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Synthesis and evaluation of organic pigments. 4. New monoarylide and diarylide pigments

Monthon Nakpathom, David Hinks *, Harold S. Freeman

Department of Textile Engineering, Chemistry and Science, North Carolina State University, Raleigh, NC 27695-8301, USA

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Abstract

The synthesis, characterization and mutagenicity of a series of diarylide pigments prepared using highly twisted nonmutagenic 2,2′-dimethyl-5,5′-dipropoxybenzidine and 2,2′-dimethoxy-5,5′-dipropoxybenzidine, and their monoarylide counterparts are reported. Five pigments in which nitro groups were incorporated into either the 3- or 4-position of the acetoacetanilide coupling component were mutagenic in either the standard *Salmonella* mutagenicity assay (Ames test) or the Prival modification. The presence of 3-trifluoromethyl or 3-acetyl groups in the acetoacetanilide moiety led to nonmutagenic pigments. Despite the high dihedral angle across the biphenyl linkage, the λ_{max} of the highly twisted diarylide pigments was significantly bathochromic relative to the corresponding monoarylide pigments.

Keywords: Pigments; Disazoacetoacetanilide; Diarylide; Monoarylide pigments; Mutagenicity; Dihedral angle

1. Introduction

Benzidine and analogs such as 3,3′-dimethylbenzidine, 3,3′-dimethoxybenzidine and 3,3′-dichlorobenzidine are widely used in the production of dyes and pigments. It is known, however, that these intermediates are mutagenic in the standard Ames test and carcinogenic in laboratory animals [1–3]. In addition, benzidine itself is one of the few known human carcinogens.

Diarylide pigments based on benzidine analogs are still widely used, despite being derived from genotoxic diamines [4,5]. However, there are regulations that limit the maximum allowed level of the diamines as unconverted reactants in pigments [6]. As a result of safety concerns, a number of approaches to the development of safe replacements for mutagenic and carcinogenic benzidines have been undertaken [5]. One notable success was the elimination of mutagenicity by incorporating bulky alkyl or alkoxy groups in the 3,3′ positions of the benzidine moiety [4,7,8].

In part one of this series, the synthesis of a number of new, nonmutagenic benzidine congeners was reported [9]. Initial work on the utility of 3,3′-disubstituted nonmutagenic benzidine analogs for pigment synthesis showed that bathochromic shifts

* Corresponding author. Tel.: +1-919-515-6554; fax: +1-919-515-6532.

E-mail address: david_hinks@ncsu.edu (D. Hinks).

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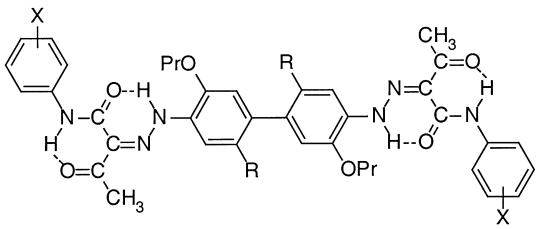
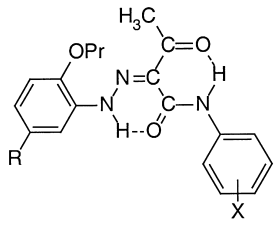
were observed relative to when 3,3′-dimethylbenzidine intermediates were used, for example [10]. Hence, the development of nonmutagenic benzidine analogs that, when converted to pigment, produced hues similar to those obtained from 3,3′-dimethylbenzidine and 3,3′-dichlorobenzidine was a goal of our research [11]. One approach utilized highly twisted tetrasubstituted diamines, such as 2,2′-dimethyl-5,5′-dipropoxybenzidine and 2,2′-dimethoxy-5,5′-dipropoxybenzidine. The large dihedral angle across the biphenyl linkage reduced π-orbital overlap, producing a hypsochromic shift in the resultant pigments relative to when hydrogen was employed in the 2,2′- positions. These two tetrasubstituted diamines were nonmutagenic in both the standard *Salmonella* mutagenicity assay (Ames test) and the Prival modification of the standard assay [9]. Following the preparation of these diamines in good yield and with minimum purification, they were employed as intermediates in the synthesis of bisazomethine, disazoacetoacetanilide, disazopyrazolone and disazobenzimidazolone pigments [11].

As an extension of the initial work, the present paper is concerned with the synthesis and evaluation of diarylide pigments (**1**–**8**) from 2,2′-dimethyl-5,5′-dipropoxybenzidine and 2,2′-dimethoxy-5,5′-dipropoxybenzidine as well as the corresponding monoarylide pigments (**9**–**16**).



Pigment	R	X
1	CH_3	3-NO_2
2	CH_3	4-NO_2
3	CH_3	3-CF_3
4	CH_3	3-$COCH_3$
5	OCH_3	3-NO_2
6	OCH_3	4-NO_2
7	OCH_3	3-CF_3
8	OCH_3	3-$COCH_3$



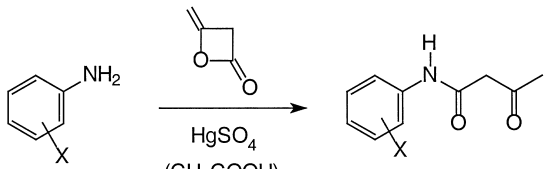
Pigment	R	X
9	CH_3	3-NO_2
10	CH_3	4-NO_2
11	CH_3	3-CF_3
12	CH_3	3-$COCH_3$
13	OCH_3	3-NO_2
14	OCH_3	4-NO_2
15	OCH_3	3-CF_3
16	OCH_3	3-$COCH_3$

2. Results and discussion

2.1. Synthesis

2.1.1. Acetoacetanilide coupling components

The reaction sequence used to prepare acetoacetanilides **18a**–**d** is shown in Scheme 1 and is based on the method of Zavialov et al. [12]. Acetoacetylation of 4-nitroaniline (**17b**) by diketene was conducted at room temperature in HOAc solution, with $HgSO_4$ catalysis, to give an 85% yield.



	X
17a	3-NO_2
b	4-NO_2
c	3-CF_3
d	3-$COCH_3$

	X
18a	3-NO_2
b	4-NO_2
c	3-CF_3
d	3-$COCH_3$

Scheme 1. Reaction sequence used to prepare acetoacetanilides **18a**–**d**.

This method was chosen instead of acetoacetylation by ethyl acetoacetate because the starting amines are weakly nucleophilic.

Acetoacetylation of 2-nitroaniline and 2-(trifluoromethyl)aniline by diketene was also undertaken, but very low yields and impure products were produced even after repeated recrystallizations.

2.1.2. Benzidine analogs

The reaction sequence used to prepare 2,2′-dimethyl-5,5′-dipropoxybenzidine (**22a**) and 2,2′-dimethoxy-5,5′-dipropoxybenzidine (**22b**) is shown in Scheme 2. Nitropropoxybenzene compounds **20a–b** were obtained in at least 95% yield from the nitrophenols **19a–b**, by alkylation with 1-bromopropane. Benzidine dihydrochlorides **21a–b** were then prepared as reported previously [9].

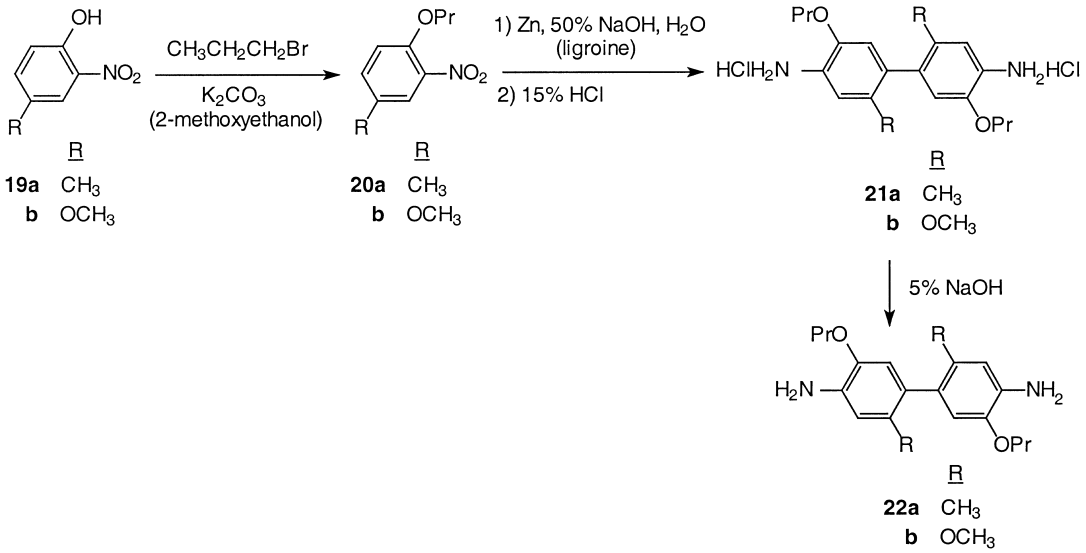
2.1.3. Diarylide pigments

The reaction sequence used to prepare diarylide pigments **1–8** is shown in Scheme 3. Tetrazotization of **22a**, for pigments **1–4**, or **22b**, for pigments **5–8**, and coupling of the resulting tetrazonium salts (**23a–b**) with acetoacetanilides **18a–d** gave the desired pigments in 84–93% yield. Only pigment **7** required recrystallization from EtOH to give a pure product (78%).

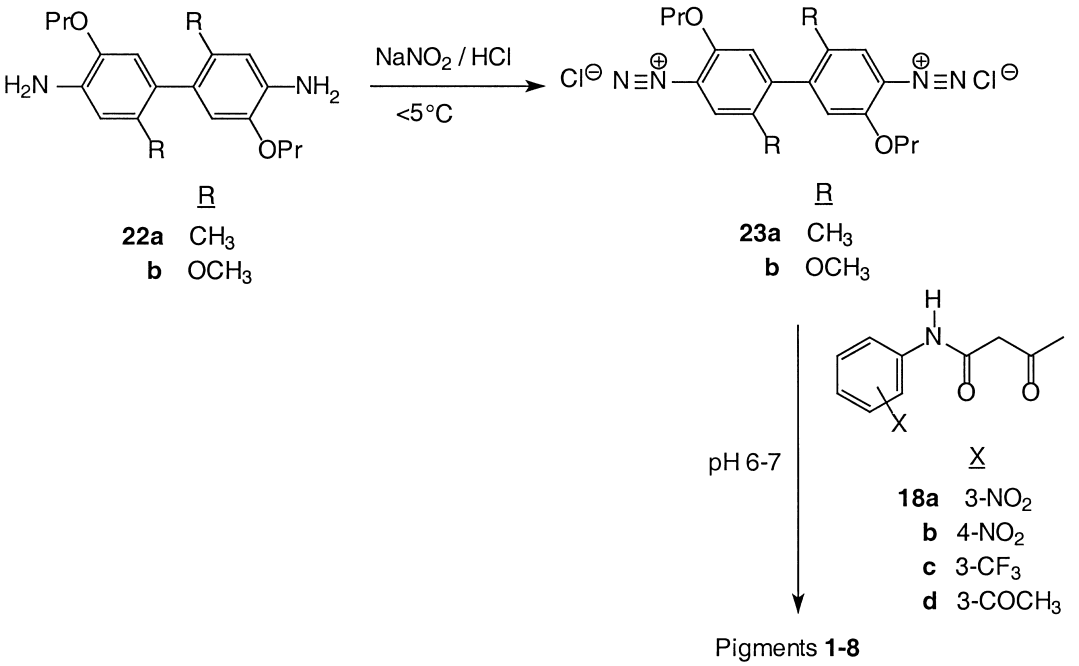
2.1.4. Monoarylide pigments

The reaction sequence used to prepare the required diazo components is shown in Scheme 4. Specifically, hydrogenation of **20a–b** using 20% $Pd(OH)_2/C$ was conducted to give the amines **24a–b**. Since these amines are subjected to air oxidation over time, they were converted to the corresponding hydrochlorides before storage.

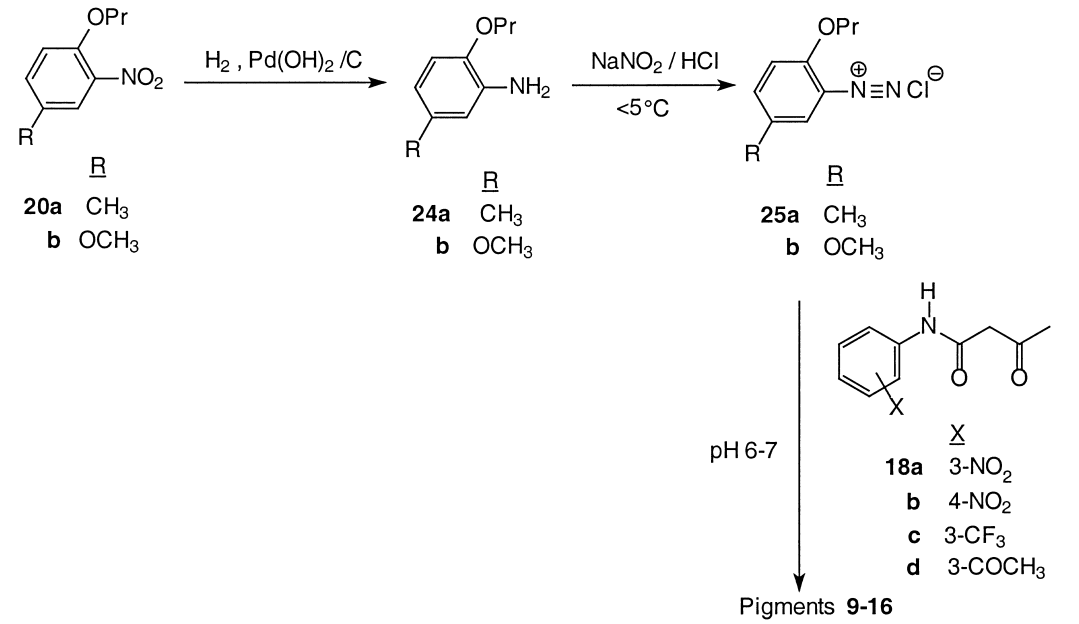
Amines **24a–b** were used to prepare monoarylide pigments (**9–16**) as shown in Scheme 4. For pigments **9–12**, the hydrochloride of **24a** was employed as a diazo component because there was a slight improvement in the brightness of pigments obtained versus those from the free amine. On the other hand, amine **24b** gave pigments **13–16** with brighter colors than its hydrochloride. Diazotization of the hydrochloride of **24a** or the amine **24b** and coupling of the resulting diazonium salts (**25a–b**) with acetoacetanilides **18a–d** gave the target pigments. Recrystallization of pigments **13** and **15** from 2-methoxyethanol and EtOH, respectively, was carried out to obtain pure products. Pigments that have a CH_3 group in the diazo component were obtained in 82–91% yield (cf. **9–12**), whereas pigments containing an OCH_3 group in the diazo component were obtained in 45–68% yield (cf. **13–16**).



Scheme 2. Reaction sequence used to prepare benzidine analogs **22a–b**.



Scheme 3. Reaction sequence used to prepare diarylide pigments **1**–**8**.



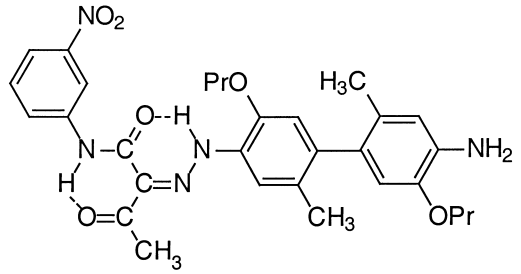
Scheme 4. Reaction sequence used to prepare monoarylide pigments **9**–**16**.

2.2. Analysis

The structures of diarylide (**1**–**8**) and monoarylide (**9**–**16**) pigments were confirmed by elemental analysis, ^{1}H NMR (except for pigments **5**–**6**, which were insoluble in NMR solvents), and field desorption mass spectrometry (FDMS).

As expected, all pigments contained singlet peaks in their ^{1}H NMR spectra in the low field region, δ 14.6–15.0 ppm and δ 11.6–12.0 ppm, that corresponded to the hydrazone and amide protons, respectively (see Fig. 1, in the case of pigment **1**).

Analysis of each diarylide pigment by FDMS showed a molecular ion peak [$M^{+\cdot}$] as the base peak and a peak at [$M^{+\cdot}/2$] in FD spectra [13]. They also contained a minor impurity that gave a peak at m/z 558–617. For example, the FD spectra of pigment **1** are shown in Figs. 2 and 3. This impurity was monoazo compound **26**.



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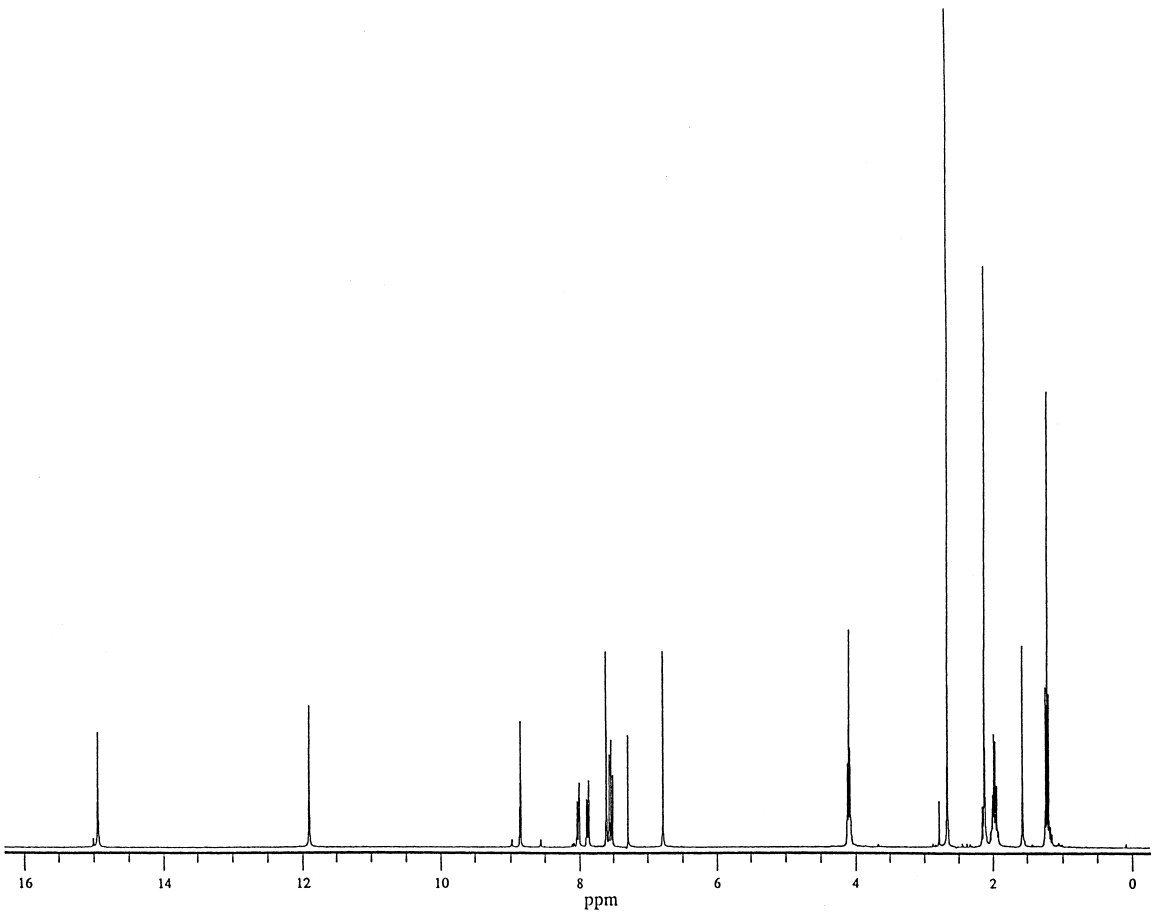

Fig. 1. ^{1}H NMR spectrum of pigment **1**.

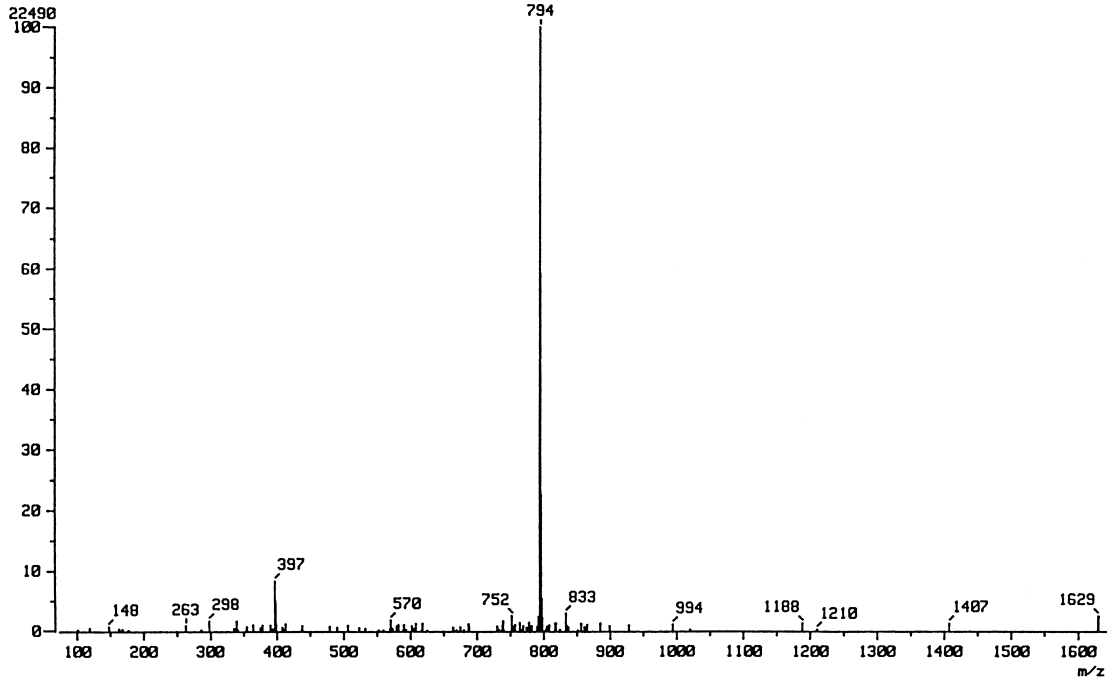

Fig. 2. FD spectrum of pigment **1**.

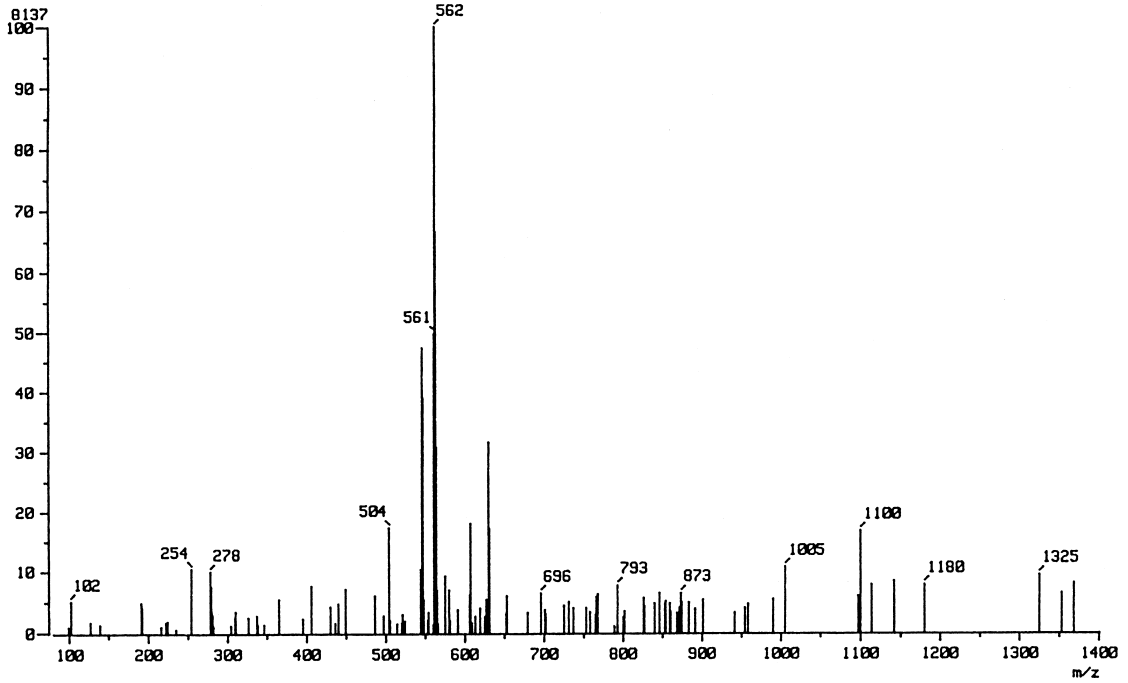

Fig. 3. FD spectrum of by-product from the synthesis of pigment **1**.

With the exception of **14** and **16**, each monoarylide pigment gave a molecular ion peak [$M^{+\cdot}$] as the base peak. In the case of pigments **14** and **16**, peaks at m/z 826 and 820 were obtained, respectively, each corresponding to a dimer.

2.3. Visible absorption spectral data

The visible absorption spectra obtained on solutions of diarylides **1**–**4** and **7**–**8** and monoarylides **9**–**16** in 1,2-dichlorobenzene are reported in Table 1. Pigments **5**–**6** were insufficiently soluble in this solvent. Formation of diarylide pigments caused a bathochromic effect, with the diarylides absorbing at 24–44 nm longer wavelength than the corresponding monoarylide pigments. Also, the diarylide pigments provided 2–3 times greater molar extinction coefficient relative to the monoarylide pigments.

Previously [11], it was reported that incorporation of methyl groups in the 2,2′-position of the biphenyl moiety of diarylide pigments produced a hypsochromic shift relative to when hydrogen was present in these positions. For example, a hypsochromic shift from 452 to 428 nm was produced when 2,2′-dimethyl-5,5′-dipropoxybenzidine was used in lieu of 3,3′-dipropoxybenzidine for the synthesis of diarylide pigments. A possible reason for the hypsochromic shift is reduction of π orbital overlap across the biphenyl linking bond due to steric strain caused by the presence of the methyl groups. Interestingly, the present work indicates that significant π orbital overlap remains across the biphenyl linking bond in diarylides when methyl is present in the 2,2′-positions, since these bis-chromophoric pigments are still significantly bathochromic relative to the corresponding monoarylides.

Table 1
Visible absorption spectral data for pigments **1**–**4** and **7**–**16**

Pigment	λ_{max} (nm)	$\varepsilon_{max} \times 10^4$ (l mol^{-1} cm^{-1})
1	431	7.47
2	438	7.26
3	430	6.07
4	429	7.73
7	458	4.00
8	457	5.15
9	406	2.47
10	414	2.68
11	407	2.41
12	405	2.68
13	418	2.15
14	423	2.72
15	414	2.21
16	413	2.27

2.4. Mutagenicity

Table 2 shows the degree of mutagenicity of all pigments synthesized in this study. Pigments **1**, **3**–**5**, **7**–**8**, **11**–**13**, and **15**–**16** were nonmutagenic under all test conditions. Four of five mutagenic pigments (**2**, **6**, **10**, and **14**) contain at least one nitro group in the 4-position in the acetoacetanilide ring, with the other mutagenic pigment (**9**) containing a 3-nitro group. Of these, the diarylide pigment (**6**), which also has a 3-methoxy group in the benzidine moiety, possessed the highest mutagenicity (see Fig. 4). Also, 3-nitro substituted pigments (**1**, **5**, **9**, and **13**) were found to be either nonmutagenic or less mutagenic than those having a 4-nitro group (**2**, **6**, **10**, and **14**) are. The introduction of 3-trifluoromethyl or 3-acetyl groups led to nonmutagenic pigments (**3**–**4**, **7**–**8**, **11**–**12**, and **15**–**16**).

In this study, all mutagenic pigments containing nitro group(s) were both direct-acting frameshift and base-pair substitution mutagens, except for pigment **9**, which was only a direct-acting frameshift mutagen. This is consistent with earlier investigations which found that other nitro group containing azo dyes were mutagenic and exhibited mutagenic activity in the unreduced form [14,15]. Also, the majority of the pigments that were mutagenic following metabolic activation in the Ames test were nonmutagenic in the Prival assay. The exception was pigment **10** which was weakly mutagenic in TA98 in the latter test. This indicates that these pigments produced mutagenic metabolites in the Ames test by pathways other than azo reduction, since the Prival assay is designed to optimize reduction of azo compounds to the corresponding aromatic amines.

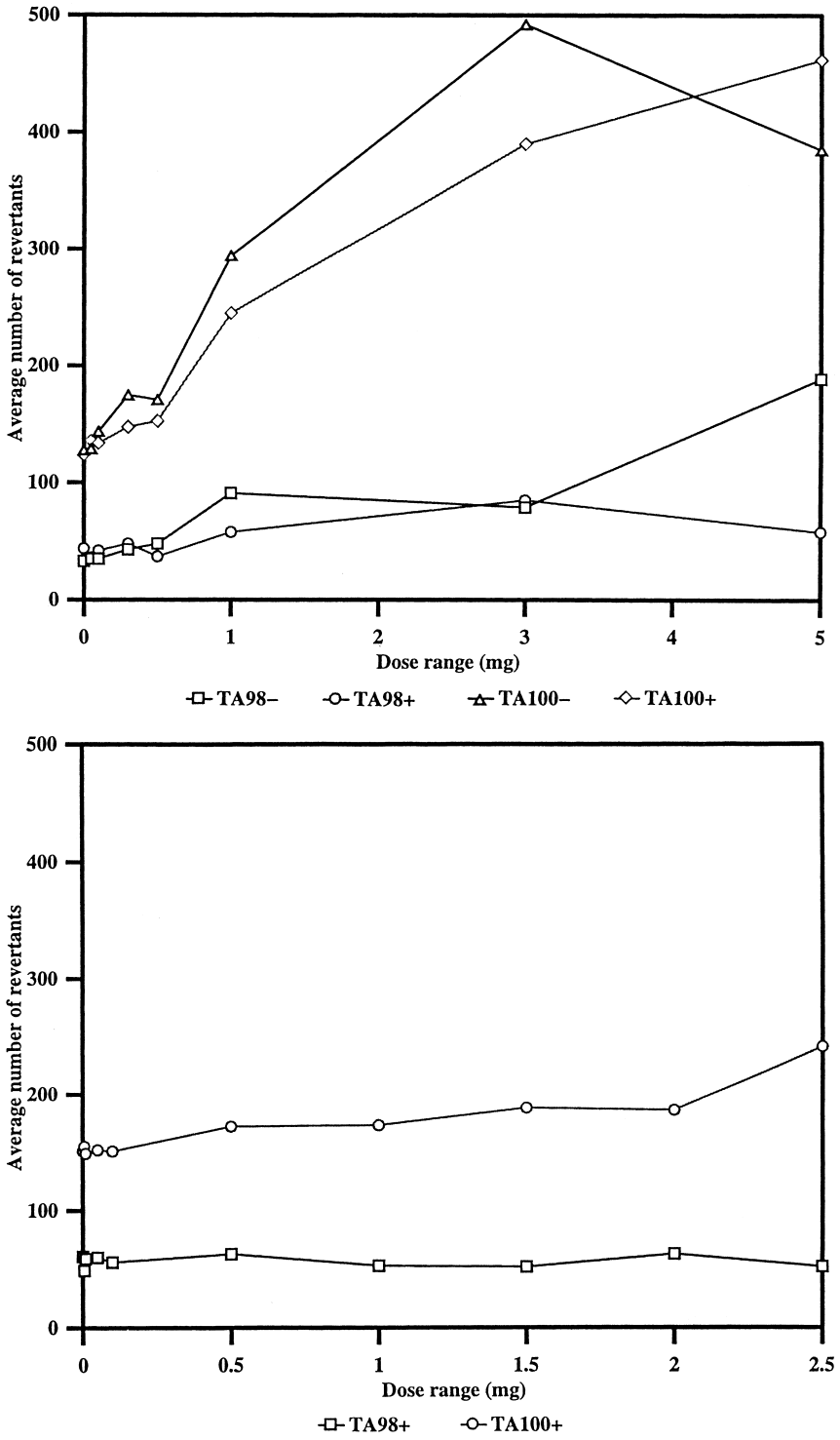

Fig. 4. Mutagenicity of pigment **6** with (+S9) and without (−S9) activation in Ames (upper) and Prival modification (lower).

3. Experimental

3.1. General

All starting materials not synthesized in this study were obtained from either Aldrich Chemical Company or Fisher Scientific Company. Melting points were determined on a Mel-Temp capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a General Electric Omega 300 MHz spectrometer using $CDCl_3$ or DMSO-d_6, and the chemical shifts are reported in ppm using tetramethylsilane (TMS) as the internal reference. Visible spectra were recorded on a Varian Cary 3 UV-Visible spectrophotometer and microanalyses were performed by Atlantic Microlab Inc., Norcross, GA. Thin layer chromatography (TLC) was conducted using Whatman 250 μm silica gel 60A plates. Field desorption mass spectra were recorded on a JEOL HX110 double-focusing mass spectrometer.

3.2. Mutagenicity testing

Mutagenicity testing of pigments **1**–**16** was performed using both the standard *Salmonella* mutagenicity assay (Ames test) and the preincubation or Prival modification, using bacterial strains TA98 and TA100 in the presence and absence of metabolic activation (S9 mix) [16,17]. Strain TA98 was used to detect frameshift mutations, while strain TA100 was used to detect base-pair substitutions. A plus or minus sign after the strain type represents the presence or absence of metabolic activation. To be designated as mutagenic, a pigment must have produced an average revertant count that was greater than two times the background average, must have shown a dose–response effect, and the test result must have been reproducible. The initial dose range for the standard mutagenicity assay was 0.05–5 mg, and was 0.005–2.5 mg for the Prival modification. Pigments that gave a positive result were retested in the active dose range in order to confirm their mutagenic activity. The degree of mutagenicity was then judged as weakly, moderately, or strongly mutagenic. Pigments giving average revertant counts two to four times the background count were judged as weakly mutagenic, those giving average revertant counts four to six times the background count were judged as moderately mutagenic, and those giving average revertant counts more than six times the background count were judged as strongly mutagenic [17].

Table 2
Summary of mutagenicity data of pigments **1**–**16**[a]

Pigment	Ames test[b]				Prival	
	TA98−	TA98+	TA100−	TA100+	TA98+	TA100+
1	N	N	N	N	N	N
2	M(4.96)	N	W(3.33)	W(2.66)	N	N
3	N	N	N	N	N	N
4	N	N	N	N	N	N
5	N	N	N	N	N	N
6	S(7.04)	W(2.07)	M(5.16)	M(5.69)	N	N
7	N	N	N	N	N	N
8	N	N	N	N	N	N
9	M(4.22)[b]	N	N	N	N	N
10	W(3.30)	M(4.07)	W(2.43)	N	W(2.15)	N
11	N	N	N	N	N	N
12	N	N	N	N	N	N
13	N	N	N	N	N	N
14	W(3.12)	N	W(3.35)	W(3.50)	N	N
15	N	N	N	N	N	N
16	N	N	N	N	N	N

[a] N = nonmutagenic, W = weakly mutagenic, M = moderately mutagenic, S = strongly mutagenic.
[b] The numbers in parentheses were calculated by dividing the highest average number of revertants obtained by the background average.

3.3. Synthesis

The synthesis of 4-methyl-2-nitropropoxybenzene (**20a**), 4-methoxy-2-nitropropoxybenzene (**20b**), 2,2′-dimethyl-5,5′-dipropoxybenzidine (**22a**), and 2,2′-dimethoxy - 5, 5′ - dipropoxybenzidine (**22b**) was reported previously [9].

3.3.1. 3-Nitroacetoacetanilide (**18a**)

A mixture of 3-nitroaniline (**17a**; 13.8 g, 0.1 mol), $HgSO_4$ (2.4 g, 0.008 mol), and 100 ml glacial HOAc was stirred at room temperature. Diketene (10.1 g, 0.12 mol) was then slowly added over 30 min, and the temperature of the reaction rose spontaneously to 45–55°C. After the addition was complete and the exotherm ended, the brown mixture was allowed to stand at room temperature for 2–3 h. The product was precipitated by dilution with water. After standing at room temperature overnight, the mixture was filtered to give 19.5 g (88%) of a pale yellow powder (**18a**), m.p. 119–120°C. Elemental analysis. Calculated for $C_{10}H_{10}N_2O_4$: C, 54.05; H, 4.54; N, 12.61. Found: C, 53.80; H, 4.58; N, 12.42. ^{1}H NMR ($CDCl_3$): δ 2.36 (s, 3H), δ 3.65 (s, 2H), δ 7.46–7.52 (t, 1H), δ 7.89–7.92 (dd, 1H), δ 7.95–7.99 (dd, 1H), δ 8.46–8.47 (t, 1H), δ 9.63 (s, 1H). TLC: $R_f = 0.51$ (PhMe:EtOAc/1:4).

The method described above for the synthesis of **18a** was used to prepare **18b**–**d**.

3.3.2. 4-Nitroacetoacetanilide (**18b**)

The crude product was purified by dissolving it in 5% NaOH, filtration and precipitation by the addition of 5% HCl to the filtrate. After standing at room temperature overnight, the mixture was filtered to give 6.3 g (49%) of a yellow powder (**18b**), m.p. 118–120°C. Elemental analysis. Calculated for $C_{10}H_{10}N_2O_4$: C, 54.05; H, 4.54; N, 12.61. Found: C, 54.13; H, 4.50; N, 12.64. ^{1}H NMR ($CDCl_3$): δ 2.36 (s, 3H), δ 3.65 (s, 2H), δ 7.72–7.76 (dd, 2H), δ 8.19–8.23 (dd, 2H), δ 9.75 (s, 1H). TLC: $R_f = 0.49$ (PhMe:EtOAc/1:4).

3.3.3. 3-(Trifluoromethyl)acetoacetanilide (**18c**)

Compound **18c** (22 g, 90%) was obtained as a pale yellow powder, m.p. 110–112°C. Elemental analysis. Calculated for $C_{11}H_{10}NO_2F_3$: C, 53.88; H, 4.11; N, 5.71. Found: C, 53.98; H, 4.09; N, 5.72. ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H), δ 3.62 (s, 2H), δ 7.36–7.38 (d, 1H), δ 7.42–7.47 (t, 1H), δ 7.72–7.75 (d, 1H), δ 7.87 (s, 1H), δ 9.42 (s, 1H). TLC: $R_f = 0.65$ (PhMe:EtOAc/1:4).

3.3.4. 3-(Acetyl)acetoacetanilide (**18d**)

Compound **18d** (6.3 g, 26%) was obtained as pale yellow needles, m.p. 94–97°C. Elemental analysis. Calculated for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.56; H, 5.93; N, 6.33. ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H), δ 2.60 (s, 3H), δ 3.62 (s, 2H), δ 7.39–7.45 (t, 1H), δ 7.69–7.71 (d, 1H), δ 7.85–7.87 (d, 1H), δ 8.07 (s, 1H), δ 9.36 (s, 1H). TLC: $R_f = 0.50$ (PhMe:EtOAc/1:4).

3.3.5. Pigments **1**–**8**

The quantity of 0.0055 mol 2,2′-dimethyl-5,5′-dipropoxybenzidine (**22a**), for pigments **1**–**4**, or 2,2′-dimethoxy-5,5′-dipropoxybenzidine (**22b**), for pigments **5**–**8**, was dispersed in 8 ml water with 5 g ice. To this was added a solution of 3 ml conc. HCl in 4 ml water and the reaction mixture was cooled to 0–5°C. $NaNO_2$ (0.77 g, 0.011 mol) in 5 ml water was added over 30 min and excess HNO_2 was destroyed by the addition of solid sulfamic acid. The tetrazonium salt solution was decolorized using activated carbon and then filtered. Acetoacetanilides **18a**–**d** (0.0116 mol, 5% excess) were dissolved in 32 ml water containing NaOH (0.32 g), with stirring and slight heating. Each anilide was precipitated by the addition of 0.8 ml glacial HOAc, followed by 4.32 g of $NaOAc.3H_2O$, and the volume was adjusted to 96 ml with water. Tetrazonium salts **23a**–**b** were added under the surface of the stirred coupling component dispersion at a rate such that excess tetrazonium salt (spot test with alkaline J-acid solution) was never observed. After completion of the coupling reaction, the pigment slurry was thrice heated to the boil and filtered, to obtain an acceptable particle size. The pigments were dried at 40–50°C.

3.3.5.1. 2,2′-[(2,2′-Dimethyl-5,5′-dipropoxybiphenyl-4,4′-diyl)dihydrazin-1-yl-2-ylidene]bis[N-(3-nitrophenyl)-3-oxobutyramide] (**1**). Pigment **1** (4.1 g, 93%) was obtained as a bright yellow powder, m.p. 273–275°C. Elemental analysis. Calculated for $C_{40}H_{42}N_8O_{10}$: C, 60.45; H, 5.33; N,

14.10. Found: C, 60.29; H, 5.41; N, 13.95. ^{1}H NMR ($CDCl_3$): δ 1.20–1.25 (t, 6H), δ 1.92–2.03 (m, 4H), δ 2.12 (s, 6H), δ 2.66 (s, 6H), δ 4.07–4.11 (t, 4H), δ 6.77 (s, 2H), δ 7.50–7.55 (t, 2H), δ 7.60 (s, 2H), δ 7.84–7.87 (dd, 2H), δ 7.98–8.01 (dd, 2H), δ 8.83–8.84 (d, 2H), δ 11.88 (s, 2H), δ 14.91 (s, 2H). TLC: $R_f = 0.44$ (PhMe:MeCN:MeOH/94:5.5:0.5). FDMS: *m*/*z* (relative intensity), 794 ([$M^{+\cdot}$] 100), 795 (59.1), 796 (22.6), 397 ([$M^{+\cdot}$/2] 8.5), 398 (5).

3.3.5.2. 2,2′-[(2,2′-Dimethyl-5,5′-dipropoxybiphenyl-4,4′-diyl)dihydrazin-1-yl-2-ylidene]bis[N-(4-nitrophenyl)-3-oxobutyramide] (**2**). Pigment **2** (4.1 g, 93%) was obtained as a bright yellow powder, m.p. 297–300°C. Elemental analysis. Calculated for $C_{40}H_{42}N_8O_{10}$: C, 60.45; H, 5.33; N, 14.10. Found: C, 60.48; H, 5.39; N, 14.03. ^{1}H NMR ($CDCl_3$): δ 1.15–1.20 (t, 6H), δ 1.89–2.01 (m, 4H), δ 2.10 (s, 6H), δ 2.64 (s, 6H), δ 4.05–4.09 (t, 4H), δ 6.75 (s, 2H), δ 7.58 (s, 2H), δ 7.84–7.87 (d, 4H), δ 8.23–8.26 (d, 4H), δ 12.00 (s, 2H), δ 14.81 (s, 2H). TLC: $R_f = 0.43$ (PhMe:MeCN:MeOH/94:5.5:0.5). FDMS: *m*/*z* (relative intensity), 794 ([$M^{+\cdot}$] 100), 795 (57.2), 796 (27.2), 397 (10.3), 398 (9.4).

3.3.5.3. 2,2′-[(2,2′-Dimethyl-5,5′-dipropoxybiphenyl-4,4′-diyl)dihydrazin-1-yl-2-ylidene]-bis[3-oxobutyramide-N-*(3-trifluoromethyphenyl)]* (**3**). Pigment **3** (4.3 g, 92%) was obtained as a bright yellow powder, m.p. 220–224°C. Elemental analysis. Calculated for $C_{42}H_{42}N_6O_6F_6$: C, 60.00; H, 5.03; N, 10.00. Found: C, 59.89; H, 5.03; N, 9.92. ^{1}H NMR ($CDCl_3$): δ 1.18–1.23 (t, 6H), δ 1.90–2.02 (m, 4H), δ 2.12 (s, 6H), δ 2.66 (s, 6H), δ 4.06–4.09 (t, 4H), δ 6.76 (s, 2H), δ 7.38–7.41 (d, 2H), δ 7.46–7.51 (t, 2H), δ 7.59 (s, 2H), δ 7.74–7.76 (d, 2H), δ 8.19 (s, 2H), δ 11.72 (s, 2H), δ 14.96 (s, 2H). TLC: $R_f = 0.65$ (PhMe:MeCN:MeOH/94:5.5:0.5). FDMS: *m*/*z* (relative intensity), 840 ([$M^{+\cdot}$] 100), 841 (99.5), 842 (26.3), 420 (4), 421 (2.8).

3.3.5.4. 2,2′-[(2,2′-Dimethyl-5,5′-dipropoxybiphenyl-4,4′-diyl)dihydrazin-1-yl-2-ylidene]bis[N-(3-acetylphenyl)-3-oxobutyramide] (**4**). Pigment **4** (3.9 g, 89%) was obtained as a bright yellow powder, m.p. 223–226°C. Elemental analysis. Calculated for $C_{44}H_{48}N_6O_8$: C, 66.99; H, 6.13; N, 10.65. Found: C, 66.92; H, 6.12; N, 10.65. ^{1}H NMR ($CDCl_3$): δ 1.18–1.23 (t, 6H), δ 1.90–2.02 (m, 4H), δ 2.12 (s, 6H), δ 2.65 (s, 6H), δ 2.66 (s, 6H), δ 4.05–4.09 (t, 4H), δ 6.76 (s, 2H), δ 7.45–7.50 (t, 2H), δ 7.60 (s, 2H), δ 7.74–7.76 (d, 2H), δ 7.85–7.88 (d, 2H), δ 8.40 (s, 2H), δ 11.69 (s, 2H), δ 14.94 (s, 2H). TLC: $R_f = 0.27$ (PhMe:MeCN:MeOH/94:5. 5:0.5). FDMS: *m*/*z* (relative intensity), 788 ([$M^{+\cdot}$] 100), 789 (49.4), 790 (19.7), 394 (6.1), 395 (4.4).

3.3.5.5. 2,2′-[(2,2′-Dimethoxy-5,5′-dipropoxybiphenyl-4,4′-diyl)dihydrazin-1-yl-2-ylidene]bis[N-(3-nitrophenyl)-3-oxobutyramide] (**5**). Pigment **5** (3.8 g, 84%) was obtained as a reddish orange powder, m.p. 292–295°C. Elemental analysis. Calculated for $C_{40}H_{42}N_8O_{12}$: C, 58.11; H, 5.12; N, 13.55. Found: C, 58.25; H, 5.10; N, 13.37. ^{1}H NMR: insoluble in $CDCl_3$ and DMSO-d_6. TLC: $R_f = 0.25$ (PhMe:MeCN:MeOH/94:5.5:0.5). FDMS: *m*/*z* (relative intensity), 826 ([$M^{+\cdot}$] 100), 827 (53.3), 828 (20), 413 (4.7), 414 (3.9).

3.3.5.6. 2,2′-[(2,2′-Dimethoxy-5,5′-dipropoxybiphenyl-4,4′-diyl)dihydrazin-1-yl-2-ylidene]bis[N-(4-nitrophenyl)-3-oxobutyramide] (**6**). Pigment **6** (3.9 g, 86%) was obtained as a red powder, m.p. > 400°C. Elemental analysis. Calculated for $C_{40}H_{42}N_8O_{12}$: C, 58.11; H, 5.12; N, 13.55. Found: C, 58.20; H, 5.19; N, 13.45. ^{1}H NMR: insoluble in $CDCl_3$ and DMSO-d_6. TLC: $R_f = 0.32$ (PhMe:MeCN:MeOH/94:5.5:0.5). FDMS: *m*/*z* (relative intensity), 827 ([$M^{+\cdot}$] 100), 826 (48.9), 828 (24.1), 829 (15.4), 413 (9.9), 414 (3.4).

3.3.5.7. 2,2′-[(2,2′-Dimethoxy-5,5′-dipropoxybiphenyl-4,4′-diyl)dihydrazin-1-yl-2-ylidene]bis[3-oxobutyramide-N-*(3-trifluoromethyphenyl)]* (**7**). This pigment was recrystallized from EtOH as an orange powder (76%), m.p. 276–280°C. Elemental analysis. Calculated for $C_{42}H_{42}N_6O_8F_6$: C, 57.80; H, 4.85; N, 9.63. Found: C, 57.55; H, 4.91; N, 9.38. ^{1}H NMR ($CDCl_3$): δ 1.15–1.20 (t, 6H), δ 1.87–1.99 (m, 4H), δ 2.62 (s, 6H), δ 3.83 (s, 6H), δ 4.04–4.08 (t, 4H), δ 6.92 (s, 2H), δ 7.35 (s, 2H), δ 7.36–7.39 (d, 2H), δ 7.44–7.49 (t, 2H), δ 7.72–7.75 (d, 2H), 8.16 (s, 2H), δ 11.67 (s, 2H), δ 14.94 (s, 2H). TLC: $R_f = 0.23$ (PhMe:MeCN:MeOH/94:5.5:0.5).

FDMS: *m/z* (relative intensity), 872 ([$M^{+\cdot}$] 100), 873 (54), 874 (19), 436 (6.8), 437 (4.4).

3.3.5.8. 2,2′-[(2,2′-Dimethoxy-5,5′-dipropoxybiphenyl-4,4′-diyl)dihydrazin-1-yl-2-ylidene]bis[N-(3-acetylphenyl)-3-oxobutyramide] (**8**). Pigment **8** (3.9 g, 88%) was obtained as an orange powder, m.p. 248–252°C. Elemental analysis. Calculated for $C_{44}H_{48}N_6O_{10}$: C, 64.38; H, 5.89; N, 10.24. Found: C, 64.11; H, 5.88; N, 10.10. ^{1}H NMR ($CDCl_3$): δ 1.16–1.21 (t, 6H), δ 1.90–1.97 (m, 4H), δ 2.62 (s, 6H), δ 2.63 (s, 6H), δ 3.83 (s, 6H), δ 4.04–4.08 (t, 4H), δ 6.92 (s, 2H), δ 7.36 (s, 2H), δ 7.43–7.48 (t, 2H), δ 7.72–7.75 (d, 2H), δ 7.84–7.86 (d, 2H), δ 8.38 (s, 2H), δ 11.64 (s, 2H), δ 14.93 (s, 2H). TLC: R_f = 0.15 (PhMe:MeCN:MeOH/94:5.5:0.5). FDMS: *m/z* (relative intensity), 821 ([$M^{+\cdot}$] 100), 822 (58.9), 823 (20.7), 410 (10.5), 411 (6).

3.3.6. 4-Methyl-2-propoxyaniline (**24a**)

In a 500-ml reduction flask was placed 4-methyl-2-nitropropoxybenzene (**20a**; 9.76 g, 0.05 mol) in 300 ml EtOH. $Pd(OH)_2/C$ (20% w/w, 0.3 g) was added and the bottle was then placed on a Parr apparatus. After the bottle was evacuated and flushed with H_2 three times, the bottle was charged with H_2 to a pressure of 40 lb/in^2. During the reduction, the bottle was repressurized to approximately 40 lb/in^2 until total H_2 consumption reached 284 lb/in^2 (0.15 mol). The bottle was then pressurized to 30–40 lb/in^2 and left shaking for 30 min. After removing residual H_2, the catalyst was removed by filtration, with the aid of a bed of celite, and the celite/catalyst residue was washed with EtOH. HCl (12 M, 6 ml) was then added to the clear colorless filtrate and the solvent was removed using a rotary evaporator. The crude hydrochloride was washed with EtOAc and dried, to give 9.2 g (91%) of white solid. The hydrochloride was treated with 10% Na_2CO_3 to give **24a**. ^{1}H NMR (DMSO-d_6): δ 0.94–0.99 (t, 3H), δ 1.66–1.73 (m, 2H), δ 2.10 (s, 3H), δ 3.79–3.84 (t, 2H), δ 4.53 (s, 2H), δ 6.26–6.29 (dd, 1H), δ 6.43–6.44 (d, 1H), δ 6.60–6.63 (d, 1H). TLC: R_f = 0.68 (PhMe:EtOAc/3:1).

3.3.7. 4-Methoxy-2-propoxyaniline (**24b**)

The synthesis was the same as that described for **24a**. The crude hydrochloride was washed with EtOAc and dried, to give 9.9 g (91%) of white solid. The hydrochloride was treated with 10% Na_2CO_3 to give **24b**. ^{1}H NMR (DMSO-d_6): δ 0.93–0.98 (t, 3H), δ 1.65–1.72 (m, 2H), δ 3.59 (s, 3H), δ 3.76–3.81 (t, 2H), δ 4.68 (s, 2H), δ 6.00–6.04 (dd, 1H), δ 6.23–6.24 (d, 1H), δ 6.62–6.65 (d, 1H). TLC: R_f = 0.57 (PhMe:EtOAc/3:1).

3.3.8. Pigments **9–16**

The quantity of 0.0125 mol 4-methyl-2-propoxyaniline hydrochloride, for pigments **9–12**, or 4-methoxy-2-propoxyaniline for pigments **13–16**, was dispersed in 25 ml water with 2 g ice. To this was added 3.1 g 30% HCl, for the hydrochloride, or 3.8 g 30% HCl, in the case of the free amine, and the reaction mixture was cooled to 0–5°C. $NaNO_2$ (0.87 g, 0.0125 mol) in 4 ml water was added over 30 min and excess HNO_2 was destroyed by the addition of solid sulfamic acid. The diazonium salt solution was decolorized using activated carbon and then filtered. Acetoacetanilides **18a–d** (0.0144 mol, 15% excess) were dissolved in 25 ml water containing 0.56 g NaOH, with stirring and slight heating. Each anilide was precipitated by the addition of 9.1 ml 12% CH_3COOH, followed by 1.5 g of $NaOAc.3H_2O$, and the volume was adjusted to 100 ml with water. Diazonium salts **25a–b** were added under the surface of the stirred coupling component dispersion at a rate such that excess diazonium salt (spot test with alkaline J-acid solution) was never observed. After completion of the coupling reaction, the pigment slurry was thrice heated to the boil and filtered, to obtain an acceptable particle size. The pigments were dried at 40–50°C.

3.3.8.1. 2-[(2-Methyl-5-propoxyphenyl-4-yl)hydrazin-1-yl-2-ylidene][N-(3-nitrophenyl)-3-oxobutyramide] (**9**). Pigment **9** (4.0 g, 83%) was obtained as a bright yellow powder, m.p. 186–188°C. Elemental analysis. Calculated for $C_{20}H_{22}N_4O_5$: C, 60.29; H, 5.57; N, 14.06. Found: C, 60.23; H, 5.54; N, 13.99. ^{1}H NMR ($CDCl_3$): δ 1.18–1.23 (t, 3H), δ 1.89–2.00 (m, 2H), δ 2.36 (s, 3H), δ 2.61 (s, 3H), δ 4.04–4.08 (t, 2H), δ 6.84–6.87 (d, 1H), δ 6.94–6.96 (t, 1H), δ 7.47–7.51 (t, 2H), δ 7.81–7.85 (dd, 1H), δ 7.95–7.98 (dd, 1H), δ 8.79 (s, 1H), δ 11.84 (s, 1H), δ 14.82 (s, 1H). TLC:

R_f = 0.55 (PhMe:MeCN:MeOH/94:5.5:0.5). FDMS: *m/z* (relative intensity), 398 ([$M^{+\cdot}$] 100), 399 (27).

*3.3.8.2. 2-[(2-Methyl-5-propoxyphenyl-4-yl)hydrazin-1-yl-2-ylidene][*N*-(4-nitrophenyl)-3-oxobutyramide]* (**10**). Pigment **10** (4.1 g, 82%) was obtained as a bright yellow powder, m.p. 206–208°C. Elemental analysis. Calculated for $C_{20}H_{22}N_4O_5$: C, 60.29; H, 5.57; N, 14.06. Found: C, 60.39; H, 5.55; N, 14.11. ^{1}H NMR ($CDCl_3$): δ 1.14–1.19 (t, 3H), δ 1.89–2.00 (m, 2H), δ 2.36 (s, 3H), δ 2.62 (s, 3H), δ 4.04–4.08 (t, 2H), δ 6.84–6.87 (d, 1H), δ 6.94–6.98 (dd, 1H), δ 7.47 (s, 1H), δ 7.82–7.86 (d, 2H), δ 8.21–8.25 (dd, 2H), δ 11.98 (s, 1H), δ 14.74 (s, 1H). TLC: R_f = 0.51 (PhMe:MeCN:MeOH/94:5.5:0.5). FDMS: *m/z* (relative intensity), 398 ([$M^{+\cdot}$] 100), 399 (21.8).

*3.3.8.3. 2-[(2-Methyl-5,5′-dipropoxybiphenyl-4,4′-yl)hydrazin-1-yl-2-ylidene][3-oxobutyramide-*N*-(3-trifluoromethyphenyl)]* (**11**). Pigment **11** (4.8 g, 91%) was obtained as a bright yellow powder, m.p. 145–148°C. Elemental analysis. Calculated for $C_{21}H_{22}N_3O_3F_3$: C, 59.85; H, 5.26; N, 9.97 Found: C, 59.71; H, 5.21; N, 9.85. ^{1}H NMR ($CDCl_3$): δ 1.15–1.20 (t, 3H), δ 1.89–1.96 (m, 2H), δ 2.35 (s, 3H), δ 2.61 (s, 3H), δ 4.03–4.07 (t, 2H), δ 6.83–6.86 (d, 1H), δ 6.92–6.95 (d, 1H), δ 7.35–7.37 (d, 1H), δ 7.42–7.47 (t, 2H), δ 7.70–7.73 (d, 1H), δ 8.15 (s, 1H), δ 11.68 (s, 1H), δ 14.87 (s, 1H). TLC: R_f = 0.70 (PhMe:acetonitrile:MeOH/94:5.5:0.5). FDMS: *m/z* (relative intensity), 421 ([$M^{+\cdot}$] 100), 422 (24.7).

*3.3.8.4. 2-[(2-Methyl-5-propoxyphenyl-4-yl)hydrazin-1-yl-2-ylidene][*N*-(3-acetylphenyl)-3-oxobutyramide]* (**12**). Pigment **12** (4.3 g, 87%) was obtained as a bright yellow powder, m.p. 145–147°C. Elemental analysis. Calculated for $C_{22}H_{25}N_3O_4$: C, 66.82; H, 6.37; N, 10.63. Found: C, 66.94; H, 6.33; N, 10.55. ^{1}H NMR ($CDCl_3$): δ 1.16–1.21 (t, 3H), δ 1.88–1.97 (m, 2H), δ 2.36 (s, 3H), δ 2.61 (s, 3H), δ 2.62 (s, 3H), δ 4.03–4.07 (t, 2H), δ 6.83–6.86 (d, 1H), δ 6.91–6.95 (dd, 1H), δ 7.41–7.47 (t, 2H), δ 7.71–7.73 (d, 1H), δ 7.81–7.84 (dd, 1H), δ 8.36–8.38 (t, 1H), δ 11.65 (s, 1H), δ 14.85 (s, 1H). TLC: R_f = 0.34 (PhMe:MeCN:MeOH/94:5.5:0.5). FDMS: *m/z* (relative intensity), 395 ([$M^{+\cdot}$] 100), 396 (24.9).

*3.3.8.5. 2-[(2-Methoxy-5-propoxyphenyl-4-yl)hydrazin-1-yl-2-ylidene][*N*-(3-nitrophenyl)-3-oxobutyramide]* (**13**). This pigment crystallized from 2-methoxyethanol as an orange powder (45%), m.p. 194–196°C. Elemental analysis. Calculated for $C_{20}H_{22}N_4O_6$: C, 57.97; H, 5.35; N, 13.52. Found: C, 57.83; H, 5.37; N, 13.35. ^{1}H NMR ($CDCl_3$): δ 1.17–1.22 (t, 3H), δ 1.90–1.97 (m, 2H), δ 2.60 (s, 3H), δ 3.83 (s, 3H), δ 4.02–4.06 (t, 2H), δ 6.66–6.70 (dd, 1H), δ 6.88–6.91 (d, 1H), δ 7.24–7.25 (d, 1H), δ 7.47–7.52 (t, 1H), δ 7.81–7.85 (dd, 1H), δ 7.95–7.99 (dd, 1H), δ 8.78–8.79 (t, 1H), δ 11.80 (s, 1H), δ 14.77 (s, 1H). TLC: R_f = 0.47 (PhMe:MeCN:MeOH/94:5.5:0.5). FDMS: *m/z* (relative intensity), 414 ([$M^{+\cdot}$] 100), 415 (32.2).

*3.3.8.6. 2-[(2-Methoxy-5-propoxyphenyl-4-yl)hydrazin-1-yl-2-ylidene][*N*-(4-nitrophenyl)-3-oxobutyramide]* (**14**). Pigment **14** (2.6 g, 50%) was obtained as a powder, m.p. 198–201°C. Elemental analysis. Calculated for $C_{20}H_{22}N_4O_6$: C, 57.97; H, 5.35; N, 13.52. Found: C, 58.07; H, 5.31; N, 13.48. ^{1}H NMR ($CDCl_3$): δ 1.13–1.18 (t, 3H), δ 1.87–1.99 (m, 2H), δ 2.59 (s, 3H), δ 3.82 (s, 3H), δ 4.01–4.06 (t, 2H), δ 6.67–6.70 (dd, 1H), δ 6.88–6.91 (d, 1H), δ 7.23–7.24 (d, 1H), δ 7.80–7.85 (dd, 2H), δ 8.20–8.24 (dd, 2H), δ 11.94 (s, 1H), δ 14.68 (s, 1H). TLC: R_f = 0.47 (PhMe:MeCN:MeOH/94:5.5:0.5). FDMS: *m/z* (relative intensity), 414 ([$M^{+\cdot}$] 100), 415 (22.3).

*3.3.8.7. 2-[(2-Methoxy-5-propoxyphenyl-4-yl)dihydrazin-1-yl-2-ylidene][3-oxobutyramide-*N*-(3-trifluoromethyphenyl)]* (**15**). This pigment recrystallized from EtOH as an orange powder (45%), m.p. 129–132°C. Elemental analysis. Calculated for $C_{21}H_{22}N_3O_4F_3$: C, 57.66; H, 5.07; N, 9.61. Found: C, 57.76; H, 5.10; N, 9.56. ^{1}H NMR ($CDCl_3$): δ 1.14–1.19 (t, 3H), δ 1.86–1.98 (m, 2H), δ 2.59 (s, 3H), δ 3.82 (s, 3H), δ 4.00–4.05 (t, 2H), δ 6.64–6.68 (dd, 1H), δ 6.87–6.90 (d, 1H), δ 7.24–7.25 (d, 1H), δ 7.35–7.38 (d, 1H), δ 7.42–7.47 (t, 1H), δ 7.70–7.73 (d, 1H), δ 8.14 (s, 1H), δ 11.64 (s, 1H), δ 14.82 (s, 1H). TLC: R_f = 0.58 (PhMe:MeCN:MeOH/94:5.5:0.5). FDMS: *m/z* (relative intensity), 437 ([$M^{+\cdot}$] 100), 438 (27.7).

*3.3.8.8. 2-[(2-Methoxy-5-propoxyphenyl-4-yl)dihydrazin-1-yl-2-ylidene][*N*-(3-acetylphenyl)-3-*

oxobutyramide] (**16**). Pigment **16** (3.5 g, 68%) was obtained as a powder, m.p. 158–161°C. Elemental analysis. Calculated for $C_{22}H_{25}N_3O_5$: C, 64.22; H, 6.12; N, 10.21. Found: C, 64.16; H, 6.13; N, 10.13. ^{1}H NMR ($CDCl_3$): δ 1.14–1.20 (t, 3H), δ 1.86–1.98 (m, 2H), δ 2.59 (s, 3H), δ 2.62 (s, 3H), δ 3.82 (s, 3H), δ 4.00–4.04 (t, 2H), δ 6.64–6.68 (dd, 1H), δ 6.86–6.89 (d, 1H), δ 7.24 (s, 1H), δ 7.41–7.47 (t, 1H), δ 7.71–7.73 (d, 1H), δ 7.81–7.84 (d, 1H), δ 8.36–8.37 (d, 1H), δ 11.61 (s, 1H), δ 14.81 (s, 1H). TLC: $R_f = 0.30$ (PhMe:MeCN:MeOH/94:5.5:0.5). FDMS: m/z (relative intensity), 411 ([$M^{+\cdot}$] 100), 412 (24.8).

4. Conclusions

It was found that FDMS is an excellent method for verifying the formation of pigments prepared in this study. Acetoacetanilides were readily obtained by acetoacetylation of the corresponding anilines by diketene in HOAc solution, with $HgSO_4$ catalysis at room temperature, but it was found that 2-mono-substituted anilines pose a limitation for this reaction.

The mutagenicity of diarylide and monoarylide pigments prepared in this study depended primarily on the presence of a nitro group in the coupling component. In addition, the mutagenic pigments in this study do not appear to owe their activity to reductive-cleavage of the azo bond, in contrast to prior reports. A nitro group in the 4-position of the coupling component appears to lead to higher mutagenicity compared with incorporation of a nitro group in the 3-position.

In case of diarylide pigments, the high dihedral angle across the biphenyl linkage caused by the presence of 2,2′-disubstitution did not have an adverse effect on color strength. The intensity of the color of diarylide pigments was more than twice that of the corresponding monoarylides. This suggests that significant π-orbital overlap exists across the biphenyl linkage despite the incorporation of bulky substituents in the 2,2′-positions.

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