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마이크로웨이브 조사와 Diisopropylcarbodiimide (DIC)/7-Aza-1-hydroxybenzotriazole (HOAt): *N*-Protected Amino Acid와 Hydrazine으로부터 다양한 Hydrazide합성을 위한 반응조건

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Microwave Irradiation and Diisopropylcarbodiimide (DIC)/7-Aza-1-hydroxybenzotriazole (HOAt): A Potent Combination for Synthesis of Variuos Hydrazide from *N*-Protected Amino Acid and Hydrazine

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요약. 마이크로웨이브 반응 장치 (Synthos 3000 Aton Paar, GmbH, 1400 W maximum magnetron)를 이용하여, diisopropylcarbodiimide (DIC)/와 1-hydroxybenzotriazoles (HOXt) (X = A or B)를 반응시켜서 amino acid hydrazide를 효율적으로 합성할 수 있는 반응 조건을 개발하였다. 일반적인 가열반응과 마이크로웨이브 반응을 반응 시간, 반응 조건 등을 비교하였을 때에, 마이크로웨이브 반응이 보다 효율적으로 진행되었으며, diisopropylcarbodiimide (DIC)와 1-hydroxybenzotriazole (HOBt) 반응에서보다는 diisopropylcarbodiimide (DIC)와 7-aza-1-hydroxybenzotriazole (HOAt)를 반응시켰을 때에 좋은 수율 (95 - 98%)로 얻어졌다.

주제어: Microwave irradiation, Carbodiimide, 7-Aza-1-hydroxybenzotriazole, Amino acid hydrazide, Peptide hydrazide

ABSTRACT. Here we describe a fast and rapid technique for preparation of amino acid hydrazide as well as peptide hydrazide derivatives using diisopropylcarbodiimide (DIC)/1-hydroxybenzotriazoles (HOXt) (X = A or B) under microwave irradiation employing a multimode reactor (Synthos 3000 Aton Paar, GmbH, 1400 W maximum magnetron). A comparison between conventional and microwave irradiation was described. The microwave methodology is rapid, convenient, proceeds under mild conditions. Diisopropylcarbodiimide (DIC)/7-aza-1-hydroxybenzotriazole (HOAt) always gave much better yield (95 - 98%) and purity than diisopropylcarbodiimide (DIC)/1-hydroxybenzotriazole (HOBt).

Keywords: Microwave irradiation, Carbodiimide, 7-Aza-1-hydroxybenzotriazole, Amino acid hydrazide, Peptide hydrazide

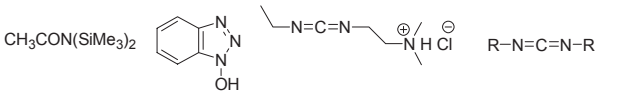
INTRODUCTION

Microwave-assisted organic synthesis has been recognized as one of the most interesting areas of current research.[1,2,3] Coupling of microwave irradiation with the use of catalysts, under solvent-free conditions, provides a clean chemical process with an enhanced reaction rates, higher yields, purities, and ease of manipulation.[4]

Acid hydrazides serve as building blocks in many syntheses,[5,6] among these were the synthesis of many heterocyclic compounds which are biologically active.[7] They can be used as ligands that form stable complexes with various transition metals.[8,9,10] They can also be oxidized to form azo compounds, which can be utilized as dyes, analytical rea-

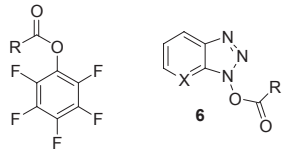
gents[11] and for storage of optical information in laser disks.[12] Acid hydrazides also proved to be useful in protein synthesis where they serve as linkers that show high stability to both acid and base.[13,14] It has been found that direct preparation of hydrazides from acids are inefficient, most reported procedures are low yielding and require chromatographic purification.[15,16]

Good yields were reported using *bis*-(trimethylsilyl)acetamide **1** as a condensing agent, but this method requires completely anhydrous conditions.[16] Some of the coupling reagents used in the preparation of the desired hydrazide, in carbodiimide-based coupling reactions, are 1-hydroxybenzotriazole (HOBt, **2**) and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC, **3**)[15,16] Disopropylcarbodiimide (DIC, **4a**) or dicyclohexyl carbodiimide (DCC, **4b**) in the presence of a base such as *N*-methylmorpholine (NMM).



1 HOBt, **2** EDC, **3** R = isopropyl; DIC, **4a** R = cyclohexyl; DCC, **4b**

Recently, mild methods were used involving formation of pentafluorophenyl esters (Pfp esters **5**) from aryl carboxylic acids, which are amenable to the preparation of symmetrical and unsymmetrical diaroyl hydrazines.[17]

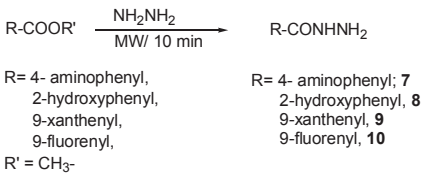


Pfp ester, **5** X = CH, OBt ester X = N, OAt ester

The present work represent an efficient, general and high yielding procedure for preparing *N*-protected amino acid hydrazide as well as dipeptide and tripeptide hydrazide derivatives from the corresponding amino acids, involving the *in situ* formation of 1-hydroxylbenzotriazole active ester (OBt-ester) or 7-aza-1-hydroxylbenzotriazole active ester (OAt-ester); **6**) using conventional method as well as microwave irradiation.

RESULTS AND DISCUSSION

Acid hydrazides were prepared according to the reported standard method 5,6 using a mixture of an ester and hydrazinium hydroxide in ethanol which were then refluxed for



***Scheme* 1.** Synthesis of acid hydrazide

***Table* 1.** Yield (**%**) of carboxylic acid hydrazides using conventional method and MW irradation.

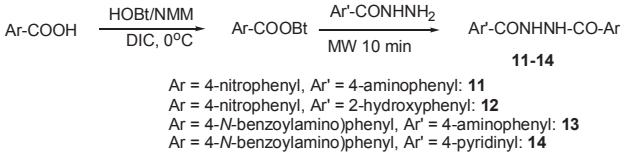
Entry	Yield (%) Conventional	Yield (%) MW
7	69	89
8	57	86
9	84	92
10	72	95

10-15 hr. The same reaction was repeated using the microwave irradiation employing a multimode reactor (Synthos 3000, Aton Paar GmbH, 1400 W maximum magnetron). The initial step was conducted with 4-Teflon vessels rotor (MF 100) that allow processing 4 reactions under the same conditions. Each carboxylic acid ester was mixed with neat hydrazine hydrate in the individual vessels and placed in the corresponding rotor, fixed by screwing down the upper rotor place, and finally the rotor was closed with a protective hood.[18] After heating the vessels for 5 min. at 120 ^{o}C and hold at the same temeprature for 5 min (~10 bar pressure, 1000 W). Cooling was complished by a fan (5 min), and the workup for the individual vessels was preformed as described in the experimental part to afford the desired product (*Scheme* 1, *Table* 1).

From *Table* 1 it is very clear that microwave-irradiated reactions give better yields and higher purities.

Full spectral analyses as well as elemental analysis for the prepared carboxylic acid hydrazides (**7-10**) were carried out to confirm the obtained structures (experimental section).

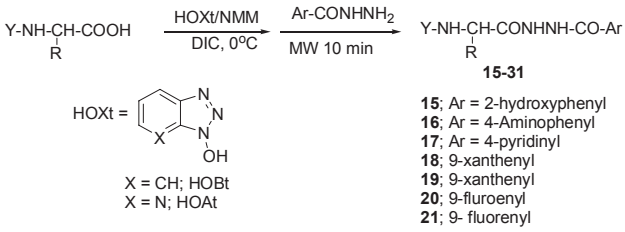
The activated carboxylic acid ester was prepared *in situ* by reacting the acid with 1-hydroxybenzotriazole (HOBt; **2**) and diisopropylcarbodiimide (DIC; **4a**) in the presence of NMM as a base in dimethylformamide (DMF) for 10 min. at 0 ^{o}C, and was added to the prepared acid hydrazide and then the reaction mixture was stirred at room temperature for 24 hours (conventional method) to afford the corresponding *N,N′*-diaroylhydrazine (**11-14**). While in case of microwave irradiation, the activated carboxylic acid ester was prepared *in situ* by reacting the acid with 1-hydroxybenzotriazole (HOBt; **2**) and diisopropylcarbodiimide (DIC; **4a**) in the presence of NMM as a base in adquate amount of DMF for 10 min. at 0 ^{o}C and then mixed with the hydrazide in the individual vessels and placed in the corresponding



***Scheme* 2.** Synthesis of *N,N′*-diaroylhydrazine.

***Table* 2.** Yield (**%**) of *N,N′*-Diaroylhydrazines (Ar'-CO-NHNH-CO-Ar) using DIC/HOBt as coupling reagent by conventional method and MW irradation.

Entry	Yield (%) Conventional	Yield (%) MW
11	62	83
12	65	88
13	58	85
14	75	93



***Scheme* 3.** Synthesis of *N*-amino acyl, *N′*-aroyl hydrazine.

***Table* 3.** Yield (%)of *N*-amino acyl, *N′*-aroyl hydrazine (Y-NH-CHRCO-NHNH-COAr) using DIC/HOXt by conventional method and MW irradation.

Entry	Amino Acid[a]	Y	Yield (%) Conventional (MW)
15	Gly	Bz-	HOBt:71 (85)
16	Gly	Bz-	HOBt:60 (88)
17	Gly	Bz-	HOBt:58 (88)
18	Val	Boc-	HOBt:87 (90)
19	Aib	Boc-	HOBt:59 (85) HOAt:80 (96)
20	Val	Boc-	HOBt:67 (83)
21	Aib	Boc-	HOBt:19 (76) HOAt:48 (89)

[a]Amino acids are abbreviated and designated following the rules of the IUPAC-IUB Commission of Biochemical Nomenclature [*J. Biol. Chem.* **1972**, *247*, 977] (see reference and notes section).

rotor, fixed by screwing down the upper rotor place, and finally the rotor was closed with a protective hood. After heating the vessels for 5 min. at 60 °C and hold at the same temeprature for 5 min to ensure a complete reaction (~10 bar pressure, 800 W). Cooling was complished by a fan for 5 min., and the workup for the individual vessels was pre-

***Table* 4.** Yield (%) of *N*-Boc-amino acyl, *N′*-aroyl hydrazine (*N*-Boc-AA-NHNH-COAr**)** using HOAt/DIC as coupling reagent by conventional methods and MW irradation.[a]

Entry	*N*-Boc-AA-NHNH-COAr	Yield (%) Conventional (MW)
22	Boc-Tyr(OBz)-NHNH-CO-Xanth	92 (98)
23	Boc-Leu-NHNH-CO-Xanth	82 (92)
24	Boc-Lys(*N*-Z)-NHNH-CO-Xanth	98 (98)
25	Boc-Glu(OBn)-NHNH-CO-Xanth	95 (96)
26	Boc-Pro-NHNH-CO-Xanth	89 (94)
27	Boc-Tyr(OBz)-NHNH-CO-Flu	94 (98)
28	Boc-Leu-NHNH-CO-Flu	93 (97)
29	Boc-Lys(*N*-Z)-NHNH-CO-Flu	92 (98)
30	Boc-Glu(OBn)-NHNH-CO-Flu	88 (95)
31	Boc-Pro-NHNH-CO-Flu	84 (96)

[a]Amino acids and peptides are abbreviated and designated following the rules of the IUPAC-IUB Commission of Biochemical Nomenclature [*J. Biol. Chem.* **1972**, *247*, 977] (see reference and notes section).

formed as described in the experimental part to afford *N,N′*-diaroylhydrazines (**11-14**) (*Scheme* 2, *Table* 2).

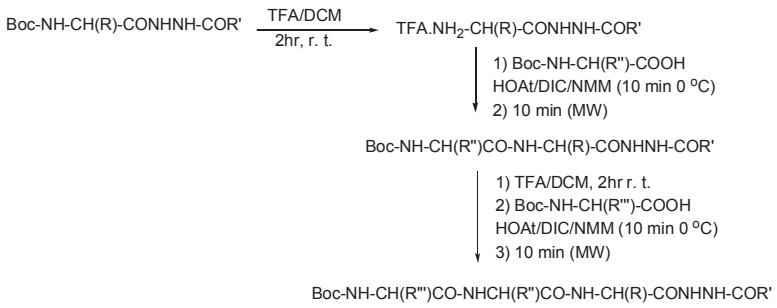
Under the same coupling conditions, *N*-protected amino acid was activated using DIC/HOXt (X = A or B) in the presence of NMM in DMF (10 min. at 0 °C). Then, the carboxylic acid hydrazide was added and heated in MW as described above for 10 min. at 60 °C (the reaction required 10 - 12 hrs at room temperature) to afford the desired product. The results are collected in *Tables* 3, 4 (*Scheme* 3).

Spectral data were obtained for the products (**15-31**) confirming the expected structures. Mass spectral analyses and elemental analyses, also confirmed the molecular formula for the obtained products.

From the results (*Table* 3 and 4) it was clear that using HOAt as an additive improved the yield and purity of the product, which indicate the fast activation and facile coupling through the (-OAt) active ester formed.[19]

The prepared *N*-Boc-amino acyl, *N*-aroyl hydrazine derivatives were treated with CH_2Cl_2/TFA at room temperature for 2 hr in order to remove the Boc-group and then the TFA salt of the amino acid hydrazide derivative was coupled with another *N*-Boc-amino acid in presence of HOAt/DIC in the presence of NMM in DMF under microwave irradiation for 10 min (as described above) to afford *N*-Boc-dipeptide hydrazide derivatives **32**, **34**, **36**. The *N*-Boc dipeptide hydrazide derivatives produced **32**, **34**, **36** were taken into a second deprotection step (using CH_2Cl_2/TFA) and then coupled with *N*-Boc-amino acid (using MW and HOAt/DIC) to afford *N*-Boc-tripeptide hydrazide derivatives **33**, **35**, **37** (*Scheme* 4, *Table* 5) results are gathered in *Table* 5.

The spectral analysis for dipeptides (**32**, **34**, **36**) and tripep-



***Scheme* 4.** Synthesis of *N*-Boc-dipeptide and Boc-tripeptide hydrazide derivatives

***Table* 5.** Yield (%) and Mp (°C) of *N*-Boc-dipeptide and tripeptide hydrazide derivatives using DIC/HOAt using microwave irradiation.[a]

Peptide (compd. No.)	Yield (%)	Mp (°C)
Boc-Try(OBn)-Leu-NHNH-CO-Xanth **32**	72	187 (dec)
Boc-Asp(OBn)-Val- NHNH-CO-Xanth **34**	85	233 (dec)
Boc-Asp(OBn)-Val-NHNH-CO-Flu **36**	82	195 (dec)
Boc-Lys(N-Z)-Try(OBn)-Leu--NHNH- CO-Xanth **33**	88	198 (dec)
Boc-Try(OBn)-Asp(OBn)-Val-NHNH-CO-Xanth **35**	95	192 (dec)
Boc-Try(OBn)-Asp(OBn)-Val-NHNH-CO- Flu **37**	87	197 (dec)

[a]Amino acids and peptides are abbreviated and designated following the rules of the IUPAC-IUB Commission of Biochemical Nomenclature [*J. Biol. Chem.* **1972**, *247*, 977] (see reference and notes section).

tides (**33**, **35**, **37**) as well as their mass spectral analysis were good evidence in proving that the coupling method used in peptide building process was highly successful, affording good yields and easily purified products.

EXPERIMENTAL

General Procedures

Normal workup from organic solvent involved drying over $MgSO_4$ and rotary evaporation. Column chromatography was performed using silica gel 60 obtained from Fluka Chemie (CH-9470, Mesh < 230 ASTM). TLC was performed using polyester-backed sheets ALBET Silica Gel 60 F254 plates using suitable solvent systems with spots being visualized by a Spectroline UV lamp (254 nm). Melting points were obtained in open capillary tubes by using a Gallenkamp melting point apparatus and were uncorrected.

Infrared spectra (IR) were recorded on a Shimadzu FTIR 8300 series instrument as KBr pellets. The absorption bands (υ_{max}) are given in wave numbers (cm^{-1}). ^{1}H-NMR and ^{13}C NMR were recorded on Bruker Avance 300 MHz spectrometer at ambient temperature. Tetramethylsilane (TMS) was used as reference for all ^{1}H-NMR spectra with chemical shifts reported as ppm relative to TMS. Mass spectra (MS) [*m*/*z* (% rel.int.)] were recorded on Shimadzu GC-MS QP5050A spectrometer by using electron impact (EI) at 70 eV. Elemental analysis was carried out at the University of Cairo Microanalytical Laboratories.

HPLC data were obtained using Jasco 1580 apparatus with a 7725I automatic injector and uv-visible multi-wavelength detector (Jasco 1510). Electronic absorption spectra in the wavelength range 200-800 nm were obtained on a Ciba-Corning 2800 spectrophotometer using 1 cm matched quartz cells. Atomic absorption data were obtained using Buck scientific atomic absorption spectrophotometer (Accusys 211) using air-acetylene flame technique. Microwave irradiation employing a multimode reactor (Synthos 3000 Aton Paar, GmbH, 1400 W maximum magnetron).

General procedure for preparation of carboxylic acid hydrazides[5,6]

Method A (conventional method): A mixture of carboxylic acid ester (1 gm), hydrazine hydrate (80%, 5 mL) in ethanol (10 mL) was refluxed for 10 - 15 hr. Then the reaction mixture was left to cool to room temperature and the solid product was filtered and recrystalized from ethanol.

Method B (microwave irradiation): Employing a multimode reactor (Synthos 3000, Aton Paar GmbH, 1400 W maximum magnetron). The initial step was conducted with 4-Teflon vessels rotor (MF 100) that allow processing 4 reactions under the same conditions. Each carboxylic acid ester (1 gm), was mixed with neat hydrazine hydrate (80%, 5 mL) in the individual vessels and placed in the corresponding rotor, fixed by screwing down the upper rotor place,

and finally the rotor was closed with a protective hood. After heating the vessels for 5 min. at 120 °C and hold at the same temeprature for 5 min (~10 bar pressure, 1000 W). Cooling was complished by a fan (5 min), the solid product was recrystalized from ethanol.

4-Aminobenzoic acid hydrazide (7)

The product was obtained as white crystals in yield (**A**: 69%, mp 218 - 220 °C; **B**: 89%, mp 219 °C), R_f : 0.22 (CH_2Cl_2/MeOH 9:1, 2 drops AcOH). IR (KBr) 3429 (NH), 3348 (NH), 3320 (NH), 3280 (NH), 3234 (NH), 1630 (CO, hydrazide) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 4.32 (br, 2H, NH_2), 5.56 (s, 2H, NH_2), 6.50 (d, 2H, aromatic), 7.50 (d, 2H, aromatic), 9.25 (s, 1H, NH) ppm.

2-Hydroxybenzoic acid hydrazide (8)

The product was obtained as beige crystals in yield (**A**: 57%, mp 148 - 150 °C; **B**: 86%, mp 151 °C), R_f : 0.48 (CH_2Cl_2/MeOH 9:1). IR (KBr) 3319 (NH), 3269 (OH), 3120 (NH), 1650 (CO, hydrazide) cm^{-1}.^{1}H-NMR (DMSO-d_6): δ 4.67 (2H, br, NH_2), 6.82-6.90 (2H, m, aromatic), 7.33-7.39 (1H, m, aromatic), 7.78 (1H, dd, aromatic), 10.05 (1H, br, NH), 12.40 (1H, br, OH) ppm.

9*H*-Xanthene-9-carboxylic acid hydrazide (9)

The product was obtained as white powder in yield (**A**: 84%, mp 215 °C dec; **B**: 92%, mp 216 °C dec.). R_f : 0.64 (CH_2Cl_2/MeOH 9:1, 2 drops AcOH). IR (KBr) 3330 (NH), 3296 (NH), 3290 (NH), 1643 (CO, hydrazide) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 4.28 (2H, s, NH_2), 4.81 (1H, s, H-9), 7.06-7.14 (4H, m, aromatic), 7.24-7.31 (4H, m, aromatic), 9.57 (1H, s, NH) ppm.^{13}C-NMR (DMSO-d_6): δ 43.62, 116.58, 120.29, 123.51, 128.83, 151.25, 170.98 ppm.

9*H*-Fluorene-9-carboxylic acid hydrazide (10)

The product was obtained as white powder in yield (**A**: 72%, mp 216 °C dec.; **B**: 95%, mp 218 °C dec.) R_f : 0.56 (CH_2Cl_2/MeOH 9:1, 2 drops AcOH). IR (KBr): 3317 (NH), 1643 (CO, hydrazide) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 4.37 (2H, br, NH_2), 4.72 (1H, s, H-9), 7.29-7.48 (6H, m, aromatic), 7.86 (2H, d, aromatic), 9.61 (1H, s, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 53.07, 120.37, 125.01, 127.43, 127.93, 141.59, 143.07, 169.47 ppm.

Anal. Calcd for $C_{14}H_{12}N_2O$ (M+, 224): C, 75.00; H, 5.35; N, 12.50. Found: C, 75.31; H, 5.49; N, 12.82.

General procedure for synthesis of *N,N'*-diaroyl hydrazines

Method A (conventional method): DIC (1 mmol) was added to an acid (1 mmol) and HOBt (1 mmol) in DMF (5 mL) at 0 °C. The reaction mixture was stirred at this temperature for 10 min and then 1 mmol of an acid hydrazide was added and the reaction mixture was stirred at room temperature overnight. Water (50 mL) was added and the precipitate was collected by filteration, dried and then recrystalized from ethyl acetate/hexane.

Method B (microwave irradiation): Employing a multimode reactor (Synthos 3000, Aton Paar GmbH, 1400 W maximum magnetron). The initial step was conducted with 4-Teflon vessels rotor (MF 100) that allow processing 4 reactions under the same conditions. Each carboxylic acid (1 mmol) was preactivated previously with DIC (1 mmol), HOBt (1 mmol) in adquate amount of DMF (1 mL) at 0 °C fro 10 min. and then mixed with neat hydrazide (1 mmol) in the individual vessels and placed in the corresponding rotor, fixed by screwing down the upper rotor place, and finally the rotor was closed with a protective hood. After heating the vessels for 5 min. at 60 °C and hold at the same temeprature for 5 min to ensure a complete reaction (~10 bar pressure, 800 W). Cooling was complished by a fan (5 min). Water was added (30 mL), filter, dried, the solid product was recrystalized from the corresponding ethylacetate/hexane.

***N*-(4-Aminobenzoyl)-*N'*-(4-nitrobenzoyl)-hydrazine (11)**

The product was obtained as an orange powder in yield (**A**: 62%, mp 271 °C dec.; **B**: 83%, mp 274 °C dec.). R_f : 0.51 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3400 (NH), 3200 (br, NH), 3190 (NH), 3180 (NH), 1630 (CO, hydrazide), 1620 (CO, hydrazide) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 5.76 (2H, s, NH_2), 6.56 (2H, d, aromatic), 7.63 (2H, d, aromatic), 8.11 (2H, d, aromatic), 8.34 (2H, d, aromatic), 10.11 (1H, s, NH), 10.66 (1H, s, NH) ppm.

Anal. Calcd for $C_{14}H_{12}N_4O_4$ (M+, 300): C, 56.00; H, 4.00; N,18.66. Found: C, 56.18; H, 4.22; N,18.90.

***N*-(4-Nitrobenzoyl)-*N'*-(2-hydroxybenzoyl)-hydrazine (12)**

The product was obtained as off white powder in yield (**A**: 65%, mp 232 °C dec.; **B**: 88%, mp 233 °C dec.). R_f : 0.57 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3250 (NH), 3200 (OH), 3130 (NH), 1620 (CO, hydrazide) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 6.94-7.00 (2H, m, aromatic), 7.44-7.48 (1H, m, aromatic), 7.91 (1H, d, aromatic), 8.14 (2H, d, aromatic), 8.36 (2H, d, aromatic), 10.81 (1H, br, NH), 11.07 (1H, br, NH), 11.83 (1H, br, OH) ppm.

Anal. Calcd for $C_{14}H_{11}N_3O_5$ (M+, 301): C, 55.81; H, 3.65; N, 13.95. Found: C, 56.06; H, 3.83; N, 14.21.

***N*-{4-[*N'*-(4-Aminobenzoyl)-hydrazinocarbonyl]-phenyl}-benzamide (13)**

The product was obtained as off white powder in yield (**A**: 58%, mp 240 °C dec.; **B**: 85%, mp 241 °C dec.) R_f : 0.56 (CH_2Cl_2/MeOH 9:1, 2 drops AcOH). IR (KBr): 3317 (NH), 3234 (NH), 1651 (CO), 1631 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 5.73 (2H, s, NH_2), 6.56 (2H, d, aromatic), 7.52-7.67 (5H, m, aromatic), 7.92-7.99 (6H, m, aromatic), 9.96 (1H, s, NH), 10.23 (1H, s, NH), 10.50 (1H, s, NH) ppm. MS (EI)**:** 238.05 (0.49, [M-$C_7H_8N_2O$]+), 162.05 (0.83, [M-$C_{13}H_{12}N_2O$]+), 120.90 (0.77, [M-$C_{14}H_{11}N_3O_2$]+).

***N*-{4-[N'-(Pyridine-4-carbonyl)-hydrazinocarbonyl]-phenyl}-benzamide (14)**

The product was obtained as a white powder in yield (**A**: 75%, mp 238 °C dec.; **B**: 93%, mp 240 °C dec.) R_f : 0.67 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3350 (NH), 3320 (NH), 3260 (NH), 1690 (CO), 1665 (CO), 1650 (CO) cm^{-1} . ^{1}H-NMR (DMSO-d_6): δ 7.52-7.62 (3H, m, aromatic), 7.82 (2H, d, aromatic), 7.91-7.99 (6H, m, aromatic), 8.79 (2H, d, aromatic), 10.53-10.90 (3H, br, 3NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 119.94, 121.67, 127.46, 128.12, 128.64, 130.00, 132.18, 135.01, 139.94, 142.86, 150.83, 164.74, 165.61, 166.28 ppm.

Anal. Calcd for $C_{20}H_{16}N_4O_3$ (M+, 360): C, 66.66; H, 4.44; N, 15.55. Found**:** C, 66.91; H, 4.60; N, 15.81.

General procedure for synthesis of *N*-protected amino acid hydrazides

Method A (conventional method): DIC (1 mmol) was added to a mixture of *N*-protected amino acid (1mmol), HOXt (X = A or B, 1mmol) , and NMM (1 mmol) in 5mL DMF at 0 °C. The reaction mixture was stirred at 0 °C for 10 min and then 1mmol of an acid hydrazide was added. The reaction mixture was stirred at room temperature overnight and then water (50 mL) was added. The precipitate was collected by filtration, dried and then recrystalized from ethylacetate/hexane.

Method B (microwave irradiation): Employing a multimode reactor (Synthos 3000, Aton Paar GmbH, 1400 W maximum magnetron). The initial step was conducted with 4-Teflon vessels rotor (MF 100) that allow processing 4 reactions under the same conditions. Each carboxylic acid (1 mmol) was preactivated previously with DIC (1 mmol), HOXt (1 mmol) in adquate amount of DMF (1 mL) at 0 °C fro 10 min. and then mixed with neat hydrazide (1 mmol) in the individual vessels and placed in the corresponding rotor, fixed by screwing down the upper rotor place, and finally the rotor was closed with a protective hood. After heating the vessels for 5 min. at 60 °C and hold at the same temepreature for 5 min to ensure a complete reaction (~10 bar pressure, 800 W). Cooling was complished by a fan (5 min). the residue was triturated with sat. Na_2CO_3 and extracted with ethylacetate. The organic solvent washed with 10% HCl, sat. NaCl, dried ($MgSO_4$), filtered and the solvent was removed under vacuum to afford the desired product.

***N*-(*N*-benzoyl glycinyl)-*N'*-(2-hydroxybenzoyl) hydrazine (15)**

The product was obtained as a white powder in yield (**A**: 71%; **B**: 85%), mp 216 °C (dec), using HOBt, as an additive), mp 216 °C (dec). R_f : 0.48 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3323 (NH), 3195 (OH), 3138 (NH), 1645 (CO, hydrazide) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 4.01 (2H, d, CH_2), 6.92-6.96 (2H, m, aromatic), 7.40-7.54 (4H, m, aromatic), 7.86-7.88 (3H, m, aromatic), 8.86 (1H, t, NH), 10.35 (1H, s, NH), 11.00 (1H, br, OH) ppm.

^{13}C-NMR (DMSO-d_6): δ 41.87, 114.97, 117.50, 119.41, 127.57, 128.60, 128.77, 131.74, 134.03, 134.35, 158.99, 166.84, 167.04, 168.15 ppm. Anal. Calcd for $C_{16}H_{15}N_3O_4$ (M+, 313): C, 61.34; H, 4.79; N, 13.42. Found: C, 61.50; H, 5.03; N, 13.21.

***N*-{2-[*N'*-(4-Aminobenzoyl)-hydrazino]-2-oxo-ethyl}-benzamide (16)**

The product was obtained as a beige powder in yield (**A**: 60%; **B**: 88%, using HOBt as an additive), mp 221 °C (dec). R_f : 0.41 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3460 (NH), 3360 (NH), 3310 (NH), 3300 (NH), 3200 (NH), 1650 (CO), 1630 (CO), 1620 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 3.97 (2H, d, CH_2), 5.71 (2H, s, NH_2), 6.52 (2H, d, aromatic), 7.44- 7.62 (5H, m, aromatic), 7.87-7.90 (2H, m, aromatic), 8.80 (1H, t, NH), 9.87 (2H, s, 2NH) ppm.

***N'*-(*N*-benzoyl glycinyl) isonicotinic hydrazide (17)**

The product was obtained as a white powder in yield (**A**: 58%; **B**: 88%, using HOBt as an additive), mp 228 °C (dec). R_f : 0.34 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3309 (NH), 3199 (NH), 1645 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 4.00 (2H, d, CH_2), 7.45-7.54 (3H, m, aromatic), 7.76 (2H, d, aromatic), 7.88 (2H, d, aromatic), 8.74 (2H, d, aromatic), 8.86 (1H, t, NH), 10.21 (1H, br, NH), 10.74 (1H, br, NH) ppm. Anal. Calcd for $C_{15}H_{14}N_4O_3$ (M+, 298): C, 60.40; H, 4.69; N, 18.79. Found: C, 60.71; H, 4.83; N, 19.10.

Boc-Val-NHNH-CO-9*H*-xanth (18)

The product was obtained as a white powder in yield (**A**: 87%; **B**: 90% using HOBt as an additive), mp 234 °C (dec). R_f : 0.50 (CH_2Cl_2/MeOH 9:1, 2 drops AcOH). IR (KBr):

3305 (NH), 3249 (NH), 1691 (CO), 1674 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 0.82 (6H, 2d, $2CH_3$), 1.36 (9H, s, $3CH_3$), 1.83 (1H, m, CH), 3.74 (1H, t, CH), 4.99 (1H, s, H-9), 6.72 (1H, d, NH), 7.09-7.15 (4H, m, aromatic), 7.28-7.35 (4H, m, aromatic), 10.05 (1H, s, NH), 10.53 (1H, s, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 18.67, 19.42, 28.35, 30.67, 43.31, 58.67, 78.42, 116.68, 119.76, 123.46, 129.01, 151.18, 155.67, 170.49, 170.71 ppm. Anal. Calcd for $C_{24}H_{29}N_3O_5$ (M+, 439): C, 65.60; H, 6.60; N, 9.56. Found: C, 65.91; H, 6.73; N, 9.82.

Boc-Aib-NHNH-CO-9*H*-Xanth (19)

The product was obtained as a white powder in yield (**A**: 59%; **B**: 85%, using HOBt as an additive), (**A**: 80%; **B**: 96%, using HOAt as an additive), mp 211 - 214 °C. R_f: 0.82 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3300 (NH), 3251 (NH), 3220 (NH), 1697 (CO), 1658 (CO), 1620 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.31 (6H, s, $2CH_3$), 1.36 (9H, s, $3CH_3$), 5.01 (1H, s, H-9), 6.80 (1H, s, NH), 7.09-7.14 (4H, m, aromatic), 7.27-7.35 (4H, m, aromatic), 9.65 (1H, s, NH), 10.42 (1H, s, NH) ppm. Anal. Calcd for $C_{23}H_{27}N_3O_5$ (M+, 425): C, 64.94; H, 6.35; N, 9.88. Found: C, 65.16; H, 6.61; N, 10.10.

Boc-Val-NHNH-CO-9*H*-Flu (20)

The product was obtained as a white powder in yield (**A**: 67%; **B**: 83%, using HOBt as an additive), mp 235 °C (dec). R_f : 0.48 (CH_2Cl_2/MeOH 9:1, 2 drops AcOH). IR (KBr): 3319 (NH), 3197 (NH), 1693 (CO), 1676 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 0.84 (6H, t, $2CH_3$), 1.37 (9H, s, $3CH_3$), 1.89 (1H, m, CH), 3.84 (1H, t, CH), 4.87 (1H, s, H-9), 6.75 (1H, d, NH), 7.31-7.60 (6H, 2m, aromatic), 7.87 (2H, d, aromatic), 10.10(1H,s, NH), 10.52(1H,s, NH) ppm. Anal. Calcd for $C_{24}H_{29}N_3O_4$ (M+, 423): C, 68.08; H, 6.85; N, 9.93. Found: C, 68.39; H, 6.99; N, 10.20.

Boc-Aib-NHNH-CO-9*H*-Flu (21)

The product was obtained as white powder in yield (**A**: 19%; **B**: 76%, using HOBt as an additive), (**A**: 48%; **B**: 89%, using HOAt as an additive), mp 249 °C (dec). R_f : 0.67 (CH_2Cl_2/MeOH 9:1, 2 drops AcOH). IR (KBr): 3400 (NH), 3300 (NH), 1701 (CO), 1637 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.34 (6H, s, $2CH_3$), 1.38 (9H, s, $3CH_3$), 4.88 (1H, s, H-9), 6.83 (1H, s, NH), 7.28-7.44 (4H, m, aromatic), 7.55 (2H, d, aromatic), 7.81 (2H, d, aromatic), 9.72(1H,s, NH), 10.45(1H, s, NH) ppm. Anal. Calcd for $C_{23}H_{27}N_3O_4$ (M+, 409): C, 67.48; H, 6.60; N, 10.27. Found: C, 67.30; H, 6.43; N, 10.50.

Boc-Tyr(OBn)-NHNH-CO-9*H*-Xanth (22)

The product was obtained as a white powder in yield (**A**: 92%, **B**: 98%), mp 188 °C (dec). R_f : 0.77 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3300 (NH), 3230(NH), 3220 (NH), 1705 (CO), 1693 (CO), 1681 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.27 (9H, s, $3CH_3$), 2.48-2.89 (2H, br.m, CH_2), 4.10 (1H, m, CH), 4.99 (1H, s, H-9), 5.03 (2H, s, CH_2), 6.89 (3H, d, NH, aromatic), 7.09-7.40 (15H, 2m, aromatic), 10.34 (1H, br, NH) ppm.

^{13}C-NMR (DMSO-d_6): δ 28.12, 37.50, 43.29, 54.79, 69.48, 78.39, 114.72, 116.69, 119.16, 120.00, 123.66, 125.00, 126.66, 127.92, 128.74, 129.14, 130.51, 135.97, 137.55, 151.19, 155.58, 157.26, 170.43, 171.28 ppm.

Anal. Calcd for $C_{35}H_{35}N_3O_6$ (M+, 593): C, 70.82; H, 5.90; N, 7.08. Found: C, 71.09; H, 6.21; N, 7.39.

Boc-Leu-NHNH-CO-9*H*-Xanth (23)

The product was obtained as a white powder in yield (**A**: 82%, **B**: 92%), mp 220 °C (dec). R_f : 0.74 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3320 (NH), 3260 (NH), 3250 (NH), 1700 (CO), 1680 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 0.56 (6H, 2d, $2CH_3$), 1.11 (11H, s, m, CH_2, $3CH_3$), 1.31 (1H, m, CH), 3.68 (1H, q, CH), 4.70 (1H, s, H-9), 6.62 (1H, d, NH), 6.81-6.88 (4H, m, aromatic), 7.01-7.18 (4H, m, aromatic), 9.7 (2H, br, 2NH) ppm. MS (EI)**:** 453.05 (31.79, [M]+), 339.05 (50.24, [M-$C_5H_8NO_2$]+), 280.05 (51.76, [M-$C_9H_{19}NO_2$]+).

Boc-Lys(Z)-NHNH-CO-9*H*-Xanth (24)

The product was obtained as a white powder in yield (**A**: 98%, **B**: 98%), mp 235 °C (dec). R_f : 0.70 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3320 (NH), 3300 (NH), 3234 (NH), 1705 (CO), 1700 (CO), 1687 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.24-1.51 (15H, m, $3CH_3$, $3CH_2$), 2.92 (2H, m, CH_2), 3.88 (1H, q, CH), 4.97 (3H, s, CH_2, H-9), 6.83 (1H, d, NH), 7.08-7.37 (14H, 2m, NH, aromatic), 10.05 (1H, br, NH), 10.70 (1H, br, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 23.04, 28.53, 29.36, 31.94, 44.16, 53.17, 65.45, 78.36, 116.67, 120.00, 123.65, 128.04, 128.68, 129.13, 137.58, 151.17, 155.61, 156.43, 170.41, 171.66 ppm. Anal. Calcd for $C_{33}H_{38}N_4O_7$ (M+, 602): C, 65.78; H, 6.31; N, 9.30. Found: C, 66.12 H, 6.53; N, 9.58.

Boc-Glu(OBn)-NHNH-CO-9*H*-Xanth (25)

The product was obtained as a white powder in yield (**A**: 95%, **B**: 96%), mp 192-195 °C. R_f : 0.59 (CH_2Cl_2/MeOH 9:1, 2 drops AcOH). IR (KBr): 3313 (NH), 3236 (NH), 1732 (CO, ester), 1693 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.36 (9H, s, $3CH_3$), 1.70-1.98 (2H, br.m, CH_2), 2.17 (2H, t, CH_2), 4.00 (1H, q, CH), 4.99 (1H, s, H-9), 5.10 (2H, d, CH_2), 7.07-7.38 (14H, 2m, NH, aromatic), 10.21 (2H, br, 2NH) ppm.

Anal. Calcd for $C_{31}H_{33}N_3O_7$ (M+, 559): C, 66.54; H, 5.90;

N, 7.51. Found: C, 66.79; H, 6.10; N, 7.80.

Boc-Pro-NHNH-CO-9*H*-Xanth (26)

The product was obtained as a white powder in yield (**A**: 89%, **B**: 94%), mp 203 °C (dec). R_f : 0.63 (CH_2Cl_2/MeOH 9:1, 2 drops AcOH). IR (KBr): 3200 (NH), 1700 (CO), 1695 (CO), 1674 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.22 (9H, d, $3CH_3$), 1.75 (2H, m, CH_2), 2.10 (2H, m, CH_2), 2.72-2.88 (2H, CH_2), 4.05 (1H, m, CH), 5.01 (1H, d, H-9), 7.07-7.13 (4H, m, aromatic), 7.26-7.33 (4H, m, aromatic), 10.24 (2H, br, 2NH) ppm. 13 C-NMR (DMSO-d_6): δ 23.44, 24.16, 28.31, 43.32, 46.71, 58.47, 78.94, 116.61, 120.29, 123.57, 128.93, 151.15, 153.54, 162.66, 170.14 ppm.

Anal. Calcd for $C_{24}H_{27}N_3O_5$ (M+, 437): C, 65.90; H, 6.18; N, 9.61. Found: C, 66.20; H, 6.40; N, 9.87.

Boc-Tyr(OBn)-NHNH-CO-9*H*-Flu (27)

The product was obtained as a white powder in yield (**A**: 94%, **B**: 98%), mp 205 °C (dec). R_f : 0.90 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3320 (NH), 3210 (NH), 3200 (NH), 1700 (CO), 1693 (CO), 1681 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.30 (9H, s, $3CH_3$), 2.66-2.97 (2H, br.m, CH_2), 4.18 (1H, q, CH), 4.92 (1H, s, H-9), 5.05 (2H, s, CH_2), 6.75 (1H, d, NH), 6.90-6.95 (2H, m, aromatic), 7.21 (2H, d, aromatic), 7.31-7.45 (9H, m, aromatic), 7.56-7.62 (2H, m, aromatic),7.88 (2H, d, aromatic), 10.31 (1H, s, NH), 10.66 (1H, s, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 28.28, 37.50, 52.96, 54.79, 69.51, 83.57, 114.70, 120.43, 124.60, 127.92, 128.08, 128.74, 130.00, 137.59, 140.77, 147.64, 157.26, 170.58, 171.12 ppm.

Anal. Calcd for $C_{35}H_{35}N_3O_5$ (M+, 577): C, 72.79; H, 6.06; N, 7.28. Found: C, 73.10; H, 6.21; N, 7.62.

Boc-Leu-NHNH-CO-9*H*-Flu (28)

The product was obtained as a white powder in yield (**A**: 93%, **B**: 97%), mp 206 °C (dec). R_f : 0.66 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3317 (NH), 3203 (NH), 1693 (CO), 1678 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 0.83 (6H, t, $2CH_3$), 1.37 (11H, s, m, CH_2, $3CH_3$), 1.62 (1H, m, CH), 4.02 (1H, q, CH), 4.87 (1H, s, H-9), 6.90 (1H, d, NH), 7.27-7.44 (4H, m, aromatic), 7.53-7.60 (2H, m, aromatic), 7.86 (2H, d, aromatic), 10.25 (2H, br, 2NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 19.30, 20.68, 22.38, 26.37, 39.67, 49.75, 76.23, 80.97, 116.71, 122.16, 124.63, 126.49, 138.25, 145.78, 153.42, 167.08, 168.62 ppm.

Anal. Calcd for $C_{25}H_{31}N_3O_4$ (M+, 437): C, 68.65; H, 7.09; N, 9.61. Found: C, 68.89; H, 7.00; N, 9.89.

Boc-Lys(Z)-NHNH-CO-9*H*-Flu (29)

The product was obtained as a white powder in yield (**A**: 92%, **B**: 98%), mp 205 °C (dec). R_f : 0.58 (CH_2Cl_2/MeOH 9:1) . IR (KBr): 3320 (NH), 3207 (NH), 1693 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.15-1.58 (15H, m, $3CH_3$, $3CH_2$), 2.95 (2H, m, CH_2), 3.93 (1H, q, CH), 4.88 (1H, s, H-9), 4.99 (2H, s, CH_2), 6.87 (1H, d, NH), 7.22-7.89 (14H, 2m, 2d, NH, aromatic), 10.25 (2H, br, 2NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 20.95, 26.52, 27.40, 30.55, 51.36, 63.50, 76.42, 81.45, 118.34, 120.00, 122.49, 126.02, 126.71, 127.36, 134.16, 135.53, 138.74, 145.85, 153.59, 154.53, 167.51, 168.63 ppm.

Anal. Calcd for $C_{33}H_{38}N_4O_6$ (M+, 586): C, 67.57; H, 6.48; N, 9.55. Found: C, 67.90; H, 6.30; N, 9.82.

Boc-Glu(OBn)-NHNH-CO-9*H*-Flu (30)

The product was obtained as a white powder in yield (**A**:, 88%, **B**: 95%), mp 197-200 °C. R_f : 0.60 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3320 (NH), 3195 (NH), 1730 (CO, ester), 1700 (CO), 1689 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.32 (9H, s, $3CH_3$), 1.80-2.04 (2H, br.m, CH_2), 2.19 (2H, m, CH_2), 4.03 (1H, q, CH), 4.90 (1H, s, H-9), 5.07 (2H, d, CH_2), 7.29-7.90 (14H, m, 3d, NH, aromatic), 10.20 (2H, br, 2NH) ppm. MS (EI)**:** 543 (6.21, [M]+), 351.05 (3.74, [M-$C_{11}H_{14}NO_2$]+), 267.05 (3.68, [M-$C_{15}H_{18}NO_4$]+).

Boc-Pro-NHNH-CO-9*H*-Flu (31)

The product was obtained as a white powder in yield (**A**: 84%, **B**: 96%), mp 165 °C (dec). R_f : 0.80 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3180 (NH), 1700 (CO, ester), 1681 (CO), 1670 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.33 (9H, d, $3CH_3$), 1.73-2.12 (4H, 2m, $2CH_2$), 3.25 (2H, m, CH_2), 4.09 (1H, m, CH), 4.93 (1H, d, H-9), 7.28-7.58 (6H, m, aromatic), 7.74-7.89 (2H, 2d, aromatic), 10.22 (2H, br, 2NH) ppm. Anal. Calcd for $C_{42}H_{27}N_3O_4$ (M+, 421): C, 68.41; H, 6.41; N, 9.97. Found: C, 68.70; H, 6.17; N, 10.25.

General method for preparation of peptide hydrazides derivatives

Boc-amino acid hydrazide (1 mmol) was treated with a solution of CH_2Cl_2 and TFA (10 mL, 1:1) at room temperature for 2 h in order to remove the Boc- group. The solvent was removed under reduced pressure and the solid product was dissolved in CH_2Cl_2 (20 mL) and then removed under reduced pressure. Diethyl ether (20 mL) was added and the solid product (TFA-amino acid hydrazide salt) was collected and dried to be used in the next step. NMM (1 mmol) was added to a solution of Boc-amino acid (1 mmol) , HOAt (1 mmol) and DIC (1 mmol) in DMF (1 mL). The reaction mixture was stirred at 0 °C for 10 min. and then 1 mmol of TFA-amino acid hydrazide salt was added followed by 1

mmol of NMM. The reaction mixture was heated in microwave employing a multimode reactor (Synthos 3000, Aton Paar GmbH, 1400 W maximum magnetron) for 5 min. at 60 °C and hold at the same temeprature for 5 min to ensure a complete reaction (~10 bar pressure, 800 W). Cooling was complished by a fan (5 min). Water (20 mL) was added to the reaction mixture and the precipitate was filtered, dried and recrystallized from ethylacetate/hexane.

Boc-Tyr(OBn)-Leu-NHNH-CO-9*H*-Xanth (32)

The product was obtained as a white powder in yield 72%, mp 187 °C (dec). R_f : 0.86 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3450 (NH), 3300 (NH), 3200 (NH), 1665 (CO), 1650 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 0.804 (6H, 2d, $2CH_3$), 1.29 (9H, s, $3CH_3$),1.43 (2H, m, CH_2), 1.61 (1H, m, CH), 2.59- 2.88 (2H, 2m, CH_2), 4.08 (1H, q, CH), 4.33 (1H, q, CH), 4.99 (1H, s, H-9), 5.04 (2H, s, CH_2), 6.80-6.86 (3H, d, NH, aromatic), 7.08-7.44 (15H, 2m, aromatic), 7.92 (1H, d, NH), 10.13 (1H, s, NH), 10.54 (1H, s, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 19.52, 20.94, 21.85, 26.06, 39.33, 40.95, 47.41, 53.79, 67.10, 76.10, 112.30, 114.31, 117.34, 121.28, 125.57, 125.73, 126.38, 126.76, 128.19, 135.19, 148.81, 153.25, 154.84, 168.09, 168.98, 169.30 ppm. MS (EI) for $C_{41}H_{46}N_4O_7$ (M+, 706): 705.90 (100, [M]+), 481 (52.23, [M-$C_{12}H_{19}NO_3$]+), 308 (41.35, [M-$C_{24}H_{32}NO4$]+).

Boc-Lys(Z)-Tyr(OBn)-Leu-NHNH-CO-9*H*-Xanth (33)

The product was obtained as a beige powder in yield 88%, mp 198 °C (dec). R_f : 0.50 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3320 (NH), 3280 (NH), 3200 (NH), 3190 (NH), 1710 (CO), 1700 (CO), 1650 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 0.78 (6H, 2d, $2CH_3$), 1.13-1.57 (18H, m, $3CH_3$, $4CH_2$, CH), 2.69-2.94 (4H, 2m, $2CH_2$), 3.76 (1H, q, CH), 4.35 (1H, q, CH), 4.50 (1H, q, CH), 4.98 (5H, s, $2CH_2$, H-9), 6.81-6.84 (3H, m, NH, aromatic), 7.08-7.40 (21H, 2m, aromatic, NH), 7.65 (1H, d, NH), 8.11 (1H, d, NH), 10.15 (1H, s, NH), 10.55 (1H, s, NH) ppm. MS (EI) for $C_{55}H_{64}N_6O_{10}$ (M+, 968): 967.8 (100, [M]+).

Boc-Asp(OBn)-Val-NHNH-CO-9*H*-Xanth (34)

The product was obtained as white powder in yield 85%, mp 233 °C (dec). R_f : 0.51 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3300 (NH), 3200 (NH), 1750 (CO, ester), 1700 (CO), 1650 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 0.779-0.988 (6H, 2d, $2CH_3$), 1.37 (9H, s, $3CH_3$), 2.57-2.65 (2H, br.m, CH_2), 2.92 (1H, m, CH), 4.22 (1H, m, CH), 4.25 (1H, q, CH), 4.47 (2H, d, CH_2), 4.99 (1H, s, H-9), 5.14 (1H, t, NH), 7.07-7.14 (4H, m, aromatic), 7.20-7.35 (9H, m, aromatic), 7.55 (1H, d, NH), 9.93(1H s, ,NH), 10.53(1H, s, NH) ppm. ^{13}C- NMR (DMSO-d_6): δ 19.21, 21.13, 27.62, 28.42, 35.18, 44.38, 49.28, 57.56, 63.23, 79.29, 116.64, 120.10, 123.62, 126.75, 126.95, 128.37, 129.12, 151.18, 155.54, 166.74, 170.63, 175.03, 176.35 ppm. MS (EI) for $C_{35}H_{40}N_4O_8$ (M+, 644): 643.95 (93.20, [M]+), 525 (76.24, [M-$C_5H_{13}NO_2$]+), 453.05 (68.57, [M-$C_{11}H_{13}NO_2$]+), 293.05 (70.52, [M-$C_{19}H_{29}NO_5$]+).

Boc-Tyr(OBn)-Asp(OBn)-Val-NHNH-CO-9*H*-Xanth (35)

The product was obtained as beige powder in yield 95%, mp. 192 °C (dec). R_f: 0.73 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3300 (NH), 3220 (NH), 1750 (CO, ester), 1700 (CO), 1650 (CO) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 0.83 (6H, t, $2CH_3$), 1.27 (9H, s, $3CH_3$), 1.89 (1H, m, CH), 2.59-2.88 (4H, br.m, $2CH_2$), 4.17 (2H, m, 2CH), 4.66 (1H, q, CH), 5.06 (5H, m, $2CH_2$, H-9), 6.82 (1H, d, NH), 6.86 (2H, d, aromatic), 7.07-7.15 (6H,m, aromatic), 7.28-7.43 (14H, m, aromatic), 8.11 (1H, d, NH), 8.33 (1H, d, NH), 10.14 (1H, s, NH), 10.55 (1H, s, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 18.46, 19.34, 21.07, 28.45, 31.00, 37.20, 43.32, 49.28, 56.70, 63.24, 66.39, 69.49, 78.38, 114.67, 116.70, 119.71, 123.63, 127.90, 128.01, 128.67, 128.74, 132.45, 136.19, 137.57, 151.19, 155.46, 157.22, 166.81, 170.15, 170.54, 171.40, 172.12, 175.54 ppm. MS (EI) for $C_{51}H_{55}N_5O_{10}$ (M+, 897): 897.90 (100, [M+1]+).

Boc-Asp(OBn)-Val-NHNH-CO-9*H*-Flu (36)

The product was obtained as a white powder in yield 82%, mp 195°C (dec). R_f : 0.69 (CH_2Cl_2/MeOH 9:1). ^{1}H-NMR (DMSO-d_6): δ 0.82 (6H, t, $2CH_3$), 1.35 (9H, s, $3CH_3$), 1.92 (1H, m, CH), 2.58-2.72 (2H, br.m, CH_2), 4.20 (1H, t, CH), 4.34 (1H, q, CH), 4.85 (1H, s, 9-CH), 5.09 (2H, s, CH_2), 7.07 (1H, d, NH), 7.30-7.44 (9H, m, aromatic), 7.54-7.59 (2H, m, aromatic), 7.87 (2H, d, aromatic), 7.95 (1H, d, NH), 10.18 (1H, s, NH), 10.48 (1H, s, NH) ppm. MS (EI) for $C_{35}H_{40}N_4O_7$ (M+, 628): 626.95 (100, [M-1]+).

Boc-Tyr(OBn)-Asp(OBn)-Val-NHNH-CO-9*H*-Flu (37)

The product was obtained as a beige powder in yield 87%, mp197 °C (dec), R_f : 0.53 (CH_2Cl_2/MeOH 9:1). IR (KBr): 3260 (NH), 3160 (NH), 1750 (CO, ester), 1700 (CO), 1650 (CO) cm^{-1} .^{1}H-NMR (DMSO-d_6): δ 0.84 (6H, t, $2CH_3$), 1.27 (9H, s, $3CH_3$), 1.91 (1H, m, CH), 2.63-2.88 (4H, br.m, $2CH_2$), 4.12 (1H, q, CH), 4.26 (1H, t, CH), 4.67 (1H, q, CH), 4.86 (1H, s, H-9), 5.03 (4H, m, $2CH_2$), 6.86 (3H, m, aromatic, NH), 7.12 (2H, d, aromatic), 7.32-7.40 (14H, m, aromatic), 7.54-7.60 (2H, m, aromatic), 7.87 (2H, d, aromatic), 8.16 (1H, d, NH), 8.34 (1H, d, NH), 10.21 (1H, s, NH), 10.51 (1H, s, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 17.15, 18.04, 26.81, 29.86, 35.74, 47.97, 51.66, 54.64, 55.29, 65.07, 68.16,

77.30, 113.34, 119.17, 123.94, 124.0, 127.42, 129.20, 134.91, 136.27, 140.40, 141.42, 154.15, 155.90, 167.87, 168.54, 170.12, 170.81 ppm. MS (EI) for $C_{51}H_{55}N_5O_9$ (M+, 881): 881 (90.67, [M]+), 807.95 (100, [M-C_4H_9O]+).

CONCLUSION

Application of microwave irradiation (MWI) accelerating the coupling of *N*-protectected amino acid and synthesis of *N*-protectecting amino acid hydrazide as well as peptide hydrazide derivatives. microwave irradiation (MWI) leads to many advantages, like the use of inexpensive reagents (HOBt and DIC), in addition to the eco-friendly "green chemistry" economical and environmental impacts. As expected, DIC/HOAt was confirmed to be superior to DIC/HOBt ones in terms of both coupling yield and purity for all cases.

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REFERENCES AND NOTES

*Abbreviations not defined in text: Aib = α-aminoisobutyric acid; Bn = Benzyl; Bz = Benzoyl; DCC = dicyclohexylcarbodiimide; DCM = dichloromethane; DIC = diisopropylcarbodiimide; DIEA = diisopropylethylamine; DMF = dimethyl formamide; HOBt = 1-hydroxybenzotriazole; HOAt = 7- aza-1-hydroxybenzotriazole; NMM = *N*-methylmorpholine; TFA = trifluoroacetic acid;TMP = 2,4,6-trimethylpyridine; Z = benzyloxycarbonyl. Flu = 9-fluorenyl; xanth = 9-xanthenyl. Amino acids and peptides are abbreviated and designated following the rules of the IUPAC-IUB Commission of Biochemical Nomenclature [*J. Biol. Chem.* **1972**, *247*, 977].

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