Supplementary information

Carboxymethylproline synthase catalysed syntheses of functionalized *N*-heterocycles

Refaat B. Hamed, [a] Jasmin Mecinović, [a] Christian Ducho,[a, ‡] Timothy D. W. Claridge[a] and Christopher J. Schofield*[a]

[a] University of Oxford, Department of Chemistry, Chemistry Research Laboratory, Mansfield Road, Oxford, OX1 3TA, United Kingdom. Fax: +44 1865 2-75674; Tel: +44 1865 2-75625; E-mail: christopher.schofield@chem.ox.ac.uk

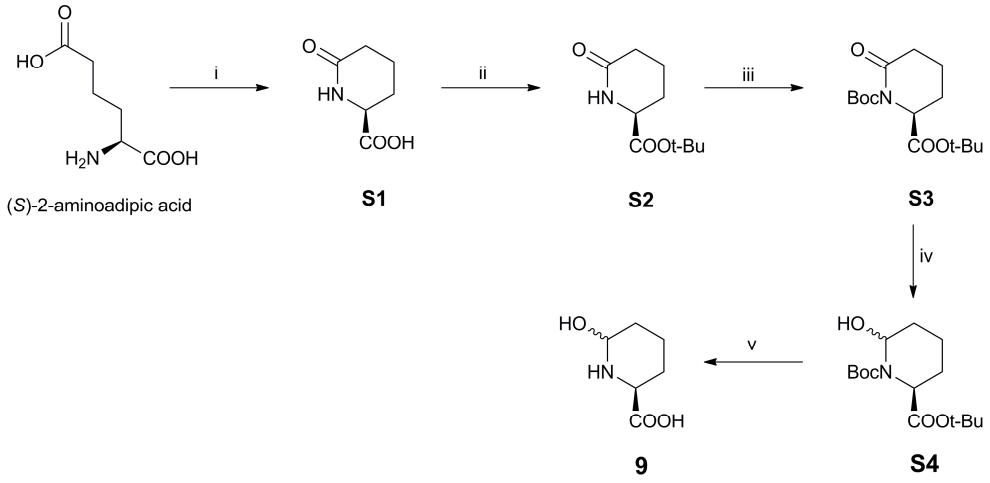
[‡] Current address: Georg-August-University Göttingen, Department of Chemistry, Institute of Organic and Biomolecular Chemistry, Göttingen, Germany.

Synthesis of L-aminoaspartate semialdehyde 11, L-glutamate semiialdehyde 4, and L-aminopimelate semialdehyde 12:

The protected forms of L-aminoaspartate semialdehyde **11**, L-glutamate semialdehyde **4** and L-aminopimelate semialdehyde **12** were prepared and deprotected as reported.[1-3]

Synthesis of L-aminoadipate semialdehyde 9:

The preparation of **9** was carried out according to Scheme S1.



Scheme S1: Synthesis of **9** from (*S*)-2-aminoadipic acid**. (i):** 20 % aqueous acetic acid, reflux, 3 h, 53%; **(ii)** *t*-BuOAc, $HClO_4$, rt, 23 h, 64 %; **(iii)** Boc_2O, 4-(*N*,*N*-dimethylamino)pyridine, NEt_3, CH_2Cl_2, rt, 5 days, 46 %; **(iv)** $LiEt_3BH$, THF, -78 °C, 5 h, 80 %; **(v)** HCOOH, H_2O, no purification**.**

(*S*)-6-oxo-pipecolic acid *tert*-butyl ester S2

To a solution of (*S*)-6-oxo-pipecolic acid) **S1**[4], (173 mg, 1.21 mmol) in *tert*-butyl acetate (2.5 mL), 70 % aqueous perchloric acid (115 µL, 134 mg $HClO_4$, 1.33 mmol) was slowly added and the mixture stirred at room temperature for 23 h. After dilution

with CH_2Cl_2, the reaction mixture was carefully added to saturated $NaHCO_3$ solution (20 mL), and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to give 155 mg (64 %) of S**2** as colourless oil which slowly crystallised. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.08 (1H, s, NH), 3.94-3.92 (1H, m, αCH), 2.41-2.27 (2H, m, CH_2), 2.17-2.11 (1H, m, CH), 1.91-1.85 (1H, m, CH), 1.79-1.69 (2H, m, CH_2), 1.46 (9H, s, $C(CH_3)_3$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.5 (CO), 170.2 (CO), 82.8 (**C**$(CH_3)_3$), 55.5 (αCH), 31.2 (CH_2), 28.1 (C(**C**$H_3)_3$), 25.7 (CH_2), 19.8 (CH_2) ppm; MS (ESI-HR) *m/z* calcd 222.1101 ($M + Na^+$), found 222.1101 ($M + Na^+$).

(*S*)-6-oxo-*N*-(*tert*-butyloxycarbonyl)-pipecolic acid *tert*-butyl ester S3

Under a nitrogen atmosphere, **S2** (130 mg, 0.652 mmol) was dissolved in dry CH_2Cl_2 (10 mL), triethylamine (0.14 mL, 0.10 g, 0.99 mmol) and 4-(*N*,*N*-dimethylamino)-pyridine (DMAP) (8.0 mg, 65 µmol) were added at room temperature. To this mixture, a solution of di-(*tert*-butyl)-dicarbonate ($(Boc)_2O$) (213 mg, 0.976 mmol) in dry CH_2Cl_2 (5 mL) was added and the resulting reaction mixture stirred at room temperature for 5 days. Subsequently, water (5 mL) was added and the mixture was stirred at room temperature for 30 min, before more water (10 mL) and CH_2Cl_2 (10 mL) were added. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The resultant crude product was purified by column chromatography (hexane/EtOAc 5 : 1) to give 91 mg (47 %) of **S3** as a colourless oil. ^{1}H NMR (500 MHz, C_6D_6) δ 4.53-4.52 (1H, m), 2.26-2.21 (1H, m), 1.95 (1H, ddd, *J* = 6.9 Hz, 10.2 Hz, 17.1 Hz), 1.73-1.67 (1H, m), 1.48 (9H, s), 1.37-1.23 (11H, m), 1.02-0.95 (1H, m) ppm; ^{13}C NMR (126 MHz, C_6D_6) δ 171.2, 169.6, 154.9, 82.9, 81.7, 59.6, 35.1, 28.4, 28.2, 26.2, 19.1 ppm; MS (ESI-HR) *m/z* calcd 322.1625 ($M + Na^+$), found 322.1629 ($M + Na^+$); R_f 0.50 (hexane/EtOAc 1 : 1).

(2*S*)-6-Hydroxy-*N*-(*tert*-butyloxycarbonyl)-pipecolic acid *tert*-butyl ester (*N*-Boc-L-2-aminoadipic acid semialdehyde *tert*-butyl ester) S4

Under a nitrogen atmosphere, **S3** (75 mg, 0.25 mmol) was dissolved in dry THF (2 mL) and the solution cooled to -78°C. At this temperature, a 1.0 M solution of lithium triethyl borohydride (Super-Hydride™) in THF (0.30 mL, 0.30 mmol) was added and the reaction mixture stirred at -78°C for 5 h. The reaction was quenched with MeOH (1 mL) and water (1 mL) and then warmed to room temperature. CH_2Cl_2 (15 mL) and water (15 mL) were added, and the aqueous phase was extracted with

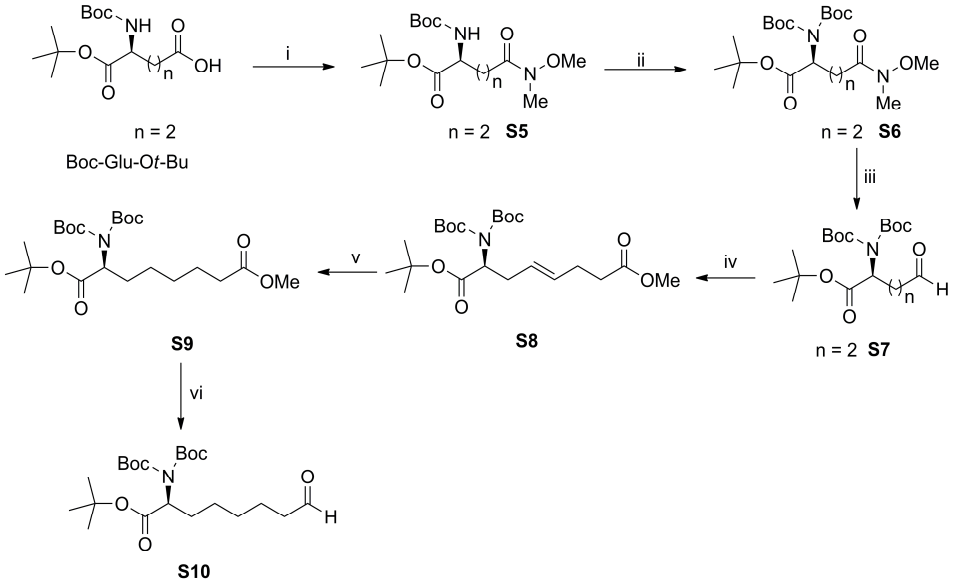
CH_2Cl_2 (2 x 15 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The resultant crude product was purified by column chromatography (hexane/EtOAc 8 : 1) to give 60 mg (80 %) of **S4** as a colourless solid. ^{1}H NMR (500 MHz, C_6D_6) δ 9.16 (0.3H, t, J = 1.2 Hz, CHO), 6.10-6.06 (0.3H, m, NCH), 5.88-5.83 (0.4H, m, NCH), 5.02 (0.4H, d, J = 5.4 Hz, αCH), 4.89 (0.3H, d, J = 6.6 Hz, αCH), 4.55-4.46 (0.3H, m, αCH), 3.87 (0.3H, s, OH), 2.04-1.64 (3H, m, CH_2 + CH), 1.47-1.07 (21H, m, CH_2 + CH, 2 x $C(CH_3)_3$) ppm; ^{13}C NMR (126 MHz, C_6D_6) δ 175.9 (CO), 174.0 (CO), 156.4 (CO), 82.5 (**C**$(CH_3)_3$), 81.8 (**C**$(CH_3)_3$), 80.8 (**C**$(CH_3)_3$), 80.5 (**C**$(CH_3)_3$), 75.5 (NCH), 74.7 (NCH), 55.5 (αCH), 32.7 (CH_2), 31.6 (CH_2), 28.7 (C(**C**$H_3)_3$), 28.6 (C(**C**$H_3)_3$), 28.3 (C(**C**$H_3)_3$), 28.1 (C(**C**$H_3)_3$), 27.0 (CH_2), 26.7 (CH_2), 14.9 (CH_2), 14.6 (CH_2) ppm; MS (ESI-HR) *m/z* calcd 324.1781 ($M + Na^+$), found 324.1784 ($M + Na^+$); R_f 0.20 (hexane/EtOAc 6 : 1).

(2*S*)-6-Hydroxy-pipecolic acid (L-2-aminoadipic acid semialdehyde, L-AASA), **9**

A mixture of **S4** (4.7 mg, 15.6 µmol) with formic acid (100 µL) and water (20 µL) was boiled for *ca.* 1-2 s and then allowed to cool again. This procedure was repeated four times. Subsequently, the reaction mixture was cooled to room temperature, diluted with water (880 µL), frozen and stored at -80°C. This solution of **9** was directly used for enzyme assays.

<u>Synthesis of L-aminosuberate semialdehyde 14</u>:

The preparation of **14** was carried out according to Scheme S2.



Scheme S2: Synthesis of **14** from Boc-Glu-OtBu**. (i):** *N*,*O*-dimethylhydroxylamine.HCl, HOBt, EDCI, Et_3N, rt, 16 h, 91%; **(ii)** $(Boc)_2O$ and DMAP, rt, 3 days, 94 %; **(iii)** Schwartz reagent in THF, rt, 20 min., 83 %; **(iv)** Ethoxycarbonylpropyltriphenylphosphinium bromide in anhydrous THF, sodium hexamethyldisilazane, -78 °C, 30 min., 37 %; **(v)** H_2, Pd-C, rt, 4 hr, 99%**; (vi)** DIBAL-H, -78 °C, 45 min, 72%.

(*S*)-*tert*-Butyl 2-(*tert*-butoxycarbonylamino)-5-(methoxy(methyl)amino)-5-oxopentanoate, **S5**

To a stirred solution of Boc-Glu-O*t*-Bu (2.42 g, 8.0 mmol) in CH_2Cl_2 (80 mL) under N_2 atmosphere were added *N*,*O*-dimethylhydroxylamine hydrochloride (858 mg, 8.8 mmol), HOBt (1.23 g, 8.8 mmol), EDCI (1.69 g, 8.8 mmol) and Et_3N (2.45 mL, 17.6 mmol). The resulting reaction mixture was then stirred for 16 hours at room temperature. After washing with 5% $KHSO_4$ (2 × 80 mL), 5% $NaHCO_3$ (2 × 80 mL), and H_2O (80 mL), the organic phase was dried over $MgSO_4$. The Crude product was purified by column chromatography (petroleum ether 40-60 ºC /EtOAc 7:3 to 4:6) to afford 2.52 g (91%) of **S5** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.19 (1H, d, *J* = 8.0 Hz, NH), 4.20-4.17 (1H, m, αCH), 3.66 (3H, s, OCH_3), 3.16 (3H, s, NCH_3), 2.56-2.44 (2H, m, CH_2), 2.16-2.10 (1H, m, CH), 1.94-1.86 (1H, m, CH), 1.45 (9H, s, $C(CH_3)_3$), 1.42 (9H, s, $C(CH_3)_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6 (CO), 155.5 (CO), 81.9 (**C**$(CH_3)_3$), 79.5 (**C**$(CH_3)_3$), 61.2 (OCH_3), 53.7 (αCH), 32.2 (NCH_3), 28.3 (C(**C**$H_3)_3$), 28.0 (C(**C**$H_3)_3$), 27.6 (CH_2) ppm.

(*S*)-*tert*-Butyl 2-(bis(*tert*-butoxycarbonyl)amino)-5-(methoxy(methyl)amino)-5-oxopentanoate S6

To a stirred solution of **S5** (2.42 g, 7.0 mmol) in acetonitrile (50 mL) under N_2 atmosphere were added $(Boc)_2O$ (7.63 g, 35 mmol) and 4-Dimethylaminopyridine (171 mg, 1.4 mmol) and the resulting mixture was stirred for 3 days at room temperature. The solvent was then evaporated and the residue redissolved in EtOAc (80 mL). The organic layer was washed with 5% $NaHSO_4$ (80 mL), 5% $NaHCO_3$ (80 mL), brine (80 mL), H_2O (80 mL), and dried over Na_2SO_4. Purification by column chromatography (petroleum ether 40-60 ºC/EtOAc 7:3 to 6:4) afforded 2.93 g (94%) of **S6** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.81 (1H, dd, ,αCH), 3.65 (3H, s, OCH_3), 3.16 (3H, s, NCH_3), 2.52-2.42 (3H, m, CH_2 + CH), 2.15-2.09 (1H, m, CH), 1.49 (18H, s, N($C(CH_3)_3$), 1.44 (9H, s, $C(CH_3)_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4 (CO), 152.3 (CO), 82.8 (**C**$(CH_3)_3$), 81.2 (**C**$(CH_3)_3$), 61.1 (OCH_3), 58.4 (αCH), 28.6 (NCH_3), 28.0 (C(**C**$H_3)_3$), 28.0 (C(**C**$H_3)_3$), 24.3 (CH_2) ppm.

(*S*)-*tert*-Butyl 2-(bis(*tert*-butoxycarbonyl)amino)-5-oxopentanoate S7

Schwartz reagent (Cp_2ZrHCl, 1.55 g, 6.0 mmol) was suspended in THF (15 mL) under N_2 atmosphere at room temperature, and a solution of **S6** (1.78 g, 4.0 mmol) in THF (10 mL) was added. The reaction mixture was stirred for 20 minutes and then quenched with H_2O (110 μL, 6.0 mmol). The solvent was removed *in vacuo* and the

crude product purified by column chromatography (petroleum ether 40-60 ºC/EtOAc 8:2) to afford 1.27 g (83%) of **S7** as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.76 (1H, s, CHO), 4.74 (1H, dd, *J* = 4.5 Hz, 9.5 Hz, αCH), 2.60-2.37 (3H, m, CH_2 + CH), 2.19-2.07 (1H, m, CH), 1.50 (18H, s, $N(C(CH_3)_3)$, 1.45 (9H, s, $C(CH_3)_3$) ppm.

(*S*,*E*)-1-*tert*-Butyl 8-methyl 2-(bis(*tert*-butoxycarbonyl)amino)oct-4-enedioate S8

Ethoxycarbonylpropyltriphenylphosphinium bromide (809 mg, 1.77 mmol) was dissolved in anhydrous THF (15 mL) and cooled to -78ºC. Sodium hexamethyldisilazide (1M in THF, 1.69 mL, 1.69 mmol) was added over 5 minutes under N_2 atmosphere, and the reaction mixture was then stirred for 30 minutes. Compound **S7** in anhydrous THF (5 mL) was then added, and the resulting mixture was allowed to warm to room temperature and stirred for 18 hours. The reaction was quenched with 10% $KHSO_4$ (20 mL) and the mixture was extracted with EtOAc (2 × 40 mL). The combined organic extracts were dried over Na_2SO_4 and purified by column chromatography (petroleum ether 40-60 ºC/EtOAc 9:1) to afford 281 mg (37%) of **S8** as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.47-5.36 (2H, m, 2 × =CH), 4.79 (1H, dd, *J* = 5.0 Hz, 10.0 Hz, αCH), 4.11 (2H, q, *J* = 7.0 Hz, OCH_2), 2.85-2.77 (1H, m, βCH), 2.72-2.66 (1H, m, βCH), 2.40-2.29 (4H, m, 2 × CH_2), 1.50 (18H, s, $N(C(CH_3)_3)$, 1.45 (9H, s, $C(CH_3)_3$), 1.24 (3H, t, *J* = 7.0 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.0 (CO), 169.4 (CO), 152.5 (CO), 130.6 (=CH), 126.6 (=CH), 82.7 ($\mathbf{C}(CH_3)_3$), 81.4 ($\mathbf{C}(CH_3)_3$), 60.3 (OCH_2), 58.7 (αCH), 34.2 (CH_2), 28.0 ($C(\mathbf{C}H_3)_3$), 27.9 ($C(\mathbf{C}H_3)_3$), 27.5 (CH_2), 22.8 (CH_2), 14.2 (CH_3) ppm.

(*S*)-1-*tert*-Butyl 8-methyl 2-(bis(*tert*-butoxycarbonyl)amino)octanedioate S9

To a stirred solution of **S8** (250 mg, 0.53 mmol) in dry EtOAc (10 mL) was added Pd-C (10%, 35 mg). The reaction mixture was then stirred for 4 hours under an H_2 atmosphere, and filtered through a pad of celite. The filtrate was evaporated and purified by column chromatography (petroleum ether 40-60 ºC/EtOAc 9:1) to give 249 mg (99%) of **S9** as colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.70 (1H, dd, *J* = 5.0 Hz, 9.5 Hz, αCH), 4.11 (2H, q, *J* = 7.0 Hz, OCH_2), 2.28 (2H, t, *J* = 7.5 Hz, CH_2COO), 2.07-2.00 (1H, m, CH), 1.89-1.82 (1H, m, CH), 1.65-1.60 (2H, m, CH_2), 1.50 (18H, s, $N(C(CH_3)_3)$, 1.44 (9H, s, $C(CH_3)_3$), 1.38-1.31 (4H, m, 2 × CH_2), 1.25 (3H, t, *J* = 7.0 Hz, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.7 (CO), 170.0 (CO), 152.5 (CO), 82.7 ($\mathbf{C}(CH_3)_3$), 81.1 ($\mathbf{C}(CH_3)_3$), 60.2 (OCH_2), 58.8 (αCH), 34.3 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 28.0 ($C(\mathbf{C}H_3)_3$), 27.9 ($C(\mathbf{C}H_3)_3$), 26.1 (CH_2), 24.8 (CH_2), 14.2 (CH_3) ppm.

(*S*)-*tert*-Butyl 2-(bis(*tert*-butoxycarbonyl)amino)-8-oxooctanoate S10

To a stirred solution of **S9** (236 mg, 0.50 mmol) in dry Et_2O (10 mL) cooled to -78ºC under N_2 atmosphere was added diisobutylaluminium hydride (1M in hexane, 0.75 mL, 0.75 mmol). The reaction mixture was stirred for 45 minutes, quenched with H_2O (65 μL, 3.6 mmol), and allowed to warm to room temperature. The mixture was then stirred for 30 minutes, dried over $MgSO_4$, and filtered through a pad of celite. Removal of the solvent of the filtrate and purification by column chromatography (petroleum ether 40-60 ºC/EtOAc 9:1 to 8:2) afforded 161 mg (72%) of **S10** as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.75 (1H, t, *J* = 2.0 Hz, CHO), 4.70 (1H, dd, *J* = 5.0 Hz, 9.5 Hz, αCH), 2.42 (2H, td, *J* = 2.0 Hz, 7.5 Hz, CH_2CO), 2.07-2.00 (1H, m, βCH), 1.89-1.82 (1H, m, βCH), 1.67-1.60 (2H, m, CH_2), 1.50 (18H, s, $N(C(CH_3)_3)$, 1.44 (9H, s, $C(CH_3)_3$), 1.39-1.33 (4H, m, 2 × CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.7 (CHO), 169.9 (CO), 152.5 (CO), 82.7 ($\mathbf{C}(CH_3)_3$), 81.1 ($\mathbf{C}(CH_3)_3$), 60.2 (OCH_2), 58.7 (αCH), 43.8 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 28.0 ($C(\mathbf{C}H_3)_3$), 27.9 ($C(\mathbf{C}H_3)_3$), 26.1 (CH_2), 21.9 (CH_2) ppm.

CarB mutagenesis:

CarB mutagenesis was performed according to the QuikChange® Site-Directed Mutagenesis Protocol (Stratagene). A pET24a/carB was used as template for PCR mutagenesis. Oligonucleotide primers were obtained from Sigma-Genosys. For CarBH229A mutant preparation, the following Oligonucleotide primers were used: 5'-ccaaagctgtcGCGaaggcagcgttccagg-3' and 5'-gaacgctgccttCGCgacagctttggaagc-3'. Successful mutagenesis was verified by DNA sequencing. Expression and purification of CarBH229A variant were as for wt CarB.

The CarB/ CarBH229A/ ThnE purification and assay:

CarB and CarB variants were purified following the reported method[5] except that glycerol was omitted from all buffers and that enzyme was buffer exchanged into 50mM 2-amino-2-hydroxymethyl-propane-1`,3-diol hydrochloride (TRIS.HCl) pH 7.5 prior to storage at -80 °C. ThnE was overexpressed and purified as described[6]. CarB, CarBH229A and ThnE incubations were performed by sequential addition of the following: 600mM TRIS.HCl pH 9.0 (35 μL), 10mM CoA derivative (8 μL), 50mM deprotected solution of semialdehyde in 10% formic acid (5 μL) and 2mM enzyme (2 μL), then incubation at 37 °C for 10 mins. An equal volume of methanol

was then added and the mixture cooled on ice for 10 mins before centrifugation at 12,500 x *g* for 10 mins. The supernatant was decanted and analysed by Liquid Chromatography/ Time Of Flight Mass spectrometry (LC/TOFMS). Control assays were performed in the same way but with substitution of 50mM TRIS.HCl pH 7.5 for the enzyme.

Small scale assay analyses:

Products from small scale assays were analysed using a Waters LCT Classic with 2790 sample/ solvent manager with a Primesep 100 column using a gradient from 5 % aqueous MeCN + 0.1 % formic acid to 100 % MeCN + 0.1% aqueous formic acid.

Large scale enzymatic product isolation and characterisation:

Products for NMR analysis were produced by scale-up of assay conditions (10x), quenching with MeOH (500 µL), centrifugation (13,000 x *g*) and freeze-drying of the supernatant. The resultant residue was re-suspended in 15 % aqueous methanol (200 µL) and purified using a mixed mode Waters Spherisorb column (250 mm x 10 mm, 5 µ) pre-equilibrated in 5 % aqueous MeOH before a gradient was run to 5-25 % aqueous MeOH (according to the polarity of the product) with 0.1% aqueous formic acid. Elution was monitored using a Waters ZMD mass spectrometer, 2700 sample manager and 600 controller. Fractions with masses corresponding to anticipated products were collected (5-10 mL) and freeze dried. The resultant residue was re-suspended in D_2O (500 µL), transferred to an Eppendorf vial and freeze-dried. The final residue was re-suspended in D_2O (12 µL), transferred into a 1mm NMR tube (Bruker) using a hand centrifuge, and analysed by NMR using a Bruker AVIII 700 with ^1H inverse cryoprobe.

CarA purification and assay:

CarA purification and analytical scale incubations were performed as reported[5]. For large scale incubations: the product of two preparative CarB/ThnE/CarBH229 assays, after purification and freeze-drying, were incubatated with the components of CarA assay (x3) for 1 hr at 37 °C. The reaction mixture was then quenched with equivalent volume of acetonitrile, incubated on ice for 10 minutes, centrifugated (13,000 x *g*) and the supernatant was purified using a preparative C18 Column (250 mm x 22 mm, 15 µ) pre-equilibrated in 5 % aqueous MeCN with 0.1% formic acid before a gradient

was run to 100 % MeCN with 0.1% formic acid over 40 min. Elution was monitored using a Waters ZMD mass spectrometer, 2700 sample manager and 600 controller. Fractions with masses corresponding to the anticipated product were collected (~10 mL), 0.1 N sodium bicarbonate was added to pH 6, and freeze-dried. The resultant residue was re-suspended in D_2O (500 μL), transferred to an Eppendorf vial and freeze-dried. The final residue was re-suspended in D_2O (75 μL), transferred into a 2mm NMR tube using a hand centrifuge, and analysed by NMR using a Bruker AVIII 700 with ^1H inverse cryoprobe.

NMR Methods:

All NMR analyses were recorded at 298 K on a Bruker AVIII 700 with an inverse TCI cryoprobe optimised for ^1H observation and running TOPSPIN 2 software. Chemical shifts are reported in ppm relative to D_2O (δ_H 4.72); the deuterium signal was used as an internal lock signal and the HDO signal was reduced by presaturation where necessary. For quantification of the carboxymethylproline synthases products of catalysis, trimethylsilane propionic acid sodium salt (TSP) was used as an external standard.

Spectroscopic identification of products of carboxymethylproline synthases (CarB, CarBH229A and ThnE) with alternate substrates:

The following general considerations apply:

1. In all cases, the LC/MS data (negative ion electrospray ionization, unless otherwise stated) supported the formation of the product as shown by observation of the molecular ion and the ion arising from decarboxylation of the product.

2. The formation of a ring structure (five, six or seven) was assigned in part from the ^{1}H-NMR chemical shift of the bridgehead proton (H-5, H-6 or H-7 in case of five, six or seven-membered rings, respectively).

3. For all the compounds reported, the stereochemical assignments of the bridgehead carbons as having the *S*-stereochemistry was in part based on the nOe data which showed no correlation between H-2 and the bridgehead proton. The nOe data between other protons within the ring system supported this assignment. The assignment assumes that the *S*-stereochemistry at C-2 is maintained during the reaction. This has been shown to be the case for CarB catalysed conversion of L-glutamate semialdehyde to (2*S*,5*S*)-5-(carboxymethyl)pyrrolidine-2-carboxylic acid.[5]

4. Some of the products differ in their stereochemistry at C-7 (in case of the 6-membered ring structures). Assignment of the stereochemistry at C-7 was, to some extent, complex (and hence is less secure) due to the free rotation about the C6-C7 bond. Careful analysis of the nOe and coupling constant data, coupled to other NMR experiments (e.g. decoupling of selected protons and TOCSY experiments) were used in these assignments.

(2*S*,6*S*)-6-(Carboxymethyl)pipecolic acid, **10**:

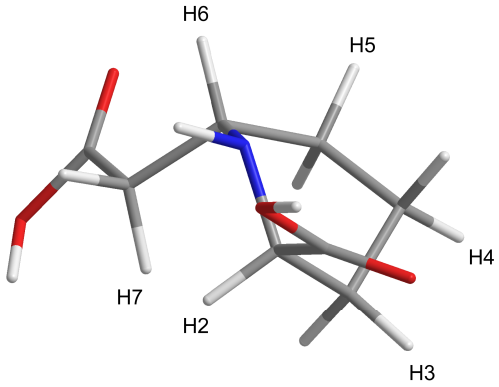


Fig. S1: Energy minimized 3D model for the proposed conformation of **10** based on the coupling constant values and the observed NOESY correlations. The 2D model of the compound was drawn in ChemBioDraw Ultra and converted into 3-D model in ChemBio3D. Energy minimization was performed by the MM2 force field computational method as implemented in the ChemBio3D version 11.0.1.[7, 8]

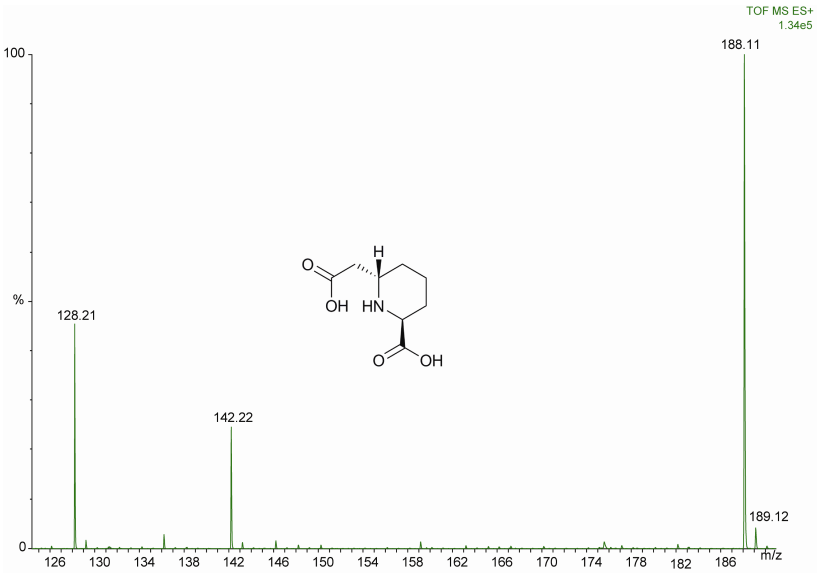


Fig. S2: MS spectrum (positive electrospray ionization) for **10**.

Proton no.	δH of **10**	Key nOe
H-2	3.83 (br.t, *J* = 5.0)	H-3, H-3'
H-6	3.62 (m)	H-5> H-5', H-7
H-7	2.47 (2H, m)	H-6, H-5 (w) H-5' (w), H-2 (w)
H-3	1.96 (br.d, *J* = 13.8)	H-3', H-4≥ H-4', H-2
H-3'	1.77 (m)	H-3, H-4, H-2
H-5	1.73 (m)	H-5', H-4', H-4
H-4	1.61 (m)	H-4', H-3'
H-5'	1.40 (m)	H-5, H-4
H-4'	1.35 (m)	H-4, H-3, H-5

Table S1: ^{1}H NMR data for **10** (700 MHz, D_2O).

For **10**: m/z (positive ion electrospray ionization) 188 [M+H$^+$], 142 [M$^+$-COOH]. The proposed boat-like conformation adopted by **10** (Fig. S1) was based on the coupling constant values and NOESY data. Although not amenable to precise measurement, the presence of large diaxial couplings (J~ 12 Hz) could be observed for protons H3', H4' and H5', in addition to geminal couplings of similar magnitude.

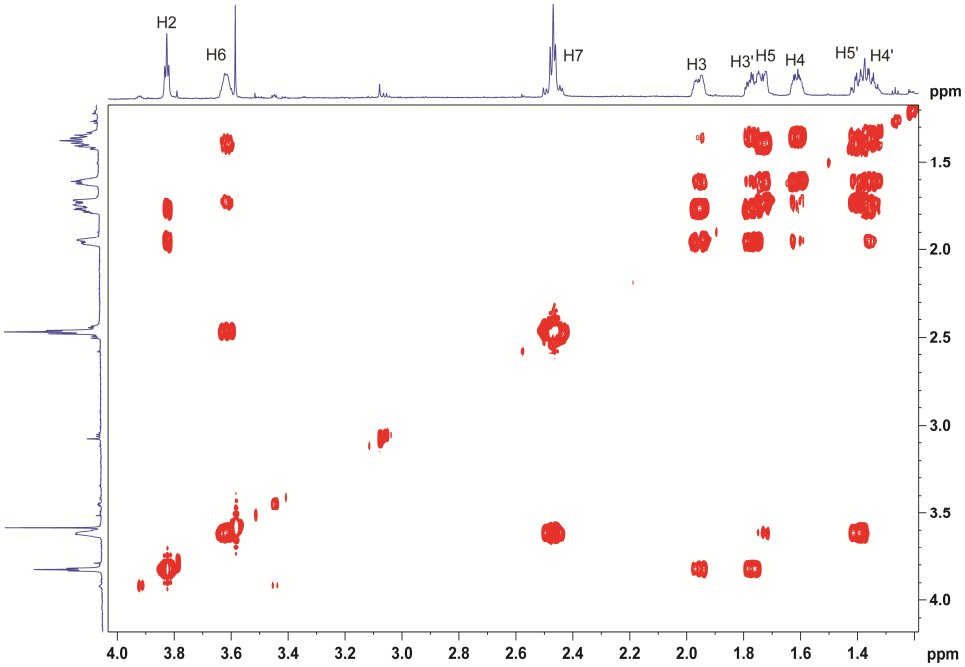


Fig. S3: 2D COSY for **10**.

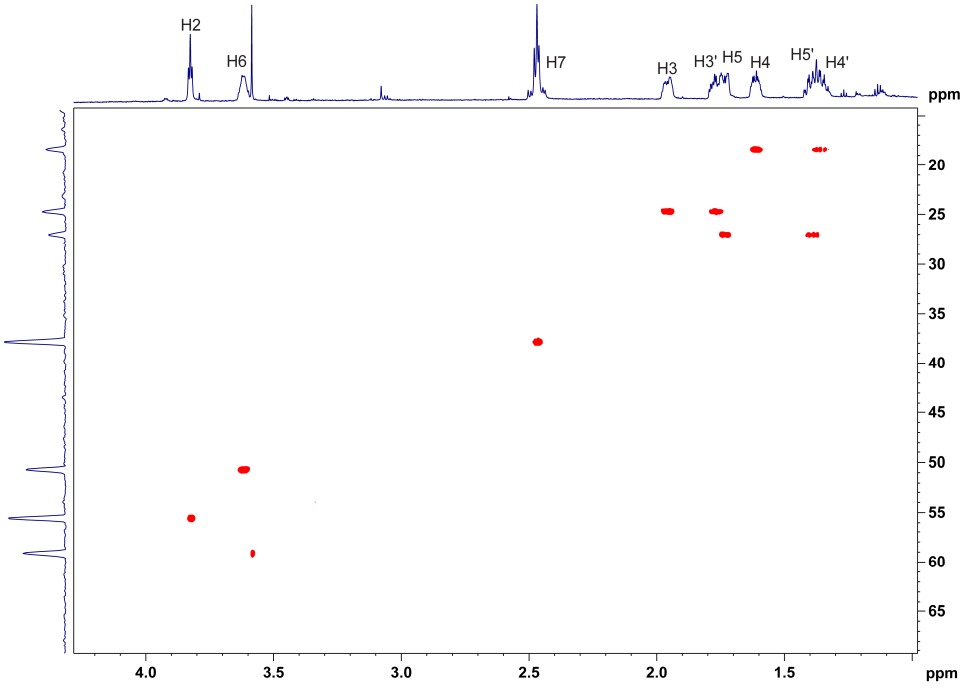


Fig. S4: 2D HSQC for **10**.

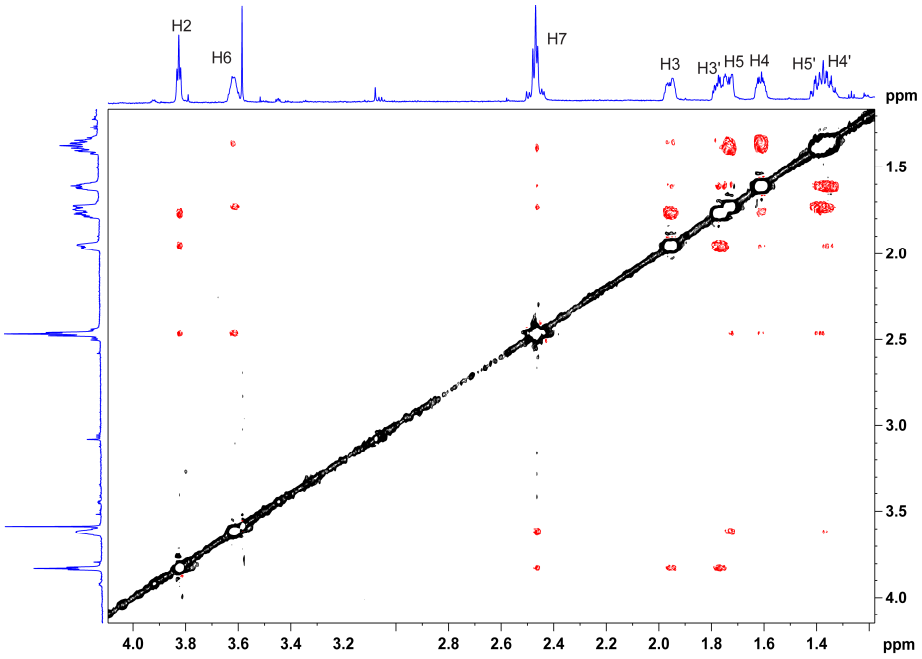


Fig. S5: 2D NOESY for **10**.

(2*S*,6*S*)-6-((*R*)-1-carboxyethyl)piperidine-2-carboxylic acid, 15a:

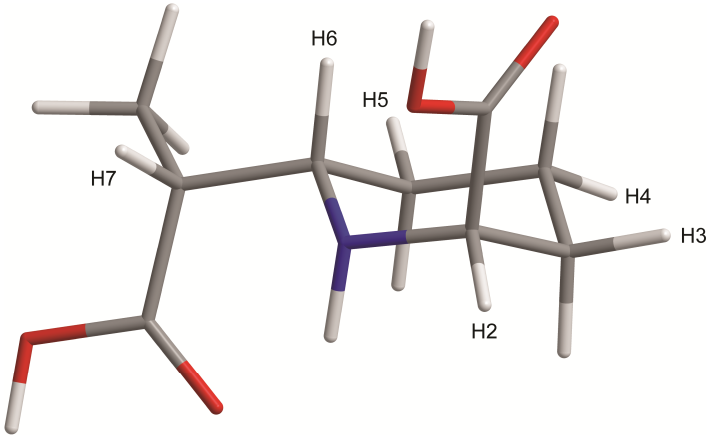


Fig. S6: Energy minimized 3D model for the proposed conformation of **15a** based on the coupling constant values and the observed NOESY correlations. The 2D model of the compound was drawn in ChemBioDraw Ultra and converted into 3-D model in ChemBio3D. Energy minimization was performed by the MM2 force field computational method as implemented in the ChemBio3D version 11.0.1.[7, 8]

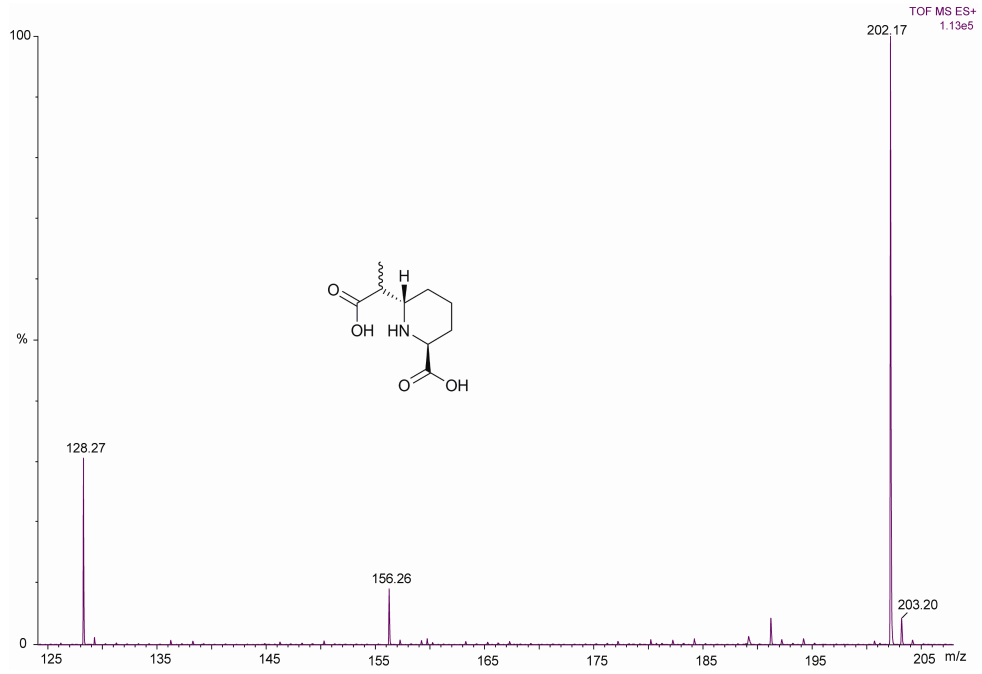


Fig. S7: MS spectrum of **15a**/ **15b**.

Proton no.	δH of **15a**	δH of **15b**
H-2	3.90 (br.d, *J* ~ 5.0 Hz)	3.91 (br.t, *J* ~ 3.9 Hz)
H-6	3.15 (m)	3.17 (m)
H-7	2.38 (dq, *J* = 4.8, 7.4 Hz)	2.34 (dq, *J* = 7.4, 8.1 Hz)
H-3	1.77 (bd, *J* = 14.4 Hz)	1.77 (bd, *J* = 16.0 Hz)
H-3'	1.38 (m)	1.41 (m)
H-5	1.34 (m)	1.44 (m)
H-4	1.28 (br.d, *J* = 13.8 Hz)	1.31(br.d, *J* = 12.8 Hz)
H-5'	0.99 (br.q)	1.02 (br.q)
H-4'	0.93 (br.q)	0.96 (br.q)
7Me	0.71 (d, *J* = 7.4 Hz)	0.75 (d, *J* = 7.4 Hz)

Table S2: ^{1}H NMR data of **15a** and **15b** (700 MHz, D_2O).

For compounds **15a** and **15b**: m/z (positive ion electrospray ionization) 202 [M+H$^+$], 156 [M$^+$-COOH]. It was not possible to, unambiguously; assign the stereochemistry of C-7 in case of **15a** and **15b** due to the free rotation about C_6-C_7. For compound **15a**, the stereochemistry of C-7 was assigned, tentatively, as *R* based on:

- The $J_{6,7}$ value of 4.8 Hz as well as the strong nOe between H-6 and H-7 indicating a predominately *gauche* relationship between the two protons.

- The nOe between H-7 and H-5> H-5' as well as the nOe between the methyl group on C-7 and H-5'~ H-5.

β-Lactam ring formation enabled unambiguous assignment of the stereochemistry at C-7 of **15a** as *R* based on the $J_{6,7}$ value of 4.8 Hz consistent with other β-lactam systems having H-6 and H-7 in *cis*-relationship[9] (Fig. S21).

(2*S*,6*S*)-6-((*S*)-1-carboxyethyl)piperidine-2-carboxylic acid, 15b:

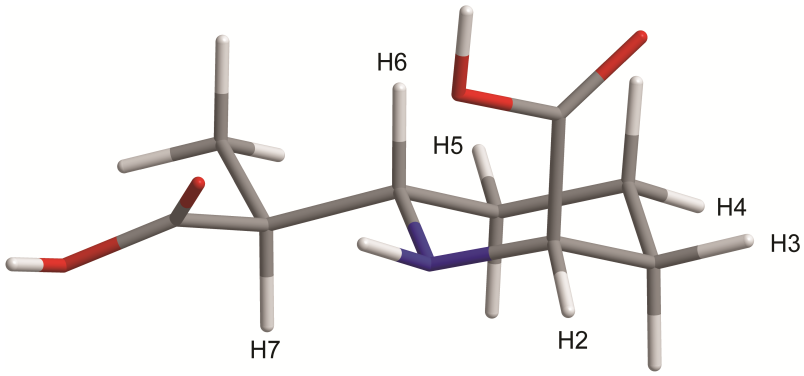


Fig. S8: Energy minimized 3D model for the proposed conformation of **15b** based on the coupling constant values and the observed NOESY correlations. The 2D model of the compound was drawn in ChemBioDraw Ultra and converted into 3-D model in ChemBio3D. Energy minimization was performed by the MM2 force field computational method as implemented in the ChemBio3D version 11.0.1.[7, 8]

The stereochemistry at C-7 was assigned as *S* based on:

- The $J_{6,7}$ value of 8.1 Hz as well as the weak nOe between H-6 and H-7 (compared to that between H-6 and the C-7 methyl group) indicating a predominantly *anti* relationship of the two protons.
- The nOe between H-7 and H-5'> H-5 as well as that between the methyl group on C-7 and H-5> H-5'.

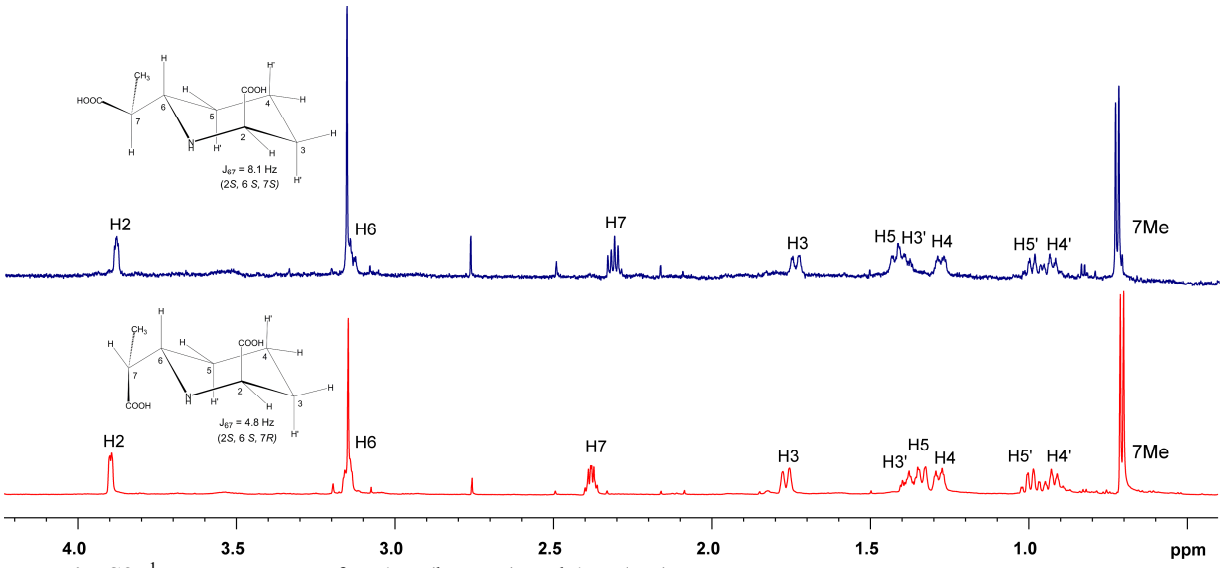


Fig. S9: ^{1}H-NMR spectra for **15a** (bottom) and **15b** (top).

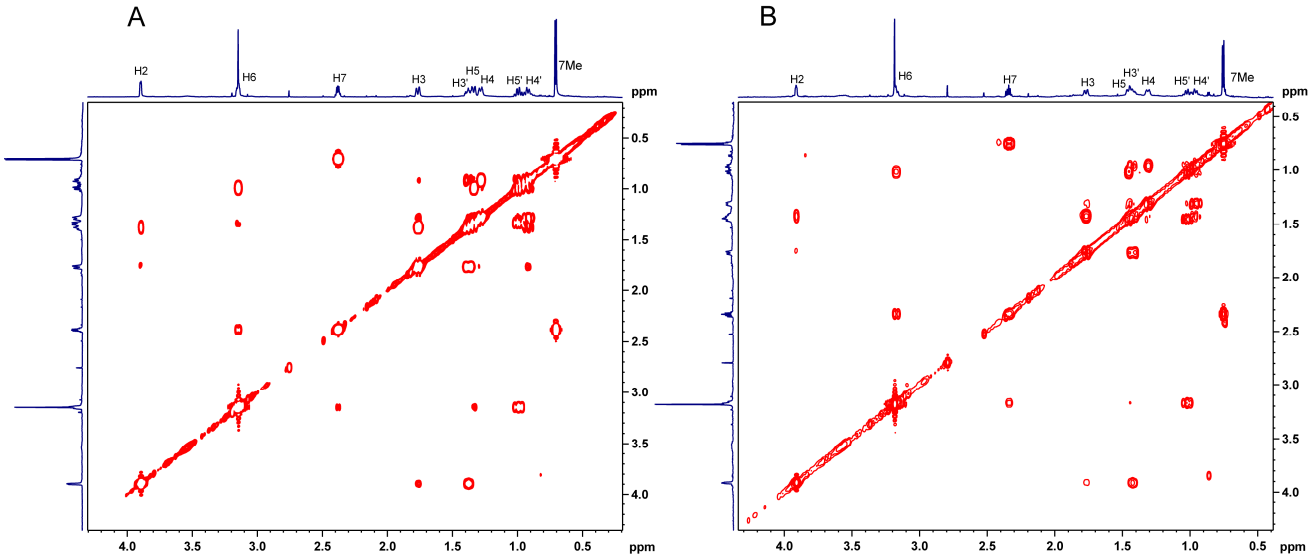


Fig. S10: 2D COSY for **15a** (A) and **15b** (B).

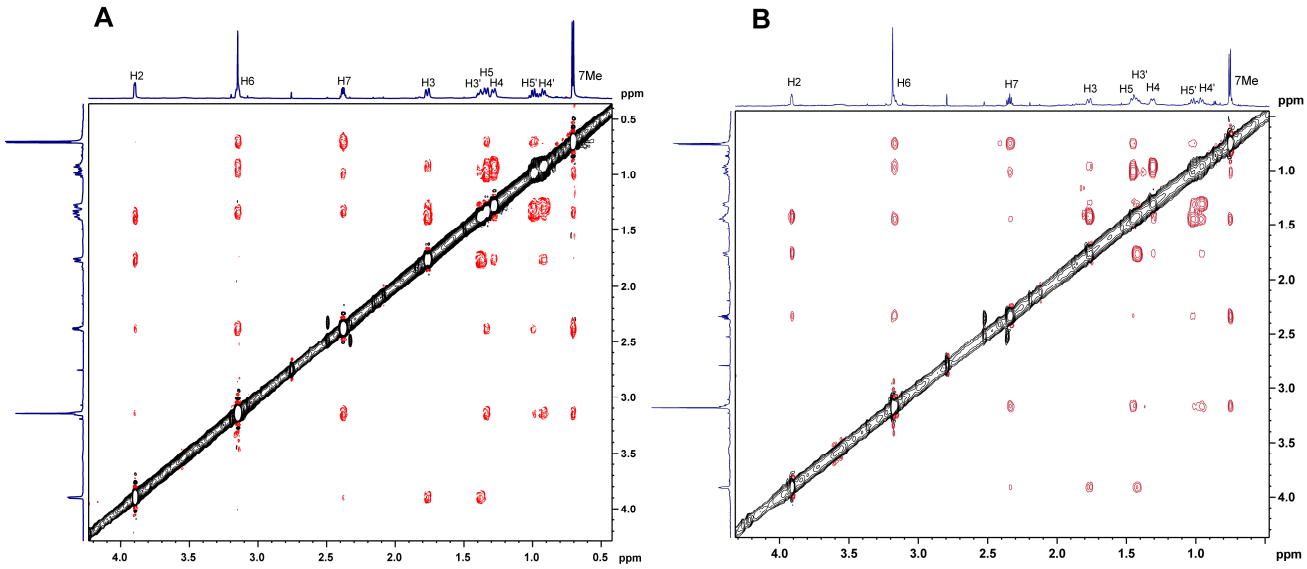


Fig. S11: 2D NOESY spectra for **15a** (A) and **15b** (B).

(2*S*,7*S*)-7-(carboxymethyl)-azepane-2-carboxylic acid, 13

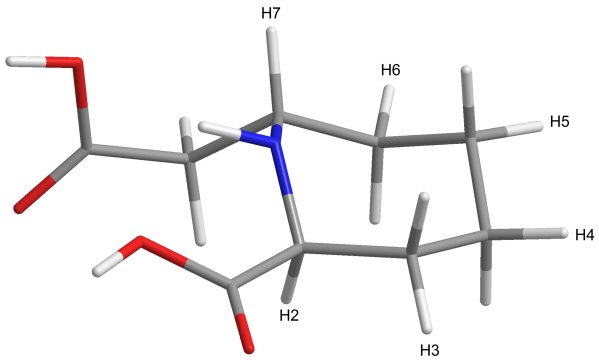


Fig. S12: Energy minimized 3D model for the proposed conformation of **13** based on the coupling constant values and the observed NOESY correlations. The 2D model of **13** was drawn in ChemBioDraw Ultra and converted into 3D model in ChemBio3D. Energy minimization was performed by the MM2 force field computational method as implemented in the ChemBio3D version 11.0.1.[7, 8]

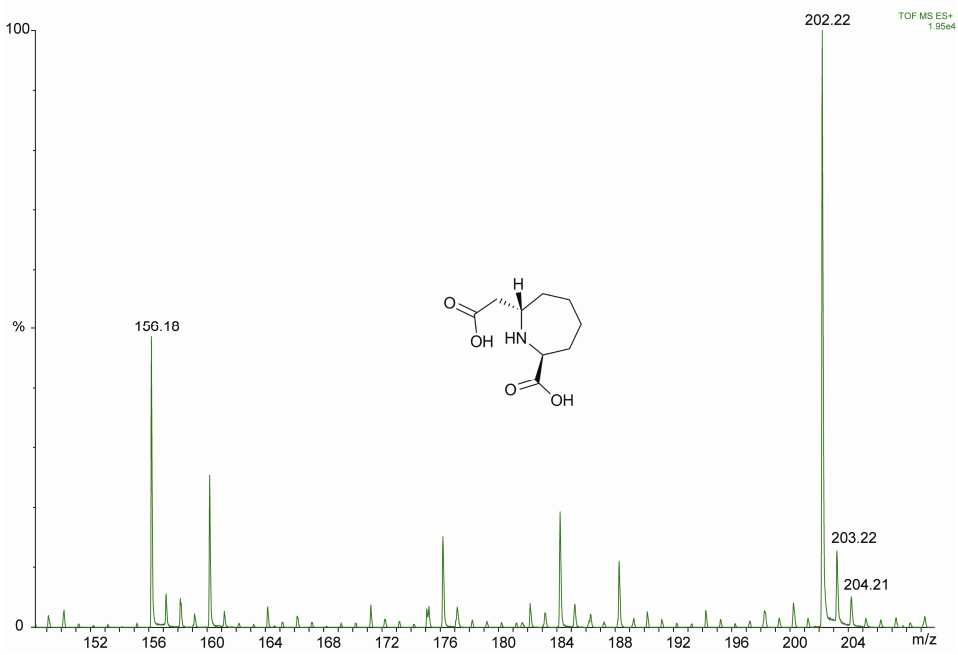


Fig. S13: MS spectrum of **13**.

Proton no.	δH of **13**
H-2	3.76 (dd, *J* = 2.8, 11.3 Hz)
H-7	3.67 (m)
H-8	2.67 (2H, m)
H-3	2.24 (m)
H-6	1.86 (m)
H-4	1.84 (m)
H-5	1.77 (m)
H-3'	1.70 (br q, *J* ~ 12 Hz)
H-6'	1.56 (br q, *J* ~ 12 Hz)
H-5'	1.37 (br q, *J* ~ 12 Hz)
H-4'	1.34 (br q, *J* ~ 12 Hz)

Table S3: ^{1}H-NMR data for **13** (700 MHz, D_2O).

For **13**: m/z (positive ion electrospray ionization) 202 [M+H$^+$], 156 [M$^+$-COOH]. The proposed chair-like conformation (Fig. S12) adopted by **13** was based on the coupling constant values (which revealed notable diaxial relationships for H2, H3', H4', H5', H6' and H7) and 2D-NOESY data.

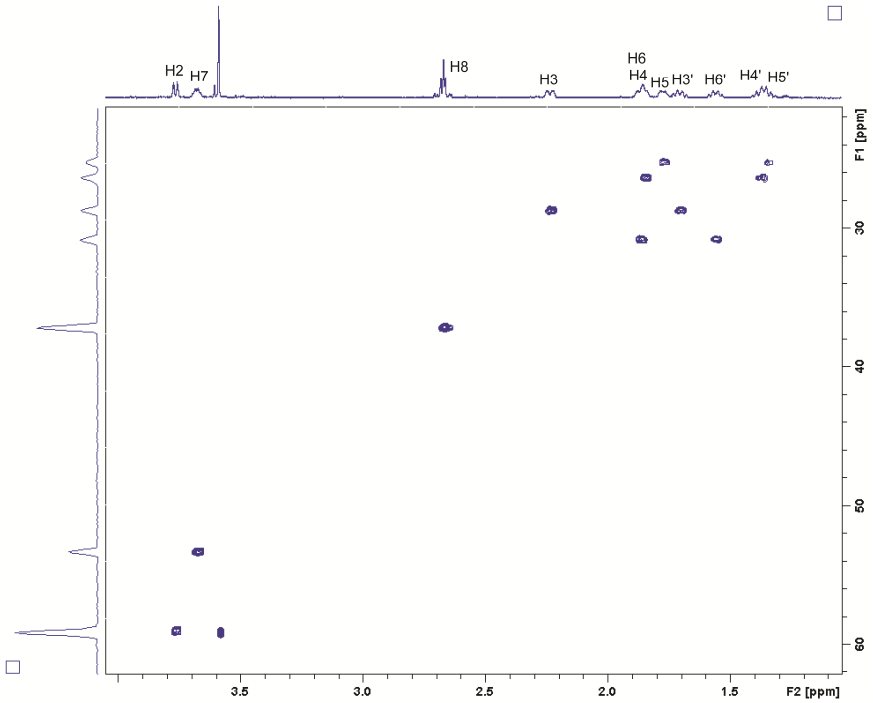


Fig. S14: 2D HSQC spectrum for **13.**

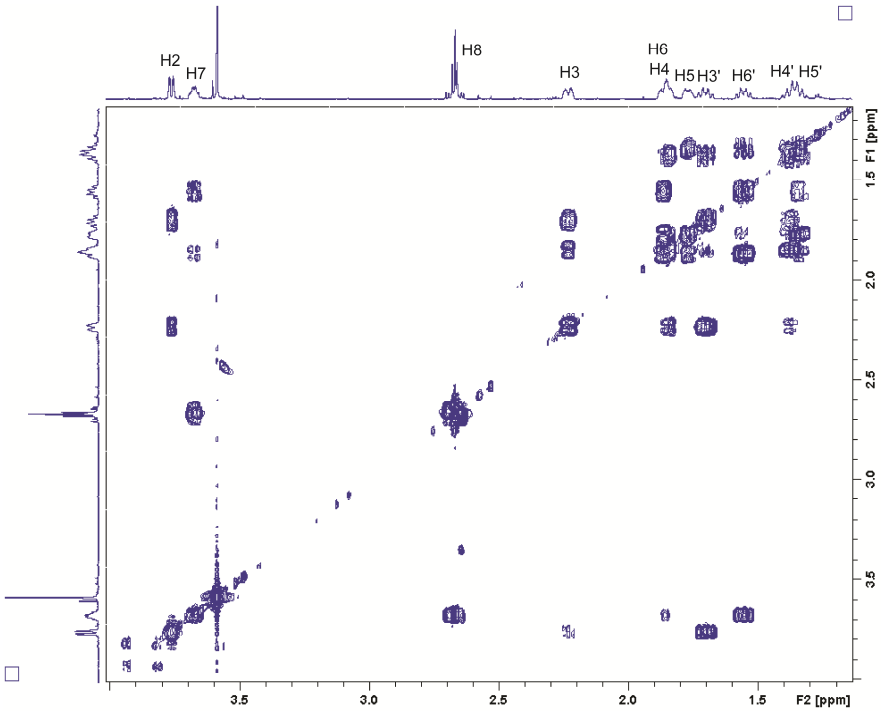


Fig. S15: 2D COSY for **13.**

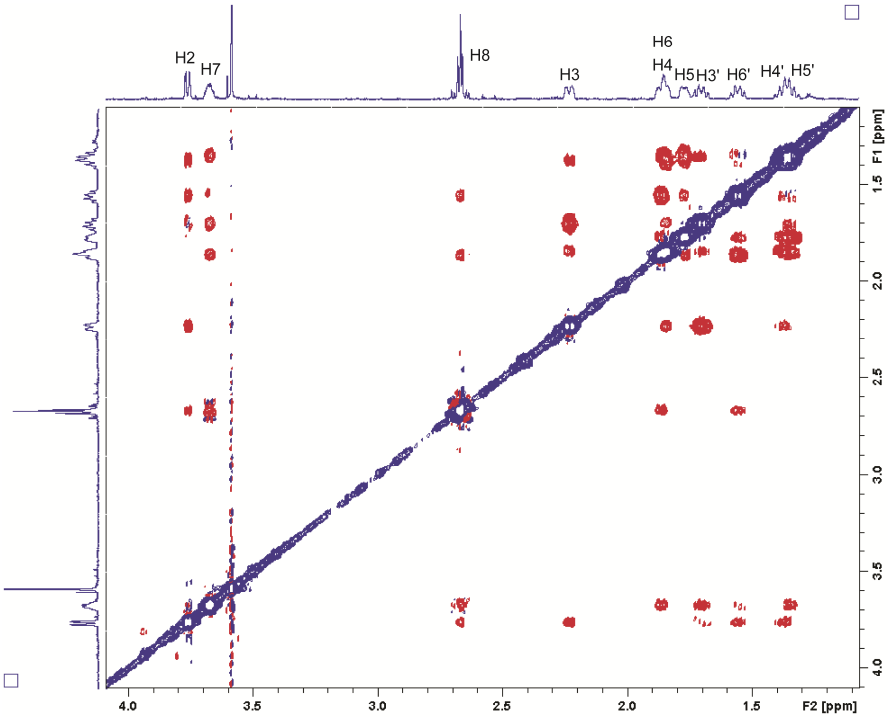


Fig. S16: 2D NOESY for **13.**

(2*S*)-7-(1-Carboxyethyl)azepane-2-carboxylic acid, **16**

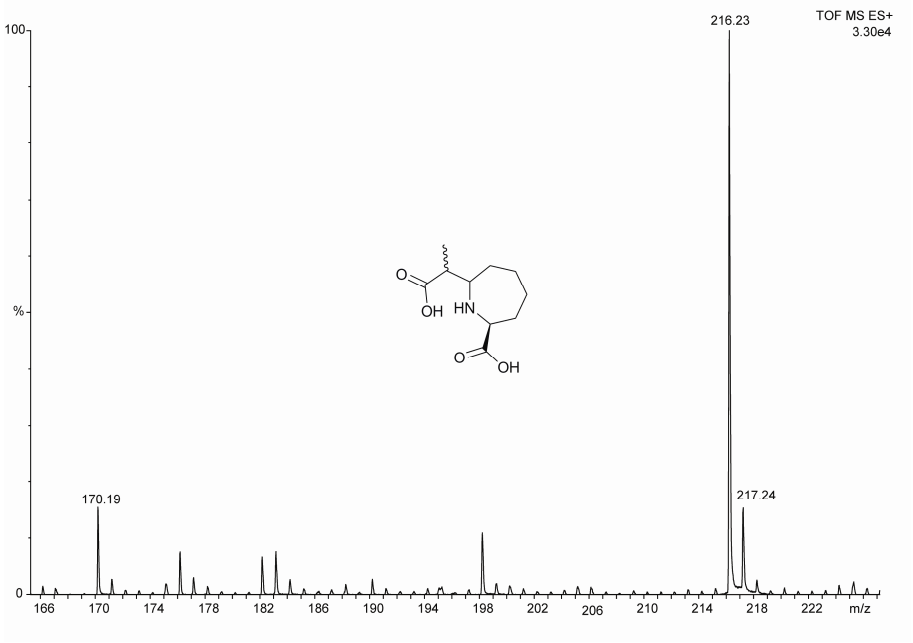


Fig. S17: MS spectrum (positive ion electrospray ionization) for **16**.

NMR analyses of compounds 20, 21, and 22 :

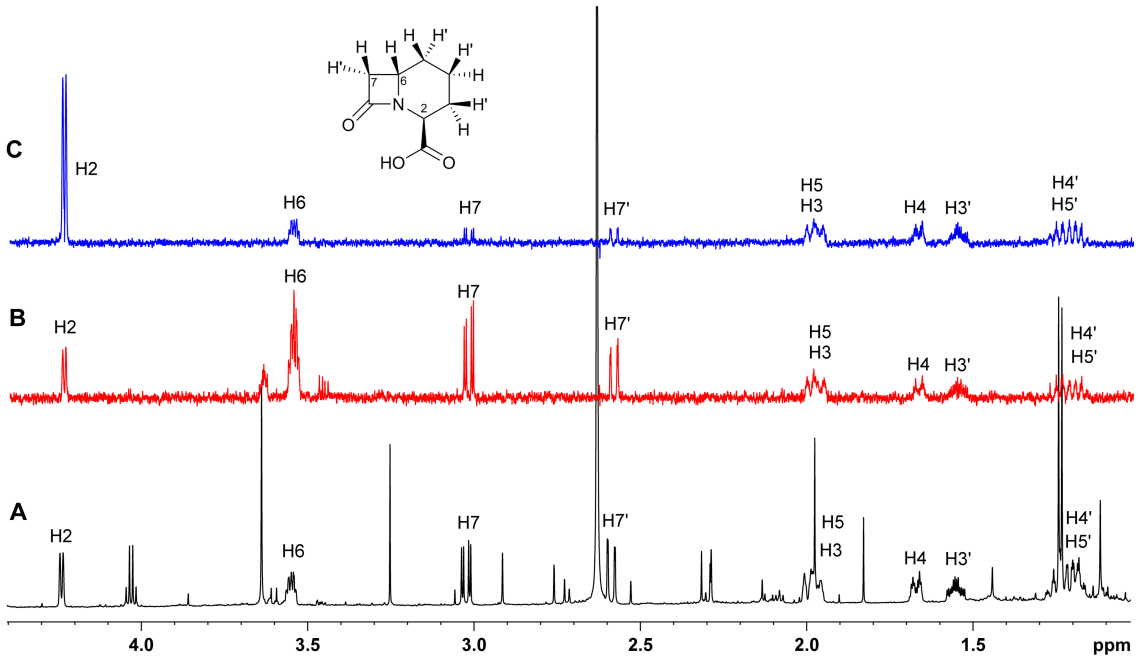


Fig. S18: A; ^{1}H NMR of (2*S*, 6*S*)-carbacepham **20** produced by CarA from **10**. B**;** 1D TOCSY (τ_m= 150 ms) of **20** obtained by selective excitation of H6. C**;** 1D TOCSY (τ_m= 150 ms) of **20** obtained by selective excitation of H2.

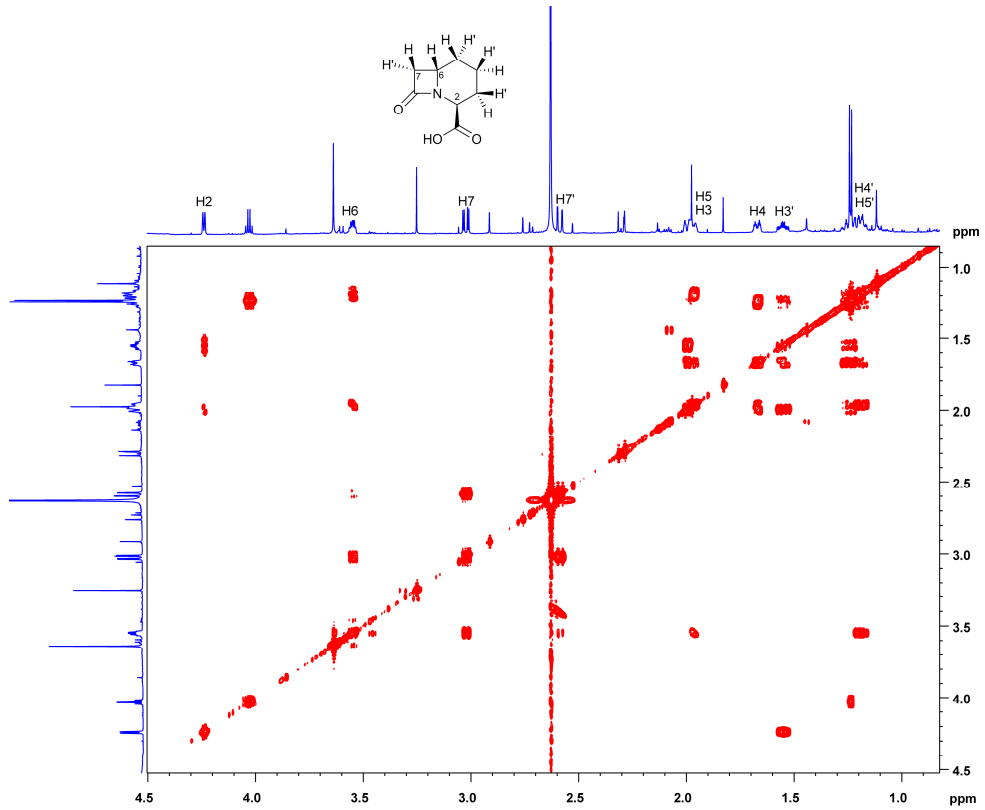


Fig. S19: 2D COSY of **20**.

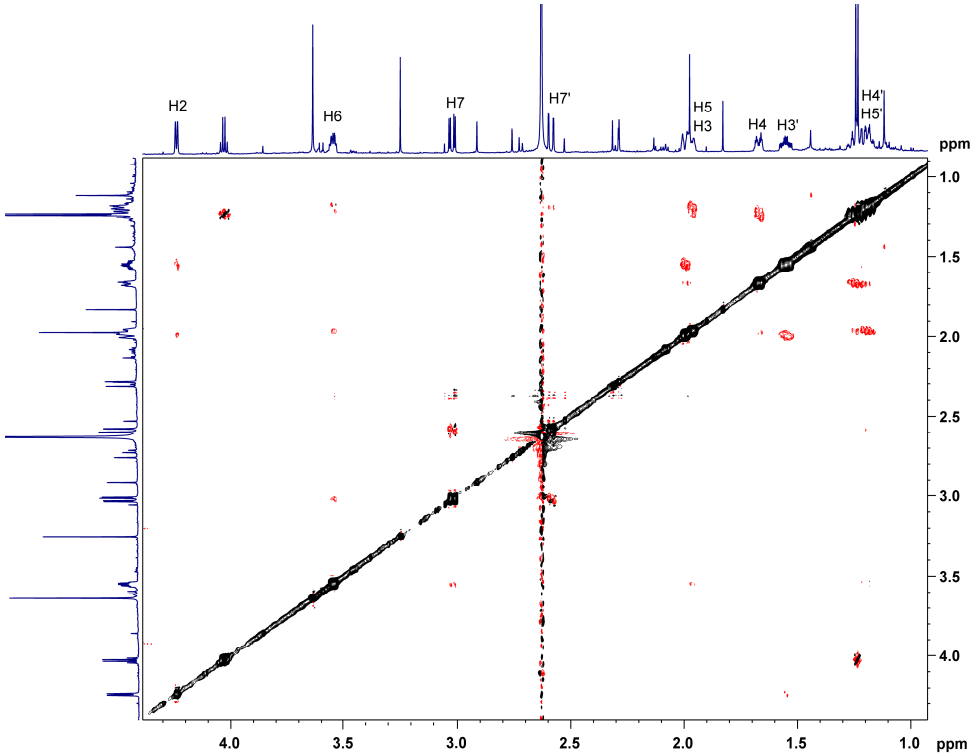


Figure S20: 2D NOESY of **20**.

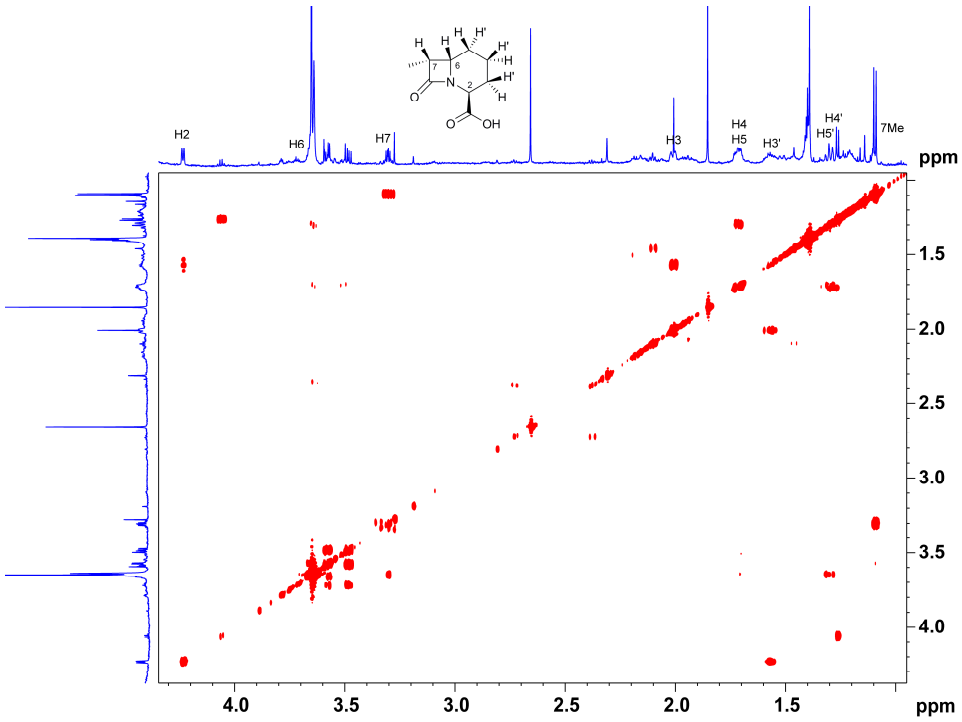


Fig. S21: 2D COSY of (2*S*, 6*S*, 7*R*)-7-methylcarbacepham **21** produced by CarA from **15a**.

For compound **20**, **21** and **22**, the β-lactam protons were the main entry point for their assignment with their characteristic chemical shifts and coupling constant pattern. For **20**, the assignment of H7 and H7' as *pro-S* and *pro-R*, respectively, was based on the following observations:

- The $J_{6,7}$ value of 4.5 Hz consistent with other β-lactam systems having H-6 and H-7 in *cis*-relationship.[9]
- The nOe correlation between H-6 and H-7 >H-7'.

For **21**, the assignment of C7 stereochemistry as *R* was based on the $J_{6,7}$ value of 4.8 Hz consistent with other β-lactam systems having H-6 and H-7 in *cis*-relationship.[9]

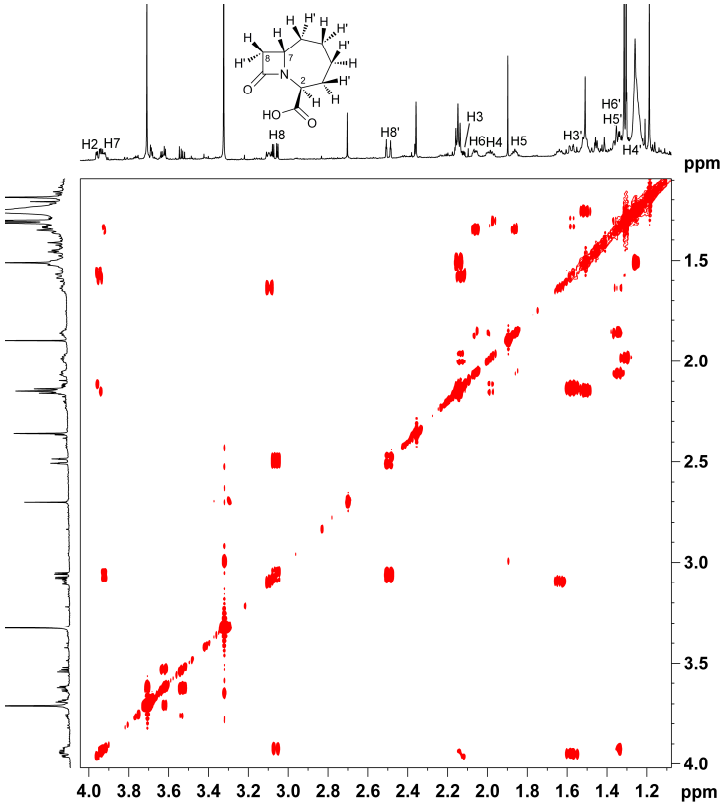


Fig. S22: 2D COSY of (2*S*, 7*S*)-9-oxo-1-azabicyclo[5.2.0]nonane-2-carboxylic acid **22** produced by CarA from **13**.

Proton no.	δH of **20**	δH of **21**
H-2	4.24 (bd, *J* = 6.6 Hz)	4.24 (bd, *J* = 6.7 Hz)
H-6	3.55 (m)	3.55 (buried under the Tris signal)
H-7	3.02 (dd, *J* = 15.0, 4.5 Hz)	3.30 (dq, *J* = 4.6, 7.6 Hz)
H-7 '	2.59 (dd, *J* = 15.0, 1.7 Hz)	-
H-3	1.98 (m)	2.02 (m)
H-5		1.71 (m)
H-4	1.67 (m)	1.73 (m)
H-3'	1.55 (m)	1.57 (m)
H-4'	1.20 (m)	1.27 (m)
H-5'		1.29 (m)
7Me	-	1.09 (d, *J* = 7.6 Hz)

Table S4: ^{1}H NMR data for **20** and **21**.

Proton no.	δH of **22**
H-2	3.95 (ddd, *J* = 1.5, 3.9, 15.2 Hz)
H-7	3.93 (m)
H-8	3.06 (dd, *J* = 4.6, 15.2 Hz)
H-8 '	2.50 (ddd, *J* = 15.2, 3.2, 1.5 Hz)
H-3	2.12 (m)
H-6	2.06 (m)
H-4	1.98 (m)
H-5	1.86 (m)
H-3'	1.58 (m)
H-5'	1.35(m)
H-6'	
H-4'	1.31 (m)

Table S5: ^{1}H NMR data for **22**.

Note: For **22**, a long range coupling have been observed between H-2 and H-8 (see Table S5) and confirmed by a decoupling experiment.

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