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SYNTHESIS OF 2-SUBSTITUTED D-TRYPTOPHAN-CONTAINING PEPTIDE DERIVATIVES WITH ENDOTHELIN RECEPTOR ANTAGONIST ACTIVITY

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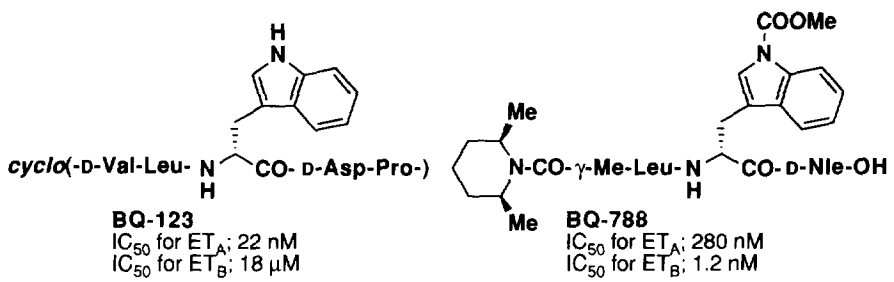
Abstract. Peptide derivatives with 2-substituted D-tryptophan analogues were synthesized. All prepared peptide derivatives showed potent affinity for ET_B receptors, while their ET_A affinity depended on the substituents of the D-tryptophanyl residue. A 2-bromo-D-tryptophan derivative **16b** (BQ-928) was a combined ET_A/ET_B receptor antagonist and a 2-cyano-D-tryptophan derivative **17c** (BQ-017) was a selective ET_B receptor antagonist.

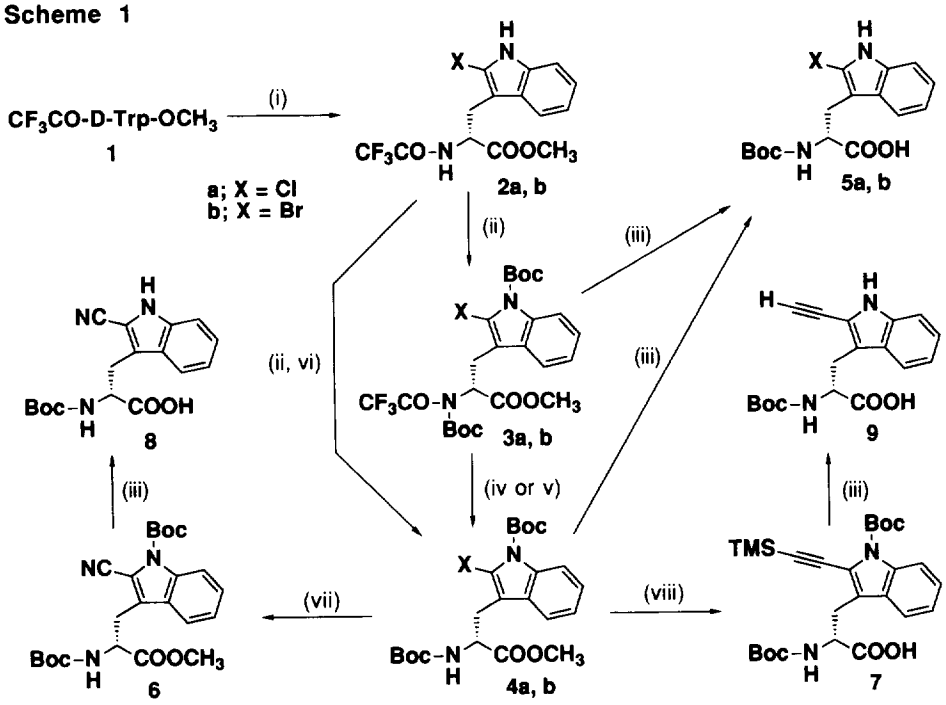
Endothelin (ET)-1, which was first isolated from the culture medium of porcine aortic endothelial cells, is a potent vasoconstrictor consisting of 21 amino acids.[1] Studies including a human genomic analysis have identified two structurally- and functionally-related isopeptides of ET-1 termed ET-2 and ET-3.[2,3,4] Several studies have demonstrated that there are at least two different ET receptor subtypes, ET_A and ET_B.[5,6,7] Since these discoveries, many efforts have been made to identify ET receptor antagonists because they may lead to the development of useful therapeutic agents.[8] We disclosed a selective ET_A receptor antagonist, BQ-123,[9] and a selective ET_B receptor antagonist, BQ-788.[10] In the course of our work on the discovery of BQ-788, one of the



most important modifications for the enhancement of the affinity for the ET_B receptor was the introduction of a methoxycarbonyl group onto the indole nitrogen of the D-tryptophanyl residue. We supposed that the structure around the indole nitrogen of the D-tryptophanyl residue was very important for the subtype selectivity between the ET_A and ET_B receptors and we planned modifications at the C-2 position of the indole ring system of the D-tryptophanyl residue. To our knowledge, the following tryptophan analogues with C-2 substituents are known: alkyl,[11,12] (hetero)aryl,[12,13] thioether,[14] carboxy,[15] carbethoxy[13] and halogen.[16] Among these tryptophan analogues, we were attracted to the 2-halo analogue because 2-halotryptophans are derived from tryptophan retaining a chirality at the C-α position. In contrast, most of the other analogues are prepared as a racemic form

and tedious optical resolution is needed for the preparation of a chiral form. In addition, if the halogen at the C-2 position can be substituted by a nucleophile, a new 2-substituted tryptophan analogue will be obtained. In this paper, we report a new and facile method for synthesizing 2-substituted D-tryptophans by substitution reaction at the C-2 position together with synthesis of 2-substituted D-tryptophan-containing tripeptide derivatives. In addition, we report the binding affinity of these compounds for both ET_A and ET_B receptors and the antagonist activities of representative compounds.

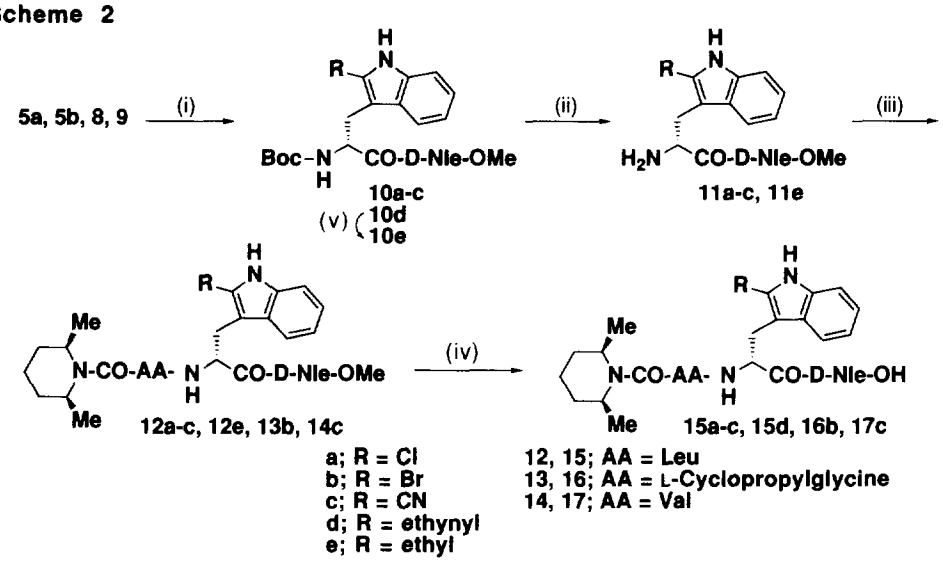
Scheme 1



Reagents: (i) see ref. 16; (ii) Boc_2O, DMAP, CH_3CN; (iii) aq. NaOH, MeOH; (iv) aq. $NaHCO_3$, MeOH; (v) NMM, H_2O, MeOH; (vi) 3-(dimethylamino)propylamine; (vii) CuCN, DMF; (viii) (trimethylsilyl)acetylene, $(Ph_3P)_4Pd$, CuI, diethylamine.

Protected 2-halo-D-tryptophans **2a** and **2b** were synthesized by radical halogenation of a protected D-tryptophan **1** according to the method reported by Phillips and Cohen.[16] They also reported that alkaline hydrolysis of the corresponding L-isomers of **2a** and **2b** did not proceed cleanly due to ionization of N^{in}-H in strongly alkaline media. They therefore employed enzymatic hydrolysis (α-chymotrypsin for the hydrolysis of the methyl ester and carboxypeptidase A for the hydrolysis of the trifluoroacetamide) to remove the protecting groups.[16] In our case, enzymatic cleavage of the protecting group could not be expected because the C-α configuration of **2a** and **2b** was 'D'. We therefore attempted N^{in}-protection. Treatment of **2a** and **2b** with an excess amount (3-5 equiv.) of di-*t*-butyl-dicarbonate (Boc_2O) in CH_3CN in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP) afforded N^{α},N^{in}-bis-Boc-protected **3a** and **3b**. Alkaline hydrolysis (aqueous NaOH in MeOH) of **3a** and **3b** proceeded cleanly to give **5a** and **5b**, in which the N^{α}-trifluoroacetyl group was

Scheme 2

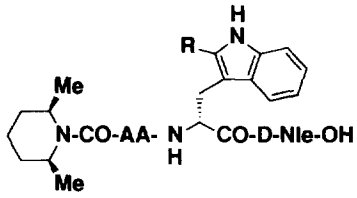


Reagents: (i) D-Nle-OMe·HCl, EDCI, HOBT, NMM, CH_2Cl_2; (ii) HCOOH then aq. $NaHCO_3$; (iii) 2,6-dimethylpiperidinocarbonyl-AA-OH, EDCI, HOBT, CH_2Cl_2; (iv) aq. NaOH, MeOH; (v) H_2, $Pd/BaSO_4$, MeOH.

also cleaved. The N^{α}-trifluoroacetyl group appeared to be easily cleaved by weaker alkaline conditions (aqueous $NaHCO_3$ in MeOH or *N*-methylmorpholine (NMM) in aqueous MeOH) to give **4a** and **4b**. Compound **4b** was also obtained from **2b** in one pot without isolation of **3b** as follows. After the initial bis-*t*-butoxycarbonylation was completed, 3-(dimethylamino)propylamine was added to the reaction mixture to cleave the N^{α}-trifluoroacetyl group together with decomposition of excess Boc_2O, giving **4b** in an almost quantitative yield. Treatment of **4a** and **4b** with aqueous NaOH in methanol brought about hydrolysis of the methyl ester together with cleavage of the N^{in}-Boc to afford **5a** and **5b**. During these protection and deprotection reactions, troublesome racemization was not observed to occur and compounds **5a** and **5b** were obtained as an optically pure form.[17] We next attempted a substitution reaction at the C-2 position of the indole ring. Reaction of **4b** with CuCN (2.5 equiv.) in DMF (85 °C, 1-2.5 h) proceeded smoothly to afford a 2-cyano derivative **6** (78-99%). In contrast, the N^{in}-unprotected compound **2b** did not react with CuCN even at 100 °C. This contrast suggested that N^{in}-Boc protection is essential to the substitution reaction at the C-2 position. Compound **4b** also reacted with (trimethylsilyl)acetylene (3 equiv.) in the presence of $(Ph_3P)_4Pd$ (10 mol%) and CuI (30 mol%) in diethylamine (40 °C, 2.5-9 h) to yield a 2-(trimethylsilyl)ethynyl derivative **7** (57-76%). These two substitution reactions proceeded cleanly without troublesome racemization at the C-α position.[17] Alkaline hydrolysis (aqueous NaOH in MeOH) of compounds **6** and **7** afforded **8** and **9**, respectively (Scheme 1).

Compounds **5a**, **5b**, **8** and **9** were coupled with D-norleucine (D-Nle) methyl ester by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBT) to give protected dipeptide derivatives **10a-d**. Deprotection of the N^{α}-Boc group in **10a-c** was achieved by formic acid to afford primary amines **11a-c**. Treatment of **10d** under the same conditions, however, afforded a complex mixture and the expected primary amine was not obtained. This compound was hydrogenated to give a 2-ethyl analogue **10e**, which was treated with formic acid to give a primary amine **11e**. Compounds **11a-c** and **11e** were coupled with

Table. Binding and Functional IC_{50} Values for 2-Substituted D-Tryptophan-Containing Tripeptide Derivatives



Compound	R	AA	Binding IC_{50} (nM) ET_A[a]	(n)[b]	ET_B[c]	(n)[b]	Functional IC_{50} (nM) ET_A[d]	(n)[b]	ET_B[e]	(n)[b]
15a	Cl	Leu	15	(3)	5.6	(3)	-		-	
15b	Br	Leu	5.6	(5)	2.5	(5)	-		-	
15c	CN	Leu	474	(1)	1.6	(1)	-		-	
15e	C_2H_5	Leu	180	(1)	5.0	(1)	-		-	
16b (BQ-928)	Br	Cprg[f]	3.8	(4)	0.81	(4)	16	(1)	0.80	(1)
17c (BQ-017)	CN	Val	2000	(4)	2.3	(3)	6100	(1)	4.0	(1)

[a] Porcine aortic smooth muscle membranes. [b] Number of IC_{50} determinations. [c] Porcine cerebellum membranes. [d] Human neuroblastoma cell line SK-N-MC cells. [e] Human Girardi heart cells. [f] L-Cyclopropylglycine.

2,6-dimethylpiperidinocarbonyl-AA-OH (AA represents an amino acid residue) by EDCI and HOBT to give tripeptide methyl esters **12a-c**, **12e**, **13b** and **14c**. Alkaline hydrolysis of the methyl esters afforded the desired peptide derivatives with 2-substituted D-tryptophans **15a-c**, **15d**, **16b** and **17c** (Scheme 2).

Compounds **15a-c**, **15e**, **16b** and **17c** were tested for their inhibitory activity on ^{125}I-labeled ET-1 binding to ET_A receptors in porcine aortic smooth muscle membranes and to ET_B receptors in porcine cerebellum membranes.[18] The results are shown in the Table. Among compounds with Leu as AA, the 2-halo-D-tryptophan-containing tripeptide derivatives, **15a** and **15b**, potently inhibited ET-1 binding to both ET_A and ET_B receptors. In contrast, the 2-cyano-D-tryptophan derivative **15c** and the 2-ethyl-D-tryptophan derivative **15e** showed potent affinity for the ET_B receptors but very weak affinity for the ET_A receptors. The replacement of Leu in **15b** with L-cyclopropylglycine increased both ET_A and ET_B affinities. Compound **16b** (BQ-928) was one of the most potent combined ET_A/ET_B receptor binding inhibitors in this series of peptide derivatives. On the other hand, the replacement of Leu in **15c** with Val resulted in a 4-fold decrease in ET_A receptor binding affinity but did not significantly alter ET_B affinity. Compound **17c** (BQ-017) exhibited 870-fold more potent binding affinity for ET_B receptors than for ET_A receptors.

Compounds **16b** and **17c** were further evaluated for their functional inhibition of the ET-1-induced $[Ca^{2+}]_i$ increase in human neuroblastoma-derived SK-N-MC cells, which express ET_A receptors, and in human Girardi heart (hGH) cells, which exclusively possess ET_B receptors.[19] Compound **16b** potently inhibited the ET-1-induced $[Ca^{2+}]_i$ increase in both SK-N-MC and hGH cells with an IC_{50} value of 16 nM and 0.80 nM, respectively. Compound **17c** exhibited potent inhibition in hGH cells (IC_{50}, 4.0 nM), but its inhibition in SK-N-MC cells was about 1500-fold less potent than that in hGH cells. These data indicate that compound **16b** is a combined ET_A/ET_B receptor antagonist and that compound **17c** is a selective ET_B receptor antagonist.

In summary, a protected 2-bromo-D-tryptophan **4b** reacted with CuCN and (trimethylsilyl)acetylene to afford the corresponding 2-substituted D-tryptophan analogues with retention of C-α chirality. Tripeptide

derivatives with 2-substituted D-tryptophans **15a-c** and **15e** all showed potent affinity for ET_B receptors, but their affinity for ET_A receptors depended on the C-2 substituents of the indole ring of the D-tryptophanyl residues. The C-2 substituent appeared to be very important for subtype selectivity between ET_A and ET_B receptors. Further modifications of 2-bromo-D-tryptophan-containing peptide **15b** and 2-cyano-D-tryptophan-containing peptide **15c** led to a potent combined ET_A/ET_B receptor antagonist **16b** (BQ-928) and a selective ET_B antagonist **17c** (BQ-017).

Typical Experimental

N^{α},N^{in}-bis(*t*-Butoxycarbonyl)-2-bromo-D-tryptophan Methyl Ester (4b)

Boc_2O (15.1 g, 69 mmol) and DMAP (0.28 g, 2.3 mmol) were added to a solution of 2-bromo-N^{α}-trifluoroacetyl-D-tryptophan methyl ester (**2b**, 9.04 g, 23.0 mmol) in dry acetonitrile (50 mL) at 0 °C. After being stirred at 0 °C for 30 min, the mixture was allowed to warm to room temperature and was stirred overnight. The mixture was cooled to 0 °C and 3-(dimethylamino)propylamine (4.3 mL, 34.5 mmol) was added. After being stirred at 0 °C for 20 min, the mixture was neutralized with 10% citric acid and concentrated under reduced pressure. The residue was taken up with EtOAc (200 mL), washed successively with 10% citric acid (100 mL), sat. $NaHCO_3$ (100 mL) and brine (100 mL), dried over $MgSO_4$ and evaporated. Trituration of the residue with hexane gave **4b** (6.70 g, 58%) as colorless crystals. The mother liquid was purified by column chromatography on silica gel eluted with EtOAc/hexane (1/3) to give **4b** (4.72 g, 41%, total 99%): mp. 58-62 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 1.40 (9 H, s, $C(CH_3)_3$), 1.70 (9 H, s, $C(CH_3)_3$), 3.10-3.40 (2 H, m, β-CH_2), 3.68 (3 H, s, $COOCH_3$), 4.52-4.71 (1 H, m, α-CH), 5.15 (1 H, d, J = 7.3 Hz, NH), 7.15-7.40 (2 H, m), 7.50 (1 H, d, J = 7.3 Hz), 8.05 (1 H, d, J = 7.3 Hz); $[\alpha]_D^{20}$ 5.83 ° (c 1.0, MeOH); MS (FAB) 496 and 498 (M^+); Anal. calcd for $C_{22}H_{29}BrN_2O_6$ C, 53.13; H, 5.88; N 5.63, found C 53.31; H 5.86; N 5.56.

N^{α},N^{in}-bis(*t*-Butoxycarbonyl)-2-cyano-D-tryptophan Methyl Ester (6)

A mixture of N^{α},N^{in}-bis(*t*-butoxycarbonyl)-2-bromo-D-tryptophan methyl ester (**4b**, 500 mg, 1.01 mmol) and CuCN (225 mg, 2.51 mmol) in dry DMF (1.0 mL) was stirred at 85 °C in an argon atmosphere for 1 h. After being cooled to room temperature, the reaction mixture was diluted with EtOAc (80 mL), washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluted with EtOAc to give **6** (442 mg, 99%) as a pale yellow amorphous: IR (KBr, cm^{-1}) 3300, 2980, 2224, 1722, 1693, 1514, 1160, 748; ^1H NMR (300 MHz, $CDCl_3$) δ 1.41 (9 H, s, $C(CH_3)_3$), 1.71 (9 H, s, $C(CH_3)_3$), 3.34 (1 H, dd, J = 5.9 Hz, 14.2 Hz, β-CH), 3.49 (1 H, dd, J = 5.9 Hz, 14.2 Hz, β-CH), 3.79 (3 H, s, $COOCH_3$), 4.67-4.74 (1 H, m, α-CH), 5.20 (1 H, d, J = 7.5 Hz, α-NH), 7.34 (1 H, t, J = 7.8 Hz), 7.50 (1 H, t, J = 7.8 Hz), 7.69 (1 H, d, J = 7.8 Hz), 8.21 (1 H, d, J = 7.8 Hz); $[\alpha]_D^{20}$ 4.26° (c 0.99, MeOH); HRMS (FAB) calcd for $C_{23}H_{29}N_3O_6$ (M^++H) 444.2135, found 444.2136.

N^{α},N^{in}-bis(*t*-Butoxycarbonyl)-2-(2-trimethylsilyl)ethynyl-D-tryptophan Methyl Ester (7)

(Trimethylsilyl)acetylene (590 mg, 6.0 mmol) was added to a mixture of N^{α},N^{in}-bis(*t*-butoxycarbonyl)-2-bromo-D-tryptophan methyl ester (**4b**, 994 mg, 2.00 mmol), CuI (115 mg, 0.60 mmol) and $(Ph_3P)_4Pd$ (232 mg, 0.20 mmol) in dry diethylamine (10 mL) in an argon atmosphere. The mixture was stirred at 40 °C for 9 h and then cooled. Ethyl ether (60 mL) was added to the reaction mixture and the resulting suspension was filtered. The filtrate was evaporated. The residue was dissolved in ethyl ether (60 mL), washed with 10% citric acid (60 mL), saturated $NaHCO_3$ (60 mL) and brine (60 mL), dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluted with EtOAc/hexane (1/10) to give **7** (782 mg, 76%) as a pale yellow

amorphous: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.32 (9 H, s, Si(CH_3)$_3$), 1.38 (9 H, s, C(CH_3)$_3$), 1.70 (9 H, s, C(CH_3)$_3$), 3.12-3.37 (2 H, m, β-CH_2), 3.69 (3 H, s, COOCH_3), 4.43-4.62 (1 H, m, α-C*H*), 5.32 (1 H, d, J = 8.1 Hz, N*H*), 7.27 (1 H, dt, J = 1.4 Hz, 7.8 Hz), 7.37 (1 H, dt, J = 1.4 Hz, 7.8 Hz), 7.55 (1 H, dd, J = 1.4 Hz, 7.8 Hz), 8.19 (1 H, dd, J = 1.4 Hz, 7.8 Hz); $[\alpha]_D^{20}$ 22.4° (c 0.85, MeOH); HRMS (FAB) calcd for $C_{27}H_{38}N_2O_6Si$ (M^+) 514.2499, found 514.2522.

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17. After coupling with D-Nle-OCH_3, the dipeptide derivatives **10a-d** were obtained as single isomers and the diastereoisomers were not detected in the reaction mixtures by TLC analysis.
18. Binding assay was performed according to the reported method. Ihara, M.; Fukuroda, T.; Saeki, T.; Nishikibe, M.; Kojiri, K.; Suda, H.; Yano, M. Biochem. *Biophys. Res. Commun.* **1991**, *178*, 132.
19. Functional assay was performed according to the method described in ref. 10.

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