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A Facile Preparation of Fmoc-Arg$^{\omega,\omega'}$(Boc)$_2$-OH and Z-Arg$^{\omega,\omega'}$(Boc)$_2$-OH, New Arginine Derivatives for Peptide Synthesis.

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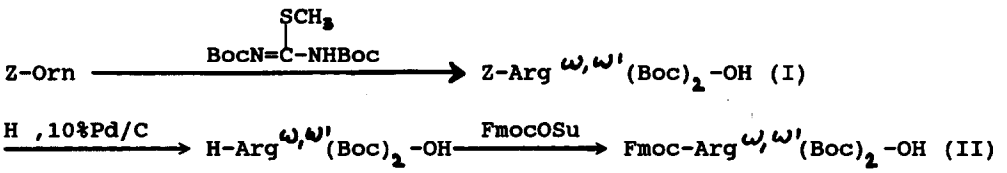
Abstract: The new arginine derivatives Fmoc-Arg$^{\omega,\omega'}$(Boc)$_2$-OH and Z-Arg$^{\omega,\omega'}$(Boc)$_2$-OH have been easily prepared in high yield starting from Z-Orn-OH and N,N'-bis (tert-butoxycarbonyl)-S-methylisothiourea.

Many side-reactions occur in peptide synthesis with the present arginine derivatives (1-4). We report here a remarkably simple preparation of the novel bisurethane derivatives Z-Arg-(Boc)$_2$-OH (I) and Fmoc-Arg$^{\omega,\omega'}$(Boc)$_2$-OH (II), with the Boc groups unambigously placed at the guanidine nitrogens. (5,6)

Scheme 1



Direct acylation of Z-Arg-OH with (Boc)$_2$O produces (I) in about 25% yield mixed with isomer Z-Arg δ,ω(Boc)$_2$-OH (50% yield) and traces of Z-Arg(Boc)-OH. (7) Contrary to N,N'-bisurethane derivatives, (I) and (II) are stable in solution, are not cleaved after deblocking of the α-amino groups and, most important for the Fmoc-t-butyl strategy of SPPS, deblocking of the guanidine side-chain occurs under mild acidolytic conditions (TFA-H_2O 95-5%(v/v), 50 min, r.t.). No racemization occurs upon activation and coupling. Ornithine formation, perhaps the most serious disadvantage of urethane-based derivatives, is completely avoided (5,8). However, δ-lactam formation has been observed during activation both in solution and in solid-phase (5,8). As severe a test for the utility of (II) in SPPS, the complex peptide sequences Ac-SYSMEHFRWGKPV-NH_2 (α-MSH) (III) and SRSASRQTRNQEQSA (IV) have been assembled on polystyrene resins using (II), Fmoc Arg (Pmc)-OH and Fmoc-Arg(Mtr)-OH (5,9,10). With derivative (II), arginine deletion peptides are the only significant impurities found in crude peptides (III) and (IV) by RPHPLC after acidolysis from the solid supports without further manipulations. Their formation, however, can be greatly reduced or even suppressed by repeated couplings.

Preparation of Z-Arg$^{\omega,\omega'}$(Boc)$_2$-OH (I). Bis Boc-S-methylisothiourea (17.76 g; 61.18 mmol) and a 35% methanolic solution of Triton-B (10.77 g; 64.40 mmol) were added under mechanical stirring to a solution of Z-Orn-OH (17.15 g; 64.40 mmol) in DMSO (200 ml) kept at 10°C in an atmosphere of nitrogen. After about 40 hrs, 1.2 l of a 2% $KHSO_4$ solution (4% $KHSO_4$ solution diluted 1:1 with H_2O/NaCl/sat.) was slowly added to obtain a precipitate which was recovered by filtration, redissolved in water and then extracted with ethyl acetate. Washings with H_2O/NaCl sat. were continued until the pH of the organic layer was between 5 and 6 before drying over Na_2SO_4. Evaporation of the solvent gave a glassy material that was kept over KOH under vacuum. 16.69g (32.82 mmol) of the residue were

dissolved in ethyl ether (80 ml) and cyclohexylamine (3.95 ml.; 34.46 mmol) added. White crystals formed on standing for about four days at 4°C. Filtration and washings with ethyl ether gave the desired product (19.02 g; yield = 90.0% overall yield).
m.p. 124-125°C; [α] = +9.34° (c=1, MeOH). El. anal.: Calcd. for $C_{30}H_{49}N_5$: C,59.29%; H,8.13%; N,11.52% Found: C,58.58%; H,8.25%; N,11.13%

Z-Arg$^{\omega,\omega'}$(Boc)$_2$-OH. CHA (33.2 g; 54.63 mmol) was partitioned between ethyl acetate (2l) and 400 ml of water containing 8.16 g of $KHSO_4$.

Washings with H_2O/NaCl sat. (200 ml) were continued until the pH of the organic layer was between 5 and 6 before drying over Na_2SO_4.

Evaporation of the ethyl acetate gave a glassy solid (27.50 g; yield 99.0%) m.p. 102°(dec); [α] +1.11° (c=1, MeOH). El. anal. Calcd. for $C_{34}H_{56}N_4$: C,56.68%; H,7.13%; N,11.02% Found: C,56.37%; H,7.14%; N,10.91%.

Preparation of Fmoc-Arg$^{\omega,\omega'}$(Boc)$_2$-OH (II). (I) (7.63 g; 15 mmol) was dissolved in MeOH (300 ml) and hydrogenated over 10% Pd on carbon in a Parr apparatus (30 psi) for about 40 min. The catalyst was removed by filtration and, following evaporation of the solvent, trituration with ethyl ether gave H-Arg$^{\omega,\omega'}$(Boc)$_2$-OH as a white powder (5.56 g; yield 99%); m.p. 230°C (dec.) [α] = -12.44° (c=1, MeOH).

El. anal.: Calcd. for $C_{16}H_{30}N_4$: C, 51.32%; H,8.08%; N, 14.96% Found: C, 49.02%; H, 8.08%; N, 14.24%.

DIPEA (686 ul; 4.0 mmol) and 9-fluorenylmethyl succinimidyl carbonate (1.31 g; 3.88 mmol) were sequentially added to H-Arg$^{\omega,\omega'}$(Boc)$_2$-OH (1.5g, 4.0 mmol) dissolved in CH_2Cl_2 under stirring. The reaction was left to stir for about 70 min, diluted with ethyl acetate (300 ml), washed repeatedly with 2% $KHSO_4$ and H_2O/Na Cl sat. until the pH reached a value between 5 and 6. After drying over Na_2SO_4 and evaporation of the solvent, the glassy residue was triturated with ethyl ether to obtain the desired product. (2.30 g; 99.6% yield).
m.p. = 115°C (dec.); [α] = + 3.2° (c = 1, MeOH).

El. anal.: Calcd. for $C_{31}H_{40}N_4$: C, 62.40%; H, 6.76%; N,9.39% Found: C, 61.75%; H, 6.76%; N, 9.07%.

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10. Three syntheses were carried out on a polystyrene resin (Rink linker) using Boc, Pmc and Mtr for Arg protection and preformed HOBt esters (DCI, 30 min) of Fmoc aminoacids for couplings. Fmoc-Arg(Pmc)-OH and Fmoc-Arg$^{\omega,\omega'}$(Boc)$_2$-OH both gave crudes containing about 80% of α-MSH, while Fmoc-Arg(Mtr)-OH gave high yields of sulfonated α-MSH derivatives, the target peptide amounting to only 19% of the total.

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