Novel γ-Aminobutyric Acid ρ_1 Receptor Antagonists; Synthesis, Pharmacological Activity and Structure−Activity Relationships

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γ-Aminobutyric acid (GABA) analogues based on 4-amino-cyclopent-1-enyl phosphinic acid (**34**−**42**) and 3-aminocyclobutane phosphinic acids (**51**, **52**, **56**, **57**) were investigated in order to obtain selective homomeric ρ_1 $GABA_C$ receptor antagonists. The effect of the stereochemistry and phosphinic acid substituent of these compounds on potency and selectivity within the GABA receptor subtypes was investigated. Compounds of high potency at $GABA_C$ ρ_1 receptors (**36**, $K_B = 0.78$ μM) and selectivity greater than 100 times (**41**, $K_B = 4.97$ μM) were obtained. The data obtained was analyzed along with the known set of $GABA_C$ ρ_1 receptor−ligands, leading to the development of a pharmacophore model for this receptor, which can be used for in silico screening.

Introduction

So ubiquitous are the γ-aminobutyric acid receptors that up to 40% of mammalian neurons use γ-aminobutyric acid (**1**, GABA,[a] Figure 1) as a neurotransmitter.[1,2] This family of receptors is of enormous medical significance, with GABA receptors mediating a variety of central processes, including cognition,[3,4] nociception,[5] vision,[6] and circadian rhythms,[7,8] and are involved in the pathophysiology of epilepsy,[9−12] schizophrenia,[13−16] and mood disorders.[17−20] The term $GABA_C$ receptor was coined to describe an ionotropic GABA receptor that was insensitive to bicuculline, benzodiazepines, neurosteroids, and sedatives[21−23] and was subsequently shown to be a homomeric ligand gated ion channel (LGIC).[24] In contrast to the $GABA_A$ and $GABA_B$ receptor subunits, the $GABA_C$ receptor subunits (designated ρ_{1-3}) have a limited distribution within the central nervous system (CNS),[25] reducing the risk of potential side effects. They are highly expressed throughout the visual system, including the retina,[26] superior colliculus[27] (SC), and hippocampus,[28,29] leading to the recent investigation of $GABA_C$ receptor antagonists in treating degenerative eye disorders.[30,31]

Clinical trials involving the nonselective $GABA_C$ antagonist 3-aminopropyl(butyl)phosphinic acid (**2**, SGS742, Figure 1), formerly CGP36742 ($GABA_B$ IC_{50} 38 μM, $GABA_C$ IC_{50} 62 μM),[20] have shown it to enhance cognition and memory.[19] While the majority of this activity has been attributed to antagonism at $GABA_B$ receptors, the magnitude of the effects observed has led to speculation about the involvement of another mechanism or receptor class.[32] The potential role of $GABA_C$ receptors in memory and cognition is also supported by the dose dependent effect observed for selective $GABA_C$ antagonist 1,2,5,6-tetrahydropyridine-4-yl-methyl-phosphinic acid (**3**, TPMPA, Figure 1) upon memory in a chick model of memory formation.[22] These findings highlight the need for more selective, orally active $GABA_C$ receptor antagonists to determine the role of $GABA_C$ receptors in cognitive processes.

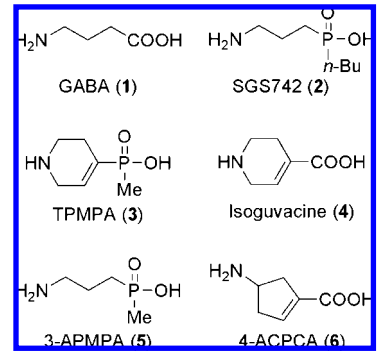

Figure 1. Structures of GABA and compounds active at GABA receptors.

The first selective $GABA_C$ receptor antagonist developed was TPMPA (**3**).[33] This ligand was designed based on the observed activities of isoguvacine (**4**, Figure 1), which is active at $GABA_A$ and $GABA_C$ receptors but not $GABA_B$ receptors, and 3-aminopropyl(methyl)phosphinic acid (**5**, 3-APMPA, Figure 1), which is active at $GABA_C$ and $GABA_B$ receptors but not $GABA_A$ receptors. Here, a similar approach is taken, with a substituted phosphinic acid moiety, known to reduce activity at $GABA_A$ receptors, used as a bioisostere of the carboxylic acid of 4-aminocyclopent-1-enecarboxylic acid (**6**, 4-ACPCA, Figure 1), a potent but nonselective $GABA_C$ receptor antagonist.[34,35] The stereochemistry at the 4-position of these compounds is an important determinant of activity for 4-ACPCA[34] and was therefore investigated along with the effect of increasing substituent size at the phosphinic acid, which has previously been observed to promote increased antagonist activity at $GABA_B$ receptors.[36]

It has been proposed that selective activity at $GABA_C$ receptors over $GABA_A$ and $GABA_B$ receptors is achieved via a more

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[a] Abbreviations: GABA, γ-aminobutyric acid; LGIC, ligand gated ion channel; CNS, central nervous system; TBDMSCl,*tert*-butyldimethylsilyl chloride; TBAF, tetrabutylammoniumfluoride; THF, tetrahydrofuran; BOC, *tert*-butyloxycarbonyl; DMF, dimethylformamide; TMS, tetramethyl silane; EI, electron impact; CI, chemical ionization; ESI, electrospray ionization; BSTFA, bis(trimethylsilyl)trifluoroacetamide; SAR, structure−activity relationship; DABCO, 1,4-diazabicyclo[2.2.2]octane; DMAP, 4-dimethylaminopyridine; DIAD, diisopropylazodicarboxylate; HMDS, hexamethyldisilazane; AIBN, azobisisobutyronitrile.

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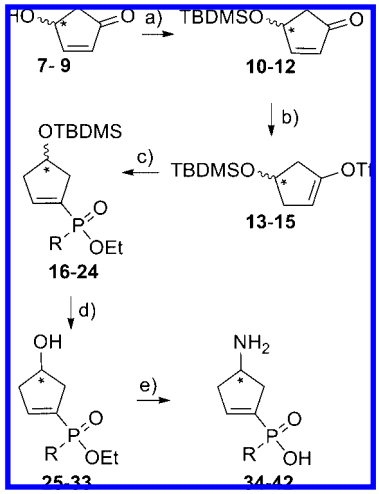
Scheme 1[a]



[a] Reagents and conditions: (a) TBDMSCl, Et_3N, DMAP, $CHCl_3$; 0 °C to room temp 24 h; **10**, (±); **11**, (*S*); **12**, (*R*); (b) L-selectride, ET_3N $PhNTf_2$, THF; −78 °C to room temp, 3 h; **13**, (±); **14**, (*R*); **15**, (*S*); (c) $Pd(PPh_3)_4$, RP(O)(OEt)H, DABCO, toluene; room temp 72 h; (d) TBAF, THF; room temp, 12 h; (e) (i) PPh_3, DIAD, HN_3; 0 °C to room temp, 12 h; 40 °C, 3 h; H_2O; 40 °C, 2 h; (ii) 6 M HCl; reflux, 36 h; **16**, **25**, and **34**, (±), R = CH_3; **17**, **26**, and **35**, (*R*), R = CH_3; **18**, **27**, and **36**, (*S*), R = CH_3; **19**, **28**, and **37**, (±), R = CH_2CH_3; **20**, **29**, and **38**, (±), R = $CH(CH_3)_2$; **21**, **30**, and **39**, (±), R = $(CH_2)_3CH_3$; **22**, **31**, and **40**, (*R*), R = $(CH_2)_3CH_3$; **23**, **32**, and **41**, (*S*), R = $(CH_2)_3CH_3$; **24**, **33**, and **42**, (±), R = $CH_2(C_6H_5)$.

folded conformation of the GABA (**1**) backbone.[37] However, little is known about the activity of cyclobutane analogues of GABA at $GABA_C$ receptors, with only two early reports outlining the synthesis and GABA-ergic activity of *cis*- and *trans*-3-aminocyclobutane carboxylic acid.[38,39]

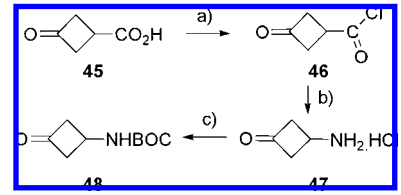
Thus, a series of cyclopentene and cyclobutane compounds were synthesized and their activity as selective ρ_1 $GABA_C$ receptor antagonists investigated. Finally, the compounds investigated were used to develop a pharmacophore model, with the potential to be utilized for the in silico screening of $GABA_C$ receptor antagonists in the future.

Chemistry. 4-*tert*-Butyldimethylsiloxy-1-cyclopenten-1-yl-trifluoromethanesulfonate (**13**, Scheme 1) was considered a key intermediate in designing the synthetic strategy, as the excellent electrophilic properties of the triflate moiety and its position α to the double bond makes this compound an ideal substrate for palladium catalyzed generation of the C−P bond. Additionally, the protected alcohol can be transformed to the required amine with complete inversion of stereochemistry, via a Mitsunobu−Staudinger reaction, to yield the desired amino phosphinic acid.

To access this intermediate, initially (±)-4-hydroxycyclopent-2-en-1-one (**7**, Scheme 1) was synthesized from 2-methyl furan as described by An et al.[40] Several enantioselective methods exist for the production of the individual enantiomers of 4-hydroxycyclopent-2-en-1-one (**8** and **9**, Scheme 1), due to its extensive use as a synthon in prostaglandin chemistry.[41] These include enzymatic,[42] chromatographic,[43] and synthetic procedures.[44−46] The method of Khanapure et al.[44] using L-tartaric acid as the source of chirality was selected due to the extremely high reported enantiomeric excess (e.e.) and the potential for multigram scale preparation. Thus (*S*)-4-hydroxycyclopent-2-en-1-one (**8**) and (*R*)-4-hydroxycyclopent-2-en-1-one (**9**) were synthesized at 99%+ e.e. as determined by optical rotation measurement.

Routine protection of the (±)-, (*S*)-, and (*R*)-4-hydroxycyclopent-2-ene-1-one (**7**, **8**, and **9**, respectively) using *tert*-butyldimethylsilyl chloride (TBDMSCl) produced compounds

Scheme 2[a]



[a] Reagents and conditions: (a) $SOCl_2$, benzene; 70 °C, 12 h; (b) (i) NaN_3, H_2O, benzene; room temp, 3 h; (ii) benzene; 60 °C, 4 h; (iii) 6 M HCl, benzene, 90 °C, 12 h; (c) BOC_2O, Et_3N, DMF; room temp, 4 h.

10−**12** (Scheme 1), and subsequent 1,4-reduction with trapping as the triflate as described by Paquette et al.[47] provided access to the desired intermediates (**13**−**15**, Scheme 1).

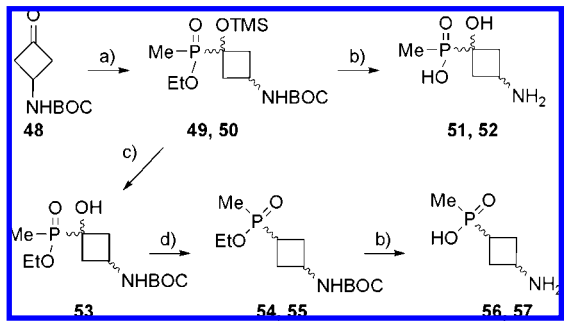
Methodology for the formation of carbon phosphorus bonds and the application of these to the synthesis of GABA analogues has been the subject of a number of studies.[33,48−50] These have reported the successful palladium catalyzed coupling of numerous alkenyl halides to alkylphosphinates and anilinium hypophosphite. Typical isolated yields for these procedures is from 30−70%. Dumond et al.[50] reported the coupling of anilinium hypophosphite to 2-trifluoromethanesulfonyloxy-1-octene with an optimized yield of 69% using $Pd(OAc)_2$ as the catalyst. More recently, a report has outlined the coupling of phosphinates to alkenyl triflates catalyzed by $Pd(OAc)_2$ under reflux for 18 h with the phosphinates generated in situ.[51] Here, we report the coupling of alkylphosphinate esters (RP(O)(OR′)H) to 4-tert-butyldimethylsiloxy-1-cyclopenten-1-yl-trifluoromethanesulfonate (**13**−**15**, Scheme 1) with 2.5 mol% $Pd(PPh_3)_4$ as the catalyst. The reaction proceeds smoothly for a variety of alkylphosphinate esters (**16**−**24**, Scheme 1) with isolated yields of 85−95% using mild conditions. While the reaction is limited by requiring long reactions times (∼72 h), as decomposition of the starting materials is observed upon heating, the high yield and ease of this coupling suggests that the leaving group properties of the triflate group make it a superior substrate to alkenyl halides. This observation may be of synthetic utility for C−P bond formation, as to the authors knowledge, it is the first demonstration of direct one-step access to esterified alkyl phosphinic acids from an alkenyl triflate and an alkylphosphinate ester.

Following C−P bond formation, the compounds are deprotected using tetrabutylammoniumfluoride (TBAF) in anhydrous tetrahydrofuran (THF), giving the alcohol (**25**−**33**, Scheme 1) and the amine generated via a one pot Mitsunobu−Staudinger reaction. Finally, the phosphinate ester is hydrolyzed using aqueous HCl and the crude product purified via ion-exchange chromatography and recrystallization to give the free amine (**34**−**42,**Scheme 1).

Mitsunobu reactions at unhindered, primary amines such as these have previously been observed to occur with complete inversion of the stereocenter.[52] To confirm this effect, the Mosher amides of compounds **35** and **36** (**43** and **44**, respectively) were prepared via the method of Smith et al.[53] Chemical shift inequivalences were observed for the P−CH_3 hydrogens via ^1H NMR and from the line fitted data (see Supporting Information) the e.e. determined to be 92−94%. As the compounds **40** and **41** were synthesized from the same starting materials using identical techniques, it was assumed that the e.e.ʼs were comparable to those determined for compounds **35** and **36**.

The synthesis of the cyclobutane compounds proceeded from 3-oxocyclobutanecarboxylic acid (**45**, Scheme 2) as the ketone and carboxylic acid moieties could be used to generate the

Scheme 3[a]



[a] Reagents and conditions: (a) HMDS, (O)PH(OEt)CH_3; 90 °C, 12 h; (b) (i) 6 M HCl; reflux, 36 h; (ii) Dowex 50 H^+; (c) $Et_3N{\cdot}3HF$, CH_3CN; 0 °C to room temp, 4 h; (d) (i) methyl oxalyl chloride, Et_3N, CH_3CN; 0 °C to room temp, 3 h; (ii) AIBN, $(Bu)_3SnH$, toluene; 90 °C, 5 h. **49**, **51**, **54**, **56** = *cis*-; **50**, **52**, **55**, **57** = *trans*-.

desired amine and phosphinic acid functionalities, respectively, by known methods. The method of Pigou and Schiesser[54] was used to generate this intermediate. After formation of the acid chloride (**46**, Scheme 2), using thionyl chloride, the amine (**47**, Scheme 2) was then generated via a Curtius rearrangement in a procedure modified from Daly and Gilheany.[55] The amine was then protected as a *tert*-butyloxycarbonyl (BOC) amide (**48**, Scheme 2) using BOC_2O and triethylamine in dimethylformamide (DMF).

C−P bond formation was achieved using the modified Pudovik reaction (Scheme 3) as outlined by Hansen and Kehler.[56] The nucleophilic addition of the silyl activated ethyl methylphosphinate[57] resulted in an approximate 1:4 ratio of cis and trans isomers (**49** and **50**, respectively, Scheme 3). The trans form predominating due to the puckered nature of the cyclobutane ring and resulting steric protection of one face by the BOC group, as previously observed for nucleophilic addition to 1,3-cyclobutanes.[57] The isomers can be separated at this stage by column chromatography and deprotected with HCl to yield the α-hydroxy amino phosphinic acid compounds (**51** and **52**, Scheme 3).

The target compounds (**56** and **57**, Scheme 3) were obtained by the modified Barton deoxygenation of Hansen and Kehler,[56] which proceeds with racemization of the substrate due to the radical mechanism of the reaction. The cis and trans BOC protected phosphinate ester isomers (**54** and **55**, respectively, Scheme 3) that are formed in approximately 1:1 ratio can be separated by column chromatography before deprotection with HCl.

Pharmacological Results. At ρ_1 $GABA_C$ receptors, dose−response curves of GABA in the presence of several concentrations of **34** (R = Me) (Figure 2(i)) were determined and the nonlinear method of Lew and Angus[58] (fitted data shown in Figure 2(ii)) used to define the interaction as competitive antagonism. From this analysis, the K_B of **34** was determined as ($pA_2 = 5.73 \pm 0.09$). The effect of increasing phosphinic acid substitution was investigated at ρ_1 $GABA_C$ receptors via the determination of apparent K_B values for compounds **37**−**39** and **42** (R = Et, *i*-Pr, Bu, and Bn, respectively). The dose response curves of GABA in absence and presence of a single concentration of antagonists are shown in Figure 2(iii). A nonsignificant decrease in activity was observed with increasing phosphinic acid substituent size, from methyl group **34** to ethyl group (**37**, $p = 0.0596$) or *n*-butyl group (**39**, $p = 0.0741$), however, statistically significant reductions in activity were observed upon substitution with an isopropyl group (**38**, $p = 0.0365$) and a benzyl group (**42**, $p = 0.0002$).

The effect of stereochemistry at the 4 position was determined in a similar manner, by determining apparent K_B values for compounds **35**(*R*), **36**(*S*), **40**(*R*), and **41**(*S*) (Figure 2 (iv)). In both cases the (*S*) enantiomer was significantly more active than the (*R*) enantiomer ($p = 0.0007$ for **35** and **36**; $p = 0.0095$ for **40** and **41**).

At $GABA_{B(1A/2)}$ receptors, compound **34** (R = Me) was found to be a partial agonist with an EC_{50} of 51.04 ± 5.6 μM and a I_{max} of 56 ± 0.5%, relative to GABA (Figure 3). The effect of stereochemistry at the 4 position was investigated by determining the dose response curves of compounds **35** (*R*) and **36** (*S*) at $GABA_{B(1A/2)}$ receptors (Figure 3), the (*S*) enantiomer (**36**, $I_{max} = 75.9 \pm 2.0\%$) was found to activate a significantly greater percentage of the receptor population compared to **35** ($I_{max} = 52.3 \pm 0.1\%$) ($p = 0.0003$) and at a lower concentration ($EC_{50} = 52.2 \pm 0.1$ μM and $EC_{50} = 71.9 \pm 0.1$ μM, respectively) than the (*R*) enantiomer ($p < 0.0001$). At these receptors, only compounds **34**−**36** (R = Me) displayed any agonist activity, with the larger substituents on the phosphinic acid of compounds **37**−**42** (R = Et, *i*-Pr, Bu, and Bn, respectively) abolishing all agonist activity and only very weak antagonist activity remaining ($IC_{50} > 300$ μM in all cases). Thus, apparent K_B values were not determined for compounds **37**−**42**, the % inhibition of 3 μM GABA by 300 μM of these compounds are given in Table 1. Compound **37** (R = Et) is significantly more active than compounds **38**, **39**, and **42** ($p = 0.0141$, 0.0106, and 0.0010, respectively), however no significant difference in inhibition is observed among compounds **38**, **39**, and **42**. For compounds **40** and **41**, the (*S*) enantiomer (**41**) is again observed to be significantly more active than the (*R*) enantiomer (**40**, $p = 0.0201$).

At $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors, all compounds were shown to be inactive or very weak antagonists at 600 μM, due the low potency observed ($IC_{50} > 300$ μM in all cases), apparent K_B values were not determined and the percentage inhibition of 30 μM GABA by these compounds at 600 μM is given in Table 1. No significant difference was observed among compounds **34** and **37**−**39**. Compound **42** (R = Bn) was significantly more potent than compounds **34** and **37**−**39** ($p < 0.0042$ in all cases). The (*R*) enantiomers **35** and **40** (R = Me and Bu, respectively) were found to be inactive at 600 μM as either agonists or antagonists.

Compounds **56** and **57** were found to be very weak antagonists ($IC_{50} > 100$ μM) at ρ_1 $GABA_C$ receptors, with the cis isomer being more active than the trans isomer (Table 1). The hydroxylated compounds **51** and **52** were found to be inactive at ρ_1 $GABA_C$ receptors at the concentrations tested. None of the compounds were active at $GABA_{B(1A/2)}$ receptors. Compounds **56** and **57** displayed some selectivity for $GABA_C$ ρ_1 receptors over $GABA_B$ receptors, where they were inactive. Because conformationally restricted phosphinic acid analogues of GABA are significantly weaker at $GABA_A$ receptors than at $GABA_C$ receptors[20] and given extremely low potencies observed for the cyclobutane compounds at $GABA_C$, the compounds were not evaluated at $GABA_A$.

$GABA_C$ ρ_1 Pharmacophore. Relatively few attempts have been made to develop computational models of the $GABA_C$ ρ_1 receptor agonists binding pocket,[59−61] and these have used primarily homology modeling[60,61] with respect to the acetylcholine binding protein for which a crystal structure exists.[62] This approach is obviously limited by the low sequence homology between the two proteins (~20%). A quantitative structure−activity relationship (SAR) model of agonist binding to the $GABA_C$ ρ_1 receptor has proposed a scheme for the

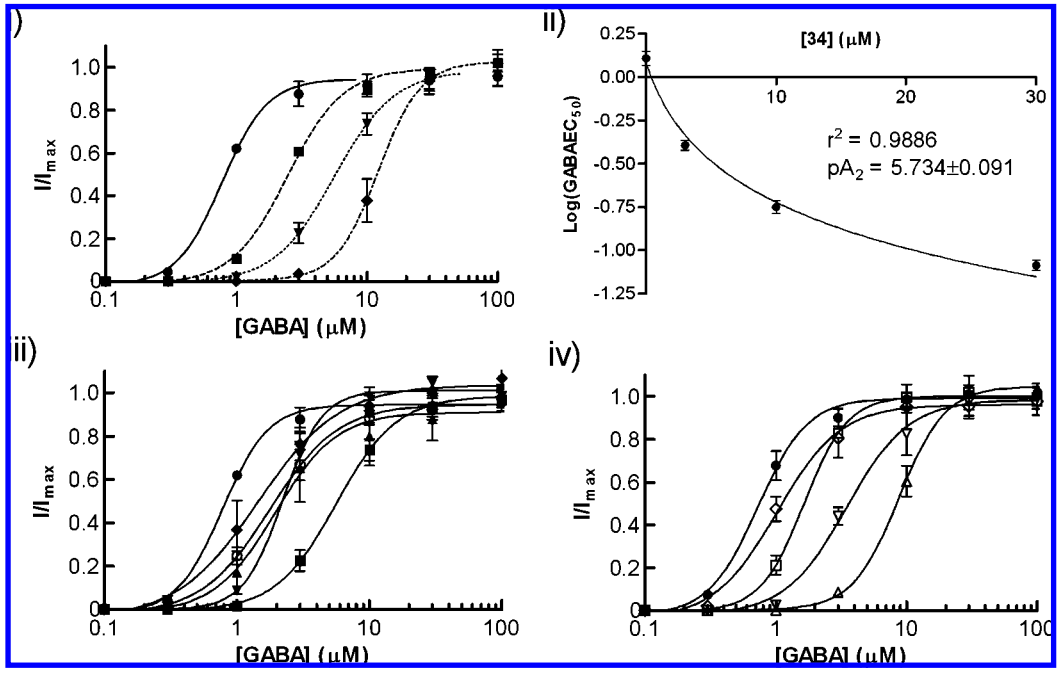


Figure 2. (i) Dose response curves of GABA alone (●, $n = 3$) and in the presence of 3 μM (■, $n = 3$), 10 μM (▼, $n = 3$), and 30 μM (◆, $n = 3$) **34**. (ii) Variation of Log(GABAEC$_{50}$) with concentration of **34** and results of nonlinear regression. (iii) Dose response curves for GABA alone (●, $n = 3$) and in the presence of 10 μM **34** (■, $n = 3$), 10 μM **37** (▼, $n = 3$), 10 μM **38** (◆, $n = 3$), 10 μM **39** (▲, $n = 3$), and 100 μM **42** (○, $n = 3$). (iv) Dose response curves of GABA alone (●, $n = 9$) and in the presence of 30 μM **35** (□, $n = 3$), 10 μM **36** (△, $n = 3$), 30 μM **40** (◇, $n = 3$), and 10 μM **41** (▽, $n = 3$). Data are mean ± SEM.

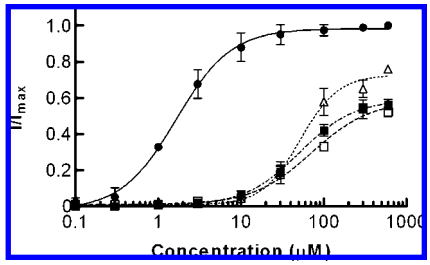


Figure 3. Dose−response curves of GABA (●, $n = 3$), **34** (■, $n = 3$), **35** (□, $n = 3$), and **36** (Δ, $n = 3$) at GABA$_{B(1A/2)}$ receptors. Data are means ± SEM.

GABA$_C$ receptor agonist binging pocket[59] but does not provide more than a conceptual outline with which to assess the potency of candidate ligands. Here, as a result of the synthesis and pharmacological evaluation of a set of new GABA$_C$ ρ_1 receptor antagonists, sufficient data now exists to allow the generation of a retrospective, ligand based GABA$_C$ ρ_1 antagonist pharmacophore. The generation of such a model will expediate efforts toward the understanding of GABA$_C$ ρ_1 receptors role in the CNS by the identification of the primary structural features responsible for antagonist activity and allowing in silico screening of potential antagonists. To this end, pharmacophore modeling was implemented using TPMPA (**3**), (*R*)-4-ACPMPA (**35**), (*S*)-4-ACPMPA (**36**), (1*R*,3*S*)-3-aminocyclopentane(methyl)phosphinic acid[63] (**60**, (1*R*,3*S*)-3-ACPMPA, Table 2), (1*S*,3*R*)-3-aminocyclopentane(methyl)phosphinic acid[63] (**62**, (1*S*,3*R*)-3-ACPMPA, Table 2), and imidazole-4-acetic acid[64] (**63**, I4AA, Table 2) as the basis set.

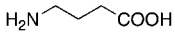
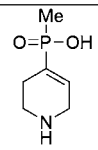
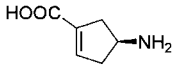
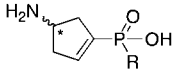
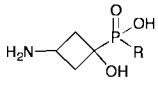
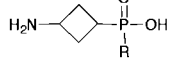
The GABA$_C$ ρ_1 pharmacophore (Figure 4a) represents a theoretical model capable of relating pharmacophore features to antagonist activity for compound **36**, discriminating it against **35**. Figure 4b shows both compounds successfully matching each of the five pharmacophore sites, however, only **36** projects the amine H-bond donor in a similar direction to that defined by the GABA$_C$ ρ_1 pharmacophore H-bond donor vector. This effect is quantitatively evident through the different vector geometry scores: 0.88 and 0.68 for **36** and **35**, respectively (Table 2), was consequently exploited during the pharmacophore optimization. The "find match to hypothesis" criteria[65] was instructed to reject hits with vector scores less than 0.76 (note: optimal vector score is 1.0).[66] As a result, the final GABA$_C$ ρ_1 pharmacophore identified only compound **36** and not compound **35** as a potent antagonist.

Upon screening a diverse set of GABA ligands (see Supporting Information for a complete list), the GABA$_C$ ρ_1 pharmacophore accurately identified only potent GABA$_C$ ρ_1 antagonists (Table 2). Moreover, it did not identify any GABA$_C$ agonists, such as GABA (**1**), or any of the potent agonists/antagonists of GABA$_A$ and GABA$_B$. The shape constraint of the pharmacophore was proven to be effective in excluding various nonpotent GABA$_C$ antagonists, for example (*R*)-4-ACPCA (*R*-**6**) (Figure 5), which was not identified as a hit, despite satisfying four of the five pharmacophore sites (DNPX), due to having excluded volume violations with spheres at the base of the shell, indicated by the red arrow.

Discussion

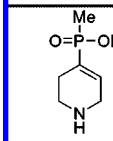
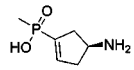
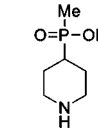
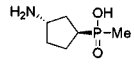
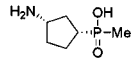
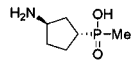
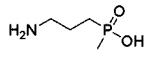
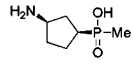
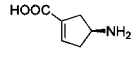
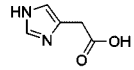
It was hypothesized that the 4-aminocyclopent-1-enyl(alkyl)-phosphinic acids (**34**−**42**) would possess the required physicochemical properties for optimal interaction with the GABA$_C$ receptor agonist binding site based on the activity of (*S*)-4-ACPCA (*S*-**6**).[35] It has previously been observed, for conformationally restricted analogues of GABA, that the use of phosphinic acids and alkyl phosphinic acids as a bioisostere of a carboxylic acid gives antagonists at ρ_1 GABA$_C$ receptors,[33,67–69] with activity comparable to or greater than the parent compound. This is reflected in the pharmacological results obtained (Table 1), with compound **36** ($K_B = 0.78$ μM) being a competitive antagonist with higher affinity for the GABA$_C$ agonist binding site than (*S*)-4-ACPCA (*S*-**6**, $K_B = 6.0$ μM).[35]

Table 1. Pharmacological Data

Structure	Config.	R	Compound	Human ρ_1 $GABA_C$ receptors		Human $GABA_{B(1A/2)}$ receptors		Human $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors	
				EC_{50} / K_B (μM)	n_H	EC_{50} (μM)/% Inhibition	n_H	EC_{50} (μM)/% Inhibition	n_H
	-	-	**1**	0.8 ± 0.1 [a]	2.5 ± 0.5 [b]	1.7 ± 0.1 [a]	1.3 ± 0.2 [b]	21.1± 0.1 [a]	1.4 ± 0.1 [b]
	-	-	**3**	K_B = 2.1 μM [33]		EC_{50} ~ 500 μM[33]		K_B = 320 μM [33]	
	(*S*)	-	**6**	K_B = 6.0 μM [35]		n.d.		ED_{50} = 1.1 μM [34]	
	(±)	CH_3	**34**	1.84 (pA_2 = 5.73±0.09) [c]		51.0 ± 5.6 [a] (56.4 ± 0.5% [d])	1.3 ± 0.2 [b]	6.2 ± 2.2% [e]	
	(*R*)	CH_3	**35**	18.9 (pA_2 = 4.74 ± 0.10) [c]		71.9 ± 0.1 [a] (52.3 ± 0.1% [d])	1.3 ± 0.3 [b]	Inactive at 600 μM	
	(*S*)	CH_3	**36**	0.78 (pA_2 = 6.12 ± 0.11) [c]		52.2 ± 0.1 [a] (75.9 ± 2.0% [d])	1.9 ± 0.4 [b]	4.1 ± 1.1 [e]	
	(±)	CH_2CH_3	**37**	3.53 (pA_2 = 5.45 ± 0.06) [c]		43.4 ± 3.9% [f]		5.2 ± 1.8% [e]	
	(±)	$CH(CH_3)_2$	**38**	13.1 (pA_2 = 4.88 ± 0.26) [c]		26.0 ± 1.5% [f]		8.8 ± 2.4 [e]	
	(±)	$(CH_2)_3CH_3$	**39**	7.24 (pA_2 = 5.14 ± 0.23) [c]		18.4 ± 3.9% [f]		23.2 ± 6.6 [e]	
	(*R*)	$(CH_2)_3CH_3$	**40**	59.3 (pA_2 = 4.25 ± 0.22) [c]		11.9 ± 2.1% [f]		Inactive at 600 μM	
	(*S*)	$(CH_2)_3CH_3$	**41**	4.97 (pA_2 = 5.42 ± 0.12) [c]		22.5 ± 1.9% [f]		24.2 ± 1.7 [e]	
	(±)	$CH_2(C_5H_6)$	**42**	48.6 (pA_2 = 4.31 ± 0.07) [c]		9.8 ± 0.3% [f]		64.1 ± 2.2 [e]	
	*cis*	CH_3	**51**	Inactive at 600 μM		Inactive at 600 μM		n.d.	
	trans	CH_3	**52**	Inactive at 600 μM		Inactive at 600 μM		n.d.	
	*cis*	CH_3	**56**	IC_{50} > 100 μM		Inactive at 600 μM		n.d.	
	trans	CH_3	**57**	IC_{50} > 100 μM		Inactive at 600 μM		n.d.	

[a] EC_{50} values of agonists. Data are mean ± SEM (n = 3−5 oocytes). [b] The Hill coefficient (n_H). Data are mean ± SEM (n = 3−5 oocytes). [c] Dissociation constants of antagonists (K_B) are calculated from the pA_2 values in brackets. Data are mean ± SEM (n = 3−5 oocytes). [d] I_{max} is the intrinsic activity calculated as a percentage of the maximum whole cell current produced by a maximum dose of GABA. Data are mean ± SEM (n = 3−5 oocytes). [e] Percentage inhibition by 600 μM of compound of the current produced by a submaximal dose of GABA (30 μM, EC_{50}). Data are mean ± SEM (n = 3−5 oocytes). [f] Percentage inhibition by 300 μM of compound of the current produced by a submaximal does of GABA (3 μM, EC_{50}). Data are mean ± SEM (n = 3−5 oocytes).

Table 2. $GABA_C$ Antagonists that Successfully Matched with the $GABA_C$ ρ_1 Pharmacophore[a]

Matched Compound	Sites Matched	Sites Not Used	Experimental Activity/ Kb(μM)	Potential Energy OPLS-2005 (kJ/mol)	Align Score	Vector Score	Volume Score	Fitness [b]
 TPMPA (**3**) [c]	5	-	2.1 [33]	-754.56	0.27	0.83	0.71	2.31
 (*S*)-4-ACPMPA (**36**) [c]	5	-	0.78	-714.66	0.41	0.88	0.69	2.23
 P4MPA (**58**)	4	X	6.0 [69]	-488.59	0.58	0.98	0.53	2.03
 (1*S*,3*S*)-3-ACPMPA (**59**)	4	X	6.6 [63]	-440.25	0.56	0.93	0.51	1.97
 (1*R*,3*S*)-3-ACPMPA (**60**) [c]	4	X	1.1 [63]	-486.04	0.62	0.97	0.49	1.94
 (1*R*,3*R*)-3-ACPMPA (**61**)	4	X	6.6 [63]	-488.74	0.58	0.91	0.51	1.94
 3-APMPA (**5**)	4	X	0.81 [86]	-771.88	0.58	0.9	0.49	1.91
 (1*S*,3*R*)-3-ACPMPA (**62**) [c]	4	X	1.1 [63]	-466.34	0.67	0.91	0.51	1.87
 (*S*)-4-ACPCA (**6**)	4	Y	6.0 [35]	-507.88	0.65	0.88	0.32	1.66
 I4AA (**63**) [c]	4	Y	1.7 [64]	-402.169	0.73	0.81	0.18	1.38

[a] Successful alignments require a minimum of four sites corresponding to exact matches of h-bond donor (D), and positive (P) and negative (N) ionizable sites, leaving the fourth site point as either the double bond (X) or an methyl/alkyl substituent (Y). [b] Fitness = 1.00 × (1.0 − align score/1.20) + 1.00 × vector score + 1.00 × volume score. [c] Indicates compound used to generate pharmacophore, **35** not shown. The fitness score is a quantitative measure of how accurately the compound matched the pharmacophore model. Specific match criteria involved applying the excluded volume shell and rejecting hits with vector scores < 0.76, volume scores < 0.00, or align scores >1.20.[65,66]

Further alkyl substitution of the phosphinic acid led to a reduction in the activity of these compounds (**37**−**39**, **42**) at ρ_1 $GABA_C$ receptors, but interestingly the effect was not a direct correlation of alkyl size and activity. The isopropyl substituted compound (**38**, K_B = 13.09 μM) was less potent than *n*-butyl substituted compound (**39**, K_B = 7.24 μM), possibly indicating an unfavorable steric interaction of the branched isopropyl chain with the binding site. The benzyl substituent (**42**) led to a major reduction in activity, indicating that large planar substituents cannot be well accommodated. Taken together, the activities

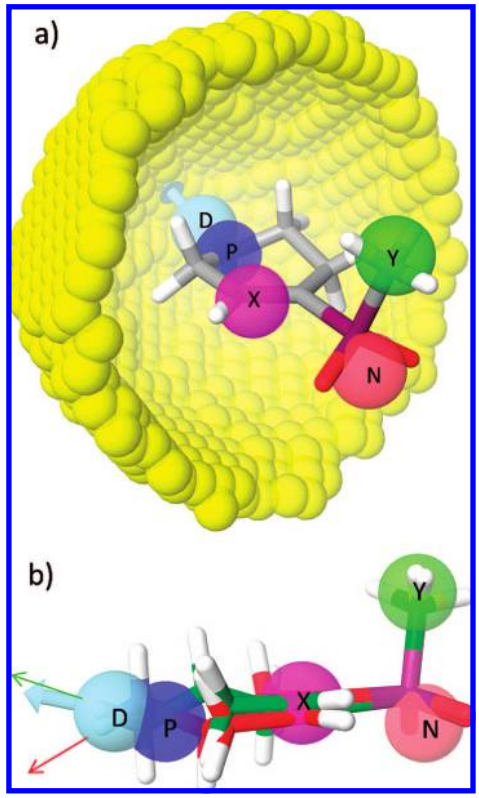


Figure 4. (a) Compound **36** mapped against the pharmacophore features and molecular shape constraint required for antagonism, indicated by the activity of compounds tested against the $GABA_C$ ρ_1 receptor. Features include, H-bond donor (D, light-blue), negative ionizable (N, red), positive ionizable (P, dark-blue), double bond (X, magenta), and methyl/alkyl (Y, green). The molecular shape constraint is comprised of excluded volume spheres (yellow). For clarity, only half of the shell is shown. (b) Pharmacophore for $GABA_C$ ρ_1 antagonism with overlay of **35** (red carbons) and **36** (green carbons), each satisfying all pharmacophore sites (DNPXY). Red and green arrows follow the projected vector path of the two amine H-bond donors for **35** and **36**, respectively.

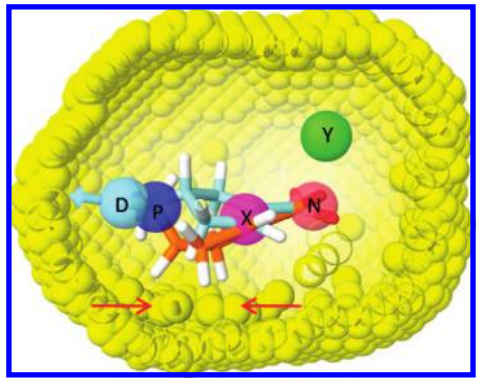


Figure 5. Pharmacophore for $GABA_C$ ρ_1 antagonism with overlay of (*S*)-4-ACPCA (*S*-**6**) (blue carbons), and (*R*)-4-ACPCA (*R*-**6**) (orange carbons). Red arrows indicate excluded volume violations.

may point to the existence of a narrow hydrophobic region near the $GABA_C$ binding site, which has previously been hypothesized.[67,70] Inspection of existing models of the $GABA_C$ ρ_1 receptor agonist binding pocket[60,61] reveals basic residues (Arg-104 and Arg-170) that are presumably the site of acid interaction. These residues are in the vicinity of hydrophobic residues (Val-155, Val-159, Met-156, Tyr-109) that are also proposed to form the agonist/antagonist binding site. These residues may interact with the phosphinic acid substituent and form its binding site.

Whereas previous modeling studies have been concerned with identifying protein structure in the area of ligand binding, this study sought to provide a tool for assessment of potential antagonists. Using the final pharmacophore to screen a large set of various GABA receptor−ligands (see Supporting Information), only potent $GABA_C$ ρ_1 receptor antagonists ($K_B \leq 10.0$ μM) matched the hypothesis, including those that were not incorporated into the original pharmacophore design: (*S*)-4-ACPCA (*S*-**6**), (piperidin-4-yl)methylphosphinic acid[69] (**58**, P4MPA, Table 2), (1*S*,3*S*)-3-aminocyclopentane(methyl)phosphinic acid[63] (**59**, (1*S*,3*S*)-3-ACPMPA, Table 2), (1*R*,3*R*)-3-aminocyclopentane(methyl)phosphinic acid[63] (**61**, (1*R*,3*R*)-3-ACPMPA, Table 2), and 3-APMPA (**5**). This indicates the $GABA_C$ ρ_1 antagonist pharmacophore has the ability to delineate the structural features pertinent for potent $GABA_C$ ρ_1 receptor antagonism and thus has the potential to explain why certain $GABA_C$ receptor congeners exhibit antagonist activity while others do not.

It is observed for compounds **35**, **36**, **40**, and **41** that the (*S*)-enantiomer is at least an order of magnitude more potent at $GABA_C$ ρ_1 receptors than the corresponding (*R*)-enantiomer, and this is in keeping with observations for (*S*)-4-ACPCA (*S*-**6**).[35] Here, the pharmacophore model effectively matches against **35** and *S*-**6** but did not match against their (*R*)-enantiomers (Table 2). This achievement is the result of two pharmacophore attributes: the use of a vector point defining the H-bond donor and the excluded volume shell. Initially it was found that both (*S*)- and (*R*)-enantiomers would satisfy the necessary sites when matched against the pharmacophore, however, as the 4 position H-bond site was defined as a vector site, it became apparent that the opposed stereochemistry of each enantiomer vastly affected the directionality of the H-bond donation. Thus, their different vector geometry scores were used as a basis for selecting only the compounds with optimal vectors. The rationale behind this decision equates to selecting compounds that possess a H-bond donor with a precise directionality toward the binding site, while those that do not are rejected.

Moreover, this distinct conformational requirement forced some weaker $GABA_C$ receptor antagonists to adopt conformation that could easily be rejected by the excluded volume. Such is the case for 4-ACPCA, for which the excluded shell sterically prohibited the conformations of the (*R*)-enantiomer while not affecting its more potent (*S*)-enantiomer. Carrying out this latter approach is in accordance with a literature $GABA_C$ ρ_1 receptor agonist pharmacophore[59] because both models hypothesize that a region of the ligand binding site is surrounded by a sterically inaccessible region.

Another critical pharmacophore feature includes the need for molecules to have *either* a methyl/alkyl substituent (a proxy for the presence of an alkyl substituted phosphinic acid) *or* a double bond. This feature is observed in the known set of competitive full $GABA_C$ antagonists, as those that do not contain an alkyl phosphinic acid contain a double bond, for example I4AA (**63**) and (*S*)-4-ACPCA (*S*-**6**). While the antagonist activity of alkyl phosphinic acids at ρ_1 $GABA_C$ receptors is well established,[68] the role of the double bond is less so. The importance of the double bond is suggested by the fact that TPMPA (**3**) is more potent than its saturated analogue P4MPA (**58**)[69] and also that compound **36** is more potent than its saturated analogues (**59**−**62**). To the authors' knowledge, no carboxylic acid full $GABA_C$ ρ_1 antagonists without a double bond α or β to the carboxylic acid have been described. The role of the double bond may be participation in a specific

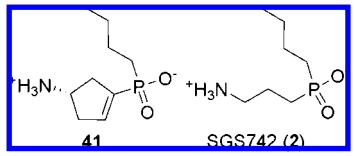


Figure 6. Comparison of the structures of SGS742 and **41**.

receptor interaction, for instance a $\pi-\pi$ interaction, or the alteration of the pK_a or orientation of the carboxylic acid.

Optimal antagonist activity is thus believed to be the result of the correct interaction of a number of ligand features with the receptor. These are the correct relative orientations of the acid group, alkyl substituent, or double bond and amine. The high affinity of compound **36** may be due to the optimal interaction of all features with the receptor. Thus, the incorporation of the H-bond donor vector site and both the double bond and alkyl substituents as pharmacophore features was considered warranted.

At the $GABA_B$ receptor, **34** is a moderately potent partial agonist in contrast with TPMPA (**3**), which is a very weak agonist, however a direct comparison of potencies is not possible as the EC_{50} of TPMPA (**3**) at $GABA_B$ receptors was assayed using rat hippocampal slices[71] as opposed to electrophysiological recordings on *Xenopus laevis* oocytes. The $GABA_B$ receptor is known to have quite strict conformational requirements for agonist binding. For analogues of GABA, substitution at the α and γ positions leads to a significant reduction in agonist activity, whereas substitution at the β carbon is well tolerated, as seen in the selective agonist baclofen and the antagonist phaclofen. In comparison to TPMPA (**3**), **34** has the steric load located closer to β carbon, which may explain its increased potency relative to TPMPA, which has more steric load around the γ position. Phosphinic acid and methyl phosphinic acid analogues of GABA have found to be potent agonists at $GABA_B$ receptors,[72,73] whereas higher order alkyl substitution gives compounds that are antagonists at $GABA_B$ receptors.[74,75] This is observed for compounds **37**−**42**, which are very weak antagonists at $GABA_B$ receptors, indicating that any phosphinic acid alkyl substituent larger than a methyl group can effectively abolish $GABA_B$ activity for conformationally restricted ligands.

At $\alpha_1\beta_2\gamma_{2L}$ containing $GABA_A$ receptors, all compounds displayed only very weak antagonist activity, which is consistent with the reported poor affinity of the $GABA_A$ receptor for phosphinic acids.[67,72,74] Interestingly, **42** (R = Bn) displays greater antagonist activity at $GABA_A$ receptors than any of the other compounds. This is in keeping with the pharmacophore model based on alkyl and aryl substituted 5-(4-piperidyl)isoxazol-3-ol compounds,[76] which includes a large hydrophobic pocket. Thus the affinity of **42** for the $GABA_A$ receptor may be enhanced by interaction with this region of the receptor.

With the exception of **42**, all cyclopent-1enyl phosphinic acid compounds are potent and selective ρ_1 $GABA_C$ receptors. While the selectivity of **34**−**36** is only moderate, the higher order alkyl substituents result in good selectivity for ρ_1 $GABA_C$ receptors over $GABA_{B(1A/2)}$ and $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors, with comparable potency and selectivity to TPMPA (**3**).

The butyl phosphinic acid analogue of GABA, SGS742 exhibits cognitive enhancing effects as mentioned previously and is also orally active.[77] The close structural similarity between SGS742 and **41** (Figure 6) but greater $GABA_C$ ρ_1 selectivity of **41** suggests it offers a means of establishing the basis of the cognition enhancement. TPMPA (**3**) is currently the best tool for elucidating the role of $GABA_C$ receptors in vivo but suffers from its lack of activity following systemic administration, thus the oral availability and effects on memory and cognition compound **41** is currently under investigation in animal systems. These results and its potential as an in vivo tool for determining the physiological roles of the $GABA_C$ receptor and will be reported in due course.

The phosphinic amino acid cyclobutane analogues **51**, **52**, **56**, **57** of GABA showed little activity at $GABA_C$ ρ_1 receptors and were inactive at $GABA_B$ receptors. Their conformationally restricted structure and relative lack of activity $GABA_C$ receptors made them useful in generating the pharmacophore model of the $GABA_C$ ρ_1 receptor. In addition, these GABA analogues are now available for testing on a variety GABA receptors, transporters, and enzymes.

Conclusion

The synthesis of novel phosphinic acid analogues of GABA is reported; this has extended the utility of palladium catalysis in the formation of C−P bonds. The activity of these compounds has been investigated at the three major GABA receptor subtypes, and those based on the cyclopent-1-enyl phosphinic acid scaffold showed excellent potency at $GABA_C$ ρ_1 receptors. The pharmacological data collected shows that (i) the nature of the alkyl substituent, (ii) the stereochemistry, and (iii) existence of a double bond α or β to the acid functionality are important in determining optimal antagonist activity and selectivity. These features have been incorporated into a $GABA_C$ ρ_1 pharmacophore that can be used for in silico screening of antagonist candidates. Compound **41** is currently under investigation in animal models of cognition and as a pharmacological tool for elucidating the role of $GABA_C$ ρ_1 receptors in vivo.

Experimental Section

All chemicals used were purchased from Aldrich Chemical Co. Ltd. (St Luois, MO) unless otherwise stated and were of highest commercially available purity. Solvents were distilled by standard techniques prior to use. Where stated, reactions were performed under inert atmosphere. ^{1}H NMR spectra were recorded at 300 MHz using a Varian (Palo Alto, CA) Gemini 300 spectrometer. Chemical shifts (δ) are quoted in parts per million (ppm), referenced externally to tetramethyl silane at 0 ppm. ^{13}C NMR were recorded at 75 MHz using a Varian (USA) Gemini 300 spectrometer. Chemical shifts (δ) are quoted in ppm, referenced internally to $CDCl_3$ at 77.0 ppm. ^{31}P NMR were recorded at 162 MHz using a Bruker (Billerica, MA) DPX300 spectrometer. Chemical shifts (δ) are quoted in ppm, referenced externally to neat trimethyl phosphite at 140.85 ppm. All coupling constants (J) are given in Hertz. Unless otherwise noted, the duplication of peaks in the NMR spectra arise from the presence of diastereomers associated with the chirality of the phosphorus atom in the phosphinate ester. Low resolution electron impact (EI) and chemical ionization (CI) MS was carried out on a ThermoFinnigan (Waltham, MA) PolarisQ Ion Trap system using a direct exposure probe. CI MS was performed using methane as the reagent gas. High resolution EI MS was carried out on a Kratos (Chestnut Ridge, NY) MS25 RFA instrument at 70 eV in magnetic scan with perfluorokerosene as standard. Low and high resolution electrospray ionization (ESI) MS was carried out using a Bruker (USA) Daltronics BioApexII with a 7T superconducting magnet and an analytical ESI source. Elemental analyses were performed at the Research School of Chemistry, Australian National University, Canberra, ACT, Australia. Thin layer chromatography was performed on Merck aluminum backed plates, precoated with silica (0.2 mm, $60F_{254}$), which were developed using one of the following techniques: UV fluorescence (254 nm), alkaline potassium permanganate solution (0.5% w/v), or ninhydrin (0.2% w/v). Flash chromatography was performed on silica gel (Merck silica gel 60H, particle size 5−40 μm). Melting points were determined using a Stuart (Stone, Staffordshire, UK) SMP10 melting point apparatus.

(±)-4-Hydroxycyclopent-2-en-1-one (7). **7** was synthesized via the method of An et al.[40] ^{1}H NMR ($CDCl_3$) δ: 7.63 (dd, 1H, J = 2.4, J = 5.6), 6.21 (dd, 1H, J = 1.3, J = 5.7), 4.25 (s, 1H), 5.02−5.05 (m, 1H), 2.76 (dd, 1H, J = 6.4, J = 18.5), 2.27 (dd, 1H, J = 2.1, J = 18.7). ^{13}C NMR ($CDCl_3$) δ: 207.8, 164.4, 134.5, 70.0, 44.1. MS (CI) m/z (%): 99.0 ([M + H]$^+$, 100), 81.0 (75), 71.1 (68), 53.0 (64).

(−)-(S)-4-Hydroxycyclopent-2-en-1-one (8). **8** was synthesized via the method of Khanapure et al.[44] $[\alpha]_D$ = −89.7 (c = 1.2, CH_3OH, lit.[44] = −90). NMR spectra identical to **7**. MS (CI) m/z (%): 99.0 ([M + H]$^+$, 100), 81.0 (80), 71.2 (72), 52.9 (75).

(+)-(R)-4-Hydroxycyclopent-2-en-1-one (9). **9** was synthesized via the method of Khanapure et al.[44] $[\alpha]_D$ = +88.31 (c = 1.1, CH_3OH, lit.[44] = +90). NMR spectra identical to **7**. MS (CI) m/z (%): 99.0 ([M + H]$^+$, 100).

(±)-4-(*tert*-Butyldimethylsiloxy)cyclopenten-1-one (10). To a stirred solution of **7** (1.7 g, 16.9 mmol), dimethylaminopyridine (DMAP, 206 mg, 10 mol%) in anhydrous CH_2Cl_2 (33 mL) at 0 °C under an atmosphere of N_2, a solution of TBDMS (3.1 g, 20.7 mmol) in anhydrous CH_2Cl_2 (11 mL) was added dropwise over 10 min. The solution was allowed to warm to room temp and stirred for 3 h, after which time deionized H_2O (100 mL) was added. The organic layer was separated, the aqueous phase extracted with CH_2Cl_2 (3 × 50 mL), and the combined organic fractions dried with anhydrous Mg_2SO_4 and concentrated in vacuo. The resulting oil was filtered through a short column of silica gel eluted with 10% ethyl acetate in hexane. The fractions containing the product were concentrated in vacuo and purified via vacuum distillation (bp 81 °C, 0.05 mm, lit.[78] 60 °C, 0.01 mm) to give **10** as a colorless oil that crystallized upon standing (3.05 g, 14.4 mmol, 85%, mp 26−28 °C lit.[79] 27−28 °C). ^{1}H NMR ($CDCl_3$) δ: 7.33 (dd, 1H, J = 2.3, J = 5.7), 6.06 (dd, 1H, J = 1.3, J = 5.6), 4.86 (m, 1H), 2.59 (dd, 1H, J = 5.9, J = 18.2), 2.12 (dd, 1H, J = 2.3, J = 18.2), 0.79 (s, 9H), 0.01 (s, 6H). ^{13}C NMR ($CDCl_3$) δ: 164.07, 135.70, 71.32, 60.65, 45.23, 25.89, 18.44, −4.43. MS (CI) m/z (%): 213.0 ([M + H]$^+$, 100), 197.1 (32).

(−)-(S)-4-*tert*-Butyldimethylsiloxy-2-cyclopenten-1-one (11). Method as for **10** (89%, mp 26−28 °C, lit.[78] 27−28 °C). $[\alpha]_D$ = −64.7 (c = 1.3, CH_3OH, lit.[78] = −65.3). NMR spectra identical to **10**. MS (CI) m/z (%): 212.9 ([M + H]$^+$, 100), 197.1 (34).

(+)-(R)-4-*tert*-Butyldimethylsiloxy-2-cyclopenten-1-one (12). Method as for **10** (87%, mp 26−28 °C, lit.[79] 27−28 °C). $[\alpha]_D$ = +64.1 (c = 0.5, CH_3OH, lit.[79] = +65.3). NMR spectra identical to **10**. MS (CI) m/z (%): 213.0 ([M + H]$^+$, 100), 197.1 (32).

(±)-4-*tert*-Butyldimethylsiloxy-1-cyclopenten-1-yl-trifluoromethanesulfonate (13). To a solution of L-selectride (1 M in THF, 4.7 mL, 4.7 mmol) in anhydrous THF (35 mL) at −78 °C under an atmosphere of N_2 was added dropwise a solution of **10** (1.0 g, 4.7 mmol) and Et_3N (0.2 mL) in THF (15 mL) over 30 min. After an additional 30 min, *N*-phenyltrifluoromethanesulfonimide (1.5 g, 4.1 mmol) was added in two portions. The resulting solution was gradually warmed to room temp overnight, the solvent removed in vacuo, and the residue partitioned between saturated $NaHCO_3$ solution (50 mL) and hexanes (100 mL) overnight. The aqueous layer was extracted with hexane (2 × 25 mL), and the combined organic fractions were washed with brine, dried with anhydrous Mg_2SO_4, and concentrated in vacuo. The residue was then purified via flash chromatography on silica gel using (hexane: ethyl acetate:Et_3N, 98:1:1) to give **13** as a colorless oil (1.59 g, 4.6 mmol, 98%). ^{1}H NMR ($CDCl_3$) δ: 5.58 (br t, 1H, J = 2.2), 4.60 (hept, 1H, J = 3.8), 2.86 (dd, 1H J = 16.3, J = 7.3), 2.73 (dd, 1H, J = 16.3, J = 7.3), 2.56 (br d, 1H, J = 16.3), 2.37 (br d, 1H, J = 16.3), 0.88 (s, 9H), 0.06 (s, 6H). ^{13}C NMR ($CDCl_3$): 146.66, 118.78 (q, J_{CF} = 320.8), 115.59, 69.81, 41.46, 39.29, 25.97, 18.26, −4.63. MS (CI) m/z (%): 346.8 ([M + H]$^+$, 34), 330.9 (52), 288.9 (54), 214.9 (100), 151.1 (22).

(+)-(R)-4-*tert*-Butyldimethylsiloxy-1-cyclopenten-1-yl-trifluoromethanesulfonate (14). Method as for **13** (78%). $[\alpha]_D$ = +1.97 (c = 1.01, CH_3OH, lit[47] = +1.69). NMR spectra identical to **13**. MS (CI) m/z (%): 346.7 ([M + H]$^+$, 33), 331.0 (40), 288.9 (40), 214.9 (100), 151.1 (21).

(−)-(S)-4-*tert*-Butyldimethylsiloxy-1-cyclopenten-1-yl-trifluoromethanesulfonate (15). Method as for **13** (89%). $[\alpha]_D$ = −1.92 (c = 1.3, CH_3OH, lit[47] = −1.69). NMR spectra identical to **13**. MS (CI) m/z (%): 346.7 ([M + H]$^+$, 34), 331.0 (41), 288.9 (40), 214.9 (100), 151.1 (21).

Ethyl (±)-4-(*tert*-butyldimethylsiloxy)cyclopent-1-enyl(methyl)phosphinate (16). To a solution of 1,4-diazabicyclo[2.2.2]octane (DABCO, 200 mg, 2.0 mmol), ethyl methylphosphinate (94 mg, 1.2 mmol) and **13** (200 mg, 0.58 mmol) in toluene (10 mL) was added $Pd(PPh_3)_4$ (17 mg, 2.5 mol%). The solution was stirred at room temp for 18 h, at which time a second portion of $Pd(PPh_3)_4$ (10 mg, 2.5 mol%) was added. The solution was stirred for a further 24 h, the toluene removed in vacuo, and the residue purified via flash chromatography on silica gel (ethyl acetate:ethanol, 90:10) to give **16** as a colorless oil (143 mg, 0.43 mmol, 78%). ^{1}H NMR ($CDCl_3$) δ: 6.62 (br dd, 1H, C(2)*H*, J = 10.8, J = 17.8), 4.63 (m, 1H, C(4)*H*), 4.10 (m, 2H, POCH_2CH$_3$), 2.72 (m, 2H, C(5)H_2), 2.45 (m, 2H, C(3)H_2), 1.49 (d, 3H, PCH_3, J_{PH} = 17.5), 1.34 (t, 3H, POCH$_2$CH_3, J = 7.1), 0.88 (s, 9H, SiC(CH_3)$_3$), 0.07 (s, 6H, Si(CH_3)$_2$). ^{13}C NMR ($CDCl_3$) δ: 145.44 and 144.83 (C(2), $^2J_{PCC}$ = 11.5, $^2J_{PCC}$ = 10.63) 134.28 (d, C(1), J_{PC} = 124.3), 72.99 and 72.94 (C(4), $^3J_{PCCC}$ = 11.6, $^3J_{PCCC}$ = 11.4), 61.77 (d, POCH$_2$CH$_3$, $^2J_{POC}$ = 6.3), 44.48 and 44.43 (C(5), $^2J_{PCC}$ = 16.3, $^2J_{PCC}$ = 15.7), 43.33 (d, C(3), $^3J_{PCCC}$ = 12.1), 25.92 (SiC(CH$_3$)$_3$), 18.25 (SiC CH$_3$)$_3$), 16.73 and 16.62 (POCH$_2C$H$_3$, $^3J_{POCC}$ = 6.2, $^3J_{POCC}$ = 6.7), 11.40 (d, PCH$_3$, J_{PC} = 144.4), −4.55 (Si (CH$_3$)$_2$). ^{31}P NMR ($CDCl_3$) δ: 38.5, 38.2. MS (CI) m/z (%): 305.1 ([M + H]$^+$, 100), 289.2 (30), 261.2 (14), 247.1 (24), 173.1 (20), 145.1 (20). HRMS (EI) m/z (%): calcd for $C_{14}H_{29}O_3PSi$ (M$^{+\cdot}$) 304.1624; found 304.1631.

Ethyl (+)-(R)-4-(*tert*-Butyldimethylsiloxy)cyclopent-1-enyl(methyl)phosphinate (17). Method as for **16**; colorless oil (79%). $[\alpha]_D$ = −5.40 (c = 1.17, CH_3OH). NMR spectra identical to **16**. MS (CI) m/z (%): 305.1 ([M + H]$^+$, 100), 289.1 (30), 261.1 (16), 247.0 (28), 195.1 (34), 173.0 (24), 145.1 (22).

Ethyl (+)-(S)-4-(*tert*-butyldimethylsiloxy)cyclopent-1-enyl(methyl)phosphinate (18). Method as for **16**; colorless oil (96%). $[\alpha]_D$ = +5.21 (c = 0.9, CH_3OH). NMR spectra identical to **16**. MS (CI) m/z (%): 305.0 ([M + H]$^+$, 100), 289.1 (28), 261.1 (12), 247.0 (22), 173.0 (22), 145.0 (22).

Ethyl (±)-4-(*tert*-Butyldimethylsiloxy)cyclopent-1-enyl(ethyl)phosphinate (19). Method as for **16**, using ethyl ethylphosphinate; colorless oil (76%). ^{1}H NMR ($CDCl_3$) δ: 6.65 (m, 1H, C(2)*H*), 4.62 (m, 1H, C(4)*H*), 4.11 (m, 2H, POCH_2CH$_3$), 2.74 (m, 2H, C(5)H_2), 2.48 (m, 2H, C(3)H_2), 1.76 (m, 2H, PCH_2CH$_3$), 1.23 (m, 6H, PCH$_2$CH_3 and POCH$_2$CH_3), 0.89 (s, 9H), 0.08 (s, 6H). ^{13}C NMR ($CDCl_3$) δ: 145.88 (apparent t, C(2), $^2J_{PCC}$ = 10.7), 133.07 (d, C(1), J_{PC} = 123.6), 73.05 and 73.04 (C(4), $^3J_{PCCC}$ = 10.9, $^3J_{PCCC}$ = 10.9), 61.71 (d, POCH$_2$CH$_3$, $^2J_{POC}$ = 6.5), 44.55 and 44.47 (C(5), $^2J_{PCC}$ = 15.3), 43.83 and 43.67 (C(5), $^3J_{PCCC}$ = 9.2, $^3J_{PCCC}$ = 8.9), 25.91 (SiC(CH$_3$)$_3$), 21.47 (d, PCH$_2$CH$_3$, J_{PC} = 102.6), 16.71 (t, POCH$_2C$H$_3$, J = 6.1), 6.79 (d, PCH$_2C$H$_3$, J = 6.8). ^{31}P NMR ($CDCl_3$) δ: 43.1, 42.9. MS (CI) m/z (%): 319.1 ([M + H]$^+$, 100), 303.2 (26), 261.1 (24), 187.1 (20), 159.1 (17). HRMS (EI): calcd for $C_{15}H_{31}O_3PSi$ (M$^{+\cdot}$) 318.1780; found 318.1789.

Ethyl (±)-4-(*tert*-Butyldimethylsiloxy)cyclopent-1-enyl(isopropyl)phosphinate (20). Method as for **16**, using ethyl isopropylphosphinate; colorless needles (mp 28−31 °C, 90%). ^{1}H NMR (CD_3OD) δ: 6.71 (bd, 1H, C(2)*H*, J = 9.9), 4.69 (m, 1H, C(4)*H*), 4.08 (m, 2H, POCH_2CH$_3$), 2.81 (m, 2H, C(5)H_2), 2.48 (m, 2H, C(3)H$_2$), 2.01 (m, 1H, PCH(CH$_3$)$_2$), 1.34 (t, 3H, POCH$_2$CH_3, J = 7.1), 1.20 (dd, 6H, PCH(CH_3)$_2$, J = 7.2, J = 18.7), 0.92 (d, 9H, SiC(CH_3)$_3$, J = 0.64), 0.12 (s, 6H, Si(CH$_3$)$_2$). ^{13}C NMR (CD_3OD) δ: 147.57 (apparent t, C(2), J = 9.6), 131.55 and 135.41 (C(1), J_{PC} = 121.0, J_{PC} = 121.1), 72.88 (d, C(4), $^3J_{PCCC}$ = 10.8), 60.91 and 60.87 (POCH_2CH$_3$, $^2J_{POC}$ = 7.0, $^2J_{POC}$ = 7.0), 44.41 and 44.34 (C(5), $^2J_{PCC}$ = 15.2, $^2J_{PCC}$ = 15.3), 44.04 and 43.90 (C(3), $^3J_{PCCC}$ = 11.3, $^3J_{PCCC}$ = 11.1), 26.81 and 26.64 (PCH (CH$_3$)$_2$, J_{PC} = 103.2, J_{PC} = 102.9), 25.11 (SiC(CH$_3$)$_2$), 17.67 (SiC(CH$_3$)$_2$), 15.68 (d, POCH$_2C$H$_3$, $^3J_{POCC}$ = 6.0), 13.77 and 13.72 (d, PCH (CH$_3$)$_2$, $^2J_{PCC}$ = 6.1, $^2J_{PCC}$ = 6.2), −5.82 (Si (CH$_3$)$_2$). ^{31}P NMR (CD_3OD) δ: 49.0, 48.9. MS (CI) m/z (%): 333.1 ([M + H]$^+$, 100), 317.2 (20),

275.1 (24), 201.0 (14). HRMS (EI): calcd for $C_{16}H_{33}O_3PSi$ ([M − $C(CH_3)_3]^{+\cdot}$) 275.1232; found 275.1228.

Ethyl (±)-4-(*tert*-Butyldimethylsiloxy)cyclopent-1-enyl(butyl)phosphinate (21). Method as for **16**, using ethyl butylphosphinate; colorless needles (94%, mp 31−34 °C). ^{1}H NMR (CD_3OD) δ: 6.69 (bd, 1H, C(2)*H*, J = 10.7), 4.69 (m, 1H, C(4)*H*), 4.06 (m, 2H, POCH_2CH$_3$), 2.82 (m, 2H, C(5)H_2), 2.47 (m, 2H, C(3)H_2), 1.82 (m, 2H, PCH_2(CH$_2$)$_2$CH$_3$), 1.54 (m, 4H, PCH$_2$(CH_2)$_2$CH$_3$), 1.36 (t, 3H, POCH$_2$CH_3, J = 3.0), 1.29 (t, 3H, P(CH$_2$)$_3$CH_3, J = 6.7), 0.92 (d, 9H, SiC(CH_3)$_3$, J = 1.2), 0.12 (s, 6H, Si(CH_3)$_2$). ^{13}C NMR (CD_3OD) δ: 146.60 (apparent t, *C*(2), $^2J_{PCC}$ = 11.8), 132.54 (d, *C*(1), J_{PC} = 124.8), 72.82 (d, *C*(4), $^3J_{PCCC}$ = 10.7), 60.79 and 60.74 (POCH_2CH$_3$, $^2J_{POC}$ = 6.5, $^2J_{POC}$ = 6.6), 44.46 and 44.41 (*C*(5), $^2J_{PCC}$ = 15.8, $^2J_{PCC}$ = 15.8), 43.52 and 43.49 (*C*(3), $^3J_{PCCC}$ = 12.1, $^3J_{PCCC}$ = 11.8), 27.17 and 26.92 (P*C*H$_2$(CH$_2$)$_2$CH$_3$, J_{PC} = 101.4, J_{PC} = 100.8), 25.13 (SiC(*C*H$_3$)$_2$), 23.59 (d, P(CH$_2$)$_2$*C*H$_2$CH$_3$, $^3J_{PCCC}$ = 23.9), 23.52 (d, PCH$_2$*C*H$_2$CH$_2$CH$_3$, $^2J_{PCC}$ = 30.3), 17.68 (s, Si*C*(CH$_3$)$_2$), 15.67 and 15.59 (POCH$_2$*C*H$_3$, $^3J_{POCC}$ = 9.0, $^3J_{POCC}$ = 8.7), 12.76 (P(CH$_2$)$_3$*C*H$_3$), −5.84 (Si(*C*H$_3$)$_2$). ^{31}P NMR ($CDCl_3$) δ: 41.6, 41.4. MS (CI) *m*/*z* (%): 347.1 ([M + H]$^+$, 100), 331.3 (21), 289.2 (18). HRMS (EI): calcd for $C_{17}H_{35}O_3PSi$ ($M^{+\cdot}$) 346.2093; found 346.2096.

Ethyl (−)-(*R*)-4-(*tert*-Butyldimethylsiloxy)cyclopent-1-enyl(butyl)phosphinate (22). Method as for **21**; colorless needles (99%, mp 30−32 °C). $[\alpha]_D$ = −2.38 (c = 1.0, MeOH).NMR spectra identical to **21**. MS (CI) *m*/*z* (%): 347.1 ([M + H]$^+$, 100), 331.2 (25), 289.1 (24).

Ethyl (+)-(*S*)-4-(*tert*-Butyldimethylsiloxy)cyclopent-1-enyl(butyl)phosphinate (23). Method as for **21**; colorless needles (92%, mp 30−32 °C). $[\alpha]_D$ = +2.22 (c = 2.5, CH_2Cl_2). NMR spectra identical to **21**. MS (CI) *m*/*z* (%): 347.1 ([M + H]$^+$, 100), 331.2 (22), 289.1 (24), 215.1 (12).

Ethyl (±)-4-(*tert*-Butyldimethylsiloxy)cyclopent-1-enyl(benzyl)phosphinate (24). Method as for **16**, using ethyl benzylphosphinate; colorless oil. ^{1}H NMR ($CDCl_3$) δ: 7.12−7.51 (m, 5H, PCH$_2$(C$_6$*H*$_5$)), 6.55 (bd, 1H, C(2)*H*, J_{PH} = 18.0), 4.65 (m, 1H, C(4)*H*), 3.95 (m, 2H, POCH_2CH$_3$), 3.23 (dd, 2H, PCH_2(C$_6$H$_5$), J = 3.2, J_{PH} = 18.0), 2.76 (m, 2H, C(5)H_2), 2.45 (m, 2H, C(3)H_2), 1.29 (t, 3H, POCH$_2$CH_3, J = 7.0), 0.90 (s, 9H, SiC(CH_3)$_3$), 0.09 (s, Si(CH_3)$_2$, 6H). ^{13}C NMR ($CDCl_3$) δ: 146.00 (apparent t, *C*(2), $^2J_{PCC}$ = 9.9), 132.37 (d, benzene *C*(1), $^2J_{PCC}$ = 10.9), 131.04 (d, *C*(1), J_{PC} = 126.96), 130.02 (d, benzene *ortho*, $^3J_{PCCC}$ = 6.6), 128.76 (d, benzene *meta*, $^4J_{PCCCC}$ = 3.1), 127.1 (d, benzene *para*, $^5J_{PCCCCC}$ = 3.7), 72.17 (d, *C*(4), $^3J_{PCCC}$ = 10.5), 62.21 and 62.15 (POCH$_2$CH$_3$, $^2J_{POC}$ = 8.2, $^2J_{POC}$ = 8.5), 44.37 and 44.33 (*C*(5), $^2J_{PCC}$ = 16.3, $^2J_{PCC}$ = 16.2), 43.85 (d, *C*(3), $^3J_{PCCC}$ = 11.0), 34.01 (d, PCH$_2$(C$_6$H$_5$), J_{PC} = 138.2), 25.92 (SiC(*C*H$_3$)$_3$), −3.32 (Si(*C*H$_3$)$_2$. ^{31}P NMR ($CDCl_3$) δ: 37.41. MS (CI) *m*/*z* (%): 281.1 ([M + H]$^+$, 100), 265.2 (14), 323.1 (22), 249.1 (12), 221.1 (16). HRMS (EI): calcd for $C_{20}H_{33}O_3PSi$ ($M^{+\cdot}$) 380.1937; found 380.1942.

Ethyl (±)-4-Hydroxy-1-cyclopent-1-enyl(methyl)phosphinate (25). To a solution of **16** (350 mg, 1.2 mmol) under an atmosphere of N_2 was added TBAF (1 M in THF, 1.4 mL). The solution was stirred at room temp for 12 h and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (ethyl acetate:ethanol, 85:15) to give **25** as a light-yellow oil (208 mg, 1.1 mmol, 98%). ^{1}H NMR ($CDCl_3$) δ: 6.62 (bd, 1H, C(2)*H*, J_{PH} = 10.6), 4.65 (m, C(4)*H*, 1H), 4.02 (m, POCH_2CH$_3$, 2H), 3.04 (m, 1H), 2.82 (d, 1H, C(5)H_2, J = 16.5), 2.55 (d, C(3)H_2, 1H, J = 18.0), 1.54 (dd, 3H, PCH_3, J = 4.3, J = 14.5), 1.33 (dt, POCH$_2$CH_3, 3H, J = 2.0, J = 7.1). ^{13}C NMR ($CDCl_3$) δ: 145.63 and 145.51 (*C*(2), $^2J_{PCC}$ = 11.4, $^2J_{PCC}$ = 11.8), 133.50 (d, *C*(1), J_{PC} = 131.6), 71.27 (d, *C*(4), $^3J_{PCCC}$ = 11.2), 60.87 and 60.85 (dd, POCH_2CH$_3$, $^2J_{POC}$ = 6.3, $^2J_{POC}$ = 6.3), 43.65 (d, *C*(5), $^2J_{PCC}$ = 16.4), 42.53 and 42.45 (*C*(3), $^3J_{PCCC}$ = 12.3, $^3J_{PCCC}$ = 12.1), 15.64 (d, POCH$_2$CH_3, $^3J_{POCC}$ = 6.6), 12.74 and 12.57 (P*C*H$_3$, J_{PC} = 102.5, J = 102.2). ^{31}P NMR (CD_3OD) δ: 42.9, 42.7. MS (EI) *m*/*z* (%): 190.1 ($M^{+\cdot}$, 9), 175.1 (14), 161.1 (96), 147.1 (41), 133.1 (100), 55.1 (37). HRMS (EI): calcd for $C_8H_{15}O_3P$ ($M^{+\cdot}$) 190.0759; found 190.0762.

Ethyl (−)-(*R*)-4-Hydroxy-1-cyclopent-1-enyl(methyl)phosphinate (26). Method as for **25**; light-yellow oil (91%). $[\alpha]_D$ = −8.9 (c = 0.53, CH_3OH). NMR spectra identical to **25**. MS (EI) *m*/*z* (%): 190.1 ($M^{+\cdot}$, 100).

Ethyl (+)-(*S*)-4-Hydroxy-1-cyclopent-1-enyl(methyl)phosphinate (27). Method as for **25**; light-yellow oil (89%). $[\alpha]_D$ = +8.7 (c = 0.72, MeOH). NMR spectra identical to **25**. MS (EI) *m*/*z* (%): 190.1 ($M^{+\cdot}$, 100).

Ethyl (±)-4-Hydroxy-1-cyclopent-1-enyl(ethyl)phosphinate (38). Method as for **25**; light-yellow oil (quant). ^{1}H NMR ($CDCl_3$) δ: 6.62 (bd, 1H, C(2)*H*, J = 10.0), 4.64 (t, 1H, C(4)*H*, J = 5.5), 4.02 (m, POCH_2CH$_3$, 2H), 2.86 (s, 1H, O*H*), 2.78 (m, C(5)H_2, 2H), 2.53 (m, C(3)H_2, 2H), 1.78 (m, PCH_2CH$_3$, 2H), 1.32 (dt, 3H, POCH_2CH$_3$ J = 2.0, J = 7.1), 1.13 (ddd, 3H, PCH$_2$CH_3, J = 2.2, J = 7.7, J = 18.6). ^{13}C NMR ($CDCl_3$) δ: 145.55 and 145.46 (*C*(2), $^2J_{PCC}$ = 10.3, $^2J_{PCC}$ = 10.2), 133.32 (d, *C*(1), J_{PC} = 124.2), 72.00 (d, *C*(4), $^3J_{PCCC}$ = 10.4), 60.64 and 60.61 (POCH_2CH$_3$, $^2J_{POC}$ = 6.4, $^2J_{POC}$ = 6.4), 44.39 (d, *C*(5), $^2J_{PCC}$ = 15.6), 43.62 and 43.57 (*C*(3), $^3J_{PCCC}$ = 11.6, $^3J_{PCCC}$ = 11.7), 21.40 and 21.37 (P*C*H$_2$CH$_3$, J_{PC} = 101.7, J_{PC} = 101.4), 16.78 (d, POCH$_2$*C*H$_3$, $^3J_{POCC}$ = 6.3), 5.85 (d, PCH$_2$*C*H$_3$, $^2J_{PCC}$ = 4.8). ^{31}P NMR ($CDCl_3$) δ: 41.6, 41.8. MS (EI) *m*/*z* (%): 204.1 ($M^{+\cdot}$, 4), 187.1 (16), 175.1(60), 147.1(100), 65.1 (38). HRMS (EI): calcd for $C_9H_{17}O_3P$ ($M^{+\cdot}$) 204.0915; found 204.0913.

Ethyl (±)-4-hydroxy-1-cyclopent-1-enyl(isopropyl)phosphinate (29). Method as for **25**; light-yellow oil (98%). ^{1}H NMR ($CDCl_3$) δ: 6.64 (bd, 1H, C(2)*H*, J = 9.2), 4.65 (m, C(4)*H*, 1H), 4.05 (m, POCH_2CH$_3$, 2H), 2.80 (m, C(5)H_2, 2H), 2.60 (s, 1H), 2.53 (m, C(3)H_2, 2H), 1.93 (m, PC*H*(CH$_3$)$_2$, 1H), 1.33 (dt, 3H, POCH$_2$CH_3, J = 1.9, J = 7.0), 1.21 (d, 3H, PCH(CH_3)$_2$, J = 7.2), 1.16 (d, 3H, PCH(CH_3)$_2$, J = 7.1). ^{13}C NMR ($CDCl_3$) δ: 146.08 (apparent t, *C*(2), $^2J_{PCC}$ = 9.4), 132.72 (d, *C*(1), J_{PC} = 119.9), 72.21 (d, *C*(4), $^3J_{PCCC}$ = 9.9), 60.73 and 60.71 (POCH_2CH$_3$, $^2J_{POC}$ = 6.8, J = 6.8), 44.37 (d, *C*(5), $^2J_{PCC}$ = 15.2), 44.23 and 44.14 (*C*(3), $^3J_{PCCC}$ = 11.0, J = 11.1), 27.46 (d, PO*C*H(CH$_3$)$_2$, J_{PC} = 102.3), 16.80 (d, POCH$_2$*C*H$_3$, $^3J_{POCC}$ = 6.3), 15.40 and 15.37 ($^2J_{PCC}$ = 25.7, $^2J_{PCC}$ = 28.0). ^{31}P NMR ($CDCl_3$) δ: 46.1, 45.8. MS (EI) *m*/*z* (%): 218.1 ($M^{+\cdot}$, 6), 203.1 (14), 201.1 (32), 189.1 (75), 175.1 (48), 161.1 (78), 147.1 (100), 129.0 (74), 101.1 (56), 65.1 (90). HRMS (EI): calcd for $C_{10}H_{19}O_3P$ ($M^{+\cdot}$) 218.1072; found 218.1076.

Ethyl (±)-4-Hydroxy-1-cyclopent-1-enyl(butyl)phosphinate (30). Method as for **25**; light-yellow oil (99%). ^{1}H NMR ($CDCl_3$) δ :6.62 (bd, 1H, C(2)*H*, J = 10.1), 4.64 (m, C(4)*H*, 1H), 4.08 (m, POCH_2CH$_3$, 2H), 2.81 (m, C(5)H_2, 2H), 2.53 (m, C(5)H_2, 2H), 1.75 (m, PCH_2(CH$_2$)$_2$CH$_3$, 2H), 1.56 (m, PCH$_2$CH_2CH$_2$CH$_3$, 2H), 1.41 (hept, 2H, P(CH$_2$)$_2$CH_2CH$_3$, J = 7.3), 1.32 (dt, 3H, POCH$_2$CH_3, J = 2.0, J = 7.0), 0.92 (t, 1H, P(CH$_2$)$_3$CH_3, J = 7.2). ^{13}C NMR ($CDCl_3$) δ: 145.31 and 145.12 (*C*(2), $^2J_{PCC}$ = 10.7, $^2J_{PCC}$ = 10.8), 133.76 (d, *C*(1), J_{PC} = 125.6), 72.01 (d, *C*(4), $^3J_{PCCC}$ = 10.5), 60.56 (d, POCH_2CH$_3$, $^2J_{POC}$ = 6.3), 44.38 (d, *C*(5), $^2J_{PCC}$ = 15.6), 43.61 and 43.55 (*C*(3), $^3J_{PCCC}$ = 11.6, $^3J_{PCCC}$ = 11.7), 28.14 (d, P*C*H$_2$(CH$_2$)$_2$CH$_3$, J_{PC} = 100.3), 23.99 (d, PCH$_2$*C*H$_2$CH$_2$CH$_3$, $^2J_{PCC}$ = 16.3), 23.86 (d, P(CH$_2$)$_2$*C*H$_2$CH$_3$, $^3J_{PCCC}$ = 3.7), 16.79 (d, POCH$_2$CH_3, $^2J_{POC}$ = 6.3), 13.83 (P(CH$_2$)$_3$*C*H$_3$). ^{31}P NMR ($CDCl_3$) δ: 42.9, 41.9. MS (EI) *m*/*z* (%): 232.1 ($M^{+\cdot}$, 5), 217.1 (79), 203.1 (50), 175.1 (92), 146.1 (100), 65.1 (60). HRMS (EI): calcd for $C_{11}H_{21}O_3P$ ($M^{+\cdot}$) 232.1228; found 232.1233.

Ethyl (−)-(*R*)-4-Hydroxy-1-cyclopent-1-enyl(butyl)phosphinate (31). Method as for **25**; light-yellow oil (92%). $[\alpha]_D$ = −7.0 (c = 0.52, CH_3OH). NMR spectra identical to **30**.

Ethyl (+)-(*S*)-4-Hydroxy-1-cyclopent-1-enyl(butyl)phosphinate (32). Method as for **25**; light-yellow oil (95%). $[\alpha]_D$ = +6.9 (c = 0.71, CH_3OH). NMR spectra identical to **30**.

Ethyl (±)-4-Hydroxy-1-cyclopent-1-enyl(benzyl)phosphinate (33). Method as for **25**; light-yellow oil (79%). ^{1}H NMR ($CDCl_3$) δ: 7.38−7.22 (m, 5H, PCH$_2$(C$_6$*H*$_5$)), 6.53 (bd, 1H, C(2)*H*, J = 10.3), 4.56−4.49 (m, C(4)*H*, 1H), 4.17−3.92 (m, POCH_2CH$_3$, 2H), 3.22 (dd, 2H, PCH_2(C$_6$H$_5$), J = 3.0, J = 17.9), 2.90−2.28 (m, C(5)H_2 and C(3)H_2, 4H), 1.31 (dt, 3H, POCH$_2$CH_3, J = 1.1, J = 7.0). ^{13}C NMR ($CDCl_3$) δ: 146.40 and 146.37 (*C*(2), $^2J_{PCC}$ = 10.7, $^2J_{PCC}$ = 10.3), 133.01 (d, *C*(1), J_{PC} = 123.3), 131.53 (d, benzene *C*(1), $^2J_{PCC}$

= 7.4), 130.16 and 130.13 (d, benzene *ortho*, $^3J_{PCCC}$ = 5.8, $^3J_{PCCC}$ = 5.9), 129.79 and 129.78 (d, benzene *meta*, $^4J_{PCCCC}$ = 2.7, $^4J_{PCCCC}$ = 2.7), 127.20 (apparent t, benzene *para*, $^5J_{PCCCCC}$ = 2.8), 72.08 (d, *C*(4), $^3J_{PCC}$ = 10.6), 61.26 and 61.25 (PO*C*H$_2$, $^2J_{POC}$ = 6.5, $^2J_{POC}$ = 6.5), 44.37 and 44.34 (*C*(5), $^2J_{PCC}$ = 16.1, $^2J_{PCC}$ = 16.0), 43.78 (d, *C*(3) $^3J_{PCCC}$ = 11.2), 37.06 and 37.03 (P*C*H$_2$(C$_6$H$_5$), J_{PC} = 95.6, $^2J_{PC}$ = 95.4), 16.75 (d, POCH$_2$*C*H$_3$, $^2J_{POC}$ = 6.2). ^{31}P NMR (CDCl$_3$): 37.5, 37.4. MS (EI) *m*/*z* (%): 266.0 (M$^{+\cdot}$), 222.0 (59), 191.0 (22), 147.0 (97), 129.0 (66), 101.0 (47), 91.1 (70), 65.1 (100). HRMS (EI): calcd for $C_{14}H_{19}O_3P$ (M$^{+\cdot}$) 266.1072; found 266.1074.

(±)-4-Amino-1-cyclopent-1-enyl(methyl)phosphinic acid (34). To a solution of **25** (150 mg, 0.85 mmol), diisopropylazodicarboxylate (DIAD, 0.7 mL, 1.9 mmol) and HN$_3$ (1.9 M in benzene, 1.1 mL, 1.7 mmol) in anhydrous THF (10 mL) at 0 °C under an atmosphere of N$_2$ was added PPh$_3$ (0.89 g, 3.4 mmol) in small portions over 1 h. The reaction mixture was allowed to warm to room temp and stirred for 12 h. The reaction mixture was then heated to 50 °C for 3 h, at which time water (0.2 mL) was added and heating continued for a further 2 h. The mixture was the cooled to room temp and the solvent removed in vacuo. The residue was partitioned between HCl (1 M, 10 mL) and CH$_2$Cl$_2$ (10 mL). The organic layer was separated and extracted with aqueous HCl (2 × 10 mL). The combined aqueous layers were washed with CH$_2$Cl$_2$ (2 × 10 mL) and concentrated in vacuo. The crude amino ester hydrochloride salt was hydrolyzed by refluxing in aqueous HCl (6 M, 15 mL) for 30 h, after which time the mixture was cooled to room temp and the solvent removed in vacuo. The residue was purified by ion exchange chromatography (Dowex 50, H$^+$), eluting first with water until the eluent was neutral and colorless, followed by pyridine (1 M). Fractions containing the product were collected and concentrated in vacuo to give **34** as a white solid (80 mg, 0.54 mmol, 64%, mp (dec.) 202−205 °C). ^{1}H NMR (D$_2$O) δ: 7.51 (m, 2H, N*H*$_2$), 6.11 (bd, 1H, C(2)*H* *J* = 9.8), 3.96 (m, C(4)*H* 1H), 2.86 (m, 2H, C(5)*H*$_2$), 2.46 (m, C(3)*H*$_2$, 2H), 1.22 (d, 3H, PC*H*$_3$, *J* = 14.1). ^{13}C NMR (D$_2$O) δ: 139.85 (d, *C*(2), $^2J_{PCC}$ = 11.5), 136.85 (d, *C*(1), J_{PC} = 127.1), 50.81 (d, *C*(4), $^3J_{PCCC}$ = 10.3), 39.09 (d, *C*(5), $^2J_{PCC}$ = 15.4), 37.99 (d, *C*(3), $^3J_{PCCC}$ = 13.5), 14.85 (d, P*C*H$_3$, J_{PC} = 101.5). ^{31}P NMR (D$_2$O) δ: 33.7. MS (EI) *m*/*z* (%): 161.1 (M$^{+\cdot}$, 5), 144.0 (100), 80.1 (62), 66.1 (32). HRMS (EI): calcd for $C_6H_{12}NO_2P$ (M$^{+\cdot}$) 161.0606; found 161.0610. Anal. ($C_6H_{12}NO_2P\cdot 0.33H_2O$) C, H, N.

(−)-(*R*)-4-Amino-1-cyclopent-1-enyl(methyl)phosphinic acid (35). Method as for **34**; white crystalline solid (77%, mp (dec.) 202 − 205 °C). $[\alpha]_D$ = −9.7 (*c* = 0.2, H$_2$O). NMR spectra identical to **34**. Anal. ($C_9H_{18}NO_2P\cdot 0.33H_2O$)C, H, N.

(+)-(*S*)-4-Amino-1-cyclopent-1-enyl(methyl)phosphinic acid (36). Method as for **34**; white crystalline solid (93%, mp (dec.) 203 − 206 °C). $[\alpha]_D$ = +10.1 (*c* = 0.20, H$_2$O). NMR spectra identical to **34**. Anal. ($C_9H_{18}NO_2P\cdot 0.33H_2O$) C, H, N.

(±)-4-Amino-1-cyclopent-1-enyl(ethyl)phosphinic acid (37). Method as for **34**. White solid (58%, mp (dec.) 201−203 °C). ^{1}H NMR (D$_2$O) δ: 7.50 (m, 2H, N*H*$_2$), 6.12 (bd, 1H, C(2)*H*, *J* = 9.2), 3.95 (m, 1H, C(4)*H*), 2.85 (bdd, 2H, C(5)*H*$_2$, *J* = 7.5, *J* = 16.7), 2.46 (dm, 2H, C(3)*H*$_2$, *J* = 15.8), 1.46 (dq, 2H, PC*H*$_2$CH$_3$, *J* = 15.3, *J* = 7.7), 0.88 (dt, 3H, PCH$_2$C*H*$_3$, *J* = 17.9, *J* = 7.7). ^{13}C NMR (D$_2$O) δ: 138.12 (d, *C*(1), J_{PC} = 123.3), 137.91 (d, *C*(2), $^2J_{PCC}$ = 10.3), 50.93 (d, *C*(4), $^3J_{PCCC}$ = 9.8), 38.93 (d, *C*(3), $^2J_{PCC}$ = 14.8), 38.55 (d, *C*(5), $^3J_{PCCC}$ = 13.0), 22.71 (d, P*C*H$_2$CH$_3$, J_{PC} = 99.67), 5.94 (d, PCH$_2$*C*H$_3$, $^2J_{PCC}$ = 4.9). ^{31}P NMR (D$_2$O) δ: 32.6; MS (EI) *m*/*z* (%): 175.1 (M$^{+\cdot}$, 2), 158.0 (100), 130.1 (43), 80.1 (71), 66.1 (44). HRMS (EI): calcd for $C_7H_{14}NO_2P$ (M$^{+\cdot}$) 175.0762; found 175.0767. Anal. ($C_7H_{14}NO_2P\cdot 5H_2O$) C, H, N.

(±)-4-Amino-1-cyclopent-1-enyl(isopropyl)phosphinic acid (38). Method as for **34**; white solid (67%, mp (dec.) 190−193 °C). ^{1}H NMR (D$_2$O) δ: 6.17 (bd, 1H, C(2)*H*, *J* = 9.1), 3.95 (m, C(4)*H* 1H), 2.86 (dd, 2H, C(5)*H*$_2$, *J* = 7.5, *J* = 16.8), 2.47 (d, 2H, C(3)*H*$_2$, *J* = 16.2), 1.63 (m, 1H, CH(CH$_3$)$_2$), 0.94 (t, 3H, PCHC*H*$_3$, *J* = 7.1), 0.88 (t, 3H, PCHC*H*$_3$, *J* = 7.2). ^{13}C NMR (D$_2$O) δ: 138.87 (d, *C*(2), $^2J_{PCC}$ = 9.3), 137.56 (d, *C*(1), J_{PC} = 120.0), 50.98 (d, *C*(4), $^3J_{PCCC}$ = 9.4), 38.98 (apparent t, *C*(3) and *C*(5), $^3J_{PCCC}$ = 13.8), 27.84 (d, P*C*H (CH$_3$)$_2$, J_{PC} = 101.1), 15.19 (dd, PCH (*C*H$_3$)$_2$, *J* = 2.3, *J* = 27.0). ^{31}P NMR (D$_2$O) δ: 35.7. MS (EI) *m*/*z* (%): 190.1 (M$^{+\cdot}$, 5), 172.0 (100), 130.0 (65), 80.1 (60), 68.1 (45), 66.1 (40). HRMS (EI): calcd for $C_8H_{16}NO_2P$ (M$^{+\cdot}$) 189.0919; found 189.0920. Anal. ($C_8H_{16}NO_2P\cdot 0.2H_2O$) C, H, N.

(±)-4-Amino-1-cyclopent-1-enyl(butyl)phosphinic acid (39). Method as for **34**; white solid (65%, mp (dec.) 192−195 °C). ^{1}H NMR (D$_2$O) δ: 7.54 (m, N*H*$_2$, 2H), 6.12 (bd, 1H, C(2)*H*, *J* = 9.2), 3.95 (m, C(4)*H*, 1H) 2.85 (dd, 2H, C(5)H$_2$, *J* = 7.7, *J* = 16.1), 2.46 (dd, 2H, C(3)H$_2$, *J* = 3.4, *J* = 18.2), 1.47 (m, PC*H*$_2$(CH$_2$)$_2$CH$_3$, 2H), 1.27 (m, PCH$_2$(C*H*$_2$)$_2$CH$_3$, 4H), 0.76 (t, 1H, P(CH$_2$)$_3$C*H*$_3$, *J* = 7.1). ^{13}C NMR (D$_2$O) δ: 137.68 (d, *C*(2), ^{2}J$_{PCC}$ = 10.2), 132.23 (d, *C*(1), J_{PC} = 121.8), 50.94 (d, *C*(4), *J* = 10.1), 38.90 (d, *C*(3), $^3J_{PCCC}$ = 14.8), 38.53 (d, *C*(5), $^2J_{PCC}$ = 12.9), 29.61 (d, P*C*H$_2$(CH$_2$)$_2$CH$_3$, *J* = 98.7), 24.24 (d, P(CH$_2$)$_2$*C*H$_2$CH$_3$, $^3J_{PCCC}$ = 3.6), 23.74 (d, PCH$_2$*C*H$_2$CH$_2$CH$_3$, $^2J_{PCC}$ = 16.1), 13.15 (s, P(CH$_2$)$_3$CH$_3$). ^{31}P NMR (D$_2$O) δ: 31.4; MS (EI) *m*/*z* (%): 204.1 (M$^{+\cdot}$, 10), 186.1(47), 80.1 (100), 68.1 (52). HRMS (EI): calcd for $C_9H_{18}NO_2P$ (M$^{+\cdot}$) 203.1075; found 203.1069. Anal. ($C_9H_{18}NO_2P\cdot 0.33H_2O$) C, H, N.

(−)-(*R*)-4-Amino-1-cyclopent-1-enyl(butyl)phosphinic acid (40). Method as for **34**; white crystalline solid (75%, mp (dec.) 193−196 °C). $[\alpha]_D$ = −7.1 (*c* = 0.5, H$_2$O). NMR spectra identical to **39**. Anal. ($C_9H_{18}NO_2P\cdot 0.5H_2O$) C, H, N.

(+)-(*S*)-4-Amino-1-cyclopent-1-enyl(butyl)phosphinic acid (41). Method as for **39**; white crystalline solid (33%, mp (dec.) 192−195 °C). $[\alpha]_D$ = +7.3 (*c* = 0.4, H$_2$O). NMR spectra identical to **39**. Anal. ($C_9H_{18}NO_2P\cdot 0.33H_2O$) C, H, N.

(±)-4-Amino-1-cyclopent-1-enyl(benzyl)phosphinic acid (42). Method as for **34**; White solid (67%, mp (dec.) 216−219 °C). ^{1}H NMR (D$_2$O) δ: 7.26−7.10 (m, 5H, PCH$_2$(C$_6$*H*$_5$)), 6.05 (bd, 1H, C(2)*H* *J* = 9.7), 3.88 (m, C(4)*H*, 1H), 2.92 (d, 2H, PC*H*$_2$(C$_5$H$_6$), *J* = 17.2), 2.84−2.62 (m, C(5)*H*$_2$ 2H), 2.47−2.29 (m, C(3)*H*$_2$, 2H). ^{13}C NMR (D$_2$O) δ: 139.38 (d, *C*(2), $^2J_{PCC}$ = 10.0), 138.07 (d, *C*(1), J_{PC} = 127.5), 135.50 (d, *C*(1) of benzene ring, $^2J_{PCC}$ = 7.1) 129.86 (d, benzene *ortho*, $^3J_{PCCC}$ = 5.3), 128.67 (d, benzene *meta*, $^4J_{PCCCC}$ = 2.9), 126.40 (d, benzene *para*, $^5J_{PCCCCC}$ = 3.3), 50.94 (d, *C*(4), $^3J_{PCCC}$ = 10.0), 38.88 (d, *C*(3), *J* = 12.8), 38.82 (d, *C*(5), *J* = 15.29), 38.63 (d, P*C*H$_2$(C$_6$H$_5$), *J* = 91.6). ^{31}P NMR (D$_2$O) δ: 26.4. MS (CI) *m*/*z* (%): 240.1 ([M + H]$^+$, 100). HRMS (ESI) calcd for $C_{12}H_{16}NNaO_2P$ ([M + Na]$^+$) 260.08163; found 260.07998. Anal. ($C_{11}H_{14}NO_2P\cdot 0.5H_2O$) C, H, N.

Methyl((*S*)-4-((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamido)cyclopent-1-enyl)phosphinic acid (43). To a suspension of **34** (4.0 mg, 24.8 μmol) in CH$_2$Cl$_2$ (1 mL) was added bis(trimethylsilyl)trifluoroacetamide (BSTFA, 19.2 mg, 74.5 μmol) and the resulting solution stirred for 18 h. Further BSTFA was added as required until the solution was clear. (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid chloride (8.0 mg, 29.8 mmol) was added to the solution and the resulting solution stirred for 40 h. Water (2 mL) was then added and the solution stirred for 30 min. The aqueous layer was then separated, washed with diethyl ether/CH$_2$Cl$_2$ (2:1), and concentrated in vacuo. The resulting solid was used without further purification for ^{1}H analysis of diastereomeric purity. ^{1}H NMR (CD$_3$OD) δ: 7.54−7.45 (m, 2H, benzene *ortho*), 7.41−7.33 (m, 3H, benzene *para* and *meta*), 6.45 (bd, *J* = 10.96, 1H, C(2)*H*), 4.69−4.55 (m, 1H, C(4)*H*), 3.59 (s, 3H, OC*H*$_3$), 2.94−2.77 (m, 2H, C(3)*H*$_2$), 2.57−2.35 (m, 2H, C(5)*H*$_2$), 1.41 (d, J_{PH} = 14.63, 3H, PC*H*$_3$). e.e. 93.8% from line fitting data.

Methyl((*R*)-4-((*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamido)cyclopent-1-enyl)phosphinic acid (44). Method as for **43**. ^{1}H NMR (CD$_3$OD) δ: 7.54−7.44 (m, 2H, benzene *ortho*), 7.40−7.33 (m, 3H, benzene *para* and *meta*), 6.43 (bd, *J* = 11.05, 1H, C(2)*H*), 4.67−4.55 (m, 1H, C(4)*H*), 3.59 (s, 3H, OC*H*$_3$), 3.09−2.75 (m, 2H, C(3)*H*$_2$), 2.60−2.31 (m, 2H, C(5)*H*$_2$), 1.45 (d, J_{PH} = 14.60, 3H, PC*H*$_3$). e.e. 92.0% from line fitting data.

3-Oxocyclobutanecarbonyl chloride (46). A solution of **45** (4.0 g, 35 mmol) and thionyl chloride (12.0 g, 104 mmol) in benzene (40 mL) was heated to 70 °C for 24 h. The solvent and excess thionyl chloride were removed in vacuo and the crude product was purified by vacuum distillation (bp 48−50 °C, 0.05 mm) to give **46** as a colorless oil (3.6 g, 24 mmol, 69%). ^{1}H NMR (CDCl$_3$) δ:

3.80−3.67 (m, 1H, C(3)*H*), 3.57 (m, 2H, C(2)*H* and C(4)*H*), 3.45 (m, 2H, C(2)*H* and C(4)*H*). ^{13}C NMR ($CDCl_3$) δ: 200.39 (*C*(1)), 174.85 (*C*(O)Cl), 52.17 (*C*(3)), 38.90 (*C*(2) and *C*(4)).

3-Aminocyclobutanone.HCl (47). A solution of **46** (1.0 g, 6.5 mmol) in benzene (8 mL) was added dropwise to a solution of NaN_3 (0.86 g, 13.2 mmol) in water (8 mL) at 0 °C. The resulting biphasic solution was allowed to warm to room temp and was stirred vigorously for 2 h. The benzene layer (caution:explosive) was then separated, washed with saturated $NaHCO_3$, water, and dried over $CaCl_2$. The benzene solution was then slowly heated to 60 °C, during which time gas evolution (N_2) was observed. After cessation of gas evolution, the solution was heated for a further 1 h before being cooled to room temp and 20% HCl (9 mL) added. The solution was then heated overnight at 90 °C, and gas evolution (CO_2) was again observed. The aqueous layer was then separated, the benzene layer washed with water, and the combined aqueous layers concentrated in vacuo to give **47** as white crystals (0.7 g, 5.8 mmol, 76%, mp (dec.) 127−130 °C), which were used without further purification in the next step. ^{1}H NMR (D_2O) δ: 4.05 (m, 1H, C(3)*H*), 3.47 (m, 2H, C(2)*H* and C(4)*H*), 3.26 (m, 2H, C(2)*H* and C(4)*H*). ^{13}C NMR (D_2O) δ: 207.11 (*C*(1)), 52.34 (*C*(3)), 36.63 (*C*(2) and *C*(4)). MS (CI) *m*/*z* (%): 86.0 (100, $[M + H]^+$). (ESI) *m*/*z* (%): 100.1 (65, $[M + Na]^+$), 86.0 (100, $[M + H]^+$).

3-(*tert*-Butoxycarbonylamino)cyclobutanone (48). To a stirred solution of **47** (1.67 g, 13.7 mmol) and BOC_2O (3.1 g, 14.0 mmol) in DMF (30 mL) at room temp was added Et_3N (1.7 g, 16 mmol) dropwise. The solution was stirred at room temp for 2 h and partitioned between water (50 mL) and diethyl ether (50 mL). The ether layer was washed with brine, dried with Mg_2SO_4, and concentrated in vacuo. The crude product was the purified via flash chromatography (hexane:ethyl acetate, 60:40) to give **48** as colorless crystals (1.38 g, 68%). ^{1}H NMR ($CDCl_3$) δ: 4.95 (bs, 1H, C(3)*H*), 4.30 (m, 1H, C(1)*H*), 3.44 (m, 2H, C(2)*H* and C(4)*H*), 3.07 (m, 2H, C(2)*H* and C(4)*H*), 1.49 (s, 9H, C(CH_3)$_3$). ^{13}C NMR ($CDCl_3$) δ: 205.19 (*C*(1)), 155.63 (*C*(O)OC(CH$_3$)$_3$), 80.41 (*C*(CH$_3$)$_3$), 54.96 (*C*(2), *C*(4)), 37.03 (*C*(3)), 28.60 (C(*C*H$_3$)$_3$). HRMS (EI): calcd for $C_9H_{15}NO_3$ ($M^{\cdot+}$) 185.1052; found 185.1054.

***tert*-Butyl 3-(ethoxy(methyl)phosphoryl)-3-(trimethylsilyloxy)-cyclobutylcarbamate (49 and 50).** Ethyl methylphosphinate (0.90 g, 4.9 mmol), **48** (1.0 g, 10.0 mmol), and hexamethyldisilazane (HMDS, 1.6 g, 0.7 mmol) were mixed under N_2 and heated to 90 °C for 24 h. The resulting mixture was diluted with CH_2Cl_2 (50 mL), washed with water (2 × 50 mL) and brine (50 mL), dried over Mg_2SO_4, and concentrated in vacuo. The crude product was purified via flash chromatography on silica gel (ethyl acetate, 1% EtOH). Separation of the cis- (minor) and *trans*- (major) isomers was possible under these conditions, the cis product eluting first and the trans later to give a combined yield was 1.4 g, 98%.

***cis-tert*-Butyl 3-(Ethoxy(methyl)phosphoryl)-3-(trimethylsilyloxy)cyclobutylcarbamate (49).** ^{1}H NMR (CD_3OD) δ: 4.45−4.32 (m, 1H, N*H*), 4.24−4.02 (m, 3H, C(3)*H* and POCH_2CH$_3$), 2.66−2.40 (m, 4H, C(2)H_2 and (C(4)H_2), 1.43 (s, 9H, C(CH_3)$_3$), 1.43 (d, 3H, PCH_3, J = 13.6), 1.34 (t, 3H, POCH$_2$CH_3, J = 7.0), 0.25 (s, 9H, Si(CH_3)$_3$). ^{13}C NMR (CD_3OD) δ: 157.44 (NH-*C*(O)OC(CH$_3$)$_3$), 80.31 (*C*(CH$_3$)$_3$), 73.41 (d, *C*(3), J_{PC} = 124.8), 62.86 (d, PO*C*H$_2$CH$_3$, $^2J_{POC}$ = 7.3), 41.09 (d, *C*(1), $^3J_{PCCC}$ = 4.6), 39.76 (d, *C*(2) and *C*(4), $^2J_{PCC}$ = 1.0), 28.77 (C(*C*H$_3$)$_3$), 17.04 (d, POCH$_2$CH$_3$, $^3J_{POCC}$ = 5.7), 8.35 (d, P*C*H$_3$, J_{PC} = 89.8), 2.00 (Si(*C*H$_3$)$_3$). ^{31}P NMR (CD_3OD) δ: 54.02. HRMS (ESI): calcd for $C_{15}H_{32}NO_5PSiNa$ (MNa^+) 388.1680; found 388.1635.

***trans-tert*-Butyl 3-(ethoxy(methyl)phosphoryl)-3-(trimethylsilyloxy)cyclobutylcarbamate (50).** ^{1}H NMR (CD_3OD) δ: 4.21−4.05 (m, 3H, POCH_2CH$_3$ and N*H*), 3.93−3.77 (m, 1H, C(1)*H*), 2.99−2.82 (m, 2H, C(2)*H* and C(4)*H*), 2.33−2.02 (m, 2H, C(2)*H* and C(4)*H*), 1.47 (d, 3H, PCH_3, J = 13.6), 1.43 (s, 9H, C(CH_3)$_3$), 1.36 (t, 3H, POCH$_2$CH_3, J = 7.1), 0.21 (s, 9H, Si(CH_3)$_3$). ^{13}C NMR (CD_3OD) δ: 157.40 (NH*C*(O)OC(CH$_3$)$_3$), 80.14 (*C*(CH$_3$)$_3$), 67.32 (d, *C*(3), J_{PC} = 120.2), 62.81 (d, PO*C*H$_2$CH$_3$, $^2J_{POC}$ = 7.2), 41.78 (d, *C*(2) and *C*(4), $^2J_{PCC}$ = 1.9), 38.44 (*C*(1)), 28.78 (C(*C*H$_3$)$_3$), 17.03 (d, POCH$_2$CH$_3$, $^3J_{POCC}$ = 5.6), 8.34 (d, P*C*H$_3$, J_{PC} = 89.6), −1.09 (Si(*C*H$_3$)$_3$). ^{31}P NMR (CD_3OD) δ: 56.33. HRMS (ESI): calcd for $C_{15}H_{32}NO_5PSiNa$ ($[M + Na]^+$) 388.1680; found 388.1633.

***cis*-3-Amino-1-hydroxycyclobutyl(methyl)phosphinic acid (51). 49** (59 mg, 161 μmol) was refluxed in HCl (6 M, 6 mL) for 36 h. The HCl was removed in vacuo to give the crude hydrochloride salt, which was purified by ion exchange chromatography (Dowex 50, H^+) to give **51** a colorless crystalline solid (26 mg, 157 μmol, 98%). ^{1}H NMR (D_2O) δ: 3.84 (p, 1H, C(1)*H*, J = 6.9), 2.45 (dd, 4H, C(2)H_2 and C(3)H_2, J = 10.7, J = 6.0), 1.12 (d, 3H, PCH_3, J = 13.4). ^{13}C NMR (D_2O) δ: 70.66 (d, *C*(1), J_{PC} = 112.9), 40.45 (d, *C*(3), $^3J_{PCCC}$ = 6.9), 35.59 (d, *C*(2) and *C*(4), $^2J_{PCC}$ = 2.6), 9.80 (d, P*C*H$_3$, J_{PC} = 92.5). ^{31}P NMR (D_2O) δ: 39.95. HRMS (ESI) calcd for $C_5H_{12}NO_3PNa$ ($[M + Na]^+$) 188.0447; found 188.0452.

***trans*-3-Amino-1-hydroxycyclobutyl(methyl)-phosphinic acid (52).** Method as for **51**; white solid (43 mg, 95%). ^{1}H NMR (D_2O) δ: 3.44 (p, 1H, C(3)*H*, J = 7.9), 2.76 (ddt, 2H, C(2)*H* and C(4)*H*, J = 10.7, J = 7.9, J = 3.0), 2.13 (dddd, 1H, C(2)*H* and C(4)*H*, J = 12.8, J = 11.3, J = 8.2, J = 2.9), 1.09 (d, 3H, PCH_3, J = 13.3). ^{13}C NMR (D_2O) δ: 67.15 (d, *C*(1), J_{PC} = 114.9), 38.00 (d, *C*(3), $^3J_{PCCC}$ = 3.4), 37.27 (d, *C*(2) and *C*(4), $^2J_{PCC}$ = 2.9), 9.94 (d, P*C*H$_3$, J_{PC} = 92.3). ^{31}P NMR (D_2O) δ: 40.41. HRMS (ESI) calcd for $C_5H_{13}NO_3P$ ($[M + H]^+$) 166.0628; found 166.0630.

***trans-tert*-Butyl 3-(ethoxy(methyl)phosphoryl)-3-hydroxycyclobutylcarbamate (53).** To a solution of **52** (0.5 g, 1.4 mmol) in acetonitrile (5 mL) was added $Et_3N \cdot 3HF$ (0.09 g, 0.55 mmol). A precipitate formed rapidly and the resulting solution stirred at room temp for a further 1 h. At this time CH_2Cl_2 (50 mL) was added and the solution washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL). Each aqueous phase was back extracted with CH_2Cl_2 (25 mL) and the combined organic phases dried over Mg_2SO_4 and concentrated in vacuo to give **53** as a white solid (0.34 g, 85%). ^{1}H NMR (CD_3OD) δ: 4.16 (m, 2H, POCH_2CH$_3$), 3.88 (m, 1H, C(1)*H*), 3.93 (m, 2H, C(2)*H* and C(4)*H*), 2.13 (m, 2H, C(2)*H* and C(4)*H*), 1.52 (d, 3H, PCH_3, J = 13.6), 1.46 (s, 9H, C(CH_3)$_3$), 1.38 (t, 3H, POCH$_2$CH_3, J = 7.0). ^{13}C NMR (CD_3OD) δ: 157.41 (*C*(O)Ot-Bu), 80.13 (*C*(CH$_3$)$_3$), 67.32 (d, C(1), J_{CP} = 120.2), 62.82 (d, PO*C*H$_2$CH$_3$, $^2J_{POC}$ = 7.3), 41.78 (d, *C*(2) and *C*(4), $^2J_{PCC}$ = 1.8), 38.43 (*C*(3)), 28.77 (C(*C*H$_3$)$_3$), 17.03 (d, POCH$_2$*C*H$_3$, $^3J_{POCC}$ = 5.6), 8.34 (d, P*C*H$_3$, J_{CP} = 89.5). ^{31}P NMR (CD_3OD) δ: 56.43. HRMS (ESI): calcd for $C_{12}H_{24}NO_5PNa$ ($[M + Na]^+$) 316.1284; found 316.1286.

***tert*-Butyl 3-(ethoxy(methyl)phosphoryl)cyclobutyl carbamate (54 and 55). 53** (0.33 g, 1.1 mmol) and 4-DMAP (0.28 g, 2.2 mmol) were suspended in anhydrous CH_3CN (10 mL) under N_2 and cooled to 0 °C. Methyl oxalyl chloride (0.21 g, 1.7 mmol) was added and the mixture allowed to warm rapidly to room temp and stirred an additional 2.5 h. After addition of ethyl acetate (40 mL), the mixture was filtered and washed with saturated $NaHCO_3$ (25 mL) and brine (25 mL), dried over $MgSO_4$, and concentrated in vacuo to give the oxalate as a slightly colored oil (0.42 g, 99%). The oil, azobisisobutyronitrile (AIBN, 46 mg, 0.28 mmol), and Bu_3SnH (0.50 g, 1.7 mmol) were dissolved in anhydrous toluene (10 mL) under N_2 and the solution heated to 90 °C for 2.5 h. The solution was concentrated in vacuo and the individual isomers isolated cis- and trans- by flash chromatography on silica gel (CH_2Cl_2, 5% CH_3OH). Combined yield 0.21 g, 68%.

***cis-tert*-Butyl 3-(Ethoxy(methyl)phosphoryl)cyclobutyl carbamate (54).** ^{1}H NMR (CD_3OD) δ: 4.18−3.93 (m, 3H, C(3) and POCH_2CH$_3$), 2.49−2.29 (m, 3H, C(2)*H*, C(4)*H* and C(1)*H*), 2.20−1.94 (m, 2H, C(2)*H* and C(4)*H*), 1.39 (s, 9H, C(CH_3)$_3$), 1.40 (d, 3H, PCH_3, J = 13.6), 1.27 (t, 3H, POCH$_2$CH_3, J = 7.0). ^{13}C NMR (CD_3OD) δ: 157.30 (*C*(O)OC(CH$_3$)$_3$), 80.22 (C(O)O*C*-(CH$_3$)$_3$), 62.15 (d, $^2J_{POC}$ = 6.66, PO*C*H$_2$CH$_3$), 44.65 (d, $^3J_{PCCC}$ = 30.1, *C*(3)), 32.21 (d, *C*(2) or *C*(4), $^2J_{PCC}$ = 3.3), 31.63 (d, *C*(2) or *C*(4), $^2J_{PCC}$ = 5.4), 28.75 (C(O)OC(*C*H$_3$)$_3$), 26.78 (d, *C*(1), J_{PC} = 102.5), 16.96 (d, POCH$_2$*C*H$_3$, $^3J_{POCC}$ = 5.9), 11.39 (d, P*C*H$_3$, J_{PC} = 92.3). ^{31}P NMR (CD_3OD) δ: 57.47. HRMS (ESI): calcd for $C_{12}H_{24}NO_4PNa^+$ ($[M + Na]^+$) 300.1335; found 300.1334.

***trans-tert*-Butyl 3-(ethoxy(methyl)phosphoryl)cyclobutyl carbamate (55).** ^{1}H NMR (CD_3OD) δ: 4.25−4.10 (m, 1H, C(3)*H*), 4.10−3.97 (m, 2H, $POCH_2CH_3$), 2.61−2.41 (m, 3H, C(2)*H*, C(4)*H* and C(1)*H*), 2.35−2.14 (m, 2H, C(2)*H* and C(4)*H*), 1.45 (d, 3H, PCH_3, J = 13.5), 1.38 (s, 9H, $C(CH_3)_3$), 1.29 (t, 3H, $POCH_2CH_3$, J = 7.0). ^{13}C NMR (CD_3OD) δ: 157.35 ($C(O)OC(CH_3)_3$), 80.16 ($C(O)OC(CH_3)_3$), 62.15 (d, $POCH_2CH_3$, $^2J_{POC}$ = 6.8), 45.26 (d, *C*(3), $^3J_{PCCC}$ = 4.6), 30.99 (d, *C*(2) or *C*(4), $^2J_{PCC}$ = 5.7), 30.92 (d, *C*(2) or *C*(4), $^2J_{PCC}$ = 4.7) 28.76 ($C(O)OC(CH_3)_3$), 27.79 (d, *C*(1), J_{PC} = 100.8), 16.99 (d, $POCH_2CH_3$, $^3J_{POCC}$ = 5.9), 11.18 (d, PCH_3, J_{PC} = 91.0). ^{31}P NMR (CD_3OD) δ: 60.34. HRMS (ESI): calcd for $C_{12}H_{24}NO_4PNa^+$ ($[M + Na]^+$) 300.1335; found 300.1338.

***cis*-3-Amino-cyclobutyl(methyl)phosphinic acid (56).** Method as for **51**; off-white solid (75 mg, 0.50 mmol, 93%, mp (dec.) 185−187 °C). ^{1}H NMR (D_2O) δ: 3.82 (d, C(3)*H*, J = 7.6), 2.54−2.36 (m, 3H, C(2)H_A, C(4)H_A and C(1)*H*), 2.26−2.06 (m, 2H, C(2)H_B and C(4)H_B), 1.27 (d, 3H, PCH_3, J = 13.8). ^{13}C NMR (D_2O) δ: 43.01 (d, *C*(3), $^3J_{PCCC}$ = 26.7), 27.31 (d, *C*(2) and *C*(4), $^2J_{PCC}$ = 4.6), 26.88 (d, *C*(1), J_{PC} = 100.6), 12.22 (d, PCH_3, J_{PC} = 91.8). ^{31}P NMR (CD_3OD) δ: 50.52. HRMS (ESI): calcd for $C_5H_{13}NO_2P$ ($[M + H]^+$) 150.0678; found 150.0682.

***trans*-3-Amino-cyclobutyl(methyl)phosphinic acid (57).** Method as for **51**; white solid (20 mg, 0.13 mmol, 93%, mp (dec.) 145−147 °C). ^{1}H NMR (D_2O) δ: 3.82 (d, 1H, C(3)*H*, J = 7.6), 2.63−2.27 (m, 5H, C(2)H_2, C(4)H_2 and C(1)*H*), 1.26 (d, 3H. PCH_3, J = 13.6). ^{13}C NMR (D_2O) δ: 44.06 (d, *C*(3), $^3J_{PCCC}$ = 6.4), 27.47 (d, *C*(1), J_{PC} = 98.6), 26.40 (d, *C*(2) and *C*(4), $^2J_{PCC}$ = 4.7), 12.20 (d, PCH_3, J_{PC} = 91.9). ^{31}P NMR (D_2O) δ: 51.15. HRMS (ESI): calcd for $C_5H_{13}NO_2P$ ($[M + H]^+$) 150.0678; found 150.0682.

Pharmacology. Expression of Recombinant Human GABA Receptors in *Xenopus* Oocytes. *Xenopus laevis* were anaesthetised with 0.17% ethyl 3-aminobenzoate and a lobe of an ovary was removed and rinsed with oocyte releasing buffer, OR2 (82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 5 mM HEPES, pH 7.5). It was then treated with Collagenase A (2 mg/ml or OR2, Bohringer Manheim) for 2 h. The released oocytes were rinsed in modified frog Ringers solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 1.8 mM $CaCl_2$, 5 mM HEPES, 2.5 mM pyruvate, 0.5 mM theophylline, 50 ng/mL gentamycin, pH 7.5). Stage V−VI oocytes were collected and stored in this buffer.

Human $GABA_C$ ρ_1 DNA cloned into the vector pcDNA1.1 (Invitrogen, CA) was donated by Dr. George Uhl (National Institute for Drug Abuse, Baltimore, MD). Human α_1, β_2, and γ_{2L} $GABA_A$ DNA cloned into pCMV6-XL5 or pCMV6-XL4 plasmid vectors were purchased from Origene Technologies Inc. (Rockville, MD). Human $GABA_{B1a}$, $GABA_{B2}$, rat $GIRK_1$, and rat $GIRK_4$ cloned into their corresponding vectors were donated by Dr. Fiona Marshall (GlaxoWellcome, Hertfordshire, UK). $GABA_{B1a}$ was cloned in the pcDNA3.1 (−) (Invitrogen, USA), $GABA_{B2}$ and rat $GIRK_1$ were cloned into pcDNA3 (Invitrogen, USA), while rat $GIRK_4$ was cloned into pBluescript KS(−) (Stratgene, USA). *Xenopus laevis* were obtained from South Africa and housed in the Department of Veterinary Science, University of Sydney. Plasmids containing the ρ_1, α_1, γ_{2L}, and $girk_4$ were linearized with restriction enzyme Xba1, similarly $GABA_{B1A}$ was linearized with BST981; $GABA_{B2}$ and girk1 were linearized with EcoR1 and not1, respectively. β_2 was expelled from the vector using a double digest of Sac1 and Sma1. cRNA was synthesized using the "mMessage mMachine" kit from Ambion (Austin, TX). cRNA was injected into defolliculated oocytes at a concentration of 50 ng/50 nL. Oocytes were stored for 1−5 days at 18 °C.

Electrophysiology. Receptor activity was measured by two electrode voltage clamp recording using a Geneclamp 500 amplifier (Axon Instruments, Foster City, CA), a MacLab 2e recorder (AD Instruments, Sydney, NSW, Australia), and Chart version 5.1 program. Oocytes were voltage clamped at −60 mV and continuously superfused with frog Ringers solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 1.8 mM $CaCl_2$, 5 mM HEPES) or in the case of $GABA_B$ receptors a buffer consisting of 45 mM KCl, 45 mM NaCl, 1 mM $MgCL_2$, 1.8 mM $CaCl_2$, and 5 mM HEPES. For receptor activation measurements, the indicated concentrations of drug were added to the buffer solution. For $GABA_A$ and $GABA_B$ containing oocytes, a washout period of 7 min was allowed to minimize desensitization.

Recombinant GABA receptors where expressed in *Xenopus laevis* oocytes with similar properties to those reported earlier, these were: homomeric ρ_1,[64] heteromeric $GABA_{B(1A/2)}$ (coexpressed with Kir 3.1 and Kir 3.4 K^+ channels),[80] and heteromeric $\alpha_1\beta_2\gamma_{2L}$ receptors.[81] The voltage potential was clamped at −60 mV, and the amplitudes of the whole-cell currents varied upon receptor opening. For all receptors, the currents ranged from 200 to 10000 nA. The maximal GABA response was attained by the application of 100 μM GABA for ρ_1 receptors, 600 μM GABA for both $GABA_{B(1A/2)}$ and $\alpha_1\beta_2\gamma_{2L}$ receptors (see Supporting Information for representative electrophysiological recordings).

Data Analysis. The amplitude of the current (I) recorded in response to each drug concentration [A] was normalized to the maximum amplitude (I_{max}) of the current response to GABA (ρ_1, 100 μM; $GABA_B$, 600 μM; $\alpha_1\beta_2\gamma_{2L}$, 600 μM). Normalized concentration−response data were analyzed using the GraphPad "Prism" version 4.00, expressed as mean ± SEM, plotted on a semilogarithmic scale and analyzed using the Gaussian distribution equation. The equation used was

$$Y = (\text{area}/[\text{SD}(2\pi)^{0.5}] \exp(-0.5(\text{X} - \text{mean})\text{SD})^2) \quad (1)$$

This data was then fit by least-squares to the Hill equation:

$$\%I = (I_{max}[A]^{n_H})/(EC_{50}{}^{n_H} + [A]^{n_H}) \quad (2)$$

where I_{max} is the maximal response, EC_{50} is the agonist concentration, which elicits 50% of the maximal response, and n_H is the Hill coefficient. The intrinsic activity of partial agonists was calculated as a persentage of the maximum current produced by a maximum dose of GABA.

pK_B values were calculated by determination of the pEC_{50} of GABA alone and in the presence of one or more concentrations of the antagonist [B], tested for statistical significance via 2-way ANOVA and fitted to the equation:

$$pEC_{50} = -\log([B] + 10^{\log(K_B)}) - P \quad (3)$$

where P is a constant. Where several antagonist concentrations were used, the data was tested to see if it was consistent with the criteria for simple competitive antagonism via an F-test using eq 3 above and:

$$pEC_{50} = -\log([B]^{\text{slope}} + 10^{\log(K)}) - P \quad (4)$$

If eq 3 fits the data better, the interaction is defined as competitive antagonism.

Molecular Modeling. All molecules investigated in this study were built with the molecular modeling and graphical user interface package, Maestro 8.0,[82] unless otherwise stated. Each structure was subsequently minimized using the OPLS_2005[83] with a constant dielectric of 1.0. All calculations were performed on an AMD64+ workstation using the SUSE10.1 Linux operating system.

Pharmacophore Perception. Pharmacophore development was carried out using PHASE 2.5 in the Maestro modeling environment in accordance with the PHASE 2.5 user manual.[66] Primary attention was placed on a small subset of potent ρ_1 $GABA_C$ receptor antagonists: TPMPA (**3**), (*S*)-4-ACPMPA (**36**), (1*R*,3*S*)-3-ACPMPA (**60**), (1*S*,3*R*)-3-ACPMPA (**63**), and I4AA (**63**), with the intention of highlighting common pharmacophore sites, while concomitantly contrasting the pharmacophore differences presented by the weak or inactive $GABA_C$ receptor antagonist, (*R*)-4-ACPMPA (**35**).

Compounds TPMPA (**3**), (*R*)-4ACPMPA (**35**), (*S*)-4ACPMPA (**36**), (1*R*,3*S*)-3-ACPMPA (**60**), (1*S*,3*R*)-3-ACPMPA (**63**), and I4AA (**63**) were divided into an active class, **3**, **36**, **60**, **62**, and **63**, and an inactive class, **35**, and thus encompassing a broad range of activity, potent and weak, respectively. A maximum of 100 conformations were generated for each molecule, sampling the torsional space that may contain possible active conformers. This was achieved using a combination of Monte Carlo Multiple

Minimum[84] and low mode conformational searching,[85] in conjugation with the OPLS_2005 force field, GB/SA solvation treatment, and a minimum atom deviation of 1.0 Å. This was conducted to exhaustively search all conformational space, in a water solvated environment. Minimized structures were filtered with a maximum relative energy difference of 10.0 kcal/mol to exclude redundant conformers.

On the basis of visual inspection and careful analysis of chemical features in the actives, pharmacophore variants considered for the models consisted of five features: H-bond donor, negatively charged feature, positively charged feature, and two custom group features representing the double bond and alkyl substituent. Thus, model variant DNPXY was preselected, as it was visually apparent that active compounds possessed DNP features with the option of additionally having either X and/or Y.

The five active antagonists were analyzed on their potential to present the DNPXY features in a common 3D spatial arrangement of functional groups (sites) and with a minimum intersite distance of 1.0 Å, suggesting a highly specific 3D pharmacophore model. Several pharmacophore hypotheses were found. The chosen hypothesis corresponded to the model, which gave the best overall PHASE scoring functions[66] for each active when screened for three sites to exact matches of D, N, and P site points, leaving the fourth site point as either the double bond (X) or methyl substituent (Y). The resulting hypothesis was deemed the preliminary "ρ_1 $GABA_C$ receptor antagonist pharmacophore" and applied in subsequent refinement procedures.

Pharmacophore Refinement: Finding Matches to Known $GABA_C$ Receptor−Ligands. Quality assurance and refinements measures were conducted on the pharmacophore model as a means of optimizing its robustness. The pharmacophore was iteratively screened against a comprehensive list of pharmacologically characterized GABA receptor−ligands (see Supporting Information) using the Find Matches to Hypothesis Panel.[65] The list included $GABA_A$ and $GABA_B$ ligands as well as five additional potent $GABA_C$ receptor antagonists that were not used in the original pharmacophore development: 3-APMPA (**5**), (*S*)-4-ACPCA (*S*-**6**), (1*R*,3*R*)-3-ACPMPA (**61**), (1*S*,3*S*)-3-ACPMPA (**59**), and P4MPA (**58**). Successful alignments to the pharmacophore require a minimum of three sites corresponding to exact matches of D, N, and P site points, leaving the fourth site point as either the double bond (X) or an alykyl substituent (Y). Distance matching tolerance was set to 2.0 Å.

The "create_xvolClash" and "create_xvolShell" Schrödinger utility scripts[65] were invoked to systematically locate regions where excluded volume spheres would cause steric violations only for inactives, in tandem with generating a shell of excluded volume spheres to cater only for the active set. A grid spacing of 0.5 Å and a buffer distance of 0.1 Å was used to create the shell. Hits with align scores greater than 1.2 and vector and volume scores less than 0.70 and 0.0, respectively, were rejected. These latter refinement steps were not performed until the models performance in identifying other $GABA_C$ active antagonists had been validated. The resulting DNPXY pharmacophore, with the excluded volume shell, was deemed the final ρ_1 $GABA_C$ antagonist pharmacophore.

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Supporting Information Available: e.e. determination, representative electrophysiological recordings, compounds used in computational analysis and elemental analysis of key compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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