 Chem. Pharm. Bull. 42(12) 2636—2640 (1994) Vol. 42, No. 12

Synthesis and Human Immunodeficiency Virus (HIV)-1 Protease Inhibitory Activity of Tripeptide Analogues Containing a Dioxoethylene Moiety[1)]

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Received April 21, 1994; accepted July 28, 1994

Tripeptide analogues 2 and 3 containing a dioxoethylene moiety were designed based on the characteristic structure of the naturally occurring human immunodeficiency virus (HIV)-1 protease inhibitors RPI-856 A, B, C and D (1). The compounds (2, 3) prepared showed high inhibitory activity, comparable to that of RPI-856 A, against HIV-1 protease *in vitro*.

Keywords human immunodeficiency virus (HIV) protease inhibitor; tripeptide analogue; dioxoethylene isostere; transition state mimic; HIV protease inhibitory activity

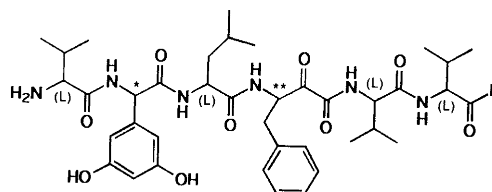
A number of human immunodeficiency virus (HIV) protease inhibitors have been designed on the basis of the concept of a transition-state mimic.[2)] Roberts and coworkers reported that incorporation of a hydroxyethylene moiety into the tripeptide fragment L-Asn–L-Phe–L-Pro in the substrate protein resulted in high inhibitory activity and that acylation at the N-terminal and amidation of the C-terminal were critical for the high activity.[3)] Furthermore, the hydroxymethylcarbonyl isosters of the tripeptide were reported to possess high inhibitory activity by Mimoto and coworkers.[4)] Tam and coworkers also described hydroxymethylcarbonyl compounds as well as a dioxoethylene analogue with potent activity.[5)] These recent studies prompted us to report our design and synthesis of tripeptide analogues containing a dioxoethylene moiety.

In our search for HIV protease inhibitors, we have screened microbial metabolites, and we previously found several novel inhibitors, RPI-856 A, B, C and D (**1**: Chart 1), in the culture filtrate of *Streptomyces* sp. AL-322.[6)] RPI-856 A, B, C and D are hexa- and heptapeptide analogues containing a characteristic dioxoethylene moiety. The fact that **1** showed very potent HIV-1 protease inhibitory activity suggested that the dioxoethylene moiety might form the hydrate at the C_2-carbonyl group and combine with carboxylic acids at the active site of the protease. The hydrated form of the dioxoethylene moiety seems to mimic the transition state of the substrate for HIV protease, and therefore, we incorporated this dioxoethylene structure into the tripeptide L-Asn–L-Phe–L-Pro in the hope of obtaining a new structural type of HIV-1 protease inhibitor.

We chose benzyloxycarbonyl (Z) and 2-quinolylcarbonyl (Q) groups as substituents at the N-terminal and *N-tert*-butylamide for the C-terminal. In this report, we describe the synthesis of the tripeptide analogues **2**[5)] and **3** (Chart 2) and their HIV-1 protease inhibitory activity *in vitro*.

Chemistry The coupling of (2*RS*,3*S*)-**4**[7)] and **5**[8)] gave a diastereomeric mixture of **6a** (more polar) and **6b** (less polar) in a ratio of *ca.* 1 : 1. The removal of the Z group of the isomers (**6a, b**) afforded the amino derivatives (**7a, b**), and these were allowed to react with **8** to give the hydroxymethylcarbonyl compounds **10a** and **10b**, respectively. The absolute configuration of C* in each isomer was not determined at this stage. The alcohols **10a, b** were each oxidized with dimethylsulfoxide–1,3-dicyclohexylcarbodiimide (DMSO–DCC) to form the same product A, which showed a substantial single peak on HPLC. Purification of A was attempted by silica gel column chromatography to remove the small amount of impurities. HPLC analysis of the product after chromatography indicated the formation of another compound B in a *ca.* 2 : 1 ratio of A/B. Silica gel-catalyzed epimerization at the methine carbon adjacent to the dioxoethylene moiety was suspected. Thus, purification of product A was carried out by preparative chromatography on an octadecyl silica column (ODS). The white powder obtained showed a single peak on HPLC (>99% de), which was confirmed to be **2** from the ^{1}H-NMR spectrum and the secondary ion mass spectrum (SI-MS). Then, the mixture of A and B (*ca.* 2 : 1) was separated into individual components by chromatography on ODS. Component B (>99% de) isolated showed the same molecular weight as that of **2** on SI-MS, and the ^{1}H-NMR and IR spectra were similar to those of **2**. On the basis of these data, compound B was assigned to be the epimer, *epi*-**2**.

As described above, individual oxidation of diastereomeric alcohols **10a, b** gave the desired compound **2** exclusively. Even when the mixture was used, compound



1 (RPI-856)
A, B : R = L-Asp
C, D : R = OH

Chart 1

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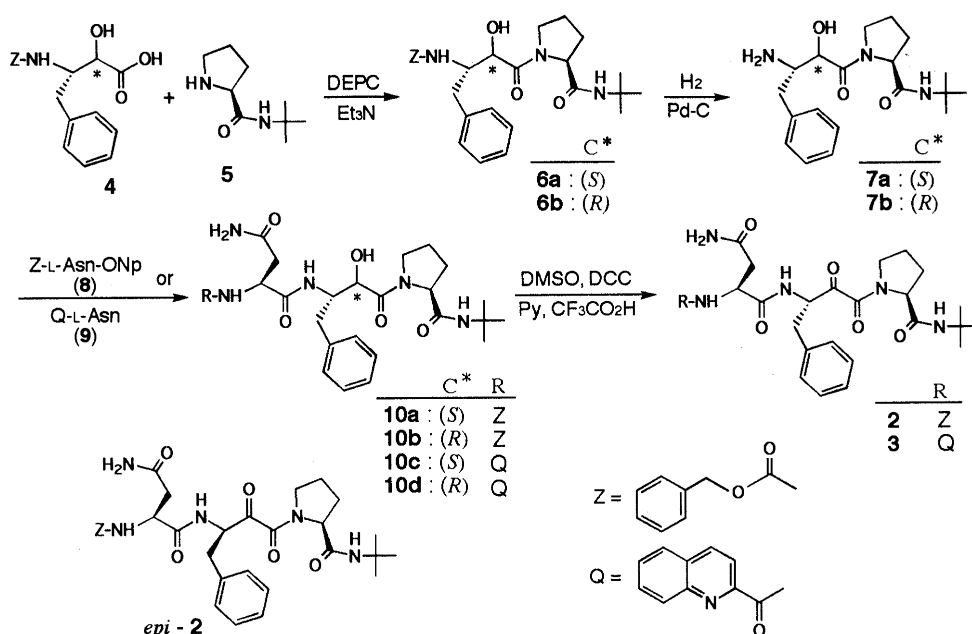

Chart 2

2 was obtained as a single product. Therefore, for the purpose of preparing **2**, separation, stereoselective preparation or assignment of the absolute configuration of the alcohols (**10a, b**) proved to be unnecessary. However, we determined the absolute configuration of C* in the precursors **10a** and **10b** by the following method. The enantiomer (2*S*,3*S*)-**4**[9] was coupled to **5**. Since the product obtained was identical to **6a**, the configuration of C* in **10a** was assigned as (*S*), and that in **10b** should be (*R*).

Next, compounds **7a** and **7b** were condensed with **9**[10] to give two diastereomers, **10c** and **10d**, respectively. As in the case of the synthesis of **2**, oxidation of the individual alcohols with DMSO–DCC gave the same product, and HPLC analysis using an ODS column showed a single peak.[11] This product was purified by chromatography on ODS to yield compound **3** in >99% de.

HIV-1 Protease Inhibitory Activity The tripeptide analogues containing a dioxoethylene moiety, **2**, *epi*-**2** and **3**, were evaluated for inhibitory activity against HIV-1 protease, and the results are shown in Table I.

Compounds **2** and **3** showed high inhibitory activities and their IC_{50} values are nearly equal to that of RPI-856 A. We have postulated that the hydrate mimics a transition state of the substrate proteins. This assumption is supported by the recent work of Ocain and Rich, who showed by examination of the ^{13}C-NMR spectrum that a dioxoethylene analogue of an amino acid forms the hydrate in a mixture of DMSO-d_6 and water (DMSO-d_6–D_2O).[12] Similarly, the ^{13}C-NMR spectrum of compound **2** in DMSO-d_6–D_2O showed a new peak at 104.9 ppm in comparison with that measured in DMSO-d_6, and this peak could be assigned to the hydrated carbonyl carbon.[13]

On the other hand, *epi*-**2** and RPI-856 B are the epimers of **2** and RPI-856 A, respectively, at the methine carbon adjacent to the dioxoethylene moiety, although the configuration of this carbon in RPI-856 A and B has not yet been determined. Compound **2**, which has the (*S*)-configuration, is five times more potent than *epi*-**2**, while RPI-856 A is seven times more potent than RPI-856 B. On the basis of the activity differences within these two sets of epimers, the configuration of the carbon (C** in **1**) adjacent to the dioxoethylene moiety in RPI-856 A is presumed to be (*S*).

Table I. HIV-1 Protease Inhibitory Activity

Compound No.	IC_{50} (nM)
2	64
epi-**2**	300
3	44
RPI-856A	37
RPI-856B	260

Furthermore, the synthetic intermediates, **10a**[14] and **10c**, also showed high activity (IC_{50}: 49 and 44 nM in our assay, respectively).[4,5,15] In contrast to this high potency, in the case of RPI-856 A it was reported that a significant decrease in activity was observed upon reduction of the dioxoethylene moiety to give the hydroxymethylcarbonyl analogue.[6]

The observations described above revealed that the dioxoethylene group can play the role of a hydrated transition state mimic not only in hexa- and heptapeptides such as **1** but also in tripeptide analogues such as **2** and **3**. Thus, incorporation of this moiety into peptide compounds could be a useful tool in designing new protease inhibitors, although the susceptibility to epimerization at the α-carbon should be carefully inspected.

Experimental

Melting points were determined using a Yanagimoto melting point apparatus and are uncorrected. IR spectra were measured with a JASCO IR-810 spectrometer. ^{1}H-NMR spectra were taken on a Varian

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Table II. Physicochemical and Spectral Data

No.	mp (°C)	Formula	Analysis (%) Calcd (Found)			^{1}H-NMR (in $CDCl_3$) δ	SIMS (m/z) MH^+	$[\alpha]_D^{25}$ (c) MeOH	IR (C=O) cm^{-1} (KBr)
			C	H	N				
6a	63—66	$C_{27}H_{35}N_3O_5 \cdot 1/2H_2O$	66.10 (66.40	7.40 7.43	8.57 8.54)	1.29 (9H, s), 1.75—2.40 (4H, m), 2.60—2.80 (2H, m), 3.60—4.25 (5H, m), 4.30—4.65 (2H, m), 4.99 (2H, s), 5.43 (1H, d, J=8.8 Hz), 6.49 (1H, s), 7.10—7.40 (10H, m)	482	−24.5° (0.4)	1705 1680
6b	114—115	$C_{27}H_{35}N_3O_5$	67.34 (67.51	7.33 7.29	8.73 8.77)	1.29 (9H, s), 1.70—2.30 (4H, m), 2.95 (2H, d, J=7.8 Hz), 3.00—3.40 (2H, m), 3.87 (1H, d, J=5.6 Hz), 3.95 (1H, d, J=3.6 Hz), 4.10 (1H, d, J=4.6 Hz), 4.22 (1H, m), 5.03 (2H, s), 5.09 (1H, br), 6.43 (1H, br), 7.20—7.50 (10H, m)	482	−82.3° (0.4)	1710 1690
7a	154.5—156	$C_{19}H_{29}N_3O_3$	65.68 (65.35	8.41 8.55	12.09 12.13)	1.29 (8H, s), 1.35 (1H, s),[a] 1.40—2.20 (2H, br), 1.70—2.40 (4H, m), 2.53 (1H, dd, J=13, 10 Hz), 2.95 (1H, dd, J=13, 3.2 Hz), 3.00—3.20 (1H, m), 3.50—3.70 (2H, m), 4.27 (1H, d, J=5.4 Hz), 4.45—4.55 (1H, m), 6.57 (1H, br), 7.10—7.40 (6H, m)	—[b]	—[b]	1650
7b	Amorphous	$C_{19}H_{29}N_3O_3$	—	—	—[b]	1.24 (2H, s), 1.30 (7H, s),[a] 1.70—2.15 (3H, m), 2.24—2.37 (1H, m), 2.65—3.00 (2H, m), 3.10—3.40 (3H, m), 3.49 (2H, s), 4.10 (1H, br), 4.20—4.40 (1H, m), 6.05 (0.1H, br), 6.58 (0.9H, br), 7.20—7.40 (6H, m)	—[b]	—[b]	1640
10a	102—103	$C_{31}H_{41}N_5O_7$	62.51 (62.20	6.94 7.06	11.76 11.66)	1.30 (9H, s), 1.75—2.85 (10H, m), 3.40—3.50 (0.2H, m), 3.59—3.66 (1.8H, br), 4.22—4.35 (0.4H, br), 4.36—4.52 (3.6H, br), 5.09 (2H, s), 5.62 (0.8H, br), 5.73 (0.2H, br), 6.10 (1H, br), 6.24 (1H, d, J=7 Hz), 6.48 (0.2H, s), 6.56 (0.8H, s), 7.09—7.38 (9H, m), 7.40—7.60 (0.8H, m), 7.65—7.75 (0.2H, m)	596	−38.7° (0.3)	1665
10b	103—108	$C_{31}H_{41}N_5O_7 \cdot 1/2H_2O$	61.57 (61.87	7.00 7.09	11.58 11.50)	1.26 (7.5H, s), 1.35 (1.5H, s),[a] 1.55—2.15 (6H, br), 2.59—2.85 (2H, m), 2.85—3.00 (2H, m), 3.05—3.36 (2H, m), 4.08—4.29 (2H, m), 4.35—4.55 (2H, m), 5.11 (2H, s), 5.60—5.80 (1H, br), 6.20 (1H, d, J=7 Hz), 6.20—6.40 (1H, br), 6.53 (1H, br), 7.10—7.55 (10H, m)	596	−84.3° (0.5)	1665
10c	Amorphous	$C_{33}H_{40}N_6O_6 \cdot H_2O$	62.45 (62.20	6.67 6.80	13.24 13.19)	(DMSO-d_6, rt): 1.24 (8H, s), 1.30 (1H, s),[a] 1.70—2.20 (4H, m), 2.44—2.82 (4H, m), 3.60—3.80 (2H, m), 4.05—4.30 (1H, br), 4.32—4.45 (1.8H, m), 4.55—4.65 (0.2H, m), 4.78—5.00 (2H, m), 6.90—7.23 (4H, m), 7.31 (2H, d, J=7 Hz), 7.40 (1H, br), 7.53 (1H, s), 7.74 (1H, t, J=8 Hz), 7.89 (1H, t, J=8 Hz), 8.08—8.21 (4H, m), 8.60 (1H, d, J=8 Hz), 9.00 (1H, d, J=8 Hz). (DMSO-d_6, 110°C): 1.24 (9H, s), 1.72—2.09 (4H, m), 2.60—2.72 (3H, m), 2.77—2.83 (1H, m), 3.52—3.72 (2H, br), 4.22—4.46 (3H, br), 4.52—4.62 (1H, br), 4.77—4.82 (1H, m), 6.70—6.90 (2H, br), 6.95—7.07 (4H, m), 7.21 (2H, br), 7.70 (1H, ddd, J=8.1, 6.8, 1.2 Hz), 7.70—7.80 (1H, m), 7.85 (1H, ddd, J=8.5, 6.8, 1.5 Hz), 8.04 (1H, d, J=8.3 Hz), 8.10 (1H, d, J=8.3 Hz), 8.14 (1H, d, J=8.6 Hz), 8.52 (1H, d, J=8.3 Hz), 8.83 (1H, d, J=8.1 Hz)	617	+1.6° (0.5)	1670
10d	Amorphous	$C_{33}H_{40}N_6O_6 \cdot 1/2H_2O$	63.34 (63.04	6.60 6.82	13.43 13.45)	(DMSO-d_6): 1.22 (8H, s), 1.27 (1H, s),[a] 1.70—2.05 (4H, m), 2.40—3.00 (4H, m), 3.25—3.50 (4H, m), 4.00—4.08 (0.2H, m), 4.11—4.24 (1.8H, m), 4.24—4.42 (0.9H, m), 4.52—4.58 (0.1H, m), 4.72—4.86 (1.9H, m), 5.15—5.20 (0.1H, m), 6.97 (1H, br), 7.08—7.31 (5H, m), 7.41 (0.9H, br), 7.58 (0.1H, br), 7.74 (1H, t, J=8 Hz), 7.89 (1H, t, J=8 Hz), 7.99—8.21 (3H, m), 8.59 (1H, d, J=8 Hz), 8.93 (1H, d, J=8 Hz)	617	−53.6° (0.4)	1670
2	88—92	$C_{31}H_{39}N_5O_7 \cdot 1/2H_2O$	61.78 (62.01	6.69 6.70	11.62 11.69)	1.28 (6H, s), 1.34 (3H, s),[a] 1.59—2.19 (4H, m), 2.46—2.90 (2H, m), 3.06—3.72 (4H, m), 4.05—4.32 (0.7H, m), 4.42—4.62 (1.3H, m), 4.98—5.10 (1H, m), 5.08 (2H, s), 5.46—5.56 (1H, br), 5.82 (0.3H, br), 6.10 (1H, br), 6.21 (0.7H, s), 6.26 (0.3H, d, J=8 Hz), 6.45 (0.7H, d, J=8 Hz), 7.14—7.30 (5.7H, m), 7.33 (5H, s), 7.40—7.45 (0.3H, m)	594	−87.1° (0.25)	1718 1660
***epi*-2**	90—94	$C_{31}H_{39}N_5O_7 \cdot 1/2H_2O$	61.78 (61.90	6.69 6.56	11.62 11.53)	1.32 (9H, s), 1.70—2.20 (4H, m), 2.38—3.11 (3H, m), 3.21—3.40 (1H, m), 3.48—3.72 (2H, m), 4.32—4.37 (1H, br), 4.48—4.60 (1H, m), 4.97—5.32 (1H, br), 5.10 (2H, s), 5.42—5.50 (1H, br), 5.58 (0.3H, br), 5.92—5.99 (1H, br), 6.27 (0.7H, br), 6.32—6.45 (1H, m), 7.14—7.30 (5.7H, m), 7.35 (5H, s), 7.72—7.78 (0.3H, m)	594	−19.4° (0.15)	1716 1660
3	110—114	$C_{33}H_{38}N_6O_6 \cdot 1/2H_2O$	63.55 (63.33	6.30 6.34	13.47 13.55)	(DMSO-d_6): 1.22 (5H, s), 1.23 (4H, s),[a] 1.55—2.20 (4H, br), 2.50—2.86 (2H, m), 2.92—3.58 (4H, m), 4.22—4.29 (0.5H, m), 4.54—4.60 (0.5H, m), 4.79—5.08 (2H, br), 6.97 (1H, br), 7.09—7.23 (5H, m), 7.43 (1H, br), 7.51 (1H, br), 7.73 (1H, t, J=7 Hz), 7.88 (1H, t, J=7 Hz), 8.10 (2H, d, J=7 Hz), 8.15 (1H, d, J=8.6 Hz), 8.53—8.65 (1H, m), 8.59 (1H, d, J=8.6 Hz), 8.93 (0.5H, d, J=8.6 Hz), 8.99 (0.5H, d, J=8.6 Hz)	615	−55.0° (0.24)	1720 1665

a) Ref. 16. *b*) Not measured.

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Gemini-200 spectrometer or a JEOL JNM-GX 400FT spectrometer with tetramethylsilane as an internal standard. The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. SI-MS were measured with a Hitachi M-80A mass spectrometer. The optical rotations were recorded with a JASCO DIP-181 or DIP-370 digital polarimeter. The physicochemical and spectral data of the compounds prepared are shown in Table II.

Reactions were run at room temperature unless otherwise noted and traced by TLC on Silica gel 60 F_{254} precoated TLC plates (E. Merck) or by HPLC using an ODS column (A-303, 4.6 mm i.d. × 250 mm, Yamamura Chemical Laboratories Co.). Standard work-up procedures were as follows. The reaction mixture was partitioned between the indicated solvent and water. Organic extracts were combined and washed in the indicated order using the following aqueous solutions; water, 10% aqueous phosphoric acid solution (aqueous H_3PO_4), 5% aqueous sodium bicarbonate solution (aqueous $NaHCO_3$) and saturated NaCl solution (brine). Extracts were dried over $MgSO_4$, filtered and evaporated *in vacuo*.

Chromatographic separations were carried out on Silica gel 60 (0.063—0.200 mm, E. Merck) or ODS (CPO-223L, pre-packed column, 22 mm × 300 mm, Kusano Kagaku Kikai Co.) using the indicated eluents.

The HIV-1 protease inhibitory activity was measured by the method described in our previous report.[6]

1-[(2*S*,3*S*)-3-Benzyloxycarbonylamino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-prolinamide (6a) and 1-[(2*R*,3*S*)-3-Benzyloxycarbonylamino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-prolinamide (6b) Diethyl phosphorocyanidate (DEPC, 2.09 g, 12.2 mmol) and Et_3N (1.7 ml, 12.2 mmol) were added to a mixture of (2*RS*,3*S*)-**4** (2 g, 6.1 mmol)[7] and **5** (1.24g, 7.3 mmol)[8] in *N*,*N*-dimethylformamide (DMF, 20 ml) at 0 °C. The resulting mixture was stirred for 1 h at 0 °C, and then stirred for 2.5 h at room temperature. Water (5 ml) was added. After being stirred for 1 h, the mixture was worked up (AcOEt: aqueous H_3PO_4, aqueous $NaHCO_3$, brine). The residue was subjected to silica gel column chromatography (AcOEt–hexane, 2 : 1) to give **6b** (0.42 g, 14%) as a white powder from the first fraction and **6a** (0.69 g, 23%) as a white powder from the second fraction. Compound **6a** was prepared from (2*S*,3*S*)-**4**[9] and **5** in a manner similar to that described above.

1-[(2*S*,3*S*)-3-Amino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-prolinamide (7a) and 1-[(2*R*,3*S*)-3-Amino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-prolinamide (7b) A solution of compound **6a** (0.3 g, 0.62 mmol) in MeOH–water (4 : 1, 10 ml) was hydrogenated for 4 h over 10% palladium carbon (Pd–C, 50%, wet, 40 mg) under ordinary pressure. The catalyst was filtered off, and washed with 50% aqueous MeOH (20 ml). The filtrate and the washing were combined and evaporated *in vacuo*. The residue was crystallized from Et_2O to give **7a** (210 mg, 98%) as a white crystalline powder. According to the procedure described above, compound **7b** was prepared as a white amorphous powder from **6b** in 93% yield.

1-[(2*S*,3*S*)-3-(N^2-Benzyloxycarbonyl-L-asparaginyl)amino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-prolinamide (10a) and 1-[(2*R*,3*S*)-3-(N^2-Benzyloxycarbonyl-L-asparaginyl)amino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-prolinamide (10b) A mixture of **8** (267 mg, 0.69 mmol)[17] and **7a** (240 mg, 0.69 mmol) in DMF (8 ml) was stirred for 1 h at 0 °C, then for 4 h at room temperature. The solvent was removed by evaporation under reduced pressure. Work-up (AcOEt; aqueous H_3PO_4, aqueous $NaHCO_3$, brine) followed by chromatography on silica gel (AcOEt–MeOH, 9 : 1) afforded **10a** (200 mg, 49%) as a white powder. According to the procedure described above, compound **10b** was obtained as a white powder in 51% yield from **7b**.

1-[(3*S*)-3-(N^2-Benzyloxycarbonyl-L-asparaginyl)amino-2-oxo-4-phenylbutyryl]-*N*-*tert*-butyl-L-prolinamide (2) Trifluoroacetic acid (0.02 ml, 0.27 mmol) and **10b** (320 mg, 0.54 mmol) were added to a mixture of DCC (332 mg, 1.6 mmol), pyridine (0.04 ml, 0.54 mmol) and DMSO (1.6 ml) in benzene (3.2 ml) at 0 °C. The mixture was stirred for 22 h, then AcOEt (5 ml) was added. The resulting insoluble substance was filtered off. The filtrate was worked up (AcOEt; water) and purified by preparative chromatography on ODS (CH_3CN–H_2O, 2 : 3) to give **2** (112 mg, 35%, >99% de) as a white powder. HPLC analysis of this product on ODS showed a single peak at 26 min under the following conditions: CH_3CN–H_2O, 2 : 3; flow rate, 0.8 ml/min; detection, UV at 262 nm. Oxidation of **10a** according to the same method as above gave the same product.

1-[(3*R*)-3-(N^2-Benzyloxycarbonyl-L-asparaginyl)amino-2-oxo-4-phenylbutyryl]-*N*-*tert*-butyl-L-prolinamide (*epi*-2) The crude product (140 mg) of oxidation as described in the synthesis of **2** was submitted to silica gel column chromatography (AcOEt–CH_2Cl_2–MeOH, 5 : 5 : 1). The white powder obtained (90 mg) was analyzed by HPLC (ODS) under the following conditions: CH_3CN–H_2O, 2 : 3; flow rate, 0.8 ml/min; detection, UV at 262 nm. Two peaks, A (compound **2**) and B, were observed at 26 and 28 min, respectively, in a *ca.* 2 : 1 ratio. This mixture (78 mg) was separated by preparative chromatography on ODS (CH_3CN–H_2O, 2 : 3). Component A, obtained as a white powder (40 mg) from the first fraction, was identical with **2**. Component B (*epi*-**2**, 10 mg, >99% de) was obtained as a white powder from the second fraction.

1-[(2*S*,3*S*)-2-Hydroxy-3-[N^2-(2-quinolylcarbonyl)-L-asparaginyl]amino-4-phenylbutyryl]-*N*-*tert*-butyl-L-prolinamide (10c) and 1-[(2*R*,3*S*)-2-Hydroxy-3-[N^2-(2-quinolylcarbonyl)-L-asparaginyl]amino-4-phenylbutyryl]-*N*-*tert*-butyl-L-prolinamide (10d) HOBt (156 mg, 1.15 mmol) and DCC (237 mg, 1.15 mmol) were added to a solution of **9** (300 mg, 1.04 mmol)[10] and **7a** (360 mg, 1.04 mmol) in DMF (6 ml) at a temperature below −5 °C and the resulting mixture was stirred for 1 h at −5 °C, then for 19 h at room temperature. The resulting precipitate was removed by filtration and the filtrate was evaporated under reduced pressure. The residue was worked up (AcOEt; aqueous H_3PO_4, aqueous $NaHCO_3$, brine) and subjected to chromatography on silica gel (AcOEt–CH_2Cl_2–MeOH, 5 : 5 : 1) to give **10c** (400 mg, 61%) as a white amorphous powder. According to the procedure described above, compound **10d** was obtained as a white amorphous powder in 74% yield from **7b** and **9**.

1-[(3*S*)-2-Oxo-3-[N^2-(2-quinolylcarbonyl)-L-asparaginyl]amino-4-phenylbutyryl]-*N*-*tert*-butyl-L-prolinamide (3) Compound **10d** (350 mg, 0.56 mmol) was converted to **3** (65 mg as a white powder, 19%, >99% de) by a method similar to that described for the synthesis of **2**. HPLC analysis of this product on ODS showed a single peak at 14 min under the following conditions: MeOH–H_2O–AcOH, 7 : 3 : 0.02; flow rate, 0.8 ml/min; detection, UV at 262 nm. Oxidation of **10c** according to the same method as above gave the same product.

Acknowledgments We thank Dr. M. Fujino for his encouragement throughout this work and Prof. S. Harada, Kumamoto University, for fruitful discussions.

References and Notes

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