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Synthesis of [^{14}C] - and [$^{13}C_6$]-labeled tipranavir and its potential hydroxyl metabolite and the glucuronide conjugate

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Tipranavir or Aptivus® is a non-peptidic protease inhibitor approved for the combination treatment with ritonavir of HIV infection. Tipranavir labeled with radioactive and stable isotopes of carbon was required for drug metabolism (excretion, distribution, and absorption) studies and to develop bioanalytical methods needed for the support of clinical studies. [7-^{14}C]-Benzoic acid and uniformly labeled benzoic acid (ring-$^{13}C_6$ 99 at% ^{13}C) were used to prepare [^{14}C]- and [$^{13}C_6$]-labeled tipranavir, respectively. Radioactively labeled tipranavir was prepared with a specific activity of 54 mCi/mmol (2GBq/mmol); it was necessary to dilute its specific activity with unlabeled tipranavir to 28 mCi/mmol (46.45 μCi/mg) because of its instability. The *N*-hydroxyl metabolite (12) and the glucuronide conjugate (13), the most abundant metabolites of tipranavir (when administered in conjunction with ritonavir) were also synthesized.

Keywords: HIV–AIDS; tipranavir; carbon14; carbon13; radiosynthesis

Introduction

Tipranavir (Aptivus®, *N*-[3-{1(*R*)- (5,6-dihydro-4-hydroxy-2-oxo-6(*R*)-phenethyl-6-propyl-2*H*-pyran-3-yl)propyl}phenyl]-5-trifluoromethylpyridine-2-sulfonamide, PNU-140690, Figure 1), acquired from Pharmacia & Upjohn in 2000 and approved for use in combination with Abbott's ritonavir by the FDA in June 2005, is the first non-peptidic protease inhibitor drug available in the US for the treatment of HIV and AIDS.[1–4] This is Boehringer's second antiretroviral drug after nevirapine (Viramune®).[5,6] Tipranavir is a very potent inhibitor of HIV protease with a Ki of 8.0 pmol.[7,8] This compound is a sulfonamide-containing dihydropyrone with two chiral centers resulting in four diastereomers. All diastereomers were active as protease inhibitors. Their inhibition potency in a tandem HIV assay, ranges from 18 pmol for (1*R*, 6*S*) to 220 pmol for tipranavir's enantiomer (1*S*, 6*S*).[7,8]

According to 2007 UNAIDS–WHO report, annually there are 2.5 million newly infected people with HIV 'ca 6800 persons daily'.[9] Over 5700 people die daily from AIDS, according to the same study mainly because of inadequate access to HIV prevention and treatment. Even when treatment is available, HIV-infected patients develop resistance to their HIV therapy. Thus, combination of two or more drugs is now the standard therapy to combat this resistance. Tipranavir is particularly potent and has excellent activity on viral strains resistant to other protease inhibitors and is metabolized by P450 3A4.[10] Its pharmacokinetic parameters were enhanced when combined with ritonavir; the metabolism in the presence of 200 mg of ritonavir was minimal. Only a few metabolites were found in plasma after administration of [^{14}C]-tipranavir to subjects that received tipranavir/ritonavir (500 mg/200 mg). The most abundant fecal metabolite was potentially identified as a hydroxyl metabolite, and the most abundant urinary metabolite was a glucuronide conjugate of tipranavir, albeit in small percentages,[11] see Figure 2. More importantly, patients taking tipranavir twice a day with ritonavir have a significant reduction of their viral load and the regiment also suppressed both wild-type and protease inhibition-resistant virus.[1,2]

Results and discussion

The goal of this work was to prepare [^{14}C]- and [$^{13}C_6$]-labeled tipranavir and its major metabolites to support research and DMPK studies (Schemes 1 and 2). Initially [7-^{14}C]benzoic acid was converted to [7-^{14}C]benzoylchloride using thionyl chloride in refluxing benzene. 1-Phenyl-1-[1-^{14}C]-propanone (**3**) was then obtained either from diethyl cadmium, prepared *in situ* from cadmium chloride and ethyl magnesium bromide or as

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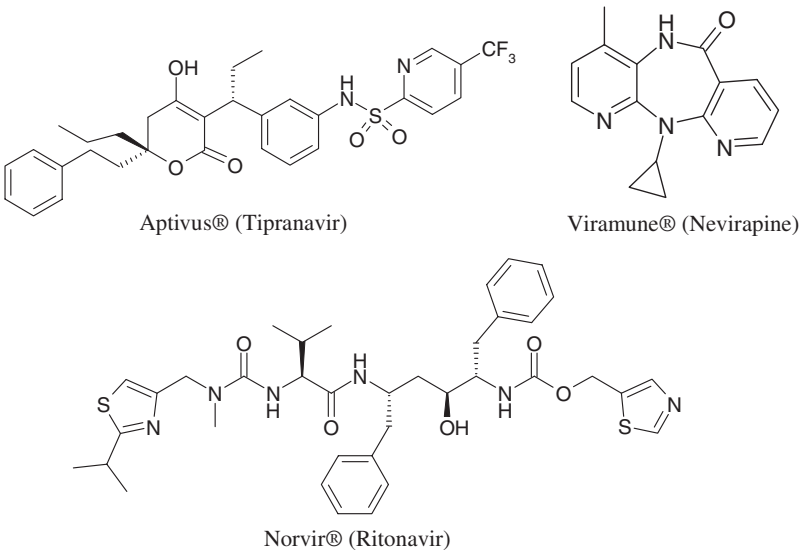


Figure 1. Structures of three HIV inhibitors.

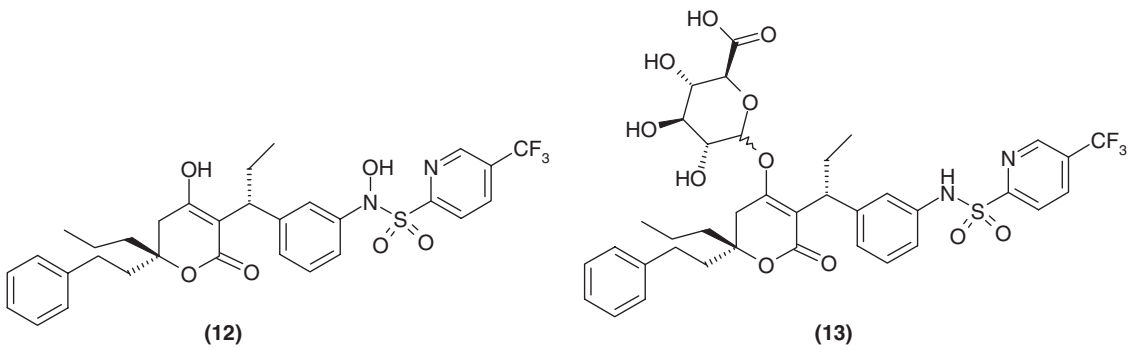
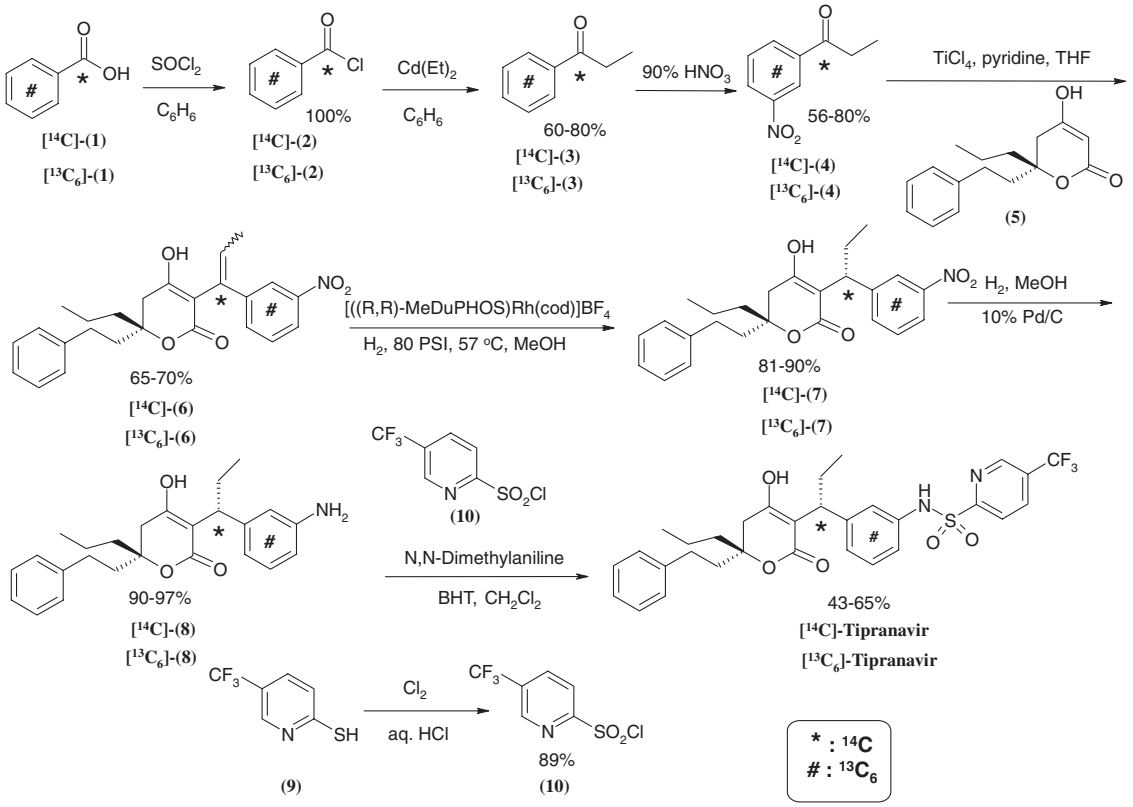


Figure 2. Tipranavir metabolites.

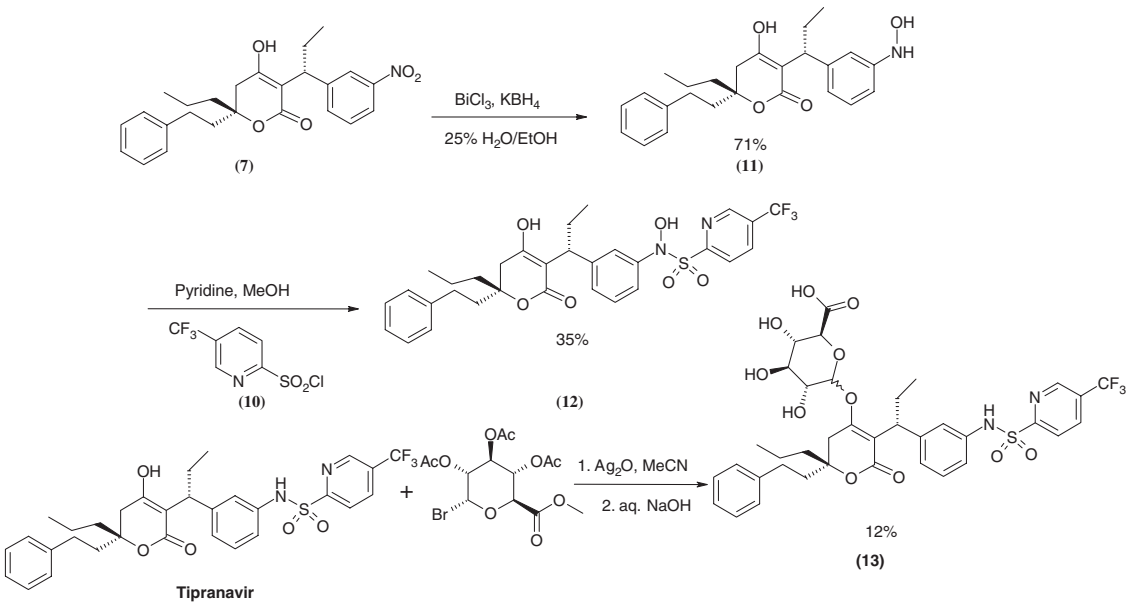
previously reported by Arisawa *et al.* from diethyl zinc and aluminum chloride in 60–70% yield.[12] Nitration with 90% nitric acid at −45°C gave 1-[3-nitrophenyl]-1-[1-^{14}C]-propanone (**4**) in 56% yield. (*R*)-4-Hydroxy-6-phenethyl-6-propyl-5,6-dihydro-2-pyran-2-one (**5**)[13–16] and the propanone (**4**) were subjected to a Kneovenagel type condensation to give compound (**6**) in 65% yield. The other chiral center was introduced via an asymmetric hydrogenation of the double bond in compound (**6**) using the chiral rhodium catalyst, (-)-1,2-bis((2*R*,5*R*)-2,5-dimethyphopholano)benzene(cyclooctadiene)-rhodium (I) tetrafluoroborate ([((*R*,*R*)-Me-DuPHOS)Rh(Cod)]BF_4) at 60°C under hydrogen in methanol and in the presence of sodium carbonate.[17,18] This hydrogenation gave a product in 94% or more enantiomeric excess as judged from chiral HPLC. Reduction of the nitro group using palladium on carbon gave the aryl amine (**8**) in 97% yield. Coupling to 5-trifluoromethyl-2-pyridinesulfonyl chloride (**10**), which was prepared by bubbling chlorine gas in a mixture of aqueous HCl solution (1.0 N) and 5-(trifluoromethyl)pyridine-2-thiol (**9**),[19] gave labeled tipranvir in 43% yield. The final step was modified from the reported procedure,[13,16] instead of using pyridine to couple the sulfonyl chloride derivative to (**8**), *N,N*-dimethylaniline and BHT (butylated hydroxytoluene) were used to give a clean reaction. [^{14}C]-Tipranavir was obtained initially with a specific activity of 54 mCi/mmol or isotopic enrichment of 86.5%. It was found that this material was not stable even when stored at −80°C. To minimize the radiolysis it was diluted with unlabeled tipranavir to a specific activity of about 28 mCi/mmol. The rate of radiolysis was slower when the compound is kept as a solution of absolute ethanol at −80°C. The chemical identity of this compound was established by comparing its ^{1}H and ^{13}C NMR and its chromatographic retention time with that of authentic tipranavir sample. The preparation of labeled 5-trifluoromethylpyridine-2-sulfonyl chloride was also considered. [^{14}C]-2-Chloro-5-trifluoromethylpyridine can be purchased from commercial suppliers and then converted in two steps to 5-trifluoromethylpyridine-2-sulfonyl chloride via a thiol-intermediate as seen in Scheme 1. The possibility that sulfonamides may undergo a glutathione-S-transferase-catalyzed cleavage has precluded us from pursuing this route.[20]

[$^{13}C_6$]-Tipranavir was obtained using the same route starting from [$^{13}C_6$]-benzoic acid with 99 at% ^{13}C.

Tipranavir metabolites (**12**) and (**13**) were prepared as described in Scheme 2. In the synthesis of (**12**), the nitro group in (**7**) was reduced to the hydroxylamine with $KBH_4/BiCl_3$[21] and then coupled with 5-trifluoromethylpyridine-2-sulfonyl chloride (**10**) in the presence of pyridine in methanol to give the desired



Scheme 1. Synthesis of [^{14}C]- and [$^{13}C_6$]-tipranavir.



Scheme 2. Synthesis of the hydroxyl-metabolite and the glucuronide conjugate.

adduct in 25% overall yield in two steps after preparative HPLC purification. The glucuronide conjugate was prepared by reacting tipranavir with the acetobromo-α-D-glucuronic acid methyl ester in the presence of silver oxide followed by basic hydrolysis. Preparative HPLC purification gave the desired (**13**) in 12% yield. No attempts were made to separate the α/β epimers.

Experimental procedures

Materials and methods

Liquid scintillation counting was accomplished using a Beckman LS5000TA and ready safe™ cocktail (Beckman, Fullerton, CA). Radio-TLC was carried out on a BIOSCAN System 200 imaging

scanner using an auto change 1000 and WinScan software version 2.1a (Bioscan Inc., Washington, DC). The quantification of the HPLC chromatograms was carried out using an HPLC system comprised of a Radiomatic A515 Flo-one\Beta radioactivity flow detector (Packard Instrument Company, Meriden, CT), two pumps (HITACHI L-6200A intelligent pump), a linear UVIS 200, Ultima Flo^{TM} AP coctail (Packard, Meriden, CT), and radiomatic 500TR V 3.60 software for data evaluation.

The analytical HPLC purity verification was carried out on a Zorbax SB300-C8 column, particle size 5 μm, (4.6 × 250 mm) fitted with an OPTI-Guard (1 mm C8) column guard. UV detection was at 220 nm. The column heater was kept at 35°C. Mobile phase: A (water), B (acetonitrile), both 10 mM TFA. Gradient: 20–100%B in 30 min, then back to 20%B in 5 min, and hold for 2 min. For enantiomeric purity, the mobile phase consisted of isocratic acetonitrile:triethylamine: acetic acid (1000:1:6 v/v/v). UV detection at 254 nm and using Astec Cyclobond I 2000 column (5 μm, 4.6 × 250 mm). Mass spectra were acquired by a Hewlett-Packard auto sampler Series 1150, connected to a Micromass LCZ mass spectrometer in the ES mode. NMR spectra were recorded with a Brucker 400 MHz DPXB and 500 MHz spectrometers using deuterated methanol, chloroform, or dimethyl sulfoxide as a solvent and tetramethyl silane as the internal standard (Cambridge Isotope Laboratories, Andover, MA). Pre-coated TLC sheets (silica gel 60 F_{254}) were obtained from EM Science (Gibbstown, NJ). [7-^{14}C]benzoic was purchased from Amersham (GE HealthCare). [$^{13}C_6$]-benzoic acid was purchased from Isotec (St. Louis, MO). 5-(Trifluromethyl)pyridine-2-thiol was obtained from Ryan Scientific, Inc. (Mt. Pleasant, SC). The catalyst [((*R,R*)-Me-DuPHOS)Rh(COD)]BF_4 was obtained from Strem (Newburyport, MA).

Synthesis

1-Phenyl-1-[1-^{14}C]-propanone ([^{14}C]-3): To a solution of [7-^{14}C]-benzoic acid (536 mg, 4.39 mmol, 250 mCi) in benzene (5 mL) was added thionyl chloride (1.7 mL, 23.3 mmol) and refluxed for 3 h. Excess thionyl chloride and benzene were then removed *in vacuo* to a stirrable volume. More benzene was added (10 mL) and distilled off to remove excess of thionyl chloride. Fresh solution of diethylcadmium was prepared from a solution of ethyl magnesium bromide (3.8 mL, 1.42 M) in diethyl ether at 0°C and cadmium chloride (521 mg, 2.84 mmol), which was added via cannulation over 10 min to the ethyl magnesium bromide. The resulting mixture was warmed to room temperature and then heated to reflux for 90 min. Diethyl ether was distilled off and swapped with benzene. This freshly prepared diethylcadmium was then mixed with [1-^{14}C]benzoyl chloride in benzene and stirred for 14 h at room temperature. The reaction was cooled in an ice bath and 6 mL of 20% v/v H_2SO_4/H_2O (cold) was added. The mixture was extracted with pentane (2 × 15 mL). The combined extracts were washed with water (10 mL), saturated $NaHCO_3$ (15 mL), brine (20 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to 4 mL. Purification by silica gel chromatography packed and eluted with 96:4 pentane:ether gave 412 mg, 175 mCi of product.

1-[3-Nitrophenyl]-1-[1-^{14}C]propanone ([^{14}C]-4): The above compound (412 mg, 3.07 mmol, 175 mCi) was cooled to −45°C and concentrated HNO_3 (4 mL) was added. The resulting yellow solution was stirred for 1 h at −45 to −35°C. The reaction was then mixed with ice chips and warmed to room temperature and extracted with ether (2 × 15 mL). The combined extracts were washed with water (10 mL), aqueous KOH (2 N, 15 mL), brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was mixed with 2 mL of absolute ethanol and cooled to −15°C for 1 h. The resulting solid was filtered, washed with cold ethanol, and dried under high vacuum at room temperature to give 307 mg of the desired product. A second crystallization from the mother liquor gave 19 mg of material. Total yield 58%, 102 mCi.

(6R)-4-Hydroxy-3-[(E)-1-(3-nitophenyl)-1-[1-^{14}C]propenyl]-6-phenethyl-6-propyl-5,6-dihydro-2H-pyran-2-one ([^{14}C]-6): To a stirred solution of the above compound (321 mg, 1.79 mmol, 102 mCi) and (**5**) (547 mg, 2.1 mmol) in dry THF (5 mL) and pyridine (0.34 mL, 4.2 mmol) under nitrogen atmosphere cooled to −10°C was added a solution of $TiCl_4$ in toluene (4 mL, 1 M). The resulting dark red solution was stirred at −10°C for 30 min, then warmed to room temperature and stirred for 21 h. The reaction was quenched by the addition of water (10 mL), and THF was removed *in vacuo*. The aqueous was extracted with CH_2Cl_2 (2 × 15 mL). The combined extracts were washed with aqueous HCl (2 N, 20 mL), water (20 mL), and brine (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in toluene (10 mL) and heated with stirring to 60°C for 3 h to equilibrate the geometric isomers. After cooling to room temperature, toluene was removed and the residue was purified by silica gel chromatography using 70% EtOAc:hexane to give 543 mg of product in 72% yield, total activity 70.58 mCi.

(6R)-4-Hydroxy-3-[(E)-1-(3-nitophenyl)-1-[1-^{14}C]propyl]-6-phenethyl-6-propyl-5,6-dihydro-2H-pyran-2-one ([^{14}C]-7): A mixture of the above compound (534 mg, 1.29 mmol), K_2CO_3 (40 mg, 0.23 mmol) in nitrogen purged methanol (1.5 mL) was placed in a stainless steel Parr bomb apparatus. The pressure bomb was purged with hydrogen, and then charged to 70 psi and heated with stirring to 57°C for 20 min. The pressure was released, and 14 mg of [((*R,R*)-Me-DuPHOS)Rh(COD)]BF_4 was added as a solution in nitrogen purged methanol (1 mL). The Parr was then purged with hydrogen and charged to 80 psi and heated with stirring at 57°C for 4 h. The pressure was released, and the contents of the flask were transferred to a round bottom flask using methanol. Methanol was removed under vacuum and the residue was partitioned between CH_2Cl_2 (25 mL) and aqueous HCl (2 N, 25 mL), and the organic layer was washed with water (20 mL), brine (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography using 1% THF/CH_2Cl_2 to give 466 mg of the desired product in 85% yield, 60.1 mCi.

(6R)-3-[(1R)-1(3-aminophenyl)-1-[1-^{14}C]propyl]-4-hydroxy-6-phenethyl-6-propyl-5,6-dihydro-2H-pyran-2-one ([^{14}C]-8): A solution of the above compound (466 mg, 1.1 mmol) and 10% Pd/C (150 mg) in nitrogen purged methanol (5 mL) was stirred under hydrogen at atmospheric pressure at room temperature. On completion, the reaction contents were filtered through a short pad of Celite® and eluted with methanol. The filtrate was concentrated *in vacuo* to give 397 mg of material in 99.9% yield, which was used without further purification.

5-trifluoromethylpyridine-2-sulfonyl chloride **(10)**: A suspension of 5-(trifluoromethyl)pyridine-2-thiol (**9**) (1.5 g, 8.0 mmol) in HCl solution (1.0 N, 40 .0 mL) was stirred at 0°C in an ice bath. Chlorine gas was then bubbled slowly for 90 min. The resulting white suspension was extracted with methylene chloride. The combined extracts were washed with a saturated solution of $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated *in vacuo* at room temperature to give 1.83 g of a white solid in 89% yield,

which was kept at −80°C. ^{1}H NMR ($CDCl_3$): δ 8.26(d, J = 8.22 Hz, 1H), 8.32(dd, J = 2.16 Hz, 8.22 Hz, 1H), 9.08(d, J = 2.16 Hz, 1H).

N-[3-{1(R)-(5,6-dihydro-4-hydroxy-2-oxo-6(R)-phenethyl-6-propyl- -2H-pyran-3-yl)-[1-^{14}C]propyl}phenyl]-5-trifluoromethylpyridine-2-sulfonamide ([^{14}C]-tipranavir): A solution of (6*R*)-3-[(1*R*)-1-(3-aminophenyl)-1-[1-^{14}C]-propyl]-4-hydroxy-6-phenyl-6-propyl-5,6-dihydro-2*H*-pyran-2-one (30 mCi, specific activity = 54 mCi/mmol, 0.55 mmol) *N,N*-dimethylaniline (80.0 μL, 0.628 mmol), 2,6-di-*tert*-butyl-4-methylphenol (25.0 mg, 0.112 mmol) in CH_2Cl_2 (3.0 mL), and water (40.0 μL) were stirred at 0°C in an ice bath. 5-Trifluoromethyl-2-pyridinesulfonyl chloride (155.5 mg, 0.63 mmol) was then added in one portion and the reaction was stirred at this temperature until deemed complete by TLC. After the workup the ethyl acetate extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography silica gel (packed in hexane) and eluted with ethyl acetate. The product was isolated in 67% radiochemical yield (225 mg, 20.6 mCi) as a fluffy white solid. A specific activity of 54 mCi/mmol was calculated by weighing 736 μg of material into a vial and dissolving it in 1.22 mL of absolute ethanol to make a 1.0 mM solution. Aliquots of 1, 2 and 5 μL were then taken, diluted in 5.0 mL of LSC Ready Safe cocktail, and counted. The total and specific activities were calculated from the average radioactivity per μL. For radioactive TLC, an aliquot of 2.0 μL of this 1.0 mM solution was spotted on a 20 cm TLC plate and developed using 30% EtOAc:Hexanes or 5% MeOH/$CHCl_3$. UV visualization showed the labeled tipranavir and the reference standard co-migrated. The plate was dried, coated with an acrylic clear film, and re-dried under a stream of nitrogen. The plate was then scanned using Bioscan. HPLC: Rad-detection, R_t = 19.30 min (98.1%); UV detection, R_t = 19.10 min (98.98%). Because of the instability of this material, it was mixed with 181.74 mg of cold tipranavir in ethyl acetate (5.0 mL). Most of the solvent was then removed under a stream of nitrogen and the residue was purified by flash chromatography to give 329 mg of a white solid, R_f = 0.25 in 40% EtOAc:Hexanes. The specific activity was counted from a solution of 0.538 mg of [^{14}C]-tipranavir in 0.892 mL of ethanol. The new specific activity was 28.86 mCi/mmol. For ^{1}H NMR, a sample of 13 mg was taken and dissolved in deuterated methanol (300 μL). The solution was placed into a Teflon tube liner (Wilmad, catalog. No. 6005-7). The tube was sealed with a Teflon plug and then inserted into a screw-cap NMR tube (Wilmad catalog No. 507-TR-8, OD 5.0 mm)). This double encapsulation was necessary to avoid contamination from the accidental breakage of the NMR tube. ^{1}H NMR (MeOH-d_4): δ: 0.82(t, J = 7.32 Hz, 3H), 0.88(t, J = 7.25 Hz, 3H), 1.28–137 (m, 2H), 1.61–1.98(m, 5H), 2.10–2.16(m, 1H), 2.50–2.68(m, 4H), 3.90(dd, J = 7.12, 9.45 Hz, 1H), 6.96–6.93(m, 1H), 6.97–7.02(m, 2H), 7.03–7.07(m, 2H), 7.10–7.15(m, 1H), 7.15-7.25(m, 3H), 8.01(d, J = 8.21 Hz, 1H), 8.20(dd, J = 2.5, 8.21 Hz, 1H), 8.94(d, J = 2.5 Hz, 1H). ^{13}C NMR (MeOH-d_4): δ:13.28, 14.67, 17.88 25.75, 30.86, 37.87, 40.49, 40.85, 43.62, 81.81, 106.19, 120.87, 122.57, 124.12, 125.63, 126.09, 129.24, 129.51, 137.00, 137.71, 142.83, 147.61, 148.06, 161.59, 166.88, 169.87.

Stereochemical purity of [^{14}C]-tipranavir

The three other diastereomers were obtained from the Analytical department for HPLC comparison. Solutions of 1.0 mg/mL of each compound were prepared using the mobile phase as the solvent (acetonitrile: triethylamine: acetic acid in 1000:1:6 v/v/v) for better separations of the diasteromers. HPLC analysis indicated that [^{14}C]-tipranavir contained about 4% of the (1*S*, 6*R*)-diastereomer, R_t = 8.17 min, and negligible amounts of the other diastereomers. The enantiomer (1*S*, 6*S*) was easily separated from tipranavir, R_t = 7.17 min for tipranavir and 9.83 min for the (1*S*, 6*S*)-enantiomer (PNU141275). The diastereomer (1*R*, 6*S*) and (1*S*, 6*S*) have the same retention times (9.83 min).

Synthesis of [$^{13}C_6$]-tipranavir

1-[$^{13}C_6$]Phenyl-1-propanone ([$^{13}C_6$]-3): To a solution of benzoic acid-$^{13}C_6$ (1.0 g, 7.8 mmol) in benzene (5 mL) was added thionyl chloride (3.1 mL, mmol) and the resulting was stirred under reflux for 5 h. After cooling to room temperature, the solution was concentrated *in vacuo* and the residue was dissolved again in benzene (5 mL) and concentrated under reduced pressure to chase excess of thionyl chloride. The remaining colorless oil was diluted in anhydrous methylene chloride (5 mL) and added at −50°C to a suspension of $AlCl_3$ (1.04 g, 7.8 mmol) in methylene chloride (7 mL). The resulting was warmed gradually to −30°C in 1 h period. A solution of diethyl zinc in toluene (4.3 mL, 1.1 M) was added dropwise in 45 min period and the resulting mixture was warmed to room temperature and stirred for 2 h. Water (30 mL) was added and the mixture was extracted with methylene chloride. The combined extracts were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 3.0 g of a colorless oil. This oil was purified by flash chromatography to give 0.88 g of colorless oil in 81% yield from benzoic acid. MS: MH^+ = 141 as the only peak.

1-[$^{13}C_6$][3-Nitrophenyl]-1-propanone ([$^{13}C_6$]-4): To the above compound (627 mg, 4.48 mmol) was added cold 90% nitric acid (7 mL) at −25°C. The resulting yellow solution was warmed to −10°C and stirred for 30 min. Ice water (15 mL) was then added dropwise. The resulting solid was filtered, washed with cold water, and dried under high vacuum at room temperature to give 450 mg of the desired product in 56% yield. MS ESI+): MH+ = 186 (100%).

(6R)-4-Hydroxy-3-[(E)-1-[$^{13}C_6$]-(3-nitophenyl)-1-propenyl]-6-phenethyl-6-propyl-5,6-dihydro-2H-pyran-2-one ([$^{13}C_6$]-6): To a stirred solution of the above compound (450 mg, 2.51 mmol) and (**5**) (850 mg, 3.26 mmol) in dry THF (10 mL) and pyridine (0.5 mL, 6.28 mmol) under nitrogen atmosphere cooled to −10°C was added a solution of $TiCl_4$ in toluene (1 M , 5 mL). The resulting dark red solution was stirred at −10°C for 30 min, then warmed to room temperature and stirred for 16 h. The reaction mixture was cooled to 0°C and water (10 mL) was added dropwise. The mixture was partitioned with ethyl acetate (10 mL), and the aqueous was extracted with ethyl acetate (20 mL). The combined organic extracts were washed with aqueous HCl (2 N, 20 mL), water (20 mL), brine (20 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was dissolved in toluene (10 mL) and heated with stirring for 3 h at 60°C to equilibrate the geometric isomers. Toluene was then distilled off and the residue was purified by silica gel chromatography to give 695 mg of the desired product in 65% yield. MS (ESI): MH^+ = 428.3 (100%).

(6R)-4-Hydroxy-3-[(E)-1-[$^{13}C_6$]-(3-nitophenyl)-1-propyl]-6-phenethyl-6-propyl-5,6-dihydro-2H-pyran-2-one ([$^{13}C_6$]-7): A mixture of the above material (695 mg, 1.65 mmol) and K_2CO_3 (40 mg, 0.29 mmol) in nitrogen purged methanol (3 mL) was placed in a 100 mL stainless steel Parr bomb apparatus and securely closed. The Parr was purged with hydrogen, and then charged to 70 psi

and heated with stirring to 57°C for 20 min. The pressure was released and 14 mg of [((*R,R*)-Me-DuPHOS)Rh(COD)]BF_4 was added via the addition port as a solution in nitrogen purged methanol (0.5 mL). The bomb was again purged with hydrogen and then charged to 80 psi and heated with stirring at 57°C for 4 h. The pressure was released and the contents of the flask were transferred to a round bottom flask using methanol. The methanol was then removed under reduced pressure and the residue was partitioned between CH_2Cl_2 (25 mL) and aqueous HCl (2 N, 25 mL). The CH_2Cl_2 layer was removed and washed with water (20 mL), brine (20 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give 715 mg in 81% yield. MS (ESI): $MH^+ = 430.3$ (100%).

(6R)-3-[(1R)-1-[$^{13}C_6$]-(3-Aminophenyl)-1-propyl]-4-hydroxy-6-phenethyl-6-propyl-5,6-dihydro-2H-pyran-2-one ([$^{13}C_6$]-8): A stirred solution of the above compound (715 mg, 1.65 mmol) and 10% Pd–C (20 mg) in nitrogen purged methanol (5 mL) was stirred under hydrogen at atmospheric pressure at room temperature. On completion the mixture was filtered through a short pad of Celite® and washed with methanol. The filtrate was concentrated under reduced pressure to give 649 mg of material in 100% yield, which was used without further purification. MS (ESI): $MH^+ = 400.3$ (100%).

[N-(3-((1R)-1-[(6R)-4-Hydroxy-2-oxo-6-phenethyl]-6-propyl-5,6-dihydroxy-2H-pyran-3-yl]propyl}[$^{13}C_6$]phenyl)-5-trifluormethyl)-2-pyridiesufonamide ([$^{13}C_6$]-tipranavir): To a stirred solution of the above material (649 mg, 1.65 mmol) in dry CH_2Cl_2 (10 mL) and pyridine (307 uL, 3.88 mmol) under nitrogen at −10°C was added 5-trifluoromethyl-2-pyridinesulfonyl chloride (467 mg, 1.9 mmol). The reaction was allowed to warm to room temperature and stirred for 3 h. The reaction was then quenched by the addition of aqueous HCl (2 N, 0.4 mL), and extracted with CH_2Cl_2 (2 × 25 mL). The combined extracts were washed with brine (25 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel chromatography using 35% ethyl acetate: hexane. The fraction containing the pure material were combined and concentrated in vacuo. The residue was crystallized from ethyl acetate/hexane in three days at −18°C and the white solid was filtered, washed with cold hexane, and dried under high vacuum at room temperature to give 426 mg of material with a chemical purity of 97.8% at 254 nm by HPLC. MS (ESI+): $MH^+ = 609.1$ and $MNa^+ = 631.38$ (100%), MS (ESI-): $M^- = 607.2$. ^{1}HNMR (d_6-DMSO): δ: 0.78(t, $J = 7.32$ Hz, 3H), 0.89(t, $J = 7.22$ Hz, 3H), 1.4(m, 2H), 1.8(m, 4H), 2.01(m, 1H), 2.6(m, 4H), 3.8(m, 1H), 6.55–7.4(m, 9H), 8.2(d, $J = 8.25$ Hz, 1H), 8.50(d, $J = 8.3$ Hz, 1H), 9.02(s, 1H), 10.7(s, 2H). ^{13}C NMR (d_6-DMSO): δ:12.64, 14.19, 16.32, 24.15, 29.17, 35.85, 40.52, 41.85, 79.34, 103.87, 117.35(t, $J_{c\text{-}c} = 57$ Hz), 120.4(t, $J_{c\text{-}c} = 53$ Hz), 123.86(t, $J_{c\text{-}c} = 48.67$ Hz), 128.20(t, $J_{c\text{-}c} = 62.5$ Hz), 135.81(t, $J_{c\text{-}c} = 55.59$ Hz), 146.06(t, $J_{c\text{-}c} = 51.18$ Hz),160.5, 164.61, 165.84.

Synthesis of the metabolites

(R)-4-Hydroxy-3-[(R)-1-(3-hydroxyamino-phenyl)-propyl]-6-phenethyl-6-propyl-5,6-dihydro-pyran-2-one (11): KBH_4 (180 mg, 3.5 mmol) was added to a mixture of (**7**) (1.0 g, 2.4 mmol) and $BiCl_3$ (150 mg, 0.48 mmol) in 25% H_2O/EtOH at 15°C. After stirring for 15 min, a second portion of KBH_4 (180 mg, 3.5 mmol) and $BiCl_3$ (150 mg, 0.48 mmol) was added. After stirring an additional 15 min, the mixture was filtered (Celite®), quenched by addition of 1% HCl. The mixture was extracted with CH_2Cl_2, dried over Na_2SO_4 and concentrated giving a yellow oil. The crude residue was purified using the Combiflash Companion (40 g column, 2% MeOH/ CH_2Cl_2) giving 700 mg, 71% yield as beige foam.

5-Trifluoromethyl-pyridine-2-sulfonic acid hydroxy-{3-[(R)-1-((R)-4-hydroxy-2-oxo-6-phenethyl-6-propyl-5,6-dihydro-2H-pyran-3-yl)-propyl]-phenyl}-amide **(12)**: 5-Trifluoromethyl-pyridine-2-sulfonyl chloride (30 mg, 0.12 mmol) in MeOH (2 mL) was added to the above compound (50 mg, 0.12 mmol) and pyridine (50 μL) in MeOH (2 mL) at 0°C. The mixture was stirred for 1 h then concentrated. The mixture was diluted with CH_2Cl_2, quenched by addition of 0.5 N HCl, extracted with CH_2Cl_2, dried over Na_2SO_4 and concentrated giving a colorless oil. The crude product was purified by preparative HPLC (Waters Masslynx, 15 to 100% MeCN/H_2O in 10 min). Collected only *m/z* 619, the combined fractions were concentrated with an E-Z evaporator giving (**12**) (23 mg, 35%) as an off white solid. LRMS *m/z* 619 (M^+). ^{1}H NMR (d_6-DMSO) δ: 0.76 (t, $J = 7.5$, 3H), 0.84 (t, $J = 7.5$, 3H), 1.25 (m, 4H), 1.74 (m, 1H), 1.63 (m, 2H), 1.86 (m, 2H), 1.99 (m, 1H), 2.57 (m, 2H), 3.82 (dd, $J = 6.5$, 9, 1H), 7.00 (m, 1H), 7.08 (s, 1H), 7.15 (m, 5H), 7.26 (m, 2H), 8.01 (d, $J = 8.01$ Hz, 1H), 8.52 (dd, $J = 2.5$, 8.01 Hz, 1H), 9.08 (s, 1H), 10.73 (s, 1H), 11.23 (s, 1H). ^{13}C NMR (d_6-DMSO) ä: 12.7, 14.3, 16.4, 24.3, 29.2, 36.8, 41.4, 79.5, 120.7, 122.1, 125.7, 125.8, 126.9, 127.5, 128.1, 128.4, 136.1, 141.6, 145.5, 146.7, 155.3, 164.9, 165.9.

(2S,3S,4S,5R)-3,4,5-Trihydroxy-6-((R)-6-oxo-2-phenethyl-2-propyl-5-{ (R)-1-[3-(5-trifluoromethyl-pyridine-2-sulfonylamino)-phenyl]-propyl}-3,6-dihydro-2H-pyran-4-yloxy)-tetrahydro-pyran-2-carboxylic acid **(13)**: A mixture of acetobromo-α-D-glucuronic acid methyl ester (1.0 g, 2.5 mmol), tipranavir (2.3 g, 3.8 mmol), and Ag_2O (880 mg, 3.8 mmol) in MeCN (15 mL) was stirred over night at room temperature. Then 1 N NaOH (15 mL) was added and the mixture was stirred for 1 h. The mixture was filtered (Celite®), then acidified with 3 N HCl. The mixture was extracted with EtOAc, dried over Na_2SO_4 and concentrated giving yellow oil. The crude oil was fractionated by filtration through a plug of silica; elution first with (75% EtOAc/Hexane) then with (20% MeOH/ CH_2Cl_2) gave 230 mg (12% yields) as white solid. A fraction (75 mg) was further purified by preparative HPLC (Waters Masslynx, 15–100% MeCN/H_2O in 10 min). Collected only *m/z* 778, the combined fractions were concentrated with an E–Z evaporator giving (**13**) (30 mg) as a white solid as a mixture of α and β epimers. LRMS *m/z* 779 (M^+). ^{1}H NMR (d_6-DMSO) δ: 0.70 (t, $J = 7.5$, 3H), 0.78 t, $J = 7.5$, 3H), 1.24 (m, 4H), 1.58 (m, 2H), 1.81 (m, 3H), 2.00 (m, 1H), 2.48 (m, 1H), 2.78 (d, $J = 18$, 1H), 2.83 (d, $J = 18$, 1H), 3.32 (m, 3H), 3.40 (t, $J = 9$, 1H), 3.87 (d, $J = 9.5$, 1H), 3.93 (t, $J = 7.5$, 1H), 5.09 (d, $J = 7.5$, 1H), 5.31 (d, $J = 4$, 1H), 5.50 (d, $J = 5$, 1H), 6.91 (m, 1H), 7.06 (m, 4H), 7.12 (m, 1H), 7.16 (m, 1H), 7.24 (m, 2H), 8.14(d, $J = 8.5$, 1H), 8.43 (dd, $J = 2.1$, 8.5, 1H), 9.12 (s, 1H), 10.61 (brs, 1H). ^{13}C NMR (d_6-DMSO) δ: 12.6, 14.2, 16.1, 23.9, 29.3, 31.7, 41.5, 71.1, 72.8, 75.3, 76.1, 80.2, 98.4, 110.5, 118.1, 120.4, 121.5, 122.9, 124.2, 124.7, 125.7, 127.5, 127.8, 128.4, 136.6, 141.4, 144.9, 147.2, 159.7, 163.5, 164.7, 169.9.

Conclusion

[^{14}C]-Tipranavir was synthesized in seven steps with a specific activity of 54 mCi/mmol. However, due to its instability the specific activity was diluted to half. The synthesis included a Kneovenagel type condensation of (*R*)-4-Hydroxy-6-phenethyl-6-propyl-5,6-dihydro-2-pyran-2-one with 1-(3-nitrophenyl-1-propanone and an asymmetric hydrogenation that gave 94% ee. A total of 245 mg of more than 98% chemical and radiochemical

pure material were obtained after flash chromatography purification. Carbon-13 labeled tipranavir was prepared in seven steps using the same route and starting from [$^{13}C_6$]-benzoic acid. The two potential metabolites found when tipranavir was administered with ritonavir, the hydroxyl-metabolite and the glucuronide conjugate were prepared to help in confirming the identities of those metabolites.

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References

[1] M. Markowitz, L. N. Slater, R. Schwartz, P. H. Kazanjian, B. Hathaway, D. Wheeler, M. Goldman, D. Neubacher, D. Mayers, H. Valdez, S. McCallister, and BI 1182.2 Study Team, *J. Acquir. Immune Defic. Syndr.* **2007**, *45*, 401–410.
[2] S. McCallister, H. Valdez, K. Curry, T. MacGregor, M. Borin, W. Freimuth, Y. Wang, D. L. Mayers, *J. Acquir. Immune Defic. Syndr.* **2004**, *35*, 376–382.
[3] E. De Clerq, *Expert Opin. Emerging Drugs* **2005**, *10*, 241–274.
[4] S. Mehandru, M. Markowitz, *Expert Opin. Invest. Drugs* **2003**, *12*, 1821–1828.
[5] (a) V. J. Merluzzi, K. D. Hargrave, M. Labadia, K. Grozinger, M. Skoog, J. C. Wu, C-K. Shih, K. Eckner, S. Hattox, J. Adams, A. S. Rosenthal, R. Faanes, R. J. Eckner, R. A. Koup, J. L. Sullivan, *Science* **1990**, *250*, 1411–1413;(b) J. C. Wu, T. C. Warren, J. Adams, J. Proudfoot, J. Skiles, P. Raghavan, C. Perry, I. Potocki, P. Farina, P. M. Grob, *Biochemistry* **1991**, *30*, 2022–2056.
[6] M. Högberg, I. Morrison, *Expert Opinion Ther Patents* **2000**, *10*, 1189–1199.
[7] S. R. Turner, J. W. Strohbach, R. A. Tommasi, P. A. Aristoff, P. D. Johnson, H. I . Skulnick, L. A. Dolak, E. P. Seest, P. K. Tomich, M. J. Bohanon, M-M. Horng, J. C. Lynn, K-T. Chong, R. R. Hinshaw, K. D. Watenpaugh, M. N. Janakiraman, S. Thaisrivongs, *J. Med. Chem.* **1998**, *41*, 3467–3476.
[8] S. Thaisrivongs, H. I. Skulnick, S. R. Turner, J. W. Strohbach, R. A. Tommasi, P. D. Johnson, P. A. Aristoff, T. M. Judge, R. B. Gammill, J. K. Morris, K. R. Romines, R. A. Chrusciel, R. R. Hinshaw, K-T. Chong, W. G. Tarpley, S. M. Poppe, D. E. Slade, J. C. Lynn, M-M. Horng, P. K. Tomich, E. P. Seest, L. A. Dolak, W. J. Howe, G. M. Howard, F. J. Schwende, L. N. Toth, G. E. Padbury, G. J. Wilson, L. Shiou, G. L. Zipp, K. F. Wilkinson, B. D. Rush, M. J. Ruwart, K. A. Koeplinger, Z. Zhao, S. Cole, R. M. Zaya, T. J. Kakuk, M. N. Janakiraman, K. D. Watenpaugh, *J. Med. Chem.* **1996**, *39*, 4349–4353.
[9] UNAIDS-WHO AIDS epidemic update December **2007**.
[10] P. J. Yeni, *Acquir. Immune Defic. Syndr.* **2003**, *34*(Suppl. 1), S91–S94.
[11] http://www.aptivus.com.
[12] M. Arisawa, Y. Torisawa, M. Kawahara, M. Yamanaka, A. Nishida, M. Nakagawa, *J. Org. Chem.* **1997**, *62*, 4327–4329.
[13] M. Sauter, O. Meyer, M. Göhlich, patent WO 02068403, **2002**.
[14] T. M. Judge, G. Philips, J. K. Morris, K. D. Lovasz, K. R. Romines, G. P. Luke, J. Tulinsky, J. M. Tustin, R. A. Chrusciel, L. A. Dolak, S. A. Mizsak, W. Watt, J. Morris, S. L. Vander Velde, J. W. Strohbach, R. B. Gammill, *J. Am. Chem. Soc.* **1997**, *119*, 3627–3628.
[15] K. S. Fors, J. R. Gage, R. F. Heier, R. F. Kelly, W. R. Perrault, N. Wicnienski, *J. Org. Chem.* **1998**, *63*, 7348–7356.
[16] B. M. Trost, N. G. Anderson, *J. Am. Chem. Soc.* **2002**, *124*, 14320–11432.
[17] F . Klingler, M. Steigerwald, R. Ehlenz, patent DE 10313118A1, **2004**.
[18] I. C. Lennon, C. J. Pilkington, *Synthesis* **2003**, *11*, 1639–1642.
[19] D. L. Romero, P. R. Manninen, F. Han, A. G. Romero, *J. Org. Chem.* **1999**, *64*, 4980–4984.
[20] Z. Zhao, K. A. Koeplinger, T. Peterson, R. A. Conradi, P. S. Burton, A. Suarato, R. L. .Heinrikson, A. G. Tomasselli, *Drug Metab. Dispos.* **1999**, *27*, 992–998.
[21] P-D. Ren, X-W. Pan, Q-H. Jin, Z-P. Yao, *Synth.Commun.* **1997**, *27*, 3497–3503.