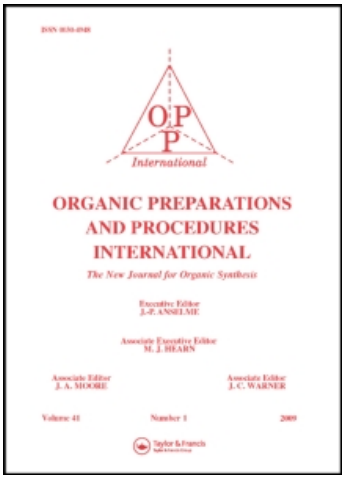

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SYNTHESIS OF A *S*-4-CARBOXYPHENYLALANINE DERIVATIVE FOR USE IN PEPTIDE SYNTHESIS

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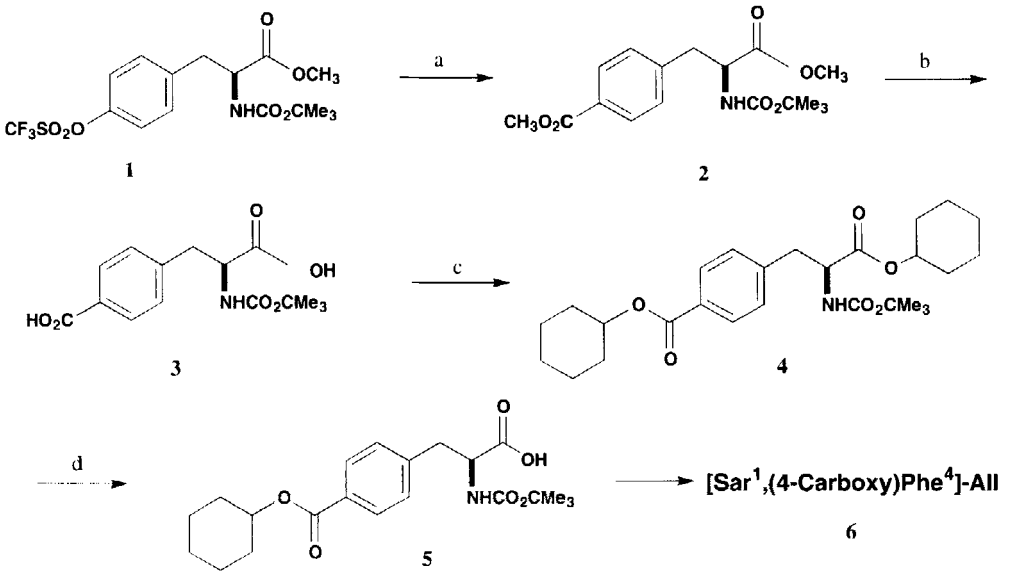
Peptide receptor agonists and antagonists often contain a tyrosine residue which is important to binding. Recently we developed a series of nonpeptide angiotensin II antagonists based on an overlay hypothesis in which the tyrosine 4-hydroxybenzyl side-chain of angiotensin II was mimicked by a 4-carboxybenzyl in the non-peptide antagonist. Therefore, we thought it would be interesting to determine if the 4-carboxyphenylalanine analog of angiotensin II retained high angiotensin II receptor affinity. The synthesis of this peptide required the preparation of an appropriately protected *S*-4-carboxyphenylalanine.

Suitable methodology involves protecting the amine and carboxyl groups in a manner amenable to the usual procedures used in standard solid-phase peptide synthesis. The *t*-butoxycarbonyl group is satisfactory for the amine since it can be removed by mild acid treatment. The aminoacid carboxyl must exist as the free acid while the aryl carboxyl must be protected by a group stable to the acidic and basic reagents used in solid-phase peptide syntheses, but removable by the standard deprotection reagent, hydrogen fluoride. The cyclohexyl ester[1] appeared to be suitable, thus suggesting compound **5** as a desirable target.

Several syntheses of racemic 4-carboxyphenylalanine have been reported,[2,3] and a small quantity of the L-enantiomer was obtained as a by-product of another synthesis.[2b] However, we wished to utilize an asymmetric synthesis to obtain the L-enantiomer directly. *N*-t-Boc-*O*-trifluoromethyl-sulfonyltyrosine methyl ester was prepared by a literature procedure.[2a] Palladium(0) catalyzed carbonylation[2,4,5] using palladium acetate as the source of catalyst gave the *bis*-methyl ester **2** which was hydrolyzed with base to the dicarboxylic acid **3**. Conversion of **3** to the *bis*-cyclohexylester **4** was achieved *via* the 2,4,6-trichlorobenzoyl anhydride as the active intermediate.[6] This somewhat circuitous route was necessary because cyclohexanol did not function as a partner when the carbonylation was attempted on the benzyl ester analog of **1**. The 2,4,6-trichlorobenzoyl anhydride esterification procedure was used when a number of other standard ester syntheses failed to effect the esterification of **3**, presumably due to the low reactivity of cyclohexanol. Finally, careful hydrolysis of

4 with potassium carbonate in methanol-water gave the desired **5** in 93% yield.



1 2 3 4 5 6

a) $Pd(OAc)_2$, Et_3N, dppp, DMSO, MeOH, CO; b) NaOH, EtOH;
c) 2,4,6-trichlorobenzoyl chloride, triethylamine, DMF, DMAP, cyclohexanol; d) K_2CO_3, MeOH, H_2O.

Compound **5** was successfully used to prepare the *L*-4-carboxyphenylalanine analog of (Sar[1])AII (**6**) by standard solid-phase peptide synthesis methodology as described in the Experimental Section. Binding assays were performed using human AT-1 receptors expressed in the stable mouse L cell line.[7] While (Sar[1])AII had an IC_{50} of 0.8 ± 0.2 nM, **6** had an IC_{50} of 2000 ± 400 nM. This result may be rationalized by considering that the strongly acidic carboxyl group in **6** may induce different low-energy conformations than those of (Sar[1])AII in which the corresponding group is a less acidic phenol.

EXPERIMENTAL SECTION

^1H NMR spectra were obtained on a Bruker AM250 MHz or AC400 MHz spectrometer. Chemical shifts are expressed in ppm downfield from internal TMS. ^{13}C GASPE NMR were obtained at 67.9 MHz on a JOEL GX 270 spectrometer. High resolution accurate mass measurements were obtained by direct chemical ionization on a VG 70 VSE at m/Δm>5000 vs PFK reference. IR spectra were obtained on a Perkin Elmer 683 spectrometer. Melting points were obtained on a Thomas Hoover melting point apparatus for temperatures below 200° and on a Mel-Temp for temperatures above 200° and are uncorrected. Chromatography was performed on Silica Gel 60, E. Merck, 230-400 mesh. Gas chromatography was performed on a Carlo Erba 4160 capillary GC; J&W DB-5, 15M, 0.32mm I.D., 0.25 μ film, He flow, FID detector. Rotations were measured on a Perkin Elmer Polarimeter Model 241 Polarimeter. ^{13}C NMR and microanalyses were performed by the SmithKline Beecham Physical and Analytical Chemistry Department.

4-(Carboxy)-*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanine (3).- A mixture of 76.49g, (156 mmol) of *N*-t-Boc-*O*-trifluoromethylsulfonyltyrosine methyl ester, palladium(II) acetate, (1.01 g, 4.50 mmol), 1,3-*bis*(diphenylphosphino)-propane, (1.86 g, 0.312 mmol), triethylamine, (31.51g, 312 mmol), 225 mL of DMSO and 175 mL of methanol was stirred while carbon monoxide gas was bubbled through. After 45 min., the mixture became homogeneous and the reaction flask was capped with two carbon monoxide filled balloons and stirred at 70°. After 25 hrs, analysis by GC of an aliquot showed the absence of starting material. The reaction mixture was poured into an ice-water mixture and extracted with ethyl acetate. The ethyl acetate solution was washed with brine, dried ($MgSO_4$), filtered, and concentrated to give 59.11 g of 4-(carbomethoxy)-*N*-[(1,1-dimethylethoxy)-carbonyl]-L-phenylalanine methyl ester **2** as an oil containing some triethylamine.

^{1}H NMR (250 MHz, $CDCl_3$): δ 7.97 (d, J = 12 Hz, 2H), 7.21 (d, J = 12 Hz, 2H), 5.10-5.00 (m, 1/2 H), 4.60-4.50 (m, 1/2H), 3.90 (s, 3H), 3.71 (s, 3H), 3.35-2.80 (m, 2H), 1.41 (s, 9H); VPC, purity 85.6%.

To this oil dissolved in 450 mL of ethanol, was added a solution of 225 mL water containing 18.72 g (468 mmol) of sodium hydroxide. The reaction mixture was stirred under an argon atmosphere at ambient temperature overnight. The major portion of the ethanol was removed under vacuum and the residue extracted with ethyl ether. The basic aqueous solution was filtered and made acidic by the addition of 3N hydrochloric acid. The initial gum (48.84 g) after two recrystallizations from 50% aqueous ethanol, gave 34.20 g (71%) of colorless crystals of **3,** mp. 301-304°, lit.[8] mp. 290-294° as hemi-hydrate.

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.28 (br s, 1H), 7.85 (d, J = 4Hz, 2H), 7.36 (d, J = 4Hz, 2H), 7.14 (d, J = 8Hz, 1H), 4.20-4.00 (m, 1H), 3.15-3.00 (m, 1H), 2.92-2.82 (m, 1H), 1.31 (s, 7.2H), 1.25 (s, 1.8H).

4-[(Cyclohexyloxy)carbonyl]-*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanine, Cyclohexyl Ester (4).- A solution of 4-(carboxyl)-*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanine (**3**), 15.0 g, (48.5 mmol) in 150 mL tetrahydrofuran to which was added triethylamine (9.79 g, 96.9 mmol) and 2,4,6-trichlorobenzoyl chloride (23.6 g, 96.9 mmol) was stirred at ambient temperature under an argon atmosphere. There was an immediate formation of a solid. The mixture was stirred for 1.75 hr, filtered and the filtrate was concentrated under vacuum to a small volume. Toluene was added and removed under vacuum; this process was repeated several times. To the residual oil diluted with 1 L of toluene, was added 23.69 g (193 mmol) of 4-dimethylaminopyridine, followed by 19.6 g (193 mmol) of cyclo-hexanol. The reaction mixture initially formed a gum, but a finely divided solid soon formed. The mixture was stirred at ambient temperature for 16 hrs, filtered, and the major portion of the toluene evaporated under vacuum from the filtrate. Ethyl ether was then added followed by ethereal HCl to precipitate dimethylaminopyridine hydrochloride which was removed by filtration. The filtrate was concentrated under vacuum (oil pump) to remove as much cyclohexanol as possible to give 20.12 g of a viscous oil. Two successive chromatographies on SiO_2 with chloroform elution gave 5.43 g (24%) of a pure fraction as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J = 8 HZ, 2H), 7.21 (d, J = 8Hz, 2H), 5.05-4.90 (m, 1H),

4.82-4.70 (m, 1/2H), 4.61-4.48 (m, 1/2H), 3.20-2.95 (m, 2H), 2.00-1.10 (m, 20H), 1.41 (s, 9H); HRMS (DCI/NH_3) Calcd *m/e* 474.2858. Found *m/e* 474.2856.

4-[(Cyclohexyloxy)carbonyl]-*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanine (5).- To a solution of 4-[(cyclohexyloxy)carbonyl]-*N*-[(1,1-dimethylethoxy)carbonyl]-L-phenylalanine cyclohexyl ester (**4**) (5.39 g, 11.34 mmol) in 100 mL methanol to which was added a solution of K_2CO_3 (1.72g, 12.45 mmol) in 50 mL water. The reaction mixture was stirred under an argon atmosphere for 16 hrs at ambient temperature. The major portion of the methanol was removed under vacuum and the solution was extracted with ethyl ether, filtered, and the pH of the filtrate was adjusted to pH 3.29 with 3N HCl. This solution was extracted with ethyl acetate and the extract was dried ($MgSO_4$), filtered, and concentrated to give 4.12g (93%) of a colorless friable glass, mp 62-62.5°. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (d, J = 8.2Hz, 2H), 7.25 (d, J = 5.1Hz, 2H), 5.05-4.92 (m, 1H), 4.95-4.90 (m, 1H), 3.32-3.10 (m, 2H), 1.95-1.35 (m, 10H), 1.42 (s, 9H); ^{1}H NMR (400MHz, $CDCl_3$ + D_2O): δ 5.05-4.95 (m, 1H), 4.74 (br s, 1H), 4.65-4.55 (m, 1H); ^{13}C NMR (67.9 MHz, $CDCl_3$) 175.5, 165.9, 155.3, 141.1, 129.8, 129.4, 80.4, 77.5, 77.0, 76.5, 73.1, 54.0, 37.8, 31.6, 28.2, 25.4, 23.6; IR (KBr) 2960, 1715, 1290, 1155 cm^{-1}. $[\alpha]^{20}D$= +9.38, c = 1, C_2H_5OH.

Anal. Calcd. for $C_{21}H_{29}NO_6$•1/4 H_2O: C, 63.76; H, 7.51; N, 3.54. Found: C, 63.80; H, 7.47; N, 3.56

H-Sar-Arg-Val-[(p-CO_2H)Phe]-Ile-His-Pro-Phe-OH (6).- The peptide was prepared by the solid-phase method on a Beckman 990-B Peptide synthesizer.[9,10] The C-terminal residue (531 mg, 2 mmol) was esterified to 1.0 g, 1 meq) of a chloromethylated copolymer of polystyrene and 2% divinylbenzene (BioRad) *via* a cesium salt procedure.[11] The degree of substitution was determined by amino acid analysis of a hydrolysate obtained by treatment of the amino acid resin with HCl-PrOH (1:1) at 120° for 3hrs.[12] Routine deprotection of Boc-amino protecting groups was accomplished with 50% TFA in CH_2Cl_2 and neutralization with 10% diisopropylethylamine in CH_2Cl_2. Coupling of each amino acid was performed with a 2.5 M excess of both *tert*-butyloxycarbonyl amino acid and DCC in CH_2Cl_2; the completeness of reaction was monitored by the ninhydrin test.[13] Side-chain protecting groups were as follows: Arg, tosyl; (*p*-CO_2R)Phe, R = cyclohexyl; His, tosyl. In most cases, coupling was complete after 2 hrs. After the last coupling and deprotection, the peptide was cleaved from the resin by treatment with HF containing 10% (v/v) anisole at 0° for 60 min. After evaporation of HF *in vacuo,* the resin was rinsed with Et_2O to remove anisole and then rinsed with glacial HOAc and filtered. The filtrate was diluted with water and lyophilized to a powder of crude peptide material (0.77 g). The crude peptide was purified to homogeneity by reverse-phase flash chromatography on C-18 YMC-Gel (Yamamura Chemical Laboratories Co., LTD, 2.5 cm X 25 cm column) using 20% CH_3CN/ 0.1% TFA/H_2O. After the first 300 ml void volume, the peptide came out in the next 500 ml of eluted material. The chromatographic fractions containing pure peptide by TLC were concentrated by rotary evaporation and then dried to a powder by lyophilization to a constant weight (0.250 g., 25% yield). Homogeneity of the peptide was determined by the following methods: (a) Amino acid analysis following 72 hrs of acid hydrolysis (6 N HCl, 110°) performed on a Beckman Model 120C analyzer. Arg 1.01; Val 1.02; Ile 1.00; His 1.03; Pro 1.05; Phe 1.02. (b) Analytical TLC on silica gel

with solvent systems A = *n*-BuOH-AcOH-H_2O-EtOAc (1:1:1:1) R_f 0.85 and B = *n*-BuOH-AcOH-H_2O-pyridine (15:5:10:10) R_f 0.90 visualizing spots with Pauly reagent.[13] (c) Analytical reversed-phase HPLC on a C_{18} silica gel column using 19% CH_3CN-0.1%TFA-H_2O mixture (K' 9.8) and 5-60% CH_3CN over 20 min., (K' 7.3) following elution by UV (220 nm detection). (d) FAB mass spectrometry performed on a VG ZAB-1F-HF mass spectrometer with a standard FAB source. $(M + H)^+$: theory 1030; found 1030; $(M - H)^-$: theory 1028; found 1028.

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