (d, 2H, $J = 7.1$ Hz), 7.71 (s, 1H), 7.47 (d, 2H, $J = 7.1$ Hz), 6.88 (d, 2H, $J = 12.1$ Hz), 4.34–4.20 (m, 1H), 4.10–3.85 (m, 3H), 3.81 (d, 1H, $J = 12.1$ Hz), 3.19 (s, 3H), 1.51 (s, 9H). MS (ESI$^+$ and ESI$^-$) *m/z* not detectable.

Preparation of 7

Compound 6 (51 mg, 0.098 mmol) was dissolved in dichloromethane (1.5 mL). To this solution was slowly added TFA (300 μL) and it was stirred for 30 min. The reaction solution was evaporated to dryness. The residue was dissolved in dichloromethane (3 × 6 mL) and saturated $NaHCO_3$ was added. The layers were separated and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give the product as a thin film (27 mg, 66%). Chiral HPLC (Method B, $R_t = 20.07$ min). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.84 (d, 2H, $J = 8.1$ Hz), 7.62 (s, 1H), 7.37 (d, 2H, $J = 8.2$ Hz), 6.71 (t, 2H, $J = 0.7$ Hz), 3.73–3.69 (m, 1H), 3.41–3.26 (m, 4H), 3.13 (s, 3H). MS ESI$^+$ $[M+H]^+ = 415.1$.

Resolution of racemic 4 by SFC

The pyrrolidine racemic mixture 4 was resolved by preparative SFC on a Chiralpak® AD-H, 3 × 25 cm, 5 μm at 35°C eluted with 30% methanol/CO_2 as the mobile phase. The conditions were: pressure 100 bar, flowrate 80 g/min, injection amount 100–300 mg, run time 15 min, and throughput 1.0 g/h. The compound 4 (5.2 g, UV purity = 99.7%) was dissolved in methanol at a concentration of 100 mg/mL and used for multiple injections. The two enantiomers were separated, collected, and concentrated to dryness. Chiral HPLC analysis (Method B) indicated that the first SFC eluted peak indeed contained the desired enantiomer 4b ($R_t = 15.65$ min) (1.30 g, yield = 23%, 99.44% ee).

(5*S*,9*R*)-*tert*-butyl 9-(4-bromophenyl)-3-(3,5-dichlorophenyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate, 9

Prepared using the same procedure as for the preparation of 5.

Preparation of Zn(^{14}CN)$_2$

To a 10-mL centrifuge tube was added $ZnCl_2$ (282 mg, 2.07 mmol) dissolved in water (2 mL). K^{14}CN (Amersham, batch CFQ30087, 100 mCi, SA = 54.9 mCi/mmol, 118.6 mg, 1.82 mmol) was dissolved in water (2 mL) and the solution was added slowly to the solution of $ZnCl_2$. Zn(^{14}CN)$_2$ precipitated out almost immediately. The reaction was stirred for 1 h. The mixture was centrifuged for 10 min at 2500 rpm. The water layer was withdrawn by pipet. The insoluble residue was washed with water (1 mL) and then with acetone (2 mL) and dried under high vacuum for 16 h to give the product as a white solid (126.5 mg, 80.4 mCi).

(5*S*,9*R*)-*tert*-butyl 9-(4-[^{14}C]-cyanophenyl)-3-(3,5-dichlorophenyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonane-7-carboxylate, 10

To a 10-mL centrifuge tube containing dry Zn(^{14}CN)$_2$ (126 mg, 1.07 mmol, 80.4 mCi) under N_2 was quickly added compound 9 (1.22 g, 2.15 mmol) and tetrakis(triphenylphosphine)palladium (0) (148.7 mg, 6 mol%) followed by the addition of the N_2 pre-purged DMF (4 mL). The mixture was carefully purged with N_2 for 15 min. It was heated to 90°C for 36 h and monitored by TLC (40% EtOAc/hexane, product $R_f = 0.3$) and HPLC (Method A, $R_t = 15.2$ min). The DMF in the suspension was carefully reduced to about 1 mL by a stream of N_2 at 60°C. The mixture was washed with 10% LiCl (20 mL) and extracted into EtOAc (3 × 30 mL). The layers were separated and the organic layer was washed with brine, dried over sodium sulfate, and concentrated to dryness. The crude product was purified by silica gel flash chromatography eluting with 10–50% EtOAc/hexane and then with 5% MeOH/dichloromethane to afford a pure white solid (463 mg), radiochemical purity = 99.4%, specific activity = 84.7 μCi/mg, total activity = 39.2 mCi, radiochemical yield = 49%. ^{1}H NMR was the same as that reported for 6.

4-[^{14}C]((5*S*,9*R*)-3-(3,5-dichlorophenyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-9-yl)benzonitrile, 11

To a solution of compound 10 (457 mg, 0.89 mmol) in CH_2Cl_2 (3 mL) was slowly added 30% TFA in CH_2Cl_2 (5 mL). The reaction was stirred at rt for 1.5 h while monitoring by TLC (5%MeOH/CH_2Cl_2, product $R_f = 0.2$). The reaction solution was evaporated to dryness. The resulting oil was dissolved in dichloromethane (3 × 20 mL) and saturated sodium bicarbonate (20 mL) was added to pH = 8–9. The layers were separated and the organic layer was washed with brine, dried over sodium sulfate, and concentrated. The reaction produced a white sticky foam (338 mg, 92%), which was used in the next reaction without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84 (d, 2H, $J = 8.1$ Hz), 7.62 (s, 1H), 7.37 (d, 2H, $J = 8.2$ Hz), 6.71 (t, 2H, $J = 0.7$ Hz), 3.73–3.69 (m, 1H), 3.41–3.26 (m, 4H), 3.13 (s, 3H). MS ESI$^+$ $[M+H]^+ = 417.1$.

5-(((5*S*,9*R*)-9-(4-[^{14}C]-cyanophenyl)-3-(3,5-dichlorophenyl)-1-methyl-2,4-dioxo-1,3,7-triazaspiro[4.4]nonan-7-yl)methyl)-thiophene-3-carboxylic acid, 12

To a 50-mL, pear-shaped flask containing a stir bar under nitrogen was charged 11 (338 mg, 0.81 mmol) and 1,2-dichloroethane (10 mL). To the homogeneous solution was added 5-formyl-3-thiophenecarboxylic acid (153 mg, 1.0 mmol) and the reaction was stirred for 15 min. To the reaction flask at rt was added sodium sulfate (338 mg, 2.4 mmol). The mixture was stirred for 20 h. To this reaction mixture was added sodium triacetoxyborohydride (310 mg, 1.46 mmol) at rt over a 5-min period. The reaction was allowed to stir at rt for 3 h during which it became slightly homogeneous. After 3 h the reaction was quenched with water (30 mL) and stirred for 15 min. The reaction was extracted with 1,2-dichloroethane (3 × 20 mL). After the layers were separated, the organic layer was washed with brine, dried over sodium sulfate, and concentrated to dryness. The dry crude product was triturated with MeOH (5 mL) and stirred for 30 min. The insoluble residue was collected by filtration to give a white solid (280 mg, 62%). TLC (5% MeOH/dichloromethane, product $R_f = 0.3$) and HPLC (Method C, $R_t = 9.1$ min, radiochemical purity = 97.3%). This material was further purified by silica gel flash chromatography eluting with 0–8% MeOH/dichloromethane to give product 12 [radiochemical purity = 98.7%, chiral HPLC (Method D), $R_t = 13.4$ min, chiral purity = 99.7%, specific activity = 87.5 μCi/mg or 48.6 mCi/mmol]. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.08 (s, 1H), 7.82 (d, 2H, $J = 8.2$ Hz), 7.60 (s, 1H), 7.37 (d, 2H, $J = 8.3$ Hz), 7.32 (s, 1H), 6.70

(s, 2H), 4.07–4.0 (m, 2H), 3.89 (d, 1H, $J = 13.2$ Hz), 3.32 (s, 2H), 3.11 (m, 4H), 2.85 (d, 1H, $J = 11.6$ Hz). MS [M−H] = 555.05.

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