Facile Synthesis of (2S,3R)-3-Amino-2-hydroxy-4(4'-hydroxyphenyl) butanoic Acid. Application to the Synthesis of Inhibitors of Aminopeptidases

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Facile methods are reported for the synthesis of optically pure derivatives of (2S,3R)-3-amino-2-hydroxy-4-(4'-hydroxyphenyl)butanoic acid. To avoid troublesome synthesis of O-benzyl-N-Boc-D-tyrosine, without the protection of phenolic OH group of tyrosine N-Boc-D-tyrosine methyl ester was reduced with DiBAL to the aldehyde. The aldehyde was converted *via* the cyanohydrin to (2S,3R)-3-amino-2-hydroxy-4 (4'-hydroxyphenyl)butanoic acid (AHpHBA). The mixture of diastereomers was converted to the corresponding Boc-AHpHBA methyl ester derivatives and separated by chromatography over silica gel. Optically active (2S,3R)-AHpHBA was used to synthesize aminopeptidase inhibitors.

Introduction

The amino acids, (2S,3R)-3-amino-2-hydroxy acids are incorporated into the structures of the natural peptide aminopeptidase inhibitors, amastatin[1] and bestatin,[2] which have remarkable biological activities.[3-5] We have encountered a need for various kinds of 3-amino-2-hydroxy acids for synthetic efforts in the peptide area in order to study the mechanism of enzyme action and to develop inhibitors with new pharmacological properties. Thus, we have initiated to the study for the synthesis of several this kind of unusual amino acids.[6] For instance we reported synthesis of (2S,3R)-3-amino-2-hydroxy-5-methylhexanoic acid (AHMHA) derivatives.[7]

In this paper we report the synthesis and characterization of (2S, 3R)-3-amino-2-hydroxy-4-(4'-hydroxyphenyl) butanoic acid (AHpHBA) and utilization of this optically pure acid to synthesize aminopeptidase inhibitors.

Results and Discussion

Synthesis of AHpHBA. AHpHBA was first synthesized from O-benzyl-N-Boc-D-tyrosine methyl ester by modifying the procedures described for the synthesis of AHMHA (Figure 1).[7] Following a modification of the reported procedure,[8] O-benzyl-N-Boc-D-tyrosine methyl ester was prepared from D-tyrosine using the following transformations: (1) protection of amino and carboxyl group by chelation with Cu; (2) alkylation of phenolic OH with benzyl bromide; (3) decomposition of copper complex with hydrogen sulfide; (4) isolation of O-benzyl-D-tyrosine; (5) treatment of O-benzyl-D-tyrosine with Boc-azide to give O-benzyl-N-Boc-D-tyrosine; (6) treatment of the latter compound with an etheral solution of diazomethane gave O-benzyl-N-Boc-Tyr-OMe.

When N,O-di-Cbz-D-Tyr-OMe was used as a starting material, the over-reduced by-product (alcohol) and the unreacted starting material were obtained as major products.

To avoid the troublesome synthesis of O-benzyl-N-Boc-D-tyrosine (this is not commercially available), DIBAL reduction of N-Boc-D-tyrosine methyl ester was attempted without the protection of phenolic OH group. The reaction conditions described for reduction of N-Boc-D-leucine methyl ester

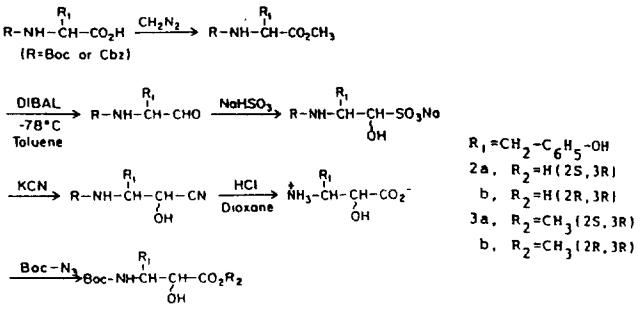


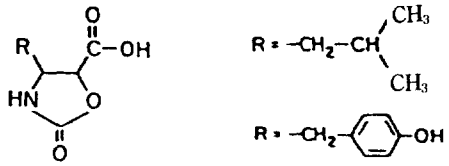
Figure 1. Synthesis of 3-amino-2-hydroxy-4-(4'-hydroxyphenyl) butanoic acid.

were modified slightly by changing solvent from toluene to toluene and THF to increase the solubility of the starting material and by increasing DIBAL by one equivalent for the free phenolic OH and by increasing reaction time to 30-45 min due to the coordination of DIBAL with THF. The aldehyde thus obtained was converted to the acid by the procedure developed for AHPBA or AHMHA. By this method, AHpHBA was obtained in 80% yield.

Separation of Diastereomers. Separation of diastereomers was achieved by converting the amino acid to a neutral derivative and chromatographing over silica gel. Thus, reaction of amino acids **1a** and **1b** with Boc-azide in aqueous dioxane gave the N-protected derivatives **2a** and **2b** as a mixture of diastereomers. Their methyl esters **3a** and **3b** were synthesized with diazomethane in a quantitative yield. Chromatography of a mixture of diastereomers **3a** and **3b** over silica gel eluting with 10% ethyl acetate in benzene or with 10% ethyl acetate in toluene gave the 2S, 3R diastereomer **3a** as a less polar and the 2R, 3R, 3b as a more polar diastereomer. Saponifications of esters **3a** and **3b** gave **2a** and **2b** which, upon treatment with trifluoroacetic acid, were converted to the free acids **1a** and **1b**. Treatement of acids **2a** and **2b** with diazomethane gave optically pure **3a** and **3b**, respectively, establishing that these saponification conditions do not racemize **3a** or **3b** at C-2.

Assignment of Stereochemistry. Assignment of stereochemistry of AHpHBA was established using Shiba's method.[9] Shiba *et al.*, reported that the relative configuration

Table 1. Chemical Shifts and Coupling Constants of 2-Oxazolidone Derivatives



2-Oxazolidone	$H_{C\text{-}2}$(δ ppm)	$J_{2\text{-}3}$(Hz)
from (2S,3R) AHMHA	4.68	4.5[a]
from (2R,3R) AHMHA	5.06	9.0[a]
from (2S,3R) AHpHBA	4.67	4.5
from (2R,3R) AHpHBA	5.12	9.0

[a]Data taken from reference 9.

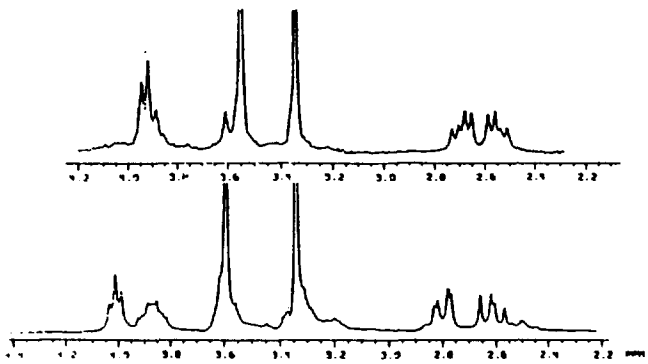


Figure 2. Comparison of 270 MHz ^{1}H-NMR spectra of AHpHBA derivatives: scale expansion of C_2-H and C_3-H region at 25℃ in DMSO-d_6. A, (2S,3R)-Boc-AHpHBA-OMe **3a** B, (2R,3R)-Boc-AHpHBA-OMe **3b**.

of α-amino-β-hydroxy acids can be determined by NMR spectrometry from the coupling constants of their oxazolidone derivatives. The coupling constants of the vicinal methine protons of the oxazolidones are distinctly different in the threo (5.0+1.0 Hz) and erythro (9.6+0.6 Hz) isomers. As shown in Table 1, the coupling constant of the vicinal methine proton of the oxazolidone derivative of (2S,3R)-AHpHBA is 4.5 Hz, while that of the diastereoisomer is 9.0 Hz. This result suggested that the configuration of (2S,3R)-AHpHBA should be threo, while that of the diastereomer should be erythro (Table 1). This results closely parallel the assignment of stereochemistry of 3-amino-2-hydroxy-5-methyl-hexanoic acid[7] (Table 1).

While analyzing the high resolution NMR spectroscopy of these acids in DMSO-d_6. It became apparent that there are some significant differences in the NMR spectra between threo (2S,3R) and erythro (2R,3R) diastereomers of the Boc-amino acid methyl esters. First, as shown in Figure 2, the Peaks (δ 3.82-4.11) of C_2-H and C_3-H of the threo isomer AHpHBA derivatives **3a** and **3c** cannot be separated With 270 MHz NMR while those of their erythro isomers are clearly separated. The same results have been shown in closely related 3-amino-2-hydroxy-phenylbutanoic acid (AHPBA) derivatives. Secondly, NH and OH of the threo isomer separate at higher field that those of the erythro isomer. Even though NH and OH are usually very sensitive to temperature and concentration, it appears that amide and hydroxy protons of the threo isomer are less exposed to the solvent than those of the erythro isomer. Finally, as shown in Table 2, in the case of AHPBA and AHpHBA derivatives, analysis of the coupling constant pattern for the side chain methylene (C_4) protons which comprise an AB region of an ABX pattern, showed that the erythro isomer has one very small and one large coupling constant, while threo isomer has two similar coupling constants.

Although this study has not been carried out in sufficient detail to assign the stereochemistry of α-hydroxy-β-amino

Table 2. Comparison of 270 MHz ^{1}H-NMR Assignment of 3-Amino-2-hydroxy Acid Derivatives

Compound	C-2	C-3	C-4	OH	NH
(2S,3R)-Boc-AHpHBA-OMe	3.82-4.11 (m)		2.46-2.75 (AB) J_{AB}=13.1 Hz J_{AX}=6.8 Hz J_{BX}=7.6 Hz	5.33 (d, J=8 Hz)	6.46 (d, J=9 Hz)
(2R,3R)-Boc-AHpHBA-OMe	3.99 (dd, J=6 Hz) J=6 Hz	3.71-3.86 (m)	2.33-2.75 (AB) J_{AB}=13.2 Hz J_{AX}=2.8 Hz J_{BX}=11.1 Hz	5.69 (d, J=6 Hz)	6.76 (d, J=9 Hz)
(2S,3R)-Boc-AHPBA-OMe	3.89-4.12 (m)		2.51-2.88 (AB) J_{AB}=13.3 Hz J_{AX}=6.8 Hz J_{BX}=8.9 Hz	5.41 (bs)	6.53 (d, J=10 Hz)
(2R,3R)-Boc-AHPBA-OMe	3.90 (dd, J=6 Hz) J=6 Hz	3.79-3.94 (m)	2.43-2.90 (AB) J_{AB}=13.8 Hz J_{AX}=3.9 Hz J_{BX}=11.2 Hz	5.77 (d, J=8 Hz)	6.79 (d, J=10 Hz)

Table 3. Physical Constants of AHpHBA Derivatives

No.	compound	mp. ℃	R_f[a]	$[\alpha]_D^{24}$ deg (C=1, MeOH)
3a	(2S,3R) Boc-AHpHBA-OMe	136-138	0.43	+85.76
3b	(2R,3R) Boc-AHpHBA-OMe	166-168	0.34	+9.68
3c	(2R,3S) Boc-AHpHBA-OMe	166-168	0.34	−7.85
3d	(2S,3S) Boc-AHpHBA-OMe	136-138	0.43	−85.39

[a]TLC solvent: 10% ethyl acetate in benzene.

acid by NMR spectroscopy itself, these NMR data, in conjunction with other data, *i.e.*, mp, R_f and optical rotation, could be used as one of the method for assignment of the stereochemistry of unknown α-hydroxy-β-amino acids. Physical constants for AHpHBA derivatives are presented in Table 3.

Synthesis of Aminopeptidase Inhibitors. The Boc 2S, 3R amino acid **2a** was used to synthesize peptides (2S,3R)-AHpHBA-Leu and (2S,3R)-AHpHBA-Gly-Gly-Phe bestatin analogs. Protected dipeptide N-Boc-(2S,3R)-AHpHBA-Leu-OMe was prepared by coupling N-Boc-(2S,3R)-AHpBA with leucine methyl ester using DCC/HOBt as coupling reagent in 70% yield. The protected peptide was converted to (2S,3R)-AHpHBA-Leu by base hydrolysis of the methyl ester followed by acid cleavage of the Boc group. Tetrapeptide N-Boc-(2S,3R)-AHpHBA-Gly-Gly-Phe-OBu^t was prepared starting from optically pure Boc-(2S,3R)-AHpHBA by stepwise addition of C-protected amino acids in 74% yield (Figure 3). Slimultaneous removal of N-Boc- and tert-butyl ester groups of the protected peptide with 4 N HCl in dioxane afforded tetrapeptide. The inhibitory activities of the synthetic dipeptide and tetrapeptide against aminopeptidase M were tested using modification of the reported procedure.[10] Dipeptide and tetrapeptide are shown to have strong inhibitory activity against aminopeptidase M ($K_i = 4.1\times10^{-7}$ M and 1.6×10^{-6} M, respectively). The kinetics of inhibition by these compounds will be reported separately.

Experimental

Melting points were determined on a Fisher-Johns melting point apparatus and are corrected. The ^{1}H-NMR spectra were recorded on a Bruker HX-90E pulse Fourier transform NMR spectrometer interfaced with a Nicolet 1080 computer and disk unit. Optical rotations were measured at the sodium D line by a Perkin-Elmer 241 polarimeter. Microanalyses were performed by Galbraith Laboratories. Thin-layer chromatography (TLC) was performed on silica gel G plates.

N-(tert-Butyloxycarbonyl)-D-tyrosinal. Boc-D-Tyr-OMe (2.95 g, 10 mmol) was dissolved in dry THF (40 m*l*). The solution was flushed with N_2 and cooled to −78℃. With vigorous stirring a solution of DiBAL in toluene (20 m*l*) was slowly added over 10 min and the reaction was allowed to stir for an additional 15 min. The excess DiBAL was destroyed with methanol (1 m*l*) and Rochelle salts solution (60 m*l*) was added immediately. The mixture was allowed to warm to room temperature and then extracted with ether (3×50 m*l*). The organic layeer was washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the titled compound (80% yield by TLC and NMR). R_f 0.20 (5% methanol in chloroform) ^{1}H-NMR ($CDCl_3$) δ 1.40 (s, 9H), 2.58-3.09 (AB, 2H), 4.20-4.60 (m, 1H) 4.75-5.25 (m, 2H), 6.53-7.08 (AA'BB', 4H), 9.60 (bs, 1H).

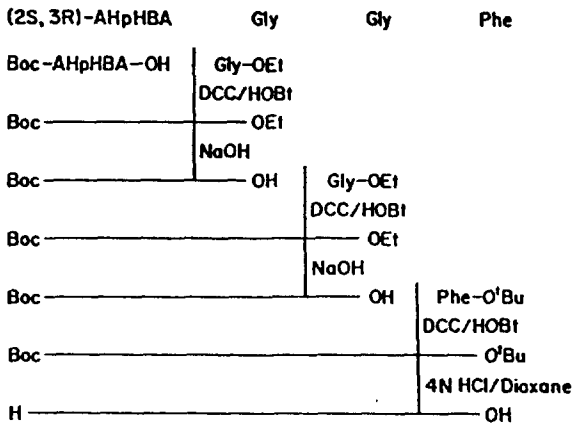


Figure 3. Synthesis of AHpBA containing aminopeptidase M inhibitor.

(2S,3R)-N-(tert-Butyloxycarbonyl)-3-amino-2-hydroxy-4-(4'-hydroxyphenyl)-butanoic acid methyl ester and (2R,3R)-N-(tert-butyloxycarbonyl)-3-amino-2-hydroxy-4-(4'-hydroxyphenyl)-butanoic acid methyl ester. Ice-cold solution of $NaHCO_3$ (0.83 g, 8 mmol in 50 m*l*) was added to amino aldehyde (10 mmol), and the mixture was stirred overnight at 5℃. To the resulting suspension of $NaHSO_3$ adduct was added ethyl acetate (150 m*l*) and a solution of KCN (0.65 g, 10 mmol in 50 m*l*). The reaction mixture was stirred for 4 hr at room temperature. The ethyl acetate layer was washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give the cyanohydrin which was dissolved in dioxane-concentrated HCl (1:1) (100 m*l*) and hydrolyzed by reflux for 12 hr. The hydrolyzate was washed with ether and concentrated under reduced pressure. The residue was dissolved in a small amount of water. Acetone (200 m*l*) was added and the mixture was adjusted to pH 5.5 with 2 N NaOH. Crystals which deposited after standing at 5℃ overnight were filtered and washed with acetone (60% yield). The mixture of esters was seperated by chromatography over silica gel, eluting with a gradient of 20-30% ethyl acetate in toluene. Physical constants for **3a** and **3b** are given in Table 3. Anal. Calcd for $C_{16}H_{23}NO_6$: C, 59.02; H, 7.13; N, 4.30. Found: C, 58.82; H, 6.97; N, 4.19.

Oxazolidone derivative of (2R,3R)-3-Amino-2-hydroxy-4-(4'-hydroxyphenyl)-butanoic acid. (2R,3R)-AHpHBA-OH (0.134 g, 0.54 mmol) was dissolved in 35 m*l* of 1 M KOH. The solution was cooled in an ice bath and then treated with 40 m*l* of cold phosgene in toluene. The mixture was stirred vigorously for 2 hr. The aqueous layer was separated, and the toluene layer washed with 1 N KOH. The combined aqueous layer was acidified with concentrated HCl and extracted with ethyl acetate. The organic extract was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Without purification, the product was dissolved in DMSO-d_6 and the solution analyzed by NMR. ^{1}H-NMR (DMSO-d_6) δ 2.68-2.97 (AB, 2H), 4.17-4.29 (m, 1H), 5.12 (d, J=9.0 Hz, 1H), 6.63-7.02 (AA'BB', 4H), 9.24 (bs, 1H).

Oxazolidone derivative of (2S,3R)-3-Amino-2-hy-

droxy-4-(4′-hydroxyphenyl)-butanoic acid. (2S,3R)-AHpHBA-OH (1.120 g, 0.48 mmol) was treated in a manner analogous to that described above for oxazolidinone derivative of (2R,3R)-AHpHBA to give the title compund. Without purification, the product was dissolved in DMSO-d_6 and analyzed by NMR. ^{1}H-NMR (DMSO-d_6) δ 2.70-2.92 (AB, 2H), 3.90-4.50 (m, 1H), 4.67 (d, J=4.5 Hz, 1H), 6.64-7.07 (AA′BB′, 4H), 9.27 (bs, 1H).

(2S,3R)-3-Amino-2-hydroxy-4-(4′-hydroxyphenyl)-butanoyl-L-leucine hydrochloride. Leucine methyl ester hydrochloride. (0.045 g, 0.25 mmol) was neutralized with triethylamine and coupled with (2S,3R)-Boc-AHpHBA-OH (0.078 g, 0.25 mmol) by using DCC (0.062 g, 0.3 mmol) and HOBt (0.058 g, 0.38 mmol) in methylene chloride overnight at 5℃. The reaction mixture was filtered and the methylene chloride layer was washed with distilled water, saturated sodium bicarbonate, and 1 N citric acid. The solution was dried ($MgSO_4$) and the solvent evaporated *in vacuo* to give the protected dipeptide. The protected peptide was purified by chromatography over silica gel eluting with 10% ethyl acetate in benzene and crystallized from ethyl acetate-Skelly B (93% yield): mp. 199-201 ℃. R_f 0.28 (20% ethyl acetate in toluene) ^{1}H-NMR (DMSO-d_6) δ 0.73-0.94 (m, 6H), 1.27 (s, 9H), 1.27-1.75 (m, 2H), 1.76-2.00 (m, 1H), 2.38-2.73 (AB, 2H), 3.60 (s, 3H), 3.72-4.00 (m, 2H), 4.20-4.55 (m, 1H), 5.52 (bd, J=9 Hz, 1H), 6.06 (bd, J=9 Hz, 1H), 6.60-7.10 (AA′BB′, 4H), 8.14 (d, J=9 Hz, 1H). Anal. Calcd for $C_{22}H_{34}N_2O_7$: C, 60.26; H, 7.82; N, 6.39. Found; C, 60.13; H, 7.93; N, 6.25.

Deprotection of the protected peptide by NaOH hydrolysis of methyl ester followed acid cleavage of Boc group gave the title compound in 95% yield: mp. 166-168℃. R_f 0.55 (n-butanol-acetic acid-water, 4:1:1) ^{1}H-NMR (CD_3OD) δ 0.96 (d, J=5 Hz, 6H), 1.71 (bd, J=5 Hz, 3H), 2.67-3.22 (AB, 2H), 3.44-3.84 (m, 1H), 4.17 (d, J=3 Hz, 1H), 4.42 (t, J=6 Hz, 1H), 6.67-7.27 (AA′BB′, 4H)

(2S,3R)-N-(tert-Butyloxycarbonyl)-3-amino-2-hydroxy-4-(4′-hydroxyphenyl)-butanoyl-glycyl-glycine ethyl ester. Glycine ethyl ester hydrochloride (0.07 g, 0.5 mmol) was neutralized with triethylamine and coupled with Boc-(2S, 3R)-AHpHBA-OH (0.156 g, 0.5 mmol) by using DCC (0.123 g, 0.6 mmol) and HOBt (0.101 g, 0.75 mmol) in methylene chloride overnight at 5℃. The reaction mixture was filtered, and the methylene chloride layer was washed with water, saturated sodium bicarbonate, and 1 N citric acid. The solution was dried ($MgSO_4$) and the solvent evaporated *in vacuo* to give the protected dipeptide. The protected dipeptide was purified by column chromatography over silica gel, eluting with 10% ethyl acetate in benzene. After hydrolysis of methyl ester with 2 N NaOH in aqueous dioxane (30 min, 25℃), the N-protected peptide acid (0.111 g, 0.3 mmol) was coupled with glycine ethyl ester (0.042 g, 0.3 mmol) as described above to give protected tripeptide: 71% yield; R_f 0.28 (10% methanol in chloroform), ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, J=7 Hz, 3H), 1.38 (s, 3H), 2.82 (bs, 2H), 3.71 (q, J=7 Hz, 2H), 3.91-4.44 (m, 6H), 5.48 (d, J=8 Hz, 1H), 6.53-7.22 (AA′BB′, 4H), 7.56 (bs, 1H), 7.87 (bs, 1H).

(2S,3R)-N-(tert-Butyloxycarbonyl)-3-amino-2-hydroxy-4-(4′-hydroxyphenyl)-butanoylglycyl-glycyl-L-phenylalanine-tert-butyl ester. The N-protected tripeptide acid, Boc-AHpHBA-Gly-Gly-OH (0.82 g, 0.19 mmol), obtained from the ethyl ester by base hydrolysis (2 N NaOH, 30 min, 25℃), was coupled to Phe-tert-butyl ester hydrochloride (0.467 g, 0.19 mmol) in DMF (1 m*l*) with DCC (0.047 g, 0.23 mmol) and HOBt (0.038 g, 0.29 mmol) as coupling reagents. The product was isolated as described above for the tripeptide. The purification of the product by column chromatography over silica gel eluting with 2% methanol in chloroform afforded the title compound (90% yield): mp. 102-104℃. R_f 0.26 (10% methanol in chloroform) ^{1}H-NMR ($CDCl_3$) δ 1.37 (s, 18H), 2.44-3.22 (m, 4H), 3.33-4.36 (m, 6H), 4.49-4.87 (m, 1H), 5.44-5.89 (m, 1H), 6.56-7.56 (AA′BB′ + m, 11H), 7.78 (bs, 1H).

Anal. Calcd for $C_{32}H_{44}N_4O_9$: C, 61.13; H, 7.05; N, 8.91. Found: C, 60.79; H, 7.24; N, 8.71.

(2S,3R)-3-Amino-2-hydroxy-4-(4′-hydroxyphenyl)-butanoyl-glycyl-glycyl-L-phenyl alanine hydrochloride. Removal of N-Boc and tert-butyl ester groups of the protected tetrapeptide (0.100 g, 0.16 mmol) was carried out using 4 N HCl in dioxane (30 min, 25℃) to give the title compound. The product was purified by preparative TLC (n-butanol-acetic acid-water=4:1:1) (12.3% yield): R_f 0.29 (n-butanol-acetic acid-water=4:1:1). ^{1}H-NMR (DMSO-d_6) δ 2.67-3.05 (m, 4H), 3.37 (s, 4H), 3.50-3.83 (m, 5H), 3.93 (bs, 1H), 4.22-4.62 (m, 1H), 6.65-6.90 (AA′BB′, 4H), 6.88 (s, 1H), 7.22 (s, 5H), 7.83-8.45 (m, 3H), 9.40 (s, 1H).

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11. The abbreviation used are as follows: DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; Boc, tert-butyloxycarbonyl; AHMHA, 3-amino-2-hydroxy-5-methylhexanoic acid; AHPBA, 3-amino-2-hydroxy-4-phenylbutanoic acid; K_i, inhibitory binding constant.