High-Potency Dipeptide Sweeteners. 2. L-Aspartylfuryl-, Thienyl-, and Imidazolylglycine Esters

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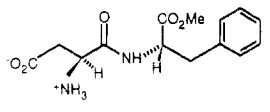
Received October 9, 1989

Eight L-aspartyl dipeptides derived from heterocyclic glycine esters were prepared and evaluated as sweeteners. The fenchyl esters of L-aspartyl-2-furyl-, 2- and 3-thienyl-, and imidazolylglycine were all potently sweet with the D-2-furylglycine (+)-β-fenchyl ester being the most potent at 16 500 times the potency of sucrose. The requirement for a planar heterocyclic group directly attached to the glycine carbon atom was demonstrated by the observation that the tetrahydrofuryl and β-thienylalanine fenchyl esters were not sweet. The heteroaromatic glycine esters join the phenylglycine esters as a novel class of dipeptide sweeteners with very high potency.

The accidental discovery of the sweet dipeptide derivative L-aspartyl-L-phenylalanine methyl ester, now known as aspartame (1), was published 19 years ago.[1] Since that



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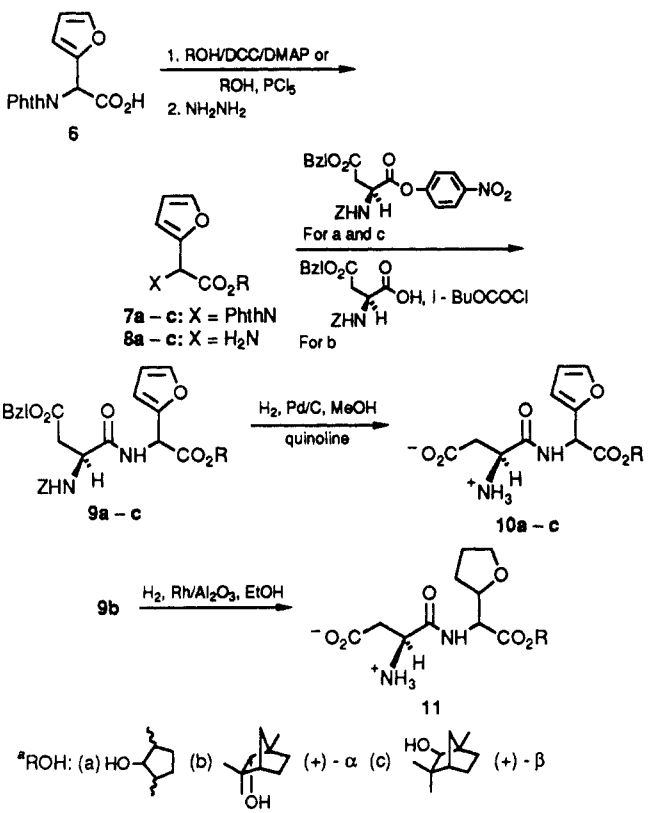
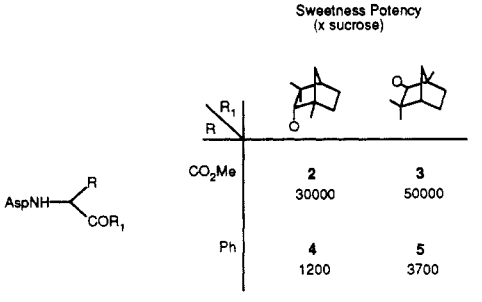
time several hundred analogues have been prepared by a variety of research groups[2] seeking more stable and more potent dipeptides. While the general structural requirements for dipeptide-type sweeteners are now well-known,[3] the preparation of highly potent (>1000 times more potent than sucrose) dipeptide sweeteners remains a major challenge.

Fujino and co-workers[4] discovered the remarkably potent L-aspartyl-D,L-aminomalonic acid diesters. The (−)-α- and (+)-β-fenchyl esters[5] **2** and **3** are 30 000 and 50 000 times as potent as sucrose and remain the most potent dipeptides known to date.[6] These sweeteners are, however, less stable than aspartame, which limits their utility. Nonetheless, they serve as benchmarks for high potency in the dipeptide class. In an earlier publication,[7] we presented our work with analogues of the Fujino sweeteners where the labile carbomethoxy group was replaced by a phenyl. Established structure–activity trends suggest that the position occupied by the carbomethoxy requires a small group for maximal potency. While there was little precedent for a group as large as phenyl in this position, the resulting L-aspartyl-D-phenylglycine esters were quite potent with the (−)-α- and (+)-β-fenchyl esters **4** and **5**

Scheme I[a]



being 1200 and 3700 times as potent as sucrose. This report expands the scope of our original work to include a variety of heteroaromatic glycine esters.[8] The heteroaromatic groups are somewhat smaller than the phenyl group and therefore we anticipated that the resulting sweeteners might be more potent.



Chemistry

The requisite heterocyclic glycines were prepared according to the literature methods described in the Ex-

(1) Mazur, R. H.; Schlatter, J. M.; Goldkamp, A. H. *J. Am. Chem. Soc.* **1969**, *91*, 2684.
(2) For reviews, see: Pavlova, L. A.; Komarova, T. V.; Davidovich, Yu. A.; Rogozhin, S. V. *Russ. Chem. Rev.* **1981**, *50*, 316. Crosby, G. A.; DuBois, G. E.; Wingard, R. E., Jr. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1979; Vol. VIII, p 215. Ariyoshi, Y. *Kagaku Kyoiku* **1983**, *31*, 12.
(3) Ariyoshi, Y. In *Food Taste Chemistry*, Boudreau, J. C., Ed.; ACS Symposium Series 115; American Chemical Society: Washington, DC, 1979; p 133. Ariyoshi, Y. *Agric. Biol. Chem.* **1976**, *40*, 983. Mazur, R. H.; Goldkamp, A. H.; James, P. A.; Schlatter, J. M. *J. Med. Chem.* **1970**, *13*, 1217. Mazur, R. H.; Reuter, J. A.; Swiatek, K. A.; Schlatter, J. M. *J. Med. Chem.* **1973**, *16*, 1284.
(4) (a) Fujino, M.; Wakimasu, M.; Tanaka, K.; Aoki, H.; Nakajima, N. *Naturwissenschaften* **1973**, *60*, 351. (b) Fujino, M.; Wakimasu, M.; Mano, M.; Tanaka, K.; Nakajima, N.; Aoki, H. *Chem. Pharm. Bull.* **1976**, *24*, 2112.
(5) L. Yinzeng; et al. *Med. Ind. (Yiyao Gongyi)* **1980**, *10*, 11.
(6) This analysis excludes aspartyl modified dipeptides which can have potencies >1000 times that of sucrose. See, for example: Nofre, C.; Tinti, J. M. U. S. Patent 4,645,678, 1987. Nofre, C.; Tinti, J. M. U. S. Patent 4,673, 582, 1987.
(7) Janusz, J. M.; Gardlik, J. M.; Young, P. A.; Burkes, R. V.; Stoll, S. J.; Estelle, A. F.; Riley, C. M. *J. Med. Chem.* **1990**, *33*, 1052.
(8) Blum, R. B.; Gardlik, J. M.; Janusz, J. M.; Rizzi, G. P. U. S. Patent 4,692,513, 1987.

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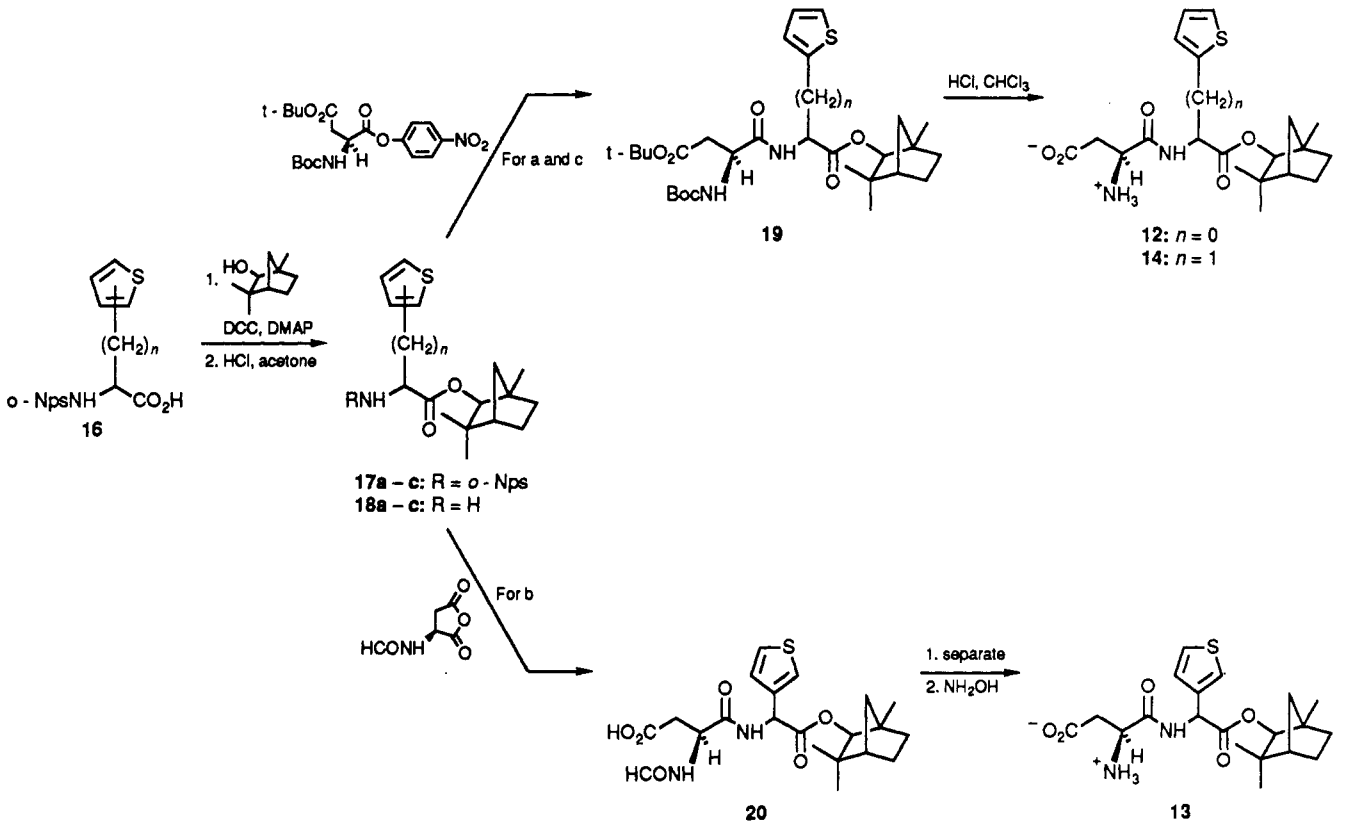
Scheme II[a]



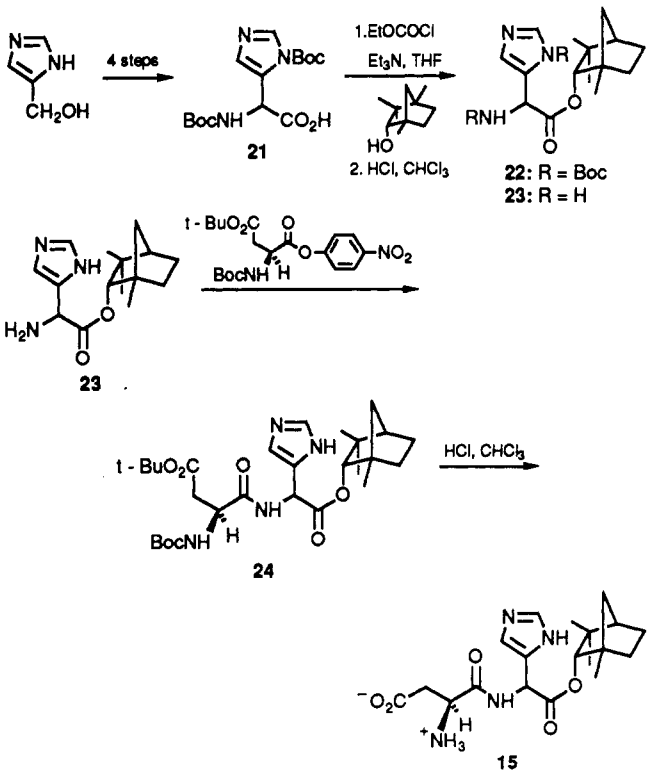
[a] (a) 2-thienyl, n = 0, (b) 3-thienyl, n = 0, (c) 2-thienylmethyl, n = 1.

perimental Section. The synthesis of the 2-furylglycine sweeteners is given in Scheme I. Protection as the phthalimide derivative[9] **6** allowed the *N,N'*-dicyclohexylcarbodiimide/4-(dimethylamino)pyridine-promoted coupling[10] of 2,5-dimethylcyclopentanol and (+)-β-fenchol to give **7a** and **7c**. (+)-α-Fenchyl compound **7b** was prepared via the acid chloride. Free amines **8a–c** were obtained by hydrazinolysis. Coupling with *N*-(benzyloxycarbonyl)-β-benzyl-L-aspartic acid *p*-nitrophenyl ester[1] gave diprotected sweeteners **9a** and **9c**. (+)-α-Fenchyl compound **9b** was prepared via the mixed anhydride. Hydrogenolysis with palladium on charcoal containing a trace of quinoline[11] gave free sweeteners **10a–c** as mixtures of diastereomers. In the case of **10c**, the diastereomers were separated by semipreparative HPLC.

2-Tetrahydrofurylglycine dipeptide **11** was prepared by hydrogenation of **9b** over 5% rhodium on alumina,[12] which afforded furan reduction as well as deprotection.

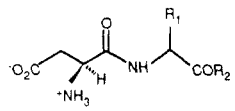
The synthesis of the thienylglycine sweeteners is given in Scheme II. *o*-Nitrophenylsulfenyl protection[13] to **16a–c**, DCC/DMAP coupling to **17a–c**, and acid hydrolysis gave the thienylglycine amines **18a–c**. D,L-2-Thienyl isomer **18a** was coupled by using *N*-(*tert*-butoxycarbonyl)-β-*tert*-butyl-L-aspartic acid *p*-nitrophenyl ester. Acid hydrolysis of **19** gave sweetener **12**. The same scheme was used to prepare β-2-thienylalanine compound **14**. D,L-3-Thienyl isomer **18b** was coupled by using *N*-formyl-L-aspartic anhydride[14] to give a mixture of α- and β-coupled products. α-Isomer **20** was readily separated by silica gel chroma-

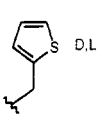
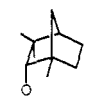
Scheme III



(9) Nefkens, G. H. L.; Tesser, G. I.; Nivard, R. J. F. *Recl. Trav. Chim. Pays—Bas* **1960**, *79*, 688. Nefkens, G. H. L. *Nature (London)* **1960**, *185*, 309.
(10) Mengel, R.; Steglich, W. *Angew. Chem.* **1978**, *90*, 556.
(11) Pesson, M.; Dupin, S.; Antoine, M.; Humbert, D.; Joannic, M. *Bull. Soc. Chim. Fr.* **1965**, 2262.
(12) Bissell, E. R.; Finger, M. *J. Org. Chem.* **1959**, *24*, 1259.
(13) Zervas, L.; Borovas, D.; Gazis, E. *J. Am. Chem. Soc.* **1963**, *85*, 3660.
(14) Bachman, G. L.; Oftedahl, M. L.; Vineyard, B. D. U. S. Patent 3,933,781, 1976.

Table I. L-Aspartyl Heterocyclicglycine Esters



cmpd	R_1	R_2	potency[a]
10a	 D,L		400
10b	 D,L	 (+)-α	630 ± 190
10c	 D	 (+)-β	16500 ± 3200
11	 D,L	 (+)-α	0
12	 D	 (+)-β	2000 ± 1300
13	 D	 (+)-β	2300 ± 370
14	 D,L	 (+)-β	0
15	 D,L	 (-)-α	350 ± 90

[a] Sweetness potency vs 5–10% sucrose. Data are means ± the 95% confidence limit. See the Experimental Section for details.

tography and converted with hydroxylamine to free sweetener **13**. The diastereomers of **12** and **13** were separated by semipreparative HPLC.

The synthesis of the imidazolylglycine sweetener is given in Scheme III. The protected N^{α},N^{Im}-diBoc-imidazolylglycine derivative **21** was prepared by modification of a published procedure for the N^{α}-Boc derivative.[15,16] Mixed anhydride coupling with (−)-α-fenchol gave Boc-amino ester **22** in low yield (25–30%). A major side product was the ethyl ester formed by the reaction of ethanol released from the mixed anhydride upon reaction with fenchol. This severe limitation in the use of mixed anhydrides for ester formation is not a problem in amide formation[17] where the nucleophilicity of the amine exceeds that of the ethanol released. Alternative coupling procedures (DCC/DMAP/THF; DCC/HOBT/DMF; carbonyldiimidazole/THF; isobutyl chloroformate/Et_3N/THF) also gave low yields. Acid hydrolysis gave amine **23**. Coupling with *N*-(*tert*-butoxycarbonyl)-β-*tert*-butyl-L-aspartic acid *p*-nitrophenyl ester to **24** followed by acid hydrolysis gave sweetener **15**.

(15) Van Batenburg, O. D.; Kerling, K. E. T.; Havinga, E. *FEBS Lett.* **1976**, *68*, 228.
(16) Van Batenburg, O. D.; Kerling, K. E. T. *Int. J. Pept. Protein Res.* **1976**, *8*, 1.
(17) Boissonnas, R. A. *Helv. Chim. Acta* **1951**, *34*, 874. Vaughan, J. R., Jr. *J. Am. Chem. Soc.* **1951**, *73*, 3547. Wieland, T.; Bernhard, H. *Ann. Chem.* **1951**, *572*, 190. Vaughan, J. R., Jr.; Osato, R. L. *J. Am. Chem. Soc.* **1952**, *74*, 676.

Results and Discussion

Table I gives the structures and sweetness potencies for the dipeptide esters. For most of our work, fenchyl esters were chosen because they proved to be the most potent for the related dipeptides **2**–**5** mentioned above.

Alicyclic 2-furylglycine 2,5-dimethylcyclopentyl ester **10a** had modest potency at 400 times that of sucrose (400×). An increase in potency was observed for (+)-α-fenchyl sweetener **10b** (630×), extending the precedent for higher potency for esters derived from appropriately substituted bicyclic versus monocyclic alcohols. Extremely high potency was observed for (+)-β-fenchyl isomer **10c** (16 500×). The relative potency of the two fenchyl sweeteners is the same as that observed for the phenylglycine ester[7] and aminomalonic acid diester series.[5] To the best of our knowledge, furylglycine sweetener **10c** is the third most potent dipeptide known, with only esters **2** and **3** being more potent.[4,5] The related 2- and 3-thienylglycine compounds **12** and **13** had nearly identical potencies (2000× and 2300×).

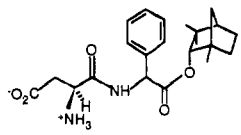
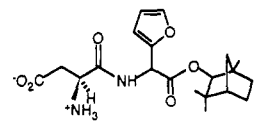
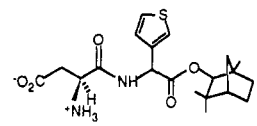
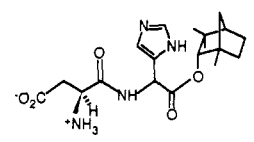
Imidazolylglycine sweetener **15** was of particular interest. While the furyl and thienylglycine sweeteners showed that heterocycles can function in place of the phenyl group of **4** and **5**, both are quite hydrophobic groups. The imidazolyl group by contrast is relatively hydrophilic. Nonetheless, sweetener **15** was quite potently sweet, which suggests more latitude in the hydrophilicity of the upper group than previously believed. On the basis of the fact that **15** was tasted as a mixture of diastereomers, and extrapolating the 3-fold increase in potency observed for the (+)-β versus (−)-α-fenchyl ester in the phenylglycine series, we estimate that the potency of the D-imidazolylglycine (+)-β-fenchyl ester should be approximately 2000× or about the same as the thienylglycine sweeteners.

The importance of the planarity of the heteroatomic groups is clearly illustrated by tetrahydrofuryl compound **11**. Reduction of the furyl sweetener **10b** (630×) to **11** results in a complete loss of sweetness. In the thienyl series, insertion of a methylene group between the glycine α-carbon and the thienyl ring in the β-thienylalanine compound **14** eliminated the sweetness of 2-thienyl dipeptide **12** (2000×). The extra volume occupied by the tetrahydrofuryl and β-thienylalanine groups may prevent access to the sweet receptor. The planarity of the furyl, thienyl, and imidazolyl groups is evidently critical for sweetness.

The taste quality of these sweeteners is generally quite good, approaching that of aspartame. For example, for **10c** the ratio of sweet/bitter/other is 86/9/5 versus >95/<5/0 for aspartame. However, the taste quality of many of our sweeteners suffers from a delayed time–intensity profile (TIP). Typically, the onset of sweetness is slightly delayed and the sweet taste persists longer than that of sucrose. The aftertaste was often described with terms such as cloying, licorice-like, or rock candy-like. We quantitatively evaluated the TIP's using a computer-aided cross modal matching technique where sweet-taste intensity was matched with sound intensity.[18] A useful way to compare the lingering of a series of sweeteners is to compare their $t_{1/2}$'s, the time for the sweetness to descend to half of maximum. These data are presented in Table II. The phenyl-, furyl-, and thienylglycine sweeteners all linger more than sucrose but much less than the protein sweetener Talin (thaumatin). In contrast, imidazolylglycine sweetener **15** has a $t_{1/2}$ identical with that of sucrose. In our earlier publication on L-aspartyl-D-phenylglycine es-

(18) Moese, S. A.; Bunger, J. R., unpublished results.

Table II. Time-Intensity Data

sweeteners	$t_{1/2}$,[a] s
sucrose	14.5[b]
	26.5
 10c	45.0
 13	44.5
 15	14.2
Talin	>50

[a] $t_{1/2}$ is the time for the sweetness intensity to decline to half maximal. [b] Sucrose ranged from 14 to 20 s over several panels.

ters,[7] we noted that the TIP appeared to be qualitatively related to the hydrophilicity of the ester group. Increasing the hydrophilicity of this group improved the TIP to more closely approximate that of sucrose. The sucrose-like TIP for **15** is consistent with this observation. However, the furyl- and thienylglycine sweeteners are more persistent than the corresponding phenylglycine sweetener shown in Table II for reasons which are not apparent.

In summary, the L-aspartyl heteroaromatic glycine esters are among the most potently sweet dipeptides known. The planarity of the heteroaromatic groups is crucial for sweetness. Their two-dimensional nature allows them to interact with the sweet receptor despite their large size. Evidently, the receptor site which interacts with the heteroaromatic group is a narrow cleft which can accommodate relatively large planar groups (e.g. phenyl, furyl) but only relatively small nonplanar groups.

Experimental Section

Melting points were obtained with a Thomas-Hoover melting point apparatus or hot-stage microscope and are uncorrected. Optical rotations were obtained at room temperature in a 1-dm cell with a Rudolph Autopol III polarimeter. Infrared spectra were obtained with a Perkin-Elmer Model 298 infrared spectrophotometer. ^{1}H NMR spectra were obtained with a Varian T-60, a JEOL FX-90Q or FX-270, or a General Electric GN-500 spectrometer with tetramethylsilane as an internal standard. Mass spectra were obtained on a Hewlett-Packard 5985B GC/MS system. High-resolution mass determinations were done with a ZAB-2F mass spectrometer in the peak-matching mode with nominal resolution of 10 000. GC analyses were obtained with a Hewlett-Packard 5830A gas chromatograph equipped with a 30-m J&W DB-1 capillary column. All TLC analyses were obtained in the indicated solvent systems with Analtech Uniplates, silica gel GF; the plates were visualized by dipping them in either a 5% solution of phosphomolybdic acid in 2-propanol or by spraying with a 0.5% solution of ninhydrin in 2-propanol and heating them on a hot plate until no further spots appeared. Flash chromatography was carried out according to the method of Still.[19] HPLC analyses were obtained with the indicated columns using a modular isocratic system consisting of an Altex Model 110A or Rainin Rabbit pump with a variable-wavelength UV detector (Isco Model 1840 or V^4 used at 210 or 254 nm). Mobile phases for all reverse-phase analyses were prepared by diluting the appropriate amount of a concentrated aqueous buffer solution (0.5 M Na_2HPO_4, pH adjusted to 6 with phosphoric acid) with the proper amount of water and methanol or acetonitrile needed to produce a final Na_2HPO_4 concentration of 0.01 M. Preparative HPLC was carried out with the same system with a Whatman Magnum 9 ODS-3 column while the eluent was monitored with a Knauer refractive index detector. The solvents were prepared as described for analytical HPLC above except that NH_4OAc was used as a volatile buffer.

Compounds were isolated by freeze-drying. Solvents for TLC and HPLC: A, 4/1/1 *n*-BuOH/HOAc/H_2O; B, 23/75/1/2 MeOH/$CHCl_3$/HOAc/H_2O; C, 55/45 MeOH/phosphate buffer; D, 60/40 MeOH/phosphate buffer; E, 65/35 MeOH/phosphate buffer; F, 65/35 MeOH/NH_4OAc buffer.

Dry THF and dry ether were obtained by distillation from sodium/benzophenone under argon immediately before use. Dry dichloromethane was obtained by distillation from calcium hydride.

Starting Materials. Alcohols. 2,5-Dimethylcyclopentanol was prepared by lithium aluminum hydride reduction (0 °C) of 2,5-dimethylcyclopentanone (Aldrich). (−)-α-Fenchol [[α]$_D$ −12.4° (*c* 3.2, EtOH) [lit.[20] [α]$_D$ −12.7° (*c* 3.0, 95% EtOH)]] was prepared from (+)-fenchone (Fluka) [[α]$_D$ +65.5° (c 5.0, EtOH)] in the same way. (+)-α-Fenchol was from Aldrich or Pfaltz & Bauer as a 94/6 α/β mixture [[α]$_D$ +10.7° (*c* 1.0, EtOH) [lit.[20] [α]$_D$ +10.5°, EtOH]]. (+)-β-fenchol was prepared by the reduction of (−)-fenchone with aluminum isopropoxide[20] or copper chromite/H_2[21] to give a 97/3 β/α ratio after silica gel chromatography as previously described in detail.[7]

Amino Acids and Derivatives. The following amino acids were commercially available: D,L-α-amino-2-thiopheneacetic acid (D,L-2-thienylglycine) and D,L-α-amino-3-thiopheneacetic acid (D,L-3-thienylglycine) were from Aldrich; β-(2-thienyl)-D,L-alanine was from Sigma. D,L-2-Furylglycine[22] was prepared from furfural via the hydantoin. D,L-Imidazoylglycine was prepared from 4-(hydroxymethyl)imidazole hydrochloride (Sigma) by oxidation with nitric acid to the aldehyde,[23] conversion to the hydantoin, and acid hydrolysis.[24] *N*-(*tert*-Butoxycarbonyl)-β-*tert*-butyl-L-aspartic acid *p*-nitrophenyl ester and *N*-(benzyloxycarbonyl)-β-benzyl-L-aspartic acid were from BaChem. The latter was recrystallized from benzene or acetic acid/water before use. *N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartic acid *p*-nitrophenyl ester was prepared by DCC coupling of the corresponding aspartic acid with *p*-nitrophenol. *N*-Formyl-L-aspartic anhydride was prepared from L-aspartic acid, formic acid, and acetic anhydride.[14]

Taste Panels. Before paneling, each sweetener was submitted for acute toxicity testing in the rat. A single oral dose of >100 times the intended human exposure, calculated on a mg/kg basis, was given. The animals were observed for two weeks for gross signs of toxicity. At the end of this period, the animals were sacrificed and necropsied. Sweeteners showing no evidence of acute toxicity were paneled.

The potencies of the aspartic acid amides were determined by taste comparisons with sucrose standards. Expert male panelists (5–10) were asked to taste and spit 10 mL of a sweetener solution and to rate the sweetness intensity and quality versus five standard sucrose solutions ranging from 0.04 M (1.4%) to 0.35 M (12.0%). The concentration of the test sweetener was generally in the range of 5–10% sucrose equivalency. The exact sucrose equivalency value is given for each sweetener following the spectral data. The ratio of the sucrose concentration perceived as equally sweet to the actual concentration of the test sweetener is the potency quoted in this report. The data are presented as the mean ± the 95% confidence limit. Informal tastings with two or three panelists were done in the same way when there was insufficient

(19) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(20) Coulombeau, A.; Rassat, A. *Bull. Soc. Chim. Fr.* **1965**, 3338.
(21) Doering, W. v. E.; Aschner, T. C. *J. Am. Chem. Soc.* **1949**, *71*, 838.
(22) Ciocca, B.; Rovesti, P.; Roccheggiani, G. *J. Soc. Cosmet. Chem.* **1959**, *10*, 77.
(23) Pyman, F. L. *J. Chem. Soc.* **1916**, 186.
(24) Schneider, F. *Z. Physiol. Chem.* **1961**, *3*, 206.

sample for a larger panel. In these cases, the potency is an estimate and is given as the mean.

Time-intensity profiling (TIP) was done using a computer-aided cross modal matching technique. Panelists described the time course of the sweet taste by matching sweetness intensity to sound intensity which they heard through earphones. Panelists controlled the intensity of a 1000-Hz sound between 0 and 90 dB by spinning a wheel on a sound-controller box. The controller delivers a digital signal to an Apple II personal computer (at up to 10 times per s) and the data is stored and then plotted. Further details will be reported separately.

Route to L-Aspartyl-D,L-2-furylglycine 2,5-Dimethylcyclopentyl Ester (10a). *N*-Phthaloyl-D,L-2-furylglycine (6). D,L-2-Furylglycine (4.5 g, 31.9 mmol) and Na_2CO_3 (4.2 g, 34 mmol) were dissolved in 45 mL of H_2O. *N*-(Ethoxycarbonyl)phthalimide (7.0 g, 32 mmol) was added and the reaction was stirred for 90 min and then was filtered. The filtrate was cooled in ice and brought to pH 3 with 6 N HCl. The precipitated product was filtered, washed twice with H_2O, and dried in a vacuum desiccator overnight. The crude material was recrystallized from EtOAc/hexane to give 6.8 g (78%) of 6.

***N*-Phthaloyl-D,L-2-furylglycine 2,5-Dimethylcyclopentyl Ester (7a).** Phthaloyl-D,L-2-furylglycine 6 (1.5 g, 5.5 mmol) and 2,5-dimethylcyclopentanol (0.65 g, 5.7 mmol) were dissolved in 50 mL of CH_2Cl_2 and 10 mL of THF and cooled to −65 °C. Dicyclohexylcarbodiimide (1.41 g, 6.8 mmol) and 4-(dimethylamino)pyridine (28 mg, 0.2 mmol) were added, and the mixture was stirred for 20 min then warmed to −23 °C for 3 h. The reaction mixture was filtered and the filtrate was washed with 0.1 N HCl, 2% $NaHCO_3$, H_2O, brine, then dried over $MgSO_4$, filtered, and evaporated. Purification by silica gel flash chromatography with 97% toluene/EtOAc gave 1.7 g (84%) of 7a.

D,L-2-Furylglycine 2,5-Dimethylcyclopentyl Ester (8a). N-Protected furylglycine ester 7a (1.5 g, 4.1 mmol) and hydrazine hydrate (0.26 mL, 5.4 mmol) in 30 mL of EtOH were refluxed for 2.5 h. The reaction was cooled, HOAc (1 mL) was added, the mixture was filtered, and the solvent was evaporated. The residue was dissolved in 1 N HCl and extracted with ether. The aqueous layer was made basic (pH 10) with 5 N NaOH and then extracted three times with ether. The combined extracts were washed with H_2O, dried over Na_2SO_4, and evaporated to give 1.0 g (quant.) of crude 8a.

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-D,L-2-furylglycine 2,5-Dimethylcyclopentyl Ester (9a).** *N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartic acid *p*-nitrophenyl ester (2.26 g, 4.7 mmol) and crude furylglycine dimethylcyclopentyl ester 8a (1.0 g, 4.2 mmol) in 50 mL of dry THF were stirred at 0 °C for 1 h and at room temperature overnight. The solvent was evaporated and the residue was dissolved in EtOAc. The organic solution was washed with H_2O, cold 4% Na_2CO_3, H_2O, and brine, dried over $MgSO_4$, and evaporated. Purification by silica gel flash chromatography with 20% EtOAc/hexane provided 2.0 g (83%) of 9a: $[\alpha]_D$ −5.7° (c 0.5, MeOH).

L-Aspartyl-D,L-2-furylglycine 2,5-Dimethylcyclopentyl Ester (10a). Diprotected aspartylfurylglycine dimethylcyclopentyl ester 9a (380 mg, 0.7 mmol) in 25 mL of MeOH was hydrogenated in a Parr hydrogenation apparatus for 3 h at 50 psi over 5% palladium on charcoal (80 mg) poisoned with quinoline (4 mg).[11] Filtration and evaporation of the solvent followed by silica gel flash chromatography with 20/1/1 EtOH/H_2O/HOAc gave 185 mg (80%) of 10a: mp 85–86 °C; $[\alpha]_D$ −1.7° (c 0.1, MeOH); TLC R_f = 0.66 (solvent A), 0.25 (solvent B); HPLC t_r = 12.9 (45%), 14.0 min (55%) (Whatman C_8, solvent C at 0.9 mL/min, 210 nm); IR (KBr) 3700–2500, 2960, 2865, 1725, 1665, 1570 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 0.64–2.30 (m, 12 H, dimethylcyclopentyl, d at 0.66, 0.68, 0.93, 1.01 J = 7.0 Hz), 2.43–2.90 (m, 2 H, Asp CH_2), 4.05–4.22 (m, 1 H, Asp CH), 4.40–4.80 (m, 1 H, CO_2CH), 5.63 and 5.66 (s, 1 H, C_4H_3OCH), 6.42 (m, 2 H, furyl, H_3 and H_4), 7.50 (m, 1 H, furyl, H_5); MS (FAB) m/z 353 $(MH)^+$, 257 $(MH - C_7H_{12})^+$; HRMS (FAB, MH^+) calcd for $C_{17}H_{25}N_2O_6$ 353.1713, found 353.1701; sweetness potency 400 (equivalent to 8% sucrose, informal tasting).

Route to L-Aspartyl-D,L-2-furylglycine (+)-α-Fenchyl Ester (10b). *N*-Phthaloyl-D,L-2-furylglycine (+)-α-Fenchyl Ester (7b). Protected furylglycine 6 (13.0 g, 48 mmol) was added to 45 mL of freshly distilled thionyl chloride and the mixture was refluxed for 1 h. Excess thionyl chloride was distilled off and the remaining solid was taken up in 40 mL of THF. The acid chloride was added to a mixture of (+)-α-fenchol (41.5 g, 270 mmol) in 40 mL of ether and 92 mL of pyridine at 0 °C. The reaction was stirred overnight at room temperature and then filtered. The filtrate was washed with 10% citric acid, 4% $NaHCO_3$, and brine, then dried over Na_2SO_4, filtered, and evaporated to give 14.8 g of crude product. Silica gel column chromatography with 97/3 toluene/EtOAc gave 11.1 g (57%) of 7b: mp 105–107.5 °C; $[\alpha]_D$ 14.3° (c 0.6, DMF).

D,L-2-Furylglycine (+)-α-Fenchyl Ester (8b). Protected furylglycine 7b (10.6 g, 26 mmol) and hydrazine hydrate (1.6 mL, 33.9 mmol) in 175 mL of EtOH were reacted as described for 8a to give 6.0 g (84%) of 8b: $[\alpha]_D$ 20.5° (c 0.9, DMF).

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-D,L-2-furylglycine (+)-α-Fenchyl Ester (9b).** *N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartic acid (2.42 g, 6.8 mmol) in 50 mL of THF was cooled to −15 °C, *N*-methylmorpholine (0.78 mL, 7.1 mmol) was added, and the mixture was stirred for 10 min. Isobutyl chloroformate (0.92 mL, 7.13 mmol) in 3 mL of THF was added dropwise and the reaction was stirred for 50 min. Furylglycine fenchyl ester 8b (2.0 g, 7.2 mmol) in 6 mL of THF was added dropwise and the reaction was stirred for 1 h and then allowed to warm to room temperature and stirred for an additional 30 min. The THF was evaporated and the solid was dissolved in 100 mL of EtOAc. The organic solution was washed with 1 N HCl, saturated Na_2CO_3, and brine, dried over Na_2SO_4, filtered, and evaporated to give 4.26 g of crude product. Silica gel chromatography with 97/3 $CHCl_3$/EtOAc gave 3.4 g (76%) of 9b.

L-Aspartyl-D,L-2-furylglycine (+)-α-Fenchyl Ester (10b). Diprotected aspartylfurylglycine 9b (0.69 g, 1.12 mmol) was hydrogenolyzed as described for 10a. The crude product was recrystallized from ether/hexane to give 0.28 g (64%) of 10b: mp 153–155 °C; $[\alpha]_D$ 1.7° (c 0.6, DMF); IR ($CHCl_3$) 3200, 1737, 1680, 1550, 1380 cm^{-1}; ^1H NMR (CD_3OD) δ 0.50 (s, 3 H, CH_3), 1.00 (s, 3 H, CH_3), 1.10 (s, 3 H, CH_3), 1.17–2.00 (m, 7 H, fenchyl), 2.67 (m, 2 H, Asp CH_2), 4.27 (m, 1 H, Asp CH), 4.43 (s, 1 H, CO_2CH), 5.77 (s, 1 H, C_4H_3OCH), 6.50 (s, 2 H, furyl), 7.53 (s, 1 H, furyl); sweetness potency 630 ± 190 (equivalent to 6% sucrose).

Route to L-Aspartyl-D-2-furylglycine (+)-β-Fenchyl Ester (10c). *N*-Phthaloyl-D,L-2-furylglycine (+)-β-Fenchyl Ester (7c). *N*-Phthaloyl-D,L-2-furylglycine 6 (6.0 g, 22.1 mmol), (+)-β-fenchol (3.41 g, 22.1 mmol), dicyclohexylcarbodiimide (5.90 g, 28.6 mmol), and 4-(dimethylamino)pyridine (110 mg, 0.9 mmol) in 50 mL of CH_2Cl_2/15 mL of THF were reacted as described for 7a. The crude product was purified by silica gel flash chromatography with 97% toluene/EtOAc to give 4.2 g (47%) of 7c: $[\alpha]_D$ −17.2° (c 0.5, DMF).

D,L-2-Furylglycine (+)-β-Fenchyl Ester (8c). N-Protected furylglycine fenchyl ester 7c (3.0 g, 7.4 mmol) and hydrazine hydrate (0.46 mL, 9.4 mmol) in 50 mL of EtOH were reacted as described for 8a to give 2.0 g (98%) of 8c.

***N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-D,L-2-furylglycine (+)-β-Fenchyl Ester (9c).** *N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartic acid *p*-nitrophenyl ester (3.0 g, 6.3 mmol) and crude furylglycine fenchyl ester 8c (1.8 g, 6.5 mmol) in 75 mL of dry THF were reacted as described for 9a. Purification by silica gel flash chromatography with 35% acetone/hexane gave 3.5 g (90%) of 9c: $[\alpha]_D$ −16.5° (c 0.5, MeOH).

L-Aspartyl-D-2-furylglycine (+)-β-Fenchyl Ester (10c). Diprotected aspartylfurylglycine fenchyl ester 9c (700 mg, 1.1 mmol) was hydrogenolyzed as described for 10a to give 402 mg (93%) of 10c. A 100-mg portion of the diastereomeric mixture was separated by semipreparative HPLC with a Whatman Magnum 9 column, Partisil 10, ODS-3, solvent F. The fractions containing the later-eluting isomer were combined and freeze-dried to give 28 mg of the sweet D-furyl isomer: mp 113 °C; $[\alpha]_D$ −63.2° (c 0.2, MeOH); TLC R_f = 0.71 (solvent A), 0.42 (solvent B); HPLC t_r = 16.8 (6%), 19.0 min (94%) (Altex Ultrasphere ODS, solvent E at 1 mL/min, 210 nm); IR (KBr) 3700–2300 (br), 2940, 1735, 1685 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 0.72 (s, 3 H, CH_3), 0.89 (s, 3 H, CH_3), 1.04 (s, 3 H, CH_3), 0.80–1.85 (m, 7 H, fenchyl), 2.39–2.75 (m, 2 H, AB part of Asp ABX, J_{AB} = 16.8 Hz, J_{AX} = 4.4 Hz, J_{BX} = 9.5 Hz, Asp CH_2), 4.08–4.24 (m, 2 H, CO_2CH, Asp CH), 5.67 (s, 1 H, C_4H_3OCH), 6.41–6.45 (m, 2 H, furyl H_3, H_4), 7.49 (s, 1 H, furyl H_5); MS (FAB) m/z 393 $(MH)^+$, 257 (MH −

$C_{10}H_{16})^+$, 137 $(C_{10}H_{17})^+$; HRMS (FAB, MH^+) calcd for $C_{20}H_{29}N_2O_6$ 393.2026, found 393.2033; sweetness potency 16500 ± 3200 (equivalent to 10% sucrose).

L-Aspartyl-D,L-2-tetrahydrofurylglycine (+)-α-Fenchyl Ester (11). Diprotected aspartylfurylglycine (+)-α-fenchyl ester **9b** (270 mg, 0.44 mmol) in 20 mL of EtOH containing 5 drops of glacial HOAc was hydrogenated in a Parr apparatus at 48 psi over 5% rhodium on alumina for 4.5 h. The catalyst was filtered off and the solvent was evaporated. The residue was purified by reverse-phase MPLC with a Lobar LiChroprep RP-8 column with 25% H_2O/MeOH followed by a silica gel column with 75/20/1/2 $CHCl_3$/MeOH/HOAc/H_2O. The resulting product was taken up in $CHCl_3$ and filtered to remove any silica gel, resulting in 93 mg (53%) of 11: mp 143–147 °C; $[\alpha]_D$ 22.6° (*c* 0.5, $CHCl_3$); TLC R_f = 0.64 (solvent A), 0.27 (solvent B); HPLC t_r = 23.2 (46%), 25.6 min (54%) (Whatman ODS-3, solvent D at 1 mL/min, 210 nm); IR (Nujol) 3700–2300, 1735, 1675, 1570 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 0.80 (s, CH_3), 0.81 (s, CH_3), 1.04 (s, 3 H, CH_3), 1.08 (s, 3 H, CH_3), 1.10–2.00 (m, 11 H, fenchyl and CH_2CH_2 of THF), 2.47–2.80 (m, 2 H, Asp CH_2), 3.71–4.24 (m, 3 H, THF $CHOCH_2$), 4.39 (d, 1 H, *J* = 2.2 Hz, CO_2CH), 4.58–4.71 (m, 1 H, C_4H_3O-C*H*); Aspartyl CH obscured by solvent peak; HRMS (FAB, MH^+) calcd for $C_{20}H_{33}N_2O_6$ 397.2239, found 397.2332; sweetness potency 0.

Route to L-Aspartyl-D-2-thienylglycine (+)-β-Fenchyl Ester (12). *N*-[(*o*-Nitrophenyl)sulfenyl]-D,L-2-thienylglycine (16a). D,L-2-Thienylglycine (9 g, 57.3 mmol) was added to 12 mL of 5 N NaOH and 40 mL of dioxane. *o*-Nitrophenylsulfenyl chloride (10.9 g, 58 mmol) was added in small portions over 10 min with the simultaneous addition of about 12 mL of 5 N NaOH to keep the pH basic. The reaction was stirred for 2 h and then diluted with 100 mL of H_2O. The mixture was filtered; the filtrate was acidified with H_2SO_4 and extracted three times with ether. The combined extracts were washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. Recrystallization of the crude product from EtOAc/hexane gave 11.4 g (64%) of **16a**: mp 148 °C dec.

***N*-[(*o*-Nitrophenyl)sulfenyl]-D,L-2-thienylglycine (+)-β-Fenchyl Ester (17a).** Crude, N-protected thienylglycine **16a** (7 g, 22.7 mmol), (+)-β-fenchol (3.5 g, 22.7 mmol), dicyclohexylcarbodiimide (5.62 g, 27.3 mmol), and 4-(dimethylamino)-pyridine (110 mg, 0.9 mmol) in 70 mL of dry THF were combined as described for **7a** and allowed to stir overnight at room temperature. Purification by silica gel flash chromatography with 60% $CHCl_3$/hexane gave 3.8 g (38%) of **17a**.

D,L-Thienylglycine (+)-β-Fenchyl Ester (18a). N-Protected thienylglycine fenchyl ester **17a** (3.8 g, 8.5 mmol) and 2.0 mL of 5 N HCl in 30 mL of acetone were stirred at 0 °C for 30 min and the solvent was evaporated. The residue was dissolved in 0.1 N HCl and extracted with ether to remove nonbasic impurities. The pH was adjusted to 10 with 1 N NaOH and the solution was extracted three times with EtOAc. The combined extracts were washed with H_2O and brine, dried over $MgSO_4$, filtered, and evaporated to give 1.2 g (48%) of **18a**.

***N*-(*tert*-Butoxycarbonyl)-β-*tert*-butyl-L-aspartyl-D,L-2-thienylglycine (+)-β-Fenchyl Ester (19a).** Thienylglycine ester **18a** (250 mg, 0.9 mmol) in 20 mL of dry THF was cooled to 0 °C and *N*-(*tert*-butoxycarbonyl)-β-*tert*-butyl-L-aspartic acid *p*-nitrophenyl ester (428 mg, 1.0 mmol) in 5 mL of THF was added. The reaction was stirred for 3 days at room temperature. The solvent was evaporated and the residue was taken up in ether. The ether was washed several times with 4% Na_2CO_3 then H_2O and brine. Drying with $MgSO_4$, filtration, and evaporation gave 630 mg of crude material, which was purified by two silica gel flash chromatographies with 20% EtOAc/hexane then 15% acetone/hexane to give 216 mg (43%) of **19a**: $[\alpha]_D$ −26.1° (*c* 0.3, MeOH).

L-Aspartyl-D-2-thienylglycine (+)-β-Fenchyl Ester (12). Diprotected aspartylthienylglycine fenchyl ester **19a** (216 mg, 0.38 mmol) was dissolved in 25 mL of $CHCl_3$ and HCl gas was bubbled through the solution for 15 min. After stirring for 2 h, the solvent was evaporated to give 140 mg of crude product. This diastereomeric mixture was separated by semipreparative HPLC as described for **10c**. The nonsweet L-2-thienyl isomer elutes first and the sweet D-2-thienyl isomer elutes second. The product was isolated by freeze-drying the fractions collected to give 43 mg (50%) of the sweet isomer: mp 179–180 °C; $[\alpha]_D$ −55.4° (*c* 0.1, MeOH); TLC R_f = 0.71 (solvent A), 0.53 (solvent B); HPLC t_r = 16.9 (8%), 18.8 min (92%) (Rainin Microsorb C18, solvent F at 1.2 mL/min, 254 nm); IR (KBr) 3700–2200, 2955, 1730, 1680 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 0.64 (s, 3 H, CH_3), 0.90 (s, 3 H, CH_3), 1.04 (s, 3 H, CH_3), 1.00–1.74 (m, 7 H, fenchyl), 2.50–2.68 (m, 2 H, AB part of Asp ABX, J_{AB} = 17.0 Hz, J_{AX} = 5.2 Hz, J_{BX} = 8.9 Hz, Asp CH_2), 4.13 (d, 1 H, *J* = 1.5 Hz, CO_2CH), 4.14–4.17 (m, 1 H, X part of Asp ABX, J_{AX} = 5.2 Hz, J_{BX} = 8.9 Hz, Asp CH), 5.64 and 5.66 (s, 1 H, C_4H_3SCH, ratio 90/10), 7.12–7.14 (dd, 1 H, *J* = 4.7 Hz, *J* = 1.5 Hz, thienyl), 7.42–7.45 (m, 2 H, thienyl); MS (FAB) *m/z* 409 $(MH)^+$, 273 $(MH - C_{10}H_{16})^+$, 137 $(C_{10}H_{17})^+$; HRMS (FAB, MH^+) calcd for $C_{20}H_{29}N_2O_5S$ 409.1797, found 409.1805; sweetness potency 2000 ± 1300 (equivalent to 8% sucrose).

Route to L-Aspartyl-D-3-thienylglycine (+)-β-Fenchyl Ester (13). *N*-[(*o*-Nitrophenyl)sulfenyl]-D,L-3-thienylglycine (16b). D,L-3-Thienylglycine (2 g, 12.7 mmol), 5 N NaOH (5.6 mL), and *o*-nitrophenylsulfenyl chloride (3.27 g, 17.2 mmol, (only 1 equiv needed)) were reacted in 25 mL of dioxane as described for **16a** to provide 3.2 g (81%) of **16b**.

***N*-[(*o*-Nitrophenyl)sulfenyl]-D,L-3-thienylglycine (+)-β-Fenchyl Ester (17b).** Crude, *N*-protected thienylglycine **16b** (3.0 g, 9.7 mmol), (+)-β-fenchol (1.5 g, 9.7 mmol), dicyclohexylcarbodiimide (2.4 g, 11.6 mmol), and 4-(dimethylamino)pyridine (49 mg, 0.4 mmol) in 75 mL of 2/1 CH_2Cl_2/THF were combined as described for **7a** to give 3.0 g (69%) of **17b**. A sample was purified by silica flash chromatography with 20% acetone/hexane: mp 99–100 °C; $[\alpha]_D$ −16.1° (*c* 0.6, MeOH).

D,L-3-Thienylglycine (+)-β-Fenchyl Ester (18b). Crude, N-protected thienylglycine fenchyl ester **17b** (3 g, 6.7 mmol) was deprotected as described for **18a** to give 1 g crude material, which was purified by silica gel flash chromatography with 30% EtOAc/hexane to give 0.55 g (28%) of **18b**.

***N*-Formyl-L-aspartyl-D,L-3-thienylglycine (+)-β-Fenchyl Ester (20).** Thienylglycine fenchyl ester **18b** (530 mg, 1.81 mmol) was dissolved in 20 mL of dry pyridine and cooled to 0 °C. *N*-Formyl-L-aspartic anhydride (375 mg, 2.62 mmol) was added and the reaction was allowed to warm to room temperature over 3 h. Water and MeOH were added and the solvents were evaporated. The residue was dissolved in EtOAc and was washed with 1 N HCl, H_2O, brine, dried over $MgSO_4$, filtered, and evaporated to give 629 mg of crude product. Purification by silica gel flash chromatography with 10/90/0.5 MeOH/$CHCl_3$/HOAc gave 240 mg of α-coupled diastereomer **20** used below (R_f = 0.47) and 244 mg of the β-coupled diastereomer (R_f = 0.28) for a combined yield of 61%.

L-Aspartyl-D-3-thienylglycine (+)-β-Fenchyl Ester (13). *N*-Formyl aspartylthienylglycine ester **20** (160 mg, 0.37 mmol, α-isomer) was dissolved in 10 mL of 1/1 pyridine/H_2O. Hydroxylamine hydrochloride (30.5 mg, 0.44 mol) was added and the reaction was stirred overnight. Additional hydroxylamine hydrochloride (90 mg, 1.3 mmol) was added and the reaction was stirred for 2 days. The solvent was evaporated. The residue was taken up in EtOAc and filtered and the solvent was evaporated. The crude product was purified by flash chromatography on silica gel with 20/80/1/2 MeOH/$CHCl_3$/H_2O/HOAc. The resulting product was taken up in $CHCl_3$/MeOH and filtered. The solvent was evaporated to give 150 mg (100%) of **13**. The diastereomers were separated by semipreparative HPLC as described for **10c**. Freeze-drying of the appropriate fractiongs gave 25 mg of the sweet diastereomer which eluted second: mp 155 °C; [α] −44.0° (*c* 5.0, MeOH); TLC R_f = 0.75 (solvent A), 0.33 (solvent B); HPLC t_r = 15.4 (13%), 17.0 min (87%) (Rainin Microsorb C18, solvent F at 1.2 mL/min, 254 nm); IR (KBr) 3700–2200, 2960, 2870, 1735, 1680 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 0.67 (s, 3 H, CH_3), 0.91 (s, 3 H, CH_3), 1.04 (s, 3 H, CH_3), 1.05–1.73 (m, 7 H, fenchyl), 2.50–2.69 (m, 2 H, AB part of Asp ABX, J_{AB} = 17.0 Hz, J_{AX} = 4.8 Hz, J_{BX} = 9.2 Hz, Asp CH_2), 4.13–4.17 (m, 2 H, CO_2CH and Asp CH), 5.78 and 5.79 (s, C_4H_3SCH, ratio 20/80), 6.99 (dd, 1 H, *J* = 5.0 Hz, 3.6 Hz, thienyl H_5), 7.12 (d, 1 H, *J* = 3.4 Hz, thienyl H_2), 7.38 (d, 1 H, *J* = 5.0 Hz, thienyl H_4); MS (FAB) *m/z* 409 $(MH)^+$, 273 $(MH - C_{10}H_{16})^+$, 137 $(C_{10}H_{16})^+$; HRMS (FAB, MH^+) calcd for $C_{20}H_{29}N_2O_5S$ 409.1797, found 409.1790; sweetness potency 2300 ± 370 (equivalent to 10% sucrose).

Route to L-Aspartyl-β-2-thienyl-D,L-alanine (+)-β-Fenchyl Ester (14). *N*-[(*o*-Nitrophenyl)sulfenyl]-β-2-thienyl-D,L-alanine (16c). β-2-Thienyl-D,L-alanine (4.0 g, 23.3 mmol), *o*-

nitrophenylsulfenyl chloride (6.0 g, 31.7 mmol), and 11 mL of 5 N NaOH were reacted in 50 mL of dioxane as described for **16a**. The crude product was recrystallized from EtOAc/hexane to give 6.8 g (90%) of **16c**: mp 120–121 °C.

***N*-[(*o*-Nitrophenyl)sulfenyl]-β-2-thienyl-D,L-alanine (+)-β-Fenchyl Ester (17c).** N-Protected thienylalanine **16c** (4.0 g, 12.3 mmol), (+)-β-fenchol (1.9 g, 12.3 mmol), dicyclohexylcarbodiimide (3.0 g, 14.5 mmol), and 4-(dimethylamino)pyridine (65 mg, 0.5 mmol) in 30 mL of dry THF were combined as described for **7a** (except that the DCC was added at 0 °C and the DMAP at −35 °C) to give 5.4 g (95%) of **17c**: $[\alpha]_D$ −6.0° (*c* 0.2, MeOH).

β-2-Thienyl-D,L-alanine (+)-β-Fenchyl Ester (18c). Crude, N-protected thienylalanine ester **17c** (5.3 g, 11.5 mmol) in 20 mL of acetone was treated with 2.3 mL of 5 HCl at room temperature for 30 min. Acid/base workup as described for **18a** provided only 300 mg of material. Most of the product was extracted into the ether from the 0.1 N HCl solution. Evaporation of these ether extracts gave 4.0 g of material, which was purified by silica gel flash chromatography with 30% EtOAc/hexane to give 1.2 g (34%) of product: $[\alpha]_D$ −14.4° (*c* 0.4, MeOH).

***N*-(*tert*-Butoxycarbonyl)-β-*tert*-butyl-L-aspartyl-β-2-thienyl-D,L-alanine (+)-β-Fenchyl Ester (19c).** *N*-(*tert*-Butoxycarbonyl)-β-*tert*-butyl-L-aspartic acid *p*-nitrophenyl ester (2.0 g, 4.9 mmol) and thienylalanine fenchyl ester **18c** (1.5 g, 4.9 mmol) were combined in 30 mL of dry THF and reacted as described for **19a**. Purification by silica gel flash chromatography with 15% EtOAc/hexane gave 1.8 g (64%) of **19c**.

L-Aspartyl-β-2-thienyl-D,L-alanine (+)-β-Fenchyl Ester (14). HCl gas was bubbled through the diprotected aspartylthienylalanine fenchyl ester **19c** (100 mg, 0.17 mmol) in 20 mL of $CHCl_3$ for 20 min. The solvent was evaporated and the residue was flash chromatographed on silica gel with 23/75/1/2 MeOH/$CHCl_3$/H_2O/HOAc to give 64 mg (89%) of **14**: mp 188–190 °C; $[\alpha]_D$ −6.8° (*c* 0.4, MeOH); TLC R_f = 0.72 (solvent A), 0.34 (solvent B); HPLC t_r = 22.4 (55%), 25.4 min (45%) (Rainin Microsorb C18, solvent E at 1.0 mL/min, 210 nm); IR (KBr) 3700–2700, 2940, 2860, 1730, 1680, 1570 (br) cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 0.82 (s, CH_3), 0.87 (s, CH_3), 1.01 (s, CH_3), 1.04 (s, CH_3), 1.06 (s, CH_3), 1.00–1.85 (m, 7 H, fenchyl), 2.42–2.81 (m, 2 H, Asp CH_2), 3.19–3.50 (m, 2 H, $C_4H_3SCH_2$), 4.00 (br s, 1 H, Asp CH), 4.18 (s, 1 H, CO_2CH), 4.75 (obscured by solvent, $C_4H_3SCH_2CH$), 6.90–6.94 (m, 2 H, thienyl), 7.24 (d, 1 H, J = 5.1 Hz, thienyl); MS (FAB) m/z 423 $(MH)^+$, 287 $(MH - C_{10}H_{16})^+$; HRMS (FAB, MH^+) calcd for $C_{21}H_{31}N_2O_5S$ 423.1954, found 423.1936; sweetness potency 0.

Route to L-Aspartyl-D,L-4-imidazolylglycine (−)-α-Fenchyl Ester (15). N^{α}-Boc-N^{im}-Boc-D,L-4-imidazolylglycine (21). The literature procedures for preparation of *N*-Boc-D,L-4-imidazolylglycine[15] and *N*-Boc-L-histidine[16] were modified to isolate the diprotected amino acid. D,L-4-Imidazolylglycine (3.5 g, 25 mmol) and di-*tert*-butyl dicarbonate (10.8 g, 50 mmol) in dioxane/water (25 mL/8 mL) were stirred for 5 h at room temperature at pH 10. The reaction was monitored by TLC (solvent A) by following the conversion of starting material to monoprotected (R_f = 0.5) and then diprotected (R_f = 0.9) product. The solvent was evaporated and the residue was taken up in H_2O and the pH was brought to 3 with 5 N HCL. The solution was extracted with EtOAc, and the combined extracts were washed with brine, dried over $MgSO_4$, filtered, and evaporated to give 5.5 g of **21** (66%), part of which was used immediately in the following reaction.

N^{α}-Boc-N^{im}-Boc-D,L-4-imidazolylglycine (−)-α-Fenchyl Ester (22). Diprotected imidazolylglycine **21** (860 mg, 2.5 mmol) in 15 mL of dry THF containing 3Å molecular sieves was cooled to 0 °C, and triethylamine (0.35 mL, 2.5 mmol) and ethyl chloroformate (0.24 mL, 2.5 mmol) were added. The mixture was stirred for 1 h and then (−)-α-fenchol (388 mg, 2.5 mmol) in 5 mL of THF was added. The reaction was allowed to warm to room temperature while stirred overnight. The solvent was evaporated and the residue was purified by silica gel flash chromatography with 30% EtOAc/hexane to give 336 mg (28%) of **22**.

D,L-4-Imidazolylglycine (−)-α-Fenchyl Ester (23). Diprotected imidazolyl fenchyl ester **22** (336 mg, 0.70 mmol) was dissolved in 10 mL of $CHCl_3$ and HCl gas was bubbled through the solution for 30 min. After 1 h the solvent was evaporated and the residue was partitioned between 1 N HCl and EtOAc. The aqueous layer was made basic and extracted twice with EtOAc. The combined extracts were washed with brine, dried over $MgSO_4$, filtered, and evaporated to give 100 mg (51%) of **23**: $[\alpha]_D$ −1.7° (*c* 0.2, MeOH).

***N*-(*tert*-Butoxycarbonyl)-β-*tert*-butyl-L-aspartyl-D,L-4-imidazolylglycine (−)-α-Fenchyl Ester (24).** Imidazolylglycine fenchyl ester **23** (100 mg, 0.36 mmol) and *N*-(*tert*-butoxycarbonyl)-β-*tert*-butyl-L-aspartic acid *p*-nitrophenyl ester (148 mg, 0.36 mmol) in 10 mL of THF were stirred overnight. The solvent was evaporated and the crude product was purified by silica gel flash chromatography with 60% EtOAc/hexane to give 152 mg (76%) of **24**: $[\alpha]_D$ −17.1° (*c* 0.2, MeOH).

L-Aspartyl-D,L-4-imidazolylglycine (−)-α-Fenchyl Ester (15). The diprotected aspartylimidazolyl fenchyl ester **24** (83 mg, 0.15 mmol) was dissolved in 5 mL of $CHCl_3$ and HCl gas was bubbled through the solution via a pipet. The reaction was stirred for 90 min, during which time a precipitate formed. The solvent was evaporated to give 66 mg of product (quant. for HCl salt): mp 245–250 °C dec; $[\alpha]_D$ −10.7° (c 0.1, MeOH); TLC R_f = 0.50 (solvent A), 0.05 (solvent B); HPLC T_r 12.1 (56%), 14.1 min (44%), (Rainin Microsorb C18, solvent C at 1 mL/min, 210 nm); IR (KBr) 3650–2500, 2960, 2870, 1730, 1680, 1560 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 0.43 (s, CH_3), 0.79 (s, CH_3), 0.83 (s, CH_3), 1.02 (s, CH_3), 1.05 (s, CH_3), 1.08 (s, CH_3), 1.00–1.90 (m, 7 H, fenchyl), 2.54–2.95 (m, 2 H, Asp CH_2), 4.12–4.17 (m, 1 H, Asp CH), 4.34 and 4.37 (d, 1 H, J = 1.5 Hz, CO_2CH), 5.61 (s, 1 H, $C_3H_3N_2$-CH), 7.16 and 7.20 (s, 1 H, Im), 7.69 (s, 1 H, Im); MS (FAB) m/z 393 $(MH)^+$; HRMS (FAB, MH^+) calcd for $C_{19}H_{29}N_4O_5$ 393.2138, found 393.2131; sweetness potency 350 ± 90 (equivalent to 6% sucrose).

Supplementary Material Available: Spectral data for **6**, **7a–c**, **8a–c**, **9a–c**, **16a–c**, **17a–c**, **18a–c**, **19a,c**, **20–24** (6 pages). Ordering information is given on any current masthead page.