115. Synthesis and NMDA Antagonistic Properties of the Enantiomers of 4-(3-Phosphonopropyl)piperazine-2-carboxylic Acid (CPP) and of the Unsaturated Analogue (*E*)-4-(3-Phosphonoprop-2-enyl)piperazine-2-carboxylic Acid (CPP-ene)

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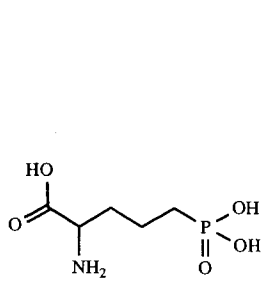
The (*R*)- and (*S*)-enantiomers of 4-(3-phosphonopropyl)piperazine-2-carboxylic acid (D- and L-CPP, resp.; **15** and **16**, resp.), and of its unsaturated analogue (*E*)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid (D- and L-CPP-ene, resp.; **13** and **14**, resp.) were prepared. The absolute configuration of the enantiomers was determined by a chemical correlation of the menthyl ester **7** with D-asparagine. The affinity of these derivatives for the NMDA receptor was determined by displacement of [^{3}H]CPP in rat cerebral cortical membranes. In two functional tests (the frog hemisected spinal cord preparation and the sodium efflux test from rat brain slices), D-CPP-ene appears to be the most potent, enantiomerically pure, competitive NMDA antagonist known to date.

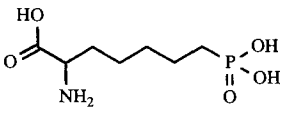
1. Introduction. – During the last decade, it has become increasingly evident that excitatory amino acids (EAA) [1–4] play an important role in brain function. Among this class of substances, L-glutamate, L-aspartate, and L-homocysteic acid [5] are the most likely candidates as endogenous neurotransmitters. EAA bind to at least four subtypes of receptors named according to the known exogenous agonists *N*-methyl-D-aspartate (NMDA), quisqualic acid (*Q*), kainic acid (K), and L-2-amino-4-phosphonobutyric acid (L-AP4). The NMDA receptor is the most intensively investigated of these receptors, due to the early discovery of selective antagonists like 2-amino-5-phosphonopentanoic acid (AP5; **1**) and 2-amino-7-phosphonoheptanoic acid (AP7; **2**). The (±)-4-(3-phosphonopropyl)piperazine-2-carboxylic acid (CPP[1]); **3**) has been described as a selective and potent NMDA antagonist [6–8], which possesses anticonvulsant [6] [8] [9] and muscle-relaxant properties [8] [9], and also has protective effects against ischemia-induced brain damage in gerbils [10].

The stereoselectivity of the NMDA receptor is surprising. For the putative endogenous agonist glutamate, the receptor prefers the L-configuration: L-Glu has been shown to inhibit the binding of [^{3}H]AP5 to rat cerebral cortex membranes with a K_i value of

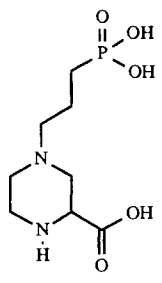
[1]) CPP is the abbreviation of the name 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid that is currently used in the literature. We prefer the nomenclature employed by *Chemical Abstracts* that considers the carboxylic-acid moiety as the principal group.



1 AP5



2 AP7



3 CPP

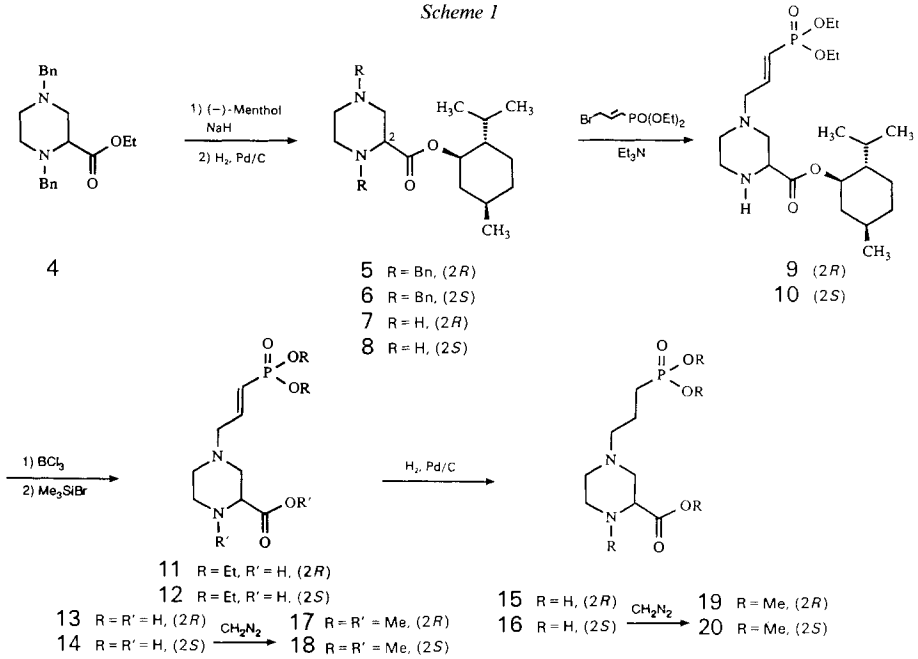
0.9 μM, compared to 49 μM for D-Glu [3]. In the case of the shorter homologue aspartate, the NMDA receptor does not differentiate between L-Asp (K_i 11 μM [3]) and D-Asp (K_i 10 μM [3]). The *N*-methylation of D-Asp to NMDA ((*R*)-configuration) does not modify the affinity for the receptor (K_i 11 μM [3]), whereas the *N*-methylation of L-Asp to NMLA ((*S*)-configuration) decreases the binding affinity (K_i 160 μM). The preference for the (*R*)-configuration has also been observed in the case of the inhibitors AP5 (**1**) and AP7 (**2**). However, the preferred absolute configuration for cyclic analogues such as CPP was not known. We present here the synthesis of the enantiomers **15** and **16** of CPP and of the unsaturated analogue (*E*)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid (CPP-ene[1]); **13** and **14**), together with the biological data showing that D-CPP-ene (**13**) is the most active, enantiomerically pure, competitive NMDA antagonist known to date.

2. Results and Discussion. – 2.1. *Synthesis.* The key step in the preparation of the pure enantiomers of CPP and CPP-ene consists in the isolation by fractional crystallization of the diastereoisomeric menthyl *N,N'*-dibenzylpiperazine-2-carboxylates **5** and **6** *(Scheme 1)*. These esters were prepared by transesterification of the known ethyl ester **4** [11] (–)-menthol and isolated as monohydrochloride **5**·HCl and dihydrochloride **6**·2 HCl in 32 and 22% yield, respectively. Hydrogenolysis of the benzyl groups of **5** and **6** gave the hydrochloride salts of the piperazine derivatives **7** and **8** in good yields. These piperazine derivatives were alkylated predominantly at N(4) by reaction, at –25°, with diethyl (*E*)-(3-bromoprop-1-enyl)phosphonate [12], to afford the triesters **9** and **10** in 60 and 83% yields, respectively.

A first trial to deprotect the triester **9** with 6N HCl (6 h at 100°) gave a partially racemized product. To overcome this problem, the triesters **9** and **10** were first treated with BCl_3 in dichloroethane to cleave the menthyl ester (→ **11** and **12**), and then with bromotrimethylsilane followed by H_2O to deprotect the phosphonic-acid function. After crystallization until constant optical rotation, D-CPP-ene (**13**) and L-CPP-ene (**14**) were obtained in 58 and 55% yield (rel. to **9** and **10**), respectively. Reduction of the double bond leads to the enantiomers **15** and **16** of CPP in high yields. This reduction was performed in a basic solution (aqueous ammonia was used as the solvent) to avoid a cleavage of the allylic side chain.

2.2. *Enantiomeric Purity.* A direct determination of the optical purity of **13–16** using ^{1}H-NMR spectroscopy and common chiral shift reagents was not possible, due to their insolubility in organic solvents. Therefore, **13–16** were permethylated with an excess of diazomethane, whereby the tetramethyl derivatives **17–20** were obtained as the main

Scheme 1



4

5 R = Bn, (2*R*)
6 R = Bn, (2*S*)
7 R = H, (2*R*)
8 R = H, (2*S*)

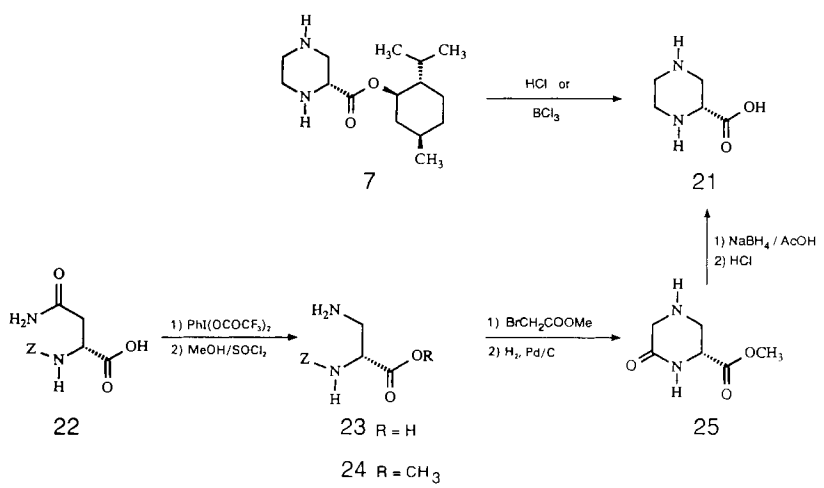
9 (2*R*)
10 (2*S*)

11 R = Et, R' = H, (2*R*)
12 R = Et, R' = H, (2*S*)
13 R = R' = H, (2*R*)
14 R = R' = H, (2*S*)
17 R = R' = Me, (2*R*)
18 R = R' = Me, (2*S*)

15 R = H, (2*R*)
16 R = H, (2*S*)
19 R = Me, (2*R*)
20 R = Me, (2*S*)

products as well as the less stable trimethyl esters, lacking the Me–N(1) group. The enantiomeric purity of the tetramethyl derivatives of CPP and CPP-ene could be assessed with ^{1}H-NMR spectroscopy using (–)-(*R*)-1-(anthr-9-yl)-2,2,2-trifluoroethanol as a chemical shift reagent in $CDCl_3$: ee > 99% for **17** (from D-CPP-ene (**13**)), ee ≥ 98% for **19** (from D-CPP (**15**)) and for **20** (from L-CPP (**16**)).

Scheme 2



7

21

22

23 R = H

24 R = CH_3

25

2.3. *Configuration.* The absolute configuration of the CPP and CPP-ene derivatives was determined by chemical correlation of menthyl piperazine-2-carboxylate (**7**) with D-asparagine *via* acid **21** *(Scheme 2)*. Z-Protected D-asparagine (**22**) was transformed into 3-amino-*N*-[(benzyloxy)carbonyl]-D-alanine (**23**) according to the procedure used for the corresponding L-enantiomer [13]. After esterification with $SOCl_2$ in MeOH (→ **24**) alkylation with methyl bromoacetate, and spontaneous cyclisation on removal of the Z-protecting group (→ **25**), reduction with $NaBH_4$ gave (*R*)-piperazine-2-carboxylic acid (**21**) as the dihydrochloride salt in low yield. The optical rotation of this sample $[\alpha]_D^{20} = +5.9$ ($c = 1.25$, H_2O) was in good agreement with the value measured for the samples of (*R*)-piperazine-2-carboxylic acid obtained by treatment of the menthyl ester **7** with HCl ($[\alpha]_D^{20} = +5.2$ ($c = 1.2$, H_2O)) or with BCl_3 ($[\alpha]_D^{20} = +5.8$ ($c = 1.1$, H_2O)). The values $[\alpha]_D^{20} = +3.9$ and -3.9 ($c = 6$, H_2O) have been reported for the neutral form of the enantiomers of piperazine-2-carboxylic acid [14]. X-Ray structure analysis of compound **13** [15] confirmed the absolute configuration established by chemical correlation.

2.4. *Biological Data.* The affinity of the enantiomers of CPP and CPP-ene for the NMDA receptor *(Table 1)* has been determined by inhibition of [^{3}H]CPP binding in rat

Table 1. *Inhibition of [^{3}H]CPP Binding in Rat Cortical Membrane* [16]

	Configuration	K_i [μM]
13, D-CPP-ene	(−)-(*R*)	0.04 ± 0.01
14, L-CPP-ene	(+)-(*S*)	0.60 ± 0.12
15, D-CPP	(−)-(*R*)	0.14 ± 0.02
16, L-CPP	(+)-(*S*)	2.33 ± 0.40
3, DL-CPP	(±)	0.28 ± 0.04
2, DL-AP7	(±)	4.2 [16]
1, D-AP5	(−)-(*R*)	0.5 [16]

cortical membranes [16]. The D-enantiomers **15** and **13** of CPP ($K_i = 0.14$ μM) and CPP-ene (0.04 μM), respectively, appear to be *ca.* 15 times more potent than their corresponding L-enantiomers **16** and **14** (2.33 and 0.60 μM, resp.). It does not seem likely that the affinity measured for the L-enantiomer is due to contamination by the D-enantiomer, the affinity measured being too strong compared to the enantiomeric purity of the samples (*ee* > 98%). The ability of the NMDA receptor to discriminate between the enantiomers of CPP and CPP-ene is similar to the selectivity observed in the case of the enantiomers of AP7 (**2**): D-AP7 displaces [^{3}H]-D-AP5 in rat cerebral cortex membranes ($K_i = 1.7$ μM) 16 times better than L-AP7 ($K_i = 28$ μM) [3]. So the NMDA receptor appears to be less selective in the case of the larger molecules AP7 and CPP than in the case of the smaller inhibitor AP5, whose D-enantiomer ($K_i = 0.62$ μM [3]) binds 90 times better than the L-enantiomer ($K_i = 55$ μM [3]).

A similar potency ratio between the enantiomeric pairs has also been measured in electrophysiological studies using the isolated hemisected spinal cord of the frog [17] *(Table 2)*. In this preparation, the D-enantiomer **15** of CPP ($pA_2 = 6.56$) is 13.5 times stronger than its L-enantiomer **16**, ($pA_2 = 5.43$) in inhibiting NMDA-induced depolarisation. In the case of the unsaturated analogue CPP-ene, the D-enantiomer **13**, ($pA_2 = 6.80$) appears to be 12 times more potent then the L-enantiomer **14** ($pA_2 = 5.73$).

Table 2. *Inhibition of the NMDA-Induced Depolarisation in the Frog Hemisected Spinal Cord* (FHS) [17] *and of the NMDA-Induced ^{22}-Na Efflux in Rat Brain Slices* [19]

	FHS			Na Efflux pA_2
	concentration		apparent pA_2	
13, D-CPP-ene	5 μM		6.6, 6.8	6.5
	10 μM		6.9, 6.9	
		mean	6.80 ± 0.14	
14, L-CPP-ene	10 μM		5.6	5.3
	20 μM		5.9	
	30 μM		5.6	
	40 μM		5.8	
		mean	5.73 ± 0.15	
DL-CPP-ene	10 μM		6.3	6.2
	20 μM		6.1	
		mean	6.20 ± 0.14	
15, D-CPP	1 μM		6.9	6.2
	5 μM		6.6	
	10 μM		6.4	
	20 μM		6.4, 6.5	
		mean	6.56 ● 0.21	
16, L-CPP	5 μM		5.2	5.0
	20 μM		5.5	
	25 μM		5.5	
	30 μM		5.5	
		mean	5.43 ± 0.15	
3, DL-CPP	20 μM		6.0, 5.6	6.0
	50 μM		5.5, 5.7	
		mean	5.70 ± 0.22	
2, DL-AP7			5.5 [17]	5.3

For all these derivatives, similar pA_2 values at all concentrations tested indicate a competitive antagonism [18].

Compounds **13**–**16** have been tested further in the sodium efflux assay described by *Teichberg* and coworkers [19]. In this model, the D-enantiomers inhibit the NMDA-induced sodium efflux from brain slices 16 times more efficiently than the L-enantiomers: $pA_2 = 6.2$ and 5.0 for D- and L-CPP (**15** and **16**), respectively, 6.5 for D-CPP-ene (**13**) and 5.3 for L-CPP-ene (**14**; *Table 2*).

The data show that D-CPP-ene (**13**), (*R*)-configuration, is the most active of these compounds. To our knowledge, these results demonstrate that D-CPP-ene is the most potent, enantiomerically pure, competitive NMDA antagonist known to date and a useful tool for the further investigation of the NMDA-receptor complex.

Experimental Part

All commercially available chemicals were used as provided by the supplier without purification. TLC: silica gel ('Kieselgel' *60 F-254* from *Merck*). Column chromatography: silica gel *60* (230-400 mesh) from *Merck* at medium pressure. M.p.: *Kofler* melting-point apparatus, *Thermopan;* uncorrected. Optical rotations: *Perkin-Elmer-241* polarimeter; cell length 10 cm; unless otherwise stated, at 20° and 589 nm. ^{1}H-NMR spectra: *Bruker*-360-MHz spectrometer, unless otherwise stated; $CDCl_3$ solns., δ in ppm with TMS as standard, coupling constants J in Hz.

Menthyl[2]) *(R)-1,4-Dibenzylpiperazine-2-carboxylate Hydrochloride Monohydrate* (**5**·HCl·H_2O). To a soln. of ethyl 1,4-dibenzylpiperazine-2-carboxylate [11] (**4**; 761 g, 2.25 mol) and (–)-menthol (458 g, 2.93 mmol) NaH (55–60% dispersion; 15 g) and toluene (1 l) were added, at 45°. *Ca.* 700 ml of the solvent were slowly removed by distillation and continuously replaced by new portions of toluene (monitoring by TLC, AcOEt/hexane 1:3). The mixture was cooled to r.t., treated with 2N aq. HCl (1.25 l) and Et_2O (6 l) and stirred thoroughly for 1 h. The crystalline precipitate was filtered off and washed with Et_2O and 0.1N aq. HCl. Recrystallization from EtOH/0.2N aq. HCl gave 364.71 g (32%) of **5**·HCl·H_2O. White crystals. M.p. > 145° (slow liquefaction and formation of new crystals with m.p. 266°). $[\alpha]_D^{20}$ = +18.5 (*c* = 1.2, $CHCl_3$). Anal. calc. for $C_{29}H_{40}N_2O_2$·HCl·H_2O (503.12): C 69.2, H 8.6, Cl 7.0, N 5.6; found: C 69.2, H 8.6, Cl 7.0, N 5.4.

For analysis, the free base **5** was isolated as a syrup after partition between Et_2O and conc. aq. NH_3. ^{1}H-NMR: 0.71 (*d*, *J* = 7, 3 H); 0.79–1.12 (*m*, 9 H); 1.39 (*ddt*, *J* = 12.0, 12.0, 3.0, 1 H); 1.44–1.58 (*m*, 1 H); 1.59–1.74 (*m*, 2 H); 1.82 (*dsept.*, 1 H); 1.98–2.08 (*m*, 1 H); 2.29–2.48 (*m*, 3 H); 2.61–2.80 (2 H); 2.94–3.05 (*m*, 1 H); 3.28 (*t*, *J* = 10.2, 1 H); 3.38 (*d*, *J* = 13.2, 1 H); 3.48–3.60 (*m*, 2 H); 3.92 (*d*, *J* = 13, 1 H); 4.75 (*dt*, *J* = 11, 4.2, 1 H); 7.18–7.38 (*m*, 10 H).

Menthyl[2]) *(S)-1,4-Dibenzylpiperazine-2-carboxylate Dihydrochloride* (**6**·2 HCl). Recrystallization (EtOH/0.2N aq. HCl) of the mother liquors obtained above gave 258 g (22%) of **6**·2 HCl as white crystals. M.p. 158–160°. $[\alpha]_D^{20}$ = –106.9 (*c* = 1.1, $CHCl_3$). Anal. calc. for $C_{29}H_{40}N_2O_2$·2 HCl (521.57): C 66.8, H 8.1, Cl 13.6, N 5.4, O 6.1; found: C 66.1, H 8.1, Cl 13.5, N 5.3, O 6.1.

For analysis, the free base **6** was isolated as mentioned above. ^{1}H-NMR: 0.78 (*d*, *J* = 7, 3 H); 0.80–0.95 (*m*, 8 H); 0.98–1.12 (*m*, 1 H); 1.32 (*ddt*, *J* = 12.0, 11.0, 3.0, 1 H); 1.42–1.56 (*m*, 1 H); 1.61–1.72 (*m*, 2 H); 1.83 (*dsept.*, *J* = 2.6, 7, 1 H); 1.93–2.01 (*m*, 1 H); 2.35–2.47 (*m*, 2 H); 2.48–2.61 (*m*, 2 H); 2.73–2.84 (*m*, 1 H); 3.09–3.22 (*m*, 1 H); 3.26 (*dd*, *J* = 5.6, 3.8, 1 H); 3.37 (*d*, *J* = 13.2, 1 H); 3.57 (*d*, *J* = 13.2, 1 H); 3.63 (br. *d*, *J* = 13, 1 H); 3.89 (*d*, *J* = 13, 1 H); 4.76 (*dt*, *J* = 11.0, 4.2, 1 H); 7.17–7.40 (*m*, 10 H).

Menthyl[2]) *(R)-Piperazine-2-carboxylate Dihydrochloride* (**7**·2 HCl). A soln. of **5** (210 g, 0.417 mol) in EtOH (2 l) was hydrogenated at r.t. and atmospheric pressure in the presence of 10% Pd/C (10.5 g). After 7 h, the mixture was filtered and evaporated. The residue was treated with HCl/EtOH, the precipitate filtered and washed with EtOH/Et_2O 1:1. Recrystallization from H_2O/MeOH/AcOEt gave 129.8 g (91%) of **7**·2 HCl. White crystals. M.p. 225–226°. $[\alpha]_D^{20}$ = –52.0 (*c* = 1.3, H_2O). Anal. calc. for $C_{15}H_{28}N_2O_2$·2 HCl·0.5 H_2O (350.33): C 51.4, H 8.9, Cl 20.2, N 8.0; found: C 51.7, H 8.9, Cl 20.5, N 8.1.

The free base **7** was isolated after partition between conc. aq. NH_3 soln. and Et_2O and concentration of the org. phase. White crystals. M.p. 50–52°. $[\alpha]_D^{20}$ = –56.7 (*c* = 1.1, $CHCl_3$). ^{1}H-NMR: 0.75 (*d*, *J* = 7.6, 3 H); 0.79–1.13 (*m*, 9 H); 1.35–1.57 (*m*, 2 H); 1.58–1.93 (*m*, 5 H; with D_2O, 3 H); 1.96–2.06 (*m*, 1 H); 2.70–2.94 (*m*, 4 H); 2.95–3.05 (*m*, 1 H); 3.23 (*dd*, *J* = 12.0, 3.2, 1 H); 3.43 (*dd*, *J* = 8.2, 3.6, 1 H); 4.74 (*dt*, *J* = 11.0, 4.0, 1 H). Anal. calc. for $C_{15}H_{28}N_2O_2$ (268.40): C 67.1, H 10.5, N 10.4; found: C 67.0, H 10.2, N 10.3.

Menthyl[2]) *(S)-Piperazine-2-carboxylate Dihydrochloride* (**8**·2 HCl). Hydrogenation of **6** (109.5 g, 0.21 mol) under the same conditions as described above gave **8** (55.6 g, 78%), after recrystallization from H_2O/MeOH/AcOEt. White crystals. M.p. 211–220°. $[\alpha]_D^{20}$ = –55.2 (*c* = 1.2, 2N aq. HCl). Anal. calc. for $C_{15}H_{28}N_2O_2$·2 HCl: Cl 20.8; found: Cl 20.8.

The free base **8** was isolated as above. M.p. 55–56°. ^{1}H-NMR: 0.76 (*d*, *J* = 7.0, 3 H); 0.80–1.14 (*m*, 9 H); 1.34–1.58 (*m*, 2 H); 1.63–1.75 (*m*, 2 H); 1.85 (*dsept.*, *J* = 7.0, 2.6, 1 H); 1.93–2.02 (*m*, 1 H); 2.36 (br. *s*, 2 H, exchanged with D_2O); 2.73–3.07 (*m*, 5 H); 3.24 (*dd*, *J* = 12.0, 3.2, 1 H); 3.48 (*dd*, *J* = 8.2, 3.6, 1 H); 4.75 (*dt*, *J* = 11.0, 4.2, 1 H).

Menthyl[2]) *(R,E)-4-[3-(Diethoxyphosphinyl)prop-2-enyl]piperazine-2-carboxylate* (**9**). To a soln. of **7** (free base; 11.3 g, 42 mmol) and Et_3N (5.9 ml, 42 mmol) in THF (90 ml) was added, at –30°, within 30 min, diethyl (*E*)-(3-bromoprop-1-enyl)phosphonate [12] (10.7 g 42 mmol) in (45 ml) THF. The soln. was strirred at –25° for 20 h. After filtration of the precipitate, the soln. was evaporated and the oil purified by chromatography on silica gel, using CH_2Cl_2 with addition of an increasing concentration of conc. aq. NH_3 soln./EtOH 1:19, reaching 10% after 2 h. The fractions containing the product of R_f 0.35 (TLC, CH_2Cl_2/conc. aq. NH_3/EtOH 200:1:19) were evaporated: **9** (11.23 g, 60%) as an oil. $[\alpha]_D^{20}$ = –56.0 (*c* = 1.4, 2N aq. HCl). ^{1}H-NMR: 0.75 (*d*, *J* = 7.0, 3 H); 0.79–1.12 (*m*, 9 H); 1.30–1.57 (*m*, 8 H); 1.64–1.74 (*m*, 2 H); 1.84 (*dsept.*, *J* = 7.0, 2.6, 1 H); 1.94–2.02 (*m*, 1 H); 2.20–2.32 (br. *m*, 2 H; with D_2O, 1 H); 2.36–2.45 (br. *m*, 1 H); 2.52–2.59 (*m*, 1 H); 2.80–2.95 (*m*, 2 H); 3.05 (*ddd*, *J* = 12.0, 4.8, 3.0, 1 H); 3.11–3.18 (*m*, 2 H); 3.54 (*dd*, *J* = 8.0, 3.0, 1 H); 4.00–4.16 (*m*, 4 H); 4.75 (*dt*, *J* = 11.0, 4.2, 1 H); 5.90 (*ddt*, *J* = 21.0, 17.0, 2.3, 1 H); 6.72 (*ddt*, *J* = 22.0, 17.0, 5.5, 1 H).

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[2]) Menthyl is used for (1*R*,2*S*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexyl.

The dihydrochloride salt **9**·2 HCl was obtained by crystallization from ethanolic HCl/Et_2O. White crystals. M.p. 157–163°. $[\alpha]_D^{20} = -48.2$ ($c = 1.4$, 2N aq. HCl). Anal. calc. for $C_{22}H_{41}N_2O_5P \cdot 2$ HCl (517.47): C 51.1, H 8.4, Cl 13.7, N 5.4, O 15.5, P 6.0; found: C 50.2, H 8.3, Cl 13.8, N 5.2, O 15.6, P 6.0.

Menthyl[2] *(S,E)-4-[3-(Diethoxyphosphinyl)prop-2-enyl]piperazine-2-carboxylate* (**10**). A soln. of **8** (22.5 g, 0.84 mol) and Et_3N (11.9 ml, 0.85 mol) in THF (160 ml) was treated as above with diethyl (*E*)-(3-bromo-prop-1-enyl)phosphonate [12] (21.6 g, 0.84 mol) in THF (42 ml). After chromatography (as above), **10** (31.1 g, 83%) was isolated as an oil (R_f 0.45, *dito*). $[\alpha]_D^{20} = -31.2$ ($c = 1.2$, 2N HCl). ^{1}H-NMR: 0.75 (*d*, $J = 7.0$, 3 H); 0.80–1.13 (*m*, 9 H); 1.23–1.60 (*m*, 8 H); 1.61–1.75 (*m*, 2 H); 1.77–2.02 (*m*, 3 H); 2.22–2.29 (*m*, 1 H); 2.31–2.43 (*m*, 1 H); 2.54–2.64 (*m*, 1 H); 2.80–2.93 (*m*, 2 H); 3.07 (*ddd*, $J = 12, 4, 2$, 1 H); 3.11–3.18 (*m*, 2 H); 3.54 (*dd*, $J = 8, 3$, 1 H); 4.00–4.18 (*m*, 4 H); 4.75 (*dt*, $J = 11.0, 4.2$, 1 H); 5.91 (*ddt*, $J = 21, 17, 2$, 1 H); 6.73 (*ddt*, $J = 22, 17, 6$, 1 H).

(R,E)-4-[3-(Diethoxyphosphinyl)prop-2-enyl]piperazine-2-carboxylic Acid (**11**). To a soln. of **9** (8.58 g, 19 mmol) in anh. CH_2Cl_2 (86 ml) was added, at −30° within 20 min, a 2.2M soln. of BCl_3 in 1,2-dichloroethane (38.6 ml). The mixture was stirred 1 h at −25° and 3.5 at 0°. At 0°, H_2O (86 ml) was added, and the mixture was brought to pH 6 by addition of aq. NaOH soln. The aq. phase was evaporated, the residue taken up in $CHCl_3$, filtered, dried (Na_2SO_4), and evaporated to give **11** (6.21 g) as an oil: R_f 0.27 (TLC, AcOEt/AcOH/H_2O 5:2:2). This crude material was used without further purification in the next step. An anal. sample was obtained by HPLC (*Nucleosil RP-8*, MeOH/H_2O 2:3): $[\alpha]_D^{20} = -18.0$ ($c = 1.1$, 2N aq. HCl). ^{1}H-NMR (DMSO, 150°): 1.24 (*t*, $J = 7.0$, 6 H); 2.26 (*ddd*, $J = 11.2, 9.0, 3.2$, 1 H); 2.35 (*dd*, $J = 11.6, 8.6$, 1 H); 2.52–2.58 (*m*, 1 H); 2.77 (*ddd*, $J = 12.0, 9.2, 3.2$, 1 H); 2.83 (*ddd*, $J = 11.6, 3.6, 1.6$, 1 H); 2.99 (*ddd*, $J = 12.0, 4.0, 3.0$, 1 H); 3.10–3.15 (*m*, 2 H); 3.33 (*dd*, $J = 8.4, 3.4$, 1 H); 3.97 (*dq*, $J = 8.6, 7.0, 4$ H); 5.50 (br. *s*, 2 H); 5.88 (*ddt*, $J = 21.0, 17.0, 2.0$, 1 H); 6.52 (*ddt*, $J = 22.0, 17.0, 5.6$, 1 H).

(R,E)-4-(3-Phosphonoprop-2-enyl)piperazine-2-carboxylic Acid Hydrate (= D-*CPP-ene* · H_2O; **13**·H_2O). A soln. of crude **11** obtained from **9** (11.61 g, 26 mmol) in abs. CH_2Cl_2 (300 ml) was treated at r.t. with bromotrimethylsilane (30.69 g, 200 mmol). After 16 h at r.t., the mixture was evaporated. The residue was partitioned between H_2O and CH_2Cl_2 and the aq. phase neutralised to pH 7–8 by addition of *Dowex 1 × 4* (OH^- form). The mixture was poured on the top of a column containing a 30-ml layer of *Dowex 1 × 4* (AcO^- form) and eluted with a gradient of aq. AcOH (0.05–0.25N). The fractions containing the product of R_f 0.15 (TLC, MeOH/H_2O/conc. NH_3 90:5:5) were concentrated and freeze-dried. The residue (6.5 g) was crystallized from H_2O/EtOH to give **13** (4.0 g, 58%) as hydrate. White crystals. M.p. 206° (dec.). $[\alpha]_D^{20} = -21.7$ ($c = 1.1$, 2N aq. HCl). ^{1}H-NMR (D_2O): 3.27–3.55 (*m*, 3 H); 3.70–3.85 (*m*, 2 H); 3.90–4.10 (*m*, 3 H); 4.17 (*dd*, $J = 11.0, 3.8$, 1 H); 6.27–6.48 (*m*, 2 H). ^{13}C-NMR (D_2O): 42.40 (*s*); 50.09 (*s*); 53.08 (*s*); 57.67 (*s*); 61.40 (*d*, $J = 24$); 133.16 (*d*, $J = 5.5$); 138.76 (*d*, $J = 172$); 171.020 (*s*). Anal. calc. for $C_8H_{15}N_2O_5P \cdot H_2O$ (268.21): C 35.8, H 6.4, N 10.4, P 11.5; found: C 35.6, H 6.4, N 10.7, P 11.5.

(S,E)-4-(3-Phosphonoprop-2-enyl)piperazine-2-carboxylic Acid Hydrate (= L-*CPP-ene* · H_2O; **14**·H_2O). The crude **12** obtained from **10** (4.7 g, 10.6 mmol) under the same conditions as for **11**, gave, after treatment with trimethylbromosilane, (10 ml), chromatography on *Dowex-1 × 4* (OH^- form) and crystallization (H_2O/EtOH), 1.55 g (55%) of **14**·H_2O. White crystals. M.p. 213° (dec.). $[\alpha]_D^{20} = +21.6$ ($c = 1.1$, 2N HCl). ^{1}H-NMR: as for **13**. Anal. calc. for $C_8H_{15}N_2O_5P \cdot H_2O$ (268.21): C 35.8, H 6.4, N 10.4, P 11.5; found: C 35.4, H 6.3, N 10.4, P 11.9.

(R)-4-(3-Phosphonopropyl)piperazine-2-carboxylic Acid Hydrate (**15**·H_2O). A soln. of **13** (1.6 g, 6.0 mmol) in conc. NH_3/H_2O 1:1 (26 ml) was hydrogenated at r.t. and normal pressure in the presence of 160 mg of 10% Pd/C. After the absorption of H_2 had ceased, the soln. was filtered. *Dowex 1 × 4* (OH^- form) was added until pH 9 and the mixture poured on the top of a column containing 10 ml of *Dowex 1 × 4* (AcO^-). Elution with an aq. AcOH gradient (0–0.2N) gave, after lyophilisation, 1.13 g (75%) of **15** that was crystallized from H_2O/MeOH. White crystals. M.p. 206° (dec.). $[\alpha]_D^{20} = -20.4$ ($c = 1.2$, 2N HCl). ^{1}H-NMR (200 MHz, D_2O): 1.56–1.79 (*m*, 2 H); 1.86–2.13 (*m*, 2 H); 3.19–3.53 (*m*, 5 H); 3.66–3.86 (*m*, 2 H); 3.92–4.05 (*m*, 1 H); 4.10 (*dd*, $J = 11.0, 3.6$, 1 H). Anal. calc. for $C_8H_{17}N_2O_5P \cdot H_2O$ (270.22): C 35.6, H 7.1, N 10.4, P 11.5; found: C 35.6, H 7.5, N 10.4, P 11.9.

(S)-4-(3-Phosphonopropyl)piperazine-2-carboxylic Acid Hydrate (**16**·H_2O). A soln. of **14** (3 g, 11.2 mmol) was reduced as above to give **16** (2.57 g, 91.1%) as a partially hydrated foam. $[\alpha]_D^{20} = +19.2$ ($c = 1.3$, 2N HCl). ^{1}H-NMR (200 MHz, D_2O): as for **15**. Anal. calc. for $C_8H_{17}N_2O_5P \cdot 0.3\ H_2O$ (257.62): C 37.3, H 6.9, N 10.9, P 12.0; found: C 37.6, H 6.9, N 10.6, P 11.7.

Methyl (R,E)-4-[3-(Dimethoxyphosphinyl)prop-2-enyl]-1-methylpiperazine-2-carboxylate (**17**). A soln. of **13** (360 mg, 1.34 mmol) in H_2O (25 ml) and MeOH (10 ml) was treated with a large excess of CH_2N_2 [20] until persistence of the yellow colour. After destruction of the excess diazomethane with AcOH and evaporation, the resulting oil was purified by chromatography on silica gel (CH_2Cl_2/EtOH/conc. NH_3 90:9:1). The product with R_f 0.45 (TCL, *dito*) was isolated to give **17** (100 mg, 24%) as an oil. ^{1}H-NMR: 2.32–2.49 (*m*, 6 H); 2.63–2.72 (*m*, 1 H); 2.76–2.85 (*m*, 1 H); 2.91–3.00 (*m*, 1 H); 3.05 (*dd*, $J = 9,4$, 1 H); 3.12–3.17 (*m*, 2 H); 3.73 (*d*, $J = 11$, 6 H); 3.75 (*s*, 3 H); 5.87 (*ddt*, $J = 21, 17, 2$, 1 H); 6.74 (*ddt*, $J = 22, 17, 6$, 1 H).

The same procedure was applied for the preparation of **18**–**20**.

Methyl (S,E)-4-[3-(Dimethoxyphosphinyl)prop-2-enyl]-1-methylpiperazine-2-carboxylate (**18**): From **14**.

Methyl (R)-4-[3-(Dimethoxyphosphinyl)propyl]-1-methylpiperazine-2-carboxylate (**19**): From **15**. ^{1}H-NMR: 1.67–1.87 (*m*, 4 H); 2.25–2.46 (*m*, 8 H); 2.61–3.06 (*m*, 4 H); 3.74 (*d*, *J* = 11, 6 H); 3.77 (*s*, 3 H).

Methyl (S)-4-[3-(Dimethoxyphosphinyl)propyl]-1-methylpiperazine-2-carboxylate (**20**): From **16**.

(R)-Piperazine-2-carboxylic Acid Dihydrochloride (**21**·2HCl). *From Methyl (R)-6-Oxopiperazine-2-carboxylate* (**25**). To a stirred mixture of **25** (553 mg, 3.5 mmol) and $NaBH_4$ (665 mg, 17.6 mmol) in anh. dioxane (14 ml), AcOH (1 ml) in dioxane (1 ml) was added at 0° within 20 min. The mixture was stirred for 0.5 h at 20° and 0.5 h at 70°. The mixture was evaporated, the residue treated with ice and extracted with CH_2Cl_2, the org. layer dried (Na_2SO_4) and evaporated, the oily residue (0.2 g) dissolved in conc. HCl soln. (0.4 ml) and H_2O (0.8 ml), and the soln. heated under reflux for 15 min. The crystals were filtered off, washed with H_2O/EtOH and then with EtOH: 55 mg of **21** as white crystals. M.p. 238–243° (dec.). $[\alpha]_D^{21} = +5.9$ (*c* = 1.25, H_2O). ^{1}H-NMR (D_2O): 3.35–3.56 (*m*, 3 H); 3.66–3.86 (*m*, 2 H); 3.96 (*dd*, *J* = 14.0, 4.0, 1 H); 4.32 (*dd*, *J* = 11.0, 4.0, 1 H). Anal. calc. for $C_5H_{10}N_2O_2 \cdot 2$ HCl (203.07): C 29.6, H 6.0, Cl 34.9, N 13.8; found: C 29.5, H 5.8, Cl 35.1, N 13.8.

From **7** *with HCl.* A soln. of **7**·2HCl (500 mg, 1.46 mmol) in 6N aq. HCl (6 ml) was heated 2 h at 120°. The soln. was cooled to r.t. and Et_2O was added. The crystalline material was filtered off and washed with EtOH and Et_2O. Recrystallization from H_2O/EtOH gave 194 mg (65%) of **21**. M.p. 252–255° (dec.). $[\alpha]_D^{20} = +5.2$ (*c* = 1.2, H_2O). ^{1}H-NMR (D_2O): as above.

From **7** *with* BCl_3. A soln. of 610 mg (2.25 mmol) of **7** (free base) in CH_2Cl_2 (6 ml) was treated at −20° with 2.2N BCl_3 in 1,2-dichloroethane (4.1 ml), kept overnight at −20°, neutralised by addition of aq. NH_3 soln., and partitioned between H_2O and CH_2Cl_2. The aq. phase was evaporated and the residue crystallized from H_2O/ethanolic HCl to afford 200 mg (43%) of **21**. M.p. 247–253° (dec.). $[\alpha]_D^{20} = +5.8$ (*c* = 1.1, H_2O). ^{1}H-NMR (D_2O): as above.

*3-Amino-*N*-[(benzyloxy)carbonyl]-*D*-alanine* (**23**) was prepared according to the procedure reported for the L-enantiomer [13]. M.p. 230–232° (dec.). $[\alpha]_D^{20} = +7.5$ (*c* = 1.3, 1N NaOH) ([13]: m.p. 228–230°, $[\alpha]_D^{20} = -7.8$ (*c* = 0.4, 1N NaOH)).

*3-Amino-*N*-[(benzyloxy)carbonyl]-*D*-alanine Methyl Ester Hydrochloride* (**24**·HCl). To a precooled (−10°) mixture of anh. MeOH (23 ml) and $SOCl_2$ (2.2 ml, 30 mmol), **23** (7.1 g, 29.8 mmol) was added. The mixture was stirred 0.5 h at 20° and 5 h at 50°. After standing overnight, the product crystallized: 7.5 g (87%). Colourless crystals. M.p. 169–171°. $[\alpha]_D^{22} = +41.9$ (*c* = 1.35, H_2O; [21]: m.p. 165–167°; $[\alpha]_D^{20} = -42.5$ (*c* = 1, MeOH) for the L-enantiomer). Anal. calc. for $C_{12}H_{16}N_2O_4 \cdot$HCl (288.73): C 49.9, H 5.9, Cl 12.3, N 9.7; found: C 50.1, H 5.6, Cl 12.4, N 9.7.

Methyl (R)-6-Oxopiperazine-2-carboxylate (**25**). To a stirred mixture of **24** (free base; 6.2 g, 24.5 mmol), $Et(i\text{-}Pr)_2N$ (3.5 g, 26.7 mmol), and anh. THF (65 ml), methyl bromoacetate in anh. THF (10 ml) was added at 0° in 15 min. The mixture was stirred for 1 h at 0° and left overnight at 20°, the suspension was filtered, and the filtrate evaporated. The residue was treated with Et_2O, filtered, and the solvent evaporated. The oily residue (7.8 g) was dissolved in EtOH (100 ml) and hydrogenated over 10% Pd/C. The catalyst was filtered off, the solvent removed, and the product crystallized from EtOH/Et_2O: 2.7 g (70%) of **25** as colourless crystals. M.p. 137–145°. $[\alpha]_D^{20} = -14.7$ (*c* = 1.25, H_2O). ^{1}H-NMR (DMSO): 3.01 (*AB* of *ABX*, *J* = 13, 2 H); 3.18 (*AB*, *J* = 17, 2 H); 3.60–3.95 (br., 1 H); 3.66 (*s*, 3 H); 4.03 (*X* of *ABX*, 1 H); 7.85 (br. *s*, 1 H). Anal. calc. for $C_6H_{10}N_2O_3$ (158.16): C 45.6, H 6.4, N 17.7, O 30.3; found: C 45.0, H 6.3, N 17.5, O 30.0.

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REFERENCES

[1] R. L. Johnson, J. F. Koerner, *J. Med. Chem.* **1988**, *31*, 2057.
[2] J. Lehmann, J. A. Schneider, M. Williams, *Annu. Rep. Med. Chem.* **1987**, *22*, 31.
[3] J. C. Watkins, H. J. Olverman, *Trends Neurosci.* **1987**, *10*, 265.
[4] T. Honore, *Med. Res. Rev.* **1989**, *9*, 1.
[5] K. Q. Do, P. L. Herrling, P. Streit, W. A. Turski, M. Cuenod, *J. Neurosci.* **1986**, *6*, 2226.
[6] J. Davies, R. H. Evans, P. L. Herrling, A. W. Jones, H. J. Olverman, P. Pook, J. C. Watkins, *Brain Res.* **1986**, *382*, 169.
[7] E. W. Harris, A. H. Ganong, D. T. Monaghan, J. C. Watkins, C. W. Cotman, *Brain Res.* **1986**, *382*, 174.
[8] J. Lehmann, J. Schneider, S. McPherson, D. E. Murphy, P. Bernard, P. C. Tsai, D. A. Bennett, G. Pastor, D. J. Steel, C. Boehm, D. L. Cheney, J. M. Liebman, M. Williams, P. L. Wood, *J. Pharmacol. Exp. Ther.* **1987**, *240*, 737.
[9] L. Turski, T. Klockgether, K. H. Sontag, P. L. Herrling, J. C. Watkins, *Neurosci. Lett.* **1987**, *73*, 143.
[10] C. A. Boast, S. C. Gerhardt, G. Pastor, J. Lehmann, P. E. Etienne, J. M. Liebman, *Brain Res.* **1988**, *442*, 345.
[11] E. Jucker, E. Rissi, *Helv. Chim. Acta* **1962**, *45*, 2383.
[12] K. Hemmi, H. Takeno, M. Hashimoto, T. Kamiya, *Chem. Pharm. Bull.* **1982**, *30*, 111.
[13] M. Waki, Y. Kitajiama, N. Izumiya, *Synthesis* **1981**, 266.
[14] E. Felder, S. Maffei, S. Pietra, D. Pitre, *Helv. Chim. Acta* **1960**, *43*, 888.
[15] M. D. Walkinshaw, J. J. Boelsterli, unpublished results.
[16] H. J. Olverman, D. T. Monaghan, C. W. Cotman, J. C. Watkins, *Eur. J. Pharm.* **1986**, *131*, 161.
[17] P. L. Herrling, *Neuroscience* **1985**, *14*, 417.
[18] T. P. Kenakin, 'Pharmacological Analysis of Drug-Receptor Interaction', Raven Press, New York, 1987.
[19] A. Luini, O. Goldberg, V. I. Teichberg, *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3250.
[20] T. J. De Boer, H. J. Backer, *Org. Synth.* **1963**, *Coll. Vol. 4*, 943.
[21] S. Moore, R. P. Patel, E. Atherton, M. Kondo, J. Meienhofer, L. Blau, R. Bittman, R. K. Johnson, *J. Med. Chem.* **1976**, *19*, 766.