Convenient preparations of azo-dye labeled amino acids and amines†

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N-(4-Arylazobenzoyl)-1*H*-benzotriazoles **3** react with amino acids **4** and amines **6** to give azo-dye labeled amino acids (**5a–m**) and amines (**7a–n**) in high yields (74–100%) with retention of chirality.

Introduction

Azobenzenes and their photochemistry are receiving increasing attention.[1] Azo-dye carboxylic acids have been widely used in the materials and life sciences fields as molecular switches based on their photoisomerization and FRET (fluorescence resonance energy transfer):[1c,d] (i) to construct photoresponsive reporters to monitor, regulate or control the activity of enzymes, with potential as inhibitors for proteases;[2] (ii) as quenchers to label oligonucleotides and phosphoramidites for the construction of molecular beacons or probes to detect specific nucleic acids, DNA or glycoconjugates/saccaride;[3] (iii) as fluorescence quenchers/reporters of various biological reactions;[4] (iv) in the photoregulation of duplex formation of DNA and ODNs (oligodeoxynucleotides);[5] and (v) as photoisomerization units in photoresponsive biomaterials.[6]

Connecting azo-dye carboxylic acids to host molecules is a key step in the synthesis of azo-photoresponsive systems. In the synthesis of photobiological switches or bioprobes, amino acids/peptides or amines are common linkages between azo-dye acyl groups and host molecules. Many azo-photoresponsive systems incorporate azo-dye labeled α(ω)-amino acids/peptides,[2a,h,4a,f,5a,6a–e] or ω-amino alcohols/amines.[3a–j,4d,5b–e]

Published synthetic methods to link azo-dye carboxylic acids to bio-moieties (Scheme 1) have used (i) coupling reagents such as DCC, EDCI, HOBT, HBTU, HATU;[2f,3f,i,5b,c,e,7] (ii) acyl chlorides;[3a,j,8a–c] and (iii) other activated azo-dye carboxylic acid intermediates, including *N*-hydroxysuccinimidoester,[3h,4b,c] 4-[(4-dimethylamino)phenylazo]benzoyl-1*H*-imidazole.[4d]

Utilization of these methods has encountered complex procedures,[2f,3a,4b,c,7] harsh reaction conditions,[4b,c,7] low yields[4c,5c,7,8b,c] and difficulties in product purification.[4b,c,f] Thus mild and efficient methods to label amino acids/peptides and amines with azo-dye carboxylic acids are desired.

N-Acylbenzotriazoles are advantageous for *N*-, *O*-, *C*-, *S*-acylation,[9] especially where the corresponding acid chlorides are unstable or difficult to prepare.[10a,b] We now report useful reactions of amino acids and amines with *N*-(4-arylazobenzoyl)-1*H*-benzotriazoles **3a** and **3b**.

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Results and discussion

1. Preparation of *N*-(4-arylazobenzoyl)-1*H*-benzotriazoles (3)

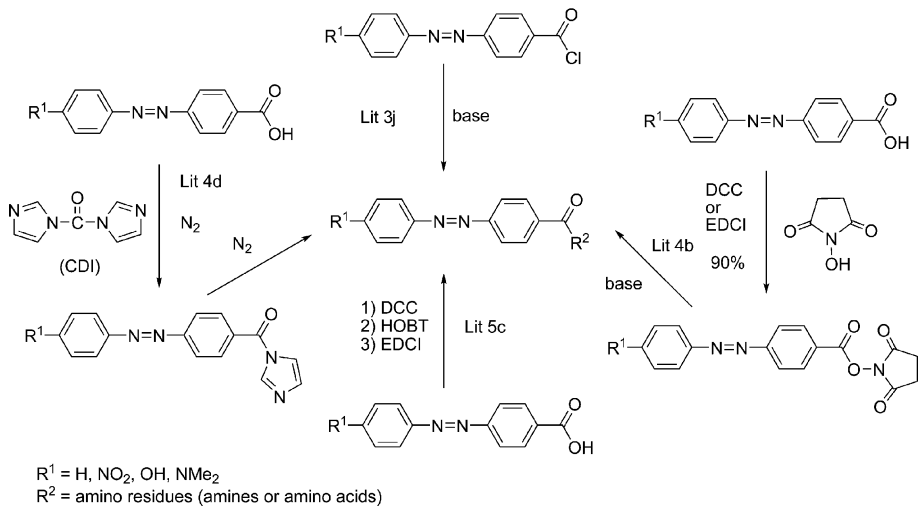
N-[[4-(*p*-Dimethylaminophenylazo)]benzoyl]-benzotriazole **3a** and *N*-(4-phenylazobenzoyl)-benzotriazole **3b** were prepared by a standard method (Scheme 2).[9c,d] Treatment of 4-(arylazo)benzoic acids **1** with 1-(methylsulfonyl)-1*H*-benzotriazole **2**[9c,d] under reflux for 5 h gave products *N*-(4-arylazobenzoyl)-1*H*-benzotriazole **3a–b** in 85–86% yield.

2. Preparation of azo-dye carboxylic acid labeled amino acid derivatives

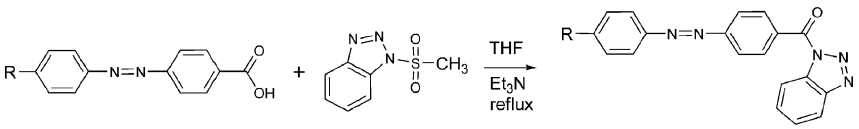
Azo-dye carboxylic acid labeled amino acid derivatives were obtained in high yields by treating *N*-(4-arylazobenzoyl)-1*H*-benzotriazole **3** with appropriate amino acids in DMF–H_2O (3 : 1, v/v) mixture in the presence of triethylamine (Scheme 3). These reactions were completed within 24 h at room temperature (monitored by TLC). Novel products were characterized by ^{1}H-NMR, ^{13}C-NMR and elemental analysis. In the preparation and purification of **5a–m**, the mild conditions retained the original chirality of the amino acids and amines, as confirmed by optical rotation and HPLC experiments. The results are summarized in Table 1. From the chiral starting materials **4a–g** and **4j,k**, the corresponding products **5a–g** and **5j–m** had the optical rotation stated; and all showed single peaks in HPLC analysis. In the case of racemic compounds **5h** and **5i**, the measured optical rotation was zero and two peaks of equal intensity were observed in HPLC analysis. For example, a single peak was obtained for the enantiomer 4-[4-(dimethylamino)phenylazo]benzoyl-L-phenylalaine **5c** at 3.04 min; but the racemic mixture 4-[4-(dimethylamino)phenylazo]benzoyl-DL-phenylalaine **5h** gave two peaks at 3.04 and 3.37 min.

3. Preparation of azo-dye carboxylic acid labeled amine derivatives

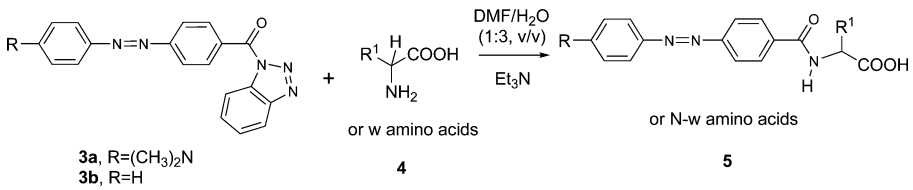
Azo-dye labeled amine derivatives were also obtained in high yields by treating *N*-(4-arylazobenzoyl)-1*H*-benzotriazole **3** with appropriate amines. Optimum conditions for the reaction of **3** with amines **6** (Scheme 4) differ according to the type of amine used, as shown in Table 2. Compound **3** and the amine **6** were mixed in the appropriate solvent (dry THF or DMF) and stirred at room temperature or with heating. Most of the products **7** were easily purified by washing with MeOH; others were obtained by chromatography on silica gel; all the novel products were characterized by ^{1}H-NMR, ^{13}C-NMR and elemental analysis. The



Scheme 1 Literature methods of labeling amines and amino acids with azo-dye carboxylic acids.



Scheme 2



Scheme 3

Table 1 Preparation of azo-dye carboxylic acid labeled amino acids

Entry	3	Amino acid 4	5, yield (%)	5, mp/°C	5, retention time/min[g]	5 $[\alpha]_D^{25}$
1	**3a**	Glycine **4a**	**5a**[a], 99	238–240	3.34	—
2	**3a**	L-Alanine **4b**	**5b**, 86	205–207	3.04	+73.3
3	**3a**	L-Phenylalanine **4c**	**5c**, 82	202–203	3.42	+119.4
4	**3a**	L-Tryptophan **4d**	**5d**, 90	220–222	3.31	+112.1
5	**3a**	L-Isoleucine **4e**	**5e**, 87	230–235	3.42	+52.2
6	**3a**	L-Methionine **4f**	**5f**, 81	205–207	3.52	+31.1
7	**3a**	L-Serine **4g**	**5g**, 88	205–207	3.04,	+60.0
8	**3a**	DL-Phenylalanine **4h**	**5h**, 82	203–204	3.37[h]	0.0
9	**3a**	DL-Phenylglycine **4i**	**5i**[b], 88	189–191	2.97,	0.0
10	**3a**	6-Aminocaproic acid **4j**	**5j**[c], 95	208–210	3.56[h]	—
11	**3b**	L-Leucine **4k**	**5k**[d], 86	120–122	—	+9.0
12	**3b**	L-Phenylalanine **4c**	**5l**[e], 95	185–186	3.19	+103.0
13	**3b**	L-Alanine **4b**	**5m**[f], 87	222–224[f]	3.23	+39.3

[a] Lit.,[4c,11] mp 232–233 °C, yield 43%. [b] Lit.,[12] mp 188–190 °C. [c] Lit.,[4b] yield 77%. [d] Lit.,[2f,8c] yield 95%, mp 173 °C. [e] Lit.,[7] mp 183–184 °C, yield 65%. [f] Lit.,[8c] mp 220 °C, yield 24%. [g] Single peak unless otherwise stated. [h] Two peaks of equal intensity.

results are summarized in Table 2, the products **7f**, **7g** from the chiral starting materials **6f**, **6g** had certain optical rotation, and showed a single peak in HPLC analysis. In contrast, for racemic compound **7n**, the optical rotation value was zero and two peaks of equal intensity were observed in HPLC analysis.

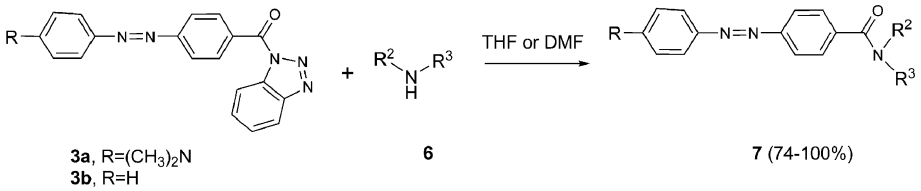
4. Utilities and advantages of our method over previous methods

In comparison with literature data, our method comprises: (1) mild and simple reaction and work-up conditions: we isolated most of the products under milder reaction conditions

Table 2 Preparation of azo-dye carboxylic acid labeled amino acid esters

Entry	3	Amine 6	7, yield (%)	7, mp/°C
1	**3a**	Morpholine **6a**	**7a**, 100	212–215
2	**3a**	*n*-Butylamine **6b**	**7b**, 93	212–214
3	**3a**	*t*-Butylamine **6c**	**7c**, 85	228–230
4	**3a**	2-(Ethylamino)-ethanol **6d**	**7d**[a], 94	176–178
5	**3a**	6-Amino-1-hexanol **6e**	**7e**[b], 93	150–152
6	**3a**	L-α-Methylbenzylamine **6f**	**7f**[c], 88	222–224
7	**3a**	L-Valine methyl ester hydrochloride **6g**	**7g**, 91	156–158
8	**3a**	*p*-Toluidine **6h**	**7h**, 86	263–265
9	**3a**	*N*-Methylaniline **6i**	**7i**, 74	185–187
10	**3a**	2-Aminopyridine **6j**	**7j**, 84	190–192
11	**3a**	Carbazole **6k**	**7k**, 81	204–206
12	**3b**	*n*-Benzylamine **6l**	**7l**[d], 91	192–193
13	**3b**	*m*-Toluidine **6m**	**7m**[e], 86	168–169
14	**3b**	DL-Valine methyl ester hydrochloride **6n**	**7n**[f], 91	131–132

[a] Lit.,[3i] yield 98%. [b] Lit.,[3a] yield 80%. [c] Lit.,[13] yield 70%. [d] Lit.,[8b] mp 194–194.5 °C, yield 50%. [e] Lit.,[8b] mp 168.5–170.0 °C, yield 42%. [f] Lit.,[14] mp130–131 °C, yield 56%.



Scheme 4

and just by washing or chromatography on silica gel, whereas reported methods gave side-products (for example, DCU in DCC method,[5c] phenylazobenzic acids in acyl chloride method,[8b] HOSu in NHS method[4b,c]) and also they needed complex purification procedures[2f,3a,i,7,8b,c,11,13,14] (entries 1, 11–13 in Table 1 and entry 4, 5, 9, 12–14 in Table 2); (2) high yields: most of our products were obtained in high yield (74–100%); whereas some old reported methods[7,8b,c,11,14] provided low yields for some compounds (for example, entries 1, 12, 13 in Table 1 (yield range 24–43%) and entry 12, 13, 14 in Table 2 (yield range 42–56%)); (3) no racemization occurred for chiral compounds in our method, but in reported methods some coupling reagents reduced the ee value of products in the coupling method[7]; (4) very high selectivity to amine over alcohol groups, which is important for the synthesis of some probes.[3a] (for example, entry 7 in Table 1 and entries 4, 5 in Table 2); (5) cost effective: our methodology is cost effective because Bt–H is cheaper than coupling reagents, such as HBTU, HATU, EDCI.

Conclusion

In conclusion, a convenient and an efficient method for the preparation of azo-dye labeled amino acids and amines has been developed by reacting *N*-(4-arylazobenzoyl)-1*H*-benzotriazole with amino acids and amines. All the azo-dye labeled products were obtained under mild and simple reaction conditions in high yields with no detectable racemization for chiral compounds. For substrates having amine and alcohol functionality, this methodology has shown high selectivity for amines to alcohol.

Experimental

General Methods

Melting points were determined on Fisher melting apparatus. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on a 300 MHz NMR spectrometer in $CDCl_3$ or DMSO-d_6. HPLC analyses were performed on Beckman system gold programmable solvent module 126, using Chirobiotic T column (4.6 × 250 mm), detection at 254 nm, flow rate of 1.0 mL min^{-1} and MeOH as eluting solvent. Elemental analyses were performed on a Carlo Erba-1106 instrument. Optical rotation values were measured with the use of sodium D line. Arylazobenzoic acids, amino acids, and amines were purchased from Fisher or Aldrich chemical companies.

General procedure for preparation of *N*-(4-arylazo)benzoyl-benzotriazoles (3a,b). Arylazobenzoic acid **1** (7.43 mmol, 2.0 g for **1a**, 1.68 g for **1b**), 1-(methylsulfonyl)-1*H*-benzotriazole **2**[9d] (1.46 g, 7.43 mmol) and Et_3N (1.5 mL, 10.4 mmol) were mixed in THF at room temperature. After refluxing for 5h, the reaction mixture was cooled to room temperature and kept overnight at room temperature, then solid was precipitated. After filtration and drying under vacuum, the corresponding product, *N*-(4-arylazo)benzoyl-benzotriazoles **3a–b**, were obtained.

***N*-[[4-(*p*-Dimethylaminophenylazo)]benzoyl]-benzotriazole (3a).** (2.24 g, 81%). Red microcrystal; mp 210.0–212.0 °C (from THF); (found: C, 68.27; H, 4.77; N, 22.59. Calc. for $C_{21}H_{18}N_6O$: C, 68.09; H, 4.90; N, 22.69%); δ_H(300 MHz; $CDCl_3$) 8.42 (1H, d, *J* 8.4, Ar*H*), 8.38 (2H, d, *J* 8.7, Ar*H*), 8.19 (1H, d, *J* 8.1, Ar*H*), 7.97 (4H, dd, $J_1 = J_2$ 8.4, Ar*H*), 7.72 (1H, t, *J* 7.2, Ar*H*), 7.56 (1H,

t, *J* 8.4, Ar*H*), 6.77 (2H, d, *J* 9.0, Ar*H*) and 3.13 (6H, s, 2 × NC*H*$_3$,); δ_C(75 MHz, $CDCl_3$) 170.0, 166.3, 156.4, 153.2, 145.9, 143.9, 133.1, 132.6, 131.1, 130.5, 126.5, 125.8, 122.1, 120.3, 115.0, 111.6 and 40.4.

General method for the preparation of carboxylic azo-dye labeled amino acids 5a–m. *N*-(4-Arylazo)benzoyl-benzotriazole **3** (200 mg, 0.54 mmol for **3a** and 0.611 mmol for **3b**, 1eq.) and amino acid **4** (1eq.) were added to a mixture of DMF and water (3 : 1,v/v), and stirred at room temperature for 24 h. After the evaporation of solvent, washed with CH_2Cl_2 and drying under vacuum, the corresponding pure products **5** were obtained with high yield of 81–99%; For **5k–m**, after the evaporation of solvent, the residue was dissolved in CH_2Cl_2 and washed with 4 N HCl.

4-[(4-Dimethylamino)phenylazo]benzoyl-glycine (5a). (175 mg, 99%). Red microcrystal; mp 238.0–240.0 °C (from CH_2Cl_2) (lit.,[4b,10] mp 232–233 °C); δ_H(300 MHz, DMSO-d_6; Me_4Si) 8.94 (1H, t, *J* 5.7, N*H*), 8.02 (2H, d, *J* 8.7, Ar*H*), 7.84 (2H, d, *J* 8.7, Ar*H*), 7.83 (2H, d, *J* 9.3, Ar*H*), 6.85 (2H, d, *J* 9.3, Ar*H*), 3.95 (2H, d, *J* 6.0, C*H*$_2$) and 3.08 (6H, s, 2 × C*H*$_3$); δ_C(75 MHz, DMSO-d_6) 171.4, 165.9, 154.1, 152.9, 142.7, 134.1, 128.4, 125.1, 121.6, 111.6, 41.4 and 39.8.

Gerenal procedure for the preparation of 7a–f. Procedure A. *N*-[[4-(*p*-Dimethylaminophenylazo)]benzoyl]-benzotriazole (**3**) (200 mg, 0.54 mmol for **3a** and 0.611 mmol for **3b**) and corresponding amines (1–3eq.) were mixed in THF (10mL) and stirred for 1–48 h at room temperature (monitored by TLC). After the evaporation of solvent, pure products **7** were obtained from the residues after simple purification procedures with high yield of 85–100%.

4-[4-(Dimethylamino)phenylazo]benzoyl-morpholine (7a). 1eq. **6a**; purified with column on silica gel eluting with ethyl acetate–hexane (1 : 2, v/v) to give **7a** as red microcrystal (184 mg, 100%); mp 212.0–215.0 °C (from EtOAc–hexane); found: C, 67.57; H, 6.66; N, 16.57. Calc. for $C_{19}H_{22}N_4O_2$: C, 67.44; H, 6.55; N, 15.56%; δ_H(300 MHz, $CDCl_3$) 7.88 (4H, t, *J* 8.1, Ar*H*), 7.52 (2H, d, *J* 8.1, Ar*H*), 6.76 (2H, d, *J* 9.0, Ar*H*), 3.85–3.40 (8H, br s, CH_2CH_2) and 3.11 (6H, s, 2 × C*H*$_3$); δ_C(75 MHz, $CDCl_3$) 170.1, 154.0, 152.8, 143.6, 135.6, 128.1, 125.4, 122.3, 111.5, 66.9, 40.3 and 29.8.

The preparation of 7g, 7h. Procedure B. *N*-[[4-(*p*-Dimethylaminophenylazo)]benzoyl]-benzotriazole (**3a**) (200 mg, 0.54 mmol) was added to the solution of amine (1–3eq.) and Et_3N (3.0 eq.) in THF (10 ml) at room temperature. The mixture was heated under reflux for 24 h. After filtration, the solvent was evaporated under reduced pressure. Pure products were obtained from the residues after simple purification procedures.

4-[4-(Dimethylamino)phenylazo]benzoyl-L-valine methyl ester (7g). 1.0eq. **6g**; purified with column on silica gel eluting with ethyl acetate–hexanes (1 : 3, v/v) to give **7g** as red microcrystal (188 mg, 90%); mp 156.0–158.0 °C (from EtOAc–hexane); $[a]_D^{25}$ +59.6 (*c* 2.8 in MeOH); retention time: 3.29; found: C, 66.05; H, 6.94; N, 14.37. Calc. for $C_{21}H_{26}N_4O_3$: C, 69.95; H, 6.85; N, 14.65%; δ_H(300 MHz, $CDCl_3$) 7.90 (6H, m, Ar*H*), 6.76 (2H, d, *J* 9.0, Ar*H*), 6.67 (1H, d, *J* 8.4, N*H*), 4.83–4.79 (1H, m, *J* 5.1, 3.6, NHC*H*), 3.79 (3H, s, OC*H*$_3$), 3.12 (6H, s, 2 × NC*H*$_3$), 2.33–2.27 (1H, m, C*H*CH$_3$) and 1.02 (6H, t, *J* 6.6, 2 × CHC*H*$_3$); δ_C(75 MHz, $CDCl_3$) 172.8, 167.0, 155.4, 152.9, 143.7, 134.2, 128.1, 125.6, 122.4, 111.6, 57.6, 52.4, 40.4, 31.8, 19.2 and 18.2.

General procedure for the preparation of 7i–k. Procedure C. *N*-[[4-(*p*-Dimethylaminophenylazo)]benzoyl]-benzotriazole (**3a**) (200 mg, 0.54 mmol) and corresponding amine (2.0–3.0 eq.), Et_3N (0.23mL, 1.62 mmol) were mixed in DMF (10mL). The mixture was heated at 150 °C for 5–10 h. After the evaporation of solvent under reduced pressure, the residue was worked up with MeOH and the products were obtained as red solids.

4-[4-(Dimethylamino)phenylazo]benzoyl-*N*-methylaniline (7i). 3 eq. **6i**; worked up with MeOH to give **7i** as red microcrystal (140 mg, 74%); mp 185.0–187.0 °C (from MeOH); found: C, 73.38; H, 6.35; N, 15.71. Calc. for $C_{22}H_{22}N_4O$: C, 73.72; H, 6.19; N, 15.63%; δ_H(300 MHz; DMSO-d_6; Me_4Si) 7.75 (2H, d, *J* 7.5, Ar*H*), 7.57 (2H, d, *J* 6.9, Ar*H*), 7.38 (2H, d, *J* 7.8, Ar*H*), 7.27 (2H, d, *J* 6.6, Ar*H*), 7. 20 (3H, d, *J* 6.3, Ar*H*), 6.82 (2H, d, *J* 7.8, Ar*H*), 3.40 (3H, s, CONC*H*$_3$) and 3.06 (6H, s, 2 × NC*H*$_3$); δ_C(75 MHz; DMSO-d_6; Me_4Si) 168.9, 152.7, 152.4, 144.4, 142.6, 136.8, 129.3, 129.1, 127.1, 126.5, 125.0, 121.0, 111.5, 39.8 and 37.8.

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