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Synthesis and anticonvulsant activities of (*R*)-(*O*)-methylserine derivatives

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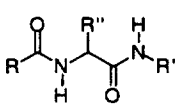
Abstract

Efficient procedures for the synthesis of (*R*)-*N*-benzyl-2-amino-3-methoxypropionamide ((*R*)-**3**), 2-acetamido-3-methoxypropionic acid (**4**), and *O*-methylserine (**5**) are described beginning from (*R*)-Cbz-serine ((*R*)-**7**). The reactions proceeded with little or no racemization and permitted the synthesis of the potent anticonvulsant (*R*)-*N*-benzyl-2-acetamido-3-methoxypropionamide ((*R*)-**2**). The anticonvulsant activities of **2–4** were determined revealing the surprising activity of (*R*)-**2**.

1. Introduction

Studies on the anticonvulsant activities of functionalized amino acids **1**[1–10] have shown that *N*-benzyl-2-acetamido-3-methoxypropionamide (**2**) provided excellent protection against maximal electroshock-induced (MES) seizures in mice and rats.[10] An important feature of the pharmacological profile for **2** was that the anticonvulsant activity principally resided in the (*R*)-enantiomer. It was observed that (*R*)-**2** was some 22-fold more potent than (*S*)-**2** in the MES-seizure test. This stereoselective profile does not exist for any currently marketed antiepileptic agent, and it distinguishes (*R*)-**2** from all other anticonvulsants.



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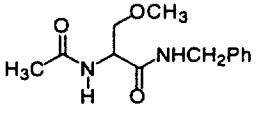

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Significantly, our investigations on the *absence* of either the *N*-acyl (RC(O)) or the *N'*-amide (N(H)R') group or both on the anticonvulsant activity of **1** were limited.[2,3] Compounds **3–5** represent analogs that

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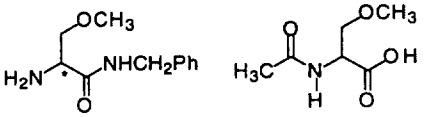
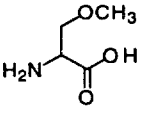
lack one or both amide linkages in (*R*)-**2** but still retain the core *O*-methylserine unit. In this paper, we report the preparation of serine derivatives (*R*)-**3**, **4**, and **5** along with (*R*)-**2**. Highlighted are synthetic procedures that permit serine modification without racemization and the surprising activity of amine (*R*)-**3**.



(*R*)-**3**

4



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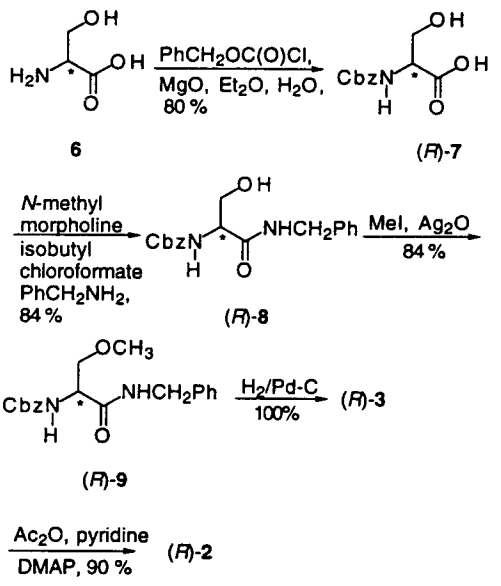
2. Results and discussion

2.1. Synthesis

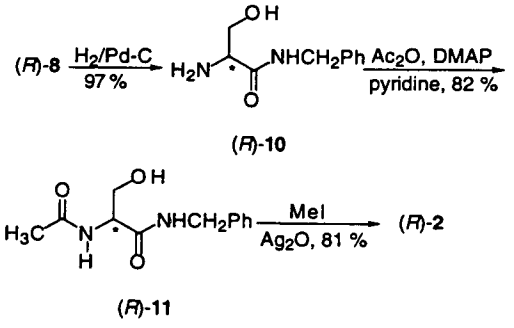
Our strategy was to prepare target compounds **3–5** through common intermediates. Synthesis of (*R*)-**3** was accomplished in four steps in 56% overall yield (Scheme 1). Treatment of (*R*)-serine ((*R*)-**6**) with benzyl chloroformate gave (*R*)-**7**,[11] which was converted to (*R*)-**8** using benzylamine and the mixed anhydride coupling (MAC) procedure.[12] Significantly, we[5,7,10] and others[12] have demonstrated that the MAC method proceeds stereospecifically. Methylation of (*R*)-**8** (MeI, Ag_2O) followed by hydrogenolysis of (*R*)-**9** afforded (*R*)-**3**. The enantiopurity of (*R*)-**3** was verified by converting (*R*)-**3** to (*R*)-**2** and then comparing the melting point and optical rotation of (*R*)-**2** with an authentic sample.[10] Additionally, we found that only a single acetyl methyl and *O*-methyl signal in the ^{1}H NMR spectrum of (*R*)-**2** was observed when the chiral resolving agent, (*R*)-(−)-mandelic acid,[13,14] was added.[10]

The overall synthesis of (*R*)-**2** (Scheme 1) proceeded in higher yields than our earlier reported method.[10] We also learned that the order of the reactions in Scheme 1 could be interchanged without losses in yield or enantiopurity (Scheme 2). Interestingly, we observed no *O*-acetylation products upon treatment of (*R*)-**10** with acetic anhydride and catalytic amounts of DMAP in pyridine.

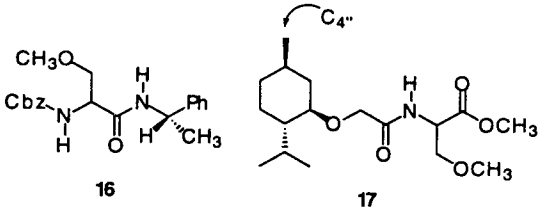
Syntheses of **4** and **5** proceeded through Cbz-serine ((*R*)-**7**) (Scheme 3). Methylation of (*R*)-**7** with MeI and Ag_2O gave methyl 2-*N*-(benzyloxycarbonyl)amino-3-methoxypropionate (**12**). Hydrogenolysis of **12** followed by immediate acetylation of the free amine **13** yielded **14**. Aqueous base (K_2CO_3) hydrolysis of **14** provided **4**. Similarly, hydrolysis (K_2CO_3) of **12** gave **15**, which was then deprotected to give **5**. NMR assessment of the enantiopurities of **4** and **5** using chiral shift reagents were unsuccessful. Accordingly, we relied upon chemical derivatization methods to determine the optical purities of all key compounds. Acid **4** was treated with benzylamine under MAC conditions to yield **2**. Optical and NMR (mandelic acid) measurements showed that **2** existed as a 65:35 mixture of the (*R*) and (*S*) enantiomers (30% ee). Correspondingly, **5** was converted back to **15** and then coupled (MAC) with the homochiral (*S*)-α-methylbenzylamine to give **16**. The ^{1}H NMR of **16** exhibited two singlets for the OCH_3 protons (δ 3.35, 3.37) in a 65:35 ratio (30% ee).



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

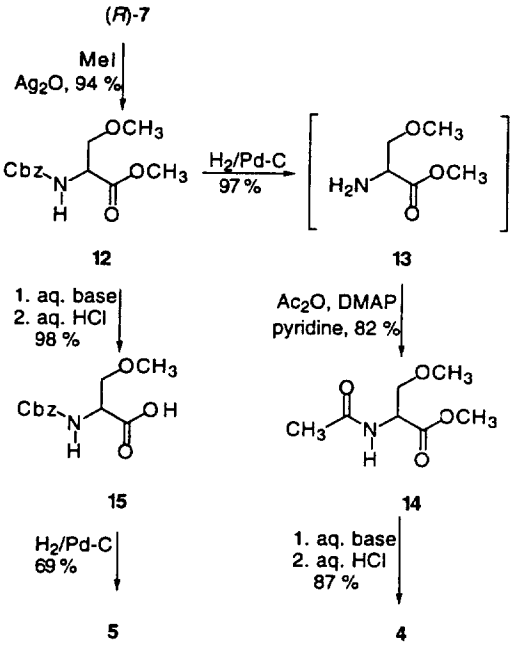


Scheme 2. Synthesis of (*R*)-**2**



Results from the analysis of optical purities of **4** and **5** clearly indicated that one or more of the steps in Scheme 3 did not proceed with stereospecificity. An investigation was undertaken to identify this step (or steps). Compound (*R*)-**7** was prepared following a literature procedure[11] and was confirmed to be optically pure. We speculated that neither the hydrogenolysis (**12**→**13**, **15**→**5**) nor the acetylation (**13**→**14**) steps proceeded with racemization. Accordingly, we focused our attention on the three base-mediated processes: the methylation of **7** using Ag_2O and the aqueous K_2CO_3 hydrolyses of esters **12** and **14**.

We first determined the optical purity of compound **13**. Accordingly, **13** was derivatized (MAC) with homochiral (−)-menthoxyacetic acid to provide diastereomeric amide **17**. The ^1H NMR spectrum of **17** exhibited two sets of doublets for the C(4″) methyl protons (δ 0.79, 0.80) in an 85:15 ratio (70% ee),



Scheme 3. Syntheses of **4** and **5**

showing that detectable levels of racemization occurred during the Ag_2O-mediated methylation step. This finding contrasted with the result observed for methylation of **11** to give **2** (Scheme 2), which proceeded without racemization under nearly identical conditions. We have attributed the partial racemization for **7** to the fact that protons adjacent to an ester moiety have increased acidity over those adjacent to an amide unit.[15] Attempts to reduce the extent of racemization that occurred during methylation of (*R*)-**7** by decreasing either the amounts of Ag_2O or the length of the reaction time led to lower overall yields of **12**, without appreciably reducing racemization.

Our finding that compounds **4** and **5** were produced in 30% ee (Scheme 3) indicated that an additional step (or steps) besides methylation of (*R*)-**7**, proceeded with partial racemization. Accordingly, we hydrolyzed **14** to **4** under milder conditions (aqueous $NaHCO_3$). The enantiopurity of **4** was assessed by conversion to **2** with benzylamine (MAC). We found that **2** was produced in 70% ee, showing that our substitution of $NaHCO_3$ for K_2CO_3 eliminated the racemization from the ester hydrolysis step. A similar effect of base strength on serine racemization was observed for hydrolysis of **12**. Use of aqueous K_2CO_3 gave **15** in 30% ee while aqueous $NaHCO_3$ provided **15** in 70% ee. The enantiopurity of **15** was determined by treatment of the acid with (*S*)-α-methylbenzylamine (MAC) to give diastereomeric amides **16**. The ^1H NMR of **16** showed the presence of two singlets for the methoxy protons (δ 3.35, 3.37) in a ratio of 85:15. The optical purity of **5** (70% ee) was further confirmed by converting **5** first to **15** and then to **16** (data not shown). These findings demonstrated that only the methylation step ((*R*)-**7**→**12**) in Scheme 3 proceeded with partial racemization, provided $NaHCO_3$ was used to hydrolyse esters **12** and **14**. At this stage, we decided neither to resolve partially racemized **12** nor to search for alternative methylation procedures (e.g., diazomethane[16]). Rather, we first measured the pharmacological activities of **4** and **5** to determine if they served as anticonvulsants.

Table 1
Pharmacological evaluation of structural analogues of (*R*)-*N*-benzyl-2-acetamido-3-methoxypropionamide ((*R*)-**2**)[a]

Compound	mice (ip)[b]			rat (po)[f]		
	MES[c], ED_{50}	TOX[d], TD_{50}	PI[e]	MES[c], ED_{50}	TOX[d], TD_{50}	PI[e]
(*R*)-**2**[g]	4.5 [0.5] (3.7-5.5)	27 [0.25]	6.0	3.9 [0.5] (2.6-6.2)	>500 [0.5]	>130
(*R*)-**3**	>30, <100	>100, <300	—	18 [4]	>500 [4]	>28
4	>300	300	—	—[h]	—[h]	—
5	>300	>300	—	—[h]	—[h]	—
phenytoin[i]	6.5 [2] (5.7-7.2)	43 [0.5] (36-48)	6.6	23 [2] (21-25)	>500 [0.25]	>22
phenobarbital[j]	22 [1] (15-23)	69 [0.5] (63-73)	3.1			
valproate[i]	290 [0.25] (240-360)	480 [0.25] (410-570)	1.7	395 [0.5] (332-441)	859 [0.5] (719-1148)	2.2

[a]The compounds were tested through the auspices of the National Institute of Neurological and Communicative Disorders and Stroke at the National Institutes of Health. ED_{50} and TD_{50} values are in mg/kg. Numbers in parentheses are 95% confidence intervals. The dose effect data were obtained at the "time of peak effect" (indicated in hours in the brackets). [b]The compounds were administered intraperitoneally to mice. [c]MES = maximal electroshock seizure test. [d]Tox = neurologic toxicity determined from rotorod test. [e]PI = protective index (TD_{50}/MES ED_{50}). [f]The compounds were administered orally to rats. [g]Reference 10. [h]Data not available. [i]Reference 18. [j]Reference 19.

2.2. *Pharmacological evaluation*

The syntheses of (*R*)-**3**, **4**, and **5** permitted us to determine their anticonvulsant activities in the MES-seizure test, using the procedure described by Stables and Kupferberg.[17] The pharmacological data for **3**–**5** are listed in Table 1, along with those found for (*R*)-**2**[10] and the proven antiepileptic agents phenytoin,[18] phenobarbital,[19] and valproate.[18]

The three simplified analogues of (*R*)-**2**, (*R*)-**3**, **4** and **5**, given intraperitonially (ip) to mice, displayed considerably reduced anticonvulsant activity compared with (*R*)-**2**. Of the three, (*R*)-**3** exhibited moderate anticonvulsant activity (MES effective dose in 50% test animals (ED_{50}) <100 mg/kg), and **4** and **5** showed no protection at concentrations of 300 mg/kg. We suspect that the observed decreased anticonvulsant activity for (*R*)-**3** over (*R*)-**2** was, in part, due to its enhanced polarity and the effect that this modification has on its ability to cross the blood–brain barrier. We speculate that a similar effect contributes to the lack of anticonvulsant activity for the two modified amino acids **4** and **5**. These findings are in agreement with previous trends.[2]

Studies have shown that MES ED_{50} values vary with the route of administration and the animal species.[18] Accordingly, we determined the anticonvulsant activity of (*R*)-**3** in the MES-induced seizure test after oral administration in rats. Significantly, (*R*)-**3** showed appreciably greater anticonvulsant activity than would have been anticipated, based solely on the mouse (ip) results. The MES ED_{50} value of (*R*)-**3** was 18 mg/kg and exceeded the value found for phenytoin (ED_{50}=23 mg/kg).[18] This finding suggests that upon oral administration, the improved activity observed for (*R*)-**3** may stem from the increased absorption into the blood stream. An additional explanation exists for the improved oral activity

of (*R*)-**3**. Primary amines undergo metabolic conversion via oxidative deamination, *N*-oxidation, and, in some instances, acetylation.[20] Acetylation may be an important metabolic pathway for (*R*)-**3**, and the potent anticonvulsant (*R*)-**2**[10] could be produced, significantly contributing to the protective effects from seizure stimulation via MES.

3. Conclusions

This study establishes an efficient, stereospecific route to the potent anticonvulsant agent (*R*)-*N*-benzyl-2-acetamido-3-methoxypropionamide ((*R*)-**2**). Moreover, the effective use of the *N*-benzyloxycarbonyl-protecting group allowed the synthesis of (*O*)-methyl serine analogues (*R*)-**3**, **4** and **5**. Analysis of each step in the syntheses of (*R*)-**2**, (*R*)-**3**, **4**, and **5** defined conditions that permitted serine modification with little or no racemization. We expect that these routes will permit future preparation of (*R*)-**2** derivatives with modified substituents that can serve as mechanistic probes (e.g., radioligands, inactivators) in receptor research. Finally, compound (*R*)-**3** exhibited modest activity in mice (ip) (ED_{50}>30, <100) but provided excellent seizure protection when given orally to rats.

4. Experimental section

Melting points were determined with a Thomas–Hoover melting point apparatus and are uncorrected. Infrared spectra (IR) were run on an ATI Mattson Genesis Series FTIR™ spectrometer. Optical rotations were obtained on a Perkin–Elmer 241 MC polarimeter. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were taken on a General Electric QE-300 NMR instrument. Chemical shifts (δ) are in parts per million (ppm) relative to tetramethylsilane and coupling constants (*J* values) are in hertz. 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).

*4.1. (*R*)-N-Benzyl-2-N-(benzyloxycarbonyl)amino-3-hydroxypropionamide ((*R*)-8)*

A dry THF solution (25 mL) containing (*R*)-**7**[11] (2.00 g, 8.4 mmol) was cooled (−78°C) and then 4-methylmorpholine (1.4 mL, 10.5 mmol) was added. After stirring (2 min), isobutyl chloroformate (1.4 mL, 10.5 mmol) was added. The reaction was stirred (2 min) and then benzylamine (1.1 mL, 10.5 mmol) was added. The reaction was stirred at −78°C (5 min), allowed to warm to room temperature and then stirred (1 h). The reaction mixture was filtered and the filtrate evaporated *in vacuo*. The residue was suspended in Et_2O (75 mL) and filtered. The crude product was purified by column chromatography (SiO_2, 10% MeOH–$CHCl_3$) to obtain 2.30 g (84%) of pure (*R*)-**8** as a white solid: mp 147–149°C; $[\alpha]_D^{23}$=+4.6 (*c*=2.0, MeOH); R_f 0.47 (10% MeOH–$CHCl_3$); IR (KBr) 3293, 1689, 1645, 1535, 1455, 1398, 1308, 1268, 1025, 754, 698 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.55–3.61 (m, CH_2OH), 4.05–4.10 (m, C*H*), 4.27 (d, *J*=5.7 Hz, NHCH_2), 4.89 (t, *J*=5.4 Hz, O*H*), 5.02 (s, CH_2OC(O)), 7.20–7.35 (m, 10 Ph*H*, OC(O)N*H*), 8.40 (t, *J*=5.7 Hz, C(O)N*H*); ^{13}C NMR (DMSO-d_6) 42.1 (*C*H_2NH), 57.4 (*C*H), 61.8 (*C*H_2OH), 65.5 (O*C*H_2Ph), 126.7 (C_4' and C_4''), 127.0 ($2C_2'$ or $2C_3'$ or $2C_2''$ or $2C_3''$), 127.8 ($2C_2'$ or $2C_3'$ or $2C_2''$ or $2C_3''$), 128.2 ($2C_2'$ or $2C_3'$ or $2C_2''$ or $2C_3''$), 128.4 ($2C_2'$ or $2C_3'$ or $2C_2''$ or $2C_3''$), 137.0 (C_1' or C_1''), 139.3 (C_1' or C_1''), 156.0 (*C*(O)O), 170.0 (*C*(O)NH) ppm; MS (+CI) (rel. intensity)

329 (M^++1, 100), 285 (81), 221 (15), 286 (12); M_r (+CI) 329.15020 [M^++1] (calcd for $C_{18}H_{21}N_2O_4$ 329.15013); anal. calcd $C_{18}H_{20}N_2O_4$ C 65.85%, H 6.10%, N 8.54%; found C 65.87%, H 6.22%, N 8.47%.

4.2. (R)-N-*Benzyl-2-*N-*(benzyloxycarbonyl)amino-3-methoxypropionamide* ((R)-**9**)

To a CH_3CN solution (50 mL) of (*R*)-**8** (1.60 g, 4.9 mmol) was successively added Ag_2O (7.20 g, 24.4 mmol) and MeI (4.0 mL, 49 mmol) at room temperature, and then the reaction mixture was stirred at room temperature (3 days). The insoluble salts were filtered and the solvent was evaporated *in vacuo*. The residue was purified by column chromatography (SiO_2, 5% MeOH–$CHCl_3$) to obtain (*R*)-**9** as a white crystalline solid (1.40 g, 84%): mp 128–130°C; $[\alpha]_D^{23}$=+2.8 (*c*=1.1, MeOH); R_f 0.77 (10% MeOH–$CHCl_3$); IR (KBr) 3294, 2880, 1688, 1641, 1534, 1458, 1397, 1314, 1233, 1128, 1054, 964, 755, 699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.37 (s, OCH_3), 3.50 (dd, *J*=2.7, 9.3 Hz, $CHH'OCH_3$), 3.87 (dd, *J*=3.9, 9.3 Hz, $CHH'OCH_3$), 4.35–4.40 (m, C*H*), 4.49 (d, *J*=6.0 Hz, $NHCH_2$), 5.13 (s, $C(O)OCH_2$), 5.65–5.75 (m, N*H*), 6.67–6.70 (m, N*H*), 7.22–7.45 (m, 10 Ph*H*); ^{13}C NMR ($CDCl_3$) 43.7 (CH_2NH), 54.5 (*C*H), 59.3 (OCH_3), 67.4 ($C(O)OCH_2$), 72.2 (CH_2OCH_3), 127.6 (C_4' and C_4''), 128.3 ($2C_2'$ or $2C_3'$ or $2C_2''$ or $2C_3''$), 128.5 ($2C_2'$ or $2C_3'$ or $2C_2''$ or $2C_3''$), 128.8 ($2C_2'$ or $2C_3'$ or $2C_2''$ or $2C_3''$), 128.9 ($2C_2'$ or $2C_3'$ or $2C_2''$ or $2C_3''$), 136.2 (C_1' or C_1''), 138.0 (C_1' or C_1''), 156.3 (*C*(O)O), 170.0 (*C*(O)NH) ppm; MS (+CI) (rel. intensity) 343 (M^++1, 100), 299 (40), 235 (31); M_r (+CI) 343.16681 [M^++1] (calcd for $C_{19}H_{23}N_2O_4$ 343.16578); anal. calcd $C_{19}H_{22}N_2O_4 \cdot 0.25H_2O$ C 65.80%, H 6.49%, N 8.08%; found C 65.67%, H 6.49%, N 8.06%.

4.3. (R)-N-*Benzyl-2-amino-3-methoxypropionamide* ((R)-**3**)

A MeOH (50 mL) solution of (*R*)-**9** (1.00 g, 2.9 mmol) was hydrogenated in the presence of 10% Pd–C (0.20 g) at room temperature (3 h). The mixture was filtered through Celite and the clear filtrate was evaporated *in vacuo* to obtain a pale yellow oil, which was purified by column chromatography (SiO_2, 10% MeOH–$CHCl_3$) to obtain (*R*)-**3** (0.61 g, 100%) as a pale yellow oil: $[\alpha]_D^{23}$=−2.0 (*c*=1.5, MeOH); R_f 0.34 (10% MeOH–$CHCl_3$); IR (liquid film) 3352, 3311, 3064, 2927, 2826, 1655, 1527, 1455, 1360, 1251, 1181, 1106, 971, 734, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.85 (br s, NH_2), 3.34 (s, OCH_3), 3.56–3.62 (m, $CHOCH_2$), 4.39 (dd, *J*=6.0, 15.2 Hz, $NHCHH'$), 4.45 (dd, *J*=6.0, 15.2 Hz, $NHCHH'$), 7.20–7.36 (m, 10 Ph*H*), 7.80–7.88 (m, N*H*); ^{13}C NMR ($CDCl_3$) 43.1 ($NHCH_2$), 54.9 (*C*H), 58.9 (OCH_3), 74.6 (CH_2OCH_3), 127.4 (C_4'), 127.6 ($2C_2'$ or $2C_3'$), 128.6 ($2C_2'$ or $2C_3'$), 138.4 (C_1'), 172.8 (*C*(O)) ppm; MS (+CI) 209 (M^++1); M_r (+CI) 209.12919 [M^++1] (calcd for $C_{11}H_{17}N_2O_2$ 209.12900); anal. calcd $C_{11}H_{16}N_2O_2 \cdot 0.15H_2O$ C 62.65%, H 7.74%, N 13.29%; found C 62.60%, H 7.78%, N 13.17%.

4.4. (R)-N-*Benzyl-2-acetamido-3-methoxypropionamide*[10] ((R)-**2**). *Determination of the enantiomeric purity of* (R)-**3**

To a solution of (*R*)-**3** (0.06 g, 0.3 mmol) in dry THF (3 mL) was added successively pyridine (0.02 mL, 0.3 mmol), DMAP (~0.005 g), and Ac_2O (0.03 mL, 0.3 mmol), and the resulting solution was stirred at room temperature (1 h). The solvents were evaporated *in vacuo* and the residue was purified by PTLC (SiO_2, 5% MeOH–$CHCl_3$) to obtain (*R*)-**2** (0.07 g, 90%) as a white solid: mp 142–143°C (lit.[10] mp 143–144°C); $[\alpha]_D^{23}$=+16.2 (*c*=1, MeOH) (lit.[10] $[\alpha]_D^{23}$=+16.0 (*c*=1, MeOH)); R_f 0.47 (10% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.05 (s, $C(O)CH_3$), 3.39 (s, OCH_3), 3.45 (dd, *J*=7.8, 9.0 Hz, $CHH'OCH_3$), 3.83 (dd, *J*=4.2, 9.0 Hz, $CHH'OCH_3$), 4.49 (d, *J*=5.7 Hz, $NHCH_2$), 4.53–4.59 (m, C*H*),

6.40–6.51 (m, NH), 6.77 (br s, NH), 7.26–7.42 (m, 5 PhH), addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**2** gave only one signal for the acetyl methyl and ether methyl protons.

4.5. (R)-N-*Benzyl-2-amino-3-hydroxypropionamide* ((R)-**10**)

To a MeOH solution (10 mL) of (*R*)-**8** (0.63 g, 1.9 mmol) was added 10% Pd–C (0.10 g) and the mixture stirred at room temperature (3 h) in the presence of H_2 gas. The catalyst was removed by filtration through Celite, and the filtrate evaporated *in vacuo* to give pure (*R*)-**10** (0.36 g, 98%) as a white solid: mp 88–90°C; $[\alpha]_D^{23}=-3.2$ (*c*=0.9, MeOH); R_f 0.13 (10% MeOH–$CHCl_3$); IR (KBr) 3289, 2950, 2359, 1649, 1555, 1453, 1399, 1240, 1058, 1035, 697 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.90 (br s, NH_2), 3.22 (t, *J*=5.6 Hz, CH), 3.37–3.53 (m, CH_2OH), 4.27 (d, *J*=5.6 Hz, $NHCH_2$), 4.76 (br s, CH_2OH), 7.18–7.30 (m, 5 PhH), 8.32 (t, *J*=5.6 Hz, NH); ^{13}C NMR (DMSO-d_6) 41.8 ($NHCH_2$), 57.0 (CH), 64.4 (CH_2OH), 126.6 (C_4'), 127.0 ($2C_2'$ or $2C_3'$), 128.2 ($2C_2'$ or $2C_3'$), 139.6 (C_1'), 173.4 (C(O)NH) ppm; MS (+Cl) (rel. intensity) 195 (M^++1, 100), 180 (4), 150 (3); M_r (+CI) 195.11322 [M^++1] (calcd for $C_{10}H_{15}N_2O_2$ 195.11335); anal. calcd $C_{10}H_{14}N_2O_2 \cdot 0.45H_2O$ C 59.38%, H 7.37%, N 13.85%; found C 59.53%, H 7.21%, N 13.64%.

4.6. (R)-N-*Benzyl-2-acetamido-3-hydroxypropionamide* ((R)-**11**)

To a THF solution (3 mL) of (*R*)-**10** (0.38 g, 2.0 mmol) was added pyridine (0.16 mL, 2.0 mmol), DMAP (~0.005 g) and Ac_2O (0.19 mL, 2.0 mmol) and the solution stirred at room temperature (1 h). The solvent and pyridine were removed *in vacuo* and the residue purified by column chromatography (SiO_2, 10% MeOH–$CHCl_3$) to obtain pure (*R*)-**11** (0.38 g, 82%) as a white solid: mp 146–147°C (lit.[10] mp 148–149°C); $[\alpha]_D^{23}=+22.0$ (*c*=0.9, MeOH) (lit.[10] $[\alpha]_D^{23}=+22.4$ (*c*=1, MeOH)); R_f 0.40 (10% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.06 (s, C(O)CH_3), 3.64 (dd, *J*=5.0, 11.6 Hz, CHH′OH), 4.18 (dd, *J*=3.2, 11.6 Hz, CHH′OH), 4.45 (d, *J*=6.0 Hz, $NHCH_2$), 4.39–4.52 (m, CH), 6.70–6.75 (m, NH), 7.24–7.39 (m, 5 PhH, NH), the signal for the OH proton was not detected; addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**11** gave only one signal for the acetyl methyl protons.

4.7. (R)-N-*Benzyl-2-acetamido-3-methoxypropionamide*[10] ((R)-**2**)

A mixture of (*R*)-**11** (0.24 g, 1.03 mmol), MeI (0.7 mL, 10.0 mmol) and Ag_2O (1.20 g, 5.0 mmol) in CH_3CN (44 mL) was stirred at room temperature (4 days). The insoluble salts were filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, 10% MeOH–$CHCl_3$) to give (*R*)-**2** (0.21 g, 81%) as a white solid: mp 142–143°C (lit.[10] mp 143–144°C); $[\alpha]_D^{23}=+16.0$ (*c*=1, MeOH) (lit.[10] $[\alpha]_D^{23}=+16.0$ (*c*=1, MeOH)); R_f 0.47 (10% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.02 (s, C(O)CH_3), 3.37 (s, OCH_3), 3.42 (dd, *J*=7.8, 9.0 Hz, CHH′OCH_3), 3.80 (dd, *J*=4.0, 9.0 Hz, CHH′OCH_3), 4.47 (d, *J*=6.0 Hz, $NHCH_2$), 4.49–4.56 (m, CH), 6.41 (br d, *J*=6.0 Hz, NH), 6.73 (br s, NH), 7.22–7.37 (m, 5 PhH), addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**2** gave only one signal for the acetyl methyl and ether methyl protons.

4.8. *Enriched* (R)-*methyl 2*-N-*(benzyloxycarbonyl)amino-3-methoxypropionate* (**12**)

To a CH_3CN solution (150 mL) of (*R*)-**7** (1.72 g, 7.2 mmol) was added successively Ag_2O (8.40 g, 36 mmol) and MeI (4.5 mL, 72 mmol) and the mixture stirred at room temperature (24 h). The mixture was filtered and the filtrate evaporated *in vacuo* to obtain an oily residue, which was purified by column

chromatography (SiO_2, 5% MeOH–$CHCl_3$) to obtain pure **12** (1.81 g, 94%) as a clear oil: $[\alpha]_D^{23}$=+9.5 (c=3.4, MeOH); R_f 0.75 (10% MeOH–$CHCl_3$); IR (liquid film) 3333, 3033, 2953, 1725, 1520, 1455, 1342, 1298, 1213, 1119, 1064, 978, 915, 776, 741, 699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.34 (s, CH_2OCH_3), 3.62 (dd, J=3.3, 9.3 Hz, $CHH'OCH_3$), 3.78 (s, $C(O)OCH_3$), 3.84 (dd, J=3.3, 9.3 Hz, $CHH'OCH_3$), 4.40–4.46 (m, CH), 5.14 (s, $PhCH_2$), 5.67 (br d, J=8.1 Hz, NH), 7.33–7.40 (m, 5 PhH); ^{13}C NMR ($CDCl_3$) 52.8 ($C(O)OCH_3$), 54.5 (CH), 59.5 (CH_2OCH_3), 67.2 ($PhCH_2$), 72.5 (CH_2OCH_3), 128.3 ($2C_2'$ or $2C_3'$), 128.4 (C_4'), 128.7 ($2C_2'$ or $2C_3'$), 136.4 (C_1'), 156.2 ($C(O)NH$), 171.0 ($C(O)O$) ppm; MS (+CI) (rel. intensity) 268 (M^++1, 100), 224 (40); M_r (+CI) 268.11835 [M^++1] (calcd for $C_{13}H_{18}NO_5$ 268.11849); anal. calcd $C_{13}H_{17}NO_5 \cdot 0.25H_2O$ C 57.46%, H 6.44%, N 5.16%; found C 57.61%, H 6.43%, N 5.08%.

4.9. Methyl 2-[N-(2-menthoxy)acetyl]amino-3-methoxypropionate (17). Determination of enantiomeric purity of 12

A MeOH (10 mL) solution of **12** (0.75 g, 2.8 mmol) was hydrogenated at room temperature (3 h) in the presence of 10% Pd–C (0.08 g). The catalyst was removed by filtration through Celite and the filtrate evaporated *in vacuo* to obtain the crude amine **13**. Using the mixed anhydride procedure described earlier for the preparation of (*R*)-**8** and utilizing (−)-menthoxyacetic acid (0.61 g, 2.8 mmol), THF (11 mL), 4-methylmorpholine (0.35 mL, 3.1 mmol), isobutyl chloroformate (0.41 mL, 3.1 mmol), and **13**, the oily product **17** was obtained (0.38 g, 40%) as a mixture of diastereomers following purification by column chromatography (SiO_2, Et_2O): R_f 0.63 (Et_2O); ^1H NMR ($CDCl_3$) δ (major diastereomer): 0.79 (d, J=6.6 Hz, $C(4'')H_3$), 0.84–1.10 (m, $C(1'')H$, $C(2'')H_3$, $C(3'')H_3$ and $C(4')H_2$), 1.25–1.45 (m, $C(3')H_2$), 1.60–1.70 (m, $C(6')H_2$), 2.00–2.10 (m, $C(5')H$), 2.10–2.25 (m, $C(2')H$), 3.13–3.20 (m, $C(1')H$), 3.35 (s, CH_2OCH_3), 3.57 (dd, J=3.6, 9.3 Hz, $CHH'OCH_3$), 3.77 (s, $C(O)OCH_3$), 3.81–3.95 (m, $CHH'OCH_3$ and $C(O)CHH'$), 4.12 (1/2 AB q, J=15.3 Hz, $C(O)CHH'$), 4.72–4.94 (m, $CHCH_2OCH_3$), 7.43 (d, J=8.1 Hz, NH); (minor diastereomer): δ 0.80 (d, J=6.6 Hz, $C(4'')H_3$), 4.11 (1/2 AB q, J=15.3 Hz, $C(O)CHH'$), all other signals for the minor diastereomer are believed to overlap with those for the major diastereomer, ^1H NMR analysis indicated the major to minor diasteromeric ratio to be 85:15. ^{13}C NMR ($CDCl_3$) (major diastereomer): 16.5 ($C(4'')$), 21.1 ($C(2'')$ or $C(3'')$), 22.4 ($C(2'')$ or $C(3'')$), 23.5 ($C(3')$ or $C(4')$), 26.3 ($C(3')$ or $C(4')$), 31.6 ($C(5')$), 34.5 ($C(1'')$), 40.3 ($C(6')$), 48.1 ($C(2')$), 52.1 ($CHCH_2OCH_3$ or $C(O)OCH_3$), 52.8 ($CHCH_2OCH_3$ or $C(O)OCH_3$), 59.5 (CH_2OCH_3), 68.1 ($OCH_2C(O)$), 72.5 (CH_2OCH_3), 81.2 ($C(1')$), 170.6 ($C(O)NH$ or $C(O)OCH_3$), 170.7 ($C(O)NH$ or $C(O)OCH_3$) ppm; (minor diastereomer): 16.4 ($C(4'')$), 26.2 ($C(3')$ or $C(4')$), 48.2 ($C(2')$), 68.0 ($OCH_2C(O)$) ppm, all other signals for the minor diastereomer are believed to overlap with those for the major diastereomer; MS (+CI) (rel. intensity) 330 (M^++1, 100), 192 (47), 175 (12); M_r (+CI) 330.22725 [M^++1] (calcd for $C_{17}H_{32}NO_5$ 330.22804).

4.10. Enriched (R)-methyl 2-acetamido-3-methoxypropionate (14)

To a MeOH solution (50 mL) of **12** (6.97 g, 26.1 mmol) was added 10% Pd–C (2.0 g) and the mixture was stirred at room temperature in the presence of H_2 gas (24 h). The catalyst was removed by filtration through Celite and the filtrate evaporated *in vacuo* to obtain the amine **13** (oil), which was acetylated without further purification. Crude amine **13** was dissolved in dry THF (15 mL) and then pyridine (2.6 mL, 31.3 mmol) and Ac_2O (3.0 mL, 31.3 mmol) were added successively and the reaction was stirred at room temperature (2 h). The solvent was removed *in vacuo* and the residue purified by column chromatography (SiO_2, gradient elution with 1% MeOH–$CHCl_3$ and then 5% MeOH–$CHCl_3$)

followed by recrystallization from Et_2O to obtain pure **14** (3.52 g, 77%): mp 76–78°C; $[\alpha]_D^{23}$=+7.8 (c=1.0, MeOH); R_f 0.54 (5% MeOH–$CHCl_3$); IR (KBr) 3299, 2945, 2360, 1742, 1644, 1551, 1436, 1379, 1343, 1216, 1117, 1064, 981 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.06 (s, $CH_3C(O)$), 3.34 (s, CH_2OCH_3), 3.61 (dd, J=3.5, 9.5 Hz, $CHH'OCH_3$), 3.77 (s, $C(O)OCH_3$), 3.81 (dd, J=3.5, 9.5 Hz, $CHH'OCH_3$), 4.72–4.78 (m, CH), 6.66 (br d, J=6.6 Hz, NH); ^{13}C NMR ($CDCl_3$) 22.9 ($CH_3C(O)$), 52.5 (CH and $C(O)OCH_3$), 59.3 (CH_2OCH_3), 72.2 (CH_2OCH_3), 170.1 ($C(O)NH$ or $C(O)OCH_3$), 170.9 ($C(O)NH$ or $C(O)OCH_3$) ppm; MS (+CI) (rel. intensity) 176 (M^++1, 100), 144 (19); M_r (+CI) 176.09251 [M^++1] (calcd for $C_7H_{14}NO_4$ 176.09228); anal. calcd $C_7H_{13}NO_4$ C 48.00%, H 7.43%, N 8.00%; found C 47.80%, H 7.43%, N 7.96%.

4.11. *Enriched (R)-2-acetamido-3-methoxypropionic acid (4)*

Method A: An aqueous (2.0 mL) solution of K_2CO_3 (0.05 g, 0.36 mmol) and **14** (0.1 g, 0.57 mmol) was stirred at room temperature (8 h). The solution was extracted with Et_2O (5 mL) and the aqueous layer was evaporated *in vacuo*. The residue was dissolved in H_2O (2 mL) and the solution adjusted to pH 3.0 (aqueous 5 N HCl). The acidified solution was evaporated to dryness *in vacuo* and the residue was suspended in $CHCl_3$ (5 mL). The insoluble salts were removed by filtration and the filtrate evaporated *in vacuo* to obtain **4** as an off-white foam (0.07 g, 78%). Compound **4** was subsequently analyzed for optical purity by its conversion to **2**.

Method B: A saturated aqueous $NaHCO_3$ (20 mL) solution containing **14** (2.00 g, 11.4 mmol) was stirred at room temperature (24 h). The solution was extracted with Et_2O (2×20 mL) and the aqueous layer was separated and evaporated to dryness *in vacuo*. The residue was dissolved in a minimal amount of H_2O (~10 mL) and adjusted to pH 3.0 using aqueous 5 N HCl. The acidic solution was evaporated *in vacuo* to dryness and the residue was suspended in $CHCl_3$ (50 mL). The insoluble salts were removed by filtration and were washed with $CHCl_3$ (2×25 mL). The $CHCl_3$ layers were combined, dried (Na_2SO_4), filtered, and evaporated *in vacuo* to obtain **4** as an off-white foam (1.60 g, 87%): $[\alpha]_D^{23}$=−16.9 (c=1.2, MeOH); R_f 0.10 (20% MeOH–$CHCl_3$); IR (KBr) 3333, 2938, 2833, 1734, 1633, 1549, 1447, 1378, 1340, 1301, 1220, 1148, 1117, 1058, 1025, 975 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.08 (s, $CH_3C(O)$), 3.36 (s, OCH_3), 3.66 (dd, J=3.0, 9.6 Hz, $CHH'OCH_3$), 3.86 (dd, J=3.3, 9.6 Hz, $CHH'OCH_3$), 4.72–4.78 (m, CH), 6.95 (d, J=8.1 Hz, NH), 11.33 (br s, C(O)OH); ^{13}C NMR ($CDCl_3$) 22.8 ($CH_3C(O)$), 52.9 (CH), 59.3 (CH_2OCH_3), 72.1 (CH_2OCH_3), 171.9 ($C(O)NH$ or $C(O)OH$), 172.7 ($C(O)NH$ or $C(O)OH$); MS (+CI) (rel. intensity) 162 (M^++1, 100), 130 (15), 120 (14); M_r (+CI) 162.07665 [M^++1] (calcd for $C_6H_{12}NO_4$ 162.07663); anal. calcd $C_6H_{11}NO_4 \cdot 0.8\ H_2O$ C 41.05%, H 7.18%, N 7.98%; found C 41.05%, H 7.23%, N 7.80%.

4.12. *N-Benzyl-2-acetamido-3-methoxypropionamide (2). Determination of enantiomeric purity of 4*

Employing the mixed anhydride procedure described previously for the preparation of (*R*)-**8** and using **4** (0.05 g, 0.3 mmol), THF (3 mL), 4-methylmorpholine (0.04 mL, 0.3 mmol), isobutyl chloroformate (0.04 mL, 0.3 mmol), and benzylamine (0.035 mL, 0.3 mmol), **2** was obtained (0.05 g, 74%) following purification by PTLC (SiO_2, 5% MeOH–$CHCl_3$): mp 122–123°C (lit.[10] mp 143–144°C); $[\alpha]_D^{23}$=+10.2 (c=1, MeOH) (lit.[10] $[\alpha]_D^{23}$=+16.0 (c=1, MeOH)); R_f 0.47 (10% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.04 (s, $C(O)CH_3$), 3.38 (s, OCH_3), 3.44 (dd, J=7.8, 9.0 Hz, $CHH'OCH_3$), 3.81 (dd, J=4.2, 9.0 Hz, $CHH'OCH_3$), 4.48 (d, J=5.7 Hz, $NHCH_2$), 4.51–4.58 (m, CH), 6.40–6.50 (m, NH), 6.75 (br s, NH), 7.23–7.40 (m, 5 PhH), addition of excess (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-**2** gave two signals each for the acetyl methyl and the ether methyl protons in a ratio of 85:15.

4.13. Enriched (R)-2-N-(benzyloxycarbonyl)amino-3-methoxypropionic acid (15)

Method A: A mixture containing **12** (0.58 g, 2.2 mmol), MeOH–H_2O (9:1, 10 mL) and K_2CO_3 (0.5 g, 3.62 mmol) was stirred at room temperature (8 h). The mixture was poured into H_2O (5 mL), adjusted to pH 3.0 (aqueous 5 N HCl) and then extracted with EtOAc (3×25 mL). The organic extracts were combined, dried (Na_2SO_4), filtered, and evaporated *in vacuo* to obtain **15** as a clear oil. Subsequent conversion of **15** to **16** allowed determination of its optical purity.

Method B: A mixture of **12** (0.09 g, 0.33 mmol), MeOH (2.5 mL) and saturated aqueous $NaHCO_3$ (2.5 mL) was stirred at room temperature (24 h) and then diluted with H_2O (20 mL). The mixture was cooled (0°C), acidified to pH 3.0 (5 N HCl) and extracted with EtOAc (3×25 mL). The EtOAc extracts were combined, dried (Na_2SO_4), filtered and evaporated *in vacuo* to obtain **15** (0.08 g, 98%) as a clear oil: $[\alpha]_D^{23}=-3.2$ (c=1.0, MeOH); R_f 0.30 (10% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.32 (s, OCH_3), 3.61 (dd, J=3.2, 9.3 Hz, CHH′OCH$_3$), 3.85 (dd, J=2.7, 9.3 Hz, CHH′OCH$_3$), 4.45–4.56 (m, CH), 5.11 (s, PhCH_2), 5.78 (d, J=8.4 Hz, NH), 7.28–7.47 (m, 5 PhH); ^{13}C NMR ($CDCl_3$) 54.2 (*C*H), 59.3 (O*C*H$_3$), 67.3 (Ph*C*H$_2$), 72.2 (*C*H$_2$OCH$_3$), 128.1 (2C_2′ or 2C_3′), 128.3 (C_4′), 128.6 (2C_2′ or 2C_3′), 136.2 (C_1′), 156.5 (*C*(O)NH), 174 (*C*(O)OH) ppm; MS (+CI) (rel. intensity) 254 (M^++1, 35), 224 (22), 210 (27), 146 (40), 118 (41), 113 (32), 91 (100); M_r (+CI) 254.10328 [M^++1] (calcd for $C_{12}H_{16}NO_5$ 254.10284); anal calcd $C_{12}H_{15}NO_5 \cdot 0.25H_2O$ C 55.92%, H 6.02%, N 5.44%; found C 56.08%, H 6.15%, N 5.24%.

4.14. Determination of optical purity of 15. Preparation of 16

Employing the mixed anhydride procedure described previously for the preparation of (*R*)-**8** and using **15** (0.08 g, 0.3 mmol), THF (3 mL), 4-methylmorpholine (0.05 mL, 0.4 mmol), isobutyl chloroformate (0.05 mL, 0.4 mmol), and (*S*)-α-methylbenzylamine (0.05 mL, 0.4 mmol), **16** was obtained (0.08 g, 71%) as an 85:15 mixture of diastereomers following purification by PTLC (SiO_2, 5% MeOH–$CHCl_3$): mp 118–124°C; R_f 0.77 (5% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$) (major diastereomer): δ 1.48 (d, J=7.2 Hz, CHCH_3), 3.37 (s, OCH_3), 3.41–3.56 (m, CHH′OCH$_3$), 3.75–3.85 (m, CHH′OCH$_3$), 4.25–4.40 (m, CHCH$_2$OCH$_3$), 5.06–5.18 (m, PhCH_2 and CHCH$_3$), 5.63–5.79 (m, OC(O)NH), 6.62–6.89 (m, C(O)NH), 7.20–7.40 (m, 10 PhH); (minor diastereomer): δ 3.35 (s, OCH_3), all other signals for the minor diastereomer are believed to overlap with those for the major diastereomer, ^{1}H NMR analysis indicated the ratio of (*R*)-**16** to (*S*)-**16** was 85:15; ^{13}C NMR ($CDCl_3$) 22.1 and 22.2 (CH*C*H$_3$, diastereomer *a* or *b*), 49.1 (*C*HCH$_3$), 54.1 and 54.2 (*C*HCH$_2$OCH$_3$, diastereomer *a* or *b*), 59.2 (CH$_2$O*C*H$_3$), 67.4 (Ph*C*H$_2$), 72.2 (*C*H$_2$OCH$_3$), 126.1 and 126.2 (2C_3′ or 2C_3″), 127.5 (C_4′ or C_4″), 128.2 and 128.3 (2C_3′ or 2C_3″, diastereomer *a* or *b*), 128.4 (C_4′ or C_4″), 128.7 (2C_2′ or 2C_2″), 128.8 (2C_2′ or 2C_2″), 136.2 (C_1″), 143.0 and 143.2 (C_1′, diastereomer *a* or *b*), 156.3 (*C*(O)O), 169.1 and 169.2 (*C*(O)NH, diastereomer *a* or *b*) ppm; MS (+CI) (rel. intensity) 357 (M^++1, 100), 268 (22), 224 (18), 222 (16); M_r (+CI) 357.18158 [M^++1] (calcd for $C_{20}H_{25}N_2O_4$ 357.18143).

4.15. Enriched (R)-O-methylserine (5)[21]

A MeOH solution (50 mL) of **15** (4.62 g, 18.3 mmol) was hydrogenated at room temperature (24 h) in the presence of 10% Pd–C (1.00 g). The catalyst was removed by filtration through Celite and the filtrate evaporated *in vacuo* to give a pale yellow solid, which was recrystallized from EtOH to give **5** as a white crystalline solid (1.50 g, 69%): mp 228–230°C dec; $[\alpha]_D^{23}=+8.7$ (c=0.7, MeOH); IR (KBr) 3433, 3061, 1629, 1586, 1500, 1412, 1350, 1308, 1196, 1122, 1102, 1004, 971 cm^{-1}; ^{1}H NMR (CD_3OD) δ 3.39 (s, OCH_3), 3.65–3.84 (m, CHCH$_2$); ^{13}C NMR (CD_3OD) 56.3 (*C*H), 59.4 (CH$_2$O*C*H$_3$), 72.2 (*C*H$_2$OCH$_3$),

172.2 (*C*(O)OH) ppm; MS (+CI) (rel. intensity) 120 (M^++1, 100), 88 (12); M_r (+CI) 120.06593 [M^++1] (calcd for $C_4H_{10}NO_3$ 120.06607); anal. calcd $C_4H_9NO_3 \cdot 0.4H_2O$ C 38.03%, H 7.76%, N 11.09%; found 38.15%, H 7.61%, N 11.04%.

Compound **5** (0.01 g) was dissolved in aqueous 5 N HCl (1 mL) and the solution evaporated *in vacuo* to dryness without the use of heat. The residue was recrystallized from EtOH–Et_2O (2×) and **5**·HCl was isolated by filtration and dried in a vacuum desiccator (24 h): mp 177–179°C dec.; $[\alpha]_D^{23}=-10.1$ (*c*=0.6, MeOH); anal. calcd $C_4H_9NO_3 \cdot HCl$ C 30.87%, H 6.43%, N 9.00%, Cl 22.83%; found C 30.97%, H 6.42%, N 8.99%, Cl 22.87%.

4.16. N-(α-Methylbenzyl)-2-N-(benzyloxycarbonyl)amino-3-methoxypropionamide (16). Determination of the enantiomeric purity of 5

Preparation of 2-*N*-(benzyloxycarbonyl)amino-3-methoxypropionic acid (**15**) from **5**: To a solution of **5** (0.02 g, 0.17 mmol) in H_2O (0.3 mL) was added MgO (0.02 g, 0.5 mmol) and Et_2O (0.15 mL). The mixture was cooled to 0°C in an ice bath and benzyl chloroformate (95%, 0.04 mL) was added dropwise. After stirring at 0°C (2 h), the mixture was allowed to warm to room temperature and stirring was continued (30 min). The mixture was filtered and the filtrate diluted with H_2O (10 mL) and extracted with Et_2O (2×10 mL). The aqueous layer was separated, cooled to 0°C and the pH carefully adjusted to 3.0 using aqueous 5 N HCl. The acidified solution was extracted with EtOAc (3×15 mL) and the organic extracts were combined, dried (Na_2SO_4), filtered and evaporated *in vacuo* to obtain **15** (0.04 g, 83%) as a clear oil; $[\alpha]_D^{23}=-3.3$ (*c*=1.4, MeOH); R_f 0.30 (10% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.33 (s, OCH_3), 3.61 (dd, *J*=3.2, 9.3 Hz, $CHH'OCH_3$), 3.85 (dd, *J*=2.7, 9.3 Hz, $CHH'OCH_3$), 4.46–4.52 (m, *CH*), 5.12 (s, $PhCH_2$), 5.75 (d, *J*=8.4 Hz, N*H*), 7.27–7.46 (m, 5 Ph*H*).

Preparation of **16**: Employing the mixed anhydride procedure described previously for the preparation of (*R*)-**8** and using **15** (0.025 g, 0.1 mmol), THF (3 mL), 4-methylmorpholine (0.014 mL, 0.1 mmol), isobutyl chloroformate (0.016 mL, 0.1 mmol), and (*S*)-α-methylbenzylamine (0.015 mL, 0.1 mmol), **16** was obtained (0.03 g, 88%) as a 85:15 mixture of diastereomers following purification by PTLC (SiO_2, 5% MeOH–$CHCl_3$): R_f 0.77 (5% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$) (major diastereomer): δ 1.47 (d, *J*=6.6 Hz, $CHCH_3$), 3.37 (s, OCH_3), 3.43–3.53 (m, $CHH'OCH_3$), 3.76–3.84 (m, $CHH'OCH_3$), 4.20–4.31 (m, $CHCH_2OCH_3$), 5.05–5.20 (m, $PhCH_2$ and $CHCH_3$), 5.60–5.75 (m, OC(O)N*H*), 6.60–6.65 (m, C(O)N*H*), 7.20–7.40 (m, 10 Ph*H*); (minor diastereomer): δ 3.35 (s, OCH_3), all other signals are believed to overlap with those for the major diastereomer, ^{1}H NMR analysis indicated the ratio of (*R*)-**16** to (*S*)-**16** was 85:15.

4.17. Pharmacology

Compounds were screened under the auspices of the National Institutes of Health for anticonvulsant activity in male albino Carthworth Farms No. 1 mice (ip route) and male albino Sprague Dawley rats (oral (po) routes). All of the compounds were administered in suspensions of 0.5% (w/v) of methylcellulose in water. The volumes are 0.01 mL/g of body weight for mice. Activity was established using the electrical (maximal electroshock or MES) test.[22] In the MES test, a drop of electrolyte solution with 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 current. A 60-cycle alternating current was administered for 0.2 s in both species, 50 mA in mice and 150 mA in rats.[23] Protection endpoints were defined as the abolition of the hind limb tonic extensor component of the induced seizure. In mice, effects of compounds on forced spontaneous motor activity were determined using the rotorod test. The inability

of animals to maintain their balance for 1 min on a 1 in. diameter knurled rod rotating at 6 rpm in three successive trials demonstrated motor impairment. Normally under these conditions, a mouse can maintain its balance indefinitely. In rats, motor impairment is assessed by observing for overt evidence of ataxia, abnormal gait and stance, and/or loss of placing response and muscle tone. In the mouse identification screening study all compounds were administered at three dose levels (30, 100, 300 mg/kg) and 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 at 100 mg/kg. In the rotorod toxicity test four animals were used at 30 and 300 mg/kg, and eight animals at 100 mg/kg (Table 1). In the rat identification screening study with po administration four animals were used at a dose of 30 mg/kg for both the MES and the rotorod toxicity tests and the activity monitored for four hours.

The quantitative determination of the median effective (ED_{50}) and toxic doses (TD_{50}) were conducted at previously calculated times of peak effect. 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 candidate substance required to produce the defined endpoint 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.[24]

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