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ENZYMATIC ASYMMETRIC SYNTHESIS OF (2R, 4R, 5S)-2-AMINO-4,5-(1,2-CYCLOHEXYL)-7-PHOSPHONOHEPTANOIC ACID, A POTENT, SELECTIVE AND COMPETITIVE NMDA ANTAGONIST[1]

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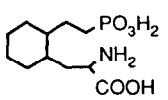
Abstract: The title compound was prepared by an efficient route which used chemoenzymatic processes to establish all absolute stereochemistry. This compound was shown to be the active isomer of the previously reported isomeric mixture, NPC 12626.

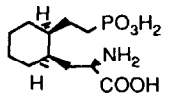
The view that L-glutamate and L-aspartate are the principal excitatory neurotransmitters in the vertebrate central nervous system has gained widespread acceptance in recent years.[2] Excitatory amino acids (EAAs) are neurotoxic, causing neuronal degeneration and ultimately cell death after local administration in brain tissue or cultured neurons.[3] Dysfunction in EAA neurotransmission has been implicated in the etiology of various mental disorders associated with neurodegeneration,[4] including epilepsy, cerebral ischemia, hypoglycaemia, Huntington's and Alzheimer's diseases and, most recently, Parkinson's disease. This growing number of disorders in which EAAs have been implicated has resulted in considerable effort toward the development of compounds which antagonize excitatory amino acid neurotransmission. Compounds which act selectively and competitively at the NMDA subclass of EAA receptor have received particular attention.

NPC 12626 (Fig. 1) is a potent and selective competitive NMDA antagonist that has been found to be an efficacious anticonvulsant against NMDA-, pentylenetetrazole- and maximal electroshock-induced seizures.[5] The compound displays anxiolytic activity in animal models and is a potent protectant against agonist- or hypoxia-induced neuronal damage. Though initially prepared and evaluated as an isomeric mixture, it was recognized that the NMDA antagonist activity was likely to reside primarily in a single isomer. We report herein the synthesis of the active constituent of NPC 12626, the 2R, 4R, 5S isomer, designated NPC 17742.

Figure 1.



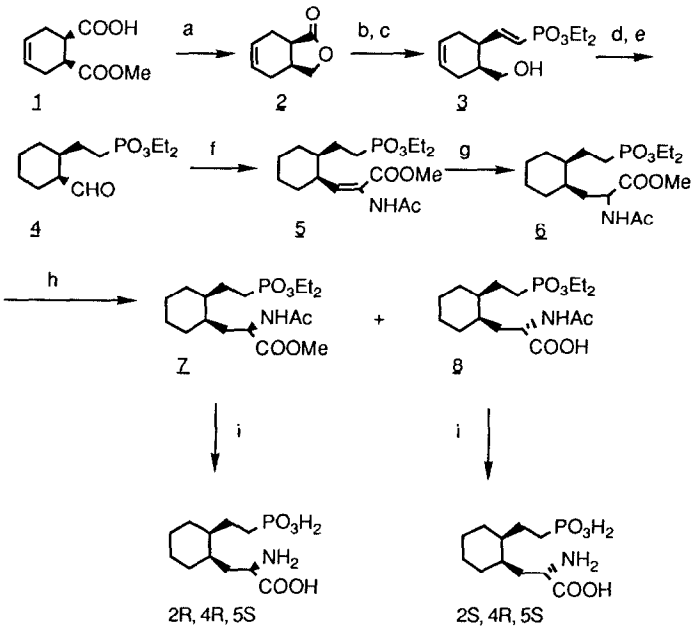
NPC 12626
(Isomeric mixture)



NPC 17742
(2R, 4R, 5S- isomer)

Our original synthesis of NPC 12626 was based on catalytic hydrogenation of the aromatic precursor; this reduction gave a mixture of eight stereoisomers typically consisting of 80% four *cis* cyclohexanes and 20% *trans* isomers. It was possible by means of high performance liquid chromatography to obtain samples of separated *cis* and *trans* isomers. Biological evaluation of these materials indicated that the *trans* isomers were inactive and that the NMDA antagonist activity resided in one or more of the *cis* isomers.

A primary goal of our work was to avoid the necessity of performing resolutions of diastereomeric mixtures to obtain optically active material; we wished to establish all absolute stereochemistry in an unambiguous and controlled fashion. Our departure point was chiral half-ester 1 (Scheme 1), readily available in high chemical and optical yield via pig liver esterase (PLE) hydrolysis of the corresponding *meso* diester.[6] This key building block was converted to opucally active lactone 2 by selective reduction of the ester functionality using lithium



Scheme I. a) Lithium triethyl borohydride, THF, -78°C then H_3O^+, b) Diisobutyl aluminum hydride, toluene, -78°C; c) Sodio tetraethyl methylenebisphosphonate, THF, -78°C to room temperature; d) H_2, 5% Pd/C, 50 psi, ethanol; e) Pyridine-SO_3, DMSO, triethylamine, r.t f) Sodio 2-acetylamino-2-(dimethoxyphosphinyl)-acetate, THF, r.t ; g) H_2, 5% Pd/C, 50psi, ethanol, h) Subtilisin A, phosphate buffer (pH 7.4); i) 6N HCl, 95°C, then ethanol, propylene oxide.

triethylborohydride, followed by acidic aqeous workup. Use of diborane as the reducing agent, effecting chemoselective reduction of the carboxylic acid group, provided the enantiomeric lactone. These lactones had optical rotations whose signs and magnitudes corresponded to those reported in the literature for these compounds.[7] For purposes of clarity, elaboration of only one of these lactones is shown in Scheme I. Introduction of the phosphonoethyl side chain was achieved by reducing lactone 2 to the corresponding lactol with diisobutylaluminum hydride followed by treatment with the sodium salt of tetraethyl methylenebisphosphonate.

Following reduction of the double bonds in diene 3 via catalytic hydrogenation, the alcohol moiety was oxidized to aldehyde 4 using DMSO and sulfur trioxide followed by triethylamine. We envisioned using the glycinate Wittig reagents of Ullrich Schmidt in order to incorporate the amino acid unit to complete the skeleton of the target. Although these reagents have proven useful in reaction with a variety of aromatic and aliphatic aldehydes,[8] in the case at hand the yields of dehydro amino acid 5 were consistently mediocre. Various combinations of bases (potassium tert-butoxide, LDA, sodium hydride) and solvents (THF, hexane, methylene chloride) were explored, but yields of 5 no greater than 15% were obtained. In addition, the purification of 5 via silica gel chromatography was complicated by the presence of significant amounts of unreacted Wittig reagent.

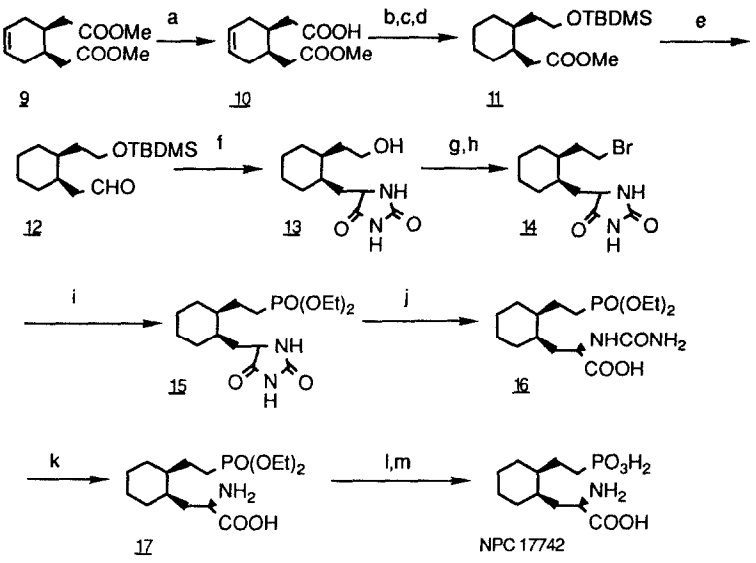
Despite the disappointing yields of dehydro amino acid 5, sufficient material was obtained to proceed through the sequence and obtain small quantities of isomerically pure material. Reduction of the double bond in 5 by catalytic hydrogenation provided a mixture of diastereomeric protected amino acids 6. This diastereomeric mixture was resolved using the protease subtilisin A, an enzyme which selectively hydrolyzes the carboxylic ester of the S-isomer of a mixture of N-acyl amino acid esters.[9] Incubation of 6 with Subtilisin A in pH 7.4 phosphate buffer for 24 hours resulted in a mixture of S-acid 7 and R-ester 8, which was readily separated by extractive techniques. The separated isomers thus obtained were hydrolytically deprotected to deliver samples of pure *cis* isomers of NPC 12626; in the specific example shown in Scheme I, the 2R, 4R, 5S and 2S, 4R, 5S isomers.

Evaluation of the four *cis* isomers in receptor binding assays indicated clearly that the 2R, 4R, 5S isomer (henceforth designated NPC 17742) was the most active antagonist of the mixture. The potency of NPC 17742 to inhibit EAA receptor ligand binding[10] (Table I), as well as its potency to block PTZ-, NMDA- and MES-induced seizures[10] (Table II), compared favorably with other known competitive NMDA antagonists such as CGP 37849,[11] D-CPP[12] and CGS 19755.[13] However, it was clear that an alternate synthetic approach would be required to obtain gram quantities of NPC 17742 for full pharmacological and toxicological characterization. We thus developed the route depicted in Scheme II.

We felt that a route beginning with diester 9,[14] in which the carbonyl groups are further from the ring junction and thus less susceptible to steric effects of the cyclohexane ring, might prove less troublesome. We therefore subjected 9 to enzymatic hydrolysis by porcine pancreas lipase as described by Nagao.[15] Monoester 10 was obtained in excellent chemical and optical yield (>97% e.e.).

After reducing the double bond in 10, the carboxylic acid was reduced to the alcohol using diborane; the alcohol was then protected as the tert-butyldimethylsilyl ether. The ester functionality was subsequently reduced to the aldehyde with diisobutylaluminum hydride. Reaction of aldehyde 12 with ammonium carbonate and sodium cyanide in a modified Strecker reaction furnished hydroxy hydantoin 13 as a white solid; the silyl group was lost

upon acidic workup. Stepwise conversion of the hydroxyl group in 13 to the mesylate and thence to the bromide, using classical conditions, delivered bromide 14. Reaction of 14 with the sodium salt of diethyl phosphite in THF then furnished hydantoinyl phosphonate 15.



Scheme II. a) Porcine pancreas lipase , phosphate buffer; b) H_2, Pd/C, EtOH; c) BH_3/THF; d) TBDMS chloride, DMAP/Et_3N, DMF; e) DIBAL, toluene, -78°C; f) NaCN/NH_4CO_3, EtOH, 90°C, sealed tube, 18 hours, then H_3O^+; g) MsCl, pyridine; h) LiBr, DMF; i) $NaPO(OEt)_2$, THF; j) D-hydantoinase, k) $NaNO_2$/HCl; l) 10N HCl, 95°C, 48 hours, then concentrate; m) Ethanol, propylene oxide

Hydantoins are 'masked' amino acids, and are susceptible to asymmetric enzymatic cleavage by enzymes known as hydantoinases.[16] We have found that hydantoin 15 is a substrate for D-hydantoinase, and may be converted in excellent chemical and optical yield to carbamoyl compound 16. By running this enzymatic reaction an an alkaline buffer, racemization of the unreacted L-isomer occurs so that the racemic mixture of hydantoins is converted efficiently to the single stereoisomer 16.[16] We have verified the absolute stereochemistry of 16 via single crystal X-ray analysis.[17] Cleavage of the carbamate moiety using nitrous acid,[16] followed by acidic aqeous hydrolysis of the phosphonate esters, provided the 2R, 4R, 5S isomer in 15% overall yield from 10. NPC 17742 is available in gram quantities via this route, and contains <1% isomeric impurities as evaluated by HPLC analysis.

In sum, we have determined that the active isomer of NPC 12626 is the 2R, 4R, 5S isomer, and have developed a synthetic scheme whereby gram quantities of the pure isomer may be prepared. Full details of the preclinical pharmacology and toxicology will be reported separately.

TABLE I
INHIBITION OF EXCITATORY AMINO ACID RECEPTOR LIGAND BINDING BY NMDA ANTAGONISTS[18]

COMPOUND	L-[^{3}H]GLUTAMATE		[^{3}H]CGS-19755	
	IC_{50} (nM)	n_H	IC_{50} (nM)	n_H
CGP-37849	25.7 ± 3.2	0.46 ± 0.02	18 ± 6	0.70 ± 0.04
D-CPP	321 ± 36	0.66 ± 0.02	86 ± 19	0.80 ± 0.03
NPC 12626	1600 ± 36	0.55 ± 0.03	976 ± 203	0.77 ± 0.04
2R, 4R, 5S isomer	607 ± 110	0.59 ± 0.04	148 ± 25	0.77 ± 0.05
2S, 4R, 5S isomer	2589 ± 577	0.60 ± 0.02	264 ± 85	0.76 ± 0.07
2R, 4S, 5R isomer	3132 ± 1767	0.59 ± 0.03	555 ± 125	0.85 ± 0.11
2S, 4S, 5R isomer	6458 ± 1611	0.69 ± 0.02	239 ± 85	0.93 ± 0.07

TABLE II
POTENCY OF VARIOUS NMDA ANTAGONISTS TO BLOCK PENTYLENETETRAZOLE-, NMDA- AND MAXIMAL ELECTROSHOCK-INDUCED SEIZURES (IP ADMINISTRATION)[18]

COMPOUND	ED_{50} (mg/kg)		
	MES	NMDA	PTZ
NPC 12626 (Mixture)	>15	12.40	6.02
NPC 17742	13.40	2.68	4.51
CGP 37849	3.64	3.41	>>10
CGS 19755	12.90	2.64	29.00

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17. Details of the structural analysis, including X-ray crystallographic data, will be reported in the full paper. All compounds reported herein had spectroscopic and elemental analysis data in accord with the given structures.

18. Details regarding the protocols used in the biological assays, as well as an in-depth description of the pharmacological profile of NPC 17742, may be found in Reference 10. All final products were obtained and tested as their free bases