α-Amino Acid Phenolic Ester Derivatives: Novel Water-Soluble General Anesthetic Agents Which Allosterically Modulate $GABA_A$ Receptors

Alison Anderson,[†] Delia Belelli,[‡] D. Jonathan Bennett,[†] Kirsteen I. Buchanan,[†] Anna Casula,[‡] Andrew Cooke,[†] Helen Feilden,[†] David K. Gemmell,[†] Niall M. Hamilton,*[,†] Edward J. Hutchinson,[†] Jeremy J. Lambert,[‡] Maurice S. Maidment,[†] Ross McGuire,[†] Petula McPhail,[†] Susan Miller,[†] Annalisa Muntoni,[‡] John A. Peters,[‡] Francis H. Sansbury,[†] Donald Stevenson,[†] and Hardy Sundaram[†]

Organon Research, Newhouse, Lanarkshire ML1 5SH, Scotland, U.K., and Neurosciences Institute, Department of Pharmacology and Neuroscience, Ninewells Hospital and Medical School, Dundee DD1 9SY, Scotland, U.K.

Received April 18, 2001

In the search for a novel water-soluble general anesthetic agent the activity of an α-amino acid phenolic ester lead, identified from patent literature, was markedly improved. In addition to improving in vivo activity in mice, good in vitro activity at $GABA_A$ receptors was also conferred. Within the series of compounds good enantioselectivity for both in vitro and in vivo activity was found, supporting a protein-mediated mechanism of action for anesthesia involving allosteric modulation of $GABA_A$ receptors. α-Amino acid phenolic ester **19**, as the hydrobromide salt Org 25435, was selected for clinical evaluation since it retained the best overall anesthetic profile coupled with improved stability and water solubility. In the clinic it proved to be an effective intravenous anesthetic in man with rapid onset of and recovery from anesthesia at doses of 3 and 4 mg/kg.

Introduction

The leading short-acting hypnotic used for induction and maintenance of general anesthesia is propofol, a formulation of 2,6-diisopropylphenol (Figure 1).[1] It exhibits the desirable characteristics of rapid onset and offset of action and can be used for long-term administration without significant accumulation. While it is an excellent intravenous anesthetic, propofol is associated with cardiovascular side effects and pain on injection.[2]

In this paper we describe a series of α-amino acid phenolic ester derivatives synthesized as part of a program aimed at identifying a new water-soluble intravenous anesthetic. The lead compound **1** (Figure 1), identified from patent literature, had the advantages of good water solubility and proven in vivo activity.[3] The in vivo activity of this lead was improved by synthetic modification, and in vitro activity at γ-aminobutyric acid A ($GABA_A$) receptors was also conferred. Allosteric modulation of $GABA_A$ receptors is now recognized as a very plausible mechanism by which many clinical agents exert their anesthetic effect[4] and has largely superseded older theories invoking perturbation of membranes or lipid bilayers.[5] The in vitro and in vivo enantioselectivity found within this series of chiral phenolic esters is compared with clinically used anesthetics and provides further support for a protein-mediated mechanism of action.

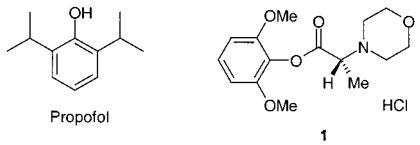


Figure 1.

Chemistry

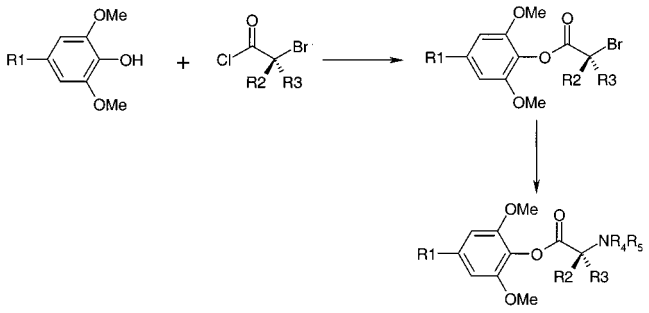
The α-amino acid phenolic ester derivatives **1**−**4**, **8**, **10**−**14**, and **17** were prepared by condensation of an appropriately substituted phenol with a 2-alkyl-2-bromoacyl chloride and subsequent displacement of the bromine atom with a secondary amine (Scheme 1). Compound **5** was prepared in an analogous way except that 2,6-dimethoxyaniline was used in place of the substituted phenol.

For compounds **9**, **15**, and **16**, an α-bromoester was first reacted with the appropriate secondary amine, and for introduction of the second α-methyl group of ester **9** the resulting amino ester was also treated with methyl iodide and lithium diisoproylamide. Ester hydrolysis of all three intermediates and condensation of the resulting α-dialkylaminocarboxylic acids with a phenol using 1,3-dicyclohexylcarbodiimide (DCC) or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) then afforded the desired products. The benzamide **6** and benzoate ester **7** were easily prepared from commercially available 2,6-dimethoxybenzoyl chloride and the appropriate N-substituted tetrahydroisoquinolinyl compound. All the target compounds were converted to water-soluble hydrochloride salts and characterized as such prior to pharmacological evaluation. Enantiomers were only prepared and tested if the racemate exhibited a good in vivo anesthetic profile. Enantiomer **1** was prepared from the racemate by an enantioselective crystallization using di-*p*-toluoyl-D-tartaric acid.[3] Analogues **3**, **4**, and **5** were prepared as shown in Scheme 1 using the corresponding chiral 2-alkyl-2-bromoacyl chlorides which were obtained via treatment of the commercially available acid with oxalyl chloride. Due to significant racemization during nucleophilic displacement of bromide

* To whom correspondence should be addressed. Tel: +44 (0)1698 736141. Fax: +44 (0)1698 736187. E-mail: n.hamilton@organon.nhe.akzonobel.nl.
[†] Organon Research.
[‡] Neurosciences Institute.

10.1021/jm010903i CCC: $20.00
Published on Web 09/14/2001

Scheme 1



with the secondary amine, an alternative strategy was required for enantiomers **18**, **19**, and **20**. These compounds were isolated by chiral HPLC or by enzymatic resolution of the respective racemates **13**, **14**, and **16**.[6] The structures of target compounds and pharmacological results are shown in Table 1.

Results and Discussion

Pharmacology. The hypnotic potency of each compound was determined upon intravenous (iv) administration to mice. The dose required to cause a loss of righting reflex (LRR) for a minimum period of 30 s in 50% of the treated mice was determined and is expressed as the HD_{50} (hypnotic dose 50) in μmol/kg. In a similar fashion, higher doses of the most potent compounds were injected to determine the LD_{50} (lethal dose 50) which was again expressed in μmol/kg. The therapeutic index (TI = LD_{50}/HD_{50}) was determined as a measure of the safety of the compound.

The in vitro effect of the target compounds at $GABA_A$ receptors was assessed through determination of their ability to inhibit [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS) binding to rat whole brain membranes. The concentration (μM) of target compound required to inhibit 50% of binding of [^{35}S]TBPS (IC_{50}) was determined. The results from the above experiments are shown in Table 1.

For potent anesthetic derivatives, the sleep time (ST) in minutes, measured at twice the HD_{50} dose, was also determined. The 'ideal' anesthetic should, like propofol, have a short duration of action (~5 min), but it should also have a good safety margin, i.e., TI ≥ 10. Determination of these parameters is crucial to the identification of a new improved anesthetic agent (see Table 2).

Further in vitro experiments involving the interactions of these compounds and propofol at $GABA_A$ receptors comprising specific subunit combinations are described later.

Structure−Activity Relationships. Previous work had demonstrated α-amino acid phenolic ester **1** to be an intravenous general anesthetic with a short duration of action.[3] Upon testing we established that the compound also afforded rapid, smooth recovery but lacked potency. We embarked on a program to improve the potency of this lead through a series of synthetic modifications and establish a mechanism of action for the anesthetic effect.

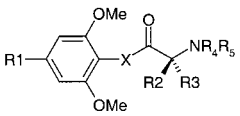
After examination of the physicochemical properties (clog P = 1.34, cpK_a = 5.98)[7] of the lead α-amino ester **1**, it was concluded that better CNS penetration would be expected from a more lipophilic compound. Replacement of the morpholinyl group of **1** with a 1,2,3,4-tetrahydroisoquinolinyl group afforded α-amino ester **2** (clogP = 3.64, cpK_a = 6.62; cf. log P = 3.8; pK_a = 5.6).[8] This modification afforded a significant increase in in vivo activity for the racemate **2** (HD_{50} = 33.2 μmol/kg) relative to the chiral lead **1** (HD_{50} = 85.9 μmol/kg) and also conferred in vitro activity in the [^{35}S]TBPS assay (IC_{50} = 4.8 μM). When racemate **2** was resolved, it was found that the *R* enantiomer **3** (HD_{50} = 20.7 μmol/kg) was 4 times more potent than the *S* enantiomer **4**. Indeed compound **3** was extensively tested in vivo and appeared to have an 'ideal' anesthetic profile in mice, i.e., rapid onset, short duration of action, and rapid recovery coupled with good potency and a reasonable therapeutic index. The main drawback of this compound was that its stability in aqueous solution was insufficient to allow easy formulation as an injectable preparation; this was a consequence of ester hydrolysis.[9]

To try to retain the desirable anesthetic profile and improve stability in aqueous solution, the amides **5** and **6** were synthesized as well as the benzoate ester **7**. Since additional steric bulk around the ester should also hinder hydrolysis, the phenolic esters **8**, **9**, and **10** were also prepared. Although anesthetic potency was largely retained when the phenolic ester was replaced, e.g., anilide **5** (HD_{50} = 44.1 μmol/kg), benzamide **6** (HD_{50} = 45.7 μmol/kg), and benzoate ester **7** (HD_{50} = 19.0 μmol/kg), the compounds were significantly slower in onset than the lead; anesthetists prefer compounds with a rapid onset of action since they are easier to titrate to effect.

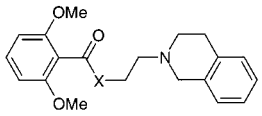
While the aqueous stability of the α-phenyl substituted derivative **8** was better than compound **2**, it was a less potent anesthetic (HD_{50} = 39.6 μmol/kg), and further testing was ruled out because of poor water solubility. Increased stability in aqueous solution was also realized with the achiral analogue **9** (HD_{50} = 53.8 μmol/kg) and the α-ethyl substituted analogue **10** (HD_{50} = 38.0 μmol/kg). The introduction of a *p*-methyl group on the aromatic ring, e.g., compound **11** (HD_{50} = 68.0 μmol/kg), caused a reduction in in vivo potency, and the time to onset of anesthesia was marginally slower than for the unsubstituted analogue **10**. Nevertheless, the realization that introduction of this methyl substituent afforded derivatives with a reasonably good anesthetic profile was important since a quinol metabolite of the lead **1** had been shown to be toxic in cats and the para substituent could prevent formation of such a metabolite.[10]

With regard to modification of the amino substituent, saturated heterocyclic derivatives tend to produce side effects or marked toxicity whereas retention of a double bond or incorporation of a heteroatom affords compounds with a good anesthetic profile.[3] Thus the tetrahydropyridinyl derivative **12** exhibited reasonably good in vitro and in vivo activity. Acyclic amino substituents incorporating a heteroatom are also well tolerated, e.g., bis(2-methoxyethyl)aminyl derivatives such as **13** (HD_{50} = 19.0 μmol/kg) and **14** (HD_{50} = 21.7 μmol/kg). Interestingly the detrimental effect of the para methyl group on anesthetic potency was reduced with these open chain amines. For these derivatives, anesthetic potency and in vitro activity remained excellent with increasing chain length, e.g., *n*-propyl **16** (HD_{50} =

Table 1. Hypnotic Activity (HD_{50}) and $GABA_A$ Modulatory Effects of α-Amino Acid Phenol Ester Derivatives



A

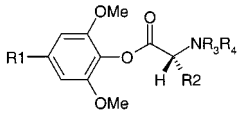


B

compd	structure	R_1	X	R_2	R_3	NR_4R_5	absolute configuration	HD_{50} (μmol/kg)[f]	TBPS IC_{50} (μM) mean ± SEM
propofol								68 (62−75)	18.0 ± 2.2 (n = 3)
1	A	H	O	H	Me	Morph[b]	R	85.9 (72.6−103.8)	>100 (n = 2)
2	A	H	O	H	Me	THQ[c]	racemic	33.2 (30.4−34.7)	4.8 ± 0.4 (n = 4)
3	A	H	O	H	Me	THQ	R	20.7 (19.0−22.6)	3.3 ± 1.0 (n = 4)
4[a]	A	H	O	H	Me	THQ	S	82.6 (69.8−98.8)	23.8 ± 6.6 (n = 6)
5	A	H	NH	H	Me	THQ	R	44.1 (39.8−48.8)	>10 (n = 4)
6	B	H	NH			THQ		45.7 (41.3−51.4)	>100 (n = 3)
7	B	H	O			THQ		19.0 (15.7−23.1)	>30 (n = 4)
8[a]	A	H	O	H	Ph	Morph	racemic	39.6 (33.1−47.8)	66 ± 17.5 (n = 3)
9	A	Me	O	Me	Me	Morph	racemic	53.8 (45.1−64.2)	>10 (n = 3)
10	A	H	O	H	Et	Morph	racemic	38.0 (31.9−45.4)	>10 (n = 4)
11	A	Me	O	H	Et	Morph	racemic	68.1 (n = 5)	ND[g]
12	A	Me	O	H	Et	THP[d]	racemic	35.1 (29.4−40.7)	17.4 ± 5.5 (n = 4)
13	A	H	O	H	Et	BME[e]	racemic	19.0 (16.3−22.2)	24.3 ± 7.2 (n = 3)
14	A	Me	O	H	Et	BME	racemic	21.7 (18.1−25.3)	29.3 ± 6.6 (n = 3)
15	A	H	O	H	^{i}Pr	BME	racemic	26.9 (22.2−32.6)	>10 (n = 3)
16	A	H	O	H	^{n}Pr	BME	racemic	12.4 (10.4−14.7)	7.7 ± 1.8 (n = 3)
17	A	H	O	H	^{n}Bu	BME	racemic	11.0 (9.4−13.6)	1.6 ± 0.4 (n = 3)
18	A	H	O	H	Et	BME	R	12.3 (11.2−13.6)	7.4 ± 1.1 (n = 6)
19	A	Me	O	H	Et	BME	R	18.0 (14.8−20.8)	16.0 ± 2.0 (n = 5)
20	A	H	O	H	^{n}Pr	BME	R	7.3 (6.1−8.7)	3.2 ± 0.5 (n = 4)

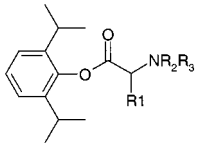
[a] CFLP mice. [b] Morpholine. [c] 1,2,3,4-Tetrahydroisoquinoline. [d] 1,2,5,6- Tetrahydropyridine. [e] Bis(2-methoxyethyl)amine. [f] 95% confidence limits. [g] Not determined.

Table 2. Sleep Time (ST) and Therapeutic Index of α-Amino Acid Phenol Ester Derivatives



compd	R_1	R_2	NR_3R_4	absolute configuration	ST (min) mean ± SEM	therapeutic index
propofol					6.30 ± 0.74	3.4
1	H	Me	Morph[b]	R	4.56 ± 0.22	6.9
2	H	Me	THQ[c]	racemic	4.26 ± 0.65	6.4
3[a]	H	Me	THQ	R	4.75 ± 0.21	7.3
19	Me	Et	BME[d]	R	5.20 ± 0.55	12

Table 3. Hypnotic Activity (HD_{50}) of 2,6-Dimethoxyphenol and Potential Propofol Prodrugs



compd	R_1	NR_2R_3	HD_{50} (μmol/kg)
propofol			68.0
2,6-dimethoxy phenol[a]			584−649
21[a]	H	Morph[b]	98.1 (90.8−106.1)
22	Et	Morph	convulsant

[a] CFLP mice. [b] Morpholine.

12.4 μmol/kg; IC_{50} = 7.7 μM) and *n*-butyl **17** (HD_{50} = 11.0 μmol/kg; IC_{50} = 1.6 μM) but decreased with branching, e.g., isopropyl **15** (HD_{50} = 27.0 μmol/kg; IC_{50} = >10 μM). Despite these advantages, excitatory side effects were much more apparent with the longer chain compounds, and the racemic α-ethyl derivatives **13** and **14** retained the best overall anesthetic profile, coupled with improved stability and water solubility. These racemates and the *n*-propyl derivative **16** were resolved, and like the lead **1**, better anesthetic activity resided with the *R* enantiomers **18**, **19**, and **20**, though the 4-fold in vivo selectivity seen with the tetrahydroisoquinolinyl derivatives **3** and **4** was no longer apparent.

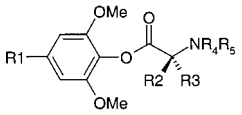
In addition to potency, the 'ideal' anesthetic must have a short duration of action (∼5 min) and a good therapeutic index. Sleep times for the more interesting α-amino ester derivatives were comparable to propofol and close to optimal, while the therapeutic index was excellent, e.g., amino ester **19** has a TI = 12.

On the basis of these results, compound **19** was profiled in other animal models and entered the clinic for evaluation in volunteers as the hydrobromide salt Org 25435.[11]

The clinical anesthetic propofol is a phenol, and prior to the aforementioned synthetic effort, we considered it prudent to check that a likely metabolite of these α-amino ester derivatives, namely 2,6-dimethoxyphenol, was not anesthetic. This phenol produced a LRR in mice only at very high doses (see Table 3). Moreover, while prodrugs of propofol have recently been reported to have interesting activity,[12] neither of the phenolic esters **21** or **22** was particularly interesting as a new anesthetic lead. Ester **21**, while retaining anesthetic activity, was less potent than propofol and very susceptible to hydrolysis in aqueous solution, whereas ester **22**, while more stable in solution, produced convulsions in mice upon iv administration. From these results and the fact that onset of action still occurred within a few seconds, we concluded that α-amino acid phenolic ester **1** is liable to have intrinsic anesthetic activity and its effect is not due to a metabolite.

Mechanism of Action: Allosteric Modulation of $GABA_A$ Receptors. Many anesthetics including bar-

Table 4. GABA-Modulatory and GABA-Mimetic Actions of α-Amino Acid Phenol Ester Derivatives and Propofol on Oocytes Expressing Recombinant Human $\alpha_1\beta_3\gamma_{2L}$ $GABA_A$ Receptors



compd	R_1	R_2	R_3	NR_4R_5	absolute configuration	GABA-modulatory action EC_{50} (μM)	GABA-mimetic action EC_{50} (μM)
propofol						4.1 ± 0.1 (n = 7)	50 ± 2 (n = 7)
1	H	H	Me	Morph[a]	R	198 ± 38 (n = 4)	ND (n = 4)[d]
2	H	H	Me	THQ[b]	racemic	5.5 ± 0.3 (n = 3)	ND (n = 3)[d]
3	H	H	Me	THQ	R	4.1 ± 0.4 (n = 4)	ND (n = 4)[d]
4	H	Me	H	THQ	S	>100 (n = 3)	ND (n = 3)[d]
19	Me	H	Et	BME[c]	R	10 ± 1.2 (n = 4)	ND (n = 4)[d]

[a] Morpholine. [b] 1,2,3,4-Tetrahydroisoquinoline. [c] Bis(2-methoxyethyl)amine. [d] Not determined: compounds were tested at concentrations of ≥100 μM.

biturates, benzodiazepines, etomidate, and propofol, as well as neuroactive steroids, are believed to act by potentiating the effects of GABA at $GABA_A$ receptors via an allosteric interaction.[13] The exact nature of the binding sites for these compounds has still to be established, but recent literature suggests the α_1 subunit is particularly important for mediating sedative activity for benzodiazepines.[14]

The α-amino acid phenolic ester derivatives were shown to modulate $GABA_A$ receptors by inhibiting the specific binding of the radioligand [^{35}S]-*tert*-butylbicyclophosphorothionate to rat whole brain membranes. As with other studies involving closely related compounds, there is no direct correlation of [^{35}S]TBPS displacement with in vivo activity for these α-amino acid phenolic esters, although in general compounds with better in vitro activity are more potent anesthetics.[4c] The in vitro results presented in Table 1 demonstrate modulation of GABAergic function by these compounds and suggest this mechanism mediates or enhances their hypnotic activity. We therefore decided to examine the effects of this series of α-amino acid ester derivatives with $GABA_A$ receptors using *Xenopus laevis* oocytes expressing recombinant human $\alpha_1\beta_3\gamma_{2L}$ GABA receptors. $\alpha_1\beta_3\gamma_{2l}$-Containing receptors are representative of $\alpha_1\beta_x\gamma_2$-containing receptors (where x = 1−3) and were selected for study since these constitute the major $GABA_A$ receptor subunit combinations present in mammalian brain. The results for esters **1**, **2**, **3**, **4**, and **19** together with propofol are shown in Table 4.

Propofol (0.1−30 μM) produced a concentration-dependent potentiation of the GABA (EC_{10})-evoked current with a calculated EC_{50} of 4.1 ± 0.1 μM and an E_{MAX} of 89 ± 7% (n = 7) produced by 30 μM of the anesthetic, where E_{MAX} is the amplitude of the response in the presence of a maximally effective concentration of the anesthetic. In the absence of GABA, propofol at concentrations greater than those required for substantial GABA modulation produced a concentration-dependent, picrotoxin-sensitive current with a calculated EC_{50} of 50 ± 2 μM (n = 7) and an E_{MAX} of 52 ± 6% occurring with 600 μM of the anesthetic.

α-Amino ester **1** produced a concentration-dependent (30 μM−1 mM) enhancement of the GABA-evoked current with an EC_{50} of 198 ± 38 μM and an E_{MAX} of 52 ± 4% (n = 4). Comparison of the EC_{50} values for GABA modulation reveal this anesthetic to be approximately 50-fold less potent than propofol, and the maximal enhancement of the GABA response produced by ester **1** is also reduced. The interaction of ester **1** with the $GABA_A$ receptor is further distinguished by a negligible 'GABA-mimetic' effect, even with high concentrations of the anesthetic.

The GABA modulatory potency of α-amino ester **2** was greatly improved in comparison with the lead compound **1**. Hence α-amino ester **2** produced a concentration-dependent (0.3−30 μM) enhancement of the GABA-evoked response with an EC_{50} of 5.5 ± 0.3 μM and an E_{MAX} of 75 ± 4% (n = 3), i.e., a 36-fold reduction of the EC_{50} and an increase of the apparent maximum compared with the lead compound **1**. In this respect α-amino ester **2** is approximately equipotent with propofol, but the interaction with the $GABA_A$ receptor was different from propofol as high concentrations (100 μM) of this compound produced only a very small 'GABA-mimetic' effect that precluded any meaningful determination of the EC_{50}.

Recent work has established that both the GABA-modulatory and central depressant effects of intravenous general anesthetics including barbiturates,[15] etomidate,[16] and isoflurane,[17] as well as neurosteroids such as 5α-pregnan-3α-ol-20-one[18] are enantioselective. Indeed, these data have been advanced to support the $GABA_A$ receptor as an important target in mediating the behavioral effects of general anesthetics. It was therefore of interest to examine the GABA-modulatory effects of the enantiomers **3** and **4**.

α-Amino ester **3** exhibited a similar concentration-dependent (0.3−30 μM) GABA-modulatory effect to that of the racemate **2** with an EC_{50} of 4.1 ± 0.4 μM and an E_{MAX} of 88 ± 7% (n = 4). The small direct effects evident for α-amino ester **2** were similar for α-amino ester **3** (100 μM = 5.5 ± 0.4%; n = 4). By contrast the GABA-modulatory effects of α-amino ester **4** were much reduced. Hence enhancement of GABA-mediated responses only became evident at concentrations of ≥10 μM with the maximum concentration tested (100 μM) producing a GABA-evoked current of only 26 ± 2% (n = 3) of the maximum response to GABA. Therefore the GABA-modulatory effects of these compounds are clearly enantioselective and consistent with their behavioral effects. The results suggest that anesthesia mediated by these esters is via an allosteric modulatory effect rather than the direct 'agonist-like' effect that is apparent only with propofol.

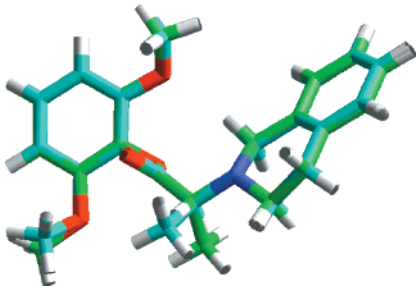


Figure 2. Overlay of the global minimum energy conformation of *R* enantiomer **3** (green carbons) and the higher local energy minimum of the *S* enantiomer **4** (blue carbons).

To rationalize the distinct pharmacology of the enantiomers **3** and **4**, some molecular modeling was done. The pharmacophore was assumed to comprise an aromatic phenolic ester, an amino substituent, and a lipophilic side chain, and binding of each enantiomer to $GABA_A$ receptors was also assumed to be similar.

Computer Modeling. A 3D structure of α-amino acid phenolic ester **3**, the more active *R* enantiomer, was generated from 2D input by Corina[19] and minimized and geometry optimized in Chem-X.[20] The optimized structure was then subjected to conformational analysis by driving each of the rotatable bonds through 360°. The lowest energy conformer was then extracted and subjected to optimization as before.

The lowest energy conformer was then reflected to give the *S* enantiomer. The *S* enantiomer was further manipulated so that all the main functionalities of the enantiomers would overlay. This involved rotation of bonds in the chain between the phenyl ring and the tetrahydroisoquinolinyl group, plus inversion of the tetrahydroisoquinolinyl nitrogen. This structure was then geometry optimized to a local energy minimum.

Overlay of both the global energy minimum structure of the *R* enantiomer (green carbons) and the local energy minimum of the manipulated *S* enantiomer (blue carbons) shows that a conformation of the *S* enantiomer can be found which has a striking similarity to the global minimum energy conformation of the more active *R* enantiomer (Figure 2). The only significant difference is on the chiral carbon where the methyls and hydrogens are, obviously, in different positions. The calculated energy for the *S* enantiomer was 1.0 $kcalmol^{-1}$ higher than for the *R* enantiomer. The RMS fit for all heavy atoms (except the methyl carbon attached to the chiral center) was 0.22 Å.

The active, binding conformation of the *R* enantiomer **3** is not known and cannot be assumed to be the global energy minimum found here. However the overlay shown in Figure 2 demonstrates that it possible to have a good match of the functional groups forming the pharmacophore for both enantiomers at a small energy cost. Moreover, since the chain atoms (between the phenyl ring and the tetrahydroisoquinolinyl ring) overlay and differ only in the position of one methyl group, such overlays are likely for additional conformations including the active conformation for receptor binding. From the above analysis and the [^{35}S]TBPS results in Table 1, it is apparent that the methyl substituent of the *S* enantiomer either hinders formation of the preferred conformation or produces an unfavorable steric interaction with the receptor.

Acting at $\alpha_1\beta_3\gamma_{2L}$ GABA receptors amino ester **19** produced a concentration-dependent enhancement (1−60 μM) of GABA (EC_{10})-evoked currents with an EC_{50} of 10 ± 1.2 μM and an E_{MAX} of 47 ± 4% (n = 4). Hence, in comparison with propofol, this compound is approximately 2-fold less active in regard to both the EC_{50} and E_{MAX}. Consistent with the other compounds examined in this chemical series, ester **19** exhibited only a limited direct effect (100 μM = 5.4 ± 1.4%; n = 4).

Given the interest in amino ester **19** as a potential new anesthetic agent, we investigated the GABAergic actions of this compound in further detail. The GABA-modulatory and GABA-mimetic effects of the general anesthetic etomidate are dependent upon the subunit composition of the $GABA_A$ receptor with a clear preference for β_2 and β_3 cf. β_1-containing receptors.[21] This β selectivity is mainly due to a single transmembrane located amino acid.[22] Acting at $\alpha_1\beta_1\gamma_{2L}$ receptors, amino ester **19** enhanced GABA-evoked currents with an EC_{50} of 23 ± 2 μM and an E_{MAX} of 34 ± 2% (n = 4). Hence, the exchange of the β_3 for the β_1 subunit had little effect on the E_{MAX} but did produce an approximately 2-fold increase in the EC_{50}, indicating that the GABA-modulatory actions of the amino ester **19** are less β-isoform selective than etomidate. Acting at $\alpha_1\beta_1\gamma_{2L}$ receptors, Org 25435 induced little or no direct current (300 μM = 1.5 ± 0.4%; n = 4).

Conclusion

Excellent anesthetic activity in mice was realized within a series of α-amino acid phenolic esters. Some of these derivatives also exhibited good activity as modulators of $GABA_A$ receptor function. The anesthetic and $GABA_A$ receptor-modulatory effects of these compounds are enantioselective, supporting a protein-mediated mechanism of action for anesthesia via allosteric modulation of $GABA_A$ receptors. Following evaluation in other animal models and pharmaceutical development, α-amino acid phenolic ester **19** was progressed to the clinic where it proved to be an effective intravenous anesthetic in man with rapid onset of and recovery from anesthesia at doses of 3 and 4 mg/kg.[11] While some side effects were noted, in particular small excitatory movements, changes in arterial blood pressure, and increased heart rate, the adverse event profile was unremarkable.

Experimental Section

Pharmacology. In Vivo Experiments. Drugs and Solutions. Propofol was obtained formulated as the emulsion Diprivan (Astra Zeneca) and was diluted in saline immediately prior to use. Stock solutions of salts of α-amino acid phenolic ester derivatives were prepared in saline, diluted as required and used immediately.

Hypnotic Potency (HD_{50}). Male MF-1 mice were used unless specified otherwise. Mice had access to food and water ad libitum and were kept on a 12 h light/dark cycle. All tests were performed during the light period. Groups of eight mice were injected intravenously (lateral tail vein) over 10 s with each dose of compound (10 mL/kg), placed in separate boxes to reduce external stimuli and tested for loss of righting reflex (LRR). Dosing was performed using a micromole/kilogram scheme. A set of dose levels, e.g., 2, 4, 8, and 16 μmol/kg, was chosen, and depending on whether LRR was observed, extra doses were introduced to provide data for LRR over a narrow dose range to allow potency calculations. From the percentage of mice in each group showing LRR for a period of

30 s or greater, a probit analysis (SAS Institute) was performed to yield an HD_{50} for each compound and 95% confidence limits.

Sleep Time (ST). Immediately after the end of the injection, the mice were tested for LRR. If immediate loss did not occur, the mice were closely observed and placed on their backs to determine the time of LRR. Once loss was noted, the duration of sleep (the interval between LRR and the return to righting reflex) was recorded. To compare the sleep time of each compound at equipotent doses, groups of 10 mice were injected with each compound at $2 \times HD_{50}$ over 10 s.

Therapeutic Index (TI). To determine an estimate of compound lethality (LD_{50}), groups of mice were injected in the same way, but over a higher dose range, and the numbers of mice dying at each dose was determined. The therapeutic index is the ratio LD_{50}/HD_{50}.

In Vitro Experiments. [^{35}S]TBPS Assay. [^{35}S]-*tert*-Butylbicyclophosphorothionate ([^{35}S]TBPS) is a potent cage convulsant which binds reversibly to the $GABA_A$ receptor at a site close to the channel. Binding of this radioligand is sensitive to both positive and negative allosteric modulators of the receptor, in addition to those compounds which directly compete for the channel binding site, such as picrotoxin.

Membrane Preparation. Male Wistar rats were decapitated and whole brains removed. Brains were homogenized in ice-cold 0.32 M sucrose solution using 10 strokes of a Potter S homogenizer set at 800 rpm. The homogenate was then centrifuged at 1000*g* for 10 min at 4 °C. The resulting pellet was discarded, and the supernatant was centrifuged at 20000*g* for 20 min at 4 °C. The pellet was resuspended and homogenized in ice-cold deionized water as previously and centrifuged at 8000*g* for 20 min at 4 °C. The tubes were gently inverted, and the 'buffy coat' was resuspended into the supernatant. The supernatant was then removed and centrifuged at 48000*g* for 20 min at 4 °C. The resulting pellet was then resuspended in assay buffer and frozen at −20 °C for 30 min. The pellets were thawed, resuspended a second time, and again centrifuged at 48000*g* for 20 min. The above freeze−thaw cycle was performed four times in total, and the final pellet was resuspended in an appropriate volume of assay buffer, dispensed into 2 mL samples, and frozen at −20 °C. Protein determinations were performed using the method of Lowry et al.[23]

Binding Assay. Assays were performed in deep well 96-well microtiter plates in a total volume of 200 μL which contained the following: [^{35}S]TBPS (2 nM final concentration), 100 μg membrane preparation, assay buffer (20 mM KH_2PO_4, 200 mM KCl, pH 7.4) containing GABA at a final concentration of 600 nM, and test drug. Incubations were initiated by addition of membranes. Nonspecific binding was determined using 200 μM picrotoxin. Incubations were carried out at room temperature (approx 22 °C) on a plate shaker for 150 min. Assays were terminated by vacuum filtration through a 96-plate cell harvester onto Whatman GF/B filters presoaked with distilled water. Filters were washed three times with 2 mL of ice-cold distilled water before being dried in an oven for 30 min at 40 °C. Scintillation fluid was added to each well and radioactivity determined using a Packard TopCount.

Electrophysiology. *Xenopus laevis* oocytes were used for experimentation 2−12 days after cRNA (human $\alpha_1\beta_3\gamma_{2L}$) injection. Electrical recordings were made from oocytes voltage-clamped at −60 mV with an Axoclamp 2A amplifier (Axon Instruments, USA), in the two electrode voltage-clamp mode. The oocytes were held in a recording chamber and continuously superfused (10 mL min^{-1}) with a buffered saline solution (composition in mM: NaCl 120, KCl 2, $CaCl_2$ 1.8, HEPES 5; adjusted to pH 7.4 with NaOH). Both the current-passing and voltage-sensing electrodes were filled with 3 M KCl and had resistances of 1−2 MΩ when measured in the buffered saline solution. Agonist-induced currents were low pass filtered at a corner frequency of 100 Hz and displayed on a chart recorder. The peak amplitude of the agonist-evoked response was measured manually. GABA and anesthetic compounds were applied via the superfusion system.

For each oocyte a maximal concentration of GABA (3 mM) was applied every 20 min until the control response was stable. Once the maximal response was stabilized, a concentration of GABA producing a peak current response 10% of the maximum obtainable (EC_{10}) was determined for each oocyte. The anesthetic was preapplied for 30−60 s before co-application with the appropriate concentration of GABA. Putative 'GABA-mimetic' effects were investigated in the absence of GABA and when evident were expressed as a percentage of the current induced by a saturating concentration of GABA. Concentration−response relationships for GABA-modulatory actions of the anesthetics were iteratively fitted by FigP 6c, with the four-parameter logistic equation

$$\frac{E}{E_{MAX}} = \frac{[A]^{n_H}}{[A]^{n_H} + [EC_{50}]^{n_H}}$$

where E is the amplitude of the GABA-evoked current in the presence of the anesthetic at concentration [A], E_{MAX} is the amplitude of the response in the presence of a maximally effective concentration of the anesthetic, EC_{50} is the concentration of anesthetic producing a half-maximal enhancement of the GABA-evoked response, and n_H is the Hill coefficient. The small magnitude of the "GABA-mimetic" effects of the novel compounds tested (compounds **1**−**4**, **19**) precluded any meaningful determination of EC_{50} for this effect. However, in agreement with previous reports, propofol produced a substantial 'GABA-mimetic' action. Propofol concentration response relationships were fitted where now E represents the current amplitude evoked by propofol concentration [A], E_{MAX} is the response in the presence of a maximally effective concentration of propofol, and EC_{50} is the concentration of propofol producing a half-maximal response. Inward currents induced by the anesthetics were only categorized as being mediated via the $GABA_A$ receptor if they were antagonized by the $GABA_A$ receptor antagonist picrotoxin (30 μM).

Experiments were conducted at ambient temperature (18−22 °C). Quantitative data are presented as the mean ± s.e.mean. The s.e.mean values associated with the EC_{50} are those derived from the fitted curve.

Chemistry. General. Melting points were taken with either a Gallenkamp capillary melting point apparatus or a Reichert hot plate apparatus and are uncorrected. Optical rotations were determined using an Optical Activity Ltd. AA-1000 polarimeter at room temperature for solutions in chloroform (unless specified otherwise), and *c* refers to concentration in grams per 100 mL. ^{1}H NMR (200 or 400 MHz) spectra were obtained using a Bruker AM200 or a Bruker DRX400 instrument; chemical shifts (δ) are relative to tetramethylsilane as internal standard. Only discrete or characteristic signals are reported. Coupling constants are given in hertz. IR spectra were obtained with a Perkin-Elmer 16PC FT-IR spectrometer. Electrospray masses were determined on a PE Sciex 150 EX spectrometer. Accurate masses were determined on a PerSeptive Biosystems Mariner Biospectrometry Workstation using TOF MS. Elemental analyses were determined on a (Perkin-Elmer 2400 CHN) elemental analyzer and are within 0.4% of theory unless otherwise noted.

Materials. Reagents were used as supplied from commercial sources.

Syntheses. (*S*)-2-Bromopropionyl chloride: A solution of oxalyl chloride (73 mL, 0.84 mol) in dry dichloromethane (70 mL) was added slowly to a stirred solution of (*S*)-2-bromopropionic acid (58.8 g, 0.38 mol) in dichloromethane (590 mL). Gas evolution was observed, and the system was allowed to stir at room temperature for 28 h. The system was concentrated in vacuo to give the title compound as an oil (65.0 g, 100%): ^{1}H NMR ($CDCl_3$) δ 1.92 (d, 3H, $J \sim 6$), 4.65 (q, 1H, $J \sim 6$).

(*R*)-2-Bromopropionyl chloride was prepared similarly. ^{1}H NMR analysis of the product was identical to that shown above.

(*S*)-2-Bromopropionic acid, 2,6-dimethoxyphenyl ester: A solution of (*S*)-2-bromopropionyl chloride (64.0 g, 0.37

mol) and 2,6-dimethoxyphenol (55.0 g, 0.36 mol) in dry toluene (150 mL) was stirred under nitrogen and cooled to −10 °C. A solution of dry pyridine (32.2 mL, 0.40 mol) was added dropwise, ensuring the temperature remained below 0 °C. After 20 min, the resulting suspension was diluted with water (500 mL), and the mixture was filtered through a dicalite pad. After the mixture was rinsed with more toluene (400 mL), the layers were separated and the organic layer washed with more water (3 × 150 mL). The organic layer was dried ($MgSO_4$) and filtered, and the solvent was removed in vacuo to give the title compound as a light yellow solid (92.6 g, 87%): ^{1}H NMR ($CDCl_3$) δ 1.98 (d, 3H, $J \sim 5.5$), 3.82 (s, 6H), 4.70 (q, 1H, $J \sim 5.5$), 6.65 (d, 2H, $J \sim 8.5$), 7.15 (t, 1H, $J \sim 8.5$).

(*R*)-2-Bromopropionic acid, 2,6-dimethoxyphenyl ester and (±)-2-bromopropionic acid, 2,6-dimethoxyphenyl ester were prepared similarly. ^{1}H NMR analysis of the compounds was identical to that shown above.

The following compounds were similarly prepared except triethylamine was used instead of pyridine:

(±)-2-Bromobutyric acid, 2,6-dimethoxy-4-methylphenyl ester: 100%; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, $J \sim 5$), 2.05−2.35 (m, 2H), 2.34 (s, 3H), 3.80 (s, 6H), 4.45 (t, 1H, $J \sim 5.5$), 6.40 (s, 2H).

(±)-2-Bromobutyric acid, 2,6-dimethoxyphenyl ester: 100%; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, $J \sim 5$), 2.10−2.35 (m, 2H), 3.82 (s, 6H), 4.47 (t, 1H, $J \sim 5.5$), 6.60 (d, 2H, $J \sim 8.5$), 7.15(t, 1H, $J \sim 8.5$).

(±)-2-Bromohexanoic acid, 2,6-dimethoxyphenyl ester: 97%; ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H, $J \sim 6$), 1.35−1.63 (m, 4H), 2.08−2.31 (m, 2H), 3.82 (s, 6H), 4.50 (t, 1H, $J \sim 6$), 6.60 (d, 2H, $J \sim 9$), 7.13 (t, 1H, $J \sim 9$).

The following compound was similarly prepared except dry diethyl ether was used instead of toluene:

(±)-α-Chlorophenylacetic acid, 2,6-dimethoxyphenyl ester: 67%; ^{1}H NMR ($CDCl_3$) δ 3.70 (s, 6H), 5.71 (s, 1H), 6.55 (d, 2H, $J \sim 8.5$), 7.11 (t, 1H, $J \sim 8.5$), 7.34−7.46 (m, 3H), 7.59−7.70 (m, 2H).

(*R*)-2-Morpholinopropionic acid, 2,6-dimethoxyphenyl ester hydrochloride (1): (±)-2-Bromopropionic acid, 2,6-dimethoxyphenyl ester (153.5 g, 0.53 mol) was heated under reflux in anhydrous toluene (730 mL) while morpholine (92.6 mL, 1.06 mol) was added over 15 min. After heating for 6 h, the solution was cooled and filtered. The filtrate was washed with 4 M sodium hydroxide solution (200 mL) then water (2 × 200 mL) and dried (Na_2SO_4), and the solvent was removed in vacuo. The crude racemic product was recrystallized from methyl *tert*-butyl ether and isolated as a white solid (82.5 g, 53%).

A solution of (±)-2-morpholinopropionic acid, 2,6-dimethoxyphenyl ester (82.5 g, 0.28 mol) in hot ethanol (225 mL) was treated with a solution of di-*p*-toluoyl-D-tartaric acid (107.9 g, 0.28 mol) in more hot ethanol (225 mL). The resulting solution was stirred and cooled in ice until crystallization was complete. The solid was filtered off, dried, and redissolved in boiling methanol (900 mL). The hot solution was filtered and then allowed to stand at ambient temperature for 3 h. The crystalline solid was filtered off and recrystallized from methanol and then 2-butanone to give (*R*)-2,6-dimethoxyphenyl-2′-morpholinopropionate di-*p*-toluoyl-D-tartrate (1:1) salt. This salt was suspended in water (50 mL), and saturated aqueous sodium bicarbonate solution (100 mL) was added. The liberated base was extracted with methyl *tert*-butyl ether (3 × 150 mL). The combined organic phase was dried (Na_2SO_4) and filtered and the solvent removed in vacuo. The residual oil was dissolved in dry diethyl ether and treated with gaseous hydrogen chloride. The resulting precipitate was filtered off and recrystallized from 2-propanol to give the title compound as a white crystalline solid (22.3 g, 24% from racemate): ^{1}H NMR ($CDCl_3$) δ 1.95 (d, 3H, $J \sim 7.5$), 3.35 (d, 1H, $J \sim 12$), 3.45 (d, 1H, $J \sim 12$), 3.66 (br s, 2H), 3.78 (s, 6H), 3.92−4.04 (m, 2H), 4.28−4.40 (m, 2H), 4.49 (br t, 1H, $J \sim 12$), 6.64 (d, 2H, $J \sim 8.5$), 7.21 (t, 1H, $J \sim 8.5$); IR (KBr) 2947, 2410, 1777, 1610, 1482, 1261, 1177, 1071 cm^{-1}; *m*/*z* (ES) $[M+H]^+$ 296; Found: 296.1505, $C_{15}H_{22}NO_5$ requires 296.1490; $[\alpha]_D$ −8.9° (*c* 0.98; H_2O), lit $[\alpha]_D$ −9.6° (H_2O).[3] Anal. ($C_{15}H_{22}ClNO_5$) C, H, N, Cl.

The following compound was similarly prepared but isolated as a racemate:

(±)-2-[2(1,2,3,4-Tetrahydroisoquinolinyl)]propionic acid, 2′,6′-dimethoxyphenyl ester hydrochloride (2): 25%; mp 167−173 °C; ^{1}H NMR ($CDCl_3$) δ 2.07 (d, 3H, $J \sim 7$), 2.89−3.11 (m, 1H), 3.63−3.75 (m, 2H), 3.77 (s, 6H), 3.86−4.02 (m, 1H), 4.41−4.71 (m, 2H), 4.72−4.89 (m, 1H), 6.14 (d, 2H, $J \sim 9$), 7.05−7.31 (m, 5H); IR (KBr) 2953, 2433, 1771, 1617, 1480, 1264, 1172, 1117 cm^{-1}; *m*/*z* (ES) $[M+H]^+$ 342; Found: 342.1713, $C_{20}H_{24}NO_4$ requires 342.1697. Anal. ($C_{20}H_{24}ClNO_4$) C, H, N, Cl.

The following compounds were similarly prepared except chiral 2-bromopropionate esters were used with acetone as solvent. Chiral purity was achieved by recrystallization from 2-propanol.

(*R*)-2-[2(1,2,3,4-Tetrahydroisoquinolinyl)]propionic acid, 2′,6′-dimethoxyphenyl ester hydrochloride (3): 36%; ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 1.80 (d, 3H, $J \sim 7$), 3.02−3.14 (m, 1H), 3.18−3.36 (m, 2H), 3.40−3.49 (m, 1H), 3.78 (s, 6H), 4.15 (q, 1H, $J \sim 7$), 4.28 (d, 1H, $J \sim 15$), 4.33 (d, 1H, $J \sim 15$), 6.60 (d, 2H, $J \sim 8.5$), 7.05−7.12 (m, 1H), 7.12−7.23 (m, 4H), 8.58 (br s, 1H); IR (KBr) 2945, 2437, 1769, 1614, 1485, 1267, 1117 cm^{-1}; *m*/*z* (ES) $[M+H]^+$ 342; Found: 342.1708, $C_{20}H_{24}NO_4$ requires 342.1697; $[\alpha]_D$ +44.4° (*c* 0.12). Anal. ($C_{20}H_{24}ClNO_4$) C, H, N, Cl.

(*S*)-2-[2(1,2,3,4-Tetrahydroisoquinolinyl)]propionic acid, 2′,6′-dimethoxyphenyl ester hydrochloride (4): 48%; ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 1.80 (d, 3H, $J \sim 7$), 3.02−3.14 (m, 1H), 3.18−3.36 (m, 2H), 3.40−3.49 (m, 1H), 3.78 (s, 6H), 4.15 (q, 1H, $J \sim 7$), 4.28 (d, 1H, $J \sim 15$), 4.33 (d, 1H, $J \sim 15$), 6.60 (d, 2H, $J \sim 8.5$), 7.05−7.12 (m, 1H), 7.12−7.23 (m, 4H), 8.58 (br s, 1H); IR (KBr) 2945, 2437, 1769, 1614, 1485, 1267, 1117 cm^{-1}; *m*/*z* (ES) $[M+H]^+$ 342; Found: 342.1691, $C_{20}H_{24}NO_4$ requires 342.1697; $[\alpha]_D$ −45.8° (*c* 0.67). Anal. ($C_{20}H_{24}ClNO_4$) C, H, N, Cl.

(*R*)-α-Methyl-[2(1,2,3,4-tetrahydroisoquinolinyl)]-2′,6′-dimethoxyacetanilide hydrochloride (5): Diisopropylethylamine (20.5 mL, 0.11 mol) was added dropwise to a stirred solution of 2,6-dimethoxyaniline hydrochloride (11.0 g, 58.0 mmol) and *S*-(−)-2-bromopropionyl chloride (10.5 g, 61.1 mmol) in dry dichloromethane (220 mL) cooled to 0 °C. The temperature was not allowed to rise above 5 °C during addition. After 20 min the solution was washed with water (2 × 200 mL). The organic layer was dried ($MgSO_4$) and filtered and the solvent removed in vacuo to give the required α-bromoamide as an oil (15.5 g, 93%) which was used without further purification. The α-bromoamide (3.0 g, 10.4 mmol) and 1,2,3,4-tetrahydroisoquinoline (2.30 mL, 18.1 mmol) were heated at reflux in acetone (30 mL) for 1 h. The solvent was evaporated in vacuo, and the residue was partitioned between water (50 mL) and ethyl acetate (50 mL). The organic layer was then washed with 2 N hydrochloric acid (2 × 50 mL). The combined aqueous phase was basified (4 N NaOH) and then extracted with more ethyl acetate (3 × 50 mL). The combined organic phase was dried ($MgSO_4$) and filtered and the solvent removed in vacuo. The resulting residue was redissolved in dichloromethane and excess dry hydrogen chloride gas added. The hydrochloride salt was precipitated with diethyl ether, filtered, and dried in vacuo to give the title compound (1.85 g, 47%): mp 221−225 °C; ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 1.56 (d, 3H, $J \sim 7$), 3.08−3.28 (m, 4H), 3.79 (s, 6H), 3.83−3.96 (m, 1H), 4.05 (d, 1H, $J \sim 15$), 4.26 (d, 1H, $J \sim 15$), 6.56 (d, 2H, $J \sim 8.5$), 7.02−7.10 (m, 1H), 7.11−7.23 (m, 4H), 9.05 (br s, 1H); IR (KBr) 3476, 2947, 1692, 1593, 1482, 1263, 1118 cm^{-1}; *m*/*z* (ES) $[M+H]^+$ 341; Found: 341.1864, $C_{20}H_{25}N_2O_3$ requires 341.1857; $[\alpha]_D$ −0.4° (*c* 0.61; MeOH). Anal. ($C_{20}H_{25}ClN_2O_3 \cdot 0.36\ H_2O$) C, H, N, Cl.

(±)-α-Morpholinophenylacetic acid, 2,6-dimethoxyphenyl ester hydrochloride (8): (±)-α-Chlorophenylacetic acid, 2,6-dimethoxyphenyl ester (6.13 g, 0.02 mol) in toluene (80 mL) was added to a stirred mixture of triethylamine (2.79 mL, 0.02 mol), morpholine (1.74 mL, 0.02 mol), and lithium

bromide (200 mg, 2.30 mmol) in toluene (50 mL). The mixture was heated to 100 °C for 72 h and then, after cooling, was washed with water (2 × 50 mL), dried (Na_2SO_4), and filtered, and the solvent was removed in vacuo to leave a residue which was purified by chromatography on basic alumina using toluene:ethyl actetate (9:1) as eluant. The free base was dissolved in dichloromethane and excess dry hydrogen chloride gas added. The salt was precipitated with diethyl ether, filtered, and recrystallized from dichloromethane/diethyl ether. This gave the title compound as a white crystalline solid (2.43 g, 31%): ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 2.59−2.69 (m, 2H), 2.69−2.80 (m, 2H), 3.66 (s, 6H), 3.75−3.87 (m, 4H), 4.41 (s, 1H), 6.55 (d, 2H, $J \sim 8.5$), 6.78 (br s, 1H), 7.10 (t, 1H, $J \sim 8.5$), 7.33−7.44 (m, 3H), 7.60−7.68 (m, 2H); IR (KBr) 3421, 2954, 2378, 1771, 1608, 1483, 1260, 1169, 1112 cm^{-1}; *m/z* (ES) $[M+H]^+$ 358; Found: 358.1655, $C_{20}H_{24}NO_5$ requires 358.1648. Anal. ($C_{20}H_{24}ClNO_5$) C, H, N.

The following compounds were similarly prepared in the absence of lithium bromide and, where stated, without triethylamine:

(±)-2-Morpholinobutyric acid, 2′,6′-dimethoxyphenyl ester hydrochloride (10): No triethylamine used, 45%; ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 1.11 (t, 3H, $J \sim 7$), 1.93−2.11 (m, 2H), 2.92−3.10 (m, 4H), 3.58 (dd, 1H, $J \sim 9$, 7.5), 3.82 (s, 6H), 3.83−3.97 (m, 4H), 6.62 (d, 2H, $J \sim 8.5$), 6.64 (br s, 1H), 7.16 (t, 1H, $J \sim 8.5$); IR (KBr) 3454, 2935, 2224, 1759, 1611, 1482, 1305, 1176, 1114 cm^{-1}; *m/z* (ES) $[M+H]^+$ 310; Found: 310.1663, $C_{16}H_{24}NO_5$ requires 310.1644. Anal. ($C_{16}H_{24}ClNO_5$·0.2 H_2O) C, H, N, Cl.

(±)-2-Morpholinobutyric acid, 2′,6′-dimethoxy-4′-methylphenyl ester hydrochloride (11): 27%; ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 1.12 (t, 3H, $J \sim 7$), 1.92−2.17 (m, 2H), 2.35 (s, 3H), 2.99−3.14 (m, 4H), 3.60 (dd, 1H, $J \sim 9$, 7.5), 3.79 (s, 6H), 3.84−4.00 (m, 4H), 6.43 (s, 2H); IR (KBr) 3499, 2970, 2386, 1760, 1610, 1509, 1473, 1247, 1172, 1139 cm^{-1}; *m/z* (ES) $[M+H]^+$ 324; Found: 324.1809, $C_{17}H_{26}NO_5$ requires 324.1803. Anal. ($C_{17}H_{26}ClNO_5$) C, H, N, Cl.

(±)-2-(1,2,5,6-Tetrahydropyridyl)butyric acid, 2′,6′-dimethoxy-4′-methylphenyl ester hydrochloride (12): No triethylamine used, 85%; ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 1.18 (t, 3H, $J \sim 7.5$), 2.01−2.19 (m, 1H), 2.36 (s, 3H), 2.41−2.58 (m, 2H), 2.68−2.86 (m, 1H), 3.31−3.44 (m, 2H), 3.72−3.84 (m, 7H), 3.90−4.05 (m, 2H), 5.71 (br d, 1H, $J \sim 13$), 5.97 (br d, 1H, $J \sim 13$), 6.43 (s, 2H); IR (KBr) 2946, 2106, 1763, 1605, 1471, 1247, 1158, 1134 cm^{-1}; *m/z* (ES) $[M+H]^+$ 320; Found: 320.1851, $C_{18}H_{26}NO_4$ requires 320.1857. Anal. ($C_{18}H_{26}ClNO_4$) C, H, N, Cl.

(±)-2-[*N*-Bis(2-methoxyethyl)amino]butyric acid, 2′,6′-dimethoxyphenyl ester hydrochloride (13): 13%; ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 1.13 (t, 3H, $J \sim 7.5$), 1.96−2.09 (m, 2H), 3.08−3.17 (m, 2H), 3.20−3.30 (m, 2H), 3.38 (s, 6H), 3.56−3.65 (m, 2H), 3.66−3.75 (m, 2H), 3.76−3.89 (m, 7H), 6.60 (d, 2H, $J \sim 8.5$), 7.14 (t, 1H, $J \sim 8.5$); IR (KBr) 3507, 2946, 2531, 1762, 1606, 1483, 1264, 1174, 1112 cm^{-1}; *m/z* (ES) $[M+H]^+$ 356; Found: 356.2062, $C_{18}H_{30}NO_6$ requires 356.2068. Anal. ($C_{18}H_{30}ClNO_6$·1.0H_2O) C, H, N, Cl.

(±)-2-[*N*-Bis(2-methoxyethyl)amino]butyric acid, 2′,6′-dimethoxy-4′-methylphenyl ester hydrochloride (14): 29%; ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 1.13 (t, 3H, $J \sim 7.5$), 1.92−2.13 (m, 2H), 2.34 (s, 3H), 3.10−3.21 (m, 2H), 3.23−3.35 (m, 2H), 3.37 (s, 6H), 3.56−3.65 (m, 2H), 3.68−3.81 (m, 8H), 3.82−3.91 (m, 1H), 6.42 (s, 2H); IR (KBr) 2944, 2278, 1768, 1607, 1470, 1248, 1196, 1119 cm^{-1}; *m/z* (ES) $[M+H]^+$ 370; Found: 370.2217, $C_{19}H_{32}NO_6$ requires 370.2227. Anal. ($C_{19}H_{32}ClNO_6$) C, H, N, Cl.

(±)-2-[*N*-Bis(2-methoxyethyl)amino]hexanoic acid, 2′,6′-dimethoxyphenyl ester hydrochloride (17): 61%; ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 0.95 (t, 3H, $J \sim 7$), 1.34−1.48 (m, 2H), 1.49−1.60 (m, 2H), 1.97 (q, 2H, $J \sim 9$), 3.06−3.18 (m, 2H), 3.18−3.30 (m, 2H), 3.37 (s, 6H), 3.55−3.64 (m, 2H), 3.65−3.74 (m, 2H), 3.80 (s, 6H), 3.87−3.96 (m, 1H), 6.05 (br s, 1H), 6.60 (d, 2H, $J \sim 8.5$), 7.12 (t, 1H, $J \sim 8.5$); IR (KBr) 2839, 1760, 1262, 1114 cm^{-1}; *m/z* (ES) $[M+H]^+$ 384; Found: 384.2370, $C_{20}H_{34}NO_6$ requires 384.2386. Anal. ($C_{20}H_{34}ClNO_6$) C, H, N.

***N*-(2-Aminoethyl)tetrahydroisoquinoline:** 2-Bromoethylamine hydrobromide (10.0 g, 48.8 mmol) and 1,2,3,4-tetrahydroisoquinoline (21.4 mL, 168 mmol) in toluene (100 mL) were heated at reflux for 9 h and then left to cool overnight. The reaction mixture was filtered and the filtrate washed with water (100 mL). The aqueous layer was then saturated with sodium chloride and washed with more toluene (3 × 100 mL). The combined organic layers were dried (Na_2SO_4) and filtered, and the solvent was removed in vacuo to give a residue which was purified by chromatography on alumina using dichloromethane:methanol:ammonia (9:1:0.2) as eluant. The title compound was isolated as an oil (1.54 g, 18%): ^{1}H NMR ($CDCl_3$) δ 1.52 (br s, 2H), 2.61 (t, 2H, $J \sim 5.5$), 2.77 (t, 2H, $J \sim 5.5$), 2.82−2.99 (m, 4H), 3.64 (s, 2H), 6.95−7.31 (m, 4H).

The following compound was similarly prepared except that 2-bromoethanol was used instead of 2-bromoethylamine hydrobromide:

***N*-(2-Hydroxyethyl)tetrahydroisoquinoline:** 20%; ^{1}H NMR ($CDCl_3$) δ 2.56 (br s, 1H), 2.66−2.76 (m, 2H), 2.77−2.87 (m, 2H), 2.88−2.98 (m, 2H), 3.63−3.77 (m, 4H), 6.94−7.20 (m, 4H).

***N*-2-[2-(1,2,3,4-Tetrahydroisoquinolinyl)]ethyl-2′,6′-dimethoxybenzamide hydrochloride (6):** A solution of *N*-(2-aminoethyl)tetrahydroisoquinoline (1.50 g, 8.50 mmol) in toluene (15 mL) was added to a stirred mixture of 2,6-dimethoxybenzoyl chloride (1.88 g, 90%, 8.43 mmol) and triethylamine (1.20 mL, 8.60 mmol) in toluene (15 mL). The reaction mixture was stirred at room temperature for 2.5 h and was then washed with water (2 × 25 mL), dried (Na_2SO_4), and filtered, and the solvent was removed in vacuo. The residue was dissolved in dichloromethane, and excess dry hydrogen chloride gas was added. Diethyl ether was added, and the precipitate was isolated by filtration and dried in vacuo. The title compound was isolated as a white solid (1.28 g, 40%): ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 3.21−3.33 (m, 2H), 3.38−3.58 (m, 4H), 3.76 (s, 6H), 3.96−4.06 (br s, 2H), 6.54 (d, 2H, $J \sim 8.5$), 7.11 (d, 1H, $J \sim 7$), 7.18−7.34 (m, 4H), 7.55 (br s, 1H); IR (KBr) 3242, 2939, 2538, 1657, 1596, 1474, 1252, 1109 cm^{-1}; *m/z* (ES) $[M+H]^+$ 341; Found: 341.1864, $C_{20}H_{25}N_2O_3$ requires 341.1857. Anal. ($C_{20}H_{25}ClN_2O_3$·0.2H_2O) C, H; N: calcd, 7.32; found, 6.86; Cl: calcd, 9.26; found, 8.27.

The following compound was similarly prepared except that the reaction mixture was heated at reflux for 4 h prior to workup:

***N*-2-[2(1,2,3,4-Tetrahydroisoquinolinyl)]ethyl 2′,6′-dimethoxybenzoate hydrochloride (7):** 20%; mp 172−177 °C; ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 3.19−3.28 (m, 2H), 3.40−3.51 (m, 4H), 3.77 (s, 6H), 4.32 (s, 2H), 4.85−4.93 (m, 2H), 6.57 (d, 2H, $J \sim 8.5$), 7.05 (d, 1H, $J \sim 7$), 7.14−7.28 (m, 3H), 7.28−7.37 (m, 1H); IR (KBr) 3440, 2951, 2490, 1731, 1597, 1476, 1432, 1298, 1257, 1109 cm^{-1}; *m/z* (ES) $[M+H]^+$ 342; Found: 342.1702, $C_{20}H_{24}NO_4$ requires 342.1697. Anal. ($C_{20}H_{24}ClNO_4$) C; H: calcd, 6.40; found, 5.77; N: calcd, 3.71; found, 3.12.

Ethyl 2-morpholinopropionate: Ethyl 2-bromopropionate (32.6 mL, 0.25 mol) and morpholine (55.1 mL, 0.63 mol) in toluene (150 mL) was stirred at room temperature for 2 h. The precipitate was filtered off and the filtrate evaporated in vacuo to give an oily residue that was purified by vacuum distillation. The title compound was isolated as a colorless oil (28.4 g, 60%): bp 80−82 °C at 1.2 mmHg (lit. 69−70 °C at 1 mmHg);[24] ^{1}H NMR ($CDCl_3$) δ 1.29 (t, 6H, $J \sim 7$), 2.62 (dd, 4H, $J \sim 5$, 4), 3.24 (q, 1H, $J \sim 7$), 3.73 (dd, 4H, $J \sim 5$, 4), 4.20 (q, 2H, $J \sim 7$).

Ethyl α-methyl-2-morpholinopropionate: Ethyl 2-morpholinopropionate (28.2 g, 0.15 mol) in dry THF (83 mL) was added over 40 min, under nitrogen, to a 2 M solution of lithium diisopropylamide in toluene (83.3 mL, 0.17 mol) at −65 °C. Stirring was continued for 1 h whereupon methyl iodide (19.6 mL, 0.32mol) was added. The mixture was warmed to room temperature and after a further 30 min was poured onto iced water (1.20 L). The product was extracted with diethyl ether (3 × 500 mL), and the combined organic phase was then washed with brine, dried (Na_2SO_4), and filtered and the solvent removed in vacuo to give the title compound as an oil (28.6 g,

94%): ^{1}H NMR ($CDCl_3$) δ 1.24−1.36 (m, 9H), 2.57−2.65 (m, 4H), 3.68−3.77 (m, 4H), 4.20 (q, 2H, $J \sim 7$).

α-Methyl-2-morpholinopropionic acid hydrochloride: Ethyl α-methyl-2-morpholinopropionate (24.4 g, 0.12 mol) was suspended in 5 N hydrochloric acid (570 mL), and the mixture was heated at reflux for 18 h. After cooling, the ethanol and water were removed in vacuo, and the product precipitated by the addition of acetone. The title compound was filtered off and isolated as a white solid (21.4 g, 84%): ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 1.76 (s, 6H), 3.42 (br s, 4H), 4.13 (br s, 4H).

(±)-2-[*N*-Bis(2-methoxyethyl)amino]-3-methylbutyric acid, ethyl ester: A mixture of ethyl 2-bromoisovalerate (10.0 g, 47.8 mmol), bis(2-methoxyethyl)amine (7.06 mL, 47.8 mmol), and diisopropylethylamine (8.32 mL, 47.8 mmol) in xylene (50 mL) was heated to 175 °C in a glass autoclave for 18 h. After cooling, the solution was diluted with ethyl acetate (50 mL) and washed with water (2 × 50 mL). The organic layer was extracted with 5 N hydrochloric acid (2 × 25 mL) and the aqueous layer basified with 4 N sodium hydroxide solution. This was extracted with ethyl acetate (3 × 100 mL), the combined organic phase was dried (Na_2SO_4) and filtered, and the solvent was removed in vacuo. The resulting oily residue was purified by chromatography on alumina using toluene: ethyl acetate (9:1) as eluant to give the title compound as a clear oil (1.40 g, 11%): ^{1}H NMR ($CDCl_3$) δ 0.87 (d, 3H, $J \sim 7$), 0.99 (d, 3H, $J \sim 7$), 1.28 (t, 3H, $J \sim 7$), 1.86−2.09 (m, 1H), 2.59−3.01 (m, 5H), 3.32 (s, 6H), 3.36−3.50 (m, 4H), 4.19 (q, 2H, $J \sim 7$).

The following compound was similarly prepared except that ethyl 2-bromovalerate was used instead of ethyl 2-bromoisovalerate and the reaction was carried out at reflux in toluene for 9 h:

(±)-2-[*N*-Bis(2-methoxyethyl)amino]pentanoic acid, ethyl ester: 91%; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H, $J \sim 7.5$), 1.27 (t, 3H, $J \sim 7$), 1.31−1.72 (m, 4H), 2.75−2.83 (m, 2H), 2.87−2.95 (m, 2H), 3.33 (s, 6H), 3.34−3.48 (m, 5H), 4.13 (q, 2H, $J \sim 7.5$).

(±)-2-[*N*-Bis(2-methoxyethyl)amino]-3-methylbutyric acid: (±)-2-[*N*-Bis(2-methoxyethyl)amino]-3-methylbutyric acid, ethyl ester (2.4 g, 9.2 mmol) was added to ethanol:water (1:1, 60 mL) and 4 N sodium hydroxide solution (5 mL). The mixture was refluxed for 6.5 h and then allowed to cool. The solution was evaporated to dryness in vacuo, redissolved in water, and adjusted to pH 6.3 with 1 N hydrochloric acid. The solution was evaporated to dryness once more and the resulting solid extracted with chloroform. After filtration and removal of the solvent in vacuo, the title compound was isolated as a gum (1.74 g, 81%): ^{1}H NMR ($CDCl_3$) δ 1.01 (t, 6H, $J \sim 7$), 2.05−2.15 (m, 1H), 2.89−3.08 (m, 5H), 3.36 (s, 6H), 3.40−3.55 (m, 4H), 7.80 (br s, 1H).

The following compound was similarly prepared except that the product was isolated as the hydrochloride salt:

(±)-2-[*N*-Bis(2-methoxyethyl)amino]pentanoic acid hydrochloride: 69%; ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 1.00 (t, 3H, $J \sim 7$), 1.54−1.72 (m, 2H), 1.87−1.97 (m, 1H), 2.00−2.11 (m, 1H), 3.37 (s, 6H), 3.38−3.52 (m, 4H), 3.68−3.77 (m, 2H), 3.87−3.99 (m, 3H).

α-Methyl-2-morpholinopropionic acid, 2′,6′dimethoxy-4′-methylphenyl ester hydrochloride (9): 4-(Dimethylamino)pyridine (0.03 g, 0.25 mmol) was added to a stirred solution of α-methyl-2-morpholinopropionic acid hydrochloride (5.25 g, 25.0 mmol), DCC (5.17 g, 25.0 mmol), 2,6-dimethoxy-4-methylphenol (4.21 g, 25.0 mmol), *N*-hydroxybenzotriazole (3.42 g, 25.0 mmol), and triethylamine (3.50 mL, 25.0 mmol) in DMF (105 mL). The reaction mixture was stirred at room temperature for 7 d whereupon the solvent was removed in vacuo. Toluene (50 mL) was added to the residue, and any insoluble material was filtered off and washed with more toluene. The solvent was removed once more to give an oil which was purified by chromatography on alumina using gradient elution with toluene−toluene:ethyl acetate (19:1) as eluant. The main component was dissolved in dichloromethane and excess hydrogen chloride gas added. The resulting salt was precipitated by the addition of diethyl ether. After filtering and drying in vacuo, the title compound was isolated as a white solid (3.76 g, 42%): ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 1.84 (s, 6H), 2.35 (s, 3H), 3.29−3.37 (m, 4H), 3.79 (s, 6H), 4.03−4.14 (m, 4H), 6.43 (s, 2H); IR (KBr) 3392, 2947, 2247, 1763, 1611, 1511, 1465, 1248, 1164, 1118 cm^{-1}; *m/z* (ES) $[M+H]^+$ 324; Found: 324.1821, $C_{17}H_{26}NO_5$ requires 324.1803. Anal. ($C_{17}H_{26}ClNO_5$) H, N; C: calcd, 56.74; found, 56.11.

The following compounds were similarly prepared except that no *N*-hydroxybenzotriazole or triethylamine was used and EDC replaced DCC for the synthesis of (**16**).

(±)-2-[*N*-Bis(2-methoxyethyl)amino]-3-methylbutyric acid, 2,6-dimethoxyphenyl ester hydrochloride (15): 14%; mp 115−117 °C; ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 1.02−1.11 (m, 6H), 2.03−2.18 (m, 1H), 2.84−2.95 (m, 2H), 2.97−3.09 (m, 2H), 3.18 (d, 1H, $J \sim 13.5$), 3.36 (s, 6H), 3.43−3.52 (m, 4H), 3.79 (s, 6H), 6.42 (br s, 1H), 6.60 (d, 2H, $J \sim 8.5$), 7.12 (t, 1H, $J \sim 8.5$); IR (KBr) 3424, 2939, 2449, 1763, 1608, 1482. 1311, 1262, 1172, 1113 cm^{-1}; *m/z* (ES) $[M+H]^+$ 371; Found: 370.2237, $C_{19}H_{32}NO_6$ requires 370.2227. Anal. ($C_{19}H_{32}ClNO_6 \cdot 0.3H_2O$) C, H, N.

(±)-2-[*N*-Bis(2-methoxyethyl)amino]pentanoic acid, 2,6-dimethoxyphenyl ester hydrochloride (16): 8%; ^{1}H NMR ($CDCl_3$ + C_5D_5N) δ 1.00 (t, 3H, $J \sim 7.5$), 1.53−1.67 (m, 2H), 1.88−1.99 (m, 2H), 3.05−3.14 (m, 2H), 3.17−3.28 (m, 2H), 3.37 (s, 6H), 3.55−3.72 (m, 4H), 3.80 (s, 6H), 3.90 (t, 1H, $J \sim 7.5$), 6.60 (d, 2H, $J \sim 8.5$), 7.12 (t, 1H, $J \sim 8.5$), 7.21 (br s, 1H); IR (KBr) 2934, 2113, 1750, 1607, 1484, 1309, 1265, 1174, 1114 cm^{-1}; *m/z* (ES) $[M+H]^+$ 370; Found: 370.2240, $C_{19}H_{32}NO_6$ requires 370.2227. Anal. ($C_{19}H_{32}ClNO_6$) C, H, N, Cl.

The (*R*)-enantiomers **18**, **19**, and **20** of racemates **13**, **14**, and **16**, respectively, were isolated after enantiospecific enzyme hydrolysis of the corresponding (*S*)-isomer.[6] Alternatively, **19** could be isolated from chiral hplc separation of **14** using a Chiracel OJ semi-prep column (96:4 hexane:IPA + 0.1% Et_2NH; flow rate, 1.5 mL/min; UV detection at 230 nm; column temperature, 30 °C). The X-ray crystal structures[6] confirmed (*R*)-configuration:

(*R*)-2-[*N*-Bis(2-methoxyethyl)amino]butyric acid, 2′,6′-dimethoxyphenyl ester hydrochloride (18): $[\alpha]_D$ +5.24° (*c* 0.76); mp 53.5−54.5 °C; Found: 356.2078, $C_{18}H_{30}NO_6$ requires 356.2068. Anal. ($C_{18}H_{30}ClNO_6 \cdot 0.5H_2O$) C, H, N, Cl.

(*R*)-2-[*N*-Bis(2-methoxyethyl)amino]butyric acid, 2′,6′-dimethoxy-4′-methylphenyl ester hydrochloride (19): $[\alpha]_D$ +7.03° (*c* 0.77); mp 108−109 °C; Found: 370.2224, $C_{19}H_{32}NO_6$ requires 370.2227. Anal. ($C_{19}H_{32}ClNO_6 \cdot 0.38\ H_2O$) C, H, N, Cl.

(*R*)-2-[*N*-Bis(2-methoxyethyl)amino]pentanoic acid, 2,6-dimethoxyphenyl ester hydrochloride (20): $[\alpha]_D$ −5.88° (*c* 0.20; MeOH); mp 85.5−86.5 °C; Found: 370.2239, $C_{19}H_{32}NO_6$ requires 370.2227. Anal. ($C_{19}H_{32}ClNO_6$) C. H: calcd, 6.40; found, 5.77; N: calcd, 3.71; found, 3.12.

Molecular Modeling. 2D to 3D structure conversion was performed using Corina[19] to convert a 2D SD file to a 3D SD file. Energy minimization and conformational searching used the default Chem-X[20] charges, parameters, and convergence criteria throughout. For the conformational search, each of the rotatable bonds were systematically driven through 360° in 30° increments, except for bonds to the OCH_3 groups which were incremented in 60° steps. This gave a total of 746 496 conformations.

Calculated energy of the global energy minimum of the *R* enantiomer **3** (Figure 2, green carbons) was 48.7 kcal mol^{-1}, while the calculated energy of the *S* enantiomer **4** in the depicted conformation (Figure 2, blue carbons) was 49.7 kcal mol^{-1}.

Fitting and RMS fit calculations were also performed in Chem-X.

Acknowledgment. We are grateful to Organon Teknika for financial support and to Professor Howard Stevens and Dr. Ijeoma Uchegbu of the University of Strathclyde, Glasgow, for formulation work on Org 24048 (compound **3**). Physical chemistry data and

synthetic improvements were provided by the Department of Analytical Chemistry and Resupply Chemistry, Organon Laboratories Ltd.; in particular we thank Dr. Brian Montgomery, Mr. Robert Roy, and Mr. Jack Pick for their assistance.

References

(1) James, R.; Glen, J. B. Synthesis, biological evaluation, and preliminary structure−activity considerations of a series of alkylphenols as intravenous anesthetic agents. *J. Med. Chem.* **1980**, *23*, 1350−1357.

(2) Trapani, G.; Altomare, C.; Sanna, E.; Biggio, G.; Liso, G. Propofol in anesthesia. Mechanism of action, structure−activity relationships, and drug delivery. *Curr. Med. Chem.* **2000**, *7*, 249−271.

(3) Bamford, D. G.; Biggs, D. F.; Lee, G. E.; Owen, A. J.; Pulsford, D. W.; Wragg, W. R. Morpholine derivative. British Patent Specification 1160468 (*Chem. Abstr.* **1969**, *71*, 3391).

(4) (a) Krasowski, M. D.; Harrison, N. L. General anesthetic actions on ligand-gated ion channels. *Cell. Mol. Life Sci.* **1999**, *55*, 1278−1303. (b) Belelli, D.; Pistis, M.; Peters, J. A.; Lambert, J. J. General anaesthetic action at transmitter-gated inhibitory amino acid receptors. *Trends Pharmacol. Sci.* **1999**, *20*, 496−502. (c) Anderson, A.; Boyd, A. C.; Clark, J. K.; Fielding, L.; Gemmell, D. K.; Hamilton, N. M.; Maidment, M. S.; May, V.; McGuire, R.; McPhail, P.; Sansbury, F. H.; Sundaram, H.; Taylor, R. Conformationally constrained anesthetic steroids that modulate $GABA_A$ receptors. *J. Med. Chem.* **2000**, *43*, 4118−4125.

(5) Little, H. J. How has molecular pharmacology contributed to our understanding of the mechanism(s) of general anaesthesia? *Pharmacol. Ther.* **1996**, *69*, 37−58.

(6) Bennett, D. J.; Buchanan, K. I.; Cooke, A.; Epemolu, O.; Hamilton, N. M.; Hutchinson, E. J.; Mitchell, A. Enantiospecific enzyme-catalysed resolution of novel *N*,*N*-disubstituted α-amino acid phenol ester derivatives using pig liver esterase. *J. Chem. Soc., Perkin Trans.* **2001**, *1*, 362−365.

(7) PALLAS for Windows, PrologP 5.1, CompuDrug Chemistry Ltd.

(8) Sirius Analytical Instruments Ltd. *Applications and Theory Guide to pH-Metric pK_a and logP Determination*, Rev. 1; 1995, pp 67−70.

(9) Ross, A.; Uchegbu, I. F.; Stevens, H. N. E. Intravenous formulation studies on Org 24048. Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow G1 1XW, March 1998.

(10) Loveless, A. H.; Maxwell, D. R. The prevention by sulphydryl compounds of the toxicity in the cat of 2,6-dimethoxyphenol and its morpholinopropionyl ester. *Br. J. Pharmacol.* **1975**, *53*, 93−98.

(11) (a) Sneyd, J. R.; Tsubokawa, T.; Andrews, C. J. H.; Curnow, J.; Cross, M.; Lytle, J.; Visser, L.; Lunn, D. V.; Houwing, N. S.; Boen, P.; van Maanen, R.; Vijn, P. C. M. First administration to man of Org 25435, a new intravenous anaesthetic agent. *Br. J. Anaesth.* **2001**, *86*, 323P. (b) Gemmell, D. K.; Byford, A.; Sundaram, H.; Lambert, J. J.; Hamilton, N. Org 25435 − A new water-soluble intravenous anaesthetic. *Anesthesiology* **2000**, *93*, A749.

(12) (a) Stella, V. J.; Zygmunt, J. J.; Georg, I. G.; Safadi, M. S. Water-soluble prodrugs of hindered alcohols or phenols. WO 0008033 (*Chem. Abstr.* **2000**, *132*, 171119). (b) Hendler, S. S.; Sanchez, R. A.; Zielinski, J. Water-soluble prodrugs of propofol. WO 9958555 (*Chem. Abstr.* **1999**, *131*, 356098).

(13) (a) Lambert, J. J.; Belelli, D.; Shepherd, S.; Muntoni, A.-L.; Pistis, M.; Peters, J. A. The $GABA_A$ receptor: An important locus for intravenous anesthetic action. *Spec. Publ. − R. Soc. Chem.* **1998**, *220 (Gases in Medicine)*, 121−137. (b) Olsen, R. W. The molecular mechanism of action of general anesthetics: structural aspects of interactions with $GABA_A$ receptors. *Toxicol. Lett.* **1998**, *100−101*, 193−201. (c) Lambert, J. J.; Belelli D.; Hill-Venning, C.; Peters, J. A. Neurosteroids and $GABA_A$ receptor function. *Trends Pharmacol. Sci.* **1995**, *16*, 295−303.

(14) (a) Rudolph, U.; Crestani, F.; Benke, D.; Brunig, I.; Benson, J. A.; Fritschy, J.-M.; Martin, J. R.; Bluethmann, H.; Mohler, H. Benzodiazepine actions mediated by specific γ-aminobutyric $acid_A$ receptor subtypes. *Nature* **1999**, *401*, 796−800. (b) McKernan, R. M.; Rosahl, T. W.; Reynolds, D. S.; Sur, S.; Wafford, K. A.; Atack, J. R.; Farrar, S.; Myers, J.; Cook, G.; Ferris, P.; Garrett, L.; Bristow, L.; Marshall, G.; Macaulay, A.; Brown, N.; Howell, O.; Moore, K. W.; Carling, R. W.; Street, L. J.; Castro, J. L.; Ragan, C. I.; Dawson, G. R.; Whiting, P. J. Sedative but not anxiolytic properties of benzodiazepines are mediated by the $GABA_A$ receptor α_1 subtype. *Nat. Neurosci.* **2000**, *3*, 587−592.

(15) Tomlin, S. L.; Jenkins, A.; Lieb, W. R.; Franks, N. P. Preparation of barbiturate optical isomers and their effects on $GABA_A$ receptors. *Anesthesiology* **1999**, *90*, 1714−1722.

(16) (a) Tomlin, S. L.; Jenkins, A.; Lieb, W. R.; Franks, N. P. Stereoselective effects of etomidate optical isomers on gamma-aminobutyric acid type A receptors and animals. *Anesthesiology* **1998**, *88*, 708−717. (b) McGurk, K. A.; Pistis, M.; Belelli, D.; Hope, A. G.; Lambert, J. J. The effect of a transmembrane amino acid on etomidate sensitivity of an invertebrate GABA receptor. *Br. J. Pharmacol.* **1998**, *124*, 13−20.

(17) Jones, M. V.; Harrison, N. L. Effects of volatile anesthetics on the kinetics of inhibitory postsynaptic currents in cultured rat hippocampal neurons. *J. Neurophysiol.* **1993**, *70*, 1339−1349.

(18) (a) Covey, D. F.; Nathan, D.; Kalkbrenner, M.; Nilsson, K. R.; Hu, Y.; Zorumski, C. F.; Evers, A. S. Enantioselectivity of pregnanolone-induced γ-aminobutyric $acid_A$ receptor modulation and anesthesia. *J. Pharmacol. Exp. Ther.* **2000**, *293*, 1009−1016. (b) Hu, Y.; Wittmer, L. L.; Kalkbrenner, M.; Evers, A. S.; Zorumski, C. F.; Covey, D. F. Neurosteroid analogues. Part 5. Enantiomers of neuroactive steroids and benz[*e*]indenes: total synthesis, electrophysiological effects on $GABA_A$ receptor function and anesthetic actions in tadpoles. *J. Chem. Soc., Perkin Trans.* **1997**, *1*, 3665−3671.

(19) Gasteiger, J.; Rudolph, C.; Sadowski, J. Automatic generation of 3D-atomic coordinates for organic molecules. *Tetrahedron Comput. Methodol.* **1990**, *3*, 537−547.

(20) Chem-X, CDL, Oxford Molecular Group, Oxford.

(21) Belelli, D.; Lambert, J. J.; Peters, J. A.; Wafford, K.; Whiting, P. J. The interaction of the general anesthetic etomidate with the γ-aminobutyric acid type A receptor is influenced by a single amino acid. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11031−11036.

(22) Hill-Venning, C.; Belelli, D.; Lambert, J. J.; Peters, J. A. Subunit-dependent interaction of the general anesthetic etomidate with the γ-aminobutyric acid type A receptor. *Br. J. Pharmacol.* **1997**, *120*, 749−756.

(23) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. S. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

(24) Rips, R.; Derappe, C.; Dupont, M.; Crucifix, M. Chemical structure and pharmacological activity of 1,3,4-oxadiazoles. *Chim. Ther.* **1971**, *6*, 45−47.

JM010903I