ORGANIC LETTERS

2003
Vol. 5, No. 12
2115−2118

Tentoxin as a Scaffold for Drug Discovery. Total Solid-Phase Synthesis of Tentoxin and a Library of Analogues

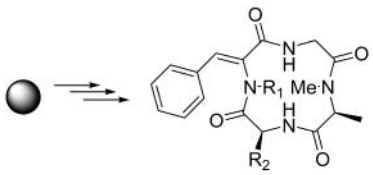
Jose C. Jiménez,[§,‡] Bibiana Chavarría,[‡] Àngel López-Macià,[‡,||] Miriam Royo,[‡,⊥] Ernest Giralt,[§,‡] and Fernando Albericio*[,§,‡]

Barcelona Biomedical Research Institute and Combinatorial Chemistry Unity, Barcelona Science Park, University of Barcelona, Josep Samitier 1, 08028-Barcelona, Spain, Department of Organic Chemistry, University of Barcelona, Martí i Franqués 1, 08028-Barcelona, Spain, and PharmaMar, Avda Reyes Católicos 1, 28770-Colmenar Viejo, Madrid, Spain

albericio@pcb.ub.es

Received March 26, 2003

ABSTRACT



A solid-phase method for the synthesis of tentoxin has been developed. Two key steps—dehydration and *N*-alkylation—are carried out while the peptide is anchored to the resin. The method, which has been validated by the preparation of a library of tentoxin analogues, should be applicable to the generation of further libraries that have the tentoxin scaffold structure, as well as other structures containing *N*-alkylated didehydroamino acids.

The recent developments in genomics, proteomics, and molecular biology are providing a large number of new targets associated with the most important diseases.[1] Although computational molecular modeling methods have evolved enormously in the past few years, rational drug design methods based on these are far from being routinely implemented.[2] 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.[3] Scaffolds or templates can be prepared through de novo design. Furthermore, an alternative and attractive strategy involves taking advantage of the rich source of structural diversity and biological activity shown by natural products as a result of evolution and natural selection.[4,5] In this sense, a number of natural products can be considered as *privileged structures*—as defined by Evans and co-workers[6]—because their function may be related to binding to more than one protein, different from that involved in their biosynthesis.[7] Natural products with contrasting biological activities could therefore be considered as unique scaffolds for the discovery of new drugs aimed at targets that differ from their primary activity. The

[§] Barcelona Biomedical Research Institute
[‡] Department of Organic Chemistry
[||] PharmaMar
[⊥] Combinatorial Chemistry Unity

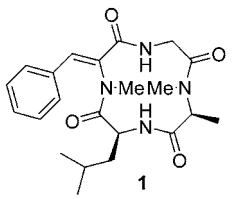
(1) Hruby, V. J. *J. Pept. Res.* **2002**, *60*, 305−306.
(2) Shimada, J.; Ekins, S.; Elkin, C.; Shakhnovich, E. I.; Wery, J.-P. *Targets* **2002**, *1*, 196−205.
(3) Tan, D. S.; Foley, M. A.; Stockwell, B. R.; Shair, M. D.; Schreiber, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 9073−9087.
(4) Henkel, T.; Brunne, R. M.; Müller, H.; Reichel, F. *Angew. Chem., Int. Ed.* **1999**, *38*, 643−647.
(5) Wrigley, S. K.; Hayes, M. A.; Thomas, R.; Chrystal, E. J. T.; Nicholson, N., Eds. *Biodiversity. New Leads for the Pharmaceutical and Agrochemical Industry*; Royal Society of Chemistry: Cambridge, UK, 2000.
(6) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J. *J. Med. Chem.* **1988**, *31*, 2235−2246.
(7) Breinbauer, R.; Vetter, I. R.; Waldmann, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 2878−2890.

10.1021/ol0345273 CCC: $25.00
Published on Web 05/09/2003

drug discovery process can be facilitated further by modifying these scaffolds by exploiting combinatorial techniques.[8]

Tentoxin (**1**), which is a phytotoxic metabolite isolated from the pathogenic fungus *Alternaria tenuis* Ness,[9,10] can be assessed as a scaffold because it fits all of the requirements described above. For example, tentoxin induces chlorosis in many dicotyledone plants, except cereals, tomatoes, and members of the species cruciferae and graminae,[11] thus making it a potential selective herbicide.[12] Furthermore, it has been demonstrated that tentoxin has at least two sites that are capable of forming strong interactions with substrates.[13−16] The first site, which shows great affinity, is the cause of the chlorosis effect whereas the second, which has a lower affinity constant, is related to an effect involving growth stimulation.

On the other hand, with the exception of diketopiperazines, cyclic tetrapeptides are the smallest cyclic peptides that can be synthesized.[17] These compounds have a molecular weight below 500, which makes them suitable for modification and to fulfill the Lipinski[18] and Tice[19] rules. Finally, NMR studies have shown that the tentoxin cycle is rather rigid.[20,21]



1

Tentoxin also contains motifs of other therapeutically interesting peptides, including the presence of *N*-methyl residues and the didehydroamino group present in cyclosporine,[22] thiocoraline,[23] or kahalalide F.[24]

Tentoxin exhibits structural features not commonly found in natural peptides: (i) the presence of a didehydroamino acid (DDAA), (ii) the strained 12-membered-ring system, and (iii) the fact that two of the four residues are methylated, including the nonproteinogenic didehydroamino acid. These factors all contribute to the challenge for this system as a synthetic target.

To adopt the tentoxin structure as a scaffold in a combinatorial program it is necessary to have a rapid and efficient method for its synthesis, preferably with the potential for automization. Although the preparation of compound libraries may be conducted in solution or in solid phase, the latter method is often preferred for reasons highlighted by the syntheses of biomolecules such as peptides[25] and oligonucleotides.[26] The first objective of the present work was therefore to develop a solid-phase method for the synthesis of tentoxin. Syntheses described in the literature are mainly carried out in solution,[10] with the exception of the example reported by Rich and Mathiaparanam, in which the linear tetrapeptide containing a precursor of the DDAA was prepared in the solid phase before the dehydration and methylation stages.[27]

The key steps of the strategy presented here are (i) the formation of the DDAA, (ii) the *N*-methylation of the DDAA, (iii) the choice of the linear precursor, and (iv) cyclization.

(i) The synthesis of didehydropeptides (DDP) such as tentoxin by the introduction of the protected N^{α}-DDAA cannot be carried out. This is because the weak nucleophilicity of the corresponding enamine function prevents an efficient elongation in the *C* to *N* direction when the N^{α}-protecting group is removed.[28,29] The synthesis of DDP can only be accomplished by forming the double bond once the peptide sequence has been assembled or by incorporation of a shorter peptide, preferably a dipeptide, containing the DDAA.[30] However, in this case the second strategy suffers from several drawbacks. For example, it would involve carrying out the dehydration step in solution, which is not appropiate in a combinatorial scheme if different analogues are required. Furthermore, selective protection of the carboxyl group of the β-hydroxyphenylalanine [phenylserine, Phe(β-OH)] in the presence of amino and hydroxyl groups must be carried out.

In the present work the DDP was prepared in the solid phase by a method developed in our laboratory.[31] The route

(8) Nicolaou, K. C.; Hanko, R.; Hartwig, W., Eds. *Handbook of Combinatorial Chemistry*; Wiley-VCH: Weinheim, Germany, 2002; Vols. 1 and 2.

(9) Fulton, N. D.; Bollenbacher, K.; Templeton, G. E. *Phytopathology* **1965**, *55*, 49−51.

(10) Cavelier, F.; Verducci, J.; Andre, F.; Haraux, F.; Sigalat, C.; Traris, M.; Vey, A. *Pestic. Sci*. **1998**, *52*, 81−89 and references therein.

(11) Durbin, R. D.; Uchytil, T. F. *Phytopathology* **1977**, *67*, 602−603.

(12) Lax, A. R.; Shepherd, H. S.; Edwards, J. V. *Weed Technol*. **1988**, *2*, 540−544.

(13) Steele, J. A.; Uchytil, T. F.; Durbin, R. D.; Bhatnagar, P.; Rich, D. H. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2245−2248.

(14) Hu, N.; Mills, D. A.; Huchzermeyer, B.; Richter, M. L. *J. Biol. Chem*. **1993**, *268*, 8536−8540.

(15) Dahse, I.; Pezennec, S.; Girault, G.; Berger, G.; Andre, F.; Liebermann, B. *J. Plant Physiol*. **1994**, *143*, 615−620.

(16) Sigalat, C.; Pitard, B.; Haraux, F. *FEBS Lett.* **1995**, *368*, 253−256.

(17) In the literature, only the synthesis of a cyclotripeptide containing concatenated α-amino acids through the α-function [*c*(D-Phe-L-Pro-L-Pro), which contains two Pro and a D residue that favors the cyclization] has been described. Rothe, M.; Faehnle, M.; Maestle, W. In *Peptides: Synthesis, Structure, Function, Proceedings of the American Peptide Symposium, 7th*; Rich, D. H., Gross, E. Eds.; Pierce Chem. Co.: Rockford, IL, 1981; pp 89−92.

(18) Lipinski, C. A.; Fiese, E. F.; Korst, R. J. *Quant. Struct.-Act. Relat*. **1991**, *10*, 109−117.

(19) Tice, C. M. *Pest Manag. Sci*. **2001**, *57*, 3−16.

(20) Rich, D. H.; Bhatnagar, P. K. *J. Am. Chem. Soc*. **1978**, *100*, 2212−2218.

(21) Pinet, E.; Neumann, J.-M.; Dahse, I.; Girault, G.; Andre, F. *Biopolymers* **1995**, *36*, 135−152.

(22) Borel, J. F.; Kis, Z. L.; Beveridge, T. *Search Anti-Inflammatory Drugs* **1995**, 27−63.

(23) Romero, F.; Espliego, F.; Perez Baz, J.; Garcia de Quesada, T.; Gravalos, D.; De la Calle, F.; Fernandez-Puentes, J. L. *J. Antibiot.* **1997**, *50*, 734−737.

(24) Hamann, M. T.; Scheuer, P. J. *J. Am. Chem. Soc*. **1993**, *115*, 5825−5826.

(25) Lloyd-Williams, P.; Albericio, F.; Giralt, E. In *Chemical Approaches to the Synthesis of Peptides and Proteins*; CRC Press: Boca Raton, FL, 1997.

(26) Bellon, L.; Wincott, F. Oligonucleotide Synthesis. In *Solid-Phase Synthesis. A Practical Guide*; Kates, S. A., Albericio, F., Eds.; Marcel Dekker: New York, 2000; pp 475−528.

(27) Rich, D. H.; Mathiaparanam, P. *Tetrahedron Lett*. **1974**, *15*, 4037−4040.

(28) Moriya, T.; Yoneda, N.; Miyoshi, M.; Matsumoto, K. *J. Org. Chem*. **1982**, *47*, 94−98.

(29) Shin, C. G.; Yonezawa, Y.; Yamada, T. *Chem. Pharm. Bull*. **1984**, *32*, 3934−3944.

(30) Bayó, N.; Jiménez, J. C.; Rivas, L.; Nicolás, E.; Albericio, F. *Chem., Eur. J*. **2003**, *9*, 1096−1103.

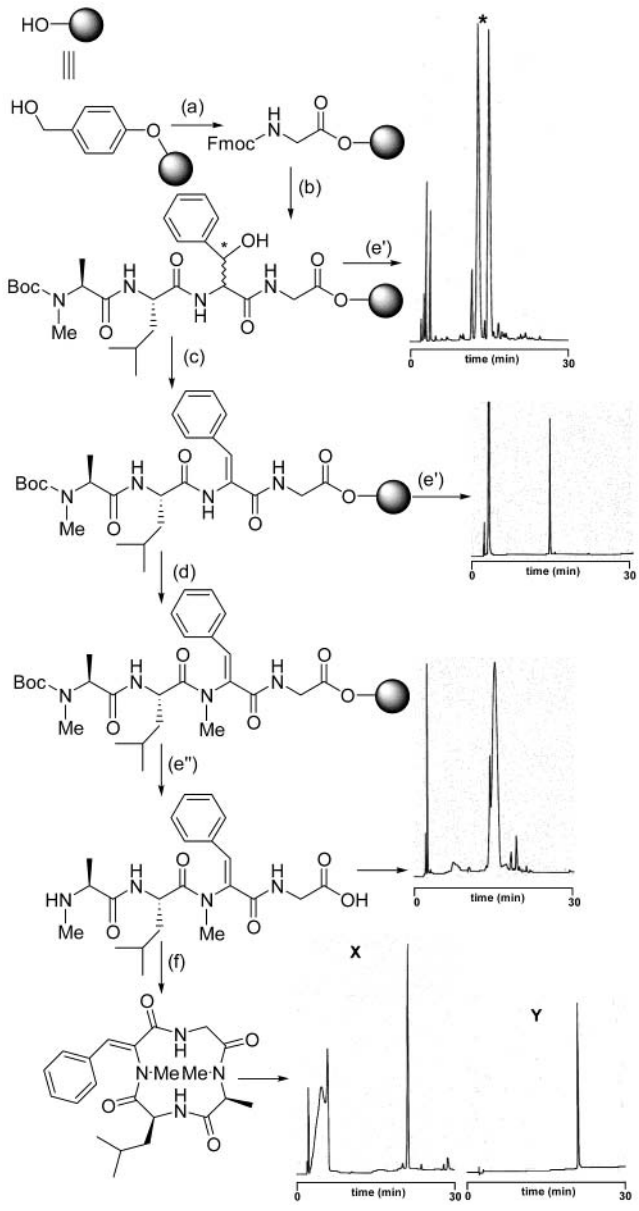
Table 1. Linear Peptides Enclosed in the Tentoxin Sequence

	sequence	risk of epimerization[a]	difficulty of cyclization[a]	DKP formation[b] *(%)*
I	H-L-MeAla-L-Leu-L/D-Phe(βOH)-Gly-O-Resin	none	moderate	34
II	H-Gly-L-MeAla-L-Leu-L/D-Phe(βOH)-O-Resin	none	high	15
III	H-L-Leu-L/D- Phe(βOH)-Gly-L-MeAla-O-Resin	high	low	48
IV	H-L/D-Phe(βOH)-Gly-L-MeAla-L-Leu-O-Resin	-	very high	not evaluated

[a] Assumed. [b] Calculated by amino acid analysis (AAA) of the peptide resin before and after the incorporation of the third residue.

involves the preparation of the peptide with the precursor of the didehydro residue, in this case [L/D-Phe(β-OH)], activation of the hydroxy group with 3-(3′-dimethylaminopropyl)-1-ethylcarbodiimide (EDC, WSC) in the presence of CuCl,[32] and concomitant elimination. Although the linear sequence was obtained with the diastereomeric mixture of Phe(β-OH), this method gave exclusively the *Z* isomer, which is the thermodynamically more stable product.

Scheme 1. Solid-Phase Strategy Developed for the Preparation of Tentoxin[a]



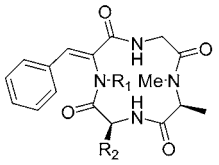
[a] Reagents and conditions: (a) Fmoc-Gly-OH, DIPCDI/DMAP, DCM; (b) SPPS; (b′) piperidine/DMF (2:8); (b″) Fmoc/Boc-aa-OH, DIPCDI/HOBt, DMF; (c) EDC·HCl, CuCl, DCM/DMF (9:1); (d) MeI, K_2CO_3, 18-crown-6, DMF; (e′) = (e″) TFA/H_2O (19:1); (f) DIPCDI/HOBt/DIEA, DCM/DMF (99:1).

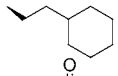
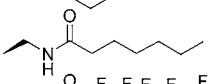
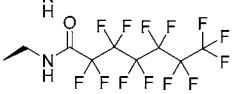
(ii) The *N*-methylation of the didehydrophenylalanine residue was also carried out on the solid phase with MeI as the alkylating agent and K_2CO_3 as the base in the presence of 18-crown-6 as a phase transfer reagent in DMF. These conditions led to regioselective methylation of the didehydro residue due to the greater acidity of the corresponding amide bond. The absence of 18-crown-6 or the use of 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) as base instead of K_2CO_3 did not give the *N*-methylated derivative in any case. As expected, the Fmoc group of the *N*-terminal residue was unstable under these conditions and therefore these residues have to be introduced while protected with the Boc group.

(iii) In this case, the choice is limited for several reasons. As mentioned above, the DDAA or its precursor cannot be situated at the *N*-terminal position (Sequence IV, Table 1). On the other hand, tentoxin contains an additional *N*-methylated residue (Ala) and a glycine, which the Ala residue can epimerizase if it is at the *C*-terminal position during the cyclization[25] and both the Ala and glycine are prone to induce diketopiperazine (DKP) formation if they are at the first two positions with respect to the *C*-terminal.[33] Synthesis of the different possible peptides was carried out and DKP formation was evaluated. Table 1 shows the results and highlights problematic sequences along with the corresponding DKP formation data. Sequence I contains an *N*-MeAla as *N*-terminal and this would be expected to make the cyclization step more difficult. The Gly at the *C*-terminal should favor both cyclization and DKP formation. Sequence II contains a Gly at the *N*-terminal that should favor cyclization. Furthemore, the didehydro residue could be advantageous because epimerization is not applicable in this case, although the carboxylic groups of these residues have poor reactivity.[34] Sequence III should suffer from a high risk of racemization because *N*-MeAla is at the *C*-terminal, but will have a high probability of cyclization because the Leu at the *N*-terminal

(31) Royo, M.; Jiménez, J.; López-Macià, À.; Giralt, E.; Albericio, F. *Eur. J. Org. Chem.* **2001**, 45−48.
(32) Fukase, K.; Kitazawa, M.; Sano, A.; Shimbo, K.; Horimoto, S.; Fujita, H.; Kubo, A.; Wakamiya, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 2227−2240.
(33) Alsina, J.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1996**, *37*, 4195−4198 and references therein.
(34) López-Macià, À.; Jiménez, J. C.; Royo, M.; Giralt, E.; Albericio, F. *J. Am. Chem. Soc.* **2001**, *123*, 11398−11401.

Table 2. Tentoxin Analogues Synthesized by Using the Approach Outlined in Scheme 1[37]



#	R_1	R_2	MS (M+H)$^+$ calcd.	found
2	CH_3		468.6	470.3
3	CH_3		499.6	500.3
4	CH_3		733.5	734.2
5			490.3	492.4
6	CH_3		414.2	414.3
7			428.4	430.3
8			440.5	442.3

is not a hindered residue. Finally, it was demonstrated that the risk of DKP formation was high because the two *C*-terminal residues were prone to promote this side reaction. Although sequence II initially appeared to be a promising candidate, it was ruled out because the dehydration step carried out on the solid phase did not give the target compound due to the formation of several byproducts and early cleavage from the resin.[35]

Tentoxin was ultimately synthesized by using sequence I (Scheme 1).

(35) Jiménez, J. C.; Bayó, N.; Chavarría, B.; López-Macià, À.; Royo, M.; Nicolás, E.; Giralt, E.; Albericio, F. *Lett. Pept. Sci.* In press.

Tentoxin was obtained with a nonoptimized overall yield of 25% calculated from the starting Wang resin [60% for steps a−e″ (Scheme 1) and 41% for cyclization and purification steps].

The compounds shown in Table 2 were synthesized by following a similar strategy. The preparation of compounds **3** and **4** was carried out with 1,2-L-diaminopropionic acid (instead of Leu) bearing Fmoc and Alloc groups for the protection of the α and β amino groups, respectively. The Alloc group was removed after the alkylation step by a process involving an allyl transfer to $PhSiH_3$ in the presence of $Pd(PPh_3)_4$, followed by acylation of the β-amino function by the DIPCDI/HOBt method.[36,37]

In conclusion, the synthetic method reported here can be applied to generate libraries of compounds that have tentoxin as the scaffold structure, as well as other structures containing *N*-alkylated didehydroamino acids. All reactions except for the final cyclization take place on the solid phase.

Acknowledgment. M.R. is a Ramon y Cajal fellow (MCyT, Spain). This work was partially supported by CICYT (BQU2000-0235, BQU2002-02047, and BIO2002-02301), Generalitat de Catalunya [Grup Consolidat, and Centre de Referència en Biotecnologia].

Supporting Information Available: Experimental procedures for the synthesis of tentoxin. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0345273

(36) Although tentoxin and its analogues were obtained after cyclization with excellent purity, similar to that shown by tentoxin (HPLC profile X, Scheme 1), further purification was carried out in all cases by reversed-phase HPLC to give compounds with a purity greater than 95%.

(37) For analogues 2 and 3, Alloc removal was performed with $Pd(PPh_3)_4$ (0.1 equiv) and $PhSiH_3$ (10 equiv) in anhydrous DCM (3 × 15 min) under an Ar atmosphere.