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The synthesis of artificial sweeteners (phenylglycine-analogues of aspartame) in order to evaluate changes in the γ-glycophore component

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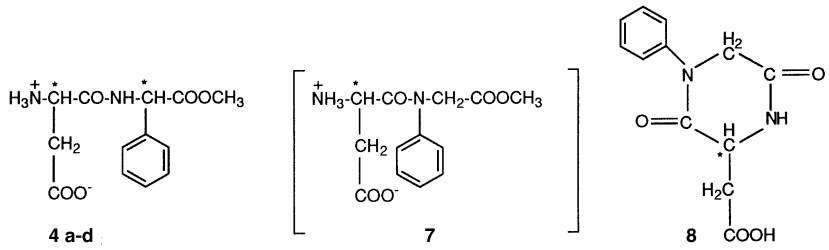
In order to assess the role and function of the third component (X, or γ) in the AH, B glycophore that is a prerequisite for sweet taste, its identity and position in a series of dipeptides was studied. Four isomers of aspartyl-α-phenylglycine methyl ester 4 (isomers 4a, 4b, 4c and 4d) were synthesised and their taste reveals (1) the effect of the size of γ; (2) the effect of the position of γ; and (3) the effect of the nature of γ.



Sensory evaluation showed that, as expected, only the S,S isomer, 4b, tasted sweet while the other three isomers tasted bitter. These results agree with a theory proposed by Goodman (1987). Attempts to produce the analogous *S*-aspartyl-*N*-phenylglycine methyl ester, 7, by a similar route unexpectedly yielded a non-sweet dioxopiperazine derivative, 8, with a sour/bitter taste.

INTRODUCTION

One of the most pressing challenges in the field of taste is elucidation of the nature of the initial chemistry of the phenomenon. Two basic tenets prevail and they are: (1) recognition chemistry; and (2) lock/key binding chemistry with G-protein activation etc. Both tenets suffer from the fact that there is no certain way to determine if cascades of subsequent biochemical events are related to the chemistry that elicits the sweetness modality or are caused by some other biological activities.

Efforts to isolate and characterise sweet taste receptors have been ongoing for decades, without significant success. However, some empirical models (Walters, 1995) have been developed and used to predict the way these receptors operate. Until there is an objective measure of sweetness, all such theories are only speculation, but some of these proposed models are more suitable for the synthetic design of new sweeteners than others:

- The hypothesis introduced by Naim *et al.* (1994) and Bernhardt *et al.* (1996) suggesting the direct G-protein activation does not provide any synthetic guideline.
- The multi-point attachment theory (MPA, Tinti and Nofre, 1991) is too complicated to serve as a useful tool for molecular design. As far as we know, none of the commercially available high-potency sweeteners has been synthesised following a sweetness predicting blueprint.
- The AH–B theory (Shallenberger and Acree, 1967) advanced by Kier (1972), Shallenberger and Lindley (1977) postulating the AH–B–γ, the three-point attachment theory and Goodman's L-shape model (1987), as well as the α-helical receptor protein theory by Suami and Hough (1991), still seems to provide the most useful construction pattern for new sweet-tasting compounds. The original 'AH–B' theory proposed by Shallenberger and Acree (1967) suggests that all sweet-tasting compounds contain a hydrogen bond donor (AH) and a hydrogen bond acceptor

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(B), separated by a characteristic distance. The theory proposed that the receptor must contain a complementary AH–B pair that forms two hydrogen bonds when the sweetener interacts with the receptor. The γ-position in the three-point attachment theory of sweetness is represented by a nonpolar, hydrophobic group with a space-filling requirement. The dimensional requirements of that group are so far unknown.

In order to further investigate the role of γ, as a component of the AH–B–γ theory, we considered several structural and positional changes in γ to vary its influence on the AH–B unit.

MATERIALS AND METHODS

Mps are uncorrected. IR spectra (KBr, NaCl) are reported in cm^{-1}. ^{1}H-NMR spectra were recorded on a Bruker DRX 500 (500.13 MHz), C^{13}-NMR were recorded at 125.6 MHz. Chemical shifts are given in δ (ppm) downfield from TMS. Optical rotations were obtained on a Perkin Elmer 141 polarimeter (c = 1; H_2O). Elemental analysis was obtained by a Perkin Elmer 240 C Elemental Analyzer.

N-(Benzyloxy)carbonyl-(*S*) or (*R*)-aspartic acid anhydride 1(a,b)

Acetic anhydride (3.0 ml) was added to *N*-((Benzyloxy)-carbonyl)-(*R*) or *N*-((Benzyloxy)carbonyl)-(*S*)-aspartic acid (4.0 g, 15 mmol) and the mixture was heated at 50–60°C for 2.5 h. The excess acetic anhydride was removed *in vacuo* and the residue was triturated with diethyl ether and the resulting solid was recrystallised from diethyl ether/petroleum (40–60°C), 3.18 g (85%) yield, mp 106–107°C (John *et al.*, 1954).

(R) or (S)-α-Phenylglycine methyl ester hydrochloride 2(a,b)

α-Phenylglycine (7.55 g, 50 mmol) was refluxed in a solution of thionyl chloride (6.4 g, 50 mmol) in methanol (70 ml) for 3–4 h. A crude product was obtained by distilling off the residual methanol and purified by crystallising from methanol/diethyl ether. The isolated yield was 9.07 g (90%), mp 198–199°C dec; IR (KBr): n 2848 br. (NH_3^+), 1742 (OMe).

N-((Benzyloxy)carbonyl)-aspartyl-phenylglycine methyl ester 3(a-d)

α-Phenylglycine methyl ester hydrochloride 2(a,b) (2.22 g, 11 mmol) was suspended in dichloromethane (15 ml) and treated with triethylamine (1.11 g, 11 mmol) in dichloromethane (7 ml). This was treated dropwise, by stirring, with a solution of *N*-((Benzyloxy)-carbonyl)-aspartic acid anhydride 1(a,b) (2.74 g, 11 mmol) in tetrahydrofuran (10 ml). After 24 h at ambient temperature the solvent was evaporated *in vacuo*, the residue dissolved in a mixture of ethyl acetate and tetrahydrofuran (3:1) (250 ml), then washed three times with water, once with brine, and dried over magnesium sulfate. After evaporation of the solvent, the solid material was triturated with warm diethyl ether to remove impurities and filtered off.

3a: S,R 3.87 g (85%), mp 184.5–185.5°C dec, IR (KBr): ν 3310 (NH), 1736 (OMe), 1695 (COOH), 1655 (CON). 3b: S,S 2.96 g (65%), mp. 129–130°C dec, IR (KBr): ν 3308 (NH), 1737 (OMe), 1699 (COOH), 1655 (CON). 3c: R,R 3.24 g (71%), mp 128–130°C dec, IR (KBr): ν 3311 (NH), 1736 (OMe), 1695 (COOH), 1654 (CON). 3d: R,S 3.42 g (75%), mp 184–186°C dec, IR (KBr): ν 3310 (NH), 1736 (OMe), 1695 (COOH), 1654 (CON).

Aspartyl-α-phenylglycine methyl ester, 4(a-d)

A solution of *N*-((Benzyloxy)carbonyl)-aspartyl-α-phenylglycine methyl ester 3(a-d) (2.96 g, 7 mmol) in methanol (50 ml) containing 10% palladium on charcoal (350 mg) was hydrogenated at 3 atm for 30 min. The catalyst was removed by filtration, the methanol evaporated *in vacuo*, and the residue was recrystallised from isopropanol/H_2O to yield:

4a: S,R 1.47 g (75%), mp 175–176°C dec; anal calcd for $C_{13}H_{16}N_2O_5 \cdot H_2O$ (298.29): C, 52.35; H, 6.08; N, 9.39. Found: C, 52.60; H, 5.56; N, 9.24; IR (KBr): ν 3341 (NH), 1741 (OMe), 1669 (COOH), 1628 (CON); ^{1}H-NMR (DMSO-d_6) δ 2.61–3.08 (m, 2H, CH_2), 3.60 (s, 3H, CH_3), 3.92 (m, 1H, C*H*, phenylgl.), 4.25 (m, 1H, C*H*), 7.38 (s, 5H, arom.), 9.39 (d, 1H, NH); C^{13} (DEPT, DMSO-d_6) δ 35.53 (CH_2), 51.00 (CH, asp), 52.26 (CH, phenylgl), 56.85 (CH_3), 128.25 (CH, arom), 129.17 (CH, arom), 129.55 (CH, arom), 135.92 (C, arom), 169.76 (CO_2^-), 170.92 (CON), 170.73 (COOMe), $[\alpha]^{20}_D = -98.56°$.

4b: S,S 1.53 g (78%), mp 173–174°C dec; anal calcd for $C_{13}H_{16}N_2O_5 \cdot H_2O$ (298.29): C, 52.35; H, 6.08; N, 9.39. Found: C, 52.52; H, 5.70; N, 9.19; IR (KBr): ν 3306 (NH), 1741 (OMe), 1670 (COOH), 1630 (CON); ^{1}H-NMR (DMSO-d_6) δ 2.65–3.10 (m, 2H, CH_2), 3.65 (s, 3H, CH_3), 4.00 (m, 1H, C*H*, phenylgl), 4.32 (m, 1H, C*H*), 7.42 (s, 5H, arom), 9.42 (d, 1H, NH); C^{13} (DEPT, DMSO-d_6) δ 36.53 (CH_2), 51.38 (CH, asp), 53.26 (CH, phenylgl), 57.20 (CH_3), 128.55 (CH, arom), 129.32 (CH, arom), 129.66 (CH, arom), 136.00 (C, arom), 170.34 (CO_2^-), 171.22 (CON), 171.73 (COOMe); $[\alpha]^{20}_D = -106.58°$.

4c: R,R 1.39 g (71%), mp.174–175°C dec; anal calcd for $C_{13}H_{16}N_2O_5 \cdot H_2O$ (298.29): C, 52.35; H, 6.08; N, 9.39. Found: C, 52.42; H, 5.80; N, 9.15.; IR (KBr): ν 3310 (NH), 1741 (OMe), 1670 (COOH), 1630 (CON); ^{1}H-NMR (DMSO-d_6) δ 2.65–3.10 (m, 2H, CH_2), 3.65 (s, 3H, CH_3), 4.00 (m, 1H, C*H*, phenylgl), 4.32 (m, 1H, C*H*), 7.42 (s, 5H, arom), 9.42 (d, 1H, NH); C^{13} (DEPT, DMSO-d_6) δ 36.53 (CH_2), 51.38 (CH, asp), 53.26 (CH, phenylgl), 57.20 (CH_3), 128.55 (CH, arom.), 129.32

(CH, arom.), 129.66 (CH, arom.), 136.00 (C, arom), 170.34 (CO_2^-), 171.22 (CON), 171.73 (COOMe); $[\alpha]^{20}_D = +104.62°$.

4d: R,S 1.35 g (75%), mp 176–177°C dec; anal calcd for $C_{13}H_{16}N_2O_5 \cdot H_2O$ (298.29): C, 52.35; H, 6.08; N, 9.39. Found: C, 52.60; H, 5.56; N, 9.24. IR (KBr): ν 3341 (NH), 1741 (OMe), 1669 (COOH), 1628 (CON); ^{1}H-NMR (DMSO-d_6) δ 2.61–3.08 (m, 2H, CH_2), 3.60 (s, 3H, CH_3), 3.92 (m, 1H, C*H*, phenylgl), 4.25 (m, 1H, C*H*), 7.38 (s, 5H, arom), 9.39 (d, 1H, NH); C^{13} (DEPT, DMSO-d_6) δ 35.53 (CH_2), 51.00 (CH, asp), 52.26 (CH, phenylgl), 56.85 (CH_3), 128.25 (CH, arom), 129.17 (CH, arom), 129.55 (CH, arom), 135.92 (C, arom), 169.76 (CO_2^-), 170.92 (CON), 170.73 (COOMe); $[\alpha]^{20}_D = +99.01°$.

The ^{13}C-NMR spectral assignments were made by standard DEPT methods. As a consequence of the two chiral centres, several of the signals were doublets; these were the benzylic carbon at the phenylglycine and the chiral carbon of the aspartic acid moiety. The adjacent amide carbonyl carbons were also split as was the ester methyl. In the aromatic ring the *o-, m-* and *p*-carbons were also split, though the ring carbon attached to the glycine moiety was not. The explanation for these observations appears to be related to the diastereotopic nature of these centres.

N-((Benzyloxy)carbonyl)-S-aspartyl-*N*-phenylglycine methyl ester, 6

N-Phenylglycine methyl ester hydrochloride, 5 (3.02 g, 15 mmol) was suspended in dichloromethane (20 ml) and treated with triethylamine (1.52 g, 15 mmol) in dichloromethane (10 ml). This was treated dropwise, by stirring, with a solution of *N*-((Benzyloxy)carbonyl)-S-aspartic acid anhydride 1(a) (3.74 g, 15 mmol) in tetrahydrofuran (15 ml). After 24 h at room temperature the solvent was evaporated *in vacuo*, the residue was dissolved in a mixture of ethyl acetate and tetrahydrofuran (3:1) (250 ml), washed three times with water, once with brine, and was then dried over magnesium sulfate. After evaporation of the solvent, the oily residue 5.71 g (92%) was used directly in the next step. IR (NaCl): ν 3312 (NH), 1731 (OMe), 1667 (CON).

1-*N*-Phenyl-2,5-dioxopiperazinyl-3-acetic acid, 8

A solution of *N*-((Benzyloxy)carbonyl)-aspartyl-N-phenylglycine methyl ester, 6 (1.8 g, 4.34 mmol) in methanol (20 ml) containing 10% palladium on charcoal (250 mg) was hydrogenated at 3 atm for 30 min at room temperature. The catalyst was removed by filtration, the solvent was evaporated *in vacuo*. The viscous residue was recrystallised from chloroform. mp 182–183°C; anal calc for $C_{12}H_{12}N_2O_4$ (248.24): C, 48.82; H, 4.87; N, 11.29. Found: C, 48.52; H, 4.76; N, 11.20; IR (KBr): ν 3298, 3214 (NH), 1718 (COOH), 1697 (CON), 1658 (CO); ^{1}H-NMR (DMSO-d_6) δ 2.78 (dd, 2H, CH_2), 4.25-4.50 (m, 3H, CH_2, CH), 7.20–7.60 (m, 5H, arom.), 8.38 (d, 1H, N*H*); ^{13}C: δ 37.41 (CH), 51.60 (CH_2), 125.51, 126.61, 128.79 (C arom), 141.00 (C arom), 165.03 (CO_2^-), 166.18 (CO), 171.72 (CO).

Taste assessment

Taste tests were carried out by a 'sip and spit' assessment of solutions of the substances using a five-member taste panel. The compound S-Aspartyl-S-α-phenylglycine methyl ester, 4b, was tested in water at room temperature without any pH adjustment, starting at 2000 ppm concentration. The test solution was diluted as necessary in order to match a 200 ppm aspartame solution used as the standard. Conversion to sucrose values was made on the basis of the sweetness potency of aspartame (150 × sucrose).

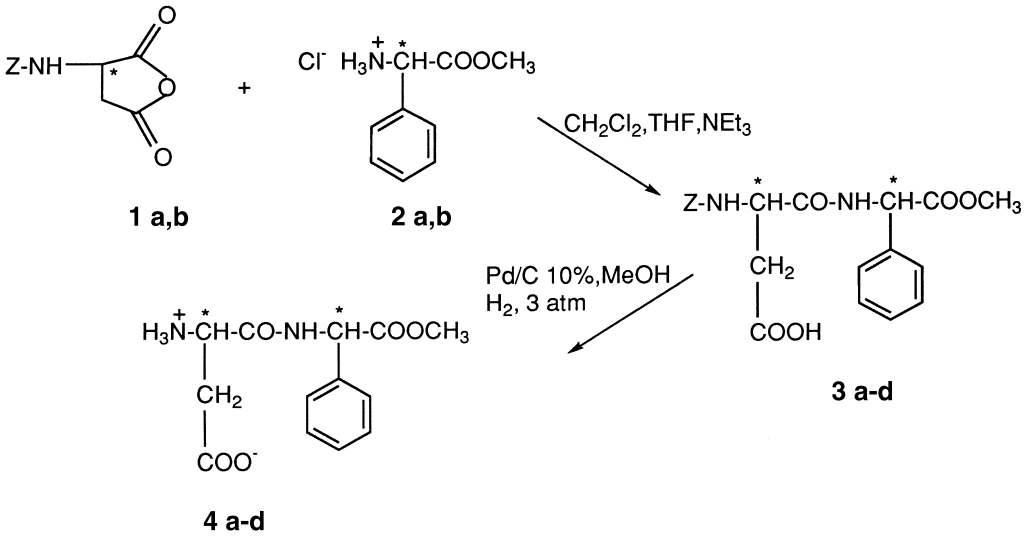
RESULTS AND DISCUSSION

Objective I: structural changes in γ

Starting off with aspartame as our paradigm it was first planned to shorten the side chain of the second amino acid by replacing the phenylalanine methyl ester by the phenylglycine methyl ester. The S, S isomer of this type, 4, (S-aspartyl-S-phenylglycine methyl ester) is already mentioned as a high-potency sweetener in 1976 in a US Patent granted to Moriarty and Tritsch. The synthesis was carried out by coupling of phenylglycine methyl ester and Z-protected asparagine. To obtain the final product, they claimed to have cleaved the primary amide of the asparagine moiety to yield the acid by treatment with dilute hydrochloric acid at elevated temperatures.

Careful theoretical examination of the reported synthetic method suggests that this route would be unlikely to yield the desired product, because the molecule should be susceptible to cleavage under acidic conditions at the positions listed in order of susceptibility: (1) methyl ester group (most likely); (2) amide function; and (3) peptide bond. This previous work was repeated and it was found that the dominating product from this series of reactions was not the anticipated one but, instead, S-asparaginyl-S-phenylglycine hydrochloride. Not surprisingly this has a sour taste with a bitter aftertaste. The report of the patented high-potency sweetener is therefore difficult to understand.

Synthesis of all four isomers of aspartyl-α-phenylglycine methyl ester, 4(a-d) in high yield (Scheme 1) was achieved using a new approach. Moisture-sensitive, freshly prepared Z-protected S- or R-aspartic acid anhydried 1(a, b) was reacted with R- or S-α-phenylglycine methyl ester which had been released from the corresponding hydrochloride 2(a,b). The Z-protected compounds 3(a–d) showed gradual racemisation in DMSO which was discovered and followed by ^{1}H-NMR. This is due to oxo-enol tautomerism via the ester group of the phenylglycine moiety in the presence of DMSO. The final products 4(a–d) were obtained by deprotection.



4 a-d
a: S-Aspartyl-(R)-(-)-phenylglycine methyl ester
b: S-Aspartyl-(S)-(+)-phenylglycine methyl ester

c: R-Aspartyl-(S)-(+)-phenylglycine methyl ester
d: R-Aspartyl-(R)-(-)-phenylglycine methyl ester

Scheme 1

An important task was to determine, if the aspartic acid anhydride had reacted at the α- or at the β-carboxylic acid function. In order to confirm that the desired α-carboxylic acid derivative was obtained, as an example, the S, S isomer, 4b, was synthesise by an alternative route.

This time the Z-S-aspartic acid β-benzyl ester and S-α-phenylglycine methyl ester hydrochloride were coupled with the aid of DCC (Mazur *et al.*, 1969), followed by removal of both of the protecting benzyl groups by hydrogenolysis. This experiment confirmed that identical products were obtained following either procedure.

Sensory evaluation showed that only the S,S-isomer, 4b, tastes sweet (100 × sucrose). However, S,R and R,S are bitter and R,R has a hot, bitter, slightly sweet taste. The claim by Janusz, (1987) that the S,R isomer is intensely sweet is questionable, based on the findings of the current study. It should be pointed out that omitting the methylene group in the side chain of the phenylalanine moiety in aspartame retains similar high-potency sweetness since the receptor requirements are still fulfilled.

The tastes obtained also agree with the 'L shape structure theory' proposed by Goodman (Goodman *et al.*, 1987). The S, S isomer of aspartyl-phenylglycine methyl ester was modelled, energy-minimised using a Silicon graphics workstation, and the software package Insight II (Builder, Discover), and the conformation with the lowest energy content showed the expected L shape (Fig. 1). According to Goodman's theory, only the S-asp-(S)-α-phenylglycine-OCH_3 isomer can adopt the requisite 'L' shaped structure with the zwitterionic aspartyl moiety as the stem of the 'L' on the $+ y$ axis and the hydrophobic phenyl side chain as the base of the 'L' on the $+ x$ axis, while the S-asp-(R)-α-phenylglycine-OCH_3 isomer will adopt a 'reversed L' structure where the phenyl side chain orients towards the $-x$ axis.

The two other isomers (R-asp-(S)- and (R)-α-phenylglycine-OCH_3 are completely unable to bind at the sweet taste receptor, resulting in a bitter taste.

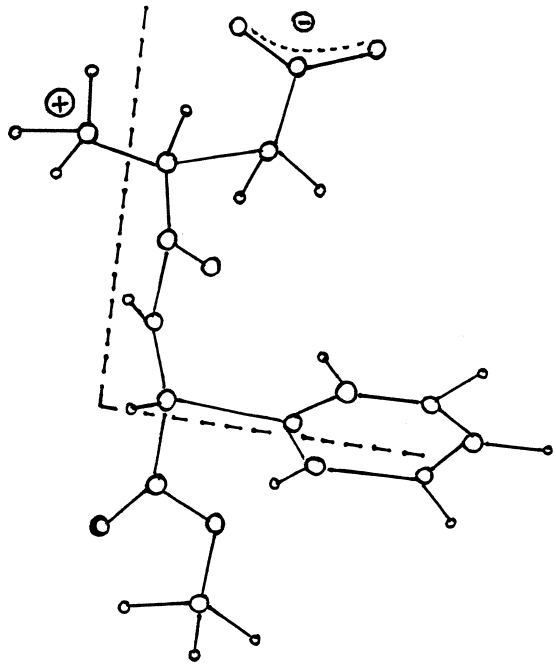
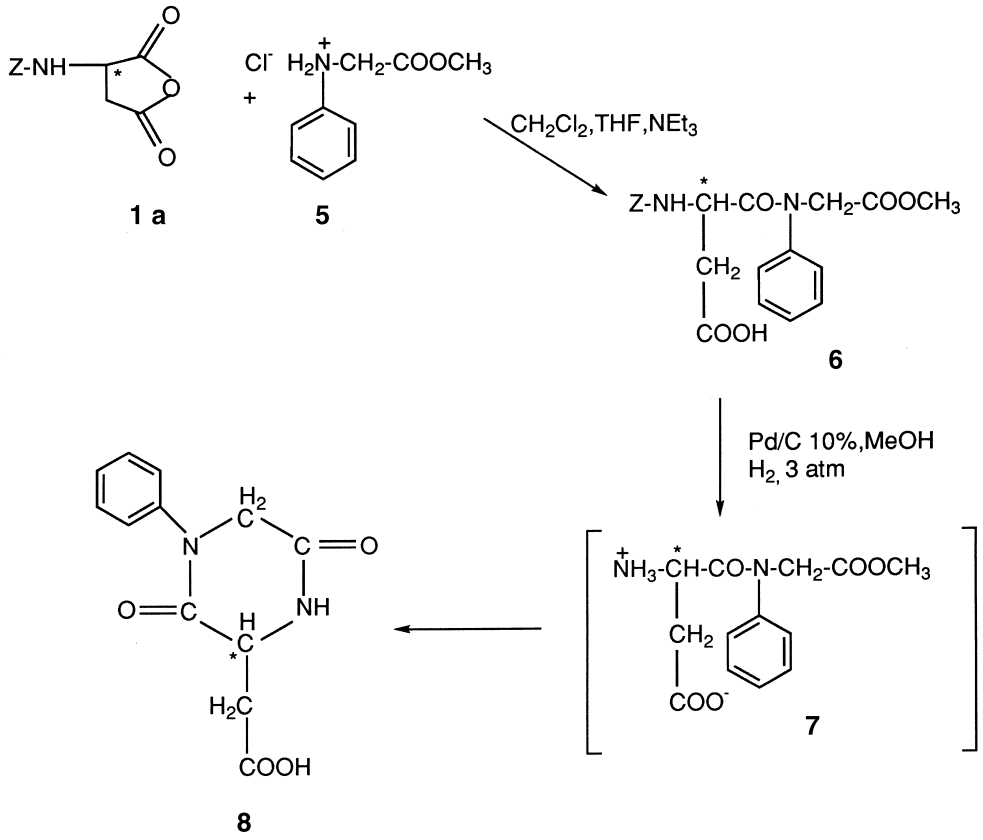


Fig. 1. Required L-shaped molecule for sweetness.



Scheme 2

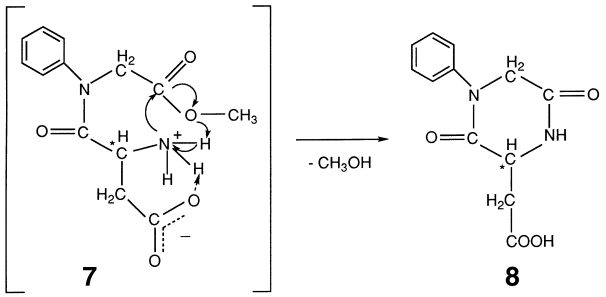


Fig. 2. Formation of cyclic dioxopiperazine, **8**.

Objective II: positional changes in γ

To develop this idea, the synthesis of the pure enantiomer of S-aspartyl-N-phenylglycine methyl ester, 7, (Scheme 2) was attempted, where the γ function is placed closer to the AH-B unit. The new approach employed Z-S-aspartic acid anhydride, 1a, and *N*-phenylglycine methyl ester hydrochloride 5. This preparation series was designed to yield S-aspartyl-*N*-phenylglycine methyl ester but resulted instead in a compound which surprisingly showed no methyl ester function (IR-, ^{1}H-NMR-spectra). The most likely explanation is that cyclisation of 7 occurred intramolecularly following the hydrogenation to form the cyclic dioxopiperazine 8 (Fig. 2).

The decrease in steric hindrance and increase in conformational mobility of the ester group by shifting the phenyl substituent to the *N*-atom favours the attack of the intermediate ammonium ion, 7. The elimination of methanol and simultaneous closure to a six-membered ring follow in consequence. A similar cyclisation does not appear in 4(a-d) because of the proximity of the bulky phenyl group to the ester.

Sensory evaluation of the purified compound, 8, showed it to have a sour/bitter taste. Also, this compound does not meet the structural requirements for the AH-B-γ system; thus it cannot adopt the required conformations.

CONCLUSIONS

- The exchange of the benzyl moiety in the aspartame structure for phenyl results in: a high potency sweetener (S,S), bitter (S,R and R,S) and a hot, bitter slightly sweet tasting (R,R) compound.
- The sweet taste receptor is stereoselective since only the S,S isomer tastes sweet (in accordance with aspartame), as only this isomer can adopt the requisite L-shape and thereby bind at the receptor.
- A change in the size in γ is possible without omitting its ability to interact with or affect the ability of AH,B to interact with the B, HA at the receptor. This is possibly because of an unaltered electron distribution at the components of the AH-B dipole.

- The stereoelectronic effect of the chosen γ (phenyl) seems to be applied equally to each component of that dipole.
- The question of how variations in the distance between AH-B and γ will influence the sweet properties of a compound remains an object of further investigation.

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