Vitamin K Dependent Carboxylation: Synthesis and Biological Properties of Tetrazolyl Analogues of Pentapeptidic Substrates[1]

Joëlle Dubois, Sonia Bory, Michel Gaudry, and Andrée Marquet*

Laboratoire de Chimie Organique Biologique (ERA CNRS No. 823), Université P. et M. Curie, 75230 Paris Cedex 05, France.
Received December 13, 1983

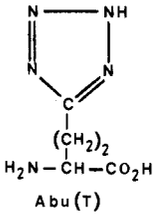
The pentapeptides Phe-Leu-X-Glu-Val and Phe-Leu-X-X-Val, where X = 4-(5*H*-tetrazolyl)-2-aminobutyric acid (tetrazolyl analogue of glutamic acid), were synthesized by addition of tri-*n*-butyltin azide to the corresponding nitrile-containing peptides. These tetrazolyl peptides and a dinitrile precursor were tested as possible substrates and inhibitors of the vitamin K dependent carboxylation. Phe-Leu-X-Glu-Val was carboxylated (40% of the reference peptide Phe-Leu-Glu-Glu-Val, K_m = 20 mM) exclusively on the glutamyl residue, whereas the dinitrile precursor and Phe-Leu-X-X-Val were not carboxylated. The latter was a competitive inhibitor with an affinity (K_i = 3 mM) close to that of the reference peptide (K_m = 3 mM).

The last step of the biosynthesis of coagulation factors (II, VII, IX, X) of the plasma proteins C, S, and Z or of bone proteins, the carboxylation of glutamyl residues into γ-carboxyglutamyl residues, is dependent on vitamin K.[2-5] Although this new carboxylation gave rise to many mechanistic studies, several aspects of the reaction remain obscure, as for instance the question of the abstraction of the hydrogen α to the carboxyl group. Two hypothetical mechanisms have been suggested,[6] involving either an anionic intermediate or a radical.

The abstraction of a hydrogen α to a carboxyl group without activation is chemically unfavorable. Usually, biochemical activation is achieved through the transformation of the carboxyl group into AMP esters or coenzyme A thioesters such as in biotin-dependent carboxylations.[7] However, so far no requirement for ATP[2-5] or for coenzyme A[8] has been demonstrated in vitamin K dependent carboxylations.

We decided to investigate the question of carboxyl activation by replacing the carboxyl group by a tetrazolyl group. Both groups are isosteric and have very similar pK values.[9,10] Numerous tetrazolyl analogues of carboxylic acids have been synthesized[9-26] and tested in biological processes; frequently they are either substrates/agonists[10,18-23] or inhibitors/antagonists.[24-26] We anticipated that if the vitamin K dependent carboxylation occurred also on the tetrazolyl-containing substrate analogues, this would strongly suggest that the reaction takes place on the free carboxyl group since the tetrazolyl group cannot undergo a similar activation process.

Scheme I. Abu(T) and Tetrazolyl Peptides



Phe-Leu-Glu-Glu-Val 1

Phe-Leu-Abu(T)-Glu-Val 2

Phe-Leu-Abu(T)-Abu(T)-Val 3

We describe here the syntheses and the properties of two tetrazolyl analogues of the pentapeptidic substrate Phe-Leu-Glu-Glu-Val, **1**, which is currently used in place of the endogenous precursors to study the vitamin K dependent carboxylation.[27-29]

In the first analogue **2**, the Abu(T) residue (tetrazolyl analogue of glutamic acid) has been incorporated into position 3, the position very predominantly carboxylated,[28,29] whereas in **3**, both glutamyl residues have been replaced by their tetrazolyl analogues (Scheme I).

Results and Discussion

Addition of trialkyltin azide to nitriles constitutes one of the more convenient accesses to tetrazoles,[30] and the

(1) Symbols and abbreviations are in accordance with the recommendations of the IUPAC–IUB commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 977). Other abbreviations used are as follows: Abu(CN), 4-cyano-2-aminobutyric acid; Abu(T), 4-(5*H*-tetrazolyl)-2-aminobutyric acid; DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; NMM, *N*-methylmorpholine; Boc, *tert*-butyloxycarbonyl; ONSu, *N*-hydroxysuccinimido; DMF, *N,N*-dimethylformamide; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance.

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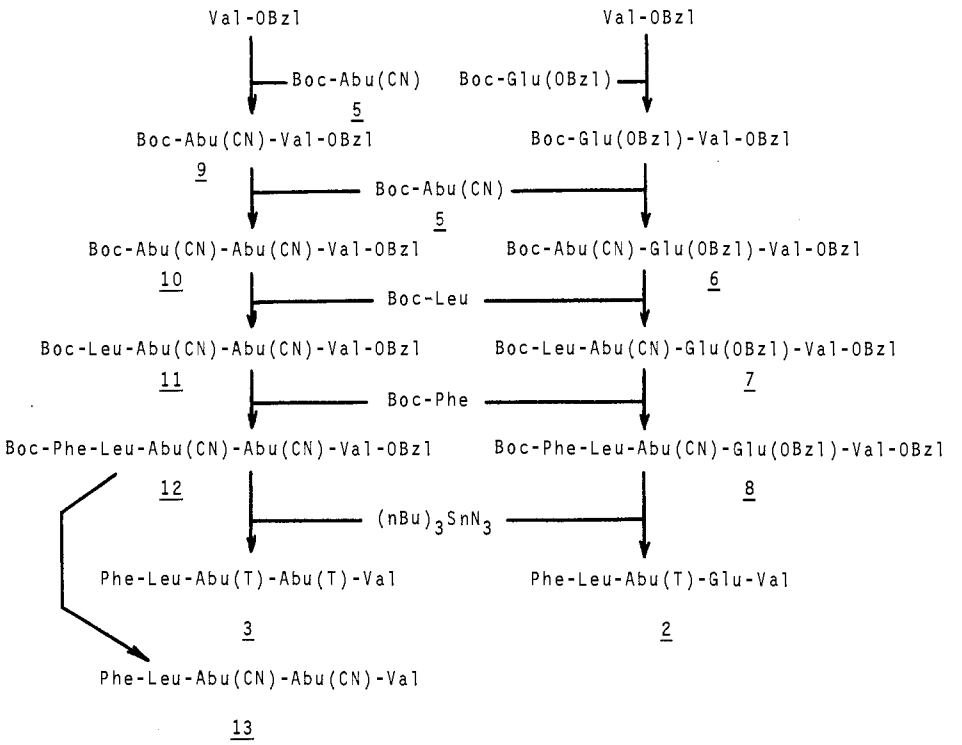
Scheme II. Syntheses of Tetrazolyl Peptides 2 and 3 and Dinitrile Peptide 13



synthesis of tetrazolyl-containing peptides can be achieved by two different strategies: either by synthesizing the tetrazolyl analogue of the amino acid followed by incorporation into the peptide or by synthesizing a suitable nitrile-containing peptide followed by the reaction with the azide to build the tetrazolyl moiety during the last step of the synthesis. The first method has been applied successfully to the syntheses of C-terminal tetrapeptidic sequences of gastrins,[10,20,21] glutathione, ophthalmic and norophthalmic acids,[11] and dipeptides.[13,14,16,23] Although criticized by Grzonka,[13] the second method has been applied to the syntheses of dipeptides[14] and gastrin analogues.[19,21]

We started using the first method with benzyl for the protection of the carboxyl group and the *tert*-butyloxycarbonyl group for the protection of the amino group instead of the benzyloxycarbonyl group as previously described.[20,21] However, the yield of recovery of products was very low at each coupling step. We thus decided to use the second strategy even though the introduction of two tetrazolyl moieties into a peptide has so far not been reported and the proximity of these moieties could have led to some difficulties. We also took advantage of the synthesis scheme to test the dinitrile-containing peptide **13**. Such dinitrile analogues have previously never been prepared or tested.

Boc-L-Abu(CN) **4** was prepared by dehydration of Boc-L-glutamine in pyridine with DCC according to the method of Ressler and Ratzkin,[31] and the cyano-containing peptides were easily synthesized by using solution-phase technology (Scheme II). Conversion to tetrazolyl-containing peptides upon reaction with tri-*n*-butyltin azide followed by deprotection and purification on ion-exchange columns yielded pure peptides **2** and **3**. The yield of this reaction (61%) was satisfactory for the ditetrazolyl peptide **3**.[32]

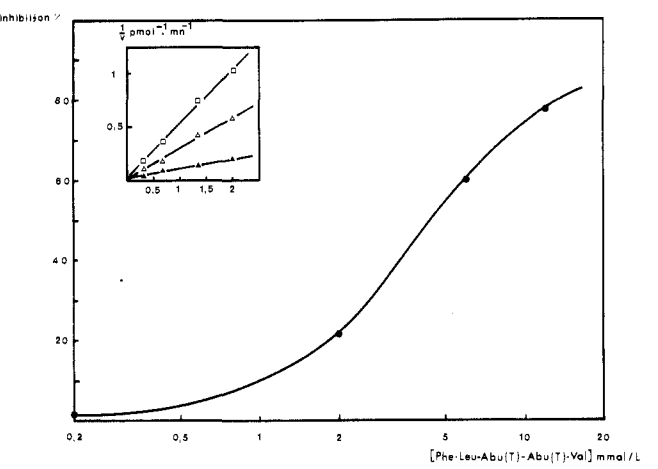


Figure 1. Inhibition of vitamin K dependent carboxylation of pentapeptide **1**, by ditetrazolyl peptide **3**. Carboxylation ([1] = 1 mM) was assayed as previously described.[33,34] Insert: competitive inhibition. The inhibitor concentrations (mM) were as follows: (▲) 0, (△) 6, (□) 12.

Both tetrazolyl peptides **2** and **3** and the dicyano peptide **13** were tested for carboxylation. Compounds **3** and **13** were not carboxylated, but **2** was significantly carboxylated (40% of the reference **1** at 1 mM). Analysis of data according to Lineweaver–Burk gave a K_m value of 20 mM; that is 5–10 times the K_m of reference **1**. Further analysis of the carboxylation product after hydrolysis (during which we checked that no transformation of Abu(T) into Glu occurred) and careful separation of Glu and Abu(T) by ion-exchange column chromatography revealed that 50% of the radioactivity initially incorporated in the peptide was recovered in the Glu residue, proving that within experimental error carboxylation occurred exclusively at the glutamyl residue. Recently Dubois et al.[33] and Gaudry et

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(32) The yield for **2** was unexpectedly low (27%) due to the formation of a methyl ester during the hydrogenolysis step (see Experimental Section).

al.[34] have reported such instances in which the reaction occurs at a position not usually carboxylated in reference peptide 1.

The lack of reactivity of the Abu(T) residue observed in 2 is consistent with the lack of reaction of peptide 3.

Peptides 3 and 13 were tested for their capacity to inhibit carboxylation of reference peptide 1. Dicyano-containing peptide 13 exhibited no inhibitory properties at 5 mM ([1] = 1 mM), whereas peptide 3 proved to be a good competitive inhibitor (Figure 1) with a K_i value of 3.1 mM, which under our carboxylation conditions was equal to the K_m of peptide 1. This affinity is not as high as that of the γ-methylglutamyl-containing peptide of Gaudry et al.[34] but is very close to that of the phosphoserine-containing analogue described by Suttie et al.[35]

Conclusion

We have shown that the synthesis of the tetrazolyl moiety during the last step of the synthesis of peptides is a very convenient method that can easily be extended to the synthesis of polytetrazolyl derivatives, even when the tetrazolyl groups are in very close proximity.

The Abu(T) is not carboxylated at the γ position and therefore this leaves open the question of the activation of the carboxyl group in vitamin K dependent carboxylation.

A number of interesting conclusions can be drawn concerning the affinity of substrates. It is clear that the presence of negatively charged groups is important since the dicyano peptide 13 does not bind to the active site, whereas the ditetrazolyl 3 and the normal substrate 1 have very similar affinities. However, this factor, although necessary, is not sufficient as illustrated by the lower affinity for the monotetrazolyl analogue 2. It is likely that conformational problems interfere in this case.

Experimental Section

Methods. ^{1}H NMR spectra were recorded on a Varian HA100 spectrometer and chemical shifts (ppm) are reported with tetramethylsilane as internal reference. $[\alpha]_D$ were measured by using an automatic Perkin-Elmer 141 polarimeter, and infrared spectra were recorded on a Perkin-Elmer 457 spectrophotometer. Melting points were measured by using a Kofler apparatus and are uncorrected. Elemental analyses were performed by the "Service Central d'Analyse du CNRS". All gave satisfactory results (±0.4%) and are reported as analytical data. ($C_xH_yO_zN_w$). Amino acid analyses were carried out by Dr. J. L. Morgat (CEA-Saclay). The purity of peptides was checked by HPLC (C_{18} μBondapak, 0.39 × 30 cm, isocratic mode, eluent: 0.25 M triethylammonium phosphate, 14% acetonitrile, pH 3.0). TLC analyses were performed on Kieselgel 60 F_{254} 0.25-mm plates from Merck. Eluent systems were as follows: A, hexane–ethyl acetate, 3:7; B, hexane–ethyl acetate, 1:1; C, chloroform–methanol, 24:1; D, chloroform–methanol, 97:3; E, 1-butanol–acetic acid–water, 4:1:5 (upper phase); F, 1-butanol–0.1 M acetic acid, 1:1 (upper phase); G, ammonium acetate linear gradient 10 mM pH 4.5 → 0.4 M pH 6.5; H, 0.1 M acetic acid; I, 0.05 M acetic acid. Radioactivity was estimated by using an Intertechnique SL 30 scintillation spectrometer, and results were corrected for quenching by using the double-channel method.

Chemicals. Dicyclohexylcarbodiimide (DCC), *N*-methylmorpholine (NMM), and isobutyl chloroformate were from Fluka (Switzerland). Amino acids and derivatives were from Bachem (Switzerland). Trifluoroacetic acid, acetic acid, and solvents were from Prolabo (France). Silica gel (Kieselgel 60, 70–230 mesh) was from Merck, and DE 52 cellulose and CM 32 cellulose were from Whatman S.A. (France). Tri-*n*-butyltin azide was prepared according to Kricheldorf and Leppert.[36]

(*S*)-(*tert*-Butoxycarbonyl)-4-cyano-2-aminobutyric Acid (Boc-L-Abu(CN)) (4). DCC (4.65 g, 22.6 mmol) in pyridine (14 mL) was added to a stirred, cooled (t < 16 °C) solution of Boc-L-Gln (5 g, 20.3 mmol) in pyridine (28 mL). After 3 h, DCU was removed by filtration and pyridine eliminated under vacuum. The oily residue was dissolved in ethyl acetate, washed with 10% citric acid, dried over sodium sulfate, and evaporated to dryness, yielding 4 (4.44 g, 96%): ^{1}H NMR ($CDCl_3$) δ 5.26 (m, 1 H, NH), 4.34 (m, 1 H, CHN), 2.46 (m, 2 H, CH_2), 2.16 (m, 2 H, CH_2), 1.44 (s, 9 H, $(CH_3)_3C$); IR cm^{-1} (neat) 2225 (CN); $[\alpha]^{20}_D$ 8° (*c* 1, $CHCl_3$). Dicyclohexylammonium salt: mp 155–157 °C (crystallized twice in methylene chloride–diethyl ether mixture); $[\alpha]^{20}_D$ 4° (*c* 0.5, $CHCl_3$). Anal. ($C_{22}H_{39}O_4N_3$).

Boc-L-Abu(CN)ONSu (5). DCC (14.6 mmol), HONSu (1.86 g, 16.2 mmol), and 4 (3.34 g, 14.6 mmol) in CH_2Cl_2 (37.3 mL) were stirred at −10 °C for 3 h followed by 20 h at 4 °C. Unreacted DCC was destroyed with acetic acid (1 mL) and DCU removed by filtration. After concentration, the residue was dissolved in ethyl acetate and washed with 1 M sodium bicarbonate and subsequently with water. Crystallization from ether yielded 5 (mp 113–115 °C, 3.59 g, 76%): ^{1}H NMR ($CDCl_3$) δ 5.39 (d, 1 H, NH), 4.75 (m, 1 H, CHN), 2.9 (s, 4 H, $(CH_2)_2$(ONSu), 2.58 (m, 2 H, CH_2), 2.3 (m, 2 H, CH_2), 1.49 (s, 9 H, $(CH_3)_3C$).

Boc-Glu(OBzl)-Val-OBzl. Val-OBzl, *p*-tosylate (3.05 g, 8.2 mmol), and NMM (0.905 mL) in DMF (10 mL) were added to a cooled (0 °C) solution of Boc-Glu(OBzl)ONSu (3.33 g, 6.3 mmol) in DMF (15 mL). After stirring at room temperature for 20 h and elimination of solvents under vacuum, the crude product was dissolved in ethyl acetate and washed with cold 10% citric acid, 1 M sodium bicarbonate, and water, yielding the dipeptide (3.4 g, 6.07 mmol, 80%): mp 85–87 °C; $[\alpha]^{20}_D$ −28.5° (*c* 1, methanol).

Boc-Abu(CN)-Glu(OBzl)-Val-OBzl (6). After the removal of the Boc protection (trifluoroacetic acid–methylene chloride, 1:1; 30 min) from Boc-Glu(OBzl)-Val-OBzl (2.5 g, 4.75 mmol), the crude product was dissolved in CH_2Cl_2/DMF (25 mL, 4:1, v/v) and cooled to 0 °C. The pH was adjusted to 7 with NMM before addition of 5 (1.2 g, 3.69 mmol) in CH_2Cl_2 (12 mL). After stirring at room temperature for 16 h and elimination of solvents under vacuum, the crude product was dissolved in ethyl acetate and washed with cold 10% citric acid, 1 M sodium bicarbonate, and water. Purification on a silica gel column (200 g (A)) yielded pure 6 (2.1 g, 69%): R_f 0.38 (B).

Boc-Leu-Abu(CN)-Glu(OBzl)-Val-OBzl (7). Deprotection of 6 (1.73 g, 2.72 mmol) and coupling with Boc-LeuONSu (1.35 g, 3.85 mmol) as above followed by purification on a silica gel column (200 g (A)) yielded pure 7 (1.66 g, 69%): R_f 0.46 (C).

Boc-Phe-Leu-Abu(CN)-Glu(OBzl)-Val-OBzl (8). After deprotection of 7 (1.43 g, 1.9 mmol), the tetrapeptide was solubilized in CH_2Cl_2/DMF (6 mL; 1:2, v/v) and neutralized with NMM. This solution was added to a cooled solution of Boc-Phe-O-CO-*i*-Bu (Boc-Phe-OH (0.74 g, 2.8 mmol), in CH_2Cl_2/DMF (24 mL, 2:1), isobutyl chloroformate (0.365 mL, 2.8 mmol), NMM (0.31 mL, 2.8 mequiv) 45 min, −15 °C) and stirred overnight. Treatment as above and purification on silica gel column (150 g (D)) yielded 8 (1.34 g, 78%): R_f 0.38 (D); mp 208–210 °C (recrystallized twice in ethyl acetate–hexane); $[\alpha]^{20}_D$ −44.7° (*c* 1, $CHCl_3$); Anal. ($C_{49}H_{64}O_{10}N_6$).

Phe-Leu-Abu(T)-Glu-Val (2). Compound 8 (0.3 g, 0.335 mmol) and tri-*n*-butyltin azide (0.208 mL, 0.67 mmol, freshly distilled) in dry tetrahydrofuran (2 mL) were heated under argon in a sealed tube (80 °C, 96 h). The precipitate obtained after addition of diethyl ether was washed and suspended in dry ether. Dry hydrogen chloride was bubbled through the suspension (1 h) and the precipitate was hydrogenolyzed over Pd/C (10%, 0.135 g, 20 h under atmospheric pressure in a methanol–acetic acid mixture (6 mL; 9:1, v/v). Purification on a DE52 column (2.5 × 23 cm, acetate form (G), 1.7 L) yielded 2 (between 585 and 670 mL; 61 mg, 27%): $[\alpha]^{20}_D$ −25.6° (*c* 0.5, 1% acetic acid); R_f 0.54 (E), R_f 0.14 (F). Purity (>98%) was checked by HPLC. Abu(T) and glutamic residues can be easily separated on an AG1X2

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column (vide infra). However, the two residues could not be separated during the amino acid analyses. With use of an AG1X2 column, it was checked that no Abu(T) was transformed into glutamic acid during acid hydrolysis. Both residues were titrated together during the amino acid analysis. Found: Glu + Abu(T), 2.16; Val, 0.94; Leu, 1.00; Phe, 0.93.

During the purification on the DE52 column, elution between 360 and 380 mL yielded 53 mg of a product corresponding to a methyl ester of **2** (presence of an OCH_3 group detected by NMR, same amino acid composition as **2**) produced by transesterification during hydrogenolysis.

Boc-Abu(CN)-Val-OBzl (9). Compound **5** (2.5 g, 7.69 mmol) and Val-OBzl tosylate (4.39 g, 11.6 mmol) were mixed together in CH_2Cl_2 (50 mL) containing NMM (1.10 g, 10.9 mmol). After stirring at room temperature for 20 h and removal of the solvent under vacuum, the crude product was dissolved in ethyl acetate and washed with 10% citric acid, 1 M sodium bicarbonate, and finally with water. Purification on a silica gel column (160 g, (C)) yielded pure **9** (3 g, 94%): R_f 0.52 (C).

Boc-Abu(CN)-Abu(CN)-Val-OBzl (10). After removal of the Boc protection of **9** (2.5 g, 5.9 mmol), the crude product was dissolved in CH_2Cl_2/DMF (30 mL; 2:1, v/v) cooled to 0 °C and the pH adjusted to 7 with NMM before addition of **5** (2.1 g, 6.46 mmol) in CH_2Cl_2 (12 mL). After 16 h the reaction mixture was treated as above and then chromatographed on a silica gel column (200 g (A)), yielding **10** (1.81 g, 57%): R_f 0.41 (A).

Boc-Leu-Abu(CN)-Abu(CN)-Val-OBzl (11). After removal of the Boc protection of **10** (1.5 g, 2.4 mmol), the crude product was dissolved in CH_2Cl_2/DMF (22 mL, 9:1, v/v) cooled at 0 °C and the pH adjusted to 7 with NMM before addition of Boc-Leu-ONSu (1.35 g, 4.12 mmol) in CH_2Cl_2 (10 mL). After 21 h at room temperature the reaction mixture was treated as above and chromatographed on a silica gel column (200 g (A)), yielding **11** (1.35 g, 74%, mp 184–186 °C (ethyl acetate–hexane)): R_f 0.50 (A).

Boc-Phe-Leu-Abu(CN)-Abu(CN)-Val-OBzl (12). After deprotection of **11** (1.3 g, 2.03 mmol), the tetrapeptide was solubilized in CH_2Cl_2/DMF (6 mL; 1:1, v/v) and neutralized with NMM. This solution was added to a cooled solution of Boc-Phe-O-CO-*i*-Bu (Boc-Phe-OH (0.77 g, 2.91 mmol), in CH_2Cl_2/DMF (24 mL; 2/3, v/v), isobutyl chloroformate (0.322 mL, 2.90 mmol), NMM (0.322 mL, 2.91 mmol), 45 min, −17 °C). After 1 h at −15 °C, the temperature was allowed to rise gradually to room temperature and the mixture was stirred overnight. After treatment as above, purification on a silica gel column (140 g (D)) yielded **12** (1.2 g, 92%, mp 204–206 °C (ethyl acetate–hexane)): R_f 0.34 (D); $[\alpha]^{20}_D$ −14.7° (*c* 1, $CHCl_3$). Anal ($C_{42}H_{57}O_8N_7$).

Phe-Leu-Abu(T)-Abu(T)-Val (3). Compound **12** (0.30 g, 0.381 mmol) and tri-*n*-butyltin azide (0.246 mL, 0.762 mmol, freshly distilled) in dry tetrahydrofuran (1.2 mL) were heated under argon in a sealed tube (80 °C, 96 h). The precipitate obtained after addition of diethyl ether was washed and suspended in dry ether (10 mL). Dry hydrogen chloride was bubbled through the suspension (1 h) and the precipitate was hydrogenolyzed over Pd/C (10%, 0.129 g) for 20 h under atmospheric pressure in methanol–acetic acid (6 mL, 9:1). Purification on a DE 52 column (2.5 × 23 cm, acetate form (G)) yielded **3** (between 800 and 990 mL, 160 mg, 61%): $[\alpha]^{20}_D$ −18.4° (*c* 0.5, 1% acetic acid); R_f 0.52 (E), R_f 0.145 (F). Purity (>98%) was checked by HPLC. Acid hydrolysis was in hydrochloric acid at 100 °C for 24 h. Found: Abu(T), 2.16; Val, 1.19; Leu, 1.0; Phe, 0.95.

Phe-Leu-Abu(CN)-Abu(CN)-Val (13). After the removal of the Boc protection from **12** (0.206 g, 0.261 mmol), the crude product was hydrogenolyzed over Pd/C (10%, 40 mg, 2 h, atmospheric pressure, methanol–acetic acid (3 mL, 9:1)). Purification was achieved on a CM 32 column (2.5 × 25 cm (G), 800 mL). **13** was eluted between 100 and 150 mL and further purified by partition chromatography (Sephadex G25, 2.3 × 90 cm; equilibration (F), lower phase; elution (F), upper phase). **13** (19 mg, 0.03 mmol, 12%) was eluted between 280 and 340 mL. Purity was 95% (HPLC); $[\alpha]^{20}_D$ −31.6° (*c* 0.5, 1% acetic acid). Acid hydrolysis (the cyano groups were transformed into carboxylic groups) was in 6 M hydrochloric acid at 110 °C for 24 h. Found: Glu, 1.95; Val, 1.00; Leu, 1.00; Phe, 0.94.

Biological Assay: Carboxylation of Peptides. Biological assays of peptides (carboxylation and inhibition) were performed as previously described.[33,34] Concentrations are provided in the legend of Figure 1.

Carboxylation of Phe-Leu-Abu(T)-Glu-Val (2). Identification of the Carboxylated Residue. After carboxylation, the supernatant containing carboxylated **2** (679 800 dpm) was desalted over a Sephadex G25 column (2.3 × 26 cm (H)), yielding a single radioactivity peak (between 50 and 83 mL; 582 200 dpm). An aliquot (11 600 dpm) was hydrolyzed and decarboxylated in a sealed tube (6N hydrochloric acid, 110 °C, 24 h). After addition of Abu(T) (4 mg) and Glu (4 mg) as carriers, the separation was achieved on an AG1X2 column (acetate form, 1 × 80 cm (I)). Abu(T) was eluted between 420 and 500 mL and, as expected, contained no radioactivity, whereas Glu was eluted between 540 and 660 mL and contained 5325 dpm, corresponding to 46% of the input radioactivity.

Acknowledgment. We thank Dr. J. L. Morgat for the amino acid analyses and we are grateful to Dr. P. Decottignies-Le Marechal for the preparation of microsomes.

Registry No. 1, 61037-79-4; **2**, 91126-92-0; **2** carboxylated deriv., 91126-93-1; **3**, 91126-94-2; 4, 45172-42-7; 4 dicyclohexylammonium salt, 24277-36-9; **5**, 91126-95-3; **6**, 91126-96-4; **7**, 91126-97-5; **8**, 91126-98-6; **9**, 91126-99-7; **10**, 91127-00-3; **11**, 91127-01-4; **12**, 91127-02-5; **13**, 91156-80-8; Boc-L-Gln, 13726-85-7; HONSu, 6066-82-6; Boc-Glu(OBzl)-Val-OBzl, 91156-81-9; Val-OBzl *p*-tosylato, 16652-76-9; Boc-Glu(OBzl)ONSu, 32886-40-1; H-Glu(OBzl)-Val-OBzl, 91127-03-6; Boc-Leu-ONSu, 3392-09-4; Boc-Phe-O-CO-*i*-Bu, 91127-04-7; $(n\text{Bu})_3\text{SnN}_3$, 17846-68-3; Boc-Phe-Leu-Abu(T)-Glu(OBzl)-Val-OBzl, 91156-82-0; H-Leu-Abu(CN)-Glu(OBzl)-Val-OBzl, 91156-83-1; H-Abu(CN)-Val-OBzl, 91127-05-8; H-Abu(CN)-Abu(CN)-Val-OBzl, 91127-06-9; H-Leu-Abu(CN)-Abu(CN)-Val-OBzl, 91127-07-0; Boc-Phe-OH, 13734-34-4; Boc-Phe-Leu-Abu(T)-Abu(T)-Val-OBzl, 91127-08-1; vitamin K, 12001-79-5.