Synthesis of Deuterium-Labeled Azo Dyes of the Sudan Family

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Received 2 August 2007; revised 6 November 2007

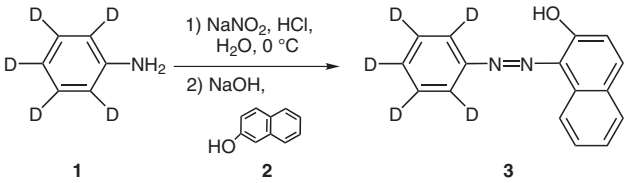
Abstract: Carcinogenic amines are formed in vivo during the metabolism of Sudan azo dyes. The identification and assay of these polluting agents requires the availability of deuterated analogues. Accordingly, two series of derivatives labeled on the phenylazo and naphthol moieties were synthesized. The new compounds were obtained in satisfactory chemical yields and with an excellent degree of labeling. The structures were confirmed by ^{1}H NMR spectroscopy and high-resolution mass spectrometry.

Key words: labeled azo dyes, labeled amines, azo compounds, coupling, deuterium labeling

The development of high-throughput methods represents one of the modern aspects of physical organic chemistry.[1] Azo dyes are widely used as colorants in cosmetics,[2] foods,[3] textiles,[4] commodities,[5] and, also, even in tattooing.[6] Their metabolism by intestinal bacteria has been described,[7] as well as the effectiveness of human skin bacteria[8] in the formation of carcinogenic amines from the same species. Information on the enzymatic mechanisms has been presented,[9,10] together with proofs of the dangerous effects on human health caused by the ingestion of azo dyes of the Sudan families.[11]

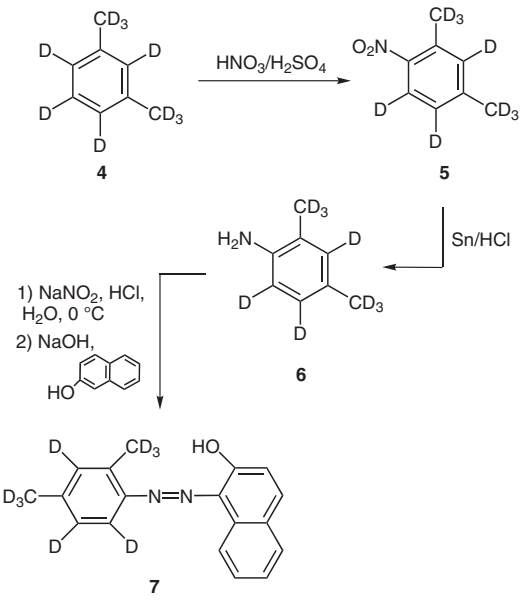
Accordingly, the synthesis of two sets of Sudan colorant, i.e. I, II, III, IV, and Para Red, labeled with deuterium on the phenylazo (**3**, **7**, **10**) or on the β-naphthol (**18**–**21**, **25**)[12] moieties of the molecules is presented (Schemes 1– 5), the aim being to provide reference compounds for simultaneous and fast screening of these dangerous substances in various matrices.[13]

Sudan I-d_5 (**3**) was obtained from aniline-d_5 (**1**) and β-naphthol (**2**) (Scheme 1), by an adaptation of a known procedure,[14] in nearly quantitative yield as a crude reddish solid, which could be further purified by crystallization from ethyl acetate.



Scheme 1

Labeled Sudan II-d_9 (**7**) was obtained from the commercially unavailable 2,4-dimethylaniline-d_9 (**6**) (Scheme 2). The latter was synthesized in two steps from the fully deuterated 1,3-xylene (**4**), which was nitrated by an established method,[14] to afford 2-nitroxylene-d_9 (**5**) in good yield. Subsequent reduction of **5** to 2,4-dimethylaniline-d_9 (**6**) by tin and hydrochloric acid, followed by coupling of aniline **6** with β-naphthol (**2**) gave the required compound **7** in 85% overall yield (Scheme 2). The isotopic purity of Sudan II-d_9 (**7**) was determined by ^{1}H NMR spectroscopy. Nevertheless, the gaseous protonated species undergoes fast H–D rearrangement prior to its dissociation, accounting for the observed deuterium distribution (see experimental procedures).

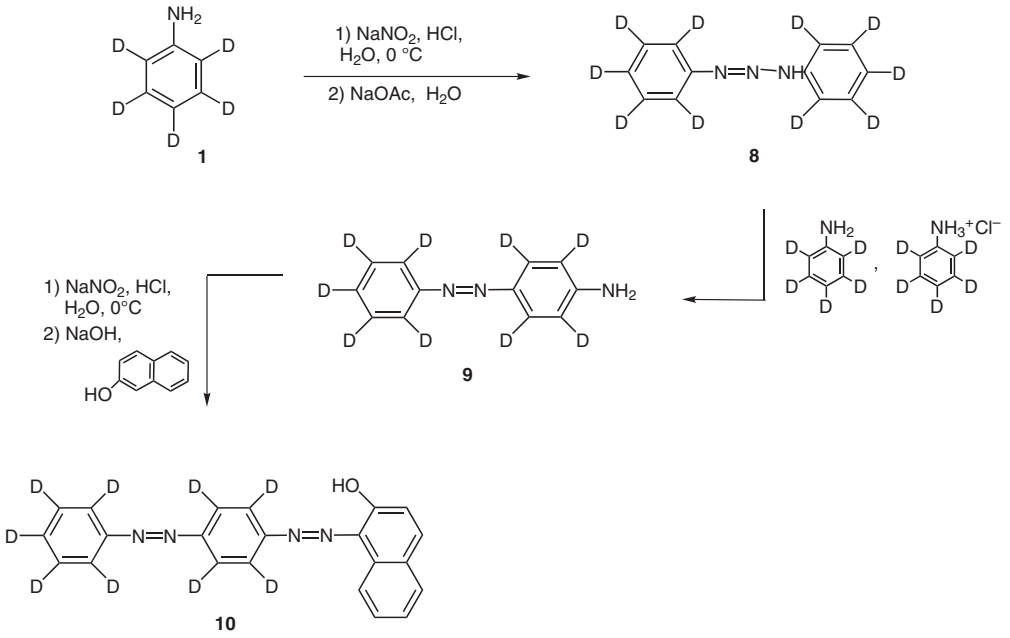


Scheme 2

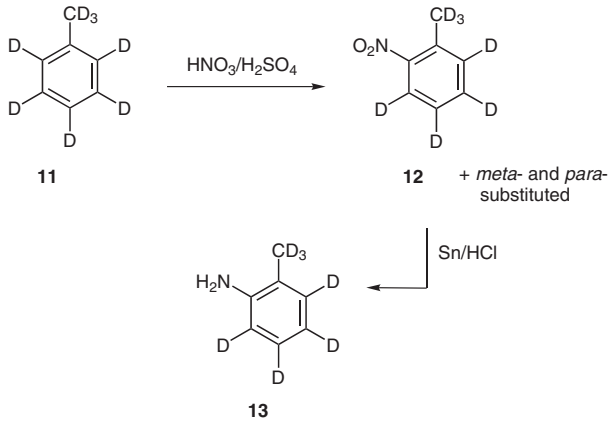
Sudan III-d_9 (**10**) was obtained from 4-(phenylazo)aniline-d_9 (**9**) (Scheme 3). The latter, in the unlabeled form, derives from the rearrangement of diazoaminobenzene, which can be obtained under suitable experimental conditions during the diazotization of aniline.[14] Accordingly, aniline-d_5 (**1**) was transformed into **9** by the steps shown in Scheme 3. The last coupling step, performed under standard conditions, afforded Sudan III-d_9 (**10**), in quantitative yield (Scheme 3).

The formation of Sudan IV-d_{13} by the same synthetic scheme used for analogue **10** (Scheme 3) failed. The nitration of toluene-d_8 (**11**) (Scheme 4) afforded a mixture of

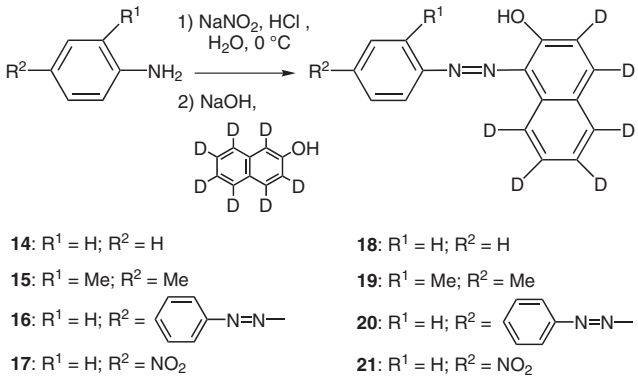
SYNTHESIS 2008, No. 3, pp 0459–0463
Advanced online publication: 10.01.2008
DOI: 10.1055/s-2008-1032036; Art ID: T12307SS



Scheme 3



Scheme 4



Scheme 5

the three possible isomers, including **12**, which were difficult to isolate, and the amino derivative **13** did not yield the expected deuterated compound in acceptable yields (Scheme 4).

Another approach to deuterated Sudan dyes is the formation of analogues labeled on the naphthol moiety. A set of products can be prepared by coupling of the common reagent β-naphthol-d_7 with different unlabeled amines (Scheme 5). Accordingly, Sudan I-d_6 (**18**), Sudan II-d_6 (**19**), Sudan III-d_6 (**20**), and Para Red-d_6 (**21**) were obtained in satisfactory yields from commercially available amines (Scheme 5).

Sudan IV-d_6 (**25**) could be obtained easily from β-naphthol-d_7 and 2-methyl-4-(*o*-tolylazo)aniline (**24**) (Scheme 6). The latter, as previously described, can be synthesized during the formation of the diazonium salt of 2-methylaniline (**22**) in slightly acidic media (Scheme 6).

The azo dyes, which were fully characterized by ^{1}H NMR spectroscopy, showed a high degree of labeling by ESI-tandem mass spectrometry. All the ^{1}H NMR spectra, when compared with those of the unlabeled isomers, showed the absence of protons pertaining to the phenylazo or naphthol moieties, as appropriate.

A typical example is shown in Figure 1, in which the ^{1}H NMR spectrum of Sudan II (A), is compared with those of Sudan II-d_9 (B) and Sudan II-d_6 (C). The complete replacement of hydrogen with deuterium atoms in the phenylazo moiety was clearly showed by the modification of the signal multiplicity in the δ = 6.86–8.65 region (Figure 1B), whereas the presence of two trideuteromethyl groups is evident by the absence of the resonances at δ = 2.50 and δ = 2.35 in spectrum B (Figure 1).

The structure of Sudan II-d_6 was confirmed, among other evidence, by the disappearance in the spectrum of all the resonances of the β-naphthol protons (Figure 1C). NMR spectra of all the new labeled Sudan azo dyes confirm that hydrogen–deuterium rearrangement does not take place during the synthetic procedure.

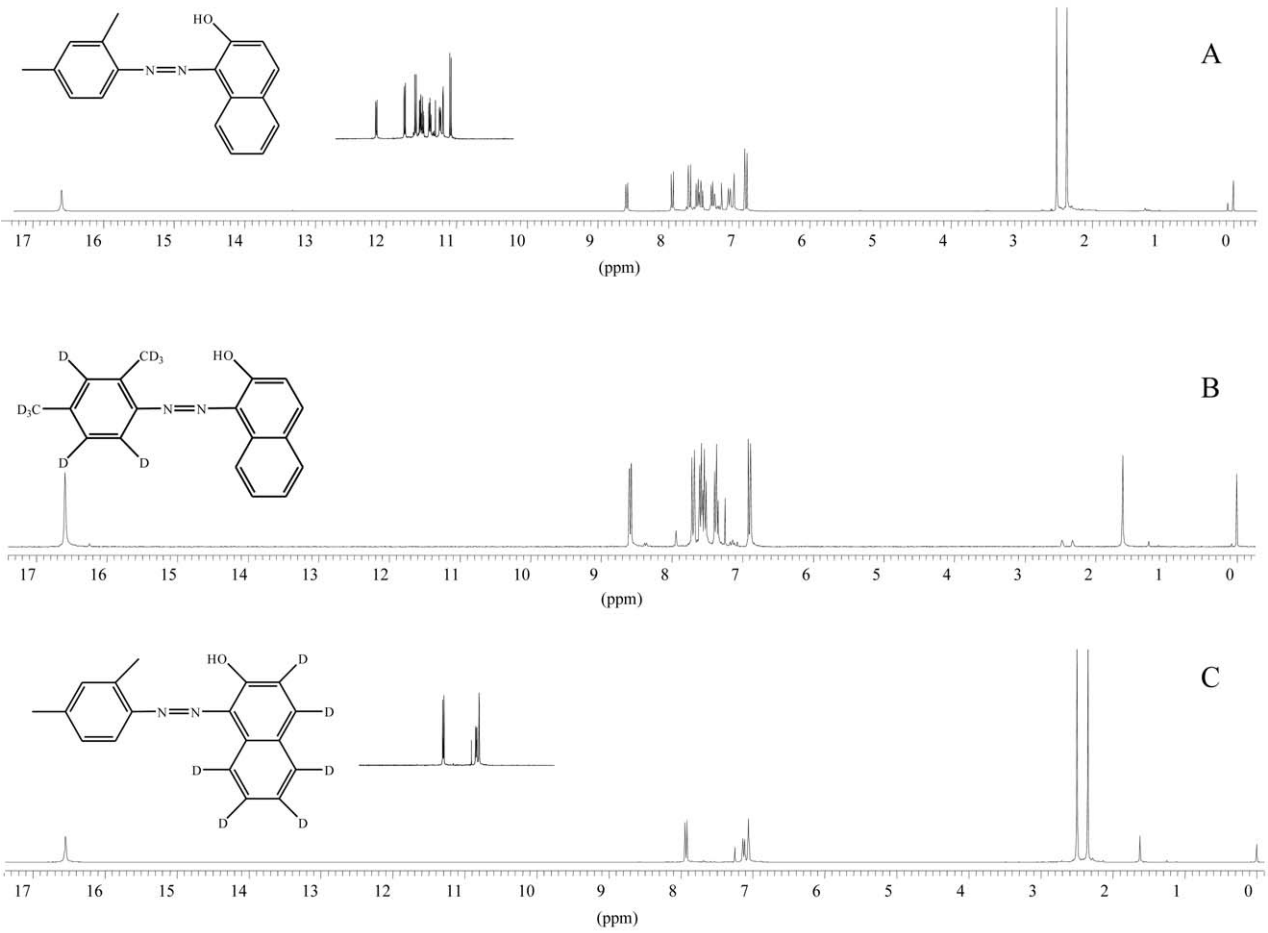
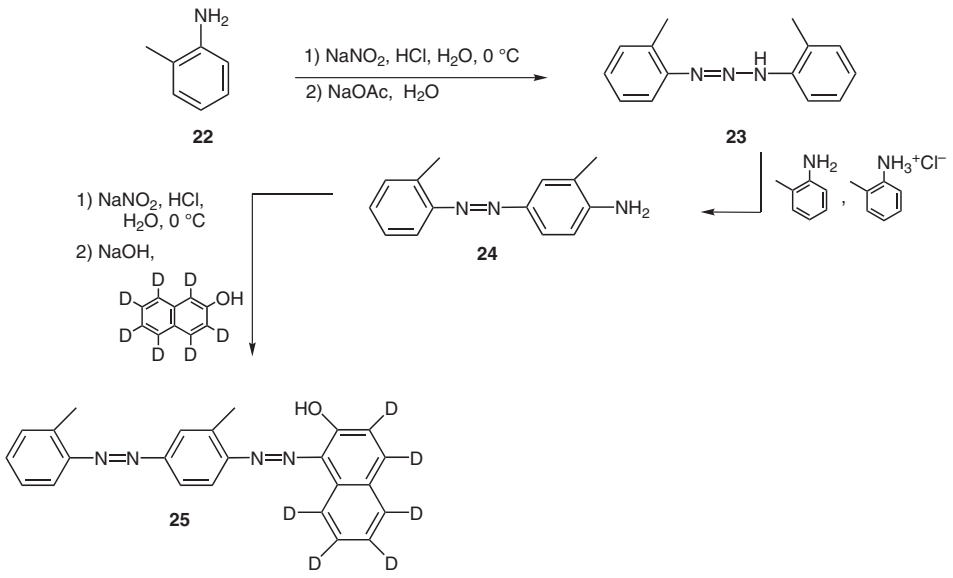


Figure 1 ^{1}H NMR (300 MHz) spectra of unlabeled Sudan II (A), Sudan II fully deuterated on the aniline moiety (B), and Sudan II fully deuterated on the naphthol moiety (C)



Scheme 6

Solvents and reagents were purified by standard procedures and were distilled or crystallized prior to use. The labeled compounds **5**, **6**, **8**, and **9**, and the unlabeled compounds **23** and **24** were obtained by general, published procedures.[14] ^{1}H NMR spectra, of samples in $CDCl_3$ with TMS as internal standard, were recorded at 300 MHz on a Bruker ACP 300 MHz spectrometer. The ^{1}H NMR spectra of the unlabeled, commercially available Sudan azo dyes are reported below to allow easy comparison with those of the synthesized labeled analogues. The ESI-HRMS experiments were carried out in a hybrid Q-Star Pulsar-i mass spectrometer (MDS Sciex Applied Bio-

systems, Toronto, Canada) equipped with an ion-spray ionization source.

4-Nitro-*m*-xylene-d_9 (5)
HNO_3 (4.66 mL) and H_2SO_4 (1 mL) were added to *m*-xylene-d_{10} (**4**; 2 mL, 16 mmol), and the reaction mixture was stirred at r.t. When the reaction was completed (after ca. 5 h), a soln of sat. aq $NaHCO_3$ (3 mL) was added and the mixture was extracted with Et_2O (3 × 10 mL). The organic extract was dried (Na_2SO_4), the solvent was removed, and the residue was distilled under vacuum.

Yield: 1.74 mL (75%); colorless liquid; bp 113 °C/15 Torr.

2,4-Dimethylaniline-d_9 (6)
Concd HCl (1.51 mL) was added in three portions to a mixture of 4-nitro-*m*-xylene-d_9 (**5**; 1.51 g, 9.4 mmol) and granular Sn (2.3 g), vigorously stirred after each addition. EtOH (ca. 7.5 mL) was added until **5** was completely solubilized. The reaction mixture was kept in a water bath at 70 °C for 1 h, then cooled to r.t. and treated with a 20% NaOH soln (4 mL) until the tin hydroxide precipitate was solubilized. The crude mixture was extracted with Et_2O (3 × 10 mL), dried (Na_2SO_4), and evaporated to dryness, and the residue was purified by vacuum distillation (120 °C/1 Torr); this afforded **6**.

Yield: 1.15 g (94%); colorless liquid.

Diazoaminobenzene-d_{10} (8)
Aniline-d_5 (**1**; 1 mL, 10 mmol) was added to a soln of H_2O (5 mL) and concd HCl (1.33 mL). The reaction mixture was vigorously shaken and put in an ice bath (0–5 °C). A soln of $NaNO_2$ (0.346 g, 5 mmol) in H_2O (3 mL) was then slowly added, while the bath temperature was maintained at 0–5 °C. The soln was allowed to stand with frequent shaking for 15 min, and then NaOAc (1.4 g, 17 mmol) in H_2O (3 mL) was added over 5 min. A yellow precipitate of compound **8** formed instantaneously, and was filtered on a Buchner funnel, washed with a little cold H_2O, and dried.

Yield: 1.88 g (91%).

4-(Phenylazo)aniline-d_9 (9)
Diazoaminobenzene-d_{10} (**8**; 0.200 g, 0.96 mmol) was dissolved in aniline-d_5 (0.534 mL, 0.576 g) and then aniline-d_5HCl (0.096 g) was added. The mixture was warmed at 40–45 °C for 1 h. Then it was left to stand for 30 min, and a soln of glacial AcOH, diluted with an equal volume of H_2O, was added until an orange precipitate was observed. The mixture was allowed to stand for 15 min, and then it was filtered on a Buchner funnel, washed with a little cold H_2O, and dried.

Yield: 0.161 g (81%).

1,3-Di-*o*-tolyltriazene (23)
Compound **23** was prepared from *o*-toluidine (**22**) by the same procedure as that used for the preparation of **8**.

Yield: 72%; yellow precipitate.

2-Methyl-4-(*o*-tolylazo)aniline (24)
Compound **24** was prepared from **23** by the same procedure as that used for the preparation of **9**.

Yield: 71%; orange precipitate.

Azo Compounds 3, 7, and 10 by Coupling of Deuteroamines 1, 6, and 9 and β-Naphthol (2); General Procedure
A cooled soln of $NaNO_2$ (11 mmol) in H_2O (4 mL) was added dropwise to a soln containing the appropriate deuterated amine **1**, **6**, or **9** (11 mmol) in concd HCl (3.25 mL) and H_2O (3 mL), kept at 0–5 °C. The bath temperature of the diazonium salt soln thus formed was raised to 10 °C, and a mixture of β-naphthol (**2**; 11 mmol) in 10% aq NaOH (9.15 mL) was slowly added. The reaction mixture was allowed to stand in an ice bath for 30 min with occasional stirring. The reddish precipitate that had formed was collected by filtration, washed with cold H_2O, and dried. The precipitate was purified by crystallization from EtOAc; this gave the azo dyes **3**, **7** and **10**.

Sudan I
^{1}H NMR (300 MHz, $CDCl_3$): δ = 16.24 (s, 1 H, OH), 8.54 (d, *J* = 8.31 Hz, 1 H, H*), 7.80–7.20 (m, 9 H, H* + H**), 6.85 (d, *J* = 9.44 Hz, 1 H, H*); H* = naphthol protons; H** = phenylazo protons.

Sudan I-d_5 (3)
Yield: 95%; red crystals; mp 134–133 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 16.27 (s, 1 H, OH), 8.52 (d, *J* = 8.25 Hz, 1 H, H*), 7.79–7.06 (m, 6 H, H*), 6.84 (d, *J* = 9.62 Hz, 1 H, H*); H* = naphthol protons.

ESI-HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{16}H_8D_5N_2O^+$: 254.1342; found: 254.1354; 5.42% d_4, 94.58% d_5.

Sudan II
^{1}H NMR (300 MHz, $CDCl_3$): δ = 16.59 (s, 1 H, OH), 8.58 (d, *J* = 8.25 Hz, 1 H, H*), 7.94 (d, *J* = 8.25 Hz, 1 H, H**), 7.77–7.09 (m, 6 H, H* + H**), 6.90 (d, *J* = 9.17 Hz, 1 H, H*), 2.50 (s, 3 H, CH_3), 2.35 (s, 3 H, CH_3); H* = naphthol protons; H** = dimethylphenylazo protons.

Sudan II-d_9 (7)
Yield: 85%; red crystals; mp 162–161 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 16.58 (s, 1 H, OH), 8.54 (d, *J* = 8.24 Hz, 1 H, H*), 7.75–7.30 (m, 4 H, H*), 6.89 (d, *J* = 9.63 Hz, 1 H, H*); H* = naphthol protons;

ESI-HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{18}H_8D_9N_2O^+$: 286.1906; found: 286.1925; 1.95% d_6, 7.31% d_7, 25.37% d_8, 65.37% d_9.

Sudan III

^{1}H NMR (300 MHz, $CDCl_3$): δ = 16.36 (s, 1 H, OH), 8.51 (d, *J* = 8.25 Hz, 1 H, H*), 8.10–7.35 (m, 13 H, H* + H**), 6.79 (d, *J* = 9.62 Hz, 1 H, H*); H* = naphthol protons; H** = diphenylazo protons.

Sudan III-d_9 (10)
Yield: 95%; red crystals; mp 208–207 °C.

^{1}H NMR (300 MHz, $CDCl_3$) 16.35 (s, 1 H, OH), 8.50 (d, *J* = 8.71 Hz, 1 H, H*), 7.72–7.35 (m, 6 H, H*), 6.78 (d, *J* = 9.62 Hz, 1 H, H*); H* = naphthol protons.

ESI-HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{22}H_8D_9N_4O^+$: 362.1967; found: 362.1978; 6.78% d_8, 93.22% d_9.

Azo Compounds 18–21 and 25 by Coupling of Amines 14–17 and 24 and β-Naphthol-d_7; General Procedure
Compounds **18**–**21** and **25** were prepared by coupling of amines **14**–**17** and **24** with β-naphthol-d_7 by the same procedure as that used for the preparation of **3**, **7**, and **10** by the coupling of deuteroamines with β-naphthol.

Sudan I-d_6 (18)
Yield: 98%; red crystals; mp 132.5–132 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 16.23 (s, 1 H, OH), 7.80–7.15 (m, 5 H, H**); H** = phenylazo protons.

ESI-HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{16}H_7D_6N_2O^+$: 255.1404; found: 255.1388; 7.05% d_5, 92.95% d_6.

Sudan II-d_6 (19)
Yield: 81%; red crystals; mp 161–160 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 16.54 (s, 1 H, OH), 8.00–7.01 (m, 3 H, H**), 2.49 (s, 3 H, CH_3), 2.34 (s, 3 H, CH_3); H** = dimethylphenylazo protons.

ESI-HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{18}H_{11}D_6N_2O^+$: 283.1787; found: 283.1799; 7.63% d_5, 92.37% d_6.

Sudan III-d_6 (20)
Yield: 89%; red crystals; mp 205–204 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 16.33 (s, 1 H, OH), 8.06–7.42 (m, 9 H, H**); H** = diphenylazo protons.

ESI-HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{22}H_{11}D_6N_4O^+$: 359.1779; found: 359.1801; 7.88% d_5, 92.12% d_6.

Para Red
^{1}H NMR (300 MHz, $CDCl_3$): δ = 16.13 (s, 1 H, OH), 8.50–7.38 (m, 9 H, H* + H**), 6.69 (d, *J* = 9.62 Hz, 1 H, H*); H* = naphthol protons; H** = nitrophenylazo protons.

Para Red-d_6 (21)
Yield: 83%; red crystals; mp 263–263.5 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 16.14 (s, 1 H, OH), 8.32 (d, *J* = 9.16 Hz, 2 H, H*), 7.69 (d, *J* = 9.16 Hz, 2 H, H**); H** = nitrophenylazo protons.

ESI-HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{16}H_6D_6N_3O_3^+$: 300.1255; found: 300.1261; 7.47% d_5, 92.53% d_6.

Sudan IV
^{1}H NMR (300 MHz, $CDCl_3$): δ = 16.58 (s, 1 H, OH), 8.50 (d, *J* = 7.79 Hz, 1 H, H*), 8.20–7.15 (m, 11 H, H* and H**), 2.73 (s, 3 H, CH_3), 2.57 (s, 3 H, CH_3); H* = naphthol protons; H** = methyldiphenylazo protons.

Sudan IV-d_6 (25)
Yield: 72%; red crystals; mp 188–189 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 16.59 (s, 1 H, OH), 8.20–7.21 (m, 7 H, H**), 2.73 (s, 3 H, CH_3), 2.58 (s, 3 H, CH_3); H** = methyldiphenylazo protons.

ESI-HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{24}H_{15}D_6N_4O^+$: 387.2092; found: 387.2079; 6.78% d_5, 93.22% d_6.

Acknowledgment

The work is supported by funds from the University of Calabria and by the project PON-avviso-68 of the Italian Ministry of University and Research (MIUR).

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