Structural Modifications of the Active Site in Teicoplanin and Related Glycopeptides. 2. Deglucoteicoplanin-Derived Tetrapeptide

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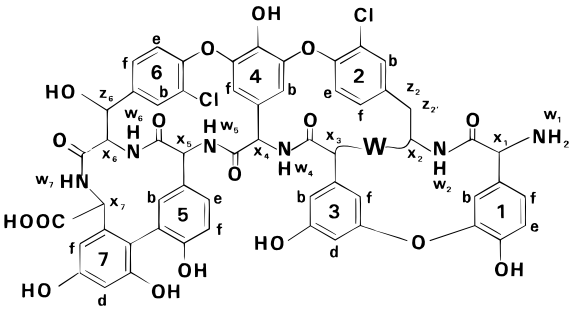
The deglucoteicoplanin-derived tetrapeptide (**TDTP**), a key synthon suitable for the synthesis of modified glycopeptide antibiotics differing in the structure of the active site, was prepared from the product (**RH-TD**) of reductive hydrolysis of the 2,3-peptide bond of deglucoteicoplanin (**TD**) upon selective oxidation of the newly formed hydroxymethyl group and following simultaneous removal of amino acids 1 and 3 by *double* Edman degradation. The oxidation of the alcohol function of residue 2 in **RH-TD** was accomplished (Jones reagent) after protection of the two free amino groups as *tert*-butyl BOC carbamates and of most of phenolic hydroxy groups as benzyl CBZ carbonates. Esterification of the C-terminal carboxy group of intermediate **di-BOC-RH-TD** allowed the formation at the end of the process of the tetrapeptide (**TDTP-Me**) protected at one carboxy group as methyl ester. Selective protection of the primary N^4- and N^2-amino groups of **TDTP-Me** as BOC and CBZ carbamates, respectively, followed by removal of the BOC function, afforded a more suitable intermediate (**N^2-CBZ-TDTP-Me**) for the synthesis of new glycopeptides.

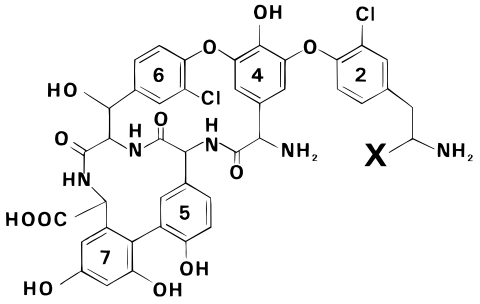
The mechanism of action of glycopeptide antibiotics[1] in part depends on the structure of amino acids 1 and 3. A current strategy to overcome the emerging resistance to glycopeptides in pathogenic bacteria[2] is based on the replacement of these amino acids with new amino acids or other moieties suitably selected to interact with the modified target[3] present in resistant organisms. The recent discovery of a selective method for the reductive hydrolysis of the amide bond between amino acids 2 and 3 in teicoplanin, its pseudoaglycons and aglycon (**TD**, Figure 1), and related glycopeptides, with sodium borohydride in EtOH/H_2O 35/65 solution,[4] provided the opportunity to remove amino acids 1 and 3 by Edman degradation. The resulting tetrapeptide derivatives are potential synthons for the synthesis of new families of glycopeptides differing in the structure of their active site.[5]

In this paper, the preparation of the deglucoteicoplanin-derived tetrapeptide (**TDTP**, Figure 2) from the product (**RH-TD**) of reductive hydrolysis of the 2,3-peptide bond of **TD** is described. Suitable methods for the selective protection and deprotection of the two primary amino groups and of the C-terminal carboxy group of the tetrapeptide chain are also reported.



TD: W = -NH-CO-; RH-TD: W = -NH_2 $HOCH_2$-

Figure 1. Structure of TD and RH-TD (with proton nomenclature).



TDTP: X = -COOH; TDTPA: X = CH_2OH

Figure 2. Structure of TDTP and TDTPA.

Results and Discussion

Two different synthetic pathways could be followed to prepare **TDTP** from **RH-TD**.

(i) Procedure A (Scheme 1): the amino and phenolic hydroxy groups of **RH-TD** are properly protected before oxidation of the primary alcohol function of residue 2. After deprotection, the resulting pentapeptide derivative (**TDPP**) is submitted to a *double* Edman degradation[6] step for the simultaneous removal of amino acid fragments 1 and 3.

(ii) Procedure B (Scheme 2): it consists of Edman degradation of **RH-TD** as a preliminary step. The resulting tetrapeptide alcohol (**TDTPA**, Figure 2) is then oxidized after proper protection of the susceptible amino and phenolic functions. Final deprotection steps give **TDTP**.

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(1) Parenti, F.; Cavalleri, B. *Drugs Future* **1990**, *15*, 57.

(2) Johnson, A. P.; Uttley, A. H. C.; Woodford, N.; George, R. C. *Clin. Microbiol. Rev.* **1990**, *3*, 280.

(3) Bugg, T. D. H.; Walsh, C. T. *Nat. Prod. Rep.*, **1992**, *9*, 199.

(4) Malabarba, A.; Ciabatti, R.; Kettenring, J.; Ferrari, P.; Vékey, K.; Bellasio, E.; Denaro, M. *J. Org. Chem.* Submitted for publication.

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(6) Edman, P. *Acta Chem. Scand.* **1950**, *4*, 277.

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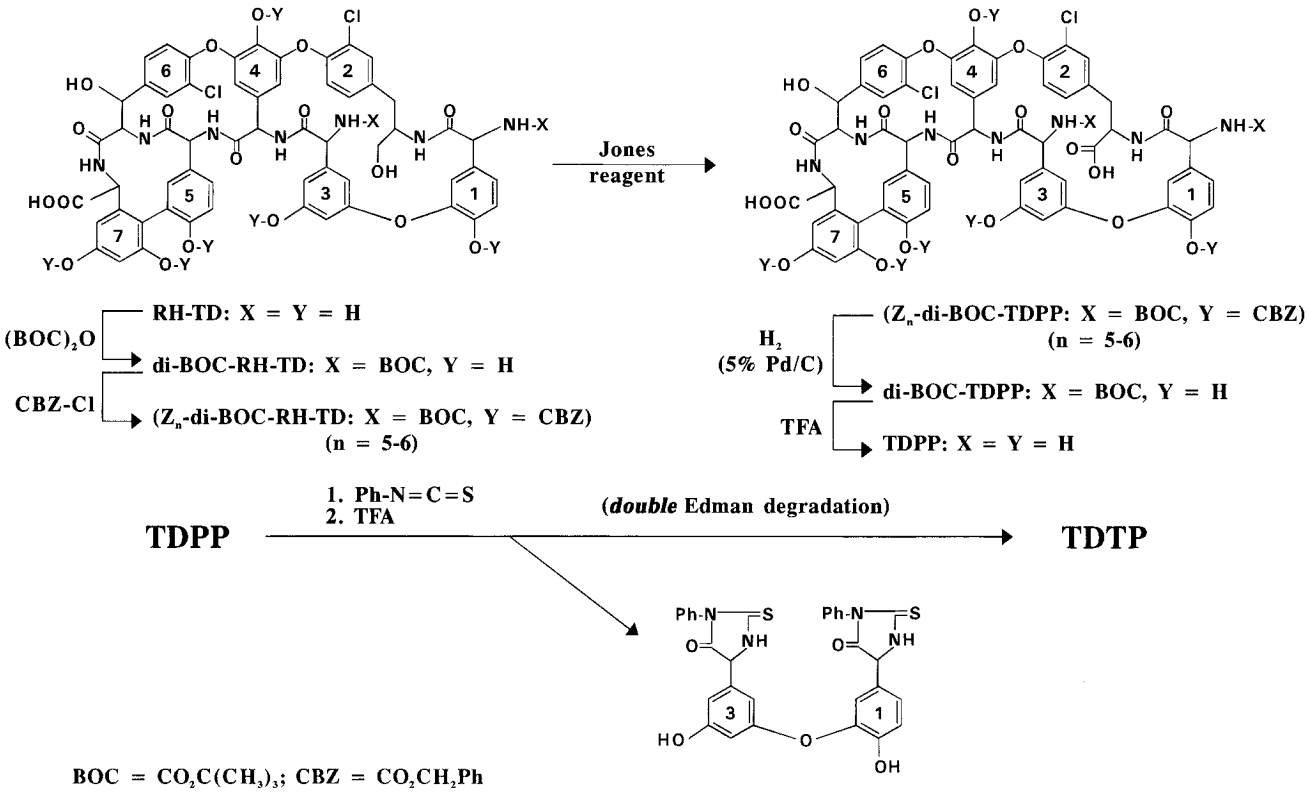
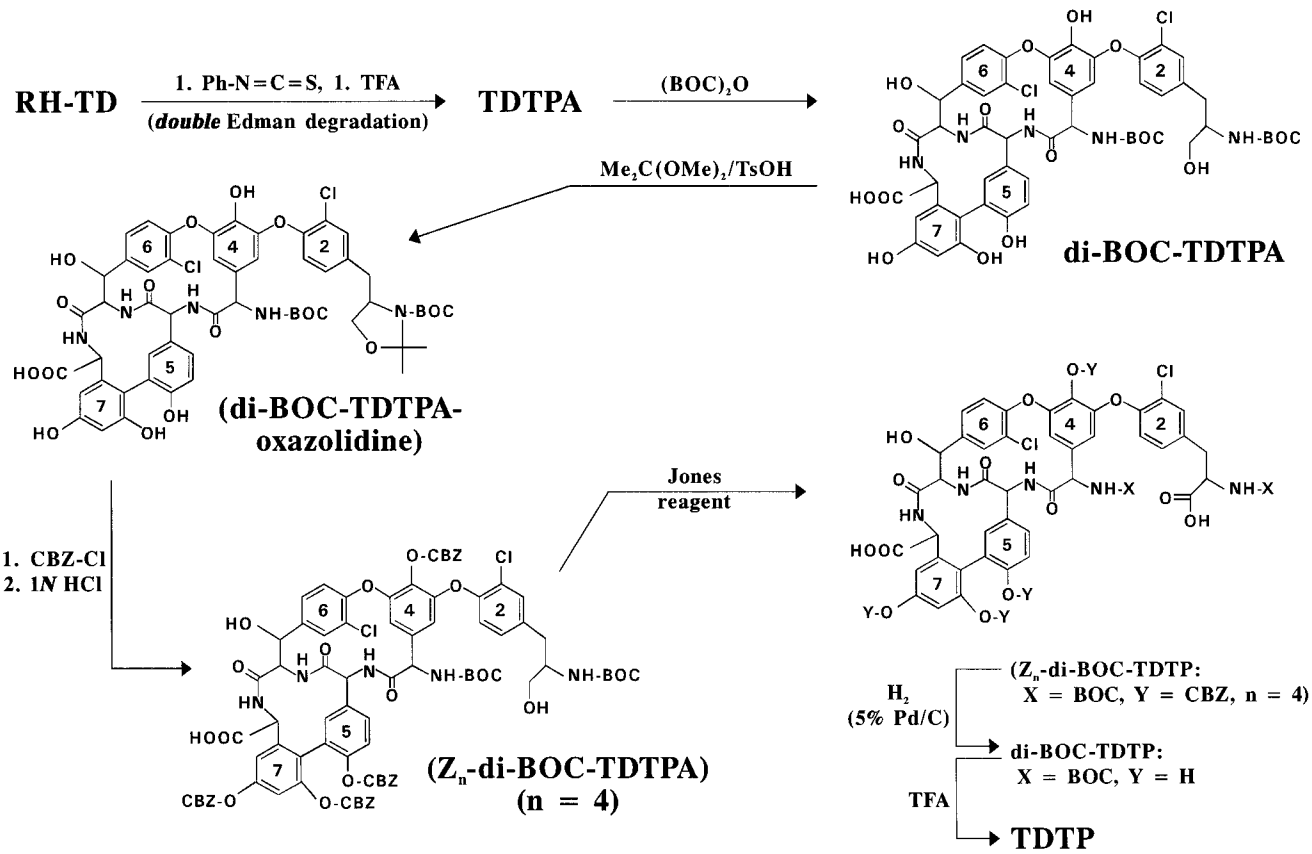
Scheme 1. Synthesis of TDTP (Procedure A)



BOC = $CO_2C(CH_3)_3$; CBZ = CO_2CH_2Ph

Scheme 2. Synthesis of TDTP (Procedure B)



BOC = $CO_2C(CH_3)_3$; CBZ = CO_2CH_2Ph

As reported below, although these approaches were both successful, procedure A was more suitable to prepare **TDTP** when the phenolic hydroxy groups were protected as benzyl carbonates.

Protection of the Oxidation-Sensitive Functional Groups in RH-TD and TDTPA. In **RH-TD** and **TDTPA** there are several functions, besides the hydroxymethyl moiety of residue 2, which are potentially

susceptible to oxidation: the two primary amino groups, the benzylic OH of residue 6, and all the phenolic hydroxy groups. Preliminary tests carried out on ***N*¹-BOC-TD** methyl ester,[7] suitably permethylated[8] at the phenolic OH's, showed that the benzylic OH of residue 6 was resistant to oxidation under both basic and acidic conditions and that only five of the six phenolic OH's needed protection.[9]

The two free amino groups were protected as *tert*-butyl carbamates by reaction of **RH-TD** (Scheme 1) or **TDTPA** (Scheme 2) with di-*tert*-butyl dicarbonate [$(BOC)_2O$] at room temperature in dioxane/water 1/1 solution, in the presence of $NaHCO_3$. The BOC protective group was resistant to oxidation, and in the final deprotection step, it was removed from **di-BOC-TDPP** or **di-BOC-TDTP** by mild acidic treatment (TFA), giving **TDPP** or **TDTP** with highest yields. The benzyloxycarbonyl (CBZ) group was found to be the most appropriate for the selective protection of the phenolic OH's of **di-BOC-RH-TD** (Scheme 1). It was resistant to oxidation with the Jones reagent[10] and easily removed from resulting per-protected compound (**Z_n-di-BOC-TDPP**) under hydrogenolysis conditions (1 atm, 5% Pd/C) which were well tolerated by the rest of the molecule. The per-CBZ-protected derivative (**Z_n-di-BOC-RH-TD**) was prepared by reaction of **di-BOC-RH-TD** with a large excess of benzyl chloroformate (CBZ-Cl) at room temperature in dioxane/water 1/1 solution, in the presence of Cs_2CO_3.[11] The CBZ group was also used to protect the phenolic OH's in **di-BOC-TDTPA** (Scheme 2), but in this case[12] a preliminary protection of the primary alcohol of residue 2 was necessary to prevent coupling of the hydroxymethyl function with the CBZ group. This was accomplished by forming an oxazolidine ring upon reaction of the *N*-BOC-ethanolamine moiety of residue 2 with 2,2-dimethoxypropane in anhydrous Me_2CO at room temperature in the presence *p*-toluenesulfonic acid (Ts-OH) as the condensation catalyst. Protection of the phenolic-OH's with CBZ-Cl under anhydrous conditions (DMSO) in the presence of Cs_2CO_3 yielded the per-CBZ phenyl carbonate derivative **Z_n-di-BOC-TDTPA-oxazolidine**. The hydroxymethyl group was then regenerated by mild acidic treatment (1 N HCl, 25 °C, 90 min) to give **Z_n-di-BOC-TDTPA** which was amenable to oxidation (Jones reagent) to afford **Z_n-di-BOC-TDTP**. In contrast to the pentapeptide (**di-BOC-TDPP**), tetrapeptide **di-BOC-TDTP** once formed was in part (~20%) susceptible to dechlorination under hydrogenation conditions (1 atm, 5% Pd/C) used to remove the CBZ groups from **Z_n-di-BOC-TDTP**.

It follows that procedure A is more suitable than B, in terms of overall yields and number of reaction steps, for preparing **TDTP** when using the CBZ as phenol-protecting group. Although the CBZ moiety could be also used for the protection of the amino groups, the BOC group was preferred in this case as it was more easily removed from the *N*-carbamate derivatives (**di-BOC-TDPP** or **di-BOC-TDTP**) than the CBZ group.[13,14]

Oxidation of the Hydroxymethyl Function to Carboxy Group. Several conditions were tried before selecting the most suitable method for the oxidation of the hydroxymethyl group of residue 2 of **Z_n-di-BOC** derivatives of **RH-TD** and **TDTPA**. Due to the protection of the phenolic OH's as benzyl carbonates, basic conditions had to be avoided. Different oxidation reagents were investigated under the more appropriate acidic conditions. When CrO_3 in glacial or aqueous AcOH was used, a large excess of oxidizer was necessary to complete the reaction. The best results were obtained by treatment of the above protected tetra- or pentapeptide−alcohol derivatives with the Jones reagent ($H_2CrO_4-H_2SO_4/H_2O$)[15] at room temperature in THF solution. Under these conditions, the oxidation was fast and occurred without deprotection/decomposition of amino groups or phenolic OH's.

Edman Degradation of RH-TD and TDPP. The diphenyl ether 1,3-diamino acid was removed from **RH-TD** or **TDPP** by a *double* Edman procedure. Accordingly, reaction of **RH-TD** or **TDPP** with 2.5 equiv of phenyl isothiocyanate in pyridine/water 1/1 afforded the corresponding mixed N^1,N^3-di-isothiourea derivative which, upon treatment with dry TFA at room temperature, gave tetrapeptide **TDTPA** or **TDTP** in high yields.[16]

Selective Protection of One Carboxy and the Two Primary Amino Groups in TDTP. TDTP is a potential intermediate for the synthesis of new families of glycopeptides by introduction of appropriate amino acids in positions 1 and 3. This process would require protection of one amino group of **TDTP** prior to coupling of the other primary amine with the carboxy function of a N-protected amino acid. The protection of the carboxy group of residue 7 of **TDTP** is also necessary to prevent intermolecular side reactions in the final macrocyclization step to give a new hexapeptide or heptapeptide compound.

A **TDTP** derivative (***N*²-CBZ-*N*⁴-BOC-TDTP-Me**) selectively protected at the C-terminus of the tetrapeptide chain as methyl ester and at the amino groups of residues 2 and 4 as CBZ and BOC carbamates, respectively, was obtained according to the procedure of Scheme 3. Esterification of the carboxy group of **di-BOC-RH-TD** with MeI in DMF in the presence of $KHCO_3$ provided a methyl

(7) Malabarba, A.; Trani, A.; Ferrari, P.; Pallanza, R.; Cavalleri, B. *J. Antibiot.* **1987**, *40*, 1572.

(8) The phenolic-OH's of ***N*¹-BOC-TD** methyl ester were protected as methyl ethers using a large excess of MeI in DMF, in the presence of K_2CO_3.

(9) The number of phenolic-OCH_3's present in permethylated ***N*¹-BOC-TD** methyl ester was determined by NMR. The position of the unprotected phenolic group resistant to oxidation was not determined.

(10) (a) Bowden, K.; Heibron, I. M.; Jones, E. R. H.; Weedon, B. C. L. *J. Chem. Soc.* **1946**, 39. (b) Bladon, P.; Fabian, J. M.; Henbest, H. B.; Koch, H. B.; Wood, G. W. *J. Chem. Soc.* **1951**, 2402. (c) Bowers, A.; Halsall, T. G.; Jones, E. R. H.; Lemin, A. J. *J. Chem. Soc.* **1953**, 2548.

(11) Suitable protection of the phenolic groups was also obtained in anhydrous conditions, in DMSO/THF 4/1 solution, using a large excess of K_2CO_3 and/or TEA as the acid-acceptor agents, but with longer reaction times. The use of K_2CO_3 in aqueous media was unsuitable since the CBZ moiety on ring 4 was susceptible, once formed, to hydrolysis under the resulting basic reaction conditions. Another advantage of the Cs_2CO_3 method is that the reaction mixture dioxane/water is the same used in the preparation of di-BOC carbamates, thus allowing a one-pot synthesis of **Z_n-di-BOC-RH-TD** from **RH-TD**.

(12) In **di-BOC-RH-TD** the protection of the hydroxymethyl group of residue 2 was unnecessary.

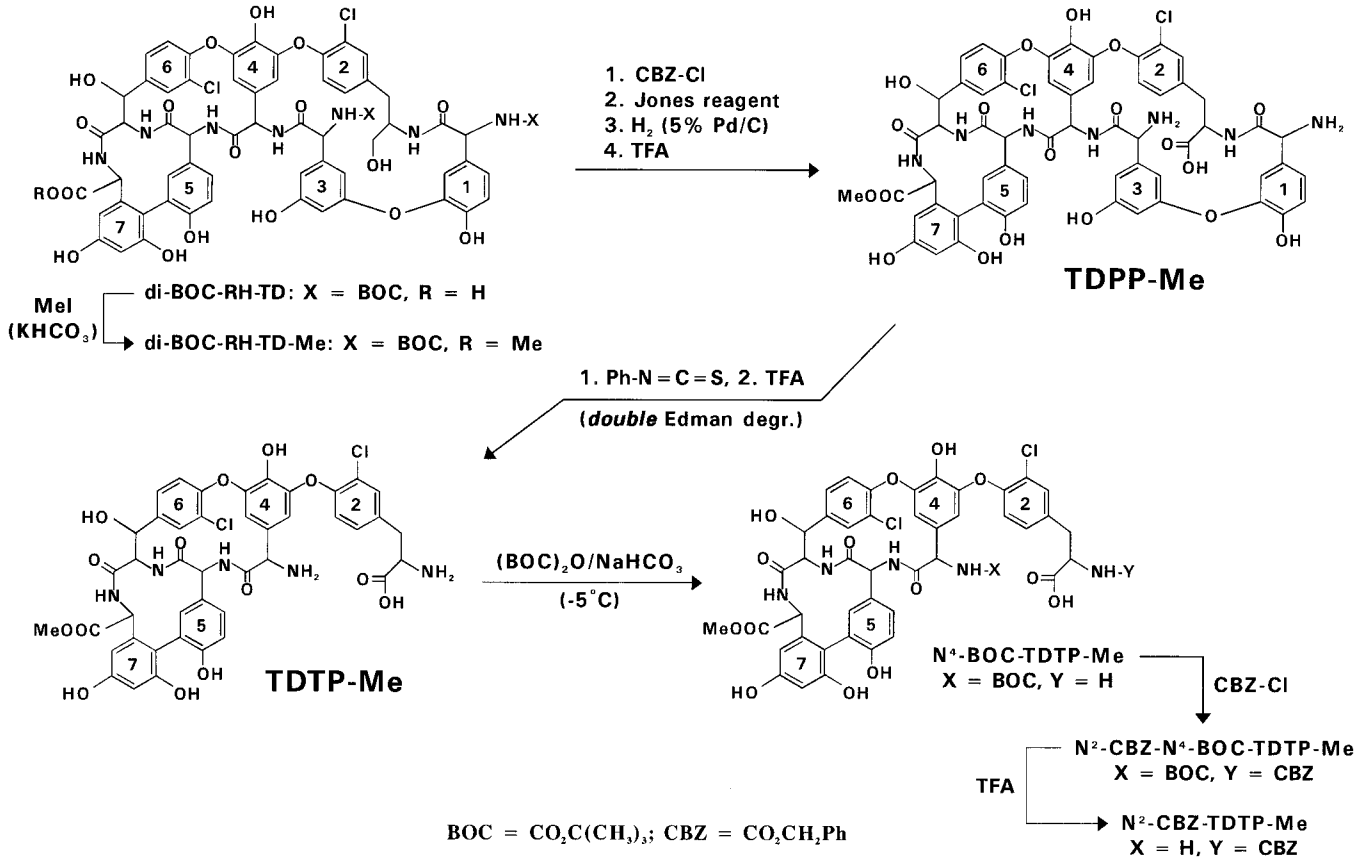
(13) Under the conditions used (H_2, 1 atm., 10% Pd/C) in the hydrogenolysis of the *N*′,*N*″-di-CBZ derivatives of **TDPP** and **TDTP** the chlorine atoms on rings 2 and 6 were also affected, to a greater extent in **TDTP** than **TDPP**, leading to dechlorinated byproducts.

(14) The number of phenolic-OH's which could be protected as BOC carbonates was insufficient, under all tried conditions, for the oxidation step.

(15) The Jones reagent, "standard" solution, was prepared according to the procedure described in Fieser & Fieser: *Reagents for Organic Synthesis*; Vol. 1: John Wiley & Sons, Inc.: New York, 1967; Vol. 1, p 142.

(16) Although the 1,3-diphenyl ether dihydantoine moiety was not isolated, its formation can be inferred according to the well-known mechanism of Edman degradation.

Scheme 3. Synthesis of N^2-CBZ-N^4-BOC-TDTP-Me, N^4-BOC-TDTP-Me and N^2-CBZ-TDTP-Me



ester derivative (**di-BOC-RH-TD-Me**) which, after protection of the phenolic functions as benzyl carbonates, oxidation of the residue 2-CH_2OH, and deprotection of the phenolic and amino groups, gave the pentapeptide monomethyl ester **TDPP-Me**. Edman degradation of **TDPP-Me** afforded the tetrapeptide monomethyl ester **TDTP-Me**. The amino group of residue 4, more nucleophilic[17] than that of residue 2, was selectively protected as BOC carbamate upon treatment of **TDTP-Me** with an equimolecular amount of $(BOC)_2O$ at −5 °C in a water/dioxane 1/1 mixture in the presence of $NaHCO_3$. Under these conditions, the mono-N^4-BOC derivative (***N*⁴-BOC-TDTP-Me**) was obtained with high (>95%) yields, while the disubstituted ***N*²,*N*⁴-di-BOC-TDTP-Me** was the main product (>50%) when the reaction was carried out at room temperature. Final triprotected derivative ***N*²-CBZ-*N*⁴-BOC-TDTP-Me** was prepared by reaction of ***N*⁴-BOC-TDTP-Me** with CBZ-Cl (1 equiv, room temperature) in DMF in the presence of $KHCO_3$. The selective removal of the BOC protective group from ***N*²-CBZ-*N*⁴-BOC-TDTP-Me** (TFA, 0 °C) afforded a further diprotected compound (***N*²-CBZ-TDTP-Me**) particularly suitable for the selective introduction of new amino acids in position 3.[18]

Structure Elucidation. The structures of **TDTP** and **TDTP-Me**, their synthetic precursors **TDTPA**, **TDPP**, and **TDPP-Me**, and the **BOC** and **CBZ** derivatives of **TDTP-Me** were determined by NMR spectroscopy and FAB-MS spectrometry. Among protected intermediates, only the **di-BOC** derivatives of **TDPP** and **TDTP** were purified and their structures confirmed by ^1H NMR and FAB-MS.[19]

The most characteristic ^1H NMR signals of the core molecules and their protecting groups, when present, are given in Table 1 in comparison with the proton assignments for **TD** hydrochloride. With respect to the **TD** spectrum, strong changes are found, as expected, in the right-hand part of the structure of **TDTP**, in particular in the chemical shift values of protons x_2, x_4, 4b, and to a lesser extent, 4f and x_5. The remaining left-hand part of the molecule of **TDTP** appears to be substantially unchanged. The differences in their ^1H NMR spectra between **TDTP** and **TDTPA** are mainly shown by a strong chemical shift change of proton x_2 and by a minor variation in the chemical shift of proton 4b. Significant changes are also present in the spectrum of **TDPP** with respect to that of **TD**, in the region from x_1 to x_4 and 4b. The attributions were based on the phase sensitive double quantum filter (Bruker COSYPHDQ microprogram) and on the well-established spectra−structure correlations in the teicoplanin field.[20] The FAB MS

(17) In **TDTP-Me**, as well as **TDTP**, the residue 4-NH_2 appeared to be generally more susceptible to acylation than the residue 2-NH_2 upon treatment with different acylating agents. In some cases, this allowed the selective coupling of the residue 4-NH_2 of **TDTP-Me** or **TDTP** with activated N-protected amino acid esters without protection of the residue 2-NH_2 (data not reported).

(18) Malabarba, A.; Ciabatti, R. Int. Patent WO94−26780, Nov 24, 1994 (to Gruppo Lepetit S.p.a.).

(19) The structure of **di-BOC-RH-TD**, which was obtained sufficiently pure for the next reaction steps but was not further purified for analysis, was confirmed by FAB MS only. The per-protected Z_n compounds and the oxazolidine derivatives of **TDTPA**, as well as the diisothiourea intermediates of the Edman degradation procedures, were only characterized on the basis of their HPLC retention time (t_R). Their chromatographic behavior is in accordance with the proposed structures.

(20) (a) Barna, J. C. J.; Williams, D. H.; Stone, D. J. M.; Leung, T.-W. C.; Doddrell, D. M. *J. Am. Chem. Soc.* **1984**, *106*, 4895. (b) Hunt, A. H.; Molloy, R. M.; Occlowitz, J. L.; Marconi, G. C.; Debono, M. *J. Am. Chem. Soc.* **1984**, *106*, 4891. (c) Malabarba, A.; Ferrari, P.; Gallo, G. G.; Kettenring, J.; Cavalleri, B. *J. Antibiot.* **1986**, *39*, 1430.

Table 1. Assignments of Significant ^{1}H NMR Signals in DMSO-d_6 in Comparison with TD (TMS, Internal Reference, δ in ppm)

proton	TD	di-BOC-TDPP	TDPP	TDPP-Me	TDTPA	di-BOC-TDTP	TDTP	TDTP-Me	N^4-BOC-TDTP-Me	N^2-CBZ-TDTP-Me	N^2-CBZ-N^4-BOC-TDTP-Me
Phe-CH_2	2.87, 3.35	2.97, 2.84	3.12, 3.00	3.12, 3.04	2.90	2.99, 2.80	3.07	3.06	3.07	3.06	3.05, 2.82
x_2	4.92	3.88	4.18	4.17	3.34	4.07	4.20	4.03	4.10	4.20	4.19
x_6	4.10	4.20	4.23	4.23	4.20	4.19	4.22	4.20	4.20	4.20	4.19
x_7	4.42	4.39	4.44	4.51	4.46	4.46	4.45	4.53	4.52	4.54	4.54
x_5	4.33	4.57	4.62	4.62	4.62	4.53	4.62	4.61	4.52	4.60	4.54
z_6	5.10	5.10	5.11	5.11	5.12	5.10	5.12	5.16	5.12	5.15	5.12
x_4	5.60	5.22	5.69	5.72	5.16	5.42	5.18	5.12	5.42	5.17	5.46
4f	5.08	5.51	5.48	5.46	5.55	5.46	5.58	5.55	5.50	5.56	6.09
7f	6.24				6.28	6.26	6.28	6.09	6.12	5.98	6.44
7d	6.39				6.43	6.42	6.41	6.42	6.48	6.42	6.53
4b	5.50		6.27	6.27	6.95	6.50	6.87	6.87	6.52	6.85	
w_2	8.10				8.05		8.35	8.45			
w_4	7.53				8.53		8.45	8.45			
w_7	8.40				8.53		8.45	8.67			
w_5	8.38				9.09		9.12	9.16			
$COOCH_3$				3.70				3.70	3.71	3.71	3.69
CH_2(OH)					2.90						
t-Bu-CH_3		1.37, 1.33				1.33, 1.26			1.27		1.24
CBZ-CH_2										5.13	5.11
x_1	5.47		4.91								

spectra of the above compounds were in accordance with the proposed structures, as shown in Table 2.

Experimental Section

The ^{1}H NMR experiments were recorded at 500 MHz in DMSO-d_6 solution, added with or without TFA. The positive ion low-resolution (RP = 2000; 10% valley definition) FAB MS spectra were obtained using 8 kV accelerating voltage. The samples were dissolved in a DMSO/thioglycerol 1/1 mixture. Reaction products were purified by reversed-phase column chromatography on silanized silica gel (0.063−0.2 mm), according to the following procedure: 1 g of crude compound was dissolved in 20−30 mL of a MeCN/H_2O (1/1) mixture, the solution was adjusted at pH 5.5 with solid HCO_2NH_4, and then H_2O was added dropwise under stirring until precipitation started. After a few drops of MeCN were added, the resulting cloudy solution was loaded on a column of 50 g of silanized silica gel in the same solvent mixture. Elution was carried out according to a linear gradient from 5−10% to 40−70% of MeCN in H_2O, in 10−15 h, at a flow rate of 200−300 mL/h, while collecting 20 mL fractions; those containing pure compound were pooled, and enough 1-BuOH was added to obtain, after evaporation of most solvent at 40 °C under reduced pressure, a concentrated dry butanol solution (or suspension). Upon addition of Et_2O the precipitated solid was collected, washed with Et_2O, and dried *in vacuo* at room temperature overnight. Reactions, column eluates, and final products were checked by HPLC performed on a column (125 × 4 mm) prepacked with LiChrospher RP-8 (5 μm). Chromatograms were recorded at 254 nm. Elutions were carried out by mixing eluent a, MeCN, with eluent b, 0.2% aqueous HCO_2NH_4, according to linear gradients programmed as follows:

	time (min)	0	10	20	30	35	40	45
method A:	% b in a	5	23	26	35	75	35	5
method B:	% b in a	20	33	47	60	75	75	20
method C:	% b in a	40	52	64	75	85	85	40

Pure deprotected pentapeptide (**TDPP** and **TDPP-Me**) and tetrapeptide (**TDTPA**, **TDTP**, and **TDTP-Me**) derivatives were analyzed for C, H, N, and Cl on samples previously dried at 140 °C under N_2 atmosphere. The analytical results obtained for the above elements were within ±0.4% of the theoretical values. The solvent content (<7%, mainly water) and inorganic residue (<0.2%) were determined by thermogravimetry (TG), at 140 °C, and after the samples were heated at 900 °C in O_2 atmosphere, respectively.

Preparation of TDTP Following Procedure A (Scheme 1). Di-BOC-RH-TD. To a stirred solution of 24 g (∼20 mmol) of **RH-TD** (HPLC, method A, t_R 11.6 min) in 400 mL of a dioxane/water 1/1 mixture was added 20 mL of a 1 M aqueous solution of $NaHCO_3$ at room temperature followed by a solution of 9 g (∼40 mmol) of $(BOC)_2O$ in 100 mL of the same above dioxane/water 1/1 mixture. After the mixture was stirred at room temperature for 5 h, 250 mL of H_2O was added, and the resulting solution was adjusted at pH 4 with 1 N HCl and then extracted with EtOAc (2 × 200 mL). The organic layer was washed with H_2O (2 × 100 mL), dried over Na_2SO_4, and concentrated at room temperature under reduced pressure to a small volume (∼50 mL). On adding Et_2O (450 mL), the precipitated solid was collected and dried *in vacuo* at room temperature overnight to give 27 g (95% yield) of the title compound: HPLC, method B, t_R 11.3 min.

Table 2. Analytical Data

compd	formula	MW (av)	FAB MS $[MH]^+$
di-BOC-RH-TD	$C_{68}H_{65}Cl_2N_7O_{22}$	1403.2244	1402.4 ± 0.1
di-BOC-TDPP	$C_{68}H_{63}Cl_2N_7O_{23}$	1417.2078	1416.3 ± 0.1
TDPP	$C_{58}H_{47}Cl_2N_7O_{19}$	1216.9714	1216.2 ± 0.1
TDPP-Me	$C_{59}H_{49}Cl_2N_7O_{19}$	1230.9984	1230.3 ± 0.1
TDTPA	$C_{42}H_{37}Cl_2N_5O_{13}$	890.7036	890.2 ± 0.1
di-BOC-TDTP	$C_{52}H_{51}Cl_2N_5O_{18}$	1104.9235	1104.3 ± 0.1
TDTP	$C_{42}H_{35}Cl_2N_5O_{14}$	904.687	904.2 ± 0.1
TDTP-Me	$C_{43}H_{37}Cl_2N_5O_{14}$	918.7141	918.2 ± 0.1
N^4-BOC-TDTP-Me	$C_{48}H_{45}Cl_2N_5O_{16}$	1018.8324	1018.2 ± 0.1
N^2-CBZ-TDTP-Me	$C_{51}H_{43}Cl_2N_5O_{16}$	1052.8498	1052.2 ± 0.1
N^2-CBZ-N^4-BOC-TDTP-Me	$C_{56}H_{51}Cl_2N_5O_{18}$	1152.9681	1152.3 ± 0.1

Di-BOC-TDPP. A solution of 22.4 g (∼16 mmol) of the above compound and of 16 g (∼50 mmol) of Cs_2CO_3 in 1 L of a dioxane/water 1/1 mixture was stirred at room temperature for 1 h, and then a solution of 23 mL (∼160 mmol) of CBZ-Cl in 100 mL of dry THF was added dropwise over 30 min. After being stirred at room temperature overnight, the reaction mixture was poured into a stirred mixture of EtOAc/H_2O 1/1 (1.5 L). The aqueous phase was adjusted at pH 3 with 1 N HCl, the organic layer was separated and dried over Na_2SO_4, and then the solvent was evaporated at room temperature under reduced pressure to give an oily residue which was slurried with Et_2O. The resulting solid material was collected to yield 27 g of per-CBZ derivative **Z_n-di-BOC-RH-TD** as a crude mixture of two main compounds (HPLC, method C, t_R 29.8, 32.3 min).[21] This product was dissolved in 400 mL of THF, and 90 mL of Jones reagent, "standard" solution,[15] was added dropwise under vigorous stirring, in 1.5 h, while the temperature was maintained at 20−25 °C. After 30 min, the

(21) It was assessed that all per-CBZ derivatives of **di-BOC-RH-TD** with t_R > 20 min (HPLC, method C) were suitable for the next oxidation step, while more hydrophilic homologous compounds were degraded upon treatment with the Jones reagent.

resulting dark suspension was poured into 2.5 L of a stirred mixture EtOAc/H_2O 1/1. The organic layer was separated, washed several times with a 1 N solution of $Na_2S_2O_5$ (to complete decomposion of peroxides), and then dried over Na_2SO_4. Evaporation of solvents at room temperature under reduced pressure yielded 25 g of **Z_n-di-BOC-TDPP** as a crude mixture of two main compounds (HPLC, method C, t_R 21.5, 24.7 min). A solution of this product in 1 L of a MeOH/DMF/AcOH 5/2/2 mixture was hydrogenated (1 atm, 25 °C) in the presence of 12.5 g of 5% Pd/C. About 1.5 L of H_2 was absorbed within 2 h; afterwards, the catalyst was filtered off and MeOH was evaporated at room temperature under reduced pressure. The resulting solution was diluted with 2.5 L of H_2O and extracted with 2.5 L of 1-BuOH. The organic layer was separated, washed with H_2O (2 × 1 L), and then concentrated at 45 °C under reduced pressure to a final volume of ~100 mL. Upon addition of Et_2O (500 mL), the precipitated solid was collected (~15 g) and purified by reversed-phase column chromatography, yielding 5.6 g (~25%) of the title compound: HPLC, method B, t_R 6.0 min.

TDPP. To remove the BOC protective groups, the above compound (5.5 g) was dissolved in 50 mL of dry TFA and the resulting solution was stirred at room temperature for 1.5 h. Then, the solvent was evaporated at room temperature under reduced pressure and the oily residue was slurried with EtOAc to obtain a solid product which was collected, washed several times with Et_2O, and dried *in vacuo* at room temperature (over KOH) to give the title compound (5.5 g, ~100%), as the ditrifluoroacetate: HPLC, method A, t_R 9.0 min.

TDTP (by *double* Edman degradation of TDPP). To a stirred solution of the above product (5.5 g, ~4 mmol) in 75 mL of a pyridine/water 1/1 mixture was added 1.1 mL (~9 mmol) of phenyl isothiocyanate at room temperature. After 3 h, the reaction mixture was poured into 200 mL of H_2O, and the resulting cloudy solution was adjusted at pH 3 with 1 N HCl and then was extracted with EtOAc (2 × 200 mL). The organic layer was discarded, and the aqueous phase was extracted again with 1-BuOH (200 mL). The butanol layer was separated, washed with H_2O (2 × 200 mL), and then concentrated at 40 °C under reduced pressure to a small volume (~20 mL). Upon addition of Et_2O (100 mL), the precipitated solid was collected (5.8 g, crude diisothiourea: HPLC, method A, t_R 11.3 min) and redissolved in 100 mL of dry TFA. The resulting solution was stirred at room temperature for 1.5 h, and then the solvent was evaporated at 30 °C under reduced pressure. The oily residue was purified by reversed-phase chromatography under the usual conditions, yielding 0.95 g (~23%) of the title compound: HPLC, method A, t_R 6.8 min.

Preparation of TDTP Following Procedure B (Scheme 2). TDTPA (by *double* Edman degradation of RH-TD). To a stirred solution of 13.5 g (~11 mmol) of **RH-TD** in 270 mL of a pyridine/water 1/1 mixture was added 2.8 mL (~23 mmol) of phenyl isothiocyanate at room temperature. After 7 h, the reaction mixture was poured into 500 mL of H_2O, and the resulting cloudy solution was adjusted at pH 3 with 1 N HCl and then extracted with EtOAc (2 × 500 mL). The organic layer was discarded, and the aqueous phase was extracted again with 1-BuOH (500 mL). The butanolic layer was separated, washed with H_2O (2 × 300 mL), and then concentrated at 40 °C under reduced pressure to a small volume (~50 mL). Upon addition of Et_2O (300 mL), the precipitated solid was collected (14.3 g, crude diisothiourea: HPLC, method A, t_R 13.5 min) and redissolved in 200 mL of dry TFA. The resulting solution was stirred at room temperature for 1.5 h, and then the solvent was evaporated at 30 °C under reduced pressure. The oily residue was purified by reversed-phase chromatography under the usual conditions, yielding 7.5 g (~75%) of the title compound: HPLC, method A, t_R 8.3 min.

Di-BOC-TDTPA. To a stirred solution of 7.2 g (~8 mmol) of the above compound and 11 g of $NaHCO_3$ in 250 mL of a dioxane/water 1/1 mixture was added a solution of 4 g (~18.5 mmol) of $(BOC)_2O$ in 30 mL of dioxane dropwise at 0−5 °C. Stirring was continued at room temperature for 5 h, and then the reaction mixture was adjusted at pH 4 with 1 N HCl and extracted with 300 mL of EtOAc. The organic layer was separated, washed with H_2O (2 × 250 mL), dried over Na_2SO_4, and then concentrated at 30 °C under reduced pressure to a small volume (~25 mL). Upon addition of Et_2O (250 mL), the precipitated solid was collected and dried *in vacuo* at room temperature overnight to yield 8.7 g (100%) of title compound pure enough for the next step: HPLC, method B, t_R 12.2 min.

Di-BOC-TDTP. To a stirred suspension of 6 g (~5.5 mmol) of the above compound in 500 mL of dry Me_2CO were added 120 mL of 2,2-dimethoxypropane and 0.27 g (~1.4 mmol) of *p*-toluenesulfonic acid. After the mixture was stirred at room temperature for 1.5 h, a solution of 0.24 g of $NaHCO_3$ in 4 mL of H_2O was added and solvents were evaporated at 35 °C under reduced pressure. The solid residue (**di-BOC-TDTPA-oxazolidine**, 6.4 g: HPLC, method b, t_R 17.9 min) was collected and dissolved in 300 mL of a dioxane/water 1/1 mixture, and 5.5 g (~17 mmol) of Cs_2CO_3 was added. The resulting solution was stirred at room temperature for 1 h, and then a solution of 7.5 mL (~50 mmol) of CBZ-Cl in 50 mL of dry THF was added dropwise in 45 min while the mixture cooled to 5−10 °C. After being stirred at room temperature overnight, the reaction mixture was poured into a stirred mixture EtOAc/H_2O 1/1 (500 mL). The aqueous phase was adjusted at pH 4.3 with glacial AcOH, the organic layer was separated and dried over Na_2SO_4, and then the solvent was evaporated at room temperature under reduced pressure. The oily residue was suspended in 250 mL of MeCN, and 25 mL of 1 N HCl was added at room temperature. After being stirred at room temperature for 1.5 h, the reaction mixture was poured into 500 mL of H_2O and the resulting suspension was extracted with EtOAc (500 mL). The organic layer was washed with 1 N $NaHCO_3$ until the pH of the aqueous washings was neutral, and then it was washed with H_2O (2 × 250 mL), dried over Na_2SO_4, and concentrated at 30 °C under reduced pressure to a small volume (~30 mL). Upon addition of Et_2O (300 mL), the precipitated solid was collected (**Z_n-di-BOC-TDTPA**, 5.7 g: HPLC, method C, t_R 27.1 min) and oxidized with the Jones reagent as described above (procedure A), obtaining 5.3 g of crude (85% HPLC titer) per-CBZ derivative **Z_n-di-BOC-TDTP** (HPLC, method C, t_R 18.7 min). Removal of the CBZ protective groups under the previously described conditions (1 atm, 25 °C, 2.5 g of 5% Pd/C) yielded a 80/15/5 mixture (3.5 g) of crude (~85%, by HPLC) **di-BOC-TDTP** and corresponding mono- and didechlorinated derivatives, respectively. Purification by reversed-phase chromatography gave 1.1 g (~17%) of pure title compound: HPLC, method b, t_R 7.9 min).

TDTP. A solution of the above compound (1.1 g) in 25 mL of dry TFA was stirred at room temperature for 1.5 h, and then the solvent was evaporated at 30 °C under reduced pressure. The oily residue was slurried with EtOAc (50 mL) and then with Et_2O (100 mL), obtaining a solid product which was collected, washed with Et_2O, and dried *in vacuo* at room temperature overnight, yielding 1.1 g (~100%) of pure title compound as the bistrifluoroacetate: HPLC, method B, t_R 6.8 min.

Preparation of N^2-CBZ-TDTP-Me (Scheme 3). TDPP-Me. To a stirred solution of 30 g (~21 mmol) of **di-BOC-RH-TD** in 200 mL of DMF was added 2.5 g of $KHCO_3$ followed by a solution of 22 mL (~23 mmol) of MeI in 30 mL of DMF. After being stirred at room temperature overnight, the reaction mixture was poured into 1 L of H_2O. The resulting cloudy solution was adjusted at pH 3 with 1 N HCl and extracted with 1 L of a EtOAc/1-BuOH 1/1 mixture. The organic layer was separated, washed several times with H_2O to neutral pH, and then concentrated at 40 °C under reduced pressure to a small (~50 mL) volume. Upon addition of Et_2O (300 mL), the precipitated solid was collected and dried *in vacuo* at room temperature overnight, yielding 30 g (~100%) of **di-BOC-RH-TD-Me** pure enough for the next step. HPLC, method B, t_R 16.1 min. The above compound was protected at the phenolic hydroxy groups following the same procedure described previously for **Z_n-di-BOC-RH-TD**, obtaining 36 g of **Z_n-di-BOC-RH-TD-Me** as crude mixture of two main compounds (HPLC, method C, t_R 30.3, 32.5 min), which was submitted to oxidation with the Jones reagent as described above, yielding 34.3 g of crude **Z_n-di-BOC-TDPP-Me** (HPLC, method C, t_R 22.3, 25.8 min). Then the CBZ groups were removed from this product

by hydrogenolysis (1 atm, room temperature, in the presence of 15 g of 5% Pd/C added in two portions), obtaining 24.5 g of crude **di-BOC-TDPP-Me** (HPLC, method B, t_R 8.3 min). This compound was dissolved in 300 mL of TFA, and the resulting solution was stirred at room temperature for 30 min. Evaporation of the solvent under reduced pressure at 30 °C yielded an oily residue which was slurried with EtOAc to give a solid (∼21 g) which was purified by reversed-phase column chromatography, obtaining 9.7 g (∼38%, overall yield) of the title compound (HPLC, method A, t_R 11.3 min).

TDTP-Me. Edman degradation of 6.5 g of **TDPP-Me** (6.5 g) was carried out as described previously for **TDTP**, yielding 1.35 g (∼25%) of title compound (HPLC, method A, t_R 10.3 min), as the bistrifluoroacetate.

N^4-BOC-TDTP-Me. A solution of 2.8 g (∼3 mmol) of **TDTP-Me** (2TFA) and of 0.75 g of $NaHCO_3$ in 100 mL of a dioxane/water 1/1 mixture was stirred at −5 °C while a solution of 0.62 g of (∼3 mmol) of $(BOC)_2O$ was added dropwise, in 1 h, in 20 mL of dioxane. Stirring was continued at −5 °C for an additional 7 h, and then the reaction mixture was poured into 300 mL of H_2O. The resulting cloudy solution was adjusted at pH 3 with 1 N HCl and extracted with 150 mL of EtOAc. The organic layer was separated, dried over Na_2SO_4, and evaporated at 30 °C under reduced pressure, obtaining 0.9 g of **di-BOC-TDTP-Me** (HPLC, method A, t_R 21.5 min) which was treated with 10 mL of TFA to regenerate starting **TDTP-Me**. The aqueous phase was extracted with 100 mL of 1-BuOH, obtaining, after evaporation of butanol at 45 °C under reduced pressure, 1.6 g (∼50%) of pure title compound (HPLC, method A, t_R 15.0 min).

***N^2*-CBZ-*N^4*-BOC-TDTP-Me**. To a stirred solution of 1.55 g (∼1.5 mmol) of **N^4-BOC-TDTP-Me** in 50 mL of a dioxane/water 1/1 mixture was added 1.4 g of $NaHCO_3$ followed by a solution of 0.23 mL of CBZ-Cl in 5 mL of dioxane (added dropwise at room temperature in 5 min). After 10 min, the reaction mixture was poured into 200 mL of a stirred H_2O/EtOAc 1/1 mixture. After the aqueous phase was adjusted at pH 3 with 1 N HCl, the organic layer was separated, dried over Na_2SO_4, and concentrated at 30 °C under reduced pressure to a small (∼20 mL) volume. Upon addition of Et_2O (100 mL), the precipitated solid was collected, obtaining 1.75 g (∼100%) of pure title compound (HPLC, method B, t_R 12.7 min).

***N^2*-CBZ-TDTP-Me**. A solution of 0.6 g of the above compound in 10 mL of TFA was stirred at room temperature for 15 min. Afterwards, the solvent was evaporated at 30 °C under reduced pressure. The oily residue was slurried with Et_2O, and the solid which separated was collected, washed with Et_2O, and dried *in vacuo* at room temperature overnight to yield 0.65 g (∼100%) of pure title compound (HPLC, method B, t_R 8.0 min), as the trifluoroacetate.

Supporting Information Available: Combustion analysis data (1 page). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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