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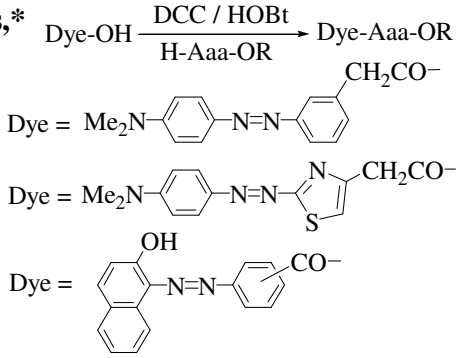

Chromophoric Azo Reagents for Amino Acid and Peptide Labeling

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

Introduction

The use of dyes in chemistry, biology and medicine is continuously growing, with new applications in the diagnostic 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 natural sample´s auto fluorescence or fluorescence created during sample preparation. This problem is avoided by the use of non-fluorescent dyes. Second, the need of 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 coloured 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] capillary[12] and polyacrylamide gel[13,14] electrophoresis.

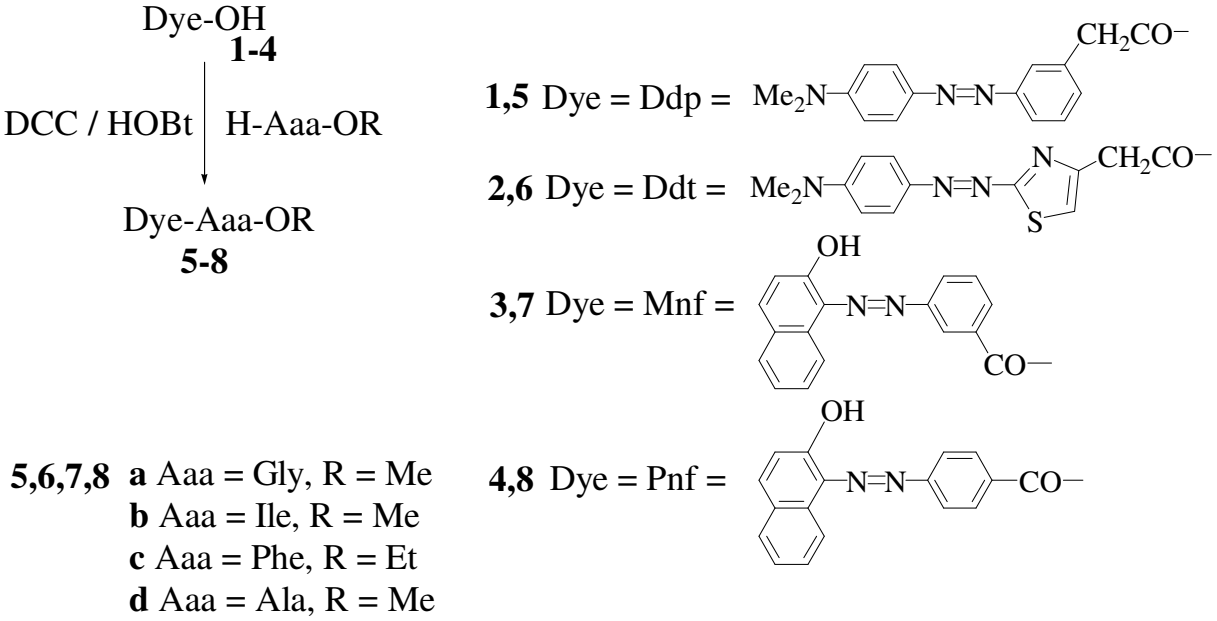
A method for staining proteins prior to polyacrylamide gel electrophoresis with Remazol Brilliant Blue R[15] and Drimarene Brilliant Blue,[16] two reactive dyes containing an ethyl sulfone and a difluorochloropyrimidyl group, respectively, was described. Non-fluorescent benzotriazole[17] and quinoline[18] 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 coloured peptide libraries labeled with monocarboxylic blue anthraquinone and red azo dyes.[19,20]

Having this in mind and following 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 labeling.

Results and Discussion

The new chromophores used (Scheme 1) were obtained by diazotation of 3-aminophenyl acetic acid, 2-amino-4-thiazoleacetic acid, 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 α-amine group of various amino acid esters by coupling them with the aid of carbodiimide (DCC) assisted by hydroxybenzotriazole (HOBt) under standard conditions. After purification by chromatography (dry or flash) on silica gel followed by recrystallisation, 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 recrystallised from acetone.



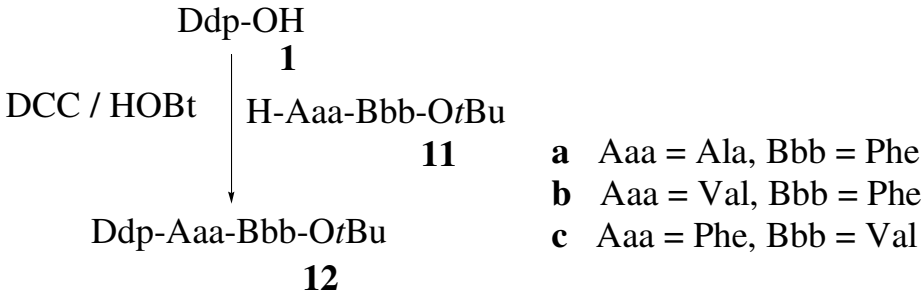
Scheme 1. Synthesis of labeled amino acid derivatives (**5-8**)

All labeled amino acids (**5-8**) were obtained as solid materials in yields ranging from 56 to 99% (Table 1) and were characterised by elemental analyses, by NMR (^{1}H and ^{13}C), IR and visible spectroscopy. The visible spectra of compounds **5** showed λ_{max} falling within 409 nm (**5d**) and 446 (**5b**), with ε values 31644 and 17282, respectively. Thiazole ring, compared to the benzene ring, produced a bathochromic shift as exemplified with compound **6d** where λ_{max} was 495 nm. When β-naphthol was used as coupling component instead of *N,N*-dimethylaniline, the resulting amino acid esters (**7, 8**) showed absorption peaks at λ_{max} 475 and 480, respectively.

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

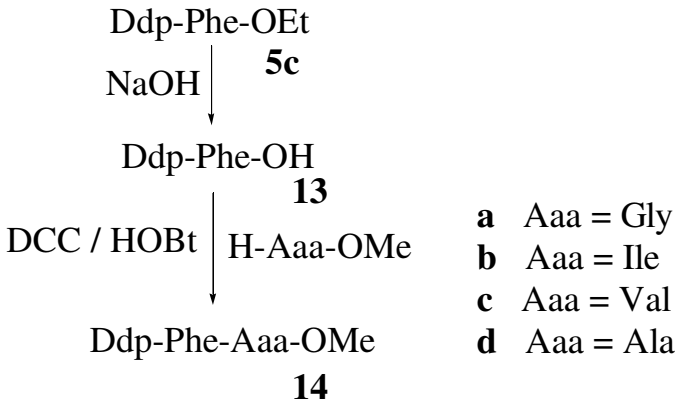
Product (compound no.)	Yield (%)
Ddp-Gly-OMe (**5a**)	77
Ddp-Ile-OMe (**5b**)	79
Ddp-Phe-OEt (**5c**)	91
Ddp-Ala-OMe (**5d**)	71
Ddt-Gly-OMe (**6a**)	85
Ddt-Ile-OMe (**6b**)	75
Ddt-Phe-OEt (**6c**)	77
Mnf-Gly-OMe (**7a**)	59
Mnf-Ile-OMe (**7b**)	56
Mnf-Phe-OEt (**7c**)	58
Pnf-Gly-OMe (**8a**)	75
Pnf-Ile-OMe (**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), coloured peptides **12** were obtained by acylation at their N-terminus with chromophore **1** (Scheme 2, Table 2).



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

Furthermore, dipeptides **14** were obtained in yields 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 to much as 5%. Previous work has shown a racemization of 50% when the benzoyl azo derivative was used.[22] As a result this marker represents a potential advancement to benzoyl azo derivatives in stepwise syntheses.



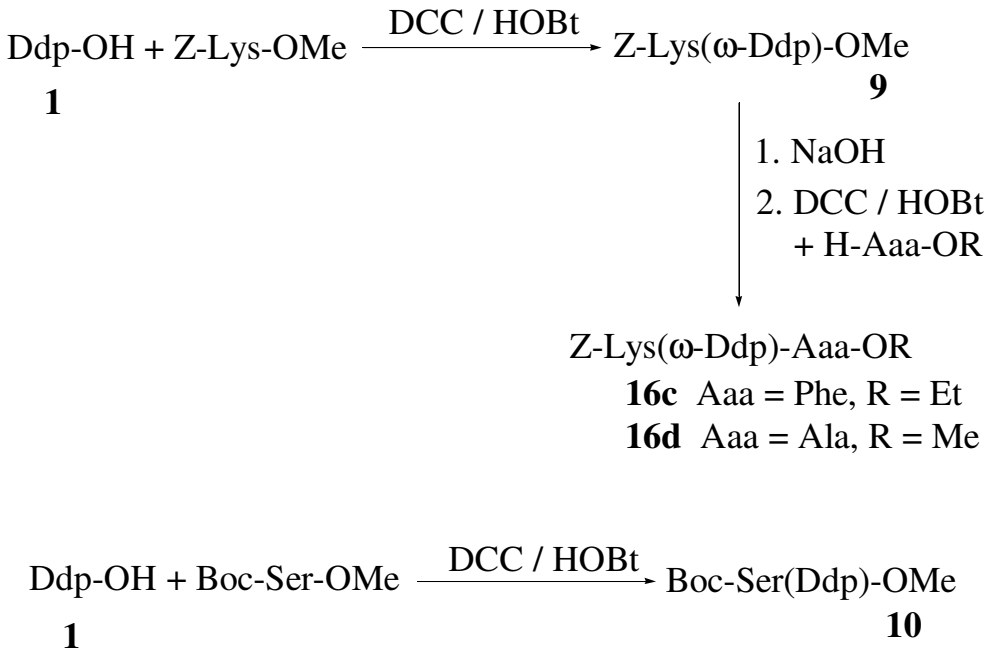
Scheme 3. Synthesis of labeled dipeptides **14**

Table 2. Yields obtained in the synthesis of labelled 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

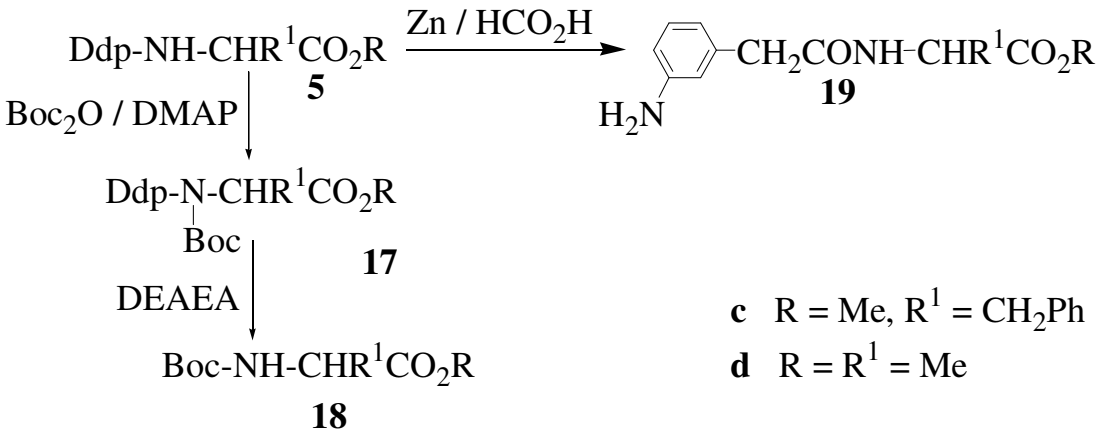
In addition to labeling amino acids or peptides at their N-terminus, an alternative acylation at a ω-amine group was also investigated. Thus, the methyl ester of *N*-benzyloxycarbonyl lysine was reacted with **1** (Scheme 4) under the conditions reported above, and the product (**9**) saponified quantitatively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

Another approach for side chain labeling was undertaken by reacting *tert*-butyloxycarbonylserine methyl ester with dye **1** to yield 86% of the corresponding ester derivative **10**. Coloured compounds were characterised as above and the visible spectra of all labeled peptides showed λ_{max} falling within 409 nm (ε 18532) (**14a**) and 415 (ε 21587) (**14d**). All products were stable on storage at room temperature without further precautions.



Scheme 4. Synthesis of labeled lysine and serine residues

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 reacted in fair yields with di-*tert*-butyl pyrocarbonate (Boc_2O) in the presence of *N,N*-dimethylamino pyridine (DMAP) to give compounds **17**. 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 colourless materials in yields 50 and 58%, 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 submitted to treatment with zinc powder and thus converted into the corresponding colourless 3-aminophenylacetyl derivatives **19** (Scheme 5, Table 3). Despite difficulties in isolating the required products, the latter method to convert the labeled compounds into colourless materials proved to lead to better yields than the former.

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

Conclusion

It was possible to obtain suitable coloured amino acid esters with maximum absorption peaks ranging from 400 to 500 nm by choosing the appropriate azo chromophore. Acetyl analogues such as those obtained from 3-aminophenyl acetic 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 shows that these chromophores can be used for peptide and protein labeling.

Experimental Section

General Remarks: All melting points are uncorrected and 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 visualised under UV or by exposure to vaporised 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 DMSO-d_6 (DMSO) solution at 25 ºC. All chemical shifts are given in δ ppm using δ_H Me_4Si = 0 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 in 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 hydroclorides were prepared with 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 synthesised 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): To a suspension of 3-aminophenyl acetic acid (3.11 g, 20 mmol) in 1 M HCl (82 mL) 6 M HCl (4.6 mL) was added with

stirring, followed by a cold aqueous solution (14 mL) of sodium nitrite (2.69 g; 30 mmol), the mixture being kept stirring at low temperature (< 5 °C) for 20 minutes. 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) the diazonium salt was added at room temperature and the mixture kept stirring for 2h. 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 $dm^3\ mol^{-1}\ cm^{-1}$). IR (KBr 1%): ν = 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* = 7.5Hz, 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 ºC). *N,N*-Dimethylaniline (1.9 mL) in acetic acid (53 mL) was added the diazonium solution slowly, keeping external cooling at 10 ºC 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 ºC 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 ºC. TLC (chloroform/methanol, 4:2): R_f = 0.69. UV/Vis (MeOH): λ_{max} = 490 nm (ε = 26855 $dm^3\ mol^{-1}\ cm^{-1}$). IR (film): ν = 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 acylation with dyes 1 to 4

Carboxylic dye (**1**-**4**) was reacted in 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 and dry (or column) chromatography on silica gel and recrystallisation from ethyl acetate-hexane, the required acetyl derivative (**5, 6**) was obtained. Compounds **7** and **8** were precipitated with water and recrystallised from acetone.

***N*-{3-[(*N,N*-Dimethylaminophenyl)-4'-diazenyl]phenylacetyl}glycine methyl ester (5a):** The product of reaction of **1** with glycine methyl ester hydrochloride (144 mg, 1.12 mmol) was chromatographed using 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 $dm^3\,mol^{-1}\,cm^{-1}$). IR (KBr 1%): ν = 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 reaction of **1** with isoleucine methyl ester hydrochloride (203 mg, 1.12 mmol) was chromatographed using 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 $dm^3\,mol^{-1}\,cm^{-1}$). IR (film): ν = 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 reaction of **1** with phenylalanine ethyl ester hydrochloride (257 mg, 1.12 mmol) was chromatographed using 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 $dm^3\,mol^{-1}\,cm^{-1}$). IR (KBr 1%): ν = 3320, 2931, 1730, 1642, 1602, 1542, 1518, 1455, 1362, 1344, 1231, 1154, 1115, 1039 cm^{-1}. ^{1}H 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 reaction of **1** with alanine methyl ester hydrochloride (156 mg, 1.12 mmol) was chromatographed using chloroform-methanol 5.5: 0.5 as the eluent to give the ester **5d** (293 mg, 71%). M.p. 138.6-140.0 ºC. TLC (chloroform/methanol, 5.5:0.5): R_f = 0.86. UV/Vis (MeOH): λ_{max} = 409 nm (ε = 31644 $dm^3\ mol^{-1}\ cm^{-1}$). IR (KBr 1%): ν = 3334, 2928, 2847, 1754, 1745, 1650, 1604, 1531, 1434, 1406, 1366, 1217, 1163, 1156, 1056, 825 cm^{-1}. ^{1}H 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 reaction of **2** with glycine methyl ester hydrochloride (144 mg, 1.12 mmol) was chromatographed using chloroform-methanol 6: 0.1 as the eluent to give the ester **6a** (344 mg, 85%). M.p. 161.2-162.7 ºC. TLC (chloroform/methanol, 6:0.1): R_f = 0.83. UV/Vis (MeOH): λ_{max} = 495 nm (ε = 26806 $dm^3\ mol^{-1}\ cm^{-1}$). IR (KBr 1%): ν = 3433, 3330, 2926, 2845, 1759, 1725, 1654, 1622, 1607, 1551, 1518, 1436, 1372, 1306, 1229, 1202, 1163, 979, 823 cm^{-1}. ^{1}H 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 reaction of **2** with isoleucine methyl ester hydrochloride (203 mg, 1.12 mmol) was chromatographed using chloroform-methanol 5.8: 0.2 as the eluent to give the ester **6b** (350 mg, 75%). M.p. 117.3-117.6 ºC. TLC (chloroform/methanol, 5.8:0.2): R_f = 0.68. UV/Vis (MeOH): λ_{max} = 493 nm (ε = 22253 $dm^3\ mol^{-1}\ cm^{-1}$). IR (KBr 1%): ν = 3250, 2726, 1741, 1735, 1698, 1654, 1601, 1540, 1523, 1458, 1371, 1305, 1258, 1148, 1131, 821, 666 cm^{-1}. ^{1}H 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 reaction of **2** with phenylalanine ethyl ester hydrochloride (257 mg, 1.12 mmol) was chromatographed using ethyl acetate/hexane 7:3 as the eluent to give the ester **6c** (401 mg, 77%). M.p. 124.0-124.5 ºC. TLC (ethyl acetate/hexane, 7:3): R_f = 0.71. UV/Vis (MeOH): λ_{max} = 495 nm (ε = 25927 $dm^3\ mol^{-1}\ cm^{-1}$). IR (KBr 1%): ν = 2921, 1723, 1718, 1691, 1643, 1606, 1558, 1542, 1523, 1466, 1451, 1370, 1339, 1288, 1157, 1125, 1109, 1034, 826 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.22 (t, *J* = 7.0 Hz, 3 H, $OCH_2C\mathit{H}_3$), 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, $OC\mathit{H}_2CH_3$), 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 ($OCH_2\mathit{C}H_3$), 37.8 (β-C Phe), 39.6 (CH_2), 40.3 (NMe_2), 53.5 (α-C Phe), 61.3 ($O\mathit{C}H_2CH_3$), 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 (CO_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 mol^{-1} cm^{-1}). ^{1}H 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 mol^{-1} 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 $dm^3\ mol^{-1}\ cm^{-1}$). ^{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 $dm^3\ mol^{-1}\ cm^{-1}$). ^{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 $dm^3\ mol^{-1}\ cm^{-1}$). ^{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 $dm^3\ mol^{-1}\ cm^{-1}$). ^{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 (1 H, s, NH) ppm. The assignments were supported by spin decoupling-double resonance. ^{13}C NMR (75.4 MHz, DMSO): δ = 14.4 ($OCH_2\mathit{C}H_3$), 36.7 (β-C Phe), 54.9 (α-C Phe), 61.0 ($O\mathit{C}H_2CH_3$), 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 ($\mathit{C}O_2CH_2CH_3$), 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 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 using 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 $dm^3\ mol^{-1}\ cm^{-1}$). ^{1}H 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, 2H, 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 ressonance 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 ($\mathit{C}O_2$ Z), 170.9 (CONH), 172.8 ($\mathit{C}O_2$Me) 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 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 using 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 $dm^3\ mol^{-1}\ cm^{-1}$). IR (KBr 1%): ν = 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}. ^{1}H 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 (CMe_3), 40.2 (NMe_2), 40.9 (CH_2), 52.7 (OMe), 52.8 (α-C Ser), 64.7 (β-C Ser), 80.2 (CMe_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 (CO_2CMe_3), 170.1 (CONH), 170.7 (CO_2CH_2), 171.3 (CO_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 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 using 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 $dm^3\ mol^{-1}\ cm^{-1}$). ^{1}H 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 ressonance 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 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 using diethyl ether-hexane 4:6 as the eluent to give the labeled dipetide **12b** (348 mg, 66%). M.p. 187.5-188.8 ºC. TLC (diethyl ether/hexane, 6:4): R_f = 0.63. UV/Vis (MeOH): λ_{max} = 414 nm (ε = 29146 $dm^3\ mol^{-1}\ cm^{-1}$). ^{1}H 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 ressonance 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 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 using 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 ºC. TLC (diethyl ether/hexane, 6:4): R_f = 0.69. UV/Vis (MeOH): λ_{max} = 411 nm (ε = 25029 dm^3 mol^{-1} cm^{-1}).^{1}H 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, 1H, 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 ressonance technique.^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 17.6 (γ-C Val), 18.7 (γ-C Val), 28.0 (*C*Me_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 (C*Me*$_3$), 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, *C*$O_2$$CMe_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):** To the fully protected amino acid **5c** (375 mg; 0.82 mmol) in 1,4-dioxane (4.10 mL) 1 M NaOH (1.23 mL, 1.23 mmol) was added at low temperature. The solution was stirred for 6 hours at 0 ºC 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): ν = 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 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 using chloroform-methanol 5.8:0.2 as the eluent to give dipeptide **14a** (284 mg, 81 %). M.p. 171.5-173.6 ºC. TLC (chloroform/methanol, 5.8:0.2): R_f = 0.51. UV (MeOH): λ_{max} = 409 nm (ε = 18532 $dm^3\ mol^{-1}\ cm^{-1}$). ^1H 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* = 5.1Hz, 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* = 8.1Hz, 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 ressonance 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 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 using chloroform-methanol 5.8:0.2 as the eluent

to give dipeptide **14b** (73 mg, 65%). M.p. 165.5-167.0 ºC. TLC (chloroform/methanol, 5.8:0.2): R_f = 0.65. UV/Vis (MeOH): λ_{max} = 410 nm (ε = 24986 $dm^3\ mol^{-1}\ cm^{-1}$). ^{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* = 7.3Hz, 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 ressonance 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 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 using diethyl ether-hexane 6:4 as the eluent to give dipeptide **14c** (152 mg, 60%). M.p. 164.0-165.9 ºC. TLC (diethyl ether/hexane, 8:2): R_f = 0.63. UV/Vis (MeOH): λ_{max} = 411 nm (ε = 20256 $dm^3\ mol^{-1}\ cm^{-1}$). ^{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 ressonance 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 (*C*O_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 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 using chloroform-methanol 5.8:0.2 as the eluent to give dipeptide **14d** (252 mg, 84%). M.p. 166.8-168.5 ºC. TLC (diethyl ether/hexane, 6:4): R_f = 0.77. UV/Vis (MeOH): λ_{max} = 415 nm (ε = 21587 $dm^3\ mol^{-1}\ cm^{-1}$). ^{1}H 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* = 8.1Hz, 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 ressonance 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 (*C*O_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):** To the fully protected amino acid **9** (187 mg, 0.34 mmol) in 1,4-dioxane (1.7 mL) 1 M NaOH (0.5 mL, 0.5 mmol) was added at low temperature. The solution was stirred at 0 ºC 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 ºC. ^{1}H 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 ressonance 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 (NH*C*O_2CH_2Ph), 171.7 (CONH Lys), 174.5 (*C*O_2Me) ppm.

***N*-Benzyloxycarbonyl-ω-{3-[(*N,N*-Dimethylaminophenyl)-4'-diazenyl]phenylacetyl}lysylphenylalanine ethyl ester (16c):** The product of 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 using 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 ºC. TLC (chloroform/methanol, 5.8:0.2): R_f = 0.44. UV/Vis (MeOH): λ_{max} = 412 nm (ε = 18704 dm^3 mol^{-1} cm^{-1}). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.20 (t, *J* = 6.9 Hz, 3 H, $OCH_2C\mathit{H}_3$), 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, $OC\mathit{H}_2CH_3$), 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 ressonance 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 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 using 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 ºC. TLC (chloroform/methanol, 5.8:0.2): R_f = 0.30. UV/Vis (MeOH): λ_{max} = 414 nm (ε = 22069 dm^3 mol^{-1} cm^{-1}). ^{1}H 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 ressonance 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):** To a solution of **5c** (161 mg, 0.35 mmol) in dry acetonitrile (5.0 mL) DMAP (4.3 mg, 0.035 mmol) was added followed by di-*tert*-butyl pyrocarbonate (368 mg, 1.68 mmol) under rapid stirring over two days at room temperature, the reaction being monitored by TLC. Evaporation under reduced pressure followed by dry chromatography on silica gel with diethyl ether-hexane 6:4 as the eluent to give ester **17c** (91 mg, 46%). TLC (diethyl ether/hexane, 6:4): R_f = 0.53. UV/Vis (MeOH): λ_{max} = 416 nm (ε = 17646 $dm^3\ mol^{-1}\ cm^{-1}$). IR (film): ν = 3252, 3000, 1749, 1732, 1714, 1692, 1676, 1602, 1520, 1456, 1371, 1355 cm^{-1}. ^{1}H 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 ºC. TLC (diethyl ether/hexane, 6:4): R_f = 0.53. UV/Vis (MeOH): λ_{max} = 417 nm (ε = 23090 $dm^3\ mol^{-1}\ cm^{-1}$). ^{1}H 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 coloured 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 data compared well with that 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 to give ester **18d** (11.8 mg, 58%). TLC (ethyl acetate/hexane, 2:8): R_f = 0.67. ^1H NMR data compared well with that 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): ν = 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): ν = 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 ressonance 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 trifluoracetic acid. To the fully protected amino acid **5d** (113 mg; 0.31 mmol) were added 0.56 mL of trifluoracetic acid under rapid stirring over 14 hours. Evaporation under reduced pressure gave a red solid (113 mg; 100%). ^1H NMR confirmed the structure of the compound.

Acidolysis with hydrochloric acid. To the fully protected amino acid **5d** (50 mg; 0.14 mmol) were added 6 M HCl (0.50 mL) under rapid stirring over 25 minutes. Evaporation under reduced pressure gave a red solid (47.6 mg; 84%). ^1H NMR confirmed the structure of the compound.

Catalytic hydrogenation. A solution of **5d** (100 mg; 0.28 mmol) in methanol (3mL) and 1,4-cyclohexadiene ($92x10^{-3}$ 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 under reduced pressure. Recrystallisation

from ethyl acetate and hexane afforded the compound as a red solid (100 mg, 100%). ^{1}H NMR was well compared with the starting material.

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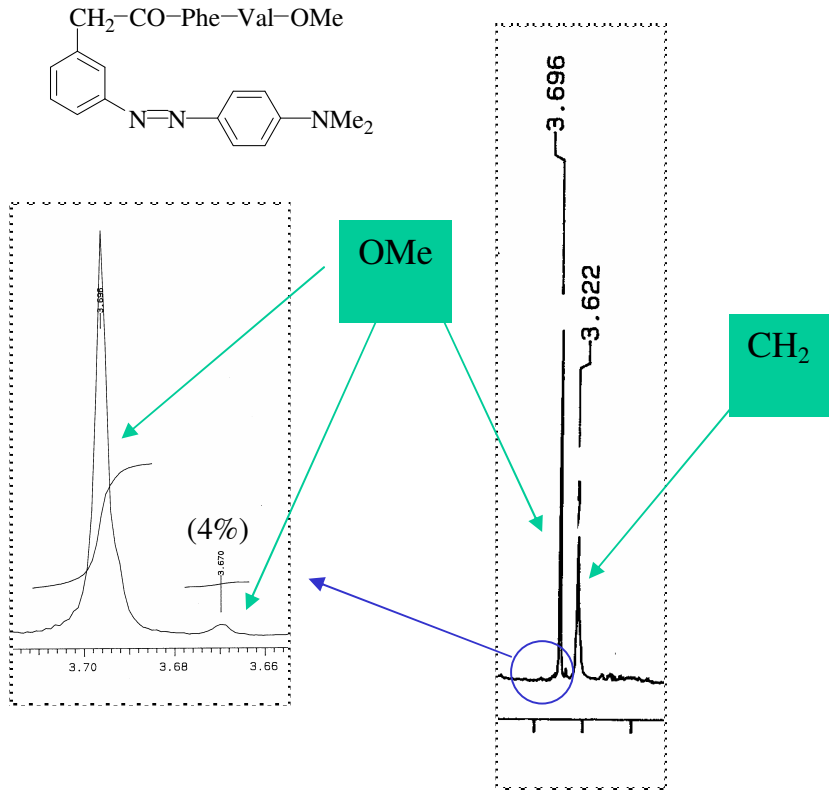

Figure 1. ^{1}H NMR spectrum of Ddp-Phe-Val-OMe (**14c**) before purification

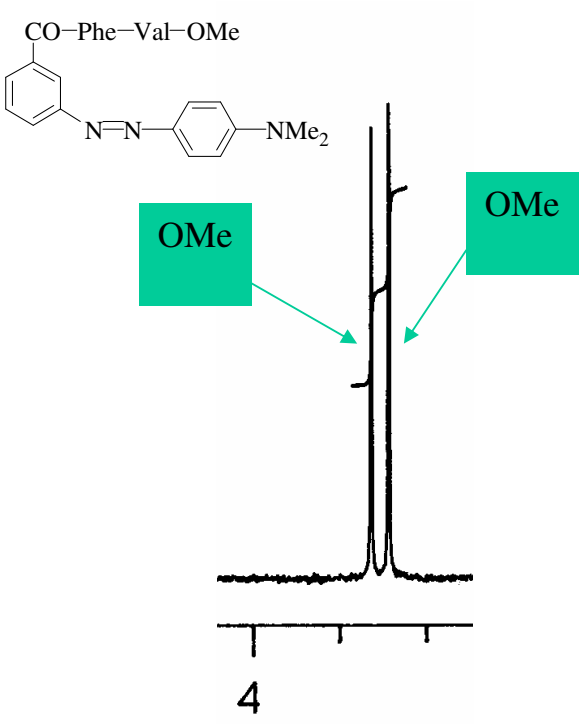

Figure 2. ^{1}H NMR spectrum of
Dpa-Phe-Val-OMe before purification