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Heterocyclic azodyes of the pyridylazonaphthol and pyridylazoresorcinol series containing tuned hydrophobic chains have been prepared. Due to their chelating properties, the dyes are suitable candidates for preconcentration and selective separation of transition metal ions. After a discussion on the choice of the best synthetic pathway, physical and spectral uv/visible properties are presented and correlated with the structural features. A detailed analysis of nmr spectra allowed the clarification of currently unresolved signal assignments.

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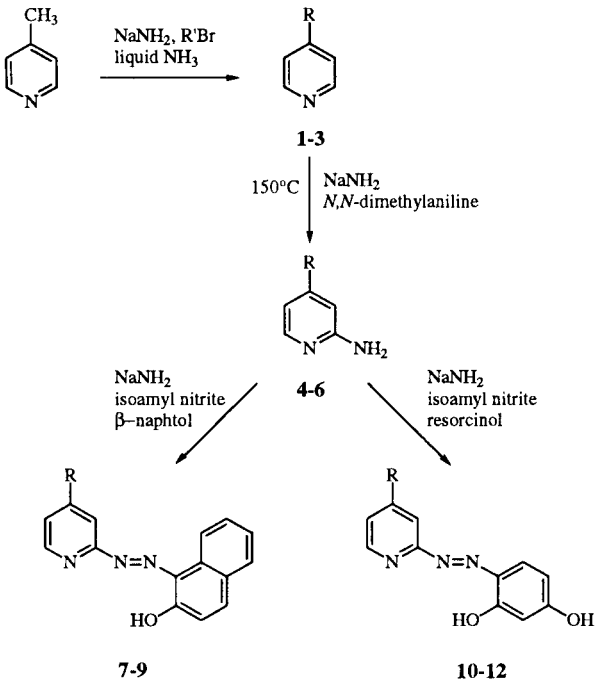
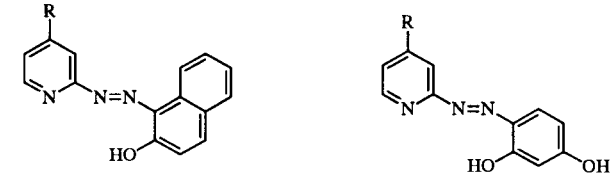
Micellar-mediated techniques afford novel chemical separation procedures with undoubted advantages, in terms of low cost and low toxicity of the aqueous surfactants (compared to organic solvents) and low alteration and/or denaturation of biomolecules [2]. In particular, micellar extraction and micellar-enhanced ultrafiltration are simple tecniques that allow efficient separation of dissolved organic and inorganic micro components from aqueous solutions without the use of organic solvents or solid absorbents [3,4]. In these techniques the combined application of surfactants and amphiphilic or hydrophobic ligands is required.

Scheme 1



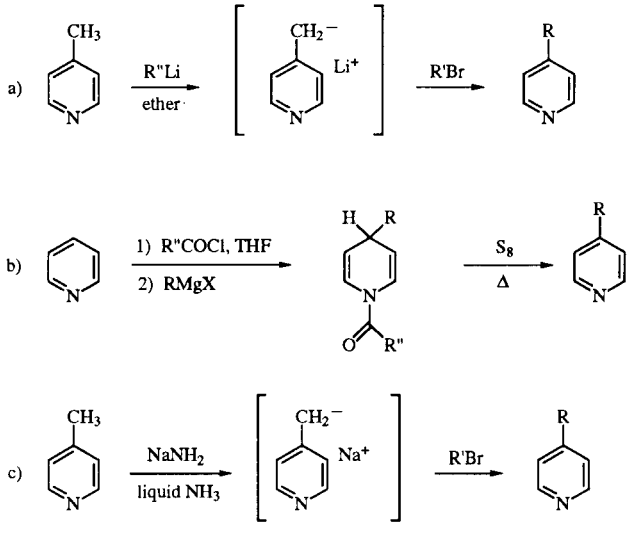

Simple heterocyclic azo dyes, such as pyridylazonaphthol (**I**, R = H), and pyridylazoresorcinol (**II** R = H), are well known chelating agents, widely used for analytical purposes [5].




**I** **II**

For enhancing their solubility in micellar media, we have inserted a tuned hydrophobic chain (R = $C_4H_9$, $C_8H_{17}$, $C_{16}H_{33}$) on the pyridine ring, thus obtaining viable candidates for the preconcentration and selective separation of

Scheme 2



transition metal ions through micellar extraction and micellar-enhanced ultrafiltration techniques. A preliminary study on the binding constants, $K_B$, of the above series with Triton X-100 micelles indicates that, in neutral or moderately acid solution, the hydrophobic ligands are strongly bound, thus ensuring a ligand-free permeate. For example, compound **7** (formula **I** R = $C_4H_9$) shows a $K_B$ value about five times greater than the parent dye (formula **I** R = H) [6].

The present paper refers to the synthesis of these novel dyes and to a discussion of their spectral data.

The reaction pathway, reported in Scheme 1, starts from 4-methylpyridine whose chain is lengthened to give 4-alkylpyridines **1-3**. With the Chichibabin reaction [7] the corresponding 2-amino-4-alkylpyridines **4-6** are obtained. By diazotization and coupling, chelating dyes **7-9** and **10-12** are synthesized. All the above reactions are simple and well known, though some attention is required in the work-up of hydrophobic products containing alkyl chains. The first step, *viz.* the alkylation, could have been formally achieved in three different ways, indicated by Scheme 2.

Route (a), the alkylation of 4-methylpyridine with lithioalkyl reagents, *e.g.* butyllithium [8] or lithium diisopropylamide [9], was discarded because of the hazards involved in the use of these expensive reagents. The alkylation of pyridine with Grignard reagents *via N*-acyl derivatives (route b) [10] was also discarded because of (i) the need for an additional step, (ii) the formation of the unwanted 2-isomer, and (iii) the unpleasant smell consequent to the use of both pivaloylchloride and sulfur during the oxidative step. Furthermore, both methods (a) and (b) require the use of anhydrous and expensive solvents. We preferred route (c), involving the preparation of the intermediate carbanion of 4-methylpyridine by sodium amide in liquid ammonia [11]. With a simple laboratory plant the reaction has been carried out in one step on a Kg scale.

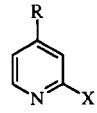
Tables 1 and 2 report physical and spectroscopic data on compounds **1-12**.

Bases **1** and **2** are liquids whereas compound **3** is a solid; the relative values of their boiling and melting points follow the variation of their molecular weights. The amines **4-6** melt at higher temperature, possibly because of intermolecular hydrogen bonds. The melting points of the dyes **7-12** are the highest in the whole series, presumably because of the occurrence of both high molecular weights and the formation of intermolecular hydrogen bonds. The chromatographic data are only comparable within subsets of sets of three compounds, having been obtained with different eluents. In each, as the hydrophobicity increases the retention by the polar substrate decreases, thus resulting in higher $R_f$ values.

The electronic spectra of the dyes and intermediates are not affected by the length of the alkyl chain in the 4-position, as has been observed previously [12]. The bases **1-3** show a main absorption at 254 nm with two shoulders, one on each side (251 and 263 nm) [13]. The presence of a 2-amino substituent causes an ipso-hyperchromic shift of the main transition and the appearance of a band at 292 nm [14]. Dyes **7-12** show a broad, intense band in the visible region, typical of the azo chromogen: in the pyridylazonaphthol series, **7-9** the absorption maximum is at 466 nm [15], whereas in the pyridylazoresorcinol series, **10-12** a marked ipso-iperchromic shift is observed [16].

Table 1

Analytical and Spectroscopic Data of Compounds **1-6**



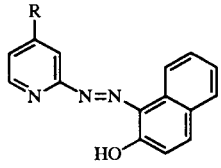

| Compound number | Structure R | X | M.p. or B.p. °C | R.f. | Empirical formula | Elemental Analysis Calcd./Found C | H | N | Yield % | Electronic Absorption Spectra λmax in nm (log ε) [a] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | $C_4H_9$ | H | 92 $_{15\ mm\ Hg}$ [b] | 0.65 | $C_9H_{13}N$ | 79.95 | 9.69 | 10.36 | 69 | s 263 (3.25) | 254 (3.40) | s 251 (3.33) |
| | | | | | | 80.06 | 9.64 | 10.33 | | | | |
| **2** | $C_8H_{17}$ | H | 175 $_{15\ mm\ Hg}$ [c] | 0.74 | $C_{13}H_{21}N$ | 81.62 | 11.06 | 7.32 | 71 | s 263 (3.23) | 254 (3.38) | s 251 (3.35) |
| | | | | | | 81.64 | 10.98 | 7.38 | | | | |
| **3** | $C_{16}H_{33}$ | H | 27-28 | 0.77 | $C_{21}H_{37}N$ | 83.10 | 12.29 | 4.61 | 62 | s 263(3.23) | 254 (3.40) | s 251 (3.34) |
| | | | | | | 83.20 | 12.26 | 4.63 | | | | |
| **4** | $C_4H_9$ | $NH_2$ | 44-45 | 0.72 | $C_9H_{14}N_2$ | 71.96 | 9.39 | 18.65 | 60 | 292 (3.61) | 235 (4.02) | |
| | | | | | | 72.00 | 9.45 | 18.58 | | | | |
| **5** | $C_8H_{17}$ | $NH_2$ | 65-66 [d] | 0.77 | $C_{13}H_{22}N_2$ | 75.68 | 10.75 | 13.58 | 56 | 292 (3.60) | 235 (3.99) | |
| | | | | | | 75.64 | 10.67 | 13.59 | | | | |
| **6** | $C_{16}H_{33}$ | $NH_2$ | 85-87 | 0.84 | $C_{21}H_{38}N_2$ | 79.18 | 12.02 | 8.79 | 61 | 292 (3.60) | 235 (4.00) | |
| | | | | | | 79.17 | 11.97 | 8.85 | | | | |

[a] s = shoulder. [b] 98 °C at 20 mm Hg [8]. [c] 91 °C at 0.1 mm Hg [8]. [d] 64°C [22].

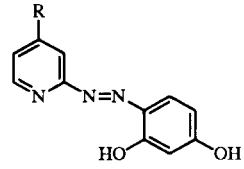
Table 2

Analytical and Spectroscopic Data of Compounds **7-12**
General Structure

Compounds **7-9**



Compounds **10-12**



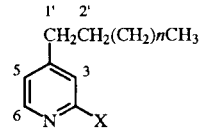
| Compound number | R | M.p. °C | R.f. | Empirical formula | Elemental Analysis Calcd./Found | | | Yield % | Electronic Absorption Spectra λmax in nm (log ε) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | C | H | N | | | | | |
| **7** | $C_4H_9$ | 90-91 | 0.88 | $C_{19}H_{19}N_3O$ | 74.73<br>74.85 | 6.27<br>6.31 | 13.76<br>13.84 | 49 | 297 (3.98) | 305 (3.99) | s 424 (4.11) | 466 (4.22) |
| **8** | $C_8H_{17}$ | 78-81 | 0.89 | $C_{23}H_{27}N_3O$ | 76.42<br>76.48 | 7.53<br>7.42 | 11.62<br>11.55 | 38 | 297 (3.98) | 305 (3.98) | s 424 (4.11) | 466 (4.21) |
| **9** | $C_{16}H_{33}$ | 83-85 | 0.92 | $C_{31}H_{43}N_3O$ | 78.60<br>78.44 | 9.15<br>9.09 | 8.87<br>8.86 | 43 | 297 (3.98) | 305 (3.99) | s 424 (4.11) | 466 (4.22) |
| **10** | $C_4H_9$ | 168 dec | 0.62 | $C_{15}H_{17}N_3O_2$ | 66.40<br>66.53 | 6.32<br>6.30 | 15.49<br>15.54 | 35 | s 256 (3.81) | | 387 (4.36) | |
| **11** | $C_8H_{17}$ | 165-168 | 0.64 | $C_{19}H_{15}N_3O_2$ | 69.70<br>69.51 | 7.70<br>7.68 | 12.83<br>12.73 | 39 | s 256 (3.81) | | 387 (4.36) | |
| **12** | $C_{16}H_{33}$ | 74-75 | 0.68 | $C_{27}H_{41}N_3O_2$ | 73.76<br>73.84 | 9.40<br>9.47 | 9.56<br>9.48 | 37 | s 256 (3.81) | | 387 (4.36) | |

The $^1H$ nmr spectra are fully consistent with the expected structures. (Tables 3 and 4).

However, some spectroscopic details are worthy of consideration. The spectrum of the reference pyridylazonaphthol (**I**, R = H), (recorded on a spectrometer operating at 100 MHz) has already been reported [17], but the peak assignments do not agree with our results. Furthermore, the two triplets at 7.50 and 7.62 ppm of compound **7** cannot be unambigously assigned simply on the basis of their chemical shift values. However, a complete and reliable spectral assignement can be obtained by higher magnetic field strength (400 MHz proton Larmor frequency), selective decoupling [18] or proton 2D NOESY techniques [19]. This latter experiment offers a means of determining spatial relationships between nuclei in a molecule by allowing the observation of nuclear Overhauser effect (NOE) cross peaks between neighboring nuclei. In figure 1 the aromatic region (6.40-8.85 ppm) of the 2D NOESY spectrum of compound **7** is reported. The singlet at 7.79 ppm can safely be attributed to pyridine $H_3$, because only the $H_3$ proton can give a signal without ortho coupling constant; it shows a cross peak with the doublet at 8.43 ppm, which can be assigned to naphtalene $H_{14}$. This latter doublet shows another cross peak with the triplet at 7.62 ppm, thus allowing its assignment to $H_{13}$. Support for these assignments was gained by a selective decoupling

Table 3

$^1H$ NMR Data of Compounds **1-6**



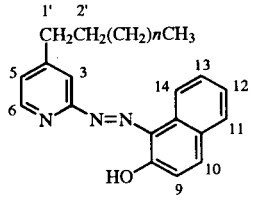
| Compound number | Structure *n* | X | Chemical shifts (δ, ppm) $H_2$ | $H_3$ | $H_5$ | $H_6$ | $^{1'}CH_2$ | $^{2'}CH_2$ | $(CH_2)_n$ | $CH_3$ | $NH_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 1 | H | 8.46 | 7.20 | 7.20 | 8.46 | 2.57 | 1.57 | 1.28 | 0.88 | —— |
| **2** | 5 | H | 8.45 | 7.21 | 7.21 | 8.45 | 2.59 | 1.59 | 1.27 | 0.87 | —— |
| **3** | 13 | H | 8.46 | 7.22 | 7.22 | 8.45 | 2.58 | 1.57 | 1.28 | 0.87 | —— |
| **4** | 1 | $NH_2$ | —— | 6.26 | 6.34 | 7.78 | 2.41 | 1.51 | 1.30 | 0.87 | 5.72 |
| **5** | 5 | $NH_2$ | —— | 6.25 | 6.33 | 7.77 | 2.40 | 1.52 | 1.28 | 0.86 | 5.72 |
| **6** | 13 | $NH_2$ | —— | 6.25 | 6.33 | 7.77 | 2.40 | 1.51 | 1.27 | 0.86 | 5.72 |

Table 4

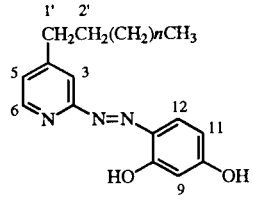
$^1$H NMR Data of Compounds **7-12**

General Structure

Compounds **7-9**




Compounds **10-12**




| Compound number | $n$ | $^1$H NMR spectra (δ, ppm) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $H_3$ | $H_5$ | $H_6$ | $H_9$ | $H_{10}$ | $H_{11}$ | $H_{12}$ | $H_{13}$ | $H_{14}$ | $1'CH_2$ | $2'CH_2$ | $^n(CH_2)_4$ | $CH_3$ |
| **7** | 1 | 7.79 | 7.14 | 8.32 | 6.69 | 7.94 | 7.71 | 7.50 | 7.62 | 8.43 | 2.75 | 1.66 | 1.38 | 0.95 |
| **8** | 5 | 7.81 | 7.13 | 8.31 | 6.69 | 7.93 | 7.71 | 7.49 | 7.60 | 8.45 | 2.77 | 1.65 | 1.39 | 0.96 |
| **9** | 13 | 7.80 | 7.14 | 8.31 | 6.70 | 7.93 | 7.71 | 7.50 | 7.60 | 8.43 | 2.76 | 1.66 | 1.38 | 0.95 |
| **10** | 1 | 7.25 | 6.81 | 8.07 | 5.17 | — | 6.00 | 6.68 | — | — | 2.58 | 1.58 | 1.22 | 0.83 |
| **11** | 5 | 7.24 | 6.81 | 8.08 | 5.18 | — | 5.99 | 6.68 | — | — | 2.58 | 1.59 | 1.23 | 0.83 |
| **12** | 13 | 7.25 | 6.81 | 8.07 | 5.18 | — | 6.00 | 6.69 | — | — | 2.59 | 1.57 | 1.22 | 0.84 |

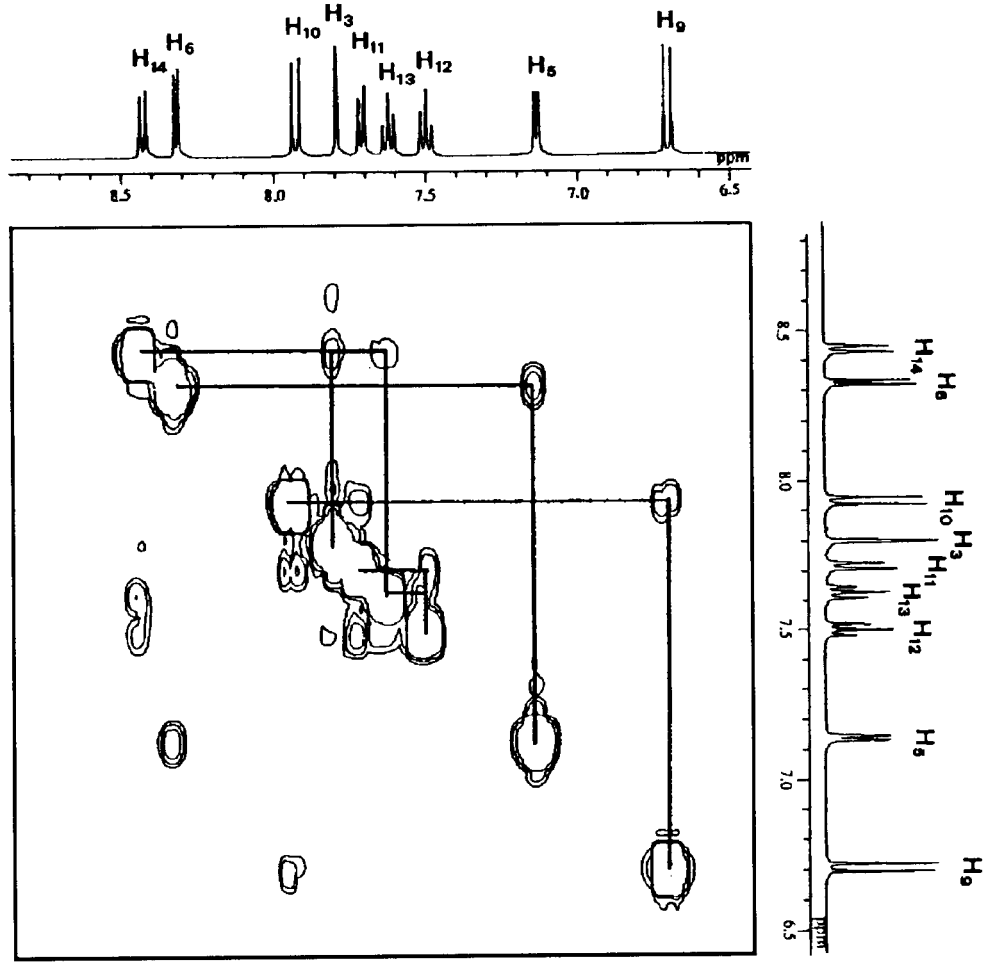



Figure 1. Aromatic region of the 2D-NOESY spectrum (400 MHz, 25°C) of compound **7**.

experiment (Figure 2).

When the peak at 8.43 ppm is irradiated, the triplet at 7.62 ppm simplifies to a doublet. The remaining triplet at 7.50 ppm has then to be assigned to $H_{12}$ and therefore the doublet at 7.71 ppm, showing a cross peak with $H_{12}$ in the NOESY spectrum, to $H_{11}$. The doublets at 7.14 and 8.32 ppm can be attributed to the pyridine ring on the basis of their chemical shifts and J values. Moreover, the presence of a small meta coupling ($^4J$ = 1.20 Hz) for the high field doublet allows its assignment to $H_5$. The remaining doublets at 7.92 and 6.69 ppm show a cross peak and are assignable to $H_{10}$ and $H_9$ respectively, on the basis of their chemical shifts.

In order to achieve a better spectral resolution and to allow a better comparison with literature data [20], the $^1H$ nmr spectra of compounds **10-12** have been recorded in a 70:30 mixture of DMSO/deuterium oxide (0.01 *M* sodium carbonate). The chemical shift values reported in Table 4 correspond to the monosodium salt **13**. It is worth noting that δ values for the benzene ring protons well agree with those reported for the unsubstituted dye at the 4-position of the pyridine (**II**, R = H) [20].

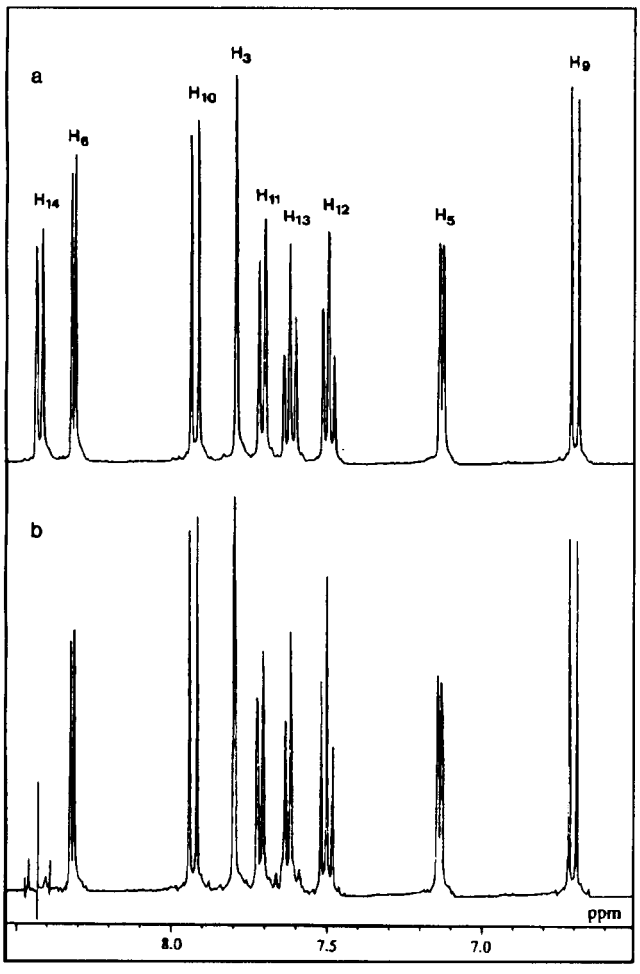
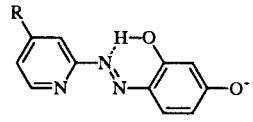



Figure 2. Aromatic region of the 400 MHz $^1H$ spectrum of compound **7** without (a) and with (b) selective decoupling of the low field doublet.




**13**

For both pyridylazonaphthol and pyridylazoresorcinol series no signal was detected for the hydroxy groups because of the exchange process with residual water (broad peak at 3.40 ppm) present in the deuterated solvent.

## EXPERIMENTAL

Electronic spectra in ethanol were recorded on a Pye Unicam SP 8-100 spectrophotometer. The $R_f$ values were determined on silica gel 60 $F_{254}$ tlc plates (Merck) using as eluent: compounds **1-6**, 40 ml *n*-butanol + 10 ml acetic acid + 50 ml water; compounds **7-9**, 50 ml 1-propanol + 20 ml ethyl-acetate + 30 ml water + 10 ml ammonia; compounds **10-12**, 60 ml *n*-butanol + 20 ml absolute ethanol + 20 ml ammonia (30%).

The $^1H$ nmr spectra were obtained with a Jeol EX 400 spectrometer in DMSO-$d_6$ solution (3%) at 25±0.5°C; for the pyridylazoresorcinol series a mixture (70:30) of DMSO/deuterium oxide (0.01 *M* sodium carbonate) was used. The two-dimensional NOESY experiment was performed with a spectral width of 4300 Hz over 1024 K. The mixing time was set at 800 ms. The spectrum (8 scans) was obtained after multiplying the data with a sine square bell function in both dimensions. All the spectra have been referenced to the solvent signal ($\delta_H$ = 2.52 ppm)

Compounds **1-3** were prepared using a 4 l five-necked flask, surrounded by a 10 l Dewar flask, containing acetone cooled with liquid nitrogen at -40°C and equipped with a glycerol-sealed stirrer, a dropping funnel, an ammonia inlet and double coiled condenser, cooled with circulating liquid nitrogen cooled acetone at -40°C. About 2.0 l of liquid ammonia was introduced and 0.5 g of finely powdered ferric nitrate and 56 g (2.4 g-atoms) of sodium in small pieces were added. Stirring was continued until the blue color disappeared. 4-Picoline (2 moles) was added drop-wise and after 5 minutes alkyl bromide (2 moles) was added with vigorous stirring. After an additional half hour of stirring, the mixture was allowed to stand at room temperature until the ammonia had evaporated. The residue was treated with 100 ml of ethanol followed by 800 ml of water. The mixture was extracted with ethyl ether and the combined organic layer was dried with anhydrous potassium carbonate, filtered and distilled to separate the volatile components. The crude product **1** was purified by vacuum distillation and crude compounds **2** and **3** by flash chromatography on 230-400 mesh ASTM silica gel 60 (Merck) using acetone/petroleum ether (10:90) as eluent (compound **2** Rf = 0.40, compound **3** Rf = 0.44).

Compounds **4-6** were prepared by the Chichibabin reaction of the corresponding 4-alkylpyridine (0.50 mole) with finely divided sodium amide (0.50 mole) in 300 ml of dried *N,N*-dimethylaniline at 150°C for six hours. During the reaction, three more additions (0.50 mole each) were made to complete

the reaction. Water was added and the mixture extracted with ethyl ether. The combined organic phases were dried with anhydrous potassium carbonate, filtered and vacuum distilled to separate the reaction products from the extraction solvents. The crude material was slurried in petroleum ether and crystallized from ethyl acetate/ligroin.

Compounds **7-9** and **10-12** were prepared by the Chichibabin method [21]. To a solution of 2-amino-4-alkylpyridine (0.05 mole) in anhydrous benzene (150 ml) was added an equimolar quantity of sodium amide under a slight flow of nitrogen. Vigorous stirring was maintained at 40°C. After ammonia evolution had ceased (about 1 hour) isopentylnitrite (0.05 mole) was added and stirring continued for another 90 minutes under a nitrogen atmosphere at 40°C. The precipitated sodium diazotate was filtered, washed with ethyl ether and immediately dissolved in cold ethanol (25 ml). An ethanolic solution of sodium diazotate was added to a solution of β-naphthol or resorcinol (an equimolar quantity) with carbon dioxide passing through the solution and with cooling in an ice-bath. Carbon dioxide was passed until nearly all of the ethanol was evaporated. The crude products were purified by crystallization from ethanol or an ethanol/water mixture.

## REFERENCES AND NOTES

[1] This work was supported by a contribution of Progetto Finalizzato Chimica Fine of Consiglio Nazionale delle Ricerche (CNR) and of Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST) of Italy.

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