Brief Articles

Active Conformations of Neotame and Other High-Potency Sweeteners

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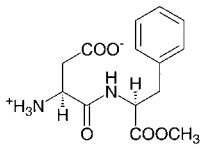
We carried out extensive conformational analysis of three high-potency sweeteners: neotame, superaspartame, and SC-45647. We then identified six possible pharmacophore features (carboxylate, two hydrophobic groups, and three NH groups) and wrote a computer program to exhaustively compare intramolecular distances among all possible sets of five-point pharmacophores (carboxylate + two hydrophobic groups + two NH groups) for the three compounds. The best pharmacophore model superimposes low-energy conformers of the three compounds in such a way that the five pharmacophore points match well both sterically and with respect to orientation of hydrogen bond donors and acceptors.

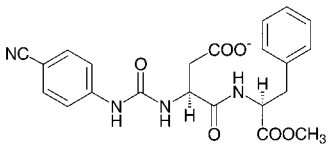
Introduction

How do high-potency sweeteners interact with their receptors? Since the receptors have not yet been identified or characterized, we are currently able to address this question only by studying the ligands which activate these receptors. It is likely that multiple receptor types mediate sweet taste,[1] so we have chosen to focus on structurally related compounds which have a high probability of acting at a common receptor site.

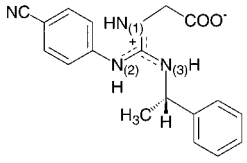
Aspartame (**1**) is a dipeptide methyl ester which was found in 1965 to taste sweet, with a potency of about 180 times that of sucrose (i.e., a 0.011% solution of aspartame has the same level of sweetness as a 2% solution of sucrose).[2] Structure−activity studies led to the discovery of several analogues with even higher potencies. Tinti and Nofre showed that the arylurea-substituted dipeptide **2** (which they named "superaspartame") has a potency 14 000 times that of sucrose.[3,4] Subsequently they discovered sweet guanidines such as **3** (given the identification code SC-45647), which has a potency 28 000 times that of sucrose.[4,5] Most recently, they reported *N*-alkyl-substituted aspartame derivatives such as neotame (**4**), which has a potency 10 000 times that of sucrose.[6] We expect that these compounds all act at a common receptor site, since they share the following structural features: a carboxylate group, two or three polar hydrogen atoms attached to nitrogen atoms, one or two hydrophobic substituents, and, in the superaspartame series and the guanidines, electronegative aryl substituents such as cyano and nitro groups, which increase potency substantially.



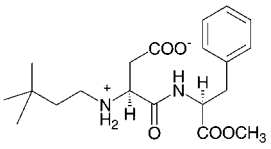
1, Aspartame



2, Superaspartame



3, SC-45647



4, Neotame

Further, as we show in this paper, all of these compounds can be superimposed, in accessible, low-energy conformations, in ways which present common patterns of pharmacophoric groups (carboxylate, polar hydrogens, hydrophobic groups) to the receptor. All molecules were modeled in their most populated ionization states at pH 7. Neotame and SC-45647 were modeled as zwitterions and superaspartame as an anion. Although superaspartame does not have a formal positive charge, Tinti and Nofre showed[3] that sweetness potency is heavily dependent on the electron-withdrawing strength of the para-substituent on the arylurea ring; strongly electronegative substituents such as *p*-cyano further polarize the already polar aryl-NH group.

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Our approach in this work has been, first, to carry out complete conformational analysis of each compound. For each conformation of each compound, we calculated all intramolecular distances between pharmacophoric groups (carboxylate to hydrophobic-1 and hydrophobic-2, carboxylate to NH-1, NH-2, and NH-3; hydrophobic-1 to hydrophobic-2 and to each NH; hydrophobic-2 to each NH; each NH to each other NH). We then wrote a computer program which compared distance patterns for each conformation of one compound with those of all conformations of a second compound. In each comparison, we required a carboxylate-to-carboxylate match; we required two hydrophobic-to-hydrophobic matches (hydrophobic-1 of the first molecule could match with either hydrophobic-1 or hydrophobic-2 of the second molecule, and vice versa); we required two NH-to-NH matches, and we examined all possible combinations of NH-to-NH match to find the best set in each case.

Methods

Conformational analysis was carried out using the MacroModel program.[7] We used the MMFF force field[8] with the planar sp^2 nitrogens option. Conformational searching was carried out using the Monte Carlo multiple minimum method,[9] followed by truncated Newton conjugate gradient minimization. Monte Carlo searching continued until extended runs produced no new conformations. For neotame, 137 600 conformers were examined; for SC-45647, only 2 200 were required, since it has fewer rotatable bonds; for superaspartame, 41 000 conformations were examined.

The GB/SA solvation model[10] for water was tried in initial studies of aspartame conformations, but this produced a family of conformations which were not consistent with NMR spectroscopy.[11] For example, aspartate and phenylalanine side chains are expected to have trans and gauche rotamers populated, but we found few of these and many eclipsed or nearly eclipsed rotamers arising from the conformational search/minimization. Therefore, all calculations reported in this paper were carried out without solvent terms.

Identification of Matching Conformers. At the completion of conformational analysis, we identified 874 neotame conformers, 34 SC-45647 conformers, and 390 superaspartame conformers. We selected six possible pharmacophoric groups in each molecule.

For neotame: (1) carboxylate group (measured from the centroid between the two oxygen atoms), (2) hydrophobic-1 (measured from the centroid of the neohexyl atoms), (3) hydrophobic-2 (measured from the centroid of the -CH($COOCH_3$)-CH_2-phenyl atoms), (4) NH-1 (defined as one of the hydrogen atoms on the Asp-nitrogen), (5) NH-2 (defined as the other hydrogen atom on the Asp-nitrogen), (6) NH-3 (the amide hydrogen).

For SC-45647: (1) carboxylate group (measured from the centroid between the two oxygen atoms), (2) hydrophobic-1 (measured from the centroid of the cyanophenyl atoms), (3) hydrophobic-2 (measured from the centroid of the α-phenethyl atoms), (4) NH-1 (defined as the hydrogen atom attached to the N-CH_2-COO^- nitrogen), (5) NH-2 (defined as the hydrogen atom attached to the N-cyanophenyl nitrogen), (4) NH-3 (defined as the hydrogen atom attached to the N-phenethyl nitrogen).

For superaspartame: (1) carboxylate group (measured from the centroid between the two oxygen atoms), (2) hydrophobic-1 (measured from the centroid of the cyanophenyl atoms), (3) hydrophobic-2 (measured from the centroid of the -CH($COOCH_3$)-CH_2-phenyl atoms), (4) NH-1 (the Asp-NH), (5) NH-2 (the cyanophenyl-NH), (6) NH-3 (the amide hydrogen).

In comparing a given conformer of one molecule with a conformer of another molecule, we looked for the best match in which (a) carboxylate groups were matched; (b) two hydrophobic groups were matched, either H-1 to H-1 and H-2 to H-2 or H-1 to H-2 and H-2 to H-1; (c) any two NH hydrogens of one molecule matched any two NH hydrogens of the other molecule. We carried out the comparison in the following way. Using the MacroModel program, we calculated for each conformer a matrix of all 15 intramolecular distances between pairs of the six pharmacophore points.

Distance...	between points
1	1 and 2
2	1 and 3
3	1 and 4
4	1 and 5
5	1 and 6
6	2 and 3
7	2 and 4
8	2 and 5
9	2 and 6
10	3 and 4
11	3 and 5
12	3 and 6
13	4 and 5
14	4 and 6
15	5 and 6

For each molecule, we could compare 12 different sets of five points, represented by 10 intramolecular distances, which met the three criteria described above:

Set of points	Set of intramolecular distances to be compared
{1,2,3,4,5}	{1,2,3,4,6,7,8,10,11,13}
{1,2,3,4,6}	{1,2,3,5,6,7,9,10,12.14}
{1,2,3,5,4}	{1,2,4,3,6,8,7,11,10,13}
{1,2,3,5,6}	{1,2,4,5,6,8,9,11,12,15}
{1,2,3,6,4}	{1,2,5,3,6,9,7,12,10,14}
{1,2,3,6,5}	{1,2,5,4,6,9,8,12,11,15}
{1,3,2,4,5}	{2,1,3,4,6,10,11,7,8,13}
{1,3,2,4,6}	{2,1,3,5,6,10,12,7,9,14}
{1,3,2,5,4}	{2,1,4,3,6,11,10,8,7,13}
{1,3,2,5,6}	{2,1,4,5,6,11,12,8,9,15}
{1,3,2,6,4}	{2,1,5,3,6,12,10,9,7,14}
{1,3,2,6,5}	{2,1,5,4,6,12,11,9,8,15}

To calculate the similarity of one conformer of one molecule with a conformer of another molecule, then, we carried out 144 comparisons (e.g., 12 different sets of pharmacophore points for molecule 1 were each compared to 12 different sets of pharmacophore points for molecule 2). For each comparison, we summed up the difference between intramolecular distances. For example, to compare the set of points {1,2,3,4,6} of molecule 1 with the set of points {1,3,2,5,4} of molecule 2, we summed up the following differences:

mol-1 distance-1 vs mol-2 distance-2
mol-1 distance-2 vs mol-2 distance-1
mol-1 distance-3 vs mol-2 distance-4
mol-1 distance-5 vs mol-2 distance-3

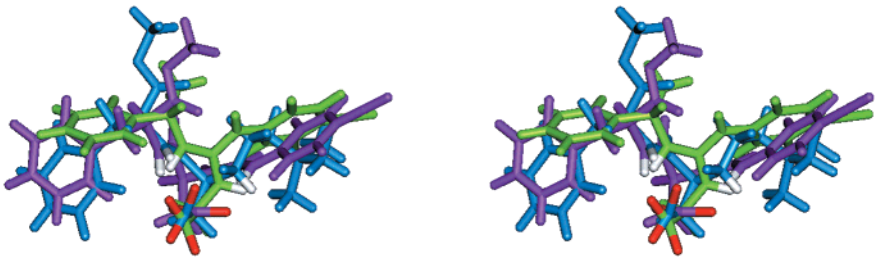

Figure 1. Superposition of SC-45647 conformer **21** (green), neotame conformer **254** (blue), and superaspartame conformer **297** (purple). Key pharmacophore atoms are colored red (carboxylate oxygens) or white (NH hydrogens).

mol-1 distance-6 vs mol-2 distance-6
mol-1 distance-7 vs mol-2 distance-11
mol-1 distance-9 vs mol-2 distance-10
mol-1 distance-10 vs mol-2 distance-8
mol-1 distance-12 vs mol-2 distance-7
mol-1 distance-14 vs mol-2 distance-13

A computer program was written to carry out the distance comparisons. The set which gave the lowest sum-of-differences was considered the best match between the given conformer of molecule 1 and the given conformer of molecule 2. We could then systematically find, for each conformer of molecule 1, the best-match conformer of molecule 2, along with the best pharmacophore pattern match.

Since SC-45647 had the fewest conformations (34), we first compared each SC-45647 conformation with the best-matching neotame conformation identified. The matches identified by the program were examined visually on a 3D computer graphics terminal, so that we could evaluate not only the superposition of points as described above but also the orientations of carboxylate groups, orientations of NH groups, and overall similarities of hydrophobic groups. Eight of the 34 pairs appeared to be reasonable:

SC-45647 conformer 2 and neotame conformer 832
SC-45647 conformer 3 and neotame conformer 108
SC-45647 conformer 4 and neotame conformer 850
SC-45647 conformer 13 and neotame conformer 571
SC-45647 conformer 20 and neotame conformer 31
SC-45647 conformer 21 and neotame conformer 211
SC-45647 conformer 24 and neotame conformer 11
SC-45647 conformer 34 and neotame conformer 22

Next we ran the program to compare SC-45647 conformers with superaspartame conformers and, in a separate run, compared neotame conformers with superaspartame conformers. For each of the eight pairs listed above, we carried out a visual comparison with the best-matching superaspartame conformers. Superimposed sets were evaluated with respect to orientations of carboxylate groups, orientations of NH groups, and overall similarities of hydrophobic groups.

Results and Discussion

Among the eight sets of three-compound superpositions, one was clearly superior to the others. This was the set consisting of SC-45647 conformer **21**, neotame conformer **211**, and superaspartame conformer **297**, shown in Figure 1. The other sets had either a poor steric overlap of one of the hydrophobic groups or, in two cases, one of the NH groups oriented in a direction almost opposite to that of the other two molecules. It can be seen in Figure 1 that carboxylate groups, NH groups, and hydrophobic groups superimpose quite well. The torsion angles which describe these conformers are listed in Table 1.

It is interesting to compare the neotame and superaspartame conformers. The phenylalanine methyl ester portions of these molecules are in very similar conformations, with the phenylalanine side chain in a gauche$^-$ (g$^-$) orientation. However, the N-substituted aspartyl portions must adopt different conformations in order to superimpose NH and hydrophobic substituents. This is because the arylurea and aliphatic substituents have much different conformational restraints. The aspartyl Φ, Ψ, and χ_1 torsions of neotame and superaspartame differ by 118°, 87°, and 48°, respectively, but both compounds are able to place the carboxylate and flanking NH groups in comparable positions. It is also noteworthy that a majority of the low-energy conformers of both neotame and superaspartame place the carboxylate side chain in position to hydrogen bond to both the amide NH and one of the amine or urea NH groups.

Our conformational analysis was helped substantially by the inclusion of SC-45647. The dipeptide derivatives have hundreds of accessible conformations, and many of the neotame conformers can superimpose well on superaspartame conformers. By themselves, such compounds would not provide sufficient information to identify a single active conformation. The high-potency guanidine derivatives array the same key functional groups (carboxylate, NH's, hydrophobics) on a much different skeleton. While the peptides provide a linear

Table 1. Torsion Angles of Receptor-Active Conformers

angle	degrees
(a) SC-45647 Conformer **21**	
$N_{(3)}-C-N_{(1)}-CH_2$	−1
$C-N_{(1)}-CH_2-COO^-$	−62
$N_{(1)}-C-N_{(2)}-C_{CNphenyl}$	26
$C-N_{(2)}-C_{CNphenyl}-CH_{CNphenyl}$	25
$N_{(1)}-C-N_{(3)}-CH$	−166
$C-N_{(3)}-CH-C_{phenyl}$	170
$N_{(3)}-CH-C_{phenyl}-CH_{phenyl}$	75
(b) Neotame Conformer **211**	
Asp-Φ	−50
Asp-Ψ	−64
Asp-χ_1	53
amide	175
Phe-Φ	−84
Phe-Ψ	164
Phe-χ_1	−70
ester	180
$C\alpha_{(Asp)}-N_{(Asp)}-C1-C2$	−74
$N_{(Asp)}-C1-C2-C3$	−85
C1−C2−C3−C4	178
(c) Superaspartame Conformer **297**	
Asp-Φ	68
Asp-Ψ	−151
Asp-χ_1	101
amide	−174
Phe-Φ	−75
Phe-Ψ	139
Phe-χ_1	−69
ester	−177
$C\alpha_{(Asp)}-N_{(Asp)}-C(=O)-N$	8
$N_{(Asp)}-C(=O)-N-C1_{(CNphenyl)}$	−163
$C(=O)-N-C1_{(CNphenyl)}-C2_{(CNphenyl)}$	−173

scaffold, the guanidines form a compact, planar, radial platform, with far fewer degrees of conformational freedom.

Aspartame and other dipeptides have been the subject of extensive conformational studies (computational, crystallographic, and spectroscopic) over the past 30 years. Temussi and co-workers have proposed an active conformation of aspartame, based on NMR and conformational energy calculations and on comparison to a conformationally rigid heterocycle.[11,12] The NMR results show clearly that the peptides are very flexible. All three side-chain rotamers of both the aspartate and phenylalanine side chains are substantially populated, and backbone torsions typically have 120° ranges (except amide and ester torsions). It is not clear that the comparison of dipeptides to a naphthimidazolesulfonic acid is a reasonable choice, since the latter compound differs substantially from the peptide sweeteners: (a) the carboxylate is replaced by a sulfonate; (b) there is only one NH group; (c) the single NH is not able to adopt a position anywhere close to the sulfonate. Therefore we suspect that this compound is acting at a receptor site different from the one used by the dipeptides and guanidine derivatives.

Goodman and co-workers have published extensively on their proposed "L-shape" conformation of aspartame, neotame, and related analogues.[13−15] Their proposal is based on NMR, X-ray crystallography, and conformational energy calculations of dipeptides and retro-inverso peptides. All three side-chain rotamers of both the aspartate and phenylalanine side chains have been observed crystallographically. While X-ray crystallographic conformations are interesting, they are not necessarily good indicators of active conformation, since they are heavily influenced by crystal packing interactions with neighboring molecules (especially for zwitterionic species). Their NMR results also show the flexibility of the dipeptides.

Our proposed active conformation differs from both the Temussi and Goodman models, but it is not inconsistent with their experimental or computational results. Significantly, neither the Temussi model nor the Goodman model is consistent with the high potencies of the guanidine series. Their proposed active conformations cannot superimpose well upon low-energy conformers of the highly potent guanidine series. This is because the Temussi and Goodman models use fairly extended conformers of aspartame, while the guanidines and our proposed active dipeptide conformers are quite compact.

Summary

Although the dipeptide derivatives have hundreds of accessible conformations, most of these cannot superimpose well upon stable conformations of the high-potency guanidine. We have used systematic comparisons of intramolecular pharmacophore distances to identify the likely receptor-active conformations of the high-potency dipeptide and guanidine sweeteners.

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