Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 1145–1148

TETRAHEDRON LETTERS

A convenient strategy of dimerization by microwave heating and using 2,5-diketopiperazine as scaffold

Vincenzo Santagada,[a,*] Ferdinando Fiorino,[a] Elisa Perissutti,[a] Beatrice Severino,[a] Sara Terracciano,[a] Giuseppe Cirino[b] and Giuseppe Caliendo[a]

[a]*Dipartimento di Chimica Farmaceutica e Tossicologica, Università di Napoli 'Federico II', Via D. Montesano, 49, 80131 Naples, Italy*

[b]*Dipartimento di Farmacologia Sperimentale, Università di Napoli 'Federico II', Via D. Montesano, 49, 80131 Naples, Italy*

Received 8 October 2002; accepted 16 December 2002

Abstract—A novel and convenient microwave-assisted dimerization of an active peptide compound using the DKPs as scaffold is described. The key reaction giving rise to the diketopiperazine scaffold is the intermolecular coupling. No epimerization was detected in the reactions used. Conventional and microwave heating of the reactions are compared. Synthesis by microwave irradiation gave the desired compounds in higher yields and in shorter reaction times than those obtained by conventional heating.

In both lead identification and lead optimization processes there is an acute need for new organic small molecules. Traditional methods of organic synthesis are orders of magnitude too slow to satisfy the demand for these compounds. The fields of combinatorial and automated medicinal chemistry (i.e. the simultaneous or parallel synthesis of libraries)[1] have been developed to meet the increasing requirement of new compounds for drug discovery; within these fields, speed is of the essence. The efficiency of microwave flash-heating chemistry in dramatically reducing reaction times (reduced from days and hours to minutes and seconds) has recently been proven in several different fields of organic chemistry.[2–7] The preponderance of heterocyclic frameworks in historical samplings of known drug structures provides strong rational for the inclusion of these scaffolds in lead discovery programs. In fact, one current strategy for the design of new drugs consists of the display, on a suitable rigid scaffold, of functional groups that are known to be involved in interactions with the corresponding receptor. Among several heterocycles, 2,5-diketopiperazines (DKPs) have attracted considerable attentions in the recent years.[8] They can be considered as the smallest cyclic peptides, derived from the folding head-to-tail of a linear unprotected dipeptide.[9] DKPs scaffolds are a common motif in several natural products with therapeutic properties. Included are inhibitors of the mammalian cell cycle,[10] of the plasminogen activator-1,[11] and of the topoisomerase I,[12] as well as competitive antagonists to Substance P at the neurokinin-1 receptor.[13] Furthermore, DKPs have been shown to be useful scaffolds for rational design of several drugs[14] since are typically stable to proteolysis, one important consideration when designing potential drug scaffolds. DKPs are often formed by a side reaction, arising from intramolecular aminolysis at the dipeptidyl level, in both solution and solid-phase synthesis.[15] DKPs formation has been established to be either acid[15b,e,16] or base catalysed[15a,c–g] and it is strongly dependent on the nature and the sequence of the amino acids.[15b] Several methods for the intentional synthesis of 2,5-diketopiperazines on solid support have been proposed and they are based mainly on the cleavage-induced cyclization of linear dipeptides.[8,17] Dimerization of an active compound often results in enhanced binding and improved pharmacological properties. This potency is attributed to a higher concentration of pharmacophores in proximity to recognition sites. Usually, this bivalent ligand approach supposes to use a symmetrical bifunctional linker X to anchor substrates P, giving rise to the general structure P-X-P.[18,19] Knowing the importance of the DKPs in medicinal chemistry our attention has turned to improve the reaction of dimerization[19] of an active peptide compound using the DKPs as scaffold and

Keywords: synthesis of diketopiperazine; dimerization; microwave irradiation in solution.

* Corresponding author. Tel.: +0039-81-678648; fax: +0039-81-678649; e-mail: santagad@unina.it

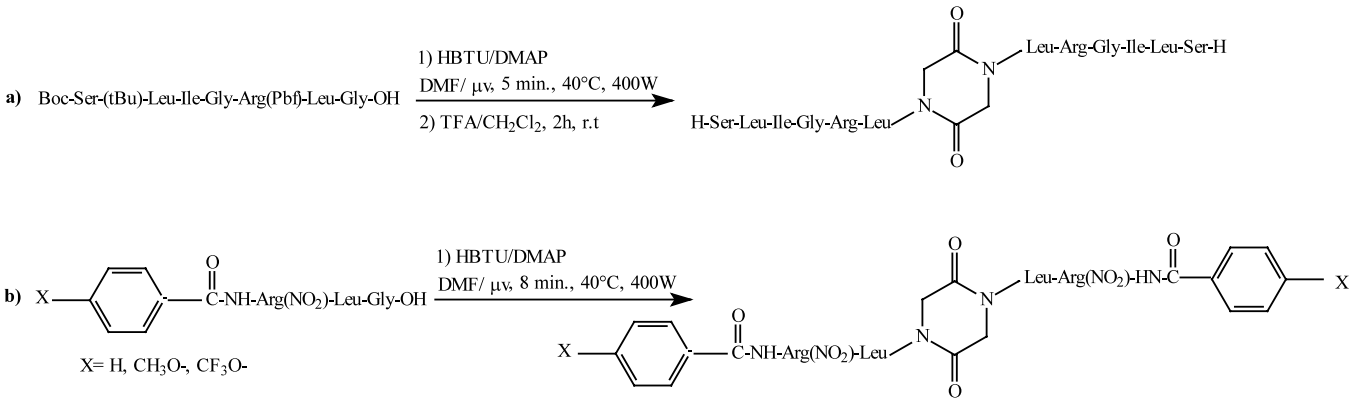
0040-4039/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
PII: S0040-4039(02)02836-8

bearing in mind new synthetic methodologies. The application of microwave energy to organic compounds for conducting synthetic reactions at highly accelerated rates is an emerging technique.[2–7] These observations led us to check into the possibility for improvement in methods used for the synthesis on solution of the substituted 2,5-DKP. The challenge was to build different dimeric structures, involving a Gly-Gly diketopiperazine scaffold, by activating C-terminal glycine monomers (Scheme 1). Our strategy started with introducing a C-terminal glycine moiety on a pharmacophore, followed by an activation step to provide the diketopiperazine bearing the two substrates. Two pathways have to be considered depending on the nature of the active substances (Scheme 1a and b). In fact, as substrate we considered the synthetic hexapeptide (Scheme 1a) corresponding to the proteolitically revealed amino terminal sequence Ser-Leu-Ile-Gly-Arg-Leu (PAR-2 AP) of rat PAR-2, that is able to efficiently activate the PAR-2 receptor,[20] and some dipeptide derivatives obtained by us[21] corresponding the following general structure N^{α}-benzoyl-Arg(NO_2)-Leu-NH_2 (Scheme 1b) and containing the minimal structural requirements for agonist activity of PAR-2 receptor. The pathophysiological role of PAR-2 is not clearly established, and only recently this receptor has been recognized to play a role in inflammatory,[22,23] gastrointestinal,[23] and vascular[24] diseases. The targeted heptapeptide was synthesized by standard solution methods, as a C-terminal glycine, by 3+4 fragment condensation.[25] α-Amino functions were protected by Fmoc group except for the N-terminal amino acid that was protected by Boc group. The side chain protecting group for arginine was Pbf (Scheme 1a). DCC/HOBt was employed for the coupling method.[26] The different masking groups, Boc, Pbf and Fmoc were removed, respectively, by TFA/CH_2Cl_2[27] and 20% piperidine in DMF.[28] The tripeptide derivatives N^{α}-benzoyl-Arg(NO_2)-Leu-Gly-OH and analogues were prepared by standard liquid phase peptide synthesis.[29] All final compounds were purified by preparative RP-HPLC[25] and analysed on a LCQ Ion-Trap mass spectrometer, yielding mass values in agreement with the expected chemical composition within 10 ppm accuracy. In both cases, the key reaction giving rise to the diketopiperazine scaffold is the intermolecular coupling.[30] In fact the intermolecular coupling was influenced by the particular activator employed (DCC/DMAP and HBTU/DMAP). DCC/DMAP method produced no cyclization when the reaction was performed both with conventional heating or microwave heating. In contrast, the intermolecular coupling under microwave heating was obtained with high yields using HBTU/DMAP (Scheme 1a, 90%, 5 min, μV, 400 W, 40°C; Scheme 1b, range between 85 and 92%, 8 min, μV, 400 W, 40°C) while the yield was lower when the reaction was performed in the same conditions by conventional heating (Scheme 1a, 33%, 24 h, 40°C and Scheme 1b, range between 35 and 42%, 24 h, 40°C). No epimerization was detected in the reactions above described. The conditions utilized with the microwave energy represents the optimum profile of power/time and temperature. The temperature of the stirred reaction mixture was monitored directly by a microwave-transparent fluoroptic probe inserted into the solution. The overall reaction times of the reactions performed by microwave irradiation were dramatically reduced. Final compounds were tested for its ability to relax isolated rat aorta as described previously.[21] The ability to relax pre contracted rat aorta with phenylephrine was tested using the final compound in a dose varying between 10^{-7} and 10^{-4} M. The tested compound did not cause any relaxation up to a dose of 10^{-4} M.

In conclusion, we have shown that the application of microwave irradiation improves the intermolecular coupling yields and significantly reduces reaction times in the solution synthesis of dimerization using DKPs as scaffold and HBTU/DMAP as coupling reagents.

Acknowledgements

This work was supported by a grant from Regione Campania ai sensi della L. R. 31 dicembre 1994, N° 41, art. 3, 1° comma.



Scheme 1. Dimerization of PAR-2AP and dipeptide derivatives (N^{α}-benzoyl-Arg(NO_2)-Leu-NH_2 and analogues) using DKPs as scaffold.

References

1. (a) *Combinatorial Libraries. Synthesis, Screening and Application Potential*; Cortese, R., Ed.; Walter de Gruyter & Co.: Berlin, 1996; (b) *Molecular Diversity and Combinatorial Chemistry. Libraries and Drug Discovery*; Chaiken, I. M.; Janda, K. D., Eds.; ACS Books: Washington, 1996; (c) *Combinatorial Chemistry: Synthesis and Applications*; Wilson, S. H.; Czarnik, A. W., Eds.; ACS Books: Washington, 1997.
2. Varma, R. S. *Green Chem.* **1999**, 43–55.
3. (a) Santagada, V.; Perissutti, E.; Fiorino, F.; Vivenzio, B.; Caliendo, G. *Tetrahedron Lett.* **2001**, *42*, 2397–2400; (b) Santagada, V.; Fiorino, F.; Perissutti, E.; Severino, B.; De Filippis, V.; Vivenzio, B.; Caliendo, G. *Tetrahedron Lett.* **2001**, *42*, 5171–5173; (c) Caliendo, G.; Fiorino, F.; Perissutti, E.; Severino, B.; Gessi, S.; Cattabriga, E.; Borea, P. A.; Santagada, V. *Eur. J. Med. Chem.* **2001**, *36*, 873–886; (d) Santagada, V.; Perissutti, E.; Caliendo, G. *Curr. Med. Chem.* **2002**, *9*, 1251–1283.
4. Loupy, A.; Petit, A.; Hamelin, J.; Texier-Boullet, F.; Jacquault, P.; Mathe, D. *Synthesis* **1998**, 1213–1234.
5. Galema, S. A. *Chem. Soc. Rev.* **1997**, *26*, 233–238.
6. Caddick, S. *Tetrahedron* **1995**, *51*, 10403–10432.
7. Larhed, M.; Hallberg, A. *Drug Discov. Today* **2001**, *6*, 406–416.
8. (a) Smith, R. A.; Bobko, M. A.; Lee, W. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2369–2374; (b) Szardenings, A. K.; Burkoth, T. S.; Lu, H. H.; Tien, D. W.; Campbell, D. A. *Tetrahedron* **1997**, *53*, 6573–6593; (c) Kowalski, J.; Lipton, M. A. *Tetrahedron Lett.* **1996**, *37*, 5839–5840; (d) Gordon, D. W.; Steele, J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 47–50; (e) Scott, B. O.; Siegmund, A. C.; Marlowe, C. K.; Pei, Y.; Spear, K. L. *Mol. Diver.* **1995**, *1*, 125–134.
9. (a) Anteunis, M. J. *Bull. Soc. Chim. Belg.* **1978**, *87*, 627–650; (b) Herbert, R.; Kelleher, F. *Tetrahedron Lett.* **1994**, *35*, 5497–5500.
10. (a) Cui, C.-B.; Kakeya, H.; Osada, H. *Tetrahedron* **1996**, *52*, 12651–12666; (b) Cui, C.-B.; Kakeya, H.; Osada, H. *J. Antibiot.* **1996**, *49*, 534–540.
11. Charlton, P. A.; Faint, R. W.; Bent, F.; Bryans, J.; Chicarelli-Robinson, I.; Mackie, I.; Machin, S.; Bevan, P. *Thromb. Haemost.* **1996**, *75*, 808–815.
12. Funabashi, Y.; Horiguchi, T.; Linuma, S.; Tanida, S.; Harada, S. *J. Antibiot.* **1994**, *47*, 1202–1218.
13. Barrow, C. J.; Musza, L. L.; Cooper, R. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 377–380.
14. (a) Weng, J. H.; Bado, A.; Garbay, C.; Roques, B. P. *Reg. Pept.* **1996**, *65*, 3–9; (b) Goodfellow, V. S.; Laudeman, C. P.; Gerrity, J. I.; Burkard, M.; Strobel, E.; Zuzack, J. S.; McLeod, D. A. *Mol. Diver.* **1996**, *2*, 97–102; (c) Pons, J. F.; Fauchère, J. L.; Lamaty, F.; Molla, A.; Lazaro, R. *Eur. J. Org. Chem.* **1998**, 853–859.
15. (a) Goodman, M.; Stueben, K. C. *J. Am. Chem. Soc.* **1962**, *84*, 1279–1283; (b) Gisin, B. F.; Merrifield, R. B. *J. Am. Chem. Soc.* **1972**, *94*, 3102–3106; (c) Khosla, M. C.; Smeby, R. R.; Bumpus, F. *J. Am. Chem. Soc.* **1972**, *94*, 4721–4724; (d) Rothe, M.; Mazanek, J. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 293; (e) Giralt, E.; Eritja, R.; Pedroso, E. *Tetrahedron Lett.* **1981**, *22*, 3779–3782; (f) Barany, G.; Albericio, F. *J. Am. Chem. Soc.* **1985**, *107*, 4936–4942; (g) Pedroso, E.; Grandas, A.; de las Heras, X.; Giralt, E. *Tetrahedron Lett.* **1986**, *27*, 743–746; (h) Fischer, P. M.; Solbakken, M.; Undheim, K. *Tetrahedron* **1994**, *50*, 2277–2288; (i) Lloyd-Williams, P.; Merzouk, A.; Guibè, F.; Albericio, F.; Giralt, E. *Tetrahedron Lett.* **1994**, *35*, 4437–4440.
16. (a) del Fresno, M.; Alsina, J.; Royo, M.; Barany, G.; Albericio, F. *Tetrahedron Lett.* **1998**, *39*, 2639–2642; (b) Sabatino, G.; Ghelli, M.; Mazzucco, S.; Ginanneschi, M.; Papini, A. M. *Tetrahedron Lett.* **1999**, *40*, 809–812.
17. Bianco, A.; Sonksen, C. P.; Roepstorff, P.; Briand, J. P. *J. Org. Chem.* **2000**, *65*, 2179–2187.
18. Erez, M.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1982**, *25*, 847–849.
19. (a) Giraud, M.; Bernad, N.; Martinez, J.; Cavalier, F. *Tetrahedron Lett.* **2001**, *42*, 1895–1897; (b) Cavelier, F.; Giraud, M.; Bernad, N.; Martinez, J. *Peptides: The Wave of the Future, Proceedings of the Second International and the Seventeenth American Peptide Symposium*; San Diego, CA, USA, June 9–14, 2001; pp. 152–154.
20. Cirino, G.; Bucci, M.; Cicala, C.; Napoli, C. *Trends Pharmacol. Sci.* **2000**, *21*, 170–172.
21. Santagada, V.; Caliendo, G.; Severino, B.; Perissutti, E.; Fiorino, F.; Cicala, C.; De Filippis, V.; Cirino, G. *Bioorg. Med. Chem. Lett.* **2002**, *7*, 21–24.
22. Steinhoff, M.; Vergnolle, N.; Young, S. H.; Tognetto, M.; Amatesi, S.; Ennes, H. S.; Travisani, M.; Hollenberg, M. D.; Wallace, J. L.; Cughey, G. H.; Mitchell, S. E.; Williams, L. M.; Geppetti, P.; Mayer, E. A.; Bunnett, N. W. *Nature Med.* **2000**, *6*, 151–158.
23. Vergnolle, N. *Aliment. Pharmacol. Ther.* **2000**, *14*, 257–266.
24. Napoli, C.; Cicala, C.; Fallace, J. L.; de Nigris, F.; Santagada, V.; Caliendo, G.; Franconi, F.; Ignarro, L. J.; Cirino, G. *Proc. Natl. Acad. Sci. USA* **2000**, *87*, 3678.
25. *Synthesis of protected heptapeptide*: The heptapeptide was synthesized from 9-fluorenylmethoxycarbonyl (Fmoc) amino acids derivatives according to the conventional procedure as a C-terminal glycine, by 3+4 fragment condensation. The residue C-terminal of the tetrapeptide fragment was protected as methylester while the side chain protecting group of the Arg was Pbf. The N-terminal amino acid of the tripeptide was protected by Boc group. The tripeptide and tetrapeptide fragments were synthesized step by step using DCC/HOBt as coupling reagent.[26] The N^{α}-Fmoc group was removed by 20% piperidine in DMF.[28] The condensation of the fragments was performed by DCC/HOBt. The removal of the methylester group of the C-terminal residue of the protected heptapeptide was performed by saponification treating 1 mmol of heptapeptide in MeOH (8 ml) with 1.2 equiv. 1N NaOH for 3 h at room temperature. Final heptapeptide was purified by preparative RP-HPLC and the homogeneity of the purified product was assessed by analytical RP-HPLC with a Vydac C18-column (5 μm, 4.6×250 mm, spherical). Analytical determinations were carried out by two solvent systems: A: acetonitrile in 0.1% TFA, B: H_2O in 0.1% TFA (linear gradient from 20% A to 80% A over 45 min, UV detection at 220 nm, flow rate 1 mL/min). Preparative RP-HPLC was performed by the same gradient used for the analytical determinations. The operational flow rate was 60 mL min^{-1}. The final heptapeptide was characterized by mass spectrometry (LCQ Thermoquest-Ion Trap) and the data were consistent with the considered structure.

26. *DCC/HOBt method*: To a solution of the carboxylic component (1 mmol) in DMF (5 mm) at 0°C were added the amino acid component (1 mmol), 1-hydroxybenzotriazole (HOBt) (1 mmol), and DCC (1.1 mmol) in the above order. The reaction mixture was stirred for 2 h at 0°C and for 24 h at room temperature. Then *N*,*N*′-dicyclohexylurea (DCU) was filtered off, and the solution was diluted with EtOAc (100 ml). The solution was washed consecutively with brine, 0.5N $KHSO_4$, brine, 5% $NaHCO_3$, and brine. The organic phase was dried ($MgSO_4$), was crystallized from appropriate solvents or purified by column chromatography.
27. *Removal of Boc- and Pbf-protecting groups*: The protected compound (1 mmol) was treated with TFA (2.5 ml) for 0.5 h at room temperature. Et_2O was added to the solution until the product precipitated. The crude product was purified by preparative RP-HPLC as reported in Ref. 25.
28. *Removal of Fmoc-protecting group*: Standard cleavage procedure was used to remove Fmoc protecting group. The peptide (1 mmol) was dissolved in 20% piperidine/DMF (15 ml) and the reaction was allowed to proceed at room temperature for 45 min. The solvent was evaporated in vacuo, the residue was triturated with diethyl ether (25 ml), the solid was collected, washed with diethyl ether, and dried in vacuo.
29. *General procedure for acyl-tripeptide derivatives*: The starting dipeptide Boc-Leu-Gly-OCH_3 was produced from Boc-Leu-OH and HCl·H-Gly-OCH_3 by the DCC/HOBt method.[26] Successively the Boc protecting group of the N-terminal residue was removed by TFA/CH_2Cl_2[27] and coupled with the appropriate N^{α}-benzoyl-Arg(NO_2)-OH derivatives by DCC/HOBt method to obtain the protected tripeptides that were purified by crystallization (diethylether/hexane, 8:2). The resulted compounds were characterized and converted to their carboxylic form by saponification with 1N NaOH,[25] successively purified by RP-HPLC and characterized by LCQ Ion-Trap mass spectrometer.
30. *General procedure for intermolecular cyclization*: Synthesis was performed using a microwave oven ETHOS 1600, Milestone especially designed for organic synthesis. The experiments were carried out in standard Pyrex glassware chamber. A solution of the free carboxylic heptapeptide or N^{α}-benzoyl-Arg(NO_2)-Leu-Gly-OH derivatives (3.16 mmol), DMAP (4-(dimethylamino)pyridine, 3.47 mmol) and HBTU (3.47 mmol) in 6 mL anhydrous DMF was treated by microwave irradiation for 5 or 8 min, respectively (UV, 400 W, 40°C) or by conventional heating for 24 h at 40°C, respectively. The reaction mixture was separated from the solvent, the crude residue was taken up in ethyl acetate and washed successively three times with citric acid (5%), sodium bicarbonate (5%), and a saturated solution of sodium chloride. The final crude products, after removal of the Boc and Pbf protecting groups (Scheme 1a), were purified and characterized by preparative RP-HPLC as reported in Ref. 25.