Design of New Photoactivatable Amino Acids: Stereoselective Synthesis of N-Protected Phenylalanine Derivatives as Precursors of *p*-Diazocyclohexadienone-Containing Peptides†

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Received October 24, 1994®

4-Diazocyclohexa-2,5-dienone-based amino acids *m*-Dip and *o*-Dip were designed for building up photoactivatable peptides. Their stable precursors L-3-[3-(benzyloxy)-6-nitrophenyl]alanine (L-*m*-Nip(Bn)) and L-3-[2-(benzyloxy)-5-nitrophenyl]alanine (L-*o*-Nip(Bn)) were synthesized by stereoselective alkylation of the corresponding benzyloxynitrobenzyl iodides by a chiral glycine equivalent. Alkylation was carried out using either butyllithium in dry organic solvents or a phase transfer procedure. Alkylation, hydrolysis of the adduct, and protection as Boc and Fmoc derivatives were achieved in 57–73% overall yields and led to 97–99% optically pure material. Boc or Fmoc-*m*/*o*-Nip(Bn) was inserted in model dipeptides Ac-*m*/*o*-Nip(Bn)-Ala-OMe or tripeptides Ala-L-*m*-Nip-(Bn)-Lys and Ala-L-*o*-Nip(Bn)-Arg, respectively, by homogeneous solution procedure and by solid-phase peptide synthesis. Deprotection and diazotization of the resulting *p*-hydroxyanilines gave the corresponding photoactivatable 4-diazocyclohexa-2,5-dienones containing peptides in quantitative yields. Such photoprobes are stable for several hours in the dark but are rapidly photolyzed at 350 nm or at 295 nm by a tryptophan-mediated energy transfer activation process.

Introduction

Photolabeling[1] is a well-suited approach for isolation, identification, and characterization of receptors and enzymes.[2] A wide variety of photoprobes was synthesized and successfully used for investigating ligand-binding sites on complex proteins.[3] Many of them are peptides containing one or several photoactivatable amino acids.[4] In most cases, hydrophobic azidophenylalanine[5] and benzoylphenylalanine[6] were employed and, at present, few hydrophilic photoreactive residues have been used in photolabeling experiments.[7] Among several photoreactive groups recently described such as trifluoromethyldiazirine[8] or polyfluoroaryl azides,[9] the 4-diazocyclohexa-2,5-dienone (DCD) derivatives developed by Hirth and co-workers[10] are moderately hydrophilic[11] and possess interesting characteristics: they are resistant to various conditions in the dark including mild nucleophiles and weak alkali and strong acidic media[12] in which the azido group is unstable;[13] they are less bulky and react much more easily than benzophenone-related compounds; they strongly absorb at 350 nm ($\epsilon > 25\,000\ M^{-1}\cdot cm^{-1}$); upon irradiation they give a highly reactive carbene which inserts in many chemical groups including C–H bonds.[10–12] The carbene does not rearrange, whereas nitrenes resulting from decomposition of arylazido derivatives are less reactive and unpredictably rearrange to didehydroazepines;[14] moreover, this short-lived intermediate avoids the use of millimolar concentrations of scavengers in photolabeling experiments.[15] In addition, *p*-diazocyclohexadienones may undergo an energy trans-

† This paper is dedicated to Pr. Christian Hirth (deceased May 28, 1992).

® Abstract published in *Advance ACS Abstracts,* January 1, 1995.

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fer activation by a protein-derived tryptophan residue: irradiation at 290–295 nm (ϵ_{DCD} = 500–1000 $M^{-1}cm^{-1}$) induces a fluorescence emission of tryptophan at 349 nm which remarkably increases the labeling selectivity.[16] Until now, information concerning the behavior of DCD compounds toward biological targets has been scarce; however, first results are encouraging: butyrylcholinesterase from the human serum was inactivated up to 60% by a photosuicide DCD derivative;[17] Kessler et al. reported the 80% irreversible inhibition of acetylcholinesterase by an acetylcholine derivative bearing a DCD;[12] in a recent paper, Goeldner and co-workers showed that $GABA_A$ receptor is partially photoinactivated by a bromo derivative of DCD.[17]

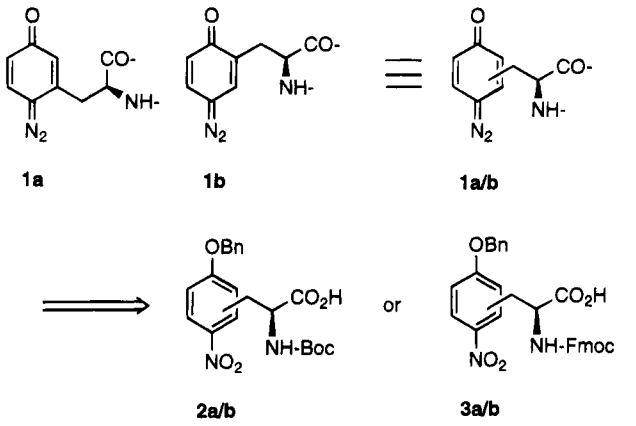
All these reasons prompted us to undertake the development of *p*-diazocyclohexadienone-based amino acids *m*-Dip (**1a**) and *o*-Dip (**1b**) (noted **1a/b**), as a new category of photoprobes. Althought compounds **1a/b** have nonaromatic structures and are sensibly more hydrophilic than tyrosine,[11] their highly conjugated six-membered ring and their moderate steric hindrance make them tightly related to these residues. On the basis of their hydrophilic properties, DCD could also be substituted to noncharged hydrophilic amino acids such as asparagine or glutamine.[11] On this assumption, DCD-derived residues are proposed as versatile photoactivatable amino acids and should be substituted to various amino acids in peptides. Moreover, the reactive position is located very close to the peptidic backbone (three or four bonds between C_α and the carbene) and should restrict the parisitic long-range labeling.

Due to their high sensitivity to light and nucleophiles, DCD-containing amino acids are not consistent with traditional synthesis; however, they can be generated from their corresponding stable precursors, the N-protected L-3-[3-(benzyloxy)-6-nitrophenyl]alanine (*m*-Nip(Bn) **2a** and **3a**) and L-3-[2-(benzyloxy)-5-nitrophenyl]-alanine (*o*-Nip(Bn), **2b** and **3b**) (Scheme 1). In this paper we wish to describe the stereoselective synthesis of compounds **2a/b** and **3a/b**, their spectral characteristics, and their insertion into model peptides by Boc and Fmoc homogeneous solution procedures and by solid-phase peptide synthesis.

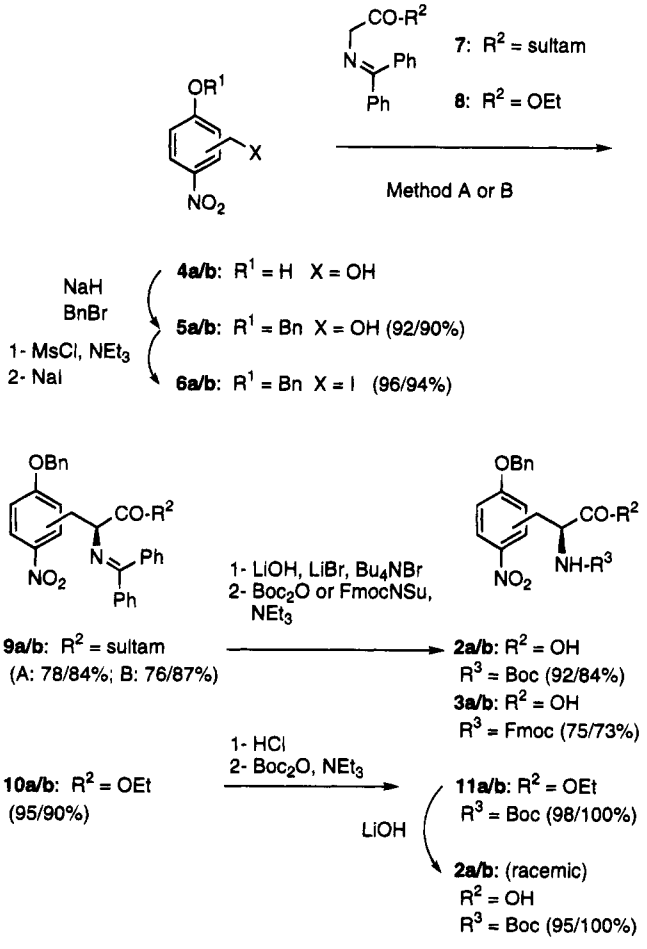
Results and Discussion

Synthesis of Protected Amino Acids. Diastereoselective alkylation of N-protected glycine bornane–sultam derivatives according to Oppolzer and co-workers is an easy and efficient route to natural and unnatural orthogonally protected L or D amino acids: satisfactory chemical yields and high enantiomeric excesses have been reported especially with bulky benzyl-related electrophiles.[18] **2a** and **2a/b** were stereoselectively synthesized in three steps by the route depicted in Scheme 2. Iodides **6a/b** were synthesized from **4a/b** by selective benzylation of the phenate[19] and by a two-step activation of the corresponding benzyl alcohols into iodides[20] (Scheme

Scheme 1. Structures of *o*-Dip and *m*-Dip Residues Obtained by Catalytic Hydrogenation and Diazotization of Their N-Protected Precursors *o*-Nip(Bn) and *m*-Nip(Bn)



Scheme 2. Synthesis of Boc and Fmoc-L-*m*/*o*-Nip(Bn)[a]



[a] Sultam = (1*R*)-(+)-2,10-camphor sultam. All yields are calculated from analytically pure material. Method A: BuLi/THF–HMPA. Method B: Bu_4NHSO_4, 1 N NaOH.

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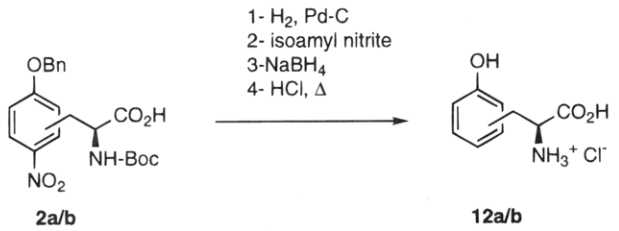
Scheme 3. Chemical Correlation of Boc-L-*m*/*o*-Nip(Bn) with L-*m*/*o*-Tyr



2a/b **12a/b**

2). The chiral glycine equivalent **7** was prepared from the commercially available ethyl ether **8** according to Josien et al.[21] The diastereoselective alkylations of **7** by **6a/b** were carried out in good yields using either Oppolzer's method[18,21] (method A: butyllithium in THF–HMPA, overnight at 20 °C; 78/84%) or a phase transfer reaction following O'Donnell's procedure[22] (method B: 1 N sodium hydroxide, tetrabutylammoniumhydrogen sulfate in dichloromethane; 76/87%). Saponification of adducts **9a/b** in biphasic conditions[21] led directly to the free amino acids which were protected as Boc[23] and Fmoc[24] derivatives **2a/b** and **3a/b**, respectively, in 57–73% overall yields. In order to evaluate the diastereoselectivity of the kinetically controlled alkylation, trimethylaluminum-catalyzed transamidation of D,L-ethyl esters **10a/b** with (+)-2,10-boranesultame failed whether **10a/b** was unreactive ($AlMe_3$/toluene, 50–110 °C, 16 h) or decomposed in previously described conditions ($AlMe_3$/xylene, 135 °C, 16 h).[25] Finally, both enantiomers of **2a/b** were separated on a chiral HPLC analytical column (Nucleodex γ-PM). Elution of the optically active compounds **2a** and **2b** indicated that alkylations were achieved with strong diastereoisomers excesses (typically 99%). Recrystallization of **9a/b** did not afford diffracting crystals. In order to confirm the L configuration of the title compounds, **2a/b** were reduced into *p*-hydroxyanilines and diazotized (Scheme 3). One-pot reduction of the resulting *p*-diazocyclohexadienones with excess sodium borohydride[26] and acidic hydrolysis led to previously described *m*- and *o*-tyrosines[27] **12a** and **12b** (described: **12a** $[\alpha]^{25}_D = -7.9°$; **12b** $[\alpha]^{25}_D = -26.8°$ ($c = 2$, 1 N HCl); observed: **12a** $[\alpha]^{25}_D = -9.6°$; **12b** $[\alpha]^{25}_D = -25.9°$ ($c = 2$, 1 N HCl)). This unambiguously confirmed that alkylation of Oppolzer's glycine proceeded with an almost total *Si* face approach of the transition state enolate, even with multifunctionalized electrophiles.

Effects of Other Amino Acids on Diazotization of *p*-Hydroxyanilines. Little information was available concerning the influence of potentially nucleophilic amino acid side chains on diazotization of *p*-hydroxyanilines. Kessler et al. showed that addition of 100 equiv of amino acids with respect to *p*-hydroxyaniline (2×10^{-5} M, pH 7.2) only slightly increased the spontaneous decomposition of DCD.[12] We observed that diazotization of *p*-hydroxyaniline using 1 equiv of isoamyl nitrite[28] did not affect either lysine, arginine, or histidine (8 equiv relative to *p*-hydroxyaniline 10^{-2} M), which are positively charged in acidic medium (Figure 1). In all cases more than 90% of the initial DCD remained after 24 h in acetic acid at room temperature. DCD was a bit less stable in diluted solution (pH 7.6, $c = 10^{-3}$ M, 10–15% chimiolysis). Althought cysteine was not supposed to interfere in diazotization, it definitely reacted with DCD: almost 35% of the DCD was quenched after 24 h at pH 7.6. This reflected the relative sensitivity of this photoactivatable group to alkali and strong nucleophiles. Assuming that 4-diazocyclohexa-2,5-dienone is much unstable than its substituted derivatives, these preliminary results suggested that DCD can be generated in totally deprotected peptides and could be stored in the dark at least several hours in neutral or slightly alkaline buffers and several days in acetic acid without reliable loss of material.

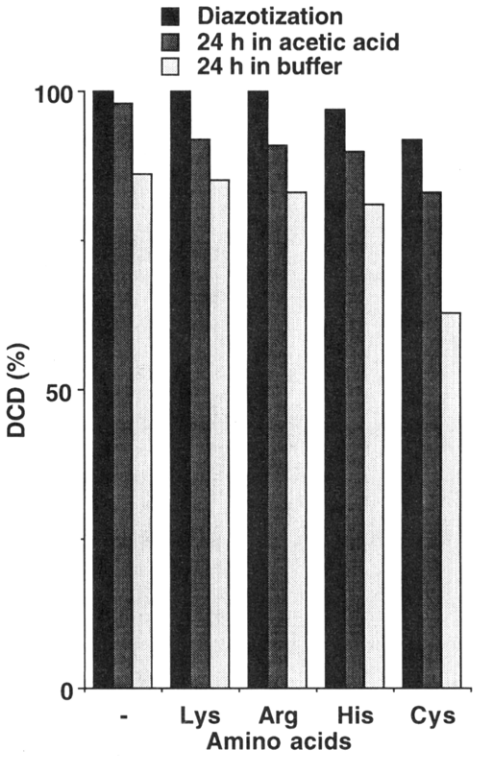


Figure 1. Remaining DCD in percent after diazotization (1 equiv of isoamyl nitrite in glacial acetic acid), 24 h in acetic acid ($c = 10^{-2}$ M) or 24 h in a Tris–HCl buffer (0.1 M, pH 7.6, $c = 10^{-3}$ M) in the presence of 8 equiv of lysine, arginine, histidine, or cysteine at 25 °C.

Synthesis of Model Peptides. In order to ensure that Boc and Fmoc-*m*/*o*-Nip(Bn) can be introduced in peptidic probes by standard peptide synthesis and to confirm that diazotization of *p*-hydroxyanilines is consistent with peptide integrity, two series of model peptides were built up (Scheme 4). Compounds **2a/b** and **3a/b** were inserted into hydrophobic dipeptides Ac-*m*/*o*-Nip(Bn)-Ala-OMe (**13a/b**) using the standard homogeneous solution procedure. Hydrophilic tripeptides Ala-*m*-Nip-Lys (**15**) and Ala-*o*-Nip-Arg (**16**) were almost quantitatively obtained from **2a/b** by solid phase peptide synthesis and anhydrous fluorohydric acid deprotection. *p*-(Benzyloxy)nitrophenyl- and *p*-nitrophenol-containing peptides can be stored at least 3 months in the dark at −20 °C without detectable degradation. In contrast, the corresponding *p*-hydroxyanilines proceeding from catalytic hydrogenation of the protected peptides are highly

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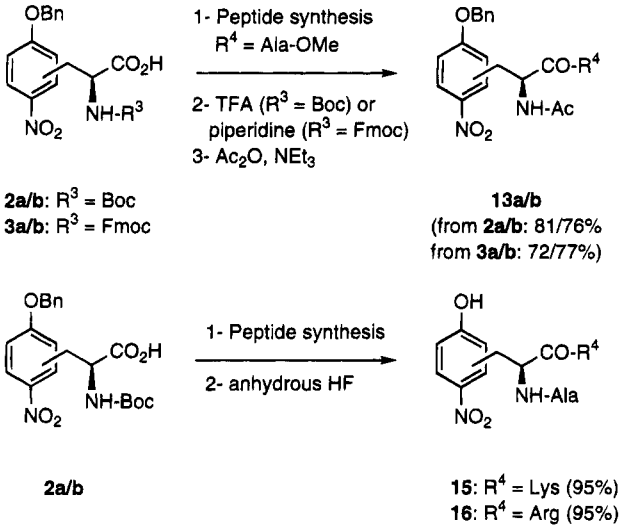
Scheme 4. Synthesis of Model Peptides Ac-L-*m*/*o*-Nip-Ala-OMe, Ala-L-*m*-Nip-Lys* and Ala-L-*o*-Nip-Arg*[a]



[a] Yields are calculated from analytically pure peptides or (*) evaluated by HPLC (analytical column Bondasorb C_{18} 10 μm, TFA 0.1%, 1 mL·min^{-1}).

oxidizable even in acidic solutions and must be diazotized immediately.

Diazotization of Peptides and Photolysis Experiments. Diazotization of reduced peptides was carried out in the dark at room temperature using 1 equiv of isoamyl nitrite, and the DCD-derived peptides **14a/b**, **17**, and **18** were purified by HPLC (Table 1). High molar absorbances (32 000–35 000 M^{-1}·cm^{-1}) were measured around 350 nm. These results are consistent with values usually reported for analogous compounds and confirmed that diazotization did not affect any other part of the peptide. DCD-containing peptides presented satisfactory stabilities in the dark (typically less than 1% degradation at 10 °C, 3% at 25 °C, and 5% at 40 °C after 24 h in a Tris–HCl buffer, 0.1 M, pH 7.6). This indicated that such photoprobes can be incubated for several hours at physiological pH and temperature when optimal interaction with the target requires such conditions. On the other hand, peptides **14a/b**, **17**, and **18** were readily photolyzed by irradiation at 350 nm ($E = 10^{-2}$ mW·cm^{-2}), (Table 1; Figure 2). Despite slight shifts of λ_{max} (**13a**: +13 nm; **13b**: +7 nm), no significant modification of photolysis rates was observed in octanol, which is commonly accepted as a mine of intramembranar area. This showed that hydrophobic environment does not sensibly modify DCD reactivity. At 295 nm ($E = 10^{-2}$ mW·cm^{-2}) photolysis of peptides was five to six times slower but was accelerated at least three times by addition of 1 equiv of *N*-acetyltryptophan ($c = 10^{-5}$ M) (Table 2). The tryptophan-mediated energy transfer process is more efficient in a hydrophobic environment and is less effective with hydrophilic peptides in buffer.

Conclusion

We synthesized two new photoactivatable amino acids bearing a DCD in 57–73% overall yields and in high enantiomeric excesses (typically more than 99%). These residues, conveniently protected for Boc and Fmoc peptide synthesis, are fully consistent with standard homogeneous solution or solid phase procedures. Stability of model peptides was satisfactory even at physiological temperatures as long as they were protected from natural light. All the spectral characteristics of *m*-Dip and *o*-Dip illustrate the promising potentials of such highly reactive photoactivatable amino acids in photolabeling experiments.

Experimental Section

General Procedures. All reagents employed were of analytical grade and were purchased from Aldrich Chemical Co., Lancaster Synthesis Ltd., and SDS Co. Amino acids were purchased from Bachem and Novabiochem. THF was distilled before use from sodium–benzophenone, and DMF was dried over activated molecular sieves. HMPA was distilled from sodium and kept over molecular sieves. All other solvents were of analytical grade and were used without further purification. Flash chromatography was performed on a 40–60 μm (230–400 mesh) SDS gel. NMR J values are given in Hz. Mass combustion analyses were carried out by the "Service Central d'Analyse" of the CNRS (Vernaison, France).

5-(Benzyloxy)-2-nitrobenzyl Alcohol/2-(Benzyloxy)-5-nitrobenzyl Alcohol (5a/b). Compound **4a/b** (8.7 g, 50 mmol) was treated 30 min by 80% sodium hydride (1.5 g, 50 mmol) in THF–DMF 80–20 (125 mL) at 30–40 °C. Benzyl bromide (6.07 mL, 50 mmol) was added slowly, and the mixture was stirred for 2 h at room temperature. After removal of the solvent under reduced pressure, the product was poured into 10% citric acid and was extracted with ethyl acetate. The organic layer, washed three times with saturated sodium carbonate, water, and brine, was dried over sodium sulfate. Removal of the solvent under reduced pressure and purification by silica gel flash–chromatography (eluent: ethyl acetate–cyclohexane 0–100 then 50–50) and recrystallization in cyclohexane gave the following compounds.

5a (11.96 g): 92% yield as a pale yellow solid (mp = 94–95 °C); IR ($CHCl_3$) 3620, 3020 + 3005, 2920, 1605 + 1575, 1510, 1335 + 1325, 1285 + 1255; ^{1}H NMR (C_6D_6O) δ 8.16 (d, J = 9.0, 1H), 7.49 (m, 6H), 7.10 (dd, J = 2.5, J' = 9.0, 1H), 5.29 (s, 2H), 5.03 (d, J = 5.4, 2H), 4.73 (d, J = 5.4 1H); ^{13}C NMR (C_6D_6O) δ 163.9, 142.9, 140.7, 137.1, 129.2, 128.8, 128.4, 128.0, 114.4, 113.7, 70.9, 61.7; MS (EI) m/z = 259 (M). Anal. Calcd for $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40. Found: C, 65.12; H, 5.11; N, 5.51.

5b (11.64 g): 90% yield as a white solid (mp = 96–97 °C); IR ($CHCl_3$) 3600, 3020 + 3010, 2930, 1615 + 1595, 1515, 1340, 1265 + 1250; ^{1}H NMR (C_6D_6O) δ 8.41 (d, J = 2.9, 1H), 8.16 (dd, J = 2.9, J' = 9.0 1H), 7.43 (m, 5H), 7.25 (d, J = 9.0, 1H), 5.33 (s, 2H), 4.79 (broad s, 2H), 4.70 (d, J = 7.9, 1H); ^{13}C NMR (C_6D_6O) δ 161.2, 142.4, 137.2, 133.3, 129.4, 128.9, 128.3, 124.7, 122.9, 112.1, 71.2, 59.0; MS (EI) m/z = 259 (M). Anal. Calcd for $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40. Found: C, 64.88; H, 5.18; N, 5.66.

5-(Benzyloxy)-2-nitrobenzyl Iodide/2-(Benzyloxy)-5-nitrobenzyl Iodide (6a/b). Compound **5a/b** (10.4 g, 40 mmol) was treated (1 h at 0 °C with freshly distilled methanesulfonyl chloride (3.50 mL, 44 mmol) and triethylamine (6.8 mL, 48 mmol) in dry THF (200 mL). The reaction was quenched by addition of cold 10% citric acid, and the product was extracted with ethyl acetate. The organic layer was washed with brine and dried over sodium sulfate, and the volume was reduced under vacuum. The crude product was treated for 2 h at room temperature by sodium iodide (26 g, 160 mmol) in dry acetone (220 mL). The solvent was removed under reduced pressure at room temperature, and the red product was dissolved in ethyl acetate and washed with cold 10% citric acid and 10% sodium thiosulfate until the color faded. Removal of the solvent under reduced pressure gave the following compounds.

6a (14.11 g): 96% yield as a yellow solid (mp = 91–93 °C); IR ($CHCl_3$) 3020 + 3010, 2920, 1600 + 1575, 1515, 1335, 1290 + 1255; ^{1}H NMR ($CDCl_3$) δ 8.11 (d, J = 9.1, 1H), 7.42 (bs, 5H), 7.01 (d, J = 2.7, 1H), 6.94 (dd, J = 2.7, J' = 9.1, 1H), 5.14 (s, 2H), 4.77 (d, J = 5.4, 2H); ^{13}C NMR ($CDCl_3$) δ 162.5, 137.8, 135.2, 129.1, 128.8, 128.6, 128.5, 127.5, 117.7, 114.5, 70.7, 1.0; MS (EI) for $C_{14}H_{12}INO_3$ m/z = 369 (M).

Table 1: Spectra Properties and Half-Lives of Diazotized Peptides in the Dark (T = 40 °C) and at 350 nm (T = 10 °C); c = 5 × 10^{-5} M, E = 10^{-2} mW × cm^{-2} in a Tris–HCl Buffer (0.1 M, pH 7.6) or ([b]) in Octanol. DCD-Containing Peptides Were Obtained by Diazotization with Isoamyl Nitrite in Acetic Acid (14a/b) or in 1 N HCl (17 and 18)

protected peptides (sequences)	diazotized peptides (sequences)	λ_{max} (nm)	ϵ (M^{-1}·cm^{-1})	$t_{1/2}$ (days) (dark)	$t_{1/2}$ (s) (350 nm)
13a (Ac-L-*m*-Nip-OMe)	**14a** (Ac-L-*m*-Dip-OMe)	351 364[b]	33 500 32 500[b]	>8 ND[b]	60 60[b]
13b (Ac-L-*o*-Nip-OMe)	**14b** (Ac-L-*o*-Dip-OMe)	354 361[b]	32 000 29 500[b]	8 ND[b]	40 60[b]
15 (Ala-L-*m*-Nip-Lys)	**17** (Ala-L-*m*-Dip-Lys)	350	35 000	7	70
16 (Ala-L-*o*-Nip-Arg)	**18** (Ala-L-*o*-Dip-Arg)	354	34 500	>8	45

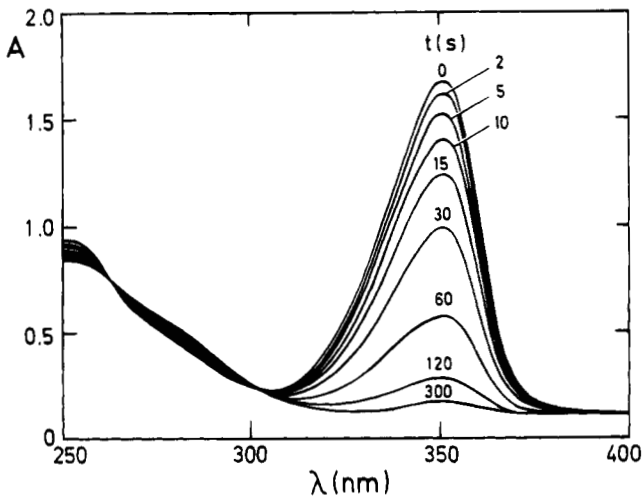


Figure 2. Photolysis of peptide **14a** in a Tris–HCl buffer (0.1 M, pH 7.6) at 350 nm (c = 5 × 10^{-5} M, E = 10^{-2} mW·cm^{-2}).

Table 2: Half-Lives of Diazotized Peptides at 295 nm without (–Ac-Trp) or with (+ Ac-Trp) 1 Equiv of *N*-Acetyltaryptophan; Peptide and Ac-Trp: c = 5 × 10^{-5} M in a Tris–HCl buffer (0.1 M, pH 7.6) or ([b]) in octanol at 25 °C

diazotized peptide	–Ac–Trp–OH $t_{1/2}$ (s)	+ Ac-Trp-OH $t_{1/2}$ (s)	ratio
14a	360	100	3.6
	330[b]	70[b]	4.7[b]
14b	385	125	3.1
	310[b]	70[b]	4.4[b]
17	365	160	2.3
18	330	150	2.2

6b (13.87 g): 94% yield as a yellow (mp = 127–129 °C); IR ($CHCl_3$) 3020 + 3010, 2930, 1610 + 1590, 1515, 1345, 1275 + 1260; ^{1}H NMR ($CDCl_3$) δ 8.25 (d, J = 2.7, 1H), 8.15 (dd, J = 2.7, J' = 9.1, 1H), 7.46 (m, 5H), 6.96 (d, J = 9.1, 1H), 5.29 (s, 2H), 4.48 (bs, 2H); ^{13}C NMR ($CDCl_3$) δ 160.9, 141.2, 135.2, 133.5, 128.8, 128.5, 128.1, 127.3, 125.5, 111.8, 71.0, –2.1; MS (EI) for $C_{14}H_{12}INO_3$ m/z = 242 (M – Bn – I).

Imines 9a/b Method. Butyllithium (1.6 M, 3.30 mL, 5.25 mmol) was slowly added to a solution of **7** (2.26 g, 5 mmol) in dry THF–HMPA 75–25 (20 mL) at –78 °C. After 15 min at –78 °C, **6a/b** (2.8 g, 7.5 mmol) in dry THF (20 mL) was added dropwise and the temperature was allowed to warm to room temperature. The mixture was stirred overnight at room temperature. The reaction was quenched with acetic acid (0.5 mL). The mixture was poured in diethyl ether and was washed with saturated ammonium chloride (six times). Drying over sodium sulfate, removal of the solvent under reduced pressure, and purification by silica gel flash chromatography (eluent: ethyl acetate–cyclohexane 25–75) gave the following compounds.

9a (2.70 g): 78% yield as a white solid (mp = 91–92 °C, dec), $[\alpha]^{25}_D$ = –73.6° (c = 1, $CHCl_3$); IR ($CHCl_3$) 3020 + 3000, 2960 + 2920, 1705 + 1695, 1600 + 1575, 1515, 1335, 1290, 1255; ^{1}H NMR ($CDCl_3$) δ 7.83 (d, J = 9.0, 1H), 7.60 (bd, J = 8.2, 2H), 7.33 (m, 11H), 6.84 (m, 2H), 5.13 (dd, J = 5.4, J' = 7.7, 1H), 4.86 (s, 2H), 3.87 (m, 1H), 3.74 (dd, J = 8.4, J' = 13.1, 1H), 3.59 (dd, J = 5.4, J' = 13.1, 1H), 3.34 (s, 2H), 2.01 (dd, J = 8.3, J' = 13.0, 1H), 1.83 (bm, 2H), 1.79 (bm, 2H), 1.36 (m, 2H), 0.89 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 171.2, 163.8, 161.5, 143.3, 139.3, 135.5, 134.9, 132.4, 130.4, 130.0, 129.0, 128.6, 128.5, 128.3, 128.2, 128.0, 127.9, 127.6, 127.0, 117.3, 114.7, 70.3, 66.2, 65.1, 53.0, 48.4, 47.7, 44.5, 38.2, 37.1, 32.7, 26.4, 20.6, 19.7. Anal. Calcd for $C_{39}H_{39}N_3O_6S$: C, 69.11; H, 5.80; N, 6.20. Found: C, 68.85; H, 5.86; N, 6.04.

9b (2.90 g): 84% yield as a white solid (mp = 104–106 °C dec); $[\alpha]^{25}_D$ = –74.6° (c = 1, $CHCl_3$); IR ($CHCl_3$) 3020 + 3005, 2980 + 2960, 1705 + 1695, 1620 + 1615 + 1590, 1515 = 1485, 1390, 1265; ^{1}H NMR ($CDCl_3$) δ 7.99 (dd, J = 2.8, 1H), 7.92 (dd, J = 2.8, J' = 9.0, 1H), 7.62 (m, 2H), 7.29 (m, 11H), 7.12 (m, 2H), 6.63 (d, J = 9.0, 1H), 5.30 (dd, J = 6.7, J' = 7.7, 1H), 4.90 (AB, J = 13.6, ν_A = 4.94, ν_B = 4.86, 2H), 3.91 (dd, J = 4.6, J' = 7.9, 1H), 3.37 (dd, J = 7.7, J' = 12.6, 1H), 3.20 (dd, J = 6.7, J' = 12.6, 1H), 2.04 (dd, J = 7.8, J' = 11.0, 1H), 1.81 (m, 2H), 1.71 (m, 2H), 1.31 (m, 2H), 0.84 (s, 3H), 0.58 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 171.7, 162.3, 161.4, 140.9, 139.5, 136.1, 135.8, 135.2, 130.3, 130.0, 128.9, 128.7, 128.6, 128.3, 128.1, 128.0, 127.9, 127.6, 126.9, 116.9, 111.4, 70.5, 68.4, 65.3, 53.1, 48.1, 47.5, 44.6, 38.1, 35.6, 32.8, 26.3, 20.2, 19.7.

Method B. A solution of **9** (4.53 g, 10 mmol) and **6a/b** (4.06 (g, 11 mmol) in dichloromethane (70 mL) was vigorously stirred overnight with 10% sodium hydroxide (30 mL) and tetrabutylammonium hydrogen sulfate (4.1 g, 12 mmol). The organic layer was isolated, washed with 10% citric acid, and dried over sodium sulfate. Removal of the solvent under reduced pressure and purification as reported above led to **9a** (5.30 g), $[\alpha]^{25}_D$ = –74.4° (c = 1, $CHCl_3$) and to **9b** (6.03 g), $[\alpha]^{25}_D$ = –68.4° (c = 1, $CHCl_3$) in, respectively, 76% and 87% yields.

Imines 10a/b. Compound **8** (2.27 g, 5 mmol) was treated as reported above (method B) and gave the following compounds.

10a (2.42 g): 95% yield as a yellow solid; IR ($CHCl_3$) 3060 + 3020 + 3010, 2930, 1740, 1600 + 1570 + 1545, 1510, 1440, 1335, 1255; ^{1}H NMR ($CDCl_3$) δ 7.95 (d, J = 9.0, 1H), 7.62 (dd, J = 1.8, J' = 6.9, 2H), 7.31 (m, 11H), 6.90 (d, J = 2.8, 1H), 6.84 (dd, J = 2.8, J' = 9.0, 1H), 6.59 (bs, 2H), 4.94 (AB, J = 11.6, ν_A = 5.00, ν_B = 4.89, 2H), 4.50 (dd, J = 3.6, J' = 9.7, 1H), 4.20 (m, 2H), 3.85 (dd, J = 3.6, J' = 12.9, 1H), 3.35 (dd, J = 9.7, J' = 12.9, 1H), 1.27 (t, J = 7.1, 3H); ^{13}C NMR ($CDCl_3$) δ 171.2, 161.5, 142.4, 139.0, 136.6, 135.5, 132.4, 130.5, 130.0, 128.7, 128.2, 128.1, 127.4, 119.2, 114.0, 70.3, 64.8, 61.2, 37.3, 14.1; MS (EI) m/z = 509 (M). Anal. Calcd for $C_{31}H_{28}N_2O_5$: C, 73.21; H, 5.55; N, 5.51. Found: C, 73.31; H, 5.55; N, 5.67.

10b (2.29 g): 90% yield as a white solid (mp = 151–152 °C); IR ($CHCl_3$) 3000, 2920, 1730, 1590, 1515, 1340, 1255; ^{1}H NMR ($CDCl_3$) δ 8.06 (m, 2H), 7.51 (dd, J = 1.5, J' = 6.6, 2H), 7.29 (m, 9H), 7.14 (m, 2H), 6.79 (d, J = 8.7, 1H), 6.69 (bd, J = 6.5, 2H), 4.84 (AB, J = 11.8, ν_A = 4.92, ν_B = 4.76, 2H), 4.51 (dd, J = 4.1, J' = 9.4, 1H), 4.19 (m, 2H), 3.45 (dd, J = 4.1, J' = 13.2, 1H), 3.24 (dd, J = 9.4, J' = 13.2, 1H), 1.25 (t, J = 7.1, 3H); ^{13}C NMR ($CDCl_3$) δ 171.5, 162.5, 149.7, 140.9, 139.2, 136.1, 135.5, 130.4, 128.8, 128.6, 128.1, 127.8, 127.5, 122.0, 110.8, 70.4, 64.2, 61.1, 34.3, 14.2; MS (EI) m/z = 509 (M). Anal. Calcd for $C_{31}H_{28}N_2O_5$: C, 73.21; H, 5.55; N, 5.51. Found: C, 72.76; H, 5.67; N, 5.07.

Boc-*m/o*-Nip(Bn)-OEt (11a/b). Compound **10a/b** (2.034 g, 4 mmol) was treated for 10 min at room temperature by 1 N acid (25 mL, excess) in THF (100 mL). The volume was reduced under vacuum, and the resulting hydrochloride was precipitated in diethyl ether, filtrated, and dried. The hydrochloride was treated with di-*tert*-butyldicarbonate (970 mg, 4.4 mmol) and triethylamine (620 μL after neutralization, 4.4 mmol) in methanol (80 mL) for 4 h at room temperature. After evaporation of the solvent, the product, dissolved in ethyl acetate, was washed with 10% citric acid and with brine and was dried over sodium sulfate. Purification by silica gel flash chromatography (eluent: ethyl acetate–cyclohexane 25–75) afforded the following compounds.

11a (1.738 g): 98% yield as a white solid (mp = 149–150 °C); IR ($CHCl_3$) 3430, 3005, 2980 + 2930, 1730 + 1710, 1575, 1515 + 1500, 1335, 1255; ^{1}H NMR ($CDCl_3$) δ 8.06 (d, J = 9.3, 1H), 7.39 (m, 5H), 6.92 (m, 2H), 6.56 (bs, 1H), 5.22 (bd, J = 7.9, 1H), 5.12 (s, 2H), 4.65 (dd, J = 8.6, J' = 14.2, 1H), 4.17 (dq, J = 3.5, J' = 7.9, 2H), 3.55 (dd, J = 5.6, J' = 13.4, 1H), 3.28 (dd, J = 8.4, J' = 13.4, 1H), 1.37 (s, 9H); ^{13}C NMR ($CHCl_3$) δ 171.5, 162.1, 155.0, 142.7, 135.6, 135.2, 128.7, 128.4, 127.8, 127.5, 118.6, 113.6, 79.9, 70.6, 61.6, 54.0, 36.4, 28.2, 14.0; MS (EI) m/z = 388 (M − C_4H_8). Anal. Calcd for $C_{23}H_{28}N_2O_7$: C, 62.15; H, 6.35; N, 6.30. Found: C, 62.24; H, 6.34; N, 6.36.

11b (1.768 g): 100% yield as a white solid (mp = 109–110 °C); IR ($CHCl_3$) 3430, 3000, 2980 + 2930, 1730, + 1715, 1705, 1595, 1515 + 1500, 1340, 1255; ^{1}H NMR ($CDCl_3$) δ 8.14 (dd, J = 2.8, J' = 9.1, 1H), 8.05 (d, J = 2.8, 1H), 7.42 (m, 5H), 6.98 (d, J = 9.1, 1H), 5.22 (AB, J = 12.0, ν_A = 5.26, ν_B = 5.19, 2H), 5.13 (bd, J = 8.4, 1H), 4.63 (dd, J = 4.9, J' = 8.5, 1H), 4.13 (m, 2H), 3.33 (dd, J = 4.9, J' = 13.5, 1H), 2.98 (dd, J = 8.5, J' = 13.5, 1H), 1.35 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.8, 161.9, 155.0, 141.3, 135.5, 128.8, 128.5, 127.2, 126.9, 124.8, 111.4, 79.9, 71.1, 61.5, 53.3, 33.8, 28.2, 14.1; MS (EI) m/z = 388 (M − C_4H_8). Anal. Calcd for $C_{23}H_{28}N_2O_7$: C, 62.15; H, 6.35; N, 6.30. Found: C, 62.29; H, 6.31; N, 6.31.

Boc-L-*m/o*-Nip(Bn) (2a/b). From 9a/b. Compound **9a/b** (3.47 g, 5 mmol) in acetonitrile (60 mL) was treated for 3 h at room temperature with lithium hydroxide (430 mg, 20 mmol), lithium bromide (2.63 g, 25 mmol), and tetrabutylammonium bromide (650 mg, 2 mmol) in water (20 mL). The solvent was removed under reduced pressure, and the product was adsorbed on silica gel. Washing with ethyl acetate–cyclohexane 50-50 and elution with butanol–acetic acid–water 60-20-20 gave the partially pure amino acids which were used in further steps without purification. Amino acids were treated with di-*tert*-butyl dicarbonate (606 mg, 2.75 mmol) and triethylamine (386 mL) in methanol (50 mL) overnight at room temperature. After removal of the solvent, the crude product dissolved in ethyl acetate was washed with 10% citric acid and brine and was dried over sodium sulfate. Purification by silica gel flash chromatography (eluent: chloroform–methanol–acetic acid 93-5-2) gave the following compounds.

2a (960 mg): 93% yield as a white solid (mp = 190 °C, dec); $[\alpha]^{25}_D$ = +6.8° (c = 1, $CHCl_3$); IR ($CHCl_3$) 3430, 3010, 2980 + 2930, 1710, 1600 + 1585 + 1575, 1510, 1420, 1335; ^{1}H NMR ($CDCl_3$) δ 8.07 (d, J = 9.2, 1H), 7.40 (m, 5H), 6.93 (m, 2H), 5.29 (bd, J = 8.1, 1H), 5.12 (s, 2H), 4.70 (m, 1H), 3.55 (dd, J = 4.8, J' = 13.5, 1H), 3.31 (dd, J = 8.9, J' = 13.5, 1H), 1.37 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.7, 161.9, 155.0, 141.3, 135.4, 128.8, 128.4, 127.2, 126.9, 124.7, 111.4, 79.9, 71.0, 53.2, 33.7, 28.2; MS (EI) m/z = 360 (M − C_4H_8). Anal. Calcd for $C_{21}H_{24}N_2O_7$: C, 60.57; H, 5.81; N, 6.73. Found: C, 60.39; H, 5.84; N, 6.47.

2b (872 mg): 84% yield as a white solid (mp = 170 °C); $[\alpha]^{25}_D$ = +12.6° (c = 1, $CHCl_3$); IR ($CHCl_3$) 3430, 3020 + 3000, 2980 + 2930, 1750, 1710, 1590, 1515 + 1500, 1340, 1255; ^{1}H NMR ($CDCl_3$) δ 8.15 (d, J = 2.8, 1H), 8.10 (dd, J = 2.8, J' = 9.1, 1H), 7.39 (m, 5H), 6.97 (d, J = 9.1, 1H), 6.56 (bs, 1H), 5.22 (AB, J = 12.1, ν_A = 5.25, ν_B = 5.19, 2H), 4.66 (m, 1H), 3.39 (dd, J = 4.8, J' = 13.7, 1H), 3.00 (dd, J = 8.8, J' = 13.7, 1H), 1.35 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 175.8, 161.8, 155.3, 141.3, 135.3, 128.8, 128.4, 127.2, 127.0, 126.4, 124.9, 111.4, 80.4, 70.9, 53.1, 33.1, 28.1; MS (EI) m/z = 360 (M − C_4H_8). Anal. Calcd for $C_{21}H_{24}N_2O_7$: C, 60.57; H, 5.81; N, 6.73. Found: C, 60.65; H, 5.87; N, 6.68.

From 11a/b. 11a/b (1.554 g, 3.5 mmol) was treated with 1 N lithium hydroxide (10 mL, excess) in THF (60 mL) 30 for min at room temperature. The reaction was quenched by addition of 10% citric acid, and the product was extracted with ethyl acetate. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure. Purification as reported above gave **2a** (1.39 g) (mp = 181–182 °C) and **2b** (1.45 g) (mp = 149–150 °C), respectively, in 95% and 100% yield.

Evaluation of the Enantiomeric Excess of 2a/b. Racemic **2a** and **2b** were separated by HPLC on a Nucleodex γ-PM 5 mm chiral analytic column (200 × 4 mm). The products were detected at 300 nm. **2a**: 0.1% triethylammonium acetate–methanol 55–65, 0.7 mL·min^{-1}, 195 HPa; racemate at 16.90 min (46%) and 18.52 min (54%); **2a** from method A at 17.11 min (98.5%) and 18.59 min (1.5%) (ee = 97%); **2a** from method B at 17.14 min (100%) (ee > 99%). **2b**: 1% triethylammonium acetate-methanol 30-70, 0.7 mL·min^{-1}, 155 Hpa; racemate at 4.38 min (54%) and 5.08 min (46%); **2b** from method A at 5.30 min (100%) (ee > 99%); **2b** from method B at 5.29 min (100%) (ee > 99%).

L-*m/o*-Tyr (12a/b). Compound **2a/b** (208 mg, 0.5 mmol) in methanol (10 mL) was treated with 10% palladium on carbon (40 mg, 20% w/w) under hydrogen (4.5 HPa) for 2 h at room temperature. Addition of acetic acid (0.5 mL) followed by fast removal of the catalyst by filtration and reduction of the volume under reduced pressure gave a colorless oil which rapidly turned dark red. The crude product was treated with isoamyl nitrite (70 μL) in acetic acid (10 mL) for 30 min at room temperature in the dark. The solvent was evaporated at 20–30 °C under vacuum. Excess 0.5 M ethanolic sodium borohydride was added, and the mixture was stirred for 1 h at room temperature in the dark. The solvent was removed under reduced pressure, and residual hydride was destroyed by cautions addition of 2 N hydrochloric acid (50 mL). Purification by HPLC (semipreparative column Bondasorb C_{18} 10 μm; eluent: 0.1% TFA–acetonitrile 95-5, 4mL·min^{-1}; detection at 275 nm; **12a**: rt = 5.25 min; **12b**: rt = 7.11 min) and freeze-drying gave the following compounds.

12a: $[\alpha]^{25}_D$ = −9.6° (c = 2, 1 N HCl) (lit.[27] $[\alpha]^{25}_D$ = −7.9°); ^{1}H NMR (D_2O) δ 7.26 (t, J = 7.8, 1H), 6.82 (m, 3H), 3.94 (dd, J = 5.1, J' = 8.0, 1H), 3.24 (dd, J = 5.1, J' = 14.5, 1H), 3.02 (dd, J = 8.0, J' = 14.5, 1H).

12b: $[\alpha]^{25}_D$ = −25.9° (c = 2, 1 N HCl) (lit.[27] $[\alpha]^{25}_D$ = −26.8°); ^{1}H NMR (D_2O) δ 7.20 (m, 2H) + 6.92 (m, 2H), 4.02 (dd, J = 4.9, J' = 7.9, 1H), 3.32 (dd, J = 4.9, J' = 14.3, 1H), 3.01 (dd, J = 7.9, J' = 14.3, 1H).

Fmoc-D,L-*m/o*-Nip(Bn) (3a/b). Compound **9a/b** was deprotected as reported above. The free amino acid was treated with (fluorenyloxycarbonyl)-*N*-hydroxysuccinimide (970 mg, 2.75 mmol) and triethylamine (386 mL) in methanol (50 mL) for 8 h at room temperature. After removal of the solvent, the crude product dissolved in ethyl acetate was washed with 10% citric acid and brine and was dried over sodium sulfate. Purification by silica gel flash chromatography (eluent: chloroform–methanol–acetic acid 93-5-2) gave the following compounds.

3a (1.08 g): 75% yield as a colorless oil; $[\alpha]^{25}_D$ = −13.9° (c = 1, $CHCl_3$); IR ($CHCl_3$) 3410, 3050 + 3020 + 3000, 2950 + 2930, 1720, 1605 + 1575, 1510, 1450, 1335, 1290, 1250; ^{1}H NMR ($CDCl_3$) δ 8.03 (d, J = 9.0, 1H), 7.70 (d, J = 7.4, 2H) + 7.43 (m, 2H) + 7.30 (m + bs, 9H), 6.91 (d, J = 2.6, 1H), 6.85 (dd, J = 2.6, J' = 9.0, 1H), 5.78 (bd, J = 8.3, 1H), 4.99 (s, 2H), 4.75 (dd, J = 5.5, J' = 13.1, 1H), 4.25 (d, J = 7.0, 2H), 4.10 (t, J = 7.0, 1H), 3.63 (dd, J = 5.5, J' = 13.1, 1H), 3.35 (dd, J = 9.6, J' = 13.1, 1H); ^{13}C NMR ($CDCl_3$) δ 175.0, 162.3, 156.1, 143.6, 142.5, 141.2, 135.3, 134.9, 128.7, 128.4, 127.9, 127.7, 127.5, 127.0, 125.1, 119.9, 118.5, 113.8, 70.5, 67.3, 54.4, 46.9, 35.6; MS (EI) m/z = 252 (M − Bn − $C_{14}H_{11}O$).

3b (986 mg): 73% yield as a colorless oil; $[\alpha]^{25}_D$ = +12.0° (c = 1, $CHCl_3$); IR ($CHCl_3$) 3420, 3060 + 3020, 2950 + 2930, 1715, 1590, 1510, 1450, 1340, 1260; ^{1}H NMR ($CDCl_3$) δ 8.10 (m, 2H), 7.72 (d, J = 7.4, 2H) + 7.35 (m, 6H) + 7.34 (bs, 5H), 6.92 (d, J = 8.6, 1H), 5.46 (bd, J = 8.3, 1H), 5.10 (AB, J = 11.9, n_A = 5.13, n_B = 5.06, 2H), 4.73 (dd, J = 4.8, J' = 8.7, 1H), 4.27 (m, 2H), 4.09 (t, J = 6.8, 1H), 3.38 (dd, J = 4.8, J' = 13.8, 1H), 3.05 (dd, J = 8.7, J' = 13.8, 1H); ^{13}C NMR ($CDCl_3$) δ 175.5, 161.7, 155.9, 143.6, 141.2, 135.1, 128.8, 128.4, 127.7, 127.2,

127.0, 126.8, 126.1, 125.2, 125.0, 119.9, 111.4, 70.9, 67.0, 53.6, 46.9, 32.8; MS (EI) m/z = 252 (M − Bn − $C_{14}H_{11}O$). Anal. Calcd for $C_{31}H_{26}N_2O_7$: C, 69.14; H, 4.87; N, 5.20. Found: C, 69.25; H, 4.81; N, 4.85.

Ac-L-*m*-Nip(Bn)-Ala-OMe (13a)/Ac-L-*o*-Nip(Bn)-Ala-OMe (13b). From 2a/b. Compound **2a/b** (208 mg, 0.5 mmol) in dichloromethane (10 mL) was treated with *N*-hydroxysuccinimide (60 mg, 0.5 mmol) and 1 M DCC in dichloromethane (550 mL, 0.55 mmol) for 2 h at −10 °C following the standard procedure. The resulting *N*-hydroxysuccinimide ester was added to a mixture of alanine methyl ester hydrochloride (142 mg, 1 mmol) and *N*-methylmorpholine (140 μL, 1 mmol after neutralization) in DMF (5 mL). The reaction was carried out overnight. The obtained Boc-dipeptide was directly deprotected in TFA−dichloromethane 50-50 (10 mL) and was capped with 20% acetic anhydride in methanol (10 mL) and triethylamine (70 μL, 0.5 mmol after neutralization). Removal of the solvent under reduced pressure, usual workup, and purification by silica gel flash chromatography (eluent: chloroform−methanol 95-5) aforded the following compounds.

13a (180 mg): 81% yield as a white solid (mp = 195 °C, dec); $[\alpha]^{25}_D$ = +26.8° (c = 1, $CHCl_3$); IR ($CHCl_3$) 3430, 3020, 2930, 17350, 1670, 1600, 1515, 1425 + 1420, 1340; ^{1}H NMR ($CDCl_3$) δ 8.10 (d, J = 9.1, 1H), 7.41 (m, 5H), 6.96 (d, J = 2.7, 1H), 6.91 (dd, J = 2.7, J' = 9.1, 1H), 6.70 (bd, J = 7.1, 1H) + 6.50 (bd, J = 7.8, 1H), 5.12 (AB, J = 11.7, ν_A = 5.15, ν_B = 5.10, 2H), 4.84 (m, 1H) + 4.54 (m, 1H), 3.74 (s, 3H), 3.57 (dd, J = 4.3, J' = 13.6, 1H), 3.11 (dd, J = 9.8, J' = 13.6, 1H), 1.90 (s, 3H), 1.29 (d, J = 7.2, 3H); ^{13}C NMR ($CDCl_3$) δ 170.4, 170.2, 162.4, 142.5, 135.7, 135.5, 128.8, 128.6, 128.2, 127.5, 118.4, 114.0, 70.6, 53.7, 52.5, 48.2, 36.9, 23.0, 18.0; MS (EI) m/z = 397 (M − NO_2). Anal. Calcd for $C_{23}H_{25}N_3O_7$: C, 59.59; H, 5.68; N, 9.48. Found: C, 59.07; H, 5.60; N, 9.43.

13b (162 mg): 76% yield as a white solid; $[\alpha]^{25}_D$ = −9.0° (c = 1, $CHCl_3$); IR ($CHCl_3$) 3410, 3020 + 3000, 2950 + 2930, 1740, 1685 + 1670, 1600, 1585 + 1575, 1510, 1450, 1375, 1335, 1255; ^{1}H NMR ($CDCl_3$) δ 8.11 (dd, J = 2.8, J' = 9.1, 1H), 8.05 (d, J = 2.8, 1H), 7.43 (m, 5H), 7.0 (d, J = 9.1, 1H), 6.91 (bd, J = 8.1, 1H) + 6.68 (bd, J = 7.4, 1H), 5.23 (s, 2H), 4.83 (dq, J = 4.6, J' = 9.0, 1H), 4.50 (m, 1H), 3.70 (s, 3H), 3.24 (dd, J = 4.6, J' = 13.7, 1H), 2.99 (dd, J = 9.0, J' = 13.7, 1H), 1.85 (s, 3H), 1.29 (d, J = 7.2, 3H); ^{13}C NMR ($CDCl_3$) δ 172.7, 170.8, 161.6, 141.2, 135.4, 129.0, 128.7, 127.5, 126.8, 124.8, 111.0, 71.1, 53.0, 52.5, 48.0, 33.6, 22.7, 17.8; MS (EI) m/z = 443 (M). Anal. Calcd for $C_{23}H_{25}N_3O_7$: C, 59.59; H, 5.68; N, 9.48. Found: C, 59.23; H, 5.67; N, 9.36.

From **3a/b**. **3a/b** (269 mg, 0.5 mmol) was treated as reported above. Triethylamine (141 mL, 0.5 mmol) was used in place of *N*-methylmorpholine during the coupling. Deprotection was carried out for 1 h at room temperature with 20% piperidine in dichloromethane (10 mL). After evaporation of the solvent under vacuum, the crude product was treated with 20% acetic anhydride in methanol (10 mL). Workup and purification gave **12a** (160 mg) $[\alpha]^{25}_D$ = +26.1° (c = 1, $CHCl_3$) in 72% yield or **12b** (171 mg) $[\alpha]^{25}_D$ = −6.0° (c = 1, $CHCl_3$) in 77% yield.

Ac-L-*m*-Dip-Ala-OMe (14a)/Ac-L-*o*-Dip-Ala-OMe (14b). Diazotization was carried out on 22 mg (50 mmol) of **13a/b**: **13a/b** was treated by 10% palladium on carbon (5 mg) in methanol−glacial acetic acid 75-25 (10 mL) under hydrogen (4 HPa) overnight at room temperature in attenuated light. The reaction mixture was rapidly filtered under argon, and the volume was reduced under vacuum. The product was treated with isoamyl nitrite (10 mL, excess) in glacial acetic acid (0.5 mL) at room temperature. Purification by HPLC (semipreparative column Bondasorb C_{18} 10 μm, 300 × 7.5 mm, solvent: isocratic 0.1% TFA−acetonitrile 90-10, 4 mL·min^{-1}, diazonium forms of **14a/b** were detected at 318 nm; **14a**: t_R = 5.18 min, 98%, **14b**: t_R = 5.06 min, 99%) and freeze-drying gave **14a** (6.2 mg) and **14b** (3.7 mg).

14a: UV DCD form, λ_{max} = 351 nm, ϵ = 33 500 M^{-1}·cm^{-1} (5 × 10^{-5} M in Tris−HCl 0.1 M, pH 7.6), diazonium form, λ_{max} = 317 nm, ϵ = 16 500 M^{-1}·cm^{-1} (5 × 10^{-5} M in TFA 0.1%); ^{1}H NMR (CD_3OD) δ 8.38 (d, J = 9.1, 1H), 7.08 (d, J = 2.2, 1H), 7.05 (dd, J = 2.2, J' = 9.1, 1H), 4.35 (m, 1H), 3.68 (s, 3H), 3.24 (m, 1H), 3.05 (dd, J = 1.8, J' = 7.4, 1H), 2.01 (s, 3H), 1.34 (d, J = 7.3, 3H).

14b: UV DCD form, λ_{max} = 354 nm, ϵ = 32 000 M^{-1}·cm^{-1} (5 × 10^{-5} M in Tris−HCl 0.1 M, pH 7.6), diazonium form, λ_{max} = 319 nm, ϵ = 16 000 M^{-1}·cm^{-1} (5 × 10^{-5} M in TFA 0.1%); ^{1}H NMR (CD_3OD) δ 8.45 (dd, J = 2.4, J' = 9.2, 1H), 8.16 (d, J = 2.4, 1H), 7.04 (d, J = 2.4, J' = 9.4, 1H), 4.64 (t, J = 7.4, 1H) + 4.38 (m, 1H), 3.72 (s, 3H), 3.02 (m, 2H), 1.95 (s, 3H), 1.35 (d, J = 7.4, 3H).

Ala-L-*m*-Nip-Lys (15)/Ala-L-*o*-Nip-Arg (16). Peptides were synthesized by manual solid support peptide synthesis on Boc-Lys(ClZ)OCH_2−PAM resin (0.1 mmol, 0.61 mmol·g^{-1}) or Boc-Arg(Tos)OCH_2−PAM resin (0.1 mmol, 0.61 mmol·g^{-1}) using a DCC−HOBT standard procedure. Deprotection of Boc was carried out using TFA−dichloromethane 50-50 (2 X 6 mL) 30 min at room temperature. The resin was washed with diisopropylethylamine−dichloromethane 50-50 (2 X 6 mL). The Boc amino acid (**2a/b**: 83.5 mg, 0.2 mmol; Boc-Ala: 76 mg, 0.4 mmol) in NMP (3 mL) was added, and the mixture was treated with 1 M HOBT in NMP (220 μL, 0.22 mmol) and 1 M DCC in NMP (220 μL, 0.22 mmol) 3 h (**2a/b**) or 1.5 h (Boc-Ala). Residual amino acid was eliminated by washing the resin with dichloromethane (X6). After removal of the terminal Boc group, the resin precipitated with methanol and was dried. The peptide was cleaved from the support and totally deprotected by anhydrous HF (5 mL, 1 h at 0 °C) using m-cresol (100 μL) as scavenger. The peptide was precipitated in cooled diethyl ether, filtered, and washed with diethyl ether. After dissolution in 15% acetic acid, the product was freeze dried. Purity of the crude peptide was evaluated by analytical HPLC (analytical column Bondasorb C_{18} 10 μm, 300 × 4 mm, solvent: linear gradient 0.1% TFA−acetonitrile 100-0 to 50-50 in 30 min, 2 mL·min^{-1}); elution was monitored at 230 and 325 nm (**15**: t_R = 7.55 min, 98%; **16**: t_R = 5.37 min, 99%).

15: UV (0.1% TFA) 213, 232, 324 nm: ^{1}H NMR (D_2O) δ 8.18 (d, J = 9.3, 1H), 6.95 (broad d, J = 9.3, 1H), 6.78 (broad s, 1H), 4.38 (dd, J = 5.4, J' = 8.9, 1H), 4.18 (t, J = 6.4, 1H), 3.62 (dd, J = 5.1, J' = 12.9, 1H), 3.32 (m, 1H), 2.94 (m, 2H), 1.63 (broad m, 4H), 1.51 + 1.31 (m, 3H); MS (FAB) 426 (M + H$^+$).

16: UV (0.1% TFA) 213, 233, 320 nm; ^{1}H NMR (D_2O) δ 8.08 (m, 2H), 6.99 (d, J = 8.9), 4.16 (m, 3H), 3.10 (broad m, 4H), 2.03 (m, 2H), 1.66 (m, 2H), 1.37 (d, J = 7.2); MS (FAB) 454 (M + H$^+$).

Ala-L-*m*-Dip-Lys (17)/Ala-L-*o*-Dip-Arg (18). Compounds **15** and **16** were treated as reported above. Diazotization was carried out in 1 N HCl (final concentration: 10^{-2} M in peptide) using 1.1 equiv of isoamylnitrite. The peptides purities were evaluated by HPLC (analytical column Bondasorb C_{18} C 10 μm, 300 × 4 mm, solvent: 0.1% TFA, 1 mL·min^{-1}); elution was monitored at 230 and 315 nm (**17**: t_R = 6.08 min, 95%; **18**: t_R = 4.96 min, 100%).

17: UV DCD form, λ_{max} = 350 nm, ϵ = 35 000 M^{-1}·cm^{-1} (5 × 10^{-5} M in Tris−HCl 0.1 M, pH 7.6), diazonium form, λ_{max} = 319 nm (in TFA 0.1%).

18: UV DCD form, λ_{max} = 354 nm, ϵ = 34 500 M^{-1}·cm^{-1} (5 × 10^{-5} M in Tris−HCl 0.1 M, pH 7.6), diazonium form, λ_{max} = 320 nm (in TFA 0.1%).

Irradiations. Irradiation experiments were carried out at 16 °C in a 1 mL thermostated UV cell using a 1000 W Müller Lax 1000 Xe/Hg lamp connected to a monochromator. The resulting light beam was focused with a lens to a spot about 10 mm high and 2 mm wide, and the resulting energy was measured with an International Light IL 1700 radiometer. The apparatus was calibrated in order to provide a constant energy of 10^{-2} mW·cm^{-2}. Standard 5 × 10^{-5} M solutions were prepared from pure **14a/b**, **17**, **and 18**. The proportion of remaining DCD-derived peptide was evaluated by measuring the optical density at the predetermined λ_{max}.

Acknowledgment. Dr. P. Kessler, Dr. G. Mourier, Dr. J. Cotton (DIEP, CE Saclay), and Dr. H. Josien (University Pierre et Marie Curie, Paris) are gratefully acknowledged for helpful advice and discussions. I express my thanks to Dr. A. Ménez (DIEP, CE Saclay) for critical reading of the manuscript.

JO9417840