

Figure 1. Protein Sequence Alignment

PDB files downloaded from the RCSB Protein Databank.
Alignment performed using ClustalW2 (EBI tools) with default settings.

Input sequences

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>1GL3 (E. coli)
MKNVGFIGWRGMVGSVLMQRMVEERDFDAIRPVFFSTSQLGQAAPSFGGTGTGLQDAFDLEALKALDIIIVTCQGGDYTNEIYPKLRESGWQGYWIDAASSLRMKDD
AIIILDPVNQDVITDGLNNGIRTFVGGNCTVSLMLMSLGGFLFANDLVDWVSVATYQAASGGGARHMRRELLTQMGLHYGHVADELATPSSAILDIERKVTTLTRSGE
LPVDNFGVPLAGSLIPWIDKQLDNGQSREEWKQAETNKILNTSSVIPVDGLCVRVGALRCHSQAFTIKLKKDVS IPTVEELLA AHNFPWAKVVPNDREITMRELT
AAVTGTLTTPVGRRLRKLNMGPFLSAFTVGDQLLWGAAEPLRRMLRQLA
>1T4B (E. coli)
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AIIILDPVNQDVITDGLNNGIRTFVGGNCTVSLMLMSLGGFLFANDLVDWVSVATYQAASGGGARHMRRELLTQMGLHYGHVADELATPSSAILDIERKVTTLTRSGE
LPVDNFGVPLAGSLIPWIDKQLDNGQSREEWKQAETNKILNTSSVIPVDGLCVRVGALRCHSQAFTIKLKKDVS IPTVEELLA AHNFPWAKVVPNDREITMRELT
AAVTGTLTTPVGRRLRKLNMGPFLSAFTVGDQLLWGAAEPLRRMLRQLA
>1NWH (H. influenzae)
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DAIIVLDPVNQHVI SEGLKKGIKTFVGGNCTVSLMLMAIGGLFEKDLVEWISVATYQAASGAGAKNMRELLSQMGLLEQAVSSELKDPASSILDIERKVTAKMRAD
NFPTDNFGAALGGSLLIPWIDKLLPETGQTKEEWKGYAETNKILGLSDNPVPDGLCVRIGALRCHSQAFTIKLKKDLPLEEIEQIIASHNEWVKVIPNDKEITLRE
LTPAKVTGTLSPVGRRLRKLAMGPEYLAFTVGDQLLWGAAEPVRRILKQLVA
>2GZ3 (S. pneumoniae)
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GMGAILETQRELREVLNDGVKPCDLHAEILPSGGDKKHYPFALNPQIDVFTDNDYTYEEMKMTKETKKIMEDDSIAVS
ATCVRIPLSAHSESVYIETKEVAPIEEVKAAIAAFPGAVLEDDVAHQIYPQAINAVGSRDTFVGRIRKDLDAEKGIHWM
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>1YS4 (M. jannaschii)
MSKGEKMKIKVGVIGATGSVGRFVQQLADHPMFELTALAASERSAGKKYKDCYWFQDRDIPENIKDMVVIPTDPKHEEFEDVDIVFSALPSDLAKKFEPEFAKE
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Alignment Scores

SeqA	Name	Len (aa)	SeqB	Name	Len (aa)	Score
1	1GL3	367	2	1T4B	367	99
1	1GL3	367	3	1NWH	371	72
1	1GL3	367	4	2GZ3	366	24
1	1GL3	367	5	1YS4	354	12
2	1T4B	367	3	1NWH	371	71
2	1T4B	367	4	2GZ3	366	24
2	1T4B	367	5	1YS4	354	12
3	1NWH	371	4	2GZ3	366	22
3	1NWH	371	5	1YS4	354	12
4	2GZ3	366	5	1YS4	354	27

Alignment

CLUSTAL 2.0.12 multiple sequence alignment

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1GL3      -----MKNVGFIGWRGMVGSVLMQRMVEERDFDAIRPVFFST---SQLGQAAPSFGG- 49
1T4B      -----MQNVGFIGWRGMVGSVLMQRMVEERDFDAIRPVFFST---SQLGQAAPSFGG- 49
1NWH      -----MKNVGFIGWRGMVGSVLMRMSQENDFENLNPVFFTT---SQAGQKAPVFGGK 50
2GZ3      -----MGYTVAVVGATGAVG-AQMIKMLEESTLPIDKIRYLAS---ARSAGKSLKFKD- 49
1YS4      MSKGEKMKIKVGVIGATGSVGRFVQQLADHPMFELTALAASERSAGKKYKDCYWFQDR 60
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1GL3      -----TTGTLQDAFDLEALKALDIIIVTCQGGDYTNEIYPKLRESGWQGYWIDAASSLR 102
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1NWH      -----DAGDLKSAFDIEELKKLDIIIVTCQGGDYTNEVYPKLKATGWDGYWVDAASALR 103
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1NWH      MKDDAIIIVLDPVNQHVI SEGLKKGIK-----TFVGGNCTVSLMLMAIGGLFEKDLVEWI 157
2GZ3      QNPDVPLVPEVNAHALDA--HNGII-----ACP--NCSTIQMMVALEPVRQKWGLDRI 149
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1YS4      FIATMQAVSGAG-----YNGVPS----- 196
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1T4B      NFGVPLAGSLIPWIDKQL--DNGQSREEWKQAETNKILNTS--SVIPVDGLCVRVGALRCH 274
1NWH      NFGAALGGSLLIPWIDKLLPETGQTKEEWKGYAETNKILGLSDNPVPDGLCVRIGALRCH 277
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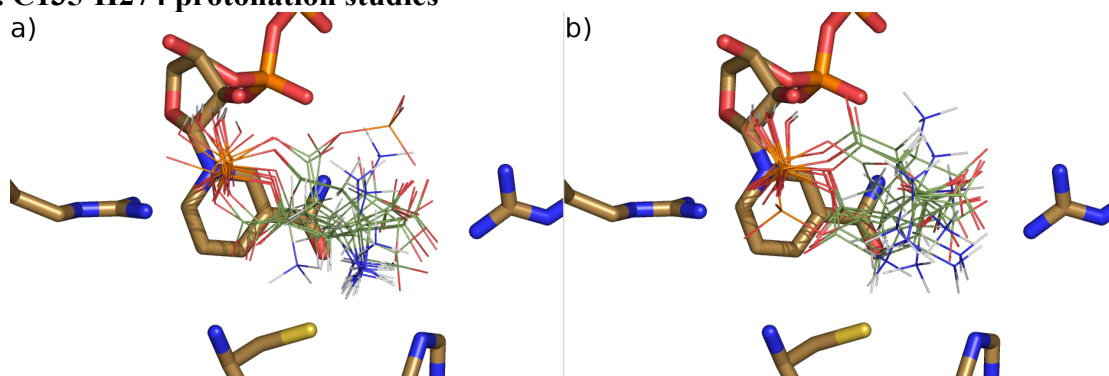
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1YS4      TESIFVKTKEGAEPEEIKEVMDKFDPLKDLNLPTYAKPIVIREEIDRPQPRLDRNEGNGM 310
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1NWH      SVPVGRLRKLAMGPEYLAFTVGDQLLWGAAEPVRRILKQLVA----- 371
2GZ3      DTFVGRIKDLDAEKGIHMMWVSDNLLKGAAWNSVQIAETLHERGLVRPTAELKFELKLE 360
1YS4      SIVVGRIKRD--PIFDVKYTALEHNTIRGAAGASVLNAEYFVKKYI----- 354
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1T4B      -----
1NWH      -----
2GZ3      HHHHHH 366
1YS4      -----

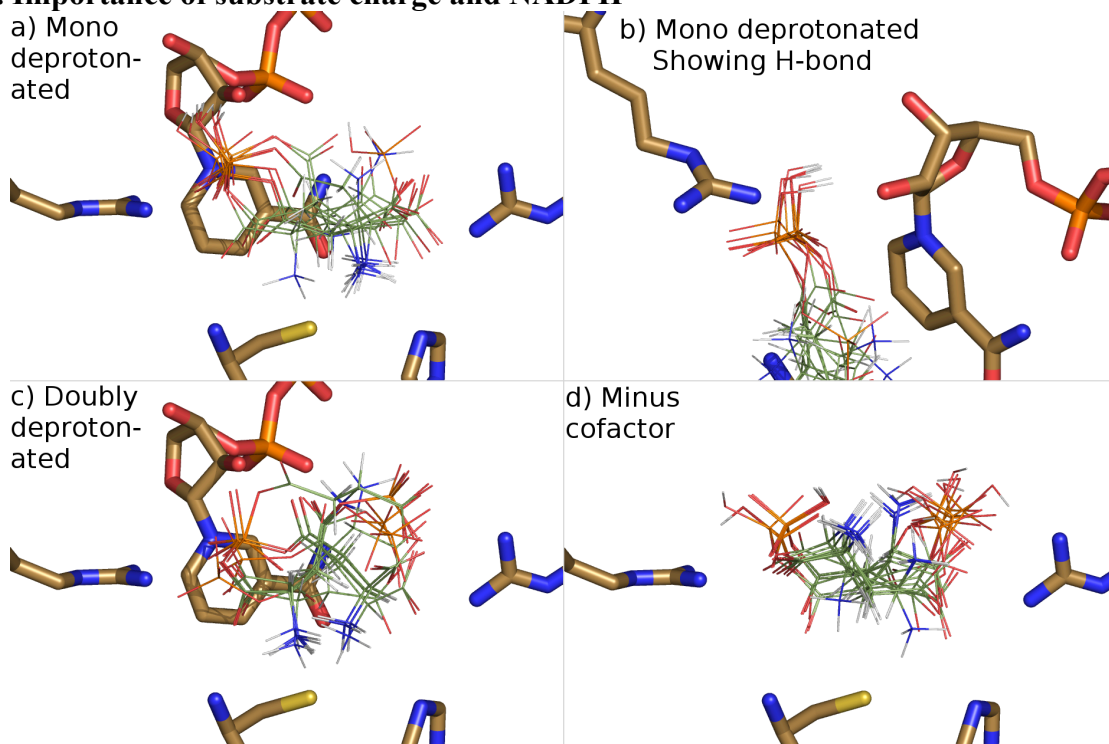
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Figure 2. C135-H274 protonation studies



Comparison of Cys135-His274 as (a) the charged pair and (b) the neutral pair. Residues left to right are Arg102, Cys135, His274 and Arg267 with NADPH behind.

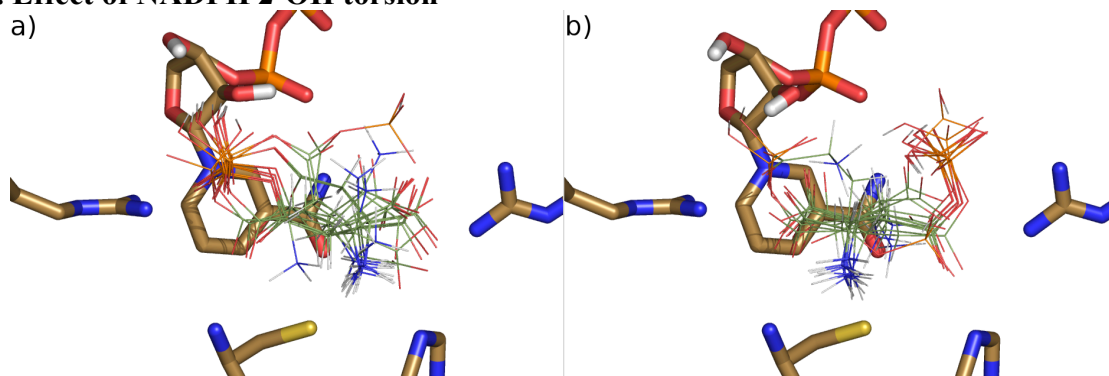
Figure 3. Importance of substrate charge and NADPH



Without a hydrogen bond between substrate and NADPH the alignment, and hence overall binding of substrate, is adversely affected;

(a) BAP as the mono deprotonated phosphate; (b) BAP as the mono deprotonated phosphate with the view rotated to highlight a hydrogen bond to NADPH; (c) BAP as the doubly deprotonated phosphate – loss of bonding interaction and alignment; (d) BAP as the mono deprotonated phosphate, docked without NADPH present – loss of bonding interaction and alignment. Residues left to right are Arg102, Cys135, His274 and Arg267 with NADPH behind.

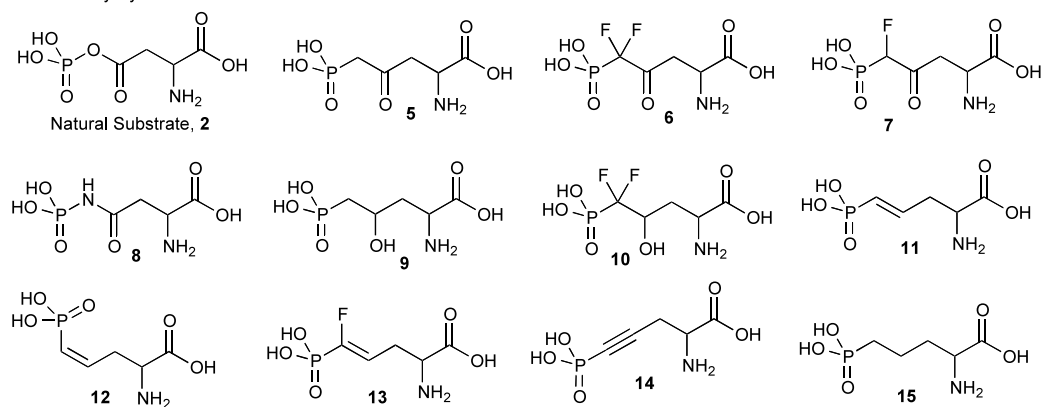
Figure 4. Effect of NADPH 2'OH torsion



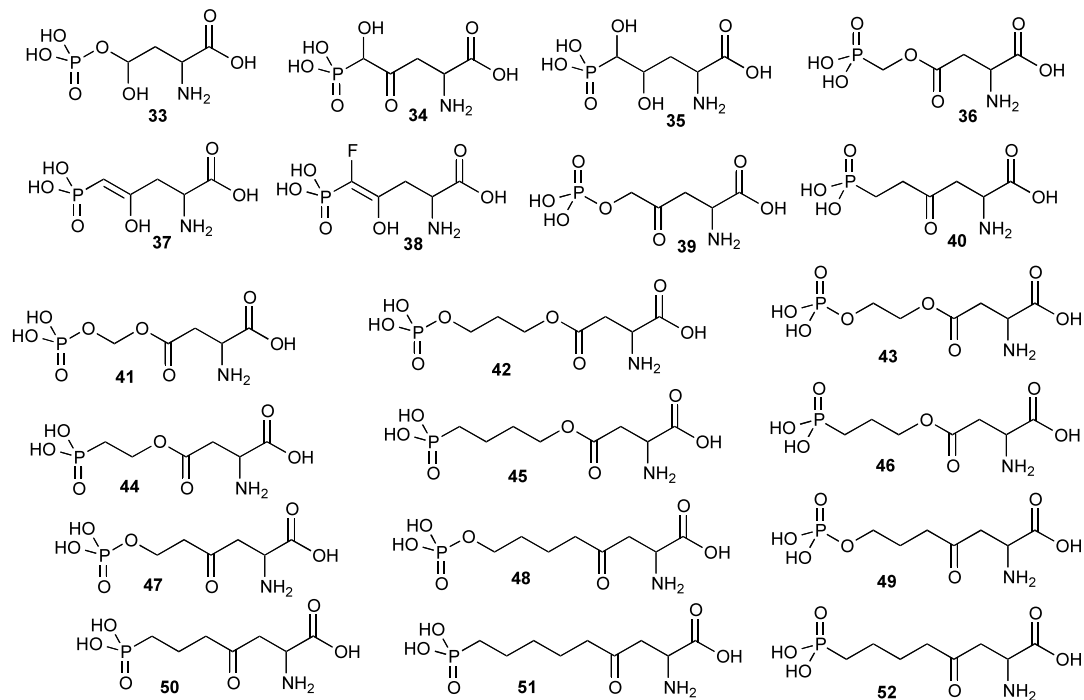
The torsion of the ribose 2' O–H must be so that it acts as a hydrogen bond acceptor rather than a hydrogen bond donor; (a) Orientation away from phosphate binding site (b) Orientation towards phosphate binding site – complete loss of alignment. Residues left to right are Arg102, Cys135, His274 and Arg267 with NADPH behind.

Figure 5. List of inhibitor structures used in library

Previously Synthesised Inhibitors:



Putative Linear Inhibitors



Putative Cyclic Inhibitors

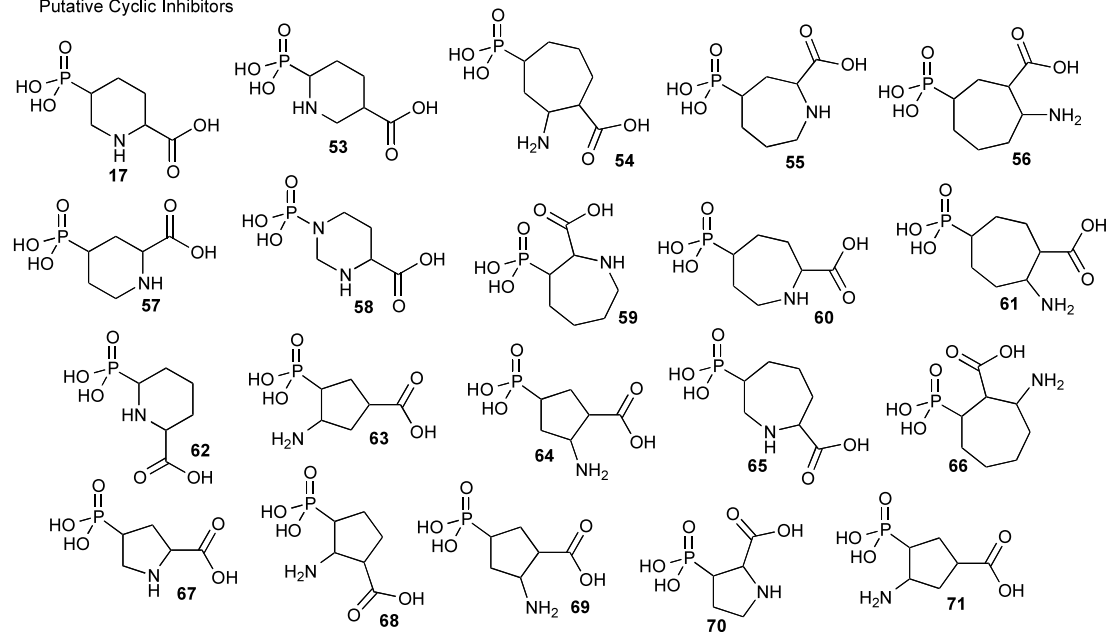


Figure 6. Top scoring poses in simulated docking of 5-membered rings.

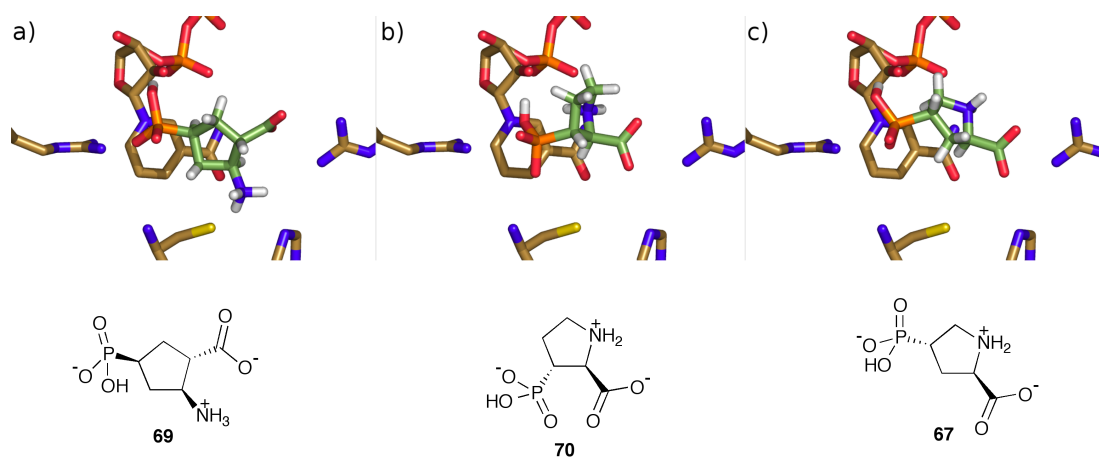
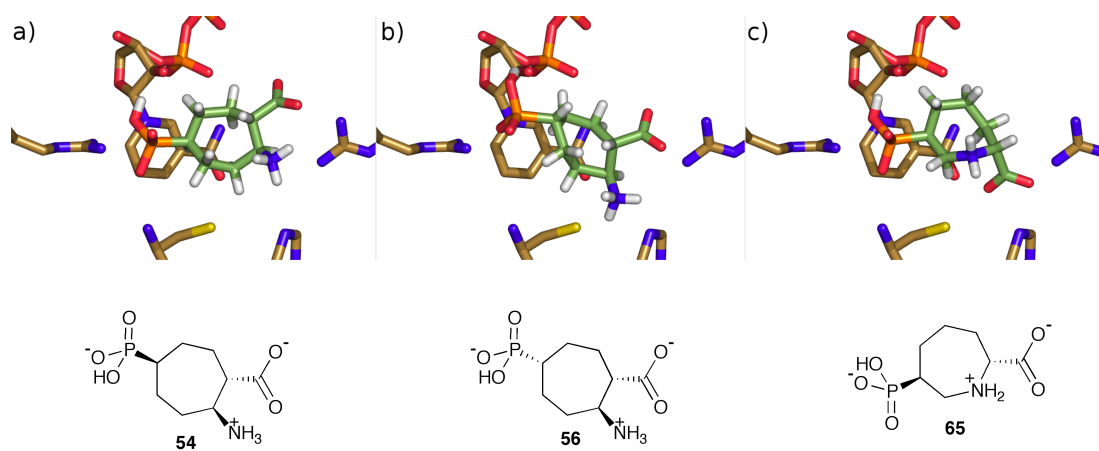


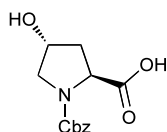
Figure 7. Top scoring poses in simulated docking of 7-membered rings.



Reproduction of literature syntheses

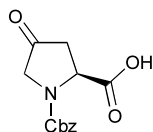
All reactions were performed under a nitrogen atmosphere with dry solvent under anhydrous conditions, unless otherwise noted. Dry, deoxygenated diethyl ether (Et_2O), tetrahydrofuran (THF), acetonitrile (MeCN), toluene, dichloromethane (DCM) and hexane were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Anhydrous ethanol was obtained by distillation from Mg / I_2 . Anhydrous methanol was purchased commercially (HPLC grade). All water used was previously deionised. Yields refer to chromatographically and spectroscopically (^1H NMR) homogeneous materials. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) performed on 0.2mm Merck silica gel glass plates (60F-254) and visualised using ultraviolet light (254 nm) and by potassium permanganate and heat as developing agents. Merck silica gel (60, particle size 0.035–0.070 mm) was used for flash column chromatography. NMR spectra were recorded on JEOL delta / GX270 ($\Delta 270$), JEOL delta / GX400 ($\Delta 400$), JEOL lambda300 ($\Delta 300$), Eclipse+ 400 (E400), Varian 400 (V400) and Varian 500 (V500) instruments. NMR spectra were obtained at 298 K unless otherwise stated and samples run as a dilute solution of the stated solvent. All NMR spectra were referenced to the residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High resolution mass spectra (HRMS) were recorded on a magnetic sector VG Autospec using EI (electron ionisation) and CI (chemical ionisation). High resolution mass spectra were recorded on a Bruker Apex FT-ICR-MS using ESI (electrospray ionisation). Infrared (IR) spectra were recorded on a Perkin-Elmer FT-IR Paragon 1000 spectrometer under neat conditions using a universal attenuated total reflection (UATR) attachment. Melting points were obtained on an electrothermal melting point apparatus and are uncorrected. Optical rotations were measured with a Bellingham + Stanley ADP220 polarimeter using a 1dm optical cell. Elemental analyses were performed in the microanalytical laboratories of the University of Bristol. GC-MS (gas chromatography – mass spectrometry) data were obtained using an Agilent 6890 series gas chromatograph system with an Agilent 5973 network mass selective detector (EI mode). A 30m \times 0.25mm, 0.25 μm film Gamma Dex column was used as the stationary phase. Injection performed with injector at 200 $^\circ\text{C}$, oven temperature started and held at 70 $^\circ\text{C}$ for 1 min, ramped at 25 $^\circ\text{C}\cdot\text{min}^{-1}$ to 150 $^\circ\text{C}$, ramped at 45 $^\circ\text{C}\cdot\text{min}^{-1}$ to 250 $^\circ\text{C}$, held at 250 $^\circ\text{C}$ for 3 min, ramped at 45 $^\circ\text{C}\cdot\text{min}^{-1}$ to 300 $^\circ\text{C}$ and held at 300 $^\circ\text{C}$ for 3 min. Total ion current trace was obtained for positive electron ionisation (EI+). LC-MS (high performance liquid chromatography – mass spectrometry) was performed using a Micromass Platform LC (ESI+ mode). The stationary phase was a 25 cm \times 4.6 mm Phenomenex Luna 5 μ column with 5 μm C_{18} packing. The solvent system started at 75% $\text{H}_2\text{O} / 25\%$ MeCN, ramped to 95% MeCN over 13 min and held for a further 2 min. All cyclic structures presented are numbered with priority given to the amino acid moiety: the α -amino carbon is consistently numbered '1'.

Trans-4-hydroxy-*N*-Cbz-L-proline. Error! Bookmark not defined.



To a 500 ml three neck flask with a reflux condenser and magnetic stirring, under an inert atmosphere (N_2), was added *trans*-4-hydroxy-L-proline **19** (15.0 g, 114.4 mmol). The solid was suspended in DCM (245 ml) and diisopropylethylamine (65.6 ml, 377.5 mmol) was added in one portion. Chlorotrimethylsilane (65.6 ml, 514.8 mmol) was added in one portion, and the solution was heated at reflux for 1.5 h. The flask was cooled to room temperature and then transferred to an ice bath. Benzyl chloroformate (15.6 ml, 108.7 mmol) was added in one portion by syringe and the stirred solution was allowed to warm to room temperature overnight. The reaction mixture was concentrated giving a dark red oil which was then dissolved in 1000 ml of 2.5% aqueous NaHCO_3 and transferred to a separatory funnel. The solution was washed with ether (3 \times 200 ml). The ether washes were combined and back extracted with water (2 \times 200 ml). The combined aqueous layers were acidified (pH 2) with 2N HCl. This solution was extracted with EtOAc (4 \times 250 ml). The EtOAc layers were washed with brine (3 \times 250 ml), dried over Na_2SO_4 , and filtered. The solvent was evaporated *in vacuo*, yielding the target material *trans*-4-hydroxy-*N*-Cbz-L-proline as a pale yellow oil (30.2 g, quantitative yield), and used without further purification. 1:1 mixture of rotamers; δH ($\Delta 400$, 400 MHz, d_6 -DMSO) 12.69 (1H, br s, COOH), 7.39-7.27 (5H, m, ArH), 5.13 (1H, br s, OH), 5.11-5.00 (2H, m, benzyl CH_2), 4.27 (1.5H, t overlapped with m, $J = 7.8$), 4.20 (0.5H, t, $J = 8.1$), 3.47 (0.5H, dd, $J = 11.0$, 3.9), 3.43 (0.5H, dd, $J = 11.0$, 4.2), 3.38 (t masked by H_2O , $J = 1.7$), 2.22-2.10 (1H, m), 2.00-1.87 (1H, m); δC ($\Delta 400$, 100 MHz, d_6 -DMSO) 174.5 (carbamate CO), 174.1 (carbamate CO), 154.8 (acid CO), 154.4 (acid CO), 137.4, 137.4, 69.1, 68.3, 66.5, 58.4, 57.9, 55.5, 55.1, 38.5; ν_{max} (neat) cm^{-1} 3400 (br, overlap of acid O-H and alcohol O-H), 2951 (C-H), 1674 (br, acid C=O); EIMS m/z 265 ($[\text{M}]^+$ 2%), 221 (2%), 220 ($[\text{M}-\text{COOH}]^+$ 8%), 176 ($[\text{M}-\text{Bn}]^+$ 5%), 130 ($[\text{M}-\text{Cbz}]^+$ 9%), 91 ($[\text{Bn}]^+$ 100%).

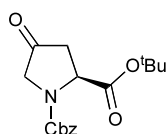
N-Cbz-4-oxo-L-proline. Error! Bookmark not defined.



Jones reagent (8N, 63 ml) was prepared by adding CrO_3 pellets (16.8 g, 168 mmol) to a solution of H_2SO_4 (14.5 ml) and H_2O (25 ml) and topping up to 63 ml with H_2O ; stirred until fully dissolved. (All waste and washings were quenched in acidified acetone / IPA solution and sent for professional disposal.) To a 2000 ml Erlenmeyer flask equipped with magnetic stirring, was added *trans*-4-hydroxy-*N*-Cbz-L-proline (16.4 g, 61.8 mmol) and acetone (1200 ml). The flask was submersed in a water bath maintained at 20 $^\circ\text{C}$. To this solution was added the Jones reagent (63 ml, 168 mmol) dropwise with stirring over a period of approximately 5 min. Stirring

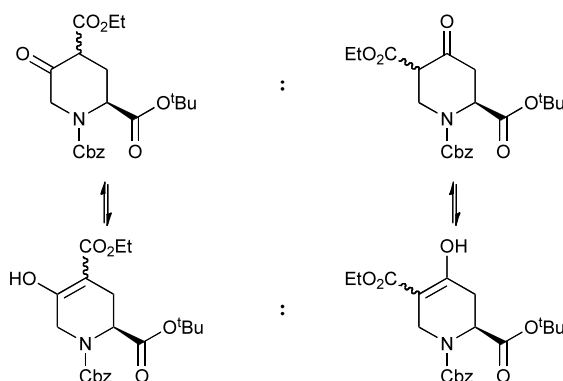
was continued for 30 min. Over this time the solution color changed from a bright red to a dark brown. The excess oxidant was quenched by dropwise addition of isopropyl alcohol (20 ml), the solution turned from dark brown to dark green and a thick green precipitate was formed. The precipitate was removed by filtration through a fine frit sintered glass funnel. The filtrate was concentrated *in vacuo* to around 300 ml, diluted with EtOAc (900 ml), and transferred to a separatory funnel. This solution was washed with brine (6 × 250 ml) and the organic layer was dried over MgSO₄ and filtered. The solvent was evaporated *in vacuo*, yielding the target material *N*-Cbz-4-oxo-L-proline as a yellow oil (turned crystalline on thorough drying; 13.6g, 51.7mmol, 75%), used without further purification. 1:1 mixture of rotamers; δ H (Δ 400, 400 MHz, d₆-DMSO) 13.09 (1H, br s, COOH), 7.41-7.29 (5H, m, ArH), 5.13 (2H, s, benzyl CH₂), 4.72 (0.5H, dd, *J* = 10.6, 2.7), 4.63 (0.5H, dd, *J* = 10.5, 2.3), 3.94 (1H, dd, *J* = 24.0, 18.3), 3.76 (1H, dd, *J* = 28.4, 18.1), 3.16 (1H, dt, *J* = 19.3, 10.7), 2.60-2.53 (1H, m masked by DMSO); δ C (Δ 400, 100 MHz, d₆-DMSO) 209.3 (ketone CO), 208.8 (ketone CO), 173.7 (carbonate CO), 173.6 (carbonate CO), 154.9 (acid CO), 154.3 (acid CO), 137.0, 67.0, 60.3, 56.6, 56.5, 55.4, 53.1, 52.8, 41.5, 21.6, 21.3, 14.6; ν max (neat) cm⁻¹ 3025 (br; acid O-H), 2965 (C-H), 1753 (ketone C=O), 1644 (acid C=O); EIMS *m/z* 263 ([M]⁺ 0.8%), 218 ([M-COOH]⁺ 2%), 176 (4%), 174 ([M-Bn]⁺ 2%), 128 ([M-Cbz]⁺ 4%), 91 ([Bn]⁺ 100%).

***N*-Cbz-4-oxo-*O*-*tert*-butyl-L-proline, 20.** Error! Bookmark not defined.



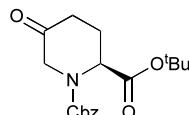
To a 500 ml flask equipped with magnetic stirring was added *N*-Cbz-4-oxo-L-proline (26.1 g, 99.3 mmol) followed by DCM (200 ml). The solution was cooled to 0 °C using an ice bath, and concentrated sulfuric acid (979 μ l) was added with stirring. Isobutylene was bubbled into the solution until the volume of the mixture had increased by approximately 50%. The reaction mixture was stirred overnight and allowed to warm to room temperature. The solution was then transferred to a separatory funnel with an additional volume of DCM (350 ml) and washed with a solution of half-saturated aqueous Na₂CO₃ (500 ml). The organic layer was washed further with water (2 × 250 ml), dried (MgSO₄) and filtered. The solvent was evaporated *in vacuo* to give a brown oil (38 g). Purification was performed by column chromatography (2:1 petrol/EtOAc used as eluent, R_F 0.40) to give the pure target material **20** (23.3 g, 73 mmol, 73%) as a pale yellow oil. δ H (Δ 400, 400 MHz, CDCl₃) 7.40-7.31 (5H, m, ArH), 5.27-5.13 (2H, ArCH₂, m), 4.75 (0.5H, dd, *J* = 10.3, 2.8), 4.71 (0.5H, dd, *J* = 10.5, 1.8), 4.04-3.89 (2H, m), 3.01-2.86 (1H, m), 2.56 (1H, dd, *J* = 18.9, 2.1), 1.45 (4.5H, s, 0.5 × C(CH₃)₃), 1.37 (4.5H, s, 0.5 × C(CH₃)₃); δ C (Δ 400, 100 MHz, CDCl₃) 208.2, 207.6, 170.6, 170.6, 155.0, 154.3, 136.2, 136.0, 128.6, 128.3, 128.1, 128.1, 82.7, 67.6, 56.9, 52.8, 52.6, 41.3, 40.7, 27.9, 27.8, 27.5; ν max (neat) cm⁻¹ 2980 (C-H), 1766 (C=O), 1736 (C=O), 1708 (C=O); EIMS *m/z* 319 ([M]⁺ 0.006%), 263 ([M-^tBu]⁺ 5%), 218 ([M-^tBu-COOH]⁺ 11%), 174 ([M-^tBu-Bn]⁺ 14%), 128 ([M-^tBu-Cbz]⁺ 7%), 91 ([Bn]⁺ 100%).

***N*-Cbz-4-ethoxycarbonyl-5-oxo-(*S*)-*tert*-butylpipercolate, 21 / *N*-Cbz-4-oxo-5-ethoxycarbonyl-(*S*)-*tert*-butylpipercolate, 22.** Error! Bookmark not defined.



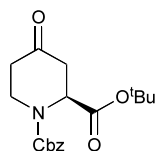
To a stirred solution of **20** (11.0 g, 34.48 mmol) in ether (130 mmol), under an inert atmosphere (N₂), was added dropwise boron trifluoride-diethyl etherate (4.59 ml, 36.2 mmol), followed by addition of ethyl diazoacetate (5.41 ml, 51.7 mmol) at 5 °C and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with ether (126 ml) and washed with saturated aqueous NH₄Cl solution (180 ml) and brine (150 ml). The organic layer was separated, dried (MgSO₄), filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (4:1 petrol/EtOAc used as eluent, 2:1 petrol/EtOAc R_F 0.50 and 0.57) to give **21** and **22** as a regioisomeric mixture of a yellow oil (10.54 g, 26 mmol, 79%). δ H (Δ 400, 400 MHz, CDCl₃) 12.07 (1H, s), 7.41-7.29 (5H, m, ArH), 5.28-5.18 (2H, m), 5.14-5.09 (0.5H, m), 5.02-4.94 (0.5H, m), 4.27-7.20 (2H, m), 4.07-3.84 (1H, m), 2.97 (0.66H, t, *J* = 14.1), 2.81 (0.33H, t, *J* = 14.8), 2.74-2.64 (0.33H, m), 2.50 (0.66H, ddt, *J* = 15.9, 6.2, 1.8), 1.42 (1.5H, s, C(CH₃)₃), 1.39 (3H, s, C(CH₃)₃), 1.38 (1.5H, s, C(CH₃)₃), 1.37 (3 H, s, C(CH₃)₃); δ C (Δ 400, 100 MHz, CDCl₃) 171.3, 171.2, 170.5, 170.4, 169.8, 169.1, 167.7, 167.1, 166.8, 166.4, 156.2, 155.8, 155.4, 155.3, 136.6, 136.4, 136.3, 136.2, 128.6, 128.6, 128.3, 128.1, 128.1, 128.1, 127.9, 94.9, 94.6, 94.5, 94.1, 82.5, 82.5, 82.3, 82.2, 68.2, 67.8, 67.6, 67.2, 60.9, 60.8, 60.4, 53.5, 53.4, 52.9, 52.8, 52.4, 43.7, 43.6, 39.1, 38.8, 30.4, 30.3, 28.0, 27.9, 27.7, 24.1, 23.8, 21.1, 15.1, 14.3; ν max (neat) cm⁻¹ 2980 (C-H), 1733 (C=O), 1708 (C=O), 1665, 1628; EIMS *m/z* , 405 ([M]⁺, 0.5%), 390 ([M-Me]⁺ 2%), 346 (8304 ([M-^tBu-COOH]⁺ 6%), 260 ([M-^tBu-Bn]⁺ 8%), 214 ([M-^tBu-Cbz]⁺ 18%), 168 (5%), 91 ([Bn]⁺ 100%).

***N*-Cbz-5-oxo-(*S*)-*tert*-butylpipicolate, **23**.** Error! Bookmark not defined.



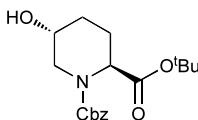
To a 250 ml round-bottom flask with magnetic stirring was added the isomeric mixture of **21** and **22** (5.04 g, 12.4 mmol), followed by DMSO (12.4 ml). NaCl (725 mg, 12.4 mmol) and water (246 μ l, 13.7 mmol) were added and the reaction mixture was heated to 140 °C (internal temperature). The reaction mixture turned from a clear pale yellow to a cloudy brown and gentle effervescence was observed. The reaction was stirred until complete by TLC (2 h) and cooled to room temperature. The reaction mixture was diluted with DCM (50 ml) and washed with NaCl solution (50% saturated; 3 \times 70 ml). The organic layer was dried (MgSO₄), filtered and reduced *in vacuo* to give a brown oil. Purification was performed by column chromatography (20% EtOAc in hexane used as eluent, R_F in 33% EtOAc:hexane **23** = 0.27 **24** = 0.24) to give ketone **23** as a pale yellow oil (1.49 g, 4.47 mmol, 36%). 1:1 mixture of rotamers; δ H (Δ 400, 400 MHz, CDCl₃) 7.39-7.29 (5H, m, ArH), 5.15 (1H, s, 0.5 \times ArCH₂), 5.16 (1H, AB-quartet, *J* = 12.3, 0.5 \times ArCH₂), 4.78 (0.5H, dd, *J* = 6.5, 5.9, 0.5 \times H-2), 4.62 (0.5H, dd, *J* = 6.5, 5.9, 0.5 \times H-2), 4.46 (0.5H, d, *J* = 19.0, 0.5 \times H-6_{eq}), 4.36 (0.5H, d, *J* = 18.8, 0.5 \times H-6_{eq}), 4.00 (0.5H, d, *J* = 18.8, 0.5 \times H-6_{ax}), 3.93 (0.5H, d, *J* = 19.0, 0.5 \times H-6_{ax}), 2.53-2.04 (4H, m, H-3 and H-4), 1.47 (4.5H, s, 0.5 \times C(CH₃)₃), 1.38 (4.5H, s, 0.5 \times C(CH₃)₃); δ C (Δ 400, 100 MHz, CDCl₃) 204.8 & 204.6 (ketone C=O), 170.3 & 170.2 (ester C=O), 155.2 & 155.0 (carbamate C=O), 135.8 (quaternary ArC), 128.3, 128.2, 128.0, 128.0, 127.8, 127.7, 82.0 (C(CH₃)₃), 67.5 & 67.4 (ArCH₂), 54.2 & 53.8 (C²), 51.9 & 51.5 (C⁶), 35.5 & 35.3 (C³), 27.7 & 27.6 (C(CH₃)₃), 23.6 & 23.4 (C⁴); ν max (neat) cm⁻¹ 2977 (C-H), 1729 (ketone C=O), 1703 (ester and carbamate C=O); CIMS *m/z*, 368.2 (19%), 334 ([M]⁺H⁺ 15%), 278 ([M-^tBu+H]⁺ 31%), 234 (34%), 91([Bn]⁺ 100%).

***N*-Cbz-4-oxo-(*S*)-*tert*-butylpipicolate, **24**.** Error! Bookmark not defined.



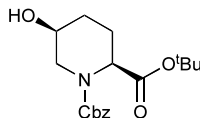
Ketone **24** obtained as a pale yellow oil (1.40 g, 4.20 mmol, 34%) 1:1 mixture of rotamers; δ H (Δ 400, 400 MHz, CDCl₃) 7.40-7.27 (5H, m, ArH), 5.18 (1H, s, 0.5 \times ArCH₂), 5.16 (1H, AB-quartet, *J* = 12.3, 0.5 \times ArCH₂), 5.06-5.00 (0.5H, m, 0.5 \times H-2), 4.89-4.83 (0.5H, m, 0.5 \times H-2), 4.16-4.06 (1H, m, H-6_{eq}), 3.76-3.64 (1H, m, H-6_{ax}), 2.83-2.43 (4H, m, H-3 and H-4), 1.43 (4.5H, s, 0.5 \times C(CH₃)₃), 1.36 (4.5H, s, 0.5 \times C(CH₃)₃); δ C (V400, 100 MHz, CDCl₃) 205.7 (ketone C=O), 169.9 (ester C=O), 155.7 (carbamate C=O), 136.2 (quaternary ArC), 130.1, 128.7, 128.1, 82.9 (C(CH₃)₃), 68.0 (ArCH₂), 55.3 (C²), 41.6 & 41.32 (C⁶), 40.4 & 40.32 (C³), 39.7 (C⁵); 28.0 (C(CH₃)₃), ν max (neat) cm⁻¹ 3065 (ArC-H), 3034 (ArC-H), 2978 (C-H), 2936(C-H), 1729 (ketone C=O), 1700 (ester and carbamate C=O); CIMS *m/z*, 368 (28%), 334 ([M]⁺H⁺ 10%), 324 (11%), 316 (9%), 278 ([M-^tBu+H]⁺ 18%), 234 (30%), 232 ([M-CO₂^tBu]⁺ 23%), 91([Bn]⁺ 100%).

***N*-Cbz-5-(*R*)-hydroxy-(*S*)-*tert*-butylpipicolate, **25a**.** Error! Bookmark not defined.



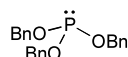
To an anhydrous 250 ml round-bottom flask, under an inert atmosphere (N₂) and with magnetic stirring, was added the 5-keto pipicolate **23** (1.0 g, 3.00 mmol). The flask was evacuated and nitrogen backfilled three times. Anhydrous THF (30 ml) was added and the flask was cooled to -45 °C (internal temperature). L-selectride (lithium tri-*sec*-butyl(hydrido)-borate); 1M in THF; 3.60 ml, 3.60 mmol) was added dropwise over 5 min, over which time the reaction changed from pale yellow to dark yellow. Stirring was continued at -45 °C for 30 min and then the reaction was warmed to room temperature. The reaction was quenched with NH₄Cl(aq) (saturated; 15 ml) causing a thick white precipitate to form. EtOAc (50 ml) and water (20 ml) were added and the layers separated. The organic layer was dried (MgSO₄), filtered and reduced *in vacuo*. Purification was performed by column chromatography (45% EtOAc in hexane, R_F **25a** = 0.22, R_F **25b** = 0.24) to give the *trans*-alcohol **25a** (222 mg, 0.66 mmol, 22.2%) as a pale yellow oil. [α]_D¹⁹ = -24.4 (*c* 0.1, DCM); 1:1 mixture of rotamers; δ H (Δ 400, 400 MHz, CDCl₃) 7.38-7.29 (5H, m, ArH), 5.16 (1H, AB-quartet, *J* = 12.6, 0.5 \times ArCH₂), 5.14 (1H, AB-quartet, *J* = 12.5, 0.5 \times ArCH₂), 4.87 (0.5H, d, *J* = 5.5, 0.5 \times H-2_{eq}), 4.74 (0.5H, d, *J* = 6.4, 0.5 \times H-2_{eq}), 4.12 (0.5H, d, *J* = 14.2, 0.5 \times H-6_{eq}), 4.09 (0.5H, d, *J* = 14.9, 0.5 \times H-6_{eq}), 4.04-4.01 (0.5H, m, 0.5 \times H-5_{eq}), 3.96-3.92 (0.5H, m, 0.5 \times H-5_{eq}), 2.87 (0.5H, dd, *J* = 14.2, 0.5 \times H-6_{ax}), 2.77 (0.5H, dd, *J* = 14.9, 0.5 \times H-6_{ax}), 2.21-2.09 (1H, m, H-3_{eq}), 2.10-1.70 (1H, br s, OH), 2.08-2.14 (1H, m, H-3_{ax}), 2.01-1.93 (1H, dm, *J* = 14.3, H-4_{eq}), 1.32-1.19 (1H, dm, *J* = 14.3, H-4_{ax}); 1.45 (4.5H, s, 0.5 \times C(CH₃)₃), 1.41 (4.5H, s, 0.5 \times C(CH₃)₃); δ C (Δ 400, 100 MHz, CDCl₃) 170.3 (ester C=O), 156.8 & 156.6 (carbamate C=O), 136.3 & 136.2 (quaternary ArC), 128.2 (2 \times ArC), 127.72, 127.50 (2 \times ArC), 81.5 & 81.4 (C(CH₃)₃), 67.1 & 67.0 (ArCH₂), 63.2 & 63.0 (C⁵), 54.6 & 54.3 (C²), 47.4 & 47.4 (C⁶), 27.7 & 27.7 (C(CH₃)₃), 26.7 & 26.4 (C³), 20.0 & 20.0 (C⁴); ν max (neat) cm⁻¹ 3444 (br, O-H), 2975 (C-H), 2940 (C-H), 1731 (ester C=O), 1686 (carbamate C=O); CIMS *m/z*, 336 ([M]⁺H⁺, 10%), 326 (36%), 236 (100%), 234 ([M-CO₂^tBu]⁺ 68%), 190 (71%), 91 ([Bn]⁺ 98%); ESIMS *m/z*, 358 ([M]⁺Na⁺ 100%), 302 ([M-O^tBu+H]⁺Na⁺ 61%), HRMS ESI calcd. for C₁₈H₂₅NO₅Na ([M]⁺Na⁺) 358.1625, found 358.1626.

***N*-Cbz-5-(*S*)-hydroxy-(*S*)-*tert*-butylpipecolate, 25b.** Error! Bookmark not defined.



Cis-alcohol **25b** (405.4 mg, 1.209 mmol, 40.3%) isolated as a pale yellow oil. $[\alpha]_D^{19} = -23.3$ (c 0.2, DCM); 1:1 mixture of rotamers; δ H (Δ 400, 400 MHz, CDCl_3) 7.38-7.28 (5H, m, ArH), 5.29 (1H, AB-quartet, $J = 12.4$, $0.5 \times \text{ArCH}_2$), 5.16 (1H, s, $0.5 \times \text{ArCH}_2$), 4.78 (0.5H, d, $J = 5.1$, $0.5 \times H-2_{\text{eq}}$), 4.65 (0.5H, d, $J = 5.1$, $0.5 \times H-2_{\text{eq}}$), 4.25 (0.5H, dd, $J = 13.0$, 4.7, $0.5 \times H-6_{\text{eq}}$), 4.18 (0.5H, dd, $J = 13.0$, 4.7, $0.5 \times H-6_{\text{eq}}$), 3.69-3.59 (1H, m, $H-5_{\text{ax}}$), 2.87 (0.5H, dd, $J = 13.0$, 10.5, $0.5 \times H-6_{\text{ax}}$), 2.77 (0.5H, dd, $J = 13.0$, 10.5, $0.5 \times H-6_{\text{ax}}$), 2.32-2.22 (1H, m, $H-3_{\text{eq}}$), 2.20-2.12 (0.5H, br s, $0.5 \times \text{OH}$), 2.01-1.93 (1H, m, $H-4_{\text{eq}}$), 1.87-1.82 (0.5H, br s, $0.5 \times \text{OH}$), 1.76-1.63 (1H, m, $H-3_{\text{ax}}$), 1.46 (4.5H, s, $0.5 \times \text{C}(\text{CH}_3)_3$), 1.42 (4.5H, s, $0.5 \times \text{C}(\text{CH}_3)_3$), 1.32-1.19 (1H, m, $H-4_{\text{ax}}$); δ C (Δ 400, 100 MHz, CDCl_3) 169.8 (ester C=O), 155.9 & 155.7 (carbamate C=O), 136.1 & 136.0 (quaternary ArC), 128.1 ($2 \times \text{ArC}$), 127.72, 127.49 ($2 \times \text{ArC}$), 127.38, 81.6 & 81.5 ($\text{C}(\text{CH}_3)_3$), 67.5 & 67.4 (ArCH_2), 66.7 & 66.5 (C^5), 53.8 & 53.6 (C^2), 47.8 & 47.7 (C^6), 29.8 & 29.4 (C^3), 27.6 & 27.6 ($\text{C}(\text{CH}_3)_3$), 24.8 & 24.6 (C^4). ν_{max} (neat) cm^{-1} 3451 (br, O-H), 2976 (C-H), 2938 (C-H), 2869 (C-H), 1732 (ester C=O), 1705 (carbamate C=O); CIMS m/z 336 ($[\text{M}]^+$ 5%), 326 (43%), 280 (20%), 236 (88%), 234 ($[\text{M}-\text{CO}_2^t\text{Bu}]^+$ 61%), 218 (15%), 190 (69%), 91 ($[\text{Bn}]^+$, 100%).

Tribenzylphosphite.¹



To an anhydrous 250 ml round-bottom flask, under an inert atmosphere (N_2) and with magnetic stirring, was added anhydrous Et_2O (50 ml) and PCl_3 (870 μl , 10 mmol). The reaction mixture was cooled to -10°C (internal temperature) and Et_3N solution was added (2.16 ml, 15.5 mmol in 2.1 ml anhydrous Et_2O). A white vapour and white precipitate formed. BnOH solution (1.55 ml, 15.0 mmol in 1.8 ml anhydrous Et_2O) was added dropwise over 5 min. The reaction was allowed to warm to room temperature overnight and the resulting solution was filtered through celite under an inert atmosphere (nitrogen) and washed with hexane. The solvent was removed *in vacuo* to give tribenzylphosphite as a clear, colourless liquid (3.15 g, 8.95 mmol, 90%) which was used without further purification. δ H (Δ 400, 400 MHz, neat), 6.38-6.12 (15H, m, ArH), 3.91-3.82 (6H, d, $J = 7.9$, $3 \times \text{ArCH}_2$); δ C (E400, 100 MHz, neat) 138.4 (d, $J = 5.4$, quaternary ArC), 128.4, 127.7, 127.5, 64.2 (d, $J = 10.8$, ArCH_2); δ P (E400, 162 MHz, neat) 139.6.

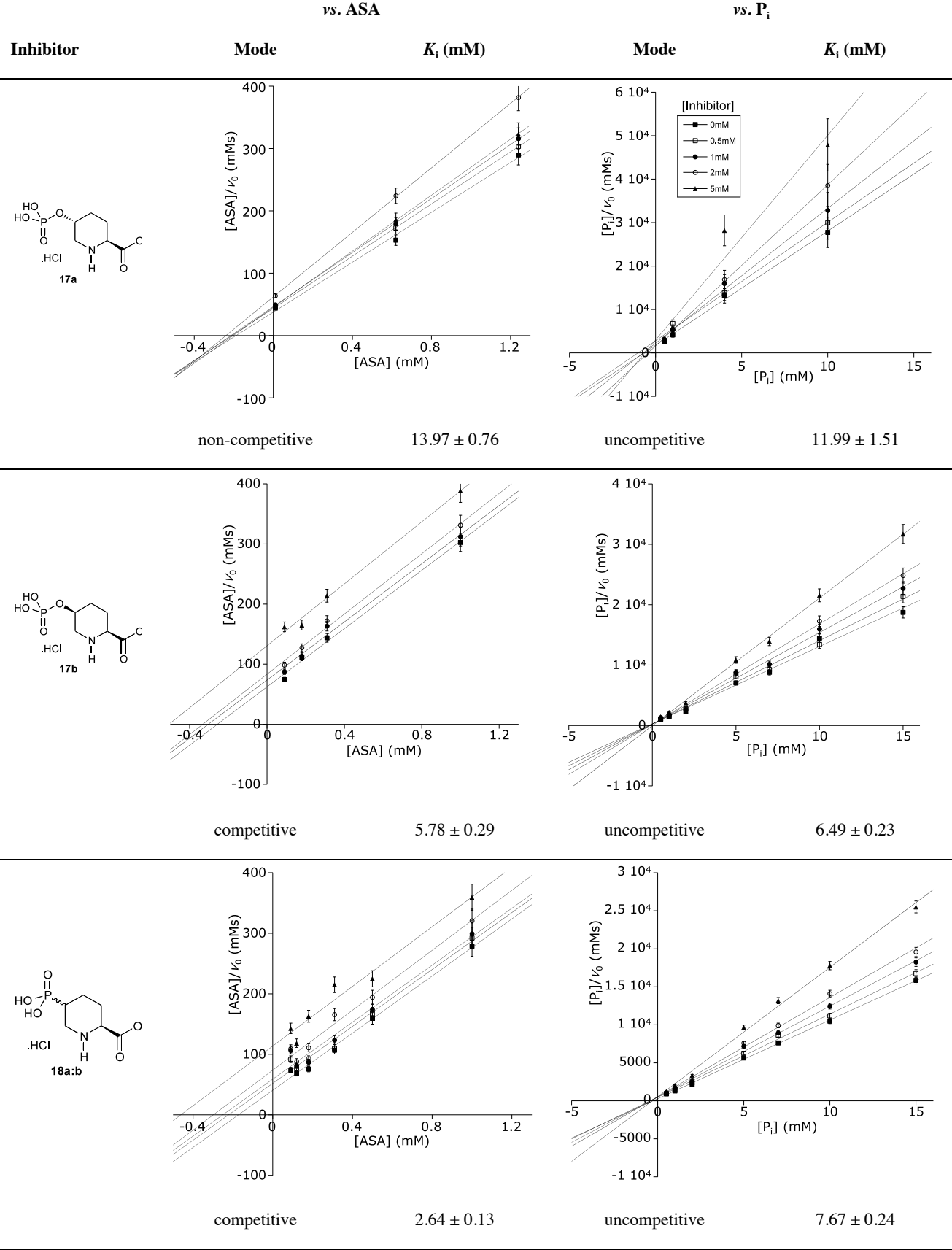
Dibenzyl phosphoryl iodide, 26.



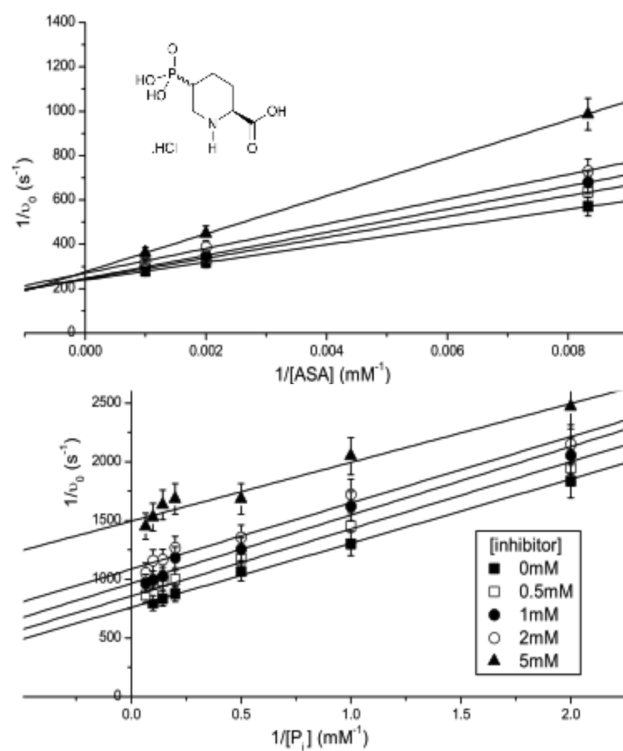
The required number of moles of iodine were weighed into an anhydrous flask, under an inert atmosphere (N_2) with magnetic stirring, and DCM (0.18 M) was added, leading to a dark violet solution. The solution was cooled to 0°C and tribenzylphosphite was titrated in, dropwise, until the solution turned colourless. The solution was then used without further purification.

Kinetic Data

Hanes-Wolf Plots



Lineweaver-Burk Plots



References

1. R. J. Cox, A. de Andrés-Gómez and C. R. A. Godfrey, *Org. Biomol. Chem.*, 2003, **1**, 3137-3177.