A Facile Method for the Side-Chain Protection of α-Methyl-β-3,4-dihydroxyphenyl-L-alanine (αMeDopa) for Solid-Phase Peptide Synthesis

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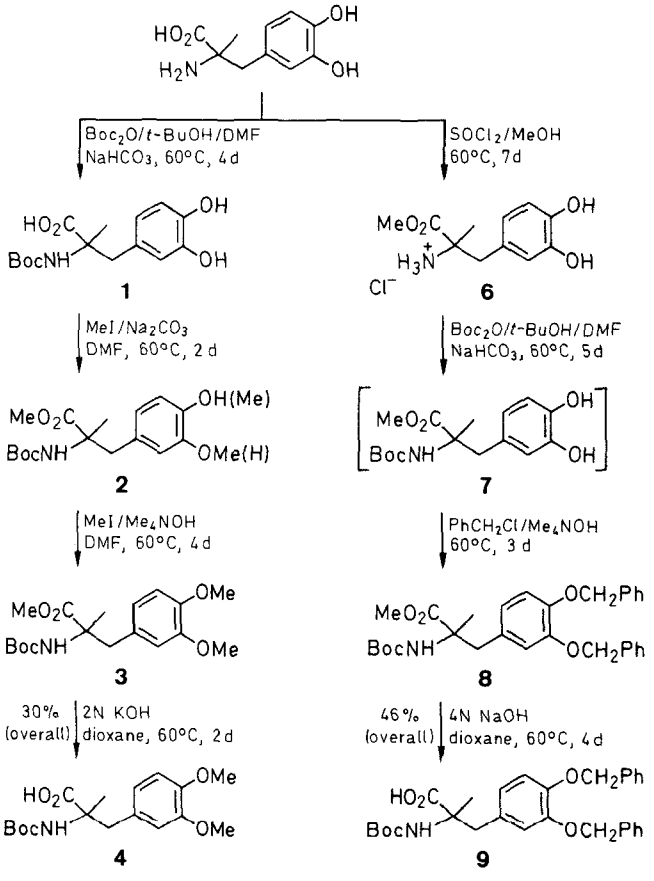
Two approaches have been used to prepare N^{α}-*tert*-butoxycarbonyl-α-methyl-β-3,4-dimethoxy-, or dibenzyloxyphenyl-L-alanine, N^{α}-Boc-α-MeDopa(R)$_2$, where R is the methyl or benzyl ether. In the first approach, α-methyl-β-3,4-dihydroxyphenyl-L-alanine (αMeDopa) is reacted with di-*tert*-butyl dicarbonate (Boc_2O) in the presence of sodium bicarbonate under anhydrous conditions. The resultant *N*-protected derivative (Boc-αMeDopa) is methylated with methyl iodide/sodium carbonate, followed by reaction with methyl iodide/tetramethylammonium hydroxide, and saponification with potassium hydroxide in aqueous dioxane to give N^{α}-*tert*-butoxycarbonyl-α-methyl-β-3,4-dimethoxyphenyl-L-alanine, Boc-αMeDopa(Me)$_2$, in 30% overall yield. In the second approach, methyl ester of α-methyl-β-3,4-dihydroxyphenyl-L-alanine is prepared by the thionyl chloride/methanol procedure, followed by *N*-protection with di-*tert*-butyl dicarbonate/sodium bicarbonate, side-chain protection with benzyl chloride/tetramethylammonium hydroxide, and saponification with sodium hydroxide in aqueous dioxane to give N^{α}-*tert*-butoxycarbonyl-α-methyl-β-3,4-dibenzyloxyphenyl-L-alanine, Boc-αMeDopa(CH_2Ph)$_2$, in 46% overall yield. The latter derivative has been incorporated into angiotensin II under stepwise solid-phase peptide synthesis and solution coupling conditions.

The 3-(3,4-dihydroxyphenyl)-2-methyl-L-alanine (α-methyldopa, aldomet^{R}) is a commercially available amino acid, with established antihypertensive activity.[1] Its catechol ring is more π-electron enriched than the phenol group of tyrosine, and the α-methyl substitution is known to render proteolytic resistance to amide bonds immediately neighboring to the sterically hindered α-position.[2] In spite of these interesting properties, α-methyldopa has rarely been used for structure–activity-relationship studies, possibly due to the rapid autoxidation of the catechol group to eumelanin under aqueous alkaline conditions.[3] To circumvent this oxygen radical-mediated reaction, the OH groups of catechol are often protected under anhydrous and/or acidic conditions. A number of α-methyldopa derivatives have been reported, including the N^{α}-*tert*-butoxycarbonyl-derivatized Boc-αMeDopa,[2] the N^{α}-benzyloxycarbonyl and catechol-protected *O*-diphenylmethylene-*Z*-αMeDopa,[1] and the catechol- and carboxylic-benzylated αMeDopa(CH_2Ph)$_2$-OCH_2Ph.[4] The last compound has been incorporated into position 8 of angiotensin II (Asp-Arg-Val-Tyr-Val/Ile-His-Pro-Phe, AII).[4]

However, because of the stepwise addition of amino acids from the C- to the N-terminus of the solid-phase peptide synthesis (SPPS) strategy,[5] the most useful intermediate for α-methyldopa should possess: 1) an *N*-protection easily removed by acidolyis or piperidine treatment such as the commonly used *tert*-butoxycarbonyl (Boc) or fluorenylmethoxycarbonyl (Fmoc) group; 2) a side-chain protection stable to the above deprotecting reagents, such as the benzyl group which can be removed by catalytic hydrogenation or liquid hydrogen fluoride cleavage; and 3) a free carboxylic moiety. For this reason, neither the above amino-free but *O*-tribenzylated derivative,[4] nor the C-terminal free N^{α}-benzyloxycarbonyl-*O,O*-diphenylmethylene-αMeDopa[1] and N^{α}-Boc-αMeDopa[2] can provide the differential protection of the α-amino and side chain functional groups of α-methyldopa useful for the exhaustive C-activating conditions employed in the stepwise solid-phase peptide synthesis.

To address this need, we explored the possibility of selective protection of the amino (NH_2), carboxylate (CO_2^-) and catecholate groups of α-methyldopa based on their differential nucleophilicity and basicity. For example, in the presence of a mild base (sodium bicarbonate) in its anhydrous solid form, only the highly nucleophilic amino group reacted with di-*tert*-butyl dicarbonate (Boc_2O). However, subsequent reaction of the resultant Boc-αMeDopa with methyl iodide in the presence of sodium carbonate, in analogy to reported conditions,[4] gave more than one product, with the major product displaying an orange color with the Pauly diazonium reagent.[6] Since the Pauly reagent reacts with the highly π-enriched, unprotected catechol of αMeDopa to produce an intense brown color, as compared with a light yellow color for the less electron-enriched tyrosine ring and a negative response with *O*-protected tyrosine, the orange colorimetric response indicates the presence of an



unreacted aromatic hydroxyl group in the methylation reaction product. Consistent with this finding, ^{1}H-NMR study of model experiments of methylation (methyl iodide/sodium carbonate), or benzylation (benzyl chloride/sodium carbonate), of the methyl ester of α-methyldopa indicated the incorporation of only one methyl or benzyl ether function. Further treatment of the above methylation reaction product with methyl iodide in the presence of tetramethylammonium hydroxide, an organic base we found especially effective in promoting S_N2 displacement involving lipophilic or sterically hindered amino acids,[7] produced the fully methylated product negative to the Pauly reagent. However, this reaction condition also promotes esterification of the carboxylic function,[7] and requires subsequent saponification of the product to give *N*$^{\alpha}$-*tert*-butoxycarbonyl-α-methyl-β-dimethoxyphenyl-L-alanine, Boc-αMeDopa(Me)$_2$ **(4)**, which clearly shows the presence of a 6-proton singlet for the two methyl ether groups in NMR analysis.

Because the carboxylate and the catecholate ions do not differ enough in their S_N2 reactivity toward alkyl halide to permit selective catechol protection, the synthesis of *N*$^{\alpha}$-*tert*-butoxycarbonyl-α-methyl-β-dibenzyloxyphenyl-L-alanine, Boc-αMeDopa(CH$_2$Ph)$_2$ was accomplished using a different approach. Thus, thionyl chloride mediated esterification of α-methyldopa[8] was followed by amino protection with di-*tert*-butyl dicarbonate in the presence of anhydrous sodium bicarbonate. Subsequent catechol protection by benzyl chloride in the presence of tetramethylammonium hydroxide, followed by saponification with sodium hydroxide in aqueous dioxane gave Boc-αMeDopa(CH$_2$Ph)$_2$ **9**. Although its NMR spectrum showed a 10-proton double singlet ($\delta = 7.36$) and a 4-proton singlet ($\delta = 5.10$) for the two benzyl ether groups, the fully derivatized catechol ring of this compound, unexpectedly, reacted with the Pauly diazonium reagent to give a yellow color, suggesting a more electron-enriched nature for the dibenzylated than for the dimethylated derivative.

In addition to monitoring the completeness of catechol protection, the Pauly reagent is useful for detecting α-methyldopa-containing peptides, and the characteristic brown to greenish brown color provides a sensitive method for substantiating the presence of the easily oxidizable catechol ring in the peptide structure. This is especially important because α-methyldopa has a very low color yield with ninhydrin in standard amino acid analysis, approximately 1/38 of that of tyrosine. In addition, α-methyldopa decomposes during peptide hydrolysis by 6N hydrochloric acid. Thus, amino acid analysis, traditionally used to verify the polymeric structure of the peptide hydrolysate, is not a sensitive tool for α-methyldopa detection, for which the complementary evidence by Pauly reaction and fast-atom-bombardment mass-spectroscopy is needed to conclusively demonstrate the presence of α-methyldopa in the appropriate peptide sequence.

Boc-αMeDopa(CH$_2$Ph)$_2$ availability permitted assessment of its incorporation into peptides using stepwise solid-phase peptide synthesis. While it has been recognized that sterically hindered dialkylglycines, to which category α-methyldopa also belongs, are difficult to incorporate by normal coupling methods,[9] recent reports indicate that the phosphorous-activated bis(2-oxo-3-oxazolidinyl)phosphinic chloride, BOP-Cl, is particularly useful in coupling the hindered sequence.[10] However, initial condensation of Boc-α-MeDopa(CH$_2$Ph)$_2$ (3 molar excess) to Val-His(CH$_2$Ph)-Pro-2-Ind-resin by BOP-Cl (3 molar excess) in the presence of triethylamine (6 molar excess) was incomplete even after prolonged heating at 60 °C for 3 days, with the unreacted peptide resin producing an intense color reaction in the Kaiser ninhydrin test.[11]

Subsequent study utilized dicyclohexylcarbodiimide (DCC, 3 molar excess) coupling of Boc-α-MeDopa(CH$_2$Ph)$_2$ (3 molar excess) to Val-His(CH$_2$Ph)-Pro-resin at 60 °C for 9 days until the peptide resin was negative to the Kaiser test. Acidolytic (25% trifluoroacetic acid in methylene chloride) removal of the Boc-group gave αMeDopa(CH$_2$Ph)$_2$-Val-His(CH$_2$Ph)-Pro-resin, which displayed an intense blue color under the anhydrous conditions of the Kaiser test (in contrast to the very low color yield of α-methyldopa with aqueous ninhydrin in standard amino acid analysis), and coupled sequentially with Boc-Val, Boc-Arg(NO$_2$), Boc-Sar by DCC in the normal fashion. Hydrogenation[12] of the resultant Boc-Sar-Arg(NO$_2$)-Val-αMeDopa(CH$_2$Ph)$_2$-Val-His(CH$_2$Ph)-Pro-resin cleaved the benzyl ester linkage of peptide-resin and gave the Boc-Sar-Arg-Val-αMeDopa-Val-His-Pro heptapeptide, [Boc-Sar^1, αMeDopa^4, des-Phe^8]AII, with unprotected side chains.

It is worth noting that the absence of the α-proton in α-methyldopa eliminates the risk of racemization (via proton abstraction or oxazalone formation) during peptide bond formation under the vigorous solid-phase peptide coupling conditions necessitated by the sterically hindered α-methyl amio acid. On the other hand, the prolonged C-activation needed for α-methyldopa incorporation into peptides requires a fully protected Boc-αMeDopa(CH$_2$Ph)$_2$ in order to avoid side-chain branching. However, solution coupling of α-methyldopa-containing peptides can be accomplished with the unprotected catechol side chain. For example, condensation of the above heptapeptide [Boc-Sar^1, αMeDopa^4, des-Phe^8]AII with the benzyl ester of 2-indan amino acid (2-Ind-OCH$_2$Ph) by dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole (DCC/HOBt) proceeded smoothly to the octapeptide product. Further *N*- and *C*-deprotection by acidolysis and hydrogenation gave Sar-Arg-Val-αMeDopa-Val-His-Pro-2-Ind, which retained its catechol moiety (as demonstrated by the Pauly reaction) through ion-exchange chromatography eluted by ammonium acetate solution, followed by countercurrent–distribution separation with the acidic mixture of butanol/acetic acid/water.

Compared to its Tyr-containing counterpart Sar-Arg-Val-Tyr-Val-His-Pro-2-Ind, which displayed potent inhibition of angiotensin II actions in both peripheral and central nervous systems, the αMeDopa-containing analogue was selectively active in the brain.[13] Since the latter

analogue had high affinity for both central and peripheral receptors in binding studies, α-methyldopa substitution for tyrosine in angiotensin appears to affect receptor activation, possibly through aromatic–aromatic interaction within the ligand or via such interaction between the ligand and an aromatic/lipophilic region of the receptor. Thus, α-methyldopa substitution for tyrosine or phenylalanine may offer an interesting opportunity to examine such interaction, which is believed to occur widely in proteins, contributing to the stability of protein structures.[14] In addition, the approach taken in this study should be generally applicable to the protection of other phenolic side chains, superior to the laborious cupric complex procedure usually used for tyrosine and α-methyltyrosine.[15]

All chemicals were of reagent grade. NH_4OAc, $PhCH_2Cl$, bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl), di-*tert*-butyl dicarbonate (Boc_2O), 1,3-dicyclohexylcarbodiimide (DCC), 3-(3,4-dihydroxyphenyl)-2-methyl-L-alanine sesquihydrate (αMeDopa), 1-hydroxybenzotriazole hydrate (HOBt), MeI, $Pd(OAc)_2$, 10% palladium on activated carbon (Pd-C), Et_3N, and CF_3CO_2H were purchased from Aldrich Chemical Co. N^{α}-*tert*-Butyloxycarbonyl-L-amino acids were supplied by Bachem, Inc. SP-Sephadex C-25 and Sephadex G-25 were obtained from Pharmacia. Melting points (Thomas-Hoover Uni-melt) are uncorrected. ^{1}H-NMR in $CDCl_3$ containing TMS were obtained on a Variant-60 (60 MHz) or a Jeol FX90-Q (90 MHz). Elemental analyses were performed by Desert Analytics. Mass spectra were obtained using a VG model 7070E/HF spectrometer with FAB ionization.

Homogeneity of amino acid derivatives and of the synthetic peptides was assessed by TLC on Merck precoated silica gel glass plates (type G60-F254) in the solvent systems of (I) 8:1:2:9 of BuOH/pyridine/AcOH/H_2O (upper phase), (II) 100:44 of 2-butanol/3% NH_4OH. The products were identified by a combination of UV, ninhydrin, chlorox-KI, and Pauly sprays. Peptide samples were hydrolyzed for 48 h at 110°C in 6N HCl + 0.2% phenol containing D-alanine as an internal standard. Amino acid analyses were performed on a Beckman Model 120 analyzer equipped with an integrator.

N^{α}-*tert*-Butoxycarbonyl-α-methyl-β-3,4-dimethoxyphenyl-L-alanine, Boc-αMeDopa(Me)$_2$ (4):

The 3-(3,4-dihydroxyphenyl)-2-methyl-L-alanine sesquihydrate (αMeDopa; 2.98 g, 12.5 mmol) and $NaHCO_3$ (3.68 g, 43.75 mmol) are suspended in DMF/*t*-BuOH (1:1, 20 mL), to which di-*tert*-butyl dicarbonate (Boc_2O; 6.83 g, 31.3 mmol) in DMF/*t*-BuOH (1:1, 10 mL) is added. The mixture is stirred at 60°C for 4 d, cooled to r.t. and diluted with Et_2O/EtOAc (1:4, 75 mL). The organic solution is washed with 2N HCL (3 × 25 mL), dried (Na_2SO_4), and the solvent is evaporated under reduced pressure to give the white solid of Boc-αMeDopa **1**, which darkens to a purple syrup upon standing.

The crude product **1** is dissolved in DMF (12 mL). To this solution, MeI (4.67 mL, 75 mmol) and Na_2CO_3 (7.95 g, 75 mmol) are added, and the suspension is stirred at 60°C for 2 d. The mixture is cooled to r.t., diluted with Et_2O/EtOAc (1:4, 75 mL) and washed with 2N HCl (4 × 25 mL). The organic phase is dried (Na_2SO_4), and the solvent is evaporated under reduced pressure to give a brown oil **2**, which displays an orange color to Pauly spray.

^{1}H-NMR ($CDCl_3$) studies show that model experiments with the methyl ester of αMeDopa under similar conditions for Boc-addition and methyl or benzyl protection, followed by saponification of the ester (δ = 3.70, 3 H-singlet), result in products containing only one methyl (δ = 3.77, 3 H-singlet) or benzyl ether (δ = 7.27, 5 H-singlet) relative to the Dopa signal (δ = 6.63, 3 H), indicating incomplete catechol reaction using the inorganic base of Na_2CO_3.

Further reaction of **2** in DMF (12 mL) with MeI (3.2 mL, 50 mmol) in the presence of the organic base of Me_4NOH (20% w/v in MeOH; 22.8 mL, 50 mmol) at 60°C for 4 d results in a Pauly-negative product. The mixture is cooled to r.t., diluted with Et_2O/EtOAc (1:4, 50 mL), and washed with 3N HCl (3 × 40 mL), followed by 2N NaOH (3 × 40 mL). The organic solvent is evaporated under reduced pressure, and the resultant oil **3** is saponified with 2N KOH (50 mL, 100 mmol) in dioxane (20 mL) at 60°C for 2 d. The mixture is evaporated under reduced pressure to remove dioxane, and the remaining solution (about 30 mL) is washed with petroleum ether (bp 35–60°C, 2 × 20 mL), acidified to pH 1 with 4N HCl, and extracted into Et_2O/EtOAc (1:4, 4 × 40 mL). The organic layer is dried (Na_2SO_4) and concentrated at reduced pressure. The resulting oil is chromatographed on a dry-packed column of silica gel (2 × 40 cm), eluted with $CHCl_3$ (100 mL), followed by $CHCl_3$/MeOH (49:1, 250 mL, 24:1, 250 mL). The appropriate fractions are combined, and the solvent is evaporated under reduced pressure to give a pale yellow, hygroscopic solid **4**; yield: 1.29 g (30% overall); mp 63–65°C; R_f values: (I) 0.66, (II) 0.44; positive to UV, ninhydrin, chlorox-KI, but negative to Pauly detection.

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.47 (s, 12 H, *t*-C_4H_9, αCH$_3$), 3.20 (s, 2 H, βCH$_2$), 3.80 (s, 6 H, 2OCH$_3$), 6.63 (s, 3 H, C_6H_3), 10.73 (s, 1 H, CO_2H).

A sample of **4** (170 mg, 0.5 mmol) is dissolved in Et_2O (5 mL) and neutralized with dicyclohexylamine (DCHA) in petroleum ether (1:9). Upon standing, colorless needles of Boc-αMeDopa(Me)$_2$ · DCHA **5** appear; yield: 228 mg (88% recovery); mp 201.5–202.5°C.

$C_{17}H_{25}NO_6 \cdot C_{12}H_{23}N$	calc.	C 66.89	H 9.29	N 5.38
(339.4 + 181.3)	found	66.94	9.47	5.29

N^{α}-*tert*-Butoxycarbonyl-α-methyl-β-3,4-dibenzyloxyphenyl-L-alanine, Boc-αMeDopa(CH$_2$Ph)$_2$ (9):

To the suspension of αMeDopa (10.3 g, 43 mmol) in MeOH (49 mL, 1.2 mol), $SOCl_2$ (36.4 mL, 498 mmol) is added dropwise. The resulting clear, yellow solution is stirred at 60°C for 7 d. The solvent is evaporated under reduced pressure, and excess $SOCl_2$ is decomposed with H_2O and evaporated to dryness repeatedly from acetone (3 ×). The residue is dried (P_4O_{10}) under vacuum for 30 min to give the white solid of αMeDopa-OMe · HCl **6**. The methyl ester **6** is dissolved in DMF/*t*-BuOH (1:1, 30 mL), to which Boc_2O (18.8 g, 86 mmol) in DMF/*t*-BuOH (1:1, 10 mL) and $NaHCO_3$ (12.6 g, 150.5 mmol) are added. The mixture is stirred at 60°C for 5 d, and used directly for the next step without separating the Boc-αMeDopa-OMe **7** product. To the above mixture is added $PhCH_2Cl$ (24.8 mL, 215 mmol) and Me_4NOH (20% in MeOH; 98 mL, 215 mmol). Stirring of the mixture at 60°C for 3 d results in the disappearance of the Pauly-positive (brown color) **7**. The mixture is evaporated under reduced pressure to a small volume, diluted with Et_2O/EtOAc (1:1, 150 mL), washed with 4N HCl (4 × 50 mL), and 4N NaOH (3 × 50 mL). The organic solvent is evaporated to dryness, and the resulting Boc-αMeDopa(CH$_2$Ph)$_2$-OMe **8** is dissolved in dioxane (50 mL), and saponified with 4N NaOH (161 mL, 645 mmol) at 60°C for 4 d. The mixture is acidified to pH 1 with 4N HCl, and extracted with Et_2O/EtOAc (1:1, 4 × 50 mL). The organic phase is washed with 3N HCl (3 × 50 mL), and evaporated under reduced pressure. The resulting Boc-αMeDopa(CH$_2$Ph)$_2$ **9** is chromatographed on a dry-packed column of silica gel (2 × 30 cm), eluted with $CHCl_3$/MeOH (49:1, 250 mL, 9:1, 300 mL). The appropriate fractions are combined, and the solvent is evaporated under reduced pressure. The residue is dissolved in Et_2O (50 mL), and neutralized with dicyclohexylamine/petroleum ether (20%). The Boc-αMeDopa(CH$_2$Ph)$_2$ · DCHA precipitate **10** is collected and recrystallized from MeOH/Et_2O/petroleum ether (50:100:150 mL) to give 7.77 g of colorless needles. A second crop of 5.54 g is obtained from $CHCl_3$/MeOH/Et_2O/petroleum ether (40:20:20:140 mL); total yield: 13.31 g (46% overall); mp 170–171°C.

$C_{28}H_{33}NO_6 \cdot C_{12}H_{23}N$	calc.	C 72.69	H 8.54	N 4.24
(479.6 + 181.3)	found	72.98	8.45	4.03

Suspension of **10** in Et_2O/EtOAc (1:1), followed by washing with

2N HCl (3×) and drying (Na_2SO_4), regenerates the white hygroscopic solid of Boc-αMeDopa(CH_2Ph)$_2$ **9**; mp 58–60°C; R_f values: (I) 0.73, (II) 0.48, positive to UV, ninhydrin, chlorox-KI, and Pauly (yellow color) detection.

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.44 (s, 12H, t-C_4H_9, αCH_3), 3.16 (s, 2H, βCH_2), 5.10 (s, 4H, 2 benzylic CH_2), 6.62–6.88 (m, 3H, C_6H_3), 7.24–7.47 (2 m, 10H, 2 benzylic C_6H_5), 9.20 (s, 1H, CO_2H).

Alternative preparation of Boc-αMeDopa(CH_2Ph)$_2$ through: a) $SOCl_2$-mediated esterification of αMeDopa; b) Boc_2O reaction in the presence of $NaHCO_3$ for *N*-protection; c) partial catechol protection by $PhCH_2Cl$ addition in the presence of Na_2CO_3, followed by $PhCH_2Cl/Me_4NOH$ addition to effect full protection of the 2 catechol OH groups; d) saponification of the methyl ester by KOH in aqueous dioxane; and e) silica gel chromatography purification of the product, gives a product identical to **9** in 36% overall yield. Work up of the products from steps b)–e) consists of diluting the mixture with Et_2O/EtOAc (1:4), followed by acid washing (2N HCl).

[Boc-Sar^1, αMeDopa^4, des-Phe^8]angiotensin II (12):
Boc-Sar-Arg-Val-αMeDopa-Val-His-Pro **12** is prepared by stepwise solid-phase synthesis,[5,7] starting from Boc-Pro-resin (2.5 mmol) by attaching Boc-Pro to Merrifield's chloromethylated resin. Sequential addition of 3-molar excess (7.5 mmol) of Boc-His(CH_2Ph) in the presence of 1-hydroxybenzotriazole (HOBt, to suppress racemization), Boc-Val, Boc-αMeDopa(CH_2Ph)$_2$ **9**, Boc-Val, Boc-Arg(NO_2), Boc-Sar gives the fully protected Boc-Sar-Arg(NO_2)-Val-αMeDopa(CH_2Ph)$_2$-Val-His(CH_2Ph)-Pro-resin **11**. Except for the dicyclohexylcarbodiimide (DCC) coupling of the α-methyl amino acid **9**, which requires prolonged heating at 60°C for 9 d, all other DCC-mediated coupling reactions are completed overnight at r.t., as determined by the Kaiser test.[11] After the coupling step, 1-acetylimidazole is introduced to terminate any truncated sequence. Hydrogenation of a portion of the peptide-resin **11** (1.5 mmol) with $Pd(OAc)_2$ (0.68 g, 3 mmol) in DMF (25 mL) under 50 psi of H_2 at 50°C for 2 d, followed by ion-change chromatography of the product on SP-Sephadex (2×45 cm) eluted with a gradient of 0.1 N AcOH adjusted to pH 4.5 and 4.8 with NH_4OH. The appropriate fractions are combined to give **12**; yield: 280 mg (19.6%); R_f values: (I) 0.28, (II) 0.14, positive to UV, ninhydrin, chlorox-KI, and Pauly (a brown color) detection.

Amino acid analysis of a peptide hydrolysate gives the ratios of: Sar 0.85, Arg 1.00, Val 1.00, αMeDopa 0.54, Val 1.00, His 1.06, and Pro 0.95. Sar emerges at 23.1 min in comparison to 26.1 min for Pro, and has 1/5.7 of the color yield of the latter. αMeDopa emerges at 52.0 min in comparison to 55.8 min for Tyr, and has 1/38.3 of the color yield of the latter. Its decomposition during peptide hydrolysis is accompanied by the partial disappearance of the 52.0 min band and the appearance of a new band at 74.0 min.

$C_{45}H_{70}N_{12}O_{12}$ (970.5)
MS (FAB): m/z = 972 ($M^+ + 1$).

[Sar^1,αMeDopa^4,2-Ind^8]angiotensin II (14):
Sar-Arg-Val-αMeDopa-Val-His-Pro-2-Ind **14** is synthesized via solution coupling of **12** (96 mg, 0.1 mmol) with 2-Ind-$OCH_2Ph \cdot HCl$[7] (the HCl salt of 2-indan amino acid benzyl ester; 66 mg, 0.2 mmol) in the presence of HOBt (31 mg, 0.2 mmol) by DCC (52 mg, 0.25 mmol) in DMF (5 mL) at 60°C for 5 d. The mixture is evaporated to dryness under reduced pressure.

The residue is washed with EtOAc (10 mL) and extracted with 30% AcOH (4×5 mL). The AcOH solution is evaporated to dryness, and the residue is gel-filtered on Sephadex G-25 (2×98 cm) eluted with 0.1 N AcOH. The appropriate fractions are combined to give Boc-Sar-Arg-Val-αMeDopa-Val-His-Pro-2-Ind-OCH_2Ph **13**. This is dissolved in DMF (6 mL) and hydrogenated with Pd-C (60 mg) under 40 psi of H_2 at r.t. for 4 d. The catalyst is removed by filtration. The filtrate is evaporated to dryness under reduced pressure, and the residue is lyophilized, followed by acidolysis with 25% CF_3CO_2H/CH_2Cl_2 for 45 min and evaporation to dryness. Ion-exchange chromatography of the product on SP-Sephadex (2×45 cm) eluted with 0.4 N NH_4OAc, followed by lyophilization of the appropriate fractions, gives the pale purple powder of **14**. Subsequent purification of the product by countercurrent-distribution between the mixture of BuOH/AcOH/H_2O (4:1:5) for 100 transfers does not remove the color. Since the colored peptide turns colorless in acidic solution, the αMeDopa chromophore may be inherently colored under non-acidic conditions; yield: 20 mg (19.4%); R_f values: (I) 0.20, (II) 0.13, positive to UV, ninhydrin, chlorox-KI, and Pauly (a brown color in I, and a green-brown color in II) detection.

Amino acid analysis of a hydrolysate gives the ratios of: Sar 1.02; Arg 1.01; Val 1.03; αMeDopa (some αMeDopa is present, with the majority decomposed to the 74.0 min band); Val 1.03; His 0.93; Pro 0.99; 2-Ind 0.90.

$C_{50}H_{71}N_{13}O_{11}$ (1029.5)
MS (FAB): m/z = 1031 ($M^+ + 1$).

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