8.3 (br signal, 3 H, $NH_3$); mass spectrum, *m/e* (relative intensity) 179 (9), 149 (100). Anal. ($C_{10}H_{13}NO_2$·HCl) C, H, N.

**(±)-4-(Aminomethyl)-4,6-dimethoxychroman Hydrochloride (14a).** Compound **14a** was prepared from **13a** in 72% yield by the same method employed for the synthesis of **14b**: mp 168–170 °C (dec) after recrystallization from a methanol–$Et_2O$ mixture; NMR ($Me_2SO$-$d_6$) δ 2.1 (m, 2 H, $CH_2$), 2.85 (s, 3 H, $OCH_3$), 3.05 (s, 2 H, $CH_2N$), 3.55 (s, 3 H, $ArOCH_3$), 4.05 (t, 2 H, $OCH_2$), 6.2 (s, 3 H, ArH), 8.2 (br signal, 3 H, $NH_3$); mass spectrum, *m/e* (relative intensity) 223 (5), 193 (100). Anal. ($C_{12}H_{17}NO_3$·HCl) C, H, N.

**(±)-4-(Aminomethyl)-4-methoxychroman Chloride (14b).** Dry HCl gas was bubbled into a solution of **13b** (2 g, 9.28 mmol) in MeOH (100 mL) at 0 °C for 2 min. The exothermic reaction was cooled so as to not allow the temperature to exceed 30 °C; stirring at room temperature was continued for 2 h. The MeOH was evaporated under reduced pressure, and the resultant product was recrystallized from a MeOH–$Et_2O$ mixture to give 1.83 g (71%) of **14b** as white crystals: mp 183–185 °C dec; NMR ($Me_2SO$-$d_6$) δ 2.15 (m, 2 H, $CH_2$), 3.0 (s, 3 H, $OCH_3$), 3.25 (s, 2 H, $CH_2N$), 4.3 (m, 2 H, $OCH_2$), 6.8–7.45 (m, 4 H, ArH), 8.35 (br signal, 3 H, $NH_3$). Anal. ($C_{11}H_{15}NO_2$·HCl) C, H, N.

**(±)-4-(Aminomethyl)-4-ethoxychroman Hydrochloride (14c).** Compound **14c** was prepared in the same manner as **14b** except that absolute EtOH replaced the MeOH as reaction solvent. Recrystallization from an absolute EtOH–$Et_2O$ mixture gave an 88% yield of **14c** as white crystals, mp 187–189 °C dec. Compound **14c** was used without further purification for the preparation of **4b**: NMR ($Me_2SO$-$d_6$) δ 1.4 (t, 3 H, $CH_2CH_3$), 2.4 (m, 2 H, $CH_2$), 3.4 (s, 2 H, $CH_2N$), 3.55 (m, 2 H, $CH_2CH_3$), 4.5 (m, 2 H, $OCH_2$), 7.1–7.85 (m, 4 H, ArH), 8.6 (br signal, 3 H, $NH_3$).

**(±)-1-(Aminomethyl)-6-methoxychroman Hydrochloride (15).** A solution of **4a** (0.15 g, 6.6 mmol) in MeOH (25 mL), to which 150 mg of 10% Pd on C had been added, was shaken under an atmosphere of $H_2$ (40 psig) for 4 h. The solution was filtered and evaporated to dryness, and the crude product was recrystallized from 2-propanol to yield 80 mg (53%) of **15**, mp 177–179 °C. Anal. ($C_{11}H_{15}NO_2$·HCl) C, H.

**4-Cyanochromene (16).** Trimethylsilylcyanide (2.5 mL) was added via syringe to a mixture of 4-chromanone (3 g, 20.25 mmol) and a catalytic amount of $ZnI_2$ in $CH_2Cl_2$, under a nitrogen atmosphere. The mixture was stirred at 50–55 °C (oil bath temperature) for 5 h and then cooled to room temperature, 3 M hydrochloric acid (30 mL) was added, and stirring was continued for an additional 4 h. The solution was extracted three times with $Et_2O$ (25 mL), and the ether portions were combined, dried ($MgSO_4$), and evaporated to dryness. The crude cyanochromanol was dissolved in benzene, to which tosic acid (0.5 g) had been added, and the solution was heated at reflux for 2 h. The solvent was removed under reduced pressure, and the product was distilled (Kugelrohr) to yield 2.55 g (80%) of **16** as a clear colorless liquid, which crystallized upon standing, mp 35–37 °C. Anal. ($C_{10}H_7NO$) C, H, N.

**Affinity Assay Studies.** Male Sprague–Dawley rats weighing 200–300 g were used; the stomach fundus was dissected and prepared according to the procedure described by Vane.[12] Two strips were cut from the same tissue and were used in parallel 8-mL muscle baths; the muscle baths and wash (Tyrodes) solution were aerated with 95% $O_2$–5% $CO_2$ and were maintained at 37 °C. The relative sensitivity of the two strips was determined, after a 1-h equilibration period, by exposure to a dose of 5-HT, which resulted in submaximal contractions. Only one compound was examined per preparation. The ability of each compound to inhibit the contractile response to 5-HT was determined by obtaining cumulative dose–response curves to 5-HT, first in the absence and then in the presence of several increasing concentrations of the agent in question. The $ED_{50}$ for each of the curves was determined, and the apparent affinities were calculated as $pA_2$ values by the method of Arunlakshana and Schild.[13] The $pA_2$ value is actually the negative logarithm of the molar concentration of an antagonist which effectively reduces the effect of the agonist by a factor of two. Determinations of $pA_2$ values are valid as long as the interaction is of a competitive nature; although the ideal slope of a Schild plot is −1.0 for a competitive antagonist, the interaction is assumed to be competitive when slopes are between −0.8 and −1.2. The number of Schild plots ($pA_2$ determinations), the number of dose–response curves, and the slopes of the Schild plots are shown in Table I.

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# Peptide Sweeteners. 5. Side-Chain Homologues Relating Zwitterionic and Trifluoroacetylated Amino Acid Anilide and Dipeptide Sweeteners

Masao Kawai,[1] Rolf Nyfeler, Judd M. Berman, and Murray Goodman*

*Department of Chemistry, B-014, University of California, San Diego, La Jolla, California 92093.* *Received September 8, 1981*

Side-chain homologues of sweet trifluoroacetyl-α-L-aspartyl-*p*-cyanoanilide have been synthesized and tasted. Removal of the trifluoroacetyl group only changes the potency of sweet taste, not the taste property. These results have been compared with the structure–taste relationships of dipeptide sweeteners. An informative discontinuity of taste effects was found to exist with novel aminomalonyl dipeptide derivatives. The results are explained on topochemical grounds.

An extremely wide variety of structural features are found in sweet tasting compounds. Attempts to determine general characteristics from the great diversity of the structures of sweet compounds have been made, and molecular theories of sweet taste have been proposed.[2,3] Recently, the relationship between structure and taste has been quantitatively analyzed by correlating the potency of sweet taste of L-aspartyl dipeptide analogues to steric, electronic, and hydrophobic parameters.[4] Derivatives

(1) Visiting research chemist from Mitsubishi-Kasei Institute of Life Sciences, Machida-shi, Tokyo 194, Japan.

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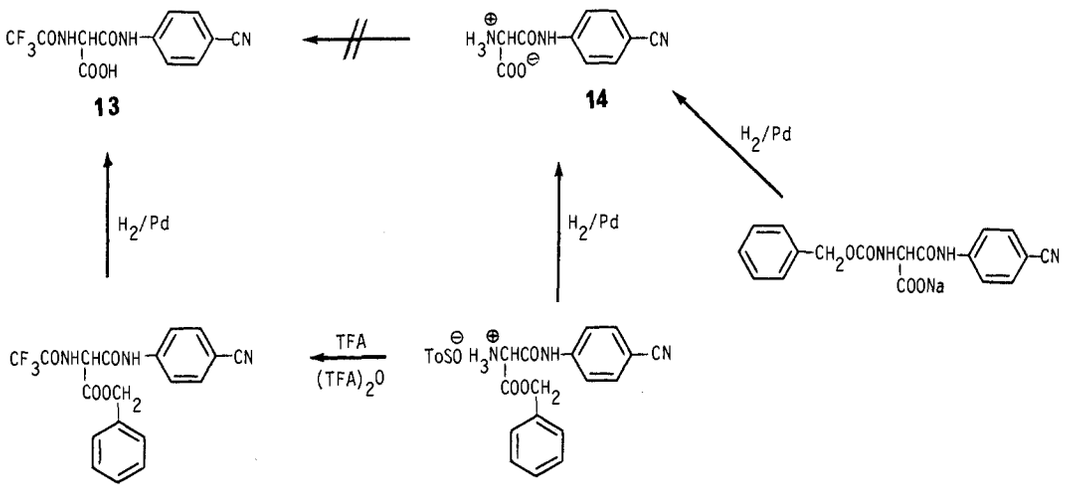
Scheme I




based on L-aspartyl-L-phenylalanine methyl ester (1) have been extensively studied.[5-13] A structurally similar class of L-aspartic acid derivatives, namely, trifluoroacetyl-L-aspartylanilides, has been reported by Lapidus and Sweeney.[14]

In the present study, side-chain homologues of trifluoroacetyl-L-aspartyl-*p*-cyanoanilide (2), L-aspartyl-*p*-cyanoanilide (3), and trifluoroacetyl-L-aspartyl-L-phenylalanine methyl ester (4), including novel aminomalonyl homologues, were synthesized and tasted.

**Synthesis of Compounds.** The procedure described by Lapidus and Sweeney[14] was followed to prepare the parent compound, trifluoroacetyl-α-L-aspartyl-*p*-cyanoanilide (2), starting from trifluoroacetyl-L-aspartic anhydride. The structural isomer of 2, i.e., trifluoroacetyl-β-L-aspartyl-*p*-cyanoanilide (5), was obtained from the mother liquor of the crystallization of 2. The same procedure was employed to synthesize enantiomeric analogues, trifluoroacetyl-α- and -β-D-aspartyl-*p*-cyanoanilides (6 and 7).

The higher homologue of 2, trifluoroacetyl-α-L-glutamyl-*p*-cyanoanilide (8), was synthesized from *tert*-butoxycarbonyl-L-glutamic acid γ-benzyl ester to avoid ambiguity in the structure of the compound obtained. Reaction of trifluoroacetyl-L-glutamic anhydride[15] and *p*-cyanoaniline gave two products: trifluoroacetyl-α- and -γ-L-glutamyl-*p*-cyanoanilides (8 and 9). Their optical isomers, trifluoroacetyl-α- and -γ-D-glutamyl-*p*-cyanoanilides (10 and 11), were synthesized similarly, starting from trifluoroacetyl-D-glutamic anhydride, followed by chromatographic separation of the isomers. In a like manner, α-DL-(α-aminoadipyl)-*p*-cyanoanilide (12), was synthesized from DL-α-aminoadipic acid δ-benzyl ester by trifluoroacetylation and coupling with *p*-cyanoaniline, followed by removal of the δ-benzyl ester group.

Synthesis of aminomalonyl-*p*-cyanoanilides took into account the instability of aminomalonic acid derivatives. Aminomalonic acid and its esters and amides are known to decarboxylate very easily to give the corresponding glycyl compounds.[16,17] However, salts of aminomalonic acid are described as being quite stable.[18] Indeed, either hydrogenation of *N*-(benzyloxycarbonyl)aminomalonyl-*p*-cyanoanilide sodium salt or hydrogenation of aminomalonyl-*p*-cyanoanilide benzyl ester tosylate in the presence of sodium hydroxide afforded the sodium salt of 14, which was converted to the zwitterion by careful acidification in aqueous solution (Scheme I).

Trifluoroacetylation of aminomalonyl-*p*-cyanoanilide using various methods always led to formation of trifluoroacetylglycyl-*p*-cyanoanilide. However, trifluoroacetylation of aminomalonyl-*p*-cyanoanilide benzyl ester and subsequent hydrogenation afforded the desired compound 13.

The synthesis of the lower homologue of 4, trifluoroacetylaminomalonyl-L-phenylalanine methyl ester (18), could not be achieved by trifluoroacetylation of aminomalonyl-L-phenylalanine methyl ester, since decarboxylation occurred as in the case of the *p*-cyanoanilide 14. However, selective deprotection of *N*-(benzyloxycarbonyl)benzyl-DL-aminomalonyl-L-phenylalanine methyl ester followed by trifluoroacetylation and hydrogenation, afforded the desired compound 18.

## Results and Discussion

The much studied structure–taste relationships of L-aspartyl-L-phenylalanine methyl ester have involved modifications to the N-terminal zwitterionic portion,[5,7,8] to the C-terminal hydrophobic portion,[5,6,8-12] and to the peptide bond.[13] As for the zwitterionic portion, the free β-carboxyl group and L configuration are required for sweet taste. Only aminomalonic acid can replace aspartic acid. Also, any modification of the peptide bond results in tasteless analogues.[13] The C-terminal portion is more flexible in its spatial requirements and can accommodate

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Table I. Taste Evaluation of Side-Chain Homologues of Zwitterionic and Trifluoroacetylated Amino Acid Anilides and Dipeptides

$CF_3CONHCHCONH\text{-}p\text{-}C_6H_4CN$ $H_2NCHCONH\text{-}p\text{-}C_6H_4CN$
$(CH_2)_nCOOH$ $(CH_2)_nCOOH$

| no. | confign | n | taste[a] | no. | confign | n | taste[a] |
|---|---|---|---|---|---|---|---|
| 13 | DL | 0 | 0 | 14 | DL | 0 | 0 |
| 2 | L | 1 | +++[b] | 3 | L | 1 | +[b] |
| 8 | L | 2 | +++ | 16 | L | 2 | + |
| 12 | DL | 3 | 0 | | | | |

$CF_3CONHCHCONHCHCOOCH_3$ $H_2NCHCONHCHCOOCH_3$
$(CH_2)_nCOOH$ $(CH_2)_nCOOH$
$CH_2C_6H_5$ $CH_2C_6H_6$

| no. | confign[e] | n | taste[a] | no. | confign[e] | n | taste[a] |
|---|---|---|---|---|---|---|---|
| 18 | DL | 0 | 0 | 20 | DL | 0 | ++[c] |
| 4 | L | 1 | ++[b] | 1 | L | 1 | ++[d] |
| 17 | L | 2 | 0 | 19 | L | 2 | 0[d] |

[a] +, ++, and +++ correspond to 15-30, 150-200, and 2500-3000, respectively, taking the sweetness of sucrose as 1 on a weight basis. 0 = not sweet. [b] The taste intensities of these compounds are taken from ref 14. [c] The taste intensity of this compound is taken from ref 8. [d] The taste intensity of this compound is taken from ref 5. [e] Configuration refers to the N-terminal amino acid. Phenylalanine always has the L configuration.

a large variety of amino acid esters and other structures.[5,9-12] The configuration and size of groups attached to the C-terminal $\alpha$-carbon atom are important in determining potency of sweet taste. However, some amides of *achiral* amines have been reported as sweet, e.g., L-aspartyl-*n*-hexylamide.[6]

A series of acylated L-aspartylanilides and -amides were synthesized by Lapidus and Sweeney.[14] These researchers established that a strong electronic effect influences the taste of the anilides. Only the trifluoroacetyl- and the trichloroacetyl-L-aspartylanilides are intensely sweet. The maximum sweetness for these compounds occurs with electron-withdrawing groups, such as *p*-cyano- or *p*-haloanilides. Tinti et al.[19] showed that the analogous *p*-nitroanilide is also intensely sweet. Lapidus and Sweeney found that sweetness remains after removal of the trifluoroacetyl group from **2**, although the taste intensity was greatly reduced.

We discovered that trifluoroacetyl-L-glutamyl-*p*-cyanoanilide (**8**) is also intensely sweet, comparable to the aspartyl compound **2**. This was recently independently observed by Tinti et al.[19] We found that L-glutamyl-*p*-cyanoanilide (**16**) is less sweet than its trifluoroacetyl analogue. Thus, the aspartyl and glutamyl compounds manifest the same structure–sweet taste relationship. This finding is in contrast to results published by Tinti et al.[19] They report L-glutamyl-*p*-cyanoanilide as a tasteless compound (mp 208–210 °C), while we find this compound (mp 160–161 °C) to be 12 times sweeter than sucrose. It is possible that Tinti et al.[19] may have actually isolated L-pyroglutamyl-*p*-cyanoanilide.

We have also prepared the higher and lower homologues, namely, trifluoroacetylaminoadipyl-*p*-cyanoanilide (**12**) and trifluoroacetylaminomalonyl-*p*-cyanoanilide (**13**). Both these compounds were found to be tasteless (see Table I). The lower homologue of **3**, aminomalonyl-*p*-cyanoanilide (**14**), was also determined to be tasteless. We conclude that trifluoroacetylation of homologues of L-aspartyl-*p*-cyanoanilide influences taste intensity but not taste character. Both classes of *p*-cyanoanilides (i.e., trifluoroacetylated and zwitterionic amino acid derivatives) most likely create their sweet taste by interacting with the same receptor site.

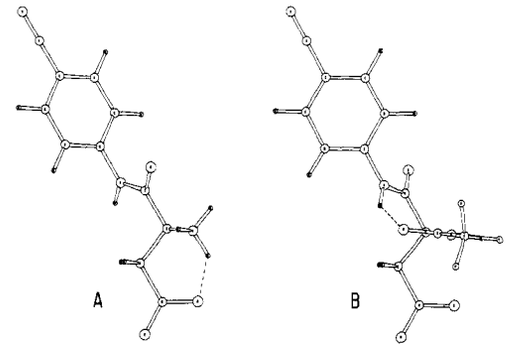


**Figure 1.** Comparative arrays of (A) L-aspartyl-*p*-cyanoanilide (**3**) and (B) trifluoroacetyl-L-aspartyl-*p*-cyanoanilide (**2**). The orientation and location of the carboxylate are fixed. Hydrogen bonds are indicated by dashed lines.

The synthesis of aminomalonyl-, L-aspartyl-, and L-glutamyl-L-phenylalanine methyl ester has been described.[5,7,8] The aminomalonyl and aspartyl dipeptides are sweet, while the glutamyl dipeptide is tasteless. Trifluoroacetyl-L-aspartyl-L-phenylalanine methyl ester (**4**) was also reported to be sweet.[14] We prepared trifluoroacetylaminomalonyl- and trifluoroacetyl-L-glutamyl-L-phenylalanine methyl ester. Neither of these compounds elicited sweet taste. Thus, the introduction of the trifluoroacetyl group in the sweet aminomalonyl-L-phenylalanine methyl ester resulted in a tasteless compound. Here we encounter the first discontinuity between a trifluoroacetyl derivative and its analogous zwitterionic compound. This observation is different from that found in the *p*-cyanoanilide series where both the zwitterionic and trifluoroacetylaminomalonyl-*p*-cyanoanilides are tasteless.

Our approach to understanding the sweet taste of the cyanoanilides and the dipeptides is based on the assumption that the sweet molecules, L-aspartyl-*p*-cyanoanilide and its trifluoroacetylated derivative, are essentially rigid. Examination of molecular models and ORTEP II drawings shows that all of the torsional angles for these molecules are relatively frozen. A hydrogen bond can be postulated within the zwitterionic ring or between the carboxylate and the *trans*-trifluoroacetamide. With this part of the molecule fixed, the remaining portion is totally determined. In order to compare these molecules with respect to each other, the carboxylate groups are placed in an identical orientation as shown in Figure 1. This seems quite reasonable, since the carboxylate group is common to all of these sweeteners. Its presence is essential for eliciting sweet taste, since replacement of the N-terminal amino acid by glycine in compounds 1–4 results in complete loss of sweetness.

The dipeptide L-aspartyl-L-phenylalanine methyl ester is sweet. It is not rigid; however, it can be arrayed in the orientation presented by the sweet *p*-cyanoanilides. The same orientation can be achieved by the sweet aminomalonyl-L-phenylalanine methyl ester (Figure 2A). However, the trifluoroacetylated analogue of this dipeptide derivative cannot be arrayed as the sweet compounds (Figure 2B). The array of the molecule is essentially perpendicular to those shown by the sweet compounds (Figures 1A,B and 2A). As noted above, trifluoroacetylaminomalonyl-L-phenylalanine methyl ester is not sweet.

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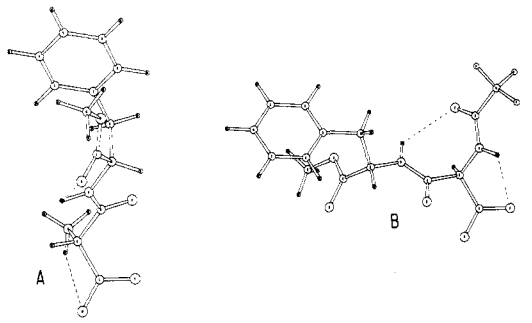



**Figure 2.** Comparative arrays of (A) aminomalonyl-L-phenylalanine methyl ester (**20**) and (B) trifluoroacetylaminomalonyl-L-phenylalanine methyl ester (**18**). The orientation and location of the carboxylate are fixed. Hydrogen bonds are indicated by dashed lines.

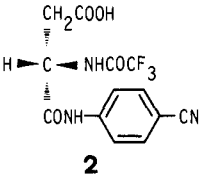
Table II. Taste Evaluation of Trifluoroacetylaspartyl-*p*-cyanoanilides

| structure | no. | config | taste[a] |
|---|---|---|---|
| $CF_3CONHCHCONH$-*p*-$C_6H_4CN$ | 2 | L | +++ [b] |
| $CH_2COOH$ | 6 | D | 0 |
| $CF_3CONHCHCOOH$ | 5 | L | 0 |
| $CH_2CONH$-*p*-$C_6H_4CN$ | 7 | D | 0 |

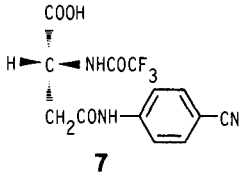
[a] +, ++, and +++ correspond to 15-30, 150-200, and 2500-3000, respectively, taking the sweetness of sucrose of 1 on a weight basis. 0 = not sweet. [b] The taste intensity of this compound is taken from ref 14.

Further insight into the molecular basis of sweet taste came from a comparison of the structure–sweet taste relationships of the zwitterionic and the trifluoroacetylated amino acid anilide and dipeptides. The *p*-cyanoanilides are sweet when $n = 1$ or 2 (see Table I), while the dipeptide derivatives are sweet when $n = 0$ or 1, the exception is trifluoroacetylaminomalonyl-L-phenylalanine methyl ester. The sweet *p*-cyanoanilides have carboxylate side chains one methylene longer than the sweet homologues in the dipeptide sweeteners. This shift is required to allow the proper arrangement of the conformationally highly restricted hydrophobic site; it gives the two classes of compounds the same overall rodlike array. The strikingly similar steric and structural requirements of these two classes of compounds are strong evidence that they interact with the same receptor site.

The situation is even more subtle than is indicated by the above discussion of the number of methylenes in the side chain of the sweet compounds. Isomers of L-aspartyl-L-phenylalanine methyl ester (**1**), such as β-L-aspartyl-L-phenylalanine methyl ester and α-D-aspartyl-L-phenylalanine methyl ester, are not sweet.[5] We found that neither the D-aspartyl, the D-glutamyl, nor the β and γ isomers of the trifluoroacetylated amino acid *p*-cyanoanilides are sweet (see Tables II and III). Lack of sweetness of trifluoroacetyl-β-D-aspartyl-*p*-cyanoanilide (**7**) is worth noting, considering that **7** and the parent compound **2** differ only in the position of one methylene group.




**2**




**7**

## Conclusion

Our results lead to the conclusion that trifluoroacetylation of α-L-aspartyl- or α-L-glutamyl-*p*-cyanoanilides and L-aspartyl dipeptide esters does not change taste properties but only taste intensity. With aminomalonyl-L-phenylalanine methyl ester, we note a change in taste property upon trifluoroacetylation; the former is sweet while the latter is tasteless. This we attribute to the significant alteration in the orientation of the trifluoroacetylaminomalonyl-L-phenylalanine methyl ester as compared to its zwitterionic analogues (Figure 2). The aminomalonyl-*p*-cyanoanilide and its trifluoroacetylated analogue possess the same orientation as the trifluoroacetylaminomalonyl-L-phenylalanine methyl ester when the carboxylate is arrayed as in Figure 1 or 2. It is therefore understandable that both of these molecules are not sweet. There is a great similarity in the structure–taste relationships of zwitterionic and trifluoroacetylated amino acid *p*-cyanoanilide and dipeptide sweeteners. This lends strong support for the existence of a single receptor site that accommodates both the zwitterionic and the trifluoroacetylated sweet compounds. Thus, we now believe that all four classes of sweet compounds, the zwitterionic *p*-cyanoanilides, their trifluoroacetylated derivatives, the zwitterionic dipeptides, and the trifluoroacetyl-L-aspartyl-L-phenylalanine methyl ester, all interact with the same receptor.

Table III. Taste Evaluation of Trifluoroacetylglutamyl-*p*-cyanoanilides

| structure | no. | config | taste[a] |
|---|---|---|---|
| $CF_3CONHCHCONH$-*p*-$C_6H_4CN$ | 8 | L | +++ |
| $CH_2CH_2COOH$ | 10 | D | 0 |
| $CF_3CONHCHCOOH$ | 9 | L | 0 |
| $CH_2CH_2CONH$-*p*-$C_6H_4CN$ | 11 | D | 0 |

[a] +, ++, and +++ correspond to 15-30, 150-200, and 2500-3000, respectively, taking the sweetness of sucrose as 1 on a weight basis. 0 = not sweet.

## Experimental Section

Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 with a 10-cm water-jacketed cell. All elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Where analyses are indicated by symbols of the elements, the analytical values are within ±0.4% of the theoretical values.

Analytical TLC plates were purchased from E. Merck: silica gel 60 F-254, aluminum backed. Analytical reversed-phase TLC plates were purchased from Whatman: $KC_{18}$, glass backed. Preparative TLC plates were purchased from Analtech: silica gel GF, 2000 μm, glass backed. The plates were developed with ninhydrin, $Cl_2$/tolidine reagent, or UV light (254 nm). The following chromatography systems were used: (A) $CHCl_3$/MeOH/HOAc, 85:10:5; (B) EtOAc/hexanes, 4:5; (C) $CH_3Cl$/MeOH, 9:1; (D) cyclohexane/$CHCl_3$/HOAc, 45:45:10; (E) *n*-BuOH/HOAc/$H_2O$, 4:1:1; (F) $CH_3CN$/EtOH, 95:5; (G) *n*-BuOH/HOAc/pyridine/$H_2O$, 4:1:1:2; (H) EtOH/$H_2O$, 60:40.

Usual workup refers to dissolving the residue in a suitable organic solvent, successive washings with 2 M $NaHSO_4$ and saturated $NaHCO_3$, and removal of the solvent under reduced pressure.

These compounds were taste tested by three volunteers from our laboratories. The panel was able to achieve reporducible taste intensities involving sucrose solutions and the new compounds. Compounds were dissolved in doubly distilled water (pH 5) and related to an 8% sucrose solution. At least three double-blind tests were performed by the panel on each compound.

**Trifluoroacetyl-α- and -β-L-aspartyl-*p*-cyanoanilides (2 and 5).** *p*-Cyanoaniline (0.59 g, 5 mmol) was treated with trifluoroacetyl-L-aspartic anhydride (1.05 g, 5 mmol) in tetrahydrofuran as described in the literature.[14] TLC (A) of the crude product showed two spots, a major spot at $R_f$ 0.3 and a minor spot at $R_f$ 0.15. Crystallization from acetonitrile gave the α isomer (**2**) as colorless needles: yield 0.58 g (35%); TLC $R_f$ (A) 0.3; mp

187–189 °C (lit.[14] 187–188 °C); $[\alpha]^{24}_D$ −40.4° (c 1.1, acetone).

The mother liquor from the crystallization of **2** was subjected to preparative TLC (A). The slower moving band ($R_f$ 0.15) was collected and extracted with ethyl acetate/acetone. Recrystallization from isopropyl alcohol gave colorless needles of the β-anilide **5**: mp 148–156 °C (shrinking at 120–125 °C); $[\alpha]^{24}_D$ +18.4° (c 0.7, acetone). Anal. ($C_{13}H_{10}N_3O_4F_3 \cdot 0.5H_2O$) C, H, N.

**Trifluoroacetyl-α- and -β-D-aspartyl-*p*-cyanoanilides (6 and 7).** In a manner similar to the synthesis of the corresponding L compounds, **6** and **7** were prepared starting with trifluoroacetyl-D-aspartic anhydride.

The α-anilide **6** crystallized from acetonitrile as colorless needles: mp 187–188 °C; $[\alpha]^{24}_D$ +40.6° (c 1.1, acetone). Anal. ($C_{13}H_{10}N_3O_4F_3 \cdot 0.5H_2O$) C, H, N.

The β-anilide **7** crystallized from isopropyl alcohol as colorless needles: mp 157–160 °C (shrinking at 120–125 °C); $[\alpha]^{24}_D$ −18.0° (c 1.0, acetone). Anal. ($C_{13}H_{10}N_3O_4F_3 \cdot 0.5H_2O$) C, H, N.

**Trifluoroacetyl-α-L-glutamyl-*p*-cyanoanilide (8).** To a cooled solution (−15 °C) of *tert*-butoxycarbonyl-L-glutamic acid γ-benzyl ester (1.01 g, 3 mmol) and triethylamine (0.42 mL, 3 mmol) in tetrahydrofuran (6 mL) was added isobutyl chloroformate (0.39 mL, 3 mmol), and the reaction mixture was stirred for 15 min at −15 °C. *p*-Cyanoaniline (0.47 g, 4 mmol) was added, and stirring continued at 0 °C for 1 h and then at room temperature overnight. Evaporation under reduced pressure and usual workup gave *tert*-butoxycarbonyl-α-L-glutamyl-*p*-cyanoanilide γ-benzyl ester as a colorless oil (0.98 g, 75%). The protected anilide (0.98 g, 2.25 mmol) was dissolved in methanol (2.5 mL), 1 N NaOH (2.25 mL) was added, and the mixture was stirred for 0.5 h at room temperature.

The mixture was concentrated under reduced pressure, dissolved in 5% $NaHCO_3$, washed with ethyl acetate, and acidified. Extraction with ethyl acetate, followed by silica gel column chromatography using 2% methanol in chloroform as eluent, gave *tert*-butoxycarbonyl-α-L-glutamyl-*p*-cyanoanilide as a colorless oil (0.49 g, 63%). The saponified anilide (0.17 g, 0.5 mmol) was treated with trifluoroacetic acid (2 mL). The product was dissolved in methanol and a large excess of methyl trifluoroacetate[20] was added to the solution. Triethylamine was added to keep the solution neutral. After 0.5 h, volatile components were removed by evaporation under reduced pressure, and the residue was dissolved in ethyl acetate and washed with 0.5 N HCl. The product was extracted into 5% $NaHCO_3$, acidified, and extracted with ethyl acetate; crystallization from ethyl acetate/hexane afforded **8** as colorless needles (0.11 g, 64%): mp 190–191 °C; $[\alpha]^{24}_D$ −11.8° (c 1.0, acetone). Anal. ($C_{14}H_{12}N_3O_4F_3$) C, H, N.

**Trifluoroacetyl-γ-L-glutamyl-*p*-cyanoanilide (9).** Trifluoroacetyl-L-glutamic anhydride[15] (0.45 g, 2 mmol) and *p*-cyanoaniline (0.59 g, 5 mmol) were dissolved in tetrahydrofuran and stirred for 4 days at room temperature. After the usual workup (no $NaHCO_3$ extraction), the oily crude product (0.8 g) showed two major spots ($R_f$ 0.5 and 0.2) on TLC (A). The mixture was subjected to preparative TLC (A). The higher $R_f$ value compound was identified as α-anilide (**8**) by comparison with the authentic sample synthesized as above. The lower $R_f$ value compound was crystallized from ethyl acetate/hexane to give the γ isomer (**9**) as a colorless powder: mp 186–187 °C; $[\alpha]^{24}_D$ −6.0° (c 0.7, acetone). Anal. ($C_{14}H_{12}N_3O_4F_3$) C, H, N.

**Trifluoroacetyl-α- and -γ-D-glutamyl-*p*-cyanoanilides (10 and 11).** These compounds were synthesized from trifluoroacetyl-D-glutamic anhydride and *p*-cyanoaniline as described for the synthesis of **9**.

The α-anilide **10** crystallized from ethyl acetate/hexane as colorless needles: mp 187-192 °C; $[\alpha]^{24}_D$ +11.8° (c 1.2, acetone). Anal. ($C_{14}H_{12}N_3O_4F_3$) C, H, N.

The γ-anilide **11** crystallized from ethyl acetate/hexane as a colorless powder: mp 186–188 °C; $[\alpha]^{24}_D$ +5.5° (c 0.9, acetone). Anal. ($C_{14}H_{12}N_3O_4F_3$) C, H, N.

**Trifluoroacetyl-α-DL-(α-aminoadipyl)-*p*-cyanoanilide (12).** A mixture of DL-α-aminoadipic acid γ-benzyl ester (376 mg, 1.5 mmol; mp 186–187 °C; prepared as described in the literature for the preparation of the L compound, mp 198 °C[21]) and trifluoroacetic acid (4 mL) was cooled to −10 °C, and trifluoroacetic anhydride (0.28 mL, 2 mmol) was added. After stirring for 2 h at room temperature, the volatile components were removed by evaporation. To the residue was added oxalyl chloride (2 mL). After the solution was stirred overnight at room temperature, excess oxalyl chloride was evaporated, and the resulting trifluoroacetyl-DL-α-aminoadipic acid α-chloride δ-benzyl ester was dissolved in tetrahydrofuran (4 mL). *p*-Cyanoaniline (0.5 g, 3 mmol) and triethylamine (0.28 mL, 2 mmol) were added to the solution and stirred for 0.5 h at room temperature. Usual workup gave trifluoroacetyl-α-DL-(α-aminoadipyl)-*p*-cyanoanilide δ-benzyl ester as a semisolid (0.8 g, 86%), which was treated with HF–anisole at 0 °C for 0.5 h. After the usual workup (no $NaHCO_3$ extraction), the product was chromatographed over Sephadex LH-20 using methanol as eluent to afford 12 yield 0.2 g (32%); colorless needles from isopropyl alcohol; mp 148–149 °C. Anal. ($C_{15}H_{14}N_3O_4F_3 \cdot 0.5H_2O$) C, H, N.

**Ethyl *tert*-Butoxycarbonyl-DL-aminomalonate.** Sodium hydroxide, 1 N (100 mL, 0.1 mol) was added to an ice-cold solution of diethyl aminomalonate hydrochloride (21.2 g, 0.1 mol) in dioxane (240 mL) and water (120 mL). After the addition of di-*tert*-butyl dicarbonate (29 g, 0.11 mol), the reaction mixture was stirred at room temperature for 45 min. Evaporation of the solvents under reduced pressure and the usual workup gave pure diethyl *tert*-butoxycarbonyl-DL-aminomalonate as a liquid: yield 27.2 g; TLC $R_f$ (B) 0.5; $R_f$ (C) 0.8. This liquid (26.3 g) was dissolved in acetone (105 mL) and ethanol (95 mL) and cooled to 0 °C. NaOH, 1 N (96 mL, 0.096 mol), was added, and the reaction mixture was stirred at room temperature for 1 h and filtered. The filtrate was concentrated in vacuo, extracted with ether/hexanes (1:1), and acidified with 2 N HCl. Extraction into ethyl acetate and recrystallization from ether/hexanes yielded ethyl *tert*-butoxycarbonyl-DL-aminomalonate: yield 14 g (68%); TLC $R_f$ (A) 0.4; mp 93–95 °C.

***tert*-Butoxycarbonyl-DL-aminomalonyl-*p*-cyanoanilide.** Ethyl *tert*-butoxycarbonyl-DL-aminomalonate (7.1 g, 29 mmol) was dissolved in $CH_3CN$ (80 mL). Imidazole (7.8 g, 0.11 mol) and phosphorus trichloride (1.7 mL, 19 mmol) were added at −5 °C, and the reaction mixture was stirred for 15 min. A solution of *p*-cyanoaniline (13 g, 0.11 mol) in $CH_3CN$ (20 mL) was added, and stirring was continued overnight at room temperature.[22] After evaporation and the usual workup, a mixture of *tert*-butoxycarbonyl-DL-aminomalonyl-*p*-cyanoanilide ethyl ester and *p*-cyanoaniline was obtained. The material was dissolved in dioxane (40 mL) and ethanol (445 mL). NaOH, 1 N (28 mL, 28 mmol), was added, and the mixture was stirred for 80 min at room temperature. Evaporation, finally at high vacuum, afforded a solid material, which was treated twice with ether and filtered. The hygroscopic sodium salt was then dissolved in water (100 mL) and acidified, and the free acid was extracted with ether. The ether layer was washed with water and dried. Dicyclohexylamine (DCHA; 4.84 mL, 24 mmol) in ether (50 mL) was added at 0 °C. Filtration gave the DCHA salt (11.2 g, 78%), mp 194–196 °C. The free acid was obtained by liberating the salt with $KHSO_4$ solution and extraction into ethyl acetate. Recrystallization from ether/hexanes yielded *tert*-butoxycarbonyl-DL-aminomalonyl-*p*-cyanoanilide: yield 5.3 g (58%); TLC $R_f$ (A) 0.4; $R_f$ (D) 0.36; mp 202–203 °C dec.

**DL-Aminomalonyl-*p*-cyanoanilide Benzyl Ester Tosylate.** *tert*-Butoxycarbonyl-DL-aminomalonyl-*p*-cyanoanilide (4.79 g, 15 mmol), benzyl alcohol (1.55 mL, 15 mmol), and 4-(dimethylamino)pyridine (0.18 g, 1.5 mmol) were dissolved in tetrahydrofuran (60 mL). *N,N'*-Dicyclohexylcarbodiimide (3.3 g, 16 mmol) was added, and the reaction mixture was stirred for 6 h at room temperature and filtered. Evaporation of the filtrate, followed by the usual workup, yielded an oil, which was taken up in acetone (10 mL), cooled, and filtered. Evaporation of the filtrate gave crude *tert*-butoxycarbonyl-DL-aminomalonyl-*p*-cyanoanilide benzyl ester (6.3 g). This oily material was treated with ice-cold tri-

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fluoroacetic acid (50 mL) and left at room temperature for 1.5 h. The reaction mixture was then poured into cold isopropyl ether (400 mL). The precipitate was filtered and the hygroscopic material was dissolved in 5% $NaHCO_3$/ether. The aqueous layer was reextracted with ether, and the combined ether layers were washed with water and dried. A slurry of *p*-toluenesulfonic acid (2.28 g, 12 mmol) in ether was added. Filtration of the precipitate gave the tosylate salt of DL-aminomalonyl-*p*-cyanoanilide benzyl ester: yield 4.8 g (64%); TLC $R_f$ (E) 0.8; $R_f$ (A) 0.4; mp 222 °C dec.

**Trifluoroacetyl-DL-aminomalonyl-*p*-cyanoanilide Benzyl Ester.** DL-Aminomalonyl-*p*-cyanoanilide benzyl ester tosylate (0.5 g, 1 mmol) was dissolved in ice-cold trifluoroacetic acid (5 mL) and treated with trifluoroacetic anhydride (1.44 mL, 10 mmol). After 1 h at room temperature, another 1.4 mL of the anhydride was added. After 4 h, solvent and reagent were removed by evaporation. The residue was taken up in ether and washed with 5% $NaHCO_3$ and water until neutral. The ether layer was dried and evaporated, and the residue was treated with isopropyl ether and finally recrystallized from ether/isopropyl ether: yield 0.32 g (79%); TLC $R_f$ (A) 0.7; $R_f$ (C) 0.6; mp 151–152 °C. Anal. ($C_{19}H_{14}N_3O_4F_3$) C, H, N, F.

**Trifluoroacetyl-DL-aminomalonyl-*p*-cyanoanilide (13).** Trifluoroacetyl-DL-aminomalonyl-*p*-cyanoanilide benzyl ester (0.35 g, 0.9 mmol) was dissolved in methanol (60 mL) at 10 °C and subjected to hydrogenation in the presence of palladium black (18 mg) at room temperature under atmospheric pressure for 3 h. After filtration, the solvent was removed by evaporation. The residue was dissolved in methanol (0.5 mL), and isopropyl ether (50 mL) was added. Filtration, followed by evaporation of the filtrate under reduced pressure and crystallization from isopropyl ether, yielded 13: yield 0.22 g (81%); TLC $R_f$ (E) 0.75; $R_f$ (F); mp 117 °C dec. Anal. ($C_{12}H_8N_3O_4F_3$) C, H, N, F.

**DL-Aminomalonyl-*p*-cyanoanilide (14).** DL-Aminomalonyl-*p*-cyanoanilide benzyl ester tosylate (0.4 g, 0.8 mmol) was dissolved in methanol (40 mL) and 2-propanol (20 mL) and subjected to hydrogenation in the presence of palladium black at room temperature under atmospheric pressure. After 3 h, 0.8 mL of 1 N NaOH was added. Filtration and evaporation gave the crude sodium salt, which was triturated with ethyl acetate and filtered. The solid material was dissolved in water and brought to pH 5 with 0.5 N HCl. After 1 h at 4 °C, the precipitate was filtered and washed with cold water, ethanol/ethyl acetate, and ether to yield 14: yield 0.11 g (63%); TLC $R_f$ (A) 0.2; $R_f$ (G) 0.5; reversed phase TLC $R_f$ (H) 0.5; mp 113 °C dec. Anal. ($C_{10}H_9N_3O_3 \cdot 0.25H_2O$) C, H, N.

**Benzyl-DL-aminomalonyl-L-phenylalanine Methyl Ester Tosylate.** To *N*-(benzyloxycarbonyl)benzyl-DL-aminomalonyl-L-phenylalanine methyl ester[7] (1 g, 1.98 mmol) was added 4 mL of 2 N HBr in HOAc. After 45 min, 50 mL of ethyl acetate was added and the mixture was washed successively with saturated $NaHCO_3$ and $H_2O$. The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was dissolved in 60 mL of ether, and *p*-toluenesulfonic acid (400 mg in 100 mL of ether) was added. The mixture was cooled and filtered to give benzyl-DL-aminomalonyl-L-phenylalanine methyl ester tosylate as a white solid: yield 530 mg (51%); TLC $R_f$ (A) 0.45; mp 178–180 °C dec; $[\alpha]^{25}_D$ 8.5° (*c* 1, MeOH).

***N*-(Trifluoroacetyl)benzyl-DL-aminomalonyl-L-phenylalanine Methyl Ester.** To benzyl-DL-aminomalonyl-L-phenylalanine methyl ester tosylate (348 mg, 0.64 mmol) in 3.5 mL of trifluoroacetic acid was added trifluoroacetic anhydride (1.8 mL, 12.8 mmol) at room temperature. The reaction mixture was stirred for 4 h and then evaporated under reduced pressure. The residue was dissolved in 60 mL of ether and washed successively with $H_2O$, saturated $NaHCO_3$, brine, and $H_2O$. The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was treated with ethyl acetate/hexane to give *N*-(trifluoroacetyl)benzyl-DL-aminomalonyl-L-phenylalanine methyl ester as a white solid: yield 211 mg (80%); mp 120–122 °C; TLC $R_f$ (B) 0.47; $[\alpha]^{25}_D$ 2.3° (*c* 1.1, MeOH). Anal. ($C_{22}H_{21}N_2O_6F_3$) C, H, N, F.

***N*-(Trifluoroacetyl)-DL-aminomalonyl-L-phenylalanine Methyl Ester (18).** *N*-(Trifluoroacetyl)benzyl-DL-aminomalonyl-L-phenylalanine methyl ester (100 mg, 0.26 mmol) was dissovled in methanol (10 mL) and subjected to hydrogenation in the presence of palladium black (5 mg) at room temperature under atmospheric pressure for 2 h. The catalyst was removed by filtration, and the filtrate was evaporated under reduced pressure. The residue was recrystallized from ether/hexanes to give 18 as a white powder: yield 60.4 mg, (75%); mp 109–111 °C; TLC $R_f$ (A) 0.20; $R_f$ (G) 0.67; $[\alpha]^{25}_D$ 6.7° (*c* 1, MeOH). Anal. ($C_{15}H_{15}N_2O_6F_3$) C, H, N, F.

**α-L-Glutamyl-*p*-cyanoanilide (16).** *tert*-Butoxycarbonyl-α-L-glutamyl-*p*-cyanoanilide (1.1 g, 3.2 mmol), prepared as described in the synthesis of 8, was treated with trifluoroacetic acid. After evaporation of trifluoroacetic acid, the residue was dissolved in methanol, and triethylamine was added to neutralize trifluoroacetic acid. Concentration of the solution gave a colorless powder, which was collected by filtration and crystallized from water to give 16 as colorless prisms: yield 0.58 g (74%); mp 160–161 °C; $[\alpha]^{24}_D$ 57.5 °C (*c* 1.2, HOAc). Anal. ($C_{12}H_{13}N_3O_3 \cdot 1.5H_2O$) C, H, N.

**Trifluoroacetyl-L-glutamyl-L-phenylalanine Methyl Ester (17).** *tert*-Butoxycarbonyl-L-glutamic acid γ-benzyl ester (1.01 g, 3 mmol) and L-phenylalanine methyl ester hydrochloride (0.71 g, 3.3 mmol) were dissolved in chloroform/tetrahydrofuran/*N*,*N*-dimethylformamide (2:1:1). The solution was cooled to −10 °C, and to the solution were added triethylamine (0.46 mL, 3.3 mmol), *N*,*N*′-dicyclohexylcarbodiimide (0.69 g, 3.3 mmol), and 1-hydroxybenzotriazole (0.14 g, 1 mmol). The reaction mixture was stirred at −10 °C and then overnight at room temperature. Usual workup gave *tert*-butoxycarbonyl-γ-benzyl-L-glutamyl-L-phenylalanine methyl ester as a colorless oil (1.47 g, 98%), which solidified on standing (mp 78–80 °C). The protected dipeptide (0.73 g, 1.45 mmol) was treated with trifluoroacetic anhydride in trifluoroacetic acid. The usual workup, followed by silica gel column chromatography using chloroform as eluent, afforded trifluoroacetyl-γ-benzyl-L-glutamyl-L-phenylalanine methyl ester (0.32 g, 45%). The trifluoroacetyl derivative (0.27 g, 0.55 mmol) was subjected to hydrogenolysis in methanol under atmospheric pressure of hydrogen in the presence of palladium on charcoal catalyst. The usual workup and crystallization from chloroform gave 17 as colorless needles: yield 0.20 g (90%); mp 137–138 °C; $[\alpha]^{24}_D$ −5.4° (*c* 1.0, acetone). Anal. ($C_{17}H_{19}N_2O_6F_3$) C, H, N.

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