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The Structure−Activity Relationship of the 3-Oxy Site in the Anticonvulsant (*R*)-*N*-Benzyl 2-Acetamido-3-methoxypropionamide

Pierre Morieux,[†] Christophe Salomé,[†] Ki Duk Park,[†] James P. Stables,[‡] and Harold Kohn*[,†,§]

[†]*Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599-7568,* [‡]*Epilepsy Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, 6001 Executive Boulevard, Suite 2106, Rockville, Maryland 20892, and* [§]*Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599-3290*

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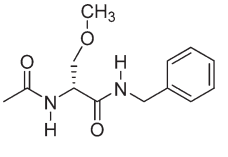
Lacosamide ((*R*)-*N*-benzyl 2-acetamido-3-methoxypropionamide, (*R*)-**1**) is a low molecular weight anticonvulsant recently introduced in the United States and Europe for adjuvant treatment of partial-onset seizures in adults. In this study, we define the structure−activity relationship (SAR) for the compound's 3-oxy site. Placement of small nonpolar, nonbulky substituents at the 3-oxy site provided compounds with pronounced seizure protection in the maximal electroshock (MES) seizure test with activities similar to (*R*)-**1**. The anticonvulsant activity loss that accompanied introduction of larger moieties at the 3-oxy site in (*R*)-**1** was offset, in part, by including unsaturated groups at this position. Our findings were similar to a recently reported SAR study of the 4′-benzylamide site in (*R*)-**1** (*J. Med. Chem.* **2010**, *53*, 1288−1305). Together, these results indicate that both the 3-oxy and 4′-benzylamide positions in (*R*)-**1** can accommodate nonbulky, hydrophobic groups and still retain pronounced anticonvulsant activities in rodents in the MES seizure model.

Epilepsy is a common neurological disorder that affects 0.5−1% of the world population.[1] It is a broad term that encompasses many different human seizure types.[2,3] The treatment mainstay for patients with epilepsy has been the long-term and consistent administration of anticonvulsant drugs, but these agents may be either poorly tolerated or ineffective.[4,5] Elucidation of new biological targets for seizure control are expected to provide new treatment strategies and lead to a better understanding of the pharmacological mechanisms underlying epileptic disorders.

Lacosamide[6] ((*R*)-**1**) is a novel antiepileptic drug (AED[a]) that was recently introduced in the United States and Europe for adjuvant treatment of partial-onset seizures in adults.[7] Whole animal pharmacology studies (e.g., maximal electroshock seizure (MES), chemoconvulsant seizure tests, Frings mouse model, hippocampal kindled rat test) have revealed a distinctive profile for (*R*)-**1** that differentiated it from all other antiseizure drugs.[8] Studies have appeared concerning the mechanism of action of (*R*)-**1**. Radioligand displacement studies using (*R*)-**1** (10 μM) and more than 100 potential pharmacological targets failed to show significant binding,[9,10] and efforts to detect selective binding in rat brain homogenates with radiolabeled (*R*)-**1** were unsuccessful.[11] These findings suggest that either (*R*)-**1** binds to its target(s) with modest affinity or its binding partner(s') levels are too low to be detected or a combination of both factors. Electrophysiology experiments in neuroblastoma cells demonstrated that (*R*)-**1** selectively enhanced sodium channel slow inactivation in a time- and voltage-dependent manner without affecting fast inactivation.[12] Similarly, use of recombinant human $Na_V1.3$ and $Na_V1.7$ channels and $Na_V1.8$-type tetrodotoxin-resistant (TTX-R) currents from dorsal root ganglion (DRG) neurons demonstrated that (*R*)-**1** selectively interacted with the slow inactivation state in each of these sodium channel subtypes.[13] Use of (*R*)-**1** analogues designed to irreversibly modify (*R*)-**1** targets preferentially modified collapsin response mediator protein 2 (CRMP2) in brain lysates.[9,14] While CRMP2 is a signaling protein involved in neuronal differentiation and axonal guidance,[15] its importance in (*R*)-**1** function has not been determined. Thus, the pharmacological actions of (*R*)-**1** remain elusive.



(*R*)-**1**

In 2009, we advanced a strategy to identify molecular drug targets within the proteome where binding is modest.[14] Our approach required attaching an affinity bait (AB) and a chemical reporter (CR) moiety to the drug framework

*To whom correspondence should be addressed. Phone: 919-843-8112. Fax: 919-966-0204. E-mail: harold_kohn@unc.edu.

[a] Abbreviations: AED, antiepileptic drug; MES, maximal electroshock; Na_v,voltage-gated sodium channel; TTX, tetrodotoxin; DRG, dorsal root ganglion; CRMP2, collapsin response mediator protein 2; AB, affinity bait; CR, chemical reporter; AB&CR, affinity bait and chemical reporter; SAR, structure−activity relationship; ASP, Anticonvulsant Screening Program; NINDS, National Institute of Neurological Disorders and Stroke; DTPP, diethoxytriphenylphosphorane; DTPP-F_6, bis(2,2,2-trifluoroethoxy)triphenylphoshorane; DMTMM, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride; HRMS, high resolution mass spectrometry; scMet, subcutaneous metrazol; IBCF, isobutylchloroformate; NMM, *N*-methylmorpholine.

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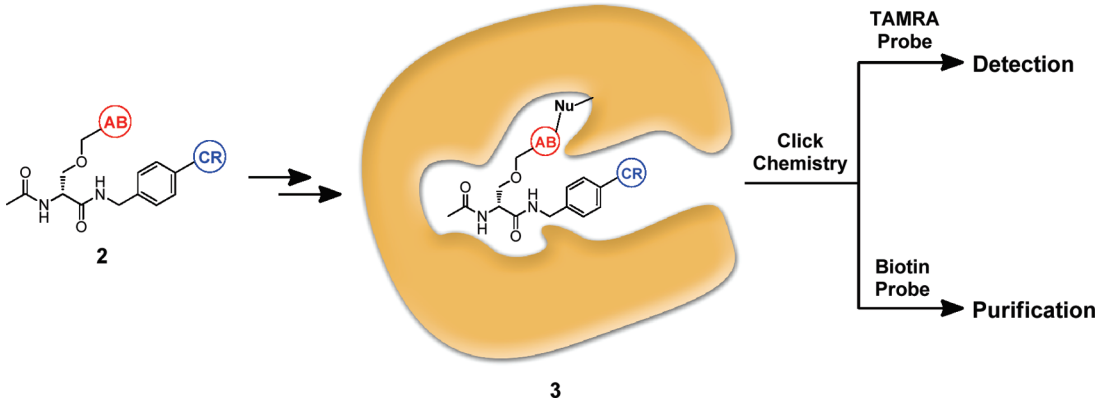
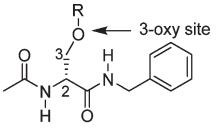
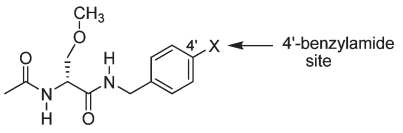


Figure 1. Use of the AB&CR strategy to identify potential drug receptors.

(Figure 1, **2**). The AB group is designed to irreversibly modify the potential target site(s), and the CR unit permits either the detection (e.g., by fluorescence) or isolation (e.g., by biotin conjugation) of the covalently labeled receptor **3** after reaction with a bioorthogonal probe. We outlined the critical requirements for the AB and CR units.[14] Key to the success of this strategy is that the AB and CR groups in **2** do not impede binding to the cognate receptor(s) of the medicinal agent that elicit drug function.

We have applied this strategy to search for molecular targets in the rodent brain proteome by installing AB and CR groups at select sites in (*R*)-**1**.[14,16] Initial structure−activity relationship (SAR) studies[6] showed that small structural changes at the 3-oxy site in (*R*)-**1** led to modest reductions in anticonvulsant activity, indicating that including either an AB or a CR moiety at this position was possible. Here, we report an in-depth SAR of the 3-oxy site in (*R*)-**4** and show the importance of size, hydrophobic interactions, and polar modifications of this site on anticonvulsant activity in the MES seizure test[17] in rodents. We include in our SAR possible AB and CR moieties. We demonstrate that placing nonpolar, nonbulky substituents at the 3-oxy site provided compounds with pronounced seizure protection in the MES seizure test. This study complements a recently reported SAR study of the 4′-benzylamide site in (*R*)-**5**.[18] Together, these investigations serve as the structural basis for the design and use of (*R*)-**1** AB&CR agents that can be employed in target-based search studies. Moreover, they provide important information about the structural elements that can be incorporated at these (*R*)-**1** sites while still retaining significant protection in animal tests.



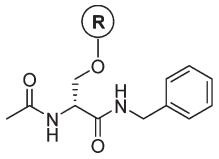


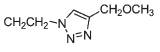
Results and Discussion

Choice of Compounds. Table 1 lists the (*R*)-**4** 3-oxy substituted compounds that constitute the SAR. Our previous findings show that the anticonvulsant activity for (*R*)-**1**[6] and similar analogues[18−21] principally reside in the D-amino acid derivative, and thus, we synthesized only the (*R*)-enantiomer for **4**. The compounds were evaluated for seizure protection at the Anticonvulsant Screening Program (ASP) of the National Institute of Neurological Disorders and Stroke (NINDS) at the National Institutes of Health. We examined several potential interactions that might affect drug binding. To test for the effect of steric size, we replaced the (*R*)-**1** *O*-methyl unit with larger alkyl groups ((*R*)-**6**−(*R*)-**9**). We introduced unsaturated aliphatic and (hetero)aromatic systems ((*R*)-**10**−(*R*)-**16**, (*R*)-**18**, (*R*)-**19**, (*R*)-**26**) to look for potential hydrophobic, $\pi-\pi$, and cationic$-\pi$ interactions that might facilitate binding.[22] Within this set, we varied the length of the methylene spacer between several of these groups and the 3-oxy site in (*R*)-**4**. Not to limit our study to hydrophobic interactions, we prepared derivatives that contained a polar side chain ((*R*)-**24**, (*R*)-**27**, (*R*)-**28**). These compounds could accept and/or donate a hydrogen bond(s) to a suitable amino acid residue within the putative drug binding pocket(s). Finally, we evaluated (*R*)-**4** oxy-substituted analogues that contained either an AB ((*R*)-**20**−(*R*)-**23**) or a CR group ((*R*)-**12**, (*R*)-**18**, (*R*)-**25**). In those cases where little or no anticonvulsant activity was observed for the AB, we prepared the corresponding isostere (i.e., (*R*)-**16** for (*R*)-**21**, (*R*)-**17** for (*R*)-**22**, (*R*)-**18** for (*R*)-**23** and (*R*)-**25**) to see if either metabolic factors or structural constraints contributed to the observed activity loss.

Chemistry. We have reported a versatile method for the introduction of 3-oxy substituents within the (*R*)-**4** framework (Scheme 1).[23] The method was adopted from earlier reports showing that *N*-substituted aziridines carboxylate esters serve as valuable intermediates in the synthesis of *O*-substituted serine amino acid derivatives.[23,24−31] To expedite the synthesis of the central aziridine intermediate (*R*)-**32** from D-serine ((*R*)-**29**), we adopted Evans' and co-workers' cyclodehydration protocol using dialkoxytriphenylphosphoranes ($PPh_3(OR)_2$).[32−36] Accordingly, serine methyl ester hydrochloride ((*R*)-**30**) was sequentially treated with Et_3N and diethoxytriphenylphosphorane ($PPh_3(OCH_2CH_3)_2$, DTPP)[37] to give aziridine (*R*)-**31a** and (*R*)-**31b** as a mixture of methyl and ethyl esters,[23] respectively. The 24 h reaction provided good yields (50−70%) of (*R*)-**31** after bulb-to-bulb distillation and was used to prepare multiple grams of (*R*)-**31** in a single experiment. We found that the reaction only proceeded if (*R*)-**30** was isolated as the free amine.[23] Acetylation of (*R*)-**31a** and (*R*)-**31b** with acetic anhydride and catalytic amounts of DMAP afforded (*R*)-**32a** and (*R*)-**32b**. Treatment of the (*R*)-**32a** and (*R*)-**32b** mixture with alcohols under Lewis acid-catalyzed conditions produced the desired 3-oxy substituted serine esters, (*R*)-**33a** and (*R*)-**33b**,

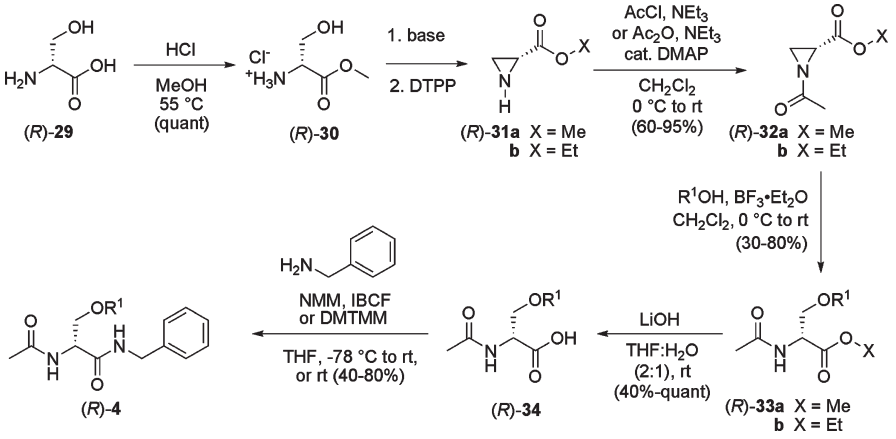
Table 1. Structure−Activity Relationship of the (*R*)-**4**[a]



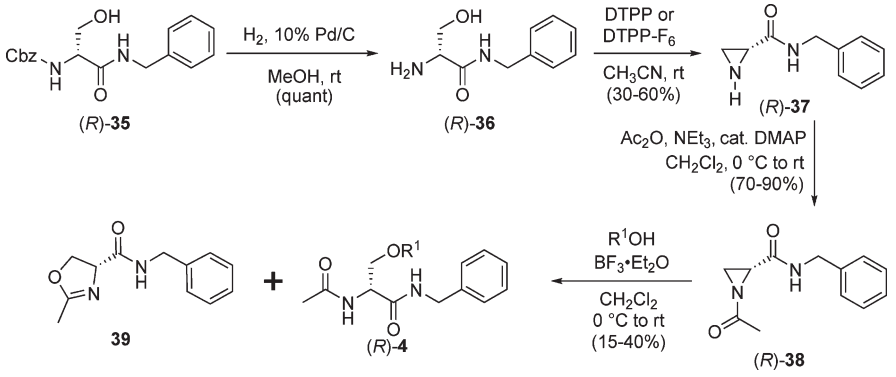
			Mice (ip)[b]				Rat (po)[c]		
No.	R	mp (°C)	MES,[d] ED_{50}	6 Hz, ED_{50}[e]	Tox,[f] TD_{50}	PI[g]	MES,[d] ED_{50}	Tox,[h] TD_{50}	PI[g]
(*R*)-**1**[i]	CH_3	142–143	4.5 [0.5] (3.7 – 5.5)		27 [0.25] (26 – 28)	6.0	3.9 [0.5] (2.6 – 6.2)	>500	>128
(*R*)-**1**-d_3	CD_3	142–143					5.2 [0.5] (4.3 – 5.7)	~200	~38
(*R*)-**6**[j]	CH_2CH_3	129–130	7.9 [0.25] (5.3 – 10)		44 [0.25] (37 – 54)	5.6	5.6 [0.25] (2.5 – 16)	>500	>89
(*R*)-**7**[j]	$CH(CH_3)_2$	151–153	23 [0.25] (20 – 26)	23 [0.25] (16 – 28)	77 [0.25] (66 – 96)	3.3	8.6 [0.25] (6.9 – 13)	>500	>58
(*R*)-**8**[j]	$C(CH_3)_3$	126–127	30-100 [0.5]		~300 [0.5]				
(*R*)-**9**	C_6H_{11}	134–135	100-300 [0.5]		~300 [0.5]		~30 [0.25]	>30	
(*R*)-**10**[j]	C_6H_5	169–170	100-300 [0.5]		>600 [0.5]				
(*R,S*)-**11**[j]	$CH_2CH{=}CH_2$	76–77	30–100		30–100				
(*R*)-**12**[k]	$CH_2C{\equiv}CH$	149	16 [0.25] (13 – 19)	29 [0.5] (21 – 40)	59 [0.25] (55 – 66)		7.9 [0.5] (4.7 – 11)	>500	>63
(*R*)-**13**	$CH_2C{\equiv}CCH_3$	149–151	30-100 [0.5]		30-100 [0.5]		6.4 [1] (3.8 – 9.3)	>500	
(*R*)-**14**	$CH_2C_6H_{11}$	143–144	100-300 [0.5]		~300 [0.5]				
(*R*)-**15**	$CH_2C_6H_5$	145–146	64 [0.25] (56 – 76)		200 [0.25] (160 – 300)	3.2	>30	>30	
(*R*)-**16**	$CH_2CH_2CH{=}CH_2$	103–104	30-100 [0.5]		~100 [0.5]		17 [0.5] (13 – 21)	>500	>29
(*R*)-**17**	CH_2CH_2	97–99	46 [0.25] (42 – 50)		85 [0.25] (69 – 105)	1.9	>30	>30	
(*R*)-**18**	$CH_2CH_2C{\equiv}CH$	111–113	30–100 [0.5]		30–100 [0.5]		11 [0.5] (7-16)	~250 [0.5]	>23
(*R*)-**19**	$CH_2CH_2C_6H_5$	90–92	100-300 [0.5]		100-300 [0.5]		>30	>30	
(*R*)-**20**	CH_2CH_2 	120–121	30-100 [0.5]		100-300 [0.5]		44 [1] (28–65)	>500	>11
(*R*)-**21**	$CH_2CH_2C(O)H$	120–121	>300 [0.5]		>300 [0.5]		>100		
(*R*)-**22**	CH_2CH_2	104–110	>300 [0.5]		>300 [0.5]				
(*R*)-**23**[k]	CH_2CH_2NCS		>300 [0.5]		100-300 [0.5]		<30 [4]	>30 [4]	
(*R*)-**24**	$CH_2CH_2NHC(O)CH_3$	166–168	>300 [0.5]		>300 [0.5]		>30	>30	
(*R*)-**25**[k]	$CH_2CH_2N_3$	111–113	100-300 [0.5]	44 [0.25] (32 – 65)			>40 [0.25] (po) 5.7 [0.25] (ip) (3.6 – 8.4)	78 [0.25] (ip) (72 – 83)	>13
(*R*)-**26**[k]		127–129	>300 [0.5]	>100	>300 [0.5]				
(*R*)-**27**	$CH_2CH_2OCH_3$	109–110	30-100 [0.5]		~300 [0.5]				
(*R*)-**28**[j]	$(CH_2CH_2O)_2CH_3$	48–52	>300 [0.5]	>100	>300 [0.5]		>30	>50	
	phenytoin[l]		9.5 [2] (8.1–10)		66 [2] (53–72)	6.9	30 [4] (22–39)	[m]	>100
	phenobarbital[l]		22 [1] (15 – 23)		69 [0.5] (63 – 73)	3.2	9.1 [0.5] (7.6 – 12)	61 [0.5] (44 – 96)	6.7
	Valproic acid[l]		270 [0.25] (250 – 340)		430 [0.25] (370 – 450)	1.6	490 [0.5] (350 – 730)	280 [0.5] (190 – 350)	0.6

[a] All compounds tested corresponded to the (*R*)-enantiomer except **11**. The compounds were tested through the auspices of the NINDS ASP. [b] The compounds were administered intraperitoneally. ED_{50} and TD_{50} values are in milligrams per kilogram. [c] The compounds were administred orally unless otherwise indicated. ED_{50} and TD_{50} values are in mg/kg. [d] MES = maximal electroshock seizure test. [e] The 6 Hz test was carried out at 32 mA. [f] TD_{50} value determined from the rotorod test. [g] PI = protective index (TD_{50}/ED_{50}). [h] Tox = behavioral toxicity. [i] Ref 6. [j] Ref 23. [k] Ref 14. [l] Ref 51. [m] No ataxia observed up to 3000 mg/kg.

Scheme 1. Synthetic Pathway to Enantiopure (*R*)-**4** Derivatives Using *N*-Acetyl Aziridine Ester (*R*)-**32** Ring-Opening



Scheme 2. Alternative Synthetic Pathway to *O*-Substituted Derivatives (*R*)-**4**



with the same C(2) stereochemistry as its precursor.[30] The (*R*)-**33a** and (*R*)-**33b** esters were hydrolyzed with LiOH to the free acid (*R*)-**34** and then coupled with benzylamine using either the mixed anhydride coupling method[38] or the reagent 4-(4,6-dimethoxy-1,3,5-triazine-2-yl)-4-methyl-morpholinium chloride (DMTMM).[39] Using this protocol, we prepared (*R*)-**6**−(*R*)-**10**, (*R*)-**12**−(*R*)-**14**, (*R*)-**16**, (*R*)-**17**, and (*R*)-**20**−(*R*)-**28**.

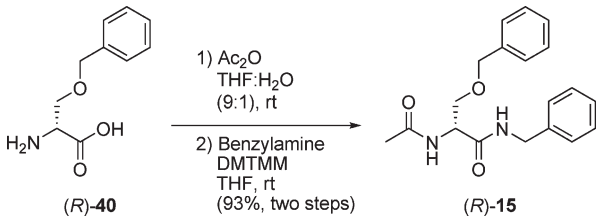
Although DTTP facilitated the production of aziridine esters (*R*)-**31**, its preparation was tedious and required an initial centrifugation step (8000 rpm, 10 min) to remove finely divided NaBr, and then three or four evaporation/filtration steps to precipitate $PPh_3(O)$, a byproduct of the reaction.[34] Accordingly, we examined the use of a hexafluoro derivative of DTPP, $PPh_3(OCH_2CF_3)_2$ (DTPP-F_6),[40] to generate aziridine (*R*)-**31**. DTPP-F_6 formation proceeded faster than DTPP and required only filtration and evaporation to yield the cyclodehydration reagent.[41] Unfortunately, we found that compared with DTPP yields (50−70%), DTPP-F_6 provided lower yields (25−30%) of aziridine (*R*)-**31a** after bulb-to-bulb transfer.

We examined an alternative method to prepare the 3-oxy substituted (*R*)-**4** analogues, wherein the *N*-benzyl amide group was installed at an early stage of the synthesis (Scheme 2). (*R*)-*N*-Benzyl 2-amino-3-hydroxypropionamide[42] ((*R*)-**36**) was obtained by catalytic hydrogenation of (*R*)-**35**[42] and then reacted with either DTPP or DTPP-F_6 to give (*R*)-*N*-benzyl aziridine-2-carboxamide ((*R*)-**37**).[14] Following acetylation of (*R*)-**37** to give (*R*)-**38**, the aziridine was ring-opened with 3-butyn-1-ol and phenethyl alcohol to yield (*R*)-**18**, and (*R*)-**19**, respectively. Paralleling the formation of aziridine esters (*R*)-**31a** and (*R*)-**31b**, greater yields of (*R*)-**37** were obtained with DTPP (60−70%) over DTPP-F_6 (30−35%). Several factors, however, made this synthetic route (Scheme 2) inconvenient. First, the low volatility of (*R*)-**37** prevented its bulb-to-bulb distillation, thus requiring silica gel flash chromatography for purification. Second, we observed in the final step a byproduct that was not readily separated from the desired product by column chromatography. Accordingly, the pure 3-oxy-substituted (*R*)-**4** derivatives were obtained by recrystallization. We have tentatively identified the additional product as the acid-catalyzed rearranged[31] 2-oxazoline **39** on the basis of high resolution mass spectrometry (HRMS) and the characteristic oxazoline methyl resonance (δ 13.4 ppm) in the ^{13}C NMR spectrum.[43] Using this method, we prepared (*R*)-**18** and (*R*)-**19** on a sufficient scale for the pharmacological studies.

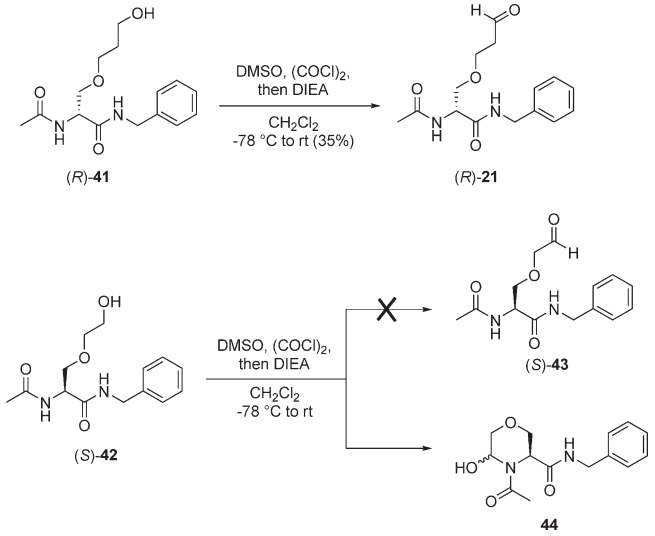
Synthesis of the *O*-benzyl analogue (*R*)-**15** was simplified by the commercially availability of (*R*)-*O*-benzylserine ((*R*)-**40**). Treatment of (*R*)-**40** with acetic anhydride followed by amide coupling with benzylamine and DMTMM gave (*R*)-**15** in 93% overall yield (Scheme 3).

For several of the 3-oxy-substituted (*R*)-**4** derivatives, the 3-oxy group was modified in the last step of the synthesis. Aldehyde (*R*)-**21** was prepared from alcohol (*R*)-**41** using Swern oxidation conditions (Scheme 4).[44] Attempts to prepare the corresponding aldehyde AB derivative containing a single methylene group between the 3-oxy site and the aldehyde group were unsuccessful. Using the (*S*)-enantiomer (*S*)-**42**, Swern oxidation gave the cyclized hemiaminal **44** as a ~9:1 mixture of diastereoisomers with no trace of the desired product (*S*)-**43** (Scheme 4). We have attributed the difference in Swern oxidation products **44** and (*R*)-**21** to the entropic

Scheme 3. Synthesis of (*R*)-**1** Derivative (*R*)-**15**



Scheme 4. Synthesis of (*R*)-**21** and **44**



ease in formation of a six-membered versus the corresponding seven-membered ring system.[45]

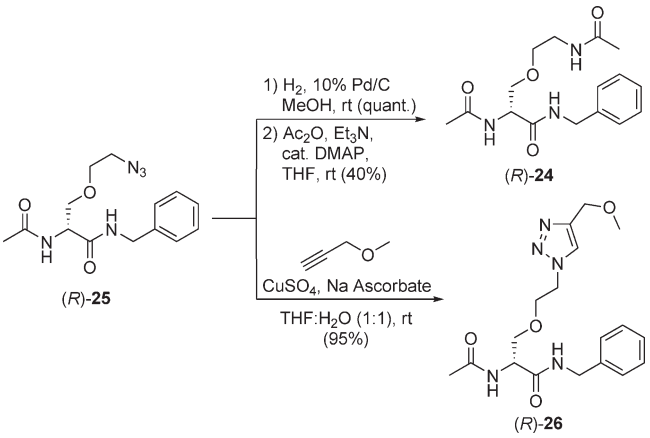
We used azide (*R*)-**25** to synthesize (*R*)-**24** and (*R*)-**26** (Scheme 5). Catalytic reduction (10% Pd/C, H_2) of (*R*)-**25** gave the terminal amine, which was treated directly with acetic anhydride and base to give amide (*R*)-**24**. When azide (*R*)-**25** was treated with propargyl methyl ether under Cu(I)-mediated cycloaddition conditions,[46] we obtained the triazolyl derivative (*R*)-**26**.[14]

The enantiopurity of (*R*)-**6**−(*R*)-**10** and (*R*)-**12**−(*R*)-**28** were assessed by the detection of a single acetyl methyl signal in the ^{1}H NMR spectrum for each compound when a saturated solution of (*R*)-(−)-mandelic acid was added.[47]

We report in the Experimental Section the details (synthetic procedure, characterization) of the final step for all new compounds evaluated in the seizure models. In the Supporting Information, we provide a synthetic scheme for each new compound tested and the experimental procedures, physical, and full spectroscopic properties for all new synthetic compounds prepared in this study.

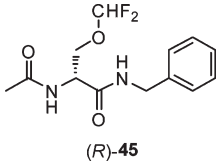
Pharmacological Activity. Compounds (*R*)-**6**−(*R*)-**28** were tested for anticonvulsant activity at the NINDS ASP using the procedures described by Stables and Kupferberg.[48] The pharmacological data from MES[48,49] and 6 Hz[50] seizure tests are summarized in Table 1, along with clinical AEDs phenytoin,[51] valproic acid,[51] and phenobarbital.[51] All compounds were administered intraperitoneally (ip) to mice and orally (po) to rats, unless otherwise indicated. The table lists the values determined to be protective in blocking hind limb extension induced in the MES seizure model from the rodent studies. For compounds that showed significant activity, we report the 50% effective dose (ED_{50}) obtained in quantitative screening evaluations. Using the rotorod test[52] in mice

Scheme 5. Synthesis of (*R*)-**24** and (*R*)-**26**



and the behavioral toxicity effects observed in rats, we determined the median doses for 50% neurological impairment (TD_{50}). TD_{50} values were determined for compounds that exhibited significant activity in the MES seizure test. The protective index ($PI = TD_{50}/ED_{50}$) for these analogues are also listed. Select compounds were evaluated in the psychomotor 6 Hz (32 mA) seizure models (mice, ip).[50] When the 3-oxy (*R*)-**4** derivatives were evaluated in the subcutaneous metrazol (scMet) seizure model,[48] none provided protection at 300 mg/kg doses at the times (0.5 and 4 h) tested (data not shown), a finding similar to (*R*)-**1**[6] and structurally related compounds.[6,14,19−21,53−55]

At the beginning of our studies, little was known about the SAR of the 3-oxy site in (*R*)-**4**. Only the anticonvulsant activities of the *O*-ethyl ((*R*,*S*)-**6**) and the *O*-allyl (*R*,*S*)-**11**) **4** analogues were reported.[6] Both compounds exhibited significant activity but were 2−8-fold less active then (*R*,*S*)-**1** in mice (MES $ED_{50} = 8.3$ mg/kg).[6] Recently, a (*R*)-**4** analogue was prepared wherein the (*R*)-**1** methyl moiety was replaced by the difluoromethyl moiety to give (*R*)-**45**.[56] This fluorinated derivative displayed excellent protection and prolonged duration of action (rat, po) in the MES seizure test ($ED_{50} = 3.0$ mg/kg (0.5 h), 4.2 mg/kg (4 h)) when compared with (*R*)-**1** (($ED_{50} = 1.8$ mg/kg (0.5 h), 8.3 mg/kg (4 h)) under the same test conditions.[56] Our development of a general, versatile method to prepare enantiomerically pure 3-oxy-substituted (*R*)-**4** derivatives through *N*-acetyl aziridine (*R*)-**32**[23] permitted us to explore the SAR of the 3-oxy position.



In total, 23 *O*-substituted (*R*)-**4** analogues were prepared and evaluated for anticonvulsant activity at the NINDS ASP (Table 1). We observed a steady increase in activity in mice (ip) as the steric size of the (*R*)-**4** R substituent decreased from cyclohexyl ((*R*)-**9**, $ED_{50} = 100-300$ mg/kg) and *tert*-butyl ((*R*)-**8**, $ED_{50} = 30-100$ mg/kg) to isopropyl ((*R*)-**7**, $ED_{50} = 23$ mg/kg), to ethyl ((*R*)-**6**, $ED_{50} = 7.9$ mg/kg), and to methyl ((*R*)-**1**, $ED_{50} = 4.5$ mg/kg), indicating that nonbulky 3-oxy substituted groups in (*R*)-**4** analogues provided the highest anticonvulsant activity.[23] Interestingly, we observed

a similar increase in activity as the size of the 3-oxy substituent decreased when the compounds were administered orally to rats, but the range of activities was narrower. For example, the *O*-cyclohexyl derivative (*R*)-**9** exhibited an ED_{50} of ~30 mg/kg, while *O*-isopropyl (*R*)-**7** (ED_{50} = 5.6 mg/kg), *O*-ethyl (*R*)-**6** (ED_{50} = 5.2 mg/kg), and *O*-methyl (*R*)-**1** (ED_{50} = 3.9 mg/kg) all displayed excellent seizure protection. (*R*)-**6** and (*R*)-**7** showed no behavioral neurotoxicity in the rat at the highest dose (500 mg/kg) tested, leading to high PI values for both compounds ((*R*)-**6**: PI > 89; (*R*)-**7**: PI > 58). We concluded that including bulky alkyl substituents at the 3-oxy site in (*R*)-**4** adversely affected seizure protection in the MES seizure test.

Inserting one methylene (CH_2) group between the oxygen and the cyclohexyl ring in (*R*)-**9** (ED_{50} = 100−300 mg/kg) to give (*R*)-**14** (ED_{50} = 100−300 mg/kg) did not improve activity in mice (ip). However, we observed a pronounced increase in potency in mice (ip) going from *O*-phenyl (*R*)-**10** (ED_{50} = 100−300 mg/kg)[23] to *O*-benzyl (*R*)-**15** (ED_{50} = 64 mg/kg).

We also found notable anticonvulsant activity for other derivatives that contained an unsaturated substituent one methylene group separated from the 3-oxy site in (*R*)-**4**. The *O*-allyl[6] ((*R*,*S*)-**11**, ED_{50} = 30−100 mg/kg), *O*-propargyl ((*R*)-**12**, ED_{50} = 16 mg/kg),[14] and *O*-but-2-ynyl ((*R*)-**13**, ED_{50} = 30−100 mg/kg) (*R*)-**1** analogues all provided pronounced seizure protection in mice (ip). When the *O*-propargyl ((*R*)-**12**, ED_{50} = 7.9 mg/kg) and the *O*-but-2-ynyl ((*R*)-**13**, ED_{50} = 6.4 mg/kg) (*R*)-**4** derivatives were tested in the rat (po), we observed excellent seizure protection, which was comparable with phenytoin (ED_{50} = 30 mg/kg)[51] and phenobarbital (ED_{50} = 9.1 mg/kg),[51] and no behavioral neurological toxicity at 500 mg/kg. Significantly, the propargyl unit was shown to be a superior CR unit in bioorthogonal Cu(I)-mediated cycloaddition reactions.[14,57] Our finding that (*R*)-**12** and (*R*)-**13** exhibited excellent activity in the MES seizure model indicated that this CR unit in (*R*)-**1** AB&CR agents would not likely impact the unit's binding to its cognate receptor(s).

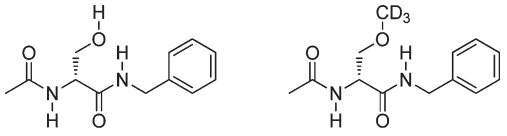
We next explored the effect of adding a second methylene unit between the unsaturated unit and the (*R*)-**4** 3-oxy site to determine if the location of the unsaturated site affected anticonvulsant activity. When we added the second methylene group to *O*-propargyl (*R*)-**12** (ED_{50} = 16 mg/kg) and *O*-benzyl (*R*)-**15** (ED_{50} = 64 mg/kg) to give *O*-3-butynyl (*R*)-**18** (ED_{50} = 30−100 mg/kg) and *O*-2-phenylethyl (*R*)-**19** (ED_{50} = 100−300 mg/kg) (*R*)-**4** derivatives, we saw a decrease in anticonvulsant activity in mice (ip). Like the *O*-phenylethyl derivative (*R*)-**19** (ED_{50} = 100−300 mg/kg), the *O*-triazolylethyl (*R*)-**26** compound (ED_{50} = > 300 mg/kg) did not display activity in mice (ip) at the doses tested. Nonetheless, when tested orally in rats, the *O*-3-butynyl (*R*)-**18** (ED_{50} = 11 mg/kg) provided excellent seizure protection. These results indicated that the site of unsaturation at the 3-oxy site in (*R*)-**4** may be important for anticonvulsant activity. We observed one exception to this pattern, the *O*-allyl ((*R*,*S*)-**11**, ED_{50} = 30−100 mg/kg) and the *O*-3-butenyl ((*R*)-**16**, ED_{50} = 30−100 mg/kg) (*R*)-**4** derivatives showed similar anticonvulsant activity in mice (ip). Like *O*-3-butynyl (*R*)-**18**, the activity of the *O*-3-butenyl (*R*)-**16** derivative improved from mice (ip) (ED_{50} = 30−100 mg/kg) to rat (po) (ED_{50} = 17 mg/kg). Collectively, these findings suggested that a favorable interaction with the 3-oxy π system in (*R*)-**4** at the target site(s) may foster binding, provided the group was not large and the unsaturated system was correctly positioned at the 3-oxy site.

The effect of polar substituents at the C(2) site in (*R*)-**4** was assessed by incorporating either an ethylenoxy or an acetamidoethoxy unit two methylene units removed from the 3-oxy site. When we attached one ethylenoxy group to give methyl ether (*R*)-**27**, we observed noticeable seizure protection (ED_{50} = 30−100 mg/kg) in mice (ip). Adding a second ethylenoxy spacer to (*R*)-**27** to give (*R*)-**28** produced no detectable activity under comparable test conditions (ED_{50} = > 300 mg/kg). Similarly, the acetamidoethyl analogue (*R*)-**24** (ED_{50} = > 300 mg/kg) was inactive at the doses tested. The limited data do not allow us to speculate on the factors (e.g., (*R*)-**4** biodistribution in the brain, metabolism, drug binding) responsible for the loss of anticonvulsant activity of (*R*)-**24** and (*R*)-**28**. Nonetheless, our findings indicated that introducing polar substituents at the 3-oxy site in (*R*)-**4** did not improve seizure protection and provided valuable information for the installation of both AB and CR units in the (*R*)-**1** framework and for future drug design efforts.

We tested (*R*)-**4** derivatives, (*R*)-**21**−(*R*)-**23**, that contained AB groups at their 3-oxy sites. The electrophilic groups aldehyde (*R*)-**21** (ED_{50} = > 300 mg/kg) and epoxide (*R*)-**22** (ED_{50} = > 300 mg/kg) exhibited no anticonvulsant activity and no neurological toxicity at the doses tested in mice (ip). The lack of neurotoxicity may suggest that the compounds did not cross the blood−brain barrier. The aliphatic isothiocyanate (*R*)-**23** was inactive in mice (ED_{50} = > 300 mg/kg), active in rats (ED_{50} = < 30 mg/kg), but neurologically toxic (TD_{50} = 30−100 mg/kg, mice (ip); TD_{50} = > 30 mg/kg, rat (po)). We suspect that several of these AB groups may have undergone change or been metabolized under the test conditions. Indeed, aldehydes and epoxides are known substrates for a variety of enzymes (e.g., aldehyde reductases, epoxide hydrolases).[58−63] Because we planned to use these AB groups for in vitro experiments where metabolism would less likely be an issue, we evaluated, where possible, the corresponding AB isostere to determine if the steric size of the AB group precluded their use. We were gratified to find that the *O*-but-3-enyl isostere (*R*)-**16** (ED_{50} = 30−100 mg/kg, mice (ip); ED_{50} = 17 mg/kg, rat (po)) for aldehyde (*R*)-**21** and the *O*-cyclopropyl isostere (*R*)-**17** (ED_{50} = 46 mg/kg, mice (ip)) for epoxide (*R*)-**22** displayed pronounced-to-excellent anticonvulsant activity. Thus, we have not attributed the absence of activity for aldehyde (*R*)-**21** and epoxide (*R*)-**22** to steric factors. Similar to the *O*-cyclopropyl (*R*)-**17**, the photolabile methyldiazirine AB derivative (*R*)-**20** exhibited pronounced animal protection in the MES seizure test (ED_{50} = 30−100 mg/kg, mice (ip); ED_{50} = 44 mg/kg, rat (po)).

Several (*R*)-**4** compounds were prepared that contained a CR unit at the 3-oxy site ((*R*)-**12**, (*R*)-**18**, (*R*)-**25**). The alkyne (*R*)-**12** and azide (*R*)-**25** CR compounds displayed excellent anticonvulsant activity (ED_{50} = < 10 mg/kg) in the rat upon po and ip administration, respectively. These findings supported the use of these CR units in (*R*)-**1** proteomic target searches. Of all the (*R*)-**4** 3-oxy substituted analogues prepared, the whole animal pharmacology of *O*-azidoethyl analogue (*R*)-**25** was the most intriguing. (*R*)-**25** anticonvulsant activity was dependent on the animal, the seizure test, and the route of administration. While (*R*)-**25** displayed poor MES seizure protection in mice (ip) (ED_{50} = 100−300 mg/kg), it proved potent in the 6 Hz (32 mA) test[50] when also administered ip to mice (ED_{50} = 44 mg/kg). Furthermore, we observed that (*R*)-**25** exhibited exceptional activity in the MES seizure test in the rat when administered ip (ED_{50} = 5.7 mg/kg), but that activity dropped with po administration (MES ED_{50} = > 40 mg/kg). When administered ip to the rat, the TD_{50} for (*R*)-**25** was 78 mg/kg, providing a PI greater than 13.

Finally, we asked whether that a methyl-d_3 analogue of (*R*)-**1**, (*R*)-**1**-d_3, would display enhanced anticonvulsant activity over (*R*)-**1**. Recently, pharmaceutical companies have prepared deuterated versions ("heavy drugs") of marketed medicinal agents.[64] The greater strength of C−D bonds compared with C−H bonds is predicted to confer increased metabolic stability for the deuterated version of the drug.[64] Significantly, the major metabolite for (*R*)-**1** in humans is the desmethyl analogue (*R*)-**46**.[8] The metabolic conversion of (*R*)-**1** to (*R*)-**46** has not been attributed to a specific metabolic enzyme.[65] Nonetheless, (*R*)-**46** accounts for ∼30% of the (*R*)-**1** excreted in human urine,[66] and it exhibits little anticonvulsant activity (ED_{50} = 100−300 mg/kg).[6] Accordingly, we reasoned that replacing the 3-oxy methyl group in (*R*)-**1** with the perdeuterated methyl unit to give (*R*)-**1**-d_3 might reduce metabolic processes and thus lead to increased bioavailability of the AED. We prepared (*R*)-**1**-d_3 using a method we reported for (*R*)-**1**[42] and substituting CD_3I for CH_3I. The deuterated derivative (*R*)-**1**-d_3 (ED_{50} = 5.2 mg/kg) showed an anticonvulsant activity in the rat (po) comparable with (*R*)-**1** (ED_{50} = 3.9 mg/kg), without exceeding it, and exhibited greater neurological toxicity ((*R*)-**1**-d_3, TD_{50} = ∼200 mg/kg; (*R*)-**1**, TD_{50} = > 500 mg/kg).



(*R*)-**46** (*R*)-**1**-d_3

Conclusions

This SAR study demonstrated that replacing the 3-oxy methyl group in (*R*)-**1** with small, nonbulky, hydrophobic groups provided derivatives with pronounced anticonvulsant activity in the MES seizure model. We showed that substituting the 3-oxy methyl group in (*R*)-**1** with larger alkyl groups diminished activity, but the loss could be offset, in part, by the incorporating hydrophobic, unsaturated moieties. Our SAR findings for the 3-oxy site in (*R*)-**4** were similar to that observed for the 4′-benzylamide position in (*R*)-**5**, where nonbulky, hydrophobic groups provided derivatives with excellent anticonvulsant activities[18] (Figure 2). Most important, these findings support our use of (*R*)-**1** AB&CR agents in proteomic searches wherein either the AB or the CR unit is installed at the 3-oxy site.

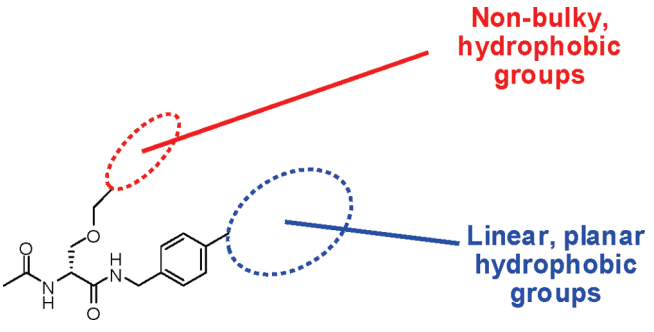


Figure 2. Structural sites in (*R*)-**1**, where incorporation of nonpolar substituents lead to pronounced anticonvulsant activity in the MES seizure model.

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 ±40% of the theoretical value. The TLC, NMR, and the analytical data confirmed the purity of the products was ≥95%.

General Procedure for the Pd-Catalyzed Hydrogenation Reaction. Method A. A MeOH suspension of the starting material ([C] ∼0.01−0.2 M) and 10% Pd/C (10% w/w) was vigorously stirred under an atmosphere of H_2 (balloon) at room temperature (16 h). The mixture was filtered through a bed of celite. The bed was washed with MeOH and CH_2Cl_2, and the washings were collected and evaporated in vacuo. The amine was used without further purification.

General Procedure for the Ester Hydrolysis of (*R*)-33 with LiOH. Method B. To a THF solution of ester (*R*)-**33** (2 volumes, [C] ∼0.1 M) was added an aqueous solution (1 volume) of LiOH (1 equiv). The homogeneous solution was stirred at room temperature (60 min), after which time Et_2O (2 volumes) was added. The aqueous layer was recovered and washed with Et_2O. The remaining aqueous layer was acidified (pH ∼1) by the dropwise addition of aqueous concentrated HCl, saturated with NaCl, and extracted with EtOAc until no further product was detected (TLC analysis). The combined organic layers were combined, dried (Na_2SO_4), and evaporated to an oily residue that was used directly for the next step or recrystallized from EtOAc and hexanes to provide an analytical sample.

General Procedure for the DMTMM Amide Coupling Reaction.[39] **Method C.** To a THF solution of acid (*R*)-**34** ([C] ∼0.1 M) at room temperature was added the desired benzylamine (1.2 equiv). The solution was stirred (5−10 min) until the benzylammonium carboxylate precipitated. With stirring, DMTMM (1.2 equiv) was added all at once, and the resulting suspension was stirred at room temperature (3−12 h). In those cases where a salt did not precipitate, DMTMM was added after 15 min to the solution. The salts were removed by filtration and washed with THF, and the solvent was removed in vacuo. The residue obtained was purified by flash column chromatography to afford the benzylamide and then recrystallized from EtOAc and hexanes.

General Procedure for the Aziridine Ring-Opening of (*R*)-32 and (*R*)-38 with Alcohols. Method D. To a cooled CH_2Cl_2 solution (ice bath) of either (*R*)-**32** or (*R*)-**38** ([C] ∼ 0.5−1 M) and the appropriate alcohol (1−5 equiv) was added $BF_3 \cdot Et_2O$ (1 equiv) dropwise while stirring. After addition, the mixture was warmed to room temperature and stirred (30 min), and then an equal volume of saturated aqueous $NaHCO_3$ was added. The reaction was vigorously stirred (15 min) and the organic layer separated. The aqueous layer was extracted with CH_2Cl_2 until no additional product could be detected (TLC analysis). All the organic layers were then combined, dried (Na_2SO_4), and concentrated in vacuo to yield a residue that was either purified by flash column chromatography, used directly for the next step or recrystallized from EtOAc and hexanes.

(*R*)-*N*-Benzyl 2-Acetamido-3-(methoxy-d_3)propionamide ((*R*)-1-d_3). Using method A, (*R*)-*N*-benzyl 2-*N*-(benzyloxycarbonyl)-amino-3-(methoxy-d_3)propionamide (1.5 g, 4.25 mmol) and 10% Pd/C (200 mg) in MeOH (50 mL) gave after evaporation an oily residue that was directly dissolved in CH_2Cl_2 (50 mL). While stirring, Et_3N (590 μL, 4.25 mmol), DMAP (25 mg, 212 μmol), and Ac_2O (400 μL, 4.25 mmol) were successively added and the reaction was stirred at room temperature (1 h). The organic layer was washed with aqueous 0.1 M H_2SO_4 (20 mL). The aqueous layer was extracted with CH_2Cl_2 (4 × 20 mL). The organic layers were combined, washed with brine (50 mL), dried (Na_2SO_4), and the solvents were removed under vacuum. The obtained solid was recrystallized from EtOAc and hexanes to give (*R*)-**1**-d_3 as a white solid (888 mg, 82% over 2 steps): mp 142−143 °C;

$[\alpha]^{25}_D$ +16.0° (*c* 0.7, MeOH); R_f = 0.31 (5/95 MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H), 3.47 (dd, *J* = 6.6, 9.0 Hz, 1H), 3.84 (dd, *J* = 4.0, 9.0 Hz, 1H), 4.34−4.52 (m, 2H), 4.55−4.69 (m, 1H), 6.72 (d, *J* = 7.0 Hz, 1H), 7.05−7.16 (m, 1H), 7.20−7.38 (m, 5H). M_r (+ESI) 276.1 $[M + Na]^+$ (calcd for $C_{13}H_{15}D_3N_2O_3Na^+$ 276.1). Anal. ($C_{13}H_{15}D_3N_2O_3$): C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3-(2-cyclohexyloxy)propionamide ((*R*)-9). Using method B, a ∼1:1 mixture of (*R*)-methyl 2-acetamido-3-(cyclohexyloxy)propionate and (*R*)-ethyl 2-acetamido-3-(cyclohexyloxy)propionate (2.10 g, 8.4 mmol) in THF (80 mL) and LiOH (202 mg, 8.4 mmol) in H_2O (40 mL) gave upon workup the corresponding acid (1.62 g, 7.1 mmol, 84%) as a yellow viscous oil (M_r (+ESI) 252.1212 $[M + Na]^+$ (calcd for $C_{11}H_{19}NO_4Na^+$ 252.1204)) that was directly dissolved in THF (70 mL). Using method C, addition of benzylamine (930 μL, 8.5 mmol) and DMTMM (2.4 g, 8.5 mmol) gave (*R*)-**9** as a white solid (1.17 g, 65%) after purification by flash chromatography (1:2 hexanes/EtOAc to 1/9 MeOH/CH_2Cl_2) followed by recrystallization from EtOAc and hexanes: mp 134−135 °C; $[\alpha]^{25}_D$ −33.5° (*c* 1.2; $CHCl_3$); R_f = 0.52 (EtOAc). ^{1}H NMR ($CDCl_3$) δ 1.10−1.34, 1.42−1.88 (m, 10H), 2.03 (s, 3H), 3.24−3.36 (m, 1H), 3.43 (app t, *J* = 9.0 Hz, 1H), 3.86 (dd, *J* = 3.6, 9.0 Hz, 1H), 4.38−4.56 (m, 3H), 6.48−6.58 (br d, 1H), 6.92−7.02 (br t, 1H), 7.22−7.38 (m, 5H). M_r (+ESI) 341.3 $[M + Na]^+$ (calcd for $C_{18}H_{26}N_2O_3Na^+$ 341.3). Anal. ($C_{18}H_{26}N_2O_3$): C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3-(but-2-yn-1-yloxy)propionamide ((*R*)-13). To a cooled (−78 °C) solution of (*R*)-2-acetamido-3-(but-2-ynyloxy)propionic acid (1.15 g, 5.78 mmol) in THF (50 mL) were successively added *N*-methylmorpholine (NMM) (0.95 mL, 8.67 mmol), stirred (2 min), isobutylchloroformate (IBCF) (0.95 mL, 7.28 mmol), stirred (15 min), and benzylamine (0.75 mL, 6.94 mmol).[38] The reaction was allowed to warm to room temperature and further stirred (2 h), filtered, and concentrated in vacuo. Purification by flash chromatography (1/9 MeOH/$CHCl_3$) gave 1.53 g (92%) of (*R*)-**13** as a white solid: mp 149−151 °C; $[\alpha]^{25}_D$ −34.2° (*c* 0.5, $CHCl_3$); R_f = 0.45 (1/9 MeOH/$CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.83 (t, *J* = 2.4 Hz, 3H), 2.03 (s, 3H), 3.61 (dd, *J* = 7.2, 9.3 Hz, 1H), 3.90 (dd, *J* = 4.2, 9.3 Hz, 1H), 4.08−4.21 (m, 2H), 4.41−4.54 (m, 2H), 4.57−4.63 (m, 1H), 6.48−6.51 (br d, *J* = 6.6 Hz, 1H), 6.77−6.84 (br m, 1H), 7.25−7.37 (m, 5H). M_r (+ESI) 288.1 $[M + H]^+$ (calcd for $C_{16}H_{20}N_2O_3H^+$ 288.1). Anal. ($C_{16}H_{20}N_2O_3$) C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3-(2-cyclohexylmethoxy)propionamide ((*R*)-14). Using method B, a ∼1:4 mixture of (*R*)-methyl 2-acetamido-3-(cyclohexylmethoxy)propionate and (*R*)-ethyl 2-acetamido-3-(cyclohexylmethoxy)propionate (2.32 g, 8.6 mmol) in THF (85 mL) and LiOH (207 mg, 8.6 mmol) in H_2O (40 mL) gave upon workup the corresponding acid (1.88 g, 7.7 mmol, 90%) as a yellow viscous oil that was directly dissolved in THF (75 mL). Using method C, addition of benzylamine (1.0 mL, 9.2 mmol) and DMTMM (2.54 g, 9.2 mmol) gave (*R*)-**14** as a white solid (1.50 g, 59%) after purification by flash chromatography (1:2 hexanes/EtOAc to 1/9 MeOH/CH_2Cl_2) followed by recrystallization from EtOAc and hexanes: mp 143−144 °C; $[\alpha]^{25}_D$ +6.7° (*c* 1.6; MeOH); R_f = 0.39 (5/95 MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 0.74−0.90, 1.00−1.26, 1.40−1.72 (m, 11H), 2.02 (s, 3H), 3.16−3.32 (m, 2H), 3.42 (app. t, *J* = 9.0 Hz, 1H), 3.79 (dd, *J* = 3.6, 9.0 Hz, 1H), 4.38−4.50 (m, 2H), 4.51−4.60 (m, 1H), 6.53 (d, *J* = 6.3 Hz, 1H), 6.88−7.00 (br t, 1H), 7.22−7.38 (m, 5H). M_r (+ESI) 355.1998 $[M + Na]^+$ (calcd for $C_{19}H_{28}N_2O_3Na^+$ 355.1997). Anal. ($C_{19}H_{28}N_2O_3$): C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3-(benzyloxy)propionamide ((*R*)-15). To a stirred suspension of *O*-benzyl-D-serine (Astatech Inc.) (1.00 g, 5.13 mmol) in a 9:1 THF:H_2O mixture (50 mL) was added Ac_2O (1.45 mL, 15.3 mmol) at room temperature, and the reaction was stirred at room temperature (3 h). The solvents were removed in vacuo to yield a viscous clear residue (1.22 g, 5.13 mmol, quant) that was directly dissolved in THF (60 mL). Using method C, benzylamine (670 μL, 6.2 mmol) and DMTMM (1.71 g, 6.2 mmol) gave (*R*)-**15** as a white solid (1.55 g, 93% for 2 steps) upon purification by flash chromatography (EtOAc to 1/9 MeOH/CH_2Cl_2): mp 145−146 °C; $[\alpha]^{25}_D$ −28.7° (*c* 0.7; $CHCl_3$); R_f = 0.49 (EtOAc). ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3H), 3.51 (dd, *J* = 7.8, 9.0 Hz, 1H), 3.90 (dd, *J* = 4.2, 9.0 Hz, 1H), 4.38−4.64 (m, 5H), 6.40−6.52 (br d, 1H), 6.72−6.84 (br t, 1H), 7.18−7.38 (m, 10H). M_r (+ESI) 349.2 $[M + Na]^+$ (calcd for $C_{19}H_{22}N_2O_3Na^+$ 349.2). Anal. ($C_{19}H_{22}N_2O_3$): C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3-(but-3-enyloxy)propionamide ((*R*)-16). Using method C, (*R*)-2-acetamido-3-(but-3-en-1-yloxy)-propionic acid (1.32 g, 6.57 mmol), benzylamine (860 μL, 7.88 mmol), and DMTMM (2.18 g, 7.88 mmol) gave (*R*)-**16** (1.30 g, 67%) as a white solid after purification by flash chromatography (EtOAc) and further recrystallization from EtOAc and hexanes: mp 103−104 °C; $[\alpha]^{25}_D$ +51.3° (*c* 0.6; MeOH); R_f = 0.37 (5/95 MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 3H), 2.22−2.36 (m, 2H), 3.40−3.62 (m, 3H), 3.85 (dd, *J* = 4.2, 9.0 Hz, 1H), 4.45 (d, *J* = 7.2 Hz, 2H), 4.48−4.57 (m, 1H), 4.92−5.06 (m, 2H), 5.62−5.74 (m, 1H), 6.42−6.56 (m, 1H), 6.84−6.96 (br t, 1H), 7.20−7.36 (m, 5H). M_r (+ESI) 313.1528 $[M + Na]^+$ (calcd for $C_{16}H_{22}N_2O_3Na^+$ 313.1528). Anal. ($C_{16}H_{22}N_2O_3$): C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3-(2-cyclopropylethoxy)propionamide ((*R*)-17). Using method B, a ∼1:1 mixture of (*R*)-methyl 2-acetamido-3-(2-cyclopropylethoxy)propionate and (*R*)-ethyl 2-acetamido-3-(2-cyclopropylethoxy)propionate (1.60 g, 6.6 mmol) in THF (60 mL) and LiOH (158 mg, 6.6 mmol) in H_2O (30 mL) gave upon workup the corresponding acid (1.27 g, 5.9 mmol, 90%) as a yellow oil (M_r (+ESI) 238.1056 $[M + Na]^+$ (calcd for $C_{10}H_{17}NO_4Na^+$ 238.1054)) that was directly dissolved in THF (60 mL). Using method C, addition of benzylamine (740 μL, 7.13 mmol) and DMTMM (2.0 g, 7.13 mmol) gave (*R*)-**17** as a white solid (1.17 g, 65%) after purification by flash chromatography (1:2 hexanes/EtOAc to 1/9 MeOH/CH_2Cl_2) followed by recrystallization from EtOAc and hexanes: mp 97−99 °C; $[\alpha]^{25}_D$ −32.1° (*c* 1.5; MeOH); R_f = 0.52 (EtOAc). ^{1}H NMR ($CDCl_3$) δ −0.08−0.01 (m, 2H), 0.30−0.38 (m, 2H), 0.50−0.64 (m, 1H), 1.32−1.48 (m, 2H), 2.02 (s, 3H), 3.40−3.62 (m, 3H), 3.84 (dd, *J* = 3.9, 9.0 Hz, 1H), 4.38−4.58 (m, 3H), 6.48−6.58 (br d, 1H), 6.90−7.00 (br t, 1H), 7.20−7.36 (m, 5H). M_r (+ESI) 327.2 $[M + Na]^+$ (calcd for $C_{17}H_{24}N_2O_3Na^+$ 327.2). Anal. ($C_{17}H_{24}N_2O_3$): C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3-(but-3-ynyloxy)propionamide ((*R*)-18). Using method D, compound (*R*)-**38** (1.14 g, 5.22 mmol), homopropargyl alcohol (2.0 mL, 26.1 mmol), and $BF_3 \cdot Et_2O$ (500 μL, 3.97 mmol) in CH_2Cl_2 (30 mL) gave (*R*)-**18** as a white solid (450 mg, 30%) upon workup, purification by flash chromatography (2/1 EtOAc/CH_2Cl_2), and subsequent recrystallization from EtOAc and hexanes: mp 120−122 °C; $[\alpha]^{25}_D$ −49.1° (*c* 0.8, $CHCl_3$); R_f = 0.38 (2/1 EtOAc/CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 1.80 (t, *J* = 2.7 Hz, 1H), 2.04 (s, 3H), 2.38−2.46 (m, 2H), 3.46−3.74 (m, 3H), 3.90 (dd, *J* = 3.9, 9.0 Hz, 1H), 4.38−4.58 (m, 3H), 6.48−6.56 (m, 1H), 6.92−7.06 (m, 1H), 7.20−7.38 (m, 5H).; M_r (+ESI) 421.1 $[M + Cs]^+$ (calcd for $C_{16}H_{20}N_2O_3Cs^+$ 421.1). Anal. ($C_{16}H_{20}N_2O_3$): C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3-phenethoxypropionamide ((*R*)-19). Using method D, compound (*R*)-**38** (2.00 g, 9.2 mmol), phenethyl alcohol (6.0 mL, 49.2 mmol), and $BF_3 \cdot Et_2O$ (1.0 mL, 8.0 mmol) in CH_2Cl_2 (30 mL) gave (*R*)-**19** as a white solid (392 mg, 10%) upon workup and purification by flash chromatography (2/1 EtOAc/CH_2Cl_2) and recrystallization from EtOAc and hexanes: mp 90−92 °C; $[\alpha]^{25}_D$ −29.2° (*c* 0.5, $CHCl_3$); R_f = 0.44 (3/1 EtOAc/CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H), 2.84 (d, *J* = 6.3 Hz, 2H), 3.40 (app d, *J* = 8.1 Hz, 1H), 3.62−3.92 (m, 3H), 4.20−4.38 (m, 2H), 4.40−4.50 (m, 1H), 6.32−6.40 (m, 1H), 6.50−6.62 (m, 1H), 7.12−7.38 (m, 10H). M_r (+ESI) 363.2 $[M + Na]^+$ (calcd for $C_{20}H_{24}N_2O_3Na^+$ 363.2). Anal. ($C_{20}H_{24}N_2O_3$): C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3-(2-(3-methyl-3*H*-diazirin-3-yl)-ethoxy)propionamide ((*R*)-20). Using method C, (*R*)-2-acetamido-3-(2-(3-methyl-3*H*-diazirin-3-yl)ethoxy)propionic acid (500 mg, 2.2 mmol), benzylamine (285 μL, 2.6 mmol), and DMTMM

(723 mg, 2.6 mmol) in THF (22 mL) gave (*R*)-**20** as a white solid (565 mg, 81%) after purification by flash chromatography (3/2 hexanes/EtOAc to EtOAc to 3/2 EtOAc/acetone): R_f = 0.43 (EtOAc); mp 120−121 °C; $[\alpha]^{24.1}_D$ −33.4° (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 0.98 (s, 3H), 1.65 (t, *J* = 6.0 Hz, 2H), 2.07 (s, 3H), 3.14−3.22 (m, 1H), 3.33−3.44 (m, 2H), 3.84 (dd, *J* = 3.6, 9.0 Hz, 1H), 4.44−4.58 (m, 3H), 6.63 (d, *J* = 6.3 Hz, 1H), 6.97−7.05 (br t, 1H), 7.24−7.37 (m, 5H). M_r (+ESI) 319.1769 $[M + H]^+$ (calcd $C_{16}H_{22}N_4O_3H^+$ 319.1770). Anal. ($C_{16}H_{22}N_4O_3$): C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3-(3-oxopropoxy)propionamide ((*R*)-21). To a cooled solution (dry ice/acetone bath) of oxalyl chloride (432 μL, 4.92 mmol) in CH_2Cl_2 (12 mL) was added dropwise a solution of DMSO (720 μL, 9.84 mmol) in CH_2Cl_2 (24 mL). After stirring at −78 °C (15 min), a solution of (*R*)-*N*-benzyl 2-acetamido-3-(3-hydroxypropoxy)propionamide (1.20 g, 4.08 mmol) in CH_2Cl_2 (12 mL) was added dropwise at −78 °C and the reaction was stirred at −78 °C (1 h). DIEA (3.55 mL, 14.4 mmol) was then added dropwise, stirred (20 min), warmed to room temperature, and then stirred (30 min). Aqueous 10% citric acid (50 mL) was added to the solution, and the CH_2Cl_2 layer was separated. The aqueous layer was extracted with CH_2Cl_2 (6 × 50 mL), the combined organic layers were washed with brine (50 mL), dried (Na_2SO_4), and evaporated to give an oily residue that was recrystallized from EtOAc and hexanes to afford (*R*)-**21** as white needles: mp 120−121 °C; $[\alpha]^{25}_D$ −22.1° (*c* 0.9, $CHCl_3$); R_f = 0.34 (5/95 MeOH/$CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 2.04 (s, 3H), 2.60−2.80 (m, 2H), 3.48 (dd, *J* = 7.5, 9.3 Hz, 1H), 3.70−3.78 (m, 1H), 3.80−3.87 (m, 1H), 3.90 (dd, *J* = 3.6, 9.3 Hz, 1H), 4.46 (d, *J* = 6.0 Hz, 2H), 4.51−4.58 (m, 1H), 6.61 (d, *J* = 6.3 Hz, 1H), 6.85−7.04 (br t, 1H), 7.22−7.38 (m, 5H), 9.71 (t, *J* = 1.4 Hz, 1H). M_r (+ESI) 315.1323 $[M + Na]^+$ (calcd for $C_{15}H_{20}N_2O_4Na^+$ 315.1321). Anal. ($C_{15}H_{20}N_2O_4$): C, H, N.

(2*R*)-*N*-Benzyl 2-Acetamido-3-(2-(oxiran-2-yl)ethoxy)propionamide ((*R*)-22) (Mixture of Diastereomers). A solution of *m*CPBA (77 wt %, 420 mg, 1.88 mmol) in CH_2Cl_2 (3 mL) was stirred in the presence of Na_2SO_4 (~50 mg, 5 min), and (*R*)-**16** (320 mg, 1.10 mmol) was added at once. The suspension was stirred at room temperature (15 h), filtered, concentrated under vacuum to give (*R*)-**22** (265 mg, 72%) as a ~1:1 mixture of diastereoisomers after purification by flash chromatography (15/85 acetone/EtOAc): mp 104−110 °C; $[\alpha]^{25}_D$ −29.6° (*c* 0.3; $CHCl_3$); R_f = 0.31 (15/85 acetone/EtOAc). ^{1}H NMR ($CDCl_3$) δ 1.52−1.64 (m, 1H), 1.86−2.00 (m, 1H), 2.03, 2.04 (s, 3H), 2.41−2.46 (m, 1H), 2.66−2.71 (m, 1H), 2.82−2.96 (m, 1H), 3.44−3.52 (m, 1H), 3.54−3.76 (m, 2H), 3.84−3.93 (m, 1H), 4.39−4.52 (m, 2H), 4.50−4.58 (m, 1H), 6.56−6.68 (m, 1H), 6.88−6.98, 6.99−7.08 (m, 1H), 7.22−7.36 (m, 5H). M_r (+ESI) 329.1478 $[M + Na]^+$ (calcd for $C_{16}H_{22}N_2O_4Na^+$ 329.1477). Anal. ($C_{16}H_{22}N_2O_4$): C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3-(2-acetamidoethoxy)propionamide ((*R*)-24). Using method A, (*R*)-**25** (1.14 g, 3.7 mmol) and 10% Pd/C (100 mg) in MeOH (25 mL) gave upon filtration and evaporation a residue that was dissolved in CH_2Cl_2 (50 mL). Et_3N (570 μL, 4.1 mmol), DMAP (1 mg, catalytic), and Ac_2O (390 μL, 4.1 mmol) were then successively added and the reaction was stirred at room temperature (30 min), filtered, and the solvents were removed under vacuum. The crude residue was purified by flash chromatography (1/9 MeOH/CH_2Cl_2) to give an oily residue that was dissolved in warm THF (25 mL). The white solid that precipitated upon cooling was filtered to give (*R*)-**24** (490 mg, 40% overall yield for two steps): mp 166−168 °C; $[\alpha]^{25}_D$ +11.7° (*c* 0.6, MeOH); R_f = 0.30 (5/95 MeOH/CH_2Cl_2). ^{1}H NMR (DMSO-d_6) δ 1.79, 1.88 (s, 6H), 3.10−3.24 (m, 2H), 3.36−3.46 (m, 2H), 3.52−3.64 (m, 2H), 4.29 (d, *J* = 7.0 Hz, 2H), 4.42−4.52 (m, 1H), 7.20−7.36 (m, 5H), 7.80−7.88 (m, 1H), 8.07 (d, *J* = 7.0 Hz, 1H), 8.50 (t, *J* = 6.0 Hz, 1H). M_r (+ESI) 344.2 $[M + Na]^+$ (calcd for $C_{16}H_{23}N_3O_4Na^+$ 344.2). Anal. ($C_{16}H_{23}N_3O_4$): C, H, N.

(*R*)-*N*-Benzyl 2-Acetamido-3-(2-methoxyethoxy)propionamide ((*R*)-27). Using method B, a ~5:95 mixture of (*R*)-methyl 2-acetamido-3-(2-methoxyethoxy)propionate and (*R*)-ethyl 2-acetamido-3-(2-methoxyethoxy)propionate (4.00 g, 17.4 mmol) in THF (170 mL) and LiOH (415 mg, 17.3 mmol) in H_2O (80 mL) gave 1.10 g (31%, 5.36 mmol) of a crude viscous yellow oil upon workup (M_r (+ESI) 228.0848 $[M + Na]^+$ (calcd for $C_8H_{15}NO_5$-Na^+ 228.0848)). Using method C, the oil was directly dissolved in THF (60 mL), and then benzylamine (732 μL, 6.7 mmol) followed by DMTMM (1.90 g, 6.7 mmol) were added. Purification by flash chromatography (1/9 hexanes/EtOAc to 1/9 MeOH/CH_2Cl_2) followed by recrystallization from EtOAc and hexanes afforded (*R*)-**27** (750 mg, 45%) as a white solid: mp 109−110 °C; $[\alpha]^{25}_D$ +10.3° (*c* 0.5; MeOH); R_f = 0.43 (1/9 MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3H), 3.18 (s, 3H), 3.43−3.53 (m, 3H), 3.62−3.78 (m, 2H), 3.85 (dd, *J* = 4.2, 9.6 Hz, 2H), 4.37−4.51 (m, 2H), 4.52−4.58 (m, 1H), 6.65 (d, *J* = 6.6 Hz, 1H), 7.21−7.38 (m, 6H). M_r (+ESI) 317.1478 $[M + Na]^+$ (calcd for $C_{15}H_{22}N_2O_4Na^+$ 317.1477). Anal. ($C_{15}H_{22}N_2O_4$): C, H, N.

Pharmacology. Compounds were screened under the auspices of the National Institutes of Health's Anticonvulsant Screening Program. Experiments were performed in male rodents (albino Carworth Farms no. 1 mice (intraperitoneal route, ip), albino Spague-Dawley rats (oral route, po)). Housing, handling, and feeding were in accordance with recommendations contained in the "Guide for the Care and Use of Laboratory Animals". Anticonvulsant activity was established using the MES test,[53,54] 6 Hz,[55] and the scPTZ test,[53] according to previously reported methods.[6]

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Supporting Information Available: Synthetic procedures for the intermediates leading to the preparation of (*R*)-**1**-d_3, (*R*)-**9**, (*R*)-**13**−(*R*)-**22**, (*R*)-**24**, (*R*)-**27**, and **44**, elemental analyses, ^{1}H and ^{13}C NMR spectra of compounds (*R*)-**1**-d_3, (*R*)-**9**, (*R*)-**13**−(*R*)-**22**, (*R*)-**24**, and (*R*)-**27** evaluated in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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