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Synthesis and Characterization of 4′-Amino and 4′-Nitro Derivatives of 4-*N,N*-Dimethylaminotriphenylmethane as Precursors for a Proximate Malachite Green Metabolite

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Abstract—This paper describes the preparation of 4′-amino (**2**) and 4′-nitro (**3**) derivatives of 4-*N,N*-dimethylaminotriphenylmethane as precursors for presumed DNA-binding metabolites of malachite green. The primary amine **2** was synthesized via a condensation of 4-lithiated bis-(*N,N*-trimethylsilyl)aniline and 4-(dimethylamino)benzophenone. A simple nitro-dediazoniation reaction of **2** failed to afford **3**, but gave exclusively a mixture derived from hydro-dediazoniation followed by nitration and *N*-dealkylative-*N*-nitrosation. Direct nitration of *N,N*-dimethylaminotriphenylmethane, however, afforded **3** as well as other nitro isomers. Published by Elsevier Science Ltd.

Introduction

Malachite green (MG, Fig. 1), an *N*-methylated diaminotriphenylmethane dye, is a widely used antifungal agent in commercial fish hatcheries.[1] The antifungal activity of MG stems from inhibition of intracellular enzymes and interactions with DNA and cellular membranes. The nonpolar reduced form of the dye, leucomalachite green (LMG, Fig. 1), has been shown to accumulate in the tissues[2–4] and eggs[5] of fish treated with MG. Human exposure to MG, through the consumption of treated fish and occupational exposure in fabric dyeing, has also been documented.[1,4,6] Although MG is not approved for use, it has been widely used due to its low cost, ready availability, and high antifungal efficacy.

In short term feeding studies,[7] we have shown that MG is sequentially *N*-demethylated to secondary and primary aromatic amines in rats and mice both before and after reduction to LMG. Similar sequential demethylation was also observed in a thyroid peroxidase-catalyzed reaction of LMG.[8] Preliminary ^{32}P-postlabeling results from our laboratory suggest that MG and its *N*-demethylated MG and LMG derivatives are capable of forming DNA adducts in vivo, with the binding being consistently greater with the ionic MG derivatives. In addition, exposure to LMG causes

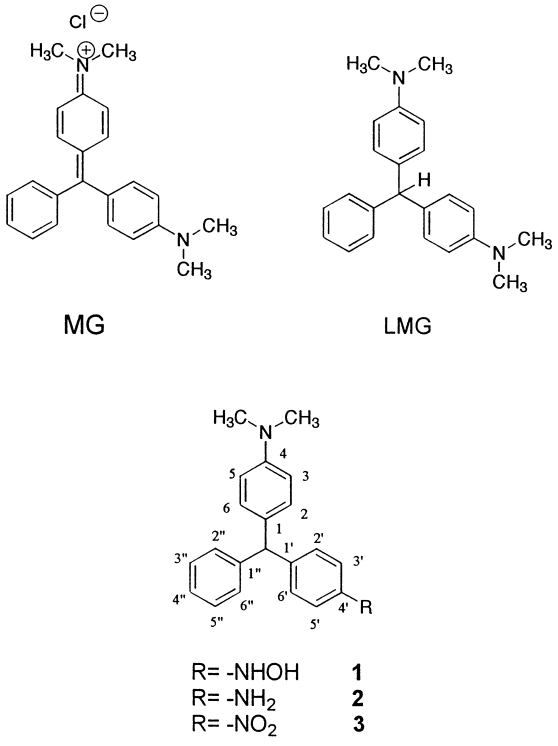

Figure 1. Structures and the numbering system for malachite green (MG) and leucomalachite green (LMG) and their derivatives.

Keywords: malachite green; nitration; nitro-dediazoniation; hydro-dediazonation; nitrosamines.

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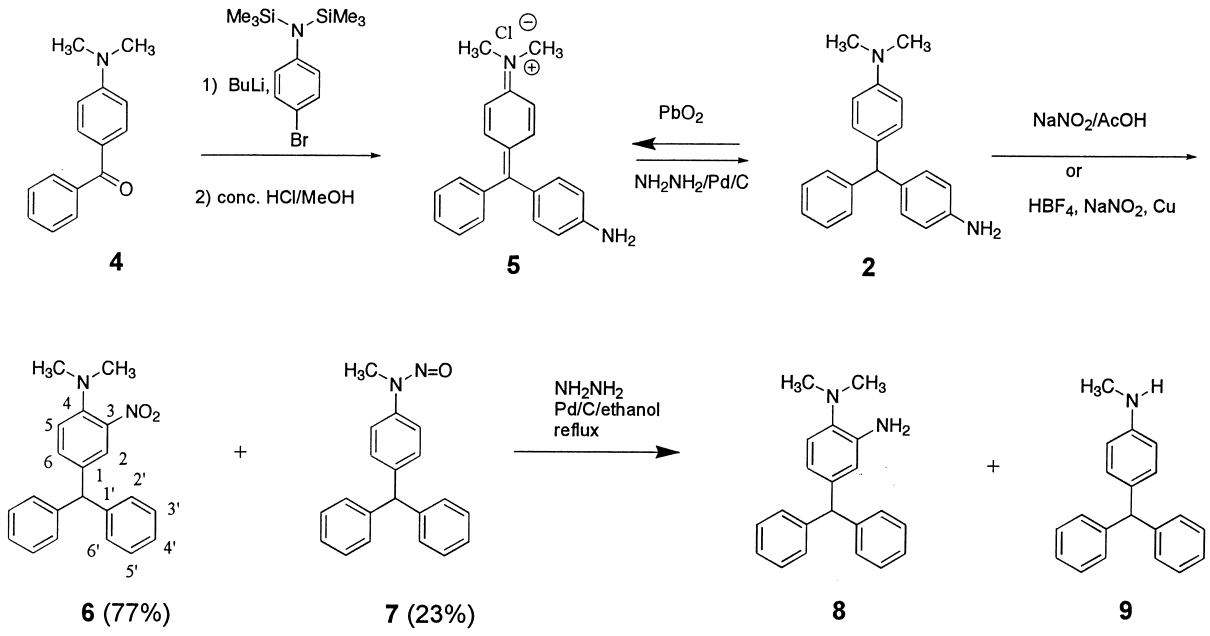


Scheme 1.

apoptosis in the transitional epithelium of the urinary bladder of mice and thyroid follicular epithelial cells in rats. Taken together, these data suggest that the *N*-demethylated amine metabolites of MG may undergo metabolic activation in a manner similar to that observed with carcinogenic aromatic and heterocyclic aromatic amines, i.e. an oxidation to *N*-hydroxylamines that may bind covalently to cellular DNA, either directly or after esterification, to form stable adducts.[9]

To elucidate the metabolic activation pathways of MG, we focused on 4-*N*,*N*-dimethylamino-4′-hydroxylaminotriphenylmethane (**1**, Fig. 1) as one of the presumed proximate MG metabolites responsible for the DNA adducts formed in vivo. *N*-Hydroxylamines are usually prepared by partial reduction of the corresponding nitro compounds. To explore this possibility, the preparation of the amino and nitro analogs, **2** and **3**, respectively, was required (Fig. 1). This paper reports the synthesis and full characterization of the target compounds and their analogs.

Results

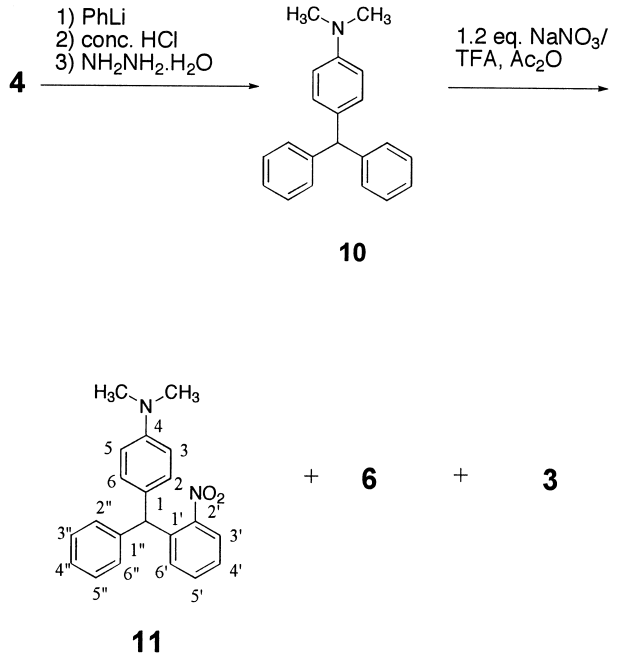
Synthesis of 2

The amino compound **2** was synthesized in 30% overall yield via a three-step reaction sequence as shown in Scheme 1. The initial reaction was patterned partially after the procedure of McDonald et al.[10] for the preparation of demethylated gentian violet metabolites, with some modification in the isolating procedure. It entailed an addition reaction of an aryllithium reagent derived from 4-bromo-bis-(*N*,*N*-trimethylsilyl)aniline with 4-(dimethylamino)-benzophenone (**4**), followed by treatment of the resulting carbinol with concentrated HCl in refluxing methanol. After evaporation of the solvent, the dark blue residue was partitioned between ether and H_2O. The ionic condensation product **5** was isolated from the aqueous layer and then purified further by a column chromatography on silica. This modified procedure was more efficient and gave a cleaner product than the literature method, which involved a laborious salt-induced precipitation. The positive ion mode electrospray ionization (ESI) mass spectrum of the product showed an intense signal at *m*/*z* 301, corresponding to the cationic portion of the molecule. The ^{1}H NMR spectrum showed two methyl signals at 3.34 ppm and a total of 13 expected aromatic protons in the 7.05–7.74 ppm range, in good agreement with the assigned structure of **5**. The UV spectrum exhibited an absorption maximum at 595 nm, 23 nm lower than that of MG.

The chromatic form **5** was reduced to the leuco form **2** by treatment with $NH_2NH_2{\cdot}H_2O$ and 10% Pd/C in refluxing absolute ethanol. The ESI mass spectrum of the product gave a distinctive $(M+H)^+$ ion at *m*/*z* 303 and product ions at *m*/*z* 288 (M^+-CH_3+1), 211 ($M^+-Ph-CH_3+1$ or $M^+-C_6H_4NH_2+1$), which were in good agreement with the assigned structure. The notable features in the ^{1}H NMR spectrum were the presence of a broad D_2O exchangeable singlet for NH_2 protons observed at 4.44 ppm and a benzylic proton at 5.30 ppm. Additional features include seven well-separated sets of signals for 13 aromatic protons and a sharp singlet at 2.87 ppm for the two methyl groups. Complete ^{1}H NMR spectral assignments of **2** have been made by homonuclear decoupling experiments and the results were fully consistent with the assigned structure. In addition, **2** was readily converted back to the chromatic **5** following oxidation using HPLC with a post-column reactor containing 10% PbO_2 on Celite,[11] further confirming the structure.

Attempted nitro-dediazoniation of 2 using $NaNO_2$/acetic acid

A 50% acetic acid solution of **2** was treated with excess $NaNO_2$ according to the nitro-dediazoniation procedure of



Scheme 2.

Grivas (Scheme 1).[12] The reaction afforded a bright orange solid that consisted of two products in an approximately 3:1 mixture. The two compounds were separated by HPLC and their structures were determined based on spectral (UV, MS, ^{1}H and ^{13}C NMR) and elemental analyses and characterization of subsequently reduced products.

The mono-nitro nature of the red crystalline major product (t_R16.4 min, system 2) was confirmed by the presence of an intense $(M+H)^+$ ion at *m/z* 333 in the ESI mass spectrum. NMR data, however, indicated that the product was the 3-nitro analog **6**, not the desired compound **3** (Scheme 1). Compound **6** showed two UV absorption maxima at 253 and 440 nm. A notable feature in the ^{1}H NMR spectrum was the lack of an upfield AX spectral pattern (usually below 7.00 ppm), which is characteristic for the aromatic protons *ortho* ($H_{3,5}$) and *meta* ($H_{2,6}$) to the *N*,*N*-dimethylamino group. This was consistent with nitration within the dimethylanilino ring. This conclusion was supported by the presence of six rather than seven sets of magnetically equivalent signals for the 13 aromatic protons in the ^{1}H NMR spectrum. A doublet at 7.46 ppm that was assigned as the proton *ortho* (H_2) to the nitro group showed a small coupling (J=2.3 Hz) and became narrowed upon irradiation of the benzylic proton at 5.62 ppm. The same signal, however, was not affected by irradiation of the methyl protons. These NMR data clearly indicated a single nitro substitution at the 3 position rather than at the 2 position. As expected, the hydrazine reduction of **6** afforded the corresponding amine derivative **8** (Scheme 1). The ESI mass spectrum of **8** displayed a $(M+H)^+$ ion at *m/z* 303 and a product ion spectrum consistent with the assigned structure, *m/z* 288 (M^+-CH_3+1) and 211 (M^+-CH_3-Ph+1). The ^{1}H NMR spectrum showed the presence of a broad singlet at 4.40 ppm for two NH_2 exchangeable protons. Complete ^{1}H NMR spectral assignments for **6** and **8** are fully consistent with the assigned structures.

The minor product (t_R 14.5 min, system 2) was isolated as a white solid and exhibited a single UV absorption at 272 nm. The most revealing aspect in the ^{1}H NMR spectrum was the presence of a single methyl proton signal at 3.43 ppm that was significantly deshielded compared to that (2.87 ppm) of the starting material **2**. The observed spectral pattern for 14 aromatic protons was relatively simple, suggesting a symmetrical nature for the molecule. Similarly, the ^{13}C NMR spectrum exhibited only five aromatic methine and three quaternary carbon signals and a highly shielded methyl carbon signal at 31.4 ppm. These NMR data suggest that the product is monodemethylated, with no additional substitution in the triphenylmethane ring system. The CI mass spectrum showed a M+H signal at *m/z* 303, while the direct probe El mass spectrum gave a low intensity signal at *m/z* 302 along with a base peak at *m/z* 272. These data are consistent with the *N*-nitroso structure for **7**. We were unable to obtain a satisfactory elemental analysis for **7**, but the CI mode HRMS gave the correct mass. Reduction ($NH_2NH_2\cdot H_2O$) of **7** was also conducted for additional characterization (Scheme 1). The ESI mass spectrum of the reduction product showed an intense $(M+H)^+$ ion at *m/z* 274 and readily recognizable fragment ions at *m/z* 259 (M^+-CH_3+1), and 182 (M^+-CH_3-Ph+1). The ^{1}H NMR spectral pattern was virtually identical to that of *N*,*N*-dimethyltriphenylmethane (**10**)(see below), except for the presence of one exchangeable proton at 4.81 ppm and a slightly shielded methyl signal at 2.74 ppm. The methyl signal (30.6 ppm) in the ^{13}C NMR spectrum was highly shielded as well. These spectral data are fully consistent with the structure of 4-*N*-methylaminotriphenylmethane (**9**). Taken all together, the structure of the minor product was assigned to the nitrosamine structure **7**.

Attempted nitro-dediazoniation of 2 using $HBF_4/NaNO_2$

Diazotization of **2** was also conducted by reacting the amine with 1 equiv. of $NaNO_2$ in aqueous HBF_4. The resulting diazonium tetrafluoroborate salt was then treated with excess $NaNO_2$ in the presence of copper powder (Scheme 1). HPLC/ESI-MS and ^{1}H NMR analyses indicated that the crude reaction product also contained approximately a 3:1 mixture of **6** and **7**.

Synthesis of 10

The synthesis involved a 3-step addition, dehydration, and reduction sequence similar to that used for the synthesis of **2**, starting from 4-(dimethylamino)benzophenone (**4**) and phenyllithium (Scheme 2). The overall yield by this sequence was 11%. Alternatively, **10** could be prepared by a similar sequence (not shown) using benzophenone and 4-bromo-*N*,*N*-dimethylaniline as starting materials, but with a poor overall yield (~2%). The ESI mass spectrum showed a $(M+H)^+$ ion at *m/z* 288. Not surprisingly, the ^{1}H NMR spectrum was very simple, showing only five groups of aromatic signals for fourteen aromatic protons. Complete ^{1}H spectral analysis and other spectra data (UV, ^{13}C NMR) were consistent with the structure of **10**.

Nitration of 10

The nitration was carried out with 1.2 equiv. of $NaNO_3$

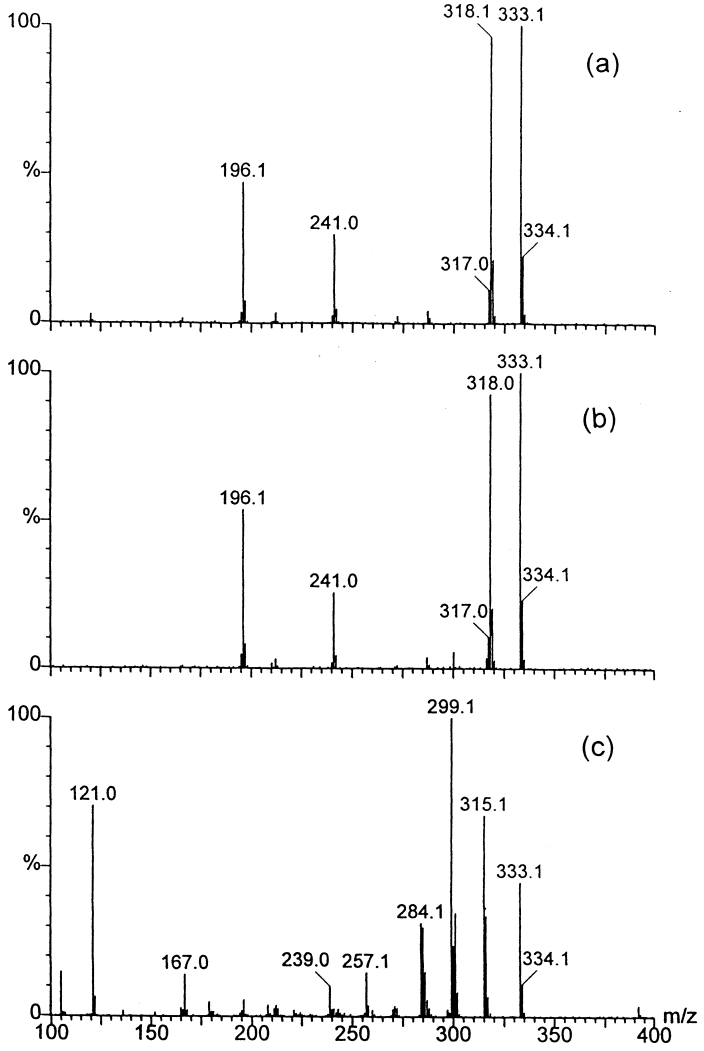
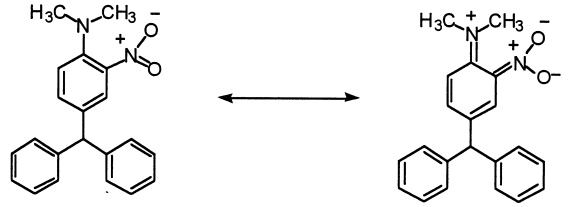


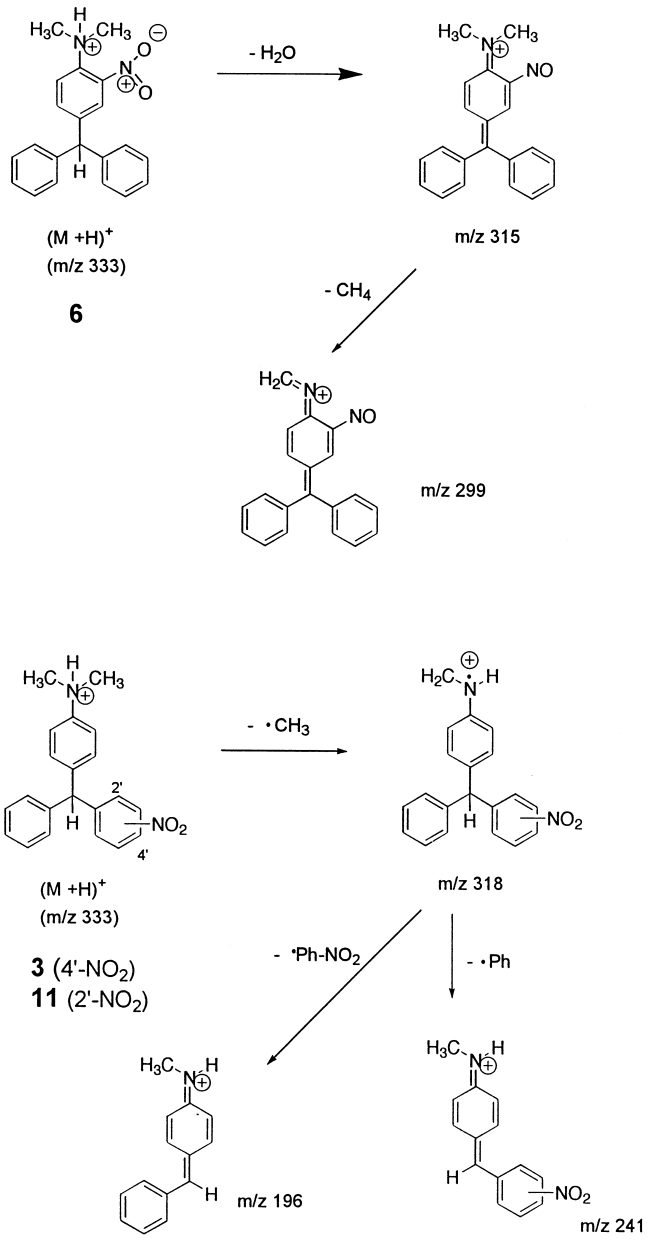
Figure 2. Electrospray ionization mass spectra of (a) **3**, (b) **11** and (c) **6**.

using trifluoroacetic acid/acetic anhydride as a mixed solvent (Scheme 2). HPLC analysis of the crude reaction residue showed the presence of four compounds: **6**, **11**, **3** and the unreacted starting material **10**, in decreasing order of polarity (t_R 5.2, 6.1, 6.7, 7.8 min, respectively) using HPLC system 1 (see Experimental). Their relative yields after HPLC separation were 28, 3, 39, 30%, respectively. The most polar product exhibited identical spectral (UV, ^{1}H and ^{13}C NMR, ESI-MS) characteristics as those of **6** obtained from the $NaNO_2$/acetic acid procedure described above. The next two eluting compounds showed the same $(M+H)^+$ ion at *m*/*z* 333 and similar product ion spectra (Fig. 2), but different NMR characteristics. The ^{1}H NMR spectra showed that they both retained the upfield $H_{3,5/2,6}$ AX pattern characteristic for the *N*,*N*-dimethylanilino ring, but differed in their general spectral patterns. The overall symmetric ^{1}H NMR spectral pattern of **3** was similar to that of its amino analog **2**, except for the lack of exchangeable protons and the unique deshieldings of the $H_{3',5'}$ (−1.59 ppm) and benzylic (−0.37 ppm) protons due to the electron withdrawing effect of the nitro group. These NMR data are consistent with the assigned structure of **3**. In contrast, the ^{1}H NMR spectrum of **11** exhibited complex aromatic proton signals for the same number (13) of aromatic protons. These results are in good agreement with an isomer in which a nitro group is attached to the *ortho* (H_2) position of one of the phenyl rings. Complete ^{1}H NMR assignments are consistent with the structure of **11**.



6 **6a**

The UV spectrum of **6** showed the presence of an extended n−π absorption at 440 nm, which is unique among the other mononitro isomers (**11** and **3**). While the ESI mass spectra of **3**, **6**, and **11** all showed the anticipated molecular $(M+H)^+$ ion at *m*/*z* 333, the product ion spectra of **6** vs **3** and **11** are significantly different (Fig. 2). The proposed fragmentation pathways are shown in Scheme 3.



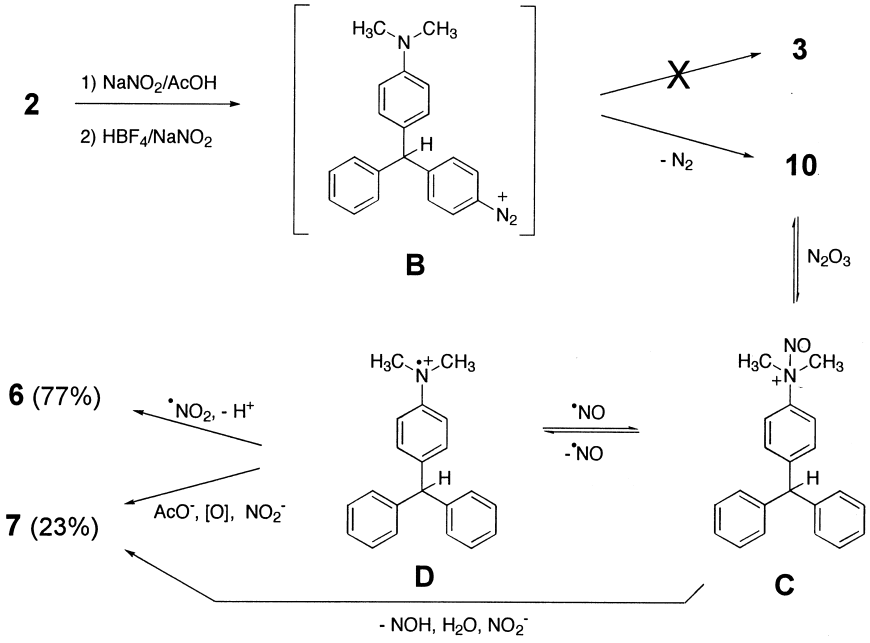
Scheme 3.

Compounds **3** and **11** showed an initial demethylation (*m*/*z* 318) followed by a loss of phenyl (*m*/*z* 241) or nitrophenyl (*m*/*z* 196) ring moieties. For **6**, the ions at *m*/*z* 315 and 299 are daughter ions derived from the $(M+H)^+$ ion. The *m*/*z* 315 fragment is consistent with intramolecular reduction involving the overall loss of H_2O (Scheme 3). Subsequent loss of a methyl group (plus H) is consistent with the *m*/*z* 299 daughter ion. The product ion spectra reflected clearly the difference in their nitro substitution pattern. Taken together, these spectroscopic data are consistent with **6a** being the major resonance contributor due to the electronic effect of the *ortho* nitro group.

Discussion

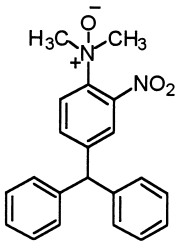
While a number of methods exist for the preparation of nitroaromatics, direct oxidation of the amines has been the method of choice for a variety of carcinogenic aromatic and heterocyclic nitro derivatives.[12–16] Presumably, this is due to the simplicity of the procedure and the ready availability of amino precursors. Accordingly, we tried initially to prepare **3** by a one step oxidation of the amino precursor **2**. Our attempts to convert **2** to the corresponding nitro derivative **3** by several oxidative systems (*m*-CPBA/CH_2Cl_2, dimethyldioxirane/THF, and H_2O_2/Na_2WO_4) under varying conditions were all unsuccessful.

We then turned our attention to a procedure reported by Grivas,[12] which involves a simple nitro-dediazoniation reaction of the amines with excess $NaNO_2$ in 50% acetic acid (Scheme 1). HPLC/ESI mass spectral analysis of the crude worked-up residue showed a prominent signal at *m*/*z* 333, presumably the $(M+H)^+$ ion for the nitro product. A weak signal observed at *m*/*z* 349 was tentatively assigned to the *N*-oxide (**A**). A bright orange solid obtained after passing



Scheme 4.

the residue through a short silica gel column, consisted of approximately a 3:1 mixture of **6** and **7**. Similar results were obtained when the amine was treated with $NaNO_2$ in aqueous fluoroboric acid, followed by $NaNO_2$ and powdered copper, the so-called nitro-Sandmeyer reaction.[17] In the latter case, however, no *N*-oxide compound (**A**) was detected. There was no evidence for the formation of the desired product **3** in either reaction. The two compounds (**6** and **7**) were separated by HPLC and their structures were fully characterized by UV, ^1H and ^{13}C NMR, ESI-MS and elemental analyses (see Results).



A

It appears that the reaction proceeds via a sequential process as depicted in Scheme 4; i.e. an initial exclusive hydrodeazoniation of the diazonium intermediate (**2→B→10**) followed by either nitration (**6**) or *N*-nitrosative dealkylation (**7**). It is well known that the reactions of *N*,*N*-dialkyl aromatic amines with nitrous acid give nitro compounds and nitrosamines, but the mechanisms of these transformations have not been well understood.[18–23] Loeppky et al.,[24,25] however, have recently presented strong evidence for the involvement of a radical cation clarifying the paths to the products of these reactions. Our data presented in Scheme 4 are consistent with their mechanistic interpretation. Thus, compound **10** reacts with NO to give a nitrosammonium ion **C**, which either undergoes reversible homolysis to form a radical cation intermediate **D** or loses NOH ultimately to provide the nitrosamine **7** by the establish hydrolysis/nitrosation mechanism. The radical cation **D** can then react with NO_2 to give **6** as the major and the sole nitro product (77%). Alternatively, *N*-α-CH deprotonation of **D** with subsequent oxidative generation of an immonium ion affords **7** as the minor product (23%). All three pathways are linked through the reversible homolysis of **C** to NO and the radical cation **D**.[24] The nitro compound **6** may also be formed by C-nitrosation of **10** followed by an oxidation, but is slow compared to the radical cation route.

The complete absence of the desired product **3** in both the Grivas ($NaNO_2$/acetic acid)[12] and $HBF_4/NaNO_2$ conditions implies that the diazonium group in **B** is replaced far more efficiently with hydrogen (hydro-dediazoniation) than with nitrite ion (nitro-dediazoniation, Scheme 4). This reaction preference is remarkable in view of numerous literature examples that the Sandmeyer type reaction is generally known to occur in high yields through a free radical process, particularly when Cu is used.[17,26,27] It is possible, however, that because of the facile oxidation potential of the substrate, an intermolecular hydride transfer from the central CH to the carbon losing the N_2 could occur.

Another intriguing fact is the observed regio-specificity, i.e. an exclusive formation of **6** with no trace of **3**. This is in contrast with the fact that nitration of **10** with $NaNO_3$/trifluoroacetic acid/acetic anhydride afforded all three possible nitration products (**6**, **3**, **11**, Scheme 2). Thus, the question is whether **10** is actually involved as an intermediate as depicted in Scheme 4. To clarify this, **10** was treated separately under the Grivas reaction conditions. HPLC analysis of the reaction mixture indicated an exclusive formation of **6** and a trace of **11**, but again, no **3** was detected. These results support the involvement of **10** as an intermediate in the reaction. It has been shown that the regioselectivity in nitration of active substrates like aniline-type compounds is strongly influenced by the

nitrating conditions.[17,28] The species undergoing nitration under strong acidic conditions (i.e. trifluoroacetic acid) is actually the conjugate acid of **10**, thus suppressing nitration at the *ortho* position (H_3). This explains why the nitration occurred also in less activated positions, 2′ and 4′, affording **11** and **3**. However, when the conditions are mildly acidic or neutral, the free amine underwent electrophilic substitution to provide **6** exclusively.

In summary, this paper describes the preparation of **2** and **3** as precursors for the presumed DNA-binding metabolites of malachite green. A simple nitro-dediazoniation reaction of **2** under mild conditions did not provide the desired product **3**. Instead, products derived from hydro-dediazoniation followed by nitration (**6**) and *N*-dealkylative nitrosation (**7**) were obtained. This unexpected result is obviously due to the unique structural features associated with **2**. The target nitro product **3**, however, was prepared by direct nitration of *N*,*N*-dimethylaminotriphenylmethane (**10**). The seemingly simple nitro-dediazoniation procedure should be used with caution for certain functionalized arylamines and heterocyclic amines. It should be mentioned, however, that the chemistry of the heterocyclic system used by Grivas,[12] may differ sufficiently from that expected in the aromatic rings of an easily oxidizable tiphenylmethane derivative. We are currently attempting to convert **3** into the *N*-hydroxyl derivative that would subsequently be reacted with DNA to form adducts. The results of these studies will be reported elsewhere.

Experimental

Materials and methods

Tetrahydrofuran (THF) was distilled from $LiAlH_4$, followed by a fresh distillation from sodium/benzophenone prior to use. All reagents were purchased from Aldrich (Milwaukee, WI). 4-Bromo-bis(*N*,*N*-trimethylsilyl)aniline was prepared according to the literature procedure by Pratt et al.[29] All organic solvents were purchased from Fisher Scientific (Pittsburgh, PA). Melting points were determined on a Thermoelectrical melting point apparatus and are uncorrected. Elemental analyses were obtained from M-H-W Laboratories, Phoenix, AZ. High-resolution mass mass spectra (HRMS) were obtained at the University of Illinois Mass Spectrometry Laboratory, Urbana-Champaign, IL. All ^1H and ^{13}C NMR spectra were recorded in acetone-d_6 on a Bruker AM500 spectrometer operating at 500 and 126 MHz, respectively. Chemical shifts are expressed in ppm with respect to the internal standard tetramethylsilane: s, singlet; bs, broad singlet; d, doublet; dd, doublet of doublets; m, multiplet; C_q (quaternary carbon). Complete ^1H NMR assignments were obtained by conducting homonuclear decoupling experiments. HPLC separations with a photodiode array detector were conducted using the following systems: system 1 (Ultrasphere C18, 4.6×250 mm, 5 μm, isocratic 90% methanol/10% H_2O, 1 mL/min); system 2 (Ultrasphere C18, 4.6×250 mm, 5 μm, isocratic 75% methanol/25% H_2O, 1 mL/min); system 3 (Spherisorb CN, 4.6×250 mm, 5 μm, 30 min gradient, 10 to 90% acetonitrile/l00 mM ammonium acetate, pH 4.5, 1 mL/min). The LC separation for mass spectrometry was performed using an ODS-3 column (4.6×250 mm, 5 μm, 10 min gradient, 50 to 100% acetonitrile/50 mM ammonium acetate, pH 4.5, 1 mL/min). For direct infusion studies, the solvent was delivered at about 10 μL/min using a syringe pump. A Platform II single quadrupole or Quattro LC triple quadrupole mass spectrometer, equipped with either an atmospheric pressure chemical ionization (with heated nebulizer probe at 550°C) or electrospray (capillary potential 3 kV) interface were used with an ion source temperature of 150°C. For MS measurements, HPLC was used for analysis of positive ions acquired in full scan (*m*/*z* 100–400 in 1 s cycle time). For MS/MS measurements, direct infusion of pure analytes was performed and product ion spectra were acquired using a collision cell gas pressure (Ar) of 4×10^{-3} mbar and collision energies of between 15–35 eV. The mass spectrometers were calibrated over the mass range *m*/*z* 85–1200 using a solution of polyethylene glycols.

***N*,*N*-Didesmethylmalachite green chloride (5).** Butyllithium (4.8 mL, 2.5 M in hexane, 12 mmol) was added via a syringe to a solution of 4-bromo-*N*,*N*-bis(trimethylsilyl)aniline (3.15 g, 10 mmol) in a dry THF (100 mL) at −78°C under an argon atmosphere. The mixture was stirred at the same temperature for 5 h and then a solution of 4-(dimethylamino)benzophenone (**4**, 4.5 g, 10 mmol) in 80 mL of THF was added dropwise. The reaction mixture was warmed slowly to room temperature and stirred overnight. The reaction was quenched by addition of H_2O and the residual THF was evaporated. The resulting aqueous residue was extracted with ether (3×80 mL) and the combined ether extracts were washed with H_2O and dried over anhydrous $MgSO_4$. Concentration by a roto-evaporator gave 4.26 g of the carbinolic intermediate as a green viscous residue. The residue was transferred to a flask containing 100 mL of methanol and 1 mL of concentrated HCl. The light green solution turned dark blue immediately upon contact with the acid solution. The mixture was refluxed for 1 h. After cooling, the solution was concentrated in vacuo to give a viscous, blue solid that was subsequently partitioned between 20 mL of H_2O and 50 mL of ether in a 100-mL separatory funnel. The aqueous layer was washed repeatedly with ether until TLC of the ether washings showed no starting materials. The aqueous layer was then concentrated to dryness via a Speedvac overnight. The residue was subsequently purified by column chromatography on silica using chloroform:methanol (9:1) as an eluent to afford 1.94 g of **5** (58%) as shiny dark blue crystals: mp>160°C (dec); UV λ_{max} (log ϵ) 312 nm (4.27), 411 nm (4.30), 595 nm (4.98); ESI-MS *m*/*z* 301 (M^+-Cl); HPLC (system 3) t_R 22.4 min; ^1H NMR δ 3.34 (s, 6, dimethyl), 7.05 (d, 2, $H_{3,5}$, J=9.2 Hz), 7.25 (d, 2, $H_{2,6}$, J=8.9 Hz), 7.35–7.41 (m, 5), 7.61 (m, 2), 7.74 (m, 2); Anal. Calcd for $C_{21}H_{21}N_2Cl$: C, 74.88, H, 6.28, N, 8.32. Found: C, 75.03; H, 6.18, N, 8.33.

4-(*N*,*N*-Dimethylamino)-4′-aminotriphenylmethane (2). Compound **5** (1.625 g, 4.8 mmol) was treated with $NH_2NH_2\cdot H_2O$ (800 μL, 15.5 mmol) and 10% Pd/C (250 mg) in absolute ethanol (150 mL) and the mixture was heated to reflux in an oil-bath. The dark blue solution turned colorless upon refluxing for about 10 min. The refluxing continued for another hour and the solution was

filtered to remove the catalyst. The filtrate was concentrated and partitioned between H_2O and ether. The aqueous layer was extracted with ether (3×50 mL) and the combined ether extracts were washed with water. The ether layer was dried over anhydrous $MgSO_4$ and the solution was filtered and evaporated. The resulting oily residue was applied to a silica gel column and eluted with ethyl acetate and hexane (1:4). Appropriate portions were pooled, concentrated and recrystallized from ethyl acetate and hexane to give 894 mg (62%) of an analytically pure product **2**. Mp 114–115°C; UV λ_{max} (log ϵ) 255 nm (4.36), 292 nm (shoulder, 3.57); HPLC (system 3) t_R 18.8 min; ESI-MS *m/z* 303 $(M+H)^+$, 288 (M^+-CH_3+1), 211 $(M^+-Ph-CH_3+1$ or $M^+-C_6H_4NH_2+1)$; ^{1}H NMR δ 2.87 (s, 6, dimethyl), 4.44 (bs, 2, NH_2, D_2O exchangeable), 5.30 (s, 1, benzylic), 6.57 (d, 2, $H_{3,5}$, J=8.5 Hz), 6.66 (d, 2, $H_{3',5'}$, J=8.9 Hz), 6.80 (d, 2, $H_{2,6}$, J=8.4 Hz), 6.92 (d, 2, $H_{2',6'}$, J=8.8 Hz), 7.12 (m, 2, $H_{2'',6''}$), 7.14 (m, 1, $H_{4''}$), 7.24 (m, 2, $H_{3'',5''}$); ^{13}C NMR δ 40.7 (dimethyl), 56.0 (benzylic), 113.2, 115.0, 120.0 (C_q), 126.5, 128.7, 128.9 (C_q), 130.0, 130.5, 130.6, 133.6 (C_q), 133.8 (C_q), 146.7 (C_q); Anal. Calcd for $C_{21}H_{22}N_2$: C, 83.40; H, 7.33; N, 9.26. Found: C, 83.51; H, 7.26; N, 9.13.

Attempted nitro-dediazoniation of 2

Method A—using Grivas' conditions. A solution of **2** (604 mg, 2.0 mmol) in 50% acetic acid (10 mL) was added dropwise to a solution of $NaNO_2$ (1.38 g, 20 mmol) in H_2O (25 mL) in an ice-bath. The solution turned initially to an orange color and then a dark brown viscous residue. After 30 min, the mixture was neutralized by addition of saturated $NaHCO_3$. The aqueous solution was then extracted with chloroform three times. The combined organic extracts were washed subsequently with saturated $NaHCO_3$ and H_2O, and dried over anhydrous $MgSO_4$. The extracts were evaporated and the residue was subjected to column chromatography on silica using ethyl acetate and hexane (1:9) as the eluant. The distinct yellow band was collected and concentrated to give 225 mg of a bright orange solid, which is an approximately 3:1 mixture of **6** and **7**. The mixture was separated by semi-prep HPLC using system 2. **6** (128 mg): mp 98–99°C (ethyl acetate/hexane); UV λ_{max} (log ϵ) 253 nm (4.44), 440 nm (3.45); HPLC t_R 16.4 min (system 2); ESI-MS *m/z* 333 $(M+H)^+$, 315 (M^+-H_2O+1), 299 $(M^+-H_2O-CH_4+1)$; ^{1}H NMR δ 2.84 (s, 6, dimethyl), 5.62 (s, 1, benzylic), 7.14 (d, 1, H_5, J=8.7 Hz), 7.17 (m, 4, $H_{2',6',2'',6''}$), 7.23 (m, 2, $H_{4',4''}$), 7.27 (dd, 1, H_6, J=8.7, 2.3 Hz), 7.32 (m, 4, $H_{3',5',3'',5''}$), 7.46 (d, 1, H_2, J=2.3 Hz); ^{13}C NMR δ 42.6 (dimethyl), 56.0 (benzylic), 119.5, 127.1, 127.4, 129.3, 130.1, 134.9, 135.2 (C_q), 144.5 (C_q), 145.5 (C_q); Anal. Calcd for $C_{21}H_{20}N_2O_2$: C, 75.88; H, 6.07; N, 8.43. Found: C, 76.03; H, 5.92; N, 8.60. **7** (38 mg): mp 138–139°C (methanol/H_2O); direct probe EI-MS *m/z* 302 (M^+, 10%), 272 (M^+-NO, 100%); HPLC t_R 14.5 min (system 2); UV λ_{max} (log ϵ) 272 nm (3.94); ^{1}H NMR δ 3.43 (s, 3, methyl) 5.71 (s, 1, benzylic), 7.19 (m, 4, $H_{2'',6''}$), 7.25 (m, 2, $H_{4',4''}$), 7.29 (m, 2, $H_{2,6}$), 7.33 (m, 4, $H_{3'',5''}$), 7.57 (m, 2, $H_{3,5}$); ^{13}C NMR δ 31.4 (methyl), 56.9 (benzylic), 112.0, 127.3, 129.3, 130.1, 131.1, 141.7 (C_q), 144.1 (C_q), 144.7 (C_q); HRMS (CI mode using methane gas) calcd for $C_{20}H_{19}N_2O$ $(M+H)^+$ 303.1497, found 303.1491.

Method B—using HBF_4/$NaNO_2$. Compound **2** (284 mg, 0.94 mmol) was dissolved in 0.5 mL of 48% aqueous HBF_4 and placed in an ice-bath. An ice-cold solution of $NaNO_2$ (65 mg, 0.94 mmol) was added dropwise with stirring. The resulting brown viscous residue was filtered and washed with H_2O and transferred to a new flask containing excess $NaNO_2$ (200 mg) and copper powder (10 mg) in 2 mL of H_2O. After stirring for 10 min, the mixture was treated with 10 mL of 1.0 M NaOH and extracted with chloroform. The combined organic extracts were washed with H_2O, dried over anhydrous $MgSO_4$, and evaporated. The crude residue was passed through a short silica gel column using ethyl acetate/hexane (1:9) as the eluent. A bright yellow band was collected to give 120 mg of an orange solid. According to ^{1}H NMR and HPLC/ESI-MS analyses using HPLC system 3, the product contained an approximately 3:1 mixture of **6** and **7**.

4-(*N*,*N*-Dimethylamino)-3-aminotriphenylmethane (8). Compound **6** (100 mg, 0.3 mmol) was treated with $NH_2NH_2.H_2O$ (100 μL, 2 mmol) and 10% Pd/C (20 mg) in 20 mL of absolute ethanol and refluxed for 1 h. The warm solution was filtered, concentrated, and extracted with ether. The ether extracts were washed with H_2O, dried over anhydrous $MgSO_4$, and evaporated. The residue was purified by a silica chromatography (ethyl acetate/hexane=1:4) to give an analytically pure product **8** as a viscous residue (78 mg, 86%). UV λ_{max} (log ϵ) 250 nm (4.49), 297 nm (4.28); HPLC (system 2) t_R 12.4 min; ESI-MS *m/z* 303 $(M+H)^+$, 288 (M^+-CH_3+1), 211 (M^+-CH_3-Ph+1); ^{1}H NMR δ 2.60 (s, 6, dimethyl), 4.40 (bs, 2, NH_2, D_2O exchangeable), 5.41 (s, 1, benzylic), 6.36 (dd, 1, H_6, J=8.1, 2.1 Hz), 6.51 (d, 1, H_2, J=2.1 Hz), 6.87 (d, 1, H_5, J=8.1 Hz), 7.14 (m, 4, $H_{2',6',2'',6''}$), 7.18 (m, 2, $H_{4',4''}$), 7.27 (m, 4, $H_{3',5',3'',5''}$); ^{13}C NMR δ 43.7 (dimethyl), 57.3 (benzylic), 116.5, 119.1, 119.3, 126.8, 128.9, 130.1, 139.3 (C_q), 140.3 (C_q), 142.9 (C_q), 145.5 (C_q); Anal. Calcd for $C_{21}H_{22}N_2$: C, 83.40; H, 7.33; N, 9.26. Found: C, 83.19; H, 7.43; N, 9.11.

4-Methylaminotriphenylmethane (9). Compound **7** (39 mg, 0.13 mmol) was reduced as described above for **8**. A usual workup followed by silica column purification gave a pure product (30 mg, 85 %) as a viscous residue. UV λ_{max} (log ϵ) 253 nm (4.24), 298 nm (3.41); HPLC (system 2) t_R 10.9 min; ESI-MS *m/z* 274 $(M+H)^+$, 259 (M^+-CH_3+1), 182 (M^+-CH_3-Ph+1); ^{1}H NMR δ 2.74 (s, 3, methyl), 4.81 (bs, 1, NH, D_2O exchangeable), 5.45 (s, 1, benzylic), 6.53 (d, 2, $H_{3,5}$, J=8.6 Hz), 6.87 (d, 2, $H_{2,6}$, J=8.5 Hz), 7.13 (m, 4, $H_{2',6',2'',6''}$), 7.17 (m, 2, $H_{4',4''}$), 7.27 (m, 4, $H_{3',5',3'',5''}$); ^{13}C NMR δ 30.6 (methyl), 56.9 (benzylic), 112.6, 126.8, 128.9, 130.1, 130.7, 132.4 (C_q), 146.0 (C_q), 150.0 (C_q); Anal. Calcd for $C_{20}H_{19}N$: C, 87.87; H, 7.01; N, 5.12. Found: C, 87.64; H, 7.02; N, 5.10.

***N*,*N*-Dimethyltriphenylmethane (10).** *Route A:* Phenyllithium (5.6 mL, 1.8 M in cyclohexane/ether, 10 mmol) was added dropwise to a solution of 4-(dimethylamino)-benzophenone (**4**, 2.25 g, 10 mmol) in dry ether at −78°C under an argon atmosphere. Upon addition of phenyllithium, the milky white solution turned sequentially to green, then yellow, and finally clear. The mixture was warmed to room temperature overnight with stirring. The mixture was worked up as described for **5** to give 3.69 g of a

viscous residue that was converted to the leuco product **10** by a sequence similar to that used for the synthesis of **2** described above. The carbinolic intermediate was treated with concentrated HCl (1 mL) in 50 mL of refluxing methanol that turned the solution dark red. The chromatic intermediate (570 mg) was obtained using the usual extraction procedure. Reduction with $NH_2NH_2{\cdot}H_2O$ and 10% Pd/C followed by silica column purification and recrystallization from pentane afforded 310 mg (11% overall) of an analytically pure **10** as a white solid. Mp 131–132°C; UV λ_{max} (log ϵ) 260 nm (4.39), 411 nm (3.46); HPLC (system 1) t_R 7.81 min; ESI-MS *m/z* 288 $(M+H)^+$, 273 (M^+-CH_3+1), 196 (M^+-CH_3-Ph+1); ^{1}H NMR δ 2.89 (s, 6, dimethyl), 5.48 (s, 1, benzylic), 6.68 (d, 2, $H_{3,5}$, J=8.8 Hz), 6.95 (d, 2, $H_{2,6}$, J=8.8 Hz), 7.13 (m, 4, $H_{2',6',2'',6''}$), 7.18 (m, 2, $H_{4',4''}$), 7.26 (m, 4, $H_{3',5',3'',5''}$); ^{13}C NMR δ 40.6 (dimethyl), 56.7 (benzylic), 113.3, 126.8, 128.9, 130.1, 130.6, 132.6 (C_q), 145.8 (C_q), 150.2 (C_q); Anal. Calcd for $C_{21}H_{21}N$: C, 87.76; H, 7.37; N, 4.87. Found: C, 87.54; H, 7.32; N, 4.97.

Route B: Butyllithium (1.6 M in hexane, 12.5 mL, 20 mmol) was added to a dry THF solution of 4-bromo-*N*,*N*-dimethylaniline (4.0 g, 20 mmol) at −78°C. A milky white solution was obtained and stirred for additional 4 h at the same temperature. Benzophenone (3.64 g, 20 mmol) in dry THF (100 mL) was added dropwise under an argon atmosphere. The milky solution turned clear yellow upon the addition of benzophenone. The solution was warmed to room temperature overnight and the reaction was worked up as previously described to give 6.74 g of a yellow viscous residue. The carbinolic intermediate was converted to **10** using a similar sequence as in Route A to give 130 mg (2.3% overall) of a pure product **10**.

Nitration of 10

$NaNO_3$ (116 mg, 1.35 mmol) was added in small portions to a solution of **10** (316 mg, 1.11 mmol) in trifluoroacetic acid (6 mL) and acetic anhydride (5 mL). The solution turned red and then dark red. After stirring at room temperature for 2 h, the mixture was quenched by adding ice and 1 mL of H_2SO_4 and stirred for 10 h to ensure complete hydrolysis of acetic anhydride. The mixture was extracted with ethyl acetate. The combined organic extracts were washed with 1 M NaOH, saturated $NaHCO_3$, and H_2O and dried over anhydrous $MgSO_4$. The crude residue (407 mg) obtained after evaporating the solvent was passed through a short silica column and subjected to semi-prep HPLC separation using system 2 (t_R/isolated amount): **6** (16.3 min/41 mg), **11** (19.4 min/5 mg), **3** (21.4 min/56 mg) and the recovered starting material **10** (25.1 min/43 mg). The structures of **6** and **10** were confirmed by comparison of spectral and chromatographic data with the authentic samples prepared above.

4-(*N*,*N*-Dimethylamino)-2′-nitrotriphenymethane (11). UV λ_{max} (log ϵ) 263 nm (4.74); HPLC (system 1) t_R 6.1 min; ESI-MS *m/z* 333 $(M+H)^+$, 318 (M^+-CH_3+1), 241 (M^+-CH_3-Ph+1); ^{1}H NMR δ 2.90 (s, 6, dimethyl), 5.70 (s, 1, benzylic), 6.71 (d, 2, $H_{3,5}$, J=8.8 Hz), 6.99 (d, 2, $H_{2,6}$, J=8.8 Hz), 7.17 (dd, 2, $H_{2'',6''}$, J=8.0, 1.6 Hz), 7.23 (m, 1, $H_{4''}$), 7.32 (m, 2, $H_{3'',5''}$), 7.60 (m, 2, $H_{4',6'}$), 7.99 (m, 1, $H_{5'}$), 8.09 (m, 1, $H_{3'}$); ^{13}C NMR δ 40.6 (dimethyl), 56.1 (benzylic), 113.4, 122.0, 124.5, 127.3, 129.3, 130.1, 130.3, 130.6, 131.2 (C_q), 136.5,144.6 (C_q), 148.4 (C_q), 150.5 (C_q), 153.8 (C_q); HRMS calcd for $C_{21}H_{20}N_2O_2$ 332.1525, found 332.1526.

4-(*N*,*N*-Dimethylamino)-4′-nitrophenylmethane (3). Mp 72–73°C; UV λ_{max} (log ϵ) 265 nm (4.77); HPLC (system 1) t_R 6.7 min; ESI-MS, *m/z* 333 $(M+H)^+$, 318 (M^+-CH_3+1), 241 (M^+-CH_3-Ph+1); ^{1}H NMR δ 2.90 (s, 6, dimethyl), 5.67 (s, 1, benzylic), 6.70 (d, 2, $H_{3,5}$, J=8.9 Hz), 6.97 (d, 2, $H_{2,6}$, J=8.7 Hz), 7.16 (d, 2, $H_{2'',6''}$, J=7.3 Hz), 7.23 (m, 1, $H_{4''}$), 7.31 (d, 2, $H_{3'',5''}$, J=7.8 Hz), 7.40 (d, 2, $H_{2',6'}$, J=8.8 Hz), 8.17 (d, 2, $H_{3',5'}$, J=8.8 Hz); ^{13}C NMR δ 40.6 (dimethyl), 56.4 (benzylic), 113.4, 124.1, 127.3, 129.3, 130.1, 130.6, 131.1 (C_q), 131.2, 144.5 (C_q), 150.5 (C_q), 153.8 (C_q); Anal. Calcd for $C_{21}H_{20}N_2O_2$: C, 75.88; H, 6.07; N, 8.43. Found: C, 75.72; H, 5.84; N, 8.37.

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