Phosphinic Acid Analogues of GABA. 1. New Potent and Selective $GABA_B$ Agonists†

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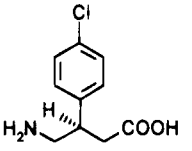
The antispastic agent and muscle relaxant baclofen **1** is a potent and selective agonist for bicuculline-insensitive $GABA_B$ receptors. For many years efforts to obtain superior $GABA_B$ agonists were unsuccessful. We describe the syntheses and biological properties of two new series of $GABA_B$ agonists, the best compounds of which are more potent than baclofen *in vitro* and *in vivo*. They were obtained by replacing the carboxylic acid group of GABA or baclofen derivatives with either the phosphinic acid or the methylphosphinic acid residue. Surprisingly, ethyl- and higher alkylphosphinic acid derivatives of GABA yielded novel $GABA_B$ antagonists, which are described in part 2 of this series. Structure–activity relationships of the novel $GABA_B$ agonists are discussed with respect to their affinities to $GABA_B$ receptors as well as to their effects in many functional tests *in vitro* and *in vivo* providing new muscle relaxant drugs with significantly improved side effect profiles.

Introduction

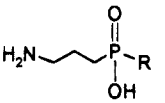
γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system.[1] It interacts with two types of receptors designated $GABA_A$ and $GABA_B$ by Hill and Bowery.[2] The distribution and density of both types of receptors in the mammalian brain is similar in many brain regions. $GABA_A$ receptors outnumber $GABA_B$ receptors in the frontal cortex of rats, whereas $GABA_B$ receptors predominate in the molecular layer of the cerebellum, the thalamus, and the dorsal horns of the spinal cord.[3] $GABA_A$ receptors are coupled to chloride ion channels and mediate fast synaptic inhibition. They contain multiple allosteric binding sites for benzodiazepines, picrotoxinin, barbiturates, centrally active steroids, avermectin, and propofol.[4] The bicuculline-insensitive $GABA_B$ receptors are coupled through G-proteins to neuronal potassium and calcium channels and mediate slow synaptic inhibition by increasing potassium and decreasing calcium conductances.[5,6] A major function of $GABA_B$ receptors is the modulation of the release of several neurotransmitters, such as glutamate,[7] dopamine,[8] noradrenaline,[8] serotonin,[8] substance P,[9] cholecystokinin,[10] and somatostatin[11] via presynaptic $GABA_B$ binding sites.

$GABA_B$ receptors are stereoselectively activated by the (*R*)-(−)-enantiomer of the antispastic agent and muscle relaxant baclofen **1** (Table 1),[12] a lipophilic derivative of GABA, synthesized for the first time in 1962. Since then hundreds of analogues of baclofen have been prepared,[13] including 3-heteroaryl-GABA analogues[14] or compounds mimicking a possible bioactive conformation,[15] none of which produced $GABA_B$ agonists displaying superior affinity to $GABA_B$ receptors in comparison to baclofen. However, more potent and selective $GABA_B$ receptor agonists were discovered, when the carboxylic acid moiety of GABA was replaced by various phosphinic acid residues.[16]

Table 1. Inhibition of Binding of [^{3}H]Baclofen to $GABA_B$ Receptors of Cat Cerebellum and of [^{3}H]Muscimol to $GABA_A$ Receptors of Rat Cortex



(*R*)-(-)-Baclofen



2 R = H CGP27492
42 R = Me CGP35024
3 R = Et CGP36216

compd	IC_{50} (μM) $GABA_B$	IC_{50} (μM) $GABA_A$	selectivity $GABA_B/GABA_A$
GABA	0.025	0.128	5
1, (*R*,*S*)-baclofen	0.035	1047	29600
(*R*)-(−)-baclofen	0.015	964	64000
(*S*)-(+)-baclofen	1.77	1564	880
2	0.0024	1.7	700
42	0.0066	inactive[a]	
3	1.35	inactive[a]	

[a] Measured at a concentration of 10^{-5} M.

Table 1 compares the affinities of the two GABA mimics, (3-aminopropyl)phosphinic acid (**2**, CGP27492) and (3-aminopropyl)methylphosphinic acid (**42**, CGP35024, identical with SK&F97541[17]) to $GABA_B$ and $GABA_A$ receptors, respectively, with the affinities of the endogenous neurotransmitter GABA and of baclofen and its enantiomers, as measured by displacement of [^{3}H]baclofen from synaptosomal membranes of cat cerebellum and by displacement of [^{3}H]muscimol from rat cortex. Due to its 15 times higher potency, its high specific binding, and the possibility to carry out filtration assays, [^{3}H]CGP27492 has now replaced [^{3}H]-baclofen as a radioligand for $GABA_B$ receptor binding assays.[18]

† W. Froestl would like to dedicate this work to his highly respected teacher, Prof. Karl Schloegl, University of Vienna, Austria, on the occasion of his 70th birthday.

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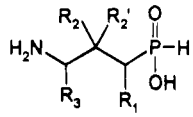
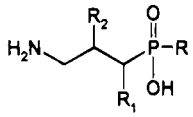
Table 2. Inhibition of Binding of [^{3}H]Baclofen to $GABA_B$ Receptors of Cat Cerebellum



compd	R_1	R_2	R_2'	R_3	IC_{50} (μM)
2	H	H	H	H	0.0024
7	CH_3	H	H	H	0.92
8	H	CH_3	H	H	0.78
9	H	H	H	CH_3	0.5
10	C_6H_5	H	H	H	10%[a]
11	4-ClC_6H_4	H	H	H	42%[a]
12	H	4-ClC_6H_4	H	H	0.039
13	H	4-FC_6H_4	H	H	0.36
14	H	C_6H_5	H	H	0.88
15	H	4-$CF_3C_6H_4$	H	H	66%[a]
16	H	OH	H	H	0.018
17	H	H	H	4-ClC_6H_4	29%[a]
21	H	OH	H	(*R*)-CH_3	12.8[b]
27	H	4-ClC_6H_4	OH	H	0.065[b]
29	$R_1 + R_2 =$ 		H	H	0.28[b]
70	$R_1 + R_2 =$ 		H	H	4.41[b]

[a] Percent inhibition at a concentration of 10^{-5} M. [b] Inhibition of binding of [^{3}H]CGP27492 to $GABA_B$ receptors of rat cortex.

Table 3. Inhibition of Binding of [^{3}H]CGP27492 to $GABA_B$ Receptors of Rat Cortex



compd	R	R_1	R_2	IC_{50} (μM)
30[a]	OH[a]	H	H	6.81[a]
42	CH_3	H	H	0.016
43	CHF_2	H	H	0.089
44	CH_2OH	H	H	1.05
45	CH_3	CH_3	H	0.14
47	CH_3	C_2H_5	H	18.5
50	CH_3	CF_3	H	67.6
52	CH_3	OH	H	1.16
55	CH_3	H	OH	0.077
56	CHF_2	H	OH	0.213
63	CH_3	H	(*S*)-OH	0.045
64	CH_3	H	(*R*)-OH	0.152
68	CH_3	$R_1 + R_2 =$ 		0.665
69	CH_3	$R_1 + R_2 =$ 		16.58

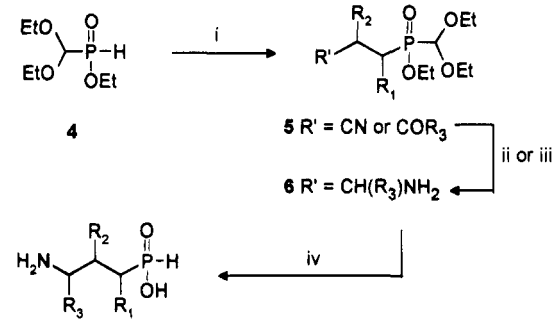
[a] $GABA_B$ antagonist.

Later it was discovered[19] that in this series of $GABA_B$ ligands only phosphinic acid analogues of GABA (Table 2) and methylphosphinic acid derivatives (Table 3) with the exception of [3-amino-2-(4-chlorophenyl)propyl]-methylphosphinic acid (**71**)[20] (Chart 3) and [3-amino-2-(4-chlorophenyl)-2-hydroxypropyl]methylphosphinic acid (**72**)[20] (Chart 3) were potent $GABA_B$ agonists. Electrophysiological experiments demonstrated that (3-aminopropyl)ethylphosphinic acid **3** (Table 1) and homologous alkylphosphinic acids ($R \geq C_2H_5$) were $GABA_B$ antagonists, as is described in part 2 of this series.[20]

Chemistry

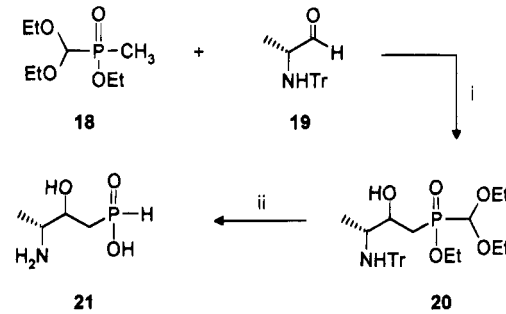
Dingwall et al.[21,22] described an efficient synthetic route for the preparation of phosphinic acid analogues of (natural) α-, β-, and γ-amino acids. Although the phosphinic acid analogue of GABA, **2**, displayed a significantly higher affinity to $GABA_B$ receptors than

Chart 1[a]



[a] Reagents and conditions: (i) $R_1CH{=}C(R_2)CN$, cat. NaOEt, EtOH, 0 °C → rt, 4 h or (a) HMDS, reflux, 3 h; (b) $R_1CH{=}C(R_2)COR_3$, 50 °C, 1 h → rt, H_2O; (ii) H_2, Raney nickel, 10% NH_3 in EtOH, 1 bar; (iii) $NaCNBH_3$, NH_4OAc, MeOH, rt, 48 h; (iv) conc. HCl, reflux 1–4 h; propylene oxide, EtOH, rt.

Scheme 1[a]



[a] Reagents and conditions: (i) LDA, THF, −78 °C → room temperature, 24 h; (ii) 2 M HCl, reflux, 4 h; propylene oxide, EtOH, room temperature, 24 h.

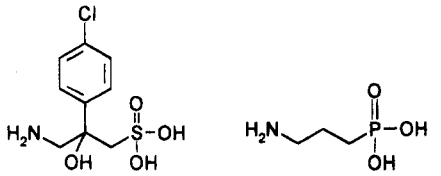
the endogenous neurotransmitter *in vitro*, it was of little therapeutic value, because it did not show the pronounced muscle relaxant effects of baclofen *in vivo* (Table 8). This prompted us to start a medicinal chemistry program to improve on the muscle relaxant effects of substituted (3-aminopropyl)phosphinic acids.

Several monosubstituted phosphinic acid analogues of GABA had been already synthesized by Dingwall et al.[22] (Chart 1). Conjugate addition of ethyl (diethoxymethyl)phosphinate (**4**)[23] to acrylonitrile (or in case of 3-substituted derivatives to vinyl alkyl ketones) gave cyano (or keto) ester **5**, which upon hydrogenation (or reductive amination) yielded amino ester **6**. After acidic hydrolysis, **2** and **7–17** (Table 2) were obtained.

We extended this series to disubstituted derivatives (Scheme 1). Thus, reaction of the anion of ethyl (diethoxymethyl)methylphosphinate (**18**)[22] with (*R*)-2-(*N*-tritylamino)propanal (**19**) gave secondary alcohol **20**, which after hydrolysis yielded [3(*R*)-amino-2(*R,S*)-hydroxybutyl]phosphinic acid (**21**) as a 1:1 mixture of diastereoisomers.

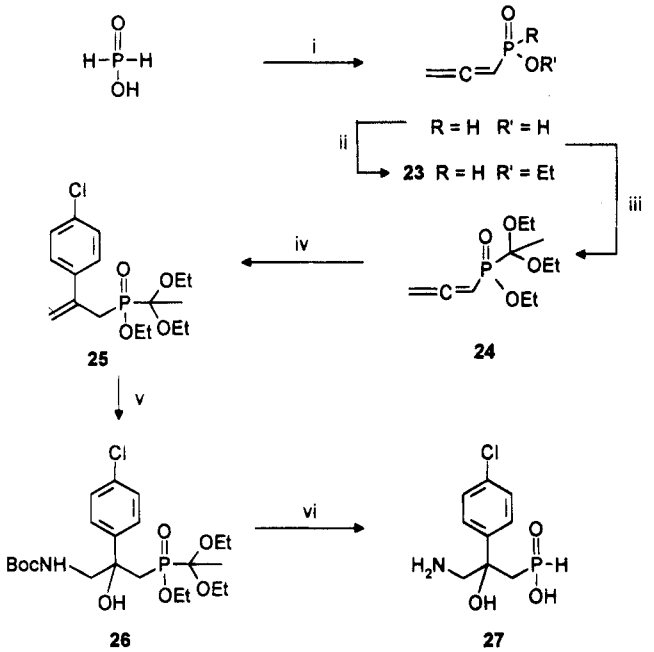
An interesting disubstitution pattern was reported by Kerr et al.[24] with the novel $GABA_B$ antagonist 2-hydroxysaclofen (**22**) (Chart 2). The synthesis of its phosphinic acid analogue **27** is shown in Scheme 2. 1,2-Propadienylphosphinic acid[25] was esterified by treatment with ethyl chloroformate to give **23**. Protection of the P–H bond by Lewis acid-catalyzed reaction with triethyl orthoacetate gave the key intermediate **24**, a very reactive Michael acceptor. Addition of (4-chlorophenyl)cuprate gave the allylic compound **25** without rearrangement provided that the temperature during the alkylation was maintained strictly at −78 °C. The

Chart 2



22 30

Scheme 2[a]



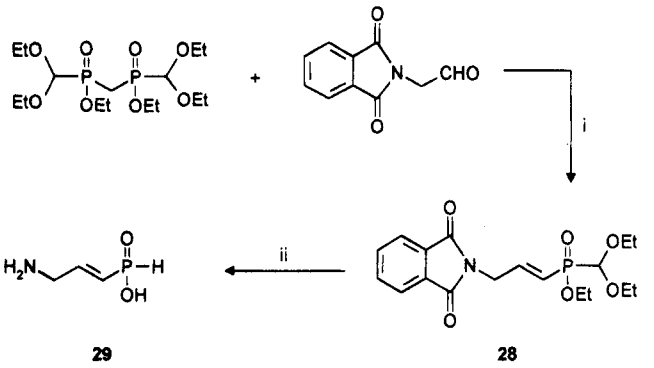
[a] Reagents and conditions: (i) $HC{\equiv}CCH_2OH$, toluene, reflux, 24 h; (ii) ClCOOEt, Et_3N, DCM/THF, 2:1, −5 °C, 2 h, → room temperature, 16 h; (iii) $MeC(OEt)_3$, $BF_3{\cdot}Et_2O$, room temperature, 24 h; (iv) 4-$ClC_6H_4MgI{\cdot}CuBr{\cdot}Me_2S$, Et_2O, −45 °C, 3 h, −20 °C, 2 h; (v) $BocNH_2$, MeOH, 0 °C, *t*-BuOCl, NaOH, MeOH, $AgNO_3$, 1 mol % OsO_4, MeCN, *t*-BuOH, H_2O, room temperature, 24 h; (vi) Me_3SiBr, DCM, room temperature, 24 h; propylene oxide, MeOH, room temperature, 24 h.

regiospecific introduction of the 3-amino and 2-hydroxy groups was achieved using the osmium-catalyzed oxyamination procedure described by Sharpless et al.[26] to give Boc-protected ester **26** as a 1:1 mixture of diastereoisomers. All three protecting groups were cleaved simultaneously using trimethylsilyl bromide to give **27**.

To examine the effects of conformational restriction on the structure activity relationship in this series, the unsaturated derivative **29** was prepared (Scheme 3). Horner–Emmons condensation of diethyl methylenebis(diethoxymethyl)phosphinate[27,28] with 2-phthalimidoacetaldehyde[29] gave **28**, which was hydrolyzed to [(*E*)-3-amino-1-propenyl]phosphinic acid (**29**).

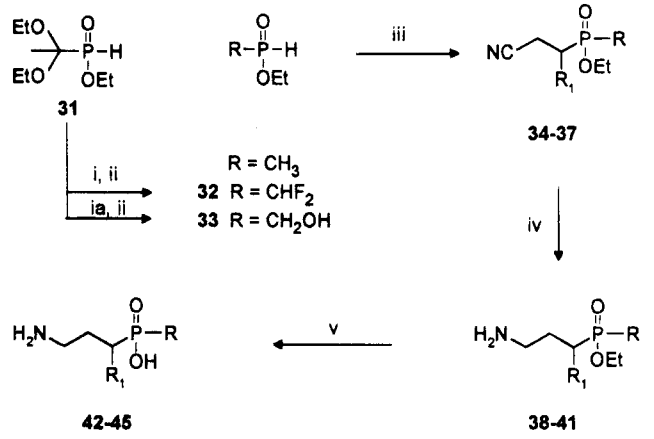
However, the various mono- and disubstituted (3-aminopropyl)phosphinic acid derivatives did not elicit therapeutically useful activities *in vivo* significantly superior to those of **2**. One reason could be the metabolic lability of the P–H bond, which may be oxidized to the corresponding phosphonic acid under *in vivo* conditions. The oxidation product of **2**, i.e., (3-aminopropyl)phosphonic acid (**30**) (Chart 2) showed properties of a weak $GABA_B$ antagonist in the $GABA_B$ binding assay (Table 3) and in electrophysiological experiments. For this reason we abandoned this class of compounds and turned our attention to methylphosphinic acids.

Scheme 3[a]



[a] Reagents and conditions: (i) NaH, THF, room temperature, 15 min; 2-phthalimidoacetaldehyde, 4 °C, 15 min; (ii) concentrated HCl, reflux, 24 h, ion exchange chromatography on Dowex 50 W X 2.

Scheme 4[a]



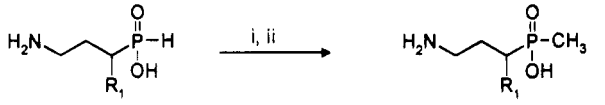
34, 38, 42: R = CH_3 R_1 = H **36, 40, 44**: R = CH_2OH R_1 = H

35, 39, 43: R = CHF_2 R_1 = H **37, 41, 45**: R = CH_3 R_1 = CH_3

[a] Reagents and conditions: (i) NaH, THF, 4 °C, 1 h, $ClCHF_2$, −10 °C → 4 °C, 1.5 h; (ia) $(CH_2O)_n$, Et_3N, 130 °C, 2 h; (ii) 1.5 equiv of TMSCl, 10% EtOH/DCM, 4 °C, 48 h; (iii) NaOEt, EtOH, 4 °C, $R_1CH{=}CHCN$, 4 °C, 0.5–1 h → 20 °C, 24 h; (iv) H_2, Raney nickel, 10% NH_3 in EtOH, 1 bar, room temperature, 4 h; (v) concentrated HCl, reflux, 15 h; propylene oxide, MeOH, room temperature, 16 h.

(3-Aminopropyl)methylphosphinic acid (**42**) was first described in 1972 in the patent literature as a flame retardant.[30] The synthetic sequence for **42** and its analogues is outlined in Scheme 4: conjugate addition of deprotonated ethyl methylphosphinate[31] to acrylonitrile gave **34**, which was hydrogenated to amino ester **38** and hydrolyzed to **42** under acidic conditions. The new intermediates ethyl (difluoromethyl)phosphinate (**32**) and ethyl (hydroxymethyl)phosphinate (**33**) were prepared by condensation of ethyl (1,1-diethoxyethyl)-phosphinate[32] (**31**), the homologue of ethyl (diethoxymethyl)phosphinate[23] **4** (Chart 1), with chlorodifluoromethane and formaldehyde, respectively. The advantage of reagent **31** over **4** is that the masked P–H group in various (1,1-diethoxy*ethyl*)phosphinates can be liberated under very mild conditions (1.5 equiv of TMSCl, 10% ethanol in dichloromethane at room temperature) giving the phosphinic acid esters directly. Cleavage of the P–H protecting group in (diethoxy*methyl*)phosphinates requires reflux in 6 M HCl. Under these conditions the ester functionality is cleaved as well to give the corresponding phosphinic acids, which, in many

Scheme 5[a]

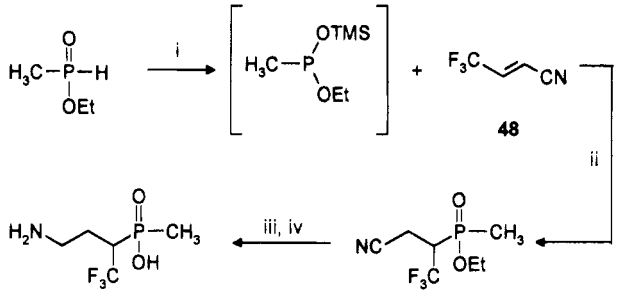


2 R_1 = H
46 R_1 = Et

42 R_1 = H
47 R_1 = Et

[a] Reagents and conditions: (i) HMDS, reflux, 24 h; (ii) diglyme, Hünig's base, MeI, room temperature, 96 h; propylene oxide, MeOH, room temperature, 16 h.

Scheme 6[a]

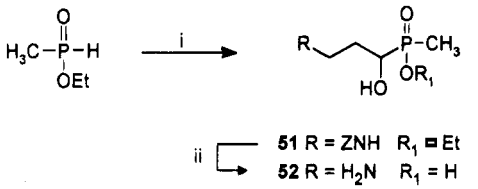


48

50 **49**

[a] Reagents and conditions: (i) Me_3SiCl, Et_3N, THF, 4 °C → room temperature, 16 h; (ii) THF, 65 °C, 24 h; (iii) H_2, CF_3CO_2H, PtO_2, 1 bar, room temperature, 2 h; (iv) Me_3SiBr, CH_3CN, room temperature, 24 h; propylene oxide, MeOH, room temperature, 2 h.

Scheme 7[a]



51 R = ZNH R_1 = Et
52 R = H_2N R_1 = H

[a] Reagents and conditions: (i) $PhCH_2OCONHCH_2CH_2CHO$, Et_3N, 100 °C, 2 h; (ii) 6 M HCl, reflux, 17 h.

instances, have to be re-esterified for subsequent P–C bond formation reactions.

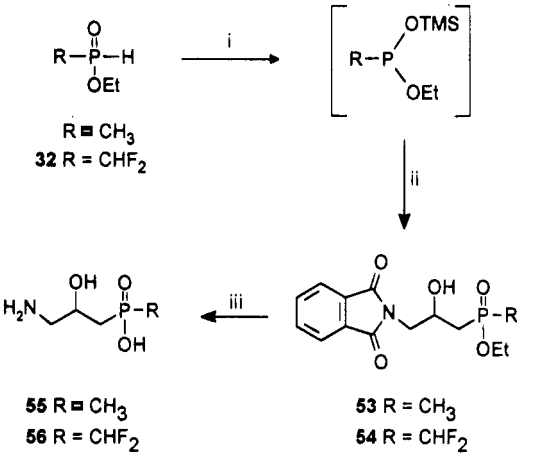
Methylphosphinic acids were also prepared directly from phosphinic acids (Scheme 5): Persilylation of **2** or **46** converted their pentavalent phosphorus into highly reactive reactive P(III) intermediates, which underwent an Arbusov reaction with methyliodide to give the corresponding methylphosphinic acids **42** or **47**.

An advantage of the P(III) intermediate (Scheme 6) is that reactions with electrophiles proceed under neutral conditions. Thus even very base-sensitive compounds as (*E*)-4,4,4-trifluorobut-2-enenitrile (**48**) can be induced to react with the trimethylsilylderivative of ethyl methylphosphinate[31] to give **49** in good yield. Further conversion of **49** to [3-amino-1-(trifluoromethyl)propyl]methylphosphinic acid **50** was achieved by catalytic hydrogenation using platinium oxide followed by ester cleavage with trimethylsilyl bromide.

Phosphinic acid esters react with aldehydes, e.g., 3-[*N*-(benzyloxycarbonyl)amino]propanal[33] under mildly basic conditions to give compounds with a substituent R_1 equal to hydroxy (Scheme 7). Thus obtained **51**, a 1:1 mixture of diastereoisomers, was hydrolyzed under acidic conditions to **52**.

Trivalent phosphorus species also react readily with epoxides under Lewis acid catalysis to produce compounds with substituent R_2 equal to hydroxy (Scheme 8). Ethyl methylphosphinate[31] or **32** were converted to the corresponding silylated P(III) intermediates by reaction with trimethylsilyl chloride in the presence of triethylamine. Reaction with *N*-(2,3-epoxypropyl)-phthalimide catalyzed by small amounts of zinc chloride in the absence of solvent gave, regiospecifically, the trimethylsilyl ethers of the 2-hydroxy-3-phthalimidopropyl phosphinic acid esters **53** and **54** as 1:1 mixtures of diastereoisomers. Under these conditions the trimethylsilyl group is transferred from the intermediate ethyl trimethylsilyl phosphonite to the newly formed secondary hydroxy group. Acidic hydrolysis furnished racemates **55** and **56**.

Scheme 8[a]



R = CH_3
32 R = CHF_2

55 R = CH_3
56 R = CHF_2

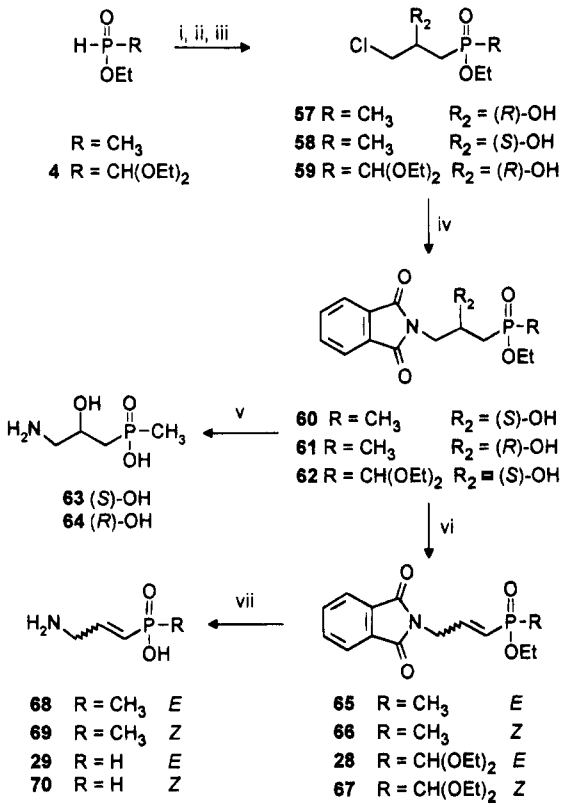
53 R = CH_3
54 R = CHF_2

[a] Reagents and conditions: (i) Me_3SiCl, Et_3N, THF, 15 °C → room temperature, 24 h; (ii) *N*-(2,3-epoxypropyl)phthalimide, 15 mol % $ZnCl_2$, 85 °C, 24 h; 1% HOAc/MeOH, room temperature, 24 h; (iii) concentrated HCl, 100 °C, 24 h; propylene oxide, MeOH, room temperature, 16 h.

This silyl transfer reaction is particularly important for the reaction of silylated phosphonites with (*R*)- or (*S*)-epichlorohydrin (Scheme 9). Several apects of this reaction are noteworthy: The reaction of the P(III) species is regiospecific, occurring only at the terminal end of the epoxide to give the trimethylsilyl ethers of **57**–**59**. No displacement of the chlorine atom was observed. The stereochemical integrity of the chiral center is preserved, i.e., no epoxide reclosure took place. This finding may be explained by the efficient silyl transfer reaction, which prevents ring closure. The trimethylsilyl ethers were hydrolyzed under very mild conditions, i.e., by stirring in methanolic solutions containing 1% acetic acid at room temperature to give the chlorohydrins **57**–**59**. Under these conditions no ring closure to epoxide derivatives occurred. Displacement of the chlorine atom with potassium phthalimide proceeded via a clean S_N2 process yielding phthalimides **60**–**62**. The 1:1 mixtures of diastereoisomers **60** and **61** were hydrolyzed under acidic conditions to yield (3-amino-2(*S*)-hydroxypropyl)- and (3-amino-2(*R*)-hydroxypropyl)methylphosphinic acids **63** and **64**, respectively. Each compound was optically pure according to HPLC analysis on chiral Crownpack columns. The X-ray analysis of the methanesulfonate salt of **63** (Figure 1) provided an unambiguous proof of its absolute stereochemistry.

Elimination of water from the 1:1 mixture of diastereoisomers **61** under Mitsonobu conditions gave (*E*)- and (*Z*)-phthalimidoesters **65** and **66** in a 1:1 ratio. After separation of the isomers by chromatography **65** was hydrolyzed under acidic conditions to [(*E*)-3-aminopro-

Scheme 9[a]



[a] Reagents and conditions: (i) Me_3SiCl, Et_3N, THF, room temperature, 24 h; (ii) (*R*)- or (*S*)-epichlorohydrin, 15 mol % $ZnCl_2$, 70 °C, 4 h; (iii) 1% HOAc/MeOH, 20 °C, 24 h; (iv) potassium phthalimide, 18-crown-6, toluene, 60 °C, 120 h; (v) concentrated HCl, reflux, 24 h; propylene oxide, MeOH, room temperature, 16 h; (vi) Ph_3P, EtOOCN=NCOOEt, THF, toluene, 5 °C → room temperature, 16 h; (vii) concentrated HCl, reflux, 5 h; propylene oxide, MeOH, room temperature, 5 °C, 16 h.

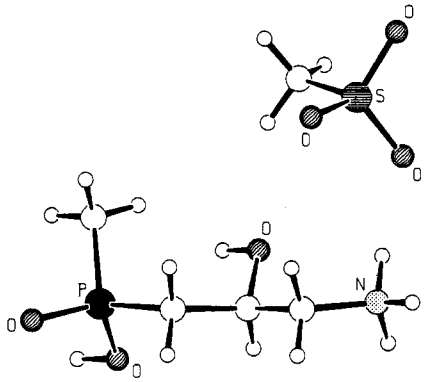


Figure 1. X-ray crystal structure showing the absolute stereochemistry of **63** methanesulfonate salt.

pen-1-yl]methylphosphinic acid (**68**) in good yield. Likewise, hydrolysis of **66** gave the (*Z*)-isomer **69**.

Elimination of water from the (diethoxymethyl)-phosphinic acid ester **62** under Mitsonobu conditions gave a 1:1 mixture of (*E*)- and (*Z*)-phthalimido esters **28** and **67**. After chromatographic separation and acidic hydrolysis of the *N*-, *O*-, and *P*-protecting groups [(*E*)- and (*Z*)-3-aminopropen-1-yl]phosphinic acids **29** and **70** were obtained. The overall yield of **29** via this procedure was significantly superior to the Horner–Emmons route of Scheme 3.

Biology

Affinity to $GABA_B$ Receptors and Structure–Activity Relationships. The (3-aminopropyl)phosphinic acids listed in Table 2 were tested for their ability to inhibit the binding of the selective $GABA_B$ agonist ligand [^{3}H]baclofen from membranes obtained from cat cerebellum tissue. The methylphosphinic acids listed in Table 3 were examined for their ability to inhibit the binding of the novel $GABA_B$ agonist ligand [^{3}H]-CGP27492 from membranes obtained from rat cerebral cortex tissue. The half maximal concentration of inhibition (IC_{50}) of each compound is shown in Tables 2 and 3. Table 4 shows the IC_{50} values of eight compounds, same batch, in both binding paradigms. Depending on the radioactive ligand, the brain tissue and the animal species used the variations of the IC_{50} values were within a very narrow range and differed at the most by a factor of 3. Therefore, the impact of this change of parameters on the discussion of the effects of various substituents on the structure activity relationships is small.

Table 4. Direct Comparison of Effects of Selected Compounds (Same Batch) on the Inhibition of Binding of [^{3}H]Baclofen to $GABA_B$ Receptors of Cat Cerebellum to the Inhibition of Binding of [^{3}H]CGP27492 to $GABA_B$ Receptors of Rat Cortex

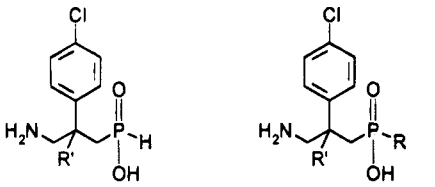
	IC_{50} (nM)	
compd	[^{3}H]baclofen	[^{3}H]CGP27492
GABA	25	17
1, (*R*,*S*)-baclofen	35	107
(*R*)-(−)-baclofen	15	32
2	2.4	5.4
3	1356	1979
42	6.6	16.3
45 (CGP35582)	166	142
55 (CGP34938)	29	77

The most potent $GABA_B$ agonists *in vitro* are the unsubstituted (3-aminopropyl)phosphinic acid (**2**) and (3-aminopropyl)methylphosphinic acid (**42**). Therefore, both phosphinic acid groups may be considered as bioisosteric replacements for the carboxylic acid group of GABA. **2** and **42** showed a higher affinity to $GABA_B$ receptors than the endogenous neurotransmitter, whereas their affinities to $GABA_A$ receptors were weaker by several orders of magnitude (Table 1). It appears that the $GABA_B$ receptors may contain functional groups capable of interacting with the small phosphinic acid residues, which are presumably not present in the $GABA_A$ receptors. The molecular basis of this finding can be explained only, when information about the precise structure of $GABA_B$ receptors becomes available. Sustained efforts to isolate and characterize $GABA_B$ receptors are underway.[34,35]

The fluorine atoms of (3-aminopropyl)(difluromethyl)-phosphinic acid (**43**) (CGP47656) may be considered as a bioisosteric replacement of the hydrogen atoms in **42**, producing a compound with high affinity to $GABA_B$ receptors. In depth biochemical investigations, *vide infra*, revealed that **43** displayed the biological properties of a $GABA_B$ receptor partial agonist. In other neurotransmitter release experiments in rat neocortex synaptosomes Raiteri et al.[36] could show that **43** acted as an antagonist on presynaptic $GABA_B$ autoreceptors increasing the release of GABA but acted as a full agonist at presynaptic $GABA_B$ heteroreceptors inhibiting the release of somatostatin. Larger substituents at the phosphorus atom, e.g., replacing methyl by hydroxymethyl as in **44**, caused a loss of affinity to $GABA_B$ receptors by a factor of 65.

Substitution of the 3-aminopropyl side chain by an alkyl group in either position R_1, R_2, or R_3, as in **7**–**9** or

Chart 3



12 R' = H
27 R' = OH

71 R = CH_3 R' = H
72 R = CH_3 R' = OH
73 R = OH R' = H

21 (Table 2), or in **45**, **47** and **50** (Table 3) provoked a substantial loss of affinity, which is true also for aryl substituents R_1 and R_3 as exemplified by **10**, **11**, and **17**. A hydroxy group R_1 as shown in **52** caused a loss of affinity to $GABA_B$ receptors by a factor of about 70 in comparison to unsubstituted **42** (Table 3).

However, as was the case with baclofen, a *p*-chlorophenyl substituent at carbon-2 of the 3-aminopropyl side chain produced potent $GABA_B$ agonists (**12**). A *p*-chloro substituent proved to be superior to other substituents, consistent with findings in the baclofen series[13] (Cl > F > H > CF_3, **12**–**15**), suggesting an additional interaction of the chloro substituent with the $GABA_B$ receptors. As described in the next chapter electrophysiological experiments revealed that **12** and **27** were $GABA_B$ agonists, whereas both of the corresponding homologous methylphosphinic acids **71** and **72** (Chart 3) displayed properties of $GABA_B$ antagonists. The syntheses and biological effects of **71** and **72** are described in part 2 of this series of papers.[20]

2-Hydroxy-substituted compounds **16**, **55**, **56**, **63**, and **64** emerged as potent $GABA_B$ agonists. The (*S*)-(−)-enantiomer **63** showed a higher affinity to $GABA_B$ receptors than the (*R*)-(+)-enantiomer **64** by a factor of three. Krogsgaard-Larsen et al.[37] obtained similar results with a series of hydroxylated GABA derivatives. They reported IC_{50}'s for 4-amino-3-(*R*)-(−)-hydroxybutyric acid and 4-amino-3-(*S*)-(+)-hydroxybutyric acid of 0.35 and 2.4 μM, respectively, i.e., inhibition of binding of [^{3}H]GABA from rat brain after blockade of the $GABA_A$ receptors by isoguvacine. Despite opposite suffixes the absolute stereochemical orientations of the more potent compounds in both series are identical, because in the Cahn Ingold Prelog nomenclature the phosphinic acid assumes a higher priority than the carboxylic acid.

Double bonds in the 3-aminopropyl side chain introduce a severe conformational restriction leading to a drastic loss of affinity, which was significantly more pronounced for derivatives with *Z* geometry in comparison to compounds with *E* stereochemistry. Compounds with an *E* double bond were weaker by factors of 40–50 in comparison to the saturated (3-aminopropyl)-phosphinic or methylphosphinic acids. In the series of phosphinic acids **29** with *E* geometry showed an IC_{50} of 280 nM in comparison to 5.4 nM for **2** (inhibition of binding of [^{3}H]CGP27492, Table 4). In the series of methylphosphinic acids **68** with *E* stereochemistry showed an IC_{50} of 665 nM in comparison to 16 nM for **42**.

In contrast, compounds with a *Z* double bond were weaker by factors of 800–1000 in comparison to the saturated (3-aminopropyl)phosphinic or methylphosphinic acids. In the series of phosphinic acids **70** with *Z* geometry showed an IC_{50} of 4.4 μM in comparison to 5.4 nM for **2** (inhibition of binding of [^{3}H]CGP27492, Table 4). In the series of methylphosphinic acids **69** with *Z* geometry showed an IC_{50} of 16.58 μM in comparison to 16 nM for **42**.[65]

Table 5. Percent Inhibition of Binding of [^{3}H]Ligands to Different CNS Receptors by **2**, **42**, and **63** at a Concentration of 10^{-5} M

receptor	[^{3}H]ligand	2	42	63
$GABA_A$	muscimol	65	0	0
benzodiazepine	flunitrazepam	24	0	0
musc. ACh.	CMD	27	0	0
α_1-adrenergic	prazosine	16	6	0
α_2-adrenergic	clonidine	nt[a]	9	0
β-adrenergic	DHA	0	0	9
5-HT_1	5-HT	13	46	7
5-HT_2	ketanserine	0	0	36
5-HT_3	BRL 43 694	nt[a]	0	0
histamine 1	doxepine	0	30	22
adenosine 1	N-6-CA	2	6	16
μ-opiate	naloxone	0	0	0
NK-1	substance P	9	2	7

[a] nt = not tested.

Selectivity for $GABA_B$ Receptors. The novel $GABA_B$ agonists displayed high affinity to $GABA_B$ receptors only. Table 5 shows the percentage of inhibition of binding of selective [^{3}H]ligands to $GABA_A$ and 12 other receptors present in the central nervous system by **2**, **42**, and **63** at a standard concentration of 10^{-5} M.

Agonistic Effects at $GABA_B$ receptors *in Vitro*. Binding experiments yield only information about the affinity of compounds to the receptor but do not differentiate between agonists or antagonists. These aspects were studied in a variety of functional tests:

2 inhibited the electrically evoked twitch contraction of guinea pig ileum and of rat anococcygeus muscle preparations being 5–7 times more potent than the prototype $GABA_B$ agonist baclofen.[40,41] Biochemical[42] and electrophysiological[43] experiments indicated that **2** may act as a partial agonist. Whole-cell patch-clamp recordings from rat hippocampal slices provided information that **2** and **42** are potent $GABA_B$ agonists at both pre- and postsynaptic $GABA_B$ receptors.[44–47]

Bath applied baclofen **1** at a concentration of 10 μM hyperpolarized hippocampal CA1 pyramidal neurons by opening potassium channels and inhibited cell firing in those neurons that were spontaneously active.[48] The novel $GABA_B$ agonists produced qualitatively similar effects at the following concentrations: at 6 μM, **27** (CGP 49713; effects are shown in comparison to **1** in Figure 2) and **43**, **55**, and **64**; at 10 μM, **12**, **44**, **52**, and **63**; at 100 μM, **42**. Surprisingly, **2** did not show inhibiting effects in this paradigm. This effect may be due to a possible oxidation of **2** to the corresponding phosphonic acid **30** (Chart 2).

When $GABA_B$ agonists interact with presynaptic $GABA_B$ autoreceptors, they cause an inhibition of the electrically induced release of [^{3}H]GABA from rat cortical slices. This effect is more pronounced at lower than at higher frequencies of electrical stimulation.[49,50] The half-maximal concentration of inhibition (IC_{50}) for **2**, **42**, **43**, **63**, and **64** in comparison to both enantiomers of baclofen, determined at a stimulation frequency of 0.125 Hz, are shown in Table 6. The most potent compound, **2**, inhibited GABA release about 3 times more effectively than did (*R*)-(−)-baclofen, whereas **42**, **63**, and **64** were about equipotent to (*R*)-(−)-baclofen.

The IC_{50} values for inhibition of GABA release were 4 (for **64**) to 30-fold (for **42**) lower than those with respect to $GABA_B$ binding. However, a direct comparison between the data of the $GABA_B$ binding and GABA

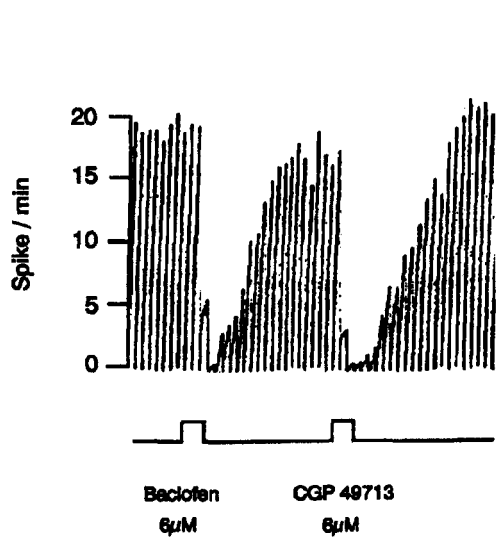


Figure 2. Intracellular recordings from CA1 pyramidal neurons. Bath application of 6 μM baclofen and 6 μM **27** induced hyperpolarization of the membrane potential.

Table 6. Inhibition of Release of [^{3}H]GABA from Rat Cortical Slices after Electrical Stimulation (Frequency, 0.125 Hz)

compd	IC_{50} (μM)
(*R*)-(−)-baclofen	0.33
(*S*)-(+)-baclofen	213
2	0.106
42	0.45
43	10.6
63	0.40
64	0.60

release experiments is not correct. The IC_{50} values of the binding experiments represent an integral of interactions of the $GABA_B$ agonists with several pre- and postsynaptic subtypes of $GABA_B$ receptors, whereas, on the other hand, the IC_{50} values for the inhibition of GABA release reflect the interaction of the $GABA_B$ agonists with one single, presynaptically located, $GABA_B$ receptor subtype. Additionally the $GABA_B$ autoreceptor is already activated by at least 50% at the low stimulation frequency of 0.125 Hz both by basally released GABA and by GABA released by the electrical stimulation.[51]

Interestingly, **43** inhibited [^{3}H]GABA release, when the slices were stimulated at a frequency of 0.125 Hz (IC_{50} = 10.6 μM), whereas upon stimulation at a frequency of 2 Hz it increased the release of [^{3}H]GABA (EC_{150} = 62 μM, i.e., the concentration causing a 50% increase with respects to controls). This pattern is indicative of the properties for a partial $GABA_B$ receptor agonist. For **43** the differences of the IC_{50} value for inhibition of GABA release and the IC_{50} of binding to $GABA_B$ receptors were particulary large, by a factor of 140, which is compatible with partial agonism

Therapeutic Potential of $GABA_B$ Agonists. The potent $GABA_B$ agonist baclofen is generally considered to be the drug of choice for the treatment of spasticity.[52] Its mechanism of action is the suppression of mono- and polysynaptic reflex pathways in the spinal cord due to blockade of the release of the excitatory amino acids glutamate and aspartate[12,53] via inhibition of Ca^{2+} currents in the presynaptic terminals of primary afferent fibers.

The activity of selected potent $GABA_B$ agonists, **2**, **42**, and the enantiomers **63** and **64**, on the blockade of monosynaptic reflexes in the hemisected neonatal rat spinal cord preparation is shown in Table 7 in comparison to the effects of (*R*)-(−)-baclofen. **42** was 6 times more potent than (*R*)-(−)-baclofen; **63** was twice as potent and approximately 3 times more potent than its (*R*)-enantiomer **64**. **2** was much less effective to block the monosynaptic reflexes in the spinal cord, which may be due to the metabolic lability of the P−H bond. **2**, under *in vivo* conditions, may be oxidized to the corresponding phosphonic acid **30** (Chart 2), which was identified as a weak $GABA_B$ antagonist in electrophysiological paradigms.

Table 7. Blockade of the Monosynaptic Reflexes in the Hemisected Neonatal Spinal Cord

compd	IC_{50} (μM)
(*R*)-(−)-baclofen	0.325
2	60
42	0.050
63	0.161
64	0.425

Table 8. Deterioration of Rotarod Performance of Rats after Treatment with Selected $GABA_B$ Agonists (EC_{50}s in μmol/kg, sc and po Administration)

compd	sc[a]	po[b]
1, (*R*,*S*)-baclofen	24	52
(*R*)-(−)-baclofen	9	16
(*S*)-(+)-baclofen	>200	>200
12	19	>215
2	>813	nt[c]
42	2	9
43	22	101
63	2	36
64	13	135

[a] As observed 0.5 to 1 h after sc drug administration (i.e., at time point of maximal effect = minimal EC_{50}). [b] As observed 1.5 to 2 h after po drug administration (i.e., at time point of maximal effect = minimal EC_{50}). [c] Not tested.

Additionally, it was observed that the inhibition of the spontaneous electrical activity of the spinal cord of **63** was significantly longer lasting than that of (*R*)-(−)-baclofen: A 4 min application of a 0.5 μM solution of **63** induced complete inhibition of the spontaneous activity for 9.5 min as compared to a duration of 6.9 min caused by application of a 0.5 μM solution of (*R*)-(−)-baclofen. The duration of the effect was 37% longer for **63** in comparison to (*R*)-(−)-baclofen.

The rotarod test, where rodents have to keep their balance on a rotating cylinder, was used to assess the antispastic and muscle relaxant effects of the novel $GABA_B$ agonists. The results are presented in Table 8 for sc and po administration. [3-Amino-2-(*p*-chlorophenyl)]propylphosphinic acid **12** (CGP35832) was equipotent to **1**, when administered subcutaneously, but was inactive after oral administration. This finding may be due to oxidation of **12** under *in vivo* conditions to the corresponding phosphonic acid **73**, i.e., phaclofen,[54] a weak $GABA_B$ antagonist (Chart 3).

2, although very potent in a large variety of *in vitro* paradigms, did not show muscle relaxant effects even after subcutaneous administration. The most potent compound in the rotarod test was **42**. However, toxic effects were observed at relatively low doses after sc and po administrations. The partial $GABA_B$ agonist **43** also produced muscle relaxant effects comparable to those of baclofen. Again, toxic side effects precluded further evaluation.

The requirements of a valuable antispastic agent were fulfilled by **63**, which proved to be more potent than baclofen. With respect to the time course of the muscle relaxant activity after po administration, **63** showed a slightly delayed onset of action but considerably exceeded the duration of action of baclofen. At twice the EC_{50} the duration of action of **63** was 9 h versus 3 h for baclofen. **63** showed a significantly larger therapeutic window than any other new $GABA_B$ agonist.

In cases of severe spasticity the doses of baclofen required by some individuals exceed the tolerated doses. The most frequent side effects at high doses of baclofen are sedation, vertigo, nausea, and gastrointestinal side effects such as vomiting and diarrhoea. Extensive comparative studies in three groups of four Rhesus monkeys each (single blind paradigm, baclofen *versus* **63** *versus* placebo) demonstrated that **63** produced pronounced muscle relaxation but no sedation. The monkeys were capable of reacting fully to external stimuli such as noise or offered food. They did not show any gastrointestinal side effects.

In conclusion, considerably stronger muscle relaxation could be achieved with **63** than with baclofen without the occurrence of sedation, reduced vigilance, vomiting or diarrhoea. **63** was selected as a development compound for the treatment of spasticity.

Experimental Section

Melting points were determined on a Reichert Kofler block and are uncorrected. Thin layer chromatography (TLC) was performed on precoated silica gel plates (E. Merck, silica gel 60 F_{254}, 0.25 mm), and components were visualized by UV light of λ 254 nm, by iodine vapor, or by spraying with ninhydrin solutions. Column flash chromatography was performed on silica gel 60, 0.040–0.063 mm (230–400 mesh, ASTM, E. Merck), eluting under a positive pressure of approximately 20 psi of nitrogen ensuring a flow rate of about 5 mL/min. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a 10 cm cuvette. Optical purities were measured on a Hewlett-Packard HPLC series 1050 using Crownpack CR 100 × 4 mm columns and refractive index detection. Elemental combustion analyses were performed on a Perkin-Elmer PE-240 or a Leco CHN-800. Elemental analyses were within ±0.4% of the calculated values, unless stated otherwise. ^{1}H NMR spectra were recorded on Varian-60, Varian-90, Varian-Gemini-200/300, Varian-Gemini-250 or on Bruker-AM-360 spectrometers. Chemical shifts δ are expressed in parts per million (ppm) relative to tetramethylsilane as internal standard. Coupling constants J are reported in hertz. ^{13}C NMR and ^{31}P NMR spectra were recorded on a Bruker-AM-360 spectrometer. Mass spectra were obtained with a Varian CH7/MAT212. High-resolution FAB mass spectra were measured using a Finnigan MAT-90 or a Visons Instruments VG70-SE. The X-ray crystallographic analysis was carried out using a Enraf-Nonius CAD4 diffractometer with graphite monochromated CuKα radiation.

2(*R*)-(*N*-Tritylamino)propanal (19). A solution of 13.2 g (40 mmol) of *N*-trityl-D-alanine[55] in 200 mL of dry THF was cooled to 5 °C under argon, and 200 mL of a 1 M solution of diborane in THF (0.2 mol) was added dropwise over 30 min while the temperature was maintained at 5 °C. The solution was stirred for 24 h at room temperature and quenched with 50% aqueous THF. Potassium carbonate (10 g, 72 mmol) was added and the suspension stirred for 10 min. The THF layer was removed and the aqueous layer extracted twice with 10 mL of THF. The combined organic layers were evaporated to give an oil. Chromatography on silica gel using 7:3:1 toluene–petroleum ether (40–60)–ethyl acetate as eluant gave 10.5 g (83%) of 2(*R*)-(*N*-tritylamino)propanol: mp 84–86 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.66–7.09 (m, 15H, aromatic CH), 3.20–3.05 (m, 2H, CH_2O), 3.00–2.60 (m, 1H, CH), 0.67 (d, J = 4 Hz, 3H, CH_3).

Oxalyl chloride (15 g, 11 mmol) was dissolved in 25 mL of DCM and cooled to −78 °C under argon. A solution of 1.8 g (22 mmol) of DMSO in 10 mL of DCM was added slowly over 2 min and the resulting solution stirred for 5 min at −78 °C. A solution of 3.1 g (10 mmol) of 2(*R*)-(*N*-tritylamino)propanol in 10 mL of DCM was added slowly over 2 min and the reaction stirred for 15 min at −78 °C. A solution of 5 g (50 mmol) of triethylamine in 15 mL of DCM was added over 2 min upon which a precipitate formed. The reaction was stirred for 10 min at −78 °C and warmed to room temperature. Water (50 mL) was added and the organic layer separated. The aqueous layer was extracted twice with 50 mL of DCM, the combined organic layers were dried over magnesium sulfate and filtered, and the solvent was removed to give a pale yellow oil. Filtration through silica gel with toluene as eluant gave 2.7 g (87%) of **19** as an oil: ^{1}H NMR (90 MHz, $CDCl_3$) δ 8.50 (d, $J \leq 2$ Hz, 1H, CHO), 7.60–7.10 (m, 15H, aromatic CH), 3.45–3.25 (m, 1H, CH), 1.00 (d, J = 4.5 Hz, 3H, CH_3).

[3(*R*)-Amino-2(*R,S*)-hydroxybutyl)phosphinic Acid (21). A solution of 9.6 mmol of lithium diisopropylamide in dry THF was cooled to −78 °C under argon, and a solution of 1.7 g (8 mmol) of **18**[22] in 15 mL of dry THF was added over 2 min by syringe. The mixture was stirred for 1 h at −78 °C, and 2.5 g (8 mmol) of **19** in 15 mL of dry THF was added over 2 min. The resulting mixture was warmed to room temperature and stirred overnight. The solvent was evaporated and the residue partitioned between saturated aqueous ammonium chloride and ethyl acetate. The organic layer was removed, dried over magnesium sulfate, and filtered and the solvent removed to give 4.1g of an oil. Chromatography on silica gel using 2:1 ethyl acetate–petroleum ether (40–60) as eluant gave 3.1 g (74%) of ester **20** as a 1:1 mixture of diastereoisomers: ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.60–7.10 (m, 15H, aromatic CH), 4.75 (d, J = 6 Hz, 1H, CHP), 4.35–3.95 (m, 3H, CH_2OP and CHOH), 3.90–3.60 (m, 4H, CH_2), 2.80–2.30 (m, 1H, CHN), 2.10–1.75 (m, 2H, CH_2P), 1.45–1.05 (m, 6H), 0.65 and 0.35 (d, J = 4 Hz, total 6H, CH_3). Anal. ($C_{30}H_{40}NO_5P$) C, H, N, P.

A mixture of 1.5 g (2.8 mmol) of **20** and 50 mL of 2 M hydrochloric acid was heated to reflux for 4 h. The reaction mixture was cooled to room temperature and extracted three times with 25 mL of DCM. The aqueous layer was evaporated to dryness to give a viscous oil which was coevaporated with 3 × 100 mL water and 3 × 100 mL of absolute ethanol to give a solid. This was dissolved in 30 mL of ethanol and 10 mL of propylene oxide added slowly. The resulting suspension was stirred at room temperature for 24 h and the solid collected by filtration and dried in high vacuum to give 0.3 g (70%) of **21** as a 1:1 mixture of diastereoisomers: mp 207–210 °C; ^{1}H NMR (90 MHz, D_2O) δ 9.00 (d, J = 550 Hz, 1H, PH), 4.02–3.95 (m, 1H, C*H*OH), 3.32–3.21 (m, 1H, CHN), 2.00–1.55 (m, 2H, CH_2P), 1.29–1.20 (m, 3H, CH_3). Anal. ($C_4H_{12}NO_3P$) C, N, P; H: calcd, 7.90; found, 7.49.

Ethyl (1,1-Diethoxyethylpropa-1,2-dienyl)phosphinate (24). A solution of 153 g (1.46 mol) of 1,2-propadienylphosphinic acid[25] in 750 mL of DCM/THF (2:1) was cooled to −5 °C under argon, and 151 g (1.5 mol) of triethylamine was added dropwise. An exothermic reaction occurred, the solution was recooled to −5 °C, and 162.74 g (1.5 mol) of ethyl chloroformate was added dropwise over 2 h. Gas evolution was observed, and the reaction became exothermic with the appearance of a white precipitate. After the addition was complete, the reaction was warmed to room temperature, stirred for 16 h, and filtered. The filtrate was evaporated *in vacuo* to about 500 mL and washed with saturated aqueous ammonium chloride solution. The organic phase was dried and the solvent removed to give an oil. Distillation under high vacuum afforded 50 g (26%) of **23**: bp 53–55 °C/10^{-1} mbar; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.35 (dd, J = 588 and 5 Hz, 1H, P-H), 5.43 (tdd, J = 13, 5, and 1.5 Hz, 1H, C=CHP), 5.12 (d, J = 13 Hz, 1H, CH=C), 5.04 (d, J = 13 Hz, 1H, CH=C), 4.14 (q, J = 7 Hz, 2H), 1.36 (t, J = 7 Hz, 3H).

A solution of 40 g (0.303 mol) of **23** in 100 mL of triethyl orthoacetate was treated with 1.5 mL of boron trifluoride diethyl etherate and stirred at room temperature for 24 h. The reaction mixture was diluted with DCM and washed with 10% aqueous sodium bicarbonate solution. The organic layer was removed, dried over magnesium sulfate, and filtered and the

solvent removed *in vacuo* to give 68.5 g (91%) of **24** as an oil which was used without further purification, as extensive losses occurred upon distillation: ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.41 (q, J = 12 Hz, 1H), 5.00 (dd, J = 12 and 6 Hz, 2H), 4.20 (q, J = 6 Hz, 2H), 3.80–3.60 (m, 4H), 1.50 (d, J = 12 Hz, 3H), 1.33 (t, J = 6 Hz, 3H), 1.20 (t, J = 6 Hz, 6H).

Ethyl [2-(4-Chlorophenyl)propen-2-yl](1,1-diethoxyethyl)phosphinate (25). Magnesium turnings (2.43 g, 0.1 mol) were placed in a two-necked flask under argon, and a solution of 23.85 g (0.1 mol) of 4-chloroiodobenzene in 100 mL of dry diethyl ether was added until the magnesium was just covered. The reaction was initiated by gentle warming and the rest of the 4-chloroiodobenzene solution added at such a rate so as to maintain reflux. After the addition was complete, reflux was continued for 1 h after which time a brown solution had formed. This solution was cooled to 0 °C and added slowly to a suspension of 20.56 g (0.1 mol) of copper(I) bromide dimethyl sulfide complex in 30 mL of diethyl ether and dimethyl sulfide (3:2 v/v) at −45 °C under argon. The resulting suspension was stirred at −45 °C for 3 h, warmed to −20 °C, stirred for 2 h, and recooled to −45 °C before addition of a solution of 24.85 g (0.1 mol) of **24** in 10 mL of diethyl ether over a period of 15 min. The temperature was allowed to rise to −20 °C and the reaction mixture stirred at this temperature for 1 h and quenched with saturated aqueous ammonium chloride solution. The mixture was partitioned between DCM and water, the organic phase dried over magnesium sulfate and filtered, and the solvent removed to give an orange-brown oil. Chromatography on silica gel using hexane–ethyl acetate, initially 2:1 then 1:1 as eluants gave 15.5 g (42%) of **25**: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.50–7.42 (m, 2H, aromatic CH), 7.32–7.25 (m, 2H, aromatic CH), 5.52 (d, J = 6 Hz, 1H, CH=C), 5.35 (d, J = 6 Hz, 1H, CH=C), 4.05 (q, J = 6 Hz, 2H, ester CH_2), 3.85–3.58 (m, 4H, acetal CH_2), 3.05 (d, J = 15 Hz, 2H, CH_2P), 1.50 (d, J = 12 Hz, 3H, $PCCH_3$), 1.26–1.17 (m, 6H), 1.15 (t, J = 6 Hz, acetal CH_3).

Ethyl [3-[(*tert*-Butoxycarbonyl)amino]-2-(4-chlorophenyl)-2-hydroxypropyl](1,1-diethoxyethyl)phosphinate (26). A solution of 0.351 g (3 mmol) of *tert*-butyl carbamate in 1.5 mL of methanol was cooled to 0 °C under argon, 0.324 g (3 mmol) of *tert*-butyl hypochlorite was added slowly, and the resulting solution was stirred for 15 min at 0 °C. A solution of 0.126 g (3 mmol) of sodium hydroxide in 1.5 mL of methanol was added and the cooling bath removed. The solution was stirred for 10 min and the solvent evaporated. The residue was dried in high vacuum, stirred with ether, and filtered. The beige crystals were suspended in 8 mL of acetonitrile at room temperature and treated with 0.339 g (2 mmol) of silver nitrate followed by 0.716 g (2 mmol) of **25** in 2 mL of acetonitrile. This suspension was then treated with 0.203 mL of a 2.5% solution of osmium tetraoxide in *tert*-butyl alcohol (0.02 mmol) and 0.162 mL (9 mmol) of water. The black suspension was stirred for 24 h at room temperature and filtered and the filtrate treated with 4 mL 5% aqueous sodium bisulfite solution. This mixture was refluxed for 3 h and the acetonitrile removed *in vacuo*. The residue was diluted with water and extracted with chloroform. The organic phase was dried over magnesium sulfate and filtered and the solvent removed to give a yellow oil. Chromatography on silica gel using hexane–ethyl acetate, initially 4:1 and increasing to 1:1 as eluants, allowed separation of the diastereoisomers of **26** in 41% total yield.

Less polar diastereoisomer: 0.23 g; R_f = 0.71 (ethyl acetate–hexane, 1:1); ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.43–7.34 (m, 2H, aromatic CH), 7.28–7.20 (m, 2H, aromatic CH), 5.75 (s, 1H, exch. D_2O, OH), 5.05 (br t, J = 6 Hz, 1H, exch. D_2O, NH), 3.69–3.51 (m, 5H, 2 acetal CH_2 and POCH), 3.48–3.28 (m, 2H, POCH and CHN), 3.06–3.00 (m, 1H, CH-N), 2.55 (dd, J = 18 and 6 Hz, 1H, CHP), 2.12 (dd, J = 18 and 12 Hz, 1H, CHP), 1.44–1.25 (m, 12H, *t*-Bu and $PCCH_3$), 1.20–1.05 (m, 6H, acetal CH_3), 0.77 (t, J = 6 Hz, 3H, CH_3); ^{13}C NMR (360 MHz, $CDCl_3$) δ 156.0 (C=O), 142.5 (aromatic tertiary C), 132.9, (CCl), 127.9 and 127.2 (aromatic C), 100.0 (d, $PCCH_3$), 79.5 (Me_3-*C*-O), 75.3 (COH), 61.5 ($POCH_2$), 58.0 and 57.8 (CH_2OC), 52.2 (CH_2N), 32.0 (d, CH_2P), 28.1 (3 CH_3), 19.8 (d, $PCCH_3$), 15.7 ($POCH_2CH_3$), 15.2 (acetal CH_3); ^{31}P NMR (360 MHz, $CDCl_3$) δ +51.

More polar diastereoisomer: 0.17 g; R_f = 0.52 (ethyl acetate–hexane, 1:1); ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.43–7.34 (m, 2H, aromatic CH), 7.32–7.27 (m, 2H, aromatic CH), 5.45 (br s, 1H, exch. D_2O, OH), 4.98 (br t, 1H, exch. D_2O, NH), 4.33–4.12 (m, 2H, CH_2OP), 3.73–3.50 (m, 6H, 2 acetal CH_2 and CH_2N), 2.47 (dd, J = 15 and 9 Hz, 1H, CHP), 2.22 (dd, J = 15 and 12 Hz, 1H, CHP), 1.45–1.27 (m, 15H, *t*-Bu, CH_3, and CH_3-CP), 1.22–1.07 (m, 6H, 2 acetal CH_3); ^{13}C NMR (360 MHz, $CDCl_3$) δ 156.5 (C=O), 143.3 (aromatic tertiary C), 133.3 (CCl), 128.5 and 127.2 (aromatic C), 101.0 (d, $PCCH_3$), 79.6 (Me_3CO), 75.5 (COH), 62.6 ($POCH_2$), 58.5 and 58.2 (CH_2OC), 50.8 (CH_2N), 35.5 (CH_2P), 28.5 (3 CH_3), 21.0 ($PCCH_3$), 16.9 ($POCH_2CH_3$), 15.6 (acetal CH_3); ^{31}P NMR (360 MHz, $CDCl_3$) δ +45.

[3-Amino-2(*R,S*)-(4-chlorophenyl)-2-hydroxypropyl]-phosphinic Acid (27). A solution of 0.155 g (0.315 mmol) of the diastereoisomeric mixture **26** in 3 mL of DCM was treated with 0.241 g (1.575 mmol) of bromotrimethylsilane at room temperature for 24 h. The volatile material was removed *in vacuo* and the residue dissolved in methanol containing 1% water. After the mixture was stirred for 30 min at room temperature the solvent was removed, and the residue was redissolved in 1 mL of methanol and treated with propylene oxide until neutral, after which a white precipitate appeared, which after stirring overnight at room temperature was collected by filtration and dried to give 0.055 g (73%) of **27**: mp 208–209 °C; ^{1}H NMR (360 MHz, D_2O + 1 drop trifluoroacetic acid) δ 7.50–7.25 (m, 4H), 6.75 (d, J = 568 Hz, 1H), 3.18 (AB q, J = 18 and 4 Hz, 2H, CH_2N), 2.42 (AB q, J = 12 and 7 Hz, 2H, CH_2P); high-resolution FAB MS (matrix: glycerin saturated with CsI) calcd for $C_9H_{12}ClNO_3P$ $(M - H)^-$ 248.0243; found 248.0232 ± 0.0003 (n = 10).

((*E*)-3-Aminopropen-1-yl)phosphinic Acid (29). A solution of 5.0 g (12.37 mmol) of diethyl methylenebis(diethoxymethyl)phosphinate[27,28] in 50 mL of dry THF was added to a suspension of 0.29 g (12.37 mmol) of sodium hydride in 20 mL of dry THF under argon at room temperature. After stirring for 15 min a clear pale yellow solution had formed. This was then added, via syringe, to a solution of 2.34 g (12.37 mmol) of 2-phthalimidoacetaldehyde[29] in dry THF at 4 °C. After the addition was complete the mixture was stirred at 4 °C for 15 min. The red reaction mixture was quenched with saturated aqueous ammonium chloride solution and extracted with diethyl ether. The organic extract was dried over magnesium sulfate and filtered and the solvent removed. The oily residue was purified by chromatography on silica gel using ethyl acetate as the eluant to give 0.6 g (13%) of **ethyl (*E*)-(3-*N*-phthalimidopropen-1-yl)(diethoxymethyl)phosphinate (28)** as a viscous oil: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.90–7.81 (m, 2H, aromatic CH), 7.76–7.70 (m, 2H, aromatic CH), 6.80 (ddt, J = 19, 17, and 5 Hz, 1H, C=C*H*CH_2N), 5.90 (ddt, J = 21, 17, and 2 Hz, 1H, PCH=C), 4.62 (d, J = 7 Hz, 1H, PCH), 4.45 (ddd, J = 5, 5, and 2 Hz, 2H, CH_2N-phthalimide), 4.15–4.05 (m, 2H, ester CH_2), 3.95–3.84 (m, 2H, acetal CH_2), 3.82–3.78 (m, 2H, acetal CH_2), 1.30–1.19 (m, 9H, ester CH_3 and 2 acetal CH_3); ^{31}P NMR (360 MHz, $CDCl_3$) δ +30.5.

A solution of 0.6 g (1.57 mmol) of **28** in 50 mL of concentrated hydrochloric acid was heated to reflux for 24 h. After cooling the acid was removed by coevaporation with water (5 × 50 mL). The crude product was purified by dissolution in 5 mL of water and ion exchange chromatography on Dowex 50 W X 2 acidic resin, eluting with 1 M ammonium hydroxide solution. The ninhydrin positive fractions were combined and evaporated to give 0.12 g (63%) of **29**: mp 234–236 °C; ^{1}H NMR (360 MHz, CH_3OD) δ 7.12 (d, J = 517 Hz, 1H, PH), 6.30 (ddt, $J_{b,P}$ = 21.0 Hz, $J_{a,b}$ = 17.2 Hz, $J_{b,c}$ = 5.4 Hz, 1H, NCH_2-CH_b=CP), 6.10 (ddt, $J_{a,P}$ = 17.2 Hz, $J_{a,b}$ = 17.2 Hz, $J_{a,c}$ = 1.5 Hz, 1H, PCH_a=C), 3.60 (dd, $J_{b,c}$ = 5.4 Hz, J_{ac} = 1.5 Hz, 2H, CH_2N); ^{31}P NMR (360 MHz, CH_3OD) δ +13.2; high-resolution FAB MS (matrix: glycerin saturated with CsI) calcd for $C_3H_9NO_2P$ $(M + H)^+$ 122.0371, found 122.0375 ± 0.0016.

Ethyl (1,1-Diethoxyethyl)phosphinate[32] (31). An aqueous solution of phosphinic acid (300 mL, 50%, 2.75 mol) was concentrated *in vacuo* at a temperature ≤ 40 °C to give 190 g of 95% pure acid, which slowly crystallized. Triethyl orthoacetate 2.05 L (11.2 mol) was cooled to 5 °C, under argon, and 50 mL (0.4 mol) of boron trifluoride–diethyl etherate was

added slowly. The mixture was recooled to −5 °C and 190 g of phosphinic acid added slowly. After the addition was complete the mixture was warmed to room temperature over 3 h. A 1.2 M solution of disodium hydrogen phosphate decahydrate (500 mL) was added and the mixture extracted with 4 × 200 mL of dichloromethane. The combined organic layers were washed with 1 L of water, dried over magnesium sulfate, and filtered, and the solvent was removed to give 500 g of a yellow oil. Molecular distillation under high vacuum gave 338 g (60%) of **31**: bp 65 °C/10^{-3} mbar; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.35 (d, J = 588 Hz, 1H), 4.14 (q, J = 6 Hz, 2H, CH_2P), 3.87−3.65 (m, 4H, acetal CH_2), 1.36 (d, J = 15 Hz, 3H, $PCCH_3$), 1.25 (t, J = 6 Hz, 3H), 1.17 (t, J = 6 Hz, 6H).

Ethyl (Difluoromethyl)phosphinate (32). A suspension of 2.64 g (55 mmol) of 50% sodium hydride in oil in 30 mL of anhydrous THF was cooled to 4 °C under argon and a solution of 11.2 g (50 mmol) of **31** in 10 mL of anhydrous THF added dropwise over 20 min while the temperature was maintained at 4 °C. The mixture was stirred for 1 h at 4 °C and cooled to −10 °C, and 13 g (150 mmol) of chlorodifluoromethane was introduced. The thick suspension was stirred for 1.5 h at ice-bath temperature. Ice-cold water was added slowly, and the volatile material was removed by evaporation. The residue was extracted 3 × with 50 mL of chloroform, and the combined organic phases were dried over magnesium sulfate, filtered, and evaporated to give 12.3 g (95%) of ethyl (difluoromethyl)-(1,1-diethoxyethyl)phosphinate as a colorless oil, which was used without further purification: ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.04 (td, J = 40 and 20 Hz, 1H, $PCHF_2$), 4.33−4.18 (m, 2H, ester CH_2), 3.91−3.64 (m, 4H, 2 acetal CH_2), 1.53 (d, J = 10 Hz, 3H, $PCCH_3$), 1.35 (t, J = 5 Hz, 3H), 1.21 (t, J = 5 Hz, 6H). A solution of 8.23 g (32 mmol) of ethyl (difluoromethyl)(1,1-diethoxyethyl)phosphinate in 500 mL of dichloromethane containing 50 mL of absolute ethanol was treated with 60 mL (47 mmol) of trimethylchlorosilane at 4 °C. The reaction was stirred for 48 h at 4 °C, and the volatile materials were removed to give 4.6 g (100%) of **32** as an oil, which was used without further purification: ^{1}H NMR (360 MHz, $CDCl_3$) δ 9.0 (d, J = 520 Hz, 1H, PH), 5.90 (td, J = 40 and 25 Hz, 1H), 4.38−4.15 (m, 2H, ester CH_2), 1.35−1.20 (m, 3H, ester CH_3).

Ethyl (Hydroxymethyl)phosphinate (33). A mixture of 60 g (0.285 mol) of **31**, 28.8 g (0.285 mol) of triethylamine, and 60 g (2 mol) of paraformaldehyde was heated to 130 °C for 2 h. The mixture was then diluted with 500 mL of a DCM/water (1:1) mixture and filtered. The organic phase was separated and the aqueous layer re-extracted with a further 200 mL of DCM. The combined organic layers were dried over magnesium sulfate and filtered, and the solvent was removed to give an oil. Molecular distillation gave 35.2 g (51%) of ethyl (hydroxymethyl)(1,1-diethoxyethyl)phosphinate: ^{1}H NMR (360 MHz, CD_3Cl) δ 4.25 (q, J = 9 Hz, 2H, ester CH_2), 3.98 (AB qd, J = 18 and 5 Hz, 2H, PCH_2OH), 3.81−3.59 (m, 4H, 2 ketal CH_2), 1.57 (d, J = 12 Hz, 3H, $PCCH_3$), 1.35 (t, J = 6 Hz, 3H, ester CH_3), 1.21 (t, J = 6 Hz, 6H, 2 ketal CH_3). Treatment of this intermediate with chlorotrimethylsilane in dichloromethane and ethanol as described above for **32** gave **33** in 100% yield, which was used without further purification: ^{1}H NMR (360 MHz, CD_3Cl) δ 7.08 (d, J = 546 Hz, PH), 5.05 (br s, exch. D_2O, OH), 4.25−4.05 (m, 2H, ester CH_2), 4.01−3.90 (m, 2H, CH_2-OH), 1.17 (t, J = 6 Hz, 3H, ester CH_3); ^{31}P NMR (300 MHz $CDCl_3$) δ +35.8.

Ethyl (2-Cyanoethyl)methylphosphinate (34). Sodium (1.60 g, 69 mmol) was dissolved in 25 mL of absolute ethanol, and the solution was cooled to 4 °C under argon. A mixture of 15.0 g (139 mmol) of ethyl methylphosphinate[31] and 7.37 g (139 mmol) of acrylonitrile in 25 mL of absolute ethanol was added dropwise. The mixture was warmed to room temperature and stirred for 24 h. Glacial acetic acid (4.14 g, 69 mmol) was then added and the solvent removed. The residue was dissolved in 200 mL of DCM and washed with water. The organic layer was separated and the aqueous layer re-extracted with DCM. The combined organic layers were dried over magnesium sulfate and filtered, and the solvent was removed to give a pale yellow oil. Distillation in high vacuum afforded 18.5 g (83%) of **34** as a colorless oil: bp 140 °C/2 × 10^{-1} mbar; ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.15−3.98 (m, 2H, ester CH_2), 2.72−2.66 (m, 2H, CH_2N), 2.10−2.00 (m, 2H, CH_2P), 1.60 (d, J = 15 Hz, 3H, PCH_3), 1.30−1.20 (m, 3H); ^{31}P NMR (360 MHz, $CDCl_3$) δ +50.5.

Ethyl (2-Cyanoethyl)(difluoromethyl)phosphinate (35). **35** was prepared as for **34** from 4.62 g (32.1 mmol) of **32** but purified via chromatography on silica gel, using 7:3 ethyl acetate−hexane as eluant gave 2.59 g (41%) of **35** as an oil: ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.01 (td, J = 42.5 and 20 Hz, 1H, CHF_2), 4.39−4.11 (m, 2H, ester CH_2), 2.73−2.55 (m, 2H, CH_2CN), 2.31−2.11 (m, 2H, CH_2P), 1.41 (t, J = 5 Hz, 3H, ester CH_3); MS m/e 197 (M^+).

Ethyl (2-Cyanoethyl)(hydroxymethyl)phosphinate (36). **36** was obtained as for **34** without further purification in 93% yield: ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.30−4.10 (m, 2H, ester CH_2), 4.10−3.96 (m, 2H, CH_2OH), 2.95−2.80 (m, 2H, CH_2CN), 2.40−2.36 (m, 2H, CH_2P), 1.37 (t, J = 5 Hz, 3H, CH_3).

Ethyl 2-Cyano-1-methylethyl-methylphosphinate (37). **37** was prepared as for **34** using crotononitrile: 40% yield; bp 110 °C/10^{-1} mbar; ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.10−3.98 (m, 2H, ester CH_2), 2.84−2.69 (m, 2H, CH_2CN), 2.40−2.32 (m, 1H, CH), 1.64 (d, J = 15 Hz, 3H, PCH_3), 1.23 (t, J = 5 Hz, 3H, ester CH_3), 1.10−0.95 (m, 3H, alkyl CH_3); ^{31}P NMR (360 MHz, $CDCl_3$) δ +55.9 and 55.5.

Ethyl (3-Aminopropyl)methylphosphinate (38). A solution of 18.0 g (0.112 mol) of **34** in 200 mL of absolute ethanol containing 16 g of ammonia was treated with 15 mL of a Raney nickel slurry and the suspension hydrogenated at normal pressure until hydrogen uptake ceased (4 h). The mixture was filtered and the filtrate concentrated to give a light green oil. Distillation in high vacuum gave 13.86 g (75%) of **38** as a colorless oil: bp 130 °C/10^{-2} mbar; ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.05−3.95 (m, 2H, ester CH_2), 3.03 (t, J = 6 Hz, 2H, CH_2N), 2.10−1.64 (m, 4H), 1.30 (d, J = 18 Hz, 3H, P-CH_3), 1.18 (t, J = 6 Hz, 3H, ester CH_3); ^{31}P NMR (360 MHz, $CDCl_3$) δ +50.5.

Ethyl (3-Aminopropyl)(difluoromethyl)phosphinate (39). Hydrogenation of 2.59 g (13.15 mmol) of **35** and workup as described above followed by chromatography of the residue on silica gel eluting with DCM−methanol−ammonia (80:19:1) gave 1.39 g (53%) of **39** as a colorless oil: ^{1}H NMR (360 MHz, DMSO-d_6) δ 6.38 (td, J = 40 and 20 Hz, 1H, C=CHF), 4.10−4.00 (m, 2H, ester CH_2), 2.60 (t, J = 5 Hz, 2H, CH_2N), 2.00−1.90 (m, 2H, CH_2P), 1.70−1.52 (m, 2H), 1.22 (t, J = 5 Hz, 3H); MS m/e 202 (M^+).

Ethyl (3-aminopropyl)(hydroxymethyl)phosphinate (40): 80% yield; bp 140−145 °C/3.2 × 10^{-2} mbar; ^{1}H NMR (300 MHz, CD_3OD) δ 4.21−4.00 (m, 4H, ester CH_2 and CH_2OH), 2.62 (t, J = 5 Hz, 2H, CH_2N), 1.90−1.65 (m, 4H), 1.32 (t, J = 6 Hz, 3H, ester CH_3).

Ethyl (3-amino-1-methylpropyl)methylphosphinate (41): 84% yield; bp 100 °C/10^{-2} mbar; ^{31}P NMR (360 MHz, $CDCl_3$) δ +58.9 and 58.4.

(3-Aminopropyl)methylphosphinic Acid (42). A solution of 10.0 g (60.61 mmol) of **38** in 60 mL of concentrated hydrochloric acid was heated to reflux for 15 h. The reaction mixture was cooled to room temperature and evaporated to dryness. The residue was coevaporated with water (5 × 50 mL) and absolute ethanol (5 × 50 mL) and the resulting white solid dried in high vacuum. This was dissolved in 50 mL of hot propanol and filtered. The filtrate was triturated with acetone and slowly cooled to 4 °C and the solid collected by filtration. Drying in high vacuum gave 9.29 g (87%) of the hydrochloride salt of **42**: mp 135−137 °C; ^{1}H NMR (300 MHz, D_2O), δ 2.90 (t, J = 6 Hz, 2H, $CH_2NH_3^+$), 1.82−1.60 (m, 4H), 1.32 (d, J = 15 Hz, PCH_3). Anal. ($C_4H_{12}NO_2P$·HCl·0.14H_2O) C, H, Cl, N, P, H_2O.

The hydrochloride salt (9 g) was dissolved in 200 mL of methanol and treated with 40 mL of propylene oxide at room temperature. After the mixture was stirred overnight, the precipitated solid was collected by filtration and dried to give 5.6 g (80%) of **42** as a white solid: mp 280−285 °C; ^{1}H NMR (360 MHz, D_2O) δ 3.20 (t, J = 6 Hz, 2H, CH_2NH_2), 2.10−1.90 (m, 4H), 1.6 (d, J = 15 Hz, 3H, $PCCH_3$); ^{31}P NMR (360 MHz, D_2O) δ +42.1. Anal. ($C_4H_{12}NO_2P$) C, H, N, P.

(3-Aminopropyl)(difluoromethyl)phosphinic Acid (43). A solution of 1.39 g (6.92 mmol) of **39** in 20 mL of concentrated hydrochloric acid was heated to reflux for 3 h. The reaction mixture was evaporated to dryness and the residue azeotroped with toluene to give 2.4 g of a light brown resin which was

dissolved in 20 mL of methanol and treated with 10 mL of propylene oxide. A white suspension formed, which was stirred overnight at room temperature and filtered. The semicrystalline solid was dissolved in 50 mL of hot methanol and filtered. Methanol was slowly evaporated until the solution just became opaque. This was left to crystallize at 4 °C overnight. The product was collected by filtration and dried in high vacuum to give 1.0 g (54%) of **43** as a white solid: mp 261 °C dec; ^{1}H NMR (360 MHz, DMSO-d_6) δ 5.72 (td, J = 45 and 15 Hz, 1H, CHF_2), 2.50 (t, J = 6 Hz, 2H, CH_2N), 1.62–1.40 (m, 2H), 1.38–1.25 (m, 2H); MS m/e 174 $(M + H)^+$. Anal. ($C_4H_{10}F_2NO_2P$) C, H, N; F: calcd, 21.95; found, 21.45.

(3-Aminopropyl)(hydroxymethyl)phosphinic acid (44): 50% yield; mp 275 dec; ^{1}H NMR (360 MHz, D_2O) δ 3.95 (d, J = 6 Hz, 2H, CH_2OH), 3.02 (t, J = 6 Hz, 2H, CH_2N), 1.90–1.80 (m, 2H, CH_2P), 1.67–1.57 (m, 2H, CH_2). Anal. ($C_4H_{12}NO_3P$) C, H, N, P.

(3-Amino-1(*R,S*)-methylpropyl)methylphosphinic acid (45): 48% yield; mp 68–75 °C; ^{1}H NMR (360 MHz, D_2O) δ 3.00 (t, J = 6 Hz, 2H, CH_2N), 1.80–1.70 (m, 1H), 1.55–1.45 (m, 5H), 0.9 (d, J = 7 Hz, 3H); ^{31}P NMR (360 MHz, D_2O) δ +46.5; high-resolution FAB MS (matrix: glycerin saturated with CsI) calcd for $C_5H_{15}NO_2P$ $(M + H)^+$ 152.0840; found, 152.0843 ± 0.0004.

(3-Aminopropyl)methylphosphinic Acid (42). A suspension of 49.3 g (0.4 mol) of **2** in 410 mL (2 mol) of HMDS was heated to reflux for 18 h. The colorless solution was cooled to room temperature, treated with 400 mL of diglyme, and heated to reflux for 19 h. After the mixture was cooled to room temperature, 205 mL (1.2 mol) of Hünig's base were added followed by dropwise addition of 75 mL (1.2 mol) of methyl iodide, while the temperature was maintained at 25 °C with good cooling in an ice bath. After the reaction mixture was stirred for 24 h at room temperature, the white precipitate was filtered, the filtrate evaporated *in vacuo*, and the residue dissolved in 800 mL of DCM and extracted with 3 × 600 mL of 2 M HCl. The aqueous phases were washed with 800 mL of DCM and 400 mL of diethyl ether and evaporated. The residue was dissolved in 500 mL of hot 1-propanol, filtered, and treated with 800 mL of acetone. After 24 h at 4 °C the crystals were filtered and dried at 50 °C *in vacuo* overnight to give 56.38 g (81%) of **42** hydrochloride salt: mp 139–140 °C; ^{1}H NMR (300 MHz, D_2O) δ 2.90 (t, J = 7 Hz, 2H, CH_2N), 1.82–1.60 (m, 4H), 1.32 (d, J = 15 Hz, 3H, PCH_3). Anal. ($C_4H_{13}ClNO_2P$) C, H, Cl, N, P.

(3-Amino-1(*R,S*)-ethylpropyl)phosphinic Acid (46). Sodium (2.90 g, 0.126 mol) was dissolved in 72 mL of absolute ethanol and the solution cooled to 4 °C under argon. A mixture of 58.5 g (0.3 mol) of ethyl (diethoxymethyl)phosphinate,[23] and 42.3 mL (0.3 mol) of pent-2-enenitrile in 24 mL of ethanol was added dropwise over 6 h while the temperature was maintained at 4 °C. The reaction mixture was warmed to room temperature and stirred for 24 h, cooled to 10 °C, and treated with 7.5 mL (0.13 mol) of glacial acetic acid to give a yellow suspension. The solvent was removed by evaporation and the residue suspended in ethyl acetate and washed with water. The organic phase was dried over magnesium sulfate and filtered and the solvent removed to give 73.4 g (88%) of ethyl [1-(cyanomethyl)propyl](diethoxymethyl)phosphinate as a 1:1 mixture of diastereoisomers which was used without further purification: ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.73 (d, J = 8 Hz, 1H, $PCH(OEt)_2$), 4.25–4.15 (m, 2H, ester CH_2), 3.90–3.86 (m, 2H, acetal CH_2), 3.75–3.65 (m, 2H, acetal CH_2), 2.95–2.85 (m, 1H, CHCN), 2.73–2.65 (m, 1H, CHCN), 2.25–2.10 (m, 1H), 2.11–1.95 (m, 1H), 1.85–1.70 (m, 1H), 1.35 (t, J = 6 Hz, 3H), 1.26 (t, J = 6 Hz, 6H), 1.10 (t, J = 6 Hz, 3H).

A solution of 73.4 g (0.26 mol) of the above nitrileester in 770 mL of absolute ethanol containing 8% ammonia was treated with 15 g of Raney nickel. The suspension was hydrogenated at normal pressure and 45 °C for 9 h and the catalyst removed by filtration. The filtrate was evaporated to dryness to give 61.88 g (83%) of ethyl (3-amino-1-ethylpropyl)phosphinate, which was used without further purification: ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.73 (d, J = 8 Hz, 1H, $PCH(OEt)_2$), 4.25–4.15 (m, 2H, ester CH_2), 3.90–3.86 (m, 2H, acetal CH_2), 3.75–3.65 (m, 2H, acetal CH_2), 2.95–2.85 (m, 1H, CHCN), 2.73–2.65 (m, 1H, CHCN), 2.25–2.10 (m, 1H), 2.11–1.95 (m, 1H), 1.85–1.70 (m, 1H), 1.35 (t, J = 6 Hz, 3H), 1.26 (t, J = 6 Hz, 6H), 1.10 (t, J = 6 Hz, 3H).

To 220 mL of concentrated hydrochloric acid was added dropwise 61.88 g (0.22 mol) of the above amino ester. An exothermic reaction occurred. The mixture was heated to reflux for 6 h, cooled to room temperature, and evaporated to dryness. The residue was coevaporated with water (3 × 100 mL) and absolute ethanol (4 × 100 mL) and the crystalline mass dried under high vacuum. This was dissolved in 100 mL of methanol and treated with 500 mL of propylene oxide. The white suspension was stirred overnight and filtered to give 24.3 g (73%) of **46** as a hygroscopic solid: ^{1}H NMR (360 MHz, D_2O) 6.68 (d, J = 510 Hz, 1H, PH), 3.10–2.85 (m, 2H), 1.80–1.30 (m, 3H), 1.30–1.20 (m, 2H), 0.80 (t, J = 6 Hz, 3H); high-resolution FAB MS (matrix: glycerin saturated with CsI) calcd for $C_5H_{15}NO_2P$ $(M + H)^+$ 152.0840; found, 152.0842 ± 0.0004. Anal. ($C_5H_{14}NO_2P{\cdot}H_2O$) C, H, N, P.

(3-Amino-1(*R,S*)-ethylpropyl)methylphosphinic Acid (47). A suspension of 4.53 g (30 mmol) of **46** in 30 mL of HMDS was heated at reflux for 24 h under argon, after which time a clear solution resulted, diglyme (15 mL) was added, and reflux continued for a further 2 h. The mixture was cooled to 100 °C, and 26 mL (152 mmol) of Hünig's base was added. The mixture was then cooled to room temperature, and 9.34 mL (150 mmol) of methyl iodide was added over 15 min. An exothermic reaction resulted, and the temperature was kept below 25 °C with ice-bath cooling. A precipitate formed during the addition. When the addition was complete the mixture was stirred at room temperature for 4 days and the precipitate removed by filtration. The volatile material was removed *in vacuo* and the residue dissolved in DCM and washed with 2 M hydrochloric acid. The aqueous layer was removed and concentrated *in vacuo* to give a solid which was coevaporated with 100 mL of ethanol and dried under high vacuum. The solid was dissolved in 1-propanol triturated with acetone and left to crystallize. The crystals were collected by filtration and dried. Dissolution in methanol followed by treatment with propylene oxide gave 1.76 g (22%) of **47** as an oil: ^{1}H NMR (360 MHz, D_2O) δ 3.20–2.10 (m, 2H, CH_2N), 2.00–1.65 (m, 4H), 1.45–1.35 (m, 1H), 1.25 (d, J = 15 Hz, 3H), 1.00 (t, J = 5 Hz, 3H). Anal. ($C_6H_{16}NO_2P{\cdot}0.5H_2O$) H, N, P, H_2O; C: calcd, 41.37; found, 40.90.

(*E*)-4,4,4-Trifluorobut-2-enenitrile (48). A solution of 102 g (1.2 mol) of cyanoacetic acid in 250 mL of pyridine was treated with 186.6 g (1.2 mol) of 63% trifluoroacetaldehyde hydrate. A slight exothermic reaction occurred, and the mixture was heated. At about 70 °C gas evolution was observed, and the mixture was heated at 85 °C for 24 h. The reaction was cooled to room temperature and the pyridine removed by evaporation *in vacuo* to give 116.6 g of a light brown oil. Vacuum distillation gave 72.1 g (43%) of 3-hydroxy-4,4,4-trifluorobutyronitrile: bp 112–116 °C/17 mbar.

A mixture of 72.1 g (0.52 mol) of this intermediate, 1.0 g of sodium acetate and 200 mL of acetic anhydride was heated to 135 °C for 5 h. After cooling to room temperature, the mixture was poured onto 1 L of ice water and extracted three times with 300 mL of diethyl ether, and the combined ether extracts were washed with water, dried over magnesium sulfate, and filtered, and the solvent was removed to give 65 g of a brown oil. Fractional vacuum distillation gave 51.8 g (55%) of 3-acetoxy-4,4,4-trifluorobutyronitrile: bp 86 °C/20 mbar. Anal. ($C_6H_6F_3NO_2$) C, H, F, N.

A mixture of 46.4 g (0.255 mol) of this intermediate and 50 mL of quinoline was heated to 170 °C and the acetic acid formed removed by fractional distillation to give 26.3g (85%) of **48**: bp 110–120 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.61 (dq, J = 15 and 7.5 Hz, 1H), 6.12 (dq, J = 15 and 3.5 Hz, 1H); ^{13}C NMR (250 MHz, $CDCl_3$) δ 137.3 (dqd, J_{CF} = 36 Hz), 120.7 (qdd, J_{CF} = 271 Hz, CF_3), 113.8 (d, CN), 108.9 (dqd, J_{CF} = 8 Hz).

Ethyl [2-Cyano-1-(trifluoromethyl)ethyl]methylphosphinate (49). A solution of 2.16 g (20 mmol) of ethyl methylphosphinate[31] in 50 mL of anhydrous THF containing 4.05 g (40 mmol) of triethylamine was cooled to 4 °C under argon and treated with 4.35 g (40 mmol) of trimethylchlorosilane. A white precipitate formed immediately and the mixture stirred overnight at room temperature to give a solution of the reactive P(III) intermediate. A solution of 2.42 g (20 mmol)

of **48** in 15 mL of anhydrous THF was added and the reaction heated to reflux for 24 h. The reaction was cooled to room temperature, poured onto ice water, and extracted with dichloromethane. The organic phase was dried over magnesium sulfate and filtered and the solvent removed. Chromatography of the residue on silica gel eluting with chloroform afforded 2.3 g (50%) of **49** as an oily mixture of diastereoisomers: ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.27–4.18 (m, 2H), 3.18–2.81 (m, 3H), 1.72 (d, J = 15 Hz, 3H, PCH_3), 1.39 (t, J = 6 Hz, 3H). Anal. ($C_7H_{11}F_3NO_2P$) C, H, N, P; F: calcd, 24.87; found, 22.70.

[3-Amino-1(*R,S*)-(trifluoromethyl)propyl]methylphosphinic Acid (50). A solution of 2.205 g (9.62 mmol) of **49** was dissolved in 190 mL of trifluoroacetic acid, and 2.4 g of platinum oxide was added. The suspension was hydrogenated at room temperature and normal pressure for 2 h and the catalyst removed by filtration. The solvent was removed and the residue chromatographed on reverse phase silica gel (Opti-Up C-18) with acetonitrile to give 3.4 g (100%) of ethyl [3-amino-1-(trifluoromethyl)propyl]methylphosphinate trifluoroacetate salt: ^{1}H NMR (300 MHz, D_2O) δ 4.25–4.05 (m, 2H, ester CH_2), 3.35–3.10 (m, 3H, $CHCF_3$), 2.24–2.05 (m, 2H), 1.75 (d, J = 15 Hz, diastereoisomeric PCH_3), 1.35 (t, J = 6 Hz, 3H, ester CH_3). Anal. ($C_9H_{16}F_6NO_4P{\cdot}0.37H_2O$) C, H, F, N, P, H_2O.

A solution of 0.347 g (1 mmol) of this intermediate in 10 mL of acetonitrile was treated with 0.78 mL (6 mmol) of bromotrimethylsilane. The reaction mixture was stirred for 24 h at room temperature and the volatile material removed by evaporation. The orange residue (340 mg) was dissolved in 2 mL of methanol and 1 mL of propylene oxide added. After a few minutes crystallization began, the suspension was stirred at room temperature for 2 h, and the solid was collected by filtration and dried to give 0.16 g (78%) of **50**: mp 225–227 °C; ^{1}H NMR (360 MHz, D_2O) δ 3.30–3.05 (m, 2H, CH_2N), 2.75–2.40 (m, 1H, $CHCF_3$), 2.30–2.05 (m, 2H, CH_2), 1.33 (d, J = 15 Hz, 3H, PCH_3); ^{13}C NMR (300 MHz, D_2O) δ 127.2 (q, J_{CF} = 279 Hz, CF_3), 44.1 (dq, J_{CF} = 26 Hz, J_{CP} = 83 Hz, CH), 39.3 (d, J_{CP} = 8 Hz, CH_2N), 22.6 (m, CH_2), 16.3 (dt, J_{CP} = 99 Hz, CH_3). Anal. ($C_5H_{11}F_3NO_2P$) C, F, N, P; H: calcd, 5.41; found, 4.90.

Ethyl [3-[*N*-(Benzyloxycarbonyl)amino]-1-hydroxypropyl]-methylphosphinate (51). A mixture of 5.18 g (25 mmol) of ethyl methylphosphinate,[31] 2.53 g (25 mmol) of triethylamine, and 2.7 g (25 mmol) of [3-[*N*-(benzyloxycarbonyl)amino]propanal[33] was heated to 100 °C for 2 h. After this time the mixture was cooled to room temperature, and the volatile material removed by evaporation, and the residue chromatographed on silica gel to using 99:1 rising to 95:5 DCM–methanol as eluant to give 6.66 g (85%) of **51** as an oily mixture of diastereoisomers in a 1:1 ratio: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.40–7.23 (m, 5H, aromatic CH), 5.56–5.37 (m, 1H, exch. D_2O, NH), 5.10 (AB q, $J \leq 2$ Hz, 2H, CH_2Ph), 4.47 and 4.30 (dd, J = 15 and 5 Hz, 1H, exch. D_2O, OH), 4.10–4.00 (m, 2H), 3.90–3.76 (m, 1H, C*H*OH), 3.63–3.46 and 3.36–3.23 (m, 2H, CH_2N), 2.04–1.89 and 1.87–1.66 (m, 2H), 1.48 (d, J = 15 Hz, 3H, PCH_3), 1.30 (t, J = 6 Hz, 3H).

(3-Amino-1(*R,S*)-hydroxypropyl)methylphosphinic Acid Hydrochloride (52). A solution of 4.0 g (12.7 mmol) of **52** in 50 mL of 6 M hydrochloric acid was heated to reflux for 17 h. The mixture was cooled and extracted twice with 50 mL of DCM. The aqueous layer was removed and evaporated to dryness. The residue was coevaporated with water (4 × 100 mL) and absolute ethanol (4 × 100 mL) to give a semicrystalline solid. This was dissolved in 15 mL of hot 1-propanol and left to crystallize at 4° C overnight. The solid was collected by filtration and dried to give 2.33 g (97%) of **52**: mp 115–116.5 °C; ^{1}H NMR (360 MHz, D_2O) δ 3.95 (dt, J = 10 and 5 Hz, 1H, C*H*OH), 3.33–3.13 (m, 2H, CH_2N), 2.13–1.98 (m, 2H), 1.49 (d, J = 15 Hz, 3H, PCH_3). Anal. ($C_4H_{12}NO_3P{\cdot}HCl{\cdot}0.13H_2O$) H, Cl, N, P, H_2O; C: calcd, 25.03; found, 25.71.

Ethyl (3-*N*-Phthalimido-2-hydroxypropyl)methylphosphinate (53). A solution of 13.2 g (0.12 mol) of ethyl methylphosphinate[31] in 150 mL of anhydrous THF containing 13.55 g (0.132 mol) of triethylamine under argon was cooled to 15 °C and treated with 14.3 g (0.132 mol) of trimethylchlorosilane. A white precipitate formed immediately and the suspension was stirred overnight at room temperature. The precipitate was removed by filtration under argon and the filtrate concentrated at 30 °C/200 mbar to give an oil. This oil was treated sequentially with 24.36 g (0.12 mol) of *N*-(epoxypropyl)phthalimide and 1 g (7.34 mmol) of anhydrous zinc chloride. An exothermic reaction resulted, and the mixture was heated to 85 °C for 24 h. After cooling to room temperature and dilution with 100 mL of DCM, the solution was extracted with 3 × 100 mL of water. The organic phase was dried over magnesium sulfate and filtered and the solvent removed to give the trimethylsilyl ether of **53**. This was dissolved in methanol containing 1% acetic acid and stirred at room temperature for 24 h. The volatile material was removed and the residue chromatographed on silica gel using 19:1 ethyl acetate–ethanol as eluant to give 11.9 g (41%) of **53** as an oily 1:1 mixture of diastereoisomers: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.90–7.80 (m, 2H), 7.75–7.68 (m, 2H), 4.50–4.37 (m, 1H, C*H*OH), 4.24 and 4.14 (d, 1H, exch. D_2O, OH), 4.11–3.91 (m, 2H), 3.90–3.60 (m, 2H), 2.10–1.85 (m, 2H), 1.55 (d, J = 15 Hz, 3H, PCH_3), 1.32 (t, J = 6 Hz, 3H, ester CH_3). Anal. ($C_{14}H_{18}NO_5P{\cdot}0.15H_2O$) C, H, N, P, H_2O.

Ethyl (3-*N*-phthalimido-2-hydroxypropyl)(difluoromethyl)phosphinate (54): 61% yield (1:1 mixture of diastereoisomers); ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.90–7.80 (m, 2H), 7.75–7.65 (m, 2H), 5.89 (dt, J = 40 and 15 Hz, 1H, CHF_2), 4.55–4.45 and 4.30–4.10 (m, 1H, C*H*OH), 4.18–4.05 (m, 2H), 3.90–3.76 (m, 2H), 2.30–1.90 (m, 2H, CH_2P), 1.30 (t, J = 6 Hz, 3H, ester CH_3).

(3-Amino-2(*R,S*)-hydroxypropyl)methylphosphinic Acid (55). A suspension of 8.4 g (27 mmol) of **53** in 84 mL of concentrated hydrochloric acid was heated to reflux for 24 h. A precipitate formed, and the suspension was cooled to 0 °C and the solid removed by filtration. The aqueous filtrate was washed with DCM and evaporated to dryness. The residual pale yellow oil was co-evaporated with water (3 × 100 mL) followed by absolute ethanol (3 × 100 mL) and dried *in vacuo* for 15 h at 50 °C. The resulting solid was dissolved in 250 mL of hot methanol, treated with active charcoal, and filtered. After the mixture was cooled to room temperature, 50 mL of propylene oxide was added slowly with vigorous stirring. The resulting suspension was stirred overnight at room temperature, an additional 50 mL of propylene oxide was added, and the solid was collected by filtration. Drying in high vacuum gave 3.01 g (73.4%) of **55** as a white solid: mp 223–226 °C; ^{1}H NMR (360 MHz, D_2O) δ 4.20–4.15 (m, 1H, C*H*OH), 3.22 (dd, J = 14 and 5 Hz, 1H, CHN), 2.95 (dd, J = 14 and 5 Hz, 1H, CHN), 1.95–1.60 (m, 2H, CH_2P), 1.28 (d, J = 15Hz, 3H, PCH_3). Anal. ($C_4H_{12}NO_3P$) C, H, N, P.

(3-Amino-2(*R,S*)-hydroxypropyl)(difluoromethyl)-phosphinic acid (56): 60% yield; mp 231–236 °C; ^{1}H NMR (360 MHz, D_2O) δ 5.88 (dt, J = 50 and 22 Hz, 1H, CHF_2), 4.30–4.16 (m, 1H, C*H*OH), 3.23 (dd, J = 15 and 5 Hz, 1H, CHN), 2.98 (dd, J = 15 and 12 Hz, 1H, CHN), 2.12–1.85 (m, 2H, CH_2P). Anal. ($C_4H_{10}F_2NO_3P{\cdot}0.02H_2O$) C, H, N, P, H_2O.

Ethyl (3-Chloro-2(*R*)-hydroxypropyl)methylphosphinate (57). A solution of 27.5 g (0.25 mol) of ethyl methylphosphinate[31] in 250 mL of anhydrous THF containing 27.8 g (0.275 mol) of triethylamine was treated at room temperature under argon with 29.9 g (0.275 mol) of trimethylchlorosilane. A white precipitate formed, and the suspension was stirred overnight at room temperature. The precipitate was removed by filtration under argon and the filtrate concentrated at 30 °C/200 mbar to give the reactive P(III) species as an oil. This oil was treated sequentially with 18.5 g (0.25 mol) of (*R*)-epichlorohydrin and 2.5 g (18.34 mmol) of anhydrous zinc chloride. A very exothermic reaction resulted, which was allowed to subside before heating to 70 °C for 4 h. After cooling to room temperature, the reaction mixture was diluted with 300 mL of DCM and extracted 100 mL of water. The organic phase was dried over magnesium sulfate and filtered and the solvent removed to give 53.8 g (99%) of the trimethylsilyl ether of **57**, which was dissolved in 100 mL of methanol containing 1% acetic acid and stirred for 24 h at room temperature. The volatile material was removed and the residue chromatographed on silica gel, eluting with ethyl acetate to give 45.1 g (90%) of **57** as an oily 1:1 mixture of diastereoisomers: ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.38–4.27 and 4.25–4.18 (m, 1H, C*H*OH), 4.10–4.04 (m, 2H, ester CH_2), 3.70–3.53 (m, 2H, CH_2-

Cl), 2.17–1.92 (m, 2H, CH_2P), 1.59 (d, J = 15 Hz, 3H, P-CH_3), 1.33 (t, J = 7.5 Hz, 3H, ester CH_3); $[\alpha]^{20}_D$ = +25.3 ± 0.7° (c = 1.483, $CHCl_3$).

Ethyl (3-Chloro-2(*S*)-hydroxypropyl)methylphosphinate (58). 85% yield after two steps, $[\alpha]^{20}_D$ = −24.1 ± 0.7° (c = 1, $CHCl_3$).

Ethyl (3-Chloro-2(*R*)-hydroxypropyl)(1,1-diethoxymethyl)phosphinate (59). 51% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.70 (d, J = 8 Hz, 1H, CH-P), 4.42–4.38 (m, 1H, C*H*OH), 4.29–4.18 (m, 2H, ester CH_2), 3.894–3.82 (m, 2H, acetal CH_2), 3.80–3.72 (m, 2H, acetal CH_2), 3.68–3.57 (m, 2H, CH_2Cl), 2.32–1.97 (m, 3H, CH_2P and OH, becomes 2H on D_2O exchange), 1.35 (t, J =7 Hz, 3H), 1.28 (t, J = 7 Hz, 6H); $[\alpha]^{20}_D$ = +16.0 ± 1.2° (c = 0.860 in $CHCl_3$).

Ethyl (3-*N*-Phthalimido-2(*S*)-hydroxypropyl)methylphosphinate (60). To a solution of 12.0 g (60 mmol) of **57** in 60 mL of toluene were added 2.3 g (8.9 mmol) of 18-crown-6 and 16.6 g (89 mmol) of potassium phthalimide, and the resulting suspension was stirred at 60 °C for 5 days. The yellow suspension was then cooled to 0 °C and the precipitated solid removed by filtration. The filtrate was evaporated to dryness and the residue partitioned between DCM and water. The aqueous layer was extracted five times with 100 mL of DCM, the combined organic layers were dried over magnesium sulfate and filtered, and the solvent was removed to give an oil. This oil was chromatographed on 600 g of silica gel using ethyl acetate–ethanol (95:5) as eluant. After evaporation of the product-containing fractions, a pale yellow crystalline solid was obtained, which was suspended in diethyl ether and stirred for 24 h at room temperature. Collection of the solid by filtration and washing with ether afforded 8.4 g (45%) of **60** as a 1:1 mixture of diastereoisomers: ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.90–7.80 (m, 2H, aromatic CH), 7.75–7.68 (m, 2H, aromatic CH), 4.50–4.27 (m, 1H, C*H*OH), 4.11–3.93 (m, 2H, ester CH_2), 3.90–3.70 (m, 2H, CH_2N-phthalimide), 12.10–1.84 (m, 2H, CH_2P), 1.53 (d, J = 15 Hz, 3H, PCH_3), 1.29 (t, J = 7 Hz, 3H, ester CH_3); $[\alpha]^{20}_D$ = +1.6 ± 0.5° (c = 1, $CHCl_3$); $[\alpha]^{20}_D$ = −2.9 ± 0.7° (c = 1, CH_3OH).

Ethyl (3-*N*-phthalimido-2(*R*)-hydroxypropyl)methylphosphinate (61): 40% yield as a 1:1 mixture of diastereoisomers; $[\alpha]^{20}_D$ = −1.5 ± 0.7° (c = 1, $CHCl_3$); $[\alpha]^{20}_D$ = +3.1 ± 1.0° (c = 1, CH_3OH).

Ethyl (3-*N*-phthalimido-2(*S*)-hydroxypropyl)(1,1-diethoxymethyl)phosphinate (62): 32% yield as a 1:1 mixture of diastereoisomers; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.90–7.83 (m, 2H, aromatic CH), 7.78–7.69 (m, 2H, aromatic CH), 4.69 (d, J = 7 Hz, 1H, PCH), 4.53–4.40 (m, 1H, C*H*OH), 4.28–4.08 (m, 2H, ester CH_2), 3.95–3.60 (m, 6H, 2 acetal CH_2 and CH_2NPhthal), 2.20–1.92 (m, 2H, CH_2P), 1.40–1.20 (m, 9H, 2 acetal CH_3 and ester CH_3); $[\alpha]^{20}_D$ = +1.18 ± 0.5° (c = 1.02, $CHCl_3$); $[\alpha]^{20}_D$ = −8.1 ± 0.7° (c = 1.085, CH_3OH).

(3-Amino-2(*S*)-hydroxypropyl)methylphosphinic Acid (63). A suspension of 8.4 g (27 mmol) of **60** in 84 mL of concentrated hydrochloric acid was heated to reflux for 24 h. After the mixture was cooled to 0 °C, the solid was removed by filtration. The aqueous filtrate was extracted three times with 100 mL of DCM and then evaporated to dryness. The residual pale yellow oil was coevaporated with water (3 × 100 mL) followed by absolute ethanol (3 × 100 mL) and dried *in vacuo* for 15 h at 50 °C. The resulting solid was dissolved in 250 mL of hot methanol, treated with active charcoal, and filtered. This methanol solution was allowed to cool to room temperature, and 50 mL of propylene oxide was added slowly with vigorous stirring. After stirring overnight at room temperature a further 50 mL of propylene oxide was added and the solid was collected by filtration. Drying in high vacuum gave 3.01 g (73%) of **63** as a white solid: >99% ee; mp 220–223 °C; ^{1}H NMR (360 MHz, D_2O) δ 4.21–4.03 (m, 1H, C*H*OH), 3.14 (ddd, J = 13, 8, and 4 Hz, 1H, CHN_2), 2.88 (ddd, J = 13, 8, and 4 Hz, 1H, CHN_2), 1.95–1.60 (m, J = 14, 14, and 6 Hz, 2H, CH_2P), 1.26 (d, J = 14 Hz, 3H, P-CH_3); ^{31}P NMR (360 MHz, D_2O) δ +36.8; $[\alpha]^{20}_D$ = −6.4 ± 0.9° (c = 1, H_2O). Anal. ($C_4H_{12}NO_3P$·0.03H_2O) C, H, N, P, H_2O.

Method for Determination of Enantiomeric Excess (ee). Analysis conditions: HPLC on Crownpack CR 100 × 4 mm column, 995.7 mL of H_2O, 4.3 mL of 70% perchloric acid, flow rate 0.1 mL/min at 5 °C, detection via refractive index, t_R = (*R*)-**64**, 19.75 min; (*S*)-**63**, 21.51 min.

(3-Amino-2(*S*)-hydroxypropyl)methylphosphinic Acid Methanesulfonate Salt (63). A hot solution of 918 mg (6 mmol) of **63** and 634 mg (6.6 mmol) of methanesulfonic acid in 15 mL of methanol was treated dropwise with diethyl ether until the solution became cloudy. After 3 h at 4 °C the white crystals were collected by filtration to give 1.42 g (95%) of **63** methanesulfonate salt: mp 121–122 °C; ^{1}H NMR (360 MHz, D_2O) δ 4.28–4.17 (m, 1H, C*H*OH), 3.12 (dd, J = 13 and 3 Hz, 1H, CHN), 2.98 (dd, J = 13 and 9.5 Hz, 1H, CHN), 2.78 (s, 3H, CH_3SO_3), 2.05 (AB dd, J = 15, 8, and 5 Hz, 1H, PCH_2), 1.97 (AB dd, J = 15, 8, and 5 Hz, 1H, PCH_2), 1.47 (d, J = 15 Hz, 3H, PCH_3); $[\alpha]^{20}_D$ = −3.7 ± 0.5° (c = 0.595, MeOH). Anal. ($C_4H_{12}NO_3P$·CH_3SO_3H) C, H, N, P, S.

X-ray Crystallographic Analysis of 63 Methanesulfonate Salt. A colorless platelet-shaped crystal, obtained after two additional crystallizations from acetone, of $C_4H_{13}NO_3P^+$·$CH_3SO_3^-$ of molecular weight 249.22 having approximate dimensions 0.45 × 0.27 × 0.08 mm was mounted on a glass fiber. All measurements were made on a Enraf-Nonius CAD4 diffractometer with graphite monochromated Cu Kα radiation of wavelength 1.5418 Å. The crystal belongs to the orthorhombic space group $P2_12_12_1$ with a = 5.525(1) Å, b = 7.145(1) Å, c = 27.258(3) Å, V = 1076.0(5) Å^3, Z = 4, D_{calc} = 1.538 g/cm^3. The intensities were corrected for Lorentz and polarization effects but not for absorption. A total of 2604 independent intensities were measured of which 2109 were classified as observed with $I > 3\sigma(I)$. The structure was solved by direct methods using the computer program SHELXS86[56] All hydrogen atom positions were located from difference Fourier maps or calculated assuming normal geometry. The structure was refined using full matrix least squares calculations with anisotropic displacement parameters for non-hydrogen atoms. Hydrogen atom parameters were not refined. The absolute configuration was determined by measuring Friedel pairs and calculationg the Flack parameter x[57] which refined to a value of 0.003(9). The final R-factor for 128 variables was 0.061 and the goodness of fit was 1.86. The highest peak in the final difference Fourier map was 0.53 e/Å^3. Positional and thermal parameters, bond lengths, bond angles, and atomic coordinates are available as supporting information.

(3-Amino-2(*R*)-hydroxypropyl)methylphosphinic acid (64): 55% yield; >99% ee; mp 222–225 °C; $[\alpha]^{20}_D$ = +5.6 ± 0.9° (c = 1.161, H_2O). Anal. ($C_4H_{12}NO_3P$) C, H, N, P.

Ethyl (*E*)- and (*Z*)-(3-*N*-Phthalimidopropen-1-yl)methylphosphinate (65 and 66). A solution of 3.11 g (10 mmol) of **61** and 5.25 g (20 mmol) of triphenylphosphine in 200 mL of tetrahydrofuran and 200 mL of toluene was cooled in an ice bath to 0–5 °C and treated dropwise with a solution of 3.87 g (20 mmol) ethyl azodicarboxylate (90% pure) in 100 mL of toluene. After addition the reaction mixture was stirred for additional 16 h at room temperature, and the solvent was evaporated *in vacuo*. Then, 150 mL of diethyl ether was added and the solution treated with *n*-pentane until it became turbid. Crystallization was induced with seed crystals of triphenylphosphine oxide. After the mixture was left to stand overnight at 5 °C, 4.97 g of a mixture of triphenylphosphine oxide and ethyl hydrazinodicarboxylate were collected by filtration. The filtrate was evaporated *in vacuo* to give 8.4 g of a yellow oil. Chromatography on silica gel using toulene–ethyl acetate, initially 1:1, increasing to 2:3 and 1:4, then using ethyl acetate followed by ethyl acetate–ethanol (9:1) as eluants allowed separation of the geometrical isomers as oils, which crystallized from diethyl ether–*n*-pentane to give 945 mg (32%) of the (*E*)-isomer **65** [R_f = 0.41 (ethyl acetate–ethanol, 9:1); mp 73–75 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.92–7.85 (m, 2H, aromatic CH), 7.79–7.72 (m, 2H, aromatic CH), 6.74 (ddt, $J_{b,P}$ = 19.2 Hz, $J_{a,b}$ = 17.23 Hz, $J_{b,c}$ = 4.92 Hz, 1H, C=CH_b-CH_2NPhthal), 5.81 (ddt, $J_{a,P}$ = 21.7 Hz, $J_{a,b}$ = 17.23 Hz, $J_{a,c}$ = 1.9 Hz, 1H, P-CH_a=C), 4.44 (ddd, $J_{b,c}$ = 4.92 Hz, $J_{a,c}$ = 1.9 Hz, $J_{c,P}$ = 1.9 Hz, 2H, CH_2NPhthal=H_c), 1.47 (d, $J_{P,CH3}$ = 14.8 Hz, PCH_3); ^{31}P NMR (360 MHz, D_2O) δ +39.5. Anal. ($C_{14}H_{16}NO_4P$) C, H, N, P] and 770 mg (26%) of the (*Z*)-isomer **66**: R_f = 0.49 (ethyl acetate–ethanol, 9:1); mp 109–110 °C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.90–7.82 (m, 2H, aromatic CH), 7.76–7.70 (m, 2H, aromatic CH), 6.33 (dddd, $J_{b,P}$ = 41.8 Hz, $J_{a,b}$ = 12.8 Hz, $J_{b,c}$ = 6.3 Hz, $J_{b,c'}$ = 5.8 Hz, 1H, C=$CH_b$$CH_2$-NPhthal), 5.76 (ddt, $J_{a,P}$ = 20 Hz, $J_{a,b}$ = 12.8 Hz, $J_{a,c}$ = 2 Hz,

1H, PCH_a=C), 5.00 (AB dd, $J_{c,c'}$ = 16.2 Hz, $J_{b,c}$ = 6.3 Hz, $J_{a,c}$ = 2 Hz, $J_{c,P}$ = 2 Hz, 1H, CH_cNPhthal), 4.86 (AB dd, $J_{c,c}$ = 16.2 Hz, $J_{b,c'}$ = 5.8 Hz, $J_{a,c'}$ = 2.5 Hz, $J_{c',P}$ = 2.5 Hz, 1H, $CH_{c'}$-NPhthal), 4.20–4.05 (m, 2H, ester CH_2), 1.64 (d, $J_{P,CH3}$ = 14.8 Hz, PCH_3), 1.40 (t, J = 7 Hz, ester CH_3); ^{31}P NMR (360 MHz, D_2O) δ +40.3. Anal. ($C_{14}H_{16}NO_4P$) C, H, N, P.

(*E*)-(3-Aminopropen-2-yl)methylphosphinic Acid (68). A suspension of 293 mg (1 mmol) of **65** in 10 mL of concentrated hydrochloric acid was heated to reflux for 5 h. The mixture was cooled to room temperature, diluted with 20 mL of water, and extracted three times with 25 mL of DCM. The aqueous layer was evaporated to dryness. The residue was coevaporated with water (3 × 25 mL) and absolute ethanol (3 × 25 mL) and dried *in vacuo* for 15 h. The residue was recristallized from acetone to give 166 mg (96%) of the hydrochloride salt of **68**: mp 147–150° C.

The hydrochloride salt (156 mg) was dissolved in 3 mL of methanol and treated with 5 mL of propylene oxide at room temperature. The turbid solution was left to crystallize overnight at 4 °C. The crystals were collected by filtration and dried in high vacuum to give 115 mg (93%) of **68**: mp 204–207 °C; ^{1}H NMR (360 MHz, CH_3OD) δ 6.33 (ddt, $J_{b,P}$ = 16.7 Hz, $J_{a,b}$ = 16.7 Hz, $J_{b,c}$ = 5.9 Hz, 1H, NCH_2CH_b=CP), 6.13 (ddt, $J_{a,P}$ = 16.7 Hz, $J_{a,b}$ = 16.7 Hz, $J_{a,c}$ = 1.5 Hz, 1H, PCH_a=C), 3.60 (ddd, $J_{b,c}$ = 5.9 Hz, $J_{a,c}$ = 1.5 Hz, $J_{c,P}$ = 1.5 Hz, 2H, CH_2N=H_c), 1.25 (d, $J_{P,CH3}$ = 14.3 Hz, 3H, PCH_3); ^{31}P NMR (360 MHz, CH_3OD) δ +26.0; high-resolution FAB MS (matrix: glycerin saturated with CsI) calcd for $C_4H_{11}NO_2P$ (M + H)$^+$ 136.0527, found, 136.0545 ± 0.0010.

(*Z*)-(3-Aminopropen-2-yl)methylphosphinic Acid (69). From 293 mg (1 mmol) of **66** was obtained 165 mg (96%) of the hydrochloride salt of **69**: mp 132–134 °C. Treatment with propylene oxide as above gave 115 mg (93%) of **69**: mp 190–191 °C; ^{1}H NMR (360 MHz, CH_3OD–C_6D_6, 1/10) δ 6.09 (ddt, $J_{a,P}$ = 18.2 Hz, $J_{a,b}$ = 13.3 Hz, $J_{a,c}$ = 1 Hz, 1H, PCH_a=C), 5.89 (ddt, $J_{b,P}$ = 35.5 Hz, $J_{a,b}$ = 13.3 Hz, $J_{b,c}$ = 6.4 Hz, 1H, NCH_2-CH_b=CP), 3.45 (ddd, $J_{b,c}$ = 6.4 Hz, $J_{c,P}$ = 2 Hz, $J_{a,c}$ = 1 Hz, 2H, CH_2N=H_c), 1.32 (d, J_{P,CH_3} = 14.3 Hz, 3H, PCH_3); ^{31}P NMR (360 MHz, CH_3OD–C_6D_6, 1/10) δ +26.2; high-resolution FAB MS (matrix: glycerin saturated with CsI) calcd for $C_4H_{11}NO_2P$ (M + H)$^+$ 136.0527, found 136.0543 ± 0.0010.

Ethyl (*E*)- and (*Z*)-(3-*N*-Phthalimidopropen-1-yl)(1,1-diethoxymethyl)phosphinate (28 and 67). Analogous to the preparation and separation of **65** and **66** from 1.6 g (4 mmol) of **62** were obtained as oils 620 mg (40%) of the (*E*)-isomer **28** [R_f = 0.18 (ethyl acetate); ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.90–7.81 (m, 2H, aromatic CH), 7.76–7.70 (m, 2H, aromatic CH), 6.80 (ddt, $J_{b,P}$ = 18.7 Hz, $J_{a,b}$ = 17.2 Hz, $J_{b,c}$ = 5 Hz, 1H, C=CH_bCH_2N), 5.90 (ddt, $J_{a,P}$ = 20.7 Hz, $J_{a,b}$ = 17.2 Hz, $J_{a,c}$ = 2 Hz, 1H, PCH_a=C), 4.62 (d, $J_{d,P}$ = 7 Hz, 1H, PCH_d), 4.45 (ddd, $J_{c,P}$ = 5 Hz, $J_{b,c}$ = 5 Hz, $J_{a,c}$ = 2 Hz, 2H, CH_2N-phthalimide=H_c), 4.15–4.05 (m, 2H, ester CH_2), 3.95–3.84 (m, 2H, acetal CH_2), 3.82–3.78 (m, 2H, acetal CH_2), 1.30–1.19 (m, 9H, ester CH_3 and 2 acetal CH_3); ^{31}P NMR (360 MHz, $CDCl_3$) δ +30.5] and 440 mg (29%) of the (*Z*)-isomer **67**: R_f = 0.42 (ethyl acetate); ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.89–7.81 (m, 2H, aromatic CH), 7.76–7.69 (m, 2H, aromatic CH), 6.44 (ddt, $J_{b,P}$ = 42.3 Hz, $J_{a,b}$ = 13.3 Hz, $J_{b,c}$ = 6 Hz, 1H, C=CH_bCH_2N), 5.84 (ddt, $J_{a,P}$ = 19.2 Hz, $J_{a,b}$ = 13.3 Hz, $J_{a,c}$ = 2 Hz, 1H, PCH_a=C), 5.17–4.95 (AB dd, $J_{c,c'}$ = 16.8 Hz, $J_{b,c}$ = 6 Hz, $J_{c,P}$ = 3.5 Hz, $J_{c',P}$ = 2.5 Hz, $J_{a,c}$ = 2 Hz, 2H, CH_2N-phthalimide=$H_{c,c'}$), 4.80 (d, J_d = 7.4 Hz, 1H, PCH_d), 4.23–4.10 (m, 2H, ester CH_2), 3.82–3.70 (m, 2H, acetal CH_2), 3.65–3.56 (m, 2H, acetal CH_2), 1.22–1.11 (m, 9H, ester CH_3 and 2 acetal CH_3); ^{31}P NMR (360 MHz, $CDCl_3$) δ +30.7.

(*E*)-(3-Aminopropen-1-yl)phosphinic Acid (29). From 589 mg (1.545 mmol) **28** were obtained 200 mg (82%) of the hydrochloride salt of **29** as an oil, which on treatment with propylene oxide in methanol gave 130 mg (84%) of **29**: mp 225–230 °C dec; ^{1}H NMR (360 MHz, CH_3OD) δ 7.12 (d, J = 517 Hz, 1H, PH), 6.30 (ddt, $J_{b,P}$ = 21.0 Hz, $J_{a,b}$ = 17.2 Hz, $J_{b,c}$ = 5.4 Hz, 1H, NCH_2CH_b=CP), 6.10 (ddt, $J_{a,P}$ = 17.2 Hz, $J_{a,b}$ = 17.2 Hz, $J_{a,c}$ = 1.5 Hz, 1H, PCH_a=C), 3.60 (dd, $J_{b,c}$ = 5.4 Hz, J_{ac} = 1.5 Hz, 2H, CH_2N); ^{31}P NMR (360 MHz, CH_3OD) δ +13.2; high-resolution FAB MS (matrix: glycerin saturated with CsI) calcd for $C_3H_9NO_2P$ (M + H)$^+$ 122.0371; found, 122.0375 ± 0.0016.

(*Z*)-(3-Aminopropen-1-yl)phosphinic Acid (70). From 330 mg (0.865 mmol) of **67** were obtained 115 mg (84%) of the hydrochloride salt of **70** as an oil, which was treated with propylene oxide in methanol. Chromatography on silica gel using methanol–water (95:5) gave 45 mg (51%) of **70** as a slightly yellow foam: ^{1}H NMR (360 MHz, CH_3OD) δ 7.25 (dt, $J_{d,P}$ = 518 Hz, $J_{a,d}$ = 1 Hz, $J_{b,d}$ = 1 Hz, 1H, PH_d), 6.20 (dddt, $J_{b,P}$ = 37.4 Hz, $J_{a,b}$ = 13.3 Hz, $J_{b,c}$ = 6.9 Hz, $J_{b,d}$ = 1 Hz, 1H, NCH_2CH_b=CP), 6.07 (dddt, $J_{a,P}$ = 18.2 Hz, $J_{a,b}$ = 13.3 Hz, $J_{a,c}$ = 1 Hz, $J_{a,d}$ = 1 Hz, 1H, PCH_a=C), 3.83 (ddd, $J_{b,c}$ = 6.9 Hz, $J_{c,P}$ = 2.2 Hz, J_{ac} = 1 Hz, 2H, CH_2N); ^{31}P NMR (360 MHz, CH_3OD) δ +8.6; high-resolution FAB MS (matrix: glycerin saturated with CsI) calcd for $C_3H_9NO_2P$ (M + H)$^+$ 122.0371, found 122.0380 ± 0.0012.

Radioreceptor Binding Protocol. Method for the $GABA_B$ Receptor Assay Using [^{3}H]Baclofen as Radioligand. Preparation of the membranes: three male cats were decapitated under anesthesia, the brains removed and the cerebella separated. These were homogenized in 10 volumes of ice-cold 0.32 M sucrose solution containing $MgCl_2$ (1 mmol/L) and K_2HPO_4 (1 mmol/L) with a glass/Teflon homogenizer. The membrane suspension was centrifuged at 1000*g* for 10 min. The supernatant was centrifuged at 20000*g* for 15 min. It was suspended in the same solution, *vide supra*, and aliquots of 5 mL were centrifuged on a density gradient (25 mL of 0.8 M sucrose containing 1 mmol/L K_2HPO_4 at pH 7.2) at 40000*g* for 30 min. The supernatants were removed and the pellets suspended in 25 mL water at 4 °C and kept for 30 min at 4 °C. The suspensions were centrifuged at 40000*g* for 10 min. The supernatants were removed, the pellets covered with 2 mL of Krebs–Henseleit buffer, containing 20 mmol/L Tris at pH 7.4, frozen at −20 °C, and kept for 2 days. The frozen homogenates were thawed at room temperature and kept for 45 min. The pellets were suspended in 10 volumes of Krebs–Henseleit buffer and centrifuged at 12000*g* for 10 min. The pellets were frozen and stored in liquid nitrogen. For the assays the pellets were thawed at 37 °C, suspended in 10 volumes of Krebs–Henseleit buffer, and centrifuged at 12000*g* for 10 min. Resuspension and centrifugation were repeated three times. The suspension was kept at 4 °C overnight. The next day the centrifugation-resuspension procedure was repeated twice.

The radioreceptor assay was performed in 1 mL of Krebs–Henseleit buffer containing 20 mmol/L Tris, pH 7.4, 200–300 μg of membrane protein, 10 nmol/L [^{3}H]baclofen (7.5 Ci/mmol, prepared at CIBA-GEIGY, Horsham, UK), and the compound to be tested. The incubation was performed at 20 °C for 10 min and terminated by centrifugation at 16000*g* for 5 min. The supernatants were removed and the pellets dissolved in 1 mL of tissue solubilizer. To the dissolved tissue was added 10 mL of Irgascint A300 scintillator solution (CIBA-GEIGY) and the radioactivity counted. Incubations were performed in triplicates, which varied by less than 5%, and nonspecific binding was determined in the presence of 10 μM of (*R*)-(−)-baclofen. IC_{50} values were obtained by computer-aided curve fitting, according to a single site model.

Measurements of the inhibition of binding of [^{3}H]CGP27492 from $GABA_B$ receptors on membranes obtained from rat cerebral cortex were carried out according to refs 18, 48. Incubations were performed in triplicates, which varied by less than 5%, and nonspecific binding was determined in presence of 10 μM of (*R*)-(−)-baclofen. IC_{50} values were obtained by computer-aided curve fitting, according to a single site model.

Measurements of the inhibition of binding of [^{3}H]ligands from $GABA_A$, benzodiazepine, muscarinic acetylcholine, α_1-adrenergic, α_2-adrenergic, 5-HT_1, histamine 1, and adenosine 1 receptors according to ref 48. For other central nervous system receptors the following radioligands were used: [^{3}H]-dihydroalprenolol for β-adrenoceptors,[58] [^{3}H]ketanserin from 5-HT_2 receptors,[59] [^{3}H]BRL 43 694 for 5-HT_3 receptors,[60] [^{3}H]-naloxone for opiate receptors (μ-type),[61] and [^{3}H]substance P for NK-1 receptors.[62]

Hyperpolarization of hippocampal slices: according to ref 48.

Inhibition of GABA release: according to ref 50. All experiments were performed in quadruplicate. IC_{50} values were obtained by computer-aided curve fitting. The experi-

mental errors for the release experiments listed in Table 6 were as follows: log IC_{50} (mol) (*R*)-(−)-baclofen, −6.487 ± 0.073; (*S*)-(+)-baclofen, −3.672 ± 0.193; **2**, −6.973 ± 0.147; **42**, −6.344 ± 0.288; **43**, −4.975 ± 0.289; **63**, −6.400 ± 0.008; **64**, −6.224 ± 0.221.

Blockade of monosynaptic reflexes in the hemisected spinal cord preparation of the rat: according to ref 48. The experimental errors of the experiments on compounds listed in Table 7 at various concentrations. (*R*)-(−)-Baclofen: 0.1 μM, 22 ± 15% (n = 3); 0.5 μM, 82 ± 4.5% (n = 3); 1 μM, 89 ± 3% (n = 3). **2**: 50 μM, 33 ± 18% (n = 6); 100 μM, 39 ± 19% (n = 6); 500 μM, 91 ± 5% (n = 3). **42**: 0.05 μM, 71 ± 28% (n = 3); 0.1 μM, 84 ± 7% (n = 5); 0.5 μM, 96 ± 1% (n = 5). **63**: 0.05 μM, 15% ± 9% (n = 9); 0.1 μM, 30% ± 16% (n = 10); 0.3 μM, 78% ± 4% (n = 4); 0.5 μM, 86% ± 4% (n = 12); 1 μM, 88% ± 4% (n = 6). **64**: 0.1 μM, 15% ± 7% (n = 4); 0.5 μM, 62% ± 20% (n = 6); 1 μM, 83% ± 11% (n = 4); 5 μM, 91% ± 6% (n = 3).

Rotarod Performance of Rats. Eight male Sprague–Dawley rats (Tif:RAlf (SPF)) of 120–140 g body weight were used per dose. All compounds were dissolved in water and injected orally (5 mL/kg) or subcutaneously into the neck (2 mL/kg). Rotarod performance was determined during the light phase according to the established methodology[63] using a commercially available rotarod apparatus (UGO Basile, Milano). Each rat was trained to stay for 300 s on the rotating cylinder (6 cm diameter; constant speed of rotation, 11 rpm), located 30 cm above the table. After drug administration the length of time each rat remained on the cylinder (maximally 300 s) was recorded. EC_{50} values (i.e., the concentration in μmol/kg at which 50% of the rats fell off the cylinder within ≤150 s) were estimated on the basis of a qualitative Probit model.[64]

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Supporting Information Available: Single crystal X-ray crystallographic data, bond lengths, bond angles, and atomic coordinates for **63** methanesulfonate salt and the logarithms of IC_{50} values of $GABA_B$ binding experiments, their respective standard errors, and the numbers of independent experiments (8 pages). Ordering information is given on any current masthead page.

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(65) *trans*-4-Aminocrotonic acid[38] was measured to display a high affinity to $GABA_A$ receptors (IC_{50} = 80 nM, i.e., inhibition of binding of [^{3}H]GABA;[37] IC_{50} = 90 nM for inhibition of binding of [^{3}H]muscimol) comparable to the affinity of GABA (IC_{50} = 33 nM).[37] Its affinity to $GABA_B$ receptors was found to be at least 75 times weaker (IC_{50} = 2.3 μM for inhibition of binding of [^{3}H]-GABA;[37] IC_{50} = 3.25 μM for inhibition of binding of [^{3}H]-CGP27492) in comparison to GABA (IC_{50} = 30 nM for inhibition of binding of [^{3}H]GABA;[37] IC_{50} = 17 nM for inhibition of binding of [^{3}H]CGP27492, Table 4). In contrast, the analogous phosphinic acids with *E* stereochemistry, i.e., **29** and **68** showed only weak affinities to $GABA_A$ receptors (for **29**: IC_{50} = 6.8 μM, inhibition of binding of [^{3}H]muscimol) or no affinity at all (**68** was inactive at a concentration of 100 μM). *cis*-4-Aminocrotonic acid,[38] a selective agonist of the putative $GABA_C$ receptors[39] showed a significantly weaker affinity for $GABA_A$ receptors than the corresponding *trans* isomer (IC_{50} = 2.8 μM, inhibition of binding of [^{3}H]muscimol). Its interaction with $GABA_B$ receptors was very weak (43% inhibition of binding of [^{3}H]CGP27492 at a concentration of 100 μM). The interaction of the analogous phosphinic acid **70** to $GABA_A$ receptors was in the same order of magnitude (IC_{50} = 6.6 μM) as for *cis*-4-aminocrotonic acid. The analogous methylphosphinic acid **69** interacted only very weakly with $GABA_A$ receptors (IC_{50} = 242 μM).

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