Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 693–701.
Published online in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1395

JLCR

Research Article

The synthesis of three isotopomers of 2-methyl-2-(4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]phenoxy)propionic acid, a potent and selective peroxisome proliferator-activated receptor alpha agonist

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Received 10 January 2007; Revised 4 February 2007; Accepted 5 February 2007

Abstract: Although fenofibrate (**1a**) is commercially available and clinically effective in lowering serum triglycerides, its activity and sub-type selectivity at the PPARα receptors are only moderate; therefore, there exists a need for more potent and sub-type selective PPARα agonists. To that end, discovery efforts have identified 2-methyl-2-(4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]phenoxy)propionic acid (**2**), a potent and selective human PPARα receptor agonist. In support of pre-clinical ADME studies and bioanalysis, three isotopomers of **2** have been synthesized. The results of these efforts are described below. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: PPARα; C-14; tritium; SLIS; dyslipidemia

Introduction

In the developing world, cardiovascular disease is the leading cause of death.[1] Among the associated risk factors of this disease are hypercholesterolemia and hypertriglyceridemia. The fibrates (e.g. fenofibrate, **1a**, Figure 1) have been widely used in the treatment of dyslipidemia and are the current treatment of choice for hypertriglyceridemia.[2] These agents effectively reduce serum triglycerides and effectively increase HDL-cholesterol in humans because of their agonist activity at the PPARα (peroxisome proliferator-activated receptors) receptor. The PPAR's are members of the nuclear receptor hormone super family of ligand-activated transcription factors. Three mammalian PPAR isoforms have been identified (PPARα, PPARδ and PPARγ) and each receptor sub-type displays distinct biological actions.[3] Although **1b**, the active metabolite of **1a** is a ligand for the PPARα receptor, its activity (IC_{50}= 24 000 *nM* versus hPPARα) and sub-type selectivity are moderate. Xu *et al.* recently reported on a SAR which delivered 2-methyl-2-(4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]phenoxy)-propionic acid (**2**), a potent and selective human PPARα receptor agonist (IC_{50}= 24 nM versus hPPARα, 6500 nM versus hPPARγ and 3800 nM versus hPPARδ), which has been selected for clinical studies.[4] Preliminary pharmacokinetic studies conducted in Beagle dogs and Fischer 344 rats (Table 1) indicated that **2** had good oral bioavailability (79 and 100%) as well as a reasonably long half-life (6.1 and 6.8 h, respectively). Further drug disposition studies in laboratory animals and humans (a preliminary report of the use of **2-[^{14}C]** in humans has been published)[5] required C-14-labeled material. In addition, the tritiated isotopomer (**2-[^{3}H]**) was synthesized for use in the determination of protein binding. A stable labeled isotopomer was synthesized for use as a stable labeled internal standard (SLIS) for LC–MS–MS bioanalysis of **2** in biological fluids. The syntheses of the isotopomers of **2** will be detailed in this paper.

Discussion

Bromination of **2** with bromine/acetic acid afforded **2-Br** in nearly quantitative yield. Treatment of **2-Br** with deuterium gas in the presence of 10% Pd–C/Et_3N yielded **2-D** with incorporation of deuterium in the aromatic ring as well as the aryl methyl moiety. From the ^{1}H-NMR spectrum, it was found that **2-D** was a

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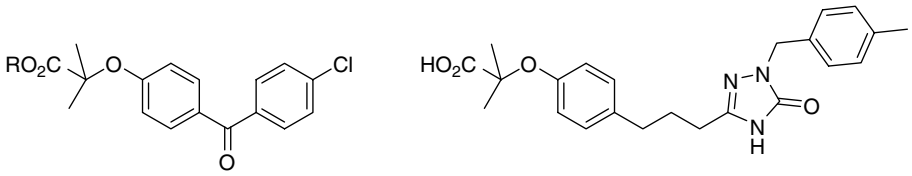


1a; R = *i*-Pr
1b; R = H

2

Figure 1 Structures of early PPAR-alpha agonists.

Table 1 Pharmacokinetic properties of **2** in Fischer 344 rats and Beagle dogs

Compound 2	F344 rat	Beagle dog
Bioavailability (%)	100	79
$T_{1/2}$ (h)	6.8	6.1
CL (mL/min/kg)	8.4	10.8
Volume of distribution	4.9	5.8

mixture of compounds **2a–c** in which there were either 2, 3 or 4 deuteriums (83% incorporation in the aryl moiety and 38% exchange in the aromatic methyl group). Indeed, when treated with tritium gas under the same conditions, the ^{3}H-NMR (Figure 2) of **3** showed three peaks in the methyl region; a singlet at δ 2.21 (single tritium), a doublet at 2.23 (J= 16.1 Hz, two tritia), a triplet at 2.26 (J= 15.9 Hz, three tritia), and a singlet at 6.80 ppm corresponding to the position *ortho* to the oxygen. By comparing the ^{1}H-NMR of the tritiated material, there appeared to be 47% tritiation on the aromatic ring and 12% on the methyl group. The specific activity as determined by mass spectrometry was 22 Ci/mmol and the radiochemical purity (RCP) was 99.7% (Scheme 1).

Bargellini described the synthesis of α-alkoxy-*iso*-butyric acids by the reaction of chloroform–acetone with alcohols in the presence of base.[6] This procedure was later examined in further detail by Weizmann *et al.* and was found to involve the intermediacy of the α-lactone of α-hydroxy-*iso*-butyric acid formed by the reaction of chloroform–acetone with NaOH.[7] Nucleophilic attack of the lactone by either alkoxide or phenoxide formed the α-alkoxy- or phenoxy-*iso*-butyric acid. Corey *et al.* reported that treatment of a mixture of cresol and acetone with powdered NaOH, followed by the addition of $CHCl_3$, yielded α-tolyloxy-*iso*-butyric acid in 45% yield.[8] Reduction of **2** with BH_3–THF reduced the carboxyl group to yield **4**. Treatment of **4** with BBr_3 in CH_2Cl_2 at −78°C yielded phenol **5**. Reaction of **5** with powdered NaOH in acetone was stirred at room temperature. The reaction was chilled to −15°C and chloroform was added to yield **2** after purification. Repeating this sequence with acetone-[UL-^{13}C] and $^{13}CHCl_3$ yielded **2-[^{13}C$_4$]** in 50% yield (Scheme 2).

To support the ADME studies, a carbon-14-labeled material was required with the tracer labeled at a metabolically stable position, preferably at the backbone of the molecule. The commercially available 3-(4-methoxyphenyl)-propan-1-ol (**6**) was mesylated followed by treatment with KCN-[^{14}C] to give the corresponding nitrile-[^{14}C] **7**. Hydrolysis of the nitrile under basic conditions gave the carboxylic acid **8**. Demethylation[9] of **8** followed by re-alkylation of the resulting phenol **9** with ethyl bromo-*iso*-butylate (EBIB) afforded the carboxylic acid **10** (Scheme 3).[4,10]

The carboxylic acid **10** was converted to an acyl chloride **11** and condensed with a mesylate salt of hydrazine **12** to give an intermediate **13**, which without isolation was cyclized under acidic conditions (using excess of camphorsulfonic acid) to a triazolone **14.** Saponification of **14** gave the desired product **2-[^{14}C]** (Scheme 4).[4,10]

Conclusion

Methods were developed for labeling of a leading candidate 2-methyl-2-(4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]phenoxy)propionic acid (**2**), a potent and selective human PPARα receptor agonist. Three isotopomers have been synthesized to support its use in ADME studies and bioanalysis.

Experimental

To monitor the deuterium labeling, LC–MS analysis was run on LCQ Classic Mass Spectrometer with inline PDA UV detector, using a Metachem Polaris C18-A column (150 × 2 mm, 5 μ). The NMR spectra were obtained on a Varian Mercury-400 spectrometer at 400 (^{1}H) and 100 (^{13}C) MHz. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. ES-MS was run on a Waters Micromass ZQ single quadrapole mass spectrometer. Exact masses were determined on a Micromass High Resolution Q-TOF II mass spectrometer (Manchester, UK). Flash chromatography was performed on Biotage system, or as described by Still *et al.*, using E.M. Science silica gel 60 (230–400 mesh). RCP was

DOI: 10.1002.jlcr

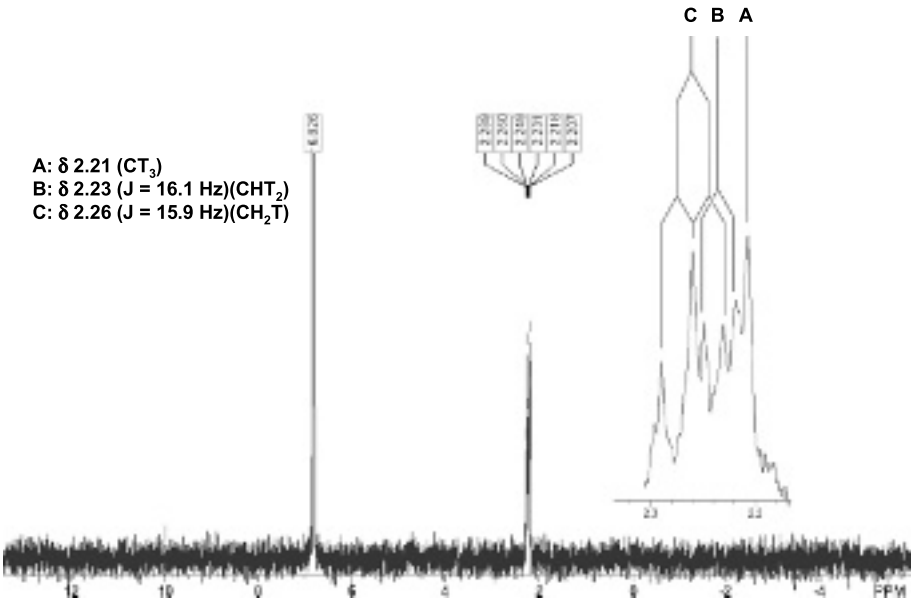
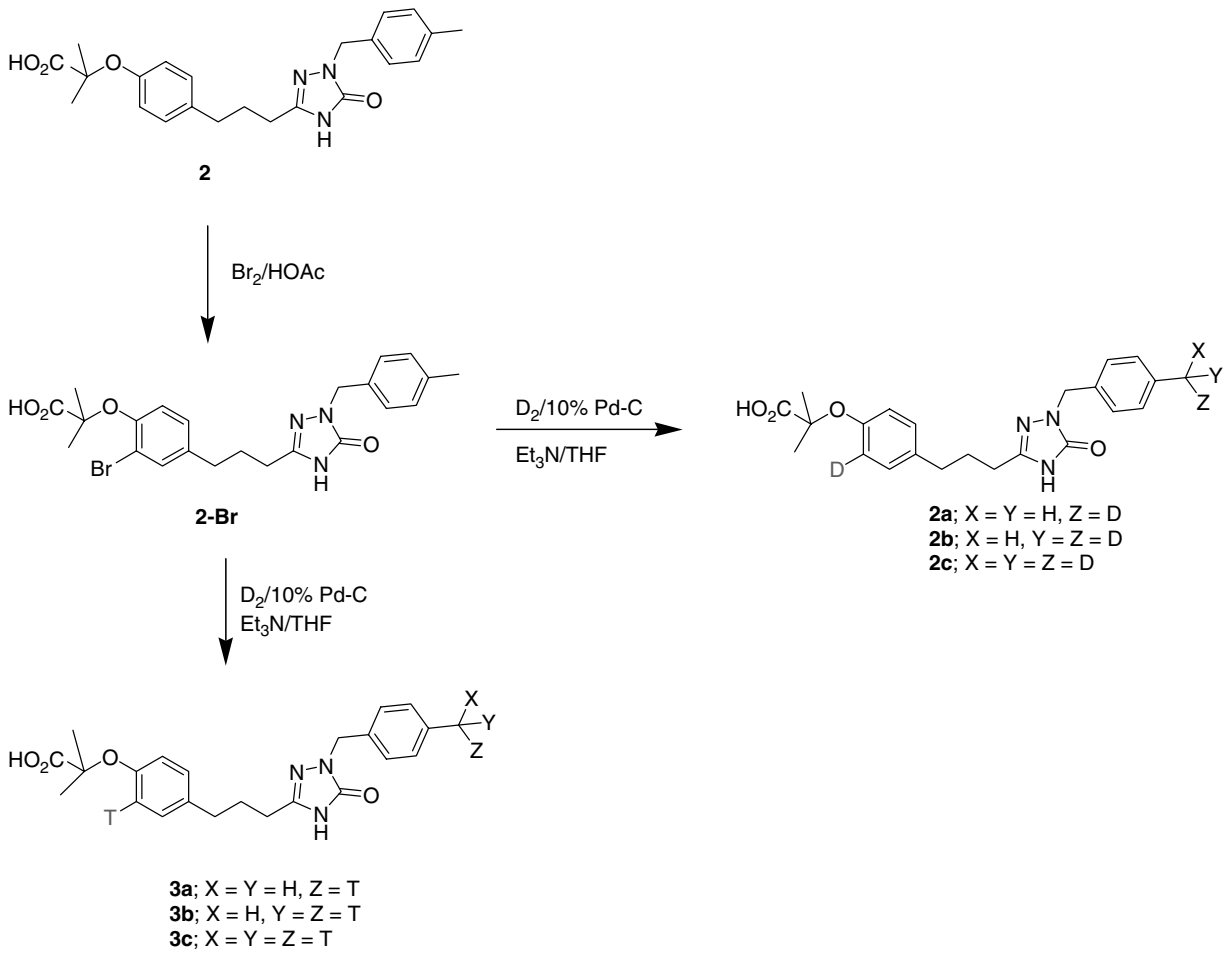
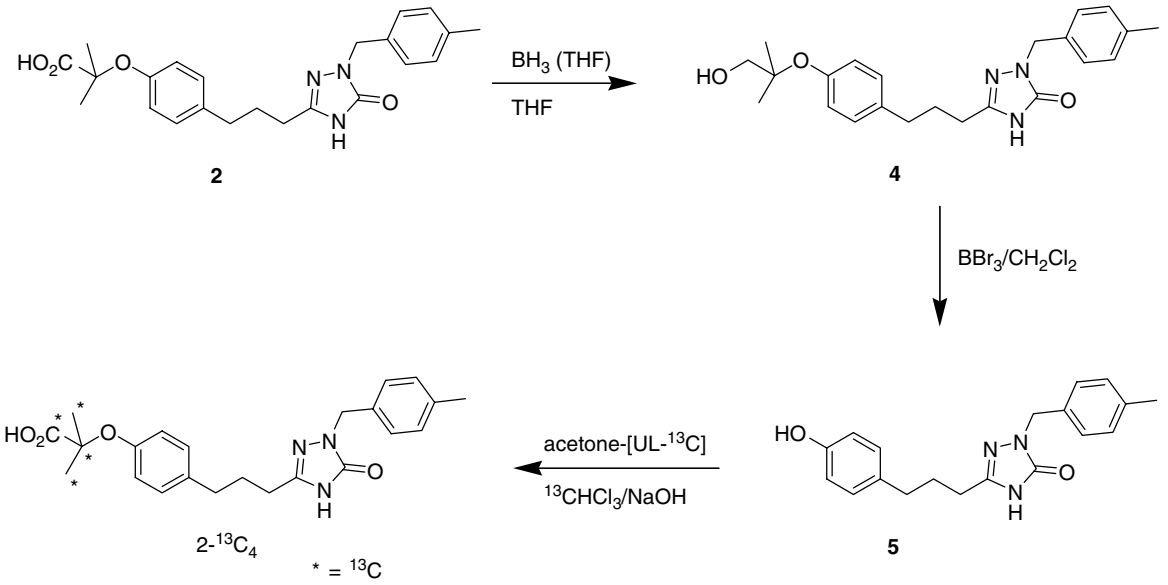


Figure 2 Tritium NMR of **3**-[^{3}H] in CD_3OD. Inset is the expanded region from δ 2.1 to 2.3 ppm. Figure available in colour online at www.interscience.wiley.com

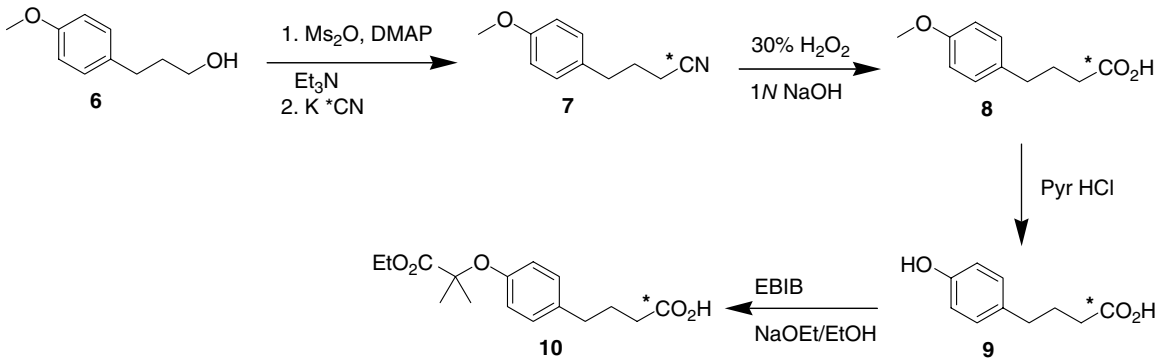


Scheme 1 Scheme available in colour online at www.interscience.wiley.com

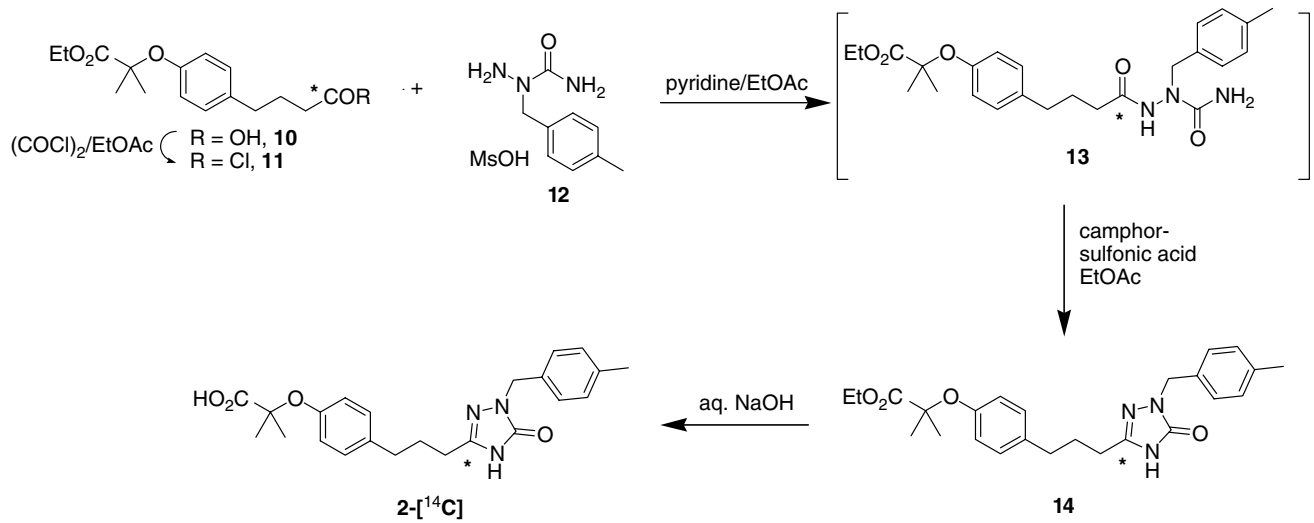
DOI: 10.1002.jlcr



Scheme 2



Scheme 3



Scheme 4

DOI: 10.1002.jlcr

assessed by autoradiography. As a further check of the RCP, the sample was subjected to radio-HPLC.

Synthesis of 2-(2-bromo-4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]phenoxy)-2-methylpropionic acid, 2-Br

An acetic acid solution (6 mL) of 2-methyl-2-(4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]phenoxy)propionic acid (**2**, 0.527 g, 1.286 mmol) was stirred and treated dropwise with bromine (100 μL, 1.940 mmol, 1.5 eq). The reaction was followed by HPLC (*vide infra*); the R_T (**2**) = 7.65 and R_T (**2-Br**) = 8.12. After 30 min, the reaction was approximately 50% complete. An additional 33 μL of bromine was added and stirring was continued for 15 min. HPLC showed the reaction to be 77% complete. Additional bromine (33 μL) was added twice more for a total of 199 μL (3.86 mmol, 3 eq). Three hours after the final addition of bromine (total reaction time was *ca.* 6 h), HPLC showed a single peak corresponding to **2-Br**. The reaction mixture was chilled in an ice bath and treated with Na_2SO_3 (0.42 g, 3.31 mmol) in 15 mL of water whereupon a white gum was deposited. The mixture was filtered and the residue was washed with water (3 × 5 mL) and then hexanes (5 mL). The semi-solid residue was dissolved in Et_2O (5 mL) and stirred at 5°C for 1 h. A white crystalline precipitate formed which was collected by filtration, washed with fresh Et_2O (3 × 3 mL) and dried *in vacuo* to yield 2-(2-bromo-4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]phenoxy)-2-methylpropionic acid (**2-Br**, 0.612 g, 97%). ES-MS: $[M + H]^+$, m/z = 488/490; $[M + Na]^+$, m/z = 512/514. In MS–MS, the base peak was 105, corresponding to CH_3–$PhCH_2^+$ which confirms the location of the site of the bromination. ^{1}H-NMR (acetone/d_6) δ 1.59 (s, 6H, O–C(CH_3)$_2$), 1.94 (dt, 2H, J = 7.4 and 8 Hz, CH_2**CH_2**CH_2), 2.29 (s, 3H, $ArCH_3$), 2.50 (dd, 2H, J = 7.2 and 7.4 Hz, triazole–CH_2), 2.61 (dd, 2H, J = 7.4 and 7.7 Hz, ROPh–CH_2), 4.79 (s, 2H, $ArCH_2$), 6.94 (d, 1H, J= 8.3 Hz, 5′-ArOR), 7.10 (dd, 1H, J = 2 and 7.5 Hz, 6′-ArOR), 7.13 (d, 2H, J= 7.7 Hz, 3′, 5′-Ar–triazole), 7.21 (d, 2H, J= 7 Hz, 2′, 6′-Ar–triazole), 7.43 (d, 1H, J= 2 Hz, 3′-ArOR) and 10.44 (bs, 1H, NH).

HRMS (*QTOF*): Calculated for $C_{23}H_{26}BrN_3O_4$: 487.1107. Found: 487.1099.

Synthesis of 2-methyl-2-(4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]phenoxy)propionic-[2-phenoxy-^{2}H] acid, 2a–c

A three-necked flask was purged with dry nitrogen; 2-(2-bromo-4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]phenoxy)-2-methylpropionic acid (0.0313 g, 0.064 mmol), 10% Pd–C (0.032 g) and THF were added (3 mL). A deuterium-filled balloon was added and the reaction mixture was cooled in a dry ice/acetone bath. The flask was evacuated and re-filled with deuterium (this was repeated twice). The cooling bath was removed and the mixture was stirred over an atmosphere of deuterium. The progress of the reaction was followed by HPLC (*vide infra*) and after stirring for 2.75 h, Et_3N (45 μL, 0.323 mmol, 5 eq) was added and the mixture was stirred overnight. The mixture was filtered through Celite. The filter cake was washed with THF (3 × 1 mL) and the filtrate was concentrated *in vacuo*. The residue was re-dissolved in CH_3OH and concentrated (1 mL, repeated four times) to remove any exchangeable deuterium. The residue was partitioned between Et_2O and water (1:1); concentration of the Et_2O extract and crystallization from Et_2O yielded 2-methyl-2-(4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]phenoxy)propionic-[2-phenoxy-methylphenyl-^{2}H] (0.023 g, 87%) as a white solid which was 98% pure by HPLC. ES-MS: $[M + H]^+$, m/z = 411, 412, 413, 414; ^{1}H-NMR (acetone/d_6) δ 1.41 (s, 6H, O–C(CH_3)$_2$), 1.79 (dt, 2H, J= 7.7 Hz, CH_2**CH_2**CH_2), 2.15 (s, 1.86H, $ArCH_3$, diminished in size and broadened because of the H–D coupling), 2.35 (t, 2H, J= 7.45 Hz, triazole–CH_2), 2.45 (t, 2H, J= 7.42 Hz, ROPh–CH_2), 4.67 (s, 2H, $ArCH_2$), 6.68 (d, 1H, J= 8.9 Hz, 5′-ArOR), 6.94 (m, 1.17 H, 2′6′-ArOR), 6.99 (d, 2H, J= 8.02 Hz, 3′, 5′-Ar–triazole), 7.07 (d, 2H, J= 8.02 Hz, 2′, 6′-Ar–triazole) and 10.56 (bs, ^{1}H, NH).

Integration of the NMR showed about 83% incorporation in the aromatic ring and 38% exchange in the aromatic methyl group.

HPLC method: Zorbax SB-phenyl column (4.6 × 250 mm) with gradient elution at 2 mL/min (Solvent A = CH_3CN with 0.1% TFA; Solvent B=0.1% aqueous TFA) and UV detection at 230 nm. Gradient: 100% of solvent B to 100% of solvent A in 10 min; hold at 100% A for 5 min, then gradient to 100% B in 1 min.

Synthesis of 2-methyl-2-(4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]phenoxy)propionic-[2-phenoxy-^{3}H] acid, 3a–c

A mixture of 2-(2-bromo-4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]-phenoxy)-2-methylpropionic acid (0.0054 g, 0.011 mmol) and 10% Pd–C (0.0087 g) in DMF (1.5 mL) containing *i*-Pr_2NEt (0.05 mL) was stirred under tritium gas (5 Ci) for 2 h. The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was re-dissolved in EtOH and subsequently concentrated to remove the labile tritium (this was repeated twice).

DOI: 10.1002.jlcr

The residue was purified by reversed-phase HPLC on an Ultrasphere ODS column and gradient elution similar to the one used above. The pure 2-methyl-2-(4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl] propyl]-phenoxy)propionic-[2-phenoxy-methylphenyl-^{3}H] was concentrated *in vacuo* and re-dissolved in EtOH at 1 mCi/mL.

^{3}*H-NMR* (*CD_3OD*): δ 2.21 (s); 2.23 (d, J= 16.1 Hz) 2.26 (t, J= 15.9 Hz), 6.80 (s) (Figure 2). By comparing the ^{1}H-NMR of the tritiated material, there appeared to be 47% tritiation on the aromatic ring and 12% on the methyl group. The specific activity as determined by mass spectrometry was 22 Ci/mmol and the RCP was 99.7%, showing a single peak by HPLC on a Spherisorb Phenyl column (4.6 × 250 mm) with gradient elution at 1 mL/min; 0% B to 100% B over 2 min (Solvent A = 1% TFA in water; Solvent B = 1% TFA in CH_3CN). UV detection was at 230 nm. FAB-MS: $[M + H]^+$, $m/z = 410$, 412, 414.

Synthesis of 5-[3-[4-(2-hydroxy-1,1-dimethylethoxy) phenyl]2-(4-methylbenzyl)-2,4-dihydro[1,2,4]triazol-3-one, 4

A THF solution (25 mL) solution of 2-methyl-2-(4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]phenoxy)propionic acid (2.50 g, 6.11 mmol) was cooled in an ice bath under nitrogen and treated dropwise (over 10 min) with borane–THF complex (30 mL, 1 M in THF, 30 mmol). The resulting clear solution was stirred in the cold for 1.5 h and then allowed to warm to room temperature. After 90 h, the THF solution was slowly decanted into a stirred mixture of ice/water and then extracted with Et_2O (100 mL). The aqueous layer was re-extracted with Et_2O (3 × 50 mL). The combined Et_2O extracts were washed with water (2 × 100 mL) and then $NaHCO_3$ (2 × 50 mL). The Et_2O extract was dried (anhydrous $MgSO_4$) and concentrated *in vacuo* to yield 2.65 g of a yellow viscous oil. The oil was triturated with pentane, methyl *tert.* butyl ether to yield 5-[3-[4-(2-hydroxy-1, 1-dimethylethoxy)-phenyl]2-(4-methylbenzyl)-2,4-dihydro[1,2,4]triazol-3-one as a white gummy solid (2.40 g, 99.3%). HPLC (*vide supra*) showed that this material was slightly impure but free of starting material.

Synthesis of 5-[3-(4-hydroxyphenyl)propyl]-2-(4-methylbenzyl)-2,4-dihydro-[1,2,4]triazol-3-one, 5

A methylene chloride solution (25 mL) of 5-[3-[4-(2-hydroxy-1,1-dimethylethoxy)-phenyl]2-(4-methylbenzyl)-2,4-dihydro[1,2,4]triazol-3-one as a white gummy solid (2.40 g, 6.07 mmol) was cooled in an ice bath under nitrogen and stirred; boron tribromide (7 mL, 1 M in CH_2Cl_2, 7.0 mmol) was added dropwise over 15 min. The resulting mixture was stirred at 5°C for 0.5 h and then quenched by the dropwise addition of methanol (5 mL). After 5 min, the quenched mixture was poured into a mixture of ice and water and stirred for 0.5 h. The resulting white precipitate was collected by filtration, washed with water and then hexanes (3 × 25 mL). HPLC (*vide supra*) showed the material to be pure (R_T= 7.18 min). Air drying afforded 5-[3-(4-hydroxyphenyl)-propyl]-2-(4-methylbenzyl)-2,4-dihydro-[1,2,4] triazol-3-one as a white solid (1.70 g, 86.7%): ^{1}H-NMR (acetone/d_6) δ 1.90 (dt, 2H, J = 7.4 and 7.7 Hz, $CH_2\mathbf{CH_2}CH_2$), 2.29 (s, 3H, $ArCH_3$), 2.47 (dd, 2H, J = 7.0 and 8.1 Hz, triazole–CH_2), 2.56 (dd, 2H, J = 7.7 and 7.8 Hz, $ArCH_2$), 4.81 (s, 2H, $CH_3Ph\mathbf{CH_2}$), 6.74 (d, 2H, J= 8.4 Hz, 3′, 5′-HOAr), 7.01 (d, 2H, J= 8.3 Hz, 2′,6′-HOAr), 7.13 (d, 2H, J= 7.3 Hz, 3′, 5′-CH_3Ar), 7.21 (d, 2H, J= 8.5 Hz, 2′, 6′-CH_3Ar), 8.14 (s, ^{1}H, OH) and 10.47 ppm (bs, ^{1}H, NH); ES-MS: $[M + H]^+$, $m/z = 324$, $[M + Na]^+$, $m/z = 346$.

HRMS (*QTOF*): Calculated for $C_{19}H_{21}N_3O_2$: 323.1634. Found: 323.162.

Synthesis of 2-methyl-2-(4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]phenoxy) propionic-[2-methyl-2-propionyl-UL-^{13}C] acid, 2-^{13}C

Sodium hydroxide (0.866 g, 21.64 mmol, 11.5 eq) was ground in a mortar and pestle and added to a flask containing 5-[3-(4-hydroxyphenyl)-propyl]-2-(4-methylbenzyl)-2,4-dihydro-[1,2,4]triazol-3-one (0.605 g, 1.88 mmol). Acetone-[UL-^{13}C] (4.0 g, 65.5 mmol, 35 eq) was added and the resulting mixture was stirred at room temperature under a balloon of nitrogen for 20 min. A gummy precipitate was formed; the mixture was cooled to *ca.* −15°C and $CHCl_3$–[^{13}C] (1.08 g, 8.97 mmol, 4.8 eq) was added dropwise over 4 min. After stirring at −15°C for 2 h, the reaction mixture was allowed to warm to room temperature and stirring was continued overnight. The excess acetone–[UL-^{13}C] was distilled off; the residue was cooled in an ice bath and diluted with water (5 mL) and EtOAc (10 mL). The mixture was acidified to pH 1 with HCl (2 mL, 5 N) and the EtOAc layer was separated and filtered through anhydrous $MgSO_4$. The filtrate was concentrated *in vacuo*. HPLC of the residue showed the desired compound (36.5%) as well as several smaller peaks due to impurities. The residue was dissolved in CH_3OH and divided into three 1.5 mL aliquots. Each was purified by preparative HPLC (TAI Gel-ODS column) eluting with CH_3CN/H_2O (each containing 0.01% TFA, 47:53) at 8 mL/min. The combined eluate containing the desired product was concentrated *in vacuo* and extracted with EtOAc (4 × 25 mL). The combined extracts were dried (anhy-

DOI: 10.1002.jlcr

drous $MgSO_4$) and concentrated *in vacuo*. The residue was crystallized from Et_2O to yield 2-methyl-2-(4-[3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl]propyl]-phenoxy)propionic-[2-methyl-2-propionyl-UL-^{13}C] acid (0.387 g, 50%) as a white solid which was >97.5% pure by HPLC ($R_T = 7.69$, *vide infra*). ES-MS: $[M+H]^+$, $m/z = 414$ (judged to be >95% $^{13}C_4$ and 5% $^{13}C_3$); ^{1}H-NMR (acetone/d_6) δ 1.41 (ddd, 6H, $J_{13C-H} = 128.7$, 4.1, 3.9 Hz, $^{13}C(^{13}CH_3)_2$), 1.92 (dt, 2H, $J = 7.7$ and 7.4 Hz, $CH_2\mathbf{CH_2}CH_2$), 2.16 (s, 3H, $ArCH_3$), 2.48 (t, 2H, $J = 7.5$ Hz, triazole–CH_2), 2.59 (t, 2H, $J = 7.5$ Hz, $ArCH_2$), 4.78 (s, 2H, $CH_3Ph\mathbf{CH_2}$), 6.82 (d, 2H, $J = 8.3$ Hz, 3′, 5′-HOAr), 7.08 (d, 2H, $J = 8.3$ Hz, 2′,6′-HOAr), 7.13 (d, 2H, $J = 7.7$ Hz, 3′, 5′-CH_3Ar), 7.21 (d, 2H, $J = 7.7$ Hz, 2′, 6′-CH_3Ar),10.38 (bs, ^{1}H, NH) and 11.2 ppm (bs, ^{1}H, CO_2H); ^{13}C-NMR (acetone/d_6) δ 24.7 (d, $^{13}CH_3$), 78.54 (m, $^{13}\mathbf{C}(^{13}CH_3)_2$) and 174.71 (d, $^{13}CO_2H$).

HRMS (*QTOF*): Calculated for $C^{13}_{19}C_4H_{27}N_3O_4$: 413.4393. Found: 413.2136.

Synthesis of 4-(4-methoxyphenyl)butyric acid-[^{14}C], 8

To a colorless, clear solution of mesylate (734 mg, 3 mmol, prepared from corresponding alcohol **6**) in anhydrous DMF (10 mL) was added KCN–[^{14}C] (100 mCi from ARC, 55 mCi/mmol), KCN (100 mg), and the resulting heterogeneous solution was heated in an oil bath (about 90°C). The oil temperature continued to go up to about 95°C. To avoid overheating, the flask was removed from the oil bath, and was replaced when the oil temperature dropped to 80°C. The solution was stirred at that temperature overnight (about 27 h), then cooled to room temperature, and stirred overnight. To work up the reaction, the solution was taken into a separatory funnel containing water (75 mL) and ethyl acetate (50 mL). The layers were separated. The organic layer was washed with water (50 mL), and concentrated with ethanol (20 mL) under reduced pressure to give a crude product **7** (about 550 mg as a light yellowish liquid). Without further purification, the crude product was taken into ethanol (10 mL). To the solution was added 1 N NaOH (10 mL), followed by 30% H_2O_2 (0.5 mL) at room temperature with stirring. The solution was stirred at 40–50°C (oil bath) for 4.5 h, then refluxed overnight (about 21.5 h). After cooling to room temperature, the solution was diluted with water, and then concentrated *in vacuo* to remove most of ethanol. The remaining aqueous solution was extracted with ethyl ether (2 × 10 mL) to remove any neutral species, adjusted to about pH 2, and extracted with ethyl acetate (2 × 10 mL). The combined ethyl acetate extracts were washed with water (2 × 10 mL), and concentrated *in vacuo* with absolute EtOH (20 mL). The residue was further dried *in vacuo* overnight to give the desired acid **8** as beige solid (440 mg, 2.26 mmol, 97.8% chemical purity by HPLC). The crude product **8** was used in the next step without further purification.

HPLC conditions: (Zorbax SB-Phenyl, 4.6 × 250 mm). UV at 220 nm, temperature at rt, flow rate at 1 mL/min. Solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in ACN. Gradient: A/B = 70:30 to A/B = 50:50 in 15 min, then maintain at 50:50 for 10 min. Product is at R_t = 11.27 min.

^{1}H-NMR of mesylate ($CDCl_3$): 7.10 (d, 2H), 6.84 (d, 2H), 4.10 (t, 2H), 3.79 (s, 3H), 3.00 (s, 3H), 2.67 (t, 2H), 2.05 (tt, 2H). MS: $[M+NH_4]^+$=262.2. ^{1}H-NMR of unlabeled acid **8** ($CDCl_3$): 7.08 (d, 2H), 6.82 (d, 2H), 3.89 (s, 3H), 2.60 (t, 2H), 2.35 (t, 2H), 1.94 (tt, 2H). MS: $[M+NH_4]^+ = 212.2$.

Synthesis of 4-[4-(1-ethoxycarbonyl-1-methylethoxy) phenyl]-butyric acid-[^{14}C], 10

The crude acid **8** (440 mg, 2.26 mmol) was mixed with pyridinium HCl salt (1.2 g) in absolute EtOH (15 mL) to give a colorless, clear solution. The solution was concentrated *in vacuo* to remove most of the ethanol to give a residue as a wet solid containing residual ethanol. The flask containing the wet solid was heated in an oil bath (about 100°C) with stirring to melt the solid to give a colorless clear solution. The resulting solution was then heated at 190–200°C (oil temperature) overnight (about 21 h) under an argon atmosphere. After cooling to room temperature, the crude product was stirred with MTBE (20 mL) and 1 N HCl (20 mL). The layers were separated. The aqueous layer was extracted with MTBE (2 × 10 mL), and the combined MTBE extracts were washed once with 1 N HCl (20 mL) and with water (2 × 20 mL). The solution was concentrated *in vacuo*. The resulting residue was taken into absolute EtOH (20 mL) and re-concentrated *in vacuo*. The residue was further dried *in vacuo* overnight to give a crude product **9** as a beige solid (392 mg, 2.15 mmol). Without further purification, the crude compound **9** (390 mg) was dissolved in anhydrous EtOH (12 mL) containing anhydrous EtOAc (0.2 mL) to give a clear solution. To the solution was added a solution of 21% sodium ethoxide in EtOH (2 mL), and the resulting solution was heated to reflux for 70 min. To the refluxing solution was added ethyl bromo-*iso*-butyrate (1 mL, 3 eq) via a syringe, and 0.6 mL more of it was added after refluxing for about 3.75 h. The resulting solution was then refluxed overnight (about 15 h). The solution was cooled in an ice bath, acidified with conc. H_3PO_4 (1 mL) in water (10 mL), and concentrated

DOI: 10.1002.jlcr

in vacuo to remove most of ethanol. To the remaining aqueous solution containing an oily product was added Na_2CO_3 solution (3.3 g in 30 mL of water). Some white salt precipitated. Water was added to dissolve the salt, and the resulting clear aqueous solution was extracted with ethyl ether (3×20 mL) to remove any neutral species. The aqueous layer was adjusted to about pH 2–3 with concentrated H_3PO_4 (about 3.6 mL) in the presence of ethyl acetate (30 mL). The layers were separated, and the aqueous layer was extracted once with ethyl acetate (20 mL). The combined ethyl acetate solution was washed with water (2×30 mL), and concentrated *in vacuo* with ethanol (30 mL). The residue was dissolved in a minimum amount of CH_2Cl_2, and purified by Biotage (SiO_2, 100:10:1=CH_2Cl_2:EtOAc:HOAc). The desired fractions were pooled, concentrated *in vacuo* with ethanol (10 mL) and toluene (10 mL) in sequence. The desired product **10** was obtained as viscous oil initially; further drying *in vacuo* gave a white crystalline solid (511 mg, 1.72 mmol, 80%). For cold **9**: ^{1}H-NMR (acetone-d_6): 7.04 (d, 2H), 6.75 (d, 2H), 2.56 (t, 2H), 2.28 (t, 2H), 1.83 (tt, 2H). MS: $[M + NH_4]^+ = 198.2$. For cold **10**: ^{1}H-NMR ($CDCl_3$): 7.02 (d, 2H), 6.77 (d, 2H), 4.23 (q, 2H), 2.60(t, 2H), 2.37 (t, 2H), 1.93 (tt, 2H), 1.56 (s, 6H), 1.27 (t, 3H). MS $(M + NH_4)^+ = 312.3$.

Synthesis of ethyl 2-[4-[3-[2,5-dihydro-1-[(4-methylphenyl)-methyl]-5-oxo-1*H*-1,2,4-triazol-3-yl]propyl]-phenoxy]-2-methylpropanoate-[^{14}C], 14

To a clear solution of the acid **10** (510 mg, 1.72 mmol) in anhydrous EtOAc (4.5 mL) was added three drops of anhydrous DMF, followed by adding of oxalyl chloride (175 μL, 1.15 eq) dropwise during a period of 36 min while the solution was cooled in an ice bath. The resulting solution was stirred for another 30 min in a water bath to generate the corresponding acyl chloride **11**. Meanwhile, in another flask, compound **12** (0.5 g, 1 eq) was stirred with anhydrous pyridine (0.6 mL) in anhydrous EtOAc (10 mL) as a heterogeneous solution for about 60 min. The above acyl chloride solution of **11** was concentrated *in vacuo* to remove the solvent and the excess oxalyl chloride. The residue was re-dissolved in anhydrous EtOAc (4.5 mL). Some solid was insoluble. The supernatant was taken out via syringe and added dropwise to the heterogeneous solution containing **12** during a period of 30 min while the reaction was cooled in an ice bath. After the addition, the resulting solution was stirred at room temperature for about 3 h. Most of the starting material (acyl chloride of **10**) was consumed, and a new compound, presumably the intermediate **13**, was generated as detected by HPLC. To the reaction solution was added anhydrous EtOAc (10 mL), followed by camphorsulfonic acid (2.32 g). The resulting solution was refluxed overnight (about 16 h), then cooled and kept at room temperature for another 24 h. The solution was then stirred with 1 N HCl (10 mL). The organic layer was separated, washed with 1 N HCl (2×25 mL), sat. Na_2CO_3 (3×30 mL), water (2×20 mL), and concentrated with ethanol (20 mL) *in vacuo* to give a crude product as viscous oil (0.75 g). The crude product was purified by Biotage (SiO_2, 80:20:1 = CH_2Cl_2:EtOAc:HOAc) to give the desired product **14** as a viscous oil (0.65 g, 1.48 mmol, 86%). For cold **14**: ^{1}H NMR (CD_3OD): 7.14 (AB q, 4 H), 7.02 (d, 2H), 6.74 (d, 2H), 4.81 (s, 2H), 4.20 (q, 2H), 2.57 (t, 2H), 2.46 (t, 2H), 2.30 (s, 3H), 1.90 (dd, 2H), 1.52 (s, 6H), 1.27 (q, 3H). MS $[M + H]^+ = 438.3$.

Synthesis of 2-[4-[3-[2,5-dihydro-1-[(4-methylphenyl)-methyl]-5-oxo-1*H*-1,2,4-triazol-3-yl]propyl]phenoxy]-2-methylpropanoic acid-[^{14}C], 2-[^{14}C]

The compound **14** (310 mg, 0.7 mmol) was taken into toluene (10 mL), concentrated *in vacuo*, and re-dissolved in toluene (3 mL). To the solution was added 1 N NaOH (1.4 mL) and water (1.7 mL), and the resulting heterogeneous solution was stirred at room temperature overnight (about 17 h). A small amount of **14** remained as monitored by TLC (SiO_2, CH_2Cl_2:EtOAc:HOAc = 80:25:1). About 0.5 mL of 1 N NaOH was added to the solution, and the reaction was stirred for another 3.5 h. The solution was then diluted with water (15 mL), and the aqueous layer was extracted with toluene (3×15 mL) to remove any neutral species. The aqueous solution was then adjusted to about pH 2 with conc. HCl, and extracted with ethyl acetate (20 mL). The ethyl acetate extract was washed with water (3×20 mL), and concentrated *in vacuo* with ethanol (20 mL) to give a viscous residue. The residue was re-dissolved in ethyl acetate (10 mL), and any insoluble particles were filtered off. The filtrate was concentrated and dried *in vacuo* again to give viscous oil (220 mg). The viscous oil was dissolved in 1 mL of ethyl acetate, seeded with cold **2**, and stirred at room temperature until some white solid formed. The solution was stored in refrigerator over the weekend. After stirring in an ice bath for about 40 min, the white solid was filtered, rinsed with cold ethyl acetate (3 mL), and dried *in vacuo* at room temperature. The desired product **2-[^{14}C]** was obtained as a white solid (137 mg, 0.334 mmol) at 64.3 μCi/mg with 99.3% RCP by HPLC. For cold **2**: ^{1}H-NMR (CD_3OD): 7.15 (AB q, 4H), 7.03 (d, 2H), 6.80 (d, 2H), 4.80 (s, 2H), 2.57 (t, 2H), 2.46 (t, 2H), 2.30 (s, 3H), 1.90 (dd, 2H), 1.52 (s, 6H). MS: $[M + H]^+ = 410.2$.

DOI: 10.1002.jlcr

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