Stereoselective Synthesis of Novel Bestatin Analogs

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Abstract
Two new analogs of bestatin were prepared from D-leucine and D-valine in a stereoselective and efficient way. An aminopeptidase inhibitor bestatin shows significant biological effects on immunomodulation and is marketed for the treatment of acute myelocytic leukemia. The key intermediates, trans-oxazolidine methyl esters 2a and 2b, were obtained with more than 20 to 1 stereoselectivity in a one-pot procedure by the three cascade reactions between N-hydroxymethyl protected α-amino aldehydes (4a and 4b) and phenylsulfonylnitromethane (PhSO2CH2NO2) and the following in-situ ozonolysis. Basic hydrolysis of 2a and 2b, and then the peptide coupling with L-Leu-OMe produced the protected derivatives of two new bestatin analogs, 3a and 3b, respectively. The new isobutyl and isopropyl analogs of bestatin (1a and 1b) were produced in overall 51% and 38% yields, respectively, with high stereoselectivity from the corresponding protected α-amino aldehydes 4 in a six-step process.

Keywords: bestatin analogs, aminopeptidase inhibitor, β-amino-α-hydroxy acid, intramolecular conjugate addition.

1. Introduction
Aminopeptidase N (APN), a metal-dependent membrane-bound protease, has been studied as a useful clinical marker because its overexpression affects the protein activation, degradation, and regulation that are closely related to the inflammatory diseases and cancers[1-4]. In order to control the overexpression of APN, several naturally occurring aminopeptidase inhibitors have been developed from microbial culture filtrates. Bestatin, first isolated from a culture filtrate of Streptomyces olivoreticuli (MD976-C7)[5], has been extensively stud-

ied due to its multiple effects on the immune system, and it has been used to treat acute myelocytic leukemia under a trade name of Ubenimex[6]. For the structure-activity relationship, its analogs have been synthesized (Figure 1(a))[7-10], which has revealed that the threo-β-amino-α-hydroxy acid moiety in bestatin interacts with the active site of aminopeptidase N[11]. The bioactive vicinal amino hydroxy acid unit is also widely found in other naturally occurring aminopeptidase inhibitors. For example, amastatin isolated also from the culture filtrate of Streptomyces sp.[12] contains (2R,3S)-3-amino-2-hydroxy-5-methylhexanoic acid (AHMHA) at the N-terminus (Figure 1(b)), and 3-amino-2-hydroxy-4-methylpentanoic acid (AHMPA) is embedded in lapstatin although its stereochemistry has not been established yet (Figure 1(c))[13]. In order to demonstrate the effects of the side chain at the threo-vicinal amino hydroxy acid unit on the bioactivity, we planned to substitute the benzyl group at the N-terminus of bestatin with an isobutyl...
or isopropyl group, which are present in amastatin or lapstatin, respectively (Figure 1). To the best of our knowledge, this is the first synthetic report for isobutyl or isopropyl substituted bestatin analogs (Figure 2).

2. Experimental

2.1. General

Materials were obtained from commercial suppliers and were used without further purification. Methylene chloride was distilled from calcium hydride immediately prior to use. Air or moisture sensitive reactions were conducted under nitrogen atmosphere using oven-dried glassware and standard syringe/septa techniques. The reactions were monitored with a SiO2 TLC plate under UV light (254 nm) and by visualization with a ninhydrin staining solution. Column chromatography was performed on silica gel 60 (70-230 mesh). Melting point was measured on a Meltemp apparatus in open capillary tubes. Optical rotations were determined at ambient temperature with a digital polarimeter and are the average of ten measurements. 1H and 13C NMR spectra were referenced with the 77.16 resonance of CDCl3, 49.00 resonance of MeOH-d4. Low and high resolution mass spectra were measured by the CI or FAB ionization method and analyzed by magnetic sector mass analyzer.

2.2. General procedure for trans-oxazolidine methyl esters 2

To α-amino aldehyde 4a (R1 = i-Bu, 646 mg, 2.63 mmol) in THF (2 mL) was added phenylsulfonylnitromethane (636 mg, 3.16 mmol) and DMAP (482 mg, 3.95 mmol). The reaction mixture was stirred at room temperature for 2 days with vigorous stirring until the starting material 4a disappeared. The mixture was diluted with THF (5 mL) and methanol (5 mL), to which was added DBU (1.19 mL, 7.90 mmol) at room temperature. Then, the reaction mixture was cooled to -78 °C, and ozone was bubbled through over 30 min. After quenching the reaction with acetic acid (1 mL), the resulting mixture was warmed up to room temperature. After removing the solvent under reduced pressure, the residue was partitioned between EtOAc (20 mL) and an aqueous saturated solution of NH4Cl (30 mL). The aqueous layer was extracted with EtOAc (20 mL × 3), and the combined organic layers were dried over MgSO4, filtered, and concentrated under reduced pressure. The organic residue was purified by silica gel chromatography (hexane : EtOAc = 8 : 1) to afford the desired ester 2a (546 mg, 1.90 mmol, 72%) as a colorless oil.

2a : 3-tert-Butyl 5-methyl (4R,5S)-4-isobutyloxazolidin-3,5-dicarboxylate : Yield 72% (546 mg); colorless oil; [α]20D = +5.8 (c = 2.8, CHCl3); 1H NMR δ 0.99 (d, 3H, J = 6.4), 1.01 (d, 3H, J = 6.4), 1.44-1.50 (m, 1H), 1.48 (s, 9H), 1.60 (m, 1H), 1.69 (m, 1H), 2.79 (s, 3H), 4.24 (br s, 1H), 4.37 (d, 1H, J = 1.6), 4.85 (d, 1H, J = 3.6), 5.28 (s, 1H); 13C NMR δ 22.1, 22.8, 25.2, 28.3, 42.2, 52.3, 57.8, 79.0, 79.0, 80.6, 152.8, 171.4.

2b : 3-tert-Butyl 5-methyl (4R,5S)-4-isobutyloxazolidin-3,5-dicarboxylate : 50% (415 mg), colorless oil; [α]20D = +10.5 (c = 0.64, CHCl3); 1H NMR δ 0.94 (d, 3H, J = 7.0), 0.96 (d, 3H, J = 7.0), 1.44 (s, 9H), 1.48 (m, 1H), 3.46 (s, 3H), 3.74 (br s, 1H), 4.44 (s, 1H), 4.80 (d, 1H, J = 3.2), 5.23 (br s, 1H); 13C NMR δ 17.8, 18.9, 28.3, 31.0, 52.4, 64.6, 76.8, 80.0, 80.8, 153.3, 171.9.

2.3. General procedure for dipeptide derivatives 3

To 2a (R1 = i-Bu, 753 mg, 2.62 mmol) in THF (6 mL) at 0 °C was added 2 N NaOH (6 mL). After the reaction mixture was stirred at room temperature for 1 h, it was acidified with 2 N aq. HCl to pH 1 at 0 °C. The resulting mixture was then partitioned between EtOAc (20 mL) and brine (5 mL). The aqueous phase was extracted with EtOAc (20 mL × 2), and the combined organic layers were dried over MgSO4, filtered and concentrated under reduced pressure to give crude 2a. To crude 2a in THF (10 mL) at 0 °C, HOBT (203 mg, 1.50 mmol) and t-Leu-OMe (250 mg, 1.38 mmol) were added. Then, EDC-HCl (288 mg, 1.50 mmol), and N,N-diisopropylethylamine (44 µL, 0.23 mmol) were added to the reaction mixture. The mixture was stirred at room temperature overnight. After the removal of the solvent, the residue was partitioned between a saturated aq. solution of NH4Cl (20 mL) and EtOAc (20 mL). The aqueous phase was extracted with EtOAc (20 mL × 2), and the combined organic layers were dried over MgSO4, filtered and concentrated under reduced pressure. The organic residue was purified by silica gel chromatography (hexane : EtOAc = 8 : 1) to afford the dipeptide intermediate 3a as a colorless oil.

3a : [3-tert-Butyl 5-methyl (4R,5S)-4-isobutyloxazolidin-5-carboxylic acid]-t-leucine methyl ester : Yield 74% (370 mg); [α]20D = -17.4 (c = 0.88, CHCl3); colorless oil; 1H NMR δ 0.94 (dd, 6H, J = 2.8, 6.2), 1.00 (t, 6H, J = 6.4), 1.47 (s, 9H), 1.54-1.63 (m, 3H), 1.66-1.77

Figure 2. New bestatin analogs.
propanoic acid: colorless film; Yield quant. (223 mg); Mp. 61
(20 mL × 2), and the combined organic layers were dried over MgSO4,
mL) and brine (5 mL). The aqueous phase was extracted with EtOAc
room temperature for 1 h, it was acidified with 2 N HCl to pH 1 at
added 2 N NaOH (6 mL). After the reaction mixture was stirred at

HRMS (CI) calcd for C20H37N2O6 401.2652 ([M+H]+), found 401.2651.

41.4, 42.7, 50.3, 52.3, 57.9, 78.2, 80.9, 81.7, 152.9, 170.4, 172.9;
13C NMR (MeOH-d6) δ 21.9, 22.4, 22.8, 24.9, 25.0, 28.3, 41.4, 42.7, 50.3, 52.3, 57.9, 78.2, 80.9, 81.7, 152.9, 170.4, 172.9;
HRMS (CI) calcd for C20H37N2O6 401.2652 ([M+H]+), found 401.2651.

4b : [3-tert-Butyl 5-methyl (4R,5S)-4-isopropylloxazolidine-5-carbox-
ylic acid]-l-leucine methyl ester : Yield 76% (264 mg); [α]D = -13.7
(c = 0.81, CHCl3); colorless oil; 1H NMR δ 0.92 (dd, 6H, J = 1.6, 6.4), 0.99 (t, 6H, J = 7.0), 1.45 (s, 9H), 1.54-1.61 (m, 2H), 1.63-1.69
(m, 1H), 1.94-1.99 (m, 1H), 3.74 (s, 3H), 4.10 (dd, 1H, J = 2.2, 6.6),
4.34 (d, 1H, J = 2.8), 4.59-4.65 (m, 1H), 4.74 (d, 1H, J = 4.8), 5.31
(br s, 1H), 6.91 (d, 1H, J = 8.4); 13C NMR δ 18.5, 18.9, 22.0, 23.2, 26.1, 29.3, 41.3, 52.5, 65.9, 78.3, 81.0, 153.4, 171.0, 173.0;
HRMS (FAB) calcd for C19H35N2O6 387.2495 ([M+H]+), found 387.2496.

2.4. General procedure for bestatin analogs 1
To 3a (R1 = i-Bu, 370 mg, 0.92 mmol) in THF (6 mL) at 0 °C
was added 2 N NaOH (6 mL). After the reaction mixture was stirred at
room temperature for 1 h, it was acidified with 2 N HCl to pH 1 at
0 °C. The reaction mixture was then partitioned between EtOAc (20
mL) and brine (5 mL). The aqueous phase was extracted with EtOAc
(20 mL × 2), and the combined organic layers were dried over MgSO4,
filtered and concentrated under reduced pressure.

To the obtained crude in CH2Cl2 was added trifluoroacetic acid (39
μL, 5.15 mmol) and the reaction mixture was reacted at room temperature
overnight. After the removal of the solvents, the reaction mixture
was diluted with water (10 mL). The aqueous layer was washed with
EtOAc (10 mL) three times and then the aqueous layer was condensed
under reduced pressure to afford bestatin analog 1a as a white solid
in 95% yield.

1a : (2S,3R)-3-Amino-2-hydroxy-5-methylhexanamindo-4-methyl-
penantoic acid : Yield 95% (235 mg); Mp. 131 °C; [α]D = -20.4 (c
= 2.1, H2O); 1H NMR (MeOH-d6) δ 0.98 (m, 12H), 1.50 (m, 1H),
1.63-1.77 (m, 5H), 3.50 (br s, 1H), 4.22 (br s, 1H), 4.43-4.46 (m, 1H);
13C NMR (MeOH-d6) δ 22.0, 22.3, 22.9, 23.3, 25.2, 26.1, 29.3, 39.2, 41.3,
52.2, 53.3, 71.1, 173.6, 175.7; HRMS (FAB) calcd for C19H35N2O6

1b : (2S,3R)-3-Amino-2-hydroxy-5-methylhexanamindo-4-methyl-
propanoic acid : colorless film; Yield quant. (222 mg); Mp. 61 °C;
[α]D = -28.4 (c = 2.2, H2O); 1H NMR (MeOH-d6) δ 0.97 (dd, 6H,
or unnatural bioactive γ-amino-β-hydroxy acids, such as (-)-statine[21], 3-aminodeoxystatin[22], threo-β-hydroxy-L-glutamic acid[23], and (3R,4S)-AHPPA derivatives[24].

Contrast to the previous syntheses of γ-amino-β-hydroxy acids, the phenylsulfonylnitroolefin group was selected as a suitable Michael acceptor for the synthesis of β-amino-α-hydroxy acids because the phenylsulfonylnitromethyl group on 5 (Scheme 2), resulted from the intramolecular conjugate addition of the N-hydroxymethyl group to the phenylsulfonylnitroolefin group of 7 (see below, Schemes 3), could be readily converted to the methyl ester group by ozonolysis in a methanolic solution via an oxidative Nef reaction[25].

Thus, the condensation reactions between configurationally stable α-amino aldehydes 4 and phenylsulfonylnitromethane (PhSO₂CH₂NO₂) under the weakly basic conditions[26] yielded the diastereomeric mixture of trans-oxazolidines 5, which were oxidized in-situ to give the desired β-amino-α-hydroxy acid derivatives, 2a and 2b, with an excellent stereoselectivity[18]. No minor cis-stereoisomers of 2a and 2b were observed on their ¹H NMR spectra. The desired absolute stereochemistry of 2a and 2b was derived from the corresponding D-amino acids, whereas the previous synthesis of threo-β-amino-α-hydroxy acids from the L-amino acids gave the enantiomers of 2a and 2b[18].

Here, the different reactivity between 4a and 4b needs to be mentioned. Most of a valinal derivative 4b remained intact after the reaction with phenylsulfonylnitromethane at room temperature, whereas a leucinal derivative 4a smoothly reacted to produce the condensation product 5a under the same reaction conditions, which was converted into the corresponding methyl ester 2a in a 72% overall yield. The disappointing result with 4b was solved by simply raising the reaction temperature to 50 °C, from which the methyl ester 2b was obtained in a 50% overall yield after the in-situ ozonolysis.

Formation of the condensation products 5 could be explained by the three cascade reactions as shown in Scheme 3[18]. The initial nitroalcohols 6 resulted from the nitroaldol reaction between 4 and PhSO₂CH₂NO₂ were dehydrated in-situ to result in the phenylsulfonylnitroolefin intermediates 7, which underwent the intramolecular conjugate addition to yield the cyclized adducts 5. The excellent stereoselectivity (>20:1) for trans-oxazolidines 5 could be rationalized by the favored H-epiplanar conformation of 7[27-28]. The slower reactivity by the isopropyl analog 4b (R = i-Pr) might be also explained from the proposed mechanism (Scheme 3). The dehydration step of 6b (R = i-Pr) with the bulky isopropyl group would be slower compared to that of 6a (R = i-Bu) because the increased allylic strains (both A₁,3 and A₁,2 strains) between the R group the substituents (-NO₂ or -SO₂Ph, and -CH=C) on the double bond.

With trans-oxazolidine methyl esters 2a and 2b in hand that were properly protected forms of β-amino-α-hydroxy acids, the peptide coupling of 2a and 2b with L-Leu-OMe afforded the corresponding dipptide precursors, 3a and 3b, in 74% and 76% yields, respectively, after the basic hydrolysis of methyl esters 2 to the corresponding carboxylic acids (not shown). Finally, the global deprotection under the sequential basic and acidic hydrolysis conditions produced the desired isobutyl or isopropyl substituted bestatin analogs, 1a and 1b, in more than 95% yields.

4. Conclusions

We have reported an efficient and stereoselective synthesis of two new alkyl substituted bestatin analogs 1 from the appropriately protected β-amino-α-hydroxy acid derivatives 2, which were in turn successfully prepared with more than 20:1 stereoselectivity from stable α-amino aldehydes 4 via the stereoselective condensation reactions with phenylsulfonylnitromethane followed by the in-situ ozonolysis. Therefore, the isobutyl and isopropyl analogs of bestatin, 1a and 1b, were reported for the first time. The new isobutyl and isopropyl analogs of bestatin, 1a and 1b, were produced in overall 51% and 38% yields with high stereoselectivity from the corresponding protected α-amino aldehydes 4 in 6 steps, respectively. Further biological tests of the two bestatin analogs will be performed soon.

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References


