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ARTICLE

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Primary Amino Acid Derivatives: Compounds with Anticonvulsant and Neuropathic Pain Protection Activities

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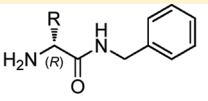
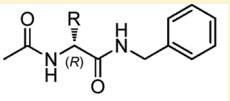
Supporting Information

ABSTRACT: Pharmacological management remains the primary method to treat epilepsy and neuropathic pain. We have advanced a novel class of anticonvulsants termed functionalized amino acids (FAAs). In this study, we examine FAA derivatives from which the terminal acetyl moiety was removed and termed these compounds primary amino acid derivatives (PAADs). Twenty-seven PAADs were prepared; the central C(2) R-substituent was varied, including C(2) stereochemistry, and the compounds were tested in rodent models of seizures and neuropathic pain. C(2)-Hydrocarbon *N*-benzylamide PAADs were potent anticonvulsants and excellent anticonvulsant activity (mice, ip; rat, po) was observed for C(2) R-substituted PAADs in which the R group was ethyl, isopropyl, or *tert*-butyl, and the C(2) stereochemistry conformed to the D-amino acid configuration ((*R*)-stereoisomer). These values surpassed the activities of several clinical antiepileptic drugs. The C(2) (*R*)-ethyl and C(2) (*R*)-isopropyl PAADs also displayed excellent activities in the mouse (ip) formalin neuropathic pain model. Significantly, unlike the FAA structure−activity relationship, PAAD anticonvulsant activity increased upon substitution of a methylene unit for a heteroatom in the R-substituent that was one atom removed from the C(2) site, suggesting that these PAADs function by a different pathway than FAAs.



PAAD



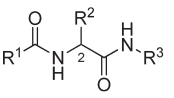
FAA

R	PAAD MES ED_{50} (mg/kg)	FAA MES ED_{50} (mg/kg)
CH_2OCH_3	48	4.5
$CH(CH_3)_2$	15	>100, <300
$C(CH_3)_3$	14	>300

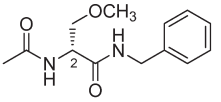
INTRODUCTION

Epilepsy is a serious neurological disorder affecting up to 1% of the world's population, including more than 2 million Americans.[1,2] Currently, its estimated annual medical cost exceeds $15 billion, and approximately 140,000 new cases were diagnosed in the US in 2010.[1] Epilepsy is characterized by recurring, unprovoked seizures that result from neuronal hyperexcitability and hypersynchronous neuronal firing[2] and is commonly, but incorrectly, defined as a singular disease. More appropriately, epilepsy is a heterogeneous mixture of disorders with the commonality of neuronal dysregulations that result from varying external, brain developmental, or genetic causes.[3]

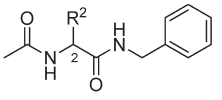
We have advanced a novel class of anticonvulsants termed functionalized amino acids (FAAs, **1**).[4−15] The structure−activity relationship (SAR) for FAAs provided distinctive trends that permitted our discovery of the lead FAA lacosamide ((*R*)-**2**),[5] which is marketed in the US and Europe for the adjuvant treatment of partial-onset seizures in adults.[16] We found that FAA **3**, wherein R^1 was methyl and R^3 was benzyl, provided excellent anticonvulsant activity in the maximal electroshock seizure (MES) model. Most important, activity improved when we incorporated a substituted heteroatom in R^2 that was one atom removed from the C(2) carbon and whose activity principally resided in the (*R*)-stereoisomer.[4,5,10−12]



1 (FAAs)



(*R*)-**2**



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Neuropathic pain affects up to 8% of the world's population, and results from dysfunctioning neuronal pathways within the peripheral nervous system (PNS), the central nervous system (CNS), or both.[17−19] Thus, it is not surprising that several antiepileptic drugs (AEDs) are used to manage neuropathic pain.[20,21]

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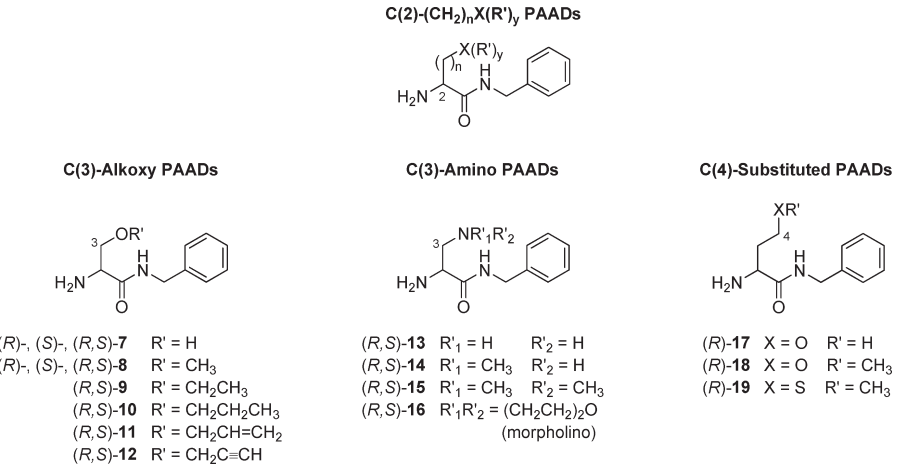


Figure 1. C(2)-$(CH_2)_nX(R')_y$ PAADs.

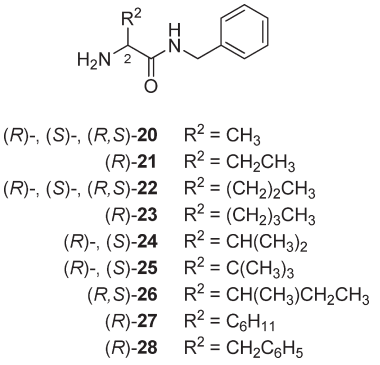
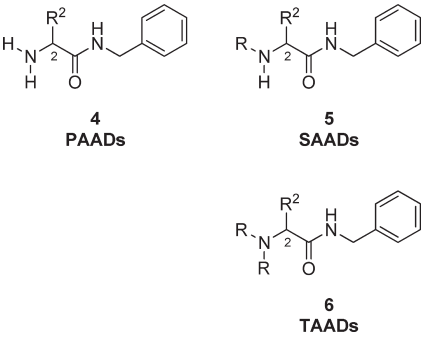


Figure 2. C(2)-Hydrocarbon PAADs.

(*R*)-**2** has shown significant protection in neuropathic models for inflammatory pain, osteoarthritis pain, diabetic neuropathy, bone cancer pain, chemotherapy-induced pain, and spinal-cord nerve injury.[22−28]

Pharmacological management remains the primary method to treat epilepsy and neuropathic pain disorders. Although newer generation AEDs have improved patient care, it is estimated that 30% of epilepsy patients fail at least two first-line AED treatments, so their epilepsy is deemed to be pharmacoresistant. Additionally, 40% of the patients who do respond to AEDs experience adverse side effects.[29,30] Similarly, neuropathic pain medications provide 30−50% pain reduction in approximately 50% of patients.[31] There remains, therefore, a need for potent new neurological agents with improved efficacy, decreased toxicity, and favorable physiochemical and pharmacokinetic properties to better treat these disorders.

We, and others, have briefly examined FAA derivatives from which the terminal acetyl moiety was removed.[32−38] We named these compounds primary amino acid derivatives (PAADs, **4**), secondary amino acid derivatives (SAADs, **5**), or tertiary amino acid derivatives (TAADs, **6**), depending on the terminal amine substitution pattern. We saw potential therapeutic benefits in these amine derivatives. Chief among these was the protonation of the terminal amine at physiological pH values to give the corresponding ammonium species. We anticipated amine protonation would allow these compounds to exhibit improved water solubility while permitting greater diversity for the C(2) R^2 and *N*-benzylamide substituents. A preliminary SAR study of **4**−**6** examined the importance of the C(2) R^2-group and the terminal amine substituents. While representative **4**−**6** displayed excellent activity in the MES model, the limited data did not provide clear trends to warrant further development.[33] In this investigation, we restricted our compound set to **4**s that had an unsubstituted *N*-benzylamide unit and focused on the central C(2) R^2-substituent, including C(2) stereochemistry. We demonstrated that select PAADs exhibited outstanding activities in established animal models for seizures and neuropathic pain. Significantly, PAADs and FAAs did not share similar SARs; PAADs that contained a branched hydrocarbon at the C(2) position displayed excellent activities while the corresponding FAAs were largely inactive. The implications of these findings are discussed.



RESULTS

Choice of Compounds. We used PAAD **4**, which contained an unsubstituted *N*-benzylamide moiety, as our structural template and systematically modified the C(2) R^2-position according to two categories: heteroatom-containing substituents (PAADs **7**−**19**) (Figure 1) or hydrocarbon substituents (PAADs **20**−**28**) (Figure 2). Heteroatom-containing substituents correspond to the general formula $(CH_2)_nX(R')_y$, where *n* equals the number of methylene carbons, X is an oxygen, nitrogen, or sulfur, R′ is an alkyl group, and *y* is the number of R′ groups. For select compounds in this series, we compared the (*R*)-, (*S*)-, and (*R*,*S*)-stereoisomers.

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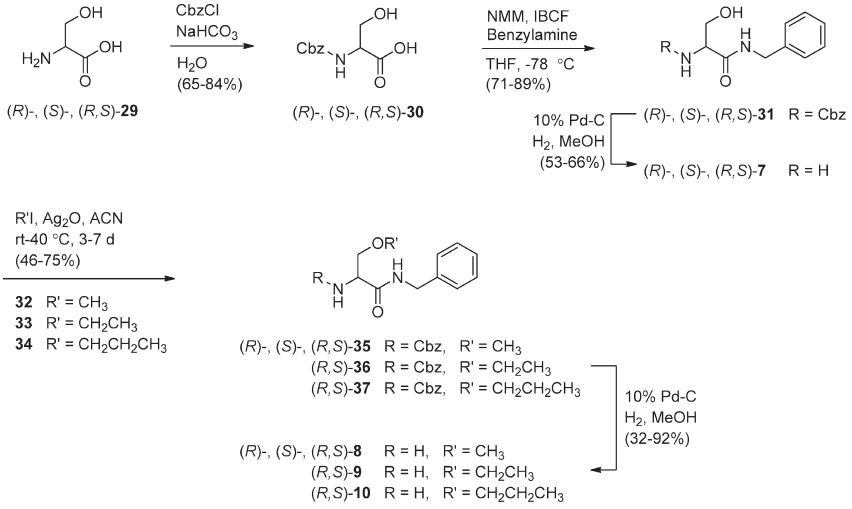
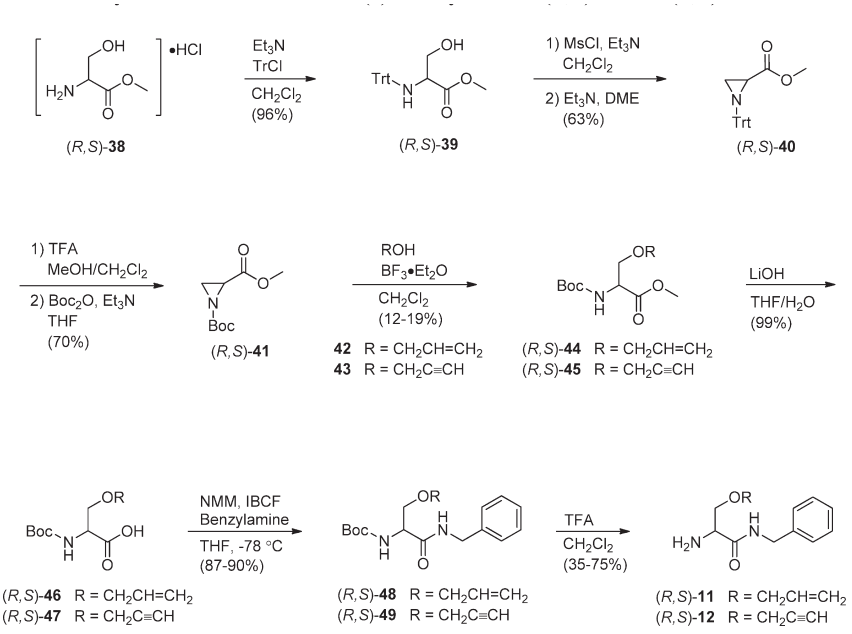
Scheme 1. Synthesis of C(3)-Hydroxy PAAD 7 and C(3)-Alkoxy PAADs 8−10



Scheme 2. Synthesis of Unsaturated C(3)-Alkoxy PAADs (*R,S*)-11 and (*R,S*)-12



The heteroatom-containing PAADs were further classified into three subcategories (Figure 1): C(3)-alkoxy PAADs (**7**−**12**), C(3)-amino PAADs (**13**−**16**), and C(4)-substituted PAADs (**17**−**19**). In the C(3)-alkoxy series, R′ was successively replaced with a methyl, ethyl, propyl, allyl, and propargyl group. All C(3)-alkoxy PAADs were synthesized in the (*R,S*)-configuration, with the exception of the C(3)-hydroxy PAAD **7** and the C(3)-methoxy PAAD **8**, which were synthesized in the (*R*)-, (*S*)-, and (*R,S*)-configurations. In the C(3)-amino series, we prepared the parent C(3)-amino PAAD **13**, the *N*′,*N*′-dimethylamino PAAD **15** ($y = 2$), and morpholino **16**. Efforts to prepare the *N*′-monomethyl PAAD **14** were unsuccessful. All C(3)-amino PAADs were synthesized in the (*R,S*)-configuration. In the C(4)-substituted series, X is oxygen or sulfur, and R′ is either a hydrogen or methyl group (**17**−**19**). All C(4)-substituted PAADs were synthesized in the (*R*)-configuration. Efforts to prepare (*R*)-**18** on a scale sufficient for testing were unsuccessful.

In the C(2)-hydrocarbon PAADs **20**−**28**, R^2 is a linear alkyl group (**20**−**23**; methyl, ethyl, propyl, and *n*-butyl), a branched alkyl group (**24**−**26**; isopropyl, *tert*-butyl, and 2-methylpropyl), or a cyclic group (**27**−**28**; cyclohexyl and benzyl) (Figure 2). Because the C(2)-$(CH_2)_nX(R')_y$ PAADs revealed a preference for stereochemistry in the D-amino acid configuration, all hydrocarbon PAADs were synthesized as the (*R*)-stereoisomer, except PAAD **26**, which contained two chiral centers and was a mixture of four diastereomers. The excellent anticonvulsant activity of PAADs (*R*)-**24** and (*R*)-**25** prompted the synthesis of the corresponding (*S*)-stereoisomers to determine if pharmacological activity paralleled the C(2)-$(CH_2)_nX(R')_y$ PAADs, where activity preferentially resided in the C(2) D-amino acid configuration.

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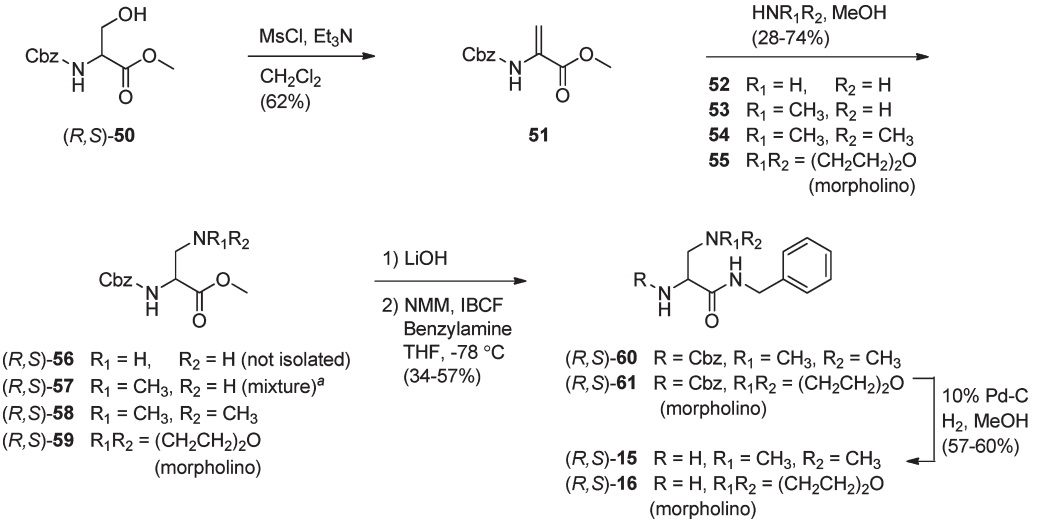
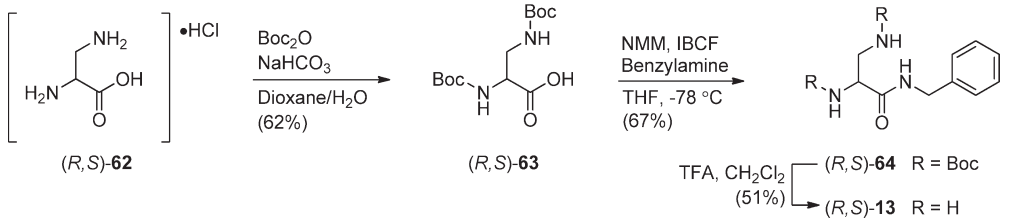
Scheme 3. Pathway for the Synthesis of N',N'-Disubstituted C(3)-Amino PAADs (*R*,*S*)-15 and (*R*,*S*)-16 Utilizing Cbz-Protected Dehydroalanine Methyl Ester 51[a]



[a] Mixture = mixture of (*R*,*S*)-**57** and (*R*,*S*)-*N*-methyl 2-N''-(benzyloxycarbonyl)amino-3-(N''-methylamino)propionamide.

Scheme 4. Synthesis of (*R*,*S*)-*N*-Benzyl 2,3-Diaminopropionamide ((*R*,*S*)-13)



Chemistry. C(3)-Hydroxy and C(3)-alkoxy PAADs **7**−**10** were synthesized in 3−4 steps using commercially available reagents and established synthetic procedures (Scheme 1). Treatment of (*R*)-, (*S*)-, and (*R*,*S*)-serine (**29**) with benzyl chloroformate under basic conditions gave (*R*)-, (*S*)-, and (*R*,*S*)-**30**,[39] which were then converted to the amides (*R*)-, (*S*)-, and (*R*,*S*)-**31** using the mixed anhydride coupling (MAC) procedure,[40] followed by subsequent hydrogenolysis to obtain the corresponding PAADs (*R*)-, (*S*)-, and (*R*,*S*)-**7**. Alkylation of (*R*)-, (*S*)-, and (*R*,*S*)-**31** using methyl iodide (**32**) and Ag_2O gave (*R*)-, (*S*)-, and (*R*,*S*)-**35**[39] before hydrogenolysis to the corresponding PAADs (*R*)-, (*S*)-, and (*R*,*S*)-**8**.[32,33] Similarly, treatment of (*R*,*S*)-**31** with either ethyl iodide (**33**) or propyl iodide (**34**) and Ag_2O gave (*R*,*S*)-**36** and (*R*,*S*)-**37**, followed by hydrogenolysis to the PAADs (*R*,*S*)-**9** and (*R*,*S*)-**10**. We observed an increase in reaction time and temperature, as well as a decrease in yield, as the alkyl iodide went from methyl to ethyl to propyl. A similar finding was observed for the corresponding FAAs.[5]

We attempted to synthesize (*R*,*S*)-**11** and (*R*,*S*)-**12** using allyl iodide and propargyl iodide under the alkylation conditions from Scheme 1, but no reaction was observed. Therefore, we prepared PAADs (*R*,*S*)-**11** and (*R*,*S*)-**12** via an aziridine intermediate (Scheme 2). First, we executed a 3-step procedure beginning with the *N*-tritylation of commercially available (*R*,*S*)-**38**, followed by cyclodehydration to give (*R*,*S*)-**40**.[41] Completion of the remaining steps required consideration of the sensitivity of the *N*-protecting group to $BF_3 \cdot Et_2O$ and of the sensitivity of the unsaturated alkenyl or alkynyl group to the final deprotection conditions. Aziridines exhibit modest reactivity toward ring-opening by oxygen nucleophiles, so we incorporated an electron-withdrawing group at the nitrogen site to enhance ring-opening.[42] Thus, deprotection of (*R*,*S*)-**40** under acidic conditions was followed by reprotection with Boc_2O under basic conditions to give aziridine (*R*,*S*)-**41**.[43] Ring-opening with allyl alcohol (**42**) or propargyl alcohol (**43**) in the presence of $BF_3 \cdot Et_2O$ gave (*R*,*S*)-**44** and (*R*,*S*)-**45**,[44] which were hydrolyzed to give (*R*,*S*)-**46** and (*R*,*S*)-**47** and then directly used for the MAC procedure to give amides (*R*,*S*)-**48** and (*R*,*S*)-**49**. Finally, amides (*R*,*S*)-**48** and (*R*,*S*)-**49** were trifluoroacetic acid (TFA) deprotected to the corresponding PAADs (*R*,*S*)-**11** and (*R*,*S*)-**12**.

We prepared the N',N'-disubstituted C(3)-amino PAADs (*R*,*S*)-**15** and (*R*,*S*)-**16** using a procedure similar to our earlier synthesis of racemic 2,3-diaminopropionic acid derivatives.[45] Accordingly, we generated dehydroalanine **51**[46] via the mesylation and elimination of (*R*,*S*)-**50** using Et_3N (Scheme 3). Michael addition of dimethylamine (**54**) or morpholine (**55**) to **51** gave (*R*,*S*)-**58** and (*R*,*S*)-**59**, which were subsequently hydrolyzed and directly used in the MAC procedure to give the corresponding amides (*R*,*S*)-**60** and (*R*,*S*)-**61**. Deprotection of amides (*R*,*S*)-**60** and (*R*,*S*)-**61** by hydrogenolysis gave PAADs (*R*,*S*)-**15** and (*R*,*S*)-**16**. Use of ammonia (**52**) in this protocol was unsuccessful. We

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observed a competing side reaction when methylamine (**53**) was employed and could not isolate sufficient quantities of (*R*,*S*)-**57**.[47]

To prepare (*R*,*S*)-**13**, we treated commercially available diamine (*R*,*S*)-**62** with Boc_2O under basic conditions to give (*R*,*S*)-**63** (Scheme 4).[48] Using the MAC procedure, (*R*,*S*)-**63** was coupled with benzylamine to give amide (*R*,*S*)-**64**, followed by TFA deprotection to give PAAD (*R*,*S*)-**13**.

Evaluation of (*R*)-, (*S*)-, and (*R*,*S*)-**8** indicated that the pharmacological activity resided largely in the D-amino acid configuration (see Pharmacological Activity). Therefore, the C(4)-substituted PAADs were synthesized as the (*R*)-stereoisomer. Synthesis of (*R*)-**19** was achieved in 3 steps, starting with the *N*-*t*Boc protection of commercially available D-methionine ((*R*)-**65**),[49] followed by coupling with benzylamine using the MAC procedure to provide (*R*)-**67**, and finally, TFA deprotected to give (*R*)-**19** (Scheme 5).

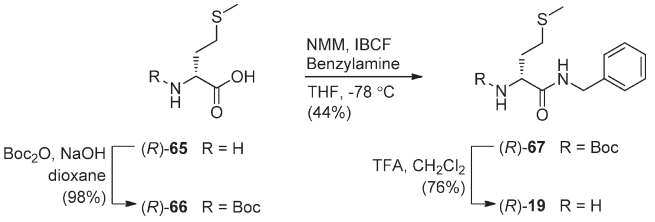
To synthesize the oxygen equivalent of (*R*)-**19** (*S* → *O*, (*R*)-**18**), we attempted to follow the synthetic route depicted in Scheme 1 using commercially available D-homoserine ((*R*)-**68**) to obtain the C(4)-hydroxy PAAD (*R*)-**17** and the C(4)-methoxy PAAD (*R*)-**18**. However, lactonization during *N*-Cbz protection of (*R*)-**68** gave (*R*)-**69**, so we modified our route to take advantage of (*R*)-**69** (Scheme 6).[50] Treatment of (*R*)-**69** under basic conditions with benzylamine gave amide (*R*)-**70**, followed by hydrogenolysis to give PAAD (*R*)-**17**. Several attempts were made to obtain (*R*)-**18**, but large scale efforts to methylate (*R*)-**70** were unsuccessful.

C(2)-Hydrocarbon PAADs were prepared using a 3-step procedure beginning with commercially available amino acids (Scheme 7). (*R*)-**71**−**74**, (*S*)-**73**−**74**, (*R*,*S*)-**75**, and (*R*)-**76** were *N*-*t*Boc protected following standard procedures, coupled with benzylamine using the MAC method, and then TFA deprotected to give PAADs (*R*)-**21**, (*R*)-**23**−**25**, (*S*)-**24**−**25**, (*R*,*S*)-**26**, and (*R*)-**27**. PAADs (*R*)-, (*S*)-, (*R*,*S*)-**22** and (*R*)-**28** were prepared using a *N*-Cbz protecting group similar to that used to synthesize (*R*)-, (*S*)-, and (*R*,*S*)-**7** (Scheme 1). Accordingly, (*R*)-, (*S*)-, and (*R*,*S*)-norvaline (**89**) were used to prepare the corresponding *N*-Cbz intermediates (*R*)-, (*S*)-, and (*R*,*S*)-**91** and (*R*)-, (*S*)-, and (*R*,*S*)-**93**. Similarly, (*R*)-phenylglycine ((*R*)-**90**) was used to prepare the corresponding *N*-Cbz intermediates (*R*)-**92** and (*R*)-**94** (Scheme 8).

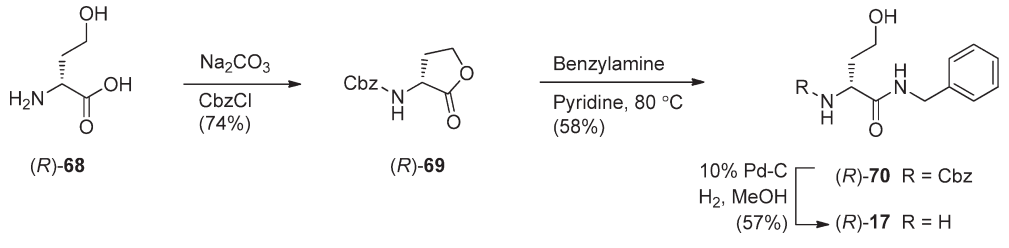
We detail in the Experimental Section the final step (synthetic procedure and characterization) for all compounds evaluated in the animal models. In the Supporting Information, we provide our experimental procedures for all compounds prepared and their physical and spectroscopic properties.

Pharmacological Activity. PAADs **7**−**13**, **15**−**17**, and **19**−**28** were evaluated for anticonvulsant activity using the MES test at UCB Pharma, following the procedures reported by Klitgaard,[51] or at the National Institute of Neurological Disorders and Stroke Anticonvulsant Screening Program (NINDS ASP), following the procedures reported by Stables and Kupferberg,[52] or at both. Anticonvulsant activity using the 6 Hz test was performed at UCB Pharma, following the procedures reported by Kaminski and co-workers (44 mA),[53] or at the NINDS ASP, following the procedures reported by Stables and Kupferberg (32 mA),[52] or at both. Several PAADs evaluated at UCB Pharma were tested in the formalin model of neuropathic pain.[54,55] Recently, Visser and co-workers demonstrated a good correlation between findings in the second phase of the formalin test and results for cold allodynia in the chronic constriction injury (CCI) model for both rats ($r = 0.72$) and gerbils ($r = 0.68$) using drugs with pain-attenuating effects in humans.[56] Therefore, the formalin model has an advantage over other well-characterized models of neuropathic pain (e.g., CCI) due to its ease of administration and standardization, and is an effective tool to prescreen compounds for neuropathic pain protection. All compounds were administered intraperitoneally (ip) to mice at UCB Pharma or ip to mice and orally (po) to rats at the NINDS ASP. For compounds that exhibited significant activity, we report the effective dose (50%, ED_{50}) values obtained in quantitative screening evaluations. Also provided are the median doses for neurological impairment (50%, TD_{50}) in mice using the rotorod test[57] and the behavioral toxicity effects observed in rats. TD_{50} values were determined for compounds that exhibited significant activity in the MES test. The pharmacological data from the MES, 6 Hz, and formalin tests are summarized in Tables 1−3. The MES activities of PAADs are compared with those of their corresponding FAAs in Tables 4, 5, and Supporting Information Table S1. The pharmacological data from PAADs synthesized as individual isomers ((*R*), (*S*), and (*R*,*S*)) are reported in Tables 1−3 and then summarized in Supporting Information Table S2. Several compounds were evaluated at both UCB Pharma and the NINDS ASP and displayed comparable activities (Supporting Information Table S3). The protective indices (PI = TD_{50}/ED_{50}) are provided, when applicable. PAADs tested at the

Scheme 5. Synthesis of (*R*)-*N*-Benzyl 2-Amino-4-(methylthio)butanamide ((*R*)-19)



Scheme 6. Synthesis of (*R*)-*N*-Benzyl 2-Amino-4-hydroxybutanamide ((*R*)-17)



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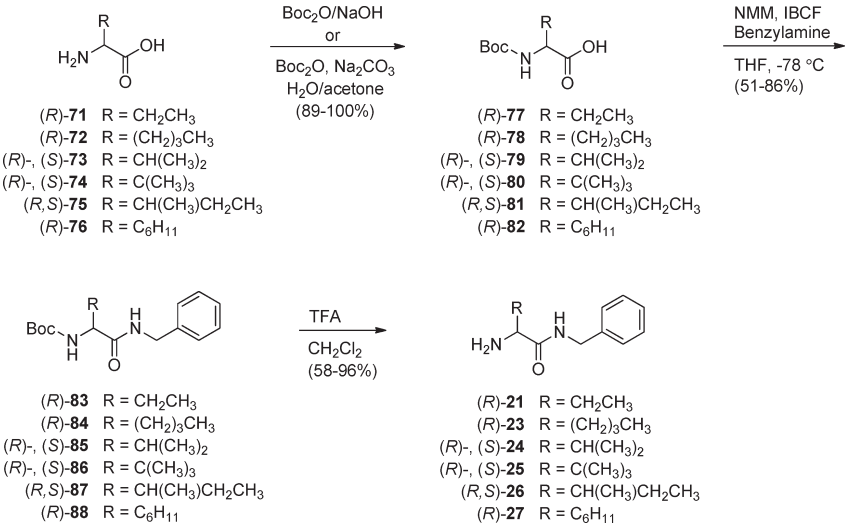
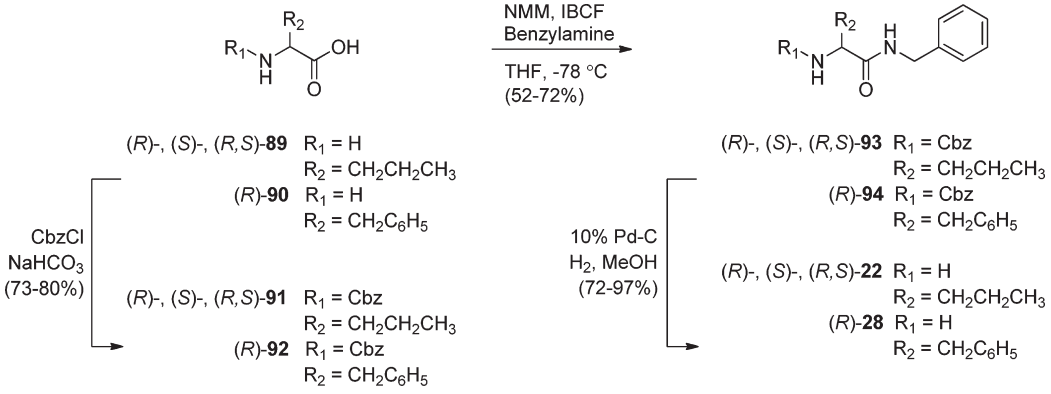
Scheme 7. Synthesis of C(2)-Hydrocarbon PAADs (*R*)-21, (*R*)-23−25, (*S*)-24−25, (*R,S*)-26, and (*R*)-27



Scheme 8. Synthesis of (*R*)-, (*S*)-, (*R,S*)-*N*-Benzyl 2-Aminopentanamide ((*R*)-, (*S*)-, (*R,S*)-22) and (*R*)-*N*-Benzyl 2-Amino-3-phenylpropionamide ((*R*)-28)



NINDS ASP were evaluated in the subcutaneous Metrazol (scMet) seizure model, but little or no protection was observed at the doses (30, 100, 300 mg/kg) and times (0.5 and 4 h) tested (data not shown).

Table 1 lists the neurological activities for the first category of C(2)-substituted PAADs, which included the C(3)-alkoxy, C(3)-amino, and C(4)-substituted derivatives. Beginning with the C(3)-oxy *N*-benzylamide PAADs (*R*)-, (*S*)-, and (*R,S*)-**7**, (*R*)- and (*S*)-**8**, and (*R,S*)-**9**−**12** in mice, we systematically evaluated the effect of a hydrogen, methyl, ethyl, propyl, allyl, and propargyl group placed at the C(3)-oxy terminus (R′) on anticonvulsant activity and pain attenuation. The clinical AEDs (*R*)-**2**,[5] phenytoin,[58] phenobarbital,[58] and valproate[58] served as the reference compounds. The MES activities of the C(3)-alkoxy PAADs (*R*)-, (*S*)-, and (*R,S*)-**8**, and (*R,S*)-**9**−**12** remained relatively constant throughout the series, but we observed a slight increase in activity as we went from a saturated alkyl group ((*R,S*)-**10**, ED_{50} = 69 mg/kg) to unsaturated hydrocarbon groups (ED_{50} (mg/kg): (*R,S*)-**11**, 45; (*R,S*)-**12**, 46). (*R*)-**8** displayed the highest anticonvulsant activity in this series (ED_{50} = 34 mg/kg), but it was an approximate 10-fold drop in activity from the corresponding FAA (*R*)-**2** (ED_{50} = 3.3 mg/kg).[5] PAAD **8** showed an approximate 2-fold preference, in regard to MES activity, for the (*R*)-isomer. This selectivity was considerably lower than that exerted for (*R*)-**2**, where we saw a >22-fold difference in activity between the (*R*) and (*S*) isomers in mice.[5] Overall, comparing the MES activities of PAADs **7**−**9**, **11**, and **12** with their corresponding FAAs (Table 4) revealed a consistent drop in activity (3−10-fold) as we went from the FAA to the PAAD. In all instances, the MES activity was greater than the 6 Hz activity (44 and 32 mA), and no significant pain attenuation was observed in the formalin assay.

Table 1 also lists the pharmacological activities for the C(3)-amino *N*-benzylamide PAADs. Here, we evaluated the unsubstituted C(3)-amino PAAD ((*R,S*)-**13**), the corresponding substituted C(3)-amino PAAD with two methyl groups ((*R,S*)-**15**), and the PAAD in which the C(3)-amino was embedded within a morpholino moiety ((*R,S*)-**16**). No significant neurological activity was observed in either anticonvulsant models or the pain model, and toxicity was not evaluated because there was no activity. A similar finding was observed for the C(3)-amino substituted FAAs (Table 4).

The next compounds listed in Table 1 were the C(4)-substituted *N*-benzylamide PAADs (*R*)-**17** and (*R*)-**19**. PAADs

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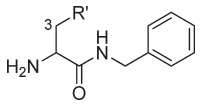
Table 1. Pharmacological Activities of Primary Amino Acid Derivatives (PAADs) in Mice (mg/kg) at UCB and the NINDS ASP



			mice (ip)[a]					
compd no.	test site	R′	MES,[b] ED_{50}	6 Hz,[c] ED_{50}	formalin, ED_{50}	Tox,[d] TD_{50}	PI,[e] MES	PI,[e] form
(*R*)-**7**	UCB	OH	ND[f]	>62	>62	>110		
(*S*)-**7**	UCB	OH	ND[f]	>62	>62	>110		
(*R*,*S*)-**7**	UCB	OH	ND[f]	>62	>62	>110		>1.8
(*R*)-**8**	UCB	OCH_3	34	>67	>67	>120	>3.5	>1.8
(*R*)-**8**	NINDS	OCH_3	48 [0.25] (40−61)	ND[f]	ND[f]	>30, <100 [0.25]		
(*S*)-**8**	UCB	OCH_3	64	>70	120	63	1.0	0.5
(*R*,*S*)-**9**	UCB	OCH_2CH_3	73	120	71[g] (19%)	ND[f]		
(*R*,*S*)-**10**	UCB	$OCH_2CH_2CH_3$	69	>130	>130	ND[f]		
(*R*,*S*)-**11**	UCB	$OCH_2CH{=}CH_2$	45	130 (MAD)[h]	75[g](12%)	ND[f]		
(*R*,*S*)-**12**	UCB	$OCH_2C{\equiv}CH$	46	130 (MAD)[h]	74[g] (27%)	47	1.0	
(*R*,*S*)-**13**	UCB	NH_2	>68	ND[f]	ND[f]	ND[f]		
(*R*,*S*)-**15**	UCB	$N(CH_3)_2$	94	>110	83[g](14%)	ND[f]		
(*R*,*S*)-**16**	UCB	$N(CH_2CH_2)_2O$	89	>84	69	ND[f]		
(*R*)-**17**	UCB	CH_2OH	>160	>120	>67	ND[f]		
(*R*)-**19**	UCB	CH_2SCH_3	75	130 (MAD)[h]	>76	ND[f]		
(*R*)-**22**	UCB	CH_2CH_3	21	66 (MAD)[h]	35	57	2.8	1.6
(*R*)-**23**	UCB	$(CH_2)_2CH_3$	23	93	>71	ND[f]		
lacosamide ((*R*)-**2**)	UCB		3.3	10	15	19	5.8	1.3
lacosamide[i] ((*R*)-**2**)	NINDS		4.5 [0.5] (3.7−5.5)	10	ND[f]	27 [0.25] (26−28)	6.0	
phenytoin[j]			9.5 [2.0] (8.1−10)			66 [0.5] (53−72)		
phenobarbital[j]			22 [1.0] (15−23)			69 [0.5] (63−73)		
valproate[j]			270 [0.25] (250−340)			430 [0.25] (370−450)		

[a] The compounds were administered either intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose−response curve was generated for all compounds that displayed sufficient activity and the dose−effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA, UCB; 32 mA, NINDS ASP). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] ND = not determined. [g] Single dose experiments where the mg/kg used is followed by the percentage protected in parentheses. [h] MAD = minimal active dose. [i] Reference 5. [j] Reference 58.

containing an oxygen ((*R*)-**17**) or a sulfur ((*R*)-**19**) two atoms from the C(2) carbon did not display appreciable anticonvulsant activity in the MES test (ED_{50} (mg/kg): (*R*)-**17**, >160; (*R*)-**19**, 75). (*R*)-**17** and (*R*)-**19** were also inactive in the 6 Hz and formalin tests (ED_{50} = >60 mg/kg), therefore, the inactivity did not justify toxicity studies.

Because the substituted heteroatom one atom removed from the C(2) carbon in R^2 led to increased FAA anticonvulsant activity, we compared the activities of (*R*)-**8** and (*R*,*S*)-**9** with their R^2-hydrocarbon equivalents (*R*)-**22** and (*R*)-**23** (Table 1). Significantly, methylene substitution of the C(3)-oxy moiety provided PAADs with appreciable anticonvulsant activity (ED_{50} (mg/kg): (*R*)-**22**, 21; (*R*)-**23**, 23) that surpassed their heteroatom counterparts (ED_{50} (mg/kg): (*R*)-**8**, 48; (*R*,*S*)-**9**, 73). (*R*)-**22** also displayed significant activity in the formalin test (ED_{50} = 35 mg/kg). We concluded that C(2)-acyclic PAADs containing a heteroatom one and two atoms removed from the C(2) center in R^2 did not exhibit notable seizure protection and that including a substituted heteroatom in R^2 did not increase activity. The diminished activity of the C(3)-alkoxy PAADs versus their hydrocarbon counterparts distinctly differentiates the PAAD SAR from the corresponding FAA SAR.

The significant activities of (*R*)-**22** and (*R*)-**23** prompted us to expand our study of C(2)-hydrocarbon *N*-benzylamide PAADs. Table 2 lists the neurological activities in mice (ip) for C(2)-hydrocarbon *N*-benzylamide PAADs (*R*)-, (*S*)-, and (*R*,*S*)-**20**, (*R*)-**21**, (*R*)-, (*S*)-, and (*R*,*S*)-**22**, (*R*)-**23**, (*R*)- and (*S*)-**24**, (*R*)- and (*S*)-**25**, (*R*,*S*)-**26**, (*R*)-**27**, and (*R*)-**28**. The C(2)-hydrocarbon PAADs displayed significant anticonvulsant activity in the MES test and, to a lesser degree, in the 6 Hz test. MES activity decreased slightly as the length of the alkyl chain increased (ED_{50} (mg/kg): ethyl ((*R*)-**21**, 16) > propyl ((*R*)-**22**, 21) > *n*-butyl ((*R*)-**23**, 23)), but the MES activity improved slightly with an increase in branching (ED_{50} (mg/kg): ethyl ((*R*)-**21**, 16) < isopropyl ((*R*)-**24**, 15) < *tert*-butyl ((*R*)-**25**, 13)). Substituting the terminal methyl group of (*R*)-**21** (ED_{50} = 16 mg/kg) with a phenyl group ((*R*)-**28**, ED_{50} = 40 mg/kg) resulted in a 2.5-fold decrease in MES activity. The same trends were observed for anticonvulsant activity in the 6 Hz test, although 3−5-fold less sensitive. The C(2)-hydrocarbon PAADs (*R*)-**20**,[33] (*R*)-**21**, (*R*)-**24**, and (*R*)-**25**

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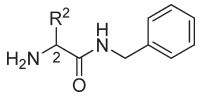
Table 2. Pharmacological Activities of C(2)-Hydrocarbon *N*-Benzylamide PAADs in Mice (mg/kg) at UCB and the NINDS ASP



			mice (ip)[a]					
compd no.	test site	R^2	MES,[b] ED_{50}	6 Hz,[c] ED_{50}	formalin, ED_{50}	Tox,[d] TD_{50}	PI,[e] MES	PI,[e] form
(*R*)-**20**[f]	NINDS	CH_3	>10, <30	ND[g]	69	>100, <300		
(*S*)-**20**[f]	NINDS	CH_3	>300	ND[g]	ND[g]	>300		
(*R,S*)-**20**[f]	NINDS	CH_3	>100, <300	ND[g]	ND[g]	>300		
(*R*)-**21**	UCB	CH_2CH_3	16	62 (MAD)[h]	22	ND[g]		
(*R*)-**21**	NINDS	CH_2CH_3	18 [0.25] (10−25)	∼50 [0.5]	ND[g]	80 [0.25] (65−95)	4.4	
(*R*)-**22**	UCB	$(CH_2)_2CH_3$	21	66 (MAD)[h]	35	57	2.8	1.6
(*S*)-**22**	UCB	$(CH_2)_2CH_3$	>37	>210	100	ND[g]		
(*R,S*)-**22**	UCB	$(CH_2)_2CH_3$	39	120 (MAD)[h]	68[i] (17%)	ND[g]		
(*R*)-**23**	UCB	$(CH_2)_3CH_3$	23	93	>71	ND[g]		
(*R*)-**24**	UCB	$CH(CH_3)_2$	16 (MAD)[h]	74	20	47		2.4
(*R*)-**24**	NINDS	$CH(CH_3)_2$	15 [0.25] (13−18)	<100 [0.25−1.0]	15 [0.25]	70 [0.25] (63−80)	4.7	
(*S*)-**24**	NINDS	$CH(CH_3)_2$	>300 [0.5]	ND[g]	ND[g]	>300 [0.5]		
(*R*)-**25**	UCB	$C(CH_3)_3$	13	>71	>22	ND[g]		
(*R*)-**25**	NINDS	$C(CH_3)_3$	14 [0.25] (11−17)	∼30 [0.25−0.5]	ND[g]	66 [0.25] (58−73)	4.7	
(*S*)-**25**	NINDS	$C(CH_3)_3$	42 [0.25] (37−46)	ND[g]	ND[g]	100 [0.25] (100−110)		
(*R,S*)-**26**	UCB	$CH(CH_3)CH_2CH_3$	46 (MAD)[h] 46[i] (100%)	>120	>22	ND[g]		
(*R*)-**27**	UCB	C_6H_{11}	28	140 (MAD)[h]	25 (inactive) 79[i] (95%)	ND[g]		
(*R*)-**28**	UCB	$CH_2C_6H_5$	40	81 (MAD)[h]	>37	ND[g]		

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose−response curve was generated for all compounds that displayed sufficient activity and the dose−effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] 6 Hz test = psychomotor seizure model (44 mA, UCB; 32 mA, NINDS ASP). [d] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [e] PI = protective index (TD_{50}/ED_{50}). [f] Reference 33. [g] ND = not determined. [h] MAD = minimal active dose. [i] Single dose experiments where the mg/kg used is followed by the percentage protected in parentheses.

all exhibited excellent activity in the rat (po) (MES ED_{50} = 11−19 mg/kg) (Table 3) and the values exceeded those of phenytoin (MES ED_{50} = 30 mg/kg).[58] The MES ED_{50} values for (*R*)-**21**, (*R*)-**24**, and (*R*)-**25** were the same (11 mg/kg), but interestingly, the time of peak efficiency increased as the size of the C(2)-hydrocarbon moiety decreased (0.25−2.0 h). None of the three compounds exhibited neurotoxic effects, resulting in high PI values (>45).

All of the C(2)-hydrocarbon *N*-benzylamide PAADs that were tested as individual enantiomers (**20**, **22**, **24**, and **25**) revealed a >1.8−20-fold increase in anticonvulsant activity in the MES test for the (*R*)-isomer compared with the (*S*)-isomer (Table 2). The isopropyl group at the C(2) carbon displayed the largest (*R*)- versus (*S*)-selectivity (ED_{50} (mg/kg): (*R*)-**24**, 15; (*S*)-**24**, >300), but the stereoselectivity was diminished to 3-fold using the *tert*-butyl group (ED_{50} (mg/kg): (*R*)-**25**, 14; (*S*)-**25**, 42). The stereoselectivity of (*R*)-**24** resembles that of (*R*)-**2**, wherein (*R*)-**2** is >22-fold more potent than its (*S*)-stereoisomer.[5] The large variation in (*R*)- versus (*S*)-selectivity with respect to MES activity in the C(2)-hydrocarbon series was unexpected. In the FAAs, the eudismic ratio[59] (the ratio of the affinity between the more tightly bound isomer [eutomer] and the less tightly bound isomer [distomer]) ranged between 10 and 22. We are uncertain why this ratio varied within the PAAD series but suspect that multiple factors (e.g., site selectivity, metabolism, transport, efflux) affected the observed anticonvulsant activities for the individual stereoisomers. The PI values in the MES test in mice of (*R*)-**24** (PI = 4.7) and (*R*)-**25** (PI = 4.7) are similar to (*R*)-**2** (PI = 6.0).[5] When (*R*)-**24** was tested in the rat, no apparent behavioral toxicity (TD_{50} = >500 mg/kg) was observed, providing a PI >45 (Table 3). Remarkably, the anticonvulsant activities in the MES test (mice, ip) of PAADs (*R*)-**24** (ED_{50} = 15 mg/kg) and (*R*)-**25** (ED_{50} = 14 mg/kg) greatly surpassed those of their FAA counterparts, (*R*)-**105** (ED_{50} = >100, <300 mg/kg) and (*R*)-**106** (ED_{50} = >300 mg/kg) (Table 5), and exceeded the MES activity of the traditional antiepileptic phenobarbital (ED_{50} = 22 mg/kg).[58] The observed increase in anticonvulsant activity in proceeding from FAA (*R*)-**105** and (*R*)-**106** to PAAD (*R*)-**24** and (*R*)-**25**, respectively, exceeded 6.7-fold, and the trend was opposite to the pattern observed for the C(3)-alkoxy PAADs compared with their corresponding FAA counterparts (Table 4).

PAADs (*R*)-**21**, (*R*)-**22**, and (*R*)-**24** displayed excellent activity in the formalin test (ED_{50} (mg/kg): (*R*)-**21**, 22; (*R*)-**22**, 35; (*R*)-**24**, 20) and approached the formalin activity of (*R*)-**2** (ED_{50} = 15 mg/kg) (Table 2). We did not observe a correlation between the C(2)-alkyl length and formalin activity, as seen in the MES test. There was an initial 3-fold increase in activity from methyl to ethyl (ED_{50} (mg/kg): (*R*)-**20**, 69; (*R*)-**21**, 22), but then a steady decrease in activity was observed as the C(2)-alkyl length increased (ED_{50} (mg/kg): ethyl ((*R*)-**21**, 22) > propyl

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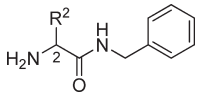
Table 3. Pharmacological Activities of C(2)-Hydrocarbon *N*-Benzylamide PAADs in Rats (mg/kg) at the NINDS ASP



		rat (po)[a]		
compd no.	R^2	MES,[b] ED_{50}	Tox,[c] TD_{50}	PI[d]
(*R*)-**20**[e]	CH_3	19 [2.0] (13−25)	>30	>1.5
(*S*)-**20**[e]	CH_3	>80	>80	
(*R,S*)-**20**[e]	CH_3	14 [1.0] (7−22)	>500	>36
(*R*)-**21**	CH_2CH_3	11 [2.0] (7.8−16)	>500	>45
(*R*)-**24**	$CH(CH_3)_2$	11 [0.25] (9.1−13)	>500	>45
(*R*)-**25**	$C(CH_3)_3$	11 [0.25] (8.7−15)	>500	>45
(*S*)-**25**	$C(CH_3)_3$	>30 [0.25−4.0]	>30 [0.25−4.0]	
lacosamide[f] ((*R*)-**2**)		3.9 [0.5] (2.6−6.2)	>500 [0.5]	>130
phenytoin[g]		30 [4.0] (22−39)	>3000	>100
phenobarbital[g]		9.1 [5.0] (7.6−12)	61 [0.5](44−96)	6.7
valproate[g]		490 [0.5] (350−730)	280 [0.5] (190−350)	0.6

[a] The compounds were administered orally to adult male albino Sprague−Dawley rats. ED_{50} and TD_{50} values are in mg/kg. A dose−response curve was generated for all compounds that displayed sufficient activity and the dose−effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = behavioral toxicity. [d] PI = protective index (TD_{50}/ED_{50}). [e] Reference 33. [f] Reference 5. [g] Reference 58.

((*R*)-**22**, 35) > *n*-butyl ((*R*)-**23**, >71). We observed an activity increase in the formalin test upon branching. A three carbon C(2) substituent displayed greater activity when it was in the isopropyl configuration ((*R*)-**24**, ED_{50} = 20 mg/kg) compared with the *n*-propyl configuration ((*R*)-**22**, ED_{50} = 35 mg/kg).

DISCUSSION

In this study, we asked whether C(2)-substituted PAADs exhibit potent anticonvulsant activities and neuropathic pain protection in animal models. We assumed that the close structural correspondence of FAAs and PAADs would facilitate our identification of active PAAD agents and prepared 27 PAADs containing C(2) R^2-substituents (i.e., CH_2OR', $CH_2N(R')R'$, CH_2CH_2XR', hydrocarbon) that tested the hallmarks of the FAA SAR[4−13] with respect to anticonvulsant activity. Accordingly, we investigated the importance for activity of a heteroatom in the R^2 group that was one atom removed from the C(2) center, the need for heteroatom substitution (R′), and the stereochemical preference at the C(2) position.

For many PAADs, anticonvulsant activity was determined in both the MES and the 6 Hz psychomotor seizure assays. Each model has shown different sensitivities to distinct classes of antiepileptic agents. A prime example is the efficacy of levetiracetam in the 6 Hz test but not in the MES test.[51] Conversely, traditional antiepileptic agents are active in the MES test but are largely inactive in the 6 Hz test.[60] Including the MES test in our studies proved beneficial in determining the anticonvulsant activity of PAADs because many were largely insensitive to the 6 Hz test. We employed the formalin model for neuropathic pain and report values for the late (chronic) inflammation phase. Because the collective animal studies were conducted at two testing facilities, we determined the pharmacological activities of several active PAADs ((*R*)-**8**, (*R*)-**21**, (*R*)-**24**, (*R*)-**25**) at both sites (Supporting Information Table S3). We found that the MES activities corresponded to each other and there was a modest variance among the neurological toxicities, resulting in different PI values.

The anticonvulsant activities observed for the PAADs suggested that there were reasonable CNS concentrations for many of these compounds. Nonetheless, we determined the relative plasma and brain concentration levels for two hydrophilic PAADs, (*R*)-**7** and (*R*)-**8**, 30 min after ip administration (data not shown). Appreciable brain-to-plasma (B:P) ratios were observed in mice ((*R*)-**7**, 1.2:1; (*R*)-**8**, 1.2:1). These findings indicated that the PAADs may exert their function by targeting sites in the PNS, or the CNS, or both.

We found that the C(2)-hydrocarbon *N*-benzylamide PAADs were the most potent anticonvulsants (Table 2). Hydrocarbon PAADs (*R*)-**21**, (*R*)-**22**, (*R*)-**24**, and (*R*)-**25** exhibited excellent anticonvulsant activity in the MES test (mice, ip) (ED_{50} (mg/kg): (*R*)-**21**, 16; (*R*)-**22**, 21; (*R*)-**24**, 15; and (*R*)-**25**, 13) that surpassed the activity observed for the traditional AED phenobarbital (ED_{50} = 22 mg/kg)[58] (Table 2). Similarly, superb activities in the MES test were observed for the C(2)-hydrocarbon PAADs when tested in the rat (po) (Table 3). The MES ED_{50} value for (*R*)-**21**, (*R*)-**24**, and (*R*)-**25** were all 11 mg/kg. Surprisingly, we observed that activity increased upon substitution of a methylene unit for a heteroatom in R^2 that was one atom removed from the C(2) site (ED_{50} (mg/kg): (*R*)-**22**, 21; (*R*)-**8**, 34). This finding was unexpected because in the FAA series activity increased with the incorporation of a substituted heteroatom at this site,[4−6,10−12,39] and this structural determinant proved critical in our optimization of this class of compounds[5] (Figure 3). For example, FAA anticonvulsant activity in the MES test (mice, ip) increased going from (*R,S*)-**104**[39] to (*R,S*)-**2**[5] (ED_{50} (mg/kg): (*R,S*)-**104**, 38; (*R,S*)-**2**, 8.3). Similarly, incorporation of a nitrogen in the C(2) aromatic ring of (*R,S*)-**107**[6] to give (*R,S*)-**108**[4] led to an improvement in anticonvulsant activity (ED_{50} (mg/kg): (*R*)-**107**, 20; (*R*)-**108**, 11). Finally, we showed that for C(2)-thiophene FAAs,[11] the 2-thienyl FAA (*R,S*)-**109** was more active than the isomeric 3-thienyl FAA

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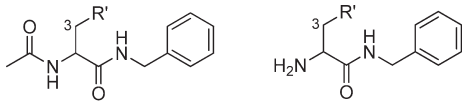
Table 4. Comparison of the Pharmacological Activities of C(3)-Alkoxy and C(3)-Amino *N*-Benzylamide FAAs and Their PAAD Counterparts in Mice (mg/kg)



	FAA				PAAD			
			mice (ip)[a]				mice (ip)[a]	
R′	FAA compd no.	FAA Test Site	FAA MES,[b] ED_{50}	FAA Tox,[c] TD_{50}	PAAD compd no.	PAAD test site	PAAD MES,[b] ED_{50}	PAAD Tox,[c] TD_{50}
OH	(*R*)-**95**[d]	NINDS	53 [2.0] (38−67)	>500 [2.0]	(*R*)-**7**	UCB	>62	>110
OH	(*R,S*)-**95**[d]	NINDS	>100, <300	<300	(*R,S*)-**7**	UCB	>62	>110
OCH_3	(*R*)-**2**[d]	NINDS	4.5 [0.5] (3.7−5.5)	27 [0.25] (26−28)	(*R*)-**8**	NINDS	48 [0.25] (40−61)	>30, <100 [0.25]
OCH_3	(*S*)-**2**[d]	NINDS	>100, <300	>300	(*S*)-**8**	UCB	64	63
OCH_3	(*R,S*)-**2**[d]	NINDS	8.3 [0.5] (7.9−9.8)	43 [0.25] (38−47)	(*R,S*)-**8**[e]	NINDS	84 [0.25] (65−97)	290 [0.25] (240−320)
OCH_2CH_3	(*R,S*)-**96**[d]	NINDS	17 [0.25] (15−19)	78 [0.25] (64−90)	(*R,S*)-**9**	UCB	73	ND[g]
$OCH_2CH{=}CH_2$	(*R,S*)-**97**[d]	NINDS	>30, <100	>30, <100	(*R,S*)-**11**	UCB	45	ND[g]
$OCH_2C{\equiv}CH$	(*R,S*)-**98**[f]	NINDS	16 [0.25] (13−19)	59 [0.25] (55−66)	(*R,S*)-**12**	UCB	46	47
NH_2	(*R,S*)-**99**[h]	NINDS	>100	>100	(*R,S*)-**13**	UCB	>68	ND[g]
$N(CH_3)_2$	(*R,S*)-**100**[h]	NINDS	>30, <100	>100, <300	(*R,S*)-**15**	UCB	94	ND[g]
morpholino	(*R,S*)-**101**[h]	NINDS	>100, <300	300	(*R,S*)-**16**	UCB	89	ND[g]

[a] The compounds were administered either intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose−response curve was generated for all compounds that displayed sufficient activity and the dose−effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [d] Reference 5. [e] Reference 33. [f] Reference 41. [g] ND = not determined. [h] Choi, D. et al., unpublished results.

(*R,S*)-**110** (ED_{50} (mg/kg): (*R*)-**109**, 45; (*R*)-**110**, 88). This difference in the PAAD and FAA SARs led us to compare the effect of the structural size of the C(2)-hydrocarbon substituent on anticonvulsant activity for both series of compounds. For FAAs, we found that anticonvulsant activity in the MES test (mice, ip) was higher for R^2 nonbulky alkyl substituents (ED_{50} (mg/kg): (*R*)-**102**,[9] 55; (*R*)-**104**,[39] 38) compared with the more sterically bulky R^2 moieties (ED_{50} (mg/kg): (*R*)-**105**, 100−300; (*R*)-**106**, >300). The opposite was true for the PAADs, where the more bulky R^2 alkyl substituents exhibited slightly improved anticonvulsant activities (ED_{50} (mg/kg): (*R*)-**24**, 15; (*R*)-**25**, 14) compared with PAADs with less sterically bulky R^2 groups (ED_{50} (mg/kg): (*R*)-**20**, 10−30; (*R*)-**21**, 18). Together these two structural features (i.e., effect of heteroatom substitution, steric size of the R^2-substituent) differentiated C(2)-hydrocarbon PAADs from all previously reported FAAs.

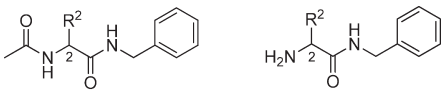
Evaluation of the individual stereoisomers of C(2)-hydrocarbon PAADs **24** and **25** demonstrated that the (*R*)-stereoisomer consistently exhibited greater anticonvulsant activity than the (*S*)-stereoisomer (Supporting Information Table 2). A high eudismic ratio was observed for **24** (>20), but for **25**, this value decreased to 3. (*R*)-**24** displayed significant activity in the formalin test (ED_{50} = 20 mg/kg); however, the lack of data from the corresponding (*S*)-stereoisomer prevented any generalized conclusions concerning the importance of C(2) stereochemistry for activity in this animal model.

Where possible, we evaluated other C(2)-hydrocarbon PAADs for neuropathic pain protection using the formalin test. PAADs (*R*)-**21** and (*R*)-**22** displayed significant activity (ED_{50} (mg/kg): (*R*)-**21**, 22; (*R*)-**22**, 35). For **22**, both stereoisomers were evaluated in the formalin test with the (*R*)-stereoisomer being 2.8-fold more active (ED_{50} (mg/kg): (*R*)-**22**, 35; (*S*)-**22**, 100).

Results for the C(3)-alkoxy and the C(4)-substituted *N*-benzylamide PAADs (Table 1) were different. The C(3)-alkoxy PAADs exhibited moderate anticonvulsant activity in the MES model. While we observed a similar trend in the MES activities for the C(3)-alkoxy PAADs and the corresponding FAAs (Table 4), PAAD activity decreased 3−10-fold compared with the FAAs. Moreover, the degree of separation between (*R*)- and (*S*)-specificity for the C(3)-alkoxy PAADs was typically 1.9-fold, compared with the 10 to >22-fold difference in the FAAs.[5,8,9,11] The C(3)-alkoxy PAADs provided little or no appreciable protection in the formalin assay (ED_{50} (mg/kg): (*R*)-**8**, >67; (*S*)-**8**, 120; (*R,S*)-**9**, >71; (*R,S*)-**10**, >120; (*R,S*)-**11**, >75; (*R,S*)-**12**, >74). The poor activity of the C(3)-methoxy PAAD (*R*)-**8** (ED_{50} = >67 mg/kg) in the formalin test contrasted with the activity of its FAA counterpart (*R*)-**2** (ED_{50} = 15 mg/kg). The C(3)-amino and the C(4)-substituted PAADs were largely inactive in the anticonvulsant models, with (*R*)-**19** (MES ED_{50} = 75 mg/kg) displaying the greatest activity in this class of PAADs. Similarly, we observed little or no protection in the formalin assay for the C(3)-amino PAADs (ED_{50} (mg/kg): (*R,S*)-**15**, >83; (*R,S*)-**16**, 69) and the C(4)-substituted PAADs (ED_{50} (mg/kg): (*R*)-**17**, >67; (*R*)-**19**, >76).

Does the FAA SAR correlate with the corresponding PAAD bioactivity? Implicit in this question is whether FAAs and PAADs

Table 5. Comparison of the Pharmacological Activities of C(2)-Hydrocarbon FAAs and Their PAAD Counterparts in Mice (mg/kg)



FAA PAAD

	FAA				PAAD			
			mice (ip)[a]				mice (ip)[a]	
R^2	FAA compd no.	FAA Test Site	FAA MES,[b] ED_{50}	FAA Tox,[c] TD_{50}	PAAD compd no.	PAAD Test Site	PAAD MES,[b] ED_{50}	PAAD Tox,[c] TD_{50}
CH_3	(*R*)-**102**[d]	NINDS	55 [0.5] (50−60)	210 [0.5] (150−260)	(*R*)-**20**[e]	NINDS	>10, <30	>100, <300
CH_3	(*S*)-**102**[d]	NINDS	550 [0.5] (460−740)	840 [0.5] (690−950)	(*S*)-**20**[e]	NINDS	>300	>300
CH_3	(*R,S*)-**102**[d]	NINDS	76 [0.5] (67−89)	450 [0.5] (420−500)	(*R,S*)-**20**[e]	NINDS	>100, <300	>300
CH_2CH_3	(*R*)-**103**	ND[f]	ND[f]	ND[f]	(*R*)-**21**	NINDS	18 [0.25] (10−25)	80 [0.25] (65−95)
CH_2CH_3	(*R,S*)-**103**[g]	NINDS	>100, <300	>300	(*R,S*)-**21**	ND[f]	ND[f]	ND[f]
$CH_2CH_2CH_3$	(*R,S*)-**104**[h]	NINDS	38 [0.25] (35−45)	160 [0.25] (150−170)	(*R,S*)-**22**	UCB	39	ND[f]
$CH(CH_3)_2$	(*R*)-**105**	NINDS	>100, <300	>300	(*R*)-**24**	NINDS	15 [0.25] (13−18)	70 [0.25] (63−80)
$C(CH_3)_3$	(*R*)-**106**	NINDS	>300	>300	(*R*)-**25**	NINDS	14 [0.25] (11−17)	66 [0.25] (58−73)

[a] The compounds were administered intraperitoneally to adult male NMRI mice under the auspices of UCB or administered intraperitoneally to adult male albino CF-1 mice under the auspices of the NINDS ASP. ED_{50} and TD_{50} values are in mg/kg and were determined 30 min after ip administration (UCB) or a dose−response curve was generated for all compounds that displayed sufficient activity and the dose−effect data for these compounds was obtained at the "time of peak effect" (indicated in hours in the brackets) (NINDS ASP). Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] Tox = neurological toxicity. TD_{50} value determined from the rotorod test. [d] Reference 9. [e] Reference 33. [f] ND = not determined. [g] Reference 65. [h] Reference 39.

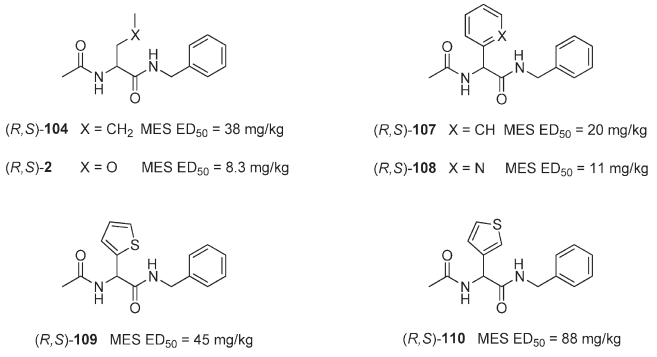


Figure 3. The effect of heteroatom substitution on anticonvulsant activity.

function by similar pathways. We can only compare the anticonvulsant activity for these two series in the MES test because we have limited data in the formalin test. For the C(3)-alkoxy *N*-benzylamide PAADs **7**−**9**, **11**, and **12**, we saw a 3−10-fold reduction in activity from the FAAs to the PAADs; for the C(3)-amino PAADs **13**, **15**, and **16**, both the FAAs and PAADs displayed modest activity; and for the C(2)-linear hydrocarbon PAADs **20** and **22**, both FAAs and PAADs showed comparable activity (Tables 4 and 5). These differences do not allow us to speculate if most PAADs exert their anticonvulsant activities by pathway(s) similar to FAAs or if the observed differences may be attributed to changes in bioavailability, metabolism, and physiochemical properties. Only for the C(2)-branched hydrocarbon PAADs **24** and **25** (ED_{50} (mg/kg): (*R*)-**24**, 15; (*R*)-**25**, 14 mg/kg) did we see a dramatic difference compared with their FAA counterparts (*R*)-**105** and (*R*)-**106** (ED_{50} (mg/kg): (*R*)-**105**, >100, <300; (*R*)-**106**, >300) (Table 5), where the PAADs exhibited excellent activity and the corresponding FAAs were minimally active. These results suggest that these PAADs and other C(2)-hydrocarbon PAADs possibly operate by different mechanism(s) than FAAs. The excellent activities of the C(2)-hydrocarbon PAADs (*R*)-**21**, (*R*)-**24**, and (*R*)-**25**, and the water solubility of their corresponding salts, makes these new compounds worthy of further study.

CONCLUSIONS

Our data indicated that small hydrocarbon substituents at the C(2) center of PAADs afforded excellent anticonvulsant activity in the MES test (mice, ip; rats, po) and that activity principally resided in the (*R*)-stereoisomer. Additionally, C(2)-hydrocarbon PAADs significantly attenuated pain in the formalin model (mice, ip) of neuropathic pain. Most notably, incorporation of a heteroatom that was one atom removed from the C(2) center was detrimental to anticonvulsant activity. The dramatic improvement in MES protection for PAADs (*R*)-**24** and (*R*)-**25**, compared with their FAA counterparts (*R*)-**105** and (*R*)-**106**, suggested that C(2)-hydrocarbon PAADs may function, in part, by different pharmacological pathways than other PAADs and the FAA class. PAADs (*R*)-**21**, (*R*)-**24**, and (*R*)-**25** emerged as the lead compounds in this series.

EXPERIMENTAL SECTION

General Methods. The general methods used in this study were identical to those previously reported[14] and are summarized in the Supporting Information. All compounds were checked by TLC, ^1H and ^{13}C NMR, MS, and elemental analyses. The analytical results are within ±0.40% of the theoretical value. The TLC, NMR, and the analytical data confirmed the purity of the products was ≥95%.

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General Procedure for the Conversion of Cbz-Protected PAADs to PAADs Using Pd-Catalyzed Hydrogenation (Method A). A MeOH solution of Cbz-protected PAAD (0.05−0.1 M) was hydrogenated (1 atm) in the presence of 10% Pd−C at room temperature (3 h−7 d). The mixture was filtered through a bed of Celite, the filtrate was evaporated in vacuo, and the product was purified by column chromatography (SiO_2).

General Procedure for the Conversion of *t*Boc-Protected PAADs to PAADs Using TFA Deprotection (Method B). TFA (15 equiv) was added to an anhydrous CH_2Cl_2 solution of the *N*-*t*-butoxycarbonyl *N*′-benzylamide (0.3 M) at room temperature. The solution was stirred (1 h), and then the solvent was evaporated in vacuo. The crude product was diluted with CH_2Cl_2 and extracted with aqueous 1 M HCl (3×). The combined aqueous layers were washed with CH_2Cl_2 (2×), basified (pH 10−12) with aqueous 4 M NaOH, and extracted with CH_2Cl_2 (3×). The combined organic layers were washed with brine (2×), dried (Na_2SO_4), evaporated in vacuo, and purified by column chromatography (SiO_2).

(*R*)-*N*-Benzyl 2-Amino-3-hydroxypropionamide ((*R*)-7). Utilizing method A with (*R*)-**31** (1.82 g, 5.53 mmol), 10% Pd−C (180 mg), and MeOH (200 mL) (8 h) gave the crude product that was purified by medium pressure liquid chromatography to give the desired product (0.56 g, 53%) as a white solid: mp 95−96 °C; $[\alpha]^{25}_D$ −0.48° (*c* 1.5, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.84 (br s, 2H), 3.25 (dd, *J* = 4.8, 4.8 Hz, 1H), 3.42−3.56 (m, 2H), 4.29 (d, *J* = 6.0 Hz, 2H), 4.72−4.86 (br s, 1H), 7.20−7.33 (m, 5H), 8.35 (t, *J* = 6.0 Hz, 1H). HRMS (ESI) 217.0953 [M + Na^+] (calcd for $C_{10}H_{14}N_2O_2Na^+$ 217.0953). Anal. ($C_{10}H_{14}N_2O_2$): C, H, N.

(*S*)-*N*-Benzyl 2-Amino-3-hydroxypropionamide ((*S*)-7). The previous procedure was repeated using (*S*)-**31** (1.46 g, 4.44 mmol), 10% Pd−C (140 mg), and MeOH (200 mL) (8 h) to give the desired product (0.54 g, 63%) as a white solid: mp 91−92 °C; $[\alpha]^{25}_D$ +4.88° (*c* 1.5, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.27 (dd, *J* = 5.1, 5.1 Hz, 1H), 3.42−3.57 (m, 2H), 4.30 (d, *J* = 6.2 Hz, 2H), 7.19−7.34 (m, 5H), 8.37 (t, *J* = 6.2 Hz, 1H). HRMS (ESI) 217.0953 [M + Na^+] (calcd for $C_{10}H_{14}N_2O_2Na^+$ 217.0953). Anal. ($C_{10}H_{14}N_2O_2$): C, H, N.

(*R,S*)-*N*-Benzyl 2-Amino-3-hydroxypropionamide ((*R,S*)-7). The previous procedure was repeated using (*R,S*)-**31** (1.33 g, 4.07 mmol), 10% Pd−C (130 mg), and MeOH (200 mL) (8 h) to give the desired product (0.52 g, 66%) as a white solid: mp 90−91 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.14 (br s, 2H), 3.27 (dd, *J* = 4.5, 4.8 Hz, 1H), 3.42−3.57 (m, 2H), 4.30 (d, *J* = 6.5 Hz, 2H), 4.62−4.88 (br s, 1H), 7.19−7.34 (m, 5H), 8.37 (t, *J* = 6.5 Hz, 1H). HRMS (ESI) 217.0953 [M + Na^+] (calcd for $C_{10}H_{14}N_2O_2Na^+$ 217.0953). Anal. ($C_{10}H_{14}N_2O_2$): C, H, N.

(*R*)-*N*-Benzyl 2-Amino-3-methoxypropionamide ((*R*)-8).[32] Utilizing method A with (*R*)-**35** (2.00 g, 5.85 mmol), 10% Pd−C (0.2 g), and MeOH (100 mL) (5 h) gave the crude product that was purified by flash column chromatography (SiO_2; 1:10 MeOH/$CHCl_3$). The resulting oil was dissolved in CH_2Cl_2 (20 mL) and extracted with aqueous 0.1 N HCl (3 × 20 mL). The aqueous layers were combined and washed with CH_2Cl_2 (2 × 60 mL). The aqueous layer was basified to pH 10−12 with aqueous 0.1 N NaOH and then extracted with CH_2Cl_2 (3 × 100 mL). The CH_2Cl_2 layers were combined, dried ($MgSO_4$), and concentrated in vacuo to give the desired product (0.89 g, 73%) as a waxy solid: mp 39−40 °C; $[\alpha]^{25}_D$ −1.5° (*c* 1.6, MeOH) (lit.[32] $[\alpha]^{23}_D$ −2.0° (*c* 1.5, MeOH)); R_f 0.26 (1:20 MeOH/$CHCl_3$). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.82 (s, 2H), 3.25 (s, 3H), 3.35−3.44 (m, 3H), 4.23−4.36 (m, 2H), 7.20−7.34 (m, 5H), 8.36−8.44 (br t, 1H). Anal. ($C_{11}H_{16}N_2O_2 \cdot 0.18H_2O$): C, H, N.

(*S*)-*N*-Benzyl 2-Amino-3-methoxypropionamide ((*S*)-8).[39] The previous procedure was repeated using (*S*)-**35** (2.00 g, 5.85 mmol), 10% Pd−C (0.2 g), and MeOH (100 mL) to give the desired product (1.12 g, 92%) as a waxy solid: mp 39−40 °C; $[\alpha]^{25}_D$ +1.7° (*c* 1.5, MeOH) (lit.[39] $[\alpha]^{23}_D$ +1.8° (*c* 0.8, MeOH)); R_f 0.39 (1:20 MeOH/$CHCl_3$). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.84 (s, 2H), 3.25 (s, 3H), 3.35−3.44 (m, 3H), 4.23−4.36 (m, 2H), 7.20−7.34 (m, 5H), 8.36−8.45 (br t, 1H). Anal. ($C_{11}H_{16}N_2O_2 \cdot 0.15H_2O$): C, H, N.

(*R,S*)-*N*-Benzyl 2-Amino-3-methoxypropionamide ((*R,S*)-8). The previous procedure was repeated using (*R,S*)-**35** (2.00 g, 5.85 mmol), 10% Pd−C (0.2 g), and MeOH (100 mL) to give the desired product (0.42 g, 32%) as a pale-yellow oil: R_f 0.37 (1:20 MeOH/$CHCl_3$). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.82 (br s, 2H), 3.25 (s, 3H), 3.37−3.44 (m, 3H), 4.23−4.36 (m, 2H), 7.19−7.34 (m, 5H), 8.36−8.44 (br t, 1H). Anal. ($C_{11}H_{16}N_2O_2$): C, H, N.

(*R,S*)-*N*-Benzyl 2-Amino-3-ethoxypropionamide ((*R,S*)-9). Utilizing method A and (*R,S*)-**36** (1.00 g, 2.81 mmol), 10% Pd−C (0.1 g), and MeOH (30 mL) (18 h) gave the crude product that was purified by flash column chromatography (SiO_2; 1:10 MeOH/CH_2Cl_2). The resulting oil was dissolved in CH_2Cl_2 (10 mL) and was extracted with aqueous 0.1 N HCl (3 × 10 mL). The aqueous layers were combined and washed with CH_2Cl_2 (2 × 30 mL). The aqueous layer was basified to pH 10−12 with aqueous 0.1 N NaOH and then extracted with CH_2Cl_2 (3 × 60 mL). The CH_2Cl_2 layers were combined, dried ($MgSO_4$), and concentrated in vacuo to give the desired product (0.42 g, 68%) as a pale-yellow oil: R_f 0.33 (1:1 EtOAc/hexanes). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10 (t, *J* = 7.2 Hz, 3H), 1.82 (s, 2H), 3.29−3.49 (m, 5H), 4.22−4.37 (m, 2H), 7.20−7.33 (m, 5H), 8.36−8.45 (1H). HRMS (ESI) 223.1450 [M + H^+] (calcd for $C_{12}H_{18}N_2O_2H^+$ 223.1447). Anal. ($C_{12}H_{18}N_2O_2 \cdot 0.06CH_2Cl_2$): C, H, N.

(*R,S*)-*N*-Benzyl 2-Amino-3-propoxypropionamide ((*R,S*)-10). Utilizing method A and (*R,S*)-**37** (1.42 g, 3.85 mmol), 10% Pd−C (0.15 g), and MeOH (50 mL) (6 h) gave the crude product that was purified by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2). The resulting oil was dissolved in CH_2Cl_2 (10 mL) and extracted with aqueous 0.1 N HCl (3 × 10 mL). The aqueous layers were combined and washed with CH_2Cl_2 (2 × 30 mL). The aqueous layer was basified to pH 10−12 with aqueous 0.1 N NaOH and then extracted with CH_2Cl_2 (3 × 60 mL). The second set of CH_2Cl_2 layers were combined, dried ($NaSO_4$), and concentrated in vacuo to give the desired product (0.66 g, 72%) as a pale-orange oil: R_f 0.52 (1:100 MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (t, *J* = 7.8 Hz, 3H), 1.44−1.56 (m, 2H), 1.82 (br s, 2H), 3.31−3.46 (m, 5H), 4.23−4.37 (m, 2H), 7.19−7.32 (m, 5H), 8.36−8.45 (br t, 1H). Anal. ($C_{13}H_{20}N_2O_2$): C, H, N.

(*R,S*)-*N*-Benzyl 2-Amino-3-allyloxypropionamide ((*R,S*)-11). Utilizing method B with (*R,S*)-**48** (1.17 g, 3.50 mmol), TFA (3.90 mL, 52.52 mol), and CH_2Cl_2 (12 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:100−1:10 MeOH/CH_2Cl_2) to give the desired product (285 mg, 35%) as a pale-yellow oil: R_f 0.44 (1:20 MeOH/CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61−1.73 (br s, 2H), 3.62 (t, *J* = 5.6 Hz, 1H), 3.66−3.74 (m, 2H), 4.00 (app t, *J* = 1.2 Hz, 1H), 4.02 (app t, *J* = 1.6 Hz, 1H), 4.41−4.51 (m, 2H), 5.17−5.29 (m, 2H), 5.84−5.93 (m, 1H), 7.24−7.35 (m, 5H), 7.73−7.81 (br t, 1H). HRMS (ESI) 235.1452 [M + H^+] (calcd for $C_{13}H_{18}N_2O_2H^+$ 235.1447). Anal. ($C_{13}H_{18}N_2O_2 \cdot 0.20H_2O$): C, H, N.

(*R,S*)-*N*-Benzyl 2-Amino-3-(prop-2-ynyloxy)propionamide ((*R,S*)-12). Utilizing method B and using (*R,S*)-**49** (973 mg, 2.93 mmol), TFA (3.26 mL, 43.94 mol), and CH_2Cl_2 (10 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:100−1:10 MeOH/CH_2Cl_2) to give the desired product (512 mg, 75%) as a pale-yellow oil: R_f 0.29 (1:100 MeOH/CH_2Cl_2). ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.64−1.72 (br s, 2H), 2.45 (t, *J* = 2.4 Hz, 1H), 3.62 (dd, *J* = 4.0, 6.2 Hz, 1H), 3.76 (dd, *J* = 6.2, 9.2 Hz, 1H), 3.84 (dd, *J* = 4.0, 9.2 Hz, 1H), 4.13−4.24 (m, 2H), 4.41−4.51 (m, 2H), 7.24−7.35 (m, 5H), 7.73−7.81 (br t, 1H). HRMS (ESI) 233.1294 [M + H^+] (calcd for $C_{13}H_{16}N_2O_2H^+$ 233.1290). Anal. ($C_{13}H_{16}N_2O_2 \cdot 0.09CH_2Cl_2$): C, H, N.

(*R,S*)-*N*-Benzyl 2,3-Diaminopropionamide ((*R,S*)-13). Utilizing method B and using (*R,S*)-**64** (1.71 g, 4.35 mmol), TFA (4.86 mL, 65.23 mmol), and CH_2Cl_2 (15 mL) gave the crude product after workup

that was further purified by recrystallization from hot EtOAc/hexanes to give the desired product (427 mg, 51%) as a white solid: mp 119−120 °C; R_f 0.17 (1:10 MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, CD_3OD) δ 2.70−2.95 (m, 2H), 3.35−3.42 (m, 1H), 4.40 (s, 2H), 7.21−7.35 (m, 5H). HRMS (ESI) 216.1107 [M + Na^+] (calcd for $C_{10}H_{15}N_3ONa^+$ 216.1113). Anal. ($C_{10}H_{15}N_3O\cdot H_2O$): C, H, N.

(*R,S*)-*N*-Benzyl 2-Amino-3-(*N′*,*N′*-dimethyl)aminopropionamide ((*R,S*)-15). Utilizing method A and using (*R,S*)-**60** (1.08 g, 3.03 mmol), 10% Pd−C (0.1 g), and MeOH (30 mL) gave a crude oil that was purified by flash column chromatography (SiO_2; 1:100−1:20 MeOH/CH_2Cl_2) to give the desired product (0.40 g, 60%) as a pale-orange oil: R_f 0.26 (1:10 MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.86 (s, 2H), 2.26 (s, 6H), 2.42 (dd, J = 8.8, 12.3 Hz, 1H), 2.60 (dd, J = 6.0, 12.3 Hz, 1H), 3.50 (dd, J = 6.0, 8.8 Hz, 1H), 4.38−4.52 (m, 2H), 7.24−7.36 (m, 5H), 8.20−8.34 (br t, 1H). HRMS (ESI) 244.1434 [M + Na^+] (calcd for $C_{12}H_{19}N_3ONa^+$ 244.1426). Anal. ($C_{12}H_{19}N_3O_2\cdot 0.33H_2O$): C, H, N.

(*R,S*)-*N*-Benzyl 2-Amino-3-morpholinopropionamide ((*R,S*)-16). Utilizing method A with (*R,S*)-**61** (2.50 g, 6.29 mmol), 10% Pd−C (0.25 g), and MeOH (60 mL) gave the crude product that was further purified by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2) to give the desired compound (0.94 g, 57%) as a white solid: mp 84−85 °C; R_f 0.24 (1:20 MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.83 (s, 2H), 2.37−2.55 (m, 5H), 2.67 (dd, J = 5.7, 12.3 Hz, 1H), 3.53−3.69 (m, 5H), 4.43 (d, J = 5.7 Hz, 2H), 7.24−7.36 (m, 5H), 8.06−8.18 (br t, 1H). HRMS (ESI) 286.1533 [M + Na^+] (calcd for $C_{14}H_{21}N_3O_2Na^+$ 286.1532). Anal. ($C_{14}H_{21}N_3O_2$): C, H, N.

(*R*)-*N*-Benzyl 2-Amino-4-hydroxybutanamide ((*R*)-17). Utilizing method A and using (*R*)-**70** (1.50 g, 4.38 mmol), 10% Pd−C (0.15 g), and MeOH (45 mL) gave the crude product that was further purified by flash column chromatography (SiO_2; 1:100−1:10 MeOH/CH_2Cl_2) to give the desired product (0.53 g, 57%) as a white solid: mp 88−89 °C; R_f 0.40 (1:10 MeOH/CH_2Cl_2); $[\alpha]^{25}_D$ +4.8° (*c* 1.1, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.79−1.90 (m, 1H), 1.93−2.19 (m, 3H), 3.61 (t, J = 6.6 Hz, 1H), 3.76−3.88 (2H), 4.46 (d, J = 6.3 Hz, 2H), 7.25−7.38 (m, 5H), 7.56−7.64 (br t, 1H). HRMS (ESI) 209.1288 [M + H^+] (calcd for $C_{11}H_{16}N_2O_2H^+$ 209.1290). Anal. ($C_{11}H_{16}N_2O_2$): C, H, N.

(*R*)-*N*-Benzyl 2-Amino-4-(methylthio)butanamide ((*R*)-19). Utilizing method B and using (*R*)-**67** (1.64 g, 4.85 mmol), TFA (5.40 mL, 72.74 mol), and CH_2Cl_2 (15 mL) gave the crude product that was further purified by flash column chromatography (SiO_2; 1:100−1:10 MeOH/CH_2Cl_2) to give the desired product (0.87 g, 76%) as a pale-yellow oil: R_f 0.58 (1:20 MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 2H), 1.73−1.87 (m, 1H), 2.10 (s, 3H), 2.16−2.27 (m, 1H), 2.62 (t, J = 7.4 Hz, 2H), 3.55 (dd, J = 4.4, 8.3 Hz, 1H), 4.45 (d, J = 6.0 Hz, 2H), 7.25−7.36 (m, 5H), 7.57−7.64 (br t, 1H). HRMS (ESI) 239.1224 [M + H^+] (calcd for $C_{12}H_{18}N_2OSH^+$ 239.1218). Anal. ($C_{12}H_{18}N_2OS$): C, H, N, S.

(*R*)-*N*-Benzyl 2-Aminobutanamide ((*R*)-21). Utilizing method B and using (*R*)-**83** (4.87 g, 16.67 mmol), TFA (18.57 mL, 0.25 mol), and CH_2Cl_2 (55 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:100−1:10 MeOH/CH_2Cl_2) to give the desired compound (1.86 g, 58%) as a pale-yellow oil: R_f 0.53 (1:20 MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, J = 7.2 Hz, 3H), 1.38−1.46 (br s, 2H), 1.51−1.66 (m, 1H), 1.83−1.97 (m, 1H), 3.35 (dd, J = 4.5, 7.8 Hz, 1H), 4.44 (d, J = 5.7 Hz, 2H), 7.26−7.35 (m, 5H), 7.61−7.73 (br t, 1H). HRMS (ESI) 193.1348 [M + H^+] (calcd for $C_{11}H_{16}N_2OH^+$ 193.1341). Anal. ($C_{11}H_{16}N_2O\cdot 0.06CH_2Cl_2$): C, H, N.

(*R*)-*N*-Benzyl 2-Aminopentanamide ((*R*)-22). Utilizing method A and using (*R*)-**93** (2.00 g, 5.88 mmol), 10% Pd−C (0.2 g), and MeOH (60 mL) gave the crude product that was further purified by flash column chromatography (SiO_2; 1:100 MeOH/CH_2Cl_2). The resulting oil was dissolved in CH_2Cl_2 (10 mL) and was extracted with aqueous 1 M HCl (3 × 10 mL). The aqueous layers were combined and extracted with CH_2Cl_2 (2 × 30 mL). The aqueous layer was basified to pH 10−12 with aqueous 1 M NaOH, and then extracted with CH_2Cl_2 (3 × 60 mL). The CH_2Cl_2 layers were combined, dried ($NaSO_4$), and concentrated in vacuo to give the desired product (0.95 g, 79%) as a pale-yellow oil: $[\alpha]^{25}_D$ −9.4° (*c* 1.0, MeOH); R_f 0.64 (1:10 MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, J = 7.4 Hz, 3H), 1.19−1.42 (m, 3H), 1.47−1.61 (m, 1H), 1.78 (br s, 2H), 3.15−3.20 (app t, 1H), 4.28 (d, J = 5.7 Hz, 2H), 7.20−7.34 (m, 5H), 8.34 (t, J = 5.7 Hz, 1H). HRMS (ESI) 229.1317 [M + Na^+] (calcd for $C_{12}H_{18}N_2ONa^+$ 229.1317). Anal. ($C_{12}H_{18}N_2O$): C, H, N.

(*S*)-*N*-Benzyl 2-Aminopentanamide ((*S*)-22). The previous procedure was repeated using (*S*)-**93** (3.00 g, 8.82 mmol), 10% Pd−C (0.3 g), and MeOH (100 mL) to give the desired product (1.76 g, 97%) as a pale-yellow oil: $[\alpha]^{25}_D$ +9.4° (*c* 1.5, MeOH); R_f 0.65 (1:10 MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, J = 6.6 Hz, 3H), 1.22−1.42 (m, 3H), 1.48−1.61 (m, 1H), 1.78 (br s, 2H), 3.17 (t, J = 6.9 Hz, 1H), 4.28 (d, J = 6.0 Hz, 2H), 7.20−7.34 (m, 5H), 8.34 (t, J = 4.8 Hz, 1H). LRMS (ESI) 207.12 [M + H^+] (calcd for $C_{12}H_{18}N_2OH^+$ 207.12). Anal. ($C_{12}H_{18}N_2O\cdot 0.32H_2O$): C, H, N.

(*R,S*)-*N*-Benzyl 2-Aminopentanamide ((*R,S*)-22). The previous procedure was repeated using (*R,S*)-**93** (2.00 g, 11.76 mmol), 10% Pd−C (0.2 g), and MeOH (60 mL) to give the desired product (0.88 g, 72%) as a pale-yellow oil: R_f 0.65 (1:10 MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, J = 6.6 Hz, 3H), 1.24−1.42 (m, 3H), 1.48−1.61 (m, 1H), 1.74 (br s, 2H), 3.15−3.18 (app t, 1H), 4.28 (d, J = 5.9 Hz, 2H), 7.20−7.34 (m, 5H), 8.36 (t, J = 5.9 Hz, 1H). HRMS (ESI) 229.1320 [M + Na^+] (calcd for $C_{12}H_{18}N_2ONa^+$ 229.1317). Anal. ($C_{12}H_{18}N_2O\cdot 0.18H_2O$): C, H, N.

(*R*)-*N*-Benzyl 2-Aminohexanamide ((*R*)-23). Utilizing method B and using (*R*)-**84** (3.33 g, 9.78 mmol), TFA (10.9 mL), and CH_2Cl_2 (33 mL) gave crude product that was further purified by flash column chromatography (1:10 MeOH/CH_2Cl_2) to give the desired product (1.83 g, 80%) as a clear oil: R_f 0.55 (1:10 MeOH/CH_2Cl_2); $[\alpha]^{25}_D$ −3.5° (*c* 2.6, EtOH). ^{1}H NMR ($CDCl_3$) δ 0.87−0.92 (br t, 3H), 1.31−1.60 (m, 7H), 1.80−1.95 (br m, 1H), 3.31−3.43 (m, 1H), 4.42 (d, J = 6.0 Hz, 2H), 7.25−7.32 (m, 5H), 7.59−7.78 (br s, 1H). HRMS (ESI) 221.1655 [M + H] $^+$ (calcd for $C_{13}H_{21}N_2O^+$ 221.1654). Anal. ($C_{13}H_{20}N_2O\cdot 0.15H_2O$): C, H, N.

(*R*)-*N*-Benzyl 2-Amino-3-methylbutanamide ((*R*)-24). Utilizing method B and using (*R*)-**85** (4.00 g, 13.06 mmol), TFA (14.56 mL, 0.20 mol), and CH_2Cl_2 (45 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:100−1:10 MeOH/CH_2Cl_2) to give the desired compound (2.25 g, 83%) as a pale-yellow oil: R_f 0.70 (1:20 MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (d, J = 6.8 Hz, 3H), 0.97 (d, J = 6.8 Hz, 3H), 1.31 (s, 2H), 2.24−2.35 (m, 1H), 3.23 (d, J = 3.9 Hz, 1H), 4.35−4.49 (m, 2H), 7.21−7.33 (m, 5H), 7.72−7.81 (br t, 1H). HRMS (ESI) 207.1501 [M + H^+] (calcd for $C_{12}H_{18}N_2OH^+$ 207.1497). Anal. ($C_{12}H_{18}N_2O\cdot 0.04CH_2Cl_2$): C, H, N.

(*S*)-*N*-Benzyl 2-Amino-3-methylbutanamide ((*S*)-24).[61] The previous procedure was repeated using (*S*)-**85** (2.57 g, 8.39 mmol), TFA (9.35 mL, 0.13 mol), and CH_2Cl_2 (28 mL) to give the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (1.65 g, 96%) as a white solid: mp 52−53 °C; $[\alpha]^{28.5}_D$ −27.1° (*c* 0.6, CH_2Cl_2) (lit.[61] $[\alpha]^{20}_D$ −27.2° (*c* 0.5, CH_2Cl_2)); R_f 0.47 (1:20 MeOH/CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (d, J = 6.8 Hz, 3H), 0.99 (d, J = 6.8 Hz, 3H), 1.43 (s, 2H), 2.28−2.40 (m, 1H), 3.27 (d, J = 4.0 Hz, 1H), 4.42 (dd, J = 6.2, 14.8 Hz, 1H), 4.48 (dd, J = 6.0, 14.8 Hz, 1H), 7.24−7.34 (5H), 7.62−7.70 (br t, 1H). HRMS (ESI) 207.1502 [M + H^+] (calcd for $C_{12}H_{18}N_2OH^+$ 207.1497). Anal. ($C_{12}H_{18}N_2O\cdot 0.30H_2O$): C, H, N.

(*R*)-*N*-Benzyl 2-Amino-3,3-dimethylbutanamide ((*R*)-25).[62] Utilizing method B and using (*R*)-**86** (3.80 g, 11.87 mmol), TFA (13.22 mL, 0.18 mol), and CH_2Cl_2 (40 mL) to give the crude product

after workup that was further purified by flash column chromatography (SiO_2; 1:20 EtOAc/hexanes followed by 1:10 MeOH/CH_2Cl_2) to give the desired compound (2.17 g, 83%) as a pale-yellow solid: mp 65−66 °C (lit.[62] mp 53−54 °C); $[\alpha]^{28.5}_D$ +21.8° (*c* 0.5, CH_2Cl_2); R_f 0.48 (1:20 MeOH/CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (s, 9H), 1.45 (s, 2H), 3.12 (s, 1H), 4.42 (dd, *J* = 4.0, 12.8 Hz, 1H), 4.46 (dd, *J* = 4.0, 12.8 Hz, 1H), 7.05−7.13 (br m, 1H), 7.24−7.34 (m, 5H). HRMS (+ESI) 221.1654 $[M + H]^+$ (calcd for $C_{13}H_{20}N_2OH^+$ 221.1653). Anal. ($C_{13}H_{20}N_2O$): C, H, N.

(*S*)-*N*-Benzyl 2-Amino-3,3-dimethylbutanamide ((*S*)-25).[61,63] The previous procedure was repeated using (*S*)-**86** (2.50 g, 7.81 mmol), TFA (8.70 mL, 0.12 mol), and CH_2Cl_2 (26 mL) to give the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:100−1:10 MeOH/CH_2Cl_2) to give the desired compound (1.58 g, 92%) as a white solid: mp 65−66 °C (lit.[63] mp 53−54 °C); $[\alpha]^{25}_D$ −15.2° (*c* 0.51, CH_2Cl_2) (lit.[61] $[\alpha]^{20}_D$ −17.5° (*c* 0.56, CH_2Cl_2); R_f 0.19 (100% EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (s, 9H), 1.47 (s, 2H), 3.14 (s, 1H), 4.44 (d, *J* = 5.7 Hz, 2H), 7.04−7.12 (br t, 1H), 7.24−7.36 (m, 5H). HRMS (ESI) 221.1652 $[M + H^+]$ (calcd for $C_{13}H_{20}N_2OH^+$ 221.1654). Anal. ($C_{13}H_{20}N_2O$): C, H, N.

(*R*,*S*)-*N*-Benzyl 2-Amino-3-methylpentanamide ((*R*,*S*)-26). Utilizing method B and using (*R*,*S*)-**87** (4.00 g, 12.49 mmol), TFA (13.92 mL, 0.19 mol), and CH_2Cl_2 (40 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2; 1:100−1:10 MeOH/CH_2Cl_2) to give the desired compound (2.24 g, 81%) as a pale-yellow oil and as a 1:1 mixture of diastereomers: R_f 0.73 (1:20 MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.77−0.96 (m, 6H), 1.00−1.46 (m, 4H), 1.92−2.17 (m, 1H), 3.26 (d, *J* = 3.6 Hz, 0.5H), 3.36 (d, *J* = 3.6 Hz, 0.5H), 4.36−4.49 (m, 2H), 7.21−7.33 (m, 5H), 7.74−7.82, 7.83−7.91 (1H). HRMS (ESI) 221.1663 $[M + H^+]$ (calcd for $C_{13}H_{20}N_2OH^+$ 221.1654). Anal. ($C_{13}H_{20}N_2O \cdot 0.03CH_2Cl_2$): C, H, N.

(*R*)-*N*-Benzyl 2-Amino-2-cyclohexylacetamide ((*R*)-27). Utilizing method B and using (*R*)-**88** (1.10 g, 3.2 mmol), TFA (2.40 mL, 31.8 mmol), and CH_2Cl_2 (50 mL) gave the crude product after workup that was further purified by flash column chromatography (SiO_2; 0:95:5−5:90:5 MeOH/EtOAc/Et_3N) to give the desired product (530 mg, 68%) as a yellow solid: mp 94−96 °C; $[\alpha]^{27}_D$ +24.0° (*c* 0.5, $CHCl_3$); R_f 0.55 (95:5 EtOAc/Et_3N). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92−1.34 (m, 1H), 1.45−1.71 (m, 5H), 1.85−1.94 (m, 5H), 3.20 (d, *J* = 3.9 Hz, 1H), 4.39 (d, *J* = 5.7 Hz, 2H), 7.17−7.30 (m, 5H), 7.49−7.62 (br m, 1H). HRMS (ESI) 247.1810 $[M + H^+]$ (calcd for $C_{15}H_{22}N_2OH^+$ 247.1810). Anal. ($C_{15}H_{22}N_2O \cdot 0.08H_2O$): C, H, N.

(*R*)-*N*-Benzyl 2-Amino-3-phenylpropionamide ((*R*)-28). Utilizing method A and using (*R*)-**94** (3.50 g, 9.02 mmol), 10% Pd−C (0.35 g), and MeOH (90 mL) gave the crude product that was purified by flash column chromatography (SiO_2; 1:10 MeOH/CH_2Cl_2) to give the desired compound (2.22 g, 97%) as a pale-yellow solid: mp 66−67 °C; $[\alpha]^{25}_D$ +63.3° (*c* 1.4, CH_2Cl_2); R_f 0.43 (1:20 MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98−1.62 (br s, 2H), 2.75 (dd, *J* = 9.0, 13.7 Hz, 1H), 3.29 (dd, *J* = 4.2, 13.7 Hz, 1H), 3.65 (dd, *J* = 4.2, 9.0 Hz, 1H), 4.37−4.50 (m, 2H), 7.20−7.35 (m, 10H), 7.56−7.64 (br t, 1H). HRMS (ESI) 277.1321 $[M + Na^+]$ (calcd for $C_{16}H_{18}N_2ONa^+$ 277.1317). Anal. ($C_{16}H_{18}N_2O$): C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3-methylbutanamide ((*R*)-105). (*R*)-*N*-Benzyl 2-amino-3-methylbutanamide hydrochloride (0.79 g, 3.26 mmol) was dissolved in CH_2Cl_2 (60 mL), and then Et_3N (1.4 mL, 9.78 mmol) and acetyl chloride (0.28 mL, 3.91 mmol) were carefully added at 0 °C. The resulting solution was stirred at room temperature (2 h). Aqueous 10% citric acid (60 mL) was added, the layers separated, and the organic layer was washed with a saturated aqueous $NaHCO_3$ solution (60 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 60 mL). All of the organic layers were combined, dried (Na_2SO_4), and concentrated in vacuo. The residue was recrystallized with EtOAc to give the desired compound as a white solid: mp 223−224 °C; $[\alpha]^{26}_D$ +32.8° (*c* 0.5, $CHCl_3$); R_f 0.53 (100% EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94−0.97 (m, 6H), 1.98 (s, 3H), 2.04−2.11 (m, 1H), 4.25 (app t, *J* = 8.2 Hz, 1H), 4.37 (1/2 AB_q, *J* = 5.4, 14.8 Hz, 1H), 4.47 (1/2 AB_q, *J* = 5.8, 14.8 Hz, 1H), 6.18−6.21 (br d, 1H), 6.42−6.50 (br t, 1H), 7.24−7.34 (m, 5H), addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**105** gave only one signal for the acetyl methyl protons. LRMS (ESI) 271.16 $[M + Na^+]$ (calcd for $C_{14}H_{20}N_2O_2Na^+$ 271.14). Anal. ($C_{14}H_{20}N_2O_2$): C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3,3-dimethylbutanamide ((*R*)-106). (*R*)-*N*-Benzyl 2-amino-3,3-dimethylbutanamide hydrochloride (0.64 g, 2.5 mmol) was dissolved in CH_2Cl_2 (50 mL), and then Et_3N (1.0 mL, 7.50 mmol) and acetyl chloride (0.2 mL, 3.00 mmol) were carefully added at 0 °C and the resulting solution was stirred at room temperature (2 h). Aqueous 10% citric acid (50 mL) was added, the layers separated, and the organic layer was washed with a saturated aqueous $NaHCO_3$ solution (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). All the organic layers were combined, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by chromatography (SiO_2; 30:70−70:30 EtOAc/hexanes) to give crude product that was recrystallized with EtOAc to give the desired product as a white solid: mp 199−200 °C; $[\alpha]^{25}_D$ −6.1° (*c* 0.5, $CHCl_3$); R_f 0.19 (30:70 EtOAc/hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (s, 9H), 1.86 (s, 3H), 4.29 (1/2 AB_q, *J* = 5.5, 14.8 Hz, 1H), 4.43 (1/2 AB_q, *J* = 5.8, 14.8 Hz, 1H), 4.45 (d, *J* = 9.4 Hz, 1H), 6.45 (d, *J* = 9.4 Hz, 1H), 7.18 (br t, *J* = 5.4 Hz, 1H), 7.23−7.32 (m, 5H), addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**106** gave only one signal for the acetyl methyl protons. LRMS (ESI) 263.20 $[M + H^+]$ (calcd for $C_{15}H_{22}N_2O_2H^+$ 263.18). Anal. ($C_{15}H_{22}N_2O_2$): C, H, N.

Pharmacology. Compounds were screened under the auspices of UCB Pharma (Braine L'Alleud, Belgium) and the NINDS ASP (Rockville, MD). Housing, handling, and feeding were in full accordance with recommendations contained in the *Guide for the Care and Use of Laboratory Animals*.[64] Pharmacological evaluation by UCB Pharma consisted of four assays using male NMRI mice (ip): the 6 Hz test[53] and the MES test[51] to assess anticonvulsant activity, the formalin test[54,55] to assess neuropathic pain attenuation, and the rotorod test[51] to assess neurological toxicity. Pharmacological evaluation by the NINDS ASP utilized male albino Carworth Farms No. 1 mice (ip) or male albino Sprague−Dawley rats (po) and consisted of the MES test (mice and rats) and the subcutaneous pentylenetetrazol (Metrazol) (scMet) seizure threshold test (mice) to assess anticonvulsant activity, the rotorod test (mice) to assess neurological toxicity, and the positional sense test or gait and stance test (rats) to assess behavioral toxicity.[52]

■ ASSOCIATED CONTENT

Supporting Information. Synthetic procedures and spectral properties (IR, ^{1}H and ^{13}C NMR, MS) for the synthetic intermediates, the PAADs, (*R*)-**105**, and (*R*)-**106**; table of elemental analyses and table of MS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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White, and K. Wilcox. Harold Kohn has a royalty-stake position in (*R*)-**2**.

■ ABBREVIATIONS USED

AED, antiepileptic drug; ASP, Anticonvulsant Screening Program; B:P, brain-to-plasma; CCI, chronic constriction injury; CNS, central nervous system; ED_{50}, effective dose (50%); FAA, functionalized amino acid; ip, intraperitoneally; MAC, mixed anhydride coupling; MES, maximal electroshock seizure; NINDS, National Institute of Neurological Disorders and Stroke; PAAD, primary amino acid derivative; PI, protective index; po, orally; PNS, peripheral nervous system; SAAD, secondary amino acid derivative; SAR, structure−activity relationship; scMet, subcutaneous Metrazol; TAAD, tertiary amino acid derivative; TD_{50}, neurological impairment (toxicity, 50%); TFA, trifluoroacetic acid

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