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## Supplementary information

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# Discovery of potent small-molecule inhibitors of lipoprotein(a) formation

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Supplementary Information for  
**Discovery of potent small-molecule inhibitors of lipoprotein(a) formation**

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## Supplementary Methods

### Synthesis and characterization of the compounds described.

#### General notes:

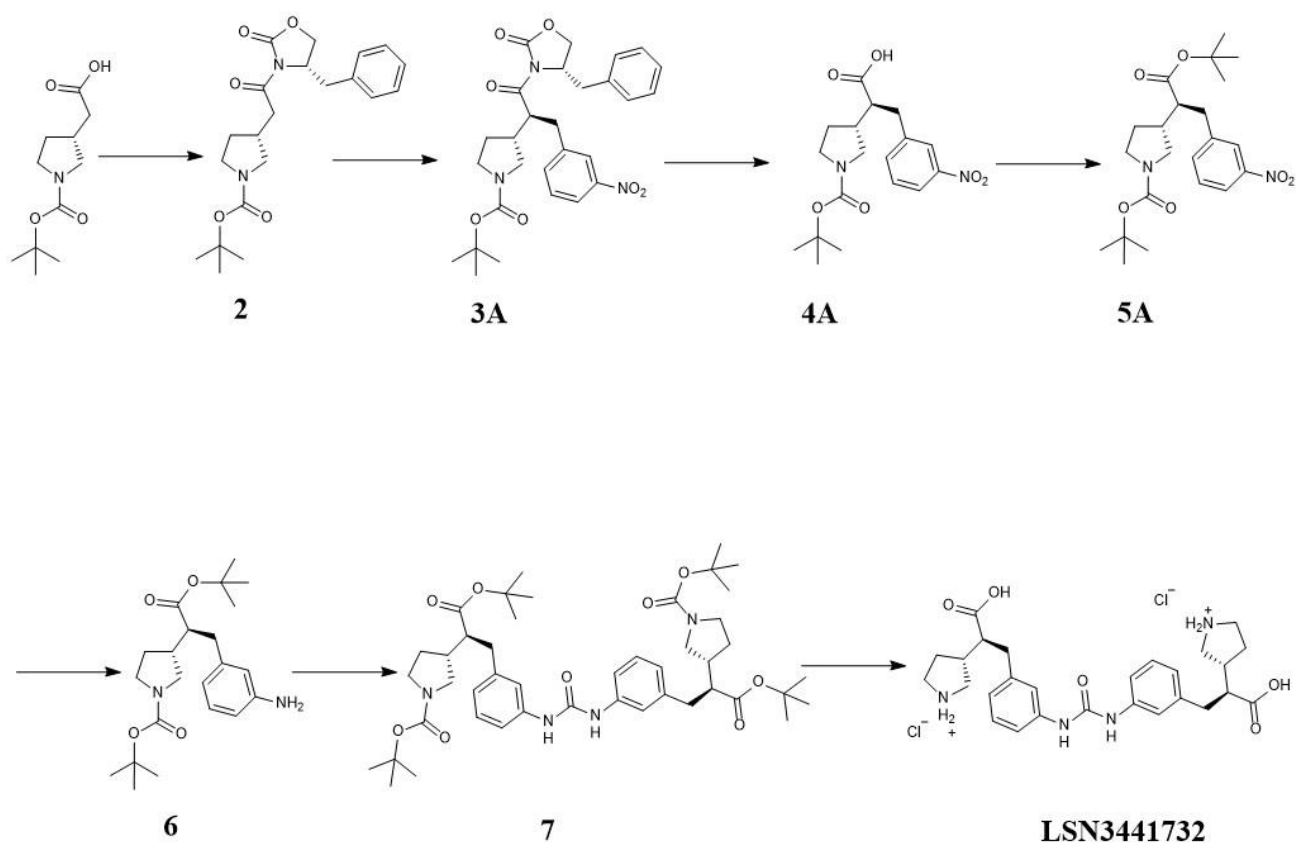
Synthesis of <sup>3</sup>H-LSN3374443 is described in WO 2020/247429 A1. Synthesis and characterization of LSN3441732, LY3473329, LSN3353871 and LSN3374443 was carried out at Lilly Research Laboratories. Enantiomeric excess of LY3473329 was determined by HPLC at Asymchem. The synthesis of radiolabeled materials was carried out at Pharmaron UK.

All solvents and starting materials were obtained from commercial sources and used as received. <sup>1</sup>H-nuclear magnetic resonance (<sup>1</sup>H-NMR) spectra were recorded using Bruker NMR spectrometer 400 MHz instrument and <sup>13</sup>C-nuclear magnetic resonance (<sup>13</sup>C-NMR) spectra were recorded on Bruker 100 MHz instrument. Chemical shifts were assigned in ppm relative to respective solvent signals (e.g., signal of residual CHCl<sub>3</sub> present in CDCl<sub>3</sub> for <sup>1</sup>H-NMR). The following abbreviations are used to describe signal splitting patterns: singlet (s), doublet (d), triplet (t), and multiplet (m).

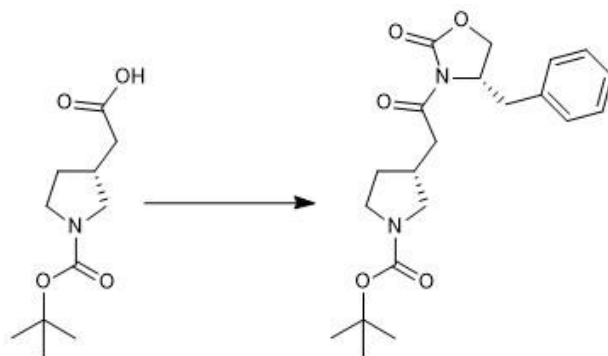
LCMS (ESI) analyses for sample characterization were performed using an Agilent LCMS 1260 instrument, equipped with DAD and MS detectors. LC analyses were obtained after injection of the sample onto a XBridge C18 column (3.5 μm, 2.1 x 50 mm). Chromatographic method in basic media using 10mM ammonium bicarbonate pH 9.0 in channel A and CH<sub>3</sub>CN in channel B. Flow Rate: 1.2mL/min; T<sup>a</sup> 50°C; gradient mode: from 5 to 95% B in 1.5 min, hold 0.5 min at 95%B. UV detection was registered at 214 and 300 nm. MS-ESI detection was acquired between 100 and 800 amu. High-resolution mass spectra (HRMS) were recorded using an electrospray ion source (ESI). ESI analysis was performed using an Agilent Accurate-Mass QTOF LCMS instrument, model 6530, equipped with a JetStream ESI Source with two nebulizers. The main nebulizer was hyphenated to a HPLC Agilent 1290 system for sample introduction, and the second one to an isocratic HPLC pump for a continuous introduction of external standards to be used for continuous calibration of the spectra. Diastereomeric excesses (de) were determined by <sup>1</sup>H-NMR, high-performance liquid chromatography (HPLC) or supercritical fluid chromatography (SFC) in an

Agilent 1200 Analytical SFC-MS system. The retention times ( $t_R$ ) described in the chromatograms are those obtained under the conditions described in each case and for a particular equipment configuration; any change may result in modifications to the retention time. Optical rotations were measured on an Anton Paar MCP 500 polarimeter with  $[\alpha]_D^{23}$  values reported in degrees; concentration (c) is in g/100 mL.

### Synthesis of compound LSN3441732

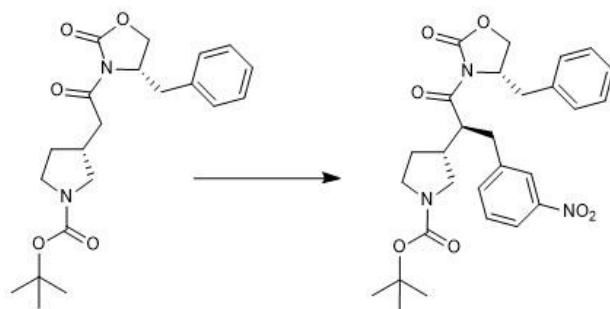


tert-Butyl (R)-3-(2-((S)-4-benzyl-2-oxooxazolidin-3-yl)-2-oxoethyl)pyrrolidine-1-carboxylate (**2**)



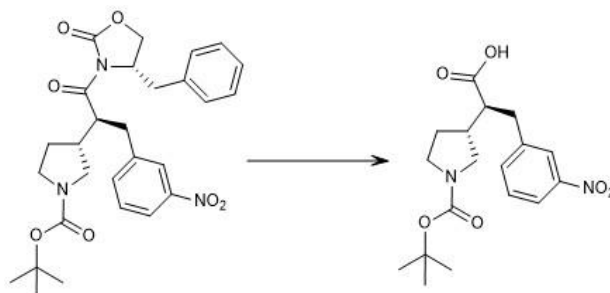
Add triethylamine (56.5 g, 77.9 mL, 559 mmol, 2.5 equivalent) to a solution of (*R*)-2-(1-(tert-butoxycarbonyl)pyrrolidin-3-yl)acetic acid (53.8 g, 235 mmol, 1.05 equivalent) in THF (540 mL) maintained at 10 °C. After 5 min, add pivaloyl chloride (33.7 g, 34.2 mL, 279 mmol, 1.25 equivalent). After 15 min, add lithium chloride (11.8 g, 279 mmol, 1.25 equivalent) in THF (540 mL) and (4*S*)-4-benzyloxazolidin-2-one (40 g, 223 mmol, 1 equivalent). Allow the mixture to warm to room temperature and stir for 24 h. After 24 h, add 1N aqueous HCl (500 mL) and separate the organic phase from the aqueous phase. Wash the organic phase with 1N aqueous NaOH (500 mL) and saturated aqueous NaCl (500 mL). Separate the layers and dry the organic layer over MgSO<sub>4</sub>, filter, and concentrate the solution in vacuo. Suspend the residue in a mixture of MeOH/water (1:2, 575 mL) and stir at room temperature overnight. Filter off the solid, wash with hexanes (2 × 150 mL), and dry the solid to give the title compound (65.7 g, 76%). The diastereomeric excess was determined to be >95% by <sup>1</sup>H-NMR spectroscopy; the diastereomeric proton resonances of the minor isomer (separated from the proton signals of the major isomer) were not observed in the <sup>1</sup>H-NMR spectra of the final product. LCMS (ESI): *m/z* 333.2 [M+H-*tert*-butyl]<sup>+</sup>. <sup>1</sup>H-NMR (400.13 MHz, CDCl<sub>3</sub>) δ 7.38-7.28 (m, 3H), 7.23-7.17 (m, 2H), 4.73-4.63 (m, 1 H), 4.27-4.16 (m, 2H), 3.75-3.61 (m, 1H), 3.55-3.39 (m, 1H), 3.38-3.25 (m, 2H), 3.13-2.91 (m, 3 H), 2.84-2.73 (m, 1H), 2.73-2.61 (m, 1H), 2.16-2.06 (m, 1H), 1.64-1.58 (m, 1H), 1.47 (s, 9H).

*tert*-Butyl (R)-3-((S)-1-((S)-4-benzyl-2-oxooxazolidin-3-yl)-3-(3-nitrophenyl)-1-oxopropan-2-yl)pyrrolidine-1-carboxylate (**3A**)



Add lithium bis(trimethylsilyl)amide (1.0 mol/L in THF, 46.33 mL, 46.33 mmol, 1.2 equivalent) to a solution of *tert*-butyl (*R*)-3-(2-((*S*)-4-benzyl-2-oxooxazolidin-3-yl)-2-oxoethyl)pyrrolidine-1-carboxylate (15 g, 38.61 mmol, 1 equivalent) in THF (5 mL/g, 75 mL) at  $-20^{\circ}\text{C}$ . Stir the mixture at  $-20^{\circ}\text{C}$  for 20 min. Add a solution of 1-(bromomethyl)-3-nitro-benzene (9.17 g, 42.47 mmol, 1.1 equivalent) in THF (3 mL/g, 45 mL). Stir the solution for 2 h and allow it to warm to room temperature. Dilute the mixture with MTBE and quench with a saturated solution of  $\text{NH}_4\text{Cl}$ , separate the phases, and extract the aqueous phase with MTBE (1 $\times$ ). Combine the organic extracts and sequentially wash the organic extracts with water and brine. Separate the layers and dry the organic layer over  $\text{Na}_2\text{SO}_4$ , filter, and concentrate the filtrate under reduced pressure. Triturate the residue with a mixture of MeOH/ $\text{H}_2\text{O}$  (2:1, 150 mL). Stir the slurry overnight. Filter to collect the solid and wash with hexanes. Dry the solid under vacuum at  $40^{\circ}\text{C}$  to give the title compound (19 g, 88%). LCMS (ESI):  $m/z$  468.1  $[\text{M}+\text{H}-\text{tert-butyl}]^+$ .  $^1\text{H-NMR}$  (400.13 MHz,  $\text{CDCl}_3$ )  $\delta$  8.18-8.04 (m, 2H), 7.68-7.58 (m, 1H), 7.52-7.42 (t,  $J = 7.9$  Hz, 1H), 7.30-7.21 (m, 3H), 7.04-6.97 (m, 2H), 4.66-4.57 (m, 1H), 4.52-4.42 (m, 1H), 4.16-3.99 (m, 2H), 3.75-3.44 (m, 2H), 3.29-2.96 (m, 5H), 2.62-2.46 (m, 1H), 2.30-2.13 (m, 1H), 2.00-1.90 (m, 1H), 1.80-1.64 (m, 1H), 1.46 (s, 9H).

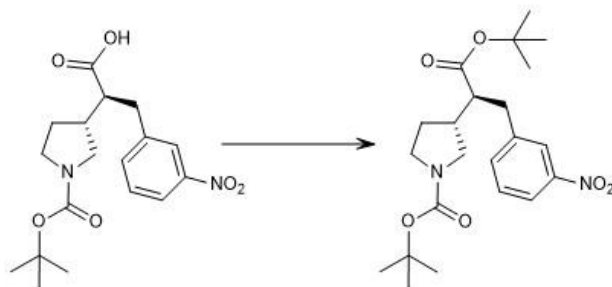
(*S*)-2-((*R*)-1-(*tert*-Butoxycarbonyl)pyrrolidin-3-yl)-3-(3-nitrophenyl)propanoic acid (**4A**)



Add lithium hydroxide (11.46 mL, 11.46 mmol, 1.5 equivalent, 1M in water) and hydrogen

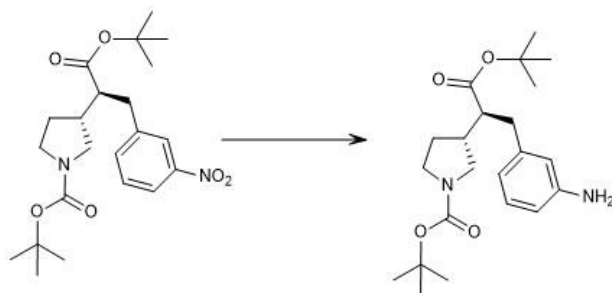
peroxide (5.9 mL, 61.12 mmol, 8 equivalent, 35 mass %) to a solution of *tert*-butyl (*R*)-3-((*S*)-1-((*S*)-4-benzyl-2-oxooxazolidin-3-yl)-3-(3-nitrophenyl)-1-oxopropan-2-yl)pyrrolidine-1-carboxylate (4 g, 7.639 mmol, 1 equivalent) in THF (25 mL). Stir at room temperature for 3 h. Add 1N aqueous NaOH and extract the crude with MTBE and discard organics. Add 1N aqueous HCl until acidic pH and extract with EtOAc (x2). Combine organic phases, wash with water and brine, dry over MgSO<sub>4</sub>, filter and concentrate under reduced pressure to give the title compound (2.5 g, 85%). LCMS (ESI): *m/z* 265.0 [M+H-BOC]<sup>+</sup>. <sup>1</sup>H-NMR (400.13 MHz, CDCl<sub>3</sub>) δ 8.12-8.01 (m, 2H), 7.55-7.48 (m, 1H), 7.47-7.41 (m, 1H), 3.78-3.43 (m, 2H), 3.32-2.85 (m, 4H), 2.68-2.58 (m, 1H), 2.50-2.35 (m, 1H), 2.06-1.96 (m, 1H), 1.79-1.64 (m, 1H), 1.46 (s, 9H).

*tert*-Butyl (R)-3-((S)-1-(*tert*-butoxy)-3-(3-nitrophenyl)-1-oxopropan-2-yl)pyrrolidine-1-carboxylate (5A)



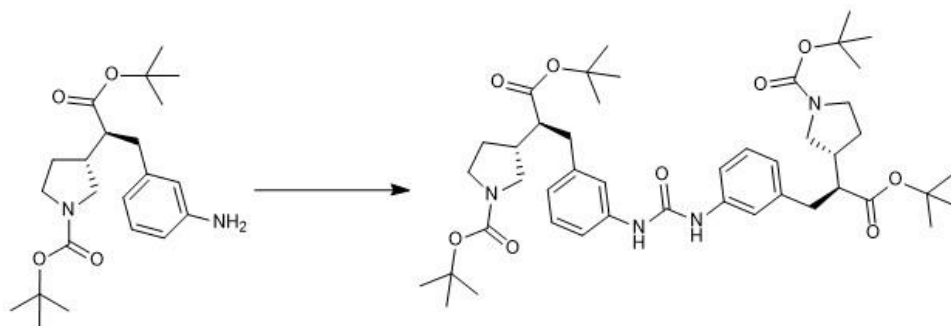
Add 1,1-di-*tert*-butoxy-*N,N*-dimethyl-methanamine (18.3 mL, 76.1 mmol, 4 equivalent) to a solution of (*S*)-2-((*R*)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl)-3-(3-nitrophenyl)propanoic acid (6.9 g, 19.0 mmol, 1 equivalent) in toluene (190 mL, 10 mL/mmol). Stir and heat the reaction for 6 h at 80 °C. Add 1,1-di-*tert*-butoxy-*N,N*-dimethyl-methanamine (18.3 mL, 76.1 mmol, 4 equivalent) again and stir and heat the reaction overnight at 80 °C. Concentrate the mixture under reduced pressure. Subject the residue to silica gel chromatography eluting with a 5–20% gradient of EtOAc in hexanes to give the title compound (5.6 g, 70%). LCMS (ESI): *m/z* 309.0 [M+H-2x *tert*-butyl]<sup>+</sup>. <sup>1</sup>H-NMR (400.13 MHz, DMSO-*d*<sub>6</sub>) δ 8.10-8.08 (m, 2H), 7.70 (d, *J* = 7.7 Hz, 1H), 7.59 (t, *J* = 8.1 Hz, 1H), 3.57-3.50 (m, 1H), 3.39-3.34 (m, 1H), 3.22-3.11 (m, 1H), 3.05 (t, *J* = 10.0 Hz, 1H), 2.96-2.84 (m, 2H), 2.64-2.60 (m, 1H), 2.39-2.27 (m, 1H), 1.86 (dd, *J* = 5.6, 11.1 Hz, 1H), 1.67-1.53 (m, 1H), 1.41 (s, 9H), 1.21 (s, 9H).

*tert*-Butyl (R)-3-((S)-3-(3-aminophenyl)-1-(*tert*-butoxy)-1-oxopropan-2-yl)pyrrolidine-1-carboxylate (6)



To a stirred solution of *tert*-butyl (R)-3-((S)-1-(*tert*-butoxy)-3-(3-nitrophenyl)-1-oxopropan-2-yl)pyrrolidine-1-carboxylate (3 g, 7.134 mmol) in EtOAc (0.1 M) under nitrogen atmosphere add palladium on carbon (0.05 equivalent, 0.3567 mmol, 10 mass %). Purge the mixture with hydrogen and stir under a hydrogen atmosphere (balloon) at room temperature overnight. Filter the reaction mixture over a pad of diatomaceous earth. Concentrate the filtrate under reduced pressure to give the title compound (2.62 g, 94%). LCMS (ESI):  $m/z$  291.2  $[M+H-BOC]^+$ .  $^1H$ -NMR (400.13 MHz,  $CDCl_3$ )  $\delta$  7.03 (t,  $J = 7.4$  Hz, 1H), 6.59-6.45 (m, 3H), 3.74-3.40 (m, 2H), 3.30-3.17 (m, 1H), 3.07-2.91 (m, 1H), 2.82-2.63 (m, 2H), 2.50-2.29 (m, 2H), 1.99-1.88 (m, 1H), 1.73-1.60 (m, 1H), 1.46 (s, 9H), 1.31 (s, 9H).

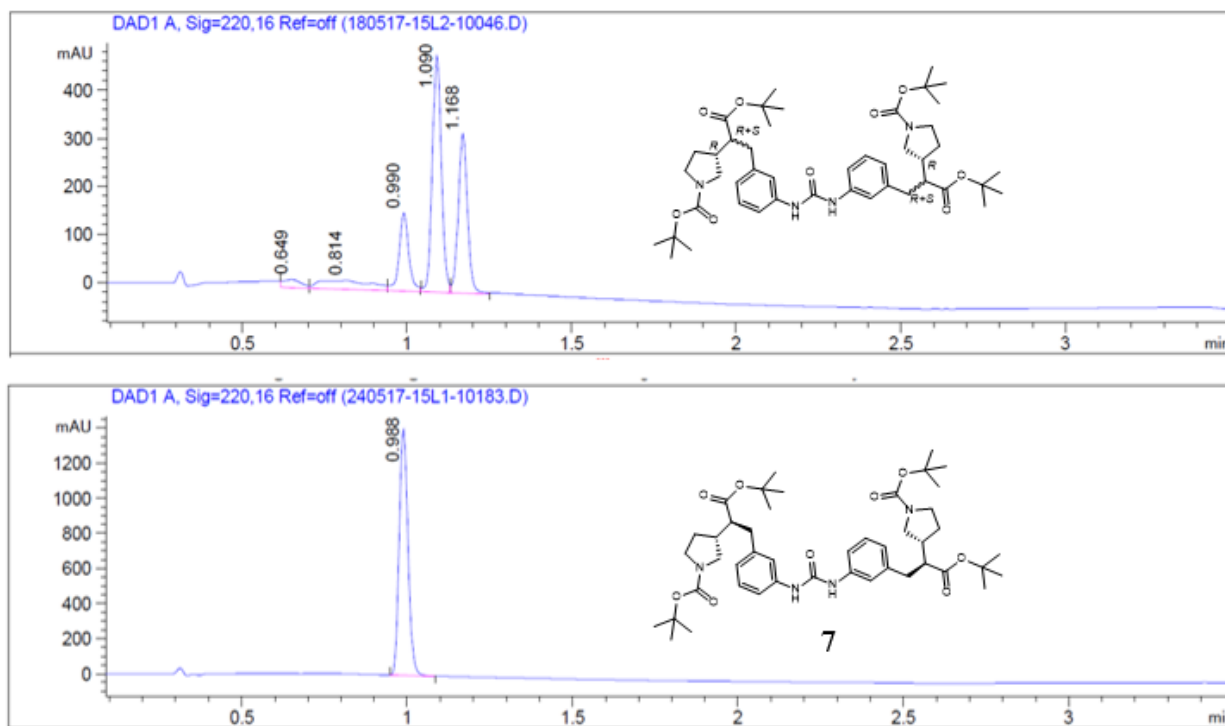
di-*tert*-Butyl 3,3'-((2S,2'S)-((carbonylbis(azanediyl))bis(3,1-phenylene))bis(3-(*tert*-butoxy)-3-oxopropane-1,2-diyl))(3R,3'R)-bis(pyrrolidine-1-carboxylate) (7)



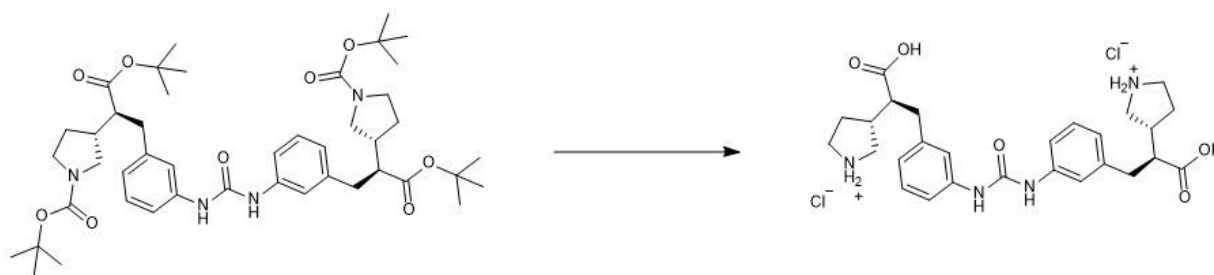
Combine *tert*-butyl (R)-3-((S)-3-(3-aminophenyl)-1-(*tert*-butoxy)-1-oxopropan-2-yl)pyrrolidine-1-carboxylate (57.80 g, 148.010 mmol, 2 equivalent) and 1,1'-carbonyldiimidazole (12 g, 74 mmol, 1.0 equivalent) in 2-methyl tetrahydrofuran (20 mL/g, 240 mL). Stir and heat the mixture

at 70 °C overnight. Cool the mixture to room temperature and add water (250 mL) and 2-methyl tetrahydrofuran (250 mL). Separate the layers, discard the aqueous layer, dry the organic layer over MgSO<sub>4</sub>, filter, and concentrate the filtrate under reduced pressure to provide the crude material. Triturate the crude material at 60 °C in a mixture of IPA/water 1/1 (600 mL) for 4 h and then at room temperature overnight. Filter to collect the resulting solid, wash the solid with IPA/water 1/1. Dry the solid under reduced pressure at 40 °C to give the title compound (58 g, 97%). LCMS (ESI): *m/z* 707.2 [M+H-BOC]<sup>+</sup>. <sup>1</sup>H-NMR (400.13 MHz, DMSO-d<sub>6</sub>) δ 8.52 (s, 2H), 7.34-7.20 (m, 4H), 7.15 (t, *J*= 7.8 Hz, 2H), 6.7 (d, *J*= 7.3 Hz, 2H), 3.57-3.46 (m, 2H), 3.40-3.27 (m, 2H), 3.22-3.07 (m, 2H), 2.97 (t, *J*= 10.0 Hz, 2H), 2.78-2.62 (m, 4H), 2.52-2.42 (m, 2H), 2.36-2.20 (m, 2H), 1.91-1.79 (m, 2H), 1.68-1.51 (m, 2H), 1.40 (s, 18H), 1.25 (s, 18H).

The diastereomeric excess (de) was determined by HPLC, comparing with the diastereomeric mixture di-*tert*-butyl 3,3'-(((carbonylbis(azanediyl))bis(3,1-phenylene))bis(3-(*tert*-butoxy)-3-oxopropane-1,2-diyl))(3*R*,3'*R*)-bis(pyrrolidine-1-carboxylate) under the assumption that only the stereogenic center in α to the carbonyl will be susceptible to epimerization. SFC analysis (Chiralpak IB 4.6x100mm,5um; gradient from 15 to 55% B in 2 min, hold 1 min at 55% B (A: CO<sub>2</sub>, B: MeOH-0.2%IPAm), 4 mL/min; 120 bar outlet P/ 40°C, 220 nm) indicated >98% de: *t<sub>R</sub>* = 0.99 min. The *t<sub>R</sub>* for the other isomers are: *t<sub>R</sub>* = 1.09 min and *t<sub>R</sub>* = 1.16 min. SFC chromatograms included below.

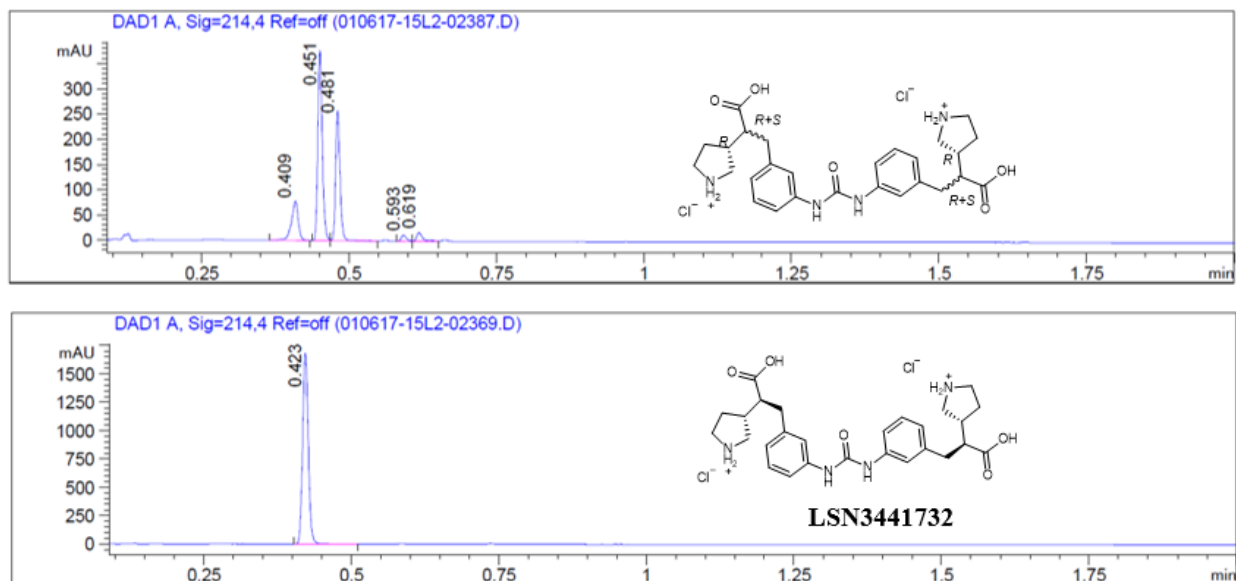


(3R,3'R)-3,3'-((1S,1'S)-((Carbonylbis(azanediyl))bis(3,1-phenylene))bis(1-carboxyethane-2,1-diyl))bis(pyrrolidin-1-ium) chloride (LSN3441732)

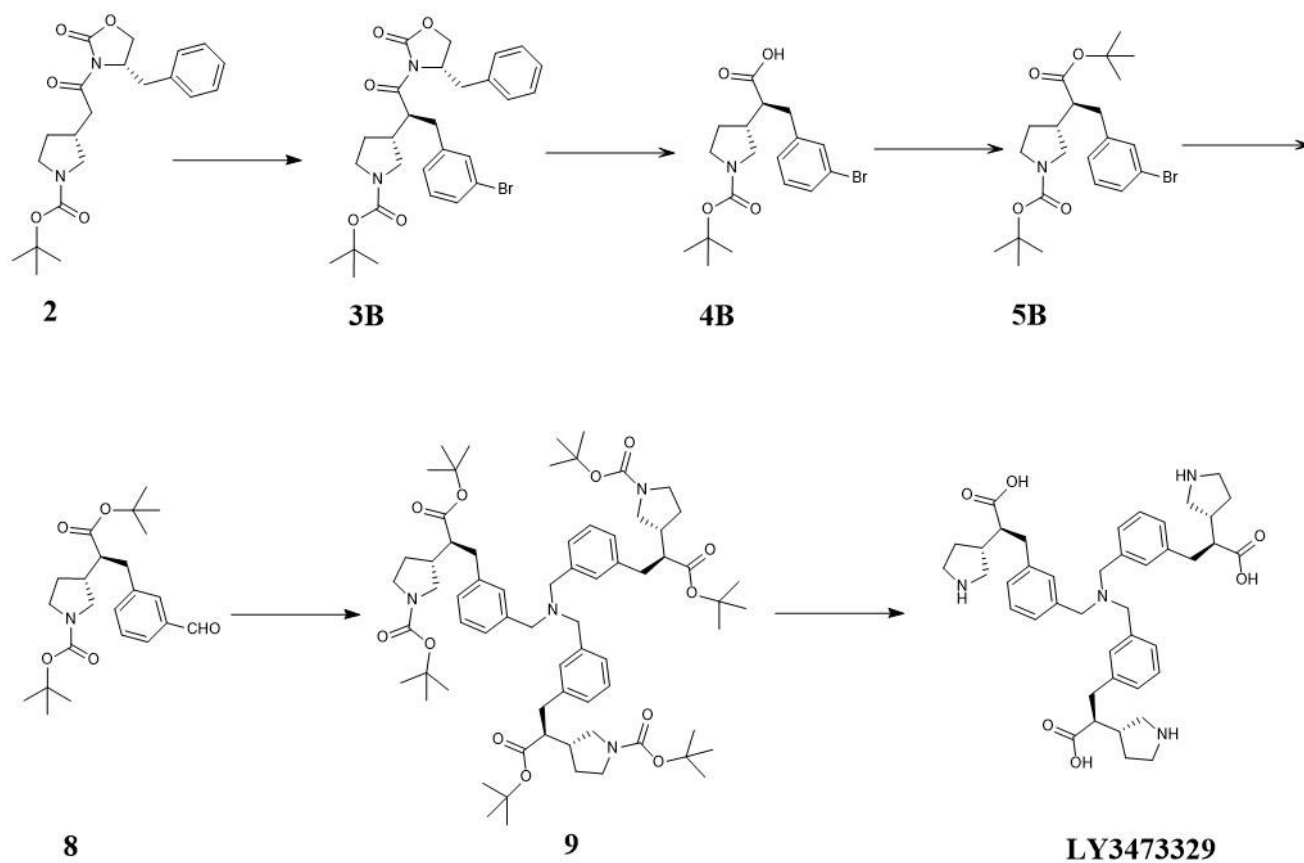


Add HCl solution in dioxane (4.0 mol/l, 170 mL, 693.9 mmol, 10 equivalent) to a mixture of di-*tert*-butyl 3,3'-((2S,2'S)-((carbonylbis(azanediyl))bis(3,1-phenylene))bis(3-(*tert*-butoxy)-3-oxopropane-1,2-diyl))(3R,3'R)-bis(pyrrolidine-1-carboxylate) (56 g, 69.39 mmol, 1 equivalent) in 1,4-dioxane (56 mL). Stir the mixture at room temperature overnight. Filter to collect the resulting solid. Wash the solid with dioxane. Dry the resulting solid under reduced pressure at 40 °C. Triturate the material in *tert*-amyl alcohol (600 mL) overnight. Filter to collect the solid and wash with *tert*-amyl alcohol. Dry the solid under reduced pressure at 40 °C to give the title compound (39.0 g, 99%). LCMS (ESI):  $m/z$  494.8  $[M+H]^+$ .  $^1\text{H-NMR}$  (400.13 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.32 (t,  $J$  = 7.8 Hz, 2H), 7.66-7.18 (m, 4H), 7.02 (d,  $J$  = 7.6 Hz, 2H), 3.58 (dd,  $J$  = 7.9, 11.8 Hz, 2H), 3.48-3.38

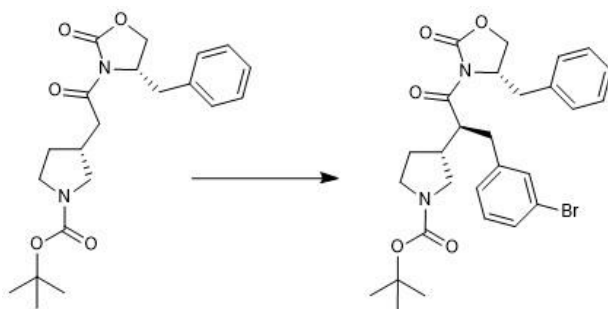
(m, 2H), 3.32-3.21 (m, 2H), 3.06 (dd,  $J = 10.2, 11.6$  Hz, 2H), 2.98-2.73 (m, 6H), 2.65-2.50 (m, 2H), 2.26-2.14 (m, 2H), 1.84-1.70 (m, 2H).  $^{13}\text{C}$  NMR (100.62 MHz,  $\text{D}_2\text{O}$ )  $\delta$  177.7, 155.2, 139.3, 138.1, 129.3, 124.1, 120.8, 118.9, 50.2, 48.1, 45.1, 39.5, 36.7, 28.0. HRMS (DART-TOF)  $m/z$  calculated for  $\text{C}_{27}\text{H}_{34}\text{N}_4\text{O}_5$   $[\text{M}+\text{H}]$ : 495.2602, found 495.2600.  $[\alpha]_{\text{D}}^{23} = -33.6$  ( $c = 0.1$ ,  $\text{H}_2\text{O}$ ). The diastereomeric excess (de) was measured by HPLC, comparing with the diastereomeric mixture (3*R*,3'*R*)-3,3'-(((carbonylbis(azanediyl))bis(3,1-phenylene))bis(1-carboxyethane-2,1-diyl))bis(pyrrolidin-1-ium) chloride under the assumption that only the stereogenic center in  $\alpha$  to the carbonyl will be susceptible to epimerization. HPLC analysis (Column XBridge C18 3.5 $\mu\text{m}$ , 2.1 x 50mm; gradient mode: from 5 to 95% B in 1.5min, hold 0.5min at 95%B (A: 10mM Ammonium Bicarbonate pH:9.0. B:  $\text{CH}_3\text{CN}$ ); 1.2mL/min; 214 nm) indicated >98% de:  $t_{\text{R}} = 0.41$  min. The  $t_{\text{R}}$  for the other isomers are:  $t_{\text{R}} = 0.45$  min and  $t_{\text{R}} = 0.48$  min. HPLC chromatograms included below.



## Synthesis of compound LY3473329



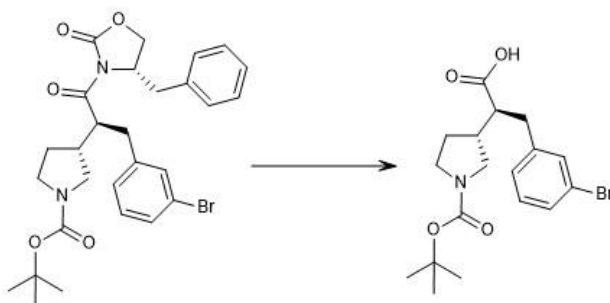
tert-Butyl (R)-3-((S)-1-((S)-4-benzyl-2-oxooxazolidin-3-yl)-3-(3-bromophenyl)-1-oxopropan-2-yl)pyrrolidine-1-carboxylate (**3B**)



**3B** was prepared following essentially the same procedure as **3A**, using 1-bromo-3-(bromomethyl)benzene.

LCMS (ESI):  $m/z$  501.0/503.0  $[M+H\text{-}tert\text{-butyl}]^+$ .  $^1\text{H-NMR}$  (400.13 MHz,  $\text{CDCl}_3$ )  $\delta$  7.43-7.32 (m, 2H), 7.31-7.11 (m, 5H), 7.02 (d,  $J = 6.6$  Hz, 2H), 4.66-4.56 (m, 1H), 4.50-4.37 (m, 1H), 4.14-3.96 (m, 2H), 3.78-3.40 (m, 2H), 3.29-3.17 (m, 1H), 3.17-2.86 (m, 4H), 2.60-2.44 (m, 1H), 2.27-2.12 (m, 1H), 1.99-1.89 (m, 1H), 1.80-1.61 (m, 1H), 1.46 (s, 9H).

(2S)-3-(3-Bromophenyl)-2-((3R)-1-*tert*-butoxycarbonylpyrrolidin-3-yl)propanoic acid (**4B**)

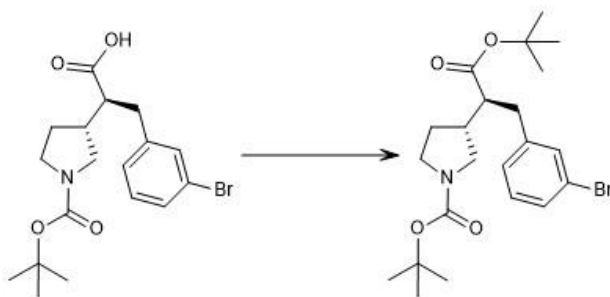


**4B** was prepared following essentially the same procedure as **4A**.

LCMS (ESI):  $m/z$  341.8/343.8  $[M+H\text{-}tert\text{-butyl}]^+$ .  $^1\text{H-NMR}$  (400.13 MHz,  $\text{CDCl}_3$ )  $\delta$  7.38-7.29 (m, 2H), 7.19-7.04 (m, 2H), 3.75-3.42 (m, 2H), 3.31-3.19 (m, 1H), 3.12-2.74 (m, 3H), 2.66-2.55 (m, 1H), 2.48-2.31 (m, 1H), 2.05-1.93 (m, 1H), 1.78-1.62 (m, 1H), 1.46 (s, 9H).

The diastereomeric excess was determined to be >95% by NMR spectroscopy as the diastereomeric proton resonances for the minor isomer (which appeared separated from the proton signals of the major isomer) were not observed in the  $^1\text{H-NMR}$  spectra of the reaction product.

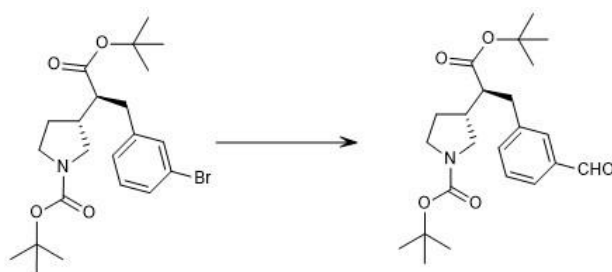
*tert*-Butyl(*R*)-3-((*S*)-3-(3-bromophenyl)-1-(*tert*-butoxy)-1-oxopropan-2-yl)pyrrolidine-1-carboxylate (**5B**)



**5B** was prepared following essentially the same procedure as **5A**.

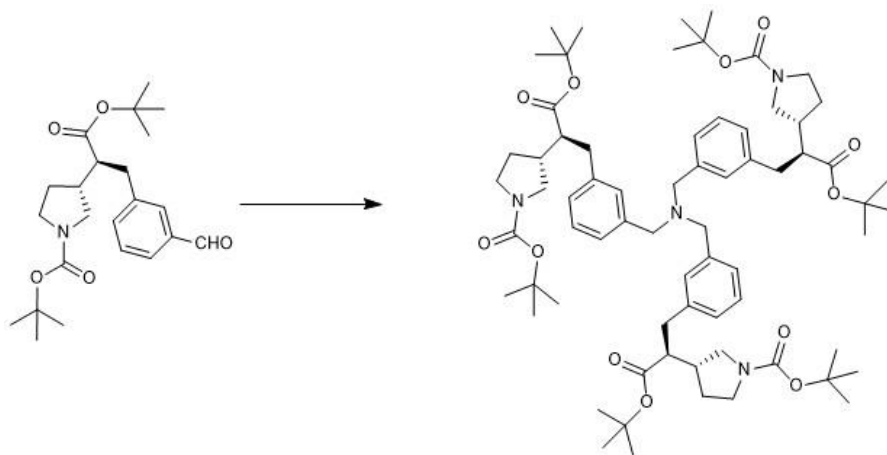
LCMS (ESI):  $m/z$  341.8/343.8  $[M+H-2\times\text{tert-butyl}]^+$ .  $^1\text{H-NMR}$  (400.13 MHz,  $\text{CDCl}_3$ )  $\delta$  7.37-7.29 (m, 2H), 7.18-7.05 (m, 2H), 3.75-3.39 (m, 2H), 3.32-3.18 (m, 1H), 3.10-2.90 (m, 1H), 2.89-2.69 (m, 2H), 2.50-2.29 (m, 2H), 2.00-1.89 (m, 1H), 1.75-1.58 (m, 1H), 1.47 (s, 9H), 1.30 (s, 9H).

*tert*-Butyl(*R*)-3-((*S*)-1-(*tert*-butoxy)-3-(3-formylphenyl)-1-oxopropan-2-yl)pyrrolidine-1-carboxylate (**8**)



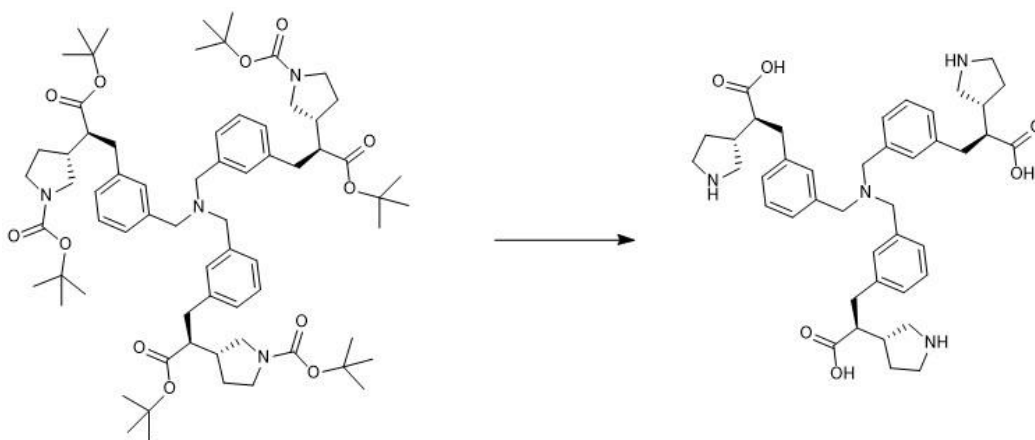
Add *tert*-butyl (*R*)-3-((*S*)-3-(3-bromophenyl)-1-(*tert*-butoxy)-1-oxopropan-2-yl)pyrrolidine-1-carboxylate (48 g, 105.6 mmol, 1.0 equivalent), degassed toluene (0.12 M, 880 mL), *n*-butyl-di-1-adamantylphosphine (0.1 mol/mol, 10.56 mmol, 95 mass %, 3.987 g, 0.1 equivalent), palladium(II) acetate (0.05 mol/mol, 5.282 mmol, 1.186 g, 0.05 equivalent) and *N,N,N',N'*-tetramethylethylenediamine (1.5 equivalent, 158.5 mmol, 23.9 mL, 1.5 equivalent) to a 2L Parr reactor. Pressurize the Parr reactor with 70 psi  $\text{CO}/\text{H}_2$  1:1. Stir the mixture at 100 °C overnight. Cool the mixture to room temperature and evaporate the solvent to dryness. Add water (500 mL) to the crude and extract the mixture with EtOAc (500 mL). Wash organic layer with 1M aqueous  $\text{KHSO}_4$  (200 mL), dry over  $\text{MgSO}_4$ , filter through silica gel and concentrate to dryness to afford the title compound (42 g, 98% yield) as a yellow oil. LCMS (ESI):  $m/z$  426.0  $[M+\text{Na}]$ .  $^1\text{H-NMR}$  (400.13 MHz,  $\text{CDCl}_3$ )  $\delta$  9.98 (s, 1H), 7.76-7.67 (m, 2H), 7.48-7.41 (m, 2H), 3.79-3.41 (m, 2H), 3.33-3.20 (m, 1H), 3.11-2.80 (m, 3H), 2.57-2.47 (m, 1H), 2.45-2.31 (m, 1H), 2.00-1.91 (m, 1H), 1.76-1.56 (m, 1H), 1.47 (s, 9H), 1.25 (s, 9H).

tri-*tert*-Butyl 3,3',3''-((2*S*,2'*S*,2''*S*)-((nitrilotris(methylene))tris(benzene-3,1-diyl))tris(3-(*tert*-butoxy)-3-oxopropane-1,2-diyl))(3*R*,3'*R*,3''*R*)-tris(pyrrolidine-1-carboxylate) (**9**)



Add *tert*-butyl (*R*)-3-((*S*)-1-(*tert*-butoxy)-3-(3-formylphenyl)-1-oxopropan-2-yl)pyrrolidine-1-carboxylate (222 g, 550.2 mmol, 1.0 equivalent), 2-propanol (4 mL/g, 888 mL), ammonia (2 mol/L in 2-propanol, 302.6 mL, 1.1 equivalent) to a round bottom flask. Cool the mixture to 0–5 °C with an ice water bath. Add sodium triacetoxyborohydride (550.2 mmol, 1.0 equivalent) in four portions at 40 min intervals. Stir the mixture at room temperature overnight. Evaporate the solvent to dryness. Add water (200 mL) and aqueous 2M K<sub>2</sub>HPO<sub>4</sub> (300 mL) and extract with MTBE (2 × 500 mL). Dry the organic layer over MgSO<sub>4</sub>, filter, and concentrate under reduced pressure. Subject the residue to silica gel chromatography eluting with a 20–80% gradient of EtOAc in hexanes to give the title compound (57.8 g, 27%). <sup>1</sup>H-NMR (400.13 MHz, CDCl<sub>3</sub>) δ 7.30-7.18 (m, 6H), 7.14-7.06 (m, 3H), 7.5-6.98 (m, 3H), 3.75-3.34 (m, 12H), 3.30-3.17 (m, 3H), 3.10-2.92 (m, 3H), 2.89-2.70 (m, 6H), 2.55-2.28 (m, 6H), 1.98- 1.88 (m, 3H), 1.74-1.57 (m, 3H), 1.45 (s, 27H), 1.20 (s, 27H).

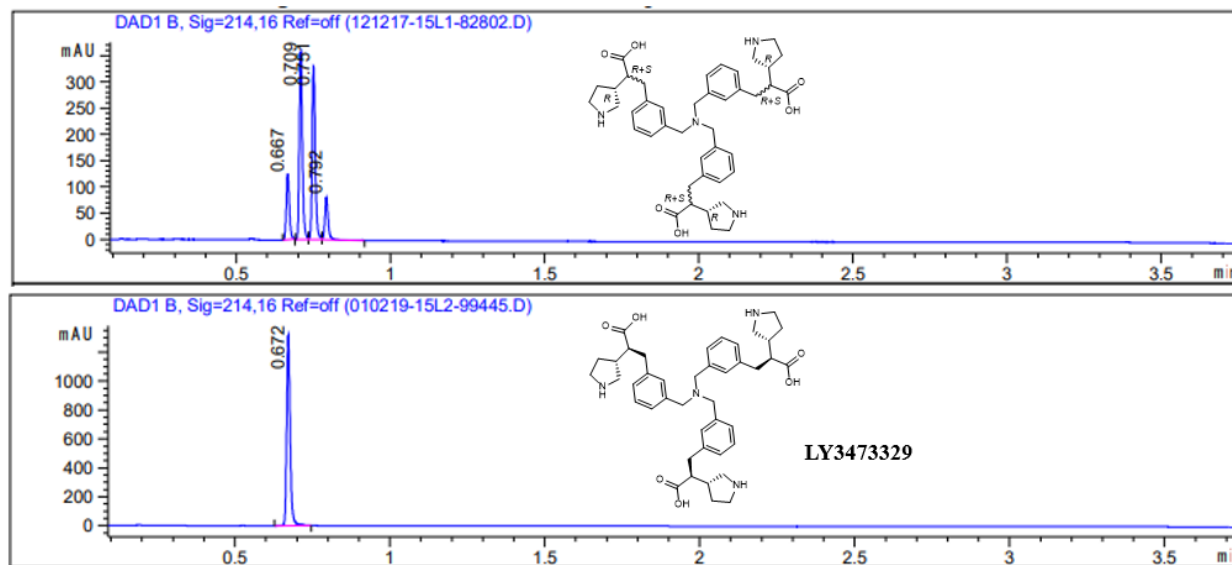
(2*S*,2'*S*,2''*S*)-3,3',3''-((Nitrilotris(methylene))tris(benzene-3,1-diyl))tris(2-((*R*)-pyrrolidin-3-yl)propanoic acid) (LY3473329)



To a round bottom flask, add tri-*tert*-butyl 3,3',3''-((2*S*,2'*S*,2''*S*)-((nitrilotris(methylene))tris(benzene-3,1-diyl))tris(3-(*tert*-butoxy)-3-oxopropane-1,2-diyl))(3*R*,3'*R*,3''*R*)-tris(pyrrolidine-1-carboxylate) (499.3 g, 423.3 mmol), 1,4-dioxane (1,997 mL), and a solution of hydrochloric acid (12 M in water, 529.1 mL, 15 equivalent). Stir the mixture at 40 °C for 1 h and then concentrate the mixture under reduced pressure to remove 1,4-dioxane, resulting in an aqueous slurry. Filter the mixture through a propylene filter to eliminate insoluble particles. Adjust the pH of the filtrate to 9–10 using a solution of NaOH (2M in water). Stir the mixture at room temperature overnight. Collect the resulting solid using a paper filter. Wash the solid with water and dry under reduced pressure at 45 °C to obtain the title compound (281 g, 88%) as a white crystalline solid.

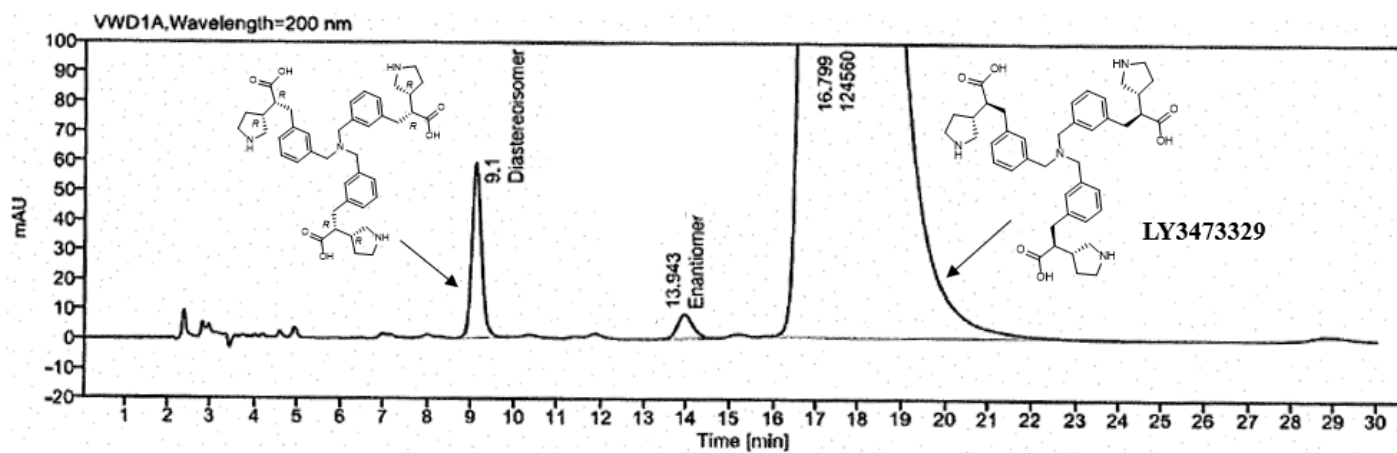
LCMS (ESI):  $m/z$  711.4  $[M+H]^+$ .  $^1\text{H-NMR}$  (400.13 MHz,  $\text{D}_2\text{O}$  + 1% DCl)  $\delta$  7.36-7.25 (m, 6H), 7.15-7.09 (m, 6H), 4.28-4.15 (m, 6H), 3.51 (dd,  $J$  = 7.9, 11.6 Hz, 3H), 3.37-3.28 (m, 3H), 3.21-3.10 (m, 3H), 2.99 (dd,  $J$  = 10.1, 11.6 Hz, 3H), 2.89 (dd,  $J$  = 4.5, 13.1 Hz, 3H), 2.84-2.68 (m, 6H), 2.59-2.45 (m, 3H), 2.17-2.05 (m, 3H), 1.77-1.63 (m, 3H).  $^{13}\text{C NMR}$  (100.62 MHz,  $\text{D}_2\text{O}$  + 1% DCl)  $\delta$  177.6, 139.4, 131.7, 130.5, 129.6, 129.5, 129.4, 57.3, 50.4, 48.1, 45.2, 39.6, 36.5, 28.1. HRMS (DART-TOF)  $m/z$  calculated for  $\text{C}_{42}\text{H}_{54}\text{N}_4\text{O}_6$   $[M+H]^+$ : 711.4116, found 711.4109.  $[\alpha]_{\text{D}}^{23}$  = -34.7 ( $c$  = 0.1,  $\text{H}_2\text{O}$ ). The diastereomeric excess (de) was measured by HPLC, comparing with the diastereomeric mixture 3,3',3''-((nitrilotris(methylene))tris(benzene-3,1-diyl))tris(2-((*R*)-pyrrolidin-3-yl)propanoic acid) under the assumption that only the stereogenic center in  $\alpha$  to the carbonyl will be susceptible to epimerization. HPLC analysis (Column XBridge C18 3.5 $\mu\text{m}$ , 2.1 x 50mm; gradient mode: from 10 to 100% B in 3 min; hold 0.75min at 100%B (A: 10mM Ammonium Bicarbonate pH:9.0, B:  $\text{CH}_3\text{CN}$ ); 1.0mL/min; 214 nm) indicated >98% de:  $t_{\text{R}}$  = 0.67

min. The  $t_R$  for the other isomers are:  $t_R = 0.71$  min,  $t_R = 0.75$  min, and  $t_R = 0.79$  min. HPLC chromatograms included below.

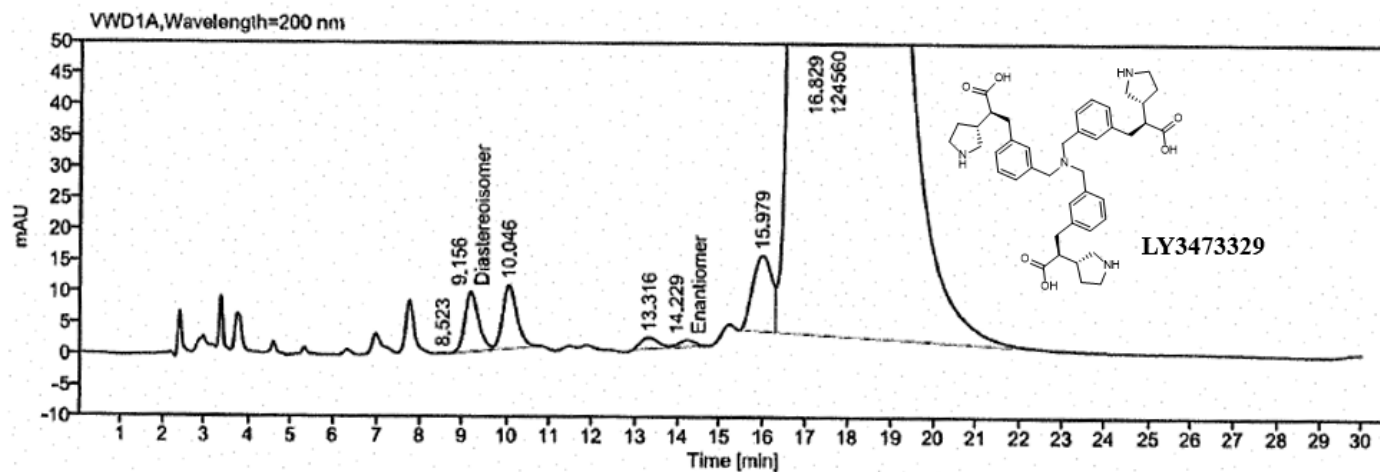


HPLC analysis (Agilent InfinityLab Poroshell 120 Chiral-V, 150x4.6 mm, 2.7 mm P/N:683975-604; gradient mode: 70A:30B Isocratic (A: 20 mM ammonium formate in water (pH=4.0±0.2), B: 60 MeOH:40 CH<sub>3</sub>CN); 0.7 mL/min, 200 nm) indicated >99% ee and de:  $t_R$  (major enantiomer)= 16.79 min,  $t_R$  (minor enantiomer)=13.94 min,  $t_R$  (diastereoisomer)= 9.16. % Chiral impurities (%w/w) (sum of enantiomer/diastereoisomer)= 0.1%

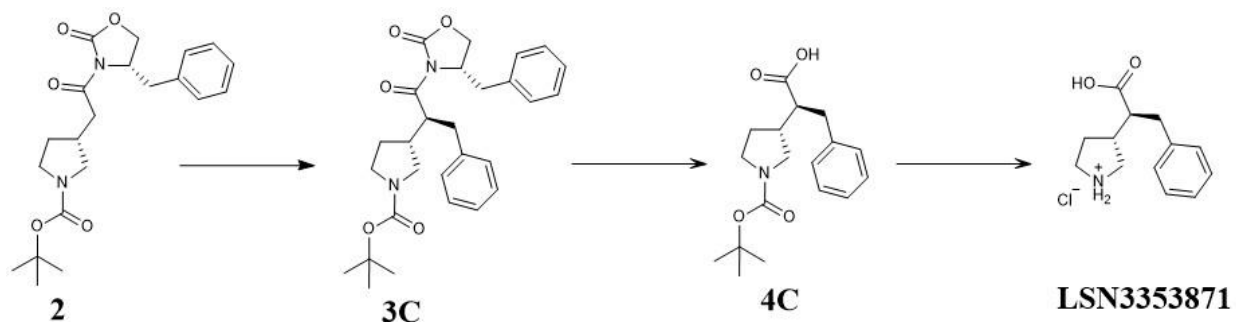
## Typical Chromatogram of Marker Solution



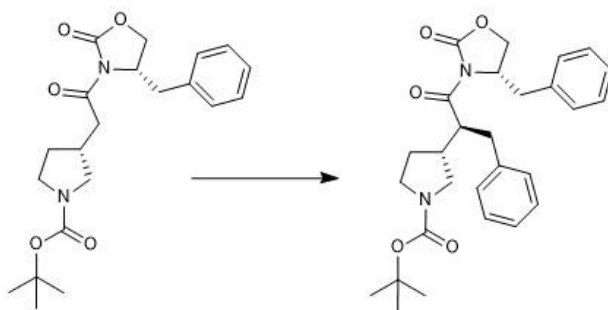
## Typical Chromatogram of Sample Solution



## Synthesis of compound LSN3353871



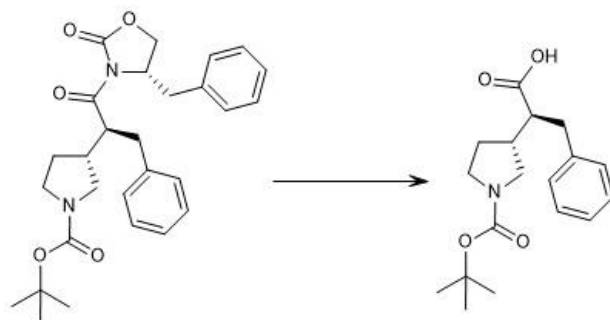
*tert*-Butyl (R)-3-((S)-1-((S)-4-benzyl-2-oxooxazolidin-3-yl)-1-oxo-3-phenylpropan-2-yl)pyrrolidine-1-carboxylate (**3C**)



**3C** is prepared following essentially the same procedure as **3A**, using (bromomethyl)benzene.

LCMS (ESI):  $m/z$  423.2  $[M+H-tert\text{-butyl}]^+$ .

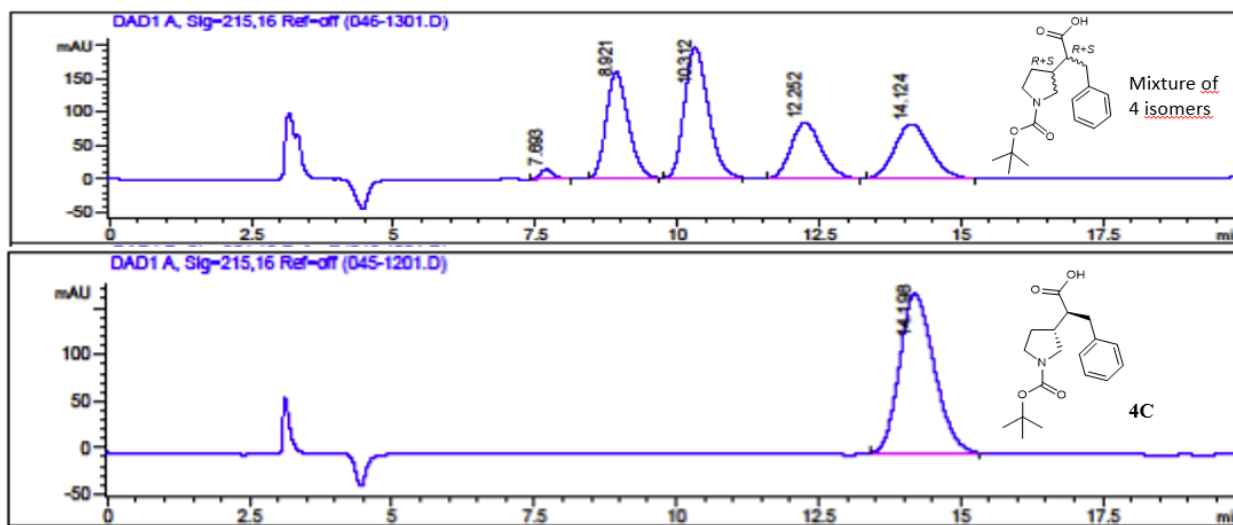
(S)-2-((R)-1-(*tert*-Butoxycarbonyl)pyrrolidin-3-yl)-3-phenylpropanoic acid (**4C**)



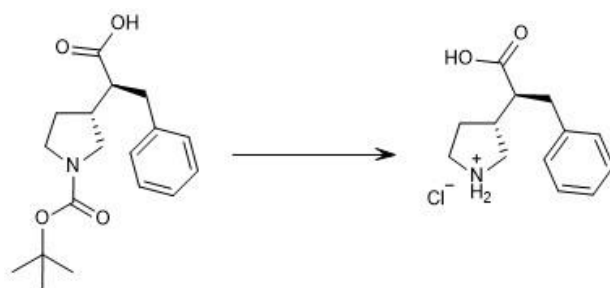
**4C** is prepared following essentially the same procedure as **4A**.

LCMS (ESI):  $m/z$  264.2  $[M + H\text{-}tert\text{-butyl}]^+$ .  $^1\text{H-NMR}$  (400.13 MHz,  $\text{CDCl}_3$ )  $\delta$  7.33-7.07 (m, 5H), 3.77-3.40 (m, 2H), 3.30-3.16 (m, 1H), 3.12-2.77 (m, 3H), 2.71-2.54 (m, 1H), 2.47-2.30 (m, 1H), 2.06-1.90 (m, 1H), 1.77-1.57 (m, 1H), 1.45 (s, 9H).

HPLC (Chiralcel OJ 4.6 x 250mm, 10 $\mu\text{m}$ ; gradient mode: 7% B Isocratic mode (A: Hexane-TFA 0.05%, B: EtOH; 1 mL/min, 215 nm) indicates de and ee >98%. HPLC chromatograms included below.



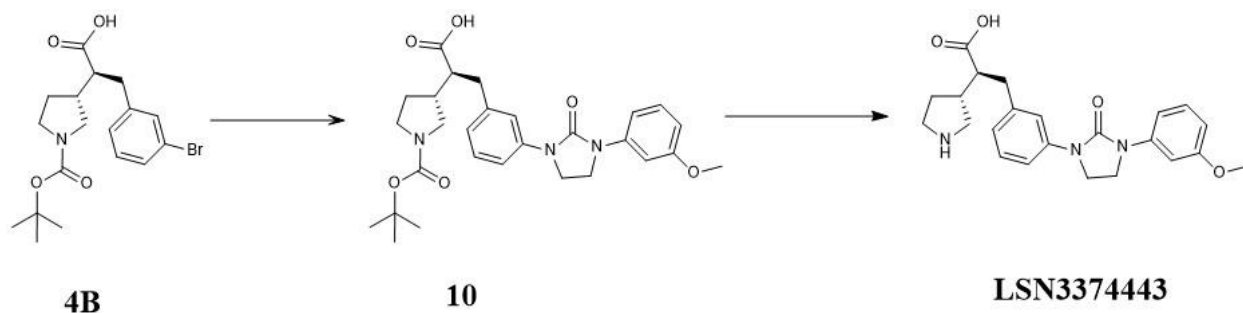
(R)-3-((S)-1-Carboxy-2-phenylethyl)pyrrolidin-1-ium chloride (LSN3353871)



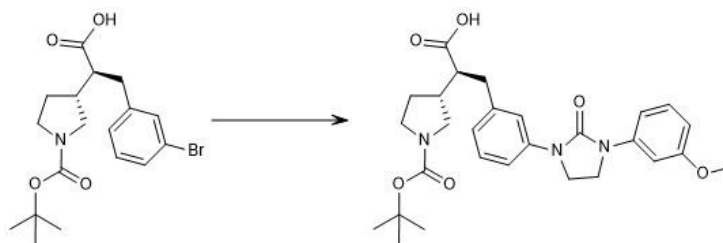
BOC deprotection is carried out following essentially the same procedure as described for the synthesis of **LSN3441732**.

LCMS (ESI):  $m/z$  220.2  $[M + H]^+$ .  $^1\text{H}$  NMR (400.13 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.40 – 7.34 (m, 2H), 7.33 – 7.24 (m, 3H), 3.59 (dd,  $J = 11.8, 7.9$  Hz, 1H), 3.48-3.38 (m, 1H), 3.33-3.20 (m, 1H), 3.07 (dd,  $J = 11.8, 10.0$  Hz, 1H), 2.99 – 2.84 (m, 3H), 2.66-2.51 (m, 1H), 2.25-2.13 (m, 1H), 1.84-1.70 (m, 1H).  $^{13}\text{C}$  NMR (100.62 MHz,  $\text{D}_2\text{O}$ )  $\delta$  178.0, 138.3, 128.9, 128.8, 127.0, 50.5, 48.2, 45.2, 39.5, 36.8, 28.2. HRMS (DART-TOF)  $m/z$  calculated for  $\text{C}_{13}\text{H}_{17}\text{NO}_2$   $[M+H]$ : 220.1332, found 220.1317.  $[\alpha]_{\text{D}}^{23} = -35.1$  ( $c = 0.1$ ,  $\text{H}_2\text{O}$ ). The diastereomeric excess was determined to be >95% by NMR spectroscopy as the diastereomeric proton resonances of the minor isomer that appeared separated from the proton signals of the major isomer were not observed in the  $^1\text{H}$ -NMR spectra of the reaction product.

## Synthesis of compound LSN3374443



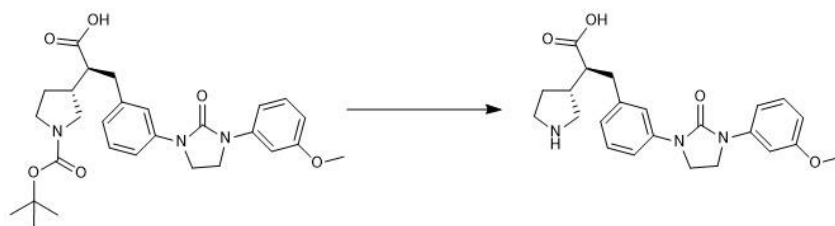
(S)-2-((R)-1-(tert-Butoxycarbonyl)pyrrolidin-3-yl)-3-(3-(3-(3-methoxyphenyl)-2-oxoimidazolidin-1-yl)phenyl)propanoic acid (**10**)



Mix (S)-3-(3-bromophenyl)-2-((R)-1-(tert-butoxycarbonyl)pyrrolidin-3-yl)propanoic acid (190 mg, 0.4770 mmol), 1-(3-methoxyphenyl)imidazolidin-2-one (0.2751 g, 1.431 mmol, 3 equivalent), [(2-di-*tert*-butylphosphino-2',4',6'-triisopropyl-1,1'-biphenyl)-2-(2'-amino-1,1'-biphenyl)]palladium(II) methanesulfonate (*t*BuXPhos-Pd-G3, 0.0379 g, 0.0477 mmol, 0.1 equivalent) and sodium *tert*-butoxide (0.1418 g, 1.431 mmol, 3 equivalent) in 1,4-dioxane (4.8 mL) and stir the mixture under a nitrogen atmosphere at 100 °C overnight. Dilute the mixture with EtOAc and acidify with an aqueous solution of HCl (1N). Filter the mixture through a pad of diatomaceous earth, separate the layers, and dry the organics over MgSO<sub>4</sub>. Filter and concentrate the organics, then purify the residue by reverse-phase flash chromatography (silica-bound C18 column) using a gradient of 40–70% acetonitrile in aqueous NH<sub>4</sub>CO<sub>3</sub> (pH 9) to give the title compound (70 mg, 28%) as a white solid. LCMS (ESI): *m/z* 510.2 [M+H]<sup>+</sup>. <sup>1</sup>H-NMR (400.13 MHz, DMSO-*d*<sub>6</sub>) δ 7.50-7.44 (m, 2H), 7.32 (t, *J* = 2.2 Hz, 1H), 7.30-7.23 (m, 2H), 7.17-7.13 (m, 1H), 6.91 (d, *J* = 7.6 Hz, 1H), 6.68-6.64 (m, 1H), 3.97-3.93 (m, 4H), 3.77 (s, 3H), 3.56-3.44 (m,

1H), 3.21-3.07 (m, 1H), 3.01-2.91 (m, 1H), 2.86-2.71 (m, 2H), 2.60-5.53 (m, 2H), 2.34-2.23 (m, 1H), 1.92-1.82 (m, 1H), 1.69-1.53 (m, 1H), 1.40 (s, 9H).

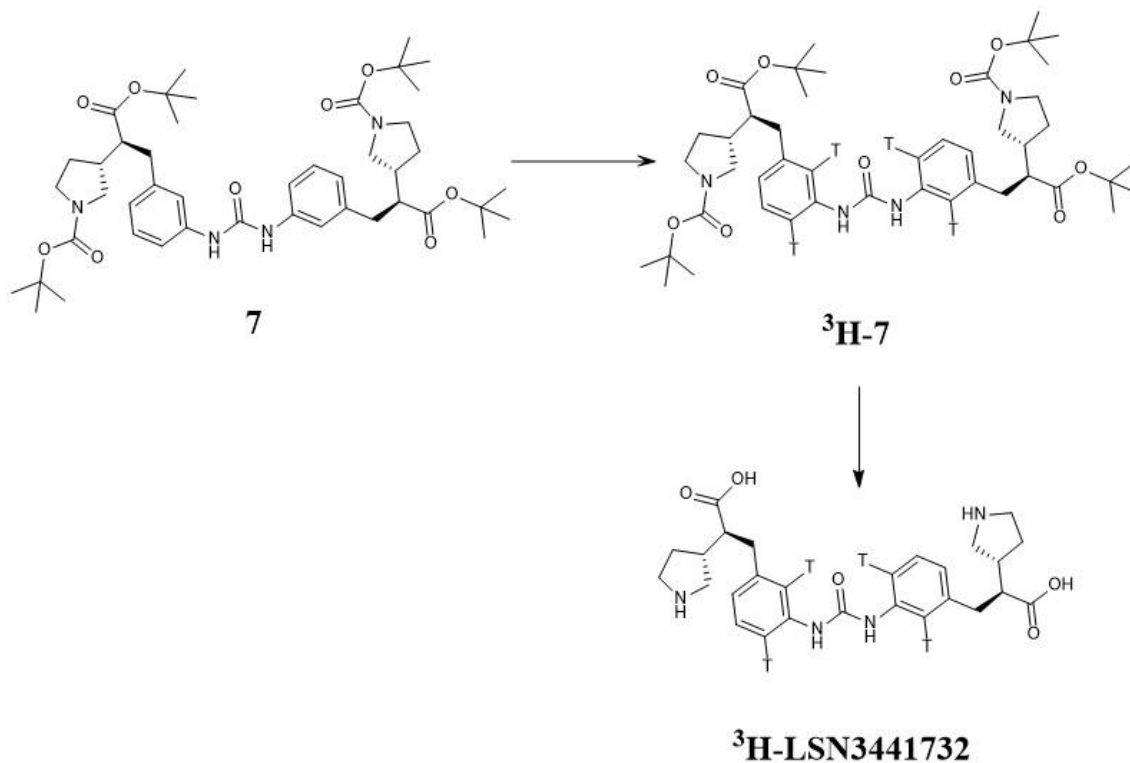
(S)-3-(3-(3-(3-Methoxyphenyl)-2-oxoimidazolidin-1-yl)phenyl)-2-((R)-pyrrolidin-3-yl)propanoic acid (LSN3374443)



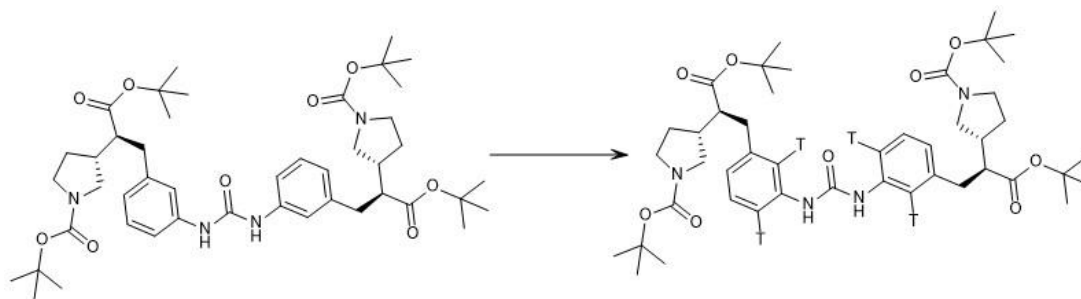
BOC deprotection is carried following essentially the same procedure as described for the synthesis of LSN3441732. Purify by reverse-phase flash chromatography (silica-bound C18 column) using a gradient of 5–50% acetonitrile in aqueous  $\text{NH}_4\text{CO}_3$  (pH 9) to give the title compound.

LCMS (ESI):  $m/z$  410.2  $[\text{M}+\text{H}]^+$ .  $^1\text{H}$  NMR (400.13 MHz,  $\text{D}_2\text{O}$  + 1% DCl)  $\delta$  7.14 – 7.05 (m, 4H), 6.95 (t,  $J$  = 2.3 Hz, 1H), 6.79 (ddd,  $J$  = 12.8, 7.9, 3.0 Hz, 2H), 6.49 (dd,  $J$  = 8.3, 2.4 Hz, 1H), 3.55 (s, 3H), 3.43-3.33 (m, 6H), 3.16 (ddd,  $J$  = 11.7, 9.8, 7.2 Hz, 1H), 2.91 (dd,  $J$  = 11.8, 10.0 Hz, 1H), 2.65-2.63 (m, 3H), 2.43 (m, 1H), 2.10-2.03 (m, 1H), 1.66 (m, 1H).  $^{13}\text{C}$  NMR (100.62 MHz,  $\text{D}_2\text{O}$  + 1% DCl)  $\delta$  177.4, 159.2, 155.6, 140.6, 139.4, 139.1, 129.8, 129.1, 123.7, 118.6, 116.7, 110.8, 108.5, 104.3, 55.1, 50.1, 48.0, 45.2, 41.8, 41.7, 39.6, 36.8, 28.0. HRMS (DART-TOF)  $m/z$  calculated for  $\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_4$   $[\text{M}+\text{H}]$ : 410.2074, found 410.2053.

## Synthesis of compound <sup>3</sup>H-LSN3441732

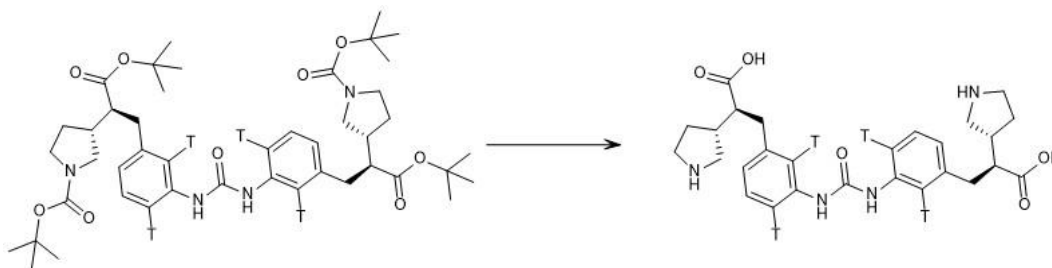


di-tert-Butyl 3,3'-((2S,2'S)-((carbonylbis(azanediyl))bis(3,1-phenylene-2,4-t<sub>2</sub>))bis(3-(tert-butoxy)-3-oxopropane-1,2-diyl))(3R,3'R)-bis(pyrrolidine-1-carboxylate) (<sup>3</sup>H-7)



Combine **7** (5 mg), Crabtree's catalyst (10 mg), and dichloromethane (1 mL) in a tritiation flask and stir under 5 Ci of tritium gas for 16 h. Remove labile tritium by repeated evaporations to dryness from ethanol. Dissolve the residues in ethanol.

(2*S*,2'*S*)-3,3'-((Carbonylbis(azanediyl))bis(3,1-phenylene-2,4-*t*<sub>2</sub>))bis(2-((*R*)-pyrrolidin-3-yl)propanoic acid) (<sup>3</sup>H-LSN3441732)



Dry <sup>3</sup>H-7 to dryness in a small round bottom flask. Add 2-methyl-2-butanol (2 mL). Stir the slurry and add 4M hydrochloric acid in dioxane (1 mL) was added dropwise under a nitrogen atmosphere. The resulting solution is stirred at 45 °C for 4 h then overnight at room temperature.

Crude <sup>3</sup>H-LSN3441732 was purified by HPLC as follows:

Column: Gemini C18 25 × 1 cm

Eluent A: water + 0.1% TFA

Eluent B: acetonitrile + 0.1% TFA

Gradient: 5–100% B over 2 h

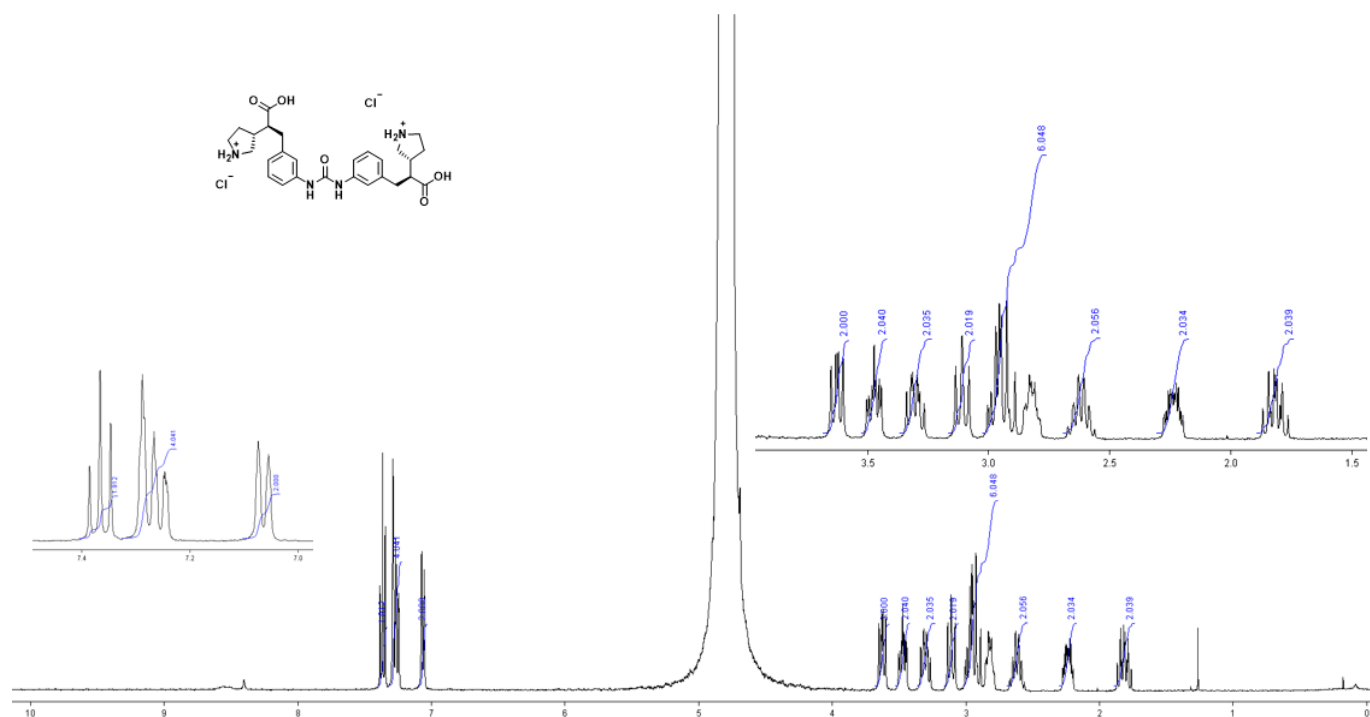
Flow: 3 mL/min

<sup>3</sup>H-LSN3441732 was collected, rotary evaporated to dryness, and dissolved in ethanol.

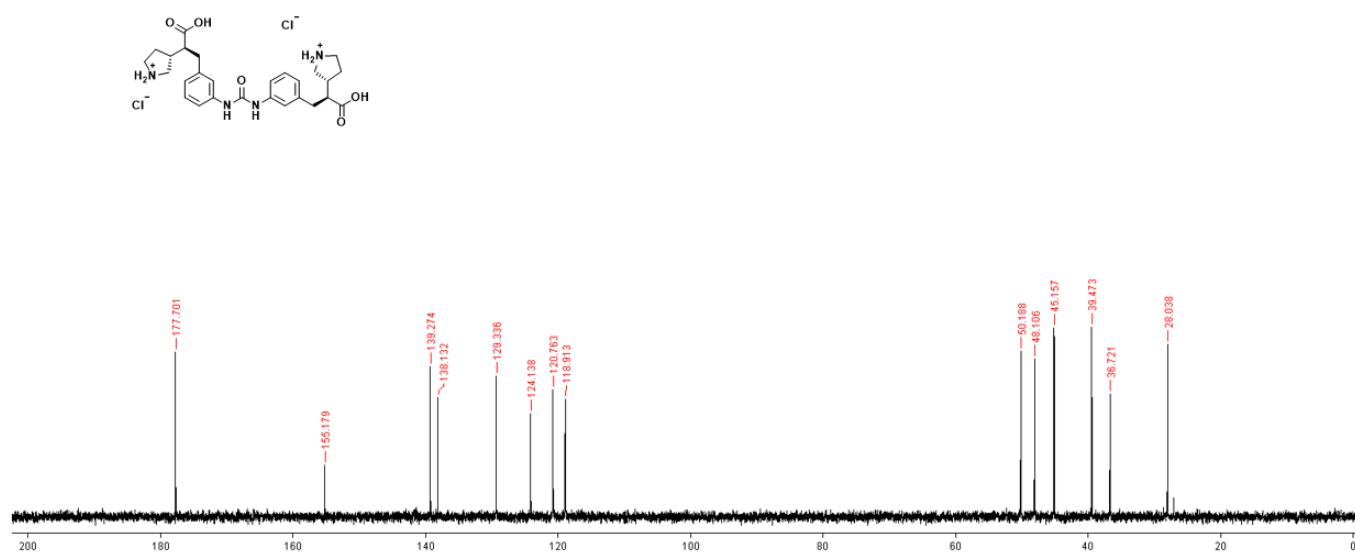
The radiochemical purity of <sup>3</sup>H-LSN3441732, determined by high-performance liquid chromatography was 98%. Mass spectrometry of <sup>3</sup>H-LSN3441732 gave a spectrum that was consistent with the inactive material LSN3441732 and a specific activity of 40 Ci/mmol. Tritium NMR spectroscopy of <sup>3</sup>H-LSN3441732 was consistent with the structure. Assignment of the tritium NMR is based on the results of a deuteration experiment in the protected intermediate 7.

## Spectra of the compounds described

LSN 3441732 ( $^1\text{H}$  NMR in  $\text{D}_2\text{O}$ )

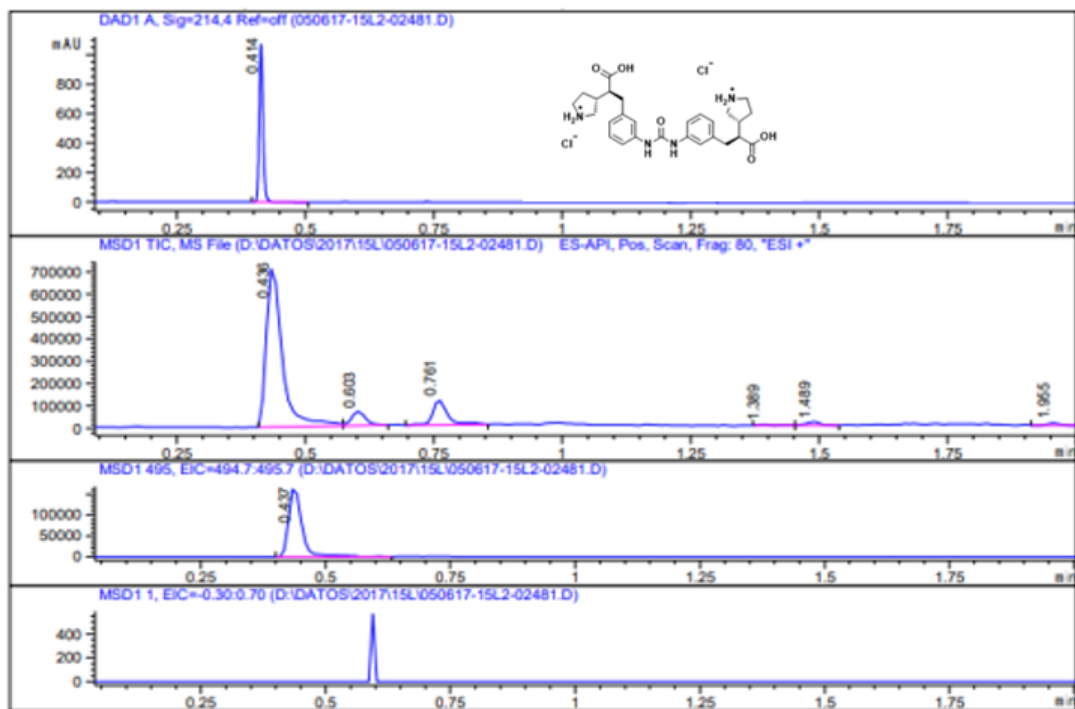


LSN 3441732 ( $^{13}\text{C}$  NMR in  $\text{D}_2\text{O}$ )



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 Method info: Column XBridge C18 3.5um, 2.1 x 50mm; UV: 214 and 300; MS-ESI  
 100-800 A: 10mM Ammonium Bicarbonate pH:9.0. B: CH3CN  
 Flow Rate: 1.2ml/min; T# 50°C; Gradient mode: From 5 to 95% B  
 in 1.5min. Hold 0.5min at 95%B.

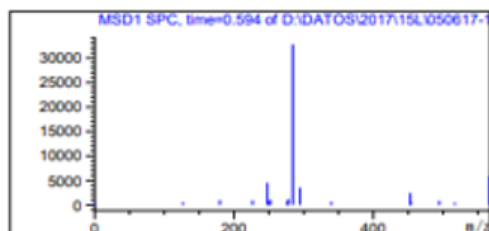
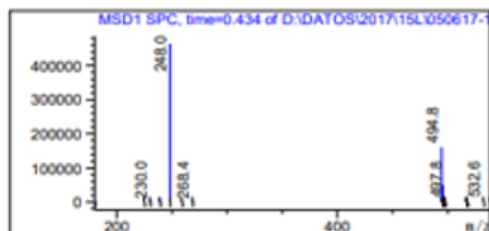
Alcobendas AT



Area % Table of Signal DAD1 A, Sig=214,4

| RET.T | Height | Area | Area%  |
|-------|--------|------|--------|
| 0.414 | 982    | 567  | 100.00 |

Target 1 : 494.00  
 UV RT T1 : 0.414  
 Purity T1 : 100.00  
 PosThr T1 : 167453



# High Resolution Mass Spectrometry Report: LSN3441732

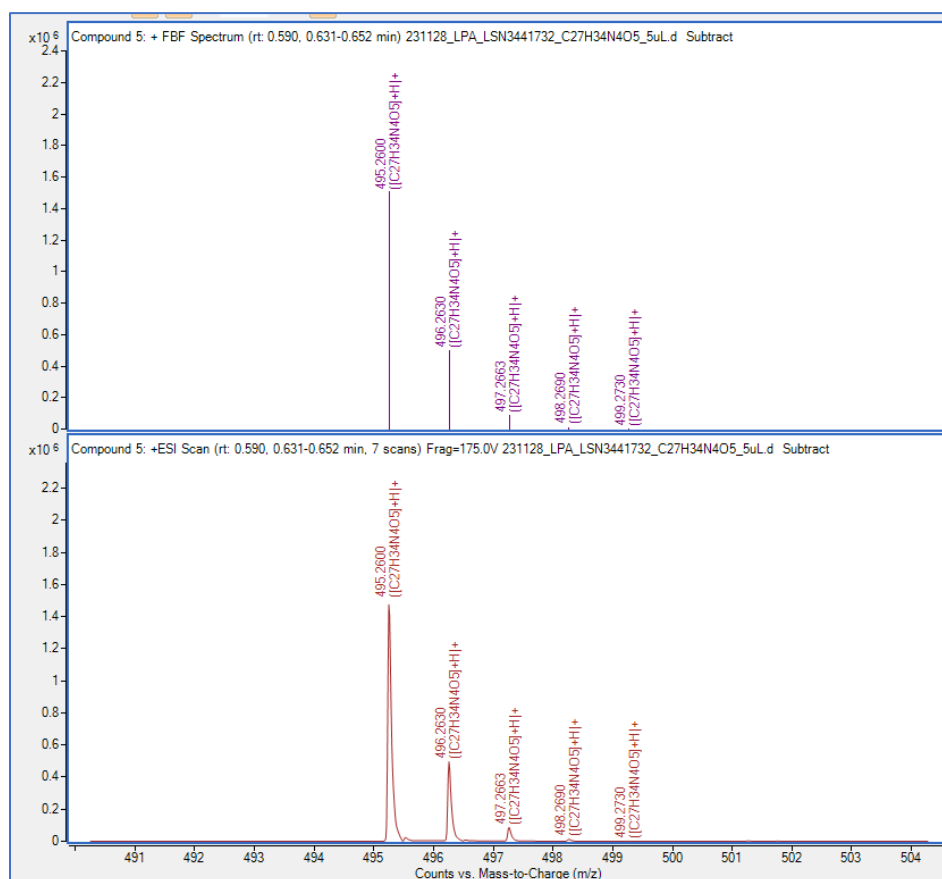
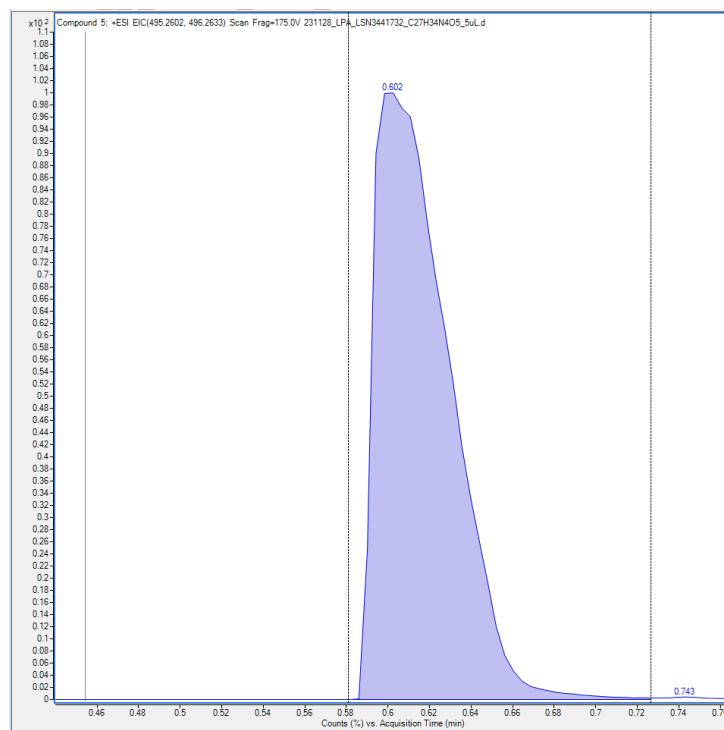
Acquisition method: Column: X-Bridge C18 3.5 $\mu$ M 2.1x50mm. Mobile phase: A= 0.1% FA in water; B= 0.1% FA in CH<sub>3</sub>CN. Run length: 2 min. Column temperature: 50°C. Acquisition mode: ES+. Injection volume: 1 $\mu$ L

| Time [min] | $\Delta$ | A [%] | B [%] | Flow [mL/min] | Max. Pressure Limit [bar] |
|------------|----------|-------|-------|---------------|---------------------------|
| 0.00       |          | 95.00 | 5.00  | 1.200         | 1000.00                   |
| 0.25       |          | 95.00 | 5.00  | ---           | ---                       |
| 1.25       |          | 5.00  | 95.00 | ---           | ---                       |
| 1.75       |          | 5.00  | 95.00 | ---           | ---                       |
| 2.00       |          | 95.00 | 5.00  | ---           | ---                       |

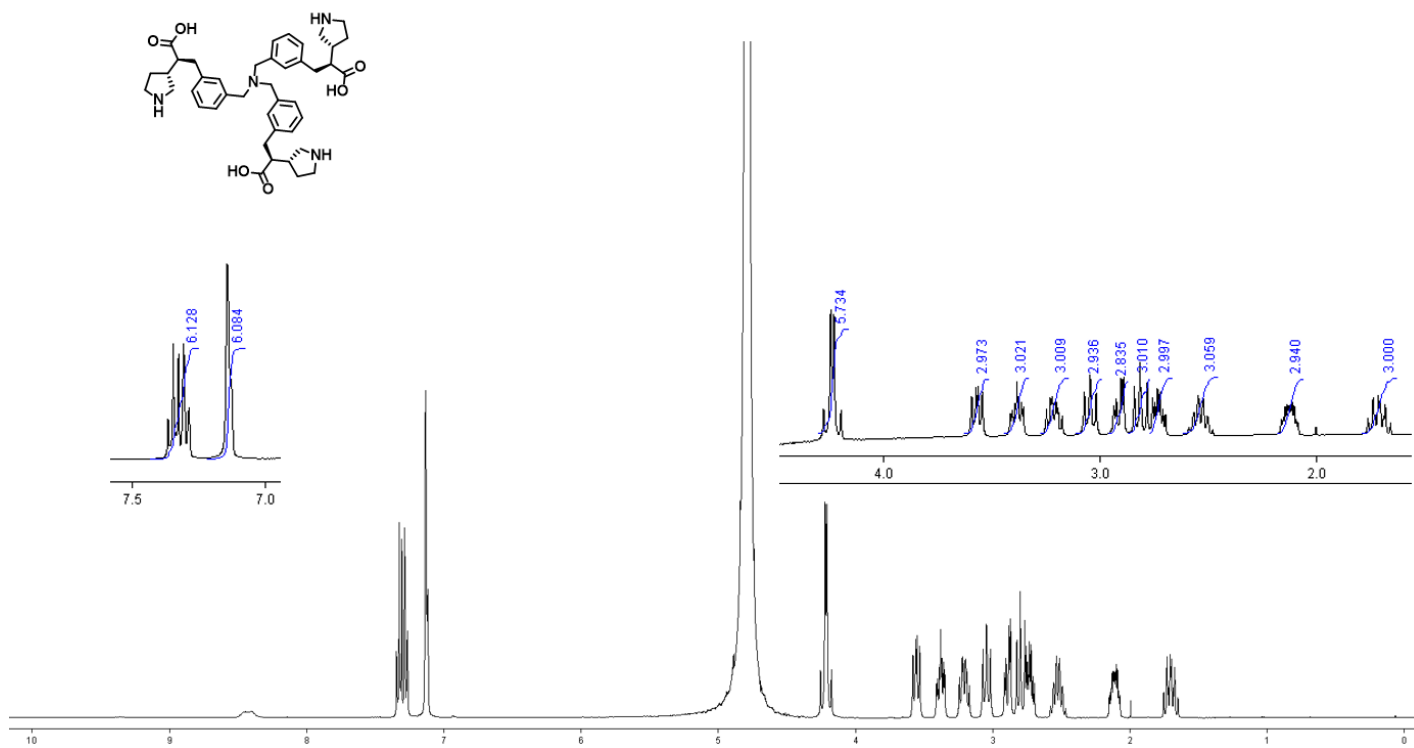
Ref. Standards from Agilent's Internal Reference Mass Kit (p/ G1969-85001)

- Purine (m/z 121.05087)
- HP-921 (m/z 922.00980)

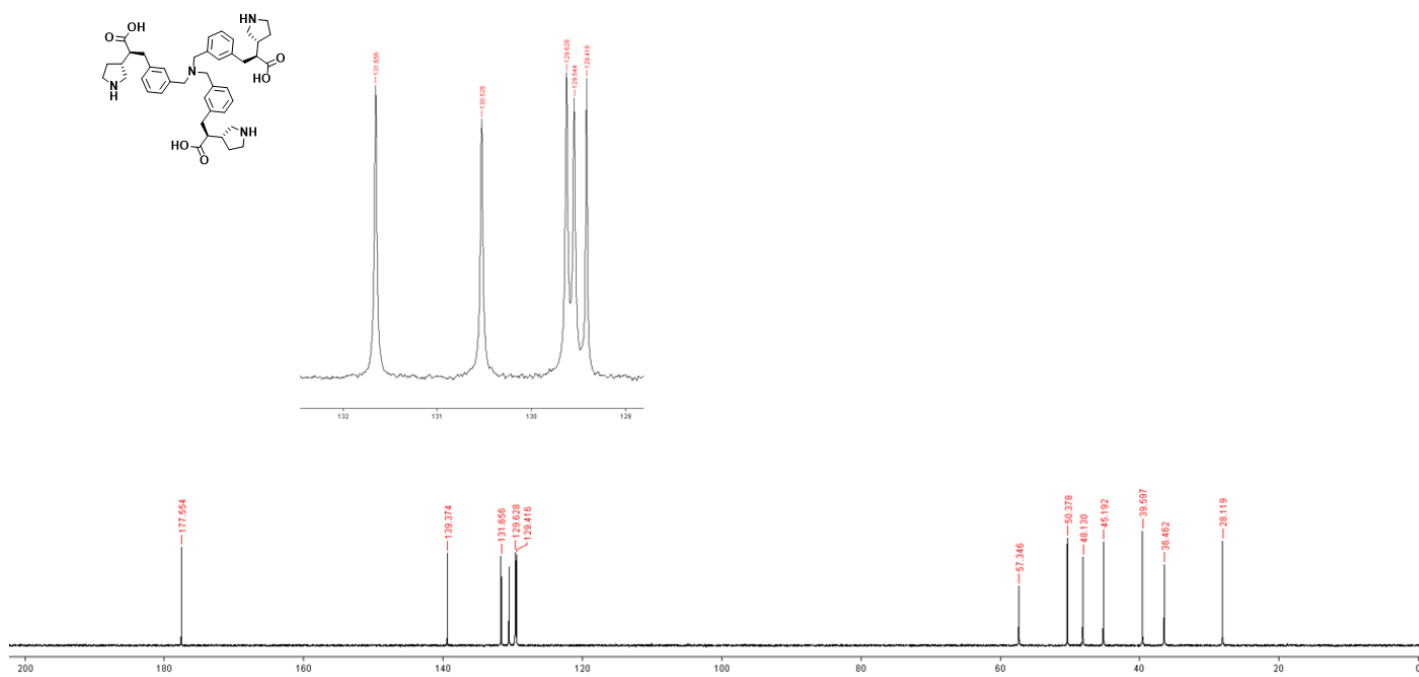
| Sample                                    |                 |
|-------------------------------------------|-----------------|
| LSN                                       | <b>3441732</b>  |
| Molecular Formula                         | C27H34N4O5      |
| Exact Mass                                | 494.2529        |
| Exact Mass (M+H)                          | 495.2602        |
| Experimental Results                      |                 |
| Measured Exact Mass of (M+H) <sup>+</sup> | <b>495.2600</b> |
| ppm error with expected Exact Mass:       | <b>-0.36</b>    |



LY 3473329 (<sup>1</sup>H NMR in D<sub>2</sub>O + 1% DCl)

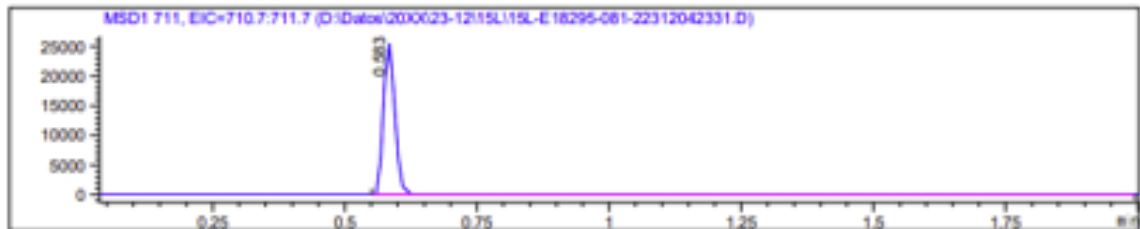
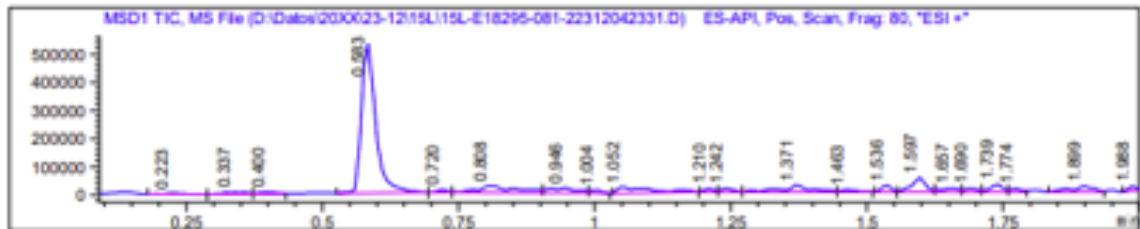
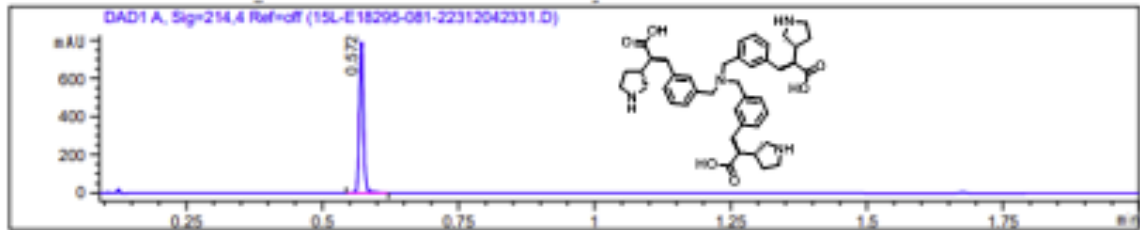


LY 3473329 (<sup>13</sup>C NMR in D<sub>2</sub>O + 1% DCl)



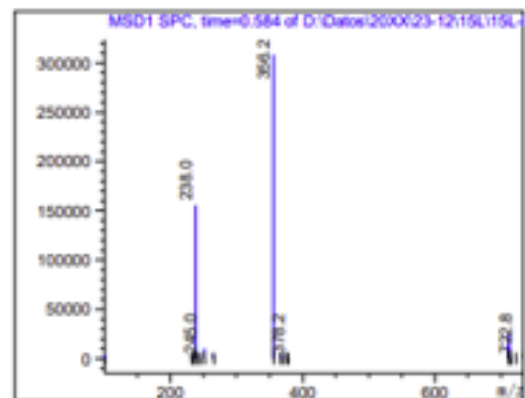


Operator: Admin Comment: CHECK  
 FILE NAME: D:\Datos\20XX\23-12\15L-E18295-081-22312042331.D  
 INJ. METHOD: HIGHPH.M Vial: P1-C5  
 Method info: Column XBridge C18 3.5um, 2.1 x 50mm; UV: 214 and 300; MS-ESI  
 100-800 A: 10mM Ammonium Bicarbonate pH:9.0. B: CH3CN  
 Flow Rate: 1.2ml/min; T° 50°C; Gradient mode: From 5 to 95% B  
 in 1.5min. Hold 0.5min at 95%B.



Area % Table of Signal DAD1 A, Sig=214,4

| RET.T | Height | Area | Area%  |
|-------|--------|------|--------|
| 0.572 | 754    | 489  | 100.00 |



Target 1 : 710  
 UV RT T1 : 0.572  
 Purity T1 : 100.00

### High Resolution Mass Spectrometry Report: LY3473329

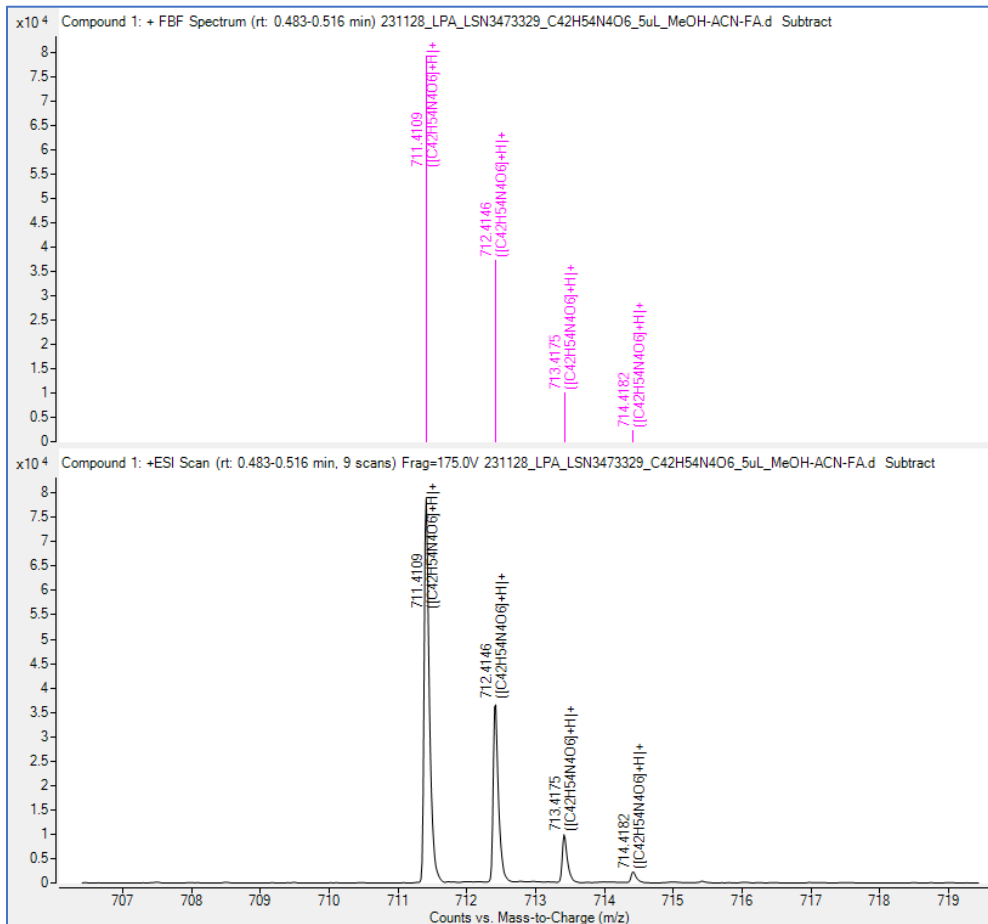
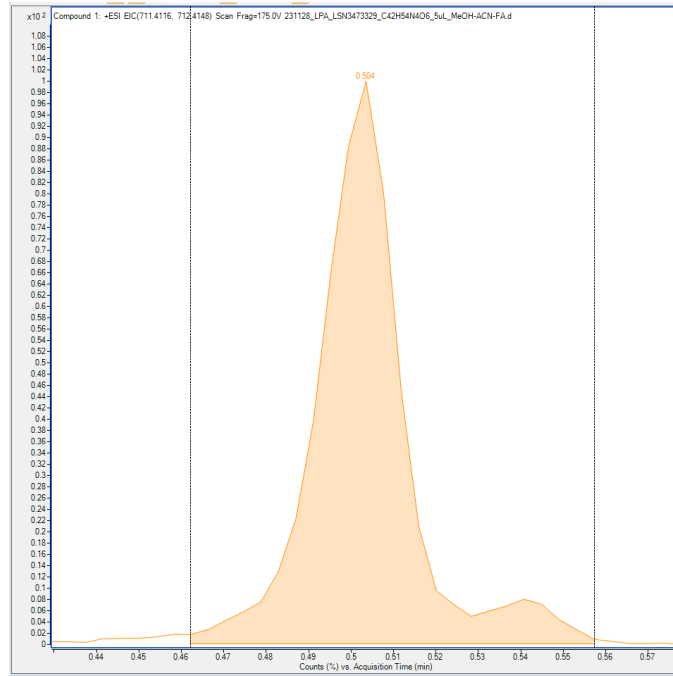
Acquisition method: Column: X-Bridge C18 3.5 $\mu$ M 2.1x50mm. Mobile phase: A= 0.1% FA in water; B= 0.1% FA in CH<sub>3</sub>CN. Run length: 2 min. Column temperature: 50°C. Acquisition mode: ES+. Injection volume: 1 $\mu$ L

| Time [min] | △ | A [%] | B [%] | Flow [mL/min] | Max. Pressure Limit [bar] |
|------------|---|-------|-------|---------------|---------------------------|
| 0.00       |   | 95.00 | 5.00  | 1.200         | 1000.00                   |
| 0.25       |   | 95.00 | 5.00  | ---           | ---                       |
| 1.25       |   | 5.00  | 95.00 | ---           | ---                       |
| 1.75       |   | 5.00  | 95.00 | ---           | ---                       |
| 2.00       |   | 95.00 | 5.00  | ---           | ---                       |

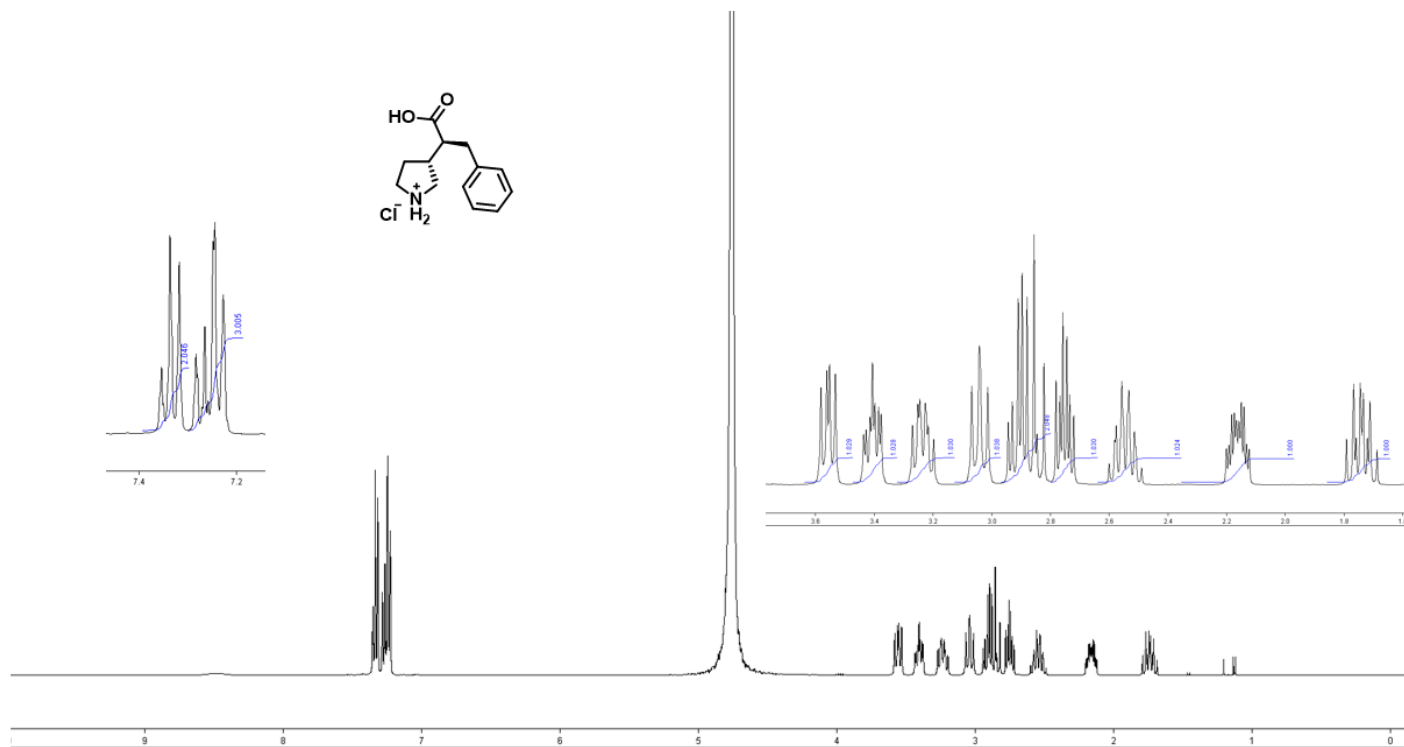
Ref. Standards from Agilent's Internal Reference Mass Kit (p/ G1969-85001)

- Purine (m/z 121.05087)
- HP-921 (m/z 922.00980)

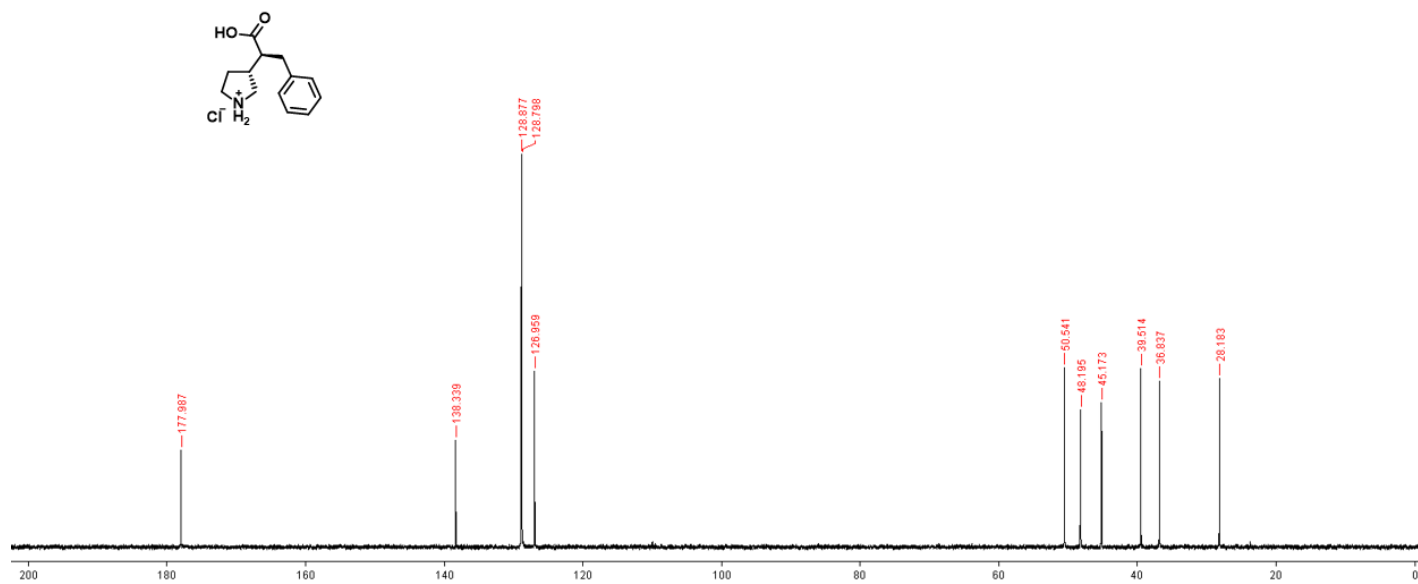
| Sample                                          |                 |
|-------------------------------------------------|-----------------|
| LSN                                             | <b>3473329</b>  |
| Molecular Formula                               | C42H54N4O6      |
| Exact Mass                                      | 710.4043        |
| Exact Mass (M+H)                                | 711.4116        |
|                                                 |                 |
| <b>Experimental Results</b>                     |                 |
| <b>Measured Exact Mass of (M+H)<sup>+</sup></b> | <b>711.4109</b> |
| ppm error with expected Exact Mass:             | <b>-0.79</b>    |



LSN 3353871 ( $^1\text{H}$  NMR in  $\text{D}_2\text{O}$ )

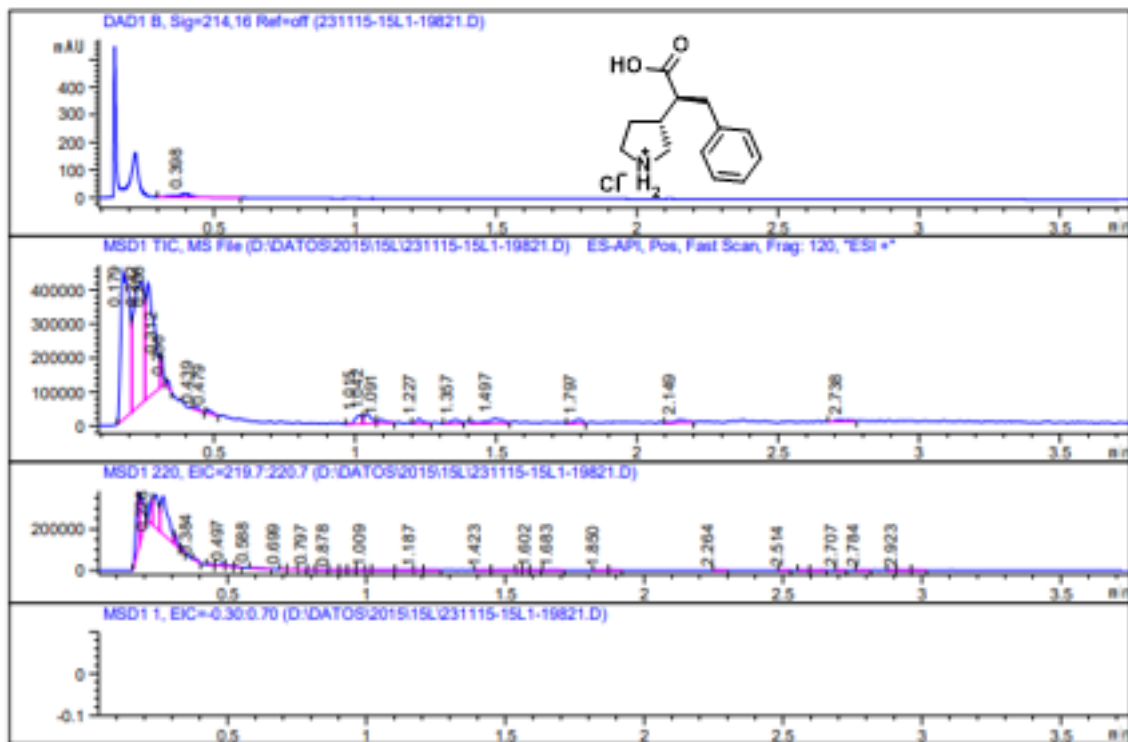


LSN 3353871 ( $^{13}\text{C}$  NMR in  $\text{D}_2\text{O}$ )



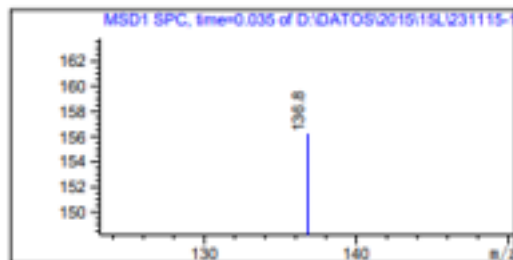
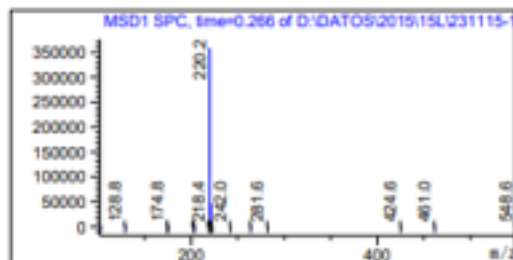


Operator: 15L Comment: PRECIPITATE  
 FILE NAME: D:\DATOS\2015\15L\231115-15L1-19821.D  
 INJ. METHOD: C:\Chem32\1\METHODS\NEW\_OA\MC\_BASIC.M Vial: P1-E-08  
 Method info: Column XBridge C18 3.5um, 2.1 x 50mm; UV: 214 & 300; MS ESI(+/-)  
 ) 150-700 A: 10mM Ammonium Bicarbonate pH:9.0. B: CH3CN  
 Flow Rate: 1.0ml/min; T\* 50°C; Gradient mode: From 10 to 100%  
 B in 3 min. Hold 0.75min at 100%B.



Area % Table of Signal DAD1 B, Sig=214,16

| RET.T | Height | Area | Area%  |
|-------|--------|------|--------|
| 0.398 | 13     | 50   | 100.00 |



Target 1 : 219.00  
 UV RT T1 : 0.000  
 Purity T1 : 0.00  
 PosThr T1 : 181150

## High Resolution Mass Spectrometry Report: LSN3353871

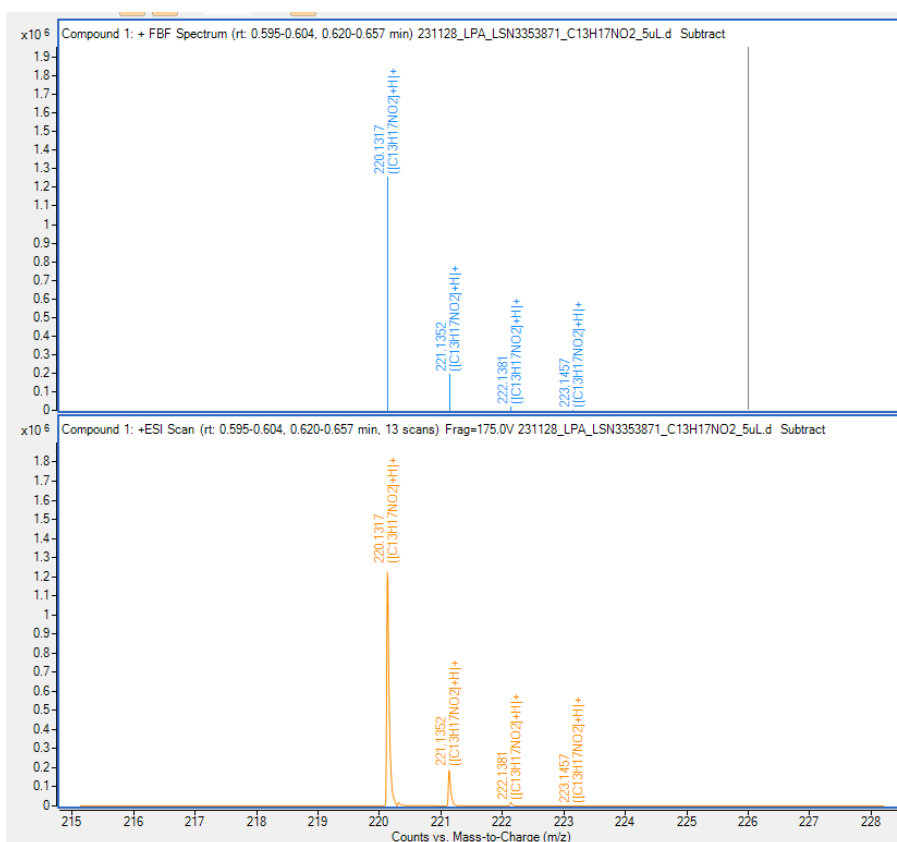
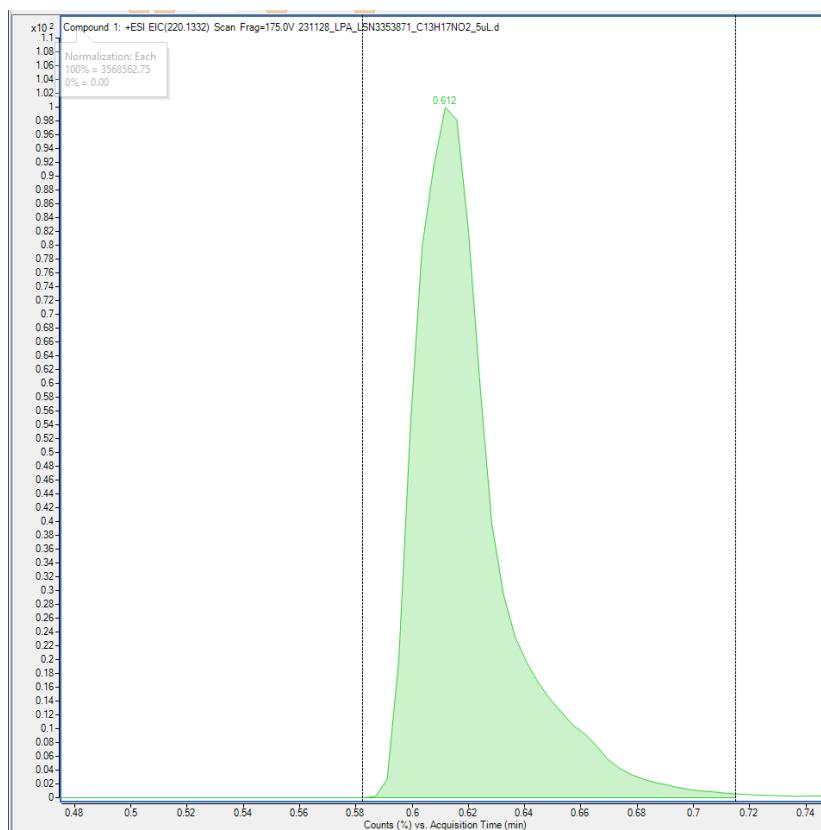
Acquisition method: Column: X-Bridge C18 3.5 $\mu$ M 2.1x50mm. Mobile phase: A= 0.1% FA in water; B= 0.1% FA in CH<sub>3</sub>CN. Run length: 2 min. Column temperature: 50°C. Acquisition mode: ES+. Injection volume: 1 $\mu$ L

| Time [min] | $\Delta$ | A [%] | B [%] | Flow [mL/min] | Max. Pressure Limit [bar] |
|------------|----------|-------|-------|---------------|---------------------------|
| 0.00       |          | 95.00 | 5.00  | 1.200         | 1000.00                   |
| 0.25       |          | 95.00 | 5.00  | ---           | ---                       |
| 1.25       |          | 5.00  | 95.00 | ---           | ---                       |
| 1.75       |          | 5.00  | 95.00 | ---           | ---                       |
| 2.00       |          | 95.00 | 5.00  | ---           | ---                       |

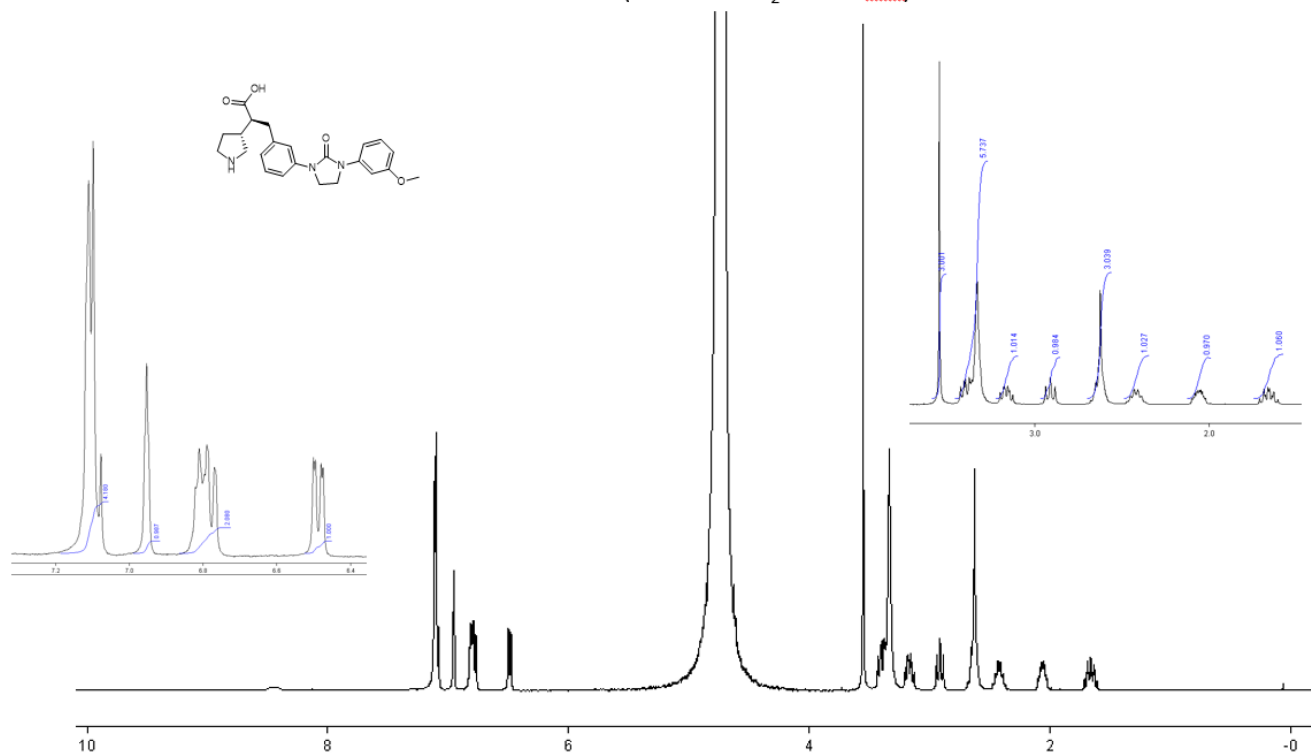
Ref. Standards from Agilent's Internal Reference Mass Kit (p/ G1969-85001)

- Purine (m/z 121.05087)
- HP-921 (m/z 922.00980)

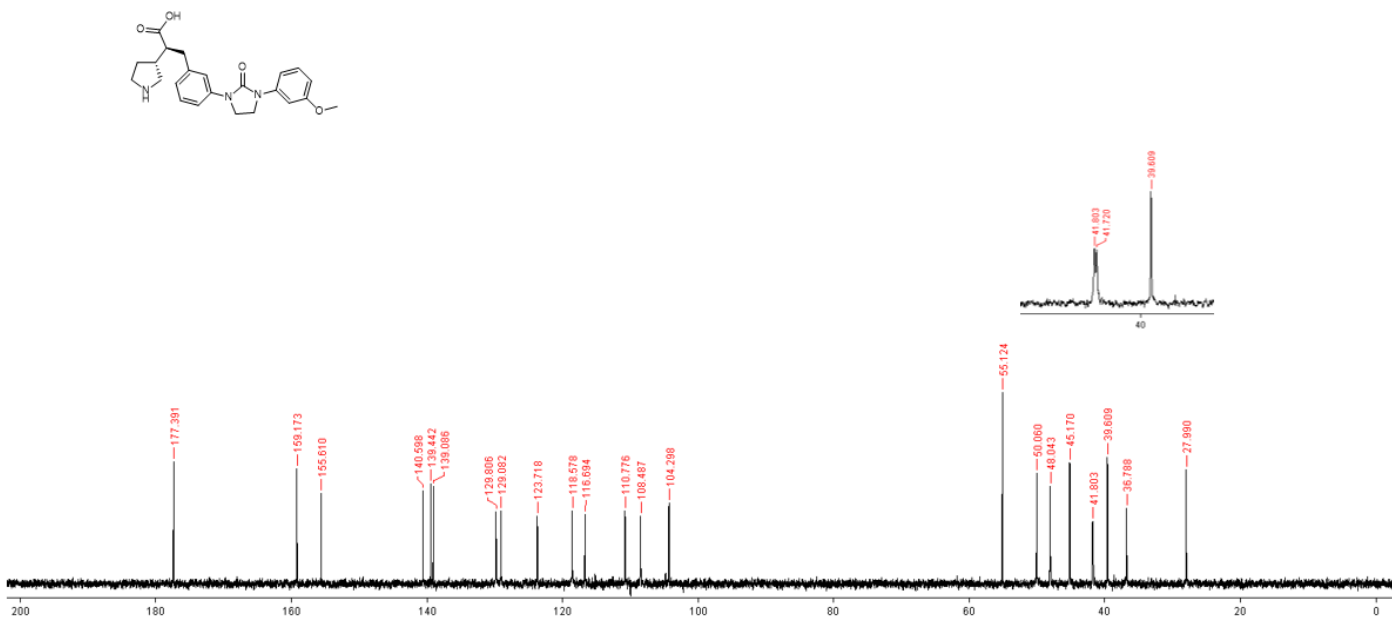
| Sample                                          |                                                 |
|-------------------------------------------------|-------------------------------------------------|
| LSN                                             | <b>3353871</b>                                  |
| Molecular Formula                               | C <sub>13</sub> H <sub>17</sub> NO <sub>2</sub> |
| Exact Mass                                      | 219.1259                                        |
| Exact Mass (M+H)                                | 220.1332                                        |
|                                                 |                                                 |
| <b>Experimental Results</b>                     |                                                 |
| <b>Measured Exact Mass of (M+H)<sup>+</sup></b> | <b>220.1317</b>                                 |
| ppm error with expected Exact Mass:             | <b>-5.72</b>                                    |



LSN 3374443 ( $^1\text{H}$  NMR in  $\text{D}_2\text{O}$  + 1% DCl)

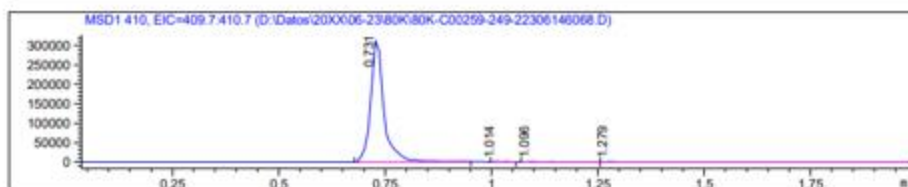
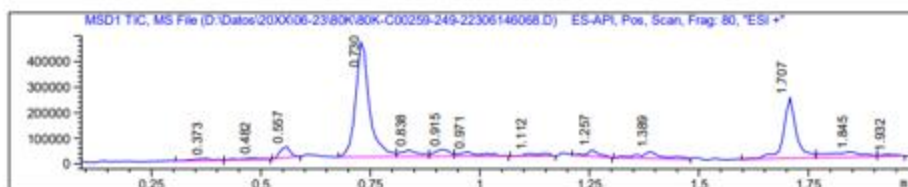
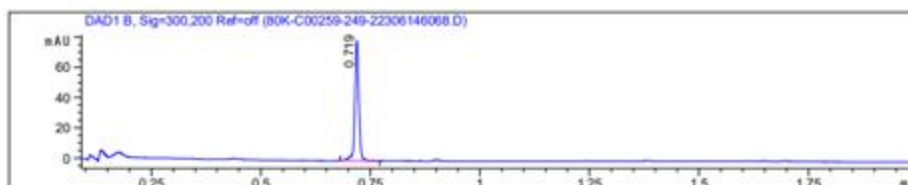


LSN 3374443 ( $^{13}\text{C}$  NMR in  $\text{D}_2\text{O}$  + 1% DCl)



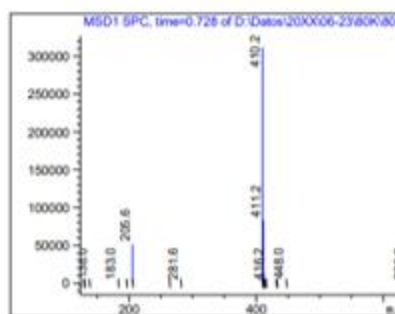


Operator: Admin Comment: PURE ISCO REV ->  
 FILE NAME: D:\Datos\20XX\06-23\80K\80K-C00259-249-22306146068.D  
 INJ. METHOD: HIGHPH.M Vial: P1-D6  
 Method info: Column XBridge C18 3.5um, 2.1 x 50mm; UV: 214 and 300; MS-ESI  
 100-800 A: 10mM Ammonium Bicarbonate pH:9.0. B: CH3CN  
 Flow Rate: 1.2ml/min; T\* 50°C; Gradient mode: From 5 to 95% B  
 in 1.5min. Hold 0.5min at 95%B.



Area % Table of Signal DAD1 A, Sig=214.4

| RET.T | Height | Area | Area%  |
|-------|--------|------|--------|
| 0.719 | 291    | 196  | 100.00 |



Target 1 : 409  
 UV RT T1 : 0.719  
 Purity T1 : 100.00  
 PosThr T1 : 318046

### High Resolution Mass Spectrometry Report: LSN3374443

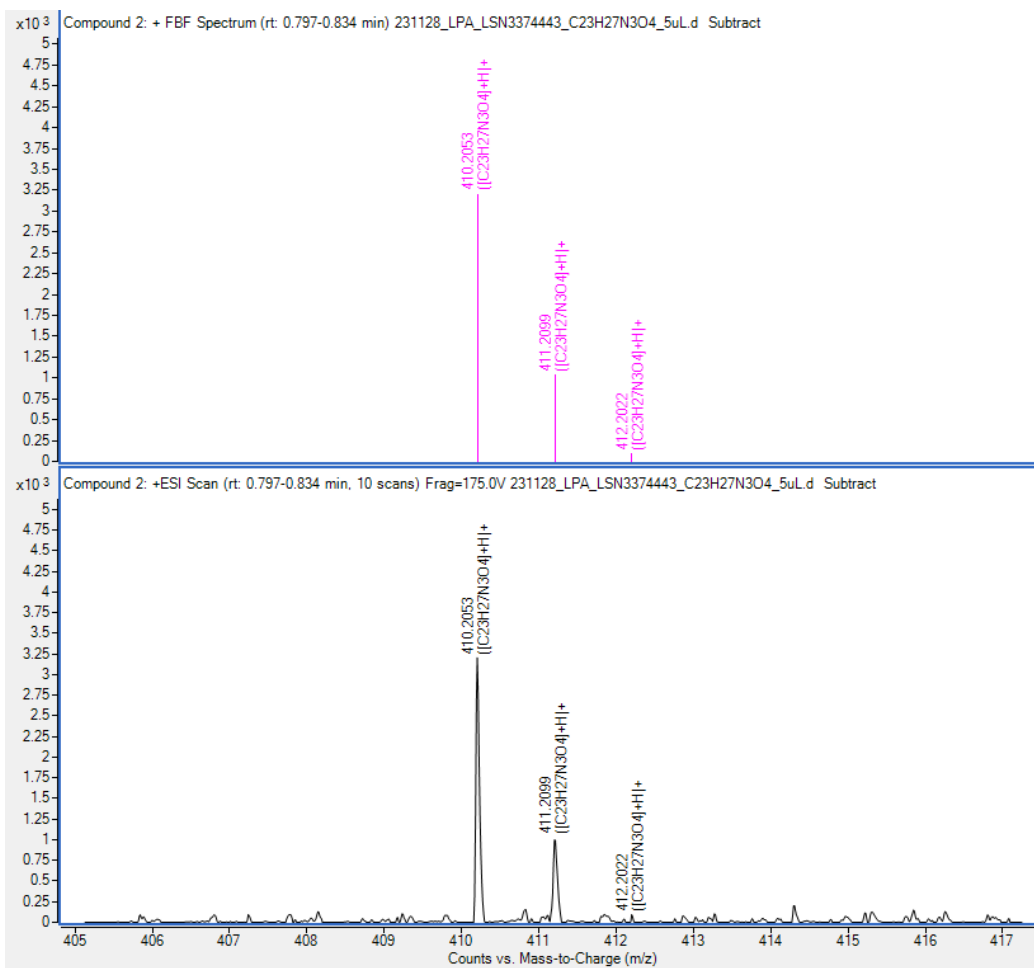
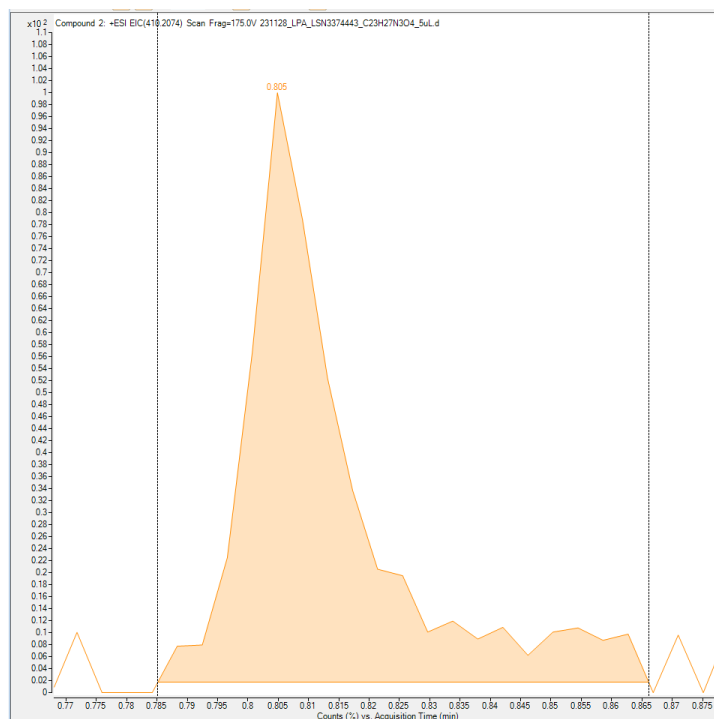
Acquisition method: Column: X-Bridge C18 3.5 $\mu$ M 2.1x50mm. Mobile phase: A= 0.1% FA in water; B= 0.1% FA in CH<sub>3</sub>CN. Run length: 2 min. Column temperature: 50°C. Acquisition mode: ES+. Injection volume: 1 $\mu$ L

| Time [min] | A [%] | B [%] | Flow [mL/min] | Max. Pressure Limit [bar] |
|------------|-------|-------|---------------|---------------------------|
| 0.00       | 95.00 | 5.00  | 1.200         | 1000.00                   |
| 0.25       | 95.00 | 5.00  | ---           | ---                       |
| 1.25       | 5.00  | 95.00 | ---           | ---                       |
| 1.75       | 5.00  | 95.00 | ---           | ---                       |
| 2.00       | 95.00 | 5.00  | ---           | ---                       |

Ref. Standards from Agilent's Internal Reference Mass Kit (p/ G1969-85001)

- Purine (m/z 121.05087)
- HP-921 (m/z 922.00980)
- 

| Sample                                          |                 |
|-------------------------------------------------|-----------------|
| LSN                                             | <b>3374443</b>  |
| Molecular Formula                               | C23H27N3O4      |
| Exact Mass                                      | 409.2002        |
| Exact Mass (M+H)                                | 410.2074        |
|                                                 |                 |
| <b>Experimental Results</b>                     |                 |
| <b>Measured Exact Mass of (M+H)<sup>+</sup></b> | <b>410.2053</b> |
| ppm error with expected Exact Mass:             | <b>-4.82</b>    |



# TRQ41776 [3H]LSN3441732

Injection Date/Time: 02/10/2017 17:41:27

Injection Volume: 8 µL

Sample info: Active

Column: Inertsil ODS3 5 µm 250 x 4.6 mm

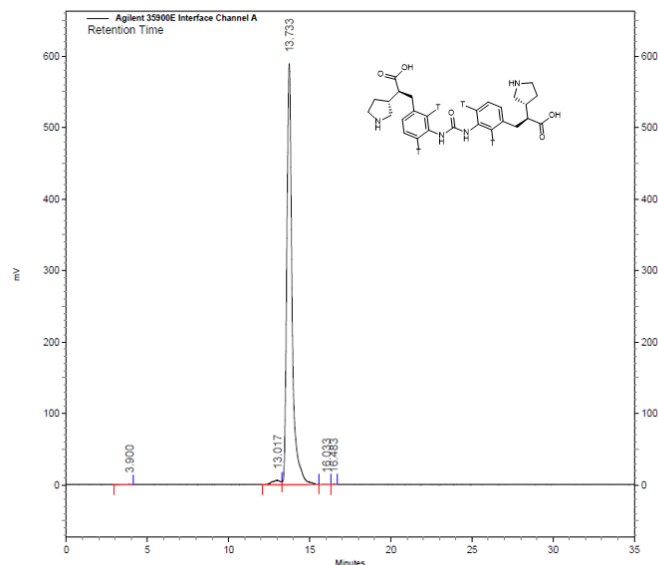
Column temperature: 22 °C

Eluent A: Water:trifluoroacetic acid (1000:1)

Eluent B: Acetonitrile:trifluoroacetic acid (1000:1)

Gradient T(min):%B: 0/10, 20/40, 21/100, 31/100, 32/10, 35/10 @ 1 mL/min

## Radiochemical Signal



## TRQ41776 [3H]LSN3441732

Agilent 35900E  
Interface Channel A  
Results

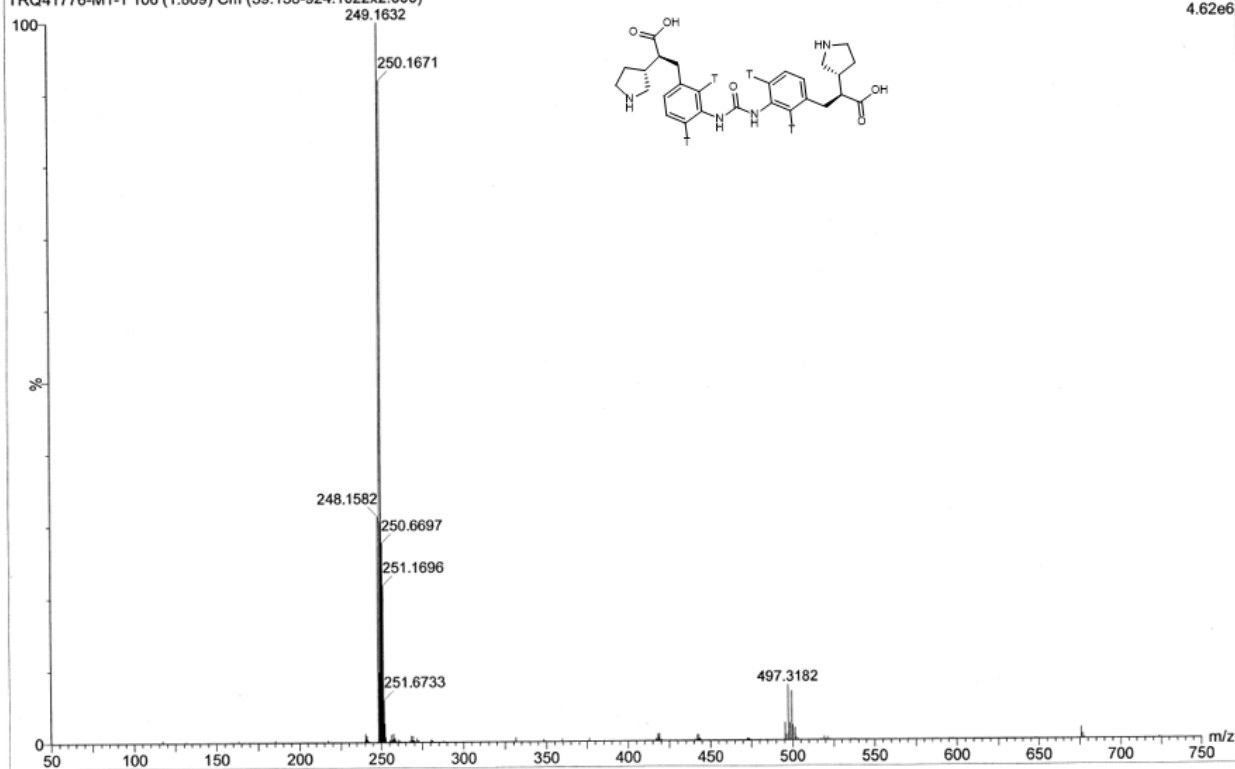
| Name   | Retention Time | Area       | Area Percent |
|--------|----------------|------------|--------------|
|        | 3.900          | 2396238    | 0.2          |
|        | 13.017         | 22328988   | 1.7          |
|        | 13.733         | 1323533567 | 98.0         |
|        | 16.033         | 1983292    | 0.1          |
|        | 16.483         | 758097     | 0.1          |
| Totals |                | 1351000182 | 100.0        |

TRQ41776 [3H]LSN3441732

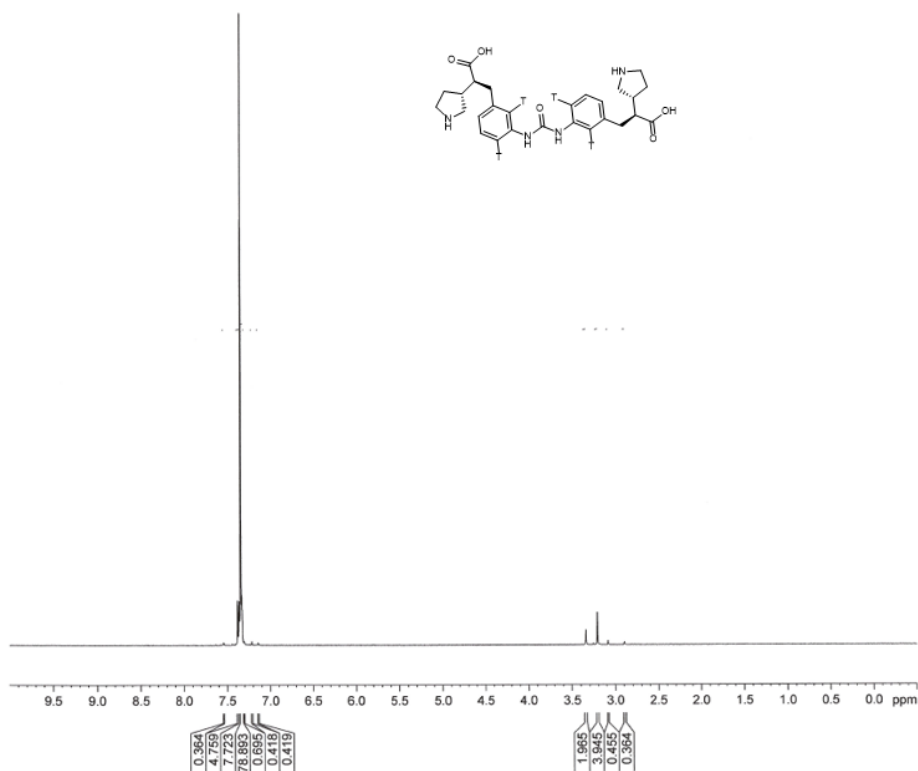
02-Oct-2017 14:15:37

TRQ41776-M1-1 106 (1.809) Cm (39:138-924:1022x2.000)

TOF MS ES+  
4.62e6



TRQ41776 [3H]LSN3441732



```

NAME TRQ41776-N1-1.171004
EXPNO 2
PROCNO 1
Date_ 20171004
Time 15:55
INSTRUM spect
PROBHD 5 mm DUX 3H-1H
PULPROG zgpg
TD 32000
SOLVENT DMSO
NS 8448
DS 0
SWH 8270.145 Hz
FIDRES 0.258992 Hz
AQ 1.9225500 sec
RG 1625.5
DW 60.400 usec
DE 6.00 usec
TE 298.0 K
D1 5.00000000 sec
D11 0.03000000 sec
TDO 33

===== CHANNEL f1 =====
NUC1 3H
P1 13.85 usec
PL1 -1.00 dB
PL1W 2.5262211 W
SFO1 426.795930 MHz

===== CHANNEL f2 =====
PCPDG2 waltz16
NUC2 1H
PCPD2 101.00 usec
PL2 -2.00 dB
PL12 18.00 dB
PL2W 8.96165594 W
PL12W 0.11545108 W
SFO2 400.1324710 MHz
SI 32768
SF 426.7945590 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
    
```

**Table with abbreviations**

|          |                                                                               |
|----------|-------------------------------------------------------------------------------|
| BOC      | <i>tert</i> -butoxycarbonyl                                                   |
| C        | Celsius                                                                       |
| Ci       | Curie                                                                         |
| d        | doublet                                                                       |
| DMSO     | Dimethylsulfoxide                                                             |
| DAD      | diode-array detection                                                         |
| ESI      | Electrospray ionization                                                       |
| EtOAc    | Ethyl Acetate                                                                 |
| g        | gram                                                                          |
| h        | hour                                                                          |
| HPLC     | High Performance Liquid Chromatography                                        |
| HRMS     | High-resolution mass spectrometry                                             |
| DART-TOF | High-resolution mass spectrometry direct analysis in real time-time of flight |
| de       | Diastereomeric excesses                                                       |
| ee       | Enantiomerically excesses                                                     |
| IPA      | Isopropylalcohol                                                              |
| LC       | Liquid chromatography                                                         |
| LCMS     | Liquid chromatography mass spectrometry                                       |
| m        | multiplet                                                                     |
| M        | Molar                                                                         |
| MHz      | Megahertz                                                                     |
| mL       | milliliter                                                                    |
| mmol     | millimol                                                                      |
| MS       | mass spectrometry                                                             |
| MTBE     | methyl <i>tert</i> -butyl ether                                               |
| N        | Normal                                                                        |
| NMR      | Nuclear magnetic resonance                                                    |
| ppm      | parts per million                                                             |

|                |                                    |
|----------------|------------------------------------|
| psi            | Pounds per square inch             |
| °C             | Degree Celsius                     |
| QTOF           | Quadrupole Time-of-Flight          |
| s              | singlet                            |
| SFC            | Supercritical Fluid Chromatography |
| t              | triplet                            |
| t <sub>R</sub> | Retention time                     |
| TFA            | Trifluoroacetic acid               |
| THF            | Tetrahydrofuran                    |
| UV             | Ultraviolet                        |