Dyes and Pigments **8** (1987) 431–447

An Approach to the Design of Non-Mutagenic Azo Dyes: 2. Potential Replacements for the Benzidine Moiety of Some Mutagenic Azo Dyestuffs

Frank Calogero, Harold S. Freeman,*
James F. Esancy, Wilson M. Whaley

Textile Chemistry Department, North Carolina State University,
Raleigh, NC 27695-8302, USA

and

Betty J. Dabney†

International Business Machines Corporation,
Boulder, CO, USA

(Received 5 January 1987; accepted 11 February 1987)

SUMMARY

This paper describes the synthesis of 5,5'-diamino-2,2'-bipyridine and its subsequent evaluation as a possible replacement for the benzidine residue in some well-known carcinogenic benzidine-based azo dyes. The results of this investigation suggest that this diaminobipyridine is much less genotoxic than benzidine itself, and that certain dyes derived from it are also less genotoxic than their benzidine counterparts.

The compounds prepared in this study were evaluated in the standard Ames mutagenicity test, and in an interesting modification developed by Prival and coworkers. Each compound was found to be more active in the latter protocol.

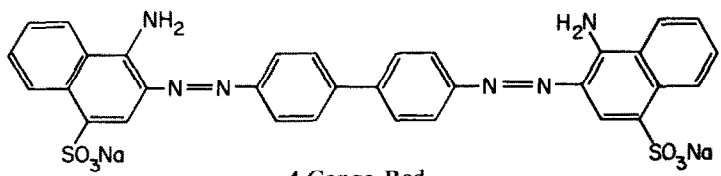
The synthesis of the diamine and of four azo dyes derived from it is described.

* To whom correspondence should be addressed.
† Present address: Healthgenics, Inc., San Antonio, TX 78238, USA.

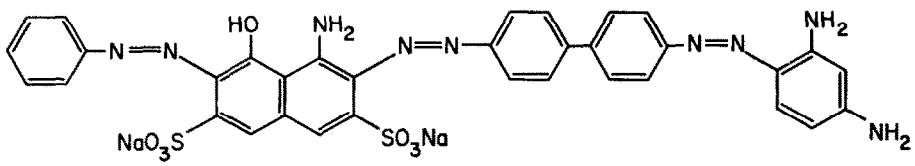
Dyes and Pigments 0143-7208/87/$03·50

1 INTRODUCTION

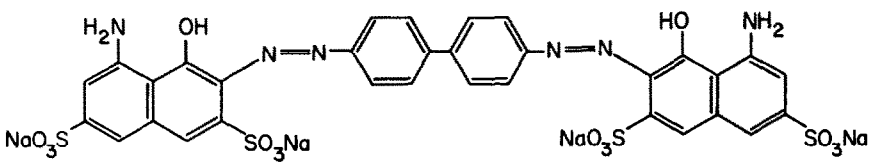
It is well known that benzidine (**1**) is both a genotoxic amine and a human carcinogen.[1–4] Prior to the realization of these facts, numerous dyestuffs had been synthesized which employed benzidine or a benzidine derivative such as *o*-tolidine (**2**) or *o*-dianisidine (**3**) as a key ingredient. Benzidine and dyes derived from it (e.g. **4**–**6**) and its derivatives (e.g. **7**–**9**) are no longer widely used in the USA; however, an EPA report[5] indicated that as late as 1981, some 22 benzidine-based dyes were still commercially available in this country. The literature which describes the toxicity testing of benzidine and dyes derived from it will not be extensively reviewed at this time. We will, however, describe some of the more recent papers which are relevant to the present investigation.



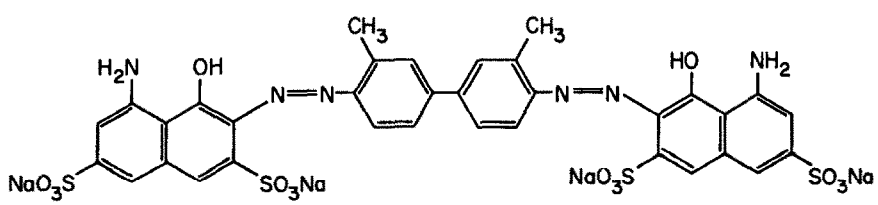
4 Congo Red



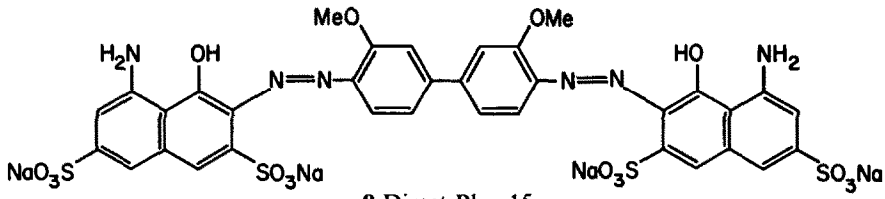
5 Direct Black 38



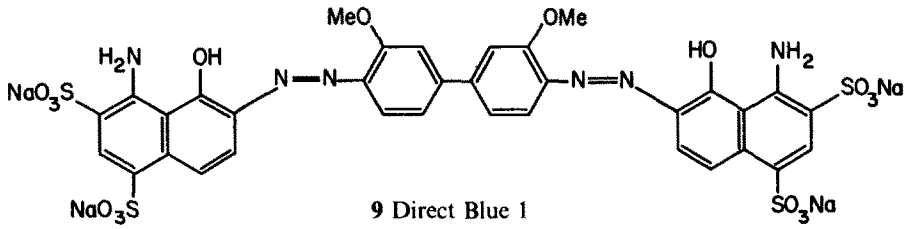
6 Direct Blue 6



7 Direct Blue 14 (Trypan Blue)

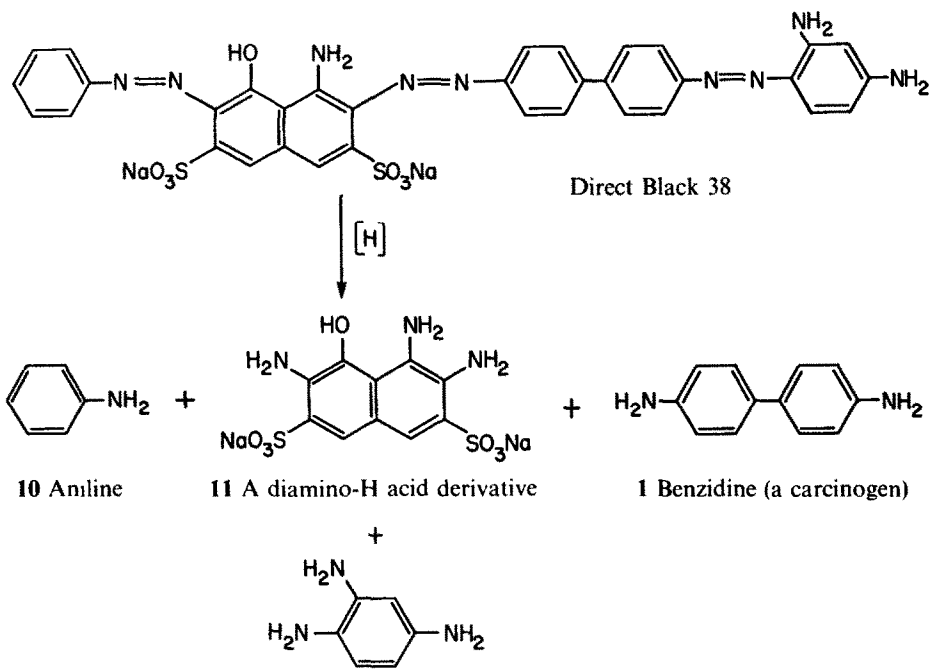


8 Direct Blue 15



9 Direct Blue 1

Although much of the human exposure to benzidines which led to bladder cancer was the result of handling the intermediate itself during the course of tetrazotization, an appreciable level of exposure to these intermediates resulted from the use of crude dyestuffs which contained a free benzidine as an unreacted starting material. Also, it is now well established that the ability of azo dyes to undergo reductive cleavage of the azo linkage (see Scheme 1)



Direct Black 38

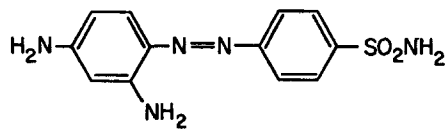
10 Aniline + **11** A diamino-H acid derivative + **1** Benzidine (a carcinogen)

+

12 1,2,4-Triaminobenzene (a carcinogen)

Scheme 1. The metabolism of an azo dye via a reductive-cleavage reaction.

could lead to an indirect route of exposure to an established carcinogen. Indeed, researchers have used gut microflora,[6-8] liver enzymes,[9] certain bacteria,[10] and tissues containing the enzyme azo reductase[11] to demonstrate that such a process characterizes the metabolism of azo dyes. Ironically, it was the ability of the azo dye Protosil (**13**) to undergo *in vivo* reductive cleavage[12] to produce the antibacterial sulfanilamide (**14**) which led to the prodrug approach to chemotherapy.



13 Prontosil

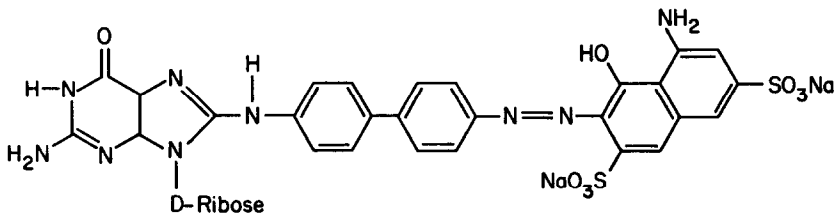
In other *in vivo* work,[13] certain benzidine-based dyes were found to undergo metabolism in Rhesus monkeys to produce free benzidine. Similarly, benzidine and dianisidine have been detected[14] in the urine of some workers who were exposed to dyes derived from those intermediates. Related studies led to a report[15] which concludes that all benzidine-based dyes should be recognized as potential human carcinogens.

The literature contains three papers which describe work involving short-term testing of Direct Black 38 (**5**):(1) to determine the mutagenicity of some proposed metabolites[16] of this dye; and (2) to establish a correlation between Ames testing and carcinogenicity testing.[17,18] Closely related work has been published which involves an attempt to correlate the rate of *N*-hydroxylation of aminoazo dyes with their carcinogenic activity in the rat.[19] A number of recent papers have been published[20-26] which describe the development of *in vitro* assays to improve the correlation between test results from the mutagenicity testing and the carcinogenicity testing of benzidine-based dyes.

It should be pointed out that at least one group of researchers have demonstrated[27] that the reductive cleavage of a benzidine-based azo dye to benzidine itself is not necessary for covalent binding of the dye to rat liver DNA. The DNA adducts of Direct Blue 6 (**6**) and Congo Red (**4**) were among the metabolites characterized during the course of that work. It was suggested that the existence of the adduct **15** might explain the observed potent carcinogenicity of the benzidine dye **6**.

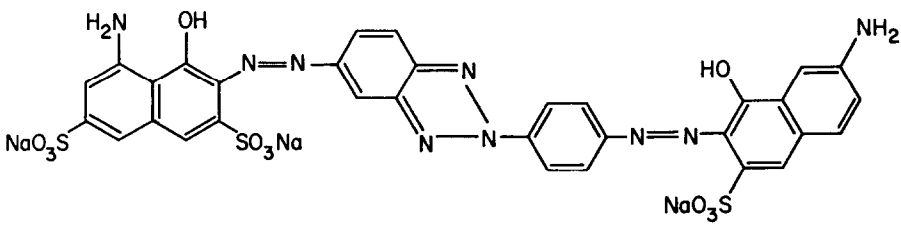
Similarly, genotoxic metabolites have been isolated[28] from the urine of animals which had been fed azo dyes like **5** and **7** to determine the effect of *in vivo* metabolism on the mutagenicity of such dyes.

The literature contains little information concerning attempts to find suitable replacements for benzidine and the conversion of such compounds

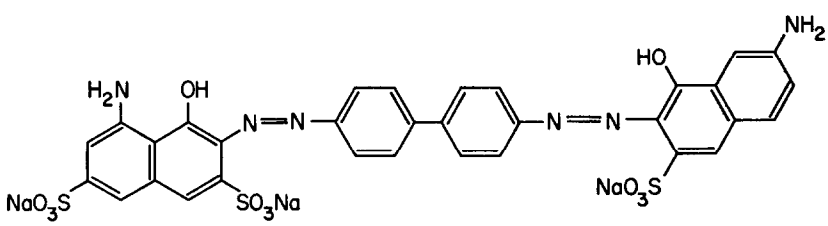


15

to dyes. We were able to identify one paper[29] which described some previous research that has been conducted on this subject. Dye **16** has been synthesized as a possible replacement for the genotoxic dye **17**. Although **16** appears to have similar dyeing properties, its hue and brightness are slightly different.



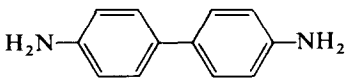
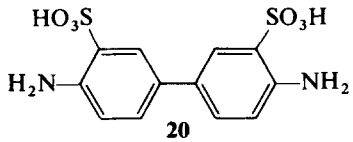
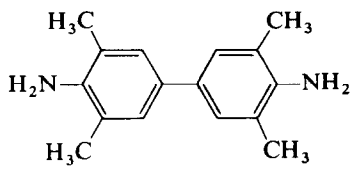
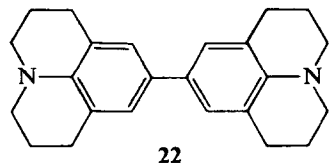
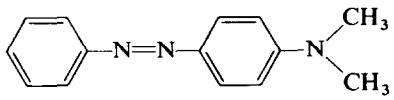
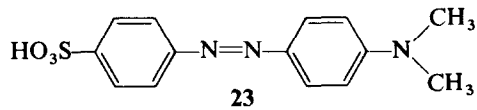
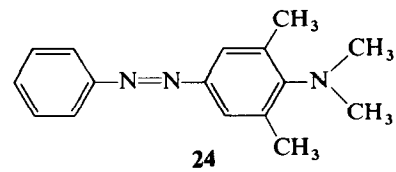
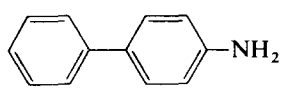
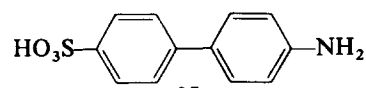
16 Direct Blue 2 analog



17 Direct Blue 2

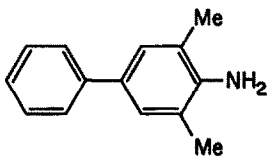
In a series of papers by Ashby and coworkers, research leading to the removal of the carcinogenicity of some azo dyes and dyestuff intermediates by altering their molecular structure is described. For example, it was suggested [30-32] that the sulfonation of Butter Yellow (**18**), benzidine (**1**), and 4-aminobiphenyl (**19**) produces either a non-carcinogen or a weak carcinogen (**20**, **23**, **25** in Table 1). The sulfonated intermediate, however, would be expected to produce dyes of inferior wetfastness. The conversion of **1**, **18**, and **19** to certain sterically hindered amines (**21** and **22**, **24**) lowers significantly or removes mutagenicity.[33] On the other hand, the hindered amines **26** and **27** are quite genotoxic.

TABLE 1
The Conversion of Some Toxic Compounds to Non-Toxic Analogs

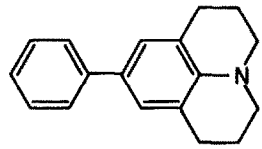
Mutagen/carcinogen	*Non-mutagen/non-carcinogen*
 **1**	 **20**
	 **21**
	 **22**
 **18**	 **23**
	 **24**
 **19**	 **25**

The work of Prival and coworkers[23] indicates that the formation of copper complexes of certain *o*-dianisidine-based dyes reduces or removes their genotoxicity. For example, while Direct Blue 15 (**8**) is a mutagen, its bis-copper complex Direct Blue 218 (**28**) is not. Such complexes, apparently, prevent or inhibit the reductive cleavage of the azo linkage.

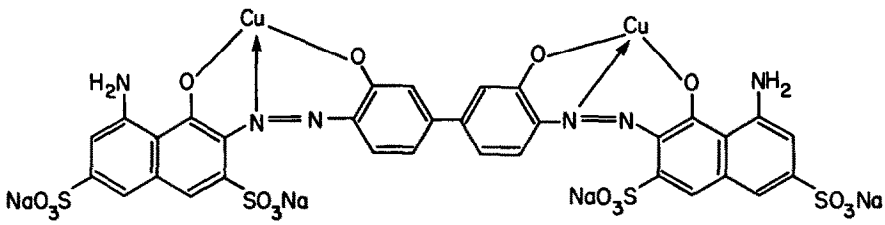
The present paper is concerned with the synthesis and evaluation of 5,5′-diamino-2,2′-bipyridine (**29**) and the closely related phenylpyridine **30** as possible replacements for benzidine in disazo dyes. Our long-term interest in



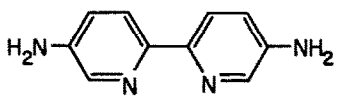
26



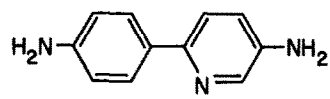
27



28 Direct Blue 218

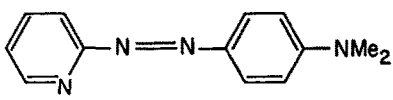


29

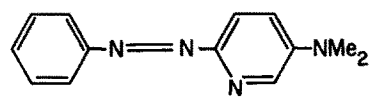


30

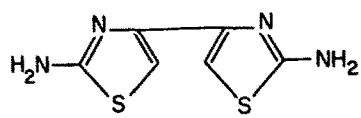
these two particular compounds was further stimulated in part by reports which indicate that the carcinogenicity of Butter Yellow has been removed by preparing the pyridine analogs **31** and **32**,[34,35] and that the three isomers of aminopyridine are less genotoxic than aniline.[36] Compounds **31** and **32** produced no liver tumors in rats over a 12-month period. It is also known[37] that replacing the two phenyl rings of benzidine with thiazyl rings produces the non-genotoxic diamine **33**.



31



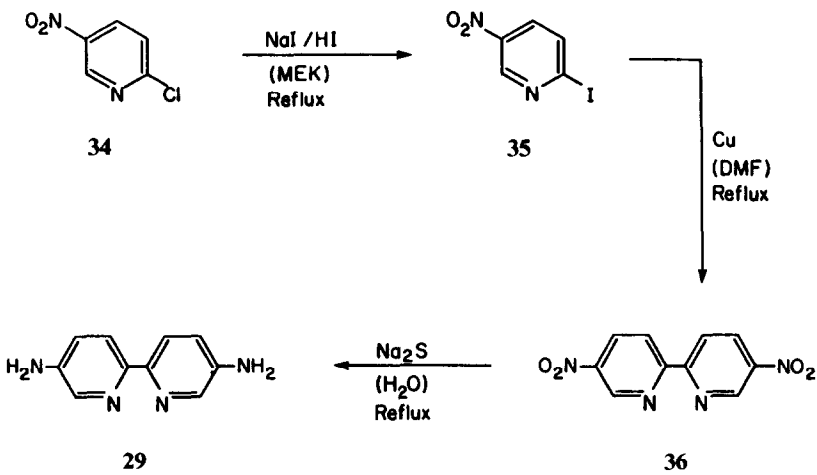
32



33

2 RESULTS AND DISCUSSION

The target diamines were synthesized according to the routes outlined in Schemes 2 and 3. The synthesis of **29** was accomplished in three steps from 2-chloro-5-nitropyridine (**34**). Those steps involved a halogen exchange

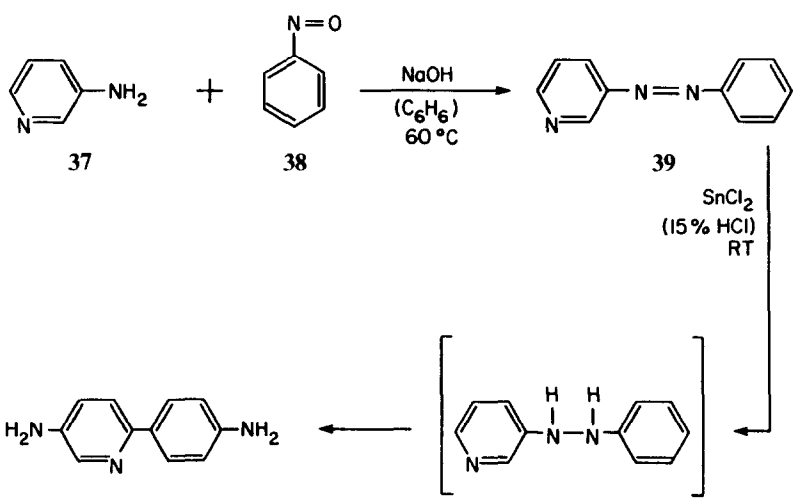


34 35 29 36

Scheme 2. The synthetic route to the dye intermediate **29**.

reaction and an Ullmann reaction[38] to give **36**, and a sodium sulfide reduction of the nitro groups of the intermediate **36**. The preparation of **30** required two steps: condensation of 3-aminopyridine (**37**) with nitrosobenzene (**38**) followed by reaction of the phenylazopyridine **39** with stannous chloride ($SnCl_2$). The generation of **30** no doubt proceeds through the intermediate **40**, via a benzidine-type rearrangement.

When the two nitrogen analogs of benzidine were evaluated in the standard Ames test,[40] **29** was non-mutagenic (2.2 Rev. μmol^{-1}) and **30** was

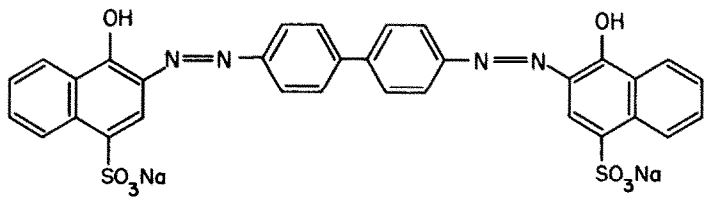


37 38 39 30 40

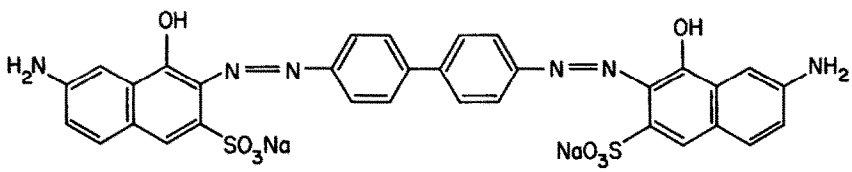
Scheme 3. The synthetic route to the phenylpyridine **30**.

weakly active. An examination of the two compounds in the Prival modification[22] revealed that both were mutagenic (2000 Rev. μmol^{-1} for **29**, and 7352 Rev. μmol^{-1} for **30**). On the basis of these results, our work with compound **30** was terminated. We did, however, prepare a few dyes from the diamine **29**, and then evaluated them for mutagenicity.

The benzidine moiety of the dyes Congo Red (**4**), Direct Violet 43 (**41**), Direct Black 29 (**42**), and Direct Black 38 (**5**) was replaced with the



41 Direct Violet 43

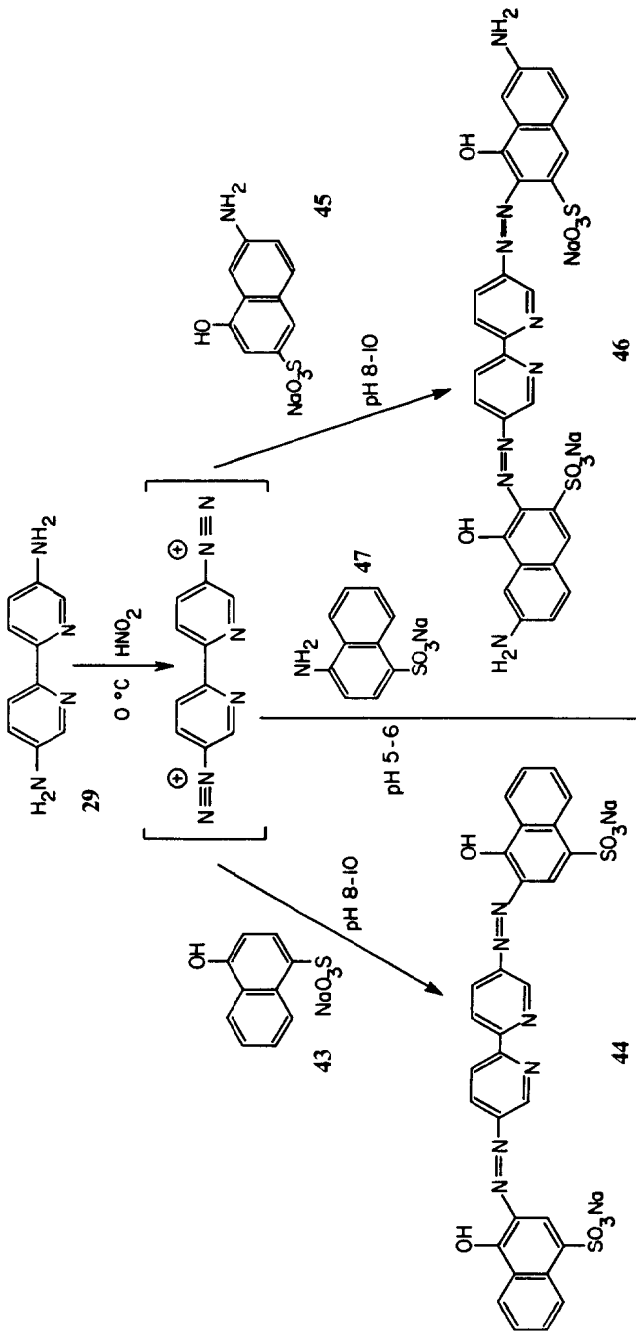


42 Direct Black 29

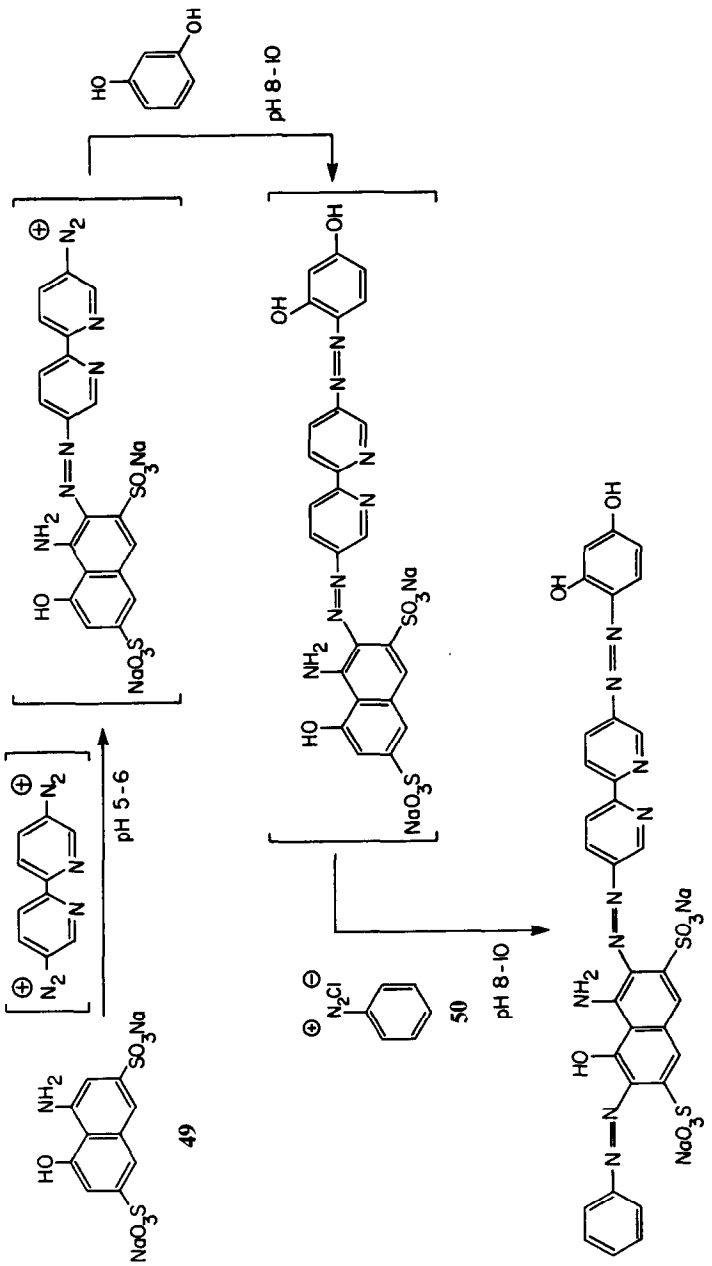
diaminobipyridine analog according to the methods outlined in Schemes 4 and 5. Nevile–Winther acid (**43**), gamma acid (**45**), and naphthionic acid (**47**) were coupled twice to the tetrazonium salt of **29** to produce the disazo dyes **44**, **46**, and **48**. Compounds **44** and **48** were purified by chromatography, and dye **46** was purified by dialysis.

The trisazo dye **51** was prepared in three steps from H-acid (**49**; Scheme 5), but its purification proved difficult. A sample of only 90% purity was obtained after column chromatography and countercurrent chromatography. This sample was employed in the biological analyses without further purification. The impurities present were subsidiary dyes; none of the reactants (e.g. **49**, **50**) could be detected by HPLC analysis.

Each of the four dyes was non-mutagenic by the standard Ames test. Table 2 shows the results of the mutagenicity testing in the standard Ames assay and the Prival modification. Although each dye exhibits some activity in the second test, each also shows less genotoxicity than the corresponding benzidine-based dye. For example, we found that Congo Red produces approximately 2300 Rev μmol^{-1}, which makes it twice as active as the nitrogen isolog **48**.



Scheme 4. The synthetic route to the diazo Dyes **44**, **46**, and **48**.



Scheme 5. The synthetic route to the Direct Black 38 analog **51**.

TABLE 2
Mutagenicity Data of Some Azo Dyes and Dyestuff Intermediates

Test compound	*Mutagenicity (revertants μmol^{-1})*	
	Standard assay + S9	*Prival modifications*
1	216	23 789
4	Negative	2 300
29	2·2	2 000
30	175	7 352
44	Negative	587
46	Negative	—
48	Negative	1 093
51	Negative	444

The data in Table 2 suggest that the intact dyes are safe compounds, but that they produce weakly genotoxic metabolites as a result of the reductive cleavage of the azo linkages. The data provide further support for the idea that azo dyes are most appropriately examined in an *in vitro* (short-term) test which permits the evaluation of the reductive-cleavage products. The actual mutagens which result from the metabolic breakdown of the dyes in this investigation have not been identified. An examination of these dyes *in vivo* would be required before it is possible to conclude whether it is safe to use the diaminopyridine as a dye intermediate. The data in this investigation, however, suggest that it probably is safe to employ in azo dye synthesis.

3 EXPERIMENTAL

3.1 General

The chemicals employed in this study were obtained from Aldrich Chemical Co., Mobay Chemical Co., or from Fisher Scientific Co. The TLC data were taken from silica gel plates (Whatman MK 6F) obtained from Bodman Chemical Co. NMR spectra were recorded on a Bruker 250 MHz instrument, and HPLC analyses were conducted with the aid of a Waters HPLC instrument. Melting points were recorded on a Mel-Temp melting point apparatus and are uncorrected. The silica gel used in the dye purifications was silica gel for dry column chromatography from Bodman Chemical Co., or silica gel 60 from Fisher Scientific. The dialyses were conducted with the aid of Spectra/Par 7 membrane material, Fisher Scientific (Cat. No. 08-680-2C).

3.2 Synthesis

2-Iodo-5-nitropyridine (**35**)
A mixture of NaI (60 g, 0·4 mol), 57% HI (19 ml), and methyl ethyl ketone (MEK, 400 ml) was stirred at 50°C until the NaI dissolved. To this solution was added a solution of 2-chloro-5-nitropyridine (25 g, 0·157 mol) in 200 ml MEK. The reaction was stirred under a reflux for 16 h, cooled to room temperature, and filtered. The filter cake was washed with three 50 ml portions of MEK, and the combined filtrates were concentrated. The purple solid obtained was suspended in 750 ml H_2O and stirred as the pH of the solution was made alkaline using 10% NaOH. Excess iodine was destroyed by adding 25 g $NaHSO_3$. The solid was collected by filtration and washed with H_2O. Recrystallization of the crude product from acetone/H_2O afforded 31 g of pure **35**, m.p. 164–165°C (lit.[37,42,43] 164–166°C). NMR (DMSO-d_6): δ8·15, doublet (1H); δ8·23, doublet of doublets (1H); δ9·12, doublet (1H).

5,5'-Dinitro-2,2'-bipyridine (**36**)
A mixture of 2-iodo-5-nitropyridine (15 g, 0·06 mol), 450 ml of DMF, and Cu powder (50 g; Aldrich Chemical Company, Cat. no. 20 778-0) was stirred under a reflux in an N_2 environment for 7 h. The hot mixture was filtered to remove the copper, and the filtrate was poured into 1 litre of H_2O. The brownish-gray solid which formed was collected by vacuum filtration and dried at room temperature. The resulting solid was purified in two steps; first, it was dissolved in benzene via a seven-day soxhlet extraction, and once the benzene was removed the solid was recrystallized from acetone/H_2O (50:50), to give 4·0 g (33%) of pure **36** (yellowish-gold prisms), m.p. 245–247°C (lit.[41,42] 244–250°C). NMR (DMSO-d_6): δ8·70, doublet (2H); δ8·81, doublet of doublets (2H); δ9·54, doublet (2H).

5,5'-Diamino-2,2'-bipyridine (**29**)
A mixture of compound **35** (1 g, 0·004 mol) and Na_2S (10 g, 0·05 mol) in 100 ml of hot water was stirred under reflux for 3 h. The reaction was filtered hot and the filtrate was allowed to stand for two days in the refrigerator. The solid which precipitated was recrystallized from H_2O to give 0·45 g of colorless needles, m.p. 208°C (lit.[41] 208–210°C). NMR (DMSO-d_6): δ5·39, singlet (4H); δ7·01, doublet of doublets (2H); δ7·92, doublet (2H); δ7·97, doublet (2H).

3-Phenylazopyridine (**39**)
A mixture of 3-aminopyridine (9·6 g, 0·01 mol), 50% NaOH (400 ml), and benzene (50 ml) was stirred at 60°C as nitrosobenzene (10·7 g, 0·1 mol) was

added over a 30-min period. The reaction was maintained near 60°C for 16 h. The reaction mixture was cooled and the organic (upper) layer was collected, charcoaled, and evaporated to 50 ml. The components of the resulting oil were separated by dry column chromatography[44,45] using PhMe as the eluent. The orange-red band of the column afforded an orange solid which crystallized from hexane as orange prisms. Pure **39** (8·0 g, 40%) was obtained, m.p. 49°C (lit.[46] 48–52°C).

5-Amino-2-(4-aminophenyl)pyridine (**30**)
A solution of 3-phenylazopyridine (**39**; 10 g, 0·058 mol) and $SnCl_2$ (40 g, 0·17 mol) in 15% HCl (300 ml) was stirred overnight (16 h) under reflux. The cooled yellow solution was made alkaline with 10% NaOH, and 10% Na_2S solution (20 ml) was added to break up the tin complex which formed. The resulting solution was refrigerated for two days, during which time a brown precipitate formed. The crude solid was eluted through a silica gel column using Et_2O/acetone/hexane (1:1:1), and the resulting yellow solid was recrystallized from H_2O to give a 20% yield of pure **30** as colorless needles, m.p. 186–188°C (lit.[47] 186°C).

Dye **44**
A solution of **29** (1·86 g, 0·01 mol) in 10% HCl (50 ml) was cooled to 0°C and stirred as 2M-$NaNO_2$ solution (10 ml) was added over a 10-min period. The resulting solution was stirred at 0°C for 1 h, during which time a color change from red to pale yellow occurred. Compound **43** (7·38 g, 0·03 mol) was dissolved in 0·5M-Na_2CO_3 (100 ml); the solution was cooled to 5°C and added to the tetrazonium salt at a rate such that the reaction temperature did not exceed 5°C. The pH of the reaction was maintained at 8–9 by the periodic addition of 0·5M-Na_2CO_3. The solution was kept cold for 4 h and then allowed to warm to room temperature over the next 4 h. The dye was precipitated by adjusting the pH of the solution to 2, with the aid of 10% HCl. The crude dye was collected by vacuum filtration and purified by chromatography on silica gel using 1-propanol/acetone/NH_4OH/H_2O (4:4:2:1) as the eluent. The pure bluish-purple dye had $R_f = 0·3$ and was obtained in 40% yield. ^{1}H-NMR (DMSO-d_6): δ7·49, triplet (2H); δ7·60, triplet (2H); δ7·80, singlet (2H); δ8·28, doublet (2H); δ8·60, multiplet (6H); δ9·26, singlet (2H); δ9·71, singlet (2H).

Dye **46**
Compound **29** (1·0 g, 0·0054 mol) was tetrazotized as described above and then stirred into a solution of **45** (2·57 g, 0·011 mol) in 100 ml of H_2O which had been brought to pH 10 with 10% NaOH. The reaction was stirred at 0°C

for 24 h. The dye was precipitated by lowering the pH to 2, and was collected by filtration. The still-moist solid was suspended in 250 ml of H_2O and the pH adjusted to 7 with 10% NaOH. The resulting solution was evaporated to dryness to give 3·3 g of crude dye. This solid was subjected to dialysis for four weeks leaving 1·3 g (33%) of pure **46**, $R_f = 0{\cdot}44$ on silica gel with 1-butanol/MeOH/NH_4OH/pyridine (4:1:3:2). NMR (DMSO-d_6: δ7·16, doublet (2H); δ7·26, singlet (2H); δ7·55, singlet (2H); δ7·85, doublet (2H); δ8·07, doublet (2H); δ8·61, doublet (2H); δ9·12, singlet (2H); δ12·56, singlet (2H).

Dye **48**

Compound **29** (1·86 g, 0·01 mol) was tetrazotized by the procedure described above for preparation of **44**. The tetrazonium salt solution was then added to a solution of naphthionic acid (1·4 g, 0·03 mol) in 100 ml of 0·5M-$NaHCO_3$. The reaction was kept cold (0–5°C) for 4 h, and then stirred at room temperature for 24 h. The dye was precipitated by adding NaCl, collected by filtration, and dried over P_2O_5. Chromatography on silica gel with 1-propanol/acetone/NH_4OH/H_2O (4:4:2:1) as the eluent afforded pure **48**, $R_f = 0{\cdot}3$. NMR (DMSO-d_6): δ7·54, triplet (2H); δ7·70, triplet (2H); δ8·32, singlet (2H); δ8·51, doublet (2H); δ8·64, multiplet (6H); δ9·19, singlet (2H).

Dye **51**

To a solution of **49** (1·96 g, 0·0054 mol) in 10 ml H_2O was added a solution of 0·0054 mol of the tetrazonium salt of **29**, at 0–5°C. After 20 h at pH 2, the pH was raised to 4 using 2M-Na_2CO_3 solution, and a solution of resorcinol (0·6 g, 0·0054 mol) was then added. The pH was raised to 8–9 by the addition of 2M-Na_2CO_3 solution, and the reaction was stirred for 12 h. After a solution of benzenediazonium chloride (0·8 g, 0·0054 mol) in 5 ml of H_2O was added, the reaction was stirred overnight (16 h) at 0–5°C and pH 8–9. The pH was adjusted to 2 using 2M-HCl and the precipitated dye was isolated by centrifugation. The resulting solid was suspended in 200 ml of H_2O, the pH increased to 7 with 10% NaOH, and the resulting greenish-black solution was evaporated to dryness to produce a mixture of dye and salt. The dye mixture was eluted through a silica gel column with 1-butanol/acetone/H_2O (5:5:3) to produce a crop of dye which was rich in the desired component, and free of salt. The sample was further purified with the aid of countercurrent chromatography,[48–50] using 1-butanol/H_2O/MeOH (1:1:0·1) as the solvent system. The resulting dye had a major component with $R_f = 0{\cdot}51$ on silica gel TLC plates, using 1-butanol/MeOH/NH_4OH/pyridine (4:1:3:2) as the eluent. The yield of this dye (which also showed weakly intense yellow, blue, and purple colors) was less than 10%. HPLC suggested a purity of 90%.

4 CONCLUSIONS

It has been shown through this investigation that 5,5′-diamino-2,2′-bipyridine (**29**) is a possible replacement for the benzidine moiety of certain known carcinogenic azo dyes. This diamine is considerably less genotoxic than benzidine, undergoes tetrazotization readily, and generates essentially the same hues exhibited by the benzidine dyes, when converted to disazo dyes such as **44**, **46**, and **48**. It remains to be established whether the resulting dyes will possess satisfactory dyeing properties; however, it is clear that the dyes themselves (as well as their reductive-cleavage products) are also appreciably less genotoxic than the corresponding benzidine-based dyes.

Later work with **29** *in vivo* for carcinogenesis will determine its actual future as a benzidine replacement for the preparation of azo dyes.

ACKNOWLEDGEMENTS

This research was part of a study which was supported by a grant from International Business Machines Corporation.

REFERENCES

1. *IARC monographs on the evaluation of the carcinogenic risk of chemicals to man*, Vol. 8, Lyon, IARC, (1975).
2. R. A. M. Case, *Brit. J. Ind. Med.*, **11**, 75 (1954).
3. M. Barsotti and E. C. Vigliani, *AMA Arch. Ind. Hyg. Occup. Med.*, **5**, 234 (1952).
4. T. S. Scott, *Brit. J. Ind. Med.*, **9**, 27 (1952).
5. Environmental Protection Agency *Fed. Regist.*, **46**, 55004, 55006, (5 Nov., 1981).
6. K. T. Chung, G. E. Fulk and M. Egan, *Appl. Environ. Microbiol.*, **35**, 558 (1978).
7. K. T. Chung, *Mutat. Res.*, **114**, 269 (1983).
8. C. P. Hartmann, G. E. Fulk and A. W. Andrews, *Mutat. Res.*, **58**, 125 (1978).
9. Ph. Manchon and R. Lowry, *Food Cosmet. Toxicol.*, **3**, 783 (1965).
10. E. Idaka, T. Ogawa, H. Horitsu and M. Tomoyeda, *J. Soc. Dyers Colour.*, **94**, 91 (1978).
11. M. R. Juchau, J. Krasner and S. J. Yaffe, *Biochem. Pharmacol.*, **17**, 1969 (1968).
12. J. H. Weisburger and E. K. Weisburger, *Food Cosmet. Toxicol.*, **6**, 235 (1968).
13. E. Rinde and W. Troll, *J. Nat. Cancer Inst.*, **55**, 181 (1975).
14. V. A. Genin, *Vopr. Onkol.*, **23**(9), 50 (1977).
15. DHEW (NIOSH) Publication No. 80-109 (January 1980).
16. E. J. Lazear, J. G. Shaddock, P. R. Barren and S. C. Louie, *Toxicol. Lett.*, **4**, 519 (1979).
17. A. R. Gregory, J. Elliot and P. Kluge, *J. Appl. Toxicol.*, **1**(6), 308 (1981).
18. J. C. Kennelly, P. J. Hertzog and C. N. Martin, *Carcinogenesis*, **3**(8), 947 (1982).
19. T. Kimura, M. Kodama and C. Nagata, *Carcinogenesis*, **3**(12), 1393 (1982).

20. C. E. Cerniglia, J. P. Freeman, W. Franklin and L. D. Pack, *Carcinogenesis*, **3**(11), 1255 (1982).
21. J. A. Robertson, W. J. Harris and D. B. McGregor, *Carcinogenesis*, **3**(1), 21 (1982).
22. M. J. Prival and V. D. Mitchel, *Mutat. Res.*, **97**, 103 (1982).
23. M. J. Prival, S. J. Bell, V. D. Mitchell, M. D. Peiperl and V. L. Vaughan, *Mutat. Res.*, **136**, 33 (1984).
24. B. W. Manning, C. E. Cerniglia and T. W. Federle, *Appl. Environ. Microbiol.*, **50**(1), 10 (1985).
25. T. M. Reid, K. C. Morton, C. Y. Wang and C. M. King, *Environ. Mutagen.*, **6**, 705 (1984).
26. J. P. Brown and P. S. Dietrich, *Mutat. Res.*, **116**, 305 (1983).
27. J. C. Kennelly, A. Shaw and C. N. Martin, *Toxicology*, **32**, 315 (1984).
28. F. Joachim, A. Burrell and J. Anderson, *Mutat, Res.*, **156**, 131 (1985).
29. R. Anliker, *Rev. Prog. Coloration*, **8**, 60 (1977).
30. F. J. DeSerres and J. Ashby (eds), *Evaluation of short-term tests for carcinogens*, Amsterdam, Elsevier North-Holland (1981).
31. J. Ashby, *Br. J. Cancer*, **37**, 904 (1978).
32. J. Ashby, J. A. Styles and D. Paton, *Br. J. Cancer*, **38**, 34 (1978).
33. J. Ashby, D. Paton, P. A. Leveure, J. A. Styles and F. L. Rose, *Carcinogenesis*, **3**(11), 1277 (1982).
34. E. Brown, A. F. Smetana and A. A. Hamdan, *J. Org. Chem.*, **8**, 252 (1965).
35. K. Wakabayshi, T. Yahagi, M. Nagao and T. Suginura, *Mutat. Res.*, **105**, 205 (1982).
36. C. Y. Wang, K. Muraoka and G. T. Bryan, *Cancer Res.*, **35**(12), 3611 (1975).
37. F. Setliff, *Organic. Prep. and Proceed. Int.*, 305 (1973).
38. P. Fanta, *Chem. Rev.*, **64**, 613 (1964).
39. T. Cohen and J. Wood, *Tetrahedron Lett.*, **50**, 3555 (1974).
40. B. N. Ames, J. McCann and E. Yamasaki, *Mutat. Res.*, **31**, 347 (1975).
41. K. Kurita and R. Williams, *J. Polym. Sci. Polym. Chem. Ed.*, **11**, 3125 (1973).
42. F. Case, *J. Amer. Chem. Soc.*, **68**, 2574 (1946).
43. J. Reinheimer and J. Gerig, *J. Amer. Chem. Soc.*, **84**, 2770 (1962).
44. B. Loev and M. M. Goodman, *Prog. Separ. Purif.*, **3**, 73 (1978).
45. H. S. Freeman, C. S. Williard and W. N. Hsu, *Dyes and Pigments*, **7**, 397 (1986).
46. N. Campbell, A. Henderson and D. Taylor, *J. Chem. Soc. (London)*, 1281 (1953).
47. Z. T. Allan and J. Podstata, *Collec. Czech. Chem. Commun.*, **36**, 3053 (1971).
48. H. M. Fales, L. K. Plannell, E. A. Sokoloski and P. Carmeci, *Anal. Chem.*, **57**(1), 376 (1985).
49. J. L. Sandline and Y. Ito, *J. Liq. Chromatog.*, **8**(12), 2153 (1985).
50. H. S. Freeman and C. S. Williard, *Dyes and Pigments*, **7**, 407 (1986).