I. Synthesis of the reaction intermediate

(3): To a solution of compound (2) (10 g, 53 mmol) in water/dioxane (2/1) (170 mL) was added sodium bicarbonate (13 g, 159 mmol) and over a two hours period, a solution of benzyl chloroformate (11.8 mL, 69 mmol) in dioxane (70 mL). The reaction mixture was stirred overnight, then washed with ethyl acetate (3 x 200 mL), and the pH was brought to 2 with 6N HCl at 0°C. The aqueous phase was extracted with ethyl acetate (3 x 200 mL). Organic phases were combined and washed successively with brine (200 mL) and distilled water (200 mL), then dried over anhydrous magnesium sulphate and concentrated to obtain the acid intermediate as a colourless oil (16.7 g, 97%).

MS (ES⁺): [M+H]⁺, 324.1; [M+H - tBu]⁺, 268.1

¹H NMR (CDCl₃, 300 MHz): 1.45 (s, 9H); 2.95 (dd, 2H, J = 4.5 et 14 Hz); 4.55 (q, 1H, J = 4.3Hz); 5.10 (s, 2H); 5.8 (d, 1H, J = 8 Hz); 7.35 (m, 5H); 10.7 (sl, 1H).

¹³C NMR (CDCl₃, 75 MHz): 27.8; 36.7; 50.8; 67.1; 82.8; 128.1; 128.2; 128.5; 135.1; 156.06; 169.5; 176.3.

To a suspension of the resulting oil (15 g, 46.4 mmol) and BOP (27 g, 60.4 mmol) in anhydrous THF was added DIEA (10.5 mL, 60.4 mmol). The reaction mixture was stirred for 10 minutes until it turned yellow, and cooled down to 0°C for 15 minutes. Then NaBH₄ (2.3 g, 60.4 mmol) was added portionwise. The reaction mixture was stirred overnight. The solvent was evaporated in vacuo and the crude was dissolved in ethyl acetate (250 mL). The organic layer was washed successively with brine (200 mL) and distilled water (200 mL). The organic layer was washed over anhydrous magnesium sulphate and concentrated to afford a colourless oil. Compound (3) was purified by chromatography on silica gel (petroleum ether/ether: 3/7) to afford a colourless oil (13.95 g, 96%).
RF: (petroleum ether /ether: 3/7) = 0.42

MS (ES+): [M+H]+, 310.2; [M+H - tBu]+, 254.1

1H NMR (CDCl3, 300 MHz): 1.43 (s, 9H); 2.09 (dd, 2H, J = 4.6 et 15.2 Hz); 3.48 (s, 1H); 3.8 (m, 2H); 4.45 (m, 1H); 5.10 (s, 2H); 5.87 (d, 1H, J = 8 Hz); 7.35 (m, 5H).

13C NMR (CDCl3, 75 MHz): 27.9; 35.5; 52.0; 58.4; 67.0; 82.4; 128.07; 128.1; 128.3; 129.0; 136.1; 156.8; 169.5; 171.7.

(4): To a solution of compound (3) (5.8 g, 18.8 mmol) in anhydrous DCM (260 ml) under argon atmosphere were added iodine (14.3 g, 56.4 mmol), triphenylphosphine (14.8 g, 56.4 mmol) and after 10 minutes, imidazole (9.6 g, 141 mmol). The reaction mixture was refluxed for 3 hours. The reaction mixture was cooled down to room temperature and washed successively with a molar solution of potassium sulphate (3 x 150 mL), a saturated solution of sodium bicarbonate (3 x 150 mL), a 5% sodium thiosulfate solution (3 x 150 mL), and with brine (3 x 150 mL). The organic layer was dried over anhydrous magnesium sulphate and concentrated to afford a yellow oil. The compound (4) was obtained after silica gel chromatography (petroleum ether/ethyl acetate: 10/0 to 8/2) as yellow oil (6.2 g, 78%).

RF: (petroleum ether/ethyl acetate: 8/2) = 0.65

MS (ES+): [M+Na]+, 442.0; [M+H]+, 420.1; [M+H - tBu]+, 364.2

1H NMR (CDCl3, 300 MHz): 1.45 (s, 9H); 2.15-2.45 (2m, 2H); 3.15 (m, 2H); 4.35 (m, 1H); 5.10 (s, 2H); 5.87 (d, 1H, J = 8 Hz); 7.35 (m, 5H).

13C NMR (CDCl3, 75 MHz): 16.1; 28.4; 38.5; 54.2; 56.2; 67.9; 83.6; 128.95; 129.03; 129.2; 129.3; 129.4; 137.0; 156.7; 171.0.

(6): To a solution of tert-butyl glutamate chlorhydrate salt (5) (0.62 g, 2.10 mmol) in anhydrous DCM (20 mL) at 0°C was added TEA (0.29 mL, 2.10 mmol). The solution was filtrated and TEA was added (0.60 mL, 4.41 mmol) and 2,2,5,6,7-pentamethylchromane sulfonyl chloride (0.7 g, 3.30 mmol) dissolved in anhydrous DCM (4 mL) on a two hour period with a syringe pump. After overnight stirring, the reaction mixture was concentrated and dissolved in ethyl acetate (20 mL). The organic layer was successively washed with a 10 % citric acid solution (3 x 20 mL), a saturated solution of sodium bicarbonate (3 x 20 mL) and distilled water (2 x 20 mL), then dried on anhydrous magnesium and concentrated. Compound (6) was obtained after silica gel chromatography (petroleum ether /ether 8/2) as a colorless oil (1 g, 91%).

RF: (petroleum ether/ether 6/4) = 0.30
**MS (ES⁺):** [M+H]⁺, 526.3 ; [M+H - tBu]⁺, 470.2 ; [M+H- 2 tBu]⁺, 414.2

**¹H NMR** (CDCl₃, 300 MHz) : 1.14-1.24 (s, 9H, 1 tBu); 1.22-1.24 (2s, 6H, 2 CH₃); 1.36 (s, 9H, 1 tBu); 1.70-1.99 (m, 4H, CH₂m, CH₂β); 2.03 (s, 3H, CH₃); 2.27-2.33 (m, 2H, CH₂γ); 2.47-2.49 (2s, 6H, 2 CH₃α); 2.54-2.59 (t, 2H, J = 6.8 et 6.8 Hz, CH₂); 3.66-3.67 (m, 1H, CHα); 5.20-5.23 (d, 1H, J = 9.4 Hz, NH).

**¹³C NMR** (CDCl₃, 75 MHz) : 11.4; 12.2; 14.1; 17.3; 18.3; 19.4; 20.4; 21.5; 22.6; 26.5; 26.8; 27.6; 28.0; 28.6; 29.0; 29.7; 31.0; 32.7; 55.1; 73.9; 76.6; 80.5; 82.3; 118.3; 124.6; 128.4; 136.4; 136.6; 154.6; 171.1; 172.0.

(7): to a solution of (6) (0.81 g, 1.54 mmol) in anhydrous ACN (20 mL) was added cesium carbonate (0.75 g, 2.31 mmol). The reaction mixture was vigorously stirred for 30 minutes, and heated to 55°C. Then was added dropwise with a syringe pump (4) (0.64 g, 1.54 mmol) in solution in anhydrous ACN (5 mL). After stirring overnight at 55°C, the reaction mixture was concentrated and the crude was dissolved in ethyl acetate (20 mL). The organic layer was successively washed with brine (3 x 20 mL) and distilled water (2 x 20 mL), then dried on anhydrous magnesium and concentrated. Compound (7) was obtained after silica gel chromatography (petroleum ether/ether 7/3 then DCM/ether 99/1 to 98/2) as a colorless oil (1g, 80 %).

**Rf:** (petroleum ether/ether 6/4) = 0.57

**MS (ES⁺):** [M+Na]⁺, 839.6 ; [M+H]⁺, 817.6 ; [M+H - tBu]⁺ ; 761.5 ; [M+H- 2 tBu]⁺, 705.5 ; [M+H- 3 tBu]⁺, 649.5

**¹H NMR** (CDCl₃, 300 MHz) : 1.17-1.36 (5s, 33H, 3 tBu, 2 CH₃); 1.70-2.00 (m, 6H, CH₂, CH₂β, CH₂γ); 2.03 (s, 3H, CH₃); 2.17-2.20 (m, 2H, CH₂γ); 2.41-2.42 (2s, 6H, 2 CH₃α); 2.52-2.56 (t, 2H, J = 6.8 et 6.3 Hz, CH₂); 3.27-3.52 (m, 2H, CH₂γ); 4.01-4.06 (m, 1H, CHα); 4.10-4.13 (t, 1H, CHα'); 5.02-5.03 (s, 2H, CH₂Ph); 5.19-5.25 (1H, J = 6 Hz, NH); 7.24-7.29 (m, 5H, arom).

**¹³C NMR** (CDCl₃, 75 MHz) : 11.1; 16.0; 17.0; 18.2; 20.3; 21.4; 24.4; 25.4; 25.5; 26.6; 26.6; 26.8; 27.8; 28.4; 30.6; 31.3; 31.4; 39.4; 51.6; 57.0; 65.6; 72.8; 75.4; 79.3; 80.7; 81.0; 117.2; 123.5; 126.4; 126.8; 127.2; 135.2; 136.5; 136.7; 153.8; 154.8; 169.1; 169.6; 170.2.

(1): To compound (7) (354 mg, 0.44 mmol) was added a solution of 45% hydrogen bromide in glacial acetic acid (1.5 mL). The reaction mixture was vigorously stirred for 45 min and then quenched with ether (5 mL). The precipitate was filtrated and washed with ether (5 x 5 mL). The white solid was dissolved in 5 mL of a 0.01N HCl solution and freeze dried to afford the product (1) as a solid (140 mg).
Supplementary Material (ESI) for Chemical Communications

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**MS (ES^+):** [M+H]^+, 249.1 ; [M+H - H2O]^+, 231.1

**HRMS:** for C9H17N2O6: calculated: 249.1087 observed: 249.1079

**1H NMR** (CDCl₃, 300 MHz): 2.04-2.43 (m, 4H, 2CH₂β); 2.47-2.62 (m, 2H, CH₂γ); 3.20-3.39 (m, 2H, CH₂γ'); 4.00-4.10 (dd, 1H, J = 5.0 and 3.0 Hz, CHα); 4.10-4.20 (dd, 1H, J = 5.6 and 3.0 Hz, CHα').

**13C NMR** (D₂O, 75 MHz): 23.7; 26.3; 29.2; 43.0; 50.3; 59.1; 170.35; 170.40; 176.0.
Synthesis and trapping of a reaction intermediate in an archaeal thermoNicotianamine synthase.

Contents:

**General methods:**

**Compound (1):**
- MS ESI⁺: 7
- HRMS ESI⁺: 7
- ¹H NMR: 8
- ¹³C NMR: 8

**Compound (3):**
- MS ESI⁺: 9
- ¹H NMR: 9
- ¹³C NMR: 10

**Compound (4):**
- MS ESI⁺: 11
- ¹H NMR: 11
- ¹³C NMR: 12

**Compound (6):**
- MS ESI⁺: 13
- ¹H NMR: 13
- ¹³C NMR: 14
- COSY: 14

**Compound (7):**
- MS ESI⁺: 15
- ¹H NMR: 15
- ¹³C NMR: 16
- COSY: 17
General methods:

All reactions involving air-sensitive reagents were performed under nitrogen or argon. Solvents and reagents were purchased from Aldrich and Fluka. THF was freshly distilled from benzophenone/sodium prior to use. Merck silica gel 60 F-254 plates were used for TLC. Column chromatographies were performed using silica gel (Merck 60, 230-400 mesh). $^1$H NMR and $^{13}$C NMR spectra were recorded at 300 and 75 MHz, respectively. All chemical shifts were recorded as values (ppm) relative to internal tetramethylsilane when CDCl$_3$ was used as solvent. The $^{13}$C standard in D$_2$O was calculated from the chemical shift of water in $^1$H NMR and the apparatus frequency (SF1=BF1). Low resolution mass spectrometry (MS) was performed using electrospray ionization (ESI) on a Micromass Platform II spectrometer. HRMS were measured on a JEOL JMS-SX-102A spectrometer or on a Micromass QTof spectrometer. High pressure liquid chromatography (HPLC) analysis were performed on a Waters Alliance 2796 apparatus with a Chromolith SpeedROD (50 x 4.6 mm) column and a diode array detector.

Content: NMR of all compounds are in chloroform (CDCl$_3$) except (1) in D$_2$O.

- $^1$H NMR (Bruker spectrometer Advance 300, 300 MHz)
- $^{13}$C NMR (Bruker spectrometer Advance 300, 75 MHz)
- MS ESI$^+$ specters (Micromass Platform II spectrometer)
- HRMS ES$^+$ (Micromass QTof spectrometer)
Compound (I):

MS ESI⁺:

HRMS ES⁺:

Single Mass Analysis
Tolerance = 3.0 mDa / DBE: min = -10.0, max = 100.0
Isotope cluster parameters: Separation = 1.0  Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
95 formula(e) evaluated with 3 results within limits (all results (up to 1000)) for each mass

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$^1$H NMR:

![$^1$H NMR Spectrum](image1)

$^{13}$C NMR:

![$^{13}$C NMR Spectrum](image2)
Compound (3):

MS ESI⁺:

\[ \text{Compound (3)} \]

$^1$H NMR:
$^{13}$C NMR:
**Compound (4):**

**MS ESI⁺:**

![MS ESI⁺ spectrum](image)

**¹H NMR:**

![¹H NMR spectrum](image)
$^{13}$C NMR:

**Compound (4)**
Compound (6):

MS ESI⁺:

\(^1\)H NMR:
$^{13}$C NMR:

COSY:
Compound (6):

MS ESI⁺:

1H NMR:
13C NMR:
COSY:

![COSY spectrum of Compound (7)](image-url)
II. Crystallization and structure determination of MtNAS in complex with the reaction intermediate.

Expression and purification of MtNAS-E81Q mutant was already described. Crystals of the MtNAS-E81Q – Reaction Intermediate complex was obtained by mixing the purified protein solution (10 mg/ml) with equal volumes of the reservoir solution (100 mM Bis-tris-propane pH 8.5, 20% (w/v) PEG3350, 200 mM NaBr) supplemented with 5 mM 1. Before data collection, the crystal was soaked briefly in paraffin oil then flash-cooled directly in liquid nitrogen. Diffraction data was collected at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). Data sets were processed with MOSFLM2 and scaled using SCALA from the CCP4 suite3. The crystal belonged to space group $P_{2_1}2_12_1$ (see Supplementary Table 1 for more details). The structure of the MtNAS-E81Q – Reaction Intermediate complex was solved by molecular replacement with the MtNAS-tNA structure as the search model and refined at 1.7 Å resolution to an $R$-factor of 18.8% ($R_{free} = 23.9\%$) (Supplementary Table 1). The model contains amino acid residues 2-265, two reaction intermediate and 961 water molecules. Graphics were generated by using the program PYMOL (www.pymol.org).

Supplementary references


### Data collection

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### Refinement

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*Supplementary Table 1. Data collection and refinement statistics.*