Synthesis W. Moormann et al. Paper

Solvent-Free Synthesis of Diazocine

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Dedicated to Professor Herbert Mayr on the occasion of his 70th birthday

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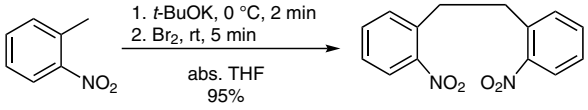
Abstract A convenient two-step synthesis of diazocine starting from 2-nitrotoluene is described. The first step, the oxidative dimerization of 2-nitrotoluene, is improved to 95% yield. The second step, the reductive azo cyclization, is performed as a solvent-free reaction with lead powder in a ball mill (51% yield). As a reference, the previously described azo cyclization with Zn/$Ba(OH)_2$ is investigated in detail. The results explain why in previous experiments the yields are low and extremely dependent on the reaction conditions. In view of potential applications in photopharmacology, we checked the stability under reducing conditions. Diazocine does not react with glutathione, indicating intracellular stability.

Key words bridged azobenzene, diazocine, reductive azo-cyclization, solvent-free synthesis, oxidative C–C coupling, lead, azoxy, hydrazine, glutathione

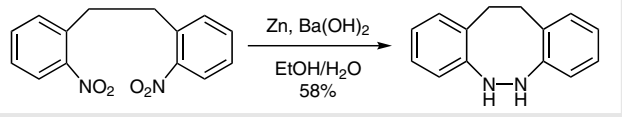
Diazocines have been known for more than 100 years, however, it was only recently discovered that they exhibit extraordinary photophysical and photochemical properties.[1,2] As compared to the most frequently used photoswitch, azobenzene, the parent diazocine has higher switching efficiencies (100% *E*→*Z*, 96% *Z*→*E*), and higher quantum yields (50% *E*→*Z*, 72% *Z*→*E*). Moreover, diazocines can be isomerized with visible light (400 nm *E*→*Z*, 500 nm *Z*→*E*), and the photodynamics are ultrafast.[3] Most importantly, in contrast to azobenzene, diazocine is more stable in its sterically demanding *Z* form than in the slender *E* configuration. Hence, diazocines are ideal switches in photopharmacology.[4,5] Most drugs equipped with an azobenzene unit are active in their more stable and less hindered *E* configuration. Biological activity is switched off upon isomerization with UV light because the bent *Z* isomer no longer fits into the binding site of the receptor. Diazocines, however, are more stable in their *Z* state, and could be administered in their inactive form. At the site of illness, the drug would be switched on with light with high spatiotemporal control. Several examples prove the practicability of diazocines in biochemical applications. Peptides and DNA strands with switchable conformations have been prepared by incorporation of diazocines, and spatiotemporal regulation of DNA functions has been suggested.[6,7] However, the synthesis of diazocines proceeds with notoriously poor yields and low reproducibility. We therefore optimized and investigated in detail the most frequently used preparation method, i.e., the oxidative coupling of 2-nitrotoluene and the reduction of 2,2′-dinitrodibenzyl with Zn powder and $Ba(OH)_2$. Based on the mechanistic information, we have developed a convenient, reliable, and solvent-free synthesis of diazocine.

The synthesis of diazocine started with dimerization of 2-nitrotoluene using *t*-BuOK as a base, and air as the oxidizing agent. According to our observations, 2,2′-dinitrodibenzyl is formed in low yields, even in the absence of additional oxidizing agents, because part of the nitro compound is sacrificed in the oxidation step (and reduced). We therefore screened the effect of external oxidizing agents on the reaction time, temperature and yields. The yield reported by Chaudhuri and Ball[8] (65%) was increased to 95% by addition of bromine as the oxidizing agent (Scheme 1). Thereby, the reaction time was reduced from several hours to 7 minutes.



Scheme 1 Improved method for the oxidative coupling of 2-nitrotoluene to 2,2′-dinitrodibenzyl

The majority of procedures describing the synthesis of diazocines are based on the reduction of 2,2′-dinitrodibenzyl with Zn and $Ba(OH)_2$, as published by Duval (Scheme 2).[1,2,6,9–12] The reported yields vary substantially.



Scheme 2 Reduction of 2,2′-dinitrodibenzyl with Zn and $Ba(OH)_2$ as reported by Duval et al.

According to our observations the process is strongly dependent on the reaction conditions. We therefore investigated the mechanism in detail by monitoring the intermediates and their concentrations during the reduction (Figure 1).

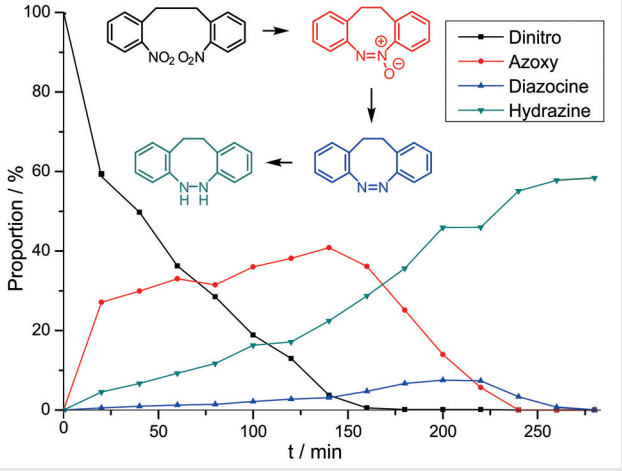
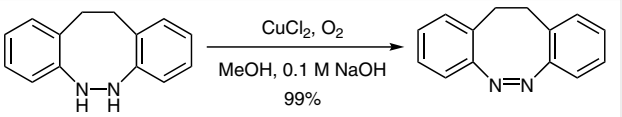


Figure 1 Fractions of products and intermediates during reduction of 2,2′-dinitrodibenzyl with Zn/$Ba(OH)_2$ as a function of time

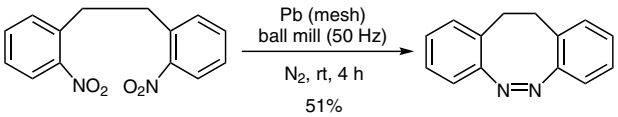
Several facts are worth noting. The azoxy compound is the first isolable reduction product, and it is obviously an intermediate with a peak concentration of 41% after about 2 hours (16 equiv of Zn powder, 3 equiv $Ba(OH)_2$, ethanol, reflux). The azoxy compound is further reduced to the diazocine (peak concentration ~8% after ~3 h) which again is reduced to the hydrazine (~58%). Further reduction to the diamine is slow (if no further Zn is added). We checked this mechanistic hypothesis by resubmitting the intermediate azoxy compound to the same reduction conditions, and observed an analogous concentration dependence of diazocine and hydrazine as shown in Figure 1. The sum of azoxy compound, diazocine, and hydrazine remains constant during reduction. There is no loss of material after formation of the azoxy compound. By-products, such as the dimer and higher oligomers, are formed in the first reductive ring-closure step of the dinitrodibenzyl. We conclude that reduction to the hydrazine and reoxidation is the most convenient method to prepare the diazocine giving the highest yields. The reaction can be easily monitored by TLC. Diazocine gives rise to a yellow spot which can be switched to a red color upon irradiation with UV light (and back to yellow with green light). The colorless hydrazine spot is converted into the yellow diazocine upon very short exposure to fuming nitric acid vapor, and thus easily identified by subsequent switching with UV light. Oxidation of the hydrazine to diazocine with air and $CuCl_2$ is an almost quantitative reaction (Scheme 3).



Scheme 3 Formation of diazocine by oxidation of the corresponding hydrazine

A reason for the varying yields in previous syntheses might be the fact that upon standing in air the hydrazine autoxidizes to the diazocine. By-products during reduction are the cyclic dimer (16-membered ring) and oligomers.[13]

Ring-closure reactions are favored over oligomerization under high dilution conditions, or if performed as heterogeneous reactions keeping the local concentration of the reactant low.[14,15] Besides reduction with Zn/$Ba(OH)_2$, reactions with lead under basic conditions (pH 9.5) in methanol and formic acid as a proton source have been successfully performed.[7,10] Under these reaction conditions the cyclic azoxy compound is formed, which subsequently is reduced with PCl_3 or $Ph_3P/MoCl_2O_2(dmf)_2$. Hence, both procedures reported in the literature are two-step reactions. To avoid over-reduction to the hydrazine [Zn/$Ba(OH)_2$], or incomplete reduction to the azoxy compound (Pb/Et_3N/HCOOH), we investigated the one-step, solvent-free reduction with lead powder in a ball mill (Scheme 4).[16] Avoiding moisture (dry nitrogen atmosphere), or other additional proton sources should prevent formation of the hydrazine or diamine. Various substituted azobenzenes have been prepared on small scale by Suzuki et al. using this method.[17]



Scheme 4 Solvent-free reduction of 2,2′-dinitrodibenzyl with lead powder in a ball mill

We mixed 2,2′-dinitrodibenzyl with a 10-fold molar excess of lead powder, and agitated the mixture in a ball mill under a nitrogen atmosphere. At regular intervals, probes were taken and analyzed. As in the case of Zn/$Ba(OH)_2$ reduction, the cyclic azoxy compound is the first isolable compound with a peak concentration of 45% after about 2 hours (vibrational frequency 50 Hz). Upon further milling,

the diazocine is formed at the expense of the azoxy compound concentration (Figure 2). The isolated yield of diazocine after 3.5 hours is 51%. No further reduction takes place after extended periods of milling. The reaction time depends on the scale of the reaction and the milling frequency.

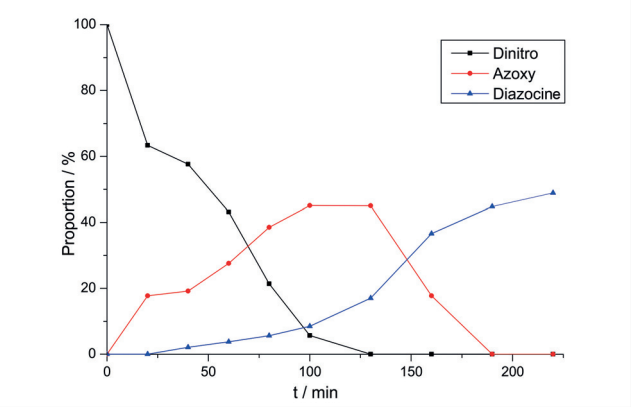


Figure 2 Fractions of products and intermediates during reduction of 2,2′-dinitrodibenzyl with lead powder in a ball mill as a function of time

The product is separated from the lead powder by extraction with acetone and filtration. Lead should be removed quickly, or protic solvents or moisture should be carefully avoided. Upon standing of the suspension with lead under air, further reduction to the hydrazine was observed. Small amounts (~6%) of the dimer (16-membered ring with both azo groups in *E* configuration) and higher cyclic oligomers are formed as well.[13]

Under physiological conditions, a number of azobenzenes (particularly amino-substituted azobenzenes) are reduced *in vivo* to the corresponding anilines, some of which are carcinogenic.[18] Good correlations have been observed between the glutathione-inducing effect of azo dyes in the liver and their carcinogenic activities.[19] For applications in photopharmacology, the stability of the switchable drugs toward reduction by glutathione inside cells is essential.

We therefore subjected diazocine to reduction by glutathione. For solubility reasons, the experiments were performed in a 1:1 acetonitrile/PBS buffer. After incubation of diazocine for 24 hours, no change in UV–vis absorption was detected. Furthermore, the diazocine was switched 22 times between the *Z* and the *E* configuration with light of 385 and 530 nm in the presence of glutathione. No fatigue, or side products were detected (Figure 3). Our experiments indicate that diazocine is stable toward reduction by glutathione. Besides photopharmacology, diazocines have been proposed as substitutes for azobenzenes in demonstration experiments, because they can be switched with visible light and they exhibit a distinct color change.[20] The negative glutathione test gives a first indication that diazocine is physiologically benign.

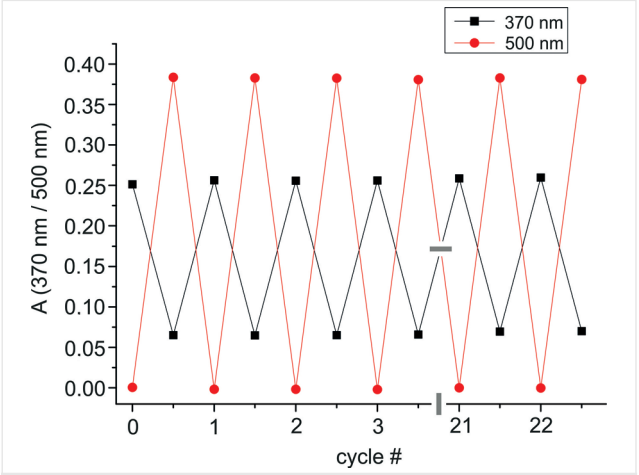


Figure 3 Switching of diazocine with light of 385 and 530 nm in an alternating sequence in the presence of glutathione in acetonitrile/water (1:1) after incubation for 24 h. Absorption at 370 and 500 nm was recorded after each switching cycle. No fatigue was observed.

In conclusion, we optimized the synthesis of parent diazocine. The yield of the first step (oxidative dimerization of 2-nitrotoluene) was improved to 95% by using bromine as the oxidizing agent (instead of air). The eight-membered ring in diazocines is rather strained (ring strain ~16 kcal mol^{-1}). Reductive azo-cyclization of the dinitro precursor, therefore, competes with cyclic oligomer and polymer formation. Further problems on the reaction pathway to diazocine arise from incomplete reduction (azoxy compound), or over-reduction (hydrazine), or further reductive ring cleavage (diamino compound). We minimized oligomer formation, and avoided over-reduction by performing the reduction of the dinitro compound under dry and solvent-free conditions with metallic lead in a ball mill. In contrast to a number of azobenzenes, diazocine is stable towards reduction by glutathione which is an important prerequisite for applications in photopharmacology.

Milling reactions were carried out in a 15 mL stainless steel grinding cup using stainless steel balls of 10 mm diameter in a laboratory ball milling apparatus (Pulverisette 23, Fritsch GmbH, Idar-Oberstein, Germany). Test for stability to glutathione reduction: Diazocine was incubated (24 h, 27 °C) in 1:1 acetonitrile/PBS (1X phosphate-buffered saline:137 mM NaCl, 2 mM KCl, 8 2 mM Na_2PHO_4, 2 mM KH_2PO_4, pH = 7.4) in a 10 mM solution of glutathione. A reaction control was performed with the use of an Agilent Technologies 1100 Series HPLC and Agilent Zorbax 5 µm Eclipse XDB-C8, 4.6 × 150 mm column. TLC plates (Polygram Sil G/UV254 Macherey–Nagel) were used for reaction control. Flash column chromatography was carried out with a Biotage® Isolera system using Biotage® SNAP ULTRA (HP-Sphere™ 25 µm) columns. Melting points were obtained using a Büchi, Melting Point M-560 apparatus. IR spectra were recorded with a Perkin–Elmer 1600 series FT-IR spectrophotometer, using a Golden Gate Diamond ATR unit, A531-G. NMR spectra were recorded using a Bruker DRX 500 spectrometer [^{1}H NMR (500.1 MHz), ^{13}C NMR (125.8 MHz)]

without an internal standard. Mass spectra were recorded on a Finnigan MAT 8230 (EI, 70 eV) spectrometer. UV–Vis spectra were recorded with a Lambda 14 UV/Vis spectrometer, Perkin–Elmer. Irradiation was performed with LED light sources [385 nm: 12 × Nichia NCSU034A, FWHM = 9 nm, P(opt) = 12 × 340 mW, 530 nm: 16 × Luxeon LXML-PM01-0080, FWHM = 33 nm, P(opt) = 16 × 200 mW], Sahlmann Photochemical Solutions.

2,2′-Dinitrodibenzyl

[CAS Reg. No. 16968-19-7]

Under a nitrogen atmosphere, 2-nitrotoluene (2.00 g, 15.0 mmol) was dissolved in dry THF (90 mL), cooled to 0 °C, followed by addition of potassium butoxide. The reaction was stirred for 2 min before addition of bromine (3.12 g, 19.5 mmol). After further stirring for 5 min, the reaction was added to 500 mL of ice/water. The precipitate was filtered and the filtrate extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with saturated sodium thiosulfate solution and saturated sodium chloride solution, then dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by crystallization (H_2O/EtOH, 1:2) to give 2,2′-dinitrodibenzyl as a white solid (1.94 g, 7.13 mmol, 95%); mp 121 °C; R_f = 0.33 (*n*-pentane/CH_2Cl_2, 1:1).

IR (ATR): 2962, 2854, 2343, 1608, 1576, 1509, 1443, 1344, 1310, 1262, 1201, 1164, 1126, 1073, 1041, 959, 859, 787, 749, 704, 666, 567, 531, 421 cm^{-1}.

^1H NMR (500.1 MHz, $CDCl_3$, 300 K): δ = 7.96 (dd, *J* = 8.2 Hz, *J* = 1.2 Hz, 2 H, *H*-3), 7.54 (pseudo t, 2 H, *H*-5), 7.42 (dd, *J* = 7.7 Hz, *J* = 1.3 Hz, 2 H, *H*-6), 7.38 (pseudo t, 2 H, *H*-4), 3.25 (s, 4 H, CH_2).

^{13}C NMR (125.8 MHz, $CDCl_3$, 300 K): δ = 149.37, 136.01, 133.30, 132.49, 127.56, 124.84, 34.44.

MS (EI, 70 eV): *m*/*z* (%) = 273 (1) $[M + H]^+$, 255 (8), 237 (9), 178 (21), 136 (100), 120 (70), 92 (85).

5,6,11,12-Tetrahydrodibenzo[*c*,*g*][1,2]diazocine (Hydrazine)

[CAS Reg. No. 2225-55-0]

To a solution of 2,2′-dinitrodibenzyl (201 mg, 738 µmol) in EtOH (40 mL) were added an aqueous solution of barium hydroxide [$Ba(OH)_2{\cdot}8H_2O$] (695 mg, 2.20 mmol) in H_2O (20 mL) and zinc powder (778 mg, 11.9 mmol), and the mixture was stirred for 5 h under reflux. The reaction mixture was filtered through Celite and the solvent was removed under reduced pressure. The crude product was dissolved in CH_2Cl_2, filtered through Celite, and the solvent evaporated. The residue was purified by flash column chromatography (cyclohexane/EtOAc, 3:1, R_f = 0.72) to afford the product as a colorless solid (90.0 mg, 429 µmol, 58%); mp 151 °C.

IR (ATR): 3337, 3327, 3049, 2939, 2902, 1606, 1580, 1495, 1454, 1441, 1406, 1234, 1101, 935, 864, 746, 717, 542, 484, 441, 405 cm^{-1}.

^1H NMR (500 MHz, acetone-d_6, 300 K): δ = 7.03 (d, *J* = 7.3 Hz, 2 H, *H*-6), 6.99 (dt, *J* = 7.6 Hz, *J* = 1.5 Hz, 2 H, *H*-2), 6.83–6.76 (m, 4 H, *H*-3, *H*-5), 6.55 (s, 2 H, N-*H*), 3.18 (s, 4 H, CH_2).

^{13}C NMR (125 MHz, acetone-d_6, 300 K): δ = 148.9, 133.8, 131.3, 127.0, 122.0, 117.8, 32.0.

MS (EI, 70 eV): *m*/*z* (%) = 210 (100) $[M]^+$.

HRMS (EI, 70 eV): *m*/*z* $[M]^+$ calcd for $C_{14}H_{14}N_2$: 210.1157; found: 210.1154.

(*Z*)-11,12-Dihydrodibenzo[*c*,*g*][1,2]diazocine

[CAS Reg. No. 1194317-15-1]

By Reduction/Reoxidation of 2,2′-Dinitrodibenzyl: To a solution of 2,2′-dinitrodibenzyl (201 mg, 738 µmol) in EtOH (40 mL) were added an aqueous solution of barium hydroxide [$Ba(OH)_2{\cdot}8H_2O$] (695 mg, 2.20 mmol) in H_2O (20 mL) and zinc powder (778 mg, 11.9 mmol), and the mixture was stirred for 5 h under reflux. The reaction mixture was filtered through Celite, and the solvent was removed under reduced pressure. The crude product was dissolved in CH_2Cl_2 and filtered through Celite, and the solvent was removed under reduced pressure. The crude product was dissolved in 0.1 M methanolic NaOH solution (50 mL), $CuCl_2$ (4 mg, 29.8 µmol) was added, and air was bubbled through the solution until completion of the reaction. The reaction was neutralized with 1 M HCl solution. After addition of saturated sodium bicarbonate solution, the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (cyclohexane/EtOAc, 3:1, R_f = 0.51) to afford the product as a yellow solid (89.1 mg, 428 µmol, 58%); mp 103–105 °C.

IR (ATR): 3058, 2898, 1813, 1568, 1522, 1480, 1438, 1153, 1083, 1037, 949, 924, 864, 805, 763, 747, 682, 631, 596, 566, 535, 481, 459, 422, 405 cm^{-1}.

^1H NMR (500.1 MHz, $CDCl_3$, 300 K): δ = 7.13 (pseudo t, 2 H, H-4), 7.02 (pseudo t, 2 H, H-5), 6.98 (d, *J* = 7.7 Hz, 2 H, H-6), 6.83 (d, *J* = 7.8 Hz, 2 H, H-3), 2.87 (m_c, 4 H, CH_2).

^{13}C NMR (125.8 MHz, $CDCl_3$, 300 K): δ = 155.49, 129.60, 128.08, 127.01, 126.66, 118.70, 31.65.

MS (EI, 70 eV): *m*/*z* (%) = 208 (19) $[M]^+$, 179 (100), 165 (61).

HRMS (EI, 70 eV): *m*/*z* $[M]^+$ calcd for $C_{14}H_{12}N_2$: 208.1001; found: 208.1004.

UV–Vis (MeCN): λ_{max} (log ε) = 401 (2.99), 281 (3.48), 245 (3.96) nm.

By One-Step Reduction of 2,2′-Dinitrodibenzyl with Pb in a Ball Mill: Under a nitrogen atmosphere, 2,2′-dinitrodibenzyl (320 mg, 1.18 mmol), lead mesh (2.44 g, 11.8 mmol; Alfa Aesar lead powder, 200 mesh, 99.9%), and two stainless steel balls (10 mm diameter) were added to a stainless steel grinding cup (15 mL). The reaction vessel was shaken at a rate of 50 Hz for 4 h. The black pasty product was extracted twice with acetone (200 mL). The extracts were filtered over Celite and the solvent was evaporated. The crude residue was purified by flash column chromatography (cyclohexane/EtOAc, 3:1, R_f = 0.51) to afford the product as a yellow solid (125 mg, 602 µmol, 51%).

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