Design and Evaluation of Affinity Labels of Functionalized Amino Acid Anticonvulsants

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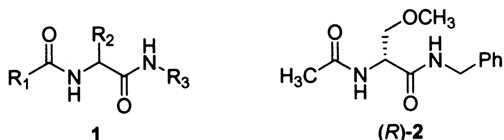
Studies have shown that functionalized amino acids (FAA) exhibit outstanding activity in the maximal electroshock-induced seizure (MES) test in rodents. Affinity labels patterned in part after the potent antiepileptic (*R*)-*N*-benzyl-2-acetamido-3-methoxypropionamide ((*R*)-**2**) have been prepared as mechanistic probes to learn the pharmacological basis for FAA function. The chemical reactivity of the affinity labels with nucleophiles was assessed, and the labels were evaluated in in vitro radioligand assays and in the MES tests in rodents. The affinity labels did not bind to receptors known to effect seizure spread. Three affinity labels, (*R*,*S*)-*N*-benzyl-2-acetamido-6-isothiocyanatohexanamide ((*R*,*S*)-**5**), (*R*)-*N*-(4-isothiocyanatobenzyl)-2-acetamido-3-methoxypropionamide ((*R*)-**6**), and (*R*)-*N*-(3-isothiocyanatobenzyl)-2-acetamido-3-methoxypropionamide ((*R*)-**7**), possessed excellent in vivo anticonvulsant activity and exhibited maximal activity at later time periods than typically observed for FAA. The anticonvulsant activity of **6** and **7** resided primarily in the (*R*)-enantiomer and the activity of (*R*)-**6** and (*R*)-**7** in rats (po) exceeded that of phenytoin. The chemical properties, pharmacological profile, and marked stereospecificity associated with **6** and **7** anticonvulsant activity make these compounds useful pharmacological tools for the study of the mode of action of FAA.

Functionalized amino acids (FAA, **1**) are a new class of potent anticonvulsants.[1–3] More than 250 compounds



have been prepared and tested in animal model systems.[1–3] Representative FAA have been shown to have activity comparable with or exceeding that of phenytoin (dilantin) in the maximal electroshock (MES)-induced seizure test in mice.[1e–h,j] The MES test is a model of generalized tonic-clonic seizures and identifies compounds that prevent seizure spread,[4,5] and phenytoin is considered the prototypical agent in this model.[6] (*R*)-*N*-Benzyl-2-acetamido-3-methoxypropionamide[1j] ((*R*)-**2**) has emerged as the lead FAA and has entered phase II clinical trials for the treatment of epilepsy and neuropathic pain under Schwarz Pharma sponsorship.

Little is known about the mechanism of action of FAA. The preclinical pharmacological profile for **2** and **1** differed from established agents such as phenytoin, carbamazepine, lamotrigine, gabapentin, felbamate, valproic acid, clonazepam, and ethosuximide.[7] Information concerning function is vital for maximizing the therapeutic potential of these agents.

Affinity labeling has emerged as a powerful technique useful for elucidating the structural site(s) for drug action.[8] An affinity label consists of two functionally different groups: an affinity group, which structurally resembles the biological substrate or drug, and a reactive group, which covalently modifies amino acid residues at the drug binding site. An essential premise in this concept is that the close structural correspondence of the drug and the affinity group permit the affinity label to target the correct binding site.

In this study, we describe the synthesis of a family of FAA-based electrophilic affinity labels designed to identify the site(s) of FAA function. We report on the reactivity of the FAA affinity labels with nucleophiles and summarize their activities in in vivo and in vitro pharmacological assays. The biological properties documented that the placement of electrophilic tags at select sites within the FAA does not lead to noticeable losses in anticonvulsant activity, and they provided evidence that these compounds may aid future mechanistic studies.

Results and Discussion

1. Choices of Substrates. Four criteria were established in our design of FAA affinity labels. First, the compounds needed to conform to the SAR observed for **1**.[1] When possible, we fashioned the affinity label after **2**. Second, the reactive unit within the affinity label must have documented success in previous receptor site inactivation studies.[8] Accordingly, we selected the isothiocyanate,[9] the α-bromoacetamide,[10] and the acrylamide[11] electrophilic tags. These functional groups display an

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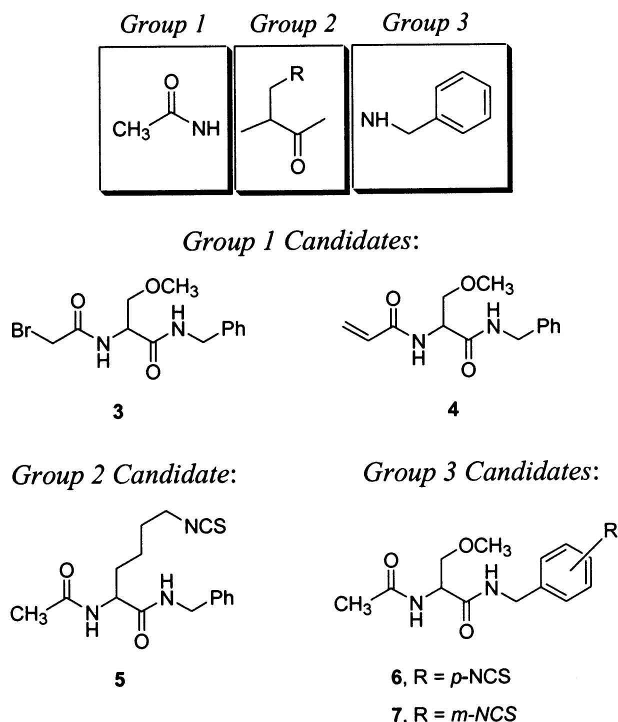


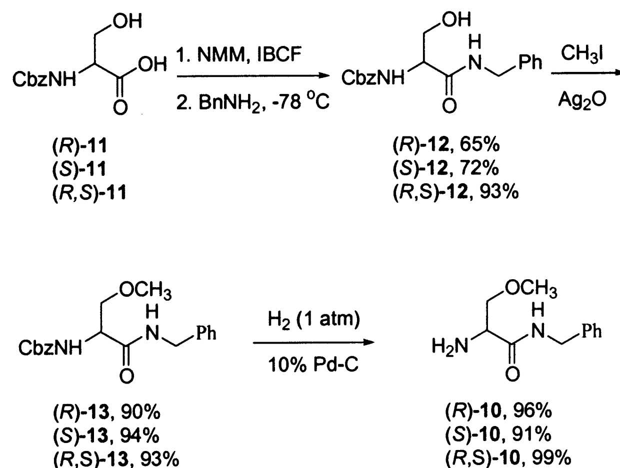
Figure 1. Design of functionalized amino acid affinity labels.

excellent balance between reactivity and stability; they react with nucleophiles (e.g., thiols, amines) but do not readily react with hydroxylic solvents, thus allowing their dissolution in aqueous buffer solutions.[8] Third, we required syntheses that permitted preparation of both enantiomeric forms of the affinity label. A distinguishing feature of **1** is the pronounced anticonvulsant activity of the (*R*)-stereoisomer compared with the (*S*)-isomer.[1c–e,g,j] Since enantioselective irreversible inactivation is an important measure of receptor site specificity,[12] we have prepared and tested both isomers in select cases. Fourth, the type and placement of the reactive group in the FAA was varied. We incorporated this criterion to satisfy the possible structural requirements for affinity label–receptor site adduction[8] and to maximize the chances of identifying multiple sites of FAA function.[13]

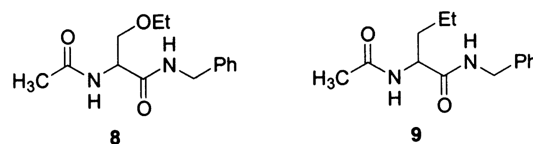
Five different FAA affinity labels (**3**–**7**) were evaluated (Figure 1). For **3**, **6**, and **7**, we prepared both the (*R*)- and (*S*)-enantiomers for a total of eight test compounds. The five affinity labels were divided by the site of the electrophilic tag. In group 1 (compounds **3** and **4**), the label was placed at the N terminus of the FAA.[14] In group 2 (compound **5**) we incorporated the tag at the C(2) position, and in group 3 (compounds **6** and **7**) the electrophilic unit was positioned at the FAA C terminus.

Including electrophilic tags in the core structure affected the size and electronic properties of the FAA. Our earlier structure–activity relationship (SAR) studies showed that in several cases, increasing the R_1 group in **1** from methyl to higher alkyl gave compounds with significant anticonvulsant activities, suggesting the use of either an α-bromoacetamide unit (**3**) or an acrylamide moiety (**4**) as an electrophilic tag.[1b] The SAR for **1** further predicted that inclusion of an isothiocyanate unit within the R_3 benzyl unit would lead to small

Scheme 1. Group 1 Affinity Label Candidates: Preparation of (*R*)-, (*S*)-, and (*R*,*S*)-**10**



pharmacological changes compared with the corresponding unsubstituted R_3 benzyl FAA.[1b,e,g,j] Finally, compound **5** was structurally similar to (*R*,*S*)-**8**[1j] (MES ED_{50} = 17 mg/kg (mice, ip)) and (*R*,*S*)-**9**[17] (MES ED_{50} = 38 mg/kg (mice, ip)). The excellent anticonvulsant



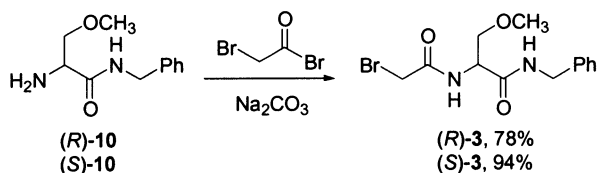
activities for **8** and **9** suggested that **5** may serve as an effective affinity label to target the site(s) of FAA function.

2. Syntheses. The synthetic schemes adopted for affinity labels **3**–**7** considered the reactivity of the electrophilic tags. The isothiocyanate and α-bromoacetamide moieties were not expected to survive the amide coupling and functional group deprotection reactions. Accordingly, we introduced the electrophilic unit at a late stage in the synthesis.

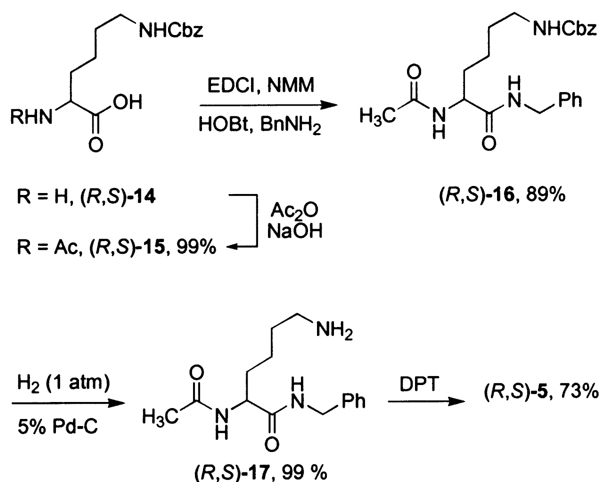
2.1. Group 1 Affinity Label Candidates. Affinity labels **3** and **4**[17] were prepared from the common intermediate **10** (Scheme 1). The procedure previously employed for (*R*)-**10**[18] and (*R*,*S*)-**10**[15] was also used to synthesize (*S*)-**10**. Synthesis of (*R*)-, (*S*)-, and (*R*,*S*)-**10** began with readily available Cbz-D-, Cbz-L-, and Cbz-DL-serine ((*R*)-, (*S*)-, and (*R*,*S*)-**11**), respectively.[19] Compound **11** was converted to **12** using benzylamine and the mixed-anhydride coupling (MAC) procedure.[20] Methylation of **12** (MeI, Ag_2O) followed by hydrogenolysis of **13** afforded (*R*)-, (*S*), and (*R*,*S*)-**10**, which were the precursors for group 1 affinity label candidates. Treatment of either (*R*)-**10** or (*S*)-**10** with α-bromoacetyl bromide afforded (*R*)-**3** and (*S*)-**3**, respectively (Scheme 2). The overall yield for affinity labels (*R*)-**3**, (*S*)-**3**, and (*R*,*S*)-**4** was 44–71% beginning with (*R*)-**11**, (*S*)-**11**, or (*R*,*S*)-**11**.

Three methods were used to assess the enantiopurity of (*R*)-**13**, (*S*)-**13**, (*R*)-**3**, and (*S*)-**3**. These were the use of NMR and a chiral resolving agent, optical rotation, and melting point. In particular, we detected in the ^1H NMR only a single *O*-methyl ether signal for (*R*)-**13** and (*S*)-**13** and a single signal for the *O*-methyl ether and

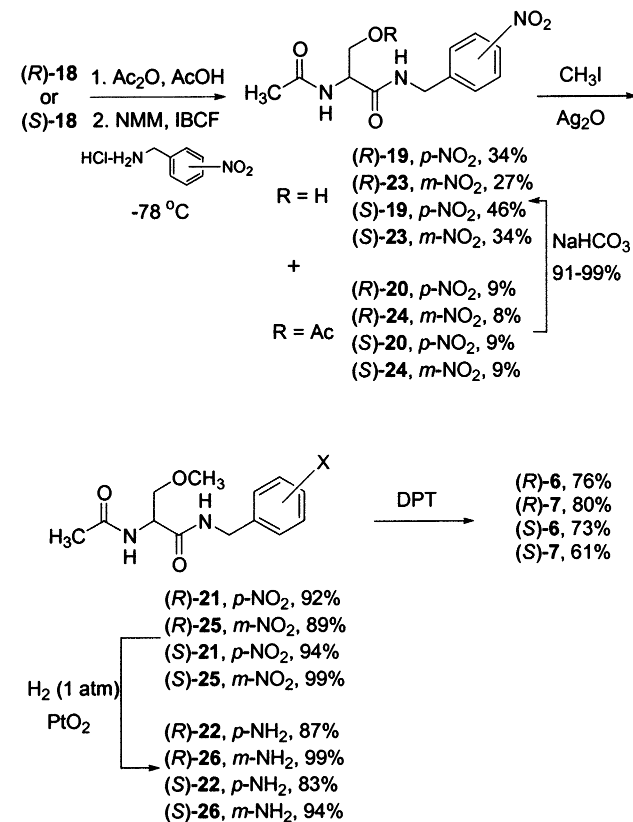
Scheme 2. Group 1 Affinity Label Candidates: Preparation of (*R*)-**3** and (*S*)-**3**



Scheme 3. Group 2 Affinity Label Candidates: Preparation of (*R*,*S*)-**5**



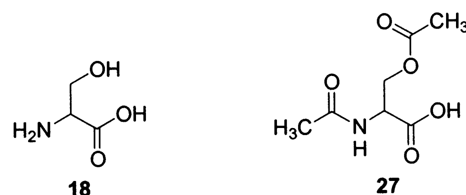
Scheme 4. Group 3 Affinity Label Candidates: Preparation of (*R*)-**6**, (*S*)-**6**, (*R*)-**7**, and (*S*)-**7**



bromoacetyl methylene peaks for (*R*)-**3** and (*S*)-**3** when the chiral resolving agent, (*R*)−(−)-mandelic acid,[21] was added to the $CDCl_3$ solutions of these compounds.[1j] We also verified the enantiopurity of (*R*)-**10**, (*R*)-**12**, and (*R*)-**13** by comparison with reported data.[18]

2.2. Group 2 Affinity Label Candidates. Commercially available N^{ϵ}-Cbz-DL-lysine (Novabiochem) ((*R*,*S*)-**14**) served as the starting material for the synthesis of the affinity label (*R*,*S*)-**5** (Scheme 3). Using a previously reported procedure,[22] we acetylated (*R*,*S*)-**14** to get (*R*,*S*)-**15** in near-quantitative yield. Acid (*R*,*S*)-**15** was coupled with benzylamine using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDCI)/1-hydroxybenzotriazole (HOBt) to give (*R*,*S*)-**16** (89% yield). Hydrogenolysis (H_2, Pd/C in MeOH)[18,23] of the Cbz protecting group provided lysine derivative (*R*,*S*)-**17** in a near-quantitative yield. Conversion of the amine (*R*,*S*)-**17** to the affinity label (*R*,*S*)-**5** was accomplished with di-2-pyridyl thionocarbonate[24] (DPT) (64% overall yield from (*R*,*S*)-**14**). In agreement with our structural assignment for isothiocyanate (*R*,*S*)-**5**, we observed a signal at 130.2 ppm in the ^{13}C NMR spectrum[25] and two absorption bands at 2184 and 2110 cm^{-1} in the FT-IR[26] spectrum for this moiety.

2.3. Group 3 Affinity Label Candidates. The preparation of the group 3 affinity label candidates (*R*)-**6**, (*S*)-**6**, (*R*)-**7**, and (*S*)-**7** is outlined in Scheme 4. Our protocol is similar to that used by Choi and Kohn[1j] for the synthesis of (*R*)-**2** except that *p*- or *m*-nitrobenzylamine hydrochloride (Aldrich) was substituted for benzylamine. Accordingly, beginning with enantiopure serine ((*R*)-**18**, (*S*)-**18**) acetylation and then treatment with either *p*- or *m*-nitrobenzylamine hydrochloride using MAC[20] methodology gave binary mixtures of amides **19** and **20** or amides **23** and **24**, respectively. The diacetylated products (**20**, **24**) likely resulted from the formation of *N*,*O*-diacetyl serine (**27**) in the first step. The binary mixtures (**19** + **20**, **23** + **24**) formed in



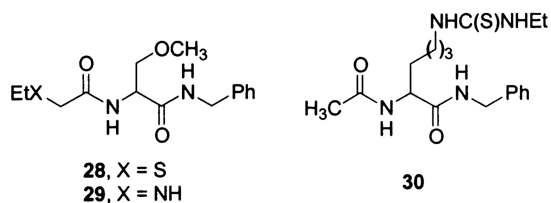
each of these reactions were separated by column chromatography. Furthermore, **20** and **24** were converted (91−99% yield) to **19** and **23**, respectively, without racemization upon treatment with basic MeOH. Methylation of **19** and **23** with MeI and Ag_2O provided **21** and **25**, respectively. Catalytic reduction (H_2, PtO_2) of the aromatic nitro groups in **21** and **25** gave amines **22** and **26**, respectively, which served as immediate precursors for the affinity labels. Treatment of THF solutions containing (*R*)-**22**, (*S*)-**22**, (*R*)-**26**, or (*S*)-**26** with DPT[24] efficiently produced (*R*)-**6**, (*S*)-**6**, (*R*)-**7**, or (*S*)-**7**, respectively. The overall yield for group 3 affinity labels was 21−29% beginning with either D- or L-serine (**18**) (five steps). Spectroscopic evidence for the isothiocyanate unit in **6** or **7** was obtained both by detecting the NCS carbon resonance (135.6−135.8 ppm) in the ^{13}C NMR[25] and by observing the NCS absorption band (2125−2183 cm^{-1}) in the FT-IR.[26] We documented the enantiopurity of group 3 affinity labels (*R*)- and (*S*)-**6** and **7** and their synthetic precursors ((*R*)- and (*S*)-**19**−**26**) by determining their NMR chemical shift values in the presence of mandelic acid (e.g., *N*-acetyl methyl group, *O*-methyl group), optical activities, and melting points.

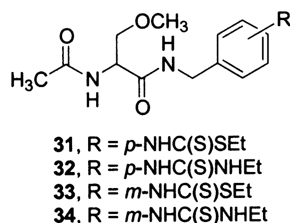
Table 1. Selected Physical and Pharmacological Data for FAA Affinity Labels and Their Reference Compounds

		mice (ip)[a]			rat (po)[b]		
compd	mp[c]	MES,[d] ED_{50}	Tox,[e] TD_{50}	PI[f]	MES,[d] ED_{50}	Tox,[e] TD_{50}	PI[f]
(*R*,*S*)-**2**	121–122	8.3 [0.5] (7.9–9.8)	43 [0.25] (38–47)	5.2	3.8 [2] (2.9–5.5)	37 [1] (320–520)	101
(*R*)-**2**	143–144	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
(*S*)-**2**	143–144	>100, <300	>300		>30	>30	
(*R*,*S*)-**9**	138–139	38 [0.25] (35–45)	160 [0.25] (150–170)	4.2	~30	>30	
(*R*)-**3**	169–171 (dec)	>300	>30, <100		*g*	*g*	
(*S*)-**3**	169–171 (dec)	>30, <100	>30, <100		*g*	*g*	
(*R*,*S*)-**4**	138–139	>100, <300	>300		>30	>30	
(*R*,*S*)-**5**	98–99 (dec)	>30, <100	>30, <100		>30	>30	
(*R*)-**6**	172–173	24 [0.5] (21–27)	47 [0.25] (43–50)	2.0	4.2 [4] (2.4–8.0)	>250	>59
(*S*)-**6**	171–172	>100, <300	>30, <100		>180	>30	
(*R*)-**7**	161–163	74 [1] (61–85)	120 [0.25] (93–170)	1.6	28 [4] (19–40)	>250	>9.0
(*S*)-**7**	162–163	>100, <300	>100, <300		>150	>150	
phenytoin[h]		9.5 [2] (8.1–10)	66 [2] (53–72)	6.9	30 [4] (22–39)	*i*	>100
phenobarbital[h]		22 [1] (15–23)	69 [0.5] (63–73)	3.2	9.1 [5] (7.6–12)	61 [0.5] (44–96)	6.7
valproate[h]		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] The compounds were administered intraperitoneally. ED_{50} and TD_{50} values are in mg/kg. Numbers in parentheses are 95% confidence intervals. The dose effect data was obtained at the "time of peak effect" (indicated in hours in the brackets). [b] The compounds were administered orally. [c] Melting points (°C) are uncorrected. [d] MES = maximal electroshock seizure test. [e] Tox = neurologic toxicity determined from rotorod test. [f] PI = protective index (TD_{50}/ED_{50}). [g] Not determined. [h] Reference 31. [i] No ataxia observed up to 3000 mg/kg.

3. Chemical Reactivity. A key criterion for affinity labels **3**–**7** is that they readily, efficiently, and selectively react with biological nucleophiles. Accordingly, we have treated each compound with surrogate nucleophiles to test whether the reactive label functioned as designed and to predict the reaction conditions necessary for irreversible inactivation of the receptor site(s). We chose to use ethanethiol, ethylamine, and imidazole as mimics for cysteine-, lysine-, and histidine-containing peptides, respectively. The affinity labels were treated with these nucleophiles at room temperature (0.5–20 h) in aqueous buffer solutions (0.1 M KH_2PO_4/NaOH, pH 7.4), and the reaction was monitored by TLC. For those compounds (**3**, **6**, **7**) for which the two enantiomers had been individually prepared, we chose one of the stereoisomers for evaluation. Probes **3**, **6**, and **7** reacted with ethanethiol and ethylamine. For **5**, we used only ethylamine. In each case, the products (**28**–**34**) were isolated and identified (see Supporting Information). For **4**, we observed no reaction with any of the test nucleophiles, and we did not detect any product formation when **3** and **5**–**7** were treated with imidazole. We found that **3**–**7** were moderately stable in hydroxylic solvents (aqueous KH_2PO_4/KOH buffer, pH 7.4) with approximate half-lives of 2 h for **5** and >1 day for **3**, **4**, **6**, and **7**.





4. Pharmacological Evaluation. All the affinity labels (Table 1) were tested for anticonvulsant activity using the procedure described by Stables and Kupferberg.[4] We wished to learn if their in vivo pharmacological profile followed the general profile previously observed for FAAs (**1**).[1] Moreover, we anticipated that whole animal pharmacological studies would provide preliminary information concerning their utility as affinity labels. First, we expected that useful agents would likely display significant anticonvulsant activities in the MES-induced seizure test upon administration to mice (ip) and rats (po). Second, we hypothesized that if the affinity label irreversibly modified the receptor site(s), then the duration of action of the affinity label may exceed that of the reference FAA.[8] We understood that both of these experimental predictions hinged upon the assumption that the affinity labels survived administration and passage to the receptor site. The results were compared with findings previously reported for **2**,[1j] **9**,[17] and the proven antiepileptic agents phenytoin, phenobarbital, and valproate.[27]

The FAA affinity labels were administrated intraperitoneally (ip) to mice and orally (po) to rats. Table 1 lists the results obtained from qualitative testing in mice (ip) along with quantitative mice (ip) and rat (po) evaluations. We include in this table the MES ED_{50} values required to prevent tonic extension of the limbs and the median neurologically impairing dose (TD_{50}) values using the rotorod test.[28] Finally, the protective

index (PI = TD_{50}/(MES ED_{50})) for these adducts, when appropriate, is shown.

4.1. Group I Affinity Label Candidates. Inspection of the composite data in Table 1 revealed that group 1 (**3**, **4**) affinity label candidates displayed moderate or poor anticonvulsant activity in the MES-induced seizure test. The estimated ED_{50} value for these compounds was >30 mg/kg in mice (ip) and rats (po). The low activity observed for **3** and **4** was in contrast to the potent activity reported for **2**.[1j] Even more surprising was the difference in the anticonvulsant activities (mice, ip) for (*R*)-**3** (ED_{50} > 300 mg/kg) and (*S*)-**3** (30 mg/kg < ED_{50} < 100 mg/kg). This finding is opposite the stereochemical pattern learned in our SAR studies for FAAs.[1c−e,g,j]

4.2. Group II Affinity Label Candidates. Compound (*R*,*S*)-**5** displayed moderate protection against MES-induced seizures in mice (ip) (30 mg/kg < ED_{50} < 100 mg/kg). Significantly, (*R*,*S*)-**5** showed only modest loss of anticonvulsant activity compared with the reference anticonvulsant FAA (*R*,*S*)-**9** (ED_{50} = 38 mg/kg). Further, (*R*,*S*)-**5** showed significant inhibitory activity at 4 h (mice, ip). By comparison, the "time of peak effect" (TPE) for (*R*,*S*)-**9** was 0.25 h. Additional whole-animal pharmacological testing was not conducted because of the high neurological toxicity observed in mice (30 mg/kg < TD_{50} < 100 mg/kg) and the death of both test animals at the 300 mg/kg dose.

4.3. Group III Affinity Label Candidates. Evaluation of the group 3 affinity labels **6** and **7** showed that placement of an isothiocyanate moiety at either the 4′- or the 3′-phenyl sites, respectively, within the benzyl unit provided compounds effective for the control of MES-induced seizures in mice (ip). We found, of note, that the (*R*)-stereoisomer in both series was more potent than the (*S*)-isomer and that the activity of (*R*)-**6** (ED_{50} = 24 mg/kg) matched that of phenobarbital (ED_{50} = 22 mg/kg).

The pharmacological activities of (*R*)-**6** and (*R*)-**7** warranted further evaluation. Table 1 lists the MES ED_{50}, TD_{50}, and PI values for these compounds as a result of oral administration (po) to rats. Significantly, the ED_{50} values for (*R*)-**6** (ED_{50} = 4.2 mg/kg) and (*R*)-**7** (ED_{50} = 28 mg/kg) compared favorably with phenytoin (ED_{50} = 30 mg/kg). The excellent activity of (*R*)-**6** coupled with its low neurological toxicity (>250 mg/kg) led to a PI value greater than 59. A distinguishing pharmacological feature for FAAs is their stereochemical differences in anticonvulsant activities.[1c−e,g,j] For **2**, the eudismic ratio[29] was >7.6 in rats. We observed similar high eudismic ratios for **6** and **7** with values exceeding 43 and 5, respectively.

The in vivo pharmacological data documented that (*R*)-**6** and (*R*)-**7** were the most effective anticonvulsants within our family of affinity labels. Significantly, we observed that the TPE for (*R*)-**6** and (*R*)-**7** was 4 h (rats, po), which was 8 times longer than that for (*R*)-**2** (0.5 h). Protective activity for (*R*)-**2** was highest at 30 min and then gradually diminished. Correspondingly, (*R*)-**6** showed maximal activity after 4 h with three out of four animals protected after 2 and 6 h at 13 mg/kg.[30] Similarly, we observed that (*R*)-**7** afforded full protection after 4 h at 30 mg/kg with 25% of the animals protected after 0.5 h (we do not have the length of full protection provided by this compound, since testing was ended after 4 h). When the dose was 20 mg/kg, 25% of the animals were still protected after 6 h. These results demonstrated that maximal anticonvulsant activity for (*R*)-**6** and (*R*)-**7** was achieved at a later time period than our lead compound (*R*)-**2** and that seizure protection was likely maintained for longer time periods. At least four hypotheses can be offered to account for these findings. First, (*R*)-**6** and (*R*)-**7** acted as affinity labels and covalently modified the putative receptor providing prolonged seizure protection. Second, the site and function of affinity label candidates (*R*)-**6** and (*R*)-**7** differed from those of (*R*)-**2** and other FAA. Third, (*R*)-**6** and (*R*)-**7** underwent oral bioactivation, metabolism, or both to a species that exhibits increased biological activity, and the TPE reflects this change. Finally, (*R*)-**6** and (*R*)-**7** have different absorption properties compared with (*R*)-**2**. Our data do not permit us to distinguish among these possibilities, but this aspect of the pharmacological data warrants future attention.

FAA constitute a novel class of anticovulsant agents whose mode of action remains unknown. Prior to this study, we reported the anticonvulsant activities of four pair of stereoisomers.[1c−e,g,j] In each case, the (*R*)-enantiomer was more potent (10× to 22×) than the (S)-isomer. This finding led to our suggestion that FAA function, in part, was associated with stereoselective binding to a receptor or enzyme.[1d] Other factors may account for these differences, and so we tested all the FAA affinity labels and (*R*)- and (*S*)-**2** against receptors and channels known to be involved in seizure processes. We chose $GABA_A$ (agonist), $GABA_A$ (bzp), NMDA (PCP), NMDA (MK-801), Na^+ (type 2), and Ca^{2+} (type L) sites. The tests were conducted through the auspices of the National Institute of Mental Health (NIMH) Psychoactive Drug Screening Program. None of the affinity labels or (*R*)- and (*S*)-**2** displaced the radioligand in these receptor assays when tested at 10 μM concentrations. These findings indicate that the receptors are probably not involved in the mechanism of FAA action.

Conclusions

We have prepared three FAA affinity labels ((*R*,*S*)-**5**, (*R*)-**6**, and (*R*)-**7**) that exhibit significant anticonvulsant activities in animal models of MES-induced seizures. The three compounds readily reacted with either sulfur- or nitrogen-containing nucleophiles and underwent a slow change in hydroxylic solvents. For **6** and **7**, we demonstrated that the principal anticonvulsant activity resided in the (*R*)-stereoisomer. The chiral specificity for **5** anticonvulsant activity has not been determined, but both enantiomers should be accessible from commercial materials using the methodology outlined in Scheme 3. The FAA and (*R*)- and (*S*)-**2** were tested at receptors previously identified to be important for controlling seizure spread using in vitro radioligand binding assays. No significant binding was observed for any of the test compounds, a finding in line with the preclinical pharmacological profile that showed **1** differed from established antiepileptic agents.[7] Mechanistic studies concerning FAA function are underway in which FAA and affinity labels **4**−**7** serve as important test substrates.

Experimental Section

General Methods. Melting points were determined in open capillary tubes using a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (IR) were run on an ATI Mattson Genesis FT-IR spectrometer. Absorption values are expressed in wavenumbers (cm^{-1}). Optical rotations were obtained on Perkin-Elmer 241 MC and Jasco P-1030 polarimeters at the sodium D line (589 nm) using a 1 dm path length cell. Nuclear magnetic resonance spectra were measured at 300 MHz for ^1H NMR and at 75 MHz for ^{13}C NMR either on General Electric QE-300 NMR or Varian Gemini 2000 spectrometers. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane. Low-resolution mass spectra (CI+) were obtained with a Varian MAT CH-5 spectrometer by Dr. M. Moini at the University of Texas—Austin. The high-resolution chemical ionization mass spectrum was performed on a Finnigan MAT TSQ-70 by Dr. M. Moini at the University of Texas—Austin. Microanalyses were provided by Atlantic Microlab, Inc. (Norcross, GA). Analytical thin-layer chromatography (TLC) and preparative TLC (PTLC) were performed on precoated silica gel slides (20 cm × 20 cm; Sigma Z12272-6). All column chromatography separations were performed on Merck silica gel (SiO_2) (grade 9385, 230−400 mesh, 60 Å). CH_2Cl_2 was distilled from CaH_2, and THF was distilled from blue sodium benzophenone ketyl. Yields reported are for purified products and were not optimized.

General Procedure for the Preparation *N*-Benzylamide Amino Acids Derivatives Using Mixed-Anhydride Coupling (MAC) (Method A).[1j,20] A dry THF solution of the carboxylic acid (∼0.5−2.0 M) was cooled to −78 °C under Ar, and 4-methylmorpholine (NMM) (1.1−2.2 equiv) was added. After the solution was stirred (2 min), isobutyl chloroformate (IBCF) (1.1−1.25 equiv) was added, leading to the precipitation of a white solid. The reaction was allowed to proceed for an additional 2 min, and then benzylamine (1.1−1.25 equiv) was added at −78 °C. The reaction mixture was allowed to stir at room temperature (30 min to 3 h), and then the insoluble salts were filtered. The organic layer was concentrated in vacuo, and the product was purified by column chromatography on SiO_2 gel.

(*S*)-*N*-Benzyl-2-*N*-(benzyloxycarbonyl)amino-3-hydroxypropionamide ((*S*)-12). Utilizing method A, (*S*)-**11**[19b,c] (2.00 g, 8.4 mmol), NMM (1.4 mL, 12.7 mmol), IBCF (1.4 mL, 10.8 mmol), and benzylamine (1.1 mL, 11.6 mmol) gave crude (*S*)-**12**. The product was purified by column chromatography (SiO_2; 1:9 MeOH/$CHCl_3$) to obtain 1.97 g (72%) of pure (*S*)-**12** as a white solid: mp 148−149.5 °C; $[\alpha]^{23}_D$ −5.4° (*c* 1.04, MeOH); R_f = 0.51 (1:9 MeOH/$CHCl_3$); IR (KBr) 3294, 1689, 1645, 1535 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.53−3.63 (m, C**H₂**OH), 4.03−4.10 (m, C**H**), 4.27 (d, *J* = 5.7 Hz, C**H₂**NH), 4.88 (t, *J* = 5.1 Hz, O**H**), 5.02 (s, C**H₂**OC(O)), 7.17−7.35 (m, 10 Ph**H** and N**H**C(O)O), 8.41 (t, *J* = 5.7 Hz, N**H**C(O)); ^{13}C NMR (DMSO-d_6) δ 42.0 (**C**H_2NH), 57.3 (**C**H), 61.8 (**C**H_2OH), 65.5 (O**C**H_2Ph), 126.6, 127.0, 127.7, 128.1, 128.3, 136.9, 139.3 (2 **C₆**H_5), 155.9 (**C**(O)O), 170.1 (**C**(O)NH); MS (+CI) *m*/*z* (rel intensity) 330 (19), 329 (M^+ + 1, 87), 285 (19), 221 (100), 203 (13), 191 (18); M_r (+CI) 329.149 18 [M^+ + 1] (calcd for $C_{18}H_{21}N_2O_4$ 329.150 13). Anal. ($C_{18}H_{20}N_2O_4$) C, H, N.

General Procedure for the Preparation of 3-Methoxy-2-amidopropionamide Derivatives (Method B). To a CH_3CN solution of alcohol (∼0.05−0.1 M) was successively added Ag_2O (5 equiv) and MeI (10 equiv) at room temperature. The reaction mixture was maintained at room temperature (1−3 days) and filtered, and the solvent was evaporated in vacuo. The residue was purified by column chromatography on SiO_2.

(*S*)-*N*-Benzyl-2-*N*-(benzyloxycarbonyl)amino-3-methoxypropionamide ((*S*)-13). Utilizing method B, (*S*)-**12** (3.96 g, 12.1 mmol), Ag_2O (14.0 g, 60 mmol), and MeI (7.5 mL, 0.12 mol) gave crude (*S*)-**13** after 3 days. The product was purified by column chromatography (SiO_2; 1:9 MeOH/$CHCl_3$) to obtain 3.86 g (94%) of (*S*)-**13** as a white solid: mp 130−132 °C; $[\alpha]^{24}_D$ −3.3° (*c* 1.1, MeOH); R_f = 0.31 (1:1 hexanes/EtOAc); IR (KBr) 3295, 3059, 3030, 2880, 1688, 1641, 1541 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.34 (s, OC**H₃**), 3.49 (dd, *J* = 6.6, 9.2 Hz, C**H**H′OCH₃), 3.84 (dd, *J* = 3.9, 9.2 Hz, CH**H′**OCH₃), 4.31−4.36 (m, C**H**), 4.46 (d, *J* = 5.7 Hz, C**H₂**NH), 5.10 (s, C**H₂**OC(O)), 5.65−5.75 (m, N**H**), 6.68−6.75 (m, N**H**), 7.22−7.33 (m, 10 Ph**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*S*)-**13** gave only one signal for the ether methyl protons. ^{13}C NMR ($CDCl_3$) δ 43.7 (**C**H_2Ph), 54.6 (**C**H), 59.2 (O**C**H_3), 67.4 (**C**H_2-OC(O)), 72.2 (**C**H_2OCH_3), 127.5, 128.2, 128.3, 128.6, 128.8, 136.1, 138.0 (2 **C₆**H_5), 156.1 (**C**(O)O), 169.9 (**C**(O)NH); MS (+CI) *m*/*z* (rel intensity) 344 (20), 343 (M^+ + 1, 100), 299 (23); M_r (+CI) 343.166 41 [M^+ + 1] (calcd for $C_{19}H_{23}N_2O_4$ 343.165 78). Anal. ($C_{19}H_{22}N_2O_4$·0.2H_2O) C, H, N.

(*S*)-*N*-Benzyl-2-amino-3-methoxypropionamide ((*S*)-10). A methanolic solution (100 mL) of (*S*)-**13** (3.85 g, 11.2 mmol) was hydrogenated (1 atm) in the presence of 10% Pd/C (0.60 g) at room temperature (4 h). The mixture was filtered through a bed of Celite, and the clear filtrate was evaporated in vacuo. Purification of the product by column chromatography (SiO_2; 1:9 MeOH/$CHCl_3$) gave (*S*)-**10** (2.15 g, 91%) as a pale-yellow oil: $[\alpha]^{23}_D$ +1.8° (*c* 0.8, MeOH); R_f = 0.33 (1:9 MeOH/$CHCl_3$); IR (liquid film) 3312, 3063, 2926, 2894, 2825, 1660, 1524 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.69 (br s, N**H₂**), 3.38 (s, OC**H₃**), 3.59−3.69 (m, C**H** and C**H₂**), 4.43 (dd, *J* = 6.0, 14.7 Hz, C**H**H′NH), 4.49 (dd, *J* = 6.0, 14.7 Hz, CH**H′**NH), 7.25−7.36 (m, 5 Ph**H**), 7.75−7.85 (m, N**H**); ^{13}C NMR ($CDCl_3$) δ 43.1 (**C**H_2NH), 54.9 (**C**H), 58.8 (O**C**H_3), 74.6 (**C**H_2OCH_3), 127.3, 127.5, 128.6, 138.4 (**C₆**H_5), 172.8 (**C**(O)NH); MS (+CI) *m*/*z* (rel intensity) 210 (13), 209 (M^+ + 1, 100); M_r (+CI) 209.129 34 [M^+ + 1] (calcd for $C_{11}H_{17}N_2O_2$ 209.129 00). Anal. ($C_{11}H_{16}N_2O_2$·0.22H_2O) C, H, N.

(*R*)-*N*-Benzyl-2-(2-bromo)acetamido-3-methoxypropionamide ((*R*)-3). A mixture of (*R*)-**10** (1.33 g, 6.39 mmol) in CH_2Cl_2 (70 mL), aqueous saturated $NaHCO_3$ (70 mL), and aqueous 2 N Na_2CO_3 (15 mL) was cooled to 5 °C and treated with α-bromoacetyl bromide (0.85 mL, 9.76 mmol). The reaction mixture was allowed to warm to room temperature, stirred (10 min), and then poured into H_2O (200 mL). The organic layer was removed, and the aqueous layer was washed with CH_2Cl_2 (2 × 50 mL). The combined CH_2Cl_2 extracts were washed with aqueous saturated $NaHCO_3$ (50 mL) and brine (50 mL). The solvent was evaporated in vacuo, and the residue recrystallized (acetone) to give 1.64 g (78%) of (*R*)-**3** as a white solid: mp 169−171 °C (dec); $[\alpha]^{23}_D$ +3.5° (*c* 1.5, DMSO); R_f = 0.55 (EtOAc); IR (KBr) 3284, 3075, 2953, 1634, 1551 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.40 (s, OC**H₃**), 3.46 (dd, *J* = 8.1, 9.0 Hz, C**H**H′OCH₃), 3.83 (dd, *J* = 4.1, 9.0 Hz, CH**H′**OCH₃), 3.88 (s, C**H₂**Br), 4.43−4.57 (m, C**H** and C**H₂**NH), 6.62−6.72 (m, N**H**), 7.25−7.37 (m, 5 Ph**H** and N**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**3** gave only one signal for the bromoacetyl methylene protons and one signal for the ether methyl protons. ^{13}C NMR ($CDCl_3$) δ 28.8 (**C**H_2Br), 43.9 (**C**H_2NH), 53.1 (**C**H), 59.4 (O**C**H_3), 71.5 (**C**H_2OCH_3), 127.7, 127.8, 128.9, 137.9 (**C₆**H_5), 166.1 (**C**(O)CH_2Br), 169.4 (CH**C**(O)); MS (+CI) *m*/*z* (rel intensity) 332 (16), 331 (86), 330 (21), 329 (M^+ + 1, 100), 251 (11); M_r (+CI) 329.048 54 [M^+ + 1] (calcd for $C_{13}H_{18}{}^{79}BrN_2O_3$ 329.050 08). Anal. ($C_{13}H_{17}BrN_2O_3$) C, H, N.

(*S*)-*N*-Benzyl-2-(2-bromo)acetamido-3-methoxypropionamide ((*S*)-3). Using (*S*)-**10** (1.85 g, 8.89 mmol), α-bromoacetyl bromide (1.3 mL, 14.8 mmol), and the preceding procedure gave after recrystallization (acetone) 2.75 g (94%) of (*S*)-**3** as a white solid: mp 169−171 °C (dec); $[\alpha]^{23}_D$ −2.9° (*c* 2.04, DMSO); R_f = 0.55 (EtOAc); IR (KBr) 3287, 3074, 2953, 1633, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.39 (s, OC**H₃**), 3.47 (dd, *J* = 8.8, 8.8 Hz, C**H**H′OCH₃), 3.82 (dd, *J* = 4.1, 8.8 Hz, CH**H′**OCH₃), 3.87 (s, C**H₂**Br), 4.42−4.56 (m, C**H** and C**H₂**NH), 6.65−6.78 (m, N**H**), 7.25−7.37 (m, 5 Ph**H** and N**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*S*)-**3** gave only one signal for the bromoacetyl methylene protons and one signal for the ether methyl protons. Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**3** and (*S*)-**3** (1:1 ratio) gave two signals for the bromoacetyl methylene protons (δ 3.85 and 3.86) and two signals for the ether methyl protons (δ 3.36 and 3.37). ^{13}C NMR (DMSO-d_6) δ 29.4 (**C**H_2Br), 42.0

(**C**H$_2$NH), 53.0 (**C**H), 58.2 (O**C**H$_3$), 71.9 (**C**H$_2$OCH$_3$), 126.6, 126.9, 128.1, 139.0 (**C$_6$**H$_5$), 166.0 (**C**(O)CH$_2$Br), 168.9 (CH**C**(O)). The structural assignments were in agreement with the HETCOR experiment. MS (+CI) *m/z* (rel intensity) 332 (12), 331 (74), 330 (17), 329 (M$^+$ + 1, 100); M_r (+CI) 329.049 24 [M$^+$ + 1] (calcd for $C_{13}H_{18}{}^{79}BrN_2O_3$ 329.050 08). Anal. ($C_{13}H_{17}BrN_2O_3 \cdot 0.2C_3H_6O$)) C, H, N.

(*R,S*)-2-Acetamido-6-*N*-(benzyloxycarbonyl)aminohexanoic Acid ((*R,S*)-15).[22b] (*R,S*)-6-*N*-(Benzyloxycarbonyl)-DL-lysine ((*R,S*)-**14**) (3.17 g, 11.3 mmol) was dissolved in aqueous 0.5 N NaOH (28 mL) and cooled in an ice bath, and Ac_2O (1.52 mL, 15.8 mmol) and TEA (3.2 mL, 32.2 mmol) were added in two portions over 30 min. The solution was stirred at 0 °C (1 h) and then at room temperature (1 h). The reaction solution was washed with Et_2O (15 mL), and the layers were separated. The pH of the aqueous layer was adjusted to 2 with an aqueous 3 N HCl solution, and then the layer was extracted with EtOAc (100 mL). The organic layer was washed with brine (20 mL), dried (Na_2SO_4), and evaporated in vacuo to give 3.65 g (99%) of (*R,S*)-**15** as a white solid: mp 119−120 °C (lit.[22b] 115−116 °C); R_f = 0.22 (95:4:1 $CHCl_3$/MeOH/AcOH); ^{1}H NMR (CD_3OD) δ 1.38−1.46 (m, C(4)**H$_2$**), 1.48−1.55 (m, C(5)**H$_2$**), 1.63−1.76 (m, C(3)**H**H′), 1.79−1.91 (m, C(3)H**H′**), 1.98 (s, C**H$_3$**C(O)), 3.12 (t, *J* = 6.9 Hz, C(6)**H$_2$**), 4.34 (dd, *J* = 4.9, 8.8 Hz, C**H**), 5.06 (s, C**H$_2$**OC(O)), 7.27−7.35 (m, 5 Ph**H**); ^{13}C NMR (CD_3OD) δ 22.4 (**C**H$_3$C(O)), 24.1 (**C**(4)H$_2$), 30.5 (**C**(5)H$_2$), 31.3 (**C**(3)H$_2$), 41.6 (**C**(6)H$_2$), 53.7 (**C**H), 67.4 (O**C**H$_2$Ph), 128.8, 129.0, 129.5, 138.5 (**C$_6$**H$_5$), 159.0 (**C**(O)O), 173.4, 175.5 (2 **C**(O)).

(*R,S*)-*N*-Benzyl-2-acetamido-6-(benzyloxycarbonyl)aminohexanamide ((*R,S*)-16). To a dry DMF solution (30 mL) of (*R,S*)-**15** (2.06 g, 6.39 mmol) were successively added under Ar EDCI (1.47 g, 7.7 mmol), HOBt (951 mg, 7.0 mmol), and NMM (700 μL, 7.7 mmol). After the mixture was stirred (5 min), benzylamine (840 μL, 7.7 mmol) was added dropwise and the reaction mixture was stirred at room temperature (12 h). The mixture was diluted with 1 M HCl and extracted with EtOAc (2 × 50 mL). The organic layers were combined, washed with brine (50 mL), and concentrated in vacuo. The product was purified by column chromatography (SiO_2; 1:19 MeOH/$CHCl_3$) and recrystallized (EtOH) to obtain 2.35 g (89%) of pure (*R,S*)-**16** as a white solid: mp 157−158 °C; R_f = 0.31 (1:19 MeOH/$CHCl_3$); IR (KBr) 3287, 3063, 3032, 2923, 1688, 1632, 1553 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.33−1.43 (m, C(4)**H$_2$**), 1.46−1.52 (m, C(5)**H$_2$**), 1.60−1.70 (m, C(3)**H**H′), 1.73−1.85 (m, C(3)H**H′**), 1.98 (s, C**H$_3$**C(O)), 3.10 (t, *J* = 6.9 Hz, C(6)**H$_2$**), 4.30 (dd, *J* = 5.7, 8.4 Hz, C**H**), 4.37 (s, C**H$_2$**Ph), 5.06 (s, C**H$_2$**OC(O)), 7.20−7.36 (m, 10 Ph**H**). The structural assignments were in agreement with the ^{1}H−^{1}H COSY experiment. ^{13}C NMR (CD_3OD) δ 22.4 (**C**H$_3$C(O)), 24.1 (**C**(4)H$_2$), 30.5 (**C**(5)H$_2$), 32.8 (**C**(3)H$_2$), 41.5 (**C**(6)H$_2$), 44.0 (NH**C**H$_2$Ph), 55.0 (**C**H), 67.3 (O**C**H$_2$Ph), 128.2, 128.5, 128.8, 128.9, 129.4, 129.5, 138.4, 139.9 (2 **C$_6$**H$_5$), 159.0 (**C**(O)O), 173.4, 174.4 (2 **C**(O)). The ^{13}C NMR structural assignments were in agreement with the DEPT experiment. MS (+CI) *m/z* (rel intensity) 413 (24), 412 (M$^+$ + 1, 100), 343 (22), 305 (29), 304 (15); M_r (+CI) 412.223 01 [M$^+$ + 1] (calcd for $C_{23}H_{30}N_3O_4$ 412.223 63). Anal. ($C_{23}H_{29}N_3O_4$) C, H, N.

(*R,S*)-*N*-Benzyl-2-acetamido-6-aminohexanamide ((*R,S*)-17). Utilizing a similar procedure employed for the synthesis of (*S*)-**10** and using (*R,S*)-**16** (2.10 g, 5.10 mmol) and a catalytic amount of 5% Pd/C gave, after filtration over a bed of Celite, (*R,S*)-**17** (1.41 g, 99%) as a pale-yellow oil: R_f = 0.01 (2:1 EtOAc/hexanes); IR (liquid film) 3987, 3064, 3033, 2934, 2962, 1650, 1553 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.29−1.55 (m, C(4)**H$_2$** and C(5)**H$_2$**), 1.60−1.71 (m, C(3)**H**H′), 1.72−1.86 (m, C(3)H**H′**), 1.99 (s, C**H$_3$**C(O)), 2.63 (3.04) (t, *J* = 7.1 (6.3) Hz, C(6)**H$_2$**), 4.33 (dd, *J* = 5.9, 8.6 Hz, C**H**), 4.38 (s, C**H$_2$**Ph), 7.20−7.33 (m, 5 Ph**H**). ^{1}H NMR analysis indicated the major and minor conformational isomers existed in a 90:10 ratio in CD_3OD, the number in parentheses corresponds to the value observed for the minor conformer; all other signals for the minor conformer are believed to overlap with those for the major conformer. ^{13}C NMR (CD_3OD) δ 22.7 (**C**H$_3$C(O)), 24.1 (**C**(4)H$_2$), 32.5, 32.9 (**C**(3)H$_2$ and **C**(5)H$_2$), 41.9 (**C**(6)H$_2$), 43.8 (**C**H$_2$Ph), 54.9 (**C**H), 128.1, 128.3, 129.4, 139.9 (**C$_6$**H$_5$), 173.0, 174.3 (2 **C**(O)); MS (+CI) *m/z* (rel intensity) 279 (15), 278 (M$^+$ + 1, 100); M_r (+CI) 278.186 30 [M$^+$ + 1] (calcd for $C_{15}H_{24}N_3O_2$ 278.186 85). Anal. ($C_{15}H_{23}N_3O_2 \cdot H_2O$) C, H, N.

(*R,S*)-*N*-Benzyl-2-acetamido-6-isothiocyanatohexanamide ((*R,S*)-5). A dry THF/DMF (2:1, 60 mL) solution of (*R,S*)-**17** (1.37 g, 4.94 mmol) was added (10 min) to a THF solution (25 mL) of DPT (1.15 g, 4.94 mmol). The reaction solution was stirred at room temperature (1 h), and then the solvent was evaporated in vacuo. The product was purified by column chromatography (SiO_2; 4:1 EtOAc/hexanes) to give 1.15 g (73%) of pure (*R,S*)-**5** as a white solid: mp 98−99 °C (dec); R_f = 0.36 (4:1 EtOAc/hexanes); IR (KBr) 3274, 3084, 2935, 2184, 2110, 1638, 1554 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27−1.45 (m, C(4)**H$_2$**), 1.54−1.80 (m, C(3)**H$_2$** and C(5)**H$_2$**), 1.89 (s, C**H$_3$**C(O)), 3.39 (t, *J* = 6.3 Hz, C(6)**H$_2$**), 4.28 (dd, *J* = 5.7, 14.8 Hz, C**H**H′Ph), 4.37 (dd, *J* = 5.5, 14.8 Hz, CH**H′**Ph), 4.59−4.67 (m, C**H**), 7.19−7.28 (m, 5 Ph**H**), 7.45 (d, *J* = 8.4 Hz, N**H**CH), 8.01 (app br t, *J* = 5.7 Hz, N**H**CH$_2$); ^{13}C NMR ($CDCl_3$) δ 22.6 (**C**(4)H$_2$), 22.8 (**C**H$_3$C(O)), 29.5 (**C**(5)H$_2$), 31.9 (**C**(3)H$_2$), 43.3 (**C**H$_2$Ph), 44.8 (**C**(6)H$_2$), 52.7 (**C**H), 127.3 (**C$_{4'}$**), 127.5, 128.6 (**C$_{2'}$** and **C$_{3'}$**), 130.2 (N**C**S), 138.1 (**C$_{1'}$**), 170.7, 172.1 (2 **C**(O)). The ^{1}H NMR and ^{13}C NMR structural assignments were in agreement with the HETCOR and DEPT experiments. MS (+CI) *m/z* (rel intensity) 321 (21), 320 (M$^+$ + 1, 100); M_r (+CI) 320.142 00 [M$^+$ + 1] (calcd for $C_{16}H_{22}N_3O_2S$ 320.143 27). Anal. ($C_{16}H_{21}N_3O_2S$) C, H, N.

(*R*)-*N*-(4-Nitrobenzyl)-2-acetamido-3-hydroxypropionamide ((*R*)-19) and (*R*)-*N*-(4-Nitrobenzyl)-2-acetamido-3-acetoxypropionamide ((*R*)-20). Compounds (*R*)-**19** and (*R*)-**20** were prepared utilizing a procedure comparable to the synthesis of (*R*)-**3** and using D-serine ((*R*)-**18**) (10.00 g, 95 mmol), Ac_2O (9.9 mL, 0.11 mol), NMM (20.9 mL, 0.19 mol), IBCF (24.6 mL, 0.19 mol), and 4-nitrobenzylamine hydrochloride (25.00 g, 0.13 mol). The 4-nitrobenzylamine hydrochloride was solubilized in DMF (50 mL) upon addition of NMM (14.3 mL, 0.13 mol), and then the generated free amine was added dropwise by cannulation to the mixed anhydride at −78 °C. After evaporation of the solvent, the crude product was purified by column chromatography (SiO_2; EtOAc) to yield 9.12 g (34%) of (*R*)-**19** and 2.64 g (9%) of (*R*)-**20** as white solids.

(*R*)-19: mp 164−166 °C; $[\alpha]^{27}_D$ +8.14° (*c* 1.03, DMSO); R_f = 0.15 (1:9 MeOH/$CHCl_3$); IR (KBr) 3370, 3352, 3281, 2954, 1657, 1638, 1546, 1520 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, C**H$_3$**C(O)), 3.21 (dd, *J* = 3.4, 9.7 Hz, O**H**), 3.58−3.68 (m, C**H**H′OH), 4.23 (dt, *J* = 3.4, 11.4 Hz, CH**H′**OH), 4.22−4.46 (m, C**H**), 4.53 (d, *J* = 6.3 Hz, NHC**H$_2$**), 6.66 (d, *J* = 6.6 Hz, N**H**CH), 7.42 (d, *J* = 8.9 Hz, 2 C$_{2'}$**H**), 7.45−7.51 (m, CH$_2$N**H**), 8.20 (d, *J* = 8.9 Hz, 2 C$_{3'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**19** gave only one signal for the acetyl methyl protons. ^{13}C NMR (DMSO-d_6) δ 22.7 (**C**H$_3$C(O)), 41.7 (**C**H$_2$NH), 55.4 (**C**H), 61.6 (**C**H$_2$OH), 123.3 (**2C$_{3'}$**), 128.0 (**2C$_{2'}$**), 146.4, 147.7 (**C$_{1'}$** and **C$_{4'}$**), 169.5, 170.7 (2 **C**(O)); MS (+CI) *m/z* (rel intensity) 283 (12), 282 (M$^+$ + 1, 100), 264 (11), 252 (10), 130 (29), 119 (11), 132 (32); M_r (+CI) 282.108 41 [M$^+$ + 1] (calcd for $C_{12}H_{16}N_3O_5$ 282.109 00). Anal. ($C_{12}H_{15}N_3O_5$) C, H, N.

(*R*)-20: mp 177−180 °C (dec); $[\alpha]^{26}_D$ +13.5° (*c* 1.0, DMSO); R_f = 0.30 (1:9 MeOH/$CHCl_3$); IR (KBr) 3285, 3114, 1733, 1647, 1556, 1515 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.02, 2.06 (s, 2 C**H$_3$**C(O)), 4.29 (dd, *J* = 5.0, 11.6 Hz, C**H**H′OAc), 4.46 (dd, *J* = 5.9, 11.6 Hz, CH**H′**OAc), 4.50 (dd, *J* = 5.5, 15.2 Hz, C**H**H′NH), 4.57 (dd, *J* = 5.8, 15.2 Hz, CH**H′**NH), 4.76−4.80 (m, C**H**), 6.56 (d, *J* = 7.5 Hz, N**H**CH), 7.32−7.37 (t, *J* = 5.3 Hz, N**H**CH$_2$), 7.42 (d, *J* = 8.7 Hz, 2 C$_{2'}$**H**), 8.17 (d, *J* = 8.7 Hz, 2 C$_{3'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**20** gave only one signal for each of the two acetyl methyl protons. ^{13}C NMR (DMSO-d_6) δ 20.6, 22.5 (2 **C**H$_3$C(O)), 41.7 (**C**H$_2$NH), 51.7 (**C**H), 63.5 (**C**H$_2$OAc), 123.4, 127.9, 128.0, 146.4, 147.4 (**C$_6$**H$_4$), 169.3, 169.6, 170.0 (3 **C**(O)); MS (+CI) *m/z* (rel intensity) 325 (18), 324 (M$^+$ + 1, 100); M_r (+CI) 324.119 75 [M$^+$ + 1] (calcd for $C_{14}H_{18}N_3O_6$ 324.119 56). Anal. ($C_{14}H_{17}N_3O_6$) C, H, N.

(*S*)-*N*-(4-Nitrobenzyl)-2-acetamido-3-hydroxypropionamide ((*S*)-19) and (*S*)-*N*-(4-Nitrobenzyl)-2-acetamido-3-

acetoxypropionamide ((*S*)-20). Compound (*S*)-**19** and (*S*)-**20** were prepared utilizing the preceding procedure and L-serine ((*S*)-**18**) (4.50 g, 42.8 mmol), Ac_2O (4.6 mL, 48 mmol), NMM (5.9 mL, 53.5 mmol), IBCF (6.90 mL, 53.5 mmol), and 4-nitrobenzylamine hydrochloride (6.04 g, 54.9 mmol). The 4-nitrobenzylamine hydrochloride was solubilized in DMF (30 mL) upon addition of NMM (6.0 mL, 54.9 mmol). After evaporation of the solvent, the crude product was purified by column chromatography (SiO_2; EtOAc) and then recrystallized (EtOH) to yield 5.48 g (46%) of (*S*)-**19** and 1.19 g (9%) of (*S*)-**20** as white solids.

(*S*)-19: mp 162−164 °C; $[\alpha]^{27}_{D}$ −8.0° (*c* 1.07, DMSO); R_f = 0.15 (1:9 MeOH/$CHCl_3$); IR (KBr) 3371, 3352, 2955, 2939, 1662, 1638, 1547, 1519 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, C**H$_3$**C(O)), 3.16 (dd, J = 3.5, 9.6 Hz, O**H**), 3.59−3.68 (m, C**H**H′OH), 4.23 (dt, J = 3.5, 11.4 Hz, CH**H′**OH), 4.14−4.46 (m, C**H**), 4.53 (d, J = 6.0 Hz, C**H$_2$**NH), 6.65 (d, J = 6.9 Hz, N**H**CH), 7.41 (d, J = 8.7 Hz, 2 C$_{2'}$**H**), 7.45−7.50 (m, CH$_2$N**H**), 8.20 (d, J = 8.7 Hz, 2 C$_{3'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*S*)-**19** gave only one signal for the acetyl methyl proton. Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**19** and (*S*)-**19** (1:1 ratio) gave two signals for the acetyl methyl protons (δ 2.07 and 2.08). ^{13}C NMR (DMSO-d_6) δ 22.6 (**C**H$_3$C(O)), 41.7 (**C**H$_2$NH), 55.3 (**C**H), 61.6 (**C**H$_2$-OH), 123.3 (**2C$_{3'}$**), 128.0 (**2C$_{2'}$**), 146.3, 147.6 (**C$_{1'}$** and **C$_{4'}$**), 169.5, 170.6 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 283 (11), 282 (M$^+$ + 1, 100), 264 (33), 246 (22); M_r (+CI) 282.109 60 [M$^+$ + 1] (calcd for $C_{12}H_{16}N_3O_5$ 282.109 00). Anal. ($C_{21}H_{15}N_3O_5 \cdot 0.46H_2O$) C, H, N.

(*S*)-20: mp 179−180 °C (dec); $[\alpha]^{26}_{D}$ −13.2° (*c* 1.03, DMSO); R_f = 0.30 (1:9 MeOH/$CHCl_3$); IR (KBr) 3286, 3113, 2954, 1736, 1643, 1558, 1516 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.02, 2.05 (s, 2 C**H$_3$**C(O)), 4.29 (dd, J = 5.1, 11.4 Hz, C**H**H′OAc), 4.46 (dd, J = 6.0, 11.4 Hz, CH**H′**OAc), 4.49 (dd, J = 6.1, 16.1 Hz, C**H**H′NH), 4.55 (dd, J = 6.4, 16.1 Hz, CH**H′**NH), 4.76−4.83 (m, C**H**), 6.56 (d, J = 7.2 Hz, N**H**CH), 7.34−7.38 (m, N**H**CH$_2$), 7.42 (d, J = 8.7 Hz, 2 C$_{2'}$**H**), 8.17 (d, J = 8.7 Hz, 2 C$_{3'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*S*)-**20** gave only one signal for each of the two acetyl methyl protons. Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**20** and (*S*)-**20** (1:1 ratio) gave two signals for each acetyl methyl proton (δ 1.94, 1.98, 2.00, and 2.01). ^{13}C NMR (DMSO-d_6) δ 20.6, 22.5 (2 **C**H$_3$C(O)), 41.7 (**C**H$_2$NH), 51.7 (**C**H), 63.5 (**C**H$_2$OAc), 123.4 (**2C$_{3'}$**), 128.0 (**2C$_{2'}$**), 146.4, 147.4 (**C$_{1'}$** and **C$_{4'}$**), 169.3, 169.7, 170.1 (3 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 324 (M$^+$ + 1, 100), 282 (11), 264 (49), 172 (12), 106 (20); M_r (+CI) 324.120 00 [M$^+$ + 1] (calcd for $C_{14}H_{18}N_3O_6$ 324.119 56). Anal. ($C_{14}H_{17}N_3O_6$) C, H, N.

(*R*)-*N*-(4-Nitrobenzyl)-2-acetamido-3-methoxypropionamide ((*R*)-21). Utilizing method B, (*R*)-**19** (2.65 g, 9.40 mmol), Ag_2O (10.90 g, 47.0 mmol), and MeI (5.85 mL, 94.0 mmol) gave 2.55 g (92%) of pure (*R*)-**21** as a white solid after purification by column chromatography (SiO_2; EtOAc): mp 163−164 °C; $[\alpha]^{27}_{D}$ +9.6° (*c* 1.35, MeOH); R_f = 0.32 (1:9 MeOH/$CHCl_3$); IR (KBr) 3275, 3096, 2939, 1639, 1544, 1517 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.04 (s, C**H$_3$**C(O)), 3.40 (s, OC**H$_3$**), 3.49 (dd, J = 7.1, 9.2 Hz, C**H**H′OCH$_3$), 3.82 (dd, J = 4.1, 9.2 Hz, CH**H′**OCH$_3$), 4.48−4.58 (m, C**H$_2$**NH), 4.59−4.66 (m, C**H**), 6.55 (d, J = 6.6 Hz, N**H**CH), 7.24 (t, J = 5.4 Hz, N**H**CH$_2$), 7.42 (d, J = 8.7 Hz, 2 C$_{2'}$**H**), 8.17 (d, J = 8.7 Hz, 2 C$_{3'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**21** gave only one signal for the acetyl methyl and one signal for the ether methyl protons. ^{13}C NMR ($CDCl_3$) δ 23.3 (**C**H$_3$C(O)), 42.9 (**C**H$_2$NH), 52.8 (**C**H), 59.3 (O**C**H$_3$), 71.7 (**C**H$_2$OCH$_3$), 124.0 (**2C$_{3'}$**), 128.0 (**2C$_{2'}$**), 145.8, 147.3 (**C$_{1'}$** and **C$_{4'}$**), 170.6, 170.7 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 297 (16), 296 (M$^+$ + 1, 100); M_r (+CI) 296.124 34 [M$^+$ + 1] (calcd for $C_{13}H_{18}N_3O_5$ 296.124 65). Anal. ($C_{13}H_{17}N_3O_5$) C, H, N.

(*S*)-*N*-(4-Nitrobenzyl)-2-acetamido-3-methoxypropionamide ((*S*)-21). Utilizing method B, (*S*)-**19** (2.50 g, 8.89 mmol), Ag_2O (10.30 g, 44.4 mmol), and MeI (5.50 mL, 89.0 mmol) gave 2.46 g (94%) of pure (*S*)-**21** as a white solid after purification by column chromatography (SiO_2; 1:17 MeOH/$CHCl_3$): mp 163−164 °C; $[\alpha]^{27}_{D}$ −10.0° (*c* 1.35, MeOH); R_f = 0.32 (1:9 MeOH/$CHCl_3$); IR (KBr) 3278, 3082, 2935, 1639, 1547, 1516 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05 (s, C**H$_3$**C(O)), 3.41 (s, OC**H$_3$**), 3.47 (dd, J = 7.1, 9.3 Hz, C**H**H′OCH$_3$), 3.84 (dd, J = 3.9, 9.3 Hz, CH**H′**OCH$_3$), 4.50−4.62 (m, C**H$_2$**NH and C**H**), 6.35−6.37 (m, N**H**CH), 6.96 (br s, N**H**CH$_2$), 7.42 (d, J = 8.7 Hz, 2 C$_{2'}$**H**), 8.19 (d, J = 8.7 Hz, 2 C$_{3'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*S*)-**21** gave only one signal for the acetyl methyl and one signal for the ether methyl protons. Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**21** and (*S*)-**21** (1:1 ratio) gave two signals for the acetyl methyl protons (δ 2.02 and 2.03) and two signals for the ether methyl protons (δ 3.34 and 3.38). ^{13}C NMR ($CDCl_3$) δ 23.4 (**C**H$_3$C(O)), 43.0 (**C**H$_2$NH), 52.8 (**C**H), 59.4 (O**C**H$_3$), 71.6 (**C**H$_2$OCH$_3$), 124.1 (**2C$_{3'}$**), 128.1 (**2C$_{2'}$**), 145.7, 147.6 (**C$_{1'}$** and **C$_{4'}$**), 170.6, 170.7 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 297 (16), 296 (M$^+$ + 1, 100), 254 (22); M_r (+CI) 296.124 97 [M$^+$ + 1] (calcd for $C_{13}H_{18}N_3O_5$ 296.124 65). Anal. ($C_{13}H_{17}N_3O_5$) C, H, N.

(*R*)-*N*-(4-Aminobenzyl)-2-acetamido-3-methoxypropionamide ((*R*)-22). A methanolic solution (200 mL) of (*R*)-**21** (2.50 g, 8.47 mmol) was hydrogenated (1 atm) in the presence of a catalytic amount of PtO_2 (200 mg) at room temperature (1.5 h). The catalyst was filtered over a bed of Celite, and the solvent was evaporated in vacuo to give 1.97 g (87%) of pure (*R*)-**22** as a white solid: mp >176 °C (dec); $[\alpha]^{25}_{D}$ −4.1° (*c* 1.21, DMSO); R_f = 0.39 (1:9 MeOH/$CHCl_3$); IR (KBr) 3435, 3356, 3236, 3071, 2926, 1678, 1642, 1519 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.03 (s, C**H$_3$**C(O)), 3.36 (s, OC**H$_3$**), 3.41 (dd, J = 7.2, 9.1 Hz, C**H**H′OCH$_3$), 3.65 (br s, N**H$_2$**), 3.79 (dd, J = 4.1, 9.1 Hz, CH**H′**OCH$_3$), 4.35 (d, J = 5.7 Hz, C**H$_2$**NH), 4.47−4.53 (m, C**H**), 6.43 (d, J = 6.0 Hz, N**H**CH), 6.59 (br s, N**H**CH$_2$), 6.65 (d, J = 8.4 Hz, 2 C$_{3'}$**H**), 7.05 (d, J = 8.4 Hz, 2 C$_{2'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**22** gave only one signal for the acetyl methyl protons and one signal for the ether methyl protons. ^{13}C NMR ($CDCl_3$) δ 23.4 (**C**H$_3$C(O)), 43.4 (**C**H$_2$NH), 52.6 (**C**H), 59.2 (O**C**H$_3$), 71.9 (**C**H$_2$OCH$_3$), 115.4 (**2C$_{3'}$**), 127.8 (**C$_{1'}$**), 129.0 (**2C$_{2'}$**), 146.0 (**C$_{4'}$**), 169.9, 170.4 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 266 (M$^+$ + 1, 37), 106 (100); M_r (+CI) 265.141 46 [M$^+$] (calcd for $C_{13}H_{19}N_3O_3$ 265.142 64). Anal. ($C_{13}H_{19}N_3O_3$) C, H, N.

(*S*)-*N*-(4-Aminobenzyl)-2-acetamido-3-methoxypropionamide ((*S*)-22). Using the preceding procedure and using (*S*)-**21** (2.45 g, 8.30 mmol) and PtO_2 (~200 mg) gave 1.82 g (83%) of pure (*S*)-**22** as a pale-yellow solid after purification by column chromatography (SiO_2; 1:17 MeOH/$CHCl_3$): mp >176 °C (dec); $[\alpha]^{25}_{D}$ +4.3° (*c* 1.30, DMSO); R_f = 0.39 (1:9 MeOH/$CHCl_3$); IR (KBr) 3437, 3352, 3244, 3066, 2927, 1678, 1520 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.02 (s, C**H$_3$**C(O)), 3.37 (s, OC**H$_3$**), 3.41 (dd, J = 7.2, 9.2 Hz, C**H**H′OCH$_3$), 3.67 (br s, N**H$_2$**), 3.79 (dd, J = 3.9, 9.2 Hz, CH**H′**OCH$_3$), 4.34 (d, J = 5.4 Hz. NHC**H$_2$**), 4.47−4.54 (m, C**H**), 6.44 (d, J = 6.3 Hz, N**H**CH), 6.60−6.67 (m, N**H**CH$_2$ and 2 C$_{3'}$**H**), 7.05 (d, J = 8.7 Hz, 2 C$_{2'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*S*)-**22** gave only one signal for the acetyl methyl protons and one signal for the ether methyl protons. Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**22** and (*S*)-**22** (1:1 ratio) gave two signals for the acetyl methyl protons (δ 1.93 and 1.94) and two signals for the ether methyl protons (δ 3.24 and 3.28). ^{13}C NMR ($CDCl_3$) δ 23.5 (**C**H$_3$C(O)), 43.5 (**C**H$_2$-NH), 52.6 (**C**H), 59.3 (O**C**H$_3$), 71.9 (**C**H$_2$OCH$_3$), 115.4 (**2C$_{3'}$**), 127.8 (**C$_{1'}$**), 129.1 (**2C$_{2'}$**), 146.1 (**C$_{4'}$**), 169.9, 170.5 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 266 (M$^+$ + 1, 28), 106 (100); M_r (+CI) 266.149 54 [M$^+$ + 1] (calcd for $C_{13}H_{20}N_3O_3$ 266.150 47). Anal. ($C_{13}H_{19}N_3O_3 \cdot 0.18H_2O$) C, H, N.

(*R*)-*N*-(4-Isothiocyanatobenzyl)-2-acetamido-3-methoxypropionamide ((*R*)-6). To a dry THF solution (50 mL) of (*R*)-**22** (1.30 g, 4.9 mmol) was added dropwise a solution of DPT (1.73 g, 7.43 mmol) in THF (20 mL). The reaction mixture was stirred under Ar at room temperature (2 h). The solvent was removed in vacuo, and the product was purified by column chromatography (SiO_2; EtOAc) to give 1.14 g (76%) of pure (*R*)-**6** as a white solid: mp 172−173 °C; $[\alpha]^{23}_{D}$ +0.55° (*c* 2.56, acetone); R_f = 0.24 (1:9 acetone/EtOAc); IR (KBr) 3276, 3075, 2929, 2181, 2130, 1638, 1543 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05

(s, C**H3**C(O)), 3.40 (s, OC**H3**), 3.46 (dd, J = 7.7, 9.2 Hz, C**H**H′OCH$_3$), 3.82 (dd, J = 4.1, 9.2 Hz, CH**H′**OCH$_3$), 4.38–4.55 (m, C**H2**NH), 4.56–4.60 (m, C**H**), 6.50 (d, J = 5.4 Hz, N**H**CH), 6.95 (br s, N**H**CH$_2$), 7.19 (d, J = 8.6 Hz, 2 C$_{2'}$**H** or 2 C$_{3'}$**H**),), 7.26 (d, J = 8.6 Hz, 2 C$_{2'}$**H** or 2 C$_{3'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a CDCl$_3$ solution of (*R*)-**6** gave only one signal for the acetyl methyl protons and one signal for the ether methyl protons. ^{13}C NMR (CDCl$_3$) δ 23.4 (**C**H$_3$C(O)), 43.1 (**C**H$_2$NH), 52.7 (**C**H), 59.3 (O**C**H$_3$), 71.6 (**C**H$_2$OCH$_3$), 126.2 (**2C$_{3'}$**), 128.7 (**C$_{2'}$**), 130.6 (**C$_{4'}$**), 135.7 (N**C**S), 137.5 (**C$_{1'}$**), 170.3, 170.8 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 336 (14), 309 (16), 308 (M$^+$ + 1, 100), 148 (11), 144 (11); M_r (+CI) 308.106 03 [M$^+$ + 1] (calcd for $C_{14}H_{18}N_3O_3S$ 308.106 89). Anal. ($C_{14}H_{17}N_3O_3S$) C, H, N.

(*S*)-*N*-(4-Isothiocyanatobenzyl)-2-acetamido-3-methoxypropionamide ((*S*)-6). Using the preceding procedure, (*S*)-**22** (1.46 g, 5.50 mmol), and DPT (1.28 g, 5.50 mmol) gave 1.23 g (73%) of pure (*S*)-**6** as a white solid after purification by column chromatography (SiO$_2$; 3:17 acetone/EtOAc): mp 171–172 °C; $[\alpha]^{24}_{D}$ −0.76° (*c* 2.17, acetone); R_f = 0.24 (1:9 acetone/EtOAc); IR (KBr) 3274, 3070, 2927, 2183, 2125 (br), 1639, 1543, 1504 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.04 (s, C**H3**C(O)), 3.39 (s, OC**H3**), 3.44 (dd, J = 7.4, 9.2 Hz, C**H**H′OCH$_3$), 3.81 (dd, J = 4.1, 9.2 Hz, CH**H′**OCH$_3$), 4.42 (dd, J = 5.6, 15.2 Hz, C**H**H′Ar), 4.48 (dd, J = 6.1, 15.2 Hz, CH**H′**Ar), 4.51–4.57 (m, C**H**), 6.41 (d, J = 6.0 Hz, N**H**CH), 6.84 (br s, N**H**CH$_2$), 7.18 (d, J = 8.6 Hz, 2 C$_{2'}$**H** or 2 C$_{3'}$**H**), 7.24 (d, J = 8.6 Hz, 2 C$_{2'}$**H** or 2 C$_{3'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a CDCl$_3$ solution of (*S*)-**6** gave only one signal for the acetyl methyl protons and one signal for the ether methyl protons. Addition of excess (*R*)-(−)-mandelic acid to a CDCl$_3$ solution of (*R*)-**6** and (*S*)-**6** (1:1 ratio) gave two signals for the acetyl methyl protons (δ 1.99 and 2.00) and two signals for the ether methyl protons (δ 3.32 and 3.34). ^{13}C NMR (CDCl$_3$) δ 23.4 (**C**H$_3$C(O)), 43.1 (**C**H$_2$NH), 52.7 (**C**H), 59.3 (O**C**H$_3$), 71.7 (**C**H$_2$OCH$_3$), 126.2 (**2C$_{3'}$**), 128.7 (**2C$_{2'}$**), 130.6 (**C$_{4'}$**), 135.6 (N**C**S), 137.6 (**C$_{1'}$**), 170.3, 170.6 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 309 (17), 308 (M$^+$ + 1, 100), 247 (20); M_r (+CI) 308.106 82 [M$^+$ + 1] (calcd for $C_{14}H_{18}N_3O_3S$ 308.106 89). Anal. ($C_{14}H_{17}N_3O_3S$) C, H, N.

(*R*)-*N*-(3-Nitrobenzyl)-2-acetamido-3-hydroxypropionamide ((*R*)-23) and (*R*)-*N*-(3-Nitrobenzyl)-2-acetamido-3-acetoxypropionamide ((*R*)-24). Compounds (*R*)-**23** and (*R*)-**24** were prepared utilizing the procedure for the synthesis of (*R*)-**19** and (*R*)-**20**, respectively, and using D-serine ((*R*)-**18**) (17.80 g, 0.169 mol), Ac$_2$O (19.8 mL, 0.203 mol), NMM (23.2 mL, 0.21 mol), IBCF (27.4 mL, 0.21 mol), and 3-nitrobenzylamine hydrochloride (35.00 g, 0.19 mol). The 3-nitrobenzylamine hydrochloride was solubilized in DMF (100 mL) upon addition of NMM (20.4 mL, 0.19 mol). After evaporation of the solvent, the crude product was purified by column chromatography (SiO$_2$; EtOAc) to yield a mixture of 12.95 g (27%) of (*R*)-**23** and 4.36 g (8%) of (*R*)-**24** as white solids.

(*R*)-23: mp 137–140 °C; $[\alpha]^{27}_{D}$ +12.7° (*c* 1.45, DMSO); R_f = 0.20 (1:9 MeOH/CHCl$_3$); IR (KBr) 3312, 3271, 3079, 1646, 1635, 1553, 1528 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.10 (s, C**H3**C(O)), 3.26 (dd, J = 3.3, 9.6 Hz, O**H**), 3.60–3.69 (m, C**H**H′OH), 4.23 (br d, J = 11.4 Hz, CH**H′**OH), 4.41–4.47 (m, C**H**), 4.49 (dd, J = 6.3, 15.5 Hz, C**H**H′Ph), 4.60 (dd, J = 6.6, 15.5 Hz, CH**H′**Ph), 6.68–6.71 (m, N**H**CH), 7.49–7.61 (m, C$_{5'}$**H**, C$_{6'}$**H**, and N**H**CH$_2$), 8.09–8.15 (m, C$_{2'}$**H** and C$_{4'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a CDCl$_3$ solution of (*R*)-**23** gave only one signal for the acetyl methyl protons. ^{13}C NMR (DMSO-d_6) δ 22.6 (**C**H$_3$C(O)), 41.4 (**C**H$_2$NH), 55.3 (**C**H), 61.6 (**C**H$_2$OH), 121.5, 121.7 (**C$_{2'}$** and **C$_{4'}$**), 129.7 (**C$_{5'}$**), 133.7 (**C$_{6'}$**), 142.0 (**C$_{1'}$**), 147.8 (**C$_{3'}$**), 169.5, 170.6 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 282 (M$^+$ + 1, 100), 264 (13), 130 (64); M_r (+CI) 282.108 37 [M$^+$ + 1] (calcd for $C_{12}H_{16}N_3O_5$ 282.109 00). Anal. ($C_{12}H_{15}N_3O_5$) C, H, N.

(*R*)-24: mp 152–154 °C; $[\alpha]^{26}_{D}$ +14.6° (*c* 1.0, DMSO); R_f = 0.39 (1:9 MeOH/CHCl$_3$); IR (KBr) 3282, 3097, 2970, 1743, 1639, 1562, 1523 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.00, 2.04 (s, 2 C**H3**C-(O)), 4.28 (dd, J = 5.3, 11.3 Hz, C**H**H′OAc), 4.44 (dd, J = 5.8, 11.3 Hz, CH**H′**OAc), 4.52 (d, J = 5.8 Hz, C**H2**NH), 4.80–4.85 (m, C**H**), 6.71 (d, J = 7.5 Hz, N**H**CH), 7.42 (t, J = 8.2 Hz, C$_{5'}$**H**), 7.59–7.67 (m, N**H** and C$_{6'}$**H**), 8.10–8.12 (m, C$_{2'}$**H** and C$_{4'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a CDCl$_3$ solution of (*R*)-**24** gave only one signal for each of the two acetyl methyl protons. ^{13}C NMR (DMSO-d_6) δ 20.5, 22.6 (2 **C**H$_3$C(O)), 41.5 (**C**H$_2$NH), 51.6 (**C**H), 63.4 (**C**H$_2$OAc), 121.5, 121.7 (**C$_{2'}$** and **C$_{4'}$**), 129.7 (**C$_{5'}$**), 133.7 (**C$_{6'}$**), 141.7 (**C$_{1'}$**), 147.7 (**C$_{3'}$**), 169.2, 169.5, 170.0 (3 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 324 (M$^+$ + 1, 100), 264 (24); M_r (+CI) 324.119 85 [M$^+$ + 1] (calcd for $C_{14}H_{18}N_3O_6$ 324.119 56). Anal. ($C_{14}H_{17}N_3O_6$) C, H, N.

(*S*)-*N*-(3-Nitrobenzyl)-2-acetamido-3-hydroxypropionamide ((*S*)-23) and (*S*)-*N*-(3-Nitrobenzyl)-2-acetamido-3-acetoxypropionamide ((*S*)-24). Compounds (*S*)-**23** and (*S*)-**24** were prepared utilizing the preceding procedure, L-serine ((*S*)-**18**) (5.00 g, 47.6 mmol), Ac$_2$O (5.20 mL, 54.7 mmol), NMM (6.5 mL, 59.5 mmol), IBCF (7.7 mL, 59.5 mmol), and 3-nitrobenzylamine hydrochloride (11.22 g, 59.5 mmol). The 3-nitrobenzylamine hydrochloride was solubilized in DMF (30 mL) upon the addition of NMM (6.5 mL, 59.5 mmol). After evaporation of the solvent, the crude product was purified by column chromatography (SiO$_2$; 1:25 MeOH/CHCl$_3$) to yield a mixture of 4.54 g (34%) of (*S*)-**23** and 1.38 g (9%) of (*S*)-**24** as white solids.

(*S*)-23: mp 136–138 °C; $[\alpha]^{27}_{D}$ −12.5° (*c* 0.50, DMSO); R_f = 0.20 (1:9 MeOH/CHCl$_3$); IR (KBr) 3391, 3359, 3310, 3065, 1642, 1551, 1531 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.10 (s, C**H3**C(O)), 3.24–3.30 (m, O**H**), 3.59–3.68 (m, C**H**H′OH), 4.20–4.25 (m, CH**H′**OH), 4.45–4.50 (m, C**H**), 4.49 (dd, J = 6.4, 15.8 Hz, C**H**H′Ph), 4.59 (dd, J = 6.1, 15.8 Hz, CH**H′**Ph), 6.67–6.75 (m, N**H**CH), 7.48–7.61 (m, C$_{5'}$**H**, C$_{6'}$**H**, and N**H**CH$_2$), 8.09–8.15 (m, C$_{2'}$**H** and C$_{4'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a CDCl$_3$ solution of (*S*)-**23** gave only one signal for the acetyl methyl protons. Addition of excess (*R*)-(−)-mandelic acid to a CDCl$_3$ solution of (*R*)-**23** and (*S*)-**23** (1:1 ratio) gave only one signal for the acetyl methyl protons. ^{13}C NMR (DMSO-d_6) δ 22.6 (**C**H$_3$C(O)), 41.4 (**C**H$_2$NH), 55.3 (**C**H), 61.6 (**C**H$_2$OH), 121.6 (**C$_{2'}$** and **C$_{4'}$**), 129.6 (**C$_{5'}$**), 133.7 (**C$_{6'}$**), 142.0 (**C$_{1'}$**), 147.8 (**C$_{3'}$**), 169.5, 170.6 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 283 (13), 282 (M$^+$ + 1, 100); M_r (+CI) 282.108 69 [M$^+$ + 1] (calcd for $C_{12}H_{16}N_3O_5$ 282.109 00). Anal. ($C_{12}H_{15}N_3O_5$) C, H, N.

(*S*)-24: mp 151–154 °C; $[\alpha]^{26}_{D}$ −14.4° (*c* 1.00, DMSO); R_f = 0.39 (1:9 MeOH/CHCl$_3$); IR (KBr) 3282 (br), 3097, 1743, 1639, 1562, 1523 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.00, 2.04 (s, 2 C**H3**C-(O)), 4.28 (dd, J = 5.1, 11.4 Hz, C**H**H′OAc), 4.43 (dd, J = 6.0, 11.4 Hz, CH**H′**OAc), 4.52 (d, J = 5.7 Hz, C**H2**NH), 4.79–4.86 (m, C**H**), 6.72 (d, J = 7.5 Hz, N**H**CH), 7.46–7.53 (m, C$_{5'}$**H**), 7.59–7.66 (m, N**H**CH$_2$ and C$_{6'}$**H**), 8.10–8.12 (m, C$_{2'}$**H** and C$_{4'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a CDCl$_3$ solution of (*S*)-**24** gave only one signal for each of the two acetyl methyl protons. Addition of excess (*R*)-(−)-mandelic acid to a CDCl$_3$ solution of (*R*)-**24** and (*S*)-**24** (1:1 ratio) gave only one signal for each acetyl methyl proton. ^{13}C NMR (DMSO-d_6) δ 20.5, 22.5 (2 **C**H$_3$), 41.5 (**C**H$_2$NH), 51.6 (**C**H), 63.4 (**C**H$_2$OAc), 121.5, 121.7 (**C$_{2'}$** and **C$_{4'}$**), 129.7 (**C$_{5'}$**), 133.7 (**C$_{6'}$**), 141.7 (**C$_{1'}$**), 147.7 (**C$_{3'}$**), 169.2, 169.6, 170.0 (3 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 324 (M$^+$ + 1, 100), 264 (26), 233 (47), 172 (21); M_r (+CI) 324.119 78 [M$^+$ + 1] (calcd for $C_{14}H_{18}N_3O_6$ 324.119 56). Anal. ($C_{14}H_{17}N_3O_6$) C, H, N.

(*R*)-*N*-(3-Nitrobenzyl)-2-acetamido-3-methoxypropionamide ((*R*)-25). Utilizing method B, (*R*)-**23** (3.00 g, 10.40 mmol), Ag$_2$O (12.41 g, 53.6 mmol), and MeI (6.7 mL, 0.11 mol) gave 2.81 g (89%) of pure (*R*)-**25** as a white solid after purification by column chromatography (SiO$_2$; EtOAc): mp 152–153 °C; $[\alpha]^{26}_{D}$ +18.3° (*c* 1.31, MeOH); R_f = 0.40 (1:9 MeOH/CHCl$_3$); IR (KBr) 3308, 3093, 2941, 2839, 1633, 1558, 1530 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 2.05 (s, C**H3**C(O)), 3.42 (s, OC**H3**), 3.49 (dd, J = 7.4, 9.0 Hz, C**H**H′OCH$_3$), 3.82 (dd, J = 4.1, 9.0 Hz, CH**H′**OCH$_3$), 4.56–4.64 (m, C**H** and C**H2**NH), 6.49 (d, J = 6.3 Hz, N**H**CH), 7.14 (br s, N**H**CH$_2$), 7.48–7.52 (m, C$_{5'}$**H**), 7.61 (d, J = 7.5 Hz, C$_{6'}$**H**), 8.05–8.15 (m, C$_{2'}$**H** and C$_{4'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a CDCl$_3$ solution of (*R*)-**25** gave only one signal for the acetyl methyl protons and one signal for the ether methyl protons. ^{13}C NMR (CDCl$_3$) δ 23.4 (**C**H$_3$C(O)), 42.8 (**C**H$_2$NH), 52.8 (**C**H), 59.4 (O**C**H$_3$), 71.7 (**C**H$_2$OCH$_3$), 122.1, 122.6 (**C$_{2'}$** and **C$_{4'}$**), 129.7 (**C$_{5'}$**), 133.6 (**C$_{6'}$**),

140.5 (**C**$_{1'}$), 148.7 (**C**$_{3'}$), 170.6, 170.7 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 296 (M^+ + 1, 100), 144 (69), 116 (15); M_r (+CI) 296.123 82 [M^+ + 1] (calcd for $C_{13}H_{18}N_3O_5$ 296.124 65). Anal. ($C_{13}H_{17}N_3O_5$) C, H, N.

(*S*)-*N*-(3-Nitrobenzyl)-2-acetamido-3-methoxypropionamide ((*S*)-25). Utilizing method B, (*S*)-**23** (2.50 g, 8.89 mmol), Ag_2O (10.30 g, 44.4 mmol), and MeI (5.5 mL, 89.0 mmol) gave 2.60 g (99%) of pure (*S*)-**25** as a white solid after purification by column chromatography (SiO_2; 1:17 MeOH/$CHCl_3$): mp 152−153 °C; $[\alpha]^{26}_D$ −18.4° (*c* 1.20, MeOH); R_f = 0.40 (1:9 MeOH/$CHCl_3$); IR (KBr) 3310, 3089, 2941, 2837, 1639, 1531 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05 (s, C**H$_3$**C(O)), 3.42 (s, OC**H$_3$**), 3.49 (dd, J = 7.4, 8.9 Hz, C**H**H′OCH$_3$), 3.84 (dd, J = 3.9, 8.9 Hz, CH**H′**OCH$_3$), 4.54−4.64 (m, C**H** and C**H$_2$**NH), 6.51 (d, J = 6.2 Hz, N**H**CH), 7.16 (br s, N**H**CH$_2$), 7.49−7.53 (m, C$_{5'}$**H**), 7.61 (d, J = 7.2 Hz, C$_{6'}$**H**), 8.10−8.13 (m, C$_{2'}$**H** and C$_{4'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*S*)-**25** gave only one signal for the acetyl methyl protons and one signal for the ether methyl protons. Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**25** and (*S*)-**25** (1:1 ratio) gave two signals for the acetyl methyl protons (δ 1.99 and 2.00) and two signals for the ether methyl protons (δ 3.33 and 3.36). ^{13}C NMR ($CDCl_3$) δ 23.3 (**C**H$_3$C(O)), 42.7 (**C**H$_2$NH), 52.9 (**C**H), 59.4 (O**C**H$_3$), 71.9 (**C**H$_2$OCH$_3$), 122.0, 122.5 (**C**$_{2'}$ and **C**$_{4'}$), 129.7 (**C**$_{5'}$), 133.6 (**C**$_{6'}$), 140.6 (**C**$_{1'}$), 148.6 (**C**$_{3'}$), 170.6, 170.7 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 297 (15), 296 (M^+ + 1, 100), 254 (41), 144 (14); M_r (+CI) 296.124 78 [M^+ + 1] (calcd for $C_{13}H_{18}N_3O_5$ 296.124 65). Anal. ($C_{13}H_{17}N_3O_5$) C, H, N.

(*R*)-*N*-(3-Aminobenzyl)-2-acetamido-3-methoxypropionamide ((*R*)-26). Utilizing the procedure for (*R*)-**22** and using a methanolic solution (80 mL) of (*R*)-**25** (1.49 g, 5.05 mmol), PtO_2 (~115 mg), and H_2 (1 atm) gave, after 45 min, pure (*R*)-**26** (1.34 g, 99%) as a clear oil, which solidified upon standing: mp 183−184 °C (dec); $[\alpha]^{25}_D$ +3.59° (*c* 1.33, DMSO); R_f = 0.28 (1:9 MeOH/$CHCl_3$); IR (KBr) 3356, 3308, 3290, 2922, 1650, 1622, 1560, 1544 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.04 (s, C**H$_3$**C(O)), 3.38 (s, OC**H$_3$**), 3.43 (dd, J = 7.5, 9.1 Hz, C**H**H′OCH$_3$), 3.64−3.69 (br s, N**H$_2$**), 3.81 (dd, J = 4.1, 9.1 Hz, CH**H′**OCH$_3$), 4.38 (d, J = 5.7 Hz, C**H$_2$**NH), 4.51−4.57 (m, C**H**), 6.43 (d, J = 5.4 Hz, N**H**CH), 6.58 (s, C$_{2'}$**H**), 6.60−6.69 (m, C$_{4'}$**H**, C$_{6'}$**H**, and N**H**CH$_2$), 7.11 (br t, J = 8.1 Hz, C$_{5'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**26** gave only one signal for the acetyl methyl protons and one signal for the ether methyl protons. ^{13}C NMR (DMSO-d_6) δ 22.6 (**C**H$_3$C(O)), 42.3 (**C**H$_2$NH), 52.5 (**C**H), 58.2 (O**C**H$_3$), 72.2 (**C**H$_2$OCH$_3$), 112.5, 112.7 (**C**$_{2'}$ and **C**$_{4'}$), 114.6 (**C**$_{6'}$), 128.7 (**C**$_{5'}$), 139.7 (**C**$_{1'}$), 148.5 (**C**$_{3'}$), 169.3, 169.5 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 267 (13), 266 (M^+ + 1, 100), 123 (21), 119 (13); M_r (+CI) 266.149 70 [M^+ + 1] (calcd for $C_{13}H_{20}N_3O_3$ 266.150 47). Anal. ($C_{13}H_{19}N_3O_3$) C, H, N.

(*S*)-*N*-(3-Aminobenzyl)-2-acetamido-3-methoxypropionamide ((*S*)-26). Utilizing the preceding procedure and using a methanolic solution (80 mL) of (*S*)-**25** (1.89 g, 6.40 mmol), MeOH (180 mL), PtO_2 (~180 mg), and H_2 (1 atm) gave 1.59 g (94%) of pure (*S*)-**26** as a clear oil, which solidified upon standing: mp 184−185 °C (dec); $[\alpha]^{25}_D$ −3.71° (*c* 1.30, DMSO); R_f = 0.28 (1:9 MeOH/$CHCl_3$); IR (KBr) 3356, 3308, 3091, 2884, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.04 (s, C**H$_3$**C(O)), 3.38 (s, OC**H$_3$**), 3.45 (dd, J = 7.7, 9.0 Hz, C**H**H′OCH$_3$), 3.60−3.70 (br s, N**H$_2$**), 3.81 (dd, J = 4.1, 9.0 Hz, CH**H′**OCH$_3$), 4.38 (d, J = 5.7 Hz, C**H$_2$**NH), 4.52−4.57 (m, C**H**), 6.44 (br d, J = 6.6 Hz, N**H**CH), 6.58 (s, C$_{2'}$**H**), 6.60−6.65 (m, C$_{4'}$**H**, C$_{6'}$**H**), 6.65−6.71 (m, N**H**CH$_2$), 7.11 (br t, J = 8.1 Hz, C$_{5'}$**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*S*)-**26** gave only one signal for the acetyl methyl protons and one signal for the ether methyl protons. Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**26** and (*S*)-**26** (1:1 ratio) gave two signals for the acetyl methyl proton (δ 1.99 and 2.00) and two signals for the ether methyl protons (δ 3.33 and 3.36). ^{13}C NMR (DMSO-d_6) δ 22.5 (**C**H$_3$C(O)), 42.2 (**C**H$_2$NH), 52.4 (**C**H), 58.1 (O**C**H$_3$), 72.1 (**C**H$_2$OCH$_3$), 112.4, 112.6 (**C**$_{2'}$ and **C**$_{4'}$), 114.6 (**C**$_{6'}$), 128.6 (**C**$_{5'}$), 139.6 (**C**$_{1'}$), 148.5 (**C**$_{3'}$), 169.3, 169.4 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 267 (14), 266 (M^+ + 1, 100); M_r (+CI) 266.150 72 [M^+ + 1] (calcd for $C_{13}H_{20}N_3O_3$ 266.150 47). Anal. ($C_{13}H_{19}N_3O_3$·0.12H_2O) C, H, N.

(*R*)-*N*-(3-Isothiocyanatobenzyl)-2-acetamido-3-methoxypropionamide ((*R*)-7). Utilizing the procedure for (*R*)-**6** and using (*R*)-**26** (431 mg, 1.62 mmol), CH_2Cl_2 (40 mL), and DPT (378 mg, 1.62 mmol) gave 400 mg (80%) of pure (*R*)-**7** as a white solid after purification by column chromatography (SiO_2; EtOAc): mp 161−163 °C; $[\alpha]^{27}_D$ +5.7° (*c* 1.77, acetone); R_f = 0.46 (1:9 MeOH/$CHCl_3$); IR (KBr) 3281, 3120, 2929, 2165, 2139, 1633, 1543 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05 (s, C**H$_3$**C(O)), 3.44 (s, OC**H$_3$**), 3.53 (dd, J = 7.1, 9.2 Hz, C**H**H′OCH$_3$), 3.83 (dd, J = 4.4, 9.2 Hz, CH**H′**OCH$_3$), 4.43 (dd, J = 6.0, 15.5 Hz, C**H**H′Ar), 4.51 (dd, J = 6.2, 15.5 Hz, CH**H′**Ar), 4.54−4.59 (m, C**H**), 6.90 (br d, J = 6.0 Hz, N**H**CH), 7.14−7.36 (m, 4 Ar**H** and N**H**CH$_2$). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**7** gave only one signal for the acetyl methyl protons and one signal for the ether methyl protons. ^{13}C NMR ($CDCl_3$) δ 23.4 (**C**H$_3$C(O)), 43.0 (**C**H$_2$NH), 52.7 (**C**H), 59.4 (O**C**H$_3$), 71.8 (**C**H$_2$OCH$_3$), 124.7, 124.8 (**C**$_{2'}$ and **C**$_{4'}$), 126.4 (**C**$_{6'}$), 130.0, 131.8 (**C**$_{3'}$ and **C**$_{5'}$), 135.8 (N**C**S), 140.2 (**C**$_{1'}$), 170.4, 170.6 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 336 (M^+ + 1, 15), 309 (14), 308 (100), 144 (12); M_r (+CI) 308.105 68 [M^+ + 1] (calcd for $C_{14}H_{18}N_3O_3S$ 308.106 89). Anal. ($C_{14}H_{17}N_3O_3$·0.45H_2O) C, H, N.

(*S*)-*N*-(3-Isothiocyanatobenzyl)-2-acetamido-3-methoxypropionamide ((*S*)-7). Utilizing the preceding procedure, (*S*)-**26** (2.50 mg, 9.43 mmol), THF (110 mL), and DPT (2.20 g, 9.43 mmol) gave 1.77 g (61%) of pure (*S*)-**7** as a white solid after purification by column chromatography (SiO_2; 1:9 acetone/EtOAc): mp 162−163 °C; $[\alpha]^{26}_D$ −5.8° (*c* 1.77, acetone); R_f = 0.46 (1:9 MeOH/$CHCl_3$); IR (KBr) 3281, 3099, 2928, 2138 (br), 1637, 1545 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05 (s, C**H$_3$**C(O)), 3.41 (s, OC**H$_3$**), 3.46 (dd, J = 7.5, 9.0 Hz, C**H**H′OCH$_3$), 3.83 (dd, J = 3.9, 9.0 Hz, CH**H′**OCH$_3$), 4.46 (J = 6.0 Hz, C**H$_2$**Ar), 4.54−4.60 (m, C**H**), 6.45 (d, J = 5.7 Hz, N**H**CH), 6.88−6.91 (m, N**H**CH$_2$), 7.13−7.33 (m, 4 Ar**H**). Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*S*)-**7** gave only one signal for the acetyl methyl protons and one signal for the ether methyl protons. Addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**7** and (*S*)-**7** gave two signals for the acetyl methyl protons (δ 2.02 and 2.03) and two signals for the ether methyl protons (δ 3.36 and 3.38). ^{13}C NMR ($CDCl_3$) δ 23.4 (**C**H$_3$C(O)), 43.0 (**C**H$_2$NH), 52.7 (**C**H), 59.4 (O**C**H$_3$), 71.8 (**C**H$_2$OCH$_3$), 124.7, 124.8 (**C**$_{2'}$ and **C**$_{4'}$), 126.4 (**C**$_{6'}$), 130.0, 131.8 (**C**$_{3'}$ and **C**$_{5'}$), 135.8 (N**C**S), 140.2 (**C**$_{1'}$), 170.4, 170.6 (2 **C**(O)); MS (+CI) *m*/*z* (rel intensity) 336 (M^+ + 1, 24), 309 (22), 308 (100), 165 (12); M_r (+CI) 308.105 88 [M^+ + 1] (calcd for $C_{14}H_{18}N_3O_3S$ 308.106 89). Anal. ($C_{14}H_{17}N_3O_3S$) C, H, N.

Pharmacology. 1. In Vivo Studies. Compounds were screened under the auspices of the National Institutes of Health's Anticonvulsant Screening Project. Experiments were performed in male rodents (albino Carworth Farms No. 1 mice (intraperitoneal route, ip), albino Sprague−Dawley rats (oral route, po)). The mice weighed between 18 and 25 g, while rats were between 100 and 150 g. All animals had free access to feed and water except during the actual testing period. Housing, handling, and feeding were all in accordance with recommendations contained in the "Guide for the Care and Use of Laboratory Animals". All of the test compounds were administrated in suspensions of 0.5% (w/v) of methylcellulose in water. The volumes administered were 0.01 mL/g of body weight for mice and 0.2 mL/10 g for rats. Anticonvulsant activity was established using the maximal electroshock (MES) test.[4,31] For the MES test, a drop of electrolyte solution with an anesthetic (0.5% butacaine hemisulfate in 0.9% sodium chloride) was placed in the eyes of the animals prior to positioning the corneal electrodes and delivery of a nonlethal current. A 60-cycle alternating current was administered for 0.2 s in both species, utilizing 50 mA in mice and 150 mA in rats. Protection endpoints were defined as the abolition of the hind limb tonic extensor component of the induced seizure.[5] In mice, effects of compounds on forced spontaneous motor activity were determined using the rotorod test.[28,32] The inability of experimental mice to maintain their balance for 1

min on a 1 in. diameter knurled rod rotating at 6 rpm in three successive trials was interpreted as a demonstration of motor impairment. Under these conditions, mice can normally maintain their balance indefinitely. Motor impairment in rats was assessed by observing the overt evidence of ataxia, abnormal gait and stance, and/or loss of placing response and muscle tone. In the mouse identification screens, all compounds were administered at three dose levels (30, 100, 300 mg/kg) and in two time periods (0.5 and 4 h). Typically, in the MES seizure test, one animal was used at 30 and 300 mg/kg and three animals were used at 100 mg/kg. In the rotorod toxicity test, four animals were used at 30 and 300 mg/kg and eight animals were used at 100 mg/kg (Table 1). Oral rat identification screening was performed using four animals at a fixed dose of 30 mg/kg for both the MES and the rotorod toxicity tests over 5 time periods ranging from $^{1}/_{4}$ to 4 h postdrug administration. The quantitative determinations of the median effective (ED_{50}) and toxic doses (TD_{50}) were conducted at previously calculated time of peak effect using the ip route in mice and the oral route in rats. Groups of at least eight animals were tested using different doses of test compound until at least two points were determined between 100% and 0% protection and minimal motor impairment. The dose of the candidate substance required to produce the desired endpoint (abolition of hindlimb tonic extensor component) in 50% of the animals in each test and the 95% confidence interval were calculated by a computer program based on methods described by Finney.[33]

2. Receptor Screen. Assays[34] for the following receptors were performed by the NIMH Psychoactive Drug Screening Program: (1) $GABA_A$, agonist ([^{3}H] muscimol), (2) $GABA_A$, bzp ([^{3}H] flunitrazepam], (3) NMDA, PCP ([^{3}H] TCP), (4) NMDA, MK-801 ([^{3}H] MK-801), (5) Na^+, type 2 ([^{3}H]batrachotoxin), and (6) Ca^{2+}, type L ([^{3}H]nifedipine). Detailed on-line protocols for the binding assays are described at http://meds20785.cwru.edu./myweb/protocol.htm. For screening purposes, 10 μM of each compound (dissolved in 10% DMSO) was incubated with the appropriate receptor preparation and the percent inhibition was determined for duplicate determinations each performed in duplicate.

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Supporting Information Available: Experimental details on the saponification of (*R*)-**20**, (*S*)-**20**, (*R*)-**24**, and (*S*)-**24** and the chemical reactivity of (*S*)-**3**, (*R*,*S*,)-**5**, (*R*,*S*)-**6**, and (*R*)-**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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