Structure−Activity Relationships of Pregabalin and Analogues That Target the α_2-δ Protein

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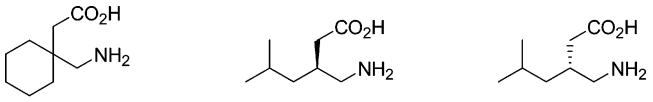
Pregabalin exhibits robust activity in preclinical assays indicative of potential antiepileptic, anxiolytic, and antihyperalgesic clinical efficacy. It binds with high affinity to the α_2-δ subunit of voltage-gated calcium channels and is a substrate of the system L neutral amino acid transporter. A series of pregabalin analogues were prepared and evaluated for their α_2-δ binding affinity as demonstrated by their ability to inhibit binding of [^{3}H]gabapentin to pig brain membranes and for their potency to inhibit the uptake of [^{3}H]leucine into CHO cells, a measure of their ability to compete with the endogenous substrate at the system L transporter. Compounds were also assessed in vivo for their ability to promote anxiolytic, analgesic, and anticonvulsant actions. These studies suggest that distinct structure activity relationships exist for α_2-δ binding and system L transport inhibition. However, both interactions appear to play an important role in the in vivo profile of these compounds.

Introduction

The γ-amino acids gabapentin **1** and pregabalin **2** (Figure 1) both have anticonvulsant, anxiolytic-like, and analgesic actions.[1] Originally developed as an add-on therapy for the treatment of partial seizures, gabapentin has shown efficacy in the treatment of postherpetic neuralgia (a type of neuropathic pain) and has shown activity in several preclinical models of neuropathic pain.[2−4] Pregabalin has more potent and robust activity than gabapentin in preclinical models of epilepsy,[5−7] neuropathic pain,[4] and anxiety.[8] In placebo-controlled clinical studies, pregabalin reduces the incidence of partial seizures, reduces pain from post-herpetic neuralgia[9] and tooth extraction,[10] and reduces symptoms of generalized anxiety disorder[11] with modest side effects including dizziness and somnolence.[12] Preliminary analyses suggest more linear oral bioavailability, greater potency, and superior efficacy for pregabalin compared to gabapentin in these clinical conditions.

Although both compounds contain the γ-amino butyric acid (GABA) functional group, there is little evidence to suggest gabapentin or pregabalin exert their pharmacological activity through direct interactions with $GABA_A$ channels, $GABA_B$ or $GABA_C$ receptors, GABA transaminase, or GABA reuptake sites, and recent studies indicate that neither compound alters brain concentration of GABA in rats.[13] While a single group has published that gabapentin is a subtype selective agonist at $GABA_B$ gb-1a-gb-2 receptor heterodimers,[14,15] a number of labs, including our own, have been unable to replicate these findings.[16,17] The precise mechanism through which gabapentin and pregabalin exhibit their pharmacological action has been the subject of many studies in recent years.[18]



1 2 3

Figure 1.

Both compounds have affinity for the large neutral amino acid system L amino acid transporter,[19] as evidenced by their ability to inhibit the uptake of [^{3}H]-leucine in CHO cells. This high volume molecular transport mechanism allows endogenous amino acids such as leucine, isoleucine, and valine to cross the blood−brain barrier. The ability of pregabalin and gabapentin to be transported by the system L amino acid transporter is thought to be important for allowing these zwitterionic molecules to cross the blood−brain barrier.[20]

Both pregabalin and gabapentin bind with high affinity to whole brain membranes.[21] Purification of solubilized pig brain isolates with enhanced binding to [^{3}H]gabapentin and subsequent protein expression studies revealed that this binding is due to the α_2-δ subunit of voltage-gated calcium channels.[22] Electron microscopy studies suggest that this protein is closely associated with the alpha pore forming subunit of calcium channels.[23] Coexpression of α_2-δ with the alpha pore-forming subunit increases calcium flux across cell membranes compared to cells expressing the pore-forming subunit alone.[24] Subsequent cloning and expression of the isoforms of this subunit have revealed that gabapentin and pregabalin bind with high affinity to both the α_2-δ-1 and α_2-δ-2 subtypes, but have negligible affinity for the α_2-δ-3 or α_2-δ-4 subtypes.[25−27]

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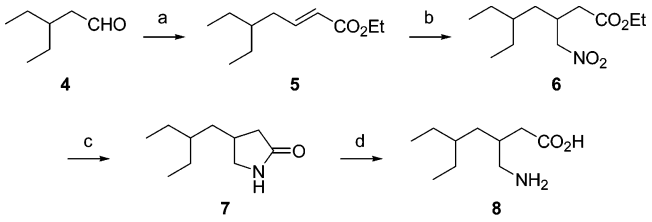
Scheme 1[a]



[a] Reagents and conditions: (a) $(EtO)_2P(O)CH_2CO_2Et$, NaH, DME (67%); (b) CH_3NO_2, DBU, CH_3CN (62%); (c) H_2, RaNi, MeOH (72%); (d) 6 N HCl (49%).

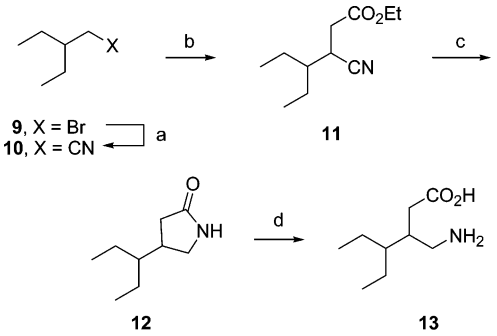
It is currently proposed that gabapentin and pregabalin binding to the α_2-δ protein mediates the functional effects these molecules have on calcium currents in activated neurons[28−30] and on stimulated neurotransmitter release.[31,32] Reduced release of excitatory neurotransmitters and peptide neuromodulators (particularly under conditions of hyperexcitability) is then thought to mediate the analgesic, anxiolytic, and anticonvulsant effects of these compounds. Consistent with this hypothesis, a series of ring-substituted analogues of gabapentin demonstrated that the ability of compounds to bind to α_2-δ (as evidenced by their ability to displace [^{3}H]gabapentin from pig brain membranes) was proportional to their activity in a model of epilepsy.[33] More recently a report of novel ligands for α_2-δ with gabapentin-like in vivo activity have been disclosed.[34] As part of a program aimed at developing a new generation of α_2-δ ligands as well as testing the α_2-δ hypothesis, a series of analogues of pregabalin were prepared and tested for their α_2-δ affinity in a [^{3}H]-gabapentin binding assay and for their ability to interact with the system L transporter.

Previous studies with analogues of gabapentin have shown the subtle nature of structure−activity relationships (SAR) in this class of compounds.[1] The interaction of molecules of this class with the α_2-δ protein from porcine brain homogenates is subject to the regio- and stereochemical influence of substituents.[33] Herein, the addition of alkyl substituents to the pregabalin scaffold and their effects on biological activity is described. The three goals of these studies were as follows: first, to study the effect of modification of the pregabalin structure with simple alkyl groups on interaction with the α_2-δ subunit; second, to study the effect of these modifications on system L transporter affinity that is believed to be the principal carrier system for the active uptake of these agents into the CNS; and finally, to study the effect of modifying the pregabalin backbone on in vivo pharmacological activity.

Synthesis

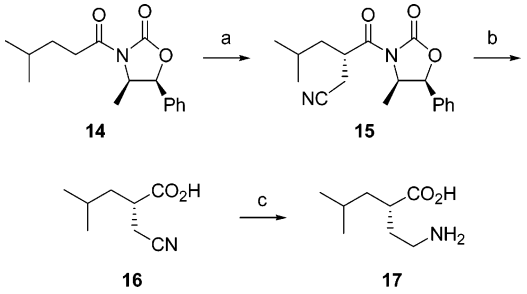
GABA derivative **8** was prepared using a nitromethane conjugate addition approach.[35] Hence, aldehyde **4**[36] underwent Horner−Emmons homologation with triethylphosphonoacetate to afford an unsaturated ester **5**, which was warmed with nitromethane and DBU in acetonitrile to give the nitroester **6** (Scheme 1). The nitroester was reduced in the presence of Raney nickel, providing a lactam **7** that was subsequently hydrolyzed in refluxing aqueous 6 N HCl. Concentration and recrystallization from acetonitrile furnished the desired racemic amino acid **8**.

Scheme 2[a]



[a] Reagents and conditions: (a) KCN, EtOH/H_2O (62%); (b) LDA, 2,2′-dipyridyl, ethyl bromoacetate, THF (24%); (c) H_2, RaNi, Et_3N, THF (44%); 6 N HCl (95%).

Scheme 3[a]



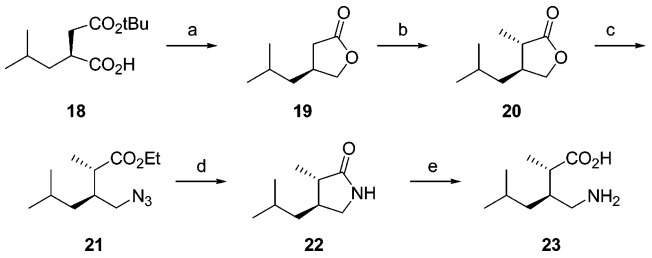
[a] Reagents and conditions: (a) LDA, bromoacetonitrile, THF (41%); (b) LiOH, H_2O_2, THF/H_2O, (100%); (c) H_2, RaNi, H_2O (30%).

Truncation of the side chain of **8** by one methylene unit was achieved via a nitrile anion alkylation process. Commercially available 2-ethylbromobutane **9** was treated with potassium cyanide in refluxing aqueous ethanol to yield nitrile **10** (Scheme 2). Deprotonation of the nitrile with LDA was followed by alkylation with ethyl bromoacetate in the presence of 2,2′-dipyridyl[37] to afford **11** in moderate yield. The addition of the bidentate ligand was critical for obtaining alkylated material, as none of the desired product was isolated without added bipyridine. Hydrogenation of the nitrile ester afforded the lactam **12**, which was subjected to hydrolysis under acidic conditions to provide amino acid **13**.

Repositioning the isobutyl backbone on the GABA template to afford **17** was accomplished via the introduction of acetonitrile to an imide enolate. As such, acyl oxazolidinone **14**[38] was selectively cyanomethylated using LDA and bromoacetonitrile (Scheme 3). Subsequent hydrolysis of the oxazolidinone **15** with lithium peroxide provided the nitrile acid **16** as a single enantiomer. Reduction of the nitrile function in the presence of Raney nickel afforded amino acid **17** substituted α to the carboxylate. The enantiomeric purity was determined by derivatization with Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) and subsequent HPLC analysis.[39]

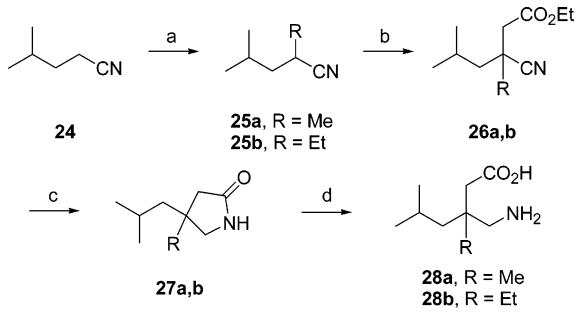
A series of pregabalin analogues were prepared that contained small alkyl substituents along the GABA backbone. Hence, the α-methyl analogue **23** (Scheme 4) was prepared from the known mono-*tert*-butyl diacid **18**.[40] Reduction and subsequent cyclization resulted in lactone **19** which could be smoothly methylated to give trans-disposed lactone **20**. Although the ratio of trans:cis isomers obtained in the alkylation step was 4.5:1,

Scheme 4[a]



[a] Reagents and conditions: (a) i. BH_3–DMS; ii. TsOH, THF (66%); (b) LDA, MeI, THF (69%); c) i. HBr, EtOH (75%); ii. NaN_3, DMSO (92%); (d) H_2, RaNi (90%); (e) 6 N HCl (49%).

Scheme 5[a]



[a] Reagents and conditions: (a) LDA, 2,2′-dipyridyl, R–I, THF; (b) LDA, 2,2′-dipyridyl, ethyl bromoacetate, THF (25%, two steps); (c) H_2, RaNi, Et_3N, THF (65–95%); (d) 6 N HCl (24–27%).

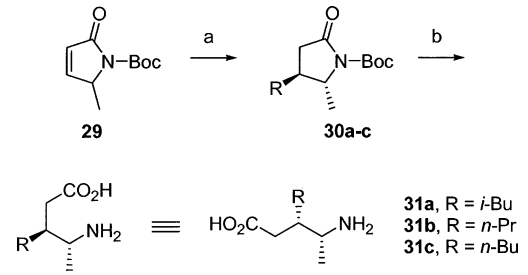
the major trans isomer could be cleanly obtained after simple chromatography. Similar trans selectivities (10–12:1 trans:cis) were observed for lactone enolate methylations in a recent synthesis of the peperomin family of natural products.[41] Ring opening of **20** was achieved by exposure to an anhydrous ethanolic solution of HBr, and the resultant alkyl bromide was displaced to provide azidoester **21**. Azide reduction and hydrolysis of the lactam **22** were effected, providing amino acid **23**.

The 3-alkyl-3-isobutyl GABA derivatives **28a**,**b** were prepared starting from isocapronitrile **24.** The cyano compound **24** was first deprotonated in the presence of 2,2′-dipyridyl and then treated with either iodomethane or iodoethane to give aliphatic nitriles **25** (Scheme 5). Repeating the deprotonation step on the crude product, this time followed by quench with ethyl bromoacetate, provided the quaternary cyanoesters **26** in 25% yield. Hydrogenation and hydrolysis of the lactams **27** as before afforded the 3,3-disubstituted amino acids **28a** and **28b**.

The introduction of a methyl group α to the amine on the GABA scaffold could be accomplished using unsaturated lactam **29**.[42] Addition of a cuprate generated from a Grignard reagent and copper(I) bromide–dimethyl sulfide complex to **29** furnished disubstituted lactams **30** (Scheme 6). The stereochemistry of the adduct was presumed to have a trans orientation of the methyl and newly installed alkyl chain on the pyrrolidinone ring based on analogy from similar conjugate lactam additions reported previously.[43] The products were exhaustively hydrolyzed to cleave the carbamate and the lactam, providing amino acids **31** containing the syn-oriented alkyl groups on the amino acid backbone.

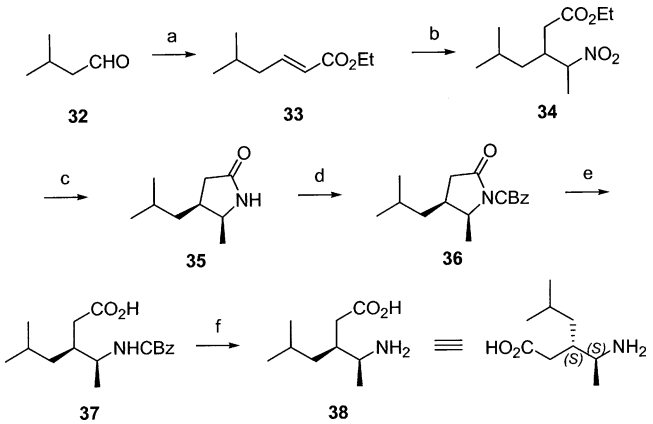
Alternately, it was found that trans-oriented alkyl substituents (on the amino acid backbone) could be installed using a variant of the nitroalkane addition

Scheme 6[a]



[a] Reagents and conditions: (a) RMgX, CuBr–DMS, THF (39–95%); (b) 6 N HCl (32–61%).

Scheme 7[a]

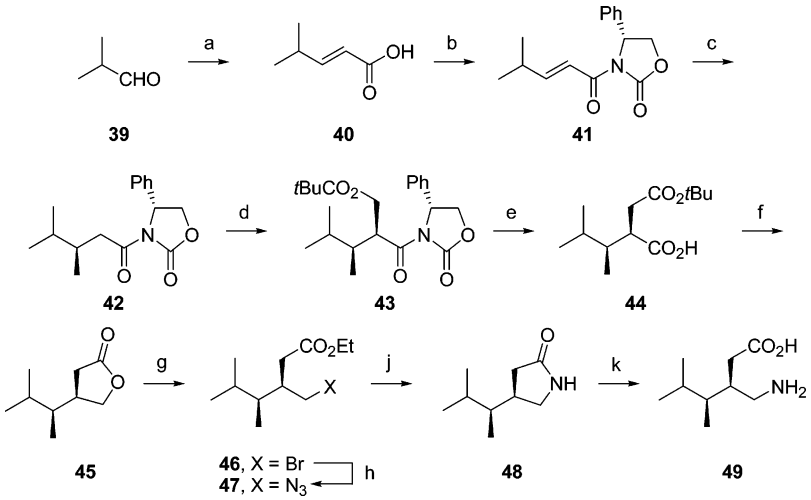


[a] Reagents and conditions: (a) $(EtO)_2P(O)CH_2CO_2Et$, NaH, DME (100%); (b) $CH_3CH_2NO_2$, DBU, CH_3CN (43%); (c) i. H_2, RaNi, MeOH (23%); ii. recrystallization; (d) NaH, benzyl chloroformate, THF (68%); (e) LiOH–H_2O, THF (21%, recrystallized); (f) H_2, Pd/C, THF/EtOH (60%).

chemistry previously employed to generate **8**. Hence, isovaleraldehyde **32** was subjected to Horner–Emmons homologation and the resultant unsaturated ester **33** exposed to nitroethane and DBU (Scheme 7). The nitroethane Michael addition resulted in a 1:1 mixture of (racemic) diastereomeric products **34**. The lactam product of nitro reduction **35** was purified by chromatography and subsequent recrystallization, resulting in an enriched (3:1) diastereomeric ratio. Carbonylbenzyloxy protection of the lactam nitrogen **36** was followed by hydrolysis under basic conditions to generate the *N*-CBz-amino acid **37**. Finally, reductive cleavage of the *N*-CBz followed by recrystallization afforded amino acid **38**. The ^1H NMR spectra (D_2O) of the hydrochloride salts of **38** and isomer **31a** were compared and determined to differ only in the region between 2.1 and 2.3 ppm. A sample containing a mixture of both **31a** and **38** showed unambiguously that the two compounds were indeed diastereomeric.

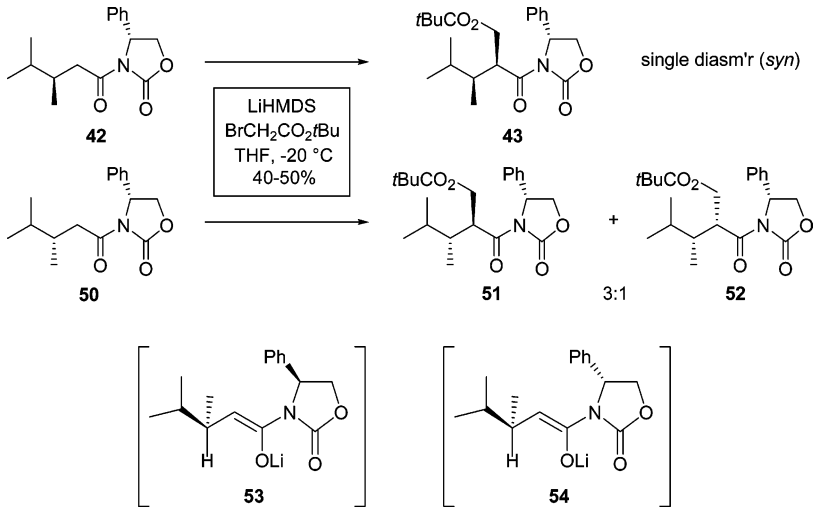
The stereocontrolled introduction of a methyl group α-to the GABA scaffold itself proved to be a formidable challenge. An auxiliary-mediated Michael addition approach was initiated through formation of α,β-unsaturated imide **41** in a two-step process from isovaleraldehyde **39** (Scheme 8). The critical introduction of the methyl group via a cuprate addition proceeded smoothly, furnishing **42** as a single diastereomer in 80% isolated yield. The masked amino acid stereocenter was then installed via an enolate generation/bromoacetate quench sequence, again giving a single diastereomer **43** in moderate yield. The absolute and relative stereochem-

Scheme 8[a]



[a] Reagents and conditions: (a) $(EtO)_2P(O)CH_2CO_2H$, ^{n}BuLi, THF (84%); (b) (*S*)-(+)-4-phenyl-2-oxazolidinone, PivCl, Et_3N, LiCl (30%); (c) MeMgCl, CuBr−DMS, THF (80%); (d) LiHMDS, *tert*-butyl-bromoacetate, THF (49%); (e) LiOH, H_2O_2, THF/H_2O (66%); (f) i. BH_3−DMS, THF; ii. TsOH, THF (84%); (g) HBr, EtOH (96%); (h) NaN_3, DMSO (71%); (j) H_2, Pd/C (quant.); (k) 6 N HCl (47%).

Scheme 9

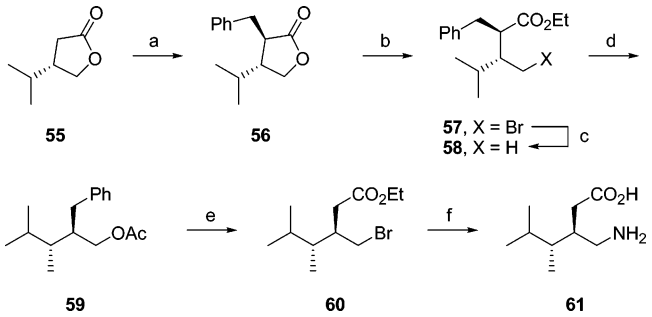


istry of **43** was confirmed by X-ray crystallography details of which are provided in the Supporting Information. Cleavage of the oxazolidinone to **44**, followed by reduction of the carboxylic acid, resulted in spontaneous cyclization to lactone **45**. Ring opening of **45** was achieved by exposure to an anhydrous ethanolic solution of HBr, and the resultant alkyl bromide **46** was displaced to provide azidoester **47**. Azide reduction and hydrolysis of lactam **48** were effected, providing amino acid **49** possessing the syn-oriented methyl and acetate groups on the aminopentane backbone.

The initial attempt to fashion the anti-orientated analogue of **49** resulted in an unexpected finding. The first step involved generating the opposite configuration (*S*) at the peripheral stereocenter, which was successfully accomplished by adding isopropyl cuprate to the imide derived from crotonic acid and (*R*)-(−)-4-phenyl-2-oxazolidinone as reported previously.[44] With the diastereomerically pure **50** in hand, the introduction of the masked amino acid stereocenter was examined (Scheme 9). Disappointingly, alkylation of the lithium enolate with *tert*-butyl bromoacetate proceeded with diminished selectivity, affording a 3:1 mixture of **51** and **52**. The ratio of isomers was rationalized by considering the hypothetical transition states **53** and **54**. Transition state **53**, produced from imide **42**, would adopt a configuration that places the bulkier isopropyl substituent on the same face of the enolate as the directing phenyl on the auxiliary. As a consequence, these two groups work in concert to facilitate entry of the electrophile syn to the methyl substituent (cf. **43**). In contrast, approach of the electrophile to enolate **54** (from imide **50**) is partially hindered by the isopropyl function, resulting in reduced facial preference. A similar alkylation result was observed in the synthesis of mycophenolic acid analogues that was rationalized as being due to 1,3-allylic strain effects on the enolate.[45] As the mixture of alkylated products **51** and **52** were found to be inseparable, an alternate route was devised.

An alternate strategy to obtain the analogue of **49** containing the anti substitution was designed to exploit the lactone ring opening reaction used previously, however in a slightly different context. Employing chiral lactone **55**,[46] a latent carboxylate function was installed in the form of a phenyl ring (Scheme 10). Hence, stereoselective enolate benzylation to **56**, followed by lactone opening (HBr/anhydrous EtOH), furnished bromoester **57**. The resulting bromomethyl group was

Scheme 10[a]



[a] Reagents and conditions: (a) LiHMDS, $PhCH_2I$, THF (58%); (b) HBr, EtOH; (c) H_2, Pd/C (45%, two steps); (d) i. LAH, THF; ii. Ac_2O, pyridine (99%); (e) i. $RuCl_3$, $NaIO_4$, $H_2O/CH_3CN/CCl_4$; ii. K_2CO_3, MeOH; iii. HBr, EtOH (66%); (f) i. NaN_3, DMSO; ii. H_2, Pd/C; iii. 6 N HCl (31%).

hydrogenated to give **58**, and further reduction of the ester followed by protection afforded acetate **59**. The carboxylate was then revealed through oxidative cleavage of the phenyl ring, and lactonization was effected followed by ring opening with HBr as before to give the trans-disposed bromoester **60**. Conversion to the amino acid **61** was carried out in an analogous fashion for the preparation of the syn isomer **49**.

Results and Discussion

Compounds were tested for their ability to displace [^{3}H]gabapentin from whole brain tissue similar to previously described methods.[21] In vivo evaluation of compounds was carried out for their ability to promote analgesic, anticonvulsant, and anxiolytic-like effects in rodent models. The antiepileptic activities of compounds were measured by their ability to prevent audiogenically induced seizures in the DBA/2 strain of mice.[47,48] Activity in this assay is expressed as a percentage of mice protected from seizure. Antihyperalgesic activity was assessed by the ability of compounds to increase paw withdrawal latency in the carageenan-induced thermal hyperalgesia model in rats.[49] Data are expressed as a percentage of unsensitized paw withdrawal latency. The anxiolytic potential of compounds was assessed by measuring their ability to reverse shock-induced suppression of drinking in the Vogel water lick conflict assay in rats.[50] Activity in this assay is expressed as a percentage of pregabalin's ability to restore punished drinking behavior at a dose of 30 mg/kg. Unless otherwise stated, the test compounds were administered orally at a dose of 30 mg/kg.

Consistent with earlier reports, pregabalin and gabapentin were both found to bind to α_2-δ (as evidenced by their ability to potently inhibit [^{3}H]gabapentin binding to pig brain membranes) and were able to inhibit [^{3}H]-leucine uptake by the system L amino acid transporter. Both compounds were active in the antiepileptic, antihyperalgesia, and anxiolytic in vivo models. Interestingly, the enantiomer of pregabalin, (*R*)-3-aminomethyl-5-methylhexanoic acid (**3**), which lacks activity in in vivo animal models of antiepileptic, antihyperalgesic, or anxiolytic activity, has significantly weaker affinity for α_2-δ[6] and the system L transporter (Table 1).

It was found that substitution of the isopropyl group in pregabalin with the diethyl moiety **8** led to a >15-fold decrease in affinity for α_2-δ while the affinity for system L transporter was retained (Table 2). However, the 1-ethylpropyl analogue **13** was approximately 2.5-fold weaker at α_2-δ but had similar potency to pregabalin at inhibiting system L transport of [^{3}H]leucine. Transposition of the isobutyl side chain in pregabalin **2** to the C2 position gave a compound **17** with reduced affinity for both the α_2-δ subunit and the system L transporter. Each of these changes led to a significant loss of in vivo activity in the epilepsy, pain, and anxiety models (Table 2).

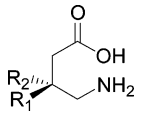
Incorporation of substituents at positions along the hexanoic acid backbone had varying effects on the affinities at α_2-δ or system L transporter. Substitution of a methyl group α to the carboxylic acid of pregabalin (compound **23**) resulted in a complete loss of affinity for the α_2-δ subunit but had relatively little effect on system L transporter affinity. A similar pattern of activity was seen with compounds **28a** and **28b** which had methyl and ethyl substituents respectively at the C-3 position of pregabalin. This substitution caused a 40 to 100-fold drop in α_2-δ affinity but a 3−5-fold increase in affinity for the system L transporter. The decrease in α_2-δ affinity observed with these molecules was also accompanied by significant losses of in vivo activity.

In contrast to substitutions at the positions α and β to the carboxylic acid, the installation of a methyl group α to the amine group and trans to the 3-isobutyl group gave compound **31a** with 2-fold enhancement of α_2-δ affinity but with a sharp decrease in system L transport potency (Table 3). Even though the loss of affinity for the system L transporter protein was considered detrimental at the time, we further evaluated the beneficial effect of the incorporation of the methyl group α-to the amine in pregabalin **2** by modifying the side chain at the C3 position. These efforts revealed that the original isobutyl side chain was the optimum side chain installed at the C3 position, as each other modification (**31b**, **31c**) led to a drop in affinity for both the α_2-δ and the system L transporter proteins. Introduction of a methyl α to the amine in a syn orientation to the 3-isobutyl group (compound **38**) resulted in a 2-fold increase in potency at the α_2-δ subunit but also led to a complete loss of activity at the system L amino acid transporter. This compound was also inactive in the in vivo models.

Taken together, these results indicate that separate structure−activity relationships exist for α_2-δ affinity and system L transporter inhibition (Figure 2). Target compounds **3**−**39** (Tables 1−3) lacked significant activity in the in vivo models of epilepsy, neuropathic pain, or anxiety at a dose of 30 mg/kg, a dose at which pregabalin showed robust activity. One explanation of this lack of activity is that both α_2-δ binding affinity and system L transporter activity are required for in vivo pharmacological activity.

It was found that the stereochemical orientation of substitution at the C4′ position of the isobutyl chain had a profound influence on the activity at α_2-δ. Introduction of a methyl group in the 4′*S* orientation of pregabalin (compound **49**) resulted in more than a 20-fold loss of affinity for α_2-δ but had an approximately 5-fold increase in system L transporter inhibitory potency. This compound lacked activity in the in vivo assays. By contrast, introduction of a methyl group into the 4′*R* position of pregabalin (compound **61**) resulted in a 4-fold

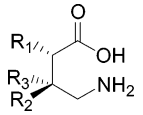
Table 1.



compd	R1	R2	stereochemistry	[^{3}H]GBP IC_{50} μM[a]	Sys. L IC_{50} μM[b]	DBA/2% protection[c]	CITH 1 h[d]	WLC PRA[e]
1	-cyclohexyl-		–	0.070 ± 0.002	30 ± 8	100	48 ± 5	60
2	isobutyl	H	*S*	0.080 ± 0.007	158 ± 35	100	52 ± 5	100 (*p < 0.05)
3	H	isobutyl	*R*	0.95 ± 0.04	355 ± 55	0	NA[f]	NA

[a] IC_{50} is the concentration (μM) producing half-maximal inhibition of the specific binding of [^{3}H]gabapentin binding to pig brain membranes. [b] IC_{50} is the concentration (μM) producing half-maximal inhibition of the uptake of [^{3}H]leucine into CHO cells. [c] % protection is the fraction of DBA/2 mice (out of five animals) protected from audiogenically induced tonic seizures by a 30 mg/kg dose of the test compound. [d] Inhibition of carageenan-induced thermal hyperalgesia in rats was assessed 1 h after administration of a 30 mg/kg dose of test compound. [e] The ability of an oral dose of test compounds (30 mg/kg) to restore punished drinking behavior (as evidenced by number of shocks taken) in rats is expressed as the percent reference activity (PRA) compared to a 30 mg/kg dose of pregabalin which is defined as 100%. [f] NA = not active, i.e., not statistically different from controls.

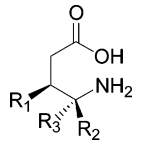
Table 2.



compd	R1	R2	R3	stereochemistry	[^{3}H]GBP IC_{50} μM[a]	Sys. L IC_{50} μM[b]	DBA/2% protection[c]	CITH 1 h[d]	WLC PRA[e]
8	H	2-ethylbutyl	H	*R/S*	1.33 ± 0.06	142 ± 9	0	NA[f]	NA
13	H	1-ethylpropyl	H	*R/S*	0.203 ± 0.02	146 ± 51	20	NA	NA
17	isobutyl	H	H	*R/S*	2.06 ± 0.95	>10000	0	–	NA
23	methyl	isobutyl	H	*R/S*	>10	117 ± 28	0	–	NA
28a	H	isobutyl	methyl	*R/S*	3.3 ± 0.26	89 ± 13	0	NA	NA
28b	H	isobutyl	ethyl	*R/S*	8.56 ± 1.94	60 ± 13	20	–	NA

[a–f] See Table 1.

Table 3.



compd	R1	R2	R3	stereochemistry	[^{3}H]GBP IC_{50} μM[a]	Sys. L IC_{50} μM[b]	DBA/2% protection[c]	CITH 1 h[d]	WLC PRA[e]
31a	isobutyl	H	Me	*R/SR/S*	0.48 ± 0.17	>100000	0	NA[f]	NA
31b	*n*-propyl	H	Me	*R/S*	1.29 ± 0.46	>8300	0	NA	NA
31c	*n*-butyl	H	Me	*R/S*	1.03 ± 0.20	>8300	0	NA	NA
38	isobutyl	Me	H	*R/S*	0.039 ± 0.008	>10000	0	NA	NA
49	3-methyl-2-butyl	H	H	3*R*,4′*S*	1.7 ± 0.06	28[g]	20	NA	NA
61	3-methyl-2-butyl	H	H	3*R*,4′*R*	0.021± 0.003	157 ± 3	100	71 ± 7	152 (*p < 0.05)

[a–f] See Table 1. [g] IC_{50} from single determination.

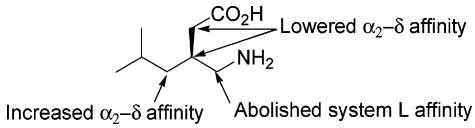


Figure 2. Summary of SAR of pregabalin substitution.

increase in α_2-δ affinity and similar system L transporter potency to pregabalin. Compound **61** had potent in vivo activity across a variety of assays in which pregabalin is also active. At a dose of 30 mg/kg, compound **61** had complete protection in the DBA2 anticonvulsant model. In the carageenan-induced thermal hyperalgesia model, **61** showed an increase in paw withdrawal latency. The compound was also active in the Vogel conflict assay predictive of anxiolytic activity.

Conclusions

Previous studies have shown that pregabalin has robust activity in preclinical models predictive of clinical efficacy in anxiety, epilepsy, and neuropathic pain, and that the molecule interacts with the α_2-δ subunit of calcium channels and is a substrate for the system L transporter. A study of a series of pregabalin analogues demonstrates distinct structure−activity relationships for these two putative molecular targets. Changes to the size and stereochemical orientation of substituents on the pregabalin framework had a dramatic effect on in vitro affinities for α_2-δ and System L. These data indicate that the size and precise stereochemical orientation of substituents are critical to interactions at these two proteins as summarized in Figure **2**. Because it had optimal properties at both α_2-δ and the system L transporter, compound **61** had activity across the same range of in vivo models for anxiety, epilepsy, and neuropathic pain as pregabalin.

The correlation between the in vitro and in vivo activities for these compounds is demonstrated in the three-dimensional plot of α_2-δ binding affinity (*X*-axis)

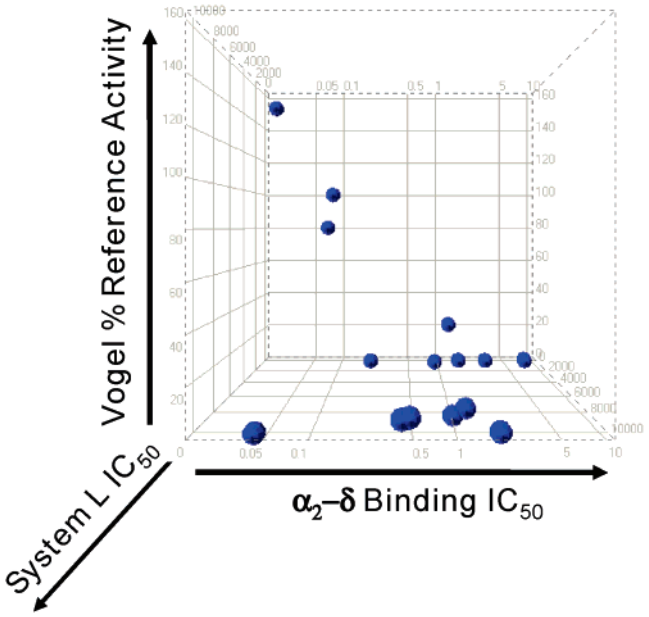


Figure 3. Correlation of in vitro and in vivo activities.

and System L inhibitory potency (*Z*-axis) with activity in the Vogel conflict model of anxiety (*Y*-Axis) as shown in Figure 3. While the correlation of in vitro activity with this in vivo effect is not perfectly linear possibly due to factors such as differences in oral absorption or clearance rates, analogues that show increased α_2-δ binding affinity and System L inhibitory potency (toward the left and back part of the grid) are associated with activity in the anxiety model.

Taken together, these results suggest that the system L amino acid transporter is required to allow these zwitterionic molecules to gain access to the CNS. Subsequent interaction of drug molecules with the α_2-δ subunit of calcium channels is required for the observed pharmacological activity of pregabalin.

Experimental Section

Unless otherwise indicated, all reagents were purchased from Aldrich chemical company and used without purification. All reactions were monitored by thin-layer chromatography on Merck glass plates precoated with 0.25 mm of silica gel. Chromatography for purification was done with Merck silica gel (230−400 mesh) or Elution Solution Flashelute cartridges packed with 32−63 μM silica gel. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Mass spectra were obtained on a Micromass Platform LC mass spectrometer and are chemical ionization spectra. Proton NMR spectra were obtained on either a Varian 300 or a Varian 400 spectrometer with tetramethylsilane as an internal standard. Multiplicity is indicated as follows: s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets, t = triplet, q = quartet, and m = multiplet. Combustion analyses were performed by either Robertson Microlit Laboratories or QTI Laboratories.

5-Ethylhept-2-enoic Acid Ethyl Ester 5. Sodium hydride (60% dispersion in mineral oil, 3.0 g, 0.75 mmol) was washed with hexane and then taken up in DME (150 mL). To the slurry was added triethylphosphonoacetate (80 mL, 0.4 mol) slowly. After the addition was complete, the mixture was stirred for 20 min at which time a solution of 3-ethyl-pentanal **4**[36] (8.4 g, 74 mmol) in 50 mL of DME was added. The mixture was refluxed 3 h, cooled, and concentrated. The residue was partitioned between hexanes and water, and the phases were separated. The aqueous phase was extracted twice with hexanes, and the combined organic phases were washed twice with brine, dried (Na_2SO_4), and concentrated to furnish 8.6 g (67%) of 5-ethylhept-2-enoic acid ethyl ester **5** as a colorless oil which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 6.90 (m, 1H), 5.78 (d, J = 15.6 Hz, 1H), 4.12 (m, 2H), 2.12 (m, 2H), 1.29 (m, 8H), 0.83 (m, 6H).

5-Ethyl-3-nitromethylheptanoic Acid Ethyl Ester 6. To a solution of 5-ethylhept-2-enoic acid ethyl ester **5** (8.5 g, 49 mmol) in 35 mL of acetonitrile were added nitromethane (15 g, 246 mmol) and DBU (7.6 g, 50 mmol). The mixture was heated at 65 °C for 4 h, cooled, and concentrated. The residue was partitioned between Et_2O and sat. NH_4Cl (aq), and the phases were separated. The aqueous phase was extracted twice with Et_2O, and the combined organic phases were washed with 0.1 N HCl, twice with water, and twice with brine, dried (Na_2SO_4), and concentrated. The crude oil was filtered through a plug of silica gel, eluting first with hexanes and then with 30% EtOAc/hexanes, to afford 7.5 g (62%) of 5-ethyl-3-nitromethylheptanoic acid ethyl ester **6** as a light brown oil: ^{1}H NMR ($CDCl_3$) δ 4.46 (dd, J = 12.2, 6.6 Hz, 1H), 4.39 (dd, J = 12.5, 5.9 Hz, 1H), 4.11 (m, 2H), 2.64 (m, 1H), 2.39 (d, J = 6.4 Hz, 2H), 1.26 (m, 10H), 0.82 (m, 6H).

4-(2-Ethylbutyl)pyrrolidin-2-one 7. To a solution of 5-ethyl-3-nitromethylheptanoic acid ethyl ester **6** (3.1 g, 12.6 mmol) in 100 mL of MeOH was added RaNi (2 g), and the mixture was hydrogenated in a Parr shaker at 48 psi for 15 h. Additional RaNi (1 g) was added and the hydrogenation continued for 3 h. The mixture was filtered and concentrated. The residue was filtered through a silica gel plug eluting with EtOAc to provide 1.55 g (72%) of 4-(2-ethylbutyl)pyrrolidin-2-one **7** as an amber oil: ^{1}H NMR ($CDCl_3$) δ 5.65 (br s, 1H), 3.44 (m, 1H), 2.96 (m, 1H), 2.51 (m, 1H), 2.38 (dd, J = 16.6, 8.6 Hz, 1H), 1.95 (dd, J = 16.8, 8.6 Hz, 1H), 1.35 (m, 2H), 1.27 (m, 4H), 1.17 (m, 1H), 0.81 (m, 6H).

3-Aminomethyl-5-ethylheptanoic Acid Hydrochloride 8. 4-(2-ethylbutyl)pyrrolidin-2-one **7** (1.5 g, 8.9 mmol) was taken in 100 mL of 6 N HCl and the mixture refluxed 18 h. The mixture was cooled and washed twice with Et_2O. The aqueous phase was concentrated and the residue crystallized from EtOAc. Recrystallization from acetonitrile afforded 0.98 g (49%) of 3-aminomethyl-5-ethylheptanoic acid hydrochloride **8** as a colorless solid, mp 105−170 °C (starts melting at 105 °C, a clear liquid at 170 °C): ^{1}H NMR (DMSO-d_6) δ 2.69 (d, J = 6.6 Hz, 2H), 2.38 (dd, J = 16.1, 5.6 Hz, 1H), 2.15 (dd, J = 16.1, 6.8 Hz, 1H), 2.02 (m, 1H), 1.15 (m, 7H), 0.75 (app t, J = 6.8 Hz, 6H). MS (m/z) 188 (M + 1). Anal. ($C_{10}H_{21}NO_2$·HCl) C, H, N, Cl.

3-Ethylpentanenitrile 10. A solution of 2-ethylbromobutane **9** (Eastman, 5.0 g, 30.3 mmol) and KCN (2.2 g, 33.3 mmol) in 50 mL of 15% H_2O/EtOH was warmed to reflux overnight. The reaction mixture was cooled to room temperature and diluted with 100 mL of water. The organic products were extracted into CH_2Cl_2 (3 × 50 mL) and the combined extracts dried over Na_2SO_4. Evaporation of the solvent afforded the product containing residual CH_2Cl_2 which was removed by repeated (3×) addition and evaporation of hexane (50 mL) to yield 2.1 g (62%) of 3-ethylpentanenitrile **10**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (d, J = 5.9 Hz, 2H), 1.53 (m, 1 H), 1.42 (m, 4 H), 0.86 (m, 6 H).

3-Cyano-4-ethylhexanoic Acid Ethyl Ester 11. A solution of freshly prepared LDA (18.9 mmol) in 50 mL of THF was added to a solution of 3-ethylpentanenitrile **10** (2.1 g, 18.9 mmol) and 2,2′-dipyridyl (0.03 g, 0.19 mmol) in 50 mL of THF at −78 °C. After 1.5 h, the solution was transferred via cannula to a solution of ethyl bromoacetate (6.3 g, 37.8 mmol) in 50 mL of THF at −40 °C. The reaction mixture was allowed to warm to room temperature overnight and then quenched by addition of 50 mL of 1.0 N HCl. The THF was evaporated, the residue was extracted with CH_2Cl_2 (3 × 50 mL), and the combined extracts were dried over Na_2SO_4. Evaporation of the solvents followed by chromatography of the remaining oil provided 0.9 g (24%) of 3-cyano-4-ethylhexanoic acid ethyl ester **11**. ^{1}H NMR ($CDCl_3$) δ 4.22 (q, J = 4.4 Hz, 2H), 3.19 (m, 1H),

2.69 (dd, J = 16.5, 8.6 Hz, 1H), 2.5 (dd, J = 16.6, 6.0 Hz, 1H), 1.42 (m, 5H), 1.28 (t, J = 7.1 Hz, 3H), 0.9 (t, J = 8.3 Hz, 6H).

4-(1-Ethylpropyl)pyrrolidin-2-one 12. 3-Cyano-4-ethylhexanoic acid ethyl ester **11** (0.9 g, 4.5 mmol) was hydrogenated over RaNi in THF at room temperature. When the theoretical amount of H_2 was taken up, the catalyst removed by filtration, and the filtrate evaporated. The remaining oil was chromatographed on silica gel in EtOAc to give 0.31 g (44%) of 4-(1-ethylpropyl)pyrrolidin-2-one **12** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.43 (s, 1H), 3.40 (t, J = 8.7 Hz, 1H), 3.04 (t, J = 8.8 Hz, 1H), 2.43 (m, 1H), 2.33 (m, 1H), 2.02 (dd, J = 16.5, 10.1 Hz, 1H), 1.42 (m, 1H), 1.35 (m, 1H), 1.26 (m, 3H), 0.82 (t, J = 7.5 Hz, 6H).

3-Aminomethyl-4-ethylhexanoic Acid Hydrochloride 13. 4-(1-Ethylpropyl)pyrrolidin-2-one **12** (0.31 g, 2.0 mmol) was heated to reflux in 50 mL of 6.0 N HCl for 8 h. The reaction mixture was cooled and concentrated. The remaining solid was triturated with EtOAc and dried under vacuum at room temperature with P_2O_5 to afford 0.4 g (95%) of 3-aminomethyl-4-ethylhexanoic acid hydrochloride **13** as a colorless solid, mp171−173 °C: ^{1}H NMR (DMSO-d_6) δ 7.82 (m, 1H), 2.72 (m, 1H), 2.63 (m, 1H), 2.23 (m, 2H), 2.15 (m, 1H), 1.15 (m, 4H), 0.82 (m, 6H). MS (m/z) M^+ = 174. Anal. ($C_9H_{19}NO_2$·HCl) C, H, N.

5*S*-Methyl-3-(4*R*-methyl-2-oxo-5*S*-phenyloxazolidine-3-carbonyl)hexanenitrile 15. To diisopropylamine (1.3 g, 0.013 mol) in THF (40 mL) at −78 °C was added n-BuLi (1.6 M in hexanes, 8.25 mL, 0.013 mol). After 15 min oxazolidinone **14** (3 g, 0.011 mol) in THF (25 mL) was added over 5 min. After 40 min bromoacetonitrile (6.5 g, 0.055 mol) in THF (15 mL) was added in one portion, and after 3.5 h the reaction was quenched with sat. NH_4Cl (aq). The phases were separated, the aqueous phase was extracted with ether, and the combined organics were dried ($MgSO_4$) and concentrated. Flash chromatography of the residue (7:1 hexanes/EtOAc) afforded 5*S*-methyl-3-(4*R*-methyl-2-oxo-5*S*-phenyloxazolidine-3-carbonyl)hexanenitrile **15** (1.41 g, 41%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.38 (m, 3H), 7.28 (m, 2H), 5.67 (d, J = 7.1 Hz, 1H), 4.74 (m, 1H), 4.18 (m, 1H), 2.62 (m, 2H), 1.71 (m, 1H), 1.61 (m, 2H), 1.47 (m, 1H), 0.94 (m, 9H). IR (KBr, cm^{-1}): 2967, 2935, 1774, 1696, 1345, 1194.

(*S*)-2-(2-Aminoethyl)-4-methylpentanoic Acid 17. To nitrile **15** (0.81 g, 2.5 mmol) in THF (10 mL) at −10 °C was added LiOH (83 mg) in H_2O (4 mL). The solution was stirred at −10 °C for 6 h and the solution diluted with water and concentrated to ca. half the volume. The mixture was extracted with CH_2Cl_2 and the aqueous phase acidified to pH 1 with concentrated HCl. The aqueous phase was extracted with EtOAc, and the combined organic phases were dried ($MgSO_4$) and concentrated. The crude acid **16** (670 mg, 4.3 mmol) was treated with NH_4OH (5 mL), EtOH (45 mL), and Raney Nickel (0.5 g) under an atmosphere of hydrogen for 24 h. The mixture was filtered and the filtrate concentrated and tritiated with 1 M HCl in ether. The residue was subjected to ion-exchange chromatography (Dowex 50WX8−200) and then recrystallized from MeOH/EtOAc to give 0.20 g (30%) of (*S*)-2-(2-aminoethyl)-4-methylpentanoic acid **17** as a white solid: $[\alpha]_D$ +4.6 (c 1.0, MeOH). ^{1}H NMR (CD_3OD) δ 2.89 (t, J = 7.3 Hz, 2H), 2.35 (m, 1H), 1.70 (m, 2H), 1.63 (m, 2H), 1.15 (d, J = 5.6 Hz, 1H), 0.91 (dd, J = 9.8, 6.4 Hz, 6H). MS (m/z) M^+ = 160. Anal. ($C_8H_{18}NO_2$) C, H, N. The enantiopurity was determined by mixing 1 mL of **17** (2 mg/mL in 1:1 CH_3CN:H_2O), 0.5 mL of 5 mg/mL 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide in CH_3CN, and 0.1 mL of 84 mg/mL $NaHCO_3$ in water. The mixture was sealed and heated to 50 °C for 1 h. The solution was cooled to ambient temperature and injected onto a Phenomenex Luna phenylhexyl column (150 mm × 4.6 mm). A gradient of 20% A:B (A = 0.1% formic acid in water, B = 0.1% formic acid in CH_3CN) at 0.8 mL/min was increased to 80% B at 30 min. The Marfey's derivative of **17** was detected at λ = 330 nm (confirmed by MS) as a single peak with a retention time of 20.38 min. The peak corresponding to excess Marfey's reagent was verified by running a blank sample containing only the reagent, prepared as above in the absence of amino acid.

(4*S*)-Isobutyldihydrofuran-2-one 19. To a solution of (2*S*)-isobutylsuccinic acid *tert*-butyl ester **18** (5.0 g, 21.7 mmol) in 170 mL of THF at 0 °C was added borane−dimethyl sulfide complex (2.0 M solution in THF, 10.9 mL, 21.7 mmol). The mixture was allowed to warm slowly to room temperature after addition and stirred 16 h at which time methanol (160 mL) was added. The mixture was stirred 1 h and then concentrated to 5.0 g of a clear oil. The crude hydroxyester was taken up in 60 mL of THF, p-toluenesulfonic acid (0.07 g) was added and the mixture heated to reflux for 4 h. The mixture was cooled to room temperature and concentrated to remove THF. The residue was partitioned between Et_2O and sat. Na_2CO_3 (aq), the phases were separated, and the organic phase was washed with brine, dried ($MgSO_4$), and concentrated. Flash chromatography of the residue (20% EtOAc/hexanes) afforded 1.96 g (66%) of (4*S*)-isobutyldihydrofuran-2-one **19** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 4.38 (m, 1H), 3.85 (m, 1H), 2.59 (m, 2H), 2.12 (m, 1H), 1.54 (m, 1H), 1.32 (m, 2H), 0.88 (app t, J = 6.3 Hz, 6H).

(4*S*)-Isobutyl-(3*S*)-methyldihydrofuran-2-one 20. To a solution of diisopropylamine (1.4 mL, 11.0 mmol) in 10 mL of THF at 0 °C was added n-butyllithium (1.6 M solution in hexanes, 6.9 mL, 11.0 mmol) quickly dropwise. The solution was stirred at 0 °C 5 min and then cooled to −78 °C at which time (4*S*)-isobutyldihydrofuran-2-one **19** in 5 mL of THF was added over 10 min. The mixture was stirred at −78 °C for 20 min at which time iodomethane (1.7 g, 12.0 mmol) was added in one portion. The mixture was allowed to warm to room temperature, stirred 2 h, and then partitioned between Et_2O and sat. NH_4Cl (aq). The phases were separated, and the organic phase was washed with brine, dried ($MgSO_4$), and concentrated to provide a crude 4.5:1 mixture of diastereomers. The residue was subjected to flash chromatography (20% EtOAc/hexanes) to provide 0.98 g (69%) of (4*S*)-isobutyl-(3*S*)-methyldihydrofuran-2-one **20** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 4.36 (m, 1H), 3.72 (m, 1H), 2.13 (m, 2H), 1.55 (m, 1H), 1.45 (m, 1H), 1.27 (m, 1H), 1.21 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 2.9 Hz, 3H), 0.88 (d, J = 2.9 Hz, 3H).

(3*S*)-Azidomethyl-(2*S*,5)-dimethylhexanoic Acid Ethyl Ester 21. A solution of (4*S*)-isobutyl-(3*S*)-methyldihydrofuran-2-one **20** (0.97 g, 6.4 mmol) in anhydrous EtOH (30 mL) was cooled to 0 °C and saturated with hydrogen bromide. The mixture was allowed to warm slowly to room temperature at which time the mixture was again saturated with HBr. The saturation process was continued until complete by GC analysis. The mixture was partitioned between hexanes and brine, the phases were separated, and the organic phase was dried (Na_2SO_4) and concentrated to afford 1.28 g of a pale yellow oil. The residue was dissolved in DMSO (6.0 mL), sodium azide (0.34 g, 5.2 mmol) was added, and the mixture was warmed to 60 °C for 20 h. The mixture was partitioned between hexanes and water, the phases were separated, and the organic phase was washed with brine, dried (Na_2SO_4), and concentrated to furnish 1.04 g (69%, two steps) of (3*S*)-azidomethyl-(2*S*,5)-dimethylhexanoic acid ethyl ester **21** as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 4.11 (m, 2H), 3.30 (dd, J = 12.5, 5.1 Hz, 1H), 3.23 (dd, J = 12.5, 7.3 Hz, 1H), 2.60 (m, 1H), 2.01 (m, 1H), 1.55 (m, 1H), 1.23 (t, J = 7.1 Hz, 3H), 1.06 (d, J = 7.1 Hz, 3H), 0.84 (m, 6H).

(4*S*)-Isobutyl-(3*S*)-methylpyrrolidin-2-one 22. To a solution of (3*S*)-azidomethyl-(2*S*,5)-dimethylhexanoic acid ethyl ester **21** (1.04 g, 4.4 mmol) in methanol (50 mL) was added Raney nickel (1.0 g), and the mixture was hydrogenated in a Parr shaker at 48 psi for 4 h. The mixture was filtered and concentrated. Flash chromatography of the residue (20% EtOAc/CH_2Cl_2) gave 0.61 g (90%) of (4*S*)-isobutyl-(3*S*)-methylpyrrolidin-2-one **22** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.63 (br s, 1H), 3.39 (app t, J = 8.1 Hz, 1H), 2.87 (app t, J = 8.8 Hz, 1H), 2.01 (m, 2H), 1.56 (m, 1H), 1.42 (m, 1H), 1.28 (m, 1H), 1.15 (d, J = 6.8 Hz, 3H), 0.88 (app t, J = 7.3 Hz, 6H).

(3*S*)-Aminomethyl-(2*S*,5)-dimethylhexanoic Acid Hydrochloride 23. To (4*S*)-isobutyl-(3*S*)-methylpyrrolidin-2-one **22** (0.60 g, 3.9 mmol) was added 6 N HCl (15 mL) and the mixture heated to reflux 20 h, cooled, and concentrated. The

residue was triturated with EtOAc and filtered, and the solid recrystallized from MeOH/EtOAc/heptane to provide 0.40 g (49%) of (3*S*)-aminomethyl-(2*S*,5)-dimethylhexanoic acid hydrochloride **23** as a colorless crystalline solid, mp 167−169 °C: $[\alpha]_D$ +12.7 (*c* 0.22, H_2O). ^{1}H NMR (DMSO-d_6) δ 12.3 (br s, 1H), 8.00 (br s, 3H), 2.65 (m, 3H), 2.11 (m, 1H), 1.51 (m, 1H), 1.07 (m, 1H), 0.96 (m, 1H), 0.91 (d, J = 7.1 Hz, 3H), 0.79 (app t, J = 6.3 Hz, 6H). Anal. ($C_9H_{19}NO_2$·HCl) C, H, N, Cl.

2,4-Dimethylvaleronitrile 25a. Prepared according to the procedure outlined for **25b** below. Physical and spectroscopic properties of **25a** were identical to those described in the literature.[51]

2-Ethyl-4-methylpentanenitrile 25b. A solution of 4-methylpentanenitrile **24** (10.0 g, 103 mmol) and 2,2′-dipyridyl (1.6 g, 10.2 mmol) in 50 mL of THF was added to a solution of freshly generated LDA in 100 mL of THF at −78 °C. After 10 min, iodoethane (16.0 mL, 200 mmol) was added and the mixture stirred at −78 °C for 1 h. The reaction was quenched by addition of 150 mL of 1.0 N HCl and warmed to room temperature. The THF was evaporated and the mixture extracted with Et_2O (3 × 50 mL). The combined extracts were washed with 1.0 N HCl (3 × 50 mL) and sat. $NaHCO_3$ (2 × 50 mL). Drying over Na_2SO_4 followed by evaporation of the solvent and atmospheric distillation (bp 170−178 °C) yielded 10.8 g (84%) of 2-ethyl-4-methylpentanenitrile **25b** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.58 (m, 1H), 1.85 (m, 1H), 1.58 (m, 3H), 1.30 (m, 1H), 1.08 (t, J = 7.3 Hz, 3H), 0.94 (m, 6H).

3-Cyano-3,5-dimethylhexanoic Acid Ethyl Ester 26a. Prepared as described above for compound **11** using 2,4-dimethylvaleronitrile **25a**. Yield of **26a** was 25%: ^{1}H NMR ($CDCl_3$) δ 4.13 (q, J = 7.1 Hz, 2H), 2.58 (m, 1H), 2.44 (d, J = 15.4 Hz, 1H), 1.81 (dt, J = 13.0, 6.6 Hz, 1H), 1.60 (m, 2H), 1.40 (s, 3H), 1.22 (t, J = 7.1 Hz, 3H), 0.97 (dd, J = 6.6, 4.6 Hz, 6H).

3-Cyano-3-ethyl-5-methylhexanoic Acid Ethyl Ester 26b. Prepared as described above for compound **11** using 2-ethyl-4-methylpentanenitrile **25b** (10.8 g, 86.2 mmol). Yield of **26b** 5.5 g (30%): ^{1}H NMR ($CDCl_3$) δ 4.14 (q, J = 7.1 Hz, 2H), 2.57 (s, 2H), 1.83 (ddd, J = 12.6, 6.6, 6.4 Hz, 1H), 1.73 (t, J = 7.5 Hz, 2H), 1.60 (m, 1H), 1.52 (m, 1H), 1.24 (t, J = 7.1 Hz, 3H), 1.01 (ddd, J = 14.8, 7.3, 7.2 Hz, 9H).

4-Methyl-4-isobutylpyrrolidin-2-one 27a. Prepared as described above for 4-(1-ethyl-propyl)pyrrolidin-2-one **7**, using 3-cyano-3,5-dimethylhexanoic acid ethyl ester **26a** (1.95 g, 9.9 mmol). Yield of **27a** 1.0 g (65%): mp = 59−61 °C.

4-Ethyl-4-isobutylpyrrolidin-2-one 27b. Prepared as described above for 4-(1-ethyl-propyl)pyrrolidin-2-one **7**, using 3-cyano-3-ethyl-5-methylhexanoic acid ethyl ester **26b** (3.0 g, 14.2 mmol). Yield of **27b** 2.2 g (95%): ^{1}H NMR ($CDCl_3$) δ 5.87 (s, 1H), 3.10 (s, 2H), 2.12 (m, 2H), 1.66 (dt, J = 12.9, 6.5 Hz, 1H), 1.49 (q, J = 7.2 Hz, 2H), 1.35 (s, 2H), 0.86 (m, 9H).

3-Aminomethyl-3,5-dimethylhexanoic Acid 28a. Prepared as described above for compound **13** using 4-isobutyl-4-methylpyrrolidin-2-one **27a** (1.0 g, 6.4 mmol). Yield of **28a** 0.27 g (24%), mp 144−146 °C (dec): ^{1}H NMR (DMSO-d_6) δ 7.87 (m, 2H), 3.57 (s, 1H), 2.89 (m, 1H), 2.40 (s, 1H), 2.3 (s, 1H), 1.64 (m, 1H), 1.26 (m, 2H), 0.98 (s, 3H), 0.87 (m, 6H). MS (*m/z*) M^+ = 174. Anal. ($C_9H_{19}NO_2$·0.15 H_2O) C, H, N.

3-Aminomethyl-3-ethyl-5-methylhexanoic Acid Hydrochloride 28b. Prepared as described above for compound **13** using 4-ethyl-4-isobutylpyrrolidin-2-one **27b** (2.2 g, 12.9 mmol). Yield of **28b** 27%, mp 133−135 °C: ^{1}H NMR (DMSO-d_6) δ 7.69 (s, 2H), 2.89 (d, J = 13.7 Hz, 1H), 2.78 (d, J = 13.7 Hz, 1H), 2.27 (s, 2H), 1.61 (s, 1H), 1.36 (s, 2H), 1.22 (s, 2H), 0.85 (m, 6H), 0.73 (d, J = 7.3 Hz, 3H). MS (*m/z*) M^+ = 188. Anal. ($C_{10}H_{21}NO_2$·1.1HCl) C, H, N, Cl.

3-Isobutyl-2-methyl-5-oxopyrrolidine-1-carboxylic Acid *tert*-Butyl Ester 30a. To a suspension of copper(I) bromide−dimethyl sulfide complex (5.14 g, 25 mmol) in 150 mL of THF at −10 °C was added isobutylmagnesium bromide (2.0 M soln. in Et_2O, 25 mL, 50 mmol). The mixture was cooled to −40 °C at which time a solution of 2-methyl-5-oxo-2,5-dihydropyrrole-1-carboxylic acid *tert*-butyl ester **29**[42] (4.93 g, 25 mmol) was added over 1.5 h. After the addition was complete, the reaction was quenched with 350 mL of sat. NH_4Cl (aq) and concentrated to remove THF. The residue was extracted three times with Et_2O, and the combined organic phases washed with sat. NH_4Cl (aq), brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (3:1 hexanes:EtOAc) to yield 6.1 g (95%) of 3-isobutyl-2-methyl-5-oxopyrrolidine-1-carboxylic acid *tert*-butyl ester **30a** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 3.82 (m, 1H), 2.73 (dd, J = 17.6, 8.4 Hz, 1H), 2.15 (dd, J = 17.6, 3.6 Hz, 1H), 1.90 (m, 1H), 1.61 (m, 1H), 1.54 (s, 9H), 1.33 (d, J = 6.5 Hz 3H), 1.31 (m, 2H), 0.90 (m, 6H). Anal. ($C_{14}H_{25}NO_3$) C, H, N.

2-Methyl-5-oxo-3-propylpyrrolidine-1-carboxylic Acid *tert*-Butyl Ester 30b. Prepared in 59% yield using *n*-propylmagnesium chloride as described above for the 3-isobutyl derivative **30a**: ^{1}H NMR ($CDCl_3$) δ 3.80 (m, 1H), 2.69 (dd, J = 17.6, 8.5 Hz, 1H), 2.12 (dd, J = 17.8, 3.7 Hz, 1H), 1.78 (m, 1H), 1.49 (s, 9H), 1.44 (m, 1H), 1.28 (d, J = 6.3 Hz 3H), 1.31 (m, 3H), 0.88 (app t, J = 7.1 Hz, 3H).

3-Butyl-2-methyl-5-oxopyrrolidine-1-carboxylic Acid *tert*-Butyl Ester 30c. Prepared in 39% yield using *n*-butylmagnesium chloride as described above for the 3-isobutyl derivative **30a**: ^{1}H NMR ($CDCl_3$) δ 3.81 (m, 1H), 2.69 (dd, J = 17.8, 8.5 Hz, 1H), 2.13 (dd, J = 17.8, 3.7 Hz, 1H), 1.75 (m, 1H), 1.49 (s, 9H), 1.45 (m, 1H), 1.28 (d, J = 6.4 Hz 3H), 1.23 (m, 5H), 0.86 (app t, J = 6.8 Hz, 3H).

3-(1-Amino-ethyl)-5-methylhexanoic Acid 31a. To 3-isobutyl-2-methyl-5-oxopyrrolidine-1-carboxylic acid *tert*-butyl ester **30a** (4.45 g, 17.4 mmol) was added 350 mL of 6 N HCl (aq), and the mixture was heated to reflux for 30 h, cooled, and concentrated. The residue was triturated several times with Et_2O, and the remaining solid residue was recrystallized from acetonitrile to afford 1.18 g (32%) of 3-(1-aminoethyl)-5-methylhexanoic acid **31a** as a white solid, mp 136.5−138.5 °C: ^{1}H NMR (DMSO-d_6) δ 3.20 (m, 1H), 2.19 (m, 2H), 2.06 (m, 1H), 1.51 (m, 1H), 1.23 (m, 1H), 1.06 (d, J = 6.6 Hz 3H), 1.00 (m, 1H), 0.81 (m, 3H). MS (*m/z*) 174 (M + 1). Anal. ($C_9H_{19}NO_2$·HCl) C, H, N, Cl.

3-(1-Amino-ethyl)hexanoic Acid 31b. Prepared in 55% yield from 2-methyl-5-oxo-3-propylpyrrolidine-1-carboxylic acid *tert*-butyl ester **30b** as described above for the isobutyl derivative **31a**. Obtained as a white solid, mp 129−131 °C: ^{1}H NMR (DMSO-d_6) δ 3.25 (m, 1H), 2.27 (m, 2H), 2.06 (m, 1H), 1.45 (m, 1H), 1.34 (m, 1H), 1.27 (m, 1H), 1.13 (m, 1H), 1.12 (d, J = 6.8 Hz 3H), 0.87 (app t, J = 7.2 Hz, 3H). MS (*m/z*) 160 (M + 1). Anal. ($C_8H_{17}NO_2$·HCl) C, H, N, Cl.

3-(1-Amino-ethyl)heptanoic Acid 31c. Prepared in 61% yield from 3-butyl-2-methyl-5-oxopyrrolidine-1-carboxylic acid *tert*-butyl ester **30c** as described above for the isobutyl derivative **31a**. Obtained as a white solid, mp 107−109 °C: ^{1}H NMR (DMSO-d_6) δ 3.19 (m, 1H), 2.21 (m, 2H), 1.96 (m, 1H), 1.42 (m, 1H), 1.20 (m, 4H), 1.12 (m, 1H), 1.06 (d, J = 6.6 Hz 3H), 0.81 (app t, J = 6.8 Hz, 3H). MS (*m/z*) 174 (M + 1). Anal. ($C_9H_{19}NO_2$·HCl) C, H, N, Cl.

5-Methylhex-2-enoic Acid Ethyl Ester 33. Sodium hydride (60% dispersion in mineral oil, 16.0 g, 0.41 mol) was washed with hexane and then taken up in DME (250 mL) and cooled to 0 °C. To the slurry was added triethylphosphonoacetate (80 mL, 0.4 mol) in 50 mL of DME slowly over 25 min. After the addition was complete, the ice bath was removed and a solution of isovaleraldehyde **32** (129 mL, 1.2 mol) in 100 mL of DME was added. The mixture was refluxed 16 h and then concentrated. The residue was partitioned between hexanes and water, the aqueous phase was extracted with hexanes, and the combined organic phases were washed twice with brine, dried (Na_2SO_4), and concentrated to afford 68 g (~100%) of crude 5-methylhex-2-enoic acid ethyl ester **33** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 4.21 (m, 1H), 2.55 (m, 1H), 2.41 (m, 1H), 2.13 (m, 1H), 1.60 (m, 1H), 1.49 (s, 9H), 1.28 (d, J = 6.3 Hz, 3H).

5-Methyl-3-(1-nitroethyl)hexanoic Acid Ethyl Ester 34. To 5-methylhex-2-enoic acid ethyl ester **33** (66 g, 0.42 mol) in 200 mL of acetonitrile were added DBU (63.9 g, 0.42 mol) and nitroethane (157.5 g, 2.1 mol). The mixture was heated to 60

°C for 4 h, cooled, and concentrated. The residue was partitioned between Et_2O and sat. NH_4Cl. The aqueous phase was extracted with Et_2O, and the combined organic phases were washed twice with water and twice with brine, dried (Na_2SO_4), and concentrated to a brown oil. Flash chromatography of the residue (hexanes) provided 42 g (43%) 5-methyl-3-(1-nitroethyl)hexanoic acid ethyl ester **34** as a 1:1 mixture of diastereomers: ^{1}H NMR ($CDCl_3$) δ 4.70 (m, 1H), 4.11 (m, 2H), 2.45 (m, 2H), 2.27 (m, 1H), 1.58 (m, 1H), 1.46 (m, 3H), 1.23 (m, 3H), 1.15 (m, 2H), 0.87 (m, 9H).

4-Isobutyl-5-methylpyrrolidine-2-one 35. To a solution of 5-methyl-3-(1-nitroethyl)hexanoic acid ethyl ester **34** (42 g, 0.18 mol) in 500 mL of MeOH was added RaNi (10 g), and the mixture was hydrogenated in a Parr shaker at 48 psi for 21 h, filtered, and concentrated. Flash chromatography of the residue (3:1 hexanes:EtOAc) followed by recrystallization from pentane furnished 6.3 g (23%) of 4-isobutyl-5-methylpyrrolidine-2-one **35** as colorless crystals, mp 47−50 °C. The ^{1}H NMR spectrum of the product showed a 3:1 cis:trans isomer ratio. Anal. ($C_9H_{17}NO$) C, H, N.

3-Isobutyl-2-methyl-5-oxopyrrolidine-1-carboxylic Acid Benzyl Ester 36. Sodium hydride (60% dispersion in mineral oil, 1.76 g, 0.04 mol) was washed with hexane and then taken up in THF (100 mL) and cooled to 0 °C. To this suspension was added 4-isobutyl-5-methylpyrrolidine-2-one **35** (6.2 g, 0.04 mol) slowly resulting in H_2 evolution. The mixture was allowed to warm to ambient temperature and then recooled to 0 °C at which time benzyl chloroformate (0.68 g, 0.04 mol) was added slowly. After stirring at ambient temperature overnight, the reaction was quenched by addition of MeOH (20 mL) and water (300 mL), and the THF was concentrated. The residue was extracted with Et_2O (3×), and the combined organics were washed with sat. $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (4:1 hexanes:EtOAc) to yield 7.9 g (68%) of 3-isobutyl-2-methyl-5-oxopyrrolidine-1-carboxylic acid benzyl ester **36** as a colorless oil. The ^{1}H NMR spectrum of the product showed a 3:1 cis:trans isomer ratio.

3-(1-Benzyloxycarbonylaminoethyl)-5-methylhexanoic Acid 37. To a solution of 3-isobutyl-2-methyl-5-oxopyrrolidine-1-carboxylic acid benzyl ester **36** (7.8 g, 0.027 mol) in 200 mL of THF was added a solution of lithium hydroxide monohydrate (1.42 g, 0.034 mol) in 100 mL of water, and the mixture was stirred at ambient temperature 15 h. The mixture was acidified to pH 2 with 4 N HCl (aq) and extracted with ether (3×). The combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated. The residue was washed with pentane (3×) and then recrystallized from Et_2O/hexane to yield 1.72 g (21%) of 3-(1-benzyloxycarbonylaminoethyl)-5-methylhexanoic acid **37** as colorless crystals, mp 110−111 °C. The ^{1}H NMR spectrum was complex owing to the presence of rotomers. Anal. ($C_{17}H_{25}NO_4$) C, H, N.

3-(1-Aminoethyl)-5-methylhexanoic Acid 38. To a solution of 3-(1-benzyloxycarbonylaminoethyl)-5-methylhexanoic acid **37** (0.85 g, 2.77 mmol) in 50 mL of THF:EtOH (1:1) was added 20% Pd/C (0.08 g), and the mixture was hydrogenated in a Parr shaker at 48 psi for 1.5 h. The mixture was filtered, the catalyst washed with water, THF, and EtOH, and the filtrate was concentrated. The residue was washed with Et_2O and then recrystallized from MeOH/Et_2O to afford 0.29 g (60%) of 3-(1-aminoethyl)-5-methylhexanoic acid **38** as a colorless solid, mp 185−186 °C (dec): ^{1}H NMR (D_2O) δ 3.23 (m, 1H), 2.12 (dd, J = 15.4, 7.1 Hz, 1H), 2.03 (dd, J = 15.4, 6.3 Hz, 1H), 1.91 (m, 1H), 1.41 (m, 1H), 1.01 (d, J = 6.8 Hz, 3H), 0.97 (m, 2H), 0.68 (d, J = 6.6 Hz, 3H), 0.66 (d, J = 6.6 Hz, 3H). MS (m/z) 174 (M + 1). Anal. ($C_9H_{19}NO_2$) C, H, N.

4-Methylpent-2-enoic Acid 40. (Diethoxyphosphoryl)-acetic acid (47.1 g, 240 mmol) was added to a solution of *n*-BuLi (1.6 M in hexanes, 300 mL, 480 mmol) in 400 mL of THF at −70 °C at such a rate that the internal temperature did not rise above −65 °C. Isobutyaldehyde **39** (15.7 g, 218 mmol) was then added over 15 min and the mixture kept at −70 °C for 2 h and then warmed to room temperature and stirred overnight. The reaction was quenched with 200 mL of of H_2O and the THF evaporated. The residue was cooled to 0 °C and acidified to pH = 1 with concentrated HCl. The mixture was extracted with Et_2O (3 × 200 mL) and dried over Na_2SO_4. Evaporation of the solvent gave 20.9 g (84%) of 4-methylpent-2-enoic acid **40** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.04 (dd, J = 8.0, 6.6 Hz, 1H), 5.75 (d, J = 5.8 Hz, 1H), 2.46 (m, 1H), 1.06 (d, J = 6.8 Hz, 6H).

3-(4-Methylpent-2-enoyl)-4*R*-phenyloxazolidin-2-one 41. Pivaloyl chloride (18.5 g, 153.2 mmol) was added to a solution of 4-methylpent-2-enoic acid **40** (17.5 g, 153.2 mmol) and triethylamine (38.8 g, 383 mmol) in 300 mL of THF at −20 °C. After 1 h, LiCl (7.1 g, 168.5 mmol) was added followed by (4*R*)-phenyloxazolidin-2-one (25.0 g, 153.3 mmol). The reaction mixture was stirred at −20 °C for 1.5 h, then warmed to room temperature overnight. The solids were removed by filtration and the THF evaporated. The crude product was adsorbed onto a silica gel pad and the pad eluted with 800 mL of EtOAc. Evaporation of the eluate followed by crystallization from hexane afforded 13.3 g (30%) of 3-(4-methylpent-2-enoyl)-4*R*-phenyloxazolidin-2-one **41**: ^{1}H NMR ($CDCl_3$) δ 7.36 (m, 5H), 7.20 (s, 1H), 7.06 (m, 1H), 5.49 (dd, J = 8.7, 3.9 Hz, 1H), 4.70 (t, J = 8.8 Hz, 1H), 4.29 (d, J = 12.8 Hz, 1H), 2.51 (m, 1H), 1.06 (s, 6H).

3-(3*R*,4-Dimethylpentanoyl)-4*R*-phenyloxazolidin-2-one 42. Methylmagnesium chloride (3.0M in Et_2O, 29.3 mL, 87.9 mmol) was added to a suspension of CuBr−SMe_2 in 100 mL of THF at −20 °C. After 20 min, a solution of 3-(4-methylpent-2-enoyl)-4*R*-phenyloxazolidin-2-one **41** (11.4 g, 44 mmol) in 100 mL of THF was added to the cuprate solution while maintaining the temperature at −20 °C. When the addition was complete (about 30 min), the reaction mixture was warmed to −5 °C for 0.5 h. The reaction was quenched by addition of 200 mL of sat. NH_4Cl and the THF evaporated. The aqueous solution was extracted with Et_2O (3 × 200 mL), and the combined ether extracts were washed with brine (2 × 100 mL). Drying over Na_2SO_4 and evaporation of the solvent furnished a white solid which was recrystallized from hexane to give 9.7 g (40%) of 3-(3*R*,4-dimethylpentanoyl)-4*R*-phenyloxazolidin-2-one **42**, mp 72−75 °C: ^{1}H NMR ($CDCl_3$) δ 7.34 (m, 5H), 5.43 (m, 1H), 4.68 (t, J = 8.8 Hz, 1H), 4.26 (d, J = 8.8 Hz, 1H), 2.90 (m, 1H), 2.80 (m, 1H), 1.92 (m, 1H), 1.54 (m, 2H), 0.81 (m, 8H).

(3*R*)-(2-Acetoxy-1*R*-phenylethylcarbamoyl)-4*S*,5-dimethylhexanoic Acid *tert*-Butyl Ester 43. LiHMDS (1.0M soln in THF, 36.9 mL, 36.9 mmol) was added to a solution of 3-(3*R*,4-dimethylpentanoyl)-4*R*-phenyloxazolidin-2-one **42** (9.7 g, 35.2 mmol) in 200 mL of THF at −65 °C under N_2. The mixture was stirred at −65 °C for 25 min and then at −30 °C for 0.5 h. *tert*-Butyl bromoacetate (7.8 mL, 52.8 mmol) was added and the reaction mixture stirred at −30 °C for 1 h and then at room temperature for 0.5 h. The reaction was quenched by addition of sat. KH_2PO_4 and the THF evaporated. The mixture was extracted with Et_2O (3 × 100 mL). The combined Et_2O extracts were washed with brine (50 mL) and dried over Na_2SO_4. Evaporation of the solvent and recrystallization from hexane (150 mL) provided 6.7 g (49%) of 3*R*-(2-acetoxy-1*R*-phenylethylcarbamoyl)-4*S*,5-dimethylhexanoic acid *tert*-butyl ester **43**: ^{1}H NMR ($CDCl_3$) δ 7.34 (d, J = 4.4 Hz, 5H), 5.39 (d, J = 5.4 Hz, 1H), 4.67 (t, J = 8.7 Hz, 1H), 4.41 (d, J = 11.7 Hz, 1H), 4.24 (dd, J = 8.8, 3.2 Hz, 1H), 2.65 (dd, J = 17.1, 11.7 Hz, 1H), 2.25 (d, J = 16.9 Hz, 1H), 1.64 (m, 1H), 1.45 (m, 1H), 1.24 (s, 9H), 1.02 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 6.6 Hz, 3H), 0.80 (d, J = 7.1 Hz, 3H).

2*R*-(1*S*,2-Dimethylpropyl)succinic Acid 4-*tert*-Butyl Ester 44. A solution of LiOH·H_2O (1.3 g, 32.3 mmol) and H_2O_2 (4.4 g, 129.4 mmol) in 30 mL of H_2O was stirred at −3 °C for 20 min at which time 3*R*-(2-acetoxy-1*R*-phenylethylcarbamoyl)-4*S*,5-dimethylhexanoic acid *tert*-butyl ester **43** (6.3 g, 16.2 mmol) was added as a solution in 200 mL of THF. The mixture was stirred at −3 °C for 30 min and then at room temperature overnight. The reaction was quenched by addition of solid $NaHSO_3$ until test paper showed no peroxide present and the THF was evaporated. The solution was made basic with 1.0 N NaOH (aq) and washed with CH_2Cl_2 (3 × 100 mL). The

aqueous phase was made acidic (pH = 4) with saturated KH_2PO_4 and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were dried over Na_2SO_4 and concentrated. The residue was chromatographed in 50% EtOAc/hexane to give 2.6 g (66%) of 2*R*-(1*S*,2-dimethylpropyl)succinic acid 4-*tert*-butyl ester **44**: ^{1}H NMR ($CDCl_3$) δ 5.30 (s, 1H), 2.97 (m, 1H), 2.55 (m, 1H), 2.29 (dd, J = 16.6, 3.4 Hz, 1H), 1.70 (m, 1H), 1.52 (m, 1H), 1.43 (s, 9H), 0.92 (dd, J = 15.6, 6.6 Hz, 6H), 0.83 (d, J = 6.8 Hz, 3H).

4*R*-(1*S*,2-Dimethylpropyl)dihydrofuran-2-one 45. Borane−dimethyl sulfide complex (10.0 M soln in THF, 1.06 mL, 10.6 mmol) was added dropwise over 10 min to a solution of 2*R*-(1*S*,2-dimethylpropyl)succinic acid 4-*tert*-butyl ester **44** (2.6 g, 10.6 mmol) in THF at 0 °C. The mixture was stirred at 0 °C for 20 min and at room temperature overnight. The mixture was cooled to 0 °C, and 50 mL of MeOH was added. After warming to room temperature, the solvent was evaporated and the crude oil treated with 0.1 g of *p*-toluenesulfonic acid in refluxing THF for 6 h. The solution was cooled to room temperature and the THF evaporated. The residue was taken up in 100 mL of Et_2O and washed with 1.0 N NaOH (3 × 10 mL) and brine. Drying over Na_2SO_4 and evaporation of the solvent gave 1.4 g (84%) of 4*R*-(1*S*,2-dimethylpropyl)dihydrofuran-2-one **45** as a white solid: ^{1}H NMR ($CDCl_3$) δ 4.42 (m, 1H), 3.93 (t, J = 9.0 Hz, 1H), 2.53 (m, 2H), 2.21 (m, 1H), 1.59 (dq, J = 10.2, 3.5 Hz, 1H), 1.43 (m, 1H), 0.92 (d, J = 6.8 Hz, 3H), 0.82 (d, J = 7.0 Hz, 6H).

3*R*-Bromomethyl-4*S*,5-dimethylhexanoic Acid Ethyl Ester 46. A solution of 4*R*-(1*S*,2-dimethylpropyl)dihydrofuran-2-one **45** (1.4 g, 9.0 mmol) in 20 mL of EtOH was saturated with HBr at 0 °C and stirred at room temperature overnight. Dilution with 50 mL of water and extraction with hexane (3 × 50 mL) followed by evaporation furnished 2.3 g (96%) of 3*R*-bromomethyl-4*S*,5-dimethylhexanoic acid ethyl ester **46**: ^{1}H NMR ($CDCl_3$) δ 4.14 (q, J = 7.1 Hz, 2H), 3.58 (m, 1H), 3.47 (m, 1H), 2.44 (m, 1H), 2.28 (m, 2H), 1.69 (m, 1H), 1.52 (m, 1H), 1.27 (t, J = 7.1 Hz, 3H), 0.89 (m, 6H), 0.78 (d, J = 6.8 Hz, 3H).

3*R*-Azidomethyl-4*S*,5-dimethylhexanoic Acid Ethyl Ester 47. 3*R*-Bromomethyl-4*S*,5-dimethylhexanoic acid ethyl ester **46** (2.3 g, 8.7 mmol) and NaN_3 (1.1 g, 16.5 mmol) were heated to 60 °C in 20 mL of DMSO under N_2 for 18 h. The mixture was cooled, diluted with 100 mL of water and extracted with Et_2O (4 × 50 mL). The combined extracts were washed with 50 mL of brine and dried over Na_2SO_4. Evaporation of the solvent afforded 1.4 g (71%) of 3*R*-azidomethyl-4*S*,5-dimethylhexanoic acid ethyl ester **47** as an oil: ^{1}H NMR ($CDCl_3$) δ 4.15 (q, J = 7.1 Hz, 2H), 3.2−3.5 (m, 2H), 2.0−2.4 (m, 3H), 1.56 (m, 1H), 1.36 (m, 1H), 1.26 (t, J = 7.1 Hz, 3H), 0.91 (dd, J = 20.3, 6.6 Hz, 6H), 0.79 (d, J = 6.2 Hz, 3H),

4*R*-(1*S*,2-Dimethylpropyl)pyrrolidin-2-one 48. 3*R*-Azidomethyl-4*S*,5-dimethylhexanoic acid ethyl ester **47** (1.4 g, 6.2 mmol) was hydrogenated as before for compound **22**. The catalyst was removed by filtration and the filtrate evaporated to give 1.1 g (100%) of 4*R*-(1*S*,2-dimethylpropyl)pyrrolidin-2-one **48**: ^{1}H NMR ($CDCl_3$) δ 5.60 (s, 1H), 3.37 (t, J = 8.2 Hz, 1H), 3.00 (t, J = 8.9 Hz, 1H), 2.32 (m, 3H), 1.98 (dd, J = 16.2, 10.1 Hz, 1H), 1.55 (m, 2H), 1.31 (m, 1H), 0.85 (d, J = 6.8 Hz, 3H), 0.74 (d, 6H).

3*R*,4*S*-3-Aminomethyl-4,5-dimethylhexanoic Acid 49. 4*R*-(1*S*,2-Dimethylpropyl)pyrrolidin-2-one **48** (1.1 g, 7.1 mmol) was heated to reflux in 25 mL of 6.0 N HCl overnight. The reaction mixture was cooled and concentrated. The remaining solution was filtered through Celite and the filtrate placed on a column of Dowex-50 ion-exchange resin. The column was eluted with water until the eluate was neutral pH. It was then eluted with 300 mL of 10% NH_4OH and the alkaline eluate collected. Evaporation gave a solid which was taken up in 50 mL of MeOH. The solid was removed by filtration and the filtrate concentrated to 20 mL. EtOAc (20 mL) was added and the mixture allowed to stand at room temperature for 2 h. The solid was collected to give 0.41 g (33%) of 3*R*,4*S*-3-aminomethyl-4,5-dimethylhexanoic acid **49** as a colorless solid: $[\alpha]_D$ +14.9 (*c* 0.65, H_2O). ^{1}H NMR (CD_3OD) δ 2.91 (m, 2H), 2.41 (m, 1H), 2.16 (m, 2H), 1.54 (m, 1H), 1.24 (m, 1H), 0.91 (dd, J = 15.9, 6.6 Hz, 6H,), 0.80 (d, J = 6.8 Hz, 3H). MS (*m*/*z*) M^+ = 173. Anal. ($C_9H_{19}NO_2$) C, H, N.

3*R*-Benzyl-4*R*-isopropyldihydrofuran-2-one 56. To a solution of 4*S*-isopropyldihydrofuran-2-one **55**[44] (11.7 g, 91 mmol) in 100 mL of THF at −78 °C was added lithium bis-(trimethylsilyl)amide (1.0 M solution in THF, 92 mL, 92 mmol) over 5 min. The solution was stirred for 1 h at −78 °C at which time benzyl iodide (21.9 g, 100 mmol) was added. Stirring was continued for 90 min at −78 °C at which time brine was added. The mixture was warmed to room temperature and extracted with EtOAc. The organic phase was separated, dried ($MgSO_4$), and concentrated. Flash chromatography of the residue (10% Et_2O in hexanes) afforded 11.6 g (58%) of 3*R*-benzyl-4*R*-isopropyldihydrofuran-2-one **56**: ^{1}H NMR ($CDCl_3$) δ 7.19 (m, 5H), 4.02 (app t, J = 8.0 Hz, 1H), 3.87 (dd, J = 9.3, 6.8 Hz, 1H), 2.98 (d, J = 5.9 Hz, 2H), 2.57 (q, J = 6.1 Hz, 1H), 2.05 (m, 1H), 1.55 (m, 1H), 0.81 (d, J = 6.8 Hz, 3H), 0.72 (d, J = 6.8 Hz, 3H).

2*R*-Benzyl-3*R*-bromomethyl-4-methylpentanoic Acid Ethyl Ester 57. To a solution of 3*R*-benzyl-4*R*-isopropyldihydrofuran-2-one **56** (6.5 g, 29.8 mmol) in 80 mL of absolute EtOH at 0 °C was added anhydrous HBr gas for 1 h. After saturation with HBr the mixture was stirred at room temperature overnight and then poured into an ice cold mixture of hexanes and brine. The organic phase was separated and the aqueous phase extracted with hexanes. The combined organic phases were washed with water, dried ($MgSO_4$), and concentrated to provide 7.0 g 2*R*-benzyl-3*R*-bromomethyl-4-methylpentanoic acid ethyl ester **57** in ca. 80% purity: ^{1}H NMR ($CDCl_3$) δ 7.27 (m, 5H), 4.10 (m, 1H), 3.93 (m, 1H), 3.80 (q, J = 7.1 Hz, 2H), 3.40 (br s, 2H), 3.05 (d, J = 5.9 Hz, 2H), 2.66 (q, J = 6.0 Hz, 1H), 2.11 (m, 1H), 1.63 (m, 1H), 1.29 (t, J = 6.8 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H), 0.79 (d, J = 6.8 Hz, 3H).

2*R*-Benzyl-3*R*,4-dimethylpentanoic Acid Ethyl Ester 58. To a solution of 2*R*-benzyl-3*R*-bromomethyl-4-methylpentanoic acid ethyl ester **57** (7.0 g, ca. 80% pure) and 3.2 mL of triethylamine in 100 mL of EtOH was added 1.0 g 20% Pd/C. The mixture was hydrogenated in a Parr shaker at 48 psi overnight. The mixture was filtered through Celite, washed with EtOH, and concentrated. To the residue was added Et_2O, resulting in a colorless precipitate (Et_3N·HCl). The mixture was filtered, the precipitation process repeated, and the resultant product purified by flash chromatography on silica gel (hexanes) to provide 3.35 g (45% from **56**) 2*R*-benzyl-3*R*,4-dimethylpentanoic acid ethyl ester **58**: ^{1}H NMR ($CDCl_3$) δ 7.21 (m, 5H), 3.95 (m, 2H), 2.85 (m, 2H), 2.64 (m, 1H), 1.85 (m, 1H), 1.62 (m, 1H), 1.05 (t, J = 7.1 Hz, 3H), 0.95 (d, J = 6.8 Hz, 3H), 0.82 (m, 6H). Also recovered was 2.25 g (35%) of lactone **56**.

Acetic Acid 2*R*-benzyl-3*R*,4-dimethylpentyl Ester 59. To a solution of 2*R*-benzyl-3*R*,4-dimethylpentanoic acid ethyl ester **58** (3.20 g, 12.9 mmol) in 25 mL of Et_2O was added lithium aluminum hydride (0.50 g, 13.2 mmol), and the suspension was stirred at room temperature overnight. Excess LAH was destroyed by addition of EtOAc to the cooled reaction mixture. Saturated Na_2SO_4 was added cautiously to coagulate the alumina which separated at room temperature as a white precipitate. The reaction mixture was diluted with CH_2Cl_2, anhydrous Na_2SO_4 was added, and the mixture was filtered and concentrated. To the residue in 30 mL of CH_2Cl_2 were added 2.5 mL of triethylamine, 0.2 g of DMAP, and 1.5 mL of acetic anhydride. The mixture was stirred at room temperature for 3 h and then partitioned between Et_2O and water. The organic phase was washed with 1 N HCl, sat. $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated to provide 3.16 g (99%) of acetic acid 2*R*-benzyl-3*R*,4-dimethylpentyl ester **59**: ^{1}H NMR ($CDCl_3$) δ 7.19 (m, 5H), 4.03 (m, 2H), 2.69 (m, 2H), 2.09 (m, 1H), 2.02 (s, 3H), 1.68 (m, 1H), 1.23 (m, 1H), 0.87 (d, 3H), 0.84 (d, 3H), 0.81 (d, 3H).

3*R*-Bromomethyl-4*R*,5-dimethylhexanoic Acid Ethyl Ester 60. To a solution of 2*R*-benzyl-3*R*,4-dimethylpentyl ester **59** (5.0 g, 20.2 mmol) in 240 mL of 2:1:1 H_2O:CH_3CN:CCl_4 was added sodium periodate (86.2 g, 403 mmol), followed by

ruthenium(III) chloride (0.41 g, 2.0 mmol). The mixture was stirred vigorously overnight at room temperature, diluted with 400 mL of CH_2Cl_2, and filtered through a pad of Celite. The organic phase was separated and the aqueous phase extracted with CH_2Cl_2. The combined organic phases were concentrated, and the residue was dissolved in Et_2O and applied to a Florisil column. Elution with 3% MeOH in Et_2O furnished a paste that was dissolved in 100 mL of MeOH. Potassium carbonate (8.0 g) was added and the mixture heated to reflux for 6 h. The mixture was cooled and concentrated. The residue was dissolved in H_2O and cooled to 0 °C, and the mixture was adjusted to pH 2 with concentrated HCl. Chloroform (200 mL) was added to the solution, and stirring was continued overnight at room temperature. The phases were separated, and the aqueous phase was extracted with $CHCl_3$. The combined organic phases were dried (Na_2SO_4) and concentrated. To the crude lactone in 25 mL of EtOH at 0 °C was added a stream of HBr gas for 45 min. The mixture was allowed to stand at room temperature overnight and then partitioned between ice cold brine and hexanes. The organic phase was dried ($MgSO_4$) and concentrated. Flash chromatography of the residue (10% Et_2O in hexanes) afforded 3.54 g (66%) of 3*R*-bromomethyl-4*R*,5-dimethylhexanoic acid ethyl ester **60**: ^{1}H NMR ($CDCl_3$) δ 4.14 (q, 2H), 3.60 (dd, 1H), 3.41 (dd, 1H), 2.54 (dd, 1H), 2.44 (dd, 1H), 2.22 (m, 1H), 1.67 (m, 1H), 1.37 (m, 1H), 1.26 (t, 3H), 0.94 (d, 3H), 0.81 (d, 3H), 0.79 (d, 3H).

3*R*-Aminomethyl-4*R*,5-dimethylhexanoic Acid 61. To a solution of 3*R*-bromomethyl-4*R*,5-dimethylhexanoic acid ethyl ester **60** (3.54 g, 13.3 mmol) in 8.0 mL of DMF was added sodium azide (1.04 g, 16.1 mmol), and the mixture was stirred at room temperature overnight. The mixture was partitioned between hexanes and H_2O, the phases were separated, and the aqueous phase was extracted with hexanes. The combined organic phases were dried ($MgSO_4$) and concentrated. The residue was dissolved in EtOH, 10% Pd/C was added, and the mixture hydrogenated at 48 psi in a Parr shaker overnight. The mixture was filtered and concentrated, and the residue was refluxed overnight in 6 N HCl (aq). The mixture was cooled, concentrated, and then azeotroped with toluene. The crude residue was dissolved in the minimum amount of H_2O and loaded onto DOWEX 50WX8−100 ion-exchange resin. The column was eluted with H_2O until neutral and then with 5% NH_4OH (aq). The alkaline fractions were concentrated, and the crude product was crystallized from MeOH to furnish 0.72 g (31%) of 3*R*-aminomethyl-4*R*,5-dimethylhexanoic acid **61**, mp 163−165 °C: $[\alpha]_D$ −5.3 (*c* 1.9, MeOH). ^{1}H NMR (CD_3OD) δ 3.04 (dd, 1H), 2.82 (dd, 1H), 2.52 (dd, 1H), 2.40 (dd, 1H), 2.07 (m, 1H), 1.67 (m, 1H), 1.35 (m, 1H), 0.97 (d, 3H), 0.88 (d, 3H), 0.83 (d, 3H). MS (*m*/*z*) 174 (M + 1). Anal. ($C_9H_{19}NO_2$) C, H, N.

[^{3}H]Gabapentin Binding Assay Using Pig Brain Membranes. Pig brain tissue is homogenized, centrifuged, and washed extensively in a 10 mM HEPES buffer. Following determination of protein concentration, aliquots are stored at −70 °C until the day of testing. The binding assay is setup in a 96-well format using deep-well polypropylene plates. In a total volume of 500 μL, the following additions are made: 250 mL of buffer (10 mM HEPES, pH 7.4 by KOH), 25 μL of [^{3}H]-gabapentin (10 nM final concentration), 200 μL of thawed tissue (~40 μg protein per well), and 25 μL of test compound. Nonspecific binding is defined by the addition of 10 μM cold (nonradiolabeled) pregabalin. Following incubation for 45 min at room temperature, the contents of the wells are filtered using a Brandel harvester onto presoaked (in 0.1% of polyethylenimine, or PEI) GF/B filter and then washed (4 × 1 mL) with chilled 50 mM Tris HCl buffer (pH 6.9 by KOH). Filters are placed into plastic pouches, scintillation cocktail is added, the pouches are sealed and radioactivity for each sample is counted. IC_{50} values are then determined using the program Prism. The results reported are the average of 2-3 individual determinations. The standard error of the mean is also reported.

System L Transport Assay. CHO cells were used in this study since System L transport has been well characterized in CHO cells.[52] CHO-K1 cells were maintained in minimum essential medium alpha medium (Gibco #32571-036) supplemented with 5% fetal bovine serum (Gibco #10082−139) and 1% penicillin/streptomycin (Gibco #15140−122). Cells were typsinized, diluted, and plated into 96-well tissue culture microplates (Perkin-Elmer, Isoplate TC) the day prior to running the transport assay. One hundred microliters of a cell suspension at a density of 3×10^5 cells/mL was added to each well. This resulted in 90−100% confluent cell monolayers 24 h later. Protein analysis (~10 μg/well) consistently showed ≤5% difference between all wells on a plate (data not shown). The System L transporter assays were run in sodium-free buffer (PBC) containing (in mM): choline chloride (137), KCl (2.7), choline phosphate (10.6), KH_2PO_4 (1.5), d-glucose (5.6), $MgCl_2$ (0.49), and $CaCl_2$ (0.9). A [^{3}H]L-leucine uptake solution consisting of PBC with 2 μCi/mL L-[4,5-^{3}H]leucine (Amersham #TRK 510, 1 mCi/mL, 120−190 Ci/mmol) added was made. L-Leucine is a protypical substrate of the System L transporter. Test compound dilutions ranging from 10 mM to 300 nM in half log increments were made in duplicate for generation of IC_{50}s. A 96-well drug addition plate was prepared with these 10 drug dilutions in the tritiated L-leucine uptake solution. The drug plate also had two control wells for each test compound dilution set, one with no drug added and one with saturating L-leucine (10 mM) added. The osmolarities of all the uptake mixtures were held constant by varying the amounts of choline choride. To start the uptake assay, culture plates were washed with Na^+-free buffer two times for 20 min each at 37 °C. This step depleted endogenous amino acids and washed out the residual sodium. The following steps of the assay were done on an automated Beckman Multimek 96-well pipettor. One hundred microliters from each of the wells on the test compound plate was added to corresponding wells on the culture plate simultaneously. After 2 min at room temperature (RT−22 °C) the uptake of [^{3}H]L-leucine was terminated by washing three times with cold (4 °C) PBC. Preliminary experiments had indicated that [^{3}H]L-leucine uptake was linearly dependent on incubation time up to at least 5 min at 22 °C (data not shown). Plate wells were aspirated and then shaken out completely dry. One hundred and fifty microliters of scintillation cocktail (Perkin-Elmer-Trisafe) was then added to each well. This scintillation cocktail lysed the cells, and then the plates could be counted directly on a liquid scintillation plate counter (Wallac-Trilux). IC_{50}s were calculated by nonlinear least squares regression analysis (Sigmaplot 2001; SPSS, Inc, Chicago, IL) using a three-parameter Logistic fit formula: where $f = a/(1 + \mathrm{abs}(x/x_0)^b)$, a = max. y (dependent variable), b = width of transition, (x_0 = x (independent variable) value at 50% of functions amplitude (IC_{50}), and abs is the absolute value. Except as noted, the data reported is the product of three individual determinations; the standard error of the mean is also reported.

DBA/2 Seizure Model. All procedures were carried out in compliance with the NIH Guide for the Care and Use of Laboratory Animals under a protocol approved by the Pfizer Animal Use Committee. Male DBA/2 mice 3 weeks old (7−12 g at time of testing) were obtained from Jackson Laboratories, Bar Harbor, ME. Immediately before anticonvulsant testing, mice were placed upon a wire mesh, 4 in. square, suspended from a steel rod 12 to 18 in. from the top of a table. The square was slowly inverted through 180° and mice observed for 30 s. Any mouse falling from the wire mesh was scored as ataxic.[53] Mice were placed into an enclosed acrylic plastic chamber (21 cm height, approximately 30 cm diameter) with a high-frequency speaker (4 cm. diameter) in the center of the top lid. An audio signal generator (Protek Model B-810) was used to produce a continuous sinusoidal tone that was swept linearly in frequency between 8 and 16 kHz once each 10 ms. The average sound pressure level (SPL) during stimulation was approximately 100 dB at the floor of the chamber. Mice were placed within the chamber and allowed to acclimate for 1 min. DBA/2 mice in the vehicle-treated group responded to the sound stimulus (applied until tonic extension occurred, or for a maximum of 60 s) with a characteristic seizure sequence consisting of wild running followed by clonic seizures, and later

by tonic extension, and finally by respiratory arrest and death in 80% or more of the mice. In vehicle-treated mice, the entire sequence of seizures to respiratory arrest lasted approximately 15 to 20 s. The incidence of all the seizure phases in the drug-treated and vehicle-treated mice was recorded, and the occurrence of tonic seizures was used for calculating anticonvulsant activity.[54] Groups of DBA/2 mice ($n = 5$) were tested for sound-induced seizure responses at previously determined time of peak effect after oral (PO) drug administration. All compounds in the present study were dissolved in distilled water and given orally by gavage and in a volume of 10 mL/kg of body weight. Vehicle treatment consisted of water only, given by oral gavage. Testing for sound-induced seizures for each group was done at a single time after oral dosing.

Carrageenan-Induced Thermal Hyperalgesia in the Rat. Thermal hyperalgesia was assessed using the rat plantar test following a modified method.[45] Rats were habituated to the apparatus, which consisted of three individual Perspex boxes on an elevated glass table. A mobile radiant heat source located under the table was focused onto the desired paw, and paw withdrawal latencies (PWL) were recorded. PWL were taken three times for both hind paws of each animal, the mean of which represented baselines for right and left hind paws. At least 5 min were allowed between each PWL for an animal. The apparatus was calibrated to give a PWL of approximately 10 s. There was an automatic cutoff point of 20 s to prevent tissue damage. After baseline PWL's were determined, animals received an intraplantar injection of carrageenan (100 μL of 20 mg/mL) into the right hind paw. PWLs were reassessed following the same protocol as above 2-h postcarrgeenan (this time point represented the start of peak hyperalgesia) to ascertain that hyperalgesia had developed. Test compounds were administered orally (in a volume of 1 mL/kg) at 2.5 h after carrageenan. PWLs were reassessed 1 h after drug administration. Results are expressed as a percentage of the maximal reversal of hyperalgesia.

Vogel Conflict Model of Anxiety. The water source was removed, and the housing rack was drained 24 h before training. On training day, water-deprived naïve animals are moved into the test room and allowed to acclimate for 1 h. Training was conducted in the light between 8 and 11am-2days/week The test room is equipped with two white noise generators used to mask routine operator movement and distractions near the test room. Following the acclimation period, experimental subjects were placed into the test chambers facing the sidewall opposite the drink tube. Animals were allowed to explore the chamber and drink during a 10 min training session (no-shock condition). Drinking was limited to 500 responses from the drink tube, which resulted in approximately 5 mL of water. Training subjects that did not emit at least 200 responses during the training session were excluded from day 2 testing. Immediately following the no-shock training session, rats were returned to their home cages, deprived of water for an additional 24 h, and were food deprived. On test day all trained animals were moved into the test room and allowed to acclimate for 1 h. Experiments were conducted in the light between 8 am and 3 pm. The test room is equipped with two white noise generators used to mask routine operator movement and distractions near the test room. On test day 2, rats ($n = 10$) were dosed with pregabalin or test compounds (30 mg/kg, po) 120 min prior to testing. After the pretreatment period, rats were placed into a test chamber and allowed to drink for 10 min. After every 10 licks, rats received a 1-s shock (1 mA) through the drink tube. All data were analyzed using a Kruskal-Wallis One-Way Analysis of Variance on Ranks and Mann−Whitney Rank Sum Tests. Results were expressed as a percent of activity compared to a reference dose of pregabalin at 30 mg/kg

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Supporting Information Available: X-ray structure data of compound **43**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bryans, J. S.; Wustrow, D. J. 3-Substituted GABA analogues with central nervous system activity: A review. *Med. Res. Rev.* **1999**, *19*, 149−177.
(2) Xiao, W. H.; Bennett, G. J. Gabapentin has an antinociceptive effect mediated via a spinal site of action in a rat model of painful peripheral neuropathy. *Analgesia* **1996**, *2*(5/6), 267−273.
(3) Hwang, J. H.; Yaksh, T. L. Effect of subarachnoid gabapentin on tactile-evoked allodynia in a surgically induced neuropathic pain model in the rat. *Reg. Anesth.* **1997**, *22*, 249−256.
(4) Field, M. J.; Oles, R. J.; Lewis, A. S.; McCleary, S.; Hughes, J.; Singh, L. Gabapentin (Neurontin) and S−(+)-3-isobutylgaba represent a novel class of selective antihyperalgesic agents. *Br. J. Pharmacol.* **1997**, *121*, 1513−1522.
(5) Silverman, R. B.; Andruszkiewicz, R.; Nanavati, S. M.; Taylor, C. P.; Vartanian, M. G. 3-Alkyl-4-aminobutyric acids: The first class of anticonvulsant agents that activates L-glutamic acid decarboxylase. *J. Med. Chem.* **1991**, *34*, 2295−8.
(6) Taylor, C. P.; Vartanian, M. G.; Yuen, P. W.; Kanter, G. D.; Bigge, C.; Suman-Chauhan, N.; Hill, D. R. Potent and stereospecific anticonvulsant activity of 3-isobutyl GABA relates to in vitro binding at a novel site labled by tritiated gabapentin. *Epilepsy Res.* **1993**, *14*, 11−15.
(7) Yuen, P. W.; Kanter, G. D.; Taylor, C. P.; Vartanian, M. G. Enantioselective synthesis of PD144723: A potent stereospecific anticonvulsant. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 823−6.
(8) Singh, L.; Field, M. J.; Ferris, P. Hunter, J. C. Oles, R. J.; Williams, R. G.; Woodruff, G. N. The antiepileptic agent gabapentin (Neurontin) possesses anxiolytic-like and antinociceptive actions that are reversed by D-serine. *Psychopharmacology* **1996**, *127*, 1−9.
(9) Dworkin, R. H. P.; Corbin, A. E. M.; Young, J. P. J.; Sharma, U.; LaMoreaux, L.; Bockbrader, H.; Garofalo, E. A. M.; Poole, R. M.; Pregabalin for the treatment of postherpetic neuralgia: A randomized, placebo-controlled trial. *Neurology* **2003**, *60*, 1274−83.
(10) Hill, C. M.; Balkenohl, M.; Thomas, D. W.; Walker, R.; Mathe, H.; Murray, G. Pregabalin in patients with postoperative dental pain. *Eur. J. Pain* **2001**, *5*, 119−24.
(11) Pande, A. C.; Crockatt, J. G.; Feltner, D. E.; Janney, C. A.; Smith, W. T.; Weisler, R.; Londborg, P. D.; Bielski, R. J.; Zimbroff, D. L.; Davidson, J. R. Pregabalin in generalized anxiety disorder: a placebo-controlled trial. *Am. J.. Psychiatry* **2003**, *160*, 533−40.
(12) Lauria-Horner, B. A.; Pohl, R. B. Pregabalin: A new anxiolytic. *Expert Opin. Invest. Drugs* **2003**, *12*, 663−672.
(13) Errante, L. D.; Petroff, O. A. C. Acute effects of gabapentin and pregabalin on rat forebrain cellular GABA, glutamate, and glutamine concentrations. *Seizure* **2003**, *12*, 300−6.
(14) Ng, G.; Bertrand, S.; Sullivan, R.; Ethier, N.; Wang, J.; Yergey, J.; Belley, M.; Trimble, L.; Bateman, K.; L Alder, L.; Smith, A.; Mckernan, R.; Metters, K.; O′Neill, G.; Lacaille, J.-C.; Hebert, T. E. γ-Aminobutyric acid type B receptors with specific heterodimer composition and postsynaptic actions in hippocampal neurons are targets of anticonvulsant gabapentin action. *Mol. Pharmacol.* **2001**, *59*, 144−152.
(15) Bertrand, S.; Ng, G.; Purisai, M. G.; Wolfe, S.; Severidt, M.; Nouel, D.; Robitaille, R.; Low, M.; O′Neill, G.; Metters, K.; Lacaille, J.-C.; Chronwall, B.; Morris, S. The anticonvulsant, antihyperalgesic agent gabapentin is an agonist at brain γ-aminobutyric acid type B receptors negatively coupled to voltage-dependent calcium channels. *J. Pharmacol. Exp. Ther.* **2001**, *298*, 15−24.
(16) Jensen, A.; Mosbacher, J.; Elg, S.; Lingenhoehl, K.; Lohmann, T.; Johansen, T.; Abrahamsen, B.; Mattsson, J.; Lehmann, A.; Bettler, B.; Brauner-Osborne, H. The anticonvulsant gabapentin (Neurontin) does not act through γ-aminobutyric acid-B receptors. *Mol. Pharmacol.* **2002**, *6*, 1377−1384.
(17) Lanneau, C.; Green, A.; Hirst, W.; Wise, A.; Brown, J.; Donnier, E.; Charles, K.; Wood. M.; Davies C.; Pangalos, M. Gabapentin is not a GABAB receptor agonist. *Neuropharmacology* **2001**, *41*, 965−975.
(18) Scott, R. H.; Martin, D. J.; McClelland, D. Cellular actions of gabapentin and related compounds on cultured sensory neurones. *Curr. Neuropharmacol.* **2003**, *1*, 219−235.

(19) Su, T.-Z.; Lunney, E.; Campbell, G.; Oxender, D. Transport of gabapentin, a γ-amino acid drug, by system L α-amino acid transporters: a comparative study in astrocytes, synaptosomes, and CHO cells. *J. Neurochem.* **1995**, *64*, 2125−2131.
(20) Feng, M. R.; Turluck, D.; Burleigh, J.; Lister, R.; Fan, C.; Middlebrook, A.; Taylor, C.; Su, T.-Z. Brain microdialysis and PK/PD correlation of pregabalin in rats. *Eur. J. Drug Metab. Pharmacokinet.* **2001**, *26*, 123−128.
(21) Suman-Chauhan, N.; Webdale, L.; Hill, D. R.; Woodruff, G. N. Characterization of [3H]gabapentin binding to a novel site in rat brain: Homogenate binding studies. *Eur. J. Pharmacol., Mol. Pharmacol. Sect.* **1993**, *244*, 293−301.
(22) Gee N. S.; Brown J. P.; Dissanayake V. U.; Offord J.; Thurlow R.; Woodruff G. N. The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha-2d-elta subunit of a calcium channel. *J. Biol. Chem.* **1996**, *271*, 5768−5776.
(23) Wolf, M.; Eberhart, A.; Glossmann, H.; Striessnig, J.; Grigorieff, N. Visualization of the Domain Structure of an L-type Ca2+ Channel Using Electron Cryo-microscopy. *J. Mol. Biol.* **2003**, *332*, 171−182.
(24) Klugbauer, N.; Marais, E.; Hofmann, F. Calcium Channel $\alpha_2\delta$ Subunits: Differential Expression, Function, and Drug Binding. *Journal of Bioenerg. Biomembr.* **2003**, *35*, 639−647.
(25) Gong, H. C.; Hang, J.; Kohler, W.; Li, L.; Su, T.-Z. Tissue-specific expression and gabapentin-binding properties of calcium channel $\alpha_2\delta$ subunit subtypes. *J. Membr. Biol.* **2001**, *184*, 35−43.
(26) Marais, E.; Klugbauer, N., Hofmann, F. Calcium channel alpha-2-delta subunits − Structure and gabapentin binding. *Mol. Pharmacol.* **2001**, *59*, 1243−48.
(27) Qin, N.; Yagel, S.; Momplaisir, M.; Codd, E.; D′Andrea, M. Molecular cloning and characterization of the human voltage-gated calcium channel α(2)δ-4 subunit. *Mol. Pharmacol.* **2002**, *62*, 485−496.
(28) Martin D. J.; McClelland, D.; Herd, M. B.; Sutton, K. G.; Hall, M. D.; Lee, K.; Pinnock, R. D.; Scott, R. H. Gabapentin-mediated inhibition of voltage-activated Ca^{2+} channel currents in cultured sensory neurones is dependent on culture conditions and channel subunit expression. *Neuropharmacology* **2002**, *42*, 353−366.
(29) Sutton, K. G.; Martin, D. J.; Pinnock, R. D.; Lee, K.; Scott, R. H. Gabapentin inhibits high-threshold calcium channel currents in cultured rat dorsal root ganglion neurones. *Br. J. Pharmacol.* **2002**, *135*, 257−265.
(30) Stefani, A.; Hainsworth, A. H. Gabapentin-mediated effects on voltage- and ligand-gated currents. *Curr. Neuropharmacol.* **2003**, *1*, 237−244.
(31) Dooley D. J.; Donovan, C. M.; Pugsley, T. A. Stimulus-dependent modulation of [3H]norepinephrine release from rat neocortical slices by gabapentin and pregabalin. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 1086−1093.
(32) Maneuf, Y. P.; Hughes, J.; McKnight, A. T. Gabapentin inhibits the substance P-facilitated K^+-evoked release of [3H]glutamate from rat caudal trigeminal nucleus slices. *Pain* **2001**, *93*, 191−196.
(33) Bryans J. S.; Davies N.; Gee N. S.; Dissanayake V. U.; Ratcliffe G. S.; Horwell D. C.; Kneen C. O.; Morrell A. I.; Oles R. J.; O'Toole J. C.; Perkins G. M.; Singh L.; Suman-Chauhan N.; O'Neill J. A. Identification of novel ligands for the gabapentin binding site on the alpha2delta subunit of a calcium channel and their evaluation as anticonvulsant agents. *J. Med. Chem.* **1998**, *41*, 1838−45.
(34) Sterns, B. A.; Anker, N.; Arruda, J. M.; Campbell, B. T.; Chen, C.; Cramer, M.; Hu, T.; Jiang, X.; Park, K.; Ren, K. K.; Salabad, M.; Santini, A.; Schaffhauser, H.; Urban, M. O.; Munoz, B. Synthesis and biological evaluation of 6-aryl-6H-pyrrolo[3,4-d]-pyridazine derivatives: high affinity ligands to the $\alpha_2\delta$ subunit of voltage gated claclium channels. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1295−1298.
(35) Andruszkiewicz, R.; Silverman, R. B. A convenient synthesis of 3-alkyl-4-aminobutanoic acids. *Synthesis* **1989**, *12*, 953−5.
(36) Arai, S.; Shioiri, T. Catalytic asymmetric Darzens condensation under phase-transfer-catalyzed conditions. *Tetrahedron Lett.* **1998**, *39* (15), 2145−8.
(37) Rychnovsky, S. D.; Mickus, D. E. Synthesis of *ent*-cholesterol, the unnatural enantiomer. *J. Org. Chem.* **1992**, *57*, 2732−6.
(38) Thaisrivongs, S.; Tomasselli, A. G.; Moon, J. B.; Hui, J.; McQuade, T. J.; Turner, S. R.; Strohbach, J. W.; Howe, W. J.; Tarpley, W. G.; Heinrikson, R. L. Inhibitors of the protease from human immunodeficiency birus: Design and modeling of a compound containing a dihydroxyethylene isostere insert with high binding affinity and effective antiviral activity. *J. Med. Chem.* **1991**, *34* (8), 2344−56.
(39) For a review, see: B'Hymer, C.; Montes-Bayon, M.; Caruso, J. A. Marfey's reagent: past, present, and future uses of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide. *J. Sep. Sci.* **2003**, *26* (1/2), 7−19.
(40) Hoekstra, M. S.; Sobieray, D. M.; Schwindt, M. A.; Mulhern, T. A.; Grote, T. M.; Huckabee, B. K.; Hendrickson, V. S.; Franklin, L. C.; Granger, E. J.; Karrick, G. L. Chemical development of CI-1008, an enantiomerically pure anticonvulsant. *Org. Process Res. Dev.* **1997**, *1* (1), 26−38.
(41) Sibi, M. P.; Johnson, M. D.; Punniyamurthy, T. Enantioselective synthesis of peperomins A, C, D, and analogs − Examination of diastereoselective cuprate conjugate additions to *N*-enoyl-4-diphenylmethyl-2-oxazolidinones. *Can. J. Chem.* **2001**, *79*, 1546−1555.
(42) Zanardi, F.; Battistini, L.; Rassu, G.; Cornia, M.; Casiraghi, G. γ-Substituted pyrrole-based silyl dienol ethers as α-amino acid enolate equivalents: A versatile entry to racemic α-substituted α-amino acids. *J. Chem. Soc., Perkin Trans. 1* **1995**, *19*, 2471.
(43) Nagashima, H.; Ozaki, N.; Washiyama, M.; Itoh, K. Conjugate addition of organocopper reagents to *N*-tosylated α,β-unsaturated amides. *Tetrahedron Lett.* **1985**, *26* (5), 657.
(44) Williams, D. R.; Kissel, W. S.; Li, J. J. Diastereoselection in the conjugate additions of organocopper reagents to *N*-enoyloxazolidinones. *Tetrahedron Lett.* **1998**, *39*, 8593−8596
(45) Fernandez-Zertuche, M.; Robledo-Perez, R.; Meza-Avina, M. E.; Ordonez-Palacios, M. Allylic strain effects on the stereochemistry of the alkylation reaction of mycophenolic acid chiral enolates. *Tetrahedron Lett.* **2002**, *43*, 3777.
(46) King, C. R.; Poulter, C. D. Model studies of terpene biosynthesis. Intermolecular 1′-2 electrophilic condensation of 3-methyl-2-butenyl acetate. *J. Am. Chem. Soc.* **1982**, *104*, 1413−20.
(47) Schlesinger, K.; Greik, B. J. The genetics and biochemistry of audiogenic seizures. In *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype*; Lindzey, G.; Theissen, D. D.; Eds. Appleton-Centruy-Crofts: New York, 1970; pp 219−257.
(48) Seyford, T. N. Audiogenic Seizures in Mice *Fed. Proc.* **1979**, *38*, 2399−2404
(49) Hargreaves K.; Dubner R.; Brown F.; Flores C.; Joris J A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* **1988**, *32* (1), 77−88.
(50) Milan, M. J. The neurobiology and control of anxious states. *Prog. Neurobiol. (Amsterdam, Netherlands)* **2003**, *70*, 83−244.
(51) Okimoto, M.; Chiba, T. Electrochemical oxidation of ketone acylhydrazones and their HCN adducts in NaCN-MeOH. Transformation of ketones to nitriles. *J. Org. Chem.* **1990**, *55*, 1070−1076.
(52) Shotwell M. A.; Jayme D. W.; Kilberg M. S.; Oxender D. L. Neutral amino acid transport systems in Chinese hamster ovary cells. *J. Biol. Chem.* **1981**, *256* (11), 5422−7.
(53) Coughenour L. L.; McLean J. R.; Parker R. B. A new device for the rapid measurement of impaired motor function in mice. *Pharm. Biochem. Behav.* **1977**, *6*, 351−3.
(54) Litchfield J. T. Jr; Wilcoxon F. A simplified method of evaluating dose−effect experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99−113.

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