

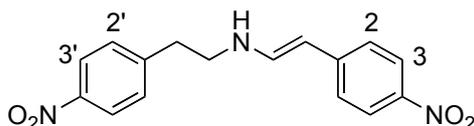
Supplementary Information

1- General information

The solvents and chemicals were purchased from Sigma Aldrich and Fluorochem, and were used without further purification. NMR spectra were recorded on a Bruker Avance 500. Chemical shifts (in ppm) were referenced to acetone- d_6 . Proton coupling patterns have been described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br).

HPLC analyses were performed on an Agilent 1260 Infinity provided with a 1260 VWD detector. Mass spectrometry analyses were performed at the UCL Chemistry Mass Spectrometry Facility using a Finnigan MAT 900XP double focusing hybrid (EBqQ) mass spectrometer with a direct insertion probe. Gas phase ions were generated in EI-volume and detected by a PATRIC (positron and time resolved ion counter) scanning array detector. The instrument resolution was 10,000 Da. Accurate mass determination was performed by the peak-matching method.

2- Reaction scale-up using **1** to characterize (*E*)-*N*-(4-nitrophenethyl)-2-(4-nitrophenyl)ethen-1-amine **5**



The enzymatic reaction was performed using 2-(4-nitrophenyl)ethan-1-amine hydrochloride **1** (50.7 mg, 0.25 mmol; 25 mM) as amine donor, benzaldehyde **2** (10.6 mg, 11 μ L, 0.10 mmol; 10 mM) as amine acceptor, pyridoxal-5'-phosphate (PLP) (0.2 mM), potassium phosphate (KPi) buffer (100 mM, pH 7.5), and cell lysate of CV-TAm (0.4 mg mL⁻¹), all in a total volume of 10 mL at 30 °C and 500 rpm for 18 h. The reaction was started by the addition of amine donor **1**. The mixture was centrifuged (16,000 rpm) and the red precipitate washed with water (3 \times 2 mL). The precipitate was then solubilized in acetone and the sample was centrifuged (16,000 rpm). The supernatant was collected and evaporated to afford **5** with a purity \geq 85% (11 mg, 35%) as the major component. λ_{max} 440 nm (H₂O); ¹H NMR (500 MHz; acetone- d_6) δ 3.12 (2H, t, J = 7.1 Hz, CH₂N), 3.50-3.55 (2H, m, CH₂Ar), 5.50 (1H, d, J = 14.1 Hz, =CHAr), 5.96 (1H, br s, NH), 7.28 (2H, d, J = 8.5 Hz, 2 \times 3'-H), 7.36-7.42 (1H, m, HC=CHAr), 7.61 (2H, d, J = 8.2 Hz, 2 \times 2-H), 7.98 (2H, d, J = 8.5 Hz, 2 \times 2'-H), 8.18 (2H, d, J = 8.2 Hz, 2 \times 3-H); ¹³C NMR (125 MHz; acetone- d_6) δ 35.9 (CH₂), 45.7 (CH₂), 95.9 (alkene CH), 123.2 (CH), 124.2 (CH), 125.0 (CH), 130.8 (CH), 141.5 (alkene CH), 143.1 (C), 147.5 (C), 148.7 (C), 149.8 (C); m/z (EI+) 313 (M⁺, 15%), 177 (100), 150 (28), 104 (18), 91 (15); HRMS (EI+) found [M]⁺ 313.1061; C₁₆H₁₅N₃O₄ requires 313.1064.

3- Assay limit of sensitivity

The enzymatic reactions were performed (in triplicate) in 96 well-plate with a total volume of 200

μL containing 2-(4-nitrophenyl)ethan-1-amine hydrochloride **1** (25 mM) as amine donor, benzaldehyde **2** (10 mM) as amine acceptor, PLP (0.2 mM), KPi buffer pH 7.5 (100 mM), TAM cell lysate at different concentrations (0.004 to 0.5 mg mL⁻¹ of enzyme) at 30 °C and 500 rpm for 90 min. The reaction was started by the addition of amine donor **1**. Reactions were then quenched with 10 μL of TFA (10% in water). Reaction mixtures were centrifuged to remove the protein, diluted and analyzed by HPLC. Two negative controls were also performed at the same time, one without benzaldehyde and another without enzyme.

4- Strains and plasmids

Strains and plasmids used in this work are listed in Table 1. *Escherichia coli* BL21 (DE3) was used for bioconversions. The plasmid pET-29a(+) was used as expression vector. Recombinant strains were obtained by introduction of plasmid DNA into the host strains via heat-shock. Transformants were selected via their antibiotic resistance. Cultivations were carried out at 37 °C or 30 °C in 2xTY medium (16 g L⁻¹ tryptone, 10 g L⁻¹ yeast extract and 5 g L⁻¹ NaCl). For cultivation, an Innova® 44/44R orbital shaker (Eppendorf, Hamburg, Germany) was used at 200 rpm. Kanamycin (Km) was added in concentrations of 50 $\mu\text{g mL}^{-1}$. Solid 2xTY media were prepared accordingly containing 1.5% agar.

Table 1. Strains and Plasmids used in this work.

	Characteristics	Reference
Strain		
<i>E. coli</i> BL21 (DE3)	F ⁻ , <i>ompT</i> , <i>hdsSB</i> ($r_B^- m_B^-$), λ (DE3 [<i>lacI lacUV5 T7 gene 1 Sam7 $\Delta nin5$</i>])	[1]
Plasmid		
pQR427	pET-29a(+), T7 promoter <i>lacI</i> , Km ^r , PP0596 encoding the Pp-TAm gene from <i>Pseudomonas putida</i> KT2440	[2]
pQR801	pET-29a(+), T7 promoter <i>lacI</i> , Km ^r , CV2025 encoding the CV-TAm gene from <i>Chromobacterium violaceum</i> DSM30191	[3]
pQR1006	pET-29a(+), T7 promoter <i>lacI</i> , Km ^r , KPN_00799 encoding the Kp-TAm gene from <i>Klebsiella pneumoniae</i>	[4,5]
pQR1049	pET-29a(+), T7 promoter <i>lacI</i> , Km ^r , synthetic gene encoding the ArRMut11 variant from <i>Arthrobacter</i> sp.	[6]

5- Growth and expression

For pre-cultivation, 5 mL 2xTY medium were inoculated with a single colony and cultivated over-

night. Subsequently, 100 mL 2xTY culture was inoculated with 100 μ L of the overnight culture. The cells were grown until the suspension reached an optical density at 600 nm (OD_{600}) of 0.9 and induced with 1 mM isopropyl- β -thiogalactopyranoside (IPTG) for 6 h at 30 °C before they were harvested via centrifugation (4650 g, 15 min, 4 °C). The supernatant was discarded and the cell pellet frozen in liquid nitrogen for 15 min and freeze dried overnight. Freeze dried cells were used either directly for cell lysis or stored at -20 °C until usage.

6- Preparation of cell lysates

Freeze dried *E. coli* BL21 (DE3) TAmS were resuspended in KPi buffer (100 mM, pH 7.5) to a concentration of 30 g L⁻¹ cell dry weight (g_{CDW}). Cell lysis was performed by sonication using a Soniprep 150 plus sonicator with microprobe tip (MSE Ltd., London, UK) at a power of 16 Watts for 10 cycles of 15 s on/15 s off. The sonicated suspension was centrifuged (13,000 g, 4 °C, 20 min) and the pellet discarded. The clarified cell lysate was diluted to a final protein concentration of 4 mg mL⁻¹ and used either immediately in bioconversions or was frozen at -20 °C for a maximum of 1 month.

7- Plate assay

A 5 mL 2xTY medium was inoculated with a single colony of *E. coli* BL21 (DE3) CV-TAm and cultivated for 8 h at 37 °C. The cell suspension was diluted 1:10,000 in 2xTY medium. A sample of 100 μ L of the diluted cell suspension were plated on LB agar, which was covered with a Hybond N-nylon membrane (GSK Healthcare Life Sciences, Buckinghamshire, UK). The cells were cultivated for 16 h at 30 °C. The membrane was transferred to LB agar containing 1 mM IPTG and induction was carried out at 30 °C for 5 h. The membrane was transferred on blotting filter paper soaked with KPi buffer (100 mM, pH 7.5) and stored for 18 h at 4 °C. Wild type *E. coli* BL21 (DE3) not containing a recombinant transaminase were plated and cultivated in the same manner as a control. The transamination assay was initiated by transferring the nylon membrane on top of a filter paper soaked with a solution of KPi buffer (100 mM, pH 7.5), PLP (0.2 mM), 2-(4-nitrophenyl)ethan-1-amine hydrochloride **1** (25 mM or 12.5 mM) and benzaldehyde **2** (10 mM or 5 mM). The assay was carried out for 30 min at 30 °C. An assay lacking benzaldehyde was performed in parallel to determine background transamination activity.

8- *In silico* docking studies

Visual inspection of the crystal structure of CV-TAm in the holo-form with PLP forming a Schiff's base to K288 (PDB identifier: 4AH3) was performed using PYMOL.⁷ Covalently bound PLP was removed from the model in PYMOL. Side chains B and D were removed to obtain the CV-TAm structure in dimeric form, using AutoDockTools4 (MGL-tools 1.5.4).⁸ Docking of an intermediate of PLP and the amino donors 2-(4-nitrophenyl)ethan-1-amine **1**, 4-(2-aminoethyl)benzotrile hydrochloride **12**, and 5-nitro-2,3-dihydro-1H-inden-2-amine hydrochloride **13**, respectively, into the

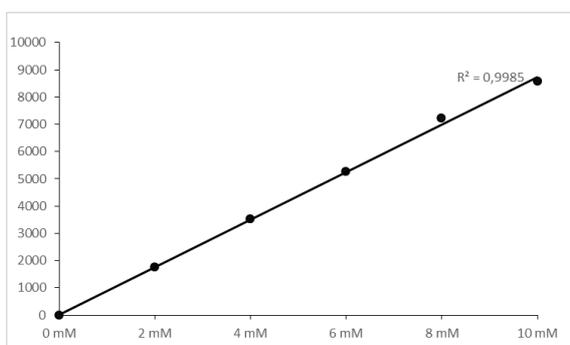
CV-TAm structure was performed using AUTODOCK vina.⁹ A cubic grid centered at (x= 14, y= – 14, z= 16) with sides of 18 Å was used. Defaults were used for docking each substrate. Visual analysis of docked conformations was carried out in PYMOL.

9- Fluorodinitrobenzene (FDNB) derivatisation

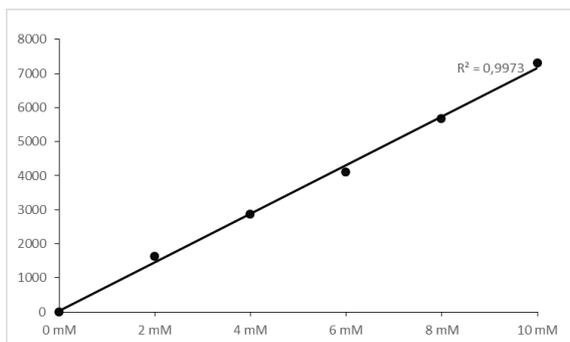
The enzymatic reaction or control reaction (40 µL), 3% FDNB solution in ethanol (160 µL) and NaHCO₃ solution (1 M, 20 µL) were stirred in a closed 1.5 mL eppendorf at 37 °C, 1000 rpm for 90 minutes. Dilute HCl solution (1 M, 50 µL) was added and a sample of 80 µL was withdrawn, filtered and analyzed by HPLC.

10-Calibration Curves: showing concentration versus absorption units at 210 nm (a,b) and 350 nm (c-f)

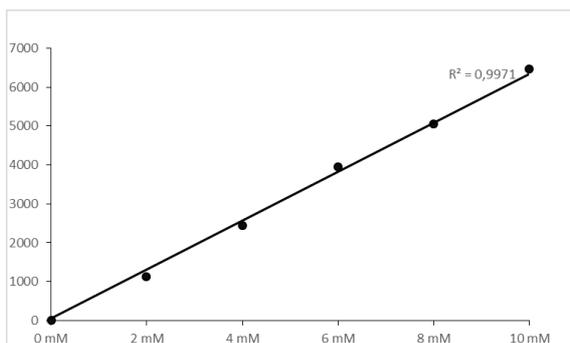
a- Benzylamine



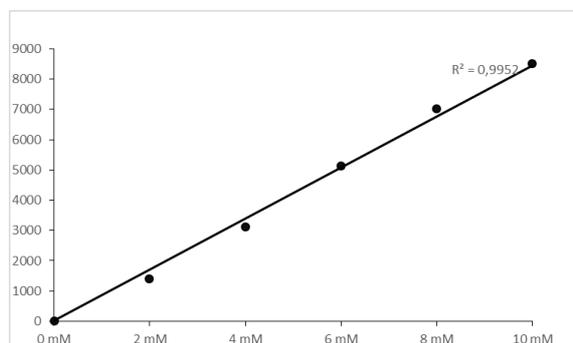
b- α-Methylbenzylamine



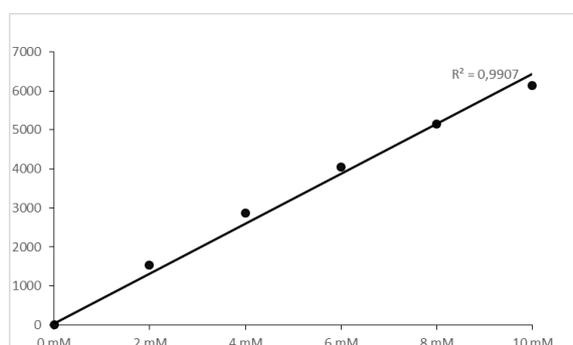
c- DNP-butylamine



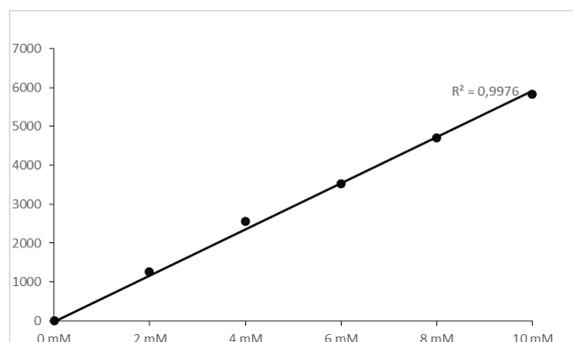
d- DNP-sec-butylamine



e- DNP-cyclohexylamine



f- DNP-cyclohexylmethylamine



11-Screening

Enzyme screening with 6 aldehydes or ketones with 2-(4-nitrophenyl)ethan-1-amine hydrochloride **1** as amine donor were performed with four different enzymes (CV-TAm, Pp-TAm, Kp-TAm and ArRmut11). The enzymatic reactions were performed in 96 well-plate with a total volume of 200 μL containing 2-(4-nitrophenyl)ethan-1-amine hydrochloride **1** (25 mM) as amine donor, an aldehyde or a ketone (10 mM) as amine acceptor, PLP (0.2 mM), KPi buffer (100 mM, pH 7.5), cell lysate (0.4 mg mL⁻¹) at 30 °C and 500 rpm for 90 min. For substrates **2** and **7**, reactions were then quenched with 10 μL of TFA (10% in water), and reaction mixtures were centrifuged to remove the protein. A 150 μL sample of the reaction mixture was diluted with 450 μL of water and then analyzed by HPLC. For substrates **8**, **9**, **10** and **11**, reaction mixtures were derivatized with FDNB

and then analyzed by HPLC. Two negative controls were performed at the same time, one without aldehyde or ketone and another without enzyme.

12-Table 2

Table 2. HPLC analysis of the aldehyde/ketone (10 mM) screening with 1 (25 mM) as amine donor using selected TAMs

Substrate	CV-TAM		Pp-TAM		ArRMut11	
	conv. ^[a] /%	well ^[b]	conv. ^[a] /%	well ^[b]	conv. ^[a] /%	well ^[b]
9	1	A1	1	B1	1	D1
2	54	A2	16	B2	19	D2
10	4	A3	3	B3	1	D3
7	87	A4	22	B4	16	D4
11	54	A5	2	B5	13	D5
8	41	A6	17	B6	7	D6

Reactions were carried out in triplicate. ^[a]Calculated based on HPLC analysis of the amine formed for **2** and **9** at 210 nm and after derivatization with fluorodinitrobenzene (FDNB) for substrates **7**, **8**, **10**, **11** at 350 nm.

^[b]Wells referring to Figure 1.

13-Other amine donors 12 and 13

The enzymatic reaction was performed in 96 well-plate with a total volume of 200 μ L containing 4-(2-aminoethyl)benzotrile hydrochloride **12** or 5-nitro-2,3-dihydro-1H-inden-2-amine hydrochloride **13** (25 mM) as amine donor, benzaldehyde **2** (10 mM) as amine acceptor, PLP (0.2 mM), KPi buffer (100 mM, pH 7.5), cell lysate (protein 0.4 mg mL⁻¹) at 30 °C and 500 rpm for 90 min. Two negative controls were performed at the same time, one without aldehyde or ketone and another without enzyme.

14-HPLC conditions

A Benzylamine and α -methylbenzylamine

Samples were analyzed by HPLC (Agilent 1260 Infinity) using an ACE C18 column (3 μ m, 4.5 x 150 mm) with a gradient of 15-72% eluent B (MeCN) and eluent A (H₂O + 0.1% TFA) over 10 min at 1 mL min⁻¹ at 30 °C. Detection was by UV at 210 nm.

Benzylamine retention time (Rt): 2.9 min; α -methylbenzylamine Rt: 3.86 min

B DNP-butylamine, DNP-sec-butylamine, DNP-cyclohexylamine and DNP-cyclohexylmethylamine

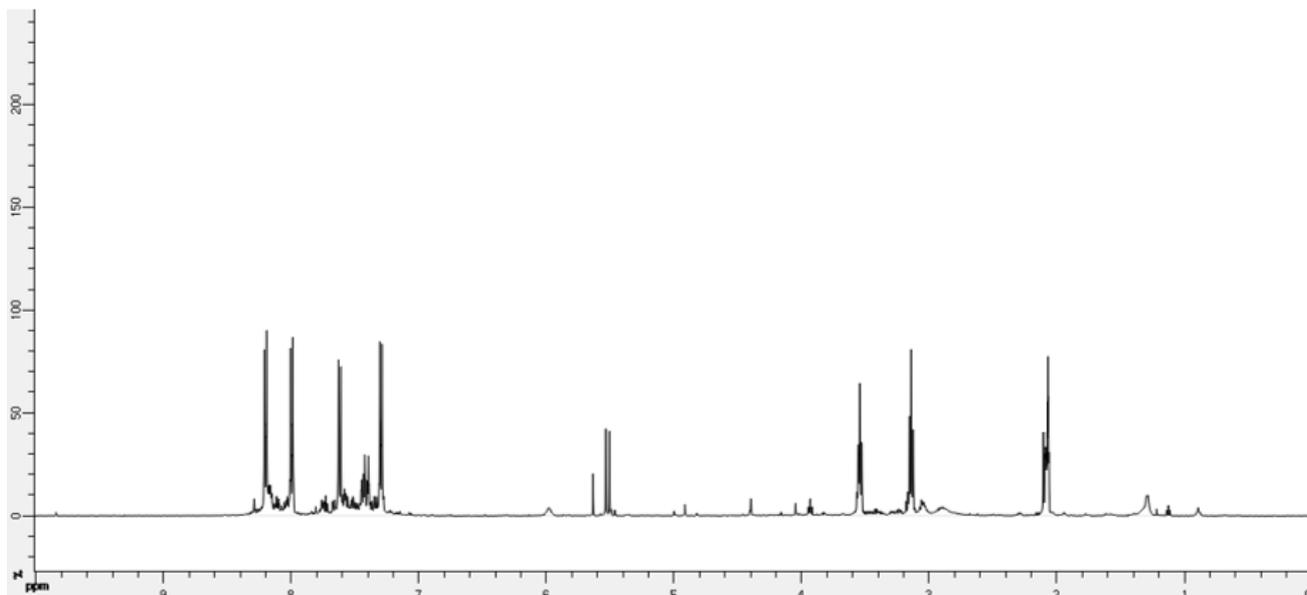
Samples were analyzed by HPLC (Agilent 1260 Infinity) using an ACE C18 column (3 μ m, 4.5 x

150 mm) with a gradient of 20-100% eluent B (MeCN) and eluent A (H₂O + 0.1% TFA) over 19 min at 1 mL min⁻¹ at 30 °C. Detection was by UV at 350 nm.

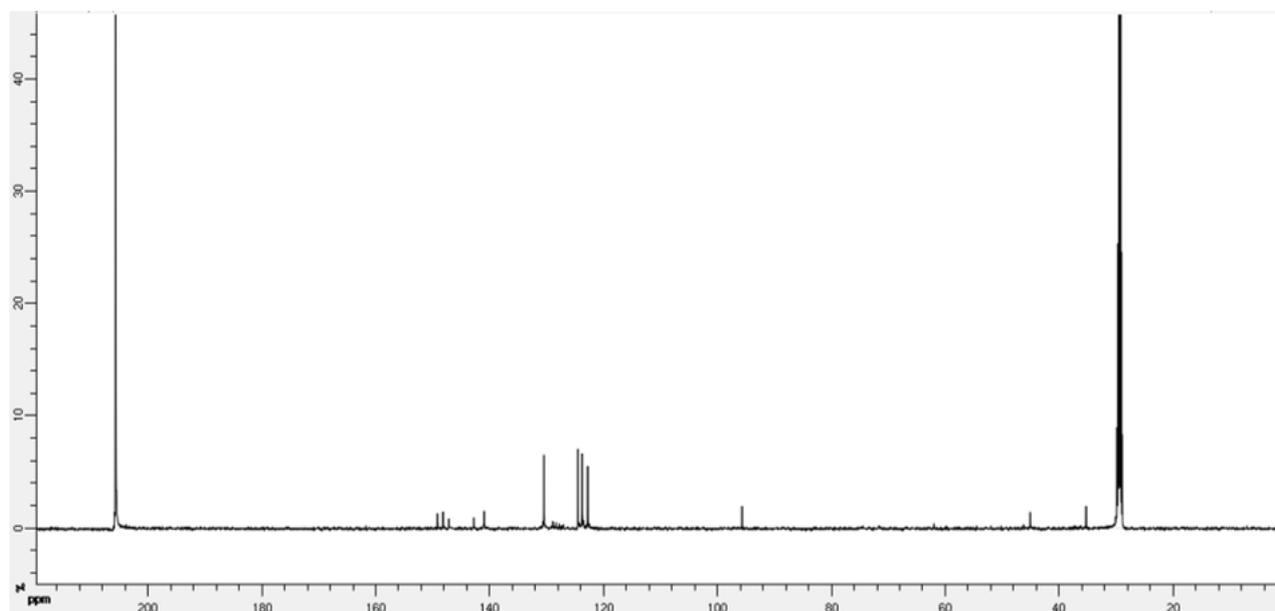
DNP-butylamine Rt: 14.6 min; DNP-*sec*-butylamine Rt: 14.4 min; DNP-cycloxyamine Rt: 16.1 min; DNP-cyclohexylmethylamine Rt: 17.0 min

15-NMR Spectra

¹H NMR (500 MHz; acetone-d₆)

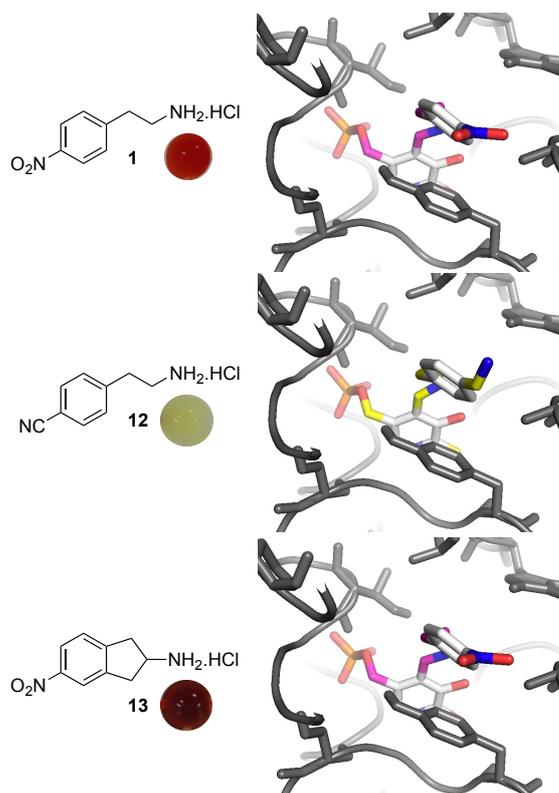


¹³C NMR (125 MHz; acetone-d₆)



16- Figure 1.

Figure 1. Assay coloration and docking of amino donors 1, 12 and 13 bound to PLP in the active site of holo-CV-TAm (PDB ID: 4AH3) using Autodock Vina⁹



References

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