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Chemoenzymatic Synthesis of Optically Active 1,4-Dihydropyridine Derivatives via Enantioselective Hydrolysis and Transesterification

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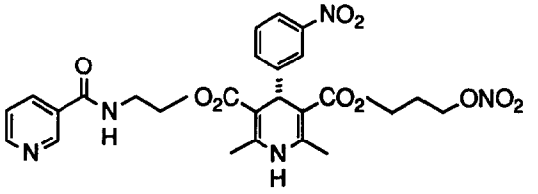
Abstract: (4*R*)-(-)-2-(Nicotinoylamino)ethyl 3-nitrooxypropyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate (**1**), a new calcium antagonist, was synthesized via both enantioselective hydrolysis and transesterification of prochiral bis[2-(nicotinoylamino)ethyl]ester •2HCl (**5**) by using enzymes. Hydrolysis of **5** by proteases originated from *Aspergillus melleus* and *Bacillus licheniformis* etc. in aqueous media afforded (4*R*)-(-)-3-[2-(nicotinoylamino)-ethoxycarbonyl]-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylic acid (**6**) in more than 99% e.e., which was converted to **1** by esterification with 3-nitrooxypropyl bromide. Enzymatic transesterification of **5** with 3-nitrooxypropanol gave **1** in more than 99.5% e.e. directly.

Since the discovery of nifedipine in 1971, many 1,4-dihydropyridine calcium antagonists have been developed and used widely for the treatment of hypertension, angina pectoris and peripheral and cerebral vascular diseases.[2] The compounds having different esters at the C-3 and C-5 in the 1,4-dihydropyridine ring possess a stereogenic carbon at the C-4, and in many cases the enantiomers have been revealed to have different pharmacological properties.[3]

(4*R*)-(-)-2-(Nicotinoylamino)ethyl 3-nitrooxypropyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate (**1**), a new calcium antagonist, has long lasting antihypertensive, selective coronary vasodilating, negative chronotropic and nitrate-like effects in anesthetized dogs, and has been under evaluation as a drug for hypertension and angina pectoris.[4] Compound **1** is more active than its enantiomer, (4*S*)-(+)-form. This paper describes a chemoenzymatic synthesis of **1** via both enantioselective hydrolysis and transesterification of a prochiral diester.



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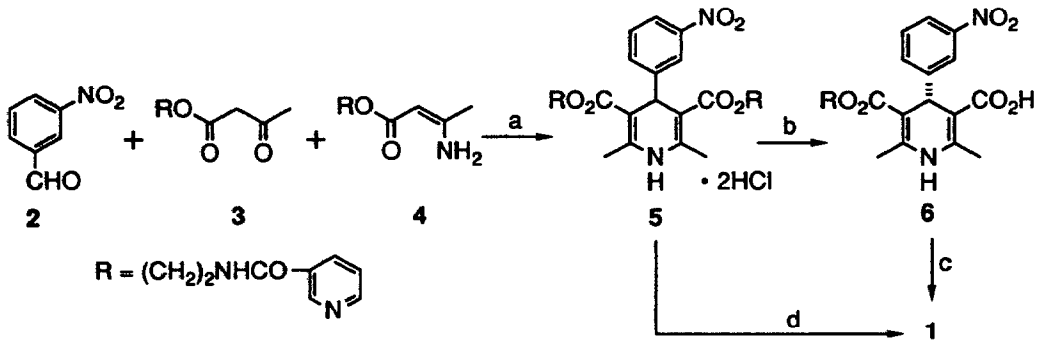
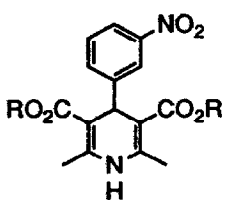
The prochiral bis[2-(nicotinoylamino)ethyl]ester•2HCl (**5**), prepared by way of a *Hantzsch* cyclization[5] from 3-nitrobenzaldehyde (**2**), 2-(nicotinoylamino)ethyl acetoacetate (**3**) and 2-(nicotinoylamino)ethyl 3-aminocrotonate (**4**) (Scheme), was tested for hydrolysis by using 84 commercially available hydrolytic enzymes including lipase, esterase and protease in aqueous media. Compound **5** was hydrolyzed using various kinds of enzymes originated from fungi and bacteria, and the results are shown in Table 1. Proteases originated from *Aspergillus melleus*, *Aspergillus sojae*, *Basillus licheniformis* and *Streptomyces griseus* showed hydrolysis activities. Acylase 30,000[6] and Deamizyme®[6] from *Aspergillus* sp. also showed these activities. Among them, Protease P6 and Seaprose S showed the highest hydrolytic activities.[7] Fortunately, the desired (4*R*)-(-)-3-[2-(nicotinoylamino)ethoxycarbonyl]-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylic acid (**6**) was obtained in an extremely high optical yield (>99% e.e.) by all the enzymes listed in Table 1. It is interesting to note that the monocarboxylic acid (**6**) was obtained in an excellent yield and further hydrolysis to the dicarboxylic acid (**7**) hardly occured. The prochiral bis(3-nitrooxypropyl)ester (**8**)[8], synthesized by a similar method to **5**, could not be hydrolyzed because of its insolubility in aqueous media.



Scheme Reagent : a) piperidinium acetate in IPA; b) Protease P6 in H_2O; c) 3-nitrooxypropyl bromide, K_2CO_3 in DMF; d) Protease P6, 3-nitrooxypropanol in H_2O.



7 R = H

8 R = $(CH_2)_3ONO_2$

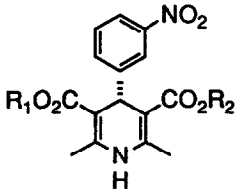
Protease P6 was chosen for preparative scale hydrolysis of **5**, and the reaction conditions were examined. Optimum pH and temperature were 7-8 and 30-35 °C, respectively. There were, however, the following problems; 1) The solubility of **5** in the reaction media (pH 7.5) was very low; if the substrate precipitated in the reaction media, the hydrolysis did not proceed; 2) A large amount of Protease P6 was needed to achieve a good yield. In order to solve these problems, the substrate was added continuously keeping the reaction mixture clear and also maintaining a high ratio of the enzyme/substrate. Thus, **5** (4.0g) was hydrolyzed at 30 °C for 27 h by using Protease P6 (8.0g) in water (1L) maintained at pH 7.5 by the addition of 1N NaOH with a pH-stat to afford **6** (2.14g, 79% yield). Esterification of **6** with 3-nitrooxypropyl bromide[9] in the presence of K_2CO_3 gave optically pure **1** in 77% yield.

The absolute configuration of **6** was determined to be *R*. Esterifications of the two enantiomers of 3-methoxycarbonyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylic acid[3a], (4*S*)-(+)-isomer (**9**)

Table 1. Enantioselective Hydrolysis of **5**

Enzyme	Origin	Yield (**6**, %)[a]	E.e. (%)[b]
Seaprose S (Amano)	*Aspergillus melleus*	99	>99
Protease P6 (Amano)	*Aspergillus melleus*	99	>99
Acylase 30,000 (Amano)	*Aspergillus sp.*	90	>99
Subtilisin Carlsberg (Protease Type VIII, Sigma)	*Bacillus licheniformis*	41	>99
Deamizyme® (Amano)	*Aspergillus sp.*	24	>99
Pronase E (Protease Type XIV, Sigma)	*Streptomyces griseus*	10	>99
Protease Type XIX (Sigma)	*Aspergillus sojae*	7	>99

All reactions were performed in 20 mM phosphate buffer (pH 7.5, 2 mL) at 30 °C for 24 h; **5**, 1mg; enzyme, 20 mg. [a] Yield was determined by HPLC analysis using a column packed with C_{18} ODS (methanol/water= 1/1). [b] The enantiomeric excesses (e.e.'s) were determined by HPLC analysis using a column of Ultron ES-OVM (i-propanol/20 mM KH_2PO_4 = 1/9).



9 R_1 = H, R_2 = CH_3

10 R_1 = CH_3, R_2 = H

11 R_1 = $(CH_2)_2NHCO$- 
R_2 = CH_3

12 R_1 = H
R_2 = $(CH_2)_2NHCO$- 

and (4*R*)-(-)-isomer (**10**), with 2-(nicotinoylamino)ethanol gave the diesters, (4*R*)-isomer **11** ($[\alpha]^{25}_D$=-62.2 (c=1, EtOH)) and (4*S*)-isomer **12** ($[\alpha]^{25}_D$ =+61.2 (c=1, EtOH)), respectively. Methylation of **6** with diazomethane afforded **11** in 84% yield, and its specific rotation ($[\alpha]^{25}_D$=-59.1 (c=1, EtOH)) was in exellent agreement with that of the compound **11** derived from **9**.

When we investigated solvates of the substrate, it was found that the transesterification products of **5** were obtained by the addition of alcohols in the reaction media. By the addition of 5-10% methanol and ethanol, methyl and ethyl 2-(nicotinoyl-amino)ethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylates were mainly obtained, respectively, with a small amount of the monocarboxylic acid. These results led us to investigate tansesterification of **5** with 3-nitrooxypropanol[10] to synthesize **1** directly. Several enzymes were screened, and the results are shown in Table 2. Seaprose S, Protease P6 showed high activities, and Acylase 30,000 and Deamizyme® showed moderate activities. Transesterification using these enzymes afforded optically pure **1** (>99.5% e.e.). Subtilisin Carlsberg, Pronase E and Protease Type XIX having the hydrolytic activity also showed transesterification activities, but the yields of **1** were only 2-4%. As the monocarboxylic acid **6** was obtained as a by-product in this method, the reaction conditions were investigated and it was found that the amount of 3-nitrooxypropanol in the reaction media affected the ratio of the products. The results are shown in Table 3. By the addition of 3-nitrooxypropanol (1%, v/v of the reaction media), the amount of the diester (**1**) was about equal to that of the monocarboxylic acid. By the addition of 3-nitrooxypropanol (8%), transesterification became predominant over hydrolysis and monocarboxylic acid was hardly detected. However, 3-nitrooxypropanol (more than 10%) inhibited the transesterification. In preparative scale experiments, transesterification of **5** (200mg) with Protease P6 (2g) in

water (50mL) containing 3-nitrooxypropanol (2.5mL) at pH 7.5 at 30 °C for 24 h gave **1** in 77% yield.

Table 2. Enantioselective Transesterification of **5** with 3-Nitrooxypropanol

Enzyme	Yield (%)[a]		E.e.[b] of **1**
	1	**6**	(%)
Seaprose S	71	5	>99.5
Protease P6	50	3	>99.5
Acylase 30,000	26	2	>99.5
Deamizyme®	19	2	>99.5

All transesterification reactions were performed in 20 mM phosphate buffer (pH 7.5, 1.9 mL) at 30 °C for 24 h; **5**, 4 mg; enzyme, 20 mg; 3-nitrooxypropanol, 0.1 mL. [a] Yield was determined by HPLC analysis using a column packed with C_{18} ODS (methanol/water=1/1). [b] The enantiomeric excesses (e.e.'s) were determined by HPLC analysis using a column of Chiralcel OJ (n-hexane/ethanol= 17/3).

Table 3. Enantioselective Transesterification of **5** with 3-Nitrooxypropanol by Protease P6

3-Nitrooxypropanol (%)	HPLC analysis (%)[a]		
	1	**6**	**5**
1	47	49	3
2	70	30	-
4	84	15	1
6	88	8	4
8	79	-	21
10	35	-	65

All transesterification reactions were performed in 10 mM phosphate buffer (pH 7.5) at 30 °C for 24 h; **5**, 4 mg; Protease P6, 40 mg; 3-nitrooxypropanol, 1% - 10% in the reaction media. Total volume of the reaction media was 2 mL.
[a] HPLC analysis was performed by using a column of C_{18} ODS (methanol/ water=1/1).

Recently the synthesis of optically active 1,4-dihydropyridine derivatives has been achieved by lipase catalyzed hydrolysis of bis(acyloxymethyl)esters in organic solvents.[11] The acyloxymethyl group was suggested to be hydrolyzed in two steps, enzymatic hydrolysis of the acyl group and successive cleavage of the hydroxymethyl group.[11b] It was also reported that the ester groups on the 1,4-dihydropyridine ring were sterically hindered and methyl, ethyl, allyl and phenyl esters could not be hydrolyzed. We also thought that the 2-(nicotinoylamino)ethyl group was hydrolyzed in two steps, hydrolysis of the nicotinoyl group and the 2-aminoethyl group. However, it was revealed that the proteases hydrolyzed the ester group of **5** directly from the

following results: 1) A small amount of 2-(nicotinoylamino)ethanol was detected in the reaction media. 2) Transesterification products were obtained when 3-nitrooxypropanol was added to the reaction mixture.

Optically active 1,4-dihydropyridine derivatives have been synthesized via both enantioselective hydrolysis and transesterification of a prochiral bis[2-(nicotinoylamino)ethyl]ester using hydrolytic enzymes in aqueous media. It is noteworthy that the proteases showed higher activities than lipases and esterases for hydrolysis and transesterification of the 1,4-dihydropyridine diester. Porcine pancreas lipase (PPL) and porcine liver esterase (PLE) commonly used in the hydrolysis of many organic compounds showed no activities. It has been known that proteases (subtilisin Carlsberg, α-chymotrypsin etc.) hydrolyze various kinds of esters as well as peptide bonds.[12] Recently, these enzymes have been used as catalysts for esterification and transesterification in anhydrous organic solvents.[13] Subtilisin Carlsberg and proteases from *Aspergillus melleus*[7] belong to a group of serine protease. The mechanisms of these enzymes seem to be similar in hydrolysis and transesterification of a prochiral bis[2-(nicotinoylamino)ethyl]ester.

Glazer reported that subtilisin Carlsberg catalized transesterification of L-tyrosine ethyl ester in 5% n-butanol-water to give L-tyrosine n-butyl ester.[14] The yield of the n-butyl ester obtained was lower than that of the hydrolyzed compound, L-tyrosine. In our present studies, the transesterification product (**1**) was obtained in a fairly high yield compared to the hydrolyzed compound (**6**) in spite of using aqueous media. Transesterification reaction competes usually with hydrolysis in the alcohol-water mixture. Proteases from *Aspergillus melleus* seem to have a property to incorporate alchols in their active sites. Much investigation is necessary for understanding the enzymatic mechanism.

EXPERIMENTAL

The ^1H spectra were recorded at 300 MHz and 200 MHz with a Varian VXR-300 and Varian VXR-200 spectrometer, respectively. The ^{13}C NMR spectra were recorded at 75 MHz with a Varian XL-VXR300 spectrometer. Melting points were measured using a Yanaco micromelting point apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer 1760 FT-IR spectrometer. Mass spectra were measured on a Jeol JMS-SX 102 spectrometer equipped with a Jeol JMA-DA 6000 data system using a FAB techniques. Optical rotations were measured on a Jasco DIP-350 polarimeter.

Bis[2-(nicotinoylamino)ethyl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate•2HCl (5)

The solution of 3-nitrobenzaldehyde (**2**, 15.1 g, 100 mmol), 2-(nicotinoylamino)ethyl acetoacetate (**3**, 25.0 g, 100 mmol), 2-(nicotinoylamino)ethyl-3-aminocrotonate (**4**, 24.9 g, 100 mmol) and piperidinium acetate (1.45 g, 10 mmol) in isopropyl alcohol (250 mL) was refluxed for 8 h. The reaction mixture was concentrated in vacuo and dissolved in AcOEt (300mL). The solution was washed with water and brine, and dried over $MgSO_4$. To the solution was added 4N HCl-AcOEt (25 mL), and the resulting precipitate was filtered, recrystallized from MeOH-Acetone to give 43.2 g (63%) of **5**: ^1H NMR ($(CD_3)_2SO$) δ 2.27 (s, 6H), 3.4-3.6 (m, 4H), 4.0-4.2 (m, 4H), 4.98 (s, 1H), 7.35(t, J=8 Hz, 1H), 7.60 (d, J=8 Hz, 1H), 7.85 (d, J=8 Hz, 1H), 7.9-8.0 (m, 3H), 8.69 (d, J=8 Hz, 2H), 8.96 (d, J=5 Hz, 2H), 9.1-9.3 (m, 4H), 9.5-11.0 (m, 2H); IR (KBr) 3435, 3232, 3086, 1696, 1672, 1532, 1482, 1303, 1271, 1204, 1136, 1115, 1097, 1056, 1020, 687 cm^{-1}; MS (FAB)

m/z=615(MH^+-HCl); Anal. Calcd for $C_{31}H_{32}Cl_2N_6O_8$: C, 54.16; H, 4.69; N, 12.22: Found: C, 53.67; H, 4.66; N, 12.05.

(4*R*)-(-)-3-[2-(Nicotinoylamino)ethoxycarbonyl]-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylic acid (6)

The solution of Protease P6 (8 g) in water (1 L) was adjusted to pH 7.5 with 0.5N aqueous NaOH. To the above stirred solution was continuously added **5** (4.0 g, 5.8 mmol) in water (40 mL) at 30 °C for 27 h. The reaction mixture was maintained at pH 7.5 by the addition of 0.5N NaOH with a pH-stat. After the completion of the reaction, the reaction mixture was adjusted to pH 5.0 by the addition of 0.5N H_3PO_4 and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was crystallized from acetonitrile to afford 2.14 g (79%) of **6**: mp 124-130°C (decomposed); $[\alpha]^{30}_D$ -90.7 (*c*=1.0, EtOH); ^{1}H NMR (CD_3OD) δ 2.32 (s, 3H), 2.34 (s, 3H), 3.56-3.73 (m, 2H), 4.20 (ddd, J=5.0, 6.2, 11.4 Hz, 1H), 4.31 (ddd, J=5.0, 6.2, 11.4 Hz, 1H), 5.11(s, 1H), 7.32 (dd J=7.6, 8.2 Hz, 1H), 7.51 (ddd, J=0.8, 5.0, 8.0 Hz, 1H), 7.63 (ddd, J=1.0, 1.3, 7.6 Hz, 1H), 7.88 (ddd, J=1.0, 2.3, 8.2 Hz, 1H), 8.08 (dd, J=1.3, 2.3 Hz, 1H), 8.16 (ddd, J=1.7, 2.2, 8.0 Hz, 1H), 8.66 (dd, J=1.7, 5.0 Hz, 1H), 8.89 (dd, J=0.8, 2.2 Hz, 1H); ^{13}C NMR (CD_3OD) δ 18.7(q), 18.8(q), 40.4(t), 40.9(d), 63.0(t), 102.6(s), 103.7(s), 122.0(d), 123.5(d), 125.1(d), 130.0(d), 131.9(s), 135.2(d), 137.0(d), 147.8(s), 148.9(s), 149.1(d), 149.5(s), 151.6(s), 152.7(d), 167.9(s), 169.0(s), 171.1(s); IR (KBr) 3335, 3081, 2954, 1676, 1652, 1527, 1484, 1349, 1308, 1213, 1123, 1097, 1019, 703 cm^{-1}; MS (FAB) *m/z*=467(MH^+).

(4*R*)-(-)-Methyl 2-(Nicotinoylamino)ethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate (11)

(a) To the solution of **6** (466 mg, 1 mmol) in THF (10 mL) was added diazomethane derived from 1-methyl-3-nitro-1-nitrosoguanidine (294 mg, 2 mmol) in ether (5 mL) at room temperature. The reaction mixture was allowed to stand at room temperature for 3 h and evaporated under reduced pressure. The residue was chromatographed on a silica gel column with $CHCl_3$/MeOH/conc. NH_4OH (50/1/0.1) and crystallized from AcOEt-diisopropyl ether to afford **11** (404 mg, 84 %): mp 139-141 °C; $[\alpha]^{25}_D$ -59.1 (*c*=1.0, EtOH); ^{1}H NMR (CD_3OD) δ 2.31 (s, 3H), 2.33 (s, 3H), 3.58(s, 3H), 3.56-3.73 (m, 2H), 4.20 (ddd, J=4.9, 6.3, 11.4 Hz, 1H), 4.31 (ddd, J=4.9, 6.3, 11.4 Hz, 1H), 5.07(s, 1H), 7.32 (dd, J=7.9, 8.2 Hz, 1H), 7.52 (ddd, J=0.9, 4.9, 7.9 Hz, 1H), 7.60 (ddd, J=1.0, 1.4, 7.9 Hz, 1H), 7.88 (ddd, J=1.0, 2.4, 8.2 Hz, 1H), 8.05 (dd, J=1.4, 2.4 Hz, 1H), 8.16 (ddd, J=1.7, 2.3, 7.9 Hz, 1H), 8.67 (dd, J=1.7, 4.9 Hz, 1H), 8.90 (dd, J=0.9, 2.3 Hz, 1H); ^{13}C NMR (CD_3OD) δ 18.7(q), 18.8(q), 40.3(t), 40.8(d), 51.5(q), 63.0(t), 102.8(s), 103.3(s), 122.1(d), 123.4(d), 125.1(d), 130.1(d), 131.9(s), 135.1(d), 137.0(d), 148.0(s), 148.7(s), 149.1(d), 149.5(s), 151.5(s), 152.7(d), 167.9(s), 168.9(s), 169.5(s); IR (KBr) 3366, 1702, 1665, 1652, 1530, 1487, 1349, 1221, 1121, 1099, 1025, 705 cm^{-1}; MS (FAB) *m/z*=481(MH^+); Anal. Calcd for $C_{24}H_{24}N_4O_7$: C, 60.0; H, 5.03; N, 11.66: Found: C, 59.74; H, 5.20; N, 11.36.

(b) The mixture of (4*S*)-(+)-3-methoxycarbonyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylic acid (**9**, 1.0g, 3 mmol) and acetic anhydride (20 mL) was stirred for 2 h at 70 °C. The reaction mixture was evaporated in vacuo, then the resulting residue was dissolved in DMF (20 mL). To the solution was added (2-nicotinoylamino)ethanol p-toluenesulfonate (1.53 g, 4.5 mmol) at rt. The solution was stirred for 8 h at rt. The reaction mixture was poured into 10% NaOH solution and extracted with AcOEt. The organic

layer was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Silica gel column chromatography of the crude product with AcOEt/n-Hexane (1/1) and crystallization from diisopropyl ether/ CH_2Cl_2 gave 0.93 g of **11** (64%): mp 133-135 °C; $[\alpha]^{25}_D$ -62.2 (*c*=1.0, EtOH).

(4*S*)-(+)-Methyl 2-(nicotinoylamino)ethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate (12)

Esterification of (4*R*)-(-)-3-methoxycarbonyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylic acid (**10**, 1.0g, 3 mmol) with 2-(nicotinoylamino)ethanol by the similar method to the synthesis of **11** gave 0.80 g of **12** (55%): mp 134-136 °C; $[\alpha]^{25}_D$ +61.2 (*c*=1.0, EtOH).

(4*R*)-(-)-(2-Nicotinoylamino)ethyl 3-nitrooxypropyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate (1)

(a) To the solution of **6** (250 mg, 0.54 mmol) in DMF (10mL) was added 3-nitrooxypropyl bromide (120 mg, 0.65 mmol) and K_2CO_3 (90 mg) at rt. The reaction mixture was stirred at rt for 10 h, poured into water and extracted with AcOEt. The organic layer was washed with water and aqueous $NaHCO_3$ solution, and dried over $MgSO_4$. After evaporation of the solvent, the resulting residue was crystallized from isopropyl acetate to give 240 mg (79%) of **1**: mp 126-128 °C; $[\alpha]^{24}_D$ -39.6 (*c*=1.0, EtOH); Anal. Calcd for $C_{26}H_{27}N_5O_{10}$: C, 54.83; H, 4.77; N, 12.29: Found: C, 54.65; H, 4.74; N, 12.15.

(b) To the solution of Protease P6 (2 g), 3-nitrooxypropanol (2.5 mL) in water (45 mL) was added **5** (200 mg, 0.29 mmol) dissolved in water (5 mL), and the reaction mixture was shaken at 30 °C for 24 h. The reaction mixture was maintained at pH 7.0 - 7.5 by the addition of 0.5N NaOH. After the completion of the reaction, the reaction mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was chromatographed on a silica gel column with $CHCl_3$/MeOH/conc.NH_4OH (50/1/0.1) to give 123 mg (77%) of **1** as a glass. Crystallization from AcOEt/ diisopropyl ether provided an analytical sample: mp 126-128 °C; $[\alpha]^{24}_D$ -39.1 (*c*=1.0, EtOH); ^1H NMR (CD_3OD) δ 1.97(dt, J=6.3, 12.6 Hz, 2H), 2.32 (s, 3H), 2.33 (s, 3H), 3.57-3.75 (m, 2H), 4.05 (dt, J=6.1, 11.4 Hz, 1H), 4.15 (dt, J=6.1, 11.4 Hz, 1H), 4.21 (ddd, J=4.7, 6.2, 11.0 Hz, 1H), 4.33 (ddd, J=4.7, 6.2, 11.0 Hz, 1H), 4.34(t, J=6.4 Hz, 2H), 5.07(s, 1H), 7.34 (dd, J=7.9Hz, 8.2Hz, 1H), 7.51 (ddd, J=0.9, 4.9, 8.0 Hz, 1H), 7.62 (ddd, J=1.0, 1.2, 7.9 Hz, 1H), 7.89 (ddd, J=1.0, 2.3, 8.2 Hz, 1H), 8.07 (dd, J=1.2, 2.3 Hz, 1H), 8.16 (ddd, J=1.6, 2.3, 8.0 Hz, 1H), 8.67 (dd, J=1.6, 4.9 Hz, 1H), 8.90 (dd, J=0.9, 2.3 Hz, 1H); ^{13}C NMR (CD_3OD) δ 18.8(q), 18.9(q), 27.5(t), 40.3(t), 40.9(d), 61.3(t), 63.1(t), 71.5(t), 103.0(s), 103.1(s), 122.2(d), 123.5(d), 125.2(d), 130.2(d), 131.9(s), 135.2(d), 137.0(d), 148.5(s), 148.6(s), 149.1(d), 149.5(s), 151.7(s), 152.7(d), 167.9(s), 168.7(s), 168.9(s); IR (KBr) 3366, 1699, 1665, 1650, 1635, 1531, 1488, 1349, 1279, 1218, 1119, 1023, 870, 705; MS (FAB) *m/z*=570(MH^+); Anal. Calcd for $C_{26}H_{27}N_5O_{10}$: C, 54.83; H, 4.77; N, 12.29: Found: C, 55.03; H, 4.81; N, 12.08.

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