Preparation and Properties of N^{α}-Di-*tert*-Butoxycarbonyl Amino Acids. Applicability in the Synthesis of Leu-Enkephalin

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The synthesis of N^{α}-di-*tert*-butoxycarbonyl amino acids starting from the corresponding mono-derivatives via a three-step route is reported. The latter were converted into a suitable ester and then exhaustively *t*-butoxycarbonylated, after which the ester was selectively cleaved. The intermediates and products are characterised together with some active esters. The Boc_2-amino acids are stable, crystalline compounds which can be used for coupling in peptide synthesis. They react more slowly than the corresponding Boc-amino acids owing to steric hindrance. Their applicability in peptide synthesis is demonstrated in the synthesis of Leu-enkephalin. In the coupling of Boc_2-Tyr(Bzl)-ONp to Gly-Gly-Phe-Leu-OBzl, a large amount of a hydantoin derivative was obtained as a by-product. Some factors influencing the formation of hydantoins were studied. Boc_2-amino acids seem to enhance the risk of formation of such compounds in comparison with the ordinary monosubstituted amino acids.

The principle of temporary protection of amino functions by their conversion into urethanes, pioneered by Bergmann and Zervas,[1] has found innumerable applications in organic synthesis. Their original benzyloxycarbonyl (Z)* together with the *t*-butoxycarbonyl (Boc)[2-4] and, more recently, the fluorenylmethoxycarbonyl (Fmoc)[5] groups are the most important for the protection of the α-amino function in peptide synthesis. These protecting groups are stable in many synthetic routes and are easily removed in the presence of each other under mild conditions.

A general method for exhaustive *t*-butoxycarbonylation of amides[6] has recently been described. In this context, *N*-Boc_2- and *N*-Boc(Z)-derivatives of amines were also prepared.[6-8] This prompted us to start the present investigation, from which we have already briefly reported the synthesis of Boc_2- and Boc(Z)-amino acids.[9] These derivatives represent a new type of protection for the amino function of amino acids with two alkoxycarbonyl groups on the same nitrogen (such a derivative has also been reported by Chen and Benoiton[10]). On activation, such imidodicarbonates cannot even theoretically form oxazolones, and consequently cannot racemize through such an intermediate.

In this paper, the general synthetic pathways to these compounds are discussed in detail, and several novel intermediates and derivatives are described. Finally, the applicability of Boc_2-amino acids in the synthesis of Leu-enkephalin is presented.

* Abbreviations: the symbols of the amino acids, peptides and protecting groups are in accordance with the 1983 Recommendations of the IUPAC–IUB Joint Commission on Biochemical Nomenclature [*Eur. J. Biochem. 138* (1984) 9]. In addition, OAll stands for allyl esters.

Results and discussion

Synthesis of Boc_2-amino acids. The synthesis of Boc_2-amino acids was accomplished by a three-step route (Scheme 1) from Boc-amino acids (**1**). Thus, the second Boc-group was introduced on the urethane nitrogen of a Boc-amino acid ester (**2**). This procedure eliminates the potential risk of generating mixed anhydrides as well as salt formation with the basic catalyst. Our first choice for the carboxyprotection was the benzyl ester which can be introduced smoothly and the final removal is easily accomplished by catalytic hydrogenation. For the amino acids with non-functionalized side-chains, this was the obvious method.

For some trifunctional amino acids, the benzyl group was already in use for the protection of the side-chain and a carboxy-protecting group that could be removed by alternative means was therefore sought. Such an ester would also be required for the synthesis of Boc(Z)-amino acids. Our first attempt was with the base-labile fluorenylmethyl ester[11,12] which has been reported to be removable with 15 % piperidine. This approach gave acceptable results for the smallest amino acids, such as Z-Gly and Z-Ala, but with Z-Phe, Boc-Ser(Bzl) and Boc-Tyr(Bzl), the desired products were not obtained, evidently due to steric hindrance. For the serine and tyrosine derivatives, the acylation step could not be driven to completion, whereas for the phenylalanine analogue, the cleavage by piperidine was very slow. In a model experiment with Z-Gly, the phenacyl ester[13] was tried but its cleavage with sodium thiophenoxide also to some extent removed the Z-group.

The allyl esters[14,15] gave satisfactory results in both the acylation reaction and the cleavage step, and became the ester of choice. They were easily prepared via the corre-

$$\underset{\mathbf{1}}{\text{Boc–NH–CHR–COOH}} \rightarrow \underset{\mathbf{2}}{\text{Boc–NH–CHR–COOX}} \rightarrow \underset{\mathbf{3}}{\text{Boc}_2\text{–N–CHR–COOX}} \rightarrow \underset{\mathbf{4}}{\text{Boc}_2\text{–N–CHR–COOH}}$$

Scheme 1. Synthesis of N^{α}-di-*tert*-butoxycarbonyl amino acids.

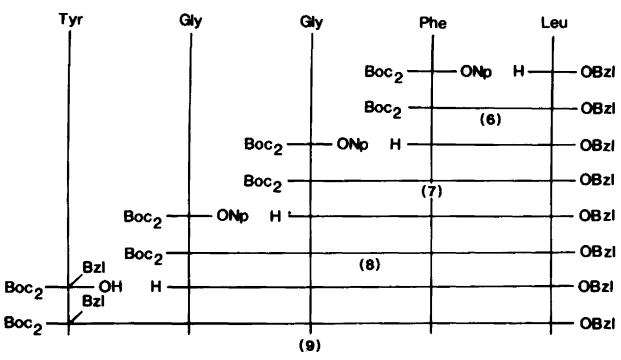
sponding caesium salts. Due to the moderate size of the allyl group, the subsequent acylation step generally proceeded smoothly although in the presence of bulky side-chains, as in the case of *O*-benzyltyrosine, longer reaction times and repeated additions of Boc_2O and DMAP were needed for complete reaction. Time was saved if the crude reaction mixture was taken to dryness and redissolved in fresh acetonitrile before the addition of further reagents. The specific cleavage of the allyl ester function with tris-(triphenylphosphine)rhodium(I) chloride at 70 °C presented no unexpected problems.[14] For the more sterically hindered derivatives, prolonged reaction times were required, in extreme cases more than 24 h. Addition of the catalyst (altogether up to 0.1 equiv.) in smaller portions shortened the reaction time needed. This indicates that the catalyst is consumed or poisoned under the conditions applied. In most cases, the work-up procedure presented no difficulties. However, the lipophilic derivative Boc_2-Tyr(Bzl), and, to some extent, Boc_2-Ser(Bzl) and Boc_2-Glu(OBzl) were incompletely extracted into the aqueous bicarbonate phase. For tyrosine, less than 5 % was extracted under normal conditions. Changing the extraction conditions did not improve the yield, and the tyrosine derivative had to be purified by chromatography.

Properties of Boc_2-amino acids. The Boc_2-amino acids seem to be stable, crystalline compounds with high melting points. Their optical rotations are considerably higher than those of the corresponding Boc-derivatives. They are resistant to moderately strong bases such as piperidine and 2-ethylaminoethylamine, and Boc_2-Gly remained essentially intact even when treated with NaH in dioxane. Infrared spectra of Boc_2-amino acids exhibit an additional absorption band in the carbonyl region, whereas the amide II bond at ca. 1550 cm^{-1} is absent. To mention a few examples, strong $\nu_{C=O}$ bands are observed in KBr for Boc_2-Gly at 1757, 1737 and 1720 cm^{-1} (Boc-Gly: 1749, 1669 and 1538 cm^{-1}), for Boc_2-Leu at 1735, 1715 and 1698 cm^{-1} (Boc-Leu: 1718, 1677 and 1543 cm^{-1}), for Boc_2-Tyr(Bzl) at 1749, 1724 and 1707 cm^{-1} [Boc-Tyr(Bzl): 1713, 1644 and 1517 cm^{-1}]. In the ^1H NMR spectrum, the α-protons of Boc_2-amino acids appear 0.4–0.8 ppm downfield of the corresponding shifts of the mono-derivatives (90 MHz).

Earlier experiments had revealed that Boc_2-amino acids could be coupled to amino acid esters with both active esters and with carbodiimide.[9] The coupling of Boc_2-Gly to Phe-Leu-OBzl by the mixed anhydride method has now also been carried out successfully (see the Experimental), but when the same procedure was applied for the synthesis of **6** (see Scheme 2 and the Experimental), only 50 % of the product was obtained after chromatography. In addition, a substantial amount of *i*-butoxycarbonyl-Leu-OBzl was isolated, accounting for more than 30 % of the Leu. The same product ratio was observed when the activation time was extended from one to five minutes. With respect to the application of Boc_2-amino acids in solid-phase peptide synthesis, competitive coupling experiments with both alanine and valine resins were carried out under standard conditions (DCC, CH_2Cl_2) and evaluated by amino acid analysis after hydrolysis.[16] In this test system, in competition with ordinary Boc-amino acids, only Boc_2-Gly coupled to a measurable extent, giving values corresponding to 40–50 % of those for Boc-Leu, whereas four others, including Boc_2-Ala, did not, suggesting severe steric hindrance for all of them. Nevertheless, a solid-phase experiment where Boc_2-Phe was coupled to a Leu-polymer (2.5 times excess, 60 min coupling time) gave 86 % incorporation of phenylalanine.

Synthesis of Leu-enkephalin. To investigate the applicability of these new derivatives, a model peptide, Leu-enkephalin,[17] was synthesized. The synthesis was carried out stepwise from the carboxy end using *p*-nitrophenyl esters (Scheme 2). We first attempted the coupling of Boc_2-Phe-ON*p* to Leu-OBzl in ethyl acetate. This reaction was, however, very slow. A comparison of the reaction rates in ethyl acetate and DMF was made by monitoring the release of *p*-nitrophenol at its absorption maximum at ca. 310 nm. In DMF, 50 % of the active ester was consumed within 3 h, confirming an earlier observation that *p*-nitrophenyl ester couplings are fast in this solvent,[18] whereas in ethyl acetate, as much as two days were needed for 50 % reaction. Consequently, we chose to carry out the reactions in DMF instead.

The syntheses up to the tetrapeptide presented no particular difficulties. The coupling of Boc_2-Gly-ON*p* with **6a** to give **7** was fast and complete within a few hours, whereas the formation of **6** required at least 24 h, in accordance



Scheme 2. Synthesis of Leu-enkephalin.

with the observation described in the preceding paragraph. In the coupling of Boc_2-Tyr(Bzl)-ON*p* to **8a**, the crude product was inhomogeneous and gave remarkably low values in the amino acid analysis for Gly and Tyr, although the former value was excellent in **8**. ^1H NMR spectroscopy showed two Boc-singlets but together they integrated for only 60 % of the expected value. Chromatography of the reaction mixture gave two components, the major of which was assigned the hydantoin structure **10**, and the minor the desired peptide **9**. The result was reproducible even when the reaction conditions were changed to eliminate basic components. The Boc-protons of **10** gave a characteristic shift in ^1H NMR ($\delta = 1.61$ compared with $\delta = 1.38$ for **9**). In addition, the ^{13}C NMR spectrum for **10** showed a new carbonyl signal outside the range previously encountered at 148.2 ppm, in excellent agreement with the corresponding shifts of the three model compounds **13–15** (see the Experimental). Furthermore, according to TLC analysis, **10** is formed in parallel with **9** from the beginning of the experiment. On amino acid analysis, pure **10** analysed for 0.2 Tyr and 1.3 Gly, showing that the hydantoin does not open cleanly in the hydrolysis step. The chromatographically isolated **9** shows no tendency to cyclize when allowed to stand in $CDCl_3$ in an NMR tube at room temperature for months. This compound also gave the expected values on amino acid analysis after hydrolysis.

At this stage, we decided to use another coupling method for the last step in the synthetic scheme. Coupling with DCC in DMF in the presence of *N*-hydroxysuccinimide gave **9** in up to 50 % yield after chromatography. In addition, Boc_2-Tyr(Bzl)OSu (**11**) was isolated in this case. The total amount of **9** and **11** accounted for more than 80 % of the tyrosine used. An aliquot of **11** was treated with **8a** (small excess) in DMF overnight. TLC indicated that the product contained both **9** and **10** in the ratio 1:1 as estimated from ^1H NMR spectroscopy (this was also supported by amino acid analysis). The *N*-hydroxysuccinimide ester is evidently enriched in this coupling experiment and does not undergo further reaction since the crude product does not contain noticeable amounts of hydantoin. A small-scale DCC coupling experiment without *N*-hydroxysuccinimide gave exclusively the *N*-acylurea product.

Studies on the hydantoin formation. A number of small-scale coupling experiments were carried out in a study of the factors influencing the formation of hydantoin. In each case, the product was analysed by ^1H NMR spectroscopy and amino acid analysis. The amount of hydantoin formed was estimated by integration of the hydantoin Boc protons at δ ca. 1.60 and the Boc_2 protons at δ ca. 1.40. First, Boc_2-Tyr(Bzl)-ON*p* was exchanged for the corresponding phenylalanine and glycine derivatives. In the experiment with Boc_2-Phe-ON*p* and **8a**, the product ratio was about the same as for the tyrosine derivative whereas in the latter, the amount of hydantoin formed was only ca. 10 %, indicating some influence of the active ester on the outcome of the reaction. When Boc_2-Tyr(Bzl)-ON*p* was coupled instead to **7a**, ca. 20 % of the hydantoin analogue was formed, while with Gly-OAll and Gly-OBzl, the pure dipeptides were obtained in quantitative yield with no traces of hydantoin.

It is known that hydantoins can be formed on ammonolysis or saponification of Z-peptide esters when glycine is the second residue.[19] These experiments indicate that with Boc_2-amino acid *p*-nitrophenyl esters, hydantoins are also formed under milder basic conditions in the synthesis of such glycine peptides. Obviously, a bulky side-chain in position 1 and another glycine in position 3 enhance the cyclization as no by-products of this type could be detected in **8**. Furthermore, since no hydantoin formation was previously noticed in connection with the synthesis of Leu-enkephalin, Boc_2-amino acids seem to be subject to an enhanced risk of such formations in comparison with ordinary Boc-amino acids.

Experimental

All amino acids used (except for glycine) were of L-configuration. Melting points were determined on a Gallenkamp apparatus and are uncorrected. Optical rotations were measured using a 1 dm cell in a Perkin-Elmer 141 polarimeter at 25 °C. All solvents used as reaction media were dried over molecular sieves (4 A). TLC analyses were performed on 0.25 mm thick, precoated silica plates (Merck DC-Fertigplatten Kieselgel F_{254}) in the following systems: CH_2Cl_2–acetone–HOAc 40:10:1 (A); toluene–CH_3CN 2:1 (B); CH_2Cl_2 (C); CH_2Cl_2–MeOH 95:5 (D); CH_2Cl_2–acetone 15:1 (E); $CHCl_3$–MeOH–HOAc–H_2O 30:20:4:6 (F); EtOAc–pyridine–HOAc–H_2O 60:20:6:11 (G); n-BuOH–HOAc–H_2O 4:1:1 (H). Spots were visualized by inspection under UV light at 254 nm, and/or, after brief heating, by exposure to Cl_2 followed by dicarboxidine spray. Preparative chromatography was carried out using silica gel 60 (70–230 mesh, Merck). Analytical HPLC was performed on a standard apparatus equipped with a Pharmacia PepRPC column. NMR spectra were recorded routinely in $CDCl_3$ with tetramethylsilane as an internal standard on a JEOL FX 90Q instrument at 90 MHz or on a Varian XL 300 instrument at 300 MHz (^1H) or at 75 MHz (^{13}C). Amino acid analyses were performed, after acid hydrolysis, by the Central Amino Acid Analysis Laboratory, Institute of Biochemistry, Uppsala. Tyrosine-containing peptides were hydrolyzed in the presence of phenol (10 g l^{-1}). Elemental analyses were made by Mikro Kemi AB, Uppsala. Except where stated otherwise, organic extracts of evaporated reaction mixtures were washed three times each with one third of the volume of 1 M $KHSO_4$, 1 M $NaHCO_3$ and saturated NaCl solution, and then dried over $MgSO_4$.

General procedures for the synthesis of N,N-*di*-tert-*butoxycarbonyl amino acids. A. Synthesis via benzyl esters (I)* The Boc-amino acid was converted into its Cs salt and treated with benzyl bromide in DMF overnight according to a

described procedure.[20] Most of the solvent was stripped off (*T* ca. 45 °C), and the residue was dissolved in diethyl ether, washed and dried. Removal of the solvent generally gave a solid which, after trituration with light petroleum, gave pure benzyl ester.

(II) To a solution of the Boc-amino acid benzyl ester and DMAP (0.1 equiv.) in CH_3CN (1–2 ml $mmol^{-1}$), was added a suspension of Boc_2O in CH_3CN (1.1 equiv. in 0.5 ml $mmol^{-1}$) and the mixture was stirred at room temperature overnight. The reaction was monitored by TLC (B or C). In some cases, starting material remained after 4–6 h and more Boc_2O (0.5 equiv.) was added. If necessary, this procedure was repeated until all starting material had been consumed. The coloured reaction mixture was taken to dryness and the residue was dissolved in EtOAc, washed and dried. Evaporation generally afforded an oil. In some cases, excess Boc_2O had to be removed under high vacuum overnight. The purity was checked by TLC (B or C) and ^1H NMR spectroscopy.

(III) The Boc_2-amino acid benzyl ester was hydrogenated with 5 % (w/w) of Pd/C (5 %) in methanol (NTP). The reaction was monitored by TLC (A), and was complete after 1–2 h. The catalyst was filtered off, and the filtrate taken to dryness. The residue crystallized spontaneously when allowed to stand. The product was recrystallized from ether/light petroleum or EtOAc/light petroleum.

B. Synthesis via allyl esters (I) The Boc-amino acid – in which the side chain was protected with Bzl when required – was converted into the corresponding Cs salt. The salt was dissolved in DMF (1–3 ml $mmol^{-1}$) and treated with distilled allyl bromide (1.0 equiv.) at room temperature overnight. Most of the DMF was evaporated, and the residue was dissolved in EtOAc, washed and dried. Removal of the solvent under reduced pressure afforded the pure allyl ester. The purity was checked by TLC (A) and ^1H NMR spectroscopy.

(II) A solution of the allyl ester in CH_3CN (1–3 ml $mmol^{-1}$) was treated with DMAP (0.1 equiv.) and Boc_2O (1.5 equiv.) at room temperature overnight. When necessary, more Boc_2O (0.5 equiv.) was added. This procedure was repeated until TLC showed that the reaction was complete. The volatile components were removed under reduced pressure and the yellow residue was partitioned between EtOAc and 1 M $KHSO_4$. The organic extract was washed, dried and evaporated. The product was obtained as an oil, pure by TLC and ^1H NMR spectroscopy.

(III) The allyl ester was dissolved in 90 % ethanol (5 ml $mmol^{-1}$) and treated with tris(triphenylphosphine)-rhodium(I) chloride (0.1 equiv.) at 70 °C for 2–3 h.[14] Prolonged reaction times were required for the bulkier derivatives and it was noted that the reaction was more efficient if the catalyst was added in several smaller portions. When TLC (A) showed that the reaction was complete, the reaction mixture was cooled to room temperature. Some solid material was filtered off and the filtrate evaporated to dryness. The residue was partitioned between EtOAc and 1 M $NaHCO_3$ (1:1) and the organic phase was further extracted with 2–5 portions of 1 M $NaHCO_3$. The combined aqueous extracts were acidified to pH 3 with solid $KHSO_4$ and extracted with three portions of EtOAc, washed with sat. NaCl solution (two portions) and dried. The solvent was evaporated and the residue recrystallized from EtOAc/light petroleum. Tyrosine required a different work-up procedure as the product could not be extracted into 1 M $NaHCO_3$ and was therefore chromatographed on silica in CH_2Cl_2. After some unknown by-products had been eluted, the solvent was changed to diethyl ether to give the pure product as an oil that solidified upon treatment with light petroleum. About 50 % was lost on a column large enough to provide separation. For yields and properties, see Table 1 and Ref. 9.

Synthesis of Boc(Z)-Gly (**4k**) *via the fluorenylmethyl ester (I)* To a suspension of fluorenylmethanol (11 mmol) and imidazole (18 mmol) in toluene, was added solid Z-Gly-ON*p* (10 mmol) and the reaction was stirred at room temperature overnight.[11] The solvent was removed, and the residue taken up in EtOAc and washed with 1 M $KHSO_4$, 1 M Na_2CO_3, and saturated NaCl solution. After drying, evaporation gave an oil that crystallized from EtOAc/light petroleum to give pure Z-Gly-OFm (**2i**).

(II) Compound **2k** (1.95 g, 5 mmol) and DMAP (61 mg, 0.5 mmol) were suspended in CH_3CN and Boc_2O (1.2 g, 6 mmol) was added. After 3 h at room temperature, TLC (B) showed that the reaction was complete. The solution was evaporated and the residue taken up in EtOAc. The usual work-up procedure gave an oil that was chromatographed on a short column* first in toluene, then in toluene–CH_3CN 4:1, to give the pure **3k** in 83 % yield.

(III) Compound **3i** (1.0 g, 2.1 mmol) was treated with a 15 % solution of piperidine in DMF (10 ml) for 2 h, after which TLC (A) indicated complete reaction. The reaction mixture was partitioned between EtOAc (100 ml) and 1 M $KHSO_4$ (30 ml). The organic phase was washed with further portions of 1 M $KHSO_4$ (2×30 ml), and then extracted with 1 M $NaHCO_3$ (3×30 ml). The combined aqueous extracts were acidified to pH ca. 3 with solid $KHSO_4$, and extracted with EtOAc (3×30 ml). The organic extract was washed with sat. NaCl solution and dried. Evaporation gave the pure product **4k** as a colourless oil in 64 % yield.

Preparation of p*-nitrophenyl esters: general procedure.* The Boc_2-amino acid and *p*-nitrophenol (1.2 equiv.), were dissolved in EtOAc (1–5 ml $mmol^{-1}$) and stirred in an ice-bath. A solution of DCC (1 equiv.) in EtOAc (1 ml $mmol^{-1}$) was added and the solution was stirred at 0 °C for 30 min and at room temperature overnight. The dicyclohexylurea was filtered off and the filtrate evaporated to dryness. The product was recrystallized from hot ethanol to give the pure *p*-nitrophenyl ester in good yield (Table 1).

* On a long column, the product partially decomposed.

Table 1. Spectral and other data on intermediates in the synthesis of Boc_2-amino acids including a number of active ester derivatives made according to Scheme 1.

Compound	R	X	Yield (%)[a]	Recryst. solvent[b]	M.p. /°C[a]	$[\alpha]_D$[a]	^1H NMR ($CDCl_3$ rel. TMS) (δ-values)[c]
2a	H	Bzl	92 (97)[22]	A	74–75 (72–73)[22]	–	7.35 (s, arom.), 5.18 (s, CH_2-Bzl), 3.94 (d, CH_2-Gly), 1.44 (s, Boc)
2b	CH_3	Bzl	88 (75)[23]	A	25.5–26 (oil)[23]	−32.8 *c* 1, DMF (−40.5 *c* 1, MeOH)[23]	7.34 (s, arom.), 5.17 (s, CH_2-Bzl), 4.35 (q, α-CH), 1.43 (s, Boc)
2c	*i*-C_4H_9	Bzl	98	Oil	– (oil)[24]	–	7.34 (s, arom.), 5.16 (s, CH_2-Bzl), 4.3 (br signal, α-CH), 1.43 (s, Boc)
2d	$CH_2C_6H_5$	Bzl	93 (95)[22]	B	63–63.5[d] (64–65)[22]	−11.8 *c* 1, EtOH (−12.8 *c* 2, MeOH)[22]	7.33 (s, arom.), 5.13 (s, CH_2-Bzl), 4.6 (br signal, α-CH), 1.40 (s, Boc)
2e	CH_2OBzl	All	92	Oil	–	–	5.6–6.1 (m, CH=), 5.1–5.4 (m, CH_2=), 4.64 (pert. d, CH_2O), 1.44 (s, Boc)
2f	CH_2CH_2-COOBzl	All	47	C	63–64	−20.6 *c* 1, MeOH	5.6–6.15 (m, CH=), 5.1–5.5 (m, CH_2=), 4.62 (pert. d, CH_2O), 4.3 (br signal, α-CH), 1.43 (s, Boc)
2g	$CH_2C_6H_4$-OBzl	All	87	A	47–48	−2.3 *c* 1.03, MeOH	5.6–6.1 (m, CH=), 5.1–5.4 (m, CH_2=), 4.59 (pert. d, CH_2O), 4.5 (br signal, α-CH), 1.41 (s, Boc)
2h[e]	H	All	89 (96)[25]	C	36.5–37 (36–37)[25]	–	7.34 (s, arom.), 5.6–6.1 (m, CH=), 5.1–5.5 (m, CH_2=), 5.13 (s, CH_2-Z), 4.5–4.7 (m, CH_2O), 4.00 (d, CH_2-Gly)
2i[e]	CH_3	All	84	Oil	–	–	7.35 (s, arom.), 5.8–6.0 (m, CH=), 5.2–5.4 (m, CH_2=), 5.11 (s, CH_2-Z), 4.5–4.7 (m, CH_2O), 4.3–4.5 (m, α-CH)
2j	H	Fm	67	C	72.5–73.5	–	7.20–7.81 (m, -Fm), 3.90–4.50 (m, CH_2 + 9 H, -Fm; CH_2-Gly), 1.46 (s, Boc)
2k[e]	H	Fm	81	C	93–94	–	7.21–7.81 (m, -Fm), 7.34 (s, arom.), 5.13 (s, CH_2-Z), 4.0–4.5 (m, CH_2 + 9 H, -Fm; CH_2-Gly)
2l[e]	CH_3	Fm	72 (65)[12]	C	96.5–97 (90–91)[12]	−29.8 *c* 1, MeOH (−38.4 *c* 1, MeOH)[12]	7.27–7.83 (m, -Fm), 7.36 (s, arom.), 5.13 (s, CH_2-Bzl), 4.1–4.6 (m, CH_2 + 9 H, -Fm; α-CH)
3a	H	Bzl	99 (95)[7]	A	31–31.5 (30.5–31)[7]	–	7.34 (s, arom.), 5.18 (s, CH_2-Bzl), 4.37 (s, CH_2-Gly), 1.46 (s, Boc)
3b	CH_3	Bzl	94	Oil	–	–	7.33 (s, arom.), 5.15 (s, CH_2-Bzl), 5.00 (q, α-CH), 1.46 (s, Boc)
3c	*i*-C_4H_9	Bzl	99	Oil	–	–	7.33 (pert. s, arom.), 5.15 (s, CH_2-Bzl), 4.96 (dd, α-CH), 1.44 (s, Boc)
3d	$CH_2C_6H_5$	Bzl	100	Oil	–	–	7.34 (s, arom.), 5.21 (dd, α-CH), 5.19 (s, CH_2-Bzl), 1.35 (s, Boc)
3e	CH_2OBzl	All	98	Oil	–	–	5.6–6.15 (m, CH=), 5.1–5.5 (m, CH_2=), 4.50–4.75 (m, CH_2O), 1.47 (s, Boc)
3f	CH_2CH_2-COOBzl	All	71	Oil	–	–	5.6–6.1 (m, CH=), 5.1–5.4 (m, CH_2=), 4.9 (br signal, α-CH), 4.61 (pert. d, CH_2O), 1.48 (s, Boc) (+ 1–2 % impurity at 1.43)
3g	$CH_2C_6H_4$-OBzl	All	99	Oil	–	–	5.7–6.2 (m, CH=), 5.1–5.5 (m, CH_2=), 5.0 (br signal, α-CH), 4.64 (pert. d, CH_2O), 1.39 (s, Boc) (+ up to 5 % impurities at 1.54 and 1.47)
3h[e]	H	All	99	Oil	–	–	7.35 (s, arom.), 5.6–6.1 (m, CH=), 5.1–5.4 (m, CH_2=), 5.24 (s, CH_2-Z), 4.61 (pert. d, CH_2O), 4.43 (s, CH_2-Gly), 1.46 (s, Boc)
3i[e]	CH_3	All	99	Oil	–	–	7.36 (s, arom.), 5.6–6.1 (m, CH=), 5.0–5.4 (m, CH_2=), 5.24 (s, CH_2-Z), 5.06 (q, α-CH), 4.51–4.58 (m, CH_2O), 1.45 (s, Boc)

contd.

Table 1. contd.

Compound	R	X	Yield (%)[a]	Recryst. solvent[b]	M.p. /°C[a]	$[\alpha]_D$[a]	^{1}H NMR ($CDCl_3$ rel. TMS) (δ-values)[c]
3j	H	Fm	40	Oil	–	–	7.20–7.81 (m, -Fm), 4.1–4.6 (m, CH_2 + 9 H, -Fm; CH_2-Gly), 1.51 (s, Boc)
3k[e]	H	Fm	94	Oil	–	–	7.20–7.81 (m, -Fm), 7.35 (s, arom.), 5.24 (s, CH_2-Z), 3.95–4.6 (m, CH_2 + 9 H, -Fm; CH_2-Gly), 1.46 (s, Boc)
3l[e]	CH_3	Fm	60	Oil	–	–	7.20–7.79 (m, -Fm), 5.24 (s, CH_2-Z), 4.92 (q, α-CH), 4.0–4.5 (m, CH_2 + 9 H, -Fm), 1.40 + 1.43 + 1.46 (3 s, Boc)
4g[f]	$CH_2C_6H_4$-OBzl	H	48	D	94–95	−100.1 *c* 1.02, MeOH	5.17 (dd, α-CH), 1.39 (s, Boc)
5a[f]	H	Np	84	E	80.5–81.5	–	8.28 (pert. d, 3′,5′), 7.31 (pert. d, 2′,6′), 4.62 (s, CH_2-Gly), 1.53 (s, Boc)
5b[f]	CH_3	Np	61	E	115.5–116.5	−70.6 *c* 1.08, DMF	8.27 (pert. d, 3′,5′), 7.30 (pert. d, 2′,6′), 5.21 (q, α-CH), 1.53 (s, Boc)
5c	*i*-C_4H_9	Np	51	Oil	–	–	8.26 (pert. d, 3′,5′), 7.28 (pert. d, 2′,6′), 5.16 (dd, α-CH), 1.52 (s, Boc)
5d[f]	$CH_2C_6H_5$	Np	72	E	122.5–124	−107.7 *c* 1.05, MeOH	8.28 (pert. d, 3′,5′), 7.31 (pert. d, 2′,6′), 5.41 (dd, α-CH), 1.42 (s, Boc)
5g[f]	$CH_2C_6H_4$-OBzl	Np	64	E	99–99.5	−102.7 *c* 1.01, MeOH	8.27 (pert. d, 3′,5′), 7.3 (obscured, 2′,6′), 5.36 (dd, α-CH), 1.42 (s, Boc)
5l[e]	CH_3	Np	52	Oil	–	–	8.23 (pert. d, 3′,5′), 7.32 (s, arom.), 7.17 (pert. d, 2′,6′), 5.28 (s, CH_2-Z), 5.28 (q, α-CH), 1.47 (s, Boc)
5m	H	NSu	74	F	183–184	–	4.69 (s, CH_2-Gly), 2.85 (s, CH_2CH_2), 1.52 (s, Boc)
5n[f]	CH_3	NSu	72	F	109–110	−2.0 *c* 1.07, dioxane	5.36 (q, α-CH), 2.82 (s, CH_2CH_2), 1.53 (s, Boc)

[a]Literature values are given in brackets. [b]A = light petroleum; B = *n*-heptane; C = ethyl acetate–light petroleum; D = diethyl ether–light petroleum; E = ethanol, F = 2-propanol. [c]The amino acid sidechains gave the expected shifts and multiplicities. All peaks integrated for the expected value. [d]An analytical sample was recrystallized three times to a constant m.p. Schnabel reported the m.p. 82 °C.[26] [e]Starting from a Z-amino acid. [f]Satisfactory elemental analysis, C ± 0.2, H ± 0.2, N ± 0.1. Pert. = perturbed.

Boc_2-Phe-Leu-OBzl (**6**). To a solution of Leu-OBzl (1.33 g, 6.0 mmol) in DMF (20 ml) was added solid Boc_2-Phe-ON*p* (2.43 g, 5.0 mmol) at room temperature and the reaction mixture was stirred for 2 days. After evaporation, the residue was dissolved in EtOAc (120 ml) and washed with 1 M $KHSO_4$ (3×30 ml), 1 M Na_2CO_3 (8×20 ml), and saturated NaCl solution (3×30 ml), and then dried. Evaporation gave a yellow oil that solidified when triturated with light petroleum to give the product **6** (2.15 g, 76%), pure by TLC (A, B); m.p. 62.5–63 °C; $[\alpha]_D$ −68° (*c* 0.98, MeOH). δ_H 7.43 + 7.21 (s + pert. s, 10 H, arom.), 6.2 (br signal, 1 H, NH), 5.16 (s, 2 H, CH_2, Bzl), 4.97 (dd, 1 H, α-CH, Phe), 4.68 (br signal, 1 H, α-CH Leu), 3.0–3.6 (m, 2 H, CH_2, Phe), 1.1–1.9 (m) and 1.40 (s, together 21 H, CH_2-CH, Leu, + Boc), 0.86 (pert. d, 6 H, CH_3, Leu). Amino acid analysis: $Phe_{1.03}Leu_{0.97}$. (Found: C, 67.1; H, 7.6; N, 5.0. $C_{32}H_{44}N_2O_7$ requires C, 67.57; H, 7.80; N, 4.95%).

Boc_2-Gly-Phe-Leu-OBzl (**7**). *A. Deprotection step.* Compound **6** (2.27 g, 4.0 mmol) was treated with TFA (10 ml) at 0 °C for 1 h. After evaporation, the residue was distributed between EtOAc (100 ml) and 1 M $NaHCO_3$ (50 ml). The organic layer was washed with 1 M $NaHCO_3$ (30 ml) and saturated NaCl solution (2×30 ml), and dried (Na_2SO_4). Evaporation gave the amine (**6a**) in quantitative yield as an oil, which was used directly in the synthesis of compound **7**.

B. Coupling step: synthesis with nitrophenyl ester. Boc_2-Gly-ON*p* (1.67 g, 4.2 mmol) was added to a solution of **6a** (4.0 mmol) in DMF (20 ml) at room temperature, and the yellow mixture was stirred overnight. After 21 h, TLC (B) indicated that all of the amine had been consumed. 2-Diethylaminoethylamine (DEAEA) (100 µl, ca. 0.2 equiv.) was added, and the solution was left for another 6 h. TLC (B) showed no remaining nitrophenyl ester and the DMF was evaporated. The product was worked up and

purified as described for **6** to give **7** as white crystals (2.09 g). TLC (B, E) showed two weak spots except for the main component. The product was chromatographed in CH_2Cl_2–acetone 15:1 to give pure **7** (TLC B, E) as a white powder (1.64 g, 64 %); m.p. 107–107.5 °C; $[\alpha]_D$ −16.4° (*c* 1.00, MeOH). δ_H 7.34 + 7.21 (s + s, 10 H, arom.), 6.0–6.5 (2 br signals, 2 H, 2 NH), 5.12 (s, 2 H, CH_2, Bzl), 4.4–4.9 (2 m, 2 H, 2 α-CH), 4.21 (2 H, CH_2, Gly), 2.8–3.3 (m, 2 H, CH_2, Phe), 1.2–1.9 (m) and 1.48 (s, together 21 H, CH_2CH, Leu, + Boc), 0.85 (pert. d, 6 H, CH_3, Leu). Amino acid analysis: $Gly_{0.99}Phe_{1.00}Leu_{1.01}$. (Found: C, 65.2; H, 7.3; N, 6.7. $C_{34}H_{47}N_3O_8$ requires C, 65.24; H, 7.57; N, 6.74 %).

Synthesis with the mixed anhydride method. A solution of Boc_2-Gly (275 mg, 1.0 mmol) in THF (3 ml) with *N*-methylmorpholine (110 µl, 1.0 mmol) was cooled to −15 °C. Activation was initiated by the addition of isobutyl chloroformate (132 µl, 1.0 mmol). After 60 s, a precooled suspension of **6a** (1.0 mmol) in DMF (2.0 ml), neutralized with TEA (140 µl, 1.0 mmol) was introduced. The temperature was maintained at −15 °C for 20 min, after which the reaction mixture was allowed to reach room temperature. After 5 h, the reaction was complete as could be judged by TLC (A). The normal work-up procedure gave a solid containing less than 2 % of contaminants (^{1}H NMR). Recrystallization from diethyl ether/light petroleum gave **7** as needles, 69 % yield, TLC (A, B); m.p. 105–106 °C, ^{1}H NMR and amino acid analysis were in accordance with the product obtained with the synthesis above.

Boc_2-Gly-Gly-Phe-Leu-OBzl (**8**). Compound **7** (2.02 g, 3.24 mmol) was deprotected, coupled to Boc_2-Gly-ON*p*, and worked up as described for the synthesis of **6**. The crude product was obtained as a white foam, which crystallized nicely when triturated with diethyl ether to give compound **8** (1.88 g, 86 %), pure by TLC (A, B); m.p. 135.5–136 °C; $[\alpha]_D$ −17.9° (*c* 1.01, MeOH). δ_H 6.8–7.4 (m), 7.17 (s) + 7.33 (s, together 13 H, arom. + 3 NH), 5.13 (s, 2 H, CH_2, Bzl), 4.4–4.9 (2 m, 2 H, 2 α-CH), 4.28 (s, CH_2, *Gly*Gly), 3.92 (d, 2 H, CH_2, *Gly*Phe), 2.8–3.2 (m, 2 H, CH_2, Phe), 1.1–1.9 (br signal) + 1.49 (s, together 21 H, CH_2CH, Leu, + Boc), 0.86 (pert. d, 6 H, CH_3, Leu). Amino acid analysis: $Gly_{2.00}Phe_{1.01}Leu_{0.99}$. (Found: C, 63.5; H, 7.3; N, 8.2. $C_{36}H_{50}N_4O_9$ requires C, 63.30; H, 7.38; N, 8.24 %.

*Attempted synthesis of Boc_2-Tyr(Bzl)-Gly-Gly-Phe-Leu-OBzl using Boc_2-Tyr(Bzl)-ON*p. Compound **8** (175 mg, 0.25 mmol) was deprotected as described above. The amine (**8a**) was dissolved in DMF (2 ml) and solid Boc_2-Tyr(Bzl)-ON*p* (155 mg, 0.26 mmol) was added. The reaction mixture was stirred at room temperature overnight. After 22 h, DEAEA (25 µl) was added and the solution was left for 4 h, when TLC (B) showed that no nitrophenyl ester remained. The product was processed as described for (**6**), to afford a yellow powder (166 mg) that gave two spots on TLC (A, D) in the ratio ca. 1:4. Amino acid analysis of the crude product gave $Tyr_{0.44}Gly_{1.39}Phe_{1.00}Leu_{0.99}$. Chromatography on silica in CH_2Cl_2–MeOH 95:5 separated two components. The first-eluted compound **10** (ca. 70 %), pure by TLC (A, D), gave amino acid analysis $Tyr_{0.20}Gly_{1.27}Phe_{0.99}Leu_{1.01}$ and was assigned a hydantoin structure (see the Discussion); the other one (minor component) analysed for $Tyr_{0.90}Gly_{1.94}Phe_{1.00}Leu_{0.99}$ (**9**). This experiment was also carried out with a slight excess of **8a** in the absence of DEAEA, to give **9** and **10** in the same ratio (^{1}H NMR spectroscopy).

Boc_2-Tyr(Bzl)-Gly-Gly-Phe-Leu-OBzl (**9**). *DCC-coupling experiment.* Compound **8** (341 mg, 0.50 mmol) was treated with TFA (3 ml) at 0 °C for 1 h. The residue was dissolved in DMF (3 ml) and the solution was neutralized with triethylamine. Boc_2-Tyr(Bzl)-OH (235 mg, 0.50 mmol) and *N*-hydroxysuccinimide (115 mg, 1.0 mmol) were added, and the reaction mixture was cooled to 0 °C before the introduction of a solution of DCC in DMF (2.0 ml, 0.25 M). The reaction was stirred at room temperature overnight. TLC (A) indicated that all of the Boc_2-Tyr(Bzl)-OH had been consumed. After the urea had been filtered off, the reaction mixture was dissolved in EtOAc and washed and dried. Evaporation gave a sticky solid. To remove any remaining dicyclohexyl urea (TLC A, D), the protected pentapeptide was chromatographed on silica in CH_2Cl_2–MeOH 95:5 to give pure **9** (TLC A, D) as an oil in 37 % yield; ^{1}H NMR showed all the expected peaks, amino acid analysis $Tyr_{0.96}Gly_{2.00}Phe_{1.03}Leu_{1.01}$. (Found: C, 66.3; H, 7.2; N, 7.3. $C_{52}H_{65}N_5O_{11}$ requires C, 66.70; H, 7.00; N, 7.51 %).

Another component, eluting before **9**, was isolated and identified by ^{1}H and ^{13}C NMR spectroscopy as Boc_2-Tyr(Bzl)-OSu (**11**) (130 mg, 0.23 mmol). In a duplicate experiment, 50 % **9** was obtained.

Tyr-Gly-Gly-Phe-Leu (**12**). The fully protected pentapeptide (77 mg, 82 µmol) was dissolved in 80 % AcOH. 50 mg of 5 % Pd/C were introduced and the solution was hydrogenated. The reaction was monitored by removal of a small sample for treatment with HCl in dioxane (see below), for subsequent analysis by HPLC (with 0.1 M triethylammonium formate pH 2.8 (A) and MeOH (B) as the solvents, using a 30 min gradient 10–60 % B; flow rate 1.0 ml min^{-1}; detection at 280 nm). After 5 h, the catalyst was filtered off and the filtrate was taken to dryness. The residue was treated with 2.2 M HCl in dioxane (10 ml) at room temperature for 1 h. After evaporation, the product was chromatographed on a G-15 column (1.4×140 cm) in 50 % AcOH. This system gave one symmetrical peak. Lyophilization furnished the peptide as a white fluffy powder (32 mg, 70 %); pure by TLC (F, G, H) and HPLC (system as above); $[\alpha]_D$ −24.3° (*c* 1.00, DMF; lit.[21] −23.4°); amino acid analysis: $Tyr_{0.99}Gly_{1.98}Phe_{1.02}Leu_{1.02}$.

Experiment with Boc_2-Tyr(Bzl)-OSu. Deprotected **8** (26 μmol) was dissolved in DMF (300 μl) and neutralized with triethylamine (ca. 10 μl) before introduction of **11** (21 μmol in 200 μl DMF). The reaction mixture was stirred at room temperature. After 30 min, some succinimide ester remained (TLC A) but after 4 h this material had been consumed. The mixture was left at room temperature overnight, then dissolved in EtOAc and washed and dried. TLC with the appropriate references showed that again both the pentapeptide and the hydantoin were formed as described above for the corresponding ON*p*-ester. The ratio as estimated by ^{1}H NMR spectroscopy was 1:1. Amino acid analysis: $Tyr_{0.42}Gly_{1.59}Phe_{1.01}Leu_{0.99}$.

1-Boc-5-methylimidazolidine-2,4-dione (1-Boc-5-methylhydantoin) (**13**). Boc_2-Ala-ON*p* (200 mg, 0.5 mmol) was treated with a solution of ammonia in methanol, saturated at 0 °C (2.0 ml) overnight. The volatile components were evaporated and the residue was chromatographed in CH_2Cl_2–acetone 4:1 to give the product as a white powder in 84 % yield; m.p. 135.5–136 °C; $[\alpha]_D$ + 30.5° (*c* 1.00, MeOH); δ_H 8.54 (br s, 1 H, NH), 4.43 (q, 1 H, CH), 1.60 (d, 3 H, CH_3), 1.56 (s, 9 H, Boc); δ_C 171.7 (C-4), 151.6 (CO, Boc), 148.2 (C-2), 84.5 [$C(CH_3)_3$], 57.1 (CH), 28.0 [$C(CH_3)_3$], 16.8 (CH_3CH). (Found: C, 50.5; H, 6.6; N, 12.9. $C_9H_{14}N_2O_4$ requires C, 50.43; H, 6.58; N, 13.13 %).

1-Boc-5-isobutylimidazolidine-2,4-dione (1-Boc-5-isobutylhydantoin) (**14**). Boc_2-Leu-ON*p* was reacted similarly and chromatographed in CH_2Cl_2–acetone 15:1 to give a pure oil (^{1}H NMR). Trituration with light petroleum gave a white powder, m.p. 113–115 °C; δ_H 8.19 (br s, 1 H, NH), 4.44 (dd, 1 H, H-5), 2.0–1.6 (m, 3 H, CH_2CH), 1.56 (s, 9 H, Boc), 0.96 (d, 6 H, CH_3); δ_C 171.3 (C-4), 151.6 (CO, Boc), 148.1 (C-2), 84.5 [$C(CH_3)_3$], 59.7 (C-5), 28.0 [$C(CH_3)_3$], 39.3, 23.9, 23.6 and 22.1 (other C). (Found: C, 55.9; H, 8.0; N, 10.8. $C_{12}H_{20}N_2O_4$ requires C, 56.21; H, 7.86; N, 10.97 %).

1-Boc-3-benzyl-5-methylimidazolidine-2,4-dione (1-Boc-3-benzyl-5-methylhydantoin) (**15**)- Boc_2-Ala-ON*p* (820 mg, 2.0 mmol) was dissolved in methanol (8 ml) and treated with benzylamine (4 ml) overnight. The reaction mixture was partitioned between ethyl acetate (20 ml) and 1 M $KHSO_4$ (10 ml) and the organic layer was washed with 1 M $KHSO_4$ (2×5 ml), 1 M Na_2CO_3 (7×5 ml) and saturated NaCl solution (3×5 ml), and dried. Evaporation gave an oil which crystallized from diethyl ether/light petroleum to give the product (448 mg, 74 %); m.p. 69.5–70.5 °C; $[\alpha]_D$ −0.4° (*c* 1.00, MeOH); δ_H 7.45–7.25 (m, 5 H, arom.), 4.67 (s, 2 H, CH_2), 4.38 (q, 1 H, H-5), 1.56 (d, 3 H, CH_3), 1.55 (s, 9 H, Boc); δ_C 171.4 (C-4), 151.9 (CO, Boc), 148.4 (C-2), 135.5, 128.9, 128.7, 128.2 (arom.), 84.3 [$C(CH_3)_3$], 55.8 (C-5), 42.6 (CH_2), 28.0 [$C(CH_3)_3$], 16.8 (CH_3CH). (Found: C, 63.2; H, 6.7; N, 9.2. $C_{16}H_{20}N_2O_4$ requires C, 63.12; H, 6.62; N, 9.24 %).

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