Novel Cyclopropyl β-Amino Acid Analogues of Pregabalin and Gabapentin That Target the α_2-δ Protein

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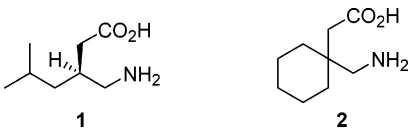
As part of a program aimed at generating compounds with affinity for the α_2-δ subunit of voltage-gated calcium channels, several novel β-amino acids were prepared using an efficient nitroalkane-mediated cyclopropanation as a key step. Depending on the ester that was chosen, the target amino acids could be prepared in as few as three steps. The cyclopropyl amino acids derived from ketones proved to be potent binders of the α_2-δ subunit of voltage-gated calcium channels, but did not interact with the large neutral amino acid system L (leucine) transporter. Anticonvulsant effects were observed in vivo with compound **34** but only after intracerebroventricular (icv) administration, presumably due to inadequate brain concentrations of the drug being achieved following oral dosing. However, pregabalin **1** was active in the DBA/2 model after oral (and icv) dosing, supporting a hypothesis that active transport is a prerequisite for such zwitterionic species to cross the blood−brain barrier.

Introduction

Pregabalin **1** has demonstrated potent and robust activity in preclinical models of epilepsy, neuropathic pain, and anxiety and is currently approved or in clinical development for these conditions.[1] Both pregabalin **1** and gabapentin **2**[2] have been shown to bind potently to the α_2-δ subunit of voltage-gated calcium channels.[3,4] As a consequence of binding to α_2-δ, calcium influx is modulated at nerve terminals[5] which in turn reduces the release of several neurotransmitters including glutamate,[6] norepinephrine,[7] and substance P.[8] It is through the inhibition of calcium flux and subsequent attenuation of neurotransmitter release that **1** and **2** are postulated to exert their therapeutic effects.[9] Consistent with this hypothesis, it has been demonstrated that the affinity of analogues of **1** and **2** for α_2-δ (as evidenced by the displacement of [^{3}H]-gabapentin from pig brain membranes) was proportional to their activity in an in vivo seizure model.[10−12] As part of a program to generate SAR around additional α_2-δ ligands,[13−16] we were interested in the possibility of installing alkyl substituents at the position α- to the amino function of gabapentin and examining the effect on α_2-δ binding affinity. To accomplish this task, unsaturated cyanoester **3** derived from cyclohexanone was treated with nitroethane and DBU at ambient temperature. Instead of isolating the expected Michael adduct **4**, however, we were surprised to find that a facile cyclization had occurred, furnishing spirocyclopropane **5**. Indeed, on inspection of the literature we found several methods available for effecting this nitroalkane-mediated cyclopropanation, including potassium carbonate,[17] potassium *tert*-butoxide,[18] and KF/alumina.[19] However, to our knowledge no reports concerning the conversion of these cyclic cyanoesters to the corresponding β-amino acids have appeared. We felt that novel compounds of this type, having amine and carboxylate functionalities in similar proximity to those of gabapentin and pregabalin, albeit with a more rigid framework, might also be ligands for the α_2-δ subunit. The preparation and biological testing of such constrained analogues thus encompass the subject of this report.[20,21]



1 2

Figure 1. Structures of pregabalin **1** and gabapentin **2**.

Chemistry

It was envisioned that use of a benzyl ester in the Knoevenagel reaction would, after cyclopropanation with nitromethane, result in a substrate that could be transformed in one operation to the desired amino acids. Gratifyingly, this proved to be the case (Scheme 1). Hence, condensation of 2-ethylbutyraldehyde **6** or 4-heptanone **7** with benzyl cyanoacetate and subsequent cyclization with nitromethane afforded cyclopropane cyanoesters **10** and **11** in good overall yield. In the cases where an aldehyde was employed, only a single diastereomer of the cyclopropane was obtained, presumably placing the nitrile and the alkyl group in the cis-orientation in accordance with literature precedent.[18] Although the Michael addition occurred at ambient temperature, the aldehyde-derived substrates required heating to effect the ring closing process. Exhaustive reduction of the benzyl ester and nitrile was effected by hydrogenation catalyzed by PtO_2. Unfortunately,

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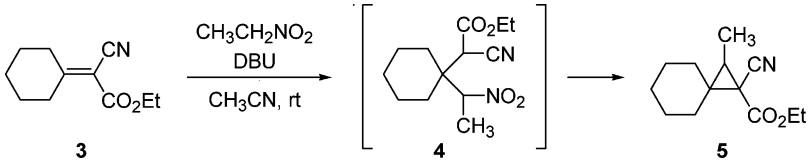
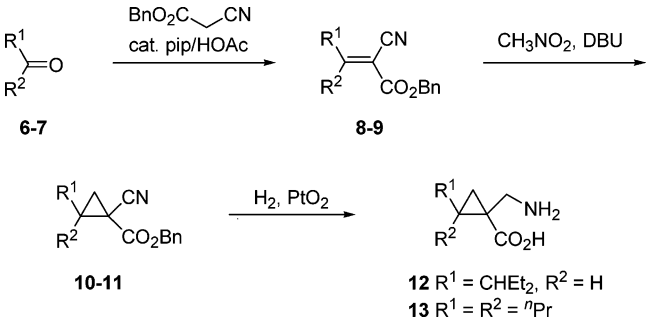


Figure 2. Facile cyclopropanation of **3** with nitroethane.

Scheme 1



although the route proved expeditious, the yield of the reduction step was quite low after purification of the amino acids **12** and **13** (11−18%). As a result, we set out to develop a more efficient and scalable process to obtain the target amino acids.

The second-generation synthesis, typified as follows, commenced with the condensation of cyclopentanone with methyl cyanoacetate under azeotropic dehydration conditions (Scheme 2). Nitromethane addition and subsequent cyclization occurred smoothly at ambient temperature, providing spirocyclopropane **16** in 62% yield. Chemoselective reduction of the nitrile function was effected using the sodium borohydride/Co(II) reagent system.[22] The aminoester **17** was subsequently hydrolyzed under basic conditions (39%) to give amino acid **18** after acidification.

In an attempt to expand the synthetic utility of the process, we were interested in generating differentially protected amino acid core structures that would also be amenable to further elaboration (i.e. insertion into a peptide sequence). Thus, the requisite carbonyl substrates (cyclohexanone **19**, isobutyraldehyde **20**, isovaleraldehyde **21**) were first converted in the usual way to the cyclopropane derivatives (Scheme 3). Although the cyclopropanation of **22** with nitromethane proceeded at ambient temperature (DBU, CH_3CN), we found the KF−alumina conditions[19] to be most appropriate for the aldehyde-derived unsaturated cyanoesters **23** and **24**. Following reduction of the nitrile, the amine was capped with a *tert*-butoxycarbonyl group, leaving open the possibility for further manipulation at either terminus. To demonstrate this point, aminoesters **28**−**30** could be hydrolyzed to give the *N*-protected carboxylic acids **31**−**33**. These valuable synthetic building blocks could also be easily converted (63−88%) to the corresponding β-amino acids **34**−**36** upon exposure to hydrochloric acid in dioxane.

As a further test of this methodology, we set out to prepare novel amino acids containing multiple cyclopropane units.[23] Hence, cyclopropanecarboxaldehyde **37** underwent smooth condensation with ethyl cyanoacetate to provide cyanoester **38** (Scheme 4). Completion of the sequence (under nonoptimized conditions) afforded the bis-cyclopropanated amino acid **40**.[24]

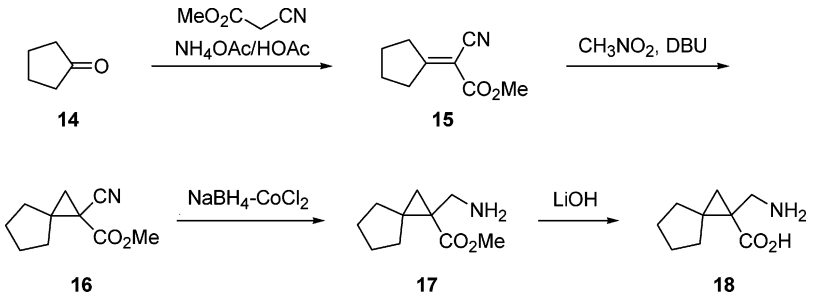
Finally, we wished to prepare the aldehyde-derived cyclopropane amino acids bearing the opposite relative configuration (i.e. alkyl group cis to carboxylate). To achieve this objective while taking advantage of the readily accessible intermediate cyclopropyl cyanoesters described above, the ester would need to be converted to an aminomethyl moiety and the nitrile to a carboxylic acid. Such utility of the cyanoester functionality has recently been demonstrated by Díaz-de-Villegas and Gálvez in an enantioconvergent synthesis of (*S*)-α-benzyl-α-methyl-β-alanine.[25] Hence, preparation of the cyclopropyl cyanoesters **43** (R = ^{i}Bu) and **44** (R = $CHEt_2$) by the usual method was followed by borohydride reduction of the ester to provide cyano alcohols **45** and **46** (Scheme 5). Surprisingly, the alcohols were not converted to the mesylate on treatment with methanesulfonyl chloride, but rather the chloronitriles **47** and **48**.[26] This was of no consequence as the purified chloronitriles could be smoothly displaced by azide anion to afford azidonitriles **49** and **50** in nearly quantitative yield. Finally, hydrogenation and hydrolysis of the resultant aminoesters furnished the desired amino acids **53** and **54** after purification. Compound **53** was diastereomeric with **36** and compound **54** was diastereomeric with **12**.

Pharmacology

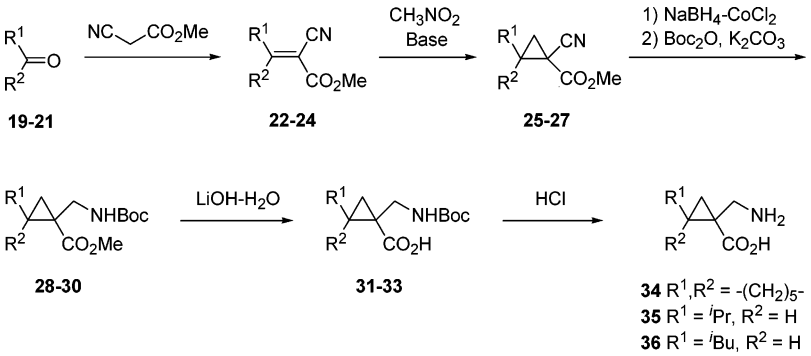
Of the compounds disclosed in this report, in general the ketone-derived substrates showed greater potency for the α_2-δ subunit of voltage-gated calcium channels (as evidenced by their ability to potently inhibit [^{3}H]-gabapentin binding to pig brain membranes, see Table 1). For instance, the dipropyl- (**13**), cyclopentyl- (**18**), and cyclohexyl-substituted (**34**) cyclopropyl β-amino acids all bound with K_i values (equilibrium dissociation constants) less than or equal to 0.2 μM, demonstrating high potency. In contrast, none of the aldehyde-derived amino acids containing the alkyl and carboxylate in the trans-orientation about the cyclopropane ring showed comparably potent (<0.2 μM) affinity for α_2-δ. However, when the relative orientation of alkyl and carboxylate groups in **36** was reversed as in **53** (cis-orientation), a 10-fold increase in α_2-δ binding affinity was observed. This did not prove to be the case for switching the relative orientation of the 1-ethyl-propyl group of **12**, as the isomer **54** was found to have similar potency to **12**. Noteworthy is the fact that unsubstituted 1-aminomethylcyclopropane carboxylic acid **55**[27] did not bind to α_2-δ or system L, and as a point of reference, pregabalin **1** demonstrated 0.019 μM binding affinity to α_2-δ (K_i) and 158 μM to system L (IC_{50}) as a single enantiomer.

As can also be seen from Table 1, unlike pregabalin **1**, none of the cyclopropyl amino acids were able to significantly inhibit [^{3}H]leucine uptake by the system L amino acid transporter.[28] The ability of pregabalin and gabapentin to be transported by system L is thought to play a pivotal role in allowing these zwitterionic mol-

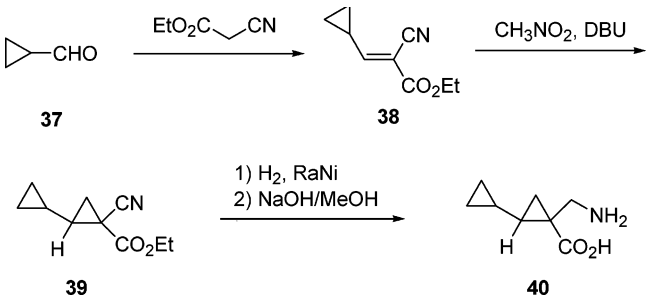
Scheme 2



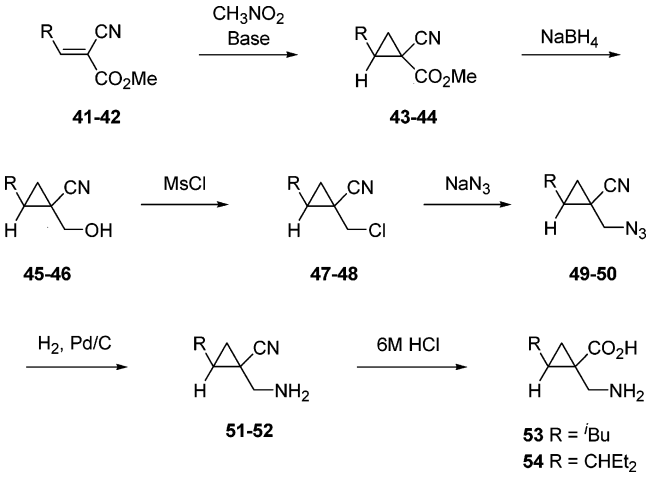
Scheme 3



Scheme 4



Scheme 5

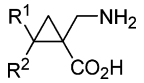


ecules to enter the systemic circulation following oral dosing and cross the blood−brain barrier. This notion was borne out through in vivo mouse anticonvulsant testing, whereby the antiepileptic activity of compounds was measured by their ability to prevent audiogenically induced seizures in the DBA/2 strain of mice.[29] Activity in this assay is expressed as a percentage of mice protected from seizure (Graph 1). Whereas pregabalin showed robust anticonvulsant activity when dosed orally or by injection into the cerebral ventricles (icv), compound **34** only protected DBA/2 mice from audiogenic seizures when the compound was introduced directly into the brain through icv dosing. This observation served to further implicate the system L transporter in mediating the brain uptake of amino acid-based α_2-δ ligands. This point is further illustrated by examining the brain and plasma levels achieved by both compounds at 2 h following 30 mg/kg po dosing in Sprague−Dawley rats (Table 2). Not only was the brain to plasma (b/p) ratio of **34** significantly smaller than that shown with pregabalin **1** (0.054 vs 0.22, respectively), but the absolute plasma concentrations for **34** were also significantly lower, which may suggest the active role of system L (or another transporter) in improving brain penetration and increasing the extent of absorption of these zwitterionic amino acids by the gastrointestinal tract. Further studies to elucidate the mechanism by which these compounds are absorbed and distributed to the brain are in progress.

Conclusion

Using an efficient cyclopropanation reaction of electron-deficient olefins, several novel β-amino acids were prepared efficiently and in good overall yields. The most expeditious route used only three steps but the efficiency was somewhat compromised. As a result, a more reliable method was developed, which also allowed access to differentially substituted termini of the amino acid products. The ketone-derived cyclopropyl amino acids were shown to be potent substrates for the α_2-δ subunit, unlike the less hindered aldehyde-derived substrates which in two cases were prepared with the opposite relative stereochemistry. However, none of the compounds were substrates for the system L transporter, thought to be required for efficient brain penetration. This was supported by in vivo anticonvulsant studies which showed that activity could only be achieved if the

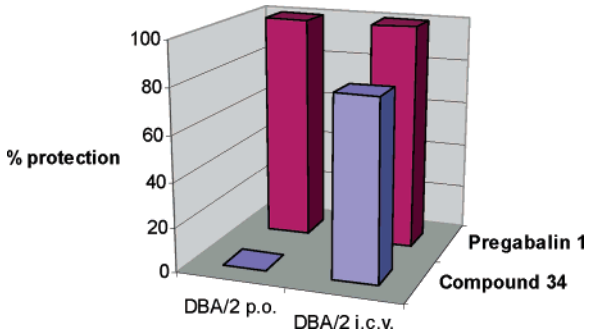
Table 1. α_2-δ and System L Transporter Affinity of Cyclopropyl-β-Amino Acids



compd	R^1	R^2	α_2-δ binding affinity (K_i, μM)[a]	system L binding affinity (IC_{50}, μM)[b]
1	- -	- -	0.019 ± 0.003	158 ± 35
12	$CH(CH_2CH_3)_2$	H	0.56 ± 0.11	>8300
13	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	0.20 ± 0.04	>2490
18	$-CH_2CH_2CH_2CH_2-$		0.023 ± 0.007	>8300
34	$-CH_2CH_2CH_2CH_2CH_2-$		0.013 ± 0.001	>2490
35	$CH(CH_3)_2$	H	0.34 ± 0.10	>8300
36	$CH_2CH(CH_3)_2$	H	0.33 ± 0.013	>2490
40	*c*-C_3H_5	H	0.20 ± 0.055	>2490
53	H	$CH_2CH(CH_3)_2$	0.037 ± 0.004	>8300
54	H	$CH(CH_2CH_3)_2$	0.63 ± 0.08	>2490
55	H	H	>10	>8300

[a] See Experimental Section. Data reported as mean ± SEM (N = 4 experiments). [b] IC_{50} is the concentration (μM) producing half-maximal inhibition of the uptake of [^{3}H]leucine into CHO cells.

Chart 1. Anticonvulsant Activity of Pregabalin **1** and Compound **34** in DBA/2 Mice[a]



[a] % protection is the fraction of DBA/2 mice (N = 10 animals) protected from audiogenically induced tonic seizures at 2 h after dosing by a 30 mg/kg po dose of the test compound, or a 30 μg icv dose using saline as vehicle (see Experimental Section).

Table 2. Comparison of Brain and Plasma Levels for Pregabalin **1** and Compound **34** at 2 Hours Following 30 mg/kg po Dosing in Sprague−Dawley Rats

compd	system L (IC_{50}, μM)	brain levels (ng/mL)	plasma levels (ng/mL)	*B/P*
1	158 ± 35	4330 ± 347	19367 ± 2021	0.22
34	>2490	385 ± 88.7	7070 ± 572	0.054

compounds were administered by injection into the cerebral ventricles.

Experimental Section

Unless otherwise indicated, all reagents were purchased from Sigma-Aldrich Corporation and used without purification. All reactions were monitored by thin-layer chromatography on Merck glass plates precoated with 0.25 mm of silica gel. Chromatography for purification was done with Merck silica gel (230−400 mesh) or Biotage cartridges packed with 32−63 μM silica gel. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Mass spectra were obtained on a Micromass Platform LC mass spectrometer and are chemical ionization spectra. NMR spectra were obtained on either a Varian 300 or a Varian 400 spectrometer with tetramethylsilane as an internal standard. Combustion analyses were performed by QTI Laboratories.

2-Cyano-4-ethylhex-2-enoic Acid Benzyl Ester 8. To a mixture of 2-ethylbutyraldehyde **6** (2.0 mL, 16.2 mmol) and benzyl cyanoacetate (2.85 g, 16.2 mmol) at 0 °C were added acetic acid (0.1 mL, 1.62 mmol) and then piperidine (0.16 mL, 1.62 mmol) dropwise. The ice bath was removed and stirring continued for 10 min. Additional acetic acid (0.1 mL) and piperidine (0.16 mL) were added, followed by the addition of oven-dried 4 Å molecular sieves such that stirring was not impeded. The mixture was stirred 1.5 h and then partitioned between EtOAc and sat. $NaHCO_3$ (aq). The phases were separated, and the organic phase was washed with brine, dried ($MgSO_4$), and concentrated to provide 3.96 g (95%) of 2-cyano-4-ethylhex-2-enoic acid benzyl ester **8** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.39 (m, 6H), 5.28 (s, 2H), 2.60 (m, 1H), 1.62 (m, 2H), 1.40 (m, 2H), 0.88 (t, J = 7.6 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 168.7, 161.4, 135.0, 128.9, 128.8, 128.5, 114.1, 109.9, 68.1, 46.4, 27.3, 12.0. IR (neat) 2233, 1729 cm^{-1}. LRMS: *m/z* 256.1 (M-1). Anal. ($C_{16}H_{19}NO_2$) C, H, N.

2-Cyano-3-propyl-hex-2-enoic Acid Benzyl Ester 9. Prepared as above for compound **8** from 4-heptanone **7** (2.0 mL, 14.3 mmol) and benzyl cyanoacetate (2.51 g, 14.3 mmol) to provide 1.03 g (27%) of 2-cyano-3-propyl-hex-2-enoic acid benzyl ester **9** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 5.24 (s, 2H), 2.71 (m, 2H), 2.52 (m, 2H), 1.60 (m, 2H), 1.49 (m, 2H), 1.00 (t, J = 7.3 Hz, 3H), 0.95 (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 182.5, 161.7, 135.4, 128.8, 128.6, 128.2, 115.9, 104.8, 67.3, 40.8, 35.8, 22.4, 21.9, 14.5, 14.3. LRMS: *m/z* 272.1 (M + 1). IR (neat) 2223, 1729 cm^{-1}. Anal. ($C_{17}H_{21}NO_2$) C, H, N.

1-Cyano-2-(1-ethylpropyl)cyclopropanecarboxylic Acid Benzyl Ester 10. To a solution of 2-cyano-4-ethylhex-2-enoic acid benzyl ester **8** (3.76 g, 14.6 mmol) in 80 mL of acetonitrile was added nitromethane (3.95 mL, 73 mmol), followed by DBU (2.18 mL, 14.6 mmol), resulting in an orange solution. The reaction was heated to 60 °C for 16 h and then cooled and partitioned between Et_2O and 1 N HCl (aq). The phases were separated, and the organic phase was washed with brine, dried ($MgSO_4$), and concentrated. Flash chromatography of the residue (5→10% EtOAc/hexanes) afforded 2.63 g (66%) of 1-cyano-2-(1-ethylpropyl)cyclopropanecarboxylic acid benzyl ester **10** as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 5.22 (m, 2H), 1.86 (dd, J = 4.6, 9.0 Hz, 1H), 1.72 (m, 1H), 1.45−1.57 (m, 4H), 1.42 (dd, J = 4.6, 8.3 Hz 1H), 1.14 (m, 1H), 0.93 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 168.2, 135.1, 128.9, 128.7, 128.2, 117.8, 68.3, 42.4, 36.7, 26.2, 26.0, 25.2, 19.3, 11.2, 10.8. LRMS: *m/z* 272.1 (M + 1). IR (neat) 2245, 1734 cm^{-1}. Anal. ($C_{17}H_{21}NO_2$) C, H, N.

1-Cyano-2,2-dipropylcyclopropanecarboxylic Acid Benzyl Ester 11. To a solution of 2-cyano-3-propylhex-2-enoic acid benzyl ester **9** (0.91 g, 3.35 mmol) in 50 mL of acetonitrile was added nitromethane (0.91 mL, 16.8 mmol), followed by DBU (0.50 mL, 3.35 mmol), resulting in an orange solution. The reaction was stirred at room temperature for 16 h and then partitioned between Et_2O and 1 N HCl (aq). The phases were separated, and the organic phase was washed with brine, dried ($MgSO_4$), and concentrated. Flash chromatography of the residue (5→10% EtOAc/hexanes) afforded 0.71 g (81%) of 1-cyano-2,2-dipropylcyclopropanecarboxylic acid benzyl ester

11 as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 5.21 (dd, J = 12.4, 18.3 Hz, 2H), 1.79 (d, J = 5.1 Hz, 1H), 1.74 (m, 1H), 1.52 (m, 3H), 1.43 (d, J = 5.1 Hz, 1H), 1.36 (m, 3H), 1.04 (m, 1H), 0.95 (t, J = 7.1 Hz, 3H), 0.73 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 166.4, 135.2, 128.8, 128.7, 128.5, 118.6, 68.2, 41.3, 36.5, 30.3, 29.8, 24.9, 19.7, 19.7, 14.2, 14.1. IR (neat) 2240, 1734 cm^{-1}. Anal. ($C_{18}H_{23}NO_2$) C, H, N.

1-Aminomethyl-2-(1-ethylpropyl)cyclopropanecarboxylic Acid 12. To a solution of 1-cyano-2-(1-ethylpropyl)-cyclopropanecarboxylic acid benzyl ester **10** (1.76 g, 6.5 mmol) in 25 mL of THF was added 20% Pd/C (0.2 g). The mixture was hydrogenated in a Parr shaker at 48 psi for 3 h. ^{1}H NMR analysis indicated the benzyl ester had been completely cleaved. The mixture was filtered, concentrated hydrochloric acid (2 g) was added along with PtO_2 (0.42 g), and the mixture was hydrogenated for an additional 16 h until the nitrile was reduced. The mixture was filtered and concentrated. Flash chromatography of the residue on silica gel (0.25:1.25:3.5 concentrated NH_4OH (aq)/MeOH/CH_2Cl_2), followed by ion-exchange chromatography on a DOWEX-50WX8−100 resin, provided 0.13 g (11%) of 1-aminomethyl-2-(1-ethylpropyl)-cyclopropanecarboxylic acid **12** as a colorless solid, mp 255−257 °C: ^{1}H NMR (D_2O) δ 3.53 (d, J = 13.2 Hz, 1H), 2.50 (d, J = 13.2 Hz, 1H), 1.30 (m, 3H), 1.08−1.23 (m, 4H), 0.74 (t, J = 7.6 Hz, 3H), 0.65 (t, J = 7.6 Hz, 3H), 0.54 (m, 1H). LRMS: *m/z* 184.1 (M-1). Anal. ($C_{10}H_{19}NO_2$) C, H, N.

1-Aminomethyl-2,2-dipropylcyclopropanecarboxylic Acid 13. To a solution of 1-cyano-2,2-dipropylcyclopropane-carboxylic acid benzyl ester **11** (0.35 g, 1.79 mmol) in 15 mL of MeOH were added concentrated HCl (0.2 g) and platinum dioxide (PtO_2, 0.035 g). The mixture was hydrogenated in a Parr shaker at 48 psi for 21 h. The mixture was filtered and concentrated. Flash chromatography of the residue on silica gel (0.25:1.25:3.5 concentrated NH_4OH (aq)/MeOH/CH_2Cl_2), followed by ion-exchange chromatography on a DOWEX-50WX8−100 resin provided 0.09 g (18%) of 1-aminomethyl-2,2-dipropylcyclopropanecarboxylic acid **13** as a colorless solid, mp 255−257 °C: ^{1}H NMR (CD_3OD) δ 3.26 (d, J = 12.9 Hz, 1H), 3.03 (d, J = 12.9 Hz, 1H), 1.69 (m, 1H), 1.56 (m, 2H), 1.28−1.50 (m, 4H), 1.26 (d, J = 4.4 Hz, 1H), 1.17 (m, 1H), 0.93 (t, J = 7.3 Hz, 3H), 0.85 (t, J = 7.3 Hz, 3H), 0.54 (d, J = 4.6 Hz, 1H). LRMS: *m/z* 198.1 (M − 1). Anal. ($C_{11}H_{21}NO_2$) C, H, N.

Cyanocyclopentylideneacetic Acid Methyl Ester 15. To a solution of cyclopentanone **14** (84.1 g, 1.0 mol) in dry benzene (100 mL) were added methyl cyanoacetate (99.1 g, 1.0 mol), ammonium acetate (10 g), and glacial acetic acid (20 mL). The reaction mixture was heated to reflux using Dean−Stark apparatus for 12 h and allowed to cool to room temperature. Excess solvent was removed in vacuo and the residue dissolved in EtOAc (400 mL). The organic phase was washed with water, dried (Na_2SO_4), and evaporated to give the product as pale yellow oil. Further purification of the pale yellow oil by distillation (10 mmHg, 140−145 °C) provided cyanocyclopentylideneacetic acid methyl ester **15** (130 g, 79%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80 (s, 3H), 3.0 (t, 2H), 2.80 (t, 2H), 1.80 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.3, 162.6, 115.8, 100.7, 52.5, 38.1, 35.8, 26.9, 25.4.

1-Cyanospiro[2.4]heptane-1-carboxylic Acid Methyl Ester 16. To a solution of cyanocyclopentylideneacetic acid methyl ester **15** (20.8 g, 126 mmol) in acetonitrile (500 mL) was added nitromethane (34 mL, 630 mmol) followed by dropwise addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (18.8 mL, 126 mmol). The reaction solution turned orange upon the addition of DBU. The reaction mixture was stirred at room temperature for 16 h. Another portion of DBU (1.0 mL) was added and the mixture stirred for 1 h. The reaction mixture was partitioned between ether (1 L) and 1 N HCl (400 mL), and the phases were separated. The organic phase was washed with 1 N HCl (2 × 300 mL) and brine (2 × 200 mL), dried over sodium sulfate, filtered, and evaporated. The residue was chromatographed on a wet-packed silica gel column eluting with 4→6% EtOAc/hexanes to furnish 14.0 g (62%) of 1-cyanospiro[2.4]heptane-1-carboxylic acid methyl ester **16** as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.82 (s, 3H), 2.1−2.19 (m, 1H), 2.14 (d, J = 5.0 Hz, 1H), 1.70−1.88 (m, 7H), 1.64 (d, J = 5.0 Hz, 1H). MS (APCI) *m/z* 180 [M + H]$^+$.

1-Aminomethylspiro[2.4]heptane-1-carboxylic Acid Methyl Ester 17. To a solution of 1-cyanospiro[2.4]heptane-1-carboxylic acid methyl ester **16** (3.45 g, 19.3 mmol) in methanol (240 mL) was added cobalt chloride hexahydrate ($CoCl_2$-$6H_2O$, 9.16 g, 38.5 mmol) to give a deep purple colored solution. Sodium borohydride (7.30 g, 193 mmol) was added portionwise over 10 min with caution to control the evolution of hydrogen and the exothermic reaction that ensued to give a black solution. The reaction mixture was stirred for 30 min under nitrogen after the addition was completed and then quenched carefully by addition of 0.5 N HCl (aq, 1.3 L). The solution was made alkaline (pH ∼ 9) by the addition of concentrated NH_4OH (aq). The mixture was extracted with ethyl acetate (4 × 400 mL). The combined organic phases were dried (Na_2SO_4), and concentrated to afford 3.07 g (87%) of 1-aminomethylspiro[2.4]heptane-1-carboxylic acid methyl ester **17** as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.48 (s, 3H), 3.16 (d, J = 13.8 Hz, 1H), 2.58 (d, J = 13.8 Hz, 1H), 1.9 (m, 1H), 1.4−1.7 (m, 10H), 0.74 (br d, 1H). MS (APCI) *m/z* 184 [M + H]$^+$.

1-Aminomethylspiro[2.4]heptane-1-carboxylic Acid 18. To a solution of 1-aminomethylspiro[2.4]heptane-1-carboxylic acid methyl ester **17** (3.40 g, 18.6 mmol) were added methanol (75 mL) and lithium hydroxide monohydrate (LiOH−H_2O) (1.55 g, 37.1 mmol). The mixture was heated to reflux under nitrogen for 48 h. The solvent was removed by evaporation under reduced pressure, and the residue was dissolved in water (50 mL). With ice bath cooling, concentrated HCl (∼2.5 mL) was added until the pH was adjusted to 6. A white precipitate was isolated by filtration and dried under vacuum to yield 1.22 g (39%) of 1-aminomethylspiro[2.4]heptane-1-carboxylic acid **18** as an off-white solid: mp 231−235 °C (dec); ^{1}H NMR (300 MHz, D_2O) δ 3.43 (d, J = 13.2 Hz, 1H), 2.86 (d, J = 13.2 Hz, 1H), 1.54−1.77 (m, 7 H), 1.40 (m, 1H), 1.32 (d, J = 4.7 Hz, 1H), 0.78 (d, J = 4.7 Hz, 1H). MS (APCI) *m/z* 170 [M + H]$^+$. Anal. ($C_9H_{15}NO_2$·H_2O) C, H, N.

Cyanocyclohexylidene-acetic Acid Methyl Ester 22. To a solution of cyclohexanone **19** (84.1 g, 1.0 mol) in dry benzene (100 mL) were added methyl cyanoacetate (99.1 g, 1.0 mol), ammonium acetate (10 g) and glacial acetic acid (20 mL). The reaction mixture was heated to reflux using Dean−Stark apparatus for 12 h and allowed to cool to room temperature. Excess solvent was removed in vacuo and the residue dissolved in EtOAc (400 mL). The organic phase was washed with water, dried over sodium sulfate, and evaporated to give cyanocyclohexylideneacetic acid methyl ester **22** as pale yellow oil. Further purification of the pale yellow oil by distillation (10 mmHg, 150−155 °C) gave 110 g (61%) of **22** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80 (s, 3H), 3.00 (t, 2H), 2.70 (t, 2H), 1.70−1.85 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.8, 162.7, 115.8, 101.9, 52.8, 37.2, 31.9, 28.9, 28.6, 25.9.

2-Cyano-4-methylpent-2-enoic Acid Methyl Ester 23. To a solution of isobutyraldehyde **20** (18.2 mL, 200 mmol) in dry benzene (20 mL) were added methyl cyanoacetate (18 mL, 200 mmol), ammonium acetate (2 g), and glacial acetic acid (4 mL). The reaction mixture was stirred at 60 °C for 30 min and allowed to cool to room temperature. Excess solvent was removed in vacuo and the residue dissolved in EtOAc (200 mL). The organic phase was washed with water, dried (Na_2SO_4), and concentrated. Further purification of the pale yellow oil by distillation (7 mmHg) gave 27.4 g (56%) of 2-cyano-4-methylpent-2-enoic acid methyl ester **23** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 10.5 Hz, 1H), 3.87 (s, 3H), 2.96−3.04 (m, 1H), 1.16 (d, J = 6.6 Hz, 6H).

2-Cyano-5-methylhex-2-enoic Acid Methyl Ester 24. To a solution of isovaleraldehyde **21** (86.1 g, 1 mol) in dry benzene (100 mL) were added methyl cyanoacetate (99.1 g, 1 mol), ammonium acetate (10 g), and glacial acetic acid (20 mL). The reaction mixture was stirred at 0 °C for 1 h and allowed to cool to room temperature. Excess solvent was removed in vacuo and the residue dissolved in EtOAc (400 mL). The organic

phase was washed with water, dried over sodium sulfate and evaporated to give the product as pale yellow oil. Further purification of the pale yellow oil by distillation (10 mmHg) gave 100 g (60%) of 2-cyano-5-methylhex-2-enoic acid methyl ester **24** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (t, J = 8.0 Hz, 1H), 3.89 (s, 3H), 2.47 (dd, J = 7.9, 6.8 Hz, 2H), 1.91 (m, 1H), 1.00 (d, J = 6.7 Hz, 6H).

1-Cyanospiro[2.5]octane-1-carboxylic Acid Methyl Ester 25. To a solution of cyanocyclohexylideneacetic acid methyl ester **22** (20.0 g, 112 mmol) in acetonitrile (400 mL) was added nitromethane (30 mL, 558 mmol) followed by dropwise addition of DBU (16.9 mL, 113 mmol) over 5 min. The solution went from clear to orange and was stirred at room temperature for 5.5 h. The solution was diluted with ether (1 L), washed with 1 N HCl (2 × 250 mL) and then brine (2 × 250 mL). The organic layer was dried over sodium sulfate, filtered, and evaporated. The residue was chromatographed on a wet-packed silica gel column (6.5 × 40 cm) eluting with 5% EtOAc/hexanes to furnish 18.6 g (86%) of 1-cyanospiro[2.5]octane-1-carboxylic acid methyl ester **25** as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.83 (s, 3H), 1.82 (d, J = 5.0 Hz, 1H), 1.5−1.75 (m, 9H), 1.49 (d, J = 5.0 Hz, 1H), 1.35 (m, 1H).

1-Cyano-2-isopropylcyclopropanecarboxylic Acid Methyl Ester 26. To a solution of 2-cyano-4-methylpent-2-enoic acid methyl ester **23** (10.5 g, 68.3 mmol) in dry acetonitrile (60 mL) were added nitromethane (5.5 mL, 103 mmol) and a portionwise addition of alumina-supported potassium fluoride (40 wt %, 22 g). The reaction mixture was stirred under reflux for 2 h and cooled to room temperature. The solid was removed by filtration through a short pad of Celite and washed with acetonitrile. Excess solvent was removed in vacuo and the residue dissolved in ether. The ether phase was washed with water and brine, dried (Na_2SO_4), and concentrated. The crude product was purified by flash column chromatography (20% EtOAc/hexanes) to afford 7.7 g (68%) of 1-cyano-2-isopropylcyclopropanecarboxylic acid methyl ester **26** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.81 (s, 3H), 1.81 (dd, J = 8.9, 4.4 Hz, 1H), 1.65−1.74 (m, 1H), 1.39−1.45 (m, 2H), 1.13 (t, J = 7.2 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.7, 117.6, 53.7, 39.3, 31.2, 25.3, 21.9, 21.8, 19.6.

1-Cyano-2-isobutylcyclopropanecarboxylic Acid Methyl Ester 27. To a solution of 2-cyano-5-methylhex-2-enoic acid methyl ester **24** (20.2 g, 121 mmol) in dry acetonitrile (110 mL) were added nitromethane (9.8 mL, 181 mmol) and a portionwise addition of alumina-supported potassium flouride (40 wt %, 39.3 g). The reaction mixture was stirred under reflux for 2 h and cooled to room temperature. The solid was removed by filtration through a short pad of Celite and washed with acetonitrile. Excess solvent was removed in vacuo and the residue dissolved in ether. The ether phase was washed with water and brine, dried (Na_2SO_4), and concentrated. The crude product was purified by flash column chromatography (20% EtOAc/hexanes) to give 15 g (69%) of 1-cyano-2-isobutylcyclopropanecarboxylic acid methyl ester **27** as colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.81 (s, 3H), 1.79−1.94 (m, 3H), 1.65 (m, 1H), 1.35−1.42 (m, 2H), 1.00 (d, J = 3.0 Hz, 3H), 0.98 (d, J = 2.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 117.3, 53.4, 39.0, 30.1, 27.9, 25.5, 22.4, 22.3, 19.3.

1-(*tert*-Butoxycarbonylaminomethyl)-spiro[2.5]octane-1-carboxylic Acid Methyl Ester 28. To a solution of 1-cyanospiro[2.5]octane-1-carboxylic acid methyl ester **25** (18.6 g, 96.2 mmol) in methanol (480 mL) was added $CoCl_2-H_2O$ (22.8 g, 96.2 mmol) to give a deep purple color. Sodium borohydride (14.5 g, 385 mmol) was added portionwise over 10 min with ice bath cooling to give a black colored solution. After 40 min, the reaction was quenched carefully with 2 N HCl (aq). The pH was adjusted to 9 with 2 N NaOH (aq) and the solution was extracted 4x with CH_2Cl_2. To the combined organic phases were added saturated K_2CO_3 (aq) and di-*tert*-butyl dicarbonate (26.2 g, 120 mmol). After 1 h, the phases were separated. The organic phase was dried (Na_2SO_4), and concentrated to yield 35.7 g (>100%) of 1-(*tert*-butoxycarbonylaminomethyl)spiro[2.5]octane-1-carboxylic acid methyl ester **28** as an off-white solid. The material was used without further purification in the next step.

1-(*tert*-Butoxycarbonylaminomethyl)-2-isopropylcyclopropanecarboxylic Acid Methyl Ester 29. To a solution of 1-cyano-2-isopropylcyclopropanecarboxylic acid methyl ester **26** (7.7 g, 46.1 mmol) and cobaltous chloride hexahydrate (21.9 g, 92.2 mmol) in MeOH (560 mL) was added sodium borohydride (17.4 g, 461 mmol) in portions. The black precipitate formed was stirred for 1 h at room temperature and was quenched with 0.5N HCl (aq, 200 mL). After the black precipitate dissolved, excess solvent was removed and the aqueous phase made alkaline by addition of 2 N NaOH (aq). The alkaline solution was added slowly to a solution of di-*tert*-butyl dicarbonate (20.1 g, 92.2 mmol) in dichloromethane (400 mL). The reaction mixture was stirred at room-temperature overnight, and the organic phase was separated, washed with brine, and dried (Na_2SO_4). Evaporation of solvent followed by purification of the residue by column chromatography (10% EtOAc/hexanes) furnished 10.9 g (88%) of 1-(*tert*-butoxycarbonylaminomethyl)-2-isopropylcyclopropanecarboxylic acid methyl ester **29** as a colorless oil: ^{1}H NMR (300 MHz, CD_3OD) δ 3.69 (s, 3H), 3.57 (d, J = 14.3 Hz, 1H), 3.23 (d, J = 14.3 Hz, 1H), 1.43 (s, 9H), 1.26−1.31 (m, 3H), 1.05 (d, J = 6.3 Hz, 3H), 0.97 (d, J = 6.3 Hz, 3H), 0.76 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 177.1, 158.5, 80.5, 53.0, 41.1, 38.1, 29.2, 23.6, 23.4, 20.5. MS (APCI) m/z 172 $[M + H\text{-}100(Boc)]^+$.

1-(*tert*-Butoxycarbonylaminomethyl)-2-isobutylcyclopropanecarboxylic Acid Methyl Ester 30. Prepared as above for compound **29** from 1-cyano-2-isobutylcyclopropanecarboxylic acid methyl ester **27** (13.0 g, 71.8 mmol) to afford 16 g (78%) of 1-(*tert*-butoxycarbonylaminomethyl)-2-isobutylcyclopropanecarboxylic acid methyl ester **30** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.67 (s, 3H), 5.27 (m, 1H), 3.33−3.46 (m, 2H), 1.64−1.70 (m, 1H), 1.51−1.58 (m, 2H), 1.44 (s, 9H), 1.37 (dd, J = 8.5, 3.8 Hz, 1H), 1.20−1.28 (m, 1H), 0.93 (t, J = 6.5 Hz, 6H), 0.75 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 156.1, 79.1, 52.1, 40.2, 37.8, 28.9, 28.7, 28.6, 26.7, 22.9, 22.4, 21.1. MS (APCI) m/z 186 $[M + H - 100(Boc)]^+$.

1-(*tert*-Butoxycarbonylaminomethyl)spiro[2.5]octane-1-carboxylic acid 31. To a solution of crude 1-(*tert*-butoxycarbonylaminomethyl)spiro[2.5]octane-1-carboxylic acid methyl ester **28** (35.0 g, ~96.2 mmol) in MeOH (400 mL) was added lithium hydroxide monohydrate (12.3 g, 294 mmol) followed by the addition of water (75 mL). The mixture was heated to reflux. After 48 h, an additional portion of $LiOH-H_2O$ (6.15 g, 146 mmol) was added and refluxed for an additional 24 h. The solvent was removed under reduced vacuum and the residue partitioned between water and ether. The aqueous phase was acidified to pH 2−3 with 2 N HCl (aq) with ice bath cooling with a layer of CH_2Cl_2 present. The phases were separated, and the aqueous phase was extracted 3x with CH_2Cl_2. The combined organic phases were dried (Na_2SO_4), filtered, and evaporated to give 17.0 g (63%, two steps) of 1-(*tert*-butoxycarbonylaminomethyl)spiro[2.5]octane-1-carboxylic acid **31** an off-white solid.

1-(*tert*-Butoxycarbonylaminomethyl)-2-isopropylcyclopropanecarboxylic acid 32. To a solution of 1-(*tert*-butoxycarbonylaminomethyl)-2-isopropylcyclopropanecarboxylic acid methyl ester **29** (10.9 g, 40.2 mmol) in MeOH (320 mL) was added a solution of lithium hydroxide monohydrate (4.2 g, 100 mmol) in water (98 mL). The reaction mixture was heated to reflux for 3 h and cooled to room temperature. Excess solvent was removed, and the residue was dissolved in water (100 mL). The aqueous solution was washed with ether, acidified to pH = 3 with 2 N HCl (aq), and extracted with EtOAc (2 × 150 mL). The combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated to provide 9.0 g (87%) of 1-(*tert*-butoxycarbonylaminomethyl)-2-isopropylcyclopropanecarboxylic acid **32** as a colorless oil: ^{1}H NMR (300 MHz, CD_3OD) δ 3.54 (d, J = 14.3 Hz, 1H), 3.23 (d, J = 14.3 Hz, 1H), 1.42 (s, 9H), 1.28−1.36 (m, 3H), 1.06 (d, J = 6.2 Hz, 3H), 0.99 (d, J = 6.2 Hz, 3H), 0.72 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 178.5, 158.1, 80.2, 40.8, 37.6, 30.2, 28.8, 23.3, 23.0, 20.0. MS (APCI) m/z 258 $[M + H]^+$.

1-(*tert*-Butoxycarbonylaminomethyl)-2-isobutylcyclopropanecarboxylic Acid 33. To a solution of 1-(*tert*-butoxycarbonylaminomethyl)-2-isobutylcyclopropanecarboxylic acid methyl ester **30** (9.1 g, 31.9 mmol) in MeOH (260 mL) was added a solution of lithium hydroxide monohydrate (3.34 g, 79.7 mmol) in water (80 mL). The reaction mixture was heated to reflux for 3 h and cooled to room temperature. Excess solvent was removed and the residue dissolved in water (100 mL). The aqueous solution was washed with ether, acidified to pH = 3 with 2 N HCl (aq), and extracted with EtOAc (2 × 150 mL). The combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated to give 8.4 g (97%) of 1-(*tert*-butoxycarbonylaminomethyl)-2-isobutylcyclopropanecarboxylic acid **33** as a colorless oil: ^{1}H NMR (300 MHz, CD_3OD) δ 3.40 (d, J = 14.2 Hz, 1H), 3.23 (d, J = 14.2 Hz, 1H), 1.64−1.69 (m, 2H), 1.49−1.55 (m, 1H), 1.43 (s, 9H), 1.35 (m, 1H), 0.95 (t, J = 6.4 Hz, 6H), 0.71 (dd, J = 6.4, 4.0 Hz, 1H).

1-Aminomethylspiro[2.5]octane-1-carboxylic Acid Hydrochloride 34. To a solution of 1-(*tert*-butoxycarbonylaminomethyl)spiro[2.5]octane-1-carboxylic acid **31** (200 mg, 0.706 mmol) in 1,4-dioxane (3 mL) was added 4 N HCl in 1,4-dioxane (3 mL). The reaction was stirred for 18 h at room temperature. Ether (20 mL) was added, and the precipitate was isolated by vacuum filtration. The solid was dried in a vacuum oven at 48 °C to give 133 mg (86%) of 1-aminomethylspiro[2.5]octane-1-carboxylic acid hydrochloride **34** as a white solid: mp 240−242 °C; ^{1}H NMR (300 MHz, D_2O) δ 3.61 (d, J = 14.0 Hz, 1H), 3.12 (d, J = 14.0 Hz, 1H), 1.4−1.6 (m, 10H), 1.40 (d, J = 5.2 Hz, 1H), 0.92 (d, J = 5.2 Hz, 1H). MS (APCI) *m/z* 184 $[M + H]^+$. Anal. ($C_{10}H_{17}NO_2$·HCl) C, H, N, Cl.

1-Aminomethyl-2-isopropylcyclopropanecarboxylic Acid Hydrochloride 35. To a solution of 1-(*tert*-butoxycarbonylaminomethyl)-2-isopropylcyclopropanecarboxylic acid **32** (2.05 g, 8.0 mmol) in dry 1,4-dioxane (40 mL) was added 4 N HCl (40 mL, in 1,4-dioxane). The reaction mixture was stirred at room-temperature overnight and ether was added (100 mL). The white solid was collected and dried to afford 1.35 g (88%) of 1-aminomethyl-2-isopropylcyclopropanecarboxylic acid hydrochloride **35**, mp 245−246 °C: ^{1}H NMR (300 MHz, D_2O) δ 3.83 (d, J = 13.7 Hz, 1H), 2.85 (d, J = 13.7 Hz, 1H), 1.49−1.59 (m, 2H), 1.23−1.31 (m, 1H), 1.05 (d, J = 6.4 Hz, 3H), 0.96 (m, 1H), 0.94 (d, J = 6.5 Hz, 3H). ^{13}C NMR (75 MHz, D_2O) δ 179.6, 41.7, 39.6, 30.8, 27.8, 23.9, 22.3. MS (APCI) *m/z* 158 $[M + H]^+$. Anal. ($C_8H_{15}NO_2$·HCl) C, H, N, Cl.

1-Aminomethyl-2-isobutylcyclopropanecarboxylic Acid Hydrochloride 36. To a solution of 1-(*tert*-butoxycarbonylaminomethyl)-2-isobutylcyclopropanecarboxylic acid **33** (2.8 g, 10.3 mmol) in dry 1,4-dioxane (60 mL) was added 4 N HCl (40 mL, in 1,4-dioxane). The reaction mixture was stirred at room-temperature overnight, and then ether was added (100 mL). The white solid was collected and dried to furnish 1.6 g (76%) of 1-aminomethyl-2-isobutylcyclopropanecarboxylic acid hydrochloride **36**, mp 254−255 °C: ^{1}H NMR (300 MHz, D_2O) δ 3.58 (d, J = 13.8 Hz, 1H), 3.06 (d, J = 13.8 Hz, 1H), 1.51−1.80 (m, 4H), 1.21−1.28 (m, 1H), 0.96 (m, 1H), 0.95 (t, J = 6.2 Hz, 6H). ^{13}C NMR (75 MHz, D_2O) δ 178.1, 40.2, 37.1, 28.6, 28.1, 25.3, 22.2, 21.9, 21.7. MS (APCI) *m/z* 172 $[M + H]^+$. Anal. ($C_9H_{17}NO_2$·HCl) C, H, N.

2-Cyano-3-cyclopropylacrylic Acid Ethyl Ester 38. To a mixture of cyclopropanecarboxaldehyde **37** (2.4 mL, 32.1 mmol) and ethyl cyanoacetate (3.8 mL, 35.3 mmol) at 0 °C were added acetic acid (0.2 mL) and then piperidine (0.3 mL) dropwise. The ice bath was removed, and stirring was continued for 10 min. Additional acetic acid (0.2 mL) and piperidine (0.3 mL) were added, followed by the addition of oven-dried 4 Å molecular sieves such that stirring was not impeded. The mixture was stirred for 12 h, and then partitioned between Et_2O and sat. $NaHCO_3$ (aq). The phases were separated, and the organic phase washed with brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the residue (3→5→9% EtOAc/hexanes) furnished 3.71 g (70%) of 2-cyano-3-cyclopropylacrylic acid ethyl ester **38** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 6.97 (d, J = 11.2 Hz, 1H), 4.27 (q, J = 7.2 Hz, 2H), 2.09 (m, 1H), 1.32 (m, 5H), 0.95 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 169.0, 162.0, 114.8, 106.0, 62.4, 16.3, 14.4, 11.7. LRMS: *m/z* 166.0 (M + 1). Anal. ($C_9H_{11}NO_2$) C, H, N.

2-Cyano-bicyclopropyl-2-carboxylic Acid Ethyl Ester 39. To a solution of 2-cyano-3-cyclopropylacrylic acid ethyl ester **38** (2.0 g, 12.1 mmol) in 40 mL of acetonitrile was added nitromethane (3.3 mL, 60.9 mmol) followed by DBU (1.8 mL, 12.0 mmol). The mixture was stirred for 2 d, then at 50 °C for 12 h. The mixture was cooled and partitioned between Et_2O and 1 M HCl (aq), and the organic phase was separated, washed with brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the residue (1→5% EtOAc/hexanes) furnished 0.30 g (14%) of 2-cyano-bicyclopropyl-2-carboxylic acid ethyl ester **39** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.19 (m, 2H), 1.74 (m, 2H), 1.39 (m, 1H), 1.28 (t, J = 7.1 Hz, 3H), 0.89 (m, 1H), 0.70 (m, 1H), 0.62 (m, 1H), 0.34 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 168.0, 117.6, 62.9, 35.3, 23.7, 20.0, 14.3, 10.1, 4.8, 4.0. LRMS: *m/z* 180.0 (M + 1). Anal. ($C_{10}H_{13}NO_2$) C, H, N.

2-Aminomethylbicyclopropyl-2-carboxylic Acid 40. To a solution of 2-cyanobicyclopropyl-2-carboxylic acid ethyl ester **39** (0.14 g, 0.78 mmol) in 48 mL EtOH was added RaNi (0.18 g). The mixture was hydrogenated in a Parr shaker at 48 psi for 16 h, filtered, and concentrated. To the crude aminoester were added 5 mL of MeOH and then 5 mL of 10% NaOH (aq), and the mixture was stirred 12 h. The mixture was acidified to pH 2 by addition of 6 M HCl (aq) and then concentrated to remove MeOH. The residue was diluted with water and loaded onto DOWEX-50WX8−100 resin. Elution with 50 mL of water and then 50 mL 5% NH_4OH (aq) and concentration of the alkaline fractions provided a solid that was recrystallized from MeOH/EtOAc to afford 0.036 g (30%) of 2-aminomethylbicyclopropyl-2-carboxylic acid **40** as a colorless solid. ^{1}H NMR (D_2O) δ 3.22 (d, J = 13.4 Hz, 1H), 2.95 (d, J = 13.4 Hz, 1H), 1.24 (m, 1H), 0.99 (dd, J = 8.8, 4.6 Hz, 1H), 0.54 (m, 1H), 0.49 (dd, J = 6.6, 4.9 Hz, 1H), 0.33 (m, 2H), 0.05 (m, 2H). LRMS: *m/z* 156.0 (M + 1). Anal. Calcd for $C_8H_{13}NO_2$: C, 61.91; H, 8.44; N, 9.03. Found: 59.21; H, 8.26; N, 8.32.

1-Cyano-2-isobutylcyclopropanecarboxylic Acid Methyl Ester 43. To a solution of 2-cyano-5-methylhex-2-enoic acid methyl ester **41**[18] (6.7 g, 40 mmol) in 40 mL acetonitrile were added nitromethane (2.2 mL, 40 mmol) and then DBU (6.0 mL, 40 mmol) resulting in a red-orange solution. The mixture was heated to reflux 6 h, cooled, and concentrated. The residue was partitioned between Et_2O and 1 N HCl (aq). The aqueous phase was extracted with Et_2O, and the combined organics were washed with brine, dried ($MgSO_4$), and concentrated. Flash chromatography of the residue (10→15→20% EtOAc/hexanes) afforded 4.38 g (60%) of 1-cyano-2-isobutylcyclopropanecarboxylic acid methyl ester **43** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3H), 1.85 (m, 3H), 1.64 (m, 1H), 1.44 (m, 2H), 0.97 (d, J = 6.6 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 168.8, 117.7, 53.8, 39.2, 30.5, 28.1, 25.9, 22.7, 22.5, 19.5.

1-Cyano-2-(1-ethylpropyl)cyclopropanecarboxylic Acid Methyl Ester 44. To a solution of 2-cyano-4-ethyl-hex-2-enoic acid methyl ester **42**[30] (6.3 g, 35 mmol) in 35 mL of acetonitrile were added nitromethane (3.0 mL, 5 mmol) and then DBU (5.2 mL, 35 mmol), resulting in a red-orange solution. The mixture was heated to reflux 3 h, cooled, and concentrated. The residue was partitioned between Et_2O and 1 N HCl (aq). The aqueous phase was extracted with Et_2O, and the combined organics were washed with brine, dried ($MgSO_4$), and concentrated. Flash chromatography of the residue (10→15% EtOAc/hexanes) afforded 5.1 g (75%) of 1-cyano-2-(1-ethyl-propyl)-cyclopropanecarboxylic acid methyl ester **44** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3H), 1.84 (dd, J = 9.0, 4.6 Hz, 1H), 1.71 (m, 1H), 1.54 (m, 3H), 1.46 (m, 1H), 1.41 (dd, J = 8.1, 4.4 Hz, 1H), 1.12 (m, 1H), 0.93 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 168.9, 118.0, 53.8, 42.6, 36.7, 26.2, 26.0, 25.2, 19.1, 11.2, 11.0.

1-Hydroxymethyl-2-isobutylcyclopropanecarbonitrile 45. To a solution of 1-cyano-2-isobutylcyclopropanecarboxylic acid methyl ester **43** (3.6 g, 20 mmol) in 40 mL of THF at ambient temperature was added a mixture of sodium borohydride in THF:H_2O (5:1, 30 mL). Additional $NaBH_4$ (1.5 g) and 1.5 mL of water was added and stirred overnight and

quenched cautiously with 1 N HCl (aq), resulting in vigorous gas evolution and exotherm. The mixture was concentrated to remove THF and then partitioned between Et_2O/1 N HCl (aq). The organic phase was washed with sat. $NaHCO_3$ (aq) and brine, dried ($MgSO_4$), and concentrated to provide 2.66 g (86%) of 1-hydroxymethyl-2-isobutylcyclopropanecarbonitrile **45** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 3.65 (d, J = 11.7 Hz, 1H), 3.57 (d, J = 11.7 Hz, 1H), 1.98 (br s, 1H), 1.77 (m, 1H), 1.54 (m, 1H), 1.35 (m, 1H), 1.20 (m, 1H), 1.12 (m, 1H), 0.97 (d, J = 6.6 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H), 0.92 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 121.5, 66.5, 39.7, 28.4, 22.8, 22.7, 22.5, 19.1, 18.7.

2-(1-Ethyl-propyl)-1-hydroxymethylcyclopropanecarbonitrile 46. To a solution of 1-cyano-2-(1-ethyl-propyl)-cyclopropanecarboxylic acid methyl ester **44** (4.5 g, 23 mmol) in 40 mL of THF at ambient temperature was added a mixture of sodium borohydride (4.0 g, 115 mmol) in THF/H_2O (5:1, 40 mL). Additional THF and H_2O was used to completely transfer the borohydride. The mixture was stirred 3 d and then quenched cautiously with 1 N HCl (aq). The mixture was concentrated to remove THF and then partitioned between Et_2O/1 N HCl (aq). The organic phase was washed with brine, dried ($MgSO_4$), and concentrated to afford 3.5 g (91%) of 2-(1-ethyl-propyl)-1-hydroxymethylcyclopropanecarbonitrile **46** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 3.65 (d, J = 11.7 Hz, 1H), 3.56 (d, J = 11.7 Hz, 1H), 1.52 (m, 5H), 1.43 (m, 1H), 1.13 (m, 1H), 1.02 (m, 1H), 0.97 (t, J = 7.6 Hz, 3H), 0.91 (t, J = 7.6 Hz, 3H).

1-Chloromethyl-2-isobutylcyclopropanecarbonitrile 47. To a solution of 1-hydroxymethyl-2-isobutylcyclopropanecarbonitrile **45** (2.65 g, 17.3 mmol) in 50 mL of CH_2Cl_2 at ambient temperature were added pyridine (5.0 mL, 62 mmol) and then methanesulfonyl chloride (5.0 mL, 64 mmol) quickly dropwise. The mixture was stirred 2 h and then poured into brine and shaken. The organic phase was separated, dried ($MgSO_4$), and concentrated. To the residue was added 20 mL DMF and sodium azide (2.0 g, 35 mmol) in one portion. On heating to 100 °C the mixture turned brown. It was then heated 90 min, cooled to room temperature, and partitioned between Et_2O and water. The organic phase was washed with water and brine, dried $MgSO_4$, and concentrated. Flash chromatography of the residue (5→10% EtOAc/hexanes) yielded 1.43 g (48%) of 1-chloromethyl-2-isobutylcyclopropanecarbonitrile **47** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 3.60 (d, J = 12.0 Hz, 1H), 3.45 (d, J = 11.7 Hz, 1H), 1.79 (m, 1H), 1.53 (m, 1H), 1.38 (m, 1H), 1.31 (m, 1H), 1.24 (m, 1H), 1.13 (m, 1H), 0.97 (d, J = 6.8 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 120.1, 48.7, 39.7, 28.2, 26.0, 22.8, 22.3, 21.9, 19.3.

1-Chloromethyl-2-(1-ethylpropyl)cyclopropanecarbonitrile 48. To a solution of 2-(1-ethyl-propyl)-1-hydroxymethylcyclopropanecarbonitrile **46** (3.5 g, 21 mmol) in 85 mL of CH_2Cl_2 at ambient temperature were added pyridine (5.0 mL, 62 mmol) and then methanesulfonyl chloride (5.0 mL, 64 mmol) quickly dropwise. The mixture was stirred 2 h and then poured into brine and shaken. The organic phase was separated, dried ($MgSO_4$), and concentrated. Flash chromatography of the residue (10→15% EtOAc/hexanes) gave 0.22 g (6%) of 1-chloromethyl-2-(1-ethyl-propyl)cyclopropanecarbonitrile **48** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 3.63 (d, J = 11.7 Hz, 1H), 3.40 (d, J = 11.7 Hz, 1H), 1.55 (m, 3H), 1.45 (m, 1H), 1.23 (m, 2H), 1.11 (m, 2H), 0.96 (t, J = 7.6 Hz, 3H), 0.91 (t, J = 7.3 Hz, 3H).

1-Azidomethyl-2-isobutylcyclopropanecarbonitrile 49. To a solution of 1-chloromethyl-2-isobutylcyclopropanecarbonitrile **47** (0.67 g, 3.9 mmol) in 10 mL of DMF was added sodium azide (0.54 g, 8.3 mmol), and the mixture was heated to 100 °C for 1 h. The mixture was cooled and partitioned between Et_2O and water. The organic phase was separated and washed with brine, dried ($MgSO_4$), and concentrated to provide 0.68 g (99%) of 1-azidomethyl-2-isobutylcyclopropanecarbonitrile **49** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 3.42 (d, J = 13.2 Hz, 1H), 3.28 (d, J = 13.2 Hz, 1H), 1.78(m, 1H), 1.55 (m, 1H), 1.37 (m, 1H), 1.22 (m, 1H), 1.16 (m, 1H), 1.03 (m, 1H), 0.97 (d, J = 6.8 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H).

1-Azidomethyl-2-(1-ethylpropyl)cyclopropanecarbonitrile 50. To a solution of 1-chloromethyl-2-(1-ethyl-propyl)-cyclopropanecarbonitrile **48** (0.21 g, 1.1 mmol) in 5 mL of DMF was added sodium azide (0.18 g, 2.8 mmol), and the mixture heated to 100 °C for 1.5 h. The mixture was cooled and partitioned between Et_2O and water. The organic phase was separated, washed with brine, dried ($MgSO_4$), and concentrated to furnish 0.22 g (100%) of 1-azidomethyl-2-(1-ethyl-propyl)cyclopropanecarbonitrile **50** as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 3.45 (d, J = 12.9 Hz, 1H), 3.28 (d, J = 13.2 Hz, 1H), 1.55 (m, 4H), 1.45 (m, 1H), 1.16 (m, 1H), 1.10 (m, 1H), 1.04 (m, 1H), 0.98 (t, J = 7.6 Hz, 3H), 0.92 (t, J = 7.3 Hz, 3H).

1-Aminomethyl-2-isobutylcyclopropanecarbonitrile 51. To a solution of 1-azidomethyl-2-isobutylcyclopropanecarbonitrile **49** (0.68 g, 3.8 mmol) in 50 mL of methanol was added 10% palladium on carbon (0.15 g), and the mixture was hydrogenated at 48 psi in a Parr shaker for 8 h, filtered, and concentrated to provide 0.56 g (96%) of 1-aminomethyl-2-isobutylcyclopropanecarbonitrile **51** as a gray oil. ^{1}H NMR ($CDCl_3$) δ 2.71 (d, J = 13.9 Hz, 1H), 2.62 (d, J = 13.7 Hz, 1H), 1.75 (m, 1H), 1.47 (m, 1H), 1.26 (m, 1H), 1.20 (m, 1H), 1.10 (m, 1H), 0.95 (d, J = 7.3 Hz, 3H), 0.95 (d, J = 6.6 Hz, 3H), 0.86 (m, 1H).

1-Aminomethyl-2-(1-ethylpropyl)cyclopropanecarbonitrile 52. To a solution of 1-azidomethyl-2-(1-ethyl-propyl)-cyclopropanecarbonitrile **50** (0.22 g, 1.1 mmol) in 4 mL of methanol was added 10% palladium on carbon (0.05 g). The mixture was hydrogenated at 48 psi in a Parr shaker for 2 h, filtered, and concentrated to provide 0.19 g (100%) of 1-aminomethyl-2-(1-ethyl-propyl)cyclopropanecarbonitrile **52** as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.76 (d, J = 13.9 Hz, 1H), 2.60 (d, J = 13.9 Hz, 1H), 1.52 (m, 5H), 1.14 (m, 1H), 0.99 (m, 8H).

1-Aminomethyl-2-isobutylcyclopropanecarboxylic Acid 53 (isobutyl and carboxylate groups cis). To 1-aminomethyl-2-isobutylcyclopropanecarbonitrile **51** (0.56 g, 3.7 mmol) was added 10 mL of 6 M HCl (aq) and the mixture refluxed for 5 d, cooled, and concentrated. The solid residue was dissolved in water and washed with Et_2O. The aqueous phase was separated and loaded onto DOWEX-50WX8−100 ion-exchange resin. The column was eluted with 100 mL of water and then 100 mL of 5% NH_4OH (aq). The alkaline fractions were concentrated to an off-white solid. ^{1}HNMR of the residue showed a trace amount of aminonitrile present. To the solid was added hot methanol and water until it was mostly dissolved. The liquid was withdrawn by pipet, filtered through a glass wool plug, and concentrated to give 0.13 g (20%) of 1-aminomethyl-2-isobutylcyclopropanecarboxylic acid **53** as a colorless solid. ^{1}H NMR (D_2O) δ 2.98 (d, J = 13.2 Hz, 1H), 2.70 (d, J = 13.2 Hz, 1H), 1.40 (m, 1H), 1.23 (m, 1H), 1.11 (m, 1H), 0.99 (m, 1H), 0.89 (m, 1H), 0.74 (m, 1H), 0.71 (d, J = 6.6 Hz, 3H), 0.67 (d, J = 6.6 Hz, 3H). Anal. Calcd. for $C_9H_{17}NO_2$: C, 63.13; H, 10.01; N, 8.18. Found: C, 62.52; H, 9.97; N, 8.15.

1-Aminomethyl-2-(1-ethylpropyl)cyclopropanecarboxylic Acid 54 [(1-ethylpropyl) and carboxylate groups cis]. To 1-aminomethyl-2-isobutylcyclopropanecarbonitrile **52** (0.56 g, 3.7 mmol) was added 10 mL of 6 M HCl (aq) and the mixture refluxed for 4 d, cooled, and concentrated. The solid residue was dissolved in water and washed with Et_2O. The aqueous phase was separated and loaded onto DOWEX-50WX8−100 ion-exchange resin. The column was eluted with 100 mL of water and then 100 mL of 5% NH_4OH (aq). The alkaline fractions were concentrated to an off-white solid. The solid was dissolved in the minimum amount of hot MeOH and water and filtered while hot through a glass wool plug. Concentration afforded 56 mg (26%) of 1-aminomethyl-2-(1-ethyl-propyl)-cyclopropanecarboxylic acid **54** as a colorless solid. ^{1}H NMR (D_2O) δ 2.97 (d, J = 12.9 Hz, 1H), 2.68 (d, J = 13.2 Hz, 1H), 1.27 (m, 1H), 1.14 (m, 3H), 1.07 (m, 3H), 0.78 (m, 1H), 0.72 (t, J = 7.6 Hz, 3H), 0.61 (t, J = 7.6 Hz, 3H). Anal. Calcd for $C_{10}H_{19}NO_2$: C, 64.83; H, 10.34; N, 7.56. Found: C, 63.47; H, 10.32; N, 7.88.

[^{3}H]-Gabapentin Binding Assay Using Pig Brain Membranes. Pig brains were purchased from Pel-Freez Biologicals

(Rogers, AZ). The cerebral cortex was stripped, deveined, and stored at −80 °C before use. Tissue samples were thawed and then homogenized on ice using a glass/Teflon homogenizer in 10 volumes (weight/volume) of Buffer A (10 mM HEPES, 1 mM EDTA, 1 mM EGTA, 320 mM sucrose, 100 μM phenylmethylsulfonyl fluoride (PMSF), pH 7.4 using KOH). The cell debris was pelleted by centrifugation (1000*g* for 10 min) using a Beckman centrifuge (JA17 rotor). The supernatants were collected and centrifuged at 40 000*g* for 20 min at 4 °C. The membrane pellet was resuspended in 10 volumes of Buffer B (10 mM HEPES, 1 mM EDTA, 1 mM EGTA, 100 μM PMSF, pH 7.4 using KOH), stirred on ice for 30 min, and then centrifuged at 40 000*g* for 20 min at 4 °C. The pellet was washed twice in 10 volumes of Buffer B before being resuspended in 10 mM HEPES, pH 7.4 and stored at −80 °C. The membrane protein concentration was determined by the Pierce bicinchonic acid method using bovine serum albumin (BSA) as the standard.

The SPA binding assay was performed in Costar 3632 96-well, clear bottom assay plates using wheat germ agglutinin coated polyvinyl toluene scintillation proximity assay (SPA) beads (Amersham Biosciences) using [^{3}H]gabapentin. Pig cortical membranes (10−20 μg protein per well) and SPA beads (0.5 mg per well) were mixed with 30 nM [^{3}H]gabapentin (specific activity = 44.15 Ci/mmol) in 10 mM HEPES/10 mM $MgSO_4$ assay buffer, pH 7.4 using KOH. The final well volume was 200 μL, and nonspecific binding was determined in the presence of 10 μM unlabeled (cold) pregabalin. The mixture containing membrane protein with SPA beads and [^{3}H]gabapentin was incubated at room temperature overnight (15−24 h), and plates were then counted on a Wallace Trilux 1450 Microbeta scintillation counter. Curve fitting and IC_{50} values were calculated using a four-parameter, nonlinear regression equation from GraphPad Prism 4.0 software, while K_i values were determined using previously determined K_D values for pig (K_D = 20 nM) and the equation of Cheng and Prusoff.[31]

System L Transport Assay. CHO cells were used in this study since System L transport has been well characterized in CHO cells.[32] CHO−K1 cells were maintained in minimum essential medium alpha medium (Gibco #32571−036) supplemented with 5% fetal bovine serum (Gibco #10082−139) and 1% penicillin/streptomycin (Gibco #15140−122). Cells were typsinized, diluted and plated into 96-well tissue culture microplates (Perkin-Elmer, Isoplate TC) the day prior to running the transport assay. One hundred microliters of a cell suspension at a density of 3×10^5 cells/mL was added to each well. This resulted in 90−100% confluent cell monolayers 24 h later. Protein analysis (~10μg/well) consistently showed ≤5% difference between all wells on a plate (data not shown). The System L transporter assays were run in sodium-free buffer (PBC) containing (in mM): choline chloride (137), KCl (2.7), choline phosphate (10.6), KH_2PO_4 (1.5), d-glucose (5.6), $MgCl_2$ (0.49), and $CaCl_2$ (0.9). A [^{3}H]L-leucine uptake solution consisting of PBC with 2 μCi/mL L-[4,5-^{3}H]leucine (Amersham #TRK 510, 1 mCi/mL, 120−190Ci/mmol) was used. L-Leucine is a protypical substrate of the System L transporter. Test compound dilutions ranging from 10 mM to 300 nM in half log increments were made in duplicate for generation of IC_{50} values. A 96-well drug addition plate was prepared with these 10 drug dilutions in the tritiated L-leucine uptake solution. The drug plate also had two control wells for each test compound dilution set, one with no drug added and one with saturating L-leucine (10 mM) added. The osmolarities of all the uptake mixtures were held constant by varying the amounts of choline choride. To start the uptake assay, culture plates were washed with Na^+-free buffer two times for 20 min each at 37 °C. This step depleted endogenous amino acids and washed out the residual sodium. The following steps of the assay were done on an automated Beckman Multimek 96-well pipettor. One hundred microliters from each of the wells on the test compound plate were added to corresponding wells on the culture plate simultaneously. After 2 min at room temperature, the uptake of [^{3}H]L-leucine was terminated by washing three times with cold (4 °C) PBC. Preliminary experiments had indicated that [^{3}H]L-leucine uptake was linearly dependent on incubation time up to at least 5 min at 22 °C (data not shown). Plate wells were aspirated and then shaken out completely dry. One hundred and fifty microliters of scintillation cocktail (Perkin-Elmer-Trisafe) was then added to each well. This scintillation cocktail lysed the cells, and then the plates could be counted directly on a liquid scintillation plate counter (Wallac-Trilux). IC_{50}s were calculated by nonlinear least squares regression analysis (Sigmaplot 2001; SPSS, Inc, Chicago, Illinois) using a three-parameter Logistic fit formula: where $f = a/(1 + \mathrm{abs}(x/x_0)^b)$, $a = \max y$ (dependent variable), b = width of transition, ($x_0 = x$ (independent variable) value at 50% of functions amplitude (IC_{50}), and abs is the absolute value.

DBA/2 Anticonvulsant Model. All procedures were carried out in compliance with the NIH Guide for the Care and Use of Laboratory Animals under a protocol approved by the Pfizer Animal Use Committee. Male DBA/2 mice 3 weeks old (7−12 g at time of testing) were obtained from Jackson Laboratories, Bar Harbor, ME. Mice were placed into an enclosed acrylic plastic chamber (21 cm height, approximately 30 cm diameter) with a high-frequency speaker (4 cm diameter) in the center of the top lid. An audio signal generator (Protek Model B-810) was used to produce a continuous sinusoidal tone that was swept linearly in frequency between 8 and 16 kHz once each 10 ms. The average sound pressure level (SPL) during stimulation was approximately 100 dB at the floor of the chamber. Mice were placed within the chamber and allowed to acclimatize for 1 min. DBA/2 mice in the vehicle-treated group responded to the sound stimulus (applied until tonic extension occurred, or for a maximum of 60 s) with a characteristic seizure sequence consisting of wild running followed by clonic seizures, and later by tonic extension, and finally by respiratory arrest and death in 80% or more of the mice. In vehicle-treated mice, the entire sequence of seizures to respiratory arrest lasted approximately 15 to 20 s. The incidence of all the seizure phases in the drug-treated and vehicle-treated mice was recorded, and the occurrence of tonic seizures was used for calculating anticonvulsant activity. Mice were used only once for testing, and therefore different groups were used for each time point and for each dose. Groups of DBA/2 mice (N = 10) were tested for sound-induced seizure responses at previously determined time of peak effect after oral (PO) or intracerebroventricular (icv) drug administration. All compounds in the present study were dissolved in distilled water and given orally by gavage, or by icv injection and given in a volume of 10 mL/kg of body weight PO, or 5 μL total volume icv. Vehicle treatment consisted of water, given by oral gavage, or 0.09% sodium chloride given icv. Immediately before intracerebroventricular injection DBA/2 mice were restrained by the investigator before injection of 5 μL volume to the right lateral ventricle of the animal using a 10 μL Hamilton syringe. Each animal received only one icv injection before placing in chamber for anticonvulsant testing.

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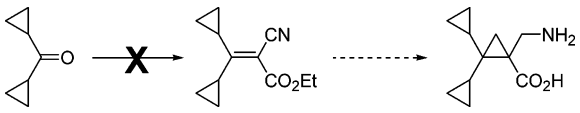
Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Lauria-Horner, B. A.; Pohl, R. B. Pregabalin: a new anxiolytic. *Expert Opin. Investig. Drugs* **2003**, *12* (4), 663.
(2) Wheeler, K. Gabapentin (Pfizer). *Curr. Opin. Investig. Drugs* **2002**, *3* (3), 470.
(3) Field, M. J.; Oles, R. J.; Singh, L. Pregabalin may represent a novel class of anxiolytic agents with a broad spectrum of activity. *Br. J. Pharmacol.* **2001**, *132*, 1−4.
(4) Gee, N. S.; Brown, J. P.; Dissanayake, V. U. K.; Offord, J.; Thurlow, R.; Woodruff, G. N. The novel anticonvulsant drug, gabapentin (Neurontin), binds to the α2δ subunit of a calcium channel. *J. Biol. Chem.* **1996**, *271* (10), 5768.

(5) Fink, K.; Dooley, D. J.; Meder, W. P.; Suman-Chauhan, N.; Duffy, S.; Clusmann, H.; Gothert, M. Inhibition of neuronal Ca^{2+} influx by gabapentin and pregabalin in the human neocortex. *Neuropharmacology* **2002**, *42* (2), 229.
(6) Dooley, D. J.; Mieske, C. A.; Borosky, S. A. Inhibition of K^+-evoked glutamate release from rat neocortical and hippocampal slices by gabapentin. *Neurosci. Lett.* **2000**, *280* (2), 107.
(7) Dooley, D. J.; Donovan, C. M.; Pugsley, T. A. Stimulus-dependent modulation of [^{3}H]norepinephrine release from rat neocortical slices by gabapentin and pregabalin. *J. Pharmacol. Exp. Ther.* **2000**, *295* (3), 1086.
(8) Maneuf, Y. P.; Hughes, J.; McKnight, A. T. Gabapentin inhibits the substance P-facilitated K^+-evoked release of [^{3}H]glutamate from rat caudal trigeminal nucleus slices. *Pain* **2001**, *93* (2), 191.
(9) McClelland, D.; Evans, R. M.; Barkworth, L.; Martin, D. J.; Scott, R. H. A study comparing the actions of gabapentin and pregabalin on the electrophysiological properties of cultured DRG neurones from neonatal rats. *BMC Pharmacology* **2004**, *4* (14).
(10) Bryans, J. S.; Davies, N.; Gee, N. S.; Dissanayake, V. U. K.; Ratcliffe, G. S.; Horwell, D. C.; Kneen, C. O.; Morrell, A. I.; Oles, R. J.; O'Toole, J. C.; Perkins, G. M.; Singh, L.; Suman-Chauhan, N.; O'Neill, J. A. Identification of novel ligands for the gabapentin binding site on the α2δ subunit of a calcium channel and their evaluation as anticonvulsant agents. *J. Med. Chem.* **1998**, *41*, 1838.
(11) Bryans, J. S.; Wustrow, D. J. 3-Substituted GABA analogues with central nervous system activity: a review. *Med. Res. Rev.* **1999**, *19* (2), 149.
(12) Belliotti, T. R.; Capiris, T.; Ekhato, I. V.; Kinsora, J. J.; Field, M. J.; Heffner, T. G.; Meltzer, L. T.; Schwarz, J. B.; Taylor, C. P.; Thorpe, A. J.; Vartanian, M. G.; Wise, L. D.; Su, T.-Z.; Weber, M. L.; Wustrow, D. J. Structure−activity relationships of pregabalin and analogues that target the α_2-δ protein. *J. Med. Chem.*, in press.
(13) For other efforts to identify α_2-δ ligands, see: Stearns, B. A.; Anker, N.; Arruda, J. M.; Campbell, B. T.; Chen, C.; Cramer, M.; Hu, T.; Jiang, X.; Park, K.; Ren, K. K.; Sablad, M.; Santini, A.; Schaffhauser, H.; Urban, M. O.; Munoz, B. Synthesis and biological evaluation of 6-aryl-6*H*-pyrrolo[3,4-d]pyridazine derivatives: high affinity ligands to the α2δ subunit of voltage-gated calcium channels. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1295, and also refs 13−15.
(14) Lim, J.; Stock, N.; Pracitto, R.; Boueres, J. K.; Munoz, B.; Chaudhary, A.; Santini, A. M.; Orr, K.; Schaffhauser, H.; Bezverkov, R. E.; Aiyar, J.; Venkatraman, S. *N*-Acridin-9-yl-butane-1,4-diamine derivatives: high affinity ligands to the α2δ subunit of voltage-gated calcium channels. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1913.
(15) Hu, T.; Stearns, B. A.; Campbell, B. T.; Arruda, J. M.; Chen, C.; Aiyar, J.; Bezverkov, R. E.; Santini, A.; Schaffhauser, H.; Liu, W.; Venkatraman, S.; Munoz, B. Synthesis and biological evaluation of 6-aryl-6*H*-pyrrolo[3,4-d]pyridazine derivatives as high affinity ligands of the α2δ subunit of voltage-gated calcium channels. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2031.
(16) Lebsack, A. D.; Gunzner, J.; Wang, B.; Pracitto, R.; Schaffhauser, H.; Santini, A.; Aiyar, J.; Bezverkov, R.; Munoz, B.; Liu, W.; Venkatraman, S. Identification and synthesis of [1,2,4]triazolo-[3,4-*a*]phthalazine derivatives as high-affinity ligands to the α2δ-1 subunit of voltage gated calcium channel. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2463.
(17) Babler, J. H.; Spina, K. P. A tandem Michael reaction-cycloalkylation utilizing 2-nitropropane: A facile route to the acid component of insecticidal pyrethroids. *Tetrahedron Lett.* **1985**, *26*, 1923.
(18) Ono, N.; Tetsuya, Y.; Isami, H.; Kamimura, A.; Kaji, A. Nitro compounds as nucleophilic alkylidene transfer reagents. *J. Org. Chem.* **1985**, *50* (15), 2806. The methyl ester of compound **5** is disclosed in this paper.
(19) Mélot, J.-M.; Texier-Boullet, F.; Foucaud, A. Cyclopropanation of electrophilic alkenes with nitroalkanes in the presence of alumina-supported potassium fluoride. *Synthesis* **1987**, 364.
(20) For other reports describing constrained gabapentin and pregabalin analogues, see: Receveur, J.-M.; Bryans, J. S.; Field, M. J.; Singh, L.; Horwell, D. C. Synthesis and biological evaluation of conformationally restricted gabapentin analogues. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2329, and references therein. See also ref 20.
(21) Ling, R.; Ekhato, I. V.; Rubin, J. R.; Wustrow, D. J. Synthesis of 4-alkyl-pyrrolidine-3-carboxylic acid stereoisomers. *Tetrahedron* **2001**, *57* (30), 6579.
(22) Satoh, T.; Suzuki, S.; Suzuki, Y.; Miyaji, Y.; Imai, Z. Reduction of organic compounds with sodium borohydride-transition metal salt systems (1). Reduction of organic nitrile, nitro, and amide compounds to primary amines. *Tetrahedron Lett.* **1969**, (52), 4555.
(23) For the preparation of related cyclopropyl amino acids see: Brandl, M.; Kozhushkov, S. I.; Yufit, D. S.; Howard, J. A. K.; de Meijere, A. Cyclopropyl building blocks for organic synthesis, 49. convenient preparation of α-amino acids with bicyclopropylidene and other methylenecyclopropane moieties. *Eur. J. Org. Chem.* **1998**, (12), 2785, and references therein.
(24) Attempts to fashion the tris-cyclopropyl amino acid failed at the Knoevenagel condensation stage with the sterically encumbered dicyclopropyl ketone.



(25) Badorrey, R.; Cativiela, C.; Díaz-de-Villegas, M. D.; Gálvez, J. A.; Gil, A. Efficient enantioconvergent synthesis of (*S*)-α-benzyl-α-methyl-*β*-alanine from (*R*)- and (*S*)-2-cyano-2-methyl-3-phenylpropanoic acid. *Tetrahedron: Asymmetry* **2003**, *14*, 2209−2214.
(26) The direct chlorination of α-methylbenzyl alcohols with methanesulfonyl chloride/pyridine was found to found to proceed in a solvent-dependent fashion: Tanaka, K.; Katsurada, M.; Ohno, F.; Shiga, Y.; Oda, M.; Miyagi, M.; Takehara, J.; Okano, K. Practical Asymmetric Synthesis of (*S*)-MA20565, a Wide-Spectrum Agricultural Fungicide. *J. Org. Chem.* **2000**, *65*, 432.
(27) Ohno, M.; Tanaka, H.; Komatsu, M.; Ohshiro, Y. Novel synthesis of 1-cyanocyclopropane-1-carboxylic acid and its application to the synthesis of amino acids containing cyclopropane rings. *Synlett* **1991**, 919.
(28) Su, T.-Z.; Lunney, E.; Campbell, G.; Oxender, D. Transport of gabapentin, a γ-amino acid drug, by system L α-amino acid transporters: a comparative study in astrocytes, synaptosomes, and CHO cells. *J. Neurochemistry* **1995**, *64*, 2125−2131.
(29) Schlesinger, K.; Greik, B. J. The genetics and biochemistry of audiogenic seizures in: *Contributions to Behavior-Genetic Analysis: The Mouse as a Prototype* Lindzey, G., Theissen, D. D., Eds. Appleton-Century-Crofts, New York **1970**, 219−257; Seyford, T. N. Audiogenic Seizures in Mice. *Fed. Proc.* **1979**, *38*, 2399.
(30) Stevens, C. V.; Van Heecke, G.; Barbero, C.; Patora, K.; De Kimpe, N.; Verhe, R. Synthesis of substituted cyclopropylphosphonates by Michael induced ring closure (MIRC) reactions. *Synlett.* **2002**, (7), 1089.
(31) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(32) Shotwell, M. A.; Jayme, D. W.; Kilberg, M. S.; Oxender, D. L. Neutral amino acid transport systems in Chinese hamster ovary cells. *J. Biol. Chem.* **1981**, *256* (11), 5422−7.

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