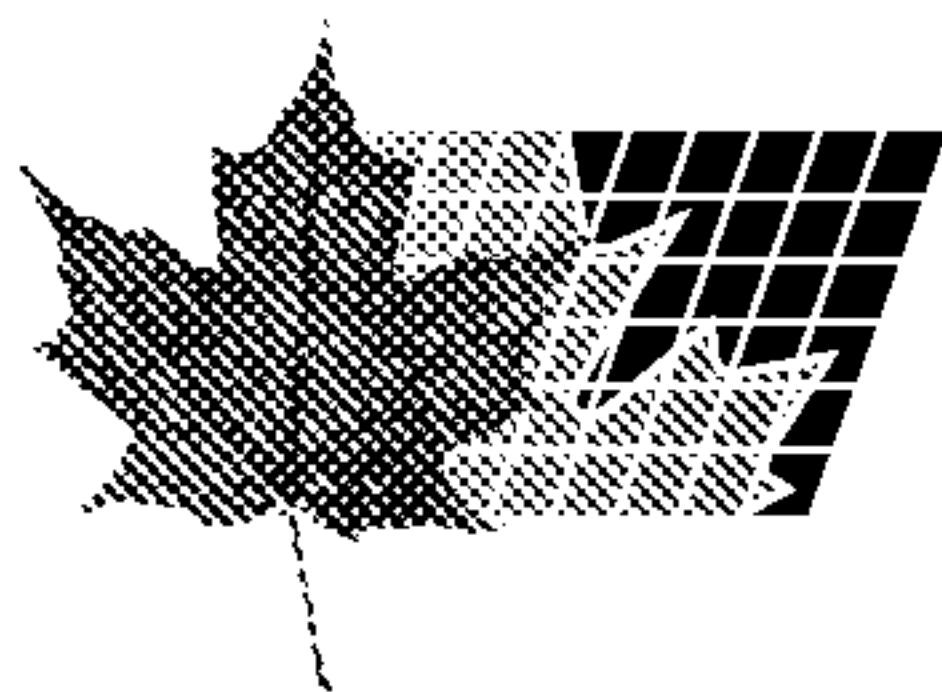




(11) (21) (C) **2,190,455**
(86) 1995/03/17
(87) 1996/09/26
(45) 2000/11/28

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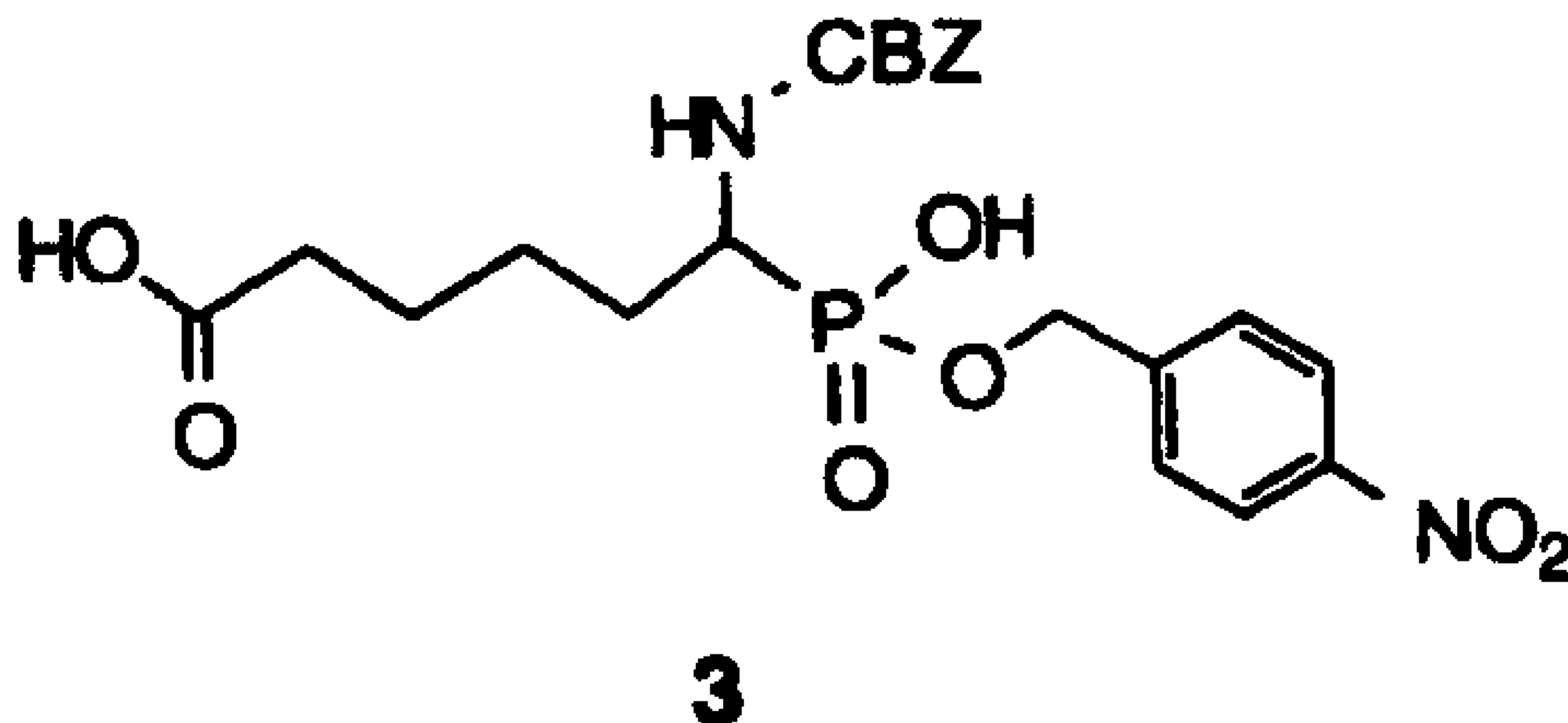
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(51) Int.Cl.⁶ C12P 41/00, C07K 16/44, C12N 5/18, C12P 13/04

(54) **ANTICORPS CATALYTIQUE QUI HYDROLYSE DES ESTERS
D'ACIDES AMINES D'UNE MANIERE ENANTIOSELECTIVE**

(54) **CATALYTIC ANTIBODIES ENANTIOSELECTIVELY
HYDROLYSING AMINO ACID ESTER DERIVATIVES**

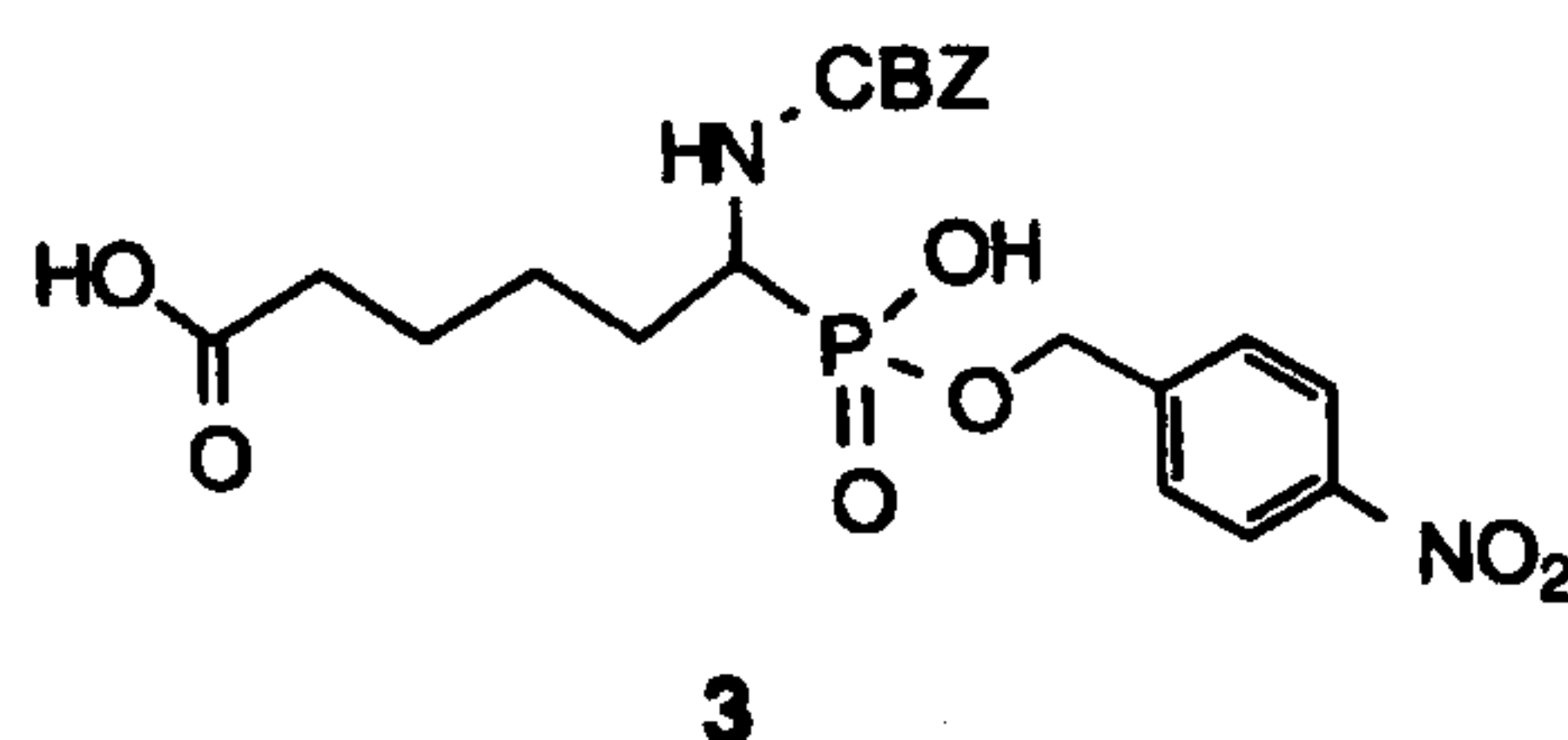


(57) The present invention is directed to a process for conducting optical resolution of a racemic mixture of an amino acid derivatives and a process for preparing an optically active amino acid using a catalytic antibody enantioselectively hydrolyzing an amino acid ester derivative. The catalytic antibody and hybridoma producing said antibody are also provided. The hybridomas in the present invention are typically produced by stimulation with an antigen comprising as a hapten a compound of the formula: (see above formula) wherein CBZ is N-benzyloxycarbonyl.

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ABSTRACT

The present invention is directed to a process for conducting optical resolution of a racemic mixture of an amino acid derivatives and a process for preparing an optically active amino acid using a catalytic antibody enantioselectively hydrolyzing an amino acid ester derivative. The catalytic antibody and hybridoma producing said antibody are also provided. The hybridomas in the present invention are typically produced by stimulation with an antigen comprising as a hapten a compound of the formula:



wherein CBZ is N-benzyloxycarbonyl.

CATALYTIC ANTIBODIES ENANTIOSELECTIVELY
HYDROLYSING AMINO ACID ESTER DERIVATIVES

The present invention belongs to the field of the art synthesizing optically active amino acids. Specifically, the present invention relates to a process for conducting enantioselective hydrolysis by utilizing catalytic antibodies. More specifically, the invention relates to processes for enantioselectively hydrolyzing amino acid ester derivatives with catalytic antibodies to effect optical resolution of a racemic mixture of amino acid derivatives and to prepare optically active amino acids. Because the present invention allows very easy production of optically active amino acids having various substituted groups, it can contribute to basic studies on the function of living bodies, development of a novel medical drug containing optically active amino acids as an essential component, and development of methodologies for synthesizing various optically and physiologically active substances or functional compounds, which methodologies use optically active amino acids in synthesizing processes.

Proteins, a primary component of the living body, are constructed with optically active amino acids, which results in a chiral environment in the living body. Therefore, many of the physiologically active substances including medical drugs are optically active substances, and it is known that bioactivity is found only in one of the enantiomers, or a difference in bioactivity is observed between the enantiomers. Accordingly, developing a

methodology for obtaining optically active substances is one of the targets of investigations.

5 The simplest process usually performed to obtain an optically active amino acids is optical resolution of a racemic mixture of amino acid derivatives. The process for optical resolution includes a process employing an enzyme (e.g. a kinetic optical resolution using aminoacylase, lipase and the like), and a process forming a diastereomer, or the like. However, the suitable process must be selected depending on the particular amino acid to be resolved,
10 because there is no common process effective for all the amino acids having a variety of substituted groups.

In such a situation as mentioned above, the inventors of the present invention have succeeded in developing a process for performing optical resolution of a racemic mixture of amino acid
15 derivatives, using catalytic antibodies, which is applicable for any amino acid. Although reactions using catalytic antibodies usually have drawbacks in that the catalytic antibodies have substrate specificity, and therefore, they have only limited applicablity, the present inventors have overcome such drawbacks by developing
20 catalytic antibodies which enantioselectively hydrolyze ester derivatives of a racemic mixture of any amino acid derivatives to produce corresponding optically active amino acids.

25 The present invention relates to a process for performing optical resolution of a racemic mixture of amino acid derivatives, comprising using a nonspecific catalytic antibodies enantioselectively hydrolyzing various amino acid ester derivatives.

In another aspect, the present invention relates to a process for preparing optically active amino acids from a racemic mixture of amino acid derivatives, characterized by using catalytic antibodies to enantioselectively hydrolyze amino acid ester derivatives.

5 The processes of the invention can be applied to the synthesis of both L- and D-isomers of amino acids and include two processes each consisting of the following two steps:

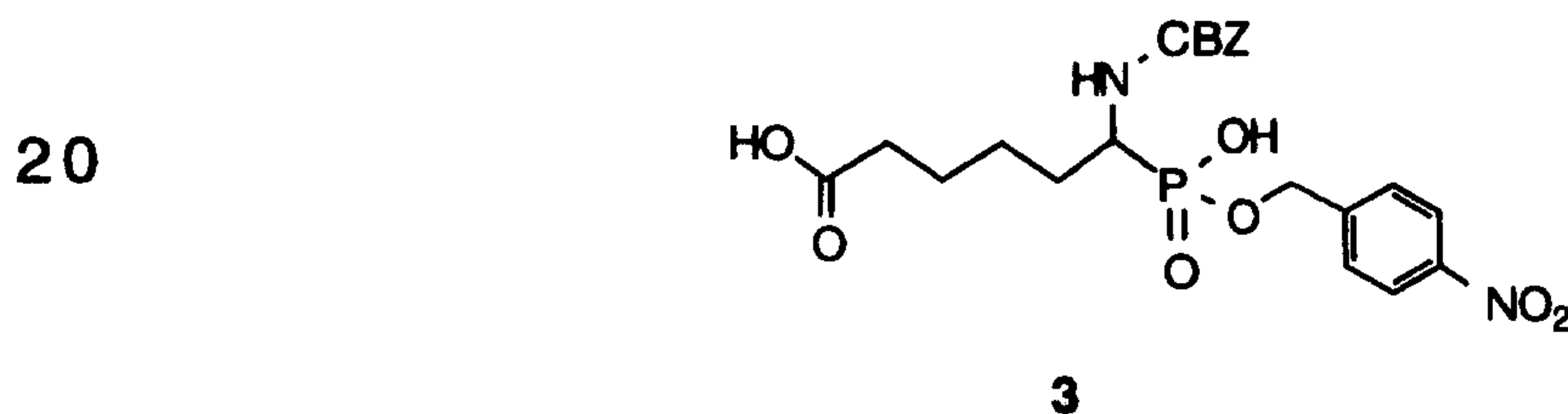
(1) preparing ester derivatives of a racemic mixture of amino acid derivatives, and then

10 (2) enantioselectively hydrolyzing only L-form of amino acid ester derivatives; and

(1) preparing ester derivatives of a racemic mixture of amino derivatives, and then

15 (2) enantioselectively hydrolyzing only D-form of amino acid ester derivatives.

The catalytic antibodies as used herein are typically prepared by immunizing a mammal with, as a hapten, a compound having the formula 3 below:



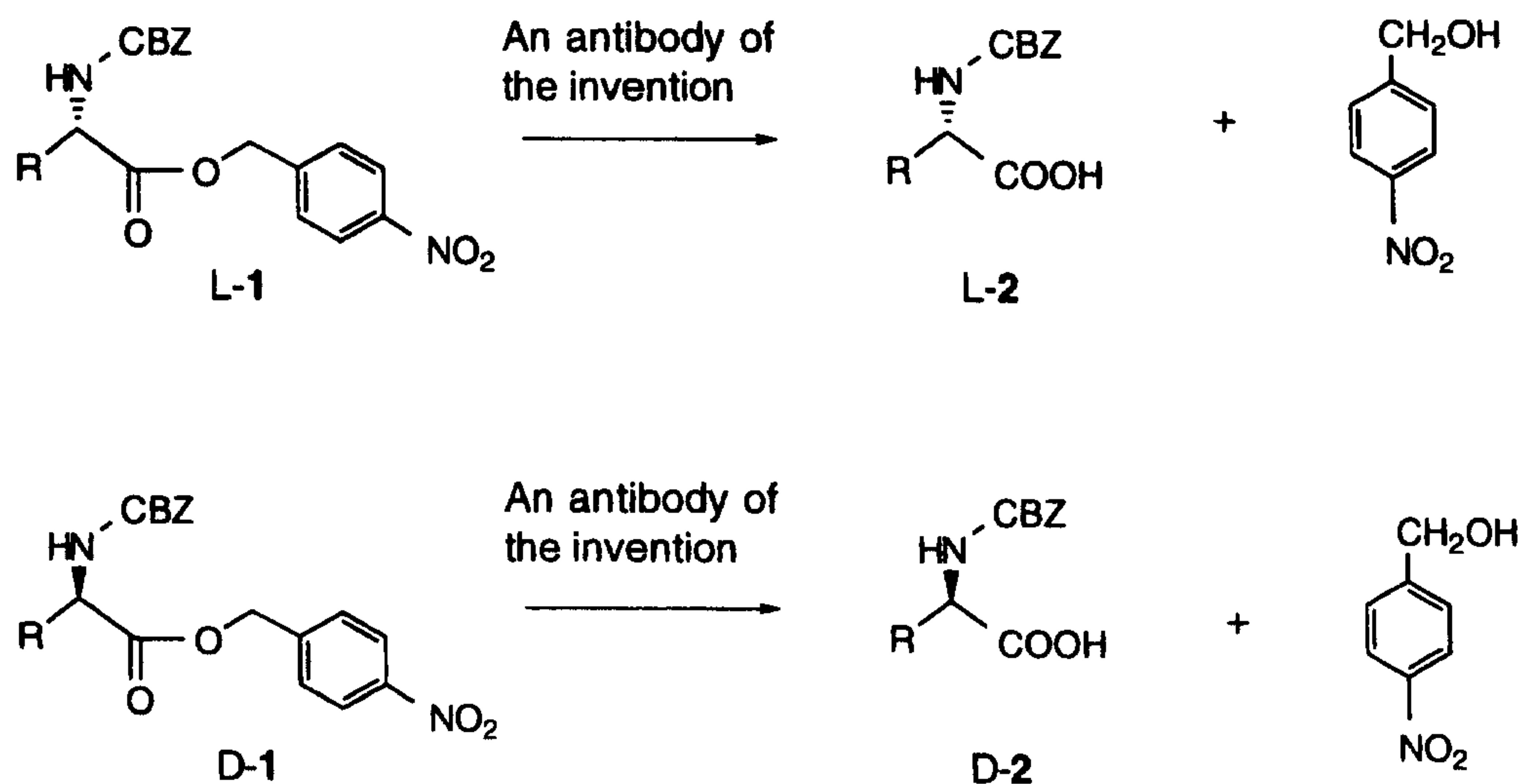
wherein CBZ is benzyloxycarbonyl.

25 Although CBZ in formula 3 is an amino-protecting group, other conventional amino-protecting groups known to those skilled in the art, for example, trichloroacetyl, benzyl, t-butoxycarbonyl, 9-fluorenylmethoxycarbonyl, or CBZ having a substituent such as lower alkyl, lower alkoxy, halogen, nitro, etc. in

the phenyl moiety can also be used instead of CBZ. The compound of the above formula 3 has a 4-nitrobenzyl ester moiety. However, the benzyl ester moiety can have one or two substituents selected from lower alkyl, lower alkoxy, nitro, halogen, or the like in the phenyl moiety. Catalytic antibodies obtained using the haptens having a substituted benzyl moiety can also hydrolyze any corresponding amino acid ester derivatives. Thus, these are also potential haptens.

In one embodiment, the invention aims at antibodies which catalyze enantioselective hydrolysis of L- and D-isomers of N-benzyloxycarbonyl amino acid ester derivatives. N-benzyloxycarbonyl amino acid is an N-protecting amino acid often used for the synthesis of peptides. Such antibodies catalyze hydrolytic reactions as shown in the following Scheme 1. Although the following explanation concerns the catalytic antibodies which are obtained by the use of the compound of the above Formula 3, those skilled in the art will understand that the following explanation is also applicable to catalytic antibodies obtained from the potential haptens mentioned above.

Scheme 1



wherein CBZ is N-benzyloxycarbonyl and

R may be substantially any substituent, and, it is typically alkyl or phenyl group which is optionally substituted with hydroxy, amino, alkylthio, acyloxy or phenyl, and is, more particularly,

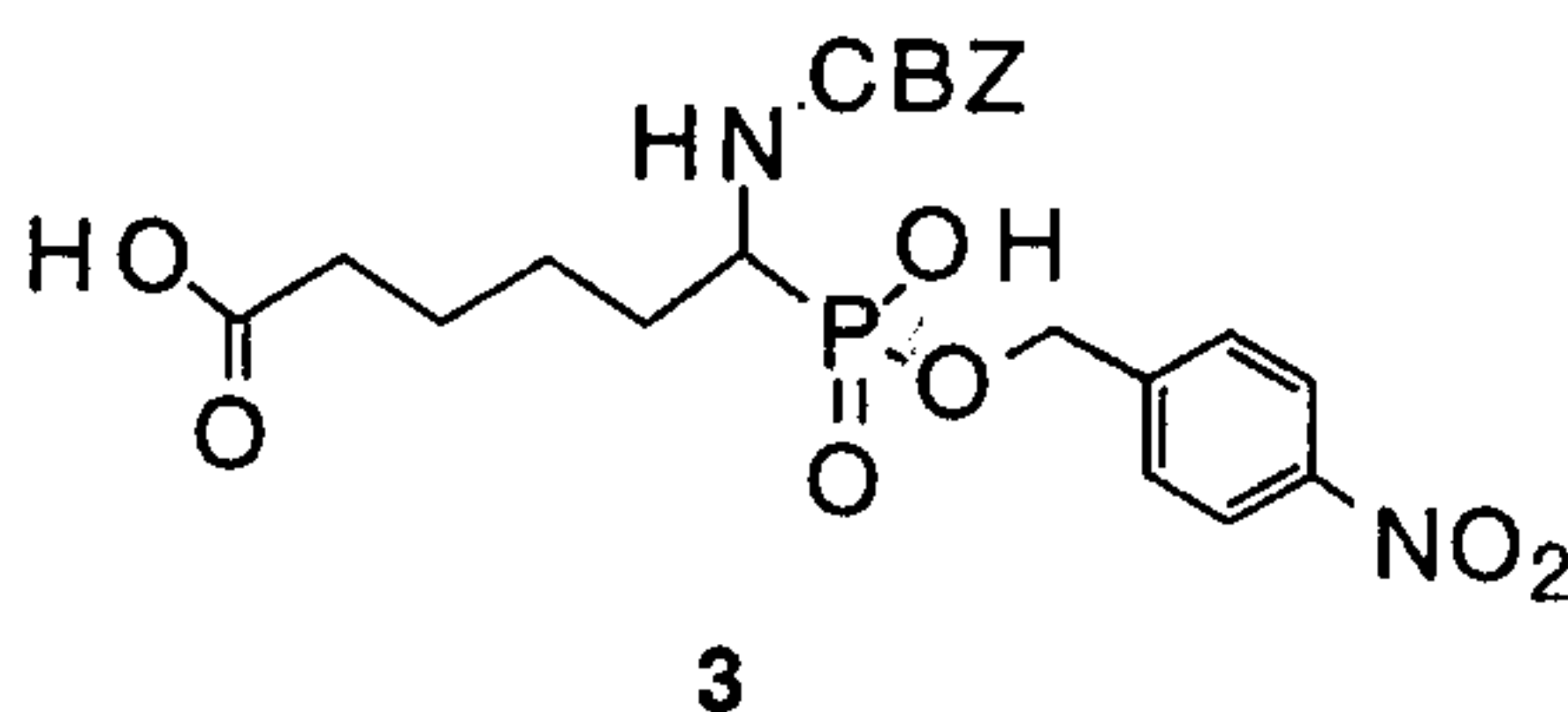
CH₃-, phCH₂-, (CH₃)₂CHCH₂-, CH₃(CH₂)₃-, CH₃SCH₂CH₂-, (CH₃)₂CH-, ph-, or 4-HOph-;

wherein ph- is phenyl group;

alkyl is typically a straight or branched C₁-C₁₀ alkyl; and

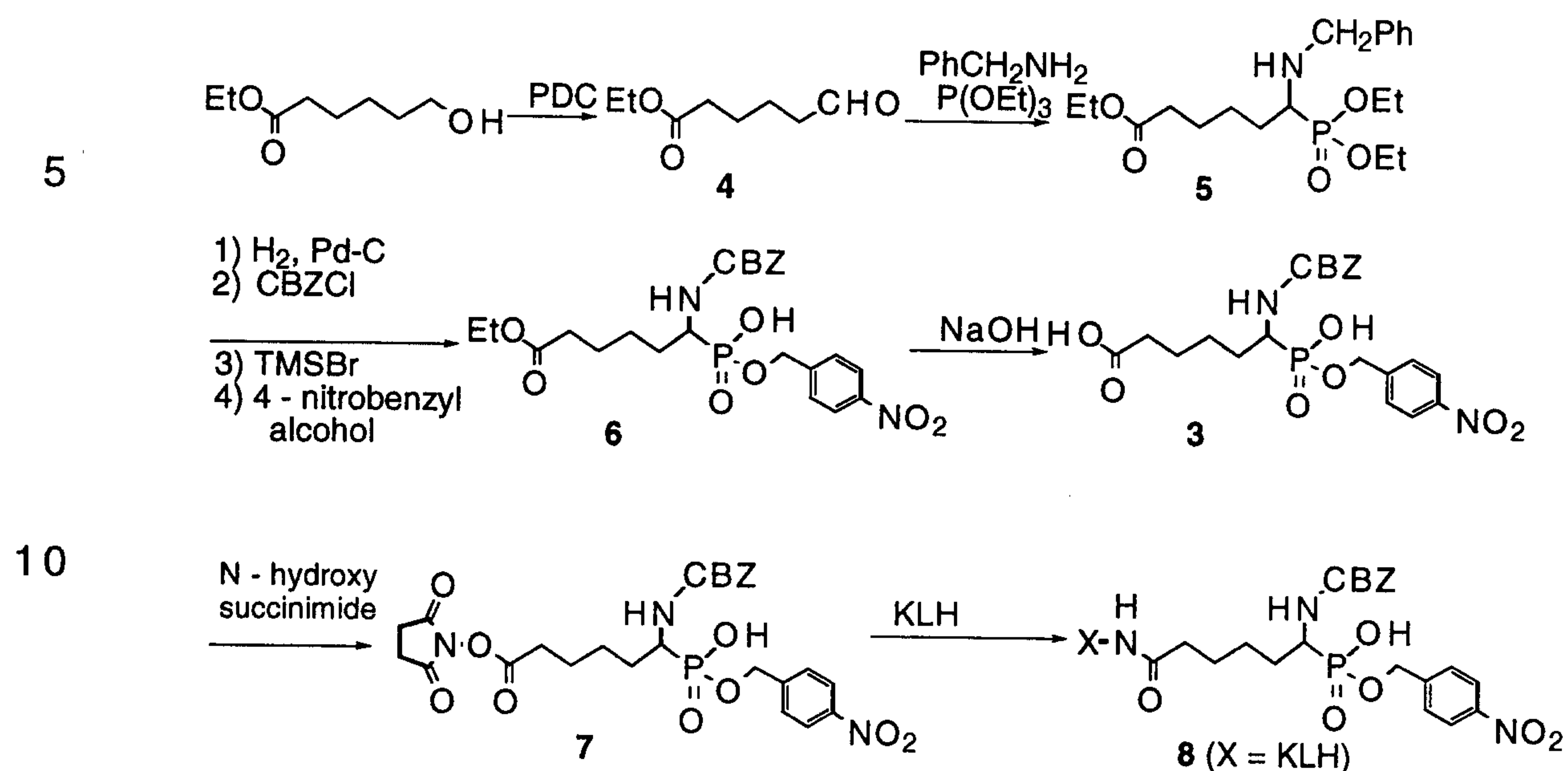
acyloxy is a group of the formula: RCOO- wherein R is alkyl.

The above-noted antibodies are prepared using a known technique for catalytic antibodies reported by Lerner, R. A. et al., Science, 252, 659, 1991. Thus, the antibodies of the invention are produced by immunizing mammals using, as a hapten, the phosphonate derivative of the following formula 3 which is a transition state analog during hydrolysis.



Preparation of Hapten

The above phosphonate derivative can be synthesized, for example, according to the synthetic route shown in the following Scheme 2.

Scheme 2

Commercially available ethyl 6-hydroxy hexanoate is oxidized with PDC (pyridinium dichromate), the resultant compound (4) is iminated with benzylamine in situ, and reacted with triethylphosphite to give compound (5). Compound (5) is then debenzylated by catalytic hydrogenation, and the amino group is subsequently benzyloxycarbonylated. After diethylphosphonate is deethylated by bromotrimethylsilane, the resultant phosphonic acid is reacted with 4-nitrobenzyl alcohol in the presence of DCC (dicyclohexylcarbodiimide) and 1H-tetrazole to obtain compound (6). An ethyl ester of compound (6) is alkali-hydrolyzed to obtain hapten (3). Hapten (3) is converted to activated ester (7) with hydroxysuccinimide and the latter is reacted with KLH (Keyhole limpet hemocyanin) to obtain antigen (8).

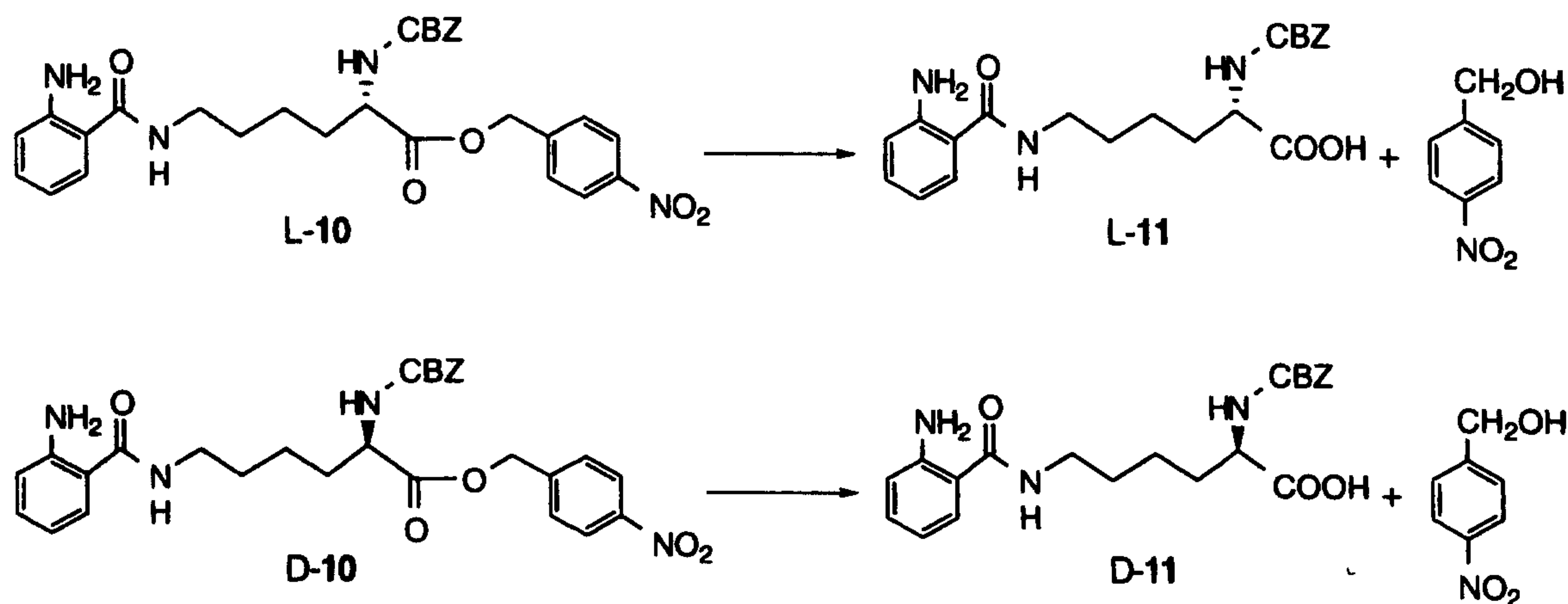
Preparation of Catalytic Antibodies

BALB/c mice are immunized with the above antigen (8) to give monoclonal antibodies.

5 A BALB/c mouse (female, 4-week old) is immunized four times with antigen (8), and spleen cells are removed. After cell fusion is performed in a conventional manner, resultant hybridomas are screened using Enzyme-Linked Immuno Sorbent Assay (ELISA) to obtain a hybridoma producing antibodies which bind to the hapten. Repeated clonings by limiting dilution are performed to obtain a monoclonal IgG-producing hybridoma. Crude antibodies precipitated
10 by adding $(\text{NH}_4)_2\text{SO}_4$ to the supernatant of the culture are purified using cation exchange chromatography and protein G affinity chromatography.

Identification of Catalytic Antibodies

15 The above-obtained antibodies are screened for catalytic activity thereof. In order to simply and quickly conduct the screening of the catalytic activity, the esters (L-10) and (D-10) shown in the following Scheme 3 are used as substrates, which permit tracing of the progress of hydrolysis by measuring the changes in fluorescence intensity. Fluorescence due to anthranilic acid
20 moiety in the esters (L-10) and (D-10) is quenched by 4-nitrobenzyl group existing in the molecules, and when the quenching is dissolved by hydrolysis, fluorescence intensity thereof increases. Namely, the esters (L-11) and (D-11) in the following Scheme 3 are fluorogenic substances.

Scheme 3

5

10

Ester (L-10) is equivalently mixed with ester (D-10) to prepare a fluorogenic substrate (DL-10) and the fluorogenic substrate is used in the screening of the catalytic activity.

15

At first, changes in fluorescent intensity of the obtained purified antibodies are traced at λ ex 340 nm and λ em 415 nm for 10 minutes using the fluorogenic substrate (DL-10). By performing the screening, antibodies hydrolyzing the fluorogenic substrate are identified. Next, a similar procedure is performed using either one of the fluorogenic substrates (L-10) and (D-10) to evaluate enantioselectivity of each antibody.

20

The antibodies having selective catalytic activities to L- or D-form, as obtained above, are examined for their substrate-specificity and enantioselectivity to various amino acid ester derivatives by HPLC analysis.

25

Antibody 7G12 (see working Examples hereinafter described) having selective catalytic activity to L-form catalyzed the hydrolysis of L-form (L-1) of substrates derived from alanine,

phenylalanine, leucine, norleucine, methionine, valine, phenylglycine, 4-hydrophenylglycine, and lysine, but the antibody 7G12 did not catalyze the hydrolysis of the corresponding D-form substrates (D-1). Namely, the antibody 7G12 enantioselectively catalyzed the hydrolysis of various
5 substrates.

Antibody 3G2 (see working Examples hereinafter described), having selective catalytic activity to D-form, catalyzed the hydrolysis of D-form (D-1) substrates derived from alanine, phenylalanine, leucine, norleucine, methionine, valine, phenylglycine, and 4-hydrophenylglycine,
10 but the antibody 3G2 did not catalyze the hydrolysis of the corresponding L-form substrates (L-1). Thus, the antibody 3G2 enantioselectively catalyzed the hydrolysis of various substrates.

The above results demonstrate that the antibodies of the invention obtained by use of the phosphonate (3) as a hapten
15 enantioselectively catalyze the hydrolysis of various amino acid ester derivatives.

The reason why the antibodies of the invention show such non-specificity to substrates in spite of being antibodies is considered to be as follows. It is known that the total maximum contacting surface area
20 between hapten and antibody is 700-800 Å², and that the contacting surface area between low molecular hapten (M.W. up to 350) and antibody is 250-400 Å². This means that the recognition site of the antibody has such size, and it is presumed that, when an antibody derived from the above hapten 3 recognizes CBZ group, 4-nitrobenzyl
25 alcohol, phosphonate, and stereochemistry of α -position of the hapten 3, recognition site of the antibody is sufficiently filled by them, and substituent (R) on α -position of the hapten gets out of the recognition

site. Thus, when the antibodies of the invention catalyze the hydrolysis of amino acid ester derivatives, α -substituent (R) thereof will be outside the recognized site, and therefore, it is believed that the antibodies catalyze every substrate irrespective of their substituents (R) so long as said substrates have the same stereochemistry as the enantiomer of the hapten recognized by the antibodies.

Antibodies 7G12 and 3G2 catalyzed indeed the enantioselective hydrolysis of a wide range of substrates according to the above-noted design (see working Examinations hereinafter described). Accordingly, it was demonstrated that immunization by the use of the hapten having a structural unit which has a size corresponding to that of the recognition sites of the antibodies, and a structural unit which may protrude outside said recognition site gave the catalytic antibodies the capability of performing the stereospecific reaction and catalyzing the wide range of substrates thanks to their high recognition abilities.

As mentioned above, the antibodies of the invention can be utilized in a process for preparing optically active amino acids from their racemic mixture. Specifically, such a process for preparing an optically active amino acid comprising the steps of:

- a) subjecting a racemic mixture of an amino acid derivatives to an ester-forming reaction;
- b) selectively hydrolyzing a desired stereoisomer, D- or L-isomer, of the ester derivative by the use of a catalytic antibody which enantioselectively hydrolyzes an amino acid ester derivative; and
- c) treating the resultant reaction mixture in a conventional manner, for example, making the mixture acidic or basic and then extracting

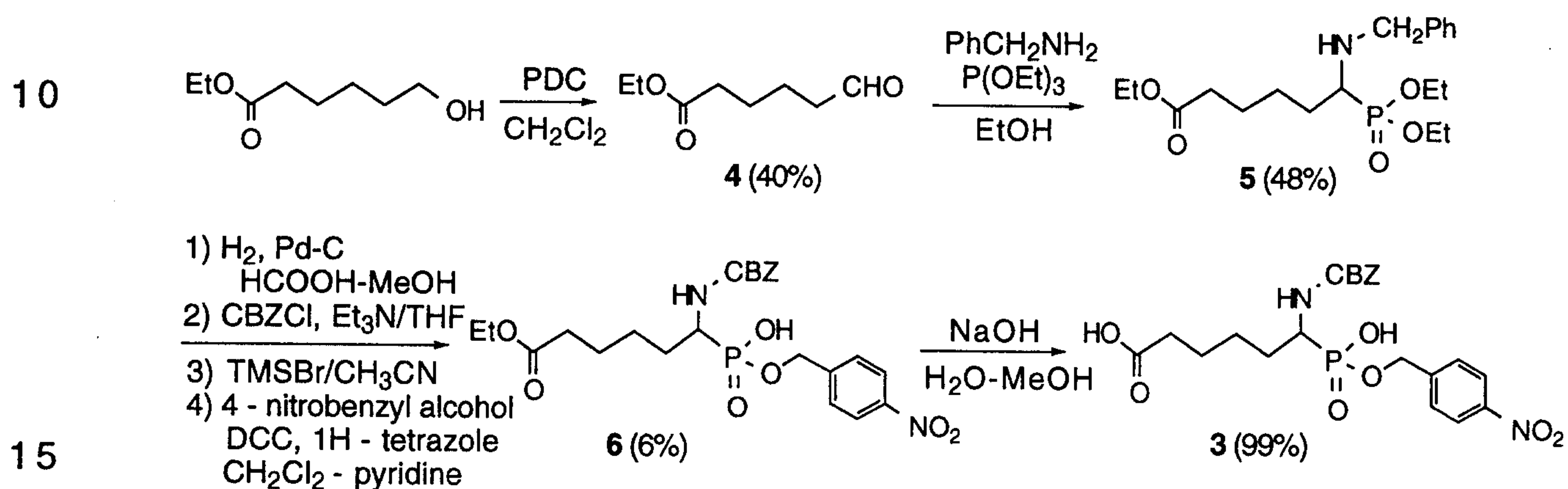
with an organic solvent to leave an aqueous layer, from which the desired hydrolyzed stereoisomer is isolated.

The present invention will be illustrated in more detail with reference to the following Examples and Examinations.

5 However, none are intended to limit the scope of the invention.

Example 1

Synthesis of hapten (3)



1. Synthesis of compound (4)

To a solution of commercially available ethyl 6-hydroxyhexanoate (2.83 g, 17.7 mmol) in methylene chloride (70ml) was added PDC (pyridinium dichromate) (9.95 g, 26.4 mmol) at room temperature. After stirring for 5 hours, the reaction mixture was purified by flash column chromatography over silica gel, eluting with ethyl acetate/hexane (1:2), to obtain compound (4) (1.1276 g, 40%).

1H-NMR (500 MHz, CDCl_3): δ 9.78 (t, $J=1.4$ Hz, 1H), 4.14 (q, $J=7.2$ Hz, 2H), 2.50-2.46 (m, 2H), 2.37-2.30 (m, 2H), 1.71-1.63 (m, 4H), 1.26 (t, $J=7.2$, 3H).

2. Synthesis of compound (5)

To a solution of compound (4) (1.1276 g, 7.13 mmol) in ethanol (2.5 mL) were added benzylamine (1.56 mL, 14.3 mmol) and triethylphosphite (2.44 mL, 14.2 mmol) at room temperature. The mixture was stirred at room temperature for 17.5 hours and then at 40°C for 6 hours. After the solvent was evaporated, the residue was purified by flash column chromatography over silica gel, eluting with ethyl acetate/isopropanol (20:1), to obtain compound (5) (1.1276 g, 40%).

¹H-NMR (500 MHz, CDCl₃): δ 7.35-7.23 (m, 5H), 4.21-4.09 (m, 6H), 3.97 (d, J=13.1 Hz, 2H), 3.88 (dd, J=13.1 Hz, 1.3 Hz, 2H), 2.85 (m, 1H), 2.30-2.25 (m, 2H), 1.83-1.53 (m, 6H), 1.34 (t, J=7.0 Hz, 6H), 1.25 (t, J=7.1 Hz, 3H).

FBMAS (fast atom bombardment mass) m/z: 386 (M⁺+N).

HR-FABMAS (high resolution-FABMAS) for C₁₉H₃₃O₅NP(M⁺+H):
Calcd.: 386.2132, Found: 386.2092.

3. Synthesis of compound (6)

To a solution of compound (5) (473.0 mg, 1.23 mmol) in methanol (3.0 mL) and formic acid (0.40 mL) was added 10% Pb-C (4.6 mg), and the mixture was stirred at room temperature for 2.5 days under hydrogen. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to obtain a colorless residue. The residue was dissolved in THF (5.0 mL), and carbobenzoxy chloride (700 μL, 4.90 mmol) and triethyl amine (1.71 mL, 12.3 mmol) were added thereto at room temperature. After stirring for 3 hours, 1N hydrochloric acid was added, and the mixture was extracted with ethyl acetate. The combined organic phase was washed with saturated brine, dried, and evaporated. The residue was purified by flush column chromatography over silica

gel, eluting with ethyl acetate to obtain colorless gum (120.5 mg). The gum (120.5 mg) was dissolved in methylene chloride (0.5 mL) and bromotrimethylsilane (222 μ L, 1.68 mmol) was added thereto at room temperature. After stirring at 35 $^{\circ}$ C for 2 hours, the solvent was evaporated off, and to the residue were added acetonitrile (1.0 mL) and water (0.2 mL). After 12 hours, the solvent was evaporated and the residue was dried. To a solution of the residue in methylene chloride (1.0 mL) and pyridine (0.5 mL) were added 4-nitrobenzyl alcohol (61.2 mg, 0.40 mmol), 1H-tetrazole (9.7 mg, 0.14 mmol) and DCC (261.5 mg, 1.27 mmol), and the mixture was stirred at 35 $^{\circ}$ C for 5 hours. After the solvent was evaporated off, acetonitrile was added to the residue, and the mixture was filtered. The filtrate was purified by HPLC (YMCA-323^{*}: C-18, 10 mm in diameter x 250 mm in length, acetonitrile/0.1% aqueous trifluoroacetic acid solution=50/50, 3.0 mL/min, 254 nm) to obtain compound (6) (34.5 mg, 6% from compound (5)).

¹H-NMR (500 MHz, CDCl₃): δ 8.22 (d, J=8.6 Hz, 2H), 7.62 (d, J=8.6 Hz, 2H), 7.38-7.28 (m, 5H), 5.18 (ABX, J_{AB}=13.5 Hz, J_{AX}=J_{BX}= 7.4 HZ, $\Delta\nu$ =16.2 Hz, 2H), 5.11 (AB, J=12.6 Hz, $\Delta\nu$ =20.2 Hz, 2H), 4.14 (q, J=7.1 Hz, 2H), 4.06 (m, 1H), 2.33 (t, J =7.3 Hz, 2H), 1.95-1.37 (m, 6H), 1.27 (t, J=7.1 Hz, 3H).

FABMAS m/z: 509 (M⁺+H).

HR-FABMAS for C₂₃H₃₀O₉N₂P(M⁺+H):

Calcd.: 509.1689, Found: 509.1688.

4. Synthesis of compound (3)

To a solution of compound (6) (15.0 mg, 0.0295 mmol) in methanol (0.2 mL) and water (0.2 mL) was added 1N NaOH (0.1 mL, 0.1 mmol) at room temperature. After 6.5 hours, the reaction

^{*}Trade mark

mixture was made acidic by addition of trifluoroacetic acid, and the mixture was purified by HPLC (YMCA-323: C-18, 10 mm in diameter x 250 mm in length, acetonitrile/0.1% aqueous trifluoroacetic acid solution=50/50, 254 nm) to obtain compound (3) (14.0 mg, 99%).

5 $^1\text{H-NMR}$ (500 MHz, CD_3OD): δ 8.22 (d, $J=8.6$ Hz, 2H), 7.61 (d, $J=8.6$ Hz, 2H), 7.37-7.28 (m, 5H), 5.18 (ABX, $J_{AB}=13.5$ Hz, $J_{AX}=J_{BX}=7.4$ Hz, $\Delta\nu=16.7$ Hz, 2H), 5.11 (AB, $J=12.5$ Hz, $\Delta\nu=26.6$ Hz, 2H), 4.07 (m, 1H), 2.32 (t, $J=7.3$ Hz, 2H), 1.97-1.38 (m, 6H).

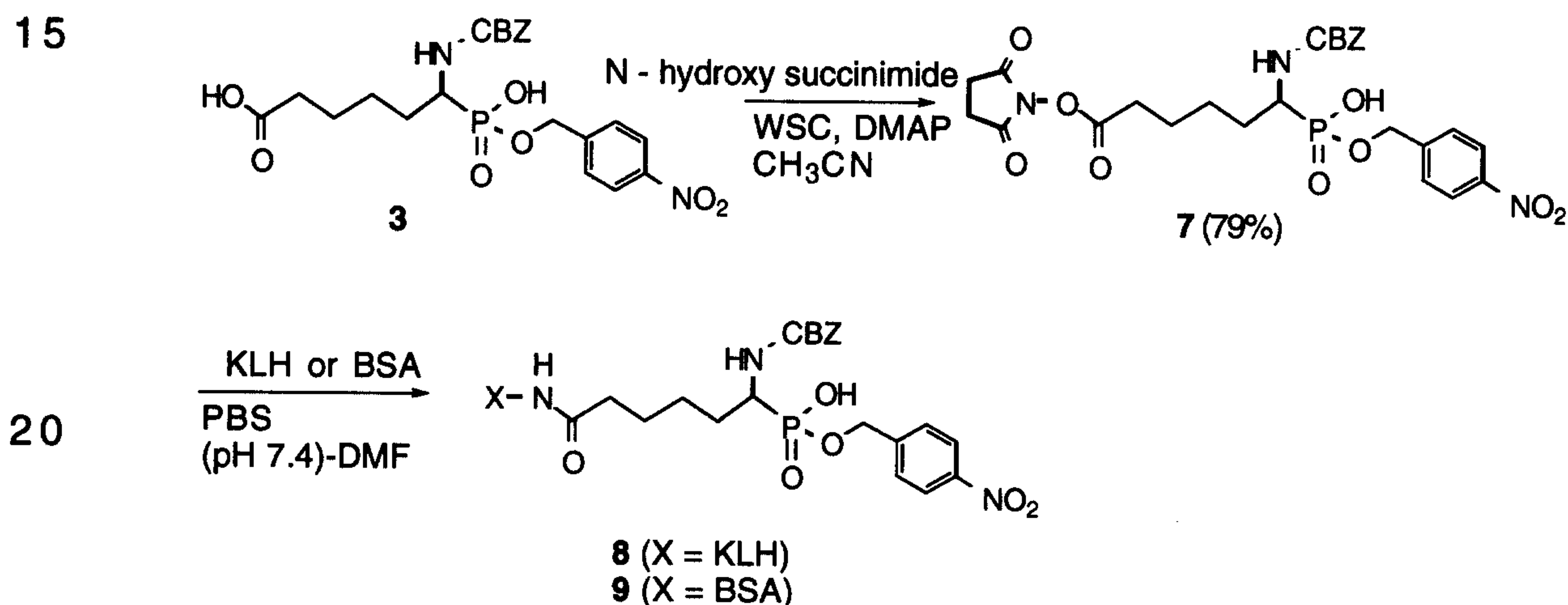
FABMAS m/z : 503 (M^+Na), 481 (M^+H).

10 HR-FABMAS for $\text{C}_{21}\text{H}_{25}\text{O}_9\text{N}_2\text{PNa}(M^+Na)$:

Calcd.: 503.1196, Found: 503.1202.

Example 2

Condensation of hapten and carrier proteins



5. Synthesis of compound (7)

25 To a solution of compound (3) (6.4 mg, 0.013 mmol) in acetonitrile (0.3 mL) were added N-hydroxysuccinimide (2.5 mg, 0.022 mmol), WSC water-soluble carbodiimide (WSC) (8.3 mg, 0.043 mmol) and dimethylaminopyridine (DMAP) (0.1 mg, 0.0008 mmol) and

the mixture was stirred for 2 hours. The reaction mixture was purified by HPLC (YMCA-323: C-18, 10 mm in diameter x 250 mm in length, acetonitrile/0.1% aqueous trifluoroacetic acid solution=50/50, 3.0 mL/min, 254 nm) to obtain compound (7) (6.1 mg, 79%).

$^1\text{H-NMR}$ (500 MHz, CD_3OD): δ 8.23 (d, $J=8.7$ Hz, 2H), 7.62 (d, $J=8.7$ Hz, 2H), 7.38-7.27 (m, 1H), 5.19 (ABX, $J_{\text{AB}}=13.4$ Hz, $J_{\text{AX}}=J_{\text{BX}}=7.5$ Hz, $\Delta\nu=15.9$ Hz, 2H), 5.11 (AB, $J=12.5$ Hz, $\Delta\nu=16.4$ Hz, 2H), 4.08 (m, 1H), 2.85 (s, 4H), 2.66 (t, $J=7.2$ Hz, 2H), 1.97-1.43 (m, 6H).

6. Synthesis of KLH-condensate (8)

To a solution of compound (7) (2.5 mg, 0.0043 mmol) in DMF (60 μL)-200 mM $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$, pH 7.4 (0.5 mL) was added a solution of KLH in 10 mM NaH_2PO_4 , pH 7.4 (11.3 mg/mL, 442 μL).

After 21 hours, the reaction mixture was purified by Sephadex* G-25M (Pharmacia, PD-10), eluting with PBS, to obtain KLH-condensate (8). The concentration of the protein therein was determined by Bradford's method (1.3 mg/mL).

7. Synthesis of BSA-condensate (9)

To a solution of compound (7) (3.0 mg, 0.0052 mmol) in DMF (40 μL) was added a solution of BSA (6.0 mg) in 200 mM $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$, pH 7.4 (0.5 mL) at room temperature. After 12 hours, the reaction mixture was purified by Sephadex G-25M (Pharmacia, PD-10), eluting with PBS, to obtain KLH-condensate (9). The concentration of the protein therein was determined according to Bradford's method (1.3 mg/mL). The compound (9) was used for ELISA analysis.

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Example 3

Immunization

A solution of 50 μ g of the antigen (KLH-condensate), prepared in Example 2, in 50 μ L of saline was mixed with an equal amount of Freund's complete adjuvant, and the mixture was intraperitoneally injected to BALB/c mice (4-week old, female). After 10 days, the mice were boosted with a mixture of the antigen-saline solution (50 μ g/50 μ L) and equal amount of Freund's incomplete adjuvant and, following 10 days, boosted again with the same mixture. After 7 days, blood was taken from the tail vein of the mouse. Antibody titer was assayed using BSA-condensate by ELISA, which uses as a secondary antibody, biotinized anti-mouse IgG antibodies, avidin, and biotinized peroxidase, and the titer measured 2.5×10^5 . One month after the second booster, the antigen-saline solution (100 μ g/100 μ L) was administered via the tail vein (final immunization).

Example 4

Preparation of hybridoma

Three days after the final immunization, the spleen of the mouse was removed, followed by cell fusion of 1.9×10^8 spleen cells and 2.7×10^7 myeloma cells (x63/Ag 8.653) using polyethyleneglycol. Using ten 96-well plates each containing HAT selection medium (RPMI medium containing 0.1 mM hypoxanthine, 0.4 μ M aminopterin, 0.016 mM thymidine, and 10% fetus bovine serum) containing 6×10^5 feeder cells (mouse thymocytes)/well, selection of the hybridomas obtained was conducted (at 37°C, under 10% CO₂). Following 8-14 days, supernatants removed from the wells in which a growth of a colony was found were subjected to screening by

ELISA. Out of 111 positive wells, 57 wells were subjected to cloning to obtain 39 clones. ELISA analysis using anti-mouse IgG H-chain antibody demonstrated that all of the 39 clones produced IgG.

5

Example 5

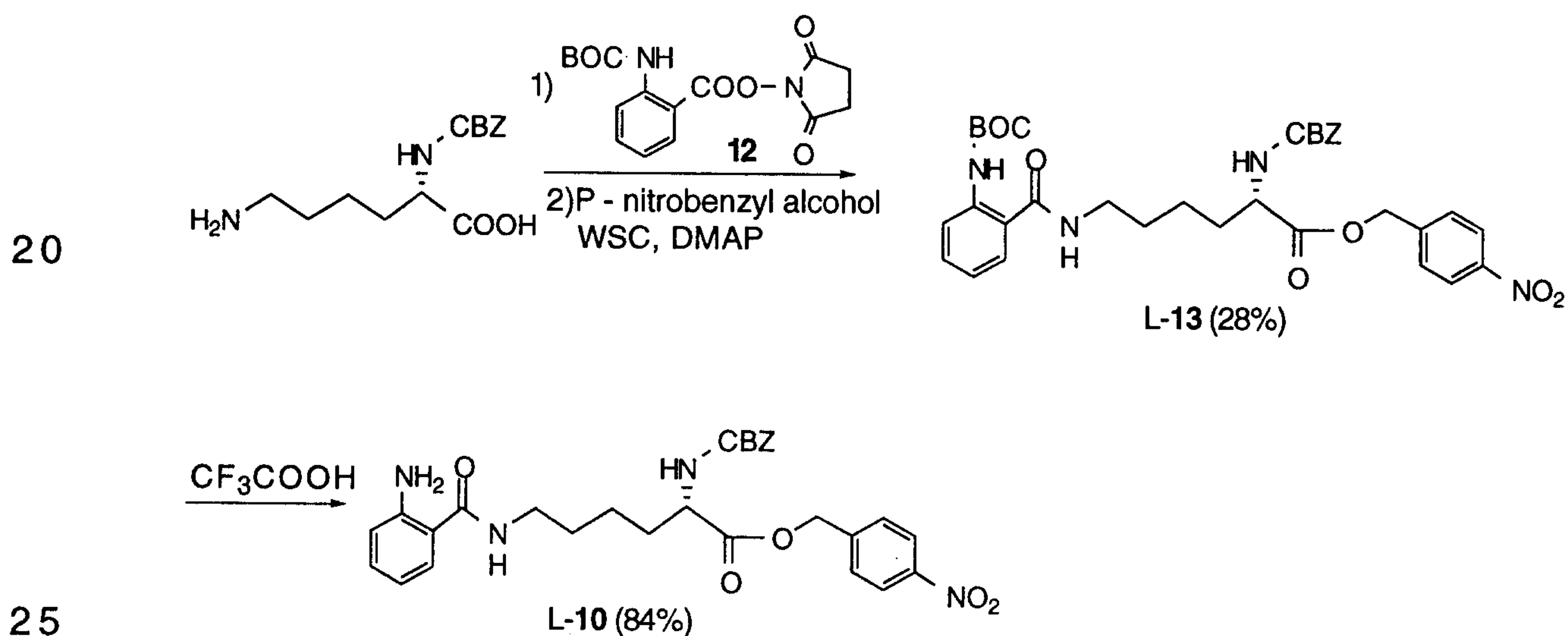
Preparation of monoclonal antibodies

Each of 39 hybridomas prepared in Example 4 was cultured in a complete medium (RPMI medium containing 10% fetus bovine serum) for about 7 days. The supernatants were precipitated with an equal volume of aqueous saturated ammonium sulfate solution and then subjected to cation exchange chromatography on a S-Sepharose* column and affinity chromatography on a protein G column to obtain 10-20 mg of the purified antibodies.

15

Preparation 1

Synthesis of fluorogenic substrate (L-10)



1. Synthesis of compound (12)

To a solution of commercially available anthranilic acid

*Trade mark

(1.3737 g, 10.0 mmol) in 0.5N sodium hydroxide (20.0 mL), dioxane (10.0 mL) and acetonitrile (2.0 mL) was added di-*t*-butyldicarbonate (3.124 g, 14.3 mmol) at 0°C. Following 8-hours of stirring at room temperature, the volatile solvent was evaporated under reduced pressure. To the reaction mixture were added ice and 10% citric acid solution and the mixture was extracted with ethyl acetate. The combined organic phase was washed with saturated brine and dried. The solvent was evaporated, and the residue was crystallized from ethyl acetate-hexane to obtain *t*-butoxycarbonyl anthranilic acid (1.71 g, 72%).

¹H-NMR (500 MHz, CDCl₃): δ 10.1 (s, 1H), 8.47 (d, *J*=8.0 Hz, 1H), 8.09 (dd, *J*=8.0 Hz, 1.4 Hz, 1H), 7.56 (dt, *J*=8.0 Hz, 1.4 Hz, 1H), 7.04 (t, *J*=8.0 Hz, 1H), 1.54 (s, 9H).

t-Butoxycarbonylanthranilic acid is known (Nichino, N.; Powers, J. C. J. Biol. Chem. 1980, 255, 3482). To a solution of *t*-butoxycarbonylanthranilic acid (378.9 mg, 1.60 mmol) in methylene chloride (2.5 mL) were added *N*-hydroxysuccinimide (225.9 mg, 1.96 mmol), WSC (565.9 mg, 2.95 mmol), DMAP (4.7 mg, 0.04 mmol) at room temperature, and the mixture was stirred for 1 hour. To the reaction mixture was added 1% citric acid solution and the mixture was extracted with methylene chloride. The combined organic phase was washed with saturated brine and dried. The solvent was evaporated and the residue was crystallized from ethyl acetate-hexane to give compound (12) (199.7 mg, 30%).

¹H-NMR (500 MHz, CDCl₃): δ 9.49 (s, 1H), 8.51 (d, *J*=8.0 Hz, 1H), 8.17 (d, *J*=8.0 Hz, 1H), 7.63 (d, *J*=8.0 Hz, 1H), , 7.07 (d, *J*=8.0 Hz, 1H), 2.93 (s, 4H), 1.52 (s, 9H).

2. Synthesis of compound (L-13)

A mixture of compound (12) (67.3 mg, 0.201 mmol), N α -benzyloxycarbonyl-L-lysine (56.4 mg, 0.201 mmol), and diisopropylethylamine (0.04 mL, 0.230 mmol) was stirred in DMF (0.2 mL), methanol (0.2 mL), ethyl acetate (0.1 mL) and water (0.1 mL) at room temperature for 16 hours. The volatile solvent was evaporated under reduced pressure and the residue was purified by silica gel flush column chromatography, eluting with ethyl acetate/isopropanol/water (9:1:0—8:2:0.2—5:3:1) to give the residue (122.2 mg). To a solution of the residue (122.2 mg) in methylene chloride (1.0 mL) and acetonitrile (0.1 mL) were added 4-nitrobenzylalcohol (34.9 mg, 0.228 mmol), WSC (58.6 mg, 0.306 mmol) and DMAP (1.4 mg, 0.011 mmol) at room temperature and stirred for 4 hours. The reaction mixture was purified by silica gel flush column chromatography, eluting with ethyl acetate/hexane (1:1.1), to give compound (L-13) (35.7 mg, 28% from compound (12)). ¹H-NMR (600 MHz, CDCl₃): δ 10.1 (s, 1H), 8.33 (d, J=8.0 Hz, 1H), 8.20 (d, J=8.0 Hz, 2H), 7.52 (d, J=8.0 Hz, 2H), , 7.44-7.38 (m, 2H), 7.35-7.28 (m, 5H), 6.95 (t, J=8.0 Hz, 1H), 6.37 (brs, 1H), 5.36 (brs, 1H), 5.24 (AB, J=13.3 Hz, $\Delta\nu$ =16.6 Hz, 2H), 5.06 (AB, J=12.3 Hz, $\Delta\nu$ =36.0 Hz, 2H), 4.46 (m, 1H), 3.43-3.37 (m, 2H), 1.95-1.40 (m, 6H).

3. Synthesis of compound (L-10)

To a solution of compound (L-13) (54.1 mg, 0.0858 mmol) in methylene chloride (0.3 mL) was added trifluoroacetic acid (0.2 mL) at room temperature and stirred for 1 hour. The solvent was evaporated under reduced pressure and purified by HPLC (YMCA-323: C-18, 10 mm in diameter x 250 mm in length, acetonitrile/0.1% aqueous trifluoroacetic acid solution=60/40, 3.0 mL/min, 254 nm) to obtain compound (L-10) (38.1 mg, 84%). The

compound (L-10) was crystallized from ethyl acetate-hexane.

$^1\text{H-NMR}$ (600 MHz, $\text{CDCl}_3\text{-CD}_3\text{OD}$): δ 8.21 (d, $J=8.0$ Hz, 2H), 7.49 (d, $J=8.0$ Hz, 2H), 7.37-7.25 (m, 6H), 7.19 (t, $J=8.0$ Hz, 1H), 6.65 (d, $J=8.0$ Hz, 1H), 6.64 (t, $J=8.0$ Hz, 1H), 5.25 (AB, $J=13.3$ Hz, 2H), 5.08 (AB, $J=12.3$ Hz, $\Delta\nu=24.6$ Hz, 2H), 4.40 (m, 1H), 3.42-3.34 (m, 2H), 1.94-1.85 (m, 1H), 1.80-1.72 (m, 1H), 1.68-1.54 (m, 2H), 1.51-1.39 (m, 2H).

Preparation 2

Synthesis of fluorogenic substrate (D-10)

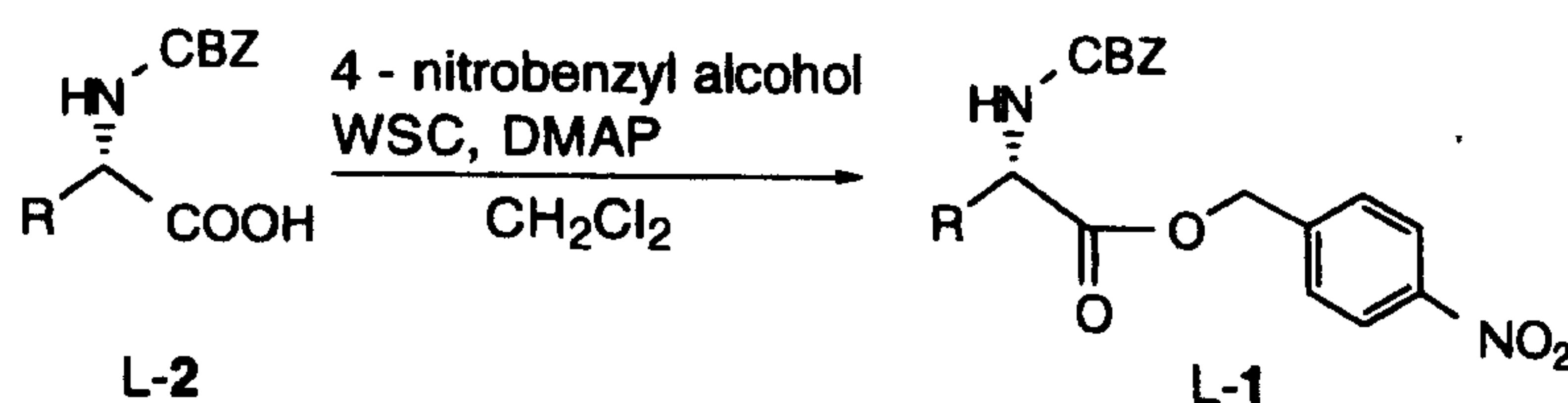
The ester (D-10) was prepared according to the same procedure as that described in Preparation 1, except that the D-form of N α -benzyloxycarbonyl-D-lysine was used.

Physico-chemical properties: $^1\text{H-NMR}$ spectrum of D-10 was the same as that of L-10.

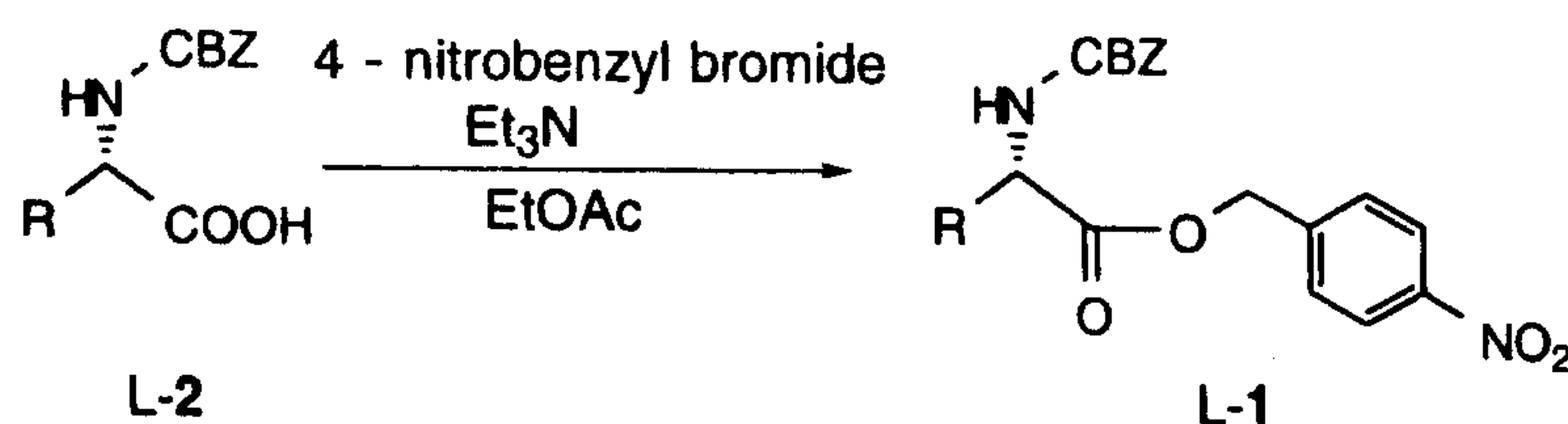
Preparation 3

Synthesis of substrate compounds (L-1) and (D-1)

Method A



Method B



General synthesis of compounds (L-1) and (D-1)

According to the following methods A and B, various amino acid ester derivatives were synthesized.

5 Method A: A solution of commercially available L- or D-carbobenzoxy amino acid (1 eq.), 4-nitrobenzylalcohol (1.1 eq.), WSC (1.3-1.6 eq.), and DMAP (0.01 eq.) in methylene chloride was stirred at room temperature for 1 hour. The solvent was evaporated under reduced pressure, and ice and 1N hydrochloric acid were added to the resultant residue, and the mixture was extracted with ethyl
10 acetate. The combined organic phase was washed with saturated brine and dried. The solvent was evaporated, and the residue was purified by silica gel flush column chromatography to obtain compound (L-1) or (D-1).

Method B: A solution of commercially available L- or D-
15 carbobenzoxy amino acid (1 eq.), 4-nitrobenzylbromide (1.5 eq.), and triethylamine (1.5 eq.) in ethyl acetate was heated under reflux for 2 hours. The reaction mixture was cooled, to which ice and 1N hydrochloric acid were added, and the mixture was extracted with ethyl acetate. The combined organic phase was washed with
20 saturated brine and dried. The solvent was evaporated, and the residue was purified by silica gel flush column chromatography to obtain compound (L-1) or (D-1).

1. Synthesis of N-(benzyloxycarbonyl)alanine 4-nitrobenzyl ester (R=CH₃)

25 According to the method A, the titled compound was synthesized, purified by silica gel flush column chromatography, eluting with ethyl acetate/hexane (2:3), and crystallized from ethyl acetate-hexane.

$^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 8.21 (d, $J=8.0$ Hz, 2H), 7.49 (d, $J=8.0$ Hz, 2H), 7.37-7.30 (m, 5H), 5.26 (s, 2H), 5.24 (brd, 1H), 5.12 (s, 2H), 4.47 (m, 1H), 1.45 (d, $J=7.2$ Hz, 3H).

FABMAS m/z : 359 ($\text{M}^+ + \text{H}$).

5 HR-FABMAS for $\text{C}_{18}\text{H}_{19}\text{O}_6\text{N}_2$ ($\text{M}^+ + \text{H}$):

Calcd.: 359.1244; Found: 359.1244.

2. Synthesis of N-(benzyloxycarbonyl)phenylalanine 4-nitrobenzyl ester ($\text{R}=\text{PhCH}_2$)

10 According to the method A, the titled compound was synthesized, purified by silica gel flush column chromatography, eluting with ethyl acetate/hexane (2:3), and crystallized from ethyl acetate-hexane.

15 $^1\text{H-NMR}$ (600 MHz, CDCl_3): δ 8.17 (d, $J=8.0$ Hz, 2H), 7.38-7.23 (m, 10H), 7.08 (m, 2H), 5.23 (brd, $J=7.5$ Hz, 1H), 5.19 (s, 2H), 5.09 (s, 1H), 4.92 (m, 1H), 3.12 (d, $J=6.8$ Hz, 2H).

3. Synthesis of N-(benzyloxycarbonyl)leucine 4-nitrobenzyl ester ($\text{R}=(\text{CH}_3)_2\text{CHCH}_2$)

20 According to the method A, the titled compound was synthesized, purified by silica gel flush column chromatography, eluting with ethyl acetate/hexane (1:2).

$^1\text{H-NMR}$ (600 MHz, CDCl_3): δ 8.22 (d, $J=8.3$ Hz, 2H), 7.50 (d, $J=8.3$ Hz, 2H), 7.40-7.30 (m, 5H), 5.25 (AB, $J=13.4$ Hz, $\Delta\nu=11.8$ Hz, 2H), 5.11 (AB, $J=11.4$ Hz, $\Delta\nu=6.1$ Hz, 2H), 5.13-5.11 (1H), 4.46 (m, 1H), 1.74-1.52 (m, 3H), 0.95 (d, $J=7.2$ Hz, 3H), 0.94 (d, $J=7.2$ Hz, 3H).

25 4. Synthesis of N-(benzyloxycarbonyl)norleucine 4-nitrobenzyl ester ($\text{R}=\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2$)

According to the method B, the titled compound was

synthesized, purified by silica gel flush column chromatography, eluting with ethyl acetate/hexane (2:5), and crystallized from ethyl acetate-hexane.

5 $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 8.22 (d, $J=8.5$ Hz, 2H), 7.50 (d, $J=8.5$ Hz, 2H), 7.38-7.30 (m, 5H), 5.26 (s, 2H), 5.20 (brd, $J=7.9$ Hz, 1H), 5.12 (s, 2H), 4.43 (m, 1H), 1.85 (m, 1H), 1.68 (m, 1H), 1.37-1.23 (m, 4H), 0.87 (t, $J=6.9$ Hz, 3H).

5. Synthesis of N-(benzyloxycarbonyl)methionine 4-nitrobenzyl ester ($\text{R}=\text{CH}_3\text{SCH}_2\text{CH}_2$)

10 According to the method A, the titled compound was synthesized, purified by silica gel flush column chromatography, eluting with ethyl acetate/hexane (2:3).

15 $^1\text{H-NMR}$ (600 MHz, CDCl_3): δ 8.22 (d, $J=8.0$ Hz, 2H), 7.51 (d, $J=8.0$ Hz, 2H), 7.39-7.32 (m, 5H), 5.42 (brd, $J=7.1$ Hz, 1H), 5.27 (AB, $J=12.9$ Hz, $\Delta\nu=12.4$ Hz, 2H), 5.12 (s, 2H), 4.59 (m, 1H), 2.53 (t, $J=7.1$ Hz, 2H), 2.19 (m, 1H), 2.07 (s, 3H), 2.01 (m, 1H).

6. Synthesis of N-(benzyloxycarbonyl)valine 4-nitrobenzyl ester ($\text{R}=(\text{CH}_3)_2\text{CH}$)

20 According to the method A, the titled compound was synthesized, purified by silica gel flush column chromatography, eluting with ethyl acetate/hexane (1:2), and crystallized from ethyl acetate-hexane.

25 $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 8.22 (d, $J=8.5$ Hz, 2H), 7.51 (d, $J=8.5$ Hz, 2H), 7.39-7.31 (m, 5H), 5.26 (s, 2H), 5.23 (brd, $J=8.8$ Hz, 1H), 5.12 (s, 2H), 4.38 (dd, $J=4.8$ Hz, 8.8 Hz, 1H), 2.20 (m, 1H), 0.98 (d, $J=6.8$ Hz, 3H), 0.88 (d, $J=6.8$ Hz, 3H).

7. Synthesis of N-(benzyloxycarbonyl)phenylglycine 4-nitrobenzyl ester ($\text{R}=\text{Ph}$)

According to the method B, the titled compound was synthesized, purified by silica gel flush column chromatography, eluting with ethyl acetate/hexane (2:5) and crystallized from ethyl acetate-hexane.

5 $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 8.12 (d, $J=8.5$ Hz, 2H), 7.39-7.30 (m, 5H), 7.27 (d, $J=8.5$ Hz, 2H), 5.77 (brd, $J=7.1$ Hz, 1H), 5.44 (d, $J=7.1$ Hz, 1H), 5.25 (AB, $J=13.4$ Hz, $\Delta\nu=27.4$ Hz, 2H), 5.11 (AB, $J=12.1$ Hz, $\Delta\nu=19.8$ Hz, 2H).

10 8. Synthesis of N-(benzyloxycarbonyl) 4-hydroxyphenylglycine 4-nitrobenzyl ester (R=4-HOPh)

According to the method B, the titled compound was synthesized, purified by silica gel flush column chromatography, eluting with ethyl acetate/hexane (1:1.25), and crystallized from ethyl acetate-hexane.

15 $^1\text{H-NMR}$ (600 MHz, CDCl_3): δ 8.14 (d, $J=8.5$ Hz, 2H), 7.38-7.31 (m, 5H), 7.30 (d, $J=8.5$ Hz, 2H), 7.22 (d, $J=8.5$ Hz, 2H), 6.81 (d, $J=8.5$ Hz, 2H), 5.70 (brd, $J=7.1$ Hz, 1H), 5.36 (d, $J=7.1$ Hz, 1H), 5.24 (AB, $J=13.9$ Hz, $\Delta\nu=24.2$ Hz, 2H), 5.11 (AB, $J=12.0$ Hz, $\Delta\nu=21.1$ Hz, 2H), 5.10 (s, 1H).

20 9. Synthesis of $\text{N}\alpha$ -(benzyloxycarbonyl)- $\text{N}\epsilon$ -(tert-butoxycarbonyl)lysine 4-nitrobenzyl ester

According to the method B using $\text{N}\alpha$ -(benzyloxycarbonyl)- $\text{N}\epsilon$ -(tert-butoxycarbonyl)lysine, the titled compound was synthesized, purified by silica gel flush column chromatography, eluting with ethyl acetate/hexane (2:3), and
25 crystallized from ethyl acetate-hexane.

$^1\text{H-NMR}$ (600 MHz, CDCl_3): δ 8.22 (d, $J=8.3$ Hz, 2H), 7.50 (d, $J=8.3$ Hz, 2H), 7.38-7.30 (m, 5H), 5.40 (brd, $J=6.6$ Hz, 1H), 5.25 (AB, $J=12.6$ Hz,

$\Delta\nu=8.1$ Hz, 2H), 5.11 (AB, $J=12.2$ Hz, $\Delta\nu=12.0$ Hz, 2H), 4.55 (m, 1H), 4.41 (m, 1H), 3.12-3.07 (m, 2H), 1.88 (m, 1H), 1.72 (m, 1H), 1.60-1.31 (m, 4H), 1.42 (s, 9H).

10. Synthesis of $N\alpha$ -(benzyloxycarbonyl)-D-lysine 4-nitrobenzyl ester ($R=H_2NCH_2CH_2CH_2CH_2$)

To a solution of $N\alpha$ -(benzyloxycarbonyl)- $N\epsilon$ -(tert-butoxycarbonyl)-D-lysine 4-nitrobenzyl ester (210.5 mg, 0.408 mmol) in chloroform (1.0 mL) was added trifluoroacetic acid (0.3 mL) at room temperature and the mixture was stirred for 8 hours. The solvent was evaporated under reduced pressure and the residue was crystallized from ethyl acetate-hexane.

^1H -NMR (500 MHz, $CDCl_3$ - CD_3OD): δ 8.22 (d, $J=8.5$ Hz, 2H), 7.51 (d, $J=8.5$ Hz, 2H), 7.40-7.26 (m, 5H), 5.27 (AB, $J=13.3$ Hz, $\Delta\nu=19.9$ Hz, 2H), 5.11 (AB, $J=12.3$ Hz, $\Delta\nu=19.1$ Hz, 2H), 4.35 (m, 1H), 2.87 (t, $J=7.2$ Hz, 2H), 1.88 (m, 1H), 1.77-1.56 (m, 3H), 1.50-1.37 (m, 2H).

Examination 1

Identification of monoclonal antibodies having catalytic activity

1. Identification of monoclonal antibodies having hydrolyzing activity by measuring fluorescence

To a solution of each of 39 purified antibodies, prepared in Example 5, in 50 mM Tris-buffered solution, pH 8.0 (360 μ L), was added a mixture (DL-10) of equal amounts of compounds (L-10) and (D-10), which were respectively prepared in Preparations 1 and 2, in 150 μ M DMSO solution (40 μ L) at 25 °C, and they were mixed with stirring to obtain a reaction mixture containing 1.5 μ M antibodies and 15 μ M substrate. Fluorescence intensity was measured

immediately after and ten minutes after the mixing at λ_{ex} 340 nm and λ_{em} 415 nm to determine the change of intensity in ten minutes. Based on the magnitude of the change in ten minutes, 14 antibodies having the catalytic activity were selected. The results obtained are shown in the following Table 1.

Table 1

<u>Antibodies</u>	<u>10-minute change in fluorescence intensity</u>
7G12	12.54
10C8	9.40
6C4	8.00
6B12	7.20
10B2	7.10
7H1	6.40
2E1	5.04
4G9	4.08
8H10	3.49
8A11	3.40
5H2	2.88
3G2	2.31
9F9	2.20
6G10	2.08

2. Determination of enantioselectivity of catalytic antibodies by measuring fluorescence

To a solution of each of 14 antibodies, which were found to be a catalytic antibody, in 50 mM Tris-buffered solution, pH 8.0 (360 μL), was added a solution of one of the compounds (L-10) and

(D-10), which were respectively prepared in Preparations 1 and 2, in 150 μ M DMSO solution (40 μ L) at 25 °C, and they were mixed with stirring to obtain the reaction mixture containing 1.5 μ M antibody and 15 μ M substrate. Fluorescence intensity was immediately after and ten minutes after the mixing, at λ ex 340 nm and λ em 415 nm to determine the change of the intensity in 10 minutes. Based on the magnitude of the change in ten minutes, 10 antibodies having the catalytic activity selective to the L-form and 4 antibodies having a catalytic activity selective to the D-form were identified. The results obtained are shown in the following Tables 2 and 3.

Table 2

<u>Antibodies selective to L-form</u>	<u>10-min. change in fluorescence intensity</u>	
	<u>L-10</u>	<u>D-10</u>
7G12	23.29	0.80
10C8	30.45	0.48
6C4	10.4	0.77
6B12	9.67	0.90
10B2	7.90	0.96
7H1	8.00	1.03
2E1	7.80	1.28
4G9	5.67	1.12
8H10	5.05	0.72
6G10	3.00	0.62

Table 3

<u>Antibodies selective to D-form</u>	<u>10-min. change in fluorescence intensity</u>	
	<u>L-10</u>	<u>D-10</u>
3G2	0.38	4.32
5H2	0.40	5.16
8A11	0.30	4.62
9F9	0.48	4.44

Hybridomas producing antibodies 7G12 and 3G2 were named hybridomas ZAA 7G12 and ZAA 3G2, respectively.

Both hybridomas 7G12 and 3G2 were deposited at Fermentation Research Institute Agency of Industrial Science and Technology, Japan, on December 21, 1994, under the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure, with accession Nos. FERM BP-4947 and FERM BP-4946, respectively.

DNA-base sequences corresponding to variable regions of 7G12 and 3G2 antibodies, in which antigen-binding sites exist, were determined using DyeDeoxy Terminator Cycle Sequencing kits (Applied Biosystems) and the corresponding amino acid sequences were also determined.

For antibody 7G12, DNA-base sequence corresponding to and the amino acid sequence for the variable region of the heavy chain are shown in SEQ ID NOs. 1 and 2, respectively, and those for variable region of the light chain in SEQ ID NOs. 3 and 4, respectively. For antibody 3G2, DNA-base sequence and the amino acid sequence for the variable region of the heavy chain are shown in SEQ ID NOs. 5 and 6, respectively, and those for variable region of the light chain in SEQ ID NOs. 7 and 8, respectively.

Examination 2Determination of specificity to substrate and enantioselectivity of monoclonal antibodies having catalytic activity

5 1. Determination of specificity to substrate and enantioselectivity of monoclonal antibody 7G12 by HPLC

To a solution of antibody 7G12 in 50 mM Tris-buffered solution, pH 8.0 (90 μ L), was added a solution of a substrate compound, N-(benzyloxycarbonyl)-L-phenylalanine 4-nitrobenzyl ester (L-1) ($R=PhCH_2$), synthesized in Preparation 3, in 2mM DMSO solution (10 μ L) at 25°C, and they were mixed with stirring to obtain the reaction mixture containing 10.8 μ M antibody and 200 μ M substrate. By means of HPLC (YMCAM-303: C-18, 10 mm in diameter x 250 mm in length, an aqueous solution of acetonitrile/0.1% trifluoroacetic acid=35/65 (0-7 minutes), 35/65-90/10 (7-15 minutes), 90/10 (15-17 minutes), 1.0 mL/minute, 278 nm), the amount of 4-nitrobenzyl alcohol (retention time: 5.9 minutes) produced in the reaction mixture was traced in time course for the purpose of determining initial rate. Next, the same procedure as mentioned above was repeated using N-(benzyloxycarbonyl)-D-phenylalanine 4-nitrobenzyl ester (D-1) ($R=PhCH_2$) instead of N-(benzyloxycarbonyl)-L-phenylalanine 4-nitrobenzyl ester (L-1) ($R=PhCH_2$) to determine the initial rate. Background hydrolytic reaction rate was also determined based on the amount of 4-nitrobenzyl alcohol produced by spontaneous degradation in the reaction solution free from antibody 7G12.

In the above-experiment where antibody 7G12 was used, background hydrolytic reaction rate was subtracted from the initial hydrolytic reaction rate for L- and D-forms of the substrate ($R=PhCH_2$) to determine the net initial hydrolytic reaction rate of antibody 7G12. The antibody 7G12 did not catalyze the hydrolysis of

D-form of substrate (D-1) ($R = \text{PhCH}_2$). Thus, the antibody 7G12 enantioselectively catalyzed the hydrolysis of the substrate.

The specificity to substrate and enantioselectivity of the antibody 7G12 were extensively studied, according to the same procedure as mentioned above, using other substrates derived from alanine, leucine, norleucine, methionine, valine, phenylglycine, 4-hydroxyphenylglycine and lysine, synthesized in Preparation 3, and it was found that the antibody 7G12 enantioselectively catalyzed the hydrolysis of L-form of the substrates. The following Table 4 shows the initial hydrolytic rate as well as background reaction rate constants for L-form of the substrates.

Table 4

Hydrolysis of L-amino acid ester derivatives by antibody 7G12

Substrate L-1 (R-)	Background reaction rate constants k_{uncat} (min^{-1})	Concentration for reaction		Initial rate V ($\mu\text{M}/\text{min.}$)
		Antibody 7G12 (μM)	L-1 (μM)	
CH_3-	1.06×10^{-4}	10	200	3.49×10^{-1}
$(\text{CH}_3)_2\text{CHCH}_2-$	1.26×10^{-5}	10	200	6.20×10^{-1}
$\text{CH}_3(\text{CH}_2)_3-$	1.71×10^{-5}	5	100	3.74×10^{-2}
$\text{CH}_3\text{SCH}_2\text{CH}_2-$	3.83×10^{-5}	10	200	7.73×10^{-1}
PhCH_2-	2.24×10^{-5}	10	200	3.85×10^{-1}
$(\text{CH}_3)_2\text{CH}-$	6.47×10^{-6}	5	100	5.54×10^{-3}
$\text{Ph}-$	5.62×10^{-3}	5	100	7.83×10^{-1}
4-HOPh-	1.84×10^{-4}	5	200	$3.33 \times 10^{-1a)}$
$\text{H}_2\text{N}(\text{CH}_2)_4-$	1.40×10^{-4}	5	100	4.36×10^{-1}

a) Reacted with DL-1 as a substrate.

2. Determination of specificity to substrate and enantioselectivity of monoclonal antibody 3G2 by HPLC

Specificity to substrate and enantioselectivity of antibody 3G2 were determined according to the same procedure as that used for antibody 7G12, and it was found that said antibody enantioselectively catalyzed the hydrolysis of D-form of substrates derived from alanine, phenylalanine, leucine, norleucine, methionine, valine, phenylglycine, and 4-hydroxyphenylglycine, synthesized in Preparation 3. The following Table 5 shows the initial hydrolytic rate for D-form of the substrates. The background reaction rate constants are the same as those shown in Table 4.

Table 5

Hydrolysis of D-amino acid ester derivatives (D-1) by antibody 3G2.

Substrate D-1 (R-)	Concentration for reaction		Initial rate V ($\mu\text{M}/\text{min.}$)
	Antibody 3G2 (μM)	D-1 (μM)	
$\text{CH}_3\text{-}$	5	100	9.31×10^{-2}
$(\text{CH}_3)_2\text{CHCH}_2\text{-}$	10	100	1.16×10^{-1}
$\text{CH}_3(\text{CH}_2)_3\text{-}$	10	100	9.12×10^{-2}
$\text{CH}_3\text{SCH}_2\text{CH}_2\text{-}$	10	100	2.15×10^{-1}
$\text{PhCH}_2\text{-}$	5	50	1.54×10^{-2}
$(\text{CH}_3)_2\text{CH-}$	10	100	1.96×10^{-3}
Ph-	5	100	2.91×10^{-2}
4-HOPh-	10	100	4.08×10^{-1}

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT:

(A) NAME: Protein Engineering Research Institute

(B) STREET: 2-3, Furuedai 6-chome

(C) CITY: Suita-shi

(D) STATE: Osaka

(E) COUNTRY: Japan

(ii) TITLE OF INVENTION: CATALYTIC ANTIBODIES

ENANTIOSELECTIVELY HYDROLYSING AMINO ACID ESTER DERIVATIVES

(iii) NUMBER OF SEQUENCE: 8

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER:

(vi) PRIORY APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 640

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AGGACGTCAG ACTGGAGATG TGAGACTCGT CGAGTCACTG ACAGGGGAGG TCGTGGACCG GGTCGCTCTG

570 580 590 600 610 620 630
CGTCACCTGC AACGTTGCC ACCCGGCCAG CAGCACCAAG GTGGACAAGA AAATTGTGCC CAGGGATTGT
GCAGTGGACG TTGCAACGGG TGGGCCGGTC GTCGTGGTTC CACCTGTTCT TTTAACACGG GTCCCTAACA

640
25 ACTAGT
TGATCA

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 212

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

40 Leu Glu Ser Gly Thr Glu Leu Ala Lys Pro Gly Ala Ser Val Lys MET Ser Cys

Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Ile His Trp Val Lys Gln Arg

45 Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Thr Asp Tyr Thr

Glu Tyr Ile Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser

50 Ser Thr Ala Tyr MET Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr

Tyr Cys Val MET Lys Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
5 Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln
Thr Asn Ser MET Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro
10 Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro
Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser
15 Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser
Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Thr Ser
20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 640
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

35	10	20	30	40	50	60	70
	GAGCTCGTGA	TGACCCAGAC	TCCAGCAATC	ATGTCTGCAT	CTCCAGGGGA	GAAGGTCACC	ATGACCTGCA
	CTCGAGCACT	ACTGGGTCTG	AGGTCGTTAG	TACAGACGTA	GAGGTCCCCT	CTTCCAGTGG	TACTGGACGT
40	80	90	100	110	120	130	140
	GTGCCAGCTC	AAGTATAAGT	TACATGCACT	GGTACCAGCA	GAAGCCAGGC	ACCCCCCCCA	AAAGATGGAT
	CACGGTCGAG	TTCATATTCA	ATGTACGTGA	CCATGGTCTG	CTTCGGTCCG	TGGGGGGGGT	TTTCTACCTA
45	150	160	170	180	190	200	210
	TTATGGCACA	TCCAAACTGA	CTTCTGGAGT	CCCTGCTCGC	TTCAGTGGCA	GTGGGTCTGG	GACCTCTTTT
	AATACCGTGT	AGGTTTGACT	GAAGACCTCA	GGGACGAGCG	AAGTCACCGT	CACCCAGACC	CTGGAGAAAA
50	220	230	240	250	260	270	280
	TCTCTCACAA	TCAGCAGCAT	GGAGGCTGAA	GATGCTGCCA	CTTATTACTG	CCATCAGCGG	AGTAGTTACC
	AGAGAGTGTT	AGTCGTCGTA	CCTCCGACTT	CTACGACGGT	GAATAATGAC	GGTAGTCGCC	TCATCAATGG
	290	300	310	320	330	340	350
	CGACGTTTCG	TGGAGGCACC	AAGCTGGAAA	TCAAACGGGC	TGATGCTGCA	CCAAGTGTAT	CCATCTTCCC
	GCTGCAAGCC	ACCTCCGTGG	TTCGACCTTT	AGTTTGCCCC	ACTACGACGT	GGTTGACATA	GGTAGAAGGG

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35

5 360 370 380 390 400 410 420
ACCATCCAGT GAGCAGTTAA CATCTGGAGG TGCCTCAGTC GTGTGCTTCT TGAACAACTT CTACCCCAAA
TGGTAGGTCA CTCGTCAATT GTAGACCTCC ACGGAGTCAG CACACGAAGA ACTTGTTGAA GATGGGGTTT

5 430 440 450 460 470 480 490
GACATCAATG TCAAGTGGAA GATTGATGGC AGTGAACGAC AAAATGGCGT CCTGAACAGT TGGACTGATC
CTGTAGTTAC AGTTCACCTT CTAACCTACCG TCACTTGCTG TTTTACCGCA GGACTTGTCA ACCTGACTAG

10 500 510 520 530 540 550 560
AGGACAGCAA AGACAGCACC TACAGCATGA GCAGCACCCCT CACGTTGACC AAGGACGAGT ATGAACGACA
TCCTGTCGTT TCTGTCGTGG ATGTCGTACT CGTCGTGGGA GTGCAACTGG TTCCTGCTCA TACTTGCTGT

15 570 580 590 600 610 620 630
TAACAGCTAT ACCTGTGAGG CCACTCACAA GACATCAACT TCACCCATTG TCAAGAGCTT CAACAGGAAT
ATTGTCGATA TGGACACTCC GGTGAGTGTT CTGTAGTTGA AGTGGGTAAC AGTTCTCGAA TGTCTTACT

20 640 650
GAGTGTTAAT TCTAGA
GTCACAATTA AGATCT

(2) INFORMATION FOR SEQ ID NO:4:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 213
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

35 Glu Leu Val MET Thr Gln Thr Pro Ala Ile MET Ser Ala Ser Pro Gly Glu Lys
Val Thr MET Thr Cys Ser Ala Ser Ser Ser Ile Ser Tyr MET His Trp Tyr Gln
40 Gln Lys Pro Gly Thr Pro Pro Lys Arg Trp Ile Tyr Gly Thr Ser Lys Leu Thr
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Phe Ser Leu
45 Thr Ile Ser Ser MET Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Arg
Ser Ser Tyr Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp
50 Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly

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Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val

5

Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr

Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser MET Ser Ser Thr Leu Thr Leu Thr

10

Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr

Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys . Phe .

15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

20

(A) LENGTH: 656

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

30

10	20	30	40	50	60	70
CTCGAGTCTG	GACCTGAGCT	GGTGAAGCCT	GGGGGCTCAG	TGACGATTTC	CTGCAAAGCT	TCTGGCTACG
GAGCTCAGAC	CTGGACTCGA	CCACTTCGGA	CCCCCGAGTC	ACTGCTAAAG	GACGTTTCGA	AGACCGATGC

35

80	90	100	110	120	130	140
GATTCACTAC	CTCTTGGATG	AACTGGGTGA	GGCAGAGGCC	TGGACAGGGT	CTTGAGTGGA	TTGGACGGAT
CTAAGTGATG	GAGAACCTAC	TTGACCCACT	CCGTCTCCGG	ACCTGTCCCA	GAATCACCT	AACCTGCCTA

40

150	160	170	180	190	200	210
TTATCCTGGA	AGTGGGGATA	ATAATTACAA	TGGGAAGTTC	AAGGTCAAGG	CCACATTGAC	TGCAGAGAGA
AATAGGACCT	TCACCCCTAT	TATTAATGTT	ACCCTTCAAG	TTCCAGTTCC	GGTGTAAGTG	ACGTCTCTCT

45

220	230	240	250	260	270	280
TCCTCCACCA	CAGTCTACCT	GCACCTCAGC	AGCCTGACCT	CTGTAGATTTC	TGCGGTCTAT	TTCTGTGCAA
AGGAGGTGGT	GTCAGATGGA	CGTGGAGTCG	TCGGACTGGA	GACATCTAAG	ACGCCAGATA	AAGACACGTT

50

290	300	310	320	330	340	350
GATTTCACTA	TGATTATCGT	CGTTCCTATG	CTATGGACTA	CTGGGGTCAA	GGAACCTTCAG	TCACCGTCTC
CTAAAGTGAT	ACTAATAGCA	GCAAGGATAC	GATACCTGAT	GACCCCAAGTT	CCTTGAAGTC	AGTGGCAGAG

360	370	380	390	400	410	420
CTCAGCCAAA	ACGACACCCC	CATCTGTCTA	TCCACTGGCC	CCTGGATCTG	CTGCCCAAAC	TAACCTCCATG
GAGTCGGTTT	TGCTGTGGGG	GTAGACAGAT	AGGTGACCGG	GGACCTAGAC	GACGGGTTTG	ATTGAGGTAC

430	440	450	460	470	480	490
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GTGACCCTGG GATGCCTGGT CAAGGGCTAT TTCCCTGAGC CAGTGACAGT GACCTGGAAC TCTGGATCCC
 CACTGGGACC CTACGGACCA GTTCCCGATA AAGGGACTCG GTCACTGTCA CTGGACCTTG AGACCTAGGG

5 500 510 520 530 540 550 560
 TGTCCAGCGG TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACTCTGAGCA GCTCAGTGAC
 ACAGGTCGCC ACACGTGTGG AAGGGTCGAC AGGACGTCAG ACTGGAGATG TGAGACTCGT CGAGTCACTG

10 570 580 590 600 610 620 630
 TGTCCCCTCC AGCACCTGGC CCAGCGAGAC CGTCACCTGC AACGTTGCCC ACCCGGCCAG CAGCACCAAG
 ACAGGGGAGG TCGTGGACCG GGTCGCTCTG GCAGTGGACG TTGCAACGGG TGGGCCGGTC GTCGTGGTTC

15 640 650 660
 GTGGACAAGA AAATTGTGCC CAGGGATTGT ACTAGT
 CACCTGTTCT TTTAACACGG GTCCCTAACA TGATCA

(2) INFORMATION FOR SEQ ID NO:6:

20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 210
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

30 Leu Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Gly Ser Val Thr Ile Ser Cys Lys
 Ala Ser Gly Tyr Gly Phe Thr Thr Ser Trp MET Asn Trp Val Arg Gln Arg Pro Gly
 35 Gln Gly Leu Glu Trp Ile Gly Arg Ile Tyr Pro Gly Ser Gly Asp Asn Asn Tyr Asn
 Gly Lys Phe Lys Val Lys Ala Thr Leu Thr Ala Glu Arg Ser Ser Thr Thr Val Tyr
 40 Leu His Leu Ser Ser Leu Thr Ser Val Asp Ser Ala Val Tyr Phe Cys Ala Arg Phe
 His Tyr Asp Tyr Arg Arg Ser Tyr Ala MET Asp Tyr Trp Gly Gln Gly Thr Ser Val
 45 Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser
 50 Ala Ala Gln Thr Asn Ser MET Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro
 Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe

Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser
Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr
Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Thr Ser

10 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 652

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

	10	20	30	40	50	60	70
	GAGCTCGTGA	TGACCCAGAC	TCCATCTTCC	ATGTATGCAT	CTCTAGGAGA	GAGAGTCACT	ATCACTTGCA
25	CTCGAGCACT	ACTGGGTCTG	AGGTAGAAGG	TACATACGTA	GAGATCCTCT	CTCTCAGTGA	TAGTGAACGT
	80	90	100	110	120	130	140
	AGGCGAGTCA	GGACATTAAT	ATCTATTTAA	GTTGGTTCCA	GCAGAAACCA	GGGAAATCTC	CTAAGGCCCT
	TCCGCTCAGT	CCTGTAATTA	TAGATAAATT	CAACCAAGGT	CGTCTTTGGT	CCCTTTAGAG	GATTCCGGGA
30	150	160	170	180	190	200	210
	GATCTATCGT	ACAAACGGAT	TGGTAGATGG	GGTCCCATCA	AGGTTTCAGTG	GCAGTGGATC	TGGGCAAGAT
	CTAGATAGCA	TGTTTGCCTA	ACCATCTACC	CCAGGGTAGT	TCCAAGTCAC	CGTCACCTAG	ACCCGTTCTA
35	220	230	240	250	260	270	280
	TATTCTCTCA	CCATCAGCAG	CCTGGAATAT	GAAGATATGG	GAATTTATTA	TTGTCTACAG	TATGATGAGT
	ATAAGAGAGT	GGTAGTCGTC	GGACCTTATA	CTTCTATACC	CTTAAATAAT	AACAGATGTC	ATACTACTCA
40	290	300	310	320	330	340	350
	TTCCGTACAC	GTTTCGGAGGG	GGGACCAAGC	TGGAAATAAA	ACGGGCTGAT	GCTGCACCAA	CTGTATCCAT
	AAGGCATGTG	CAAGCCTCCC	CCCTGGTTTCG	ACCTTTATTT	TGCCCCGACTA	CGACGTGGTT	GACATAGGTA
45	360	370	380	390	400	410	420
	CTTCCCACCA	TCCAGTGAGC	AGTTAACATC	TGGAGGTGCC	TCAGTCGTGT	GCTTCTTGAA	CAACTTCTAC
	GAAGGGTGGT	AGGTCACCTCG	TCAATTGTAG	ACCTCCACGG	AGTCAGCACA	CGAAGAACTT	GTTGAAGATG
50	430	440	450	460	470	480	490
	CCCAAAGACA	TCAATGTCAA	GTGGAAGATT	GATGGCAGTG	AACGACAAAA	TGGCGTCCTG	AACAGTTGGA
	GGGTTTCTGT	AGTTACAGTT	CACCTTCTAA	CTACCGTCAC	TTGCTGTTTT	ACCGCAGGAC	TTGTCAACCT
	500	510	520	530	540	550	560
	CTGATCAGGA	CAGCAAAGAC	AGCACCTACA	GCATGAGCAG	CACCCTCACG	TTGACCAAGG	ACGAGTATGA
	GACTAGTCCT	GTCGTTTCTG	TCGTGGATGT	CGTACTCGTC	GTGGGAGTGC	AACTGGTTCC	TGCTCATACT

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570 580 590 600 610 620 630
ACGACATAAC AGCTATACCT GTGAGGCCAC TCACAAGACA TCAACTTCAC CCATTGTCAA GAGCTTCAAC
TGCTGTATTG TCGATATGGA CACTCCGGTG AGTGTCTCTGT AGTTGAAGTG GGTAACAGTT CTCGAAGTTG

5

640 650
AGGAATGAGT GTTAATTCTA GA
TCCTTACTCA CAATTAAGAT CT

10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 204

(B) TYPE: amino acid

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

20

Glu Leu Val MET Thr Gln Thr Pro Ser Ser MET Tyr Ala Ser Leu Gly Glu Arg Val

25

Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Ile Tyr Leu Ser Trp Phe Gln Gln

Lys Pro Gly Lys Ser Pro Lys Ala Leu Ile Tyr Arg Thr Asn Gly Leu Val Asp Gly

30

Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser

Ser Leu Glu Tyr Glu Asp MET Gly Ile Tyr Tyr Cys Leu Gln Tyr Asp Glu Phe Pro

35

Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr

40

Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser Val Val

Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly

45

Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser

Thr Tyr Ser MET Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn

50

Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser Phe

Asn Arg Asn Glu Cys . Phe .

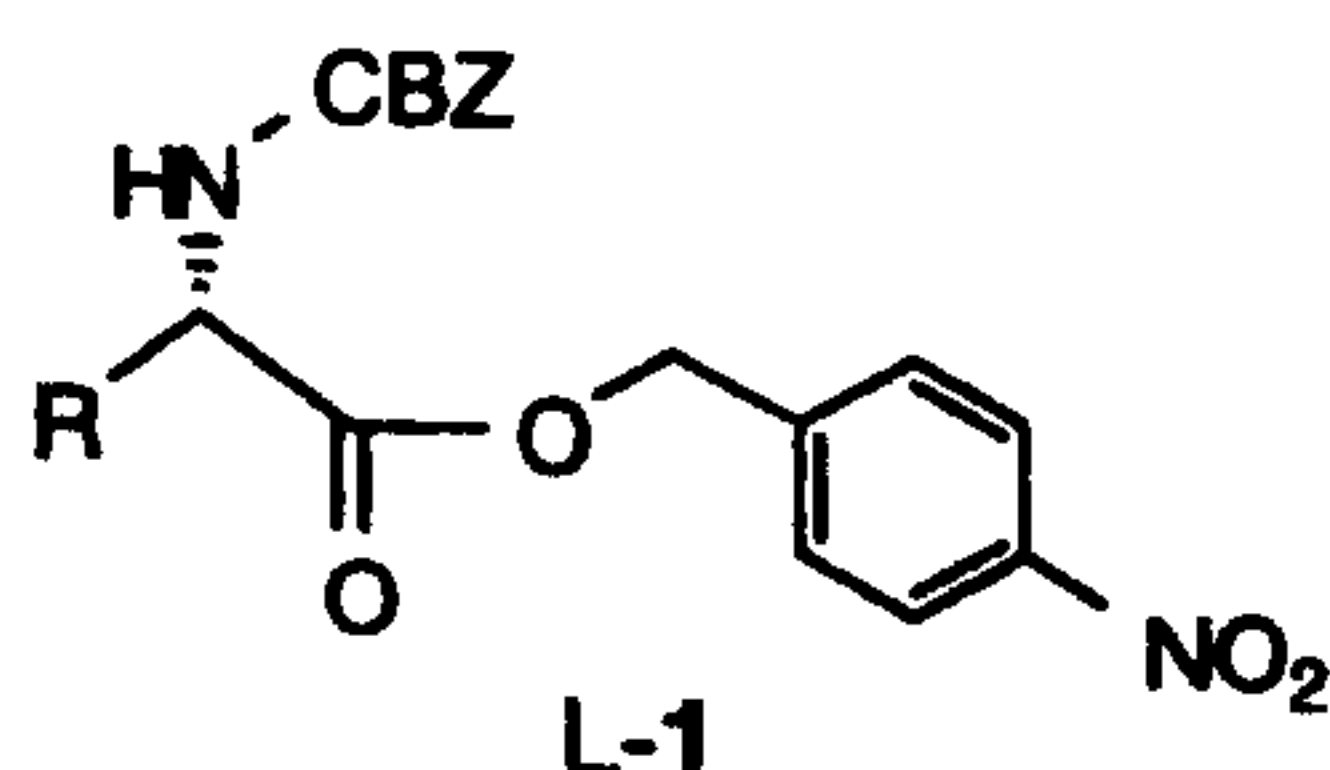
CLAIMS

1. A process for optically resolving a racemic mixture of amino acid derivatives, comprising using a nonspecific catalytic antibody which enantioselectively hydrolyzes various amino acid ester derivatives.

5 2. The process of Claim 1 wherein the amino acid ester derivative is a 4-nitrobenzyl ester.

3. The process of Claim 1 or 2 wherein the amino acid ester derivative is amino-protected.

10 4. The process of Claim 1, 2 or 3 wherein the amino acid ester derivative has the formula (L-1):

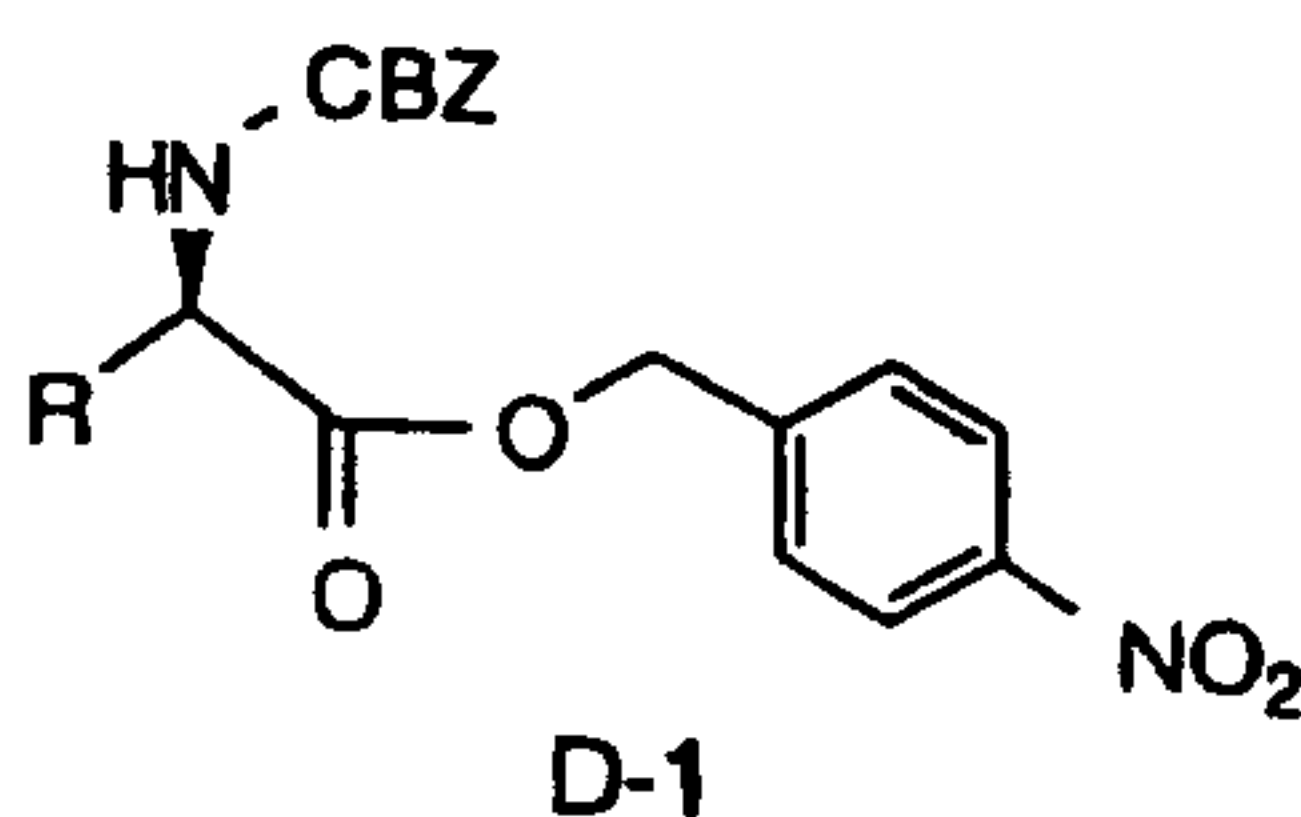


wherein CBZ is N-benzyloxycarbonyl and

15 R is an alkyl or phenyl group which is unsubstituted or substituted with hydroxy, amino, alkylthio, acyloxy, or phenyl.

5. The process of Claim 4 wherein the R of the formula (L-1) is CH_3 -, phCH_2 -, $(\text{CH}_3)_2\text{CHCH}_2$ -, $\text{CH}_3(\text{CH}_2)_3$ -, $\text{CH}_3\text{SCH}_2\text{CH}_2$ -, $(\text{CH}_3)_2\text{CH}$ -, ph -, or 4-HOph-.

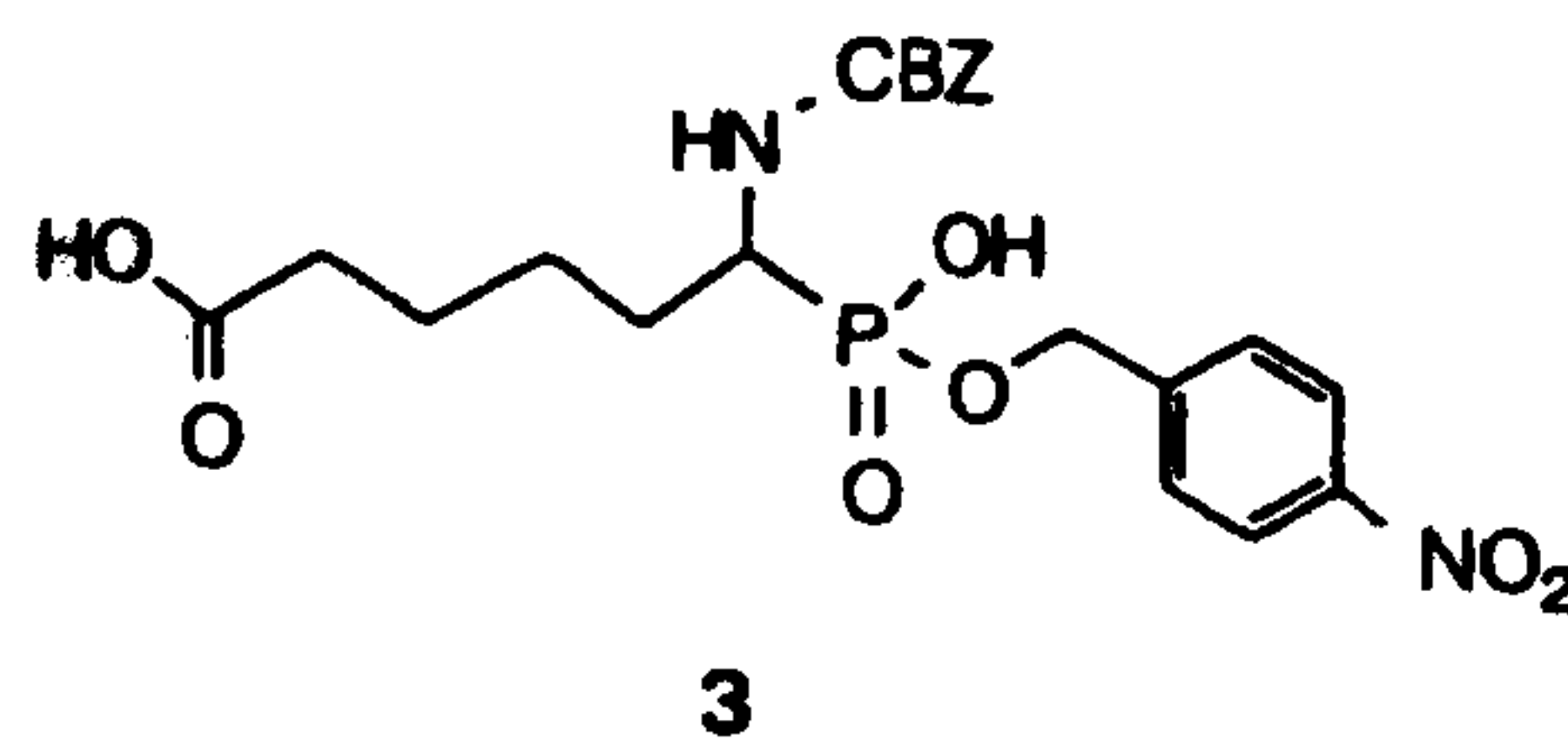
20 6. The process of Claim 1, 2 or 3 wherein the amino acid ester derivative has the formula (D-1):



wherein CBZ is N-benzyloxycarbonyl and R is an alkyl or phenyl group which is optionally substituted with hydroxy, amino, alkylthio, acyloxy, or phenyl.

7. The process of Claim 6 wherein the R of the formula (D-1): is CH_3 -, phCH_2 -, $(\text{CH}_3)_2\text{CHCH}_2$ -, $\text{CH}_3(\text{CH}_2)_3$ -, $\text{CH}_3\text{SCH}_2\text{CH}_2$ -, $(\text{CH}_3)_2\text{CH}$ -, ph -, or 4-HOph-.

8. The process of Claims 1 or 2 wherein the catalytic antibody is produced by stimulation with an antigen comprising a compound of the formula (3):



wherein CBZ is N-benzyloxycarbonyl.

9. The process of Claim 8 wherein the catalytic antibody is produced by hybridoma ZAA 7G12.

10. The process of Claim 8 wherein the catalytic antibody is produced by hybridoma ZAA 3G2.

11. A process for preparing an optically active amino acid comprising the steps of:

- a) subjecting a racemic mixture of amino acid derivatives to an ester forming reaction to form amino acid ester derivatives;
- b) selectively hydrolyzing a desired stereoisomer, D- or L-isomer, of the amino acid ester derivatives with a nonspecific catalytic antibody which enantioselectively hydrolyzes various amino acid ester derivatives; and

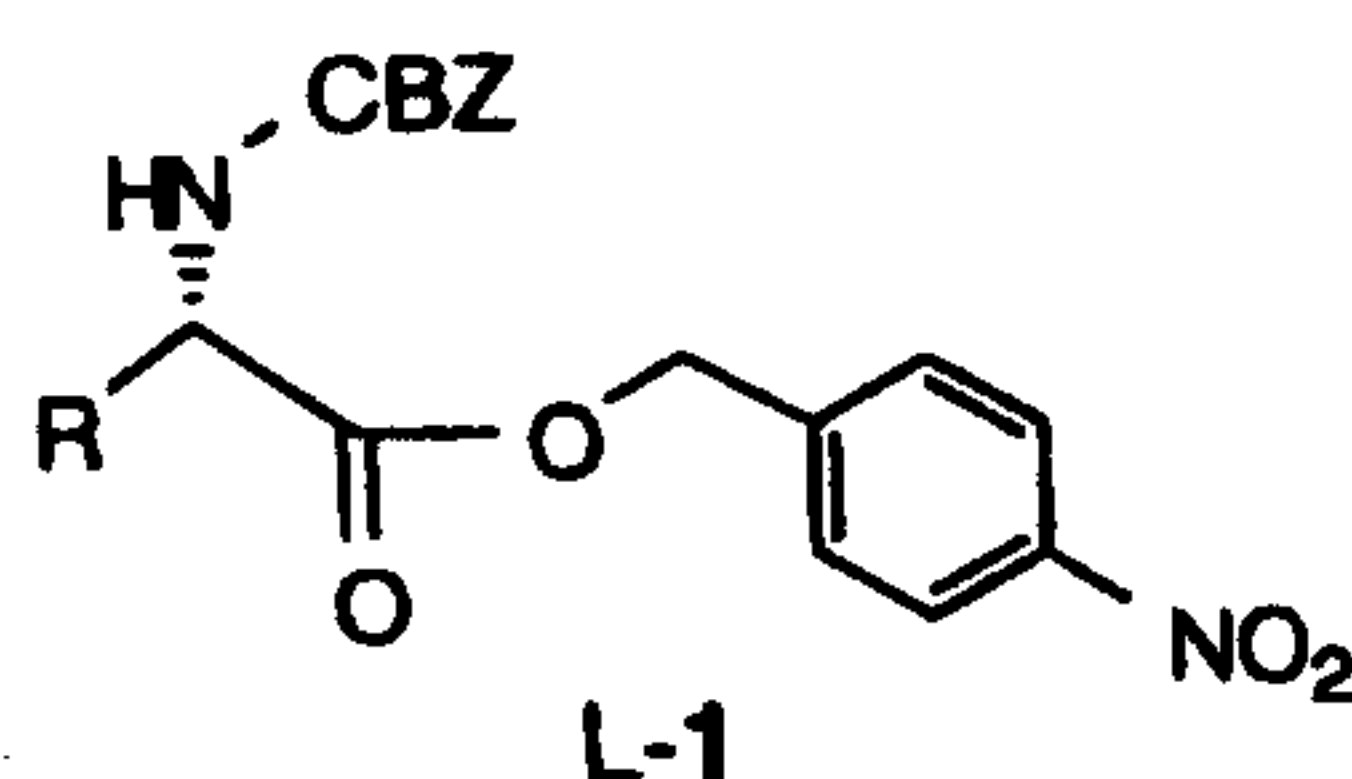
c) treating the resulting hydrolyzed mixture to isolate the desired hydrolyzed stereoisomer of the amino acid ester derivatives thereby preparing the optically active amino acid.

12. The process of Claim 11 wherein the resulting hydrolyzed mixture is treated by being made acidic or basic, followed by extracting with an organic solvent to obtain an aqueous layer from which the desired hydrolyzed stereoisomer is isolated.

13. The process of Claim 11 or 12 wherein the amino acid ester derivative is 4-nitrobenzyl ester.

14. The process of Claim 11 or 12 wherein the amino acid ester derivative is amino-protected.

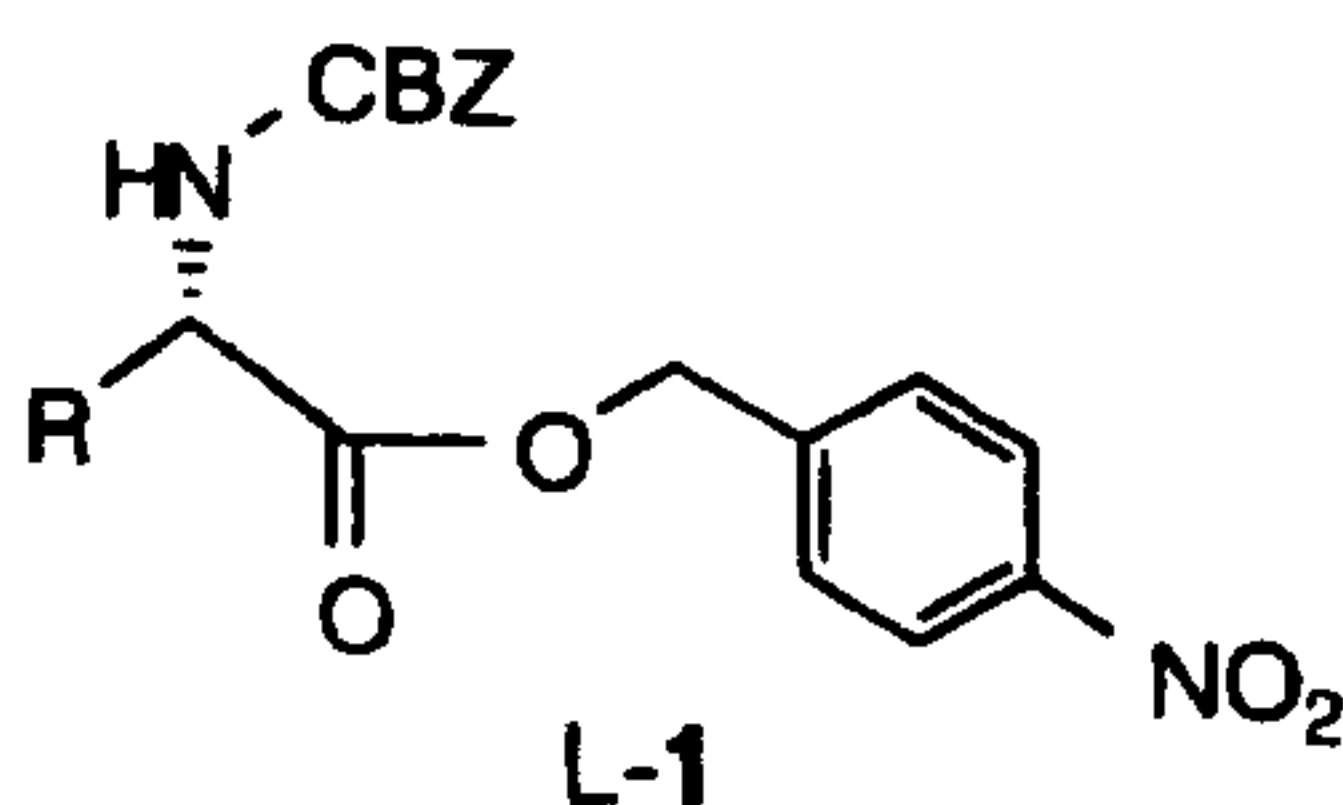
15. The process of Claim 11, 12, 13 or 14 wherein the amino acid ester derivative is a compound having the formula (L-1):



wherein CBZ is N-benzyloxycarbonyl and

R is an alkyl or phenyl group which is unsubstituted or substituted with hydroxy, amino, alkylthio, acyloxy, or phenyl.

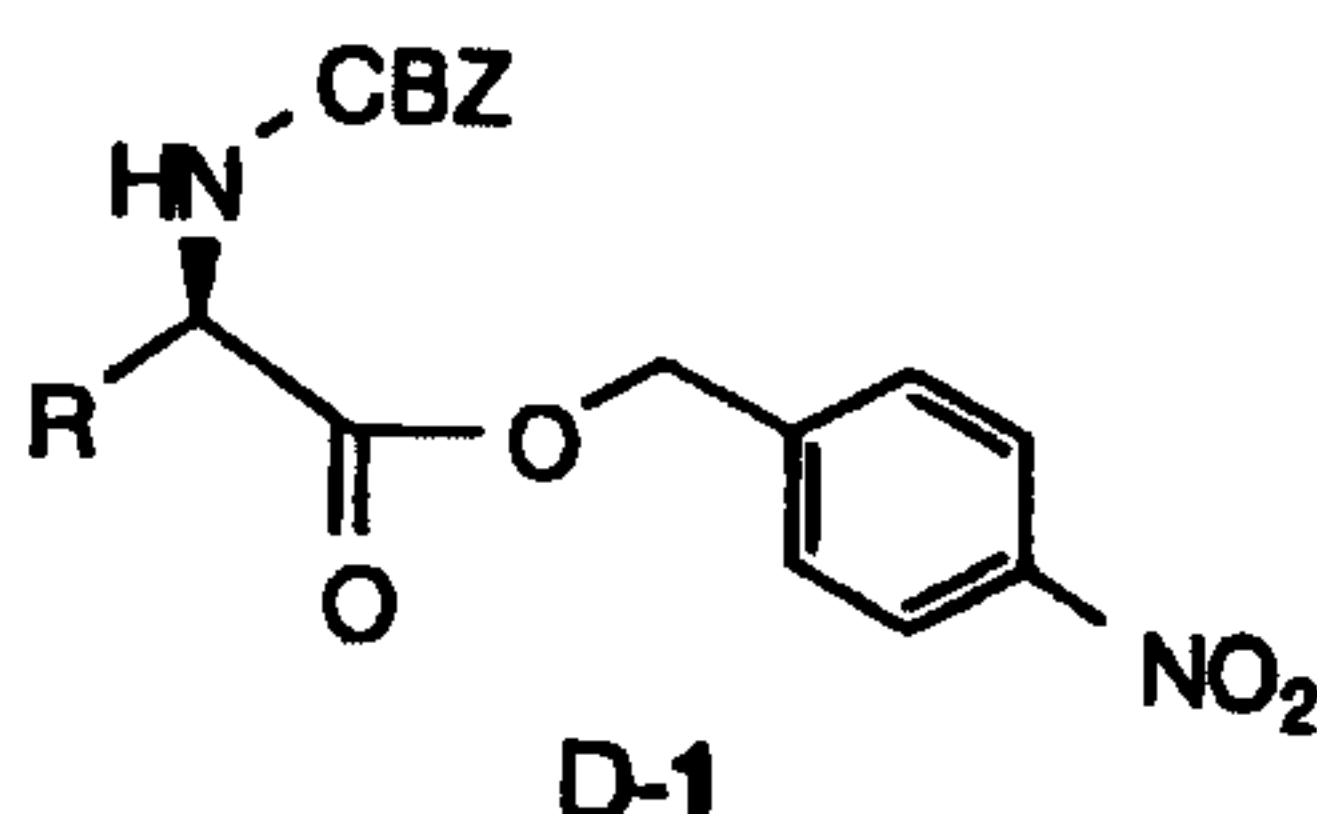
16. The process of Claim 15 wherein the amino acid ester derivative has the formula (L-1):



wherein CBZ is N-benzyloxycarbonyl and

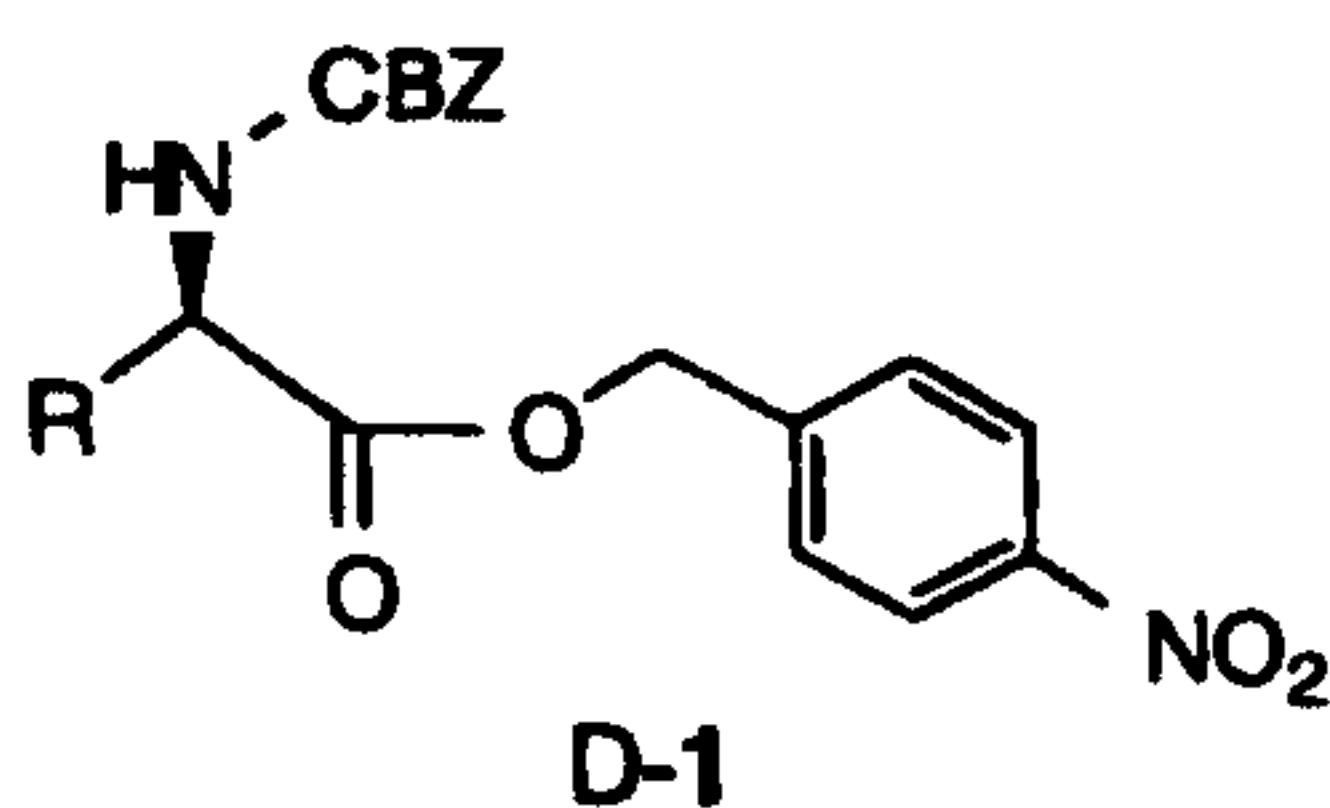
R is CH_3 -, phCH_2 -, $(\text{CH}_3)_2\text{CHCH}_2$ -, $\text{CH}_3(\text{CH}_2)_3$ -, $\text{CH}_3\text{SCH}_2\text{CH}_2$ -, $(\text{CH}_3)_2\text{CH}$ -, ph -, or 4-HOph-.

17. The process of Claim 11, 12, 13 or 14 wherein the amino acid ester derivative has the formula (D-1):



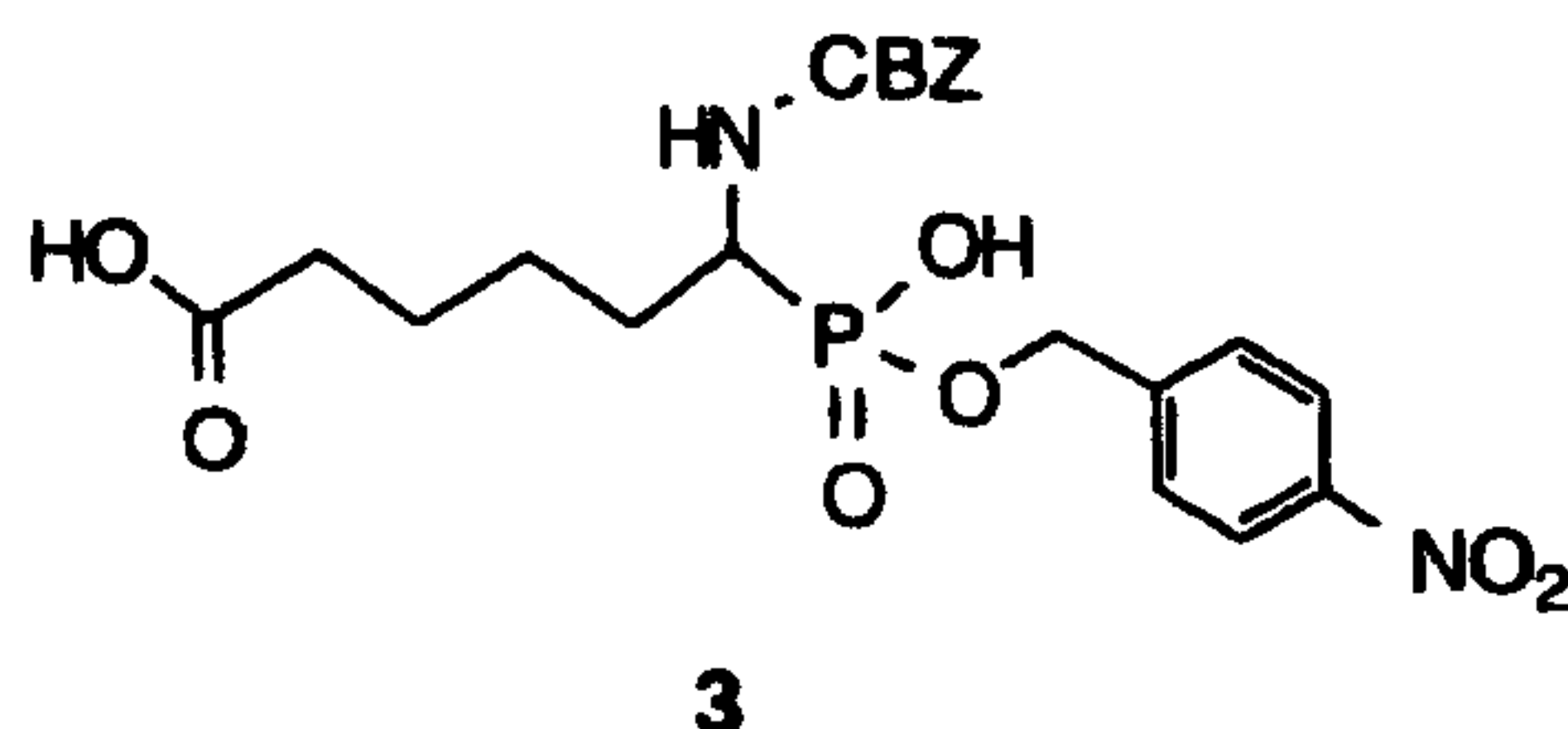
wherein CBZ is N-benzyloxycarbonyl and R is an alkyl or phenyl group which is optionally substituted with hydroxy, amino, alkylthio, acyloxy, or phenyl.

18. The process of Claim 17 wherein the amino acid ester derivative has the formula (D-1):



wherein CBZ is N-benzyloxycarbonyl and R is CH_3 -, phCH_2 -, $(\text{CH}_3)_2\text{CHCH}_2$ -, $\text{CH}_3(\text{CH}_2)_3$ -, $\text{CH}_3\text{SCH}_2\text{CH}_2$ -, $(\text{CH}_3)_2\text{CH}$ -, ph -, or 4-HOph-.

19. The process of Claims 11 or 13 wherein the catalytic antibody is produced by stimulation with an antigen comprising a compound of the formula:

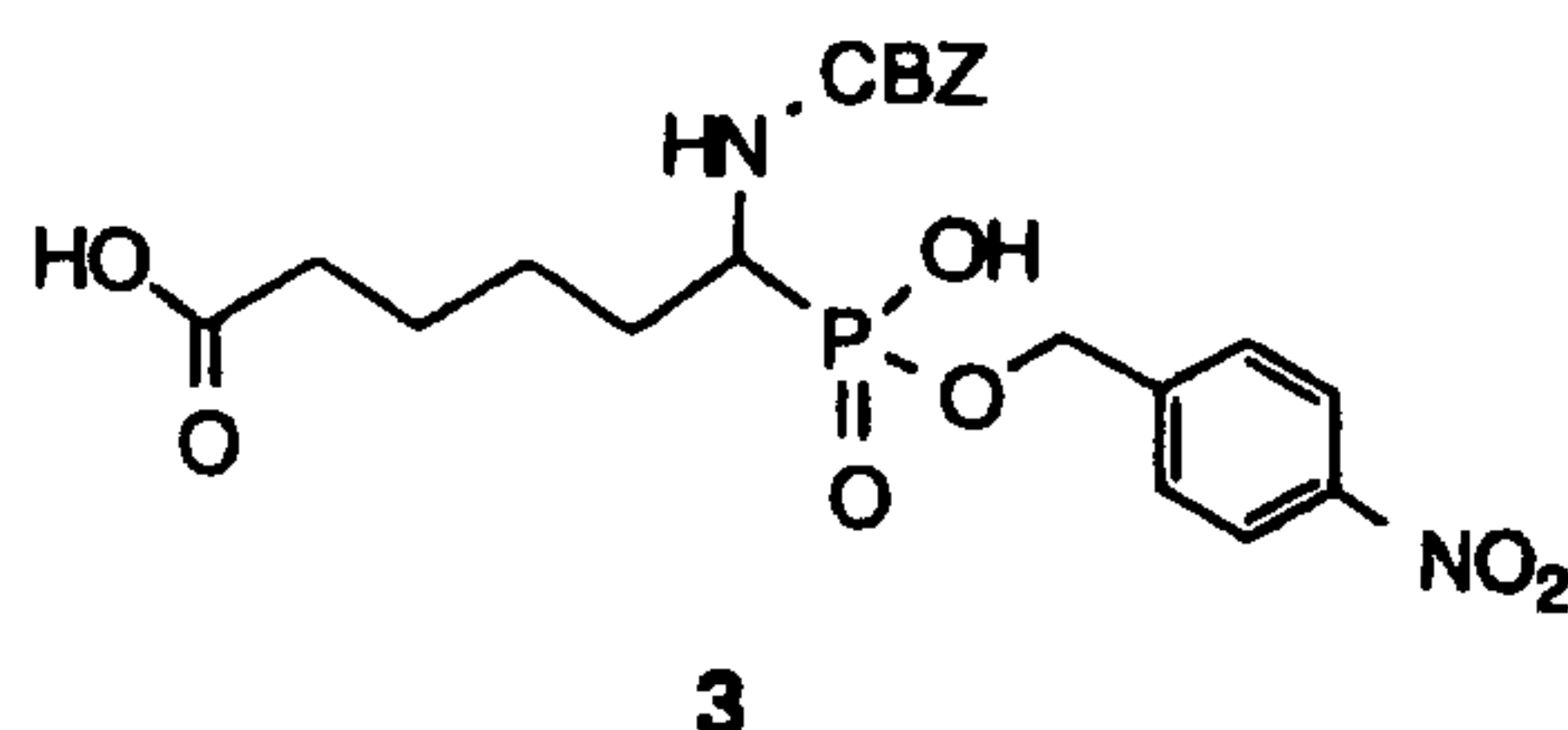


wherein CBZ is N-benzyloxycarbonyl.

20. The process of Claim 19 wherein the catalytic antibody is produced by hybridoma ZAA 7G12.

21. The process of Claim 19 wherein the catalytic antibody is produced by hybridoma ZAA 3G2.

22. A catalytic antibody capable of enantioselectively hydrolyzing various amino acid ester derivatives, produced by stimulation with an antigen comprising a compound of the formula:



wherein CBZ is N-benzyloxycarbonyl.

23. The catalytic antibody of Claim 22 produced by hybridoma ZAA 7G12.

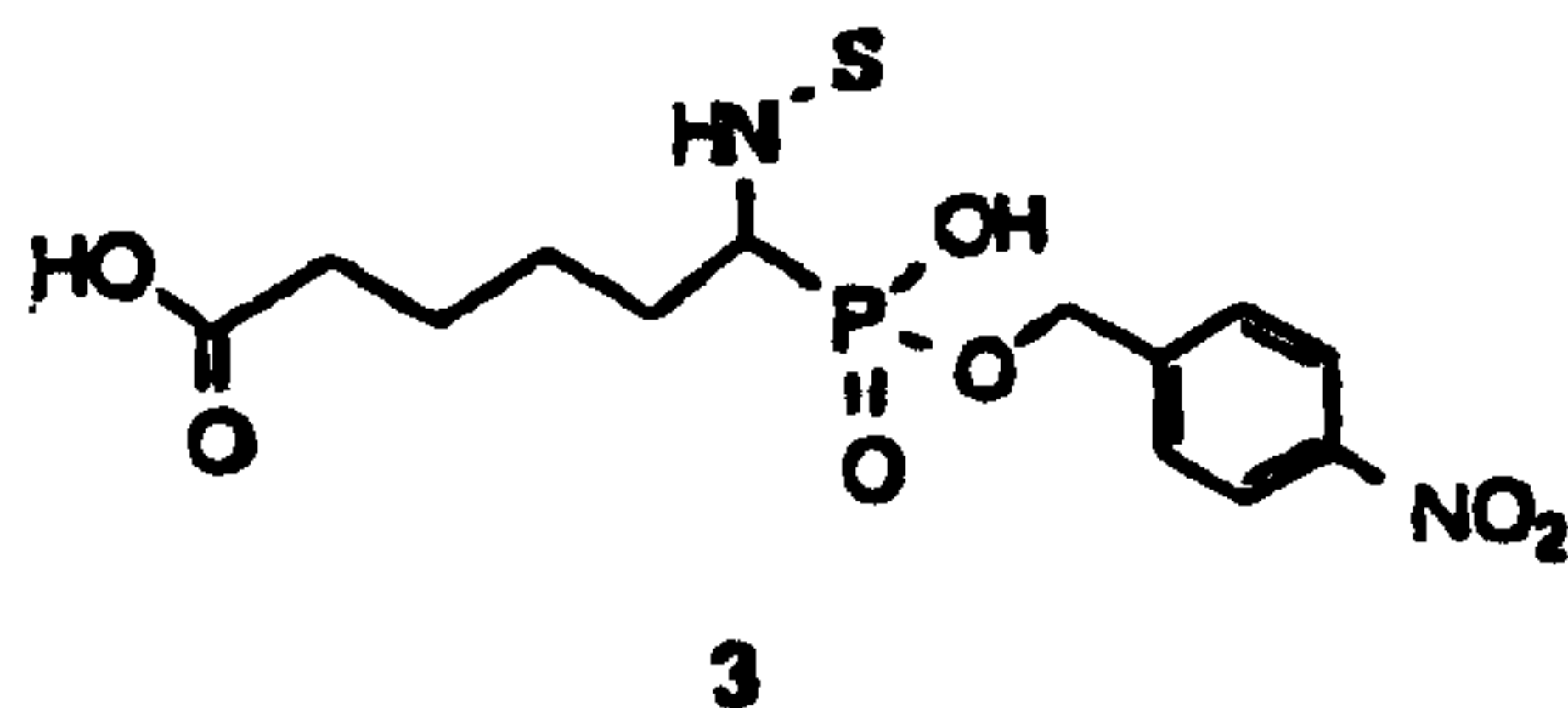
24. The catalytic antibody of Claim 22 produced by hybridoma ZAA 3G2.

25. A hybridoma producing the catalytic antibody of Claim 22.

26. The hybridoma of Claim 25 designated ZAA 7G12.

27. The hybridoma of Claim 25 designated ZAA 3G2.

28. A catalytic antibody produced by stimulation with an antigen comprising a compound of the formula:

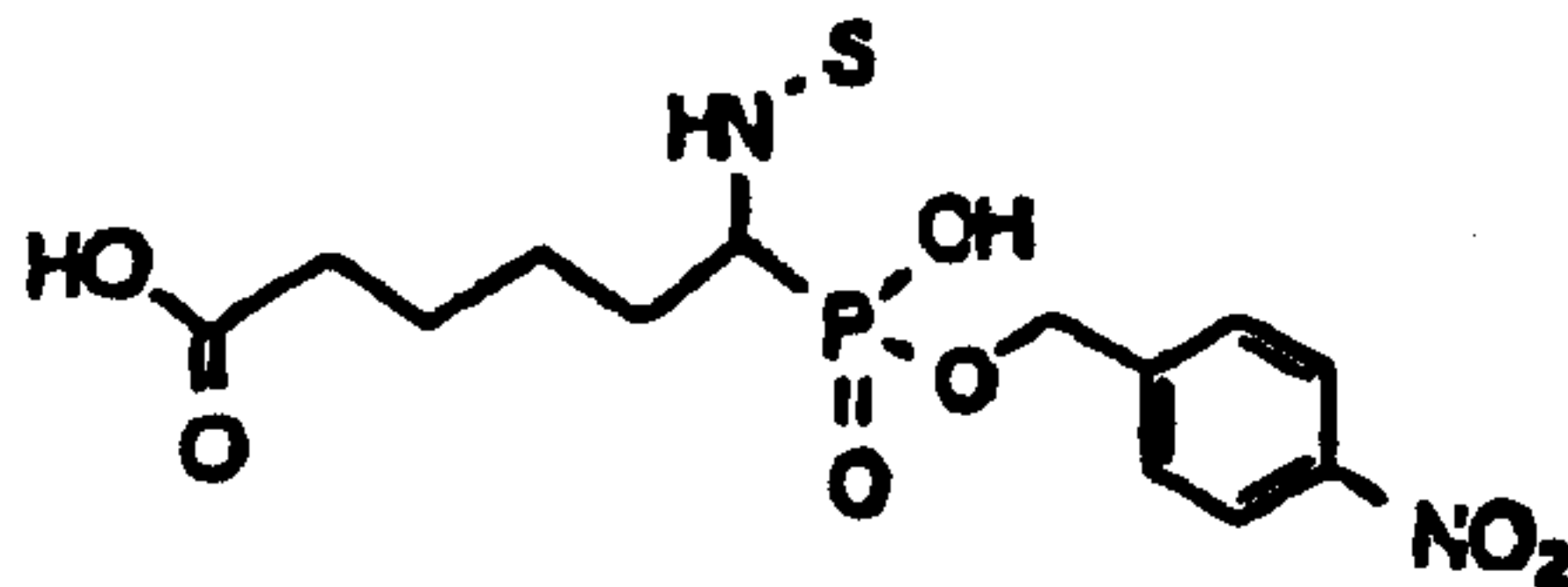


wherein S is an amino protecting group.

29. The catalytic antibody of Claim 28 wherein S is trichloroacetyl, benzyl, t-butoxycarbonyl, or 9-fluorenylmethoxycarbonyl.

30. A catalytic antibody produced by stimulation with an antigen comprising a compound of the formula:

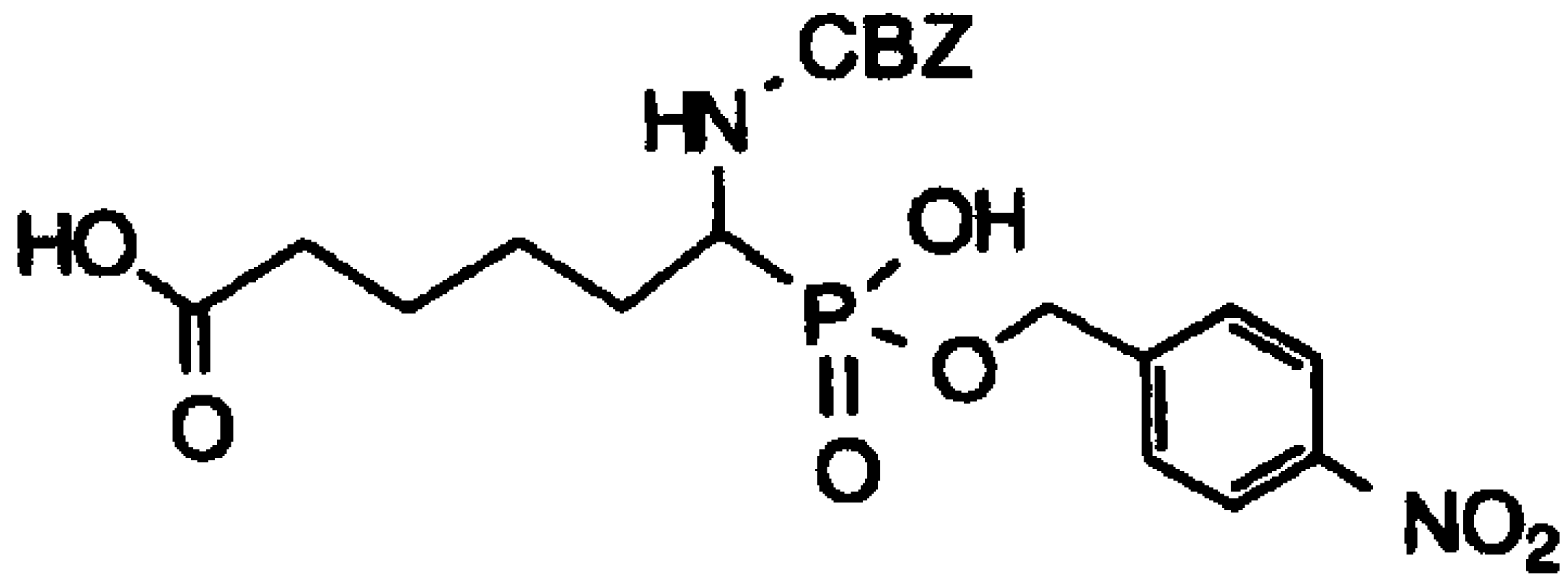
5



3

wherein S is a phenyl moiety which is substituted with lower alkyl, lower alkoxy, halogen or nitro.

10



3