Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Synthesis

General. All reagents were commercially available and used without purification unless specified otherwise. Thin-layer chromatography was carried out on precoated silica gel (60 F_{254}) plates obtained from EMD. Silica gel (35-70 *i*m) from EM Science was used for column chromatography. All NMR spectra were acquired on a Bruker DPX-300 or a Bruker DRX-400 instrument unless otherwise noted. Mass spectral data was acquired by Nonka Sevova at Notre Dame University unless specified otherwise. Labeled ethyl bromoacetate, acetyl chloride and tyrosine were purchased from Cambridge Isotope Laboratories. Labeled isoleucine was obtained from Sigma-Aldrich. Tyrosine decarboxylase from *Streptococcus faecalis* was obtained from Sigma-Aldrich.

Synthesis of (4-methoxy)phenyl [1,2- $^{13}C_2$*]acetic acid***(7)**. To a dry flask was added palladium acetate (40.4 mg, 0.18 mmol), tri-1-naphthyl phosphine (218.6 mg, 0.53 mmol), K_3PO_4 (6.261 g, 29.5 mmol), ethyl bromoacetate (1 g, 5.9 mmol), 4-methoxyphenyl boronic acid (1.793 g, 11.8 mmol) and anhydrous tetrahydrofuran (25 mL)under argon atmosphere. The reaction was quenched with 45 mL water after 16hrs at room temperature and the mixture was extracted with dichloromethane (3×20mL). The combined organic layers were dried over $MgSO_4$, the solvent was removed *in vacuo* and the product was purified via silica gel chromatography (19:1 hexane-ethyl acetate, R_f 0.25) to give an oil (0.658 g, 57.4%). ^{1}H NMR ($CDCl_3$) δ 1.20-1.25 (t, 3H, J=7.2Hz), 3.30-3.76 (dd, 2H, J=7.8Hz, $^1J_{CH}$=129Hz), 3.78 (s, 3H), 4.08-4.16 (dq, 2H, J=7.2, $^3J_{CH}$=3Hz), 6.83-

6.86 (d, 2H, J=11.7Hz), 7.16-7.20 (dd, 2H, J=11.7Hz, $^{3}J_{CH}$=3Hz) ^{13}C NMR δ 13.9, 39.8-40.6 (d, J=57.6Hz), 55.0, 60.5, 113.6, 113.7, 129.9, 130.0, 158.3, 171.3-172.1 (d, J=57.6Hz) FAB-MS ($M+H^{+}$): 196.1032.

Ethyl 2-(4-methoxy phenyl) acetate (1.891 g, 9.6 mmol) was stirred in 19.2mL 1N NaOH and 19.2mL methanol for 2 hours at room temperature. The solution was then extracted with ethyl acetate (3x10mL). The organic layers were combined, dried over $MgSO_4$, and the solvent was removed in vacuo to give a white solid (1.485 g, 92%). ^{1}H NMR ($CDCl_3$) δ 3.34-3.80 (dd, 2H, J=7.5Hz, $^{1}J_{CH}$=129Hz), 3.78 (s, 3H), 6.83-6.86 (d, 2H, J=8.4Hz), 7.16-7.20 (dd, 2H, J=8.4Hz, $^{3}J_{CH}$=4.2Hz) ^{13}C NMR δ 39.3-40.0 (d, J=55.4Hz), 55.0, 113.7, 113.8, 130.1, 158.6, 176.4-177.1 (d, J=55.4Hz) FAB-MS ($M+H^{+}$): 168.0671.

Synthesis of diethyl (4-methoxy)phenyl [1,2- $^{13}C_2$*]acetyl phosphonate* **(8)**. Thionyl chloride (0.7 mL, 9.7 mmol) was added to a suspension of (4-benzyloxy)phenyl *[1,2-* $^{13}C_2$*]*acetic acid (1.485 g, 8.8 mmol) in $CHCl_3$ (20 mL) containing DMF (0.1 mL). The mixture was stirred for 4 hours at reflux. The solvent and excess thionyl chloride were removed *in vacuo* yielding yellow oil. The product was used without isolation for the subsequent step.

The acyl chloride (1.642 g, 8.8 mmol) was dissolved in 5 mL THF and chilled to 0^{o}C. Triethyl phosphite (1.53 mL, 8.8 mmol) was added dropwise under anhydrous conditions. When the addition was complete, the reaction was warmed to 70^{o}C and continued stirring for 15 minutes. The reaction was evaporated to obtain **(8)**, which was used without isolation in the following step.

Supplementary Material (ESI) for Chemical Communications

Synthesis of (*R*)-1-amino-2-(4-hydroxyphenyl) *[1,2-* $^{13}C_2$*]*ethylphosphonic acid **(2)**. To a suspension of hydroxylammonium chloride (0.799g, 11.5mmol) in 1 mL pyridine and 2 mL ethanol was added slowly 2.536g **(8)** in 2 mL ethanol. After stirring for 12 hours the clear solution was evaporated and the oily residue treated with 10 mL 2% HCl. The mixture was extracted with dichloromethane. The organic layers were combined, dried and concentrated to yield a yellow oil (2.2735 g, 85.2%). The product was used in the next reaction without further purification or characterization.

To a suspension of zinc (1.9611 g, 30 mmol) in 7.5mL formic acid was added the oxime(2.2735 g, 7.5 mmol) under argon. The reaction was allowed to proceed overnight. The suspension was filtered and the filtrate was evaporated to give yellow oil (100%). ^{1}H NMR ($CDCl_3$) δ 1.20-1.39 (m, 6H), 2.80-2.92 (m, 1H), 3.10-3.21 (m, 1H), 3.48-3.52 (m, 1H), 3.78 (s, 3H), 4.09-4.20 (m, 4H), 6.84-6.86 (d, 2H, J=8.6Hz), 7.14-7.18 (dd, 2H, J=8.6Hz, $^3J_{CH}$=4.0Hz) ^{13}C NMR δ 16.0, 34.1-34.5 (d, J=32.7Hz), 47.6-50.1 (dd, J=32.7Hz, $^1J_{CP}$=155Hz), 55.0, 63.3, 63.4, 114.0, 130.1, 158.7 ^{32}P NMR δ 25.3-26.5 (d, $^1J_{CP}$=155Hz) FAB-MS ($M+Na^+$): 312.124574.

The amine (2.1696 g, 7.5 mmol) was dissolved in 100mL 48% HBr and refluxed for 2 hours. The solvent was evaporated and the resulting yellow residue was dissolved in 1mL 1N NaOH solution, run through a Dowex 50WX4-100 column (25mm ID × 100mm) and eluted with 100 mL water. Ninhydrin positive fractions were combined to give AHEP (940 mg, 57.2%). ^{1}H NMR (D_2O) δ 2.40-

2.51 (m, 1H), 2.86-3.24 (m, 1H), 3.35-3.65 (m, 1H), 6.74-6.79 (d, 2H, J=8.4Hz), 7.08-7.12 (dd, 2H, J=8.4Hz, $^{3}J_{CH}$=4Hz) ^{13}C NMR δ 32.7-33.2 (d, J=32.7Hz), 49.5-51.8 (dd, J=32.7Hz, $^{1}J_{CP}$=141Hz), 115.5, 115.6, 130.2, 154.3 ^{32}P NMR δ 14.4-15.5 (d, $^{1}J_{CP}$=141Hz) FAB-MS (M+H^{+}): 220.0666.

Synthesis of [1,2- $^{13}C_2$]N-acetyl [1- $^{13}C_1$]isoleucine. Isoleucine (0.5 g, 3.8 mmol) was dissolved in 4N NaOH solution (2.85 mL, 11.4 mmol) and chilled to 0^{o}C. Acetyl chloride (0.31 mL, 4.2 mmol) was added in 5 batches over 50 min. The reaction was acidified to pH 1.5 with concentrated HCl and extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and concentrated resulting in a white solid. The solid was recrystallized from ethyl acetate/hexane to give *N*-acetyl isoleucine (167 mg, 25%). ^{1}H NMR (DMSO) δ 0.80-0.84 (m, 6H), 1.10-1.23 (m, 1H), 1.31-1.42 (m, 1H), 1.66-1.72 (m, 1H), 1.61-2.06 (dd, 3H, $^{1}J_{CH}$=127Hz, $^{2}J_{CH}$=6Hz), 4.09-4.16 (m, 1H), 7.94-7.97 (m, 1H) ^{13}C NMR δ 11.3, 15.6, 22.0-22.7 (d, J=50.8Hz), 24.7, 36.4, 55.2, 169.0-169.6 (d, J=50.8Hz), 173.2 FAB-MS (M+H^{+}): 177.1216.

Synthesis of [ring-D_4]tyramine **(10)**. The protocol of decarboxylation of *d_4*-tyrosine as described by Battersby *et al*[1] was followed with a few modifications. Specifically, tyrosine (100mg) was dissolved in 100 mL H_2O by microwave heating. A suspension of tyrosine decarboxylase (2mg, 0.05unit/mg) from *Streptococcus faecalis* and pyridoxal-5-phosphate (7mg) in acetate buffer (4mL, 0.1M, pH 5.5) was added to the cooled tyrosine solution and the mixture was incubated at 37^{o}C for 3hrs with shaking. The reaction was stopped by heating to boiling. Tyramine was purified using Millipore Ultra 5000 MWCO centrifugal filter

devices (3450g, 30min). The filtrate was concentrated and passed through a Dowex 1-X8 anion exchange column. Evaporation of eluent (H_2O) gave tyramine as a tan powder (74mg, 97%). ^{1}H NMR (D_2O) δ 2.747 (t, 2H, *J* = 7.2Hz), 3.058 (t, 2H, *J* = 7.2Hz) ^{13}C NMR δ 155.33, 129.80 (t, *J*= 19Hz), 127.68, 115.66 (t, *J*= 19Hz), 40.79, 31.89.

Stable isotopic incorporation experiments

Fermentation. The K-26 producing strain (*Astrosporangium hypotensionis*) was obtained from the Agricultural Research Service (NRRL 12379). The production protocol suggested by Yamato *et al*[2] was followed with minor modifications. The seed medium consisted of dextrose, 0.1g/L; Difco soluble starch, 0.1g/L; Bacto beef extract, 0.05g/L; Bacto yeast extract, 0.05g/L; Bacto tryptone, 0.05g/L; and $CaCO_3$, 0.02g/L dissolved in distilled water and was adjusted to pH 7.2 before autoclaving. Fermentation was initiated by aseptically inoculating one loop of *Astrosporangium hypotensionis* mycelia grown on an agar plate into a sterile 50-mL Falcon tube containing 10 mL seed medium (Phase I). The Falcon tube was incubated for 10-12 days at 28^{o}C in a shaker incubator. In the second phase, 3 mL of the phase I seed culture were transferred into a 300-mL flask containing 30 mL of seed medium. The flask was incubated for 3-4 days at 28^{o}C in a shaker incubator. In phase III, 30 mL of phase II seed culture were transferred into a 3000-mL Fernbach flask containing 300 mL production medium. The flask was incubated for 5-6 days at 28^{o}C in a shaker incubator. The production medium contained Difco soluble starch, 0.4g/L; soy bean meal (Wild

Oat), 0.3g/L; corn steep liquor (Sigma), 0.05g/L; K_2HPO_4, 5mg/L; $MgSO_4$, 2.4mg/mL; KCl, 3mg/L; and $CaCO_3$, 0.03g/L dissolved in distilled water and was adjusted to pH 7.8 before autoclaving.

Pulse feeding experiments. Millimolar amounts (based on the culture volume) of each precursor (0.3mM for AHEP, 0.4mM for isoleucine and *N*-acetylisoleucine, and 1mM for tyramine) were dissolved in 5 mL water and administered separately to phase III culture through a sterile syringe filter in every 24 hours for 4 days.

K-26 purification. The production culture was centrifuged and the supernatant was acidified to pH 3.0. To one liter of supernatant were added thirty grams of the hydrophobic resin Diaion HP-20 (activated by stirring with methanol and rinsing with water) and the suspension was stirred for 30 minutes. The resin was filtered and washed with water (2x30mL). The washed resin beads were placed in a 50% methanol-50% water solution and stirred for 10 min. The suspension was then filtered, washed with 50% methanol (2x30 mL), and discarded. The filtrate was concentrated to 40 mL and neutralized. K-26 was further purified using Millipore Ultra 5000 MWCO centrifugal filter devices and centrifuging at 3450*g* for 30 minutes. The filtrate was concentrated to ca. one mL by rotary evaporation.

Mass spectrometry

General. Mass spectrometry was performed using ThermoFinnigan (San Jose, CA) TSQ® Quantum triple quadrupole mass spectrometer equipped with a

standard electrospray ionization source outfitted with a 100-μm I.D. deactivated fused Si capillary. Data acquisition and spectral analysis were conducted with Xcalibur™ Software, version 1.3, from ThermoFinnigan (San Jose, CA), on a Dell Optiplex GX270 computer running the Microsoft® Windows 2000 operating system. The source spray head was oriented at an angle of 90^{o} to the ion-transfer-tube. Nitrogen was used for both the sheath and auxiliary gas. The sheath and auxiliary gases were set to 33 and 14 (arbitrary units) respectively. Samples were introduced by HPLC. A Surveyor® Autosampler and a Surveyor® MS Pump from ThermoFinnigan (San Jose, CA) were used. The injection volume was 10μL. K-26 was separated from co-metabolites using a Jupiter™ minibore 5μm C18 column (2.0mm × 15cm) with a linear water-acetonitrile gradient (ranging from 95:5 to 5:95 H_2O:CH_3CN) containing 10mM ammonium acetate. The flow rate was 0.2mL/min.

$^{13}C_2$-AHEP. The mass spectrometer was operated in the negative ion mode and the electrospray needle was maintained at 4200V. The ion transfer tube was operated at -35V and 350^{o}C. The tube lens voltage was set to -150V. Source CID (offset voltage between skimmer and the first ion guide, Q00) was used at 15V. The selected reaction monitoring (SRM) mode was used. Ions were collisionally activated with argon at an indicated pressure of 1.4mT. The mass-spectral resolution was set to a peak width (full width at half maximum, FWHM) of 0.50u and 0.50u for precursor and product ions respectively. Mass transitions at the specified collision energy (m/z 534→216; 35eV), (m/z 536→218; 35eV) and (m/z 540→220; 35eV) were monitored for AHEP, AHEP+2, and AHEP+4,

respectively. Transitions (m/z 534→379; 35eV), (m/z 536→381; 35eV), and (m/z 538→383; 35eV) were monitored for Tyr-AHEP, Tyr-AHEP+2, and Tyr-AHEP+4, respectively. The scan width for product ions was 1.000u and the cycle time for each ion was 0.15 seconds. The electron multiplier gain was set to 2×10^6. Data were acquired in profile mode. Both a $^{13}C_2$-AHEP enriched sample and an unenriched sample were scanned. The unenriched sample was used to create theoretical curves of ratios of mass isotopomer abundance in K-26 to correct the isotopomer distribution of the enriched sample. Similar calculations as described earlier[3] were used and the incorporation of $^{13}C_2$-AHEP in K-26 was calculated as 85%. No ^{13}C enrichment was observed in the central tyrosine.

Ring-d_4-tyramine. The mass spectrometer was operated in the negative ion mode and the electrospray needle was maintained at 4200V. The ion transfer tube was operated at -35V and 350^oC. The tube lens voltage was set to -150V. Source CID (offset voltage between skimmer and the first ion guide, Q00) was used at 15V. The selected reaction monitoring (SRM) mode was used. Ions were collisionally activated with argon at an indicated pressure of 1.4mT. The mass-spectral resolution was set to a peak width (full width at half maximum, FWHM) of 0.50u and 0.50u for precursor and product ions respectively. Mass transitions at the specified collision energy (m/z 534→216; 35eV), (m/z 538→220; 35eV) and (m/z 538→220; 35eV) were monitored for unenriched K-26, enrichment in the central tyrosine and enrichment in AHEP, respectively. The scan width for product ions was 1.000u and the cycle time for each ion was 0.25 seconds. The electron multiplier gain was set to 2×10^6. Data were acquired in profile mode.

Both a ring-d_4-tyramine enriched sample and an unenriched sample were scanned. The mass spectral data for the two samples was almost identical, indicating no d_4 enrichment of K-26.

$^{13}C_3$-N-acetyl isoleucine and $^{13}C_1$-isoleucine. The mass spectrometer was operated in the negative ion mode and the electrospray needle was maintained at 4200V. The ion transfer tube was operated at -35V and 350^{o}C. The tube lens voltage was set to -150V. Source CID (offset voltage between skimmer and the first ion guide, Q00) was used at 15V. The selected reaction monitoring (SRM) mode was used. Ions were collisionally activated with argon at an indicated pressure of 1.4mT. The mass-spectral resolution was set to a peak width (full width at half maximum, FWHM) of 0.50u and 0.50u for precursor and product ions respectively. Transitions (m/z 534→379; 35eV), (m/z 535→379; 35eV), (m/z 536→379; 35eV), and (m/z 537→379; 35eV) were monitored for *N*-Ac-Ile, *N*-Ac-Ile+1, *N*-Ac-Ile+2, and *N*-Ac-Ile+3, respectively. The scan width for product ions was 1.000u and the cycle time for each ion was 0.15 seconds. The electron multiplier gain was set to 2 × 10^6. Data were acquired in profile mode. An unenriched sample was used to create theoretical curves of ratios of mass isotopomer abundance in K-26 to correct the isotopomer distribution of the enriched sample. Similar calculations as described earlier[3] were used and the incorporation of $^{13}C_3$-N-acetyl isoleucine and $^{13}C_1$-isoleucine in K-26 were calculated as 43% and 24%, respectively. In the case of *N*-acetyl isoleucine, there was only enrichment of the M+1 peak and not of the M+3, indicating incorporation of the amino acid occurred after deacetylation.

References

[1] Battersby, A.R.; Chrystal, E.J.; Staunton, J. *J. Chem. Soc., Perkin Trans. 1* **1980**, *1*, 31-42.

[2] Yamato, M.; Koguchi, T.; Okachi, R.; Yamada, K.; Nakayama, K.; Kase, H.; Karasawa, A.; Shuto, K. *J Antibiot* **1986**, *39*, 44-52.

[3] Ntai, I.; Manier, M.L.; Hachey, D.L.; Bachmann, B.O.; *Organic Letters*, **2005**, *7*, 2763.