Synthesis and Pharmacological Evaluation of Novel γ-Aminobutyric Acid Type B ($GABA_B$) Receptor Agonists as Gastroesophageal Reflux Inhibitors

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We have previously demonstrated that the prototypical $GABA_B$ receptor agonist baclofen inhibits transient lower esophageal sphincter relaxations (TLESRs), the most important mechanism for gastroesophageal reflux. Thus, $GABA_B$ agonists could be exploited for the treatment of gastroesophageal reflux disease. However, baclofen, which is used as an antispastic agent, and other previously known $GABA_B$ agonists can produce CNS side effects such as sedation, dizziness, nausea, and vomiting at higher doses. We now report the discovery of atypical $GABA_B$ agonists devoid of classical $GABA_B$ agonist related CNS side effects at therapeutic doses and the optimization of this type of compound for inhibition of TLESRs, which has resulted in a candidate drug (*R*)-**7** (AZD3355) that is presently being evaluated in man.

Introduction

It has been demonstrated that the prototypical γ-aminobutyric acid type B ($GABA_B$[a]) receptor agonist baclofen inhibits transient lower esophageal sphincter relaxations (TLESRs) in ferrets, dogs and humans.[1] TLESRs account for the majority of reflux episodes in both healthy individuals and patients with gastroesophageal reflux disease (GERD).[1c,d] However, high doses of baclofen and other previously known $GABA_B$ agonists produce central nervous system (CNS) related side effects like sedation, dizziness, nausea, and vomiting, which would not be acceptable for a drug intended for treatment of GERD. Thus, $GABA_B$ agonists effective in inhibiting TLESRs but devoid of CNS side effects would have a major therapeutic potential. Since $GABA_B$ receptors are expressed both within and outside the CNS in the reflex pathway underlying TLESRs,[2] one strategy was to find agonists that would affect peripheral $GABA_B$ receptors selectively.

Here, we report the syntheses and some pharmacological characteristics of a series of $GABA_B$ agonists from which a candidate drug (*R*)-**7** (AZD3355[3]) has been selected and submitted to studies in humans (for structure, see Figure 1). In contrast to baclofen, compound (*R*)-**7** lacks CNS side effects at doses effective in inhibiting TLESRs in animal models.

We decided to explore derivatives of 3-aminopropylphosphinic acid (**1**, CGP27492), since this compound has been demonstrated to be a potent and selective $GABA_B$ agonist.[4] As compound **1** has been described in the literature as being inactive in vivo (as a muscle relaxant), speculatively because of metabolic lability of the P−H bond,[4,5] we soon focused on derivatives of 3-aminopropyl(methyl)phosphinic acid (**2**, SK&F97541[4]) to avoid this metabolic liability.[6] However, as it proved difficult to significantly improve the therapeutic window for derivatives of **2**, we eventually decided to re-evaluate the alleged metabolic lability of **1** and its P−H congeners. Surprisingly,[4,5] we found that it was possible to design new P−H phosphinic acids that showed a high metabolic stability (see data for (*S*)-**3** in Results and Discussion). Since **1** has been found to be inactive at CNS $GABA_B$ receptors after systemic administration, our hypothesis was that 3-aminopropylphosphinic acids may inhibit TLESR through a peripheral site of action without carrying the burden of CNS side effects. In fact, almost all efforts to find therapeutically useful $GABA_B$ agonists have been done in the CNS field, which explains the lack of interest in exploring analogues of **1**. Therefore, we decided to explore this compound class further, aiming at increased therapeutic window and maintained potency.[3]

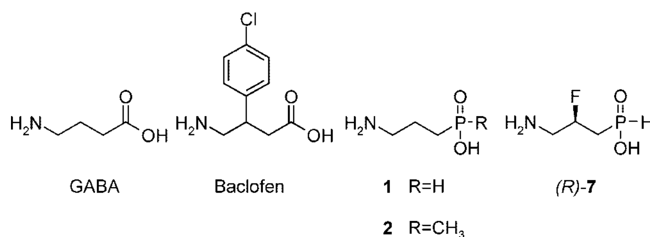

Figure 1. Structure of the novel $GABA_B$ agonist (*R*)-**7** together with structures of some known $GABA_B$ agonists.

Chemistry

The syntheses of the known $GABA_B$ agonists **1**−**3** (CGP35583), (*S*)-**4** (CGP44532), (*R*)-**4** (CGP44533), and **6** [(3-amino-2-oxopropyl)(methyl)phosphinic acid] were all performed in accordance with those procedures already being described in the literature[7] (for structures, see Table 1). All compounds that were isolated as single enantiomers were synthesized by routes using optically pure starting materials (single enantiomers of serine or epichlorohydrin). Since the reactions are known to be

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[a] Abbreviations: $GABA_B$, γ-aminobutyric acid type B; TLESR, transient lower esophageal sphincter relaxation; GERD, gastroesophageal reflux disease; CNS, central nervous system; EC_{50}, 50% effective concentration; ED_2, effective dose producing 2 °C decrease in body temperature; IC_{50}, 50% inhibition concentration; FLIPR, fluorescence imaging plate reader.

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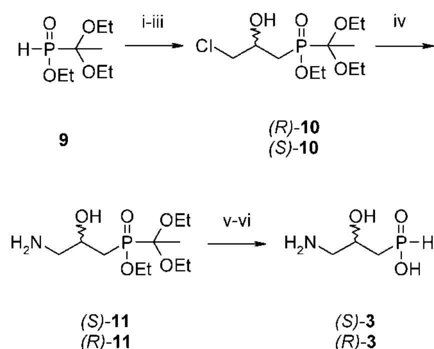
Table 1. Binding Affinities and Agonistic Properties of Compounds **1**−**8**

compd	R_1	R_2	$GABA_B$ affinity K_i[a] ± SEM (*n*) (nM)	$GABA_B$ agonism EC_{50}[a,b] ± SEM (*n*) (nM)
baclofen			220 ± 50 (6)	750 ± 150 (6)
1	H	H	15 ± 3.2 (11)	19 ± 3.0 (3)
2	CH_3	H	33 ± 2.3 (87)	41 ± 6.3 (7)
3	H	OH	94 ± 32 (3)	220 ± 29 (3)
(*S*)-**3**	H	OH	50 ± 8.1 (11)	130 ± 17 (7)
(*R*)-**3**	H	OH	2100 ± 570 (2)	1100 (1)
(*S*)-**4**	CH_3	OH	180 ± 20 (3)	150 ± 50 (3)
(*R*)-**4**	CH_3	OH	840 ± 330 (3)	600 ± 200 (2)
5	H	=O	48 ± 8.8 (12)	81 ± 6.3 (3)
6	CH_3	=O	200 ± 50 (11)	270 ± 25 (2)
7	H	F	10 ± 1.9 (12)	15 ± 1.5 (5)
(*S*)-**7**	H	F	70 ± 7.1 (5)	250 ± 10 (2)
(*R*)-**7**	H	F	5.1 ± 1.2 (10)	8.64 ± 0.77 (4)
8	CH_3	F	14 ± 3.2 (9)	23 ± 1.9 (2)
(*S*)-**8**	CH_3	F	480 ± 30 (3)	1700 ± 240 (3)
(*R*)-**8**	CH_3	F	4.3 ± 1.3 (3)	14 ± 3.2 (3)

[a] Binding affinities (K_i) and agonistic properties (EC_{50}) of compounds at the $GABA_B$ receptor were measured as detailed in the Experimental Section. Data are the mean ± SEM of *n* experiments. [b] All compounds shown in the table had an intrinsic activity of 1.

Scheme 1[a]



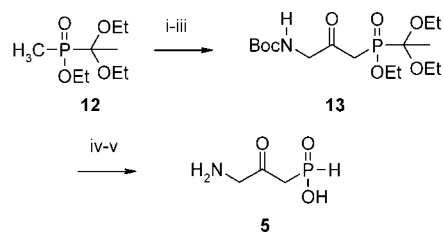
[a] Reagents: (i) HMDS, reflux, 3 h; (ii) (*R*)- or (*S*)-epichlorohydrin, $ZnCl_2$, 60 °C, 15 h; (iii) 1% HOAc/MeOH, 15 h; (iv) 9% NH_4OH, room temp, 4 days, then 60 °C, 24 h; (v) concentrated HCl, reflux 2 h; (vi) propylene oxide, MeOH, 5 h.

stereospecific, the absolute configuration of compounds (*S*)-**3**, (*R*)-**3**, (*S*)-**7**, (*R*)-**7**, (*S*)-**8**, and (*R*)-**8** could be determined from the knowledge of stereochemistry of each starting material. There has been no sign of any racemization in the reaction steps, and thus, the compounds are stated to be optically pure. In addition, benzamide derivatives of compounds (*S*)-**7** and (*R*)-**7** have been analyzed by chiral HPLC and no opposite enantiomer has been detected (see Experimental Section).

The optically pure 2-hydroxy analogues, (*S*)-**3** and (*R*)-**3**, were prepared as shown in Scheme 1. Ethyl (diethoxyethyl)phosphinate (**9**)[4] was transformed to the corresponding P(III) intermediate followed by reaction with (*R*)- and (*S*)-epichlorohydrin, respectively. The two enantiomers of the formed trimethylsilylated ethers were then hydrolyzed to yield chlorohydrins (*R*)-**10** and (*S*)-**10**, respectively, which then were transformed to aminoalcohols (*S*)-**11** and (*R*)-**11**, respectively. The phosphinic acid ester moiety was finally subjected to acid hydrolysis to afford (*S*)-**3** and (*R*)-**3**, respectively.

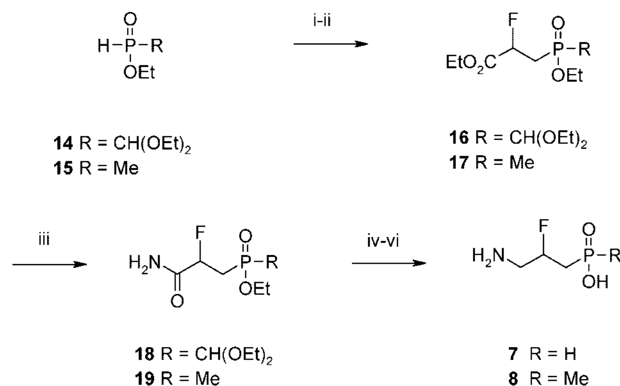
The 2-oxo analogue **5** was prepared by the synthetic route diagrammed in Scheme 2. Ethyl (diethoxyethyl)(methyl)phosphinate (**12**)[8] was condensed with *N*-Boc-glycine methyl ester

Scheme 2[a]



[a] Reagents: (i) LDA, THF, −78 °C, 1 h; (ii) Boc-Gly-OMe, −78 °C, 45 min; (iii) HOAc, −78 °C, then room temp, 45 min; (iv) 3 M HCl, room temp, 14 h; (v) MeOH, propylene oxide, room temp, 12 h.

Scheme 3[a]



[a] Reagents: (i) HMDS, reflux, 2 h; (ii) ethyl 2-fluoroacrylate, 60 °C, 3 days for **16** (room temp, 60 h for **17**); (iii) NH_4OH, EtOH, room temp, 16 h; (iv) BH_3−THF, THF, reflux, 2.5 h; (v) 6 M HCl, reflux, 2.5 h; (vi) DOWEX 50WX-8-200, H^+ form.

to afford ketone **13**, which then was completely deprotected by a hydrolytic reaction to give **5**.

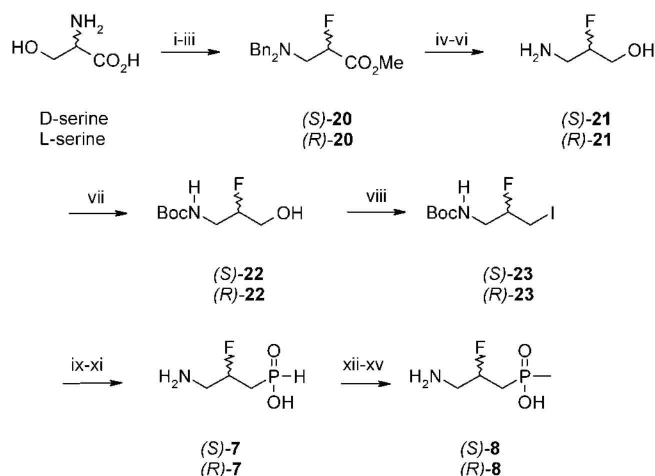
The preparation of the racemic 2-fluoro analogues **7** and **8** was achieved as shown in Scheme 3. Ethyl (diethoxymethyl)phosphinate (**14**[9]) and ethyl (methyl)phosphinate (**15**[10]) were both transformed to the corresponding trimethylsilylated P(III) intermediates (structures not shown), which on addition to ethyl 2-fluoroacrylate[11] afforded fluoropropanoates **16** and **17**, respectively. The carboxylic acid ester moiety of **16** and **17** was converted to the corresponding amide, yielding **18** and **19**, respectively, which then were reduced and finally subjected to acidic hydrolyses to afford **7** and **8**, respectively.

The syntheses of the enantiomerically pure 2-fluoro analogues (*S*)-**7**, (*R*)-**7**, (*S*)-**8**, and (*R*)-**8** employed either D-serine or L-serine as starting materials (see Scheme 4). The stereospecific transformations of the β-hydroxy-α-amino acids to the α-fluoro-β-amino acid derivatives (*S*)-**20** and (*R*)-**20** were performed in accordance to Somekh and Shanzer.[12] Reduction of the ester group was followed by removal of the benzyl groups, which afforded amino alcohols (*S*)-**21** and (*R*)-**21**, respectively. The amino group was Boc-protected, which gave carbamates (*S*)-**22** and (*R*)-**22**, and then the hydroxy group was replaced by an iodine atom to give the iodo intermediates (*S*)-**23** and (*R*)-**23**, respectively. Subsequent introduction of the phosphinic acid head was accomplished by treating the alkyl iodides with 10 equiv of bis(trimethylsilyl) phosphonite.[13] Boc deprotection afforded the $GABA_B$ receptor ligands (*S*)-**7** and (*R*)-**7**, respectively, which optionally were P-methylated to afford (*S*)-**8** and (*R*)-**8**, respectively.

Results and Discussion

Generally, only minor modifications are tolerated in the parent 3-aminopropyl(methyl)phosphinic and 3-aminopropylphosphinic

Scheme 4[a]



[a] Reagents: (i) $SOCl_2$, MeOH; (ii) $PhCH_2Br$, $NaHCO_3$, DMSO, THF; (iii) DAST, THF, room temp; (iv) $LiBH_4$, THF, −15 °C, then room temp, 17 h; (v) aqueous NH_4Cl, 0 °C; (vi) H_2, $Pd(OH)_2$−C, EtOH, 55 psi, room temp, 6 h; (vii) $(Boc)_2O$, K_2CO_3, H_2O, dioxane, room temp, 17 h; (viii) PPh_3, I_2, imidazole, CH_2Cl_2, 0 °C, then room temp, 17 h; (ix) $HP(OTMS)_2$, 10 equiv, CH_2Cl_2, room temp, 18 h; (x) $MeOH/H_2O$; (xi) DOWEX 50WX-8-200, H^+ form; (xii) HMDS, reflux, 16 h; (xiii) diglyme, reflux, 6 h; (xiv) *N*,*N*-diisopropylethylamine, MeI, room temp, 24 h; (xv) DOWEX 50WX-8-200, H^+ form.

acids, in both of which the 2-position is the most accommodating site. For example, as has been shown previously,[4] introduction of a hydroxy group in the 2-position is quite well-tolerated. As for the aminopropyl(methyl)phosphinic acids, it was shown that the *S*-isomer was more potent than the corresponding *R*-isomer.[4] We have found that this is also true for the corresponding P−H analogues. Thus, it seems that a hydroxy group in the 2-position is involved in a stereospecific interaction with the $GABA_B$ receptor. Interestingly, by introducing a fluorine atom in the 2-position of both of the compound classes, we discovered $GABA_B$ agonists with potencies that exceed that of any previously known $GABA_B$ agonist (see data for **7**, (*R*)-**7**, and (*R*)-**8**). In contrast to what has been established for the 2-hydroxy-substituted analogues, the *R*-isomer of the 2-fluoro derivatives was unexpectedly found to be more potent than the *S*-isomer (cf. data for (*R*)-**7** and (*S*)-**7**, and (*R*)-**8** and (*S*)-**8**, respectively).

In general, as has also been shown previously,[4] 3-aminopropyl-(methyl)phosphinic acids have in vitro potency in the same range at the $GABA_B$ receptor compared to the corresponding 3-aminopropylphosphinic acids. However, these two compound classes behave very differently in animal studies with regard to inhibition of TLESRs as well as to possible side effects. While those 3-aminopropyl(methyl)phosphinic acids that are potent $GABA_B$ agonists in vitro have an in vivo profile similar to that of baclofen, the corresponding P−H analogues have an in vivo profile that is considerably different with regard to TLESR inhibition as well as to side effects. Thus, while both compound classes act as inhibitors of TLESRs in a dose-dependent way, where the in vivo potency correlates with the affinity for the $GABA_B$ receptor in vitro, 3-aminopropyl(methyl)phosphinic acids can produce CNS side effects such as sedation[14] close to therapeutic doses, whereas the P−H analogues are devoid of side effects even at high doses.[15] For instance, (*S*)-**4** has an EC_{50} for stimulation of human recombinant $GABA_B$ receptors amounting to 150 nM (Table 1), an ED_{50} for TLESR inhibition in the dog of about 0.17 μmol/kg, and an ED_2 in the mouse approximating 5.4 μmol/kg (ED_2, which is used to assess CNS side effects, is defined as the subcutaneous dose producing a 2 °C decrease in body temperature). In contrast, the corresponding values for (*S*)-**3** are 130 nM (Table 1), 7 μmol/kg, and >8000 μmol/kg, respectively. Consequently, if therapeutic index is defined as the ratio of ED_2 to ED_{50}, 3-aminopropylphosphinic acids with a small substituent or no substituent in the 2-position have significantly better therapeutic index than the corresponding P−Me analogues. Also 3-aminopropylsulfinic acids[16] with a small substituent or no substituent in the 2-position behave as 3-aminopropylphosphinic acids; i.e., they have considerably better therapeutic index than baclofen and 3-aminopropyl-(methyl)phosphinic acids. A more detailed SAR for 3-aminopropylphosphinic acids and 3-aminopropyl(methyl)phosphinic acids regarding effects on TLESR and CNS side effects will be the subject of a forthcoming publication.[15] Also, the mechanisms underlying the in vivo differences between these two compounds classes are beyond the scope of the current work and will be published separately.[15]

Despite the fact that the molecules contain a P−H bond, it is notable that the novel 3-aminopropylphosphinic acid derivatives have metabolic properties making them useful as drugs even when given perorally. This finding was unexpected because contradictory results of the compound class have previously been reported in the literature.[4,5] The discovery is exemplified by the fact that the bioavailability of compound (*S*)-**3** is 88% in dog and 78% in rat.

Conclusions

In summary, we have discovered that $GABA_B$ agonists can be divided into two groups with respect to inhibition of TLESR and production of CNS side effects. Tentatively, we have attributed this differentiation to a difference in availability of the compounds to CNS $GABA_B$ receptors. This discovery enabled us to select (*R*)-**7** as a candidate drug, devoid of typical $GABA_B$ receptor related CNS side effects despite its high affinity for the $GABA_B$ receptor. This compound is presently being evaluated in man with respect to inhibition of TLESR and reflux and may be exploited in the treatment of GERD.

Experimental Section

General Methods. Melting points are uncorrected. ^{1}H NMR spectra were recorded at 300 or 500 MHz, ^{19}F NMR spectra at 282 MHz, and ^{31}P NMR at 121 MHz. Elemental analyses were performed at Quantitative Technologies Inc. (Whitehouse, NJ). All reactions were performed under a dry N_2 or argon atmosphere. Analytical TLC was performed on silica gel GF (Analtech) or silica gel 60 F_{254} (EM Science) plates. Flash column chromatography was performed with silica gel 60 (230−400 mesh, EM Science). Baclofen used in this work was racemic, i.e., (±)-β-(aminomethyl)-4-chlorobenzenepropanoic acid, and purchased from Aldrich-Sigma.

Ethyl (2*R*)-3-Chloro-2-hydroxypropyl(1,1-diethoxyethyl)phosphinate ((*R*)-10). A mixture of ethyl (diethoxyethyl)phosphinate (**9**)[4] (15.0 g, 71 mmol) and toluene was evaporated to dryness. The residue and 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (13.2 g, 82 mmol) were heated to reflux for 3 h under an argon atmosphere. The mixture was cooled to room temperature and evaporated. (*R*)-Epichlorohydrin (6.6 g, 71 mmol) and anhydrous zinc chloride (2.5 g, 18 mmol) were added, and the reagents were heated to 60 °C overnight under an argon atmosphere. The mixture was cooled to room temperature and diluted with methylene chloride and water. The organic layer was washed with water, dried over $MgSO_4$, filtered, and evaporated to give 20.7 g of a yellow oil. The residue was dissolved in methanol (150 mL) containing 1% acetic acid, and the solution was stirred overnight. The solvent was removed to give 17.7 g (82%) of (*R*)-**10** as a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.37 (m, 1H), 4.23 (m, 2H), 3.57−3.86 (m, 6H), 1.92−2.37 (m, 2H), 1.53 (dd, J = 2.3, 11.4 Hz, 3H), 1.32−1.37 (m, 3H), 1.18−1.24 (m, 6H).

Ethyl (2*S*)-3-Amino-2-hydroxypropyl(1,1-diethoxyethyl)phosphinate ((*S*)-11). A solution of (*R*)-**10** (5.0 g, 17 mmol) in ethanol containing 9% of ammonia was stirred in an autoclave at room temperature for 4 days and at 60 °C for 1 further day. The solution was evaporated, and the residue was purified by chromatography on a wet-packed silica gel column eluting with methylene chloride/methanol (5−8% MeOH) containing 5% triethylamine. The appropriate fractions were combined, evaporated, and diluted with methylene chloride and water. The aqueous layer was pH adjusted to the alkaline side by the addition of a few milliliters of 10% aqueous Na_2CO_3 and repeatedly extracted with methylene chloride. The combined organic layers were dried over Na_2SO_4 and evaporated to give 1.2 g (26%) of (*S*)-**11** as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.40−4.55 (m, 1H), 4.10−4.30 (m, 2H), 3.55−3.80 (m, 4H), 3.20−3.30 (m, 1H), 3.00−3.10 (m, 1H), 2.00−2.40 (m, 2H), 1.45−1.53 (dd, J = 3.4, 11.7 Hz, 3H), 1.30−1.40 (m, 3H), 1.15−1.25 (m, 6H).

(2*S*)-3-Amino-2-hydroxypropylphosphinic Acid ((*S*)-3). A mixture of (*S*)-**11** (1.0 g, 3.5 mmol) and concentrated HCl (50 mL) was heated to reflux for 2 h. The solution was cooled to room temperature and evaporated. The residue was dissolved in methanol (100 mL) and treated with propylene oxide (2 mL) at room temperature. After the mixture was stirred for 5 h, the precipitated solid was collected by decanting off the solvent. The solid was dried with a stream of argon to give 220 mg (45%) of (*S*)-**3** as a white solid: mp 220−225 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.08 (dt, J = 1.2, 522 Hz, 1H), 4.21 (m, 1H), 2.94−3.23 (m, 2H), 1.72−1.99 (m, 2H); ^{31}P NMR (121 MHz, D_2O) δ 24.2 (d, J = 522 Hz); FABMS m/z = 140 (M + H)$^+$; $[\alpha]_D^{25}$ +8° (0.5% in 0.1 M HCl). Anal. ($C_3H_{10}NO_3P$) C, H, N.

Ethyl [3-[*N*-(*tert*-Butoxycarbonyl)amino]-2-oxopropyl](1,1-diethoxyethyl)phosphinate (13). To a solution of diisopropylamine (195 mL, 1.39 mol, 3.5 equiv) in THF (300 mL) at −10 °C was added dropwise (about 3.5 h) *n*-BuLi (1.6 M in hexanes, 867 mL, 1.39 mol, 3.5 equiv). After 10 min, the mixture was cooled to −78 °C and a solution of ethyl (diethoxyethyl)(methyl)phosphinate[8] (**12**, 178 g, 0.793 mol, 2 equiv) in THF (400 mL) was added dropwise (about 30 min). After the addition, the solution was stirred at −78 °C for 1 h. A solution of *N*-Boc-glycine methyl ester (75.0 g, 0.396 mol, 1 equiv) in THF (450 mL) was added dropwise (about 45 min). After the addition was complete, the mixture was stirred for 45 min. Acetic acid (79.5 mL, 1.39 mol) was added, and the mixture was warmed to room temperature. Brine (200 mL) was added to the reaction mixture, and the organic layer was separated. The aqueous layer was extracted once with ethyl acetate (300 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to remove the solvent. The residue was purified by chromatography on 5 kg of silica gel eluting with ethyl acetate/hexanes (3/2). The appropriate fractions were collected to give 129 g (85%) of **13** as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.48 (s, 1H), 4.10−4.30 (m, 2H), 4.17 (d, 2H), 3.60−3.80 (m, 4H), 3.01−3.30 (m, 2H), 1.52 (d, 3H), 1.43 (s, 9H), 1.32 (t, 3H), 1.19 (t, 6H).

(3-Amino-2-oxopropyl)phosphinic Acid (5). Compound **13** (153 g, 400 mmol) was dissolved in 3 N HCl (2400 mL) that was previously deoxygenated by bubbling argon through the solution. The mixture was stirred for 14 h at room temperature and then concentrated. The residue was coevaporated with methanol. The residue was then dissolved in methanol (450 mL), and propylene oxide was added (225 mL). The mixture was stirred for 12 h and the resulting precipitate isolated by filtration. The solid was washed with cold methanol and dried under vacuum at 50 °C to give 41.8 g of solid. The solid was dissolved in water (350 mL), and the insoluble impurities were removed by filtration. The filtrate was diluted further with water (400 mL). To this aqueous solution, acetone was added with stirring until the completion of the precipitation (1600 mL of acetone). The resulting precipitate was isolated by filtration. The solid was washed with acetone and dried under vacuum at 50 °C to give 39.1 g of **5** (71%) as a tan solid: mp 143−145 °C; R_f = 0.45 (85:15 methanol/water); ^{1}H NMR (300 MHz, D_2O) δ 7.13 (d, J = 551 Hz, 1H), 4.14 (s, 2H), 3.14 (d, J = 18 Hz, 2H); FABMS: m/z = 138 (M + H)$^+$. Anal. ($C_3H_8NO_3P$) C, H, N, P.

Ethyl 3-[(Diethoxymethyl)(ethoxy)phosphoryl]-2-fluoropropanoate (16). A mixture of ethyl (diethoxymethyl)phosphinate (**14**)[9] (26.0 g, 133 mmol) and HMDS (28 mL, 133 mmol) was heated to reflux for 2 h under an argon atmosphere. The mixture was cooled to room temperature, and ethyl 2-fluoroacrylate[11] (10.5 g, 89.0 mmol) was added. The reagents were heated to 60 °C for 3 days under an argon atmosphere. The mixture was cooled to room temperature, diluted with ethyl acetate (300 mL), and washed with 1 N HCl (2 × 150 mL) and saturated sodium chloride (100 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to give 32.0 g of a yellow oil. The residue was purified by chromatography on a wet-packed silica gel column (6 cm × 30 cm) eluting with 97:3 methylene chloride/methanol. The appropriate fractions were combined and evaporated to give 16.0 g (57%) of **16** as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.32 (dm, 1H), 4.67−4.77 (m, 1H), 4.18−4.32 (m, 4H), 3.58−3.91 (m, 4H), 2.30−2.62 (m, 2H), 1.20−1.41 (m, 12H).

Ethyl (3-Amino-2-fluoro-3-oxopropyl)(diethoxymethyl)phosphinate (18). To a solution of phosphinate **16** (16.0 g, 51.1 mmol) in ethanol (22 mL) was added concentrated ammonium hydroxide (14.8 N, 3.5 mL, 51.1 mmol). The solution was stirred for 16 h and evaporated. The residue was purified by chromatography on a wet-packed silica gel column (7 cm × 37 cm) eluting with 96.5:3.5 methylene chloride/methanol. The appropriate fractions were combined and evaporated to give 3.43 g (27%) of **18** as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.43 (s, 1H), 5.70 (s, 1H), 5.21−5.49 (dm, 1H), 4.7 (dd, 1H), 4.18−4.31 (m, 2H), 3.65−3.91 (m, 4H), 2.21−2.81 (m, 2H), 1.30−1.40 (m, 3H), 1.20−1.28 (m, 6H).

(3-Amino-2-fluoropropyl)phosphinic Acid (7). To an ice bath cooled solution of amide **18** (3.43 g, 13.5 mmol) in THF (15 mL) was added 1 M BH_3−THF (8.7 mL, 8.7 mmol) while under an argon atmosphere. After 10 min, the solution was heated to reflux for 2.5 h. The solution was cooled to room temperature, and 6 N HCl (200 mL) was added slowly. The THF was removed by evaporation in vacuo and the aqueous layer refluxed for 2.5 h. The solution was cooled and evaporated. The residue was purified by ion exchange chromatography (DOWEX 50WX-8-200, H$^+$ form, 3.5 cm × 4.0 cm). The ion-exchange resin was washed with 2:1 methanol/water (400 mL). The crude product dissolved in 1:1 methanol/water was loaded onto the column and washed with 1:1 methanol/water (400 mL). The eluent was changed to 3:1 methanol/concentrated ammonium hydroxide. Two fractions (150 mL) were combined and evaporated to give 645 mg (34%) of **7** as a white solid: mp 203−207 °C; R_f = 0.37 (60:40:1 $CH_3OH/CH_2Cl_2/NH_4OH$); ^{1}H NMR (300 MHz, D_2O) δ 7.11 (d, J = 528 Hz, 1H), 5.18 (dm, J = 54 Hz, 1H), 3.28−3.45 (m, 2H), 1.65−2.23 (m, 2H); ^{31}P NMR (121 MHz, D_2O) δ 17.5 (d, J = 21 Hz); ^{19}F NMR (282 MHz, D_2O) δ −184 (d, J = 22.6 Hz); APIMS m/z 142 [M + H]$^+$. Anal. ($C_3H_9FNO_2P\cdot 0.5H_2O$) C, H, N. H: calcd, 6.72; found, 6.28.

(2*R*)-3-(Amino)-2-fluoro-1-propanol ((*R*)-21). Lithium borohydride (5.3 g, 0.24 mol) was suspended in THF (200 mL) under a nitrogen atmosphere and cooled to −15 °C with stirring. Ester (*R*)-**20**[12] (56.6 g, 0.190 mol) was suspended in THF (250 mL) and added dropwise to the mixture over 1 h; the internal temperature was maintained below −10 °C during the addition. On completion of addition, the reaction mixture was allowed to warm to room temperature and stirred at this temperature for 17 h. TLC analysis indicated complete consumption of starting material. The reaction mixture was cooled to 0 °C and cautiously quenched with a saturated aqueous solution of ammonium chloride (300 mL). The mixture was extracted with ethyl acetate (2 × 200 mL), and the organic phase was concentrated under reduced pressure. The crude residue was dissolved in 2 N hydrochloric acid (200 mL, pH 2 approximately), and the aqueous phase was washed with ether (2 × 200 mL). The aqueous phase was basified (pH 10 approximately) with 80% ammonium hydroxide in brine, extracted with ethyl acetate (3 × 200 mL), dried over anhydrous sodium sulfate (10 g),

filtered, and concentrated under reduced pressure to afford 48 g of a yellow oil of which 29 g was dissolved in ethanol (300 mL). An amount of 10 wt % palladium(II) hydroxide on carbon (5.0 g) was added and the mixture placed on a Parr shaker and shaken under a hydrogen atmosphere (55 psi) for 6 h. When no further hydrogen uptake was observed, the mixture was filtered through a pad of Celite (20 g). A fresh batch of palladium(II) hydroxide (5 g) was added to the ethanol mixture and resubjected to the hydrogenation conditions described above for 17 h. The crude mixture was filtered through a pad of Celite and concentrated under reduced pressure to afford 9.6 g of (*R*)-**21** (96%) as a pale-yellow oil: ^{1}H NMR (300 MHz, CD_3OD) δ 4.78−5.00 (br s, 3H), 4.49−4.62 (m, 0.5H), 4.32−4.46 (m, 0.5H), 3.54−3.70 (m, 2H), 2.70−2.96 (m, 2H).

***tert*-Butyl [(2*R*)-2-Fluoro-3-hydroxypropyl]carbamate ((*R*)-22).** Amino alcohol (*R*)-**21** (4.6 g, 49 mmol) was dissolved in 25% aqueous dioxane (160 mL). Potassium carbonate (7.1 g, 51 mmol) was added and the mixture cooled to 0 °C. Di-*tert*-butyl dicarbonate (11.6 g, 53.0 mmol) was added in two portions. The mixture was then allowed to warm to room temperature overnight. The crude mixture was concentrated to dryness. Then water (150 mL) was added followed by saturated aqueous potassium hydrogen sulfate (until pH 3 approximately). The organic material was extracted with methylene chloride (2 × 150 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford 9.5 g (100%) of (*R*)-**22** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.82−5.04 (br s, 1H), 4.62−4.72 (m, 0.5H), 4.48−4.58 (m, 0.5H), 3.62−3.72 (m, 2H), 3.32−3.62 (m, 2H), 3.20−3.44 (br s, 1H), 1.48 (s, 9H).

***tert*-Butyl (2*R*)-2-Fluoro-3-iodopropylcarbamate ((*R*)-23).** Imidazole (26.6 g, 0.39 mol) was dissolved in methylene chloride (400 mL) at room temperature. Iodine (99 g, 0.39 mol) was added, and the reaction mixture was stirred for 10 min at room temperature and then cooled to 0 °C. Triphenylphosphine (102 g, 0.39 mol) was added portionwise over 10 min such that the internal temperature remained below 10 °C. A solution of (*R*)-**22** (60.4 g, 0.31 mol) in methylene chloride (100 mL) was added dropwise. Upon completion of addition, additional methylene chloride (200 mL) was added. The reaction mixture was allowed to warm to room temperature, and stirring was continued for 17 h. The mixture was filtered through a pad of Celite (50 g) and washed with additional methylene chloride. The filtrate was concentrated under reduced pressure and purified by silica gel column chromatography, eluting with methylene chloride. This procedure afforded 64.7 g (68%) of (*R*)-**23** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.80−5.10 (br s, 1H), 4.58−4.72 (m, 0.5H), 4.42−4.56 (m, 0.5 H), 3.48−3.70 (m, 1H), 3.20−3.46 (m, 3H), 1.48 (s, 9H).

(2*R*)-(3-Amino-2-fluoropropyl)phosphinic Acid ((*R*)-7). Ammonium hypophosphite (73.8 g, 0.89 mol) was added to a three-necked 2 L flask equipped with a mechanical stirrer, thermometer, addition funnel, and an argon bubbler. The flask was placed in a water bath at room temperature, and *N,O*-bis(trimethylsilyl)acetamide (215 mL, 0.87 mol, BSA) was added at such a rate that the internal temperature was maintained below 38 °C (30 min approximately) using ice cooling. During the addition ammonia gas was evolved (during the first 15 min this was rather rapid). Upon completion of the addition of BSA, the reaction mixture was heated to 45−48 °C and maintained at this temperature for 1 h (during this time the reaction became viscous). The mixture was cooled to room temperature, and a solution of iodide (*R*)-**23** (27.3 g, 0.090 mol) in methylene chloride (300 mL) was added to the reaction mixture. The mixture was then allowed to stir at room temperature for 18 h. The reaction mixture was cooled to 0 °C and was cautiously quenched with methanol (275 mL) and then with water (32 mL). The reaction mixture was stirred for 30 min after which the mixture was filtered and the solids were washed with methanol. The filtrate was concentrated and the residue placed under high vacuum (0.1 mmHg) overnight. The crude residue was triturated with methylene chloride, methanol, and concentrated ammonium hydroxide solution (80:20:1) and was filtered. The filtrate was concentrated under reduced pressure, and the trituration was repeated. The crude concentrate was transferred to a 2 L flask, dissolved in methanol (375 mL), and placed in a water bath at room temperature. A saturated solution of hydrogen chloride gas in ethyl acetate (500 mL) was added and the mixture stirred for 3 h. The reaction mixture was filtered, and the solids were washed with a mixture of methanol and ethyl acetate (90:10). The filtrate was concentrated under reduced pressure, and the crude product was passed through a Dowex 50WX8-200 mesh H$^+$ form (500 g, 8 cm × 15 cm) column, eluting with 1:1 methanol/water until no further material was detected by TLC analysis (chloroform, methanol, concentrated ammonium hydroxide solution, 6:3:1, ninhydrin development, R_f((*R*)-**23**) = 0.9, R_f(*R*)-**7**) = 0.3). The crude product was then eluted with 1:3 concentrated ammonium hydroxide solution/methanol. The product was further purified by silica gel column chromatography, eluting with chloroform, methanol, concentrated ammonium hydroxide solution (6:3:1) to afford (*R*)-**7** as a white solid (3.12 g, 24%): mp = 183−185 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.90 (s, 0.5 H), 6.15 (s, 0.5 H), 5.12−5.29 (m, 0.5 H), 4.92−5.10 (m, 0.5 H), 3.12−3.42 (m, 2H), 1.74−2.26 (m, 2H); $[\alpha]_D^{25}$ −4.0° (*c* 1.0, H_2O); APIMS *m/z* 142 [M + H]$^+$. Anal. ($C_3H_9FNO_2P \cdot 0.25H_2O$) C, H, N.

(2*R*)-(3-Amino-2-fluoropropyl)(methyl)phosphinic Acid ((*R*)-8). A suspension of compound (*R*)-**7** (1.0 g, 7.1 mmol) in HMDS (7.47 mL, 35.4 mmol) was heated to reflux for 16 h. The mixture was cooled to room temperature, treated with diglyme (8.0 mL), and heated to reflux for 6 h. After the mixture was cooled to room temperature, *N,N*-diisopropylethylamine (1.23 mL, 7.10 mmol) was added followed by dropwise addition of methyl iodide (1.32 mL, 21.2 mmol). The reaction mixture was stirred for 24 h, and then it was diluted with methylene chloride and extracted with 2 N HCl solution. The aqueous layer was washed with methylene chloride and diethyl ether and then evaporated under reduced pressure. The crude product was passed through a Dowex 50WX8-200 mesh H$^+$ form column, eluting with 1:1 methanol/water until no further material was detected by TLC analysis (chloroform, methanol, concentrated ammonium hydroxide solution, 6:3:1, ninhydrin development, R_f((*R*)-**7**) = 0.3, R_f((*R*)-**8**) = 0.2). The requisite crude (*R*)-**8** was then eluted with 1:3 concentrated ammonium hydroxide solution/methanol. The crude product (*R*)-**8** was further purified by column chromatography, eluting with methylene chloride, methanol, concentrated ammonium hydroxide solution (6:3:1) to afford (*R*)-**8** as a white solid (720 mg, 65%): mp = 210−214 °C; ^{1}H NMR (300 MHz, D_2O) δ 5.20 (m, 0.5H), 5.03 (m, 0.5H), 3.20−3.42 (m, 2H), 1.80−2.22 (m, 2H), 1.30 (d, J = 14.0 Hz, 3H); CIMS *m/z* 156 [M + H]$^+$.

In Vivo Studies. The potency of GABA$_B$ agonists as inhibitors of TLESRs was determined using a dog model described by Lehmann et al.[1a] Hypothermic effects in mice were assessed as reported by Quéva et al.[17]

Supporting Information Available: Experimental details for synthesis of (*R*)-**3**, (*S*)-**7**, **8**, (*S*)-**8**, and the *N*-benzamide derivatives of (*S*)-**7** and (*R*)-**7**; procedure for determination of the optical purity of (*S*)-**7** and (*R*)-**7**; description of measurement of $[Ca^{2+}]_i$ in a fluorescence imaging plate reader (FLIPR); elemental analysis results of compounds (*S*)-**3**, **5**, **7**, (*S*)-**7**, (*R*)-**7**, and (*S*)-**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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