

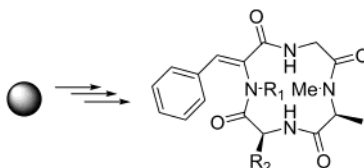
Tentoxin as a Scaffold for Drug Discovery. Total Solid-Phase Synthesis of Tentoxin and a Library of Analogues

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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 dihydroamino 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-workers⁶—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

(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.

[§] Barcelona Biomedical Research Institute

[†] Department of Organic Chemistry

^{||} PharmaMar

[⊥] Combinatorial Chemistry Unity

(1) Hruby, V. J. *J. Pept. Res.* **2002**, *60*, 305–306.

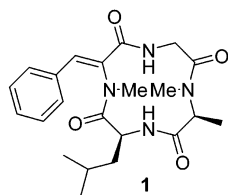
(2) Shimada, J.; Ekins, S.; Elkin, C.; Shakhnovich, E. I.; Wery, J.-P. *Targets* **2002**, *1*, 196–205.

(3) Tan, D. S.; Foley, M. A.; Stockwell, B. R.; Shair, M. D.; Schreiber, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 9073–9087.

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 gramineae,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 Lipinski¹⁸ and Tice¹⁹ rules. Finally, NMR studies have shown that the tentoxin cycle is rather rigid.^{20,21}



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 automatization. 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 peptides²⁵ 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 Mathiaraman, 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 appropriate 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) Cavellier, 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.; Uchytel, 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.; Uchytel, 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.; Pezenec, 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 cyclotriptide 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.; Faehle, 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.; Fieser, 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.; Giral, 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.; Mathiaraman, 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.

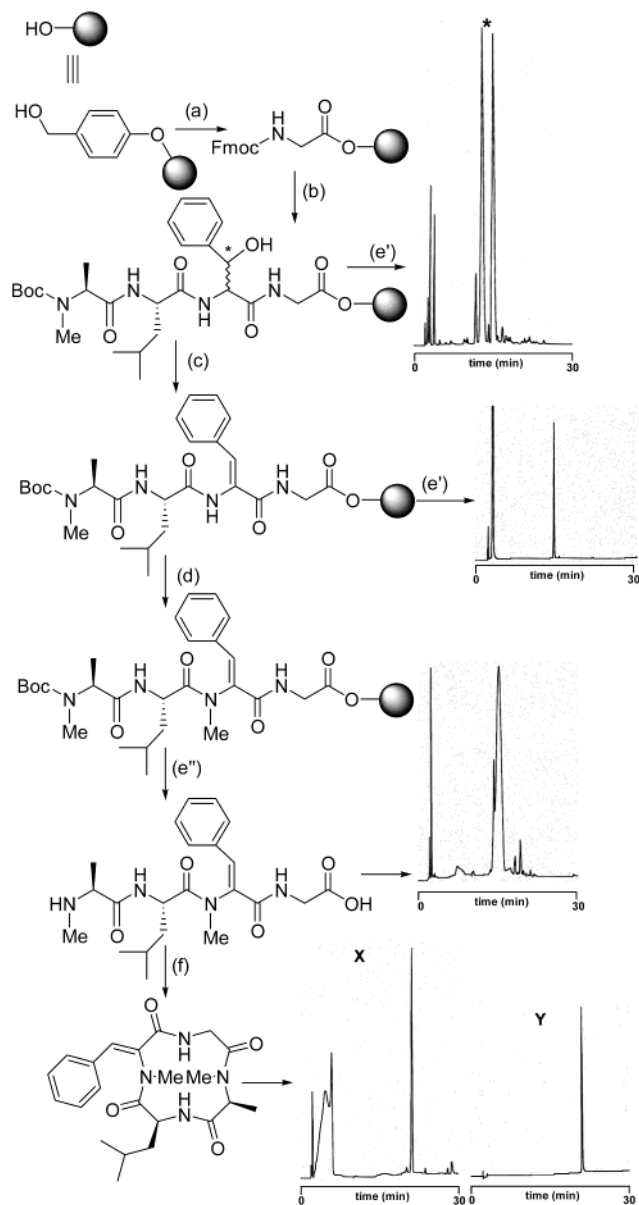
(30) Bayó, N.; Jiménez, J. C.; Rivas, L.; Nicolás, E.; Albericio, F. *Chem., Eur. J.* **2003**, *9*, 1096–1103.

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(β OH)-Gly-O-Resin	none	moderate	34
II	H-Gly-L-MeAla-L-Leu-L/D-Phe(β OH)-O-Resin	none	high	15
III	H-L-Leu-L/D-Phe(β OH)-Gly-L-MeAla-O-Resin	high	low	48
IV	H-L/D-Phe(β OH)-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 dihydro residue, in this case [L/D-Phe(β -OH)],

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

^a Reagents and conditions: (a) Fmoc-Gly-OH, DIPCIDI/DMAP, DCM; (b) SPPS; (b') piperidine/DMF (2:8); (b'') Fmoc/Boc-aa-OH, DIPCIDI/HOBt, DMF; (c) EDC·HCl, CuCl, DCM/DMF (9:1); (d) MeI, K₂CO₃, 18-crown-6, DMF; (e') = (e'') TFA/H₂O (19:1); (f) DIPCIDI/HOBt/DIEA, DCM/DMF (99:1).

activation of the hydroxy group with 3-(3'-dimethylamino-propyl)-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.

(ii) The *N*-methylation of the dihydrophenylalanine residue was also carried out on the solid phase with MeI as the alkylating agent and K₂CO₃ 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 dihydro 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 K₂CO₃ 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 epimerize if it is at the *C*-terminal position during the cyclization²⁵ 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. Furthermore, the dihydro 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

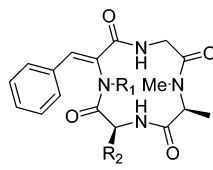
(31) Royo, M.; Jiménez, J.; López-Macià, À.; Giralt, E.; Albericio, F. *Eur. J. Org. Chem.* **2001**, 45–48.

(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 1³⁷



#	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.; Chavarria, 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 PhSiH₃ in the presence of Pd(PPh₃)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 dihydroamino acids. All reactions except for the final cyclization take place on the solid phase.

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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>.

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(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(PPh₃)₄ (0.1 equiv) and PhSiH₃ (10 equiv) in anhydrous DCM (3 \times 15 min) under an Ar atmosphere.