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Synthesis and Application of *N,N*-Bis-(1-adamantyloxycarbonyl) Amino Acids

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The preparation of novel bis-(1-adamantyloxycarbonyl) amino acid derivatives has been undertaken and their properties studied. Among them, the *p*-nitrophenyl esters were subsequently applied to the stepwise synthesis of Leu-enkephalin. In the last coupling step, some hydantoin formation was encountered but it could be nearly completely overcome by working with more concentrated solution. The preparation of a tyrosine derivative presented special problems owing to the existence of the phenolic group in the precursor. The relative stability of 1-adamantyloxycarbonyl as *N*- and *O*-protecting groups was also studied.

Recently it was demonstrated that *N,N*-bis-Boc-amino acids[1] are stable, easily made derivatives of practical application and that they can also be used in peptide synthesis.[2] In that context it was noticed that they are more prone to hydantoin formation than the corresponding mono-Boc-derivatives. To gain further insight into this phenomenon and in continuation of some preliminary work on the adamantyloxycarbonylation of amides and carbamates,[3] the preparation of a number of *N,N*-bis-1-adamantyloxycarbonyl amino acids, $Adoc_2$-amino acids, has now been undertaken and compared with the corresponding Boc_2-derivatives.

This paper also reports on the specific behaviour of *N*-mono-protected tyrosine caused by the presence of its competing functional groups under the experimental conditions used. Finally, the applicability of $Adoc_2$-amino acids is demonstrated in the synthesis of Leu-enkephalin.

Results and discussion

Synthesis of $Adoc_2$-amino acids. α-Mono Adoc-amino acids have previously been prepared by a straightforward procedure from 1-adamantyl chloroformate in water–dioxane in up to 89% yields but for most non-hydrophobic amino acids the yields were quite modest.[4] In this work such derivatives **1** were prepared by reacting the corresponding amino acid benzyl ester tosylate salts with 1-adamantyl chloroformate under anhydrous conditions. The introduction of the second α-Adoc group into **1** to give **2** was accomplished by the use of $Adoc_2CO_3$[5] as the acylating reagent under DMAP catalysis (Scheme 1).[6] $Adoc_2CO_3$ could be used directly under anhydrous conditions similarly to the corresponding dicarbonate.[3] Under these circumstances the yields were nearly quantitative (92–98%) except for tyrosine. The free acids **3** were obtained by standard catalytic hydrogenolysis. They could subsequently be converted into *p*-nitrophenyl esters **4**.

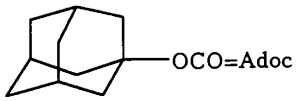
H_2N-CH(R)-COOBzl

$\xrightarrow{i}$ Adoc-HN-CH(R)-COOBzl **1**

$\xrightarrow{ii}$ $Adoc_2N$-CH(R)-COOBzl **2**

$\xrightarrow{iii}$ $Adoc_2N$-CH(R)-COOH **3**

$\xrightarrow{iv}$ $Adoc_2N$-CH(R)-COON*p*



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Scheme 1. Reagents: i, Adoc-Cl; ii, $Adoc_2CO_3$/DMAP; iii, H_2–Pd/C; iv, HON*p*/DCC.

Yields and physical data for all new compounds **1–4** are collected in Table 1.

The IR carbonyl region of these derivatives exhibited an additional absorption band around 1765 (glycine) and 1750 cm^{-1} (leucine and phenylalanine) (Table 2). This corresponds to an 8–10 cm^{-1} frequency increase compared with their Boc_2-analogues.[2] In the ^1H NMR spectra, characteristic signals of the Adoc-groups were observed for non-aromatic amino acids as singlets at 2.1 and 1.6 ppm (Table 2). For aromatic derivatives, the signal at low field was split into two or three (e.g. $Adoc_2$Phe: 2.18 and 2.04). α-Protons were shifted down-

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Table 1. Yields and physical data for novel Adoc-amino acid derivatives.

Compd.	R	Yield (%)	M.p./°C (solvent)[a]	$[\alpha]_D^{25}$ (c)[b]
1a	H	78	74–75 (EE/LP)	—
1b	C_4H_9	87	Oil	−14.3 (0.23)
1c	CH_2Ph	84	Oil	+7.6 (0.50)
1d	$CH_2C_6H_4OH(p)$	85	47–49 (EE/LP)	−6.0 (0.20)
1e	$CH_2C_6H_4OAdoc(p)$	79	54–55 (EE/LP)	−6.4 (0.11)
2a	H	98	94–95 (EE/LP)	—
2b	C_4H_9	93	Oil	−23.5 (0.20)
2c	CH_2Ph	96	Oil	−53.5 (0.23)
2d	$CH_2C_6H_4OAdoc(p)$	83	76–77 (EE/LP)	−52.7 (0.11)
3a	H	91	198–199 (EE)	—
3b	C_4H_9	95	88–89 (EE)	−23.8 (0.02)
3c	CH_2Ph	71	178–179 (EE)	−74.5 (0.21)
3d	$CH_2C_6H_4OAdoc(p)$	79	174–175 (toluene)	−62.5 (0.06)
4a	H	78	150–151 (EtOH)	—
4c	CH_2Ph	72	182–183 (EtOH)	−92.5 (0.15)
4d	$CH_2C_6H_4OAdoc(p)$	61	223–224 (EtOH)	−81.4 (0.14)

[a] Abbreviations used for recrystallization solvents: EE = diethyl ether, LP = light petroleum. [b] $CHCl_3$.

field by 0.1–0.6 ppm in comparison with their mono Adoc-analogues.

Mono- and bis-Adoc amino acid benzyl esters were difficult to obtain as crystals and when crystallization occurred, the resulting products had low melting points (47 to 97°C) whereas the corresponding free acids readily crystallized with higher melting points (up to 199°C). Derivatives obtained in this way were readily soluble in most organic solvents but *not* in alcohols like ethanol or methanol, in which they instead tended to form gums, suggesting that the second Adoc-group contributed to increase the hydrophobicity of these derivatives.

Experiments with tyrosine derivatives. When an excess of 1-adamantyl chloroformate was allowed to react with tyrosine benzyl ester, the expected compound **1d** was isolated in 84% yield along with 16% of Adoc-Tyr(Adoc)-OBzl (**1e**). The reactivity of the tyrosine phenolic group was also observed during the introduction of the second Adoc-group into the product **1d** using $Adoc_2CO_3$/DMAP (Scheme 2).

Monitoring the latter reaction by ^1H NMR spectroscopy, residual NH-groups could still be detected in the complex mixture after 24 h. Even when two equivalents of $Adoc_2CO_3$ were used and the reaction time extended to 48 h, no improvement was noticed. When the mixture was worked up, compound **1e** was identified as the major component, indicating that, in this case, the phenolic group is more reactive than is the urethane function. The minor component isolated corresponded to the tris-Adoc derivative **2d**.

In subsequent experiments starting with compound **1d**, one equivalent of $Adoc_2CO_3$ was used in the preparation of **1e** which, after isolation, was converted into $Adoc_2$-Tyr(Adoc)-OBzl (**2d**) in satisfactory yield. Various attempts to introduce two Adoc-groups simultaneously into **1d** by a one-pot procedure resulted in intractable mixtures. The reason for this is not well understood at present.

Adoc-Tyr-OBzl
1d
$\xrightarrow{i}$ Adoc-Tyr(Adoc)-OBzl
1e
$\underset{ii}{\overset{i}{\rightleftharpoons}}$ $Adoc_2$Tyr(Adoc)-OBzl
2d

Scheme 2. Reagents: i, $Adoc_2CO_3$/DMAP; ii, TFA.

From the above experiments it can be concluded that the phenolic group of tyrosine not only interferes with the $Adoc_2CO_3$/DMAP reagent in its reaction with NH functions but can also itself be protected almost quantitatively in this way. It is worth mentioning that the Boc-group has been successfully introduced into phenols by the use of Boc_2O under phase transfer catalysis conditions[7] but our experiment constitutes the first case of this type in which the reactivities of phenols and urethanes have been compared.

Our attention was then focussed on the relative stability of the Adoc-groups in the tris-Adoc derivative **2d**. For this purpose it was treated with trifluoroacetic acid (TFA), as a result of which compound **1e** was isolated in 70% yield, showing that the Adoc–O bond is more stable to TFA than the second Adoc–N bond. Similarly easy cleavage of imidodicarbonates has previously been reported by Åkermark *et al.*[8]

Synthesis of Leu-enkephalin. Bearing in mind the increased risk of hydantoin formation induced by the Boc_2-amino acids,[2] to investigate the applicability to

Table 2. FT-IR and ^{1}H NMR data on compounds **1**–**4**.

Compd.	FT-IR[a] (ν/cm^{-1})	^{1}H NMR
1a	3337 1753 1678	7.35 (5 H, Ph), 5.18 (2 H, OCH_2), 5.01 (1 H, NH), 3.94 (d, 2 H, J=5.7 Hz, CH_2Ph), 2.09 and 1.64 (15 H, Adoc)
1b	3373 1742 1714	7.35 (5 H, Ph), 5.15 (2 H, OCH_2), 4.88 (1 H, NH), 4.33 (1 H, H_α), 2.08 and 1.64 (15 H, Adoc), 1.29 (3 H), 0.92 (d, 6 H, J=5 Hz, 2 × Me)
1c	3373 1746 1698	7.86 (5 H, Ph), 7.73 (5 H, Ph), 5.65 (2 H, OCH_2), 5.45 (1 H, NH), 5.08 (1 H, H_α), 3.60 (m, 2 H, H_β), 2.11, 2.10 and 1.73 (15 H, Adoc)
1d	3374 1746 1712 1688	7.33 (5 H, Ph), 6.83 and 6.63 (d, 4 H, J=8.7 Hz), 5.13 (2 H, OCH_2), 5.05 (1 H, NH), 4.52 (m, 1 H, H_α), 2.98 (m, 2 H, H_β), 2.06 and 1.62 (15 H, Adoc)
1e	3387 1755 1715	7.33 (5 H, Ph), 7.02 (m, 4 H, Tyr), 5.12 (2 H, OCH_2), 4.98 (1 H, NH), 4.67 (m, 1 H, H_α), 3.06 (m, 2 H, H_β), 2.19, 2.06 and 1.66 (30 H, Adoc)
2a	1764 1709	7.35 (5 H, Ph), 5.18 (2 H, OCH_2), 4.36 (2 H, CH_2), 2.10 and 1.64 (30 H, Adoc)
2b	1747 1703	7.34 (5 H, Ph), 5.15 (2 H, OCH_2), 4.95 (m, 1 H, H_α) 2.08 and 1.63 (30 H, Adoc), 1.8–1.3 (m, 3 H), 0.93 (d, 6 H, J=6.1 Hz, 2 × Me)
2c	1746 1698	7.33 (5 H, Ph), 7.26 (m, 5 H, Ph), 5.18 (2 H, OCH_2), 5.16 (dd, 1 H, J=9.4 and 5.5 Hz, H_α), 3.35 (dd, 1 H, J=14 and 5.5 Hz, H_β), 3.20 (dd, 1 H, J=14 and 9.4 Hz, H_β), 2.11 and 1.64 (30 H, Adoc)
2d	1754 1698	7.34 (5 H, Ph), 7.12 (m, 4 H, Tyr), 5.18 (2 H, OCH_2), 5.14 (m, 1 H, H_α), 3.30 (m, 2 H, H_β), 2.18, 1.99 and 1.61 (30 H, Adoc)
3a	3200 1721	10.73 (1 H, OH), 4.38 (2 H, CH_2), 2.15 and 1.66 (30 H, Adoc)
3b	3378 1753 1722	4.9–4.8 (m, 1 H, H_α), 2.15–1.66 (33 H, 2 × Adoc + 3 H), 0.93 (d, 6 H, J=6.5 Hz, 2 × Me)
3c	3200 1782 1733 1679	7.31–7.17 (5 H, Ph), 5.21 (dd, 1 H, J=5.1 and 10.0 Hz, H_α), 3.42 (dd, 1 H, J=5.1 and 14.0 Hz, H_β), 3.23 (dd, 1 H, J=10.0 and 14.0 Hz, H_β), 2.15, 2.04 and 1.63 (30 H, Adoc)
3d	3318 1753 1725	7.19 and 7.08 (d, 4 H, 8.6 Hz), 5.21 (dd, 1 H, J=5.2 and 9.8 Hz, H_α), 3.40 (dd, 1 H, J=5.2 and 14.1 Hz, H_β), 3.22 (dd, 1 H, J=9.8 and 14.1 Hz, H_β), 2.20, 2.18 and 1.67 (*O*-Adoc), 2.15, 2.05 and 1.63 (*N*-Adoc)
4a	1756 1707	8.24 and 7.28 (d, 4 H, J=9.2 Hz), 4.76 (2 H, CH_2), 2.17, 2.07 and 1.65 (30 H, Adoc)
4c	1765 1733 1674	8.24 and 7.29 (d, 4 H, J=9.4 Hz), 7.37 (5 H, Ph), 5.28 (m, 1 H, H_α), 3.60 (m, 2 H, H_β), 2.17 and 1.67 (30 H, Adoc)
4d	1758 1734	8.24 and 7.28 (d, 4 H, J=9.2 Hz), 7.19 and 7.08 (d, 4 H, J=8.7 Hz), 5.38 (dd, 1 H, J=5.8 and 8.9 Hz, H_α), 3.36 (m, 2 H, H_β), 2.19, 2.09 and 1.66 (45 H, Adoc)

[a] FT-IR spectra of solids and oils were run as KBr pellets and films, respectively.

peptide synthesis of these new derivatives, we again selected Leu-enkephalin[9] as the model peptide. Its two consecutive glycines[10] seem further to magnify this side reaction. The synthesis was carried out from the carboxy end using *p*-nitrophenyl esters.

Up to the protected tetrapeptide, the syntheses proceeded without difficulty and using a small excess of active esters the reactions were complete in DMF[11] within 24–48 h, giving crystalline intermediates (**5**–**7**) in relatively good yields (63–72%). Signals characteristic of the bis-Adoc groups were observed in the ^{1}H NMR spectra of all protected peptides (30 H, δ 2.15–1.60, cf. also second paragraph under Results and discussion). This supported the fact that all coupling reactions had proceeded without loss of the amino-protecting group.

The fully protected pentapeptide (**8**) was successfully obtained in three independent experiments under slightly different conditions. In this context it should be mentioned that, in order to avoid the use of any extra base that might induce side reactions, the deprotected tetrapeptide TFA salt was used without further neutralization.

In the first attempt, using 1 equiv. of the deprotected tetrapeptide and 1.3 equiv. of $Adoc_2$-Tyr(Adoc)-ON*p* (**4d**) in 3 ml of DMF, 69% of the expected peptide **8** was isolated. In this case the minor components were not collected. When 1 equiv. of the tetrapeptide was coupled with 1.2 equiv. of **4d** in 10 ml of DMF, 59% of the pentapeptide **8** was isolated along with 29% of the corresponding hydantoin[12] **9**. Finally, the same operation with stoichiometric amounts of the reactants in 3 ml of DMF gave 67% of the desired product **8** with only 2% of the hydantoin **9**.

These results suggest that the concentration of the reactants may be important in inducing hydantoin formation even though the cage nature of the Adoc substituents perhaps also minimizes the bulkiness of these derivatives compared with the branched *tert*-butyl residue in Boc-amino acid derivatives.

Unlike Boc_2-amino acids which seem to enhance the risk of hydantoin formation, $Adoc_2$ analogues gave insignificant amounts of hydantoin in a more concentrated solution. Owing to their extreme hydrophobicity, they might be useful synthons in peptide synthesis, particularly to increase the solubility in organic solvents.[13]

Experimental

All amino acids used (except glycine) were of L-configuration. M.p.s. were determined on a Gallenkamp apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ using a 1 dm cell in a Perkin-Elmer 141 or 241 polarimeter at 25°C. TLC analyses were performed on 0.25 mm thick, precoated silica plates (Merck DC-Fertigplatten, Kieselgel 60 F_{254}). Spots were visualized by inspection under UV light at 254 nm and after brief heating, by exposure to Cl_2 followed by dicarboxidine spray. Acetonitrile (analytical grade), used as the reaction medium, was dried over molecular sieves (4A). All

glassware was dried in an open flame immediately before use. ^{1}H NMR spectra were recorded on a Jeol FX90Q at 90 MHz or a Jeol JNM-EX270 instrument at 270 MHz (Me_4Si, $CDCl_3$). FT-IR spectra were recorded on a Mattson Polaris spectrometer. Amino acid analyses were performed by the Central Amino Acid Analysis Laboratory, Institute of Biochemistry, Uppsala and elemental analyses by Mikro Kemi AB, Uppsala, Sweden.

Preparation of 1-adamantyl chloroformate. To a 20% solution of phosgene in toluene (150 ml, 30 g) was added a solution of 1-adamantanol (7.05 g, 46.3 mmol) and pyridine (7.30 g, 92.3 mmol) in toluene (100 ml) dropwise, with stirring and cooling in an ice-bath. When the addition was complete, the reaction mixture was stirred for 1 h at 0°C and 4 h at room temperature, when the precipitate was filtered off and the filtrate poured into ice–water. The toluene layer was collected, dried (Na_2SO_4) and evaporated *in vacuo* to afford 7.60 g (77%) of a semisolid white residue, which was used as such in the subsequent syntheses.

General procedure for the preparation of N^{α}*-Adoc-amino acid benzyl esters.* To the amino acid benzyl ester tosylate salt (1 mequiv.), dissolved in dry pyridine (3 ml/mequiv.) and $CHCl_3$ (10 ml/mequiv.), was added dropwise over 1 h with stirring and cooling (−5°C) Adoc-Cl (1.5 mequiv.) in $CHCl_3$ (10 ml/equiv.). The mixture was kept until TLC (light petroleum–Et_2O 2 : 1) indicated complete reaction (generally 6 h was enough). After attaining room temperature, the reaction mixture was washed several times with brine and dried (Na_2SO_4). Evaporation gave oils which were purified by column chromatography (CH_2Cl_2–acetone 97 : 3) on silica. The corresponding tyrosine derivative (**1d**) was prepared from the free benzyl ester.

Adoc-Tyr-OBzl (**1d**). This compound was made from Tyr-OBzl (3.00 g, 11 mmol) and Adoc-Cl (3.60 g, 16.8 mmol) essentially as described above. After work-up and column chromatography compound **1d**, obtained as an oil, which by treatment with ether could be converted into an amorphous solid (4.20 g, 84%), was isolated along with **1e** (1.10 g, 16%).

General procedure for the preparation of N^{α},N^{α}*-bis-Adoc amino acid benzyl esters.* To a solution of Adoc-amino acid benzyl ester (1 mequiv.) and DMAP (0.1 mequiv.) in dry CH_3CN (3–10 ml mmol^{-1}), was added $Adoc_2CO_3$[5] (1.1–1.5 mequiv.) and the reaction mixture was stirred at room temperature for 3–16 h with TLC monitoring (CH_2Cl_2 or CH_2Cl_2–acetone 100 : 2). The solvent was evaporated off and the residue dissolved in Et_2O (50 ml), washed twice with 1 M $KHSO_4$, 1 M $NaHCO_3$ and brine (30 ml each time) and dried ($MgSO_4$). After evaporation to dryness, the residue was chromatographed on silica (CH_2Cl_2–acetone 100 : 2).

Adoc-Tyr(Adoc)-OBzl (**1e**). This compound was prepared from **1d** (1.00 g, 2.23 mmol) and $Adoc_2CO_3$ (1.00 g, 2.4 mequiv.) in the presence of DMAP (30 mg, 0.2 mequiv.). Work-up as previously gave 1.10 g of **1e** (79%).

Partial deprotection of $Adoc_2$*-Tyr(Adoc)-OBzl.* Compound **2d** (15 mg, 18 μmol) was treated with an equivalent amount of TFA in CH_2Cl_2 (1 ml) and the reaction monitored by TLC. After 48 h only traces of the starting material had been consumed. Addition of 0.5 equiv. of TFA, allowing a reaction time of 24 h with TLC monitoring twice, followed by reaction for an additional 48 h indicated only traces of the starting material. The solvent was evaporated off and the residue distributed between EtOAc (15 ml) and 1 M $NaHCO_3$ (10 ml). After chromatography on silica (CH_2Cl_2), 10 mg (78%) of pure compound **1e** could be isolated, the ^{1}H NMR spectrum of which agreed with that of the authentic material.

General procedure for the preparation of free $Adoc_2$*-amino acids.* $Adoc_2$-amino acid-OBzl, dissolved in EtOH or toluene (20 ml g^{-1}), after flushing with nitrogen was hydrogenolysed over 5% Pd–C with TLC monitoring (CH_2Cl_2–acetone 100 : 2) until no more starting material remained (6–18 h). The catalyst was filtered off, the solution evaporated and the product chromatographed on silica (CH_2Cl_2–acetone–HOAc 40 : 10 : 1) to afford the pure material.

General procedure for the preparation of $Adoc_2$*-amino acid p-nitrophenyl esters.* The free $Adoc_2$-amino acids [or $Adoc_2$-Tyr(Adoc)] as obtained from the hydrogenolyses of the corresponding benzyl esters above were used without further purification. $Adoc_2$-amino acid (1 equiv.) and *p*-nitrophenol (1.2 equiv.) were dissolved in EtOAc (5–15 ml mmol^{-1}) with stirring in an ice bath and a solution of DCC (1 equiv.), also in EtOAc (5–10 ml mmol^{-1}), was added and allowed to react for 30 min at 0°C and then for 24 h at room temperature. The precipitate was filtered off and the filtrate evaporated to dryness. The yellow residue was directly purified by chromatography on silica (CH_2Cl_2–acetone 100 : 1) to give the pure material which could be recrystallized from alcohol or Et_2O.

Peptide synthesis.

$Adoc_2$-Phe-Leu-OBzl (**5**). Compound **4c** (625 mg, 0.95 mmol) was added to a solution of Leu-OBzl (110 mg, 0.5 mmol) in DMF (10 ml) and left for 24 h when TLC ($CHCl_3$–acetone 100 : 3) indicated that the reaction was complete. The solvent was removed using an oil pump and the residue was dissolved in EtOAc (30 ml) and successively washed with 1 M $KHSO_4$, 1 M Na_2CO_3 (3 times) and brine (20 ml each) and dried ($MgSO_4$). Chromatography on silica (CH_2Cl_2–acetone 100 : 2) afforded 260 mg (72%) of a white solid; m.p. 51–52°C

(from Et_2O–light petroleum); $[\alpha]_D^{25} - 60.9$ (*c* 0.049, $CHCl_3$) ^1H NMR: δ 7.348 (s, 5 H, $PhCH_2$), 7.217 (m, 5 H, $PhCH_2$), 6.211 (br d, 1 H, $J = 8.1$ Hz, CONH), 5.160 (s, 2 H, OCH_2Ph), 4.950 (m, 1 H, H_α-Leu), 4.760 (m, 1 H, H_α-Phe), 3.366 (m, 2 H, H_β-Phe), 2.141, 2.033, 1.628 (3 s, together 30 H, Adoc), 1.800–1.325 (m, 3 H, $\beta + \gamma$-CH-Leu), 0.891 (d, 6 H, $J = 5.2$, Me-Leu); IR (KBr): 3375 (NH), 1740, 1691 (CO) cm^{-1}; amino acid analysis: $Phe_{1.00}$ and $Leu_{1.00}$ (Found: C, 72.6; H, 8.0; N, 4.0. $C_{44}H_{56}N_2O_7$ requires C, 72.9; H, 7.8; N, 3.9%).

$Adoc_2$-Gly-Phe-Leu-OBzl (**6**). Compound **5** was deprotected by treating this material (230 mg) with TFA (3 ml) under stirring for 1.5 h. Evaporation to dryness and distribution between EtOAc (30 ml) and 1 M $NaHCO_3$ (20 ml), further washing twice with $NaHCO_3$ (20 ml) and brine (30 ml) and drying (Na_2SO_4) followed by evaporation left an oil (80 mg). This was used as such in the following coupling step with **4a** (120 mg, 0.218 mmol) in DMF (4 ml). Work-up as for **5** (chromatography in CH_2Cl_2–acetone 100 : 3) gave pure **6** (114 mg, 67%); m.p. 94–95°C (Et_2O–light petroleum); $[\alpha]_D^{25} - 19.1$ (*c* 0.115, $CHCl_3$); ^1H NMR: δ 7.345 (s, 5 H, CH_2Ph), 7.226 (s, 5 H, CH_2Ph), 6.306 (d, 1 H, $J = 7.6$ Hz, NH), 6.159 (d, 1 H, $J = 8.3$ Hz, NH), 5.124 (s, 2 H, OCH_2Ph), 4.633 (m, 2 H, H_α-Leu + Phe), 4.191 (s, 2 H, Gly), 3.195 (dd, 1 H, $J = 13.6$ and 5.5, H_β-Phe), 2.951 (dd, 1 H, $J = 13.6$ and 7.5, H_β-Phe), 2.116 and 1.610 (s, 30 H, 2 Adoc), 1.5–1.3 (m, 3 H, $H_{\beta+\gamma}$-Leu), 0.855 (d, 6 H, $J = 6$ Hz, $2 \times CH_3$-Leu); IR (KBr): 3295 (NH), 1745, 1694, 1654 (CO) cm^{-1}; amino acid analysis: $Gly_{0.97}Phe_{1.03}Leu_{1.01}$. (Found: C, 70.6; H, 7.8; N, 5.2. $C_{46}H_{59}N_3O_8$ requires C, 70.5; H, 7.8; N, 5.3%).

$Adoc_2$-Gly-Gly-Phe-Leu-OBzl (**7**). The deprotected tripeptide (72.4 mg, 0.17 mmol) was allowed to react with **4a** (140 mg, 0.25 mmol) in DMF (4 ml) for 48 h when TLC (toluene–CH_3CN 2 : 1) indicated complete reaction. Work-up as for **5** (chromatography in CH_2Cl_2–acetone 10 : 1) gave a residue which crystallized from Et_2O–light petroleum (90 mg, 63%); m.p. 175–176°C; $[\alpha]_D^{25} - 11.22$ (*c* 0.129, $CHCl_3$); ^1H NMR: δ 7.341 (s, 5 H, CH_2Ph), 7.204 (s, 5 H, CH_2Ph), 6.725 (d, 1 H, $J = 7.6$ Hz, NH), 6.475 (d, 1 H, $J = 7.3$ Hz, NH), 5.124 (s, 2 H, OCH_2Ph), 4.523 (m, 2 H, H_α-Leu + Phe), 4.240 (s, 2 H, Gly), 3.859 (d, 2 H, $J = 5.5$ Hz, Gly), 3.107 (m, 2 H, H_β-Phe), 2.150 and 1.645 (s, 30 H, 2 Adoc), 1.8–1.286 (m, 3 H, $H_{\beta+\gamma}$-Leu), 0.876 (d, 6 H, $J = 5.9$ Hz, $2 \times CH_3$-Leu); IR (KBr): 3411 and 3299 (NH), 1746, 1694 and 1648 (CO) cm^{-1}; amino acid analysis: $Gly_{1.99}Phe_{1.01}Leu_{1.00}$. (Found: C, 68.4; H, 7.4; N, 6.6. $C_{48}H_{62}N_4O_9$ requires C, 68.5; H, 7.7; N, 6.7%).

$Adoc_2$-Tyr(Adoc)-Gly-Gly-Phe-Leu-OBzl (**8**). This peptide was synthesized in three separate experiments (A–C) at different concentrations. Deprotection of compound **7** (370 mg) with TFA at 0°C, essentially as before but using $CHCl_3$ instead of EtOAc for liberation of the free base, gave a semisolid (250 mg) which was used in the subsequent experiments.

(A) The free tetrapeptide base (77 mg, 0.16 mmol) was coupled with **4d** (178 mg, 0.213 mmol) in DMF (3 ml) for 48 h, when TLC (toluene–CH_3CN 2 : 1 or CH_2Cl_2–MeOH 9 : 1) showed that the coupling was complete. Work-up as previously, including chromatography of the crude product on silica (CH_2Cl_2–MeOH 50 : 1), gave compound **8** (130 mg, 69%) as a white powder (no attempt was made to isolate and characterize the side product in this case); m.p. 124–125°C (Et_2O–light petroleum); $[\alpha]_D^{25} - 76.3$ (*c* 0.08, $CHCl_3$); amino acid analysis: $Tyr_{0.96}Gly_{2.03}Phe_{0.99}Leu_{1.03}$ (Found: C, 69.0; H, 7.3; N, 5.8. $C_{68}H_{85}N_5O_{13}$ requires C, 69.2; H, 7.3; N, 5.9%).

(B) In this experiment the free base (75 mg) was treated with **4d** (150 mg, 0.18 mmol) in DMF (10 ml) under otherwise identical conditions. Two main components were isolated after chromatography: fully protected **8** (110 mg, 60%); $[\alpha]_D^{25} - 77.5$; amino acid analysis: $Tyr_{0.98}Gly_{2.02}Phe_{1.00}Leu_{1.06}$ and the corresponding *hydantoin* **9** (9.6 mg, 29%); amino acid analysis: $Tyr_{0.21}Gly_{1.20}Phe_{1.01}Leu_{0.99}$.

(C) Finally the free base (215 mg, 0.446 mmol) was treated with **4d** (400 mg, 0.478 mmol) in DMF (3 ml) as described above. In this case only 7 mg (2%) of the *hydantoin* **9** were obtained together with 354 mg (67%) of the peptide **8**; $[\alpha]_D^{25} - 77.5$; ^1H NMR (270 MHz): δ 7.528 (m, 1 H, NH), 7.325 (s, 5 H, CH_2Ph), 7.172 (m, 5 H, Phe), 7.153 and 7.078 (AA'BB' system, $J = 8.6$ Hz, 4 H, Tyr), 5.122 (s, 2 H, CH_2Ph), 5.058 (dd, 1 H, $J = 4.8$ and 10.5 Hz, H_α-Tyr), 4.867 (dd, 1 H, $J = 6.4$ and 14.4 Hz, H_α-Leu), 4.568 (m, 1 H, H_α-Phe), 4.1–3.8 (m, 4 H, CH_2–Gly), 3.420 (dd, 1 H, $J = 4.9$ and 14.8 Hz, H_β-Tyr), 3.264 (dd, 1 H, $J = 10.5$ and 14.8 Hz, H_β-Tyr), 3.148 (dd, 1 H, $J = 6.5$ and 13.2 Hz, H_β-Phe), 3.022 (dd, 1 H, $J = 8.2$ and 13.2 Hz, H_β-Phe), 2.25–1.5 (m, 48 H, 3 Adoc + $H_{\beta+\gamma}$-Leu), 0.867 (mixed d, 6 H, 2 Me-Leu); IR (KBr): 3372 and 3328 (NH), 1753, 1696 and 1685 (CO) cm^{-1}.

Tyr-Gly-Gly-Phe-Leu (Leu-enkephalin) (**10**). The fully protected pentapeptide **8** (152 mg, 0.129 mmol) was hydrogenolysed in 50% HOAc (20 ml) over 5% Pd–C (50 mg) for 7 h, when according to TLC (CH_2Cl_2–acetone–HOAc 40 : 10 : 1), no starting material remained. After evaporation of the filtrate an oil was obtained which solidified in ether. This was treated with 50% TFA–CH_2Cl_2 (5 ml) for 1 h after which time the reaction was complete. After evaporation, the oily residue was applied to a Sephadex G-15 column (1.4 × 140 cm) which was eluted with 50% HOAc. The relevant fractions were collected and lyophilized to give the pentapeptide **10** as a white powder (68.0 mg, 72%); pure by TLC (*n*-BuOH–HOAc–H_2O 4 : 1 : 1 and $CHCl_3$–MeOH–HOAc–H_2O 15 : 10 : 2 : 3); amino acid analysis: $Tyr_{1.00}Gly_{1.96}Phe_{1.02}Leu_{1.02}$.

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