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A temporary marker for biological applications

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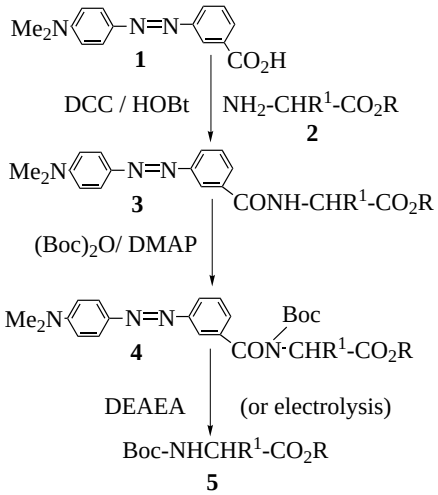
Abstract—Having in mind the development of new colour labelled amino acid derivatives, a carboxyl azo dye was coupled to amino acid esters to give the corresponding orange *N*-acyl derivatives, which were in turn further acylated at their N-terminus with Boc for investigation of the conditions of possible cleavage of the chromophore by electrolysis or with nucleophiles. While difficulties were met with electrolysis owing to competitive reduction of the azo group, cleavage with *N*,*N*-diethylaminoethylamine (DEAEA) gave satisfactory results. This allows the use of the chromophore as a temporary marker.

In recent years one has witnessed a fast growth of new areas of dye chemistry, mainly in connection with biomedical applications.[1] Diazo coupling has been employed for the first time in the field of protein chemistry[2,3] as early as 1915, finding applications in biology, forensics, diagnostic and immunology. Determination of enzyme activity[4] is one of its especially useful applications, which accounts for the development of new methodologies and new improvements in this field often leading to new patents.[5,6] The use of azobenzene moieties[7] and that of colour labelling related to the investigation of biological activity[8] are examples of recent applications in the field of peptide chemistry.

Following our previous work with reactive azo dyes for wool and polyamide fibres,[9] and in connection with our programme to explore new methods for cleavage of known, and to develop new, protecting groups,[10] we decided to investigate the use of azo dyes to prepare coloured derivatives of α-amino acids. Now, we report our first results of the preparation and characterisation of several orange α-amino acid esters bearing a 3-



a R = Me, NH-CHR^1-CO = Pro

b R = Me, R^1 = H

c R = Me, R^1 = CH_3

d R = Me, R^1 = $CH(CH_3)_2$

e R = Me, R^1 = $CH(CH_3)(CH_2CH_3)$

f R = Me, R^1 = $CH_2CH(CH_3)_2$

g R = Me, R^1 = $CH_2CH_2SCH_3$

h R = Et, R^1 = Ph-CH_2

Scheme 1.

Keywords: acylation; amino acid; cleavage reactions; azo compounds; dyes.

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[(*N*,*N* - dimethylaminophenyl) - 4′ - diazenyl] - benzoyl group (Dpa) at their N-terminus and also of the investigation of the cleavage of this group.

One equivalent of a carboxyl azo dye (**1**) derived from 3-aminobenzoic acid and *N*,*N*-dimethylaniline was reacted with amino acid methyl (**2a**–**g**) or ethyl esters (**2h**) in DMF by a DCC/HOBt coupling[11] (Scheme 1).[12] After purification by dry or flash chromatography on silica gel followed by recrystallisation, the orange 3-[(*N*,*N*-dimethylaminophenyl)-4′-diazenyl]-benzoylamino acid esters (**3a**–**h**) were obtained as solid materials with yields within the range 56–99% (Table 1) and characterised by ^{1}H and ^{13}C NMR, FTIR and visible spectroscopy, and also by elemental analysis. All products were stable on storage in the air and at room temperature. Their ^{1}H and ^{13}C NMR ($CDCl_3$) as well as their FTIR spectra agreed with the expected structure. The visible spectra showed λ_{max} at 415 nm with ε values falling between 17000 (**3a**) and 33145 (**3g**).[13]

Table 1. Results obtained in the synthesis of compounds **3**

Compound		Yield (%)
No.	Formula	
3a	Dpa-Pro-OMe	56
3b	Dpa-Gly-OMe	99
3c	Dpa-Ala-OMe	91
3d	Dpa-Val-OMe	81
3e	Dpa-Ile-OMe	79
3f	Dpa-Leu-OMe	59
3g	Dpa-Met-OMe	70
3h	Dpa-Phe-OEt	68

Table 2. Results obtained in the synthesis of coloured compounds **4**

Compound		Yield (%)
No.	Formula	
4b	Dpa-Gly(*N*-Boc)-OMe	90
4c	Dpa-Ala(*N*-Boc)-OMe	99
4d	Dpa-Val(*N*-Boc)-OMe	85
4e	Dpa-Ile(*N*-Boc)-OMe	56
4f	Dpa-Leu(*N*-Boc)-OMe	99
4g	Dpa-Met(*N*-Boc)-OMe	60
4h	Dpa-Phe(*N*-Boc)-OEt	99

Table 3. Results obtained in the selective cleavage of compounds **4** with DEAEA

Starting material	Product		
Compound no.	No.	Formula	Yield (%)
4b	**5b**	Boc-Gly-OMe	56
4c	**5c**	Boc-Ala-OMe	63
4d	**5d**	Boc-Val-OMe	40
4e	**5e**	Boc-Ile-OMe	72
4f	**5f**	Boc-Leu-OMe	49
4g	**5g**	Boc-Met-OMe	78
4h	**5h**	Boc-Phe-OEt	71

Having in mind that the acyl group under study is a derivative of the benzoyl group and that the latter can be cleaved selectively from Boc-acylamino acid and peptide derivatives under mild conditions[14,15] and that cleavage of the dye moiety would give back colourless compounds, the deacylation of the materials described above was then investigated. For this purpose, compounds **3b**–**h** were converted (Scheme 1) into the corresponding Boc-acylamide esters (**4b**–**h**) by treatment at room temperature with *tert*-butyl pyrocarbonate in dry acetonitrile and in the presence of catalytic amounts of 4-(*N*,*N*-dimethylamino)-pyridine (DMAP). After purification by dry chromatography, the coloured reaction products were obtained in yields within the range 56–99% (Table 2) and characterised as above. The visible spectra showed λ_{max} within 418 nm (**4b**) and 470 nm (**4e**), with ε values falling between 7723 (**4d**) and 24589 (**4g**).[16]

The Boc-acylamides (**4b**–**h**) were then treated with *N*,*N*-diethylaminoethyl-amine (DEAEA) in dry acetonitrile and at room temperature, and the expected Boc-amino acid esters (**5b**–**h**) isolated as colourless materials in yields within the range 40–78% (Table 3).[16] In all preparations a coloured by-product was shown by TLC; isolation and characterised by IR and ^{1}H and ^{13}C NMR spectroscopy revealed that this was the transamidation product resulting from transfer of the dye moiety to DEAEA.

Compound **4h** was electrolysed at a constant potential of 1.81 V in acetonitrile containing Et_4NCl as the supporting electrolyte and Et_3NHCl as a proton donor.[15] A 6 h reaction, which was stopped when 88% of the starting material had been consumed as shown by HPLC, yielded 32% of the expected cleavage product (**5h**) and also 32% of *N*-(3-aminobenzoyl)-*N*-*tert*-butyloxycarbonyl–phenylalanine ethyl ester resulting from competitive reductive cleavage of the azo group;[17] as one would expect, both compounds were colourless. We are now investigating the conditions required for electrolysis of the latter compound having in mind to improve the yield of **5h**.

As model compounds for peptides and proteins, the coloured amino acid derivatives mentioned above could be obtained usually in good yields and can be converted back into colourless materials in fair yields by treatment with DEAEA whenever this may be required. Thus, our results show that the azo dye investigated can be used as a temporary marker for peptide and protein labelling, certainly with applications both in chemistry and in biology.

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13. Typical experiment: 3-[(*N*,*N*-dimethylaminophenyl)-4′-diazenyl]-benzoic acid (**1**) was reacted in a 1.12-mmolar scale with alanine methyl ester hydrochloride (**2c**) in DMF by a standard DCC/HOBt coupling. After dry chromatography on silica gel (diethyl ether/*n*-hexane 9:1) and recrystallisation from ethyl acetate-*n*-hexane, **3c** was obtained as an orange solid in a 91% yield. Mp 132.4–134.1°C. R_f 0.56 (diethyl ether-*n*-hexane, 9:1). UV–vis (MeOH): λ_{max} 415 (28453) nm. FTIR (KBr, 1%): ν_{max} 3282, 2921, 1752, 1637, 1605, 1538, 1442, 1398, 1372, 1344, 1202, 1150, 1109, 1066 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.57 (3H, d, *J* 7.5 Hz, α-CH_3), 3.12 (6H, s, $N(CH_3)_2$), 3.82 (3H, s, OCH_3), 4.80–4.85 (1H, m, α-H), 6.79 (2H, d, *J* 9.3 Hz, 2×*ortho*-ArH $N(CH_3)_2$), 6.85 (1H, d, *J* 7.2 Hz, NH), 7.57 (1H, t, *J* 7.8 Hz, 5-H), 7.86–7.94 (3H, m, 2×*meta*-ArH $N(CH_3)_2$, 4 or 6-H), 8.0 (1H, dt, *J* 8.7 Hz, 1.2 Hz, 6- or 4-H), 8.22 (1H,t, *J* 1.8 Hz, 2-H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 18.5 (α-CH_3), 40.2 (N-CH_3), 48.5 (α-C), 52.4 (O-CH_3), 111.4 (2′-C, 6′-C), 120.2 (2-C), 125.1 (3′-C, 5′-C), 125.3 (4-C), 128.0 (5-C), 129.2 (6-C), 134.7 (1-C), 143.3 (4′-C), 152.6 (3-C), 153.1 (1′-C), 166.4 (O=C-N), 173.5 (O-C=O). The assignments were supported by the Dept 45 technique. Anal. calcd for $C_{19}H_{22}N_4O_3$: C, 64.39; H, 6.26; N, 15.81. Found: C, 64.65; H, 6.21; N, 15.68.
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16. To a solution of **3c** in dry acetonitrile (47 mmol dm^{-3}) 0.3 equiv. of DMAP were added under rapid stirring at room temperature, followed by 3.6 equiv. of *tert*-butyl pyrocarbonate. The reaction was stirred overnight and monitored by TLC (diethyl ether-*n*-hexane, 9:1). Evaporation under reduced pressure gave a residue that was purified by dry chromatography on silica (diethyl ether–light petroleum, 9:1) and crystallisation from ethyl acetate-*n*-hexane to give **4c** as an orange solid in a 99% yield. Mp 101.8–103.4°C. R_f 0.74 (diethyl ether-*n*-hexane, 8:2). UV–vis (MeOH): λ_{max} 450 (17282) nm. FTIR (neat): ν_{max} 2976, 2929, 2856, 1747, 1732, 1681, 1602, 1562, 1520, 1446, 1398, 1367, 1311, 1255, 1197, 1148, 1066, 1039, 946, 902, 848, 823, 750 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.16 (9H, s, $C(CH_3)_3$), 1.68 (3H, d, *J* 7.0 Hz, α-CH_3), 3.12 (6H, s, $N(CH_3)_2$), 3.80 (3H, s, OCH_3), 5.18 and 5.21 (1H, 2×d, *J* 7.0 Hz, α-H), 6.77 (2H, d, *J* 9.3 Hz, 2×*ortho*-ArH $N(CH_3)_2$), 7.53 (1H, t, *J* 7.8 Hz, 5-H), 7.63 (1H, dt, *J* 7.0 Hz, 1.2 Hz, 4- or 6-H), 7.90 (2H, d, *J* 9.3 Hz, 2×*meta*-ArH $N(CH_3)_2$), 7.99 (1H, dt, *J* 7.8 Hz, 1.2 Hz, 6- or 4-H), 8.06 (1H, t, *J* 1.8 Hz, 2-H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 15.2 (α-CH_3), 27.3 ($C(CH_3)_3$), 40.2 (($CH_3)_2$), 52.4 (α-C), 53.5 (O-CH_3), 83.8 ($C(CH_3)_3$), 111.4 (2′-C, 6′-C), 120.9 (2-C), 125.1 (3′-C, 5′-C), 125.4 (4-C), 128.1 (5-C), 128.8 (6-C), 138.1 (1-C), 143.3 (4′-C), 152.4 (3-C), 152.6 (1′-C, O=*C*-OC$(CH_3)_3$), 171.4 (O=C-N), 172.3 (O=*C*-OCH_3). The assignments were supported by the Dept 135 technique. Anal. calcd for $C_{24}H_{30}N_4O_5$: C, 63.42; H, 6.65; N, 12.33. Found: C, 63.52; H, 6.55; N, 12.21. Compound **4c** was treated with DEAEA according to the procedure described in Ref. 14 above to give **5c** in a 63% yield, which compared well with a genuine sample prepared by a different procedure.
17. *N*-(3-Aminobenzoyl)-Phe(*N*-Boc)-OEt was obtained as an oil in a 32% yield. FTIR (neat): ν_{max} 3378, 2984, 2979, 1732, 1682, 1602, 1497, 1447, 1368, 1258, 1149, 1030, 886, 842, 727, 698 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.04 (9H, s, $C(CH_3)_3$), 1.30 (3H, t, *J* 7.0 Hz, OCH_2CH_3), 3.40–3.60 (2H, m, $CH_2C_6H_5$), 4,20–4.35 (2H, m, OCH_2CH_3), 5.42, 5.45 (1H, 2×d, *J* 6.9 Hz, α-H), 7.15 (1H, d, *J* 7.6 Hz, 4-H), 7.20–7.30 (6H, m, 5-H, C_6H_5), 7.40 (1H, d, *J* 7.6 Hz, 6-H), 7.45 (1H, s, 2-H). ^{13}C NMR ($CDCl_3$; 75.4 MHz): δ 14.2 (OCH_2CH_3), 27.2 $C(CH_3)_3$), 35.4 (CH_2-C_6H_5), 58.7 (α-C), 61.6 (OCH_2CH_3), 83.3 ($C(CH_3)_3$), 123.8 (2-C), 126.6 (6-C), 127.4 (4-C), 127.8 (4′-C), 128.5 (3′-C, 5′-C), 129.5 (2′-C, 6′-C), 130.9 (5-C), 137.5 (1′-C, 1-C), 152.7 (3-C, O=*C*-O-C$(CH_3)_3$), 170.2 (O=C-N) 173.0 (O=*C*-O-CH_2CH_3).