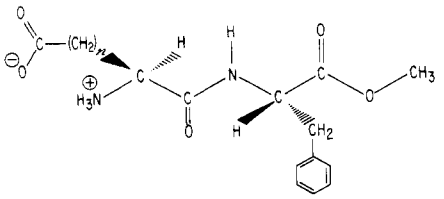
Peptide Sweeteners. 3. Effect of Modifying the Peptide Bond on the Sweet Taste of L-Aspartyl-L-phenylalanine Methyl Ester and Its Analogues

Scott A. MacDonald, C. Grant Willson,[1a] Michael Chorev,[1b] Fred S. Vernacchia,[1c] and Murray Goodman*

Department of Chemistry, University of California, San Diego, La Jolla, California 92093. *Received September 21, 1979*

A series of analogues designed to assess the importance of the amide bond in the dipeptide sweetener L-aspartyl-L-phenylalanine methyl ester has been synthesized and tested. The peptide bond was methylated, replaced by an ester bond, or reversed. All of these modifications produced compounds that did not have a sweet taste. We conclude that the steric, electronic, and directional characteristics of the amide bond are essential for biological activity in the dipeptide sweeteners.

The artificial sweetener L-aspartyl-L-phenylalanine methyl ester [Asp-Phe-OMe (**1a**)] is not only 150 times



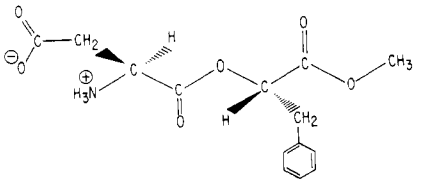
1 a, $n=1$; b, $n=0$

sweeter than sucrose but also possesses excellent taste properties. A large number of analogues have been synthesized in an attempt to improve upon these qualities and to understand which structural features are necessary to elicit a sweet taste. It has been demonstrated that, while the N-terminal residue of sweet analogues is restricted to aspartic acid[2a] or aminomalonic[2b] acid, the C-terminal residue can be successfully replaced by alkyl- and arylamines,[3] several D or L amino acid esters,[4] or a variety of aminomalonyl diesters.[5] All of the previous studies were aimed at determining which structural characteristics of the side chains were important for biological activity. In this paper, we present a series of Asp-Phe-OMe analogues designed to examine the relationship between the amide bond and the compound's sweet taste.

Two important characteristics of an amide bond are its ability to participate in hydrogen bonding and its directionality. We have prepared two compounds (**2** and **3**) in which the possibility of hydrogen bonding from the amide nitrogen to the receptor has been eliminated and two others (**4** and **10**) in which the capacity for hydrogen bonding has been retained but the direction of the amide bond has been reversed. These analogues were then tested for biological activity.

Results

To study the effect of hydrogen bonding in Asp-Phe-OMe, we have synthesized the ester and *N*-methyl analogues. The depsipeptide L-aspartyl-(*S*)-phenyllactic acid methyl ester (**2**) was prepared by coupling *N*-(benzyloxy-



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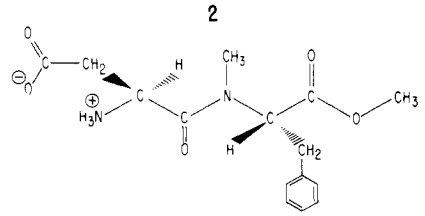

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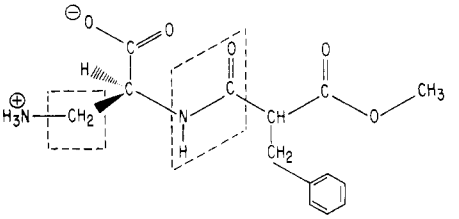
carbonyl)-L-aspartic acid β-benzyl ester[6] to (*S*)-phenyllactic acid methyl ester with carbonyldiimidazole.[7] The methyl ester of (*S*)-phenyllactic acid (Plac) was prepared by esterification of (*S*)-phenyllactic acid[8] with *O*-methyl-*N*,*N*′-diisopropylisourea[9] following a general method for preparing carboxylic acid alkyl esters.[10] The L-aspartyl-*N*-methyl-L-phenylalanine methyl ester (**3**) was prepared by coupling the methyl ester to the same protected aspartic acid derivative, using *N*,*N*′-dicyclohexylcarbodiimide (DCC). The *N*-methylphenylalanine ester used in this preparation was obtained by Fischer esterification. The protecting groups were removed from the depsipeptide by catalytic hydrogenation and from the *N*-methyl dipeptide by HBr–trifluoroacetic acid to yield compounds **2** and **3**, respectively. Neither compound was sweet.

We then prepared an analogue in which the amide bond was reversed but could still participate in hydrogen bonding. The first analogue with this reversed structure was N^{α}-[(*RS*)-α-(methoxycarbonyl)-β-phenylpropionyl]-L-α,β-diaminopropionic acid (**4**). The preparation of this



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analogue starts with the selective benzyloxycarbonylation[11]

(1) (a) Address: IBM Research, San Jose, Calif. 95193; (b) Address: Department of Pharmaceutical Chemistry, School of Pharmacy, The Hebrew University, Jerusalem, Israel; (c) Address: University of California, San Francisco, School of Medicine, San Francisco, Calif. 94143.

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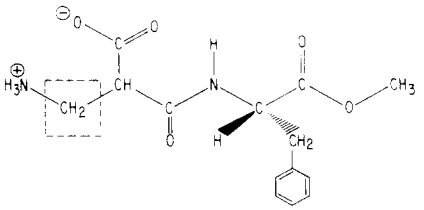
of the less basic β-amino function in L-α,β-diaminopropionic acid.

The N^{β}-(benzyloxycarbonyl)-L-α,β-diaminopropionic acid *N*-carboxyanhydride, prepared according to a procedure developed in our laboratories,[12] was reacted with an excess of benzyl alcohol to yield benzyl N^{β}-(benzyloxycarbonyl)-L-α,β-diaminopropionate. Coupling of this ester to methyl (*RS*)-2-benzylmalonate with DCC gave the fully protected dipeptide. Hydrogenation produced the dipeptide ester **4**, which was tasteless.

In compound **4** we reversed the direction of the amide bond while preserving the zwitterionic ring found in the parent structure **1a**. Previous studies had indicated that this zwitterionic ring was essential for sweetness,[1] and, in order to maintain its position, we moved the aspartyl β-methylene from between the α carbon and the carboxylic acid to between the α carbon and the amine. Thus, analogue **4** contains two modifications relative to compound **1a**: reversal of the amide bond and relocation of the aspartyl methylene. In order to assess the effect of each change, it was necessary to prepare the two compounds that contained single modifications. First, we sought to isolate the effect of moving the β-methylene by preparing 2-(aminomethyl)malonyl-L-phenylalanine methyl ester (**5**).



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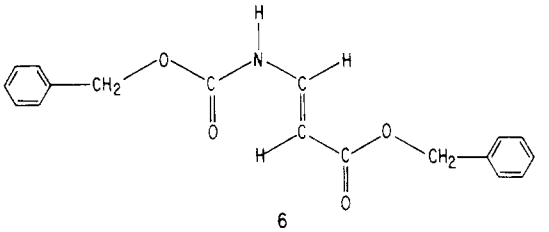
The benzyl *N*-(acetoxymethylene)carbamate, prepared from benzyl *N*-(hydroxymethylene)carbamate,[13] was used to alkylate benzyl *tert*-butyl malonate. The *tert*-butyl ester was removed with trifluoroacetic acid, and the resulting carboxylic acid was coupled to L-phenylalanine methyl ester with DCC. A rapid catalytic hydrogenation removed the benzyloxycarbonyl group and the benzyl ester to yield **5**. The compound was not sweet.

The second modification, reversal of the amide bond, produced the retro-inverso analogue in which the side chains are topochemically equivalent to those found in the parent peptide.[14] Our general synthetic approach to these isomers involved the transformation of a substituted α-amino acid into a *gem*-diamine. This was accomplished by preparing the amino acid hydrazide, converting the hydrazide to the azide with nitrosyl chloride,[15] and rearranging the azide to the corresponding isocyanate. The isocyanate was then allowed to react with the appropriate alcohol to yield a urethane, which was deprotected and incorporated into the peptide. Unfortunately, our two synthetic routes to the retro-inverso analogue of **1a** were unsuccessful.

In our first attempt, benzyloxycarbonyl-β-benzyl-L-aspartate was easily converted to the corresponding isocyanate, but the addition of *t*-BuOH to this isocyanate was very slow. The major product was not the desired urethane, but rather a β-aminoacrylate (**6**). By changing the aspartic acid protecting groups to *tert*-butyloxycarbonyl



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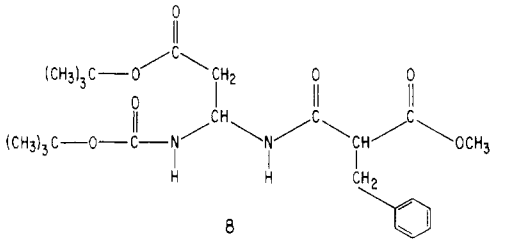

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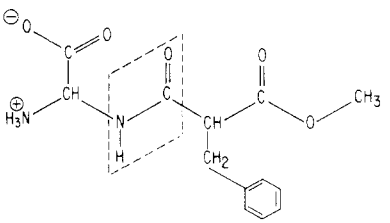
and β-*tert*-butyl ester, we prepared the completely blocked *gem*-diamino residue (**7**). This compound was deprotected by catalytic hydrogenation and coupled to methyl 2-benzylmalonate to yield the protected retro-inverso analogue **8**. Unfortunately, we were unable to convert this compound to the zwitterion and abandoned the synthesis. The synthesis of this desired retro-inverso analogue failed because the *gem*-diamino residue contains two protons on the β carbon. As a result, this residue was prone to the elimination of either isocyanic acid or ammonia, producing an α,β-unsaturated ester.

We decided to prepare the retro isomer of the intensely sweet dipeptide (*RS*)-α-aminomalonyl-L-phenylalanine methyl ester[2] (**1b**), which does not contain the β-methylene in the zwitterionic portion of the molecule. The synthesis of the retro-analogue **10** is outlined in Scheme I. The fully



10

blocked compound **9** was deprotected by catalytic hydrogenation to give *N*-[(*RS*)-α-(methoxycarbonyl)-β-phenylpropionyl]-α,α-diaminoacetic acid (**10**). This analogue was characterized by spectroscopy and tasted. It was not sweet.

These analogues were assayed for sweetness both in the solid state and as a 0.5% aqueous solution by a volunteer taste panel from our laboratories. The term "not sweet" is used when the analogue has a detectable taste that is not identifiable as sweet, sour, bitter, or salty.

Discussion

Two distinct views on the importance of the peptide backbone in determining the biological activity of a peptide have been proposed.[16] The topochemical approach treats the peptide backbone as a support which carries the amino acid side chains to the receptor. Only the nature of the side chains and the particular sequence in which they are arranged would determine the peptide's three-dimensional character. According to this view, the side chains are the only molecular structures which interact with the biological target. The other approach relates part of the interaction of the target site to the amide function in the peptide

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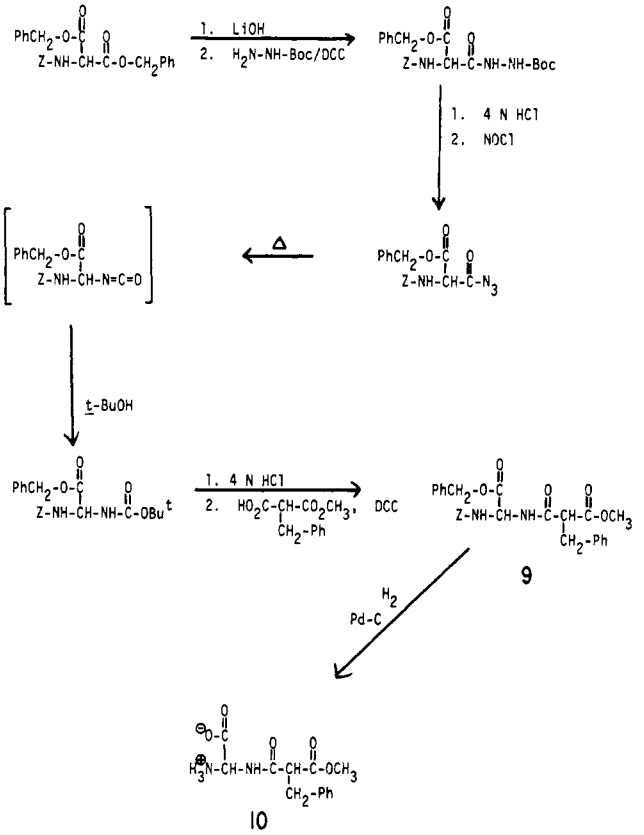
Scheme I



backbone.[17] One way for the amide bond to interact with the receptor would be through hydrogen bonding. This could occur either from the amide N–H to the receptor, from the receptor to the amide carbonyl, or both.

The first two analogues of **1a**, methyl L-aspartyl-(*S*)-phenyllactate (**2**) and L-aspartyl-*N*-methyl-L-phenylalanine methyl ester (**3**), cannot involve hydrogen bond donation from the internal amide group of the sweetener analogue to the receptor. In the depsipeptide **2**, the replacement of the amide NH by an oxygen produced an isosteric analogue. This type of modification has been studied in angiotensin[18] and bradykinin[19] and was found to affect substantially the biological activity of these peptides. In the *N*-methyl amide analogue **3**, this structural change not only caused the loss of hydrogen bonding potential but also introduced a steric factor. This modification has been studied in aspartylphenethylamide,[3] lysine-vasopressin,[20] oxytocin,[21] and in other biologically active peptides where it was found to reduce and even abolish biological activity. Both compounds **2** and **3** were not sweet, which suggests that some biologically important function related to the peptide bond was deleted by these modifications.

The most isosteric and isoelectronic modification to which the amide bond in a peptide backbone can be subjected is the reversal of its direction. This modification was initially applied to cyclic peptide and depsipeptide antibiotics and was found to produce analogues with full biological activity.[22] Studies that attempted to extend this approach to linear peptides were less successful.[14] In a recent review, we introduced the term retro-inverso isomers (previously called "retro-enantiomers" or "retro-all-D-analogues") to indicate that they differ from the parent peptides by having a reversed amide structure.[14] Thus, the retro-inverso analogue for the dipeptide sweeteners are composed of *gem*-diamines and malonic acid derivatives (see compound **10**). The ultimate goal of this modification is to produce an analogue with a side-chain orientation that is almost indistinguishable from that found in the parent peptide. In the retro-inverso isomer, as compared to the *N*-methyl or depsipeptide analogues, the overall potential for hydrogen bonding is maintained.

We have initiated a program to study the conformational effects of retro-inverso segments that are incorporated into biologically active peptides.[23] The conformational consequences of introducing such modifications can obviously be substantial. We examined the retro analogues of the dipeptide sweeteners as if they were highly flexible molecules and, therefore, adopted the premise that only the primary structure would be significant in determining biological activity. This first approximation can be refined when we obtain more conformational information.

We prepared N^{α}-[(*RS*)-α-(methoxycarbonyl)-β-phenylpropionyl]-L-α,β-diaminopropionic acid (**4**), in which two modifications were introduced simultaneously. The peptide bond was reversed and the β-methylene was relocated to between the α carbon and the side-chain amine. This preserved the size of the zwitterionic ring, but the carboxylate was attached to the α carbon and the amine was joined to the β-methylene. Compound **4** is not sweet.

It has been shown that having both the carboxylate and ammonium ion functions attached to the α carbon, as in α-aminomalonyl-L-phenylalanine methyl ester (**1b**), produces an intensely sweet compound.[2b] Therefore, it was impossible to relate the loss of biological activity to one of the two simultaneous modifications. Thus, we prepared two different analogues, each containing a single modification. The preparation of 2-(aminomethyl)malonyl-L-phenylalanine methyl ester (**5**) was designed to isolate the effect of moving the nitrogen to the β-methylene. The dipeptide **5** is not sweet. This observation does not specifically relate to the primary emphasis of this paper, which deals with the effect of the peptide bond on the sweet taste; however, from the result with compound **5** we can state that alterations in the orientation of the substituents on the zwitterionic ring can destroy the sweet taste. The other analogue with the single variation would have been one in which the amide bond was reversed while leaving the remaining portion of the molecule unchanged. Our attempts to synthesize the retro-inverso analogue of **1a** were unsuccessful due to the limited stability of this particular gem residue. We attributed this instability to the facile elimination of either isocyanic acid or ammonia, which produced high yields of the substituted β-aminoacrylates.

Fortunately, we were able to prepare *N*-[(*RS*)-α-(methoxycarbonyl)-β-phenylpropionyl]-β,β-diaminoacetic acid (**10**), a retro-inverso analogue of the dipeptide sweetener **1b**. This topochemical analogue was tasteless. Since both of the residues in **10** were derived from racemic starting

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materials, the resulting analogue was a complex diastereomeric mixture. It has been shown that the wrong stereochemistry at either of the two chiral centers in Asp-Phe-OMe gives rise to tasteless or even bitter tasting analogues.[2a] However, analogues have been reported in which an (*RS*)-α-aminomalonyl residue was incorporated into the dipeptide sweetener either as the N-terminal residue, such as in **1b**, or as the C-terminal residue, such as in L-aspartyl (*RS*)-α-aminomalonic acid diester,[24] to produce intensely sweet compounds. We prepared (*RS*)-α-aminomalonyl-DL-phenylalanine methyl ester[25] and *N*-[(*RS*)-α-aminomalonyl]-(*RS*)-α-aminomalonic acid adamantyl methyl ester[26] and found them to be intensely sweet. Thus, diastereomeric mixtures of intensely sweet dipeptide derivatives remain sweet.

The retro-inverso analogue **10** is not sweet. Our work indicates that the nature of the zwitterion, compound **5**, and the direction of the peptide bond are both essential for maintaining the sweet taste. We can now conclude that replacement of the peptide bond with an ester linkage, methylation of the amide nitrogen, alteration of the orientation of the charge in the zwitterion, or simple reversal of the bond direction completely destroys the perception of sweet taste.

Summary

Five analogues (**2–5** and **10**) of the dipeptide sweeteners **1a** and **1b** were synthesized and found not to be sweet. Any modification of the peptide bond, i.e., conversion to an ester (**2**), N-methylation (**3**), or reversal (**4** and **10**), resulted in a complete loss of the sweet taste. The incorporation of the N-terminal amino acid, as in compound **5** where the orientation of the zwitterionic ring has been slightly altered, also resulted in a loss of sweet taste. This finding represents an additional factor in determining sweet taste for these peptide-derived sweeteners.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 180 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 141 with a 10-cm water-jacketed cell. High-resolution ^{1}H NMR spectra were obtained in the Fourier-transform mode by using a Varian HR-220 spectrometer at the University of California, San Diego. All chemical shifts are reported in parts per million downfield from either Me_4Si or $Me_3Si(CD_2)_3SO_3Na$. All elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Analytical TLC plates were purchased from E. Merck: silica gel, 60 F-254, glass backed. The plates were developed with ninhydrin or UV light (254 nm). The following chromatography systems were used: (A) EtOAc, (B) *n*-BuOH/HOAc/H_2O (4:1:5) upper phase, (C) MeOH/$CHCl_3$ (5:95), (D) EtOAc/HCO_2H/H_2O (17:2:1), (E) EtOAc/hexane (1:1), (F) MeCOEt/pyridine/0.1% HOAc/H_2O (70:15:15:2), (G) EtOAc/hexane (3:7), (H) $CHCl_3$/MeOH/HOAc (85:10:5), (I) $CHCl_3$/MeOH/HOAc (93:2:5).

(*S*)-Phenyllactic Acid Methyl Ester. (*S*)-Phenyllactic acid[8] (4.3 g, 26 mmol) was mixed with a solution of *O*-methyl-*N*,*N*-diisopropylisourea[9] (3.95 g, 25 mmol) in tetrahydrofuran (70 mL). The mixture was stirred at room temperature overnight. The mixture was evaporated, and the residue was filtered and washed with 140 mL of ether. The filtrate was combined with the ethereal washings and washed with a saturated solution of $NaHCO_3$, followed by H_2O. The solution was dried over $MgSO_4$ and the solvent removed under reduced pressure to give 4.2 g of crude solid, which was crystallized from hexane to yield 3.91 g (87%): TLC R_f (C) 0.51, R_f (D) 0.77; mp 47 °C; $[\alpha]^{25}_D$ −12.4° (*c* 0.7, H_2O). Anal. ($C_{10}H_{12}O_3$) C, H.

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-(*S*)-phenyllactic Acid Methyl Ester.** *N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartate (1.5 g, 4.2 mmol) and freshly prepared carbonyldiimidazole (0.68 g, 4.2 mmol) were dissolved at 0 °C in dry CH_2Cl_2 (20 mL). After 15 min at 0 °C, (*S*)-phenyllactic acid methyl ester (0.756 g, 4.2 mmol) was added and the stirring solution was allowed to reach room temperature. The reaction mixture was stirred under N_2 for 3 days; the solvent was then removed under reduced pressure and the residue taken up into ether. The ether solution was washed successively with H_2O, three portions of a saturated $NaHCO_3$ solution, and brine, dried over $MgSO_4$, and removed under reduced pressure to leave a white solid. Recrystallization from EtOAc/hexane yielded 0.70 g of fine needles (46%): TLC R_f (E) 0.52; mp 77–78 °C; $[\alpha]^{25}_D$ −8.2° (*c* 1.0, $CHCl_3$); IR (KBr) 1778, 1749, 1738, 1695 cm^{-1}; NMR ($CDCl_3$) δ 7.33 (m, 15 H, arom), 5.17 (d, *J* = 7 Hz, 1 H, NH), 5.29 (m, 1 H, Plac C_α H), 5.10 (s, 4 H, PhCH_2O), 4.71 (m, 1 H, Asp C_α H), 3.65 (s, 3 H, OCH_3), 3.16–2.58 (m, 4 H, Plac C_β H_2, Asp C_β H_2). Anal. ($C_{29}H_{29}NO_8$) C, H, N.

L-Aspartyl-(*S*)-phenyllactic Acid Methyl Ester (2). *N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-(*S*)-phenyllactic acid methyl ester (219 mg, 0.42 mmol) was dissolved in 50 mL of warm *i*-PrOH; 10% Pd/C (25 mg) was added and the suspension was hydrogenated on a Parr apparatus at 50 psi for 4 h. Methanol (50 mL) was added to the suspension, and the resulting mixture was filtered through a Celite pad. The alcohol was removed under reduced pressure to leave a white powder: yield 94 mg (75%); TLC R_f (B) 0.38, R_f (F) 0.56; mp 140 °C; $[\alpha]^{25}_D$ −16.8° (*c* 1.0, HOAc); IR (KBr) 1755, 1745, 1200 cm^{-1}; NMR (Me_2SO-d_6) δ 7.27 (s, 5 H, arom), 5.20 (m, 1 H, Plac C_α H), 3.73 (m, 1 H, Asp C_α H), 3.62 (s, 3 H, OCH_3), 3.12 (m, 2 H, Plac C_β H_2), 2.59–2.29 (m, 2 H, Asp C_β H_2). Anal. ($C_{14}H_{17}NO_6$) C, H, N.

***N*-Methyl-L-phenylalanine Methyl Ester Hydrochloride.** *N*-Methyl-L-phenylalanine[27] (4.0 g, 22 mmol) was suspended in 100 mL of dry MeOH. The suspension was saturated with anhydrous HCl gas at 0 °C, then warmed slowly to room temperature, and stirred for 12 h. The solvent was removed under reduced pressure to leave an oil, which was dissolved in 100 mL of MeOH and saturated with HCl gas. After 12 h, the solvent was removed under reduced pressure to yield a light tan solid. Recrystallization from MeOH/Et_2O gave 4.5 g (90%): TLC R_f (B) 0.56; mp 102–103 °C, lit.[28] mp 98 °C; $[\alpha]^{25}_D$ +18° (*c* 5.0, H_2O), lit.[28] $[\alpha]^{20}_D$ +16.1° (*c* 5.0, H_2O). Anal. ($C_{11}H_{16}ClNO_2$) C, H, N.

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-*N*-methyl-L-phenylalanine Methyl Ester.** To an ice-cold solution of *N*-methyl-L-phenylalanine methyl ester (0.98 g, 5.1 mmol) and *N*-(benzyloxycarbonyl)-β-benzyl-L-aspartate (1.82 g, 5.1 mmol) dissolved in CH_2Cl_2 (15 mL) was added dicyclohexylcarbodiimide (1.16 g, 5.6 mmol) dissolved in 10 mL of CH_2Cl_2. The reaction mixture was left to stir at room temperature for 20 h, after which it was treated with glacial acetic acid (1 mL), followed by stirring for an additional 3 h. The mixture was filtered, the CH_2Cl_2 was removed under reduced pressure, and the residue was taken up into EtOAc. The EtOAc solution was washed with a saturated solution of $NaHCO_3$ and H_2O and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was taken up into 5 mL of acetone and cooled to 4 °C for 12 h. The residual urea was removed by filtration, and the solvent was removed under reduced pressure to yield an oil: 2.24 g (82%). This material was purified by column chromatography on silica gel using EtOAc/hexane (1:1) to give an oil (1.33 g, 49%): R_f (E) 0.52; $[\alpha]^{25}_D$ −72.1° (*c* 1.30, $CDCl_3$); IR ($CHCl_3$) 1735, 1650 cm^{-1}; NMR ($CDCl_3$) δ 7.33 (s, 10 H, arom), 7.18 (m, 5 H, arom), 5.23 (m, 1 H, MePhe C_α H), 5.10–4.93 (m, 6 H, Z*NH*–, PhCH_2O–, Asp C_α H), 3.71 (s, 3 H, OCH_3), 3.37 (m, 1 H, MePhe C_β H), 3.05, 2.94 (m, s, 4 H, MePhe $C_{\beta'}$ H, –NCH_3), 2.78 (d of d, *J* = 16 and 7 Hz, 1 H, Asp C_β H), 2.59 (d of d, *J* = 17 and 6 Hz, 1 H, Asp $C_{\beta'}$ H). Anal. ($C_{30}H_{32}N_2O_7$) C, H, N.

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L-Aspartyl-*N*-methyl-L-phenylalanine Methyl Ester Hydrobromide (3). A solution of *N*-(benzyloxycarbonyl)-β-benzyl-L-aspartyl-*N*-methyl-L-phenylalanine methyl ester (0.5 g, 0.9 mmol) in CF_3CO_2H (25 mL) was saturated with dry HBr gas. The reaction was allowed to stir for 4 h at room temperature. The solvent was removed under reduced pressure to yield a viscous oil, which was taken up in 2.0 mL of fresh CF_3CO_2H. The slow addition of this solution to 200 mL of anhydrous ether precipitated the hydrobromide salt. The solid was collected by filtration and washed with ether to yield 140 mg (38%): mp 135–136 °C dec; $[\alpha]^{25}_D$ −74.2° (*c* 1.2, CF_3CO_2H); IR (KBr) 1730, 1653, 1578 cm^{-1}; NMR (CF_3CO_2H) δ 7.52 (m, 3 H, NH_3^+), 7.28 (m, 5 H, arom), 5.04 (m, 2 H, Asp C_α H, MePhe C_α H), 4.46 (s, 3 H, OCH_3), 3.94 (m, 2 H, Asp C_β H_2), 3.30 (m, 2 H, MePhe C_β H_2), 3.07 (s, 3 H, NCH_3). Anal. ($C_{15}H_{21}BrN_2O_5$) C, H, N.

***N*$^\beta$-(Benzyloxycarbonyl)-L-α,β-diaminopropionic Acid.** L-α,β-Diaminopropionic acid hydrochloride[29] (9.5 g, 68 mmol) was dissolved in hydrochloric acid (145 mL, 0.75 N). The pH of the solution was adjusted to 7.0 and maintained at neutrality during the reaction by the controlled addition of 1 N NaOH. The stirring mixture was cooled to 0 °C and benzyl chloroformate (9.7 mL, 68 mmol) was added at a constant rate (2.4 mL/h). After about 4 h, the solution was allowed to reach room temperature and stirring continued for an additional 15 h. The reaction mixture was filtered and the precipitate was washed successively with H_2O. The solid was washed twice with anhydrous ether (25 mL) and dried in vacuo to yield 13.3 g (83%). This material was recrystallized from H_2O: yield 11.9 g (75%); mp 239–240 °C dec, lit.[11] mp 227–229 °C dec; $[\alpha]_D$ −18.5° (*c* 1.07, 1 N HCl), lit.[11] $[\alpha]^{25}_D$ −18.7° (*c* 1.0, 1 N HCl).

Benzyl *N*$^\beta$-(Benzyloxycarbonyl)-L-α,β-diaminopropionate Hydrochloride. *N*$^\beta$-(Benzyloxycarbonyl)-L-α,β-diaminopropionic acid *N*-carboxyanhydride[12] (3.2 g, 12 mmol) and $PhCH_2OH$ (7.0 mL, 66 mmol) were added to anhydrous ether (100 mL), saturated with HCl gas, and cooled to 0 °C. The reaction mixture was stirred and warmed to room temperature. After about 1 h, crystals appeared and the stirring was continued overnight. The crystalline material was filtered and washed with anhydrous ether to yield 3.0 g (70%) of white solid, which was recrystallized from acetone: TLC R_f (B) 0.65, R_f (F) 0.76; mp 157–158 °C; $[\alpha]^{25}_D$ −6.97° (*c* 1.19, MeOH). Anal. ($C_{18}H_{21}ClN_2O_4$) C, H, N.

Benzyl *N*$^\beta$-(Benzyloxycarbonyl)-*N*$^\alpha$-[(*RS*)-α-(methoxycarbonyl)-β-phenylpropionyl]-L-α,β-diaminopropionate. Benzyl *N*$^\beta$-(benzyloxycarbonyl)-L-α,β-diaminopropionate hydrochloride (720 mg, 1.97 mmol) was suspended in 25 mL of CH_2Cl_2, cooled to 0 °C, and neutralized with Et_3N (0.275 mL, 1.97 mmol). To this cold solution was added 41 mg (2.0 mmol) of methyl 2-benzylmalonate[30] dissolved in 10 mL of CH_2Cl_2, followed by 450 mg (2.2 mmol) of dicyclohexylcarbodiimide. The reaction was stirred for 2 h at 0 °C and then allowed to warm to room temperature. Acetic acid (1 mL) was added and, after 15 min, the mixture was filtered. The filtrate was washed with a saturated solution of $NaHCO_3$, washed with H_2O, dried over $MgSO_4$, and evaporated to leave 1.0 g of crude product. Chromatography on silica gel (20 × 390 mm) with EtOAc/hexane (1:1) as the eluant gave 0.77 g (76%) of a viscous oil which crystallized from *i*-PrOH: TLC R_f (B) 0.39; mp 78–80 °C; $[\alpha]^{25}_D$ −2.22° (*c* 2.03, CH_2Cl_2); IR (KBr) 3320, 1742, 1735, 1691, 1654, 1540 cm^{-1}; NMR ($CDCl_3$) δ 7.29 (m, 15 H, arom), 7.05 (d, 1 H, CH*NH*CO), 5.15 and 5.07 (m, 2 H, OCH_2Ph; m, 2 H, OCH_2Ph; m, 0.5 H, Z-*NH*), 4.64 (m, 1 H, DAP C_α H; m 0.5 H, Z-*NH*), 3.66 (s, 3 H, OCH_3), 3.64–3.36 (m, 3 H, ZNHCH_2, *CH*CH_2Ph), 3.23 (m, 2 H, CHCH_2Ph). Anal. ($C_{29}H_{30}N_2O_7$) C, H, N.

***N*$^\alpha$-[(*RS*)-α-(Methoxycarbonyl)-β-phenylpropionyl]-L-α,β-diaminopropionic Acid (4).** Benzyl *N*$^\beta$-(benzyloxycarbonyl)-*N*$^\alpha$-[(*RS*)-α-(methoxycarbonyl)-β-phenylpropionyl]-L-α,β-diaminopropionate (212 mg, 0.43 mmol) was dissolved in a mixture composed of 20 mL of MeOH, 1 mL of H_2O, and 1 mL of HOAc. Pd/C, 10%, was added and the suspension was hydrogenated on a Parr apparatus at 40 psi for 4 h. The mixture was filtered through a Celite pad and the solvent removed under reduced pressure. The material was taken up in 2% HOAc and lyophilized to yield 98 mg of a white solid. The product was purified by partition chromatography on Sephadex G-25 (1.5 × 100 cm), *n*-BuOH/HOAc/H_2O (4:1:5), and lyophilized from 0.1% HOAc to yield 45 mg: TLC R_f (B) 0.38; mp 108–109 °C; $[\alpha]^{24}_D$ −24.6° (*c* 1.21, 1 N HOAc); IR (KBr) 3400 (br), 1740, 1630 (br) cm^{-1}; NMR (D_2O) δ 7.29 (m, 5 H, arom), 4.30 (m, 1 H, DAP C_α H), 3.89 (m, 1 H, $COCHCO_2CH_3$), 3.73 (d, *J* = 3.7 Hz, 3 H, OCH_3), 3.33–2.73 (m, 4 H, H_2NCH_2–, $PhCH_2$–). Anal. ($C_{14}H_{18}N_2O_5 \cdot H_2O$) C, H, N.

***N*-(Benzyloxycarbonyl)-*O*-benzyl-*N*-[2-(aminomethylene)malonyl]-L-phenylalanine Methyl Ester. A. Benzyl *N*-(Acetoxymethylene)carbamate.** Benzyl *N*-(hydroxymethylene)carbamate[13] (6.2 g, 34 mmol) was dissolved in dry tetrahydrofuran (50 mL) and treated with Et_3N (4.73 mL, 34.2 mmol). The solution was stirred vigorously and acetic anhydride (3.24 mL, 34.2 mmol) was added in a single portion. The reaction was stirred under N_2 at room temperature for 4 h. The solvent was evaporated and the oily residue was taken into ether and washed successively with H_2O, a saturated solution of NH_4Cl, H_2O, a saturated solution of $NaHCO_3$, and brine. The ether solution was dried over $MgSO_4$ and evaporated to yield the crude product. Purification by silica gel column chromatography (50 × 350 mm) with EtOAc/hexane (1:1) gave a colorless oil: yield 6.9 g (82%); TLC R_f (E) 0.43.

B. *N*-(Benzyloxycarbonyl)-2-(aminomethylene)malonic Acid Benzyl *tert*-Butyl Ester. Sodium hydride (0.4 g), as a 50% suspension in mineral oil, was weighed into a 50-cm^3 solid-phase reaction vessel and washed successively with hexane and dry tetrahydrofuran. The washed sodium hydride was suspended in 25 mL of dry tetrahydrofuran, protected with an atmosphere of N_2, and vigorously stirred. A solution of benzyl *tert*-butyl malonate[31] (1.01 g, 4.06 mmol) in 10 mL of dry tetrahydrofuran was added to the sodium hydride suspension and stirred for 15 min. The vessel was then pressurized with N_2, which transferred the sodium malonate into a flask containing benzyl *N*-(acetoxymethylene)carbamate, prepared as described above (0.905 g, 4.06 mmol) in tetrahydrofuran (15 mL). After stirring the mixture for 2 h, the solvent was removed under reduced pressure to leave a gel, which was taken into $CHCl_3$ and washed with H_2O, dried over $MgSO_4$, and removed under reduced pressure to leave an oily residue: yield 1.5 g (89%). The crude oil was chromatographed on a silica gel column (20 × 290 mm) with EtOAc/hexane (1:3) as the eluant. This yielded 1.3 g (79%) of an oil, which crystallized upon refrigeration but melted at room temperature: TLC R_f (E) 0.53; IR (film) 1730, 1520 cm^{-1}; NMR ($CDCl_3$) δ 7.31 (s, 10 H, arom), 5.34 (m, 1 H, NH), 5.15 (s, 2 H, OCH_2Ph), 5.08 (s, 2 H, OCH_2Ph), 3.60 (m, 3 H, C_7H_7-*CH*-, ZNHCH_2), 1.33 [s, 9 H, $(CH_3)_3C$]. Anal. ($C_{23}H_{27}NO_6$) C, H, N.

C. *N*-(Benzyloxycarbonyl)-2-(aminomethylene)malonic Acid Benzyl Ester. *N*-(Benzyloxycarbonyl)-2-(aminomethylene)malonic acid benzyl *tert*-butyl ester (5.0, 12 mmol) was dissolved in anhydrous trifluoroacetic acid (35 mL), and the resulting solution was stirred under N_2 at room temperature for 2 h. The solvent was removed under reduced pressure and the residue taken into CCl_4. The solution was washed with three portions of saturated $NaHCO_3$ and the combined aqueous washings were acidified to pH 2 with concentrated HCl. The acidified solution was extracted with three portions of $CHCl_3$, and the combined organic phase washings were dried over $MgSO_4$. The solvent was removed under reduced pressure to leave a viscous oil: yield 4.3 g (99%).

D. Coupling of *N*-(Benzyloxycarbonyl)-*O*-benzyl-2-(aminomethylene)malonic Acid Benzyl Ester to L-Phenylalanine Methyl Ester Hydrochloride. To a solution of monoacid monoester (694 mg, 1.94 mmol) in CH_2Cl_2 (10 mL) was added L-phenylalanine methyl ester hydrochloride (419 mg, 1.94 mmol) and Et_3N (268 μL, 1.94 mmol). The mixture was cooled in an ice bath and a solution of dicyclohexylcarbodiimide (399

(29) This compound was obtained from Calbiochem, La Jolla, CA 92037.

(30) Prepared by the general scheme described in "Organic Syntheses", Collect. Vol. I, Wiley, New York, 1941, p 250; *ibid.*, Collect. Vol IV, 1963, p 417.

(31) Prepared by the general scheme described by T. J. Brocksom, N. Petragnani, and R. Rodrigues, *J. Org. Chem.*, **39**, 2114 (1974).

mg, 1.94 mmol) in 1 mL of CH_2Cl_2 was added. The resulting mixture was stirred for 2 days at room temperature, followed by addition of glacial acetic acid (0.1 mL), and then stirred for an additional 0.5 h. The reaction mixture was filtered and the filtrate washed successively with 1 N H_2SO_4, brine, a saturated solution of $NaHCO_3$, and brine. The solution was dried over $MgSO_4$, and the solvent was removed under reduced pressure to leave a solid (0.91 g). The crude product was chromatographed on a silica gel column (20 × 290 mm) and eluted with EtOAc/hexane (1:1). The protected dipeptide was crystallized from EtOAc/hexane: yield 722 mg (72%); R_f (E) 0.42; mp 120–122 °C; $[\alpha]^{22}_D$ +21.0° (*c* 2.02, CH_2Cl_2); IR (KBr) 3320, 1735, 1695, 1645, 1530 cm^{-1}; NMR ($CDCl_3$) δ 7.33–6.97 (m, 16 H, arom, –CHCO*NH*CH–), 5.37 (m, 1 H, Z*NH*CH_2), 5.12, 5.08 (2 s, 4 H, Ph*CH*$_2$O–), 4.80 (m, 1 h, Phe C_α H), 3.70 (br s, 5 H, OCH_3, ZNH*CH*$_2$), 3.49 (m, 1 H, $C_7H_7O_2CCHCONH$), 3.15 (d of d, *J* = 13.9 and 5.5 Hz, 1 H, Phe C_β H), 3.01 (d of d, *J* = 14.2 and 5.5 Hz, 1 H, Phe $C_{\beta'}$ H); MS *m/e* 518 (M^+). Anal. ($C_{29}H_{30}N_2O_7$) C, H, N.

N-[2-(Aminomethylene)malonyl]-L-phenylalanine Methyl Ester (5). *N*-(Benzyloxycarbonyl)-*O*-benzyl-*N*-[2-(aminomethylene)malonyl]-L-phenylalanine methyl ester (500 mg, 0.965 mmol) was dissolved in MeOH (50 mL) and 10% Pd/C (250 mg) was added. The suspension was vigorously stirred on a vortex mixer, and a stream of H_2 gas was rapidly bubbled through the mixture for 10 min. The catalyst was removed by filtration through Celite and the alcohol was removed under reduced pressure to leave a clear glass. Lyophilization from 1 M HOAc gave 265 mg (91%): TLC R_f (B) 0.41; mp 77–80 °C dec; $[\alpha]^{23}_D$ +28.0° (*c* 1.43, HOAc); IR (KBr) 3343 (br), 3255 (br), 1745, 1659, 1532 cm^{-1}; NMR (Me_2SO-d_6) δ 8.3–7.5 (m, 3 H, NH_3), 7.22 (m, 6 H, arom, CO*NH*), 6.20 (q, 1 H, $O_2CCHCONH$), 4.52 (m, 1 H, *CH*CO_2CH_3), 3.58, 3.56 (2 s, 3 H, OCH_3), 3.38–2.94 (m, H_2O, NH_3CH_2–, Ph*CH*$_2$–). Anal. ($C_{14}H_{18}N_2O_5$·0.33HOAc·H_2O) C, H, N.

α-[(*tert*-Butyloxycarbonyl)hydrazidyl] β-Benzyl Benzyloxycarbonyl-L-asparatate. β-Benzyl benzyloxycarbonyl-L-aspartate (7.14 g, 20 mmol) and *tert*-butyl carbazate (2.64 g, 20 mmol) were dissolved in CH_2Cl_2 (70 mL) and cooled to 0 °C. Dicyclohexylcarbodiimide (4.12 g, 20 mmol) in 30 mL of CH_2Cl_2 was added to the stirring solution. The reaction was allowed to reach room temperature and stirred for an additional 12 h. Acetic acid (1 mL) was added to the mixture and stirring continued for an additional 0.5 h. The reaction mixture was filtered, the precipitate was washed with CH_2Cl_2, and the washings were combined with the filtrate, which was then washed successively with 2 M $NaHSO_4$, H_2O, saturated $NaHCO_3$, and H_2O. The solution was dried over $MgSO_4$ and the solvent was removed under reduced pressure to give 7.82 g of a foam. The residue was chromatographed on a silica gel column (45 × 350 mm) eluting with EtOAc/hexane (3:7). The product was obtained as a clear viscous oil: yield 6.3 g (67%); R_f (E) 0.35; $[\alpha]^{25}_D$ –17.7° (*c* 2.12, EtOH). Anal. ($C_{24}H_{29}N_3O_7$) C, H, N.

Attempted Preparation of *N*-(Benzyloxycarbonyl)-*N'*-(*tert*-butyloxycarbonyl)-β,β-diaminopropionic Acid Benzyl Ester. A. Deprotection. α-[(*tert*-Butyloxycarbonyl)hydrazidyl] β-benzyl benzyloxycarbonyl-L-aspartate (2.6 g, 5.5 mmol) was dissolved in 10 mL of 4 N HCl in dioxane. After 1 h, the dioxane was removed under reduced pressure and ether was added to the residue. The crystalline material was filtered and washed with ether to give a quantitative yield of the corresponding hydrazide hydrochloride salt.

B. Conversion of Hydrazide to Urethane. α-Hydrazidyl β-benzyl benzyloxycarbonyl-L-aspartate hydrochloride (330 mg, 0.8 mmol) was suspended in dry tetrahydrofuran (10 mL), cooled to 0 °C, and treated with a saturated solution of nitrosyl chloride in tetrahydrofuran until an orange color persisted for 5 min. The reaction mixture was diluted with 20 mL of cold EtOAc and washed with a saturated solution of $NaHCO_3$, followed by brine. The solution was dried over $MgSO_4$ and the solvent was removed under reduced pressure to give the azide. The azide was dissolved in 10 mL of dry toluene and heated at 80 °C under N_2 until the azide was completely converted to the isocyanate (1.5 h, monitored by IR). To the isocyanate solution was added *t*-BuOH (5 mL), and the reaction was maintained at 80 °C for 12 h. The toluene was removed under reduced pressure, which yielded a solid that was chromatographed on silica gel (20 × 290 mm) with a gradient of EtOAc (0–50%) in hexane. The major fraction gave 60 mg (24%) of benzyl *N*-(benzyloxycarbonyl)-3-aminoacrylate (**6**): TLC R_f (E) 0.57; mp 137 °C; IR (KBr) 1733, 1702, 1636 cm^{-1}; NMR ($CDCl_3$) δ 7.85 (m, 1 H, N*CH*=C), 7.36 (s, 10 H, arom), 6.93 (br d, 1 H, NH–), 5.41 (d, *J* = 14 Hz, 1 H, C=*CH*CO), 5.20, 5.17 (2 s, 4 H, Ph*CH*$_2$O–). Anal. ($C_{18}H_{17}NO_4$) C, H, N.

α-[(Benzyloxycarbonyl)hydrazidyl] β-*tert*-Butyl *tert*-Butyloxycarbonyl-L-aspartate. β-*tert*-Butyl *tert*-butyloxycarbonyl-L-aspartate dicyclohexylamine (7.0 g, 15 mmol) was dissolved in 2 N $NaHSO_4$ and extracted into ether. The ethereal solution was washed with 2 N $NaHSO_4$ and dried over $MgSO_4$, and the solvent was removed under reduced pressure to leave a clear oil (3.9 g, 14 mmol) of β-*tert*-butyl *tert*-butyloxycarbonyl-L-aspartate.

The oil (3.9 g) was dissolved in 50 mL of CH_2Cl_2, benzyl carbazate hydrochloride[32] (2.72 g, 13.5 mmol) was added, and the mixture was cooled to 0 °C. Triethylamine (1.87 ml, 13.5 mmol) and a solution of dicyclohexylcarbodiimide (2.78 g, 13.5 mmol) in CH_2Cl_2 (20 mL) were added to the stirring reaction mixture, and this was allowed to warm to room temperature. After 12 h, glacial acetic acid (0.5 mL) was added to the reaction mixture and allowed to stir for an additional 0.5 h. The mixture was filtered and the precipitate washed with CH_2Cl_2. The filtrate and the washings were combined and washed successively with 2 N $NaHSO_4$, brine, a saturated solution of $NaHCO_3$, and brine. The solution was dried over $MgSO_4$, and the solvent was removed under reduced pressure to leave a foam (6.4 g). The foam was taken into acetone (30 mL) and stored at –10 °C for 12 h. The residual urea was removed by filtration, and removal of the acetone under reduced pressure yielded 5.1 g (86%) of an amorphous solid: TLC R_f (E) 0.38; mp 41–42 °C; $[\alpha]^{25}_D$ –20.0° (*c* 2.29, EtOH). Anal. ($C_{21}H_{31}N_2O_7$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-*N'*-(benzyloxycarbonyl)-β,β-diaminopropionic Acid *tert*-Butyl Ester (7). A. Deprotection.** α-[(Benzyloxycarbonyl)hydrazidyl] β-*tert*-butyl *tert*-butyloxycarbonyl-L-aspartate (3.0 g, 6.8 mmol) was dissolved in glacial acetic acid (50 mL). To the solution was added 10% Pd/C (100 mg) and the suspension was hydrogenated in a Parr apparatus at 50 psi for 1.5 h. The suspension was filtered through a Celite pad, the solvent was removed under reduced pressure, and the amorphous residue was used as such in the subsequent synthesis.

B. Conversion of Hydrazide to Urethane. The residue from the hydrogenation was taken into dry tetrahydrofuran (30 mL), cooled in an ice bath, and treated with a solution of saturated nitrosyl chloride in tetrahydrofuran until an orange color persisted for 5 min. The reaction mixture was diluted with 100 mL of cold EtOAc, washed with a cold, saturated solution of $NaHCO_3$ and a cold brine solution, and dried over $MgSO_4$. The solvent was removed under reduced pressure to give the azide, an oil, which was taken in dry toluene (20 mL) and heated under N_2 at 60 °C for 2 h (complete disappearance of the azide band in the IR). The isocyanate solution was treated with dry $PhCH_2OH$ (5 mL, 50 mmol) and heated for 12 h at 60 °C (complete disappearance of the isocyanate band in the IR). The solvent was removed under reduced pressure to give an oil, which was crystallized from EtOAc/hexane: yield 1.45 g (55%); TLC R_f (G) 0.35; R_f (H) 0.71; mp 114–117 °C dec; $[\alpha]^{25}_D$ –3.8° (*c* 1.0, EtOH); IR (KBr) 1746, 1733, 1718, 1684 cm^{-1}; NMR ($CDCl_3$) δ 7.21 (s, 5 H, arom), 5.80 (m, 1 H, NH), 5.64 (m, 1 H, NH), 5.50 (m, 1 H, C_α H), 5.11 (s, 2 H, CH_2Ph), 2.80 (m, 2 H, C_β H_2), 1.41 [m, 18 H, $(CH_3)_3C$]. Anal. ($C_{20}H_{30}N_2O_6$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-*N'*-[(*RS*)-α-(methoxycarbonyl)-β-phenylpropionyl]-β,β-diaminopropionic Acid *tert*-Butyl Ester (8).** Compound 7 (500 mg, 1.27 mmol) was dissolved in 30 mL of tetrahydrofuran and 10% Pd/C (50 mg) was added. The suspension was hydrogenated in a Parr apparatus at 50 psi for 2 h. The suspension was filtered through a Celite pad, concentrated to a small volume (2 mL), cooled to 0 °C, and added to a solution of methyl (*RS*)-2-benzylmalonate (264 mg, 1.27 mmol) in 4 mL of tetrahydrofuran, followed by dicyclohexylcarbodiimide (261 mg, 1.27 mmol). The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 12 h. The reaction mixture was filtered and the tetrahydrofuran was removed

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under reduced pressure. The residue was taken up in EtOAc and washed successively with 2 N $NaHSO_4$, brine, a saturated solution of $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure to give a residue which was chromatographed on a silica gel column (20 × 290 mm) with EtOAc/hexane (3:7) and crystallized from EtOAc/hexane: yield 350 mg (61%); R_f (G) 0.21, R_f (I) 0.45; mp 107 °C; $[\alpha]^{25}_D$ +12.6° (c 2.0, EtOH); IR (KBr) 1736, 1695, 1662, 1551, 1521 cm^{-1}; NMR ($CDCl_3$) δ 7.24 (m, 6 H, arom, NH), 5.59 (m, 2 H, NHCHNH, NH), 3.64 (s, 3 H, OCH_3), 3.42 (m, 1 H, $COCHCO_2CH_3$), 3.20 (m, 2 H, $PhCH_2$), 2.73 (br s, 2 H, $-CH_2CO_2C_4H_9$), 1.44, 1.43 [2 s, 18 H, $(CH_3)_3C$]. Anal. ($C_{23}H_{34}N_2O_7$) C, H, N.

***N*-(Benzyloxycarbonyl)-2-aminomalonic Acid Dibenzyl Ester. A. Oximination.** Dibenzyl malonate (10.0 g, 35.2 mmol) was taken up in 1,4-dioxane (100 mL) and treated with a 40% aqueous solution of acetic acid (35 mL), followed by the slow addition (2.5 h) of solid sodium nitrate (10 g). The reaction was stirred for another 2.5 h and extracted into ether (3 × 70 mL). The organic phase was washed with a 1% solution of $NaHCO_3$ until the aqueous layer was slightly acidic (pH 5–6). The ethereal solution was dried over $MgSO_4$ and removed under reduced pressure to give an oil (10.9 g). The crude oxime was carried directly to the next step.

B. Reduction.[33] Amalgamated aluminum (obtained from 1.25 g, 0.463 g atom of aluminum foil) was covered with tetrahydrofuran (28 mL), followed by 1.9 mL of water. The reaction mixture was stirred mechanically and cooled in a dry ice–acetone bath. A solution of the crude oxime (from the previous step) in 30 mL of tetrahydrofuran was added dropwise (20 min) while the temperature was maintained between −15 and −30 °C. The ice bath was removed and a spontaneous reaction occurred, which resulted in a rapid rise in temperature (50 °C). When the evolution of heat ceased, the mixture was refluxed for 1 h, diluted with ether (100 mL), and filtered through Celite. The solvent was removed under reduced pressure to give the crude amine (7.5 g), which was taken to the following step without further purification.

A small sample (0.5 g) of the crude amine was taken up in dry ether (10 mL) and treated with HCl gas at 0 °C. The amine hydrochloride was collected by filtration, washed with ether, dried in vacuo, and recrystallized from MeOH/*i*-Pr_2O: TLC R_f (I) 0.63; mp 143 °C dec. Anal. ($C_{17}H_{18}ClNO_4$) Cl.

C. *N*-Benzyloxycarbonylation. The crude amine (7 g) was dissolved in a saturated solution of $NaHCO_3$ (200 mL) and cooled in an ice bath. Benzyl chloroformate (4.0 g, 23 mmol) was added dropwise (0.5 h) to the vigorously stirred solution. The reaction mixture was left at room temperature for 12 h, during which time the product precipitated. The product was collected by filtration, washed with water, dried in air, and recrystallized from *i*-PrOH: yield 4.8 g (52%), from dibenzyl malonate; TLC R_f (G) 0.68; R_f (I) 0.80; mp 104–106 °C. Anal. ($C_{25}H_{23}NO_6$) C, H, N.

***N*-(Benzyloxycarbonyl)-(*RS*)-2-aminomalonic Acid Benzyl Ester.** *N*-(Benzyloxycarbonyl)-2-aminomalonic acid dibenzyl ester (4.33 g, 10 mmol) was dissolved in acetone/water (4:1, 133 mL). The solution was stirred and lithium hydroxide monohydrate (0.42 g, 10 mmol) in water (11 mL) was added dropwise (1 h). The reaction mixture was stirred for 12 h at room temperature, the acetone was removed under reduced pressure, and the residue was taken up into a saturated solution of $NaHCO_3$ (60 mL) and extracted with EtOAc (3 × 100 mL). The EtOAc washings were combined, dried over $MgSO_4$, and removed under reduced pressure to give a solid, which was crystallized from EtOAc/hexane. This solid was identified as recovered starting material (1.1 g, 25.4%). The aqueous phase was acidified with 3 N HCl to pH ≃1 and extracted with $CHCl_3$ (4 × 50 mL). The combined $CHCl_3$ washings were dried over $MgSO_4$, and the solvent was removed under reduced pressure to give a residue which crystallized from *i*-PrOH: yield 2.0 g (58%); TLC R_f (G) 0.39; mp 114–116 °C. Anal. ($C_{18}H_{17}NO_6$) C, H, N.

***N*-(Benzyloxycarbonyl)-(*RS*)-2-aminomalonic Acid (*tert*-Butyloxycarbonyl)hydrazidyl Benzyl Ester.** *N*-(Benzyloxycarbonyl)-(*RS*)-2-aminomalonic acid monobenzyl ester (0.68 g, 2.0 mmol) and *tert*-butyl carbazate (0.26 g, 2.0 mmol) were dissolved in 10 mL of EtOAc. The solution was cooled in an ice bath with stirring and dicyclohexylcarbodiimide (0.41 g, 2.0 mmol) dissolved in EtOAc (4 mL) was slowly added to it. The reaction was allowed to warm to room temperature and stirred for 12 h. Acetic acid (0.5 mL) was added and the reaction stirred for another 0.5 h. The mixture was filtered, the filtrate was washed successively with 2 N $NaHSO_4$, brine, a saturated solution of $NaHCO_3$, and brine and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was taken up into acetone (30 mL) and cooled to −20 °C for 12 h. Precipitation occurred and the residual urea was removed by filtration. Removal of the acetone under reduced pressure left a residue, which was crystallized fom EtOAc/hexane: yield 0.83 g (91%); TLC R_f (E) 0.46; mp 94 °C; IR (KBr) 1755, 1713, 1696, 1640, 1577, 1542 cm^{-1}; NMR ($CDCl_3$) δ 8.35 (m, 1 H, *NH*NH-Boc), 7.33 (s, 10 H, arom), 6.52 (m, 1 H, NH*NH*-Boc), 6.07 (d, 1 H, *NH*-Z), 5.22, 5.10 (2 s, 4 H, $PhCH_2O$), 5.04 (m, 1 H, COCHCO), 1.44 [s, 9 H, $(CH_3)_3C$]. Anal. ($C_{23}H_{27}N_3O_7$) C, H, N.

Benzyl *N*-(Benzyloxycarbonyl)-*N'*-(*tert*-Butyloxycarbonyl)-α,α-diaminoacetate. A. Acidolysis. *N*-(Benzyloxycarbonyl)-(*RS*)-2-aminomalonic acid (*tert*-butyloxycarbonyl)hydrazidyl benzyl ester (0.83 g, 1.8 mmol) was dissolved in 10 mL of 4 N HCl–dioxane and stirred for 0.5 h at room temperature. Anhydrous ether was added to the reaction mixture to precipitate the product, which was cooled to −10 °C and filtered. The precipitate was washed with ether and dried in vacuo to give 0.66 g (93%) of a white solid: TLC R_f (H) 0.48. The hydrazide hydrochloride was taken to the next step without further purification.

(B) Rearrangement of Hydrazide to Urethane. The hydrazide hydrochloride (0.4 g, 0.1 mmol) was suspended in dry tetrahydrofuran (20 mL) and stirred in an ice bath. To this mixture was added a saturated solution of nitrosyl chloride in tetrahydrofuran until a red color persisted for 5 min. The reaction mixture was passed through a sinter glass funnel, and the filtrate was diluted with cold EtOAc (100 mL) and washed with a saturated solution of $NaHCO_3$ (3 × 25 mL), followed by brine. The solution was dried over $MgSO_4$ and removed under reduced pressure to give a white solid [IR (KBr) 2124 cm^{-1}; $-N_3$]. The azide was taken up into dry toluene (50 mL) and stirred at 80 °C until the rearrangement to the isocyanate was complete, 0.5 h [IR (C_7H_8) 2212 cm^{-1}; −NCO]. Dry *t*-BuOH (2 mL, 20 mmol) was added and the heating was continued for an additional 1 h (complete disappearance of isocyanate). The solvent was removed under reduced pressure and the residue was taken up into EtOAc. The solution was passed through a sinter glass funnel and the solvent was removed under reduced pressure to yield a solid. The residue was chromatographed on a silica gel column (20 × 290 mm) with EtOAc/hexane (1:1). The fast-running material was collected and recrystallized from EtOAc/hexane to give the fully protected α,α-diamino acetic acid derivative: yield 318 mg (40%), from the protected hydrazide; TLC R_f (E) 0.56; mp 129–130 °C; IR (KBr) 1747, 1685, 1521 cm^{-1}; NMR ($CDCl_3$) δ 7.33 (s, 10 H, arom), 6.21 (m, 1 H, NH), 5.94 (m, 1 H, NH), 5.21 (m, 1 H, C_α H), 5.18 (s, 2 H, $PhCH_2O$), 5.11 (s, 2 H, $PhCH_2O$), 1.41 [s, 9 H, $(CH_3)_3C$]. Anal. ($C_{22}H_{26}N_2O_6$) C, H, N.

Benzyl *N*-[(*RS*)-α-(methoxycarbonyl)-β-phenylpropionyl]-*N'*-(benzyloxycarbonyl)-α,α-diaminoacetate (9). A. Acidolysis. The protected α,α-diaminoacetic acid benzyl ester (318 mg, 0.768 mmol) was treated with 10 mL of 4 N HCl in dioxane for 0.5 h. The solvent was removed under reduced pressure at room temperature to give a clear oil, which was dried on a vacuum line for 0.5 h at 0.5 mmHg.

B. Coupling. The deprotected α,α-diamino compound was added to (*RS*)-2-benzylmalonic acid monomethyl ester (160 mg, 0.77 mmol), which was dissolved in CH_2Cl_2 (5 mL). The reaction mixture was cooled in an ice bath and stirred under N_2; triethylamine (107 μL, 0.768 mmol) was added, followed by dicyclohexylcarbodiimide (158 mg, 0.768 mmol). The ice bath was removed, and the mixture was allowed to reach room temperature and was left to stir for 12 h. The urea was removed by filtration and the solid was washed with EtOAc. The filtrate and washings were combined, the solvent was removed under reduced pressure, and the residue was chromatographed on a silica gel column (30 × 540 mm) with EtOAc/hexane (4:6): yield 124 mg (32%), from

(33) This procedure is very similar to that described in "Organic Syntheses" Collect. Vol V, Wiley, New York, 1973, p 32.

the protected α,α-diamino compound; TLC R_f (E) 0.52; mp 145–147 °C; IR (KBr) 1735, 1683, 1647, 1519 cm^{-1}; NMR ($CDCl_3$) δ 7.75 (m, 1 H, NH), 7.31, 7.27, 7.25 (3 m, 15 H, arom), 6.26 (m, 1 H, NH), 5.48 (m, 1 H, NC*H*N), 5.16, 5.07 (2 s, 4 H, PhCH_2O), 3.57, 3.52 (2 s, 3 H, CH_3), 3.51 (m, 1 H, CO*CH*CO), 3.14 (m, 2 H, C_β H_2). Anal. ($C_{28}H_{28}N_2O_7$) C, H, N.

***N*-[(*RS*)-α-(Methoxycarbonyl)-β-phenylpropionyl]-α,α-diaminoacetic Acid (10).** Benzyl *N*-[(*RS*)-α-(methoxycarbonyl)-β-phenylpropionyl]-*N'*-(benzyloxycarbonyl)-α,α-diaminoacetate (27.55 mg, 0.55 mmol) was dissolved in MeOH. To this solution was added Et_3N (600 μL, 0.43 mmol), followed by 100 mg of 10% Pd/C. The hydrogenation was performed at atmospheric pressure with vigorous stirring. After 10 min, the mixture was filtered through a Celite pad and the solvent removed under reduced pressure to yield a glass. The residue was studied in NMR and IR and was tasted: IR (KBr) 1720, 1670, 1643, 1510 cm^{-1}; NMR ($CDCl_3$) δ 8.95 and 8.88 (2 d, 1 H, NH), 7.25 (m, 5 H, arom), 4.71 (2 overlapping d, 1 H, NC*H*N), 3.95 (m, 1 H, CO*CH*CO), 3.54 (s, 3 H, OCH_3), 3.03 (m, 2 H, C_β H_2).

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Peptide Sweeteners. 4. Hydroxy and Methoxy Substitution of the Aromatic Ring in L-Aspartyl-L-phenylalanine Methyl Ester. Structure–Taste Relationships

Masao Kawai,[1] Michael Chorev,[2] Janine Marin-Rose,[3] and Murray Goodman*

Department of Chemistry, University of California, San Diego, La Jolla, California 92093. *Received September 21, 1979*

A series of analogues of the dipeptide sweetener L-aspartyl-L-phenylalanine methyl ester having hydroxy and/or methoxy substitution on the aromatic ring was synthesized and tasted. The introduction of a methoxy group in the para position of the aromatic ring of the peptide sweetener is crucial to the reduction or destruction of the sweet taste. The effects of substituents in the ortho or meta position are not as pronounced. In the case of *o*-methoxy substitution, the resulting analogue is only slightly less sweet than the parent dipeptide sweetener.

Following the accidental discovery[4] that L-aspartyl-L-phenylalanine methyl ester (1) is intensely sweet, several groups have studied the structural requirements necessary for sweet taste. The zwitterionic N-terminal function must possess either a six- or five-membered zwitterionic ring as in L-aspartyl[4] or (*RS*)-α-aminomalonyl[5,6] residues, respectively. The C-terminal residue is more flexible in its structural requirements and can accommodate a large variety of amino acid esters[4–9] and amides.[10]

In order to maximize sweetness, it is necessary to have a certain dissimilarity in size of the hydrophobic groups located on the α carbon of the C-terminal residue. The orientation of these groups should correspond to the configurational arrangement found in L-phenylalanine methyl ester (1) such that the large group corresponds to the benzyl side chain and the small group corresponds to the methoxycarbonyl structure. Our previous studies[11,12] demonstrated the importance of the peptide bond which connects the N-terminal zwitterionic part and the C-terminal hydrophobic portion. Any modification of the peptide bond results in the complete loss of sweetness.

In this paper, we present a study of the effects of hydroxy and methoxy mono- and disubstitution at various positions on the aromatic ring in the dipeptide sweetener 1. Results from these compounds should refine our present understanding of the sweetener's hydrophobic portion.

Synthesis. The general synthetic approach used in this paper was to couple β-benzyl *N*-(benzyloxycarbonyl)-L-aspartate[13] to the appropriate aromatic-substituted phenylalanine methyl esters with either *N,N'*-dicyclohexylcarbodiimide (DCC)[14] or DCC and 1-hydroxybenzotriazole.[15]

The DL-*m*-hydroxyphenylalanine was resolved by fractional crystallization of the brucine salts following the procedure of Sealock.[16] The optical resolution of DL-*o*-hydroxyphenylalanine was performed on the corresponding

(1) Visiting Research Chemist from Mitsubishi-Kasei Institute of Life Sciences, Machida-shi, Tokyo 194, Japan.
(2) Address: Department of Pharmaceutical Chemistry, School of Pharmacy, The Hebrew University, Jerusalem, Israel.
(3) Address: Cetus Corp., Berkeley, Calif. 94710.
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