Synthesis and Evaluation of Novel Substrates and Inhibitors of *N*-Succinyl-LL-diaminopimelate Aminotransferase (DAP-AT) from *Escherichia coli*

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Abstract: *N*-Succinyl-LL-diaminopimelate aminotransferase (DAP-AT) (EC 2.6.1.17), a key enzyme in the bacterial pathway to L-lysine, was purified to near homogeneity (1500-fold) in five steps from wild type *Escherichia coli* ATCC 9637. This pyridoxal phosphate (PLP) dependent enzyme has a molecular weight of 39.9 kDa, appears to form an active homodimer, and uses L-glutamate as the amino group donor for its substrate, *N*-succinyl-α-amino-ϵ-ketopimelic acid (**1a**) (K_m = 0.18 ± 0.04 mM, k_{cat} = 86 ± 5 s^{-1}). Progress of the reaction is monitored by spectrophotometric observation of decrease in NADPH concentration at 340 nm in a coupled enzyme assay with L-glutamate dehydrogenase (EC 1.4.1.4). Stereochemically pure **1a** was synthesized as its trilithium salt by ene reaction of methyl glyoxylate with methyl *N*-succinyl-L-allylglycinate (**4a**) followed by hydrogenation of the double bond, Dess−Martin oxidation of the alcohol, and careful lithium hydroxide hydrolysis. Similar approaches allowed synthesis of a series of substrate analogues **1b**−**g** having different *N*-acyl substituents, as well as derivatives missing the carboxyl group or the amide functionality (**13** and **17**, respectively). Compounds lacking the keto functionality (**18a**, **18c**, and **19**) were also prepared. Assay of DAP-AT shows that the enzyme has quite strict requirements for substrate recognition, but it will accept compounds with an aromatic ring in place of the terminal succinyl carboxyl group in **1a** (e.g. *N*-Cbz-α-amino-ϵ-ketopimelic acid (**1c**)). Reaction of substrates **1a**,**c** with hydrazine hydrate followed by $NaCNBH_3$ reduction gives 2-(*N*-(succinylamino))- (**20a**) and 2-(*N*-Cbz-amino)-6-hydrazinoheptane-1,7-dioic acids (**20c**), respectively. These are the most potent slow-binding inhibitors of any DAP-metabolyzing enzyme reported so far (K_i* for DAP-AT: **20a** is 22 ± 4 nM; **20c** is 54 ± 9 nM).

Introduction

The increased incidence of pathogenic bacteria which are resistant to conventional antibiotics currently used in treatment of infectious disease continues to stimulate interest in alternative strategies to disrupt microbial cell wall synthesis and thereby inhibit their growth.[1] The biosynthesis of the peptidoglycan layer in bacterial cells provides numerous targets for further antibiotic development.[2] The key cross-linking amino acid in this essential structural polymer can be variable but is usually *meso*-diaminopimelic acid (*meso*-DAP) in Gram negative bacteria and its biosynthetic product, L-lysine, in Gram positive organisms. Since mammals lack the DAP biosynthetic pathway and require L-lysine in their diet,[3] there has been considerable interest in specific inhibition of enzymes enroute to these metabolites.[4,5] Some of the major obstacles to antibiotic development emerging from these studies include unexpectedly high specificity of some enzymes for the structural features inherent in the substrates,[5b] the ability of key enzymes to transform certain inhibitors into normal metabolic intermediates,[6] ineffective transport into cells, and the presence of alternative routes to *meso*-DAP.

L-Lysine is formed in bacteria via three main avenues from L-tetrahydrodipicolinic acid (L-THDP) (Figure 1).[4] In *E. coli*, and many other bacteria, succinylation of L-THDP affords *N*-succinyl-α-amino-ϵ-ketopimelic acid,[7,8] which after transamination by *N*-succinyl-LL-DAP aminotransferase (EC 2.6.1.17),[9] gives *N*-succinyl-LL-DAP. Desuccinylation yields LL-DAP,[10]

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(7) For a summary of early work on the *E. coli* L-lysine pathway see: (a) Gilvarg, C. *Meth. Enzymol.* **1962**, *5*, 848−850. (b) Kindler, S. H. *Meth. Enzymol.* **1962**, *5*, 851−853. (c) Peterkofsky, B. *Meth. Enzymol.* **1962**, *5*, 853−858. (d) Work, E. *Meth. Enzymol.* **1962**, *5*, 858−864. (e) Work, E. *Meth. Enzymol.* **1962**, *5*, 864−870. (f) Berges, D. A.; DeWolf, W. E.; Dunn, G. L.; Newman, S. J.; Schmidt, J.; Taggart, J.; Gilvarg, C. *J. Biol. Chem.* **1986**, *261*, 6160−6167.

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S0002-7863(96)00640-3 CCC: $12.00

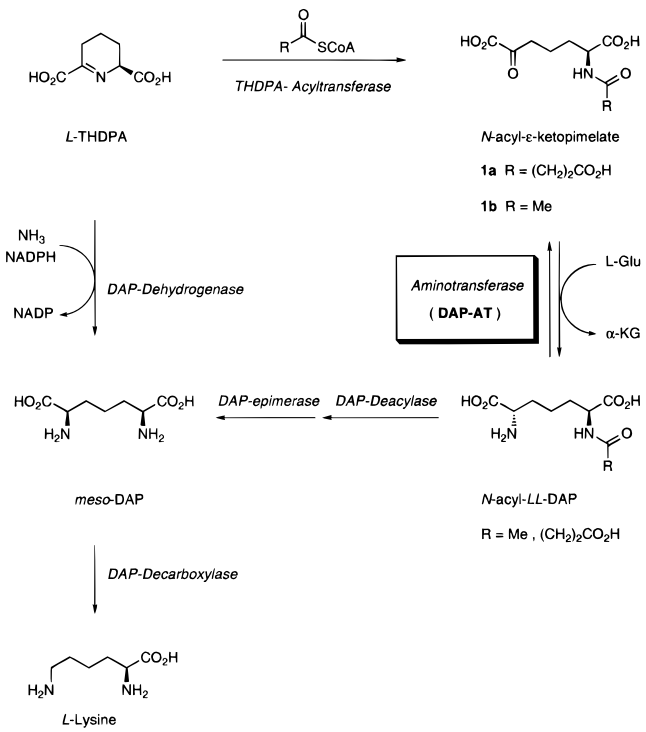

Figure 1. Biochemical routes to L-lysine in bacteria.

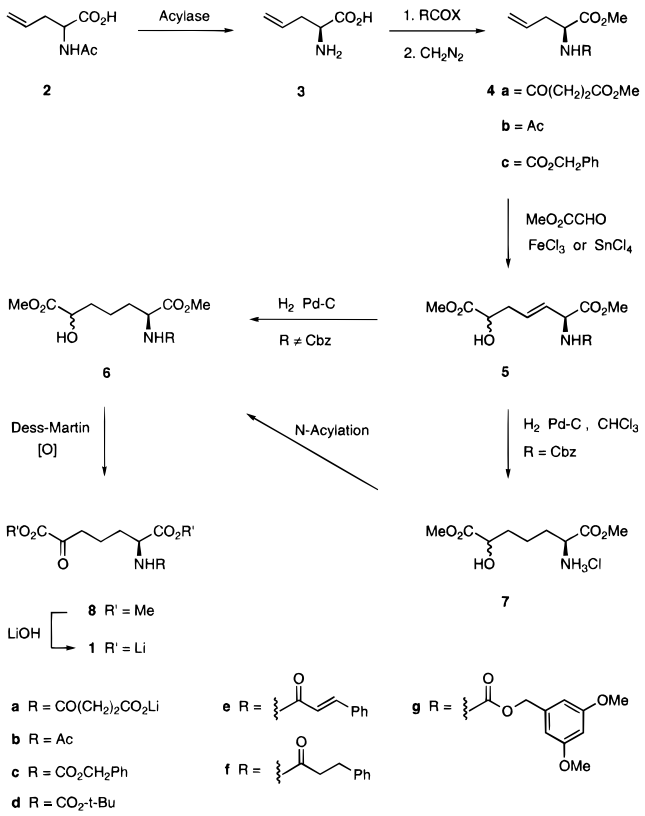

Figure 2. Syntheses of DAP-AT substrates **1a**−**g**. See Table 1 for yields.

and epimerization then generates *meso*-DAP.[11] Certain bacteria such as *Bacillus megaterium* and *B. subtilis* utilize N-acetylated intermediates instead of the N-succinylated versions,[12] whereas others such as *Bacillus sphaericus* reductively aminate L-THDPA directly in a single NADPH dependent step catalyzed by DAP dehydrogenase to generate *meso*-DAP directly.[13] Dual pathways are known to occur in *B. macerans* , which uses both the N-acetylated route and DAP dehydrogenase,[14] and also in *Mycobacterium bovis*[15] and *Corynebacterium glutamicum*,[16] both of which have the enzymes required for the N-succinylated route as well as the DAP dehydrogenase.

Despite extensive study of the enzymes and genes involved in DAP biosynthesis in bacteria and higher plants,[3,4,17] detailed examination of *N*-succinyl-LL-DAP aminotransferase has not been described since Gilvarg and co-workers achieved partial purification in the 1960s.[9] One of the difficulties has been the lack of an efficient chemical synthesis of pure substrate, *N*-succinyl-α-amino-ϵ-ketopimelic acid (**1a**), and its analogues. As part of our continuing effort to develop antimicrobial inhibitors of the DAP pathway,[5a,b,6] we now describe the chemical synthesis of pure substrates, the purification of *N*-succinyl-LL-DAP aminotransferase (EC 2.6.1.17) (DAP-AT) to apparent homogeneity, and substrate specificity studies, as well as the synthesis and testing of potent inhibitors of this enzyme.[18]

Results and Discussion

Synthesis of (*S*)-*N*-Succinyl-α-amino-ϵ-ketopimelic Acid (1a) and Substrate Analogues. The intermolecular ene reaction provides a convenient route to functionalized α-aminopimelic acid derivatives by condensation of protected allylglycines with methyl glyoxylate.[19] We initially sought to synthesize the pure L enantiomers (*S* configuration) of both *N*-succinyl and *N*-acetyl substrates **1a**,**b** in order to compare their activities with purified *N*-succinyl-LL-DAP aminotransferase (DAP-AT). Racemic *N*-acetyl allylglycine (**2**) is readily resolved with pork kidney acylase by the literature procedure,[20] with modified purification using cation exchange chromatography to generate L-allylglycine (**3**) in >99% enantiomeric excess and superior yield (>90%) (Figure 2). Acylation with either succinic or acetic anhydride in aqueous base, followed by methyl ester formation with diazomethane, and ene reaction of the resulting protected derivatives **4a,b** with methyl glyoxylate (obtained by oxidation of dimethyl tartrate) under Lewis acid conditions ($FeCl_3$) at room temperature affords the ene adducts **5a,b** (1:1 mixture of diastereomers) (Table 1). Catalytic hydrogenation proceeds smoothly to give the saturated hydroxy esters **6a,b**. Although many standard oxidation methods (e.g. Swern,[21] H_2CrO_4/SiO_2,[22] $(^nPr)_4RuO_4/NMO$,[23] $MnO_4/CuSO_4$[24])

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(22) Santaniello, E.; Ponti, F.; Manzocchi, A. *Synthesis* **1978**, 534−535.

Table 1. Isolated % Yields of Reaction Products[a]

	reacn, product				
	ene reacn, **5**	redn, **6**	acylation, **6**	oxidn, **8**	hydrolysis, **1**
a	47	91		79	99
b	46	95		70	92
c	65	99 (**7**)[b]	84	56	98
d			80	13[c]	99
e			56	64	99
f			83	60	99
g			58	63	95

[a] See Figure 2 for structures and Experimental Section for procedures. [b] Reduction removes Cbz group. [c] Not optimized.

proceed in unexpectedly low yield, a 1.2−1.5 fold excess of the Dess−Martin periodinane[25] readily affords the desired α-keto esters **8a,b**. Careful hydrolysis with 1.0 equiv/ester moiety of lithium hydroxide hydrate in 50/50 water/acetonitrile quantitatively generates the pure lithium salts of **1a,b** as stable compounds. Acidification of **1a** (e.g. pH 4) results in immediate decomposition.

Slight modification of this procedure permits preparation of a variety of *N*-acyl derivatives **1c**−**g.** Ene reaction of the N-Cbz analogue **4c** requires use of $SnCl_4$ instead of $FeCl_3$, and hydrogenation of the adduct **5c** in the presence of Boc anhydride gives the Boc-protected compound **6d** in 80% yield. Alternatively, hydrogenation of **4c** in ca. 10% $CHCl_3$ in methanol without an acylating agent present produces the expected amino alcohol as its hydrochloride salt **7** in quantitative yield. Selective N-acylation using standard conditions in the presence of pyridine followed by oxidation yields the amides **8c**−**g**, which are transformed to the corresponding substrate analogues (as lithium salts) **1c**−**g** as above.

To test the optical purity of these compounds, the lithium salts of the α-keto acids **1a**,**c** were reductively aminated[26] using ammonium acetate and sodium cyanoborohydride in methanol to produce N-protected DAP derivatives (Figure 3). Deprotection by hydrogenolysis or acid-catalyzed hydrolysis afforded mixtures of DAP stereoisomers which were converted to bis-Marfey derivatives[27] and analyzed by reverse phase HPLC. Comparison of samples obtained from **1a**,**c** with those derived from commercial DAP (statistical mixture of stereoisomers) shows the presence of only LL- and *meso*-DAP isomers in equal amounts. The lack of a detectable level of *DD*-DAP indicates that the optical purity at the 2-position is >99% and that there is no significant diastereoselectivity during the reductive amination.

In order to determine structural requirements for substrate recognition by the aminotransferase (DAP-AT), analogues missing the 2-carboxyl group (i.e. **13**), the 2-amido group (i.e. **17**), or the 6-keto group (i.e. **18a**, **18c**, **19**) were also synthesized (Figure 4). Treatment of 3-butenylamine with succinic anhydride followed by diazomethane generates **9**, which is readily transformed by the ene procedure with functional group manipulation to **13**. Similarly, ene condensation of diethyl 2-allyl-1,7-heptanedioate with methyl glyoxylate followed by hydrogenation in methanol affords a partially transesterified mixture **15**, which after oxidation and basic hydrolysis gives

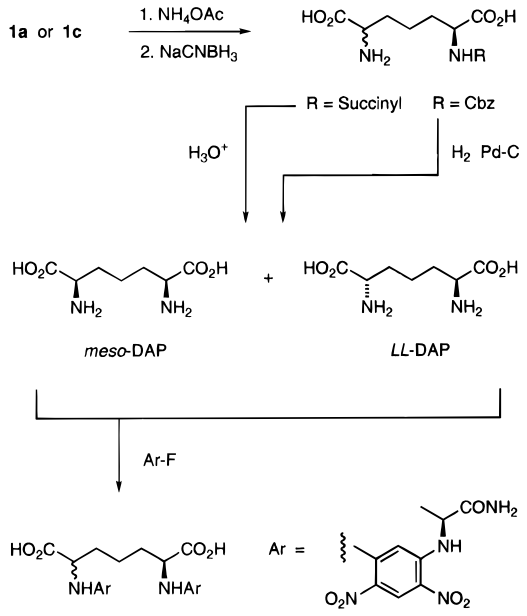

Figure 3. Stereochemical analysis of **1a**,**c** by formation of Marfey derivatives for HPLC.

17. Direct acylation of racemic α-aminopimelic acid provides access to **18a**,**c**, whereas hydrolysis of **6a** gives **19**.

Assay, Purification, and Substrate Specificity of DAP Aminotransferase. The availability of the natural substrate **1a** allows utilization of an efficient continuous assay for the pyridoxal phosphate (PLP) dependent DAP-AT through coupling of the "forward" reaction with L-glutamate dehydrogenase (EC 1.4.1.4) (Figure 5).[28] The assay mixture contains the substrate **1a**, PLP, L-glutamate as the amino group donor for the transamination, L-glutamate dehydrogenase, NH_4Cl, and NADPH. The α-ketoglutarate formed in the DAP-AT reaction is rapidly transformed back to L-glutamate by the dehydrogenase with the concomitant consumption of 1 equiv of NADPH. The progress of the reaction can be monitored by spectrophotometric observation of decrease in NADPH concentration at 340 nm.

Purification of DAP-AT from wild type *E. coli* requires five chromatographic steps (Table 2). Precipitation methods are relatively ineffective for concentrating the enzyme, but dye affinity chromatography in the "negative" mode to extract out unwanted proteins followed by "positive" mode dye affinity to bind the aminotransferase affords efficient partial purification. Hydroxylapatite chromatography, gel filtration, and HPLC anion exchange on Mono-Q gives nearly pure DAP-AT (by SDS PAGE) with an overall purification of 1500-fold.[29] MALDI TOF mass spectra and SDS PAGE indicate a subunit molecular weight of 39.9 kDa, whereas calibrated gel filtration chromatography suggests an approximate molecular weight of 82 kDa. The enzyme is therefore likely to be dimeric in its active form. Michaelis and kinetic constants obtained for the pure enzyme (for **1a**, $K_m = 0.18 \pm 0.04$ mM, $k_{cat} = 85 \pm 5$ s^{-1}, (cf. Table 3); for L-glutamate (Tris buffer), $K_m = 1.21 \pm 0.14$ mM) compare favorably with those obtained by Gilvarg and Pe-

(23) Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625−1627.

(24) Menger, F. M.; Lee, C. *J. Org. Chem.* **1979**, *44*, 3446−3448.

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(28) The procedure is a modification of that initially developed by Gilvarg and Peterkofsky: see refs 7 and 9b.

(29) Literature values for other DAP pathway enzyme purifications: (a) L-THDPA succinyltransferase 1900-fold from *E. coli*; see ref 8. (b) DAP desuccinylase (EC 3.5.1.18) 7100-fold from *E. coli*; see ref 10b. (c) DAP epimerase (EC 5.1.1.7) 6000-fold from *E. coli*; see ref 11. (d) DAP dehydrogenase (EC 1.4.1.16) 290-fold from *B. sphaericus*; see ref 13b. (d) DAP decarboxylase (EC4.1.1.20) 500-fold from *B. sphaericus*: Asada, Y.; Tanizawa, K.; Kawabata, Y.; Misono, H.; Soda, K. *Agric. Biol. Chem.* **1981**, *45*, 1513−1514. Andersen, Å. B.; Hansen, E. B. *Gene* **1993**, *124*, 105−109.

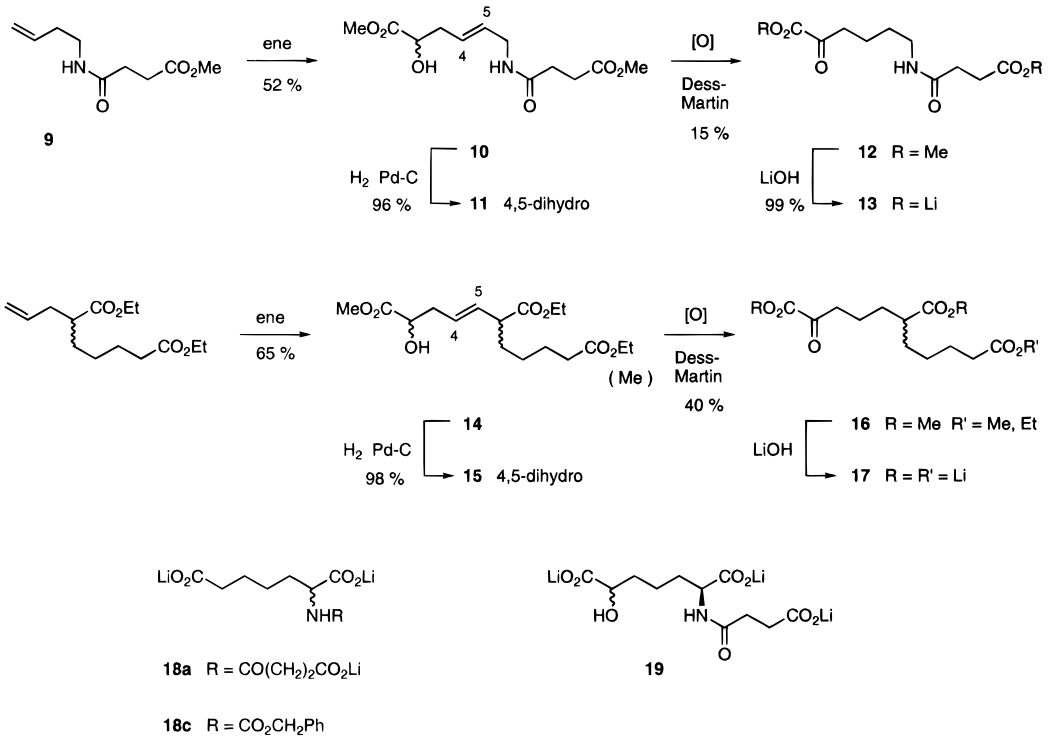


Figure 4. Syntheses of DAP-AT substrate analogues 13 and 17 and structures of **18a**,**c** and **19**.

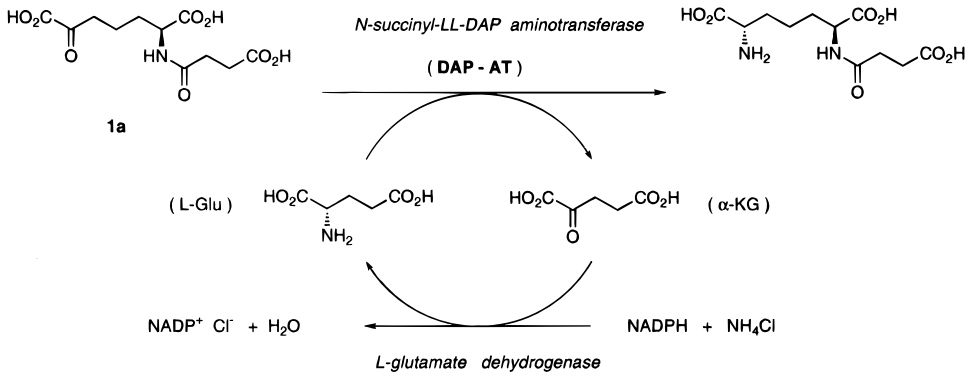


Figure 5. Assay of DAP-AT by coupled reaction with L-glutamate dehydrogenase.

Table 2. Purification of DAP-AT from *E. coli* ATCC 9637

step	% activity recovery for step	specific activity, units/mg[a]	fold purification for step	cumulative purification
crude *E. Coli* prep	-	0.089	-	1.0
brown-10 affinity column	49%	0.121	1.4	1.4
blue 3-GA affinity column	33%	0.282	2.3	3.3
hydroxyl apatite column	90%	1.60	5.7	18.6
Sephadex G-75 column	86%	3.37	2.1	39.4
Mono-Q anion exchange	95%	129	38.3	1508

[a] Enzyme unit defined as 1.0 μmol of L-*N*-succinyl-α-amino-ϵ-ketopimelate (**1a**) transformed/min at 30 °C.

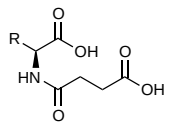
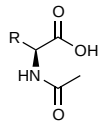
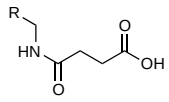
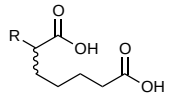
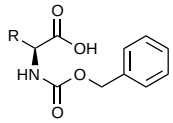
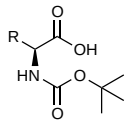
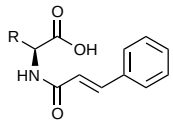
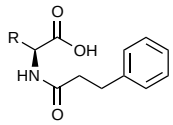
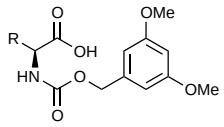
terkofsky utilizing less pure enzyme and substrates under somewhat different experimental conditions.[9b,c] The physical characteristics of *N*-succinyl-LL-DAP aminotransferase (EC 2.6.1.17) are similar to aspartate aminotransferase (EC 2.6.1.1) which is also dimeric and has a subunit molecular weight of ca. 45 kDa.[30]

In the initial studies by Gilvarg and co-workers, the relative abilities of a number of common amino acids to act as amine donors were examined for the "forward" aminotransferase reaction (generation of *N*-succinyl DAP).[9] Only L-glutamate showed activity in their studies. In the reverse direction, amino acids resembling LL-*N*-succinyl DAP were tested. No activity could be detected when *meso*-*N*-succinyl-DAP, LL-DAP, L-lysine or L-*N*-Cbz-lysine were utilized. Clearly the enzyme has high specificity for the correct stereoisomer, and the lack of reactivity of L-lysine derivatives indicates a requirement for the terminal C-7 carboxyl group.

Kinetic parameters for the substrate analogues synthesized in the current study (Table 3) reveal that only certain structural variations are tolerated by DAP-AT. The presence of the C-1 carboxyl is crucial for substrate turnover, and **13** is not accepted by the aminotransferase. Similarly, the 2-amido group is also of key importance, and even though **17** can act as a substrate, it is so poor that reliable kinetic parameters could not be obtained. The *N*-acetyl derivative **1b** is also quite ineffective with a specificity only 4.3% that of the natural substrate **1a**. The *N*-Boc derivative **1d** shows very similar behavior. Since

(30) Kirsch, J. F.; Eichele, G.; Ford, G. C.; Vincent, M. G.; Jansonius, J. N.; Gehring, H.; Christen, P. *J. Mol. Biol.* **1984**, *174*, 497−525.

Table 3. Conversion of Substrate Analogues by DAP-AT

entry	substrate[a] no.	substrate[a] structure	K_m^{app}, mM[b,c]	k_{cat}, s^{-1}	k_{cat}/K_m^{app}, s^{-1} M^{-1}	rel to **1a**, % (k_{cat}/K_m^{app})
1	**1a**		1.2	86	73	100
2	**1b**		6.9	23	3.1	4.3
3	**13**					
4	**17**		>10	<5	<0.5	<0.7
5	**1c**		3.6	62	17	23
6	**1d**		8.2	26	3.1	4.3
7	**1e**		1.2	61	51.	70.
8	**1f**		3.5	20.	5.6	7.7
9	**1g**		3.5	76	22	30

[a] R = $(CH_2)_3(CO)CO_2H$. [b] At 10 mM L-Glu; K_m for **1a** independent of L-Glu is 0.18 mM. [c] ±10%.

1b is presumably the natural substrate for the *N*-acetyl-LL-DAP aminotransferase from *B. megaterium*, the relatively poor interaction of **1b** with *N*-succinyl-LL-DAP aminotransferase supports earlier observations[31] that the two acylated pathways to *meso*-DAP are quite specific.

Interestingly, replacement of the terminal carboxyl group of the succinate moiety in **1a** with an aromatic ring produces good substrates. The specificity (k_{cat}/K_m^{app})[32] calculated for each compound and expressed as a percentage of that of the natural substrate **1a** indicates that the *N*-cinnamoyl derivative **1e** is the best of this group (specificity 70% of *N*-succinyl). The *N*-Cbz derivative **1c** and its dimethoxy-substituted analogue **1g** are also quite effective. We initially rationalized that these aromatic groups could substitute for the natural succinyl moiety by replacing a carboxylate−cation interaction with an aromatic π-cation interaction.[33] Thus the terminal carboxylate moiety of the succinyl residue of **1a** could bind to DAP-AT by an electrostatic interaction with a cation (e.g. protonated basic amino acid side chain) on the protein surface; this enzyme cation would presumably stack against and recognize the aromatic residue of the substrate analogues. However, the conformationally flexible hydrocinnamoyl derivative **1f** shows a specificity of only 7.6% that of **1a**. This suggests that increased electron donation from either carbamate oxygen (**1c**,**g**) or cinnamoyl olefin moiety (**1e**) may also be important for binding and recognition and that hydrophobic interactions of the aromatic ring could also play a critical role. PLP-dependent aminotransferases show considerable homology,[34] and enlargement of a cluster of hydrophobic residues near the active site entrance of aspartate aminotransferase[35] has been suggested[36] to be the cause of the enhanced specificity toward both hydrophobic and acidic

(31) Studies with the desuccinylase enzyme from *E. coli*[10a] demonstrate that acetylated DAP analogues are not substrates for this hydrolytic enzyme.

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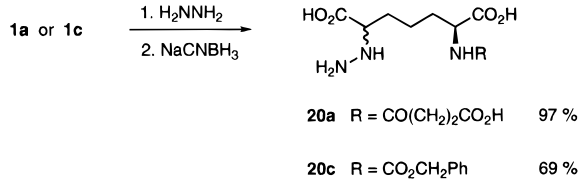

Figure 6. Syntheses of DAP-AT slow-binding inhibitors **20a**,**c**.

substrates by aromatic amino acid aminotransferase, an enzyme which shares 41% sequence identity with aspartate aminotransferase.[37]

Inhibition of DAP Aminotransferase by Analogues of 1a. Since L-glutamate and α-ketoglutarate are utilized extensively by mammalian systems, inhibitors of DAP-AT based on these compounds may be toxic, and analogues of **1a** or *N*-succinyl DAP are more attractive targets. Inhibition experiments with *N*-succinyl- and *N*-Cbz-α-aminopimelic acids (**18a**,**c**) and also with the *N*-succinyl-2-hydroxy-6-aminopimelic acid (**19**) show no detectable effect on DAP-AT at concentrations up to 10 mM. These results further support the common observation that DAP-metabolizing enzymes usually recognize the complete substrate skeleton as well as all polar functional groups (i.e. amino and carboxyl groups).[4,5b] The substrate specificity studies described above also support this concept. The α-hydrazino analogues **20a**,**c** would appear to fulfill the skeletal and functional group requirements since **1c** is a substrate. A number of α-hydrazino analogues of α-amino acids are known to inhibit PLP-dependent enzymes, presumably by reaction with the enzyme-cofactor aldimine to generate very stable hydrazones in the active site.[38] Indeed, the mono-α-hydrazino analogue of DAP is a good inhibitor of the PLP-dependent DAP decarboxylase.[39]

Compounds **20a**,**c** are readily accessible as a 1:1 mixture of diastereomers by treatment of aqueous solutions of the lithium salts of α-keto acids **1a**,**c** with hydrazine followed by removal of volatiles and immediate reduction of the crude hydrazone in methanol with sodium cyanoborohydride (Figure 6). A variety of alternative approaches, including in situ reduction of the aqueous hydrazone solution with sodium amalgam, fail due to competing hydrolysis or, in the case of **20c**, cleavage of the Cbz group. Both compounds are potent inhibitors of DAP-AT which display time-dependent behavior. Detailed analyses of interactions of **20a** or **20c** with DAP-AT show the usual characteristics of a slow-binding mechanism in addition to time dependence, namely substrate protection, reversibility upon 100-fold dilution, and progressive onset of inhibition.[40] Progress curves obtained from absorbance measurements in the standard assay were fitted using nonlinear least-squares regression analysis.[40b] The K_i* determined for **20a** is 22 ± 4 nM and for **20c** is 54 ± 9 nM, making these compounds the most potent reversible substrate analogue inhibitors of any DAP enzyme reported thus far. The $t_{1/2}$ values for release of these inhibitors from the active site of DAP-AT are 79 ± 10 and 45 ± 5 min, respectively. The actual chemical mechanism of inhibition is uncertain, but it probably involves reaction of the terminal amino group of the hydrazine with the PLP cofactor held in the aminotransferase active site (Figure 7). Although the amino

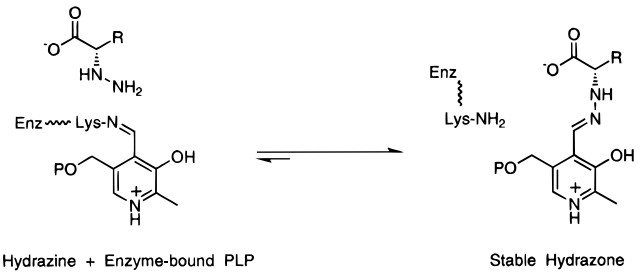

Figure 7. Possible mechanism of inhibition of DAP-AT by hydrazino analogues **20a**,**c**.

acid sequence of the enzyme is still unknown, the homology of most PLP-dependent aminotransferases[34−37] suggests that the ε-amino group of an active site lysine forms an imine with PLP. This species would presumably undergo transimination with the terminal NH_2 group of **20a** or **20c** to form the inhibitor-PLP hydrazone held by noncovalent interactions in the DAP-AT active site. Slow release of the complex and/or hydrolysis of the hydrazone double bond would regenerate active enzyme.

Initial antimicrobial tests with these inhibitors against a limited array of bacteria as described previously[5a,b] showed negligible activity at concentrations up to 100 μg/mL, possibly due to problems of transport into cells, as has been previously observed for DAP analogues.[5b]

Conclusions

The present work describes the isolation and purification of *N*-succinyl-LL-DAP aminotransferase (DAP-AT) (EC 2.6.1.17) from wild type *E. coli* to apparent homogeneity and studies the interaction of this enzyme with a series of substrate analogues and inhibitors. The sensitive substrate of this pyridoxal phosphate-dependent enzyme, (*L*)-*N*-succinyl-α-amino-ε-ketopimelic acid (**1a**), is synthesized in stereochemically pure form using an ene reaction between methyl *N*-succinyl-L-allylglycinate and methyl glyoxylate as a key step. Analogous approaches afford efficient access to a series of analogues missing the carboxyl or amido functionality or having other *N*-acyl groups in place of succinyl. The results of substrate specificity studies confirm that, like other DAP-metabolizing enzymes, this aminotransferase recognizes all polar functional groups as well as the overall skeleton of its substrates. However, replacement of the terminal carboxyl in the succinyl moiety by an aromatic ring is permitted, possibly because an aromatic π-cation interaction may partly mimic the substrate carboxylate−enzyme cation recognition. Alternatively, additional hydrophobic interactions could contribute to the binding of the aromatic ring. Derivatives **20a**,**c** in which an α-hydrazino group replaces the α-amino of the enzyme product are the most potent reversible inhibitors of any DAP enzyme reported thus far. Current studies to sequence and further characterize DAP-AT as well as to apply ene methodology to stereospecific syntheses of other DAP analogues will be reported in the future.

Experimental Section

General. General procedures and instrumentation have been previously described.[41] Ion exchange resins were obtained from BioRad (Mississauga, ON, Canada) and were washed before use according to the manufacturer's instructions. Diazomethane[42] and methyl glyoxyl-

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ate[43] were generated by standard literature procedures. The Cleland matrix for positive ion fast atom bombardment (POSFAB) mass spectra consists of a 5:1 mixture of dithiothreitol and dithioerythritol.

(2*S*)-2-(*N*-(Succinyl)amino)-6-oxoheptane-1,7-dioic Acid, Trilithium Salt Trihydrate (1a). To a stirred solution of oxo diester **8a** (338 mg, 1.02 mmol) in 1:1 MeCN:H_2O (10 mL) was added LiOH·H_2O (129 mg, 3.06 mmol). After 90 min the solvent was removed *in vacuo*, and the residue was dissolved in water (2 mL). The solution was lyophilized to afford **1a** as a powder (310 mg, 99%): mp 125 °C dec; $[\alpha]_D = +6.4°$ (c = 0.68, H_2O); IR (KBr) 3482, 2970, 1675, 1671, 1596, 1420 cm^{-1}; ^{1}H NMR (360 MHz, D_2O) δ 4.13−4.05 (m, 1H), 2.71−2.64 (m, 2H, D_2O exchanged), 2.55−2.39 (m, 4H), 1.82−1.45 (m, 4H); ^{13}C NMR (90 MHz, D_2O) δ 190.0, 180.0, 174.4, 174.3, 54.4, 38.8, 32.4, 31.6, 30.4, 18.5; MS (POSFAB, glycerol/HCl) *m/z* 307 (M^+, 3.4%), 308 (MH^+, 1.2%). Anal. Calcd for $C_{11}H_{12}NO_8Li_3$·3H_2O: C, 36.59; H, 5.02; N, 3.88. Found: C, 36.30; H, 4.71; N, 3.74.

Analysis of Enantiomeric Excess of 1a. To a suspension of **1a** (19.0 mg, 61.9 μmol) in absolute MeOH (1 mL) was added NH_4OAc (47.7 mg, 620 μmol). After 30 min, $NaCNBH_3$ (39 mg, 620 μmol) was added and the mixture was stirred for 16 h. Excess $NaCNBH_3$ was destroyed by the addition of 1 N HCl (1 mL). Solvent was removed *in vacuo*, and the residue was dissolved in concentrated HCl. The solution was stirred for 2 h at 80 °C and then evaporated to dryness. The residue was purified by cation exchange chromatography to afford 2.5 mg of a white solid. The solid was derivatized by the method of Marfey,[27] and the resultant solution was analyzed by HPLC. (Column: Chemcosorb 5-ODS-UH, 4.6 × 150 mm; solvent A 12 mM ammonium phosphate buffer + 4% DMF, pH 6.5, solvent B 70% MeCN in milli-Q water. Method: gradient 15%-35% B in 35 min, at 1 mL/min, detection at 340 nM. Retention times for Marfey derivatives: *meso*-DAP 22.0 min, LL-DAP 26.6 min, *DD*-DAP 31.6 min.) Less than 1% DD-DAP was observed confirming that **1a** had exclusively *2S* configuration within experimental error.

(2*S*)-2-(*N*-Acetylamino)-6-oxoheptane-1,7-dioic Acid, Dilithium Salt Monohydrate (1b). The procedure used for preparation of **1a** was employed to convert **8b** to **1b** in 92% yield: mp 218 °C dec; IR (KBr) 3427, 1716, 1617, 1418 cm^{-1}; ^{1}H NMR (400 MHz, D_2O + CD_3CN) δ 4.00−3.92 (m, 1H), 1.89 (s, 3H), 1.68−1.35 (m, 4H); ^{13}C NMR (90 MHz, H_2O + CD_3CN) δ 206.4, 177.4, 172.0, 169.7, 54.3, 36.6, 30.7, 21.3, 18.6; MS (POSFAB, glycerol/HCl) *m/z* 244 (MH^+, 2.7%), 243 (M^+, 4.1%), 238 ((M − Li + H)H^+, 2.7%). Anal. Calcd for $C_9H_{11}NO_6Li_2$·H_2O: C, 41.40; H, 5.02; N, 5.36. Found: C, 41.55; H, 4.87; N, 5.25.

(2*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-6-oxoheptane-1,7-dioic Acid, Dilithium Salt Dihydrate (1c). The procedure used for preparation of **1a** was employed to convert **8c** to **1c** in 98% yield: mp 67−69°C; IR (KBr) 3423, 2933, 1697, 1615, 1522, 1454, 1420 cm^{-1}; ^{1}H NMR (360 MHz, D_2O) δ 7.51−7.35 (m, 5H), 5.23−5.05 (m, 2H), 4.00−3.85 (m, 1H), 2.81−2.73 (m, 2H), 1.95-1.40 (m, 4H); ^{13}C NMR (90 MHz, D_2O + CH_3CN) δ 203.5, 181.6, 174.5, 161.8, 140.4, 132.8, 132.2, 129.9, 70.6, 60.0, 41.0, 34.8, 22.5; MS (POSFAB, glycerol/HCl) *m/z* 335 (M^+, 0.6%), 330 ((M − Li + H)H^+, 1.33%), 324 ((M − 2Li + 2H)H^+, 1.1%). Anal. Calcd for $C_{15}H_{15}NO_7Li_2$·2H_2O: C, 48.54; H, 5.16; N, 3.77. Found: C, 48.48; H, 4.91; N, 3.71.

The stereochemical analysis of **1c** was accomplished analogously to the method for **1a** described above using reductive amination and derivatization. The Marfey derivative[27] of DD-DAP was not observed, indicating pure *2S* configuration for **1c**.

(2*S*)-2-(*N*-(*tert*-butyloxycarbonyl)amino)-6-oxoheptane-1,7-dioic Acid, Dilithium Salt (1d). The procedure used for preparation of **1a** was employed to convert **8d** to **1d** in 99% yield: mp 87−90 °C; IR (KBr) 3429, 2978, 2934, 1700, 1617, 1512, 1416 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 3.72−3.58 (m, 1H), 2.62−2.54 (m, 2H, D_2O exchanged), 1.70−1.33 (m, 4H), 1.25 (s, 9H); ^{13}C NMR (90 MHz, D_2O + CH_3CN) δ 199.9, 180.0, 171.5, 157.6, 81.2, 56.0, 39.1, 31.5, 27.9, 19.5; MS (POSFAB, glycerol/HCl) *m/z* 302 (MH^+, 1.2%), 297 ((M - Li + H)H^+, 3.36%), 296 ((M − Li + H)$^+$, 2.32%).

(2*S*)-2-(*N*-(Cinnamoyl)amino)-6-oxoheptane-1,7-dioic Acid, Dilithium Salt Monohydrate (1e). The procedure used for preparation of **1a** was employed to convert **8e** to **1e** in 99% yield: mp 198 °C dec; IR (KBr) 3397, 3083, 3061, 2973, 2934, 2870, 1709, 1656, 1600, 1449, 1415 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 7.49−7.46 (m, 2H), 7.36 (d, 1H, J = 15.8 Hz), 7.38−7.29 (m, 3H), 6.54 (d, 1H, J = 15.8 Hz), 4.15−4.11 (m, 1H), 2.65 (t, 2H, J = 7.2 Hz, D_2O exchanged), 1.74−1.20 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.0, 179.2, 170.9, 168.4, 141.4, 134.7, 130.4, 129.3, 128.2, 120.4, 55.6, 38.0, 31.4, 19.5; MS (POSFAB, nitrobenzyl alcohol) *m/z* 338 (MLi^+, 3.3%), 332 (MH^+, 2.3%), 326 ((M − Li + H)H^+, 1.0%). Anal. Calcd for $C_{16}H_{15}NO_6Li_2$·H_2O: C, 55.03; H, 4.91; N, 4.01. Found: C, 55.22; H, 4.52; N, 3.80.

(2*S*)-2-(*N*-(Hydrocinnamoyl)amino)-6-oxoheptane-1,7-dioic Acid, Dilithium Salt Hemihydrate (1f). The procedure used for preparation of **1a** was employed to convert **8f** to **1f** in 99% yield: mp 99−103 °C; IR (KBr) 3406, 3086, 3063, 3028, 2934, 1709, 1605, 1496, 1453, 1417 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ 7.23−7.03 (m, 5H), 3.88−3.84 (m, 1H), 2.81−2.72 (m, 2H), 2.48−2.41 (m, 4H, (2H D_2O exchanged)), 1.55−1.27 (m, 3H), 1.15−0.98 (m, 1H); ^{13}C NMR (100 MHz, D_2O + CH_3CN) δ 206.8, 179.0, 175.1, 170.6, 140.8, 129.1, 129.0, 126.8, 55.1, 39.1, 37.8, 33.5, 31.7, 19.2; MS (POSFAB, Cleland) *m/z* 340 (MLi^+, 11.0%), 334 (MH^+, 6.8%), 333 (M^+, 1.5%). Anal. Calcd for $C_{16}H_{17}NO_6Li_2$·0.5H_2O: C, 56.16; H, 5.30; N, 4.09. Found: C, 55.99; H, 5.25; N, 4.08.

(2*S*)-2-(*N*-((3,5-Dimethoxybenzyl)oxycarbonyl)amino)-6-oxoheptane-1,7-dioic Acid, Dilithium Salt (1g). The procedure used for preparation of **1a** was employed to convert **8g** to **1g** in 95% yield: mp 189−193 °C dec; IR (KBr) 3423, 2942, 1703, 1600, 1530, 1460, 1428 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 6.46 (br, 2H), 6.32 (br s, 1H), 4.91−4.83 (m, 2H), 3.66 (s, 6H), 3.64−3.59 (m, 1H), 2.64−2.61 (m, 2H, D_2O exchanged), 1.72−1.35 (m, 4H); ^{13}C NMR (100 MHz, D_2O + CH_3CN) δ 206.9, 179.6, 170.2, 160.8, 157.9, 139.9, 106.1, 100.4, 66.7, 56.8, 55.9, 39.1, 31.8, 19.7; MS (POSFAB, Cleland) *m/z* 402 (MLi^+, 6.3%), 396 (MH^+, 5.9%), 395 (M^+, 1.4%).

(*S*)-Allylglycine (3). A stirred solution of (*RS*)-*N*-acetylallylglycine (**2**) (30.0 g, 191 mmol) in water (1.4 L) was adjusted to pH 7.8 with LiOH. Porcine kidney acylase I (Sigma, 350 mg) was added, and the solution was stirred 12 h at 38 °C. Norit A charcoal (5 g) was added, and the suspension was filtered through Celite. The solid was washed with water (200 mL) and the combined aqueous filtrate applied to BioRad AG-50 WX-8 cation exchange resin (H^+, 600 mL). The column was washed with water until the eluent pH was neutral and then with 1 M aqueous ammonia. Amine-containing fractions (ninhydrin) were evaporated *in vacuo*, and the residue was recrystallised from water/ethanol to afford pure (*S*)-allylglycine (**3**) (10.4 g 95%): mp 282−283 °C; $[\alpha]_D$ −37.3° (4% H_2O, lit.[44] −37.1°); IR (KBr) 3420, 2900, 1643, 1612, 1586, 1561, 1512, 1405 cm^{-1}; ^{1}H NMR (360 MHz, D_2O) δ 5.86−5.72 (m, 1H), 5.36−5.22 (m, 2H), 3.83 (dd, 1H, J = 7.0, 5.2 Hz), 2.74−2.55 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 173.1, 130.5, 119.5, 53.0, 33.9; MS (POSFAB, glycerol/HCO_2H) *m/z* 116 (MH^+). Anal. Calcd for $C_5H_9NO_2$: C, 52.17; H, 7.88; N, 12.17. Found: C, 52.12; H, 7.73; N, 12.23.

Dimethyl *N*-(Succinyl)-(*S*)-allylglycinate (4a). A stirred solution of **3** (2.80 g, 24.3 mmol) in aqueous K_2CO_3 (1.2M, 50mL) was treated with succinic anhydride (3.65 g, 36.5 mmol) in portions over a period of 20 min. After 40 min, the solution was acidified to pH 2.0 with concentrated HCl and extracted with EtOAc (4 × 50 mL). The combined extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was treated with excess ethereal diazomethane. After 10 min excess diazomethane was destroyed by the addition of acetic acid. The solution was evaporated *in vacuo* to afford an oil which was purified by flash chromatography (70% EtOAc in hexane, R_f 0.22) to afford the product **4a** as an oil (5.03 g, 85%): $[\alpha]_D = +36.2°$ (c = 4.35, $CHCl_3$); IR (neat) 3314, 3003, 2982, 2954, 1741, 1678, 1658, 1536, 1438 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 (d, 1H, J = 6.7 Hz), 5.75−5.62 (m, 1H), 5.17−5.09 (m, 2H), 4.72−4.66 (m, 1H), 3.80 (s, 3H), 3.65 (s, 3H), 2.75−2.47 (m, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 172.9, 171.9, 170.9, 132.1, 118.7, 52.0, 51.5, 51.4, 36.1, 30.4, 28.9; exact mass 243.1104 (M^+) (243.1107 calcd for $C_{11}H_{17}NO_5$). Anal. Calcd for $C_{11}H_{17}NO_5$: C, 54.31; H, 7.04; N, 5.76. Found: C, 54.17; H, 6.99; N, 5.78.

(43) (a) Kelly, T. R.; Schmidt, T. E.; Haggerty, J. G. *Synthesis* **1972**, 544−545. (b) Jung, M. E.; Shishido, K.; Davis, L. H. *J. Org. Chem.* **1982**, *47*, 891−892.

Methyl *N*-(Acetyl)allylglycinate (4b). Reaction of **3** and acetic anhydride analogous to preparation of **4a** gave known[44] **4b** in 91% yield (R_f 0.37, EtOAc on SiO_2): $[\alpha]_D = +45.4°$ ($c = 3.57$, $CHCl_3$); IR (neat) 3285, 3079, 2954, 1747, 1658, 1544, 1438 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.22 (s, 1H), 5.67−5.56 (m, 1H), 5.08−5.02 (m, 2H), 4.64−4.59 (m, 1H), 3.67 (s, 3H), 2.51−2.40 (m, 2H), 1.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 170.1, 132.6, 119.7, 52.8, 52.1, 37.0, 26.4, 23.7; exact mass 171.08987 (M^+) (171.08954 calcd for $C_8H_{13}NO_3$).

Methyl *N*-(Benzyloxycarbonyl)allylglycinate (4c). Reaction of **3** and CbzCl analogous to preparation of **4a** gave known **4c** in 90% yield (R_f 0.25, 30% EtOAc/Hexane on SiO_2). All spectral properties agreed with those previously reported.[5a]

Ene Reaction with $FeCl_3$. Dimethyl (2*S*,6*RS*)-2-(*N*-(Methyl succinyl)amino)-6-hydroxyhept-3-ene-1,7-dioate (5a). A stirred ice-cooled solution of freshly distilled methyl glyoxylate (1.10 g, 12.5 mmol) in CH_2Cl_2 (20 mL) under Ar was treated with anhydrous $FeCl_3$ (4.00 g, 24.6 mmol). The suspension was stirred at 0 °C for 30 min, and then a solution of alkene ester **4a** (1.0 g, 4.1 mmol) in CH_2Cl_2 (30 mL) was added dropwise over 10 min. The suspension was warmed to 20 °C and stirred 24 h. The black suspension was poured onto crushed ice (100 mL), and the mixture was extracted into CH_2Cl_2 (4 × 100 mL). The combined organic extracts were washed with 1 N HCl (100 mL) followed by saturated aqueous $NaHCO_3$ (100 mL) and then dried ($MgSO_4$) and evaporated *in vacuo*. The residue was purified by flash chromatography (EtOAc, R_f 0.33) to afford **5a** as an oil (646 mg, 47%): IR ($CHCl_3$ cast) 3140, 2950, 1738, 1656, 1531, 1437 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.22 (s, 1H), 5.82−5.72 (m, 1H), 5.62 (dd, 1H, $J = 6.0$, 15.5 Hz), 5.05 (dd, 1H, $J = 12.0$, 6.0 Hz), 4.23 (dd, 1H, $J = 6.6$, 4.5 Hz), 3.74 (s, 3H), 3.73 (s, 3H), 3.68 (s, 3H), 3.08 (s, 1H), 2.76−2.38 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 173.2, 171.3, 171.0, 128.3, 127.4, 69.8, 53.8, 52.4, 52.1, 51.6, 36.8, 30.3, 28.9; exact mass 332.1347 (MH^+) (332.1345 calcd for $C_{14}H_{22}NO_8$).

Dimethyl (2*S*,6*RS*)-2-(*N*-Acetylamino)-6-hydroxyhept-3-ene-1,7-dioate (5b). Ene reaction of **4b** following the procedure for **5a** gave **5b** as an oil in 46% yield (EtOAc, R_f 0.38): IR (CH_2Cl_2 cast) 3448, 3309, 3293, 2956, 1742, 1737, 1655, 1649, 1541, 1534, 1509, 1457, 1438 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.22 (s, 1H), 5.79−5.69 (m, 1H), 5.64−5.58 (m, 1H), 5.09−5.04 (m, 1H), 4.26−4.20 (m, 1H), 3.76 (s, 3H), 3.75 (s, 3H), 2.99 (s, 1H), 2.59−2.52 (m, 1H), 2.46−2.36 (m, 1H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 174.5, 171.5, 169.6, 128.3, 128.0, 69.5, 53.5, 52.4, 52.2, 36.8, 22.5; exact mass 259.10534 (M^+) (259.10559 calcd for $C_{11}H_{17}NO_6$).

Ene Reaction with $SnCl_4$. Dimethyl (2*S*,6*RS*)-2-(*N*-(Benzyloxycarbonyl)amino)-6-hydroxyhept-3-ene-1,7-dioate (5c). To a solution of freshly distilled methyl glyoxylate (4.2 g, 48 mmol) in CH_2Cl_2 (65 mL) stirred under Ar and cooled to −55 °C was added freshly distilled $SnCl_4$ (25.0 mL, 214 mmol) over a period of 10 min. The solution was stirred a further 10 min and then cooled to −78 °C. A solution of alkene ester **4c** (3.20 g, 12.2 mmol) in CH_2Cl_2 (20 mL) was added dropwise over 10 min. The suspension was warmed to −23 °C and stirred for 4 h and then poured onto crushed ice (200 mL) (*caution*: *vigorous reaction*). The mixture was extracted into CH_2Cl_2 (4 × 200 mL), and the combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo* to afford an oil which was purified by flash chromatography (70% EtOAc in hexane, R_f 0.30) to afford **5c** as an oil (2.79 g, 65%): IR ($CHCl_3$ cast) 3462, 3364, 2955, 1743, 1523, 1455, 1438, 1411 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.30 (m, 5H), 5.82−5.72 (m, 1H), 5.65−5.50 (m, 2H), 5.10 (s, 2H), 4.85 (m, 1H), 4.22 (m, 1H), 3.72 (s, 6H), 2.99 (s (br), 1H), 2.60−2.50 (m, 1H), 2.45−2.38 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 171.0, 155.5, 136.1, 129.0, 128.9, 128.8, 128.1, 128.0, 69.8, 67.0, 55.4, 52.6, 52.4, 39.9; exact mass 351.13162 (M^+) (351.13181 calcd for $C_{17}H_{21}NO_7$).

Dimethyl (2*S*,6*RS*)-2-(*N*-(Methyl succinyl)amino)-6-hydroxyheptane-1,7-dioate (6a). To a solution of **5a** (430 mg, 1.30 mmol) in MeOH (20 mL) was added 10% Pd/C (50 mg). The suspension was stirred under 1 atm of H_2 for 19 h and then vacuum filtered through a bed of Celite, which was washed with MeOH. Evaporation *in vacuo* afforded **6a** as an oil (395 mg, 91%): IR ($CHCl_3$ cast) 3140, 2950, 1738, 1656, 1531, 1437 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.24 (t, 1H, $J = 7.0$ Hz), 4.63−4.58 (m, 1H), 4.20−4.15 (m, 1H), 3.78 (s, 3H), 3.75 (s, 3H), 3.69 (s, 3H), 2.90 (s, 1H), 2.76−2.55 (m, 4H), 1.89−1.66 (m, 4H), 1.50-1.20 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 173.2, 172.8, 171.3, 70.0, 52.4, 52.2, 51.8, 51.7, 33.4, 31.7, 30.5, 29.0, 20.5; exact mass 333.1414 (M^+) (333.1424 calcd for $C_{14}H_{23}NO_8$).

Dimethyl (2*S*,6*RS*)-2-(*N*-(Acetylamino))-6-hydroxyheptane-1,7-dioate (6b). Alkene **5b** was hydrogenated by the procedure used for **5a** to give **6b** as an oil in 95% yield (EtOAc, R_f 0.13): IR ($CHCl_3$ cast) 3303, 2955, 1742, 1655, 1541, 1437 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.34 (d, 1H, $J = 7.9$ Hz), 4.59−4.53 (m, 1H), 4.15−4.10 (m, 1H), 3.73 (s, 3H), 3.69 (s, 3H), 3.06 (s, 1H), 1.97 (s, 3H), 1.81−1.37 (m, 6H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 175.5, 173.2, 170.4, 70.5, 52.6, 52.4, 52.3, 33.9, 32.1, 23.0, 21.1; exact mass 262.12872 (MH^+) (262.12906 calcd for $C_{11}H_{20}NO_6$).

Acylation of 7. Dimethyl (2*S*,6*RS*)-2-(*N*-(Benzyloxycarbonyl)-amino)-6-hydroxyheptane-1,7-dioate (6c). A stirred solution of amine hydrochloride **7** (455 mg, 1.78 mmol) in CH_2Cl_2 (20 mL) at 20 °C under Ar was treated with pyridine (288 μL, 282 mg, 3.56 mmol). Benzyl chloroformate (280 μL, 334 mg, 1.96 mmol) was added dropwise, and the solution was stirred 90 min before 1 N HCl (50 mL) was added. The mixture was extracted into CH_2Cl_2 (3 × 100 mL), and the combined extracts were dried ($MgSO_4$) and evaporated *in vacuo*. The residue was purified by flash chromatography (70% EtOAc in hexane, R_f 0.17) to give **6c** as an oil (527 mg, 84%): IR (CH_2Cl_2 cast) 3356, 2954, 1735, 1528, 1455, 1438 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.35−7.28 (m, 5H), 5.39 (d, 1H, $J = 7.9$ Hz), 5.05 (s, 2H), 4.36−4.28 (m, 1H), 4.14−4.08 (m, 1H), 3.75 (s, 3H), 3.70 (s, 3H), 3.05 (s, 1H), 1.88−1.35 (m, 6H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 175.8, 173.3, 156.4, 136.7, 129.0, 128.6, 128.5, 70.5, 67.4, 54.1, 52.9, 52.8, 34.1, 32.6, 21.1; exact mass 353.14775 (M^+) (353.14746 calcd for $C_{17}H_{23}NO_7$). Anal. Calcd for $C_{17}H_{23}NO_7$: C, 57.77; H, 6.56; N, 3.97. Found: C, 57.88; H, 6.89; N, 3.84.

Dimethyl (2*S*,6*RS*)-2-(*N*-(*tert*-Butyloxycarbonyl)amino)-6-hydroxyheptane-1,7-dioate (6d). To a solution of *N*-Cbz-alkene **5c** (1.42 g, 4.05 mmol) in MeOH (30 mL) was added *tert*-butyl pyrocarbonate (1.32 g, 6.0 mmol) and 10% Pd/C (200 mg). The suspension was stirred under 1 atm of H_2 for 15 h and then vacuum filtered through Celite, which was washed with MeOH. The solution was evaporated *in vacuo* and the residue was purified by flash chromatography (25% EtOAc in CH_2Cl_2, R_f 0.30) to afford **6d** as an oil (1.03 g, 80%): IR (CH_2Cl_2 cast) 3375, 2976, 2955, 2935, 1741, 1715, 1518, 1455, 1438 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 5.12 (s, 1H), 4.21−4.12 (m, 1H), 4.10−4.04 (m, 1H), 3.65 (s, 3H), 3.60 (s, 3H), 3.11 (s, 1H), 1.78−1.62 (m, 2H), 1.60−1.48 (m, 2H), 1.46−1.30 (m, 2H), 1.29 (s, 9H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 175.7, 173.6, 155.8, 80.1, 70.5, 53.6, 52.7, 52.5, 34.0, 32.6, 28.6, 21.2; MS (CI, NH_3) *m/z* 337 (16.5%, MNH_4^+), 320 (17.6%, MH^+). Anal. Calcd for $C_{14}H_{25}NO_7$: C, 52.65; H, 7.89; N, 4.39. Found: C, 52.40; H, 7.98; N, 4.19.

Dimethyl (2*S*,6*RS*)-2-(*N*-(Cinnamoyl)amino)-6-hydroxyheptane-1,7-dioate (6e). Amine hydrochloride **7** was acylated with cinnamoyl chloride as described for preparation of **6c** to give **6e** as an oil in 56% yield (50% EtOAc in hexane, R_f 0.17): IR (CH_2Cl_2 cast) 3305, 2953, 1742, 1658, 1621, 1577, 1538, 1497, 1450, 1436 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, 1H, $J = 15.6$ Hz), 7.46−7.44 (m, 2H), 7.32−7.30 (m, 3H), 6.65 (d, 1H, $J = 7.6$ Hz), 6.48 (d, 1H, $J = 15.6$ Hz) 4.75−4.72 (m, 1H), 4.17−4.14 (m, 1H), 3.72 (s, 6H), 2.00−1.43 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 171.6, 140.2, 133.1, 128.3, 127.3, 126.4, 118.5, 68.4, 51.4, 51.1, 50.8, 32.0, 30.5, 19.2; exact mass 349.15275 (M^+) (349.15253 calcd for $C_{18}H_{23}NO_6$).

Dimethyl (2*S*,6*RS*)-2-(*N*-(Hydrocinnamoyl)amino)-6-hydroxyheptane-1,7-dioate (6f). Amine hydrochloride **7** was acylated with hydrocinnamoyl chloride as described for preparation of **6c** to give **6f** as an oil in 83% yield (60% EtOAc in hexane, R_f 0.20): IR (CH_2Cl_2 cast) 3308, 3085, 3028, 2953, 2865, 1740, 1651, 1604, 1541, 1497, 1454, 1438 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.26−7.15 (m, 5H), 6.23 (d, 1H, $J = 6.2$ Hz), 4.59−4.52 (m, 1H), 4.12−4.08 (m, 1H), 3.72 (s, 3H), 3.67 (s, 3H), 3.11 (s, 1H), 2.94−2.69 (m, 2H), 2.55−2.44 (m, 2H), 1.83−1.50 (m, 4H), 1.37−1.32 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 175.7, 173.3, 172.6, 141.0, 128.8, 128.6, 126.5, 70.4, 52.7, 52.6, 52.2, 38.2, 33.8, 32.1, 31.7, 21.0; exact mass 351.1691 (M^+) (351.16818 calcd for $C_{18}H_{25}NO_6$).

(44) Black, S.; Wright, N. G. *J. Biol. Chem.* **1955**, *213*, 39−50.

Dimethyl (2*S*,6*RS*)-2-(*N*-((3,5-Dimethoxybenzyl)oxycarbonyl))-amino-6-hydroxyheptane-1,7-dioate (6g). Amine hydrochloride **7** was acylated with *p*-nitrophenyl 3,5-dimethoxybenzyl carbonate[45] as described for preparation of **6c** to give **6g** as an oil in 58% yield (50% EtOAc in hexane, R_f 0.15): IR (CH_2Cl_2 cast) 3356, 3002, 2954, 2842, 1740, 1609, 1598, 1525, 1489, 1460, 1434, 1404 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.48 (s, 2H), 6.39 (s, 2H), 5.65−5.62 (m, 1H), 5.03 (s, 2H), 4.46−4.33 (m, 1H), 4.22−4.15 (m, 1H), 3.77 (s, 6H), 3.74 (s, 3H), 3.72 (s, 3H), 3.19 (s, 1H), 1.89−1.46 (m, 6H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 175.5, 173.1, 161.3, 156.3, 138.9, 106.1, 100.5, 70.5, 67.1, 55.6, 54.1, 52.6, 52.1, 33.9, 32.4, 21.1; exact mass 413.16835 (M^+) (413.16858 calcd for $C_{19}H_{27}NO_9$).

Dimethyl (2*S*,6*RS*)-2-Amino-6-hydroxyheptane-1,7-dioate Hydrochloride (7). A suspension of *N*-Cbz-alkene **5c** (2.01 g, 5.73 mmol) and 10% Pd/C (100 mg) in MeOH (25 mL) and $CHCl_3$ (5 mL) was hydrogenated under 1 atm of H_2 for 24 h. The mixture was vacuum filtered through Celite, which was then washed with MeOH. The filtrate was evaporated *in vacuo* to afford a gum which was dissolved in CH_2Cl_2 (10 mL). Evaporation of this solution *in vacuo* gave **7** as a wax (1.46 g, 99%): IR (CH_2Cl_2 cast) 2954, 2630, 1744, 1506, 1456, 1439 cm^{-1}; ^{1}H NMR (360 MHz, D_2O) δ 4.21−4.15 (m, 1H), 4.10−4.01 (m, 1H), 3.95 (s, 3H), 3.83 (s, 3H), 2.05−1.43 (m, 6H); ^{13}C NMR (90 MHz, CD_3OD) δ 176.1, 170.8, 71.3, 54,1, 53.6, 52.4, 34.4, 31.2, 21.8; MS (CI, NH_3) *m/z* 220 (MH^+, 100%).

Dess−Martin Oxidation of α-Hydroxy Esters. Dimethyl (2*S*)-2-(*N*-(Methyl succinyl)amino)-6-oxoheptane-1,7-dioate (8a). To a stirred solution of **6a** (140 mg, 420 μmol) in dry CH_2Cl_2 (15 mL) under Ar at 20 °C was added a solution of freshly prepared Dess−Martin periodinane[25] (356 mg, 840 μmol) in dry CH_2Cl_2 (5.0 mL). After 90 min the solution was added to a saturated aqueous solution of $NaHCO_3$ (20 mL) containing $Na_2S_2O_3$ (2.0 g). The mixture was stirred vigorously for 5 min and then extracted into CH_2Cl_2 (3 × 100 mL). The dried ($MgSO_4$) extracts were evaporated *in vacuo*, and the residue was purified by flash chromatography (EtOAc, R_f 0.49) to afford **8a** as an oil (110 mg, 79%): $[\alpha]_D = +18.4°$ ($c = 4.76$, $CHCl_3$); IR (CH_2Cl_2 cast) 3350, 2956, 1734, 1678, 1660 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.26 (d, 1H, J = 7.6Hz), 4.61−4.55 (m, 1H), 3.83 (s, 3H), 3.71 (s, 3H), 3.65 (s, 3H), 2.85 (t, 2H, J = 6.9 Hz), 2.67−2.59 (m, 2H), 2.54−2.57 (m, 2H), 1.92−1.82 (m, 1H), 1.76−1.62 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 193.4, 173.2, 172.5, 171.2, 161.2, 52.7, 52.1, 51.5, 51.4, 38.3, 31.4, 30.4, 28.9, 18.3; exact mass 331.1262 (M^+) (331.1267 calcd for $C_{14}H_{21}NO_8$). Anal. Calcd for $C_{14}H_{21}NO_8$: C, 50.75; H, 6.39; N, 4.23. Found: C, 50.34; H, 6.46; N, 4.47.

Dimethyl (2*S*)-2-(*N*-Acetylamino)-6-oxoheptane-1,7-dioate (8b). Hydroxy ester **6b** was oxidized by the procedure used to form **8a** to give **8b** as an oil in 70% yield (EtOAc, R_f 0.22): IR (CH_2Cl_2 cast) 3291, 2933, 2955, 1733, 1654, 1539, 1437, 1401 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.35 (s, 1H), 4.60 (m, 1H), 3.82 (s, 3H), 3.65 (s, 3H), 2.83 (m, 2H), 1.97 (s, 3H), 1.85−1.76 (m, 1H), 1.70−1.50 (m, 3H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 193.7, 172.9, 170.4, 161.5, 53.0, 52.5, 51.9, 38.7, 31.5, 23.0, 18.9; exact mass 259.10571 (M^+) (259.10559 calcd for $C_{11}H_{17}NO_6$). Anal. Calcd for $C_{11}H_{17}NO_6$: C, 50.96; H, 6.61; N, 5.40. Found: C, 50.76; H, 6.59; N, 5.27.

Dimethyl (2*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-6-oxoheptane-1,7-dioate (8c). Hydroxy ester **6c** was oxidized by the procedure used to form **8a** to give **8c** as an oil in 56% yield (30% EtOAc in hexane, R_f 0.15): IR (CH_2Cl_2 cast) 3365, 2954, 1730, 1524, 1454, 1437, 1400 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.26−7.15 (m, 5H), 5.26 (d, 1H, J = 7.2 Hz), 4.99 (s, 2H), 4.28−4.22 (m, 1H), 3.72 (s, 3H), 3.62 (s, 3H), 2.75 (m, 2H), 1.86−1.65 (m, 1H), 1.65−1.45 (m, 3H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 193.5, 172.2, 161.0, 157.9, 137.9, 128.5, 128.3, 128.2, 65.5, 53.0, 52.5, 51.7, 37.8, 31.0, 17.6; exact mass 351.13203 (M^+) (351.13181 calcd for $C_{17}H_{21}NO_7$). Anal. Calcd for $C_{17}H_{21}NO_7$: C, 58.10; H, 6.03; N, 3.99. Found: C, 57.93; H, 6.00; N, 3.91.

Dimethyl (2*S*)-2-(*N*-(*tert*-Butyloxycarbonyl)amino)-6-oxoheptane-1,7-dioate (8d). Hydroxy ester **6d** was oxidized by the procedure used to form **8a** to give **8d** as an oil in 13% yield (10% EtOAc in CH_2Cl_2, R_f 0.25): IR ($CHCl_3$ cast) 3383, 2976, 2955, 2934, 1733, 1714, 1513, 1454, 1437 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.05 (d, 1H, J = 7.0 Hz), 4.28−4.20 (m, 1H), 3.77 (s, 3H), 3.66 (s, 3H), 2.84−2.75 (m, 2H), 1.89−1.70 (m, 1H), 1.70−1.58 (m, 3H), 1.35 (s, 9H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 193.9, 173.4, 161.7, 155.5, 80.4, 53.4, 53.3, 52.7, 38.9, 32.2, 28.7, 19.1; MS (CI, NH_3) *m/z* 335 (MNH_4^+, 21.1%), 318 (MH^+, 3.4%), 317 (M^+, 7.4%).

Dimethyl (2*S*)-2-(*N*-(Cinnamoyl)amino)-6-oxoheptane-1,7-dioate (8e). Hydroxy ester **6e** was oxidized by the procedure used to form **8a** to give **8e** as an oil in 64% yield (50% EtOAc in hexane, R_f 0.29): IR (CH_2Cl_2 cast) 3283, 2953, 1733, 1658, 1624, 1577, 1534, 1449, 1437, 1400 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.59 (d, 1H, J = 15.6 Hz), 7−45−7.42 (m, 2H), 7.31−7.26 (m, 3H), 6.67 (d, 1H, J = 8.0 Hz), 6.50 (d, 1H, J = 15.6 Hz), 4.77−4.72 (m, 1H), 3.81 (s, 3H), 3.74 (s, 3H), 2.87 (t, 2H, J = 7.0 Hz), 1.94−1.91 (m, 1H), 1.77−1.65 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.9, 173.2, 166.1, 161.6, 142.1, 135.0, 130.2, 129.2, 128.3, 120.4, 53.4, 52.9, 52.3, 38.9, 31.8, 19.0; exact mass 347.13736 (M^+) (347.13690 calcd for $C_{18}H_{21}NO_6$).

Dimethyl (2*S*)-2-(*N*-(Hydrocinnamoyl)amino)-6-oxoheptane-1,7-dioate (8f). Hydroxy ester **6f** was oxidized by the procedure used to form **8a** to give **8f** as an oil in 60% yield (50% EtOAc in hexane, R_f 0.15): IR (CH_2Cl_2 cast) 3302, 2953, 1731, 1652, 1537, 1497, 1454, 1437, 1400 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25−7.11 (m, 5H), 6.16 (d, 1H, J = 7.8 Hz), 4.60−4.53 (m, 1H), 3.82 (s, 3H), 3.66 (s, 3H), 2.93 (t, 2H, J = 6.6 Hz), 2.78 (t, 2H, J = 7.0 Hz), 2.54−2.48 (m, 2H), 1.80−1.71 (m, 1H), 1.62−1.45 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 194.0, 172.9, 172.3, 161.6, 140.9, 128.8, 128.7, 126.5, 53.3, 52.8, 51.9, 38.6, 38.4, 31.8, 31.7, 18.7; exact mass 349.15237 (M^+) (349.15253 calcd for $C_{18}H_{23}NO_6$). Anal. Calcd for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.64; N, 4.01. Found: C, 61.71; H, 6.54; N, 4.00.

Dimethyl (2*S*)-2-(*N*-((3,5-Dimethoxybenzyl)oxycarbonyl))amino)-6-oxoheptane-1,7-dioate (8g). Hydroxy ester **6g** was oxidized by the procedure used to form **8a** to give **8g** as an oil in 63% yield (50% EtOAc in hexane, R_f 0.40): IR (CH_2Cl_2 cast) 3366, 2954, 1731, 1610, 1598, 1523, 1458, 1434, 1398 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.47−6.42 (m, 2H), 6.39−6.37 (m, 1H), 5.40 (d, 1H, J = 8.0 Hz), 5.02 (s, 2H), 3.82 (s, 3H), 3.76 (s, 6H), 3.73 (s, 3H), 2.88−2.84 (m, 2H), 1.92−1.80 (m, 1H), 1.77-1.61 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 194.0, 173.1, 162.5, 162.3, 156.0, 138.6, 107.0, 101.3, 67.1, 56.0, 54.1, 53.2, 52.9, 39.0, 31.7, 19.6; exact mass 411.15226 (M^+) (411.15292 calcd for $C_{19}H_{25}NO_9$). Anal. Calcd for $C_{19}H_{25}NO_9$: C, 55.47; H, 6.12; N, 3.40. Found C, 55.30; H, 5.89; N, 3.54.

Methyl *N*-(3-Butenyl)succinamide (9). To a solution of 3-butenylamine hydrochloride[46] (3.0 g, 28 mmol) in aqueous $KHCO_3$ (6.98 g, 69.8 mmol, 50 mL) cooled to 4 °C was added succinic anhydride (5.58 g, 55.8 mmol) in portions over 20 min. After a further 40 min, the solution was acidified with concentrated HCl to pH 2.0 and extracted into EtOAc (4 × 150 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo*. The solid residue was treated with an excess of ethereal diazomethane. Excess diazomethane was destroyed by addition of glacial acetic acid. Evaporation of solvent *in vacuo* gave an oil which was purified by flash chromatography (50% EtOAc in hexane, R_f 0.22) to yield **9** as an oil (3.61 g, 77%): IR ($CHCl_3$ cast) 3310, 3078, 3002, 2980, 2955, 1740, 1649, 1550, 1435 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.15 (s, 1H), 5.83−5.69 (m, 1H), 5.12−5.05 (m, 2H), 3.68 (s, 3H), 3.34−3.28 (m, 2H), 2.66 (t, 2H, J = 6.8 Hz), 2.48 (t, 2H, J = 6.8 Hz), 2.29−2.22 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 173.3, 171.2, 135.1, 116.8, 51.6, 38.4, 33.5, 30.7, 29.2; MS (CI, NH_3) *m/z* 186 (MH^+, 100%). Anal. Calcd for $C_9H_{15}NO_3$: C, 58.36; H, 8.16; N, 7.56. Found: C, 58.20; H, 8.34; N, 7.64.

Methyl 2-Hydroxy-6-(*N*-(methyl succinyl)amino)hex-4-enoate (10). The ene reaction ($FeCl_3$ catalyzed) used to prepare **5a** was employed to convert **9** to **10** as an oil in 52% yield (EtOAc, R_f 0.25): bp 185−190 °C, 0.7 mmHg; IR (CH_2Cl_2 cast) 3298, 2953, 1737, 1650, 1544, 1438 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.87 (t, 1H, J = 5.4 Hz), 5.67−5.52 (m, 1H), 4.27−4.23 (m, 1H), 4.04 (d, 1H, J = 5.6 Hz), 3.81−3.76 (m, 2H), 3.75 (s, 3H), 3.67 (s, 3H), 2.65 (t, 2H, J = 6.9 Hz), 2.55−2.48 (m, 3H), 2.44−2.35 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.1, 173.0, 171.1, 129.5, 126.3, 69.8, 51.8, 51.3, 40.7, 36.6, 30.2, 28.7; MS (CI, NH_3) *m/z* 291 (MNH_4^+, 22.7%), 274 (MH^+, 100%). Anal. Calcd for $C_{12}H_{19}NO_6$: C, 52.74; H, 7.01; N, 5.13. Found: C, 52.49; H, 7.17; N, 5.17.

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(46) Abd El Samii, Z. K. M.; Al Ashmawy, M. I.; Mellor, J. M. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2517−2522.

Methyl 2-Hydroxy-6-(*N*-(methyl succinyl)amino)hexanoate (11). Hydrogenation of **10** for 3 h using the procedure for preparation of **6a** gave **11** as an oil in 96% yield: IR ($CHCl_3$ cast) 3320, 2952, 1737, 1651, 1550, 1437 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.32 (s, 1H), 4.22−4.17 (m, 1H), 3.77 (s, 3H), 3.68 (s, 3H), 3.47 (d, 1H, J = 5.7 Hz), 3.24 (q, 2H, J = 6.3 Hz), 2.66 (t, 2H, J = 7.1 Hz), 2.47 (t, 2H, J = 7.1 Hz), 1.81−1.41 (m, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 175.2, 173.4, 171.3, 70.1, 52.1, 51.6, 39.1, 33.6, 30.7, 29.2, 28.9, 21.9; MS (CI, NH_3) *m/z* 293 (MNH_4^+, 4.5%), 276 (MH^+, 100%).

Methyl 2-Oxo-6-(*N*-(methyl succinyl)amino)hexanoate (12). Dess−Martin oxidation as described for preparation of **8a** was employed to convert **11** to **12** as a solid in 15% yield (EtOAc, R_f 0.33): mp 61−66 °C; IR ($CHCl_3$ cast) 3300, 2955, 1733, 1650, 1400 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.76 (br s, 1H), 3.87 (s, 3H), 3.69 (s, 3H), 3.27 (q, 2H, J = 6.9 Hz), 2.88 (t, 2H, J = 6.9 Hz), 2.68 (t, 2H, J = 6.6 Hz), 2.47 (t, 2H, J = 6.9 Hz), 1.69−1.62 (m, 2H), 1.59−1.51 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 193.9, 173.5, 171.4, 161.4, 52.9, 51.8, 39.0, 38.8, 31.0, 29.4, 28.8, 20.0; MS (CI, NH_3) *m/z* 291 (MNH_4^+, 4.7%), 274 (MH^+, 100%).

2-Oxo-6-(*N*-(succinyl)amino)hexanoic Acid, Dilithium Salt (13). Careful LiOH hydrolysis as described for preparation of **1a** was employed to convert **12** to solid **13** in 99% yield: mp 146 °C dec; IR (KBr) 3430, 2931, 1636, 1417 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 3.13 (t, 2H, J = 6.7 Hz), 2.71 (t, 2H, J = 7.1 Hz), 2.39−2.33 (m, 4H), 1.58−1.42 (m, 4H); ^{13}C NMR (100 MHz, D_2O + 1,4-dioxane) δ 195.6, 170.7, 165.3, 160.4, 28.8, 22.7, 23.0, 17.9, 17.8, 11.9; MS (POSFAB, Cleland) *m/z* 258 (MH^+, 6.8%), 257 (M^+, 2.2%), 252 ((M − Li + H)H^+, 4.3%), 251 ((M − Li + H)$^+$, 1.1%).

1-Methyl 11-Ethyl 6-(Carboethoxy)-2-hydroxy-4-undecene-1,11-dioate (14). The ene reaction ($FeCl_3$ catalyzed) used to prepare **5a** was employed to convert diethyl 2-allylheptane-1,7-dioate[47] to **14** as an oil in 65% yield (33% EtOAc in hexane, R_f 0.30): IR ($CHCl_3$ cast) 3480, 2936, 1732 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.56−5.53 (m, 2H), 4.28−4.24 (m, 1H), 4.15−4.09 (m, 4H), 3.76 (s, 3H), 3.06 (d, 1H, J = 6.2 Hz), 2.99−2.93 (m, 1H), 2.56−2.52 (m, 1H), 2.45−2.38 (m, 1H), 2.29 (t, 2H, J = 7.5 Hz), 1.75−1.69 (m, 1H), 1.68−1.49 (m, 2H), 1.47−1.35 (m, 1H), 1.35−1.28 (m, 2H), 1.25 (t, 6H, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 173.9, 173.4, 131.8, 126.8, 70.1, 60.4, 60.0, 52.2, 48.8, 37.2, 33.9, 31.8, 26.3, 24.4, 14.1, 14.0; MS (CI, NH_3) *m/z* 362 (MNH_4^+, 100%), 345 (MH^+, 15.2%).

6-Carboxy-2-oxo-undecane-1,11-dioic Acid (17). Hydrogenation of **14** in MeOH for 24 h using the procedure for preparation of **6a** gave **15** as an inseparable mixture of methyl and ethyl esters at the C-11 carboxyl group in 98% yield. Dess−Martin oxidation of this mixture as described for preparation of **8a** converted it to the corresponding 2-oxo mixture of esters **16** (30% EtOAc in hexane, R_f 0.25). The mixture of esters **16** (150.5 mg) was hydrolyzed by reflux in 1 N HCl (25 mL) for 2 h. Evaporation *in vacuo* afforded a solid which was dissolved in saturated $NaHCO_3$ (25 mL). The solution was washed with CH_2Cl_2 (2 × 25 mL). The aqueous layer was acidified to pH 1.0 with 1 N HCl and extracted with diethyl ether (3 × 100 mL). The combined ether extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give **17** as a waxy solid (70.0 mg, 40% over 2 steps): IR (KBr) 3430, 2931, 1636, 1417 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 9.71 (s , 3H), 2.91−2.87 (m, 2H), 2.51−2.24 (m, 3H), 1.90−1.20 (m, 10H); ^{13}C NMR (75.5 MHz, acetone-d_6) δ 196.0, 172.3, 176.0, 175.0, 45.6, 38.8, 34.0, 32.7, 32.1, 27.5, 25.5, 21.5; MS (POSFAB, Cleland) *m/z* 274 (M^+).

2-(*N*-(Succinyl)amino)heptane-1,7-dioic Acid, Trilithium Salt Dihydrate (18a). Although this could be prepared by direct acylation of α-aminopimelic acid, a more pure product was obtained by a 2-step procedure involving formation of the corresponding *N*-succinyl trimethyl ester and LiOH hydrolysis as described above for generation of **1a**. Data for the intermediate dimethyl 2-(*N*-(methyl succinyl)amino)-heptane-1,7-dioate isolated as an oil (50% EtOAc in hexane, R_f 0.11): IR ($CHCl_3$ cast) 3323, 2997, 2953, 2865, 1739, 1679, 1676, 1654, 1539, 1437 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 6.36 (d, 1H, J = 7.9 Hz), 4.55−4.50 (m, 1H), 3.67 (s, 3H), 3.62 (s, 3H), 3.59 (s, 3H), 2.66−2.57 (m, 2H), 2.53−2.46 (m, 2H), 2.43 (t, 2H, J = 7.4 Hz), 1.79−1.73 (m, 1H), 1.63−1.52 (m, 3H), 1.34−1.20 (m, 2H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 174.2, 173.6, 173.3, 171.6, 52.8, 52.4, 52.3, 51.9, 34.1, 32.5, 31.3, 29.6, 25.1, 24.8; exact mass 317.1475 (M^+) (317.14746 calcd for $C_{14}H_{23}NO_7$).

Data for 18a: mp 89−91 °C; IR (KBr) 3420, 2920, 1650, 1610, 1600, 1410 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 3.94 (dd, 1H, J = 4.8, 8.6 Hz), 2.37−2.25 (m, 4H), 2.01 (t, 2H, J = 7.6 Hz), 1.60−1.36 (m, 4H), 1.20−1.14 (m, 2H); ^{13}C NMR (90 MHz, D_2O + CH_3CN) δ 182.4, 179.9, 178.3, 174.0, 54.3, 36.5, 32.2, 31.5, 30.7, 24.8, 24.3; MS (POSFAB, Cleland) *m/z* 310 ((M − Li + Na)H^+, 2.1%), 309 ((M − Li + Na)$^+$, 11.8%), 294 (MH^+, 0.5%), 293 (M^+, 0.3%), 288 ((M − Li + H)H^+, 1.5%), 275 ((M - 3Li + 3H)$^+$, 1.4%). Anal. Calcd for $C_{11}H_{14}NO_7Li_3{\cdot}2H_2O$: C, 40.15; H, 5.51; N, 4.26. Found: C, 40.41; H, 5.26; N, 4.31.

2-(*N*-(Benzyloxycarbonyl)amino)heptane-1,7-dioic Acid, Dilithium Salt Hemihydrate (18b). The 2-step procedure described to prepare **18a** was employed to convert α-aminopimelic acid using CbzCl to **18b** (85% over 2 steps): mp 185−186.5 °C; ^1H NMR (360 MHz, D_2O) δ 7.29−7.21 (m, 5H), 4.99−4.89 (m, 2H), 3.76−3.72 (m, 1H), 1.98 (t, 2H, J = 7.5 Hz), 1.61−1.55 (m, 1H), 1.48−1.33 (m, 3H), 1.20−1.14 (m, 2H); ^{13}C NMR (90 MHz, D_2O + CH_3CN) δ 183.8, 180.2, 158.5, 129.2, 128.7, 128.1, 67.2, 57.0, 37.9, 32.2, 26.1, 25.7; IR (KBr) 3568, 3402, 3090, 2946, 1701, 1578, 1500, 1421 cm^{-1}; MS (POSFAB, Cleland) *m/z* 322 (MH^+, 19.6%), 321 (M^+, 4.3%). Anal. Calcd for $C_{15}H_{17}NO_6Li_2{\cdot}0.5H_2O$: C, 54.54; H, 5.45; N, 4.24. Found: C, 54.75; H, 5.26; N, 4.28.

(2*S*,6*RS*)-2-(*N*-(Succinyl)amino)-6-hydroxyheptane-1,7-dioic Acid, Trilithium Salt (19). Hydrolysis of **6a** with LiOH as described for preparation of **1a** gave **19** in 92% yield: mp 114−116 °C dec; IR (KBr) 3536, 3410, 1663, 1608, 1413 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 4.05 (dd, 1H, J = 8.3, 5.0 Hz), 3.93 (dd, 1H, J = 7.6, 4.7 Hz), 2.50−2.37 (m, 4H), 1.75−1.50 (m, 4H), 1.38−1.28 (m, 2H); ^{13}C NMR (90 MHz, D_2O + 1,4-dioxane) δ 176.9, 176.6, 170.6, 61.9, 50.6, 29.1, 28.3, 27.6, 26.8, 16.5; MS (POSFAB, Cleland) *m/z* 310 (MH^+, 3.6%), 309 (M^+, 9.1%).

(2*S*,6*RS*)-2-(*N*-(Succinyl)amino)-6-hydrazinoheptane-1,7-dioic Acid (20a). To a stirred suspension of trilithium salt **1a** (21.1 mg, 68.7 μmol) in anhydrous MeOH (1 mL) was added hydrazine hydrate (688 μmol, 34.5 mg, 33.5 μL). After 60 min, $NaCNBH_3$ (43.2 mg, 688 μmol) was added, and the white suspension was stirred for 16 h at 20 °C. Excess $NaCNBH_3$ was destroyed by the addition of 1 N HCl followed by stirring for 30 min. The clear solution was applied to BioRAD AG-50-WX-8 cation exchange resin (H^+ form, 10 mL). The column was eluted with water until washings were neutral and then with 0.5 N aqueous ammonia. Amino acid-containing fractions (ninhydrin) were pooled and evaporated *in vacuo*. The residue was suspended in water (2 mL) and lyophilized to give **20a** as a solid (20.4 mg, 97%): mp 92−94 °C; IR (KBr) 3416, 3329, 2956, 1577, 1401 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 4.08−4.04 (m, 1H), 3.41−3.32 (m, 1H), 2.52−2.38 (m, 4H), 1.90−1.55 (m, 4H), 1.40-1.25 (m, 2H); ^{13}C NMR (100 MHz, D_2O + CH_3CN) δ 181.6, 179.8, 175.7, 154.5, 66.5, 55.4, 33.5, 32.0, 31.5, 29.8, 21.9; MS (POSFAB, glycerol/HCl) *m/z* 306 (MH^+, 4.4%), 305 (M^+, 1.0%).

(2*S*,6*RS*)-2-(*N*-(Benzyloxycarbonyl)amino)-6-hydrazino-1,7-dioic Acid (20c). The procedure for the preparation of **20a** was employed to convert dilithium salt **1c** to **20c**. After cation exchange and concentration, the residue was purified by reverse-phase HPLC (Column: 2× Waters RadialPak Resolve 0.5 m (8 × 100 mm); solvent A 0.1% aqueous trifluoroacetic acid, solvent B 70% aqueous MeCN + 0.1% trifluoroacetic acid. Method: ∼1.0 mg injection, gradient 30% to 56% B over 13.0 min at 2.0 mL/min, detecting at 254 nm, retention time 10.4 min.) Product-containing fractions were lyophilized to afford **20c** as a hygroscopic powder (29.8 mg, 69%): mp 53−55 °C; IR (KBr) 3424, 3150, 3049, 2856, 1701, 1618, 1534, 1499, 1439, 1404 cm^{-1}; ^1H NMR (360 MHz, D_2O + CD_3CN) δ 7.34−7.28 (m, 5H), 5.03 (m, 2H), 4.09−4.03 (m, 1H), 3.68−3.64 (m, 1H), 1.79−1.63 (m, 4H), 1.45−1.41 (m, 2H); ^{13}C NMR (75.5 MHz, D_2O + CD_3CN) δ 175.0, 173.3, 158.5, 137.2, 127.8, 127.4, 126.7, 66.1, 59.6, 52.8, 29.2, 27.8, 20.2; MS (POSFAB, glycerol/HCl) *m/z* 362.2 (MNa^+, 2.5%), 340.2 (MH^+, 87.1%), 339.1 (M^+, 6.3%).

***N*-Succinyl-LL-DAP Aminotransferase (DAP-AT) Assay**. Stock solution (100 mM Tris buffer pH 8.0 containing EDTA tetrasodium

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salt (0.1 mM), NaN_3 (5 mM), bovine serum albumin (BSA, Sigma, 1.0 mg/mL), and NH_4Cl (100 mM)) was used to prepare the assay solution (pyridoxal phosphate (PLP), 3.0 mg, and β-NADPH, 6.0 mg, made up to 40.0 mL with stock solution). The solutions were made using ACS grade reagents and Milli-Q water. Assays were performed at 30 °C and contained sufficient *N*-succinyl-LL-DAP aminotransferase to give Δ-OD_{340} of 20−100 mAU_{340}/min, 1 mM substrate **1a**, 10 mM L-glutamate, 10 units of L-glutamate dehydrogenase (EC 1.4.1.4, Sigma), and assay solution to give a final volume of 1000 μL. The decrease in β-NADPH concentration was observed at 340 nm (with a reference value observed at 520 nm) over 300 s using a Hewlett Packard 8452A diode array spectrophotometer equipped with a cuvette having a water jacket. Stock solution was kept refrigerated at 4 °C for up to 6 months; assay solution was made up fresh daily. The assay conditions were varied as outlined below for kinetic analyses.

Purification of DAP-AT. All procedures were done at 4 °C, all buffers contained 10 mg/L PLP, and protein elution during chromatographic steps was monitored at 280 nm unless otherwise stated. Buffers were made using ACS grade reagents and Milli-Q water. Centrifugations were performed using a DuPont Sorvall RC-5B centrifuge equipped with a Sorvall GSA rotor (6 × 250 mL tubes). Protein determinations were done using the BioRAD reagent kit, standardized against BSA, and activity assays were performed as described above. Wild type *E. coli* (ATCC 9637) glycerol stock solution (2 × 1 mL) was added to 2 × 500 mL of a sterilized solution containing yeast extract (3 g/L), peptone (5 g/L), and glucose (5 g/L) in 2 L Erlenmeyer flasks. The solution was shaken at 200 rpm, 37 °C, for 15.5 h and then used to innoculate 50 L of modified Davis and Mingioli medium[48] (KH_2PO_4, 13.6 g/L; $(NH_4)_2SO_4$, 2.0 g/L; $MgSO_4 \cdot 2H_2O$, 200 mg/L; $FeSO_4 \cdot 7H_2O$, 0.5 mg/L; glycerol, 2.0 g/L; PLP, 10 mg/L; yeast extract, 3.0 g/L; peptone, 5.0 g/L; glucose, 5.0 g/L). Fermentation was done at 37 °C, pH 7.0, with 200 rpm stirring, using a 76 L LH2000 fermentor until the end of logarithmic growth as determined by OD_{600} (3 h). Cells were harvested by ultrafiltration (0.3 μ filter) and washed with 30 mM phosphate buffer pH 7.5. The resultant concentrate (2 L) was centrifuged (2 × 10 000 rpm, 10 min). The cells were resuspended in 30 mM phosphate buffer, pH 7.5, and the pooled cell concentrate (600 mL) was passed six times through a continuous-flow sonicator cooled to −20 °C. The sonicated cell suspension was centrifuged (8000 rpm, 15 min) to remove cell debris and the clear supernatant (450 mL) containing soluble protein decanted.

Step 1. Reactive Brown Affinity Chromatography. A portion of the soluble protein solution (230 mL, 2 g protein) was applied to a column (60 × 300 mm) of Reactive Brown-10 (Sigma) immobilized[49] on Sepharose-6CL (Sigma), which was then eluted with 30 mM phosphate buffer, pH 7.5, at a flow rate of 2 mL/min; 20 mL fractions were collected. Active fractions (tubes 31−50, total volume 380 mL) were pooled and concentrated to a volume of 120 mL using an Amicon ultrafiltration cell fitted with an Amicon YM-10 ultrafiltration membrane under N_2 (50 psi).

Step 2. Blue-3GA Affinity Chromatography. The concentrated solution from step 1 was applied to a column (60 × 300 mm) of Cibacron Blue-3GA immobilized on cross-linked 4% Agarose (Sigma), which was then eluted with 30 mM phosphate buffer pH 6.0 at a flow rate of 2 mL/min; 20 mL fractions were collected. After 900 mL of elution a pH gradient was run to 30 mM phosphate buffer, pH 8.0, over 1000 mL. Active fractions (tubes 87−119, total volume 640 mL) were pooled and concentrated to a volume of 70 mL. This solution was dialyzed three times vs 1 L of 1.0 mM phosphate buffer pH 6.8 (2 h, 15 h, and then 2 h).

Step 3. Hydroxylapatite Chromatography. The dialyzed solution from step 2 was applied to a hydroxylapatite column (BioRAD, 25 × 170 mm) which was eluted with 1 mM phosphate buffer pH 6.8 at 600 μL/min; 6 mL fractions were collected. After initial protein elution had ceased, a concentration gradient was run to 300 mM phosphate buffer, pH 6.8, over 600 mL. Active fractions (tubes 96−110, total volume 84 mL) were pooled, and glycerol (8.5 mL) was added. This solution could be stored indefinitely at −20 °C without appreciable loss of activity.

Step 4. Gel Filtration Chromatography. A portion (32 mL) of the solution from step 3 was concentrated using Amicon Centriprep ultrafiltration-10 units to a final volume of 2.5 mL. The concentrated solution was applied to a Sephadex G-75 column (Pharmacia, 15 × 300 mm) and eluted at 100 μL/min with 200 mM phosphate buffer pH 6.8; 1.5 mL fractions were collected. Active fractions (tubes 20−25, total volume 7.5 mL, calibrated molecular weight 81.5 kDa) were pooled. Glycerol (750 μL) was added and the solution frozen.

Step 5. Mono-Q Anion Exchange Chromatography. A portion (4.0 mL) of the soution from step 4 was desalted using Amicon centricon-30 filters and the residue made up to 4.0 mL with 20 mM phosphate buffer, pH 7.0. This solution was purified by HPLC chromatography using a BioRAD gradient module and UV detector (218 nm) and a Pharmacia Resource mono-Q anion exchange column (1 mL of bed volume) eluted at 1 mL/min: Eluent A, 20 mM phosphate buffer, pH 7.0; eluent B, 20 mM phosphate buffer plus 500 mM NaCl, pH 7.0. The buffers were cooled to 0 °C and *did not* contain PLP. Injections of 200 μL were made, then 0.0−2.0 min 0% B, and then gradient 2.0−26.0 min 0−80% B, then 26.0−28.0 min 80−0% B. Peaks were collected manually as they were eluted into ice cooled polypropylene test tubes. Activity was eluted in a single peak, retention time 15.3 min. To this enzyme solution was added glycerol to 10%, and the solution was frozen at −20 °C. Thawed samples of this solution were used for kinetic analyses. The solution was desalted utilizing Amicon centricon-30 ultrafiltration units to provide samples suitable for SDS PAGE.

Kinetic Analyses and Inhibition Studies. Absolute K_m values for the natural substrate **1a** and L-glutamate were obtained by measurement of initial rates (10−30 s) for a matrix of 7 substrate **1a** concentrations by five L-glutamate concentrations. Assays were performed in a 1 mL quartz cuvette containing substrate **1a**, L-glutamate, L-glutamate dehydrogenase (10 units), and assay solution to make up 1000 μL. Reactions were initiated by the addition of *N*-succinyl-LL-DAP aminotransferase (17.9 nM) and were followed at 340 nm over 300 s. The array of data points was analyzed using the computer program Enzyme Kinetics (Trinity Software, Compton, NH). Values of K_m^{app} and V_{max} were measured for the natural substrate **1a** and substrate analogues **1b**−**g** at fixed L-glutamate concentration (10 mM). Initial reaction rates (10−30 s) were determined for reactions performed in a 1 mL quartz cuvette containing substrates **1a**−**g** at seven concentrations (0.1−10.0 mM), L-glutamate (10 mM), L-glutamate dehydrogenase (10 units), and assay solution to make 1000 μL. Reactions were initiated by the addition of *N*-succinyl-LL-DAP aminotransferase (17.9 nM) and were followed at 340 nm. Initial rates (10−30 s) were plotted vs substrate concentration and kinetic parameters (K_m^{app}, V_{max}) were calculated using the EnzFitter computer program (Elsevier Biosoft, Cambridge, U.K.).

For preliminary inhibition studies, reactions were initiated by the addition of *N*-succinyl-LL-DAP aminotransferase to standard assay mixtures containing natural substrate **1a** (1 mM), L-glutamate (10mM), L-glutamate dehydrogenase (10 units), and varying concentrations (0−10 mM) of potential inhibitor made up to 1000 μL with assay solution. No significant reduction of reaction rates could be observed for **18a**, **18c,** or **19**. For detailed examination of slow-binding inhibitors **20a**,**c**, stock solution was used to prepare assay solution as described above, except that PLP was deleted from the mixture. No inhibition of the coupling enzyme, L-glutamate dehydrogenase, was observed for **20a** or **20c**. Absorbance, time pairs were collected every 15 s over 3600 s for reactions performed in a 1 mL quartz cuvette containing substrate **1a** (1 mM), L-glutamate (10 mM), L-glutamate dehydrogenase (10 units), inhibitor at seven concentrations (0.1−20.0 mM), and assay solution to make 1000 μL. Reactions were initiated by the addition of *N*-succinyl-LL-DAP aminotransferase (1.3 nM), and reaction progress was observed at 340 nm over 3600 s. The data points were fitted to the integrated rate equation

$$P = V_s t + (V_0 - V_s)(1 - \exp(-k_{obs} t))/k_{obs} + d$$

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using the program MacCurveFit[50] to obtain estimates for the parameters V_0 (initial velocity), V_s (steady-state velocity), k_{obs} (pseudo-first-order rate constant), and d (displacement from x axis). In each case the goodness of fit (residual) of the theoretical curve to the experimental data points was >99.5%. Data analysis was performed according to the methods of Morrison.[40] For preincubation studies, inhibitor (**20a** or **20c**) at varying concentrations (0−1000 μM) was added to enzyme (660 nM) in stock solution containing 10 mM L-glutamate at time zero. Aliquots of this solution were tested for residual activity at intervals over 60 min using the standard activity assay. The experiments were repeated at 0.0 mM L-glutamate concentration for varying inhibitor **20a** or **20c** concentrations (0−5 μM). Results were plotted as % residual acivity *vs* time or as % residual activity after 60 min *vs* inhibitor concentration. For reversal of inhibition studies, a solution of enzyme was treated with 10 μM inhibitor **20a** or **20c** for 60 min in 0.0 mM L-glutamate stock solution. An aliquot of this solution was then diluted 100-fold into a standard activity assay and reaction progress observed at 340 nm over 3600 s.

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Supporting Information Available: A table giving details of enzyme purification, figures showing enzyme mass spectra and an SDS PAGE gel, and plots of kinetic data for inhibition studies (11 pages). Ordering information is given on any current masthead page.

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