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FACILE SYNTHESIS OF 3-METHOXYCARBONYL-2,2,5,5-TETRA-METHYLPYRROLIDINE-1-OXYL AND DERIVATIVES

Bunpei Hatano,* Hiroki Araya, Yutaka Yoshimura, Haruna Sato, Tomohiro Ito, Tateaki Ogata, and Tatsuro Kijima

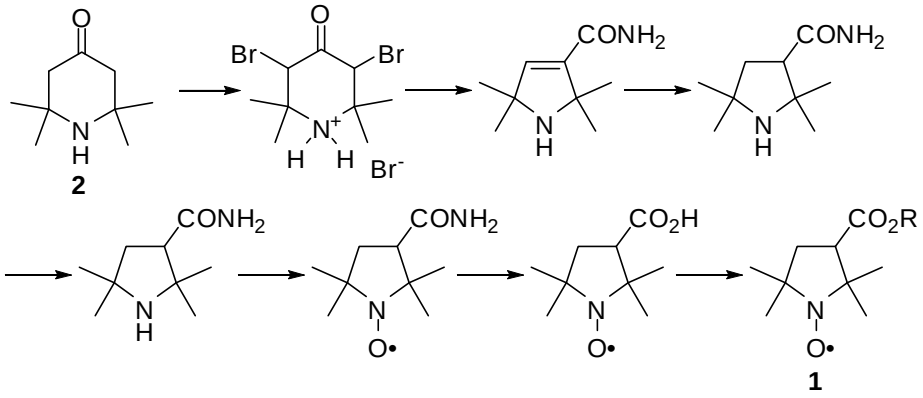
Department of Chemistry, Graduate School of Science and Engineering, Yamagata University, 4-3-16 Jonan, Yonezawa, Yamagata 992-8510, Japan
E-mail: hatano@yz.yamagata-u.ac.jp

Abstract – We have achieved an efficient alternative synthesis of blood-brain-barrier permeable nitroxyl radicals 3-methoxycarbonyl-2,2,5,5-tetra-methylpyrrolidine-1-oxyl (**1a**) and 3-ethoxycarbonyl-2,2,5,5-tetramethylpyrrol-idine-1-oxyl (**1b**), which affords **1a** and **1b** in 65% isolated yields by four steps from 2,2,6.6-tetramethyl-4-piperidone (**2**), respectively. This protocol is applicable to the synthesis of 3-isopropoxy-2,2,5,5-tetramethylpyrrolidine-1-oxyl (**1c**) and 3-carbonyl-2,2,5,5-tetramethylpyrro-lidine-1-oxyl (**6**).

INTRODUCTION

Stable heteroalicyclic nitroxyl radicals bearing piperidine and pyrrolidine ring systems are widely used as antioxidants,[1] contrast agents,[2] radiation protective agents,[3] and mediators of radical polymerization.[4] Especially in the field of biological chemistry and pathophysiology, nitroxyl radicals have also been used as probes for monitoring of the free radical reactions and the redox state in living organisms because nitroxyl radicals are observable *in vivo* using EPR spectroscopy and EPR-CT imaging.[5] Recently, nitroxyl radicals are also used to study reactive oxygen species in the brain, which are involved in many brain diseases.[6] The nitroxyl radical must pass through the blood-brain-barrier to monitor the redox reaction derived from reactive oxygen species in the brain. Three nitroxyl radicals have been prepared for use as probes in past decades,[7] but their synthetic routes and total yields were unsatisfactory (Scheme 1).[8] The development and efficient synthesis of an alternative blood-brain-barrier permeable nitroxyl radical is an attractive research area in the field of organic synthesis, extending the study of redox-reaction induced reactive oxygen species and elucidating brain diseases mechanisms. We now

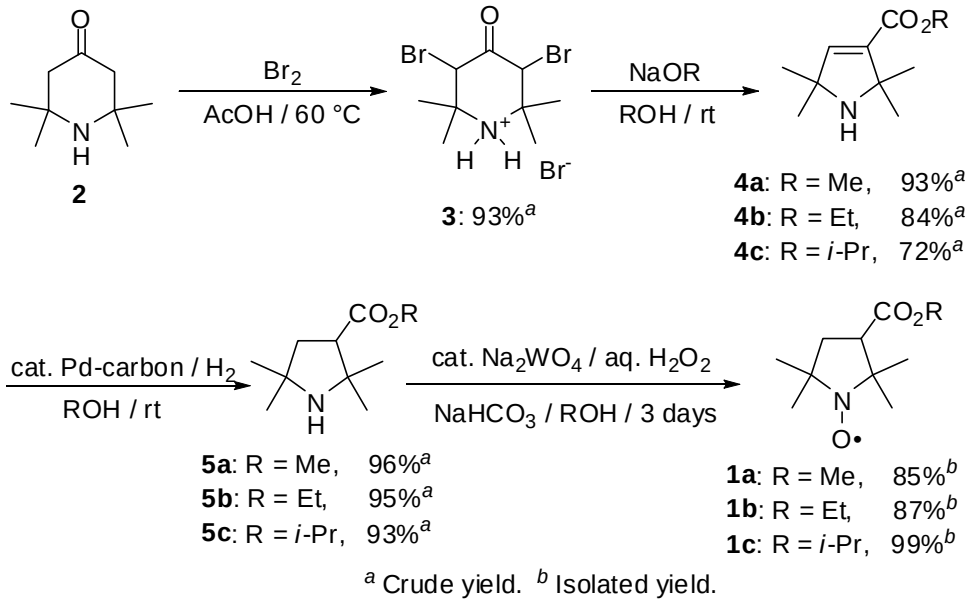
describe a new access to nitroxyl radicals 3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (**1a**) and 3-ethoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (**1b**), which are blood-brain-barrier permeable nitroxyl radicals (Scheme 2). This method involves alternative synthesis of general nitroxyl radical, 3-carbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (**6**), followed by the hydrolysis of **1a**, efficiently.



Scheme 1. General synthetic route of **1** from **2**

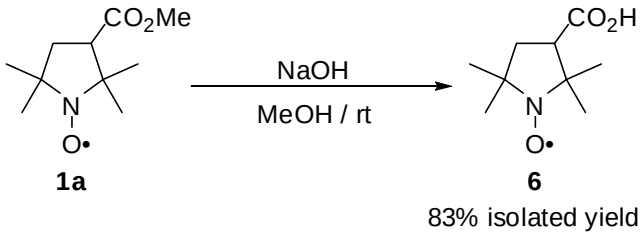
RESULTS AND DISCUSSION

Bromination of 2,2,6,6-tetramethyl-4-piperidone (**2**) in acetic acid gave 3,5-dibromo-2,2,6,6-tetramethyl-4-piperidone hydrobromide (**3**) in 93% crude yield.[9] Favorskii rearrangement of dibromide **3** with sodium methoxide in methanol afforded a 93% crude yield of almost pure grade α,β-unsaturated ester **4a**. This rearrangement of **3** was applied to other alkoxides: similar treatments of **3** with sodium ethoxide and sodium isopropoxide gave the corresponding α,β-unsaturated esters (**4b** and **4c**) in 84% and 72% crude yields, respectively.[10] The next hydrogenation step under hydrogen in the presence of a catalytic amount of palladium carbon showed no difficulty: unsaturated ester **4a** was transformed to the corresponding saturated ester **5a** in 96% crude yield. The other unsaturated esters also transformed to the corresponding saturated esters in almost identical crude yields (**5b**; 95%, **5c**; 93%, respectively). The next oxidation step was conducted using catalytic amounts of tungstate in the presence of hydrogen peroxide.[7a] Finally, the desired nitroxyl radical **1a** (3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl) was obtained in 85% isolated yield after distillation. The other derivatives (ethyl- and isopropyl-) also synthesized in this manner, giving nitroxyl radical **1b** and **1c** in 87% and 99% isolated yields, respectively. Finally, we achieved an efficient synthetic route of **1a** and **1b**, blood-brain-barrier permeable nitroxyl radical reagents, in an overall yields of 65% from low-cost commercially available 2,2,6,6-tetramethyl-4-piperidone (**2**), respectively.



Scheme 2. Synthesis of **1** from **2**

Furthermore, when 3-carboxy-2,2,5,5-tetramethylpyrrolidine-1-oxyl (**1a**) was treated with NaOH in methanol, pure **6** was obtained in 83% isolated yield after recrystallization (Scheme 3).



Scheme 3. Synthesis of **6** from **1a**

In conclusion, we achieved an efficient alternative synthesis of blood-brain-barrier permeable spin probe reagents (**1a** and **1b**), which affords **1a** and **1b** in 65% isolated yields by four steps from readily available 2,2,6,6-tetramethyl-4-piperidone (**2**), respectively. This synthesis was applied to nitroxyl radical derivatives (**1c** and **6**). More detailed studies of the synthesis of functional spin probe reagents and their utilization are now in progress.

EXPERIMENTAL

General. All melting points were determined on a Stuart melting point apparatus (SMP3) and are uncorrected. Infrared spectra were recorded using sodium chloride plates (liquid compounds) or a pressed potassium bromide disc (solid compounds) on a Jasco FT-IR 460Plus. Frequencies were given

in reciprocal centimeters (cm^{-1}) and only selected absorbance was reported. ^1H NMR spectra were recorded on a Varian 500 MHz spectrometers and the chemical shifts were reported in parts per million (δ) relative to internal standard TMS (0 ppm) for $CDCl_3$ or the center peak of residual DMSO (2.50 ppm). The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; dt, doublet of triplet; dq, doublet of quartet; ddd, doublet of doublet of doublet; dddd, doublet of doublet of doublet of doublet; m, multiplet; q, quartet. The coupling constants, J, are reported in Hertz (Hz). ^{13}C NMR spectra were obtained at 125 MHz and referenced to the internal solvent signals (central peak is 77.0 ppm in $CDCl_3$ or 39.43 ppm in DMSO-d_6). High-resolution mass spectra were obtained under electron spray ionization conditions on a JEOL JMS-T100LP. Elemental analyses were performed in the analytical section of our department on a Perkin Elmer 2400 Series II. Analytical thin layer chromatography (TLC) was carried out on aluminum sheets pre-coated with silica gel 60 F_{254} (Merck). Visualization on TLC was achieved by use of UV light (254 nm) and treatment with anisaldehyde or molybdatophosphoric acid stain followed by heating. Column chromatography was performed on silica gel (63–210 μm). All reagents and solvents are of commercial quality. MeOH, EtOH, and *i*-PrOH, which are used for the preparation of sodium alkoxide, are also of commercial quality. All reactions were carried out under nitrogen atmosphere in well-dried glassware, unless otherwise noted.

Preparation of dibromide (3). To a solution of **2** (20.0 g, 128.8 mmol) in glacial acetic acid (120 mL) was dropwise added bromine (19.8 mL, 38.6 mmol). The reaction mixture was vigorously stirred at 60 °C overnight. After cooling in an ice-water bath, the mixture was filtrated in suction. The residual solid was washed with acetic acid (100 mL × 2), water (50 mL), and Et_2O (100 mL × 2). After drying in air for 2 days, the pure dibromide **3** (47.2 g, 119.8 mmol) was obtained as a white solid in 93% crude yield. This product was suitable for use in the next step without further purification.

3,5-Dibromo-2,2,6,6-tetramethyl-4-piperidone hydrobromide (3):[9b] Registry number 19971-12-1; mp 191.4 – 192.4 °C (decomp.); ^1H NMR (500 Mz, DMSO-d_6): δ 9.35 (br, 1H), 5.49 (s, 2H), 1.71 (s, 6H), 1.35 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 188.7, 64.1, 60.2, 27.7, 22.0.

Synthesis of α,β-unsaturated ester (4a). To a solution of NaOMe in MeOH, which was prepared from sodium (2.3 g, 101.6 mmol) and MeOH (50 mL, commercial grade), was added dibromide **3** (10.0 g, 25.4 mmol) in five portions in an ice-water bath. After stirring at room temperature overnight, the resulting mixture was evaporated until almost all solvent had been removed. Then Et_2O (100 mL) was added to the residue, and the deposited salt was filtered off with suction. The filtrate was poured in a 10% aqueous K_2CO_3 solution (50 mL) and extracted with Et_2O (30 mL × 3). The combined ethernal solutions were dried over K_2CO_3. After evaporation, almost pure α,β-unsaturated ester **4a** (4.32 g, 23.6 mmol) was obtained in 93% crude yield. In this rearrangement, fresh or near fresh NaOMe is important:

the use of old NaOMe gave an unknown byproduct along with **4a**. In **4b**, a similar treatment to that described above was followed, except that MeOH was replaced by EtOH (commercial grade). The use of old NaOEt engendered the same result, giving an unknown byproduct along with **4b**. For **4c**, dibromide **3** was added slowly to NaO*i*-Pr solution at 70 °C because NaO*i*-Pr did not dissolve sufficiently in *i*-PrOH (commercial grade) less than 70 °C. These obtained unsaturated esters **4** were suitable for use in the next step without further purification. Analytic samples were obtained, respectively, using Kugelrohr distillation.

2,2,5,5-Tetramethyl-2,5-dihydropyrrole-3-carboxylic acid methyl ester (4a):[11] Registry number 70473-81-3; ^{1}H NMR (500 Mz, $CDCl_3$): δ 6.59 (s, 1H), 3.70 (s, 3H), 1.71 (br, 1H), 1.37 (s, 6H), 1.24 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.4, 149.0, 138.9, 65.8, 63.3, 51.2, 29.9, 29.8.

2,2,5,5-Tetramethyl-2,5-dihydropyrrole-3-carboxylic acid ethyl ester (4b):[11] Registry number 308106-20-9; IR (neat): ν_{max} = 3353, 2970, 1715, 1636, 1460, 1367, 1324, 1254, 1184, 1060 cm^{-1}; ^{1}H NMR (500 Mz, $CDCl_3$): δ 6.64 (s, 1H), 4.20 (q, 2H, *J* = 7.2 Hz), 1.80 (br, 1H), 1.42 (s, 6H), 1.32 (t, 3H, *J* = 7.2 Hz), 1.30 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.1, 148.8, 139.3, 65.8, 63.3, 60.1, 29.9, 29.8, 14.1.

2,2,5,5-Tetramethyl-2,5-dihydropyrrole-3-carboxylic acid isopropyl ester (4c): mp 41.5 – 43.1 °C; IR (KBr): ν_{max} = 3403, 2973, 1711, 1458, 1368, 1110, 1048, 773 cm^{-1}; ^{1}H NMR (500 Mz, $CDCl_3$): δ 6.58 (s, 1H), 5.04 (sept, 1H, *J* = 6.3 Hz), 1.70 (br, 1H), 1.38 (s, 6H), 1.25 (s, 6H), 1.25 (d, 6H, *J* = 6.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.7, 148.6, 139.7, 67.5, 65.8, 63.2, 29.9, 29.9, 21.8; HRMS (ESI) calcd for $C_{12}H_{22}N_1O_2$: 212.16505 $[M + H]^+$; Found: 212.16441.

Synthesis of saturated ester (5a). To a solution of crude **4a** (20.0 g, 109.1 mmol) in MeOH (100 mL) was added 5% Pd on carbon (500 mg). After stirring under a balloon of hydrogen at room temperature overnight, the resulting mixture was filtered. The filtrate was evaporated to give almost pure saturated ester **5a** (19.4 g, 104.7 mmol) in 96% crude yield. In **5b** and **5c**, a similar procedure to that described above was followed except that MeOH was replaced, respectively, by EtOH and *i*-PrOH. These obtained saturated esters **5** were suitable for use in the next step without further purification. Analytic samples were obtained using Kugelrohr distillation.

2,2,5,5-Tetramethylpyrrolidine-3-carboxylic acid methyl ester (5a):[11] Registry number 6910-74-3; ^{1}H NMR (500 Mz, $CDCl_3$): δ 3.65 (s, 3H), 2.86 (dd, 1H, *J* = 7.2, 11.8 Hz), 2.17 (dd, 1H, *J* = 11.8, 13.0 Hz), 1.90 (dd, 1H, *J* = 7.2, 13.0 Hz), 1.81 (br, 1H), 1.30 (s, 3H), 1.20 (s, 3H), 1.08 (s, 3H), 0.99 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 62.1, 60.1, 57.6, 55.0, 42.5, 31.5, 31.2, 31.0, 26.0, 14.2.

2,2,5,5-Tetramethylpyrrolidine-3-carboxylic acid ethyl ester (5b):[11] Registry number 908568-88-7; IR (neat): ν_{max} = 3345, 2967, 1731, 1461, 1373, 1284, 1184, 1147, 1032 cm^{-1}; ^{1}H NMR (500 Mz, $CDCl_3$): δ

4.13 (m, 2H), 2.81 (dd, 1H, *J* = 7.0, 11.5 Hz), 2.16 (dd, 1H, *J* = 11.5, 13.0 Hz), 1.89 (dd, 1H, *J* = 7.0, 13.0 Hz), 1.81 (br, 1H), 1.34 (s, 3H), 1.25 (s, 3H), 1.24 (td, 3H, *J* = 0.9, 7.3 Hz) 1.15 (s, 3H), 1.05 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 62.1, 60.1, 57.6, 55.0, 42.5, 31.5, 31.2, 31.0, 26.0, 14.2.

2,2,5,5-Tetramethylpyrrolidine-3-carboxylic acid isopropyl ester (5c): IR (neat): ν_{max} = 3344, 2968, 1725, 1460, 1374, 1110, 1062, 821, 753 cm^{-1}; ^{1}H NMR (500 Mz, $CDCl_3$): δ 4.99 (sept, 1H, *J* = 6.2 Hz), 2.79 (dd, 1H, *J* = 7.2, 11.8 Hz), 2.57 (br, 1H), 2.15 (dd, 1H, *J* = 11.8, 13.0 Hz), 1.87 (dd, 1H, *J* = 7.2, 13.0 Hz), 1.36 (s, 3H), 1.27 (s, 3H), 1.02(dd, 6H, *J* = 6.2 Hz) 1.16 (s, 3H), 1.06 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 67.4, 62.3, 57.8, 54.8, 42.3, 31.3, 31.1, 30.8, 25.7, 21.7; HRMS (ESI) calcd for $C_{12}H_{24}N_1O_2$: 214.18070 $[M + H]^+$; Found: 214.18061.

Synthesis of nitroxyl radical (1a). To a solution of crude **5a** (19.4 g, 104.7 mmol) in MeOH (200 mL) was added $NaHCO_3$ (17.6 g, 209.4 mmol) and $NaWO_4$ (6.6 g, 20 mmol). After cooling the mixture to 0 °C, the 30% aqueous H_2O_2 solution (59.3 mL, 523 mmol) was added dropwise for 30 min. The resulting yellow suspension was stirred for 3 days at room temperature. After evaporating almost all of the solvent, water (100 mL) was added to the residue. The mixture was extracted with CH_2Cl_2 (50 mL × 4), and the combined organic extracts were dried over Na_2SO_4. After evaporation, almost pure nitroxyl radical **1a** (19.2 g) was obtained. Pure nitroxyl radical **1a** (17.8 g, 88.9 mmol) was obtained using Kugelrohr distillation under vacuum conditions in 85% isolated yield. In **1b** and **1c**, a similar procedure to that described above was followed, except that MeOH was replaced, respectively, by EtOH and *i*-PrOH. In **1c**, nitroxyl radical **1c** was purified by silica gel chromatography (eluent hexane – AcOEt = 100:0, 90:10, 80:20, 100 mL each).

3-Methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (1a):[7f] Registry number 2154-33-8; IR (neat): ν_{max} = 2977, 1739, 1462, 1439, 1363, 1306, 1203, 1167, 1151 cm^{-1}; Anal. Calcd for $C_{10}H_{18}NO_3$: C, 59.98; H, 9.06; N, 6.99. Found: C, 59.87; H, 9.23; N, 6.83.

3-Ethoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (1b):[7b] Registry number 163082-99-3; IR (neat): ν_{max} = 3504, 2978, 1734, 1462, 1371, 1301, 1197, 1027 cm^{-1}; Anal. Calcd for $C_{11}H_{20}NO_3$: C, 61.66; H, 9.41; N, 6.54. Found: C, 61.43; H, 9.49; N, 6.44.

3-Isopropoxycarbonyl-2,2,5,5-tetramethylpyrrolidine-1-oxyl (1c): IR (neat): ν_{max} = 3354, 2977, 1729, 1461, 1372, 1108, 999, 825, 789 cm^{-1}; Anal. Calcd for $C_{12}H_{22}NO_3$: C, 63.13; H, 9.71; N, 6.14. Found: C, 62.86; H, 9.85; N, 5.95.

Synthesis of nitroxyl radical (6). To a solution of crude **1a** (1.00 g, 4.99 mmol) in MeOH (10 mL) was added 1M NaOH aqueous solution (10 mL) at 0 °C. After stirring for 6 h at room temperature, the reaction mixture was concentrated to half its prior volume. The residue was diluted with water (50 mL), and the solution was acidified to pH 3 with 3M HCl aqueous solution. The aqueous layer was extracted

with CH_2Cl_2 (30 mL × 3), and the combined organic extracts were dried over Na_2SO_4, and evaporated. After recrystallization from $CHCl_3$ – petroleum ether, the pure nitroxyl radical **6** (771 mg, 4.14 mmol) was obtained in 83% isolated yield.

3-Carboxy-2,2,5,5-tetramethylpyrrolidine-1-oxyl (6):[7f] Registry number 2154-68-9; mp 191.6 – 192.3 °C (decomp.); IR (KBr): ν_{max} = 3415, 2979, 1731, 1372, 1198 cm^{-1}; Anal. Calcd for $C_9H_{16}NO_3$: C, 58.05; H, 8.66; N, 7.52. Found: C, 58.26; H, 8.80; N, 7.46.

ACKNOWLEDGMENT

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8. Nitroxyl radicals **1a** and **1b** were generally synthesized from **6** by means of diazomethane (for **1a**) or classically esterification in the presence of suitable acid (for **1a** and **1b**), see: Ref. 7.
9. The dibromide **3** was prepared according to a modified protocol, see: (a) E. G. Rozantsev, 'Free Nitroxyl Radicals', ed. by H. Ulrich, Plenum Press, New York, 1970, p. 203; (b) S. W. Stork and M. W. Makinen, *Synthesis*, 1999, 1309.
10. In this rearrangement, fresh or near fresh sodium alkoxide is important: the use of old NaOMe or NaOEt gave an unknown byproduct along with **4**.
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