Nitroxides. IX.[1] Synthesis of nitroxide free radical α-amino acids

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Three methods are described for preparation of a new stable nitroxide free radical amino acid, 2-amino-3-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl) propionic acid and its derivates. This amino acid may be used as a paramagnetic amino acid synthon in studies of peptides.

LÁSZLÓ LEX, KÁLMÁN HIDEG et H. OLGA HANKOVSZKY. Can. J. Chem. **60**, 1448 (1982).

On décrit trois méthodes de préparation d'un nouveau radical libre nitroxyde stable d'un acide aminé: l'acide amino-2 (oxyl-1 tétraméthyl-2,2,5,5 pyrroline-3 yl-3)-3 propionique et ses dérivés. On utilise cet acide aminé comme synthon paramagnétique dans des études de peptides.

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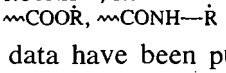
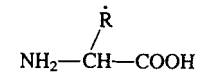
Introduction

Reactive functional groups containing nitroxides have often been used for acylating or alkylating peptides and proteins at reactive functional groups such as amino, thiol, or carboxyl groups (2–5).

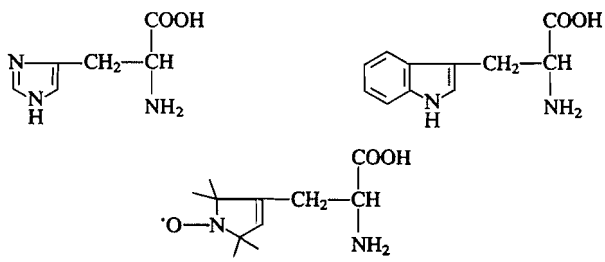
It is essential to carry out the labeling with as minimal perturbance of the biomolecule as possible to fulfill the requirements for an ideal reporter-group. That is of course not possible, because the modification of any essential function (e.g. acylating an amino group, alkylating a thiol group, or acylating a carbonyl group) may result in the loss of biological activity.



Relatively few data have been published on the synthesis of nitroxide free radical analogues of amino acids (6–8):



We wish to report methods generally applicable for synthesis of nitroxide α-amino acids, which can be used as synthons due to their structural resemblance to natural aralkyl amino acids like histidine or tryptophane:



As starting compounds, nitroxide derivatives are needed which are reactive enough and do not have a chiral centre. These requirements are fulfilled excellently by either the 1-oxyl-2,2,5,5-tetramethyl-3-bromomethyl-3-pyrroline (**1***a*) or the 1-oxyl-2,2,5,5-tetramethyl-3-methanesulfonyloxymethyl-3-pyrroline (**1***b*) (9).

Results and discussion

Three esters of α-functionalized carboxylic acids are acylated on their active methylene or methine group with **1***a* or **1***b* in alkaline media. In the first route (Method A) the dimethyl 2-acetamino malonate (**2**) is alkylated with **1***a* to **3** in the presence of sodium ethoxide. After partial hydrolysis of **3** the monomethyl ester of dicarboxylic acid (**4**) is formed. The acetamino nitroxide amino acid (**6**) can be obtained via decarboxylation of **4** and the hydrolysis of **5**. The compound **6** is suitable for reactions of its free carboxylic group with amino acids or peptides because its amino terminal is protected by an acetyl group.

The second method (Method B) utilizes a synthetic route by which the Schiff's base **7** (formed from benzophenone and glycine ester) can be alkylated with alkyl halides in a two-phase system using tetrabutylammonium hydrogen sulfate as a phase transfer catalyst (10). Following this, **7** can be alkylated with **1***a* to **8**. After acidic hydrolysis of **8** with aqueous citric acid (11) the ethyl ester of the nitroxide amino acid (**9**) is obtained. This route leads to a free α-amino group containing ester which can be acylated on its amino terminal. For longer storage the more stable tosylate salt of **9** is prepared with toluenesulfonic acid in dry acetone. On acetylation of **9** with 1-acetylimidazole, **10** is formed, which can be hydrolyzed to **6** obtained at the end of Method A.

[1]For Part VIII, see ref. 1.

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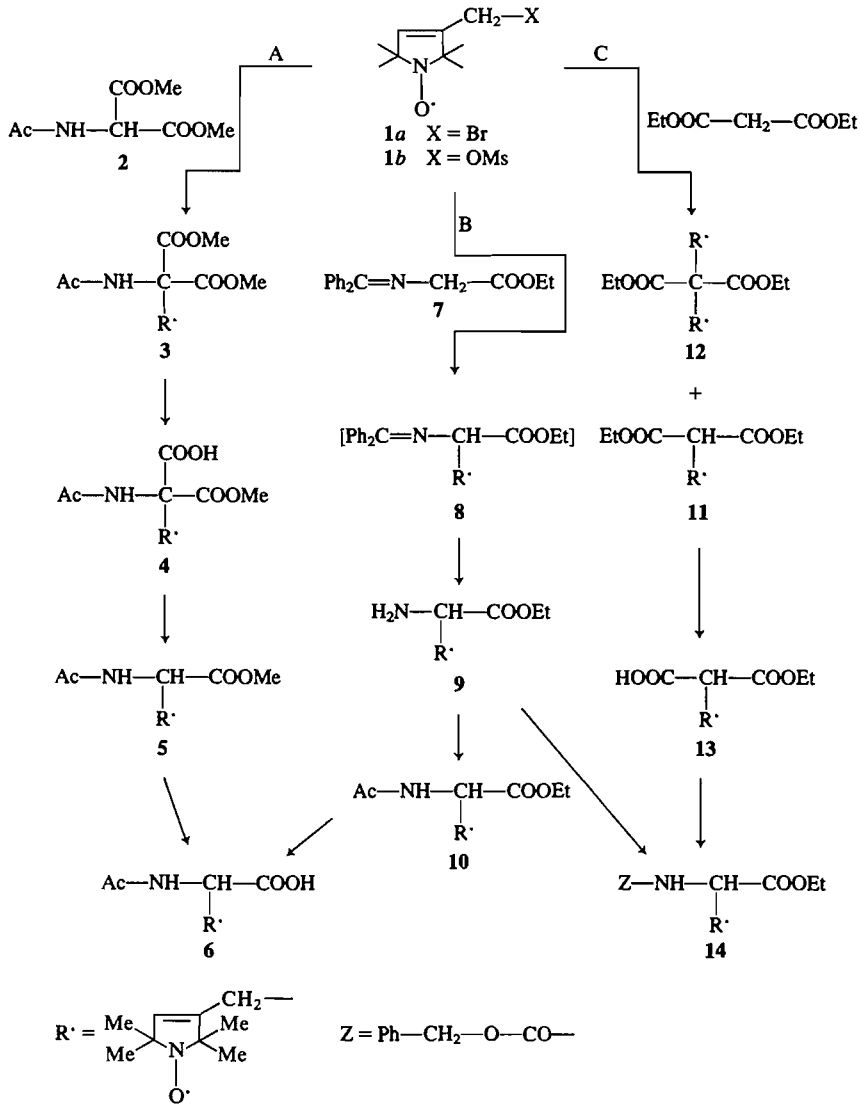

SCHEME 1

In the third route (Method C) the sodium diethyl malonate is alkylated with **1***b* (9) to **11** with formation of some bis-alkylated compound (**12**) as well. The biradical compound exhibits a 5-line esr spectrum characteristic of nitroxide biradicals.

In a partial hydrolysis of diester **11** the acid monoethyl ester (**13**) is formed. Utilizing the carbonyl-azide formation reaction with diphenyl phosphorylazide (DPPA) (12) **13** is allowed to react with DPPA and then with benzyl alcohol to obtain the desired ethylester of nitroxide amino acid (**14**) which is protected on its amino terminal by a carbobenzyloxy group (Z). This derivative can also be obtained when **9** is reacted with benzyl chloroformate in the presence of triethylamine.

These methods are equally useful for preparing derivates of nitroxide amino acids protected either on amino or carboxyl terminal groups. All of these amino acids are racemates; the experiments for resolution are in progress.

Experimental

Melting points were measured using a Boetius micro mp-determining instrument and are not corrected. The ir spectra were measured as a neat oil or in Nujol suspensions with a Zeiss Specord 75 type of instrument. The esr spectra were obtained

from 10^{-3} *M* solution using a Zeiss ER9 spectrometer. All the monoradicals exhibited three equidistant lines and the biradical showed five equidistant lines with a_N = 14.8–15.0 G. The mass spectra were taken using a Varian-MAT-SM-1 instrument. Solvents were reagent grade and dried prior to use. All organic extracts obtained during the work-up of the reaction mixture were dried over anhydrous magnesium sulfate and distilled off *in vacuo*. For column chromatography silica gel 60 was used.

*Dimethyl 2-acetamino-2-[(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)methyl] malonate (**3**)*

To a stirred solution of **2** (0.95 g, 5.0 mmol) in dry ethanol (15 mL) with sodium ethoxide (1 *M*, 5 mL, 5.0 mmol) a solution of **1***a* (1.17 g, 5.0 mmol) in dry ethanol (10 mL) was added, refluxed for 5 h, and then evaporated *in vacuo* to dryness. The residue was taken up in water (10 mL) and extracted with chloroform (3 × 10 mL). The organic phase was separated, dried, and evaporated. The solid residual was subjected to column chromatography on silica gel with tetrachloromethane/ethyl acetate (2:1). The first yellow band was unreacted **1***a*, 0.3 g (25%); the second one was compound **3**, yield: 0.9 g (53%), mp 97–98°C; ir ν_{max}: 3345 (NH), 1725 (CO), 1665 (CONH) cm^{-1}. *Anal.* calcd. for $C_{16}H_{25}N_2O_6$ (Mol. Wt. 341.4): C 56.30, H 7.38, N 8.21; found: C 56.42, H 7.37, N 8.35.

*Monomethyl ester of 2-acetamino-2-[(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)methyl] malonic acid (**4**)*

Sodium hydroxide solution (1 *M*, 2 mL, 2.0 mmol) was added to a solution of **3** (0.341 g, 1.0 mmol) in methanol (2 mL) and allowed to stay at room temperature overnight, then diluted with water (10 mL), acidified with dilute sulfuric acid (5%), and extracted with chloroform (3 × 10 mL). The organic phase was dried and evaporated to dryness. The solid residue was crystallized from chloroform/hexane to give **4**, yield: 0.27 g (82%), mp 120–121°C; ir ν_{max}: 3500–3200 (OH), 3365 (NH), 1730 (CO), 1640 (CONH) cm^{-1}. *Anal.* calcd. for $C_{15}H_{23}N_2O_6$ (Mol. Wt. 327.4): C 55.04, H 7.08, N 8.56; found: C 55.20, H 7.09, N 8.54.

*Methyl 2-acetamino-3-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)propionate (**5**)*

A suspension of the monomethyl ester (**4**) (0.327 g, 1.0 mmol) in water (10 mL) was refluxed for 3 h, then extracted with chloroform (3 × 10 mL). The organic phase was dried, evaporated, and crystallized from ether/hexane to give the pure **5**, yield: 0.22 g (78%), mp 87–88°C; ir ν_{max}: 3250 (NH), 1740 (CO), 1635 (CONH) cm^{-1}. *Anal.* calcd. for $C_{14}H_{23}N_2O_4$ (Mol. Wt. 283.4): C 59.35, H 8.18, N 9.89; found: C 59.41, H 8.08, N 9.92.

*2-Acetamino-3-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl) propionic acid (**6**)*

A solution of sodium hydroxide (2 *N*, 5 mL, 10.0 mmol) was added to a solution of **5** or **10** (5.0 mmol) in ethanol (15 mL) and left at room temperature overnight, then diluted with water (20 mL) and extracted with chloroform (3 × 10 mL). The aqueous phase was acidified with 1 *N* hydrochloric acid to pH 3, saturated with sodium chloride, and extracted with chloroform (3 × 10 mL), dried, evaporated to dryness, and crystallized from chlorform/methanol/ether to give **6**, yield: 0.99 g (74%) from **5**, and 0.96 g (72%) from **10**; mp in both cases 174–176°C; ir ν_{max}: 3330 (NH), 1700 (CO), 1620 (CONH) cm^{-1}. *Anal.* calcd. for $C_{13}H_{21}N_2O_4$ (Mol. Wt. 269.3): C 57.98, H 7.86, N 10.40; found: C 57.81, H 7.66, N 10.65.

*Ethyl 2-amino-3-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)-propionate (**9**) and its tosylate salt*

A solution of sodium hydroxide (2 *N*, 18 mL, 36.0 mmol) was added to a methylene chloride solution (15 mL) of **7** (4.0 g, 15.0 mmol), tetrabutylammonium hydrogen sulfate (TBAH) (6.1 g, 18.0 mmol), and **1***a* (4.2 g, 18.0 mmol) and stirred at room temperature for 2 days. The organic layer was separated, washed with water, dried, and evaporated to dryness to remove the residue quaternary bromide. The residue was taken up in ether and filtered. The filtrate was evaporated to dryness. The aqueous citric acid (10%, 30 mL) was added to the tetrahydrofuran (20 mL) solution of the Schiff's base **8** and stirred overnight. The reaction mixture was diluted with water (30 mL) and extracted with chloroform (2 × 25 mL) to remove the benzophenone. The aqueous phase was basified with 2 *N* sodium hydroxide solution to pH 8, extracted with chloroform (3 × 20 mL), dried, and evaporated to dryness to give **9** as a red oil, yield: 2.9 g (75%); ir ν_{max}: 3400–3200 (NH_2), 1730 (CO) cm^{-1}. *Anal.* calcd. for $C_{13}H_{23}N_2O_3$ (Mol. Wt. 255.4): C 61.15, H 9.08, N 10.97; found: C 61.05, H 9.10, N 11.16.

*The tosylate salt of **9***

A solution of *p*-toluenesulfonic acid monohydrate (0.95 g, 5.0 mmol) was added to the solution of **9** (1.27 g, 5.0 mmol) in dry acetone (10 mL) and diluted with ether until the crystallization started. The crude product was recrystallized from chloroform/ether/hexane to give the pure salt of **9**, yield: 1.8 g (84%), mp 145–146°C; ir ν_{max}: 1750 (CO) cm^{-1}. *Anal.* calcd. for $C_{20}H_{31}N_2O_6S$ (Mol. Wt. 427.5): C 56.19, H 7.31, N 6.55; found: C 56.07, H 7.02, N 6.44.

*Ethyl 2-acetamino-3-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)propionate (**10**)*

To a stirred solution of **9** (1.27 g, 5.0 mmol) in dry tetrahydrofuran (15 mL) a solution of 1-acetylimidazole (0.55 g, 5.0 mmol) was added in dry tetrahydrofuran (10 mL). The reaction mixture was allowed to stay for 2 h, then evaporated to dryness, and taken up in ethyl acetate. The ethyl acetate solution was extracted with 1 *N* hydrochloric acid solution, the organic phase was washed with saturated sodium hydrogen carbonate solution (5%), dried, evaporated to dryness, and the solid residue was crystallized from ether/hexane to give **10**, yield: 1.2 g (80%); mp 87–88°C; ir ν_{max}: 3240 (NH), 1745 (CO), 1640 (CONH) cm^{-1}. *Anal.* calcd. for $C_{15}H_{25}N_2O_4$ (Mol. Wt. 297.4): C 60.59, H 8.47, N 9.42; found: C 60.54, H 7.99, N 9.61.

*Diethyl 2-[(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)methyl]-malonate (**11**) and diethyl bis-2-[(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)methyl]malonate (**12**)*

A solution of diethyl malonate (1.6 g, 10.0 mmol) in dry ethanol (10 mL) was added to a freshly prepared solution of sodium ethoxide (1 *M*, 10 mL, 10.0 mmol) and stirred for 30 min. Then a solution of **1***b* (2.48 g, 10.0 mmol) in dry ethanol (25 mL) was added to the reaction mixture and heated at 60°C for 2 h. The reaction mixture was diluted with water (30 mL) and extracted with chloroform (3 × 30 mL). The chloroform extract was washed with water, dried, and evaporated to dryness to give a red oil. The oil was suspended in ether (10 mL) and the precipitated crystals were filtered to give as a by-product the bis-alkylated malonate (**12**), yield: 0.9 g (19%), mp 189–190°C; ir ν_{max}: 1720 (CO) cm^{-1}; esr ($CHCl_3$): 5 lines, a_N = 14.9 G. *Anal.* calcd. for $C_{25}H_{40}N_2O_6$ (Mol. Wt. 464.6): C 64.63, H 8.68, N 6.03; found: C 64.79, H 8.41, N 6.07.

The filtrate was evaporated and subjected to column chromatography on silica gel with tetrachloromethane/ethyl acetate (10:1) to give the monoalkylated product (**11**) as a red oil, yield: 1.6 g (51%); ir ν_{max}: 1735 (CO) cm^{-1}. *Anal.* calcd. for $C_{16}H_{26}NO_5$ (Mol. Wt. 312.4): C 61.52, H 8.39, N 4.48; found: C 61.25, H 8.15, N 4.89.

*Monoethyl ester of 2-[(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)methyl] malonic acid (**13**)*

A solution of potassium hydroxide (0.56 g, 10.0 mmol) was

added in ethanol (10 mL) to a stirred solution of **11** (3.12 g, 10.0 mmol) in absolute ethanol (20 mL) and refluxed for 2 h. The reaction mixture was evaporated *in vacuo* to dryness and dissolved in water (30 mL). The aqueous solution was extracted with chloroform (2 × 20 mL). The aqueous phase was acidified with 1 *N* hydrochloric acid to pH 3 and extracted again with chloroform (3 × 10 mL), dried, and evaporated to dryness. The solid residue was crystallized from ether/hexane to give the pure **13**, yield: 2.2 g (77%), mp 110–111°C; ir ν_{max}: 3200–2600 (OH), 1730 (CO) cm^{-1}. *Anal.* calcd. for $C_{14}H_{22}NO_5$ (Mol. Wt. 284.4): C 59.14, H 7.80, N 4.93; found: C 59.32, H 7.46, N 5.02.

*Ethyl 2-benzyloxycarbonylamino-3-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)propionate (**14**)*

Triethylamine (0.5 g, 5.0 mmol) and diphenylphosphoryl azide (1.38 g, 5.0 mmol) were added to a solution of **13** (1.42 g, 5.0 mmol) in dry benzene (30 mL) and refluxed for 1 h, then benzyl alcohol (0.7 g, 7.0 mmol) was added to the reaction mixture and refluxed for 2 days. The reaction mixture was evaporated to dryness *in vacuo* and chromatographed on a silica gel column with benzene/tetrahydrofuran (10:1) to give **14**, yield: 1.0 g (51%), mp 61–62°C; ir ν_{max}: 3310 (NH), 1740 (CO), 1690 (CONH) cm^{-1}; ms, *m/e*: M^+ 389(31), $M^+ + 1$ 390(8), 316(27), 224(14), 223(20), 135(19), 123(12), 91(100). *Anal.* calcd. for $C_{21}H_{29}N_2O_5$ (Mol. Wt. 389.5): C 64.76, H 7.51, N 7.14; found: C 64.74, H 7.41, N 7.48.

*Preparation of **14** from **9***

Triethylamine (0.1 g, 1.0 mmol) and benzylchloroformate (0.170 g, 1.0 mmol) were added to the solution of **9** (0.255 g, 1.0 mmol) in dry tetrahydrofuran (10 mL) and stirred at room temperature for 1 h. The reaction mixture was diluted with water (20 mL) and extracted with ethyl acetate (3 × 10 mL). The ethyl acetate phase was washed with 1 *N* hydrochloric acid, saturated sodium bicarbonate, saturated sodium chloride, dried, and evaporated to dryness *in vacuo*. The residue was crystallized from chloroform/hexane to give **14**, yield: 0.3 g (77%), mp 61–62°C.

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