Synthesis of New 2,2,5,5-Tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl Radicals and 2-Substituted-2,5,5-trimethylpyrrolidin-1-yloxyl Radicals Based α-Amino Acids

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Received 17 August 2004

Abstract: Unnatural paramagnetic α-amino acids with 2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl-3-yl radical or 2,5,5-trimethylpyrrolidin-1-yloxyl-2-yl radical side-chains, including a lysine mimic azido precursor and their derivatives, are described. The new set of paramagnetic amino acids presented in this work with different (polar, nonpolar, aliphatic, aromatic, etc.) side-chains offers a useful tool for the ESR study of the protein structure and function after incorporation, fulfilling diverse structural requirements.

Key words: amino acids, azides, free-radicals, O'Donnell synthesis, protecting groups

Unnatural amino acids have been the focus of biophysical, biochemical, and synthetic and medicinal chemical studies, particularly as they are applied to design of novel peptides.[1,2] One main group of unnatural amino acids have fluorophores or paramagnetic labels in the side chain, which allows to follow them by biophysical methods.[3–6] There are two main approaches to modifying peptides with spin labels. One approach is site-directed spin labeling, which requires synthesis of cysteine mutants which can be modified afterwards with paramagnetic methanethiosulfonates.[7] The other approach includes the incorporation of a paramagnetic amino acid in a step-by-step synthesis, e. g. Merrifield synthesis or nonsense suppression methodology.[2] For the ESR studies of proteins, a variety of paramagnetic α-amino acids,[3–5,8] β-amino acids [9] and γ-amino acids[10] have been synthesized. In several cases naturally occurring amino acids were modified by alkylation or acylation with functionalized pyrrol-1-yloxyl radicals to obtain a paramagnetic protein building block[11] and very recently paramagnetically modified cysteine and tyrosine were inserted using nonsense incorporation in Xenopus Oocytes.[12] TOAC,[3] (4-amino-1-oxyl-2,2,6,6-tetramethyl-piperidine-4-carboxylic acid) by far the most popular among the above mentioned α-amino acids, was incorporated into α-melanocyte stimulating hormone without loss of biological activity.[13] Very recently, from our laboratory, paramagnetic amino acids obtained by O'Donnell synthesis,[4] including conformationally constrained amino acids have been reported.[8] Until now, mainly 3-substituted 2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radicals have been used for the synthesis of paramagnetic amino acids. In this paper, we report the extension of the above procedure for 3,4-disubstituted 2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radicals and 2-substituted 2,5,5-trimethylpyrrolidine-1-yloxyl radicals with different alkyl and aromatic substituents and spacers, leading to second generation of paramagnetic α-amino acids. The introduction of 2-substituted 2,5,5-trimethylpyrrolidine-1-yloxyl radicals generates a new α-amino acid series with a proline-like side chain with an orientation different from amino acids containing 3-substituted 2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radicals (Figure 1).

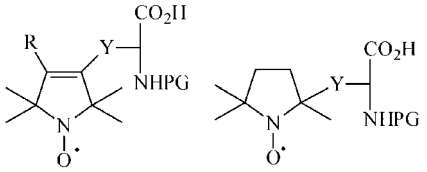
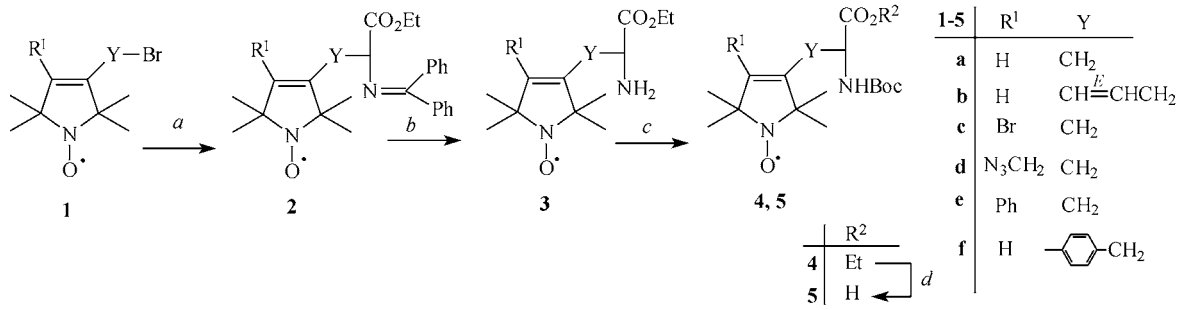


Figure 1 Chemical structure of paramagnetic amino acids.

Alkylation of ethyl *N*-diphenylmethylene glycine with paramagnetic allylic bromide **1a**,[14] **1b** obtained from the corresponding alcohol[15] **1c**,[16] **1d**[17] obtained from 3,4-bis(bromomethyl)-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical,[18] **1e**[19] and benzylic bromide **1f**[20] under phase transfer conditions[21] gave the monoalkylated product **2a**–**f**, which could be readily hydrolyzed under acidic conditions to the corresponding amine **3a**–**f**, without affecting the *N*-oxyl radical moiety. The treatment of DL-amino acid esters with *t*-butoxycarbonyl anhydride gave the corresponding protected *N*-Boc amino acid ethyl esters **4a**–**f**, which can be hydrolyzed to acids **5a**–**f** allowing utilization in Merrifield synthesis (Scheme 1). Incorporation of amino acid **5c** as a bromine containing compound is not only a spin label but may support proteomic analysis by mass spectrometry with the diagnostic unique twin peaks arising from the bromine isotopes.[22] Compound **5d** is designed to convert ε-azidobutyl side-chain to ε-aminobutyl side-chain after incorporation into a protein or it can be used for protein immobilization or aiding cross-links by Staudinger ligation,[23] while synthe-

SYNLETT 2004, No. 14, pp 2591–2593
Advanced online publication: 20.10.2004
DOI: 10.1055/s-2004-834806; Art ID: G32104ST



Scheme 1 *Reagents and conditions*: (a) $Ph_2C{=}NCH_2CO_2Et$ (1.0 equiv), 10% aq NaOH, CH_2Cl_2, Bu_4NHSO_4 (0.5 equiv), r.t., 2 h, 50–78%; (b) 5% aq H_2SO_4, EtOH, 30 min., r.t., then solid K_2CO_3 to pH = 8, 15–84%; (c) Boc_2O (1.1 equiv), THF, 40 °C, 30 min, 36–70%; (d) 10% aq NaOH, EtOH, 1 h, then aq H_2SO_4 to pH = 3, 36–53%.

sis of compounds **5e–f** were intended to mimic the natural amino acids with aromatic side chain such as phenylalanine.

The other approach to the synthesis of paramagnetic amino acids by the O'Donnell method uses 2-substituted 2,5,5-trimethylpyrrolidine-1-yloxyl radicals as alkylating agents.

These alkylating agents are readily available from 2,5,5-trimethyl-1-pyrroline *N*-oxide (TMPO)[24] by Grignard reaction of propargyl alcohol[25] or 4-(dimethoxymethyl)phenyl bromide[26] followed by functional group transformations. Although quite simple, this method has the disadvantage that a second chiral center is introduced into the molecule, necessitating a final purification step to resolve the two diastereomers. Alkylation of ethyl *N*-diphenylmethylene glycine with paramagnetic propargylic **6a**,[25] allylic **6b**[25] and benzylic bromide **6c**[26] under phase-transfer conditions gave the monoalkylated product **7a–c**, which could be readily hydrolyzed under acidic conditions to the corresponding amine **8a–c**. Treatment of racemic amino acid esters with *t*-butoxycarbonyl anhydride gave the protected *N*-Boc amino acid ethyl esters **9a–c** which can be hydrolyzed to the corresponding N-protected amino acids **10a–c** as described above (Scheme 2). The paramagnetic **10a** propargyl glycine, **10b** allyl glycine and **10c** phenylalanine with different orientation, spacer rigidity and saturation forms a novel paramagnetic amino acid series.

In conclusion, new N-protected α-amino acids[27] with paramagnetic side chains with different length, orientation, shape and polarity have been synthesized. The resolution of these new, second-generation paramagnetic amino acids with chiral chromatography as well as their incorporation into peptides are in progress as part of another ongoing project.

Acknowledgment

This work was supported by grant from Hungarian National Research Foundations (OTKA T34307) and Deutsche Forschungsgemeinschaft (EN87/12-1 for M. E., Hi 823/1-1 for K. H. and STE 640/4-3 for H.-J. S). The authors thank N. Lazsányi for elemental analyses and Mária Szabó for mass spectral measurements (ICN, Hungary).

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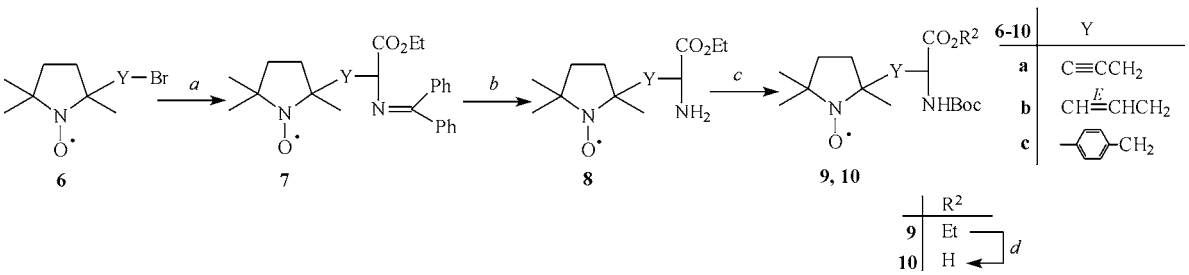

Scheme 2 *Reagents and conditions*: (a) $Ph_2C{=}NCH_2CO_2Et$ (1.0 equiv), 10% aq NaOH, CH_2Cl_2, Bu_4NHSO_4 (0.5 equiv), r.t., 2 h, 39–70%; (b) 5% aq H_2SO_4, EtOH, 30 min, r.t., then solid K_2CO_3 to pH = 8, 34–56%; (c) Boc_2O (1.1 equiv), THF, 40 ºC, 30 min, 44–61%; (d) 10% aq NaOH, EtOH, 1 h, then aq H_2SO_4 to pH = 3, 49–59%.

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(17) **Synthesis of 1d:** To a stirred solution of 3,4-bis(bromomethyl)-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (326 mg, 1.0 mmol) in acetone (10 mL) NaN_3 (65 mg, 1.0 mmol) dissolved in H_2O (2 mL) was added and the mixture was stirred for 3 h at 40 °C. The acetone was evaporated off and after adding of H_2O (5 mL) the mixture was extracted with $CHCl_3$ (2 × 10 mL). The organic layer was separated, dried ($MgSO_4$), filtered and evaporated. Purification of the residue by flash column chromatography (hexane–Et_2O) gave compound **1d** 106 mg (37%), mp 70–72 °C, R_f = 0.28 (hexane–Et_2O, 2:1). IR (nujol): ν = 2095 cm^{-1}. MS (EI): *m/z* (%) = 287/289 (10/10) [M^+], 193 (37), 152 (67), 41 (100). The side product is 3,4-*bis*(azidomethyl)-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical, 68 mg (27%), mp 90–92 °C, R_f = 0.24 (hexane–Et_2O, 2:1).
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(27) Compounds were characterized by MS, ESR, IR and elemental analysis. Spectra were consistent in each case with the assigned structures. ESR spectra of all *N*-Boc protected amino acid were taken in 10^{-4} M water solution and all monoradicals gave triplett line a_N = 15.5–15.8 G.
Representative Synthesis of Compound 5d: To stirred solution of *N*-diphenylmethylene glycine (801 mg, 3.0 mmol) and compound **1d** (864 mg, 3.0 mmol) in CH_2Cl_2 (20 mL), 10% aq NaOH (3 mL) was added followed by addition of Bu_4NHSO_4 (508 mg, 1.5 mmol) and the mixture was stirred at r.t. for 2 h. The organic phase was separated, dried ($MgSO_4$), filtered and evaporated to give compound **2d** as a yellow oil 740 mg (52%). The crude product was immediately subjected to acidic hydrolysis. Compound **2d** was dissolved in EtOH (20 mL), 5% aq H_2SO_4 (5 mL) was added, the mixture was allowed to stand at r.t. and the mixture was monitored by TLC. After consumption of compound **2d** (ca 30 min) H_2O (10 mL) was added, and the pH = 8 was adjusted by addition of solid K_2CO_3, extracted with $CHCl_3$ (2 × 20 mL). Then, the organic phase was separated, dried ($MgSO_4$), filtered, evaporated and the residue was purified by flash column chromatography ($CHCl_3$–MeOH) to give compound **3d** (203 mg, 42%) as a yellow oil. IR (nujol): ν = 3350, 3280, 2095, 1730 cm^{-1}. MS (EI): *m/z* (%) = 310 (3) [M^+], 249 (43), 233 (31), 161 (100). Anal. Cald for $C_{14}H_{24}N_5O_3$: C, 54.18; H, 7.79; N, 22.56. Found: C, 54.01; H, 7.71; N, 22.40.
To a solution of compound **3d** (310 mg, 1.0 mmol) in dry THF (15 mL) *t*-butoxycarbonyl anhydride (240 mg, 1.1 mmol) was added and the mixture was stirred at 40 °C for 30 min. After cooling, Et_2O (20 mL) was added and the organic phase was washed with brine (10 mL). Then, the organic phase was separated, dried ($MgSO_4$), filtered and evaporated to give crude **4d** as a yellow solid 279 mg (68%). This crude **4d** was dissolved in EtOH (10 mL), then H_2O (3 mL) and 10% aq NaOH (1 mL) were added and the mixture was allowed to stand at r.t. and monitored by TLC. After consumption of compound **4d** (ca 1 h) the solution was acidified to pH = 3 by cautious addition of 5% aq H_2SO_4. The aqueous phase was extracted with $CHCl_3$ (2 × 20 mL), the combined organic phase was dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography ($CHCl_3$–MeOH) to give compound **5d** as a yellow solid 101 mg (39%), mp 160–162 °C. Anal. Calcd for $C_{17}H_{28}N_5O_5$: C, 53.39; H, 7.38; N, 18.31. Found: C, 53.43; H, 7.35; N, 18.50. MS was taken with thermospray technique (TSP): *m/z* = 383 [M + H]$^+$.