Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 1453–1456
Tetrahedron Letters

An efficient approach for monosulfide bridge formation in solid-phase peptide synthesis

Pietro Campiglia,[a] Isabel Gomez-Monterrey,[a] Luigi Longobardo,[b] Teresa Lama,[a] Ettore Novellino[a] and Paolo Grieco[a,*]

[a]*Dipartimento di Chimica Farmaceutica e Tossicologica, University of Naples, 'Federico II', 80131 Naples, Italy*
[b]*Dipartimento di Scienza degli Alimenti, University of Naples 'Federico II', Naples, Italy*

Received 31 October 2003; revised 2 December 2003; accepted 9 December 2003

Abstract—An efficient approach for the synthesis of cyclic peptides containing unnatural thioether side-chain bridges, based on the use of (2*S*)-9-fluorenylmethyl-2-[(*tert*-butoxycarbonyl)amino]-4-iodobutanoate and its homologue 5-iodopentanoate, derived from Boc-L-Asp-OFm and Boc-L-Glu-OFm, respectively, is reported. The synthesis was performed by a tandem combination of solid-phase peptide synthesis and microwave-assisted cyclization strategy.

The cyclization of peptides via thioether linkages has always been of special interest, as the peptides produced are effective mimics of cysteine-linked structures.[1] In this context, the synthesis of analogues of bioactive peptides incorporating lanthionine, a monosulfide analogue of cystine and a key constituent of peptide antibiotics, has received considerable attention in recent years.[2] Compared to the labile disulfide bridge between cysteine residues in natural or unnatural cyclic peptide sequences, the monosulfide bridge provides more constrained peptide structures and greater stability toward enzymatic degradation.[3] This class of cyclic peptides has been prepared by introducing into the peptide sequence the preformed multiprotected lanthionine or nor-lanthionine building block,[4] or via direct solid-phase thioalkylation of the Cys residue with the iodide derivatives of serine side chains.[5] As a part of our research work toward the synthesis of conformationally constrained peptides, we have been developing a new approach to the synthesis of cyclic peptides with thioether side-chain linkages, based on a combination of solid-phase peptide synthesis and microwave-assisted cyclization strategies. The application of microwave irradiation allows a striking reduction in reaction times, better yields and cleaner reactions.[6,7]

In our research on new analogues of Urotensin-II-(4-11) (H-Asp[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH), we report here the methodology used in the synthesis of two new lanthionine-like analogues, H-c[Xaa-Phe-Trp-Lys-Tyr-Cys]-Val-OH where Xaa = Abu or Ape[8] (**1** and **2**, Fig. 1).

Our approach involves the exploitation of (2*S*)-9-fluorenylmethyl-2-[(*tert*-butoxycarbonyl)amino]-4-iodobutanoate and its homologue, 5-iodopentanoate, as electrophilic agents in the thioalkylation of the Cys residue. By varying the nature of the iodo derivative it is possible to modulate the ring size of the corresponding cyclopeptides. The final cyclization of the peptide chain was achieved after simultaneous piperidine mediated deprotection of the N^{α} and C-terminal residues.

The preparation of the iodide derivatives was performed via the synthetic route[9] outlined in Scheme 1, starting

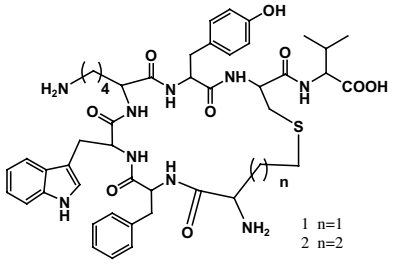
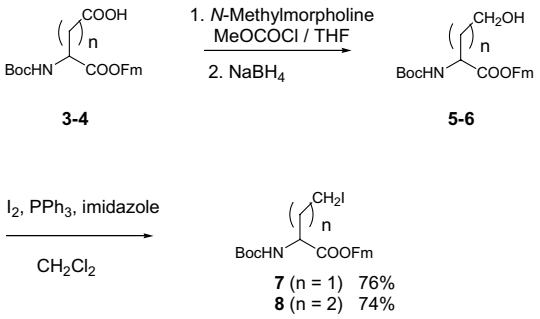


Figure 1. Urotensin-II analogues with monosulfide bridges.

Keywords: Thioether bridge; Solid-phase peptide synthesis; Macrocyclization.

* Corresponding author. Tel.: +39-081-678620; fax: +39-081-678644; e-mail: pagrieco@unina.it

0040-4039/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2003.12.036



Scheme 1. Synthesis of iodide derivatives.

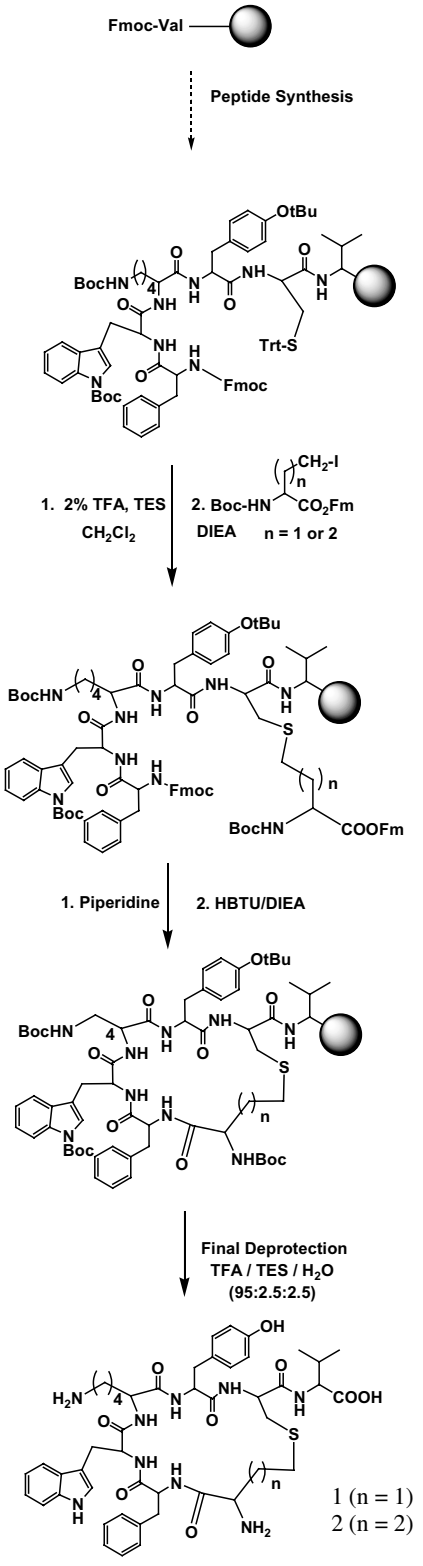
from Boc-L-Asp-OFm **3** and Boc-L-Glu-OFm **4**, respectively.[10] After the $NaBH_4$ reduction of the mixed anhydrides derived from acids **3** and **4** to the corresponding alcohols **5** and **6** (in 86% and 80% yields, respectively), a simple one-step triarylphosphine mediated iodination yielded the desired iodide derivatives **7** and **8**. The physicochemical properties and purities of the final compounds were assessed by TLC, LC–MS, analytical RP–HPLC, and ^1H NMR.[11]

Preparation of the peptides began with a series of HBTU/HOBt couplings performed using an ACT synthesizer mod 348 Ω[12] following a conventional Fmoc approach[13] to obtain the appropriate open-chain intermediates as indicated in Scheme 2. The *S*-Trt group of the Cys residue was removed with 2% trifluoroacetic acid in CH_2Cl_2 in the presence of triethylsilane. At this point, the peptide-resin and a fourfold excess of iodide derivatives (**7** or **8**) were transferred to a Milestone Ethos CombiChem microwave synthesizer. The thioalkylation reaction was performed by irradiation at 450 W, 50 °C, in DMF and DIEA for 10 min. Subsequently, the final one-step cyclization was achieved following removal of the Fmoc and OFm protecting groups.[14] The final products were isolated with good levels of purity following cleavage from the resin using a mixture of TFA/TES/H_2O (95:2.5:2.5) as was evident from HPLC analysis (Fig. 2). These conditions led to more than 73–75% conversion of the linear peptides into the cyclic peptides without decomposition or racemization of the desired compounds.

To demonstrate the real advantage of associating the microwave-assisted reaction with the solid-phase synthesis in the peptide thioalkylation step, we compared the results obtained using conventional thermal heating using identical stoichiometry.

When both microwave and conventional heating results were compared, we observed clear advantages in yield and reaction time with microwave heating, the reaction time being reduced to 10 min from 8 to 12 h. The successful results are summarized in Table 1.

The data collected in Table 1 confirm that microwave irradiation combined with the solid-phase peptide synthesis represents a powerful technique for accelerating thermal organic reactions to yield cyclic peptidomimetics.[15]



Scheme 2.

In conclusion, we report a high-speed, one-pot protocol for the generation of cyclic peptides with a thiosulfide bridge from readily available building blocks. This approach represents an attractive procedure compared with conventional methods.

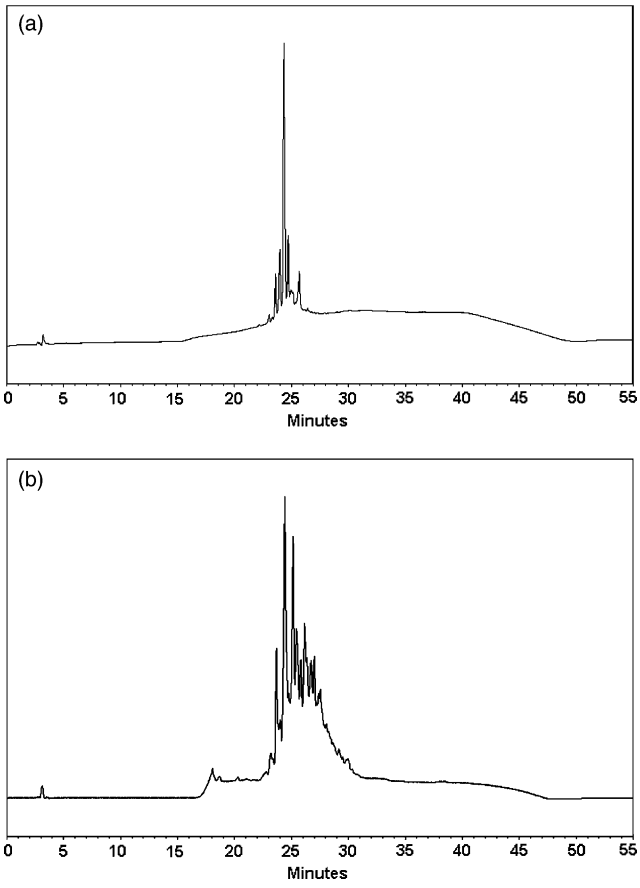


Figure 2. Reverse-phase HPLC profile of the final crude compound **1** obtained by microwave-assisted synthesis (a) and by the conventional method (b) (C_{18} column, linear gradient 10–90% MeCN/H_2O in 40 min, flow rate of 1.0 mL/min) after cleavage from the resin.

In addition, the examples reported here demonstrate that cyclic peptides containing thiosulfide bridges could be rapidly synthesized in a high-throughput fashion by combining microwave-assisted irradiation with solid-phase peptide chemistry.

Acknowledgements

The LC/MS and ^{1}H NMR spectral data were provided by Centro di Ricerca Interdipartimentale di Analisi Strumentale, Universitàdegli Studi di Napoli 'Federico II'. The assistance of the staff is gratefully appreciated.

References and notes

1. (a) Jack, R. W.; Jung, G. *Curr. Opin. Chem. Biol.* **2000**, *4*, 310–317; (b) Kaiser, D.; Jack, R. W.; Jung, G. *Pure Appl. Chem.* **1998**, *70*, 97–104.
2. (a) Li, H.; Jiang, X.; Goodman, M. *J. Pept. Sci.* **2001**, *7*, 82–91; (b) Li, H.; Jiang, X.; Howell, S. B.; Goodman, M. *J. Pept. Sci.* **2000**, *6*, 26–35; (c) Osapay, G.; Prokai, L.; Kim, H. S.; Medzihradszky, K. F.; Coy, D. H.; Liapakis, G.; Reisine, T.; Melancini, G.; Zhu, Q.; Wang, S. H. H.; Mattern, R. H.; Goodman, M. *J. Med. Chem.* **1997**, *40*, 2241–2251; (d) Zhu, X.; Schimdt, R. R. *Eur. J. Org. Chem.* **2003**, 4069–4072.
3. (a) Shao, H.; Wang, H. H.; Lee, C. W.; Osapay, G.; Goodman, M. *J. Org. Chem.* **1995**, *60*, 2956–2957; (b) Osapay, G.; Goodman, M. *J. Chem. Soc., Chem. Commun.* **1993**, 1599–1600; (c) Osapay, G.; Zhu, Q.; Shao, H.; Chadha, R. H.; Goodman, M. *Int. J. Pept. Protein Res.* **1995**, *46*, 290–301; (d) Van Kraaij, C.; Vos, W. M.; Siezen, R. J.; Kuipers, O. P. *Nat. Prod. Rep.* **1999**, *16*, 575–587; (e) Melacini, G.; Zhu, Q.; Osapay, G.; Goodman, M. *J. Med. Chem.* **1997**, *40*, 2252–2258.
4. (a) Mohd Mustapa, M. F.; Harris, R.; Bulic-Subanovic, N.; Elliott, S. L.; Bregant, S.; Groussier, M. F. A.; Mould, J.; Schultz, D.; Chubb, N. A. L.; Gaffney, P. R. J.; Driscoll, P. C.; Tabor, A. B. *J. Org. Chem.* **2003**, *68*, 8185–8192; (b) Mohd Mustapa, M. F.; Harris, R.; Esposito, D.; Chubb, N. A. L.; Mould, J.; Schultz, D.; Driscoll, P. C.; Tabor, A. B. *J. Org. Chem.* **2003**, *68*, 8193–8198, and references cited therein.
5. Rew, Y.; Malkmus, S.; Svensson, C.; Yaksh, T. L.; Chung, N. N.; Schiller, P. W.; Cassel, J. A.; DeHaven, R. N.; Goodman, M. *J. Med. Chem.* **2002**, *45*, 3743–3754.
6. (a) Strohmeier, G. A.; Kappe, O. *J. Comb. Chem.* **2002**, *4*, 154–161; (b) Lidstrom, P.; Tierney, J.; Wathey, B.; Westman, J. *Tetrahedron* **2001**, *57*, 9225–9283; (c) Finaru, A.; Berthault, A.; Besson, T.; Guillaumet, G.; Berteina-Raboin, S. *Org. Lett.* **2002**, *4*, 2613–2615.
7. (a) Lew, A.; Krutzik, P. O.; Hart, M. E.; Chamberlin, A. R. *J. Comb. Chem.* **2002**, *4*, 95–105; (b) Kappe, O. *Curr. Opin. Chem. Biol.* **2002**, *6*, 314–320; (c) Coleman, C. M.; MacElroy, J. M. D.; Gallagher, J. F.; O'Shea, D. F. *J. Comb. Chem.* **2002**, *4*, 87–93; (d) Larhed, M.; Hallberg, A. *Drug Discovery Today* **2001**, *6*, 406–416.
8. Abu = α-aminobutyric acid; Ape = α-aminopentanoic acid.
9. (a) Caputo, R.; Cassano, E.; Longobardo, L.; Palumbo, G. *Tetrahedron Lett.* **1995**, *36*, 167–168; (b) Caputo, R.; Cassano, E.; Longobardo, L.; Palumbo, G. *Tetrahedron* **1995**, *51*, 12337–12350.
10. Crozet, Y.; When, J. J.; Loo, R. O.; Andrews, P. C.; Spatola, A. F. *Mol. Diversity* **1998**, *3*, 261–276.
11. The products were prepared on 3 mmol scale and were purified by flash chromatography using a gradient of 0–30% *n*-hexane in EtOAc. **7**, yield 76%, $[\alpha]_D^{20} = -15.4°$ ($c = 0.1$, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.42 (s, 9H, Boc), 1.98–2.03 and 2.18–2.22 (m, 2H, β-CH_2), 2.95–3.09 (m, 2H, CH_2-I), 4.18–4.20 (t, $J_{\alpha,\beta} = 5.6$ Hz, 1H,

Table 1. Conventional method and microwave solid-phase experiments

	Conventional thermal heating			Microwave irradiation		
	Reaction time (h)	Temperature (°C)	Yield[a] (%)	Reaction time (min)	Power (W)	Yield[a] (%)
1	8	50	50	10	450	75
2	12	50	38	10	450	73

[a] Yield after purification by reverse-phase HPLC.

α-CH), 4.35 (br s, 1H, NH-Boc), 4.50–4.58 (m, 2H, CH_2-Fmoc), 4.99 (m, 1H, CH-Fmoc), 7.35–7.41, 7.59, and 7.78 (m, 8H, aromatic Fmoc). FABMS m/z calcd for $C_{23}H_{26}INO_4$ 507.36, found 507.79. **8**, yield, 74%, $[\alpha]_D^{20} = -16.0°$ ($c = 0.1$, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.40 (s, 9H, Boc), 1.65–1.79 (m, 4H, β and γ-CH_2), 3.01–3.12 (m, 2H, CH_2-I), 4.19 (t, $J_{\alpha,\beta} = 5.6$ Hz, 1H, α-CH), 4.32 (br s, 1H, NH-Boc), 4.50–4.59 (m, 2H, CH_2-Fmoc), 4.97 (m, 1H, CH-Fmoc), 7.35–7.41, 7.59, and 7.78 (m, 8H, aromatic Fmoc). FABMS m/z calcd for $C_{24}H_{28}INO_4$ 521.38, found 521.77.

12. The peptide was prepared on 0.1 mmol scale starting from Fmoc-Val-Wang (0.76 mmol/g).
13. Atherton, E.; Sheppard, R. C. *Solid-Phase Peptide Synthesis: A Practical Approach*; IRL: Oxford, UK, 1989.
14. General procedure: The thioalkylation reactions were carried out in a Milestone CombiChem Microwave Synthesizer with vessels of 4 mL volume, using DIEA (0.5 equiv) and DMF as solvent. In all irradiation experiments, rotation of the rotor, irradiation time, temperature, and power were monitored with the 'easyWAVE' software package. Temperature was monitored with the aid of an optical fiber inserted into one of the reaction containers. Once 50 °C was reached the reaction mixture was held at this temperature for 10 min and then cooled rapidly to room temperature. The reaction vessels were opened and the contents washed with DMF (3×10 mL) and CH_2Cl_2 (3×10 mL). The final cyclization step was perfomed, after usual piperidine Fmoc deprotection, using HBTU/HOBt as activating agent for 3 h. The resin was washed and subjected to final cleavage from the product. The crude product was precipitated using anhydrous diethyl ether and recovered by filtration. All final products were purified by reverse-phase HPLC carried out on Vydac C-18 column with the following dimensions: 25×0.46 cm for analysis and 25×2.2 cm for preparative work. Flow rate: 1.0 and 5.0 mL/min for analytical and preparative HPLC, respectively. Analysis of the purified products was performed by reverse-phase HPLC, and mass spectroscopy. Compound **1**: $C_{47}H_{61}N_9O_9S$, MW: 927.43, MS (ESI, EI^+) m/z 928.02 (M^+), HPLC: $k' = 6.80$ min; Compound **2**: $C_{48}H_{63}N_9O_9S$, MW: 941.30, MS (ESI, EI^+) m/z 942.20 (M^+), HPLC: $k' = 7.00$ min.
15. Grieco, P.; Campiglia, P.; Gomez-Monterrey, I.; Lama, T.; Novellino, E. *Synlett* **2003**, *14*, 2216–2218.