# 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

Mária Balog,[a] Tamás Kálai,[a] József Jekő,[b] Heinz-Jürgen Steinhoff,[c] Martin Engelhard,[d] Kálmán Hideg*[a]

[a] Institute of Organic and Medicinal Chemistry, University of Pécs, P. O. Box 99, 7602 Pécs, Hungary
Fax +36(72)536219; E-mail: kalman.hideg@aok.pte.hu

[b] ICN Hungary Ltd., P. O. Box 1, 4440 Tiszavasvári, Hungary

[c] Department of Physics, University of Osnabrück, Barbara Str. 7, 49069 Osnabrück, Germany

[d] Max Planck Institute of Molecular Physiology, P.O. Box 50 02 47, 44202 Dortmund, Germany

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**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).

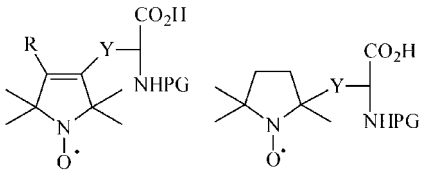


**Figure 1** Chemical structure of paramagnetic amino acids.

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-

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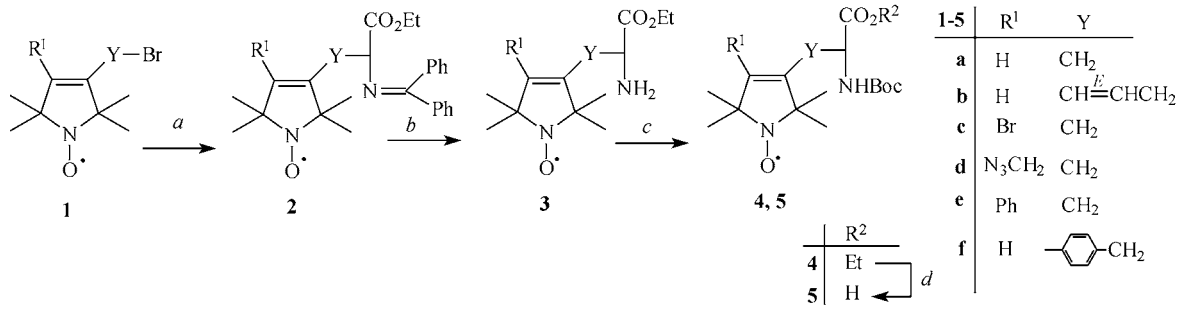


**Scheme 1** *Reagents and conditions*: (a) $Ph_2C{=}NCH_2CO_2Et$ (1.0 equiv), 10% aq NaOH, $CH_2Cl_2$, $Bu_4NHSO_4$ (0.5 equiv), r.t., 2 h, 50–78%; (b) 5% aq $H_2SO_4$, EtOH, 30 min., r.t., then solid $K_2CO_3$ to pH = 8, 15–84%; (c) $Boc_2O$ (1.1 equiv), THF, 40 °C, 30 min, 36–70%; (d) 10% aq NaOH,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

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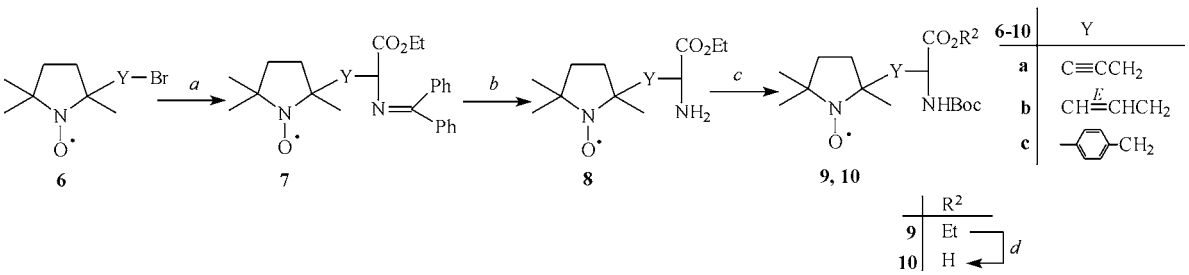
## References

(1) (a) Williams, R. M. *Synthesis of Optically Active α-Amino Acids*; Pergamon Press: Oxford, **1989**. (b) Park, K.-H.; Kurth, M. J. *Tetrahedron* **2002**, *58*, 8629. (c) Watanabe, L. A.; Jose, B.; Kato, T.; Nishino, N.; Yoshida, M. *Tetrahedron Lett.* **2004**, *45*, 491.
(2) Dougherty, D. A. *Curr. Opin. Chem. Biol.* **2000**, *4*, 645.
(3) Rassat, A.; Rey, P. *Bull. Soc. Chim. Fr.* **1967**, 815.
(4) Lex, L.; Hideg, K.; Hankovszky, H. O. *Can. J. Chem.* **1982**, *60*, 1448.
(5) Hideg, K.; Hankovszky, H. O. *Spin Labeling Theory and Applications*, In *Biological Magnetic Resonance*, Vol. 8; Berliner, L. J.; Reuben, J., Eds.; Plenum Press: New York, **1989**, 427.




**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%.

(6) Dufau, I.; Mazarguil, H. *Tetrahedron Lett.* **2000**, *41*, 6063.
(7) Hubbell, W. L.; Altenbach, C.; Hubbell, C. M.; Khorana, H. G. *Adv. Protein. Chem.* **2003**, *63*, 243.
(8) Balog, M.; Kálai, T.; Jekő, J.; Berente, Z.; Steinhoff, H.-J.; Engelhard, M.; Hideg, K. *Tetrahedron Lett.* **2003**, *44*, 9213.
(9) Wright, K.; Crisma, M.; Toniolo, C.; Török, R.; Péter, A.; Wakselman, M.; Mazaleyrat, J. P. *Tetrahedron Lett.* **2003**, *44*, 3381.
(10) Hideg, K.; Hankovszky, H. O.; Halász, H. A.; Sohár, P. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2905.
(11) (a) Cornish, V. W.; Benson, D. R.; Altenbach, C. A.; Hideg, K.; Hubbell, W. L.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2910. (b) McNulty, J. C.; Thompson, D. A.; Carrasco, M. R.; Millhauser, G. L. *FEBS Lett.* **2002**, *529*, 243. (c) Cerasi, A.; Millo, E.; Ottaviani, F. M.; Damonte, G.; Cangiotti, M.; Benatti, U.; Chiarintini, L. *Tetrahedron Lett.* **2003**, *44*, 8701. (d) Liu, J.; Zhao, M.; Wang, C.; Peng, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4065.
(12) Shafer, A. M.; Kálai, T.; Liu, S. Q. B.; Hideg, K.; Voss, J. *Biochemistry* **2004**, *43*, 8470.
(13) Barbosa, S. R.; Cilli, E. M.; Lamy-Freund, M. T.; Castrucci, A. M. L.; Nakaie, C. R. *FEBS Lett.* **1999**, *446*, 45.
(14) Hankovszky, H. O.; Hideg, K.; Lex, L. *Synthesis* **1980**, 914.
(15) Hideg, K.; Hankovszky, H. O.; Lex, L.; Kulcsár, G. *Synthesis* **1980**, 911.
(16) Kálai, T.; Balog, M.; Jekő, J.; Hideg, K. *Synthesis* **1998**, 1476.
(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).
(18) Kálai, T.; Balog, M.; Jekő, J.; Hideg, K. *Synthesis* **1999**, 973.
(19) Sár, C. P.; Jekő, J.; Hideg, K. *Synthesis* **1998**, 1497.
(20) Kálai, T.; Balog, M.; Jekő, J.; Hubbell, W. L.; Hideg, K. *Synthesis* **2002**, 2365.
(21) O'Donnell, M. J.; Boniece, J. M.; Earp, S. E. *Tetrahedron Lett.* **1978**, *30*, 2641.
(22) Hamdan, M.; Righetti, P. G. *Mass Spectrom. Rev.* **2002**, *21*, 287.
(23) Soellner, M. B.; Dickson, K. A.; Nilsson, B. L.; Raines, R. T. *J. Am. Chem. Soc.* **2003**, *125*, 11790.
(24) Delpierre, G. R.; Lamchen, M. *J. Chem. Soc.* **1963**, 4693.
(25) Bárácz, M. N.; Hankovszky, H. O.; Sár, P. C.; Jerkovich, G.; Hideg, K. *Synthesis* **1996**, 204.
(26) Gadányi, S.; Kálai, T.; Jekő, J.; Berente, Z.; Hideg, K. *Synthesis* **2000**, 2039.
(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]$^+$.