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The stereoselective synthesis of aziridine analogues of diaminopimelic acid (DAP) and their interaction with dap epimerase†

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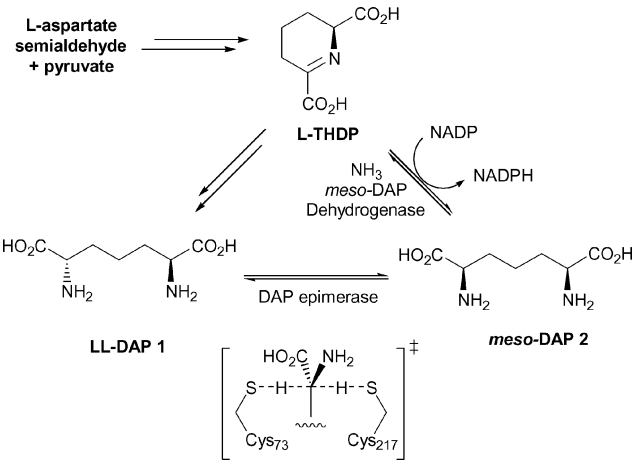
Aziridine analogues of diaminopimelic acid (DAP) have been prepared stereoselectively for the first time and evaluated as inhibitors of DAP epimerase. (2*R*,3*S*,3′*S*)-3-(3′-Aminopropane)aziridine-2,3′-dicarboxylate **4** was synthesised and shown to be a reversible inhibitor of DAP epimerase with an IC_{50} value of 2.88 mM. (2*S*,4*S*)- and (2*S*,4*R*)-2-(4-Amino-4-carboxybutyl)aziridine-2-carboxylic acid (LL-azi-DAP **14** and DL-azi-DAP **29**) were made as pure diastereomers, and both were shown to be irreversible inhibitors of DAP epimerase. LL-Azi-DAP **14** selectively binds to Cys-73 of the enzyme active site whereas DL-azi-DAP **29** binds to Cys-217 *via* attack of sulfhydryl on the methylene of the inhibitor aziridine ring. These observations are consistent with the two base mechanism proposed for the epimerisation of LL-DAP **1** and *meso*-DAP **2** by DAP epimerase.

Introduction

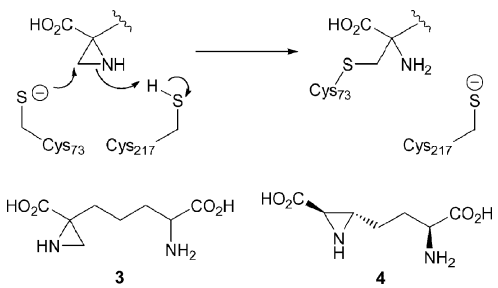
The worldwide increase in bacterial resistance has led to renewed interest in the development of new antibiotics,[1,2] including methods of inhibiting microbial cell wall biosynthesis.[3] As *meso*-diaminopimelic acid (*meso*-DAP **2**) is a precursor to L-lysine and an important component of the peptidoglycan layer of Gram-negative bacteria,[4] it has attracted considerable interest for the possible development of new antibiotics.[5] Crucially, the biosynthesis of *meso*-DAP is restricted to bacteria[5] and plants and is absent in mammals, which require L-lysine in their diet.[6] Therefore specific inhibitors of DAP enzymes are likely to be antimicrobial agents with low mammalian toxicity.

One of the key enzymes in the DAP pathway is DAP epimerase, an unusual enzyme that interconverts LL-DAP **1** and *meso*-DAP **2** without the use of co-factors, metals, or reducible keto or imino functionality (Scheme 1).[7]

This process is mechanistically analogous to the other PLP-independent amino acid racemases.[8,9] It is proposed to involve two active site cysteine residues, one of which as a thiolate, acts as a general base while the other, as a thiol, acts as a general acid.[10] Initial tritium labeling studies showed that the α-proton of DAP is exchanged during epimerisation.[7] Further investigations using 3-fluoro-DAP isomers demonstrated that the mechanism involves development of anionic character at the α-carbon and that the substrate is rigidly held in the enzyme active site.[11] In 1990, the irreversible inactivation of DAP epimerase was reported[12] using a crude mixture of all possible diastereomers of 2-(4-amino-4-carboxybutyl)aziridine-2-carboxylic acid (azi-DAP) **3**. Enzymatic digestion studies determined that Cys-73 was alkylated by the aziridino moiety, thereby demonstrating its presence in the active site (Scheme 2).[13] Recently a diastereomeric mixture of oxa analogues of azi-DAP



Scheme 1 The biosynthetic pathways to *meso*-DAP and proposed transition state for the inter-conversion of LL-DAP **1** and *meso*-DAP **2** by DAP epimerase.



Scheme 2 Proposed mechanism for the irreversible inhibition of DAP epimerase by azi-DAP **3**.

† Electronic supplementary information (ESI) available: experimental details for the preparation of **7**, **8**, **18**, **21**, **22** and **23**; 600 MHz NOE assignment of stereochemistry for **26**; 600 MHz ^{1}H NMR determination of dr's for **26** and **28**; HPLC analysis of **14** precursor; MS/MS analysis enzymatic digests of DAP epimerase inhibited with either **14** or **29**. See DOI: 10.1039/b513409a

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was found to irreversibly inhibit DAP epimerase, presumably due to thiol opening of the epoxide moiety.[14]

The crystal structure of inactive DAP epimerase from *Haemophilus influenzae* has been solved[15] and subsequently refined[16] to reveal a disulfide linkage between Cys-73 and Cys-217 at the interface of two structurally superimposable domains. Based on this observation it was proposed that in the active reduced form of the enzyme, these conserved residues are the key catalytic units required for activity. This led to detailed kinetic studies of DAP epimerase.[10] Assignment of the specific roles of Cys-217 and Cys-73 in the active site could be confirmed by the interaction of pure 3-fluoro-DAP isomers with the single DAP epimerase mutants C73A, C73S, C217A and C217S.[17]

The substrates LL-DAP **1** and *meso*-DAP **2** for DAP epimerase differ from the other PLP-independent amino acid racemases[8,18] (*e.g.* glutamate racemase,[19–21] aspartate racemase,[22] proline racemase[23,24]) in that they contain two stereocentres. DAP epimerase has very strict requirements for its substrates,[5] and only accepts DAP isomers with the L configuration at the distal site.[7,25] Neither D nor L isomers of lysine or α-aminopimelic acid are substrates or good inhibitors, thereby demonstrating that both carboxyl and amino groups must be present.[26]

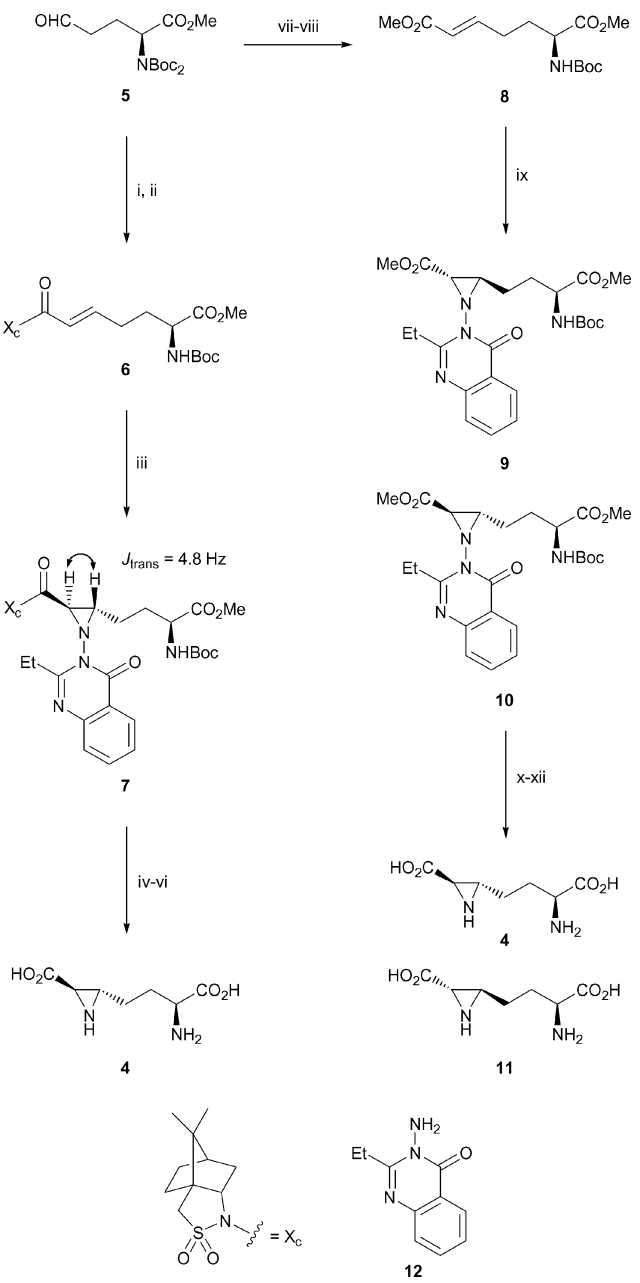
Unfortunately, the crystal structures of the inactive disulfide form of DAP epimerase[15,16] do not allow reliable modeling of the substrate in the collapsed active site. Interestingly, no crystal structures of other PLP-independent amino acid racemases have been obtained thus far with a substrate analog correctly bound in the active site to illustrate catalytic function. It therefore appeared that formation of a complex of DAP epimerase with pure diastereomers of the aziridine analogues **3** and **4** could provide further insight into the organisation of the enzyme active site. In particular, crystal structures of the DAP epimerase with stereochemically pure isomers of azi-DAP **14** and **29** could afford a detailed picture of the active site complex for each substrate isomer and the arrangement of residues for catalysis of this unusual reaction. In this report we describe the first stereoselective synthesis of highly reactive aziridine analogues **4**, **14** and **29** and analysis of their interaction with DAP epimerase.

Results

Stereoselective synthesis of DAP aziridine analogue 4

It seemed that aziridine **4** could be prepared by aziridination of a suitable α,β-unsaturated ester or amide bearing a chiral auxiliary.[27] In 1993 we reported the aziridination of *N*-enoylbornane[10,2]sultams by the oxidative addition of *N*-aminophthalimide mediated by lead tetraacetate with excellent diastereoselectivity.[28] This approach was subsequently found to be compatible with other auxiliaries such as oxoalkenyloxazolidinone[29] and camphor.[30] It appeared that a similar approach using the 3-amino-2-ethyl-3,4-dihydroquinazolin-4-one **12**[31] would lead to the stereoselective synthesis of the internal *N*-quinazolinyl aziridine **7**. The desired aziridine **4** could then be accessed by N–N bond reduction using dissolved metal in ammonia (Scheme 3).[32]

Reaction of phosphonate ester $X_cCOCH_2PO(OEt)_2$[33] with *N*,*N*-di-Boc glutamate semialdehyde **5**[34] using a Horner–Wadsworth–Emmons type coupling[35] gives exclusively the *trans*-alkene in 85% yield. One of the Boc protecting groups could then be removed by TFA to generate the aziridine precursor **6** in 94% yield. Aziridination of **6** with quinazolin-4-one **12** proceeds with optimum selectivity at a temperature of −40 °C to give a diastereomeric ratio of 9 : 1. The major isomer **7** can be isolated by recrystallisation in 72% yield. Efforts to prove the absolute stereochemistry of aziridine **7** by X-ray crystallography were unsuccessful. However, it has been demonstrated that addition to *N*-enoylbornane[10,2]sultams occurs by *syn* attack from the *re* face of the α-carbon.[28] Moreover, *syn* attack would give an aziridine with *trans* geometry which is consistent with the observed ^{1}H NMR coupling constant of 4.8 Hz for the 2′-H proton.[36] Removal of the Boc group followed by base hydrolysis and reductive cleavage of the N–N bond with Li–NH_3 affords the target molecule **4** in 40% yield over three steps (Scheme 3).



Scheme 3 The synthesis of internal aziridine DAP analogues. *Reagents and conditions*: (i) $X_cCOCH_2PO(OEt)_2$, DBU, LiCl, MeCN (85%); (ii) TFA, CH_2Cl_2 (94%); (iii) **12**, $Pb(OAc)_4$, HMDS, CH_2Cl_2 (72%); (iv) TFA, CH_2Cl_2 (99%); (v) LiOH, MeOH–H_2O (85%); (vi) Li, NH_3 (48%); (vii) $MeO_2CCH{=}PPh_3$, THF (88%); (viii) TFA, CH_2Cl_2 (88%); (ix) **12**, $Pb(OAc)_4$, HMDS, CH_2Cl_2 (83%); (x) TFA, CH_2Cl_2 (quant.); (xi) LiOH, MeOH–H_2O, (84%); (xii) Li, NH_3 (48%).

In addition to testing the single diastereomer **4** with DAP epimerase, it was desirable to examine interaction of the enzyme with another diastereomer to provide insight into stereochemical preferences for the active site. Hence, a mixture of diastereomers **4** and **11** was prepared from *N*,*N*-di-Boc glutamate semialdehyde **5** *via* the *trans*-alkene **8**[37] in an analogous manner (Scheme 3).

Inhibition study of aziridines 4 and 11 with DAP epimerase

Although several assays for DAP epimerase have been developed,[38] the activity of DAP epimerase is easily monitored

using a coupled enzyme assay. At pH 7.8, *meso*-DAP **2** is generated from LL-DAP **1** by DAP epimerase and is then transformed by DAP dehydrogenase to produce L-THDP and NADPH (Scheme 1), which can be measured spectrophotometrically at 340 nm.[7,39] The enantiopure LL-DAP **1** is prepared by the photolysis of a suitably functionalised diacyl peroxide,[40] which is then subjected to hydrolysis to remove the protecting groups.

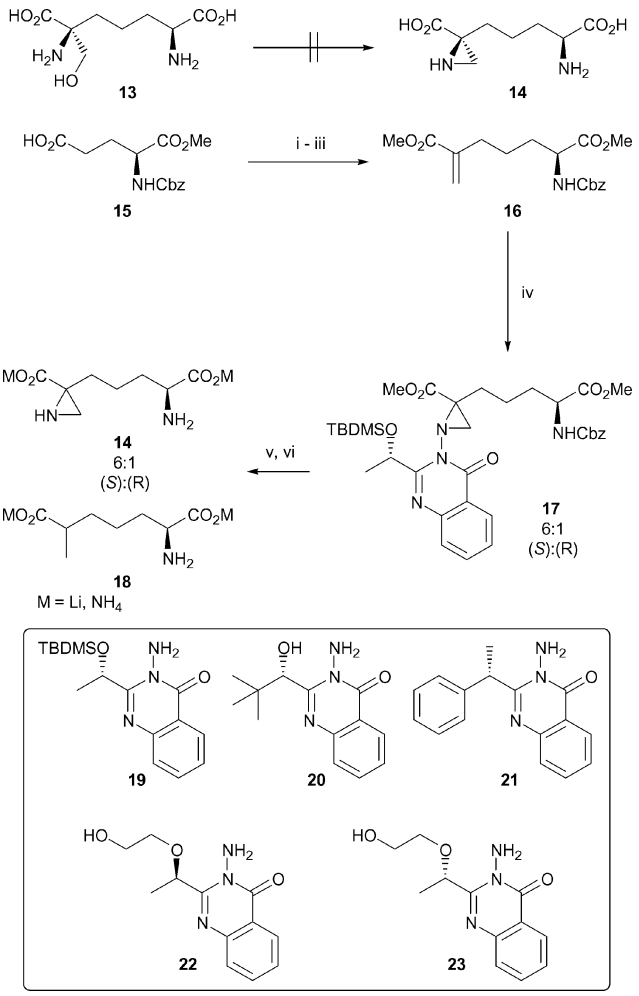
Aziridine **4** was initially tested as a substrate and as an inhibitor of DAP dehydrogenase, the enzyme used for analysis. As expected, this compound is neither a substrate nor an inhibitor of the dehydrogenase at a concentration of 1 mM. Disappointingly, aziridine **4** is also a very weak inhibitor of DAP epimerase with an IC_{50} value of only 2.88 mM. A time dependence study showed **4** to be a fully reversible inhibitor. These results show that unlike azi-DAP **3**, which rapidly inactivates DAP epimerase, presumably by cysteine attack at the methylene position of the aziridine ring, both secondary carbons at the aziridine ring positions of **4** are more stable towards the enzyme, resulting in poor reversible binding. The mixture of *trans*-stereoisomers **4** and **11** was also tested against DAP epimerase. Inhibition by this mixture is similar to the results obtained for the pure diastereomer, suggesting analogous modes of ineffective binding for both compounds.

Stereoselective synthesis of azi-DAP

Although a number of synthetic methods have been developed for the asymmetric synthesis of disubstituted aziridines,[41,42] examples of their application in the synthesis of the 1-carboxy-1-alkyl aziridine functionality required for the synthesis of azi-DAP are limited.[43–47] A number of synthetic strategies were explored in the stereospecific synthesis of azi-DAP. Wenker ring closure of (2*S*,6*S*)-2,6-diamino-6-(hydroxymethyl)pimelic acid **13**,[48,49] was unsuccessful (Scheme 4). Attempts to utilise the chiral auxiliary methodology used in the construction of the internal aziridine **4** were frustrated by problems encountered in the synthesis of the required *N*-enoylbornane[10,2]sultam precursor. This led us to investigate the use of chiral 3-acetoxyaminoquinazolinones,[31] which exert reagent controlled diastereoselectivity in the aziridination of prochiral alkenes such as **16** (Scheme 4). Boc protected unsaturated α-aminopimelic acids have been reported,[37,50] and it was proposed that aziridination of the Cbz protected analogue with chiral aminoquinazolinones **19–21** would allow the rapid preparation of a single diastereomer of the fully protected azi-DAP derivative **17**.

The required unsaturated α-aminopimelic ester **16** was constructed in one-pot by photolysis of the hydroxamate ester of *N*-Cbz-L-glutamic acid α-methyl ester **15** with methyl α-thiophenol methylacrylate using a tungsten lamp at room temperature to give a 68% yield over three steps (Scheme 4).[50]

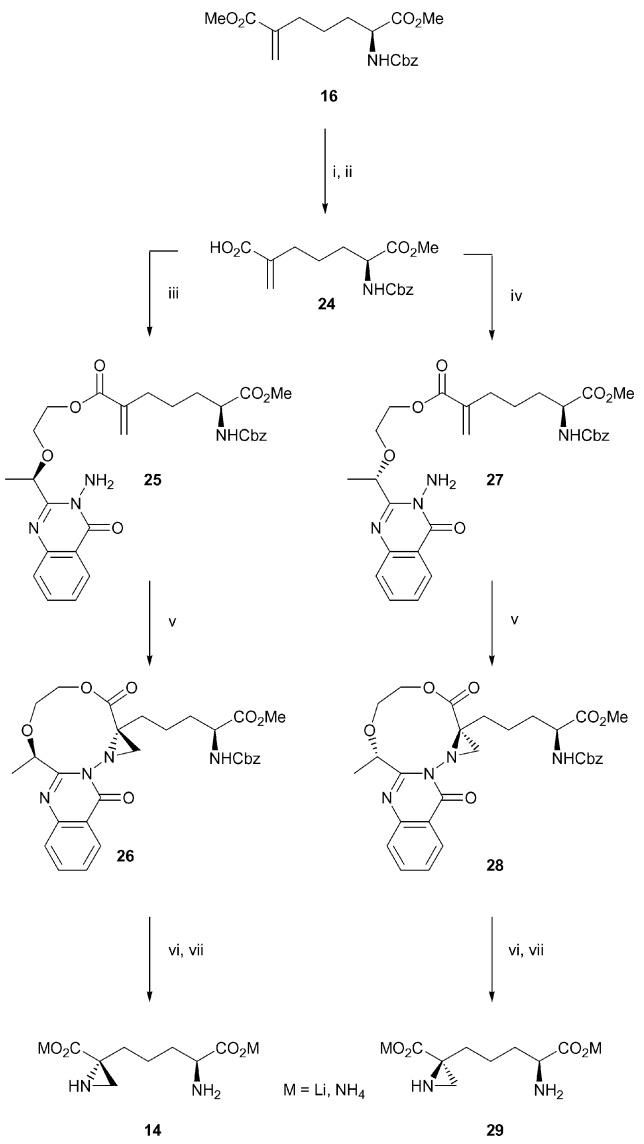
The 3-acetoxy derivatives of the known aminoquinazolin-4(3*H*)-ones **19**,[47] **20**,[51] and the previously unreported **21** were investigated as aziridinating agents. No reaction is observed during titanium mediated aziridination[31] using the hydroxy aminoquinazolin-4(3*H*)-one **20**. Aziridination using **21** and TFA[31] gives low conversion (22%) and poor selectivity (3 : 1 dr), whereas the reaction of **19** in the presence of HMDS[31] proceeds with moderate yield and selectivity (49%, dr 6 : 1). The mixture of diastereomers **17** can be saponified with base and then reduced using lithium in ammonia at −78 °C, to produce azi-DAP **14** as a 6 : 1 mixture of diastereomers at the quaternary carbon along with the unexpected fully saturated ε-methyl-α-aminopimelic acid **18** (1 : 1 dr at the carbon bearing methyl).[52] The structure of **18** was confirmed by independent synthesis (see ESI†). Separation of **14** and **18** is possible by careful preparative TLC under basic conditions (azi-DAP undergoes very rapid decomposition on exposure to acid). To our chagrin, extensive attempts to separate the minor azi-DAP diastereomer from **14**, or to purify their corresponding diacid salts or the fully protected intermediates **17** using flash chromatography, HPLC or recrystallisation all failed.



Scheme 4 The synthesis of azi-DAP. *Reagents and conditions*: (i) i-BuOCOCl, NMM, THF; (ii) NEt_3, 2-mercaptopyridine *N*-oxide, THF; (iii) methyl α-thiophenol methylacrylate, hυ (tungsten lamp), THF (68% over 3 steps); (iv) **19**, $Pb(OAc)_4$, HMDS, CH_2Cl_2 (49%); (v) LiOH, MeOH–H_2O (quant.); (vi) Li, NH_3.

To improve the selectivity of the aziridination and thus obviate the problematic isomer separation, we adopted an intramolecular variant of the aminoquinazolin-4(3*H*)-one methodology that had been reported to give complete diastereoselectivity during the aziridination of *O*-cinnamoyl esters.[53] This necessitated the modification of our synthetic strategy to couple (*R*)-aminoquinazolin-4(3*H*)-one **22** to the 1,2-disubstituted α,β-unsaturated acid **24** (Scheme 5).

The acid **24** could be synthesised in two steps from the diester **16** by basic hydrolysis and regioselective esterification of the diacid using catalytic *p*-toluene sulfonic acid (PTSA) in anhydrous methanol.[54] Although the selective esterification proceeds in >95% yield (^{1}H NMR), the isolated yields after purification by flash chromatography are significantly lower (30–60%). Use of polymer bound PTSA[55] circumvents this problem and allows the quantitative recovery of the product **24**. The coupling of acid **24** to (*R*)-aminoquinazolin-4(3*H*)-one **22** proceeds in satisfactory 62% yield using DCC–DMAP. However, several cycles of flash chromatography are required to achieve the complete removal of the unwanted urea by-product. Switching to resin bound *N*-benzyl-*N*′-cyclohexylcarbodiimide reagent (PS-DCC)[56] permits facile purification without significant loss of yield, as demonstrated by the coupling of acid **24** to the (*S*)-aminoquinazolin-4(3*H*)-one **23** to give **27** in 51% after a single pass through a plug of silica.



Scheme 5 The stereoselective synthesis of LL- and DL-azi-DAP. *Reagents and conditions*: (i) LiOH, MeOH–H_2O (82%); (ii) PS-PTSA, MeOH (95%); (iii) **22**, DCC, DMAP, CH_2Cl_2 (62%); (iv) **23**, PS-DCC, DMAP, CH_2Cl_2 (51%); (v) $Pb(OAc)_4$, HMDS, CH_2Cl_2 (**26** 55%, **28** 87%); (vi) Na_2CO_3, MeOH–H_2O (from **28** 69%); (vii) Li, NH_3 (**14** 30% over 2 steps, **29** 23%).

Intramolecular aziridination of **27** progresses rapidly at 0 °C to give the aziridine **28** with complete diastereoselectivity (>99 : 1 dr, see ESI†). The stereochemical outcome of this reaction can be determined by comparison of the observed NOE interactions with energy minimised models of the two possible reaction products (see ESI†). Preparation of the aziridine **26** under analogous conditions is slightly less selective (98 : 2 dr). Fortunately, the minor diastereomer could be removed by careful purification using flash column chromatography to give a >99 : 1 dr.

Hydrolysis of the fully protected azi-DAP **28** using lithium hydroxide or sodium hydroxide results in decomposition. However, sodium carbonate[57] in methanol–water is sufficiently mild to permit quantitative conversion to the corresponding salt of the diacid (^{1}H NMR). Final deprotection using lithium or sodium in ammonia at −78 °C results in the formation of both the desired azi-DAP isomer **29** as the major product and the fully saturated ε-methyl-α-aminopimelic acid **18** in a 5.6 : 1 ratio (^{1}H NMR). The undesired **18** is again formed as a 1 : 1 mixture of diastereomers at the carbon bearing the methyl. Reductions with sub-stoichiometric quantities of lithium on carefully purified (RP-HPLC) carboxylate salts of the diacid intermediate demonstrate that the fully saturated ε-methyl-α-aminopimelic acid **18** is formed concomitantly with azi-DAP **29**. The temperature of the reaction has little effect on the product ratio. The reaction to form **18** may proceed *via* reductive aziridine ring cleavage to generate an anion α to the carboxylate followed by elimination of nitrogen and further reduction of the resulting conjugated olefin. Separation of the crude reaction mixture could be achieved using preparative TLC under basic conditions, albeit in low isolated yield (23% for azi-DAP **29**). It was later found that HPLC purification of the diacid salt is not necessary prior to the reductive deprotection. Therefore pure azi-DAP **14** could be prepared directly from **26** with an improved yield of 30% over the final two steps. As expected, azi-DAP isomers **14** and **29** are extremely sensitive compounds that are stable in strong base, but degrade rapidly under neutral or acidic conditions. The presence of multiple internal nucleophiles (four carboxylate oxygens and the distal amino group) assists rapid intramolecular aziridine ring opening. In solid form as carboxylate salts, **14** and **29** decompose gradually at room temperature, with half lives of about 1 to 2 weeks. Although storage at −20 °C retards decomposition, they are only stable for prolonged periods under cryogenic (−80 °C) conditions. Unfortunately, lack of stability in aqueous media precludes evaluation of their antimicrobial activity.

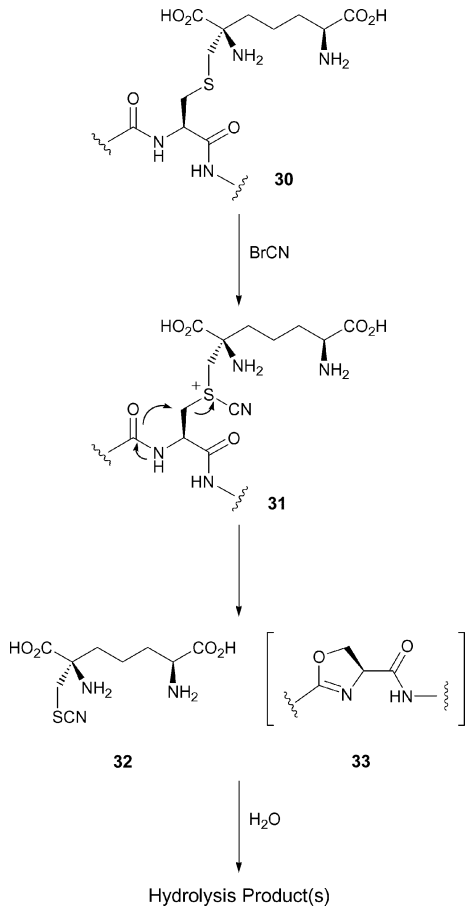
Inhibition of DAP epimerase by azi-DAP isomers 14 and 29

Inhibition studies of DAP epimerase by Higgins *et al.* using a mixture of all azi-DAP diastereomers generated *in situ* from the corresponding α-(fluoromethyl)diaminopimelic acids demonstrated that azi-DAP is an irreversible inhibitor. They were unable to determine K_i or k_i values due to the extremely fast conversion of the enzyme-inhibitor complex to the inactivated enzyme.[12,58] Using the coupled enzyme assay we were able to confirm that both diastereomers of azi-DAP are irreversible inhibitors, and show both time and concentration dependence. Furthermore, the covalent attachment of both isomers to the enzyme can be demonstrated by electrospray mass spectroscopy (MS).

The original studies by Higgins *et al.* using tryptic digests of inhibited DAP epimerase combined with Edman degradation of the purified fragments reported that the enzyme is attacked at Cys-73 by the crude mixture of all diastereomers of azi-DAP.[13] Subsequent studies suggested[17] that inhibition of DAP epimerase with pure LL-azi-DAP **14** should result in selective attachment to Cys-73, whereas DL-azi-DAP **29** would react with Cys-217. In order to test this proposal, a trypsin digestion was done on DAP epimerase inhibited with excess pure azi-DAP isomers. HPLC purification of the fragments followed by MS/MS analysis displays a surprisingly low level of sequence coverage (40% for inhibition with **14** and 32% with **29**). In each case, a fragment incorporating the Cys-73 unit (residues 62–78) could be sequenced and shown to contain free Cys-73 or Cys-73 with azi-DAP covalently attached. No fragment containing Cys-217 could be identified after HPLC of the crude digest.

In order to study the interaction of Cys-217 with azi-DAP, an alternative digestion protocol was required. As mentioned above, covalent attachment of an azi-DAP molecule to DAP epimerase could be observed for both **14** and **29** by MS. Although treatment with cyanogen bromide followed by trypsin digestion allowed the detection of a suitable fragment (residues 210–230) of the parent (not inhibited) DAP epimerase, it was found that the reaction of cyanogen bromide resulted in the cleavage of azi-DAP from the enzyme inactivated by **29**. Presumably a mechanism similar to the cleavage of the peptide chain adjacent to methionine residues occurs under these conditions. The inactivation of DAP epimerase by azi-DAP occurs by nucleophilic ring opening of the aziridine to generate **30**, which has a sulfide linkage between the enzyme and the inhibitor.[13] Nucleophilic attack of the sulfur on cyanogen bromide followed by intramolecular displacement of the resulting positively charged sulfonium species **31** from the nearby peptide

bond affords the dealkylated peptide **33**, which can subsequently undergo hydrolysis (Scheme 6).



Scheme 6 Proposed mechanism for the cleavage of covalently linked azi-DAP from cysteine residues of DAP epimerase on treatment with cyanogen bromide.

An analogous process was previously observed during cyanogen bromide cleavage of a protease inhibited by *N*-iodoacetyl peptides.[59] Fortunately, inactivation of DAP epimerase with azi-DAP **29** followed by treatment with thermolysin and trypsin provides excellent sequence coverage (89%) of the resulting fragments by MS/MS. More importantly, fragments incorporating both cysteines of interest could be analysed using this protocol (Table 1). Analysis of DAP epimerase inhibited with a large excess (10–100 fold) of azi-DAP **29** reveals that in addition to the expected alkylation of Cys-217, attack also occurs at Cys-73 to some extent. In principle, it is possible that a single diasteroisomer of azi-DAP (*i.e.* **29**) reacts with both cysteines. However, the excess of inhibitor could possibly be contaminated by very small amounts (<1.0%) of the other azi-DAP diastereomer **14**, which is not easily detectable and could react with the DAP epimerase. The use of a such a large excess of inhibitor was initially prompted by the desire to completely and rapidly inactivate the enzyme for crystallographic studies prior to its facile aerobic oxidation to form the internal disulfide between Cys-73 and Cys-217.

Table 1 MS/MS analysis of trypsin/thermolysin digest of DAP epimerase inhibited with azi-DAP **29**. Italic: undetected residues. Bold: sequence coverage. Boxed: fragments containing Cys-73 and Cys-217

1	*MQFSK*	**MHGLG**	**NDFVV**	**VDGVT**	**QNVFF**
26	**TPETI**	**RRLAN**	**RHCGI**	**GFDQL**	**LIVEA**
51	**PYDPE**	**LDFHY**	**R IFNA**	**DGSEV**	**SQCGN**
76	**GARC F**	**ARFVT**	*LKGLT*	*NKKD*I	**SVSTQ**
101	**KGNMV**	**LT***VKD*	**MNQIR**	**VNMGE**	**PIWEP**
126	**AKIPF**	*T*ANKF	**EKNYI**	**LRTDI**	**QTVLC**
151	**GAVSM**	**GNPHC**	**VVQVD**	**DIQTA**	**NVEQ***L*
176	*GPL*LE	**SHERF**	**PERVN**	**AGF***MQ*	**IINKE**
201	**HIKLR**	**VYERG**	**AGETQ**	**ACGSG**	**AC AAV**
226	**AVGIM**	**QGLLN**	**NNVQV**	**DLPGG**	**SLMIE**
251	**WNGVG**	**HP**L**YM**	**TGEAT**	**HIYDG**	**F***ITL*

Fortunately, preliminary X-ray crystallography studies of DAP epimerase separately inhibited with the individual azi-DAP isomers show LL-azi-DAP **14** bound exclusively to Cys-73, and DL-azi-DAP **29** attached only to Cys-217. In both cases, inactivation proceeds as predicted by attack of thiolate on the aziridine methylene. After complete refinement and structural analysis, these three dimensional structures will be the subject of a separate report.

In conclusion, we have described the first stereoselective synthesis of highly sensitive aziridine analogues of DAP **4**, **14** and **29**. The results show that internal aziridine analogue **4** is a reversible inhibitor of DAP epimerase. The LL-azi-DAP **14** and DL-azi-DAP **29** rapidly and irreversibly inactivate DAP epimerase, as can be seen from a coupled enzyme assay with DAP dehydrogenase as well as by MALDI-TOF MS analysis. Proteolytic digestion of the enzyme inactivated by each inhibitor with thermolysin/trypsin followed by MS/MS provides excellent sequence coverage (89%) and allows analysis of fragments incorporating both cysteines of interest. MS/MS analysis of the digest of each inactivated DAP epimerase shows that the individual azi-DAP isomers bind irreversibly to Cys-73 and Cys-217. The use of excess inhibitor to assist preparation of samples for X-ray crystallographic analysis results in some unexpected alkylation of the cysteine residue that is expected to act as the protonating thiol. This may be due to the presence of very small quantities (<1.0%; undetectable by NMR) of the "wrong" isomer of azi-DAP. Preliminary X-ray analysis of inhibited DAP epimerase crystals showed that LL-azi-DAP **14** binds selectively to Cys-73 and DL-azi-DAP **29** to Cys-217. The covalent attachment of azi-DAP is consistent with the proposed mechanism of inhibition *i.e.* attack of thiolate at the methylene position of the aziridine ring. Furthermore, the observed selectivity of azi-DAP inhibition is consistent with the two base mechanism proposed for the epimerisation of LL-DAP **1** and DL-DAP **2** by DAP epimerase, more specifically that Cys-73 acts as a thiolate base to deprotonate LL-DAP **1**, and Cys-217 in the case of *meso*-DAP **2**.[17]

Experimental

General methods

All reactions were performed under dry argon. All solvents were purified and distilled according to Perrin *et al.*[60] Flash chromatography employed silica gel 60 (Silicycle, 230–420 mesh) and was performed according to the Still procedure.[61] One or more of the following methods were used for visualisation: UV fluorescence, iodine staining, phosphomolybdic acid/ceric sulfate, potassium permangante or ninhydrin. Melting points were determined on a Büchi apparatus using open-end capillary tubes and are uncorrected. NMR spectra were recorded on Inova Varian 300, 400, 500 and 600 MHz instruments. IR spectra were determined with a Nicolet Magna 750 FT-IR spectrometer. Mass spectra (MS) were recorded with a Micromass ZabSpec Hybrid Sector-TOF instrument (electrospray ionization (ES)). Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 26 °C with a micro cell (100 mm; 0.9 mL) or a standard cell (100 mm, 8 mL) respectively. UV spectroscopy was performed on a GBC Cintra 40 UV spectrometer. $[\alpha]_D$ are given in units of 10^{-1} deg cm^2 g^{-1}. Microanalyses were completed at the University of Alberta Microanalytical Laboratory. All literature compounds had IR, ^{1}H NMR, ^{13}C NMR and mass spectra consistent with assigned structures.

Methyl (1*S*,2*R*)-*N*-{(2′*E*,6′*S*)-[*N*,*N*-di-*tert*-butoxycarbonyl-6′-amino]pentan-2′-endioate}bornane-10,2-sultam. LiCl (0.22 g,

5.2 mmol) was suspended in MeCN (10 mL). A solution of (1*S*,2*R*)-*N*-(2′-diethoxyphosphonoacetyl)bornane-10,2-sultam[33] (2.04 g, 5.2 mmol) in MeCN (15 mL) was added along with DBU (0.79 g, 5.2 mmol). Aldehyde **5** (1.49 g, 4.3 mmol) in MeCN (20 mL) was then added and the reaction mixture was allowed to stir for 2 h. The reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in EtOAc (100 mL), washed with H_2O (2 × 70 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 65 : 35 hexane–EtOAc gave the title compound (2.15 g, 85%) as a white foam. $[\alpha]_D$ −65.8 (*c* 0.5, $CHCl_3$); ν_{max}($CHCl_3$ cast)/cm^{-1} 2978, 1793, 1746, 1685, 1368, 1331, 1269, 1237, 1220, 1166, 1135; δ_H (300 MHz, $CDCl_3$) 0.96 and 1.18 (each 3H, 2 × s, 8-H_3 and 9-H_3), 1.31–1.51 (20H, m, 2 × O*t*-Bu and 5-H_2), 1.89, 2.01–2.14 and 2.26–2.39 (9H, m, 3-H_2, 4-H, 6-H_2, 4′-H_2 and 5-H_2), 3.41 (1H, d, J = 13.5 Hz, 10-*H*H), 3.50 (1H, d, J = 13.5 Hz, 10-H*H*), 3.71 (3H, s, OMe), 3.91 (1H, t, J = 6.3 Hz, 2-H), 4.86 (1H, dd, J = 9.0, 4.2 Hz, 6′-H), 6.56 (1H, d, J = 15.1 Hz, 2′-H), 7.06 (1H, dt, J = 15.1 Hz, 6.7, 3′-H); δ_C (75.5 MHz, $CDCl_3$) 19.9, 20.9, 26.5, 28.0, 28.7, 29.4, 32.9, 38.5, 44.7, 47.8, 48.5, 52.2, 53.2, 57.7, 65.2, 83.4, 121.5, 149.2, 152.0, 164.0, 170.9; *m*/*z* (ES) 607.2668 (MNa^+), $C_{28}H_{44}N_2O_9NaS$ requires 607.2665.

Methyl (1*S*,2*R*)-*N*-{(2′*E*,6′*S*)-[*N*-*tert*-butoxycarbonyl-6′-amino]-pentan-2′-endioate}bornane-10,2-sultam 6. To a solution of methyl (1*S*,2*R*)-*N*-{(2′*E*,6′*S*)-[*N*,*N*-di-*tert*-butoxycarbonyl-6′-amino]pentan-2′-endioate}bornane-10,2-sultam (1.85 g, 3.17 mmol) in CH_2Cl_2 (20 mL) was added a solution of TFA (0.47 g, 3.80 mmol) in CH_2Cl_2 (10 mL). After 4 h, the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography, eluting with 6 : 4 hexane–EtOAc gave the title compound **6** (1.44 g, 94%) as a viscous oil. $[\alpha]_D$ −41.5 (*c* 0.4, $CHCl_3$); ν_{max}($CHCl_3$ cast)/cm^{-1} 3373, 2960, 1744, 1713, 1367, 1331, 1167, 544; δ_H (300 MHz, $CDCl_3$) 0.95 and 1.14 (each 3H, 2 × s, 8-H_3 and 9-H_3), 1.32–1.44 (11H, m, O*t*-Bu and 5-H_2), 1.71–2.19 and 2.22–2.41 (9H, m, 3-H_2, 4-H, 6-H_2, 4′-H_2 and 5-H_2), 3.41 (1H, d, J = 12.2 Hz, 10-*H*H), 3.49 (1H, d, J = 12.2 Hz, 10-H*H*), 3.73 (3H, s, OMe), 3.90 (1H, dd, J = 7.2, 4.5 Hz, 2-H), 4.30 (1H, br q, J = 4.0 Hz, 6′-H), 5.04 (1H, br d, J = 7.2 Hz, NH), 6.53 (1H, dt, J = 15.2, 1.6 Hz, 2′-H), 7.00 (1H, dt, J = 15.2, 6.8 Hz, 3′-H); δ_C (75.5 MHz, $CDCl_3$) 19.9, 20.9, 26.5, 28.3, 28.4, 31.1, 32.9, 38.5, 44.8, 47.8, 48.5, 52.5, 53.1, 53.2, 65.2, 83.3, 121.7, 148.6, 155.3, 163.9, 172.8; *m*/*z* (ES) 507.2136 (MNa^+), $C_{23}H_{36}N_2O_7NaS$ requires 507.2141.

Methyl (1*S*,2*R*,2′*R*,3′*S*,3″*S*)-{1′-(2-ethyl-3,4-dihydroquinazolin-4-one)-3′-[3″-(*N*-*tert*-butoxycarbonylamino)butanoate] aziridine-2′-carboxylate} bornane-10,2-sultam 7. A solution of $Pb(OAc)_4$ (0.78 g, 1.75 mmol) in CH_2Cl_2 (10 mL) was added to a solution of quinazolin-4-one[32] **12** (0.29 g, 1.52 mmol) in CH_2Cl_2 (10 mL) at −40 °C. After 30 min, a solution of sultam **6** (0.81 g, 1.67 mmol) in CH_2Cl_2 (15 mL) along with HMDS (0.246 g, 1.52 mmol) was added. After 4 h, the reaction mixture was warmed to room temperature and filtered under gravity. The organic layer was washed with a saturated solution of $NaHCO_3$ (50 mL), H_2O (50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 7 : 3 hexane–EtOAc gave a 9 : 1 mixture of the two diastereomeric aziridines. Recrystallisation from EtOAc–hexane gave the title compound **7** (0.736 g, 72%) as a white solid. Mp 132–134 °C (EtOAc–hexane); $[\alpha]_D$ −214.6 (*c* 0.8, $CHCl_3$); ν_{max}($CHCl_3$ cast)/cm^{-1} 3320, 2975, 1741, 1713, 1675, 1595, 1520, 1472, 1456, 1367, 1334, 1272, 1240, 1165, 1136, 534; δ_H (300 MHz, $CDCl_3$) 0.98 and 1.26 (each 3H, 2 × s, 8-H_3 and 9-H_3), 1.32–1.44 (14H, m, O*t*-Bu, CH_2CH_3 and 5-H_2), 1.63–1.99 and 2.05–2.21 (9H, m, 3-H_2, 4-H, 6-H_2, 1″-H_2 and 2″-H_2), 2.74 (1H, m, C*H*HCH$_3$), 3.01 (1H, m, CH*H*CH$_3$), 3.45 (1H, d, J = 13.8 Hz, 10-*H*H), 3.57 (1H, d, J = 13.8 Hz, 10-H*H*), 3.70–3.77 (5H, m, OMe, 2-H and 3′-H), 3.84 (1H, d, J = 4.8 Hz, 2′-H), 4.35 (1H, br m, 3″-H), 5.17 (1H, br d, J = 8.0 Hz, NH), 7.30 (1H, m, 6-H(Q)), 7.58–7.68 (2H, m, 7-H(Q) and 8-H(Q)), 8.12 (1H, d, J = 8.0 Hz, 5-H(Q)); δ_C (125 MHz, $CDCl_3$) 10.7, 19.9, 20.8, 26.3, 27.2, 28.3, 28.8, 32.8, 38.0, 44.6, 46.2, 46.7, 48.8, 48.9, 52.4, 53.1, 53.3, 53.5, 65.6, 79.8, 121.3, 126.2, 126.3, 126.6, 126.9, 133.6, 146.1, 155.5, 159.9, 164.0, 172.9; *m*/*z* (ES) 694.3 (MH^+); Found: C, 58.75; H, 6.65; N, 10.04. $C_{33}H_{45}N_5O_8S$ requires C, 59.02; H, 6.71; N, 10.43%.

(2*R*,3*S*,3′*S*)-3-[3′-Aminopropane)aziridine-2,3′-dicarboxylate 4. Aziridine **7** (0.283 g, 0.47 mmol) was dissolved in CH_2Cl_2 (10 mL) and a solution of TFA (0.192 g, 1.88 mmol) in CH_2Cl_2 (5 mL) was added. After 15 h, the reaction mixture was concentrated *in vacuo*. The resulting residue was washed with 1 : 1 Et_2O–hexane (2 × 20 mL). The resulting white solid (0.287 g, 0.47 mmol) was filtered, dried ($MgSO_4$) and dissolved in MeOH (5 mL). A solution of LiOH·H_2O (0.053 g, 1.41 mmol) in H_2O (2 mL) was added. After 14 h, the reaction mixture was concentrated *in vacuo*. The resulting oil was washed with Et_2O (2 × 20 mL) producing a white solid. Purification using cellulose chromatography, eluting with 7 : 3 MeOH–H_2O gave a white solid (0.132 g, 0.35 mmol). Pre-distilled ammonia (20 mL) was added to the flask and the reaction mixture was cooled to −78 °C. Lithium (~0.05 g) was added until a deep blue colour persisted. After 1 h, the reaction mixture was quenched by the addition of NH_4Cl (~50 mg). The reaction mixture was warmed to room temperature and diluted with H_2O (20 mL). The reaction mixture was washed with EtOAc (2 × 20 mL). The aqueous layer was then concentrated *in vacuo*. Purification by flash column chromatography, eluting with 95 : 5 *n*-PrOH–NH_4OH gave the title compound **4** as a white solid (32 mg, 40% for three steps). $[\alpha]_D$ +4.0 (*c* 4.5, H_2O); ν_{max}(microscope)/cm^{-1} 2944, 1737, 1617, 1508, 1419, 1215, 1124; δ_C (300 MHz, D_2O) 1.60–1.80 (m, 2H, 1′-H_2), 1.86–2.14 (m, 2H, 2′-H_2), 4.05 (br m, 1H, 3-H), 4.11 (m, 1H, 2-H), 4.27 (m, 1H, 3′-H); δ_C (75.5 MHz, D_2O) 27.3, 28.7, 71.6, 72.2, 73.8, 172.8; *m*/*z* (ES) 211.1 (MNa^+).

Dimethyl (2*R*,3*S*,3′*S*) and (2*S*,3*R*,3′*S*)-1-(2-ethyl-3,4-dihydroquinazolin-4-one)-3-[3′-(*tert*-butoxycarbonylamino) butanoate]-aziridine-2-carboxylate 9 and 10. A solution of $Pb(OAc)_4$ (0.264 g, 0.62 mmol) in CH_2Cl_2 (5 mL) was added to a solution of quinazolin-4-one[32] **12** (0.098 g, 0.52 mmol) in CH_2Cl_2 (5 mL) at −25 °C. After 30 min, a solution of alkene[37] **8** (0.187 g, 0.62 mmol) in CH_2Cl_2 (5 mL) along with HMDS (0.084 g, 0.52 mmol) was added. After 2 h, the reaction mixture was warmed to room temperature and filtered under gravity. The organic layer was washed with a saturated solution of $NaHCO_3$ (30 mL), H_2O (30 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 7 : 3 hexane–EtOAc gave an inseparable mixture of the title compounds **9** and **10** (0.21 g, 83%) as a white foam. ν_{max}($CHCl_3$ cast)/cm^{-1} 3349, 2977, 1737, 1713, 1677, 1596, 1367, 1202, 1163; δ_H (300 MHz, $CDCl_3$) 1.37 (3H, t, J = 10.7 Hz, C*H*$_3$CH$_2$), 1.40 and 1.43 (9H, 2 × s, 2 × O*t*-Bu), 1.75–2.10 (4H, m, 1′-H_2 and 2′-H_2), 2.72 (1H, m, CH$_3$C*H*H), 3.00 (1H, m, CH$_3$CH*H*), 3.15 (1H, dd, J = 4.9, 2.5 Hz, 3-H), 3.59 (3H, m, OMe), 3.58–3.76 (4H, m, 2-H and OMe), 4.26 (1H, br q, J = 4.6 Hz, 3′-H), 5.20 (1H, 2 × br d, J = 6.9 Hz, NH), 7.38 (1H, m, 6-H(Q)), 7.56–7.68 (2H, m, 7-H(Q) and 8-H(Q)), 8.14 (1H, m, J = 8.7 Hz, 5-H(Q)); δ_C (75.5 MHz, $CDCl_3$) 10.6, 27.0, 27.4, 28.35, 29.0, 47.3, 51.7, 52.5, 52.9, 53.3, 80.9, 121.2, 126.3, 127.0, 133.7, 146.1, 156.2, 160.1, 166.8, 172.9; *m*/*z* (ES) 511.2165 (MNa^+). $C_{24}H_{32}N_4O_7Na$ requires 511.2169.

Dimethyl (2*R*,3*S*,3′*S*) and (2*S*,3*R*,3′*S*)-1-(2-ethyl-3,4-dihydroquinazolin-4-one)-3-[3′-aminobutanoate]aziridine-2-carboxylate. A mixture of aziridines **9** and **10** (0.1 g, 0.2 mmol) were dissolved in CH_2Cl_2 (5 mL) and a solution of TFA (0.26 g, 2.2 mmol) in CH_2Cl_2 (2 mL) was added. After 10 h, the reaction mixture was concentrated *in vacuo*. The resulting residue was washed with 1 : 1 Et_2O–hexane (2 × 20 mL). The resulting white solid was filtered and dried to give an inseparable mixture of the

title compounds (0.105 g, quant.). ν_{max}($CHCl_3$ cast)/cm^{-1} 2957, 1735, 1672, 1597 1202, 1134; δ_H(300 MHz, $CDCl_3$) 1.39 (3H, t, J = 7.3 Hz, CH_3CH_2), 1.90–2.21 (4H, m, 1′-H_2 and 2′-H_2), 2.91 (1H, m, CH_3CHH), 3.03 (1H, m, CH_3CHH), 3.27 (1H, t, J = 4.7 Hz, 3-H), 3.61 (3H, m, OMe), 3.71 (1H, m, 2-H), 3.76 (3H, s, OMe), 4.26 (1H, br q, J = 5.5 Hz, 3′-H), 7.44 (1H, m, 6-H(Q)), 7.68–7.82 (2H, m, 7-H(Q) and 8-H(Q)), 8.10 (1H, d, J = 8.3 Hz, 5-H(Q)); δ_C (75.5 MHz, $CDCl_3$) 10.5, 25.6, 26.6, 31.0, 51.3, 52.7, 53.5, 53.6, 59.5, 122.4, 127.2, 128.7, 136.0, 153.3, 161.8, 166.4, 169.6; m/z (ES) 389.1827. $C_{19}H_{25}N_4O_5$ requires 389.1825.

(2*R*,3*S*,3′*S*) and (2*S*,3*R*,3′*S*)-1-(2-Ethyl-3,4-dihydroquinzolin-4-one)-3-[3′-aminopropane]aziridine-2,3′-dicarboxylate. A mixture of dimethyl (2*R*,3*S*,3′*S*) and (2*S*,3*R*,3′*S*)-3-[3′-aminobutan-4′-oate]-1-(2-ethyl-3,4-dihydroquinazolin-4-one) aziridine-2-carboxylate (0.1 g, 0.2 mmol) were dissolved in MeOH (5 mL) and a solution of $LiOH{\cdot}H_2O$ (0.026 g, 0.62 mmol) in H_2O (2 mL) was added. After 14 h, the reaction mixture was concentrated *in vacuo*. The resulting oil was washed with Et_2O (2 × 20 mL) producing a white solid. Purification using cellulose chromatography, eluting with 7 : 3 MeOH–H_2O gave the title compound (0.061 g, 84%) as a white solid. ν_{max}(microscope)/cm^{-1} 2939, 1655, 1610, 1595, 1414, 1206, 558; δ_H(300 MHz, D_2O) 1.30 (3H, m, CH_3CH_2), 1.60–2.20 (4H, m, 1′-H_2 and 2′-H_2), 2.91–3.30 (2H, m, CH_3CH_2), 3.64 (1H, m, 3-H), 3.79 (1H, m, 2-H), 4.10 (1H, br m, 3′-H), 7.44 (2H, m, 6-H(Q) and 7-H(Q)), 7.80 (1H, m, 8-H(Q)), 8.12 (1H, m, 5-H(Q)); m/z (ES) 373.1674 (MH^+). $C_{17}H_{19}Li_2N_4O_5$ requires 373.1676.

(2*R*,3*S*,3′*S*) and (2*S*,3*R*,3′*S*)-3-[3′-Aminopropane)aziridine-2,3′-dicarboxylate 4 and 11. Pre-distilled ammonia (20 mL) was added to a flask containing (2*R*,3*S*,3′*S*) and (2*S*,3*R*,3′*S*)-3-[3′-aminopropane]-1-(2-ethyl-3,4-dihydroquinazolin-4-one)-aziridine-2,3′-dicarboxylate (0.12 g, 0.32 mmol) and the reaction mixture was cooled to −78 °C. Lithium (~0.05 g) was added until a deep blue colour persisted. After 1 h, the reaction mixture was quenched by the addition of solid NH_4Cl (~50 mg). The reaction mixture was warmed to room temperature and diluted with H_2O (20 mL). The reaction mixture was washed with EtOAc (2 × 20 mL). The aqueous layer was then concentrated *in vacuo*. Purification by flash column chromatography, eluting with 95 : 5 *n*-PrOH–NH_4OH gave an inseparable mixture of the title compounds **4** and **11** (29 mg, 48%) as a white solid. ν_{max}(microscope)/cm^{-1} 2943, 1737, 1617, 1508, 1419, 1215, 1124; δ_H (300 MHz, D_2O) 1.60–1.80 (2H, m, 1′-H_2), 1.86–2.14 (2H, m, 2′-H_2), 4.05 (1H, br m, 3-H), 4.11 (1H, m, 2-H), 4.27 (1H, m, 3′-H); m/z (ES) 211.1 (MNa^+).

Dimethyl (2*S*)-*N*-benzyloxycarbonyl-2-amino-6-methylhept-6-ene-dioate 16. Ester **15** (0.817 g, 2.77 mmol) was dissolved in degassed THF (20 mL) and cooled to 0 °C. NMM (0.28 g, 2.77 mmol) and isobutyl chloroformate (0.378 g, 2.77 mmol) were both added. After 15 min, a solution of *N*-hydroxy-2-thiopyridine (0.423 g, 3.33 mmol) and NEt_3 (0.464 mL, 3.33 mmol) in degassed THF (15 mL) was added and argon was bubbled through the reaction mixture. (N.B. At this stage the reaction mixture was covered with aluminium foil). After 1 h, a degassed solution of methyl α-thiophenol methylacrylate[50] (1.44 g, 6.92 mmol) in THF (4 mL) was added and the reaction mixture was irradiated with a tungsten lamp (250 W) for 30 min with cooling at 20 °C. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with a saturated solution of $NaHCO_3$ (2 × 50 mL), brine (100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 7 : 3 hexane–EtOAc gave the title compound **16** (0.66 g, 68%) as a viscous oil. $[\alpha]_D$ +8.6 (*c* 0.7, $CHCl_3$); ν_{max}($CHCl_3$ cast)/cm^{-1} 3348, 2952, 1719, 1630, 1439, 1209, 1057; δ_H (300 MHz, $CDCl_3$) 1.42–1.52 (2H, m, 4-H_2), 1.64 (1H, m, 3-*H*H), 1.83 (1H, m, 3-H*H*), 2.29 (2H, br t, J = 7.8 Hz, 5-H_2), 3.71 (6H, s, 2 × OMe), 4.38 (1H, q, J = 6.0 Hz, 2-H), 5.08 (2H, s, PhCH_2), 5.22 (1H, d, J = 7.2 Hz, NH), 5.49 (1H, s, C=C*H*H), 6.12 (1H, s, C=CH*H*), 7.36 (5H, m, Ph); δ_C (125 MHz, $CDCl_3$) 24.1, 31.4, 32.3, 51.9, 52.4, 53.7, 67.0, 125.3, 128.0, 128.1, 128.4, 136.2, 139.7, 155.7, 167.3, 172.7; m/z (ES) 372.1421 (MNa^+). $C_{18}H_{23}NO_6Na$ requires 372.1423.

Dimethyl (2*S*,4′*S*) and (2*R*,4′*S*)-1-(2-[(1*S*)-1-*tert*-butyldimethylsilyloxyethyl]-3,4-dihydroquinazolin-4-one)-2-[4′-(benzyloxycarbonylamino)pentanoate]aziridine-2-carboxylate 17. A solution of $Pb(OAc)_4$ (0.421 g, 0.94 mmol) in CH_2Cl_2 (20 mL) was added to a solution of quinazolin-4-one[51] **19** (0.263 g, 0.82 mmol) in CH_2Cl_2 (10 mL) at −40 °C. After 30 min, a solution of alkene **16** (0.33 g, 0.94 mmol) in CH_2Cl_2 (5 mL) along with HMDS (0.133 g, 0.82 mmol) was added. After 6 h, the reaction mixture was warmed to room temperature and filtered under gravity. The organic layer was washed with a saturated solution of $NaHCO_3$ (60 mL), H_2O (60 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography, eluting with 65 : 35 hexane–EtOAc gave an inseparable mixture of the title compounds **17** (0.27 g, 49%) as a viscous oil. ν_{max} ($CHCl_3$ cast)/cm^{-1} 3351, 2977, 2952, 1714, 1677, 1597, 1472, 1164; δ_H (300 MHz, $CDCl_3$) (for major diastereomer only) 0.05 (3H, s, *t*-BuSi(CH_3)CH_3), 0.23 (3H, s, *t*-BuSi(CH_3)CH_3), 0.92 (9H, s, *t*-Bu), 1.58 (3H, d, J = 6.3 Hz, CH_3CHOTBDMS), 1.62–2.04 (5H, m, 1′-HH, 2′-H_2, 3′-H_2), 2.31 (1H, m, 1′-HH), 2.74 (1H, br d, J = 4.8 Hz, 3-HH), 3.20 (1H, br s, 3-HH), 3.49 (3H, s, OMe), 3.71 (3H, s, OMe), 4.36 (1H, q, J = 3.5 Hz, 4′-H), 5.10 (2H, s, $PhCH_2$), 5.40 (1H, q, J = 6.3 Hz, CH_3CHOTBDMS), 5.44 (1H, br d, J = 6.1 Hz, NH), 7.27–7.44 (6H, m, Ph and 6-H(Q)), 7.64–7.72 (2H, m, 7-H(Q) and 8-H(Q)), 8.09 (1H, br t, J = 8.5 Hz, 5-H(Q)); m/z (ES) 689.2979 (MNa^+). $C_{34}H_{46}N_4O_8NaSi$ requires 689.2983.

(2*S*)-2-Benzyloxycarbonylamino-6-methylene-heptanedioic acid. $LiOH{\cdot}H_2O$ (300 mg, 7.2 mmol) was added to a solution of **16** (0.98 g, 2.8 mmol) in 3 : 1 MeOH–H_2O (40 mL) at room temperature and stirred for 16 h. The mixture was concentrated *in vacuo*. The residue was taken up into H_2O and was then extracted with EtOAc. The aqueous layer was then acidified to pH 2 (1 M HCl) and was then extracted with EtOAc (×4). The organic fractions were collected, dried ($MgSO_4$) and concentrated *in vacuo* to give the title compound (0.77 g, 82%). $[\alpha]_D$ −2.5 (*c* 1.3, CH_3OH); ν_{max} (CH_2Cl_2 cast)/cm^{-1} 3033, 2957, 1709, 1630, 1530, 1420, 1235, 1062, 738, 698; δ_H (500 MHz, $CDCl_3$) 1.47–1.52 (2H, m, 4-H_2), 1.77–1.85 (2H, m, 3-H*H*), 2.31 (2H, br t, J = 7.0 Hz, 5-H_2), 4.44 (1H, q, J = 6.0 Hz, 2-H), 5.09 (2H, s, PhCH_2), 5.36 (1H, d, J = 8.0 Hz, NH), 5.63 (1H, s, C=C*H*H), 6.25 (1H, s, C=CH*H*), 7.28–7.34 (5H, m, Ph); δ_C (125 MHz, $CDCl_3$)23.7, 31.5, 53.6, 67.1, 128.1, 128.2, 128.5, 136.1, 139.1, 155.8, 172.2, 177.7; m/z (ES) calcd. for $C_{16}H_{19}NO_6Na$ 344.1105 (MNa^+), found 344.1105.

(2*S*)-2-Benzyloxycarbonylamino-6-methylene-heptanedioic acid 1-methyl ester 24. Polymer bound *p*-toluenesulfonic acid (30–60 mesh, 2.0–3.5 mmol g^{-1}, 700 mg) was added to a solution of (2*S*)-2-benzyloxycarbonylamino-6-methylene-heptanedioic acid (0.77 g, 2.4 mmol) in anhydrous MeOH (20 mL) at room temperature and shaken for 16 h. The solids were removed by filtration and washed extensively with MeOH. The resulting solution was concentrated *in vacuo* to give a 95 : 5 mixture of the title compound **24** and diester **16** (0.80 g, quant.) as a colourless oil. $[\alpha]_D$ +13.3 (*c* 0.5, $CHCl_3$); ν_{max}(CH_2Cl_2 cast)/cm^{-1} 3323, 2953, 1705, 1527, 1455, 1437, 1346, 1216, 1060, 754; δ_H(500 MHz, $CDCl_3$) 1.48–1.57 (2H, m, 4-CH_2), 1.63–1.68 (1H, m, 3-CH*H*), 1.82–1.85 (1H, m, 3-C*H*H), 2.28–2.32 (2H, m, 5-H_2), 3.72 (3H, s, OCH_3), 4.39 (1H, q, J = 5.5 Hz, C*H*N), 5.09 (2H, s, PhCH_2), 5.27 (1H, d, J = 7.5 Hz, NH), 5.62 (1H, s, C=C*H*H), 6.27 (1H, s, C=CH*H*), 7.28–7.34 (5H, m, Ph); δ_C (125 MHz, $CDCl_3$) 24.0, 30.9, 32.1, 52.4, 53.6, 67.0, 127.4, 128.1, 128.2, 128.5, 136.2, 139.2, 155.9, 171.7, 172.9; m/z (ES) calcd. for $C_{17}H_{21}NO_6Na$ 358.1263 (MNa^+), found 358.1261.

(2*S*)-2-Benzyloxycarbonylamino-6-methylene-heptanedioic acid 7-{(2*R*)-2-[1-(3-amino-4-oxo-3,4-dihydroquinazolin-2-yl)-ethoxy]-ethyl} ester 1-methyl ester 25. A solution of DCC (25 mg, 0.12 mmol) and DMAP (2 mg, 160 μmol) in CH_2Cl_2 (2 mL) was added to a solution of **22** (27 mg, 100 μmol) and monoester **24** (36 mg, 100 μmol) in CH_2Cl_2 (2 mL) at room temperature and was stirred for 1 h. The resulting precipitate was removed by filtration and the mixture was concentrated *in vacuo*. Purification by flash chromatography, eluting with 2 : 1 $CHCl_3$–EtOAc gave the title compound **25** (40 mg, 62%) as a colourless oil. $[\alpha]_D$ +7.8 (*c* 1.6, CH_2Cl_2); ν_{max}(CH_2Cl_2 cast)/cm^{-1} 3326, 2933, 1717, 1676, 1599, 1526, 1454, 1299, 1248, 1213, 1179, 1109, 1058, 775, 697; δ_H(500 MHz, $CDCl_3$) 1.49–1.56 (2H, m, 4-CH_2), 1.62–1.67 (1H, m, 3-CH*H*), 1.69 (3H, d, *J* = 6.5 Hz, CH_3CH), 1.81–1.87 (1H, m, 3-C*H*H), 2.26–2.31 (2H, m, 5-H_2), 3.72 (3H, s, OCH_3), 3.83 (2H, t, *J* = 4.5 Hz, $CO_2CH_2CH_2$), 4.31–4.34 (2H, m, $CO_2CH_2CH_2$), 4.34–4.40 (1H, m, C*H*N), 5.05 (1H, q, *J* = 6.5 Hz, CH_3CH), 5.10 (2H, s, PhCH_2), 5.42 (1H, d, *J* = 8.5 Hz, NH), 5.49 (2H, s, NH_2), 5.54 (1H, s, C=C*H*H), 6.11 (1H, s, C=CH*H*), 7.28–7.34 (5H, m, Ph), 7.47–7.50 (1H, m, ArC*H*), 7.74–7.75 (2H, m, ArC*H*), 8.26–8.28 (1H, m, ArC*H*); δ_C (125 MHz, $CDCl_3$) 16.8, 24.1, 29.8, 31.4, 32.2, 52.4, 53.8, 63.9, 67.1, 75.9, 120.3, 125.7, 126.5, 127.1, 127.9, 128.2, 128.6, 134.1, 136.3, 139.7, 146.4, 153.9, 156.0, 160.6, 166.8, 172.9; *m*/*z* (ES) calcd. for $C_{29}H_{35}N_4O_8$ 567.2449 (M$^+$), found 567.2446.

(2*S*)-2-Benzyloxycarbonylamino-5-[(1*R*,7*R*)-7-methyl-2,11-dioxo-4,5,7,15-tetrahydro-3,6-dioxa-8,15*a*,15*b*-triaza-naphthyl[*a*]cyclopropa[*c*]cyclodecen-1*a*-yl]pentanoic acid methyl ester 26. $Pb(OAc)_4$ (147 mg, 0.33 mmol) was added to a solution of the alkene **25** (183 mg, 0.32 mmol) and HMDS (110 μL, 0.52 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The mixture was then stirred for 15 min. The resulting mixture was purified by passage through a plug of silica, eluting with 1 : 1 EtOAc–CH_2Cl_2 to give the title compound **26** (157 mg, 86%) as a white foam. $[\alpha]_D$ −28.6 (*c* 1.6, CH_2Cl_2); ν_{max}(CH_2Cl_2 cast)/cm^{-1} 3336, 2951, 1738, 1682, 1592, 1526, 1273, 1217, 1172, 736, 697; δ_H (500 MHz, CD_2Cl_2) 1.49 (3H, d, *J* = 6.0 Hz, CH_3CH), 1.55–1.56 (2H, m, 4-CH_2), 1.74–1.77 (1H, m, 3-CH*H*), 1.91–1.92 (1H, m, 3-C*H*H), 2.64–2.67 (2H, m, 5-H_2), 3.13 (1H, bs, CH*H*N), 3.29 (1H, bs, C*H*HN), 3.65 (1H, m, CO_2CH_2CHH), 3.74 (3H, s, OCH_3), 3.92–3.97 (1H, m, CO_2CH_2CHH), 4.04–4.10 (1H, m, CO_2CHHCH_2), 4.38–4.41 (1H, m, C*H*N), 4.82–4.86 (1H, m, CO_2CHHCH_2), 5.10 (2H, s, PhCH_2), 5.14 (1H, q, *J* = 6.0 Hz, CH_3CH), 5.38 (1H, d, *J* = 8.0 Hz, NH), 7.30–7.37 (5H, m, Ph), 7.45–7.48 (1H, m, ArC*H*), 7.65–7.67 (1H, m, ArC*H*), 7.71–7.74 (1H, m, ArC*H*), 8.17–8.19 (1H, m, ArC*H*); δ_C (125 MHz, CD_2Cl_2) 21.3, 32.2, 33.0, 45.9, 52.7, 56.1, 64.4, 67.3, 68.2, 72.1, 122.0, 126.7, 127.3, 127.6, 128.3, 128.5, 128.9, 134.3, 146.0, 155.4, 160.0, 166.8, 173.0; *m*/*z* (ES) calcd. for $C_{29}H_{35}N_4O_8$ 565.2293 (M$^+$), found 565.2299.

(2*S*)-2-[(4*S*)-4-Amino-4-carboxybutyl]aziridine-2-carboxylic acid (LL-azi-DAP) 14. A solution of Na_2CO_3 (55 mg, 0.56 mmol) in H_2O (3 mL) was added to a solution of aziridine **26** (72 mg, 0.13 mmol) in MeOH (9 mL) at room temperature. The mixture was then stirred for 48 h and then concentrated *in vacuo*. Pre-distilled ammonia (10 mL) was added to a flask and the reaction mixture was cooled to −78 °C. Lithium was added portionwise until a deep blue colour persisted. After 5 min the reaction mixture was quenched by the addition of solid NH_4Cl. The reaction mixture was warmed to room temperature and the ammonia was allowed to evaporate under an atmosphere of argon. Purification by preparative TLC (Uniplate™ silica gel HLF plates with organic binder, 5% aq NH_4OH in MeOH, products removed from silica using 20% aq NH_4OH in MeOH) was followed by concentration *in vacuo*. The residue was dissolved in water (1 mL) and filtered through a 0.45 μm filter to remove any remaining silica. Concentration *in vacuo* gave the title compound **14** (8 mg, 30%) as a white solid. $[\alpha]_D$ +3.3 (*c* 0.40, H_2O); ν_{max}(H_2O cast)/cm^{-1} 3205, 3057, 1628, 1415, 1114; δ_H (400 MHz, D_2O) 1.09–1.15 (1H, m, $CHCH_2CHH$), 1.47–1.57 (2H, m, $CHCH_2CHH$, $CHCHH$) 1.61 (1H, bs, CH*H*N), 1.80–1.90 (2H, m, $CHCH_2$), 1.92 (1H, bs, C*H*HN), 2.15–2.21 (1H, m, CC*H*H), 3.65–3.71 (1H, m, C*H*N); δ_C (100 MHz, D_2O) 21.8, 31.6, 32.3, 55.0, 62.8, 175.8, 179.8; *m*/*z* (ES) calcd. for $C_8H_{13}N_2O_4$ 201.0870 (M$^+$), found 201.0869.

(2*S*)-2-Benzyloxycarbonylamino-6-methylene-heptanedioic acid 7-{(2*S*)-2-[1-(3-amino-4-oxo-3,4-dihydroquinazolin-2-yl)-ethoxy]-ethyl} ester 1-methyl ester 27. Polymer bound *N*-benzyl-*N*′-cyclohexylcarbodiimide (1.3 mmol g^{-1}, 7.5 g) and DMAP (70 mg, 0.57 mmol) were added to a solution of **23** (760 mg, 3.0 mmol) and monoester **24** (678 mg, 2.0 mmol) in CH_2Cl_2 (60 mL) at room temperature and was shaken for 48 h. The resin was removed by filtration was washed extensively with CH_2Cl_2. The solvent was removed *in vacuo* and the residue was purified by passage through a plug of silica, eluting with 1 : 1 EtOAc–hexane to give the title compound **27** (588 mg, 51%) as a colourless oil. $[\alpha]_D$ −2.3 (*c* 4.7, CH_2Cl_2); ν_{max} (CH_2Cl_2 cast)/cm^{-1} 3324, 2927, 1716, 1674, 1623, 1599, 1530, 1246, 1213, 1180, 1109, 1058, 775, 736, 697; δ_H(500 MHz, $CDCl_3$)1.46–1.54 (2H, m, 4-CH_2), 1.62–1.67 (1H, m, 3-CH*H*), 1.68 (3H, d, *J* = 7.0 Hz, CH_3CH), 1.80–1.82 (1H, m, 3-C*H*H), 2.26–2.31 (2H, m, 5-H_2), 3.72 (3H, s, OCH_3), 3.82 (2H, t, *J* = 4.5 Hz, $CO_2CH_2CH_2$), 4.30–4.34 (2H, m, $CO_2CH_2CH_2$), 4.35–4.39 (1H, m, C*H*N), 5.06 (1H, q, *J* = 6.5 Hz, CH_3CH), 5.09 (2H, s, PhCH_2), 5.42 (3H, bs, NH, NH_2), 5.53 (1H, s, C=C*H*H), 6.10 (1H, s, C=CH*H*), 7.28–7.34 (5H, m, Ph), 7.45–7.48 (1H, m, ArC*H*), 7.73–7.74 (2H, m, ArC*H*), 8.24–8.26 (1H, m, ArC*H*); δ_C (125 MHz, $CDCl_3$) 16.8, 24.0, 31.3, 32.1, 33.9, 53.87, 63.8, 67.0, 75.8, 120.2, 125.6, 126.4, 127.0, 127.8, 128.1, 128.5, 134.1, 136.3, 139.6, 146.2, 153.9, 160.5, 166.7, 172.8; *m*/*z* (ES) calcd. for $C_{29}H_{35}N_4O_8$ 567.2449 (M$^+$), found 567.2449.

(2*S*)-2-Benzyloxycarbonylamino-5-[(1*R*,7*S*)-7-methyl-2,11-dioxo-4,5,7,15-tetrahydro-3,6-dioxa-8,15*a*,15*b*-triaza-naphthyl-[*a*]cyclopropa[*c*]cyclodecen-1*a*-yl]pentanoic acid methyl ester 28. $Pb(OAc)_4$ (442 mg, 1.0 mmol) was added to a solution of alkene **27** (545 mg, 0.96 mmol) and HMDS (0.35 mL, 1.7 mmol) in CH_2Cl_2 (25 mL) at 0 °C. The mixture was then stirred for 15 min. The resulting mixture was then purified by passage through a plug of silica, eluting with 1:1 EtOAc/CH_2Cl_2 to give the title compound **28** (300 mg, 55%) as a white foam. $[\alpha]_D$ + 191.2 (*c* 3.8, CH_2Cl_2); ν_{max} (CH_2Cl_2 cast)/cm^{-1} 3330, 2953, 1738, 1682, 1592, 1526, 1273, 1216, 1171, 736, 697; δ_H (500 MHz, CD_2Cl_2) 1.49 (3H, d, *J* = 6.0 Hz, CH_3CH), 1.55–1.60 (2H, m, 4-CH_2), 1.74–1.76 (1H, m, 3-CH*H*), 1.93–1.95 (1H, m, 3-C*H*H), 2.67–2.72 (2H, m, 5-H_2), 3.13 (1H, d, *J* = 3.5 Hz, CH*H*N), 3.29 (1H, dd, *J* = 3.5, 1.0 Hz, C*H*HN), 3.63–3.67 (1H, m, CO_2CH_2CHH), 3.74 (3H, s, OCH_3), 3.91–3.95 (1H, m, CO_2CH_2CHH), 4.10–4.14 (1H, m, CO_2CHHCH_2), 4.39–4.40 (1H, m, C*H*N), 4.84–4.87 (1H, m, CO_2CHHCH_2), 5.10 (2H, s, PhCH_2), 5.16 (1H, q, *J* = 6.5 Hz, CH_3CH), 5.42 (1H, d, *J* = 8.0 Hz, NH), 7.30–7.39 (5H, m, Ph), 7.45–7.48 (1H, m, ArC*H*), 7.65–7.67 (1H, m, ArC*H*), 7.71–7.74 (1H, m, ArC*H*), 8.17–8.19 (1H, m, ArC*H*); δ_C (125 MHz, CD_2Cl_2) 21.3, 32.3, 32.8, 46.0, 52.6, 56.1, 64.4, 67.3, 68.2, 72.1, 122.0, 126.7, 127.3, 127.6, 128.3, 128.5, 128.9, 134.3, 146.0, 155.4, 160.0, 166.8, 173.0; *m*/*z* (ES) calcd. for $C_{29}H_{35}N_4O_8$ 565.2293 (M$^+$), found 565.2292.

(2*R*)-2-[(4*S*)-4-Benzyloxycarbonylamino-4-carboxybutyl]-1-{2-[(1*S*)-1-(2-hydroxy-ethoxy)-ethyl]-4-oxo-4*H*-quinazolin-3-yl}-aziridine-2-carboxylic acid. A solution of Na_2CO_3 (108 mg, 1.1 mmol) in H_2O (4 mL) was added to a solution of aziridine **28** (140 mg, 0.25 mmol) in MeOH (12 mL) at room temperature. The mixture was then stirred for 48 h and then concentrated *in vacuo*. The residue was taken up into H_2O (50 mL) and purified by RP-HPLC [Gracevydac reverse phase polymer column (259VHP810), eluting with 9:1

i-PrOH/50 mM NH_4OH, detection at 220 nm, 450 μL injection volume of 2 mg mL^{-1} solution, flow rate 1 mL min^{-1}, t_R 7.2 min, (see supporting information)] to give the title compound (97 mg, 69%) as a white solid. $[\alpha]_D$ + 124.0 (*c* 2.1, MeOH); δ_H (500 MHz, CD_3OD) 1.44–1.48 (2H, m, 4-CH_2), 1.55 (3H, d, J = 6.0 Hz, CH_3CH), 1.60–1.64 (1H, m, 3-CH*H*), 1.72–1.74 (1H, m, 3-CH*H*), 1.89–1.93 (1H, m, 5-C*H*H), 2.56–2.61 (1H, m, 5-H_2), 2.81 (1H, d, J = 2.0 Hz, CH*H*N), 3.63 (1H, bs, C*H*HN), 3.69–3.81 (4H, m, OCH_2CH_2OH), 4.06–4.08 (1H, m, C*H*N), 5.06 (2H, s, PhCH_2), 5.16 (1H, q, J = 6.5 Hz, CH_3C*H*), 7.30–7.36 (5H, m, Ph), 7.45–7.51 (1H, m, ArC*H*), 7.63–7.64 (1H, m, ArC*H*), 7.70–7.73 (1H, m, ArC*H*), 8.11–8.13 (1H, m, ArC*H*); δ_C (125 MHz, CD_3OD) 19.7, 22.9, 33.9, 34.4, 48.5, 62.8, 67.4, 68.2, 71.6, 74.2, 122.8, 127.2, 127.4, 128.8, 128.9, 129.4, 134.8, 138.5, 147.0, 158.1, 159.9, 161.6, 172.2 179.3; *m/z* (ES) calcd. for $C_{28}H_{32}N_4O_9Na$ 591.2062 (MNa^+), found 591.2068.

(2*R*)-2-[(4*S*)-4-amino-4-carboxybutyl]aziridine-2-carboxylic acid (DL-azi-DAP) 29 and (2*S*)-2-amino-6-methyl-heptanedioic acid 18. Pre-distilled ammonia (10 mL) was added to a flask containing (2*R*)-2-[(4*S*)-4-benzyloxycarbonylamino-4-carboxybutyl]-1-{2-[(1*S*)-1-(2-hydroxy-ethoxy)-ethyl]-4-oxo-4*H*-quinazolin-3-yl}-aziridine-2-carboxylic acid (25 mg, 44 μmol) and the reaction mixture was cooled to −78 °C. Lithium was added portionwise until a deep blue colour persisted. After 5 min the reaction mixture was quenched by the addition of solid NH_4Cl (10 mg). The reaction mixture was warmed to room temperature and the ammonia was allowed to evaporate under a stream of argon. Purification by prep TLC (Uniplate™ silica gel HLF plates with organic binder, 5% aq NH_4OH in MeOH, products removed from silica using 20% aq NH_4OH in MeOH) was followed by concentration *in vacuo*. The residue was dissolved in water (1 mL) and filtered through a 0.45 μm filter to remove any remaining silica. Concentration *in vacuo* gave the title compounds **29** (2.5 mg, 28%) and **18** (~0.5 mg) as white solids. aziridine **29**: R_f 0.52; $[\alpha]_D$ − 11.3 (*c* 0.35, H_2O); ν_{max}(H_2O cast)/cm^{-1} 3401, 2940, 1628, 1411, 1348, 1197, 1147, 1122; δ_H (500 MHz, D_2O) 1.24–1.29 (1H, m, CHCH_2C*H*H), 1.53–1.58 (1H, m, CHCH_2CH*H*). 1.81–1.90 (2H, m, CHCH_2), 1.92 (1H, bs, CH*H*N), 2.19 (1H, bs, C*H*HN), 2.20–2.22 (1H, m, CC*H*H), 3.71–3.73 (1H, m, C*H*N); δ_C (125 MHz, D_2O) 21.8, 30.5, 31.7, 31.9, 54.9, 62.7, 175.9, 177.7; *m/z* (ES) calcd. for $C_8H_{13}N_2O_4$ 201.0870 (M^+), found 201.0870.

Acid **18** as a mixture of diastereomers: R_f 0.61; ν_{max}(microscope)/cm^{-1} 3050, 2940, 1693, 1583, 1513, 1441, 1408; δ_H (300 MHz, D_2O) 1.10 (3H, d, J = 11.5 Hz, CH_3), 1.32–1.51 (3H, m, C*H*H, CH_2), 1.58–1.65 (1H, m, CH*H*), 1.81–1.92 (2H, m, CH_2), 2.38–2.46 (1H, m, C*H*CH$_3$), 3.73 (1H, t, J = 6.5 Hz, C*H*N), δ_C (100 MHz, D_2O) 17.8 & 17.9, 23.2 & 23.3, 31.2 & 31.2, 33.9, 41.6 & 41.7, 55.5 & 55.6, 175.7, 185.1; *m/z* (ES) calcd. for $C_8H_{14}NO_4$ 188.0917 (M^+), found 188.0918.

L,L-Diaminopimelic acid 1

A solution of (2*S*)-4-[(4*S*)-4-benzyloxycarbonylamino-4-methoxycarbonyl-butyrylperoxy)-2-*tert*-butoxycarbonylamino-4-oxo-butyric acid *tert*-butyl ester[40] (92 mg, 0.19 mmol) in Et_2O (5 mL) was added to 6 M HCl (5 mL) and the mixture was then heated to 90 °C for 2 h with stirring. The resulting aqueous solution was concentrated *in vacuo* and purified by ion-exchange chromatography (Biorad AG 50W-X8 hydrogen form resin), loading with distilled H_2O, flushing for 6 column lengths with distilled H_2O and then eluting with 10% NH_4OH to give the title compound **1** (28 mg, 79%) as a white powder after lyophilisation. $[\alpha]_D$ + 2.2 (*c* 2.0, H_2O); ν_{max}(microscope)/cm^{-1} 2942, 1582, 1465, 1436, 1413, 1350, 1327, 1103; δ_H (500 MHz, D_2O) 1.39–1.45 (2H, m, 4-CH_2), 1.84–1.89 (4H, m, 3, 5-CH_2), 4.06–4.08 (2H, t, J = 6.0 Hz, 2 × C*H*N), δ_C (125 MHz, D_2O) 20.9, 30.9, 55.2, 175.4; *m/z* (ES) calcd. for $C_7H_{15}N_2O_4$ 191.1026 (M^+), found 191.1026.

Inhibition Studies with DAP Epimerase Using Coupled Assay

DAP epimerase activity was monitored using a coupled assay with DAP dehydrogenase which detects the production of NADPH at 340 nm. The assay was performed in a 1 mL quartz cuvette filled with buffer solution (0.1 M tris-HCl, 1 mM EDTA, 1 mM DTT, pH 7.8), 0.4 mM L,L-DAP, 0.3 mM $NADP^+$ and 0.06 units of DAP dehydrogenase. One unit of DAP epimerase activity corresponds to the production of one μmol of NADPH per minute. For inhibition studies with aziridine analogue **4**, the assay buffer contained varying concentrations of the aziridine, 0.4 mM L,L-DAP, 0.3 mM $NADP^+$, 50 mU of DAP dehydrogenase and 15 mU of DAP epimerase. These results were compared against a control performed simultaneously, where the Tris-HCl buffer replaced the inhibitor. The DAP epimerase used in the study of aziridines **4** and **11** was isolated from *Escherichia coli* mutant BL21(DE3) pLysS using a modified procedure[7,39] and DAP dehydrogenase was purified from *Bacillus sphaericus* IFO 3525 as previously reported.[62] The DAP epimerase used in the study of azi-DAP was isolated from *Haemophilus influenza* as described previously.[15]

Labeling of DAP Epimerase with azi-DAP

A solution of azi-DAP in water (1–5 mg mL^{-1}) was added in excess (10–100 equiv) to a solution of DAP epimerase (0.54 mg mL^{-1}) in buffered solution (0.1 M tris-HCl, 1 mM EDTA, 1 mM DTT, pH 7.8) at room temperature and allowed to stand for 16 h. An aliquot (50 μL) was removed and added to a 10 mM solution of DTT in ammonium bicarbonate (50 μL, pH 8) and the mixture was heated at 37 °C for 30 min. An aliquot was removed and purified by RP-HPLC using a Jupiter™ reverse phase 1 mm Microbore column (5 μ, C18, 300 Å), eluting with a linear gradient over 50 min from 0.05% aqueous TFA to 0.05% TFA in MeCN, column temperature 70 °C, detection at 210 nm, injection volume 50 μL, flow rate 100 μL min^{-1}, t_R 29 min.

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