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**Research Article**

# A method for the synthesis of 2-amino-4-fluoro-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid-[$^3H_2$]

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## Summary
A process for double deuterium labeling of an alcohol was developed. The process was utilized in the subsequent tritium labeling of a secondary alcohol with high specific activity (24 Ci/mmol) by reduction of the corresponding ketone using sodium borotritide. The starting ketone was first brominated with pyridinium tribromide; the resulting alpha bromoketone was then reduced in THF/alcohol in the presence of $Ni(OAc)_2$. The alcohol was then converted to 2-amino-4-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid-[$^3H_2$], an mGluR agonist. Copyright © 2004 John Wiley & Sons, Ltd.

**Key Words:** tritium labeling of secondary alcohol; sodium borotritide reduction; mGluR agonist

## Introduction
Metabatropic glutamate receptors (mGluR) are a family of G-protein coupled excitatory amino acid receptors. The family has eight distinctive receptor subtypes, divided into three families based on sequence similarity, primary signal transduction pathways and pharmacology. (1*R*, 2*S*, 4*R*, 5*R*, 6*R*)-2α-amino-4α-fluorobicyclo[3.1.0]-hexane-2,6-dicarboxylic acid, **20**, is a potent $mGluR_{2,3}$ agonist (Group II).[1] To support *in vitro* receptor binding studies, a tritium isotopomer with high specific activity (over 20 Ci/mmol) was required. In this report, the synthesis of this material is described. The key to the tritiation of **20** was to find a method for the incorporation of tritium into the protected alcohol **3**. Once **3** was available, its conversion to **20** was easily accomplished. Alcohol **3** was easily obtained by reduction of the

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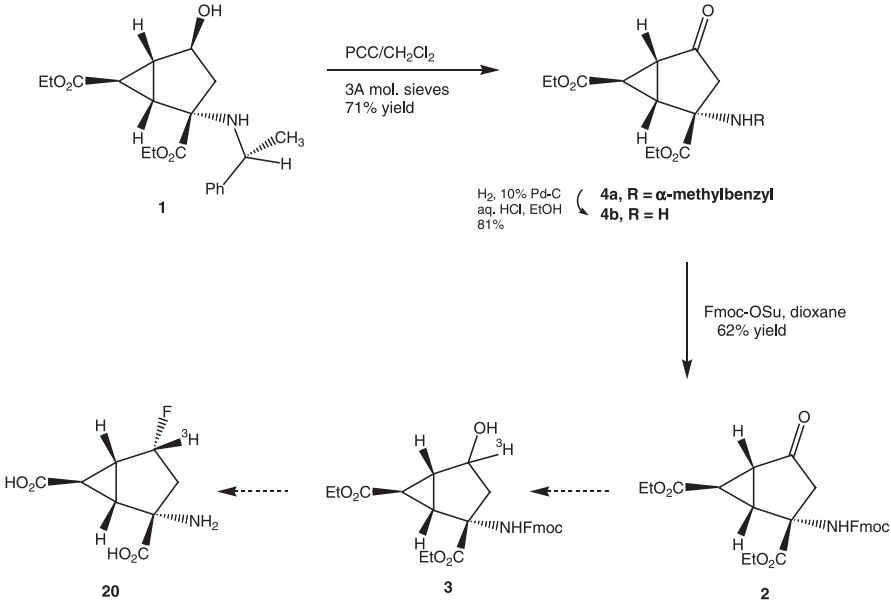
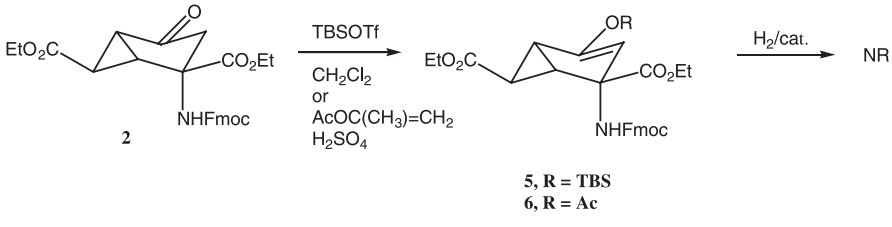
corresponding ketone **2**, which was prepared from an intermediate (**1**) used in the synthesis of **20**.

## Discussion

Direct reduction of **2** with sodium borotritide ($NaBT_4$) should give a mono-tritium labeled product **3**, but the specific activity would not meet the requirements, since commercially available $NaBT_4$ has a specific activity of 40–60 Ci/mmol, which would correspond to about 10–15 Ci/mmol in the product. Tritium hydrogen exchange at the position alpha to the ketone in **2**, followed by reduction of **2**-[$^3$**H**] with $NaBT_4$ would also yield **3**-[$^3$**H**]; however, the specific activity would still not be high enough due to the limitations of commercially available tritiated water (5–15 Ci/ml). Clearly, a new method to introduce two tritium atoms was required.




It is known that enol esters or silyl enol ethers of ketones can be reduced by hydrogenation to yield the corresponding acetates or silyl ethers,[2] which upon cleavage would give secondary alcohols containing two new hydrogen atoms. Reaction of ketone **2** with TBSOTf yielded silyl enol ether **5** (Scheme 1). No



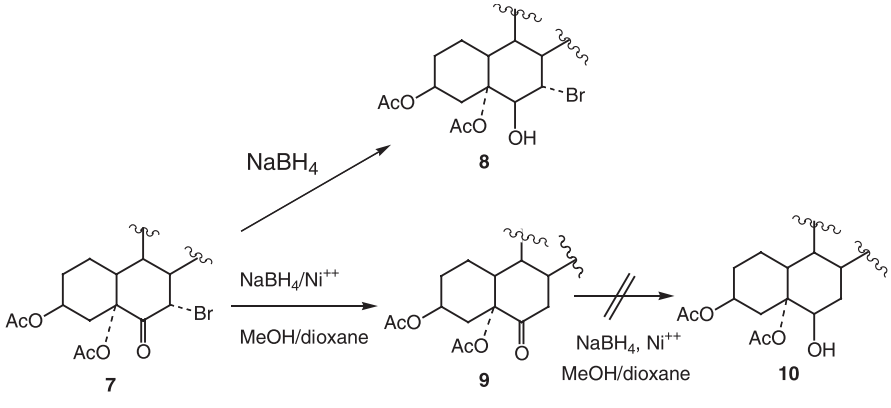

**Scheme 1.**

reaction occurred when **5** was treated with $H_2$/5% Pd-C (or 5% $Rh/Al_2O_3$) in EtOAc at room temperature.

Alternatively, ketone **2** was converted to enol acetate **6** by reaction with isopropenyl acetate. Unfortunately, no reaction occurred upon reaction of **6** with $H_2$/5% Pd-C at room temperature. Higher temperature or pressure may be required due to the steric hindrance of the molecule.

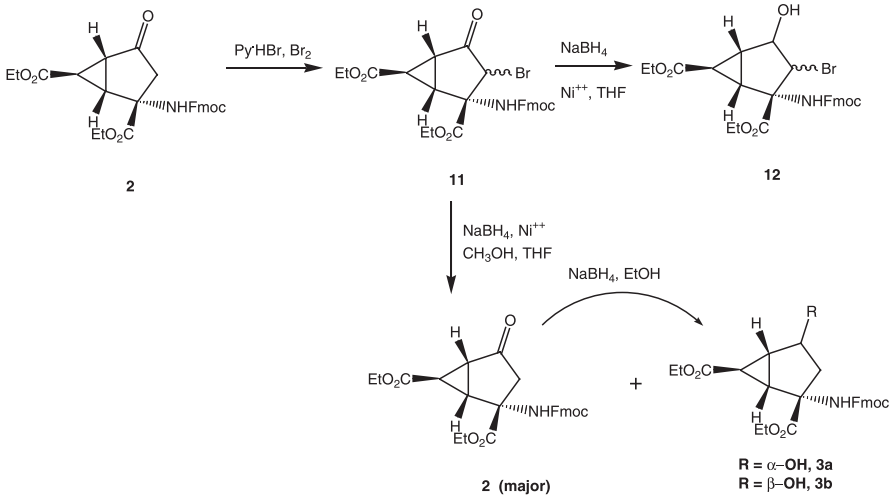
In view of the results described above, we envisioned that if a leaving group at the position alpha to the ketone in **2** could be substituted with tritium, then subsequent reduction of the ketone with $NaBT_4$ would provide **3**-[$^3$**H**] with the desired specific activity. Although the reduction of alpha-bromoketones usually yields bromohydrins, in some cases the debromination product has been reported.[3] In a detailed study, on the reduction of **7**, Goto and Kishi,[4] reported that a trace of nickel ion alters the course of the borohydride reduction of an alpha-bromoketone and yields a de-brominated product **9** instead of the expected bromohydrin **8**. Similar de-bromination of an alpha-bromoketone was observed by Sarma *et al.*[5] during their studies on the *in situ* nickel boride generated from nickel chloride and sodium borohydride (Scheme 2). It was proposed that further reduction of ketone **9** to the alcohol **10** should occur if the nickel salt could be removed from the reaction media after forming the ketone intermediate.




**Scheme 2.**

Bromoketone **11** was prepared by the bromination of ketone **2** with pyridinium tribromide.[6] The bromoketone **11** was then treated with $NaBH_4$ in a mixed solvent of 1:1 methanol/THF in the presence of nickel acetate tetrahydrate at room temperature (Scheme 3). The reaction was monitored by LC-MS. The de-bromination product **2** was detected as the major product along with minor amounts of alcohol **3** as well as a trace amount of bromohydrin **12** (and minor amounts of the methyl ester of **2**). The crude reaction mixture was passed through a short column of silica gel and eluted

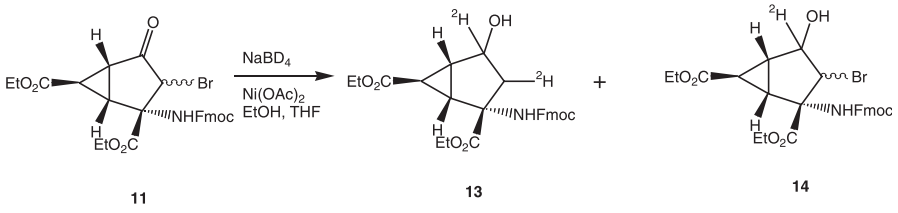



**Scheme 3.**

with EtOAc to remove the nickel salt. The crude product **2** was treated with $NaBH_4$ in EtOH to yield **3** as a mixture of diastereomer isomers in a 1:1 ratio as analyzed by LC-MS. In order to eliminate the possibility of trans-esterification, the reaction was carried out in THF alone; bromohydrin **12** became the major product, along with minor amounts of alcohol **3**, presumably because of the poor solubility of $Ni(OAc)_2 \cdot 4H_2O$ in THF.

When the reduction of **11** was carried out in 1:1 THF/EtOH using $NaBD_4$/$Ni(OAc)_2 \cdot 4H_2O$ (Scheme 4), the deuterated alcohol **13** became the major product as a mixture of two diastereomers in about a 1:1 ratio (as seen by LC-MS). Only a trace amount of the corresponding bromohydrin **14** was detected. One possible explanation might be that while nickel acetate had similar solubility in either ethanol or methanol, sodium borohydride has a 4 fold better solubility in methanol than in ethanol,[7] and it reacts with methanol at an appreciable rate and only slowly with ethanol.[8] Thus, most of the excess sodium borohydride was consumed by methanol before it could reduce the ketone, but it survived longer in ethanol and was able to reduce the ketone. That might explain why Goto and Kishi[4] did not detect the formation of alcohol **10** in their experiments when methanol was used as part of the solvent. Further analysis of the deuterium incorporation in **13** by LC-MS indicated that both diastereomers had about 80% of di-deuterium and 20% of mono-deuterium labeled, which corresponds to about 22.5 Ci/mmol of specific activity in tritium if a sodium borotritide ($NaBT_4$) of 50 Ci/mmol was used. In a similar experiment, when less $NaBD_4$ was used, the reaction stopped at the ketone stage (as shown previously). Although the ketone intermediate was further reduced to alcohol **13** with additional $NaBD_4$, the ratio of the labeling changed to 70% $D_1$ and 30% $D_2$. This suggests that H–D exchange occurred




11 13 14

**Scheme 4.**

in ketone intermediate. Very reproducible results, 70 to 80% $D_2$ and 20 to 30% $D_1$, were obtained when an excess of $NaBD_4$ was used at the onset of the reduction. Apparently, to minimize the H–D exchange, and thus maximize the deuterium incorporation, an excess of reducing agent was required in the beginning of the reaction.

Reaction of α-bromoketone **11** with $NaBT_4/Ni(OAc)_2/EtOH/THF$ at room temperature (Scheme 5),[†] yielded a mixture of the alcohols (**15**, **16**) and a trace of the bromohydrin (**17**, **18**). Purification of the reaction mixture and subsequent reaction of **15** with Deoxo-Fluor® ([*Bis*-(2-methoxyethyl)amino]-sulfur trifluoride)/$CH_2Cl_2$ yielded **19**.[1] Following HPLC purification,[‡] the radiochemical purity of **20** was 98.0%; the specific activity was 24 Ci/mmol. ES-MS showed a $[M+H]^+$ at $m/z$ 208. The $^3$H-NMR spectrum in $CDCl_3$ (Figure 1) confirms the introduction of tritium in the 3-α and 4 positions.

## Conclusion

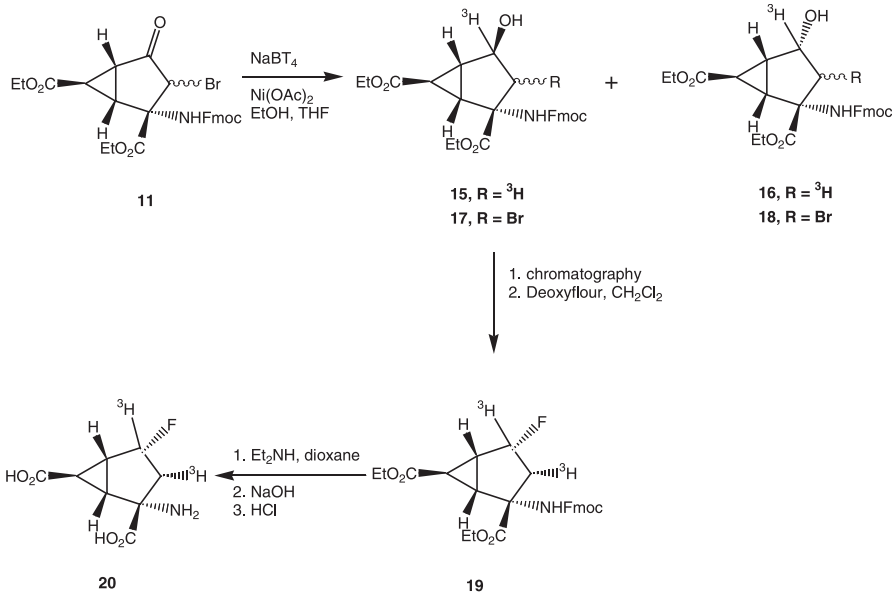
A general and simple method has been developed for the synthesis of a double-deuterium-(or tritium) labeled alcohol from the corresponding ketone **2**. Ketone **2** was brominated with pyridinium tribromide in acetic acid to give 3-bromoketone **11**, and this ketone **11** was reduced directly to the corresponding alcohol **15** or **16** by an excess of sodium borodeuteride or sodium borotritide in ethanol/THF in the presence of nickel acetate. The ketone **2** from debromination was the major product if the reaction was carried out in methanol, and bromohydrin **12** was the major product if the reaction was carried out in THF alone.

## 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). NMR spectra were obtained on a General Electric QE-400 nuclear magnetic resonance spectrometer at 400 MHz.

[†]The tritium labeling was performed by Amersham Bioscience.
[‡]HPLC conditions: Column: Hypercarb 7 μ, (100 × 4.6 mm). Solvent A: 0.1% trifluoroacetic acid in water. Solvent B: 0.1% trifluoroacetic acid in acetonitrile. Gradient: 0% B for 15 min then to 100% B over 20 min. Flow rate: 1 ml/min UV detector at 200 nm. $R_T = 7.43$ min.




**Scheme 5.**

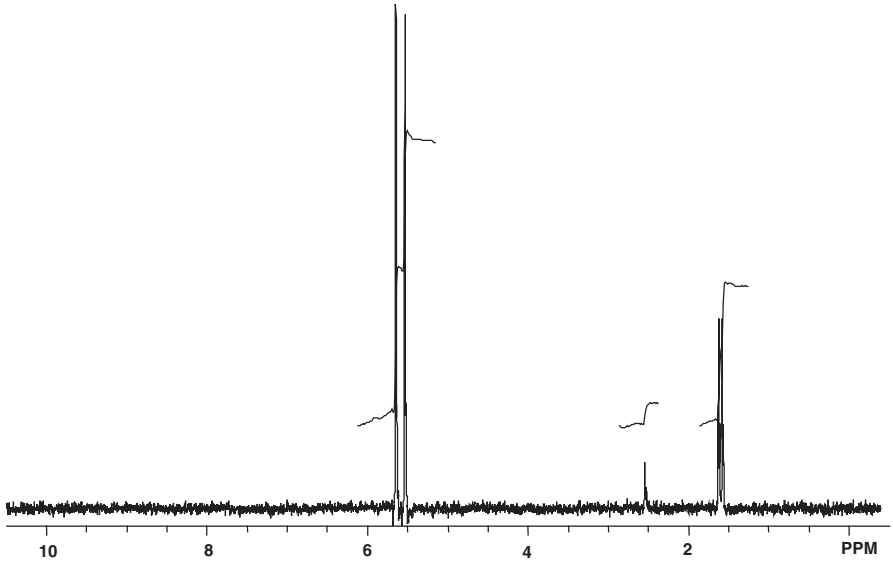



**Figure 1. $^{3}$H-NMR Spectrum for compound 20. The chemical shift for the 3$\beta$ proton occurs at $\delta$2.76 ppm**

Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Direct chemical ionization mass spectra (DCI-MS) and electron impact mass spectra (EI-MS) were recorded on a Nermag R30-10 triple stage quadrapole mass spectrometer. Flash chromatography was

performed on Biotage system, or as described by Still *et al.*, using E.M. Science silica gel 60 (230–400 mesh).

*Synthesis of (1S, 2S, 5R, 6R)-4-oxo-2-(R-1-phenylethylamino)bicyclo[3.1.0]-hexane-2,6-dicarboxylic acid, diethyl ester (**4a**)*

A mixture of **1** (0.750 g, 2.07 mmol) and crushed 3 Å molecular sieves (0.975 g) in $CH_2Cl_2$ (45 ml) was treated with pyridinium chlorochromate (PCC, 1.375 g, 6.30 mmol). The mixture was stirred for 25 h and then treated with Hi-Flo supercel. After stirring for 5 min, the mixture was filtered. The filter cake was washed with an additional 25 ml of $CH_2Cl_2$ and the combined filtrate was concentrated *in vacuo*. The crude product was crystallized from EtOH to yield **4** (0.566 g, 71%). ES-MS: $[M+Na]^+$, $m/z = 382$; $[M+H]^+$, $m/z = 360$.

HR ES-MS: Anal. Calculated for $C_{20}H_{26}NO_5$ ($[M+H]^+$) 360.1811. Found: 360.1835.

*Synthesis of (1S, 2S, 5R, 6R)-2-amino-4-oxobicyclo[3.1.0]hexane-2,6-dicarboxylic acid, diethyl ester (**4b**)*

An ethanol (80 ml) solution of **4** (0.425 g, 1.23 mmol), HCl (1 N, 1.2 ml) was treated with 10% Pd-C (0.028 g) and hydrogenated at 60 psi for 24 h. The mixture was filtered and concentrated to dryness to yield **4b** as a white solid (0.291 g, 81%). ES-MS: $[M+H]^+$, $m/z = 256$. The NMR spectrum in $CD_3OD$ was consistent with the product with no aromatic resonances.

$^1$H-NMR spectrum ($CD_3OD$) δ 1.32 (m, 7 H, ester-$CH_3$'s and H-3α), 2.52 (d, 1 H, $J = 3$ Hz, H-5), 2.70 (d, 1 H, $J = 3$ Hz, H-1), 2.75 (m, 1 H, H-6), 2.92 (m, 1 H, H-3β), 4.24 and 4.40 (t, 2 H, ester $CH_2$'s).

HR ES-MS: Anal. Calculated for $C_{12}H_{18}NO_5$ ($[M+H]^+$) 256.1185. Found: 256.1185.

*Synthesis of (1S, 2S, 5R, 6R)-2-(9H-fluoren-9-ylmethoxycarbonyl)-4-oxo-bicyclo[3.1.0]-hexane-2,6-dicarboxylic acid, diethyl ester (**2**)*

A mixture of **4b** (0.291 g, 1 mmol), Fmoc-OSu (0.337 g, 1 mmol), and NaOH (1 N, 1 ml) was dissolved in dioxane (10 ml) and stirred at room temperature for 2 days. The reaction mixture was diluted with EtOAc and washed with water. The organic layer was dried (anhydrous $MgSO_4$) and concentrated *in vacuo*. The crude product (0.570 g) was purified by Biotage chromatography on silica gel, eluting with $Et_2O$/heptane (8:2). Fractions 1–7 were combined and concentrated *in vacuo* to yield **2** as a white solid (0.496 g), which was crystallized from EtOAc/hexanes to yield crystalline **2** (0.425 g, 89%). ES-MS: $[M+H]^+$, $m/z = 478$; ES-MS: $[M+Na]^+$, $m/z = 500$.

HR ES-MS: Anal. Calculated for $C_{27}H_{27}NO_7Na$ ($[M+Na]^+$) 500.1685. Found: 500.1669.

*Synthesis of (1S, 2S, 4RS, 5R, 6R)-2-(9H-fluoren-9-ylmethoxycarbonyl)-4-hydroxy-bicyclo[3.1.0]-hexane-2,6-dicarboxylic acid, diethyl ester (**3a, 3b**)*

A DME (2 ml) solution of **2** (0.048 g, 0.10 mmol) and $NaBH_4$ (0.004 g, 0.10 mmol) was stirred at room temperature for 2 days. The reaction mixture was quenched with acetone (1 ml) and diluted with EtOAc. HCl (1 N, 1 ml) was added and the two phase mixture was stirred for 1 h. The layers were separated and the organic layer was washed with $H_2O$, dried (anhydrous $MgSO_4$), and concentrated *in vacuo*. The crude product showed 2 spots on TLC (silica gel, $Et_2O$ saturated with $H_2O$) and no unreacted starting material. ES-MS showed a $[M+H]^+$, $m/z = 480$; ES-MS: $[M+Na]^+$, $m/z = 502$. The two diastereomers of **3** were separated by HPLC (column: Discovery C18, $10 \times 250$ mm, 5 μm. Flow rate = 4 ml/min. Mobile phase A: 10 mM ammonium acetate, mobile phase B: methanol, isocratic at A/B = 30/70 for 18 min, then flush the column at A/B = 10/90) ($R_T = 12.4$ min for **$C_4$α-OH** (***3a***) isomer and $R_T = 13.7$ min for **$C_4$β-OH** (***3b***) isomer).

**3a**: $^1$H-NMR spectrum ($CD_3OD$) δ 1.22 (t, 3 H, ester-$CH_3$), 1.24 (t, 3 H, ester-$CH_3$), 1.25 (m, 1 H, 3α-H), 2.04 (m, 1 H, 6-H), 2.14 (m, 1 H, 5-H), 2.36 (m, 1 H, 1-H), 2.53 (m, 1 H, 3β-H) 4.05 (m, 2 H, Fmoc-$CH_2$), 4.10 (q, 2 H, ester-$CH_2$), 4.11 (q, 2 H, ester-$CH_2$), 4.32 (m, 1 H, Fmoc-CH), 4.61 (m, 1 H, 4-H), 7.30 (m, 2 H, 2′-H, 9′-H), 7.38 (t, 2 H, 4′-H, 7′-H), 7.65 (m, 2 H, 3′-H, 8′-H) and 7.79 ppm (d, 2 H, 5′-H, 6′-H); ES-MS: $[M+H]^+$, $m/z = 480$.

**3b**: $^1$H-NMR spectrum ($CD_3OD$) δ 1.22 (t, 3 H, ester-$CH_3$), 1.25 (t, 3 H, ester-$CH_3$), 1.58 (m, 1 H, 6-H), 1.73 (m, 1 H, 3β-H), 2.09 (m, 1 H, 5-H), 2.27 (m, 1 H, 3α-H), 4.11 (m, 2 H, Fmoc-$CH_2$), 4.16 (m, 2 H, ester $CH_2$), 4.17 (2 H, ester $CH_2$), 4.26 (m, 1 H, 4-H), 4.32 (m, 1 H, Fmoc-CH), 7.30 (m, 2 H, 2′-H, 9′-H), 7.38 (t, 2 H, 4′-H, 7′-H), 7.65 (m, 2 H, 3′-H, 8′-H) and 7.79 ppm (d, 2 H, 5′-H, 6′-H); ES-MS: $[M+H]^+$, $m/z = 480$.

HR ES-MS: Anal. Calculated for $C_{27}H_{29}NO_7Na$ ($[M+Na]^+$) 502.1842. Found: 502.1825.

*Synthesis of (1S, 2S, 4RS, 5R, 6R)-2-(9H-fluoren-9-ylmethoxycarbonyl)-4-hydroxy-bicyclo[3.1.0]-hexane-2,6-dicarboxylic-[3,4-$^2H_2$] acid, diethyl ester (**13a, 13b**)*

A solution of ketone **2** (4.8 mg) and pyridinium tribromide (4.1 mg) in 2 ml of glacial acetic acid was stirred at 70°C (oil bath) for 3 h. TLC (silica gel, 3:1 hexanes/EtOAc) showed only trace amount of the ketone **2** remaining. The solution was diluted with ethyl acetate, washed with saturated solution of $NaHCO_3$ and water. The organic layer was concentrated, and the residue was dissolved in ethanol and concentrated (this was repeated three times). The final residue was dried *in vacuo* overnight to give a crude bromoketone **11**. This crude product was used in the next step without purification. ES-MS:

$[M+H]^+ = 556/558$, two diastereomers. The crude bromoketone, **11**, was dissolved in anhydrous ethanol to a total volume of 4 ml. To a 0.5 ml aliquot of this ethanol solution was added a catalytic amount of $Ni(OAc)_2 \cdot 4H_2O$, followed by 0.5 ml of anhydrous THF. To the above solution was then added an excess of $NaBD_4$ in one portion at room temperature. After stirring for 10 min, TLC (silica gel, 3:1 hexanes/EtOAc) indicated no bromoketone **2** remained. Two new spots with lower $R_f$ were detected. The reaction was quenched by adding 10% acetic acid dropwise. The solution was passed through a short column of silica gel and concentrated. ES-MS: $[M+H]^+ = 481/482$ for $D_1$ and $D_2$ isomers in a ratio of 20 to 30% $D_1$ and 70 to 80% $D_2$ isomers. Two diasteriomeric alcohols **13** were in a ratio of about 1:1 by ES-MS. In another experiment, when less $NaBD_4$ was used, the reaction stopped at the ketone stage. Although the ketone intermediate was further reduced to alcohol with additional $NaBD_4$, the ratio of the labeling changed to 70% $D_1$ and 30% $D_2$.

*Synthesis of (1S, 2S, 4R, 5R, 6R)-2-(9H-fluoren-9-ylmethoxycarbonyl)-4-hydroxy-bicyclo[3.1.0]-hexane-2,6-dicarboxylic acid, diethyl ester (non-labeled **19**)*

To a solution of **3b** (0.050 g) in 1 ml of anhydrous $CH_2Cl_2$ at $-20^\circ C$ was added 12 µl (3 drops via a micro-syringe) of Deoxo-Fluor® ([*Bis*-(2-methoxyethyl)amino]sulfur trifluoride) in 5 min. The reaction was monitored by TLC (silica gel, 3:1 hexanes/EtOAc). After stored in a $-20^\circ C$ cooling bath for 90 min, TLC indicated some alcohol remained. Additional drops of Deoxo-Fluor® were added. No further progress was observed even after another 3 h. The solution was stored in refrigerator overnight. A solution of 15% of $Na_2CO_3$ was added dropwise to the flask to quench the reaction in an ice bath. The resulting solution was further diluted with water, and the aqueous layer was extracted twice with $CH_2Cl_2$. The combined extracts were dried over $Na_2SO_4$, filtered, and concentrated. The residue was chromatographed (silica gel, 4:1 hexanes/EtOAc with 2% of $CH_2Cl_2$) to afford a desired non-labeled product **19** as a white form (0.035 g, 72.8%). ES-MS: $[M+H]^+ = 482$.

HR ES-MS: Anal. Calculated for $C_{27}H_{28}FNO_6Na$ ($[M+Na]^+$) 504.1798. Found: 504.1786.

*Synthesis of 2α-amino-4α-fluoro-bicyclo-[3.1.0]hexanes-2,6-dicarboxylic acid*

A solution of non-labeled **19** (0.003 g in 0.5 ml of dioxane) was stirred with 0.5 ml of diethylamine at room temperature overnight. TLC (silica gel, 3:1 hexanes/EtOAc) indicated no starting material remained, ES-MS: $[M+H]^+ = 260$. The solution was concentrated *in vacuo* to remove diethylamine. The residue was taken into 0.5 ml of dioxane (or methanol) and 0.1 ml of 1 N NaOH, and the resulting solution was stirred at room temperature

overnight. The reaction was quenched by adding 0.1 ml of 1 N HCl. ES-MS indicated no starting material remained. The crude product, $[M-H]^- = 202$, co-eluted with an authentic sample[1] of LY459477 on HPLC (TosoHaas Amide 80, 4.6 × 250 mm, mobile phase: 0.1% TFA in 80:20 $CH_3CN/H_2O$, isocratic at 0.5 ml/min for 15 min, then flush the column at 90/10 $CH_3CN/H_2O$ containing 0.1% of TFA, UV at 205 nm but very weak at 1 mg/ml, $R_T = 9.5$ min). $^1$H-NMR spectrum ($D_2O$/DCl): δ 1.79 (m, 1 H, 3α-H), 2.44 (m, 1 H, 6-H), 2.52 (m, 1 H, 1-H), 2.65 (m, 1 H, 5-H), 2.76 (m, 1 H, 3β-H), 5.68 (m, 1 H, 4-H).

*Synthesis of (1S, 2S, 4S, 5R, 6R)-2-(9H-fluoren-9-ylmethoxycarbonyl)-4-hydroxy-bicyclo[3.1.0]-hexane-2,6-dicarboxylic-[3,4-$^3H_2$] acid, diethyl ester (**15-[3,4-$^3H_2$]**)*

A solution of **11** (prepared as described above from 0.033 g, 0.069 mmol of ketone **2**) in EtOH (2 ml) was treated with $NaBT_4$ (5 Ci, 64 Ci/mmol, 0.078 mmol) and stirred for 10 min.† Another 5 Ci of $NaBT_4$ was added and stirring was continued for an additional 10 min. The reaction mixture was quenched with 50% aqueous HOAc (200 µl) and the labile tritium was removed by rotary evaporations with $CH_3OH$. The product was eluted through a silica sep-pac with EtOAc/hexane (3:2) and the eluate was evaporated to dryness and re-dissolved in EtOH. This product was further purified by chromatography on silica gel, eluting with EtOAc/hexane to yield pure **15**-[*3*, *4*-$^3$**H$_2$**] (198 mCi).

*Synthesis of (1R, 2S, 4R, 5R, 6R)-2-(9H-fluoren-9-ylmethoxycarbonyl)-4-fluoro-bicyclo[3.1.0]-hexane-2,6-dicarboxylic-[3,4-$^3H_2$] acid, diethyl ester (**19-[3,4-$^3H_2$]**)*

Deoxoflour (2 µl) was added to a stirred solution of **15**-[*3*, *4*-$^3$**H$_2$**] (198 mCi) in $CH_2Cl_2$ (1.3 ml) at −20°C.† No reaction was observed after several hours and the reaction mixture was allowed to warm to room temperature and treated with additional deoxoflour (2 × 10 µl) over 2 h. After standing overnight, an additional 10 µl of deoxoflour was added. About 80% of the starting material had been consumed, so the reaction was quenched by the addition of 15% aqueous $Na_2CO_3$ (100 µl), diluted with $H_2O$ and extracted with $CH_2Cl_2$. The organic extract was washed with $H_2O$ and dried (anhydrous $Na_2SO_4$). The crude product was purified by chromatography on silica gel (18 × 250 mm), eluting with EtOAc/hexane (1:1) to yield **19**-[*3*, *4*-$^3$**H$_2$**] (55 mCi).

*(1R, 2S, 4R, 5R, 6R)-2α-amino-4α-fluorobicyclo[3.1.0]-hexane-2,6-dicarboxylic-[3,4-$^3H_2$] acid, **20***

Sodium hydroxide (1 N) was added to a methanol solution of **19**-[**3**, **4**-$^3$**H$_2$**] (55 mCi).† The reaction mixture was stirred at room temperature overnight and subsequently quenched by the addition of 2 N HCl (100 µl). The resulting

solution was diluted with $H_2O$ and washed with $Et_2O$. The aqueous solution was concentrated and re-dissolved in $H_2O$ and purified by HPLC on a Hypercarb column (7 μ, 10 × 150 mm) eluting with a gradient from $H_2O$/TFA to $CH_3CN$/TFA. The desired fractions were evaporated and the residue re-dissolved in $H_2O$/EtOH (1:1) to yield **20**-[**3**, **4**-$^{3}$**H**$_2$] (25 mCi). The radiochemical purity was 98.0%[‡] and the specific activity (determined by mass spectrometry) was 24 Ci/mmol. The $^{3}$H-NMR spectrum($D_2O$) showed two resonances at δ 1.79 (3α-$^{3}$H) and 5.68 (4-$^{3}$H) ppm.

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