Chromophoric Azo Reagents for Amino Acid and Peptide Labelling

Susana M. B. Fraga,[a] Maria S. T. Gonçalves,*[a] João C. V. P. Moura,[a] and Kavitha Rani[a]

Keywords: Amino acids / Azo dyes / Non-fluorescence / Peptides

Four carboxylic azo dyes, with spectroscopic absorption peaks ranging from 400 to 500 nm, are presented as new markers for amino acid and peptide labelling at their N-terminus. Labelling can also be performed at side-chain residues as is exemplified with lysine and serine.

Introduction

The use of dyes in chemistry, biology, and medicine is growing continuously, with many new applications in the diagnosis and treatment of disease.[1−4] In the last two decades, the sensitive detection, identification, and quantification of amino acids has been achieved through simple and fast analytical methods. Although fluorometric methods are potentially much more sensitive than colorimetric methods, the use of non-fluorescent dyes for quantitative and qualitative analyses can offer some advantages. First, histochemical applications often require reliable, sensitive, and stable detection of targets in complex samples that may have significant background from either the sample's natural auto fluorescence or fluorescence created during sample preparation. This problem can be avoided by the use of non-fluorescent dyes. Second, the need for ultra violet radiation for excitation of fluorescent dyes implies more expensive equipment than chromophoric methods. Examples of applications of non-fluorescent dyes include the use of amino acid N-derivatizing groups such as 4-[(*N*,*N*-dimethylaminophenyl)-4′-diazenyl]phenyl isothiocyanate for quantitative analysis,[5,6] 5-formyl-1*H*-pyrrole-2-carboxylic acid, which can be colored on demand by treatment with hydrocinnamoyl chloride,[7,8] and 4-[(*N*,*N*-dimethylaminophenyl)-4′-diazenyl]benzenesulfonyl chloride (DABS-Cl) for amino acid analyses by HPLC,[9−11] and capillary[12] and polyacrylamide gel[13,14] electrophoresis.

A method for staining proteins prior to polyacrylamide gel electrophoresis with Remazol Brilliant Blue R[15] and Drimarene Brilliant Blue,[16] two reactive dyes containing an ethyl sulfone and a difluorochloropyrimidyl group, respectively, has been described. Non-fluorescent benzotriazole[17] and quinoline[18] azo dyes have been suggested for the study of dye-protein interactions and sequence analysis of genes by Resonance Raman spectroscopy.

Another application of non-fluorescent dyes in peptide chemistry is the synthesis of colored peptide libraries labeled with monocarboxylic blue anthraquinone and red azo dyes.[19,20]

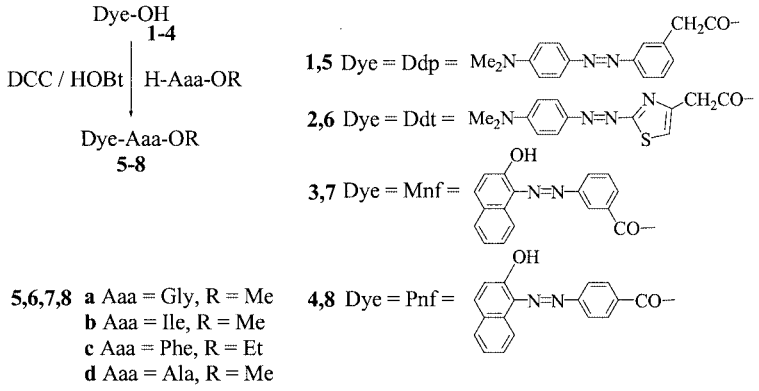
With this in mind, and following on from our previous work with a non-fluorescent dye as a temporary marker in peptide chemistry,[21,22] we decided to develop and test some new markers with spectroscopic absorption peaks ranging from 400 to 500 nm for amino acid and peptide labelling.

Results and Discussion

The new chromophores used (Scheme 1) were obtained by diazotization of 3-aminophenylacetic acid, 2-amino-4-thiazoleacetic acid, and 3- and 4-aminobenzoic acid and coupling of the resulting diazonium salt to *N*,*N*-dimethylaniline (**1**, **2**) or to β-naphthol (**3**, **4**).[23] The carboxylic azo dyes (**1**−**4**) were bonded to the α-amine group of various amino acid esters by coupling them with the aid of dicyclohexylcarbodiimide (DCC) assisted by hydroxybenzotriazole (HOBt) under standard conditions. After purification by chromatography (dry or flash) on silica gel, followed by recrystallization, the corresponding acetyl azo derivatives (**5**, **6**) were obtained; the benzoyl azo derivatives (**7**, **8**) were isolated by precipitation from the reaction mixture with water and recrystallized from acetone. All labeled amino acids (**5**−**8**) were obtained as solid materials in yields ranging from 56 to 99% (Table 1) and were characterized by elemental analysis, and by NMR (^{1}H and ^{13}C), IR, and visible spectroscopy. The visible spectra of compounds **5** show λ_{max} values of 409 (**5d**) and 446 nm (**5b**), with ε values of 31644 and 17282, respectively. The thiazole ring, com-

[a] Department of Chemistry, University of Minho,
Gualtar, 4710-057 Braga, Portugal
Fax: (internat.) +351-253678983
E-mail: msameiro@quimica.uminho.pt

  DOI: 10.1002/ejoc.200300692



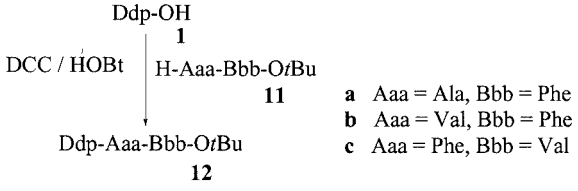
Scheme 1. Synthesis of labeled amino acid derivatives (**5**−**8**)

pared to the benzene ring, produces a bathochromic shift, as exemplified by compound **6d** where λ_{max} is 495 nm. When β-naphthol was used as the coupling component instead of *N*,*N*-dimethylaniline, the resulting amino acid esters (**7**, **8**) showed absorption peaks at 475 and 480 nm, respectively.

Table 1. Yields obtained in the synthesis of labeled amino acid derivatives (**5**−**10**)

Product (compound no.)	Yield (%)
Ddp-Gly-OMe (**5a**)	77
Ddp-Ile-OMe (**5b**)	79
Ddp-Phe-OEt (**5c**)	91
Ddp-Ala-OMe (**5d**)	71
Ddt-Gly-OMe (**6a**)	85
Ddt-Ile-OMe (**6b**)	75
Ddt-Phe-OEt (**6c**)	77
Mnf-Gly-OMe (**7a**)	59
Mnf-Ile-OMe (**7b**)	56
Mnf-Phe-OEt (**7c**)	58
Pnf-Gly-OMe (**8a**)	75
Pnf-Ile-OMe (**8b**)	99
Pnf-Phe-OEt (**8c**)	76
Z-Lys(ω-Ddp)-OMe (**9**)	70
Boc-Ser(Ddp)-OMe (**10**)	86

Following the same method (DCC/HOBt), colored peptides **12** were obtained by acylation at their N-terminus with chromophore **1** (Scheme 2, Table 2). Furthermore, dipeptides **14** were obtained in yields of between 60% and 84% by reacting labeled phenylalanine derivative **13** with several amino acid esters (Scheme 3, Table 2). ^{1}H NMR spectroscopy suggested that the racemization was as much as 5% (see Figures 1 and 2). Previous work has shown a

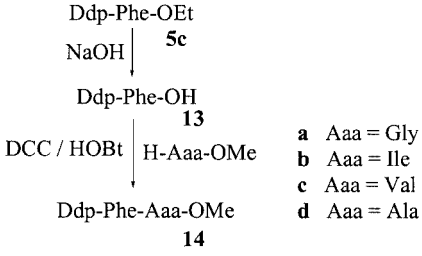


Scheme 2. Labelling of dipeptides **11** with dye **1**

racemization of 50% when the benzoyl azo derivative was used.[22] As a result this marker represents a potential improvement with respect to benzoyl azo derivatives in stepwise syntheses.

Table 2. Yields obtained in the synthesis of labeled dipeptides **12**, **14**, and **16**

Compound	Yield (%)
Ddp-Ala-Phe-O*t*Bu (**12a**)	84
Ddp-Val-Phe-O*t*Bu (**12b**)	66
Ddp-Phe-Val-O*t*Bu (**12c**)	94
Ddp-Phe-Gly-OMe (**14a**)	81
Ddp-Phe-Ile-OMe (**14b**)	65
Ddp-Phe-Val-OMe (**14c**)	60
Ddp-Phe-Ala-OMe (**14d**)	84
Z-Lys(ω-Ddp)-Phe-OEt (**16c**)	91
Z-Lys(ω-Ddp)-Ala-OMe (**16d**)	98



Scheme 3. Synthesis of labeled dipeptides **14**

In addition to labelling amino acids or peptides at their N-terminus, an alternative acylation at a ω-amine group was also investigated. Thus, the methyl ester of *N*-benzyloxycarbonyl lysine was treated with **1** (Scheme 4) under the conditions reported above, and the product (**9**) saponified quantitatively to the C-deprotected amino acid **15**, which was then coupled with alanine and phenylalanine esters to yield labeled dipeptides **16** (Table 2).

Another approach for side-chain labelling was undertaken by reacting *tert*-butyloxycarbonylserine methyl ester with dye **1** to yield 86% of the corresponding ester derivative **10**. Coloured compounds were characterized as above

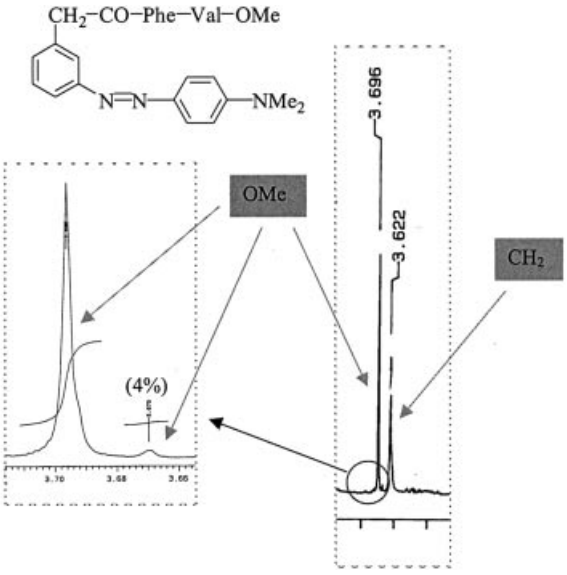


Figure 1. ^{1}H NMR spectrum of Ddp-Phe-Val-OMe (**14c**) before purification

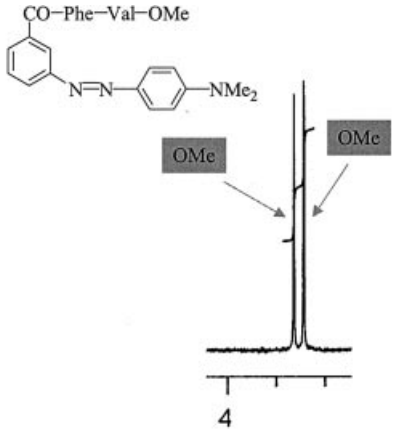
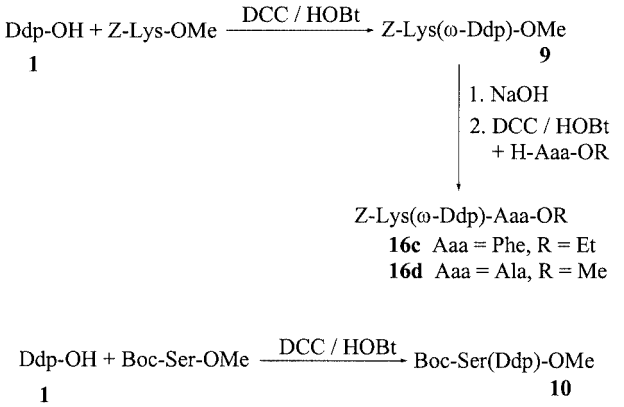


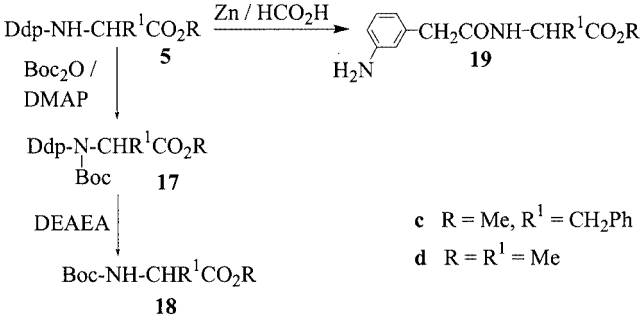
Figure 2. ^{1}H NMR spectrum of Dpa-Phe-Val-OMe before purification



Scheme 4. Synthesis of labeled lysine and serine residues

and the visible spectra of all labeled peptides showed λ_{max} falling at 409 nm (ε = 18532; **14a**) and 415 (ε = 21587; **14d**). All products were stable on storage at room temperature without further precautions.

With the aim of testing the possibility of recovering the initial amino acid esters by removal of the chromophore, two of the resulting labeled products **5** were then treated with di-*tert*-butyl pyrocarbonate (Boc_2O) in the presence of *N*,*N*-dimethylamino pyridine (DMAP) to give compounds **17** in fair yields. Aminolysis with 2-(*N*,*N*-dimethylamino) ethylamine (DEAEA) in dry acetonitrile at room temperature gave the expected Boc-amino acid esters **18c** and **18d** (Scheme 5, Table 3) isolated as colorless materials in 50% and 58% yields, respectively.



Scheme 5. Selective cleavage of labeled amino acids

Table 3. Selective cleavage of labeled amino acids

Starting material	Deprotection method	Product	Yield (%)
17c	DEAEA	**18c**	50
17d	DEAEA	**18d**	58
5c	Zn/HCO_2H	**19c**	77
5d	Zn/HCO_2H	**19d**	65

Labeled amino acid esters **5c** and **5d** were also treated with zinc powder and thus converted into the corresponding colorless 3-aminophenylacetyl derivatives **19** (Scheme 5, Table 3). Despite difficulties in isolating the required products, the latter method of converting the labeled compounds into colorless materials proved to lead to better yields than the former.

Stability tests carried out with colored alanine ester **5d** under forcing conditions similar to those usually required for cleavage of protecting groups during peptide synthesis, showed a good stability of the label to acidolysis, aminolysis and hydrogenation catalyzed by Pd/C; treatment with strong base cleaved the ester function but not the label.

Conclusion

It was possible to obtain suitable colored amino acid esters with maximum absorption peaks ranging from 400 to 500 nm by choosing the appropriate azo chromophore. Acetyl analogs such as those obtained from 3-aminophenylacetic acid and *N*,*N*-dimethylaniline strongly suggest stepwise synthesis with lower epimerization rates when coupling is carried out under similar conditions as for benzoyl azo

markers. Thus, our results show that these chromophores can be used for peptide and protein labelling.

Experimental Section

General Remarks: All melting points are uncorrected; they were measured on a Gallenkamp melting point apparatus. TLC analyses were carried out on 0.25 mm-thick precoated silica plates (Merck Fertigplatten Kieselgel $60F_{254}$) and spots were visualized under UV light or by exposure to vapourized iodine. Dry and column chromatography were carried out on Merck Kieselgel (230−240 mesh). Light petroleum refers to the fraction boiling within the range 40−60 °C. IR spectra were determined on a Perkin−Elmer FTIR-1600 and UV/Vis spectra were determined on a Hitachi U-2000 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian 300 spectrometer in 5% $CDCl_3$ or [D_6]DMSO (DMSO) solution at 25 °C. All chemical shifts are given in ppm relative to $SiMe_4$ (δ = 0 ppm) as reference and *J* values are given in Hz. Assignments were made by comparison of chemical shifts, peak multiplicities, and *J* values. ^{13}C NMR spectra were run on the same instrument but at 75.4 MHz using the solvent peak as internal reference. Spectrometric analyses were performed at the "Unidad de Espectrometria de Masas" of the University of Vigo, Spain. Elemental analyses were carried out on a Leco CHNS 932 instrument. Serine methyl ester hydrochloride and *N*-benzyloxycarbonyl lysine were commercial products. All the other amino acid ester hydrochlorides were prepared from thionyl chloride by the usual procedure. *N*-*tert*-butyloxycarbonylserine methyl ester hydrochloride was prepared with di-*tert*-butylpyrocarbonate by the usual procedure. Dipeptide *tert*-butyl esters **11** were prepared by catalytic hydrogenation of the corresponding *N*-benzyloxycarbonyldipeptides, which were synthesized by standard methods. Dyes **3** and **4** were prepared following the same procedure described before.[23]

3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetic Acid (1): HCl (6 M, 4.6 mL) was added, with stirring, to a suspension of 3-aminophenyl acetic acid (3.11 g, 20 mmol) in 1 M HCl (82 mL), followed by 14 mL of a cold aqueous solution of sodium nitrite (2.69 g; 30 mmol), the mixture was stirred at low temperature (< 5 °C) for 20 minutes. The diazonium salt was added to a solution of *N*,*N*-dimethylaniline (4.6 mL, 36 mmol) in a mixture of glacial acetic acid and water (1.5:1; 23 mL) at room temperature and the mixture kept stirring for 2 h. A solution of 2 M sodium acetate was then added to pH 4 and the red oil thus obtained dissolved in the minimum amount of 6 M HCl and precipitated with aqueous 6 M NaOH. The solid was filtered off and washed with cold water and light petroleum. The dye was obtained as an orange solid (5.55 g, 98%). M.p. 269.0−271.4 °C. TLC (chloroform/methanol, 6:1): R_f = 0.56. UV/Vis (MeOH): λ_{max} = 405 nm (ε = 19200 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). IR (KBr, 1%): $\tilde{\nu}$ = 3406, 2910, 1604, 1566, 1556, 1517, 1403, 1376, 1361, 1229, 1152, 1120, 1071 cm^{-1}. ^{1}H NMR (300 MHz, DMSO): δ = 3.04 (s, 6 H, NMe_2), 3.42 (s, 2 H, CH_2), 6.82 (d, *J* = 9.0 Hz, 2 × Ar-H *ortho* NMe_2), 7.23 (d, *J* = 7.5 Hz, 1 H, 6-H or 4-H), 7.33 (t, *J* = 7.5 Hz, 1 H, 5-H), 7.52 (d, *J* = 7.5 Hz, 1 H, 4-H or 6-H), 7.63 (s, 1 H, 2-H), 7.78 (d, *J* = 9.0 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. ^{13}C NMR (75.4 MHz, DMSO): δ = 39.9 (NMe_2), 46.0 (CH_2), 111.6 (C-2′, C-6′), 119.2 (C-4), 122.4 (C-2), 124.6 (C-3′, C-5′), 128.3 (C-5), 130.8 (C-6), 141.0 (C-1), 142.7 (C-4′), 152.1 (C-3), 152.4 (C-1′), 174.5 (CO_2H) ppm.

3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]thiazoleacetic Acid (2): Sodium nitrite (1.04 g, 15 mmol) was added to concentrated H_2SO_4 (12 mL) with external cooling (5 °C), the suspension was stirred for 10 minutes and a mixture of propionic-acetic acids 1:5 (80 mL) was added. 2-Amino-4-thiazoleacetic acid (2 g, 12.6 mmol) was added in portions and the mixture was left stirring for 20 minutes, with external cooling (5−10 °C). *N*,*N*-Dimethylaniline (1.9 mL) in acetic acid (53 mL) was added to the diazonium solution slowly, keeping external cooling at 10 °C for 4 hours. A saturated solution of ammonium acetate was added until the mixture was neutral to Congo Red paper and it was allowed to stand at 4 °C for one hour. The mixture was poured into water and the separation of an oil was noted. The oil was dissolved in 6 M HCl and the addition of 6 M NaOH gave a red solid (0.94 g, 26%). M.p. 181.3−183.3 °C. TLC (chloroform/methanol, 4:2): R_f = 0.69. UV/Vis (MeOH): λ_{max} = 490 nm (ε = 26855 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). IR (film): $\tilde{\nu}$ = 2675, 2558, 2494, 1727, 1605, 1550, 1527, 1456, 1367, 1325, 1307, 1270, 1207, 1159, 988, 943, 860, 814, 767, 747, 674 cm^{-1}. ^{1}H NMR (300 MHz, DMSO): δ = 3.11 (s, 6 H, NMe_2), 3.75 (s, 2 H, CH_2), 6.87 (d, *J* = 9.6 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.41 (s, < 1 H, C-H), 7.80 (d, *J* = 9.6 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm.

General Method for the Acylation with Dyes 1 to 4

The carboxylic dye (**1**−**4**) was reacted on a 1.12-mmolar scale with an amino acid methyl (or ethyl) ester hydrochloride in DMF by a standard DCC/HOBt coupling. After evaporation of the solvent, dry (or column) chromatography on silica gel, and recrystallization from ethyl acetate/hexane, the required acetyl derivative (**5**, **6**) was obtained. Compounds **7** and **8** were precipitated with water and recrystallized from acetone.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}glycine Methyl Ester (5a):** The product of the reaction of **1** with glycine methyl ester hydrochloride (144 mg, 1.12 mmol) was chromatographed with ethyl acetate/hexane (1:1) as the eluent to give the ester **5a** (305 mg, 77%). M.p. 140.0−142.3 °C. TLC (chloroform/methanol, 5.5:0.5): R_f = 0.81. UV/Vis (MeOH): λ_{max} = 422 nm (ε = 26929 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). IR (KBr, 1%): $\tilde{\nu}$ = 3268, 2934, 1766, 1651, 1605, 1556, 1524, 1401, 1372, 1270, 1245, 1200, 1150, 1128, 1037, 819, 692 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.11 (s, 6 H, NMe_2), 3.73 (s, 5 H, CH_2, OMe), 4.02 (d, *J* = 6.1 Hz, 2 H, CH_2 Gly), 5.95 (br. s, 1 H, α-NH Gly), 6.77 (d, *J* = 8.4 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.33 (d, *J* = 7.2 Hz, 1 H, 6-H or 4-H), 7.49 (t, *J* = 7.2 Hz, 1 H, 5-H), 7.76−7.81 (m, 2 H, 2-H, 4-H or 6-H), 7.89 (d, *J* = 8.4 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 40.2 (NMe_2), 41.2 (CH_2 Gly), 43.2 (CH_2), 52.2 (OMe), 111.4 (C-2′, C-6′), 121.6 (C-4), 122.8 (C-2), 125.0 (C-3′, C-5′), 129.5 (C-5), 130.1 (C-6), 135.2 (C-1), 143.4 (C-4′), 152.4 (C-3), 153.5 (C-1′), 170.1 (CONH), 170.5 (CO_2Me) ppm. $C_{19}H_{22}N_4O_3$ (354.40): calcd. C 64.39, H 6.26, N 15.81; found C 64.39, H 6.44, N 15.50.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}-isoleucine Methyl Ester (5b):** The product of the reaction of **1** with isoleucine methyl ester hydrochloride (203 mg, 1.12 mmol) was chromatographed with ethyl acetate/hexane (4:6) as the eluent to give the ester **5b** (362 mg, 79%). M.p. 71.6−72.9 °C. TLC (ethyl acetate/hexane, 6:4): R_f = 0.75. UV/Vis (MeOH): λ_{max} = 446 nm (ε = 17282 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). IR (film): $\tilde{\nu}$ = 3315, 2962, 1738, 1650, 1601, 1518, 1444, 1408, 1365, 1309, 1246, 1127, 1152, 1128, 1045, 945, 823 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.82−0.94 (m, 6 H, δ-CH_3 Ile, γ-CH_3 Ile), 0.98−1.40 (2 × m, 2 H, γ-CH_2 Ile), 1.80−1.90 (m, 1 H, β-CH Ile), 3.10 (s, 6 H, NMe_2), 3.70 (s, 5 H, CH_2, OMe), 4.58−4.61 (m, 1 H, α-CH Ile), 5.90 (d, *J* = 8.3 Hz, 1 H, α-NH Ile), 6.77 (d, *J* = 9.6 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.32 (d, *J* = 6.4 Hz, 1 H, 6-H or 4-H), 7.48 (t, *J* = 7.5 Hz, 1 H, 5-H), 7.77−7.80 (m, 2 H, 4-H or 6-H, 2-H) ppm. ^{13}C NMR

(75.4 MHz, $CDCl_3$): δ = 11.4 (δ-CH_3 Ile), 15.3 (γ-CH_3 Ile), 25.0 (γ-CH_2 Ile), 37.7 (β-C Ile), 40.2 (NMe_2), 43.5 (CH_2), 52.0 (OMe), 56.4 (α-C Ile), 111.4 (C-2′, C-6′), 121.6 (C-4), 122.6 (C-2), 125.0 (C-3′, C-5′), 129.5 (C-5), 130.0 (C-6), 135.4 (C-1), 143.4 (C-4′), 152.4 (C-3), 153.5 (C-1′), 170.4 (CONH), 172.2 (*C*O_2Me) ppm. $C_{23}H_{30}N_4O_3$ (410.50): calcd. C 67.29, H 7.37, N, 13.65; found C 67.22, H 7.45, N 13.40.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}-phenylalanine Ethyl Ester (5c):** The product of the reaction of **1** with phenylalanine ethyl ester hydrochloride (257 mg, 1.12 mmol) was chromatographed with chloroform/methanol (5.5:0.5) as the eluent to give the ester **5c** (467 mg, 91%). M.p. 111.8−113.1 °C. TLC (diethyl ether/hexane, 9:1): R_f = 0.48. UV/Vis (MeOH): λ_{max} = 425 nm (ε = 24797 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). IR (KBr, 1%): ṽ = 3320, 2931, 1730, 1642, 1602, 1542, 1518, 1455, 1362, 1344, 1231, 1154, 1115, 1039 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.80 (t, *J* = 7.5 Hz, 3 H, OCH_2CH_3), 3.05 (t_{ap}, *J* = 7.0 Hz, 2 H, β-CH_2 Phe), 3.10 (s, 6 H, NMe_2), 3.63 (s, 2 H, CH_2), 4.10 (q, *J* = 7.5 Hz, 2 H, OCH_2CH_3), 4.80−4.90 (m, 1 H, α-CH Phe), 5.87 (d, *J* = 7.5 Hz, 1 H, α-NH Phe), 6.78 (d, *J* = 9.3 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 6.80−6.93 (m, 2 H, 2 × Ar-H Phe), 7.11−7.19 (m, 3 H, 3 × Ar-H Phe), 7.22 (d, *J* = 8.1 Hz, 1 H, 6-H or 4-H), 7.44 (t, *J* = 7.8 Hz, 1 H, 5-H), 7.70 (br. s, 1 H, 2-H), 7.78 (d, *J* = 8.0 Hz, 1 H, 4-H or 6-H), 7.90 (d, *J* = 9.0 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 14.0 (OCH_2CH_3), 37.6 (β-C Phe), 40.2 (NMe_2), 43.5 (CH_2), 53.0 (α-C Phe), 61.4 (OCH_2CH_3), 111.4 (C-2′, C-6′), 121.6 (C-4), 122.8 (C-2), 125.2 (C-3′, C-5′), 126.9 (C-4 Phe), 128.4 (C-3 Phe, C-5 Phe), 129.1 (C-2 Phe, C-6 Phe), 129.5 (C-5), 130.1 (C-6), 135.2 (C-1), 135.1 (C-1 Phe), 143.5 (C-4′), 152.5 (C-3), 153.5 (C-1′), 170.1 (CONH), 171.1 (*C*O_2Et) ppm. $C_{27}H_{30}N_4O_3$ (458.54): calcd. C 70.72, H 6.59, N 12.22; found C 70.46, H 6.74, N 12.19.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}alanine Methyl Ester (5d):** The product of the reaction of **1** with alanine methyl ester hydrochloride (156 mg, 1.12 mmol) was chromatographed with chloroform/methanol (5.5:0.5) as the eluent to give the ester **5d** (293 mg, 71%). M.p. 138.6−140.0 °C. TLC (chloroform/methanol, 5.5:0.5): R_f = 0.86. UV/Vis (MeOH): λ_{max} = 409 nm (ε = 31644 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). IR (KBr, 1%): ṽ = 3334, 2928, 2847, 1754, 1745, 1650, 1604, 1531, 1434, 1406, 1366, 1217, 1163, 1156, 1056, 825 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.35 (d, *J* = 8.2 Hz, 3 H, β-CH_3 Ala), 3.11 (s, 6 H, NMe_2), 3.68 (s, 2 H, CH_2), 3.72 (s, 3 H, OMe), 4.52−4.70 (m, 1 H, α-CH Ala), 6.02 (d, *J* = 6.3 Hz, 1 H, α-NH Ala), 6.77 (d, *J* = 8.1 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.32 (d, *J* = 7.8 Hz, 1 H, 6-H or 4-H), 7.49 (t, *J* = 8.1 Hz, 1 H, 5-H), 7.75−7.81 (m, 2 H, 4-H or 6-H, 2-H), 7.89 (d, *J* = 8.1 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 18.2 (β-C Ala), 40.2 (NMe_2), 43.4 (CH_2), 48.1 (α-C Ala), 52.4 (OMe), 111.4 (C-2′, C-6′), 121.5 (C-4), 122.8 (C-2), 125.0 (C-3′, C-5′), 129.5 (C-5), 130.1 (C-6), 135.3 (C-1), 143.4 (C-4′), 152.4 (C-3), 153.5 (C-1′), 170.2 (CONH), 173.2 (*C*O_2Me) ppm. $C_{20}H_{24}N_4O_3$ (368.42): calcd. C 65.20, H 6.57, N 15.21; found C 65.14, H 6.74, N 14.98.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]thiazoleacetyl}-glycine Methyl Ester (6a):** The product of the reaction of **2** with glycine methyl ester hydrochloride (144 mg, 1.12 mmol) was chromatographed with chloroform/methanol (6:0.1) as the eluent to give the ester **6a** (344 mg, 85%). M.p. 161.2−162.7 °C. TLC (chloroform/methanol, 6:0.1): R_f = 0.83. UV/Vis (MeOH): λ_{max} = 495 nm (ε = 26806 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). IR (KBr, 1%): ṽ = 3433, 3330, 2926, 2845, 1759, 1725, 1654, 1622, 1607, 1551, 1518, 1436, 1372, 1306, 1229, 1202, 1163, 979, 823 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 3.16 (s, 6 H, NMe_2), 3.74 (s, 3 H, OMe), 3.84 (s, 2 H, CH_2), 4.08 (d, *J* = 5.4 Hz, 2 H, CH_2 Gly), 6.75 (d, *J* = 9.0 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.10 (s, 1 H, CH), 7.22 (br. s, 1 H, α-NH Gly), 7.95 (d, *J* = 9.3 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 39.2 (CH_2), 40.2 (NMe_2), 41.4 (CH_2 Gly), 52.3 (OMe), 111.5 (C-2′, C-6′), 116.5 (C-3′, C-5′), 126.9 (C-5), 142.4 (C-4′), 148.9 (C-4), 153.9 (C-1′), 169.6 (CONH), 170.0 (*C*O_2Me), 178.7 (C-2) ppm. The assignments were supported by the Dept 135 technique. HRMS: calcd. for $C_{16}H_{19}N_5O_3S$ [M^+] 361.1209; found 361.1214.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]thiazoleacetyl}-isoleucine Methyl Ester (6b):** The product of the reaction of **2** with isoleucine methyl ester hydrochloride (203 mg, 1.12 mmol) was chromatographed with chloroform/methanol (5.8:0.2) as the eluent to give the ester **6b** (350 mg, 75%). M.p. 117.3−117.6 °C. TLC (chloroform/methanol, 5.8:0.2): R_f = 0.68. UV/Vis (MeOH): λ_{max} = 493 nm (ε = 22253 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). IR (KBr, 1%): ṽ = 3250, 2726, 1741, 1735, 1698, 1654, 1601, 1540, 1523, 1458, 1371, 1305, 1258, 1148, 1131, 821, 666 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.85−0.95 (m, 6 H, δ-CH_3 Ile, γ-CH_3 Ile), 1.10−1.50 (m, 2 H, γ-CH_2 Ile), 1.90−2.00 (m, 1 H, β-CH Ile), 3.16 (s, 6 H, NMe_2), 3.71 (s, 3 H, OMe), 3.82 (s, 2 H, CH_2), 4.57−4.60 (2 × d, *J* = 6.3 Hz, 1 H, α-CH Ile), 6.76 (d, *J* = 9.0 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.10 (br. s, < 2 H, CH, α-NH Ile), 7.94 (d, *J* = 9.3 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 11.5 (δ-C Ile), 15.5 (γ-CH_3 Ile), 25.1 (γ-CH_2 Ile), 37.6 (β-C Ile), 39.6 (CH_2), 40.2 (NMe_2), 52.0 (OMe), 56.7 (α-C Ile), 111.5 (C-2′, C-6′), 116.2 (C-3′, C-5′), 126.8 (C-5), 142.4 (C-4′), 149.4 (C-4), 153.8 (C-1′), 169.0 (CONH), 172.1 (*C*O_2Me), 178.4 (C-2) ppm. The assignments were supported by the Dept 135 technique. HRMS: calcd. for $C_{20}H_{27}N_5O_3S$ [M^+] 417.1835; found 417.1832.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]thiazoleacetyl}-phenylalanine Ethyl Ester (6c):** The product of the reaction of **2** with phenylalanine ethyl ester hydrochloride (257 mg, 1.12 mmol) was chromatographed with ethyl acetate/hexane (7:3) as the eluent to give the ester **6c** (401 mg, 77%). M.p. 124.0−124.5 °C. TLC (ethyl acetate/hexane, 7:3): R_f = 0.71. UV/Vis (MeOH): λ_{max} = 495 nm (ε = 25927 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). IR (KBr, 1%): ṽ = 2921, 1723, 1718, 1691, 1643, 1606, 1558, 1542, 1523, 1466, 1451, 1370, 1339, 1288, 1157, 1125, 1109, 1034, 826 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.22 (t, *J* = 7.0 Hz, 3 H, OCH_2CH_3), 3.05 (t_{ap}, *J* = 7.0 Hz, β-CH_2 Phe), 3.17 (s, 6 H, NMe_2), 3.48−3.82 (m, 2 H, CH_2), 4.10, 4.18 (2 × d *J* = 7.0 Hz, 2 H, OCH_2CH_3), 4.80−4.88 (m, 1 H, α-CH Phe), 6.78 (d, *J* = 9.6 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 6.90−7.20 (m, 6 H, 5 × Ar-H Phe, CH), 7.96 (d, *J* = 9.0 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 14.0 (OCH_2CH_3), 37.8 (β-C Phe), 39.6 (CH_2), 40.3 (NMe_2), 53.5 (α-C Phe), 61.3 (OCH_2CH_3), 111.6 (C-2′, C-6′), 116.2 (C-3′, C-5′), 126.9 (C-5, C-4 Phe), 128.4 (C-3 Phe, C-5 Phe), 129.3 (C-2 Phe, C-6 Phe), 135.8 (C-1 Phe), 142.5 (C-4′), 149.3 (C-4), 153.8 (C-1′), 168.7 (CONH), 171.2 (*C*O_2Et), 178.5 (C-2) ppm. The assignments were supported by the Dept 135 technique. $C_{24}H_{27}N_5O_3S$ (465.56): calcd. C 61.91, H 5.85, N 15.04, S 6.89; found C 61.80, H 6.01, N 14.90, S 6.94.

***N*-{3-[(2-Hydroxy-1-naphthyl)-1′-diazenyl]benzoyl}glycine Methyl Ester (7a):** Reaction of **3** with glycine methyl ester hydrochloride (141 mg, 1.12 mmol) gave the ester **7a** (240 mg, 59%). M.p. 205.2−205.6 °C. TLC (ethanol): R_f = 0.80. UV/Vis (MeOH): λ_{max} = 475 nm (ε = 18080 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^1H NMR (300 MHz, DMSO): δ = 3.67 (s, 3 H, OMe), 4.02−4.10 (m, 2 H, CH_2 Gly), 6.92 (d, *J* = 9.3 Hz, 1 H, 3′-H), 7.48 (t, *J* = 7.5 Hz, 1 H, 6′-H),

7.60−7.70 (m, 2 H, 7′-H, 5-H), 7.80 (d, J = 6.9 Hz, 1 H, 5′-H), 7.85 (d, J = 6.6 Hz, 1 H, 4-H), 7.98 (d, J = 9.3 Hz, 1 H, 4′-H), 8.04 (d, J = 7.0 Hz, 1 H, 6-H), 8.31 (t_{ap}, J = 1.8 Hz, 1 H, 2-H), 8.58 (d, J = 8.0 Hz, 1 H, 8′-H), 9.19 (t_{ap}, J = 4.8 Hz, 1 H, α-NH Gly) ppm. The assignments were supported by spin decoupling-double resonance. ^{13}C NMR (75.4 MHz, DMSO): δ = 41.7 (CH_2 Gly), 52.2 (OCH_3), 117.7 (β-naphthol), 121.9 (C-2), 122.1 (β-naphthol), 124.5 (C-4), 126.5 (β-naphthol), 126.8 (β-naphthol), 128.3 (C-5), 129.4 (C-6), 129.6 (β-naphthol), 129.9 (β-naphthol), 130.4 (β-naphthol), 133.0 (β-naphthol), 135.5 (C-1), 140.9 (β-naphthol), 145.4 (β-naphthol), 157.0 (C-3), 166.2 (CONH), 170.3 (CO_2CH_3), 170.7 (CO naphthol) ppm. HRMS: calcd. for $C_{13}H_{24}N_2O$ [M^+] 363.1219; found 363.1224.

***N*-{3-[(2-Hydroxy-1-naphthyl)-1′-diazenyl]benzoyl}isoleucine Methyl Ester (7b):** Reaction of **3** with isoleucine methyl ester hydrochloride (203 mg, 1.12 mmol) gave the ester **7b** (263 mg, 56%). M.p. 201.9−203.9 °C. TLC (ethanol): R_f = 0.83. UV/Vis (MeOH): λ_{max} = 475 nm (ε = 18741 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^{1}H NMR (300 MHz, DMSO): δ = 0.92 (t, J = 6.3 Hz, 3 H, δ-CH_3 Ile), 0.98−1.40 (m, 5 H, γ-CH_3 Ile, γ-CH_2 Ile), 1.90−2.10 (m, 1 H, β-CH Ile), 3.67 (s, 3 H, OMe), 4.40 (t, J = 7.8 Hz, 1 H, α-CH Ile), 6.93 (d, J = 9.3 Hz, 1 H, 3′-H), 7.48 (t, J = 7.2 Hz, 1 H, 6′-H), 7.60−7.70 (m, 2 H, 7′-H, 5-H), 7.75−7.90 (m, 2 H, 5′-H, 4-H), 7.97 (d, J = 9.9 Hz, 1 H, 4′-H), 8.06 (d, J = 8.1 Hz, 1 H, 6-H), 8.30 (s, 1 H, 2-H), 8.56 (d, J = 8.1 Hz, 1 H, 8′-H), 8.83 (d, J = 7.8 Hz, 1 H, α-NH Ile) ppm. The assignments were supported by spin decoupling-double resonance. ^{13}C NMR (75.4 MHz, DMSO): δ = 10.9 (δ-C Ile), 15.6 (γ-CH_3 Ile), 24.5 (γ-CH_2 Ile), 35.7 (β-C Ile), 51.6 (OCH_3), 57.4 (α-C Ile), 118.0 (β-naphthol), 121.0 (C-2), 121.3 (β-naphthol), 124.0 (C-4), 126.0 (β-naphthol), 126.7 (β-naphthol), 127.8 (C-5), 128.8 (C-6), 129.1 (β-naphthol), 129.2 (β-naphthol), 129.7 (β-naphthol), 132.5 (β-naphthol), 135.1 (C-1), 140.4 (β-naphthol), 144.7 (β-naphthol), 156.5 (C-3), 165.9 (CONH), 169.8 (CO_2CH_3), 172.0 (CO naphthol) ppm. HRMS: calcd. for $C_{24}H_{25}N_3O_4$ [M^+] 419.1845; found 419.1858.

***N*-{3-[(2-hydroxy-1-naphthyl)-1′-diazenyl]benzoyl}phenylalanine Ethyl Ester (7c):** Reaction of **3** with phenylalanine ethyl ester hydrochloride (257 mg, 1.12 mmol) gave the ester **7c** (301 mg, 58%). M.p. 205.0−206.0 °C. TLC (ethyl acetate/hexane, 1:1): R_f = 0.82. UV/Vis (MeOH): λ_{max} = 475 nm (ε = 12490 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^{1}H NMR (300 MHz, DMSO): δ = 1.15 (t, J = 5.2 Hz, 3 H, OCH_2CH_3), 3.10−3.20 (m, 2 H, β-CH Phe), 4.10 (q, J = 5.2 Hz, 2 H, OCH_2CH_3), 4.60−4.72 (m, 1 H, α-CH Phe), 6.92 (d, J = 9.0 Hz, 1 H, 3′-H), 7.20 (d, J = 7.5 Hz, 1 H, 1 × Ar-H Phe), 7.28 (t, J = 7.5 Hz, 2 H, 2 × Ar-H Phe), 7.36 (d, J = 7.0 Hz, 2 H, 2 × Ar-H Phe), 7.48 (t, J = 8.1 Hz, 1 H, 6′-H), 7.59−7.68 (m, 2 H, 7′-H, 5-H), 7.78 (t, J = 8.50 Hz, 2 H, 4-H, 5′-H), 7.98 (d, J = 9.6 Hz, 1 H, 4′-H), 8.02 (d, J = 9.6 Hz, 1 H, 6-H), 8.23 (br. s,1 H, 2-H), 8.59 (d, J = 8.1 Hz, 1 H, 8′-H), 9.09 (d, J = 7.5 Hz, 1 H, α-NH Phe) ppm. The assignments were supported by spin decoupling-double resonance. ^{13}C NMR (75.4 MHz, DMSO): δ = 14.1 (OCH_2CH_3), 36.2 (β-C Phe), 54.5 (α-C Phe), 60.6 (OCH_2CH_3), 117.3 (β-naphthol), 121.5 (C-2), 124.0 (β-naphthol), 125.2 (C-4), 126.0 (C-4 Phe), 126.4 (β-naphthol), 127.6 (β-naphthol), 128.1 (C-5), 128.4 (C-3 Phe, C-5 Phe), 129.0 (C-6), 129.8 (β-naphthol), 130.6 (β-naphthol), 131.8 (β-naphthol), 132.5 (β-naphthol), 135.0 (C-1), 135.1 (C-1 Phe), 137.5 (C-2 Phe, C-6 Phe), 140.4 (β-naphthol), 144.7 (β-naphthol), 156.6 (C-3), 165.6 (CONH), 169.7 ($CO_2CH_2CH_3$), 171.4 (CO naphthol) ppm. HRMS: calcd. for $C_{28}H_{25}N_3O_4$ [M^+] 467.1845; found 467.1863.

***N*-{4-[(2-Hydroxy-1-naphthyl)-1′-diazenyl]benzoyl}glycine Methyl Ester (8a):** Reaction of **4** with glycine methyl ester hydrochloride (141 mg, 1.12 mmol) gave the ester **8a** (269 mg, 74%). M.p. 180.0−181.7 °C. TLC (ethanol): R_f = 0.89. UV/Vis (MeOH): λ_{max} = 480 nm (ε = 27752 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^{1}H NMR (300 MHz, DMSO): δ = 3.66 (s, 3 H, OMe), 4.03 (d, J = 6.5 Hz, 2 H, CH_2 Gly), 6.82 (d, J = 9.6 Hz, 1 H, 3′-H), 7.47 (dt, J = 7.0, 1.2 Hz, 1 H, 6′-H), 7.61 (t, J = 8.0 Hz, 1 H, 7′-H), 7.75 (d, J = 7.8 Hz, 1 H, 5′-H), 7.91 (t, J = 8.4 Hz, 2 H, 3-H, 5-H), 7.96 (s, 1 H, 4′-H), 8.00 (d, J = 9.6 Hz, 2 H, 2-H, 6-H), 8.51 (d, J = 7.5 Hz, 1 H, 8′-H), 9.04 (t_{ap}, J = 6.0 Hz, 1 H, α-NH Gly), 15.99 (1 H, exchangeable s, NH) ppm. The assignments were supported by spin decoupling-double resonance. ^{13}C NMR (75.4 MHz, DMSO): δ = 41.7 (CH_2 Gly), 52.1 (OCH_3), 118.0 (β-naphthol), 122.1 (β-naphthol), 125.6 (C-3, C-5), 127.0 (C-2, C-6), 128.4 (β-naphthol), 129.4 (β-naphthol), 129.8 (β-naphthol), 130.3 (β-naphthol), 131.7 (C-1), 133.1 (C-4), 142.2 (β-naphthol), 146.3 (β-naphthol), 166.2 (CONH), 170.8 (CO_2CH_3), 174.8 (CO naphthol) ppm. HRMS: calcd. for $C_{20}H_{17}N_3O_4$ [M^+] 363.1219; found 363.1206.

***N*-{4-[(2-Hydroxy-1-naphthyl)-1′-diazenyl]benzoyl}isoleucine Methyl Ester (8b):** Reaction of **4** with isoleucine methyl ester hydrochloride (203 mg, 1.12 mmol) gave the ester **8b** (465 mg, 99%). M.p. 177.0−177.8 °C. TLC (ethanol): R_f = 0.83. UV/Vis (MeOH): λ_{max} = 480 nm (ε = 25321 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^{1}H NMR (300 MHz, DMSO): δ = 0.90 (t, J = 7.2 Hz, 3 H, δ-CH_3 Ile), 0.98−1.40 (m, 5 H, γ-CH_3 Ile, γ-CH_2 Ile), 1.90−2.10 (m, 1 H, β-CH Ile), 3.66 (s, 3 H, OMe), 4.36 (t, J = 7.5 Hz, 1 H, α-CH Ile), 6.83 (d, J = 9.9 Hz, 1 H, 3′-H), 7.47 (d, J = 7.20 Hz, 1 H, 6′-H), 7.61 (d t, J = 8.4, 1.5 Hz, 1 H, 7′-H), 7.75 (d, J = 7.0 Hz, 1 H, 5′-H), 7.88 (d, J = 9.0 Hz, 2 H, 3-H, 5-H), 7.95 (d, J = 9.3 Hz, 1 H, 4′-H), 8.04 (d, J = 8.1 Hz, 2 H, 2-H, 6-H), 8.49 (d, J = 8.1 Hz, 1 H, 8′-H), 8.68 (d, J = 7.8 Hz, 1 H, α-NH Ile), 15.99 (1 H, exchangeable s, NH) ppm. The assignments were supported by spin decoupling-double resonance. ^{13}C NMR (75.4 MHz, DMSO): δ = 11.2 (δ-C Ile), 15.9 (γ-CH_3 Ile), 25.7 (γ-CH_2 Ile), 36.1 (β-C Ile), 52.0 (OCH_3), 57.8 (α-C Ile), 117.8 (β-naphthol), 122.0 (β-naphthol), 125.5 (C-3, C-5), 126.9 (C-2, C-6), 128.4 (β-naphthol), 129.5 (β-naphthol), 129.8 (β-naphthol), 130.3 (β-naphthol), 131.9 (C-1), 133.0 (C-4), 142.1 (β-naphthol), 146.3 (β-naphthol), 166.4 (CONH), 172.9 (CO_2CH_3), 174.7 (CO naphthol) ppm. HRMS: calcd. for $C_{24}H_{25}N_3O_4$ [M^+] 419.1845; found 419.1856.

***N*-{4-[(2-Hydroxy-1-naphthyl)-1′-diazenyl]benzoyl}phenylalanine Methyl Ester (8c):** Reaction of **4** with phenylalanine ethyl ester hydrochloride (257 mg, 1.12 mmol) gave the ester **8c** (399 mg, 76%). M.p. 187.5−188.2 °C. TLC (ethanol): R_f = 0.83. UV/Vis (MeOH): λ_{max} = 480 nm (ε = 20620 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^{1}H NMR (300 MHz, DMSO): δ = 1.10 (t, J = 6.5 Hz, 3 H, OCH_2CH_3), 3.00−3.20 (m, 2 H, β-CH_2 Phe), 4.10 (q, J = 6.5 Hz, 2 H, OCH_2CH_3), 4.60−4.70 (m, 1 H, α-CH Phe), 6.92 (d, J = 9.3 Hz, 1 H, 3′-H), 7.15−7.30 (m, 5 H, 5 × Ar-H Phe), 7.47 (t, J = 7.2 Hz, 1 H, 6′-H), 7.61 (t, J = 7.8 Hz, 1 H, 7′-H), 7.76 (d, J = 7.8 Hz, 1 H, 5′-H), 7.87 (d, J = 8.4 Hz, 2 H, 3-H, 5-H), 7.94 (d, J = 9.0 Hz, 3 H, 2-H, 4-H, 4′-H), 8.50 (d, J = 8.1 Hz, 1 H, 8′-H), 8.89 (d, J = 7.5 Hz, 1 H, α-NH Phe), 15.90 (s, 1 H, NH) ppm. The assignments were supported by spin decoupling-double resonance. ^{13}C NMR (75.4 MHz, DMSO): δ = 14.4 (OCH_2CH_3), 36.7 (β-C Phe), 54.9 (α-C Phe), 61.0 (OCH_2CH_3), 117.9 (β-naphthol), 122.1 (β-naphthol), 125.5 (C-3, C-5), 126.9 (C-4 Phe), 126.9 (C-2, C-6), 128.4 (β-naphthol), 128.6 (C-3 Phe, C-5 Phe), 129.5 (β-naphthol), 129.8 (β-naphthol), 130.3 (β-naphthol), 131.7 (C-1, C-1 Phe), 133.1 (C-4), 138.0 (C-2 Phe, C-6 Phe), 142.1 (β-naphthol), 146.3 (β-naphthol), 166.1 (CONH), 172.1 ($CO_2CH_2CH_3$), 174.8 (CO naphthol) ppm. HRMS: calcd. for $C_{28}H_{25}N_3O_4$ [M^+] 467.1845; found 467.1856.

***N*-Benzyloxycarbonyl-ω-{3-[(*N*,*N*-dimethylaminophenyl)-4′-diazenyl]phenylacetyl}lysine Methyl Ester (9):** The product of the reaction of **1** with *N*-(benzyloxycarbonyl)lysine methyl ester hydrochloride (231 mg, 0.82 mmol) according to the above general method for acylation with dyes **1**−**4** was chromatographed with ethyl acetate/light petroleum (mixtures of increasing polarity) as the eluent to give the ester **9** (319 mg, 70%). M.p. 130.0−132.0 °C. TLC (chloroform/methanol, 5.5:0.5): R_f = 0.65.UV/Vis (MeOH): λ_{max} = 410 nm (ε = 28430 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.40−1.50 (m, 2 H, γ-CH_2 Lys), 1.54−1.72 (m, 2 H, β-CH_2 Lys), 1.75−1.97 (m, 2 H, δ-CH_2 Lys), 3.11 (s, 6 H, NMe_2), 3.14−3.29 (m, 2 H, ε-CH_2 Lys), 3.65 (s, 2 H, CH_2), 3.72 (s, 3 H, OMe), 4.25−4.36 (m, 1 H, α-CH Lys), 5.10 (s, 2 H, CH_2 Z), 5.36 (d, *J* = 8.1 Hz, 1 H, α-NH Lys), 5.53 (br. s, 1 H, ω-NH Lys), 6.77 (d, *J* = 9.0 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.24−7.36 (2 × m, 6 H, 5 × Ar-H Z, 6-H or 4-H), 7.46 (t, *J* = 7.8 Hz, 1 H, 5-H), 7.71 (s, 1 H, 2-H), 7.77 (d, *J* = 8.1 Hz, 1 H, 4-H or 6-H), 7.88 (d, *J* = 9.0 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. The assignments were supported by spin decoupling-double resonance technique. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 22.3 (γ-C Lys), 28.9 (β-C Lys), 32.1 (δ-C Lys), 39.2 (ε-C Lys), 40.3 (NMe_2), 43.8 (CH_2), 52.4 (OMe), 53.6 (α-C Lys), 67.0 (CH_2 Z), 111.5 (C-2′, C-6′), 121.6 (C-4), 122.8 (C-3′, C-5′, C-2), 125.1 (C-3 Z, C-5 Z), 128.1 (C-4 Z), 128.5 (C-2 Z, C-6 Z), 129.7 (C-5), 130.2 (C-6), 135.7 (C-1), 136.2 (C-1 Z), 143.5 (C-4′), 152.6 (C-3), 153.6 (C-1′), 155.9 (*C*O_2 Z), 170.9 (CONH), 172.8 (*C*O_2Me) ppm. $C_{31}H_{37}N_5O_5$ (559.65): calcd. C 66.53, H 6.66, N 12.52; found C 66.37, H 6.90, N 12.31.

***N*-*tert*-Butyloxycarbonyl-*O*-{3-[(*N*,*N*-dimethylaminophenyl)-4′-diazenyl]phenylacetyl}serine Methyl Ester (10):** The product of the reaction of **1** with *N*-*tert*-butyloxycarbonylserine methyl ester hydrochloride (289 mg, 1.32 mmol) according to the above general method for acylation with dyes **1**−**4** was chromatographed with chloroform/methanol (5.5:0.5) as the eluent to give the ester **10** (464 mg, 86%). M.p. 95.6−96.6 °C. TLC (ethyl acetate/light petroleum, 4:6): R_f = 0.79. UV/Vis (MeOH): λ_{max} = 415 nm (ε = 23643 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). IR (KBr, 1%): $\tilde{\nu}$ = 3370, 2977, 2910, 2812, 1741, 1714, 1601, 1565, 1520, 1446, 1408, 1367, 1310, 1246, 1224, 1154, 1063, 1023, 946, 913, 824, 785, 732, 690 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.43 (s, 9 H, CMe_3), 3.10 (s, 6 H, NMe_2), 3.68 (s, 3 H, OMe), 3.91 (s, 2 H, CH_2), 4.35−4.50 (m, 2 H, β-CH_2 Ser), 4.51−4.60 (m, 1 H, α-CH Ser), 5.32 (d, *J* = 8.1 Hz, 1 H, α-NH Ser), 6.75 (d, *J* = 9.0 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.29 (d, *J* = 7.5 Hz, 1 H, 4-H or 6-H), 7.44 (t, *J* = 7.5 Hz, 1 H, 5-H), 7.70−7.80 (m, 2 H, 2-H, 6-H or 4-H), 7. 90 (d, *J* = 9.0 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 28.2 (C*Me*$_3$), 40.2 (NMe_2), 40.9 (CH_2), 52.7 (OMe), 52.8 (α-C Ser), 64.7 (β-C Ser), 80.2 (*C*Me_3), 111.4 (C-2′, C-6′), 121.7 (C-4), 122.3 (C-2), 124.9 (C-3′, C-5′), 129.1 (C-5), 130.0 (C-6), 134.3 (C-1),143.4 (C-4′), 152.4 (C-3), 153.2 (C-1′), 155.1 (*C*O_2CMe$_3$), 170.1 (CONH), 170.7 (*C*O_2CH$_2$), 171.3 (*C*O_2Me) ppm. $C_{25}H_{32}N_4O_6$ (484.54): calcd. C 61.97, H 6.66, N 11.56; found C 62.04, H 6.75, N 11.41.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}-alanylphenylalanine *tert*-Butyl Ester (12a):** The product of the reaction of dye **1** (342 mg, 1.12 mmol) with alanylphenylalanine *tert*-butyl ester **11a** (327 mg, 1.12 mmol) under the conditions described above for acylation with compounds **1**−**4** was chromatographed with diethyl ether/hexane (4:6) as the eluent to give the labeled dipetide **12a** (524 mg, 84%). M.p. 119.7−120.4 °C. TLC (diethyl ether/hexane, 8:2): R_f = 0.82. UV/Vis (MeOH): λ_{max} = 410 nm (ε = 28430 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.28 (d, *J* = 6.9 Hz, 3 H, β-CH_3 Ala), 1.41 (s, 9 H, CMe_3), 3.00−3.09 (m, 2 H, β-CH_2 Phe), 3.10 (s, 6 H, NMe_2), 3.63 (d, *J* = 1.5 Hz, 2 H, CH_2), 4.40−4.50 (m, 1 H, α-CH Ala), 4.64−4.74 (m, 1 H, α-CH Phe), 6.03 (d, *J* = 7.2 Hz, 1 H, α-NH Ala), 6.43 (d, *J* = 7.8 Hz, 1 H, α-NH Phe), 6.77 (d, *J* = 9.0 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.10−7.16 (m, 2 H, 2 × Ar-H Phe), 7.20−7.32 (m, 4 H, 3 × Ar-H Phe, 6-H or 4-H), 7.46 (t, *J* = 7.8 Hz, 1 H, 5-H), 7.72 (s, 1 H, 2-H), 7.78 (d, *J* = 8.1 Hz, 1 H, 4-H or 6-H), 7.89 (d, *J* = 9.3 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. The assignments were supported by spin decoupling-double resonance technique. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 18.1 (β-C Ala), 27.8 (*C*Me_3), 37.7 (β-C Phe), 40.1 (NMe_2), 43.1 (CH_2), 48.7 (α-C Ala), 53.7 (α-C Phe), 82.1 (C*Me*$_3$), 111.3 (C-2′, C-6′), 121.3 (C-4), 122.7 (C-2), 124.9 (C-3′, C-5′), 126.8 (C-4 Phe), 128.2 (C-3 Phe, C-5 Phe), 129.3 (C-2 Phe, C-6 Phe, C-5), 130.0 (C-6), 135.3 (C-1), 136.0 (C-1 Phe), 143.4 (C-4′), 152.3 (C-3), 153.4 (C-1′), 170.1 (CONH), 170.6 (CONH), 171.6 (*C*O_2CMe$_3$) ppm. $C_{32}H_{39}N_5O_4$ (557.67): calcd. C 68.92, H 7.05, N, 12.56; found C 68.95, H 7.04, N 12.37.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}-valylphenylalanine *tert*-Butyl Ester (12b):** The product of the reaction of dye **1** (255 mg, 0.90 mmol) with valylphenylalanine *tert*-butyl ester **11b** (288 mg, 0.90 mmol) under the conditions described above for acylation with compounds **1**−**4** was chromatographed with diethyl ether/hexane (4:6) as the eluent to give the labeled dipetide **12b** (348 mg, 66%). M.p. 187.5−188.8 °C. TLC (diethyl ether/hexane, 6:4): R_f = 0.63. UV/Vis (MeOH): λ_{max} = 414 nm (ε = 29146 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^1H NMR (300 MHz, $CDCl_3$): δ = 0.76 (d, *J* = 6.9 Hz, 3 H, γ-CH_3 Val), 0.86 (d, *J* = 6.9 Hz, 3 H, γ-CH_3 Val), 1.40 (s, 9 H, CMe_3), 1.93−2.05 (m, 1 H, β-CH Val), 3.04 (d, *J* = 6.9 Hz, 2 H, β-CH_2 Phe), 3.10 (s, 6 H, NMe_2), 3.68 (d, *J* = 4.8 Hz, 2 H, CH_2), 4.17−4.40 (m, 1 H, α-CH Val), 4.66−4.78 (m, 1 H, α-CH Phe), 5.97 (d, *J* = 8.7 Hz, 1 H, α-NH Val), 6.18 (d, *J* = 7.8 Hz, 1 H, α-NH Phe), 6.77 (d, *J* = 9.0 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.10−7.14 (m, 2 H, 2 × Ar-H Phe), 7.20−7.32 (m, 3 H, 3 × Ar-H Phe), 7.48 (t, *J* = 8.3 Hz, 1 H, 5-H), 7.74−7.80 (m, 2 H, 6-H, 4-H), 7.89 (d, *J* = 9.3 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. The assignments were supported by spin decoupling-double resonance technique. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 17.9 (γ-C Val), 19.1 (γ-C Val), 27.8 (*C*Me_3), 30.8 (β-C Val), 38.0 (β-C Phe), 40.2 (NMe_2), 43.5 (CH_2), 53.5 (α-C Phe), 58.3 (α-C Val), 82.2 (O*C*Me_3), 111.4 (C-2′, C-6′), 121.7 (C-4), 122.6 (C-2), 125.0 (C-3′, C-5′), 126.9 (C-4 Phe), 128.4 (C-3 Phe, C-5 Phe), 129.4 (C-2 Phe, C-6 Phe), 129.5 (C-5), 130.0 (C-6), 135.5 (C-1), 135.9 (C-1 Phe), 143.5 (C-4′), 152.4 (C-3), 153.5 (C-1′), 170.1 (CONH), 170.4 (CONH), 170.7 (*C*O_2CMe$_3$) ppm. $C_{34}H_{43}N_5O_4$ (585.72): calcd. C 69.72, H 7.40, N 11.96; found C 69.53, H 7.44, N 11.95.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}-phenylalanylvaline *tert*-Butyl Ester (12c):** The product of the reaction of dye **1** (255 mg, 0.90 mmol) with phenylalanylvaline *tert*-butyl ester **11c**[24] (288 mg, 0.90 mmol) under the conditions described above for acylation with compounds **1**−**4** was chromatographed with diethyl ether/hexane (mixtures of increasing polarity) as the eluent to give the labeled dipetide **12c** (500 mg, 94%). M.p. 124.7−126.0 °C. TLC (diethyl ether/hexane, 6:4): R_f = 0.69. UV/Vis (MeOH): λ_{max} = 411 nm (ε = 25029 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^1H NMR (300 MHz, $CDCl_3$): δ = 0.82 (dd, *J* = 6.6, 1.5 Hz, 6 H, γ-CH_3 Val), 1.46 (s, 9 H, CMe_3), 2.02−2.14 (m, 1 H, β-CH Val), 3.00 (d, *J* = 6.90 Hz, 2 H, β-CH_2 Phe), 3.11 (s, 6 H, NMe_2), 3.63 (s, 2 H, CH_2), 4.30−4.34 (m, 1 H, α-CH Val), 4.64−4.72 (m, 1 H, α-CH Phe), 5.95 (d, *J* = 7.5 Hz, 1 H, α-NH Phe), 6.39 (d, *J* = 8.1 Hz, 1 H, α-NH Val), 6.78 (d, *J* = 9.3 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.02 (dd, *J* = 7.8, 1.8 Hz, 2 H, 2 × Ar-H Phe), 7.12−7.27 (m, 4 H, 3 × Ar-H Phe, 6-H or 4-H), 7.42 (t, *J* = 7.8 Hz, 1 H, 5-H), 7.65 (t_{ap}, 1.8 Hz, 1 H, 2-H), 7.75−7.82 (m, 1 H, 4-H or 6-H), 7.90 (d,

J = 9.0 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. The assignments were supported by spin decoupling-double resonance technique.^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 17.6 (γ-C Val), 18.7 (γ-C Val), 28.0 (CMe_3), 31.2 (β-C Val), 37.4 (β-C Phe), 40.2 (NMe_2), 43.5 (CH_2), 54.2 (α-C Phe), 57.5 (α-C Val), 81.9 (CMe_3), 111.4 (C-2′, C-6′), 121.7 (C-4), 122.7 (C-2), 125.0 (C-3′, C-5′), 126.8 (C-4 Phe), 128.6 (C-3 Phe, C-5 Phe), 129.1 (C-2 Phe, C-6 Phe), 129.6 (C-5), 130.1 (C-6), 135.0 (C-1), 136.0 (C-1 Phe), 143.5 (C-4′), 152.5 (C-3), 153.5 (C-1′), 170.3 (CONH), 170.7 (CONH, CO_2CMe_3) ppm. $C_{34}H_{43}N_5O_4$ (585.72): calcd. C 69.72, H 7.40, N 11.96; found C 69.76, H 7.60, N 11.96.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}-phenylalanine (13):** NaOH (1 M; 1.23 mL, 1.23 mmol) was added to the fully protected amino acid **5c** (375 mg; 0.82 mmol) in 1,4-dioxane (4.10 mL) at 0 °C (ice bath). The solution was stirred for 6 hours at 0 °C and acidified to pH 2−3 with 1 M $KHSO_4$. After extraction with ethyl acetate and evaporation of the solvent the required acylamino acid **13** was obtained as an orange solid (353 mg; 100%). M.p. 117.9−119.0 °C. IR (film): $\tilde{\nu}$ = 3405, 2925, 2854, 1726, 1648, 1601, 1519, 1459, 1376, 1152, 944, 823, 723 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.80−3.00 (m, 2 H, β-CH_2 Phe), 3.08 (s, 6 H, NMe_2), 3.61 (s, 2 H, CH_2), 4.80−4.91 (m, 1 H, α-CH Phe), 6.17 (d, J = 7.5 Hz, 1 H, α-NH Phe), 6.76 (d, J = 9.0 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 6.90−7.00 (m, 2 H, 2 × Ar-H Phe), 7.08−7.20 (m, 4 H, 3 × Ar-H Phe, 4-H or 6-H), 7.39 (t, J = 7.8 Hz, 1 H, 5-H), 7.65 (s, 1 H, 2-H), 7.76 (d, J = 8.4 Hz, 1 H, 6-H or 4-H), 7.89 (d, J = 9.0 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 36.9 (β-C Phe), 40.3 (NMe_2), 43.4 (CH_2), 53.2 (α-C Phe), 111.5 (C-2′, C-6′), 121.7 (C-4), 122.8 (C-2), 125.1 (C-3′, C-5′), 127.1 (C-4 Phe), 128.7 (C-3 Phe, C-5 Phe), 129.2 (C-2 Phe, C-6 Phe), 129.7 (C-5), 130.2 (C-6), 134.8 (C-1), 135.3 (C-1 Phe), 143.5 (C-4′), 152.6 (C-3), 153.5 (C-1′), 171.4 (CONH), 173.8 (CO_2H) ppm.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}-phenylalanylglycine Methyl Ester (14a):** The product of the reaction of acylamino acid **13** (300 mg, 0.70 mmol) with glycine methyl ester hydrochloride (88 mg, 0.70 mmol) under the conditions described above for acylation with dyes **1**−**4** was chromatographed with chloroform/methanol (5.8:0.2) as the eluent to give dipeptide **14a** (284 mg, 81%). M.p. 171.5−173.6 °C. TLC (chloroform/methanol, 5.8:0.2): R_f = 0.51. UV (MeOH): λ_{max} = 409 nm (ε = 18532 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.02 (t, J = 6.9 Hz, 2 H, β-CH_2 Phe), 3.11 (s, 6 H, NMe_2), 3.63 (s, 2 H, CH_2), 3.72 (s, 3 H, OMe), 3.96 (t, J = 6.0 Hz, 2 H, CH_2 Gly), 4.64−4.75 (m, 1 H, α-CH Phe), 6.02 (d, J = 7.5 Hz, 1 H, α-NH Phe), 6.56 (t_{ap}, J = 5.1 Hz, 1 H, α-NH Gly), 6.78 (d, J = 9.0 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.04 (dd, J = 6.0, 1.2 Hz, 2 H, 6-H or 4-H, 1 × Ar-H Phe), 7.12−7.23 (m, 4 H, 4 × Ar-H Phe), 7.43 (t, J = 8.1 Hz, 1 H, 5-H), 7.63 (t_{ap}, J = 1.8 Hz, 1 H, 2-H), 7.78 (d, J = 8.1 Hz, 1 H, 4-H or 6-H), 7.90 (d, J = 9.0 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. The assignments were supported by spin decoupling-double resonance technique. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 37.4 (β-C Phe), 40.3 (NMe_2), 41.2 (CH_2 Gly), 43.4 (CH_2), 52.4 (OMe), 54.2 (α-C Phe), 111.5 (C-2′, C-6′), 121.8 (C-4), 122.7 (C-2), 125.1 (C-3′, C-5′), 127.0 (C-4 Phe), 128.7 (C-3 Phe, C-5 Phe), 129.1 (C-2 Phe, C-6 Phe), 129.7 (C-5), 130.1 (C-6), 134.9 (C-1), 136.0 (C-1 Phe), 143.2 (C-4′), 152.5 (C-3), 153.6 (C-1′), 169.7 (CONH), 170.9 (CONH), 171.1 (CO_2Me) ppm. $C_{28}H_{31}N_5O_4$ (501.57): calcd. C 67.05, H 6.23, N 13.96; found C 66.92, H 6.31, N 13.62.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}-phenylalanylisoleucine Methyl Ester (14b):** The product of the reaction of acylamino acid **13** (87 mg, 0.20 mmol) with isoleucine methyl ester hydrochloride (36.7 mg, 0.20 mmol) under the conditions described above for acylation with dyes **1**−**4** was chromatographed with chloroform/methanol (5.8:0.2) as the eluent to give dipeptide **14b** (73 mg, 65%). M.p. 165.5−167.0 °C. TLC (chloroform/methanol, 5.8:0.2): R_f = 0.65. UV/Vis (MeOH): λ_{max} = 410 nm (ε = 24986 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.78 (d, J = 6.6 Hz, 3 H, γ-CH_3 Ile), 0.86 (t, J = 7.50 Hz, 3 H, δ-CH_3 Ile), 0.92−1.37 (2 × m, 2 H, γ-CH_2 Ile), 1.70−1.87 (m, 1 H, β-CH Ile), 3.01 (d, J = 6.9 Hz, 2 H, β-CH_2 Phe), 3.11 (s, 6 H, NMe_2), 3.63 (s, 2 H, CH_2), 3.70 (s, 3 H, OMe), 4.42−4.50 (m, 1 H, α-CH Ile), 4.65−4.70 (m, 1 H, α-CH Phe), 6.01 (d, J = 7.0 Hz, 1 H, α-NH Phe), 6.45 (d, J = 7.3 Hz, 1 H, α-NH Ile), 6.78 (d, J = 9.3 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.04 (dd, J = 7.7, 1.5 Hz, 2 H, 2 × Ar-H Phe), 7.10−7.20 (m, 4 H, 3 × Ar-H Phe, 6-H or 4-H), 7.41 (t, J = 7.3 Hz, 1 H, 5-H), 7.62 (t_{ap}, J = 2.1 Hz, 1 H, 2-H), 7.78 (d, J = 7.3 Hz, 1 H, 4-H or 6-H), 7.90 (d, J = 9.0 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. The assignments were supported by spin decoupling-double resonance technique. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 11.5 (γ-CH_3 Ile), 15.3 (δ-C Ile), 25.0 (γ-CH_2 Ile), 37.3 (β-C Phe), 37.6 (β-C Ile), 40.3 (NMe_2), 43.5 (CH_2), 52.1 (OMe), 54.3 (α-C Phe), 56.6 (α-C Ile), 111.4 (C-2′, C-6′), 121.8 (C-4), 122.7 (C-2), 125.1 (C-3′, C-5′), 126.9 (C-4 Phe), 128.7 (C-3 Phe, C-5 Phe), 129.2 (C-2 Phe, C-6 Phe), 129.6 (C-5), 130.0 (C-6), 134.9 (C-1), 136.0 (C-1 Phe), 143.5 (C-4′), 152.5 (C-3), 153.6 (C-1′), 170.4 (CONH), 170.8 (CONH), 171.7 (CO_2Me) ppm. $C_{32}H_{39}N_5O_4$ (557.67): calcd. C 68.92, H 7.05, N 12.56; found C 68.79, H 6.83, N 12.49.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}-phenylalanylvaline Methyl Ester (14c):** The product of the reaction of acylamino acid **13** (200 mg, 0.47 mmol) with valine methyl ester hydrochloride (78 mg, 0.47 mmol) under the conditions described above for acylation with dyes **1**−**4** was chromatographed with diethyl ether/hexane (6:4) as the eluent to give dipeptide **14c** (152 mg, 60%). M.p. 164.0−165.9 °C. TLC (diethyl ether/hexane, 8:2): R_f = 0.63. UV/Vis (MeOH): λ_{max} = 411 nm (ε = 20256 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.81 (t, J = 7.5 Hz, 6 H, γ-CH_3 Val), 2.0−2.19 (m, 1 H, β-CH Val), 3.01 (d, J = 7.2 Hz, 2 H, β-CH_2 Phe), 3.11 (s, 6 H, NMe_2), 3.63 (s, 2 H, CH_2), 3.70 (s, 3 H, OMe), 4.38−4.45 (m, 1 H, α-CH Val), 4.64−4.76 (m, 1 H, α-CH Phe), 6.07 (d, J = 7.5 Hz, 1 H, α-NH Phe), 6.51 (d, J = 8.4 Hz, 1 H, α-NH Val), 6.78 (d, J = 9.3 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.05 (d, J = 6.9 Hz, 2 H, 2 × Ar-H Phe), 7.10−7.23 (m, 4 H, 3 × Ar-H Phe, 6-H or 4-H), 7.42 (t, J = 7.8 Hz, 1 H, 5-H), 7.65 (s, 1 H, 2-H), 7.78 (d, J = 8.1 Hz, 1 H, 4-H or 6-H), 7.89 (d, J = 9.0 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. The assignments were supported by spin decoupling-double resonance technique. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 17.7 (γ-C Val), 18.8 (γ-C Val), 31.0 (β-C Val), 37.3 (β-C Phe), 40.3 (NMe_2), 43.5 (CH_2), 52.1 (OMe), 54.3 (α-C Phe), 57.3 (α-C Val), 111.4 (C-2′, C-6′), 121.8 (C-4), 122.7 (C-2), 125.1 (C-3′, C-5′), 126.9 (C-4 Phe), 128.6 (C-3 Phe, C-5 Phe), 129.2 (C-6 Phe, C-2 Phe), 129.6 (C-5), 130.0 (C-6), 134.9 (C-1), 136.0 (C-1 Phe), 143.5 (C-4′), 152.5 (C-3), 153.6 (C-1′), 170.6 (CONH), 170.9 (CONH), 171.7 (CO_2Me) ppm. $C_{31}H_{37}N_5O_4$ (543.65): calcd. C 68.48, H 6.86, N 12; found C 68.27, H 6.81, N 12.88.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}-phenylalanylalanine Methyl Ester (14d):** The product of the reaction of acylamino acid **13** (254 mg, 0.59 mmol) with alanine methyl ester hydrochloride (82.4 mg, 0.59 mmol) under the conditions described above for acylation with dyes **1**−**4** was chromatographed with chloroform/methanol (5.8:0.2) as the eluent to give dipeptide **14d**

(252 mg, 84%). M.p. 166.8−168.5 °C. TLC (diethyl ether/hexane, 6:4): R_f = 0.77. UV/Vis (MeOH): λ_{max} = 415 nm (ε = 21587 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.32 (d, *J* = 7.2 Hz, 3 H, β-CH_3 Ala), 3.00 (d, *J* = 7.70 Hz, 2 H, β-CH_2 Phe), 3.10 (s, 6 H, NMe_2), 3.62 (s, 2 H, CH_2), 3.71 (s, 3 H, OMe), 4.40−4.50 (m, 1 H, α-CH Ala), 4.63−4.72 (m, 1 H, α-CH Phe), 6.17 (d, *J* = 7.5 Hz, 1 H, α-NH Phe), 6.60 (d, *J* = 7.5 Hz, 1 H, α-NH Ala), 6.76 (d, *J* = 9.3 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.01−7.08 (m, 2 H, 2 × Ar-H Phe), 7.12−7.22 (m, 4 H, 3 × Ar-H Phe, 6-H or 4-H), 7.42 (t, *J* = 8.1 Hz, 1 H, 5-H), 7.65 (t_{ap}, *J* = 1.5 Hz, 1 H, 2-H), 7.77 (d, *J* = 8.1 Hz, 1 H, 4-H or 6-H), 7.89 (d, *J* = 9.3 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. The assignments were supported by spin decoupling-double resonance technique. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 18.1 (β-C Ala), 37.7 (β-C Phe), 40.4 (NMe_2), 43.5 (CH_2), 48.1 (α-C Ala), 52.4 (OMe), 54.2 (α-C Phe), 111.5 (C-2′, C-6′), 121.7 (C-4), 122.7 (C-2), 125.0 (C-3′, C-5′), 126.9 (C-4 Phe), 128.6 (C-3 Phe, C-5 Phe), 129.2 (C-2 Phe, C-6 Phe), 129.6 (C-5), 130.1 (C-6), 135.1 (C-1), 136.0 (C-1 Phe), 143.5 (C-4′), 152.5 (C-3), 153.5 (C-1′), 170.2 (CONH), 170.8 (CONH), 172.8 (CO_2Me) ppm. $C_{29}H_{33}N_5O_4$ (515.59): calcd. C 67.55, H 6.45, N 13.58; found C 67.65, H 6.67, N 13.49.

***N*-Benzyloxycarbonyl-ω-{3-[(*N*,*N*-dimethylaminophenyl)-4′-diazenyl]phenylacetyl}lysine (15):** NaOH (1 M; 0.5 mL, 0.5 mmol) was added to the fully protected amino acid **9** (187 mg, 0.34 mmol) in 1,4-dioxane (1.7 mL) at 0 °C (ice bath). The solution was stirred at 0 °C for 6 hours and acidified to pH 2−3 with 1 M $KHSO_4$. After extraction with ethyl acetate and evaporation of the solvent, acylamino acid **15** was obtained as an orange solid (178 mg, 98%). M.p. 74.5−76.2 °C. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.30−1.50 (m, 2 H, γ-CH_2 Lys), 1.60−1.81 (m, 2 H, β-CH_2 Lys), 1.82−1.95 (m, 2 H, δ-CH_2 Lys), 3.00 (s, 6 H, NMe_2), 3.10−3.20 (m, 2 H, ε-CH_2 Lys), 3.60 (s, 2 H, CH_2), 4.27−4.40 (m, 1 H, α-CH Lys), 5.10 (s, 2 H, CH_2 Z), 5.72 (d, *J* = 8.1 Hz, 1 H, α-NH Lys), 5.92 (tap, *J* = 5.9 Hz, 1 H, ω-NH Lys), 6.73 (d, *J* = 9.3 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.20−7.40 (m, 6 H, 5 × Ar-H Z, 6-H or 4-H), 7.42 (t, *J* = 7.8 Hz, 1 H, 5-H), 7.68 (s, 1 H, 2-H), 7.73 (d, *J* = 8.1 Hz, 1 H, 4-H or 6-H), 7.86 (d, *J* = 9.3 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. The assignments were supported by spin decoupling-double resonance technique. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 21.8 (γ-C Lys), 28.5 (β-C Lys), 31.7 (δ-C Lys), 39.1 (ε-C Lys), 40.2 (NMe_2), 43.3 (CH_2), 53.5 (α-C Lys), 66.8 (CH_2 Z), 111.4 (C-2′, C-6′), 121.2 (C-4), 122.9 (C-3′, C-5′), 125.2 (C-2), 127.9 (C-3 Z, C-5 Z), 128.0 (C-4 Z), 128.4 (C-2 Z, C-6 Z), 129.6 (C-5), 130.3 (C-6), 135.5 (C-1), 136.2 (C-1 Z), 143.1 (C-4′), 152.6 (C-3), 153.3 (C-1′), 156.1 (NHCO_2CH_2Ph), 171.7 (CONH Lys), 174.5 (CO_2Me) ppm.

***N*-Benzyloxycarbonyl-ω-{3-[(*N*,*N*-dimethylaminophenyl)-4′-diazenyl]phenylacetyl}lysylphenylalanine Ethyl Ester (16c):** The product of the reaction of **15** (100 mg, 0.18 mmol) with phenylalanine ethyl ester hydrochloride (41.1 mg, 0.18 mmol) under the conditions described above for acylation with dyes **1**−**4** was chromatographed with chloroform/methanol (5.8:0.2) as the eluent to give the fully protected dipeptide **16c** (117 mg, 91%). M.p. 142.7−144.7 °C. TLC (chloroform/methanol, 5.8:0.2): R_f = 0.44. UV/Vis (MeOH): λ_{max} = 412 nm (ε = 18704 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.20 (t, *J* = 6.9 Hz, 3 H, OCH_2CH_3), 1.21−1.50 (m, 2 H, γ-CH_2 Lys), 1.52−1.70 (m, 2 H, β-CH_2 Lys), 1.71−2.00 (m, 2 H, δ-CH_2 Lys), 3.00−3.30 (2 × m, 10 H, NMe_2, ε-CH_2 Lys, β-CH_2 Phe), 3.60 (s, 2 H, CH_2), 4.00−4.20 (m, 2 H, OCH_2CH_3), 4.28−4.30 (m, 1 H, α-CH Lys), 4.74−4.88 (m, 1 H, α-CH Phe), 5.08 (s, 2 H, CH_2 Z), 5.60 (d, *J* = 7.8 Hz, 1 H, α-NH Lys), 5.80 (br. s, 1 H, ω-NH Lys), 6.64 (d, *J* = 8.3 Hz, 1 H, α-NH Phe), 6.74 (d, *J* = 9.0 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.10 (d, *J* = 6.6 Hz, 2 H, 2 × Ar-H Phe), 7.20−7.40 (m, 8 H, 5 × Ar-H Z, 3 × Ar-H Phe), 7.43 (t, *J* = 7.8 Hz, 1 H, 5-H), 7.70 (m, 2 H, 4-H, 6-H), 7.82 (s, 1 H, 2-H), 7.88 (d, *J* = 9.0 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. The assignments were supported by spin decoupling-double resonance technique. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 14.0 (OCH_2CH_3), 22.1 (γ-C Lys), 28.7 (β-C Lys), 33.8 (δ-C Lys), 37.7 (β-C Phe), 38.8 (ε-C Lys), 40.2 (NMe_2), 43.5 (CH_2), 53.2 (α-C Phe), 54.5 (α-C Lys), 61.5 (OCH_2CH_3), 66.9 (CH_2 Z), 111.4 (C-2′, C-6′), 121.3 (C-4), 122.8 (C-3′, C-5′), 125.0 (C-3 Z, C-5 Z), 127.0 (C-2), 127.9 (C-4 Phe), 128.1 (C-3 Phe, C-5 Phe), 128.4 (C-4 Z, C-2 Z, C-6 Z), 129.2 (C-2 Phe, C-6 Phe), 129.5 (C-5), 130.1 (C-6), 135.7 (C-1), 135.8 (C-1 Z), 136.1 (C-1 Phe), 143.4 (C-4′), 152.4 (C-3), 153.5 (C-1′), 156.1 (NHCO_2CH_2Ph), 171.0 (CONH Lys), 171.3 (CONH Phe, CO_2Et) ppm. $C_{41}H_{48}N_6O_6$ (720.84): calcd. C 68.31, H 6.71, N 11.66; found C 68.05, H 6.95, N 11.57.

***N*-Benzyloxycarbonyl-ω-{3-[(*N*,*N*-dimethylaminophenyl)-4′-diazenyl]phenylacetyl}lysylalanine Methyl Ester (16d):** The product of the reaction of **15** (70 mg, 0.13 mmol) with alanine methyl ester hydrochloride (17.4 mg, 0.13 mmol) under the conditions described above for acylation with dyes **1**−**4** was chromatographed with chloroform/methanol (5.8:0.2) as the eluent to give the fully protected dipeptide **16d** (77 mg, 98%). M.p. 152.6−154.6 °C. TLC (chloroform/methanol, 5.8:0.2): R_f = 0.30. UV/Vis (MeOH): λ_{max} = 414 nm (ε = 22069 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.26−1.40 (m, 3 H, β-CH_3 Ala), 1.41−1.54 (m, 2 H, γ-CH_2 Lys), 1.56−1.78 (m, 2 H, β-CH_2 Lys), 1.80−2.00 (m, 2 H, δ-CH_2 Lys), 3.10 (s, 6 H, NMe_2), 3.13−3.31 (m, 2 H, ε-CH_2 Lys), 3.63 (s, 2 H, CH_2), 3.73 (s, 3 H, OMe), 4.05−4.20 (m, 1 H, α-CH Lys), 4.48−4.60 (m, 1 H, α-CH Ala), 5.11 (s, 2 H, CH_2 Z), 5.44−5.60 (m, 1 H, α-NH Lys), 5.66−5.75 (m, 1 H, ω-NH Lys), 6.60−6.73 (m, 1 H, α-NH Ala), 6.76 (d, *J* = 9.3 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.20−7.40 (m, 6 H, 5 × Ar-H Z, 6-H or 4-H), 7.45 (t, *J* = 7.8 Hz, 1 H, 5-H), 7.70−7.80 (m, 2 H, 4-H or 6-H, 2-H), 7.87 (d, *J* = 9.3 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. The assignments were supported by spin decoupling-double resonance technique. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 17.8 (β-C Ala), 22.0 (γ-C Lys), 28.7 (β-C Lys), 33.8 (δ-C Lys), 38.8 (ε-C Lys), 40.2 (NMe_2), 43.5 (CH_2), 48.0 (α-C Ala), 52.4 (OMe), 54.4 (α-C Lys), 66.9 (CH_2 Z), 111.4 (C-2′, C-6′), 121.3 (C-4), 122.8 (C-3′, C-5′), 125.0 (C-3 Z, C-5 Z), 128.0 (C-2), 128.1 (C-4 Z), 128.4 (C-2 Z, C-6 Z), 129.5 (C-5), 130.2 (C-6), 135.8 (C-1), 136.2 (C-1 Z), 143.4 (C-4′), 152.5 (C-3), 153.4 (C-1′), 156.2 (NHCO_2CH_2Ph), 171.0 (CONH Lys), 171.4 (CONH Ala), 173.2 (CO_2Me) ppm. $C_{34}H_{42}N_6O_6$ (630.72): calcd. C 64.74, H 6.71, N 13.33; found C 64.79, H 6.70, N 13.23.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}-*N*-*tert*-butyloxycarbonylphenylalanine Ethyl Ester (17c):** DMAP (4.3 mg, 0.035 mmol) was added to a solution of **5c** (161 mg, 0.35 mmol) in dry acetonitrile (5.0 mL) followed by di-*tert*-butyl pyrocarbonate (368 mg, 1.68 mmol) and the mixture stirred vigourously for two days at room temperature, the reaction being monitored by TLC. Evaporation of the solvents under reduced pressure followed by dry chromatography on silica gel with diethyl ether/hexane (6:4) as the eluent gave ester **17c** (91 mg, 46%). TLC (diethyl ether/hexane, 6:4): R_f = 0.53. UV/Vis (MeOH): λ_{max} = 416 nm (ε = 17646 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). IR (film): $\tilde{\nu}$ = 3252, 3000, 1749, 1732, 1714, 1692, 1676, 1602, 1520, 1456, 1371, 1355 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.22 (t, *J* = 7.0 Hz, 3 H, OCH_2CH_3), 1.44 (s, 9 H, CMe_3), 3.09 (s, 6 H, NMe_2), 3.40−3.52 (m, 2 H, β-CH_2 Phe), 4.00−4.30 (m, 4 H, OCH_2CH_3, CH_2), 5.49−5.58 (m, 1 H, α-CH Phe), 6.77 (d, *J* = 9.3 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.07 (d, *J* = 8.1 Hz, 1 H, 6-H or 4-H), 7.10−7.30 (m, 5 H, 5 × Ar-H

Phe), 7.38 (t, J = 8.1 Hz, 1 H, 5-H), 7.58 (t, J = 1.5 Hz, 1 H, 2-H), 7.73 (d, J = 8.1 Hz, 1 H, 4-H or 6-H), 7.88 (d, J = 9.3 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 14.1 (OCH_2CH_3), 27.8 (CMe_3), 35.7 (β-C Phe), 40.3 (NMe_2), 44.1 (CH_2), 57.4 (α-C Phe), 61.3 (OCH_2CH_3), 84.1 (CMe_3), 111.4 (C-2′, C-6′), 121.1 (C-4), 123.0 (C-2), 124.8 (C-3′, C-5′), 126.6 (C-4 Phe), 128.4 (C-3 Phe, C-5 Phe), 129.4 (C-2 Phe, C-6 Phe), 129.5 (C-5), 130.6 (C-6), 135.6 (C-1), 137.4 (C-1 Phe), 143.6 (C-4′), 151.9 (C-3), 152.3 (C-1′), 153.1 (CO_2CMe_3), 170.2 (CONH), 173.4 ($CO_2CH_2CH_3$) ppm. HRMS: calcd. for $C_{32}H_{38}N_40_5$ [M^+] 558.2842; found 558.2818.

***N*-{3-[(*N*,*N*-Dimethylaminophenyl)-4′-diazenyl]phenylacetyl}-*N*-*tert*-butyloxycarbonylalanine Methyl Ester (17d):** The product of the reaction of **5d** (100 mg, 0.27 mmol) with DMAP and di-*tert*-butyl pyrocarbonate under the conditions reported above for compound **17c** was chromatographed with diethyl ether/hexane (6:4) as the eluent to give ester **17d** (56 mg, 48%) as an orange residue. M.p. 92.0−94.0 °C. TLC (diethyl ether/hexane, 6:4): R_f = 0.53. UV/Vis (MeOH): λ_{max} = 417 nm (ε = 23090 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.46 (s, 9 H, CMe_3), 1.48 (s, 3 H, β-CH_3 Ala), 3.09 (s, 6 H, NMe_2), 3.67 (s, 3 H, OMe), 4.39 (d, J = 1.8 Hz, 2 H, CH_2), 5.33, 5.37 (2 × d, J = 6.6, 6.0 Hz, 1 H, α-CH Ala), 6.76 (d, J = 9.3 Hz, 2 H, 2 × Ar-H *ortho* NMe_2), 7.29 (d, J = 7.2 Hz, 1 H, 6-H or 4-H), 7.43 (t, J = 8.1 Hz, 1 H, 5-H), 7.72−7.78 (m, 2 H, 4-H or 6-H, 2-H), 7.88 (d, J = 9.3 Hz, 2 H, 2 × Ar-H *meta* NMe_2) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 15.4 (β-C Ala), 27.8 (CMe_3), 40.3 (NMe_2), 44.2 (CH_2), 51.8 (α-C Ala), 52.1 (OMe), 84.2 (CMe_3), 111.5 (C-2′, C-6′), 121.1 (C-4), 122.9 (C-2), 124.9 (C-3′, C-5′), 128.8 (C-5), 130.6 (C-6), 135.6 (C-1), 143.6 (C-4′), 151.9 (C-3), 152.3 (C-1′), 153.2 (CO_2CMe_3), 171.5 (CONH), 173.2 (CO_2Me) ppm. HRMS: calcd. for $C_{25}H_{32}N_40_5$ [M^+] 468.2373; found 468.2378.

***N*-*tert*-Butyloxycarbonylphenylalanine Ethyl Ester 18c by Aminolysis of 17c:** The colored substrate **17c** (62 mg, 0.11 mmol) was treated with DEAEA (62 × 10^{-3} mL, 0.44 mmol) for two days according to the procedure of Grehn[25] et al. The product was chromatographed with diethyl ether/hexane (2:8) as the eluent to give ester **18c** (16 mg, 50%) as an oil. TLC (chloroform/methanol, 5.8:0.2): R_f = 0.90. ^1H NMR spectroscopic data compared well with those of a genuine sample.[22]

***N*-*tert*-Butyloxycarbonylalanine Methyl Ester 18d by Aminolysis of 17d:** The product of the reaction of **17d** (46 mg, 0.10 mmol) with DEAEA (60 × 10^{-3} mL, 0.43 mmol) according to the procedure described above for compound **18c** was chromatographed with diethyl ether/hexane (2:8) as eluent to give ester **18d** (11.8 mg, 58%). TLC (ethyl acetate/hexane, 2:8): R_f = 0.67. ^1H NMR spectroscopic data compared well with those of a genuine sample.[22]

***N*-(3-Aminophenylacetyl)phenylalanine Ethyl Ester 19c by Chemical Reduction of 5c:** Reduction of **5c** (180 mg, 0.39 mmol) with zinc dust (144 mg, 2.2 mmol) in methanol in the presence of formic acid according to the procedure described by Gowda[26] et al. gave the corresponding ester **19c** (97.9 mg, 77%), as an oil. TLC (ethyl acetate/hexane, 8:2): R_f = 0.53. IR (film): $\tilde{\nu}$ = 3357, 3062, 3030, 2981, 2935, 1737, 1659, 1652, 1606, 1591, 1538, 1520, 1495, 1463, 1455, 1444, 1375, 1350, 1298, 1278, 1214, 1200, 1116, 1029, 701 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.23 (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 2.98−3.12 (m, 2 H, β-CH_2 Phe), 3.46 (s, 2 H, CH_2), 4.14 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 4.80−4.90 (m, 1 H, α-CH Phe), 5.93 (d, J = 8.3 Hz,1 H, α-NH Phe), 6.51 (s,1 H, 2-H), 6.53−6.68 (m, 2 H, 4-H, 6-H), 6.9−7.0 (m, 2 H, 3-H Phe, 5-H Phe), 7.11 (t, J = 7.2 Hz, 1 H, 5-H), 7.18−7.25 (m, 3 H, 2-H Phe, 4-H Phe, 6-H Phe) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 14.0 (OCH_2CH_3), 37.6 (β-C Phe), 43.6 (CH_2), 52.9 (α-C Phe), 61.4 (OCH_2CH_3), 114.1 (C-4), 115.8 (C-2), 119.4 (C-6), 126.9 (C-4 Phe), 128.4 (C-3 Phe, C-5 Phe), 129.2 (C-5), 129.9 (C-2 Phe, C-6 Phe), 135.4 (C-1), 135.7 (C-1 Phe), 146.9 (C-3), 170.5 (CONH), 171.3 ($CO_2CH_2CH_3$) ppm. HRMS: calcd. for $C_{19}H_{22}N_2O_3$ [M^+] 326.1630; found 326.1630.

***N*-(3-Aminophenylacetyl)alanine Methyl Ester 19d by Chemical Reduction of 5d:** Reduction of **5d** (100 mg, 0.27 mmol) with zinc dust (100 mg, 1.53 mmol) according to the procedure described above for compound **19c** gave the corresponding ester **19d** (42 mg, 65%) as an oil. TLC (ethyl acetate/hexane, 8:2): R_f = 0.31. IR (film): $\tilde{\nu}$ = 3360, 3042, 2986, 2953, 1739, 1658, 1651, 1607, 1538, 1494, 1461, 1436, 1375, 1357, 1300, 1245, 1216, 1168, 1055, 773 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.34 (d, J = 7.2 Hz, 3 H, β-CH_3 Ala), 3.71 (s, 5 H, CH_2, OMe), 4.50−4.64 (m, 1 H, α-CH Ala), 6.07 (d, J = 5.4 Hz, 1 H, α-NH Ala), 6.56−6.70 (m, 3 H, 2-H, 4-H, 6-H), 7.14 (t, J = 7.5 Hz, 1 H, 5-H) ppm. The assignments were supported by spin decoupling-double resonance technique. ^{13}C NMR (75.4 MHz, DMSO): δ = 18.2 (β-C Ala), 43.6 (CH_2), 48.0 (α-C Ala), 52.4 (OMe), 114.1 (C-4), 115.9 (C-2), 119.4 (C-6), 130.0 (C-5), 135.5 (C-1), 147.0 (C-3), 170.6 (CONH), 173.3 (CO_2Me) ppm. HRMS: calcd. for $C_{12}H_{16}N_2O_3$ [M^+] 236.1161; found 236.1160.

Stability Tests with Starting Material 5d

Acidolysis with Trifluoroacetic Acid: Trifluoroacetic acid (0.56 mL) was added to the fully protected amino acid **5d** (113 mg; 0.31 mmol) and the mixture stirred vigorously for 14 hours. Evaporation of the solvents under reduced pressure gave a red solid (113 mg; 100%). ^1H NMR spectroscopy confirmed the structure of the compound.

Acidolysis with Hydrochloric Acid: HCl (6 M, 0.50 mL) was added to the fully protected amino acid **5d** (50 mg; 0.14 mmol) and the mixture stirred vigorously for 25 minutes. Evaporation of the solvents under reduced pressure gave a red solid (47.6 mg; 84%). ^1H NMR spectroscopy confirmed the structure of the compound.

Catalytic Hydrogenation: A solution of **5d** (100 mg; 0.28 mmol) in methanol (3 mL) and 1,4-cyclohexadiene (92 × 10^{-3} mL; 0.98 mmol) was mixed with 10% palladium on charcoal catalyst (56 mg) and refluxed for 12 hours with stirring. The catalyst was filtered off and washed with methanol; the combined liquids were then evaporated to dryness under reduced pressure. Recrystallization from ethyl acetate and hexane afforded the compound as a red solid (100 mg, 100%). Its ^1H NMR was identical to that of the starting material.

Acknowledgments

We thank the Foundation for Science and Technology (Portugal) for financial support to the Institute of Biotechnology and Fine Chemistry (University of Minho).

[1] G. W. Case, P. J. Bekowies, *US Patent* 5413915 (**1995**).
[2] J. C. V. P. Moura, in: *Non-Antibiotics* (Eds.: A. N. Chakrabarty, J. Molnar), NISCOM, New Delhi, **1998**, pp. 327−356.
[3] G. M. Johnson, R. J. Schaeper, *US Patent* 5464739 (**1995**).
[4] B. Altenberg-Greulich, G. Vriend, *J. Mol. Struct.* **2001**, *598*, 1−8.
[5] J. Y. Chang, E. H. Creaser, K. W. Bentley, *Biochem. J.* **1976**, *153*, 607−611.
[6] J. Y. Chang, *Anal. Biochem.* **1988**, *170*, 542−556.

[7] A. D. Abell, B. C. H. May, B. K. Nabbs, *Peptides* **2001**, 785−786.
[8] A. D. Abell, D. C. Martyn, B. C. H. May, B. K. Nabbs, *Tetrahedron Lett.* **2002**, *43*, 3673−3675.
[9] E. H. J. M. Jansen, R. H. Van Den Berg, R. Both-Miedema, L. Doorn, *J. Chromatogr.* **1991**, *553*, 123−133.
[10] V. Stocchi, G. Piccoli, M. Magnani, F. Palma, B. Biagiarelli, L. Cucchiarini, *Anal. Biochem.* **1989**, *178*, 107−117.
[11] J. Y. Chang, R. Knecht, G. Braun, *Biochem. J.* **1982**, *203*, 803−806.
[12] T. W. Garner, E. S. Yeung, *Anal. Chem.* **1990**, *62*, 2193−2198.
[13] D. Parkinson, J. D. Redshaw, *Anal. Biochem.* **1984**, *141*, 121−126.
[14] M. Tzeng, *Anal. Biochem.* **1983**, *128*, 412−414.
[15] I. P. Griffith, *Anal. Biochem.* **1972**, *46*, 402−412.
[16] H. F. Bosshard, A. Datyner, *Anal. Biochem.* **1977**, *82*, 327−333.
[17] L. Fruk, A. Grondin, W. E. Smith, D. Graham, *Chem. Commun.* **2002**, 2100−2101.
[18] B. Pal, P. K. Bajpai, T. S. B. Baul, *Spectrochimica Acta Part A* **2000**, *56*, 2453−2458.
[19] F. Sebestyén, G. Szendrei, M. Mák, M. Dóda, E. Illyés, G. Szókán, K. Kindla, W. Rapp, P. Szegö, E. Câmpian, Á. Furka, *J. Pept. Sci.* **1998**, *4*, 294−299.
[20] F. Sebestyén, K. Kindla, W. Rapp, E. Câmpian, Á. Furka, in: *Peptides: Chemistry, Structure and Biology* (Eds.: P. T. P. Kaumaya, R. S. Hodges), Mayflower Scientific Ltd., **1996**; pp. 319−320.
[21] M. S. T. Gonçalves, H. L. S. Maia, *Tetrahedron Lett.* **2001**, *42*, 7775−7777.
[22] M. S. T. Gonçalves, H. L. S. Maia, *Org. Biomol. Chem.* **2003**, *1*, 1480−1485.
[23] J. I. N. R. Gomes, J. Griffiths, H. L. S. Maia, J. C. V. P. Moura, A. M. F. O. Campos, *Dyes and Pigments* **1991**, *17*, 269−278.
[24] G. R. Pettit, *Synthetic Peptides*, Litton Education Publishing Inc., New York, **1970**, *3*, 150.
[25] L. Grehn, K. Gunnarsson, U. Ragnarsson, *Acta Chem. Scand., Ser. B* **1987**, *B41*, 18−23.
[26] S. Gowda, K. Abiraj, D. C. Gowda, *Tetrahedron Lett.* **2002**, *43*, 1329−1331.

Receiced October 30, 2003

