Pergamon

Tetrahedron Letters, Vol. 38, No. 7, pp. 1191-1194, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00061-0

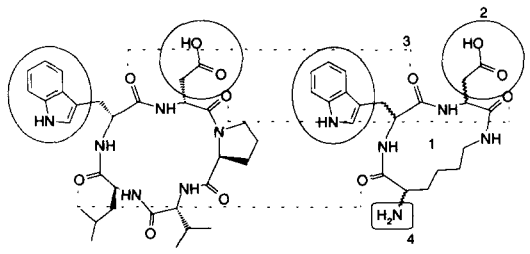
Solid-Phase Synthesis of "Head-to-Side Chain" Cyclic Tripeptides Using Allyl Deprotection

C. Flouzat *, F. Marguerite, F. Croizet *, M. Percebois, A. Monteil, M. Combourieu

R L-CERM, Centre de Recherche et Developpement, Rue H. Goudier, B. P. 140, 63203 RIOM Cédex, France

Abstract: "Head-to-side-chain" cyclic tripeptides were designed as endothelin receptor antagonists. Solid phase synthesis of cyclic peptides, using an automated allyl cleavage procedure with $Pd[P(Ph_3)]_4$ followed by cyclization was performed. Synthetic procedures were established on a continous-flow peptide synthesizer. © 1997, Published by Elsevier Science Ltd. All rights reserved.

Since their discovery by Yanagisawa et al. in 1988 [2], endothelins have been the subject of considerable research regarding their physiological function and potential pathophysiological role in cardiovascular disease [3].Two endothelin receptors, ET_A and ET_B, have been characterized in mammalian species [2,4]. Due to the implication of ET_A receptors in the mediation of vascular smooth muscle tone, pharmaceutical research has focussed on selective ET_A receptor antagonists, leading to the discovery of bioactive peptides [5]. Among them, BQ-123: cyclo(D-Asp-Pro-D-Val-Leu-D-Trp) has been studied extensively around the world [6]. Various SAR studies on BQ-123 and on family of endothelin peptides have led to the general hypothesis that a hydrophobic group (leucine residue), an indole moiety (tryptophane), and the presence of an acidic group are necessary for receptor binding affinity [7]. We chose BQ-123 as a template for designing novel cyclic tripeptide ET_A inhibitors in order to restrict the number of side chains to the essential functional groups involved in the interaction with the receptor. Molecular modelling studies have indicated that alkyl-cyclo (Lys-Trp-Asp) peptides might be good candidates for mimicking the BQ-123 structure [8].



BQ123 H-cyclo(Lys-Trp-Asp)

Our requirements for the design of these tripeptides were to preserve

1 - the overall volume of the ring ;

2 - the orientation of the tryptophane and aspartic acid side chains ;

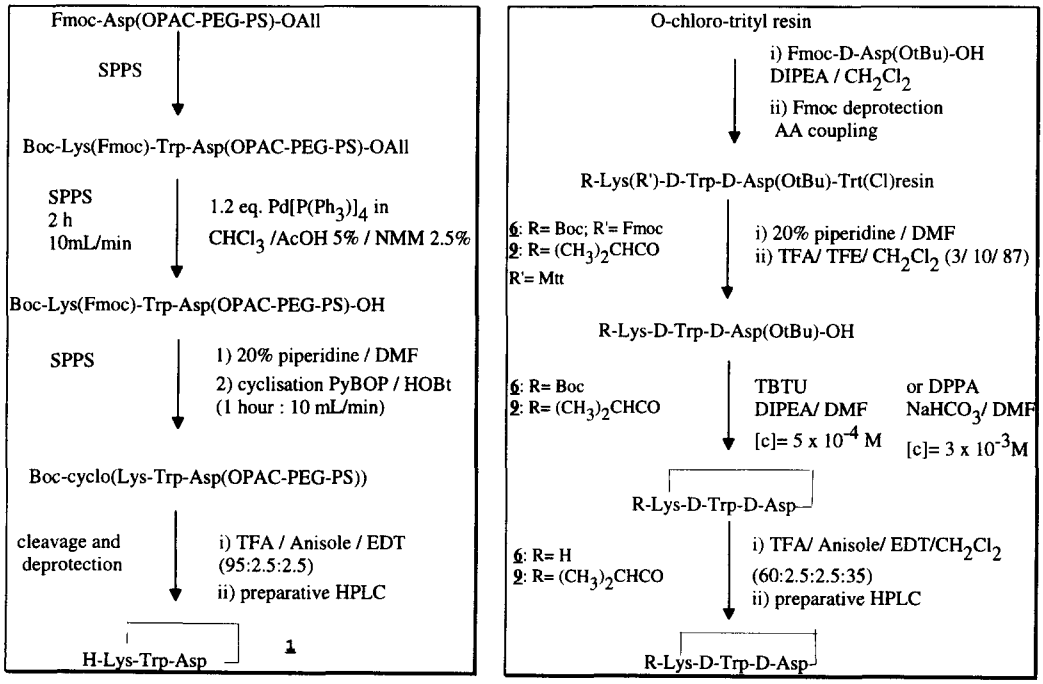
3 - the hypothetical hydrogen bonds between the receptor and the CO or NH group of the amide bonds ;

4 - the possibility of introducing hydrophobic groups on the lysine amine.

The first step was to synthezise all the possible peptides of H-cyclo(Lys-Trp-Asp) with L and D isomers of all amino-acids (DDD - DDL - DLD - DLL - LDD - LDL - LLD - LLL), and the second step was to introduce hydrophobic substituents (such as isobutylcarbonyl group) to mimick the leucine side chain on the lysine amine.

* *Fax: (33) 473 334 997, Email: f.croizet@organon.akzonobel.nl*

Chemistry: We report here the synthesis of cyclic tripeptides using two strategies: in the case of L-Asp derivatives, an automated solid-phase procedure was performed, and, in the case of D-Asp derivatives, cyclization in solution was used. In general, classical synthesis of cyclic peptides is carried out by cyclization of the partially protected linear precursor under conditions of high dilution. The synthesis of cyclic peptides on solid support favors intramolecular reactions over intermolecular side reactions [9] and has the advantage of easy washing to remove the reagents. Since the introduction of the allyloxycarbonyl (Aloc) group in 1950 as a protective group [10], the importance of aloc and allyl groups in peptide chemistry has been shown [11]. Recently, several groups have reported orthogonal strategies involving allyl cleavage on solid-phase synthesis of peptides [12]. In the context of investigating cyclic peptides with potential therapeutic properties, we have followed the strategy elaborated by Kates et al. [13] who performed continuous-flow peptide synthesis, using p-alkoxybenzylalcohol polystyrene support (PAC-PS, Perseptive Biosystem) loaded with Fmoc-Asp-OAll by side-chain anchoring. An advantage of an entirely automated strategy is a shorter reaction time than that for a cyclization in solution. After peptide elongation, allyl deprotection of the C^{α}-carbonyl group with tetrakistriphenylphosphine palladium(0), Fmoc deprotection of the lysine side chain, activation and cyclization with benzotriazol-1-yloxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) in the presence of 1-hydroxybenzotriazole (HOBt). Then, after final deprotection and cleavage to release the cyclic peptides into the solution, the crude product was obtained with an HPLC purity between 50 and 88% (see scheme below).



Compounds **1** to **4** were obtained by this synthetic route[14] . An example of this synthesis is that of the tripeptide cyclo(Lys-Tryp-Asp) **1**. The N-α-substituted lysine analogue of compound **1** was prepared by using Fmoc-Lys(Aloc)-OH during peptide elongation to yield RCO-cyclo(Lys-Trp-Asp) derivatives. D-Asp derivatives (**4** - **9**) were prepared classicaly in solution because Fmoc-D-Asp(PEG-PS)-OAll resin was not

commercially available. Ortho-chloro-trityl resin (Novabiochem) was chosen for its high esterification yields and easy cleavage of peptides, while not affecting the t-butyl type protective groups [15]. The resin was loaded with Fmoc-D-Asp(OtBu)-OH according to Barlos et al. [15]. Standard chain elongation was performed, and cleavage of the peptide from the resin with trifluoroacetic acid (TFA), trifluoroethanol (TFE), dichloromethane (3/10/87) for 5 min, yielded the partially protected linear peptide, which was cyclized under high dilution (c= 5 x 10^{-4} M) with 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) in DMF at room temperature. Deprotection of the Boc group yielded crude compounds having a HPLC purity between 52 and 75%[16]. The N-α substituted lysine derivative **9** was synthesized by using Fmoc-Lys(Mtt)-OH and isovaleric acid during peptide chain elongation, using diphenylphosphorylazide (DPPA) in DMF at 0°C (c= 3 x 10^{-3} M) according to Brady et al.[17] All peptides were purified by preparative reverse-phase HPLC.

	Compound	Purity[a]	Yield[b]		Compound	Purity[a]	Yield[b]
1	cyclo(Lys-Trp-Asp)	50%	22%	**5**	cyclo(D-Lys-D-Trp-D-Asp)	52%	13%
2	cyclo(D-Lys-Trp-Asp)	88%	14%	**6**	cyclo(Lys-D-Trp-D-Asp)	83%	27%
3	cyclo(Lys-D-Trp-Asp)	50%	8%	**7**	cyclo(D-Lys-Trp-D-Asp)	75%	36%
4	cyclo(D-Lys-D-Trp-Asp)	65%	22%	**8**	cyclo(Lys-Trp-D-Asp)	58%	27%
				9	isobutylcarbonyl-cyclo (Lys-D-Trp-D-Asp)	26%	4%

Table 1: "Head-to-side chain" cyclic tripeptides. FAB-MS for compounds **1** to **8**: calc./ found $[M+H]^+$: 429/ 430. (a) determined by HPLC (220 nm) (b) after preparative HPLC.

In conclusion, "head-to-side-chain" cyclized peptides can be efficiently and rapidly synthesized by a three-dimensional orthogonal strategy (Fmoc/ t-Butyl/ allyl) leading to libraries of cyclic peptides (in prep.). Pharmacological results with the tripeptides discussed above will be published elsewhere.

References and notes:

1) Abbreviations: All: allyl, Boc: tert-butyloxycarbonyl, DIPEA: diisopropylethylamine, DMF: dimethylformamide, EDT: ethanedithiol; Fmoc: fluorenylmethoxycarbonyl, HOBt: 1-hydroxybenzotriazole, HPLC: High-Performance Liquid Chromatography; Mtt: 4-methyltrityl; PEG-PS: polyethyleneglycol-polystyrene graft, $Pd[(PPh_3)]_4$: tetrakistriphenylphosphine palladium(O), PyBOP: benzotriazol-1-yloxy-tris(pyrrolidino)phosphonium hexafluorophosphate; SPPS: solid-phase synthesis, TBTU: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, TFA: trifluoroacetic acid; TFE: trifluoroethanol.

2) Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., Masaki, T, *Nature*, **1988**; *332*, 411-415.

3) Ferro, C. J., Webb D. J., *Drugs*, **1996**; *51*, 1, 12-27.

4) Sakurai, T., Yanagisawa, M., Masaki, T., *Trends Pharmacol.Sci.*, **1992**, *13*, 103-108.

5) a) Cody, W. L., Doherty, A. M., *Biopolymers (Peptide Science)*, **1995**, *37*, 89-104. b) Ishikawa, K. , Fukami, T. , Mase, T., Nagase, T., Hayama, T., Niiyama, K., Ihara, M., Saeki, T., Osaki, S., Nishikibe, M., Mino, N., Noguchi, K., Fukuroda, T., Yano, M., *Eur. J. Med. Chem.*, **1995**, *30 suppl(Is-688s)*, 371s-383s. c) Cheng, X. M., Nikam, S. S., Doherty, A. M. , *Curent Med. Chem.*, **1994**, *1*, 271-312.

6) a) Ishikawa, K., Fukami, T., Nagase, T., Fujita, K., Hayama, T., Niiyama, K., Mase, T., Ihara, M., Yano, M., *J. Med. Chem.* **1992**, 35, *11*, 2135-2142. b) Moreland, S., *Cardiovasc. Drug Reviews*, **1994**, *12*, 1, 48-69.

7) a)Verheyden, P., Van Assche, I., Brichard, M. H., Demaude, T., Paye, I., Scarso, A., Van Bist, G., *FEBS Lett.*, **1994**, *344*, 55-60. b) Elliot, J. D., Lago, A. M., Cousins, R. D., Gao, A., Leber, J. D., Erhard, K. F., Nambi, P., Elshourbagy, N. A., Kumar, C., Lee, J. A., Bean, J. W., DeBrosse, C. W., Eggleston, D. S. Brooks, D. P., Feuerstein, G., Ruffolo, R. R. Weinstock, J. W., Gleason, J. G., Peishoff, C. E., Ohlstein, E. H., *J. Med. Chem.*, **1994**, *37*, 1553-1557. c) Hempel, J. C., Cordova de Sintjago, T., Guner, O., Hassan, M., Koerber, S. C., Vorpagel, E. R., Hagler, A. T; *Eur. J. Med. Chem.*, **1995**, *30 suppl(Is-688s)*, 392s-395s.

8) ***Molecular Modelling:*** By varying the L and D configuration, eight different H-cyclo(Lys-Trp-Asp) peptides were built with Quanta/Charmm (Molecular Simulations). For each peptide, eight energy-minimized starting conformations were modelled by simply flipping the amide bonds above or below the average plane of the ring. Conformer generation

was performed using high-temperature dynamic runs followed by energy minimisation and cluster analysis leading to 40 low-energy conformations. The above conformers were compared with the BQ-123 solution conformation (Atkinson, A. R., Pelton J. T., *FEBS Lett.*, **1992**, *296*, 1, 1-6) using Chem-X (Chemical Design Ltd). For each conformation (out of 320), the following were fitted : average plane of the peptide rings, Trp and Asp side chains, and the CO and NH amide groups.

9) Mazur, S., Jayaleshmy, P., *J. Am. Chem. Soc.*, **1979**, *101*, 677-683.

10) Stevens C. M., and Watanabe R., *J. Am. Chem. Soc.*, **1950**, *72*, 725-727

11) a) Friedrich-Bochnitschek, S., Waldmann, H., Kunz, H., *J. Org. Chem.*, **1989**, *54*, 751-756. b) Lyttle, M. H., Hudson, D., in "Peptide-Chemistry and Biology: Proceedings of the Twelfth American Peptide Symposium", (Smith J. A., and Rivier J. Ed.), Escom Leiden, 1992, 583-584. Loffet, A., Zhang H. X. , In: "Innovation and Perspectives in Solid Phase Synthesis: Peptides, Polypeptides and Oligonucleotides", (Epton R. Ed.), Intercept Andover, **1992**, 77-82.

12) a) Alsina, J., Rabanal, F., Giralt, E., Albericio, F., *Tetrahedron Lett.*, **1994**, *35*, 9633-9636. b) Trzeciak, A., Bannwarth, W., *Tetrahedron Lett.*, **1992**, *33*, 4557-4560. Kates, S. A., Solé, N. A., Johnson ,C. R., *Tetrahedron Lett.*, **1993**, *34*, 1549-1552.

13) Kates, S. A., Daniels, S. B., Albericio, F., *Anal. Biochem.*, **1993**, *212*, 303-310.

14) ***Automated peptide synthesis for compounds 1 to 4:*** A typical procedure is described as for compound 1: (a) *Standard chain elongation*: Continuous-flow solid-phase synthesis was carried out using a Milligen 9050 Plus PepSynthesizer with Fmoc-Asp-(OPAC-PEG-PS)-OAll resin (loading: 1.6 meq /g). Fmoc group removal with piperidine/DMF (1:4) (6 min, 10 mL/min)was followed by DMF washing (5 min, 10 mL/min) and amino acid coupling (45 min, recycle: 15 mL/min); Three equivalents of amino acid were dissolved with three equivalents of HOBt until a final solution of 0.19 M in 0.6 M NMM in DMF. When amino acids were used as OPfp esters, they were dissolved in 0.37M HOBt solution of DMF with 4mM of DIPEA to a final solution of 0.15 M. (b) *Automated allyl deblocking* : $Pd(PPh_3)_4$ (1.2 eq.) was dissolved in an amino acid vial under Argon with a solution of 5% OHAc and 2.5 % NMM in $CHCl_3$ to a final concentration of 0.06 M. The solution was recycled for 2 h (10mL/min). Then, the resin was washed with a solution of 0.5% diethylditiocarbamate and 0.5% of DIPEA in DMF (10 min, 10 mL/ min). (c) *Automated cyclization*: Following Fmoc group removal, three equivalents of PyBOP and HOBt were dissolved until a final concentration of 0.19M in a solution of 0.6 M NMM in DMF, and cyclization was carried out for 1 h (recycle: 10 mL/ min) followed by DMF washing (6 min, 10 mL/min). (d) *Final cleavage* was performed with TFA-ethanedithiol-anisole (95:2.5:2.5) for 2 h at 25°C (between 59 and 91 % cleavage yield). The peptide was purified by reverse-phase HPLC[17] (purity >97%). FAB-MS (matrix: glycerol): 430 $[M+H]^+$. Amino acid analysis: Lys, 0.73; Asp, 1.00; Trp, 0.94.

15) Barlos, K., Chatzi, Olga, Gatos, D., Stavropoulos, G; *Int. J. Peptide Protein Res.* ,**1991**, *37*, 513-520.

16) ***Peptide synthesis for compounds 5 to 9***: A typical procedure is described as for compound 6 *(a) resin loading*:O-chloro-trityl resin (1g, Novabiochem) loading: 1.6 meq/g were stirred gently with Fmoc-D-Asp(OtBu)-OH (0.7eq) and DIPEA (0.41 mL, 1.6 eq) in CH_2CL_2 (10 mL) for 30 min. Then, 1 mL of MeOH was added for end-capping and stirred for 10 min. The resin was washed successively with: CH_2CL_2 (3 x 20 mL), 2-propanol (2 x 20mL), DMF (2 x 20 mL), 2-propanol (2 x 20mL), methanol (2 x 20 mL), and diethyloxide (2 x 20 mL) and dried under vaccum overnight. *(b) Standard chain elongation*: Continous flow solid-phase synthesis was carried out using a Milligen 9050 Plus PepSynthesizer with 0.91g of Fmoc-D-Asp-(OtBu)-o-chloro-trityl-resin (loading: 0.48 meq/g) and 7 g of glass beads (acid-washed, 212-300 microns, Sigma). Fmoc group removal with piperidine/DMF (1:4) (6 min, 10 mL/min) was followed by DMF washing (5 min, 10 mL/min) and amino acid coupling (45 min, recycle: 15 mL/min); three equivalents of amino acid were dissolved with three equivalents of HOBt until a final solution of 0.19 M in 0.6 M NMM in DMF. *(c) Peptide-resin cleavage and cyclization:* The protected peptide was cleaved with a solution of TFA/ TFE/ CH_2CL_2 (3:10:87) (4 x 12 mL, 2 min) . After solvent removal, the crude peptide was precipitated by diethyloxide. The cyclization step was performed with TBTU (1.3 eq) in DMF (c= 5.10^{-4} M), in the presence of DIPEA (pH= 7.5) at 25°C for 72 h. After evaporation of DMF, EtOAc was added to the crude mixture, and the organic phase was washed with saturated aqueous $NaHCO_3$, 0.05N HCl and saturated NaCl. After drying on MgSO4, the organic solvent was reduced under pressure to yield the protected cyclic peptide. *(d) Cleavage and deprotection*: The peptide was dissolved in a solution of TFA/ ethanedithiol/ anisole/ CH_2CL_2 (60: 2.5: 2.5: 35) (10 mL/100 mg of crude product) and stirred for3.5 h at 25°C. After solvent removal, the crude product was precipitated with diethyloxide and purified by reverse phase HPLC[18] (purity >99%). FAB-MS (matrix: glycerol): 430 $[M+H]^+$. Amino acid analysis: Lys, 0.99; Asp, 1.01; Trp, 0.98. Compound 9: prepared as above by using DPPA (1.2 eq) and $NaHCO_3$ (5 eq) (cyclization time: 48 h at 0°C).

17) Brady, S. F., Freidinger, R. M., Paleveda, W. J., Colton, C. D., Homnick, C. F., Whitter, W. L., Curley, P., Nutt, R. F., Veber, D. F., *J. Org. Chem.*, **1987**, *52*, 764-769

18) detection: 220 nm, on a Symmetry C8 (7 µ, 100 A) column (19 x 300 mm, flow: 24 mL/min) and linear gradient over 35 min of A (H_2O 0.1% TFA) and B (90% of CH_3CN in water) from 5% to 65% B.

(*Received in France 20 November 1996; accepted 9 January* 1997)