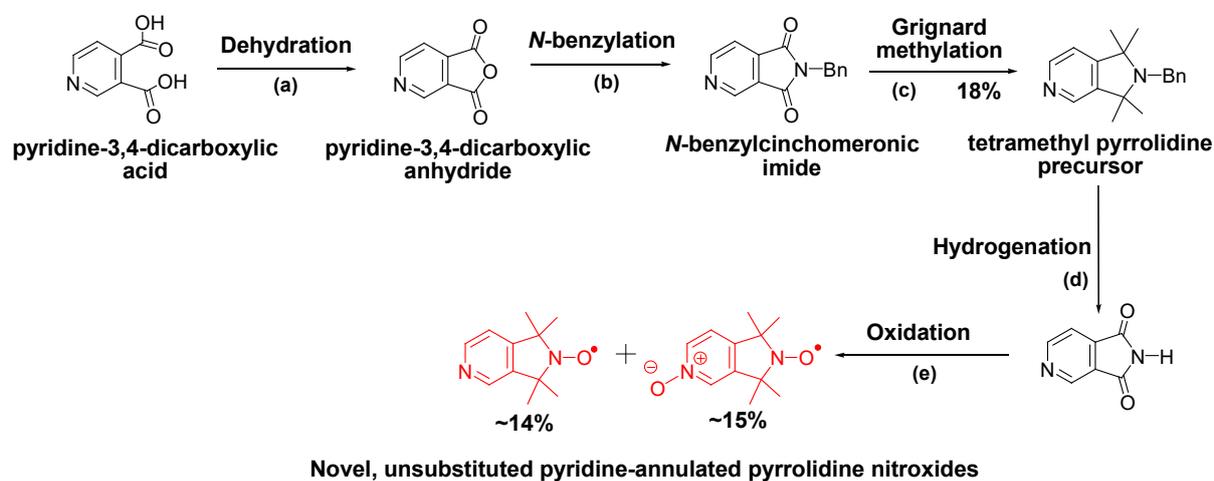


Synthesis of two novel pyridine annulated pyrrolidine nitroxides

V. C. Jayawardena



-Five-step Synthetic Route of Pyridine-Annulated Pyrrolidine Nitroxides-

Step (c): Yield limiting step due to the formation of numerous, unavoidable side products

Therefore, the overall yield of the final product has been reduced.

Highlights

- The scope was to synthesize novel pyridine-annulated heterocyclic nitroxides via a convenient route.
- Nitroxide yields were smaller due to the Grignard step in the synthetic route.
- Side product formation was the main reason for the limited yield at Grignard step.
- Two novel heterocyclic nitroxides were produced via a five-step pathway in ~15% yield.

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Abstract: Two novel, unsubstituted, pyridine-annulated pyrrolidine nitroxides; *1,1,3,3-tetramethyl-2,3-dihydro-1H-pyrrolo[3,4-c]pyridin-2-yloxy* and *1,1,3,3-tetramethyl-2,3-dihydro-1H-pyrrolo[3,4-c]pyridin-5-oxide-2-yloxy* were synthesized in overall yield of ~15% (5 steps starting from pyridine-3,4-dicarboxylic acid) via a Grignard approach. Grignard methylation of N-benzylcinchononic imide that was derived from pyridine-3,4-dicarboxylic anhydride, furnished the tetramethylpyrrolidine precursor in an isolated yield of 18%. Hydrogenation of the tetramethyl pyrrolidine precursor followed by oxidation afforded, depending on the oxidation conditions, each of the two nitroxides.

Keywords: Grignard; Heterocyclic; Pyridine-annulated; Nitroxides; Tetramethylation.

INTRODUCTION

Isoindoline nitroxides, a commercially valuable class of nitroxides have gained widespread attention mainly due to some advantages they possess over the commercially available other classes of nitroxides. If the 6-membered aromatic ring of the isoindoline skeleton is replaced by a heteroaromatic ring, the nitroxide skeleton would have been modified further with some novel vital properties. Established literature supports that monocyclic nitroxides containing heteroatoms have been synthesized in early 1980s (Keana *et al.*, 1982). Some of the uses of monocyclic heteroaromatic nitroxides such as EPR probes in biomedicine and related fields to monitor oxidative stress and reactive radical species in biological systems (Bobko *et al.*, 2012; Zhelev *et al.*, 2019), pH sensitive spin probes (Khlestkin *et al.*, 2005), contrast enhancing agents (Keana *et al.*, 1987) for magnetic resonance imaging (MRI) applications and molecular units (Vaz *et al.*, 1999; Laget *et al.*, 1998) in the synthesis of molecular magnetic materials are of notable importance. However, there are some disadvantages associated with monocyclic nitroxides containing heteroatoms as well. For instance, the electron-withdrawing effect of the heteroatom in the ring can destabilize the positive charge of the resulting oxo-ammonium cation (Hicks, 2010). Furthermore, the heteroatom can also facilitate ring opening reactions (Keana *et al.*, 1982; Gryn'ova *et al.*, 2012) in monocyclic nitroxides and thereby promote degradation.

These issues can be evaded if the heteroaromatic ring is fused to the monocyclic nitroxide skeleton. This fused heteroaromatic ring could confer some advantages to the skeleton such as rigidity and resistance towards ring opening reactions (Gryn'ova *et al.*, 2012; Hansen and Blinco, 2018) and resistance towards the alteration of functions of biomolecules when they act as spin labels (Kalai *et al.*, 2000). If the fused heteroaromatic ring is pyridine, it would further impart good σ donor capabilities as a monodentate ligand (Budzelaar, 2012). Therefore, this paper focuses on developing a novel unsubstituted heteroaromatic nitroxide with a fused pyridine ring.

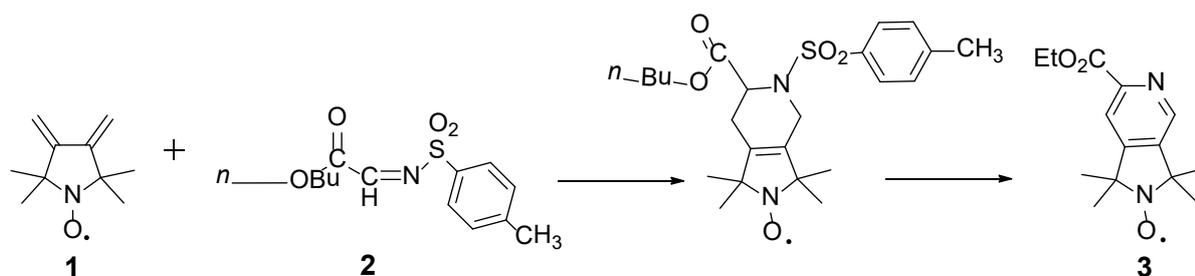
Pyridine annulated heterocyclic nitroxides have been synthesized previously via three different approaches. Hideg and co-workers synthesized a pyridine-fused paramagnetic nitroxide system **3** using hetero Diels-Alder reactions (Scheme 1) (Kalai *et al.*, 2000; Krishna *et al.*, 1996). The overall synthetic yield of this nitroxide starting from commercially available substrate was 4% via eight steps (Kalai *et al.*, 1999; Hideg *et al.*, 1988). In another approach, 5-substituted pyridine (N-oxide) fused pyrroline nitroxide **8** has been synthesized via Sonogashira coupling of aldehyde containing nitroxide **5** with phenylacetylene to give **6** of which oxime **7** spontaneously cyclized to the paramagnetic nitroxide derivative **8** upon heating (Scheme 2) (Kalai *et al.*, 2002). From commercially available starting materials, this route yields the nitroxide **8** in seven steps with an overall yield of 10 % (Kalai *et al.*, 1998; Zhdanov, 1992).

Chiusoli *et al.* (1983) have attempted to synthesize pyridine fused nitroxides using cobalt catalyzed cyclization reactions. In this method, cobalt(0) catalyzes the cyclocotrimerization of tetramethyldipropargylamine **9** with acetonitrile to give 2,3-dihydro-1,1,3,3,6-pentamethyl-1H-pyrrolo[3,4-c]pyridine (**10**) (Scheme 3) which can be oxidized to the corresponding paramagnetic nitroxide derivative. However, this methodology led to some confusion as the tetramethyl amine **10** was not converted into the corresponding paramagnetic nitroxide and the synthetic yield of **10** was confirmed only by Gas Chromatography (98%) (Chiusoli *et al.*, 1983). An alternative synthetic approach for the synthesis of pyridine fused heterocyclic nitroxides is required as all the existing approaches are low-yielding and multi-step.

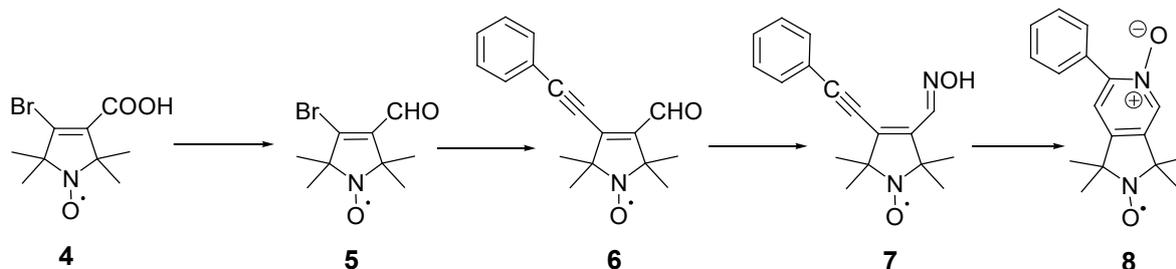
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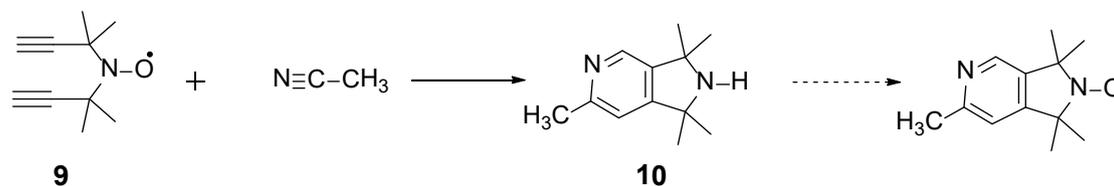




Scheme 1: Synthesis of pyridine annulated pyrroline nitroxide using hetero Diels-Alder reaction.



Scheme 2: Pyridine-fused pyrroline nitroxide synthesis using spontaneous cyclization reactions.



Scheme 3: Preparation of precursor for pyridine annulated pyrroline nitroxide using cobalt (0) catalyzed cycloaddition reactions.

Since tetraalkylation of N-benzylphthalimide provides reasonable yields and involves limited reaction steps for the formation of commercially valuable isoindoline nitroxides, 1,1,3,3-tetraethylisoindolin-2-yloxy (TEIO) and 1,1,3,3-tetramethylisoindolin-2-yloxy (TMIO) compared to other methods, the synthesis of pyridine annulated tetraalkyl nitroxides through the Grignard alkylation of N-benzylcinchomeric imide followed by hydrogenation and oxidation is an attractive approach. Herein we report a short (5 steps) and convenient approach for the synthesis of unsubstituted pyridine annulated pyrroline nitroxides with much improved yields.

EXPERIMENTAL

Chemicals & Apparatus

All chemicals used were of analytical reagent grade purchased from chemical suppliers such as Sigma-Aldrich. Dichloromethane (DCM) was freshly distilled from calcium hydride and tetrahydrofuran (THF) from sodium benzophenone ketal prior to use. Both toluene and diethyl ether were dried over sodium wire and triethylamine was dried over potassium hydroxide. All air-sensitive reactions were performed under an ultra-high purity argon atmosphere. All other reagents were purchased from commercial suppliers and used without further purification. ¹H and ¹³C NMR spectra were recorded on a 400 MHz spectrometer and referenced to the relevant solvent peak (CDCl₃; $\delta_{\text{H}}=7.26$ ppm, $\delta_{\text{C}}=77.0$ ppm). ESI-high resolution

mass spectra were obtained using a QTOF LC mass spectrometer which utilized electrospray ionisation (recorded in the positive mode) with a methanol mobile phase. Melting point values were collected on a Variable Temperature Apparatus using the capillary method and were uncorrected. Analytical HPLC was carried out on a HPLC system using an Agilent 1100 Prep-C18 scalar column (4.6 × 150 mm, 10 μm) with a flow rate of 1 mL/min in the stated mixtures of methanol and water with detection at 254 nm. In all HPLC analyses, the solvent system used was MeOH : H₂O, 65: 35 except for compound **21a** and **21b**. For compounds **21a** and **21b**, MeOH: H₂O, 70:30 was used as the solvent system. Merck Silica Gel 60 F254 TLC plates were used for analytical Thin-Layer Chromatography (TLC) while Silica Gel 60 (230-400 mesh) was used for preparative column chromatography.

Synthetic Methodologies

Pyridine-3,4-dicarboxylic anhydride (12)

A mixture of pyridine-3,4-dicarboxylic acid (15.0 g, 90.0 mmol) and acetic anhydride (60 mL, 0.635 mol, 7.0 equiv.) was refluxed for 40 min at 140 °C. The excess acetic anhydride was distilled off under high vacuum at 65 °C. The resulting brown coloured crude (13.2 g, 98%) was purified by sublimation and white crystals were obtained (12.6 g, 94%). Mp 74-76 °C (lit. 75-76 °C) (Mayor and Wentrup, 1975); ¹H NMR (400 MHz,

CDCl_3) δ 7.94-7.96 (dd, $J = 1.2$ and 4.8 Hz, 1H), 9.24 (d, $J = 5.2$ Hz, 1H), 9.38 (d, $J = 0.8$ Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 118.6, 125.3, 138.8, 147.6, 156.8, 161.15, 161.28. These ^1H NMR data were in agreement with those reported previously by Mayor and Wentrup (Mayor and Wentrup, 1975).

N-Benzylcinchomeric imide (13)

A mixture of pyridine-3,4-dicarboxylic anhydride (12.6 g, 84.0 mmol), acetic acid (65 mL) and benzylamine (18 mL, 0.170 mol, 2.0 equiv.) was refluxed at 120°C for 1 h. The hot reaction mixture was then poured in to ice/ H_2O (500 mL) and stirred. The resulting precipitate was filtered and recrystallized from 96% ethanol. The resulting needles like crystals (18.9 g, 95%) were off-white in colour. Mp 114 – 115°C (lit. 116 – 117°C) (Hunter *et al.*, 1967); ^1H NMR (400 MHz, CDCl_3) δ 4.88 (s, 2H), 7.28-7.36 (m, 3H), 7.43-7.45 (m, 2H), 7.76 (dd, $J = 0.8$ and 4.4 Hz, 1H), 9.06 (d, $J = 4.8$ Hz, 1H), 9.16 (d, $J = 0.8$ Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 41.6, 116.9, 125.9, 128.2, 128.76, 128.81, 135.7, 139.5, 144.8, 155.7, 166.5, 166.9; HRMS: calcd for $\text{C}_{14}\text{H}_{10}\text{N}_2\text{O}_2$ $[\text{M}]^+$ 238.0700, found 238.1000.

2-Benzyl-1,1,3,3-tetramethyl-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine (14)

Methyl iodide (7.3 mL, 0.118 mol, 8 equiv.) was added dropwise to a suspension of pre-dried magnesium turnings (3.57 g, 0.147 mol, 10 equiv.) in anhydrous diethyl ether (55 mL). The mixture was stirred at room temperature for 1 h and then concentrated by distillation until a temperature of 80 – 90°C was reached. The reaction mixture was allowed to cool to 64°C and a solution of *N*-benzylcinchomeric imide (13) (3.50 g, 14.7 mmol) in dry toluene (50 mL) was added. Once the addition was completed, the mixture was refluxed at 110°C for 72 h. Saturated ammonium chloride solution (30 mL) was then added and the mixture was stirred for about 1 h. The toluene layer was then separated and evaporated to dryness. The remaining brown coloured semi-solid layer was extracted to chloroform (5×50 mL). The combined chloroform layers were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The resulting residues from the toluene and chloroform layers were combined and purified by column chromatography (hexane:ethyl acetate, 1:3 and 4:1) to give four compounds (14, 15, 16 and 17) separately. Recrystallization of the crude of compound 14 from hexane gave the title compound (14) as a white solid (0.720 g, 18%). Mp 90 – 92°C ; ^1H NMR (400 MHz, CDCl_3) δ 1.31 (s, 6H), 1.37 (s, 6H), 3.99 (s, 2H), 7.09 (d, $J = 4.8$ Hz, 1H), 7.23-7.33 (m, 3H), 7.46 (d, $J = 7.6$ Hz, 2H), 8.43 (s, 1H), 8.49 (d, $J = 4.8$ Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 27.8 (CH_3), 28.3 (CH_3), 46.0 (CH_2), 64.5 (C_1 or C_3), 65.3 (C_1 or C_3), 116.7 (C_7), 126.7 (Ar-C), 128.0 (Ar-C), 128.3 (Ar-C), 142.6 (Ar-C), 143.6 (C_3 or C_7), 143.7 (C_4), 148.0 (C_6), 156.8 (C_3 or C_7); HRMS: calcd for $\text{C}_{18}\text{H}_{23}\text{N}_2$ $[\text{MH}]^+$ 267.1900, found 267.1865. All the carbons in the ^{13}C -NMR spectrum were assigned by running 2D-HSQC and DEPT spectra.

Three other compounds were also identified from this reaction. Those had been purified only by silica column chromatography:

2-Benzyl-1-ethyl-1,3,3-trimethyl-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine (15): (Colourless oil, 0.220 g, 5%). HRMS: calcd for $\text{C}_{19}\text{H}_{25}\text{N}_2$ $[\text{MH}]^+$ 281.2000, found 281.2016. Other characteristic data such as ^1H -NMR, ^{13}C -NMR were not definitively assigned due to the presence of some amount of 2-benzyl-1,1,3,3-tetramethyl-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine (14), which could not be eliminated by standard purification methods.

2-Benzyl-1-hydroxy-1,4-dimethyl-1H-pyrrolo[3,4-c]pyridin-3(2H)-one (16): (Cream coloured solid, 0.970 g, 25%). Mp 186 – 189°C ; ^1H NMR (400 MHz, CDCl_3) δ 1.54 (s, 3H), 3.08 (s, 3H), 3.98 (bs, 1H), 4.57 (d, $J = 15.6$ Hz, 1H), 4.77 (d, $J = 15.2$ Hz, 1H), 7.23-7.32 (m, 4H), 7.39 (d, $J = 7.2$ Hz, 2H), 8.46 (d, $J = 4.8$ Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 20.4, 24.4, 41.8, 87.9, 114.5, 122.9, 127.4, 128.0, 128.6, 138.1, 151.8, 157.1, 157.5, 166.3; HRMS: calcd for $\text{C}_{16}\text{H}_{16}\text{N}_2\text{NaO}_2$ $[\text{M}+\text{Na}]^+$ 291.1100, found 291.1093.

2-Benzyl-1-hydroxy-1-methyl-1H-pyrrolo[3,4-c]pyridin-3(2H)-one (17): (Cream colored solid, 1.03 g, 27%). Mp 178 – 180°C ; ^1H NMR (400 MHz, CDCl_3) δ 1.55 (s, 3H), 4.32 (bs, 1H), 4.57 (d, $J = 15.6$ Hz, 1H), 4.70 (d, $J = 15.2$ Hz, 1H), 7.26-7.33 (m, 3H), 7.37 (d, $J = 7.2$ Hz, 2H), 7.50 (d, $J = 4.8$ Hz, 1H), 8.62 (d, $J = 5.2$ Hz, 1H), 8.81 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 24.4, 41.8, 88.8, 116.8, 126.0, 127.5, 128.0, 128.6, 137.8, 144.9, 152.5, 156.7, 165.4; HRMS: calcd for $\text{C}_{15}\text{H}_{15}\text{N}_2\text{O}_2$ $[\text{MH}]^+$ 255.1100, found 255.1123; Anal. Calcd for $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_2$: C 70.85, H 5.55, N 11.02, Found C 70.85, H 5.62, N 10.82.

1,1,3,3-Tetramethyl-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine (20)

The starting material 2-benzyl-1,1,3,3-tetramethyl-2,3-dihydro-1H-pyrrolo[3,4-c]pyridine (14) (80.0 mg, 0.300 mmol) was dissolved in MeOH (15 mL) and the catalyst based on palladium at 10 wt. % on carbon was added. Argon was bubbled through the solution for 3-5 min to remove any dissolved oxygen in methanol. The reaction vessel was then sealed and hydrogen was introduced to the reaction vessel (*via* a balloon) at atmospheric pressure. The reaction was carried out at room temperature for 3-4 h with stirring and the complete conversion of the substrate was verified by means of TLC. The reaction mixture was then diluted with methanol and charcoal impurities were removed by celite filtration. The product obtained from MeOH filtrate was further purified by column chromatography (ethyl acetate: ethanol 7:1). The title compound 20 was isolated from the column as a colorless oil (50.0 mg, 95%); ^1H NMR (400 MHz, CDCl_3) δ 1.40 (s, 6H), 1.44 (s, 6H), 2.25 (bs, 1H), 7.03 (d, $J = 4.8$ Hz, 1H), 8.37 (s, 1H), 8.43 (d, $J = 4.8$ Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 31.1 (CH_3), 31.8 (CH_3), 62.2 (C_1 or C_3), 62.8 (C_1 or C_3), 116.7 (C_4 or C_6), 143.8 (C_7), 144.3 (C_3 or C_7), 148.2 (C_4 or C_6), 157.6 (C_3 or C_7); HRMS: calcd for $\text{C}_{11}\text{H}_{17}\text{N}_2$ $[\text{MH}]^+$ 177.1400, found 177.1390. Quaternary carbon peak that appeared at 144.3 was identified by a DEPT spectrum while C_4 , C_6 and C_7 were identified by 2D-HSQC spectrum.

1,1,3,3-Tetramethyl-2,3-dihydro-1H-pyrrolo[3,4-c]pyridin-2-yloxy (21a)

To a solution of **20** (30.0 mg, 0.170 mmol) in methanol (7 mL) and acetonitrile (0.5 mL) was added sodium hydrogen carbonate (11.5 mg, 0.136 mmol, 0.80 equiv.), sodium tungstate dihydrate (2.00 mg, 10.0 μ mol, 0.06 equiv.) and finally 30% aqueous hydrogen peroxide (0.10 mL, 0.561 mmol, 3.3 equiv.). The suspension was stirred at room temperature for 24 h and 0.20 mL of H₂O₂ (30% aqueous) was added again. It was stirred for another 24 h and a yellow coloured solution was observed. Then it was diluted with distilled water and extracted with dichloromethane (4 \times 30 mL). The combined organic fractions were dried with anhydrous Na₂SO₄ and evaporated to give a yellow coloured crude solid, which was purified by column chromatography (ethyl acetate: ethanol 7:1). This gave the title nitroxide **21a** (26.0 mg, 80%). Mp 88-90 °C; HRMS: calcd for C₁₁H₁₅N₂O [MH]⁺ 192.1300, found 192.1392; IR (ATR): ν_{\max} 3045 (aryl C-H), 2972 and 2929 (alkyl C-H), 1602 and 1574 (quadrant stretch), 1447 (N-O) cm⁻¹; EPR (MeOH): three lines, a_N = 1.474 mT, g = 2.0058; HPLC purity (MeOH: H₂O, 70: 30) 98%.

The other nitroxide **21b** isolated from this reaction:

1,1,3,3-Tetramethyl-2,3-dihydro-1H-pyrrolo[3,4-c]pyridin-5-oxide-2-yloxy (21b)

A solution of **20** (10.0 mg, 57.0 μ mol) in CH₂Cl₂ (6 mL) was cooled to 0 °C and treated with *m*-chloroperbenzoic acid (15.0 mg, 86.0 μ mol, 1.5 equiv.). The reaction mixture was stirred at 0 °C for 15 min and the cooling bath was removed. Then the reaction mixture was stirred for another 3 h whilst additional CH₂Cl₂ (4 mL) was added gradually in order to dissolve precipitating solids. The reaction mixture was washed with 5 moldm⁻³ NaOH (20 mL) followed by brine (20 mL), dried with anhydrous Na₂SO₄ and evaporated under reduced pressure. The title nitroxide **21b** resulted as a yellow crude solid (9.60 mg, 81%). Mp 172-174 °C; HRMS: calcd for C₁₁H₁₅N₂O₂ [MH]⁺ 208.1200, found 208.1180; Anal. calcd for C₁₁H₁₅N₂O₂:C 63.44, H 7.74, N 13.45, Found C 63.59, H 7.34, N 13.58; IR (ATR): ν_{\max} 2966 and 2929 (alkyl C-H), 1481 and 1466

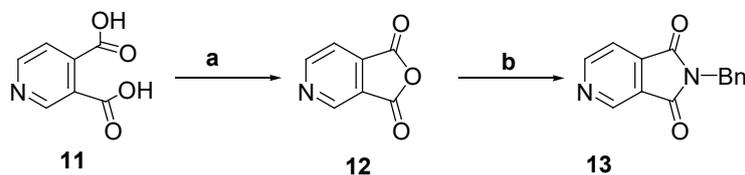
(quadrant stretch), 1428 (N-O), 1315 (N-O oxide) cm⁻¹; EPR (MeOH): three lines, a_N = 1.440 mT, g = 2.0055; HPLC purity (MeOH: H₂O 70: 30) 100%.

RESULTS & DISCUSSION

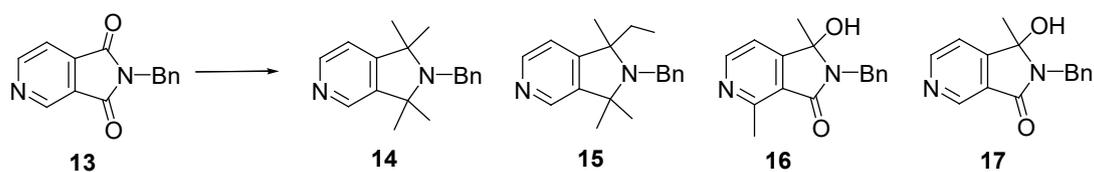
Synthesis of the starting imide, *N*-benzylcinchomeronic imide (**13**) was achieved starting from commercially available pyridine-3,4-dicarboxylic acid (**11**) via two steps (Scheme 4) with an overall yield of 89%. Next, Grignard methylation was performed on imide **13** to synthesize 1,1,3,3-tetramethyl adduct. Following Griffiths standard procedure (Griffiths *et al.*, 1983) of synthesizing 2-benzyl-1,1,3,3-tetramethylisoindoline adduct, imide **13** was treated with 6.0 equivalents of MeMgI in refluxing toluene for 3 h. Examining the reaction mixture (after the aqueous NH₄Cl work-up) by TLC and isolating the components by column chromatography showed two relatively polar products, **16** and **17**, as being formed in the mixture (Scheme 5). When the methylation reaction was undertaken on imide **13** with excess MeMgI for an extended reflux time (5 h), two relatively non-polar components appeared (Scheme 5) in the reaction mixture (by TLC and HPLC).

The desired tetramethyl adduct **14** was identified (by NMR and Mass) as being formed in the reaction mixture along with ethyltrimethyl adduct **15** (Scheme 5). Optimizing the experimental conditions of the Grignard methylation of imide **13** to improve the proportion of **14** in the reaction mixture was conducted by carrying out the Grignard methylation reactions at 110 °C under different reaction times and analyzing them by HPLC in order to calculate the relative HPLC product ratios of **14**, **15**, **16** and **17** appearing in the chromatogram (Table 1). Based on these calculations, the relative HPLC proportion of **14** was improved to 23% (Entry 4, Table 1) by increasing the amount of MeMgI from 6.0 to 8.0 equiv. and refluxing the reaction mixture for 72 h in toluene. The compounds **14**, **15**, **16** and **17** were isolated from the reaction mixture by column chromatography (hexane:ethyl acetate, 1:3, 4:1), and their yields were 18%, 5%, 25% and 27%, respectively.

Based on the relative HPLC product ratios shown in Table 1, some mechanistic insights of the Grignard



Scheme 4: Reagents & Conditions: (a) Acetic anhydride (7.0 equiv.), 140 °C, 40 minutes, 94%; (b) Bn-NH₂ (2.0 equiv.), AcOH, 120 °C, 1 h, 95%.

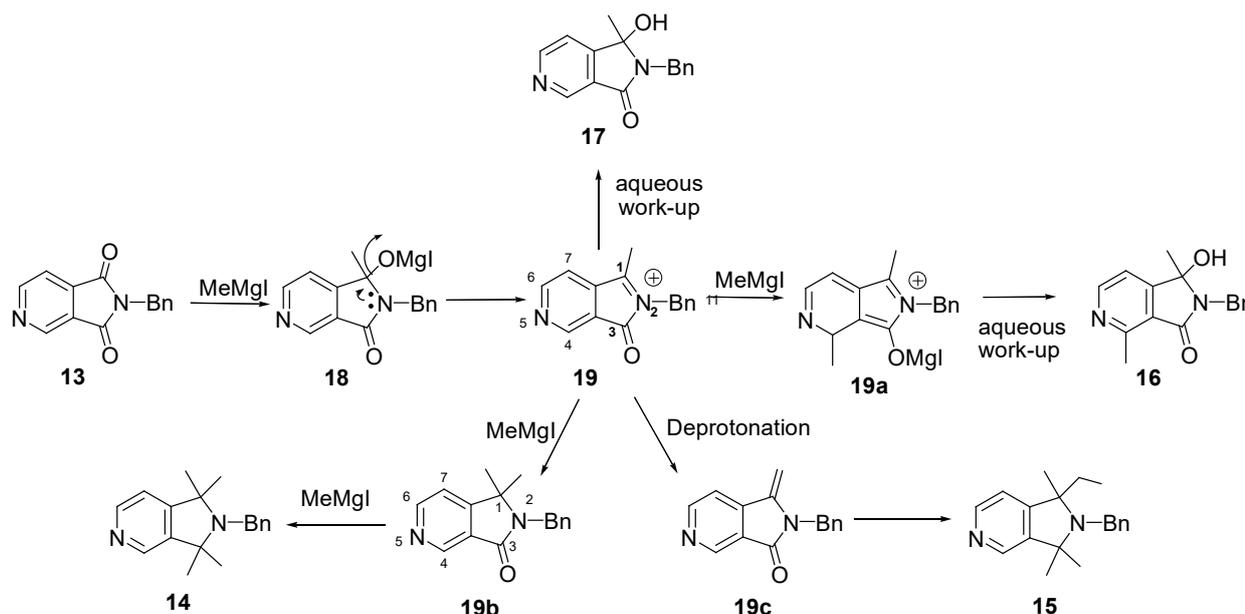


Scheme 5: Reagents & Conditions: MeMgI (6.0 equiv.), toluene, 110 °C, 5 h.

Table 1: HPLC product ratios obtained from the reaction of imide **13** with excess MeMgI.

Entry	Equiv. Mg	Equiv. MeI	Reaction time (h)	HPLC product ratio (%)			
				14	15	16	17
1	8.0	6.0	3	-	-	43	57
2	8.0	6.0	5	22	10	27	41
3	8.0	6.0	24	3	19	30	48
4	10.0	8.0	72	23	14	31	32

Each entry (1-4) corresponds to a Grignard methylation performed on **13** following the general reaction conditions described for the preparation of **14** (Experimental section).

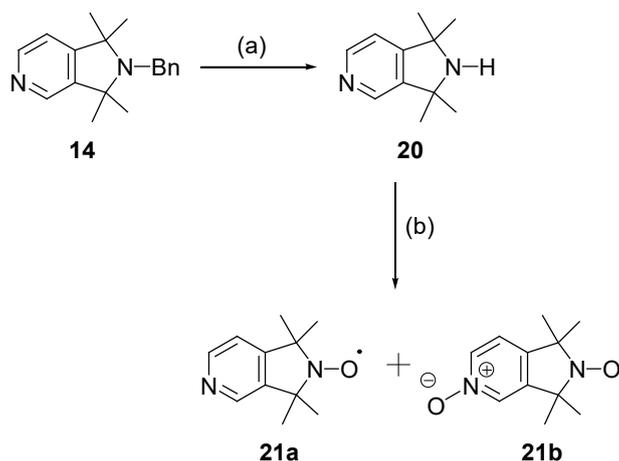
**Scheme 6:** A proposed mechanism for the formation of **14**, **15**, **16** and **17**, starting from **13** during the Grignard methylation.

methylation of imide **13** were revealed (Scheme 6). Previous literature (Jayawardena *et al.*, 2013) supports that products such as **17** could possibly arise from an iminium intermediate such as **19**, during the aqueous work-up. This intermediate **19**, sighted as the initial precursor intermediate of forming 1,1,3,3-tetraalkyl adduct during the Grignard alkylation of imides could also undergo ring methylation (at fourth position of the ring) leading to a structure like **19a** which is inert to further methylation (Colwell *et al.*, 2011). Absence of any methyl groups at the fourth position of the pyridine ring of two tetraalkyl adducts (**14** or **15**) exhibits that intermediate **19a** would remain as an inert structure for further Grignard methylation.

The variation of the HPLC product ratios in entries 1 and 2 (Table 1) is consistent with intermediate **19** as the precursor for the formation of both tetraalkyl adducts, **14** and **15**. Nonetheless, this argument is supported by the suggestion that iminium ion **19a** (precursor intermediate of **16**) would exist as an inert structure for further Grignard methylation. Extending the reaction time from 5 h to 24 h (Entry 2 & 3, Table 1) has surprisingly led to a significant decrease in the relative amount of **14** along with an increase in the relative amount of **15**. This suggests that iminium ion **19** could possibly be converted to another intermediate

like **19c** efficiently (over the long reflux time), due to the deprotonation driven by the strongly basic Grignard environment. This intermediate **19c** would eventually give rise to a higher amount of **15** by reacting with some unreacted MeI in the Grignard reagent, followed by three Grignard methylations. Previous findings suggest that exocyclic amides such as **19c** could be the possible precursors for the formation of unusual mixed alkylated adducts like **15** (Jayawardena *et al.* unpublished).

Tetramethyl adduct **14** could be derived in the reaction mixture via two possible pathways. One mechanism (Braslau and Chaplinski, 1998) is three alternative additions of MeMgI at C₁ and C₃ of intermediate **19**. The other possibility of deriving **14** is via 1,1-dimethyl intermediate (**19b**) by two methyl additions at C₃. It was earlier observed that 1,1-dimethyl amides are convertible to the 1,1,3,3-tetramethyl adduct during the Grignard methylation reactions (Jayawardena *et al.* unpublished). Based on these comparisons, it is reasonable to hypothesize that tetramethyl adduct **14** could be derived via a 1,1-dialkylamide intermediate like **19b** through 1,1-addition. This suggestion also explains why tetramethyl adduct **14** was formed during the Grignard methylation of **13**.



Scheme 7: Reagents & Conditions: (a) $\text{H}_2/\text{Pd/C}$, MeOH, rt, 3-4 h, 95%; (b) H_2O_2 (3.3 equiv.), $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ (0.03 equiv.), NaHCO_3 (0.8 equiv.), MeOH/MeCN, rt, 48 h, 21a (80%), 21b (17%).

The next step, hydrogenation of **14** was successfully achieved with stirring in MeOH (as solvent) in the presence of $\text{H}_2/\text{Pd/C}$ at room temperature (3-4 h) in a yield of 95%. Oxidation of the secondary amine **20** resulted from the hydrogenation of **14** was approached using H_2O_2 in the presence of $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}/\text{NaHCO}_3$ in a mixture of MeOH/MeCN (7:1) resulting the target nitroxide **21a** in a yield of 80% (48 h). Another novel nitroxide **21b** was also isolated from this reaction in a yield of 17%. However, an extended reaction time (120 h) generated the pyridine oxide-type-nitroxide **21b** as the major product (85%) along with some small amount of **21a** (6%). This protocol involving aqueous H_2O_2 provides milder yet higher yielding synthesis for the two novel nitroxides, **21a** and **21b**. When the oxidation of **20** was undertaken with *m*CPBA, the reaction specifically led to nitroxide **21b** within 2 h (81%). Notably, the conversion of nitroxide **21a** to **21b** was also achieved with both H_2O_2 and *m*CPBA.

This methodology provides a short and convenient pathway (5 steps) to synthesize the novel heterocyclic nitroxides in practically preparative scales.

CONCLUSIONS

The main scope of this study was to introduce the synthesis of tetraalkylated pyridine-annulated heterocyclic nitroxides via a short and convenient pathway. Finally, this approach furnished, in good overall synthetic yields (up to ~15%), two novel pyridine-annulated tetramethyl nitroxides (**21a** and **21b**) starting from pyridine-3,4-dicarboxylic acid **11**, via a short (5 steps) and convenient pathway.

Data Availability

Datasets used to characterize the compounds of this study are available upon request.

DECLARATION OF CONFLICT OF INTEREST

The author declares no competing interests.

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