Electronic Supplementary Material (ESI) for Green Chemistry.

N-Phenylputrescine (NPP): A Natural Product Inspired Amine Donor for Biocatalysis

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Contents

1 General Experimental Procedures

Commercially available chemicals were used as received. Presitagliptin ketone **19** was purchased from key organics. All buffers were prepared from standard salts and pH adjusted with NaOH or HCl before use.

Stock solutions of substrates were prepared in reaction buffer unless stated otherwise. Wild type TAs (*HEWT* & *CVTA*) were defrosted on ice and incubated with PLP (0.1 mM) for 2 h prior to assay. All commercial TAs were dissolved in reaction buffer.

NMR Spectroscopy

^{1}H NMR spectra were recorded at 400 and 600 MHz using a Bruker Ascend 400 or Bruker Ultrashield 600 spectrometer. The spectra have been referenced with the appropriate residual solvent peaks ($CDCl_3$ 7.26 ppm, CH_3OD 3.31 ppm) and the coupling constants are reported to the nearest 0.1 Hz. ^{13}C NMR spectra were taken at 126 Hz or 151 Hz using the spectrometers mentioned above and referenced from solvent peaks ($CDCl_3$ 77.16 ppm, CH_3OD 49.00 ppm). 2D NMR spectra such as COSY, HSQC and HMBC were used to assist with proton signal assignments.

Mass Spectrometry (MS)

Mass spectrometry analysis of small molecules was completed using a Bruker microTOF instrument using electron ionisation (EI+) and electrospray ionisation (ESI+).

Infrared Spectroscopy (IR)

A Shimadzu IR Affinity-1 Fourier transform IR spectrometer was used to record IR spectra of neat samples using a Pike MIRacle ATR accessory.

Protein Purification

All protein purification steps unless otherwise stated were completed on AKTA purifier Cytiva Lifesciences using a Frac-920 fraction collector.

Centrifugation

Thermoscientific Heraeus Multifuge X3R was used during protein growth steps with 8 × 50 mL rotor and 6 × 250 mL rotor at 4 °C. Benchtop centrifuge is VWR Microstar 17 and is used at rt.

Plate Reader

For colourimetric assays a Biotek Synergy HT plate reader was used.

High Performance Liquid Chromatography (HPLC)

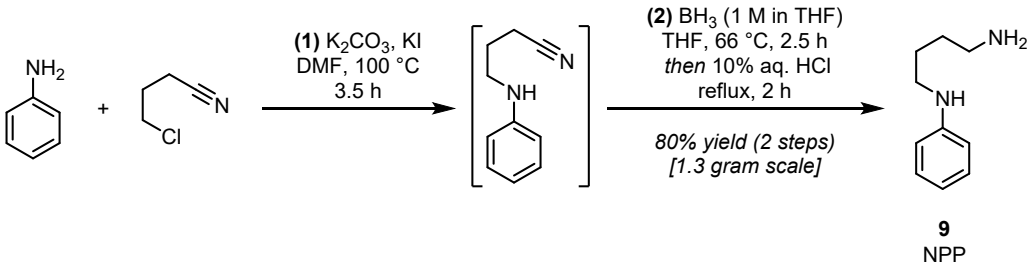
Both chiral normal phase and reverse phase HPLC methods were carried out using Shimadzu instruments. The Shimadzu HPLC used for all the reverse phase quantitative assays except with benzaldehyde **11** is fitted with an autosampler (SIL-20A HT), pump (LC-20AD), UV/visible detector (SPD-20A), system controller (CBM-20Alite) and a column oven (CTO-20A).

The Shimadzu HPLC used for the reverse phase quantitative assay with benzaldehyde **11** is fitted with an autosampler (SIL-20A HT), pump (LC-20AT) and a UV/visible detector (SPD-20A).

The Shimadzu HPLC used for determining the enantioselectivity of the NPP dimer **11** and sitagliptin **20** is fitted with an autosampler (SIL-20A HT), pump (LC-20AD), RID detector (RID-20A), UV/visible detector (SPD-20A), system controller (CBM-20A) and a column oven (CTO-20A).

2 Methods

2.1 Experimental procedure for the small-scale synthesis of NPP 9



9
NPP

Procedure adapted from Orelli and co-workers.[1]

Step 1: A solution of 4-chlorobutyronitrile (0.91 mL, 9.66 mmol) in DMF (1.0 mL) was added slowly over 2 h to a mixture of aniline (1.76 mL, 19.3 mmol, 2 eq.), potassium carbonate (2.67 g, 19.3 mmol, 2 eq.) and potassium iodide (0.40 g, 2.41 mmol, 0.25 eq.) in DMF (5.4 mL) at 100 °C with stirring. The reaction was stirred for a further 90 min after which monitoring by ^{1}H NMR spectroscopy indicated complete consumption of 4-chlorobutyronitrile. The reaction was cooled, quenched with saturated aq. NH_4Cl (40 mL) and basified to pH 14 with 5 M aq. NaOH. Ethyl acetate (50 mL) was added and the layers separated. The aqueous phase was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were washed with aq. $Na_2S_2O_3$ (20 mL). NaCl was added to the aq. thiosulfate solution and pH was adjusted to 14. The mixture was extracted with ethyl acetate (3 × 50 mL). Combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure to give a brown coloured oil (1.78 g). The crude material was used in the next step without purification.

Step 2: Crude nitrile (1.78 g) was dissolved in THF (13.8 mL) and borane (1 M in THF, 19.3 mL, 19.3 mmol, 2 eq.) was added slowly with stirring at rt. The mixture was heated at reflux for 2 h. The reaction was cooled to rt, quenched with methanol (until effervescence stopped) and concentrated under reduced pressure. The residue was heated at reflux in 10% aq. HCl (23 mL) for 2 h. The mixture was cooled and basified to pH 14 with 5 M aq. NaOH. Ethyl acetate (30 mL) was added and the layers separated. The aqueous phase was extracted with ethyl acetate (3 × 30 mL). Subsequently, NaCl was added and the aqueous phase was extracted with ethyl acetate three more times (3 × 30 mL). Combined organic layers dried over Na_2SO_4 and concentrated under reduced pressure to give a brown oil. The crude residue was dissolved in a minimal volume of CH_2Cl_2 and purified by flash column chromatography (gradient CH_2Cl_2 to 10:1 CH_2Cl_2:MeOH until TLC indicates less polar impurities removed, then 10:1:0.1 CH_2Cl_2:MeOH:*i*-$PrNH_2$ for product elution). The relevant fractions were combined and concentrated under reduced pressure to give NPP **9** as an amber oil (1.27 g, 7.75 mmol, 80% yield). All spectroscopic data matched that reported in the literature.[1]

R_f 0.38 (10:1:0.1 CH_2Cl_2:MeOH:*i*-$PrNH_2$);

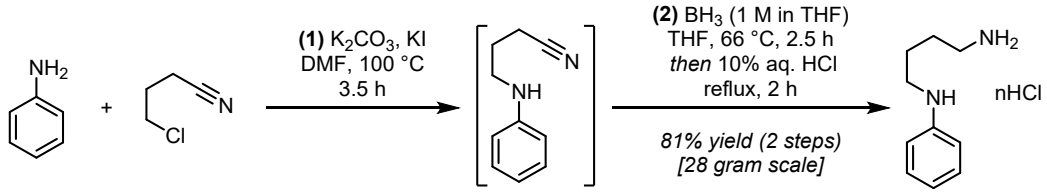
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (dd, *J* = 8.6, 7.3 Hz, 2H), 6.69 (t, *J* = 7.3 Hz, 1H), 6.60 (dd, *J* = 8.7, 1.1 Hz, 2H), 3.13 (t, *J* = 6.9 Hz, 2H), 2.75 (t, *J* = 6.9 Hz, 2H), 1.72 - 1.51 (m, 4H) ppm;

^{13}C NMR (101 MHz, $CDCl_3$) δ 148.6 (qC), 129.4 (2 × CH), 117.3 (CH), 112.8 (2 × CH), 44.0 (CH_2), 42.1 (CH_2), 31.3 (CH_2), 27.1 (CH_2) ppm;

HRMS (ESI+) $C_{10}H_{16}N_2$: 165.1403 $[M+H]^+$ (calculated 165.1386);

IR 3225, 3022, 2927, 2856, 1600, 1500 cm^{-1}.

2.2 Experimental procedure for the large-scale synthesis of NPP 9 hydrochloride salt



Step 1: A solution of 4-chlorobutyronitrile (13.7 mL, 0.145 mol) in DMF (15 mL) was added slowly over 2 h to a mixture of aniline (26.4 mL, 0.290 mol, 2 eq.), potassium carbonate (40.0 g, 0.290 mol, 2 eq.) and potassium iodide (6.0 g, 0.036 mol, 0.25 eq.) in DMF (80 mL) at 100 °C with stirring. The reaction was stirred for a further 90 min after which monitoring by ^{1}H NMR spectroscopy indicated complete consumption of 4-chlorobutyronitrile. The reaction was cooled, quenched with saturated aq. NH_4Cl (450 mL) and basified to pH 14 with concentrated aq. NaOH (100 mL). Ethyl acetate (250 mL) was added and the layers separated. The aqueous phase was extracted with ethyl acetate (3 × 250 mL) and the combined organic layers were washed with aq. $Na_2S_2O_3$ (350 mL). NaCl (30 g) was added to the aq. thiosulfate solution and pH was adjusted to 14. The mixture was extracted with ethyl acetate (3 × 200 mL). Combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. Residual aniline and DMF were removed under high vacuum (0.5 mbar, 85 °C with stirring) for 15 h to give a brown coloured oil (22.6 g). The crude material was used in the next step without further purification.

Step 2: Crude nitrile (22.6 g) was dissolved in THF (200 mL) and borane (1 M in THF, 290 mL, 0.290 mol, 2 eq.) was added slowly with stirring at rt. The mixture was heated at reflux for 2.5 h. The reaction was cooled to 0 °C, quenched with methanol (200 mL, until effervescence stopped) and concentrated under reduced pressure. The residue was heated at reflux in 10% aq. HCl (350 mL) for 2 h. The mixture was cooled to 0 °C and basified to pH 14 with saturated aq. NaOH (100 mL). Ethyl acetate (200 mL) was added and the layers separated. The aqueous phase was extracted with ethyl acetate (3 × 200 mL). Subsequently, NaCl (30 g) was added and the aqueous phase was extracted with ethyl acetate three more times (3 × 200 mL). Combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give a brown oil. The crude residue was dissolved in THF (400 mL), and HCl was bubbled through the solution for 2 h. The precipitate was filtered and rinsed several times with THF. Residual solvent was removed under reduced pressure to give NPP•nHCl (consists of 69% w/w NPP, established by ^{1}H NMR spectroscopy with 1,2,4,5-tetramethylbenzene as internal standard) as an off white solid (28.0 g, 0.118 mol of NPP, 81% yield). Note: The free base **9** can be obtained by dissolving NPP•nHCl in aq. 1 M NaOH solution and extraction with ethyl acetate.

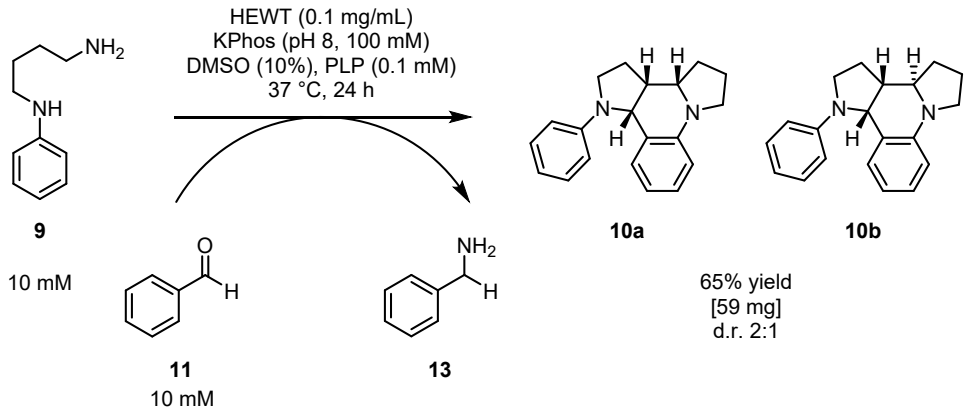
^{1}H NMR (400 MHz, Methanol-d_4) δ 7.50 – 7.44 (m, 2H), 7.38 – 7.33 (m, 2H), 7.33 – 7.28 (m, 1H), 3.43 – 3.35 (m, 2H), 2.99 (t, J = 7.2 Hz, 2H), 1.89 – 1.74 (m, 4H);

^{13}C NMR (101 MHz, Methanol-d_4) δ 140.1 (qC), 131.2 (CH), 127.7 (CH), 121.4 (CH), 50.4 (CH_2), 40.2 (CH_2), 25.7 (CH_2), 24.9 (CH_2);

HRMS (ESI+) $C_{10}H_{16}N_2$: 165.1404 $[M+H]^+$ (calculated 165.1386);

IR 2910, 2411, 1603, 1483, 1025 cm^{-1}.

2.3 Scale up of benzaldehyde 11 transamination with NPP 9: isolation of precipitate 10



A solution of *HEWT* (540 µL, 11 mg/mL) was added to KPhos buffer (60 mL, pH 8, 90% v/v aq. KPhos [100 mM], 10% v/v DMSO) containing PLP (0.1 mM), benzaldehyde (62 µL, 0.61 mmol) and NPP (100 mg, 0.61 mmol). The reaction was incubated with shaking at 37 °C for 24 h after which the precipitate was isolated by centrifugation (4 × 30 min, 3500 rpm, 4 °C). The precipitate was transferred with a minimal volume of water to a vial, dried under high vacuum to give a diastereomeric mixture of dimers **10a** and **10b** as an off white solid (59 mg, 0.20 mmol, 65% yield, d.r. 2:1).

R_f 0.48 and 0.35 (10:1 petroleum ether (40-60 °C): ethyl acetate);

^{1}H NMR (601 MHz, $CDCl_3$) δ 7.33 (dt, *J* = 7.7, 1.3 Hz, 10a, 1H), 7.30 – 7.26 (m, 10a, 2H), 7.26 – 7.23 (m, 10b, 2H), 7.20 (tt, *J* = 7.8, 1.3 Hz, 10b, 1H), 7.13 (dt, *J* = 7.5, 1.4 Hz, 10b, 1H), 7.10 (td, *J* = 7.7, 1.6 Hz, 10a, 1H), 6.84 – 6.78 (m, (10a, 2H), (10b, 1H), 6.75 (tt, *J* = 7.4, 1.1 Hz, 10b, 1H), 6.73 – 6.68 (m, (10a, 1H), (10b, 3H), 6.53 (td, *J* = 7.4, 1.2 Hz, 10a, 1H), 6.41 (dd, *J* = 8.2, 1.2 Hz, 10a, 1H), 5.13 (d, *J* = 6.9 Hz, 10a, 1H), 4.47 (d, *J* = 9.2 Hz, 10b, 1H), 3.75 (ddd, *J* = 12.7, 7.5, 3.2 Hz, 10a, 1H), 3.67 (ddd, *J* = 9.1, 7.8, 1.4 Hz, 10b, 1H), 3.47 (td, *J* = 9.0, 3.2 Hz, 10b, 1H), 3.45 (t, *J* = 8.9 Hz, 10a, 1H), 3.40 – 3.34 (m, both, 1H), 3.34 – 3.26 (m, 10a, 2H), 2.88 (q, *J* = 9.0 Hz, 10b, 1H), 2.78 (ddt, *J* = 16.1, 9.2, 7.1 Hz, 10b, 1H), 2.52 (dtd, *J* = 12.6, 6.7, 3.2 Hz, 10a, 1H), 2.50 – 2.46 (dd, 16.7, 7.1 Hz, 10b, 1H), 2.32 – 2.09 (m, 10a, 1H), 2.32 – 2.24 (m, 10b, 1H), 2.24 – 2.20 (m, 10b, 1H), 2.20 – 2.09 (m, 10b, 1H), 2.08-1.93 (m, (10a, 3H), (10b, 1H), 1.92-1.66 (m, (10a, 2H), (10b, 2H) ppm;

^{13}C NMR (151 MHz, $CDCl_3$) δ 149.3 (qC, 10b), 149.0 (qC, 10a), 147.3 (qC, 10b), 143.3 (qC, 10a), 129.5 (2 × CH, 10a), 129.2 (2 × CH, 10b), 128.9 (CH, 10a), 128.4 (qC, 10b),128.2 (CH, 10a), 127.3 (CH, 10b), 127.2 (CH, 10b), 123.1 (qC, 10a), 119.0 (CH, 10b), 116.6 (CH, 10b), 115.9 (CH, 10a), 115.5 (CH, 10a), 112.9 (2 × CH, 10b), 112.2 (CH, 10b), 111.4 (2 × CH, 10a), 110.4 (CH, 10a), 64.7 (CH, 10b), 59.6 (CH, 10b), 57.6 (CH, 10a), 56.6 (CH, 10a), 49.3 (CH_2, 10b), 48.4 (CH, 10b), 47.5 (CH_2, 10a), 47.4 (CH, 10b), 46.7 (CH_2, 10a), 40.2 (CH, 10a), 32.1 (CH_2, 10b), 30.7 (CH_2, 10b), 30.3 (CH_2, 10a), 23.4 (CH_2, 10a), 23.3 (CH_2, 10a), 22.5 (CH_2, 10b) ppm;

HRMS (EI+) $C_{20}H_{22}N_2$: 290.1781 $[M]^+$ (calculated 290.1778);

MP 129–131 °C decomposition;

IR 1600, 1502, 1448, 1361 cm^{-1}.

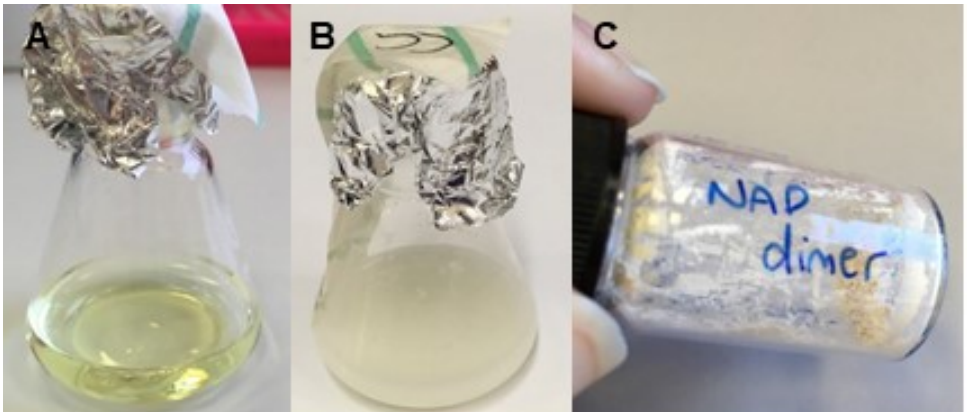


*Figure S1: A) Transamination reaction at time 0 h B) Transamination reaction at time 24 h where the formation of precipitate **10** is visible. C) Precipitate **10** after drying.*

2.4 Growth, Purification and Characterisation Protocols

2.4.1 Production of recombinant *HEWT*

HEWT Growth & Expression

The clone that expresses *Halomonas elongata* TA (*HEWT*) was kindly gifted by the Paradisi research group in a pHESPUC plasmid and has UNIPROT code E1V9I3. Growth, expression and purification procedures were adapted from their initial published characterization of *HEWT* which also details DNA sequence and molecular biology protocols.[2] The plasmid (2 µL) was transformed into BL21 (DE3) competent cells (10 µL) and selection was carried out on LB (Lysogeny broth) agar plates containing (100 µg/mL; LB/Amp100) at 37 °C overnight. LB media (250 mL) in the presence of ampicillin (100 µg/mL) was inoculated with a single colony and grown at 37 °C, 200 rpm overnight. LB media (1 L) was inoculated with the previous seed culture to an OD_{600} of 0.1. The cells were grown at 37 °C, 200 rpm until and OD_{600} of 0.6 was reached. Protein expression was induced with *iso*-propyl-β-D-1-thiogalactopyranoside (IPTG) (final concentration 1 mM) and the temperature was lowered to 20 °C, 200 rpm overnight. The cells were harvested (Thermo Scientific Multicentrifuge X3R) at 4000 x g for 1 h at 4 °C, 6 x 250 rotor. The cell pellets were resuspended in Phosphate Buffered Saline (PBS), centrifuged (Thermo Scientific Multicentrifuge X3R, 5000 x g, 30 min, 4 °C, 8 x 50 rotor) and cell pellets were stored at -20 °C.

HEWT Purification

All purification steps were carried out at 4 °C. Two cell pellets were defrosted on ice and resuspended in binding buffer (KPhos (pH 8, 50 mM), NaCl (100 mM), PLP (0.1 mM) and imidazole (30 mM)). Cell lysis was carried out by sonication (30s on 30s off for 15 cycles) and the cell debris was removed by centrifugation (9500 x g, 45 min, 4 °C, 8 x 50 rotor). The cell free extract was filtered through Millex HA filters (0.45 µm) and loaded onto a pre-equilibrated His Trap Nickel affinity column (5 mL) using an ÄKTA explorer (Cytiva Lifesciences, UK) monitoring at 280 nm. The column was washed with 20 column volumes of binding buffer (KPhos(pH 8, 50 mM), NaCl (100 mM), PLP (0.1 mM) and imidazole (30 mM)). Elution buffer (KPhos (pH 8, 50 mM), NaCl (100 mM), PLP (0.1 mM), imidazole (300 mM)) was applied with a gradient 0 to 100% over 6 min, 5 mL/min. Analaysis by 12% SDS-PAGE suggested fractions for concentration to <1 mL by vivaspin 20 Molecular Weight Cut Off (MWCO) (30 kDa, Sartorius). The concentrated protein sample was loaded onto a Hi Prep™ 16/600 superdex™ S-200 size exclusion column (SEC) (120 mL) pre-equilibrated with gel filtration buffer (KPhos (pH 8, 20 mM), NaCl (150 mM), PLP (0.1 mM)). Elution of the recombinant protein proceeded at flow rate 0.5 mL/min. Nanodrop Protein concentration was determined by nano drop (extinction coefficient *HEWT* was calculated using expasy protparum analysis software $\varepsilon = 60850\ M^{-1}\ cm^{-1}$ at 280 nm, MW monomer = 54 kDa) from an average of 10 readings as 6.95 mg/mL (13 mL protein from 2 L growth culture).

Analysis of the fractions by 12% SDS-PAGE suggested good expression of protein monomer at ≈ 50 kDa

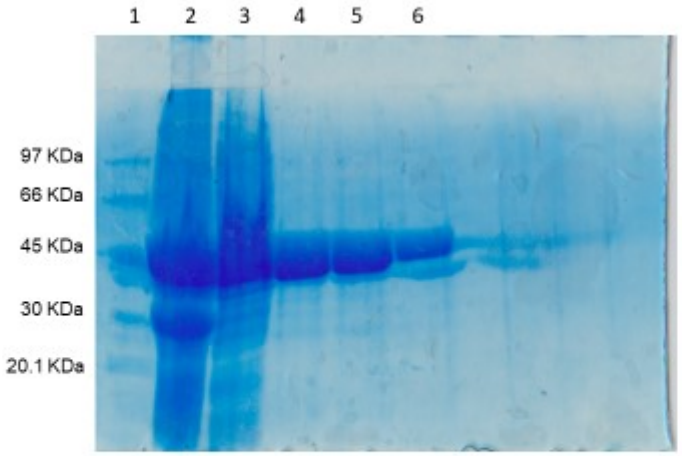


Figure S2: 12% SDS-PAGE of HEWT. 1) Low Molecular Weight (LMW) marker 2) cell pellet 3) cell free extract 4-6) fractions with high absorbance at 280 nm after His Trap.

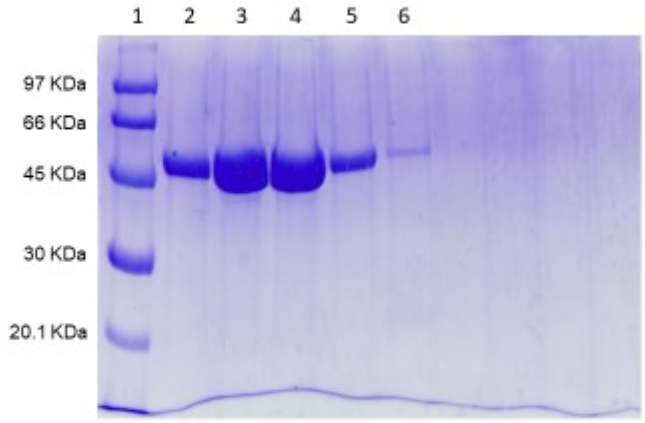
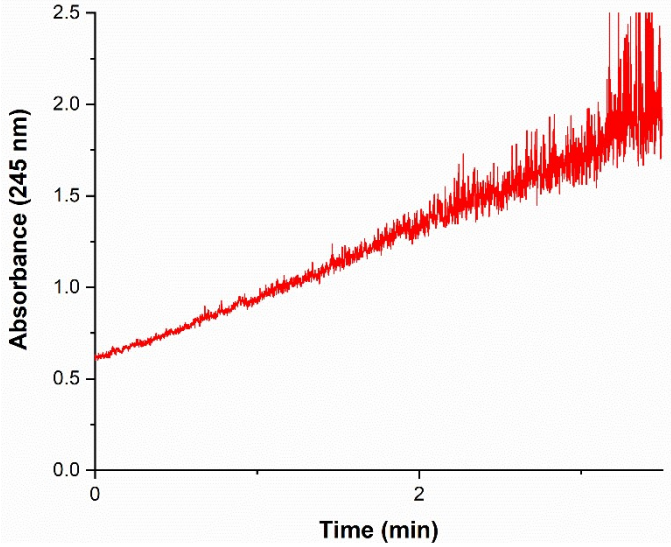


Figure S3: 12% SDS-PAGE analysis after SEC on HRS200 resin. 1) LMW marker 2-5) fractions with high UV/Vis absorbance at 280 nm.

Activity Assay

The activity of *HEWT* was confirmed using the assay described by Schätzle *et al.* which monitors the formation of acetophenone at 245 nm.[3] *HEWT* (0.1 mg/mL), sodium pyruvate (2.5 mM) and (*S*)-MBA **14** (2.5 mM) were added to a cuvette to a final volume 600 µL at 37 °C (Buffer: KPhos (pH 8, 50 mM), DMSO (10%), PLP (0.1 mM)). The rate of formation of acetophenone **12** was linear over the first 2 minutes.

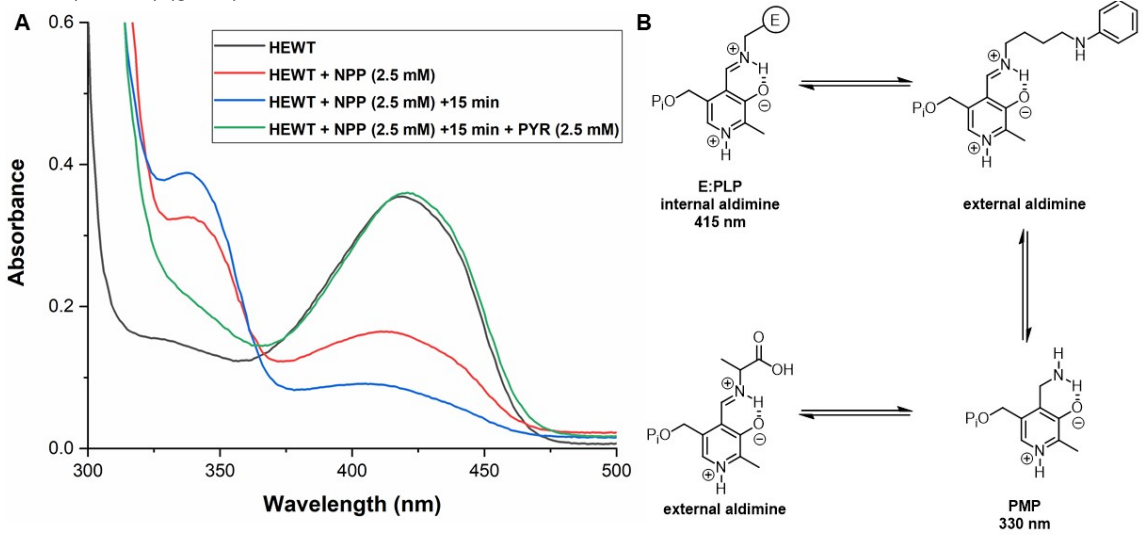
*Figure S4: Production of acetophenone **12** (245 nm) from reaction of methylbenzylamine **14** (2.5 mM) with pyruvate (2.5 mM) at 37 °C catalysed by HEWT.*



***HEWT* binding UV/Vis scans**

The UV/visible spectrum of *HEWT* was recorded after purification by desalting the enzyme using a disposable PD 10 desalting column. The assay was carried out in a CARY UV/Vis spectrometer at 37 °C. An initial spectrum was taken of *HEWT* (35 µM) in HEPES buffer (100 mM, pH 7.5). A second spectrum was recorded upon addition of NPP **9** (6 µL, 0.25 M stock) which was incubated for a further 15 min, after which a third spectrum was recorded. A final spectrum was recorded upon addition of sodium pyruvate (6 µL, 0.25 M).

*Figure S5: Spectra recorded on CARY UV/visible spectrometer at 37 °C. Initial spectrum recorded of desalted HEWT (black), NPP **9** (2.5 mM) addition (red), incubation of NPP **9** and HEWT for 15 min (blue) and addition of sodium pyruvate (2.5 mM) (green).*



2.4.2 *CVTA* Production

2.4.3 Production of recombinant *CVTA*

CVTA Growth & Expression

The clone expressing *Chromobacterium violaceum* TA (*CVTA*) was kindly gifted by the O'Reilly group in a pet28a plasmid with UNIPROT code Q7NW64. The PDB code for the holo structure is 4AH3.[4] The growth, expression and purification procedures were adapted from *CVTA* initial characterisation publication which also contains bioinformatics details.[5] *CVTA* plasmid DNA (2 µL) was transformed into BL21 (DE3) competent cells (10 µL) and selection was carried out on an LB agar plate containing kanamycin (30 µg/mL;LB/Kan 30) incubated overnight at 37 °C. One colony was used to inoculate a seed culture LB media (250 mL) containing kanamycin (30 µg/mL) and incubated overnight at 37 °C, 200 rpm. The seed culture inoculated LB media (1 L) containing kanamycin (30 µg/mL) to an OD_{600} of 0.1. The cells were grown at 37 °C, 200 rpm until an OD_{600} of 0.6-1.0 was achieved. Protein expression was induced by IPTG (final concentration 0.2 mM) and the temperature lowered to 20 °C, 200 rpm overnight. The cells were harvested by centrifugation (Thermo Scientific Multicentrifuge X3R, 4000 x g, 5 min, 4 °C, 6 x 250 rotor). The cell pellets were resuspended in PBS buffer, centrifuged (Thermo Scientific Multicentrifuge X3R, 5000 x g, 30 min, 4 °C, 8 x 50 rotor) and cell pellets were stored at -20 °C.

CVTA Purification

All purification steps were carried out at 4 °C. The cell pellet was defrosted on ice and resuspended in binding buffer (HEPES (pH 7.5, 50 mM), NaCl (300 mM), PLP (0.1 mM), imidazole (20 mM)). Cell lysis was carried out by sonication (30 s on 30 s off, 15 cycles) and the cell debris was collected by centrifugation (Thermo Scientific Multicentrifuge X3R, 9000 x g, 1 h, 4 °C, 8 x 50 rotor). The cell lysate was filtered using Millex HA filters (0.45 µm) and loaded onto a pre-equilibrated His Trap Nickel affinity column (1 mL) using an ÄKTA explorer (Cytiva Lifesciences, UK) monitoring at 280 nm. The column was washed with 20 column volumes of binding buffer (HEPES (pH 7.5, 50 mM), NaCl (300 mM), PLP (0.1 mM), imidazole (20 mM)). Elution buffer (HEPES (pH 7.5, 50 mM), NaCl (300 mM), PLP (0.1 mM), imidazole (200 mM)) was applied with a gradient of 0 to 100 over 20 min, 1 mL/min. Analysis of fractions with high UV/Vis absorbance by 12% SDS-PAGE was completed prior to concentrating the relevant fractions using viva spin MWCO (30 kDa, Sartorius) to <1mL. The concentrated protein was loaded onto a Hi Prep™ 16/600 superdex™ S-200 SEC (120 mL) pre-equilibrated with gel filtration buffer (HEPES (pH 8, 50 mM), NaCl (300 mM), PLP (0.1 mM)). Elution of the recombinant protein proceeded at flow rate 0.5 mL/min. Nanodrop Protein concentration was determined by nano drop (extinction coefficient *HEWT* was calculated using expasy protparum analysis software MW = 80245 M^{-1} cm^{-1} at 280 nm, MW monomer = 51 kDa) from an average of 10 readings as 5.28 mg/mL (6 mL protein from 1 L growth culture).

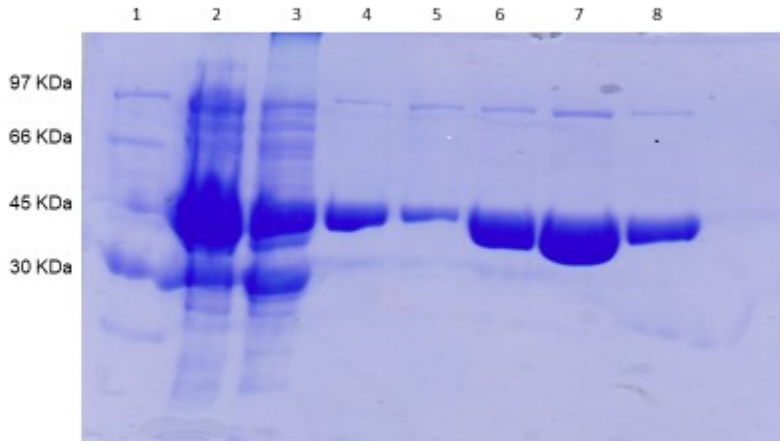


Figure S6: SDS-PAGE analysis of CVTA recombinant protein. 1) LMW marker 2) cell lysate 3) cell pellet 4) fraction from His Trap 5-8) gel filtration fractions.

Analysis of the fractions by 12% SDS-PAGE suggested good expression of protein monomer at ≈ 50 kDa.

Activity Assay

The activity of *CVTA* was confirmed by the assay described by Schätzle *et al.* which monitors the formation of acetophenone at 245 nm.[3] *CVTA* (0.1 mg/mL final concentration), sodium pyruvate (6 µL, 0.25 M) and (*S*)-methylbenzylamine **14** (6 µL, 0.25 M) were added to a cuvette to a final volume 600 µL at 37 °C (Buffer: KPhos (pH 8, 100 mM), DMSO (10%), PLP (0.1 mM)). The rate of formation of acetophenone **12** was linear over the first 30 seconds.

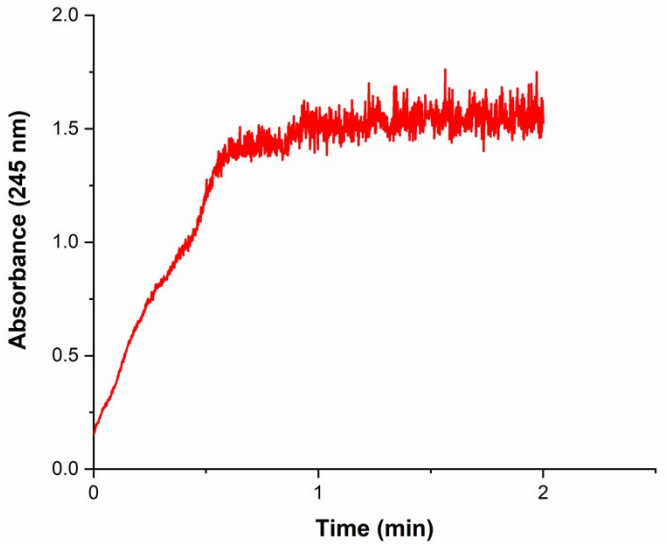


*Figure S7: Production of acetophenone **12** (245 nm) from reaction of methylbenzylamine **14** (2.5 mM) with pyruvate (2.5 mM) at 37 °C catalysed by CVTA.*

***CVTA* NPP binding assay**

The UV/visible spectrum of *CVTA* was recorded after purification by desalting the enzyme using a disposable PD 10 desalting column. The assay was carried out in a CARY UV/Vis spectrometer at rt. An initial spectrum was taken of *CVTA* (36 µM) in KPhos buffer (100 mM, pH 8). A second spectrum was recorded upon addition of NPP **9** (60 µL, 25 mM stock) which was incubated for a further 15 min, after which a third spectrum was recorded. A final spectrum was recorded upon addition of sodium pyruvate (60 µL, 25 mM).

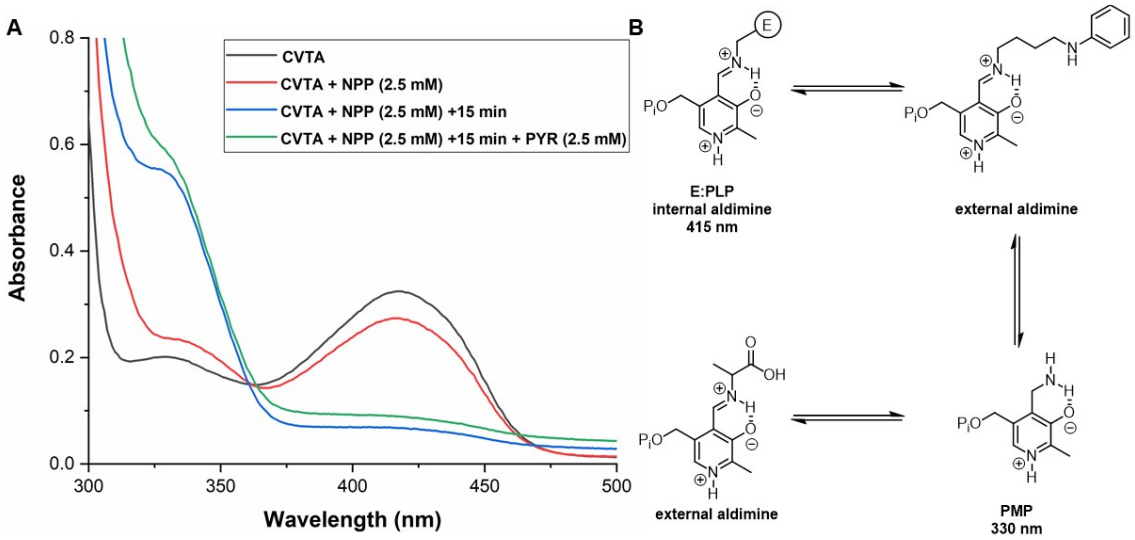
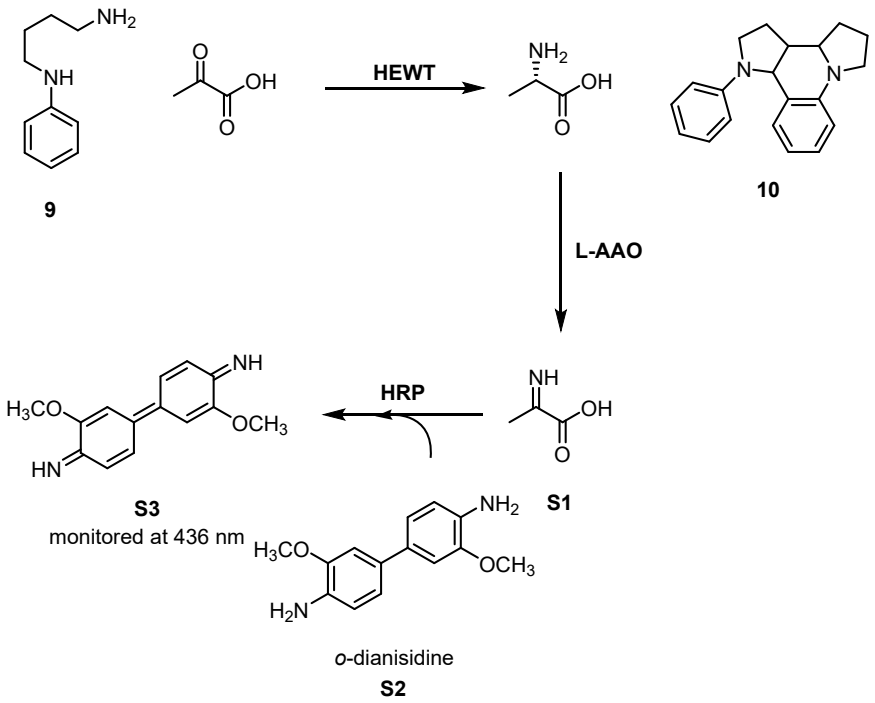


*Figure S8: Spectra recorded on CARY UV/visible spectrometer at 37 °C. Initial spectrum recorded of desalted CVTA (black), NPP **9** (2.5 mM) addition (red), incubation of NPP **9** and CVTA for 15 min (blue) and addition of sodium pyruvate (2.5 mM) (green).*

2.5 General Procedure for L-AAO coupled assay of amine donor with *HEWT*



We adapted the colourimetric assay in Sánchez-Carrón *et. al.,* and Hopwood *et. al.,* to perform kinetics of NPP **9** and methylbenzylamine **14**.[6,7] Analysis was carried out in a 96 well plate, with UV/Vis monitoring at 436 nm, preheated to 37 °C. To each well, L-AAO (280 mU/mL), HRP (10 mU/mL), *o*-dianisidine **S2** (0.1 mg/mL), sodium pyruvate (2.5 mM), *HEWT* (0.055 mg/mL) and NPP **9** (0-20 mM) were added and buffer (KPhos (pH 8, 100 mM) containing DMSO (0.25%) and PLP (1 mM)) was added to a final volume (200 µL). Prior to the addition of enzyme, the plate was shaken for 5 s and upon addition of enzyme, absorbance data was recorded for 2.5 h. The reactions were completed in triplicate and the initial rate was used to calculate the Michaelis–Menten constant.

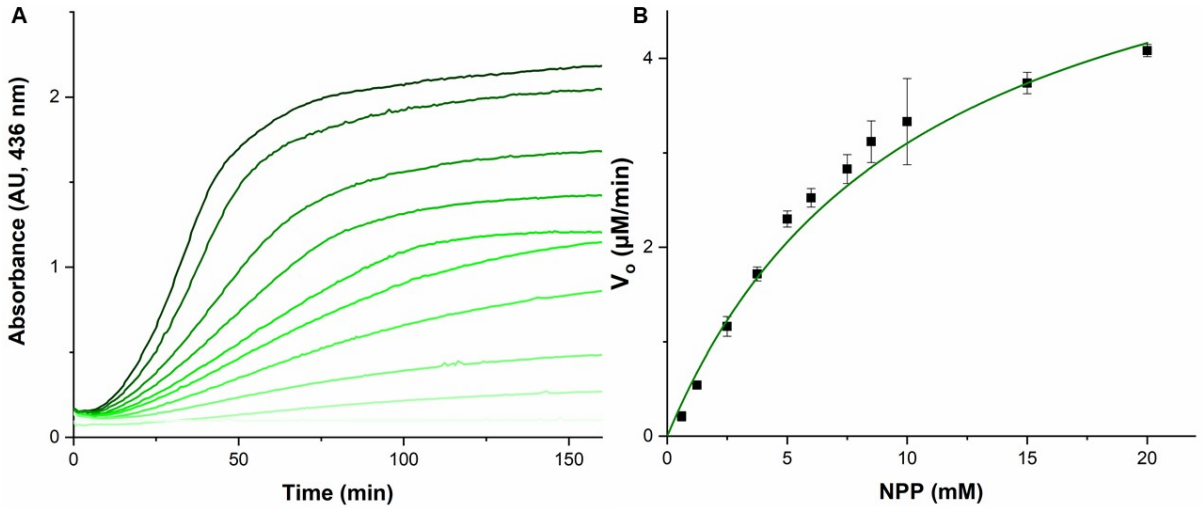


*Figure S9: A) Absorbance of o-dianisidine dye oxidation in the coupled assay of HEWT and L-AAO to determine kinetics of NPP **9** amine donor of increasing concentration from 0-20 mM. B) Michaelis–Menten kinetics of NPP **9** with HEWT and L-AAO coupled assay were 10.4 ± 1.4 mM and V_{max} 6.3 ± 0.4 were obtained. The reactions were carried out in triplicate with the error determined by the standard deviation.*

The assay was also carried out with methylbenzylamine **14** (0-60 mM) as amine donor as a comparison of acceptance of

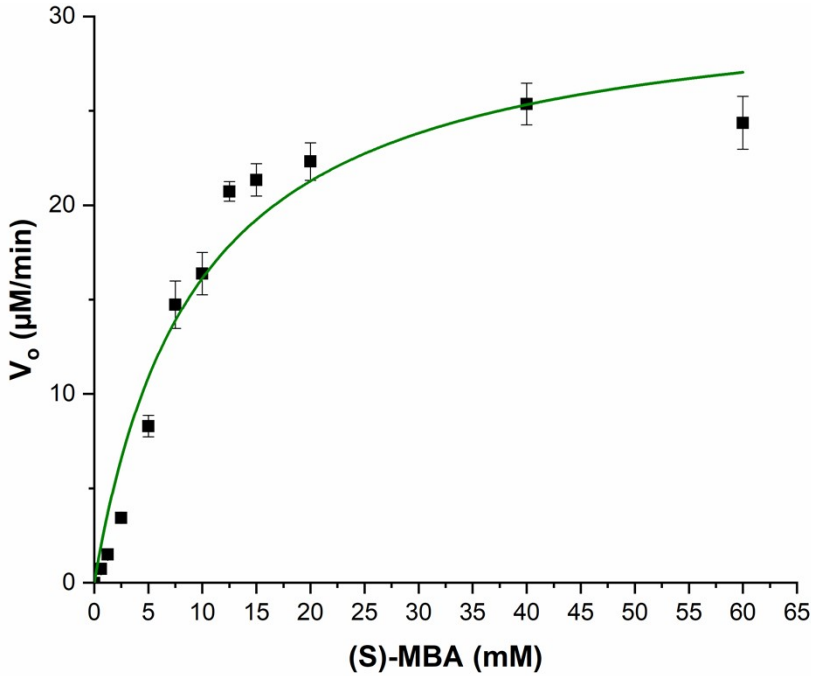


*Figure S10: Michaelis–Menten kinetics of methylbenzylamine **14** with HEWT calculated from the coupled assay with L-AAO were K_M 9.4 ± 2.2 and V_{max} 31.3 ± 2.5 were obtained. The reactions were carried out in triplicate with the error determined by the standard deviation.*

NPP **9** by *HEWT*.

2.6 General HPLC methods

Analysis by reverse phase HPLC consisted of product elution with gradient 15-72% CH_3CN in water (0.1% TFA) at 30 °C over 12 min on a Luna 5 µm C18 column (250 x 4.6 mm, 100 Å) at 210 nm. NPP **9** (RT = 10.6 min).

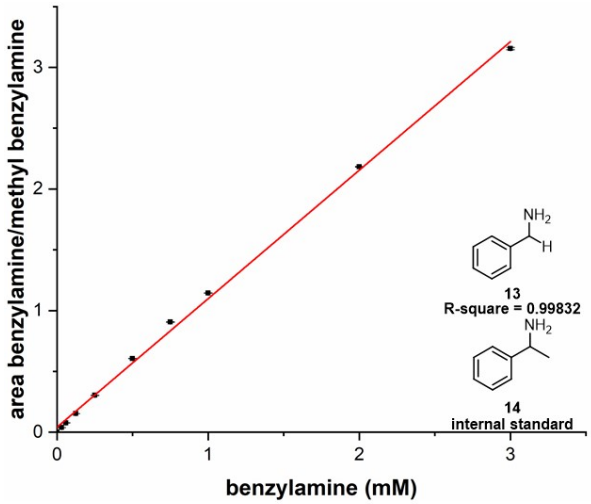
Full method details: HPLC method 0-3 min (5% CH_3CN in water (0.1% TFA)), 3-6 min (5-15% CH_3CN in water (0.1% TFA)), 6-18 min (15-72% CH_3CN in water (0.1% TFA)), 18-21 min (72% CH_3CN in water (0.1% TFA)) and 21-27 min (72-5% CH_3CN in water (0.1% TFA)). Retention time (RT) are listed below for each compound.

Benzylamine **13**

The HPLC assay measuring conversion of benzaldehyde to benzylamine was completed on an older version of the same column with this method: 0-15 min (1-75% CH_3CN in water (0.1% TFA)), 15-20 min (75-65% CH_3CN in water (0.1% TFA)) followed by 65% CH_3CN in water (0.1% TFA) for another 10 min. HPLC yield of benzylamine **13** production was calculated from an average of the area under the benzylamine **13** peak/area under methylbenzylamine **14** peak for the three samples using the equation from the calibration. (Figure S11) The retention times recorded under this method were benzaldehyde **11** RT = 17.10min, benzylamine **13** RT = 4.60 min MBA **14** RT = 5.90 min and NPP **9** RT = 3.60 min.

Retention times recorded with the newer column and reverse phase HPLC fitted with a column oven are benzylamine **13** (RT = 11.2 min) and benzaldehyde **11** (RT = 18.9 min).

*Figure S11: Graph of calibration of C18 column of benzylamine **13** with methylbenzylamine **14** (1 mM) internal standard. Values obtained were taken from an average of three samples at each concentration with the error determined as the standard deviation.*



Methylbenzylamine **14**

A calibration curve of known concentrations of methylbenzylamine **14** (0-3 mM) with benzylamine **13** internal standard was produced by HPLC (Figure S12). Methylbenzylamine **14** (RT = 12.8 min) and acetophenone **12** (RT = 19.3 min).

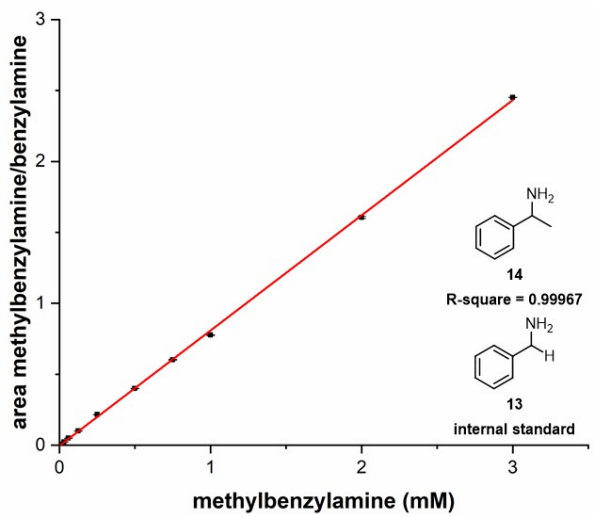


*Figure S12: Methylbenzylamine **14** calibration curve produced by HPLC analysis of methylbenzylamine **14** concentrations 0-3 mM and benzylamine **13** (1 mM) used as internal standard. Values obtained were taken from an average of three samples at each concentration with the error determined as the standard deviation.*

p-Methoxy methylbenzylamine **16**

A calibration curve of known concentrations of *p*-methoxy methylbenzylamine **16** (0-3 mM) with benzylamine **13** internal standard was produced by HPLC (Figure S13). *p*-Methoxy methylbenzylamine **16** (RT = 13.6 min) and *p*-methoxy acetophenone **15** (RT = 19.3 min).

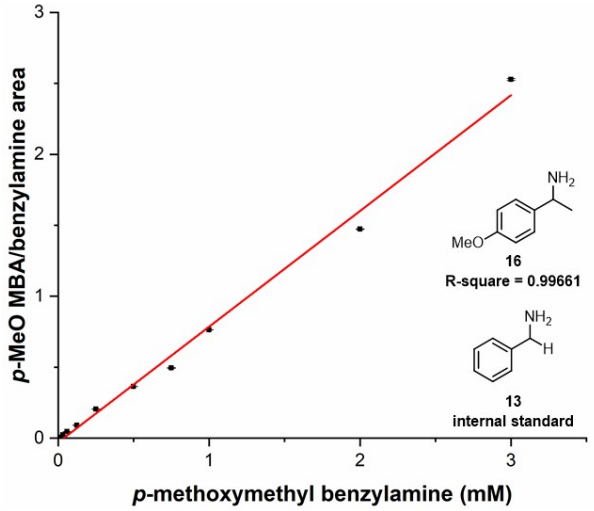


*Figure S13: Calibration curve for the quantification of p-methoxy methylbenzylamine **16** by HPLC with benzylamine **13** (1 mM) internal standard. Values obtained were taken from an average of three samples at each concentration with the error determined as the standard deviation.*

Indanamine **18**

A calibration curve of known concentrations of indanamine **18** (0-3 mM) with benzylamine **13** internal standard was produced by HPLC (Figure S14). Indanamine **18** (RT = 13.2 min) and 1-indanone **17** (RT = 18.5 min).

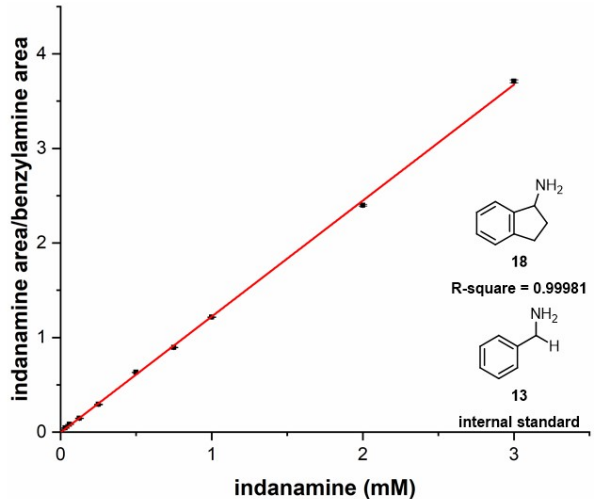


*Figure S14: Calibration for the quantification of indanamine **18** by HPLC with benzylamine **13** (1 mM) as internal standard. Values obtained were taken from an average of three samples at each concentration with the error determined as the standard deviation.*

Sitagliptin **20**

A calibration curve of known concentrations of sitagliptin **20** (0-3 mM) with methyl benzylamine **14** internal standard was produced by HPLC (Figure S15). Sitagliptin **20** (RT = 15.9 min), presitagliptin ketone **19** (RT = 19.7 min).

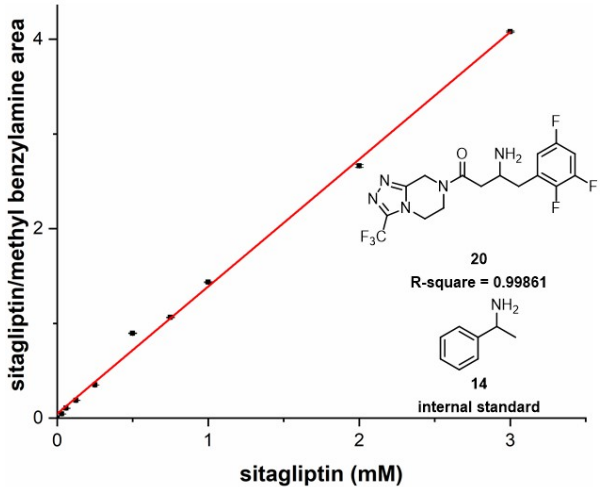
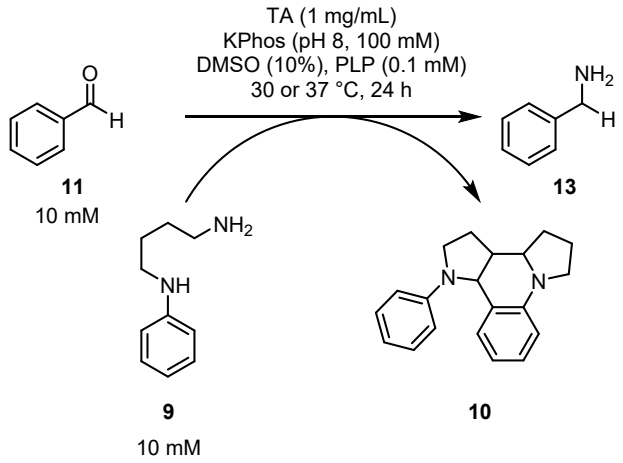
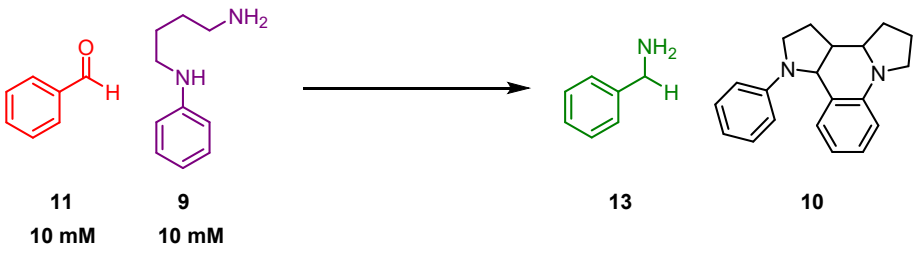


*Figure S15: Calibration for the quantification of sitagliptin **20** with methylbenzylamine **14** as internal standard. Values obtained were taken from an average of three samples at each concentration with the error determined as the standard deviation.*

2.7 General procedure for benzaldehyde 11 reaction with TAs



Benzaldehyde **11** (50 µL, 100 mM), NPP **9** (50 µL, 100 mM) and TA (50 µL, 10 mg/mL) were added to an Eppendorf (1.5 mL) and KPhos buffer (350 µL, pH 8, 100 mM) containing PLP (0.1 mM) and DMSO (10%) was added. For each TA, the reaction was set up in triplicate at 240 rpm at 37 °C for 24 h. An aliquot (200 µL) was removed, quenched with trifluoracetic acid (TFA) (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard (*S*)-methylbenzylamine **14** (4 µL, 100 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.



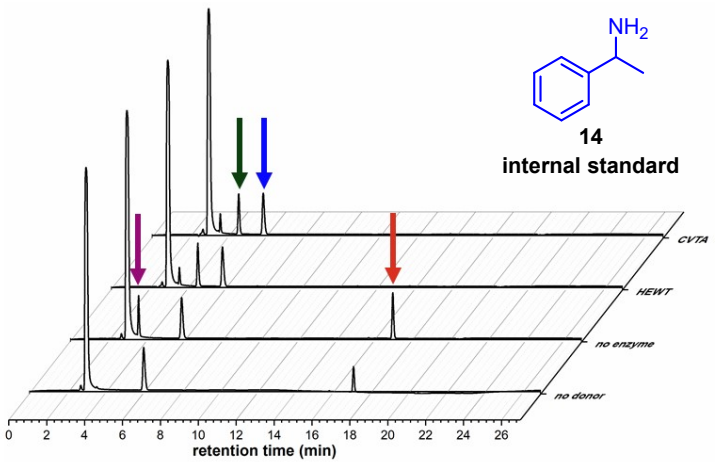


*Figure S16: HPLC chromatograms of the transamination of benzaldehyde **11** with HEWT and NPP. Amine donor NPP **9** (purple), benzylamine **13** (green), methylbenzylamine **14** (blue) as internal standard and benzaldehyde **11** (red). After 24 h, no benzaldehyde was detected in the wild-type reactions.*

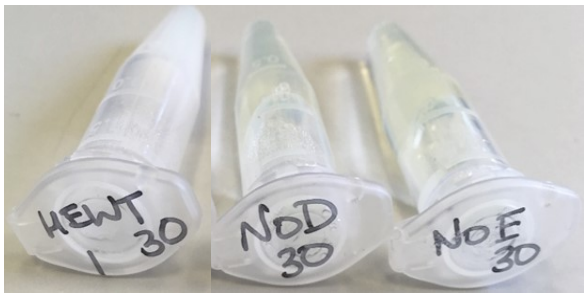
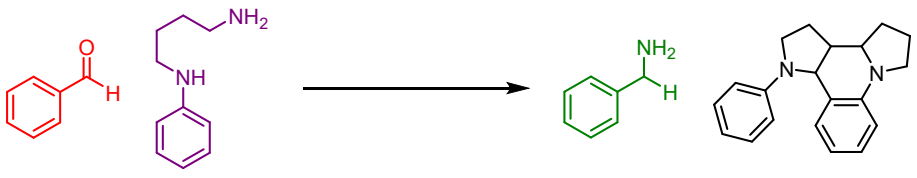


*Figure S17: Photograph of the screen of HEWT-catalysed transamination of benzaldehyde **11** with NPP **9** after 24 h. Left to right: HEWT transamination of benzaldehyde **11** by HEWT with NPP **9** as amine donor. No D is a control of the transamination with no NPP amine donor present. No E is a control of the transamination reaction with no enzyme.*



11 **9** **13** **10**

10 mM **10 mM**

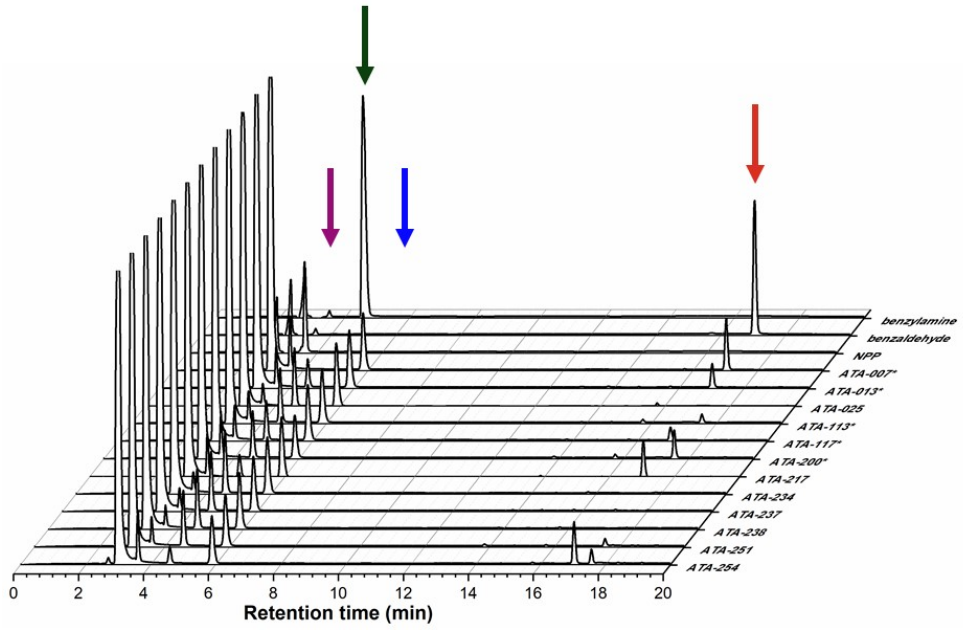


*Figure S18: HPLC chromatograms from the transamination of benzaldehyde **11** (red) with Codexis TAs, and NPP **9** (purple) amine donor after 24 h. Benzylamine **13** (green) and internal standard methylbenzylamine **14** (blue).*

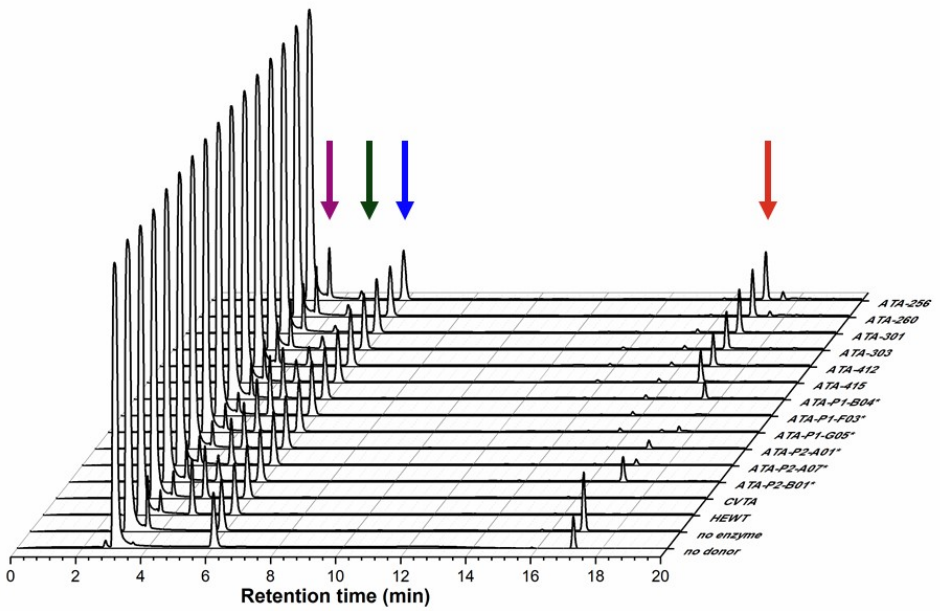
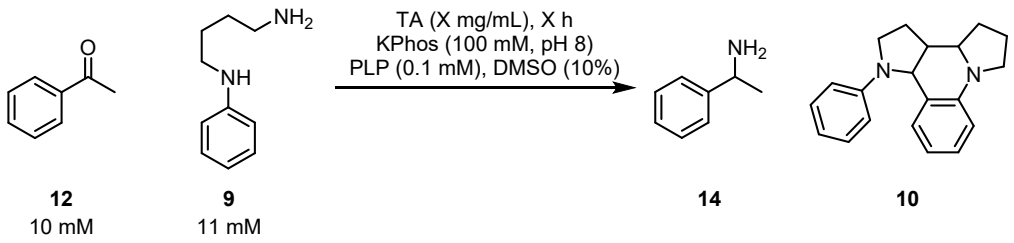


*Figure S19: HPLC chromatograms from the transamination of benzaldehyde **11** (red) with Codexis TAs, CVTA, HEWT and NPP **9** (purple) amine donor after 24 h. Benzylamine **13** (green) and internal standard methylbenzylamine **14** (blue). Two control reactions are included, one with no enzyme and the other with no donor.*

*Table 1: HPLC yields of benzylamine **13** from the transamination of benzaldehyde **11** with a variety of TAs with NPP **9** as amine donor in triplicate. Errors were determined by standard deviation.*

Transaminase	Benzylamine HPLC yield (%)	Error (±%)
ATA-007	0	0
ATA-013	29.9	2.9
ATA-025	53.3	1.4
ATA-113	58.7	1.5
ATA-117	40.6	1.0
ATA-200	65.7	0.5
ATA-217	19.8	1.0
ATA-234	64.1	0.8
ATA-237	63.1	0.1
ATA-238	63.7	1.7
ATA-251	63.2	1.3
ATA-254	18.2	1.4
ATA-256	13.3	5.5
ATA-260	10.1	1.0
ATA-301	3.1	0.3
ATA-303	9.4	0.1
ATA-412	16.2	0.4
ATA-415	21.2	1.0
ATA-P1-B04	55.4	3.7
ATA-P1-F03	62.7	0.4
ATA-P1-G05	61.1	2.0
ATA-P2-A01	49.4	0.4
ATA-P2-A07	49.8	0.8
ATA-P2-B01	25.5	0.5
CVTA	58.4	2.1
HEWT	60.8	2.3

2.8 Optimisation of acetophenone 12 transamination conditions



12 10 mM | **9** 11 mM | **14** | **10**

Acetophenone **12** (100 µL, 50 mM in 50% DMSO), NPP **9** (50 µL, 110 mM) and TA (1 mg/mL final: 50 µL, 10 mg/mL; 2 mg/mL final: 100 µL, 10 mg/mL) were added to an Eppendorf (1.5 mL) and HEPES buffer (1 mg/mL final: 300 µL; 2 mg/mL final: 250 µL; pH 7.5, 100 mM) containing PLP (0.1 mM) was added. For each TA, the reaction was set up in triplicate, at 240 rpm, 37 °C (ATA-256) and 30 °C (ATA-113) for 24 or 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard benzylamine **13** (4 µL, 100 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

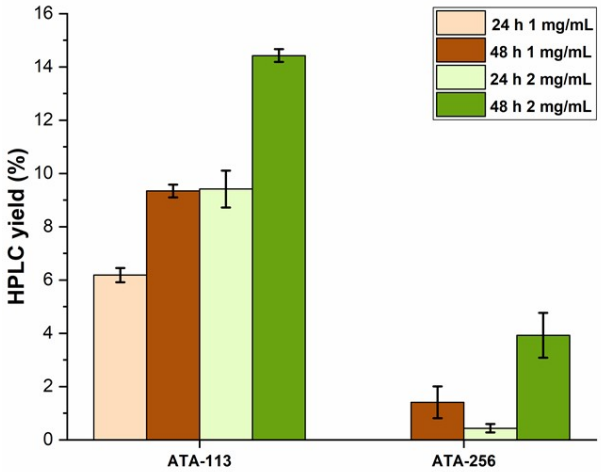
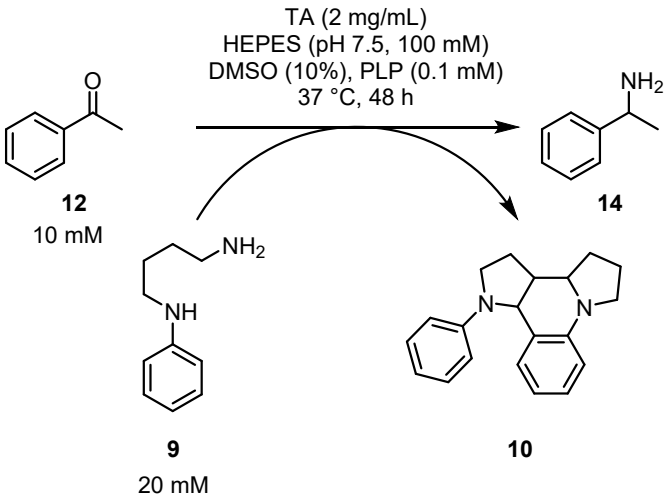
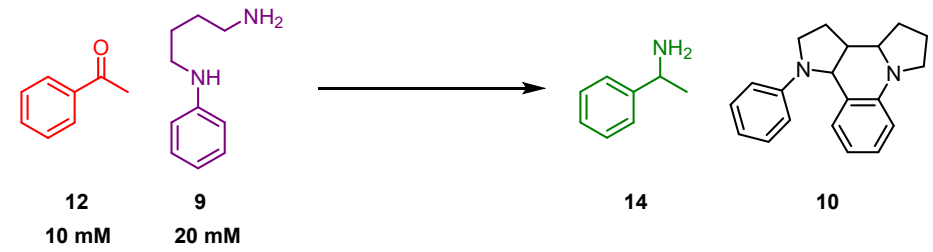


*Figure S20: Methylbenzylamine **14** HPLC yield calculated from reactions completed in triplicate at 1 mg/mL enzyme for 24 h (pale orange), 1 mg/mL 48 h (dark orange), 2 mg/mL for 24 h (pale green) and 2 mg/mL for 48 h (dark green). Reactions were carried out in triplicate and errors determined by standard deviation.*

2.9 General procedure for acetophenone 12 screening with TAs



Acetophenone **12** (50 µL, 100 mM in DMSO), NPP **9** (100 µL, 100 mM) and TA (100 µL, 10 mg/mL) were added to an Eppendorf (1.5 mL) and HEPES buffer (250 µL, pH 7.5, 100 mM) containing PLP (0.1 mM) was added. For each TA, the reaction was set up in triplicate at 240 rpm, 37 °C for 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard benzylamine **13** (4 µL, 100 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.



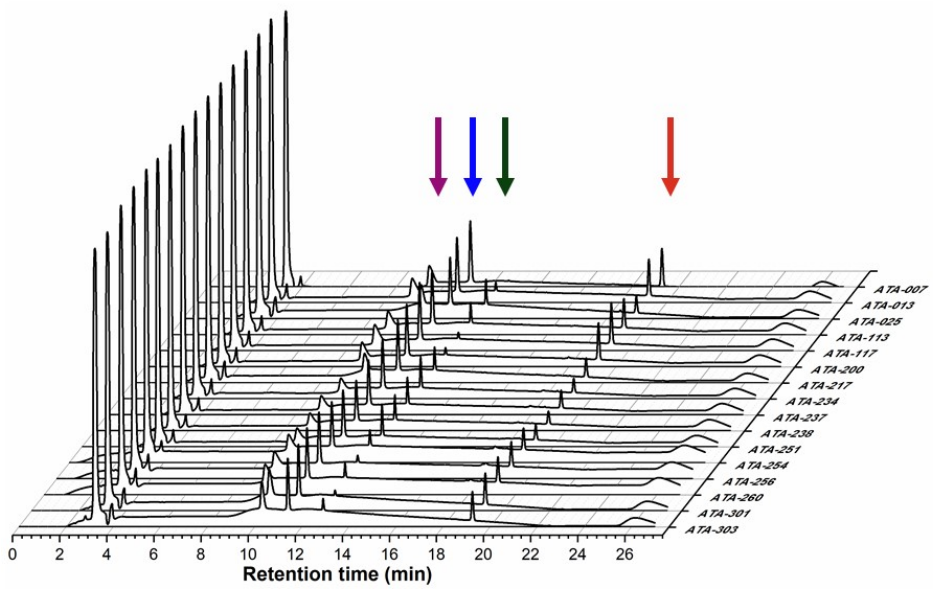


*Figure S21: HPLC chromatograms from the transamination reactions of the detailed TAs with acetophenone **12** (red) as amine acceptor, NPP **9** (purple) as amine donor, benzylamine **13** (blue) as internal standard. Methylbenzylamine **14** (green) was the observed product.*

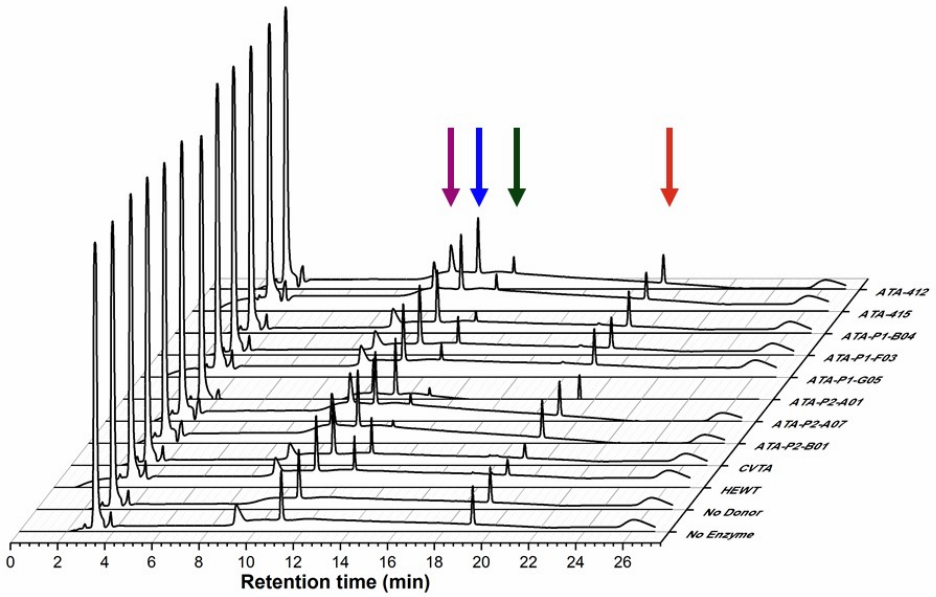


*Figure S22: HPLC chromatograms from the transamination reactions of the detailed TAs with acetopheone **12** (red) as amine acceptor, NPP **9** (purple) as amine donor, benzylamine **13** (blue) as internal standard. Methylbenzylamine **14** (green) was the observed product. Two control reactions were also included, one with no enzyme and the other with no donor.*

*Table 2: HPLC yield of methylbenzylamine **14** from the transamination of acetophenone **12** by a variety of TAs with NPP **9** as amine donor. Reactions were carried out in triplicate and errors determined by standard deviation.*

Transaminase	**Methylbenzylamine HPLC yield (%)**	**Error (±%)**
ATA-007	0.8	0.4
ATA-013	11.1	0.5
ATA-025	38.8	1.3
ATA-113	25.5	0.9
ATA-117	8.0	0.4
ATA-200	9.9	0.2
ATA-217	34.1	0.6
ATA-234	47.6	2.0
ATA-237	39.3	2.2
ATA-238	46.5	1.1
ATA-251	49.7	1.8
ATA-254	25.4	0.3
ATA-256	10.4	0.7
ATA-260	19.7	1.1
ATA-301	7.7	0.3
ATA-303	16.2	0.4
ATA-412	20.9	0.8
ATA-415	21.7	0.6
ATA-P1-B04	19.9	0.4
ATA-P1-F03	31.6	0.5
ATA-P1-G05	14.3	0.1
ATA-P2-A01	11.2	1.2
ATA-P2-A07	16.1	0.6
ATA-P2-B01	8.6	0.5
CVTA	51.2	3.2
HEWT	49.2	1.4

2.10 Temperature screen of acetophenone 12 transamination with NPP

Acetophenone **12** (100 µL, 50 mM in 50% DMSO), NPP **9** (100 µL, 100 mM) and ATA-251 (100 µL, 10 mg/mL) were added to an Eppendorf (1.5 mL) and KPhos buffer (250 µL, pH 8, 100 mM) containing PLP (0.1 mM) was added. For each TA, the reaction was set up in triplicate and shaken at 240 rpm, RT, 30, 37 and 45 °C for 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard benzylamine **13** (4 µL, 100 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

*Table 3: HPLC yield of methylbenzylamine **14** from the transamination of acetophenone **12** with 4 TAs with NPP **9** as amine donor at different temperatures. Reactions were carried out in triplicate and errors determined by standard deviation.*

Enzyme	HPLC yield (%)							
	20 °C	Error (±)	30 °C	Error (±)	37 °C	Error (±)	45 °C	Error (±)
HEWT	24.9	0.6	25.5	0.2	25.0	1.1		
CVTA	23.3	1.1	27.2	0.4	31.7	0.6		
ATA-234	14.2	0.3	18.4	0.8	20.9	0.1	21.2	0.8
ATA-251	16.7	1.3	19.5	1.1	22.7	1.5	21.8	0.9

2.11 pH screen of acetophenone 12 transamination with NPP

Acetophenone **12** (100 µL, 50 mM in 50% DMSO), NPP **9** (50 µL, 100 mM) and ATA-251 (100 µL, 10 mg/mL in 5 mM HEPES pH 8) were added to an Eppendorf (1.5 mL) and buffer (250 µL, 200 mM (KPhos (pH 6-7.4), HEPES (pH 7.5-8.5), CAPS (pH 8.5-11)), PLP (0.1 mM) were added. The reaction was set up in triplicate at 240 rpm, 37 °C for 48 h. An aliquot (200 µL) was removed and quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard benzylamine **13** (4 µL, 100 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

*Table 4: HPLC yield of methylbenzylamine **14** from the transamination of acetophenone **12** with ATA-251 with NPP **9** as amine donor at different pH. Reactions were carried out in triplicate and errors determined by standard deviation.*

Buffer	pH	HPLC yield (%)	Error (±)
KPhos	6.0	32.4	0.9
	6.4	36.6	0.7
	7.0	35.8	0.5
	7.4	32.4	1.5
HEPES	7.5	35.8	0.5
	8.0	31.2	0.8
	8.5	25.5	0.9
CAPS	8.5	29.9	1.5
	9.0	24.5	0.4
	9.5	21.5	0.7
	10.0	20.0	0.4
	10.5	20.9	0.7
	11.0	20.5	1.4

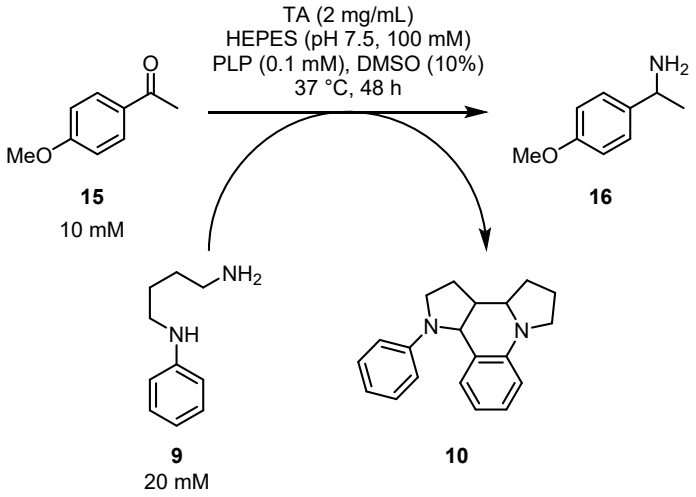
2.12 Experimental procedure for methylbenzylamine 14 production with NPP 9 and NPP hydrochloride salts

Acetophenone **12** (50 µL, 100 mM in DMSO), NPP **9** either as a free base or salt (100 µL, 100 mM), and ATA-251 (100 µL, 10 mg/mL) were added to an Eppendorf (1.5 mL) and HEPES buffer (250 µL, pH 7.5, 100 mM) containing PLP (0.1 mM) was added. The reaction was set up in triplicate at 240 rpm, 37 °C for 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard benzylamine **13** (4 µL, 100 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

*Table 5: HPLC yield of methylbenzylamine **14** from the transamination of acetophenone **12** with NPP **9** and NPP hydrochloride salts. Reactions were carried out in triplicate and errors determined by standard deviation.*

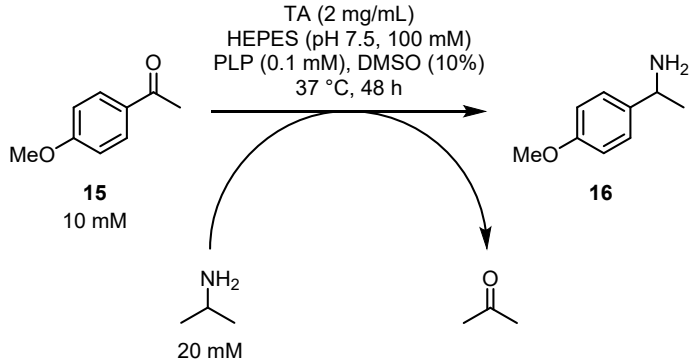
	Methylbenzylamine HPLC yield (%)	Error (±)
NPP	45.4	0.8
NPP•1HCl	47.1	0.8
NPP•2HCl	48.3	0.6
NPP•nHCl	48.1	0.9

2.13 General procedure for the screening of TA activity with *p*-methoxyacetophenone 15 with NPP 9



p-Methoxyacetophenone **15** (50 µL, 100 mM in DMSO), NPP **9** (100 µL, 100 mM) and TA (100 µL, 10 mg/mL) were added to an Eppendorf (1.5 mL) and HEPES buffer (250 µL, pH 7.5, 100 mM) containing PLP (0.1 mM) was added. For each TA, the reaction was set up in triplicate, Eppendorf tube was sealed with parafilm and shaken at 240 rpm, 37 °C for 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard benzylamine **13** (4 µL, 100 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

2.14 General procedure for the screening of TA activity with *p*-methoxyacetophenone 15 with *iso*-propylamine

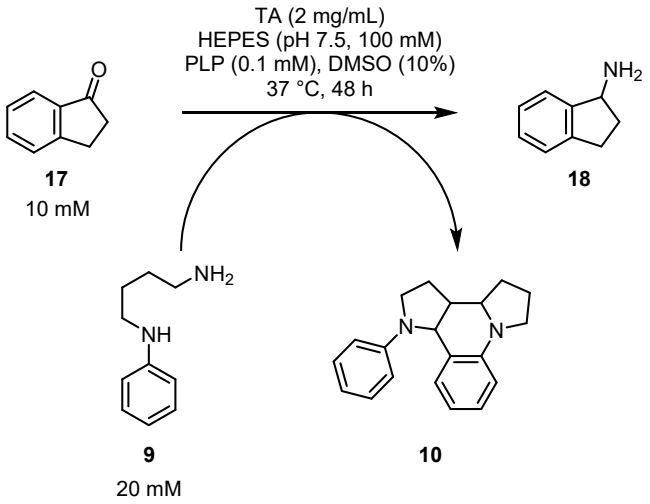


p-Methoxyacetophenone **15** (50 µL, 100 mM in DMSO), *iso*-propylamine (*i*-PrNH$_2$) (100 µL, 100 mM) and TA (100 µL, 10 mg/mL) were added to an Eppendorf (1.5 mL) and HEPES buffer (250 µL, pH 7.5, 100 mM) containing PLP (0.1 mM) was added. For each TA, the reaction was set up in triplicate, Eppendorf tube was sealed with parafilm and shaken at 240 rpm, 37 °C for 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard benzylamine **13** (4 µL, 100 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

*Table 6: p-methoxymethyl benzylamine **16** HPLC yields from transamination of p-methoxy acetophenone **15** with 3 engineered and 1 wild-type TA with NPP **9** vs i-PrNH$_2$ as amine donor. Reactions were carried out in triplicate and errors determined by standard deviation.*

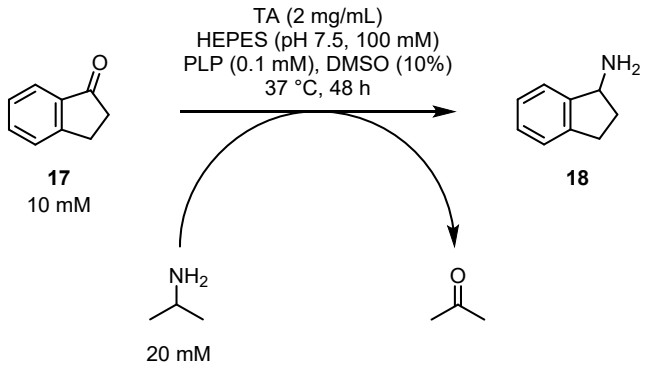
	***p*-methoxymethyl benzylamine HPLC yield (%)**			
Transaminase	**NPP**	**Error (±)**	***i*-PrNH$_2$**	**Error (±)**
ATA-234	25.0	1.1	6.3	0.1
ATA-238	18.9	1.1	4.9	0.1
ATA-251	21.3	0.7	12.7	0.3
HEWT	16.0	0.2	9.5	0.2

2.15 General procedure for the screening of TA activity with 1-indanone 17 with NPP 9



1-Indanone **17** (50 µL, 100 mM in DMSO), NPP **9** (100 µL, 100 mM) and TA (100 µL, 10 mg/mL stock) were added to an Eppendorf (1.5 mL) and HEPES buffer (250 µL, pH 7.5, 100 mM) containing PLP (0.1 mM) was added. For each TA, the reaction was set up in triplicate, Eppendorf tube was sealed with parafilm and shaken at 240 rpm, 37 °C for 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard benzylamine **13** (4 µL, 100 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

2.16 General procedure for the screening of TA activity with 1-indanone 17 with *i*-PrNH$_2$

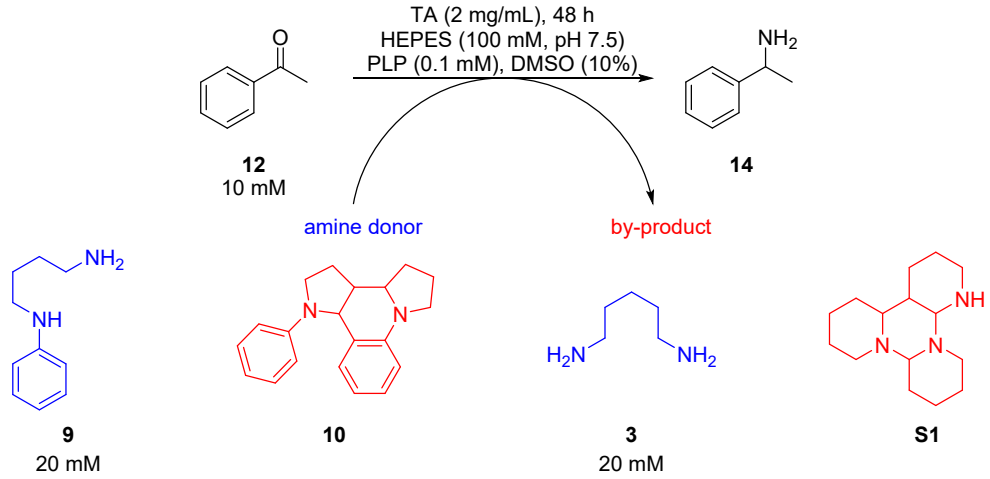


1-Indanone **17** (50 µL, 100 mM in DMSO), *i*-PrNH$_2$ (100 µL, 100 mM) and TA (100 µL, 10 mg/mL stock) were added to an Eppendorf (1.5 mL) and HEPES buffer (250 µL, pH 7.5, 100 mM) containing PLP (0.1 mM) was added. For each TA, the reaction was set up in triplicate, Eppendorf tube was sealed with parafilm and shaken at 240 rpm, 37 °C for 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard benzylamine **13** (4 µL, 100 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

*Table 7: Indanamine **18** HPLC yields from transamination of 1-indanone **17** with 3 engineered and 1 wild-type TA with NPP **9** vs i-PrNH$_2$ as amine donor. Reactions were carried out in triplicate and errors determined by standard deviation.*

	Indanamine HPLC yield (%)			
Transaminase	**NPP**	**Error (±)**	***i*-PrNH$_2$**	**Error (±)**
ATA-234	8.3	0.2	3.0	0.1
ATA-238	7.7	0.4	1.9	0.1
ATA-251	12.9	0.5	5.4	0.2
HEWT	8.0	0.6	4.6	0.1

2.17 General procedure for the screening of TA activity with acetophenone 12 and either cadaverine 3 or NPP 9 as amine donor



Acetophenone **12** (50 µL, 100 mM in DMSO), and either NPP **9** or cadaverine **3** (100 µL, 100 mM) were added to an Eppendorf (1.5 mL) with each TA (100 µL, 10 mg/mL stock (ATA-234, ATA-238, ATA-251 or *HEWT*)) and HEPES buffer (250 µL, pH 7.5, 100 mM) containing PLP (0.1 mM). For each TA, the reaction was set up in triplicate, Eppendorf tube was sealed with parafilm and shaken at 240 rpm, 37 °C for 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard benzylamine **13** (4 µL, 100 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

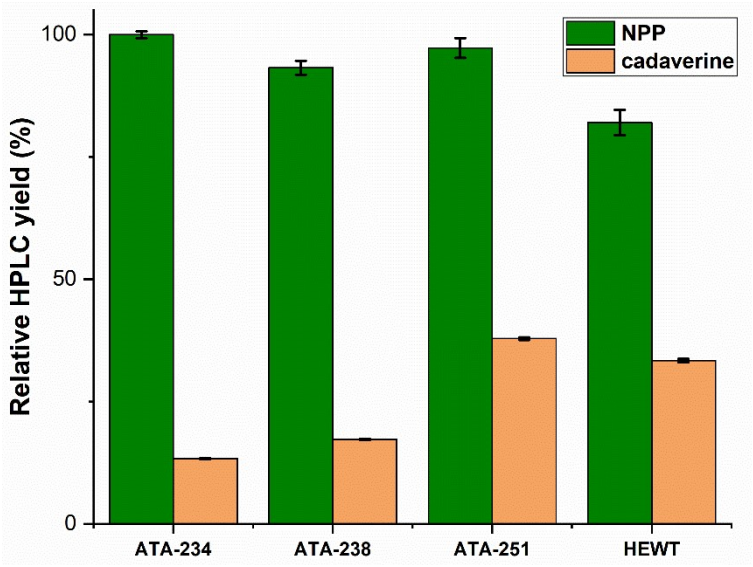


*Figure S23: Comparison of the transamination of acetophenone **12** by four TAs (ATA-234. 238, 251 and HEWT) using either cadaverine **3** or NPP **9** as amine donor. Reactions were carried out in triplicate and the error determined by the standard deviation.*

2.18 Amine donor comparison

Amine donor	*Availability (Sigma–Aldrich, 11/08/21)*	*Handling*	*Excess required*	*Demonstrated application with TAs*	*By-product*	*Applications*	*Limitations*	*Ref.*
N-phenylputrescine (NPP)	2 step synthesis from Aniline 1L £116.00, 4-chlorobutryonitrile 500 g £234.00)	Can be prepared and used as a salt	Excellent yields can be obtained with 1.1–2 eq.	Activity shown with 23 commercial TAs and 2 wild-type TAs	Precipitates from reaction and easily removed by filtration	Screening, preparative scale TA reactions	Not currently commercially available	
L-alanine	CAS: 56-41-7 (≥99%) £116.00 for 1 kg	Easily handled solid	Typically, 5–10 eq. used[10]	Widely accepted by TAs	Coupled enzymatic systems often required for pyruvate removal	TA reactions at scale or exploring kinetics using NADH dependant coupled system.	Multiple enzymes can increase cost & cooperative reaction conditions required	Shin & Kim[8]
iso-propylamine (*i*-$PrNH_2$)	CAS: 75-31-0 (≥99.5%) £293.00 for 18 L	Liquid	Large excess typically required	TA engineering often required	Acetone evaporation	Preparative scale TA reactions	Unsuitable for use in the production of volatile amines	Savile *et. al.*[9]
o-xylyenediamine dihydrochloride (OXDA)	CAS: 21294-14-4 (≥98%) £108.00 for 5 g	Easily handled solid	Excellent conversions obtained with stoichiometric quantities	Activity shown with 27 commercial TAs	Blue/black coloured polymer	Visual screening of liquid assays and colonies on agar	Expensive, polymer removal complicates use at scale	Green *et. al.*[10]
cadaverine	CAS: 462-94-2 (≥97%) £604.00 for 50 mL	Smelly, hygroscopic liquid	Excellent GC yields obtained using 1.3–3 eq.	Activity demonstrated with ATA-256	Trimeric by-product	Preparative scale TA reactions	High pH required	Gomm *et. al.*[11]
2-(4-nitrophenyl) ethan-1-amine hydrochloride	CAS: 29968-78-3 (95%) £82.10 for 5 g	Easily handled solid	Minimum of 2 eq.	Activity demonstrated with a variety of TAs identified by metagenomic screening	Red precipitate	Visual screening	Excess donor required. Conversions only determined for aldehyde substrates	Baud *et. al.*[12]
n-butylamine	CAS: 109-73-9 (99.5%) £88.40 for 2.5 L	Liquid	Excellent GC yields with 1.5 eq.	spuC wild-type TA and 2 commercial TAs	Coupled with aldehyde dehydrogenase to remove 1-butanal	Screening (NADH) by UV-Vis	Coupled system required	Slabu *et. al.*[13]
3-aminocyclohexa-1,5-dienecarboxylic acid	No reported synthesis Commercially available at publication	unknown	Excellent conversions obtained with stoichiometric quantities	Variants of *CVTA* 3 commercial TAs	Alcohol tautomerises to ketone	Preparative scale TA reactions	Not commercially available By-product removal required	Wang *et. al.*[14]
2-aminoethylaniline (2-AEA)	2 step synthesis from 2-Nitrophenylacetonitrile CAS: 610-66-2 (98%) £86.30 for 10 g	Liquid	Good conversions obtained with 2 eq.	7 wild-type TAs and 2 commercial TAs	Indole reacts with Ehrlich's reagent to give orange/red colour	Screening by UV-Vis and of colonies on agar	Not currently commercially available Multicomponent system	Cairns *et. al.*[15]
methylbenzylamine (MBA)	CAS: 2627-86-3 (98%) £79.80 for 100 g	Liquid	Good yields obtained with 2–4 eq.	Numerous examples with wild-type and commercial TAs	acetophenone	Screening by UV-Vis Preparative scale reactions	By-product removal required	Busto *et. al.*[16]

2.19 General procedure for the screening of TA activity with presitagliptin ketone 19 with NPP 9

Presitagliptin ketone **19** (100 µL, 50 mM in DMSO), NPP **9** (50 µL, 110 mM) and TA (100 µL, 10 mg/mL) were added to an Eppendorf (1.5 mL) and KPhos buffer (250 µL, pH 8, 100 mM) containing PLP (0.1 mM) was added. For each TA, the reaction was set up in triplicate at 240 rpm, 37 °C for 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard methylbenzylamine **14** (4 µL, 150 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

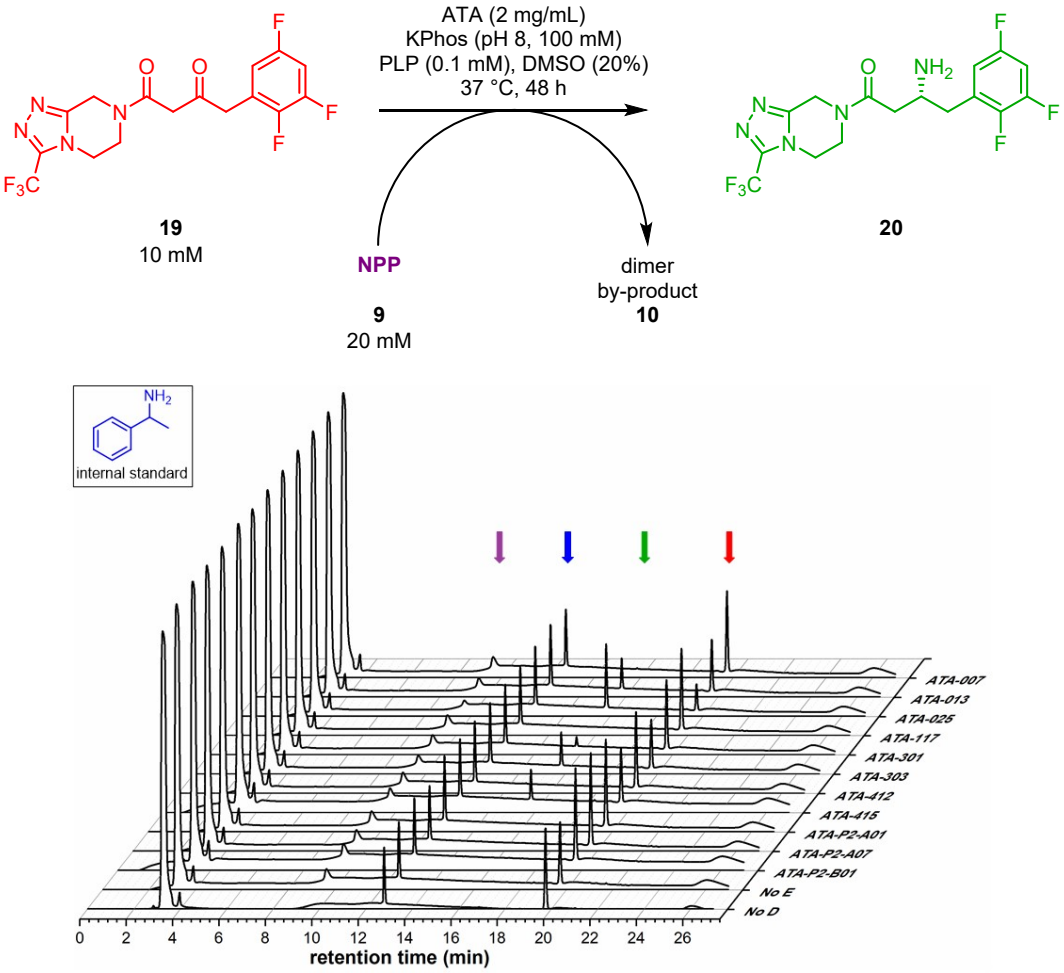


*Figure S24: HPLC chromatograms from the TAs screened with presitagliptin ketone **19.** NPP **9** (purple), presitagliptin ketone **19** (red), sitagliptin **20** (green) and methylbenzylamine **14** (blue) as internal standard. The incubation of ATA-025 shows largest peak of sitagliptin **20** production in conjunction with biggest decrease of ketone **19**.*

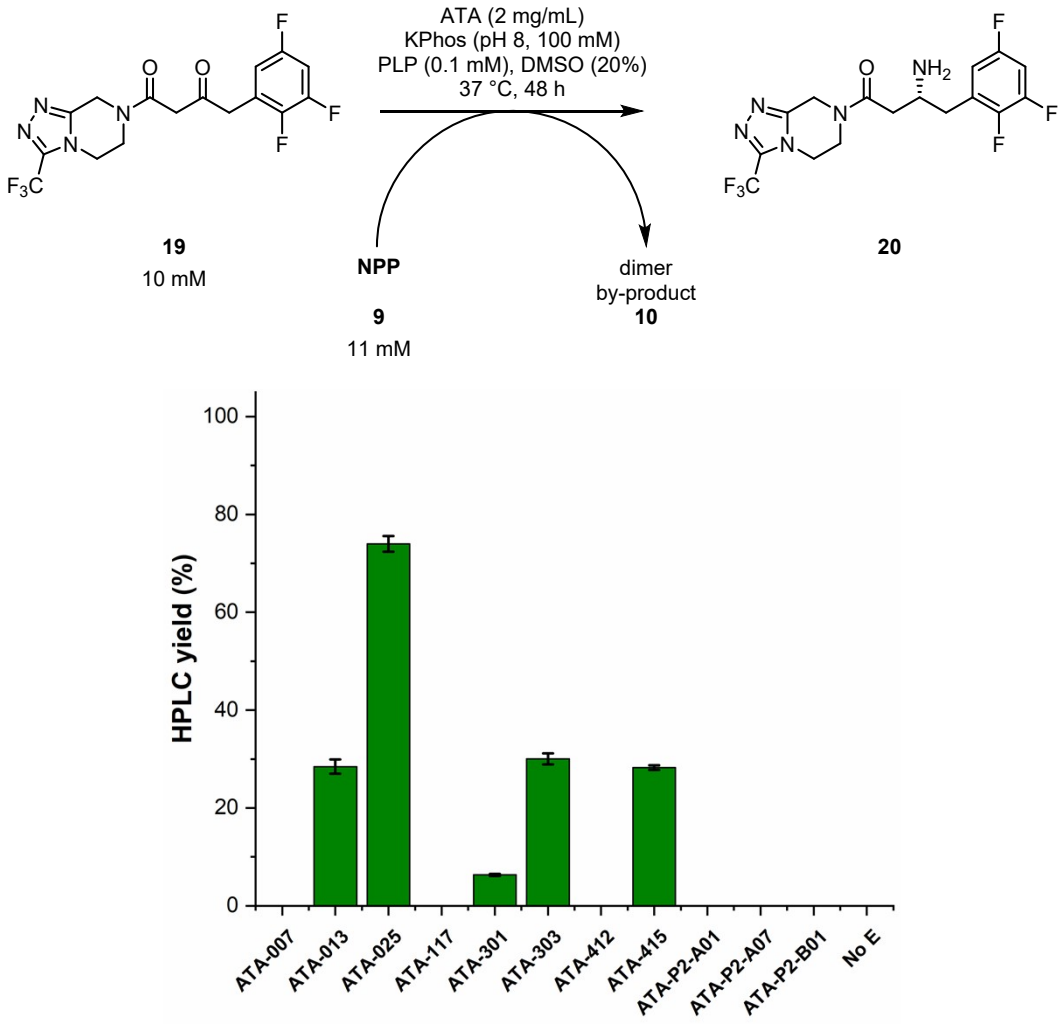


*Figure S25: HPLC yields of sitagliptin **20**. Reactions were carried out in triplicate and errors determined by standard deviation.*

2.20 pH study of NPP 9 activity with ATA-025 in production of sitagliptin 20

Presitagliptin ketone **19** (100 µL, 50 mM in DMSO), NPP **9** (50 µL, 110 mM) and ATA-025 (100 µL, 10 mg/mL) were added to an Eppendorf (1.5 mL) and buffer (250 µL, 200 mM (KPhos (pH 6.0-7.4), HEPES (pH 7.5-8.5), CAPS (pH 8.5-11.0)), PLP (0.1 mM) were added. The reaction was set up in triplicate, and shaken at 240 rpm, 37 °C for 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard methylbenzylamine **14** (4 µL, 150 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

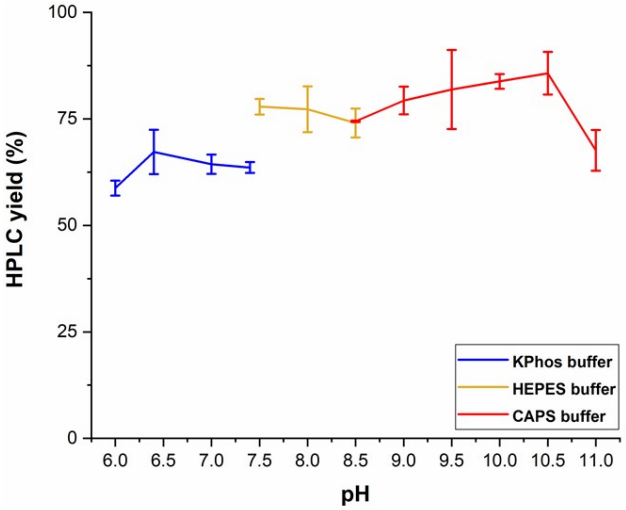


*Figure S26: Sitagliptin **20** production by transamination with NPP **9** as amine donor across pH range 6.0-11.0. KPhos pH 6.0-7.4 (blue), HEPES pH 7.5-8.5 (yellow) and CAPS pH 8.5-11.0 (red). Reactions were carried out in triplicate and errors determined by standard deviation.*

2.21 Temperature study of NPP 9 activity with ATA-025 in production of sitagliptin 20

Presitagliptin ketone **19** (100 µL, 50 mM in DMSO), NPP **9** (50 µL, 110 mM) and ATA-025 (100 µL, 10 mg/mL) were added to an Eppendorf (1.5 mL) and KPhos buffer (250 µL, pH 8.0, 100 mM) containing PLP (0.1 mM) was added. The reaction was set up in triplicate, and shaken at 240 rpm, 37 and 45°C for 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard methylbenzylamine **14** (4 µL, 150 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

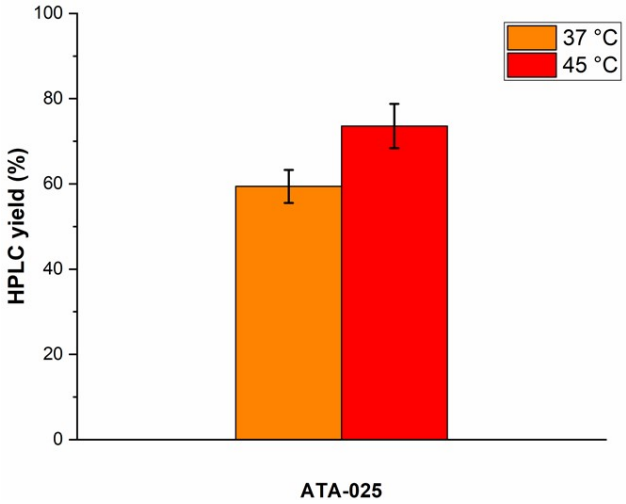


*Figure S27: HPLC yield of sitagliptin **20** from transamination of presitagliptin ketone **19** with NPP **9** amine donor at 37 (orange) and 45 (red) °C. Reactions were carried out in triplicate and errors determined by standard deviation.*

2.22 Effects of increasing equivalents of NPP 9 in production of sitagliptin 20

Presitagliptin ketone **19** (100 µL, 50 mM in DMSO), NPP **9** (1 eq 50 µL, 1.5 eq 75 µL, 2 eq 100 µL, 2.5 eq 125 µL, 3 eq 150 µL, 3.5 eq 175 µL, 4 eq 200 µL 100 mM) and ATA-025 (100 µL, 10 mg/mL) were added to an Eppendorf (1.5 mL) and KPhos buffer (pH 8.0, 100 mM) containing PLP (0.1 mM) was added to 500 µL final volume. The reaction was set up in triplicate, and shaken at 240 rpm, 37 °C for 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard methylbenzylamine **14** (4 µL, 150 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

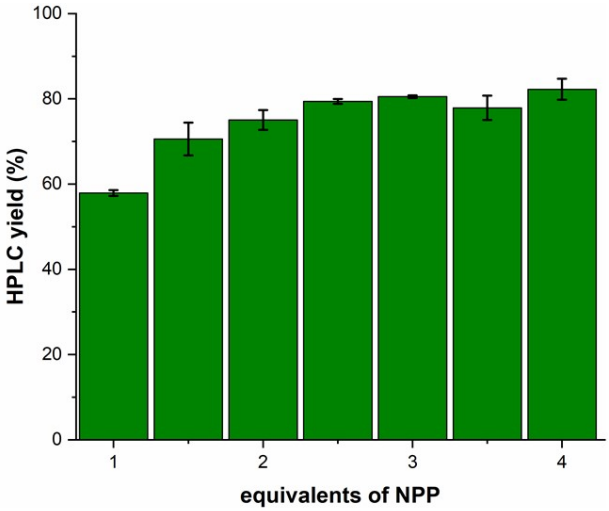


*Figure S28: Sitagliptin **20** HPLC yield for varying equivalents of NPP **9** in relation to the prochiral ketone **19**. Reactions were carried out in triplicate and errors determined by standard deviation.*

2.23 Small scale production of sitagliptin 20 with optimised reaction conditions

Presitagliptin ketone **19** (100 µL, 50 mM in DMSO), NPP **9** (100 µL, 100 mM) and ATA-025 (100 µL, 10 mg/mL) were added to an Eppendorf (1.5 mL) and HEPES buffer (200 µL, pH 8, 100 mM) containing PLP (0.1 mM) was added. The reaction was set up in triplicate at 240 rpm, 37 °C for 48 h. An aliquot (200 µL) was removed, quenched with TFA (10 µL, 10% in water). The supernatant (50 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) and diluted into water (350 µL). Internal standard methylbenzylamine **14** (4 µL, 150 mM) was added. The supernatant (100 µL) was collected by centrifugation (10 min, max speed, benchtop centrifuge) for analysis by the reverse phase HPLC method described previously.

*Table 9: Test scale sitagliptin **20** production from optimised reaction conditions. Error was determined by standard deviation.*

	Sitagliptin HPLC yield (%)			Average yield (%)	Error (±)
ATA-025 test scale	95.2	90.5	86.1	90.6	3.7

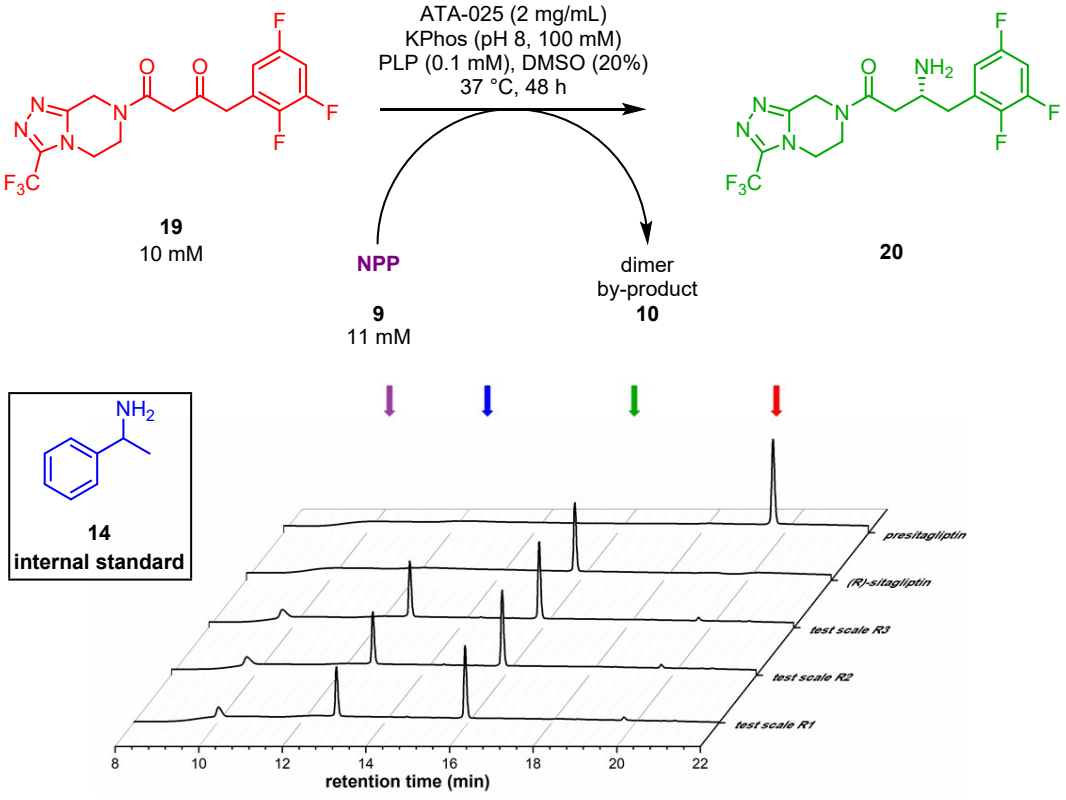
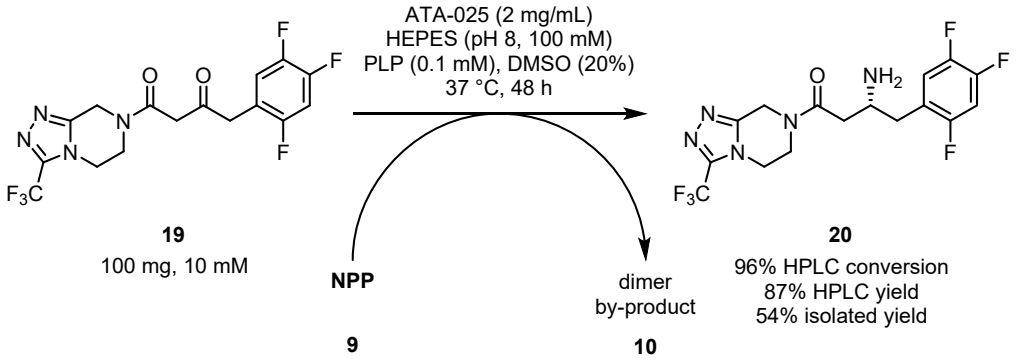


*Figure S29: HPLC chromatograms of the three test scale reactions for sitagliptin **20** (green) production. Presitagliptin ketone **19** (red) is almost completely consumed in the reaction. Methylbenzylamine **14** (blue) was used as internal standard and two equivalents of NPP **9** (purple) was used.*

2.24 Preparative scale procedure for sitagliptin 20 synthesis



Presitagliptin ketone **19** (100 mg, 2.46 mmol) was dissolved in DMSO (5 mL) and added to a HEPES pH 8.0 buffer solution (20 mL, HEPES [100 mM]) containing PLP (0.1 mM), NPP **9** (81 mg, 4.92 mmol) and ATA-025 (50 mg). The reaction was incubated at 37 °C with shaking at 240 rpm for 48 h. Aliquots (200 µL) were taken for HPLC analysis at 1, 5, 24 and 48 h. The reaction mixture was quenched by addition of concentrated aq. HCl to pH 2.0-3.0 and filtered through a pad of Celite®. The filtrate was basified to pH 12.0-14.0 with 20% aq. NaOH. The organics were extracted into CH_2Cl_2 (3 × 40 mL), washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was diluted in a minimal volume of CH_2Cl_2:7M NH_3 in methanol (1:0.03) and purified by flash column chromatography (CH_2Cl_2:7M NH_3 in methanol, 1:0.03) to give sitagliptin **20** as a clear colourless oil (54 mg, 0.13 mmol, 54% yield). In $CDCl_3$ solution, sitagliptin **20** exists as a 1.9:1 mixture of rotamers. Overlap of signals does not permit unequivocal assignment of each rotamer. Assignments are grouped (when necessary) and rotamers (major/minor/both) denoted in parentheses.

R_f (CH_2Cl_2:7M NH_3 in methanol, 1:0.03) 0.25;

^{1}H NMR (601 MHz, $CDCl_3$) δ 7.09 (m, 1H both), 6.90 (m, 1H both), 5.14 – 4.81 (m, 2H both), 4.32 – 3.82 (m, 2H & 2H both), 3.59 (br. s, 1H both), 2.76 (m, 2H both), 2.61 – 2.44 (m, 2H both);

^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6 (s, qC major), 170.2 (s, qC minor), 156.2 (dd, $^1J_{CF}$ = 245.0 Hz, $^3J_{CF}$ = 8.0 Hz, $^4J_{CF}$ = 2.5 Hz, CF both), 150.5 (overlapping m, qC minor), 149.7 (overlapping m, qC major), 147.6 & 149.7 (m & overlapping m, CF or CF both), 146.0 & 148.2 (m & m, CF or CF both), 121.6 (m, qC both), 119.2 (dd, $^2J_{CF}$ = 18.7 Hz, $^3J_{CF}$ = 6.2 Hz, CH both), 105.7 (dd, $^2J_{CF}$ = 28.7 Hz, $^2J_{CF}$ = 20.6 Hz, CH both), 48.7 (s, CNH_2 both), 43.7 (s, CH_2 minor), 43.3 (s, CH_2 major), 42.6 (s, CH_2 major), 41.8 (s, CH_2 minor), 40.0 (s, CH_2 major), 39.6 (s, CH_2 minor), 39.3 (s, CH_2 minor), 38.2 (s, CH_2 major), 36.1 (s, CH_2 major), 35.9 (s, CH_2 minor), (Note: C-CF_3 and CF_3 not identifiable);

HRMS (ESI+) $C_{16}H_{15}F_6N_5O$: 408.1251 $[M+H]^+$ (calculated 408.1254);

IR 3310, 2944, 2831, 1449, 1023 cm^{-1}.

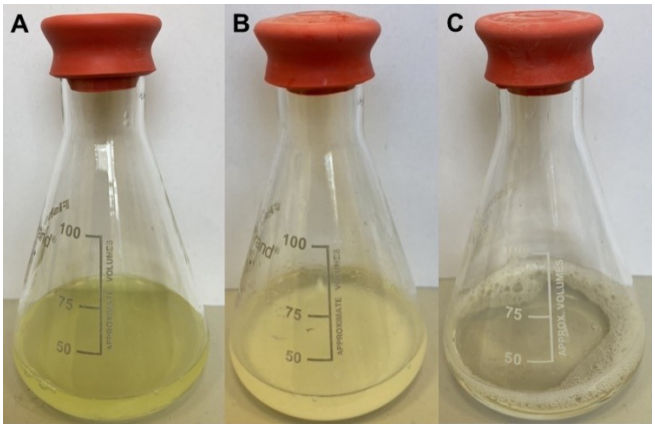


*Figure S30: Pictures taken of the scale up transamination reaction to produce sitagliptin **20** using NPP **9** as amine donor. A) Before addition of presitagliptin ketone **19** B) after 48 h reaction time were white precipitate relating to the dimer by-product **10** was observed C) after filtration through Celite® pad to remove dimer by-product **10**.*

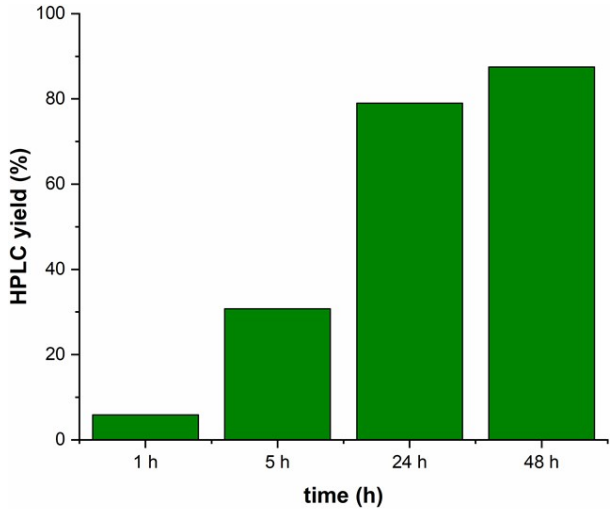


*Figure S31: Sitagliptin **20** HPLC yield from aliquots taken from scale up transamination reaction at 1, 5, 24 and 48 h.*

The enantiopurity of sitagliptin **20** was determined to be >99% ee by chiral HPLC, using a CHIRALPAK® IB N-5 analytical column (250 × 4.6 mm, 5 µm) with gradient 97:3:0.1 n-hexane: *iso*-propyl alcohol: diethylamine at 265 nm. The commercial (*R*)-sitagliptin (Sigma Aldrich) elutes at 10.4 min (black), (*S*)-sitagliptin (prepared using ATA-217 and NPP **9** elutes at 8.8 min (blue) and (*R*)-sitagliptin (prepared using ATA-025 and NPP **9** as described above) at 10.4 min (red) (Figure S31). This demonstrates that only the desired enantiomer was observed in each biocatalytic reaction.

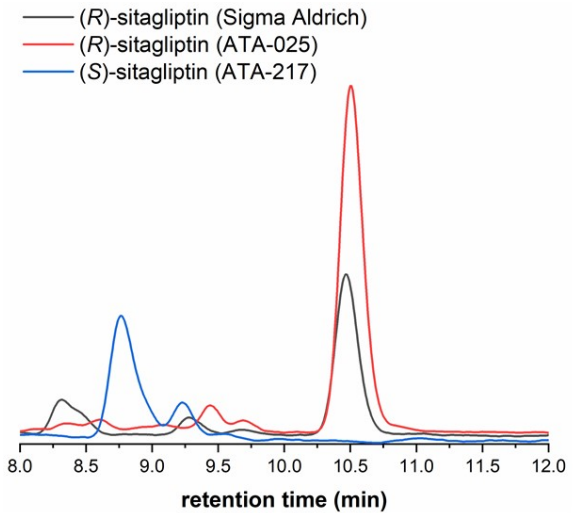


*Figure S32: Chiral HPLC chromatograms of the commercial (R)-sitagliptin (Sigma Aldrich) at 10.4 min (black), (S)-sitagliptin (prepared using ATA-217 and NPP **9** at 8.8 min (blue) and (R)-sitagliptin (prepared using ATA-025 and NPP **9** as described above) at 10.4 min (red).*

3 Spectra

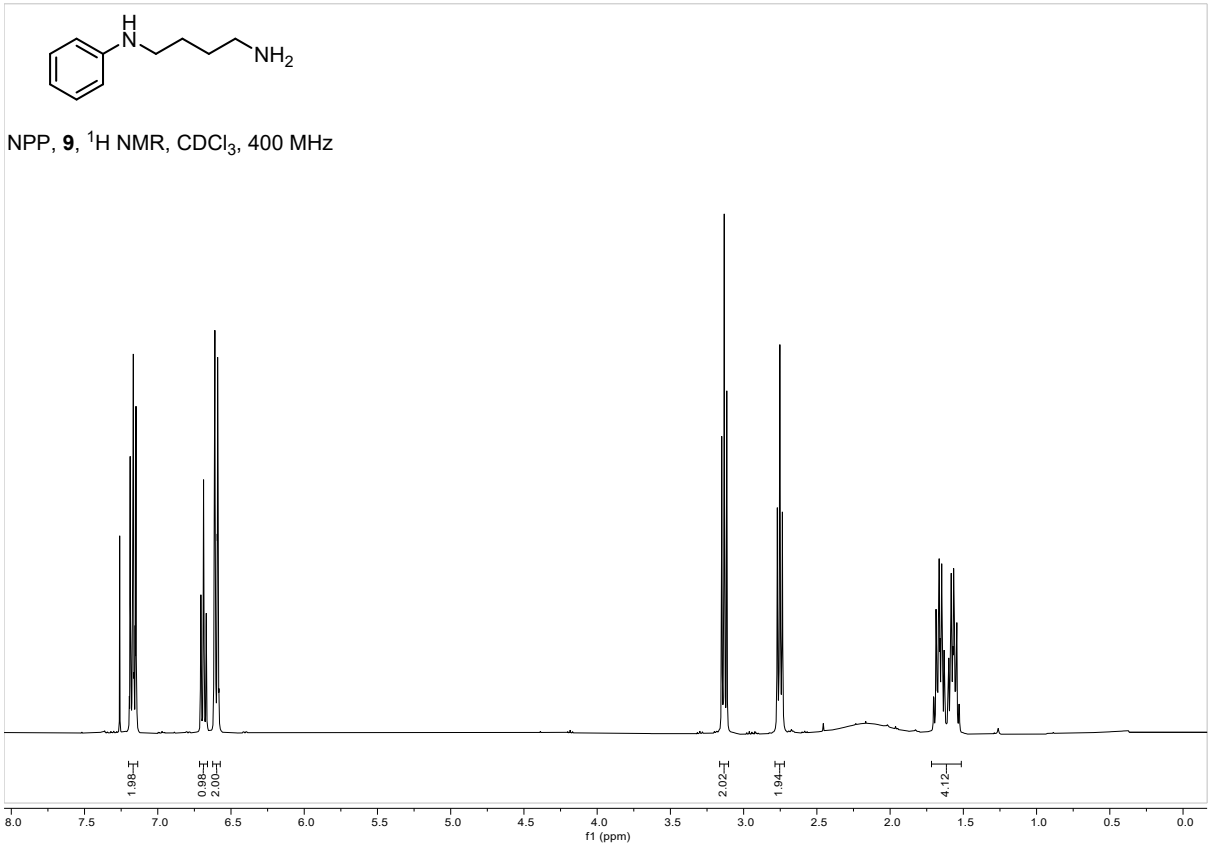


*Figure S33: ^{1}H NMR spectrum of NPP **9**.*

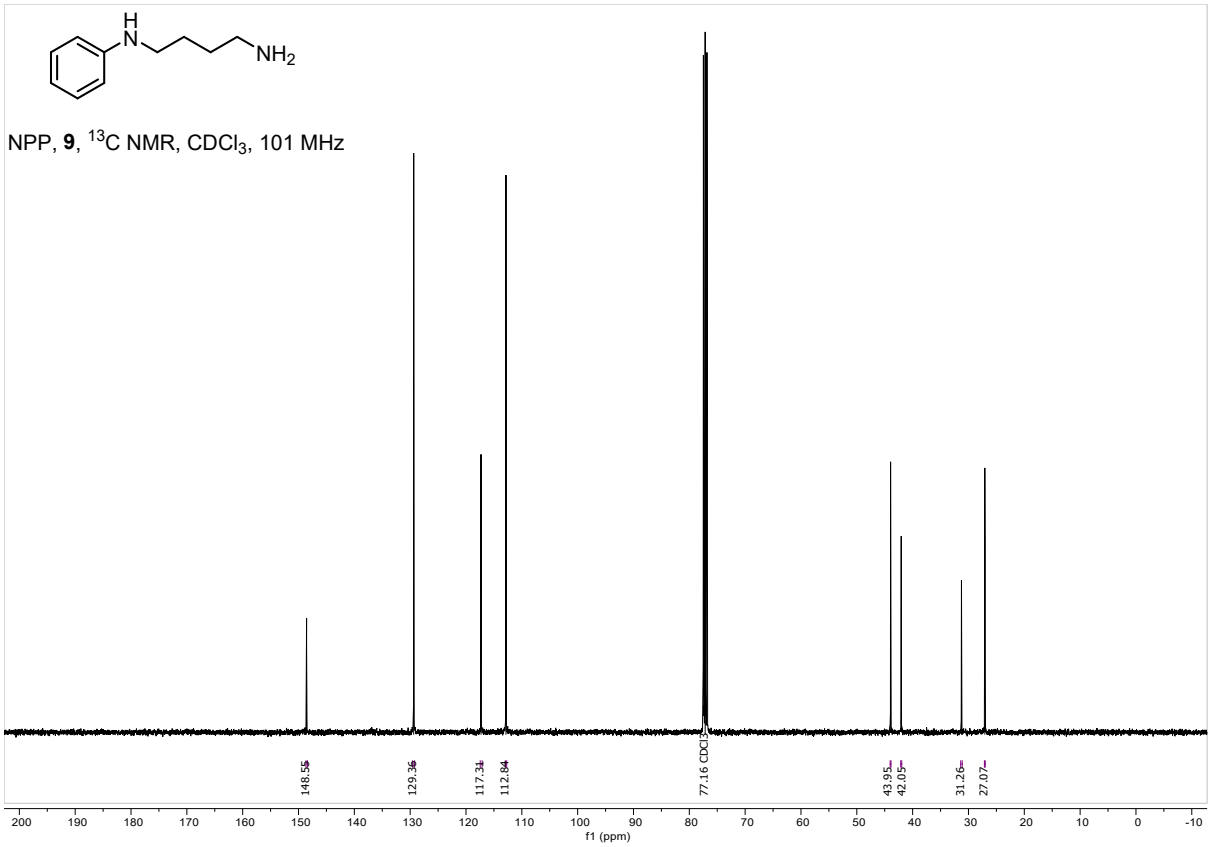


*Figure S34: ^{13}C NMR of NPP **9**.*

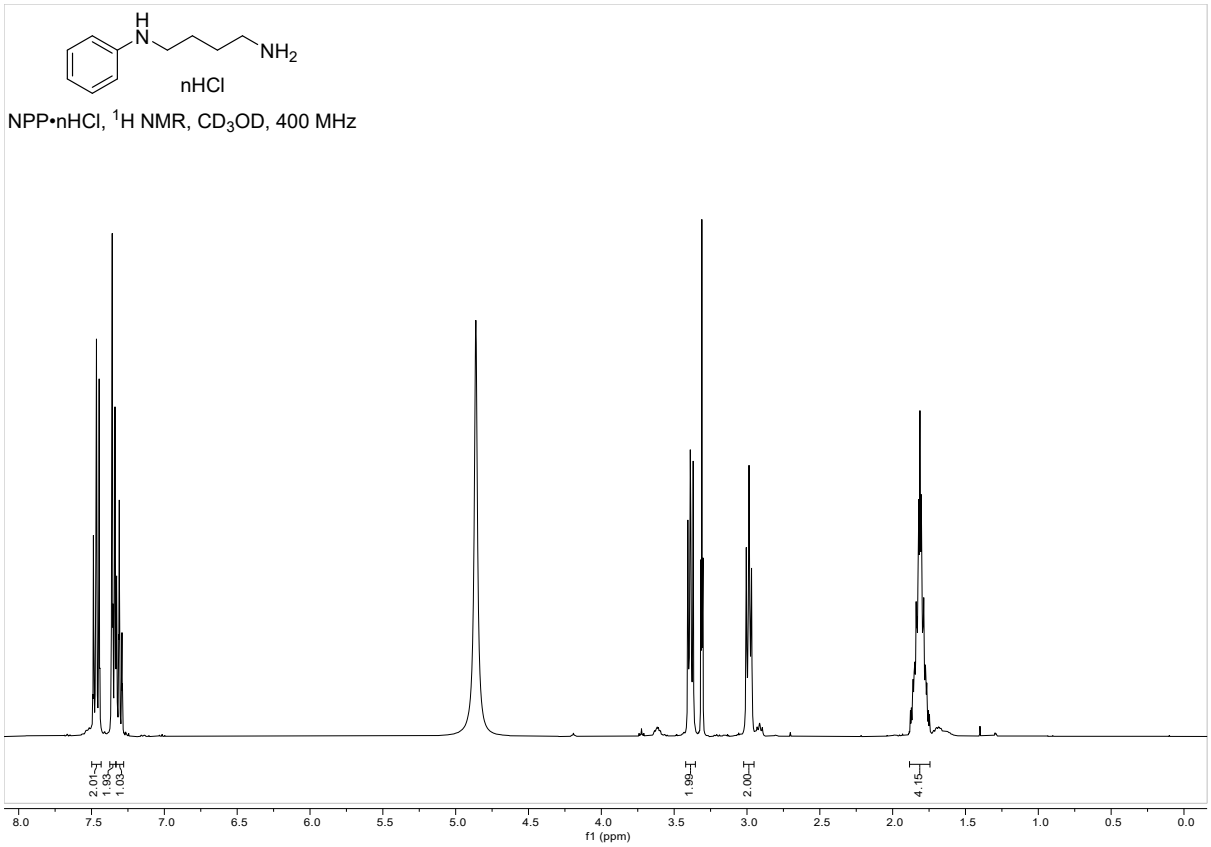


Figure S35: ^{1}H NMR spectrum of NPP•nHCl.

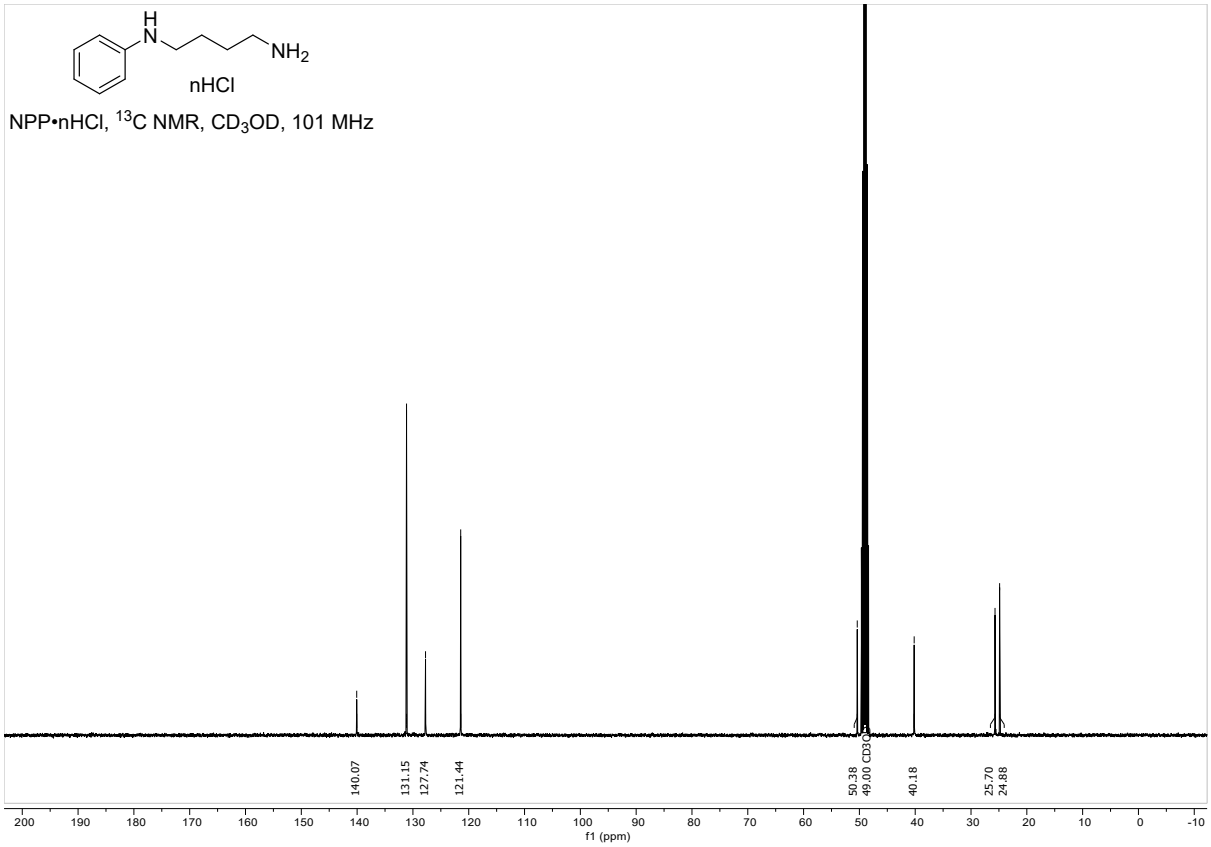


Figure S36: ^{13}C NMR spectrum of NPP•nHCl.

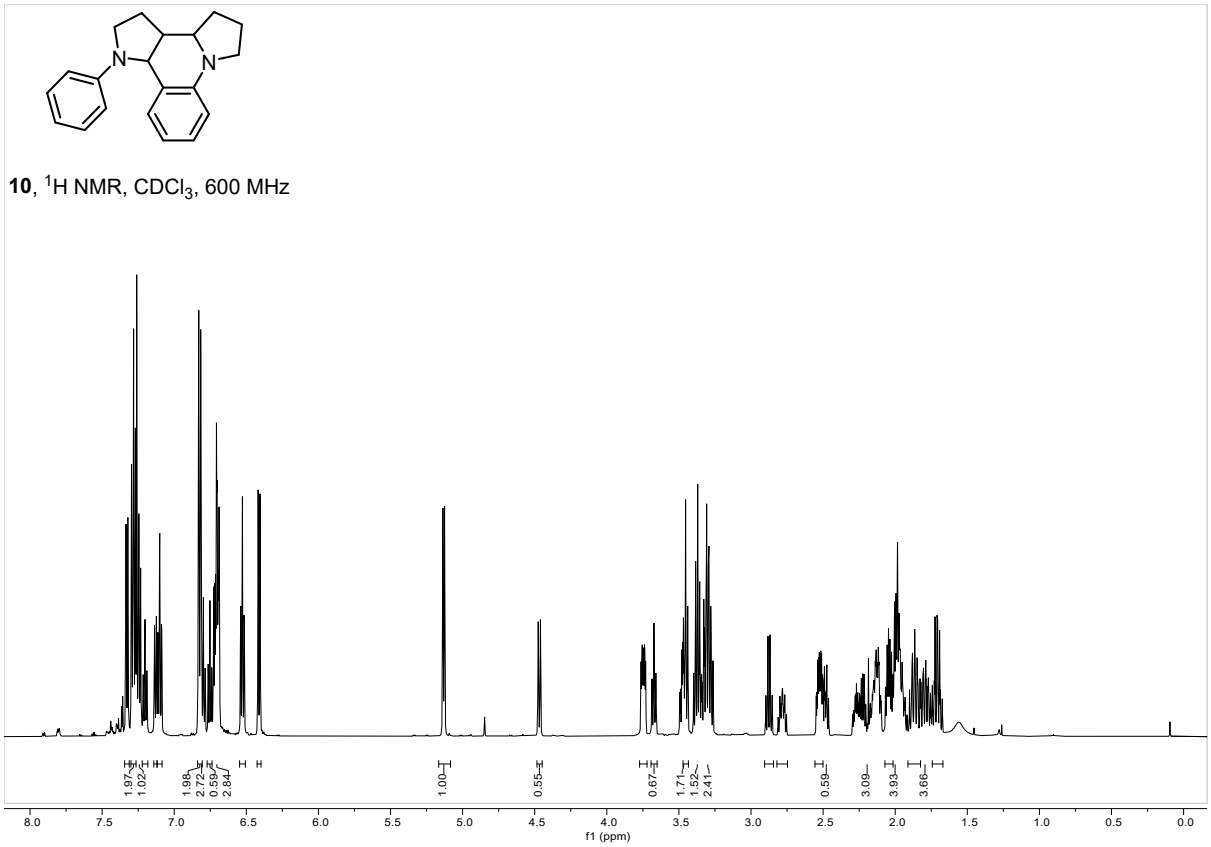
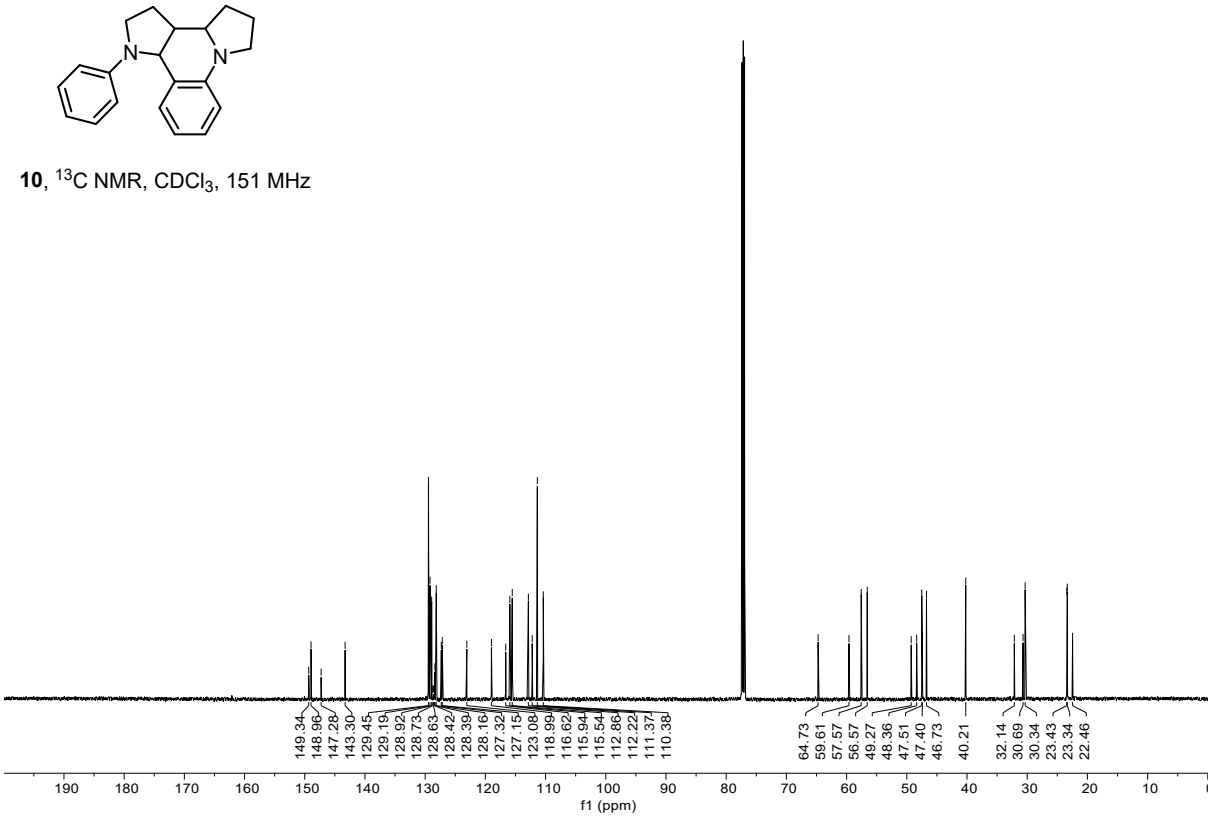


*Figure S37: [1]H NMR spectrum of dimer by-product **10**.*



10, ^{13}C NMR, $CDCl_3$, 151 MHz

*Figure S38: ^{13}C NMR spectrum of dimer by-product **10**.*

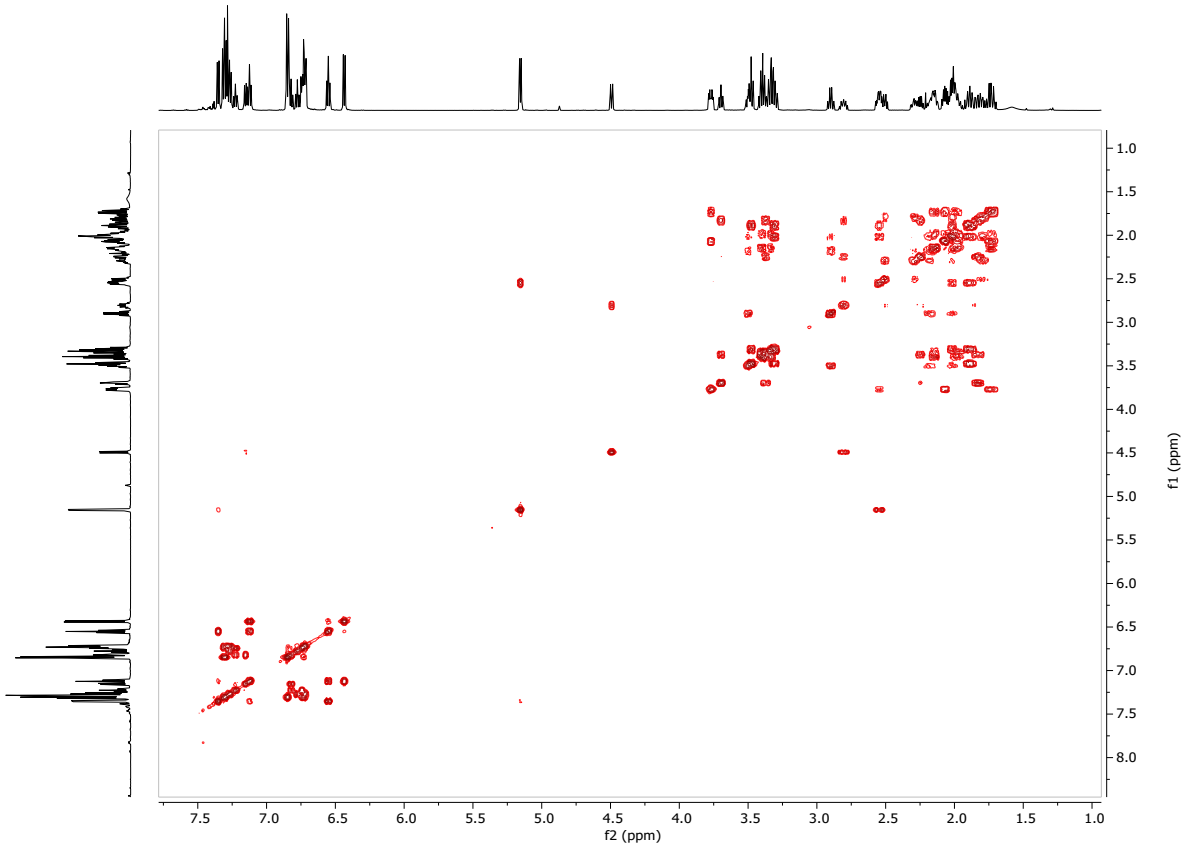


*Figure S39: COSY NMR spectrum of dimer by-product **10**.*

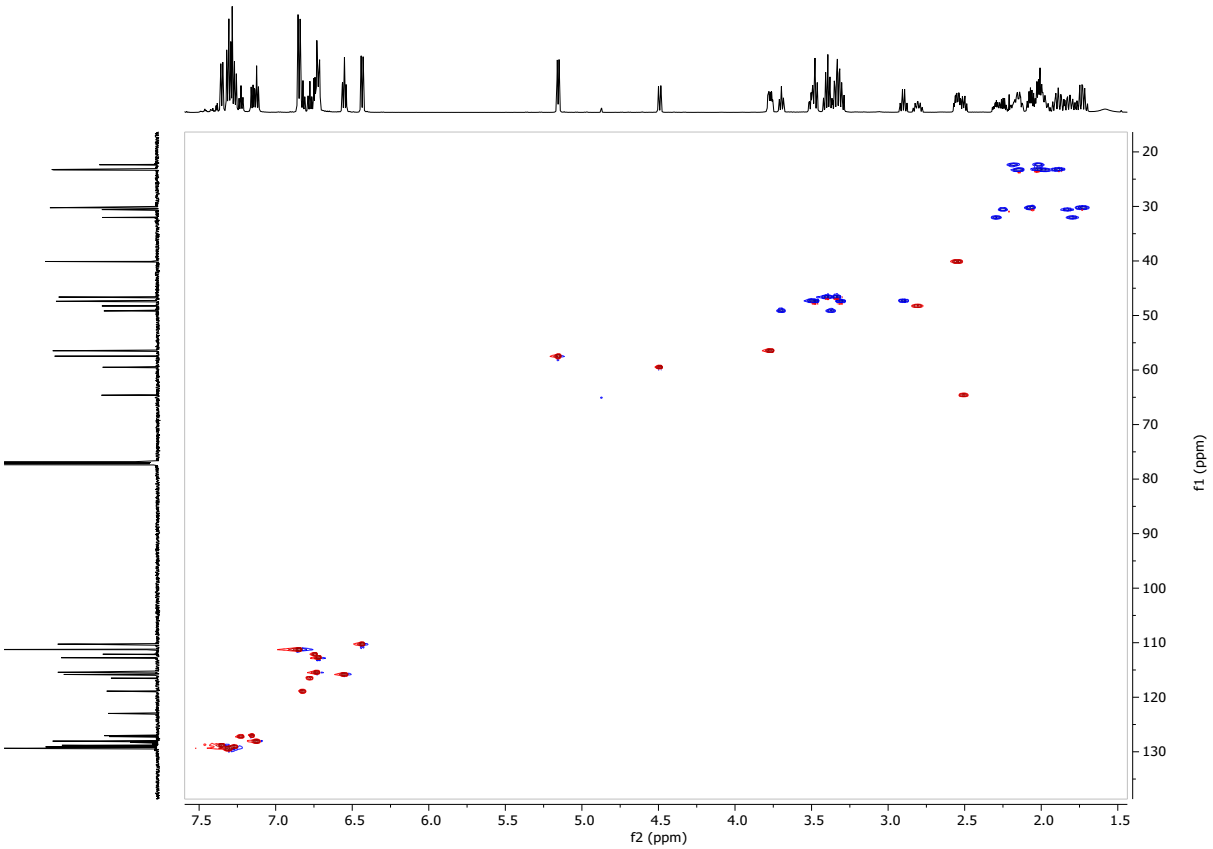


*Figure S40: HSQC NMR spectrum of dimer by-product **10**.*

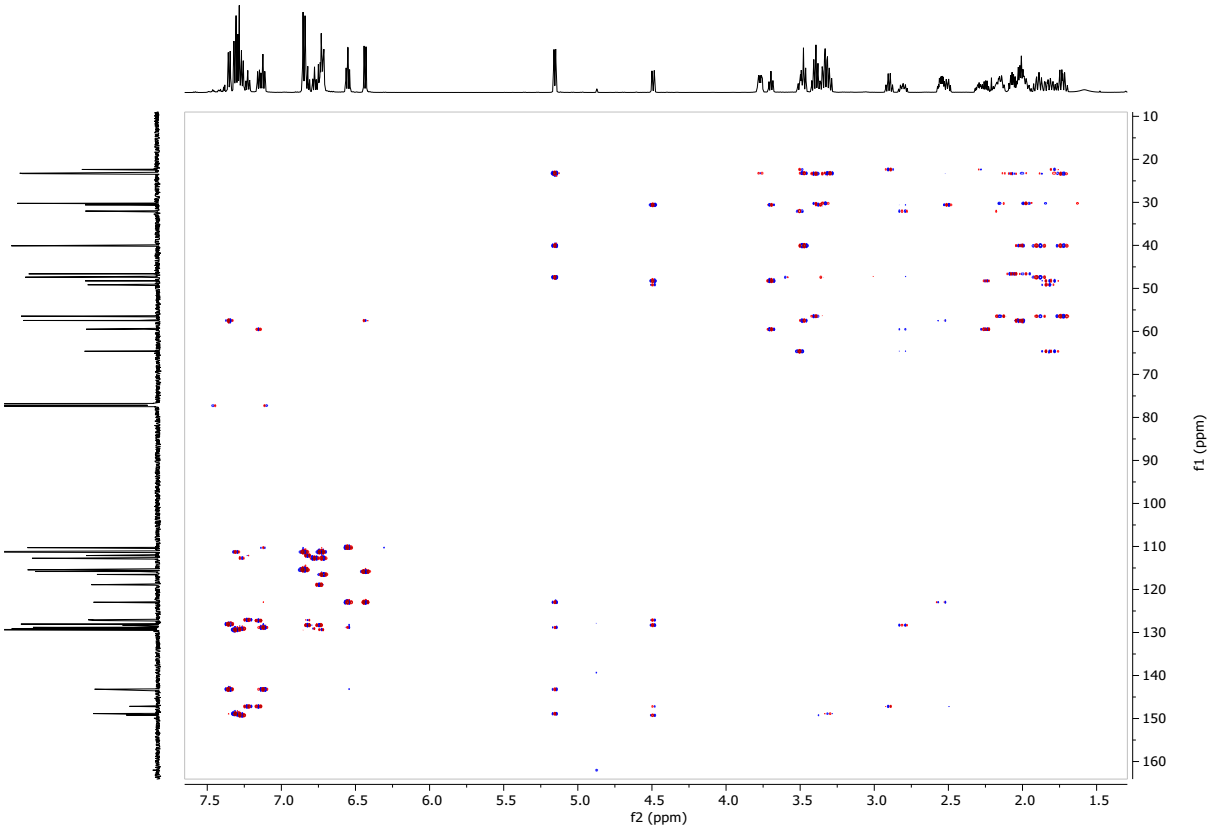


*Figure S41: HMBC NMR spectrum of dimer by-product **10**.*

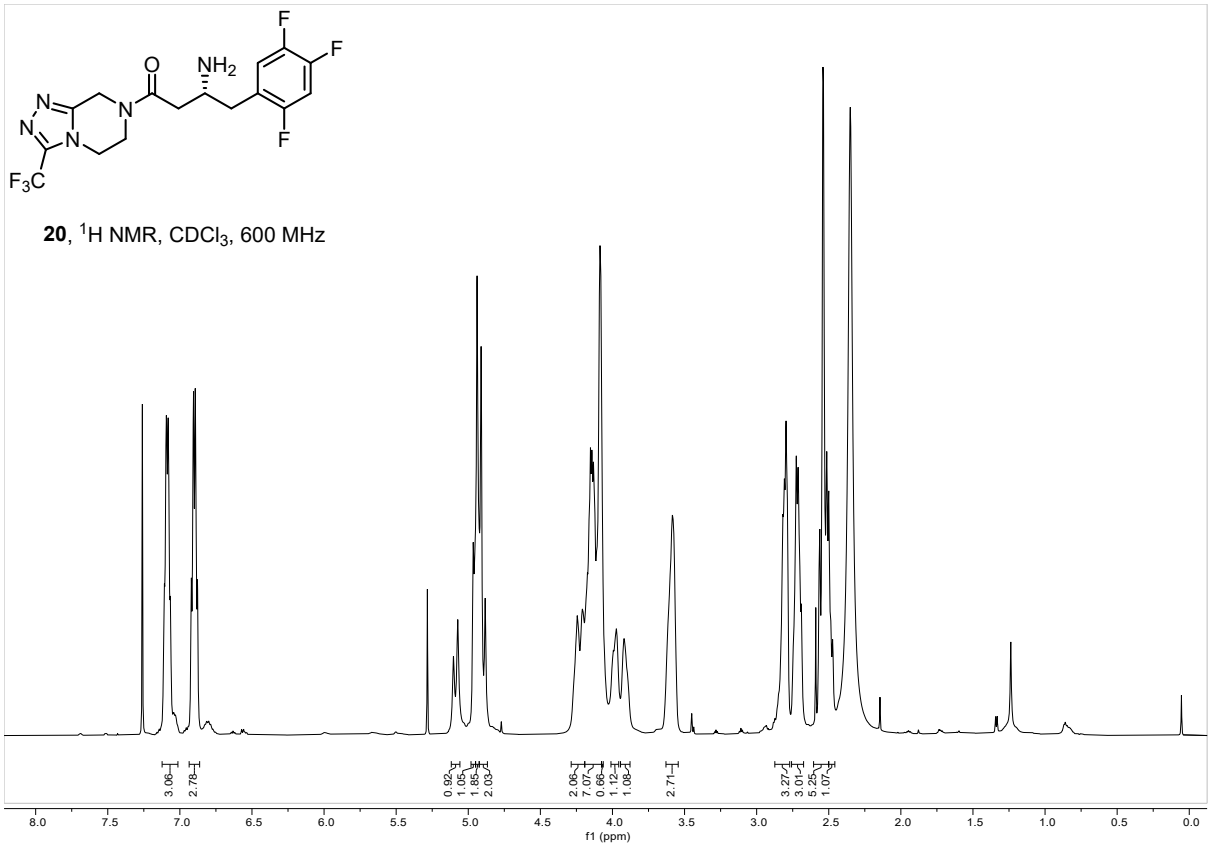


*Figure S42: [1]H NMR spectrum of sitagliptin **20**.*

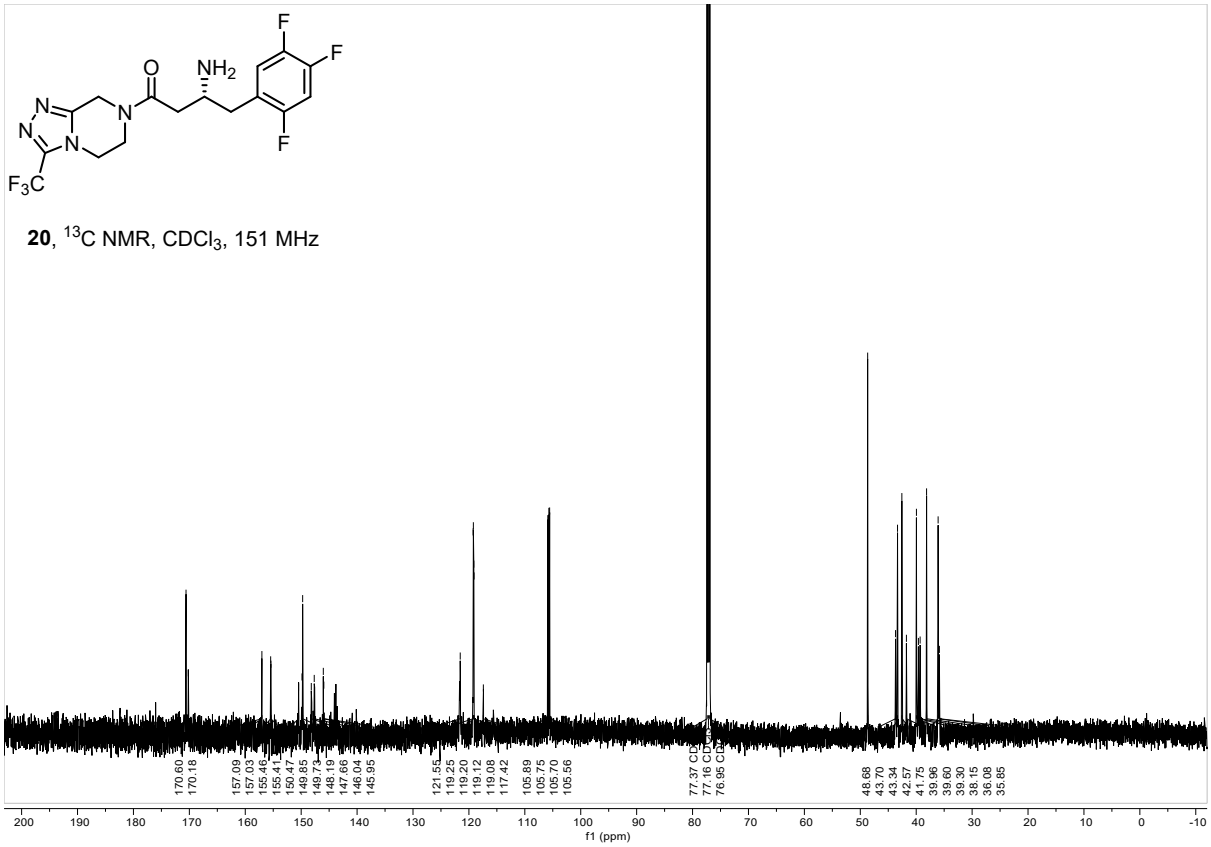


*Figure S43: [13]C NMR spectrum of sitagliptin **20**.*

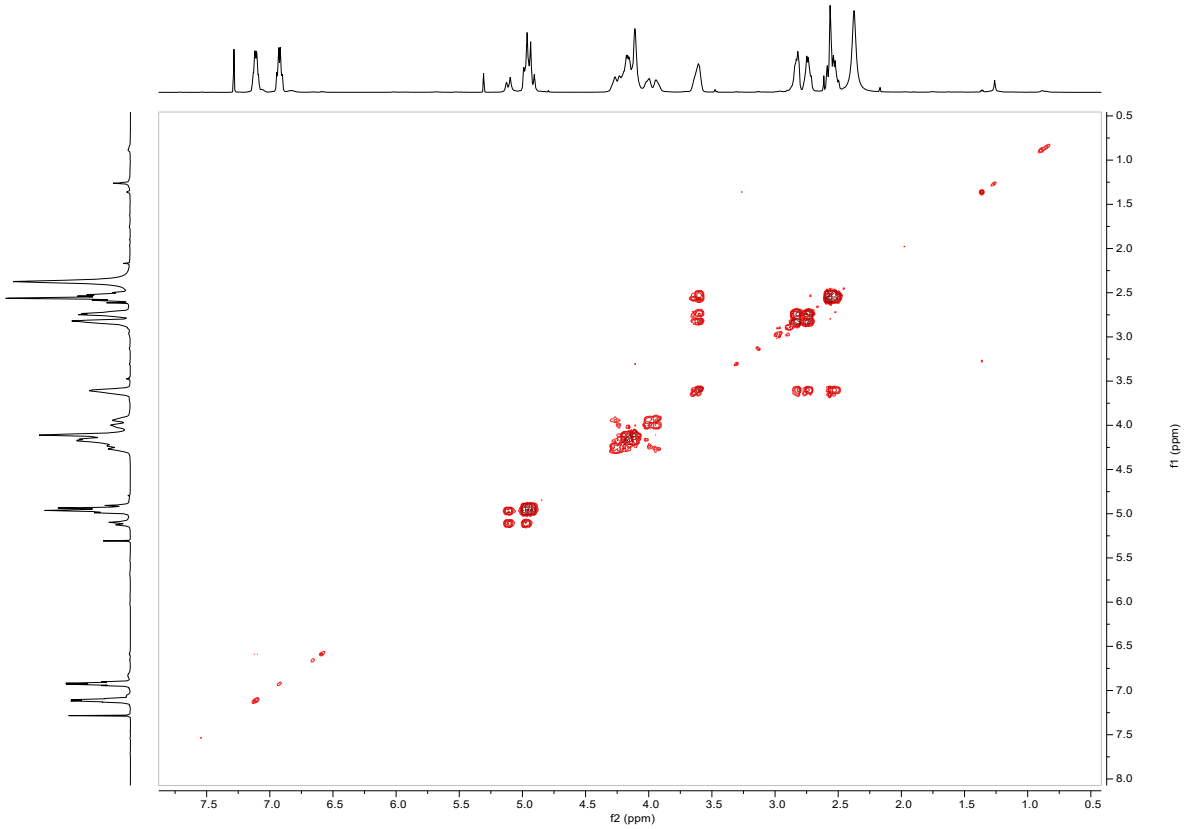


*Figure S44: COSY NMR spectrum of sitagliptin **20**.*

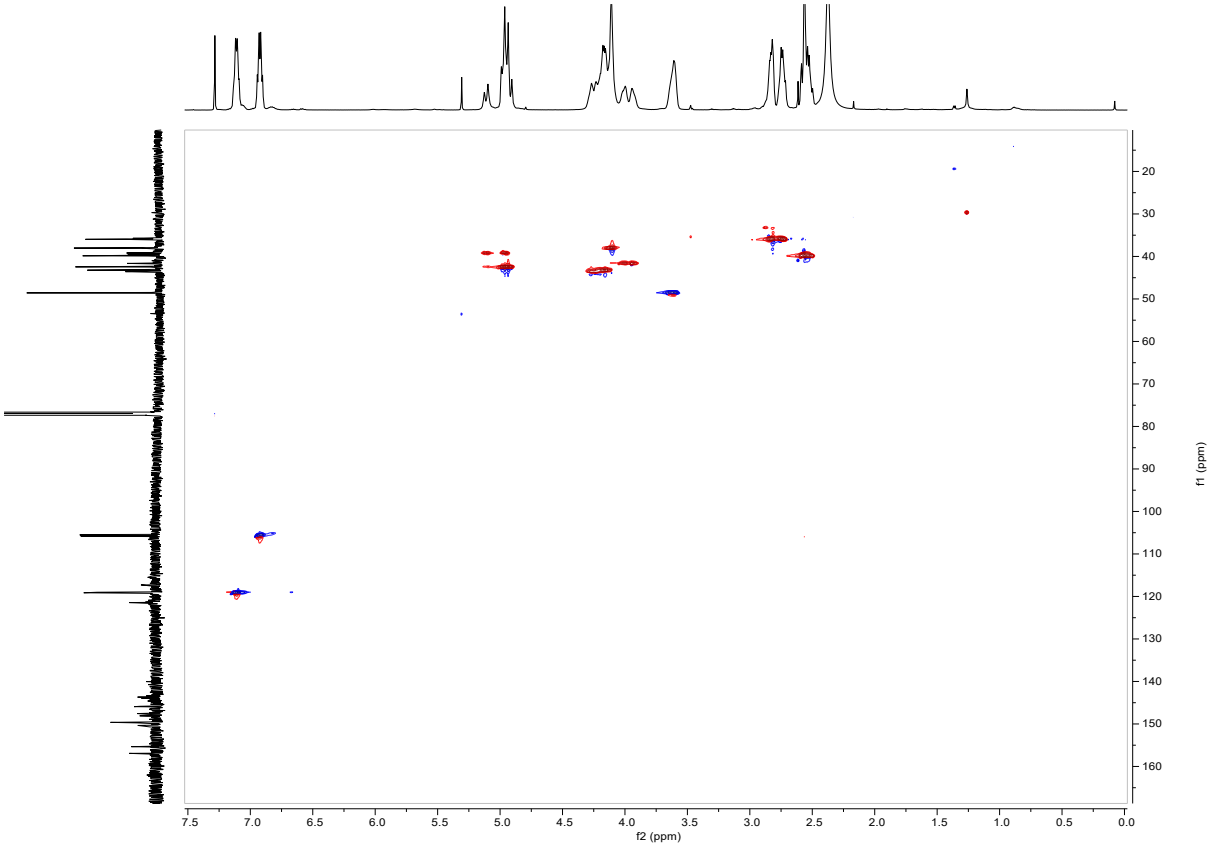


*Figure S45: HSQC spectrum of sitagliptin **20**.*

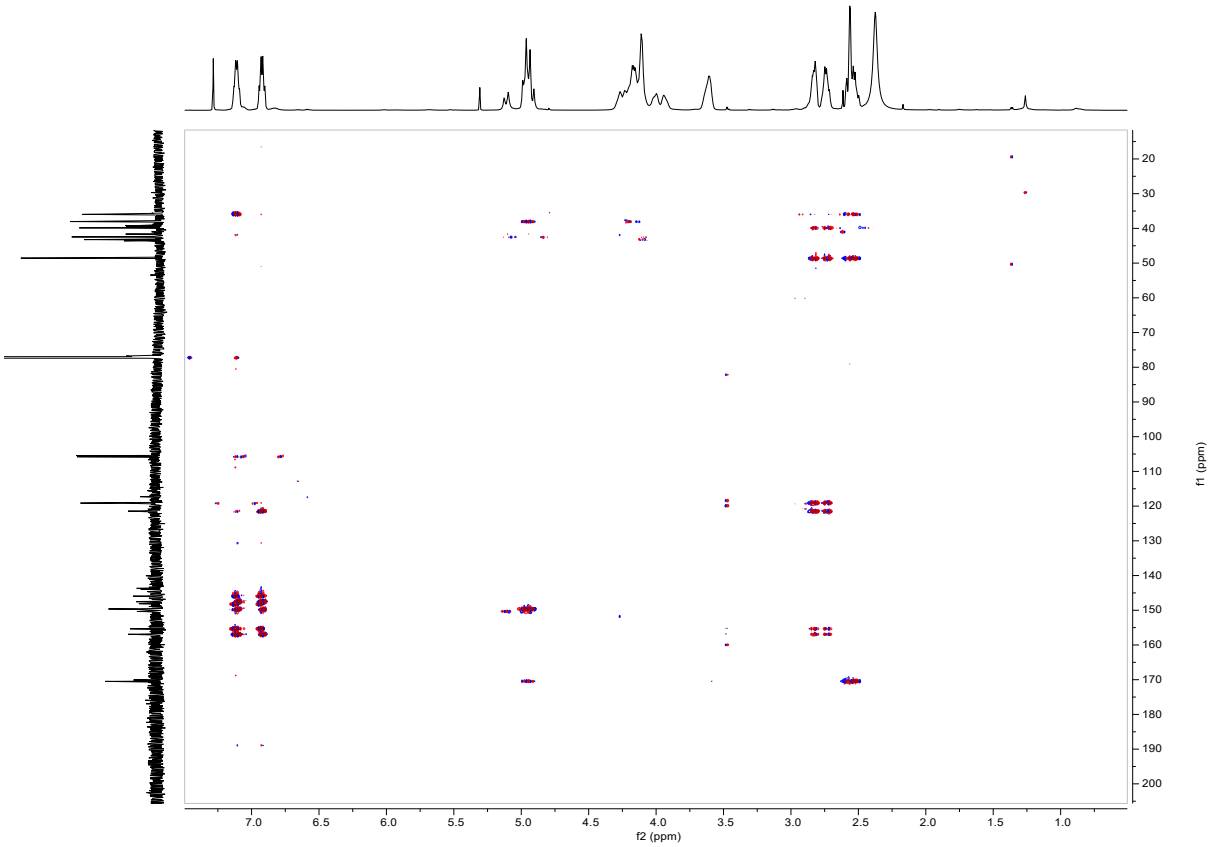
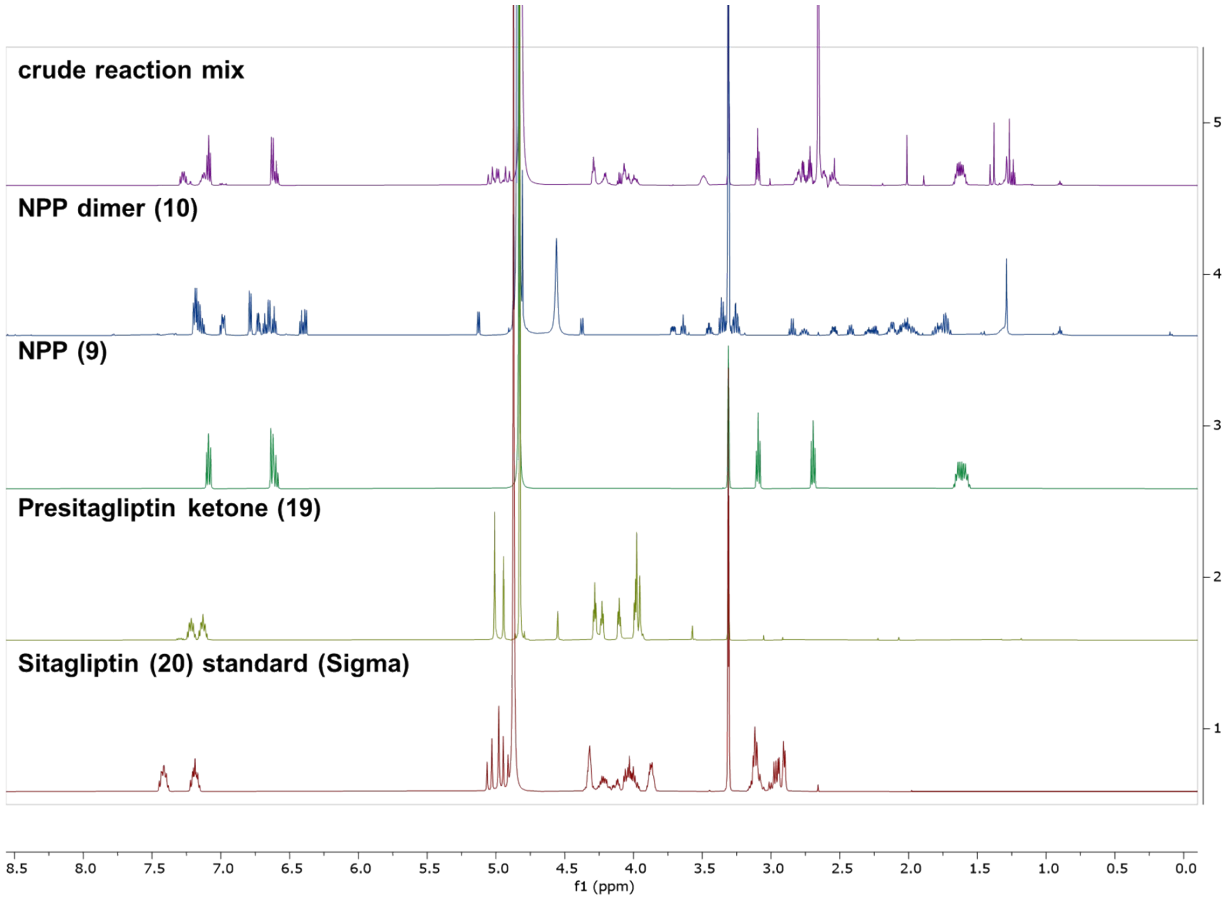


*Figure S46: HMBC NMR spectrum of sitagliptin **20**.*

(A)


crude reaction mix
NPP dimer (10)
NPP (9)
Presitagliptin ketone (19)
Sitagliptin (20) standard (Sigma)
5
4
3
2
1
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

(B)

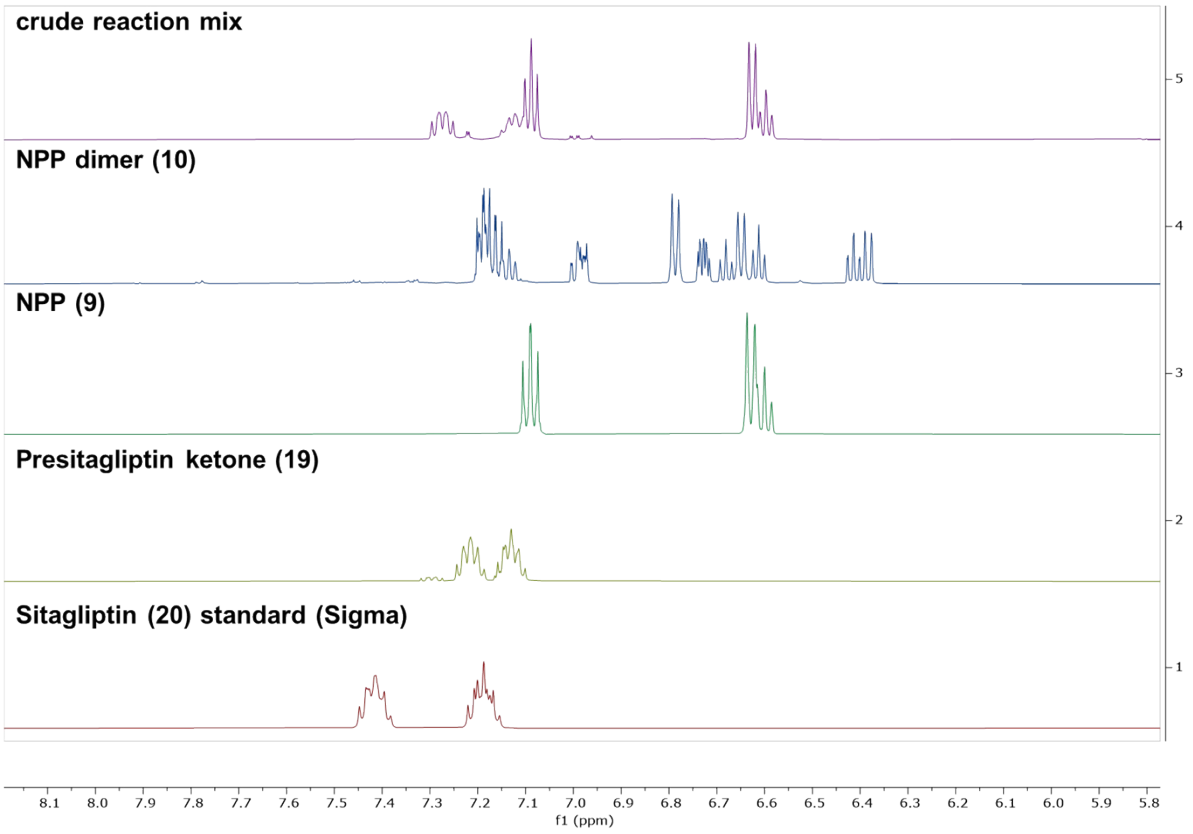


Figure S47: ***(A)*** *^{1}H NMR analysis from the scale up reaction of the biocatalytic synthesis of sitagliptin* ***19****. The stacked ^{1}H NMR from the top down are of the crude reaction mixture (purple), NPP dimer* ***10*** *(blue), NPP* ***9*** *(green), presitagliptin ketone* ***19*** *(yellow/green) and the commercially purchased standard of sitagliptin* ***20*** *(brown). Spectra were recorded in MeOD at 600 MHz.* ***(B)*** *Zoom in of the aromatic region of the same spectra.*

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