

## Conformationally Restricted Analogues of Anti-aspartame-type Sweeteners

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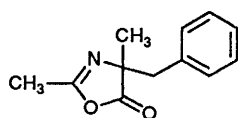
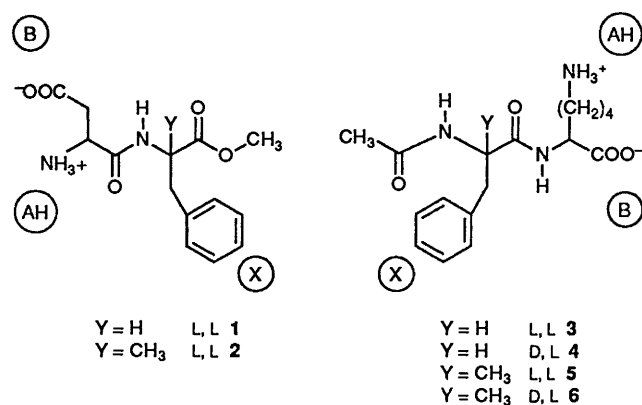
The synthesis and characterization of the two diastereomeric dipeptides Ac-L-( $\alpha$ Me)Phe-L-Lys-OH (**5**) and Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**), conformationally restricted analogues of 'anti-aspartame-type' sweeteners, are described. Both compounds are tasteless. The X-ray diffraction structure of **6**, also reported in this paper, in conjunction with the sweet perception model, as developed by Temussi, Toniolo and co-workers, is used to explain the lack of sweet taste of these compounds. The two diastereomeric synthetic intermediates Ac-L-( $\alpha$ Me)Phe-L-Lys(Z)-OBu' (**9**) and Ac-D-( $\alpha$ Me)-Phe-L-Lys(Z)-OBu' (**10**) have also been characterized by X-ray diffraction.

Aspartame (H-L-Asp-L-Phe-OMe) (**1**), a dipeptide approximately 150–200 times as sweet as sucrose on a weight-for-weight basis, may be the most thoroughly studied food additive ever approved by FDA.<sup>1,2</sup> The commercialization of aspartame has revealed the large market for a safe, low calorie synthetic sweetener with a taste profile accurately reproducing that of sucrose. However, uses of aspartame are limited by the dipeptide's lack of stability at high pH values and at high temperatures, the major degradation pathways involving the ester moiety.

Recently, a Japanese group has proposed a series of chemically stable, synthetic dipeptide sweeteners not containing the ester moiety.<sup>3,4</sup> Among these Ac-L-Phe-L-Lys-OH (**3**) was found to be promising, with a sweetness intensity 23 times that

of aspartame. For this reason these peptides are called 'anti-aspartame-type sweeteners'.

In this paper we wish to report on the synthesis, chemical characterization, and taste determination of the two diastereomeric dipeptides Ac-L-( $\alpha$ Me)Phe-L-Lys-OH (**5**) and Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**), conformationally restricted analogues<sup>5</sup> of the anti-aspartame-type sweeteners Ac-L-Phe-L-Lys-OH (**3**) and Ac-D-Phe-L-Lys-OH (**4**). A comparison between the X-ray diffraction structure of peptide **6**, also described herein, and the model of the active site of the sweet taste receptor, as proposed by Temussi, Toniolo and co-workers,<sup>6–9</sup> is used to explain the observed lack of sweet taste of these two compounds. The X-ray diffraction structures of the two diastereomeric synthetic intermediates Ac-L-( $\alpha$ Me)Phe-L-Lys(Z)-OBu' (**9**) and Ac-D-( $\alpha$ Me)Phe-L-Lys(Z)-OBu' (**10**) are also reported.



L 7  
D 8

of sucrose and a high taste quality. Interestingly, its diastereomer Ac-D-Phe-L-Lys-OH (**4**) is only slightly less sweet (16 times stronger than sucrose). In these compounds the AH-B-X trifunctional unit (AH, acidic proton; B, electronegative centre; X, hydrophobic group), considered responsible for eliciting the sweet taste,<sup>2</sup> is the reverse of that found in

### Experimental

**Materials.**—The synthesis of Ac-L-( $\alpha$ Me)Phe-OH oxazol-5(4H)-one (**7**) was carried out by treatment of H-L-( $\alpha$ Me)Phe-OH (4 mmol) (courtesy of DSM) with acetyl chloride (20 mmol) in pyridine (10 cm<sup>3</sup>) at 0 °C for 3 h. Yield 80%. Oil; [ $\alpha$ ]<sub>D</sub><sup>20</sup> –74.3° (c, 0.5 in ethyl acetate); TLC (silica gel plates 60 F-254, Merck) *R*<sub>F1</sub> (chloroform-ethanol 9:1) 0.80, *R*<sub>F2</sub> (toluene-ethanol 7:1) 0.70;  $\nu_{\max}$ (film)/cm<sup>-1</sup> 1818, 1685;  $\delta_{\text{H}}$ (400 MHz; CDCl<sub>3</sub>; Me<sub>4</sub>Si) 7.21 (5 H, m, phenyl CH), 3.06 [2 H, s, ( $\alpha$ Me)Phe  $\beta$ -CH<sub>2</sub>], 1.99 (3 H, s, acetyl CH<sub>3</sub>), 1.52 [3 H, s, ( $\alpha$ Me)Phe  $\beta$ -CH<sub>3</sub>]. The enantiomeric oxazol-5(4H)-one from Ac-D-( $\alpha$ Me)Phe-OH (**8**) was prepared by a similar procedure using H-D-( $\alpha$ Me)Phe-OH (courtesy of DSM).

The synthesis of Ac-L-( $\alpha$ Me)Phe-L-Lys(Z)-OBu' (**9**) was achieved by treatment of Ac-L-( $\alpha$ Me)Phe-OH oxazol-5(4H)-one (**7**) (3 mmol) with HCl-H-L-Lys(Z)-OBu' (4 mmol) in acetonitrile (10 cm<sup>3</sup>) at room temperature for 20 h in the presence of *N*-methylmorpholine (4 mmol). The product was isolated by 'flash chromatography' using a silica gel support. Yield 46%. M.p. 146–147 °C (from ethyl acetate-light petroleum); [ $\alpha$ ]<sub>D</sub><sup>20</sup> –65.9° (c, 0.5 in methanol); TLC *R*<sub>F1</sub> 0.60, *R*<sub>F2</sub> 0.40, *R*<sub>F3</sub> (butan-1-ol-acetic acid-water 3:1:1) 0.90;  $\nu_{\max}$ (KBr)/cm<sup>-1</sup> 3403, 3304, 1721, 1654, 1527;  $\delta_{\text{H}}$ (CDCl<sub>3</sub>) 7.30 [10 H, m, Z and ( $\alpha$ Me)Phe phenyl CH], 6.70 (1 H, d, Lys  $\alpha$ -NH), 5.80 [1 H, s, ( $\alpha$ Me)Phe NH], 5.52 (1 H, t, Lys  $\epsilon$ -NH), 5.08 (2 H, s, Z CH<sub>2</sub>), 4.45 (1 H, m, Lys  $\alpha$ -CH), 3.33 [2 H, m, ( $\alpha$ Me)Phe  $\beta$ -CH<sub>2</sub>], 3.20 (2 H, m, Lys  $\epsilon$ -CH<sub>2</sub>), 1.92 (3 H, s, acetyl CH<sub>3</sub>), 1.60 (2 H, m, Lys  $\beta$ -CH<sub>2</sub>), 1.50 [3 H, s, ( $\alpha$ Me)Phe  $\beta$ -CH<sub>3</sub>], 1.47 (9 H, s, -OBu'

**Table 1** Fractional coordinates for compound **6**

Atom	x	y	z
O <sub>0</sub>	-0.837 6(2)	0.106 520	-0.411 1(2)
O <sub>1</sub>	-0.699 6(2)	0.429 5(5)	-0.535 5(2)
O <sub>2</sub>	-1.039 4(2)	0.336 3(6)	-0.835 5(3)
O <sub>T</sub>	-0.967 9(2)	0.017 7(6)	-0.756 9(3)
N <sub>1</sub>	-0.747 4(2)	-0.133 4(5)	-0.476 2(2)
N <sub>2</sub>	-0.801 7(2)	0.185 4(5)	-0.659 1(3)
N <sub>2</sub> <sup>c</sup>	-0.886 9(2)	0.233 5(6)	-1.206 0(3)
C <sub>1</sub> <sup>δ22</sup>	-0.606 2(3)	-0.187(1)	-0.201 8(4)
C <sub>1</sub> <sup>ε22</sup>	-0.557 8(4)	-0.358(1)	-0.126 9(4)
C <sub>1</sub> <sup>β2</sup>	-0.466 3(4)	-0.413(1)	-0.123 6(5)
C <sub>1</sub> <sup>ε21</sup>	-0.424 0(4)	-0.293(1)	-0.191 9(5)
C <sub>1</sub> <sup>δ21</sup>	-0.472 0(3)	-0.120(1)	-0.263 6(4)
C <sub>1</sub> <sup>γ2</sup>	-0.564 4(3)	-0.062 6(8)	-0.272 3(3)
C <sub>1</sub> <sup>β2</sup>	-0.617 6(3)	0.120 3(8)	-0.356 2(4)
C <sub>1</sub> <sup>α</sup>	-0.677 4(2)	0.033 5(6)	-0.483 6(3)
C <sub>1</sub> <sup>β1</sup>	-0.614 2(3)	-0.074 9(8)	-0.549 7(4)
C <sub>0</sub> <sup>γ</sup>	-0.821 4(2)	-0.088 3(7)	-0.438 6(3)
C(1)	-0.884 0(3)	-0.283 5(8)	-0.432 3(4)
C <sub>1</sub> <sup>γ</sup>	-0.729 2(2)	0.235 4(6)	-0.561 7(3)
C <sub>2</sub> <sup>α</sup>	-0.872 7(2)	0.348 5(6)	-0.727 4(3)
C <sub>2</sub> <sup>β</sup>	-0.969 3(3)	0.225 1(7)	-0.777 8(4)
C <sub>2</sub> <sup>β</sup>	-0.844 2(3)	0.469 7(7)	-0.827 7(3)
C <sub>2</sub> <sup>γ</sup>	-0.843 0(3)	0.319 5(8)	-0.933 1(4)
C <sub>2</sub> <sup>δ</sup>	-0.798 4(3)	0.439 2(9)	-1.017 0(4)
C <sub>2</sub> <sup>ε</sup>	-0.792 4(3)	0.296(1)	-1.123 0(4)

CH<sub>3</sub>), 1.45 (4 H, m, Lys γ- and δ-CH<sub>2</sub>). Amino acid analysis (C. Erba model 3A 27): (αMe)Phe 1.00, Lys 1.00.

The synthesis of Ac-D-(αMe)Phe-L-Lys(Z)-OBu' (**10**) was performed as described above for the L,L-diastereomer **9**, starting from Ac-D-(αMe)Phe-OH oxazol-5(4H)-one (**8**). Yield 64%. M.p. 127–128 °C (from ethyl acetate–light petroleum); [α]<sub>D</sub><sup>20</sup> +11.0° (c, 0.5 in methanol); TLC R<sub>F1</sub> 0.60; R<sub>F2</sub> 0.40; R<sub>F3</sub> 0.90; ν<sub>max</sub>(KBr)/cm<sup>-1</sup> 3433, 3405, 3293, 1718, 1651, 1537, 1517; δ<sub>H</sub>(CDCl<sub>3</sub>) 7.25 [10 H, m, Z and (αMe)Phe phenyl CH], 6.83 (1 H, d, Lys α-NH), 5.95 [1 H, s, (αMe)Phe NH], 5.29 (1 H, t, Lys ε-NH), 5.08 (2 H, s, Z CH<sub>2</sub>), 4.42 (1 H, m, Lys α-CH), 3.29 [2 H, m, (αMe)Phe β-CH<sub>2</sub>], 3.18 (2 H, m, Lys ε-CH<sub>2</sub>), 1.92 (3 H, s, acetyl CH<sub>3</sub>), 1.67 (2 H, m, Lys β-CH<sub>2</sub>), 1.56 [3 H, s, (αMe)Phe β-CH<sub>3</sub>], 1.46 (9 H, s, -OBu' CH<sub>3</sub>), 1.40 (4 H, m, Lys γ- and δ-CH<sub>2</sub>). Amino acid analysis: (αMe)Phe 1.05, Lys 0.95.

Ac-L-(αMe)Phe-L-Lys(Z)-OH (**11**) was obtained by reaction of Ac-L-(αMe)Phe-L-Lys(Z)-OBu' (**9**) (2.3 mmol) with trifluoroacetic acid (9 cm<sup>3</sup>) in dichloromethane (3 cm<sup>3</sup>) at room temperature for 1 h. Yield 65%. M.p. 174–175 °C (from hot ethyl acetate); [α]<sub>D</sub><sup>20</sup> -81.5° (c, 0.5 in methanol); TLC R<sub>F1</sub> 0.40, R<sub>F3</sub> 0.90; ν<sub>max</sub>(KBr)/cm<sup>-1</sup> 3468, 3422, 3415, 3328, 1700, 1666, 1531; δ<sub>H</sub>(CDCl<sub>3</sub>) 7.15 [11 H, m, Lys α-NH, and Z and (αMe)Phe phenyl CH], 5.84 [1 H, s, (αMe)Phe NH], 5.15 (1 H, t, Lys ε-NH), 5.07 (2 H, s, Z CH<sub>2</sub>), 4.53 (1 H, m, Lys α-CH), 3.30 [2 H, m, (αMe)Phe β-CH<sub>2</sub>], 3.17 (2 H, m, Lys ε-CH<sub>2</sub>), 1.94 (3 H, s, acetyl CH<sub>3</sub>), 1.47 [3 H, s, (αMe)Phe β-CH<sub>3</sub>], 1.70–1.30 (6 H, m, Lys β-, γ- and δ-CH<sub>2</sub>). Amino acid analysis: (αMe)Phe 0.98, Lys 1.02.

The synthesis of Ac-D-(αMe)Phe-L-Lys(Z)-OH (**12**) was carried out as described above for the L,L-diastereomer (**11**), starting from Ac-D-(αMe)Phe-L-Lys(Z)-OBu' (**10**). Yield 60%. M.p. 156–157 °C (from hot ethyl acetate); [α]<sub>D</sub><sup>20</sup> +28.7° (c, 0.5 in methanol); TLC R<sub>F1</sub> 0.40, R<sub>F3</sub> 0.90; ν<sub>max</sub>(KBr)/cm<sup>-1</sup> 3331, 1703, 1664, 1528; δ<sub>H</sub>(CDCl<sub>3</sub>) 7.15 [11 H, m, Lys α-NH, and Z and (αMe)Phe phenyl CH], 5.93 [1 H, s, (αMe)Phe NH], 5.15 (1 H, t, Lys ε-NH), 5.07 (2 H, s, Z CH<sub>2</sub>), 4.53 (1 H, m, Lys α-CH), 3.28 [2 H, m, (αMe)Phe β-CH<sub>2</sub>], 3.17 (2 H, m, Lys ε-CH<sub>2</sub>), 1.94 (3 H, s, acetyl CH<sub>3</sub>), 1.47 [3 H, s, (αMe)Phe β-CH<sub>3</sub>], 1.70–1.30 (6 H, m, Lys β-, γ- and δ-CH<sub>2</sub>). Amino acid analysis: (αMe)Phe 1.06, Lys 0.94.

Ac-L-(αMe)Phe-L-Lys-OH (**5**) was obtained by catalytic hydrogenation with 35 mg of 10% Pd/C in methanol (30 cm<sup>3</sup>) of Ac-L-(αMe)Phe-L-Lys(Z)-OH (**11**) (0.74 mmol). Yield 95%. M.p. 230–231 °C (from methanol–diethyl ether); [α]<sub>D</sub><sup>20</sup> -61.2° (c, 0.5 in methanol); TLC R<sub>F3</sub> 0.50; ν<sub>max</sub>(KBr)/cm<sup>-1</sup> 3400, 3292, 1653, 1581, 1544; δ<sub>H</sub>([<sup>2</sup>H<sub>6</sub>]Me<sub>2</sub>SO-D<sub>2</sub>O 1:1), 7.11 [5 H, m, (αMe)Phe phenyl CH], 3.91 (1 H, m, Lys α-CH), 3.33 and 2.88 [2 H, d, (αMe)Phe β-CH<sub>2</sub>], 2.76 (2 H, t, Lys ε-CH<sub>2</sub>), 1.83 (3 H, s, acetyl CH<sub>3</sub>), 1.55 (2 H, m, Lys β-CH<sub>2</sub>), 1.48 (2 H, m, Lys δ-CH<sub>2</sub>), 1.19 (2 H, m, Lys γ-CH<sub>2</sub>), 1.15 [3 H, s, (αMe)Phe β-CH<sub>3</sub>]. Amino acid analysis: (αMe)Phe 1.03, Lys 0.97.

The synthesis of Ac-D-(αMe)Phe-L-Lys-OH (**6**) was performed as described above for the L,L-diastereomer (**5**), starting from Ac-D-(αMe)Phe-L-Lys(Z)-OH (**12**). Yield 95%. M.p. 247–248 °C (from hot methanol); [α]<sub>D</sub><sup>20</sup> +14.6° (c, 0.5 in methanol); TLC R<sub>F3</sub> 0.50; ν<sub>max</sub>(KBr)/cm<sup>-1</sup> 3335, 3254, 1650, 1573, 1556; δ<sub>H</sub>([<sup>2</sup>H<sub>6</sub>]Me<sub>2</sub>SO-D<sub>2</sub>O 1:1) 7.15 [5 H, m, (αMe)Phe phenyl CH], 4.03 (1 H, m, Lys α-CH), 3.15 and 2.96 [2 H, d, (αMe)Phe β-CH<sub>2</sub>], 2.84 (2 H, t, Lys ε-CH<sub>2</sub>), 1.88 (3 H, s, acetyl CH<sub>3</sub>), 1.58 (2 H, m, Lys β-CH<sub>2</sub>), 1.53 (2 H, m, Lys δ-CH<sub>2</sub>), 1.26 [3 H, s, (αMe)Phe β-CH<sub>3</sub>], 1.18 (2 H, m, Lys γ-CH<sub>2</sub>). Amino acid analysis: (αMe)Phe 1.03, Lys 0.97.

*Crystallographic data for Ac-L-(αMe)Phe-L-Lys(Z)-OBu' (9).* C<sub>30</sub>H<sub>41</sub>N<sub>3</sub>O<sub>6</sub>, M = 539.7. Orthorhombic, a = 34.963(3), b = 15.371(2), c = 5.638(1) Å, V = 3030.0(6) Å<sup>3</sup>, space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> [No. 19], Z = 4, D<sub>c</sub> = 1.18 g cm<sup>-3</sup>, μ = 0.48 cm<sup>-1</sup> (Mo-Kα), final R value 0.083.

*Crystallographic data for Ac-D-(αMe)Phe-L-Lys(Z)-OBu' (10).* C<sub>30</sub>H<sub>41</sub>N<sub>3</sub>O<sub>6</sub>, M = 539.7. Monoclinic, a = 17.670(2), b = 5.610(1), c = 15.334(2) Å, V = 1515.6(6) Å<sup>3</sup>, space group P2<sub>1</sub> [No. 4], Z = 2, D<sub>c</sub> = 1.18 g cm<sup>-3</sup>, μ = 0.48 cm<sup>-1</sup> (Mo-Kα), final R value 0.055.

*Crystallographic data for Ac-D-(αMe)Phe-L-Lys-OH (6).* C<sub>18</sub>H<sub>27</sub>N<sub>3</sub>O<sub>4</sub>, M = 349.4. Monoclinic, a = 14.794(2), b = 5.929(1), c = 11.584(2) Å, V = 962.4(1) Å<sup>3</sup>, space group P2<sub>1</sub> [No.4], Z = 2, D<sub>c</sub> = 1.21 g cm<sup>-3</sup>, μ = 0.51 cm<sup>-1</sup> (Mo-Kα), final R value 0.038.

*X-Ray Crystal Structure Determination of 6, 9 and 10.*—Colourless crystals of **6**, **9** and **10** were grown by slow evaporation of glycerol–water, ethyl acetate–light petroleum, and ethyl acetate–light petroleum solvent mixtures, respectively. Philips PW 1100 diffractometer, θ–2θ scan mode up to 2θ = 56°; graphite-monochromated Mo-Kα radiation (λ = 0.7107 Å); 2539, 4216 and 3856 unique reflections for **6**, **9** and **10**, respectively; 1371, 1244 and 1329 reflections with F ≥ 7σ(F) considered observed for **6**, **9** and **10**, respectively. The three structures were solved by SHELXS 86<sup>10</sup> and refined by blocked least squares with w = 1/σ<sup>2</sup> (F) + 0.0029 F<sup>2</sup> for **6**, 1/σ<sup>2</sup> (F) + 0.0019 F<sup>2</sup> for **9**, and 1/σ<sup>2</sup> (F) + 0.0006 F<sup>2</sup> for **10**. The thermal parameters were anisotropic for all non-hydrogen atoms. The hydrogen atoms of **6** were partially found on a difference-Fourier map and refined isotropically, and partially calculated; the hydrogen atoms of **9** were calculated and not refined; the hydrogen atoms of **10** were partially found on a difference-Fourier map and were not refined, and partially calculated. All calculations were performed on the IBM 370/158 computer of the University of Padova using the SHELX-76 program.<sup>11</sup>

Fractional atomic coordinates for compounds **6**, **9** and **10** are given in Tables 1–3. Tables of hydrogen atom coordinates, thermal parameters, bond lengths, bond angles, and torsion angles for **6**, **9** and **10** are available from the Cambridge Crystallographic Data Centre.\*

\* For details of the deposition scheme see 'Instructions for Authors,' *J. Chem. Soc., Perkin Trans. 2*, 1992, issue 1.

**Table 2** Fractional coordinates for compound **9**

Atom	x	y	z
O <sub>τ</sub>	0.0888(2)	0.3773(5)	0.299(2)
O <sub>2</sub>	0.0854(2)	0.4677(5)	0.618(2)
O <sub>1</sub>	0.0735(3)	0.6863(6)	0.025(2)
O <sub>0</sub>	0.1489(2)	0.7686(5)	0.349(2)
O <sub>2</sub> <sup>ε</sup>	0.2855(3)	0.6518(7)	0.280(3)
O <sub>μ</sub> <sup>1</sup>	0.2558(3)	0.7510(6)	0.497(3)
N <sub>2</sub>	0.0893(3)	0.6091(6)	0.350(2)
N <sub>1</sub>	0.0991(3)	0.7618(6)	0.595(2)
N <sub>2</sub> <sup>ε</sup>	0.2230(3)	0.6825(7)	0.225(3)
C(3)	0.1185(4)	0.289(1)	0.603(3)
C(4)	0.0475(3)	0.297(1)	0.569(3)
C(5)	0.0882(5)	0.228(1)	0.248(3)
C(2)	0.0855(4)	0.2966(8)	0.440(3)
C <sub>2</sub> <sup>1</sup>	0.0886(3)	0.4529(9)	0.406(3)
C <sub>2</sub> <sup>α</sup>	0.0963(4)	0.5288(9)	0.225(2)
C <sub>1</sub> <sup>γ</sup>	0.0779(3)	0.6829(9)	0.241(3)
C <sub>1</sub> <sup>α</sup>	0.0700(3)	0.7605(8)	0.411(2)
C <sub>1</sub> <sup>β1</sup>	0.0311(4)	0.7380(9)	0.531(3)
C <sub>0</sub> <sup>γ</sup>	0.1370(4)	0.7711(8)	0.552(3)
C(1)	0.1617(5)	0.784(1)	0.763(3)
C <sub>1</sub> <sup>β2</sup>	0.0665(3)	0.8465(9)	0.269(3)
C <sub>1</sub> <sup>γ2</sup>	0.0577(4)	0.921(1)	0.432(3)
C <sub>1</sub> <sup>ε22</sup>	0.0854(6)	0.964(1)	0.560(4)
C <sub>1</sub> <sup>ε22</sup>	0.0746(5)	1.026(1)	0.736(4)
C <sub>1</sub> <sup>32</sup>	0.0373(5)	1.052(1)	0.747(4)
C <sub>1</sub> <sup>ε21</sup>	0.0098(6)	1.013(1)	0.618(5)
C <sub>1</sub> <sup>ε21</sup>	0.0216(5)	0.947(1)	0.454(4)
C <sub>2</sub> <sup>β</sup>	0.1377(4)	0.5234(9)	0.118(3)
C <sub>2</sub> <sup>γ</sup>	0.1692(4)	0.5240(9)	0.296(3)
C <sub>2</sub> <sup>δ</sup>	0.2074(4)	0.5253(9)	0.174(4)
C <sub>2</sub> <sup>ε</sup>	0.2182(4)	0.610(1)	0.059(3)
C(13)	0.2556(4)	0.6907(8)	0.333(4)
C(12)	0.2898(6)	0.766(1)	0.652(5)
C(6)	0.2946(5)	0.857(1)	0.699(3)
C(7)	0.3173(6)	0.904(2)	0.558(5)
C(8)	0.3245(5)	0.991(2)	0.603(6)
C(9)	0.3068(5)	1.028(2)	0.792(6)
C(10)	0.2866(6)	0.983(2)	0.945(4)
C(11)	0.2811(5)	0.890(2)	0.900(4)

**Table 3** Fractional coordinates for compound **10**

Atom	x	y	z
O <sub>τ</sub>	1.012 2(3)	0.573 960	0.646 3(3)
O <sub>2</sub>	0.939 1(3)	0.246(2)	0.638 4(4)
O <sub>1</sub>	0.743 8(3)	0.825(1)	0.645 3(3)
O <sub>0</sub>	0.704 2(3)	0.500(1)	0.825 2(3)
O <sub>2</sub> <sup>ε</sup>	0.856 7(4)	0.397(2)	1.119 1(4)
O <sub>μ</sub> <sup>1</sup>	0.739 1(3)	0.318(2)	1.057 5(3)
N <sub>2</sub>	0.814 6(3)	0.492(1)	0.663 6(4)
N <sub>1</sub>	0.683 8(3)	0.257(1)	0.708 7(4)
N <sub>2</sub> <sup>ε</sup>	0.811 8(4)	0.566(2)	0.989 7(4)
C(3)	1.105 4(5)	0.297(2)	0.714 4(6)
C(5)	1.139 6(5)	0.660(2)	0.630 6(7)
C(4)	1.081 4(4)	0.312(2)	0.550 7(5)
C(2)	1.085 7(4)	0.452(2)	0.634 3(5)
C <sub>2</sub> <sup>1</sup>	0.947 2(5)	0.454(2)	0.650 4(5)
C <sub>2</sub> <sup>α</sup>	0.886 6(4)	0.619(2)	0.676 1(4)
C <sub>1</sub> <sup>γ</sup>	0.747 5(4)	0.606(2)	0.652 6(5)
C <sub>1</sub> <sup>α</sup>	0.676 9(4)	0.446(2)	0.643 2(4)
C <sub>1</sub> <sup>β1</sup>	0.606 0(4)	0.592(2)	0.654 5(5)
C <sub>0</sub> <sup>γ</sup>	0.695 7(4)	0.298(2)	0.795 1(5)
C(1)	0.701 8(5)	0.085(2)	0.853 7(6)
C <sub>1</sub> <sup>β2</sup>	0.672 4(4)	0.315(2)	0.552 7(5)
C <sub>1</sub> <sup>γ2</sup>	0.645 2(3)	0.466(1)	0.474 7(3)
C <sub>1</sub> <sup>ε22</sup>	0.571 0(3)	0.437(1)	0.438 3(3)
C <sub>1</sub> <sup>ε22</sup>	0.544 1(3)	0.578(1)	0.367 5(3)
C <sub>1</sub> <sup>32</sup>	0.591 5(3)	0.747(1)	0.333 1(3)
C <sub>1</sub> <sup>ε21</sup>	0.665 7(3)	0.775(1)	0.369 4(3)
C <sub>1</sub> <sup>ε21</sup>	0.692 6(3)	0.635(1)	0.440 2(3)
C <sub>2</sub> <sup>β</sup>	0.903 4(4)	0.709(2)	0.770 4(5)
C <sub>2</sub> <sup>γ</sup>	0.912 1(4)	0.498(2)	0.836 3(5)
C <sub>2</sub> <sup>δ</sup>	0.936 6(5)	0.575(3)	0.929 5(6)
C <sub>2</sub> <sup>ε</sup>	0.880 3(5)	0.711(3)	0.976 9(7)
C(13)	0.807 7(5)	0.422(2)	1.059 5(5)
C(12)	0.727 4(6)	0.161(3)	1.127 8(6)
C(6)	0.647 6(4)	0.089(2)	1.122 7(5)
C(7)	0.599 8(4)	0.252(2)	1.159 6(5)
C(8)	0.521 9(4)	0.210(2)	1.156 6(5)
C(9)	0.491 7(4)	0.004(2)	1.116 6(5)
C(10)	0.539 5(4)	-0.160(2)	1.079 6(5)
C(11)	0.617 4(4)	-0.117(2)	1.082 7(5)

**Taste Determination.**—Dipeptides Ac-L-( $\alpha$ Me)Phe-L-Lys-OH (**5**) and Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**) were taste checked by three volunteers from the DSM Research laboratory. The panel was able to achieve reproducible taste intensities involving solutions of sucrose and of these compounds. Sweetness intensities were determined by a ranking test, with aqueous sucrose solutions of 0.5, 2.0, 4.0 and 8.0% (w/v) as references. At least three double-blind tests were performed by the panel on each compound.

**Stereochemical Receptor Model for Sweet Taste.**—The bi-dimensional contour of the active site model, used to compare the fit of [L-( $\alpha$ Me)Phe]<sup>2</sup>-aspartame (**2**) and Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**), was derived from a combination of the mapping with saccharins and with 3-anilino-2-styryl-3H-naphth[1, 2-d]-imidazolesulfonate, as described in ref. 8. The shape of the receptor active site corresponds to the convolution of the van der Waals radii of the outer atoms of the moulds; it was obtained by positioning around the moulds fictitious 'monoatomic apolar molecules'. The main features of the receptor model can be summarized as follows: (i) The active site of the receptor is a shallow, flat cavity with the outer side accessible even during interaction with the agonist. (ii) The lower part of the cavity contains the main 'electronic features', the most important of which is the AH-B entity (this part is essential for binding). (iii) The upper part of the cavity is hydrophobic and plays an important role in the modulation of sweetness intensity.

## Results and Discussion

**Peptide Synthesis and Characterization.**—Peptide bond formation between the L- or D-( $\alpha$ Me)Phe residue and the N<sup>ε</sup>- and C-protected L-Lys residue was achieved by using the oxazol-5(4H)-one from Ac-L-( $\alpha$ Me)Phe-OH [or from Ac-D-( $\alpha$ Me)Phe-OH] (**7** and **8**, respectively). These reactive synthetic intermediates were obtained in excellent yield by treatment of the free amino acid with an excess of acetyl chloride in pyridine. The chemical and optical stabilities and the high crystallinity of the ( $\alpha$ Me)Phe oxazol-5(4H)-ones have been recently reported.<sup>12</sup> Removal of the C-protecting *tert*-butyloxy group and of the N<sup>ε</sup>-protecting benzyloxycarbonyl group was performed by mild acid hydrolysis and by catalytic hydrogenation, respectively. In addition to the usual analytical and physico-chemical characterizations, the chemical structure and chirality of the crystalline Ac-L-( $\alpha$ Me)Phe-L-Lys(Z)-OBu' (**9**) and Ac-D-( $\alpha$ Me)Phe-L-Lys(Z)-OBu' (**10**) were established by X-ray diffraction (Figs. 1 and 2, respectively, and Table 4).

**Peptide Taste Determination.**—Surprisingly, it was found that Ac-L-( $\alpha$ Me)Phe-L-Lys-OH (**5**) and Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**) are tasteless compared to sucrose, although the zwitterionic structure thought to interact with the taste receptor<sup>2</sup> would be present. With respect to the sweet taste of aspartame and its [L-( $\alpha$ Me)Phe]<sup>2</sup>-analogue,<sup>13</sup> this result is remarkable and worthwhile to study using X-ray diffraction and the sweet perception model as developed by Temussi, Toniolo and co-workers<sup>6-9</sup> (*vide infra*).

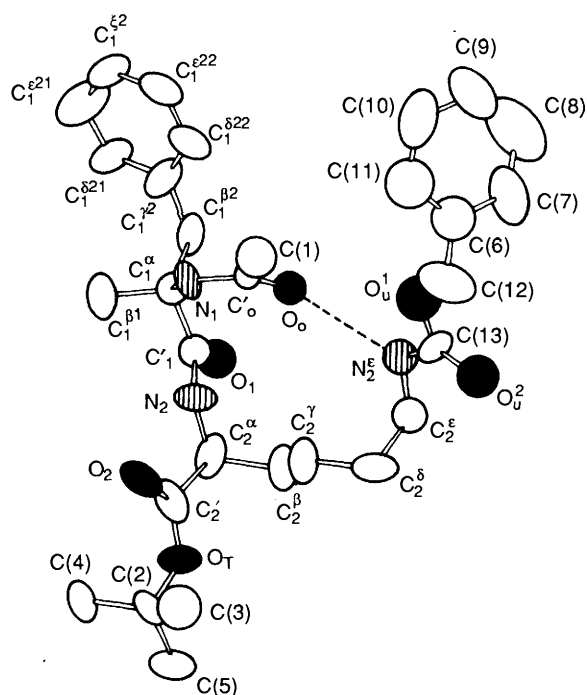


Fig. 1 Molecular structure of Ac-L-( $\alpha$ Me)Phe-L-Lys(Z)-OBu' (**9**) with atom numbering. The side chain-to-main chain intramolecular hydrogen bond is indicated as a dashed line.

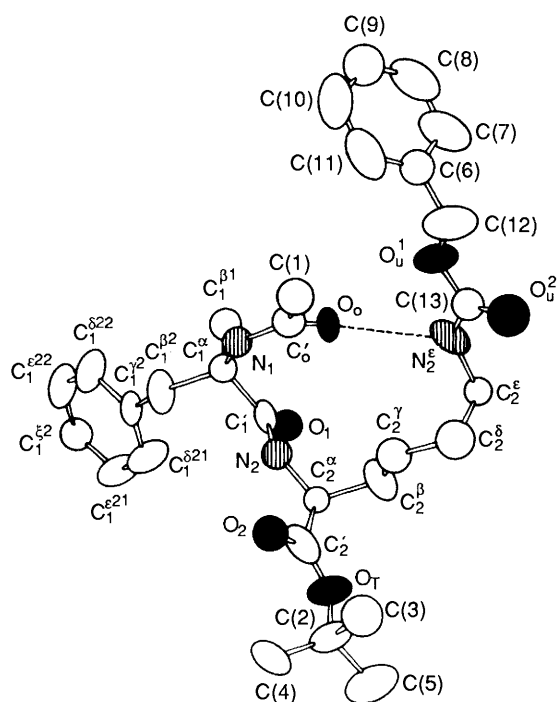


Fig. 2 Molecular structure of Ac-D-( $\alpha$ Me)Phe-L-Lys(Z)-OBu' (**10**) with atom numbering. The side chain-to-main chain intramolecular hydrogen bond is indicated as a dashed line.

**Molecular Structure of Dipeptide 6.**—The X-ray diffraction structure of Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**) with the atomic numbering scheme is shown in Fig. 3. The relevant torsion angles<sup>14</sup> are listed in Table 4.

The title compound is zwitterionic, with the  $C_2'$ - $O_2$  and  $C_2'$ - $O_T$  bond lengths 1.231(5) and 1.252(6) Å, respectively. The set of  $\phi_1$ ,  $\psi_1$  angles of the D-( $\alpha$ Me)Phe residue is indicative of a right-handed helical structure. This chirality-screw sense relationship is inverse to that commonly shown by protein amino acids, including Phe.<sup>15</sup> However, this finding is not sur-

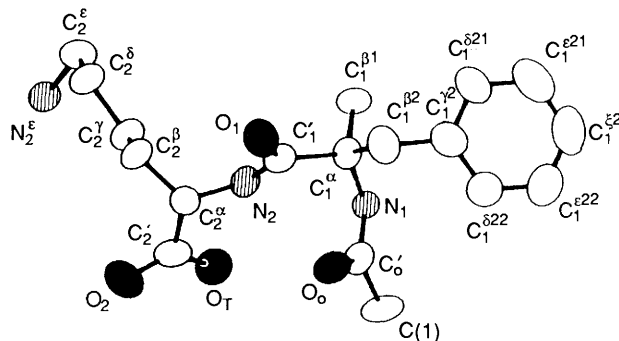


Fig. 3 Molecular structure of Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**) with atom numbering

prising in view of our recent crystallographic and conformational energy computation results on ( $\alpha$ Me)Phe peptides, which strongly support the view that the stability difference between the right- and left-handed helices formed by the two enantiomers of this  $C^\alpha$ -methylated amino acid is significantly lower than that of the two diastereomeric helices formed by its unmethylated counterpart (Phe).<sup>16</sup> The Lys residue is characterized by an extended (*trans*)  $\phi_2$  torsion angle and an almost perfectly *cis* disposition of the  $N_2$ - $C_2^\alpha$ - $C_2'$ - $O_T$  moiety. This latter torsion angle ( $\psi_T$ ) is typical of an intramolecularly hydrogen bonded  $C_5$  conformation.<sup>17,18</sup> The  $N_2 \cdots O_T$  ( $x, y, z$ ) distance is 2.556(5) Å, while the  $N_2H_2 \cdots O_T$  distance is 1.962(33) Å and the  $N_2$ - $H_2 \cdots O_T$  angle is 125.6(4)°. The amide ( $\omega_0$ ) and peptide ( $\omega_1$ ) torsion angles are *trans*,<sup>19-22</sup> with the latter deviating markedly from planarity.

The conformation of the ( $\alpha$ Me)Phe side chain ( $\chi_1^1$ ,  $\chi_1^{2,1}$  and  $\chi_1^{2,2}$  torsion angles) is that commonly observed in peptides for a Phe residue of the same configuration.<sup>22-25</sup> While the disposition of the  $N_2$ - $C_2^\alpha$ - $C_2^\beta$ - $C_2^\gamma$  moiety ( $\chi_2^1$  torsion angle) of the L-Lys residue,  $g^+$ , is different from that usually observed for this residue in peptides ( $g^-$ ), the  $\chi_2^2$  and  $\chi_2^3$  torsion angles are close to those commonly found (*trans*).<sup>22,23</sup>

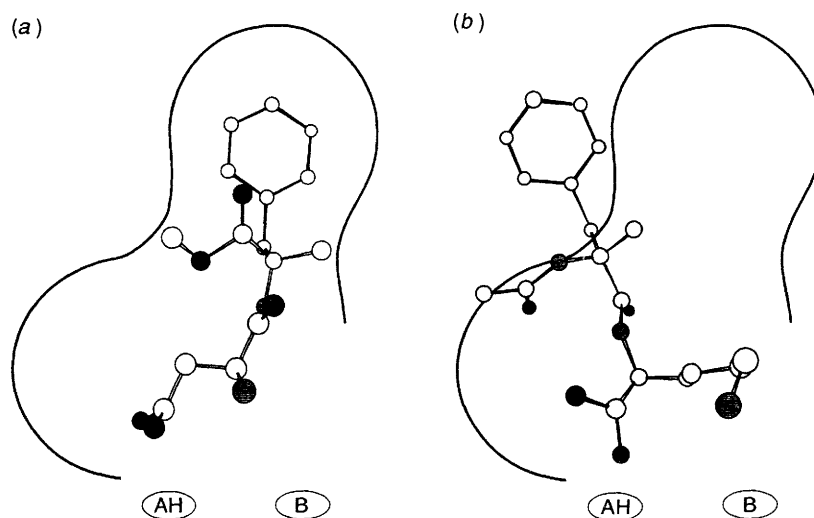
**Structure-Taste Relationships.** The high conformational flexibility of aspartame (**1**) and Ac-D-Phe-L-Lys-OH (**4**) makes any SAR very difficult. In the case of aspartame three different bioactive conformations have been proposed: one consistent with the crystal-state structure,<sup>26</sup> another based on the solution conformation,<sup>27</sup> and a third derived from energy calculations *in vacuo*.<sup>6</sup> All of them have a similar conformation for the Asp side chain and an extended backbone, but differ in the Phe side-chain conformation. The only conformer consistent with our receptor model is that derived from energy calculations, characterized by a Phe side chain *trans* to the main chain ( $\chi^1 = ca. 180^\circ$ ).

A comparison of the crystal structures of [L-( $\alpha$ Me)Phe]<sup>2</sup>-aspartame (**2**)<sup>13</sup> (as sweet as aspartame itself) and of Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**) (tasteless) offers a good opportunity for testing the sweet receptor model. The crystal-state structure of [L-( $\alpha$ Me)Phe]<sup>2</sup>-aspartame (**2**) shows that substitution of a  $\alpha$ Me group for the  $\alpha$ H atom apparently stabilizes a conformation of the benzylic side chain that is *trans* with respect to the peptide main chain, *i.e.* a conformation with  $\chi^1 = ca. 180^\circ$ . Fig. 4(a) shows the fit of the X-ray diffraction structure of [L-( $\alpha$ Me)Phe]<sup>2</sup>-aspartame (**2**) in the model of the active site of the sweet receptor.

In the case of Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**) a similar substitution has the same conformational effect, *i.e.* a stabilization of the benzylic side-chain conformation *trans* to the main chain. This conformation is now *formally* different from that of the corresponding residue in [L-( $\alpha$ Me)Phe]<sup>2</sup>-aspartame (**2**), since the  $\chi^1$  angle of ( $\alpha$ Me)Phe is = *ca.* 60°. The

**Table 4** Selected torsion angles for peptides **6**, **9** and **10** (with esds in parentheses)

Angle		<b>6</b>	<b>9</b>	<b>10</b>
C(1)-C <sub>0</sub> '-N <sub>1</sub> -C <sub>1</sub> <sup>α</sup>	(ω <sub>0</sub> )	-177.6(7)	171.5(12)	-179.8(9)
C <sub>0</sub> '-N <sub>1</sub> -C <sub>1</sub> <sup>α</sup> -C <sub>1</sub> <sup>β1</sup>		-171.0(7)	173.6(12)	-65.5(12)
C <sub>0</sub> '-N <sub>1</sub> -C <sub>1</sub> <sup>α</sup> -C <sub>1</sub> <sup>β2</sup>		66.4(10)	-63.8(16)	174.1(9)
C <sub>0</sub> '-N <sub>1</sub> -C <sub>1</sub> <sup>α</sup> -C <sub>1</sub> <sup>γ</sup>	(φ <sub>1</sub> )	-54.4(10)	60.6(15)	55.8(13)
N <sub>1</sub> -C <sub>1</sub> <sup>α</sup> -C <sub>1</sub> <sup>β2</sup> -C <sub>1</sub> <sup>γ2</sup>	(χ <sub>1</sub> <sup>1</sup> )	58.7(9)	-58.1(15)	165.1(8)
N <sub>1</sub> -C <sub>1</sub> <sup>α</sup> -C <sub>1</sub> <sup>γ</sup> -N <sub>2</sub>	(ψ <sub>1</sub> )	-39.9(9)	40.2(14)	44.4(12)
C <sub>1</sub> <sup>α</sup> -C <sub>1</sub> <sup>β2</sup> -C <sub>1</sub> <sup>γ2</sup> -C <sub>1</sub> <sup>δ21</sup>	(χ <sub>1</sub> <sup>2,1</sup> )	92.1(9)	-98.5(17)	75.3(11)
C <sub>1</sub> <sup>α</sup> -C <sub>1</sub> <sup>β2</sup> -C <sub>1</sub> <sup>γ2</sup> -C <sub>1</sub> <sup>δ22</sup>	(χ <sub>1</sub> <sup>2,2</sup> )	-86.3(9)	82.2(19)	-103.4(10)
C <sub>1</sub> <sup>α</sup> -C <sub>1</sub> <sup>γ</sup> -N <sub>2</sub> -C <sub>2</sub> <sup>α</sup>	(ω <sub>1</sub> )	164.9(7)	177.2(11)	-178.2(9)
C <sub>1</sub> <sup>γ</sup> -N <sub>2</sub> -C <sub>2</sub> <sup>α</sup> -C <sub>2</sub> <sup>γ</sup>	(φ <sub>2</sub> )	-146.8(8)	-152.4(11)	-157.5(10)
C <sub>1</sub> <sup>γ</sup> -N <sub>2</sub> -C <sub>2</sub> <sup>α</sup> -C <sub>2</sub> <sup>β</sup>		88.1(9)	84.5(15)	79.6(12)
N <sub>2</sub> -C <sub>2</sub> <sup>α</sup> -C <sub>2</sub> <sup>γ</sup> -O <sub>T</sub>	(ψ <sub>T</sub> )	-3.1(10)	170.6(10)	169.5(9)
N <sub>2</sub> -C <sub>2</sub> <sup>α</sup> -C <sub>2</sub> <sup>β</sup> -C <sub>2</sub> <sup>γ</sup>	(χ <sub>2</sub> <sup>1</sup> )	68.4(9)	63.5(15)	63.4(11)
C <sub>2</sub> <sup>α</sup> -C <sub>2</sub> <sup>β</sup> -C <sub>2</sub> <sup>γ</sup> -C <sub>2</sub> <sup>δ</sup>	(χ <sub>2</sub> <sup>2</sup> )	-170.3(7)	-175.8(12)	174.2(10)
C <sub>2</sub> <sup>β</sup> -C <sub>2</sub> <sup>γ</sup> -C <sub>2</sub> <sup>δ</sup> -C <sub>2</sub> <sup>ε</sup>	(χ <sub>2</sub> <sup>3</sup> )	178.4(7)	72.7(17)	69.3(14)
C <sub>2</sub> <sup>γ</sup> -C <sub>2</sub> <sup>δ</sup> -C <sub>2</sub> <sup>ε</sup> -N <sub>2</sub> <sup>ε</sup>	(χ <sub>2</sub> <sup>4</sup> )	63.8(9)	64.3(19)	63.2(15)
C <sub>2</sub> <sup>δ</sup> -C <sub>2</sub> <sup>ε</sup> -N <sub>2</sub> <sup>ε</sup> -C(13)		—	83.3(18)	87.5(14)
C <sub>2</sub> <sup>ε</sup> -N <sub>2</sub> <sup>ε</sup> -C(13)-O <sub>u</sub> <sup>1</sup>		—	-172.7(13)	177.7(10)
N <sub>2</sub> <sup>ε</sup> -C(13)-O <sub>u</sub> <sup>1</sup> -C(12)		—	175.5(15)	180.0(10)
C(13)-O <sub>u</sub> <sup>1</sup> -C(12)-C(6)		—	143.1(17)	172.3(10)
O <sub>u</sub> <sup>1</sup> -C(12)-C(6)-C(7)		—	-92.6(23)	-83.1(13)
O <sub>u</sub> <sup>1</sup> -C(12)-C(6)-C(11)		—	97.5(22)	94.6(14)
C <sub>2</sub> <sup>α</sup> -C <sub>2</sub> <sup>γ</sup> -O <sub>T</sub> -C(2)	(ω <sub>T</sub> )	—	174.6(10)	171.4(9)

**Fig. 4** Fit of the X-ray diffraction molecular structures of [L-( $\alpha$ Me)Phe]<sup>2</sup>-aspartame (**2**) (a) and Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**) (b) in the model of the active site of the sweet receptor

conformation of Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**) is no longer compatible with the receptor model [Fig. 4(b)] since the relative orientation of the main chain and the carboxylate group is different from that of [L-( $\alpha$ Me)Phe]<sup>2</sup>-aspartame (**2**), owing to the additional C-C single bond in the latter. This difference can be compensated for by a different conformation of the aromatic residue, as shown by the observation that both Ac-D-Phe-L-Lys-OH (**4**) and Ac-L-Phe-L-Lys-OH (**3**) are sweet. However, this does not apply to Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**), probably owing to its increased conformational rigidity.

In Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**) the terminal carboxylate group of Lys corresponds to the side-chain  $\beta$ -carboxylate group of Asp in [L-( $\alpha$ Me)Phe]<sup>2</sup>-aspartame (**2**), *i.e.* it acts as the B part of the AH-B entity, whereas the <sup>+</sup>NH<sub>3</sub> group of the Lys side chain corresponds to the Asp  $\alpha$ <sup>+</sup>NH<sub>3</sub> group, *i.e.* it represents the AH part of the AH-B entity. In this connection, it may be interesting to check the consistency of the AH-B-X intramolecular distances that, according to the theory proposed by Kier,<sup>28</sup> are responsible for eliciting the sweet taste. In [L-( $\alpha$ Me)-

Phe]<sup>2</sup>-aspartame (**2**) these distances are consistent with those predicted by Kier: AH...B 3.6 Å, AH...X 8.7 Å and B...X 9.4 Å, whereas in Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**) they are AH...B 5.7 Å, AH...X 11.6 Å and B...X 8.7 Å. The relative position of AH and B of Ac-D-( $\alpha$ Me)Phe-L-Lys-OH (**6**) is probably not critical, since the interaction with the receptor counterparts is electrostatic and can easily be adjusted at the receptor site by exploiting the great conformational flexibility of the Lys side chain. On the contrary, the relative orientation of the carboxylate group and the main chain is rigidly fixed by the constitution of the Lys residue, depending only on the N-C<sup>α</sup>-C<sup>γ</sup> bond angle. Thus, the orientation of the whole molecule in the *x, y* plane (the plane of maximum interaction in the receptor model) is also fixed. From Fig. 4(b) it can be seen that, if the Lys carboxylate group (B) interacts with the AH unit of the receptor, the ( $\alpha$ Me)Phe side chain 'invades' the upper wall of the receptor. The only possibility of fitting the receptor would be a change in the conformation of the ( $\alpha$ Me)Phe side chain, which is not allowed owing to the presence of the sterically demanding  $\alpha$ Me group.

## Conclusions

Aspartame (**1**) is a flexible molecule. Therefore, its three-dimensional structure, deduced from an X-ray diffraction analysis,<sup>26</sup> may not be representative of the conformation active at the receptor site. Recently, we have synthesized and solved the crystal structure of a conformationally constrained aspartame analogue, [L-( $\alpha$ Me)Phe]<sup>2</sup>-aspartame (**2**),<sup>13</sup> which fits with only slight modifications to the sweet receptor model proposed by Temussi, Toniolo and co-workers.<sup>6-9</sup>

The drawback of the chemical and thermal instabilities of aspartame (**1**)<sup>1,2</sup> has been successfully removed either by blocking the Asp  $\alpha$ -amino function (*N*-formylcarbamoyl-aspartame has a sweetness comparable to that of aspartame itself)<sup>29</sup> or by synthesizing the anti-aspartame-type sweeteners, e.g., Ac-L-(D)-Phe-L-Lys-OH, **3** and **4**,<sup>3,4</sup> which lack the reactive ester group. In this paper we have described the synthesis and characterization of the conformationally restricted [( $\alpha$ Me)Phe]<sup>1</sup> analogues of the anti-aspartame-type sweeteners **3** and **4**, namely diastereomers **5** and **6**. Both dipeptides proved to be tasteless. Crystalline compound **6** was subjected to an X-ray diffraction investigation.

The results of the taste determination, coupled with the information extracted from the crystal-state structural analysis, indicate that the sweet receptor model proposed by Temussi, Toniolo and co-workers<sup>6-9</sup> can be exploited to discriminate among similar agonists even when Kier's theory<sup>28</sup> is not sufficient.

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Paper 2/02403I

Received 11th May 1992

Accepted 24th June 1992