Nitroxyls: IV. Synthesis of Spin-Labelled N-(4-Piperidinoloxycarbonyl)-imidazoles and 4-Piperidinoloxycarbonyl Azides and Their Reaction with Amino Acid Derivatives

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The spin-label technique is a useful method for investigating biomolecules by E.S.R. spectroscopy. For labelling amino acids at the amino group it is desirable to prepare a spin-labelled group which resembles the t-butoxycarbonyl protective group.

We wish to describe the synthesis of N-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinoloxycarbonyl)-imidazole (2) and 1-oxyl-2,2,6,6-tetramethyl-4-piperidinoloxycarbonyl azide (4). The imidazole 2 can be prepared from 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (1) and N,N-carbonyl-2-imidazole by the method of Staub described for the synthesis of N-t-butoxycarbonylimidazole. Compound 2 is used in the form of its higher reactive tosylate (3). This salt is so reactive that it is instantaneously converted into 4 when dissolved in an aqueous solution of sodium azide. The azidoformylamide azide proved to be a convenient reagent for the preparation of spin-labelled amino acids (6) and their esters (7) under the conditions generally used in peptide chemistry in N-protection with t-butoxycarbonyl azide.

The reagent 4 may also be prepared in moderate yield from 4-nitrophenyl 1-oxyl-2,2,6,6-tetramethyl-4-piperidinol carbonate (5) and sodium azide.

It has also been found that the reactivity of N-t-butoxycarbonyl-imidazole (8) in the formation of t-butoxycarbonyl azide (10) is increased by conversion to the tosylate (9). This fact shows more generally that N-alkoxycarbonylimidazolium tosylates are convenient and reactive starting compounds for the synthesis of azidoformylamide azide.

The starting materials 1, 2, and 8 were prepared by known methods. Melting points were measured using a Boekeis micro-m.p. determining instrument and are not corrected. The I.R. spectra were measured in Nujol suspensions with a Zeiss Specord 71 type instrument. The E.S.R. spectra were obtained from 10⁻⁴ molar solutions using a Zeiss E9 spectrometer.

N-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinoloxycarbonyl)-imidazole (2):
A solution of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (1: 5.16 g, 0.03 mol) in dry ether (20 ml) is added dropwise to a stirred solution of carbonyl-2-imidazole (4.86 g, 0.03 mol) in dry tetrahydrofurane (30 ml) at room temperature. After the addition is complete, the solution is left for a further 3 h at room temperature, then extracted with water (30 ml) and saturated sodium chloride solution. The organic phase is dried with sodium sulfate and evaporated to dryness. The orange solid obtained is recrystallized from ether/90% aceton: yield: 6.1 g (76%), m.p. 62.3°.

C₁₇H₂₉N₃O₃ calc.: C 58.63 H 7.57 N 15.78 (266.3) found 58.84 7.45 15.51
E.S.R. (CHCl₃): 3 lines, δν = 15.8 Gs.
I.R. (Nujol): νmax = 1750 (COO⁻) cm⁻¹.

N-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinoloxycarbonyl)-imidazolium Tosylate (3):
A solution of compound 2 (5.32 g, 0.02 mol) in dry acetone (10 ml) is added to a solution of p-toluenesulfonic acid monohydrate (3.80 g, 0.02 mol) in acetone (10 ml). Crystallization is completed by adding ether and the product isolated by suction: yield: 7.9 g (90%), m.p. 147/149°.

C₁₈H₂₅N₃O₃S calc.: C 54.78 H 6.44 N 9.88 S 7.31 (438.5) found 54.38 6.34 9.58 7.80
E.S.R. (CHCl₃): 3 lines, δν = 15.8 Gs.
I.R. (Nujol): νmax = 1780 (COO⁻) cm⁻¹.

1-Oxyl-2,2,6,6-tetramethyl-4-piperidinoloxycarbonyl Azide (4):
Method A: The imidazolium salt 3 (4.38 g, 0.01 mol) is added at once to a stirred solution of sodium azide (1.30 g, 0.02 mol) in water (10 ml) at room temperature. The mixture is stirred for 15 min and then extracted with hexane (30 ml). The extract is dried with sodium sulfate and evaporated to a volume of 3 ml and cooled to -20°. The product separates as red crystals which are isolated by suction: yield: 2.17 g (92%), m.p. 47/48°.

C₁₉H₂₁N₄O₃ calc.: C 49.78 H 7.10 N 23.22 (281.3) found 49.78 6.70 22.73
E.S.R. (CHCl₃): 3 lines, δν = 15.8 Gs.
I.R. (Nujol): νmax = 2100, 2150 (N₃) cm⁻¹.

Method B: To 4-nitrophenyl 1-oxyl-2,2,6,6-tetramethyl-4-piperidinol carbonate (5: 5.37 g, 0.01 mol) prepared by the method of Sparrow from 1 and 4-nitrophenyl carbomethoxycarbamate in the presence of triethylamine and diethylether in acetone (40 ml) a solution of sodium azide (1.30 g, 0.02 mol) in water (10 ml) is added and
the mixture is stirred at room temperature overnight. The mixture is then diluted with water (100 ml) and extracted with hexane (3 x 30 ml). The extract is washed with 10% potassium carbonate solution (3 x 30 ml) until the aqueous layer remains colorless. The further work-up is as above in Method A; yield: 1.6 g (65%).

Ethyl N-[1-Oxy]-2,2,6,6-tetramethyl-4-piperidinoloxycarbonyl-aminooacetate (6); Typical Procedure:
A solution of compound 4 (2.41 g, 0.01 mol) in dimethylformamide (10 ml) is added to a solution of glycine ethyl ester (1.03 g, 0.01 mol) and triethylamine (1.01 g, 0.01 mol) in dimethylformamide (10 ml). The mixture is stirred for 3 h at room temperature, then evaporated to dryness under vacuum. The residue is taken up in ethyl acetate (30 ml) and the solution washed with 1 normal hydrochloric acid at 0°, 5% potassium carbonate solution and water. It is then dried with sodium sulfate and evaporated under vacuum. The residue crystallizes from ether/hexane; yield: 20 g (65%); m.p. 78-79°.

C_{14}H_{22}N_{2}O_{3} calc. C 55.80 H 8.36 N 9.29
(301.4) found 55.70 8.25 9.40
E.S.R. (CHCl₃): 3 lines; δₑₑₑ = 15.9 Gs.
I.R. (Nujol): vₓₓₓ = 3570 (NH); 1730 (COO); 1700 (O–CO–N) cm⁻¹.

N-[1-Oxy]-2,2,6,6-tetramethyl-4-piperidinoloxycarbonyl-aminooacetate (7); General Procedure:
A solution of compound 4 (2.41 g, 0.01 mol) in dioxane (20 ml) is added to a stirred suspension of the amino acid (0.01 mol) and magnesium oxide (0.08 g, 0.02 mol) in water (10 ml). The mixture is stirred for 24 h at 0°, then diluted with water (30 ml) and extracted with ethyl acetate. The aqueous phase is acidified with 1 normal hydrochloric acid at 0° and extracted with ethyl acetate. The extract is dried with sodium sulfate, and evaporated. The residue is crystallized from ether.

Labelled l-Phenylalanine (7a); yield: 2.90 g (80%); m.p. 131-132°.

C_{14}H_{15}NO_{3} calc. C 62.79 H 7.49 N 7.11
(263.1) found 62.53 7.42 7.92
E.S.R. (CHCl₃): 3 lines; δₑₑₑ = 15.9 Gs.
I.R. (Nujol): vₓₓₓ = 3250 (NH); 3500-3100 (OH); 1720 (CO); 1690 (O–CO–N) cm⁻¹.

Labelled l-Tryptophane (7b); yield: 2.90 g (72%); m.p. 110-112°.

C_{21}H_{21}N_{2}O_{3} calc. C 62.67 H 7.01 N 10.44
(402.5) found 62.33 7.26 10.53
E.S.R. (CHCl₃): 3 lines; δₑₑₑ = 15.9 Gs.
I.R. (Nujol): vₓₓₓ = 3370 (NH); 1710 (COO) cm⁻¹.

N-(t-Butoxycarbonyl)-imidazolium Tosylate (9):
To a freshly prepared solution of N-(t-butoxycarbonyl)-imidazole (8; 5.5 g, 0.03 mol) in dry acetic anhydride (30 ml), a solution of toluenesulfonic acid monohydrate (5.71 g, 0.03 mol) in dry acetic anhydride is added. Precipitation of 9 as colorless salt is completed by adding some ether. The product is isolated by suction; yield: 7.85 g (77%); m.p. 140-141°.

C_{18}H_{22}N_{2}O_{3}S calc. C 52.93 H 5.92 N 8.23 S 9.42
(454.0) found 53.14 6.13 8.57 9.54
I.R. (Nujol): vₓₓₓ = 1780 (COO) cm⁻¹.

H-N.M.R. (CDCl₃); δ = 1.62 [s, 9H, C(CH₃)₃]; 2.3 (s, 3H, CH₃); 6.7-7.9 (m, 6H, arom); 9.4 ppm (m, 1H, imidazolyl).

t-Butoxycarbonyl Azide (10, Boc-Azide):
N-(t-Butoxycarbonyl)-imidazolium tosylate (9; 3.40 g, 0.01 mol) is added in one portion to a solution of sodium azide (2.60 g, 0.04 mol) in water (10 ml) and the mixture is stirred for 15 min at room temperature. The mixture is then extracted with ether (3 x 20 ml). The extract is dried with sodium sulfate, the solvent evaporated, and the residue distilled in vacuum; yield: 1.22 g (85%); b.p. 36-38°/11 torr (Ref.³, b.p. 73-76°/70 torr (orr).
I.R. (film) vₓₓₓ = 2180, 2115 (Nₓₓ); 1740 (COO) cm⁻¹.

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