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An Approach to the Design of Nonmutagenic Azo Dyes: Analogs of the Mutagen CI Direct Black 17

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ABSTRACT

The effect on mutagenicity caused by incorporating an alkoxy substituent into the structure of a disazo hydrophilic dye has been investigated. The results of this study indicate that while bulky alkoxy groups are useful in lowering the mutagenicity of certain analogs of CI Direct Black 17, the decrease observed is less than that noted for a series of monoazo disperse dyes. The color and fastness properties of these novel disazo dyes are also described.

INTRODUCTION

It is well known that the presence of sodium sulfonate groups in the structure of an azo dye is not necessarily sufficient to render the molecule nonmutagenic. For instance, although CI Acid Red 18 (**1**) and CI Acid Red 27 (**2**) are nonmutagenic[1] even under conditions sufficient to cause reductive cleavage of the azo linkage, the structurally similar dye CI Acid Red 26 (**3**) is reported to be carcinogenic.[2,3] These results suggest that specific structural features are required in the potential reductive-cleavage products of azo dyes

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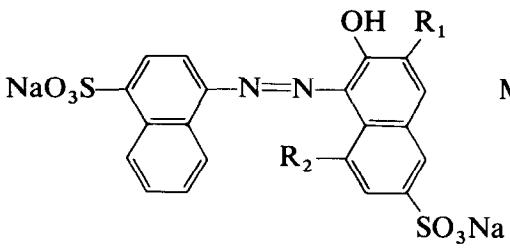
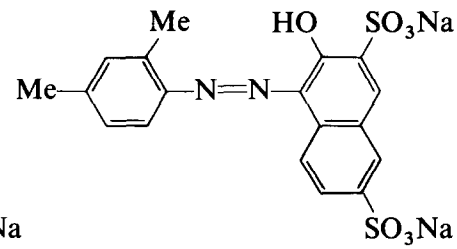
that would render the resulting aromatic amines nongenotoxic, as the reductive cleavage of dye **3** would generate the known mutagen 2,4-dimethylaniline.



	R_1	R_2
1	H	SO_3Na
2	SO_3Na	H



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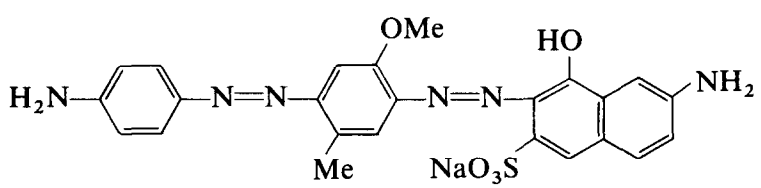
In the previous paper in this series,[4] we described the effect on genetic toxicology caused by incorporating an alkoxy substituent into the structure of a hydrophobic monoazo dye. In that work, it was clear that the more bulky alkoxy groups are quite effective in lowering the mutagenicity exhibited by the parent dye molecule. In the present paper, this approach is extended to hydrophilic dyes as a potential way to remove the mutagenicity of CI Direct Black 17 (**4**). This dye is typical of many of the direct dyes for



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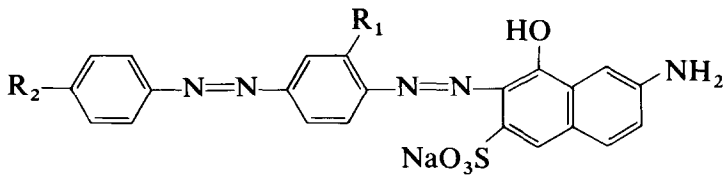
cellulosic substrates that are either banned from use or see very limited use in the United States as a consequence of established genotoxicity data. As a further extension of our work on the monoazo dyes, the disazo dyes **5–19** were synthesized and evaluated in the standard *Salmonella* test for mutagenicity. In addition to incorporating a bulky alkoxy group into the aromatic ring bearing R_1, the nature of the group in position R_2 was also varied. It was envisaged that the three amines produced as a result of the reductive cleavage of the azo linkages of **4** could be rendered nonmutagenic by the SO_3Na group of the Gamma acid residue, by a bulky alkoxy group *ortho* to the amino group of the diamine derived from the ring bearing R_1, and by alkylating the NH_2 group on the aromatic residue bearing R_2. The effects of these changes on color, lightfastness, and wetfastness were also studied.



	R_1	R_2		R_1	R_2
5	OMe	H	**13**	OBu	NEt_2
6	OEt	H	**14**	OCH_2CH_2OH	NEt_2
7	OPr	H	**15**	OMe	$N(CH_2CH_2OH)_2$
8	OBu	H	**16**	OEt	$N(CH_2CH_2OH)_2$
9	OCH_2CH_2OH	H	**17**	OPr	$N(CH_2CH_2OH)_2$
10	OMe	NEt_2	**18**	OBu	$N(CH_2CH_2OH)_2$
11	OEt	NEt_2	**19**	OCH_2CH_2OH	$N(CH_2CH_2OH)_2$
12	OPr	NEt_2			

RESULTS AND DISCUSSION

The reaction scheme used to prepare the disazo dyes is given in Fig. 1. Essentially, the dyes were prepared by diazotizing the amino group of the appropriate monoazo dye precursor and coupling the resulting diazonium salt to Gamma acid (**20**). The diazotization of the five monoazo dyes where $R_2 = H$ worked best when each dye was first powdered, then pasted with a small amount of HCl solution, and the resulting paste diluted with a solution

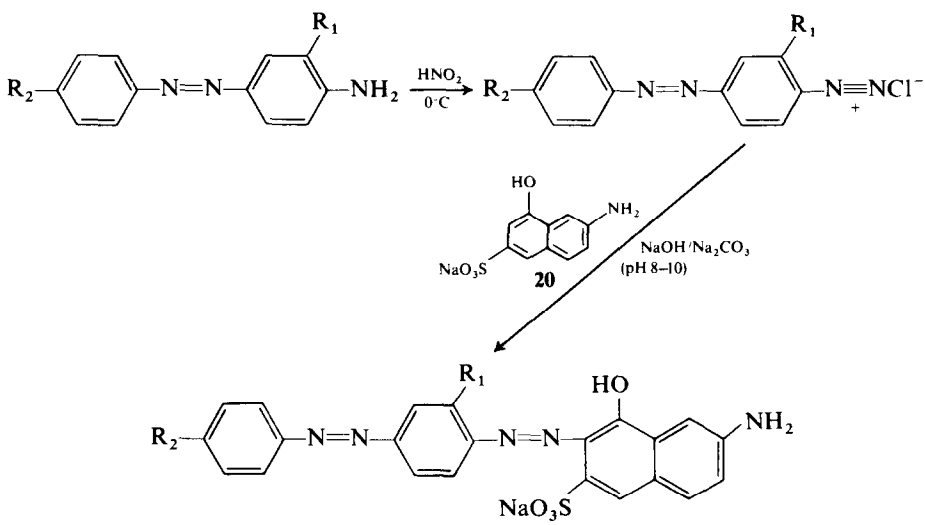


Fig. 1. Reaction sequence used to prepare dyes **5–19**.

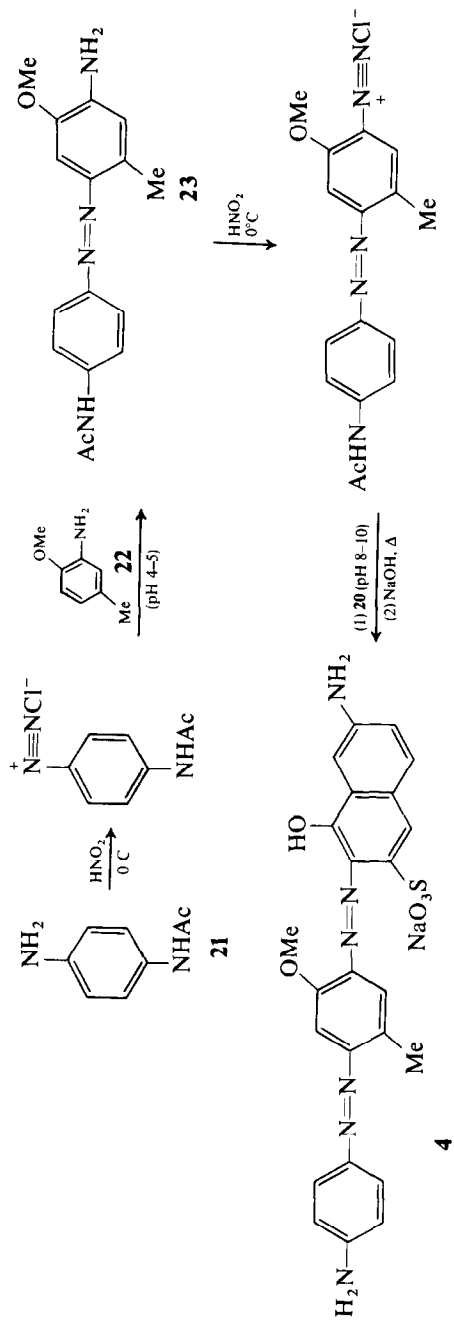


Fig. 2. Reaction sequence used to prepare Cl Direct Black 17 (**4**).

of cold water containing enough HCl to provide a total of 2·5 mol of HCl per mole of the aminoazobenzene. Diazotization was accomplished by adding $NaNO_2$ solution to the suspension of the aminoazobenzene in acid at 0°C. The resulting diazo compound was coupled to Gamma acid at pH 8–10.

The reaction scheme used to prepare the known commercial dyestuff **4** is shown in Fig. 2. The diazotization of **21** was carried out on a suspension of its finely divided hydrochloride. The resulting diazonium salt was coupled with 2-methoxy-5-methylaniline (**22**) to give dye **23**. Diazotization of **23**, coupling of the diazonium salt to Gamma acid (**20**) at pH 8–10, and subsequent hydrolysis of the acetamido group, produced CI Direct Black 17 (**4**). The structure of this compound was confirmed by ^{1}H-NMR and negative-ion FAB mass spectrometry.

Table 1 gives the visible absorption spectral data for dyes **4–19** and Fig. 3 shows representative spectra. These data suggest that the incorporation of the larger alkoxy substituents into the structure of CI Direct Black 17 does not adversely effect color or intensity. Table 2 contains the washfastness and lightfastness data recorded for the 16 dyes. As was the case with the absorption characteristics, the introduction of a bulky alkyl group has not altered the lightfastness or washfastness of these dyes.

Table 3 summarizes the test results (positive, negative or equivocal) for all of the compounds tested. The table reflects a slope value for those compounds concluded to be positive.

All of the new dyes that do not contain an *N,N*-dialkyl group require metabolic activation to produce a mutagenic response. The most active dye in this group is **6**, followed closely by dye **5**. The higher alkoxy-substituted members of this group, **7** and **8**, are less mutagenic than **5** and **6** in strains TA98 and TA1538. However, dye **9** is more mutagenic in these strains than **7**

TABLE 1
Visible Absorption Spectral Data[a] for Dyes **4–19**

Dye	λ_{max} (*nm*)	ε_{max} *liter* $(mol\,cm)^{-1}$	*Dye*	λ_{max} (*nm*)	ε_{max} *liter* $(mol\,cm)^{-1}$
4	555	31 000	**12**	593	25 100
5	548	32 600	**13**	592	28 900
6	548	26 700	**14**	592	29 800
7	548	21 900	**15**	576	33 400
8	548	24 800	**16**	576	34 700
9	547	25 600	**17**	576	41 000
10	592	23 000	**18**	576	37 300
11	592	31 600	**19**	576	43 700

[a] The dyes were dissolved in water.

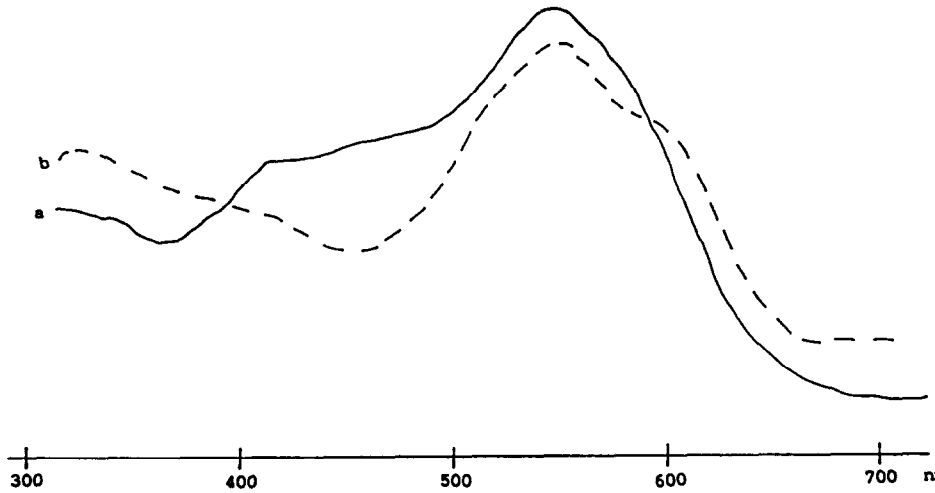


Fig. 3. Visible absorption spectrum of dyes **5** (a) and **6** (b) in water.

TABLE 2
Washfastness and Lightfastness Test Results[a] for Dyes **4–19**

Dye	*Washfastness*		*Lightfastness*
	Change in color	*Staining of cotton*	
4	2–3	2–3	3–4
5	2–3	2–3	3–4
6	2–3	2–3	3–4
7	2–3	2–3	3
8	2–3	2–3	3
9	2–3	2–3	3–4
10	2–3	2–3	3–4
11	2–3	2–3	3–4
12	2–3	2–3	3–4
13	2–3	2–3	3
14	2–3	2–3	3
15	2–3	2–3	3–4
16	2–3	2–3	3–4
17	2–3	2–3	3
18	2–3	2–3	3–4
19	2–3	2–3	4

[a] All samples were rated on a scale of 1–5 with a value of 5 representing no detectable color change or staining.

TABLE 3
Summary of the Mutagenicity Data for the Disazo Dyes Tested in This Study[a]

Compound	*TA98*[b]		*TA100*		*TA1538*	
	+S9[c]	*−S9*	*+S9*	*−S9*	*+S9*	*−S9*
4	835	Neg	221	Neg	1095	Neg
5	2920	Neg	2020	Neg	7495	Neg
6	4383	Neg	3866	Neg	8726	Neg
7	400	Neg	Neg	Neg	1035	Neg
8	668	Neg	Neg	Neg	1594	Neg
9	1348	Neg	1804	Neg	4027	Neg
10	342	Neg	Neg	Neg	448	Neg
11	580	Neg	Neg	Neg	981	Neg
12	256	?	Neg	Neg	394	Neg
13	228	Neg	Neg	Neg	463	Neg
14	148	Neg	Neg	Neg	192	Neg
15	601	Neg	Neg	Neg	881	Neg
16	1983	Neg	Neg	Neg	1377	?
17	1533	Neg	Neg	Neg	1819	Neg
18	1452	Neg	Neg	Neg	1371	Neg
19	350	Neg	Neg	Neg	809	Neg

[a] The numbers denote Rev μmol^{-1} observed and a positive result; Neg, negative result; ?, equivocal result. Slope values are derived using the model of Bernstein *et al.*[5]

[b] Designates the *Salmonella typhimurium* tester strain used.

[c] Designates whether or not the test was conducted with (+S9) or without (−S9) a mammalian microsomal activation system.

and **8**. When dyes **5–9** are tested in TA100 three of the five (**5**, **6** and **9**) are mutagenic, with **6** being the most active. Figure 4 shows a plot of the mutagenicity data recorded for these five disazo dyes in TA98 with metabolic activation.

The five disazo dyes containing an NEt_2 group (cf. **10–14**) are less mutagenic than dyes **5–9**. As observed in the series of dyes **5–9**, the ethoxy-substituted dye **11** is the most active dye in the group **10–14**. However, unlike dyes **5–9**, none of dyes **10–14** is mutagenic in TA100. Also, the butoxy-substituted dye in this group (**13**) does not differ significantly in mutagenic activity in TA98 and TA100 from the methoxy analog **10**. The least active dye in this group is compound **14**.

On the whole, the five disazo dyes containing an $N(CH_2CH_2OH)_2$ group, **15–19**, are less mutagenic than dyes **5–9** and more mutagenic than dyes **10–14**. Interestingly, dyes **15–19** do not show a stepwise decrease in mutagenicity as the size of the alkoxy group is increased.

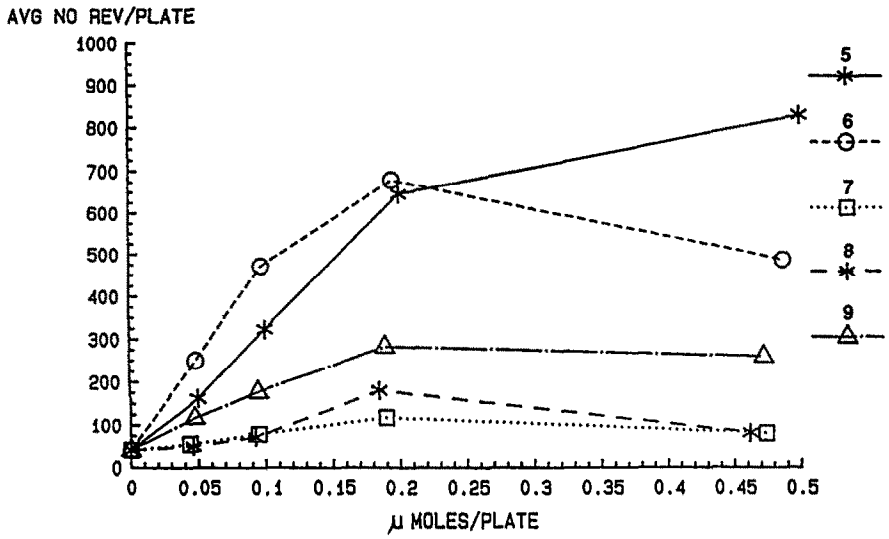


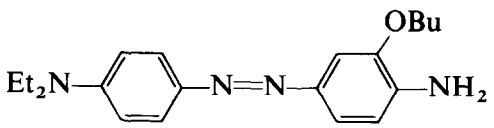
Fig. 4. Mutagenicity of dyes **5–9** in TA98 with S9 activation.

CI Direct Black 17 (**4**) is mutagenic in TA100, whereas dyes **10–19** are inactive, and it would appear that *N*-substitution is responsible for the loss of activity in TA100. The activity of **4** in TA98 and TA100 is similar to that exhibited by the analogs **10–19**. This work is consistent with the work of Venturini & Tamaro,[6] who showed **4** to be mutagenic in TA98 and TA1538 with metabolic activation. However, unlike the results obtained in the present study, those workers reported **4** to be *nonmutagenic* in TA100 with activation and *mutagenic* in TA98 and TA1538 without metabolic activation.

Many of the disazo dyes in this study are less mutagenic than the monoazo dyes from which they were derived. This is not surprising since it is known that a sulfonic acid group can reduce or remove genotoxic activity in organic compounds.[7] However, disazo dyes **15–19** were found to be *more* mutagenic in TA98 and TA1538 than their monoazo precursors. In fact, the monoazo dyes used to prepare disazo dyes **16–19** are *nonmutagenic* in TA98.

Interestingly, this study shows that disazo dyes can be rendered mutagenic by processes other than reductive cleavage. For example, **13** causes 228 Rev μmol^{-1} in TA98 with metabolic activation, whereas its reductive-cleavage products are negative, equivocal, or very weakly active under such conditions. Compound **24** produces only 1 Rev μmol^{-1} and is the only mutagenic reductive-cleavage product of **13**. Consequently, the reductive-cleavage products of **13** do not completely account for its mutagenicity.

The results of this study also indicate that although the replacement of the OMe group of the Direct Black 17 analogs **5–9** leads to a significant decrease



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in activity, the corresponding substitution in disazo dyes **10** and **15** does not lead to a similar stepwise decrease in mutagenic activity.

EXPERIMENTAL

All of the starting materials not synthesized in this work were obtained from Aldrich Chemical Co., Milwaukee, WI, USA, except for Gamma acid (**20**), which was obtained from Mobay Chemical Co., Pittsburgh, PA, USA.

The mercerized cotton print cloth (Style No. 400M) was obtained from Test Fabrics, Inc., Middlesex, NJ, USA. The apparatus used to dye the cotton fabric was an Ahiba AG (type WBRG 7) dyeing machine.

Melting points were determined using a Mel-Temp capillary melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker 250 MHz spectrometer using DMSO-d_6 as the solvent, and the chemical shifts are reported in ppm relative to TMS. Negative-ion Fast Atom Bombardment (FAB) mass spectra were recorded using a JEOL HX110HF double-focusing mass spectrometer equipped with a DA-5000 data system, and matrix ions were excluded in the calculations of relative intensity. Visible spectra were recorded on a Perkin–Elmer UV–Visible spectrophotometer model 559A. High Performance Liquid Chromatography (HPLC) data were recorded on a Econosphere C_{18} 5μm column, 4·6 mm (i.d.) × 250 mm (Alltech) using a Waters model 6000A solvent delivery system equipped with a Waters model 441 absorbance detector (254 nm).

Mutagenicity test procedures

Dyes **4–19** were examined in the Ames standard plate incorporation *Salmonella*/mammalian microsome plate incorporation assay with bacterial strains TA98, TA100, and TA1538 in the presence and absence of exogenous activation.[8] Strains TA98, TA1537, and TA1538 detect frameshift mutations while TA1535 and TA100 detect base-pair substitutions. As described in the previous paper,[4] all compounds were evaluated two or more times on separate days. The activation system used was a 9000*g* (S9) liver homogenate obtained from Aroclor 1254-induced Sprague–Dawley male rats. Where an adequate amount of test compound was available, the

chemicals were tested up to their limit of solubility, point of toxicity, or to at least 5 mg/plate at a minimum of six doses using triplicate plates. Appropriate negative (blank solvent) and positive controls were run concurrently with each assay.

Mutagenicity results were designated positive, negative, or equivocal according to the criteria of Claxton *et al.*[9] A response was judged positive (mutagenic) when there was a dose-related increase in revertant counts as determined by the statistical models of Stead *et al.*[10] and Bernstein *et al.*[5] It was not necessary for the increase to be equal to or greater than two-fold the background. A response was considered equivocal when (1) test results were not reproducible, (2) there was a reproducible low-level increase in induced mutants but no dose-related response, or (3) when an increase in induced mutants was observed at only one dose.

Washfastness and lightfastness testing[11]

AATCC test method 61–190 (colorfastness to washing, domestic; and laundering, commercial: accelerated)
Test no. IIA was employed for the cotton fabrics. The change in color was evaluated using the Grey Scale for Color Change, and staining of cotton was evaluated using the Grey Scale for Staining.

AATCC test method 16E–1982 (colorfastness to light: water-cooled xenon arc lamp, continuous light)
Note: The protocol for this test was followed except for the following modifications. The apparatus used was the air-cooled Xenotest 1200 (Original Hanau) and the test conditions were:

Black panel temperature: 64°C
Relative humidity: $30 \pm 5\%$
Chamber temperature: 50°C
Window glass filters: 310 nm cut-off
Duration of test: 20 h

No reference fabric was used and the samples were rated with the aid of the Grey Scale for Color Change. The disazo dyes (**4–19**) were exposed as a group on the same day.

Application of dyes 4–19 to cotton fabric

All dyeings were performed using a liquor ratio (LR) of 30:1 with 2% on weight of fabric dye and an Ahiba AG dyeing apparatus. The dyeings were carried out as follows. A dyebath was prepared that contained 1 g litre^{-1}

Levegal N (a levelling agent) and the required amount of dye. The cotton fabric was immersed in this bath and the dyeing was initiated at 38°C. The bath temperature was allowed to rise to 49°C and held there for 10 min. The bath temperature was then increased to 95°C over a period of 30 min, and Na_2SO_4 (20% on weight of fabric) was slowly added over the next 30 min. After a total of 60 min at 95°C, the bath was allowed to cool to 70°C. The fabric was removed, rinsed thoroughly with water, and pressed dry with a warm iron.

Synthesis of dyes 4–19

[4-(4-Amino-3-methoxy-2-methyl)phenylazo]acetanilide (**23**)
Finely powdered 4′-aminoacetanilide (**21**, 3 g, 0·02 mol), 10 ml of 2M-HCl, and 10 g of crushed ice were combined and the mixture was ground into a paste. The paste was diluted with 7·5 ml of 2M-HCl, 10 ml of H_2O, 10 g of crushed ice, and then cooled to 0°C with vigorous stirring. The amine was diazotized by adding 20 ml of 1M-$NaNO_2$. The diazonium salt was added to a cold (0°C) stirred solution of 2-methoxy-5-methylaniline (**22**, 2·47 g, 0·02 mol) and NaOAc (4·8 g, 0·06 mol) in 75 ml of H_2O. After stirring for 24 h at 0°C, the reddish precipitate that formed was collected by filtration (92%). ^{1}H-NMR, δ (ppm): 2·10 (s, 3H), 2·52 (s, 3H), 3·88 (s, 3H), 6·80 (s, 1H), 7·57 (s, 1H), 7·74–7·83 (m, 4H), 10·35 (s, 1H).

CI Direct Black 17 ***(4)***
Finely powdered **23** (5 g, 0·017 mol), 10 ml of H_2O, 2·1 ml of conc. HCl, and 10 g of ice were combined, and the mixture was blended to form a paste. The paste was diluted with 20 ml of H_2O, 2·1 ml of conc. HCl, and 10 g of crushed ice, and then cooled to 0°C with vigorous stirring. The amine was diazotized by adding 16·9 ml of 1M-$NaNO_2$. After 1 h, the mixture was filtered to remove a small amount of insoluble material. The diazonium salt was added dropwise to a well-stirred, cold (0°C) solution containing Gamma acid (**20**, 4·01 g, 0·017 mol), NaOH (0·67 g, 0·017 mol), and Na_2CO_3 (0·88 g, 0·008 mol) in 250 ml of H_2O. During the addition, the pH was kept above 8 by the periodic addition of cold (0°C) 10% NaOH. After stirring for 24 h at 0°C, the pH of the solution was adjusted to 7 with 10% HCl, and 60 g of NaCl were added to give a purple precipitate that was collected by filtration. Hydrolysis of the acetamido group was accomplished by stirring the collected solid at the boil with 46 g of NaOH in 460 ml of 95% EtOH/230 ml H_2O for 10 h. The blue solution was evaporated down to 250 ml using a rotary evaporator and acidified to pH 2 with conc. HCl to precipitate **4**. The crude dye was collected by filtration, then suspended in 300 ml of H_2O, the pH was adjusted to 7 with 10% NaOH, and the solution was concentrated to

dryness. Residual salt was removed by stirring the crude dye with dry DMF and then filtering the mixture with the aid of a fine-mesh fritted glass filter. Concentration of the filtrate gave 6·6 g (75%) of **4** as a blue solid. ^{1}H-NMR, δ(ppm): 2·63 (s, 3H), 4·03 (s, 3H), 6·71–6·74 (d, 2H), 7·09–7·11 (d, 1H), 7·36 (s, 1H), 7·43 (s, 1H), 7·49–7·52 (d, 1H), 7·58 (s, 1H), 7·69–7·73 (d, 2H), 7·94 (s, 1 H), 16·24 (s, 1 H). FAB mass spectrum (matrix = diethanolamine/DMSO), *m/e* (relative intensity): 505 ($[M - Na]^-$, 27), 401 (15), 400 (16), 253 (100), 252 (87), 251 (62), 238 (30), 237 (23). HPLC: $t_r = 2·78$ min using MeOH/H_2O, 50:50 (v/v), as the eluent.

Dyes **5–19** were prepared using essentially the same procedure as described above for the synthesis of **4**. However, it should be added that monoazo dyes containing R_2 = *N,N*-dialkyl were soluble in dilute HCl and were therefore diazotized by the direct method.[12]

*6-Amino-3-[(2-methoxy-4-phenylazo)phenylazo]-4-hydroxy-2-naphthalenesulfonic acid, sodium salt (**5**)*

Dye **5** was obtained as a bluish-purple solid (74%). ^{1}H-NMR, δ(ppm): 4·13 (s, 3H), 5·82 (s, 2H), 6·97–7·02 (dd, 1H), 7·40–7·44 (m, 3H), 7·55–7·64 (m, 4H), 7·73–7·77 (dd, 1H), 7·90–7·97 (dd, 2H), 8·15–8·19 (d, 1H), 15·98 (s, 1H). FAB mass spectrum (matrix = triethanolamine/DMSO), *m/e* (relative intensity): 499 ($[M\cdot]^-$, 9), 476 ($[M - Na]^-$, 47), 387 (40), 386 (11), 385 (8), 372 (6), 371 (6), 253 (72), 252 (57), 251 (100), 238 (12), 237 (19), 81 (16), 82 (22). HPLC: $t_r = 3·46$ min using MeOH/H_2O, 50:50 (v/v), as the eluent.

*6-Amino-3-[(2-ethoxy-4-phenylazo)phenylazo]-4-hydroxy-2-naphthalenesulfonic acid, sodium salt (**6**)*

Dye **6** was obtained as a bluish-purple solid (70%). ^{1}H-NMR, δ(ppm): 1·53–1·58 (t, 3H), 4·36–4·39 (q, 2H), 5·81 (s, 2H), 7·0–7·01 (dd, 1H), 7·39–7·47 (m, 3H), 7·55–7·63 (m, 4H), 7·72–7·75 (d, 1H), 7·89–7·92 (d, 2H), 8·14–8·17 (d, 1 H), 15·97 (s, 1 H). FAB mass spectrum (matrix = triethanolamine/DMSO), *m/e* (relative intensity): 513 ($[M\cdot]^-$, 15), 490 ($[M - Na]^-$, 50), 484 (4), 462 (3), 401 (32), 400 (26), 399 (8), 386 (6), 385 (5), 253 (49), 252 (61), 251 (100), 238 (19), 237 (22), 81 (21), 80 (32). HPLC: $t_r = 4·86$ min using MeOH/H_2O, 50:50 (v/v), as the eluent.

*6-Amino-3-[(2-n-propoxy-4-phenylazo)phenylazo]-4-hydroxy-2-naphthalenesulfonic acid, sodium salt (**7**)*

Dye **7** was obtained as a bluish-purple solid (68%). ^{1}H-NMR, δ(ppm): 1·14–1·20 (t, 3H), 1·89–1·98 (m, 2H), 4·22–4·28 (t, 2H), 5·80 (s, 2H), 7·02–7·06 (dd, 1H), 7·40–7·45 (m, 3H), 7·55–7·64 (m, 4H), 7·72–7·76 (d, 1H), 7·87–7·92 (d, 2H), 8·14–8·18 (d, 1H), 15·98 (s, 1H). FAB mass spectrum (matrix = triethanolamine/DMSO), *m/e* (relative intensity): 527 ($[M\cdot]^-$, 2), 504

([M − Na]$^-$, 36), 484 (2), 462 (2), 415 (20), 414 (16), 413 (4), 400 (6), 399 (6), 253 (33), 252 (60), 251 (100), 238 (29), 237 (32), 81 (26), 80 (45). HPLC: $t_r = 3{\cdot}04$ min using MeOH/H_2O, 60:40 (v/v), as the eluent.

*6-Amino-3-[(2-n-butoxy-4-phenylazo)phenylazo]-4-hydroxy-2-naphthalenesulfonic acid, sodium salt (**8**)*

Dye **8** was obtained as a bluish-purple solid (60%). ^{1}H-NMR, δ(ppm): 1·01–1·07 (t, 3H), 1·59–1·73 (m, 2H), 1·87–1·96 (m, 2H), 4·30–4·35 (t, 2H), 5·81 (s, 2H), 7·01–7·08 (dd, 1H), 7·39–7·44 (m, 3H), 7·55–7·64 (m, 4H), 7·71–7·75 (d, 1H), 7·86–7·93 (d, 2H), 8·14–8·17 (d, 1H), 15·97 (s, 1H). FAB mass spectrum (matrix = triethanolamine/DMSO), *m/e* (relative intensity): 541 ([M·]$^-$, 12), 518 ([M − Na]$^-$, 25), 484 (16), 462 (2), 429 (8), 428 (8), 427 (3), 414 (4), 413 (4), 253 (20), 252 (49), 251 (100), 238 (21), 237 (29), 81 (26), 80 (50). HPLC: $t_r =$ 3·98 min [MeOH/H_2O, 60:40 (v/v)].

*6-Amino-3-[(2-(2-hydroxyethoxy)-4-phenylazo)phenylazo]-4-hydroxy-2-naphthalenesulfonic acid, sodium salt (**9**)*

Dye **9** was obtained as a bluish-purple solid (70%): ^{1}H-NMR, δ(ppm): 3·93–3·95 (m, 2H), 4·35–4·39 (t, 2H), 4·99 (s, 1H), 5·75 (s, 2H), 6·97–7·02 (dd, 1H), 7·39–7·48 (m, 3H), 7·54–7·66 (m, 4H), 7·71–7·75 (dd, 1H), 7·89–7·93 (dd, 2H), 8·18–8·21 (d, 1 H), 15·96 (s, 1 H). FAB mass spectrum (matrix = diethanolamine/DMSO), *m/e* (relative intensity): 529 ([M·]$^-$, 9), 506 ([M − Na]$^-$, 23), 484 (14), 417 (7), 416 (7), 415 (4), 402 (5), 401 (6), 253 (31), 252 (63), 251 (100), 238 (22), 237 (33), 81 (16), 80 (32). HPLC: $t_r = 2{\cdot}40$ min [MeOH/H_2O, 60:40 (v/v)].

*6-Amino-3-[4-(4-diethylaminophenyl)azo)-2-methoxyphenylazo]-4-hydroxy-2-naphthalenesulfonic acid, sodium salt (**10**)*

Dye **10** was obtained as a bluish-purple solid (75%). ^{1}H-NMR, δ(ppm): 1·13–1·19 (t, 6H), 3·42–3·48 (q, 4H), 4·12 (s, 3H), 5·73 (s, 2H), 6·79–6·82 (d, 2H), 6·96–7·00 (dd, 1H), 7·39–7·44 (m, 2H), 7·51–7·58 (m, 2H), 7·78–7·82 (d, 2H), 7·95 (s, 1H), 8·10–8·13 (d, 1H), 16·20 (s, 1H). FAB mass spectrum (matrix = triethanolamine/DMSO), *m/e* (relative intensity): 570 ([M·]$^-$, 19), 555 (8), 547 ([M − Na]$^-$, 37), 541 (3), 533 (4), 519 (4), 387 (8), 386 (8), 385 (4), 372 (5), 371 (5), 253 (61), 252 (84), 251 (100), 238 (23), 237 (31), 81 (39), 80 (68). HPLC: $t_r = 10{\cdot}36$ min [MeOH/H_2O, 50:50 (v/v)].

*6-Amino-3-[4-(4-diethylaminophenylazo)-2-ethoxyphenylazo]-4-hydroxy-2-naphthalenesulfonic acid, sodium salt (**11**)*

Dye **11** was obtained as a bluish-purple solid (72%). ^{1}H-NMR, δ(ppm): 1·13–1·17 (t, 6H), 1·51–1·60 (t, 3H), 3·43–3·46 (q, 4H), 4·32–4·39 (q, 2H), 5·72 (s, 2H), 6·80–6·83 (d, 2H), 6·97–6·99 (dd, 1H), 7·39–7·44 (m, 2H), 7·50–7·58

(m, 2H), 7·77–7·81 (d, 2H), 7·94 (s, 1H), 8·11–8·13 (d, 1H), 16·22 (s, 1H). FAB mass spectrum (matrix = triethanolamine/DMSO), *m/e* (relative intensity): 584([M·]$^-$, 14), 561 ([M − Na]$^-$, 40), 555 (11), 533 (6), 401 (7), 400 (8), 399 (4), 386 (3), 385 (4), 253 (54), 252 (65), 251 (100), 238 (33), 237 (36), 81 (30) 80 (53). HPLC: $t_r = 4{\cdot}32$ min [MeOH/H_2O, 60:40 (v/v)].

*6-Amino-3-[4-(4-diethylaminophenylazo)-2-*n*-propoxyphenylazo]-4-hydroxy-2-naphthalenesulfonic acid, sodium salt (**12**)*
Dye **12** was obtained as a bluish-purple solid (67%). ^{1}H-NMR, δ(ppm): 1·0–1·05 (t, 3H), 1·13–1·17 (t, 6H), 1·74–1·89 (m, 2H), 3·43–3·46 (q, 4H), 3·97–4·02 (t, 2H), 5·71 (s, 2H), 6·78–6·82 (d, 2H), 6·97–7·00 (dd, 1H), 7·38–7·46 (m, 2H), 7·49–7·56 (m, 2H), 7·78–7·81 (d, 2H), 7·96 (s, 1H), 8·08–8·12 (d, 1H), 16·25 (s, 1H). FAB mass spectrum (matrix = triethanolamine/DMSO), *m/e* (relative intensity): 598 ([M·]$^-$, 12), 575 ([M − Na]$^-$, 38), 555 (7), 547 (5), 533 (3), 415 (9), 414 (9), 400 (6), 399 (6), 253 (55), 252 (78), 251 (100), 238 (36), 237 (32). HPLC: $t_r = 5{\cdot}46$ min [MeOH/H_2O, 60:40 (v/v)].

*6-Amino-3-[4-(4-diethylaminophenylazo)-2-*n*-butoxyphenylazo]-4-hydroxy-2-naphthalenesulfonic acid, sodium salt (**13**)*
Dye **13** was obtained as a bluish-purple solid (63%). ^{1}H-NMR, δ(ppm): 1·01–1·07 (t, 3H), 1·15–1·19 (t, 6H), 1·64–1·75 (m, 2H), 1·86–1·94 (m, 2H), 3·43–3·47 (q, 4H), 4·27–4·32 (t, 2H), 6·78–6·82 (d, 2H), 6·96–7·01 (dd, 1H), 7·38–7·46 (m, 2H), 7·52–7·59 (m, 2H), 7·77–7·81 (d, 2H), 7·96 (s, 1H), 8·08–8·11 (d, 1H), 16·17 (s, 1H). FAB mass spectrum (matrix = triethanolamine/DMSO), *m/e* (relative intensity): 612 ([M·]$^-$, 9), 589 ([M − Na]$^-$, 22), 555 (5), 429 (12), 428 (11), 427 (6), 253 (60), 252 (73), 251 (79), 238 (100), 237 (52), 81 (58), 80 (93). HPLC: $t_r = 7{\cdot}84$ min [MeOH/H_2O, 60:40 (v/v)].

*6-Amino-3-[4-(4-diethylaminophenylazo)-2-(2-hydroxyethoxy)-phenylazo]-4-hydroxy-2-napthalenesulfonic acid, sodium salt (**14**)*
Dye **14** was obtained as a bluish-purple solid (70%). ^{1}H-NMR, δ(ppm): 1·13–1·19 (t, 6H), 3·42–3·48 (q, 4H), 3·90–3·94 (t, 2H), 4·32–4·36 (t, 2H), 6·79–6·83 (d, 2H), 6·99–7·04 (dd, 1H), 7·42–7·46 (m, 2H), 7·51–7·57 (m, 2H),7·78–7·81 (d, 2H), 8·10–8·13 (d, 1H), 16·18 (s, 1H). FAB mass spectrum (matrix = triethanolamine/DMSO), *m/e* (relative intensity): 600 ([M·]$^-$, 14), 577 ([M − Na]$^-$, 44), 555 (8), 417 (7), 416 (6), 402 (6), 253 (82), 252 (77), 251 (100), 238 (27), 237 (28), 81 (25), 80 (45). HPLC: $t_r = 3{\cdot}26$ min [MeOH/H_2O, 60:40 (v/v)]).

*6-Amino-3-[4-(4-*N,N-*bis*-2-*hydroxyethylaminophenylazo)-2-methoxyphenylazo]-4-hydroxy-2-naphthalene sulfonic acid, sodium salt (**15**)*
Dye **15** was obtained as a bluish-purple solid (75%). ^{1}H-NMR, δ(ppm): 3·58–3·62 (m, 8H), 4·10 (s, 3H), 6·85–6·89 (d, 2H), 7·00–7·04 (dd, 1H), 7·42–7·59

(m, 4H), 7·77–7·81 (d, 2H), 7·95 (s, 1H), 8·10–8·12 (d, 1H), 16·20 (s, 1H). FAB mass spectrum (matrix = diethanolamine/DMSO), *m/e* (relative intensity): 602 ($[M\cdot]^-$, 1), 579 ($[M - Na]^-$, 3), 387 (2), 386 (3), 385 (3), 372 (2), 371 (2), 253 (100), 252 (44), 251 (28), 238 (5), 237 (9), 81 (11), 80 (21). HPLC: $t_r = 2{\cdot}7$ min [$MeOH/H_2O$, 50:50 (v/v)].

*6-Amino-3-[4-(4-*N,N-*bis-2-hydroxyethylaminophenylazo)2-ethoxyphenylazo]-4-hydroxy-2-naphthalenesulfonic acid, sodium salt (**16**)*
Dye **16** was obtained as a bluish-purple solid (73%). ^{1}H-NMR, δ(ppm): 1·51–1·57 (t, 3H), 3·60–3·62 (m, 8H), 4·31–4·39 (q, 2H), 6·85–6·88 (d, 2H), 7·02–7·05 (dd, 1H), 7·43–7·58 (m, 4H), 7·76–7·80 (d, 2H), 7·95 (s, 1H), 8·08–8·12 (d, 1H), 16·22 (s, 1H). FAB mass spectrum (matrix = diethanolamine/DMSO), *m/e* (relative intensity): 616 ($[M\cdot]^-$, 1), 593 ($[M - Na]^-$, 1), 401 (1), 400 (2), 399 (1), 386 (2), 385 (2), 253 (100), 252 (26), 251 (27), 238 (5), 237 (7), 81 (5), 80 (8). HPLC: t_r 2·52 min [$MeOH/H_2O$, 60:40 (v/v)].

*6-Amino-3-[4-(4-*N,N-*bis-2-hydroxyethylaminophenylazo)-2-*n-*propoxyphenylazo]-4-hydroxy-2-naphthalenesulfonic acid, sodium salt (**17**)*
Dye **17** was obtained as a bluish-purple solid (68%). ^{1}H-NMR, δ(ppm): 1·17–1·23 (t, 3H), 1·90–1·98 (m, 2H), 3·58–3·60 (m, 8H), 4·23–4·28 (t, 2H), 5·75 (s, 2H), 6·85–6·88 (d, 2H), 6·95–7·00 (dd, 1H), 7·39–7·58 (m, 4H), 7·77–7·80 (d, 2H), 7·95 (s, 1H), 8·09–8·12 (d, 1H), 16·20 (s, 1H). FAB mass spectrum (matrix = diethanolamine/DMSO), *m/e* (relative intensity): 630 ($[M\cdot]^-$, 1), 6·7 ($[M - Na]^-$, 1), 415 (1), 414 (1), 400 (2), 399 (1), 253 (100), 252 (8), 251 (7), 238 (1), 237 (1), 81 (1), 80 (2). HPLC: $t_r = 2{\cdot}46$ min [$MeOH/H_2O$, 60:40 (v/v)].

*6-Amino-3-[4-(4-*N,N-*bis-2-hydroxyethylaminophenylazo)-2-*n-*butoxyphenylazo]-4-hydroxy-2-naphthalenesulfonic acid, sodium salt (**18**)*
Dye **18** was obtained as a bluish-purple solid (60%). ^{1}H-NMR, δ(ppm): 1·01–1·07 (t, 3H), 1·64–1·70 (m, 2H), 1·73–1·92 (m, 2H), 3·57–3·60 (m, 8H), 4·28–4·32 (t, 2H), 5·75 (s, 2H), 6·85–6·88 (d, 2H), 6·96–6·99 (dd, 1H), 7·38–7·57 (m, 4H), 7·76–7·79 (d, 2H), 7·95 (s, 1H), 8·08–8·11 (d, 1H), 16·16 (s, 1H). FAB mass spectrum (matrix = diethanolamine/DMSO), *m/e* (relative intensity): 621 ($[M - Na]^-$, 1), 429 (2), 428 (1), 427 (1), 414 (1), 413 (1), 253 (100), 252 (16), 251 (14), 238 (3), 237 (5), 81 (4), 80 (7). HPLC: $t_r = 2{\cdot}44$ min [$MeOH/H_2O$, 60:40 (v/v)].

*6-Amino-3-[4-(4-*N,N-*bis-2-hydroxyethylaminophenylazo)-2-(2-hydroxyethoxy)-phenylazo]-4-hydroxy-2-naphthalenesulfonic acid, sodium salt (**19**)*
Dye **19** was obtained as a bluish-purple solid (65%). ^{1}H-NMR, δ(ppm): 3·58–3·61 (m, 8H), 3·92–3·96 (m, 2H), 4·32–4·36 (t, 2H), 4·86–4·88 (m, 2H), 4·96–5·00 (t, 1H), 5·73 (s, 2H), 6·85–6·88 (d, 2H), 6·95–6·99 (dd, 1H), 7·39–7·58 (m, 4H), 7·77–7·80 (d, 2H), 7·95 (s, 1H), 8·09–8·13 (d, 1H), 16·16 (s, 1H). FAB

mass spectrum (matrix = diethanolamine/DMSO), *m/e* (relative intensity): 632 ($[M\cdot]^-$, 2), 609 ($[M - Na]^-$, 5), 565 (1), 417 (2), 416 (2), 415 (1), 402 (9), 401 (2), 253 (100), 252 (26), 251 (27), 238 (5), 237 (7), 81 (5), 80 (8). HPLC: t_r = 2·48 min [MeOH/H_2O, 50:50 (v/v)].

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