Synthesis and Reactions of Paramagnetic Aromatic Aldehydes as Useful Synthetic Building Blocks

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Dedicated to Prof. Károly Lempert on the occasion of his 80th birthday

Abstract: Starting from paramagnetic benzaldehyde, a benzophenone-type photoactivable spin label, paramagnetic warfarin and phenindione were synthesized. Nitration of protected benzylic alcohol led to 2-nitrobenzylmethanethiosulfonate, a thiol specific spin label, and 2-nitroaldehyde, which was a key compound for paramagnetic indigo, salicylic acid and nifedipine.

Key words: anticoagulant, free radicals, drugs, indole, pyridines

The past 40 years have witnessed the more extended utilization of nitroxides on many fields in chemistry such as co-oxidants,[1] stoichiometric and catalytic antioxidants,[2] spin labels,[3] MRI agents[4] and spin traps.[5] The first and the most wide-spread utilization from the above mentioned applications is the spin label, which is used to evaluate function and structure of a protein or an enzyme. This means that a paramagnetic reagent modifies the protein side chain to get information on structure and function by EPR spectroscopy. The other approach for this kind of biological study is modification of a small molecule, actually a substrate, without significant change in its structure. In practice this requires that the characteristic functional groups must remain intact, so a carbon–carbon bond formation is needed to form in the presence of nitroxide free radical moiety. In our laboratory we have synthesized several biologically active molecules modified with stable nitroxide free radical such as flavones,[6] galactose,[7] ebselen,[8] omeprazole,[9] and menadione.[9] These paramagnetically modified biologically active compounds can be obtained through different methods. One of the most important approach is the Grignard reaction of nitrones, followed by oxidation of the *N*-hydroxylamine product.[10–12] Treatment of TMPO (2,2,5-trimethyl-3,4-dihydro-2*H*-pyrrol-1-oxide) with 4-dimethoxymethylphenylmagnesium bromide followed by removal of protecting group gave paramagnetic benzaldehyde **1**,[8] which could be oxidized or reduced for synthesis of useful substrates. This readily available spin labeled benzaldehyde was published from our laboratory earlier,[8] however, further utilization was not outlined. In this paper we report possible transformations of this compound to spin labels and biologically active paramagnetic compounds.

Treatment of **1** with phenylmagnesium bromide and oxidation of the intermediate secondary alcohol (not isolated) with MnO_2 gave the spin label **2** with the benzophenone photoactivable group.[13] Reduction of **1** with $NaBH_4$ yielded the benzyl alcohol **3**. The nitration of **3** with a mixture of nitric acid–sulfuric acid unfortunately oxidized the alcohol **3** to aldehyde **1** without nitration. The problem could be solved by protecting of benzylic hydroxyl group as its acetate.[14] To achieve this, compound **3** was treated with acetyl chloride in the presence of Et_3N to afford ester **4**. Nitration of **4** at low temperature gave the desired 2-nitro derivative without hydrolysis of protecting group and the crude product was deacetylated by Zemplen's method to give 2-nitroalcohol **5**. The alcohol was converted to the corresponding mesylate, with methanesulfonyl chloride in the presence of Et_3N and this mesylate was substituted with bromine by treatment with LiBr in acetone[15] to give compound **6**. The methanethiosulfonate reagent **7** was obtained by treatment of bromide **6** with $NaSSO_2CH_3$ in aqueous acetone. This reagent is capable of making reversible disulfide bond with SH group of cysteine in proteins,[16] while the nitro group is capable of making further secondary interactions, such as H-bonds.

Aldol condensation of **1** with excess acetone gave the paramagnetic benzalacetone **8** in the presence of base, the Michael addition of this product with 4-hydroxycoumarine in water gave the **9** paramagnetic derivative of warfarin, the most widely prescribed anticoagulant drug.[17] We hope nitroxide moiety will synergize the effect of warfarin, because nitroxides were reported to exhibit also anticoagulant effect.[18] An other paramagnetic anticoagulant compound was achieved with condensation of compound **1** with phthalide in ethanolic NaOEt solution followed by rearrangement to give indan-1,3-dione **10**, the paramagnetic phenindione.[19] Treatment of compound **10** with hydrazine hydrate followed by oxidation of hydroxylamine in pyrrolidine ring with MnO_2 afforded compound **11**[20] as a paramagnetic derivative of phthalazinone type PARP inhibitors[21] (Scheme 1).

Oxidation of compound **5** with activated MnO_2 in chloroform[22] yielded nitroaldehyde **12**, a key compound for further synthesis. To prove the substitution pattern of

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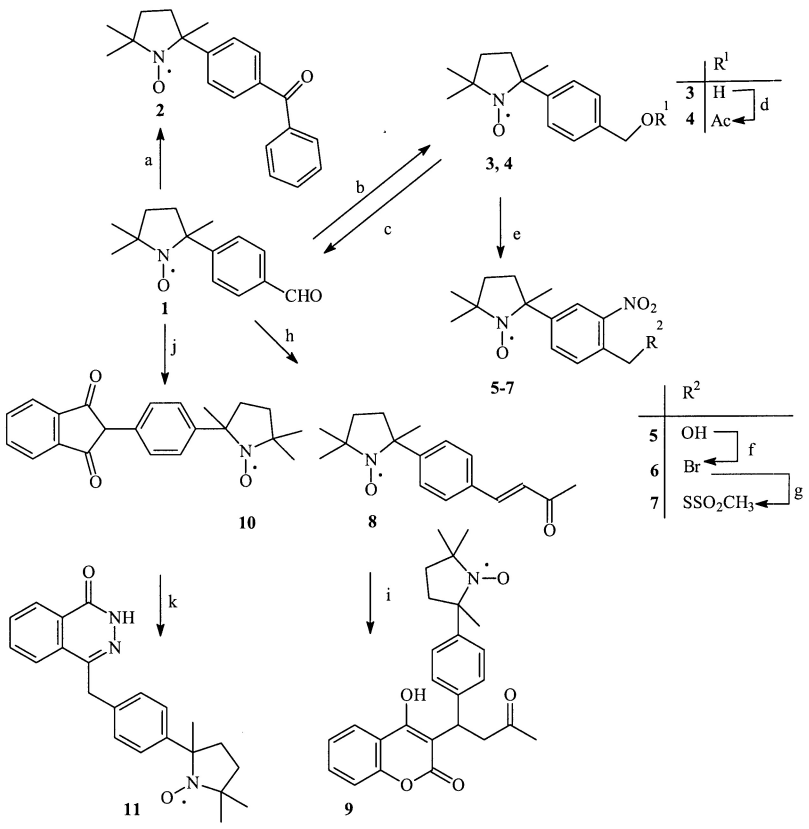


Scheme 1 *Reagents and conditions:* (a) phenylmagnesium bromide (1.1 equiv), THF, reflux 1 h, r.t., 8 h, then aq NH_4Cl, activated MnO_2 (5.0 equiv), $CHCl_3$, reflux, 1 h, 47%; (b) $NaBH_4$ (1.1 equiv), EtOH, r.t., 72%; (c) H_2SO_4–HNO_3 (2:1), 0 °C → r.t., 2 h, then K_2CO_3 to pH 8, 66%; (d) AcCl (1.1 equiv), Et_3N (1.1 equiv), CH_2Cl_2, 0 °C → r.t., 1 h, 75%; (e) for **5** H_2SO_4–HNO_3 (2:1), 0 °C → r.t., 3 h, then K_2CO_3 to pH 8, extraction with $CHCl_3$, then NaOMe (0.1 equiv), MeOH, r.t., 30 min, 42%; (f) MsCl (1.1 equiv), Et_3N (1.1 equiv), CH_2Cl_2, 0 °C → r.t., 1 h, then LiBr (2.0 equiv), acetone, reflux, 1 h, 54%; (g) $NaSSO_2CH_3$ (2.0 equiv), acetone–H_2O, 1 h, reflux, 43%; (h) acetone, aq NaOH (1.0 equiv), r.t., 12 h, 61%; (i) 4-hydroxycoumarine (1 equiv), H_2O, reflux, 12 h, 30%; (j) phthalide (1.0 equiv), MeOH, NaOMe (4 equiv), 0 °C → r.t., then reflux 5 h, 36%; (k) $N_2H_4{\cdot}H_2O$, reflux, 3 h, then H_2O, extraction with $CHCl_3$, activated MnO_2 (1 equiv), O_2, 15 min, r.t., 42%.

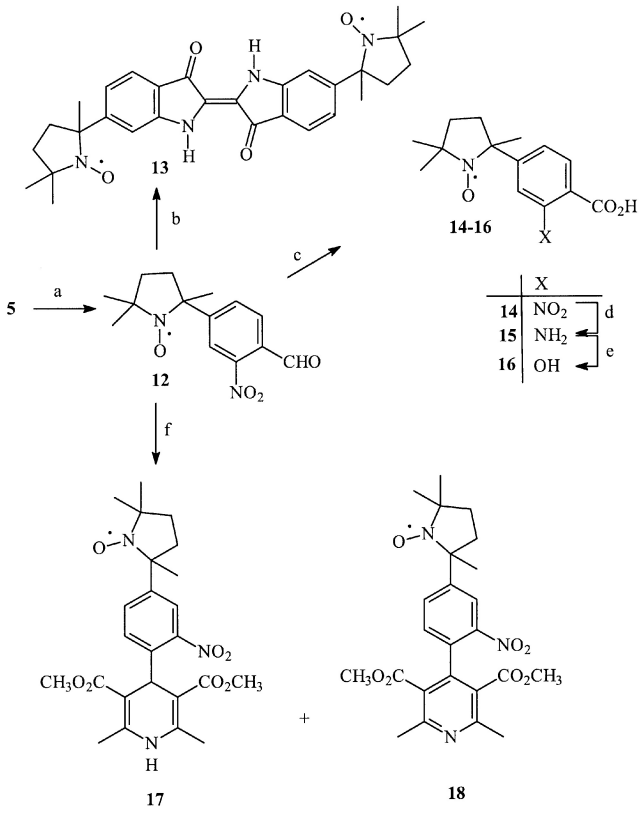
the benzene ring by NMR spectroscopy compound **12** was reduced by ascorbic acid followed by treatment with acetyl chloride in the presence of Et_3N to give the diamagnetic *O*-acetyl compound.[23] The substitution pattern of the aromatic region could be unequivocally established by the multiplicity of the aromatic protons as well as the coupling constant values determined from the ^1H NMR spectrum of diamagnetic *O*-acetyl compound.

The reaction of **12** with acetone and NaOH gave a paramagnetic indigo biradical **13** in a typical Baeyer–Drewson reaction featuring the 2-nitrobenzaldehyde.[24] Oxidation of **12** with Ag_2O in aqueous alkali solution[12] gave the 2-nitrobenzoic acid **14**, which was reduced with ammonium formate in the presence of Pd/C catalyst[25] to the paramagnetic anthranilic acid **15**. To obtain the paramagnetic salicylic acid this compound was diazotized with $NaNO_2$ in formic acid and the diazonium compound was hydrolyzed in aqueous formic acid to give paramagnetic salicylic acid **16**.[26] Reaction of **12** with ammonium acetate and methyl acetoacetate in a trimethylsilyl iodide mediated reaction under mild condition gave the dihydropyridine methyl ester **17** as a paramagnetic derivative of the nifedipine drug, which is a well known calcium channel blocker and the oxidized pyridine compound **18** as a side product (Scheme 2).[27–29]

In conclusion, paramagnetic benzaldehyde and 2-nitrobenzaldehyde are important starting materials for the synthesis for both spin labels and paramagnetically modified bioactive molecules. Further application of these molecules is under progress.

Melting points were determined with a Boetius micro melting point apparatus and are uncorrected. Elemental analyses (C, H, N, S) were performed on Fisons EA 1110 CHNS elemental analyzer. The IR (Specord 85) spectra were in each case consistent with the assigned structure. Mass spectra were recorded on a VG TRIO-2 instrument in the EI mode. NMR spectra were recorded with Varian *UNITYINOVA* 400 WB (400/100 MHz for $^1H/^{13}C$) spectrometer. Chemical shifts are referenced to Me_4Si (^1H) or to the residual solvent signal (^{13}C). Measurements were run at 298 K probe temperature in $CDCl_3$ solution. Structure elucidation was based on gradient enhanced ^{13}C-^1H HSQC and ^{13}C-^1H HMBC experiments executed using standard Varian software.

ESR spectra were taken on Miniscope MS 200 in 10^{-4} M $CHCl_3$ solution and all monoradicals gave triplett line a_N = 14.0–14.4 G, and **13** biradical gave quintet line a_{N1} = 14.0 G, a_{N2} = 7.3 G. Flash column chromatography was performed on Merck Kieselgel 60



Scheme 2 *Reagents and conditions:* (a) MnO_2 (5.0 equiv), $CHCl_3$, reflux, 1 h, 59%; (b) acetone, aq 10% NaOH (1.0 equiv), r.t., 30 min, 33%; (c) for **14** Ag_2O (2.0 equiv), 10% aq NaOH–THF, 60 °C, 2 h, then H^+, 44%; (d) HCO_2NH_4 (8.0 equiv), MeOH, 10% Pd/C (cat.), N_2, 40 °C, 2 h, then activated MnO_2, O_2, $CHCl_3$, 10 min, 32%; (e) 80% aq HCO_2H, $NaNO_2$ (1.0 equiv), 0 °C, 30 min, then H_2O, 0 °C → 40 °C, 25%; (f) methyl acetoacetate (2 equiv), NH_4OAc (2 equiv), TMSCl (1.0 equiv), NaI (1.0 equiv), MeCN, r.t., 3d, 17% for **17** and 10% for **18**.

(0.040–0.063 mm). Qualitative TLC was carried out on commercially prepared plates (20 × 20 × 0.02 cm) coated with Merck Kieselgel GF_{254}. Compounds **1**[8] and **3**[8] were prepared according to published procedures. The physical and spectral data of all new compounds are listed in Table 1.

4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzophenone Radical (2)

To a solution of phenylmagnesium bromide [prepared from bromobenzene (942 mg, 6.0 mmol) and Mg turnings (144 mg, 6.0 mmol) in THF (10 mL)], was added a solution of compound **1** (1.16 g, 5.0 mmol) in THF (10 mL) dropwise and the mixture stirred and refluxed for 1 h and allowed to stand overnight at r.t. After quenching with sat. aq NH_4Cl solution, Et_2O (10 mL) was added, the organic layer was separated, dried ($MgSO_4$), filtered and evaporated. The residue was dissolved in $CHCl_3$ (30 mL), activated MnO_2 (2.18 g, 25.0 mmol) was added and the mixture was stirred and refluxed for 1 h. After cooling, the MnO_2 was filtered off, the filtrate evaporated and the residue was purified by flash column chromatography (hexane–Et_2O, 2:1) to give compound **2** as a yellow solid; yield: 693 mg (47%); mp 87–88 °C; R_f = 0.47 (hexane–EtOAc, 2:1).

2-(4-Acetoxymethylphenyl)-2,5,5-tetramethylpyrrolidin-1-yloxy Radical (4)

To a stirred solution of compound **3** (4.68 g, 20.0 mmol) and Et_3N (2.22 g, 22.0 mmol) in CH_2Cl_2 (40 mL) was added AcCl (1.72 g, 22.0 mmol) dropwise at 0 °C. The mixture was allowed to warm to r.t. and the stirring was continued for further 1 h. The mixture was washed with brine (20 mL), the organic phase was dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography (hexane–Et_2O, 2:1) to give compound **4** as an orange oil; yield: 4.14 g (75%); R_f = 0.41 (hexane–EtOAc, 2:1).

2-(4-Hydroxymethyl-3-nitrophenyl)-2,5,5-tetramethylpyrrolidin-1-yloxy Radical (5)

To a vigorously stirred solution of 65% HNO_3 (5 mL) and 98% H_2SO_4 (10 mL) was added compound **4** (4.17 g, 15.0 mmol) dropwise at 0 °C. The mixture was stirred for further 3 h at 0 °C, then it was allowed to warm to r.t. It was poured in portions into crushed ice (100 g) in a 500 mL beaker with stirring and basified with solid K_2CO_3 to pH 8 (extensive foaming !). The solution was extracted with $CHCl_3$ (3 × 40 mL), the combined organic phases were dried ($MgSO_4$), filtered and evaporated. The residue was dissolved in MeOH (20 mL) and NaOMe [prepared freshly by dissolving Na metal (35 mg, 1.5 mmol) in MeOH (10 mL)] was added and allowed to stand for 30 min. at r.t. The MeOH was evaporated off and the residue was dissolved in $CHCl_3$ (40 mL). The $CHCl_3$ layer was washed with 5% aq H_2SO_4 solution (15 mL), brine (15 mL), dried ($MgSO_4$), filtered and evaporated and the residue was purified by flash column chromatography (hexane–EtOAc) to give compound **5** as a red oil; yield: 1.75 g, (42%); R_f = 0.39 ($CHCl_3$–Et_2O, 2:1).

2-(4-Bromomethyl-3-nitrophenyl)-2,5,5-tetramethylpyrrolidin-1-yloxy Radical (6)

To a stirred solution of alcohol **5** (2.79 g, 10.0 mmol) and Et_3N (1.1 g, 11.0 mmol) in CH_2Cl_2 (20 mL) was added $MeSO_2Cl$ (1.26 g, 11.0 mmol) dropwise at 0 °C. The mixture was allowed the warm to r.t. and stirred for further 1 h. The organic phase was washed with H_2O (2 × 10 mL), dried ($MgSO_4$), filtered and evaporated. The residue was dissolved in anhyd acetone (20 mL), LiBr (1.74 g, 20.0 mmol) was added and the mixture was stirred and refluxed for 1 h. The solvent was evaporated, the residue was dissolved in Et_2O (20 mL) and was washed with H_2O (2 × 10 mL). The organic phase was separated, dried ($MgSO_4$), filtered, evaporated and the residue was purified by flash column chromatography (hexane–Et_2O) to give compound **6** as a red solid; yield: 1.84 g (54%); mp 58–60 °C; R_f = 0.42 (hexane–EtOAc, 2:1).

2-(3-Nitro-4-methanethiosulfonylmethylphenyl)-2,5,5-tetramethylpyrrolidin-1-yloxy Radical (7)

To a solution of compound **6** (1.71 g, 5.0 mmol) in acetone (10 mL) and H_2O (5 mL) was added $NaSSO_2Me$ (1.34 g, 10.0 mmol) and the mixture was refluxed for 1 h. The solvent was evaporated, the residue dissolved in $CHCl_3$ (20 mL) and washed with brine (10 mL). The organic phase was separated, dried ($MgSO_4$), filtered, evaporated and the residue was purified by flash column chromatography (hexane–EtOAc) to give the title compound **7** as an amorphous material; yield: 803 mg (43%); R_f = 0.58 ($CHCl_3$–Et_2O, 2:1).

4-[4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)phenyl]but-3-en-2-one Radical (8)

To a solution of compound **1** (1.39 g, 6.0 mmol) in acetone (15 mL) was added NaOH (240 mg, 6.0 mmol) in H_2O (6 mL) and the mixture was stirred for 5 h at r.t. and allowed to stand overnight at r.t. The acetone was evaporated off, the aqueous phase was extracted with $CHCl_3$ (2 × 30 mL). The combined organic phases were dried ($MgSO_4$), filtered, evaporated and after purification by flash column chromatography (hexane–EtOAc) compound **8** was obtained

Table 1 Compounds **2**–**18** Prepared

Compound[a]	Yield (%)	mp °C	IR (Neat or Nujol) cm^{-1}	MS *m*/*z* (%)
2	47	87–88	1660 (C=O), 1590, 1550 (C=C)	308 (M^+, 8), 294 (26), 278 (66), 105 (100)
4	75	oil	1720 (C=O), 1600, 1550, 1500 (C=C)	276 (M^+, 10), 262 (11), 246 (100), 217 (13)
5	42	oil	3400 (OH), 1620 (C=C), 1530 (NO_2)	279 (M^+, 9), 265 (13), 234 (20), 148 (100)
6	54	58–60	1650, 1600 (C=C), 1525 (NO_2)	341/343 (M^+, 3/3), 327/329 (9/9), 311/313 (2/2), 176 (100)
7	43	semisolid	1620, 1550 (C=C), 1540 (NO_2)	373 (M^+, 6), 343 (5), 293 (30), 159 (100)
8	61	60–62	1660 (C=O), 1600, 1560 (C=C)	272 (M^+, 13), 258 (41), 242 (49), 43(100)
9	30	115–118	1710(C=O), 1650, 1620, 1570 (C=C)	434 (M^+, 6), 404 (26), 346 (13), 242 (100)
10	36	86–87	1700 (C=O), 1550 (C=C)	348 (M^+, 8), 334 (24), 318 (100), 262 (40)
11	42	201–204	3180 (NH), 1650 (C=O), 1560 (C=C)	362 (M^+, 2), 332 (100), 318 (9), 276 (8)
12	59	51–53	1680 (C=O), 1620, 1590 (C=C), 1520 (NO_2)	277 (M^+, 8), 263 (17), 232 (15), 146 (100)
13	33	192–193	1640 (C=O), 1610 (C=C)	514 (M^+, 11), 499 (20), 484 (31), 469 (100)
14	44	188–190	1660 (C=O), 1600 (C=C), 1525 (NO_2)	293 (M^+, 11), 263 (22), 177 (76), 159 (100)
15	32	197–200	3480, 3310 (NH_2), 1650 (C=O), 1620, 1590, 1550 (C=C)	263 (M^+, 15), 233 (42), 177 (80), 159 (100)
16	25	232–234	1660 (C=O), 1590, 1560 (C=C)	264 (M^+, 4), 250 (35), 234 (100), 216 (69)
17	17	115–117	3300 (NH), 1670 (C=O), 1600 (C=C), 1530 (NO_2)	TSP: 473 $[M + H]^+$
18	10	90–92	1700 (C=O), 1650 (C=C), 1540 (NO_2)	TSP: 471 $[M + H]^+$

[a] Satisfactory microanalyses obtained: C ±0.18; H ± 0.18; N ±0.17. Exception: **18**, H –0.46.

as a yellow solid; yield: 995 mg (61%); mp 60–62 °C; R_f = 0.26 (hexane–EtOAc, 2:1).

4-Hydroxy-3-{1-[4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)phenyl]-3-oxobutyl}chromen-2-one Radical (9)

A mixture of paramagnetic benzalacetone **8** (1.08 g, 4.0 mmol) and 4-hydroxycoumarine (648 mg, 4.0 mmol) was added to H_2O (10 mL), and the suspension was refluxed for 12 h. After cooling, the mixture was extracted with $CHCl_3$ (20 mL). The organic layer was separated, dried ($MgSO_4$), filtered, evaporated and after purification by flash column chromatography (hexane–EtOAc) compound **9** was obtained as a light-yellow solid; yield: 520 mg (30%); mp 115–118 °C; R_f = 0.32 ($CHCl_3$–Et_2O, 2:1).

2-[4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)phenyl]indan-1,3-dione Radical (10)

To a stirred solution of aldehyde **1** (1.16 g, 5.0 mmol) and phthalide (670 mg, 5.0 mmol) in MeOH (20 mL) was added methanolic NaOMe solution [freshly prepared by dissolving Na metal (460 mg, 20.0 mmol) in MeOH (20 mL)] dropwise at 0 °C. After warming up to r.t., the mixture was refluxed for 5 h and cooled. It was then poured into ice water (100 mL). The mixture was extracted with Et_2O (30 mL), the organic phase was discarded, and the aqueous phase was acidified with aq 2.0 M HCl and extracted with EtOAc (2 × 20 mL). The combined organic phases were dried ($MgSO_4$), filtered, evaporated and after purification by flash column chromatography (hexane–EtOAc) to give compound **10** as a pink solid; yield: 627 mg (36%); mp 86–88 °C; R_f = 0.51 ($CHCl_3$–Et_2O, 2:1).

4-[4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzyl]-2*H*-phthalazin-1-one Radical (11)

A solution of compound **10** (348 mg, 1.0 mmol) in hydrazine hydrate (7 mL) was refluxed for 3 h upon which the red solution became pale yellow. After cooling, H_2O (20 mL) was added, the mixture was saturated with solid NaCl and extracted with $CHCl_3$ (2 × 20 mL). The combined organic phases were dried ($MgSO_4$), activated MnO_2 (87 mg, 1.0 mmol) was added and O_2 was bubbled through for 15 min. The mixture was filtered, evaporated and the residue was purified by flash column chromatography ($CHCl_3$–Et_2O) to give the title compound as a pale yellow solid; yield: 152 mg (42%); mp 201–204 °C; R_f = 0.27 ($CHCl_3$–Et_2O, 2:1).

2-Nitro-4-(1-oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzaldehyde Radical (12)

To a stirred solution of alcohol **5** (5.58 g, 20.0 mmol) in $CHCl_3$ (80 mL) was added activated MnO_2 (8.70 g, 0.1 mol) and the mixture was refluxed for 1 h. The MnO_2 was filtered out the solvent was evaporated and after purification of the residue by flash column chromatography (hexane–Et_2O) compound **12** was obtained as a wine-red solid; yield: 3.26 g (59%); mp 51– 53 °C; R_f = 0.30 (hexane–EtOAc, 2:1).

2-Nitro-4-(1-acetoxy-2,5,5-trimethylpyrrolidin-2-yl)benzaldehyde for NMR Study

To a solution of radical **12** (277 mg, 1.0 mmol) in dioxane (10 mL), was added a solution of ascorbic acid (880 mg, 5.0 mmol) in H_2O (5 mL) and the mixture was stirred at 40 °C for 15 min under N_2. The pale yellow or colorless solution was extracted with $CHCl_3$ (2 × 20 mL), dried ($MgSO_4$) under N_2. First acetyl chloride (86 mg, 1.1 mmol) and then slowly Et_3N (111 mg, 1.1 mmol) were added, the temperature being kept below 10 °C. The stirring was continued for 1 h, after which the mixture was filtered and the filtrate was evaporated in vacuo to dryness. The residue was taken up in brine (10 mL) and extracted with EtOAc (2 × 15 mL). The combined organic layers were dried ($MgSO_4$), filtered, evaporated and after flash chromatography (hexane–Et_2O) the *O*-acetyl derivative was obtained as a yellow oil; yield: 112 mg (35%); R_f = 0.48 (hexane–EtOAc, 2:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.22 (s, 3 H, CH_3), 1.23 (s, 3 H, CH_3), 1.56 (s, 3 H, CH_3), 1.68 (m, 1 H, CH_2), 1.83 (m, 1 H, CH_2), 1.97 (s, 3 H, $COCH_3$), 1.98 (m, 1 H, CH_2), 2.17 (m, 1 H, CH_2), 7.82 (d, *J* = 8.1 Hz, 1 H_{arom}), 8.13 (dd, *J* = 8.1, 1.5 Hz, 1 H_{arom}), 8.43 (d, *J* = 1.5 Hz, 1 H_{arom}), 10.28 (s, 1 H, CHO).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.9 ($COCH_3$), 23.3 (CH_3), 24.1 (CH_3), 28.9 (CH_3), 35.5 (CH_2), 38.8 (CH_2), 66.0 (quat C), 69.8 (quat C), 122.3 (CH_{arom}), 129.0 (arom *C*–CHO), 129.3 (CH_{arom}), 131.6 (CH_{arom}), 149.6 (arom C–NO_2), 156.8 (quat arom C), 169.7 ($COCH_3$), 188.1 (CHO).

(*E*)-6,6′-Bis-(1-oxyl-2,5,5-trimethylpyrrolidin-2-yl)-1*H*,1′*H*-[2,2′]biindolylidene-3,3′-dione Biradical (13)

To a solution of compound **12** (277 mg, 1.0 mmol) in acetone (4 mL) was added aq 10% NaOH (0.4 mL, 1.0 mmol). The mixture was allowed to stand at r.t. for 30 min., then it was diluted with H_2O (70 mL) and the precipitated blue solid was filtered to give compound **13**; yield: 87 mg (33%); mp 192–193 °C; R_f = 0.34 ($CHCl_3$–Et_2O, 2:1).

2-Nitro-4-(1-oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzoic Acid Radical (14)

To a stirred suspension of freshly precipitated Ag_2O (4.63 g 20.0 mmol) in 10% aq NaOH solution (20 mL) was added a solution of aldehyde **12** (2.77 g, 10.0 mmol) in THF (5 mL) at 60 °C and the mixture was stirred for 2 h. After cooling, the solution was filtered through a Celite pad, and washed with MeOH (20 mL). The organic solvents were evaporated off, the residue aqueous phase was acidified with 5% aq H_2SO_4 to pH 2 and extracted with $CHCl_3$ (3 × 10 mL). The organic phase was dried ($MgSO_4$), filtered, evaporated to afford compound **14** as a yellow solid; yield: 1.29 g (44%); mp 188–190 °C; R_f = 0.12 ($CHCl_3$–MeOH, 9:1).

2-Amino-4-(1-oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzoic Acid Radical (15)

To a stirred solution of compound **14** (1.76 g, 6.0 mmol) in MeOH (40 mL) was added ammonium formate (3.0 g, 48.0 mmol) at 40 °C. After dissolution of ammonium formate, 10% Pd/C catalyst (100 mg) was added and the mixture stirred for 2 h at this temperature under N_2. After consumption of the nitro acid **14**, the mixture was filtered through a Celite pad and washed with MeOH (30 mL) and the filtrate was evaporated. The residue was dissolved in a mixture of $CHCl_3$ (30 mL) and MeOH (3 mL), and the organic solution was washed with brine, and dried ($MgSO_4$). Activated MnO_2 (869 mg, 10.0 mmol) was added and O_2 was bubbled through the solution for 10 min. The mixture was filtered, evaporated and after purification by flash column chromatography ($CHCl_3$–Et_2O, 2:1) compound **15** was obtained, as a beige solid; yield: 505 mg (32%); mp 197–200 °C; R_f = 0.26 ($CHCl_3$–MeOH, 9:1).

2-Hydroxy-4-(1-oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzoic Acid Radical (16)

To a stirred solution of compound **15** (526 mg, 2.0 mmol) in 80% aq HCO_2H (8 mL) was added a solution of $NaNO_2$ (138 mg, 2.0 mmol) in H_2O (2 mL) dropwise and the mixture was stirred at 0 °C for 30 min. The mixture was diluted with H_2O (10 mL) and slowly warmed to 40 °C and after the solvents were evaporated off to dryness, the residue was dissolved in brine (10 mL), extracted with $CHCl_3$ (2 × 15 mL). The combined organic phases were dried ($MgSO_4$), filtered, evaporated and after flash chromatography ($CHCl_3$–Et_2O, 3:1) compound **16** was obtained as a yellowish-brown solid; yield: 132 mg (25%); mp 232–234 °C; R_f = 0.26 ($CHCl_3$–MeOH, 9:1).

4-[4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)-2-nitrophenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic Acid Dimethyl Ester Radical (17) and 4-[4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)-2-nitrophenyl]-2,6-dimethylpyridine-3,5-dicarboxylic Acid Dimethyl Ester Radical (18)

To a stirred solution of compound **12** (1.38g, 5.0 mmol) in MeCN (10 mL), methyl acetoacetate (1.16 g, 10.0 mmol), and NH_4OAc (770 mg, 10.0 mmol), was added Me_3SiCl (543 mg 5.0 mmol) dropwise and then NaI (745 mg, 5.0 mmol) was added in one portion and the mixture was stirred for further 3 d at r.t. The mixture was poured into iced-water (100 mL) and extracted with EtOAc (3 × 20 mL). The combined organic phases were dried ($MgSO_4$), filtered, evaporated and after purification by flash column chromatography (hexane–EtOAc) compounds **17** as the fifth and **18** as the fourth band were obtained following aminocrotonate (R_f 0.51, hexane–EtOAc, 2:1), unreacted starting aldehyde **12** and probably a condensation product of aldehyde **12** and methyl acetoacetate (*m*/*z* = 375, M^+) (R_f = 0.19, hexane–EtOAc, 2:1).

17

Yield: 401 mg (17%); mp 115–117 °C; R_f = 0.11 ($CHCl_3$–MeOH, 9:1).

18

Yield: 235 mg (10%); mp 90–92 °C; R_f = 0.21 ($CHCl_3$–Et_2O, 2:1).

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