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Synthesis of Protected Derivatives of 3-Pyrrolylalanine

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Abstract: Protected derivatives of 3-pyrrolylalanine (3-PA) have been synthesized starting with *N*-tri(isopropyl)silylpyrrole. Following formylation and Boc protection, treatment with (±)-*N*-(benzyloxycarbonyl)-α-phosphonoglycine trimethyl ester provided a fully protected dehydro-3-pyrrolylalanine. Reduction of the double bond and removal of the methyl ester groups yielded 3-PA protected at the α- and pyrrole nitrogens. 3-PA is only transiently stable following *N*-deprotection.

Genetic engineering combined with *in vivo* bacterial protein synthesis offers a novel approach to the design and preparation of macromolecular materials, in that precise control of chain length, sequence, and stereochemistry can be achieved.[1] Since materials properties are closely tied to polymer microstructure, this synthetic strategy offers unique advantages in the creation of functionally novel biopolymers.[2-4] In addition, protein-based materials can be engineered to combine natural and artificial domains to create polymers with unique structural and biological properties.[5]

The monomers available for *in vivo* protein synthesis include the 20 natural amino acids, as well as some analogues,[6,7] which can be used to introduce novel functional groups into engineered proteins.[8-10] One of our specific goals is the synthesis of proteins containing electroactive residues, which are of interest in the development of materials for the control of cell growth,[11] drug delivery,[12] and biosensors.[13] While no translational studies of pyrrolylalanine have been reported, the ability of 3-thienylalanine[10] to be incorporated into *E. coli* proteins in place of phenylalanine suggests that 3-pyrrolylalanine (3-PA) might be similarly incorporated, and the structural similarity of 3-PA and histidine indicates that 3-PA might also act as a histidine surrogate. Regardless of its translational activity, 3-PA and its derivatives should be useful building blocks for solution- and solid-phase peptide synthesis. After incorporation into target peptide or protein, 3-PA residues should be susceptible to oxidative polymerization through the 2- and 5- positions of the heterocycle to create materials with electrochemical properties analogous to those of the polypyrroles.[11] In addition, artificial heterocyclic amino acids, such as pyrrolylalanine, are useful in the design of potential therapeutic agents.[14]

Efficient polymerization of pyrroles requires use of derivatives free of substitution in the 2- and 5- positions; 3-PA (**1**) must be used rather than the known 2-isomer.[14,15]

The initial reaction in the synthesis of 3-PA derivatives (Scheme) is the introduction of an aldehyde moiety at the 3-position of the pyrrolyl ring. While pyrrole is activated to electrophilic attack in the 2- and 5-positions, substitution can be directed to the 3-position by the introduction of a large protecting group, such as tri(isopropyl)silyl (TIPS), on the pyrrole nitrogen.[16] Reaction of *N*-tri(isopropyl)silylpyrrole with the Vilsmeier-Haack reagent, followed by aqueous workup under alkaline conditions afforded pyrrolyl-3-carboxaldehyde (**2**) in 72% yield.[17] After protection with (*tert*-butyloxycarbonyl) (Boc),[18] **3** was treated with (±)-*N*-

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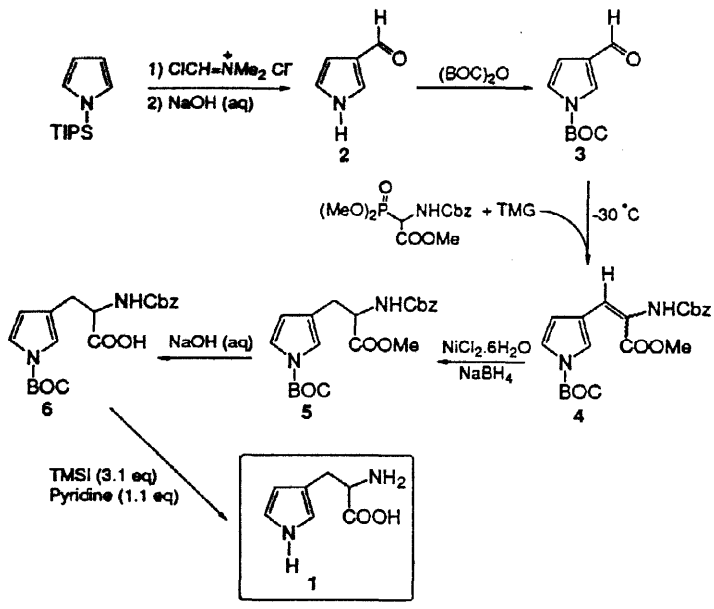
(benzyloxycarbonyl)-α-phosphonoglycine trimethyl ester in a Horner Emmons reaction to provide the dehydro-3-pyrrolylalanine derivative (**4**) in 85% yield.[19] Reduction of the double bond with sodium borohydride in the presence of catalytic nickel(II) chloride hexahydrate produced the fully protected 3-PA (**5**) in 69% yield.[20] The methyl ester protecting group was removed in aqueous sodium hydroxide to give the free carboxylic acid derivative (**6**).[21] Finally, 3-PA (**1**) was liberated from the remaining protecting groups by reaction with trimethylsilyl iodide and pyridine in 77% yield.[22,23] 3-PA decomposes rapidly in aqueous acid, less rapidly in neutral water, and negligibly in aqueous base.

Scheme



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18. A solution of di-*tert*-butyl dicarbonate (3.80 g, 17.4 mmol) in dry CH_2Cl_2 (25 mL) was added dropwise to a solution of 1*H*-pyrrole-3-carboxaldehyde (1.52 g, 16.0 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C. After 15 min, a solution of diisopropylethyl amine (3.0 mL, 17.2 mmol) and catalytic dimethylaminopyridine in dry CH_2Cl_2 (20 mL) was added dropwise over 30 min. The reaction was stirred for 3 h at 0 °C and then stirred for 1 h at room temperture. Aqueous acidic workup and purification by flash chromatography (hexanes/ethyl acetate 9:1 v/v) yielded 2.87 g (93%) of **3** as a colorless solid. ^{1}H NMR ($CDCl_3$): d 1.59 (s, 9H, C(C*H*$_3$)$_3$); 6.64 (dd, J=3.4, 1.6 1H, pyr. H); 7.26 (dd, J=3.4, 2.0, 1H, pyr. H); 7.84 (dd, J=2.0, 1.6, 1H, pyr. H); 9.83 (s, 1H, C*H*O). ^{13}C NMR ($CDCl_3$): d 27.9 (C(*C*H$_3$)$_3$); 85.5 (*C*(CH$_3$)$_3$); 109.3, 122.1, 128.2, 128.8 (4 pyr C); 147.9 (pyr-N*C*OO); 185.6 (*C*HO). mp 35-36 °C. Anal. calcd for $C_{10}H_{13}NO_3$ (195.22): C, 61.53; H, 6.71; N, 7.18; found: C, 61.44; H, 6.66; N, 7.20.

19. Tetramethylguanidine (1.41 mL, 11.2 mmol) was added dropwise to a solution of (±-*N*-(benzyloxycarbonyl)-a-phosphonoglycine trimethyl ester (3.73 g, 11.3 mmol) in dry CH_2Cl_2 (125 mL) and stirred for 20 min at room temperature. The mixture was then cooled to -41 °C (dry ice/CCl_4 bath) and a solution of **3** (2.00 g, 10.2 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise. The reaction was stirred for 1 h at -41 °C, then warmed slowly to room temperature (3 h), stirred for 18 h at room temperature, and finally refluxed for an additional 12 h. Aqueous workup and purification by flash chromatography (hexanes/ethyl acetate 8:2 v/v) yielded 3.48 g (85%) of **4** as a light yellow solid. ^{1}H NMR ($CDCl_3$): d 1.59 (s, 9H, C(C*H*$_3$)$_3$); 3.77 (s, 3H COOC*H*$_3$); 5.16 (s, 2H, C*H*$_2$Ph); 6.15-6.34 (br s, 1H, N*H*); 6.39-7.01 (m, 1H, pyr. H); 7.18 (dd, J=3.2, 2.2, 1H, pyr. H); 7.26-7.41 (m, 6H, 5 arom H, C=C*H*R); 7.45-7.49 (m, 1H, pyr. H). ^{13}C NMR ($CDCl_3$): d 27.9 (C(*C*H$_3$)$_3$); 52.3 (O*C*H$_3$); 67.4 (*C*H$_2$Ph); 84.5 (*C*(CH$_3$)$_3$); 112.0, 121.2, 121.3 (3 pyr C); 121.9 (*C*H=C); 123.4 (pyr C); 127.9, 128.2, 128.5, 136.0 (4 arom. C); 148.0 (*C*O*t*-Bu), 154.2 (*C*OOCH$_2$Ph), 165.7 (*C*OOCH$_3$). mp 86-88 °C. Anal. calcd for $C_{21}H_{24}N_2O_6$ (400.43): C, 62.99; H, 6.04; N, 7.00; found: C, 63.03; H, 6.16; N, 7.08.

20. Sodium borohydride (1.17 g, 30.9 mmol) was added in portions to a solution of **4** (2.50 g, 6.24 mmol) and nickel(II) chloride hexahydrate (0.26 g, 1.1 mmol) in MeOH (150 mL) at 0 °C. The reaction was warmed to room temperature and stirred for 16 h. After removal of the solvent *in vacuo*, the residue was redissolved in CH_2Cl_2, washed with water and dried over $MgSO_4$. Purification by flash chromatography (hexanes/ethyl acetate 7:3 v/v) yielded 1.74 g (69%) of **5** as a colorless wax. ^{1}H NMR ($CDCl_3$): d 1.57 (s, 9H, C(C*H*$_3$)$_3$); 2.96 (d, J=5.0, 2H, C*H*$_2$CH); 3.75 (s, 3H, COOC*H*$_3$); 4.53-4.63 (m, 1H, CH$_2$C*H*), 5.11 (s, 2H, PhC*H*$_2$); 5.28 (d, J=8.1, 1H, N*H*); 5.96 (dd, J=3.1, J=1.7, 1H, pyr. H); 7.00 (dd, J=2.0, 1.7, 1H, pyr. H); 7.14 (dd, J=3.1, J=2.0, 1H, pyr. H); 7.34 (m, 5H, arom H). ^{13}C NMR ($CDCl_3$): d 27.9 (C(*C*H$_3$)$_3$); 29.6 (*C*H$_2$CH); 52.3 (O*C*H$_3$); 54.1 (CH$_2$*C*H); 66.9 (*C*H$_2$Ph); 83.6 (*C*(CH$_3$)$_3$); 112.7, 118.4, 120.5, 120.7 (4 pyr. C); 128.0, 128.1, 128.5, 136.2 (4 arom. C) 148.6 (*C*O*t*-Bu), 155.7 (*C*OOCH$_2$Ph); 172.1 (*C*OOCH$_3$). Anal. calcd for $C_{21}H_{26}N_2O_6$ (402.44): C, 62.67; H, 6.51; N, 6.96; found: C, 62.75; H, 6.45; N, 6.72.

21. Aqueous NaOH (10% w/w, 20 mL) was added dropwise to a solution of **5** (1.27 g, 3.16 mmol) in MeOH (40 mL) at 0 °C. The solution was stirred for 15 min and adjusted to pH 6 with dilute HCl. After removing the solvent *in vacuo*, the residue was redissolved in CH_2Cl_2, washed with water and dilute acid, and dried over $MgSO_4$. Recrystallization from ether/hexanes gave 1.04 g (85%) of **6** as a colorless solid. ^{1}H NMR ($CDCl_3$): d 1.58 (s, 9H, C(C*H*$_3$)$_3$); 2.97-3.02 (m, 2H, C*H*$_2$CH); 4.61 (m, 1H, CH$_2$C*H*); 5.12 (s, 2H, PhC*H*$_2$); 5.23 (br d, J=8.1, 1H, N*H*); 6.03 (dd, J=3.2, J=1.7, 1H, pyr. H); 7.04-7.07 (m, 1H, pyr. H);

7.14 (dd, J=3.2, J=2.0, 1H, pyr. H); 7.33-7.36 (m, 5H, arom. H). ^{13}C NMR ($CDCl_3$): d 27.9 (C(CH_3)$_3$); 29.3 (CH_2CH); 53.8 (CH_2CH); 67.2 (PhCH_2); 83.8 (C(CH_3)$_3$); 112.8, 118.6, 120.5, 120.7 (4 pyr. C); 128.1, 128.3, 128.5, 136.0 (4 arom. C); 148.6 (*C*OO*t*-Bu), 156.0 (*C*OOCH$_2$Ph); 175.2 (*C*OOH). mp 136-137 °C. Anal. calcd for $C_{20}H_{24}N_2O_6$ (388.42): C, 61.85; H, 6.23; N, 7.21; found: C, 61.90; H, 6.25; N, 7.29.

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23. Trimethylsilyl iodide (0.24 mL, 1.7 mmol) was added dropwise to a solution of **6** (0.205 g, 0.53 mmol) and pyridine (0.046 mL, 0.57 mmol) in CH_2Cl_2 (8 mL) at 0 °C. The reaction was warmed to room temperature and stirred for 18 h. The soluble fraction of the reaction mixture was separated from the pyridine salt by-product by filtration and the solvent and other reaction by-products were removed *in vacuo*. Finally, the residue was treated with excess MeOH (10 mL) to yield 63 mg (77%) of **1** as a white precipitate. Purification (>98% as determined by ^{1}H NMR) was achieved by passing a cold solution of **1** in H_2O or D_2O through a plug of decolorizing carbon. ^{1}H NMR (D_2O): d 2.82 (dd, J=15.2, J=7.2, 1H, CH_2CH); 2.85 (dd, J=15.2, J=4.9, 1H, CH_2CH); 3.70 (dd, 1H, J=7.2, J=4.9, CH_2CH); 5.94 (dd, J=3.1, J=1.8, 1H, pyr. H); 6.96 (dd, J=2.0, J=1.8, 1H, pyr. H); 7.02 (dd, J=3.1, J=2.0, 1H, pyr. H).