# Asymmetric Synthesis of a Potent and Selective Competitive NMDA Antagonist[1]

Gregory S. Hamilton,* Zhiwen Huang, Xiao-Jing Yang, Raymond J. Patch, B. A. Narayanan, and John W. Ferkany

*Scios Nova, Inc., 6200 Freeport Centre, Baltimore, Maryland 21224-6522*

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(2*R*,4*R*,5*S*)-2-Amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid (**15**) has been prepared by an efficient nine-step route (16% overall yield) which uses chemoenzymatic processes to establish all absolute stereochemistry. This compound was found to be the most active isomer of the previously reported isomeric mixture, NPC 12626. In addition, synthetic routes were developed for the stereochemically unambiguous preparation of all the other *cis* isomers of this racemate. All of the synthetic pathways utilized enzymatic hydrolysis of a *meso* diester to prepare key optically pure building blocks. Pharmacological evaluation of the isomers indicates that **15** is one of the most potent and least toxic NMDA antagonists discovered to date.

## Introduction

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.[2,3] These excitatory amino acids (EAAs) are neurotoxic, causing neuronal degeneration and ultimately cell death after local administration in brain tissue or cultured neurons. Dysfunction in EAA neurotransmission has been implicated in the etiology of various mental disorders associated with neurodegeneration, including epilepsy, cerebral ischemia, hypoglycaemia, Huntington's and Alzheimer's diseases, and parkinsonism. This growing number of disorders in which EAAs have been implicated has resulted in considerable attention being focused on the development of compounds which antagonize excitatory amino acid neurotransmission. Compounds which act selectively and competitively at the subclass of EAA receptor selectively activated by *N*-methyl-D-aspartate (NMDA) have received particular attention.[4]

NPC 12626 (Figure 1) is a potent and selective competitive NMDA antagonist that has been found to be an efficacious anticonvulsant against NMDA-, pentylenetetrazol-, 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. The stereoselective nature of the NMDA receptor is well established, albeit not completely understood. For the endogenous agonist glutamate, the L-configuration is preferred; in the case of aspartate, there is no significant differentiation between L- and D-stereoisomers.[6] N-Methylation of D-aspartate to NMDA does not alter its affinity for the receptor, but N-methylation of L-aspartate to NMLA leads to a significant decrease in binding affinity. With regard to the protoypical antagonists AP5 and AP7, the D-configuration (i.e., *R*) at the amino acid center is preferred. This is also true of the potent acyclic AP5 analogues CGP 37849 and CGP 39551[7] and the cyclic AP5 analogue CGS 19755.[8] In the case of the cyclic AP7 analogues CPP and CPP-ene the D-amino acid stereochemistry is likewise the preferred antagonist configuration.[9] Nonetheless, it cannot be stated that antagonists invariably prefer the D-(or (*R*)-)amino acid. Ornstein et al. have recently reported[10] that the active isomer of their reduced isoquinoline analogue of AP7, LY

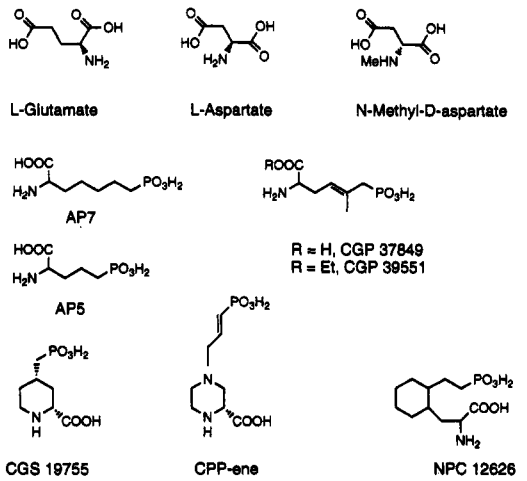


**Figure 1.** Structures of some NMDA agonists and antagonists.

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(1) Portions of this work have appeared in a preliminary account: Hamilton, G. S.; Huang, Z.; Patch, R. J.; Narayanan, B. A.; Ferkany, J. W. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 27.

(2) Mayer, M. L.; Westbrook, G. L. *Prog. Neurobiol.* **1990**, *28*, 197.

(3) Monaghan, D. T.; Bridges, R. J.; Cotman, C. W. *Rev. Pharm. Tox.* **1989**, *29*, 365.

(4) Lehman, J.; Schneider, J.; William, M. *Annu. Rep. Med. Chem.* **1987**, *22*, 31.

(5) Ferkany, J. W.; Kyle, D. J.; Willets, J.; Rzeszotarski, W. J.; Guzewska, M. E.; Ellenberger, S. R.; Jones, S. M.; Sacaan, A. I.; Snell, L. D.; Borosky, S.; Jones, B. E.; Johnson, K. M.; Balster, R. L.; Burchett, K.; Kawasaki, K.; Hoch, D. B.; Dingledine, R. *J. Pharm. Exp. Ther.* **1989**, *250*, 100.

(6) Watkins, J. C.; Olverman, H. J. *Trends Neurosci.* **1987**, *10*, 265.

(7) Fagg, G. E.; Olpe, H.-R.; Pozza, M. F.; Baud, J.; Steinmann, M.; Schmutz, M.; Portet, C.; Baumann, P.; Thedinga, K.; Bittiger, H.; Allgeier, H.; Herkendon, R.; Angst, C.; Brundish, D.; Dingwall, J. G. *Br. J. Pharm.* **1990**, *99*, 791.

(8) Hutchinson, A. J.; Williams, M.; Angst, C.; de Jesus, R.; Blanchard, L.; Jackson, R. H.; Wilusz, E. J.; Murphy, D. E.; Bernard, P. S.; Schneider, J.; Campbell, T.; Guida, W.; Sills, M. A. *J. Med. Chem.* **1989**, *32*, 2171.

(9) Aebischer, B.; Frey, P.; Haerter, H.-P.; Herrling, P. L.; Mueller, W.; Olverman, H. J.; Watkins, J. C. *Helv. Chim. Acta* **1989**, *72*, 1043.

(10) Ornstein, P. L.; Arnold, M. B.; Argenstein, N. K.; Schoepp, D. D.; Leander, J. D.; Klimkowski, V. J.; Zimmerman, D. M. The Discovery and Characterization of Competitive NMDA Antagonists as Therapeutic Agents. 201st National ACS Meeting, Atlanta, GA, April 15, 1991; Med. Chem. Division Abstract No. 6.

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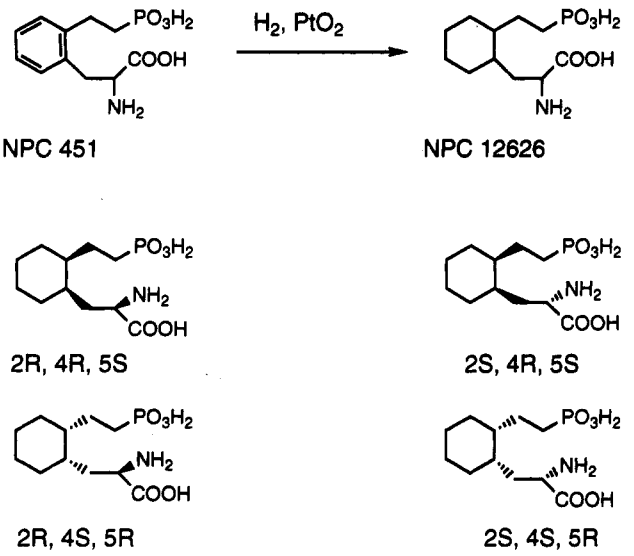



**Figure 2.** Cis isomers of NPC 12626 obtained via catalytic hydrogenation of NPC 451.

235959, has the L-(or *S*-)configuration at the amino acid center. It thus appears that the relative orientation of the functional groups in a folded conformation is a key aspect of the receptor stereoselectivity.

With the above considerations in mind, we embarked upon a program to develop a synthetic protocol which would allow us to prepare optically active isomers of NPC 12626 and elucidate the absolute stereochemistry of the active antagonist.

## Chemistry

Our original synthesis of NPC 12626 was based on catalytic hydrogenation of the aromatic precursor, NPC 451 (Figure 2).[11] This reduction gave a mixture of eight stereoisomers typically consisting of 80% *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 weakly active and that the NMDA antagonist activity resided in one or more of the *cis* isomers.

We decided at the outset to use chemoenzymatic methods for the establishment of all absolute stereochemistry. We chose as a departure point for our synthesis the preparation of an optically active *cis*-1,2-disubstituted cyclohexane ring by enantioselective enzymatic hydrolysis of a *cis* meso diester. We planned to incorporate the optically active $\alpha$-amino acid moiety by a second enzymatic transformation later in the synthesis. The half esters **2**[12,13] and **5**[14,15] are readily available in optically pure form by

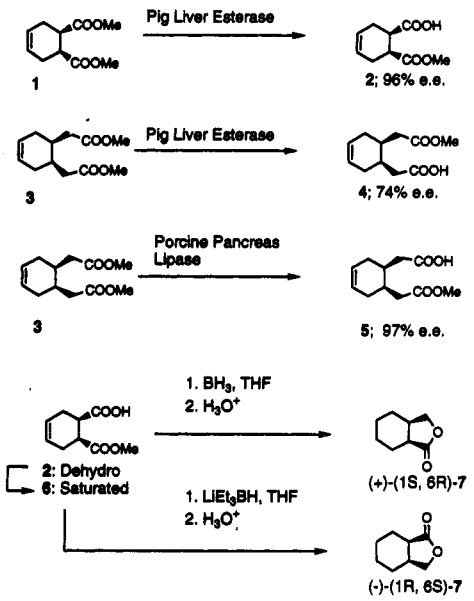
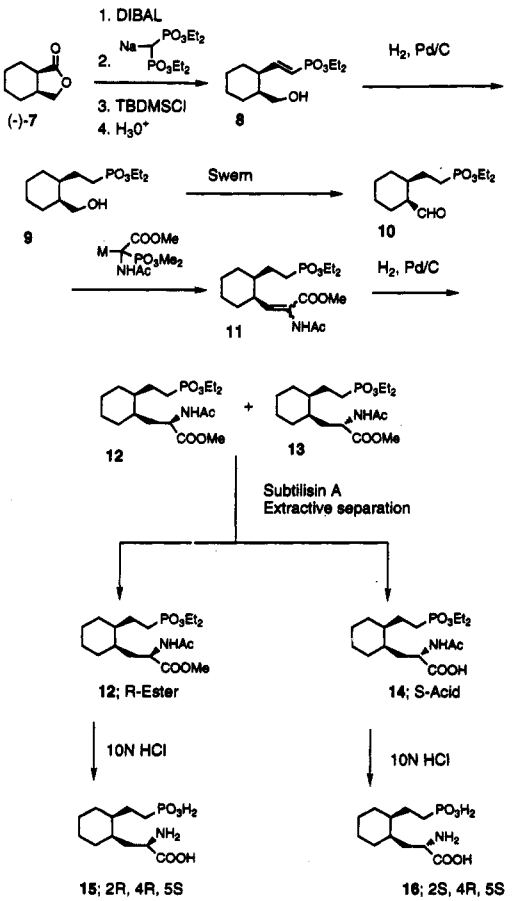



**Figure 3.**

**Scheme I**




enzymatic reduction of the meso diesters (Figure 3). Accordingly, our asymmetric synthesis commences from this point.

We intially worked with chiral half ester **2** (Scheme I). Following reduction of the double bond via catalytic hydrogenation, selective reduction of the carboxylic acid (diborane) or the methyl ester (lithium triethylborohy-

(11) Ferkany, J. W.; Kyle, D. J.; Ellenberger, W. P.; Narayanan, B. A.; Ellenberger, S. R.; Hudkins, R.; Guzewska, M. E.; Rzeszotarski, W. J.; Conti, L.; Patch, R. J.; Enna, S. In *Neurotransmitters In Epilepsy (Epilepsy Research, Supplement 8)*; Avanzini, G., Engel, J., Fariello, R., Heinemann, E., Eds.; Elsevier: Amsterdam, 1992; pp 219–227.

(12) (a) Mohr, P.; Waespe-Sareevic, N.; Tamm, C.; Gawronska, K.; Gawronski, J. K. *Helv. Chim. Acta.* **1983**, *66*, 2501. (b) Seebach, D.; Eberle, M. *Chimia* **1986**, *40*, 315. (c) Ohno, M.; Kobayashi, S.; Adachi, K. *Chimia* **1986**, *40*, 311. (d) Ohno, M.; Otsuka, M. *Organic Reactions* **1989**, *37*, 1.

(13) (a) Guanti, G.; Banfi, L.; Narisano, E.; Riva, R.; Theu, S. *Tetrahedron Lett.* **1986**, *27*, 4639. (b) Ohno, M.; Iimori, T.; Kamiyama, K.; Kobayashi, S. *Tetrahedron Lett.* **1984**, *24*, 2557.

(14) Nagao, Y.; Kume, M.; Wakabayashi, R. C.; Nakamura, T.; Ochiai, M. *The 13th Symposium of Progress in Organic Reactions and Synthesis*, Tokushima, Japan, 1986.

(15) Nagao, Y.; Kume, M.; Wakabayashi, R. C.; Nakamura, T.; Ochiai, M. *Chem. Lett.* **1989**, 239.

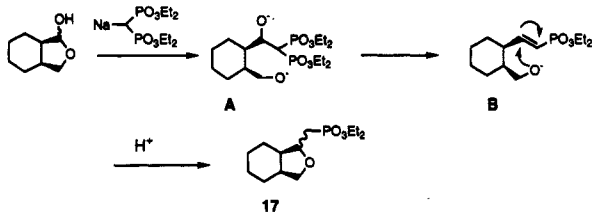


Figure 4.

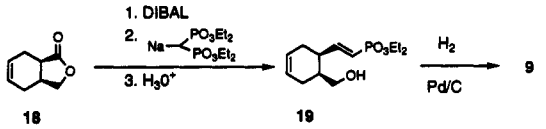


Figure 5.

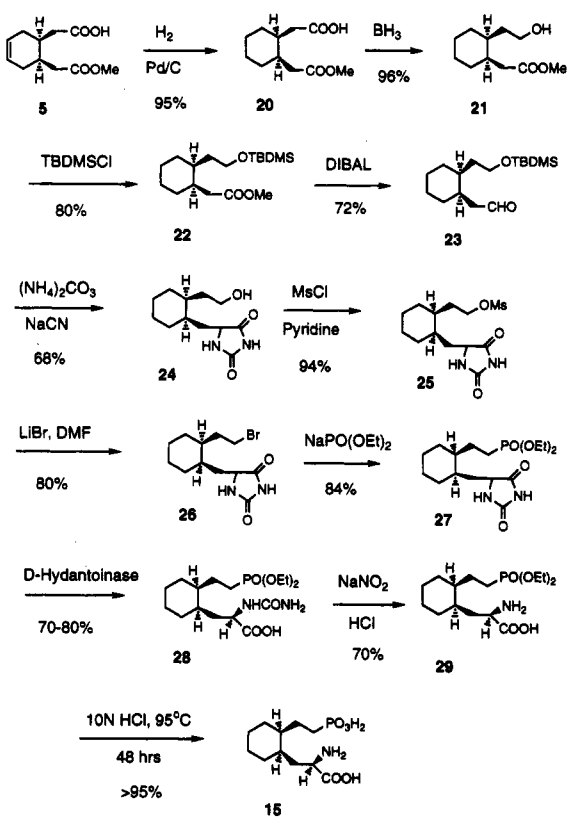
dride) furnished, after aqueous acidic workup, the (+)-(1*S*,2*R*)- and (−)-(1*R*,2*S*)-lactones (+)-7 and (−)-7, respectively.[16]

Treatment of lactone 7 with 1.2 equiv of DIBAL in toluene at −78 °C resulted in rapid and quantitative reduction to the lactol. This reduction was quite solvent sensitive; of the solvents evaluated (hexane, THF, methylene chloride, and toluene) only toluene gave consistently good results. When this lactol was reacted with the sodium salt of tetraethyl methylenebisphosphonate[17,18] only 17 was obtained (Figure 4). Apparently, the initially formed dianion **A** is converted to species **B** on warming to room temperature. Protection of the primary alkoxide with *tert*-butyldimethylsilyl chloride prevented the subsequent intramolecular Michael reaction following the elimination process. Following addition of the lactol reaction mixture to a solution of the phosphonate anion in THF at −78 °C,[19] the resulting mixture was stirred for several hours at the lowered temperature and then treated with 1 equiv of TBDMS chloride. The reaction mixture was subsequently allowed to come to room temperature overnight and worked up with aqueous acid. The silyl group was readily cleaved upon workup, and compound 8 was routinely obtained in good yield by this method. Catalytic hydrogenation of the double bond delivered 9 in quantitative yield.

Subsequent experimentation showed that the intramolecular conjugate addition could be avoided by using unsaturated lactone 18 (Figure 5). In this case there was no need to add TBDMS chloride to trap the intermediate adduct, and the reaction could be run at a higher temperature. Diene 19 obtained by this procedure was reduced by catalytic hydrogenation to obtain compound 9, identical to that obtained from saturated lactone 7.

Swern oxidation of hydroxy phosphonate 9 yielded aldehyde 10. Wittig reaction between this aldehyde and the salt of an *N*-acyl-2-(dialkoxyphosphinyl)glycine ester, as described by Schmidt et al.,[20] gave dehydro amino acid 11, albeit in poor yield. Though we were not able to

**Scheme II**



produce significant quantities of 11 by this methodology, it was possible to secure sufficient amounts to continue to the end of the sequence and obtain small amounts of the pure *cis* isomers.[21] The diastereomeric mixture of 12 and 13, obtained from hydrogenating 11, was resolved by treating the mixture with Subtilisin A in a buffered aqueous solution for 24 h[22] and separating the acid produced from the unreacted ester by conventional extractive techniques. HPLC and TLC analyses indicated that the resolution was efficient, providing 12 and 14 in greater than 98% diastereomeric excess. These two compounds were then hydrolytically deprotected with aqueous acid to provide samples of two cis isomers, 15 and 16. Evaluation of the small amounts of isomers obtained by this route led to the tentative identification of the active isomer of NPC 12626 as the 2*R*,4*R*,5*S* isomer (15). However, it was clear that an alternate synthetic approach would be required to obtain gram quantities of the materials for pharmacological and toxicological characterization.

We felt that a route beginning with diester 5 (Figure 3) might be free of some of the difficulties encountered in our original scheme. The fact that the carbonyl groups on the side chains are further out from the ring should greatly decrease reactivity problems due to unfavorable steric effects. Thus, we proceeded to elaborate 5 to the final product by the pathway shown in Scheme II. Following reduction of the double bond via catalytic hydrogenation, the carboxylic acid was reduced with diborane in THF to alcohol 21, which was protected as its *tert*-butyldimethylsilyl ether 22. Treatment of 22 with

(16) Jakovac, I. J.; Goodbrand, H. B.; Lok, K. P.; Jones, J. B. *J. Am. Chem. Soc.* **1982**, *104*, 4659.
(17) Wadsworth, W. S.; Emmons, W. D. *J. Am. Chem. Soc.* **1961**, *83*, 1733.
(18) Boland. W.; Niedermeyer, U.; Jaenicke, L.; Gorisch, H. *Helv. Chim. Acta.* **1985**, *68*, 2062.
(19) This condensation was successful when the crude lactol reaction mixture was added directly to a solution of Wittig reagent; the lactol in this case is presumably present as the aluminate. In contrast, when the lactol was first isolated and purified and then added to solutions of Wittig reagents, no reaction was observed.
(20) Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1984**, 53.
(21) A variety of bases (potassium *tert*-butoxide, lithium diisopropylamide, and sodium hydride) and solvents (hexane, THF, and methylene chloride) were investigated, and variations in amounts of reagents used and temperature of the reaction were made, but the reaction never yielded more than 10% of the desired product.
(22) Roper, J. M.; Bauer, D. P. *Synthesis* **1983**, 1041.

DIBAL in toluene furnished aldehyde **23**. In contrast to the aldehyde group of compound **10**, this aldehyde proved readily amenable to nucleophilic addition.

We wished to pursue the possibility of using a hydantoin as a synthetic equivalent for an $\alpha$-amino acid, in order to avail ourselves of known methods for the asymmetric hydrolysis of hydantoins using hydantoinase enzymes. Hydantoin derivatives have found considerable utility as precursors to $\alpha$-amino acids, to which they may be converted through the intermediacy of the corresponding ureido acids.[23] This may be done either hydrolytically, in aqueous base,[24] or enzymatically.[25] The latter procedure is particularly attractive since it may be used to prepare optically active $\alpha$-amino acids of either configuration under mild conditions. Indeed, it is frequently possible to convert a D,L-mixture of hydantoins in its entirety to a single amino acid of defined absolute configuration. Certain microorganisms which contain hydantoinases also contain a racemase[25] which racemizes the unreacted stereoisomer, resulting eventually in complete conversion of the mixture to a single ureido acid. Alternatively, the enzymatic reactions may be run in alkaline buffers; at pH 9–10, hydantoins, but not the derived ureido acids, are readily racemized, and typical hydantoinases work quite efficiently in such pH ranges. Use of such methodology thus offered the extremely attractive possibility of establishing the amino acid stereocenter with complete efficiency; no material would be discarded or recycled as is the case with conventional resolutions.

Reaction of aldehyde **23** with ammonium carbonate and sodium cyanide in ethanol/water furnished, upon acid workup, hydroxy hydantoin **24** in excellent yield. The silyl protecting group was lost during the workup, and **24** precipitated from the reaction mixture; it was readily isolated by filtering and washing with organic solvents. Direct conversion of **24** to bromide **26** was not possible with a variety of methods ($Ph_3PBr_2$, HBr, $PBr_3$, MsCl, and LiBr in a one-pot procedure). We thus used a two-step procedure, first converting **24** to mesylate **25** and then reacting **25** with LiBr in DMF to obtain **26**. Reaction of **26** with 2 equiv of the sodium salt of diethyl phosphite in THF/DMF provided **27** cleanly and in good yield as a snow-white foam. HPLC analysis of **27** demonstrated the presence of two peaks of equal size in the product mixture, corresponding to the presence of two diastereomeric compounds, epimeric at the C-5 hydantoin carbon atom.

Incubation of **27** with D-hydantoinase enzyme from a strain of *Agrobacterium* (whole cell culture), in alkaline buffer, resulted in efficient conversion to D-carbamoyl acid **28**.[25d] Yields of 70–80% of **28**, which could be recrystallized from water as long white needles, were consistently obtained using this methodology. The acquisition of well-formed crystals of **28** enabled the unambiguous determination of its absolute stereochemistry via single-crystal X-ray crystallography. The structure determination (Figure 6) clearly established the absolute stereochemistry as 2*R*,4*R*,5*S*, thus demonstrating that both enzymatic processes had proceeded with the anticipated enantiospecificity. Cleavage of the carbamate functionality of **28** using Bayer's classical methodology (HONO)[25d] delivered amino acid **29** in good (70%) yield.

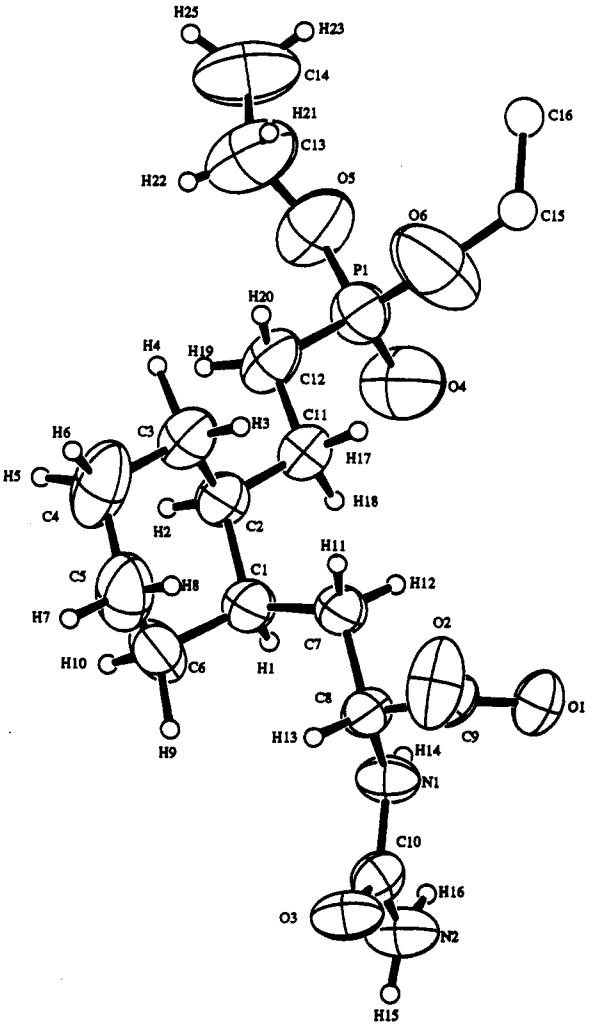



**Figure 6.** Single-crystal X-ray structure of carbamate **28**.

The hydrolytic deprotection of **29** to the final product (**15**) was carried out in aqueous HCl. Mindful of the possible loss of stereochemical integrity at the amino acid stereogenic carbon in hot aqueous acid, we carefully investigated this process using a variety of reaction times, acid strengths, and temperatures. The conversion of the diethyl phosphonate moiety of **29** to the monophosphate ("half-hydrolysis" product) and thence to the phosphonic acid (**15**) was readily monitored by HPLC analysis of aliquots withdrawn from the reaction mixtures and subjected to dansylation at the amino group. Hydrolysis of the second phosphonate ester was quite slow relative to the first; at 100 °C in 10 N HCl more than 24 h were required for complete conversion of **29** to **15**. We found that epimerization of the amino acid center to generate the diastereomeric 2*S*,4*R*,5*S* isomer (readily resolved as a separate peak by HPLC) depended more on temperature than on acid strength. In 6 N HCl, at 95 °C, the complete conversion of **29** to **15** required in excess of 70 h; in 10 N HCl at the same temperature, conversion was complete in 48 h. In neither case was a detectable amount of epimer generated during these time frames. In the latter case,

(23) (a) Ware, E. *Chem. Rev.* **1950**, *46*, 403. (b) Bateman, J. H. *Kirk-Othmer Encyclopedia of Chemical Technology*; Wiley-Interscience: New York, 1978; Vol. 12, p 692. (c) Lopez, C. A. Trigo, G. G. *Adv. Heterocycl. Chem.* **1985**, *38*, 177.

(24) (a) Johnson, T. B.; Bengis, R. *J. Am. Chem. Soc.* **1913**, *35*, 7606. (b) White, H. C. U.S. Patent 2,557,920, 1951; 2,642,459, 1953.

(25) (a) Yokozeki, K.; Nakamori, S.; Yamanaka, S.; Eguchi, C.; Mitsugi, K.; Yoshinaga, F. *Agric. Biol. Chem.* **1987**, *51*, 715. (b) Yokozeki, K.; Kubota, K. *Agric. Biol. Chem.* **1987**, *51*, 721. (c) Yokozeki, K.; Hirose, Y.; Kubota, K. *Agric. Biol. Chem.* **1987**, *51*, 737. (d) Takahashi, S.; Ohashi, T.; Kii, Y.; Kumagai, H.; Yamada, H. *J. Ferment. Technol.* **1979**, *57*, 328. (e) Olivieri, R.; Fascetti, E.; Angelini, L.; Degen, L. *Biotechnol. Bioeng.* **1981**, *23*, 2173.

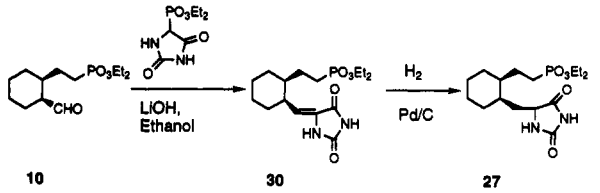
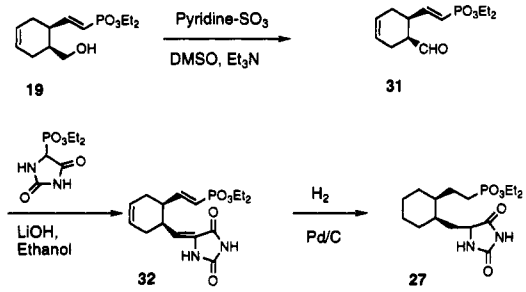



Figure 7.

**Scheme III**




the reaction time was extended to 196 h, at which point only 0.2% of epimeric product could be detected. In contrast, at 125 °C in 6 N HCl, 1–2% epimeric material had appeared after 24 h; after 120 h, the mixture contained nearly 10% diastereomeric contamination. Hydrolysis of **29** at 95 °C for 48 h in 10 N HCl consistently delivered **15** in >90% yield, free from isomeric impurities. The initially obtained HCl salt of **15** was invariably a foam. Treatment of an ethanolic solution of this salt with propylene oxide precipitated the free base of **15** as an amorphous white powder.

Encouraged by our success with the use of the hydantoin moiety as a precursor to optically active α-amino acids, we investigated the possibility of directly condensing aldehyde **10** from Scheme I with a hydantoin. Such a route would be considerably shorter and more efficient than that of Scheme II. Attempts to condense hydantoin itself, using DBU as a base in DMF, were not productive. However, reaction with diethyl 2,4-dioxoimidazolidine-5-phosphonate[26] and lithium hydroxide in ethanol led to a rapid and high-yielding conversion to dehydrohydantoin **30** (Figure 7), obtained as a mixture of *E* and *Z* isomers. Catalytic hydrogenation of **30** gave **27**, identical to that obtained from the route in Scheme II. HPLC analysis of this product indicated that no racemization at C-4 had taken place.[27]

These results, coupled with our observation that dehydro lactone **18** could be converted to **19** in a simpler and more efficient manner than the **7** to **8** conversion, led to the development of the more efficient route of Scheme III. Unsaturated alcohol **19** was successfully oxidized to aldehyde **31** using pyridine–sulfur trioxide complex in DMSO and triethylamine.[28] This procedure allowed the use of higher temperatures than the Swern protocol and consistently provided aldehyde **31** in good yield with <4% *trans* isomer as determined by HPLC analysis of the subsequent product. Condensation of **32** with the phosphonate hydantoin reagent gave **32** as a white foam. Hydrogenation of this triene over palladium-on-carbon proved difficult due to trace amounts of sulfides remaining from the oxidation step. However, hydrogenation of **32** over Raney-nickel delivered **27** in six steps and 32% overall yield from diester **1**. Analysis of samples of **27** prepared by this route showed that the material consistently contained less than 4% *trans* epimer. This minor amount of diastereomeric impurity was easily removed during the recrystallizations of compounds **28** and **29**. The overall yield of **15** by this pathway, starting from diester **1**, is 16% and requires only nine steps. Using this methodology we have prepared 100+ g quantities of **15** containing less than 0.1% isomeric impurities.

**Table I. Inhibition of Excitatory Amino Acid Receptor Ligand Binding by NMDA Antagonists**

| compd | $IC_{50}$ (nM) for ligand | |
|---|---|---|
| | [$^3$H]glutamate | [$^3$H]CGS-19755 |
| CGP-37849 | 25.7 ● 3.2 | 18 ± 6 |
| D-CPP | 321 ● 36 | 86 ± 19 |
| NPC 12626 | 1600 ● 36 | 976 ± 203 |
| 2*R*,4*R*,5*S* isomer | 607 ± 110 | 148 ± 25 |
| 2*S*,4*R*,5*S* isomer | 2589 ● 577 | 264 ● 85 |
| 2*R*,4*S*,5*R* isomer | 3132 ± 1767 | 555 ± 125 |
| 2*S*,4*S*,5*R* isomer | 6458 ± 1611 | 239 ± 85 |

## Biological Results

The potency of compounds to inhibit the specific binding of [$^3$H]CGS-19755 (specific activity 50.5 Ci/mmol, New England Nuclear, Boston, MA) to NMDA receptors was performed as described by Ferkany et al.[29] As indicated by the data in Table I, the 2*R*,4*R*,5*S* stereoisomer was found to be the most potent to inhibit NMDA-sensitive L-[$^3$H]glutamate binding to rat brain membranes; it was likewise the most potent inhibitor of the specific binding of [$^3$H]CGS 19755 to these membranes. Both the 2*R*,4*R*,5*S* isomer and its 2*S*,4*R*,5*S* diastereomer allosterically modulated the binding of [$^3$H]glycine to strychnine-insensitive sites.[29] The differential sensitivity of the glycine receptor site in the NMDA receptor supramolecular complex to various NMDA antagonists has been previously noted;[30] the present results extend these observations to a set of stereoisomers of a single compound. In agreement with the results of the receptor binding assays, the 2*R*,4*R*,5*S* isomer was determined to be the most potent to antagonize NMDA-induced convulsions when given interperitoneally.[29] The $ED_{50}$ for this isomer compares quite favorably with the competitive NMDA antagonists CGS 19755 and (±)-CPP. The 2*R*,4*R*,5*S* isomer impaired rotorod performance in test animals at doses significantly higher than the $ED_{50}$ to block agonist-induced seizures.[29]

These results demonstrate that the 2*R*,4*R*,5*S* isomer of the previously reported compound NPC 12626 is the most potent NMDA antagonist component of the isomeric

(26) Meanwell, N. A.; Roth, H. R.; Smith, E. C. R.; Wedding, D. L.; Wright, J. J. K. *J. Org. Chem.* **1991**, *56*, 6897.

(27) In order to ensure that we could monitor the presence of C-4 epimeric *trans* material by HPLC, we generated an epimeric mixture of aldehyde **10** by treatment with LDA followed by proton quench. The mixture of *cis* and *trans* aldehydes was then converted to a mixture of **30** and *epi*-**30**. The complicating presence of *E* and *Z* geometrical isomers precluded unambiguous interpretation of the HPLC traces of this mixture; however, after hydrogenation to yield a mixture of **27** and *epi*-**27**, the spectra were readily interpretable. Comparison of the chromatograms of this mixture with those of **27** obtained from Scheme II allowed identification of the *trans* epimers of **27**, which were well resolved on the HPLC column. In this way we were able to show that **27** obtained by this route contained only 1–2% *trans* isomers.

(28) von E. Doering, W.; Parikh, J. *J. Am. Chem. Soc.* **1967**, *89*, 5505.

(29) Ferkany, J. W.; Hamilton, G. S.; Patch, R. J.; Huang, Z.; Borosky, S. A.; Bednar, D. L.; Jones, B. E.; Zubrowski, R.; Willetts, J.; Karbon, E. W. *J. Pharmacol. Exp. Ther.* **1993**, *264*, 256.

(30) Kaplita, P. V.; Ferkany, J. W. *Eur. J. Pharmacol. Mol. Pharmacol.* **1990**, *188*, 175.

mixture. The results of these and further[29] studies indicate that this isomer, henceforth designated NPC 17742, is an efficacious anticonvulsant agent and possesses neuroprotectant properties against cerebral ischemia-induced neuronal damage.

## Experimental Section

**General Methods.** Melting points are uncorrected. NMR spectra were recorded at 300 MHz in deuterated solvents. Elemental analyses were performed by Atlantic Microlabs of Atlanta, GA. Air sensitive reactions were performed under an argon atmosphere in oven-dried glassware. Thin-layer chromatography (TLC) was performed on 0.25-mm layers of Merck silica gel 60F-254 on glass-backed plates. Plates were visualized by exposure to iodine vapor, viewing under UV light, or treatment with $KMnO_4$ dip. Flash chromatography was performed using Merck silica gel 60 (230–400 mesh). Solvents used for elution are noted for the specific examples. Solvent extracts were routinely dried over $MgSO_4$ and concentrated using a rotary evaporator.

Anhydrous solvents, starting materials, and reagents were purchased from Aldrich Chemical Co. and used as obtained. Pig liver esterase, porcine pancreas lipase (Type II crude), and Subtilisin A were obtained from Sigma Chemical Co.

**1-Methyl Hydrogen (1*S*,2*R*)-1,2-Cyclohexanedicarboxylate (6).** 1-Methyl hydrogen (1*S*,2*R*)-1,2-cyclohex-4-enedicarboxylate (10 g; 55.34 mmol) in ethanol (150 mL) was hydrogenated in the presence of 5% palladium on carbon for 4 h at 50 psi pressure of hydrogen. The reaction mixture was filtered through Celite and concentrated *in vacuo* to obtain the product as a white solid, 9.48 g (95%): mp 60–62 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.37–2.15 (m, 8H), 2.85 (br t, 2H), 3.70 (s, 3H), 10.15 (br, 1H); IR (neat) 1743, 1697 cm$^{-1}$.

**(−)-(1*R*,6*S*)-*cis*-8-Oxabicyclo[4.3.0]nonan-7-one ((−)-7).** Lithium triethylborohydride (1500 mL of a 1.0 M solution in tetrahydrofuran, 1.5 M, 3.1 equiv) was added dropwise to a cooled (−78 °C) solution of half ester **6** (90.0 g; 0.48 mol) in 800 mL of dry tetrahydrofuran in a 5-L flask. The reaction mixture was allowed to slowly warm to room temperature over the course of 4 h and was quenched by the addition of 1000 mL of 2 N HCl. After the mixture was stirred for an additional 1 h the layers were separated, and the aqueous layer was extracted with 2 × 500 mL of ether. The ether portions were washed with brine, dried, and freed of solvent. The crude product was distilled (90 °C at 1 mmHg) to give 60.1 g (89%) of product: $^1$H NMR ($CDCl_3$) $\delta$ 1.18–1.31 (m, 3H), 1.55–1.70 (m, 3H), 1.78–1.83 (m, 1H), 2.15 (d, 1H), 2.40–2.50 (m, 1H), 2.60–2.68 (m, 1H), 3.95–4.00 (d, 1H), 4.20 (d of d, 2H); IR (neat) 1770 cm$^{-1}$; $[\alpha]^{24}_D$ −46.0° (*c* 0.7, $CHCl_3$).

**(+)-(1*S*,6*R*)-*cis*-8-Oxabicyclo[4.3.0]nonan-7-one ((+)-7).** A solution of half ester **6** (93 g; 0.50 mol) in THF (800 mL) was cooled to 0 °C and treated with 500 mL of a 1.0 M solution of diborane in THF. After the addition was complete, the mixture was stirred for 1 h at 0 °C and 2 h at room temperature before quenching with 1 N HCl. The product was extracted into 3 × 500 mL of ether, and the residue obtained from removal of the solvent was distilled to give 40.0 g (57%) of lactone (+)-**7**, whose NMR and IR spectra were identical to (−)-**7**: $[\alpha]^{24}_D$ +45.4 (*c* 1.1, $CHCl_3$).

**(+)-(1*S*,6*R*)-8-*cis*-Oxabicyclo[4.3.0]non-3-en-7-one (18).** A solution of half ester **2** (100 g; 0.54 mol) in 500 mL of THF was cooled to −78 °C and treated with 1.74 L of a 1.0 M solution of lithium triethylborohydride in THF (1.74 mol; 3.1 equiv). The resulting reaction mixture was allowed to come slowly to room temperature and was stirred overnight. It was quenched by the addition of 450 mL of 1 N HCl; after the mixture was stirred for 30 min, the layers were separated and the aqueous layer was extracted with an additional 300-mL aliquot of ethyl acetate. The combined organic layers were washed with brine, dried, and concentrated in vacuo. The crude product was distilled (103–104 °C at 0.7 mmHg) to obtain 58.3 g (78%) of **18** as a clear oil: $^1$H NMR ($CDCl_3$) $\delta$ 1.81–1.94 (m, 1H), 2.25–2.54 (m, 3H), 2.59–2.66 (m, 1H), 2.76–2.81 (m, 1H), 3.97–4.04 (dd, 1H), 4.31–4.52 (dd, 1H), 5.75 (d, 1H); IR (neat) 3525, 3131, 2970, 2910, 2844, 1772, 1437, 1373, 1175, 995 cm$^{-1}$; $[\alpha]^{24}_D$ +46.1° (*c* 0.12, $CHCl_3$).

**(−)-(1*S*,2*R*)-[2-[(*E*)-2′-(Diethoxyphosphinyl)ethenyl]cyclohex-1-yl]methanol (8).** Tetraethyl methylenebisphosphonate (4.32 g; 14.99 mmol) was added dropwise as a tetrahydrofuran solution (10 mL) to a stirred suspension of sodium hydride (660 mg of a 60% oil dispersion; 16.5 mmol) in THF (15 mL). After gas evolution had ceased the mixture was stirred at room temperature for 2 h and then cooled to −78 °C. In a separate flask, a solution of lactone (−)-**7** (1.0 g; 7.14 mmol) in toluene (15 mL), cooled to −78 °C, was treated with diisobutylaluminum hydride in toluene via syringe (5.7 mL of a 1.5 M solution; 8.55 mmol). After this mixture was stirred for 45 min, it was quenched by the addition of 50 $\mu$L of methanol, and this mixture was added via cannula to the THF solution of the anion. The resulting mixture was stirred for 4 h at −78 °C. *tert*-Butyldimethyl silyl chloride (1.18 g; 7.5 mmol) in 15 mL of THF was then added, and the mixture was stirred overnight while slowly warming to room temperature. The reaction was quenched by the successive addition of saturated aqueous ammonium chloride (20 mL) and 1 N HCl (50 mL). The product was extracted into ethyl acetate, and the organic phase was washed with brine and freed of solvent. The product was purified on a silica gel column utilizing a gradient elution (40% hexane in ethyl acetate to pure ethyl acetate). The product was obtained as a clear viscous oil, 1.40 g (71%): $^1$H NMR ($CDCl_3$) $\delta$ 1.25–2.05 (m + t, 15H), 2.65–2.75 (m, 1H), 3.48 (d, 2H), 3.98–4.15 (m, 4H), 5.65–5.81 (m, 1H), 6.83–7.03 (m, 1H); IR (neat) 3400, 2982, 2926, 2861, 1627, 1447, 1391, 1229, 1041, 956 cm$^{-1}$; $[\alpha]^{21}_D$ −38.64° (*c* 2.60, EtOAc). Anal. Calcd for $C_{13}H_{25}O_4P\cdot 0.25H_2O$: C, 55.60; H, 9.15. Found: C, 55.57; H, 9.07.

Repetition of the above procedure using lactone (+)-**7** gave the enantiomeric (+)-(1*R*,2*S*) compound: $[\alpha]^{22}_D$ +37.82° (*c* 2.55, EtOAc). Anal. Calcd for $C_{13}H_{25}O_4P\cdot 0.25H_2O$: C, 55.60; H, 9.15. Found: C, 55.63; H, 9.07.

When TBDMS chloride was omitted from the above procedure, the major product was **17**: $^1$H NMR ($CDCl_3$) $\delta$ 1.24 (m, 6H), 1.25–1.75 (m, 8H), 1.92 (m, 3H), 2.21, 2.45 (AB m, 1H, $J$ = 12 Hz), 3.65 (dm, 1H), 3.82 (dm, 2H), 4.17 (m, 4H); IR (Nujol) 2923, 1442, 1388 cm$^{-1}$.

**(−)-(1*S*,2*S*)-[2-[2′-(Diethoxyphosphinyl)ethyl]cyclohex-1-yl]methanol (9).** A mixture of vinylphosphonate **7** (2.70 g) and 5% palladium on carbon (600 mg) in ethanol (30 mL) was hydrogenated at 50 psi pressure of hydrogen for 4 h. The reaction mixture was filtered through Celite and concentrated to obtain **8** as a clear oil, 2.70 g (99%): $^1$H NMR ($CDCl_3$) $\delta$ 1.21–1.84 (m, 20H), 2.60 (br, 1H), 3.47–3.60 (m, 2H), 4.05–4.14 (m, 4H); IR (neat) 3400, 2980, 2924, 2859, 1448, 1391, 1230, 1054, 959, 828, 784 cm$^{-1}$; $[\alpha]^{22}_D$ = −11.5° (*c* 3.8, $CHCl_3$). Anal. Calcd for $C_{13}H_{27}O_4P\cdot 0.25H_2O$: C, 55.21; H, 9.80. Found: C, 55.28; H, 9.81; (+)-(1*R*,2*R*) isomer: $[\alpha]^{21.5}_D$ +11.64° (*c* 3.6, $CHCl_3$).

**(−)-(1*S*,2*S*)-2-[2′-(Diethoxyphosphinyl)ethyl]cyclohexane-1-carboxaldehyde (10).** A solution of oxalyl chloride (1.34 g; 10.55 mmol) in methylene chloride (25 mL) was cooled to −60 °C and treated with 2 mL of DMSO (28.18 mmol). After the solution was stirred for 10 min, alcohol **9** (2.63 g; 9.46 mmol) was added as a methylene chloride solution (5 mL). After this mixture was stirred for an additional 15 min triethylamine (6.6 mL; 47.4 mmol) was added, and the mixture was stirred for 5 min at −60 °C and 30 min at room temperature. Water (50 mL) was added, the layers were separated, the organic phase was washed with brine and dried, and the solvent was removed *in vacuo*. The crude product was purified on a silica gel column eluting with ethyl acetate to obtain 2.20 g (84%) of aldehyde **10** as a clear thick oil: $^1$H NMR ($CDCl_3$) $\delta$ 1.29–1.77 (m, 13H), 2.43–2.55 (m, 1H), 4.06–4.12 (m, 4H), 9.79 (s, 1H); IR (neat) 3456, 2980, 2931, 2859, 2715, 1720, 1450, 1391, 1242, 1036, 959, 831, 789 cm$^{-1}$; $[\alpha]^{21.5}_D$ −7.46° (*c* 0.74, EtOAc). Anal. Calcd for $C_{13}H_{25}O_4P\cdot 0.85H_2O$: C, 53.5; H, 9.2. Found: C, 53.4; H, 8.8; (+)-(1*R*,2*R*)-isomer: $[\alpha]^{21.5}_D$ +10.45° (*c* 3.04, EtOAc).

***N*-Acetyl Amino Esters 12 and 13.** A solution of methyl 2-(acetylamino)-2-(dimethoxyphosphinyl)acetate (1.82 g; 7.61 mmol) in THF (50 mL) was added to a stirred slurry of sodium hydride (304 mg of a 60% oil dispersion; 7.60 mmol) in 20 mL of THF at room temperature. After this mixture was stirredfor 1 h, aldehyde **10** was added as a THF solution (20 mL). The mixture was stirred at room temperature for 24 h, quenched by the addition of 20 mL of saturated aqueous ammonium chloride, and acidified with 1 N HCl until the pH was 2. It was extracted

into ethyl acetate, and the ethyl acetate portions were washed with brine, dried, and freed of solvent. The residue was purified on a silica gel column eluting with ethyl acetate. Dehydro *N*-acetylamino ester **11** was obtained contaminated with unreacted phosphonate reagent; 640 mg of this impure product was obtained.

This impure material was hydrogenated in the presence of 5% palladium on carbon (200 mg) in 30 mL of ethanol under 50 psi pressure of hydrogen for 4 h. The mixture was filtered through Celite and freed of solvent. Purification of the crude residue on a silica gel-60 column, eluting with 5% methanol in ethyl acetate, delivered a total of 260 mg (8.7% overall) of a pure mixture of **12** and **13**: $^1$H NMR ($CDCl_3$) (**12** + **13**) $\delta$ 1.15–1.88 (m, 20H), 2.02 (s, 3H), 3.73 (s, 3H), 4.00–4.19 (m, 4H), 4.55–4.68 (m, 1H), 5.98 and 6.38 (br d's, 1H total).

**Enzymatic Resolution of 12 and 13. Compounds 12 and 14.** The mixture of esters **12** and **13** (260 mg total) was dissolved in pH 7.4 phosphate buffer and stirred at room temperature. Subtilisin A (Novo Biolabs, 30 mg) was added, and the mixture was stirred overnight. The pH was brought back to 7.4 by the dropwise addition of 0.2 M NaOH. The mixture was diluted with water and extracted with 3 × 75 mL of ethyl acetate. The ethyl acetate portions were dried and freed of solvent to deliver 100 mg of **12**: $^1$H NMR ($CDCl_3$) $\delta$ 1.24–1.88 (m, 20H), 2.04 (s, 3H), 3.74 (s, 3H), 4.03–4.15 (m, 4H), 4.57 (t of d, 1H), 6.34 (br d, 1H). The aqueous phase was made acidic (pH 1) with 1 N HCl and extracted with 3 × 75 mL of ethyl acetate. Removal of the solvent furnished 50 mg of acid **14**: $^1$H NMR ($CDCl_3$) $\delta$ 1.24–1.84 (m, 20H), 2.07 (s, 3H), 4.05–4.10 (m, 4H), 4.66–4.68 (m, 1H), 6.46 (br d, 1H).

**Hydrolysis of 12 and 14 to 2*R*,4*R*,5*S* and 2*S*,4*R*,5*S* Isomers, Respectively.** Compounds **12** and **14** were each refluxed vigorously in 6 N HCl overnight. The solutions were concentrated *in vacuo*, and in each case the crude material was dissolved in ethanol and treated with propylene oxide to obtain the free bases. Small amounts of each of the two final products were obtained. HPLC analysis of the products, using racemic NPC 12626 as a reference, confirmed their identities as two diastereomeric *cis* isomers of NPC 12626. 2*R*,4*R*,5*S* Isomer (**15**): $[\alpha]^{21.5}_D$ −16.29° (*c* 0.50, 1N HCl). 2*S*,4*R*,5*S* Isomer (**16**): $[\alpha]^{21.5}_D$ +3.32° (*c* 0.54, 1N HCl).

**(1*R*,2*S*)-Methyl Hydrogen *cis*-1,2-Cyclohexanediacetate (20).** A solution of (1*R*,2S)-methyl hydrogen 1,2-*cis*-cyclohex-4-enediacetate (41 g; 0.19 mol) in ethanol (210 mL) was treated with 6 g of 5% palladium on carbon and hydrogenated in a Parr hydrogenator for 5 h at 55 psi pressure of hydrogen. The solution was filtered through Celite and concentrated to deliver 39.4 g (95%) of the cyclohexane half ester as a colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 1.25–1.53 (m, 8H), 2.21–2.28 (m, 6H), 3.67 (s, 3H); IR (neat) 2923, 2862, 2666, 1736, 1704, 1440, 1293, 1242, 1165, 1113, 1016, 946 cm$^{-1}$; $[\alpha]^{22}_D$ +1.45° (*c* 3.96, EtOAc). Anal. Calcd for $C_{11}H_{18}O_4$: C, 61.66; H, 8.47. Found: C, 61.38; H, 8.50.

**(1*R*,2*S*)-2-(2′-Hydroxyethyl)cyclohexaneacetic Acid, Methyl Ester (21).** Diborane (187 mL of a 1.0 M solution in THF; 0.187 mol) was added in a dropwise manner to a cooled (0 °C) solution of half ester **20** (36.83 g; 0.17 mol) in THF (200 mL). After the addition was complete the reaction mixture was allowed to slowly come to room temperature overnight. The reaction was quenched by the addition of 500 mL of 1 N HCl and extracted into 3 × 300 mL of ethyl acetate. The organic layers were washed with brine, dried, and freed of solvent. The hydroxy ester was obtained as a colorless oil (32.8g; 96%): $^1$H NMR ($CDCl_3$) $\delta$ 1.23–1.76 (m, 11H), 2.12–2.27 (m, 3H), 3.70 (m, 5H); IR (neat) 3428, 2926, 2862, 1736, 1437, 1291, 1167, 1054, 1013 cm$^{-1}$; $[\alpha]^{22}_D$ +1.75° (*c* 3.41; MeOH). Anal. Calcd for $C_{11}H_{20}O_3$: C, 65.97; H, 10.07. Found: C, 66.53; H, 10.47.

**(1*R*,2*S*)-2-[2′-[(*tert*-Butyldimethylsilyl)oxy]ethyl]-cyclohexaneacetic Acid, Methyl Ester (22).** A mixture of hydroxy ester **21** (8.4 g; 42 mmol), *tert*-butyldimethylsilyl chloride (7.83 g; 50.4 mmol); (dimethylamino)pyridine (518 mg; 4.2 mmol), and triethylamine (4.67 g; 46.2 mmol) in dimethylformamide (30 mL) was stirred overnight at room temperature. Water (30 mL) was added, and the product was extracted into 3 × 50 mL of ethyl acetate. The crude product obtained from removal of the solvent was purified by column chromatography, eluting with 3–5% ethyl acetate in hexane, to obtain 11.2 g (80%) of the product as a clear liquid: $^1$H NMR ($CDCl_3$) $\delta$ 0.01 (s, 6H), 0.84 (s, 9H), 1.25–1.57 (m, 11H), 2.20 (m, 1H), 2.23 (d, 1H), 3.54–3.61 (m plus s, 5H); IR (neat) 2926, 2857, 1740, 1463, 1434, 1388, 1360, 1255, 1105, 1100, 1007, 938, 825 cm$^{-1}$; $[\alpha]^{21.5}_D$ −2.31° (*c* 2.51, $CHCl_3$). Anal. Calcd for $C_{17}H_{34}O_3Si$: C, 64.92; H, 10.90. Found: C, 65.02; H, 10.92.

**(1*R*,2*S*)-2-[2′-[(*tert*-Butyldimethylsilyl)oxy]ethyl]-cyclohexylacetaldehyde (23).** A solution of ester **22** (2.50 g; 8.0 mmol) in 40 mL of toluene was cooled to −78 °C and treated with diisobutylaluminum hydride (6.1 mL of a 1.5 M solution; 9.2 mmol). The mixture was stirred for 2 h at −78 °C and then poured into 30 mL of an ice-cold saturated aqueous solution of sodium–potassium tartrate. The layers were separated, and the aqueous layer was washed with 2 × 20 mL of ethyl acetate. The combined organic layers were washed with brine, dried, and freed of solvent, and the residue was purified on a silica gel column eluting with 1–5% ethyl acetate in hexane to obtain 1.63 g (72%) of the product as a light oil: $^1$H NMR ($CDCl_3$) $\delta$ 0.05 (s, 6H), 0.89 (s, 9H), 1.26–1.74 (m, 11H), 2.23–2.38 (m, 3H), 3.65 (m, 2H), 9.76 (d, 1H); IR (neat) 2931, 2707, 1730, 1468, 1388, 1255, 1100, 836, 776 cm$^{-1}$; $[\alpha]^{21.5}_D$ −16.22° (*c* 2.80, $CHCl_3$). Anal. Calcd for $C_{18}H_{32}O_2Si$: C, 67.55; H, 11.34. Found: C, 67.28; H, 11.27.

**(1*S*,2*R*)-1-(2′-Hydroxyethyl)-2-(5-hydantoinylmethyl)cyclohexane (24).** Aldehyde **23** (8.0 g; 28.1 mmol) was dissolved in 65 mL of ethanol, and a solution of sodium cyanide (3.09 g; 61.8 mmol) and ammonium carbonate (13.5 g; 140 mmol) in water (65 mL) was added. This mixture was sealed in a glass tube and heated in an oil bath at 90 °C for 18 h. After cooling, the reaction mixture was poured into a 500-mL beaker and acidified to pH 1 with HCl. After being stirred at room temperature for 1 h the mixture was cooled in an ice bath and the precipitate was collected by vacuum filtration. The collected solid was washed with ice-cold water until the washings were pH 5–6 and then washed with cold ether and ethyl acetate. The solid product was dried under vacuum to obtain 3.71 g (68%) of the hydroxy hydantoin as a white solid: mp 209 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.04–1.69 (m, 14H), 3.30–3.42 (m, 2H), 3.96 (d, 1H), 4.32 (t, 1H), 8.02 (d, 1H), 10.57 (br d, 1H); IR (KBr) 3309, 2928, 2757, 1730, 1715, 1421, 1314, 1203, 1059 cm$^{-1}$. Anal. Calcd for $C_{12}H_{20}N_2O_3 \cdot 0.25H_2O$: C, 58.88; H, 8.44; N, 11.44. Found: C, 59.24; H, 8.44; N, 11.52.

**(1*S*,2*R*)-1-[2′-[(*O*-Methanesulfonyl)oxy]ethyl]-2-(5-hydantoinylmethyl)cyclohexane (25).** Methanesulfonyl chloride (2.75 g; 22 mmol) was added neat in a slow, dropwise fashion to a cooled (0 °C) solution of hydroxy hydantoin **24** (4.8 g; 20 mmol) in pyridine (30 mL). After the addition was complete the reaction mixture was stirred for 15 min at 0 °C and 2 h at room temperature. The pyridine was removed *in vacuo*, and the residue was dissolved in 80 mL of chloroform. The chloroform phase was washed with 30 mL of 1 N HCl, dried, and freed of solvent to obtain 6.0 g (94%) of the product as a white solid: mp 150 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.28–1.78 (m, 14H), 3.14 (s, 3H), 3.96 (t, 1H), 4.18 (m, 2H), 8.03 (d, 1H), 10.58 (d, 1H); IR (KBr) 3286, 2928, 1769, 1416, 1357, 1177 cm$^{-1}$. Anal. Calcd for $C_{13}H_{22}N_2O_5S$: C, 49.04; H, 6.96; N, 8.80. Found: 49.14; H, 6.97; N, 8.78.

**(1*S*,2*R*)-1-(2′-Bromoethyl)-2-(5-hydantoinylmethyl)cyclohexane (26).** Mesylate **25** (6.0 g; 18.87 mmol) was dissolved in 50 mL of *N*,*N*-dimethylformamide containing 5.21 g (60 mmol) of lithium bromide. This mixture was stirred overnight at 45 °C under an argon atmosphere. After the mixture was cooled to 0 °C, cold water (80 mL) was added to the reaction mixture, and the resulting precipitate was collected by filtration and washed with 2 × 30 mL of ice-cold water and 2 × 30 mL of ether. The product was dried under vacuum to obtain 4.55 g (80%) of bromide **26** as a white solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.22–1.71 (m, 14H), 3.61 (m, 2H), 3.99 (d, 1H), 8.04 (d, 1H), 10.58 (d, 1H); IR (KBr) 3541, 3304, 3219, 2926, 2363, 1772, 1728, 1427, 774, 648 cm$^{-1}$.

**(1*S*,2*R*)-1-[2′-(Diethylphosphono)ethyl]-2-(5-hydantoinylmethyl)cyclohexane (27).** Diethyl phosphite (2.12 g; 15.34 mmol) in tetrahydrofuran (10 mL) was added dropwise to a stirred slurry of sodium hydride (506 mg of an 80% oil dispersion; 16.87 mmol) in tetrahydrofuran (20 mL). After gas evolution had ceased the mixture was stirred for 1.5 h at room temperature. Bromide **26** (1.5 g; 4.95 mmol) was added via syringe as a dimethylformamide solution (20 mL). The mixture was stirred for 1 h at room temperature and then heated to 80 °C for 24 h. After cooling, the reaction was quenched by the addition of

saturated aqueous ammonium chloride (20 mL) and extracted into 100 mL of ethyl acetate. The organic phase was washed with 3 × 50 mL of brine, dried, and freed of solvent. The residue was purified on a silica gel column, using a gradient elution from 1% methanol in methylene chloride to 10% methanol in methylene chloride. The product (1.50 g; 84%) was obtained as a white foamy glass: $^1$H NMR ($CDCl_3$) $\delta$ 1.10–2.01 (m, 22H), 4.02–4.16 (m, 5H), 7.15–7.26 (d, 1H), 9.64 (b, 1H); IR ($CDCl_3$) 3440, 3155, 2934, 2263, 1772, 1725, 1468, 1383, 1211, 1095 cm$^{-1}$. Anal. Calcd for $C_{16}H_{29}N_2O_5P$: C, 53.3; H, 8.1; N, 7.8. Found: C, 53.25; H, 8.15; N, 7.74.

**(2*R*,4*R*,5*S*)-α-[(Aminocarbonyl)amino]-2-[2′-(diethylphosphono)ethyl]cyclohexanepropionic Acid (28).** Hydantoin **27** was converted to the carbamoyl derivative by the enzyme D-hydantoinase as described in the literature.[25d] Carbamate **28** was obtained in yields of 70–80% as a white crystalline solid after recrystallization from water. X-ray diffraction analysis of this compound verified that it was of the 2*R*,4*R*,5*S* configuration: mp 189 °C; $^1$H NMR ($CDCl_3$) $\delta$ 1.27–2.01 (m, 22H), 3.49 (s, 2H), 4.17–4.22 (m, 4H), 4.48 (d, 1H), 6.58 (br, 1H); electron impact mass spectrum *m/e* 379 ($MH^+$), 364, 336, 335, 291, 263; $[\alpha]^{22}_D$ −11.70° (*c* 0.86, MeOH). Anal. Calcd for $C_{16}H_{31}N_2O_6P$: C, 50.78; H, 8.26; N, 7.40. Found: C, 50.81; H, 8.28; N, 7.43.

**(2*R*,4*R*,5*S*)-α-Amino-2-[2′-(diethylphosphono)ethyl]cyclohexanepropionic Acid (29).** *N*-Carbamoylamino acid was dissolved in aqueous HCl and treated with $NaNO_2$ in the manner described in the literature.[25d] Recrystallization of the amino acid from water afforded **29** as a white powder: mp 206 °C; $^1$H NMR ($CD_3OD$) $\delta$ 1.33 (t, 6H), 1.37–2.05 (m, 16H), 3.50 (m, 1H), 4.11 (q, 4H); IR (Nujol) 2926, 1607, 1460, 1396, 1378, 1208, 1061, 1031, 961 cm$^{-1}$; $[\alpha]^{22}_D$ +0.90° (*c* 2.9, MeOH). Anal. Calcd for $C_{15}H_{30}NO_5P$: C, 53.72; H, 9.01; N, 4.18. Found: C, 53.59; H, 9.03; N, 4.20.

**(2*R*,4*R*,5*S*)-3-[2-(2′-Phosphonoethyl)cyclohexyl]alanine (15).** A solution of **29** (50.0 g; 0.15 mol) in 10 N HCl (400 mL) was heated to 95 °C for 48 h, with stirring. After cooling, the water was removed under reduced pressure, and the resulting foam was washed with 2 × 200 mL of acetone and 2 × 100 mL of ether and dried *in vacuo* to obtain a white foam. This was dissolved in 600 mL of warm ethanol and treated with an equal volume of propylene oxide. The precipitate which formed was collected via vacuum filtration, washed with cold ethanol and ether, and dried in a vacuum oven (50 °C, 1 mmHg) overnight to obtain 42 g (100%) of a white amorphous powder: mp > 200 °C dec; $^1$H NMR ($D_2O$) $\delta$ 0.90–1.91 (m, 16H), 3.70 (m, 1H); IR (KBr) 3386, 2928, 2864, 2373, 1733, 1625, 1527, 1409, 1231, 1121, 1038, 948 cm$^{-1}$; $[\alpha]^{21}_D$ −16.30° (*c* 0.50, 1 N HCl). Anal. Calcd for $C_{11}H_{22}NPO_5$: C, 47.31; H, 7.94; N, 5.02. Found: C, 47.09, H, 7.99; N, 4.92.

**2-[2′-(Diethoxyphosphinyl)ethyl]-1-(5-dehydrohydantoinylmethyl)cyclohexane (30).** Lithium hydroxide monohydrate (450 mg; 10.71 mmol) and diethyl 2,4-dioxoimidazolidine-5-phosphonate[26] (2.14 g; 9.07 mmol) were dissolved in 10 mL of 95% ethanol. This solution was added to a solution of aldehyde **10** (2.0 g; 7.24 mmol) in 10 mL of ethanol and cooled to 0 °C, and the resulting mixture was stirred for 1 h at 0 °C and 2 h at room temperature. The solvent was removed under reduced pressure to leave a yellow foam; this was taken up in 20 mL of 1 N NaOH and washed with 3 × 20 mL of $CH_2Cl_2$. The organic extracts were backwashed with 20 mL of 1 N NaOH, and the combined aqueous fractions were made strongly acidic (pH 2) with 1 N HCl. The product was extracted into 3 × 50 mL of ethyl acetate; the combined organic extracts were washed with brine, dried, and concentrated. The product was purified on a silica gel column, eluting with 5% methanol in ethyl acetate, to obtain 1.80 g (70%) of dehydrohydantoin **30** as a snowy white foam: $^1$H (NMR) $\delta$ 1.23 (t, 6H), 1.25–1.77 (m, 13H), 2.78 (m, 1H), 4.07 (m, 4H), 4.27 (m, 1H), 5.81, 6.00 (d's, 1H total), 9.16, 9.57 (s's, 1H total); IR ($CDCl_3$) 3068, 1738 (br), 1386, 1211, 1036 cm$^{-1}$. Anal. Calcd for $C_{15}H_{27}O_5N_2P·0.25H_2O$: C, 51.34; H, 7.90; N, 7.98. Found: C, 51.11; H, 7.38; N, 7.66.

**Catalytic Hydrogenation of 30 to 27.** A mixture of **30** (1.80 g; 5.02 mmol) and 5% palladium on carbon (300 mg) in 50 mL of ethanol was hydrogenated in a shaker at 50 psi of hydrogen for 4 h. The reaction mixture was filtered through Celite, concentrated under reduced pressure, and passed through a silica gel plug, eluting with 10% methanol in ethyl acetate, to obtain 1.60 g (87%) of **27**, identical to that obtained by the previous route.

**2-[(*E*)-2′-(Diethoxyphosphinyl)ethenyl]cyclohex-4-ene-1-methanol (19).** Tetraethyl methylenebisphosphonate (10.43 g; 36.2 mmol) was added dropwise as a THF solution (25 mL) to a stirred slurry of sodium hydride (1.38 g of an 80% dispersion; 46 mmol) in 35 mL of THF. After being stirred at room temperature for 1 h the mixture was cooled to 0 °C. In a separate flask, a solution of lactone **18** (5.0 g; 36.59 mmol) in toluene (30 mL) was cooled to −40 °C and treated with diisobutylaluminum hydride (30 mL of a 1.5 M solution of DIBAL in toluene; 45 mmol) at such a rate that the internal temperature of the reaction did not rise above −35 °C. After this mixture was stirred for 1 h, it was added via cannula to the solution of phosphonate anion. The resulting mixture was stirred at 0 °C for 30 min and then heated to 60 °C for 4 h. The reaction was quenched by the addition of 1 N HCl to pH 2 and extracted into ethyl acetate. The organic phase was washed with brine, dried, and concentrated. The crude material was purified on a flash column eluting with ethyl acetate and then 5% methanol in ethyl acetate to obtain 6.90 g (70%) of **19** as a thick syrup: $^1$H NMR ($CDCl_3$) $\delta$ 1.32 (t, 6H), 1.82 (m, 1H), 2.09 (m, 3H), 2.35 (m, 1H), 2.83 (br, 1H), 3.06 (br, 1H), 3.49 (d, 2H), 4.07 (m, 4H), 5.75 (m, 3H), 6.82 (m, 1H); IR (neat) 3394, 2983, 2906, 1628, 1440, 1391, 1229, 1029, 964, 465 cm$^{-1}$; $[\alpha]^{21}_D$ −53.78° (*c* 2.50, EtOAc). Anal. Calcd for $C_{13}H_{23}O_4P·0.25H_2O$: C, 56.00; H, 8.50. Found: C, 56.22; H, 8.48.

**2-[(*E*)-2′-(Diethoxyphosphinyl)ethenyl]cyclohex-4-ene-1-carboxaldehyde (31).** Pyridine–sulfur trioxide complex (8.71 g; 54.70 mmol) in DMSO (35 mL) was added in a dropwise fashion to a cooled (−10 °C) solution of alcohol **19** (5.0 g; 18.23 mmol) and triethylamine (12.90 g; 127 mmol) in 20 mL of DMSO at such a rate that the internal temperature did not rise above −5 °C. After the addition was complete, the reaction was allowed to come to room temperature and was stirred for 4 h. It was partitioned between 1 N HCl and ethyl acetate; the aqueous layer was extracted with ethyl acetate, and the combined organic phases were washed with brine and water, dried and concentrated. The crude product was purified on a chromatography column, eluting with 5% methanol in ethyl acetate, to obtain 4.37 g of aldehyde **32**, 88%: $^1$H NMR ($CDCl_3$) $\delta$ 1.28 (t, 6H), 2.05–2.39 (m, 4H), 2.71 (m, 1H), 3.0 (m, 1H), 4.07 (q, 4H), 5.72 (m, 3H), 6.82 (m, 1H), 9.66 (s, 1H); IR (neat) 3388, 2983, 2905, 1723, 1627, 1439, 1391, 1247, 1041, 962, 848, 820, 789 cm$^{-1}$; $[\alpha]^{21}_D$ −22.29° (*c* 2.60, EtOAc). Anal. Calcd for $C_{13}H_{27}O_4P·0.4H_2O$: C, 55.87; H, 7.86. Found: C, 55.85; H, 7.79.

**2-[(*E*)-2′-(Diethoxyphosphinyl)ethenyl]-1-(5-dehydrohydantoinylmethyl)cyclohex-4-ene (32).** Lithium hydroxide monohydrate (785 mg; 18.62 mmol) and diethyl 2,4-dioxoimidazolidine-5-phosphonate[26] (4.0 g; 16.93 mmol) were dissolved in 50 mL of 95% ethanol. This solution was added to a solution of aldehyde **31** (4.19 g; 15.39 mmol) in 50 mL of ethanol and cooled to 0 °C, and the resulting mixture was stirred for 1 h at 0 °C and 2 h at room temperature. The product was worked up and purified as described for **30** to obtain 3.82 g (70%) of **33** as a white foam: $^1$H NMR ($CDCl_3$) $\delta$ 1.25 (t, 6H). 2.04–2.47 (m, 4H). 2.72 (m, 1H), 3.00 (m, 1H), 4.04 (m, 4H), 5.75 (m, 4H), 6.89 (m, 1H), 9.13 (br, 1H), 9.29 (br, 1H); IR ($CDCl_3$) 3186, 1738, 1638, 1383, 1221, 1031, 967 cm$^{-1}$.

**Catalytic Hydrogenation of 32 to 27.** A solution of triene **32** (5.0g; 14 mmol) in methanol (50 mL) was treated with Raney nickel (5.0 g wet weight of W.R. Grace 2800 grade Ra-Ni). The resulting mixture was hydrogenated in a Paar bomb at 500 psi and 65 °C for 48 h. The mixture was filtered through Celite, concentrated, and purified through a silica gel column, eluting with 10% methanol in ethyl acetate. The product was obtained as a white foam (4.80 g; 95%) whose $^1$H NMR and IR spectra were identical to **27** obtained by the earlier route.