Potent and Selective Inhibitors of 11β-Hydroxysteroid Dehydrogenase Type 1 Labeled with Carbon-13 and Carbon-14

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Abstract

(*S*)-6-(2-Hydroxy-2-methylpropyl)-3-((*S*)-1-(4-(1-methyl-2-oxo-1,2-dihydropyridin-4-yl)phenyl)ethyl)-6-phenyl-1,3-oxazinan-2-one (**1**) and (4a*R*,9a*S*)-1-(1*H*-benzo[d]midazole-5-carbonyl)-2,3,4,4a,9,9a-hexahydro-1-*H*-indeno[2,1-b]pyridine-6-carbonitrile hydrochloride (**2**) are potent and selective inhibitor of 11β-hydroxysteroid dehydrogenase type 1 enzyme. These two drug candidates developed for the treatment of type-2 diabetes were prepared labeled with carbon-13 and carbon-14 to enable drug metabolism, pharmacokinetics, bioanalytical, and other studies. In the carbon-13 synthesis, benzoic-$^{13}C_6$ acid was converted in seven steps and in 16% overall yield to [$^{13}C_6$]-(**1**). Aniline-$^{13}C_6$ was converted in seven steps to 1*H*-benzimidazole-1-2,3,4,5,6-$^{13}C_6$-5-carboxylic acid and then coupled to a tricyclic chiral indenopiperidine to afford [$^{13}C_6$]-(**2**) in 19% overall yield. The carbon-14 labeled (**1**) was prepared efficiently in two radioactive steps in 41% overall yield from an advanced intermediate using carbon-14 labeled methyl magnesium iodide and Suzuki-Miyaura cross coupling via *in situ* boronate formation. As for the synthesis of [^{14}C]-(**2**), 1*H*-benzimidazole-5-carboxylic-^{14}C acid was first prepared in four steps using potassium cyanide-^{14}C, then coupled to the chiral indenopiperidine using amide bond formation conditions in 26% overall yield.

Key words: 11β–HSD1, Type-2 diabetes, Radiosynthesis, Carbon-14, Carbon-13

Introduction:

11β-Hydroxysteroid dehydrogenase type 1 (11β–HSD1) is an NADPH-dependent enzyme expressed in key metabolic tissues.[1,2] It catalyzes the conversion of cortisone into active glucocorticoid cortisol,[3] also known as stress hormone, Figure 1.

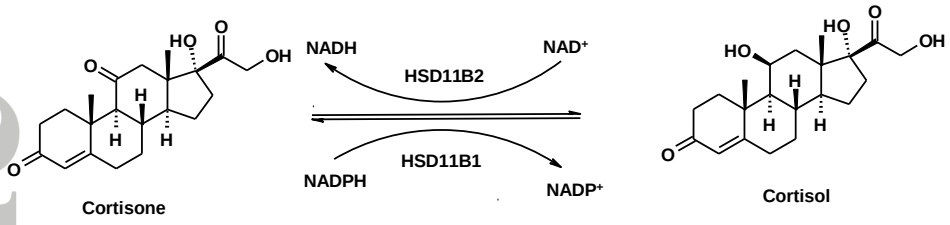


Figure 1: Conversion of cortisone to cortisol

High levels of glucocorticoids were associated with several serious clinical features including metabolic syndrome like type-2 diabetes.[4] The enzyme 11β-HSD1 is considered to be critical in the development of metabolic syndrome and thus a therapeutic target for treating type-2 diabetes.[5-11] The clinical candidates (*S*)-6-(2-hydroxy-2-methylpropyl)-3-((*S*)-1-(4-(1-methyl-2-oxo-1,2-dihydropyridin-4-yl)phenyl)ethyl)-6-phenyl-1,3-oxazinan-2-one (**1**)[12] and (4a*R*,9a*S*)-1-(1*H*-benzo[d]midazole-5-carbonyl)-2,3,4,4a,9,9a-hexahydro-1-*H*-indeno[2,1-b]pyridine-6-carbonitrile hydrochloride (**2**)[13], Figure 2, inhibit the conversion of cortisone to cortisol with nanomolar potency, an average IC_{50} of 11 nM in human adipose tissue *ex vivo* in case of (**1**).[12] Herein, we describe the synthesis of these two compounds labeled with carbon-13 and carbon-14.

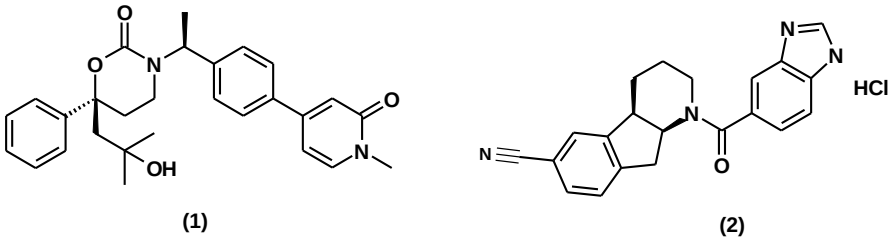
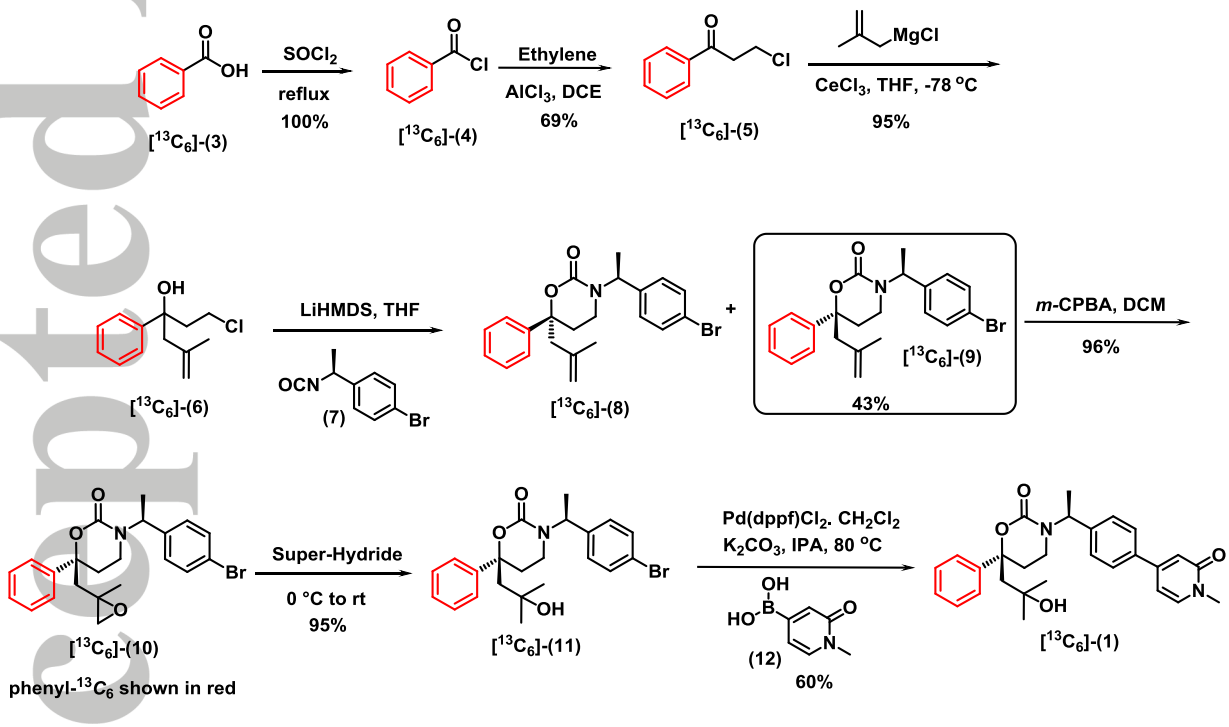


Figure 2: Structural formula of clinical candidates (**1**) and (**2**)

Results and Discussion

The separation of the molecular weights of the isotopically labeled and unlabeled drug candidate by more than two mass units is highly desirable, so one can easily distinguish between the drug candidate and its metabolites using mass spectrometry. Benzoic acid-$^{13}C_6$ and aniline-$^{13}C_6$ are suitable building blocks to access several intermediates in the synthesis of carbon-13 labeled drug candidates. The synthesis of carbon-13 labeled (**1**) was accomplished in seven steps in 16% overall yield starting form benzoic acid-$^{13}C_6$, Scheme 1. The benzoyl chloride [$^{13}C_6$]-(**4**) obtained from refluxing benzoic acid [$^{13}C_6$]-(**3**) in thionyl chloride was subjected to Friedel-Crafts reaction using aluminum trichloride in dichlorethane and a slow bubbling of ethylene gas in the reaction mixture at ambient temperature to afford carbon-13 labeled 3-chloropropiophenone [$^{13}C_6$]-(**5**) in 69% yield.[14] Grignard reaction with methylallyl magnesium chloride in the presence of cerium chloride in THF at – 78 ^{o}C gave the tertiary

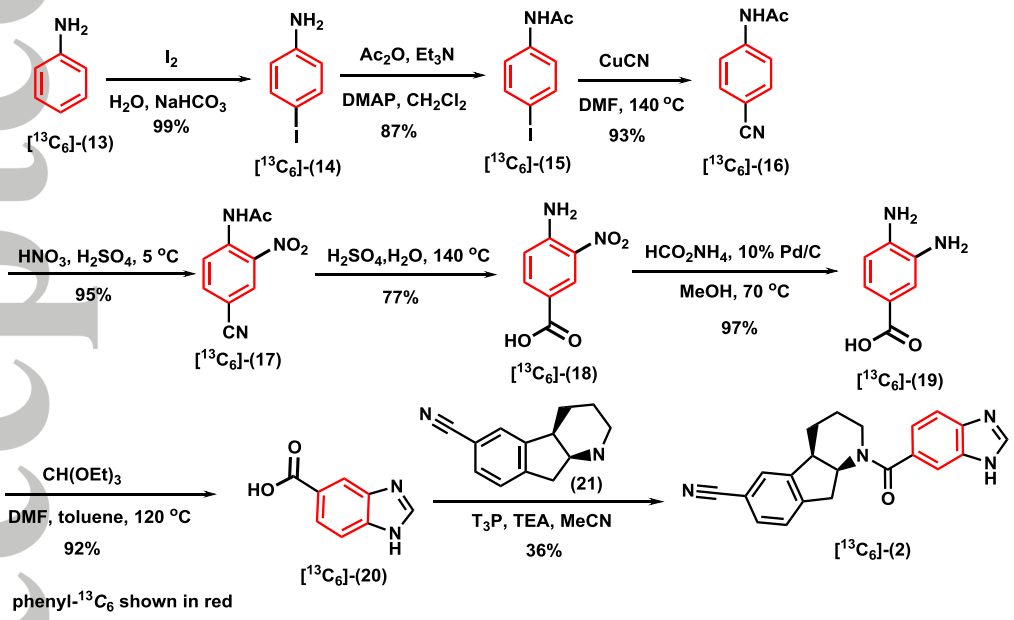
alcohol [$^{13}C_6$]-(**6**) in 95% yield. The use of cerium chloride has been reported to enhance the yields of the addition of Grignard reagents to carbonyl compounds by eliminating undesired side reactions like enolization, reduction, condensation, conjugate addition, and pinacol coupling.[15] Reaction of (*S*)-4-bromo-α-methylbenzylisocyanate (**7**) with the anion generated from treating this alcohol with lithium hexamethyldisilazane gave a 1:1 mixture of bromooxazinones [$^{13}C_6$]-(**8**) and [$^{13}C_6$]-(**9**).[16] The desired bromooxazinone [$^{13}C_6$]-(**9**) was easily separated using silica gel chromatography. The enantioselective synthesis of (**9**) using asymmetric methyllylation catalyzed by (*S*)-7,5-difluoro-BINOL has since been reported.[17] Oxidation of [$^{13}C_6$]-(**9**) with *meta*-choloroperbenzoic acid in methylene chloride to the epoxide derivative [$^{13}C_6$]-(**10**) was accomplished in 96% yield. Opening of the oxirane with super-hydride® in THF afforded alcohol [$^{13}C_6$]-(**11**) in 96% yield.[18] Finally, the transformation of this alcohol to the final compound [$^{13}C_6$]-(**1**) was achieved using Suzuki−Miyaura coupling with the boronic acid derivative (**12**) in 60% yield after silica gel purification.[17]



Scheme 1: Synthesis of [$^{13}C_6$]-(**1**)

For the synthesis of [$^{13}C_6$]-(**2**), the benzimidazole (**20**) was chosen to incorporate the carbon-13 atoms to avoid the lengthy synthesis and stereochemical issues associated with indenopiperidine (**21**), Scheme 2. Thus, aniline-$^{13}C_6$ was iodinated at the *para*-position in aqueous sodium bicarbonate with molecular iodine in quantitative yield.[19] In order to

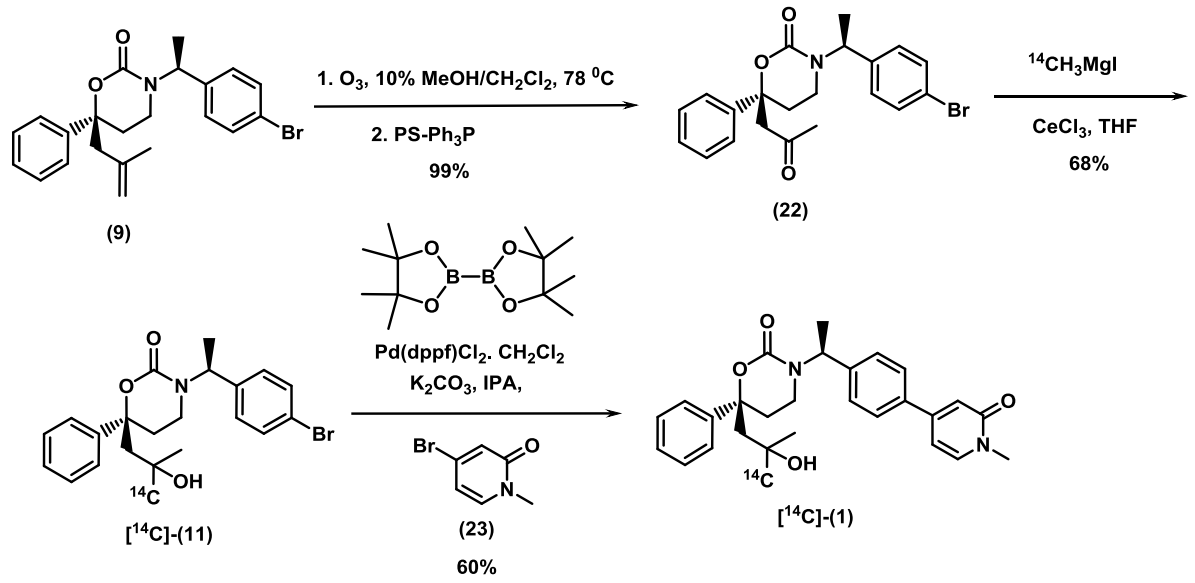
attenuate the electron density of the aromatic ring for mono-nitration, the amine was protected as the acetamide [$^{13}C_6$]-(**15**) in 87% yield using acetic anhydride in the presence of triethylamine and DMAP.[20] Reaction of [$^{13}C_6$]-(**15**) with two equivalents of copper (I) cyanide in DMF provided the benzonitrile [$^{13}C_6$]-(**16**) in 93% yield.[21] The cooperative nature of the *ortho/para* directing acetamide and *meta* directing nitrile set the stage for a selective *ortho* nitration to the acetamide group. As predicted, the nitration of [$^{13}C_6$]-(**16**) proceeded smoothly to provide [$^{13}C_6$]-(**17**) using 1.8 equivalents of nitric acid keeping the internal temperature of the reaction below 10 ºC.[22] Treatment of [$^{13}C_6$]-(**17**) with 60% H_2SO_4 at 95 ºC led to hydrolysis of the nitrile and removal of the protecting group to give 4-amino-5-nitrobenzoic acid [$^{13}C_6$]-(**18**) in 77% yield. Reduction of the nitro group to the diamino-acid [$^{13}C_6$]-(**19**) was accomplished via transfer hydrogenation with ammonium formate and 10% Pd/C in refluxing methanol in excellent yield.[23] Cyclodehydration of this compound was performed in the presence of triethyl orthoformate in refluxing toluene to give the desired 5-benzimidazole carboxylic acid [$^{13}C_6$]-(**20**) in 92% yield.[24] Finally amide bond formation using propylphosphonic anhydride solution (T_3P®, 50% solution in THF) and triethylamine in acetonitrile between this acid and (4a*R*,9a*S*)-2,3,4,4a,9,9a-hexahydro-1*H*-indeno[2,1-b]pyridine-6-carbonitrile (**21**)[25] gave [$^{13}C_6$]-(**2**) in 36% yield after silica gel chromatography.



Scheme 2: Synthesis of [$^{13}C_6$]-(**2**)

In the carbon-14 synthesis of compound (**1**), the available intermediate (**9**)[17] was first subjected to ozonolysis at -78 ºC followed by reductive work up with polystyrene-supported triphenylphospine (PS-PPh_3) to afford the ketone (**22**) in quantitative yield, Scheme 3. Carbon-14 methyl magnesium iodide was freshly prepared and added to ketone (**22**) at 0 ºC

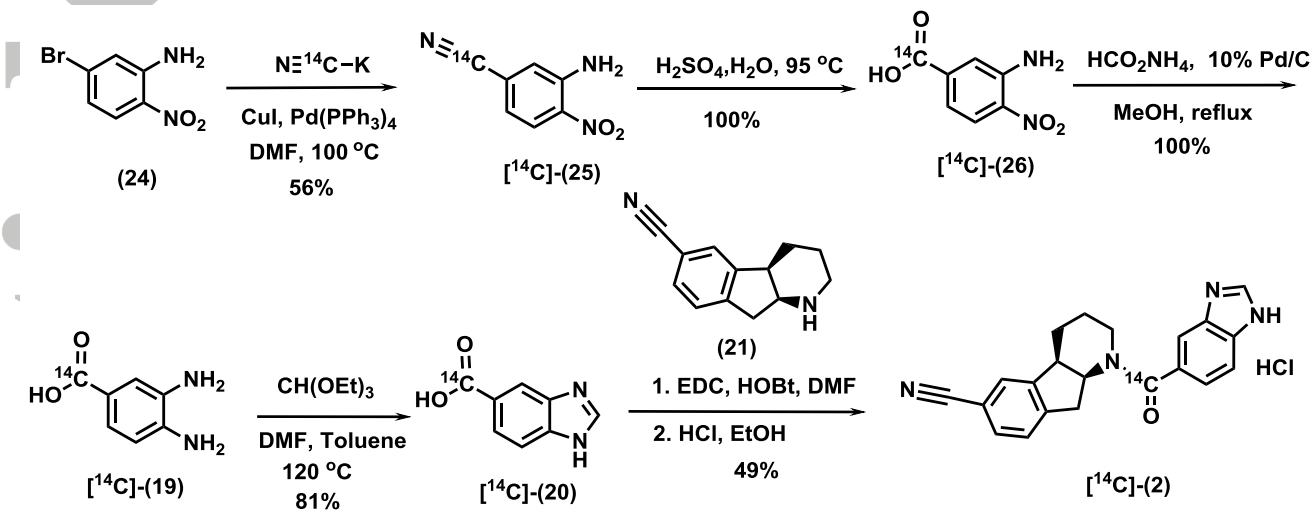
to give the tertiary alcohol [^{14}C]-(**11**) in 68% yield. Compound [^{14}C]-(**11**) was then used in the Suzuki−Miyaura coupling using *in situ* formation of the boronate[26] of 4-bromo-1-methypyridine-2-(1*H*)-one (**23**) to afford [^{14}C]-(**1**) with a specific activity of 54.7 mCi/mmol after silica gel chromatography purification followed by crystallization from acetone and water. In general, this final transformation was found to give higher amounts of the desbromo-derivative of (**11**) on larger scales than the coupling of the boronic acid (**12**) to the bromide (**11**) as described previously in the carbon-13 synthesis.[17]



Scheme 3: Synthesis of [^{14}C]-(**1**)

To prepare [^{14}C]-(**2**), Scheme 4, the cyanation of the commercially available 5-bromo-2-nitroaniline (**24**) with K^{14}CN in the presence of CuI and Pd(PPh$_3$)$_4$ proceeded to give the desired nitrile [^{14}C]-(**25**) in 56% yield. Hydrolysis of the nitrile was accomplished with 70% H_2SO_4 at 95 ºC and gave the acid [^{14}C]-(**26**) in quantitative yield. Reduction of the nitro group via transfer hydrogenation as described above proceeded quantitatively to give 3,4-diaminobenzoic acid [^{14}C]-(**19**). Treatment of the diamine with triethylorthoformate gave the desired 5-benzimidazole carboxylic-^{14}*C* acid [^{14}C]-(**20**) in 81% yield with a specific activity of 56.6 mCi/mmol. The commercially available carbon-14 labeled triethylformate would have provided a shorter synthetic route. However, the high cost of this material and the fact that at least two equivalents are needed to completed the synthesis of ^{14}C]-(**20**) was not considered for the synthesis of [^{14}C]-(**2**). Amide bond formation with the chiral (**21**) followed by hydrogen chloride salt formation gave [^{14}C]-(**2**) in 49% yield over these two final steps.

This six-step radiosynthesis of [^{14}C]-(**2**) was carried out with only one chromatographic purification, thus decreasing the amount of radioactive waste.



Scheme 4: Synthesis of [^{14}C]-(2)

Conclusion

We have described the synthesis of carbon-13 and carbon-14 labeled of two potent and selective 11β-HSD1 inhibitors. In the carbon-13 synthesis, benzoic-$^{13}C_6$ acid was converted in seven steps to [$^{13}C_6$]-(**1**) in 16% overall yield. The preparation included a Friedel-Crafts reaction using ethylene gas, a cerium-modified Grignard reaction with methylally magnesium bromide, and a Suzuki-Miyaura cross coupling with a key boronic acid derivative gave [$^{13}C_6$]-(**1**) in 60% yield with more than 99% isotopic enrichment and 99% chemical purity. Aniline-$^{13}C_6$ was converted in seven steps to 5-benzimidale carboxylic acid [$^{13}C_6$]-(**20**) in 52% yield then coupled to a chiral tricyclic indenopiperidine (**21**) using amide bond formation conditions to afford [$^{13}C_6$]-(**2**) with more than 99% chemical purity and isotopic enrichment. In the carbon-14 synthesis, the availability of the chiral 1,3-oxazinan-2-one derivative (**9**) from in house synthesis shortened the synthesis of carbon-14 labeled (**1**) to just two radioactive steps. The drug candidate [^{14}C]-(**1**) was obtained with 97.6% radiochemical purity and a specific activity of 54.7 mCi/mmol. 5-Benzimidozle carboxylic-^{14}C acid was prepared in four steps in 45% yield and then coupled under amide bond formation conditions to the chiral amine (**21**) followed by salt formation to give [^{14}C]-(**2**) with 98.6% radiochemical purity and a specific activity of 56.6 mCi/mmol.

Experimental Procedures

Experimental:

Liquid chromatography-mass spectrometry (LCMS) was acquired using a medium polar method: run time 2.0 min, gradient 95% water (0.1% TFA) and 5% MeCN (0.1%TFA) to 5% water in 1.7 min, hold to 2 min at 5% water, flow 2.5 mL/min; column: Agilent Zorbax C18 SB (4.6 mm× 30 mm, 3.5 μm). The data were acquired on Waters Acquity™ Ultra Performance LC (Milford, MA, USA). NMR spectra were recorded with a Bruker 400 MHz and 500 MHz spectrometers using deuterated chloroform or dimethyl sulfoxide as a solvent and tetramethyl silane as the internal standard (Sigma-Aldrich, USA). HPLC was performed using an HPLC system comprised of an Agilent 1200, IN/US β-RAM-4, IN-FLOW™ counting solution (LabLogic systems, Inc. Brandon, FL, USA) and using *Laura Lite* software for data evaluation for radioactive compounds. HPLC Conditions: Method (a) mobile phase: gradient A: MeCN (0.1% TFA), B: H_2O (0.1% TFA) 20% A to 100% A in 22 min, flow rate: 1 mL/min, column: Eclipse XDB C18 (4.5 mm × 150 mm, 5μm); Method (b) mobile phase gradient 30 to 98 %A, A: MeCN, B: Water 0.2% H_3PO_4/MeCN in 12 min, flow rate: 1 mL/min, column: Halo C8 (4.6 × 150 mm, 2.7 μm); Method (c) mobile phase gradient 5 to 95% MeCN in water (0.1% TFA) in 12 min, column Zorbax XDB C18 (4.6 x 150 mm, 5.0 μm); Method (d) mobile phase gradient 5 to 98% MeCN in water (0.1% H_3PO_4, 0.2% $HClO_4$) in 10 min, column: Zorbax SB-Phenyl (4.6 x 150 mm, 1.8 μm). Chiral HPLC conditions: Method (a) mobile phase A: MeCM, B: 0.1% HCO_2H in water adjusted with NH_4OH to pH 4.0, isocratic 40:60 A/B, detection UV 220 nm, column: Chiralcel OJ-RH (4.6 x 150 mm, 5μm); Method (b) mobile Phase: A: Water 0.1% AcOH, buffered to pH = 4.7 with NH_4OH, B: MeCN, Isocratic: A/B 95/5, detection: UV 230 nm, column: Chiralpak AD (4.6 x 250 mm, 5 μm). Liquid scintillation counting was carried out using a Beckman LS6500 and Ultima Gold™ cocktail (Beckman, Fullerton, CA, USA). [^{14}C]-Methyl Iodide was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). Potassium cyanide-^{14}C was obtained from Quotient Bioresearch Radiochemicals (Cardiff, UK). Benzoic-$^{13}C_6$ acid with min 99 atom % ^{13}C and aniline-$^{13}C_6$ were purchased from Isotec (Miamisburg, OH, USA).

Synthesis of [$^{13}C_6$]-(1) and [$^{13}C_6$]-(2)

Benzoyl-1,2,3,4,5,6-$^{13}C_6$ chloride, [$^{13}C_6$]-(4)*:* A solution of benzoic-ring-$^{13}C_6$ acid (2.1 g, 16.4 mmol) and thionyl chloride (6.2 mL, 85 mmol) in toluene (10 mL) was heated to reflux for 8 h. After cooling to room temperature, the resulting yellow solution was concentrated *in vacuo* and the residue was treated with toluene (10 mL) and concentrated again. This

procedure was repeated twice to remove excess thionyl chloride. The residue (2.3 g) was used as is in the following step.

3-Chloro-1-(phenyl-$^{13}C_6$)propan-1-one, [$^{13}C_6$]-(5)*:* To a suspension of aluminum chloride (2.2 g, 16.5 mmol) in 1,2-dichloroethane (10 mL) was added a solution of [$^{13}C_6$]-(**4**) (2.3 g, 16 mmol) in 1,2-dichloroethane (10 mL). The flask containing the benzoyl chloride was rinsed once with dichloroethane (5 mL) and added to the reaction flask at ambient temperature to give a brown solution. Ethylene was then bubbled slowly into this solution for 4 h and the resulting solution was stirred for 14 h. LCMS showed no starting material. A solution of 4 N aqueous HCl (45 mL) was added slowly (exothermic) to give a precipitate, which dissolved in contact with the organic layer. The aqueous phase was extracted with ether (80 mL ×3) and washed with water (50 mL ×3), aqueous saturated $NaHCO_3$ (50 mL ×3), brine (50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 2.1 g of pale yellow oil which solidified upon standing at room temperature. This crude material was purified by Combiflash using a 150 g silica gel cartridge and 100% CH_2Cl_2 to give 1.92 g of a pale yellow solid in 69% yield. TLC: 20% EtOAc:Hexanes: R_f = 0.44. HPLC method (a): 9.55 min (98%) identical to an Aldrich sample. LCMS: R_t = 1.56 min, MH^+ = 175.04 (100%) .^{1}H NMR ($CDCl_3$) δ: 8.10-8.25(m, 1H), 7.60-7.85 (m, 2.5H), 7.35-7.42(m, 0.5H), 7.20-7.34(m, 1H), 3.93(t, *J* = 7.2 Hz, 2H), 3.46(t, *J* =7.2 Hz, 2H). ^{13}C NMR($CDCl_3$) δ: 196.7(d, *J* = 57 Hz), 149.1(m), 136.5(m), 133.4(m), 128.5(m), 41.3(d), 38.6.

1-Chloro-5-methyl-3-(phenyl-$^{13}C_6$)hex-5-en-3-ol, [$^{13}C_6$]-(6): In a two-neck round bottomed flask was added cerium III chloride (1.5 g, 6.1 mmol) and anhydrous THF (10 mL). The suspension was stirred for 30 min at room temperature to give a thick slurry. To this mixture, 2-methylallylmagnesium chloride solution (12 mL, 0.5 M in THF) was added slowly to give an orange mixture. The mixture was cooled to -30 ^{o}C and [$^{13}C_6$]-(**5**) (1.6 g, 4.6 mmol) in THF (10 mL) was added dropwise keeping the internal temperature below -25 ^{o}C. A colorless mixture was obtained by the end of the addition. The mixture was stirred at -30 ^{o}C for 20 min, then warmed to 0 ^{o}C in an ice bath and stirred for 30 min. The reaction was quenched with 1N aqueous HCl (150 mL) and extracted with MTBE (200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 2.1 g of yellow oil. Purification using silica gel chromatography and CH_2Cl_2 as eluent gave 1.85 g of product in 95% yield. ^{1}H NMR ($CDCl_3$) δ: 8.15-8.27(m, 1H), 7.62-7.87(m, 2.5H), 7.37-7.444(m, 0.5H), 7.22-7.36(m, 1H), 4.92 (m, 1H), 4.77(m, 1H), 3.57(ddd, *J* = 5.5, 11, 11 Hz, 1H), 3.13(ddd, *J* = 5.5, 11, 11 Hz, 1H),

2.67(d, *J* = 13.5 Hz, 1H), 2.54(dd, *J* = 5, 13.5 Hz, 1H), 2.52(bs, 1H), 2.32(m, 2H), 1.28(s, 3H).

(*S*)-3-((*S*)-1-(4-Bromophenyl)ethyl)-6-(2-methylallyl)-6-(phenyl-$^{13}C_6$)-1,3-oxazinan-2-one, [$^{13}C_6$]-(9): A solution of [$^{13}C_6$]-(**6**) (1.66 g, 5 mmol) and (*S*)-1-bromo-4-(1-isocyanatoethyl)benzene (**7**) (1.25 g, 4.8 mmol) in anhydrous THF (15 mL) was stirred at room temperature, then cooled in an ice bath and a solution of LiHMDS in THF (1 M, 5.5 ml) was added slowly keeping the internal temperature below 10 ^{o}C. The reaction solution was warmed to room temperature and stirred for 10 h. A solution of 2 N aqueous HCl (50 mL) was added and the mixture was extracted with MTBE (100 mL ×2). The combined extracts were washed with water (100 mL), brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 2.6 g of foam as a mixture of [$^{13}C_6$]-(**8**), R_f = 0.45, and [$^{13}C_6$]-(**9**), R_f = 0.27 in 30% EtOAc:Hexanes and visualized with 10% solution of phosphomolybdic acid (PMA) in ethanol. Purification by Combiflash using 150 g disposable silica gel cartridge and up to 30% EtOAc in Hexanes gave 0.9 g of [$^{13}C_6$]-(**9**) in 43% yield. LCMS: MH^+ (420.3, 422,2 1:1). HPLC method (a), R_t = 14.89 min (98%), for [$^{13}C_6$]-(**8**), R_t = 15.24 min. ^{1}HNMR ($CDCl_3$) δ: 7.65(m, 3H), 7.47(d, *J* = 8.5 Hz, 2H), 7.10-7.40(m, 2H), 6.64(d, *J* = 8.5 Hz, 2H), 5.59(q, *J* = 7.1 Hz, 1H), 4.86(d, *J* = 8.1 Hz, 2H), 2.91(m, 2H), 2.56(dq, *J* = 2.0, 13.8 Hz, 2H), 2.1-2.4(m, 2H), 1.64(s, 3H), 1.47(d, *J* = 7.1 Hz, 3H).

(6*S*)-3-((*S*)-1-(4-Bromophenyl)ethyl]-6-((2-methyloxiran-2-yl)methyl)-6-(phenyl-$^{13}C_6$)-1,3-oxazinan-2-one, [$^{13}C_6$]-(10): To a 100 mL flask containing the alkene [$^{13}C_6$]-**(9)** (0.5 g, 0.95 mmol) and CH_2Cl_2 (15 mL) under a nitrogen atmosphere, was added *m*-CPBA (0.42 g, 1.7 mmol). The resulting colorless solution was stirred at room temperature for 2 h. HPLC, method (a), indicated the reaction was completed. The reaction was diluted with CH_2Cl_2 (20 mL) and washed with 30 wt% solution of $Na_2S_2O_3$ (15 mL), a saturated solution of $NaHCO_3$ (50 mL ×2), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 494 mg of a white foam in 96% yield. HPLC method (a): 12.30 min (98%). LCMS: R_t = 1.71 min, MH^+ = 436.1, 438.1 (1:1) ^{1}HNMR($CDCl_3$) δ: 7.54(m, 3H), 7.15-7.45(m, 2H), 7.18(d, *J* = 8.1 Hz, 2H), 7.68(d, *J* = 8.1 Hz, 2H), 5.65(q, *J* = 7.1 Hz, 1H), 2.85(m, 2H), 2.61(d, *J* = 7.1 Hz, 1H), 2.01-2.52(m, 2H), 1.75(d, 1H), 1.55(s, 3H).

(*S*)-3-((*S*)-1-(4-Bromophenyl)ethyl)-6-(2-hydroxy-2-methylpropyl)-6-(phenyl-$^{13}C_6$)-1,3-oxazinan-2-one, [$^{13}C_6$]-(11): To a solution of the epoxide [$^{13}C_6$]-**(10)** (0.38 g, 0.87 mmol) in anhydrous THF (10 mL) was added a solution of super-hydride® ($LiBHEt_3$) (1 M in THF, 1 mL) dropwise keeping the internal temperature below 25 ^{o}C. After 2 h of stirring, HPLC

showed a new product. A solution of 30% (w/w) of hydrogen peroxide in water (1 mL) was diluted in water (1 mL) and added dropwise while cooling in an ice-bath. After the addition was completed the ice bath was removed and the reaction was warmed to room temperature. Ethyl acetate (100 mL) and water (50 mL) were added and the aqueous layer was removed. The organic layer was washed twice with a 30% solution of $Na_2S_2O_3$ (40 mL ×2), brine (50 mL) and dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 0.5 g of white foam. Purification by Combiflash using a 40 g disposable silica gel cartridge and up to 10% MeOH/CH_2Cl_2 gave 0.36 g of product in 95% yield. LCMS: R_t = 1.3 min, MH^+ = 439.16 : 441.16 (1:1), 100%. HPLC method (a): R_t= 11.47 min (98%).

(*S*)-6-(2-Hydroxy-2-methyl-propyl)-3-((*S*)-1-[4-(1-methyl-2-oxo-1,2-dihydropyridin-4-yl)phenyl]ethyl)-6-(phenyl-$^{13}C_6$)-1,3-oxazinan-2-one, [$^{13}C_6$]-(1): To a solution of [$^{13}C_6$]-(**11**) (360 mg, 0.82 mmol) in isopropanol (5 mL), were added (1,1'-bis(diphenylphosphino-ferrocene)dichloropalladium II) complex with dichloromethane (1:1) ($PdCl_2$(dppf).CH_2Cl_2, 10 mg, 12 μmol), potassium acetate (246 mg, 2.5 mmol), and (**12**) (140 mg, 0.95 mmol). The mixture was degassed three times introducing nitrogen gas each time and then heated to 80 ^oC under nitrogen and stirred for 14 h. After cooling to 50 ^oC, a solution of *N*-acetyl-L-cysteine (160 mg) in water (10 mL) was added and stirred for 30 min to chelate the palladium. The mixture was then cooled to room temperature, water (30 mL) was added and extracted with EtOAc (100 mL ×2). The combined organic extracts were washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 0.85 mg of a viscous oil. The crude material was purified using 40 g disposable silica gel cartridge and up to 20% EtOAc in CH_2Cl_2 to remove non-polar impurities, then up to 20% MeOH in CH_2Cl_2 to elute the desired product (230 mg, 60% yield). LCMS: R_t = 1.34 min MH^+ = 467.29 (100%). HRMS: $[C_{22}{}^{13}C_6H_{33}N_2O_4]^+$,$(M+H)^+$, calculated: 467.26361, found 467.26360. MS/MS: 449.25329 $(M-H_2O)^+$, 423.27378 $(M-CO_2)^+$, 409.22155$(M-C_3H_6O)^+$. HPLC method (a): R_t = 7.3 min, 99.5%; chiral HPLC (method a) 99%. ^{1}H NMR ($CDCl_3$) δ: 7.32(dm, *J* = 78 Hz, 4H), 7.30(m, 3H), 7.01(d, *J* = 8.51 Hz, 2H), 6.71(d, *J* = 1.63 Hz, 1H), 6.35(dd, *J* = 2.04, 7.04 Hz, 1H), 5.69(dd, *J* = 7.04, 14.0 Hz, 1H), 3.57(s, 3H), 2.92(m, 1H), 2.39-2.47(m, 2H), 2.15-2.32(m, 6H), 1.54(d, *J* = 7.0 Hz, 3H), 1.17(s, 3H), 1.14(s, 3H). ^{13}C NMR ($CDCl_3$) δ: 163.2, 153.0, 151.2, 142.8(dt), 140.7, 138.1, 136.3, 128.8(m), 127.6(m), 126.5(m), 125.0(m), 116.7, 105.3, 84.1(m), 71.1, 53.8, 53.2, 37.4, 36.2, 33.2, 31.9, 30.4, 15.4.

4-Iodoaniline-$^{13}C_6$, [$^{13}C_6$]-(14): A 100 ml round bottomed flask was charged with aniline-$^{13}C_6$ (2.0 g, 20.2 mmol), sodium bicarbonate (2.6 g, 30.3 mmol) and 10 mL of water. The

mixture was stirred in an ice-water bath. Iodine (5.2 g, 20.2 mmol) was added portionwise in a 30 min period. The resulting deep brown color mixture was stirred vigorously for 6 h, then filtered using a Buchner funnel and the brown filter cake was washed with ice cold water (20 mL ×3) until the filtrate was colorless. The solid was dried under high vacuum for six h to give 4.5 g of [$^{13}C_6$]-(**14**) in 99% yield; HPLC (method c) 8.7 min, 98%, starting aniline (2.61 min). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.43-7.50 and 7.03-7.09(dm, *J* = 163.8 Hz, 2H), 6.28-6.24 and 6.64-6.58(dm, *J* = 158.8 Hz, 2H), 5.27(brs, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 148.3(td, *J* = 60.6, 9.3 Hz), 137.0(m), 116.5(m), 75.7(tdt, *J* = 61.4, 9.0, 1.7 Hz); LCMS (ESI$^+$) *m/z* 226.15 at 1.26 min.

***N*-(4-Iodophenyl-$^{13}C_6$)acetamide, [$^{13}C_6$]-(15): [$^{13}C_6$]-(14)** (4 g, 17.7 mmol) was dissolved CH_2Cl_2 (55 mL) to give a dark brown solution. The reaction was chilled in an ice-water bath and triethylamine (3 mL, 21.2 mmol) was added. Acetyl chloride (1.3 mL, 18.6 mmol) was then added dropwise; slight fuming was observed. The reaction mixture was stirred for 30 min and then water (100 mL) was added. The lower brown organic phase was cut. The aqueous phase was extracted with CH_2Cl_2 (50 mL). The combined organic phases were dried over $MgSO_4$ and filtered through a phase separator cartridge. The brown filtrate was concentrated *in vacuo* to a brown solid. The solid was dried under high vacuum for 3 h to afford **[$^{13}C_6$]-(15)** (4.16 g, 87%) as a brown solid. HPLC (method c): 13.23 min, 98%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.96 (1H, s, NHAc), 7.54(dm, *J* = 167.0 Hz, 2H), 7.34(dm, *J* = 163.3 Hz, 2H), 1.96(s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.4(s), 139.0(td, *J* = 62.6, 10.1 Hz), 137.2(ddd, *J* = 60.4, 55.9, 7.2 Hz), 121.1(m), 86.1(tdt, *J* = 60.6, 10.1, 2.4 Hz), 24.0(m); LCMS (ESI$^+$) *m/z* 267.00 at 1.39 min.

***N*-(4-Cyanophenyl-$^{13}C_6$)acetamide, [$^{13}C_6$]-(16):** [$^{13}C_6$]-(**15**) (4 g, 15 mmol) was dissolved in DMF (40 mL) to give a brown solution. Copper cyanide (2.7 g 29.8 mmol) was added giving a suspension. The dark brown suspension was stirred at 140 ºC for 3 h. The vessel was removed from the oil bath and cooled to room temperature. To the dark brown mixture was added 20 mL of aqueous ammonium hydroxide. The mixture was stirred vigorously for one hour then, 50 mL of ethyl acetate was added. After one hour, the mixture was filtered through a 3 cm high bed of Celite® in a 60 mL sintered glass funnel. The cake was rinsed with EtOAc (50 mL ×2). The phases were separated and the aqueous phase was extracted with EtOAc (50 mL ×2). The combined clear brown organic phases were washed with 100 mL of saturated aqueous sodium chloride (the aqueous phase turned dark blue after mixing). The organic phase was dried over $MgSO_4$ and filtered through a phase separator cartridge yielding

a clear brown filtrate. The filtrate was concentrated *in vacuo* to a brownish grey solid of [$^{13}C_6$]-(**16**) (2.32 g, 93%). HPLC (method c) 8.9 min, 98%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.31(1H, s, NHAc), 7.68(dm, *J* = 166.4 Hz, 4H), 2.02(s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.1(s), 143.4(td, *J* = 61.2, 10.5 Hz), 133.15(td, *J* = 58.9, 6.8 Hz), 118.9(td, *J* = 59.7, 6.5 Hz), 104.62(td, *J* = 59.6, 10.5 Hz), 24.23(m). LCMS (ESI$^+$) *m/z* 166.19 at 0.61 min.

***N*-(2-Nitro-4-cyanophenyl-$^{13}C_6$)acetamide [$^{13}C_6$]-(17):** To a two-neck 100 mL round bottomed flask equipped with a thermometer, was added 8 mL of sulfuric acid. The vessel was chilled in an ice-water bath with the sulfuric acid reaching 5 ^{o}C. To the sulfuric acid was added **[$^{13}C_6$]-(16)** (2.5 g, 15 mmol) portion wise. In a separate flask sulfuric acid (2.43 ml, 44.7 mmol) was added and chilled in an ice-water bath and nitric acid (2.14 ml, 33.5 mmol) was added. The contents were mixed. To the substrate-sulfuric acid mixture was added 3 mL of the sulfuric acid-nitric acid mixture dropwise via a pipet. The temperature spiked to 20 deg then cooled to 5 ^{o}C over a 20 minute period. HPLC analysis after 45 minutes showed consumption of the starting material at 8.8 min and product peak eluting at 10.6 min. LCMS showed the desired [M]$^+$ m/z 211 at 0.82 min. The dark brown mixture was poured into 200 grams of ice. Upon stirring, a brown solid precipitated, which was extracted from the aqueous phase with EtOAc (50 mL ×3). The combined organic extracts were dried over $MgSO_4$ and filtered through a phase separator cartridge. The dark brown filtrate was concentrated *in vacuo* to afford 3 g of product in 95% yield as a dark grey-brown solid. This material (87% purity by HPLC, method c) was carried forward for the next step without purification. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.50(s, 1H, NHAc), 8.50(dm, *J* = 173.7 Hz, 1H), 8.12(dq, *J* = 170.9, 8.6 Hz, 1H) 7.84(dq, *J* = 170.2, 8.6 Hz, 1H) 2.05(s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.1(s), 143.4(td, *J* = 61.2, 10.5 Hz), 138.5(td, *J* = 59.2, 6.5 Hz), 133.15 (td, *J* = 58.9, 6.8 Hz), 129.4(dt, *J* = 59.4, 6.7 Hz), 123.3(dt, *J* = 59.4, 7.1 Hz), 118.9 (td, *J* = 59.7, 6.5 Hz), 104.62 (td, *J* = 59.6, 10.5 Hz), 24.23 (m); LCMS (ESI$^+$) *m/z* 210.10 at 0.82 min.

3-Amino-4-nitrobenzoic-$^{13}C_6$ acid, [$^{13}C_6$]-(18): To a 100 mL single neck round bottomed flask was charged [$^{13}C_6$]-(**17**) (3 g, 14.2 mmol) followed by 10 mL of water. The vessel was chilled in an ice-water bath and sulfuric acid (15 ml, 275.8 mmol) was added in one portion. The suspension became homogenous and was warm to the touch. The vessel was equipped with a reflux condenser and submerged in a preheated 140 ^{o}C oil bath. The reaction mixture was stirred for three h then the vessel was cooled to room temperature and the contents poured into 200 g of ice. The mixture was agitated with a glass stir rod forming a dark black tar. The organics were extracted with EtOAc ((50 mL ×3). The combined organic phases

were dried over $MgSO_4$ and filtered through a phase separator cartridge. Prolonged drying on the rotovap gave [$^{13}C_6$]-(**18**) as solid (2.45 g, 77%). HPLC (method c) 8.5 min, 98%; LCMS (ESI^+) *m/z* 187.09 at 0.54 min.

3,4-Diaminobenzoic-$^{13}C_6$ acid, [$^{13}C_6$]-(19): To a 100 mL single neck round bottomed flask were placed [$^{13}C_6$]-(**18**) (2.45 g, 13 mmol) and 10% Palladium on carbon (277 mg) followed by methanol (20 mL). To this black suspension was added ammonium formate (4.15 g, 65.1 mmol) in one portion. After stirring for 4 h at 70 ºC, the catalyst was filtered off on a 1 cm tall bed of Celite®. The cake was rinsed with 50 mL of methanol. The filtrate was concentrated *in vacuo* to give [$^{13}C_6$]-(**19**) (2g, 97%) as a dark brown solid. HPLC (method c) 2.5 min 98%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.06(dt, *J* = 159.3, 6.5 Hz, 1H), 6.99(dt, *J* = 156.7, 6.8 Hz, 1H), 6.42(dq, *J* = 155.5, 7.3 Hz, 1H), 5.11(br s, 2H), 3.33(br s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 166.4, 140.1(dddd, *J* = 63.1, 63.0, 6.3, 3.4 Hz), 133.5(t, *J* = 64.1 Hz), 120.3(td, *J* = 58.3, 5.4 Hz), 118.3(td, *J* = 59.5, 6.9 Hz), 115.2(t, *J* = 63.0 Hz), 112.6(dddd, *J* = 63.9, 60.9, 5.4, 4.1 Hz); LCMS (ESI+) *m/z* 158.97 at 0.24 min.

1*H*-Benzo-$^{13}C_6$-[d]imidazole-5-carboxylic acid, [$^{13}C_6$]-(20): In a 100 mL single neck round bottomed flask was charged [$^{13}C_6$]-(**19**) (1.2 g, 7.53 mmol) followed by toluene (10 mL) and DMF (10 mL) to give a brown red solution. To the reaction mixture was added triethyl orthoformate (2.55 mL, 15 mmol). The vessel equipped with a reflux condenser and nitrogen inlet then submerged in a preheated 120 ºC oil bath. LCMS after 2 h showed consumption of the diamine and benzimidazole at 0.23 min with m/z 168.98. The vessel was cooled to room temperature and the solvent removed *in vacuo* leaving a dark solid that weighed 1.16 g (92%). Due to the insolubility of this material in common solvents (except DMSO), no purification was performed. The material was carried onto the amide formation step as is. HPLC 2.5 min; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 12.4(s, 1H), 8.33(d, *J* = 8.3 Hz, 1H), 8.16(dm, *J* = 163.0 Hz, 1H), 7.79(dm, *J* = 163.0 Hz, 1H), 7.61(dm, *J* = 163.0 Hz, 1H); LCMS (ESI^+) *m/z* 168.98 at 0.23 min.

(4a*R*,9a*S*)-1-(1*H*-benzo-$^{13}C_6$-[d]midazole-5-carbonyl)-2,3,4,4a,9,9a-hexahydro-1-*H*-indeno[2,1-b]pyridine-6-carbonitrile hydrochloride, [$^{13}C_6$]-(2): The amine (**21**) (0.69 g, 1.8 mmol) was diluted with MeCN (10 mL), then the acid [$^{13}C_6$]-(**20**) (0.97 g, 1.8 mmol) and Et_3N (0.72 g, 7.1 mmol) were added. T_3P® (1.1 mL, 50% solution in THF, 1.95 mmol) was added to the reaction mixture and the solution was stirred for 2 h. The reaction mixture was concentrated to a minimum volume and then quenched by addition 1N NaOH (5 mL). The mixture was diluted with EtOAc (20 mL). The organic phase was removed and washed with

water (10 mL), brine (10 mL), dried over Na_2SO_4 and concentrated to give a dark brown sticky solid. The residue was purified using by Combiflash Companion (40g silica gel cartridge, 5-10% MeOH/CH_2Cl_2). Only the pure fractions were combined and concentrated. The residue was diluted with MeCN/Water (10 mL, 1:1) and transferred to a tarred vial and lyophilized to give 220 mg as an off white solid in 36% yield. HPLC (method c) 7.6 min (99.3%); chiral HPLC (method b) 99% ee. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 12.7 (br, 1H), 8.41(t, *J* = 7.6 Hz, 1H), 8.30(S, 1H), 7.70(br, 1H), 7.58-7.66(m, 3H), 7.45(br, 1H), 7.25(br, 1H), 3.27(br, 1H), 3.11(br, 1H), 3.01(br, 2H), 1.74(br, 1H), 1.52(q, *J* =12.5 Hz, 1H), 1.30(dq, *J* = 3.0, 13.0 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 170.2, 147.5, 145.8, 143.5 (m), 138.4, 138.1 (m), 13.8 (m), 129.9, 127.1, 126.2, 120.6 (m), 119.1 (m), 114.2 (m), 109.2, 59.7, 41.2, 32.6, 32.1, 27.8, 23.1, 20.8. LCMS (ESI^+) *m/z* 349.2 (MH^+, 100%), LCMS (ESI^-) *m/z* 347.5 (M^-,100%), 0.54 min. HRMS $[C_{15}{}^{13}C_6H_{19}N_4O]^+$, MH^+ calculated 349.1755, found 349.1741. HPLC (method c): 99.3%. Chiral purity (method b): 99%.

Synthesis of [^{14}C]-(1) and [^{14}C]-(2)

(*S*)-3-((*S*)-1-(4-Bromophenyl)ethyl)-6-(2-oxopropyl)-6-phenyl-1,3-oxazinan-2-one, (22): Ozone was bubbled through a solution of (**9**) (250 mg, 0.6 mmol) in 10 % MeOH/CH_2Cl_2 (10 mL) at -78 ºC until a blue color persisted. Then O_2 was bubbled through for 10 min followed by N_2 for 5 min. PS-PPh_3 (1 g, 1.8 mmol) was added, the mixture was allowed to warm to -20 °C. The mixture was filtered and concentrated, then diluted with 50 % EtOAc/Hexane (30 mL) filtered and concentrated to give 250 mg of (**22**) in 99% yield as a light yellow solid. HPLC method (b): 6.66 min (98%). LCMS: *m/z* 416, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.35 (m, 5H), 7.17 (d, *J* = 8.5 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 5.57 (q, *J* = 7.1 Hz, 1H), 3.01 (d, *J* = 14.5 Hz, 1H), 2.92 (d, *J* = 14.5 Hz, 1H), 2.90 (m, 1H), 2.59 (m, 1H), 2.36 (m, 2H), 2.08 (s, 3H), 1.48 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 205.5, 152.9, 140.9, 138.1, 131.3, 129.0, 128.6, 128.2, 124.8, 121.2, 81.3, 55.0, 52.8, 36.1, 32.5, 29.6, 15.2.

(6*S*)-3-((*S*)-1-(4-Bromophenyl)ethyl)-6-(2-hydroxy-2-methylpropyl-2-^{14}*C*)-6-phenyl-1,3-oxazinan-2-one, [^{14}C]-(11): [^{14}C]-MeI (500 mCi, at 59 mCi/mmol, 8.5 mmol), was cooled in a dry ice bath and placed under argon. The seal of the ampule was broken, then Et_2O (8 mL) was added. The solution was transferred via cannulation into a flask containing a mixture of magnesium turnings (206 mg, 8.5 mmol) in Et_2O (5 mL) at room temperature. A cold finger was attached to the flask and the mixture was stirred for 1 h and used as is in Et_2O. In a separate reaction flask, THF (10 mL) was added to $CeCl_3$ (1.5 g, 6.3 mmol), and the mixture was stirred at 0 °C for 1 h. Compound (**22**) (2.6 g, 6.25 mmol) in THF (15 mL)

was added via syringe, and the mixture was stirred for 1h. Then, $^{14}CH_3MgI$ in Et_2O (13 mL) was added and the mixture was stirred for 0.5 h. The reaction was quenched by addition of 1N aqueous HCl (25 mL) and diluted with EtOAc (50 mL). The organic material was washed with a saturated solution of $NaHCO_3$ (20 mL), brine (20 mL), filtered through a membrane filter and concentrated to give a dark brown foam. The crude product was diluted with 20% Et_2O/Hexanes (30 mL) and stirred for 14 h. The solid was collected by filtration and washed with 20% Et_2O/Hexanes to give [^{14}C]-(**11**) (1.85 g, 252 mCi) as a solid in 68% yield. HPLC method (b): 6.56 min (98%).

(*S*)-6-(2-Hydroxy-2-methylpropyl-2-^{14}*C*)-3-((*S*)-1-(4-(1-methyl-2-oxo-1,2-dihydropyridin-4-yl)phenyl)ethyl)-6-phenyl-1,3-oxazinan-2-one, [^{14}C]-(1): [^{14}C]-(11) (81.4 mCi, 600 mg, 1.4 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (356 mg, 1.4 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (57 mg, 0.07 mmol), and KOAc (406 mg, 4.14 mmol) was diluted with IPA (10 mL) and heated to 80 °C for 3.5 h. The mixture was cooled to room temperature, then (**23**) (263 mg, 1.4 mmol) in IPA (5 mL) was added followed by K_2CO_3 (760 mg, 5.5 mmol), and H_2O (300 µL). The mixture was heated and stirred overnight at 80 °C, then cooled to room temperature, and partitioned between EtOAc (30 mL) and H_2O (30 mL). The organic layer was removed and washed with brine (20 mL), filtered through a phase separation column and concentrated giving light brown oil. The crude residue was purified using a CombiFlash (40 g silica gel cartridge, 0-10 % MeOH/CH_2Cl_2). The desired fractions were combined and concentrated to afford 383 mg of a solid (49 mCi) in 60% yield with radiochemical purity of 95%. The solid was further crystallized from acetone/water twice to afford 12 mCi of [^{14}C]-(**1**) as a white solid and with a specific activity of 54.7 mCi/mmol. HPLC method (b): 3.51 min (97.6%). ^{1}H NMR in $CDCl_3$ was identical to unlabeled material.[17]

3-Amino-4-nitrobenzonitrile-^{14}*C* [^{14}C]-(25): 5-Bromo-2-nitroaniline (**24**) (387 mg, 1.74 mmol), K^{14}CN (100 mCi at 59 mCi/mmol, 1.74 mmol), CuI (65 mg, 0.34 mmol), and $Pd(Ph_3P)_4$ (196 mg, 0.17 mmol) in DMF (5 mL) was evacuated and purged with nitrogen three times, then heated to 100 ºC for 12 h. The mixture was concentrated then diluted with EtOAc/H_2O (20 mL, 1:1), filtered through Celite®, washed with H_2O (5 mL), brine (5 mL), filtered through a membrane filter and concentrated to give a dark oil. The crude residue was purified using Combiflash Companion (40g silica gel cartridge, 25-50% EtOAc/Hexane) the pure fractions were combined and concentrated to give **[^{14}C]-(25)** (57.5 mCi, 159 mg, 56%) as a yellow solid. HPLC (method d): 5.29 min (98%).

3-Amino-4-nitrobenzoic-^{14}C acid, [^{14}C]-(26): H_2SO_4 (1.5 mL) was added to a mixture of [^{14}C]-(**25**) in H_2O (1.0 mL) at room temperature. The mixture was stirred at 95 ºC for 3h. An additional 0.5 mL of water was added and the mixture was stirred at 95 ºC for 14 h. Ice was added to the reaction mixture, and exctracted with EtOAc (5 mL ×4), the combined extracts were filtered through a membrane filter and concentrated to give [^{14}C]-(**26**) (57.5 mCi, 180 mg, 100%) as an orange solid. HPLC (method d): 4.51 min (98%).

3,4-Diaminobenzoic-^{14}C acid, [^{14}C]-(19): 10% Pd/C (20 mg) in MeOH (1 mL) was added to a solution of **[^{14}C]-(26)** (159 mg, 0.97 mmol) in MeOH (4 mL). Then, ammonium formate was added. The mixture was heated to reflux for 4h, then cooled to room temperature, filtered through Celite® and concentrated to give [^{14}C]-(**19**) (51 mCi, 150 mg, 100%) as a brown solid. HPLC (method d): 2.09 min (98%).

1*H*-Benzo[d]imidazole-5-carboxylic-^{14}C acid, [^{14}C]-(20): Triethyl orthoformate (296 mg, 2 mmol) was added to a mixture of [^{14}C]-(**19**) (150 mg, 0.97 mmol) in 1:1 DMF:toluene (4 mL). The mixture was heated at 120 ^{o}C for 2h. The mixture was concentrated to give a brown solid. The solid was diluted with H_2O (2.5 mL) and stirred for 14 h. The solid was collected by vacuum filtration, and washed with H_2O (5 mL) to give **[^{14}C]-(20)** (41.3 mCi, 130 mg, 81%) as a brown solid. HPLC (method d): 3.03 min (98%).

(4a*R*,9a*S*)-1-(1*H*-Benzo[d]midazole-5-carbonyl-^{14}C)-2,3,4,4a,9,9a-hexahydro-1-*H*-indeno[2,1-b]pyridine-6-carbonitrile hydrochloride, [^{14}C]-(2): To the amine (**21**) (159 mg, 0.8 mmol) was added acid **[^{14}C]-(20)** (130 mg, 0.8 mmol), EDC (184 mg, 0.96 mmol), HOBt (147 mg, 0.96 mmol), and DIPEA (362 mg, 2.8 mmol) in DMF (2.5 mL). The mixture was stirred for 14 h at room temperature. The mixture was diluted with water (2.5 mL) and CH_2Cl_2 (5 mL) and treated with 1N NaOH (5 mL) and the mixture was stirred for 20 min. Then 1N HCl was added to adjust the pH 9-10. The organic layer was removed and then washed with water (10 mL ×3), filtered through a membrane filter and concentrated to give an oil. This residue was diluted with EtOAc (3 mL) warmed to dissolve the oil and stirred for 14 h. The solid was collected by vacuum filtration to give a brown solid (156 mg) in 56% yield. The above material (136 mg, 0.4 mmol) was suspended in absolute ethanol (1 mL) and heated to 75 ^{o}C. A solution of HCl in ethanol (6.55 M, 63 μL, 0.41 mmol) was added and stirring was continued for 10 min. The mixture was removed from the heat after 5 min. Few seeds of (**2**) in ethanol (0.2 mL) were added and the mixture was stirred overnight. The solid was collected by vacuum filtration to give 110 mg of a light beige solid in 49% yield. Total activity 16.43 mCi at 56.6 mCi/mmol. The mother liquor was concentrated and counted to

give 4.2 mCi as a brow solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 13.84 (br, 2H), 9.42(s, 1H), 7.83(s, 2H), 7.65(s, 1H), 7.54(d, J = 7.81 Hz, 2H), 7.42(br, 1H), 4.75(br, 1H), 3.02(m, 2H), 2.02(m, 1H), 1.62(br, 1H), 1.51(m, 1H), 1.32(m, 1H), 1.25(m, 1H). Chiral purity (method b) 98.6%, and radiochemical purity 98.6%.

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Potent and Selective Inhibitors of 11β-Hydroxysteroid Dehydrogenase Type 1 Labeled with Carbon-13 and Carbon-14

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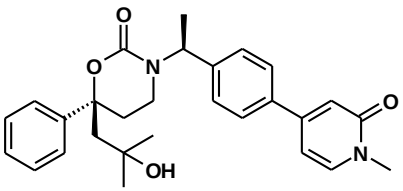
(*S*)-6-(2-Hydroxy-2-methylpropyl)-3-((*S*)-1-(4-(1-methyl-2-oxo-1,2-dihydropyridin-4-yl)phenyl)ethyl)-6-phenyl-1,3-oxazinan-2-one (**1**) and (4a*R*,9a*S*)-1-(1*H*-benzo[d]midazole-5-carbonyl)-2,3,4,4a,9,9a-hexahydro-1-*H*-indeno[2,1-b]pyridine-6-carbonitrile hydrochloride (**2**) are potent and selective inhibitor of 11β-hydroxysteroid dehydrogenase type 1 enzyme. These two drug candidates developed for the treatment of type-2 diabetes were prepared labeled with carbon-13 and carbon-14.



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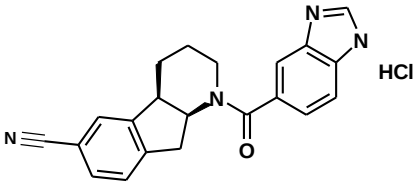

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