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Research Article

The synthesis of isotopically labeled (+)-2-amino-bicyclo[3.1.0]hexane-2,6-carboxylic acid and its 2-oxa- and 2-thia-analogs

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Dedicated to the memory of my friend and mentor, Winston S. Marshall (1938–2005)

Summary

As part of a program aimed at the design of conformationally constrained analogs of glutamic acid, (+)-2-aminobicyclo[3.1.0]hexane-2,6-carboxylic acid (**1**), identified as a highly potent, selective, group II metabotropic glutamate receptor agonist has been synthesized and studied clinically. Heterocyclic analogs of **1** were subsequently synthesized in which the C-2 methylene has been replaced by an oxygen atom (**2**) or a sulfur atom (**3**). C-14 labeled isotopomers of **1**, **2** and **3** have been synthesized to facilitate pre-clinical ADME studies. A tritium labeled isotopomer of **1** was also synthesized for use in *in vitro* experiments. A stable labeled isotopomer of ***rac*-1** was prepared for use as an internal standard for bioanalytical assays. The key step in each of these syntheses was the reaction of chiral ketone **4**, **5** or **6** with $K^{14}CN/(NH_4)_2CO_3$ using the Bucherer–Berg protocol. In the preparation of the stable labeled isotopomer, ***rac*-4**-[$^{13}\mathbf{C}_2$] was prepared in two steps from ethyl bromoacetate-[UL-$^{13}C_2$]; subsequent reaction of ***rac*-4**-[$^{13}\mathbf{C}_2$] with $K^{13}CN/^{15}NH_4Cl/Na_2CO_3$, followed by hydrolysis of the hydantoin yielded ***rac*-1**-[$^{13}\mathbf{C}_3$,$^{15}\mathbf{N}$]. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: mGlu agonist; carbon 14; tritium; Bucherer–Berg reaction

Introduction

Metabotropic glutamate receptors (mGlu) are G-protein-coupled receptors, which comprise eight different subtypes.[1] These are also divided into three

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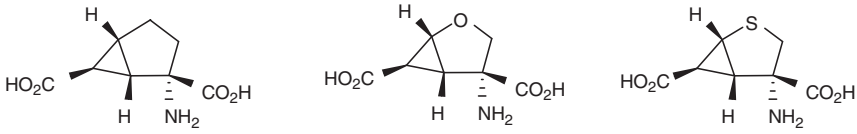
groups (Group 1, 2 and 3) on the basis of their amino acid sequence homology, pharmacology and the signal transduction pathways to which they are coupled.[2] Monn *et al.* have reported on the design, synthesis and biological evaluation of **1**, a novel, conformationally constrained analog of glutamic acid with potent $mGlu_{2,3}$ agonist activity.[3] Evaluation of **1** in humans[4] for use in the treatment of anxiety followed pre-clinical ADME studies[5] for which **1**-[^{14}**C**] as well as ***rac*-1**-[$^{13}\mathbf{C}_3$,^{15}**N**] was required. Subsequently, Monn *et al.* described the synthesis and biological evaluation of the heterocyclic analogs **2** and **3** which were very potent $mGlu_{2,3}$ agonists as well.[6,7] In preparation for pre-clinical ADME studies, carbon-14 isotopomers of **2** and **3** were also required. The syntheses of **1**-[^{14}**C**], **2**-[^{14}**C**], **3**-[^{14}**C**] and **1**-[^{3}**H**] as well as ***rac*-1**-[$^{13}\mathbf{C}_3$,^{15}**N**] have been outlined in preliminary reports.[8,9] In this manuscript, their syntheses will be discussed in further detail.

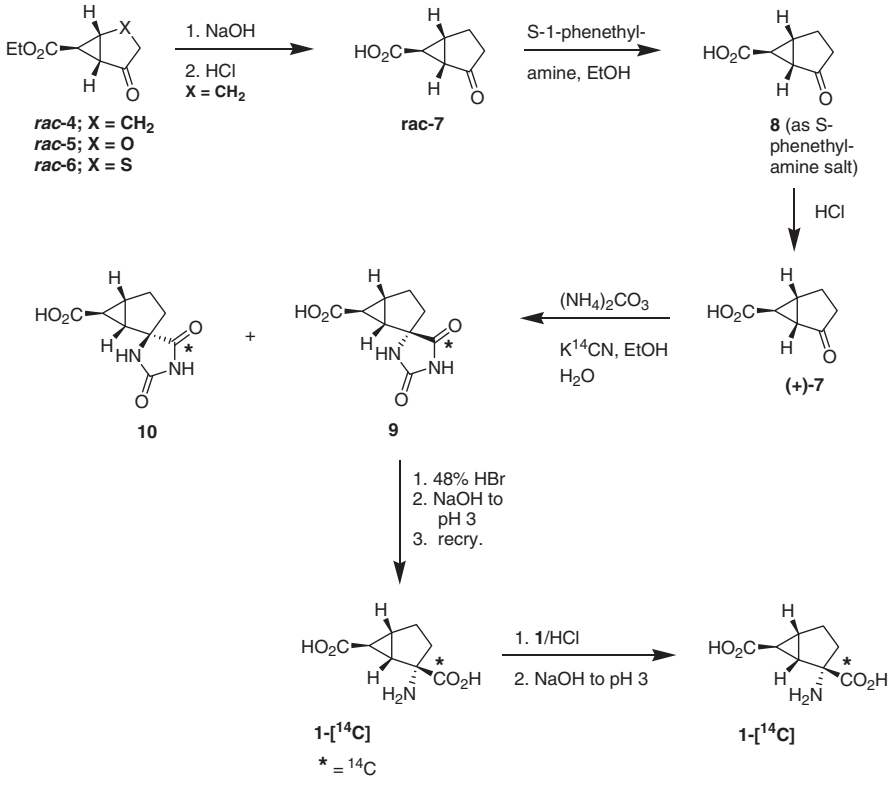


An earlier report of the synthesis of **1**-[^{3}**H**] by another method was published by Malherbe *et al.*, although no experimental details were given.[10]

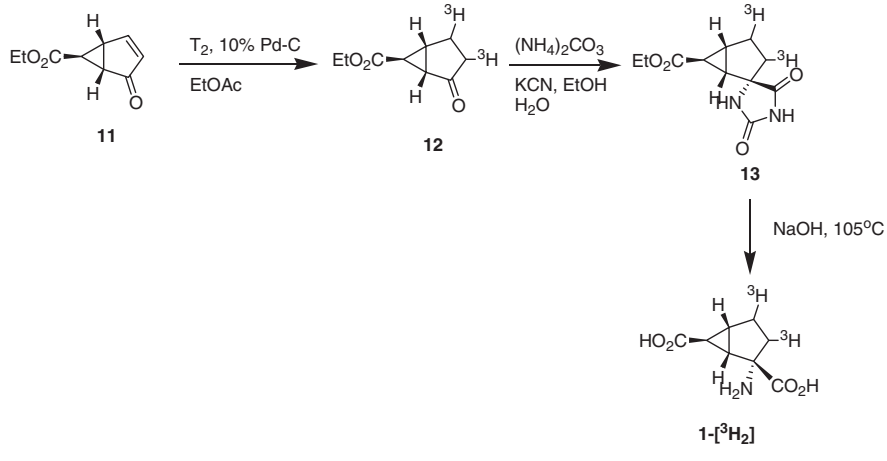
Discussion

In the preparation of **1**-[^{14}**C**], ***rac*-4**, prepared as detailed by Monn *et al.*,[3] was hydrolysed in NaOH to yield ketoacid **rac-7**, and subsequently resolved as its *S*-1-phenethylamine salt **8** (Scheme 1). Treatment of **8** with HCl yielded **(+)-7**. Reaction of (+)-**7** with $K^{14}CN/(NH_4)_2CO_3/EtOH/H_2O$ yielded the hydantoin as a mixture of diastereomers **9** and **10**. Re-crystallization of the mixture from *i*-$PrOH/H_2O$ yielded the desired chromatographically pure hydantoin **9** in 58% yield. A suspension of **9** in 48% HBr was refluxed for 20 h; the pH was adjusted to 3 by the addition of NaOH to afford crystalline **1**-[^{14}**C**] (64%, HPLC showed slight contamination with **9**). The impure material was redissolved in 1 N HCl and treated dropwise with 1 N NaOH to pH 3 whereupon a white crystalline solid formed to yield **1**-[^{14}**C**] (43% yield). This material was diluted with **1** as described above to yield **1**-[^{14}**C**] (77% yield); specific activity 37.87 μCi/mg. The radiochemical purity (RCP) was 99.81% and the material co-eluted with authentic **1** on HPLC; the chiral purity was 98.98%.

Ethyl(-)-4-oxobicyclo[3.2.1]hex-2-ene-6-carboxylate(**11**, Scheme 2) was prepared as described by Massey *et al.* from **(+)-4** by conversion, first to the TMS

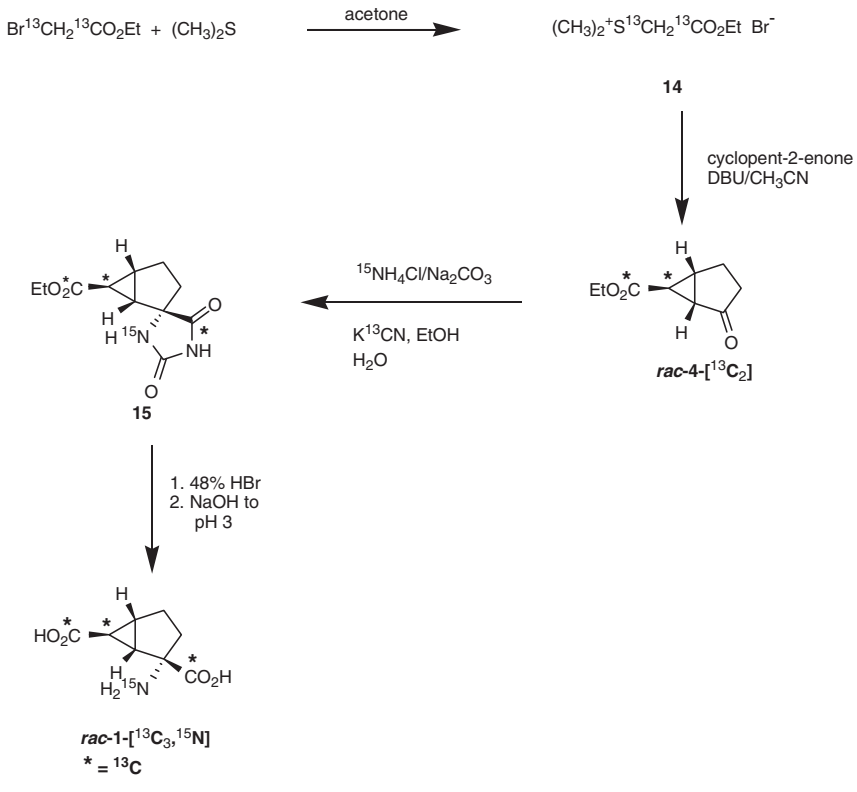


Scheme 1.



Scheme 2.

enol ether by reaction with TMSI/Et_3N, followed by treatment with $Pd(OAc)_2$.[11] Ketoester **11** is also an intermediate in the asymmetric synthesis of **1**, reported by Dominguez *et al.*[12] Treatment of **11** with tritium gas/5% Pd-C in EtOAc and HPLC purification of the crude product yielded



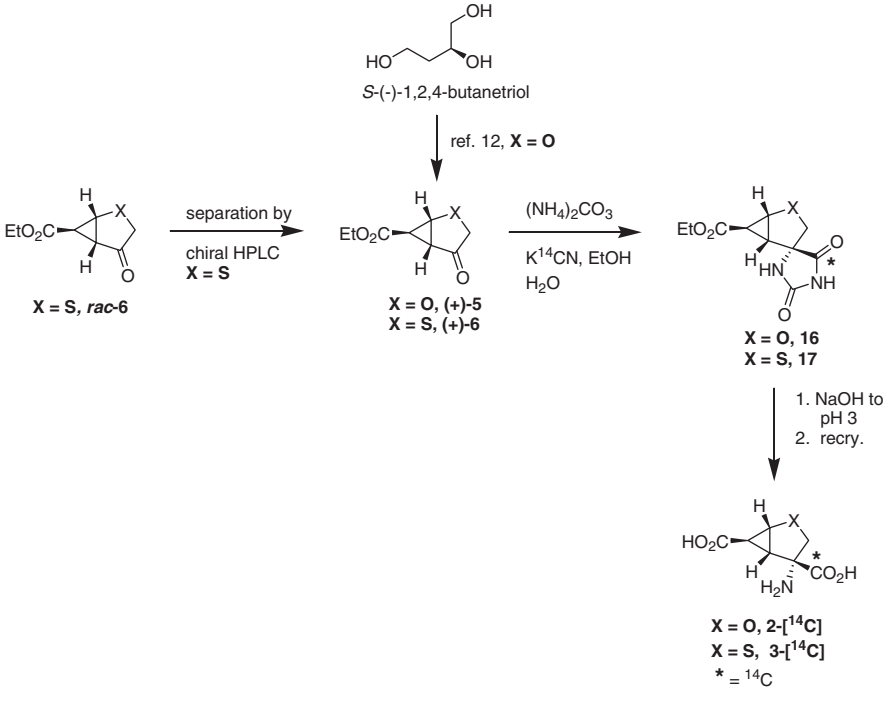
Scheme 3.

ethyl (+)-2-oxobicyclo[3.2.1]hexane-6-carboxylate-[3,4-$^3H_2$] (**12**).[†] Further reaction of **12** with $KCN/(NH_4)_2CO_3/EtOH/H_2O$ as described above and HPLC purification of hydantoin **13**, followed by NaOH hydrolysis and HPLC purification yielded **1**-[^{3}**H**$_2$] (specific activity 38 Ci/mmol).

A mixture of ethyl bromoacetate-[1,2-^{13}C] and dimethylsulfide was stirred in acetone for 44 h to yield ethyl (dimethylsulfuranylidene)acetate-[1,2-$^{13}C_2$] bromide (**14**, Scheme 3). Subsequent reaction of **14** with 2-cyclopentenone/CH_3CN/DBU yielded ***rac*-4**-[^{13}**C**$_2$] in 64% after chromatography. Further reaction of ***rac*-4**-[^{13}**C**$_2$] with a mixture of NH_4Cl-[^{15}N], Na_2CO_3, and K^{13}CN in water using the Bucherer–Berg protocol yielded a 97:3 mixture of **15** and the undesired diastereomers in 26% yield. Due to the unavailability of $(NH_4)_2CO_3$-[^{15}N], we envisioned its *in situ* preparation by the reaction of NH_4Cl-[^{15}N] and Na_2CO_3. We believe this to be the first example of using NH_4Cl-[^{15}N] in the Bucherer–Berg reaction. Hydrolysis of the hydantoin **15** in 48% HBr and subsequent adjustment of the pH of the reaction mixture to 4 with NaOH yielded ***rac*-1**-[^{13}**C**$_3$, ^{15}**N**] in 28% yield.

[†]The tritiation and subsequent chemistry was conducted at Amersham Biosciences, Cardiff, Wales, UK.

The 1-oxa-(**2**-[^{14}**C**]), and 1-thia (**3**-[^{14}**C**]), (Scheme 4) analogs were synthesized in a manner similar to that described above for **1**-[^{14}**C**]. In order to stem the losses accompanied by a resolution conducted in the last step, chiral precursors **(+)-5** and **(+)-6** were required. Chiral (+)-**5** was prepared in a six step synthesis from (*S*)-(-)-1,2,4-butanetriol as described by Massey *et al.*;[13] **(+)-6** was obtained by preparative chromatography of ***rac*-6** (prepared as described by Monn *et al.*[3]) on a 5 cm Chiralpak AD chiral column eluting with 10% ethanol in acetonitrile. As described above, reaction of (+)-**5**/(+)-**6** with $K^{14}CN/(NH_4)_2CO_3/EtOH/H_2O$ yielded the two hydantoins **16**/**17** in 52% (100% de) and 26% (99% de) yield, respectively. Hydrolysis of the hydantoins **16**/**17** in refluxing 2 N NaOH, followed by acidification to pH 3.0–3.2 yielded the crystalline **2**-[^{14}**C**] and **3**-[^{14}**C**] in 38 and 63% yields. The RCPs were 99.92% and 99.0%, respectively. The specific activity of **3**-[^{14}**C**] was 97.9 μCi/mg. There was a problem obtaining a reproducible specific activity for **2**-[^{14}**C**], which may have arisen from residual contamination by inorganics. The material was recrystallized after dilution with authentic **2** (56% yield); the specific activity was 36.89 μCi/mg and the RCP was 99.79%.



Scheme 4.

Results

We have described the syntheses of two isotopomers of **1** (**1**-[^{14}**C**] and **1**-[^{3}**H**]) as well as the isotopomer of ***rac*-1** (***rac*-1**-[$^{13}\mathbf{C}_3$,^{15}**N**]). In addition, we have described the synthesis of the isotopomers of two heterocyclic analogs of **1** (**2**-[^{14}**C**] and **3**-[^{14}**C**]). In contrast to the published syntheses of **1**, **2** and **3**, which required a resolution at the hydantoin stage, these preparations used the chiral precursors **(+)-7**, **11**, (+)-**5** and **(+)-6**, which obviated the late stage resolutions. The synthesis of ***rac*-1**-[$^{13}\mathbf{C}_3$,^{15}**N**] employed the use of *in situ* generated $(^{15}NH_4)_2CO_3$, which is not commercially available, for the Bucherer–Berg reaction.

Experimental

NMR spectra were obtained on a General Electric QE-300 or a Varian 500 MHz nuclear magnetic resonance spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Mass spectra were recorded on a Nermag R30-10 triple stage quadrupole mass spectrometer (DCI), a VG Analytical VG-ZAB3F mass spectrometer (FAB), a Varian Associates MAT 731 mass spectrometer (FD) or a Waters Micromass ZQ single quadrupole mass spectrometer (ES-MS). Flash chromatography was performed on silica gel. RCP was assessed by HPLC with radiochemical detection on a Packard Flow Scintillation Analyzer. RCP was further assessed by TLC-autoradiography. The radioactivity was detected on Kodak X-ray film BB-5; the lanes of the TLC were cut, mixed with methanol and scintillation fluid and counted. Microanalyses were conducted in the Physical Chemistry Department of the Lilly Research Laboratories.

Synthesis of (±)-2-oxobicyclo[3.1.0]-hexane-6-carboxylic acid (**rac-7**)

Ethyl 2-oxobicyclo[3.1.0]-hexane-6-carboxylate (***rac*-4**) was saponified by base hydrolysis using NaOH and subsequently acidified with HCl to yield ***rac*-7** in yields of 92–95%.[14] Several individual lots of ***rac*-7** were mixed (1150.8 g, 8.212 mol) and dissolved in 4.1 l of 1 N NaOH at 15–30°C. After stirring for 10–20 min to complete dissolution, the mixture was cooled to 15–20°C and slowly acidified to pH 1, by treatment with HCl (12 N, 800 ml). A thick slurry formed and the solid was collected by filtration and washed with water to yield (±)-2-oxobicyclo-[3.1.0]-hexane-6-carboxylic acid (***rac*-7**). The solid and NaCl (0.5 kg) was suspended in 2 l of water, stirred for 15 min and then extracted with EtOAc (4.0 l). The EtOAc solution was dried (anhydrous $MgSO_4$) and concentrated *in vacuo*. The residue was crystallized from acetone (2.5 l) to yield (±)-2-oxobicyclo[3.1.0]-hexane-6-carboxylic acid (***rac*-7**, 837 g, 72.7% yield, 97.4% by HPLC, *vida infra*).

Synthesis of (1S, 5R, 6S)-2-oxobicyclo[3.1.0]-hexane-6-carboxylic acid, (S)-α-methyl-benzylamine salt (**8**)

An EtOH/H_2O solution (4.2 l, 8:1) of (±)-2-oxobicyclo[3.1.0]-hexane-6-carboxylic acid (***rac*-7**, 837 g, 5.97 mol) at 15–20°C was treated in a portionwise manner with (*S*)-α-methylbenzylamine (724 g, 5.97 mol); the temperature was kept below 30°C by an ice bath. The resulting mixture was stirred overnight. The solid was collected by filtration and washed with acetone (4 × 0.4 l) to yield (+)-2-oxobicyclo[3.1.0]-hexane-6-carboxylic acid, (*S*)-α-methylbenzylamine salt (**8**, 323.8 g, 20.7% yield, theoretical = 50%), mp 142.5–147.5°C.

Synthesis of (1S, 5R, 6S)-2-oxobicyclo[3.1.0]-hexane-6-carboxylic acid ((+)-**7**)

(1*S*, 5*R*, 6*S*)-2-Oxobicyclo[3.1.0]-hexane-6-carboxylic acid, (*S*)-α-methylbenzylamine salt (**8**, 277.85 g, 0.863 mol) was added to a stirred solution of 1200 ml of 1 N HCl (1.2 mol, 1.13 eq.). After stirring for 15 min, the solution was extracted with EtOAc (4 × 3 l). The combined extracts were dried (anhydrous $MgSO_4$) and concentrated *in vacuo* to yield (1*S*, 5*R*, 6*S*)-2-oxobicyclo[3.1.0]-hexane-6-carboxylic acid ((+)-**7**, 157.9 g). The crude material was crystallized from water to yield (+)-**7** (108.2 g, 72.6%), mp 128–134.5°C; HPLC purity 99.7%; ^{1}H-NMR ($CDCl_3$) δ 1.98 (t, J = 3 Hz, 1H, 6-H), 2.03 (m, 1H, 3β-H), 2.11 (m, 1H, 3α-H), 2.15 (m, 1H, 4β-H), 2.24 (m, 1H, 4α-H), 2.33 (dd, J = 5.5 and 2.3 Hz, 1-H) and 2.55 (dt, J = 3.4 and 5.5 Hz, 5-H); ^{13}C-NMR ($CDCl_3$) δ 22.5 (C-4), 26.0 (C-6), 29.9 (C-5), 31.7 (C-3), 36.2 (C-1), 176.05 (carboxyl-C=O) and 211.3 (C=O); IR ($CHCl_3$) showed carbonyl resonances at 1733.91 and 1703.51 cm^{-1}; UV (95% EtOH), λ_M 277 (ε_M = 58.54); Titration (66% DMF) pK_a 4.72, 6.23; $[\alpha]_D = 74.7°$ (c = 10.06, CH_3OH); FD-MS, M^+ 140. Karl Fischer titration indicated 0.31% water. The chemical purity was 99.7% by HPLC (0.3% *S*-MBA) on a Zorbax SB Phenyl column (4.6 × 250 mm) with gradient elution at 2.0 ml/min (column temperature 40°C) and UV detection at 210 nm. R_T ((+)-**7**) = 3.05 min; R_T (*S*-MBA) = 2.35 min.

Time (min)	Solvent A	Solvent B
0	10	90
20	10	90
25	80	20
30	80	20
35	10	90

Solvent A: acetonitrile.
Solvent B: 0.1 M NaH_2PO_4, adjusted to pH 2.5 with H_3PO_4.

The chiral purity was 98.7% as determined by HPLC on a ChiralPak AD column (5 μm, 4.6 × 250 mm), eluting with 40% *n*-propanol in hexane(column temperature at 40°C) at 1.0 ml/min with UV detection at 210 nm. R_T ((+)-7) = 6.5 min; R_T ((-)-7) = 4.5 min.

Analysis calculated for $C_7H_8O_3$: C, 60.00; H, 5.75. Found: C, 60.09; H, 5.76.

Synthesis of (-)-2′,5′-dioxospiro[bicyclo[3.1.0]hexane 2,4′-imidazolidine]-6-carboxylic-[2-carbonyl-^{14}C] acid, **9**

A mixture of (1*S*, 5*R*, 6*S*)-2-oxobicyclo-[3.1.0]-hexane-6-carboxylic acid ((+)-**7**, 0.560 g, 4 mmol), potassium cyanide-[^{14}C] (200 mCi, specific activity 50 mCi/mmol, 4 mmol), and ammonium carbonate (0.920 g, 10 mmol) in 10 ml of ethanol/water (1:1) was stirred at room temperature for 68 h. The mixture was diluted with 25 ml of water and concentrated *in vacuo* to an amorphous foam. This material was re-dissolved in water and acidified to pH 2 with 1 N HCl. After stirring for 1 h at room temperature, the white crystalline material was collected by filtration, washed with water, and dried *in vacuo* to yield (-)-2′,5′-dioxospiro[bicyclo-[3.1.0]hexane-2,4′-imidazolidine]-6-carboxylic-[2-carbonyl-^{14}C] acid (**9**, 0.598 g, 71%); ES-MS: $[M + Na]^+$, $m/z = 233/235$ (major). The white solid was suspended in *i*-propyl alcohol/water (6 ml/0.4 ml) and stirred at reflux. Water was added until all the material was immersed in the solution (ca. 2 ml); the mixture was allowed to cool to room temperature and stirred for 1 h. The white solid was collected by filtration, washed with *i*-propyl alcohol, and dried *in vacuo* to yield (-)-2′,5′-dioxo-spiro-[bicyclo-[3.1.0]-hexane-2,4′-imidazolidine]-6-carboxylic-[2-carbonyl-^{14}C] acid (**9**, 0.352 g, 42%). This material co-elutes with authentic unlabeled isotopomer of **9** on HPLC (*vide infra*). The chiral purity was determined on the final product.

Synthesis of (1S, 2S, 5R, 6S)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic-[2-carboxyl-^{14}C] acid, monohydrate (1-[^{14}C] monohydrate)

A suspension of (-)-2′,5′-dioxospiro-[bicyclo[3.1.0]hexane-2,4′-imidazolidine]-6-carboxylic-[2-carbonyl-^{14}C] acid (**9**, 0.352 g, 1.68 mmol) in 48% HBr (5.5 ml) was stirred at gentle reflux for 16 h. HPLC (*vida infra*) showed >99% conversion to the desired product. Stirring at reflux was continued for an additional 4 h and the mixture was allowed to cool to room temperature. An additional 10 ml of water was added to redissolve the HBr salt; the pH was adjusted to 3 by the dropwise addition of 5 N NaOH. The solution was concentrated to ca. 8 ml *in vacuo*, seeded with authentic **1**, and stirred as a solid slowly crystallized. After stirring at room temperature for 3 h, the mixture was chilled at 4°C overnight. The white solid was collected by filtration, washed with water, and dried *in vacuo* (0.200 g, 64%). HPLC showed some

contamination with unreacted **9**. This material was dissolved in 2 ml of 1 N HCl, filtered, and treated dropwise with 5 N NaOH to pH 3. The resulting mixture was stirred until crystals were formed and 4 h thereafter. The white solid was collected by filtration, washed with water, and dried *in vacuo* to yield (1*S*, 2*S*, 5*R*, 6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic-[2-carboxyl-^{14}C] acid, monohydrate (**1**-[^{14}**C**] monohydrate, 0.134 g, 43%).

To adjust the specific activity, a mixture of **1** (0.690 g) and **1**-[^{14}**C**] (0.134 g) was dissolved in 1 N HCl (8.24 ml) at room temperature with stirring. The pH was carefully adjusted to 3.0 by the dropwise addition of 5 N NaOH (at ca. pH 2.6, material began to crystallize). After the pH adjustment was complete, the mixture was stirred at room temperature for 4 h. The white solid was collected by filtration, washed with water, and dried *in vacuo* to yield (1*S*, 2*S*, 5*R*, 6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic-[2-carboxyl-^{14}**C**] acid, monohydrate (**1**-[^{14}**C**] monohydrate, 0.631 g, 77%); ES-MS: $[M+H]^+$, $m/z = 186$ (major)/188; ^{1}H-NMR (D_2O) δ 1.49 (m, 1H, 3β-H), 1.85 (m, 1H, 2β-H) and 1.95–2.32 (m, 5H). This spectrum was superimposable with that of **1** in D_2O;[3]specific activity 37.87 μCi/mg. This material co-eluted with authentic **1** monohydrate by radio-HPLC on a Zorbax SB-phenyl column (5 μm, 4.6 × 250 mm), eluting with 0.1 N NaH_2PO_4 (pH 2.1)/acetonitrile (93:7) at 1 ml/min (column temperature 40°C), UV detection at 220 nm. The sample was dissolved at 1 mg/ml (in buffer); 20 μl was injected. The RCP using the same conditions with radiochemical detection was 99.82%. The RCP by TLC/Autoradiography (silica gel, EtOAc/CH_3CN/HOAc/H_2O, 21:7:7:9) was 99.6%. The chiral purity was 98.98% as determined by HPLC after derivatization with Marfey's reagent[15] on a Zorbax 300 SB C-18 column (4.6 × 250 mm), eluting with H_2O (pH 3.0 with H_3PO_4)/acetonitrile in the following gradient: 90:10 at 0 min, increasing to 83:17 over the next 5 min. The concentration was held at 83:17 for 40 min and then returned to the original concentration in 1 min. The flow rate was 1 ml/min at ambient temperature; UV detection at 340 nm. A 2 mg/ml sample of **1**-[^{14}**C**] was derivatized; a 20 μl sample was injected.

Synthesis of ethyl (1R, 5S, 6S)-4-oxobicyclo[3.1.0]hexane-6-carboxylate-[2,3-$^3H_2$] (**12**)

An ethyl acetate (2 ml) solution of ethyl (1*R*, 5*S*, 6*S*)-4-oxobicyclo[3.1.0]hex-2-ene-6-carboxylate (**11**, 0.0497 g) and 10% Pd-C (0.0281 g) was stirred for 5 h under tritium gas (30 Ci). The catalyst was removed by filtration and the labile tritium was removed by several evaporations from ethanol (under vacuum) to yield ethyl (1*R*, 5*S*, 6*S*)-4-oxobicyclo[3.1.0]hexane-6-carboxylate-[2,3-$^3H_2$] (**12**, 9.6 Ci). This material was purified by reversed phase HPLC on a Partisil ODS column, eluting with a water/CH_3OH gradient to yield purified **12** (6.1 Ci).

Synthesis of ethyl (-)-2′,5′-dioxospiro-[bicyclo-[3.1.0]hexane-2,4′-imidazolidine]-6-carboxylate-[2,3-$^3H_3$] (**13**)

Ethyl (1*R*, 5*S*, 6*S*)-4-oxobicyclo[3.1.0]hexane-6-carboxylate-[2,3-$^3H_2$] (**12**, 9.6 Ci, ca. 0.15 mmol) was mixed with KCN (0.011 g, 0.169 mmol), $(NH_4)_2CO_3$ (0.0296 g, 0.238 mmol), water (0.4 ml) and EtOH (1 ml) and heated at 35°C for 18 h. The reaction was quenched by the addition of TFA (0.1 ml) and concentrated *in vacuo*. The residue was re-dissolved in EtOH (3 × 1 ml) and concentrated. This material was purified by reversed phase HPLC on a Partisil ODS column, eluting with a water/CH_3CN/TFA gradient to yield ethyl (-)-2′,5′-dioxospiro-[bicyclo[3.1.0]hexane-2,4′-imidazolidine]-6-carboxylate-[2,3-$^3H_3$] (**13**, 2.78 Ci).

Synthesis of (1S, 2S, 5R, 6S)-2-aminobicyclo-[3.1.0]hexane-2,6-dicarboxylic-[2,3-$^3H_2$] acid (1-[$^3H_2$])

A NaOH (1 N, 1 ml) solution of ethyl (-)-2′,5′-dioxospiro-[bicyclo-[3.1.0]hexane-2,4′-imidazolidine]-6-carboxylate-[2,3-$^3H_3$] (**13**, 2.78 Ci) was stirred at 105°C for 1 h. TLC indicated that no starting material remained, so the reaction was quenched by the addition of HCl (12 N, 0.1 ml) and concentrated *in vacuo*. The residue was re-dissolved in 1:1 EtOH/H_2O (3 × 1 ml) and concentrated to yield (1*S*, 2*S*, 5*R*, 6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic-[2,3-$^3H_2$] acid (**1**-[^{3}**H**$_2$], 2.1 Ci). After an initial purification of a portion of the crude product by preparative TLC on KC18 plates (*n*-BuOH/H_2O/HOAc), the product was further purified by reversed phase HPLC on a Partisil ODS column, eluting with a water/CH_3CN/TFA gradient. Final purification on a Primesphere silica column, eluting with EtOAc/CH_3CN/HOAc yielded (1*S*, 2*S*, 5*R*, 6*S*)-2-aminobicyclo-[3.1.0]hexane-2,6-dicarboxylic-[2,3-$^3H_2$] acid (**1**-[^{3}**H**$_2$], 19 mCi); specific activity = 38 Ci/mmol; FAB-MS: $[M+H]^+$, $m/z = 190$. The product co-eluted on HPLC with authentic **1**; Waters Novapak C-18 column (3.9 × 150 mm) with gradient elution at 1 ml/min (0–100% B over 30 min) with UV (230 nm) and Radiochemical Detection (Solvent A = 10 mM TFA/50 mM heptane sulfonic acid (HSA), Solvent B = 10 mM TFA plus 50 mM HSA/CH_3CN, 1:3). The RCP in this same system was 95.5%. The chiral purity was 96.0% as determined by HPLC on a Chirobotic T column (5 μm, 4.6 × 250 mm), eluting with 0.1% aqueous TFA/THF (80:20) at 1 ml/min with UV detection at 230 nm.

Synthesis of ethyl (dimethylsulfuranylidene)acetate-[1,2-$^{13}C_2$] bromide (**14**)

Ethyl bromoacetate-[$^{13}C_2$] (2.0 g, 11.83 mmol) was dissolved in 5 ml of freshly opened acetone and a slight excess of dimethylsulfide (0.821 g, 0.97 ml, 13.21 mmol). The solution became cloudy after about 1 h and the reaction mixture was then stirred at ambient temperature for 44 h. The white

precipitate was collected by filtration under a stream of nitrogen, washed with cold acetone and dried *in vacuo* to yield ethyl (dimethylsulfuranylidene)acetate-[1,2-$^{13}C_2$] bromide (**14**, 2.04 g, 74.6% yield); ^{1}H-NMR (DMSO/d_6) δ 1.31 (t, 3H, CH_3), 3.50 (s, 6H, $S^+(CH_3)_2$), 4.3 (q, 2H, OCH_2Me), 5.31 (d, $J^{13}_{C-H} = 100.8$ Hz, $^{13}CH_2$).

Synthesis of ethyl rac-2-oxobicyclo[3.1.0]hexane-6-carboxylate-[carboxyl, 6-$^{13}C_2$] (rac-4-[$^{13}C_2$])

A suspension of ethyl (dimethylsulfuranylidene)acetate-[1,2-$^{13}C_2$] bromide (**14**, 2.04 g, 8.9 mmol) in CH_3CN (8 ml) was stirred and treated with DBU (1.63 g, 1.60 ml, 10.7 mmol). After 15 min, 2-cyclopenten-1-one (0.735 g, 0.75 ml, 8.95 mmol) was added and stirring was continued for 44 h. Hydrochloric acid (1 N, 10 ml) and Et_2O (25 ml) were added. The aqueous layer was re-extracted with Et_2O (3 × 25 ml) and the combined Et_2O extracts were washed with brine, dried (anhydrous $MgSO_4$) and concentrated *in vacuo*. The dark oil was purified by chromatography on silica gel (30 × 70 mm) by eluting with 500 ml of hexanes/EtOAc (60:40). The solvent was removed and the remaining oil was re-dissolved in hexanes (10 ml) and chilled to −20°C, whereupon a yellow crystalline solid formed. The crystals were collected by filtration and washed with hexanes to yield of ethyl *rac*-2-oxobicyclo[3.1.0] hexane-6-carboxylate-[carboxyl, 6-$^{13}C_2$] acid (***rac*-4-[$^{13}C_2$]**, 0.967 g, 64% yield); ^{1}H-NMR ($CDCl_3$) δ 1.30 (t, $J = 7$ Hz, 3H, CH_3), 2.0–2.4 (m, 6H), 2.48–2.55 (m, 1 H), 4.15 (q, $J = 7$ Hz, CH_2Me); ^{13}C-NMR ($CDCl_3$) δ 26.39 (^{13}CH), 169.80 ($^{13}C=O$); ES-MS, $[M+H]^+$, $m/z = 171$, $[M+NH_4]^+$, $m/z = 188$.

Synthesis of ethyl (-)-2′,5′-dioxospiro-[bicyclo[3.1.0]hexane-2,4′-imidazolidine]-6-carboxylate-[carboxyl, 5′-carbonyl, 6-$^{13}C_3$, 2′-^{15}N] **(15)**

An aqueous (1.8 ml) suspension of Na_2CO_3 (1.44 g, 9.52 mmol) and $^{15}NH_4Cl$ (1.02 g, 19.04 mmol) was sonicated for 15 min. Ethanol (5.5 ml) was added followed by ethyl *rac*-2-oxobicyclo[3.1.0]-hexane-6-carboxylate-[carboxyl, 6-$^{13}C_2$] (***rac*-4**, 0.81 g, 4.76 mmol) and the reaction mixture was heated to 40°C. After 15 min, K^{13}CN (0.347 g, 5.24 mmol) was added and heating was continued for ca. 15 h. The solution was allowed to cool to room temperature, diluted with H_2O (3.5 ml) and then chilled to 5°C. A white precipitate formed which was collected by filtration (0.472 g, inorganic salts). Three additional crops of white solid formed and collected by filtration and analysed by HPLC (see below):

A : 0.427 g (88.3 : 11.5 desired : undesired diastereomers)

B : 0.285 g (54.6 : 45.2 desired : undesired diastereomers)

C : 0.156 g (36.6 : 63.1 desired : undesired diastereomers)

Crop A (0.427 g) was suspended in EtOH (5 ml) and H_2O (0.9 ml) and heated until dissolved. Upon cooling to room temperature, some crystals formed and the mixture was diluted with 5 ml of H_2O and chilled in an ice bath. After, 2 h, the solid was collected by filtration, washed with H_2O and dried to yield ethyl (-)-2′,5′-dioxospiro-[bicyclo[3.1.0]hexane-2,4′-imidazolidine]-6-carboxylate-[carboxyl, 5′-carbonyl, 6-$^{13}C_3$, 2′-^{15}N] (**15**, 0.304 g, 26% yield, de = 94%); ^{1}H-NMR (DMSO/d_6) δ 1.14 (t, $J = 7$ Hz, 3H, CH_3), 1.30 (m, 1H, 2 eq.-H), 1.4-2.2 (m, 6H), 4.02 (q, $J = 7$ Hz, 2H, CH_2Me), 7.95 (dd, $J = 14.4$, 86.4 Hz, 2′-^{15}NH), 10.5 (bs, 1H, 4′-NH); ^{13}C-NMR (DMSO/d_6) δ 20.8 (6-^{13}C), 172.0 (5′-^{13}C = O), 177.29 (6-^{13}C = O); HPLC on a Zorbax SB phenyl column (4.6 × 250 mm), eluting with 0.1 M NaH_2PO_4 at pH 2.1/CH_3CN (80:20) at 2 ml/min with UV detection at 220 nm.

Treatment of crop B as described above only increased the de to 40%.

Synthesis of 2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic-[6, dicarboxyl-$^{13}C_3$,^{15}N] acid (rac-1-[$^{13}C_3$,^{15}N])

A mixture of ethyl (-)-2′,5′-dioxospiro-[bicyclo[3.1.0]hexane-2,4′-imidazolidine]-6-carboxylate-[carboxyl, 5′-carbonyl, 6-$^{13}C_3$, 2′-^{15}N] (**15**, 0.286 g, 1.18 mmol) and 48% HBr (15 ml) was heated at reflux for 24 h (HPLC showed 90% conversion to the desired product). The solvent was removed under vacuum and the residue was re-dissolved in H_2O (2 ml). The pH of the solution was adjusted to 3.0 by the addition of 0.1 N NaOH and then cooled in an ice bath. After 1 h, a white solid began to crystallize. The suspension was stirred in the cold for an additional 2 h. The solid was collected by filtration and washed with cold H_2O to yield 2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic-[6,dicarboxyl-$^{13}C_3$,^{15}N] acid (***rac*-1**-[^{13}**C**$_3$,^{15}**N**], 0.062 g, 28% yield); ^{13}C-NMR (D_2O) δ 24.68 (6-^{13}C), 176.74 (2-^{13}C═O), 180.52 (6-^{13}C═O); ES-MS: $[M+H]^+$, $m/z = 190$; $[M+Na]^+$, $m/z = 212$. HR-ES-MS: Analysis calculated for $C_5^{13}C_3H_{11}^{15}NO_4$: $[M + H]^+$ 190.0832. Found:190.0833.

Synthesis of ethyl (-)-2′,5′-dioxospiro-[2-oxabicyclo[3.1.0]hexane-4,4′-imidazolidine]-6-carboxylate-[5′-carbonyl-^{14}C] (**16**)

A mixture of potassium cyanide-[^{14}C] (50 mCi, specific activity 55.5 mCi/mmol, 0.9 mmol), potassium cyanide (0.171 g, 2.63 mmol), and ammonium carbonate (0.850 g, 8.50 mmol) in 4 ml of methanol was stirred at room temperature for 0.5 h. A methanol (16 ml) solution of ethyl (1*R*, 5*R*, 6*R*)-4-oxo-2-oxabicyclo[3.1.0]hexane-6-carboxylate ((+)-**5**, 0.600 g, 3.53 mmol) was added and stirring was continued at room temperature. After 24 h, the mixture was concentrated to ca. 10 ml, chilled to 0–5°C, and diluted with water (15 ml). The pH was adjusted to 7 with 5 N HCl, seeded with unlabeled product, and then stirred until crystalline and 1 h thereafter. The off-white crystalline material was collected by filtration, washed with water (2 × 10 ml), and dried *in*

vacuo to yield ethyl (-)-2′,5′-dioxospiro-[2-oxabicyclo[3.1.0]hexane-4, 4′-imidazolidine]-6-carboxylate-[5′-carbonyl-^{14}C] (**16**, 0.439 g, 52%). This material co-elutes with unlabeled product by HPLC on a Zorbax SB-phenyl column (4.6 × 250 mm) eluting with 0.1 M NaH_2PO_4/acetonitrile (97:3) at 1 ml/min; UV at 220 nm. HPLC showed that this material was 100% de.

Synthesis of (1R, 4R, 5S, 6R)-(-)-4-amino-2-oxabicyclo[3.1.0]-hexane-4,6-dicarboxylic-[4-carboxyl-^{14}C] acid, (2-[^{14}C])

A solution of ethyl (-)-2′,5′-dioxospiro-[2-oxabicyclo[3.1.0]hexane-4,4′-imidazolidine]-6-carboxylate-[5′-carbonyl-^{14}C] (**16**, 0.439 g, 1.84 mmol) in NaOH (9.2 ml, 2 N, 18.3 mmol) was stirred at just below reflux for 24 h. HPLC (see below) showed a significant amount of the corresponding hydantoin acid as well as the desired product. Stirring at reflux was continued for an additional 16 h and the mixture was allowed to cool to room temperature. HPLC showed that the slightly cloudy solution was completely converted to the desired product. The pH was adjusted to 9.2–9.3 with 5 N HCl and the precipitate was collected by filtration. The solid was washed with water (2 × 5 ml) and the filtrate was concentrated to ca. 5 ml *in vacuo*. The pH was adjusted to 3.2 with 5 N HCl (from pH 3.5, 1 N HCl was used) and stirred for 2 h (additional HCl was added as necessary to maintain the pH at 3.2). The mixture was chilled in an ice bath and stirred for an additional 2 h. The white solid was collected by filtration, washed with water, and dried *in vacuo* to yield (1*R*, 4*R*, 5*S*, 6*R*)-(-)-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic-[4-carboxyl-^{14}C] acid, (**2**-[^{14}**C**], 0.1298 g, 38%); ES-MS: $[M+H]^+$, $m/z = 186$ (major)/188. This material co-eluted with authentic **2** by radio-HPLC on a Zorbax SB-CN column (5 μm, 4.6 × 250 mm), eluting with 0.1% trifluoroacetic acid/acetonitrile (97:3) at 1 ml/min (column temperature ambient), UV detection at 220 nm. The sample was dissolved at 1 mg/ml (in buffer); 20 μl was injected. The RCP using the same conditions with radiochemical detection was 99.92%. There was a problem getting a reproducible specific activity that may have arisen from residual inorganics, so the material was recrystallized again. The white solid (0.1298 g) was mixed with **2** (0.1298 g) and dissolved in 2 N NaOH (1.5 ml, 3 mmol). The solution was diluted with water (1.1 ml) and the pH of the resulting stirred solution was adjusted to 3.5 with 5 N HCl. At pH = 4, material began crystallizing. The final adjustment of the pH to 3.2 was made with 1 N HCl. The mixture was stirred for 2 h, whereupon the white solid was collected by filtration, washed with water (2 × 2 ml), and dried *in vacuo* to yield (1*R*, 4*R*, 5*S*, 6*R*)-(-)-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic-[4-carboxyl-^{14}C] acid, (**2**-[^{14}**C**], 0.1459 g, 56%); specific activity = 36.89 μCi/mg; RCP = 99.79% (see above for HPLC details).

Preparation of ethyl (1R, 5S, 6S)-4-oxo-2-thiabicyclo[3.1.0]hexane-6-carboxylate ((+)-**6**)

An EtOH/CH_3CN (10:90, 80 ml) solution of 4-oxo-2-thiabicyclo[3.1.0]hexane-6-carboxylate (***rac*-6**, 4.0 g, 21.5 mmol) was purified by chiral HPLC on a Chiralpak AD column (5 × 24 cm) eluting with 10% EtOH/CH_3CN at 100 ml/min with UV detection at 240 nm. Eight samples of 10 ml were injected and the pure fractions of each compound was collected and concentrated *in vacuo*. The samples from the individual injections containing (+)-**6** were combined and concentrated *in vacuo* to yield ethyl (1*R*, 5*S*, 6*S*)-4-oxo-2-thiabicyclo[3.1.0]-hexane-6-carboxylate **((+)-6**, 1.7 g, 43%): ^{1}H-NMR ($CDCl_3$) δ 1.26 (t, $J = 7$ Hz, 3H, CH_3), 2.32 (t, $J = 3.5$ Hz, 1H, 6-H), 2.58 (dd, $J = 6.5$ and 3.5 Hz, 1H, 5-H), 3.20 (dd, $J = 6.5$ and 3.5 Hz, 1H, 1-H), 3.31 (bd, $J = 18$ Hz, 1H, 3β-H), 3.26 (d, $J = 18$ Hz, 1H, 3α-H and 4.15 (q, $J = 7$ Hz, 2H, CH_2Me); ^{13}C-NMR ($CDCl_3$) δ 14.1 (CH_3), 29.3 (C-1), 30.8 (C-6), 34.9 (C-3), 61.7 (CH_2), 168.8 (ester C = O) and 206.4 (ketone C = O). Upon analysis by analytical chiral HPLC on a Chiralpak AD column (4.6 × 250 mm) eluting with 10% EtOH/CH_3CN at 1.0 ml/min with UV detection at 210 nm, (+)-**6** eluted with a retention time of 5.51 min (ee = 99.9%).

In addition to the pure (+)-**6** enantiomer, (-)-**6** (1.9 g, 47%) was recovered; its retention time was 7.30 min on the analytical column (ee = 90%).

Synthesis of ethyl (-)-2′,5′-dioxospiro-2-thiabicyclo[3.1.0]hexane-4,4′-imidazolidine]-6-carboxylate-[5′-carbonyl-^{14}C] (**17**)

A mixture of ammonium carbonate (0.375 g, 2 mmol), 50 mCi of potassium cyanide-[^{14}C], and 0.083 g (1.28 mmol) of potassium cyanide in 5 ml of anhydrous methanol was stirred at room temperature for 10 min and then (1*R*, 5*S*, 6*S*)-4-oxo-2-thiabicyclo[3.1.0]hexane-6-carboxylate ((+)-**6**, 0.375 g, 2 mmol) was added as a yellow solid in one portion. The solution was stirred heterogeneously at room temperature overnight (about 23 h). The solution was diluted by adding 3 ml of water, and adjusted to pH 3.0 with 5 N HCl. A yellowish precipitate formed and was filtered, washed with 2.5 ml of water and 3 ml of *i*-PrOH, and dried in the air to give the ethyl (-)-2′,5′-dioxospiro-2-thiabicyclo[3.1.0]-hexane-4,4′-imidazolidine]-6-carboxylate-[5′-carbonyl-^{14}C] (**17**, 0.168 g, 26%) as an orange solid. HPLC analysis indicated it to be 99.2% chemical purity and 99.0% de.

Synthesis of (1R, 4S, 5S, 6S)-(-)-4-amino-2-thiabicyclo[3.1.0]-hexane-4,6-dicarboxylic-[4-carboxyl-^{14}C] acid, (3-[^{14}C])

Ethyl (-)-2′,5′-dioxospiro-2-thiabicyclo[3.1.0]hexane-4,4′-imidazolidine]-6-carboxylate-[5′-carbonyl-^{14}C] (**17**, 0.168 g) was dissolved in 4 ml of 2 N NaOH at room temperature, then heated to reflux (120°C, oil bath) for about 20 h. The

solution was cooled to room temperature, diluted with 10 ml of water, and adjusted to pH 9.3 with 5 N HCl. The white precipitate was filtered and the solid was washed with 5 ml of water. The combined filtrates were concentrated and the volume was adjusted to about 5 ml. The solution was then adjusted to pH 3.0 with 5 N HCl and stirred at room temperature until some white precipitate appeared. The solution was stirred in an ice bath for another 2 h, and the salt was then filtered, washed with 5 ml of water and 1 ml of *i*-PrOH and air dried. Further drying in the vacuum oven at 40°C for 2 h gave the desired product (1*R*, 4*S*, 5*S*, 6*S*)-(-)-4-amino-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylic-[4-carboxyl-^{14}C] acid (**3**-[^{14}**C**], 0.084 g, 63%) as a white solid; specific activity = 97.9 μCi/mg and RCP = 99.0% by HPLC and TLC-autoradiography (CH_3CN/EtOAc/HOAc/H_2O 21:7:7:9, $R_f = 0.32$). This material co-eluted with authentic **3** by radio HPLC on a Zorbax SB-phenyl column (4.6 × 250 mm), eluting with 0.1 M NaH_2PO_4 (pH 2.14)/CH_3CN (90:10, 40°C) at 1 ml/min with radiochemical and UV detection (220 nm). The retention time was 2.99 min. ES-MS: $[M + H]^+ = 204/206$.

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References

1. Hollmanm M, Heinemann S. *Annu Rev Neurosci* 1994; **17**: 31–108.
2. Conn PJ, Pin PJ. *Annu Rev Pharmacol Toxicol* 1997; **37**: 205–237.
3. Monn JA, Valli MJ, Massey SM, Wright RA, Salhoff CR, Johnson BG, Howe T, Alt CA, Rhodes GA, Robey RL, Griffey KR, Tizanno JP, Kallman MJ, Helton DR, Schoepp DD. *J Med Chem* 1997; **40**: 528–537.
4. Grillon C, Cordova J, Levine LR, Morgan CA. *Psychopharmacology (Berlin)* 2003; **168**: 446–454.
5. Johnson JT, Mattiuz EL, Chay SH, Herman JL, Wheeler WJ, Kassahun K, Swanson SP, Phillips DL. *Drug Disp Metab* 2002; **30**: 27–33.
6. Monn JA, Valli MJ, Massey SM, Hansen MM, Kress TJ, Wepsiec JP, Harkness AR, Grutsch JL, Wright RA, Johnson BG, Andis SL, Kingston A, Tomlinson R, Lewis R, Griffey KR, Tizanno JP, Schoepp DD. *J Med Chem* 1999; **42**: 1027–1040.
7. Kingston AE, O'Neill MJ, Lam A, Bates KR, Monn JA, Schoepp DD. *Eur J Pharmacol* 1999; **377**: 155–165.
8. Wheeler WJ, Monn JA, Valli MJ, O'Bannon DD. *Abstracts of the 5th International Conference for Isotopes*, Brussels, Belgium, April 2005.
9. Wheeler WJ, Spence MC, Kuo F. *Abstracts of the 5th International Conference for Isotopes*, Brussels, Belgium, April 2005.

10. Malherbe P, Knoflach F, Broger C, Ohresser S, Kratzeisen C, Adam G, Stadler H, Kemp JA, Mutel V. *Mol Pharmacol* 2001; **60**: 944–954.
11. Massey SM, Monn JA, Valli MJ. US Patent 5958960; 28 September, 1999.
12. Dominguez C, Ezquerra J, Prieto L, Espada M, Petregal C. *Tetrahedron Asymmetry* 1997; **8**: 511–514.
13. Massey SM, Monn JA, Valli MJ. US Patent 5688826; 18 November, 1997.
14. Monn JA, Schoepp DD. US Patent 5750566, 12 May, 1998.
15. B'Hymer C, Montes-Bayon M, Caruso JA. *J Sep Sci* 2003; **26**: 7–19.

