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Preparation of carbon-14 labeled (3*R*)-7-hydroxy-N-(1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl-1,2,3,4-tetrahydroisoquinolinecarboxamide (JDTic)

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Starting with [^{14}C]-D-tyrosine, carbon-14 labeled JDTic dihydrochloride with specific activity 15 mCi/mol was prepared in 5% radiochemical yield. Separation of the (3*R*)- and (3*S*)-diastereomers was carried out via the 3-phenyl-2,3,10,10a-tetrahydro-5H-imidazo[1,5-b]isoquinolin-1-ones formed by reaction with benzaldehyde.

Keywords: JDTic; κ antagonist; carbon-14

Introduction

The potent and selective κ opioid receptor antagonist (3*R*)-7-hydroxy-N-(1*S*)-1-{[(3*R*;4*R*)-4-(3-hydroxyphenyl)-3;4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (JDTic) has been found to have long lasting antinociceptive effects; mandating metabolism and distribution studies.[1] To this end the preparation of carbon-14 labeled JDTic was undertaken.

Considerations of metabolic stability precluded the labeling of pendant groups (e.g. i-propyl or methyl). Based on the synthesis of JDTic[2] it was decided to incorporate carbon-14 at the isoquinoline moiety, i.e. to use [^{14}C]-D-tyrosine as the starting point for the synthesis (Scheme 1).

Results and discussion

Conversion of tyrosine (**1**) to N-Boc-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**5**) has been carried out via 3,5-dihalogenated tyrosine derivatives using the Pictet Spengler reaction (Scheme 1).[3] Thus, 3,5-diiodotyrosine (**2a**) is reported to afford, 6,8-diiodo-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**3a**) hydrochloride in ~55% yield[3–5] while the dibromo analog **2b** gave **3b** in 30% yield.[3] Preparation of the required Boc-protected intermediate **5** has been reported to proceed in 54% yield by Boc protection of **3a** followed by dehalogenation. Experience in the synthesis of JDTic in our laboratories essentially replicated these results. Based on this information the radiochemical yield of **5** would have been ~29%, at best. In light of the expense of the starting carbon-14 labeled D-tyrosine, it was sought to improve the overall yield of **5**. After some experimentation it was found that the yield of cyclized product was significantly improved when the Pictet Spengler reaction was carried out in the presence of strong acid(s) under anhydrous conditions. Thus, cyclization of **2b** using a small excess of paraformaldehyde in the presence of hydrobromic acid in trifluoroacetic acid afforded **3b** in 89% when the reaction was carried out for 15 h at 50°C. Similar results were obtained with the diiodo analog **2a** although the purity of **3a** was somewhat lower than that of **3b**. Improved yields (>90%) were achieved in the dehalogenation reaction by replacing the triethylamine[3] (used to scavenge the acid formed in the dehalogenation) by cesium carbonate.

Based on these encouraging results the carbon-14 synthesis was carried out using commercially procured carboxyl carbon-14 labeled D-tyrosine ([^{14}C]-**1**). Bromination gave the hydrobromide salt of [^{14}C]-**2b** in 96% yield and reaction with paraformaldehyde in the presence of trifluoroacetic acid and hydrobromic acid in acetic acid for 12 h at 80°C gave [^{14}C]-**3b** hydrobromide in 88% yield. Protection followed by reductive dehalogenation at atmospheric pressure afforded the required [^{14}C]-**5** in 50% overall yield (from [^{14}C]-**1**). Coupling with 3-[1-(2*S*-amino-3-methylpropyl)-3*R*,4*R*-dimethyl-4-piperidinyl]phenol (**6**) using O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′,-tetramethyluronium hexafluorophosphate (HATU reagent) proceeded in 56% yield. Pilot deprotection of a small portion of the product [^{14}C]-**7** afforded material that was ~85% radiochemically pure. However, HPLC analysis indicated that the product consisted of a 4:1 mixture of diastereomers, which were identified as D- and

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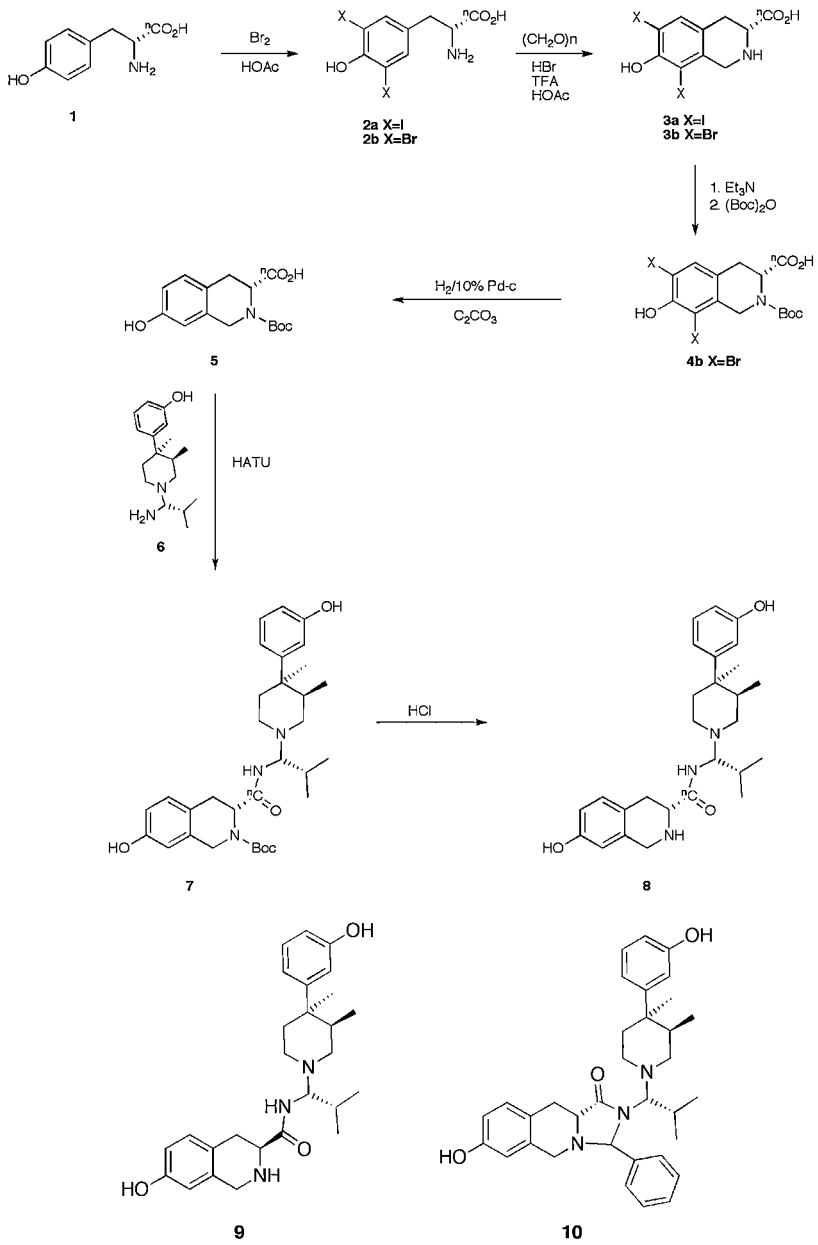

Note: Compound numbers for labeled compounds (n=14) are preceeded by [^{14}C].

Scheme 1.

L-isomers of the tetrahydroisoquinoline carboxylic acid moiety. This was surprising since it had been reported that virtually no racemization occurred when reaction of **2a** with formaldehyde was carried out at 72°C for longer time (18.5 h).[3] In subsequent studies it was found that 7% racemization occurred when the cyclization was performed at 55°C for 72 h.[6] The reason(s) for this discrepancy are unknown.

Attempts to separate the diastereomers by use of chiral acids failed, as did attempted reversed phase chromatography. During some of these investigations it was noted that treatment of the mixture of diastereomers with acetone led to the formation of two distinctly different adducts and that adduct formation was reversible. Other carbonyl containing compounds, e.g. acetophenone, cyclohexanone and benzaldehyde also reacted to give adducts. In most cases significant amounts of starting material remained unreacted and failed to react further even with addition of a large excess of the carbonyl containing compound. Condensation with benzaldehyde gave the most favorable results in that it furnished a high yield of adducts. Fractional crystallization separated an adduct that, when hydrolyzed, yielded JDTic (**8**), virtually free of JLTic (**9**) accompanied by several minor lipophilic impurities. These were readily removed by normal phase chromatography. X-ray crystallography identified the benzaldehyde adduct **10** as resulting from condensation with both the isoquinoline nitrogen and the amide nitrogen. In a tracer run carbon-14 labeled JDTic-adduct [^{14}C]-**10** was obtained in 75% yield and >95% purity. Encouraged by these results the main fraction of [^{14}C]-**7** was deprotected and the resulting carbon-14 labeled product, a mixture of 80% [^{14}C]-**8** and 20% [^{14}C]-**9** was converted to the benzaldehyde adduct. Fractional crystallization gave the pure D-isomer [^{14}C]-**10** with specific activity 57.5 mCi/mmol (53 mg,

5.5 mCi) in good agreement with the specific activity of the starting material [^{14}C]-**1**. Careful hydrolysis furnished [^{14}C]-**8** in 85% radiochemical purity and 65% chemical purity. The lipophilic benzaldehyde-related byproducts were removed by repeated chromatographies, to afford pure carbon-14 labeled JDTic ([^{14}C]-**8**) in 50% yield. The product (2.4 mCi) was diluted to specific activity 15 mCi/mmol with unlabeled **8** and then converted to the dihydrochloride salt. The diluted product **8** · 2HCl was formulated in 50% aqueous ethanol at 0.5 mCi/mL.

Conclusions

Pure (99%) JDTic ([^{14}C]-**8**) dihydrochloride was prepared in eight steps in overall 5% yield from carboxyl carbon-14 labeled tyrosine ([^{14}C]-**1**). Although the reason(s) that partially racemized product was obtained in the preparation is unknown, it has been determined that the diastereomers are separable via a cyclic benzaldehyde adduct (**10**).

Experimental

Proton NMR spectra were recorded on a Bruker Avance 300 MHz NMR spectrometer. TLC analyses were carried out on commercial pre-coated silica gel 60F254 or C18F254 glass plates (E. Merck: 5 × 10 cm and 5 × 20 cm). The plates were scanned using a Bioscan System 200 Imaging Scanner Automatic Linear Analyzer equipped with a Gateway P5-133 computer for data reduction. Chromatographies were carried out using 230–400 mesh silica gel 60 (E. Merck).

HPLC analyses were performed on a dual pump (Rainin HPX) system equipped with Rheodyne injector, variable wavelength monitor (Knauer) followed by a β-RAM detector (IN/US) using a liquid scintillate cell. Samples were counted on a Tri-Carb Scintillation Analyzer (Packard Bioscience Company model 2100TR) using Optima Gold counting cocktail.

*3′,5′-Dibromo-[^{14}C-CO_2]-D-tyrosine hydrobromide [^{14}C]-**2b***

To a suspension of [^{14}C-CO_2]tyrosine [^{14}C]-**1** (Amersham Biosciences/GE Healthcare; 50 mCi, 161 mg, 0.89 mmol; specific activity 57 mCi/mmol) in HOAc (2 mL) and H_2O (0.5 mL) at 0°C was added Br_2 (275 mg, 1.72 mmol) dropwise, with stirring, at a rate such that Br_2 was absorbed before further addition. A light yellow solution resulted after 25 min. Further Br_2 (25 mg, 0.15 mmol) in HOAc (300 μL) was added over a 10 min period. The resulting light brown solution was evaporated under a nitrogen jet at ambient temperature. After vacuum drying, [^{14}C]-**2b** was obtained as a light brown solid (48 mCi, 360 mg, 96%) which was used in the next step without purification. ^{1}H-NMR (300 MHz, MeOH-d_4) δ (ppm): 3.1–3.2 (m, 2H, CH_2), 4.2 (m, 1H, CH); 7.4 (s, 2H, ArH).

*6,8-Dibromo-1,2,3,4-tetrahydroisoquinoline-3-[^{14}C]carboxylic acid hydrobromide [^{14}C]-**3b***

To a solution of [^{14}C]-**2b** (360 mg, 0.86 mmol) in TFA (5 mL) was added paraformaldehyde (33 mg, 1.1 mmol) and 45% HBr in HOAc (160 mg, 0.89 mmol). The mixture was heated to 80°C with stirring in a teflon sealed tube. The course of the reaction was monitored by taking aliquots for ^{1}H NMR analysis after 3, 8, 10, 12 h. After 8 h more paraformaldehylde (5 mg, 0.01 mmol) was added to the reaction mixture. No change was observed after 10 h. The solvent was evaporated and, the residue was vacuum dried to give crude [^{14}C]-**3b** as a light gray solid (360 mg, 40 mCi, 83%) that was radiochemically ~88% pure based on TLC-radioscan using SiO_2, EtOAc–7% MeOH (Rf 0.16). The crude material was used in the next step without purification. ^{1}H NMR (300 MHz, MeOH-d_4) δ (ppm): 3.2–3.4 (m, 2H, H4); 4.2–4.5 (m, 3H, H1, H3); 7.5 (s, 1H, H5).

*6,8-Dibromo-N-t-butoxycarbonyl-1,2,3,4-tetrahydroisoquinoline-3-[^{14}C]carboxylic acid [^{14}C]-**4b***

A solution of crude [^{14}C]-**3b** (360 mg, 40 mCi, 0.83 mmol) in anhydrous DMF (2 mL) was diluted with H_2O (0.5 mL) and Et_3N (400 mg, 4 mmol) and di-t-butyl carbonate (380 mg, 1.7 mmol) were added. The resulting brown solution was stirred at ambient temperature for 3–4 h. The solvent was evaporated under a N_2 stream at ~40°C. The residual brown solid was partitioned between EtOAc and 1 N HCl. The aqueous layer was re-extracted with EtOAc, and the combined extract was evaporated. The residue was chromatographed on a silica gel column (10 g) using a gradient of EtOAc with 1 to 4% MeOH. This afforded [^{14}C]-**4b** as a glassy solid (370 mg, ~25 mCi, 63% yield), which was 97% radiochemically pure by TLC-radioscan (SiO_2, EtOAc–10% MeOH–1% HOAc) (Rf 0.70). ^{1}H NMR (300 MHz, MeOH-d_4) δ (ppm): 1.5 (s, 9H, $C(CH_3)_3$); 3.1 (m, 2H, H4); 4.4, 4.6 (d, dd, J = 16, 7.5 Hz, 2H, H1); 5.0 (m, 1H, H3); 7.4 (s, 1H, ArH).

*N-t-butoxycarbony-1,2,3,4-tetrahydroisoquinoline-3-[^{14}C]carboxylic acid [^{14}C]-**5***

A solution of [^{14}C]-**4b** (360 mg, 0.52 mmol) in DMF (7 mL) was treated with cesium carbonate (1.3 g), which had been vacuum dried at 150°C for 15 min. The suspension was stirred at ambient temperature under vacuum until gas evolution slowed noticeably (~20 min). Hydrogenation over 10% Pd/C (50 mg) was performed at atmospheric pressure for 8 h. The suspension was filtered through celite and, the filter cake was washed with EtOAc. The filtrate was concentrated under N_2 to ~0.5 mL and, the residue was partitioned between EtOAc and 1 N HCl. The organic layer was evaporated to a light brown glassy solid that was chromatographed on silica gel (3.5 g) using CH_2Cl_2 with a gradient of 1–10% of MeOH containing 10% NH_4OH. This furnished [^{14}C]-**5** as a white amorphous solid, (124 mg, 22.8 mCi, 91% yield). The radiochemical purity, determined by TLC-radioscan (C_{18}, CH_3CN-H_2O 1:1 with 0.1% HOAc and 0.05% Et_3N), was 94%. ^{1}H NMR (300 MHz, MeOH-d_4) δ (ppm): 1.48, 1.52 (2s, 9H, $C(CH_3)_3$); 3.05 (m, 2H, H4); 4.5 (m, 3H, H1, H3); 6.5 (m, 2H, H6, H8); 6.9 (m, 1H, H5).

*(3R)-7-Hydroxy-N-(1S)-{[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-t-butoxy-carbonylamido-3-isoquinoline-[^{14}C]carboxamide [^{14}C]-**7***

To a solution of [^{14}C]-**5** (124 mg, 0.42 mmol) in CH_2Cl_2 (3 mL) was added **6** (132 mg, 0.45 mmol) followed by O-(7-azabenzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (182 mg, 0.48 mmol). The suspension was stirred at 0°C for 1.2 h under N_2 atmosphere, then DIEA (127 mg, 1.07 mmol) was added and stirring was continued at 0°C until a clear solution resulted (~10 min). Analysis by TLC-radioscan after stirring at ambient temperature for 1.5 h showed a mixture of products (C_{18}, CH_3CN–H_2O–HOAc–Et_3N 60:40:0.1:0.05) containing ~50% of [^{14}C]-**7** (Rf 0.44). The crude product was partitioned between

H_2O and CH_2Cl_2. The aqueous phase was re-extracted with CH_2Cl_2 followed by extraction with EtOAc. The combined extract was evaporated to a light brownish yellow glass (385 mg, 15.8 mCi), which contained solvents as well as radioactive and non-radioactive materials. For purification this product was chromatographed on silica gel (7 g) conditioned with CH_2Cl_2–NH_4OH 8:2:0.2, then washed with CH_2Cl_2. Crude [^{14}C]-**7** was applied to the column in CH_2Cl_2 solution and eluted with CH_2Cl_2 (20 mL) followed by a gradient of CH_2Cl_2 with (MeOH–10% NH_4OH) up to 9% of the latter solvent. Combination of fractions with highest purity on evaporation gave an amorphous solid, which consisted of ~85% of [^{14}C]-**7** (TLC-radioscan, as above Rf 0.43). The recovered [^{14}C]-**7** (14 mCi) was used in the next step without further purification.

*(3R)-7-Hydroxy-N-(1S)-{[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinoline-[^{14}C]-carboxamide [^{14}C]-**8** dihydrochloride*

The amorphous solid [^{14}C]-**7** (140 mg, 0.25 mmol) was dissolved in THF-40% 6 N HCl (7 mL) and stored at ambient temperature for 5–6 h. The solvents were evaporated under a N_2 jet leaving a light yellow solid (11 mCi, 78% crude return), which based on HPLC analysis, [C_{18}, H_2O–0.1% Cl_2CHCO_2H–0.1% Et_3N (solvent A) and MeOH (Solvent B) using a gradient 0–20 min 20% B–60% B, 20–30 min 60% B, with a flow rate of 1 mL/min, and detection by UV at 275 nm] (R_t 17.8 min) consisted of a 4:1 mixture of [^{14}C]-**8** (R_t 16.8 min) and its L-isomer [^{14}C]-**9** (R_t 17.7 min). The mixture was dissolved in CH_2Cl_2−20% MeOH and chromatographed on silica gel (7 g) as described above. Collected fractions consisted of [^{14}C]-**8** and [^{14}C]-**9** in a ratio of 1:1 increasing to 35:1. Fractions containing ⩽20% [^{14}C]-**9** were combined, evaporated to a pale yellow-brown solid (8.7 mCi), which contained ~90% of [^{14}C]-**8**. The earlier eluting chromatography fractions containing >20% of [^{14}C]-**9** were combined and rechromatographed on silica gel. Recovery of [^{14}C]-**8** containing ~5% [^{14}C]-**9** was only 250 μCi.

Benzaldehyde adduct [^{14}C]-10

The chromatographed mixture of [^{14}C]-**8** and [^{14}C]-**9** (80–90% at a ratio of 7:1) was analyzed after storage in the freezer for ~3 months. Radiochemical purity had decreased by ~5% and chemical purity was <50% due to formation of lipophilic matter of low radioactivity. A solution of this mixture (8.7 mCi, 0.15 mmol) in MeOH (2 mL) was stirred with benzaldehyde (50 mg, 0.5 mmol) and HOTs (3 mg, 0.015 mmol) at 40°C for 2 days. The solvent was evaporated to dryness, and the residue was re-dissolved in MeOH (0.8 mL). Upon seeding, a solid crystallized. The product [^{14}C]-**10** was isolated after cooling at −20°C overnight, (53 mg 5.5 mCi, S.A 57.5 mCi/mmol, 63% yield) of mp: 215–217°C, radiochemical purity of >95%. ^{1}H NMR (MeOH-d_4) δ (ppm): 0.75–0.88 (m, 9H, CH(CH_3)$_2$; 3- CHCH_3); 1.26 (s, 3H, CCH_3); 1.5(m, 1H, C**H** CH_3); 2.0, 2.3, 2.8, 3.0, 3.2, 3.3 (6 m, 10H, $(CH_2)_3$, 3H, 4H, CH); 5.0 (s, 1H, C_6H_5–C**H**); 6.3, 6.6, 6.8, 7.0, 7.1 (5 m, 7H, C_6H_3, C_6H_4); 7.4, 7.7 (2m, 5H, C_6H_5).

*(3R)-7-hydroxy-N-(1S)-{[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinoline-[^{14}C]carboxamide [^{14}C]-**8** from hydrolysis of [^{14}C]-**10***

The benzaldehyde adduct [^{14}C]-**10** (5.5 mCi, 0.1 mmol) was dissolved by heating in MeOH (4 mL), HOTs (25 mg, 0.13 mmol) was added at ambient temperature and the mixture was stirred for 17 h. Reaction progress was monitored by TLC (SiO_2, CH_2Cl_2–MeOH–NH_3 9:1:0.1, Rf 0.70). Over a 28 h period HOTs (10 mg, 0.05 mmol) was added twice while the temperature was gradually raised to 50°C for the first 4 h of the reaction. The resulting product consisted of ~65% [^{14}C]-**8**, 2.5% [^{14}C]-**9** and several lipophilic by-products. The yellow green fluorescent solution was evaporated to a yellow glass, which was treated with a mixture of 5% $NaHCO_3$ solution and EtOAc–10% MeOH. The organic phase was separated and the aqueous phase was re-extracted with EtOAc–10% MeOH. The combined extract was evaporated to a gum that was dissolved in CH_2Cl_2–10% MeOH and chromatographed on silica gel (3 g) using a gradient of CH_2Cl_2−2 to 15% MeOH. Combination of the purest fractions furnished [^{14}C]-**8** (2.4 mCi). HPLC analysis (C_{18} column, 250 × 4.6 mm, 20 min gradient of 80% A−20% B, 40% A−60% B [A = H_2O, 0.2% dichloroacetic acid, triethylamine]; B = methanol 1 mL/min, 275 mm, β-RAM) indicated 98% chemical purity and 99% radiochemical purity. The identity of [^{14}C]-**8** was confirmed by its coelution with authentic unlabeled material (R_t 15.5 min). Impure fractions containing mostly non-polar by products were combined and re-chromatographed on silica gel. Applying the conditions described above an additional 350 μCi of [^{14}C]-**8** was recovered.

*(3R)-7-Hydroxy-N-(1S)-{[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinoline-[^{14}C]carboxamide dihydrochloride ([^{14}C]-**8** · 2HCl)*

For final formulation, 2.4 mCi of [^{14}C]-**8** (20 mg) was diluted with a solution of unlabeled **8** (55 mg) in MeOH (2 mL). The resulting solution was treated with 1 N HCl (1 mL). Evaporation of the solvent gave [^{14}C]-**8** · 2HCl as a white solid. The specific activity, determined gravimetically, was 15 mCi/mmol. The material was dissolved in 50% ethanol and stored at −20°C.

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