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Research Article

Synthesis of ^{14}C-labeled and tritiated AMPA potentiator LY450108

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Summary

Asymmetric synthesis of AMPA potentiator LY450108-[^{14}C] containing ^{14}C-label attached to the chiral center of the molecule, was accomplished based on Evans' chiral oxazolidinone auxiliary method. Diastereoselective methylation of *p*-nitrophenylacetic acid derivative was used as a key step. The auxiliary was reductively removed, and the resulting primary alcohol was converted into the corresponding amine. Its sulfonylation, reduction of the aromatic nitro group, and acylation with 3,5-difluorobenzoyl chloride led to the final product. The synthesis of tritiated LY450108 is also detailed. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: AMPA potentiator; LY450108; asymmetric synthesis; carbon-14 labeled

Introduction

Glutamic acid is the major excitatory neurotransmitter in the central nervous system. It interacts with multiple subtypes of excitatory amino acid receptors like 2-amino-3-(5-methyl-3-hydroxyisoxazol-4-yl)propanoic acid (AMPA) receptors. Signals are transformed at AMPA receptors through conductance of sodium and calcium ions into cells upon activation by glutamic acid. AMPA potentiators are compounds that enhance ion influx through AMPA receptors. These compounds are important for learning and memory processes and could be used in the development of pharmacological agents for the treatment of cognitive disorders including Alzheimer's disease and schizophrenia.[1] In the process of conducting non-clinical ADME studies, tritium and C-14 labeled LY450108[2] were required.

We report herein the asymmetric synthesis of this compound, which allowed the introduction of the ^{14}C label in a metabolically stable position, next to the

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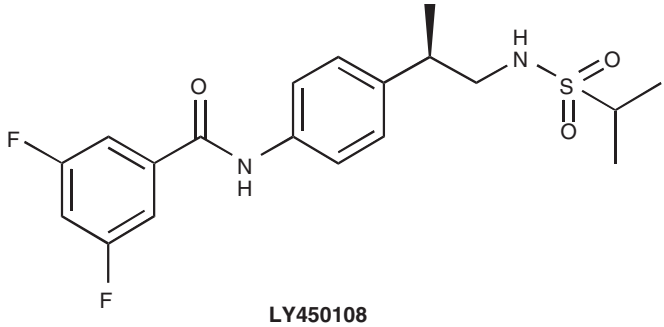
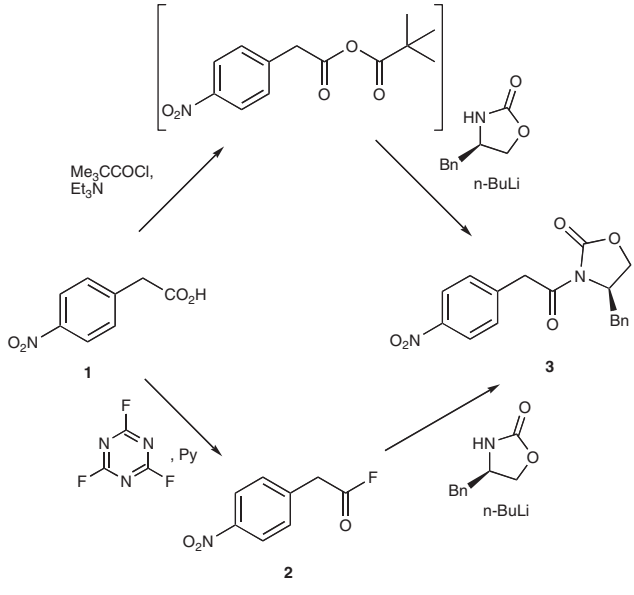
chiral center. In addition, we report on the iridium catalyzed tritiation of LY450108 using Crabtree's catalyst.



LY450108

Results and discussion

In order to generate the chiral fragment of the molecule we decided to use Evans' chiral oxazolidinone auxiliary approach.[3] In the first step (Scheme 1), acylation of the lithium derivative of (*R*)-(+)-4-benzyl-2-oxazolidinone with a mixed anhydride generated *in situ* from *p*-nitrophenylacetic acid (**1**) and pivaloyl chloride, resulted in less than 20% yield of the desired amide **3** both under standard[4] and recently modified[5] conditions. Preparation of the acyl chloride from the acid **1** and oxalyl chloride was even less efficient. The best reproducible result was obtained when the acid **1** was initially converted to *p*-nitrophenylacetyl fluoride (**2**) using cyanuric fluoride.[6]



Scheme 1.

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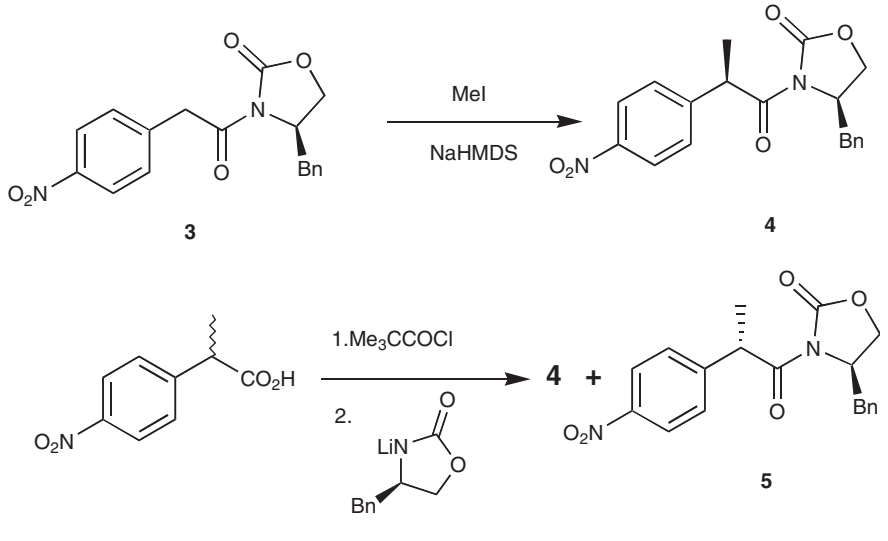
The key step was the alkylation of enolate derived from amide **3** with methyl iodide (Scheme 2). The best experimental conditions in our hands consisted of the addition of sodium hexamethyldisilazide to a mixture of equimolar amounts of **3** and methyl iodide. According to HPLC and NMR data only one stereoisomer **4** was formed, which was evident after a mixture of both isomers **4** and **5** was prepared in a separate experiment from the racemic 2-(4-nitrophenyl)-propionic acid. The diastereomers **4** and **5** appeared to be separable on silica gel, and one of them (less polar) corresponded to the above compound **4**, the absolute (*R*)-stereochemistry of which was proven by the comparison of optical rotations of the final product **LY450108** and authentic sample of this compound.

In an attempt to increase the yield in the asymmetric alkylation step we decided to replace the nitro group of **3** with dibenzylamino group in order to reduce the possibility of decomposition of *N*-acylated oxazolidinone through ketene formation under basic conditions.[5] The target substrate **9** was prepared as shown on Scheme 3 by the *bis*-benzylation of the 4-aminophenylacetic acid (**6**), subsequent hydrolysis of ester **7**, and acylation of the lithium derivative of (*R*)-(+)-4-benzyl-2-oxazolidinone with the acid chloride prepared *in situ* from **8** and oxalyl chloride.

The dibenzyl derivative **9** unexpectedly appeared to be extremely unstable under the deprotonation conditions in the presence of sodium hexamethyldisilazide. After the addition of methyl iodide, neither starting material nor desired alkylation product were found in the complex reaction mixture. Therefore the nitro compound **4** was used in the preparation of the target molecule **LY450108**.



Scheme 2.

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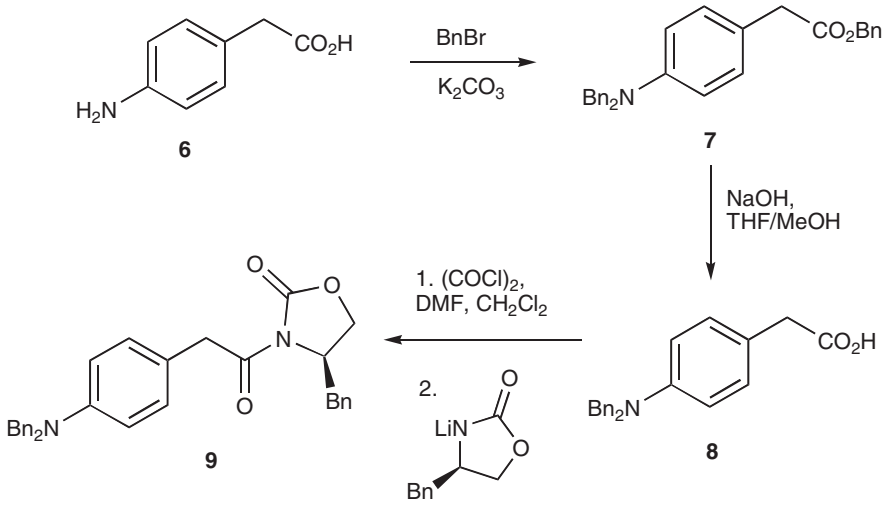

Scheme 3.

After an unsuccessful attempt of direct transamination of the oxazolidinone **4** with ammonium chloride and trimethylaluminum,[7] the reductive removal of the chiral auxiliary was accomplished using sodium borohydride[8] to give the primary alcohol **10** in a good yield. Conversion of **10** to the corresponding primary amine **13** was performed in two alternative ways. Firstly initial formation of the azide **11** using Mitsunobu reaction with hydrazoic acid[9] or diphenylphosphoryl azide (DPPA),[10] and subsequent chemoselective reduction of **11** with triphenylphosphine[11] or sodium borohydride in the presence of copper (II) sulfate.[12] The second, and more convenient approach, consisted of the reaction of the alcohol **10** with phthalimide under Mitsunobu conditions followed by hydrazinolysis[9] of the *N*-substituted imide **12** (Scheme 4).

Conversion of amine **13** into the final product **LY450108** was accomplished in three straightforward steps: sulfonylation with isopropylsulfonyl chloride, catalytic hydrogenation of nitro compound **14**, and acylation of aniline **15** with 3,5-difluorobenzoyl chloride (Scheme 5). The optical rotation of the compound thus obtained was exactly the same as the rotation of an authentic sample of **LY450108**, which was prepared by resolution of a β-methylphenethylamine salt, and subsequent nitration of the aromatic ring.

The synthesis of radiolabeled product **LY450108-[^{14}C]** (Scheme 6) was accomplished according to the sequence discussed above. Thus, diastereoselective alkylation of *N*-acyloxazolidinone **3** with methyl iodide-[^{14}C] smoothly gave methylated product **4a** with modest but expected yield. Sodium borohydride reduction of **4a** afforded the primary alcohol **10a**, which was converted by Mitzunobu reaction into the substituted phthalimide **12a**. Primary amine **13a** was obtained after treatment of **12a** with hydrazine.

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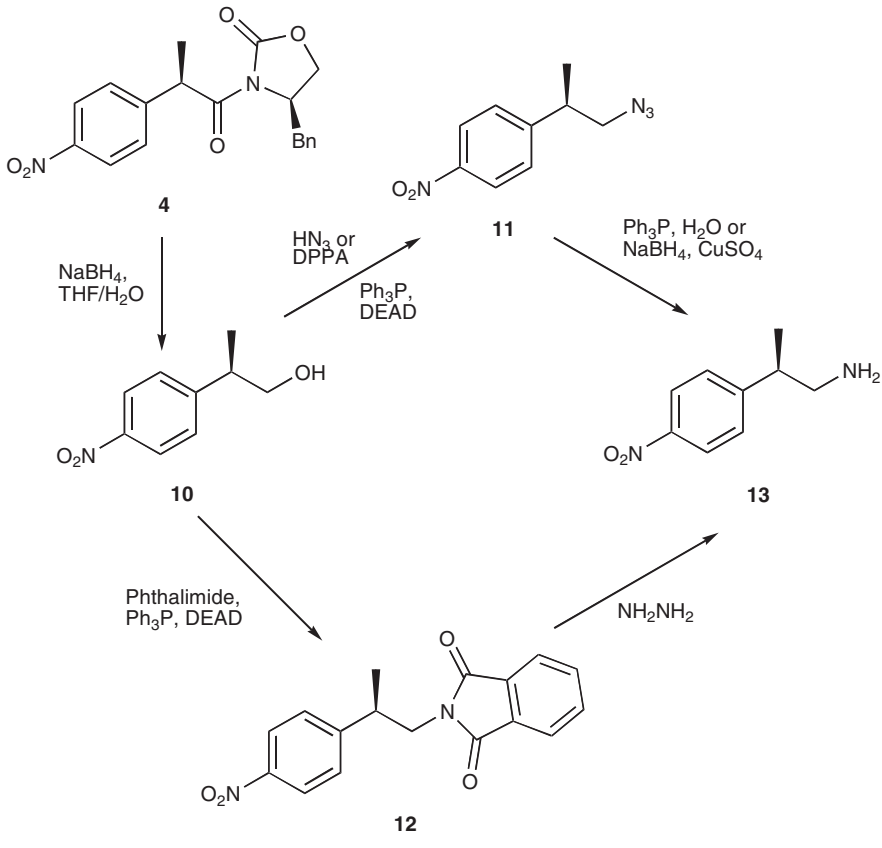

Scheme 4.

Reaction of **13a** with isopropylsulfonyl chloride led to sulfonamide **14a**, which was subjected to catalytic hydrogenation to give arylamine **15a**. Finally, acylation of **15a** with 3,5-difluorobenzoyl chloride afforded the target compound **LY450108-[^{14}C]**.

The final product **LY450108-[^{14}C]** thus obtained had the radiochemical purity of 99.4% and optical purity 99.8% (according to chiral HPLC). Its overall radiochemical yield was 17%, which exactly corresponds to the total chemical yield from methyl iodide-[^{14}C].

LY450108-[^{3}H] was prepared by tritium exchange labeling on **LY450108** in the presence of $[(cod)Ir(py)(Pcy_3)]PF_6$ (Crabtree's catalyst,[13] Scheme 7).[†] The **LY450108-[^{3}H]** had a specific activity of 96 Ci/mmol and a radiochemical purity of 98.8% as determined by HPLC. Analysis of the product by ^{3}H-NMR

[†]The tritiation of LY450108 was conducted at Amersham Biosciences, Cardiff, Wales, UK using methodology provided by the authors.

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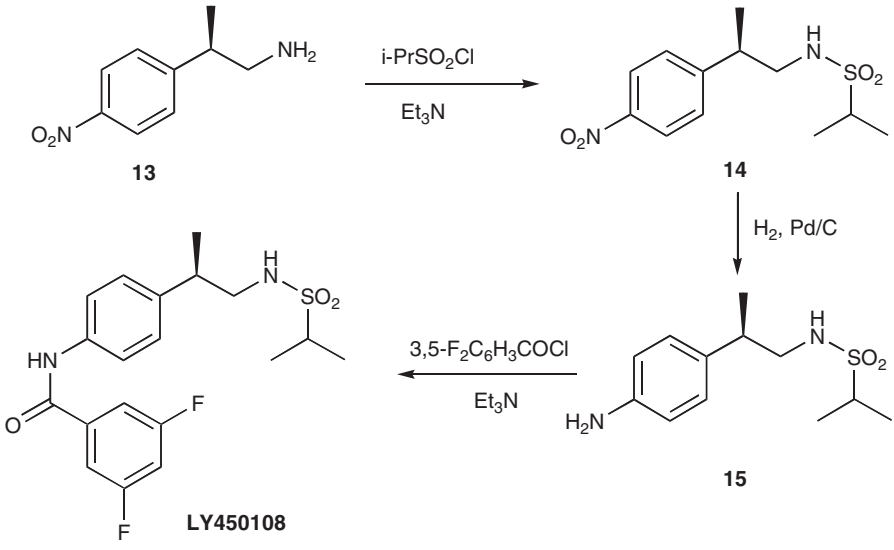

Scheme 5.

(in $CDCl_3$) showed resonances at δ 7.50 and 7.75 ppm indicating tritia in the 3,5, 2′ and 6′ positions.

Experimental

The methyl iodide-[^{14}C] was purchased from Amersham Pharmacia Biotech. The Crabtree's catalyst was obtained from Strem Chemicals. The NMR spectra were obtained in $CDCl_3$ on a Varian Mercury-400 at 400 (^{1}H) and 100 (^{13}C) MHz. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Optical rotations, IR, MS, and microanalytical data were provided by the Physical Chemistry Department of the Lilly Research Laboratories. Flash chromatography was performed on silica gel 60 (230–400 mesh). TLC was conducted on precoated plates of silica gel 60 F_{254}. All ^{14}C-compounds were identified by TLC comparison with the corresponding non-radiolabeled isotopomers. The radiochemical purity of the final material **LY450108-[^{14}C]** was determined by radio-HPLC: Zorbax SB-phenyl, 150 × 4.6 mm; mobile phase: 0.05% aqueous trifluoroacetic acid/acetonitrile 3:2; flow rate: 1 ml/min; UV detector wavelength: 225 nm.Its optical purity was determined by chiral radio-HPLC: Chiracel OD-H 250 × 4.6 mm; mobile phase: ethanol/hexane 1:9; flow rate 1 ml/min; UV detector wavelength: 275 nm; column temperature 40 °C. The radiochemical purity of LY450108-[^{3}H] was determined by radio-HPLC: Spherosorb Phenyl (5μ, 4.6 × 250 mm) with gradient elution at 1.0 ml/min (Solvent A = 0.1% TFA/water, Solvent B = 0.1% TFA/CH_3CN) from 20% B to 100% B over 30 min and simultaneous radiochemical and UV (at 225 nm) detection.

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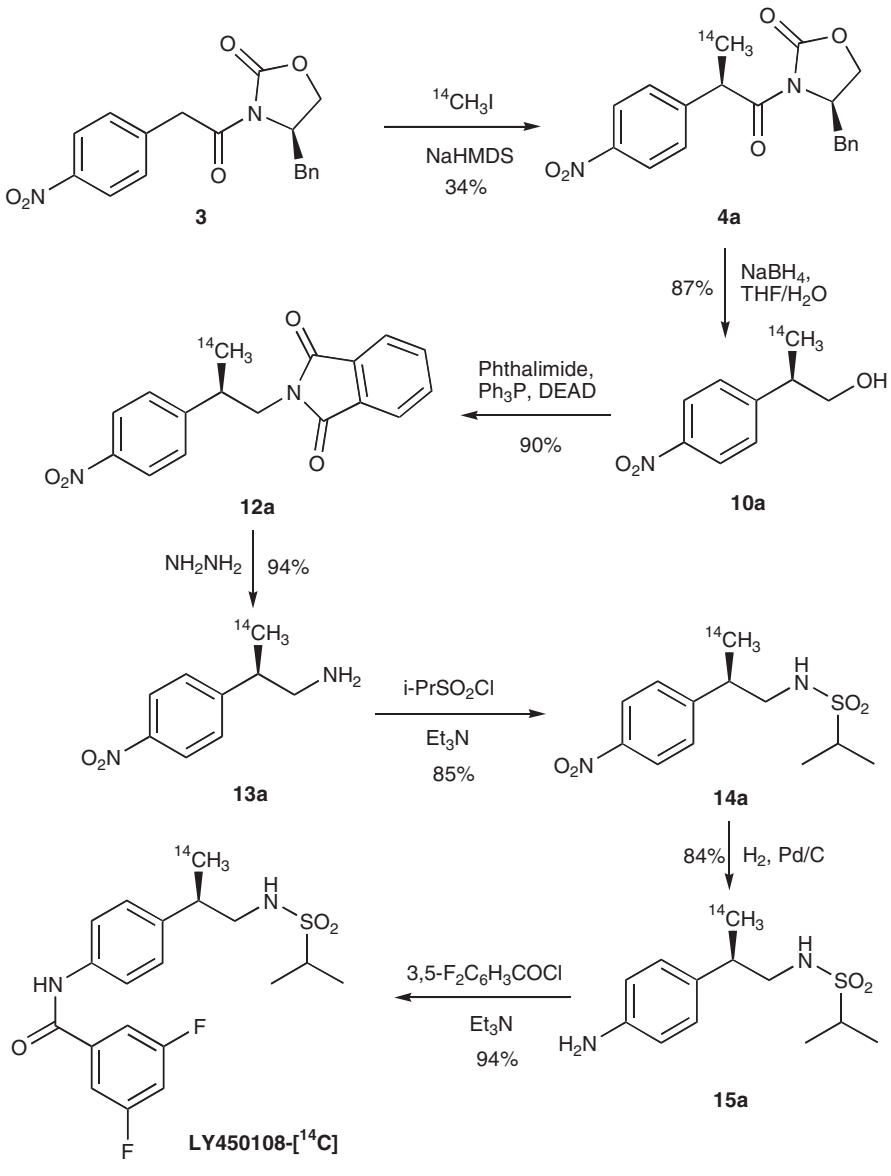

Scheme 6.

4-Nitrophenylacetyl fluoride, **2**

To a solution of **1** (4.29 g, 23.7 mmol) and pyridine (2.6 ml, 32 mmol) in acetonitrile (40 ml) was added cyanuric fluoride (2.0 ml, 23.7 mmol) dropwise. The reaction mixture was stirred at room temperature for 3.5 h, and evaporated under vacuum. The residual solid was triturated with ethyl ether (50–60 ml) and filtered. The filtrate was washed with ice water ($\sim$5 ml), dried over magnesium sulfate, and evaporated under vacuum to give **2** (4.183 g,

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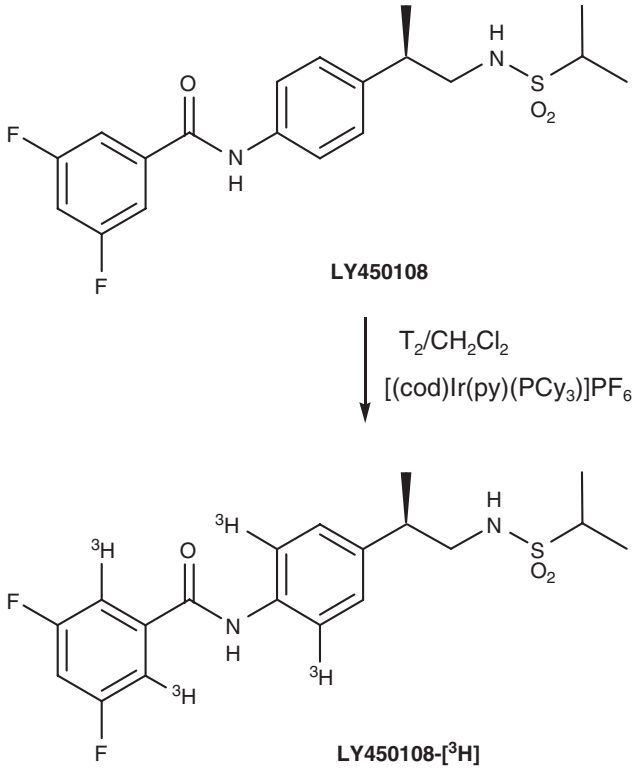

Scheme 7.

96%) as a light yellow solid. ^{1}H-NMR (δ, ppm): 3.95 (s, 2 H), 7.49 (d, $J = 8.3$ Hz, 2 H), 8.25 (d, $J = 8.3$ Hz, 2 H).

4R-Benzyl-3-(4-nitrophenylacetyl)-2-oxazolidinone, **3**

To a solution of (*R*)-(+)-4-benzyl-2-oxazolidinone (1.88 g, 10.61 mmol) and triphenylmethane (~20 mg) in tetrahydrofuran (22 ml) at −78°C was added *n*-butyllithium (1.6 M in hexane) until yellowish color persisted (*ca.* 7 ml). After 10 minutes a solution of **2** (1.94 g, 10.59 mmol) in tetrahydrofuran (8 ml) was added through cannula. The reaction mixture was stirred at −78 °C for 1 h, then diluted with saturated aqueous sodium bisulfate (10–13 ml), and extracted with ethyl acetate (*ca.* 50 ml). The extract was washed with brine (2 × 5 ml), dried over sodium sulfate, and evaporated under vacuum. Flash chromatography of the residue (column 5 × 27 cm, eluting with ethyl acetate/hexanes, 2:3, *ca.* 2000 ml) gave **3** (1.233 g, 34%) as a yellow solid, $R_f = 0.39$ (ethyl acetate/hexanes, 2:3). ^{1}H-NMR (δ, ppm): 2.77 (dd, $J = 13.2$ and 9.3 Hz, 1 H), 3.28 (dd, $J = 13.2$ and 3.4 Hz, 1 H), 4.20–4.27 (m, 2 H), 4.40 (AB dd, $J = 35.7$ and 15.6 Hz, 2 H), 4.69 (m, 1 H), 7.15 (d, $J = 7.8$ Hz, 2 H), 7.26–7.33

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(m, 3 H), 7.50 (d, $J = 8.3$ Hz, 2 H), 8.22 (d, $J = 8.3$ Hz, 2 H). IR (KBr, ν, cm^{-1}): 744, 1196, 1347, 1384, 1520, 1695, 1788. UV (EtOH, λ, nm): 268 (ε 9784). MS (ESI, %): 340 (M^+, 20), 339 (1 0 0), 194 (13), 162 (42). Analytically Calculated for $C_{18}H_{16}N_2O_5$: C, 63.53; H, 4.74; N, 8.23; Found: C, 63.71; H, 4.72; N, 8.01.

4R-Benzyl-3-(2R-(4-nitrophenyl)propionyl)-2-oxazolidinone, **4**

To a solution of **3** (681 mg, 2.0 mmol) in tetrahydrofuran (12 ml) was added a solution of methyl iodide (283 mg, 1.99 mmol) in toluene (2 ml). The mixture was cooled to −78 °C and a solution of sodium *bis*(trimethylsilyl)amide (1 M in tetrahydrofuran, 2.0 ml, 2.0 mmol) was added dropwise. The resultant dark red mixture was stirred for 15 min at *ca.* −70 °C and allowed to reach room temperature. After 3.5 h the reaction mixture was diluted with saturated aqueous sodium bisulfate (*ca.* 10 ml), and extracted with ethyl acetate (*ca.* 50 ml). The extract was washed with brine (2 × 10 ml), dried over sodium sulfate, and evaporated under vacuum. Flash chromatography of the residue (column 3 × 30 cm, eluting with ethyl acetate/hexanes, 3:7, *ca.* 500 ml) gave **4** (230 mg, 33%) as a light yellow solid, $R_f = 0.49$ (ethyl acetate/hexanes, 3:7). ^{1}H-NMR (δ, ppm): 1.57 (d, $J = 6.8$ Hz, 3H), 2.81 (dd, $J = 13.2$ and 9.3 Hz, 1H), 3.34 (dd, $J = 13.2$ and 3.4 Hz, 1H), 4.09–4.17 (m, 2H), 4.62 (m, 1H), 5.22 (q, $J = 6.8$ Hz, 1H), 7.21 (d, $J = 7.8$ Hz, 2H), 7.29–7.37 (m, 3H), 7.54 (d, $J = 8.3$ Hz, 2H), 8.18 (d, $J = 8.3$ Hz, 2H).

4R-Benzyl-3-(2R-(4-nitrophenyl)propionyl-3-[^{14}C])-2-oxazolidinone, **4a**

In the same manner as described above, starting from **2** (590 mg, 1.734 mmol) in tetrahydrofuran (10 ml), methyl iodide-[^{14}C] (100 mCi, 58 mCi/mmol, 1.724 mmol) in toluene (2 ml), and sodium *bis*(trimethylsilyl)amide (1 M in tetrahydrofuran, 1.73 ml, 1.73 mmol), **4a** (195 mg, 32%) was obtained.

The repetition of the same experimental procedure with the same amounts of the reagents gave the second lot of **4a** (209 mg, 34%). The two lots were combined and repurified by flash chromatography (column 3 × 30 cm, eluting with ethyl acetate/hexanes, 25:75, *ca.* 500 ml) to obtain **4a** (400 mg), which was used in the next step.

*4R-Benzyl-3-(2R-(4-nitrophenyl)propionyl)-2-oxazolidinone,***4***, and 4R-benzyl-1-(2S-(4-nitrophenyl)propionyl)-2-oxazolidinone,* **5**

To a solution of 2-(4-nitrophenyl)propionic acid (390 mg, 2.0 mmol) and triethylamine (335 µl, 2.4 mmol) in tetrahydrofuran (30 ml) at −78 °C was added trimethylacetyl chloride (280 µl, 2.27 mmol) dropwise. After 10 mins the reaction mixture was allowed to reach 0–5 °C and cooled back to −78 °C. In a separate flask, to a solution of (*R*)-(+)-4-benzyl-2-oxazolidinone (354 mg,

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2.0 mmol) and triphenylmethane (~10 mg) in tetrahydrofuran (8 ml) at −78 °C was added *n*-butyllithium (1.6 M in hexane) until yellowish color persisted (*ca.* 1.3 ml). After 10 mins the resulting solution was transferred through a cannula into the suspension of mixed anhydride prepared as described above. The reaction mixture was allowed to reach *ca.* −20 °C over the period of 30 minutes, then diluted with saturated aqueous sodium bisulfate (*ca.* 10 ml), and extracted with ethyl acetate (*ca.* 50 ml). The extract was washed with brine (2 × 10 ml), dried over sodium sulfate, and evaporated under vacuum. Flash chromatography of the residue (column 3 × 30 cm, eluting with ethyl acetate/hexanes, 3:7, *ca.* 500 ml followed by ethyl acetate/hexanes, 2:3, *ca.* 500 ml) gave **4** (169 mg, 24%), $R_f = 0.49$ (ethyl acetate/hexanes, 3:7), and **5** (224 mg, 32%), $R_f = 0.34$ (ethyl acetate/hexanes, 3:7). For **4**: ^{1}H-NMR is the same as described above. For **5**: ^{1}H-NMR (δ, ppm): 1.55 (d, $J = 6.8$ Hz, 3H), 2.59 (dd, $J = 13.2$ and 9.3 Hz, 1H), 3.14 (dd, $J = 13.2$ and 3.4 Hz, 1H), 4.14 (m, 1H), 4.23 (m, 1H), 4.74 (m, 1H), 5.19 (q, $J = 6.8$ Hz, 1H), 7.02 (m, 2H), 7.21–7.26 (m, 3H), 7.59 (d, $J = 8.8$ Hz, 2H), 8.21 (d, $J = 8.8$ Hz, 2H).

Benzyl 4-(dibenzylamino)phenylacetate, **7**

To a mixture of 4-aminophenylacetic acid (**6**) (1.51 g, 10.0 mmol) and potassium carbonate (14.0 g, 101 mmol) in dimethylformamide (30 ml) was added benzyl bromide (4.0 ml, 33.6 mmol). The reaction mixture was stirred for 16 h at room temperature, then diluted with water (20 ml), and extracted with ethyl acetate (*ca.* 50 ml). The extract was washed with brine (2 × 10 ml), dried over sodium sulfate, and evaporated under vacuum. Flash chromatography of the residue (column 3 × 30 cm, eluting with ethyl acetate/hexanes, 1:9, *ca.* 500 ml) gave **7** (3.772 g, 88%) as a yellow solid, $R_f = 0.42$ (ethyl acetate/hexanes, 1:9). ^{1}H-NMR (δ, ppm): 3.54 (s, 2H), 4.64 (s, 4H), 5.11 (s, 2H), 6.69 (d, $J = 8.6$ Hz, 2H), 7.08 (d, $J = 8.6$ Hz, 2H), 7.24–7.34 (m, 15H). IR (KBr, ν, cm^{-1}): 1152, 1453, 1495, 1522, 1617, 1730. UV (EtOH, λ, nm): 261 (ε 25422). MS (ESI, %): 422 (M^+, 100). Analytically Calculated for $C_{29}H_{27}NO_2$: C, 82.63; H, 6.46; N, 3.32; Found: C, 82.76; H, 6.50; N, 3.38.

4-(Dibenzylamino)phenylacetic acid, **8**

A mixture of **7** (1.0 g, 2.37 mmol) and aqueous sodium hydroxide (1 N, 5 ml, 5.0 mmol) in tetrahydrofuran (7 ml) and methanol (3 ml) was stirred at room temperature for 3 h, then acidified with aqueous hydrochloric acid (5 N, 5.2 ml), and extracted with ethyl acetate (*ca.* 40 ml). The extract was washed with brine (2 × 5 ml), dried over magnesium sulfate, and evaporated under vacuum to give a mixture of **8** with benzyl alcohol, which was removed by the azeotropic distillation with water (2 × 5 ml). The residual water, in turn, was

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removed by the azeotropic distillation with toluene (2×3 ml) to obtain **8** (527 mg, 67%) as a colorless solid, $R_f = 0.45$ (ethyl acetate/hexanes, 1:1). ^{1}H-NMR (δ, ppm): 3.52 (s, 2H), 4.64 (s, 4H), 6.69 (d, $J = 8.6$ Hz, 2H), 7.07 (d, $J = 8.6$ Hz, 2H), 7.23-7.34 (m, 10H).

4R-Benzyl-3-(4-(dibenzylamino)phenylacetyl)-2-oxazolidinone, **9**

To a solution of **8** (225 mg, 0.68 mmol) and dimethylformamide (1 drop) in dichloromethane (5 ml) at 0–5 °C was added oxalyl chloride (180 µl, 2.06 mmol) dropwise. The mixture was allowed to reach room temperature, stirred for 20 min, evaporated under vacuum, and re-evaporated with toluene (1 ml) to obtain the acid chloride. In a separate flask, to a solution of (*R*)-(+)-4-benzyl-2-oxazolidinone (120 mg, 0.68 mmol) and triphenylmethane (~5 mg) in tetrahydrofuran (2 ml) at −78 °C was added *n*-butyllithium (1.6 M in hexane) until yellowish color persisted (*ca.* 0.6 ml). After 10 mins a solution of the acid chloride in tetrahydrofuran (1.5 ml) was added through a cannula. The reaction mixture was allowed to reach room temperature over the period of 1 h, then diluted with saturated aqueous sodium bisulfate (3 ml), and extracted with ethyl acetate (*ca.* 20 ml). The extract was washed with brine (2×3 ml), dried over sodium sulfate, and evaporated under vacuum. Flash chromatography of the residue (column 3×25 cm, eluting with ethyl acetate/hexanes, 3:7, *ca.* 500 ml) gave **9** (190 mg, 57%) as a light yellow solid, $R_f = 0.45$ (ethyl acetate/hexanes, 3:7). ^{1}H-NMR (δ, ppm): 2.68 (dd, $J = 13.3$ and 9.3 Hz, 1H), 3.17 (dd, $J = 13.3$ and 3.2 Hz), 4.04–4.15 (m, 4H), 4.58 (m, 5H), 6.64 (d, $J = 8.5$ Hz, 2H), 7.06 (d, $J = 8.5$ Hz, 4H), 7.15–7.27 (m, 13H). IR (KBr, ν, cm^{-1}): 744, 1196, 1347, 1384, 1520, 1695, 1788. UV (EtOH, λ, nm): 268 (ε 9784). MS (ESI, %): 340 (M^+, 21), 339 (100), 194 (13), 162 (42). Analytically Calculated for: $C_{18}H_{16}N_2O_5$: C, 63.53; H, 4.74; N, 8.23; Found: C, 63.71; H, 4.72; N, 8.01.

R-2-(4-Nitrophenyl)-1-propanol, **10**

To a solution of **4** (229 mg, 0.646 mmol) in tetrahydrofuran (2 ml) was added a solution of sodium borohydride (100 mg, 2.64 mmol) in water (0.7 ml). The reaction mixture was stirred for 3 h at room temperature. The excess of hydride was destroyed by slow addition of aqueous hydrochloric acid (1 N, *ca.* 2.9 ml). The mixture was further diluted with water (1 ml) and extracted with ethyl acetate (*ca.* 30 ml). The extract was washed with brine (2×5 ml), dried over sodium sulfate, and evaporated under vacuum. Flash chromatography of the residue (column 3×20 cm, eluting with ethyl acetate/hexanes, 1:1, *ca.* 600 ml) gave **10** (107 mg, 91%) as a light yellow oil, $R_f = 0.43$ (ethyl acetate/hexanes, 1:1). ^{1}H-NMR (δ, ppm): 1.32 (d, $J = 7.3$ Hz, 3H), 3.09 (m, 1H), 3.78 (m, 2H), 7.42 (d, $J = 8.8$ Hz, 2H), 8.19 (d, $J = 8.8$ Hz, 2H).

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R-2-(4-Nitrophenyl)-1-propanol-3-[^{14}C], **10a**

In the same manner as described above, starting from **4a** (400 mg, 1.12 mmol) in tetrahydrofuran (3.5 ml), and sodium borohydride (173 mg, 4.57 mmol) in water (1.2 ml), **10a** (179 mg, 87%) was obtained.

R-2-(4-Nitrophenyl)-1-propyl azide, **11**

Using DPPA. To a solution of **10** (200 mg, 1.1 mmol) and triphenylphosphine (304 mg, 1.16 mmol) in tetrahydrofuran (3 ml) at 0–5 °C was added diethylazodicarboxylate (DEAD) (183 µl, 1.16 mmol) dropwise. After 5 mins diphenylphosphoryl azide (DPPA) (250 µl, 1.16 mmol) was added to the reaction mixture, which was allowed to reach room temperature, stirred for 16 h, and evaporated under vacuum. Flash chromatography of the residue (column 3×30 cm, eluting with ethyl acetate/hexanes, 1:9, *ca.* 700 ml) gave **11** as an inseparable mixture (218 mg) with DPPA in a ratio of *ca.* 2:1, $R_f = 0.34$ (ethyl acetate/hexanes, 1:9). For **11**: ^{1}H-NMR (δ, ppm): 1.36 (d, $J = 7.3$ Hz, 3H), 3.13 (m, 1H), 3.48 (d, $J = 7.3$ Hz, 2H), 7.40 (d, $J = 8.8$ Hz, 2H), 8.20 (d, $J = 8.8$ Hz, 2H).

Using hydrazoic acid. To a solution of **10** (200 mg, 1.1 mmol) and triphenylphosphine (579 mg, 2.2 mmol) in tetrahydrofuran (3.5 ml) at 0–5 °C was added a solution of hydrazoic acid[14] (*ca.* 1.2 M in toluene, 1.8 ml, *ca.* 2.2 mmol). After 1 min DEAD (346 µl, 2.2 mmol) was added to the reaction mixture, which was allowed to reach room temperature, stirred for 16 h, and evaporated under vacuum. Flash chromatography of the residue (column 3×30 cm, eluting with ethyl acetate/hexanes, 1:9, *ca.* 700 ml) gave **11** (211 mg, 93%) as a colorless oil, R_f and ^{1}H-NMR of which were identical to described above.

N-(R-2-(4-Nitrophenyl)-1-propyl)phthalimide, **12**

To a solution of **10** (105 mg, 0.58 mmol), phthalimide (90 mg, 0.61 mmol), and triphenylphosphine (228 mg, 0.87 mmol) in tetrahydrofuran (2 ml) was added DEAD (137 µl, 0.87 mmol) dropwise. The reaction mixture was stirred at room temperature for 18 h and evaporated under vacuum. Flash chromatography of the residue (column 3×23 cm, eluting with ethyl acetate/hexanes, 3:7, *ca.* 500 ml) gave **12** (159 mg, 88%) as a white solid, $R_f = 0.43$ (ethyl acetate/hexanes, 3:7). ^{1}H-NMR (δ, ppm): 1.36 (d, $J = 6.8$ Hz, 3H), 3.49 (m, 1H), 3.78–3.94 (m, 2H), 7.42 (d, $J = 8.8$ Hz, 2H), 7.70 (m, 2H), 7.79 (m, 2H), 8.12 (d, $J = 8.8$ Hz, 2H).

N-(R-2-(4-Nitrophenyl)-1-propyl-3-[^{14}C])phthalimide, **12a**

In the same manner as described above, starting from **10a** (179 mg, 0.977 mmol) in tetrahydrofuran (3 ml), phthalimide (151 mg, 1.026 mmol),

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triphenylphosphine (384 mg, 1.464 mmol), and DEAD (230 µl, 1.461 mmol), **12a** (275 mg, 90%) was obtained.

R-2-(4-Nitrophenyl)-1-propylamine, **13**

From azide **11** *with triphenylphosphine*. To a solution of **11** (58 mg, 0.28 mmol) and triphenylphosphine (90 mg, 0.34 mmol) in tetrahydrofuran (1 ml) was added water (50 µl). The mixture was heated at 50–60 °C for 3 h, and evaporated under vacuum. Flash chromatography of the residue (column 2×20 cm, eluting with chloroform/methanol/ammonium hydroxide, 100:10:1, *ca*. 200 ml) gave **13** (45 mg, 89%) as an oil, $R_f = 0.38$ (chloroform/methanol/ammonium hydroxide, 100:10:1). ^{1}H-NMR (δ, ppm): 1.14 (broad s, 2H), 1.29 (d, $J = 6.5$ Hz, 3H), 2.91 (m, 3H), 7.38 (d, $J = 8.8$ Hz, 2H), 8.18 (d, $J = 8.8$ Hz, 2H).

From azide **11** *with sodium borohydride*. To a solution of **11** (100 mg, 0.48 mmol) and copper(II) sulfate pentahydrate (12 mg, 0.048 mmol) in methanol (2 ml) was added sodium borohydride (18 mg, 0.48 mmol) in portions. After 3 h the reaction mixture was treated with saturated aqueous sodium bicarbonate (*ca*. 0.1 ml), and evaporated under vacuum. The residue was diluted with water (*ca*. 1 ml), and extracted with extracted with ethyl acetate (*ca*. 10 ml). The extract was washed with brine (2×2 ml), dried over sodium sulfate, and evaporated under vacuum. Flash chromatography of the residue (column 2×20 cm, eluting with chloroform/methanol/ammonium hydroxide, 100:10:1, *ca*. 200 ml) gave **13** (63 mg, 73%), R_f and ^{1}H-NMR of which were identical to described above.

From imide **12**. To a solution of **12** (156 mg, 0.5 mmol) in toluene (2.2 ml) was added hydrazine (220 µl, 7.0 mmol) dropwise. The reaction mixture was heated at 80–90 °C for 1 h and cooled to room temperature. The supernatant solution was decanted, and the residual solid was washed with toluene (2×1 ml). The combined solution was evaporated under vacuum to give 13 (86 mg, 95%), R_f and ^{1}H-NMR of which were identical to described above.

R-2-(4-Nitrophenyl)-1-propylamine-3-[^{14}C], **13a**

In the same manner as described above, starting from **12a** (275 mg, 0.88 mmol) in toluene (3.9 ml), and hydrazine (390 µl, 12.42 mmol), **13a** (151 mg, 94%) was obtained.

R-2-(4-Nitrophenyl)-1-(2-propanesulfonamido)-propane, **14**

To a solution of **13** (84 mg, 0.466 mmol), triethylamine (290 µl, 2.08 mmol), and 4-dimethylaminopyridine (6 mg, 0.049 mmol) in dichloromethane (2 ml) at 0–5 °C was added isopropylsulfonyl chloride (63 µl, 0.56 mmol) dropwise. The reaction mixture was stirred at room temperature for 18 h, and subjected

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directly to flash chromatography (column 2×20 cm, eluting with ethyl acetate/hexanes, 1:1, *ca.* 200 ml) to give **14** (117 mg, 88%) as a light yellow solid, $R_f = 0.36$ (ethyl acetate/hexanes, 1:1). ^{1}H-NMR (δ, ppm): 1.29 (d, $J = 6.8$ Hz, 3H), 1.31 (d, $J = 6.8$ Hz, 3H), 1.34 (d, $J = 6.8$ Hz, 3H), 3.05–3.15 (m, 2H), 3.24–3.41 (m, 2H), 3.92 (broad t, $J = 6.8$ Hz, 1H), 7.40 (d, $J = 8.8$ Hz, 2H), 8.20 (d, $J = 8.8$ Hz, 2H).

R-2-(4-Nitrophenyl)-1-(2-propanesulfonamido)-propane-3-[^{14}C], **14a**. In the same manner as described above, starting from **13a** (151 mg, 0.829 mmol) in dichlomethane (3.6 ml), triethylamine (515 µl, 3.695 mmol), 4-dimethylaminopyridine (10 mg, 0.082 mmol), and isopropylsulfonyl chloride (112 µl, 0.997 mmol), **14a** (203 mg, 85%) was obtained.

R-2-(4-Aminophenyl)-1-(2-propanesulfonamido)-propane, **15**. A mixture of **14** (114 mg, 0.398 mmol) and 10% palladium on carbon (25 mg) in ethanol (4 ml) was stirred under the balloon pressure of hydrogen for 16 h, then diluted with ethyl acetate (2 ml), filtered through hyflo supercel, and evaporated under vacuum. Flash chromatography of the residue (column 2×20 cm, eluting with ethyl acetate/hexanes, 55:45, *ca.* 200 ml) gave **15** (90 mg, 88%) as an oil, $R_f = 0.32$ (ethyl acetate/hexanes, 3:2). ^{1}H-NMR (δ, ppm): 1.24 (d, $J = 6.8$ Hz, 3H), 1.26 (d, $J = 6.8$ Hz, 3H), 1.30 (d, $J = 6.8$ Hz, 3H), 2.85 (m, 1H), 3.03–3.16 (m, 2H), 3.31 (m, 1H), 3.63 (broad s, 2H), 3.82 (broad s, 1H), 6.65 (d, $J = 8.3$ Hz, 2H), 6.99 (d, $J = 8.3$ Hz, 2H).

R-2-(4-Aminophenyl)-1-(2-propanesulfonamido)-propane-3-[^{14}C], **15a**

In the same manner as described above, starting from **14a** (203 mg, 0.704 mmol) in ethanol (7 ml), and 10% palladium on carbon (45 mg), **15a** (153 mg, 84%) was obtained.

R-2-(4-(3,5-Difluorobenzoylamino)phenyl)-1-(2-propanesulfonamido)-propane **LY450108**

To a solution of **15** (88 mg, 0.343 mmol) and triethylamine (55 µl, 0.395 mmol) in dichloromethane (3.6 ml) was added 3,5-difluorobenzoyl chloride (49 µl, 0.389 mmol) dropwise. The reaction mixture was stirred at room temperature for 16 h, then diluted with saturated aqueous sodium bisulfate (*ca.* 2 ml), and extracted with ethyl acetate (*ca.* 20 ml). The extract was washed with brine (2×3 ml), dried over sodium sulfate, and evaporated under vacuum. Flash chromatography of the residue (column 2×20 cm, eluting with ethyl acetate/hexanes, 45:55, *ca.* 200 ml) gave **LY450108** (123 mg, 90%) as a white solid, $R_f = 0.52$ (ethyl acetate/hexanes, 1:1), $[\alpha]_D = +28.8^\circ$ (c 10.4, MeOH). ^{1}H-NMR (δ, ppm): 1.28 (d, $J = 6.8$ Hz, 3H), 1.29 (d, $J = 6.8$ Hz, 3H), 1.31 (d, $J = 6.8$ Hz, 3H), 2.97 (m, 1H), 3.09 (sept, $J = 6.8$ Hz, 1H), 3.17–3.24 (m, 1H),

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3.32–3.39 (m, 1H), 3.88 (broad s, 1H), 7.00 (t, $J = 8.3$ Hz, 1H), 7.23 (d, $J = 8.8$ Hz, 2H), 7.40 (d, $J = 7.8$ Hz, 2H), 7.58 (d, $J = 8.8$ Hz, 2H), 7.82 (broad s, 1H).

R-2-(4-(3,5-Difluorobenzoylamino)phenyl)-1-(2-propanesulfonamido)-propane-3-[^{14}C], **LY450108-[^{14}C]**

In the same manner as described above, starting from **15a** (153 mg, 0.592 mmol) in dichloromethane (6.2 ml), triethylamine (95 µl, 0.682 mmol), and 3,5-difluorobenzoyl chloride (85 µl, 0.674 mmol), **LY450108-[^{14}C]** (221 mg, 94%) was obtained. A sample after dilution (1:1) with non-labeled **LY450108**, and recrystallization from ethyl acetate/hexane, 2:3, had specific activity 72.9 µCi/mmol, radiochemical purity 99.4%, and optical purity 99.8%.

R-2-(4-(3,5-Difluorobenzoylamino)phenyl)-1-(2-propanesulfonamido)-propane-3-[^{3}H], **LY450108-[^{3}H]**

LY450108 (0.005 g) and Crabtree's catalyst (0.007 g) were dissolved in CH_2Cl_2 (3 ml). The mixture was stirred under 5 Ci of tritium gas for 5 h. Labile tritium was removed by repeated rotary evaporation with EtOH. The catalyst was removed by filtration of and EtOAc solution of the residue through a silica Sep-pak. The filtrate was purified by reversed phase HPLC on an Untrasphere ODS column eluting with a H_2O/CH_3CN/TFA gradient system. Analysis of the purified ***LY*450108**–[^{3}H] by HPLC chromatography showed a radiochemical purity of 98.8%. The specific activity as determined by mass spectrometry was 96 Ci/mmol (3.55 TBq/mmol). ^{3}H-NMR ($CDCl_3$) δ 7.5, 7.75; FAB-MS: $[M + H]^+$, $m/z = 405$.

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