Efficient preparation of azodye-labeled aminoxy acids and peptides

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This paper is dedicated to Professor Julio Álvarez-Builla, on the occasion of his 65th anniversary

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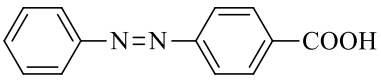
Abstract

1-(4-Arylazobenzoyl)benzotriazoles **2a**–**c** react in aqueous acetonitrile at 20 ºC with aminoxy acids **3a**–**c** to give azodye-labeled aminoxy acids **4a**–**i** in 65-80% yields. Similarly, reaction of Fmoc-protected aminoxy-dipeptides **6a**–**c** with **2a** gave azodye-labeled aminoxy peptides **7a**–**c** in 55–65% yield.

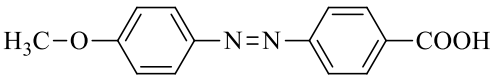
Keywords: Azodyes, aminoxy acids, peptides, acylation

Introduction

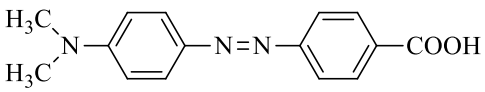
Photoisomerization and fluorescence resonance energy transfer (FRET) properties have led to extensive use of azodye carboxylic acids such as **1a**, **1b** and **1c** (Figure 1) in biology.[1-3] Azodye-labeled peptides are important for pharmaceutical and biological investigations (Figure 2). Peptide based azodye-labeled molecules show substrate specificity for prostate membrane antigens[4] and in some cases are potent inhibitors of *m*-calpain.[3] Azodye-labeled peptides may also serve as markers in biological applications.[5]



1a, 4-Paba = 4-(Phenylazo)benzoic acid



1b, 4-Mpaba = 4-(Methoxyphenylazo)benzoic acid



1c, 4-Dpaba = 4-(Dimethylaminophenylazo)benzoic acid

Figure 1. Azodye carboxylic acids.

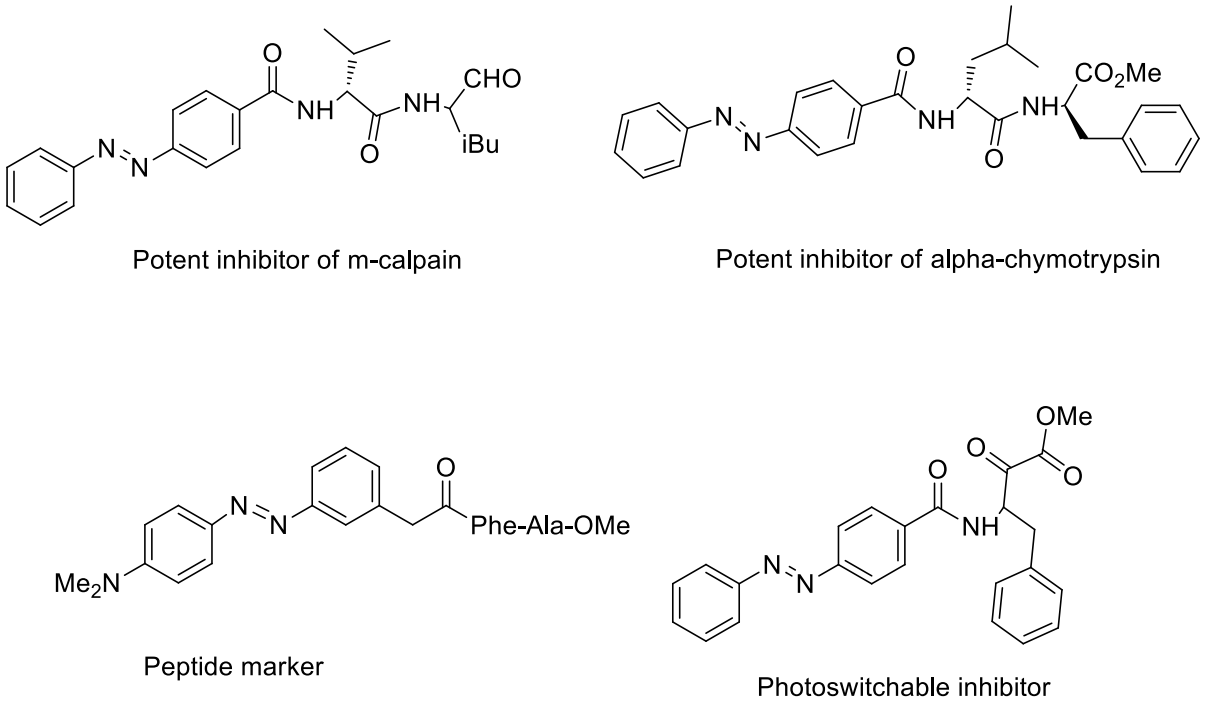


Figure 2. Azodye labeled peptides.

Attaching azodye carboxylic acids to host molecules is the key to the synthesis of azo-photoresponsive systems. Amino acids/peptides or amines acting as links between azo-dye acyl groups and host molecules are common in many photobiological switches and bioprobes. Azo-photoresponsive systems frequently incorporate azodye-labeled α(ω)-amino acids/peptides.[6]

α-Aminoxy acids are analogs of β-amino acids in which the β-carbon atom is replaced by an oxygen atom. The incorporation of α-aminoxy acids into peptidomimetics has attracted interest, since α-aminoxy acid units are more rigid than the corresponding β-amino acid units,[7] and aminoxy amide bonds RCONHOR' resist enzymatic degradation.[8] α-Aminoxy peptides have also attracted interest as novel foldamers[9] with unusual conformations and diverse bioactivity.[10a-c] Aminoxy peptides also feature strong intramolecular hydrogen bonds between adjacent residues in peptidomimetic foldamers[11] and may provide useful labels.

N-Acylbenzotriazoles are easily prepared, non-hygroscopic, chirally stable analogs of acid halides that are relatively insensitive to water,[12,13] and are therefore advantageous for N-, O-, C-, or S- acylation;[12-18] especially where the corresponding acid chlorides are unstable or difficult to prepare.[19,20] We previously acylated amino acids and amines with *N*-(4-arylazobenzoyl)-1*H*-benzotriazoles[21] and we recently synthesized azodye-labeled peptides in good yields by a milder procedure than published methods.[22]

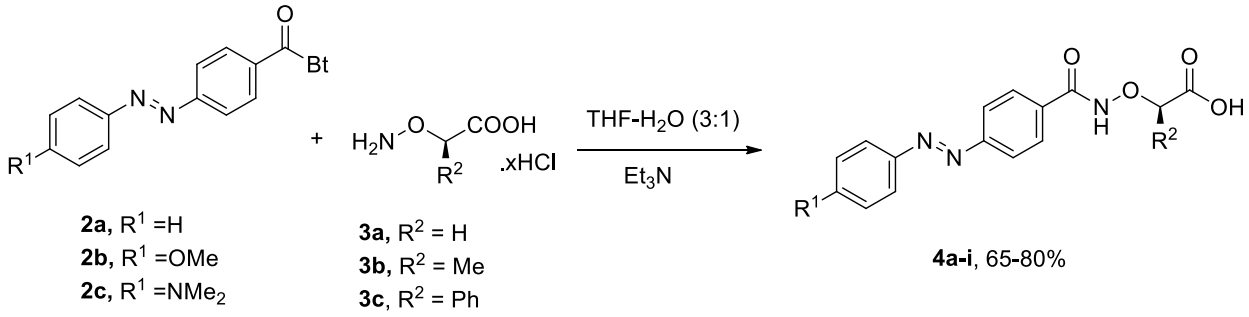
We have not located any previous syntheses of azodye-labeled aminoxy acids or peptides. Because of the interesting properties of aminoxy peptides, we now report the synthesis of azodye

labeled aminoxy acids and peptides by reaction of *N*-(4-arylazobenzoyl)-1*H*-benzotriazole with aminoxy acids and aminoxy peptides under mild conditions.

Results and Discussion

Preparation of azodye labeled aminoxy acids (4a–i)

N-(4-Arylazobenzoyl)-1*H*-benzotriazoles **2a**–**c**, which were prepared by our previously reported method,[21b] were treated with the appropriate aminoxy acids **3a**–**c** in THF-H_2O (3-1, v/v) in the presence of triethylamine for 4–8 h at 20 ºC (monitored by TLC) to afford azodye labeled aminoxy acids **4a**–**i** in yields of 65–80% (Scheme 1, Table 1). Novel products were characterized by ^{1}H-NMR, ^{13}C-NMR and elemental analysis.



Scheme 1

Table 1. Preparation of azodye carboxylic acid labeled aminoxy acids **4a**–**i**

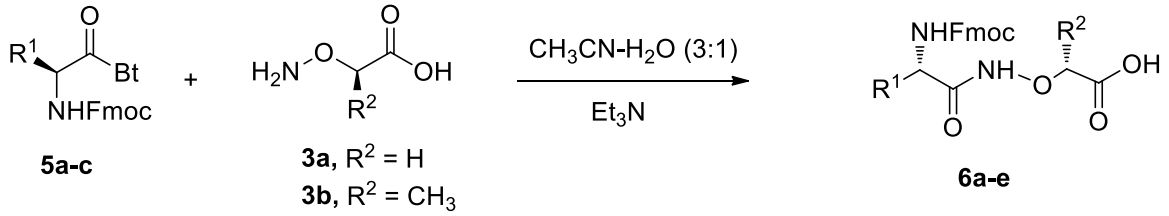
Entry	**2**	Aminoxy acid **3**[a]	**4**, yield (%)[b]	**4**, Mp ºC
1	**2a**	AO-Gly-OH **3a**	4-Paba-AO-Gly-OH **4a**, 74	200–202
2	**2a**	AO-Ala-OH **3b**	4-Paba-AO-Ala-OH **4b**, 75	188–190
3	**2a**	AO-Phe-OH **3c**	4-Paba-AO-Phe-OH **4c**, 70	178–180
4	**2b**	AO-Gly-OH **3a**	4-Mpaba -AO-Gly-OH **4d**, 65	200–202
5	**2b**	AO-Ala-OH **3b**	4-Mpaba-AO-Ala-OH **4e**, 65	182–184
6	**2b**	AO-Phe-OH **3c**	4-Mpaba-AO-Phe-OH **4f**, 75	192–194
7	**2c**	AO-Gly-OH **3a**	4-Dpaba-AO-Gly-OH **4g**, 80	189–191
8	**2c**	AO-Ala-OH **3b**	4-Dpaba-AO-Ala-OH **4h**, 70	196–198
9	**2c**	AO-Phe-OH **3c**	4-Dpaba-AO-Phe-OH **4i**, 80	222–224

[a]In our aminoxy acid nomenclature; the aminoxy acids are defined as follows: "2-(aminoxy)acetic acid **3a"** as "AO-Gly-OH **3a**"; "2-(aminoxy)propanoic acid **3b**" as "AO-Ala-OH **3b**"; "2-(aminoxy)-3-phenylpropanoic acid **3c**" as "AO-Phe-OH **3c**" and the abbreviated short names are used throughout the manuscript. [b] Isolated yield.

Preparation of Fmoc-protected aminoxy hybrid peptides (6a–e)

Fmoc protected aminoxy hybrid peptides **6a**–**e** were prepared by the reaction of *N*-(Fmoc-α-aminoacyl)benzotriazoles **5a**–**c** with aminoxy acids **3a,b** in the presence of triethylamine in

acetonitrile-water (3:1) at room temperature in 90–92% yields (Scheme 2, Table 2).



Scheme 2

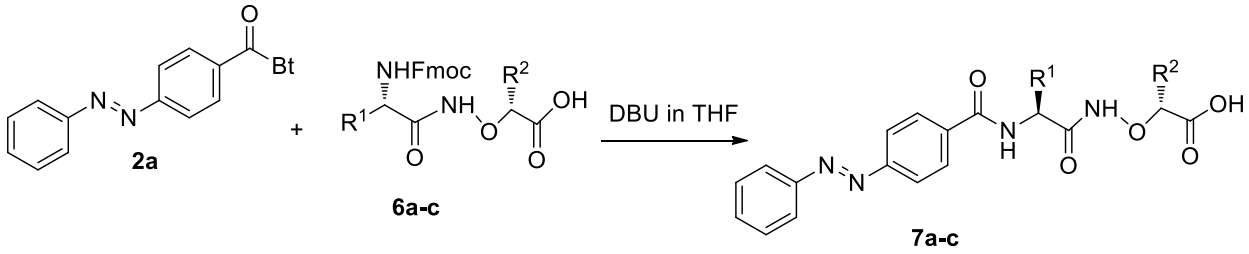
Table 2. Preparation of Fmoc-protected aminoxy hybrid peptides **6a**–**e**

Entry	**3**	**5**	**6**, yield (%)[a]	**6**, Mp °C
1	**3a**	Fmoc-L-Phe-Bt **5b**	Fmoc-L-Phe-AO-Gly-OH **6a**, 92	106–108
2	**3b**	Fmoc-L-Phe-Bt **5b**	Fmoc-L-Phe-AO-Ala-OH **6b**, 90	102–104
3	**3a**	Fmoc-L-Met-Bt **5c**	Fmoc-L-Met-AO-Gly-OH **6c,** 92	70–72
4	**3a**	Fmoc-L-Leu-Bt **5a**	Fmoc-L-Leu-AO-Gly-OH **6d**, 90	110–112
5	**3b**	Fmoc-L-Met-Bt **5c**	Fmoc-L-Met-AO-Ala-OH **6e**, 91	88–90

[a]Isolated yield.

Preparation of azodye labeled aminoxy peptides (7a–c)

Fmoc protected aminoxy dipeptides **6a**–**c** were treated with a solution of one equivalent of DBU in dry THF for 2 h at 0-5 °C, followed by treatment with 4-phenylazobenzoyl-1*H*-benzotriazole **2a** gave azodye labeled aminoxy peptides **7a**–**c** in 55-65% yields (Scheme 3, Table 3).



Scheme 3

Table 3. Preparation of azodye carboxylic acid labeled aminoxy peptides **7a**–**c**

Entry	Fmoc aminoxy dipeptide **6**	**7**, yield (%)	**7**, Mp °C
1	Fmoc-L-Phe-AO-Gly-OH **6a**	4-Paba-L-Phe-AO-Gly-OH **7a**, 55	178–180
2	Fmoc-L-Phe-AO-Ala-OH **6b**	4-Paba-L-Phe-AO-Ala-OH **7b**, 58	192–194
3	Fmoc-L-Met-AO-Gly-OH **6c**	4-Paba-L-Met-AO-Gly-OH **7c,** 65	185–187

Conclusions

In conclusion, we have synthesized novel azodye labeled aminoxy acids and aminoxy hybrid peptides in a convenient and efficient manner by reacting *N*-(4-arylazobenzoyl)-1*H*-benzotriazoles with aminoxy acids and aminoxy hybrid peptides. All the azodye labeled aminoxy acids and aminoxy hybrid peptides were obtained under mild reaction conditions in moderate to good yields. These novel azodye labeled aminoxy acids and aminoxy hybrid peptides may be useful in the preparation of peptidomimetic foldamers and in biological applications.

Experimental Section

General. Aminooxy acid **3a** was purchased commercially and used without purification. Aminooxy acids **3b**, **3c** were prepared according to our previously reported method.[23] Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. NMR spectra were recorded in acetone-d_6, $CDCl_3$ or DMSO-d_6 with TMS for ^1H (300 MHz) and ^{13}C (75 MHz) as an internal reference. Elemental analyses were performed on a Carlo Erba–1106 instrument. Mass spectrometry was done on Agilent 6210 TOF-MS with electro spray ionization (ESI). CH_2Cl_2 was dried and distilled over CaH_2, whereas THF was used after distillation over Na-benzophenone.

Synthesis of azo-dye carboxylic acid labeled aminooxy acids (4a-i)

N-(4-Arylazobenzoyl)-1*H*-benzotriazoles **2a-c** (1.0 mmol) were added to a solution of the appropriate α-aminooxy acids **3a-c** (1.2 mmol) in THF–H_2O (3:1) in the presence of Et_3N (2.0 mmol). The reaction mixture was stirred at 20 °C for about 3 h until TLC showed the absence of **2a-c**, then the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (50 mL) and the solution was washed with 4 N HCl (3 × 50 mL), saturated NaCl solution (50 mL), and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was crystallized from appropriate solvents to give **4a-i**.

4-Phenylazobenzoyl-AO-Gly-OH (4a). Orange microcrystals (74%), mp 200-202 °C. ^1H NMR (DMSO-d_6) δ 8.10-7.93 (m, 6H), 7.70-7.60 (m, 3H), 4.54 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 171.3, 163.8 153.8, 152.2, 134.6, 132.5, 130.0, 128.8, 123.1, 122.9, 72.5. Anal. Calcd for $C_{15}H_{15}N_3O_5$: C, 56.78; H, 4.76; N, 13.24. Found: C, 56.72; H, 4.68; N, 12.94.

4-Phenylazobenzoyl-AO-Ala-OH (4b). Orange microcrystals (75%), mp 188-190 °C; $[\alpha]^{23}_D$ = –12.0 (*c* 1.0, CH_3OH). ^1H NMR (DMSO-d_6) δ 12.05 (br s, 1H), 8.15 (d, *J* = 8.1 Hz, 1H), 7.97─7.91 (m, 6H), 7.60 (d, *J* = 1.8 Hz, 3H), 4.57 (q, *J* = 6.5 Hz, 1H), 1.43 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 172.7, 166.7, 151.9, 132.1, 130.6, 129.6, 128.7, 122.8, 122.6, 122.5, 78.7, 16.5. Anal. Calcd for $C_{16}H_{15}N_3O_4$: C, 61.34; H, 4.83; N, 13.41. Found: C, 61.37; H, 4.91; N, 13.24.

4-Phenylazobenzoyl-AO-Phe-OH (4c). Orange microcrystals (70%), mp 178-180 °C; $[\alpha]^{23}_D$ = –19.0 (*c* 1.0, CH_3OH). ^{1}H NMR (Acetone-d_6) δ 8.05-7.94 (m, 6H), 7.63-7.58 (m, 3H), 7.40 (d, *J* = 7.2 Hz, 2H), 7.33-7.23 (m, 3H), 4.93 (t, *J* = 5.1 Hz, 1H), 3.35-3.20 (m, 2H); ^{13}C NMR (Acetone-d_6) δ 172.0, 155.2, 153.3, 137.5, 134.1, 132.8, 131.6, 130.5, 130.2, 129.3, 129.1, 127.4, 123.8, 123.5, 85.7, 37.9. Anal. Calcd for $C_{22}H_{19}N_3O_4$: C, 67.86; H, 4.92; N, 10.79. Found: C, 67.62; H, 4.90; N, 10.79.

4-[(4-Methoxy)phenylazo]benzoyl-AO-Gly-OH (4d). Orange microcrystals (65%), mp 200-202 °C. ^{1}H NMR (DMSO-d_6) δ 12.15 (br s, 1H), 8.11 (d, *J* = 8.1 Hz, 1H), 7.95-7.86 (m, 6H), 7.15-7.13 (m, 2H), 4.52 (s, 2H), 3.87 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 170.1, 162.5 146.2, 130.6, 128.6, 124.9, 122.3, 122.2, 114.7, 71.8, 55.8. HRMS (ESI) Calcd for $C_{16}H_{15}N_3O_5$: [M – H]$^-$ 328.0939. Found: 328.0938.

4-[(4-Methoxy)phenylazo]benzoyl-AO-Ala-OH (4e). Orange microcrystals (65%), mp 182-184 °C; $[\alpha]^{23}_D$ = –6.0 (*c* 1.0, CH_3OH). ^{1}H NMR (DMSO-d_6) δ 12.03 (br s, 1H), 8.12 (d, *J* = 8.7 Hz, 1H), 7.96-7.87 (m, 5H), 7.14 (d, *J* = 8.1 Hz, 2H), 6.88 (br s, 1H), 4.56 (d, *J* = 6.9 Hz, 1H), 3.87 (s, 3H), 1.42 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 174.1, 163.4, 154.7, 147.0, 133.6, 129.3, 125.9, 123.1, 115.5, 79.8, 56.2, 17.1. Anal. Calcd for $C_{17}H_{17}N_3O_5$: C, 59.47; H, 4.99; N, 12.24. Found: C, 59.13; H, 4.86; N, 12.08.

4-[(4-Methoxy)phenylazo]benzoyl-AO-Phe-OH (4f). Orange microcrystals (75%), mp 192-194 °C; $[\alpha]^{23}_D$ = –31.0 (*c* 1.0, CH_3OH). ^{1}H NMR (Acetone-d_6) δ 11.53 (br s, 1H), 8.03-7.92 (m, 7H), 7.42-7.25 (m, 5H), 7.14 (d, *J* = 9.0 Hz, 2H), 4.98-4.93 (m, 1H), 3.93 (s, 3H), 3.37-3.17 (m, 2H); ^{13}C NMR (Acetone-d_6) δ 171.6, 167.6, 155.7, 147.7, 137.4, 133.2, 130.5, 129.5, 129.1, 127.5, 125.9, 123.2, 115.4, 86.6, 56.1, 38.0. Anal. Calcd for $C_{23}H_{21}N_3O_5$: C, 65.86; H, 5.05; N, 10.02. Found: C, 65.53; H, 5.08; N, 9.84.

4-[(4-Dimethylamino)phenylazo]benzoyl-AO-Gly-OH (4g). Brown microcrystals (80%), mp 189-191 °C. ^{1}H NMR (DMSO-d_6) δ 7.92–7.79 (m, 7H), 6.88 (d, *J* = 8.4 Hz, 2H), 4.52 (s, 2H), 3.08 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 170.1, 163.7, 153.2, 142.5, 131.8, 128.5, 126.3, 121.3, 113.0, 71.8, 40.5. Anal. Calcd for $C_{17}H_{19}ClN_4O_4$: C, 53.90; H, 5.06; N, 14.79. Found: C, 53.78; H, 5.01; N, 14.53.

4-[(4-Dimethylamino)phenylazo]benzoyl-AO-Ala-OH (4h). Brown microcrystals (70%), mp 196-198 °C; $[\alpha]^{23}_D$ = -7.0 (*c* 1.0, CH_3OH). ^{1}H NMR (DMSO-d_6) δ 8.01-7.95 (m, 2H), 7.90-7.80 (m, 4H), 6.9 (d, *J* = 9.0 Hz, 2H), 4.63 (q, *J* = 7.2 Hz, 1H), 3.13 (s, 6H), 1.47 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 173.3, 164.7, 154.1, 153.5, 142.8, 132.1, 128.8, 126.2, 121.9, 112.6, 79.2, 40.5, 16.9. Anal. Calcd for $C_{18}H_{21}ClN_4O_4$: C, 55.03; H, 5.39; N, 14.26. Found: C, 54.78; H, 5.51; N, 13.99.

4-[(4-Dimethylamino)phenylazo]benzoyl-AO-Phe-OH (4i). Brown microcrystals (80%), mp 222-224 °C; $[\alpha]^{23}_D$ = -24.0 (*c* 1.0, CH_3OH). ^{1}H NMR (DMSO-d_6) δ 8.00-7.85 (m, 7H), 7.40-7.21 (m, 5H), 7.03 (d, *J* = 9.0 Hz, 2H), 4.85 (t, *J* = 6.6 Hz, 1H), 3.22-3.08 (m, 8H); ^{13}C NMR (DMSO-d_6) δ 171.6, 153.8, 153.2, 136.7, 132.1, 129.7, 128.6, 128.2, 126.6, 126.2, 121.5, 113.2, 83.7, 45.5, 37.0. Anal. Calcd for $C_{24}H_{25}ClN_4O_4$: C, 61.47; H, 5.37; N, 11.95. Found: C, 60.69; H, 5.42; N, 11.64.

Synthesis of Fmoc-protected aminooxy peptides (6a-e)

Benzotriazole derivatives of Fmoc-amino acids **5a-c** (1 mmol) were added to a solution of the appropriate α–aminooxy acids **3a-b** (1 mmol) in MeCN–H_2O (3:1) in the presence of Et_3N (2.0 mmol). The reaction mixtures were stirred at 20 °C for about 1 h until TLC showed the absence of **5a-c** when the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (150 mL) and the solution was washed with 4 N HCl (3 × 50 mL), saturated NaCl solution (50 mL), and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was crystallized from appropriate solvents to give **6a-e**. Compounds **6a-c** were used for reaction without further purifications.

Fmoc-L-Phe-AO-Gly-OH (6a). White microcrystals (92%), mp 106-108 °C; $[\alpha]^{23}_D$ = -24.0 (*c* 1.0, CH_3OH). ^{1}H NMR (Acetone-d_6) δ 7.85 (d, *J* = 7.2 Hz, 2H), 7.68-7.61 (m, 2H), 7.41 (t, *J* = 7.2 Hz, 2H), 7.34–7.20 (m, 7H), 4.40 (br s, 2H), 4.28-4.16 (m, 3H), 3.22-2.90 (m, 2H); ^{13}C NMR (Acetone-d_6) δ 154.6, 151.7, 147.6, 139.9, 138.9, 138.2, 137.6, 137.2, 135.8, 130.4, 83.7, 77.0, 57.5, 48.2, 40.3, 29.6. Anal. Calcd for $C_{26}H_{24}N_2O_6$: C, 67.82; H, 5.25; N, 6.08. Found: : C, 67.85; H, 5.49; N, 5.55

Fmoc-L-Phe-AO-Ala-OH (6b). White microcrystals (90%), mp 102-104 °C; $[\alpha]^{23}_D$ = -30.0 (*c* 1.0, CH_3OH). ^{1}H NMR ($CDCl_3$) δ 10.04 (br s, 1H), 7.74 (d, *J* = 7.5 Hz, 2H), 7.60–7.00 (m, 12H), 5.77 (br s, 1H), 4.63–4.12 (m, 4H), 3.03 (d, *J* = 7.2 Hz, 2H), 1.44 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 171.5, 169.1, 156.4, 143.3, 141.2, 135.4, 129.2, 128.7, 127.8, 127.3, 127.1, 124.9, 120.0, 72.8, 67.7, 60.4, 53.4, 46.8, 38.8. Anal. Calcd for $C_{27}H_{26}N_2O_6$: C, 68.34; H, 5.52; N, 5.90. Found: C, 67.85; H, 5.49; N, 5.55.

Fmoc-L-Met-AO-Gly-OH (6c). White microcrystals (92%), mp 70-72 °C; $[\alpha]^{23}_D$ = -18.0 (*c* 1.0, CH_3OH). ^{1}H NMR ($CDCl_3$) δ 10.32 (br s, 1H), 7.75 (d, *J* = 7.5 Hz, 2H), 7.60-7.50 (m, 2H), 7.42-7.25 (m, 6H), 4.51 (s, 2H), 4.50-4.38 (br s, 3H), 4.22-4.18 (m, 1H), 2.60–2.40 (m, 2H), 2.20-1.80 (m, 5H); ^{13}C NMR (Acetone-d_6) δ 173.7, 170.4, 157.0, 145.0, 142.0, 128.5, 127.9, 126.1, 120.8, 74.1, 67.3, 67.1, 53.7, 52.7, 47.9, 32.2, 32.1, 30.9, 15.1. Anal. Calcd for $C_{22}H_{24}N_2O_6S$: C, 59.45; H, 5.44; N, 6.30. Found: C, 60.44; H, 5.53; N, 5.89. Compound **6c** was used for the further reaction and the final compound **7c** was fully characterized by ^{1}H, ^{13}C NMR and HRMS.

Fmoc-L-Leu-AO-Gly-OH (6d). White microcrystals (90%), mp 110–112 °C; $[\alpha]^{23}_D$ = -14.0 (*c* 1.0, CH_3OH). ^{1}H NMR ($CDCl_3$) δ 10.54 (br s, 1H), 7.73 (d, *J* = 7.5 Hz, 2H), 7.60-7.45 (m, 2H), 7.41–7.25 (m, 5H), 5.69 (d, *J* = 9.6 Hz, 1H), 4.50 (s, 2H), 4.43–4.30 (m, 3H), 4.15 (t, *J* = 6.0 Hz, 1H), 1.57 (d, *J* = 3.9 Hz, 3H), 0.89 (d, *J* = 4.5 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 171.6, 170.1, 156.7, 143.3, 141.3, 127.8, 127.1, 124.9, 120.0, 72.8, 67.7, 50.4, 46.9, 41.5, 24.5, 22.6, 22.0. Anal. Calcd for $C_{23}H_{26}N_2O_6$: C, 64.78; H, 6.14; N, 6.57. Found: C, 64.63; H, 5.87; N, 6.61.

Fmoc-L-Met-AO-Ala-OH (6e). White microcrystals (91%), mp 88-90 °C; $[\alpha]^{23}_D$ = -16.0 (*c* 1.0, CH_3OH). ^{1}H NMR ($CDCl_3$) δ 10.98 (br s, 1H), 7.84 (d, *J* = 7.8 Hz, 2H), 7.75-7.60 (m, 2H), 7.45-7.25 (m, 6H), 4.52 (d, *J* = 6.9 Hz, 1H), 4.40-4.20 (m, 4H), 2.60-2.51 (m, 3H), 2.07–2.02 (m, 4H), 1.41 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 174.3, 169.6, 156.5, 143.4, 141.1, 127.7, 127.0, 124.9, 119.9, 79.7, 67.5, 46.7, 31.7, 31.4, 29.7, 16.1, 15.1. Anal. Calcd for $C_{23}H_{26}N_2O_6S$: C, 60.25; H, 5.72; N, 6.11. Found: C, 60.05; H, 5.71; N, 5.99.

Synthesis of azodye labeled aminooxy peptides (7a-c)

Fmoc protected aminooxy dipeptide **6a-c** (1 mmol) were added to a solution of DBU (1 mmol) in dry THF (6 mL) and after stirring for 2 h at 0–5 ºC, 4-Phenylazobenzoyl-1H-benzotriazole **2a** (1 mmol) was added and the reaction mixtures were stirred at 20 ºC for another 10 h. Solvent was evaporated and residue was dissolved in CH_2Cl_2 (20 mL), washed with 3N HCl (3x10 mL) and dried over anhydrous Na_2SO_4. After evaporation of solvent, the residue was recrystalized from CH_2Cl_2/hexanes to give **7a-c**.

4-Phenylazobenzoyl-L-Phe-AO-Gly-OH (7a). Orange microcrystals (55%), mp 178-180 °C; $[\alpha]^{23}_D$ = -46.0 (*c* 1.0, CH_3OH). ^{1}H NMR (Acetone-d_6) δ 8.02 (d, *J* = 8.7 Hz, 2H), 7.99-7.91 (m, 4H), 7.58-7.56 (m, 3H), 7.35-7.17 (m, 7H), 6.85 (d, *J* = 8.7 Hz, 1H), 4.84 (br s, 1H), 4.40 (br s, 2H), 3.39-3.10 (m, 2H); ^{13}C NMR (Acetone-d_6) δ 170.2, 155.0, 153.4, 138.1, 136.9, 132.7, 130.2, 129.4, 129.2, 127.5, 123.7, 123.3, 121.1, 120.5, 74.1, 53.7, 38.0. HRMS (ESI) Calcd for $C_{24}H_{22}N_4O_5$: $[M - H]^-$ 445.1517. Found: 445.1521.

4-Phenylazobenzoyl-L-Phe-AO-Ala-OH (7b). Orange microcrystals (58%), mp 192-194 °C; $[\alpha]^{23}_D$ = -65.0 (*c* 1.0, CH_3OH). ^{1}H NMR (DMSO-d_6) δ 12.81 (br s, 1H), 8.79 (d, *J* = 8.7 Hz, 1H), 8.53 (d, *J* = 7.8 Hz, 1H), 8.15 (d, *J* = 10.8 Hz, 1H), 8.04-7.91 (m, 5H), 7.61 (s, 3H), 7.42-7.16 (m, 5H), 4.79 (br s, 1H), 4.26 (d, *J* = 6.6 Hz, 1H) 3.20–3.00 (m, 2H), 1.35 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 174.0, 171.2, 165.4, 153.2, 151.8, 138.4, 132.0, 130.6, 129.5, 129.1, 128.7, 128.0, 122.7, 122.5, 122.2, 54.7, 47.6, 37.1, 17.1. HRMS (ESI) Calcd for $C_{25}H_{24}N_4O_5$: $[M + Na]^+$ 483.1639. Found: 483.1645.

4-Phenylazobenzoyl-L-Met-AO-Gly-OH (7c). Orange microcrystals (65%), mp 185-187 °C; $[\alpha]^{23}_D$ = -32.0 (*c* 1.0, CH_3OH). ^{1}H NMR (DMSO-d_6) δ 8.10–8.00 (m, 2H), 7.88-7.82 (m, 5H), 7.55-7.45 (m, 3H), 4.45 (d, *J* = 5.7 Hz, 1H), 4.31 (s, 2H), 2.51-2.45 (m, 4H), 2.00-1.94 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 173.9, 170.4, 167.0, 154.8, 153.2, 137.1, 132.9, 130.4, 130.1, 129.9, 123.9, 123.5, 123.4, 121.8, 118.0, 114.2, 110.4, 71.2, 52.8, 50.9, 50.6, 38.3. HRMS (ESI) Calcd for $C_{20}H_{22}N_4O_5S$: $[M + Na]^+$ 453.1203. Found: 453.1215.

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