PERKIN 1

Synthesis and *in vitro* enzyme activity of aza, oxa and thia derivatives of bacterial cell wall biosynthesis intermediates†

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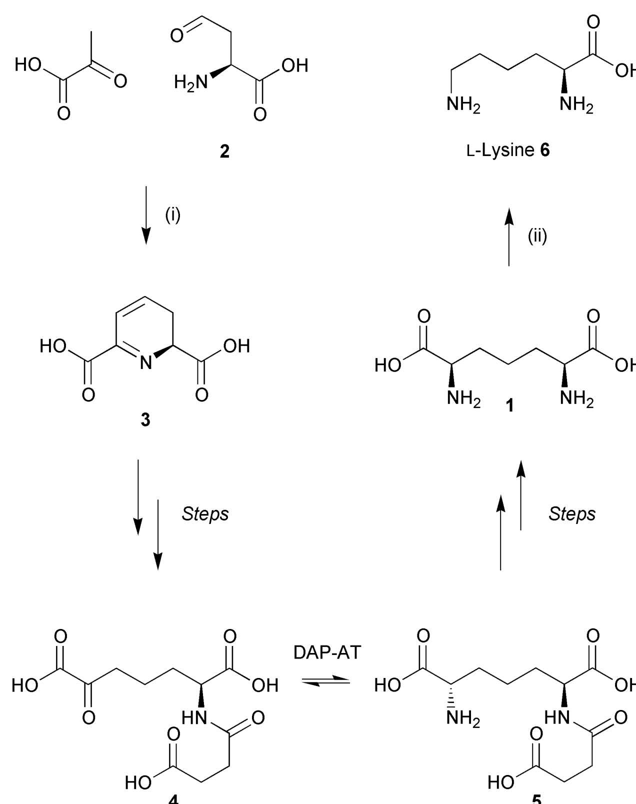
Mechanism based inhibitors of diaminopimelate aminotransferase (DAP-AT) were designed using knowledge of its substrate specificity and mechanism. Synthesis of thiolester and amide substrate analogues was achieved prior to *in vitro* inhibition studies, but ester analogues proved too unstable to isolate. Thia substrate analogues showed no inhibitory properties, but the aza substrate analogue **12a** showed reversible inhibition *vs.* DAP-AT and time dependent inhibition in the absence of the natural substrate **4**. Substrate analogue **12a** is the first example of an amide inhibitor of PLP dependent enzymes. Antibiotic properties of **12a** were also briefly assessed.

Introduction

Bacterial cell wall biosynthesis is an attractive target for the design of new antibacterial compounds.[1] Indeed many microbial organisms biosynthesize compounds such as β-lactams (*e.g.* penicillin, clavulanic acid) and glycopeptides (*e.g.* vancomycin) which target bacterial cell wall biosynthesis for evolutionary advantage over competing microorganisms. In order to achieve this, the antibiotic producing organisms have evolved parallel resistance mechanisms to avoid suicide. Genes coding for these resistance mechanisms (*e.g.* β-lactamases) can be passed between different bacterial species and in environments rich in antimicrobial compounds these mechanisms can become prevalent through natural selection.[2] Such processes may have given rise to the development of antibiotic resistant pathogens which pose a threat to public health.

We have been studying the biosynthesis of the key bacterial cell wall component diaminopimelic acid (DAP) **1** in order to develop new classes of antibacterial compounds. The bacterial biosynthesis of DAP **1** has been well studied (Scheme 1).[3] In brief, the reduction of L-aspartate gives the C_4 L-aspartate semialdehyde **2** which condenses with pyruvate to give L-dihydrodipicolinate **3** (Scheme 1). Reduction to L-tetrahydrodipicolinate followed by acylation then gives L-α-(*N*-succinylamino)-ε-oxopimelic acid **4** for most common bacteria.‡ In *Bacillus* species, and some others, the acylating group is acetyl. At this point the operation of *N*-acyl diaminopimelate aminotransferase (DAP-AT) yields LL-*N*-acyl-DAP **5** which is subjected to deacylation and epimerisation to afford *meso*-DAP **1**. In a small number of bacterial species such as *Bacillus sphaericus meso*-DAP can be synthesised directly from tetrahydrodipicolinate, catalysed by *meso*-DAP dehydrogenase.[4] The final step of the pathway is the specific decarboxylation of *meso*-DAP **1** at the D-centre to give L-lysine **6**. Since L-lysine **6** is required for protein synthesis, inhibition of the pathway could be expected to be detrimental to bacterial development. However L-lysine **6** and/or DAP **1** are also required by bacteria for the construction of the peptidoglycan layer of the cell wall. In this polymeric structure the lysine or DAP diamine plays the crucial role of cross-linking short peptides attached to the glc-*N*Ac-mur-*N*Ac polysaccharide.[5] These cross-links give the peptidoglycan structure the physical strength to resist lysis caused by the very high internal osmotic pressures of bacteria. Thus DAP pathway inhibitors could be promising new antimicrobial compounds.



Scheme 1 The bacterial synthesis of L-lysine: (i) L-dihydrodipicolinate synthase; (ii) *meso*-diaminopimelate decarboxylase.

All of the DAP pathway enzymes have been studied as potential targets for antimicrobial action. One of the most promising targets is the aminotransferase DAP-AT and we have synthesised potent slow binding inhibitors (*e.g.* **7**, **8**) of this enzyme with inhibition constants in the low nM range.[6] These compounds do show antimicrobial activity, although not at a comparable level of potency with medically useful drugs such

† Electronic supplementary information (ESI) available: ^{13}C NMR data, mass spectra and IR spectra for **11a.** See http://www.rsc.org/suppdata/p1/b1/b105117m/

‡ In this article the term succinyl refers to the group $HO_2CCH_2CH_2$-CO–, and methylsuccinyl to $MeO_2CCH_2CH_2CO$–.

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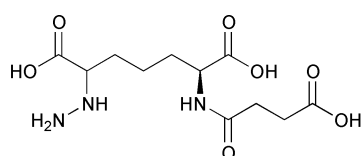
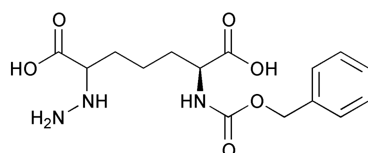
as carbenicillin.[7] One reason for this could be that compounds such as **7** and **8** are *reversible* inhibitors of the aminotransferase. One potential way of increasing *in vivo* potency could be to utilise irreversible inhibitors of DAP-AT.



7, K_I *vs.* DAP-AT 22 nM



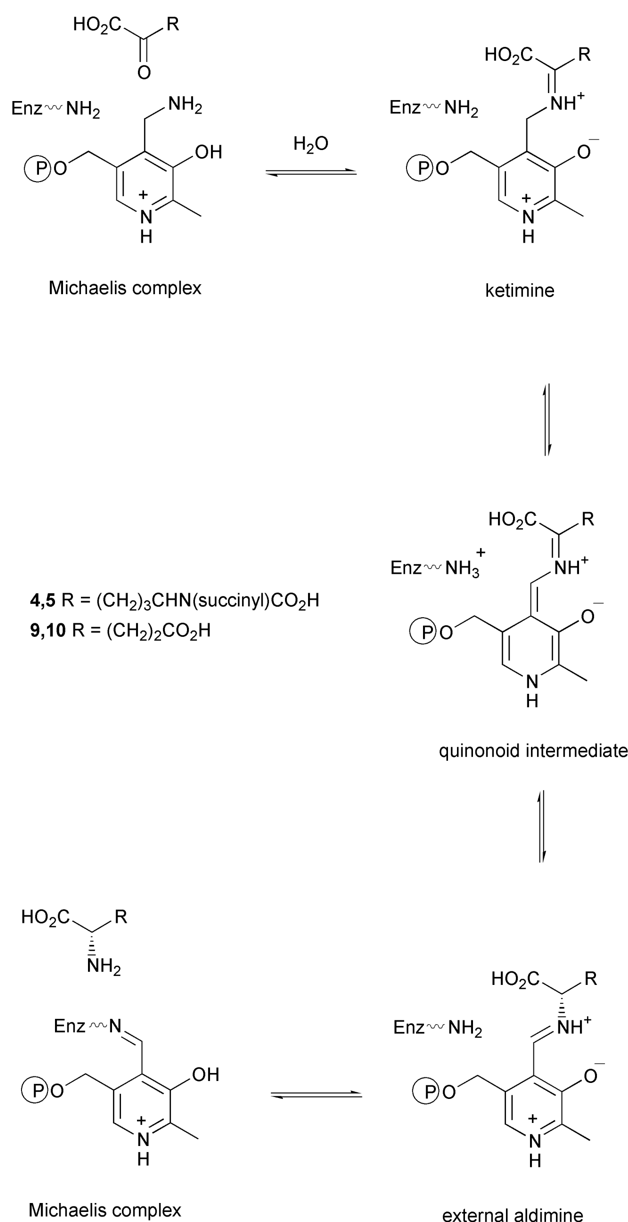
8, K_I *vs.* DAP-AT 54 nM

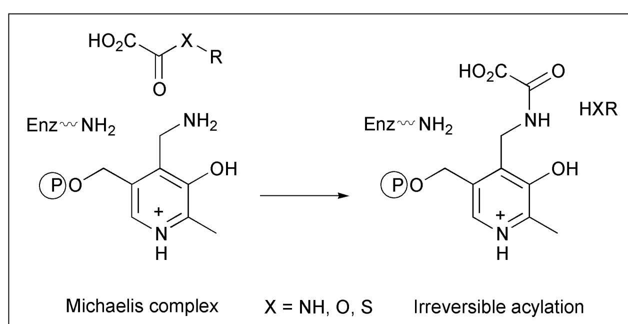
Design of potential irreversible DAP-AT inhibitors

Pyridoxal 5′-phosphate (PLP) dependent aminotransferases, such as DAP-AT, interconvert ketones and amines.[8] In the ‘forward’ direction ketones are stereoselectively reductively aminated and in the ‘reverse’ direction (and in order to regenerate the cofactor) amines are oxidised to ketones. For DAP-AT the substrate **4** is reductively aminated and *in vitro* (and presumably *in vivo*) an excess of L-glutamic acid **9** acts as an amine source to regenerate the cofactor (Scheme 2). For DAP-AT the catalytic cycle is proposed to involve attack by the nucleophilic amine of the pyridoxamine-5′-phosphate (PMP) at the substrate carbonyl to form an enzyme bound ketimine. Rearrangement of this ketimine follows, *via* the quinonoid intermediate to the aldimine. Subsequent aminolysis by an active site amine then releases the product. L-Glutamic acid **9** can then drive the reaction in reverse, yielding α-ketoglutarate **10** and the PMP form of the cofactor ready for another catalytic cycle.

Compounds such as the potent DAP-AT inhibitors **7** and **8** interrupt this process by forming (reversibly) an enzyme bound hydrazone with the electrophilic PLP form of the cofactor.[6,9] We envisaged designing compounds which could react irreversibly with the nucleophilic cofactor. Such compounds could be esters, thiolesters or possibly amides, in which the α-methylene of the substrate is replaced by oxygen, sulfur or nitrogen. These compounds, possessing a nucleofuge might be expected to acylate the PMP form of the cofactor, forming an enzyme bound amide which would be unlikely to be rapidly hydrolysed (Scheme 2). A similar strategy was recently demonstrated by Leeper who showed that the thiolester and ester substrate mimics inhibit 5-aminolaevulinic acid dehydratase – an enzyme which also operates *via* an enzyme–substrate imine intermediate.[10]

Previous substrate specificity experiments with DAP-AT revealed that for adequate recognition substrates should possess a seven-carbon backbone.[9] It is clear however that DAP-AT must also process the five-carbon skeleton of glutamate. Restrictions of choice of *N*-acyl group are less severe, with succinyl being preferred, but substitution by Cbz, for example, is tolerated well (k_{cat}/K_M *ca.* 25% wrt **4**). We sought to preserve the seven atom backbone of DAP and utilise the *N*-acyl groups succinyl and Cbz for inhibitor design. Thus our initial targets were **11**–**13**. Clearly **11a** and **11b**, containing sulfur instead of a methylene linkage could be expected to be somewhat larger than the natural substrate **4** and we also sought to synthesise the one atom shorter thia analogue **14** in order to limit the overall size of the potential inhibitor.



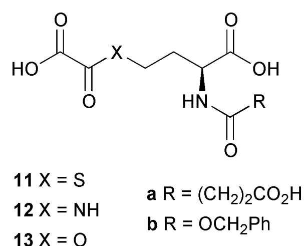


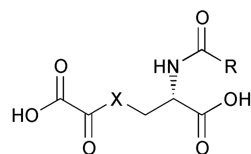
Scheme 2 The mechanism of DAP-AT. Proposed mode of action of oxalyl inhibitors (boxed).

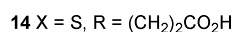
Results and discussion

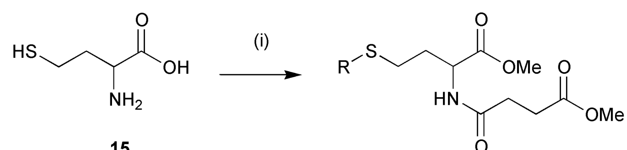
Thiol esters

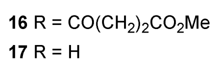
Succinylation of homocysteine **15** with succinic anhydride in saturated aqueous bicarbonate followed by rapid acidification, organic extraction and treatment with diethyl ether–diazomethane gave a 2 : 1 mixture of the methylsuccinyl thiolester **16** and the dimethyl ester thiol **17** in good yield (Scheme 3).

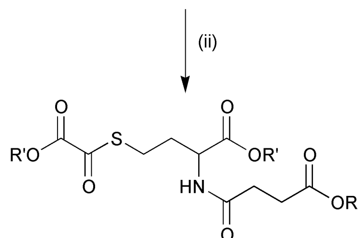


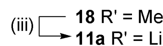


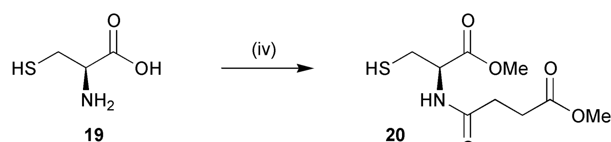


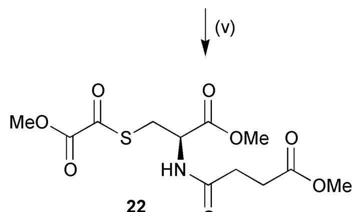


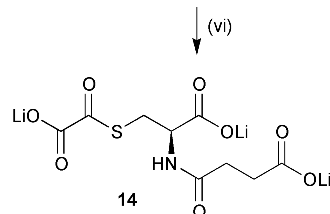










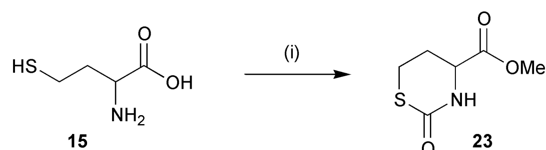


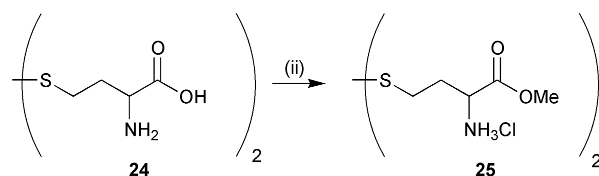
Scheme 3 Synthesis of thia substrate analogues. *Reagents and conditions*: (i) succinic anhydride, $NaHCO_3$ (aq), then CH_2N_2, 31%; (ii) MeO_2CCOCl, 2,6-lutidine, CH_2Cl_2, 49%; (iii) 3.0 eq. LiOH, CH_3-CN–H_2O, quantitative; (iv) succinic anhydride, $NaHCO_3$ (aq), then CH_2N_2, 19%; (v) MeO_2CCOCl, 2,6-lutidine, CH_2Cl_2, 72%; (vi) 3.0 eq. $LiOH{\cdot}H_2O$, CH_3CN–H_2O, quantitative.

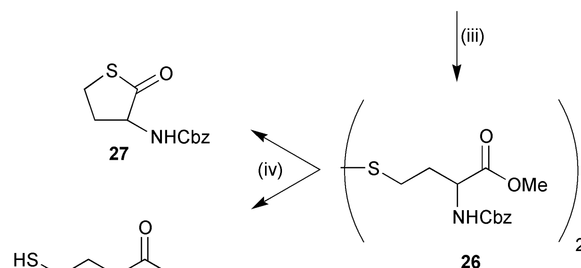
Treatment of **17** with methyl oxalyl chloride in CH_2Cl_2 in the presence of one equivalent of 2,6-lutidine then gave the expected oxalyl thiolester **18** in good yield. Final selective methyl ester hydrolysis using 3.0 equivalents of $LiOH{\cdot}H_2O$ gave the deprotected target compound **11a** with no trace of free thiol or lithium oxalate (^{1}H NMR, ^{13}C NMR, IR).

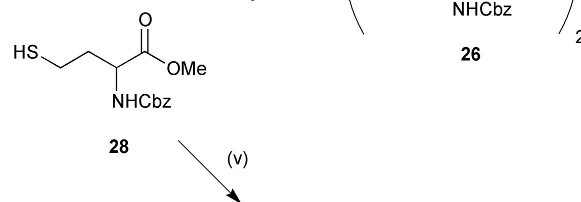
An analogous route starting with L-cysteine **19** was also followed. Succinylation and methylation yielded a mixture of the required thiol **20** and its symmetrical disulfide **21**. The purified thiol was esterified using methyl oxalyl chloride and the product **22** was separated from starting material **20** by an aqueous $CuSO_4$ wash. Final deprotection using $LiOH{\cdot}H_2O$ was difficult, but addition of solid LiOH under conditions favouring very slow dissolution (2 : 1 CH_3CN : H_2O) afforded the trilithium salt **14**. No evidence of thiolester cleavage to yield the free thiol was observed by MS or IR analysis.

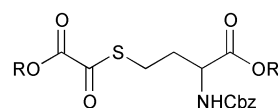
An attempted parallel synthesis of the *N*-Cbz protected homocysteine analogue began with the treatment of homocysteine **15** with CbzCl under standard Schotten–Baumann conditions. However, after acidification and treatment with diethyl ether–diazomethane only the cyclic thiocarbamate **23**[11] was isolated (Scheme 4). In order to circumvent problems caused by the nucleophilic thiol, homocystine **24** was used

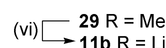




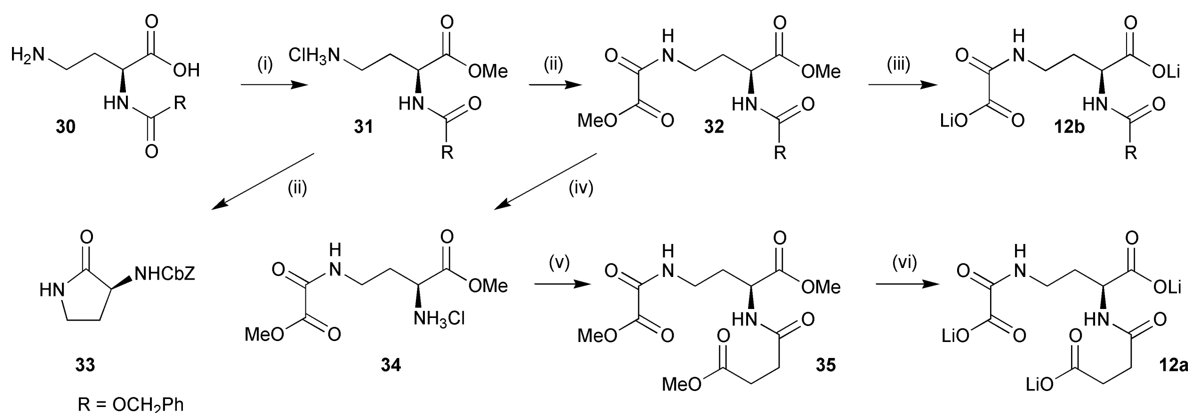








Scheme 4 Synthesis of thia substrate analogues. *Reagents and conditions*: (i) CBzCl, NaOH (aq), then H_3O^+; (ii) 10% HCl–MeOH, Δ, quant.; (iii) CbzCl, CH_2Cl_2, Et_3N, 27%; (iv) 3% Na–Hg, MeOH, 24%; (v) MeO_2CCOCl, 2,6-lutidine, CH_2Cl_2, 88%; (vi) 2.0 eq. Li-$OH{\cdot}H_2O$ CH_3CN–H_2O, quant.



Scheme 5 Synthesis of aza substrate analogues. *Reagents and conditions*: (i) 10% HCl–MeOH, Δ, 83%; (ii) MeO_2CCOCl, CH_2Cl_2, HOBT, then **31**, 53%; (iii) 2.0 eq. $LiOH\cdot H_2O$, CH_3CN–H_2O, quant.; (iv) H_2 1 atm, 10% Pd/C, MeOH, 93%; (v) succinic anhydride, $CHCl_3$, Et_3N, then CH_2N_2, 76%; (vi) 3.0 eq. $LiOH\cdot H_2O$, CH_3CN–H_2O, 97%.

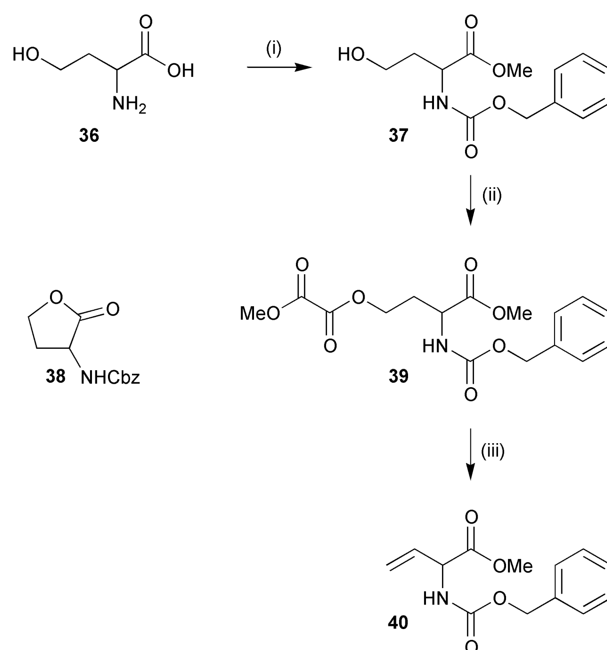
instead. Treatment of this compound with CbzCl under Schotten–Baumann conditions gave poor yields of the bis-Cbz protected product. The alternative procedure of methylating first using methanolic HCl to give the diester **25**[12,13] followed by Cbz protection in organic solvent, yielding the fully protected disulfide **26**, was preferable (Scheme 4). Disulfide cleavage by sodium borohydride in methanol was rapid, but afforded only the *N*-protected thiolactone **27**. Cleavage using sodium amalgam in methanol was slower, but afforded a 2.5 : 1 mixture of the desired thiol **28** and the thiolactone **27** which were easily separated by chromatography. Coupling the thiol **28** with methyl oxalyl chloride in the presence of 2,6-lutidine gave the thiolester **29** in high yield and the target **11b** was produced by selective methyl ester cleavage as before.

Amides

Commercially available α*N*-Cbz protected L-diaminobutyric acid **30** was treated with methanolic HCl to give a quantitative yield of the methyl ester hydrochloride **31**[14] (Scheme 5). Attempted coupling of this amine with methyl oxalyl chloride in the presence of various organic bases failed to give the desired amide **32**, yielding only the α-*N*-protected lactam **33**. The amide **32** was produced by pretreatment of methyl oxalyl chloride with hydroxybenzotriazole (HOBT) and excess base in CH_2Cl_2 before slow addition of the amine hydrochloride. Methyl ester hydrolysis then afforded the desired **12b**. Cbz protected **32** could also be deprotected under standard hydrogenolysis conditions and the resulting amine hydrochloride **34** then acylated with succinic anhydride in saturated aqueous $NaHCO_3$ followed by a diazomethane quench to afford the triester **35**, albeit in poor yield. Better yields were achieved by treating the hydrochloride salt **34** with succinic anhydride in CH_2Cl_2 in the presence of Et_3N before diazomethane treatment. Following chromatography the trimethyl ester **35** was quantitatively converted to the trilithium salt **12a** using standard procedures.

Esters

Following the successful selective LiOH deprotection of the methyl ester of **29** in the presence of the sensitive thiol ester functionality we considered synthesizing oxoaminooxapimelates by a parallel strategy to that used for the thia analogues. Homoserine **36** was thus treated with CbzCl followed by rapid acidification and treatment with excess diethyl ether–diazomethane (Scheme 6). In the case of homocysteine **15** the major product of this reaction sequence was the thiocarbamate **23**. Here, however, a mixture of the protected desired alcohol **37** and lactone **38** was formed. Rapid purification of the alcohol followed by coupling with methyl oxalyl chloride gave the expected oxopimelate **39** as well as additional lactone **38**.



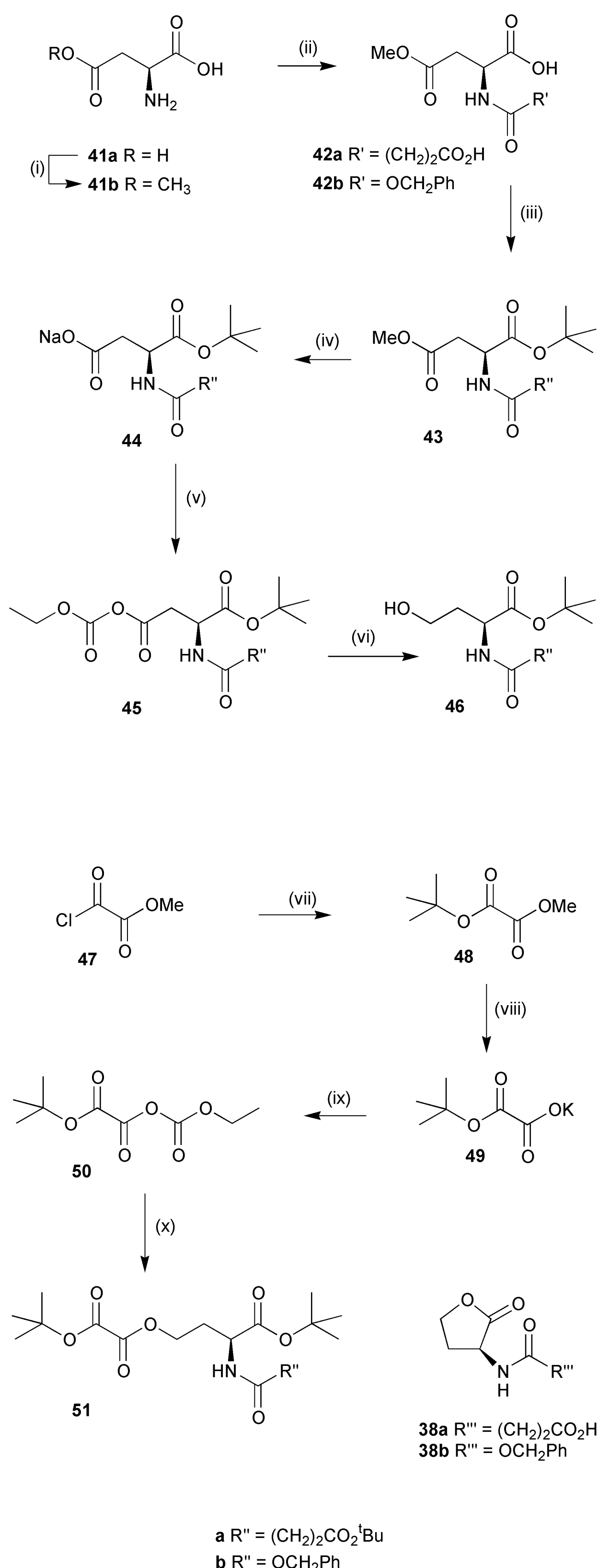
Scheme 6 Synthesis of oxa substrate analogues. *Reagents and conditions*: (i) $NaHCO_3$ (aq), CBzCl, then CH_2N_2, 95%; (ii) CH_2Cl_2, pyridine, MeO_2CCOCl, 63%; (iii) 2.0 eq. $LiOH\cdot H_2O$, CH_3CN–H_2O.

Attempted selective methyl ester hydrolysis under a number of reaction conditions (*e.g.* LiOH, various sources of I^-) resulted only in formation of mixtures of protected vinylglycine **40** and lactone **38**.

In order to avoid excessive lactone formation and to improve deprotection at the end of the synthesis we decided to utilise *tert*-butyl ester protecting groups and to utilise a masked γ-alcohol. Thus L-aspartic acid **41a** was selectively methylated at the β-carboxylate by treatment with one equivalent of thionyl chloride in methanol at 0 °C (Scheme 7). Subsequent Cbz and *tert*-butyl protection using standard procedures then gave the protected L-aspartate **43b**.[15,16] The chemistry was repeated to produce the succinyl protected material **43a**, by succinylation of the amino-monoester with succinic anhydride before treatment with isobutylene and sulfuric acid in CH_2Cl_2. Selective methyl ester hydrolysis using aqueous NaOH to give the sodium salts **44a** and **44b** was followed by formation of the mixed anhydride carbonates **45a** and **45b** and *in situ* reduction with aqueous $NaBH_4$ afforded the protected primary alcohols **46a** and **46b**. These *tert*-butyl esters did not show the propensity for lactone formation displayed by methyl ester **37**.

In order to complete the synthesis we required *tert*-butyl protected oxalate. Thus methyl oxalyl chloride **47** was added to *tert*-butanol in the presence of base to form the mixed ester **48**[17]

(Scheme 7). Selective methyl ester hydrolysis could be achieved with a variety of bases, but use of 1.0 equivalent of KOH afforded the potassium salt **49** which was conveniently soluble

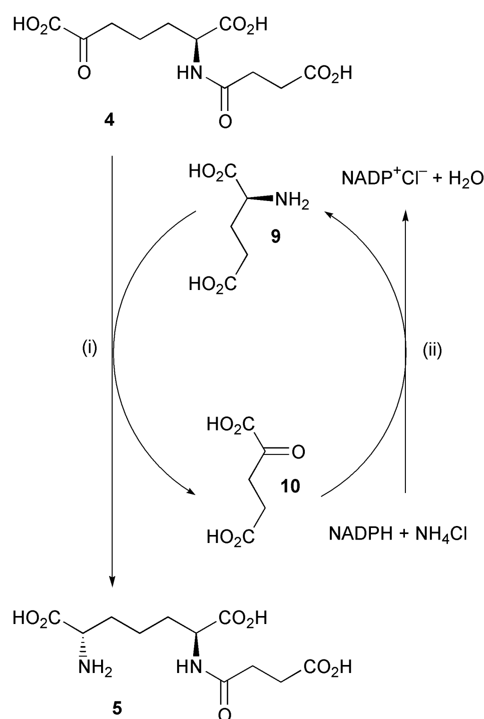


Scheme 7 Synthesis of oxa substrate analogues. *Reagents and conditions*: (i) MeOH, $SOCl_2$, quant.; (iia) succinic anhydride, $NaHCO_3$ (aq), 35%; (iib) CbzCl, $NaHCO_3$ (aq), 34%; (iii) CH_2Cl_2, −5 °C, isobutene, H_2SO_4, 88%; (iv) NaOH, H_2O–MeOH, 43–68%; (v) NMM, CH_2Cl_2, EtOCOCl; (vi) $NaBH_4$ (aq) 30–42% (two steps); (vii) ${}^tBuOH$, pyridine, Et_2O, 53%; (viii) 1.0 eq. KOH, H_2O–CH_3CN, 93%; (ix) CH_3CN, −10 °C, EtOCOCl; (x) **46a**–**46b**, CH_3CN, RT, 18–22% (two steps).

in organic media. Coupling with **46a** and **46b** was achieved by first forming the mixed anhydride carbonate **50** with ethyl chloroformate before addition of the alcohols (Scheme 7). The bis-*tert*-butyl oxooxapimelate analogues **51a** and **51b** were formed in acceptable yield. Attempted acid catalysed deprotection of **51a** and **51b** using different concentrations of TFA in CH_2Cl_2 at varied temperatures gave the lactone **38** as the only identifiable product in low yields. It is clear that synthetic approaches to these oxooxapimelates will have to be devised which do not use acidic or basic deprotection conditions.

In vitro enzyme activity

DAP-AT was isolated from *E. coli* DH5α by a procedure involving rapid sonication of whole cells, cation and anion chromatography and ultrafiltration. The enzyme fraction obtained showed high activity with the natural substrate **4** with no detectable background activity in the absence of substrate. In the standard assay DAP-AT converts the natural substrate **4** (*ca.* 1 mM) to the ε-L-amine **5** (Scheme 8).[9] This generates the



Scheme 8 Assay for DAP-AT activity: (i) DAP-AT; (ii) Glutamate dehydrogenase.

PLP form of the enzyme which then reacts in the reverse direction with an excess of glutamate **9** (10 mM) to generate α-ketoglutarate **10**. A coupling enzyme, glutamate dehydrogenase, then converts the α-ketoglutarate **10** rapidly back to L-glutamate **9** with the consumption of ammonium ions and NADPH. The fall in NADPH concentration is conveniently monitored at 340 nm in order to obtain rate data.

Substrate activity of 11a, 11b, 12a, 12b and 14

Addition of increasing concentrations of **11a**, **11b**, **12a**, **12b** and **14** to assay solutions of DAP-AT caused no observable depletion in NADPH concentration, even at elevated concentrations in the region of 20–50 mM.

Reversible inhibition

In order to measure possible reversible inhibition of DAP-AT by compounds **11a**, **11b**, **12a**, **12b** and **14**, each was added to a standard DAP-AT assay mixture simultaneously with substrate **4** at 1 mM. At concentrations up to 10 mM none of the thia-compounds (**11a**, **11b** or **14**) caused any significant reduction of

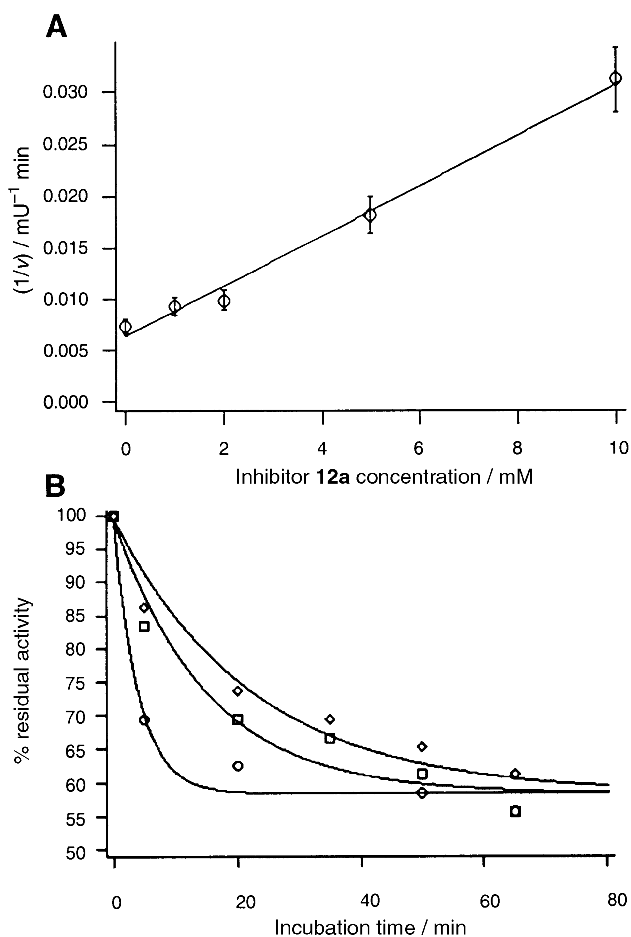


Fig. 1 **A** Reciprocal plot of inhibition of DAP-AT by **12a**. **B** Time dependent inhibition of DAP-AT at varying **12a** concentrations: ○ 20 mM; □ 10 mM; ◇ 5 mM.

rate of turn-over in comparison with control experiments lacking inhibitor. However the aza substrate analogue **12a** (*N*-succinyl) did show inhibition of DAP-AT with an estimated K_I of 2.6 ± 0.5 mM, assuming competitive inhibition (Fig. 1A). As expected the Cbz protected analogue **12b** (*N*-Cbz) showed much less activity and although inhibition was detected it was too feeble to enable accurate inhibition parameters to be determined.

Time dependent inhibition

DAP-AT was incubated with all assay components in the presence of each inhibitor **11a**, **11b**, **12a**, **12b** and **14** in the absence of substrate **4** for set lengths of time. Over this period the NADPH concentration was also monitored. For all compounds **11a**, **11b**, **12a**, **12b** and **14** no NADPH consumption was observed before addition of 1 mM substrate **4**. After addition, the rate of NADPH consumption was unchanged compared to control experiments for compounds **11a**, **11b** and **14**. However the *N*-succinyl amide **12a** did cause inhibition, with the extent of inhibition increasing with pre-incubation time (Fig 1B). Inhibition did not appear to reach 100% completion, however. This behaviour can be indicative of slow-binding inhibition.[18,19]

In order to test for potential slow binding inhibition the enzyme and all assay components were combined and the *N*-succinyl amide inhibitor **12a** included at varying concentrations. The reactions were then observed over the period of 60 min. No curvature of the plots was observed showing that in the presence of substrate **4** there is no time-dependent inhibition in these assays (data not shown). Overall it appears that **12a** can cause a time dependent inhibition of DAP-AT only in the *absence* of the natural substrate **4**.

In all the assays described here L-glutamate, the co-substrate of DAP-AT, was present at 10 mM. In the *absence* of the substrate **4** it would be expected that DAP-AT would be present almost wholly in the PMP form. Under these conditions slow inhibition occurs, perhaps by slow reversible covalent bond formation. When **4** is present it may be that this cannot happen because **12a** is displaced by substrate before bond formation can occur.

Antimicrobial properties of 12a

Sterilised 5 mm circles of Whatman number 1 paper were loaded with standard aqueous solutions of **12a** (300, 30, 3.0 and 0.3 μg mL^{-1}). The circles were placed on minimal agar plates (containing no L-lysine or DAP) which had been spread with *E. coli* DH5α. DAP-AT inhibitors such as the hydrazines **7** and **8** cause significant growth inhibition zones.[6] Compound **12a** caused no growth inhibition however, commensurate with its poor DAP-AT inhibitory properties.

Concluding remarks

Thiol ester substrate analogues showed no detectable interaction with DAP-AT, either as substrates or inhibitors. Aza analogue **12a**, however, showed reversible inhibition in the presence of natural substrate **4** and (partial) irreversible inhibition when **4** was absent. Thus **12a** is a member of a new class of inhibitors of PLP dependent enzymes. However K_I *vs.* DAP-AT for reversible inhibition was 2.6 mM and significant irreversible inhibition was manifested only at **12a** concentrations >2 mM. Kinetic analysis indicated that **12a** may inhibit DAP-AT as intended but the inhibition is much poorer than that shown by compounds such as hydrazines **7** and **8** which react preferentially with the PLP form of the enzyme. Unsurprisingly **12a** showed no detectable antibacterial activity *vs. E. coli* at the highest concentrations tested of 300 μg mL^{-1} (*ca.* 1 mM). Ester substrate analogues may yet show inhibitory properties and our synthetic efforts now focus on the use of alternative protective strategies such as the use of allyl esters which can be deprotected under neutral Pd catalysed conditions.

Experimental

All reagents and solvents were obtained from the Sigma-Aldrich chemical company and were of ACS grade and not further purified unless otherwise stated. All anhydrous solvents were purchased from Fluka and were transferred under dried N_2 gas. NMR spectra were obtained using JEOL Δ-300, Δ-270 and Δ-400 spectrometers operating at 300, 270 and 400 MHz (^1H) and 75.5, 67.9 and 100.7 MHz (^{13}C) respectively. Chemical shifts are quoted in ppm relative to TMS. Coupling constants (J) are quoted in Hz. The use of succ in the NMR assignments refers to the succinyl groups. IR spectra were obtained using a Perkin Elmer 1600 FTIR spectrometer, using KBr discs for solids and thin film between NaCl plates for oils. Melting points were obtained using a Reichert hot-stage apparatus equipped with microscope and Comark digital thermometer. Mass spectra were obtained in the indicated mode using a VG analytical autospec instrument (EI, CI, FAB, accurate mass) or Fisons VG Quattro spectrometer (ESMS). Optical rotations were obtained using a Perkin Elmer 141 polarimeter using a 1 dm cell of 1 mL capacity. Flash chromatography was performed according to the method of Still[20] or using an improvised automatic system comprising a nitrogen constant pressure head, column packed with Merck silica gel 60 (0.040–0.063 mm), Gilson Holochrome UV detector set at 254 nM and an LKB fraction collector. TLC analysis was performed using Merck glass backed 0.2 mm silica plates (F_{254}) developed with phosphomolybdic acid when necessary. Enzyme assay methods

have been reported elsewhere.[6,7,9] Diethyl ether–diazomethane was generated according to the procedure of Vogel.[21]

Purification of DAP-AT from *E. coli*

L-Broth, consisting of Difco Bacto Tryptone 10 g, Difco yeast extract 5 g, NaCl 5 g, glucose 1 g, deionised water to 1 L was dispensed into 10 × 500 mL conical flasks. The flasks were sealed with cotton wool and autoclaved at 120 °C for 20 min. *E. coli* starter culture (10 × 1 mL) was added after cooling. The flasks were shaken at 37 °C, 200 rpm for 8 h (or OD_{590} of 4–5). Cells from 1 L were collected by centrifugation (8000 rpm, 20 min, 0 °C) and resuspended in 30 mM phosphate buffer pH 7.5 (10 mL) containing 2 mg L^{-1} PLP at 0 °C. The resuspended cell pellet was disrupted by sonication at 0 °C (6 × 10 s, with 1 minute cooling intervals at 0 °C). The lysed product was centrifuged (16000 rpm, 10 min, 0 °C) and the pellet discarded. Glycerol was added to the supernatant to 10% and the protein solution frozen overnight. The frozen solution was thawed and streptomycin sulfate was added to 2% w/v at 0 °C. The mixture was stirred at 0 °C for 15 min, then centrifuged at 8000 rpm for 15 min; the pellet was discarded. The protein solution was diluted to a final volume of 21 mL with 20 mM phosphate buffer pH 7.0 and loaded onto a Q-sepharose column (5 × 20 cm). The column was eluted with a linear gradient of 20 mM phosphate buffer pH 7.0 from 0 to 500 mM NaCl and fractions were collected at 0 °C in polypropylene tubes cooled in ice. Active fractions (standard assay) were combined, desalted (by dialysis *vs.* 20 mM phosphate buffer pH 5.0, three times) adjusted to pH 5.0 if necessary and loaded onto an S-sepharose column. A linear pH gradient was run using 20 mM phosphate buffer from pH 5.0 to pH 8.0 and fractions were collected at 0 °C in polypropylene tubes. Active fractions were combined, glycerol added to 10%, and frozen.

Inhibition experiments

For reversible inhibition experiments the standard DAP-AT activity assay was used containing 1 mM substrate **4** and all other reaction components.[6,7,9] The inhibitors (**11a**, **11b**, **12a**, **12b** and **14**) were added at increasing concentrations (1–20 mM) at the same time as the assay was initiated by addition of **4**. The assays were carried out in a 1 mL quartz cuvette at 37 °C and the progress of reaction was observed at 340 nM (Pharmacia LKB ultrospec III spectrophotometer). The K_M of **4** has previously been determined[6] and the K_I of **12a** was determined from graphical plots *assuming* competitive inhibition. For preincubation experiments DAP-AT was incubated with the inhibitor at fixed concentration in assay buffer containing all components *except* **4** at 37 °C. Aliquots were removed at time points and residual activity was determined using the standard DAP-AT assay.[6,7,9] For the slow-binding assays DAP-AT was added to a standard assay mixture containing **4** at 1 mM and **12a** at fixed concentrations. DAP-AT was diluted prior to addition (10-fold) to ensure slow reaction over 3600 s. For all assays NADPH concentration was monitored at 340 nm every 2 s. Data were read into an Excel database and initial rates were calculated by the use of a best-fit straight line for the first 60 seconds of reaction.

Dilithium (2*RS*)-2-[*N*-(4-lithiooxy-4-oxobutanoyl)amino]-6-oxo-5-thiapimelate 11a

Dimethyl (1*RS*)-1-[*N*-(4-methoxy-4-oxobutanoyl)amino]-6-oxo-5-thiapimelate (**18**, 123 mg, 352 µmol) was dissolved in a mixture of CH_3CN (3 mL) and deionised water (3 mL). Lithium hydroxide monohydrate (3.0 eq., 1.06 mmol, 44.3 mg) was added and the mixture stirred at RT until all of the LiOH had dissolved (90 min). The solvent was removed *in vacuo* and the residue was lyophilized to afford *dilithium (2RS)-2-[N-(4-lithiooxy-4-oxobutanoyl)amino]-6-oxo-5-thiapimelate* **11a** as a colourless solid in quantitative yield. Mp 123–125 °C; ν_{max} (KBr disc)/cm^{-1} 3287, 2950, 1649, 1582, 1411; δ_H(300 MHz, D_2O) 4.22 (1H, m, αCH), 2.70 (2H, m, γCH_2), 2.43 (4H, m, 2 × succ CH_2), 2.15 (1H, m, βCH), 1.94 (1H, m, βCH); δ_C(75.5 MHz, D_2O + CH_3CN) 182.0 (COS), 179.9 (CO), 179.5 (CO), 176.1 (CO), 173.8 (CO), 54.8 (αCH), 35.1 (CH_2), 33.8 (CH_2), 33.1 (CH_2), 32.1 (CH_2); *m*/*z* $(FAB)^+$ 307 [$(M - 3Li + 3H)^+$, 40%], 329 [$(M - 3Li + 2H + Na)^+$, 30%], 289 [$(M - H_2O)^+$, 15%]; *m*/*z* $(FAB)^-$ 328 [$(M - 3Li + H + Na)^-$, 100%].

Dilithium (2*RS*)-2-[*N*-(benzyloxycarbonyl)amino]-6-oxo-5-thiapimelate monohydrate 11b

Dimethyl (2*RS*)-2-[*N*-(benzyloxycarbonyl)amino]-6-oxo-5-thiapimelate (**29**, 77.2 mg, 209 µmol) was dissolved in a mixture of CH_3CN (0.5 mL) and water (0.5 mL). Lithium hydroxide monohydrate (2.0 eq., 418 µmol, 17.5 mg) was added and the mixture stirred at RT until all of the LiOH had dissolved (90 min). The solvent was removed *in vacuo* and the residue was lyophilized to afford *dilithium (2RS)-2-[N-(benzyloxycarbonyl)amino]-6-oxo-5-thiapimelate monohydrate* **11b** as a colourless solid in quantitative yield. Mp 87–89 °C; ν_{max} (KBr disc)/cm^{-1} 3090, 2949, 1718, 1696, 1554, 1454, 1323, 1253, 1052; δ_H(300 MHz, DMSO-d_6) 7.67 (1H, d, *J* 8.6, NH), 7.33 (5H, m, Ph), 5.35 (2H, s, CH_2Ph), 4.37 (1H, ddd, *J* 19.5, *J* 15.6, *J* 8.6, αH), 3.37 (1H, m, γCH), 3.26 (1H, m, γCH), 2.43 (1H, m, βH), 2.08 (1H, m, βH); δ_C(75.5 MHz, DMSO-d_6) 205.6 (COS), 172.39 (CO_2Li), 172.37 (CO_2Li), 155.9 (CO_2NH), 136.8 (Ph), 128.4 (Ph), 127.8 (Ph), 126.6 (Ph), 65.3 (OCH_2), 59.9 (αCH), 29.8 (γCH_2), 26.4 (βCH_2); *m*/*z* $(FAB)^+$ 353 [$(M)^+$, 100%]; Anal. Calcd for $C_{14}H_{13}NO_7SLi_2 \cdot H_2O$: C, 45.30%; H, 4.07%; N, 3.77%; S, 8.64%. Found: C, 45.44%; H, 3.92%; N, 3.72%; S, 8.78%.

Dilithium (2*S*)-2-[*N*-(4-lithiooxy-4-oxobutanoyl)amino]-6-oxo-5-azapimelate 12a

Dimethyl (2*S*)-2-[*N*-(4-methoxy-4-oxobutanoyl)amino]-6-oxo-5-azapimelate (**35**, 20.0 mg, 60.2 µmol) was dissolved in a mixture of CH_3CN (0.5 mL) and water (0.5 mL). Lithium hydroxide monohydrate (3.0 eq., 181 µmol, 7.60 mg) was added and the mixture stirred at RT until all of the LiOH had dissolved (90 min). The solvent was removed *in vacuo* and the residue was lyophilized to afford *dilithium (2S)-2-[N-(4-lithiooxy-4-oxobutanoyl)amino]-6-oxo-5-azapimelate* **12a** as a colourless solid (18.0 mg, 58.3 µmol, 97%). Mp 240–242 °C; ν_{max} (KBr disc)/cm^{-1} 3012 (NH), 1705 (CO); δ_H(300 MHz, D_2O) 4.21 (1H, m, αCH), 3.36 (2H, m, γCH_2), 2.86 (4H, m, succ CH_2), 2.00 (2H, m, βCH_2); δ_C(67.9 MHz, D_2O) 181.5 (CO), 178.8 (CO), 175.7 (CO), 166.5 (CO), 165.4 (CO), 53.5 (αCH), 36.8 (γCH_2), 33.3 (succ CH_2), 30.9 (succ CH_2), 31.5 (βCH_2); *m*/*z* $(ES)^+$ 308.99 [$(MH)^+$, 100%], 302.99 [$(M - Li + 2H)^+$, 95%], 297.05 [$(M - 2Li + 3H)^+$, 85%].

Dilithium (2*S*)-2-[*N*-(benzyloxycarbonyl)amino]-6-oxo-5-azapimelate hydrate 12b

Dimethyl (2*S*)-2-[*N*-(benzyloxycarbonyl)amino]-6-oxo-5-azapimelate (**32**, 101 mg, 286 µmol) was dissolved in a mixture of CH_3CN (1 mL) and water (1 mL). Lithium hydroxide monohydrate (2.0 eq., 571 µmol, 24.0 mg) was added to the mixture with stirring at RT until all of the LiOH had dissolved (90 min). The solvent was removed *in vacuo* and the residue was lyophilized to afford *dilithium (2S)-2-[N-(benzyloxycarbonyl)amino]-6-oxo-5-azapimelate* **12b** as a colourless solid in quantitative yield. Mp 126–127 °C; $[\alpha]_D^{24}$ −6.11 (*c* 2.03 in H_2O); ν_{max} (KBr)/cm^{-1} 3397, 3067, 2950, 1664; δ_H(300 MHz, D_2O) 7.37 (5H, m, Ph), 5.04 (2H, m, OCH_2Ph), 3.92 (1H, m, αH), 3.25–3.15 (2H, m, γH), 1.87 (2H, m, βCH_2); δ_C(75.5 MHz, D_2O) 179.3 (CO), 171.2 (CO), 166.5 (CO), 158.4 (CO), 135.0 (Ph), 129.2 (Ph), 128.8 (Ph), 128.1 (Ph), 67.4 (OCH_2Ph), 54.3 (αCH), 37.0 (γCH_2), 31.5 (βCH_2); *m*/*z* $(FAB)^+$ 337 [$(MH)^+$,

86%]; *m/z* (ES)$^+$ 338 [(M)$^+$, 61%]; Anal. Calcd for $(C_{14}H_{14}Li_2N_2O_7)_2 \cdot 5H_2O$: C, 44.11%; H, 5.02%; N, 7.35%. Found: C, 44.30%; H, 4.97%; N, 7.49%.

Dilithium (2*S*)-2-[*N*-(4-lithiooxy-4-oxobutanoyl)amino]-5-oxo-4-thiaadipate hydrate 14

Dimethyl (2*S*)-2-[*N*-(4-methoxy-4-oxobutanoyl)amino]-5-oxo-4-thiaadipate **22** (23.5 mg, 70.2 µmol) was dissolved in CH_3CN (HPLC grade, 200 µL) in a 2 mL Wheaton vial. H_2O (deionised, 100 µL) was added and the solution was stirred at RT. $LiOH \cdot H_2O$ (8.50 mg, 3.0 eq., 210 µmol) was added in portions over 2 h. A further 250 µL H_2O was added in 50 µL portions over the same time-span until LCMS analysis indicated full substrate consumption. H_2O (2 mL) was added and the solution freeze-dried to afford *dilithium (2S)-2-[N-(4-lithiooxy-4-oxobutanoyl)amino]-5-oxo-4-thiaadipate hydrate* **14** as a pale yellow solid (22.7 mg, 69.0 µmol, 98%). Mp >220 °C; ν_{max} (KBr)/cm^{-1} 3356, 2955, 1720, 1696, 1645, 1395, 1323; δ_H(300 MHz, D_2O) 4.50 (1H, m, αH), 2.85 (2H, m, βCH_2), 2.50 (4H, m, 2 × succ CH_2); *m/z* (ES)$^+$ 293.96, [(MH)$^+$, 100%].

Methyl (2*RS*)-2-[*N*-(4-methoxy-4-oxobutanoyl)amino]homocysteinate 17

Racemic homocysteine **15** (1.80 g, 13.3 mmol) was dissolved in saturated aqueous $NaHCO_3$ (36 mL). Succinic anhydride (1.33 g, 13.3 mmol) was added in portions over 1 h. After a further 1 h the reaction was acidified by addition of 1 M aqueous HCl and extracted quickly with EtOAc (3 × 30 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo*. The residue was treated with an excess of an ethereal solution of diazomethane. Excess diazomethane was destroyed by the dropwise addition of acetic acid and solvent was removed *in vacuo*. *Methyl (2RS)-2-[N-(4-methoxy-4-oxobutanoyl)amino]homocysteinate* **17** was obtained by flash chromatography of the residue (50 : 50 EtOAc : hexane, R_f 0.16) as a colourless oil (1.10 g, 4.18 mmol, 31%). ν_{max} (thin film)/cm^{-1} 2890, 2480, 1705, 1640, 1520, 1500; δ_H(300 MHz, $CDCl_3$) 6.69 (1H, d, *J* 8.1, NH), 4.76 (1H, m, αH), 3.76 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 2.68 (2H, m, succCH_2), 2.58 (4H, m, succCH_2 + γCH_2), 2.15 (1H, m, βCH), 2.00 (1H, m, βCH), 1.63 (1H, t, *J* 8.3, SH); δ_C(75.5 MHz, $CDCl_3$) 173.3 (CO_2Me), 172.5 (CO_2Me), 171.6 (CONH), 52.6 (αCH), 51.9 (OCH_3), 51.0 (OCH_3), 36.7 (CH_2S), 30.7 (succCH_2), 29.2 (succCH_2), 20.7 (βCH_2); *m/z* (EI) 264 [(MH)$^+$, 3%], 263 [(M)$^+$, 8%], 262 [(M − H)$^+$, 18%], 230 [(M − SH)$^+$, 13%], 115 [$(CO(CH_2)_2CO_2Me)^+$, 100%]; Anal. Calcd for $C_{10}H_{17}NO_5S$: C, 45.62%; H, 6.46%; N, 5.32%; S, 12.15%. Found: C, 45.61%; H, 6.35%; N, 5.22%; S, 11.97%.

Mixed fractions containing **16** were also eluted from the column (50 : 50 EtOAc : hexane, R_f 0.13). Selected data: δ_H(300 MHz, $CDCl_3$) 6.59 (1H, d, *J* 7.9, NH), 4.65 (1H, m, αH), 3.76 (3H, s, OCH_3), 3.70 (6H, s, 2 × OCH_3), 2.91 (4H, m), 2.67 (6H, m), 2.13 (1H, m, βCH), 1.96 (1H, m, βCH); *m/z* (EI) 378 [(MH)$^+$, 1%], 262 [(M − succinyl)$^+$, 26%], 230 [(M − succinyl − SH)$^+$, 15%], 115 [$(CO(CH_2)_2CO_2Me)^+$, 100%].

Dimethyl (2*RS*)-2-[*N*-(4-methoxy-4-oxobutanoyl)amino]-6-oxo-5-thiapimelate 18

Methyl (2*RS*)-2-[*N*-(4-methoxy-4-oxobutanoyl)amino]homocysteinate (**17**, 347 mg, 1.32 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) and stirred at RT under dry N_2. 2,6-Lutidine (156 mg, 169 µL, 1.45 µmol) was added dropwise to give a bright yellow solution. Methyl oxalyl chloride (1.58 µmol, 194 mg, 146 µL) was added dropwise and the colour faded. After 30 min the reaction was extracted with a mixture of CH_2Cl_2 (10 mL) and dilute aqueous $CuSO_4$ (to remove excess thiol which co-elutes with the product during chromatography). The organic layer was dried ($MgSO_4$) and evaporated *in vacuo*. *Dimethyl (2RS)-2-[N-(4-methoxy-4-oxobutanoyl)amino]-6-oxo-5-thiapimelate* **18** was obtained by flash chromatography (50 : 50 EtOAc : hexane, R_f 0.18) as a colourless oil (227 mg, 649 µmol, 49%); ν_{max} (thin film)/cm^{-1} 3400, 3340, 2995, 1765, 1710, 1550, 1460; δ_H(300 MHz, $CDCl_3$) 6.44 (1H, d, *J* 7.9, NH), 4.69 (1H, ddd, *J* 4.9, *J* 7.7, *J* 12.6, αCH), 3.93 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.00 (2H, m, γCH_2), 2.63 (4H, m, 2 × succ CH_2), 2.21 (1H, m, βCH), 1.99 (1H, m, βCH); δ_C(75.5 MHz, $CDCl_3$) 185.1 (COS), 173.3 (CO), 171.9 (CO), 171.5 (CO), 159.3 (CON), 53.8 (αCH), 52.6 (OCH_3), 51.9, (OCH_3), 51.1 (OCH_3), 32.0 (CH_2), 30.8 (CH_2), 29.9 (CH_2), 25.2 (βCH_2); *m/z* (EI)$^+$ 350 [(MH)$^+$, 1%], 318 [(M − OMe)$^+$, 3%], 290 [(M − CO_2Me)$^+$, 3%], 262 [(M − CO_2MeCO)$^+$, 72%], 230 [(M − CO_2MeCOS)$^+$, 40%], 115 [$(COCH_2CH_2CO_2Me)^+$, 100%]; Anal. Calcd for $C_{13}H_{19}NSO_8$: C, 44.69%; H, 5.48%; N, 4.01%. Found: C, 44.76%; H, 5.63%; N, 4.31%.

Methyl (2*S*)-*N*-(4-methoxy-4-oxobutanoyl)cysteinate 20

L-Cysteine hydrochloride (2.00 g, 12.7 mmol) was dissolved in sat. aq. $NaHCO_3$ and the mixture was treated with succinic anhydride (1.50 eq., 1.90 g, 19.0 mmol). After 3 h of stirring, the reaction was acidified with conc. aq. HCl and was immediately extracted with EtOAc (5 × 40 mL). The organic extracts were dried over Na_2SO_4 and evaporated *in vacuo* to afford a clear colourless oil which was treated with an excess of diethyl ether–diazomethane. Finally, removal of solvent *in vacuo* gave a crude residue which was purified by flash chromatography (50% EtOAc in hexane, R_f 0.28) to afford *methyl (2S)-N-(4-methoxy-4-oxobutanoyl)cysteinate* **20** as a colourless solid (580 mg, 2.30 mmol, 12%); mp 73–75 °C; $[a]_D^{24}$ +35.96 (*c* 1.09 in CH_2Cl_2); δ_H(300 MHz, $CDCl_3$) 6.68 (1H, d, *J* 7.0, NH), 4.89 (1H, m, αH), 3.80 (3H, s, CO_2CH_3), 3.70 (3H, s, CO_2CH_3), 3.02 (2H, m, βCH_2), 2.65 (4H, m, 2 × succ CH_2), 1.44 (1H, t, *J* 9.0, SH); δ_C(67.9 MHz, $CDCl_3$) 173.2 (CO), 171.3 (CO), 170.6 (CO), 53.7 (αCH), 52.6 (OCH_3), 51.7 (OCH_3), 30.8 (succ CH_2), 29.2 (succ CH_2), 26.8 (βCH_2); *m/z* (EI)$^+$ 248 [(M − H)$^+$, 54%], 115 [$(COCH_2CH_2CO_2Me)^+$, 100%] (249.0671 calcd for $C_9H_{15}NO_5S$ found 249.0665); Anal. Calcd for $C_9H_{15}NO_5S$: C, 43.36%; H, 6.06%; N, 5.62%. Found: C, 43.43%; H, 5.94%; N, 5.41%.

Dimethyl (2*S*,7*S*)-2,7-bis[*N*-(4-methoxy-4-oxobutanoyl)-amino]-4,5-dithiasuberate 21. Compound **21** was obtained as a by-product from the column (50% EtOAc in hexane, R_f 0.25) as a colourless solid (970 mg, 1.96 mmol, 31%); δ_H(300 MHz, $CDCl_3$) 6.79 (2H, d, *J* 7.7, NH), 4.81 (2H, m, αH), 3.75 (6H, s, OCH_3), 3.70 (6H, s, CO_2CH_3), 3.50–3.28 (4H, m, βCH_2), 2.94–2.52 (8H, m, 4 × succ CH_2); *m/z* (ES)$^+$ 496.99 [(MH)$^+$, 100%], 247.95 [(disulfide cleavage)$^+$, 15%].

Dimethyl (2*S*)-2-[*N*-(4-methoxy-4-oxobutanoyl)amino]-5-oxo-4-thiaadipate 22

To a solution of methyl (2*S*)-*N*-(4-methoxy-4-oxobutanoyl)-cysteine **20** (500 mg, 2.01 mmol) stirred in anhydrous CH_2Cl_2 (20 mL) under N_2 was added 2,6-lutidine (1.1 eq., 261 µL, 2.21 mmol), followed by the dropwise addition of methyl oxalyl chloride (1.1 eq., 203 µL, 2.21 mmol). The solution became bright yellow and was stirred at RT under N_2 for 2 h. The reaction mixture was extracted with H_2O (3 × 40 mL) and sat. $CuSO_4$(aq) (3 × 40 mL), organic extracts were dried over Na_2SO_4 and evaporated *in vacuo*. *Dimethyl (2S)-2-[N-(4-methoxy-4-oxobutanoyl)amino]-5-oxo-4-thiaadipate* **22** was obtained by flash chromatography of the residue (50% EtOAc in hexane, R_f 0.15) as a colourless solid (483 mg, 1.44 mmol, 72%); mp 69–70 °C; $[a]_D^{24}$ +35.48 (*c* 1.26 in CH_2Cl_2); δ_H(300 MHz, $CDCl_3$) 6.70 (1H, d, *J* 7.7, NH), 4.88 (1H, m, αH), 3.94 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 3.61–3.41 (2H, m, βCH_2), 2.70–2.52 (4H, m, 2 × succ CH_2); δ_C(67.9 MHz, $CDCl_3$) 184.6 (CO), 173.2 (CO), 171.5 (CO), 170.3 (CO), 159.1 (CO), 54.0 (αCH), 53.0 (OCH_3), 51.9 (OCH_3), 51.5

(OCH_3), 31.0 (succ CH_2), 30.7 (succ CH_2), 29.2 (βCH_2); *m*/*z* $(ES)^+$ 336.08 [$(MH)^+$, 100%], 303.95, [$(M - MeOH)^+$, 100%] (336.0753 calcd for $C_{12}H_{18}NO_8S$, found 336.0767); Anal. Calcd for $C_{12}H_{17}NO_8S$: C, 43.00%; H, 5.07%; N, 4.18%. Found: C, 43.22%; H, 5.26%; N, 4.22%.

Dimethyl (2*RS*, 2′*RS*)-homocystinate dihydrochloride dihydrate 25[22]

Racemic homocystine **24** (1.00 g, 3.73 mmol) was stirred with 10% HCl in anhydrous methanol (50 mL) at room temperature for 16 h. Methanol and HCl were removed by evaporation *in vacuo* to afford a clear oil. The oil was crystallised by repeated evaporation from dry CH_2Cl_2 and then dry toluene. The dimethyl ester dihydrochloride was obtained in quantitative yield as a hydroscopic colourless powder after air drying. ν_{max} (KBr disc)/cm^{-1} 3415, 2958, 1992, 1740, 1617, 1507, 1412; δ_H(300 MHz, D_2O) 4.65 (1H, dd, *J* 6.1, *J* 6.2, αCH), 3.71 (3H, s, OCH_3), 2.72 (2H, t, *J* 7.1, γCH_2), 2.25 (2H, m, βCH_2); δ_C(75.5 MHz, D_2O) 173.5 (CO_2CH_3), 54.4 (OCH_3), 52.3 (αCH), 33.5 (γCH_2), 29.5 (βCH_2); Anal. Calcd for $C_{10}H_{11}N_2O_4S_2Cl_2{\cdot}2H_2O$: C, 29.63%; H, 6.47%. Found: C, 30.01%; H, 6.26%.

Dimethyl (2*RS*, 2′*RS*)-*N*,*N*′-bis(benzyloxycarbonyl)dihomocystinate 26[23]

Dimethyl (2*RS*, 2′*RS*)dihomocystinate dihydrochloride **25** (200 mg, 542 μmol) was dissolved in CH_2Cl_2 (10 mL) containing triethylamine (4.00 eq., 2.17 mmol, 219 mg). The solution was stirred at RT and benzyloxycarbonyl chloride (1.19 mmol, 203 mg, 170 μL) added dropwise over 2 min. After a further 90 min the solution was acidified by the addition of 1 M aqueous HCl (20 mL) and extracted into CH_2Cl_2 (3 × 50 mL). The organic extracts were combined, dried ($MgSO_4$) and evaporated *in vacuo*. Dimethyl (2*RS*, 2′*RS*)-*N*,*N*′-bis(benzyloxycarbonyl)dihomocystinate **26** was obtained by flash chromatography of the residue (50 : 50 EtOAc : hexane, R_f 0.4) as an oil (81 mg, 145 μmol, 27%). ν_{max} (thin film)/cm^{-1} 3027, 2941, 1707, 1531, 1445, 1344; δ_H(300 MHz, $CDCl_3$) 7.34 (5H, m, Ph), 5.50 (1H, br s, NH), 5.11 (2H, s, OCH_2), 4.51 (1H, m, αCH), 3.75 (3H, s, OCH_3), 2.68 (2H, m, γCH_2), 2.24 (1H, m, βCH), 2.05 (1H, m, βCH); δ_C(75.5 MHz, $CDCl_3$) 172.3 (CO_2Me), 156.0 (CO_2NH), 136.1 (Ph), 128.6 (Ph), 128.2 (Ph), 126.98 (Ph), 67.1 (OCH_2), 52.9 (αCH), 52.6 (OCH_3), 34.4 (βCH_2), 32.4 (γCH_2); *m*/*z* $(CI)^+$ 565 [$(MH)^+$, 0.3%], 457 [$(M - BnOH + H)^+$, 3%], 282 [(disulfide cleavage), 10%], 91 [$(C_7H_7)^+$, 100%].

Methyl (2*RS*)-*N*-(benzyloxycarbonyl)homocysteinate 28

Dimethyl (2*RS*,2′*RS*)-*N*,*N*′-bis(benzyloxycarbonyl)dihomocystinate (**26**, 450 mg, 798 μmol) was dissolved in anhydrous methanol (10 mL). An excess of 3% sodium amalgam (1.80 g, approx. 2.40 mmol Na) was added and the mixture stirred vigorously under dry N_2 at 0 °C until all the amalgam had reacted. Mercury was removed by paper filtration and the methanolic solution acidified by the addition of 1 M aqueous HCl. The mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo*. *Methyl (2RS)-N-(benzyloxycarbonyl)homocysteinate* **28** was obtained by flash chromatography of the residue (30 : 70 EtOAc : hexane, R_f 0.32) as a colourless solid (111 mg, 389 μmol, 24%). Mp 62–63 °C; ν_{max} (thin film from CH_2Cl_2)/cm^{-1} 3027, 2953, 2849, 2568, 1715, 1525, 1438, 1344; δ_H(300 MHz, $CDCl_3$) 7.35 (5H, m, Ph), 5.41 (1H, br d, *J* 7.9, NH), 5.11 (2H, s, OCH_2), 4.75 (1H, ddd, *J* 13.2, *J* 8.2, *J* 4.9, αH), 3.75 (3H, s, OCH_3), 2.57 (2H, m, γCH_2), 2.13 (1H, m, βCH), 1.98 (1H, m, βCH), 1.55 (1H, t, *J* 8.2, SH); δ_C(75.5 MHz, $CDCl_3$) 172.4 (CO_2Me), 156.0 (CO_2NH), 136.1 (Ph), 128.6 (Ph), 128.3 (Ph), 128.2 (Ph), 67.2 (OCH_2), 52.7 (αCH), 52.6 (OCH_3), 37.0 (CH_2S), 20.6 (βCH_2); *m*/*z* $(CI, NH_3)^+$ 283 [$(M)^+$, 0.1%], 282 [$(M - H)^+$, 1%], 91 [$(C_7H_7)^+$, 100%]; Anal. Calcd for $C_{13}H_{17}NO_4S$: C, 55.11%; H, 6.05%; N, 4.94%; S, 11.31%. Found: C, 55.28%; H, 6.21%; N, 4.72%; S, 11.18%.

(3*RS*)-3-[*N*-(Benzyloxycarbonyl)amino]-2-oxo-tetrahydrothiophene 27.[24] Compound **27** was obtained as a byproduct from the column as a colourless solid (140 mg, 558 μmol, 35%). Mp 97–100 °C, (lit.[25] 106 °C); ν_{max} (KBr disc)/cm^{-1} 3321, 3061, 2947, 1694, 1547, 1454; δ_H(300 MHz, $CDCl_3$) 7.36 (5H, m, Ph), 5.25 (1H, br s, NH), 5.12 (2H, s, OCH_2), 4.33 (1H, m, αCH), 3.28 (m, 2H, γCH_2), 2.87 (1H, m, βCH), 1.95 (1H, ddd, *J* 7.0, *J* 12.3, *J* 24.8, βCH); δ_C(75.5 MHz, $CDCl_3$) 204.7 (COS), 156.1 (CONH), 136.0 (Ph), 128.6 (Ph), 128.3 (Ph), 128.2 (Ph), 67.3 (OCH_2), 60.8 (αCH), 32.0 (γCH_2), 27.2 (βCH_2); *m*/*z* $(CI, CH_4)^+$ 252 [$(MH)^+$, 35%], 91 [$(C_7H_7)^+$, 100%]; *m*/*z* $(EI)^+$ 251.0617 $(M)^+$, calcd for $C_{12}H_{13}NO_3S$ 251.0616; Anal. Calcd for $C_{12}H_{13}NO_3S$: C, 57.35%; H, 5.21%; N, 5.57%; S, 12.76%. Found: C, 57.18%; H, 4.95%; N, 5.42%; S, 12.72%.

Dimethyl (2*RS*)-2-[*N*-(benzyloxycarbonyl)amino]-6-oxo-5-thiapimelate 29

Methyl (2*RS*)-*N*-(benzyloxycarbonyl)homocysteinate (**28**, 42.2 mg, 149 mmol) was dissolved in anhydrous CH_2Cl_2 (300 μL) and stirred under dry N_2 in a sealed dry Wheaton vial. 2,6-Lutidine (17.3 μL, 164 μmol) was added to give a bright yellow solution. Methyl oxalyl chloride (20 μL, 224 μmol) was added and the yellow colour faded. The reaction mixture was stirred at RT for 30 min and then applied directly to a flash chromatography column. *Dimethyl (2RS)-2-[N-(benzyloxycarbonyl)amino]-6-oxo-5-thiapimelate* **29** was obtained by flash chromatography (40 : 60 EtOAc : hexane, R_f 0.37) as a colourless oil (48.2 mg, 131 μmol, 88%); ν_{max} (film)/cm^{-1} 3358, 3034, 2955, 2852, 1740, 1686, 1522, 1437; δ_H(300 MHz, $CDCl_3$) 7.35 (5H, m, Ph), 5.46 (1H, d, *J* 7.9, NH), 5.12 (2H, s, CH_2Ph), 4.47 (1H, m, αCH), 3.92 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.01 (2H, m, γCH_2), 2.20 (1H, m, βCH), 1.98 (1H, m, βCH); δ_C(75.5 MHz, $CDCl_3$) 185.0 (COS), 171.9 (CO), 159.3 (CO), 157.5 (CO), 136.0 (Ph), 128.6 (Ph), 128.3 (Ph), 128.2 (Ph), 67.2 (OCH_2), 53.9 (OCH_3), 53.1 (αCH), 52.7 (OCH_3), 32.1 (γCH_2), 25.2 (βCH_2); *m*/*z* $(CI)^+$ 370 [$(MH)^+$, 30%], 91 [$(C_7H_7)^+$, 100%]; *m*/*z* $(EI)^+$ 369.0900, 369.0882 calcd for $C_{16}H_{19}NO_7S$.

Methyl (2*S*)-2-[*N*-(benzyloxycarbonyl)amino]-4-aminobutyrate hydrochloride 31

(2*S*)-2-[*N*-(Benzyloxycarbonyl)amino]-4-aminobutyric acid **30** (Fluka, 200 mg, 793 μmol) was dissolved in 10% HCl in anhydrous methanol (20 mL). The solution was stirred for 16 h at RT and the solvent was then removed by evaporation *in vacuo* to afford *methyl (2S)-2-[N-(benzyloxycarbonyl)amino]-4-aminobutyrate hydrochloride* **31** as a highly hydroscopic colourless solid (240 mg, 661 μmol, 83%). $[\alpha]_D^{24}$ −21.05 (*c* 1.2 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3380, 1705, 1532, 1439; δ_H(270 MHz, $CDCl_3$) 8.07 (3H, br s, NH_3Cl), 7.26 (5H, br s, Ph), 6.25 (1H, br s, NH), 4.95 (2H, br s, OCH_2Ph), 4.35 (1H, m, αH), 3.50 (3H, s, OCH_3), 3.10 (2H, m, γCH_2), 2.25 (1H, m, βCH), 2.10 (1H, m, βCH); δ_C(75.5 MHz, $CDCl_3$) 172.2 (CO_2Me), 156.6 (CO_2NH), 136.1 (Ph), 128.5 (Ph), 128.1 (Ph), 128.0 (Ph), 67.1 (OCH_2), 52.8 (OCH_3), 51.9 (αCH), 37.1 (γCH_2), 29.4 (βCH_2); *m*/*z* $(CI)^+$ 268 [$(MH)^+$, 30%], 267 [$(M)^+$, 100%], 250 [$(M - NH_3)H^+$, 15%], 249 [$(M - NH_3)^+$, 50%], 91 [$(C_7H_7)^+$, 60%].

Dimethyl (2*S*)-2-[*N*-(benzyloxycarbonyl)amino]-6-oxo-5-azapimelate 32

Methyl oxalyl chloride (220 μL, 2.40 mmol) was added to a solution of hydroxybenzotriazole (HOBT, 324 mg, 2.40 mmol) in anhydrous CH_2Cl_2 (3 mL). The solution was stirred at

RT under dry N_2 for 45 min. A solution of methyl (2*S*)-2-[*N*-(benzyloxycarbonyl)amino]-4-aminobutyrate hydrochloride (**31**, 242 mg, 798 μmol) in anhydrous CH_2Cl_2 (2 mL) was added dropwise with stirring. After 1 h the reaction was quenched by the addition of water (10 mL) and CH_2Cl_2 (10 mL). The mixture was extracted with 1 M aqueous HCl (1 × 25 mL), then 1 M aqueous $NaHCO_3$ (1 × 25 mL) and finally water (1 × 25 mL). The organic layer was dried ($MgSO_4$) and evaporated *in vacuo*. *Dimethyl (2S)-2-[N-(benzyloxycarbonyl)amino]-6-oxo-5-azapimelate* **32** was obtained by flash chromatography (20 : 80 Et_2O–CH_2Cl_2, R_f 0.30) as a colourless oil (150 mg, 426 μmol, 53%). $[a]_D^{24}$ −21.05 (*c* 1.53 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3328, 1693, 1531; δ_H(300 MHz, $CDCl_3$) 7.33 (5H, m, Ph), 5.76 (1H, d, *J* 8.04, NH), 5.10 (2H, m, OCH_2Ph), 4.42 (1H, m, αH), 3.87 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 3.62–3.24 (2H, m, γCH_2), 2.00 (2H, m, βCH_2); δ_C(75.5 MHz, $CDCl_3$) 172.3 (CO), 160.8 (CO), 157.9 (CO), 156.7 (CO), 136.0 (Ph), 128.6 (Ph), 128.3 (Ph), 128.1 (Ph), 67.2 (OCH_2), 53.6 (αCH), 53.5 (OCH_3), 53.1 (OCH_3), 36.0 (γCH_2), 32.2 (βCH_2); *m*/*z* (CI, NH_3)$^+$ 353 [(MH)$^+$, 58%], 91 [(C_7H_7)$^+$, 100%]. Anal. Calcd. for $C_{16}H_{20}N_2O_7$: C, 54.54%; H, 5.72%; N, 7.95%. Found: C, 54.29%; H, 5.98%; N, 7.89%.

Dimethyl (2*S*)-2-amino-6-oxo-5-azapimelate hydrochloride 34

Dimethyl (2*S*)-2-[*N*-(benzyloxycarbonyl)amino]-6-oxo-5-azapimelate (**32**, 400 mg, 1.14 mmol) was dissolved in 10% chloroform in methanol. 10% Palladium on carbon (40.0 mg) was added and the mixture was stirred overnight under hydrogen (1 atm). The solution was filtered through Celite and evaporated *in vacuo* to afford *dimethyl (2S)-2-amino-6-oxo-5-azapimelate hydrochloride* (**34**, 270 mg, 93%) as a colourless hydroscopic solid. $[a]_D^{24}$ −2.62 (*c* 0.183 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3359, 3235, 2957, 1750, 1692, 1527, 1441; δ_H(300 MHz, $CDCl_3$) 8.62 (3H, br s, NH_3Cl), 4.37 (1H, m, αH), 3.96 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.67 (2H, m, γCH_2), 2.43 (2H, m, βCH_2); δ_C(67.9 MHz, $CDCl_3$) 170.3 (CO), 160.9 (CO), 157.5 (CO), 53.7 (αCH), 51.2 (OCH_3), 50.8 (OCH_3), 36.0 (γCH_2), 29.0 (βCH_2); *m*/*z* (CI)$^+$ 219 [(MH)$^+$, 29%], 201 [(M − NH_3+H)$^+$, 70%]; *m*/*z* (EI) 219 [(MH)$^+$, 80%] (219.0981 calculated for $C_8H_{15}N_2O_5$, found 219.0981).

Dimethyl (2*S*)-2-[*N*-(4-methoxy-4-oxobutanoyl)amino]-6-oxo-5-azapimelate 35

Dimethyl (2*S*)-2-amino-6-oxo-5-azapimelate hydrochloride (**34**, 270 mg, 1.06 mmol) was dissolved in saturated aqueous $NaHCO_3$ (15 mL). Succinic anhydride (180 mg, 1.80 mmol) was added in portions over 1 h. After a further 1 h the reaction was acidified by addition of 1 M aqueous HCl and extracted quickly with EtOAc (3 × 30 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The residue was treated with an excess of an ethereal solution of diazomethane. Excess diazomethane was destroyed by the dropwise addition of acetic acid and solvent was removed *in vacuo*. *Dimethyl (2S)-2-[N-(4-methoxy-4-oxobutanoyl)amino]-6-oxo-5-azapimelate* **35** was obtained by flash chromatography of the residue (30 : 70 EtOAc–hexane, R_f 0.23) as a clear oil (25.3 mg, 76.2 μmol, 7%).

Alternative route. Dimethyl (2*S*)-2-amino-6-oxo-5-azapimelate hydrochloride **34** (138 mg, 543 μmol) was dissolved in $CHCl_3$ (10 mL). Et_3N (1.0 eq., 76.0 μL, 543 μmol) was added followed by succinic anhydride (2.0 eq., 109 mg, 1.09 mmol), in portions, over 30 min. After a further 2 h the solvent was removed *in vacuo* and the residue treated with an excess of an ethereal solution of CH_2N_2. *Dimethyl (2S)-2-[N-(4-methoxy-4-oxobutanoyl)amino]-6-oxo-5-azapimelate* **35** was obtained by flash chromatography of the residue (10% CH_3CN in EtOAc, R_f 0.5) as a clear oil (137 mg, 413 μmol, 76%). $[a]_D^{24}$ −37.3 (*c* 1.2 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 2961, 1733; δ_H(300 MHz, $CDCl_3$) 8.00 (1H, br m, NH), 6.42 (1H, d, *J* 7.2, NH), 4.60 (1H, m, αH), 3.84 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.62 (3H, s, OCH_3), 3.00 (2H, m, γCH_2), 2.52 (4H, m, succ CH_2), 2.15 (1H, m, βCH_2), 2.68 (1H, m, βCH_2); δ_C(67.9 MHz, $CDCl_3$) 174 (CO), 172.5 (CO), 171.4 (CO), 162.6 (CO), 157.2 (CO), 53.8 (αCH), 52.8 (OCH_3), 51.9 (OCH_3), 49.8 (OCH_3), 35.8 (γCH_2), 32.7 (succ CH_2), 30.9 (succ CH_2), 29.1 (βCH_2); *m*/*z* (CI) 332 [(M)$^+$, 14%], 115 [($MeO_2CCH_2CH_2CO$)$^+$, 100%]; *m*/*z* (EI)$^+$ 333 [(MH)$^+$, 2%], 115 [($MeO_2CCH_2CH_2CO$)$^+$, 100%] (318.1074 calcd for $C_{12}H_{17}N_2O_7$, found 318.1063).

Dimethyl (2*RS*)-2-[*N*-(benzyloxycarbonyl)amino]-6-oxo-5-oxapimelate 39

DL-Homoserine **36** (NovaBiochem, 201 mg, 1.69 mmol) was added to a vigorously stirred aqueous solution of $NaHCO_3$ (1 M, 8 mL) containing benzyloxycarbonyl chloride (291 μL, 2.04 mmol). The mixture was stirred for 16 h at RT. The reaction mixture was cooled to 0 °C and acidified to pH 3 with 1 M aqueous HCl. The cold solution was extracted with ethyl acetate (3 × 20 mL). The pH of the aqueous phase was readjusted to 3 after each extraction. The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo*. The colourless solid residue was treated with an excess of an ethereal solution of diazomethane and solvent was removed *in vacuo*. The resultant unstable hydroxy ester **37** (428 mg, 1.60 mmol, 95%) was not routinely purified further, but used immediately. *Methyl (2S)-2-[N-(benzyloxycarbonyl)amino]-4-hydroxybutyrate* **37** selected data: ν_{max} (thin film)/cm^{-1} 3035, 1758, 1519, 1438; δ_H(300 MHz, $CDCl_3$) 7.33 (5H, m, Ph), 5.80 (1H, br d, *J* 7.7, NH), 5.13 (1H, d, *J* 12.3, OCH*H*Ph), 5.08 (1H, d, *J* 12.3, OC*H*HPh), 4.54 (1H, m, αH), 3.74 (3H, s, OCH_3), 3.69 (2H, m, γCH_2), 3.03 (1H, br s, OH), 2.10 (1H, m, βCH), 1.75 (1H, m, βCH); δ_C(75.5 MHz, $CDCl_3$) 173.0 (CO_2Me), 156.7 (CO_2NH), 136.1 (Ph), 128.6 (Ph), 128.3 (Ph), 128.1 (Ph), 67.2 (OCH_2), 58.4 (γCH_2), 54.1 (OCH_3), 51.3 (αCH), 35.4 (βCH_2); *m*/*z* (EI)$^+$ 267 [(M)$^+$, 0.7%], 249 [(M − H_2O)$^+$, 15%], 91 [(C_7H_7)$^+$, 100%].

To a solution of the alcohol **37** as prepared above (60.0 mg, 225 μmol) in CH_2Cl_2 (2 mL) was added dry pyridine (4.0 eq., 253 μmol, 13.2 μL, 20.0 mg) and methyl oxalyl chloride (3.0 eq., 245μmol, 21.0μl, 30.0 mg). The solution was stirred for 2 h at RT, then applied directly to a flash chromatography column. *Dimethyl (2RS)-2-[N-(benzyloxycarbonyl)amino]-6-oxo-5-oxapimelate* **39** was obtained by flash chromatography (50 : 50 EtOAc–hexane, R_f 0.38) as a colourless oil (48.7 mg, 142 μmol, 63%); ν_{max} (thin film)/cm^{-1} 3034, 2957, 1746, 1523, 1439, 1319; δ_H(300 MHz, $CDCl_3$) 7.29 (5H, m, Ph), 5.52 (1H, br d, *J* 7.6, NH), 5.04 (2H, s, OCH_2Ph), 4.45 (1H, m, αH), 4.30 (2H, m, γCH_2), 3.80 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 2.26 (1H, m, βCH), 2.14 (1H, m, βCH); δ_C(75.5 MHz, $CDCl_3$) 171.8 (CO), 157.6 (CO), 157.0 (CO), 155.8 (CO), 136.0 (Ph), 128.5 (Ph), 128.2 (Ph), 128.1 (Ph), 67.1 (OCH_2Ph), 62.9 (γCH_2), 53.6 (OCH_3), 52.7 (OCH_3), 51.2 (αCH), 30.8 (βCH_2); *m*/*z* (CI, NH_3)$^+$ 354 [(MH)$^+$, 32%], 250 [(M − CH_3OCOCO_2H)$^+$, 40%], 91 [(C_7H_7)$^+$, 100%]; Anal. Calcd for $C_{16}H_{19}NO_8$: C, 54.39%; H, 5.42%; N, 3.96%. Found: C, 54.12%; H, 5.52%; N, 4.10%.

(3*RS*)-2-Oxo-3-[*N*-(benzyloxycarbonyl)amino]tetrahydrofuran 38.[26,27] Obtained as a byproduct from the column (50 : 50 EtOAc : hexane, R_f 0.29) as a colourless solid (11.0 mg, 47.0 μmol, 21%). Mp 125.5–126 °C (lit.[28] 129–130 °C); ν_{max} (KBr disc)/cm^{-1} 3306, 1779; δ_H(300 MHz, $CDCl_3$) 7.26 (5H, m, Ph), 5.58 (1H, br d, *J* 5.4, NH), 5.04 (2H, s, OCH_2Ph), 4.31 (2H, m, γCH_2), 4.12 (1H, m, αCH), 2.62 (1H, m, βCH), 2.14 (1H, m, βCH); δ_C(75.5 MHz, $CDCl_3$) 175.0 (CO_2), 156.0 (CON), 135.8 (Ph), 128.5 (Ph), 128.3 (Ph), 128.1 (Ph), 67.3 (OCH_2), 65.7 (γCH_2), 50.4 (αCH), 30.3 (βCH_2); *m*/*z* (CI, NH_3)$^+$ 236 [(MH)$^+$, 27%], 91 [(C_7H_7)$^+$, 100%].

β-Methyl hydrogen (2*S*)-aspartate hydrochloride 41b [29]

L-Aspartic acid **41a** (10.0 g, 76.9 mmol) was added to methanol (glass distilled, 50 mL) and cooled to −10 °C. Thionyl chloride (7.75 mL) was added dropwise to the mixture, the cooling bath was removed and the solution was slowly warmed to room temperature. After standing for 25 minutes, diethyl ether (50 mL) was added to the mixture and upon cooling and shaking, the product **41b** was precipitated as a colourless solid which was filtered immediately, washed with ice cold diethyl ether (20 mL) and collected as a colourless solid (9.83 g, 58.0 mmol, 75%); mp 183–184 °C (lit.[30] 186–188 °C); ν_{max} (KBr disc)/cm^{-1} 3415 (NH), 1733 (CO); δ_H(300 MHz, D_2O), 4.19 (1H, m, αH), 3.57 (3H, s, OCH_3), 3.00 (2H, m, βH); δ_C(67.9 MHz, D_2O) 172.1 (CO), 171.0 (CO), 54.1 (OCH_3), 49.6 (αCH), 34.1 (βCH_2); *m*/*z* (CI)$^+$ 148 [(MH)$^+$, 100%].

β-Methyl hydrogen (2*S*)-*N*-(benzyloxycarbonyl)aspartate 42b [31]

β-Methyl hydrogen (2*S*)-aspartate hydrochloride **41b** (4.78 g, 28.2 mmol) was dissolved at room temperature in a solution made up of water (50 mL), sodium hydroxide (4 M, 6.25 mL) and sodium bicarbonate (4.32 g). Cbz-chloride (4.38 mL, 30.7 mmol) was added dropwise over 10 minutes with vigorous stirring. The mixture was then extracted with diethyl ether (3 × 30 mL) and the ether extracts were discarded. The aqueous phase was acidified to pH 2.0 with aqueous hydrochloric acid and then extracted with toluene (2 × 30 mL). The combined organic layers were washed with water (2 × 30 mL), dried and evaporated to give the title compound as a colourless oil (2.72 g, 9.70 mmol, 34%). ν_{max}(film)/cm^{-1} 3420, 3125, 2960, 1740, 1460, 1540; δ_H(300 MHz, $CDCl_3$) 7.40 (5H, m, Ph), 6.24 (1H, d, *J* 8.00, NH), 5.32 (2H, s, OCH_2Ph), 4.80 (1H, m, αCH), 3.78 (3H, s, OCH_3), 3.20–2.90 (2H, m, βCH_2); δ_C(67.9 MHz, $CDCl_3$) 175.3 (CO), 171.2 (CO), 156.2 (CO), 129.1 (Ph), 128.6 (Ph), 128.3 (Ph), 127.0 (Ph), 67.4 (OCH_2), 52.2 (OCH_3), 50.3 (αCH), 36.3 (βCH_2); *m*/*z* (CI)$^+$ 282 [(MH)$^+$, 54%], 91 [(C_7H_7)$^+$, 100%].

β-Methyl α-*tert*-butyl (2*S*)-*N*-(4-*tert*-butyloxy-4-oxobutanoyl)-aspartate 43a

β-Methyl hydrogen (2*S*)-aspartate hydrochloride (**41a**, 2.00 g, 11.1 mmol) was dissolved in saturated sodium bicarbonate (50 mL). Succinic anhydride (1.66 g, 16.6 mmol) was added in portions over a period of 1.5 h. When reaction was complete, the solution was acidified with hydrochloric acid (conc.) to pH 4 and extracted with ethyl acetate (4 × 30 mL). The organic extract was dried ($MgSO_4$) and evaporated *in vacuo*. The resulting colourless solid (**42a**, 0.95 g) was dissolved in dichloromethane (10 mL) and cooled to −5 °C before addition of sulfuric acid (conc. 0.1 mL). Isobutene (0.5 g) was dissolved into the solution and the mixture was stirred for 24 h at RT by which time all the solid had disappeared. Dichloromethane (50 mL) was added, and the organic layer was washed with saturated aqueous sodium bicarbonate (2 × 50 mL) then water (1 × 50 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography (20% : 80% Et_2O–hexane, R_f 0.14) to give *β-methyl α-tert-butyl (2S)-N-(4-tert-butyloxy-4-oxobutanoyl)aspartate* **43a** as a colourless oil (410 mg, 3.30 mmol, 30%). ν_{max}(film)/cm^{-1} 2980, 1740; δ_H(300 MHz, $CDCl_3$) 6.70 (1H, d, *J* 7.90, NH), 4.72 (1H, m, αCH), 3.68 (3H, s, OCH_3), 2.94–2.74 (2H, m, βCH_2), 2.64–2.46 (4H, m, 2 × succ CH_2), 1.45 (18H, s, 2 × ^{t}Bu); δ_C (75.45 MHz, $CDCl_3$) 176.2 (CO), 176.0 (CO), 172.0 (CO), 169.7 (CO), 82.6 ($OC(CH_3)_3$), 80.8 ($OC(CH_3)_3$), 60.4 (OCH_3), 52.0 (αCH), 36.4 (succ CH_2), 31.6 (succ CH_2), 29.1 (βCH_2), 28.1 (2 × $OC(CH_3)_3$); *m*/*z* (CI)$^+$ 360 [(MH)$^+$, 0.06%], 57 [(^{t}Bu)$^+$, 100%]; *m*/*z* (EI)$^+$ 360 [(MH)$^+$, 8%], 57 [(^{t}Bu)$^+$, 100%] (360.2013 calcd for $C_{17}H_{30}NO_7$, found 360.2022).

β-Methyl α-*tert*-butyl (2*S*)-*N*-(benzyloxycarbonyl)aspartate 43b

A solution of β-methyl hydrogen (2*S*)-*N*-(benzyloxycarbonyl)-aspartate (**42b**, 2.33 g, 8.29 mmol) in dichloromethane (20 mL) was cooled to −5 °C and sulfuric acid (conc., 0.1 mL) was added to the solution. Isobutylene (2.0 g, 35.6 mmol) was passed into the solution which was stirred at room temperature for 24 h. Dichloromethane (50 mL) was added to the organic layer which was washed with saturated aqueous sodium bicarbonate (2 × 50 mL) then water (1 × 50 mL), then dried over sodium sulfate. The solvent was evaporated *in vacuo* to give *β-methyl α-tert-butyl (2S)-N-(benzyloxycarbonyl)aspartate* as a colourless oil (2.46 g, 7.31 mmol, 88%); ν_{max}(film)/cm^{-1} 3380, 3080, 3040, 3000, 2960, 1740, 1520; δ_H(300 MHz, $CDCl_3$) 7.70 (5H, m, Ph), 6.43 (1H, d, *J* 8.00, NH), 5.56 (2H, s, OCH_2Ph), 4.95 (1H, m, αH), 4.05 (3H, s, OCH_3), 3.40–3.20 (2H, m, βH); δ_C(67.9 MHz, $CDCl_3$) 171.4 (CO), 169.8 (CO), 157.5 (CO), 138.1 (Ph), 130.7 (Ph), 128.5 (Ph), 128.1 (Ph), 82.6 ($O\mathit{C}(CH_3)_3$), 67.0 (OCH_2), 51.8 (OCH_3), 50.9 (αCH), 36.8 (βCH_2), 27.8 ($OC(\mathit{C}H_3)_3$); *m*/*z* (CI)$^+$ 338 [(MH)$^+$, 1%], 282 [(M − ^{t}Bu + H)$^+$, 28%], 238 [(M − $CO_2{}^t$Bu + H)$^+$, 45%], 91 [(C_7H_7)$^+$, 100%].

(2*S*)-*N*-(4-*tert*-Butyloxy-4-oxobutanoyl)aspartic acid α-*tert*-butyl ester 44a

(2*S*)-*N*-(4-*tert*-Butyloxy-4-oxobutanoyl)aspartic acid α-*tert*-butyl ester β-methyl ester (**43a**, 2.28 g, 6.35 mmol) was dissolved in methanol (HPLC grade, 10 mL) and sodium hydroxide (2 M, 8 mL), the mixture was stirred at RT for 3 h. Methanol was removed at reduced pressure and the remaining aqueous layer was washed with diethyl ether (2 × 50 mL). The ether washes were discarded and water (20 mL) was added. The aqueous layer was acidified to pH 2 with hydrochloric acid (conc.) and then was extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were washed with water (50 mL), dried over sodium sulfate and evaporated *in vacuo* to yield *(2S)-N-(4-tert-butyloxy-4-oxobutanoyl)aspartic acid α-tert-butyl ester* **44a** as a pale yellow oil (950 mg, 2.76 mmol, 43%). ν_{max}(film)/cm^{-1} 2982, 1721; δ_H(300 MHz, $CDCl_3$) 9.70 (1H, s, CO_2H), 7.02 (1H, d, *J* 7.80, NH), 4.73 (1H, m, αH), 3.00–2.95 (2H, m, βH), 2.65–2.49 (4H, m, 2 × succ CH_2), 1.45 (18H, s, 2 × ^{t}Bu); δ_C(75.45 MHz, $CDCl_3$) 177.8 (CO), 175.8 (CO), 172.1 (CO), 170.0 (CO), 83.2 (2 × $O\mathit{C}(CH_3)_3$), 49.2 (αCH), 36.4 (succ CH_2), 31.6 (succ CH_2), 29.6 (βCH_2), 28.0 (2 × $OC(\mathit{C}H_3)_3$); *m*/*z* (CI)$^+$ 346 [(MH)$^+$, 0.07%], 234 [(M − 2 × ^{t}Bu + 3H)$^+$, 25%], 101 [($HO_2CCH_2CH_2CO_2H$)$^+$, 100%], 57 [(^{t}Bu)$^+$, 78%]; *m*/*z* (EI) 345 [(M)$^+$, 2%] (346.1876 calcd for $C_{16}H_{28}NO_7$, found 346.1866).

α-*tert*-Butyl sodium (*S*)-*N*-benzyloxycarbonylaspartate 44b [32]

α-*tert*-Butyl methyl (2*S*)-*N*-benzyloxycarbonylaspartate (**43b**, 2.46 g, 7.30 mmol) was dissolved in a mixture of methanol (5.1 mL) and aqueous sodium hydroxide (2 M, 3.7 mL), the mixture was stirred at room temperature for 3 h. Methanol was removed at reduced pressure and the remaining aqueous layer was washed with diethyl ether (2 × 30 mL), the ether washes were discarded and water (30 mL) was added to the aqueous layer. The aqueous layer was acidified to pH 2 with hydrochloric acid (conc.) and was extracted with ethyl acetate (2 × 50 mL). The combined organic phases were washed with water (50 mL), dried over sodium sulfate and the solvent was evaporated *in vacuo* to give the desired acid **44b** as a colourless oil (1.61 g, 5.00 mmol, 68%). ν_{max}(film)/cm^{-1} 3360, 2950, 1730; δ_H(300 MHz, $CDCl_3$) 7.35 (5H, m, Ph), 5.85 (1H, d, *J* 8.00, NH), 5.17 (2H, s, OCH_2Ph), 4.78 (1H, m, αCH), 3.09–2.80 (2H, m, βCH_2); δ_C(75.45 MHz, $CDCl_3$) 171.5 (CO), 169.5 (CO), 156.2 (CONH), 128.5 (Ph), 128.2 (Ph), 128.1 (Ph), 128.0 (Ph), 82.9 ($O\mathit{C}(CH_3)_3$), 67.3 (OCH_2), 50.9 (αCH), 36.6 (βCH_2), 27.7 ($OC(\mathit{C}H_3)_3$); *m*/*z* (EI)$^+$ 324 [(MH)$^+$, 10%], 91 [(C_7H_7)$^+$, 100%].

tert-Butyl (2S)-N-(4-tert-butyloxy-4-oxobutanoyl)homoserinate 46a

A solution of (S)-N-(4-*tert*-butyloxy-4-oxobutanoyl)aspartic acid α-*tert*-butyl ester (**44a**, 0.95 g, 2.75 mmol) in anhydrous THF (10 mL) was cooled to −10 °C and N-methylmorpholine (0.418 g, 4.13 mmol) was added to the mixture. After 1 minute at −10 °C, ethyl chloroformate (0.45 g, 4.13 mmol) was added dropwise and the mixture was stirred at −10 °C for a further 15 minutes. N-Methylmorpholine hydrochloride was removed by filtration, and the clear organic solution was added over a period of 10 minutes to a vigorously stirred suspension of sodium borohydride (300 mg) in water (3.0 mL) at 5 to 10 °C. The mixture was stirred at room temperature for 3.5 hours.

The mixture was then cooled to 5 °C, acidified to pH 2 with hydrochloric acid (conc. aq.) and extracted with ethyl acetate (2 × 50 mL). The organic extracts were washed with water (2 × 50 mL), dried over sodium sulfate and the solvent was evaporated *in vacuo* to yield the crude product which was purified by flash chromatography (35% : 60% Et_2O–hexane, R_f 0.31) to give *tert-butyl (2S)-N-(4-tert-butyloxy-4-oxobutanoyl)-homoserinate* **46a** as a colourless oil (385 mg, 1.16 mmol, 42%). δ_H(300 MHz, $CDCl_3$) 6.70 (1H, d, *J* 7.70, NH), 4.30 (1H, m, αH), 3.78–3.67 (2H, m, γCH_2), 3.08–2.96 (2H, m, βCH_2), 2.75–2.50 (4H, m, 2 × succ CH_2), 1.45 (18H, s, 2 × ^tBu); δ_C(75.45 MHz, $CDCl_3$) 176.9 (CO), 174.2 (CO), 172.1 (CO), 80.9 (2 × $OC(CH_3)_3$), 62.2 (γCH_2), 52.5 (αCH), 32.4 (succ CH_2), 31.1 (succ CH_2), 30.9 (βCH_2), 28.0 (2 × $OC(CH_3)_3$); *m/z* $(CI)^+$ 332 [$(MH)^+$, 24%], 57 [$(^tBu)^+$, 100%].

tert-Butyl (2S)-N-(benzyloxycarbonyl)homoserinate 46b [29]

A solution of α-*tert*-butyl hydrogen (S)-N-benzyloxy-carbonylasparate (**44b**, 2.22 g, 6.87 mmol) in dry THF (7.21 mL) was cooled to −10 °C and N-methylmorpholine (0.73 g, 7.22 mmol) was added to the mixture. After 1 minute at −10 °C, ethyl chloroformate (0.78 g, 7.19 mmol) was added dropwise and the mixture was stirred at −10 °C for a further 15 min. N-Methylmorpholine hydrochloride was removed by filtration, and the clear organic solution containing the mixed anhydride **45b** was added over a period of 10 minutes to a vigorously stirred suspension of sodium borohydride (600 mg) in water (3.6 mL) at 5 to 10 °C. The mixture was stirred at room temperature for 3.5 hours.

The mixture was then cooled to 5 °C, acidified to pH 2 with aqueous hydrochloric acid (conc.) and extracted with ethyl acetate (2 × 50 mL). The organic extracts were washed with water (2 × 50 mL), dried over sodium sulfate and the solvent was evaporated *in vacuo* to yield the crude product which was purified by flash chromatography (35% : 60% Et_2O–hexane, R_f 0.25) to give *tert-butyl (2S)-N-(benzyloxycarbonyl)-homoserinate* **46b** as a colourless oil (640 mg, 2.07 mmol, 30%). ν_{max}(film)/cm^{-1} 3480, 3069, 1700; δ_H(300 MHz, $CDCl_3$) 7.35 (5H, m, Ph), 5.72 (1H, d, *J* 7.50, NH), 5.12 (2H, s, OCH_2), 4.40 (1H, m, αCH), 3.70 (2H, m, γCH_2), 3.00–2.70 (2H, m, βCH_2); δ_C(74.5 MHz, $CDCl_3$) 171.6 (CO), 156.8 (CO), 136.1 (Ph), 128.5 (Ph), 128.4 (Ph), 128.2 (Ph), 82.4 ($OC(CH_3)_3$), 77.5 ($OC(CH_3)_3$), 67.1 (γCH_2), 62.0 (OCH_2), 53.4 (αCH), 35.9 (βCH_2), 27.9 ($OC(CH_3)_3$); *m/z* $(EI)^+$ 91 [$(C_7H_7)^+$, 100%].

tert-Butyl methyl oxalate 48

Methyl oxalyl chloride (1.00 g, 8.16 mmol) was added dropwise to a mixture of anhydrous pyridine (1.29 g, 16.3 mmol), *tert*-butyl alcohol (1.21 g, 16.3 mmol) and anhydrous Et_2O (60 mL). The mixture was stirred at RT for 1 h. The mixture was then washed with H_2O (20 mL), sat. $NaHCO_{3(aq)}$ (20 mL) and H_2O (20 mL), dried over Na_2SO_4 and concentrated *in vacuo* to yield *tert-butyl methyl oxalate* **48** as a colourless oil (690 mg, 4.32 mmol, 53%); ν_{max}(film)/cm^{-1} 1750 (CO); δ_H(270 MHz, $CDCl_3$) 3.80 (3H, s, OCH_3), 3.80 (9H, s, $OC(CH_3)_3$); *m/z* $(EI)^+$ 160 [$(M)^+$, 30%], 57 [$^tBu^+$ 100%].

tert-Butyl potassium oxalate 49

Methyl *tert*-butyl oxalate **48** (2.91 g, 18.2 mmol) was dissolved in a mixture of CH_3CN (HPLC grade, 10 mL) and H_2O (10 mL), followed by the addition of ground solid KOH (1.02 g, 18.2 mmol). The mixture was stirred at RT for 1 h until all KOH had dissolved. The solvent was evaporated *in vacuo* to yield *tert-butyl potassium oxalate* **49** (3.13 g, 17.0 mmol, 93%) as a colourless solid; *m/z* $(ES)^+$ 185 [$(MH)^+$, 14%], 57 [$(^tBu)^+$, 100%] (Anal. Calcd for $C_6H_9KO_4{\cdot}H_2O$: C, 35.63%; H, 5.48%. Found: C, 35.95%; H, 5.59%).

Di-tert-butyl (2S)-2-[N-(4-tert-butyloxy-4-oxobutanoyl)amino]-6-oxo-5-oxapimelate 51a

tert-Butyl potassium oxalate (**49**, 74.0 mg, 403 μmol) was dissolved in acetonitrile (15 mL) in the presence of 4 Å molecular sieves under dry nitrogen. The mixture was cooled to −10 °C and ethyl choroformate (6 eq., 2.42 mmol, 260 mg) was added dropwise and with stirring at RT for 0.5 h. *tert*-Butyl (2S)-N-(*tert*-butylsuccinyl)homoserinate (**46a**, 1.5 eq.) was added to the solution and the mixture was stirred at room temperature for 36 h. Saturated aqueous sodium bicarbonate (50 mL) was added to the mixture and the organic layer was extracted with dichloromethane (3 × 30 mL), dried (Na_2SO_4), filtered, and solvent was evaporated *in vacuo*. *Di-tert-butyl (2S)-2-[N-(4-tert-butyloxy-4-oxobutanoyl)amino]-6-oxo-5-oxapimelate* **51a** was obtained by flash chromatography of the residue (35 : 65 EtOAc–hexane, R_f 0.4) as an oil (50 mg, 109μmol, 18%). $[\alpha]_D^{24}$ −3.26 (*c* 1.9 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 2979 (NH), 1737 (CO); δ_H(300 MHz, $CDCl_3$) 6.48 (1H, d, *J* 7.7, NH), 4.27 (1H, m, αH), 2.55 (2H, m, γCH_2), 2.20 (2H, m, βCH_2), 1.55 (9H, s, ^tBu), 1.46 (9H, s, ^tBu), 1.42 (9H, s, ^tBu); δ_C(75.5 MHz, $CDCl_3$) 172.0 (CO), 171.5 (CO), 170.5 (CO), 158.3 (CO), 156.3 (CO), 85.0 ($OC(CH_3)_3$), 82.69 ($OC(CH_3)_3$), 80.72 ($OC(CH_3)_3$), 63.1 (αCH), 60.4 (γCH_2), 50.3 (βCH_2), 31.1 (succ CH_2), 30.9 (succ CH_2), 28.1 (3 × $OC(CH_3)_3$); *m/z* $(CI)^+$ 460 [$(MH)^+$ 3%], 404 [$(M - {}^tBu + 2H)^+$, 3%], 348 [$(M - 2 \times {}^tBu + 3H)^+$, 6%], 292 [$(M - 3 \times {}^tBu + 4H)^+$, 40%], 57 [$(^tBu)^+$, 100%] (460.2565 calcd for $C_{22}H_{37}NO_9$, found 460.2545).

Di-tert-butyl (2S)-2-[N-(benzyloxycarbonyl)amino]-6-oxo-5-oxapimelate 51b

18-Crown-6 (173 mg, 0.12 mmol) was added to a suspension of *tert*-butyl potassium oxalate (**49**, 131 mg, 712 μmol) in acetonitrile (HPLC grade, 15 mL) in the presence of 4 Å molecular sieves under dry nitrogen. The whole mixture was stirred until the oxalate had dissolved into the organic solvent. Ethyl chloroformate (4.00 g, 4.06 mmol) was added dropwise to the solution over a period of 5 min and then the mixture was stirred at RT for 30 minutes. *tert*-Butyl (2S)-N-(benzyloxy-carbonyl)homoserinate (**46b**, 200 mg, 1.07 mmol) was added and the solution was left to stir for 3 h. Saturated aqueous sodium bicarbonate (50 mL) was added to the mixture and the organic layer was extracted with dichloromethane (4 × 30 mL), dried over sodium sulfate and evaporated *in vacuo* to give the crude product. The crude product was purified by flash chromatography (20% : 80% Et_2O–hexane, R_f 0.31) to give *di-tert-butyl (2S)-2-[N-(benzyloxycarbonyl)amino]-6-oxo-5-oxa-pimelate* **51b** (63.5 mg, 23%) as a colourless oil. $[\alpha]_D^{24}$ −5.78 (*c* 2.23 in CH_2Cl_2); ν_{max}(film)/cm^{-1} 2979, 1739, 1523; δ_H(300 MHz, $CDCl_3$) 7.33 (5H, m, Ph), 5.48 (1H, d, *J* 8.30, NH), 5.30 (2H, s, OCH_2), 4.30 (1H, m, αH), 4.19 (2H, m, γCH_2), 2.32–2.08 (2H, m, βCH_2), 1.55 (9H, s, ^tBu), 1.53 (9H, s, ^tBu); δ_C(75.45 MHz, $CDCl_3$) 170.4 (CO), 158.3 (CO), 156.6 (CO), 155.7 (CO), 136.2 (Ph), 130.1 (Ph), 128.5 (Ph), 128.2 (Ph), 85.1 ($OC(CH_3)_3$), 82.8 ($OC(CH_3)_3$), 67.0 (OCH_2), 63.0 (γCH_2), 52.0 (βCH),

31.1 (βCH_2), 27.9 ($OC(CH_3)_3$); *m*/*z* $(CI)^+$ 438 $[(MH)^+$, 44%], 91 $[(C_7H_7)^+$, 100%] (438.2128 calcd for $C_{22}H_{32}NO_8$, found 438.2110).

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